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14. ABSTRACT TThe tasks performed and the progress made in the project included carrying out research on developing non-invasive near-infrared (NIR) diffuse optical imaging (DOI) approaches for detection of breast tumors in early stages of growth when those are more amenable to treatment, and developing a training program for physical scientists in biological aspects of cancer research. In particular, the following NIR-DOI approaches for locating absorbing, scattering and fluorescent targets, and estimating their size and optical properties were developed and evaluated:(i)time reversal optical tomography (TROT); (ii) optical tomography based on Non-negative Matrix Factorization(NMF-OT), and Principal Component Analysis (PCA-OT). Previously developed OT based on Independent Component Analysis (OPTICA) was further extended. The work on TROT was highlighted in Optics in 2012, a special issue of the Optics and Photonics News as one of the 30 most exciting peer-reviewed optics research to have emerged over the past 12 months. A graduate student working on the project defended his Ph.D. thesis. The results were disseminated in several publications and presentations. Several research proposals and pre-proposals were submitted. A breast cancer research program has been established at CCNY, a minority institution.					
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4. INTRODUCTION

This document constitutes the *final technical report* of the C /MI artnership Training Award project, “*Photonic Breast Tomography and Tumor Aggressiveness Assessment*” covering the entire period of performance, /1 /2 to /14/2 1 (which includes no-cost extension periods). The objective of the project was to establish a breast cancer training and research program at the City College of New York (CCN), a minority institution (MI) through close collaboration with the researchers at the Memorial Sloan ettering Cancer Center (MS CC), the mentoring institution.

The purpose of the *training* component of the project was to introduce the CCN researchers, who happen to be physical scientists and engineers, to cancer biology and technology of modern breast cancer research. The objectives of the *research* component of the project were: (a) to develop near-infrared (NIR) optical imaging and spectroscopic approaches for non-invasive detection and potential diagnosis of breast tumors at early growth stages, when those are more amenable to treatment; and (b) to explore the feasibility of distinguishing between aggressive and slow growing, metastatic and non-metastatic tumors, which happens to be one of the overarching challenges of the breast cancer research today.

The subsequent narrative will document the achievements of the project in both the training and research areas. The crowning achievement of the research component is the development a new, non-invasive, near-infrared light based optical imaging approach, *Time Reversal Optical Tomography* (TROT). The efficacy of the training component is demonstrated in the success of students and research personnel trained in the project in pursuing research and development careers in biomedical and cancer research.

This final report will provide a brief outline of the accomplishments, and refer to previous *annual reports* 1- , *appended Ph.D. thesis* of the graduate student supported by this project, and *publications* -1 that culminated from the project for detailed exposition where applicable. While the earlier publications were presented with the relevant annual reports, we append those in this final report for completeness and ready reference. The report will make reference to the specific aims and tasks in the *Statement of Work* (SOW) and the subsequently modified SOW. It is to be noted that TROT turned out to be a highly promising approach for early detection of breast tumors, and the *original SOW was modified* to properly focus on developing TROT instead of pursuing tasks involving animal model (*Specific Aims* 1-). References to relevant tasks in the modified SOW will be made in the report as well.

5. BODY

The report body is organized into two major sub-sections presenting activities and accomplishments in the training and research components of the project.

5.1. Training

The activities of the training component pursued through the first two years of the project were designed to familiarize the CCN researchers (physical scientists and laser spectroscopists) with the biological and biomedical aspects of cancer research. The training involved both theoretical instructions through dedicated courses; attending seminars and journal club discussions; as well as, hands-on training through laboratory rotations. We provide a brief overview of the combined course work and laboratory training below.

5.1.1. Courses, Seminars, Lectures, Workshops

The early training involved attending relevant courses (*Specific Aim 0, Task 1*), as well as, seminars, lectures, and workshops (*Specific Aim 0, Task 5*). The specialized training strategy included: access to MS CC resources; taking core courses in cell biology, biochemistry, genetics and pharmacology offered by the tri-institution consortium that includes MS CC, the Rockefeller University, and Weill-Cornell Medical College; and attending research seminars, journal clubs and clinical conferences.

Resources

All trainees (a graduate student, two research associates, a faculty member) were granted MS CC ID that provided them access to the libraries of the Hospital for Special Surgery, Rockefeller University and Weill Cornell Medical College. The trainees were on the mailing list for research seminar series and workshops.

Courses

The purpose of coursework was to prepare the trainees, whose research background is in physics and engineering with solid knowledge in biology, biochemistry, genetics, and pharmacological aspects of breast cancer research. Five courses were specifically selected from core courses in biochemistry Structural Biology, Cell Biology Genetics, and Molecular Biology (CM) programs and pharmacology program in graduate education at tri-institution consortium. The courses attended are briefly outlined below and further details on the content are presented in the *Appendix 1* of the First Annual Report 1.

Biochemistry: This is a two-quarter course in structural biology and contemporary biochemistry.

Molecular Genetics: This is a two-quarter course organized around the principles of genetic analysis, with examples chosen from organisms that best illustrate those principles.

Cell Biology and Development: This two-quarter course explored key aspects of cell and developmental biology at the molecular level.

Principles of Pharmacology: This one-quarter course was organized in three modules: general pharmacological principles, nervous and circulatory systems, and the remainder of the circulatory system along with host defense and endocrine systems.

Molecular Pharmacology of Cancer: This one-quarter course focused on the principles and applications of modern cancer therapeutic approaches.

Clinical Case Conferences and Journal Clubs

The trainees were assigned to attend the weekly early morning clinical conferences and journal clubs. The objective of participation in the clinical conferences is to teach the trainees clinical aspect of breast cancer care, which is important for developing a better understanding of the basic cancer research issues. The role of journal club is to expose the trainees to the cutting-edge research, and inculcate in them the abilities to carefully select and critically read the high-quality breast cancer research articles. The clinical conferences and journal clubs attended are as follows.

Breast Service (Surgery) Conference & Journal Club: The Breast Service Conference is held at MS CC every Tuesday morning from 8:00 - 9:00 A.M. A Journal Club for surgical trainees

follows the conference. At the conference, the multidisciplinary team consisting of breast surgeons, radiologists, pathologists, and oncologists discuss treatment for their recent cases.

Breast Cancer Medicine Service Conference and Journal Club: This conference is held on every Thursday morning from 8:00 - 9:00 A.M. followed by a Journal Club. This conference is a combination of administrative, patient care, and research planning and review.

Molecular Imaging Lectures and Journal Clubs

The trainees attended a didactic *molecular imaging lecture series* and *molecular imaging journal club*. An existing molecular imaging training grant to MS-CC from the Cancer Education and Career Development Program (R25) of the National Cancer Institute (NCI) supports the lecture series and the journal club. The lecture series is held weekly from 8:00 - 9:00 A.M. on Tuesday. It is intended as an introductory overview of the major methodologies used for experimental molecular cancer imaging, illustrated with specific examples of phenotypic and genotypic imaging. Examples are drawn from nuclear, MRI/MRS and optical imaging methodologies. The organizers agreed to accommodate CCN trainees in this lecture series.

5.1.2. Hands-on Training, Laboratory Rotations

While the first training involved accumulation of basic knowledge of the biological aspects of cancer research through course work, seminars, lectures, and workshops, the emphasis in the second year was on hands-on training through rotations in several breast cancer research laboratories at MS-CC (*Specific Aim 0, Task 2 and Task 3*), and keeping abreast of the recent developments in breast cancer research through participation in group meetings and seminars (*Specific Aim 0, Task 5*). The specialized training strategy included: training on animal handling at the Research Animal Resource Center (RARC) at MS-CC, and rotations through laboratories engaged in small animal imaging, and modern biomedical research methods. Specifics of the training received are described below.

5.1.2.1. Animal Handling Training

The trainees attended the following small animal training sessions (*Specific Aim 0, Task 2*), held by RARC at MS-CC as observers:

- *RARC Orientation* training session;
- *RARC Basic Mouse* training session that included handling and restraint; injectable anesthetics and anesthetic monitoring; parenteral administration (SC, IP, IV); blood sampling; and euthanasia;
- *RARC Rodent Survival Surgery* training session that involved surgical equipment and instruments, disinfection and sterilization, animal preparation, surgeon preparation, suture materials and wound closure, and post-operative care;
- *RARC Hazardous Material* training session that included introduction to the designated hazardous materials use areas, entry requirements, investigative staff responsibilities; animal/material manipulations, waste disposal/transportation of materials, and euthanasia.
- *RARC Xenograft* training session that focused on prevention of infectious disease transmission to laboratory researchers, animal care staff and other individuals who service the areas where these experiments are performed;

- *RARC special training on tail vein injection; and*
- *RARC special training on necropsy.*

5.1.2.2. Laboratory Rotations

The trainees rotated through the following four laboratories and received training on imaging techniques, cell culture, bioluminescence assay, Western blotting, and other key techniques used in cancer research.

Rotation I

Laboratory I: Dr. Ronald Blasberg

Laboratory background: The focus of the current research in this lab is on transgene (reporter gene) imaging using noninvasive nuclear and optical techniques.

Training Topic: *Cell culture and Bioluminescence Imaging*

- *Cell culture and bioluminescence training:* The trainees learned the basic cell culture techniques. Each of them had the responsibility to maintain two genetically modified breast cancer cell lines: MDA231-Fluc-I-F and MDA435-Fluc-I-F. Routine maintenance, such as subculture and cell counting, was performed.
- *Bioluminescence assay and imaging training:* The trainees learned the luciferase assay method for evaluating the level of expression of bioluminescence reporter gene (luciferin) and learned how to measure the linearity of bioluminescence intensity vs. cell numbers using Biospace Photon Imager. One of the trainees identified saturation problems in the imaging system, which may cause misinterpretation of experimental results for large number of cells in plates or animals. He suggested a remedy that has been implemented to correct for the problem.

Rotation II

Laboratory I: Dr. David Solit

Laboratory background: This laboratory focuses on the development of cancer therapies that target pathways responsible for cancer initiation and progression.

Training Topics: *Western Blotting and Flow Cytometry*

The techniques that the trainees learned include:

- *Western blot technique* for detecting specific proteins related to RAS/RAF/MAPK / Rk signal pathway: pRb, Rk, pM, M, Cyclin D1, p21, R, TNF, Cleaved PARP, and Caspase-3.
- *Drug (PD901) treatment and cell growth curve measurement* for OECAR, T24, and MCF cell lines.
- *Flow cytometry technique* for cell cycle analysis and Nussle nuclear preparation method for DNA content was taught.

Rotation III

Small-Animal Imaging Core

Facility Manager: Dr. Pat Zanzonico

Facility background: The imaging core facility provides MS CC investigators with unique capabilities for noninvasively detecting, localizing, and biologically characterizing primary and metastatic cancer cells *in vivo* in small-animal models.

Training Topics: Major small animal imaging techniques and systems

The trainees learned how to use the following imaging equipment and techniques:

- Micro- T T (positron emission tomography)scanners from CTI-Concorde Microsystems ;
- ioluminescence imaging system from Xenogen (Capiper) Corp.;
- Micro-CT CT (computed tomography) scanner from Imtek Corp.;
- S CT / CT scanner from amma Medica;
- ltra Sound from isual Sonics; and
- Fluorescence imaging system from CRI.

Rotation IV

Magnetic Resonance Imaging (MRI) aboratory / Small-animal MRI Core

aboratory I: Dr. Jason Koutcher

aboratory/Facility background: The MRI/MRS laboratory / facility are directed by Dr. outcher (Co- I) and equipped with a 4. T and a T magnet resonance imaging /spectroscopy systems.

Training Topics: MRI / MRSI techniques

The trainees observed the following procedures and experiments:

- Design and build radiofrequency coils for MRI/MRS experiments;
- Acquisition and reconstruction of high resolution 1- (standard) images in different orientations (axial, sagittal, and coronal) in small animals (up to cat size) and scans on mice whole-body, brain, lung, kidney, prostate, bladder cancers;
- Dynamical contrast imaging (DC) technique for analysis of perfusion;
- Magnetic resonance spectroscopy of lactate for metabolite quantization of animals.

5.1.3. Other Training Activities

The CCN and MS CC group met regularly to discuss the progress during the ear 2 training. The trainees attend the weekly MS CC MRI group meeting and selected seminars (*Specific Aim 0, Task 5*).

While the original plan was to introduce a seminar series (*Specific Aim 0, Task 4*) and a one-day symposium (*Specific Aim 0, Task 6*) at CCN under this project, such activities were implemented by a CCN -MS CC artnership for Cancer Research, Training and Community Outreach that came together with substantial support from the National Institute of ealth, and

amply served the objectives of those two tasks. The researchers involved in this project participated and benefited from the seminars and symposia organized by the CCN -MS CC artnership.

5. 2. Research

The major thrust of the research activities was on developing and testing *non-invasive near-infrared optical imaging modalities for early detection of breast cancer Specific Aim 4* . Our focus has been on developing non-iterative approaches to realize fast, near-real-time image reconstruction of small targets. Retrieving rather accurate three-dimensional location information has been another of our priorities. arly detection of breast tumor with requisite sensitivity and specificity is a challenging undertaking and we pursued developing and testing different approaches. Diffuse optical tomography (DOT) approaches may make use of the absorption, scattering, as well as, intrinsic and extrinsic fluorescence contrast between normal breast tissues and tumors. We pursued development of experimental methods and numerical algorithms for absorption, scattering and fluorescence tomography, and assessment of the relative efficacy of these three contrast-based approaches.

Another research thrust was to develop methods that enable one to noninvasively distinguishing between slow and fast growing breast tumors so that appropriate treatment protocol may be implemented. It was hypothesized that tumor growth and metastatic risk correlates with vascular permeability, vascular volume, hypoxia and lactate, and these can be studied using optical and MR techniques. While according to the modified SOW, we focused on developing TROT and other DOT techniques instead of pursuing tasks involving animal model (*Specific Aims 1-3*), we explored the feasibility of monitoring lactate using magnetic resonance (MR) spectroscopy.

The tasks performed and the progress made in the project may be broadly grouped as follows:

- Development of experimental arrangements;
- Development of the *Time Reversal Optical Tomography* (TROT) approach;
- Comparison of the efficacy of diffuse optical tomography using different *Decomposition Methods*;
- Development of *fluorescence tomography* approaches;
- Diffuse optical imaging of “realistic” model cancerous breasts using TROT, and decomposition methods;
- xploration in *finite element methods* (F M) for complementing TROT and decomposition methods; and
- *Magnetic Resonance Imaging and Spectroscopy* for lactate detection.

5.2.1. Development of Experimental Arrangement

The research activities of the project started with securing regulatory approval for carrying out experiments on *ex vivo* human breast tissues (*Specific Aim 4, Task #13*), which was followed by the development of near-infrared (NIR) optical imaging arrangements (*Specific Aim 4, Task #14*). We developed a modular experimental arrangement with sufficient flexibility for modification enabling us to carry out a variety of experiments. The core experimental arrangement, displayed schematically in Fig. 1, could be used for diffuse optical imaging based

on the absorption, scattering, and fluorescence contrasts between the target and the intervening turbid medium with simple modifications. It could provide data for diffuse optical imaging using TROT, and different decomposition methods.

The experimental arrangement used a multi-source sample excitation and multi-detector signal acquisition scheme to acquire multiple angular views of the sample. The optical sources for interrogating the sample (consisting of targets embedded in a rectangular slab of a turbid medium) included a mode-locked Ti: sapphire laser operating in the tunable wavelength range of $700\text{ nm} - 1000\text{ nm}$; and two continuous wave (CW) diode lasers, one of which operated at 780 nm and the other at 830 nm . The particular arrangement shown in Fig. 1 is for fluorescent imaging, but could be readily used for absorption or scattering contrast-based imaging by removing the narrow-band filter that blocks the interrogating pump beam and transmit the fluorescence signal.

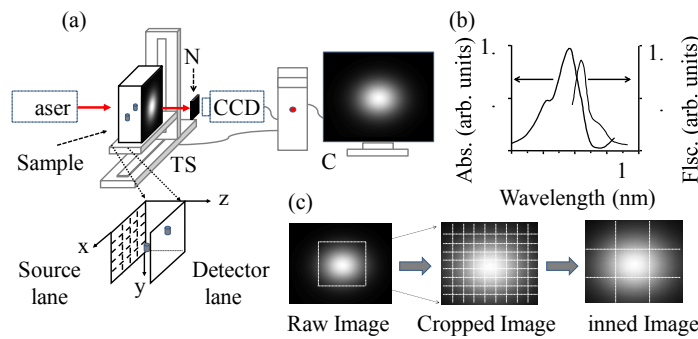


Fig.1. (a) A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium. Key: N narrow band pass filter, TS translational stage, CCD charge coupled device, C personal computer. Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam and a typical raw image is shown in the C monitor. (b) The absorption and fluorescence spectra of IC in water. (c) A typical raw image is cropped and binned.

The entrance face of the slab sample (*source plane*) was illuminated by the laser beam. The multi-source illumination scheme was realized by step scanning the sample across the laser beam in a two-dimensional x - y array of grid points using a computer-controlled translation stage. A cooled CCD camera equipped with a 50-mm focal-length camera lens collected and sensed a fraction of the forward propagating signal from the opposite face of the sample (*detection plane*). The signal, for absorptive and scattering targets, was a diffusely transmitted fraction of the incident beam which could be collected without additional filtering. For fluorescence imaging, a narrow-band (full-width-at-half-maximum (FWHM) bandwidth of 1 nm) interference filter with peak transmission around the fluorescence maximum was used to transmit the fluorescence signal and selectively block the interrogating beam. The CCD camera had 128×128 pixels with a pixel size of $24\text{ }\mu\text{m}$, and was considered as multi-detector, since each illuminated pixel could be considered as an individual detector. The scanning and data acquisition processes were controlled by a personal computer (C). Raw images were recorded by the C for each scan position, and stored for subsequent analysis. A typical image, which is a 2-D intensity distribution, is shown in the left frame of Fig. 1(c).

Further experiment specific details of experimental arrangement, laser wavelength, data acquisition scheme, scanning range and parameters are provided in References 1- and relevant chapters of Reference , as well as in published papers -1 .

5.2.2. Time Reversal Optical Tomography (TROT)

A major accomplishment of the research component of the project is the development of the *Time Reversal Optical Tomography* (TROT) approach for detection and localization of small targets embedded in a turbid medium (*Specific Aim 4, Tasks #1 and #2* of modified SOW).

TROT combines the idea of time reversal (TR) invariance, the basic symmetry that commonly holds in microscopic physics, and has been used for macroscopic imaging applications 1 -22 with the vector subspace classification method of Multiple Signal Classification (MUSIC) 2 -24 . While the TR imaging and MUSIC have been used in acoustics, ultrasonic, and radar imaging applications 1 -24 , we have developed it for NIR light-based imaging, target detection and localization applications , , 1 , 12-14, 1 .

The details of the TROT formalism and its application has been presented , , 1 , 12-14, 1 , and reported in part in earlier progress reports - . We refer to those reports and publications (appended to this document as well) for details, and present a brief overview here for completeness.

5.2.2.1. TROT formalism for absorptive and scattering targets

The propagation of a near-infrared (NIR) beam of light through a highly scattering turbid medium with embedded targets, whose optical properties are different from that of the intervening medium, may be approximated to be the diffuse transmission of light through background medium of uniform optical characteristics, with targets as perturbations. The starting point for the TROT formalism is the diffusion approximation 2 -2 of the radiative transfer equation (RT) 2 , 2 . The perturbation in the light intensity distribution due to small inhomogeneities (targets) embedded in a homogeneous medium, to the first order Born approximation, can be written as , 1

$$\begin{aligned} \Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = & - \int G(\mathbf{r}_d, \mathbf{r}) \delta\mu_a(\mathbf{r}) c G(\mathbf{r}, \mathbf{r}_s) d\mathbf{r} \\ & - \int \delta D(\mathbf{r}) c \nabla_r G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_r G(\mathbf{r}, \mathbf{r}_s) d\mathbf{r}, \end{aligned} \quad (1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a point-like source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagations from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ is the difference in absorption coefficient and δD is the difference in diffusion coefficient between the targets and the background medium; and c is the light speed in the medium.

A multi-source interrogation and multi-detector signal acquisition scheme (as described in Section 2.1) is used to acquire transillumination data, from which the difference in the light intensity distribution due to the targets, $\Delta\phi = \phi - \phi_0$, is found, where ϕ is the light intensity distribution measured on the sample boundary with targets embedded in the scattering medium and ϕ_0 is ideally the light intensity distribution without the targets, which in practice is approximated by an “average” over all the multi-source measurements. A response matrix K is constructed with $-\Delta\phi$, to describe the transport of light from different sources through the embedded objects to the array of detectors.

For small, point-like absorptive targets, the matrix elements can be rewritten in a discrete form as:

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{X}_m) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), \quad i=1,2,\dots,N_d; j=1,2,\dots,N_s, \quad (2)$$

where $\tau_m = \delta\mu_a(\mathbf{X}_m)c\delta V_m$ is the optical absorption strength of the m^{th} target, δV_m is the volume of m^{th} target, $\mathbf{r}_i, \mathbf{r}_j$ and $\mathbf{X}_m$ are locations of the i^{th} detector, j^{th} source and m^{th} target, respectively. Due to the reciprocity of light propagation in the medium, $G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r}', \mathbf{r})$. Thus,

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{X}_m, \mathbf{r}_i) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), \quad (3)$$

and

$$K = K_{ij} = \sum_{m=1}^M \mathbf{g}_d(\mathbf{X}_m) \tau_m \mathbf{g}_s^T(\mathbf{X}_m), \quad (4)$$

where $\mathbf{g}_s(\mathbf{r})$ and $\mathbf{g}_d(\mathbf{r})$ are Green's function vectors (F-s) associated with the source array and detector array, respectively. F-s are defined as

$$\mathbf{g}_s(\mathbf{r}) = [G^s(\mathbf{r}_1, \mathbf{r}), G^s(\mathbf{r}_2, \mathbf{r}), \dots, G^s(\mathbf{r}_{N_s}, \mathbf{r})]^T, \quad (a)$$

$$\mathbf{g}_d(\mathbf{r}) = [G^d(\mathbf{r}_1, \mathbf{r}), G^d(\mathbf{r}_2, \mathbf{r}), \dots, G^d(\mathbf{r}_{N_d}, \mathbf{r})]^T, \quad (b)$$

where the superscript T denotes transpose; and N_s, N_d and M are the numbers of sources, detectors and targets, respectively. The number of targets is assumed to be less than the number of sources and number of detectors, $M < \min(N_d, N_s)$. It also holds that $K^T = K_{ji}$ describes light propagation from the positions of detectors through the medium and targets to sources.

For a homogeneous background medium, the rank R of matrix K , is equal to the dimension of the source array vector space $\mathcal{S}$ spanned by $\mathbf{g}_s(\mathbf{r}_m)$, and also equal to the dimension of the detector array vector space $\mathcal{D}$ spanned by $\mathbf{g}_d(\mathbf{r}_m)$, where $\mathcal{S} \subseteq C^{N_s}$ and $\mathcal{D} \subseteq C^{N_d}$. For absorptive targets, R is equal to the number of targets M .

Similar forms of the response matrix and F-s can be obtained for scattering targets. As the dot product in the second term of eq. (1) implies, each scattering target is represented by three components coexisting at one location. The elements of the K matrix for L scattering target may be written as

$$\begin{aligned} K_{ij} &= \sum_{l=1}^L \tau_l \nabla_{\mathbf{r}} G^d(\mathbf{r}_i, \mathbf{X}_l) \cdot \nabla_{\mathbf{r}} G^s(\mathbf{X}_l, \mathbf{r}_j) \\ &= \sum_{l=1}^L \tau_l \sum_{\alpha=x,y,z} \partial_{\alpha} G^d(\mathbf{r}_i, \mathbf{X}_l) \partial_{\alpha} G^s(\mathbf{X}_l, \mathbf{r}_j), \end{aligned} \quad (5)$$

where $\tau_l = \delta D(\mathbf{X}_l)c\delta V_l$ is the optical scattering strength of the l^{th} target. The K matrix for scattering targets can be written in a manner similar to that for absorptive targets:

$$K = \sum_{l=1}^L \sum_{\alpha=x,y,z} \partial_{\alpha} \mathbf{g}_d(\mathbf{X}_l) \tau_l \partial_{\alpha} \mathbf{g}_s^T(\mathbf{X}_l). \quad (6)$$

The TR matrix may be constructed to represent light propagation from sources to detectors and back denoted by T_{SDDS} , or to represent light propagation from detector positions to source

positions and back denoted by T_{DSSD} , a consequence of the reciprocity of light propagation [2, 2]. For frequency-domain data, $T_{SDDS} = K K^T$, and $T_{DSSD} = (K^T)^T K^T = K K^T$, where response data matrix K is formed using modulated intensities, instead of the field with phase information used in the conventional TR. For CW measurements, $T_{SDDS} = K^T K$, and $T_{DSSD} = K K^T$ (K is real and only includes intensity values).

Since T_{SDDS} and T_{DSSD} are hermitian ($T = T^T$), they have complete sets of orthonormal eigenvectors v_j ($j=1, \dots, N_s$) and u_i ($i=1, \dots, N_d$), with a common set of non-negative real eigenvalues. For $M < \min(N_s, N_d)$ absorptive targets without the presence of noise, the rank of T_{SDDS} and T_{DSSD} is M . The eigenvalues $\lambda_j > 0$, when $j=1, \dots, M$, and $\lambda_j \approx 0$, when $j=M+1, \dots, N_s$ for T_{SDDS} and $j=M+1, \dots, N_d$ for T_{DSSD} . The eigen system $v_j, u_j, \lambda_j > 0$, $j=1, \dots, M$, is related to the targets. The TR matrix T_{SDDS} can be written as

$$T_{SDDS} = \sum_{m=1}^M \sum_{l=1}^M \tau_m \tau_l \langle g_d | \mathbf{X}_m, g_d | \mathbf{X}_l \rangle g_s | \mathbf{X}_m g_s^T | \mathbf{X}_l. \quad (1)$$

A similar expression follows for T_{DSSD} . The target locations are obtained as poles in the pseudo-spectra of T_{SDDS} and T_{DSSD} , as detailed in Reference [1] and Chapter 2 and Chapter 3 of Reference [1].

5.2.2.2. Application of TROT Approach and Representative Results

The efficacy of TROT for detecting and locating targets in turbid media has been tested in a variety of simulations with and without background noise, using experimental data for model samples whose size and average optical properties simulated those of a typical human breast, and using experimental data using a realistic cancerous breast formed using *ex vivo* human breast tissues with two embedded tumors. We mention below some key results and point out original sources for finding details about those results.

- In simulation, TROT could detect and accurately retrieve three-dimensional location information of six targets embedded in a 4 -mm thick uniform scattering slab. Its absorption and diffusion coefficients were $\mu_a = 1/\text{mm}$ and $D = 1/\text{mm}$, respectively [Fig. 1 and Section 4 of Ref. [1], and Chapter 2 Fig. 2.1 of Ref. [1], appended].
- In experiments involving targets embedded in a turbid sample (a 2 mm × 2 mm × 2 mm transparent plastic container filled with Intralipid-2 suspension in water, absorption coefficient $\mu_a = 1/\text{mm}$ at 660 nm, and a transport mean free path $l_t = 1$ mm, which were similar to the average values of those parameters for human breast tissue, while the cell thickness of 1 mm was comparable to thickness of a typical compressed breast) TROT formalism achieved the following results:
 - (a) Detected and located a single absorptive target (a glass sphere of diameter 1 mm which had the same scattering coefficient as the background medium and an absorption coefficient approximately 10 times higher) at depths (position along z-axis, total path length 1 mm) of 1 mm, 2 mm, 2 mm, 3 mm, 3 mm, 4 mm and 4 mm within 1 mm of the known positions [Section 2.1 of Ref. [1], and Chapter 2 Section 2.1 of Ref. [1], appended].
 - (b) Similar result was obtained for a single scattering target within 1 mm of the known positions [Section 2.2 of Ref. [1], and Chapter 2 Section 2.2 of Ref. [1], appended].

- (c) Two absorptive targets of similar characteristics as that in (a) above could be resolved even when their nearest sides were separated by only 4 mm (Section 2.2 of Ref. 1, and Chapter 2 Section 2.2 of Ref. 2, appended).
- TROT could retrieve the location and provide reasonable estimates of optical property (absorption strength and scattering strength of absorptive target and scattering target, respectively) for somewhat extended targets, but the estimate of size and shape needs further improvement. Absorption strength and scattering strength are defined in Section 2.2.1 above following eq. (2) and eq. (3), respectively (Reference 3, Chapter 4 of Reference 4, and Reference 12).
- TROT could successfully locate tumors in a realistic cancerous model breast (Section 2.2 below) and fluorescent targets (Section 2.4.1 below).

The salient feature of TROT is that it is a faster and less computation intensive approach for detecting small targets in highly scattering turbid media and determining their locations in 3-D than other inverse image reconstruction (IIR) techniques. While the dominant features in the pseudo spectrum are related to the square of the difference between the absorption (scattering) coefficient of the targets and that of the background, the approach does not directly determine these parameters. It is common for IIR approaches to estimate the optical properties of every voxel in the sample and identify target(s) from differences of these properties between the sample and the target(s), which is a considerably computation intensive undertaking. On the contrary, TROT identifies the targets as poles of the pseudo spectrum and focuses on determining their positions, which do not require as much computation time. Other IIR approaches involve iteration, while TROT is non-iterative. In TROT the data dimension is lower compared to other IIR approaches, which enables analysis and utilization of very large datasets. These two features together make TROT faster. Fast image reconstruction algorithms are of particular interest for real-time or near real-time biomedical imaging applications.

5.2.3. Diffuse Optical Imaging Using Decomposition Methods

The initial plan of the project was to use Optical Tomography using Independent Component Analysis (O-TICA), which we developed earlier (2002), for NIR image reconstruction. We extended O-TICA to include multi-wavelength probing and explored its efficacy on a more realistic model breast (*Specific Aim 4, Task #15 and Task #16*) and reported the results in an earlier Annual Report (4) and in a refereed publication (5). Diffuse optical imaging (DOI) for detection and retrieval of location information of targets in a highly scattering turbid medium may be treated as a “blind source separation” (BSS) problem (6). Independent Component Analysis (ICA) (7), the basis for O-TICA, is one of the different matrix decomposition methods for solving the BSS problem and retrieving desired information. Other decomposition methods include Principal Component Analysis (PCA) (8) and Non-negative Matrix Factorization (NMF) (9).

BSS is a general problem in information theory that involves retrieval of “component” signals from measured signals. The measured signals are weighted mixtures of the component signals contributed by the targets (“blind sources”) and may be expressed as a matrix equation:

$$X = AS, \quad (1)$$

where rows of X represent the measured mixed signals, rows of S represent the component signals, and A is the mixing matrix.

The three algorithms ICA, CA, and NMF have different assumptions, which may lead to different favored conditions. In addition to OTICA, we pursued the use of CA and NMF for solving the diffuse imaging problem, and carried out a systematic study using simulated and experimental data to compare the performance of the three decomposition methods. We first reported the initial results of this comparative study in our fourth Annual Report [4]. The study involved detection and retrieval of three-dimensional ($-D$) location information of *absorptive* and *scattering* targets in a model medium whose thickness, optical absorption coefficient, scattering coefficient, transport length, and anisotropy factor were similar to the average values of those parameters for a compressed human breast. The key result was that the approaches could detect and extract $-D$ location of small tumor-like targets within 2 mm of their known locations. The formalism and some results were presented in the fourth Annual Report [4], and subsequently we published a peer-reviewed journal article [11] presenting the experimental methods, materials, algorithms, and key results, which is appended to this report. Further details including simulation results under a variety of conditions appear in Chapter 4 of Reference [11].

It was apparent from our work on absorptive and scattering targets that the NMF formalism, because of its non-negativity constraint, may be particularly suited for detection of fluorescent targets. We explored development of NMF-based fluorescence tomography (*Specific Aim 4, Task #3 of modified SOW*) and present it in Section 2.4.2.

5.2.4. Fluorescence Tomography

Diffuse optical imaging approaches for breast cancer detection may be developed capitalizing on the absorption, scattering, and fluorescence contrasts between the tumor and the surrounding tissues. However, there has been a recent surge in interest in fluorescence tomography because of its superior detection sensitivity and specificity, higher signal-to-background ratio and better spatial resolution than other diffuse optical imaging (DOI) approaches, and potential to provide molecular information on disease-induced changes in biological tissues [4–41]. In addition, advances towards development of target-specific exogenous contrast agents [4, 42, 43], imaging instrumentation [44, 45], as well as, analytical methods and numerical algorithms [41, 46] hold the promise for noninvasive detection and characterization of tumors. The use of target-specific contrast agent endows fluorescence tomography with potentially higher contrast, and specificity. The key attributes of a clinically useful cancer imaging modality include early detection, adequate spatial resolution, speedy image reconstruction, and diagnostic potential. Fluorescence-based approaches are sensitive to molecular changes and may provide useful diagnostic information, but diffuse nature of light propagation in biological tissues impedes spatial resolution. For these reasons, we initiated the development of fluorescence tomography towards the end of the current reporting period.

We have pursued the development of fluorescence tomography approaches based on the TROT and NMF, both of which we previously explored and tested for absorptive and scattering targets [4, 11]. While other fluorescence tomography approaches are computation intensive since those try to iteratively compute fluorescence properties (such as, fluorophore concentration) of every voxel of the sample volume, both TROT and NMF are non-iterative and are expected to provide faster localization.

The experimental arrangement of Fig. 1 with the fluorescence transmitting and excitation beam blocking narrow-band filter N was used for multi-source illumination and multi-detector signal acquisition scheme to acquire multiple angular views of the sample. The input surface (source plane) of the slab sample is scanned across an external point light source (laser beam). The fluorescent target(s) embedded in the highly scattering medium are excited by diffusely propagating light of wavelength λ_x . A fraction of the forward propagating fluorescence signal emitted by the targets at wavelength λ_m , is detected on the other side of the sample by a two-dimensional detector array. For small fluorescent targets at $\mathbf{r}_j$ with volume V_j , the fluorescence signal from the target at $\mathbf{r}$ illuminated by a point source of unit power at $\mathbf{r}_s$ is given by 4

$$K = \sum_j g_d(\mathbf{r}_j, \omega) f_j(\omega) g_s^T(\mathbf{r}_j, \omega), \quad (1)$$

where $g_s(\mathbf{r}, \omega) = G_x(\mathbf{r}, \mathbf{r}_s, \omega)^T$ and $g_d(\mathbf{r}, \omega) = G_m(\mathbf{r}_d, \mathbf{r}, \omega)^T$, (the superscript T denotes transpose); $G_x(\mathbf{r}, \mathbf{r}_s, \omega)$ is a Green's function that describes the propagation of excitation light at excitation wavelength λ_x from the source at $\mathbf{r}_s$ to the target at $\mathbf{r}$; $G_m(\mathbf{r}_d, \mathbf{r}, \omega)$ is a Green's function that describes the propagation of the fluorescent light at emission wavelength λ_m from the target at $\mathbf{r}$ to the detector at $\mathbf{r}_d$; ω is the modulation angular frequency of the light; $f_j(\omega)$ is the fluorescence strength of the j^{th} target, given by

$$f_j(\omega) = \gamma(\mathbf{r}_j) c_m V_j / (1 - i\omega\tau(\mathbf{r}_j)), \quad (11)$$

γ is the fluorescence yield, c_m is the speed of light in the medium, and τ is the fluorescence lifetime. K describes that the diffuse propagation of excitation light of wavelength, λ_x from the sources through the medium to illuminate the targets, and then the propagation of emitted fluorescence of wavelength, λ_m from the targets to the detectors. In this study, continuous wave (CW) illumination is used, i.e. $\omega = 0$.

5.2.4.1. Fluorescence TROT

A time reversal matrix $T_{SDDS} = K^T K (T_{DSSD} - K K^T)$ is constructed. A set of eigenvectors u_k , $k = 1, \dots, N_d$ and v_l , $l = 1, \dots, N_s$ are calculated for T_{DSSD} and T_{SDDS} , respectively, with common eigenvalues μ_j , $j = 1, \dots, \min(N_s, N_d)$, where N_s and N_d are numbers of sources and detectors, respectively [11]. The eigenvalues $\mu_j = |f_j|^2 \|g_d(\mathbf{r}_j, \omega)\|^2 \|g_s(\mathbf{r}_j, \omega)\|^2$ are proportional to squared fluorescence strengths of the targets if the targets are well resolved; otherwise, they are linear combinations of individual fluorescence strengths of different targets [11].

By using an L -curve method with an eigenvalue threshold $\varepsilon = 4$, eigenvectors are separated into signal and noise subspaces. A pseudo spectrum associated with detector plane is calculated using multiple signal classification (MUSIC) for a test target position $\mathbf{X}_p$ in the sample volume [11]

$$P_d(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| u_j^T \frac{g_d(\mathbf{X}_p, \omega)}{\|g_d(\mathbf{X}_p, \omega)\|} \right|^2. \quad (12)$$

The locations of targets are retrieved to be the poles of the pseudo spectrum. A similar pseudo spectrum for the source plane,

$$P_s(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| v_j^T \frac{g_s(\mathbf{X}_p, \omega)}{\|g_s(\mathbf{X}_p, \omega)\|} \right|^2. \quad (13)$$

or for both source and detector planes

$$P(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j \in \mathcal{E}} \left(\left| u_j^T \frac{g_d \mathbf{X}_p, \omega}{\|g_d \mathbf{X}_p, \omega\|} \right|^2 + \left| v_j^T \frac{g_s \mathbf{X}_p, \omega}{\|g_s \mathbf{X}_p, \omega\|} \right|^2 \right). \quad (14)$$

may also be used to retrieve the target position.

The sample used in the experiment to demonstrate the approach was a 2 mm × 2 mm mm rectangular transparent plastic cell filled with Intralipid-2 (axter) suspension in distilled water with a fluorescent targets embedded inside. The thickness of the sample was comparable to that of a typical compressed breast. The concentration of Intralipid-2 was adjusted 4 to provide a transport mean free path l_t of 1mm, which happens to be about the same for both excitation and emission wavelengths, and was similar to the average value of l_t for human breast tissue at these wavelengths. The fluorescent target was a 4.2 mm inner-diameter 1 -mm long cylindrical glass tube filled with solution of IC dye (Sigma-Aldrich) water. The dye solution in the targets was prepared by dissolving IC at a concentration of 1 μM in the Intralipid-2 suspension of same concentration as the background medium to ensure that the target had the same scattering coefficient as the background medium, but a higher absorption coefficient of . 2 mm⁻¹. The water solution of IC absorbs light over the nm range with peak at and fluoresces in the nm range with peak at around 2 nm in the NIR enabling deeper penetration of light in tissues. It has been approved by Food and Drug Administration (FDA) for biomedical applications.

The target was embedded in the mid-plane (z mm) of the sample cell. The entrance face of the slab sample (*source plane*) was illuminated by a 1 -mW -nm diode laser beam. The multi-source illumination scheme was realized by scanning the sample across the laser beam in a two-dimensional x-y array of x grid points with a -mm step size using a computer-controlled translation stage. A cooled CCD camera equipped with a -mm focal-length camera lens collected and sensed a fraction of the forward propagating fluorescence signal from the opposite face of the sample (*detection plane*) through a narrow-band (full-width-at-half-maximum (FW M) bandwidth of 1 nm) interference filter with peak transmission at nm. The CCD camera had 1 24 × 1 24 pixels with a pixel size of 24 μm, and was considered as multi-detector, since each illuminated pixel could be considered as an individual detector. The N filter was chosen to select a substantial fraction of the IC fluorescence around the peak emission wavelength and block the transmitted nm pump light. The scanning and data acquisition processes were controlled by a personal computer (C). Raw images were recorded by the C for each scan position, and stored for subsequent analysis.

At every scan position, an image was also acquired with the N filter removed. In this case, the recorded images were essentially transillumination images since the fluorescence signal was negligible compared to the much more intense transmission signal (ratio of transmission signal to fluorescence signal 1). Thus correlated imaging and retrieval of target location using both fluorescence and transmission measurements were enabled in this experiment. The transmission images were analyzed to estimate the average value of the optical property of the medium $\kappa (\mu_a \mu_s')^{1/2}$, where μ_a and μ_s' are the absorption and reduced scattering coefficients at nm, respectively. In fact, the values of these optical parameters of Intralipid-2 suspension in water happened to be very close for excitation and fluorescence wavelengths.

From each fluorescence image, a region of interest was cropped out and every pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The response data matrix was constructed using the transmitted fluorescence light intensity distribution in the processed images. The TR matrix was generated by multiplying the response matrix by its transpose for our CW probing scheme. The eigenvalue equation of TR matrix was solved. Then the signal and noise subspaces were separated. M-SIC pseudo spectrum was calculated and target locations were determined using the poles in the pseudo spectrum.

For comparison, the transmission data were also analyzed using TROT as detailed in Ref. 1. In this case, the target was absorptive, and the contrast was mainly due to higher absorption of the excitation beam by the target. It should be noted that absorption measurement involves changes in the intensity of the excitation beam, and consequently the TROT analysis used the difference images between the raw transmission images and a reference image for the background medium. The reference image may be estimated as the average of the images acquired at all scan positions.

The TR matrix was constructed using the fluorescence data, and an eigenvalue equation was then solved. The eigenvector with the dominant eigenvalue was used to calculate the pseudo spectrum for all voxels in the $-D$ space of the sample. The voxel size was $.5 \text{ mm} \times .5 \text{ mm} \times 1 \text{ mm}$. Three-dimensional tomographic pseudo images were generated using the pseudo spectrum. The single target was detected, and the position of the target was determined using the peak in the pseudo spectrum and listed in Table II, with comparison to the actual position. The image at the retrieved z -coordinate of the target position ($z = 2.5 \text{ mm}$) plotted using the pseudo spectrum is shown in Fig. 2(a).

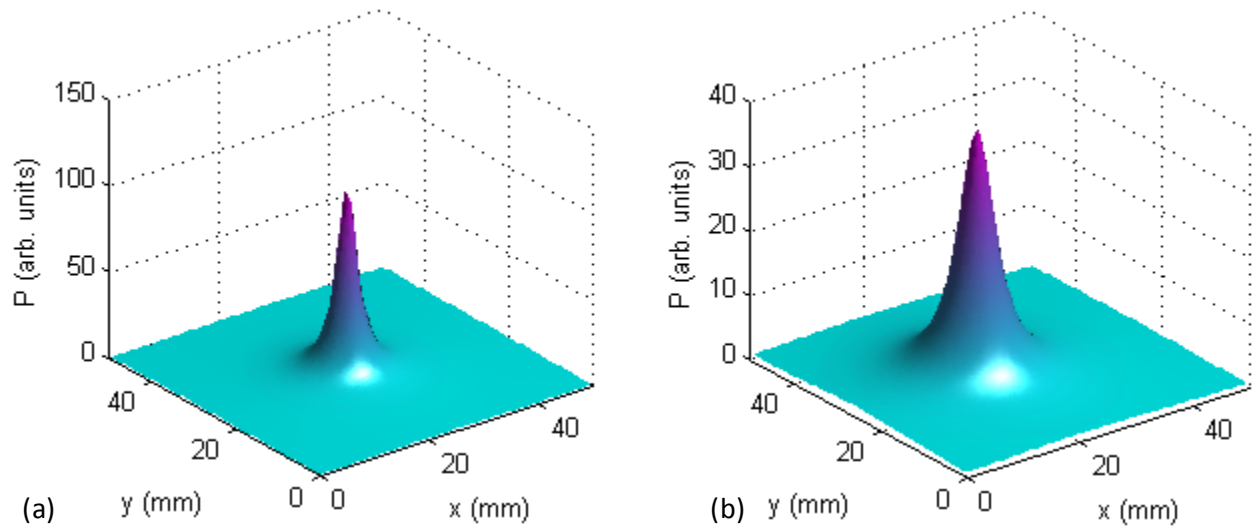


Fig. 2 TROT-reconstructed image at $z = 2.5 \text{ mm}$ using *fluorescence* data is shown in (a), and at $z = 2.5 \text{ mm}$ using *transmission* data shown in (b).

The transmission data was then analyzed for comparison. The target was also detected and its location was retrieved. The image of the target at $z = 2.5 \text{ mm}$ is shown in Fig. 2(b).

As shown in Table I, the location of target retrieved from the fluorescence data is in excellent agreement with the known position, and is consistent with that retrieved using transmission data. The pole of the pseudo image using fluorescence data is sharper than that obtained using

transmission data. The FWHM of the pole in both x and y directions in the fluorescence-TROT image is 0.5 mm and 0.5 mm in the transmission-TROT image.

Table I. Known and retrieved target positions.

	Known	Retrieved	
		Fluorescence	Transmission
Position x, y, z (mm)	20.1, 20.5, 20.5	24.1, 20.5, 20.5	21.5, 20.5, 20.5
Error $\Delta x, \Delta y, \Delta z$ (mm)	-	1.5, 0.1, 0.5	0.5, 0.1, 0.5
FWHM $\delta x, \delta y$ (mm)	-	0.5, 0.5	0.5, 0.5

The results show that *fluorescence* TROT approach could detect and retrieve the locations of a small fluorescent target embedded in a breast-simulating turbid medium with the thickness comparable to that of a realistic compressed breast. The location of targets was retrieved with an accuracy of 1 mm in all three dimensions. Further details appear in the fifth Annual Report [14], in a refereed publication [14] which is appended, and in Chapter 5 of Reference [14].

5.2.4.2. NMF-based Fluorescence Tomography

The same experimental data was further analyzed using the NMF algorithm (*Specific Aim 4, Task #3 of modified SOW*). The formalism considers the fluorescence signal to be a weighted mixture of signals arriving from the embedded targets. The fluorescence signal measured by the detector at $\mathbf{r}_d$ for illumination of the source point at $\mathbf{r}_s$ may be expressed as:

$$I(\mathbf{r}_d, \mathbf{r}_s) = \sum_j a_j(\mathbf{r}_d) s_j(\mathbf{r}_s), \quad (1)$$

where $a_j(\mathbf{r}_d)$ is the mixing vector and $a_j(\mathbf{r}_d) s_j(\mathbf{r}_s)$ represents the contribution of the j^{th} target to the signal I , and the sum is over all the targets (“fluorescent sources”). NMF retrieves $s_j(\mathbf{r}_s) = s_j(\mathbf{r}_{s1}), s_j(\mathbf{r}_{s2}), \dots, s_j(\mathbf{r}_{sN_s})$ and $a_j(\mathbf{r}_d) = a_j(\mathbf{r}_{d1}), a_j(\mathbf{r}_{d2}), \dots, a_j(\mathbf{r}_{dN_d})^T$ assuming those to be non-negative, where superscript $(^T)$ denotes transpose. We implement the alternating least squares (ALS) method [4] that steps to estimate A (or S), and use that estimate to optimize S (or A), and keep repeating the alternative steps until the desired optimization is obtained. Non-negativity is ensured by setting any negative element of A or S equal to 0. It further follows

$$s_j(\mathbf{r}_s) = \alpha_j G_x(\mathbf{r}_j, \mathbf{r}_s, \omega), \quad (1)$$

and

$$a_j(\mathbf{r}_d) = \beta_j G_m(\mathbf{r}_d, \mathbf{r}_j, \omega), \quad (1)$$

where α_j, β_j are scaling factors. One refers to $s_j(\mathbf{r}_s)$ and $a_j(\mathbf{r}_d)$ as non-negative component intensity distributions (NCIDs) on the source plane and detector plane, respectively, since those are proportional to the corresponding light intensity distributions and from reciprocity $G_x(\mathbf{r}_j, \mathbf{r}_s, \omega) = G_x(\mathbf{r}_s, \mathbf{r}_j, \omega)$. Since we used a slab sample in the experiment, the Green’s functions to be used in above equations are those for slab geometry in the diffusion approximation assuming a uniform background medium, as detailed elsewhere [14].

The task of retrieving the locations of the targets involves fitting of the NCIDs to the Green’s functions, and we use the following least square fitting for the j^{th} target

$$\arg \min_{\alpha_j, \beta_j, \mathbf{r}_j} \sum_{\mathbf{r}_s} \alpha_j^{-1} s_j(\mathbf{r}_s) - G_x(\mathbf{r}_j, \mathbf{r}_s, \omega)^2 + \sum_{\mathbf{r}_d} \beta_j^{-1} a_j(\mathbf{r}_d) - G_m(\mathbf{r}_d, \mathbf{r}_j, \omega)^2. \quad (1)$$

The fitting using eq. (1) provides optimal estimates of the two scaling factors α_j and β_j and the location $\mathbf{r}_j$ of the j^{th} target. The fluorescence strength then is

$$f_j = \alpha_j \beta_j. \quad (1)$$

Another important consideration is the size of the targets. A back projection of $U_{m_j}(\mathbf{r}_d, \mathbf{r}_s, \omega)$ from the detection plane onto the “target plane” ($z = z_j$ plane) provides an estimate of the target size [1]. The fluorescence signal due to the j^{th} target can be approximated by [1]

$$U_{m_j}(\mathbf{r}_d, \mathbf{r}_s, \omega) = \int_{z=z_j} G_m(\boldsymbol{\rho}_d - \boldsymbol{\rho}, \omega) \chi_j(\boldsymbol{\rho}) G_x(\boldsymbol{\rho} - \boldsymbol{\rho}_s, \omega) d\boldsymbol{\rho}, \quad (2)$$

where $\boldsymbol{\rho}_s$ and $\boldsymbol{\rho}_d$ are the lateral coordinates of the source and the detector, and the integration is over the $z = z_j$ plane. In the Fourier space $\chi_j(\mathbf{q})$ follows from eq. (2) as,

$$\chi_j(\mathbf{q}) = \frac{U_{m_j}(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s, \omega)}{G_m(\mathbf{q} - \mathbf{q}_s, \omega) G_x(\mathbf{q}_s, \omega)}, \quad (21)$$

where $\mathbf{q}$ and $\mathbf{q}_s$ are the spatial frequencies on the x - y plane and $*$ denotes complex conjugate. The inverse Fourier transform of $\chi_j(\mathbf{q})$ provides the cross-section image of the j^{th} target at the $z = z_j$ plane.

The NMF-based approach was used to retrieve the location and fluorescence strength of the two targets. The targets were 4.2-mm-diameter 1-mm cylindrical glass tubes filled with a solution of Indo-cyanine green (ICG) dye (Sigma-Aldrich, product I2) which fluoresces over 650–750 nm range with peak around 700 nm. The locations of both the targets were estimated within 1 mm of the known position of both the targets. The optical strengths of the two targets were also estimated within 1% of the known value.

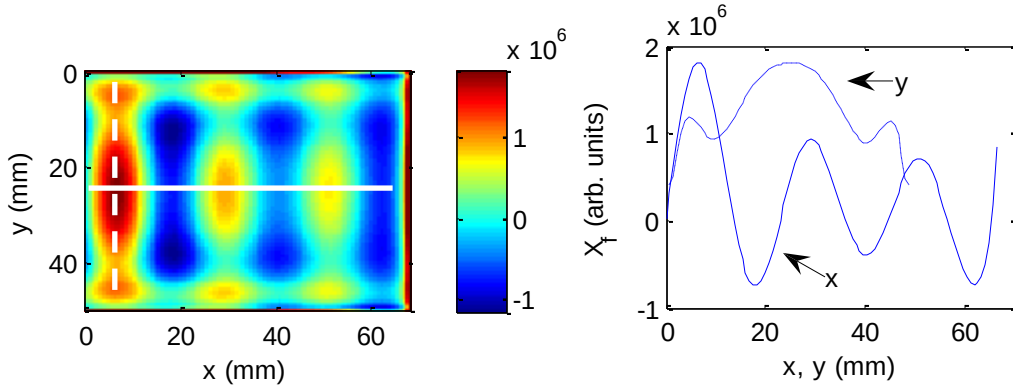


Fig. 1. (a) Cross sectional image of the left target at the $z = 1$ mm plane. (b) Spatial profiles of the cross sectional images along the x and y directions shown by the white lines. Similar image and profiles were obtained for the right target as well.

The backprojection algorithm mentioned above enables generationg cross sectional image of the targets, as shown in Fig. (a) for the the left target at the $z = 1$ mm plane. Fig. (b) shows the

spatial profiles of the cross sectional images along the x and y directions shown by the white lines. Similar cross sectional image and profiles were obtained for the right target as well. The FWHM of the spatial profiles of the cross-section images were found to be 1.0 mm and 1.1 mm for the left target, and 1.2 mm and 1.4 mm for the right target in x and y directions, respectively. The cross-section images estimate the lateral dimensions of the targets to be approximately twice their known values. We presume it a consequence of the diffusive nature of light propagation.

However, the ratio between the y and x dimensions of the targets estimated to be 2.2 and 2.1 for the left and right targets, respectively, are close to the actual value of 2.4 for both targets.

Both TROT and NMF are fast reconstruction methods, since they use data matrix with lower dimension than that used in other inverse image reconstruction (IIR) approaches, do not involve iterations of the forward model, and do not attempt to find optical properties for every voxel. The computational complexity of both approaches is less than what it is even for a single iteration of an iterative IIR method. Even though a limited number of acquisition angles were used for the slab geometry, locations of the fluorescence targets were retrieved by both approaches within 1 mm of known positions for a sample with similar thickness and average optical properties of a typical compressed human breast, which is a significant result. Further details on experiments, numerical algorithms, computational complexity and other features of fluorescence tomography are presented in Chapter 4 of Reference 13, and in Reference 14.

In summary, *fluorescence* TROT and NMF-based fluorescence tomography approaches have been developed and used to retrieve the target location and relative fluorescence strength of one and two small fluorescent targets embedded in a breast-simulating turbid medium. Locations of targets were retrieved in $-D$ with an accuracy of 1 mm under the favorable condition of well separated targets. Achievable spatial resolution is better for assessment of lateral separation between the targets than for axial separation in the forward propagation mode of signal acquisition using slab geometry. Fluorescence signal appeared to provide better resolution than transillumination signal. The results further suggest the potential of TROT and NMF-based fluorescence tomography for retrieving three-dimensional location information of contrast enhanced tumors in a human breast at early development stages.

5.2.5. Diffuse Optical Tomography of Realistic Model Cancerous Breast

An important step in testing the efficacy of the NIR Diffuse Optical Tomography (DOT) is to use “realistic” model cancerous breast assembled using *ex vivo* human breast tissue (*Specific Aim 4, Task #15 and Task #16* of original SOW; and *Specific Aim 4, Task #4* of revised SOW).

5.2.5.1 Realistic Model Cancerous Breast studied using OPTICA

An earlier study, reported in the First Annual Report 1 and published in a refereed journal article 2 used OPTICA for retrieving location of tumors and other features. The model cancerous breast used in this early study was a 100 mm X 100 mm X 10 mm slab composed of two pieces of *ex vivo* human breast tissues provided to us by National Disease Research Interchange (NDRI) under an Internal Review Board (IRB) approval at the City College of New York and SAMRMC). The larger piece was normal tissue that included mainly adipose tissue and streaks of fibro-glandular tissues. The existence of the fibro-glandular tissues was not known prior to making the measurements. The second piece was mainly a tumor (infiltrating ductal carcinoma) with a small amount of normal tissues in the margins with an overall approximate dimension of 50 mm X 50 mm X 10 mm. An incision was made in the mid-plane (along the z-axis, which was the

shorter dimension of the tissue) of the normal piece and some amount of normal tissue was removed from the central region making a small pouch. The tumor piece was then inserted into the pouch and the incision was closed by moderate compression of the composite consisting of the normal tissue and the tumor along x - y - z directions. The breast tissue slab was contained inside a transparent plastic box. One of the sides of the box could be moved to uniformly compress the tissue along the z -axis and hold it in position. The resulting specimen, a 40 mm X 40 mm X 10 mm slab, was treated as one entity in the subsequent imaging experiment. The position of the tumor within the slab was known since it was placed in position as discussed above. One of the tests of the efficacy of this imaging approach was to see how well the known position is assessed.

The experimental arrangement (shown schematically in Fig. 1) used a 200- μ m optical fiber to deliver a 640-nm, 10 mW CW light beam from a diode laser for sample illumination. The beam was collimated to a 1-mm spot onto the entrance face (the 'source plane') of the slab sample. Multiple source illumination was realized in practice by step scanning the slab sample across the laser beam in a 22 X 11 x - y array of grid points with a step size of 2.0 mm using a computer controlled translation stage. The signal from the opposite face of the sample (the 'detection plane') was collected by a camera lens and projected onto the sensing element of a cooled 1-bit, 128 X 128-pixel charged couple device (CCD) camera. Although the scanned area is 42 mm X 42 mm on the source plane, the imaged area of the detection plane was much larger, covering the entire 40 mm X 40 mm area of the model breast. Each illuminated pixel of the CCD camera could be regarded as a detector.

For illumination of each scanned point, the CCD camera recorded an image. A typical raw image is shown in Fig. 1(c) of Reference [1]. Each raw image was then cropped to select out the information-rich region, and binned to enhance the signal-to-noise ratio. All the binned images corresponding to illumination of the grid points in sequence were then stacked, and used as input for independent component analysis. The details of the analysis method, theoretical formalism, target localization algorithm, and experimental arrangement are presented in Reference [1] (appended). After optical measurements, the sample was transferred to our collaborators at the New York Eye and Ear Infirmary for pathological study and correlation.

The key results of the study are as follows.

(a) OCTICA identified three different structures (Fig. 2, Reference [1]) that include the tumor whose presence and position were known from the sample preparation process. We ascribed the other two structures to fibro-glandular tissues, since the remainder of the model breast mainly consisted of adipose tissue. Comparison with the pathology results further confirmed the identity of the tumor and the fibro-glandular tissues.

(b) The location of the tumor was determined to within 0.1 mm in all three dimensions. The locations of the fibro-glandular tissues were also estimated. The locations of the components are given in Table I of Reference [1].

(c) The FWHM of the tumor is estimated to be 1.0 mm and 0.4 mm along the x and y directions, respectively (details in Fig. 3, Reference [1]).

5.2.5.2 *Multi-wavelength study of Realistic Model Cancerous Breast using TROT and other decomposition methods*

We then carried out another set of measurements on a realistic model cancerous breast using three different wavelengths and analyzed the resulting data set using TROT, NMF-based optical tomography, and O-TICA. We then carried out MRI measurements on the same sample and compared the results (*Specific Aim 4, Task #3 and Task #4* of revised SOW). Details of this work appear in Chapter 4 of the appended Ph.D. Thesis [1], and a brief overview is presented below.

The model breast was assembled using two pieces of normal *ex vivo* female human breast tissues and two pieces of cancerous tissues provided by National Disease Research Interchange (NDRI) under an Internal Review Board approval at the City College of New York. The normal breast tissue specimens weighed 11 grams and 12 grams and consisted primarily of adipose tissue, while each tumor (infiltrating ductal carcinoma) pieces weighed approximately 1 gram. Two incisions 1 cm apart were made in the mid-plane (along the z-axis, which was the shortest dimension) of normal tissue pieces and a small amount of tissue was removed from the core region to make two small pouches. The tumor pieces were inserted into these pouches, and the incisions were closed by moderate compression of the tissue-tumor composite from all directions. The sample was placed inside a cylindrical transparent plastic container with a movable end face of diameter 11 mm, which was moved to slightly compress the tissue along the z-axis and hold it in place. The resulting slab sample filled over 90% of the lateral dimension of the cylinder and had a uniform thickness of 4 mm. It was treated as a single entity in the subsequent imaging experiments. The nominal dimension of the tumor piece located at the left side of the sample (‘left tumor’) was 10 mm × 10 mm × 10 mm and that located on the right side (‘right tumor’) was 10 mm × 10 mm × 10 mm. The positions of the tumor pieces within the sample, and the distance between the pieces were known approximately, as those were placed in position as discussed above. The axial orientation of the plastic container and sample within it was preserved for magnetic resonance imaging (MRI) experiments following the optical measurements.

The optical imaging experiments were carried out using the 690-nm, 780-nm, and 830-nm near-infrared (NIR) beams from a Ti:sapphire laser (experimental arrangement schematic presented in Fig. 1). The average beam power was maintained at 1 mW for every wavelength. The light beam at any of these wavelengths was collimated to a 1-mm spot onto the entrance face (henceforth referred to as the ‘source plane’) of the slab sample. Multiple source illumination was realized in practice by step scanning the slab sample along the horizontal (x) and vertical (y) directions across the laser beam in an x-y array of grid points using a computer controlled translation stage. A camera lens collected the diffusely transmitted light on the opposite face of the sample (henceforth referred to as the ‘detection plane’) and projected it onto the sensing element of the cooled 16-bit, 1280 × 1280 charged couple device (CCD) camera. Each illuminated pixel of the 1280 × 1280 pixels of the CCD camera could be regarded as a detector. For illumination of every scanned point on the source plane, the CCD camera recorded the diffusely transmitted intensity pattern on the detection plane. A 20 mm × 20 mm area of the source plane was scanned in a 20 × 20 array of x-y grid points with a step size of 2 mm, while the CCD camera imaged the entire detection plane.

For MRI experiments, the model breast sample in the plastic container was taken to Memorial Sloan-Kettering Cancer Center (MSKCC) small animal MRI facility. The facility

currently utilizes a 4. -T -cm bore magnet imaging/spectroscopy system (ruker ioSpin) operating at 2 M z for 1- (standard) imaging experiments for imaging. The tissue container was fixed inside the radio-frequency coil (RFC) and placed inside the bore magnet. MR images of the sample were recorded in 2. -mm slice thick sagittal slices.

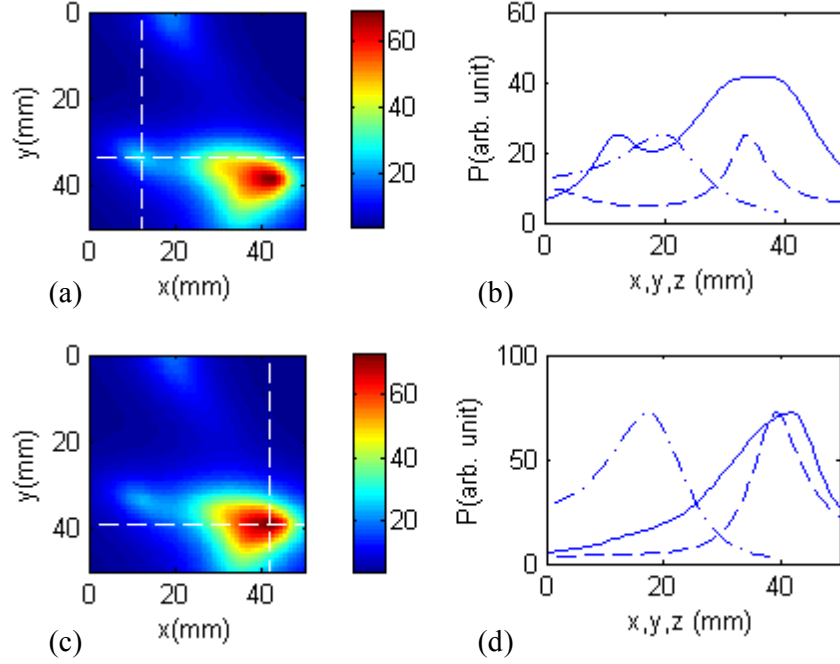


Fig.4. (a) and (c) are TROT-generated pseudo images for the left and right tumors generate using data collected at all three wavelengths; (b) and (d) are pseudo value profiles through the target along x(-), y(--), and z(- - -) directions.

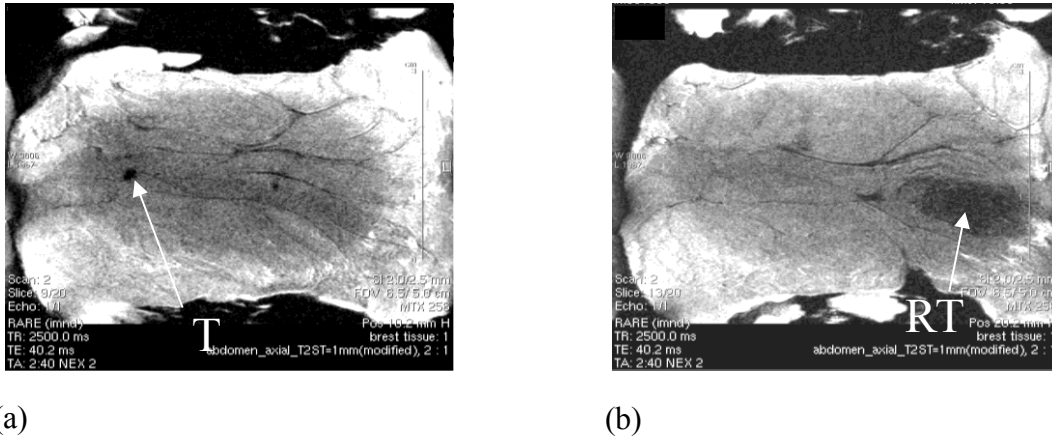


Fig. . MR images of the left tumor and the right tumor pieces (T: left tumor, RT: right tumor).

Images were also reconstructed using the NMF-OT and O TICA approaches (Figures . .12 in Reference) and corroborated with the MR images, one of which is shown in Fig. . TROT and NMF-OT-retrieved positions are within mm from the mid-plane ($z = 2$ mm), while O TICA-retrieved positions are within 2 mm from the mid-plane. This is a good agreement

between the results obtained from all three optical approaches. The results showed that multi-wavelength data may help improve the localization of targets and remove artifacts when compared with single wavelength measurements (details in Chapter of Reference). The MRI measurements corroborated the assessments of TROT, NMF-OT and O TCA in terms of the number of targets, the distance separating them, and their depth (z-position).

5.2.6. Finite Element Method for Optical Tomography

We explored the Finite Element Method (FEM) that has found considerable use in optical tomography [2], as another viable approach to optical mammography (*Specific Aim 4, Task# 17 and Task#18*). One of the objectives is to use FEM to obtain optical properties around the suspect sites that TROT can locate with high accuracy without needing long computation time. While FEM is more computation intensive than TROT, using it only over the limited suspect sites located by TROT will reduce the computation time significantly.

We pursued testing and adaptation of a program called NIRFAST developed by researchers at Dartmouth College for modeling NIR frequency domain light transport in tissue based on FEM. We have evaluated the program in simulation under different conditions that include number of targets, their location, size and optical properties, sample geometry, source and detector positions, and noise level. It is tested for both two-dimensional (2-D) and three-dimensional (3-D) absorptive and scattering targets in 2D and 3D problems. We refer to earlier Annual Reports [3,4] for our work in this area.

5.2.7. Magnetic Resonance Imaging and Spectroscopy for lactate detection

The interest in lactate detection stems from the fact that lactate level is associated with tumor aggressiveness, metastasis and treatment response [4]. As an end product of glycolysis metabolic pathway, lactate is considered as a prognostic marker of poor outcome in many tumors. Tumor lactate accumulation is caused by both aerobic glycolysis, a hallmark of tumors, and by anaerobic glycolysis in the hypoxia region of tumors. The development of non-invasive lactate detection methods is highly desirable for clinical evaluation. Magnetic resonance imaging and spectroscopy (MRIS) has been recognized as a promising technique for lactate detection.

However, the overlapping lipid and adjacent water signals obscure lactate in the conventional MRIS. The double frequency-selective multiple quantum coherence transfer (SelMTC) technique was proposed to overcome this complication [5].

The training received by CCN researchers (*Specific Aim 0, Task 2, Task 3 and Task 5*) at MSUCC prepared them for using the MRIS facilities in primary collaborating mentor (CM) Dr.

Butcher's lab at MSUCC. As a part of translation of their training to research, they explored improving the sensitivity and signal-to-noise ratio of the MRIS approaches for detection of lactate, which was hypothesized to provide a window to explore tumor aggressiveness (*Specific Aim 1*). The following work was done:

- Implementation and optimization of the SelMTC on Bruker 4. T spectrometer, and transfer the sequence to Bruker T spectrometer with higher field strength;
- Construction of necessary radiofrequency (RF) coils and optimization of the parameters for the phantoms;
- Fabricate lactate phantoms and carry measurements on the phantoms.

The results of the preliminary phantom study showed the potential for *in vivo* lactate detection with the improved RF coils, and was reported in the Third Annual Report [6].

5.3. Research Proposal Development

One of the proposed tasks (*Specific Aim 0, Task# 7*) involved developing at least one research proposal and submitting it to NCI or SAMRMC for funding. We submitted following pre-proposals/proposals for attracting funding for research.

- (1) S. S. Ayen (Initiating I), J. A. Outcher (Collaborating I), “Assessing Breast Cancer Aggressiveness using Near-Infrared and Magnetic Resonance Spectroscopic Imaging,” pre-application submitted to Breakthrough Award 2 (Collaborative Option) of DoD Breast Cancer Research Program (CR), 2011.
- (2) S. S. Ayen (Initiating I), J. A. Outcher (Collaborating I), “Multimodal nanocomposites for detection and prevention of breast cancer metastases” to the Idea Award (Collaborative Option) category of the 2012 Breast Cancer Research Program of CDMR.
- (3) S. S. Ayen (Senior personnel; I: Ben Shepard), “Optical Techniques for Actuation, Sensing, and Imaging of Biological Systems,” an NSF IRT grant (1012101) with Columbia University as the lead institution. S. S. Ayen was an investigator on this grant from CCNY and received a sub-contract for developing optical imaging techniques for breast cancer detection.
- (4) S. S. Ayen (Initiating I), J. A. Outcher (Collaborating I), “Multi-functional tumor-targeting nanocomposites and time-reversal optical imaging for early detection of breast cancer and prevention of micro-metastases,” submitted to the Idea Award (Collaborative Option) category of the 2011 Breast Cancer Research Program of CDMR.
- (5) S. S. Ayen (Initiating I), J. A. Outcher (Collaborating I), “Nanocomposite-Chemokine Mimic Conjugates for Imaging and Prevention of Breast Cancer Metastases,” submitted to the Idea Award (Collaborative Option) category of the 2011 Breast Cancer Research Program of CDMR.

6. KEY ACCOMPLISHMENTS

Key accomplishments of the project include:

- (a) Development of near-infrared (NIR) light-based diffuse optical imaging approaches: *Time Reversal Optical Tomography* (TROT), *Non-negative Matrix Factorization-based Optical Tomography* (NMF-OT), and *Optical Tomography based on Principal Component Analysis* (PCA). Previously-developed *Optical Tomography using Independent Component Analysis* (O-TICA) has been further extended and tested. These approaches are fast, noninvasive, and provide accurate position information of small tumor simulating targets embedded in breast simulating model media, and in *ex vivo* model cancerous breast. These approaches have immense potential for detection of breast tumors in early growth stages when those are more amenable to treatment.
- (b) Recognition of the TROT formalism through its highlighting in “**Optics in 2012**” a special of the *Optics and Photonics News* (published by the Optical Society of America, December 2012). The OPN noted, “Obtaining, the guest editor of the issue, and a team of seven volunteer editors have combed through the work of scientists from around the globe to identify summaries that highlight the most exciting optics research to emerge over the preceding 12 months.” The journal article presenting our work on TROT

“Time reversal optical tomography: locating targets in a highly scattering turbid medium,” *Opt. Express* **19**, 21 – 21 (2011) was summarized for this special issue.

- (c) Linlin Wu, a graduate student supported by the project, successfully defended his Ph.D. thesis and is pursuing cancer-related research as a postdoctoral research associate at the Cornell-Weill Medical College, New York.
- (d) Publication of research results in a number of peer-reviewed journal articles and conference proceedings, and presentation of results in major technical conferences including the *Era of Hope*, CDMR – CRC sponsored breast cancer conference.
- (e) Development and submission of research proposals and pre-proposals for attracting funding for breast cancer research at CCNY.
- (f) Developing a successful training program to endow physical scientists and engineers with the requisite background in cancer biology so that they can contribute to the fight against breast cancer. The training program may be emulated by others.
- (g) Together these accomplishments indicate that project helped establish a breast cancer research program at CCNY, a minority institution.

7. REPORTABLE OUTCOMES

Ph. D. Thesis

Linlin Wu, “*Time Reversal Optical Tomography and Decomposition Methods for Detection and Localization of Targets in Highly Scattering Turbid Media*,” (Advisor: Swapan Ghoshal), A dissertation submitted to the Graduate Faculty in Physics in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York, 2011.

Publications

- (1) M. Xu, M. Alrubaiee, S. Ghoshal and R. R. Alfano, “Optical diffuse imaging of an *ex vivo* model cancerous human breast using independent component analysis,” *IEEE J. Select. Topics Quantum Electron.* **14**, 4 (2008).
- (2) Linlin Wu, W. Cai, M. Alrubaiee, M. Xu and S. Ghoshal, “Three-dimensional time-reversal optical tomography,” in *Multimodal Biomedical Imaging III. Proc. SPIE*, vol. 7242, pp. 7242-1 – 7242-6 (2011).
- (3) M. Alrubaiee, Linlin Wu, M. Xu, W. Cai, and S. Ghoshal, “Multi-wavelength diffusive optical tomography using Independent Component Analysis and Time Reversal algorithms,” in *Diffuse Optical Imaging III. Proc. SPIE-OSA*, vol. 7242, pp. 7242-1 – 7242-6 (2011).
- (4) Linlin Wu, W. Cai, M. Alrubaiee, M. Xu, and S. Ghoshal, “Time reversal optical tomography: locating targets in a highly scattering turbid medium,” *Opt. Express* **19**, 21 – 21 (2011).
- (5) Linlin Wu, M. Alrubaiee, W. Cai, M. Xu, and S. Ghoshal, “Diffuse optical imaging using decomposition methods,” *International J. Opt.* **2**, 12, Article ID 1 – 4, 12 pages (2012); doi:10.1186/1546-2218-2-12-4.

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- () inlin Wu, Wei Cai, Swapan . ayen, "Time reversal optical tomography," Optics and Photonics News special issue "Optics in 2 12", December 2 12, p. 41. Also selected and published in the irtual ournal of iomedical Optics, olume , Issue 1, age 41. http://www.opticsinfobase.org/vjbo/abstract.cfm_uri_opn-2_-12-41. Although the summary was published in the December 2 12 issue of O N, the source paper was published in 2 11. (Item 4 above)
- () inlin Wu, W. Cai, S. . ayen, "Three-dimensional localization of fluorescent targets in turbid media using time reversal optical tomography," *Appl. Phys. Lett.* **101**, 2 11 (2 12).
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- (1) inlin Wu, Wei Cai, S. . ayen, "Time reversal optical tomography locates fluorescent targets in a turbid medium," *Proc. of SPIE* ol. 4, 4 N-1 N (2 1); doi: 1 .111 /12.2 2

Manuscripts

- (1) inlin Wu and S. . ayen, "Fluorescence tomography of targets in a turbid medium using nonnegative matrix factorization," submitted to *Phys. Rev. E*.
- (2) inlin Wu, M. Alrubaiee, Wei Cai, M. Xu, and S. . ayen, "Multi-wavelength diffuse optical imaging of an *ex vivo* model cancerous human breast using time reversal optical tomography and decomposition methods," in preparation.

Conference Presentations

- (1) S. . ayen, M. Alrubaiee, M. Xu, and R. R. Alfano, "Optical imaging of an *ex vivo* model cancerous human breast using independent component analysis." oster 4 - presented at the *Era of Hope*, Department of Defense reast Cancer Research rogram Meeting, une 2 -2 , 2 , altimore, Maryland. Abstract appears in p. 2 1 of Meeting roceedings.
- (2) M. Xu, M. Alrubaiee, S. . ayen, and R. R. Alfano, "Optical tomography using Independent Component Analysis," aper 42-1 presented at the *Era of Hope*, Department of Defense reast Cancer Research rogram Meeting, une 2 -2 , 2 , altimore, MD; abstract in p.2 2 of the Conference proceedings.
- () M. Alrubaiee, M. Xu, S. , ayen, . ongo, and R. R. Alfano, "Multi-wavelength optical tomography using independent component analysis." oster presented at the NI Inter-Institute Workshop on Optical Diagnostic and iophotonic Methods from ench to edside 2 , October 1-2, ethesda, MD.
- (4) inlin Wu, M. Alrubaiee, W. Cai, M. Xu and S. . ayen, "Optical imaging of objects in turbid media using rincipal Component Analysis and Time Reversal Matrix Methods," in Computational Optical Sensing and Imaging, OSA Technical Digest (CD)

(Optical Society of America, 2011), paper TuC1. Presented at the Annual Meeting of the Optical Society of America, October 11-13, 2011, San Jose, California.

- () M. Alrubaiee, Binlin Wu, W. Cai, M. Xu and S. Ayen, “Multi-wavelength diffusive optical tomography using independent component analysis and time reversal algorithms.” Paper 7212-1 presented at the SPIE/OEA European Conference on Biomedical Optics, 22-24 May, 2011, Munich, Germany.
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- () Binlin Wu, W. Cai, M. Alrubaiee, M. Xu, and S. Ayen, “Time reversal optical tomography for breast tumor detection,” Paper C114-2 presented at the 11th *Era of Hope* 2011 conference of Department of Defense (DOD) Breast Cancer Research Program (CBR), 23-25 August, Orlando, Florida.
- () Binlin Wu, W. Cai, M. Alrubaiee, M. Xu and S. Ayen, “Time-reversal optical tomography: detecting and locating extended targets in a turbid medium,” Paper 7212-1 presented at the SPIE International Symposium on Biomedical Optics, SPIE 8112/ Photonics West, 21-24 January, 2012, San Francisco, California.
- () Binlin Wu, W. Cai, and S. Ayen, “Time reversal optical tomography locates fluorescent targets in a turbid medium,” Paper 7212-1 presented at the SPIE International Symposium on Biomedical Optics, SPIE 8111/ Photonics West, 21-24 February, 2011, San Francisco, California.

Research Personnel Development

Binlin Wu, a graduate student supported by the project defended his Ph.D. Thesis in August 2011. He is now a research associate at the Cornell-Weill Medical College, NY.

Xiaohui Ni, a researcher trained and supported in part by this grant has moved to the Department of Chemistry and Chemical Biology of Harvard University as a research associate.

M. Alrubaiee, was a research associate supported in part by this grant. He is now with the Patent and Trademark Office, Alexandria, VA.

8. CONCLUSION

The training program developed in the project was effective in preparing the physical scientists and laser spectroscopists for breast cancer research and may provide a useful model for others to emulate. The research activities carried out in the project: (a) shows the potential for noninvasive detection and three-dimensional localization of a tumor within a breast with significant accuracy based on the differences in the light scattering and absorption characteristics of the tumor and normal breast tissue; (b) presents the formalism and results of fluorescence tomography approaches that may provide higher contrast and better target identification based on molecular signatures and target selectivity of exogenous contrast agents.

“So What Section”

- The National Cancer Institute (NCI) has identified the development of imaging methodologies as an extraordinary opportunity for advancement in cancer research. Since the background of the CCN team is in physical sciences and engineering, the training they received has provided them with necessary laboratory background in the biology of cancer research, and helping develop a knowledgeable multidisciplinary research force in the fight against breast cancer.
- A recent study involving 10 patients underscores the influence of primary tumor location on breast cancer prognosis, and makes it imperative that breast cancer detection modalities to obtain three-dimensional (3-D) location of the tumor relative to the axilla be developed. The optical imaging techniques (TROT, fluorescence TROT and other decomposition methods) being developed are an important steps in obtaining 3-D location of a tumor within the breast. The methods are minimally iterative, fast, and designed for locating small targets, which make those suitable for detecting tumors in early stages of development when those are more amenable to treatment.

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**Time Reversal Optical Tomography and Decomposition
Methods for Detection and Localization of Targets in Highly
Scattering Turbid Media**

by
Jinlin Wu

A dissertation submitted to the Graduate Faculty in Physics in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

Time Reversal Optical Tomography and Decomposition Methods for Detection and Localization of Targets in Highly Scattering Turbid Media

By

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Advisor: Professor S. K. Gayen

New near-infrared (NIR) diffuse optical tomography (DOT) approaches were developed to detect, locate, and image small targets embedded in highly scattering turbid media. The first approach, referred to as time reversal optical tomography (TROT), is based on time reversal (TR) imaging and multiple signal classification (MUSIC). The second approach uses decomposition methods of non-negative matrix factorization (NMF) and principal component analysis (PCA) commonly used in blind source separation (BSS) problems, and compare the outcomes with that of optical imaging using independent component analysis (OICA). The goal is to develop a safe, affordable, noninvasive imaging modality for detection and characterization of breast tumors in early growth stages when those are more amenable to treatment.

The efficacy of the approaches was tested using simulated data, and experiments involving model media and absorptive, scattering, and fluorescent targets, as well as, “realistic human breast model” composed of *ex vivo* breast tissues with embedded tumors. The experimental arrangements realized continuous wave (CW) multi-source probing of samples and multi-

detector acquisition of diffusely transmitted signal in rectangular slab geometry. A data matrix was generated using the perturbation in the transmitted light intensity distribution due to the presence of absorptive or scattering targets. For fluorescent targets the data matrix was generated using the diffusely transmitted fluorescence signal distribution from the targets. The data matrix was analyzed using different approaches to detect and characterize the targets.

The salient features of the approaches include ability to: (a) detect small targets; (b) provide three-dimensional location of the targets with high accuracy (within a millimeter or 2); and (c) assess optical strength of the targets. The approaches are less computation intensive and consequently are faster than other inverse image reconstruction methods that attempt to reconstruct the optical properties of every voxel of the sample volume. The location of a target was estimated to be the weighted center of the optical property of the target. Consequently, the locations of small targets were better specified than those of the extended targets. It was more difficult to retrieve the size and shape of a target. The fluorescent measurements seemed to provide better accuracy than the transillumination measurements. In the case of *ex vivo* detection of tumors embedded in human breast tissue, measurements using multiple wavelengths provided more robust results, and helped suppress artifacts (false positives) than that from single wavelength measurements. The ability to detect and locate small targets, speedier reconstruction, combined with fluorophore-specific multi-wavelength probing has the potential to make these approaches suitable for breast cancer detection and diagnosis.

*Dedicated to my parents,
my brothers Bintong, Binhao, and Binmeng,
my wife Xin Gao, and my son Maxwell (Yiu-Yiu)*

谨献给我的父母
兄弟彬童、彬豪、彬蒙
妻子高新
及儿子优优

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Chapter 1

Introduction

1.1. Introduction

Optical imaging of targets embedded in a highly scattering turbid medium is an important area of contemporary research because of potential applications in a variety of areas, such as, detection of breast or prostate tumors in early growth stages, earth observation through cloud or fog cover, detection of mines in shallow coastal waters, underwater imaging, harbor defense, and military surveillance. 1- Of these, biomedical imaging using near-infrared (NIR) light has received particular attention for several reasons. First, commonly used biomedical imaging techniques, such as x-ray, computed tomography (CT), ultrasound, and magnetic resonance imaging (MRI) have important limitations. X-rays are high-energy radiation that could be harmful for prolonged exposure, and x-ray based methods cannot provide diagnostic information. Ultrasound provides structural information with inadequate spatial resolution and low specificity. MRI provides diagnostic information, but is often expensive. Optical imaging usually uses either visible or near-infrared non-ionizing light as the source. It is non-invasive, and may obtain spectral and functional information. It may be used for diagnosis if the spectral characteristics of the sample are obtained.

However, optical imaging of scattering medium, in particular, biological tissues has separate sets of challenges. In a biological tissue, light encounters wavelength-dependent absorption and strong scattering at all wavelengths, which lead to poor signal-to-noise ratio, as well as loss of phase coherence and polarization. As a consequence, distinct and sharp image of the targets may not be formed directly. Light propagation through optically-thick biological tissues involves

multiple scattering by tissue ultra-structures and may be treated as a diffuse transport problem. A commonly used theoretical framework for describing this light transport problem is provided by the radiative transport equation (RT) [4], and for thick tissue, such as, a female human breast by the diffusion approximation (DA) [5] of RT. The corresponding imaging approaches are commonly referred to as diffuse optical imaging (DOI) or diffuse optical tomography (DOT) [6-10]. DOI or DOT are image reconstruction approaches, also known as inverse image reconstruction (IIR) that make use of the knowledge of characteristics of the incident probing light, measurement of the diffusely propagating light emerging from the sample boundaries, average optical properties of the intervening medium, model for light propagation through the medium, and numerical algorithms to generate a map of the sample (such as, human breast) interior with targets (such as, tumor) appearing as localized inhomogeneities within the reconstructed sample volume.

The desired requirements of a useful and effective approach for optical breast imaging (also referred to as optical mammography) include ability to: (a) detect tumors at early development stages when those are small and more amenable to treatment; (b) provide location of the tumor and assessment of its size; (c) yield diagnostic information as to whether the tumor is malignant or benign. A number of continuous wave, frequency domain, and time-resolved experimental approaches and a variety of numerical algorithms have been developed over the years [11]. Most of these approaches attempt to reconstruct optical properties of the sample, such as, scattering coefficient, absorption coefficient, anisotropy factor, and are computation intensive, and may not provide accurate target location. This thesis presents fast (less computation intensive) and accurate approaches for detection and localization of small targets.

1.2. Thesis statement

The objective of this thesis is to develop fast non-invasive diffuse optical imaging (DOI) modalities for detection, localization and potential diagnosis of tumors in human breast using near-infrared (NIR) light. Imaging of breast using light involves three components: experimental measurement of diffuse light that emerges from the breast when a part of it is illuminated, theoretical model for light propagation through biological tissues, and numerical algorithms for identifying, locating, and characterizing the tumor. In this thesis, new novel diffuse optical imaging approaches are introduced and investigated using simulations and experiments. One approach is called time reversal optical tomography (TROT), which is an optical tomography method based on time reversal imaging and subspace-based algorithm. TROT is developed for imaging both point-like and extended targets in a highly scattering turbid medium. The other approach is optical imaging using decomposition methods. Decomposition methods including independent component analysis (ICA) [12-14], principal component analysis (PCA) [15, 16] and non-negative matrix factorization (NMF) [17-19] are thoroughly studied and compared. Three approaches are evaluated using simulations and transillumination experiments in which breast-simulating tissue phantom and *ex vivo* model human breast are used. The targets are considered as absorptive or scattering targets. For the experiment using human breast tissue, the results are compared to MRI images for validation.

All these methods are suited for fluorescence imaging [20-24]. Among the decomposition methods, NMF is particularly suited for fluorescence imaging, since the fluorescence signal is naturally positive and satisfies its non-negativity constraint. TROT and NMF-based fluorescence tomography approaches are developed, and tested in experiments using breast-simulating tissue phantom with embedded fluorescence targets.

The computational complexity of these methods is compared with other algorithms to prove that these alternative DOI methods are much faster than other model-based DOI methods.

1.3. Background

Since the DOI problem involves measurement of diffuse light on the boundary of a highly scattering turbid medium, such as human breast, in this section, the background knowledge about the key parameters of highly scattering media, particularly of human breast that determine the light propagation in the media are presented. The optical imaging methods that have been developed will be reviewed briefly, and the theoretical model for the diffusive optical imaging and the imaging approaches that we have developed will be introduced.

1.3.1. Light propagation through turbid media

When light propagates through a turbid medium, absorption and scattering process occur. Absorption [1] is the process when photons of appropriate energy are absorbed and cause atoms or molecules to make a transition from ground state to an excited state. Scattering [1] deviates light from incident direction. Multiple scattering randomizes the direction of the light propagation, as well as the phase and polarization. If the medium absorbs light, another process, fluorescence [2] may occur. Fluorescence is the process of photon emission that occurs when the atoms or molecules in an excited electronic state return to the ground state.

The optical properties of turbid media of concern are absorption length (l_a), scattering length (l_s) (also referred to as scattering mean free path), transport length (l_t) (also referred to as transport mean free path), and anisotropy factor (mean cosine of the angle of scattering), g . The absorption length (l_a) is the average distance a photon travels in the medium before it is absorbed, or the distance over which the intensity of the light source beam decreases to $1/e$ due to absorption. The length also depends on the chemical species, concentration and quantum yield

of the medium 2 . The absorption length is given by

$$l_a = 1/n\sigma_a , \quad (1.1)$$

where n is number density of the absorptive molecules, and σ_a is the effective absorption cross-section of the molecule. Another commonly used parameter directly related to absorption length is the absorption coefficient μ_a , the probability that a photon will be absorbed per unit length.

$$\mu_a = 1/l_a = n\sigma_a. \quad (1.2)$$

The scattering length (l_s) is the mean distance between scattering events and is inversely dependent on the density of the scattering particles and the scattering cross-section σ_s

$$l_s = 1/n\sigma_s , \quad (1.)$$

where σ_s is the scattering cross-section. Another parameter that is directly related to l_s is scattering coefficient μ_s , the probability that a photon will be scattered per unit length.

$$\mu_s = 1/l_s = n\sigma_s. \quad (1.4)$$

σ_s depends on the shape and size of particles in comparison to the wavelength of the incident light and index of refraction with respect to the surrounding medium 2 . σ_s is given by the equation

$$\sigma_s = \int \sigma_d(\theta, \varphi) d\Omega = \int \sigma_d(\theta, \varphi) \sin \theta d\theta d\varphi. \quad (1.)$$

where $\sigma_d(\theta, \varphi)$ is the differential scattering cross-section that describes how light scatters in the direction of (θ, φ) by a particle. For example, if the particle is much smaller than the incident wavelength, the light undergoes isotropic scattering, which is called Rayleigh scattering. Rayleigh scattering 2 is strongly dependent on wavelength. Rayleigh scattering due to molecules is described by

$$I = I_0 \frac{8\pi^4 N \alpha^2}{\lambda^4 R^2} (1 + \cos^2 \theta) , \quad (1.)$$

where I_0 is the incident light intensity, N is the number of scatterers, α is the polarizability of the

molecules, λ is the wavelength, R is the distance between the detector and the particle and θ is the scattering angle. When the particle size is much larger than the incident wavelength, anisotropic scattering occurs, with more light scattered in the forward direction [2]. $\sigma_d(\theta, \varphi)$ depends strongly on the scattering angle θ when the dimension of the particle becomes close to the incident wavelength. In this case, the scattering is described by a solution to Maxwell's equations from Lorenz-Mie theory [2], which treats electromagnetic wave scatterers as spherical particles.

When light is incident on a particle whose index of refraction is different from the surrounding, the light is deflected at an angle. The angle depends on the size and shape of the particle, incident wavelength and angle of incidence. Each particle has a scattering profile or phase function $p(\theta, \varphi)$ [4], $\int_{4\pi} p(\theta, \varphi) d\Omega = 1$ which describes the probability of a photon scattering into a unit solid angle oriented at an angle (θ, φ) relative to the original direction of the photon. The anisotropic scattering of light by a particle is characterized by the anisotropy factor g , which is a measure of the extent of forward direction retained after a single scattering event. g is calculated to be the mean cosine of the scattering directions,

$$g = \langle \cos \theta \rangle = \frac{\int_{4\pi} p(\theta, \varphi) \cos \theta d\Omega}{\int_{4\pi} p(\theta, \varphi) d\Omega}, \quad (1.)$$

or

$$g = \int_0^\pi p(\theta) \cos \theta 2\pi \sin \theta d\theta, \text{ where } \int_0^\pi p(\theta) 2\pi \sin \theta d\theta = 1. \quad (1.)$$

So g is within $(-1, 1)$. If $g < 0$, it is backward scattering; $g > 0$, forward scattering; and $g = 0$, isotropic scattering. The scattering property of a particle can also be described by the reduced scattering coefficient μ'_s , that incorporates the scattering coefficient μ_s and anisotropy factor. μ'_s is the probability that a photon is scattered isotropically per unit length.

$$\mu'_s = (1 - g)\mu_s. \quad (1.)$$

Since in a biological medium, light attenuation is due to both absorption and scattering when it propagates, the total attenuation $\mu_t = \mu_a + \mu_s'$ can be used to describe the probability that a photon will either be absorbed or scattered per unit length. For a highly scattering medium, $\mu_s' \gg \mu_a$, $\mu_t \approx \mu_s'$, which is called the diffusive regime, in which scattering dominates.

The inverse of μ_s' is defined to be transport mean free path (or reduced scattering length)

$$l_t = 1/\mu_s', \quad (1.1)$$

which describes the average distance after which light completely loses memory of its initial direction before it is absorbed.

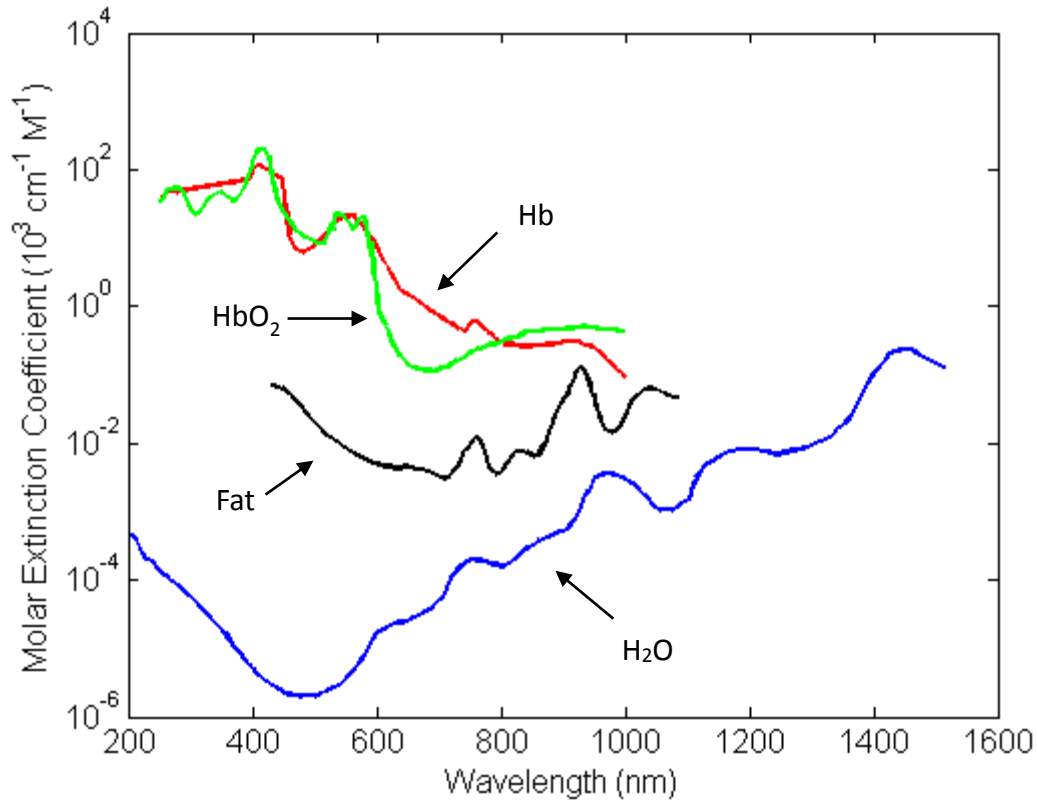


Fig. 1.1. Absorption spectra of main constituents in human breast tissue: oxy-hemoglobin (HbO_2), deoxy-hemoglobin (Hb), fat, and water (H_2O) (after Reference [1]).

In DOI, the contrast between the target and the scattering medium is based on the optical properties, such as, absorption, scattering and fluorescence of the sample. The image of a target may be generated when optical property of the target is different from the background medium.

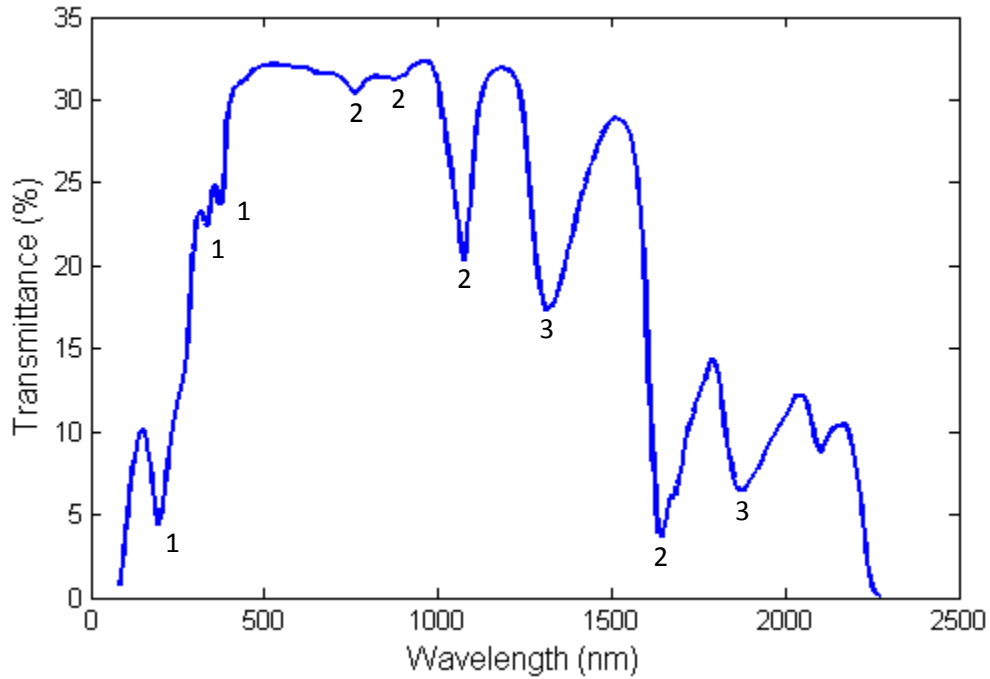


Fig. 1.2. Transmission spectrum of a 1 mm thick normal breast tissue sample showing absorption resonances of oxy-hemoglobin (1), fat (2), and water (3) superimposed on the losses due to scattering (after Reference [4]).

One main application of this work is breast cancer detection. Light may be used to measure different native parameters of tissue through which they travel, such as absorption, scattering, spectral characteristics, and fluorescence. However, due to strong absorption and scattering in biological tissue, the penetration distance of light is limited. In order to achieve good contrast and sensitivity in the optical imaging, a proper wavelength should be carefully chosen. For breast cancer detection, the illumination light with the ideal wavelength should be least absorbed or scattered by the normal breast tissue, which provides with the optimal penetration distance. Hemoglobin, proteins, water and fat are the main absorbing constituents in human breast tissue

2 . They have their lowest absorption coefficient in the NIR region around - nm as shown in Fig. 1.1.

The transmission spectrum of normal breast tissue due to both absorption and scattering (Fig. 1.2) 4 also shows that the NIR window is ideally suited for *in vivo* imaging because of minimal light absorption by hemoglobin (nm) and water (nm).

The ideal illumination light should be absorbed or scattered at a significantly different level by the breast tumors than by the normal breast tissue for adequate contrast.

1.3.2. Optical imaging of breast cancer

breast cancer incidence in women in the nited States is 1 in (about 12). In 2 1 , an estimated 2 2, 4 new cases of invasive breast cancer are expected to be diagnosed in women in the .S., along with 4, 4 new cases of non-invasive (*in situ*) breast cancer. About , 2 women in the .S. are expected to die in 2 1 from breast cancer .

The female human breast is comprised of lobules, ducts and stroma as shown in Fig. 1. . The lobules are the glands that produce milk inside the female breast. Ducts are the tiny tubes that carry the milk from the lobules to the nipple. Stroma are the fatty tissues and connective tissues surrounding the ducts and lobules, blood vessels, and the lymphatic vessels. The lymph (lymphatic) system is composed of lymph nodes that are collections of immune system cells that are connected by lymphatic vessels. The lymphatic vessels carry lymph outside the bloodstream away from the breast, which contains tissue fluid, waste products, and immune system cells.

Tumors in breast include two types, benign and malignant (cancerous). enign breast tumors are breast tissues that undergo abnormal growths, but are not invasive, and do not spread to the surrounding normal tissue and outside the breast. Most lumps in breast turn out to be not cancerous (benign), and caused by fibrosis and/or cysts. owever, malignant tumors are life

threatening. If malignant tumors are not detected and treated in time, the cancer cells may enter the lymphatic vessels, spread to and grow in the lymph nodes. These cancer cells may also spread to distant sites of the body through the lymphatic system or bloodstream system. So it is critical to detect cancer at its early growth stages, so that that treatment can be planned in time.

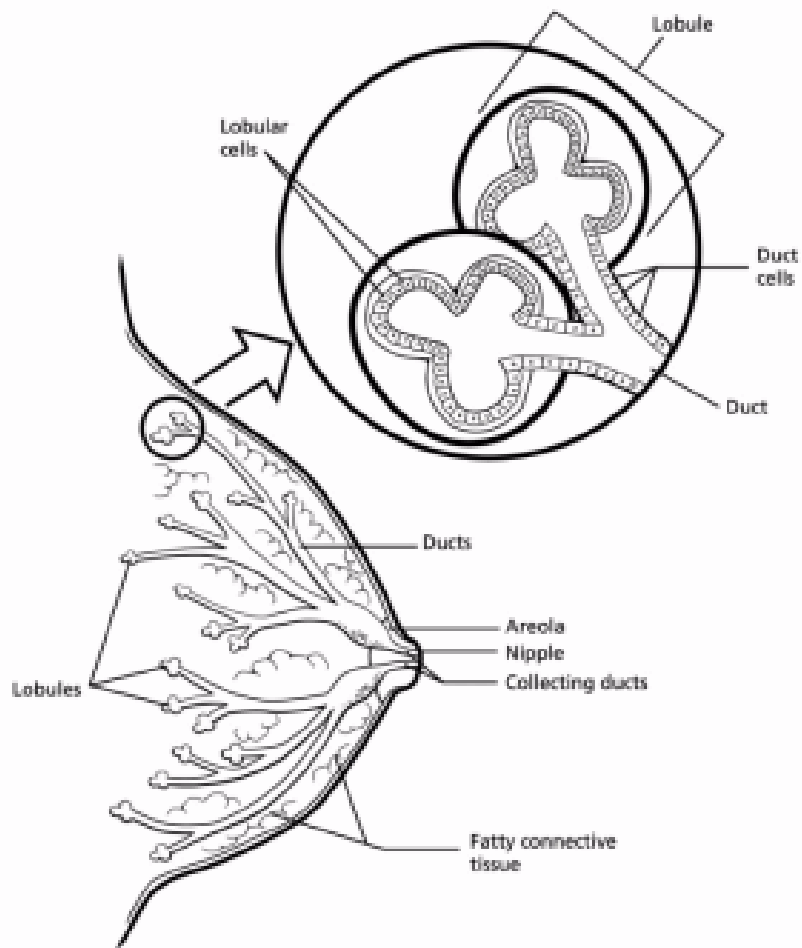


Fig. 1. Anatomy of a female breast (after Reference)

Currently used clinical techniques for detection of breast cancers include mammography, ultrasound tomography (ultrasonography), magnetic resonance imaging (MRI) etc. The drawbacks of these methods have been discussed in Section 1.1. In contrast, optical mammography as an affordable non-invasive modality could overcome these drawbacks, and has

attracted much attention over last two decades. It uses near infrared (NIR) light (wavelength range of 700 nm to 1000 nm) that is safe to human body. Optical imaging can provide functional information. Optical spectroscopic information may be obtained which has potential for diagnosis, and for distinguishing normal, benign and cancerous tissues or tumors at different stages.

Cutler first attempted optical mammography by transillumination of a female breast with a bright light source in a darkened room. He revealed that multiple scattering caused features below the surface to appear extremely blurred. Wist *et al.* demonstrated the ability to observe 1-mm details through 10 mm of breast-equivalent scattering phantoms. They also demonstrated for the first time that images of internal structures such as lesions in a tissue thicker than 1cm could be obtained. This field has since been developed [1, 2, 3, 4] over the last decades with the development of broadly tunable NIR lasers, highly sensitive detectors, charge coupled device (CCD) cameras, amplitude modulation schemes, and image reconstruction algorithms. Based on the techniques and algorithms that have been sufficiently developed, optical mammography systems attempt to use the diffuse light in frequency domain, time domain or steady-state continuous wave (CW) mode to obtain two-dimensional (2-D) projection images, or three dimensional (3-D) tomographic images of the breast using proper image reconstruction algorithms. Such techniques are referred to as diffuse optical tomography (DOT). A DOT method typically illuminates a boundary of a sample, such as human breast with a tumor inside using near-infrared (NIR) light. The illumination beam or fluorescence emitted by fluorescent targets propagates through the sample and is measured on the boundary of the sample. Different analytical or stochastic models have been used to describe the light propagation through a turbid medium. Then light intensity distribution measured on the sample boundary is used along with

the knowledge of the source and a proper forward model to reconstruct the image of the targets. The forward and inverse problems will be further explained in the next section.

1.3.3. Diffusion approximation and forward and inverse problems

Different ways are available to describe light scattering in turbid media including wave theory and transport theory. Wave theory starts with Maxwell's equation, while the transport theory which is also called radiative transfer theory deals directly with the transport of power through turbid media [41, 42]. In highly scattering media, such as biological media, light propagation is a stochastic random process. Both phase and amplitude of light vary randomly.

Phases are considered to be uncorrelated on scales larger than the transport mean free path (l_t), i.e. light loses its coherence. Radiative transfer theory has been widely used to describe light scattering in random media containing particles, with a Boltzmann-type transport equation, or radiative transfer equation (RT). RT can be written as [3, 4, 44]

$$\frac{\partial}{\partial t} \Phi(\mathbf{r}, \mathbf{s}, t) + \hat{\mathbf{s}} \cdot \nabla \Phi(\mathbf{r}, \mathbf{s}, t) + (\mu_a + \mu_s) \Phi(\mathbf{r}, \mathbf{s}, t) = S(\mathbf{r}, \mathbf{s}, t) + \mu_s \int p(\mathbf{s}, \mathbf{s}') \Phi(\mathbf{r}, \mathbf{s}', t) d^2 \mathbf{s}', \quad (1.12)$$

where $\Phi(\mathbf{r}, \mathbf{s}, t)$, the specific intensity, is the photon distribution function; c_m is the light speed in the medium; μ_a and μ_s are the absorption and scattering coefficients respectively. $p(\mathbf{s}, \mathbf{s}')$ is the phase function that represents the probability of scattering into a direction $\mathbf{s}'$ from $\mathbf{s}$. $S(\mathbf{r}, \mathbf{s}, t)$ is the radiation source term. The diffusion approximation (DA) of the RT is widely used for light propagation in highly scattering media. The DA approach is based on the fact that after undergoing a large number of scatterings, the multiply scattered light has lost its phase information, and coherence, so that any interference effect in the medium can be neglected [4]. As a result, only the light intensity needs to be considered for the propagation of light [4]. The diffusion equation due to a pulse point light source is [3, 4]

$$\frac{\partial}{\partial t} \Phi(\mathbf{r}, t) - D \nabla^2 \Phi(\mathbf{r}, t) + \mu_a \Phi(\mathbf{r}, t) = q_0(\mathbf{r}, t), \quad (1.1)$$

where $q(\mathbf{r}, t) = \delta(\mathbf{r})\delta(t)$ represents the impulse incident source at $t = 0$ and position $\mathbf{r} = \mathbf{0}$, $D = l_t/4$ is the diffusion coefficient.

When a short light pulse is scattered as it propagates, the propagating light consists of early portion (ballistic and snake-like) and diffusive portion. The diffusion equation can only describe the diffusive light, which falls in the diffusion regime, and cannot describe the early light. DA holds when the thickness of sample is at least 1 cm [44]. It can be an excellent approximation when z/l_t is large and the g factor is small. Analytical solutions to the DA exist only for a few simple geometries [4, 44], such as infinite, semi-infinite, infinite-slab, cylindrical and spherical geometries. An analytical solution to the diffusion equation when the source is a delta function is a Green's function, which may be used to derive other types of sources using convolution. The Green's function for diffusion approximation in an infinite medium is [44]

$$G(\mathbf{r}, \mathbf{r}') = \frac{1}{4\pi D} \frac{\exp(-\kappa|\mathbf{r}-\mathbf{r}'|)}{|\mathbf{r}-\mathbf{r}'|}, \quad (1.14)$$

where $\mathbf{r}$ and $\mathbf{r}'$ are the positions of two points, $\kappa = [(\mu_a - i\omega/c_m)/D]^{1/2}$. The Green's function for a slab geometry is [1]

$$G(\mathbf{r}, \mathbf{r}') = G(\rho, z, z') = \frac{1}{4\pi D} \sum_{k=-\infty}^{\infty} \left[\frac{\exp(-\kappa r_k^+)}{r_k^+} - \frac{\exp(-\kappa r_k^-)}{r_k^-} \right], \quad (1.1)$$

with an incident intensity-modulated illumination source of modulation frequency ω , where $r_k^\pm = [\rho^2 + (z \mp z' \pm 2kd)^2]^{1/2}$, $\rho = [(x - x')^2 + (y - y')^2]^{1/2}$ is the lateral distance between the two points $\mathbf{r} = (x, y, z)$ and $\mathbf{r}' = (x', y', z')$, $k = 0, \pm 1, \pm 2, \dots$, and the extrapolated boundaries of the slab are located at $z = 0$ and $z = d + L_z = 2z_e$, respectively, where L_z is the physical thickness of the slab and the extrapolated length z_e is determined from the boundary condition of the slab.

Cai *et al.* has developed an analytical solution to the transport equation [42], [43] which

provides a more accurate description of photon distribution in space as well as photon velocity direction.

Since light becomes diffusive when it goes through a thick highly scattering turbid medium and, a direct shadow image cannot be formed for a target embedded in the scattering medium. Diffuse optical tomography (DOT) attempts to detect the target using the diffuse light that comes out from the boundary of the sample. Therefore, an inverse image reconstruction (IIR) technique is needed to reconstruct the image of the target. To implement an IIR method, a forward model is employed. A forward model calculates the photon density distribution when light propagates through the medium, with given sources, detectors, and the optical properties of the sample. The analytical solution to the diffusion equation may be used in the forward model if only simple geometry is involved. In this case, a perturbation approach may be used to describe the light propagation through a homogenous medium with localized small targets, and the optical properties of the targets are different from the background medium, so that the scattered light is considered to be the sum of the original unscattered light and the perturbation in the photon density distribution due to the targets. ⁴ However, since the analytical solution to the diffusion equation is limited to only a few simple geometries, other methods have also been used in the forward model, such as finite element method (FEM), ⁴ finite difference method (FDM) - , finite volume method (FVM), and Monte Carlo methods - 2 . These methods are not limited to the sample geometry.

DOT is an inverse image reconstruction (IIR) problem, which is ill-posed ⁴ , that is, solutions obtained in the inverse problem usually lack stability with respect to small variation in the data. For IIR, it is difficult to converge to the “true solution” before it falls at a “local minimum” in the error (cost) function. Regularization is commonly employed in the IIR to

improve the stability, such as Tikhonov regularization [4]. In principle, a general unique solution to the inverse problem for the Boltzmann equation or DOT does not exist [3, 4], due to the range of the unknowns, such as absorption coefficient, scattering coefficient, anisotropy, refractive index, coupling coefficients, geometry etc. But useful results may still be obtained in situations where unique solutions do not exist. Particularly, Arridge *et al.* discussed the uniqueness of diffusion equation, and showed that the absorption and scattering coefficients cannot be separated if intensity alone is acquired in continuous wave (CW) measurements, but they can be separated if it is measured in frequency domain.

A variety of IIR methods have been developed to reconstruct a property map of the sample [3, 1, 4]. These include analytic methods such as direct inversion [5], and Fourier transform [6, 7]; or numerical image reconstruction methods including linear methods, such as, conjugate gradients, algebraic reconstruction technique (ART) and Newton methods, and non-linear methods such as Gauss-Newton method, gradient methods. Numerical methods that involve running the forward model iteratively and minimizing the difference between the calculated data using the forward model and the experimental data, are very time-consuming, particularly if Monte Carlo method is used as the forward model and billions of photons need to be simulated.

The perturbation approach is commonly used in the IIR process. In the perturbation approach, the targets are assumed to be localized weak inhomogeneities in a homogenous medium, with optical properties slightly different from the background. It is a non-linear inverse problem that uses the influence due to local perturbation in the optical properties to calculate the Jacobian of the forward model, and minimize the error norm [4]. For simplification, the problem is often linearized. Compared to the homogeneous background, the change in the

intensity due to the targets is linearized using the first order of Born approximation, or the change in the log intensity is linearized using Rytov approximation [4].

In our study, both approximations may be used. Here Born approximation is used. When illuminated by a point source of unit power, the light intensity perturbation on the detector plane (the sample surface where light exits and is collected by detector arrays such as a CCD camera) to the first order of Born Approximation can be written as [1, 4],

$$\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = - \int G(\mathbf{r}_d, \mathbf{r}) \delta\mu_a(\mathbf{r}) c_m G(\mathbf{r}, \mathbf{r}_s) d\mathbf{r} - \int \delta D(\mathbf{r}) c_m \nabla_{\mathbf{r}} G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_{\mathbf{r}} G(\mathbf{r}, \mathbf{r}_s) d\mathbf{r}, \quad (1.1)$$

where $\mathbf{r}_s$, $\mathbf{r}$, and $\mathbf{r}_d$ are the positions of the source, the inhomogeneity, and the detector, respectively; $\delta\mu_a = \mu_{a,obj} - \mu_a$ and $\delta D = D_{obj} - D$ are the differences in absorption coefficient and diffusion coefficient, respectively, between the inhomogeneity and the background; c_m is the speed of light in the medium; and $G(\mathbf{r}_d, \mathbf{r}_j)$ and $G(\mathbf{r}_j, \mathbf{r}_s)$ are Green's functions describing light propagation from an inhomogeneity to a detector and a source to the inhomogeneity respectively in the background turbid medium of absorption μ_a and diffusion coefficient D .

For absorptive inhomogeneities, the j^{th} ($1 \leq j \leq J$) one contained in volume δV_j centered at $\mathbf{r}_j$, eq. (1.1) can be re-written as [1]

$$-\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = \sum_{j=1}^J G(\mathbf{r}_d, \mathbf{r}_j) \tau_j G(\mathbf{r}_j, \mathbf{r}_s), \quad (1.1)$$

where $\tau_j = \delta\mu_a(\mathbf{r}_j) c_m \delta V_j$, is the absorptive strength of the target.

For scattering inhomogeneities, the j^{th} ($1 \leq j' \leq J'$) one contained in volume $\delta V_{j'}$ centered at $\mathbf{r}_{j'}$, a similar form of the equation can be written [1],

$$\begin{aligned} -\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = & \sum_{j=1}^{J'} g_z(\mathbf{r}_j, \mathbf{r}_d) \tau'_j g_z(\mathbf{r}_j, \mathbf{r}_s) + \\ & \sum_{j=1}^{J'} \rho_{dj} \cos \theta_d g_{\perp}(\mathbf{r}_j, \mathbf{r}_d) \tau'_j \rho_{sj} \cos \theta_s g_{\perp}(\mathbf{r}_j, \mathbf{r}_s) + \end{aligned}$$

$$\sum_{j=1}^{J'} \rho_{dj} \cos \theta_d g_{\perp}(\mathbf{r}_j, \mathbf{r}_d) \tau'_j \rho_{sj} \sin \theta_s g_{\perp}(\mathbf{r}_j, \mathbf{r}_s), \quad (1.1)$$

where $\tau'_j = \delta D(\mathbf{r}_j) c_m \delta V'_j$, is the scattering strength. One small scattering inhomogeneity is a mixture of three components.

Though the ultimate goal of breast DOT is to generate images of breast cancer, with both high resolution and specificity, many applications require rather accurate determination of location of target(s) in three dimensions. For example, a recent study involving 1 patients underscores the influence of primary tumor location on breast cancer prognosis [1], and makes it imperative that DOT for breast cancer detection be able to obtain three-dimensional (3-D) location of the tumor. While two-dimensional (2-D) IIR approaches may provide only lateral positions, 3-D IIR approaches attempt to retrieve all three position coordinates of the target(s).

Various frequency-domain, time-domain, and steady-state DOT approaches have addressed the target localization problem with different measures of success [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100]. Several groups have paid particular attention to retrieving target location. [1]epshire *et al.* developed a subsurface DOT approach to obtain location information of absorbing and fluorescent targets, but observed the sensitivity to vary nonlinearly with depth [4]. Mohajerani *et al.* reported a fluorescent tomography method for locating fluorescent targets embedded in a heterogeneous medium using partitioning of the fluorophore distribution into an object subspace and a background subspace [5]. [6]odavarty *et al.* developed another fluorescent tomography approach that used a hemispherical breast phantom, near-infrared light-induced fluorescence from a contrast agent, and finite element method-based reconstruction algorithms to obtain target information up to a depth of 2 cm from breast phantom surface [7]. [8]hao *et al.* introduced a layer-based sigmoid adjustment method to improve depth resolution of DOT and achieved positioning error within 1 mm for depths from 1 to 10 mm [8]. Optical tomography using

independent component analysis (O-TICA) approach developed by Xu *et al.* uses multi-source probing and multi-detector signal acquisition scheme and a numerical algorithm based on independent component analysis (ICA) of information theory to obtain N -D position information of absorbing, scattering and fluorescent targets embedded in highly scattering turbid media, and “model breast” assembled using *ex vivo* human breast tissue [1-1], [2], [3]. Co-registration approaches that use another modality, such as, ultrasound, magnetic resonance imaging, and x-ray mammography for locating suspect areas and DOT for obtaining images have also been introduced [4].

This thesis develops two approaches for diffuse optical imaging. One approach is called time reversal optical tomography (TROT) based on time reversal symmetry and subspace-based algorithm that have been used for ultrasound and electromagnetic waves. The other approach is based on decomposition methods that use ICA, PCA and NMF algorithms of blind source separation (BSS) problem. These tomography approaches run faster than the regular model-based IIR methods. Most other model-based image reconstruction methods discretize the sample into voxels, and attempt to find the optical properties at every voxel in the sample through an iterative process that minimize the difference between the experimental measurements and calculated light intensities on the sample boundaries, which is time consuming. On the contrary, the approaches presented in this thesis mainly focus on localization of the targets, and attempt to find some characteristics about the targets.

1.3.4. Time reversal optical tomography

The multi-source illumination and multi-detector signal acquisition scheme is used. The light intensity distribution measured on the sample boundary is used to form a data matrix. A so-called “time reversal matrix” [4], [5] is calculated which describes light propagation from the

sources through the medium and targets to the detectors and back from detectors to sources. An eigenvalue decomposition or singular value decomposition (SVD) is used to find the leading (principal) components that correspond to targets. Using a subspace-based method, multiple signal classification (MUSIC), a pseudo spectrum is calculated for all discretized voxels in the sample. The pseudo spectrum describes the likelihood of each voxel belonging to a target. The maxima in the pseudo spectrum are used to determine the positions of the targets. The pseudo spectrum is also used to estimate the dimension of the target. Though TROT can be used with various geometries (slab, cylindrical, semi-spherical etc.) and measurement domains (frequency domain, time domain, CW), transillumination slab geometry and CW laser are used in the study presented in this thesis.

1.3.5. Decomposition methods based diffuse optical tomography

The signal measured on the sample boundary is considered to be linear combination of component signals due to targets. The problem is treated as a blind source separation (BSS) problem. The experimental arrangement, uses multi-source probing and multi-detector signal acquisition. A decomposition method is used to analyze the data matrix and extract the component signals which directly correspond to light propagation from sources to targets or from targets to detectors. Decomposition methods including principal component analysis (PCA) and non-negative matrix factorization (NMF) and independent component analysis (ICA) are used and compared with each other. The component signals are then fitted to the Green's functions to retrieve the position and optical properties of the targets. Then the component signals are back projected to the axial (z) planes of the target to estimate the cross-section of the target.

1.4. Thesis organization

The thesis is organized as follows.

Chapter 2 introduces the Time Reversal Optical Tomography (TROT) approach, based on time reversal (TR) imaging and multiple signal classification (MUSIC) for point targets. This chapter is focused on localization of small targets, either absorptive or scattering, in turbid media. The efficacy of the approach is evaluated using both simulated and experimental data. The resolution of TROT is also examined for different source and detector arrangements using simulations.

In **Chapter 3**, TROT is further developed to retrieve optical strength and size of absorptive and scattering targets.

Chapter 4 introduces the decomposition methods that treat DOI as a blind source separation (BSS) problem. Various BSS methods (matrix decomposition methods) including independent component analysis (ICA), principal component analysis (PCA), and non-negative matrix factorization (NMF) are used to separate signals due to different targets and the background, which are then fitted to Green's functions to retrieve position, optical strength and cross sectional area of the targets.

Chapter 5 presents the results of an experimental study of a realistic model breast assembled using *ex vivo* breast tissue with two pieces of breast tumors embedded. TROT and NMF-based optical tomography (NMF-OT) approach are used for detection of the targets. Multiple datasets are acquired with source beams at different wavelengths to test if the use of multiple wavelengths can result in any improvement in the efficacy of TROT and NMF-OT.

Chapter 6 explores fluorescence optical imaging using experiments and relevant simulation. The sample consists of a highly scattering medium with two fluorescent targets embedded. Both TROT and NMF approaches are extended to detect and characterize the fluorescent targets. Transmission data are also acquired in the experiments, and analyzed for comparison.

Chapter 7 summarizes the results and outlines the future work that can build on the developments presented in this thesis.

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Chapter 2

Time reversal optical tomography: locating small absorptive and scattering targets

2.1. Introduction

Optical imaging of targets in a highly scattering turbid medium for biomedical applications is an active area of research where the thrust is on developing fast and accurate methods for detecting and characterizing targets, and as outlined in Chapter 1, several approaches have evolved over the years. In this chapter we report on the development of an alternative approach, time reversal optical tomography (TROT) [1-3] for NIR optical imaging of target(s) in a turbid medium, and present initial results of its efficacy using both simulated and experimental data.

Time reversal (TR) invariance, the basic symmetry that commonly holds in microscopic physics, forms the basis for macroscopic TR imaging. TR imaging using the so-called “time-reversal mirrors” (TRMs) has been used as an experimental tool in acoustics with practical applications in medicine, underwater imaging, and nondestructive testing [4-6]. The theoretical and numerical techniques involved in time reversal have been used for applications involving both acoustic waves and electromagnetic waves (radar) [7-12].

Devaney and associates developed a theoretical framework for a TR imaging method with Multiple Signal Classification (MUSIC) for finding the location of scattering targets whose size is smaller than the wavelength of acoustic waves or electromagnetic waves (radar) used for probing the homogeneous or inhomogeneous background medium in which the targets were embedded [1, 14]. While their initial focus was on *back-propagation geometry* that used coincident acoustic or electromagnetic transceiver array for collecting the back-scattered signal

from the targets, they later extended the formalism to *transmission geometry* where sources and detectors were distinct and separated [1]. They also generalized the theory which was based on distorted wave Born approximation (DWBA) to account for multiple scattering between the targets [1]. In its basic form TR-MUSIC found target location from knowledge of the response matrix K , which was constructed from multi-static data collected by the transceiver array [1, 14]. TR-MUSIC provided higher spatial resolution than the conventional TR imaging, especially in the case where targets were not well resolved [1, 14, 15].

We are adapting and extending the TR-MUSIC approach to the optical domain, *i.e.* to diffusive optical imaging for detecting and locating targets embedded in a turbid medium. In this chapter, TROT is studied in details using both simulated data and data from transillumination NIR imaging experiments in slab geometry. A TR matrix is obtained by multiplying the response matrix formed using experimental or simulated data to its conjugate matrix. The leading non-zero eigenvalues of the Hermitian TR matrix determine the signal subspace due to presence of the targets. The signal subspace is separated from the noise subspace using an L -curve method [16-18]. The vector subspace method, MUSIC, along with Green's functions calculated from an appropriate forward model for light propagation through the turbid medium is then used to determine the locations of the targets. The MUSIC algorithm judges if the calculated Green's function vector corresponding to a location in the sample is mapped into the signal subspace or the noise subspace.

Several salient features make TROT attractive and potentially more promising than other IIR methods. First, the size of the TR matrix is much smaller than those used in other IIR approaches, which makes solution of the eigenvalue problem easier and faster. Second, to determine locations of targets, TR-MUSIC approach runs the program over all voxels only once,

and there is no need to carry out an iterative procedure done by other inverse approaches. Other IIR approaches seek to determine the absorption and scattering parameters at all voxels into which the sample is divided. The process is iterative, computationally intensive, and leads to a solution of the inverse problem that is not unique because the problem is ill-posed, even when there is no noise. In contrast, TROT seeks to determine the locations of the targets first and thereafter retrieve other information, such as, the size and optical properties of the limited number of targets in the medium, which requires significantly less computation time. The focus of this chapter is on finding the locations of targets.

Our result using simulated data shows that without the presence of noise TROT determines the locations of the embedded targets accurately with high resolution. TROT exhibits promise to locate targets both in simulations and experiments even when substantial noise is present. Images of small targets obtained by this approach are sharper than that obtained by other IIR approaches.

This remainder of this chapter is organized as follows. In Section 2.2, the formalism of the TROT approach is presented. In Section 2.3, the numerical algorithm of TROT is described. In Section 2.4, the efficacy of the formalism is tested using simulated data. Section 2.5 presents the results when the formalism is applied to experimental data using Intralipid-2 suspension in water as the highly scattering turbid medium. Section 2.6 discusses the results.

2.2. Theoretical formalism

2.2.1. Diffusion approximation, perturbation method and response matrix

The starting point for the TROT formalism is the diffusion approximation [21-23] of the radiative transfer equation (RT) [24, 25]. The perturbation in the light intensity distribution due to small inhomogeneities (targets) embedded in a homogeneous medium, to the first order Born approximation, can be written as [26, 27]

$$\begin{aligned}\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = & -\int G(\mathbf{r}_d, \mathbf{r})\delta\mu_a(\mathbf{r})cG(\mathbf{r}, \mathbf{r}_s)d\mathbf{r} \\ & -\int \delta D(\mathbf{r})c\nabla_{\mathbf{r}}G(\mathbf{r}_d, \mathbf{r})\cdot\nabla_{\mathbf{r}}G(\mathbf{r}, \mathbf{r}_s)d\mathbf{r},\end{aligned}\quad (2.1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a point-like source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagations from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ is the difference in absorption coefficient and δD is the difference in diffusion coefficient between the targets and the background medium; and c is the light speed in the medium.

A multi-source interrogation and multi-detector signal acquisition scheme is used to acquire transillumination data, from which the difference in the light intensity distribution due to the targets, $\Delta\phi = \phi - \phi_0$, is found, where ϕ is the light intensity distribution measured on the sample boundary with targets embedded in the scattering medium, and ϕ_0 is ideally the light intensity distribution without the targets, which in practice is approximated by an “average” over all the multi-source measurements. A response matrix K is constructed with $-\Delta\phi$, to describe the transport of light from different sources through the embedded objects to the array of detectors $1, 1 \dots$.

For small, point-like absorptive targets, the matrix elements can be rewritten in a discrete form as:

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{X}_m)\tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), i=1, 2, \dots, N_d; j=1, 2, \dots, N_s, \quad (2.2)$$

where $\tau_m = \delta\mu_a(\mathbf{X}_m)c_m\delta V_m$ is the optical absorption strength of the m^{th} target, δV_m is the volume of m^{th} target, $\mathbf{r}_i$, $\mathbf{r}_j$ and $\mathbf{X}_m$ are locations of the i^{th} detector, j^{th} source and m^{th} target, respectively. Due to the reciprocity of light propagation in the medium, $G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r}', \mathbf{r})$. Thus,

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{X}_m, \mathbf{r}_i) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), \quad (2.3)$$

and

$$K = \{K_{ij}\} = \sum_{m=1}^M g_d(\mathbf{X}_m) \tau_m g_s^T(\mathbf{X}_m), \quad (2.4)$$

where $g_s(\mathbf{r})$ and $g_d(\mathbf{r})$ are Green's function vectors (Fields) defined as

$$g_s(\mathbf{r}) = [G^s(\mathbf{r}_1, \mathbf{r}), G^s(\mathbf{r}_2, \mathbf{r}), \dots, G^s(\mathbf{r}_{N_s}, \mathbf{r})]^T, \quad (2.5a)$$

$$g_d(\mathbf{r}) = [G^d(\mathbf{r}_1, \mathbf{r}), G^d(\mathbf{r}_2, \mathbf{r}), \dots, G^d(\mathbf{r}_{N_d}, \mathbf{r})]^T, \quad (2.5b)$$

associated with the source array and detector array, respectively, where the superscript T denotes transpose; and N_s , N_d and M are the numbers of sources, detectors and targets, respectively. It is assumed the number of targets is less than the number of sources and detectors, $M \leq \min(N_d, N_s)$. $K^T = K_{ji}$ describes light propagation from the positions of detectors through the medium and targets to sources.

For a homogeneous background medium, the rank R of matrix K , is equal to the dimension of the source array vector space $\mathcal{G}_s$ spanned by $g_s(\mathbf{r}_m)$, and also equal to the dimension of the detector array vector space $\mathcal{G}_d$ spanned by $g_d(\mathbf{r}_m)$, where $\mathcal{G}_s \subseteq C^{N_s}$ and $\mathcal{G}_d \subseteq C^{N_d}$. For absorptive targets, R is equal to the number of targets M .

Similar forms of the response matrix and Fields can be obtained for scattering targets. As the dot product in the second term of eq. (2.1) implies, each scattering target is represented by three components coexisting at one location. The elements of the K matrix for L scattering target may be written as

$$\begin{aligned}
K_{ij} &= \sum_{l=1}^L \tau_l \nabla_{\mathbf{r}} G^d(\mathbf{r}_i, \mathbf{X}_l) \cdot \nabla_{\mathbf{r}} G^s(\mathbf{X}_l, \mathbf{r}_j) \\
&= \sum_{l=1}^L \tau_l \sum_{\alpha=\{x,y,z\}} \partial_{\alpha} G^d(\mathbf{r}_i, \mathbf{X}_l) \partial_{\alpha} G^s(\mathbf{X}_l, \mathbf{r}_j),
\end{aligned} \tag{2. }$$

where $\tau_l = \delta D(\mathbf{X}_l) c_m \delta V_l$ is the optical scattering strength of the l^{th} target. The K matrix for scattering targets can be written in a manner similar to that for absorptive targets:

$$K = \sum_{l=1}^L \sum_{\alpha=\{x,y,z\}} \partial_{\alpha} g_d(\mathbf{X}_l) \tau_l \partial_{\alpha} g_s^T(\mathbf{X}_l). \tag{2. }$$

The Green's function for a slab geometry is [2, 2]

$$G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r}, \mathbf{r}') = \frac{1}{4\pi D} \sum_{k=-\infty}^{\infty} \left(\frac{e^{-\kappa r_k^+}}{r_k^+} - \frac{e^{-\kappa r_k^-}}{r_k^-} \right), \tag{2. a}$$

$$r_k^{\pm} = \left[(x - x')^2 + (y - y')^2 + (z \mp z' + 2kd)^2 \right]^{1/2}, \tag{2. b}$$

where $\kappa = \left[(\mu_a - i\omega/c)/D \right]^{1/2}$ in frequency domain with amplitude modulation frequency ω . The extrapolated boundaries of the slab are located at $z = 0$ and $z = d + L + 2z_e$, respectively, where L is the physical thickness of the slab and the extrapolation length z_e is determined from the boundary condition of the slab [2, 2].

Under ideal conditions, when all three scattering components of each of the L scattering targets are well-resolved, the rank of K contributed by L scattering targets is $3L$. In practice, four components (one for absorption and three for scattering) are calculated for each target, since the targets may have both scattering and absorptive characteristics, or the exact nature may not be known *a priori*. The dominant characteristic is used to label the target as absorptive or scattering in nature.

2.2.2. Point spread functions

If light emitted by a source of unit power at target position $\mathbf{X}$ propagates in the sample medium, the signal measured by the detector array at the sample boundary is $G_d(\mathbf{r}_i, \mathbf{X})$. The signal is then “time-reversed” and back-propagated with the Green’s function of the background medium. TR operation is phase conjugation in Fourier domain [1, 2]. So the signal evaluated at $\mathbf{r}$ is [1]

$$\begin{aligned} H_d(\mathbf{r}, \mathbf{X}) &= \sum_{i=1}^{N_d} G^d(\mathbf{r}, \mathbf{r}_i) G^d(\mathbf{r}_i, \mathbf{X}) = g_d^T(\mathbf{r}) g_d(\mathbf{X}) \\ &= g_d(\mathbf{X}) g_d(\mathbf{r}) = \langle g_d(\mathbf{X}), g_d(\mathbf{r}) \rangle, \end{aligned} \quad (2.)$$

where $*$ denotes complex conjugate, ^{T} denotes adjoint, and $\langle \cdot, \cdot \rangle$ denotes inner product. $H_d(\mathbf{r}, \mathbf{X})$ is the detector array point spread function (PSF). A source array PSF can be similarly formed as

$$H_s(\mathbf{r}, \mathbf{X}) = g_s(\mathbf{X}) g_s(\mathbf{r}) = \langle g_s(\mathbf{X}), g_s(\mathbf{r}) \rangle. \quad (2.1)$$

Due to the time reversal assumption, $H_d(\mathbf{r}, \mathbf{X})$ peaks at $\mathbf{r} = \mathbf{X}$, so it can be considered as an image of the source at $\mathbf{X}$ formed by the TR detector array. PSF vanishes when $\mathbf{r}$ is far away from $\mathbf{X}$. A similar interpretation can be used for $H_s(\mathbf{r}, \mathbf{X})$.

2.2.3. Time reversal and MUSIC

The TR matrix may be constructed to represent light propagation from sources to detectors and back denoted by T_{SDDS} , or to represent light propagation from detector positions to source positions and back denoted by T_{DSSD} , a consequence of the reciprocity of light propagation [1, 2]. For frequency domain, $T_{SDDS} = K K^T$, and $T_{DSSD} = (K^T)^T K^T = K^* K^T$, where response data matrix K is formed using modulated intensities, instead of the field with phase information used in the conventional TR. For CW measurements $T_{SDDS} = K^T K$, and $T_{DSSD} = K K^T$ (K is real and only includes intensity values).

Since T_{SDDS} and T_{DSSD} are hermitian ($T = T^T$), they have complete sets of orthonormal eigenvectors $\mathbf{v}_j$ ($j = 1, \dots, N_s$) and $\mathbf{u}_i$ ($i = 1, \dots, N_d$), with a common set of non-negative real

eigenvalues. For $M = \min(N_s, N_d)$ absorptive targets without the presence of noise, the rank of T_{SDDS} and T_{DSSD} is M . The eigenvalues $\lambda_j = \tau_j^2$, when $j = 1, \dots, M$, and $\lambda_j = 0$, when $j = M+1, \dots, N_s$ for T_{SDDS} and $j = M+1, \dots, N_d$ for T_{DSSD} . The eigen system v_j, u_j, λ_j , $j = 1, \dots, M$, is related to the targets. The TR matrix T_{SDDS} can be written as [1, 1]

$$T_{SDDS} = \sum_{m=1}^M \sum_{m=1}^M \tau_m \tau_m \langle g_d(\mathbf{X}_m), g_d(\mathbf{X}_m) \rangle g_s(\mathbf{X}_m) g_s^T(\mathbf{X}_m). \quad (2.11)$$

Subsequent formalism may be different depending on whether the targets are “well resolved” or “poorly resolved.”

2.2.3.1. Well-resolved targets

If the m^{th} and m'^{th} targets ($m \neq m'$) are well resolved, defined by the conditions: $H_s(\mathbf{X}_m, \mathbf{X}_{m'}) = 0$ and $H_d(\mathbf{X}_m, \mathbf{X}_{m'}) = 0$, i.e. the F s at $\mathbf{X}_m$ and $\mathbf{X}_{m'}$ are orthogonal, $g_d(\mathbf{X}_m), g_d(\mathbf{X}_{m'}) \in H_d(\mathbf{X}_m, \mathbf{X}_{m'}) = g_d(\mathbf{X}_m)^\perp \delta_{mm'}$. So we have

$$T_{SDDS} = \sum_{m=1}^M |\tau_m|^2 \|g_d(\mathbf{X}_m)\|^2 g_s(\mathbf{X}_m) g_s^T(\mathbf{X}_m), \quad (2.12)$$

where $\|\cdot\|$ denotes L2 norm. The eigenvectors of T_{SDDS} are proportional to the complex conjugate of the F s associated with the M targets [1, 1], i.e.

$$T_{SDDS} g_s(\mathbf{X}_m) = |\tau_m|^2 \|g_d(\mathbf{X}_m)\|^2 \|g_s(\mathbf{X}_m)\|^2 g_s(\mathbf{X}_m). \quad (2.13)$$

The eigenvectors are

$$v_j = \frac{g_s(\mathbf{X}_j)}{\|g_s(\mathbf{X}_j)\|}, \quad (2.14)$$

with eigenvalues $\lambda_j = \tau_j^2 \|g_d(\mathbf{X}_j)\|^2 \|g_s(\mathbf{X}_j)\|^2$, $j = 1, \dots, M$. Thus T_{SDDS} is a projection operator that projects a vector onto the conjugate of the source array vector space $\mathcal{G}_s$. The j^{th} non-zero eigenvalue λ_j is directly related to the optical strength τ_j of the j^{th} target. Similar equations can be

derived for T_{DSSD} , which is a projection operator for the conjugate of the detector array vector space $\mathcal{G}_d$. The eigenvectors of T_{DSSD} are

$$u_j = \frac{g_d(\mathbf{X}_j)}{\|g_d(\mathbf{X}_j)\|}, \quad (2.1 \text{ a})$$

$j = 1, \dots, M$, with the same eigenvalues as T_{SDDS} .

Therefore, for well-resolved targets, the target locations can be determined by the inner product $\langle \mathbf{v}_j, \mathbf{g}_s(\mathbf{X}_p) \rangle$, $\langle \mathbf{u}_j, \mathbf{g}_d(\mathbf{X}_p) \rangle$

$$\begin{aligned} \psi_j^s &= \langle \mathbf{v}_j, \mathbf{g}_s(\mathbf{X}_p) \rangle = \mathbf{v}_j^T \mathbf{g}_s(\mathbf{X}_p) = \frac{g_s(\mathbf{X}_j)}{\|g_s(\mathbf{X}_j)\|} g_s(\mathbf{X}_p) \\ &= \frac{1}{\|g_s(\mathbf{X}_j)\|} H_s(\mathbf{X}_p, \mathbf{X}_j), \end{aligned} \quad (2.1 \text{ a})$$

or

$$\begin{aligned} \psi_j^d &= \langle \mathbf{u}_j, \mathbf{g}_d(\mathbf{X}_p) \rangle = \mathbf{u}_j^T \mathbf{g}_d(\mathbf{X}_p) = \frac{g_d(\mathbf{X}_j)}{\|g_d(\mathbf{X}_j)\|} g_d(\mathbf{X}_p) \\ &= \frac{1}{\|g_d(\mathbf{X}_j)\|} H_d(\mathbf{X}_p, \mathbf{X}_j), \end{aligned} \quad (2.1 \text{ b})$$

where $\mathbf{X}_p$ is a test target position, which is the position of any voxel in the sample space. ψ_j^s and ψ_j^d peak when $\mathbf{X}_p$ happens to be the position of the j^{th} target. In the classical TR imaging [4], $\langle \mathbf{v}_j, \mathbf{g}_s(\mathbf{X}_p) \rangle$, $\langle \mathbf{u}_j, \mathbf{g}_d(\mathbf{X}_p) \rangle$ for ideally resolved targets, each eigenvector of the TR operator can be used to focus on one particular target. Here ψ_j^s and ψ_j^d represent focusing of signals from the source and detector planes on to the target position, respectively. Use of the eigenvectors $\mathbf{v}_j$ and $\mathbf{u}_j$, $j = 1, \dots, M$, ensures that the j^{th} target is sorted out. When T_{SDDS} and source array vector space (T_{DSSD} and detector array vector space) are used, we call the scheme SDDS (DSSD). Both source and detector arrays can be considered simultaneously to locate the target by calculating

$$\psi_j = \psi_j^d \psi_j^s = \frac{1}{\|g_s(\mathbf{X}_j)\| \cdot \|g_d(\mathbf{X}_j)\|} H_d(\mathbf{X}_p, \mathbf{X}_j) H_s(\mathbf{X}_j, \mathbf{X}_p), \quad (2.1)$$

$j = 1, \dots, M$, which is computationally equivalent to a process that light emitted from a virtual source of unit power at a test target position $\mathbf{X}_p$, propagates to the TR source array and back to a true target position $\mathbf{X}_j$; then it is re-emitted and further propagates to the TR detector array and back to the original position $\mathbf{X}_p$. ψ_j peaks when the test target position $\mathbf{X}_p$ coincides with the true target position $\mathbf{X}_j$ associated with the j^{th} eigenvector.

2.2.3.2. Poorly-resolved targets and MUSIC

When the targets are too close to each other or the sources and/or detectors are significantly sparse, the targets are considered to be poorly resolved and the $\mathbf{F}$'s at $\mathbf{X}_m$ and $\mathbf{X}_m$ are not orthogonal. In such cases, the eigenvectors $\mathbf{v}_j$ and $\mathbf{u}_j$ do not correspond one-to-one with the $\mathbf{F}$'s associated with target positions $\mathbf{X}_m$ ($m = 1, \dots, M$). The image resolution degrades because of contributions from multiple targets. To solve this problem, the subspace-based method, MUSIC was implemented with TR 1, 1, 2. MUSIC algorithm is based on the idea that although the vectors characterizing the targets are no longer orthogonal with each other, they are all located in the signal subspace, which is orthogonal to the noise subspace.

The orthonormal sets $\mathbf{v}_j^*$ ($j = 1, \dots, N_s$) and $\mathbf{u}_j^*$ ($j = 1, \dots, N_d$) span the spaces C^{N_s} and C^{N_d} associated with the source and detector arrays, respectively. While $\mathbf{v}_j^*$ and $\mathbf{u}_j^*$, with λ_j , form the signal subspaces on the source and detector arrays, $\mathcal{S}^s = \mathbf{v}_j$ and $\mathcal{S}^d = \mathbf{u}_j$ ($j = 1, \dots, M$), respectively; $\mathbf{v}_j^*$ and $\mathbf{u}_j^*$, with λ_j , form the noise subspaces, $\mathcal{N}^s = \mathbf{v}_j$ ($j = M+1, \dots, N_s$) and $\mathcal{N}^d = \{\mathbf{u}_j\}$ ($j = M+1, \dots, N_d$), respectively. Thus $C^{N_s} = \mathcal{S}^s \oplus \mathcal{N}^s$ and $C^{N_d} = \mathcal{S}^d \oplus \mathcal{N}^d$ 1, 2. Since the dimensions of the signal subspaces $\mathcal{S}^s$ and $\mathcal{S}^d$ and of the

For spaces $\mathcal{G}_s$ and $\mathcal{G}_d$ are all equal to M , $\mathcal{G}_s \equiv \mathcal{S}^s$ and $\mathcal{G}_d \equiv \mathcal{S}^d$. The vectors $g_s(\mathbf{X}_m)$ and $g_d(\mathbf{X}_m)$, $m = 1, \dots, M$, are linear combinations of v_j^* and u_j^* , $j = 1, \dots, M$, respectively. Therefore, $g_s(\mathbf{X}_m) \in \mathcal{S}^s$ and $g_d(\mathbf{X}_m) \in \mathcal{S}^d$, $m = 1, \dots, M$, associated with m^{th} target are orthogonal to $v_j \in \mathcal{N}^s$ ($j = M+1, \dots, N_s$) and $u_j \in \mathcal{N}^d$ ($j = M+1, \dots, N_d$), respectively:

$$\langle v_j, g_s(\mathbf{X}_m) \rangle = v_j^T g_s(\mathbf{X}_m) \approx 0, j = M+1, \dots, N_s, \quad (2.1 \text{ a})$$

$$\langle u_j, g_d(\mathbf{X}_m) \rangle = u_j^T g_d(\mathbf{X}_m) \approx 0, j = M+1, \dots, N_d. \quad (2.1 \text{ b})$$

The locations of targets can be determined by calculating the following squared sum of inner products:

$$Q_s(\mathbf{X}_p) = \sum_{j=M+1}^{N_s} \left| v_j^T g_s(\mathbf{X}_p) \right|^2, \quad (2.1 \text{ a})$$

$$Q_d(\mathbf{X}_p) = \sum_{j=M+1}^{N_d} \left| u_j^T g_d(\mathbf{X}_p) \right|^2. \quad (2.1 \text{ b})$$

$Q_s(\mathbf{X}_p)$ and $Q_d(\mathbf{X}_p)$ vanish when the test target position $\mathbf{X}_p$ is a true target position. Similar to eq. (2.1), $Q = Q_s + Q_d$ can be calculated with both source and detector arrays considered simultaneously. An alternative approach to accentuate a target position is to plot a pseudo spectrum defined as

$$P_s(\mathbf{X}_p) = \left\| g_s(\mathbf{X}_p) \right\|^2 / \left| Q_s(\mathbf{X}_p) \right| \quad (2.2 \text{ a})$$

associated with the source array, or

$$P_d(\mathbf{X}_p) = \left\| g_d(\mathbf{X}_p) \right\|^2 / \left| Q_d(\mathbf{X}_p) \right| \quad (2.2 \text{ b})$$

associated with the detector array, or

$$P(\mathbf{X}_p) = P_s(\mathbf{X}_p) P_d(\mathbf{X}_p) \quad (2.2 \text{ c})$$

associated with both source and detector arrays $\mathbf{1}, \mathbf{1}, \mathbf{1}, \mathbf{2}$, where $g_s(\mathbf{X}_p)^2$ and $g_d(\mathbf{X}_p)^2$ are used for normalization. The poles of the pseudo spectrum correspond to target locations. These M SIC pseudo spectra can also be used to locate well-resolved targets.

Since the dimension of the signal subspace is generally much smaller than that of the noise subspace, it is preferred that in eq. (2.1) and eq. (2.2), the signal subspace is used rather than the noise subspace for ease of computation. Using the properties of the projection operators associated with the source and detector arrays $\mathbf{1}, \mathbf{1}, \mathbf{1}, \mathbf{2}$, $Q_s(\mathbf{X}_p)$ and $Q_d(\mathbf{X}_p)$ can be calculated as

$$Q_s(\mathbf{X}_p) = \|g_s(\mathbf{X}_p)\|^2 - \sum_{j=1}^M |v_j^T g_s(\mathbf{X}_p)|^2, \quad (2.21a)$$

$$Q_d(\mathbf{X}_p) = \|g_d(\mathbf{X}_p)\|^2 - \sum_{j=1}^M |u_j^T g_d(\mathbf{X}_p)|^2. \quad (2.21b)$$

When the targets are embedded in a non-uniform medium, or when there is significant noise present, the noise or false targets contribute significantly to the eigenvalues. The near-zero and non-zero eigenvalues are not as well separated as when there are no noise. In this case, the rank of the TR matrix is larger than the number of targets M . The TR matrix may even be full rank.

However, as long as M is less than $\min(N_s, N_d)$ and eigenvalues contributed by the noise and false targets are smaller than those contributed by the real targets with a threshold ϵ , the target positions can be obtained using a pseudo spectrum $\mathbf{1}, \mathbf{2}$ associated with the source array,

$$P_s(\mathbf{X}_p) = \|g_s(\mathbf{X}_p)\|^2 / \left| Q_s(\mathbf{X}_p)_{\lambda_j \leq \epsilon} \right|, \quad (2.22)$$

where $Q_s(\mathbf{X}_p)_{\lambda_j \leq \epsilon} = \|g_s(\mathbf{X}_p)\|^2 - \sum_{\lambda_j > \epsilon} |v_j^T g_s(\mathbf{X}_p)|^2$. Pseudo spectra associated with the detector

array or with both source and detector arrays can also be obtained similarly. In practice, the

threshold is selected to separate the signal and noise subspaces using a method similar to L -curve regularization [1].

When scattering targets are concerned, the F 's $\partial_\alpha g(\alpha, x, y, z)$, associated with the test target position X_p will be used to calculate the pseudo spectrum. For a target with both absorption and scattering properties at the wavelength of probing light, one F corresponding to absorption constructed as g and three F 's corresponding to scattering target constructed with $\partial_\alpha g(\alpha, x, y, z)$, are used to calculate the pseudo spectrum over every voxel. Ideally, for an absorptive and scattering target four pseudo-values will be obtained for every target position. If the dominant value corresponds to the absorptive (any of the scattering) F the target will be identified as absorptive (scattering) in nature.

2.3. Algorithm

Implementation of TROT to locate targets embedded in a highly scattering turbid medium involves the steps outlined below. For simplicity, the sizes of source array and detector array are assumed to be the same, i.e., $N_d = N_s = N$.

- (a) A response matrix K with size $N \times N$ is constructed using experimental data (or estimated data in simulation). Data consist of the perturbations in the light intensity distribution due to the targets, $\Delta\phi = \phi - \phi_0$, where ϕ is the light intensity distribution measured on the sample boundary with targets embedded in the scattering medium and ϕ_0 is ideally the light intensity distribution without the targets. In practice, ϕ_0 is approximated by an “average” over all the multi-source measurements, while in simulation it can be estimated without such approximation.

- (b) A detector array TR matrix, $T_{DSSD} = KK^T$ with size $N \times N$ for CW measurements is constructed. All the eigenvalues and the eigenvectors of the T_{DSSD} matrix are computed. The eigenvectors are orthogonal to each other. It is to be noted that in this procedure we only deal with a matrix of dimension N , not a matrix of dimension of $N \times N$ as done in traditional inverse procedures.
- (c) The non-zero eigenvalues of T_{DSSD} belonging to the signal subspace are separated from the near-zero eigenvalues belonging to the noise subspace using the L -curve method [1-2].
- (d) M-SIC approach [1, 1, 2] is next used to determine the locations of the targets as follows. (i) The $-D$ medium is divided into a certain number of voxels. A detector array F , $g_d(\mathbf{X}_p)$, associated with an absorptive test target position $\mathbf{X}_p$ at p^{th} voxel is calculated using Diffusion Approximation of RT. Other proper forward models could be used as well. In order to check if $g_d(\mathbf{X}_p)$ is located in the signal subspace or in the noise subspace, a pseudo spectrum associated with the detector array is computed using eq. (2.2 b), where M is the dimension of the signal subspace found in step (c). If $g_d(\mathbf{X}_p)$ is located in the signal subspace, the corresponding pseudo value $P(\mathbf{X}_p)$ in eq. (2.2 b) will become a maximum. (ii) pseudo spectra are also calculated using the other three F s, $\partial_\alpha g_d(\mathbf{X}_p)$, ($\alpha = x, y, z$) for scattering property. (iii) All pseudo values are put together and sorted in a descending order. Since the leading pseudo values at $\mathbf{X}_p$ are associated with targets and specific F s, the positions of the embedded targets and their nature (absorptive or scattering) are determined. The pseudo spectrum in the whole sample space can be used to plot pseudo tomographic images.

In this approach, only a single run is needed for calculating the pseudo spectrum and no iterative procedure is involved, which makes it faster and computationally less intensive than the

traditional IIR approaches. Similar procedure can be used for application of TROT when $N_d = N_s$. The pseudo spectrum associated with either the source array, or the detector array, or both source and detector arrays, as outlined in eq. (2.2) can be used to obtain target positions.

It is instructive to compare the computational complexity of TROT formalism with that of typical iterative methods. For a typical iterative method, an equation $b = Wx$ is solved to find the inhomogeneities (targets), where W is a weight matrix with size $N_d N_s \times N$, N is the number of voxels, b is an $N_d N_s \times 1$ vector describing the perturbation in the detected light intensity due to the presence of inhomogeneities, and x is the perturbation or variation in the optical properties from the background values with dimension of $N \times 1$. The computational complexity is typically $O(N_d N_s N^2)$ for a single iteration. The computational complexity of TROT is much smaller than that for even one iteration of an iterative method. For the SDDS scheme, the complexity for TROT is $O(N_d N_s^2)$ if $N_d N_s \leq NN_k$, and $O(N_s NN_k)$ otherwise, where N_k is the dimension of the signal subspace. In the DSSD scheme, the complexity is $O(N_s N_d^2)$ if $N_d N_s \leq NN_k$, and $O(N_d NN_k)$ otherwise. TROT does not involve any iteration.

In the following sections, TROT will be tested using simulation and experimental data.

2.4. Testing TROT with simulated data

To test the efficacy of the TROT approach, we first consider a rather challenging task of detecting and locating six targets embedded in a simulated sample which is a 4 -mm thick uniform scattering slab. Its absorption and diffusion coefficients are $\mu_a = 1/\text{mm}$ and $D = 1/\text{mm}$, respectively. The incident CW beam was step-scanned in an x - y array of 41×41 grid points with a step size of 2 mm on the input plane covering an 82×82 mm area. Light transmitted from the opposite side (output plane) was recorded at 41×41 grid points covering the same area. No random noise was added.

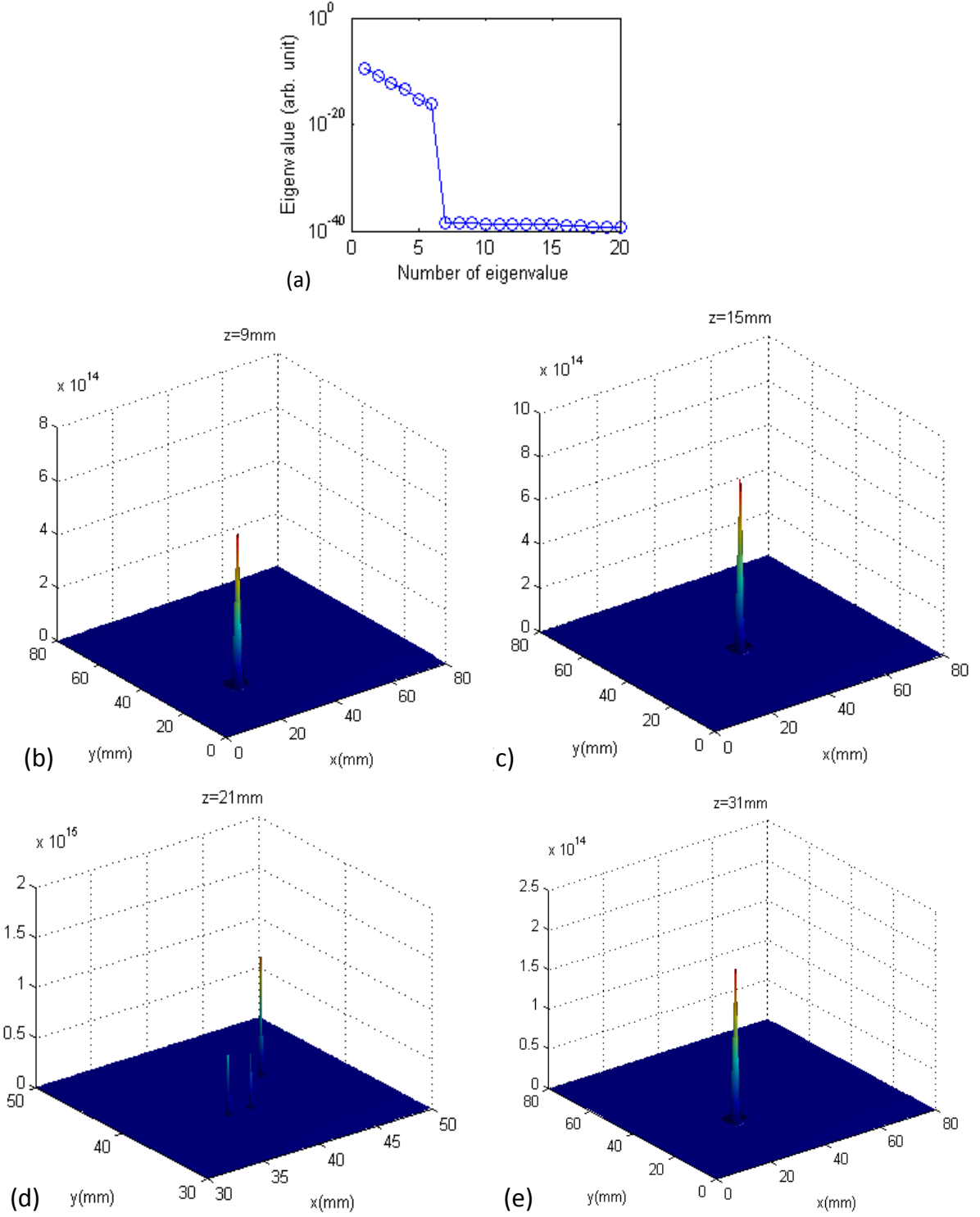


Fig. 2.1. (a) A plot of first twenty (20) eigenvalues on logarithmic scale. (b-e) 2-D slices of the pseudo spectrum on $z = 9\text{ mm}$, 15 mm , 21 mm , and 31 mm planes, respectively, showing the location of the six targets, where $z = 21\text{ mm}$ shows a zoomed-in image of the three difficult targets described in the text.

The six ($M = 6$) point-like absorptive targets, with absorption coefficient difference of $\Delta\mu_a = 1 \text{ mm}^{-1}$ from the background, were placed at $A (24 \text{ mm}, 2 \text{ mm}, 1 \text{ mm})$, $B (44 \text{ mm}, 42 \text{ mm}, 1 \text{ mm})$, $C (24 \text{ mm}, 2 \text{ mm}, 21 \text{ mm})$, $D (44 \text{ mm}, 42 \text{ mm}, 21 \text{ mm})$, $E (24 \text{ mm}, 2 \text{ mm}, 1 \text{ mm})$, and $F (44 \text{ mm}, 42 \text{ mm}, 1 \text{ mm})$, respectively. The origin ($0 \text{ mm}, 0 \text{ mm}, 0 \text{ mm}$) was located at the upper-left corner of the input boundary (source plane) of the sample. The medium was divided into $4 \times 4 \times 2$ voxels, with each voxel of size $2 \text{ mm} \times 2 \text{ mm} \times 2 \text{ mm}$. As can be seen from the assigned coordinates, targets C and D are located at two adjacent voxels, and are close to target E , and these three targets are located in the same z layer. Consequently, targets C and D are expected to be very difficult to resolve, and hard to distinguish from target E . Target A and C have the same lateral position x and y , and different depths. Target A is close to the source plane, while F is close to the detector plane.

Using the Diffusion Approximation of the RT as the model for light propagation in slab geometry, signals arising from light propagation from the source array to the detector array through medium *with* and *without* the targets were calculated. The difference between the two sets, which is the perturbation due to the targets, was used as the “simulated data”. The size of the K matrix is $N_x \times N_y \times N_z \times N_x \times N_y \times N_z = 16 \times 16 \times 2 \times 16 \times 16 \times 2$. The TR matrix $T = KK^T$ was constructed. Then, 16 eigenvalues and 16 eigenvectors of T were found.

The first seven ($M = 7$) computed eigenvalues in a descending order of magnitude are listed in the first column of Table 2.1. The leading twenty eigenvalues are plotted in Fig. 2.1(a) on a logarithmic scale. The first six ($M = 6$) eigenvalues are at least 1 orders-of-magnitude higher than the 7-th and other smaller eigenvalues. Hence, the dimension of the signal subspace and the number of targets are determined to be six. The pseudo spectrum (consisting of $4 \times 4 \times 2 \times 4 \times 4 \times 2 = 262144$ elements) was calculated using the M eigenvectors in the signal subspace. The values of

elements in the pseudo spectrum were sorted in a descending order. The seven leading pseudo values are listed in Table 2.1 with the corresponding positions of voxels. The six peaks are found to be associated with the F_s for absorptive targets, and consequently, the six targets are identified to be absorptive targets.

Table 2.1. Eigenvalues, pseudo spectrum and the corresponding positions

Leading eigenvalues	Order of pseudo Spectrum	Retrieved position (x, y, z) mm	Known position (x, y, z) mm
2.11 - 1	1.11 - 1	(44, 42, 21)	(44, 42, 21)
1.122 - 11	.14	(, , 1)	(, , 1)
4.1 - 1	.14	(, , 21)	(, , 21)
. - 14	.22 - 14	(4 , , 21)	(4 , , 21)
.22 - 1	.1 - 14	(24, 2 ,)	(24, 2 ,)
.4 - 1	2.11 - 14	(, , 1)	(, , 1)
2. -	2.4	(, , 1)	

All six large pseudo-values are located at the exact known target locations and their values are approximately orders-of-magnitude larger than those at their neighborhood locations. A 2-D slice of the pseudo spectrum on $z = 21$ mm plane is shown in Fig. 2.1(b), showing the locations of the three difficult targets.

With the highly encouraging result from simulation even for a considerably challenging task, we proceeded to test the approach for the realistic situation of detecting and locating targets from experimental data.

2.5. Testing TROT with experimental data

2.5.1. Experimental materials and methods

Three different experiments with three different samples were carried out to test the efficacy of the TROT approach in detecting and locating targets in a turbid medium. All three samples used a 2 mm × 2 mm × 2 mm transparent plastic container filled with Intralipid-2 suspension in water as the background medium. The concentration of Intralipid-2 was adjusted to provide an estimated μ_a of 0.01 mm⁻¹ at 650 nm, and a transport mean free path l_t of 1 mm, which were similar to the average values of those parameters for human breast tissue, while the cell thickness of 2 mm was comparable to thickness of a typical compressed breast.

Intralipid is an FDA-approved safe fat emulsion for human use. It is mixture of multiple types of fatty acid that can be used clinically as an intravenously administered nutrient. Since Intralipid is a highly scattering suspension with low absorption, it has also been widely used as the basis of biological tissue phantom for investigation of properties of biological tissues, light propagation in tissue and methods applied in imaging through biological tissues. The optical properties of Intralipid suspension have been well studied [4]. The scattering coefficient μ_s and the anisotropy factor g of 1% aqueous Intralipid suspension prepared by diluting 1mL Intralipid-1 with distilled water can be calculated [4] to be $\mu_s(\lambda) = 0.016\lambda^{-2.4}$ and $g(\lambda) = 1.1 - 0.58\lambda$, respectively for $0.4\mu_m < \lambda < 1.1\mu_m$, where the unit of μ_s is m⁻¹ mm⁻¹.

In the first experiment, the depth (position along z-axis) of an absorptive target was varied to explore how the accuracy of position estimate depended on depth. The target was a glass sphere of

diameter 1 mm filled with ink dissolved in Intralipid-2 suspension in water (μ_s was adjusted to be the same as that of the background medium, while $\mu_a \approx 0.1 \text{ mm}^{-1}$ was about 10 times higher than that of background medium).

In the second experiment, the separation between two absorptive targets was varied to test how close those could be and yet be resolved as separate objects. Both the targets were similar to the target in the first experiment.

In the third experiment, the depth of a scattering target was varied to explore the efficacy of TROT in locating and characterizing a scattering target. The target was a glass sphere of diameter 1 mm filled with Intralipid-2 suspension in water to provide a transport mean free path l_t of 0.2 mm, and a scattering coefficient $\mu_s \approx 11 \text{ mm}^{-1}$.

A multi-source interrogation and multi-detector signal acquisition scheme, shown in Fig. 2.2, was used to acquire data. A 10-mW 635-nm diode laser beam was used to illuminate the samples. A 1280 × 1024 pixels charge coupled device (CCD) camera equipped with a 50-mm focal-length camera lens was used on the opposite side of the sample to detect the transmitted light on the boundaries of the slab samples (detector plane). The pixel size was 24 μm . The multi-source illumination scheme was realized by scanning the sample across the laser beam in a two-dimensional x-y array of grid points using a computer-controlled translation stage. The first and third samples were scanned across the laser beam in an array of 11 × 11 grid points, and the second sample was scanned in an array of 1 × 11 grid points, with a step size of 1 mm in all cases. The scanning and data acquisition processes were controlled by a personal computer (PC). Raw transillumination images of the sample were recorded by the PC for each scan position, and stored for subsequent analysis. A typical image, which is a 2-D intensity distribution, is shown in the right side of Fig. 2.2.

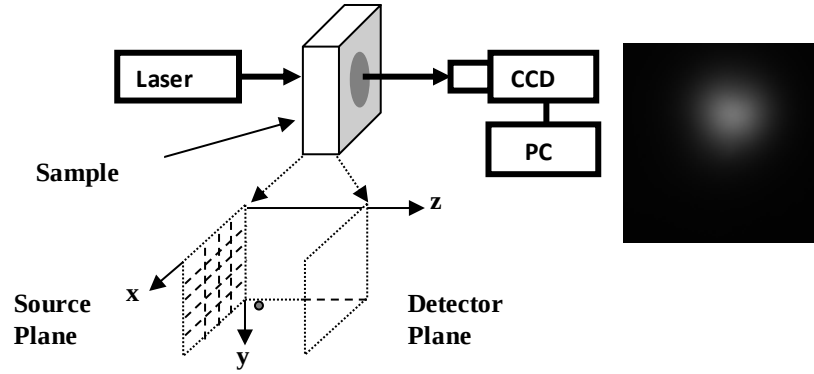


Fig.2.2. A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium. (CCD = charge coupled device, PC = personal computer) Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam, and inset (right) shows a typical raw image.

While we have scanned the sample and kept the source fixed in the experiments reported here, a more clinically relevant approach would be to scan the source and fix the sample. In the experimental arrangement, the source scanning may be accomplished by: (a) delivering the beam using an optical fiber, and translating the delivery end of the fiber in an x - y array using a computer-controlled translation stage; or (b) raster scanning the laser beam using two orthogonal (x - y) galvanometers. The main change in the processing of data would involve alignment of the images so that laser beam positions are overlapped before averaging to generate the background image.

2.5.2. Data Processing and Analysis

From each image, a region of interest was cropped out and then every $\quad$ pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The background image was generated by taking an average of the original images for all scan positions, which is a reasonable approximation since for most of the scan positions the target(s) is (are) not along the direction of the incident beam. Then the background image was also cropped and binned corresponding to the

region of interest for each scan position. Perturbation in the light intensity distribution $\Delta\phi$ due to targets in each image was found by subtracting the background image from each individual image. The response matrix was constructed using the light intensity perturbations, $-\Delta\phi$. The TR matrix was generated by multiplying the response matrix by its transpose for our continuous-wave (CW) probing scheme. The eigenvalue equation was solved and the signal subspace was selected and separated from the noise subspace. MUSIC was then used to calculate the pseudo spectrum for all voxels in the $-D$ space of the sample. For each voxel, four pseudo values, one for absorption and three for scattering as described in Algorithm step (d), were calculated. The voxel size was $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$. By sorting the pseudo spectrum in a descending order, the target(s) were located.

The voxel size to be used in reconstruction and its relation to the target size is an important consideration. In general, smaller voxels provide reconstruction of higher resolution at the cost of increased computational time. Finer details of an extended target may be obtained using smaller voxels. Decreasing the voxel size indefinitely may not improve resolution because of the diffusive nature of light propagation in the turbid medium. However, the computation time increases dramatically, which has been observed by other researchers [10]. The optimal voxel size for a given reconstruction problem will depend on factors, such as, target size, experimental geometry, and noise level.

Since the signal used in image reconstruction is taken to be the difference between the image recorded for a scan position and the background image, estimation of the background image is an important issue. This is a common problem for every diffuse optical imaging modality using perturbation method, and needs further elaboration. We accumulated data in the transillumination slab geometry, and generated the background image by averaging images for

all scan positions after proper alignment with respect to the incident source position. This averaging method for generating background image worked well for small targets that we used in our experiments, as the ratio of sample volume to target volume was quite high (10:1). This volume ratio for breast and a tumor in early stages of growth will also be substantially high for the averaging method to be applicable. Assuming a scenario where the volume ratio is substantially smaller than in above examples, a modified approach would be to select recorded images which were minimally affected by embedded targets for averaging. As long as the targets only occupy a limited volume within the host medium, a clean background image can be generated in this fashion. It should also be noted that while estimation of target optical properties, such as absorption coefficient and scattering coefficient, are sensitively dependent on background image estimation, estimation of target positions are not so sensitive.

Several alternative ways of generating background image are suggested in the literature. One experimental approach is to record image using a phantom that has the same average optical properties as the sample, such as human breast. Along the same line, image of the healthy contralateral breast taken under the same experimental conditions as that of the suspect breast may be used as background image for breast imaging [4]. Some authors have suggested acquiring data at a wavelength for which the target(s) and the background have identical optical properties for assessing the background, *e.g.*, measurement using 760-nm light for which hemoglobin and oxy-hemoglobin have the same absorption coefficient may serve as background to image hemoglobin oxygenation [41]. Still another approach is to compute the background using an appropriate forward model [42]. Any of these approaches may be employed for generating the background image for use with the TROT formalism presented here.

The geometries commonly used in DOT include slab, cylindrical, hemispherical, and semi-infinite; and different source-detector combinations have been used to record images in these geometries. As long as multiple source-detector combinations provide multiple angular views of the sample the TROT formalism can be adapted to obtain target location for these geometries. TR imaging and TR-M SIC was originally developed for reflection (backscattering) geometry that used coincident transceiver array to detect the return signal [1, 14]. With requisite modification in the experimental arrangement TROT would be suited for use in the reflection geometry.

2.5.3. Results

2.5.3.1. Single absorptive target at different depths

In the first experiment, only one target was used, the lateral (x, y) position of the target was kept the same at (2.5 mm, 24.5 mm), while seven different depths (position along z-axis) of 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm and 7 mm were used. The eigenvalue spectrum plotted on logarithmic scale for the target at z = 2.5 mm is shown in Fig. 2. Similar eigenvalue spectra were obtained for other cases.

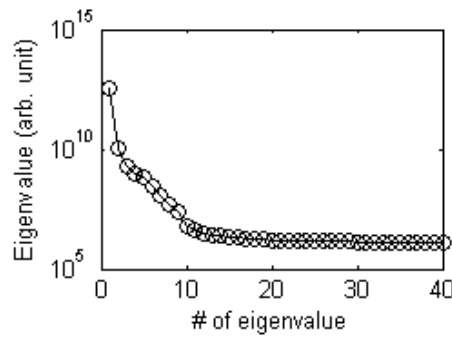


Fig. 2. A semi-log plot of eigenvalue spectrum with first 40 leading eigenvalues for the target at z = 2.5 mm

Both SDDS and DSSD pseudo spectra were calculated using eq. (2.2). The target was identified as an absorptive target. In the DSSD pseudo spectrum, the absorptive pseudo value at

the peak position is 41 times of the scattering pseudo value associated with $\partial_z g_d$, and even larger than those associated with $\partial_x g_d$, and $\partial_y g_d$, as shown in Table 2.2. Similarly in the SDDS scheme, the absorptive pseudo value at the peak position is 22 times of the scattering pseudo value with $\partial_z g_d$, and much larger than the other two.

Table 2.2. pseudo values associated with absorptive and scattering components at the peak position

Scheme with F (g)	Absorptive pseudo value	Scattering pseudo value		
		$\partial_x g$	$\partial_y g$	$\partial_z g$
DSSD (g_d)	1	1.	1.	1.
SDDS (g_s)	22	14.	1.1	1.

Three-dimensional tomographic images were generated using the whole absorption pseudo spectrum for all voxel positions in the sample. The left pane of Fig. 2.4(a) shows a tomographic image for the target at z = 4 mm. The spatial profiles in the x, y and z directions, shown in the right pane of Fig. 2.4(a) were used to assess the target location. Similar images were generated for other depths. The retrieved target positions are compared with known positions in Table 2. .

As is evident from Table 2. , when DSSD scheme was used, the TROT-assessed lateral positions (x, y) were within 0.5 mm of the known values, which is an excellent agreement. The accuracy of the z-position was found to be optimal when the target was located in the middle plane of the sample, and deteriorated when the target was closer to the source plane or the detection plane. When using SDDS scheme, the TROT-assessed lateral positions were also within 0.5 mm of the known positions, except for z = 4 mm and 4 mm, where the error in y direction was 1.2 mm and 2 mm, respectively. However, remarkable improvement in the accuracy of the z-position

estimation was observed, the error Δz being within . mm for all cases except for z mm, where the error was 1. mm. We ascribe the superior performance of the scheme using T_{SDDS} , to the much larger size of the detector array ($1 \times 24 \times 1 \times 24$) than that of the source array () used in the experimental arrangement.

Table 2. . ositions of one absorptive target located at different depths

nown ositions x, y, z (mm)	DSSD Scheme		SDDS Scheme	
	Retrieved	rror	Retrieved	rror
	ositions x, y, z (mm)	$\Delta x, \Delta y, \Delta z$ (mm)	ositions x, y, z (mm)	$\Delta x, \Delta y, \Delta z$ (mm)
2 . , 24. , 1	24. , 24.4, 1 .	. , . , 2.	24. , 2 .2, 1 .	. , . , .
2 . , 24. , 2	2 . , 24.4, 21.	.2, . , 1.	24. , 2 .2, 2 .	. , . , .
2 . , 24. , 2	2 . , 24.4, 2 .	.2, . , 1.	2 . , 24.4, 24.	.2, . , .
2 . , 24. ,	2 . , 24.4, .	.2, . , .	2 . , 2 .2, 2 .	.2, . , .
2 . , 24. ,	2 . , 2 .2, .	.2, . , 1.	24. , 24.4, .	. , . , 1.
2 . , 24. , 4	24. , 2 .2, .	. , . , .	24. , 2 . , 4 .	. , 1.2, .
2 . , 24. , 4	24. , 2 .2, .	. , . , .	24. , 2 . , 4 .	. , 2. , .

It should be noted that the choice of either DSSD or SDDS scheme depends on experimental parameters, such as, the number and density of sources and detectors, and does not depend on the characteristics of the background medium. When more detectors than sources are used and inter-detector spacing is small, SDDS would provide better resolution than DSSD, and vice versa. However, due to the diffusive nature of light propagation in the turbid medium, increasing the

numbers and decreasing the spacing of the sources/detectors beyond a limit may not always improve the results.

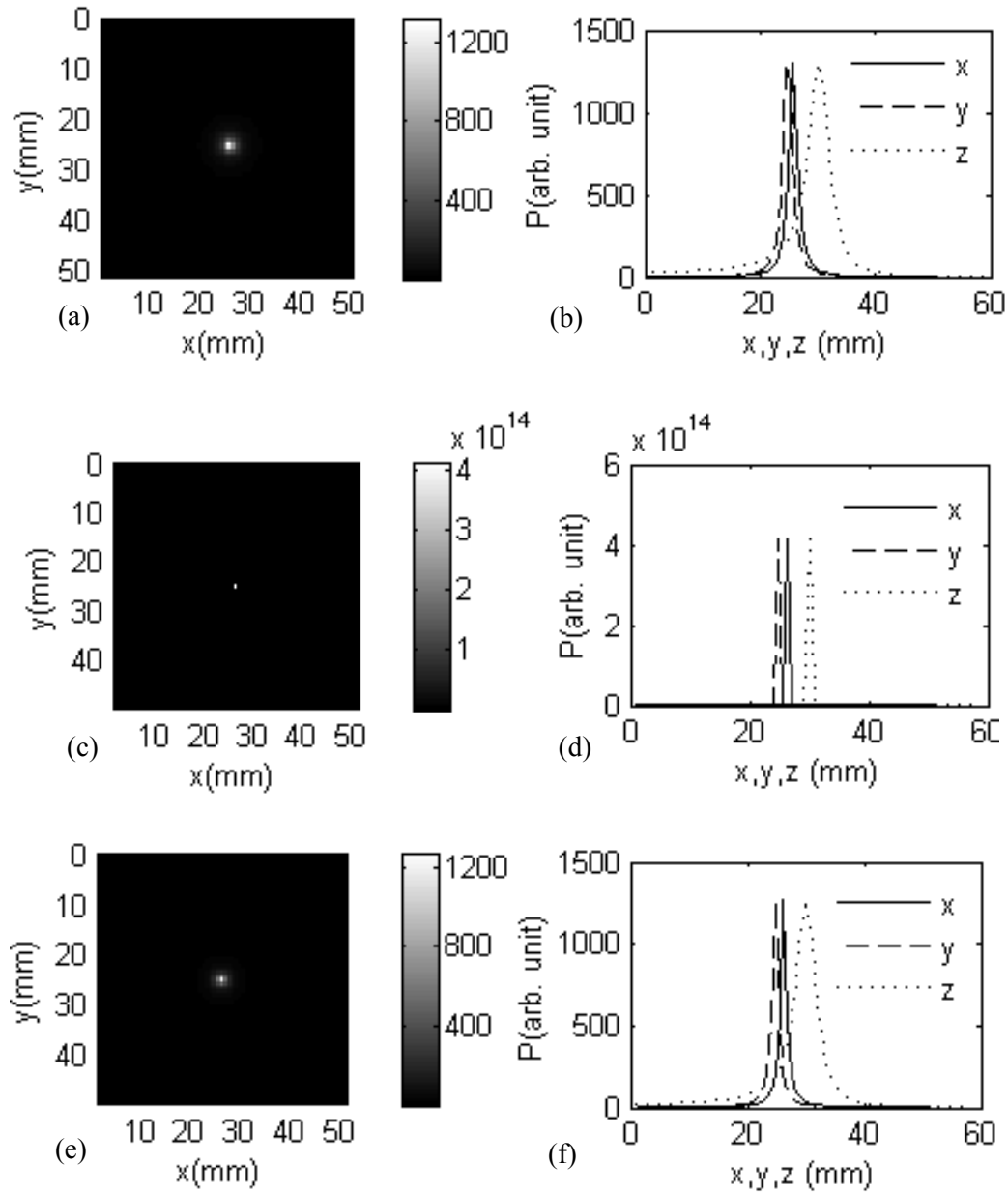


Fig. 2.4. pseudo image of the target (left pane) and corresponding spatial intensity profiles (right pane) when the target is located at $z = 25$ mm: (a) and (b) experimental data; (c) and (d) simulation without any added noise; and (e) and (f) simulation with 2% Gaussian noise added. The pseudo values are calculated using eq. (2.2).

While the target position could be obtained from the experimental data, it was observed that the difference between the smaller eigenvalues in the signal subspace and the larger eigenvalues in the noise subspace were not as pronounced as observed in the simulation in Section 4. To assess the effect of noise and to what extent noise may be present in the experimental data, we generated simulated data mimicking the experimental conditions, and added different noise levels. The lateral positions were (2 . mm, 24. mm) and all seven z-positions (depth) of 1 mm, 2 mm, 2 mm, mm, mm, 4 mm, and 4 mm were tested. Typical pseudo images generated for z mm without and with 2 aussian multiplicative noise to compare with the experimental result are shown in Fig. 2.4(b) and Fig. 2.4(c), respectively. Simulated data provided the known position coordinates.

The simulated spatial profiles with zero added noise are much sharper than the profiles obtained from experimental data, or from simulated data with 2 added aussian noise. broadening of spatial profile is an indication of the uncertainty in determination of position coordinates. Results from simulation show that uncertainty in position determination increases with added noise, and that experimental data behave in a way similar to simulated data with added noise.

2.5.3.2. Resolving two absorptive targets

In the second experiment using two targets the depth (z) and height (y) were kept same (z mm, y 2 . mm), while three different center-to-center separations, Δx of 12. mm, 1 . mm, and 2 . mm, between them along the x-axis were considered. A cross-sectional pseudo image of the targets when separated by a center-to-center distance of 2 . mm, generated using the pseudo spectrum is shown in the left pane of Fig. 2. (a). Fig. 2. (b) and 2. (c) show similar images for the separation 1 . mm and 12. mm (separation between nearest edges 4 mm). The profiles in the x, y and z directions through the right target are shown in the right panes of Fig. 2. (a) Fig.

2. (d). These profiles were used to assess locations of the targets, and the separation between the two targets. In all cases, the targets were determined to be absorptive, because peaks occurred in the pseudo spectrum with the F s corresponding to absorption property.

The known and retrieved positions from the experiments and separations Δx between the two targets appear in Table 2.4. In all the cases, the two targets were resolved, even when their center-to-center separation was 12. mm, nearest sides separated by only 4 mm. For all retrieved positions, the maximum error in the lateral positions is . mm, and the maximum error in the axial positions is 1. mm. The errors in the lateral positions increase as the targets get closer. We ascribe this increase in error in the lateral position to the crosstalk between the two targets, the peak due to one target being affected by the other. The shift in the peaks is also affected by noise. When the two targets are very close or significant noise is present, the two peaks merge, so that the two targets are not resolved. This behavior was confirmed in simulations.

The results were compared with simulated data using similar conditions. For the more challenging case of two targets located at z mm and separated by 12. mm, exact target locations were found when no noise was added. With 1 noise present, the positions of the two targets were found to be (. mm, 24. mm, 2 . mm) and (. mm, 24. mm, 2 . mm) with target separation . mm, compared to 12. mm (known) and . mm retrieved from experiment. The pseudo image and the corresponding profiles through the right target are shown in Fig. 2. (d). Similar images were also obtained for the left target. The retrieved separation between the two targets in simulation with 1 noise was smaller than the actual separation. ut the error was less than the experimental result. owever, when 2 noise was added, the two peaks merged (not shown here).

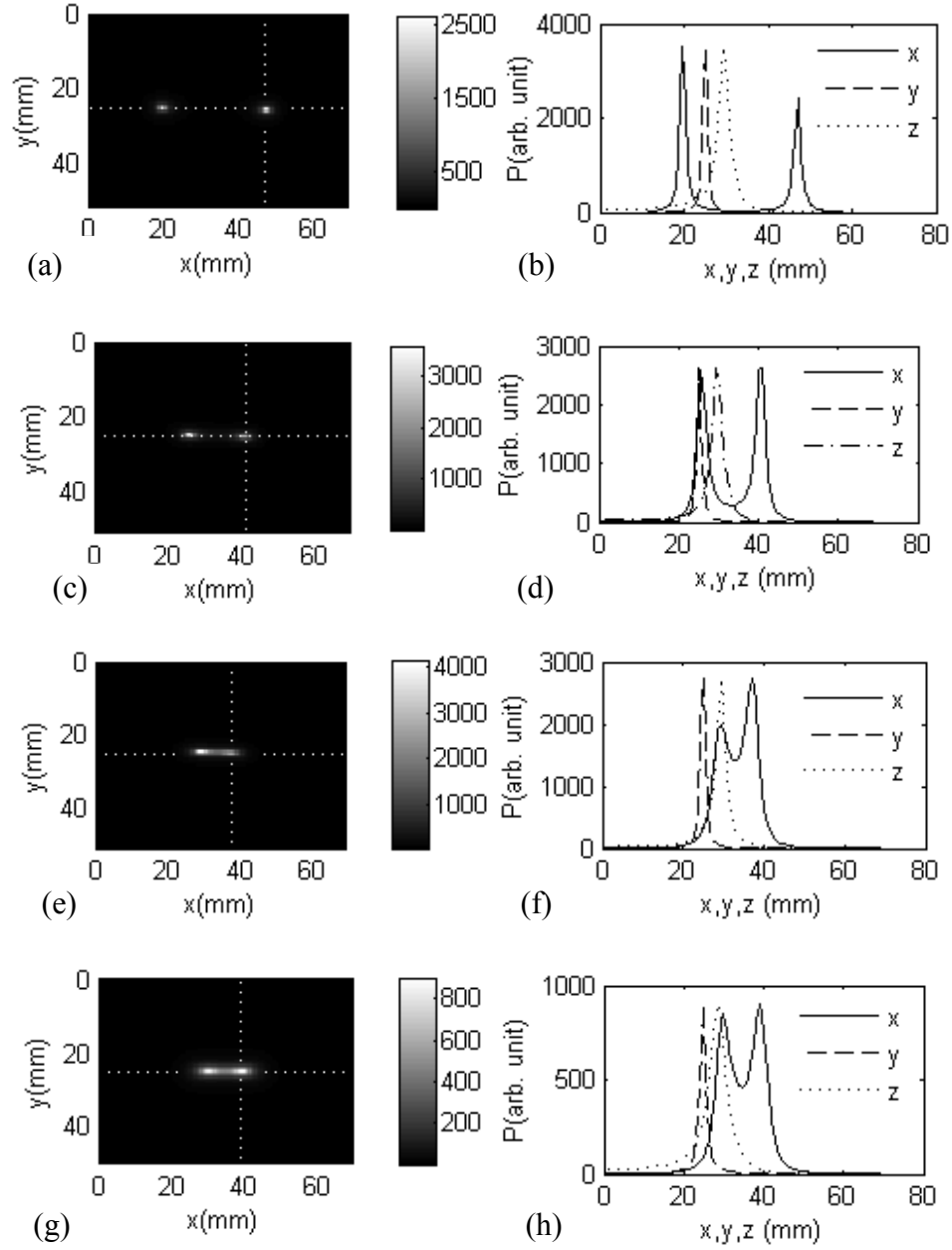


Fig. 2. (Experiment): TROT generated cross-section pseudo images when the targets are separated by 2 mm, 1 mm, and 12 mm are shown in (a), (c) and (e), respectively; and pseudo-value profiles through the right target along x, y and z directions are shown in (b), (d) and (f), respectively. (Simulation): TROT generated cross-section pseudo image when two targets are separated by 12 mm is shown in (g) and the corresponding pseudo-value profiles are plotted in (h). In simulation 1 gaussian noise is added for comparison with the experimental results. P is pseudo value calculated using eq. (2.2).

Table 2.4. Positions of two targets separated by different distances

Known Separation Δx (mm)	Target	Known position x, y, z (mm)	Retrieved position x, y, z (mm)	Error (mm)	Retrieved Separation Δx (mm)
12.	1	2.5, 2.5, .	.5, 24.4, 2.5	2.5, 1.5, 1.5	.
	2	4.5, 2.5, .	.2, 2.2, 2.5	.5, .5, .5	
14.	1	2.5, 2.5, .	2.4, 24.4, 2.5	1.5, 1.5, 1.5	14.
	2	4.5, 2.5, .	4.5, 2.2, 2.5	1.5, .5, .5	
20.	1	2.5, 2.5, .	1.5, 2.2, 2.5	.5, .5, .5	20.
	2	4.5, 2.5, .	4.5, 2.2, .5	.5, .5, .5	

The limits on the size of targets, separation between the targets, and the target-to-background contrast ratio that are needed to detect and locate the targets depend on noise level, experimental parameters (such as, number and concentration of sources and detectors), and ultimately on the diffuse nature of light propagation in the turbid medium.

2.5.3.3. Single scattering target at different depths

The experiment involving the third sample is the same as the first one except that the target was scattering in nature. The scattering target was a 1 -mm diameter glass sphere filled with Intralipid-2 suspension in water, whose concentration was adjusted to provide $l_t = .2$ mm, $\mu_s = 11.$ mm⁻¹. The same scanning and data acquisition scheme was used as for the absorptive targets and the following z-positions of the target were used: 1 mm, 2 mm, 2 mm, mm, mm, 4 mm, and 4 mm. DSSD scheme was used to calculate the pseudo spectrum. A cross-section pseudo image and the corresponding spatial profiles are displayed in Fig. 2. (a) when z

mm. It is compared to the simulation results with 2 gaussian noise (Fig. 2. (b)). The lateral (x, y) spatial profiles of the pseudo image generated using simulated data are considerably wider, while the axial (z) spatial profile is narrower than those obtained using experimental data, and the peak values from the two cases are of the same order. The retrieved target positions are listed in Table 2. . SDDS scheme was also used and provided with similar results.

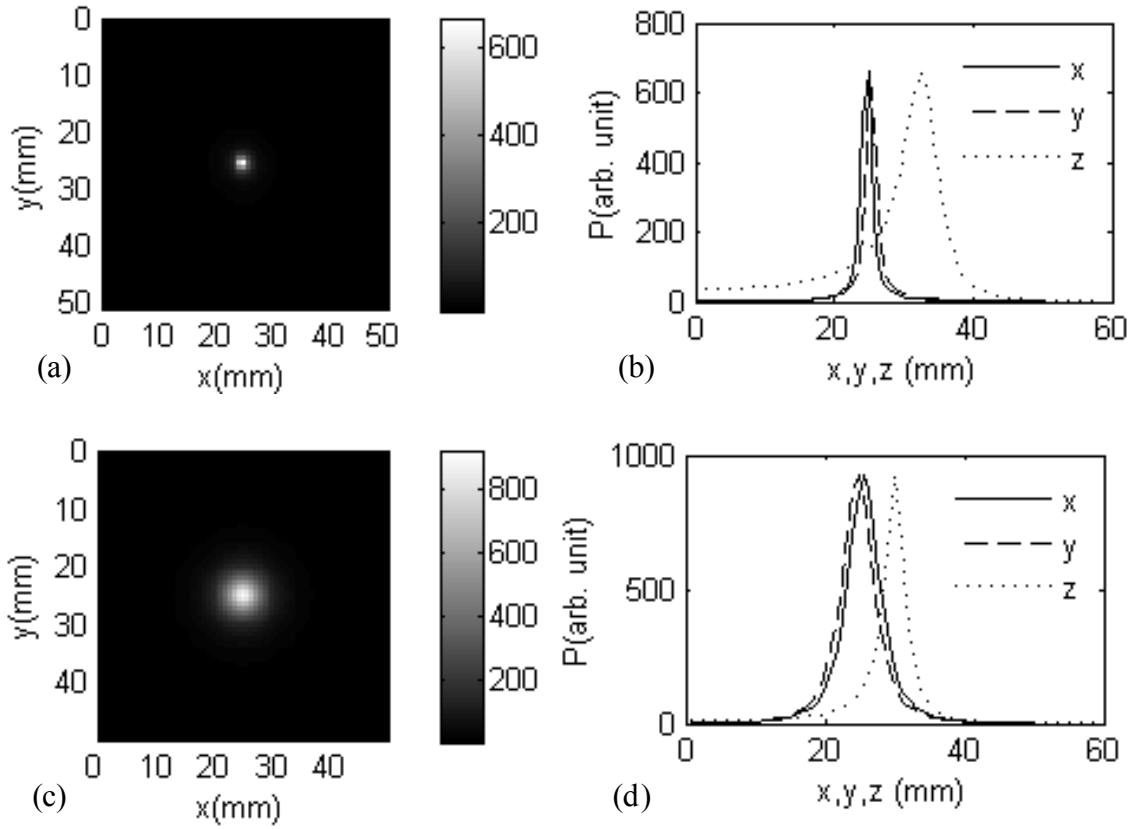


Fig. 2. . pseudo image of the target (a) and corresponding spatial intensity profiles (b) when the target is located at $z = 25$ mm: (a) and (b) experimental data; (c) and (d) simulation with 2 gaussian noise added. P is pseudo value calculated using eq. (2.2).

In Fig. 2. , and more prominently in Fig. 2. , the image resolution seems better for experimental data than simulated data. Since the peak values and bandwidth of lines (the poles)

in the pseudo spectrum depend strongly on the noise, this difference in image resolution is presumably due to lower noise level in the experiments than that used in simulations.

Table 2. Positions of one scattering target located at different depths

Known positions x, y, z (mm)	Retrieved positions x, y, z (mm)	Error (mm)
20, 24, 1	24, 20, 1	0, 1.4, 0
20, 24, 2	20.2, 20, 2	1, 2.2, 0
20, 24, 2	20, 20, 2	0, 2.2, 1
20, 24, 0	24, 20.2, 2	0, 0, 2
20, 24, 0	24, 20.2, 0	0, 0, 1
20, 24, 4	24, 20, 41	0, 1.4, 1
20, 24, 4	24, 20, 4	0, 1.4, 0

A comparison of experimental results for scattering and absorptive targets validate the common notion that it is more challenging to locate and image scattering targets than absorptive targets in a highly scattering medium. Also the lateral (x, y) positions are determined with higher accuracy than the axial (z) position. Overall the TROT-retrieved target positions are in good agreement with the known positions.

2.6. Illumination and detection schemes

In this section we will evaluate how the resolution of TROT depends on the scanning (source) and acquisition (detector) arrangements, and the difference in the resolution between SDDS and DSSD, using simulation. A 4 -mm thick uniform scattering slab was used as the background

medium. Its absorption and diffusion coefficients were taken to be $\mu_a = 0.1 \text{ mm}^{-1}$ and $D = 1/4 \text{ mm}$ (transport mean free path, $l_t = 1 \text{ mm}$), respectively. Two absorptive point targets were embedded and placed at $(-4, 4, 2) \text{ mm}$ and $(4, 4, 2) \text{ mm}$. Additive Gaussian noise was added. Sources and detectors were placed on the opposite sides of the slab. The positions and numbers of sources and detector were varied to generate different data for subsequent analysis. Two peaks in the pseudo spectrum were generated corresponding to the two targets. The results (resolution) were evaluated by comparing the retrieved positions (separation and the z position) of the two targets and the contrast, which is defined to be $(I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$, $I_{\max}$ is the average of the two peaks corresponding to the two targets, and $I_{\min}$ is the valley between the two peaks.

2.6.1. Noise

In simulated data, either additive or multiplicative noise could be added. Additive Gaussian noise is expressed as $X(\mathbf{r}) = X_0(\mathbf{r}) + \delta(\mathbf{r})$, where $X_0(\mathbf{r})$ is the pure signal at $\mathbf{r}$, δ is the random noise, the statistics of which follows Gaussian distribution; while multiplicative Gaussian noise is expressed as $X(\mathbf{r}) = X_0(\mathbf{r}) (1 + \delta(\mathbf{r}))$, where the ratio $\delta(\mathbf{r})$ is the random variable and $X_0(\mathbf{r})\delta(\mathbf{r})$ is the noise added at $\mathbf{r}$. To check the difference between the additive and multiplicative noise, additive or multiplicative Gaussian noise was added, and singular value decomposition (SVD) was used to decompose the data. The first three components retrieved by SVD are shown in Fig. 2. 1 and Fig. 2. 2 for additive and multiplicative Gaussian noise, respectively. The corresponding singular values are shown in Fig. 2. 3. When multiplicative noise was added, the third component carried some signal and showed some “pattern” in the 2D distribution. The higher order singular values (S_i) in the SVD spectrum with multiplicative noise are higher, and approaching the 2nd singular value, compared to the case with additive noise.

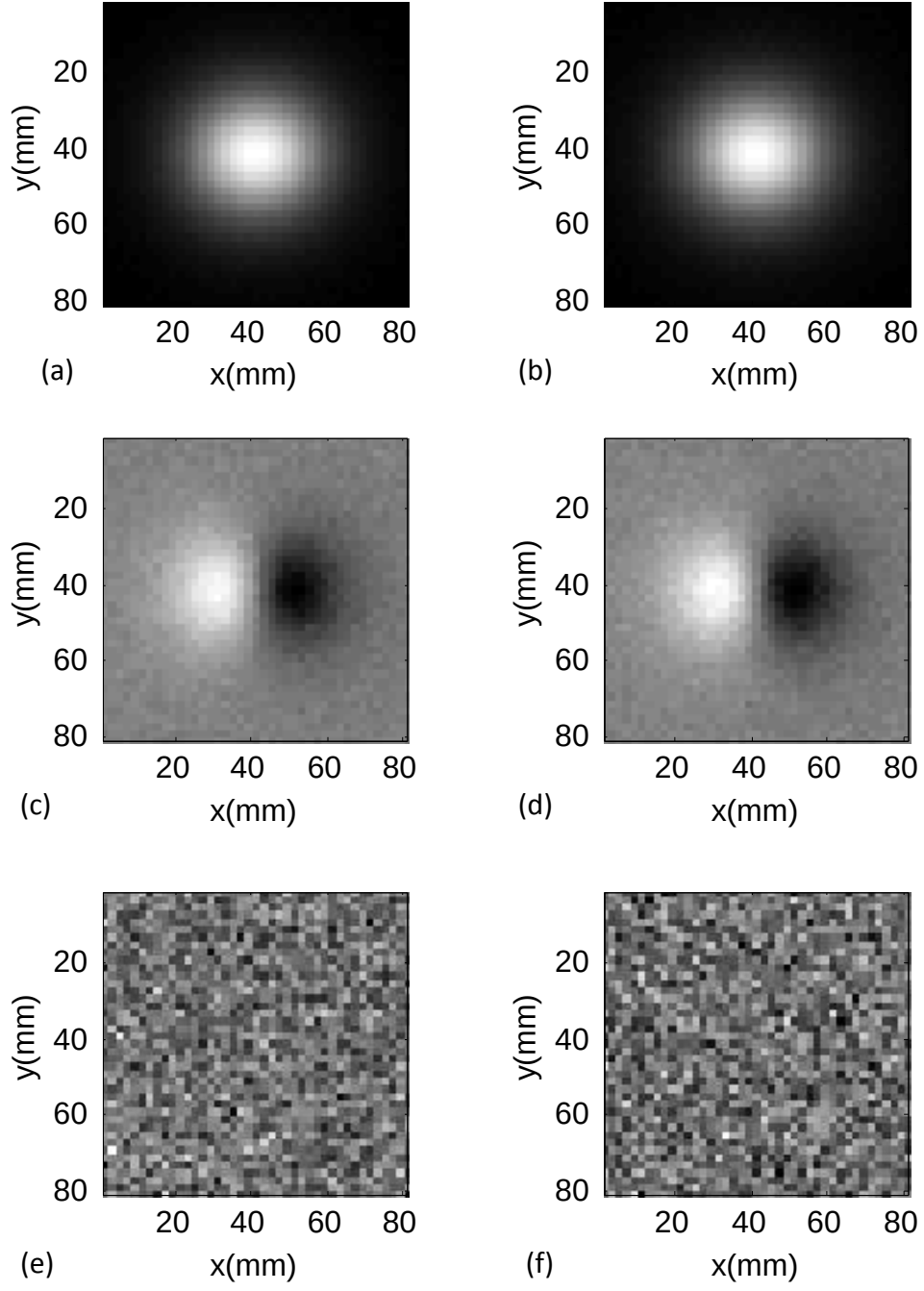


Fig. 2. . (a), (c) and (e) are the three singular components on the detector plane retrieved by S D from simulated data with additive noise; (b), (d) and (f) are the corresponding components on the source plane.

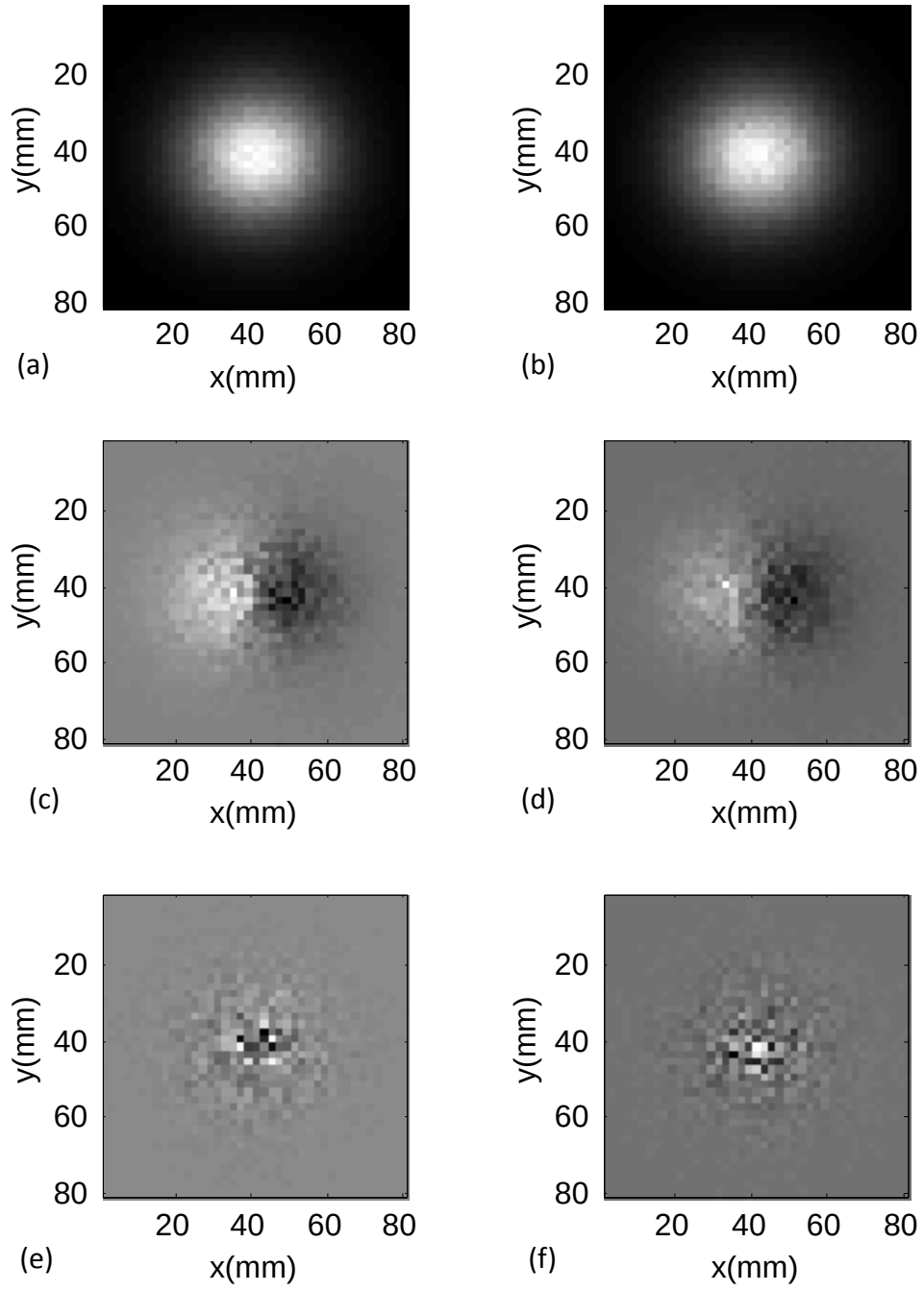


Fig. 2. . (a), (c) and (e) are the three components on the detector plane with multiplicative noise; (b), (d) and (f) are the corresponding components on the source plane.

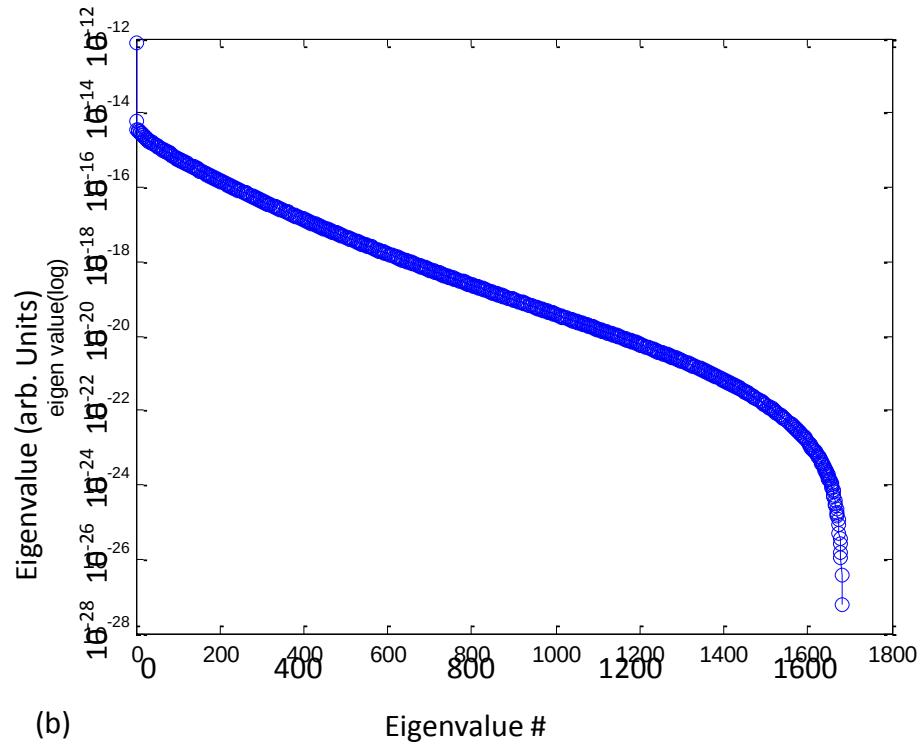
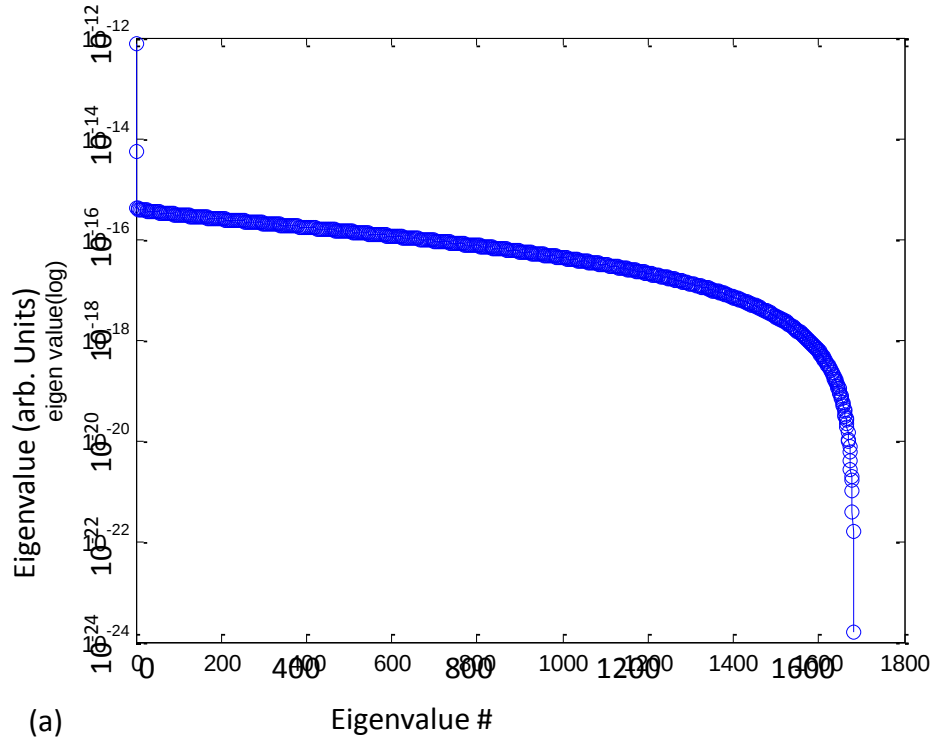


Fig. 2. . The singular values retrieved by S D from simulative data with the presence of (a) additive and (b) multiplicative noise, respectively.

Since in the experimental condition the random noise was expected to be a uniformly distributed gaussian white noise, the additive noise model was used in this study.

2.6.2. Results

Different number and spacing of sources and detectors were used to generate simulative data. Both the areas covered by the grid points where the sources and detectors are located, are centered. Both SDDS and DSSD were used to analyze for each source/detector configuration, and the results are shown in Table 2. and 2. , respectively.

Table 2. . SDDS TROT retrieved target information

Sources;Spacing (mm)	Detectors;Spacing (mm)	Left Target (x, y, z) (mm)	Right Target (x, y, z) (mm)	Separation (mm)	Contrast
41 41;2	;2.	1 , 21, 22	24, 21, 22		.1 12
1 1;1.	;2.	1 , 21, 22	2 , 21, 22		.2
1 1 ;1.2	41 41;2	N/A	N/A	Not resolved	N/A
1 1 ;	41 41;2	1 , 21, 22	2 , 21, 22		.2 1
;2.	41 41;2	1 , 21, 22	2 ,21, 22		. 1 2
41 41;1	41 41;2	1 , 21, 22	2 , 21, 22		.
41 41;2	41 41;2	1 , 21, 22	2 , 21, 22		. 4
41 41;	41 41;2	1 , 21, 22	2 , 21, 22		. 2
1 1;1.	41 41;2	1 , 21, 22	2 , 21, 22		. 4
41 41;2	41 41;1	1 , 21, 22	2 , 21, 22		. 2
41 41;2	41 41;	1 , 21, 22	2 , 21, 22		. 2 4

41 41;1	41 41;1	1 , 21, 21	2 , 21, 21	1	.
41 41;	41 41;	1 , 21, 22	2 , 21, 22		. 1 2
1 1 ;	1 1;1.	1 , 21, 22	2 , 21, 21		.4 12
;1.2	1 1;1.	1 , 21, 21	2 , 21, 22		.4 4
;2.	1 1;1.	1 , 21, 21	2 , 21, 21	1	. 41
41 41;2	1 1;1.	1 , 21, 21	2 , 21, 22		.4
;	1 1;1.	1 , 21, 21	2 , 21, 21	1	. 2
;2.	1 1; .	1 , 21, 21	2 , 21, 21	1	.
;2.	1 1; .2	1 , 21, 21	2 , 21, 22		.
1 1 ;1.2	1 1 1 1;2	1 , 21, 22	2 , 21, 22		.4 2
1 1 ;1.2	1 1 1 1; .	1 , 21, 21	2 , 21, 21	1	. 4
1 1 ;1.2	1 1 1 1; .4	1 , 21, 21	2 , 21, 21	1	. 2
1 1 ;1.2	1 1 1 1; .2	1 , 21, 21	2 , 21, 21	1	.
1 1 ;1.2	1 1 1 1; .1	1 , 21, 21	2 , 21, 21	1	. 2
1 1 ;1.2	1 1; .2	1 , 21, 21	2 , 21, 21	1	. 444
1 1 ;1.2	1 1 1 1; .	1 , 21, 21	2 , 21, 21	1	.
1 1 ;	1 1 1 1; .4	1 , 21, 21	2 , 21, 21	1	.
1 1 1 1; .4	1 1 ;2.	N/A	N/A	Not resolved	N/A

Table 2. . DSSD TROT retrieved target information

Sources;Spacing (mm)	Detectors;Spacing (mm)	Left Target (x, y, z) (mm)	Right Target (x, y, z) (mm)	Separation (mm)	Contrast
1 1 ;2.	1 1 1 1;2	N/A	N/A	Not resolved	N/A
1 1 ;2.	1 1 1 1; .	N/A	N/A	Not resolved	N/A
1 1 ;2.	1 1 1 1; .4	N/A	N/A	Not resolved	N/A
1 1 ;2.	1 1 1 1; .2	N/A	N/A	Not resolved	N/A
1 1 ;2.	1 1 1 1; .1	N/A	N/A	Not resolved	N/A
1 1 ;	1 1 1 1; .4	N/A	N/A	Not resolved	N/A
1 1 ;1.2	1 1 1 1; .4	N/A	N/A	Not resolved	N/A
;	1 1;1.	1 , 21, 2	24, 21, 2		.1 1
;2.	41 41;2	24, 21, 2	1 , 21, 2		.1 1
;2.	1 1; .2	1 , 21, 2	24, 21, 2		.1
;2.	1 1;1.	1 , 21, 2	2 , 21, 2		.1 4
;2.	1 1; .	1 , 21, 2	2 , 21, 2		.22
;1.2	1 1;1.	1 , 21, 2	2 , 21, 2		.2 1
41 41;	41 41;	1 , 21, 2	2 , 21, 2		.2 2
41 41;	41 41;2	1 , 21, 2	2 , 21, 2		. 1
41 41;2	1 1;1.	1 , 21, 2	2 , 21, 2		. 41
41 41;2	41 41;	1 , 21, 2	2 , 21, 2		. 1 4
41 41;2	41 41;2	1 , 21, 2	2 , 21, 2		. 1
41 41;2	41 41;1	1 , 21, 2	2 , 21, 2		. 4

41 41;2	;2.	1 , 21, 2	2 , 21, 2		. 42
41 41;1	41 41;2	1 , 21, 2	2 , 21, 2		.
41 41;1	41 41;1	1 , 21, 2	2 , 21, 2	1	.
1 1;1.	1 1 ;	1 , 21, 21	2 , 21, 2		.414
1 1;1.	;2.	1 , 21, 2	2 , 21, 2	1	.
1 1;1.	41 41;2	1 , 21, 2	2 , 21, 2	1	.
1 1 1 1; .4	1 1 ;2.	1 , 21, 21	2 , 21, 21	1	. 2

In general, for both the source and detector sides, the following two cases provide more information and therefore may result in better resolution: (a) the spacing between sources (detectors) stays the same, while the number of sources (detectors) increases therefore a larger source (detector) plane is covered; (b) the area of the source (detector) plane stays the same, while the spacing between sources (detectors) decreases therefore the number of sources (detectors) increases. However, due to the diffusive nature of light propagation, increase in the area of the source (detector) plane or decrease in the source (detector) spacing may not always result in improvement in the resolution. For SDDS, if the number of sources is fixed, there is a trade-off between the area covered by the detectors and the spacing between them; and it is also the case for DSSD. For example, for SDDS, when the (1 1 ;1.2) (1 rows 1 columns with 1.2 -mm spacing, covering 2 mm 2 mm area) source configuration is used, compared to the detector configuration (1 1; .2) (covering 1 mm 1 mm), both (1 1 1 1; .2) (covering 2 mm 2 mm) and (1 1 1 1; .1) (covering 1 mm 1 mm) detector configurations, which have larger area with same spacing and same area with smaller spacing, respectively, showed better results. If the number of detector is fixed to be 1 1 1 1, compared to .2mm spacing, both configurations with spacing of . mm and 2mm, which provide smaller spacing with smaller

area and larger spacing with larger area respectively, showed worse results. Tables 2. and 2. indicate that SDDS scheme strongly depends on the number and arrangement of detectors, while DSSD depends strongly on the number and arrangement of sources. If the detector side has more information than source side, SDDS has better results than DSSD, and *vice versa*. If source and detector sides have similar configuration, SDDS and DSSD provide similar results.

In general, sources and detectors that are located closer to the targets should provide signal that carries more target information. If the targets are close to each other, smaller spacing of sources (detectors) could provide with more detailed information of the region where targets are located. The target information is mainly recorded by the sources (detectors) nearby the target(s), while the sources (detectors) that are further away carry less target information, since the diffusive light decays over distance. In this case, the results may not benefit much from a larger source (detector) plane covered by more sources (detectors) with same spacing.

In a realistic experiment, if the number of sources (detectors) is given, larger area of source (detector) plane or smaller spacing of sources (detectors), which provides better resolution, varies from one case to another.

2.7. Discussion

This chapter presents the development of time reversal imaging approach with subspace classification, M SIC in the optical domain. The results from experiment and simulation show that TROT is a faster and less computation intensive approach for detecting small targets in highly scattering turbid media and determining their locations in $-D$ than other inverse image reconstruction techniques. While the dominant features in the pseudo spectrum are related to the square of the difference between the absorption (scattering) coefficient of the targets and that of the background, the approach does not directly determine these parameters. It is common for IIR

approaches to estimate the optical properties of every voxel in the sample and identify target(s) from differences of these properties between the sample and the target(s), which is a considerably computation intensive undertaking. On the contrary, TROT identifies the targets as poles of the pseudo spectrum and focuses on determining their positions, which do not require as much computation time. Other IIR approaches involve iteration, while TROT is non-iterative. In TROT the dimension of the involved matrices is lower compared to other IIR approaches, which enables analysis and utilization of very large datasets. These two features together make TROT faster. Fast image reconstruction algorithms are of particular interest.

It was observed that lateral (x, y) positions are better determined than the depth (z). Also the spatial profile is more spread out along z compared to that along x, y . We ascribe this difference to fewer data along z -direction compared to those along x - y planes. Addition of another set of data with light incident and signal collected perpendicular to the z -direction is expected to further improve resolution in this dimension. Even without that addition, TROT determines the target position well.

While we have used slab geometry and CW illumination, the TROT approach may be used for other geometries (such as, cylindrical, and spherical), different types of illumination (e.g. frequency domain and pulsed) and different models for light propagation through the medium. Application and adaption of the TROT formalism to inhomogeneous media and extended targets may require careful consideration of several factors. In a non-uniform, inhomogeneous medium, structures other than the desired targets may appear as “false targets” and may interfere with identification of “real targets”. However, as long as the contributions to the signal by any false target is smaller than that made by real targets, TROT with M-SIC will be useful in detecting and locating targets, by choosing a proper threshold to separate the signal and noise subspaces.

What is even more important, expected wavelengths dependence of the target spectroscopic properties could be used to assess the difference between the real and false targets in experiments using multi-wavelength interrogation of the sample.

The TROT formalism presented in this chapter is particularly suited for point-like targets requiring fewer eigenvectors in the signal subspace to construct a pseudo spectrum. However, for extended finite-size targets, the formalism needs to be modified and much more eigenvectors may be needed to calculate the pseudo spectrum [1, 4, 44]. These interesting problems for further study are currently being pursued.

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Chapter 3

Time reversal optical tomography: retrieval of properties of targets in turbid media

3.1. Introduction

Time Reversal Optical Tomography (TROT) has been developed [1] to detect and locate small absorptive and scattering targets embedded in an optically thick highly scattering turbid medium, as detailed in Chapter 2. The method was based on Diffusion Approximation of the Radiative Transfer equation (RTA) to describe light propagation in a highly scattering turbid medium, and a Time Reversal (TR) Multiple-Signal-Classification (MUSIC) algorithm developed by other groups in acoustics and radar applications [2-4]. In this chapter, we extend the TROT approach further to estimate size and optical properties of targets including point-like and finite-size small targets.

When an extended target is present, the dimension of the signal subspace spanned by the eigenvectors of the TR matrix is expected to be higher than one for a point target. Dou *et al.* developed algorithms with TR-MUSIC approach to detect and find the shape of an extended target using a sonar or a radar system for remote sensing applications. In one approach [4], a proper threshold was determined to separate the eigenvectors of the TR matrix in the signal subspace from the noise subspace. Then the pseudo spectrum was calculated to generate the image of the extended target. Another approach [5] used the first eigenvector of the TR matrix to calculate the MUSIC pseudo spectrum, the maximum of which provided an estimate of the position of the extended target. This approach used a level set method (LSM) [6] which is a numerical method for following evolution of interfaces (contours). A prescribed appropriate

continuous level set function $\varphi(\mathbf{r}, t)$, such as a signed-distance function, was used to describe the domain Ω or surface $\partial\Omega$ (shape of the target), where $\mathbf{r}$ is the position of any point in the space and t is (pseudo-) time: $\varphi(\mathbf{r}, t) = 0$ for the points on $\partial\Omega$, $\varphi(\mathbf{r}, t) > 0$ and $\varphi(\mathbf{r}, t) < 0$ for the interior and exterior points of Ω , respectively. The surface was updated by solving an advection level set equation (Hamilton-Jacobi equation), $\partial\varphi/\partial t + v_n|\nabla\varphi| = 0$, where v_n is the velocity function defined in the outward normal direction according to the gradient descent. Using retrieved target position as *a priori* information to initialize the level set function φ , a surface is repeatedly generated as an estimate of the boundary of the target to calculate the data matrix using the forward model. The surface was optimized by minimizing the difference (residue) between the calculated data matrix and actual data matrix which was either obtained from experiment or simulation.

Marengo and Ruber *et al.* introduced a TR-MUSIC approach using angles and distances between subspaces to find the size and shape of an extended target for radar and acoustic remote sensing [10, 11]. In one of the approaches, the target position was first determined using the maximum in the pseudo spectrum. Then contours in the pseudo spectrum were used as test boundaries of the target which corresponds to “test spaces”. The optimal surface was determined by maximizing the orthogonality between the “test spaces” and the noise subspace of the TR matrix. In another similar approach [12, 13], instead of using the contours in the pseudo spectrum, a level set method was used to generate the test surfaces.

The main difference between the approaches developed by the two groups is that different ways were used to evaluate and optimize the surfaces (contours). The shape and detailed structure of the targets were reconstructed by the groups. The problem they studied was

considered relatively easier, since there was much less scattering of the ultrasonic or electromagnetic waves used and phase information of the wave was available.

In this chapter, we report on our work to extend above-mentioned approaches to the optical domain to retrieve the size and optical properties of targets. The efficacy of the formalism was tested using simulated and experimental data. This chapter is organized as follows. Section 2 outlines the extended TROT formalism for retrieval of optical property and size of targets. Section 3, and Section 4 test the formalism using simulated data and experimental data, respectively. Section 5 provides a discussion of results and summarizes key findings.

3.2. Theoretical formalism

Similar to what we have shown in Chapter 2, in the first order Born approximation, the perturbation in the light intensity distribution due to the presence of the finite-size targets (inhomogeneities in optical properties) may be expressed using a data matrix $\mathbf{K}$ of the form:

$$\mathbf{K} \approx \left\{ \sum_{m=1}^M \sum_{l=1}^{L_m} G^d(\mathbf{r}_i, \mathbf{X}_{ml}) \tau_{ml} G^s(\mathbf{X}_{ml}, \mathbf{r}_j) \right\} = \sum_{m=1}^M \sum_{l=1}^{L_m} g_d(\mathbf{X}_{ml}) \tau_{ml} g_s^T(\mathbf{X}_{ml}), \quad (.1a)$$

for *absorptive* targets, and

$$\mathbf{K} \approx \sum_{m=1}^M \sum_{l=1}^{L_m} \sum_{\alpha=\{x,y,z\}} \partial_{\alpha} g_d(\mathbf{X}_{ml}) \tau_{ml} \partial_{\alpha} g_s^T(\mathbf{X}_{ml}), \quad (.1b)$$

for *scattering* targets, where N_s , N_d and M are the numbers of sources, detectors and targets, respectively; L_m is the number of voxels the m^{th} target is divided into ($M \leq \min(N_d, N_s)$); $g_s(\mathbf{r})$

$G^s(\mathbf{r}, \mathbf{r}_j)$, $j = 1, \dots, N_s$ and $g_d(\mathbf{r}) = G^d(\mathbf{r}_i, \mathbf{r})$, $i = 1, \dots, N_d$ are the Green's function vectors (Fields) associated with the source and detector planes, respectively; the superscript T denotes transpose; $G^s(\mathbf{r}, \mathbf{r}_s)$ and $G^d(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagations in the background medium from a source at $\mathbf{r}_s$ to a voxel (inhomogeneity) at $\mathbf{r}$ and from the voxel to a

detector at $\mathbf{r}_d$, respectively; and τ_{ml} is the *optical strength* of the l^{th} voxel in the m^{th} target. Our focus in this section is on developing a method for estimation of $\tau_m = \sum_l^{L_m} \tau_{ml}$.

Assuming the m^{th} target is homogenous, and localized within a volume V_m ; the centroid position of the target is $\mathbf{X}_m$; the optical strength τ_m is,

$$\tau_m = \sum_l^{L_m} \tau_{ml} = \sum_l^{L_m} \delta\mu_a(\mathbf{X}_{ml}) c \delta V_{ml} \approx \delta\mu_a(\mathbf{X}_m) c \Delta V_m, \quad (.2a)$$

for an *absorptive* target and

$$\tau_m = \sum_l^{L_m} \tau_{ml} = \sum_l^{L_m} \delta D(\mathbf{X}_{ml}) c \delta V_{ml} \approx \delta D(\mathbf{X}_m) c \Delta V_m, \quad (.2b)$$

for a *scattering* target, where c_m is the light speed in the medium, δV_{ml} is the volume of the l^{th} voxel in the m^{th} target, and $\delta\mu_a$ (δD) is the difference in the absorption coefficient (diffusion coefficient) between the m^{th} target and the background medium assuming the target property is homogenous.

If the target is very small, and the Green's functions for different voxels in a target may be assumed to be the same, then eqs. (.1a) and (.1b) become the same as eq. (2.4) and eq. (2.5), respectively. As shown in Chapter 2, for a small target that can be approximated to be point-like, when the eigenvalue equation of the TR matrix is solved, ideally, one absorptive target corresponds to one eigen component (eigenvalue and eigenvector), and one scattering target corresponds to three eigen components.

As shown in eq. (.1), an extended target with volume ΔV_m is considered consisting of a cluster of tiny pieces (e.g. voxels) with volume δV_{ml} located next to each other, and every piece may be considered as an individual small target as discussed in Chapter 2.

Similar to the formalism developed for point target in Chapter 2, a TR matrix is then constructed using the data matrix as $T_{SDDS} = K^\dagger K$, $T_{DSSD} = (K^T)^\dagger K^T$, $K^* K^T$ in frequency domain,

and $T_{SDDS} = K^T K (T_{DSSD} - K K^T)$ in the continuous wave (CW) illumination mode. T_{DSSD} and T_{SDDS} have a common set of eigenvalues $\lambda_j, j = 1, \dots, \min(N_s, N_d)$, and different sets of eigenvectors $u_i, i = 1, \dots, N_d$ and $v_j, j = 1, \dots, N_s$, respectively. The eigenvectors are separated into signal and noise subspaces using an L -curve method [11] with an eigenvalue threshold ε .

Since the eigenvalues and eigenvectors of T_{SDDS} and T_{DSSD} can be connected using singular value decomposition (SVD) [2, 12], i.e.

$$K = \sum_{j=1}^{\min(N_s, N_d)} u_j \sigma_j v_j^T \approx U \Sigma V^T, \quad (4.3)$$

where $V = [v_j]$ and $U = [u_j]$, corresponding to $\Sigma = \text{diag}(\sigma_j)$, $\sigma_j \geq \varepsilon$, are matrices of the signal subspaces; u_j and v_j are left and right singular vectors of K , with singular values $\sigma_j = \sqrt{\lambda_j}$. $K^T K = (U \Sigma V^T)^T U \Sigma V^T = V \Sigma^T U^T U \Sigma V^T = V \Sigma^2 V^T$ where Σ is diagonal, i.e. $K^T K v_j = \lambda_j v_j$. Similarly, $K K^T u_j = \lambda_j u_j$. Therefore, u_i and v_j are the eigenvectors of $T_{DSSD} - K K^T$ and $T_{SDDS} - K^T K$, respectively, with a common set of eigenvalues λ_j . The singular values (eigenvalues of T_{SDDS} and T_{DSSD}) follow an L -shaped curve, with the first value accounting for the most of the variance in the signal. The singular value spectrum works similarly to the L -curve method [11] used in Tikhonov regularization [1]. Hence, the singular value (eigenvalue) spectrum will also be called L -curve in this context. The ‘‘corner’’ of the L -curve is used to separate the signal and noise subspaces.

Once the signal subspace is determined, the MUSIC pseudo spectrum is calculated the same way as for a point target shown in Chapter 2. For absorptive targets, the locations are estimated using the maximum in the pseudo spectrum [1]

$$P_s(\mathbf{X}_p) = \frac{\|g_s(\mathbf{X}_p)\|^2}{\|g_s(\mathbf{X}_p)\|^2 - \sum_{\lambda_j > \varepsilon} |v_j^T g_s(\mathbf{X}_p)|^2}, \quad (4.4a)$$

associated with the source plane, or a similar form for the detector plane

$$P_d(\mathbf{X}_p) = \left\| g_d(\mathbf{X}_p) \right\|^2 / \left\| g_d(\mathbf{X}_p) \right\|^2 - \sum_{\lambda_j > \epsilon} \left| u_j^T g_d(\mathbf{X}_p) \right|^2, \quad (.4b)$$

or the product of these two,

$$P(\mathbf{X}_p) = P_s(\mathbf{X}_p) P_d(\mathbf{X}_p), \quad (.4c)$$

where $\mathbf{X}_p$ is a test target position in the sample volume.

Since the tiny pieces in an extended target are considered as non-well-resolved targets, in the signal subspace, the eigenvectors (singular vectors) are linear combinations of the $\mathbf{F}$ s associated with the tiny pieces (targets). The optical signal due to an extended target is mainly contributed by the leading eigen components, with the light intensity distribution determined by the orthonormal eigenvectors and magnitude determined by the eigenvalues (singular values). The leading eigenvalues account for the most of the optical strength of a target. So the optical strengths of targets can be estimated using the leading eigen components. However, to reconstruct the shape of a target in the pseudo image accurately, high-order components that carry details of the spatial structure are needed. In an ideal case, if all components are available, every voxel in the target acting as a tiny target will produce one large value in the pseudo spectrum, while a voxel outside the target produces a small value. In this case, a sharp pseudo image is generated. However, in a simulation with the presence of noise or under experimental conditions, the high-order components are easily buried in the noise. In this case, the tiny pieces in an extended target cannot be individually detected, *i.e.* the detailed spatial structure of the target cannot be reconstructed. The edge of the target in the pseudo image is also blurred and cannot be identified, *i.e.*, the shape of the target cannot be obtained. But with the leading components still available, the optical property (strength) of the targets may be obtained. The low-order leading components may also be used to estimate the approximate size of the target.

y comparing $q_i(\cdot)$ with $q_i(\cdot)$ and assuming the Green's functions at different voxels in the extended target are all the same, the optical property of the target can be retrieved by transforming the singular value matrix Σ via

$$\Gamma \approx (\mathbb{G}^d)^{-1} U \Sigma V^T ((\mathbb{G}^s)^T)^{-1}, \quad (4.1)$$

where $\text{diag}(\tau_m, m = 1, \dots, M; \mathbb{G}^s = g_s(\mathbf{r}_m), \mathbb{G}^d = g_d(\mathbf{r}_m))$ are matrices including $\mathbf{F}$ s associated with the retrieved target positions (centroid positions). Since $\mathbb{G}^s$ and $\mathbb{G}^d$ are usually not square matrices, pseudo inverse is calculated. For scattering targets, the $\mathbf{F}$ s g_d and g_s in eqs. (4.4) and (4.5) are replaced by ${}_x g_d$ and ${}_x g_s$, $\alpha = x, y, z$, respectively. Since the optical strength is carried by every term ($\alpha = x, y, z$), in principle, it will be retrieved three times for one target. Therefore, $\text{diag}(\tau_m, \tau_m, \tau_m, m = 1, \dots, M; \mathbb{G}^s = {}_x g_s(\mathbf{r}_m), {}_y g_s(\mathbf{r}_m), {}_z g_s(\mathbf{r}_m); \mathbb{G}^d = {}_x g_d(\mathbf{r}_m), {}_y g_d(\mathbf{r}_m), {}_z g_d(\mathbf{r}_m))$. Each term of α results in an estimate of τ_m . Since the x and y terms in the optical signal are usually much weaker than z term under realistic conditions [14], the optical strength retrieved from the z term is mainly considered with the other two terms ignored, i.e. $\text{diag}(\tau_m, m = 1, \dots, M; \mathbb{G}^s = {}_z g_s(\mathbf{r}_m), \mathbb{G}^d = {}_z g_d(\mathbf{r}_m))$.

Usually if the extended target is not too big, the first eigen component involves optical strength that is approximately the superposition of all tiny pieces in the target and the distribution that is approximately the $\mathbf{F}$ associated with the centroid position of the extended target, as shown in eqs. (4.1) and (4.2). In this case, the location and optical strength of the target can be estimated by using the first eigen component. The retrieved location of the target using the pseudo spectrum will be at the weighted center of the optical strength of the target or geometrical center if the target is homogenous, as if all the tiny pieces are co-located at one position. If higher-order components are available and used to calculate the pseudo spectrum, the full-width-at-half-maximum (FWHM) may be used to estimate the size of the target.

The size of the target may be quantitatively estimated. The target surface may be approximated to be one of the contours (an isosurface Ω when it is plotted in D) in the pseudo spectrum, or the pseudo spectrum in logarithmic scale. The optimal contour in the pseudo spectrum is selected to be the boundary of the target(s), via

$$\Omega = \arg \min_{\Omega} \|K - K_{cal}(\Omega)\|^2, \quad (4.1)$$

where K is the measured data matrix and $K_{cal}(\Omega)$ is a calculated data matrix. K is either measured in experiments or obtained in simulations using a forward model with known target information. $K_{cal}(\Omega)$ is the data matrix calculated using a forward model assuming a contour of the pseudo spectrum to be the target boundary. A contour in pseudo spectrum is a surface plotted in D . Any appropriate forward model could be used such as the analytical forward model in eq. (4.1), or a finite-element-method-based forward model to calculate $K_{cal}(\Omega)$. The Green's functions used in the calculation are those for the intervening medium. In order to search for the contour gradually, the pseudo spectrum in logarithmic scale $\log(P(X_p))$ is used instead of linear scale.

When searching for the optimal contour, starting from the maximum of the pseudo spectrum, successive contour levels are selected. When a contour Ω is selected, the volume V enclosed inside is assumed to be the target volume, and then the data matrix is calculated and compared with the measured (simulated or experimental) data. To save computation time, when the next larger volume $V + dV$ is selected, only the signal generated for the extra volume dV is calculated, and added to the signal generated for the volume V . Applying this “local contour” method, the optimal contour is found using eq. (4.1).

In summary, the framework of TROT for extended targets is as follows: a) data matrix is constructed using “experimental data” (or forward model data in simulations), and TR matrix is calculated; b) SVD is performed on the TR matrix; c) the signal subspace of the TR matrix is

determined with a proper threshold in the singular values; d) the M-SIC pseudo spectrum is calculated based on the signal subspace; e) the positions of targets are determined using the maxima in the pseudo images; f) the optical strengths of targets are retrieved by unmixing the rank-reduced data matrix using the pseudo inverse of the matrices constructed with Green's functions associated with the retrieved target positions; g) the target size/shape is estimated using contours of pseudo spectrum through an optimization process which minimizes the difference between the calculated and the actual data matrix; h) the absorption or scattering coefficient is estimated using the optical strength and the volume of a target. Unlike other inverse image reconstruction (IIR) techniques, TROT does not attempt to retrieve the optical property of every voxel. Instead, it uses Born approximation and perturbation approach, considers a target as a localized inhomogeneity in the background medium, and locate the target and retrieve size and property of the target separately. It does not consider the heterogeneity inside a target. If a target is heterogeneous, it may be considered as multiple inhomogeneities, whose locations may be determined using the same algorithm if they can be resolved in the pseudo image.

In the following sections, simulated and experimental data are used to examine the efficacy of TROT in retrieving the property and size of small targets. Localization of targets is also re-evaluated using the whole signal subspace determined in the logarithmic scale.

3.3. Simulations

The background medium was taken to be a 4 -mm thick uniform scattering slab. Its absorption and diffusion coefficients were $\mu_a = 0.1 \text{ mm}^{-1}$ and $D = 1/3 \text{ mm}$ (transport mean free path, $l_t = 1 \text{ mm}$), respectively, which are similar to the average value of those parameters for human breast tissue. The index of refraction n of the medium was taken to be 1.5. The speed of light c is $2.998 \times 10^8 \text{ m/s}$, or $2.998 \times 10^{11} \text{ mm/ns}$ in vacuum, and $c_m = 22.4 \text{ mm/ns}$ in the medium. Single or

multiple finite-size absorptive or scattering targets were embedded. The extended target was simulated with a cluster of voxels whose size was 1 mm. The simulated data were generated using analytical forward model (Eq. (1)) for subsequent TROT analysis. The retrieved optical strength has a unit of mm/ns and mm/ns for absorptive and scattering strength, respectively.

3.3.1. Singular value decomposition and determination of signal subspace

To demonstrate the relationship between the singular value (eigenvalue) spectrum and pseudo spectrum and the target size for the case of an extended target with different noise levels, a two-dimensional (2-D) absorptive target (21 mm × 21 mm) was embedded in the above scattering medium to generate simulated forward model data. The 2-D target is used for simplicity since the transillumination slab geometry has lower resolution in the axial direction. The thickness of the target is one voxel (1 mm). 41 × 41 sources and 41 × 41 detectors were placed on the source and detector planes with 2-mm spacing covering an area of 82 mm × 82 mm. The target was placed in the center of the medium, with its center position located at (41, 41, 2) mm. For simplicity, when the reconstruction result is displayed, a smaller volume of 41 mm × 41 mm × 4 mm around the center is selected. In the display coordinates, the center of the target is located at (21, 21, 2) mm.

The absorption coefficient of the targets was set to be higher than the background with $\mu_a = 1 \text{ mm}^{-1}$. The volume of the targets was 441 mm³, and the sample volume was discretized into 1 mm × 1 mm × 1 mm voxels in the forward model. The optical strength of the absorptive targets was $\mu_a c V = 4 \text{ mm/ns}$.

The forward model data were generated. The TR matrix was calculated and the SVD of the TR matrix was performed. When no noise was added into the forward model data, the singular value spectrum including 1681 singular values was plotted in linear and logarithmic scales. Fig.

.1(a) shows the first 20 values of the singular value spectrum, while Fig. .1(b) shows the singular value spectrum in logarithmic scale. As shown in Fig. .1(a), the singular value spectrum is similar to the λ -curve in Tikhonov regularization. Following the first singular value, the rest singular values dropped dramatically. The As explained above, even though the target includes 1×1 voxels, since they are non-well-resolved, the eigen components are not one-to-one related to the voxles or equivalent to each other. Instead, the eigen vectors are linear combinations of the F s associated with individual voxels. According to q. (.), the first component contributes to the signal with the first singular value as the strength of the component. The first eigen component accounts for $\sim 4\%$ variance of the signal (ratio between the first eigenvalue and the sum of all eigenvalues). The second and third components are about 1% times weaker than the first component. The higher-order components are even weaker. The first three eigen components account for $\sim 5\%$ variance of the signal.

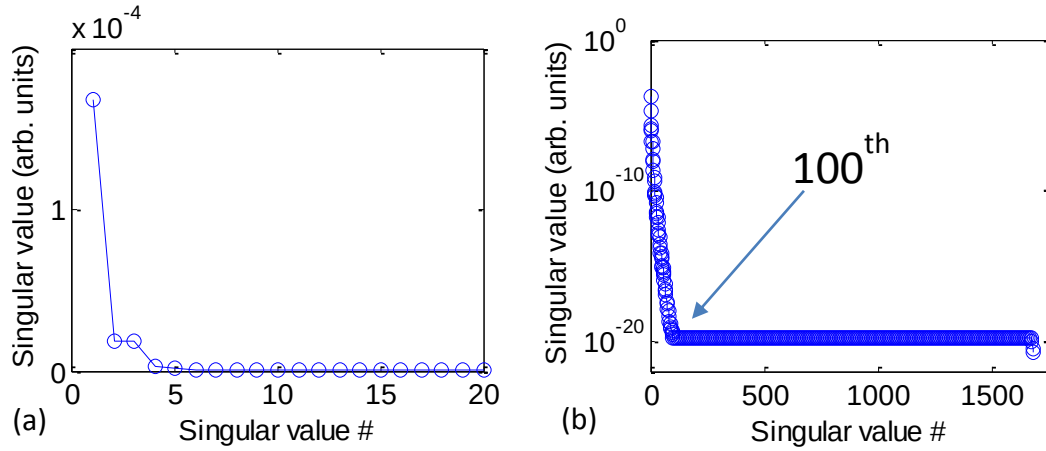


Fig. .1. (a) and (b) are singular value spectra plotted in linear and logarithmic scales.

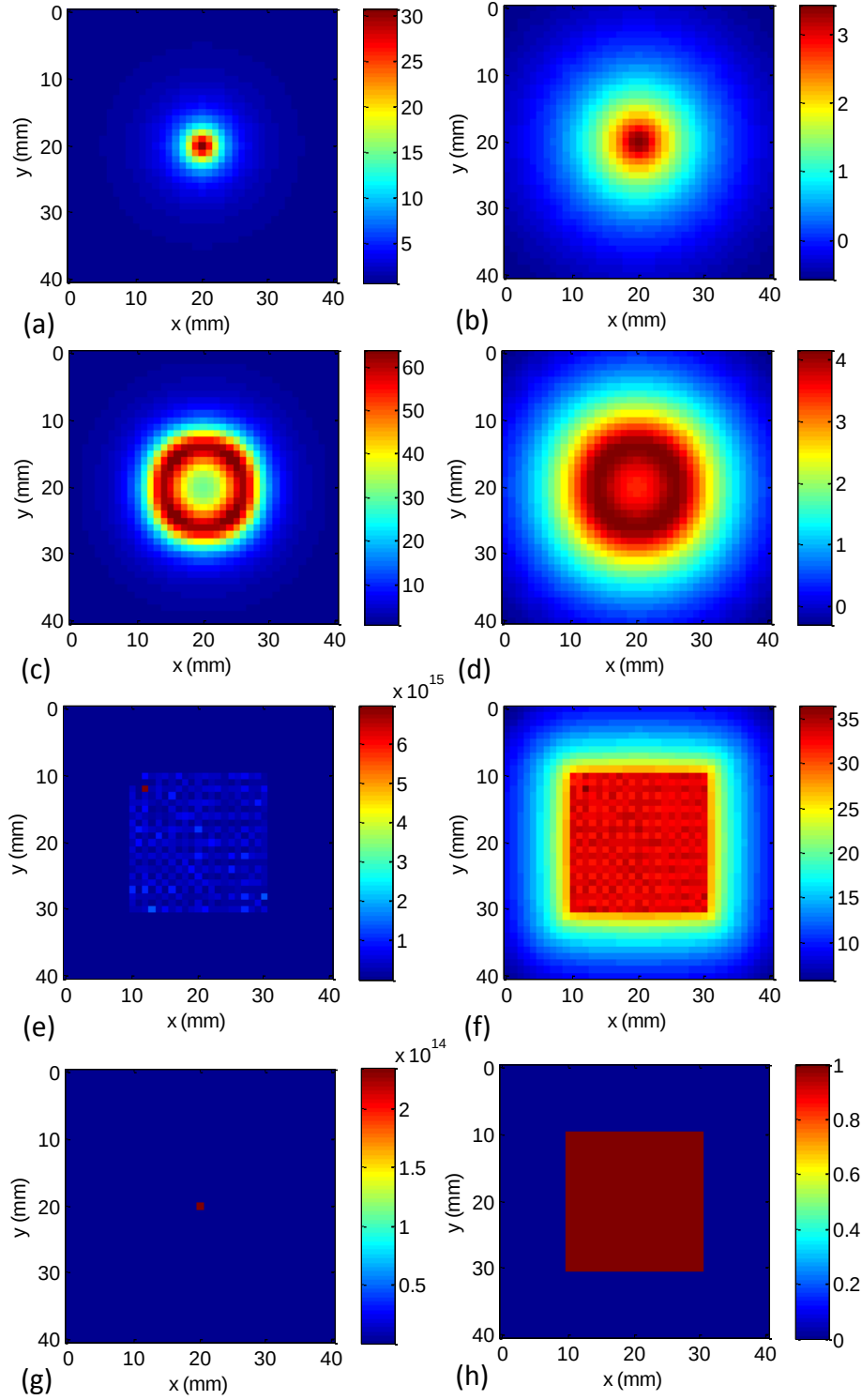


Fig. 2. The pseudo images of the target generated using pseudo spectrum with 1, and 1 eigen components shown in linear scale in (a), (c) and (e), respectively; and in logarithmic scale in (b), (d) and (f), respectively. (g) and (h) are the actual images of the point target and 2 mm 2 mm target, respectively, for comparison.

Since the high-order components carry shape information of the target, the singular value spectrum is plotted in logarithmic scale to help select more higher-order components. The Semi-log plot of the singular value spectrum is also an *L*-shaped curve. So the “corner” of the “*L*-curve” is used. Therefore, the first 1 eigen components are used to calculate the pseudo spectrum, and compared to and 1. The pseudo spectrum calculated using 1 eigen component, eigen components and 1 eigen components are shown in linear scale in Fig. .2(a), .2(c) and .2(e), respectively; and in logarithmic scale in Fig. .2(b), .2(d) and .2(f), respectively.

As shown in Fig. .2, when 1 eigen component was used, one sharp peak was generated in the pseudo image, using which the target was found to be located to be at (21, 21, 2) mm in the reconstruction coordinates. owever, no size information of the target was obtained. The reconstructed image for a point target located at (21, 21, 2) mm is show in Fig. .2(g) for comparison. When eigen components were used, the image of the target was extended. ut the shape of the image is round instead of square. The full-width-at-half-maximum (FW M) in the pseudo image of logarithmic scale was measured to be 24.4 mm in both *x* and *y* directions, which was used to estimate the size of the target. When 1 eigen components were used, all details in the image, including the edges and corners were detected. Therefore, the exact square shape was detected, as shown in Fig. .2(e). Due to the large range in the pseudo values, the image is re-plotted in logarithmic scale in Fig. .2(f) to enhance the contrast. The actual image of the target is shown in Fig. .2(h) for comparison. In all cases, the pseudo image of the target is centered at (21, 21, 2) mm.

The optical strength of the target was estimated using $q. (.)$ where the *F* was calculated using the center position of the retrieved target image. The retrieved optical strengths are shown in Table .1, and compared to the known value.

Table .1. Retrieved optical absorption strength and FW M of the pseudo spectrum with noise

		Optical strength (mm /ns)	rror ()	FW M (mm)
nown value		4.	-	2
Retrieved value using	1 eigen component	.	2 .	.2
	eigen components	.	2 .	1 .
	1 eigen components	.	2 .	2
	1 1 eigen components (original data matrix)	.	2 .	2

As shown in Table .1, when calculating the optical absorption strength, increase in the number of eigen components does not significantly improve the retrieved strength. There is 2 . error in the retrieved value no matter 1 eigen component was used or the whole original data matrix was used. We ascribe the error to the assumption that all F s related to different positions in the target are the same and correspond to the centroid position of the target. For small target, smaller error is expected. owever, the FW M of the pseudo spectrum increases as the number of components increases. When 1 components (corresponding to the “corner” of the singular value spectrum in logarithmic scale) are used, the pseudo spectrum becomes stable. The dimension of the plateau on the top of the pseudo spectrum instead of the FW M is shown in the Table, which is almost equal to the known dimension of the target.

Next the effect of noise was explored. 2 additive aussian random noise was added to the data to evaluate the efficacy of the method again. When the noise is added, the data matrix is X

$X_0 + N$, where X_0 is the simulated raw data without noise added, and N is the gaussian random noise with mean and standard deviation of 2 of the mean value of the raw data without noise.

The singular value spectrum constructed using SVD of the TR matrix is shown in Fig. 3.3.

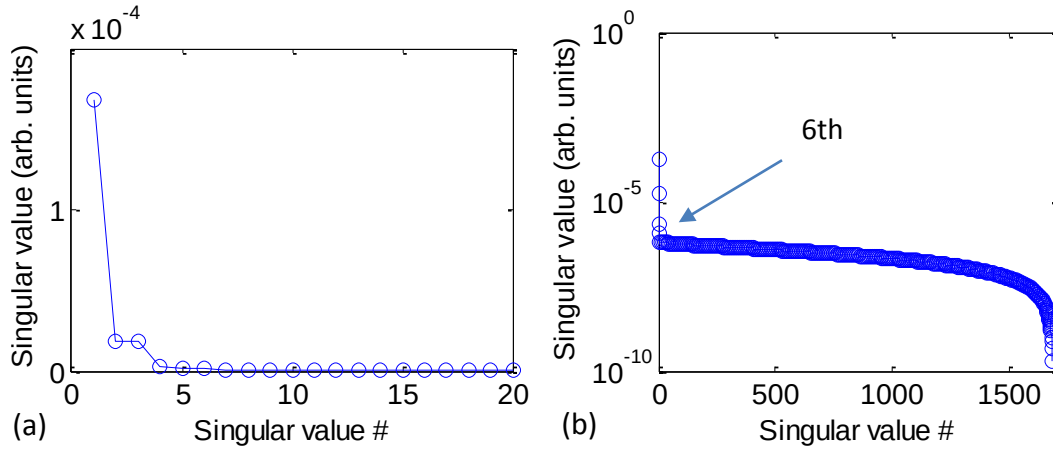


Fig. 3.3. Singular value spectrum in (a) linear scale and (b) logarithmic scale.

With noise added, the first three singular values were still prominent. As shown in the semi-log plot, most of higher-order components were then buried in the noise. The “corner” of the L -curve occurred at 6th singular value. The first 1, 3, 5, and 10 singular components are used to calculate pseudo spectrum, as shown in Fig. 3.4, for comparison.

As Fig. 3.4 shows, when 1 and 3 eigen components are used, the pseudo images shown in Fig. 3.4(a), 3.4(b), 3.4(c) and 3.4(d) are almost identical to those generated without noise. When 5 eigen components are used, the image is shown in linear scale in Fig. 3.4(e) and in logarithmic scale in Fig. 3.4(f). The size of the pseudo image of the target estimated using the FWMM is still comparable to the actual size. However, the shape of the image is not as accurate as that in the no-noise case with 1 eigen components used. Even if 10 eigen components are used to calculate the pseudo spectrum, as shown in Fig. 3.4(g) (linear) and 3.4(h) (logarithmic), the

pseudo image cannot be improved. This is because the higher-components which carry more detailed information of the target shape are buried in the noise and not available any more.

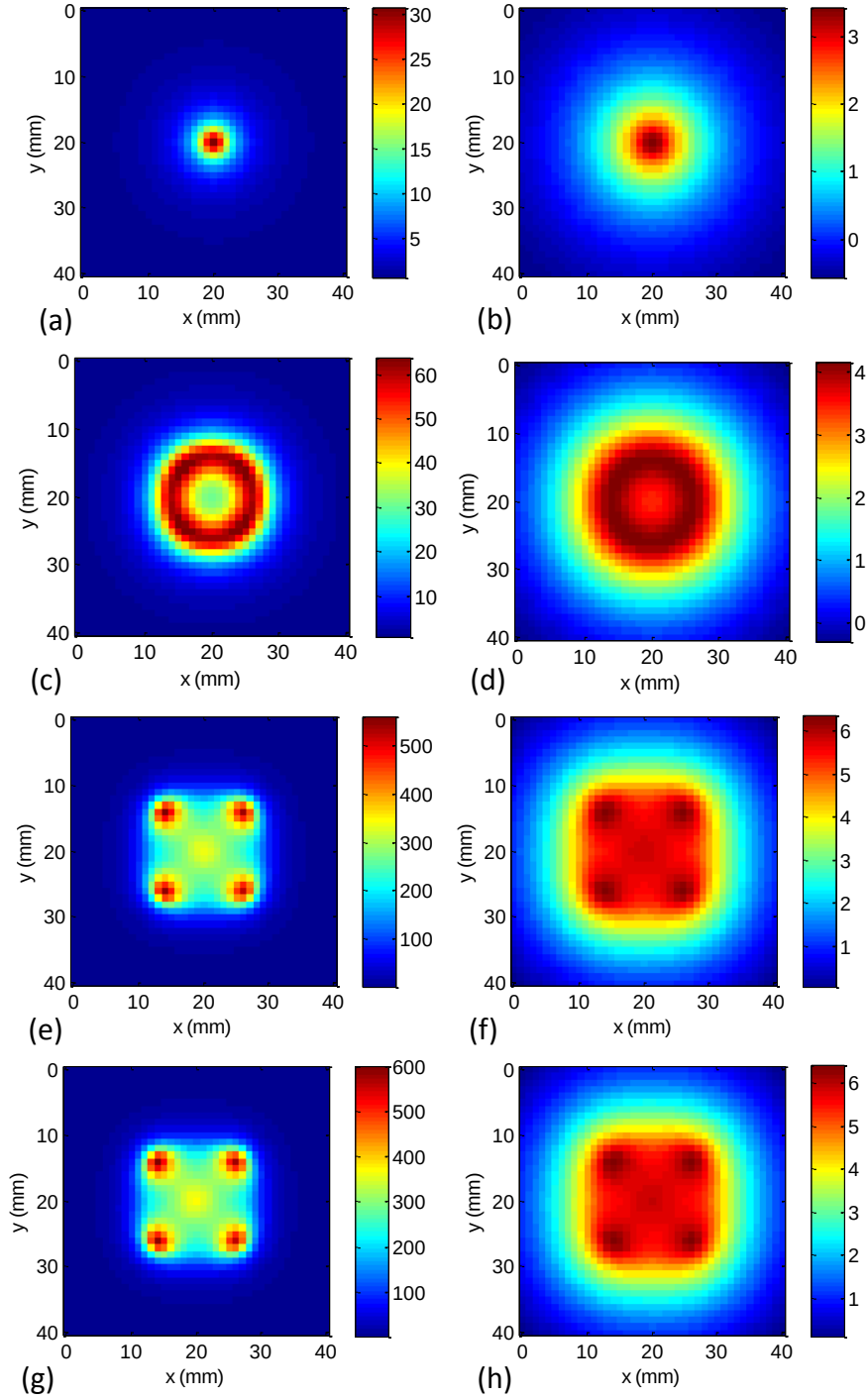


Fig. 4. The pseudo images of the target generated using 1, 2, 3, and 4 eigen components shown in linear scale in (a), (c), (e) and (g), respectively; and in logarithmic scale in (b), (d), (f) and (h), respectively.

The optical strength of the target is calculated using different number of eigen components and shown in Table .2.

Table .2. Retrieved optical absorption strength and FW M of the pseudo spectrum with 2 additive aussian noise

		Optical strength (mm /ns)	rror ()	FW M (mm)
nown value		4.	-	2
Retrieved value using	1 eigen component	.	2 .	.2
	eigen components	.	2 .	1 .
	1 eigen components	.	2 .	2
	1 1 eigen components (original data matrix)	.	2 .	2

The retrieved optical strength of the target is identical to those obtained in the non-noise case. In all cases of using different numbers of eigen components, the optical strength is found to be . mm /ns with 2 . error.

3.3.2. Localization, property and size retrieval using simulated data

In this section, we use a set of simulated datasets to evaluate the efficacy of the TROT algorithm we have developed in Section .2 in locating and retrieving optical strength and size of finite-size targets. Two approaches that use FW M in the pseudo spectrum and “local contour” will be used to estimate the target size. -D targets will be used. ven though the targets used in the simulations are not big, they are simulated using q. (.1) and treated as extended targets,

instead of point targets used in Chapter 2. Therefore, after SVD, each target contributes more than one eigen component (eigenvalue and eigenvector).

In these simulations, the same medium and source (detector) configurations were used as above simulation in Section 3.1. Three datasets were simulated. In the first simulation, one 1-mm-diameter absorptive target was centered at (4, 4, 2) mm. In the second simulation one 1-mm-diameter scattering target was centered at (4, 4, 2) mm. In the third simulation, one 1-mm-diameter and one -mm-diameter absorptive targets were located at (1, 1, 2) mm and (, , 2) mm, respectively. The absorption coefficient of all the absorptive targets was set to be higher than the background with $\delta\mu_a = 1 \text{ mm}^{-1}$, while the diffusion coefficient was taken to be the same as that of background. The diffusion coefficient of the scattering target was set to be lower than the background (higher scattering coefficient) with $\delta D = .1 \text{ mm}$ ($l_t = . \text{ mm}$), while the absorption coefficient was taken to be the same as that of the background. The volume of the 1-mm-diameter target was 1 mm^3 when the sample volume is discretized into $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$ voxels in the forward model. The volume of the -mm-diameter target was 12 mm^3 . The optical strength of the 1-mm-diameter absorptive targets was $\delta\mu_a c V = 11 \text{ mm}^3/\text{ns}$, of the -mm-diameter target $2 \times 2 \text{ mm}^3/\text{ns}$; while the optical strengths of the scattering target was $\delta D c V = -11 \times .1 \text{ mm}^3/\text{ns}$. The incident CW beam step scanned the sample at 41×41 grid points covering an 8 mm^2 area, with a step size of 2 mm. Light on the opposite side was recorded at 41×41 grid points covering the same area. Additive gaussian noise of different noise levels was added to the simulated data and compared to no-noise case. The simulated data matrix K was calculated using $q = .1$ directly, and then analyzed using TROT. The results are shown below. For simplicity, when the reconstruction result for one target is displayed, a smaller volume of $4 \text{ mm} \times 4 \text{ mm} \times 4 \text{ mm}$ around the center is selected.

3.3.2.1. Single absorptive target

The first 2 singular values of the TR matrix with noise added are plotted in Fig. . (a) and Fig. . (b) for linear scale and logarithmic scale, respectively. In the linear plot, only one eigen component is prominent. In the semi-log plot, the “corner” is right after th component. So the dimension of the signal subspace was determined to be . sing q. (.4), the pseudo spectrum was calculated. Both of axial and sagittal views of the target using the pseudo spectrum are plotted in logarithmic scale in Fig. . (c) and . (d). A “plateau” is found in the center of the image and used to estimate the size of the target. The isosurface of the “plateau” is plotted in Fig. . (e). The dimension of the target image is similar to that of the target. The image is not exactly spherical, due to the lower resolution in the z direction for the transillumination slab geometry. Since noise is an ideal case and there is a discontinuity in the derivative of the pseudo spectrum due to the plateau, target size will not be estimated using the proposed approaches. The retrieved target volumes are shown in Table . and compared with the known volume.

The target position is described by the centroid position of the pseudo image, which is found to be exactly (2 , 2 , 2) mm. sing q. (.), the optical strength was found to be 112.4 mm /ns when all eigen components in the signal subspace were used. If only the first eigen component in the signal subspace was used, the optical strength was found to be the same, which confirms the single prominent eigen component in the linear plot of singular value spectrum contributes the most to the optical strength. The retrieved optical strength and size of the target are shown in Table . .

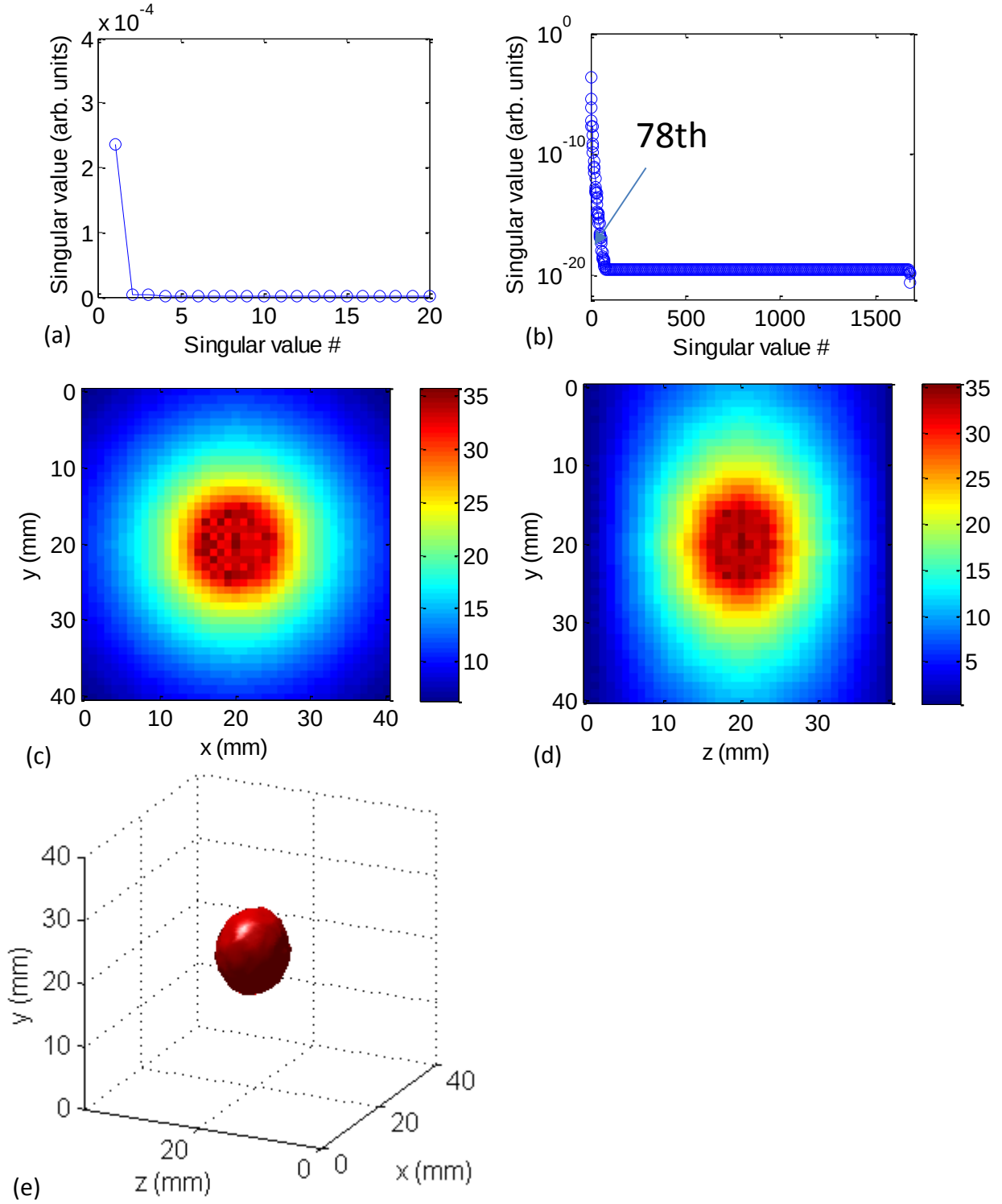


Fig. . . (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are axial and sagittal pseudo images of the absorptive target in logarithmic scale. (e) shows the isosurface for the “plateau” in the pseudo image (logarithmic scale). No noise was added to the simulated data.

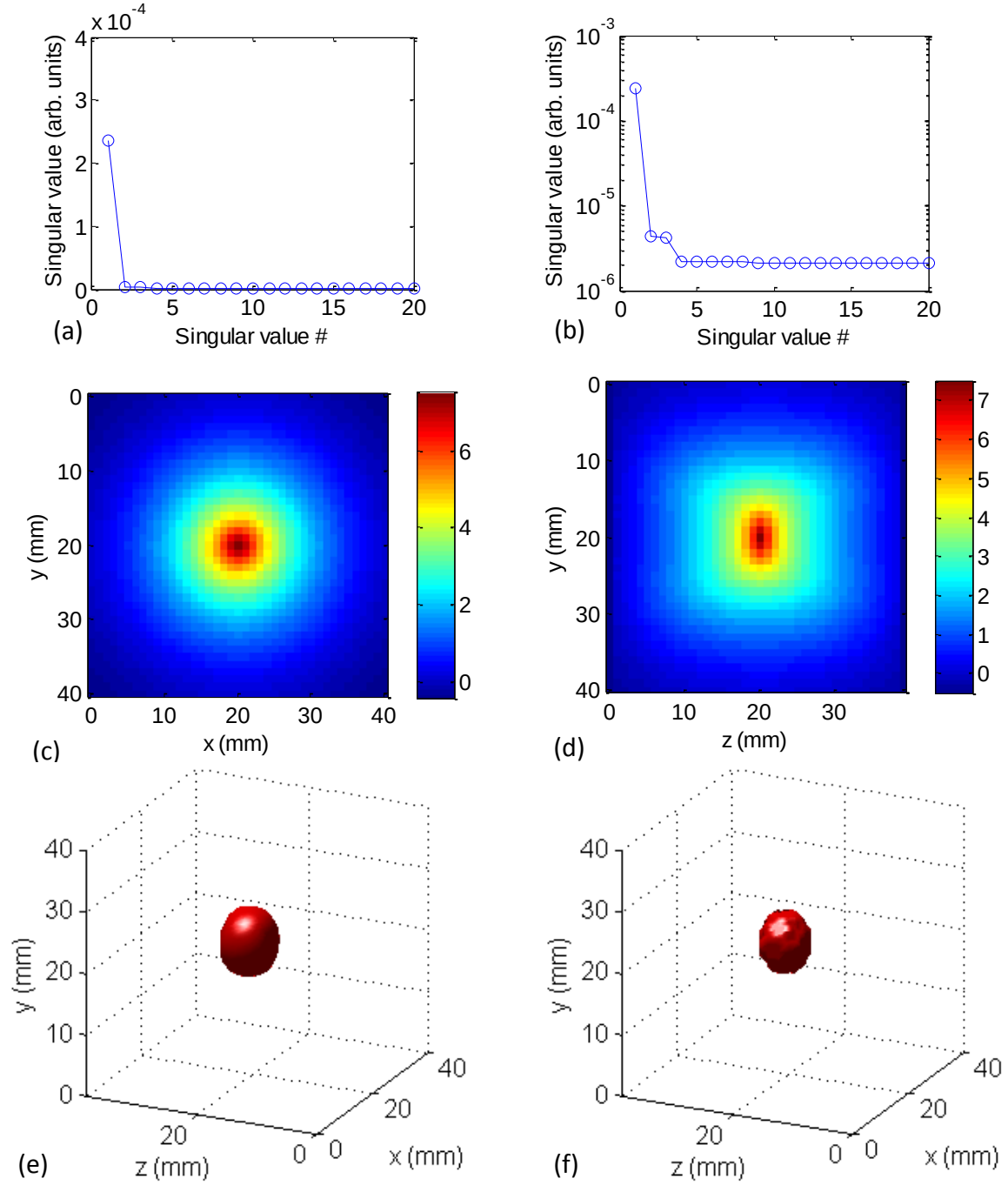


Fig. 11. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are axial and sagittal pseudo images of the absorptive target in logarithmic scale. (e) shows the isosurface for the FW M in the pseudo image (logarithmic scale). (f) is the isosurface found using the local contour method in q. (11). 2 noise was added to the simulated data.

With 2 additive gaussian noise added, the first 2 singular values were plotted in Figs. . (a) and . (b) for linear and logarithmic scales, respectively. The first singular value was still approximately the same as the one in no-noise case. In the semi-log plot, still singular values are prominent. So the dimension of the signal subspace was then determined to be . The pseudo spectrum was calculated. Both of axial and sagittal views of the target using the pseudo spectrum are plotted in logarithmic scale in Figs. . (c) and . (d). The isosurface of the FW M in the pseudo image is shown in Fig. . (e), whose volume is 1 mm . If the volume is approximated to be spherical, the estimated diameter is 1 . mm. The isosurface found using $q(\cdot)$ is shown in Fig. . (f), whose volume is mm , which is corresponding to . mm spherical diameter. The target position is obtained to be at exactly (2 , 2 , 2) mm. The absorption strength is determined to be 112.4 mm /ns, which is the same as that in the non-noise case.

Table . . Optical strength and size of an absorptive target

Noise	Optical Strength		Retrieved Size					
Level ()	Retrieved (mm /ns)	error ()	Contour (mm)	Diameter (mm)	error ()	FW M (mm)	Diameter (mm)	error ()
	112.4	.2	-	-	-	-	-	-
	112.4	.2	4	.			11.	1
1	112.4	.2	24	.	2	4	11.4	14
2	112.4	.2		.	1	1	1 .	1

Known values: volume: 1 mm , absorptive optical strength: 11 . mm /ns.

Similar simulated results are obtained for and 1 noise. In all cases, the target is accurately located. The retrieved optical strength and size of the target are shown in Table . . The retrieved optical strength is the same as that retrieved in the case of and 2 noise.

Further simulations show the optical strength can be accurately retrieved if the noise level is even higher or the target size and location are varied. The retrieved target size (diameter) using both methods is within 2% uncertainty. However, for a larger target, the error in the retrieved target size may increase significantly. The accuracy in the retrieved target size and optical property seems not to well correlate with the noise level.

3.3.2.2. Single scattering target

Similar simulations were carried out when one scattering target was embedded in the medium. Simulated data was generated and the subsequent analysis was conducted with no noise and with 1% noise and 2% noise added separately.

The first 20 eigenvalues of the TR matrix for one absorptive target with 1% noise and with 2% noise are plotted in and shown in Fig. 10 and Fig. 11. With 1% noise added, three eigen components are prominent in the linear plot of the singular value spectrum for one single scattering. In the semi-log plot, 10 eigen components are prominent, which are chosen to form the signal subspace. When 2% noise was added, the singular value spectrum in linear scale is almost identical to that for no-noise case. However, in the logarithmic scale, there are only two prominent singular values. So the dimension of the signal subspace is chosen to be 2. The pseudo spectrum was calculated using eq. (3.4). The axial and sagittal views of the target using the pseudo spectrum for 1% noise are plotted in Figs. 12 (c) and 13 (d) for linear and logarithmic scales, respectively. The axial and sagittal views of the target with 2% noise added are plotted in Figs. 14 (c) and 15 (d) for linear and logarithmic scales, respectively.

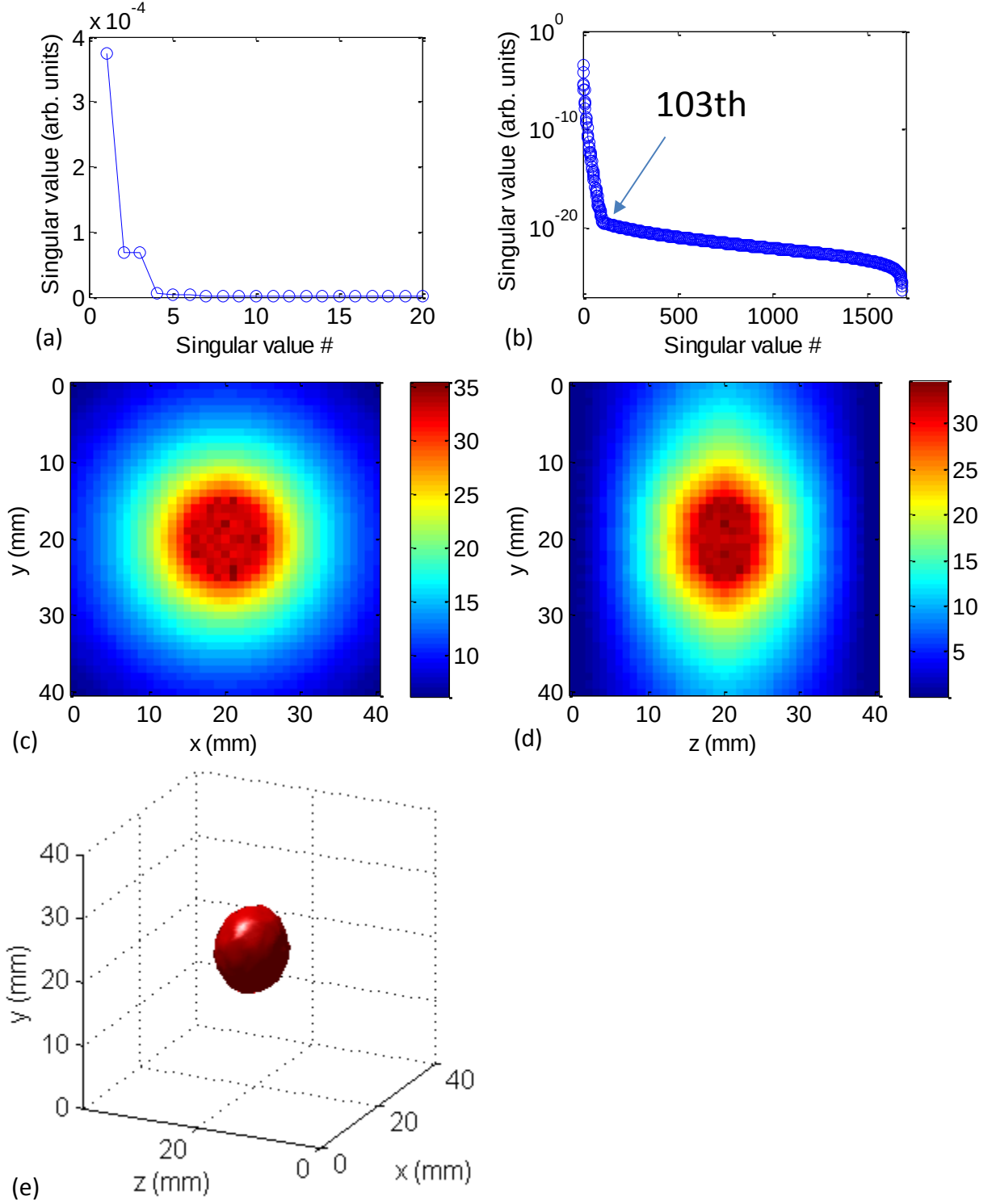


Fig. . . (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are axial and sagittal pseudo images of the scattering target in logarithmic scale. (e) shows the isosurface for the FW M in the pseudo image (logarithmic scale). No noise was added to the simulated data.

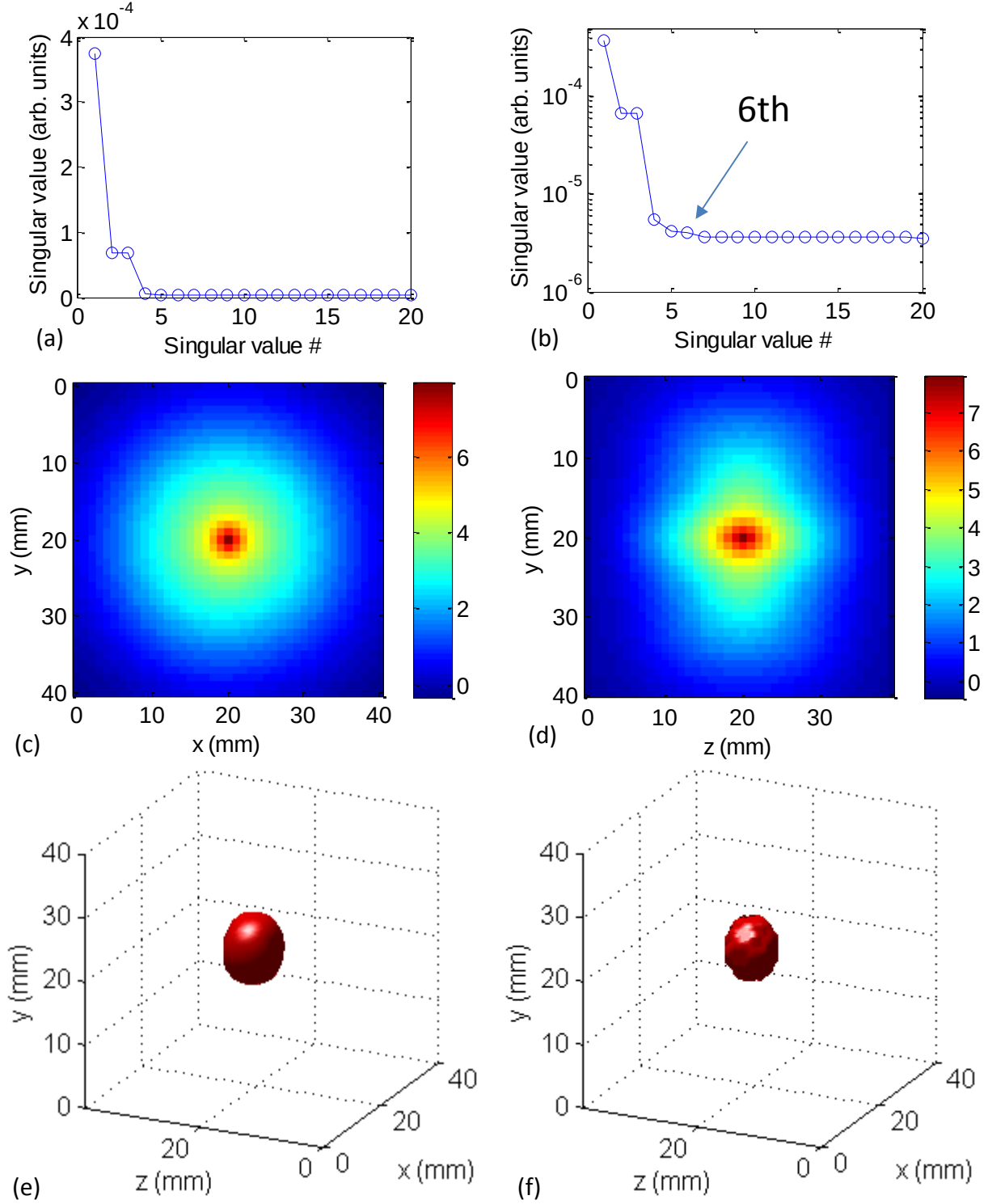


Fig. 1. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are axial and sagittal pseudo images of the scattering target in logarithmic scale. (e) shows the isosurface for the FW M in the pseudo image (logarithmic scale). (f) is the isosurface found using $q. (1). 2$ noise was added to the simulated data.

Similar to the absorptive target, with noise, there is a “plateau” shown in pseudo image. The isosurface was obtained using the “plateau” in the pseudo spectrum and plotted in Fig. 10 (e). The size of the “plateau” is similar to the actual value of the target. When 2 noise was added, the two isosurfaces appeared as plotted in Figs. 10 (e) and 10 (f), respectively. The size of the target is estimated to be 1 mm (1 mm diameter) using FW M of the pseudo spectrum and 4 mm (.4 mm diameter) using the “local contour” method (eq. (1)).

Similar images were obtained for 1 and 1 noise levels. The target location and optical strength of the target was accurately retrieved in all cases. The size in all cases was retrieved using FW M of the pseudo spectrum (logarithmic scale) and the “local contour” method (eq. (1)). The retrieved target optical strength and size are all shown in Table 4, and compared to the known values.

Table 4. Optical strength and size of a scattering target

Noise level ()	Optical Strength		Retrieved Size using					
			Local Contour			FW M		
	Retrieved (mm /ns)	error ()	Volume (mm)	error ()	Diameter (mm)	Volume (mm)	error ()	Diameter (mm)
	-1 .4	.4	-	-	-	-	-	-
	-1 .	.4		4.	1 .1	42	1 .	.
1	-1 .2	.4		2 .1	1 .	2 1	.2	.
2	-1 .2	.		11.	1 .	4	1 .	.4

Known values: volume: 1 mm , scattering optical strength: -11 .1 mm /ns

The target location was accurately found to be (2 , 2 , 2) mm for all noise levels (, , 1 and 2). The scattering optical strength of the target was retrieved within .4 error and the size of targets was found within 2 error for all noise levels.

3.3.2.3. Two absorptive targets

Next we consider the case of two spherical absorptive targets with different size (one with diameter 1 mm, the other with diameter mm) embedded in the medium, with a center-to-center separation of 14.1 mm. Simulated data are generated with different additive noise levels:

, 1 , 2 , and compared to -noise case. The singular value spectrum of the TR matrix with noise is shown in Fig. . (a) (linear scale) and in Fig. . (b) (logarithmic scale).

In linear scale, the “corner” of the “*L*-curve” is after 2th singular value, corresponding to the two targets. In logarithmic scale, the “corner” is after 11 th singular value, where the high-order components carry more details of the shape. The pseudo spectrum was calculated. The centroid positions of the two targets were determined to be exactly the same as the known positions (1 , 1 , 2) mm and (, , 2) mm, respectively. The sagittal pseudo images of the two targets at $x = 1$ mm and $x =$ mm are shown in Figs. . (c) and . (d), respectively. The axial pseudo image of the two targets at $z = 2$ mm is shown in Fig. . (e). There are two “plateaus” in the pseudo image corresponding to the two targets, whose sizes are approximately the same as the known values. The optical strengths of the two targets are estimated using q . (.). The retrieved optical strengths using 11 eigen components are shown in Table . . When 2 eigen components are used, the retrieved strengths are about the same as those retrieved using 11 eigen components. This confirms that the leading two eigen components contribute the most to the optical strengths.

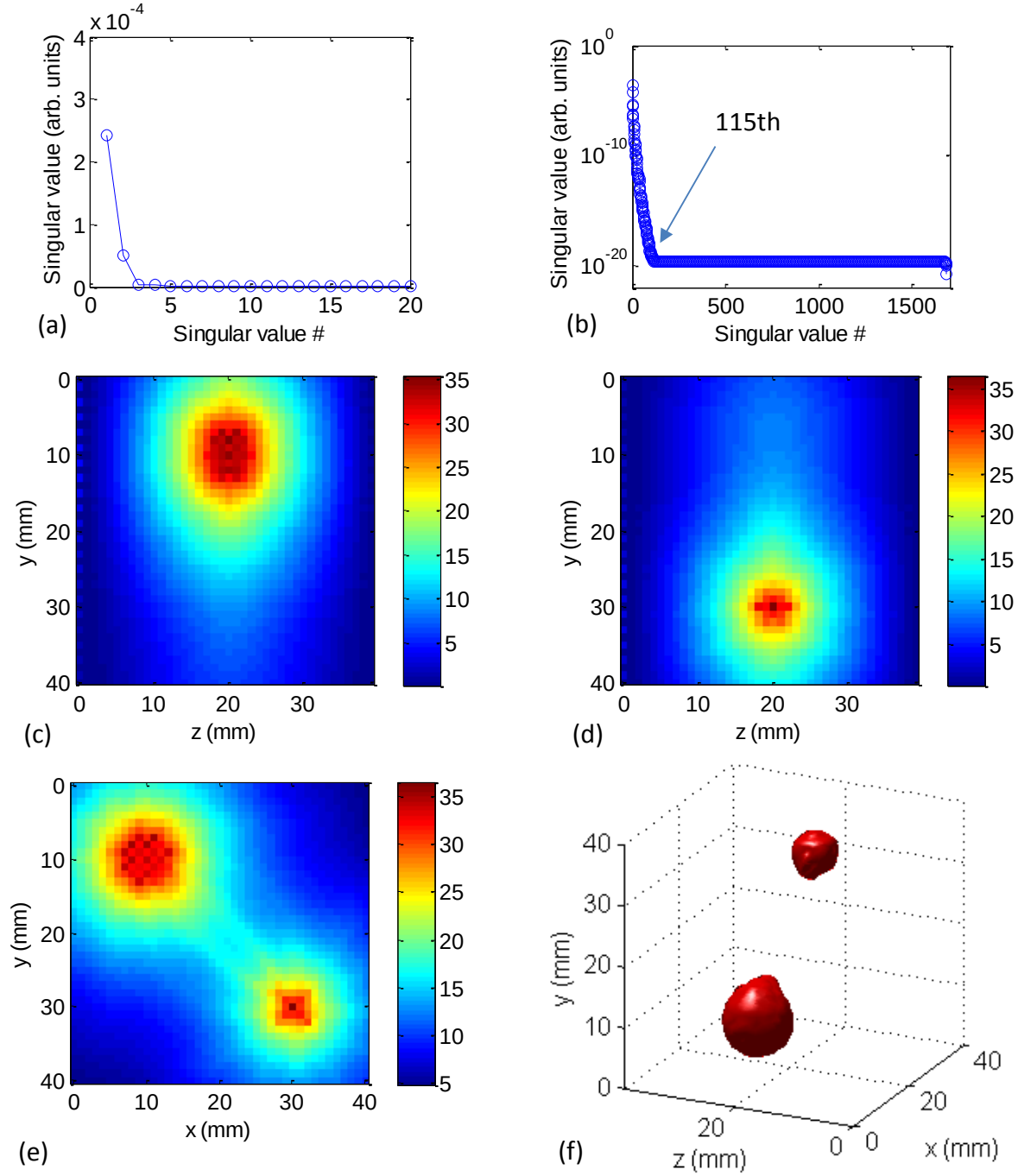


Fig. 1. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are sagittal pseudo images (logarithmic scale) of the two targets, respectively. (e) is the axial pseudo image (logarithmic scale) of the two targets at $z = 20$ mm. (f) shows the isosurface generated using the FW M in the pseudo image.

Table . . Optical strength and size of two absorptive targets

Noise level ()	Target	Optical Strength		Retrieved Size using			
				ocal contour		FW M	
		Retrieved (mm /ns)	rror ()	olume (mm)	Diameter (mm)	olume (mm)	Diameter (mm)
	eft	11 . 1	4.	-	-	-	-
	Right	2 .	. 4	-	-	-	-
	eft	11 . 1	4.	2	1 .4		11.4
	Right	2 .	. 4	4	1 .	1 1	.
1	eft	11 . 1	4.	1	1 .	1	12.1
	Right	2 .	. 4		.	2	4.
2	eft	11 . 1	4.	1	1 .4		12.1
	Right	2 . 2		4	.4		4.

nown values: for left target, volume 1 mm , optical strength 11 . mm /ns; for right target, volume 12 mm , optical strength 2 . 2 mm /ns.

The singular value spectrum when 2 noise was added is shown in Fig. .1 (a) (linear scale) and 1 (b) (logarithmic scale). In linear scale, the singular value spectrum appear to be similar to that in the noise case. When 2 noise is added, eigen components are separated to be the signal subspace. The pseudo spectrum is calculated using eigen components. The centroid locations of the two targets are exactly retrieved. The sagittal pseudo images of the two targets at $x = 1$ mm and $x =$ mm are shown in Figs. .1 (c) and .1 (d), respectively. The axial pseudo image of the two targets at $z = 2$ mm is shown in Fig. .1 (e).

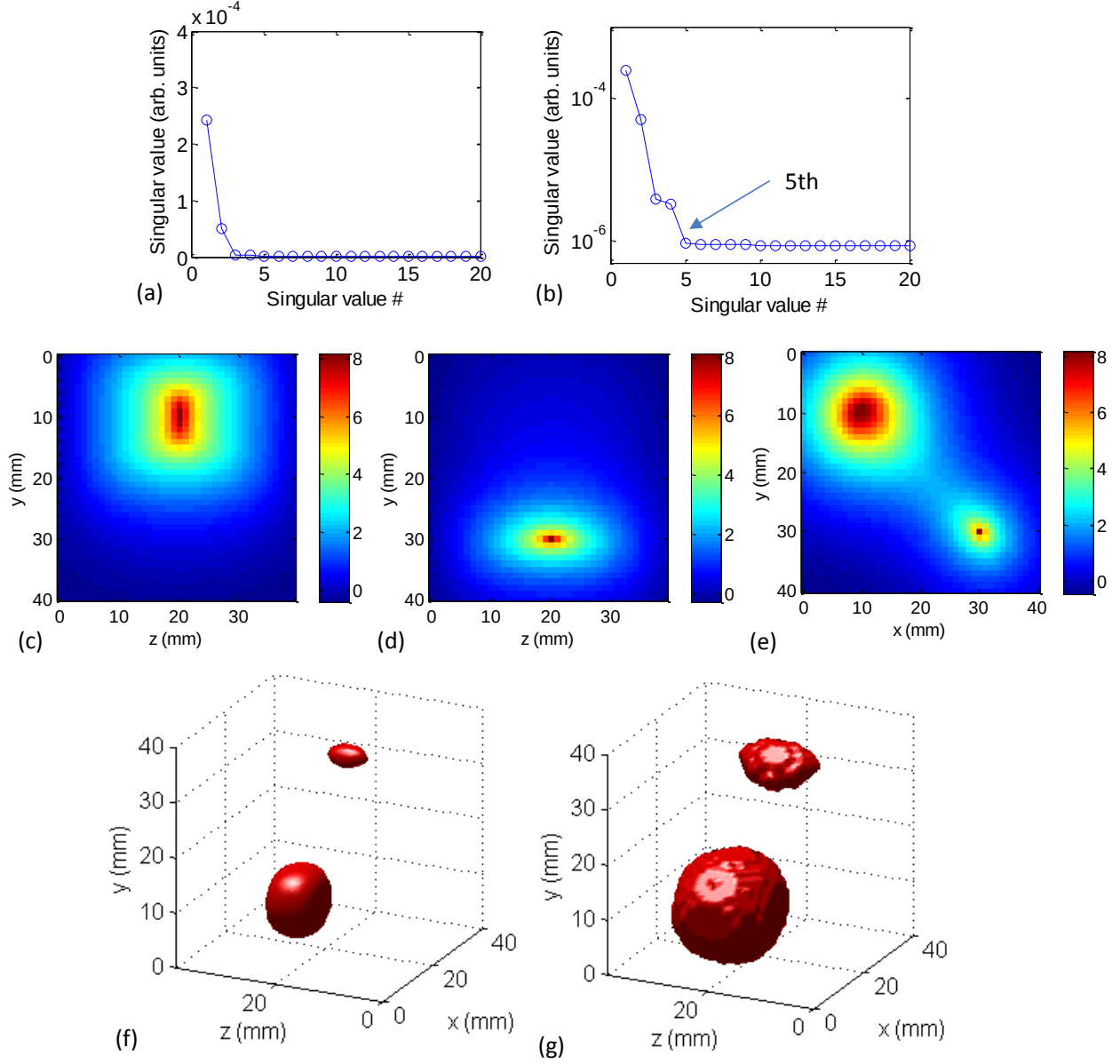


Fig. 1. (a) and (b) are singular value spectra in linear and logarithmic scales, respectively. (c) and (d) are sagittal pseudo images (logarithmic scale) of the two targets, respectively. (e) is the axial pseudo image (logarithmic scale) of the two targets at $z = 2$ mm. (f) shows the isosurface generated using the FW M in the pseudo image. (g) is the isosurface generated using the “local contour” method.

The isosurfaces are generated using the FW M in the pseudo image and the “local contour” method to estimate the size of the targets. The estimated sizes of the two targets are shown in Table 1. The volumes of the two targets estimated using the “local contour” method are .4 and

. times of the actual values. Assuming these are spherical volumes, the corresponding diameters are about 2 and 1. times of the actual values. The diameters estimated using the FW M in the pseudo spectrum have approximately 21 error.

When and 1 noises were added, similar images were obtained. The estimated sizes of the two targets are also shown in Table . . The sizes of the two targets estimated using the optimal “local contour” method are similar for different noise levels, and are not as accurate as those estimated using the FW M method. The estimated size of a target when two targets were present is not as accurate as that when only one target was present. The sizes estimated using the FW M in the pseudo spectrum with noise are the most accurate among different noise levels. The main reasons why the accuracy of size retrieval using the “local contour” method degrades may be: 1) The contours are not good estimates of the shape of the target and the actual target boundary is not available in the pseudo spectrum. In particular when the targets are not far from each other, the pseudo images of different targets are not well separated. 2) Due to the diffusive nature of light transmission, the signal is not sensitive to the shape (size) of the small target. In simulations with noise added or in experiments, when the forward model is used with the estimated target shape, the “optimal” contour may easily be a wrong contour with a “local minimum” in $q. (.)$.

The retrieved optical strength for all noise levels is approximately the same, since the optical strength is mainly determined by the leading (low order) singular components which are little affected by the noise. The retrieved strength of the left target (larger) has 4. error, and of right target almost retrieved exactly. The error may be due to the approximation of the F s for extended targets by using one single F corresponding to the centroid position. Therefore, a larger target leads to larger error in the retrieved strength.

3.4. Experiments

3.4.1. Absorptive targets

The first experiment is the same as that introduced in Section 2. . .2 of Chapter 2, except that the concentration of the ink in targets was adjusted to provide absorption coefficient μ_a . . . mm^{-1} , instead of . . . 1 mm^{-1} . The two targets were . . . mm diameter, embedded in . . . -mm transparent plastic container filled with Intralipid-2 . . . suspension in water. The absorption coefficient of the background medium is . . . mm^{-1} , and the transport mean free path l_t , 1 mm. The separation of the two targets varied to be 1 . . . mm, 2 . . . mm, and . . . mm. The sample was scanned by a 1 . . . -mW . . . -nm diode laser at 1 . . . 11 grid points with . . . mm step size. The images were acquired using a water-cooled CCD camera. The experimental setup and other details are the same as those in Chapter 2.

When the two targets are . . . 2 cm apart, the eigenvalue spectrum and the pseudo spectrum were plotted in Figs. . .11(a) and . .11(b) in linear and logarithmic scales, respectively. In linear scale, two singular values are prominent and correspond to two targets. In logarithmic scale, the “corner” is determined to be after the 1st singular value. To test the sensitivity of localization with respect to the dimension of signal subspace, the pseudo spectrum is calculated using two eigen component and 1st eigen components, and shown in Figs. . .11(c) and . .11(d), respectively. The locations of the targets are determined using the maxima in the pseudo spectra, and shown in Table . . and . . for comparison with known results. The separation between the two targets is retrieved to be 1 .4 mm. The FW M in the pseudo spectrum calculated using 1st eigen components is used to generate an isosurface and shown in Fig. . .11(e). The same pseudo spectrum is used to generate the isosurface using the “local contour” method (. . q. (. .)), as

shown in Fig. .11(f). The FW M isosurface is not available in logarithmic scale, so it is generated using pseudo spectrum in linear scale.

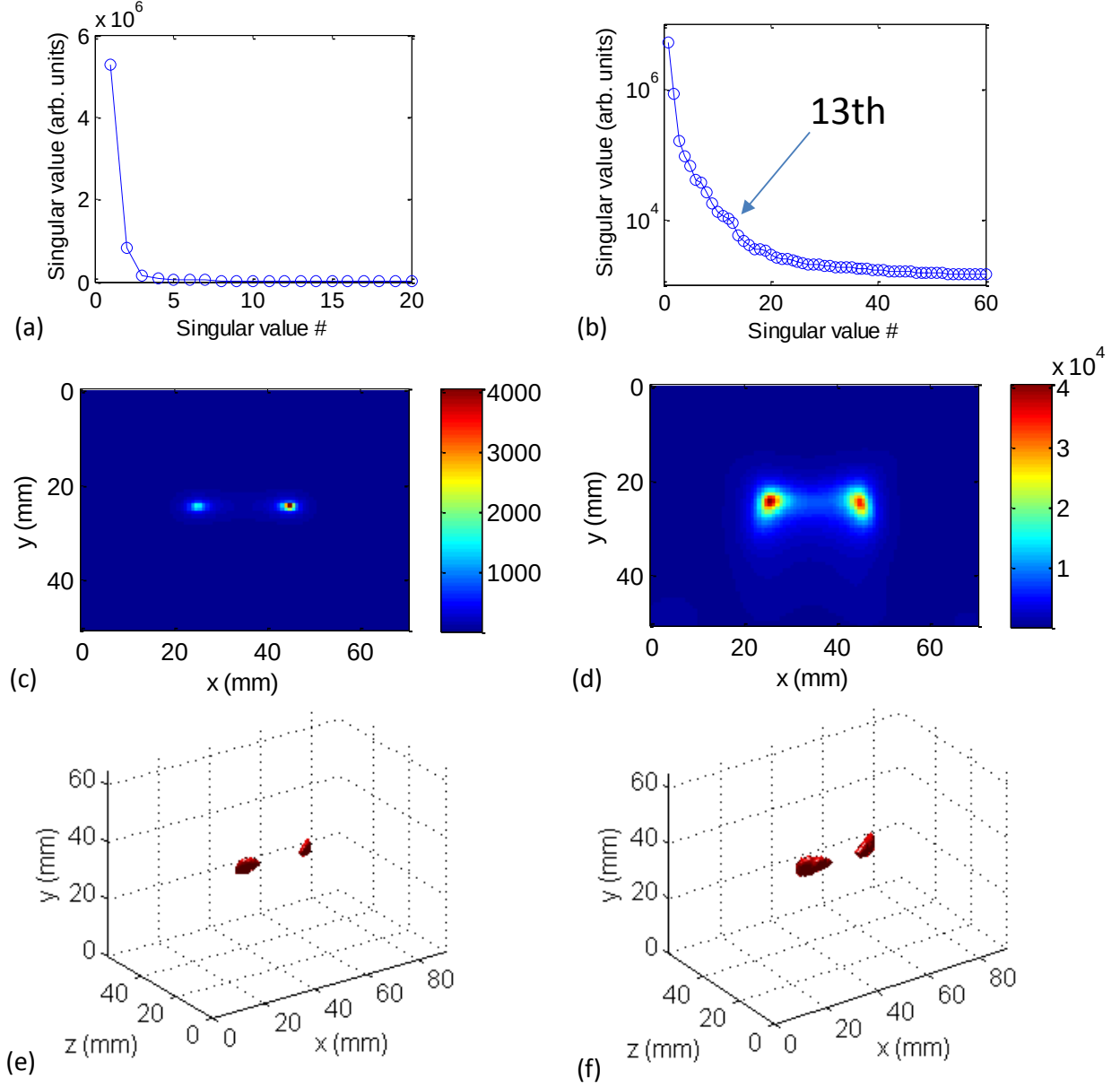


Fig. .11. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are pseudo images of the two targets generated using 2 eigen components and 1 eigen components, respectively. (e) shows the isosurface generated using the FW M in the pseudo image. (f) is the isosurface generated using the “local contour” method.

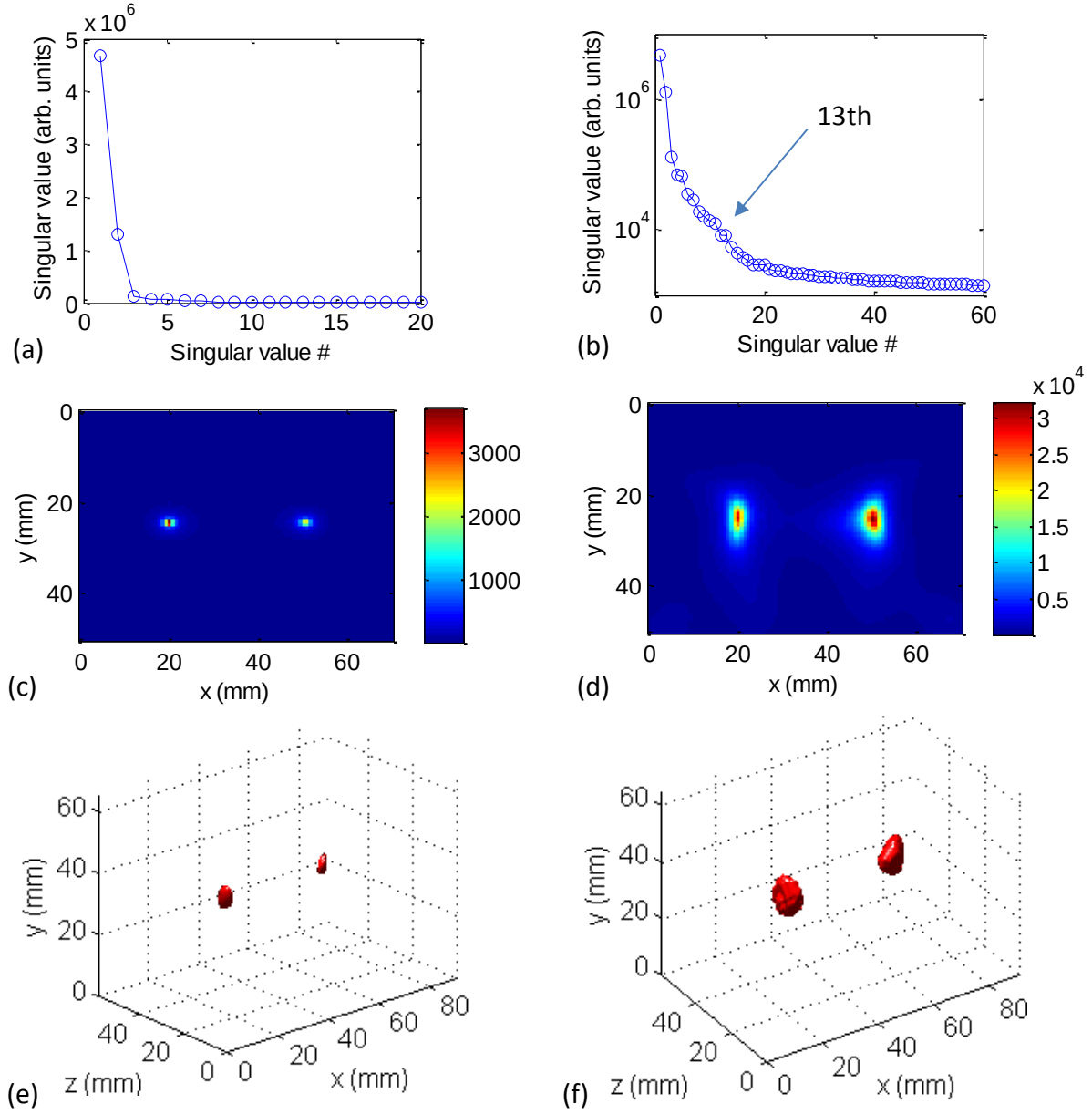


Fig. 12. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are pseudo images of the two targets generated using 2 eigen components and 1 eigen components, respectively. (e) shows the isosurface generated using the FW M in the pseudo image. (f) is the isosurface generated using the “local contour” method.

The strengths of the targets are retrieved using 2 eigen components, 1 eigen components and the original data matrix, and shown in Table 1 with comparison to the known value (q).

(.)). The sizes of the targets are estimated using the FW M in the pseudo spectrum and the “local contour” method, and shown in Table . .

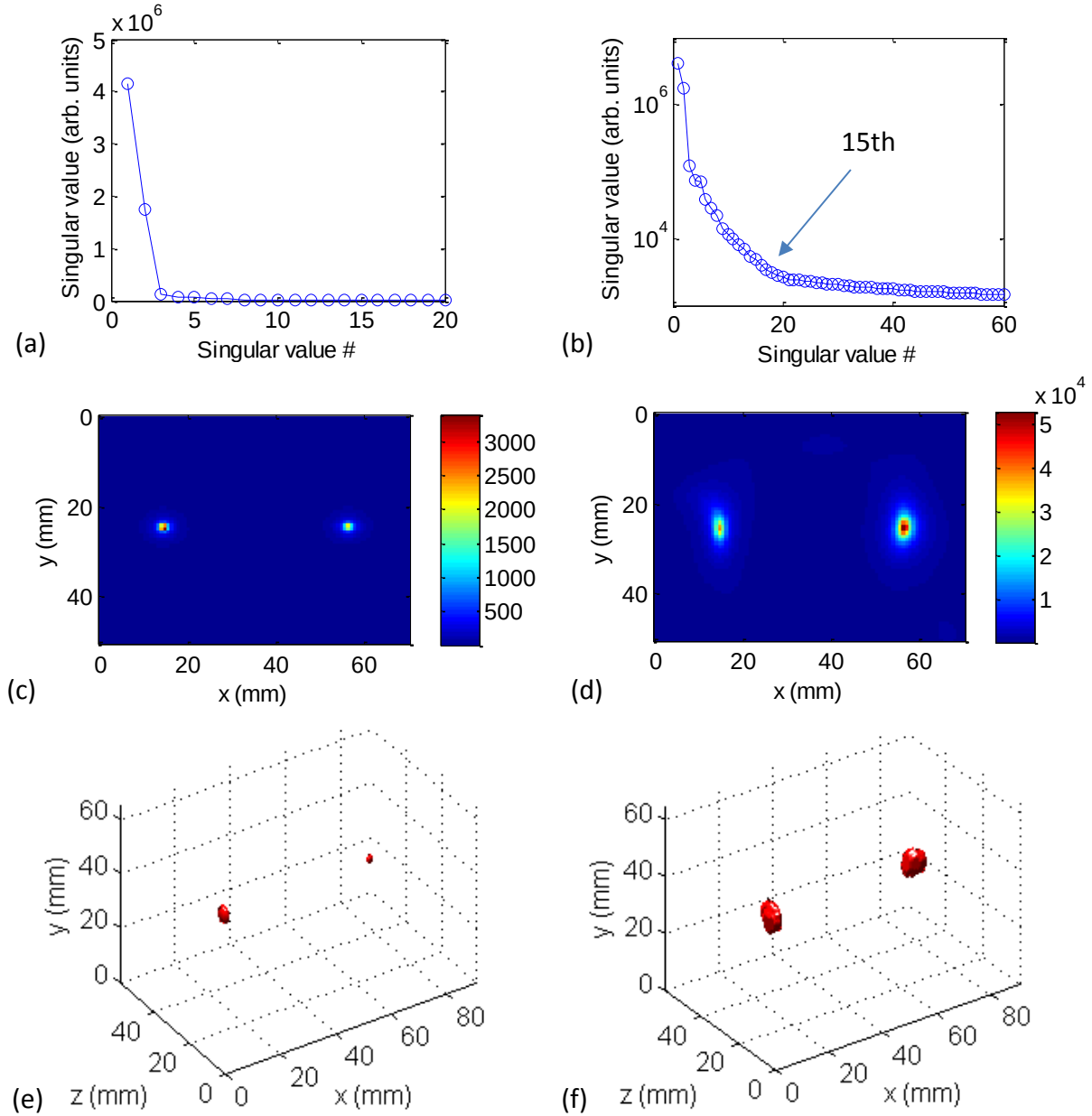


Fig. .1 . (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are pseudo images of the two targets generated using 2 eigen components and 1 eigen components, respectively. (e) shows the isosurface generated using the FW M in the pseudo image. (f) is the isosurface generated using the “local contour” method.

Table . . Retrieved target locations using 1 eigenvectors

Separation	Target	nown ositions x, y, z (mm)	Retrieved ositions x, y, z (mm)	rror (mm)
2cm	eft	2 .1, 2 .1,	2 .4, 2 .2, .	1. , .1, .
	Right	44.4, 2 .1,	44. , 2 .2, 1.	.4, .1, 1.
cm	eft	2 .1, 2 .1,	2 . , 2 .2, 1.	.2, .1, 1.
	Right	4 .4, 2 .1,	1. , 2 .2, 2.	1. , .1, 2.
4cm	eft	1 .1, 2 .1,	14. , 2 . , 1.	.2, 1. , 1.
	Right	4.4, 2 .1,	. , 2 .2, 2.	1. , .1, 2.

1 eigen components for 4-cm separation

Table . . Retrieved target locations using 2 eigenvectors

Separation	Target	nown ositions x, y, z (mm)	Retrieved ositions x, y, z (mm)	rror (mm)
2cm	eft	2 .1, 2 .1,	2 . , 24.4, 1.	. , . , 1.
	Right	44.4, 2 .1,	44. , 24.4, 2 .	.4, . , .
cm	eft	2 .1, 2 .1,	2 . , 24.4, .	.2, . , .
	Right	4 .4, 2 .1,	1. , 24.4, 2 .	1. , . , 1.
4cm	eft	1 .1, 2 .1,	14. , 2 .2, .	.2, .1, .
	Right	4.4, 2 .1,	. , 24.4, 2 .	1. , . , .

Similar analysis is also performed when the two target are 2 cm and 4 cm apart. The singular value spectra (linear and logarithmic scale), pseudo images (using 2 and 1 eigen components for 2-cm separation, and 2 and 1 eigen components for 4-cm separation), and isosurfaces generated using FW M in the pseudo images and “local contour” method are shown in Figs. 12 and 13 respectively. The retrieved target locations using 2 and 1 (1) eigen components are shown in Table 1 and 2 for comparison. For the 2-cm and 4-cm separations, the retrieved separation between the targets is 2.1 mm and 41.4 mm, respectively. The strengths of the targets with 2-cm (4-cm) separation are estimated using 2 eigen components and 1 (1) eigen components for comparison, and shown in Table 3. The sizes of the targets are estimated using the FW M in the pseudo spectrum (linear scale) and the “local contour” method and shown in Table 4.

Table 3. Retrieved optical strengths of the two targets

Separation	Retrieved τ (using 2 comps) (10^{-4} mm /ns)		Retrieved τ (using 1 comps) (10^{-4} mm /ns)		Retrieved τ (using the whole data matrix) (10^{-4} mm /ns)		Known τ (10^{-4} mm /ns)	
	eft	Right	eft	Right	eft	Right	eft	Right
2cm	2.	2.	2.	2.1	2.	2.1	4.1	4.1
2cm	.4	.2	.	.41	.	.41		
4cm	.	.4	4.	.	4.	.		

1 eigen components for 4-cm separation

Table . . . stimated size and absorption coefficient of the targets

Separation (cm)	Retrieved size using FW M (mm)		Retrieved size using “local contour” (mm)		Retrieved absorption coefficient (μ_a) (mm ⁻¹)		nown absorption coefficient (μ_a) (mm ⁻¹)	
	eft	Right	eft	Right	eft	Right	eft	Right
2		1 1	211	1 1	. 1	.	.	.
		4	4		.2	.2		
4			2	2 1	. 1	.		

nown target size (-mm-diameter sphere): 1. mm

The target locations retrieved using 2 eigen components (prominent in singular value spectrum in linear scale) and more eigen components (prominent in singular value spectrum in logarithmic scale) are both accurate.

Compared with the actual optical strengths of the targets 4.1 1⁴ (mm /ns), the retrieved values are within 2. , 1 . and . for 2-cm, -cm and 4-cm separation between the samples, respectively. The further the two targets, the more accurate results are obtained. sing the dimension of the signal subspace determined using the singular value spectrum in logarithmic scale only slightly improves the retrieved optical strengths of the targets. Further increasing the number of eigen components in q. (.) does not lead to improved accuracy in the retrieved optical strengths. So the estimate of location and optical strength of a target using TROT is not sensitive to the determination of the dimension of the signal subspace. In other words it is not critical to find the optimal threshold around the “corner” of the “L-curve” to separate the signal subspace from noise subspace so as to retrieve the location and optical

strength of the targets accurately. If there are M absorptive targets present, it is mainly the leading M eigen components that determine the locations and optical strengths of the targets. For the experiments presented above, the target locations and strengths are mainly determined by the first two eigen components. The dimension of the signal subspace determined using the singular value spectrum in logarithmic scale may be used to retrieve slightly more accurate optical strength, since more higher-order components that belong to the signal subspace will be separated from the noise subspace. This also helps in retrieving more accurate size or shape of the targets, since higher-order components carry more size information of the targets.

As shown in Table . , the sizes of targets estimated using the FW M of the pseudo spectrum in linear scale are all much smaller than the actual values. The estimated sizes using the “local contour” method are more accurate and further used to estimate the absorption coefficient of the m^{th} target using $\delta\mu_a = \tau_m/c\Delta V_m$. Compared to the known target size 1. mm , there is up to error in the retrieved sizes. Among the separations considered, the size is the most accurate for the target separation of 4-cm between the targets. The error in the estimated absorption coefficient is within 4 for the targets in all cases of separations, as shown in Table . . The forward model used in the presented work is the linearized analytical model of q. (.1), which only considers the first order of orn approximation. A forward model, such as, finite element method (F M) based forward model, which considers the non-linear effect in the diffusion process (higher orders of orn approximation) may be used to improve the estimate of target size. Since the forward model will only be run for a limited times, and there is no need to calculate the acobian matrix, it is different from other model-based inverse image reconstruction (IIR) methods which are very time-consuming.

3.4.2. Scattering target

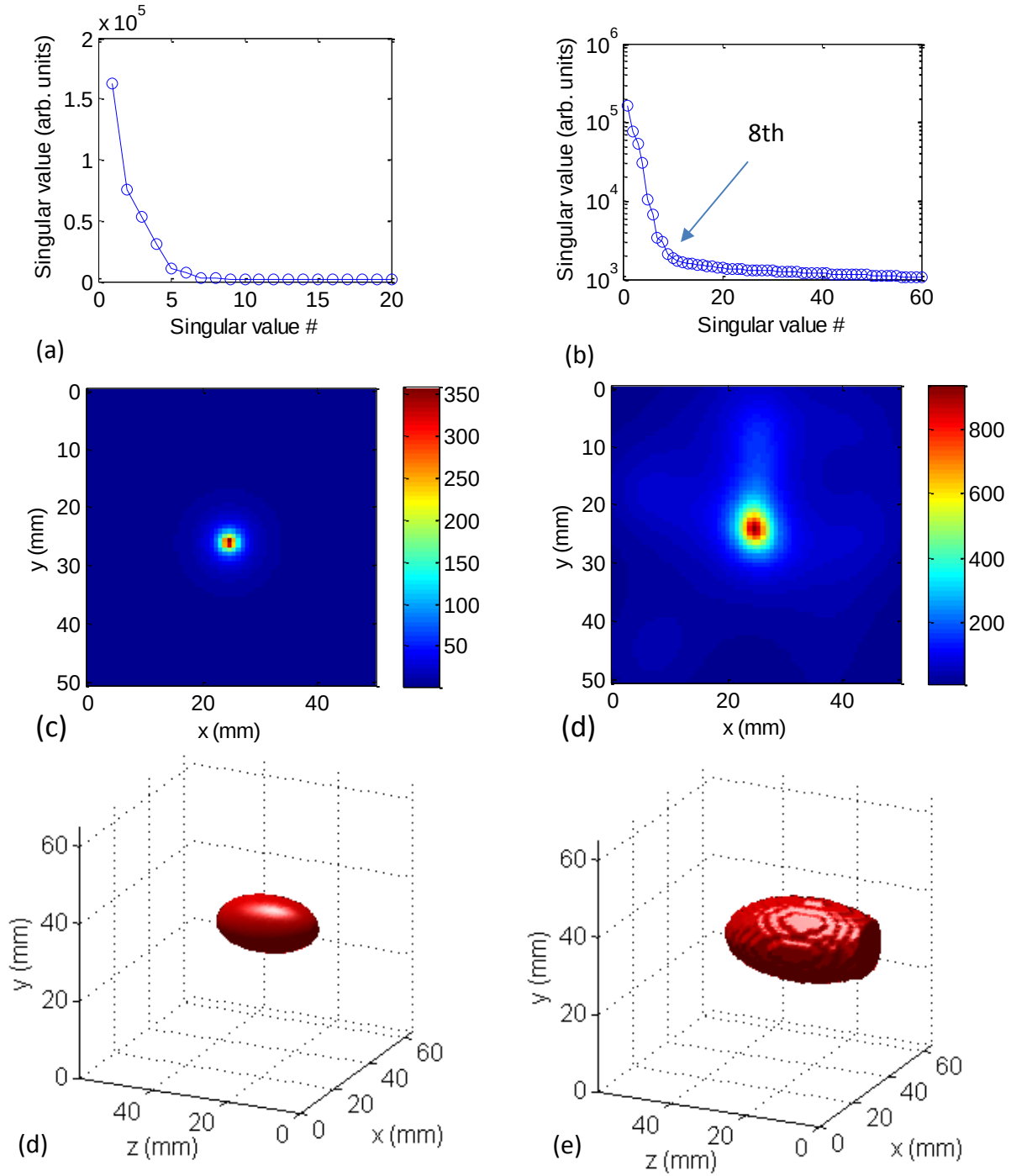


Fig. 14. (a) and (b) are singular value spectrum in linear and logarithmic scales respectively. (c) and (d) are pseudo images of the two targets generated using 1 eigen component and eigen components, respectively. (e) shows the isosurface generated using the FW M in the pseudo image (logarithmic scale). (f) is the isosurface generated using the “local contour” method.

The second experiment is the same as that presented in Section 2. . . of Chapter 2, where a scattering target was embedded in the mid-plane ($z = 0$ mm) of the scattering medium. The scattering coefficient of the target was 4 times of that of the background. All other experimental parameters are the same as in the experiments presented above. The sample was scanned by the 1 -mW -nm diode laser at grid points with mm step size. The images were acquired for TROT analysis.

The singular value spectrum of the TR matrix is shown in Figs. .14(a) (linear) and .14(b) (logarithmic).

The pseudo images generated using 1 and eigen components are shown in Figs. .14(c) and .14(d), respectively. When eigen components are used, the target image is more extended. The location of the target is accurately retrieved using the maximum in both images. However, the isosurfaces cannot be generated using either the FW M of the pseudo spectrum in logarithmic scale or the “local contour” approach. Simulations show the pseudo images for scattering targets have lower resolution and contrast than absorptive targets. Using the pseudo spectrum calculated with high order components included to estimate shape of scattering targets is more difficult. FW M of the pseudo spectrum in logarithmic scale significantly overestimated the target size. The “local contour” approach also lead to a wrong contour. So instead of including higher-order components, the isosurfaces are generated using the 1-eigen-component pseudo spectrum for both approaches, as shown in Figs. .14(e) and .14(f). The volumes are determined to be 2 21 mm and 1 mm using the two approaches, FW M of pseudo spectrum and “local contour”, respectively. The actual volume is 2 . mm . Both estimated volumes are much larger than the actual value. The optical scattering strength of the targets are also estimated using q . (.). When one eigen component is used, the optical strength is

estimated to be -1.5×10^{-4} mm /ns. When eigen components are used, the strength is estimated to be -1.5×10^{-4} mm /ns, and stays the same even if the original data matrix is used. The known value of the strength is -2.5×10^{-4} mm /ns. As expected, it is more difficult to retrieve the optical strength and size of a scattering target than an absorptive target.

3.5. Discussion

Time reversal optical tomography (TROT) was further developed to deal with finite size targets. The center position of the target(s) is determined rather accurately for both absorptive and scattering targets. Optical strengths of targets can be calculated with acceptable accuracy by unmixing the rank-reduced data matrix for small targets. For absorptive targets, it was retrieved with small error when two targets were involved with 4-cm separation, and deteriorated with less separation. However, for scattering targets, the error was large even when only one target was present. Simulation showed that the optical strength (absorption or scattering) can be retrieved for different target size, target location and noise level. However, the accuracy in the retrieved optical strength is lower for larger targets, due to the approximation of the target location being at the centroid. The retrieved location and optical strength of a target are not sensitive to the dimension of the signal subspace. The target location and optical strength are mainly determined by the prominent eigen components shown in the linear plot of the eigenvalue (singular value) spectrum. Adding more eigen components, such as the prominent ones in the semi-log plot of the eigenvalue (singular value) spectrum, may slightly improve the estimate in the locations and strengths of the targets. Further increasing the number the components does not continue to improve the results.

It is much more challenging to retrieve target size. The size of targets may be estimated by using the isosurface of the FW M in the pseudo spectrum or using an optimal contour in the

pseudo spectrum which can minimize the difference between the experimental data and calculated data using a proper forward model. Simulation shows there is no well-defined correlation between the noise level and the estimated optical strength and size of a target. Since both optical property and target shape are unknown in the forward-model, the data must be normalized before comparison without considering the absolute value of the magnitude. So only the shape of the light intensity distribution is considered. As a result, the diffuse light signal is not sensitive to target size. Due to the diffuse nature of light propagation in the medium, the exact shape of the target is not available in the contours of the pseudo spectrum. It is very difficult to find an accurate solution that matches the actual dimension of the target. In summary, both location and optical strengths of extended targets can be retrieved with confidence; while there is low confidence in the estimated target size. It may be improved if the target boundary is optimized using the orthogonality between the noise subspace and test target space generated using a level set method [10, 11].

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Chapter 4

Diffuse optical imaging using matrix decomposition methods

4.1. Introduction

Diffuse optical imaging (DOI) for detection and retrieval of location information of targets in a highly scattering turbid medium may be treated as a blind source separation (SS) problem [1, 2].

SS problem refers to the general class of problems involving retrieval of individual signals that constitute the measured mixed signal. Various matrix decomposition methods, such as, Independent Component Analysis (ICA) [3], Principal Component Analysis (PCA) [4] and Non-negative Matrix Factorization (NMF) [5], have been developed for solving the SS problem and retrieving desired information.

Min Xu *et al.* adapted ICA of information theory to develop Optical Tomography using Independent Component Analysis (OTICA) and demonstrated its application for diffuse imaging of absorptive, scattering and fluorescent targets [6-11]. ICA assumes the signals from different targets to be *independent* of each other, and optimizes a relevant measure of independence to obtain the ICs associated with different targets. The position co-ordinates of targets in three dimensions are determined from the individual components separately.

PCA assumes that the PCs contributing to the signal are *uncorrelated* and explain the most variance in the signal. PCA has been widely used in various applications, such as spectroscopy [12], face recognition [13] and neuroimaging [14]. NMF seeks to factorize a matrix into two non-negative matrices (component signals and weights) and requires the contributions to signal and the weights of the components to be *non-negative*. It does not imply any relationship between the components. NMF has also been widely used in biological analysis [15], and spectral analysis [16].

The objective of the study presented in this chapter is to test and compare the efficacy of these algorithms when used to solve the DOI problem. We present results of investigations on absorptive and scattering targets embedded in model scattering media using both simulative and experimental data.

The remainder of the chapter is organized as follows. In Section 4.2, the formalisms of the three methods are introduced. Section 4.3 evaluates the resulting imaging approaches using simulated data. The approaches are further examined in Section 4.4 for experimental data acquired using absorptive and scattering targets embedded in model scattering media. Section 4.5 discusses and summarizes the results.

4.2. Theoretical Formalism

4.2.1. Blind Source Separation problem

Blind source separation (BSS), also known as blind signal separation is a general problem in information theory that seeks to separate different individual signals from the measured signals, which are weighted mixtures of those individual signals. Assuming M individual signals, $s_m(t)$; $m = 1, \dots, M$, are linearly mixed instantaneously, the BSS problem is modeled as following. The dimension of $s_m(t)$ is N_s , the number of sampling times. In this study, t will be replaced by spatial positions of the excitation light sources. A total of N_d detectors sense N_d different mixtures of $s_m(t)$; $m = 1, \dots, M$. The mixture measured by the i^{th} detector can be represented as $x_i(t) = \sum_{m=1}^M a_{im} s_m(t)$, or $X = AS$, in a matrix notation, where $A \in R^{N_d \times M}$ is a mixing or weighting matrix, $S \in R^{M \times N_s}$, $X \in R^{N_d \times N_s}$, and $M \leq \min(N_s, N_d)$. The objective of BSS is to retrieve the signals $s_m(t)$ and their weights, a_{im} . ICA, PCA and NMF are statistical analysis methods, used to solve the BSS problem.

4.2.2. Diffuse optical imaging problem for absorptive and scattering targets

In DOI one measures the signal at the sample boundary, which includes a weighted mixture of contributions from embedded targets. One uses the diffusion approximation [1-1] of the radiative transfer equation [2, 21] as the forward model to describe light propagation in a highly scattering turbid medium. The perturbation in the light intensity distribution measured on the boundary of the sample due to the presence of the targets (which are considered to be localized inhomogeneities in the optical properties within the sample volume) may be written, in the first order Born approximation, as [22, 2]

$$\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = -\int G(\mathbf{r}_d, \mathbf{r})\delta\mu_a(\mathbf{r})c_m G(\mathbf{r}, \mathbf{r}_s)d\mathbf{r} - \int \delta D(\mathbf{r})c_m \nabla_r G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_r G(\mathbf{r}, \mathbf{r}_s)d\mathbf{r}, \quad (4.1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagation from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ and δD are the differences in absorption coefficient and diffusion coefficient between the targets and the background medium, respectively; and c_m is the light speed in the medium.

A multi-source illumination and multi-detector signal acquisition scheme is used to acquire light transmitted through a scattering medium. For small absorptive targets, a perturbation data matrix is constructed using $-\Delta\phi$ for all sources. The elements of the data matrix pertaining to absorptive targets represented by the first term in eq. (4.1) may be written in a discrete form as:

$$X_{ij} = \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{r}_m)\tau_m G^s(\mathbf{r}_m, \mathbf{r}_j), \quad (i = 1, 2, \dots, N_d; j = 1, 2, \dots, N_s), \quad (4.2)$$

where $\mathbf{r}_i$, $\mathbf{r}_j$ and $\mathbf{r}_m$ are the locations of the i^{th} detector, j^{th} source and m^{th} target, respectively; N_s , N_d and M are the numbers of sources, detectors and targets, respectively; $\tau_m = \delta\mu_a(\mathbf{r}_m)c_m\delta V_m$ is the optical absorption strength of the m^{th} target, δV_m is the volume of the target; $G^s(\mathbf{r}_m, \mathbf{r}_j)$ and $G^d(\mathbf{r}_i, \mathbf{r}_m)$

$\mathbf{r}_m$) are the Green's functions that describe light propagation from j^{th} source to m^{th} target and from m^{th} target to i^{th} detector, respectively. The number of targets is assumed to be less than that of sources and detectors, $M \leq \min(N_d, N_s)$.

The m^{th} target may be considered to be a virtual source of strength $\tau_m G^s(\mathbf{r}_m, \mathbf{r}_j)$ excited by the real light source located at $\mathbf{r}_j$. The data matrix $X = [X_{ij}]$, may be considered to be a set of combinations of light signals from all virtual sources mixed by a mixing matrix $G^d(\mathbf{r}_i, \mathbf{r}_m)$. Therefore, this problem can be treated as a SS problem.

As the second term in eq. (4.1) suggests, each scattering target is represented by three co-located virtual sources of strength: $\tau_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j)$, where $\partial_p = \partial/\partial p$, $(p = x, y, z)$, and $\tau_m = \delta D(\mathbf{r}_m) c_m / \delta V_m$, is the *optical scattering strength* of the m^{th} target. The mixing matrices become $\partial_p G^d(\mathbf{r}_i, \mathbf{r}_m)$, $(p = x, y, z)$, for the three virtual sources generated by the m^{th} target. The elements of the data matrix for scattering targets may be written as

$$X_{ij} = \sum_{m=1}^M \sum_{p=x,y,z} \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m) \tau_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j). \quad (4.)$$

Since one absorptive target is represented by one centrosymmetric virtual source, while three virtual sources (one centrosymmetric and two dumb-bell shaped) represent one scattering target, the number and patterns of virtual sources may be used, in favorable situations, to identify the target as absorptive or scattering in nature. In this chapter, only small targets are considered since all three algorithms are suited for small targets, and early detection, when the tumors are more amenable to treatment.

4.2.3. DOI as a BSS problem

The data matrix for the DOI problem may be written as

$$X = AS = \sum_{m=1}^M A_{im} S_{mj}, \quad (4.4)$$

where $A \in R^{N_d \times M}$, $S \in R^{M \times N_s}$, and $X \in R^{N_d \times N_s}$. For *absorptive* targets,

$$A_{im} = \beta_m G^d(\mathbf{r}_i, \mathbf{r}_m), \text{ and } S_{mj} = \alpha_m G^s(\mathbf{r}_m, \mathbf{r}_j), \quad (4. a)$$

while for *scattering* targets,

$$A_{im} = \beta_m \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m), \text{ and } S_{mj} = \alpha_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j). \quad (4. b)$$

S_{mj} ($j = 1, 2, \dots, N_s$) and A_{im} ($i = 1, 2, \dots, N_d$) are two-dimensional intensity distributions on the source and detector planes, respectively. Source and detector planes are the boundaries of the sample through which light enters and exits the sample volume, respectively. The scaling factors β_m and α_m are related to the target optical strength, $\tau_m = \alpha_m \beta_m$. The location of the target and the scaling factors can be retrieved using a least squares fitting via

$$\arg \min_{\alpha_m, \beta_m, \mathbf{r}_m} \left\{ \sum_j \left[\alpha_m^{-1} S_{mj} - G^s(\mathbf{r}_m, \mathbf{r}_j) \right]^2 + \sum_i \left[\beta_m^{-1} A_{im} - G^d(\mathbf{r}_i, \mathbf{r}_m) \right]^2 \right\}, \quad (4. a)$$

or,

$$\arg \min_{\alpha_m, \beta_m, \mathbf{r}_m} \left\{ \sum_p \left\{ \sum_j \left[\alpha_m^{-1} S_{mj} - \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j) \right]^2 + \sum_i \left[\beta_m^{-1} A_{im} - \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m) \right]^2 \right\} \right\}, \quad (4. b)$$

for absorptive and scattering targets, respectively. However, when a scattering target is embedded deep in a turbid medium, only the $\tau_m \partial_z G^s(\mathbf{r}_m, \mathbf{r}_j)$ virtual source remains significant. So only $p = z$ may be used for fitting in Eq. (4. b).

4.2.4. Decomposition Methods

We now provide a brief overview of three matrix decomposition methods that are commonly used in SS problems.

4.2.4.1. Independent Component Analysis (ICA)

O TICA assume that the virtual sources are *independent* of each other . So they can be retrieved through an iterative process which seeks to maximize the independence among the components. In practice, the independent components are found by maximizing some measure of independence, such as kurtosis (the fourth-order cumulant), of the unmixed components. A Matlab program for ICA was adopted from <http://sccn.ucsd.edu/eeglab/>. The location of the target can be retrieved by fitting the independent component intensity distributions (ICIDs) to reen's functions or derivatives of reen's functions using q. (4. a) and q. (4. b).

4.2.4.2. Principal Component Analysis (PCA)

CA assumes that the virtual sources are uncorrelated so that the correlation (covariance) between them is ideally zero, and minimal in practice. The covariance matrix of S , $cov(S)$ should be diagonal. The general process of CA is as follows. The data matrix $X = AS + N$, where N is random noise added to the data, and A and S are the same as defined in q. (4.4). When S is mean centered, elements of the mean-centered matrix S' are defined as

$$S'_{mj} = S_{mj} - \frac{1}{N_s} \sum_{j=1}^{N_s} S_{mj} . \quad (4. a)$$

Similarly

$$X'_{ij} = X_{ij} - \frac{1}{N_s} \sum_{j=1}^{N_s} X_{ij} . \quad (4. b)$$

CA looks for a matrix P that decomposes X into virtual sources, $S = PX$. It also holds that $S' = PX'$, since P is just a rotation matrix which does not change the center of the data.

$$cov(S) = S'S'^T = (PX')(PX')^T = PX'X'^T P^T = A , \quad (4.)$$

where $\Lambda = \text{diag}(\lambda_1, \lambda_2, \dots)$. The eigenvalues λ_m are variances in the covariance matrix. Therefore, $X'X'^TP^T = P^T\Lambda$, where P^T is orthonormal. CA is realized by eigenvalue decomposition (EVD) of the covariance matrix of X . The eigenvectors with leading eigenvalues (largest variances) are selected to be the Cs using the L -curve [24].

Since, $X = P^TS = AS$, A is determined as a matrix including only Cs. S is calculated as $S \approx (A^T A)^{-1} A^T X$. Rows of S and columns of A represent principal component intensity distributions (PCIDs) on the source plane and detector plane, respectively, and are proportional to the images of the virtual sources projected on the source and detector planes. The target positions are determined using eq. (4.1).

4.2.4.3. Nonnegative Matrix Factorization (NMF)

NMF is a group of multivariate analysis algorithms that factorize a matrix X into A and S : $X = AS$, A and S are non-negative. Unlike ICA and CA, NMF does not imply any relationship between the retrieved components; instead, it just enforces non-negativity constraints on A and S . There are various algorithms developed to solve NMF, such as the multiplicative update method [25] and alternating least squares method [26, 27].

In the multiplicative update implementation of NMF, A and S can be found by minimizing the square of Euclidean distance $\|X - AS\|^2$ as the cost function, where A and S are non-negative, using the multiplicative update rule

$$A_{ik} \leftarrow A_{ik} \frac{(XS^T)_{ik}}{(ASS^T)_{ik}}, \quad (4.1a)$$

$$S_{kj} \leftarrow S_{kj} \frac{(A^T X)_{kj}}{(A^T AS)_{kj}}. \quad (4.1b)$$

The alternating least squares (A-S) implementation of NMF uses alternate least squares steps to estimate A (or S), and use that estimate to optimize S (or A), and keep repeating the alternative steps until the desired optimization is obtained. Non-negativity is ensured by setting any negative element of A or S equal to 0.

An NMF toolbox was obtained [2] to carry out NMF computation. A built-in command *nnmf* is also available in Matlab (R2011a).

NMF algorithm requires that the non-negativity assumption must hold in the problem. In particular, for absorptive targets, when X is constructed with $-\Delta\phi$, τ_m should be positive, i.e. the targets should be more absorbing than the background. If the targets have weaker attenuation properties than the background, X needs to be constructed with $+\Delta\phi$ instead. For scattering targets, X should be treated similarly to keep its elements positive.

When NMF is applied to a scattering target, only the centrosymmetric component shows up properly, since the other two components have dumb-bell shape which includes negative values

. So without any prior knowledge or some other experimental means to assess if the target is absorptive or scattering, NMF may not distinguish between the two possibilities.

The decomposition methods can be applied with different sample geometries such as slab and cylindrical geometries, and different measurement domains such as time-resolved domain, frequency domain and continuous wave (CW). In this chapter, Green's functions for slab geometry [2] with CW measurement were used for simulation and experiments.

4.2.5. Backprojection

With the location of target(s) retrieved, the size of the target(s) may be estimated using a back-projection Fourier transform approach [11]. A back projection of $X_j(\mathbf{r}_d, \mathbf{r}_s)$ from the detection plane onto the “target plane” ($z = z_j$ plane) provides an estimate of the target dimension in the lateral

directions. However, the dimension in axial direction is collapsed with target property integrated. The perturbation signal in the light intensity distribution on the detector plane for CW illumination can be approximated by

$$X_j(\mathbf{r}_d, \mathbf{r}_s) = \int_{z=z_j} G^d(\boldsymbol{\rho}_d - \boldsymbol{\rho}) \chi_j(\boldsymbol{\rho}) G^s(\boldsymbol{\rho} - \boldsymbol{\rho}_s) d\boldsymbol{\rho}, \quad (4.1 \text{ a})$$

due to the j^{th} absorptive target, or

$$X_j(\mathbf{r}_d, \mathbf{r}_s) = \int_{z=z_j} \partial_z G^d(\boldsymbol{\rho}_d - \boldsymbol{\rho}) \chi_j(\boldsymbol{\rho}) \partial_z G^s(\boldsymbol{\rho} - \boldsymbol{\rho}_s) d\boldsymbol{\rho}, \quad (4.1 \text{ b})$$

which is the ($p = z$) component due to the j^{th} scattering target, where $\boldsymbol{\rho}_s$ and $\boldsymbol{\rho}_d$ are the lateral coordinates of the source and the detector, and the integration is over the $z = z_j$ plane. In the Fourier space $\chi_j(\mathbf{q})$ follows from eq. (4.1) as,

$$\chi_j(\mathbf{q}) = \frac{X_j(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s)}{G^d(\mathbf{q} - \mathbf{q}_s) G^s(\mathbf{q}_s)}, \quad (4.11a)$$

for the absorptive target, and

$$\chi_j(\mathbf{q}) = \frac{X_j(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s)}{\partial_z G^d(\mathbf{q} - \mathbf{q}_s) \partial_z G^s(\mathbf{q}_s)}, \quad (4.11b)$$

for the scattering target, where $\mathbf{q}$ and $\mathbf{q}_s$ are the spatial frequencies on the x-y plane. The inverse Fourier transform of $\chi_j(\mathbf{q})$ provides the cross-section image of the j^{th} target at the $z = z_j$ plane.

The calculation of $\chi_j(\mathbf{q})$ using eq. (4.11) employed Tikhonov regularization [22], with a modified L -curve method [24] to determine the optimal regularization parameter. This optimization is a tradeoff between obtaining a closer estimate of target cross section and fewer artifacts in the back-projection image. Since the positions of the targets were obtained from the previous steps, any artifacts cropping up in the back-projection process could be readily identified from their positions. So, instead of using the “corner” of the L -curve to find the optimal

regularization parameter, we settled for a lower regularization using the criterion that the highest artifact peak reaches 10% of the target peak to improve the size estimate of the cross-section images. The full width at half maximum (FWHM) of the spatial profile of the cross-section image was used as an estimate of the target size.

4.3. Simulative Study of Decomposition Methods

First we test the efficacy of the three matrix decomposition methods using simulated data.

4.3.1. One absorptive target and one scattering target with ~ 4-cm separation

The sample was considered to be a 4-cm thick uniform scattering slab with lateral dimension of 20 mm × 20 mm, as shown in Fig. 4.1. Its absorption and diffusion coefficients were taken to be $\mu_a = 0.1 \text{ mm}^{-1}$ and $D = 1/3 \text{ mm}$ (transport mean free path, $l_t = 1 \text{ mm}$), respectively, which are similar to the average value of those parameters for female human breast tissue. The index of refraction n of the medium was taken to be 1.5. The speed of light is $2.998 \times 10^8 \text{ m/s}$, or $2.998 \times 10^{11} \text{ mm/ns}$ in vacuum, and $2.2 \times 10^{11} \text{ mm/ns}$ in the medium. An absorptive and a scattering point targets were placed at $(0, 0, 1) \text{ mm}$ and $(0, 0, 2) \text{ mm}$, respectively. The absorption coefficient of the absorptive target was set to be higher than the background by $\delta\mu_a = \delta(\mathbf{r}, \mathbf{r}') \text{ mm}^{-1}$, where $\delta(\mathbf{r})$ is the Dirac delta function, while the diffusion coefficient was taken to be the same as that of background. The diffusion coefficient of the scattering target was set to be lower than the background (higher scattering coefficient) with $\delta D = -0.1 \delta(\mathbf{r}, \mathbf{r}') \text{ mm}$ ($l_t = 1.1 \text{ mm}$), while the absorption coefficient was taken to be the same as the background. The optical strengths of the absorptive and scattering targets were $\tau = \int \delta\mu_a c_m d\mathbf{r} = 1.2 \text{ mm/ns}$, and $\tau = \int \delta D c \delta\mathbf{r} = 1.2 \text{ mm/ns}$, respectively. The incident CW beam step scanned the sample at 21×21 grid points covering an 8 mm^2 area, with a step size of 4 mm. Light on the opposite side was recorded

at 41×41 grid points covering the same area. The simulated data matrix X was generated using eq. (4.2). Additive gaussian noise of σ was added to X , i.e. $X = X_0 + N$, where N is gaussian noise with mean value 0, and standard deviation σ of average of X_0 . Then the data matrix X was analyzed using the three different algorithms.

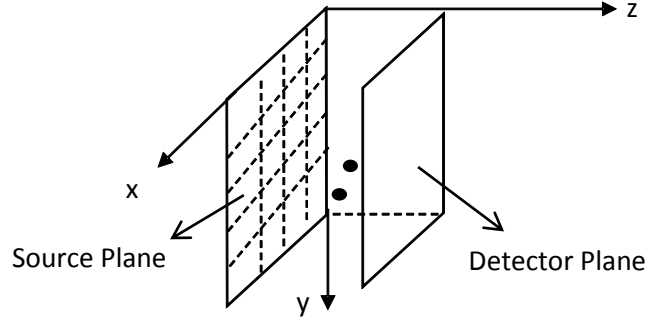


Fig. 4.1. Light intensity distribution on the detector plane is recorded when a point source scans on the source plane.

4.3.1.1. ICA Analysis

One independent component for the absorptive target and three independent components for the scattering target were retrieved by ICA. The independent component intensity distributions (ICIDs) on the detector plane are shown in Figs. 4.2(a), 4.2(c), 4.2(d), and 4.2(e). Similar ICIDs were obtained on the source plane. Fig. 4.2(g) shows the centrosymmetric ICID for the scattering target, and Fig. 4.2(i) shows the ICID for the absorbing target. Since there are more detectors than sources, the components on the detector plane have higher resolution.

The components in either the detector plane or the source plane can, in principle, be used to extract position and optical strength of the target(s). However, in our experimental arrangement signal is collected by a 124×124 pixels CCD camera, while the source plane is scanned in an x - y array of points, which is much smaller than the number of pixels in the CCD camera. Consequently, the resolution in the detector plane is much better, and the data set more robust than the source side. So, we used the images on the detector plane for retrieving target information

using experimental data. While it would not matter in simulation, to be consistent with experimental situations, we employed detector plane images when using simulated data as well for all three algorithms.

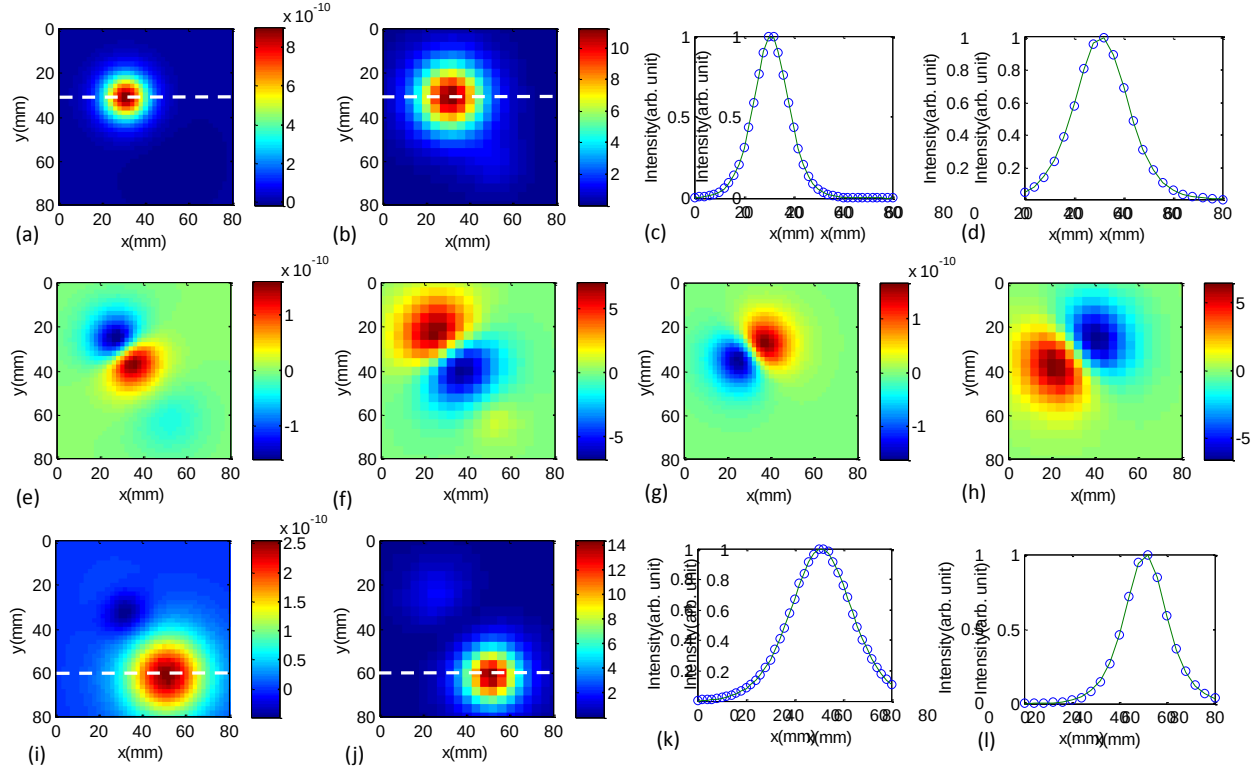


Fig. 4.2. ICA-based imaging of one absorbing and one scattering target 4-cm apart: ICA extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component, (e) and (g) dumb-bell shaped components of the scattering target; and (i) the absorptive target. Similar intensity distribution on the source plane of: (b) the centrosymmetric, (f) and (h) dumb-bell shaped components of the scattering target, and (j) the absorptive target for comparison. Fits to the spatial intensity profiles on the detector plane along the white dashed line (shown in figures) of the centrosymmetric components of the scattering target are shown in (c), and that of the absorptive target is shown in (k). Corresponding fits to spatial profiles on the source plane are displayed in (d) and (l), respectively.

Table 4.1 lists the locations and strengths of the absorptive and scattering targets retrieved by fitting the spatial intensity profile of the centrosymmetric components on the detector plane to Reen's functions and derivatives of Reen's functions using eq. 4. (a) and eq. 4. (b),

respectively, as shown in Fig. 4.2(c) and Fig. 4.2(k). Fig. 4.2(d) and Fig. 4.2(l) show the corresponding fits to the profiles on the source plane.

4.3.1.2. PCA Analysis

igenvale equation of the covariance matrix of X was solved. The eigenvalues found by CA were sorted in descending order. Fig. 4. shows a plot of leading 2 eigenvalues on a logarithmic scale.

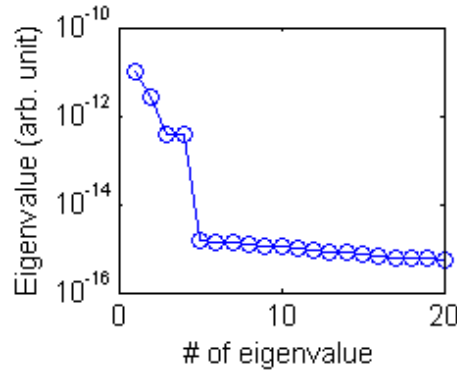


Fig.4. . A logarithmic plot of the first 2 CA eigenvalues

First four leading eigenvalues were selected as Cs. The corresponding principal component intensity distributions (CIDs) were calculated. The CIDs on the detector plane are shown in Fig. 4.4. Similar images for CIDs on the source plane were obtained. The scattering target has one centrosymmetric (Figs. 4.4(a) and 4.4(b)) component and two dumb-bell shaped (Figs. 4.4(e) 4.4(h)) components, while the absorptive target has only one component (Figs. 4(i) and 4(j)).

Figs. 4.4(c) and 4.4(k) show fits to the spatial intensity profile of the centrosymmetric component of the scattering target and that of the absorptive target on the detector plane, respectively. Figs. 4.4(d) and 4.4(l) show fits to the corresponding spatial intensity profiles on the source planes. The locations and optical strengths of the targets retrieved from the fit are also shown in Table 4.1.

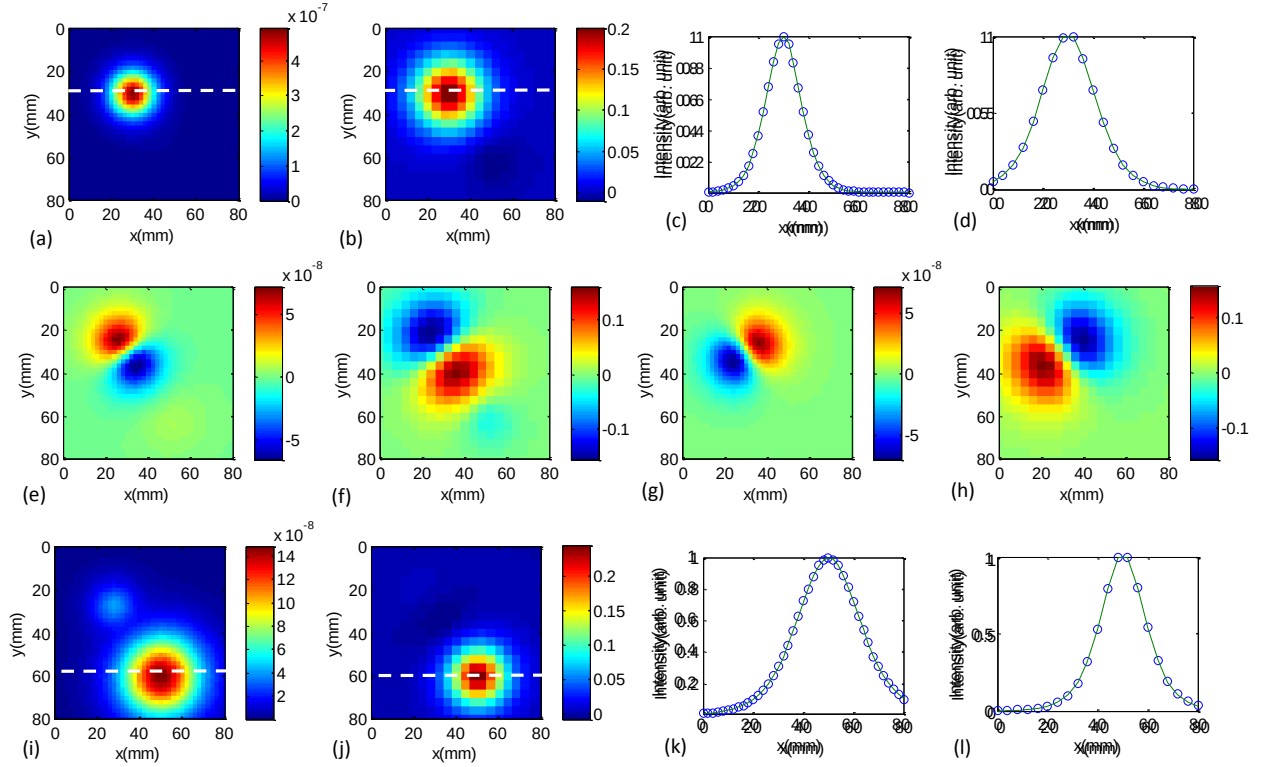


Fig. 4.4. CA-based imaging of one absorbing and one scattering target 4-cm apart: CA-extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component, (e) and (g) dumb-bell shaped components of the scattering target; and (i) the absorptive target. Similar intensity distribution on the source plane of: (b) the centrosymmetric, (f) and (h) dumb-bell shaped components of the scattering target, and (j) the absorptive target for comparison. Fits to the spatial intensity profiles on the detector plane along the white dashed line (shown in figures) of the centrosymmetric components of the scattering target are shown in (c), and that of the absorptive target is shown in (k). Corresponding fits to spatial profiles on the source plane are displayed in (d) and (l), respectively.

4.3.1.3. NMF Analysis

The mixing matrix and virtual sources were retrieved from the data matrix X using NMF as explained in Section 4.2. . . As in the other two approaches, only one component is extracted for the absorptive target. Since NMF has a non-negativity constraint, only the centrosymmetric component for the scattering target is obtained. Non-negative component intensity distributions (NCIDs) on detector planes are shown in Figs. 4. (a) and 4. (e). Similar images for NCIDs on

source plane were also obtained using the virtual sources in S and shown in Figs. 4. (b) and 4. (f). The results are also shown and compared with those obtained using the other two methods in Table 4.1.

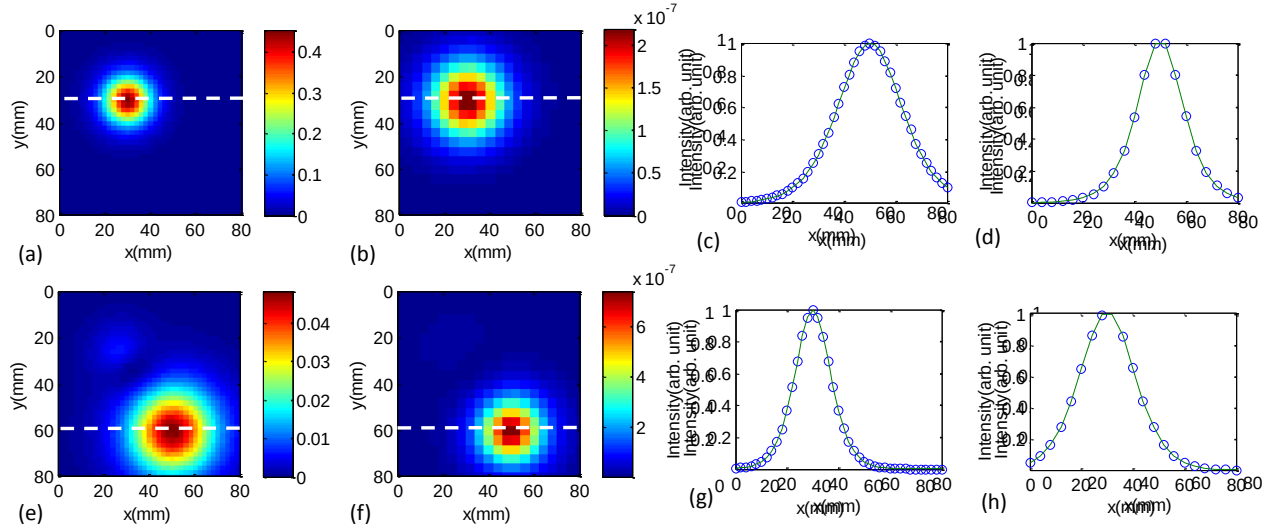


Fig. 4. . NMF-based imaging of one absorbing and one scattering target 4-cm apart: NMF-extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component of the scattering target; (e) the absorptive target; and the corresponding distribution on the source plane of: (b) the centrosymmetric component of the scattering target; (f) the absorptive target. Fits to the corresponding spatial intensity profiles along the dashed line (shown in figures) are shown in (c), (d), (g) and (h), respectively.

4.3.1.4. Results and discussions

The positions and optical strengths of the targets retrieved by ICA, CA and NMF algorithms are shown in Table 4.1, and compared to the known values. The retrieved results using all three algorithms from this simulated data are in excellent agreement with the known values. This simulation showed that all three methods could be used to accurately locate point-like targets under the conditions set in the example. CA and ICA can detect the dumb-bell-shaped components, which could, in principle be used to characterize targets as absorptive or scattering in nature. NMF cannot detect the dumb-bell shape component due to the nonnegativity constraint. However, in

realistic experimental condition, the dumb-bell-shaped components of scattering targets may not be detectable, particularly when the targets are embedded deep within the turbid medium. The above simulation did not show much difference between these decomposition methods in the efficacy of separating multiple targets and accuracy in target localization.

Table 4.1. Positions and optical strengths retrieved using ICA, CA and NMF algorithms

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)	Known strength	Fitted strength	Error ()
Sca.	(0, 0, 2)	ICA	(0.0, 0.0, 2.0)	(0, 0, 0)	-1.0 2	-1.0	0
		CA	(0.0, 0.0, 2.0)	(0, 0, 0)	-1.0 2	-1.01	0
		NMF	(0.0, 0.0, 2.0)	(0, 0, 0)	-1.0 2	-1.02	0.2
Abs.	(0, 0, 1)	ICA	(0.1, 0.2, 14.0)	(0.1, 0.2, 0.2)	1.0 2	1.024	1.2
		CA	(0.0, 0.0, 1.0)	(0, 0, 0)	1.0 2	1.0	0.1
		NMF	(0.0, 0.1, 1.0)	(0, 0.1, 0)	1.0 2	1.01	0

The unit for absorption strength of the target is mm /ns and for scattering strength is mm /ns.

4.3.2. One absorptive target and one scattering target with 2-cm separation

In order to further compare the three methods and evaluate the efficacy of decomposition for different targets, another two simulations were used with smaller separation between the two targets. In one simulation, the same two point-like targets as used in the first simulation were 2 mm apart, the absorptive target on the left and the scattering target on the right. All other parameters are the same as used in the above first simulation. The two point targets with optical strengths 1.0 2mm /ns and 1.0 2mm /ns were located at (0, 4, 2) mm and (2, 4, 2) mm, respectively. Additive Gaussian noise was added to the data. The ICIDs, CIDs and NCIDs are

shown in Fig. 4. .

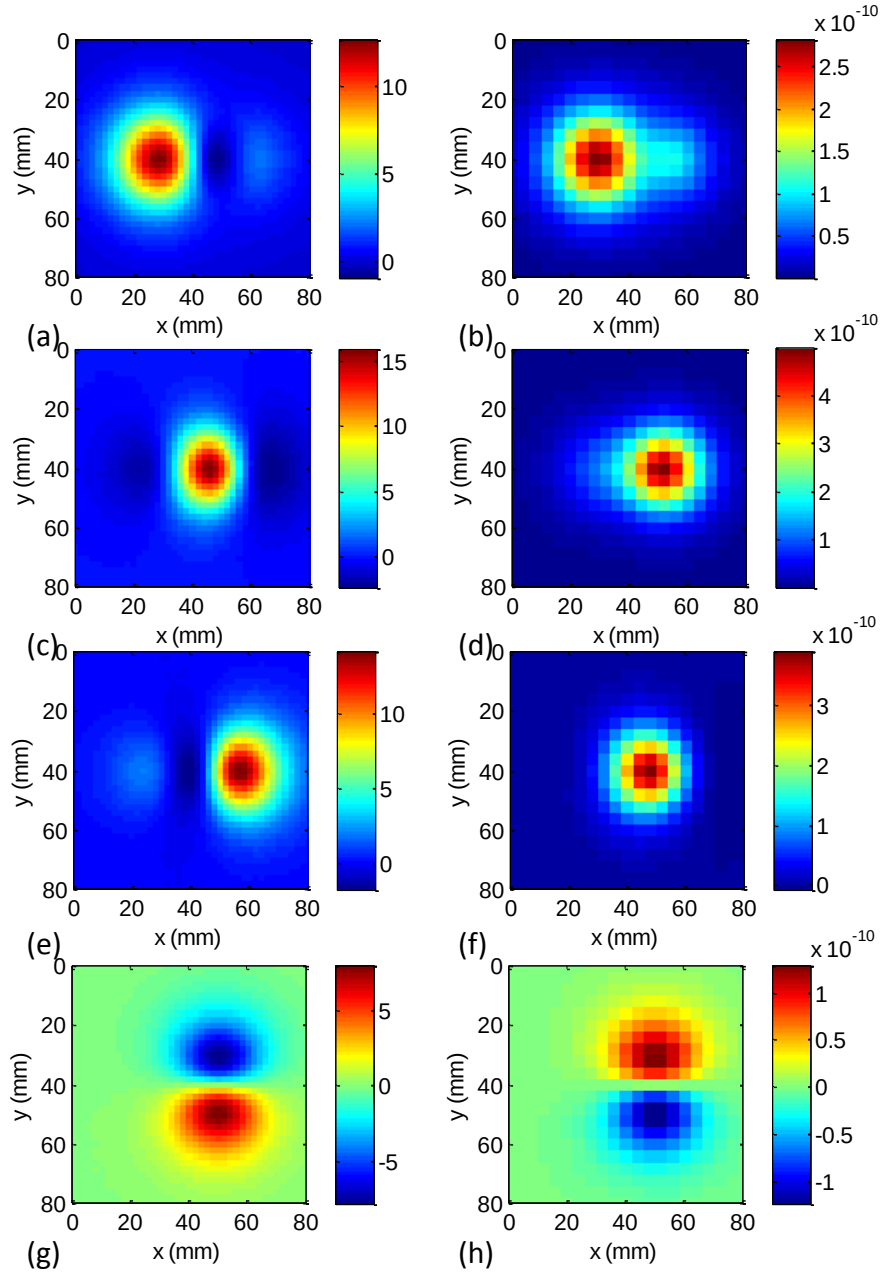


Fig. 4. . ICA-based imaging of one absorbing and one scattering target 2-cm apart: ICIDs on the detector plane of: (a) the absorptive target; (c) the centrosymmetric component; (e) and (g) dumb-bell shaped components of the scattering target; and the corresponding ICIDs on the source plane of: (b) the absorptive target; (d) the centrosymmetric component; (f) and (h) dumb-bell shaped components of the scattering target.

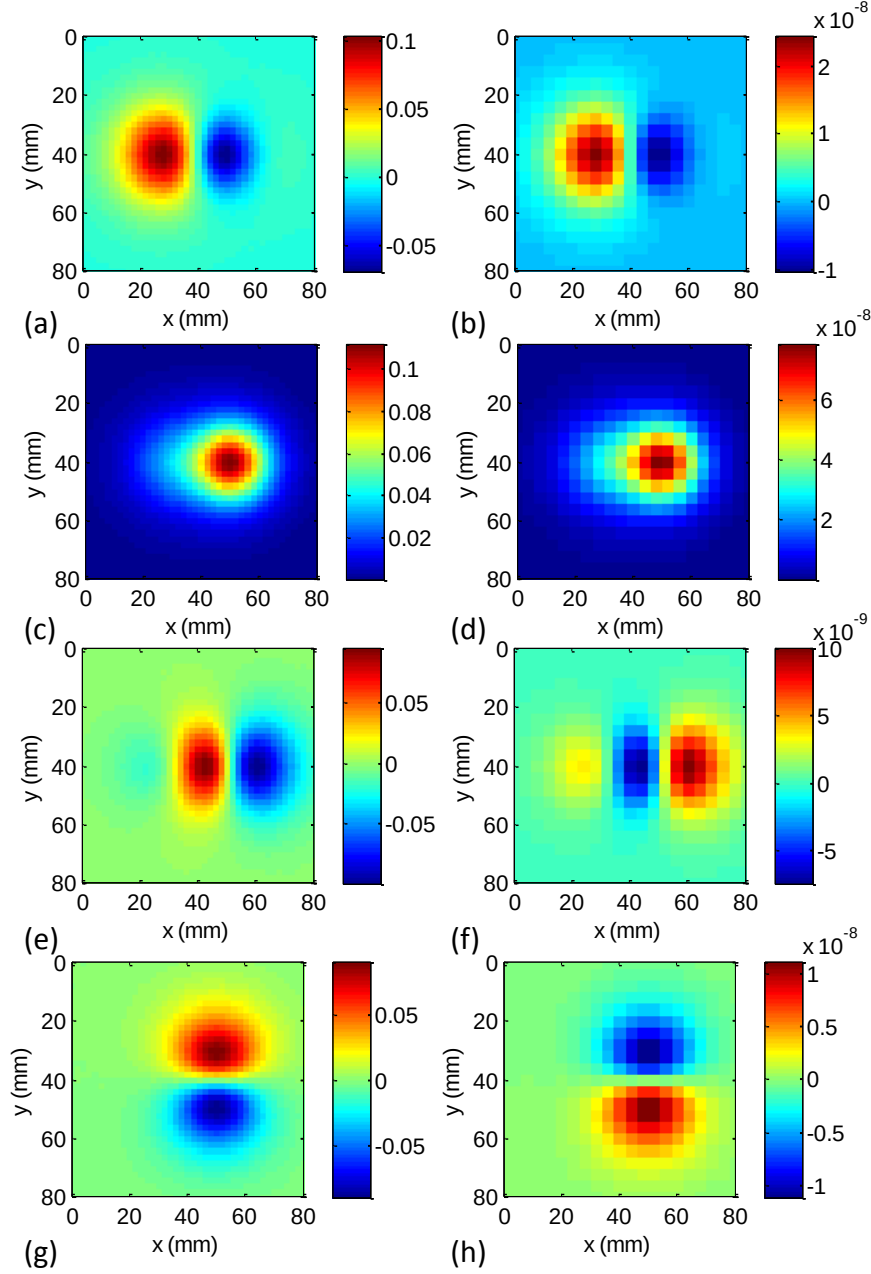


Fig. 4. . CA-based imaging of one absorbing and one scattering target 2-cm apart: and CIDs on the detector plane of: (a) the absorptive target; (c) the centrosymmetric component; (e) and (g) dumb-bell shaped components of the scattering target; and the corresponding CIDs on the source plane of: (b) the absorptive target; (d) the centrosymmetric component; (f) and (h) dumb-bell shaped components of the scattering target.

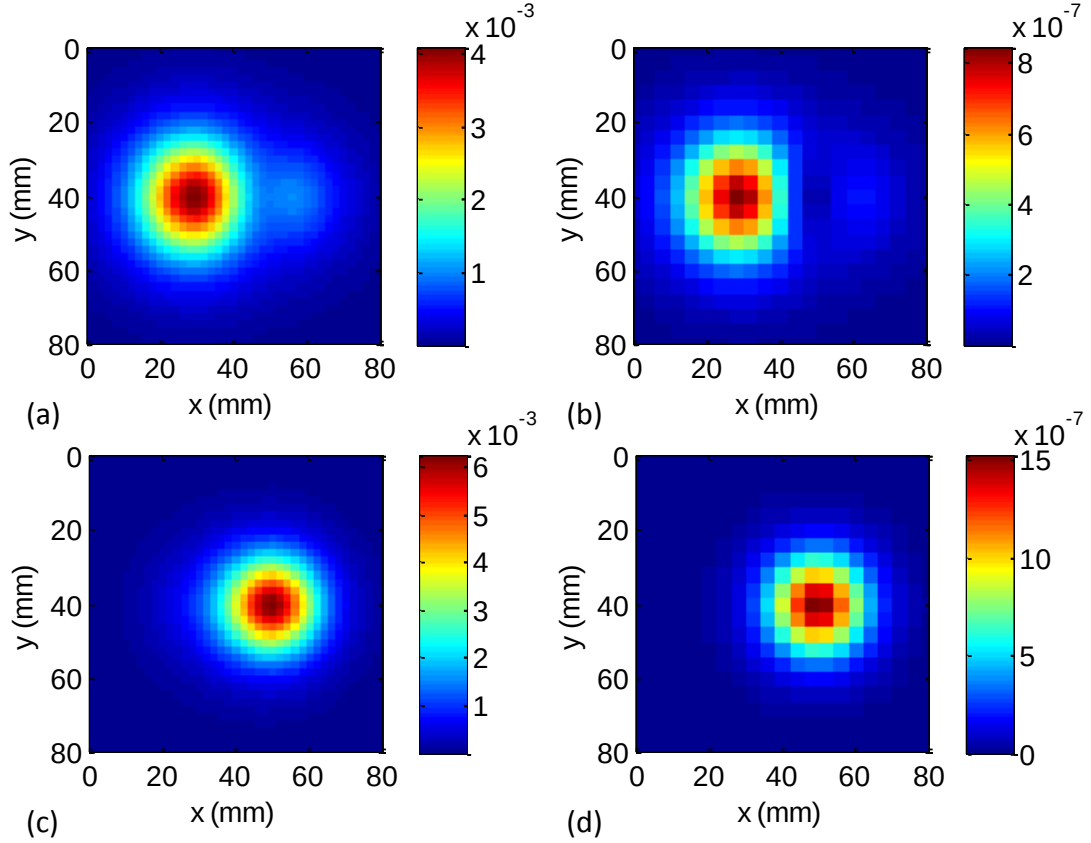


Fig. 4. . NMF-based imaging of one absorbing and one scattering target 2-cm apart: NCIDs on the detector plane of: (a) the absorptive target; (c) the centrosymmetric component of the scattering target; and the corresponding NCIDs on the source plane of: (b) the absorptive target; (d) the centrosymmetric component.

Fig. 4. shows the four ICIDs corresponding to targets. The first ICID corresponds to the left absorptive target, which has a little residue due to the right scattering target. The other three ICIDs correspond to the right scattering target. Due to the cross-talk between the two targets, the components for the scattering target are also affected due to cross-talk, and the centrosymmetric component is identified by comparing the center position in all components.

Fig. 4. shows the CIDs corresponding to the targets. The first CID corresponds to the absorptive target. Compared to the ICID, this CID has more residue due to the scattering target. The other three CIDs correspond to the scattering target, with the centrosymmetric component

more clearly recognized than that in ICIDs.

Fig. 4. shows the two NCIDs corresponding to the two targets, respectively. Even though there is also a little cross-talk between the targets, the retrieved components are much “cleaner” than those obtained by the other two methods. Similar to the previous simulated case, only one component was detected for each of the absorptive and scattering targets. No dumb-bell-shaped components were detected for the scattering target.

Table 4.2. Positions and optical strengths retrieved using simulated data and ICA, CA and NMF algorithms for one absorptive and one scattering point targets

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)	Known Strength	Fitted strength	Error ()
Abs.	(, 4 , 2)	ICA	(2 . , 4 . , 2 .4)	1. , , .4	1. 2	1.44	2 .1
		CA	(24. , 4 . , 2 .)	.1, , .	1. 2	1.4	22.4
		NMF	(2 . , 4 . , 1 .1)	1. , , .	1. 2	1.4	1 .
Sca.	(, 4 , 2)	ICA	(4 .4, 4 . , 2 .)	2. , , .	-1 . 2	-14 .	1 .
		CA	(4 .4, 4 . , 2 .2)	1. , , .2	-1 . 2	-1 .	12.4
		NMF	(4 .4, 4 . , 1 .)	. , , .2	-1 . 2	-1 2.	1.2

The unit for absorption strength of the target is mm /ns and for scattering strength is mm /ns.

All the centrosymmetric components were used to fit the positions and strengths of targets. The two dumb-bell-shaped components were used to help identify the scattering target. The retrieved positions and optical strengths of the targets are shown in Table 4.2. As expected, in these methods, NMF-retrieved values are more accurate than those retrieved using ICA and CA, since the components were better resolved using NMF. But the difference between CA and ICA is not

significant in this particular case.

4.3.3. Two absorptive targets with 2-cm separation

A similar simulation is tested using two absorptive point targets with other parameters the same as used in the above simulations. The two absorptive targets with both optical strengths $1.2 \text{ mm}^{-1}/\text{ns}$, were located at $(20, 40, 2)$ mm and $(60, 40, 2)$ mm, respectively. The two ICIDs, CIDs and NCIDs corresponding to the two targets are shown in Figs. 4. 4.11, respectively.

It is shown in Figs. 4. 4.11 that CA and ICA could not separate the two targets, while NMF clearly separated them. The positions and absorption strengths of the two targets were fitted for NCIDs only. The retrieved positions and absorption strengths of the two targets are shown in Table 4. . The lateral positions of the two targets were perfectly retrieved, while the axial positions have only 0.1 mm error. The strengths of the two targets are retrieved within 1% uncertainty.

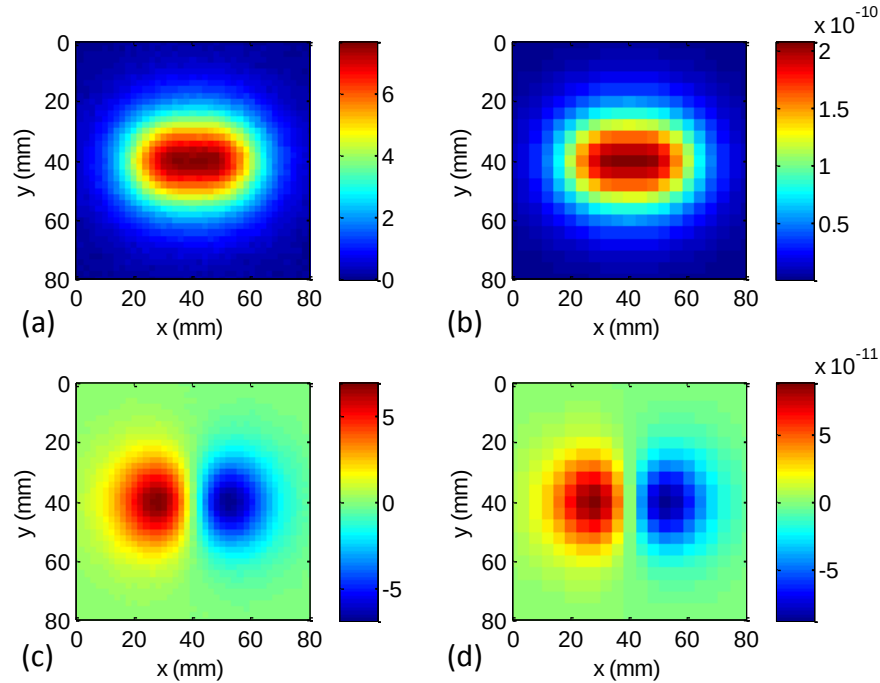


Fig. 4. . ICA-based imaging of two absorbing target 2-cm apart: (a) and (c) are the two ICIDs on the detector plane for the two targets. (b) and (d) are the two corresponding ICIDs on the source plane for the two targets.

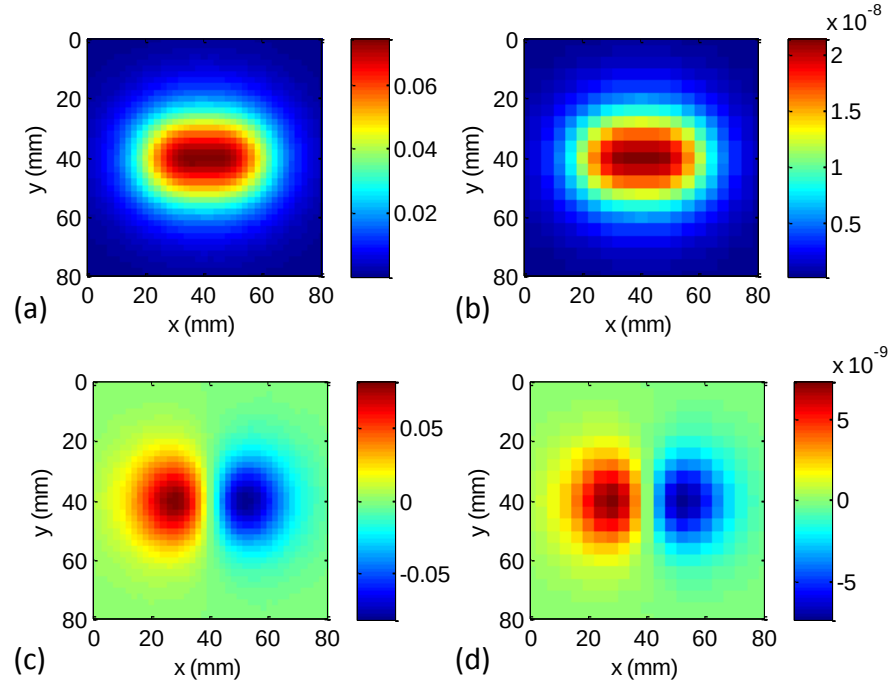


Fig. 4.1 . CA-based imaging of two absorbing target with 2-cm separation: (a) and (c) are the two CIDs on the detector plane for the two targets. (b) and (d) are the two corresponding CIDs on the source plane for the two targets.

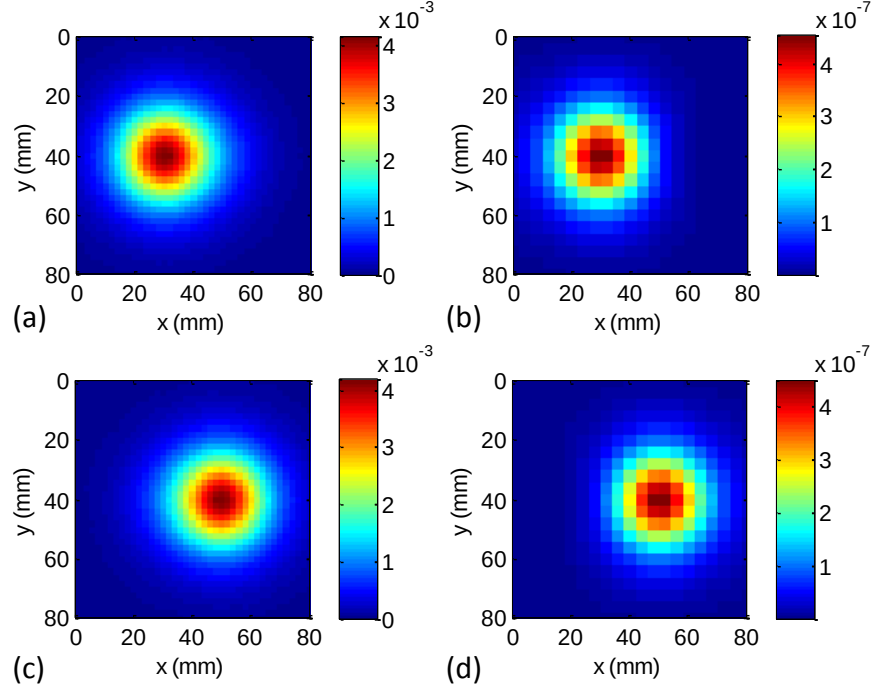


Fig. 4.11. NMF-based imaging of two absorbing target 2-cm apart: (a) and (c) are the two NCIDs on the detector plane for the two targets. (b) and (d) are the two corresponding NCIDs on the source plane for the two targets.

Table 4. Positions and optical strengths retrieved using simulated data and ICA, CA and NMF algorithms for two absorptive point targets

Target	Known position (mm)	Fitted position (mm)	Error (mm)	Known strength (mm /ns)	Fitted strength (mm /ns)	Error ()
Left	(4, 4, 2)	(4.1, 4.1, 1.1)	(0.1, 0.1, 0.1)	1.2	1.1	0.1
Right	(4, 4, 2)	(4.1, 4.1, 1.1)	(0.1, 0.1, 0.1)	1.2	1.11	0.4

4.3.4. Imaging extended targets

The imaging approaches using decomposition methods were developed for point-like targets in Section 4.2. However, in realistic condition, there is no point-like target. Any target has some finite size, either small or big. In this section, we evaluate the effect of the size of the targets, and efficacy of detecting realistic targets using the decomposition methods. Simulated data was generated using the same condition as in the previous section, except that an extended target was embedded. The centroid position of the target was used to describe the position of the target. A $2 \times 2 \times 2$ -mm target, a $1 \times 1 \times 1$ -mm (1 cm) target, and a $2 \times 2 \times 2$ -mm (1 cm) target with $\delta\mu_a = 1 \text{ mm}^{-1}$, were embedded at (4, 4, 2) mm in two separate simulations, respectively. Additive Gaussian random noise was added. For the small target, three components were retrieved by ICA and CA and shown in Fig. 4.12 and Fig. 4.13, respectively. NMF does not retrieve negative signal, so only one component was obtained and shown in Fig. 4.14. For the larger targets, ICA and CA also retrieved three components. For the $1 \times 1 \times 1$ -mm target, the ICIDs and CIDs are shown in Fig. 4.15 and Fig. 4.16, respectively. NMF retrieved one component as shown in Fig. 4.17. The components for the $2 \times 2 \times 2$ -mm target were also obtained, which were similar to those of the $1 \times 1 \times 1$ -mm target, and not shown here.

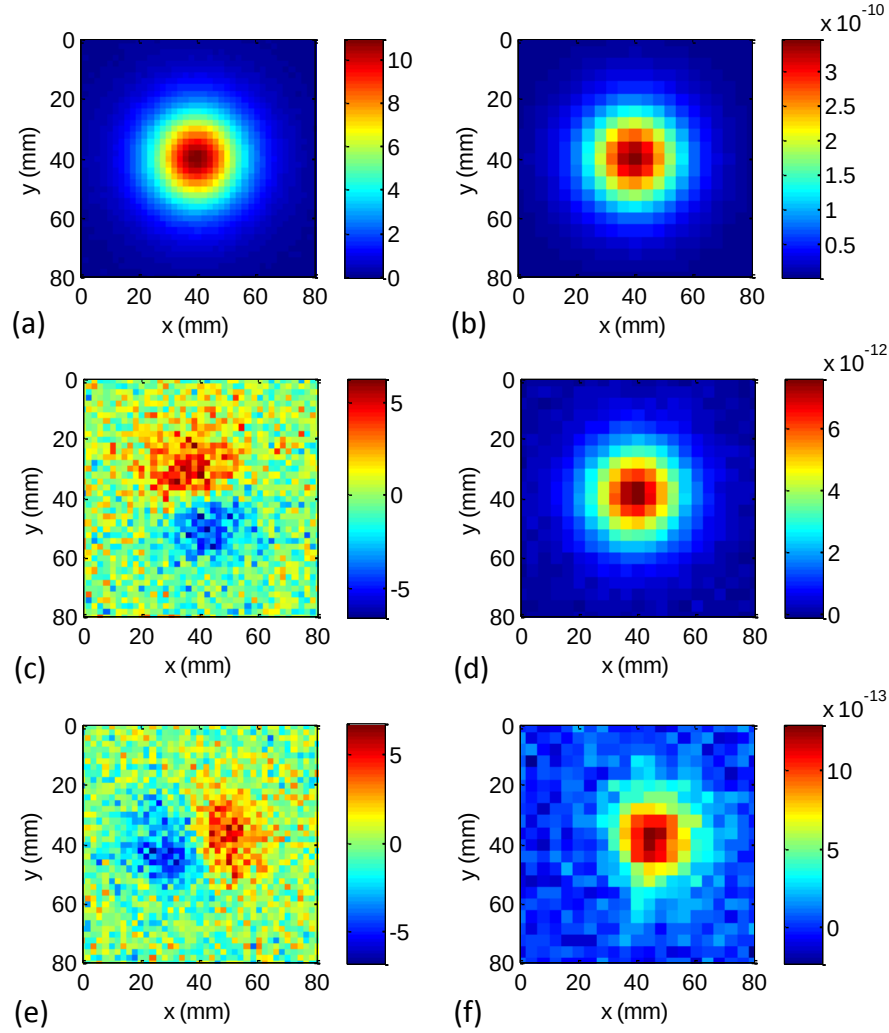


Fig. 4.12. ICA-based imaging of one $2 \times 2 \times 2$ mm absorptive target: (a), (c) and (e) are the three ICIDs on the detector plane for the target. (b), (d) and (f) are the three corresponding ICIDs on the source plane for the target.

The components in the left and right columns in the Figs. 4.12–4.1 are on the detector and source planes, respectively. As seen in the figures, ICA and CA retrieved three components for a single finite-size absorptive target, including one centrosymmetric and two dumb-bell-shaped. This is similar to a point scattering target. When the target was small, with size $2 \times 2 \times 2$ mm, the centrosymmetric component dominated and the dumb-bell-shaped components were much weaker. When the size of the target was $1 \times 1 \times 1$ mm, the dumb-bell-shaped components became more significant. When the light distribution due to a finite-size object is decomposed, the first

component corresponds to light from a point source at the “optical center” of the object, the higher-order components are due to the spatial distribution, which are analogous to the multi-pole expansion for a complex structure. The centrosymmetric components were used to fit the locations and absorption strengths of the targets, as shown in Table 4.4, 4. and 4. for the three targets, respectively.

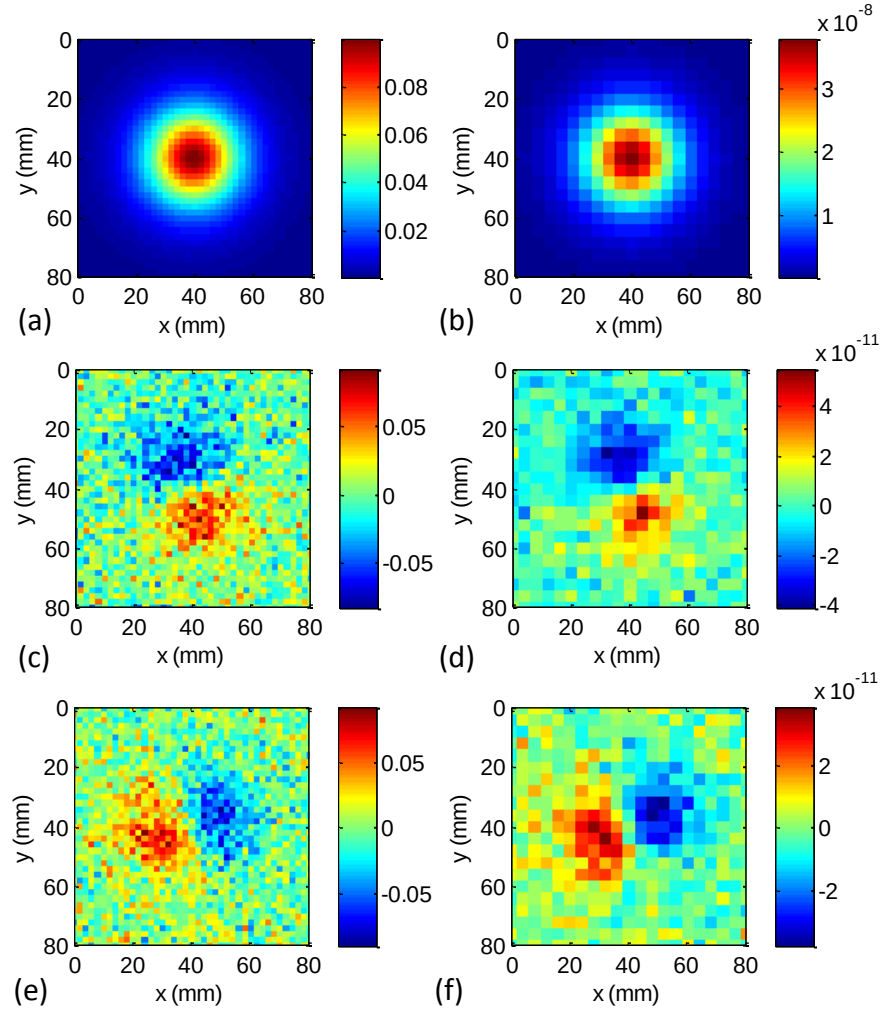


Fig. 4.1 . CA-based imaging of one 2 × 2 × 2 mm absorptive target: (a), (c) and (e) are the three CIDs on the detector plane for the target. (b), (d) and (f) are the three corresponding CIDs on the source plane for the target.

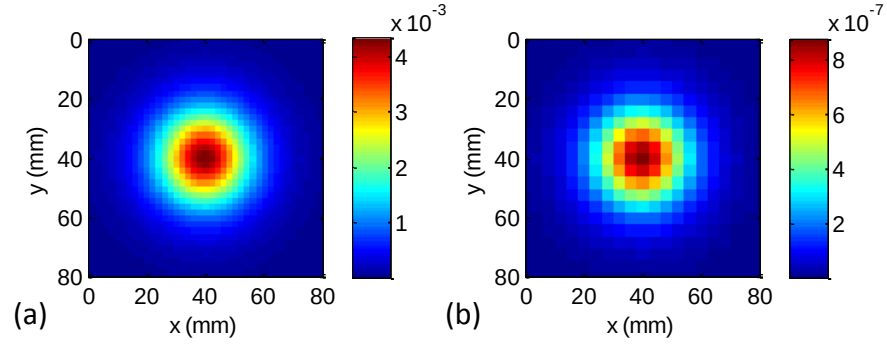


Fig. 4.14. NMF-based imaging of one 2 × 2 × 2 mm absorptive target: (a) and (b) are the NCIDs for the target on the detector plane and source plane, respectively.

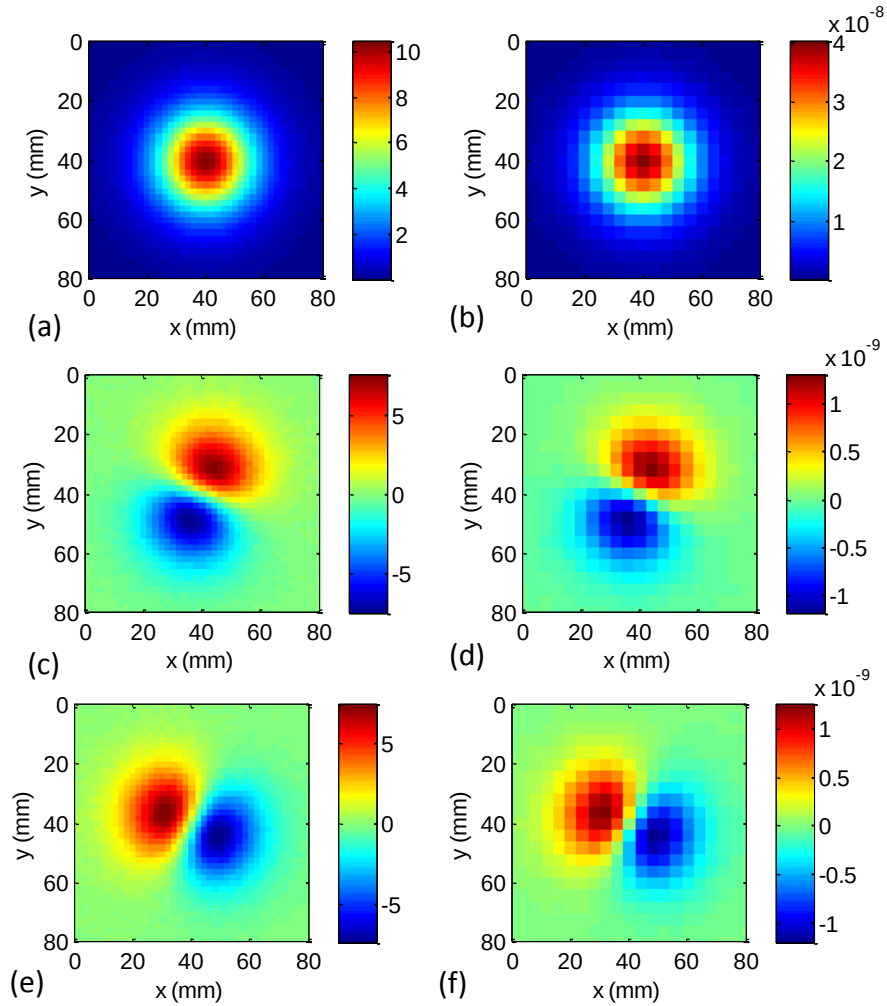


Fig. 4.15. ICA-based imaging of one 1 × 1 × 1 mm absorptive target: (a), (c) and (e) are the three ICIDs on the detector plane for the target. (b), (d) and (f) are the three corresponding ICIDs on the source plane for the target.

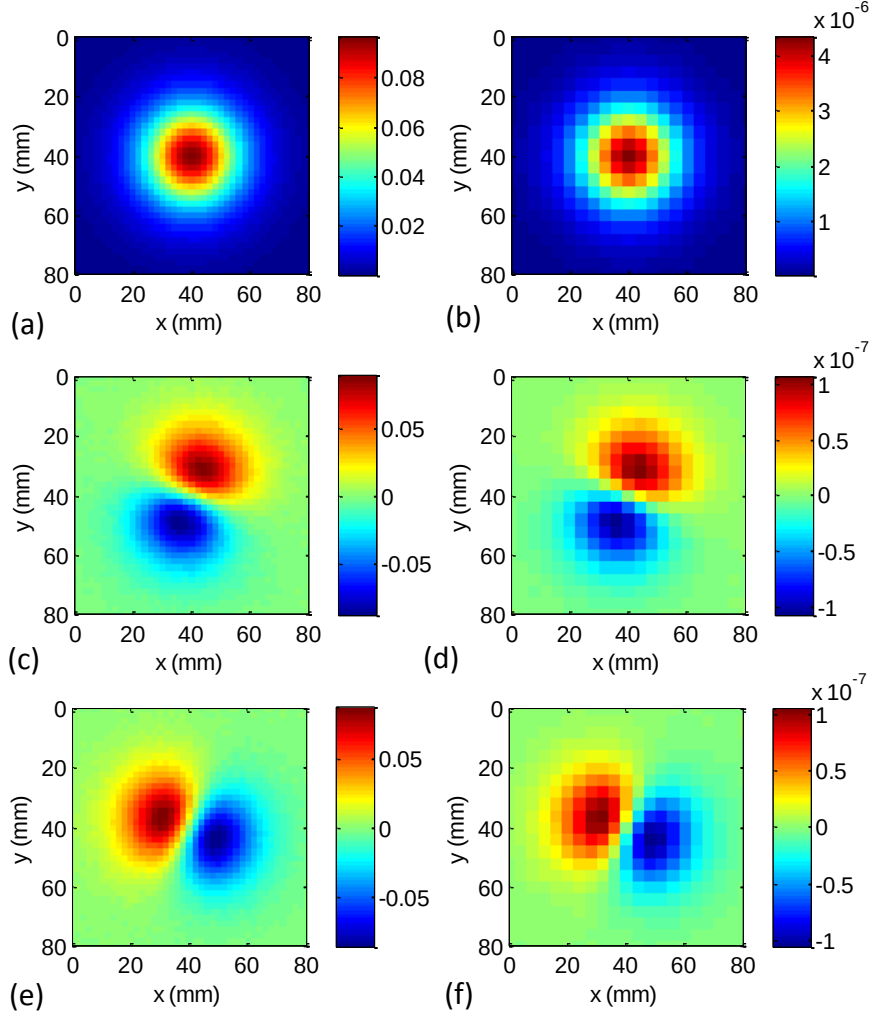


Fig. 4.1 . CA-based imaging of one 1 × 1 × 1 mm absorptive target: (a), (c) and (e) are the three CIDs on the detector plane for the target. (b), (d) and (f) are the three corresponding CIDs on the source plane for the target.

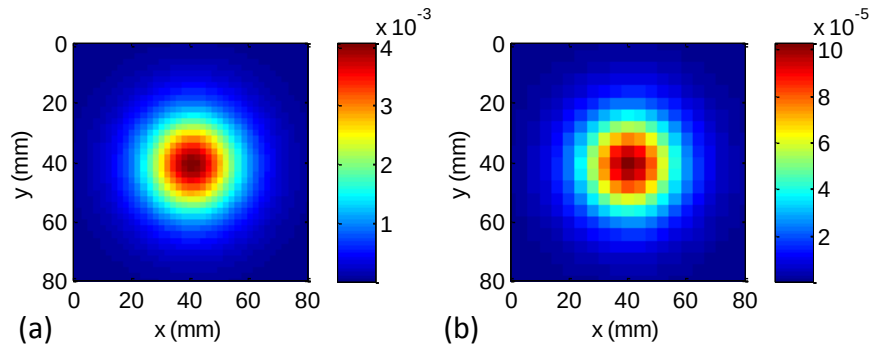


Fig. 4.1 . NMF-based imaging of one 1 × 1 × 1 mm absorptive target: (a) and (b) are the NCIDs for the target on the detector plane and source plane, respectively.

Table 4.4. Retrieved and known positions and absorption strength of the 2 2 2 mm target

	Target position x, y, z (mm)	error x, y, z (mm)	Absorption Strength (mm /ns)	error ()
known	4 . , 4 . , 2 .	-	1. 2	-
CA	4 . , 4 . , 2 .	, ,	1.	.
ICA	4 . , 4 .1, 2 .	. , .1,	1.	.4
NMF	4 . , 4 . , 2 .	, ,	1.	.

Table 4. . Retrieved and known positions and absorption strength of the 1 1 1 mm target

	Target position x, y, z (mm)	error x, y, z (mm)	Absorption Strength (mm /ns)	error ()
known	4 . , 4 . , 2 .	-	22 .4	-
CA	4 . , 4 . , 2 .	, ,	1 .	11.4
ICA	4 . , 4 . , 2 .	, ,	1 .	11.4
NMF	4 . , 4 . , 2 .	, ,	2 .	11.1

Table 4. . Retrieved and known positions and absorption strength of the 2 2 2 mm target

	Target position x, y, z (mm)	error x, y, z (mm)	Absorption Strength (mm /ns)	error ()
known	4 . , 4 . , 2 .	-	1 .2	-
CA	4 . , 4 . , 2 .	, ,	112 .	.
ICA	4 . , 4 . , 2 .	, ,	112 .	.
NMF	4 . , 4 . , 2 .	, ,	112 .4	.

The target positions were perfectly retrieved in all these cases using the centrosymmetric components, except the case when ICA was used for the $2 \times 2 \times 2$ -mm target, where the uncertainty was within ± 0.1 mm. Therefore the decomposition methods can be used to detect and locate an extended target. The position of the target is the “center of optical strength”, which is the geometric center of a target if the target is homogenous in property and symmetric in geometry.

However, the dumb-bell-shaped components cannot be used to characterize a finite-size target as scattering in nature in a realistic experiment.

Since only the first component was used to retrieve the optical strength of a target, the larger the target, the higher the uncertainty in the retrieved optical strength. For the $1 \times 1 \times 1$ -mm target, the errors in the retrieved optical strength using all three decomposition are within ± 0.4 . However, for the $1 \times 1 \times 1$ -mm target, the errors are about ± 11 , and for the $2 \times 2 \times 2$ -mm target, the errors are ± 11 .

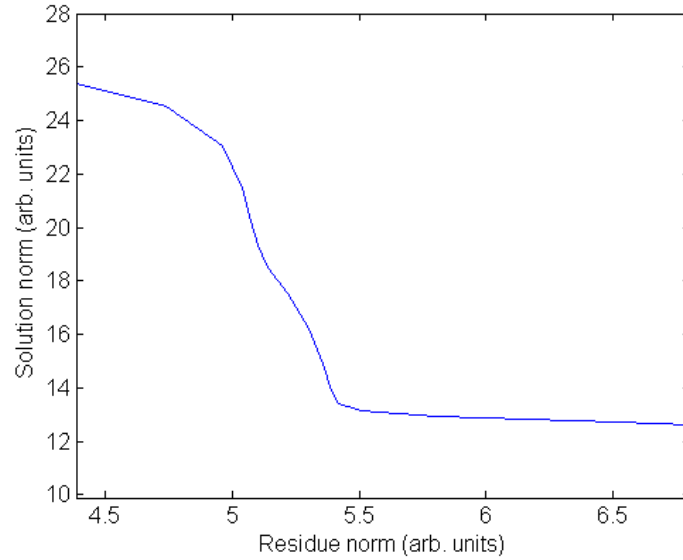


Fig. 4.1 . L-curve for back-projection of ICID, where x axis is the residue norm $\|X_j(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s) - G_d(\mathbf{q} - \mathbf{q}_s) \chi_j(\mathbf{q}) G^*(\mathbf{q}_s)\|$ derived from $\mathbf{q}$. (4.11a), and y axis is the solution norm $\chi_j(\mathbf{q})$.

A cross section image through the z-position of the target was then generated by using

backprojection of ICID, CID and NCID to estimate the dimension of the target. For the 2 × 2 mm target, when calculating (q) using ICID in eq. (4.11), the L -curve was generated as shown in Fig. 4.1 .

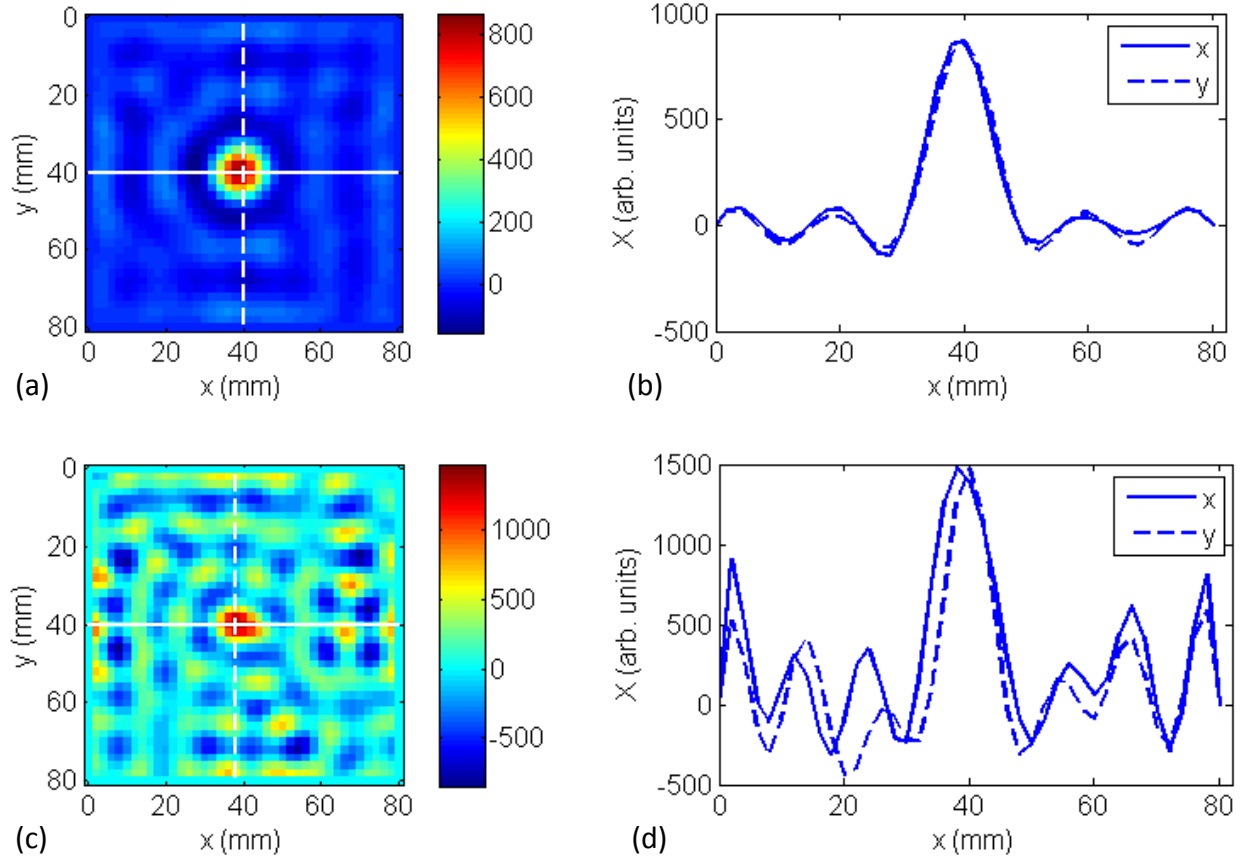


Fig. 4.1 . (a) and (c) are cross-section images through the target, generated using the regularization parameter corresponding to the “corner” of the L -curve, and an optimized regularization parameter, respectively. (c) and (d) are the corresponding profiles through the maxima in the cross-section images.

When the corner of the L -curve was used, the cross-section image through the z -position of the target was generated and shown in Fig. 4.1 (a). The profiles through maximum are plotted and shown in Fig. 4.1 (b). The full-width-at-half-maximum (FWHM) is used to estimate the dimension of the target. The FWHMs in the x and y direction were found to be 1.1 mm and 1.4 mm, respectively. The FWHMs in the cross-section image are much larger than the actual

dimension of the target (2 mm), which is due to the diffusive nature of light propagation. The back-projection was then optimized using lower regularization. The cross-section image using optimized regularization is shown in Fig. 4.1 (c), and the profiles through the maximum are shown in Fig. 4. (d). The FW Ms were found to be 1.1 mm and . mm in the x and y directions, respectively.

Similarly, optimized cross-section images were also generated using CID and NCID for back-projection, and shown in Fig. 4.2 .

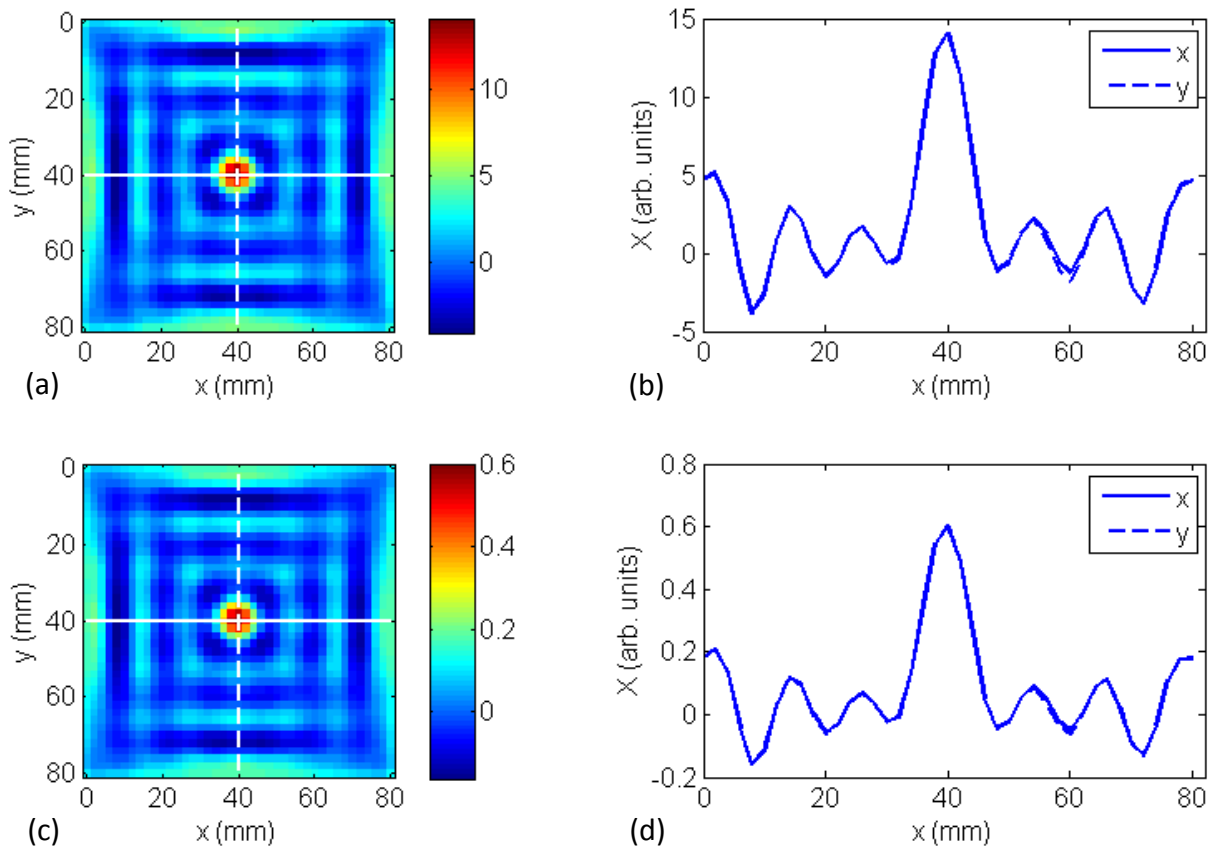


Fig. 4.2 . (a) and (c) are cross-section images through the target, generated using the CID and NCID. (b) and (d) are the corresponding profiles through the maxima in the cross-section images.

The FW Ms were also obtained from the cross-section images corresponding to CID and NCID. All the FW Ms were put in Table 4. for comparison. The FW Ms obtained using all

three decomposition methods were approximately 4 times of the actual dimension of the target.

For the 1 × 1 × 1 -mm target, similar cross-section images generated using ICID, CID and NCID are shown in Figs. 4.21(a), 4.21(c) and 4.21(e). The profiles through the maxima of the three cross-section images are shown in Figs. 4.21(b), 4.21(d) and 4.21(f), respectively.

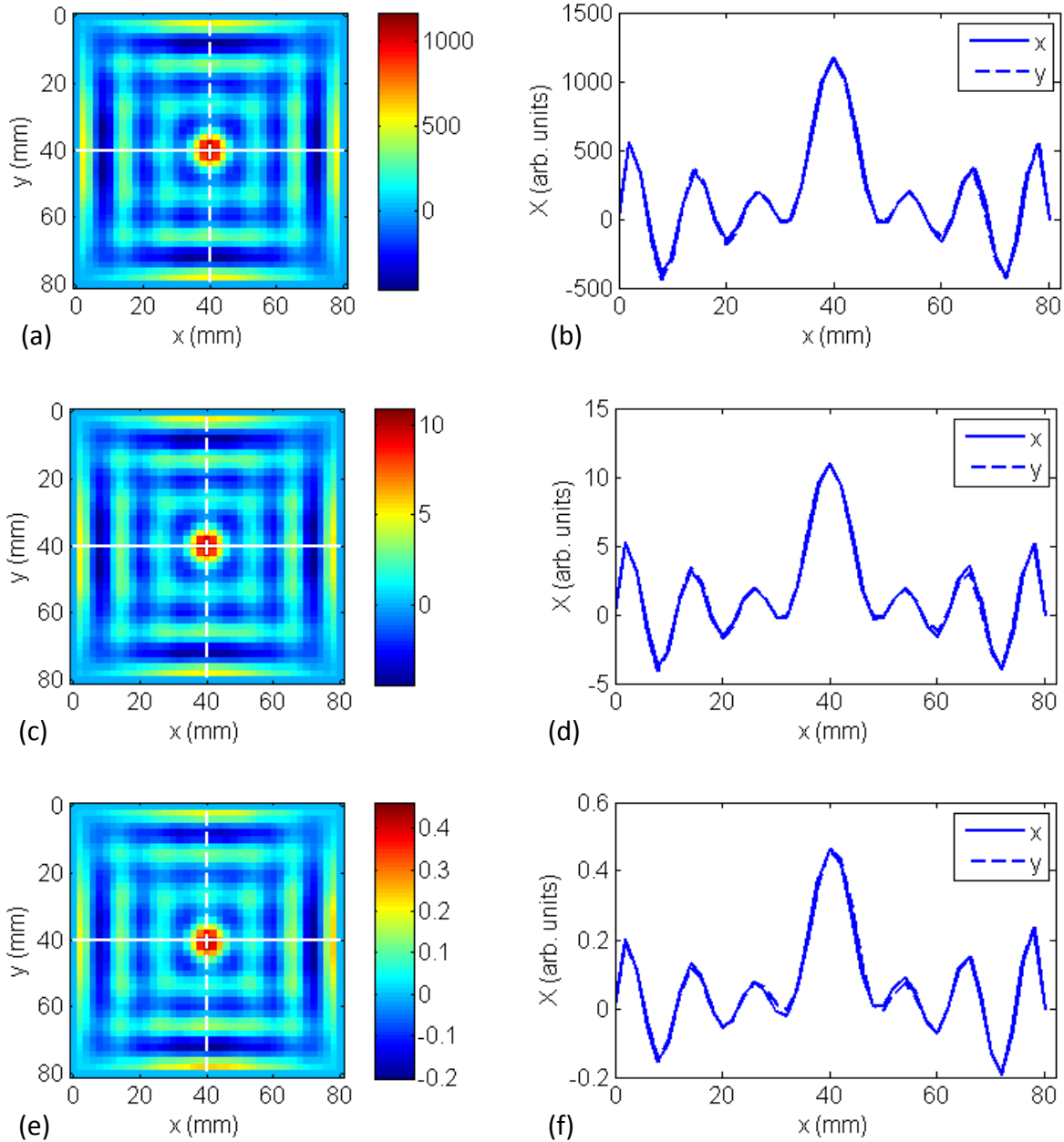


Fig. 4.21. (a), (c) and (e) are cross-section images through the target generated using ICID, CID, and NCID. (b), (d) and (f) are the corresponding profiles through the maxima in the cross-section images.

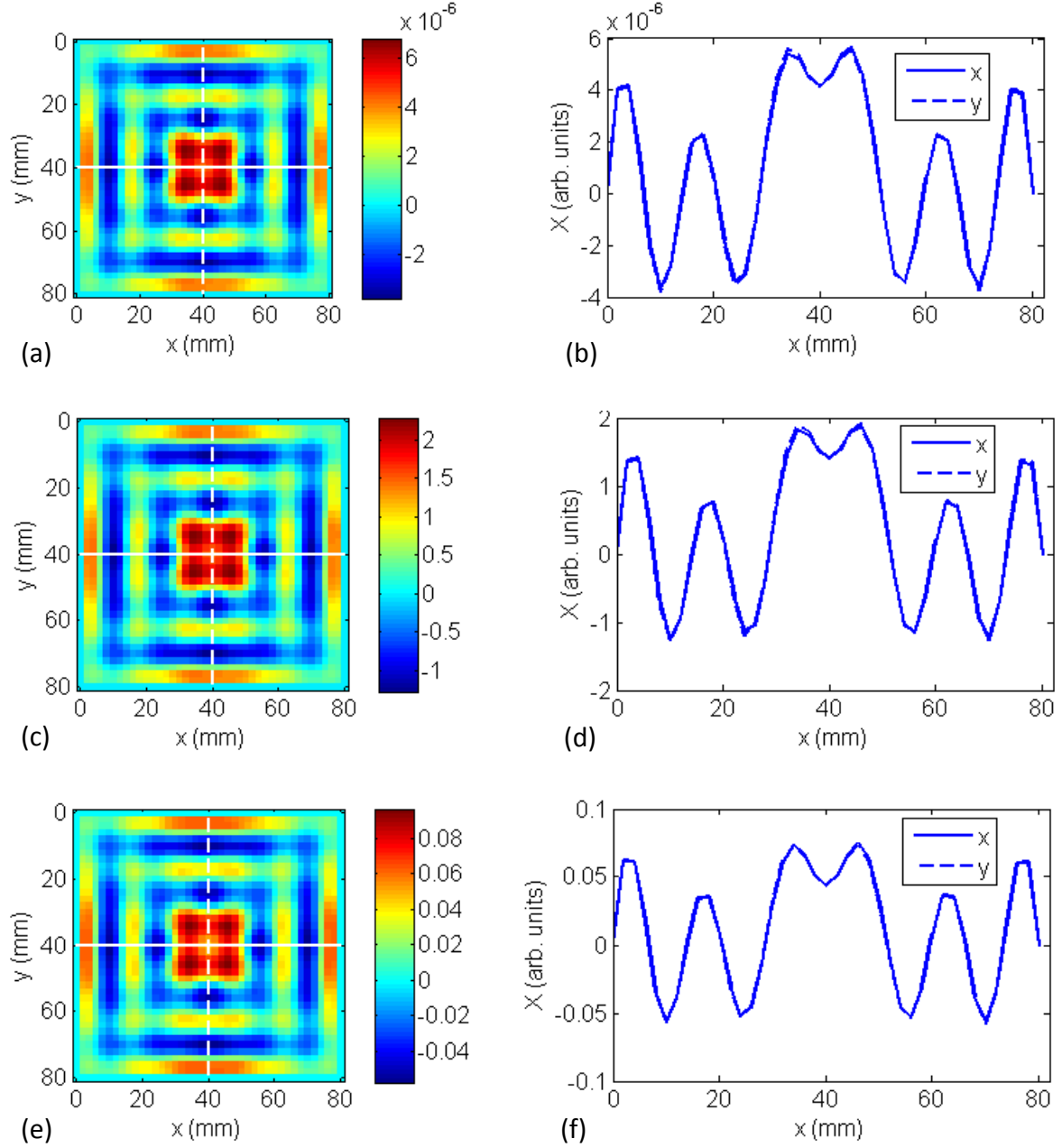


Fig. 4.22. (a), (c) and (e) are cross-section images through the target generated using ICID, CID, and NCID. (b), (d) and (f) are the corresponding profiles through the maxima in the cross-section images.

The FW Ms obtained from the cross-section images generated using ICID, CID and NCID are shown in Table 4. .

For the 2 2 2 -mm target, similar cross-section images generated using ICID, CID and NCID are shown in Figs. 4.22(a), 4.22(c) and 4.22(e). The profiles through the maxima of the

three cross-section images are shown in Figs. 4.22(b), 4.22(d) and 4.22(f), respectively.

The FW Ms obtained from the cross-section images generated using ICID, CID and NCID are shown in Table 4. .

Table 4. . FW Ms retrieved from the cross section images using ICA, CA and NMF

Target Size (mm)	FW M x, y (mm)		
	ICA	CA	NMF
2 2 2	1 .1, .	.1, .1	.1, .1
1 1 1	. , .	.4, .	. , .
2 2 2	1 . , 1 .	1 . , 1 .	2 . , 2 .

The FW Ms retrieved for the 1 1 1 -mm and 2 2 2 -mm targets are close to the actual dimensions of the targets, with less than 2-mm uncertainty for the 1 1 1 -mm target, and less than 1-mm uncertainty for the 2 2 2 -mm target. owever, due to the diffusive nature of light propagation, the cross-section image and its FW M due to the 2 2 2-mm and the 1 1 1 -mm targets are approximately the same. For one single finite-size target, ICID, CID and NCID gives rise to similar cross-section image, therefore similar estimate of target dimension. Further simulations showed that as noise increases high resolution cross-section image with low regularization may be buried in noise and cannot be obtained any more. The cross-section image obtained using the regularization at the “corner” of the L -curve is more robust with respect to the noise level. For the 2 2 2 -mm target in the above simulation with additive aussian noise, the target size estimated from the cross-section image is 1 mm if the regularization at the “corner” of the L -curve is used. If 1 noise is added, the estimated size remains approximately the same.

4.4. Experiments

4.4.1. Experimental materials and methods

In this Section, the algorithms are evaluated using experimental data for absorptive and scattering targets embedded in model scattering media whose absorption and scattering properties are adjusted to mimic the average values of those parameters for human breast tissues. Two different experiments were carried out with two different samples. The sample used in the first experiment was a 2 mm × 2 mm × 2 mm transparent plastic container filled with Intralipid-1 suspension in water as the background medium. The concentration of Intralipid-1 was adjusted to provide $\mu_a = 0.1 \text{ mm}^{-1}$, an absorption coefficient of $\mu_a = 0.1 \text{ mm}^{-1}$, and a transport mean free path $l_t = 1.4 \text{ mm}$ at 800 nm. Two absorptive targets were embedded in the medium. The targets were 1-mm diameter glass spheres filled Indocyanine green (ICG) dye dissolved in Intralipid-2 suspension in water to obtain an absorption coefficient $\mu_a = 1.1 \text{ mm}^{-1}$ at 800 nm, and to match the background scattering coefficient of 2.11 mm^{-1} . Target 1 and target 2 were placed at (0.2, 1.1, 2.1) mm and (1.1, 4.1, 2.1) mm, respectively.

The second experiment used a similar container with dimension of 2 mm × 2 mm × 2 mm filled with Intralipid-2 suspension in water. The concentration of Intralipid-2 was adjusted to provide $\mu_a = 0.1 \text{ mm}^{-1}$, and $l_t = 1 \text{ mm}$ at 800 nm. These optical parameters of the medium were selected to be similar to the average values of those parameters for human breast tissue. The thickness of the samples was also comparable to that of a typical compressed female human breast. Two scattering targets were embedded, which were also 1 mm diameter glass spheres, filled with Intralipid-2 suspension in water. The transport mean free path, l_t was adjusted to be 0.2 mm, with scattering coefficient $\mu_s = 11 \text{ mm}^{-1}$, and absorption coefficient μ_a same as the background

medium. The targets were placed in the middle plane ($z = 0$ mm) in the container with a lateral distance of 4 mm from each other (center to center).

The experimental setup is schematically shown in Fig. 4.2 . A 1 -mW -nm diode laser beam was used to illuminate the first sample, while a 1 -mW -nm diode laser beam was used for the second sample. The input surface (source plane) of the samples was scanned across the laser beam in an x - y array of grid points to realize the multi-source interrogation of the samples. The transmitted light from the exit surface (detector plane) was recorded by a 1 24 pixel 1 24 pixel (pixel size 24 m) CCD camera (hotometrics C) equipped with a -mm focal-length camera lens. Each pixel of the CCD camera can be considered to be a detector implementing the multi-detector signal acquisition arrangement. The two samples were scanned in an array of 11 12 and 11 1 grid points, respectively, with a step size of mm in both cases. The processes of scanning and data acquisition were controlled by a personal computer. At all scan positions, raw transillumination images of the samples were recorded by the computer for further analysis.

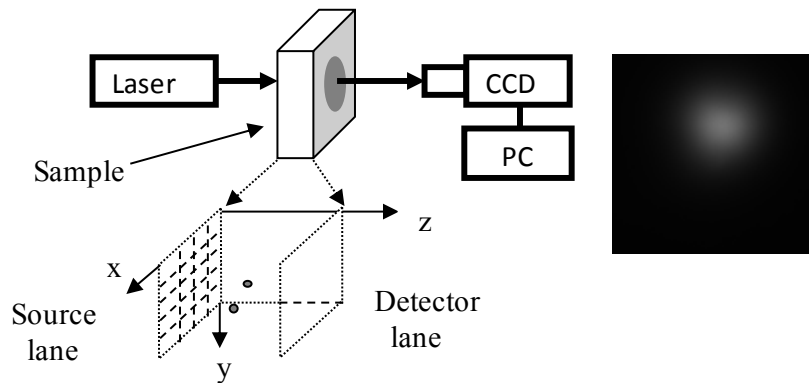


Fig. 4.2 . A schematic diagram of the experimental arrangement used for imaging objects embedded in a turbid medium. The inset at the bottom shows the 2D array in the input plane that was scanned across the incident laser beam; and the inset to the right shows a typical raw image recorded by the CCD. (CCD = charge coupled device, C = personal computer)

A region of interest (ROI) was cropped out from each image. Then every pixels in each

cropped image were binned to one pixel to enhance signal-to-noise ratio. A background image was generated by calculating an average image for all scan positions to approximate the transillumination image without target(s) embedded.

This averaging method for generating background image is suitable for small targets used in our experiments, as the ratio of the volume of the sample to that of the target was quite high (1:1). For *in vivo* imaging of tumors in early stages of growth, the breast-to-tumor volume ratio will be similarly high and the averaging method will be applicable. Alternative approaches for generating a background image include using image of (a) a phantom that has the same average optical properties as the sample [1]; (b) the healthy contralateral breast for breast imaging [2]; and (c) the sample obtained using light of wavelength for which the target(s) and the background have identical optical properties [3]. Still another approach is to compute the background using an appropriate forward model [4]. A more detailed discussion of this important issue appears in one of our earlier publications [5].

The background image was also cropped and binned corresponding to the ROI for each scan position. Perturbation in the light intensity distribution, $\Delta\phi$ due to targets in each image was found by subtracting the background image from the image. The data matrix X was then constructed using the light intensity perturbations at all scan positions. ICA, PCA, and NMF decomposition algorithms were performed on the data matrix separately. Results are shown and discussed below.

4.4.2. Experimental Results

4.4.2.1. Absorptive targets

The images on the detector plane obtained using the ICA, PCA, and NMF algorithms are shown in Fig. 4.24, Fig. 4.25, and Fig. 4.26, respectively. Similar images on the source plane were also obtained using all three algorithms.

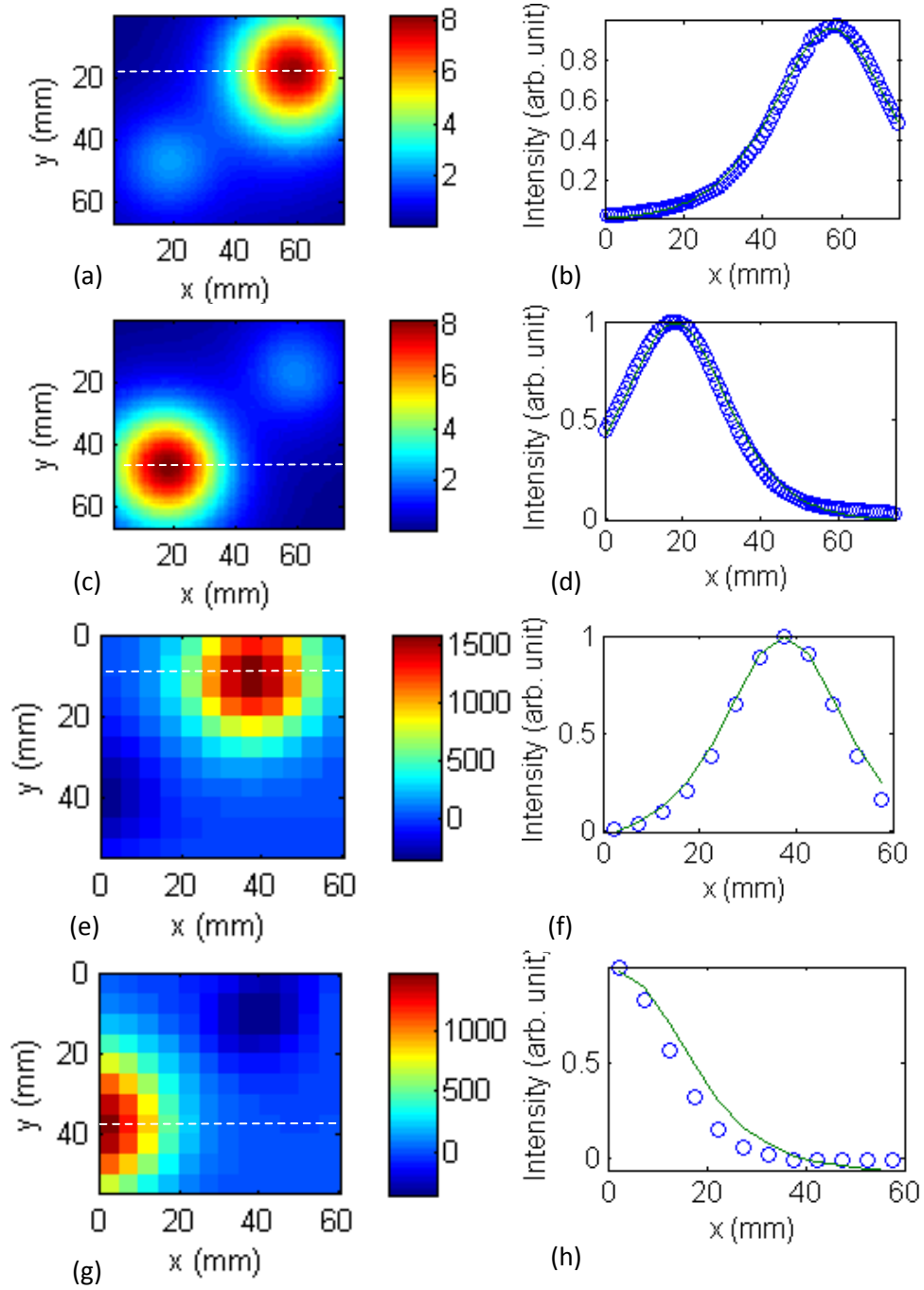


Fig. 4.24. ICA-based imaging of two absorptive targets 10-cm apart: ICA-generated ICIDs on the detector plane are shown in (a) and (c) for target 1 and 2, respectively; corresponding Green's function fits to the horizontal spatial profiles through the dashed lines are shown in (b) and (d) for target 1 and 2, respectively. ICIDs on the source plane are shown in (e) and (g) for target 1 and 2, respectively; corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (f) and (h) for target 1 and 2, respectively.

The right side of each figure shows the corresponding spatial intensity profile. Locations of the targets are extracted from fits to these spatial intensity profiles, as described in Section 4.2.2 using eq. (4.1). The results are presented in Table 4.1. In Fig. 4.24, images on the source plane are shown in (e) and (g), and Green's function fits to their spatial profiles are shown in (f) and (h) for comparison.

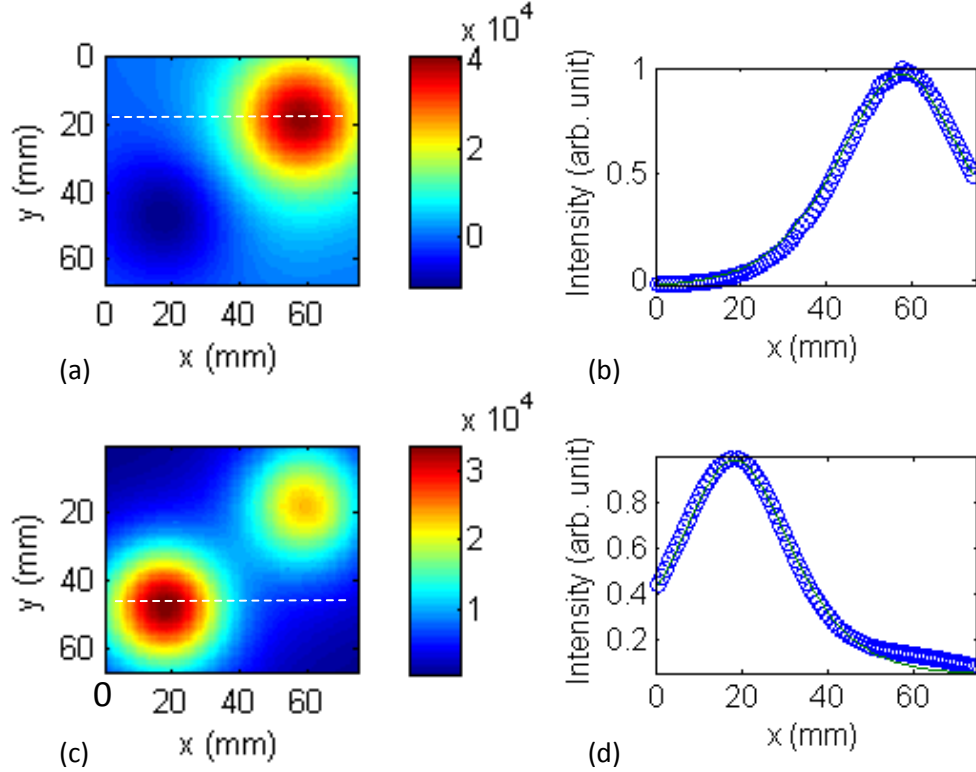


Fig. 4.2 . CA-based imaging of two absorptive targets 10-cm apart: CIDs on the detector plane are shown in (a) and (c) for target 1 and 2, respectively; and corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d) for target 1 and 2, respectively.

It follows from the comparison of in Table 4.1 that the positions retrieved by all three algorithms are in good agreement with the known positions. The errors in the retrieved locations (x, y, z) of the two targets were within 1.0 mm. The CIDs were not totally separated. Some “residue” was observed in one CID from the other. ICA and NMF separated two components from this dataset more clearly.

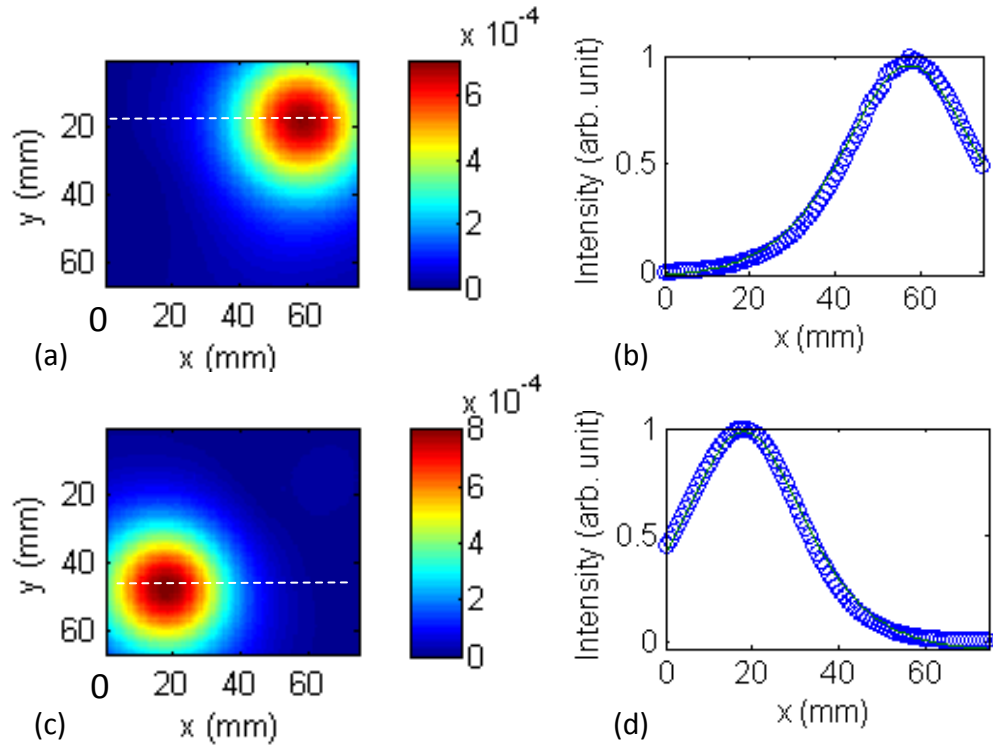


Fig. 4.2 . NMF-based imaging of two absorptive targets 1-cm apart: NCIDs on the detector plane are shown in (a) and (c) for target 1 and 2, respectively; corresponding Lorentzian function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d) for target 1 and 2, respectively.

Table 4. . Retrieved positions of the absorptive targets using ICA, PCA and NMF algorithms

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)
1	(10, 10, 10, 20)	ICA	(10.4, 10.2, 21.0)	(0.2, 0.1, 1.0)
		CA	(10.4, 10.2, 22.0)	(0.2, 0.1, 2.0)
		NMF	(10.4, 10.2, 10.0)	(0.2, 0.1, 0.0)
2	(10.0, 40.0, 10.2)	ICA	(10.2, 40.0, 24.0)	(0.2, 0.0, 14.0)
		CA	(10.2, 40.0, 20.0)	(0.2, 0.0, 10.0)
		NMF	(10.2, 40.0, 20.0)	(0.2, 0.0, 10.0)

The cross-section images of the targets were generated to estimate the size of the targets, using backprojection of the component intensity distributions, and shown in Figs. 4.2 .

The cross-section images were generated using the optimized *L*-curve method to achieve higher resolution. The FW Ms of the profiles in the cross sections are used to estimate the sizes of targets. The retrieved FW Ms using three methods are shown in Table 4. . It is shown that the retrieved sizes are all close to the actual size of the targets (1 mm diameter). owever, there are some artifacts around the target peak whose values are close to the target peak. Since most values in the other parts of the images are much weaker than the target peak, we still use these images. In the second cross-section image for the bottom-left target, the top-right target also shows up, since there was residue in the CID due to the other target.

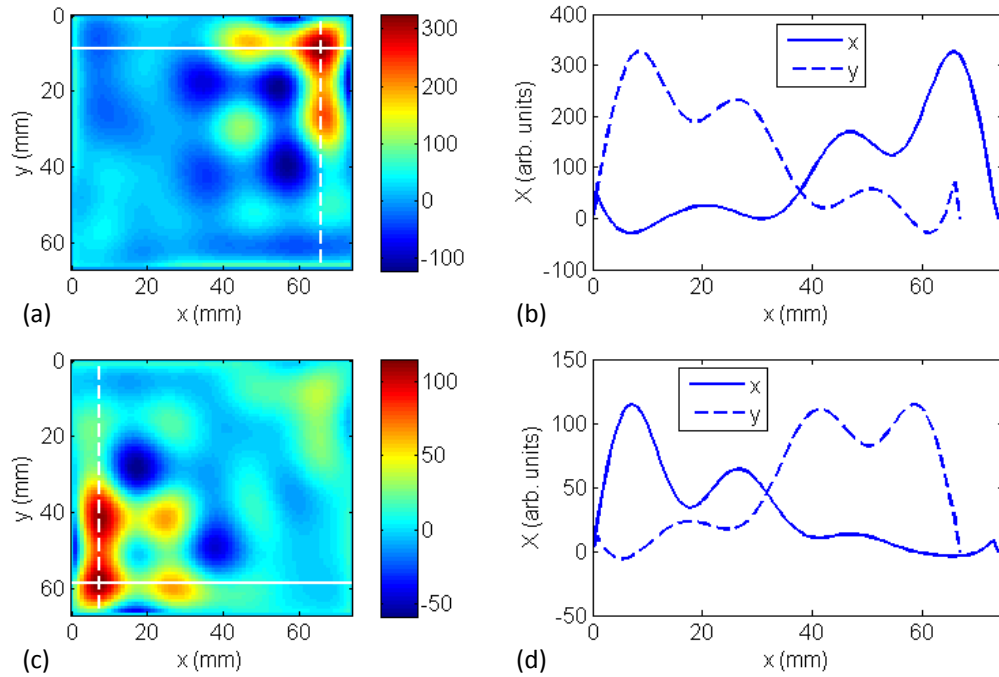


Fig. 4.2 . (a) and (c) are cross-section images of the two targets, respectively, generated using back-projection of ICIDs. (b) and (d) are the profiles through the maxima in the cross-section images, respectively.

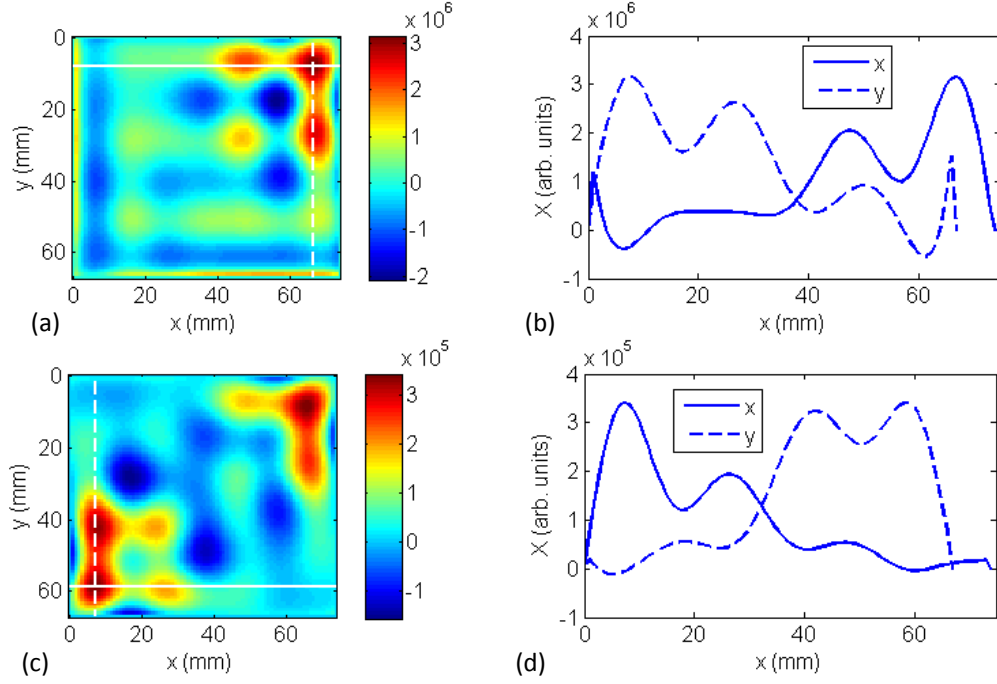


Fig. 4.2 . (a) and (c) are cross-section images of the two targets, respectively, generated using back-projection of CIDs. (b) and (d) are the profiles through the maxima in the cross-section images, respectively.

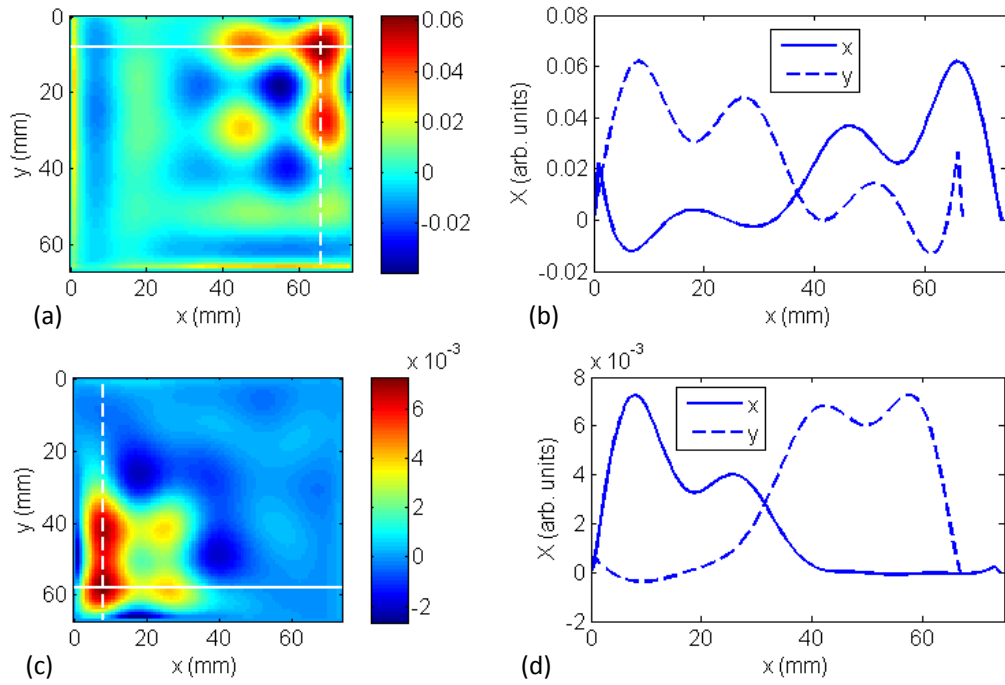


Fig. 4.2 . (a) and (c) are cross-section images of the two targets, respectively, generated using back-projection of NCIDs. (b) and (d) are the profiles through the maxima in the cross-section images, respectively.

ven though the “quality” of decomposition of NMF is slightly better than ICA, and much better than CA, the retrieved locations of targets from this experiment do not clearly show the difference among these three methods.

Table 4.1 FW Ms retrieved from the cross section images using ICA, CA and NMF

Target	FW M x, y (mm)		
	ICA	CA	NMF
1	1.1, 1.4	11.4, 11.	. , .
2	. , .	. , .	1.2, 1.2

To further compare the three approaches, we test them with the experimental data that was used in Chapter 2, where two 5-mm diameter spherical absorptive targets were embedded in the 5-mm thick medium, l_t of the medium is 1 mm, and the two targets were separated by 4 mm. The components retrieved by the three methods are shown in Figs. 4.1, 4.2 and 4.3, respectively. The two targets are still entangled in the CIDs. ICA separates two targets better than CA. Figs. 4.1(a), 4.1(c), 4.2(a), and 4.2(c) are the components on the detector plane, and correspond to the two targets retrieved by the three methods. Similarly, Figs. 4.1(b), 4.1(d), 4.2(b) and 4.2(d) are the corresponding components on the source plane. However, in each component, the residue due to the other target is still present. The fitted positions are shown in Table 4.1. Even though the axial (z) position of the targets retrieved by CA are more accurate than that retrieved by ICA, the lateral positions are significantly less accurate. Normalized mean squared error (NMS) was used to evaluate the quality of the fitting. As shown in Table 4.1, the NMS for CA and ICA are significantly larger than that for NMF. The NMS for the right target retrieved by CA is comparable to that retrieved by ICA, however, NMS for the left target retrieved by CA is significantly larger than that retrieved by ICA.

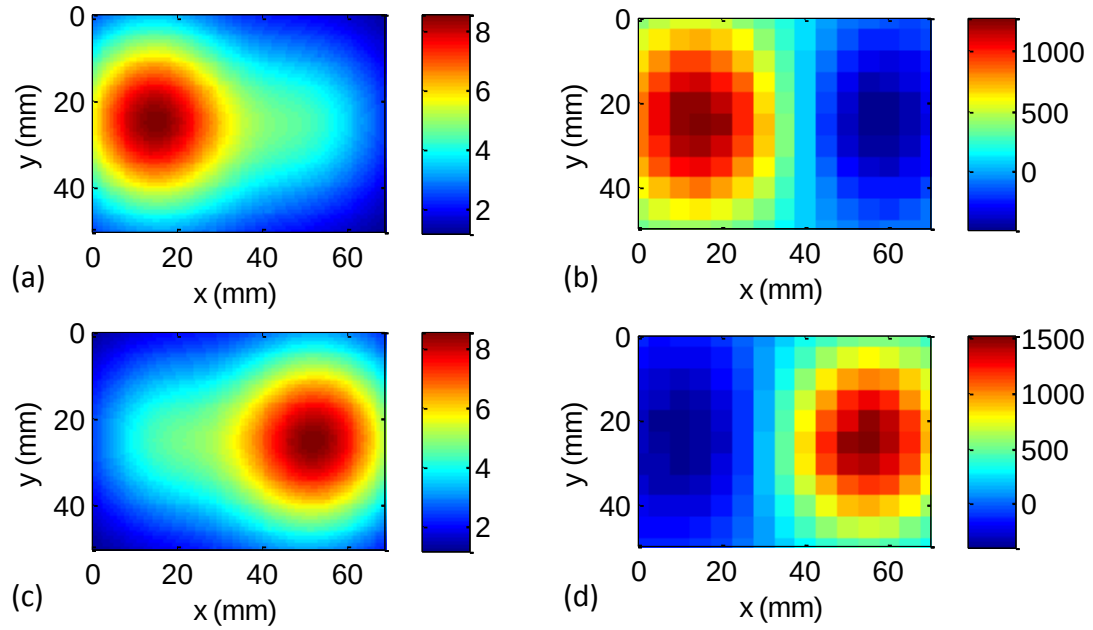


Fig. 4. ICA-based imaging of two absorptive targets 4-cm apart: (a) and (c) are the two ICIDs on the detector plane; (b) and (d) are the two ICIDs on the source plane.

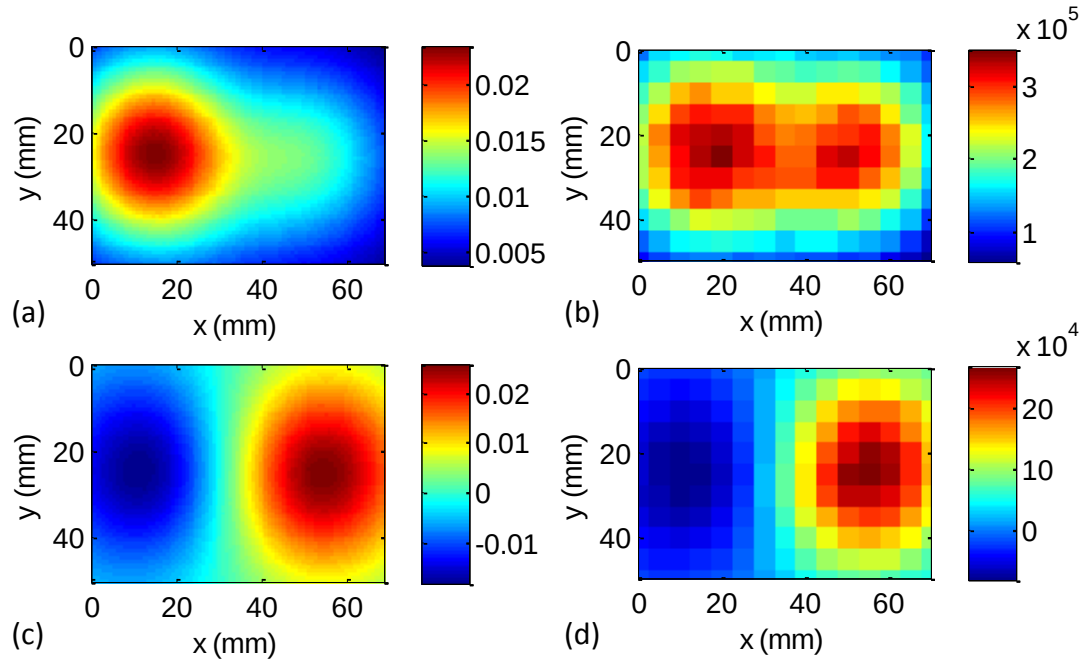


Fig. 4. 1. CA-based imaging of two absorptive targets 4-cm apart: (a) and (c) are the two CIDs on the detector plane; (b) and (d) are the two CIDs on the source plane.

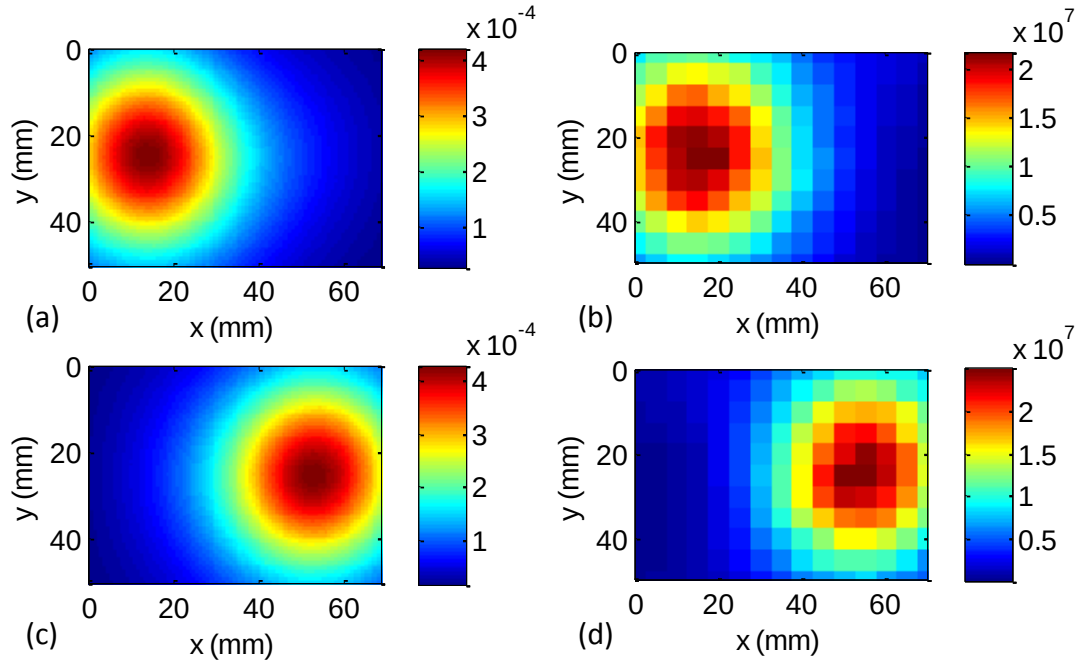


Fig. 4. 2. NMF-based imaging of two absorptive targets 4-cm apart: (a) and (c) are the two NCIDs on the detector plane; (b) and (d) are the two NCIDs on the source plane.

Table 4.1 . Retrieved positions of the absorptive targets using ICA, CA and NMF

Target	Algorithm	osition (mm)	rror (mm)	NMS
1	nown	1 .1, 2 . ,	-	-
	CA	2 .1, 24.2, .	. , 1. , .	.
	ICA	14. , 24. , .	1.1, 1. , .	.1
	NMF	1 . , 24.4, 2.4	1. , 1. , 2.4	. 1
2	nown	2. , 2 . ,	-	-
	CA	. , 2 .1, 2 .	2. , . , 1.	.14
	ICA	2.4, 2 .1, .	. , . , .	.1
	NMF	.1, 24. , 2 .	.4, 1.2, .	. 4

4.4.2.2. Scattering targets

The “images” corresponding to the centrosymmetric components of the virtual sources (targets) on the detector plane obtained using the ICA, CA, and NMF algorithms are shown in Figs. 4. , 4. 4, and 4. , respectively. Similar images on the source plane were also obtained. The right side of each figure shows the corresponding spatial intensity profile. ocations of the targets are extracted from fits to these spatial intensity profiles, as described in Section 4.2.2 using q. (4.). The results are presented in Table 4.11.

oth targets were detected by all three algorithms. The target locations retrieved by three algorithms are shown in Table 4.11, and compared with known locations. Overall, all three algorithms detect and locate the scattering and the absorptive targets with good accuracy, the maximum deviation of any one coordinate from the known value being mm.

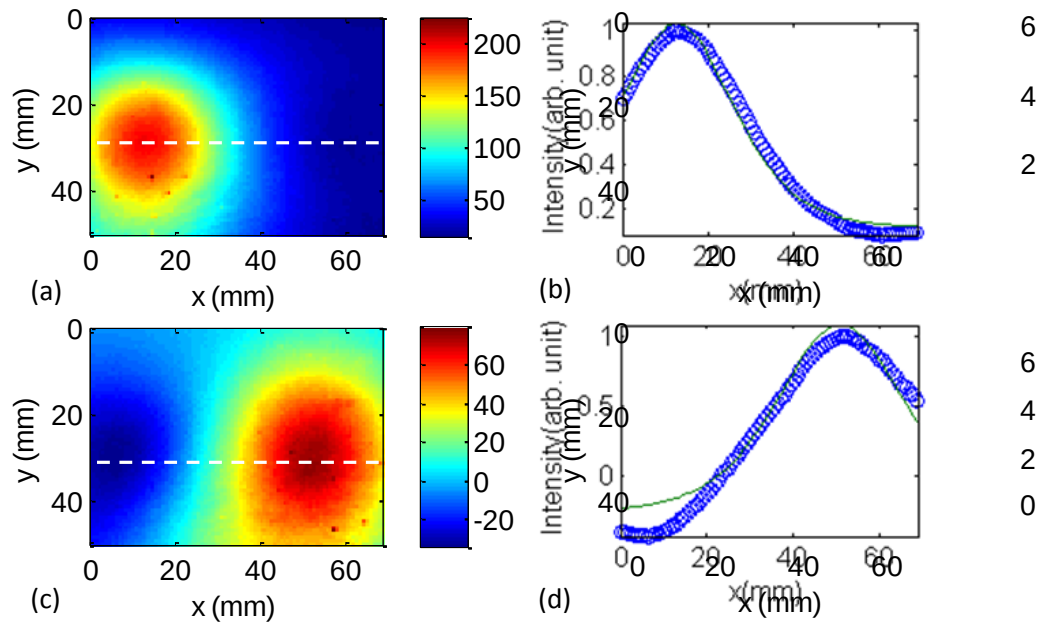


Fig. 4. . ICA-based imaging of two scattering targets 4-cm apart: ICA-generated ICIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

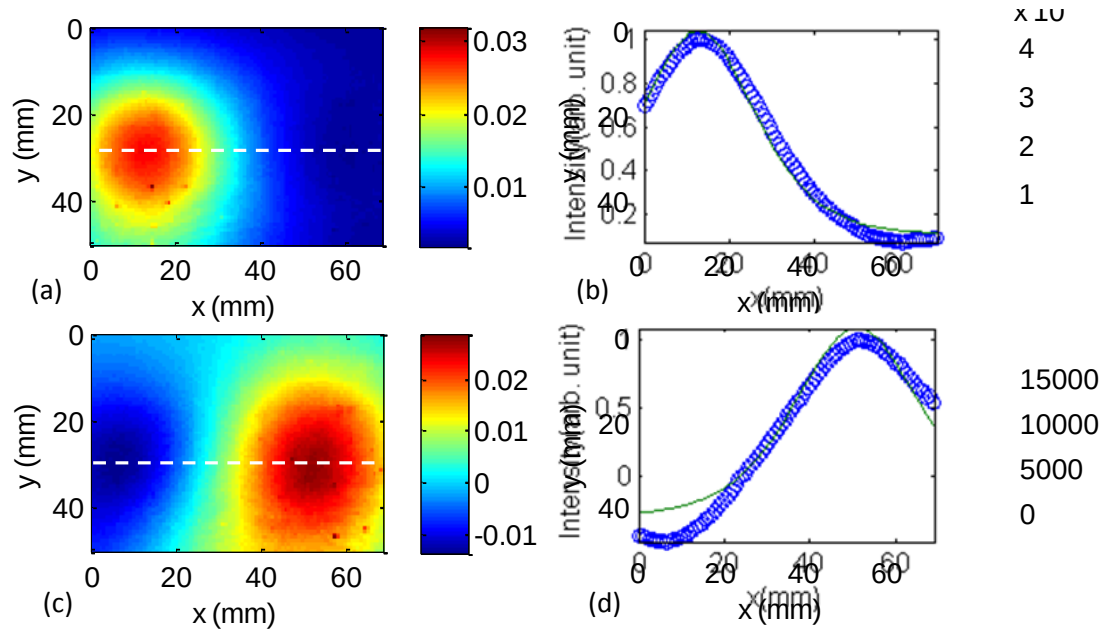


Fig. 4. 4. NMF-based imaging of two scattering targets 4-cm apart: CIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

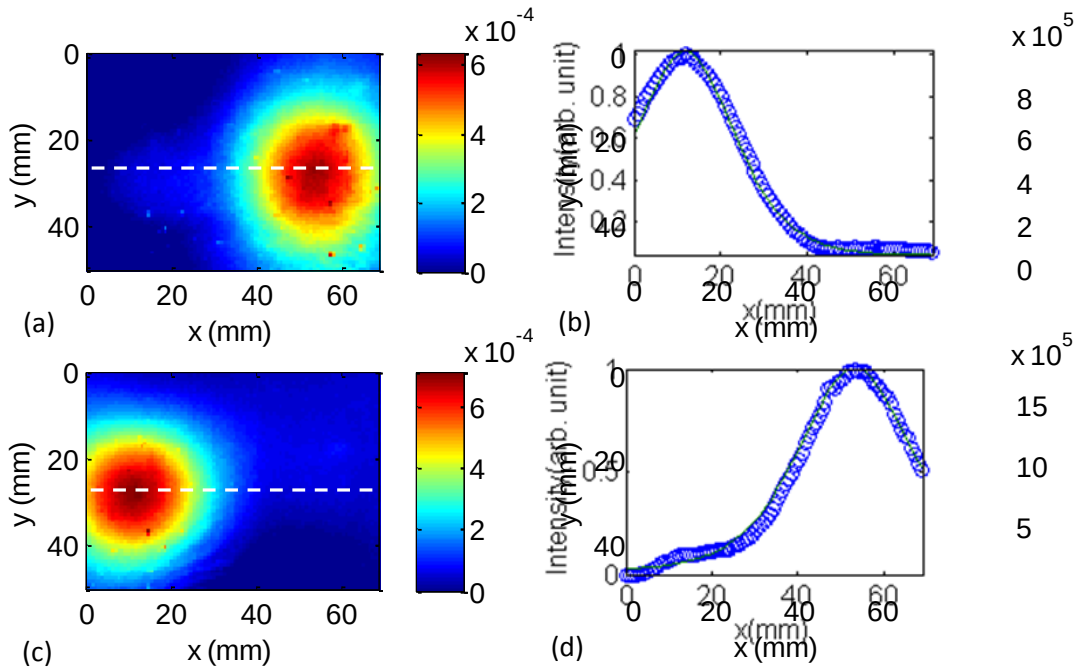


Fig. 4. 4. NCIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

Table 4.11. Retrieved positions of the scattering targets using ICA, CA and NMF algorithms

Target	Known position (mm)	Algorithm	Retrieved position (mm)	Error (mm)
1	(1.5, 2.5, 1.5)	ICA	(12.5, 2.5, 2.1)	(.4, .5, .5)
		CA	(12.5, 2.5, 2.5)	(.4, .5, 1.4)
		NMF	(12.5, 2.5, 1.5)	(1.5, .5, .5)
2	(1.5, 2.5, 1.5)	ICA	(1.5, 1.5, 2.5)	(2.5, .5, .2)
		CA	(1.5, 1.5, 2.5)	(2.4, .5, .5)
		NMF	(1.5, 2.5, 1.5)	(.5, .5, .5)

Since the maximum difference between the known and retrieved position coordinates was larger for the scattering targets, we calculated the squared correlation coefficient γ to assess the fitting quality. NMF retrieves the position coordinates better (within .5 mm) for the scattering target 2 than done by ICA and CA (deviation from known values being between 2- mm). NMF retrieved the position coordinates for target 1 with .5 mm error in z direction, which is not as good as that done by ICA and CA. But γ is .9 and .9 in the fittings for ICA and CA, respectively, as compared to .9 for NMF, indicating that the quality of the fitting is better for NMF. The quality of fitting is presumably affected by the efficacy of decomposition. The decomposed NCIDs by NMF were more “clean” than those decomposed by ICA and CA. We ascribe the observed higher errors in ICA and CA estimates of the position coordinates of the scattering target 2 than the NMF estimates to the interference from the other virtual source (corresponding to target 1) in ICA (Fig. 4. (c)) and CA (Fig. 4. 4(c)) images. It is commonly believed that errors in locating a scattering target are higher than that for locating an absorptive target, and the results of this study conform to that notion.

Since the three decomposition methods can also be used for fluorescent targets, they will be further tested and compared in Chapter .

4.5. Discussion

Diffusive optical imaging was modeled as a SS problem. ICA, CA and NMF were used to decompose the data matrix, and locate the targets embedded in a highly scattering turbid medium. Only the components corresponding to the targets were extracted from a large dataset for target detection and localization.

4.5.1. Computational complexity

It may be instructive to compare the objectives, scope and computational complexity of these decomposition methods with model-based reconstruction methods. Decomposition methods obtain the -D locations of targets (the number of targets are generally small). Based on the retrieved locations, the methods may then be further extended to retrieve size and optical property information of the targets . The common practice of model-based inverse reconstruction methods is to discretize the sample volume into $N \times N \times N$ voxels, and estimate absorption and/or scattering coefficient in each voxel iteratively. Voxels with significantly different optical properties than the surrounding are regions of interest, and may be identified as targets. While estimating the optical properties, the forward model is solved repeatedly to calculate the intensity of the multiply-scattered light on the sample boundary. The difference between the intensity of the multiply scattered light predicted by the forward model and the experimental measurements is minimized by seeking an optimal set of the optical properties of every voxel in the sample volume. The number of variables thus is on the order N . To determine location(s) of target(s) in three dimensions, the decomposition methods process the data matrix to retrieve the main components (A and S). Here A and S are two-dimensional matrices with the number of unknowns on the order

of N^2 . The number of unknowns is, hence, reduced N times in the decomposition methods compared to the model-based approaches, which leads to a substantial saving in the computational time when N is large. No repeated solution of the forward model is involved in decomposition methods. Consequently, decomposition methods are considerably faster.

A comparison of the computational complexity of these two types of approaches may shed further light on their relative computation economy. For a model-based iterative reconstruction method, an equation of the form $b = Wx$ is solved to find the targets, where W is a weight matrix of size $N_d N_s \times N_v$, N_d , N_s , and N_v are the numbers of detectors, sources and voxels, respectively, b is an $N_d N_s - 1$ vector describing the perturbation in the detected light intensity due to the presence of targets, and x is the perturbation in the optical properties from the background values with dimension of $N_v - 1$. The computational complexity is typically $O(N_d N_s N_v^2)$ for a single iteration. For the decomposition approach, b is written as a 2-D matrix X with dimension $N_d \times N_s$. To decompose matrix X , the computational complexity per iteration is typically of order $O(N_d N_k)$ for ICA [10], and $O(N_d N_s N_k)$ for NMF [11], where N_k is the number of components that relates to the number of targets and is usually a small number. For PCA using SVD, the complexity is $O(N_s^2 N_k)$ [12]. The computational complexity of the intrinsic iterative process involved in the matrix decomposition algorithms is much lower than that in the model-based inverse reconstruction methods.

4.5.2. Comparison of the decomposition methods

All three matrix decomposition methods presented in this chapter can potentially be used in *in-vivo* real-time breast cancer imaging. The three algorithms have different assumptions, which may lead to different favored conditions. In this study, the algorithms were evaluated using simulative and experimental data using model scattering media and absorptive and scattering targets. The (x ,

y, z) positions of the targets were retrieved with good accuracy. The decomposition provided by ICA is “cleaner” than that of the CA. CA did not clearly separate the two absorptive targets used in the first two experiments. NMF decomposition seems to provide residue-free “cleaner” images than the other two methods in this study. However, since NMF is based on non-negativity assumption, the results might deteriorate when such a non-negativity assumption does not hold well. Similar consistent results from experiment and simulation are shown in Chapter 4, for fluorescent targets. Since fluorescence signal is inherently positive, the non-negativity assumption automatically holds. While continuous wave measurements were used in the work presented in this chapter, the approaches could be used with frequency domain and time-domain measurements as well.

The work presented here focuses on detecting and locating small targets, which derive impetus from the need to detect tumors in early stages of growth when those are more amenable to treatment. All three methods are applicable for extended targets as well, and are expected to provide the “center of optical strength” as the location of the target.

All three approaches are applicable for both scattering and absorbing targets, and may be used in clinical setting. The contrast between a tumor and surrounding normal tissue can be due to differences in absorption, scattering, or both absorption and scattering properties and may depend significantly on the wavelength of light used. However, *a priori* knowledge of the optical characteristics (absorptive or scattering) is not crucial. As has been shown in eq. (4.2) and eq. (4.3) the expression for elements of the data matrix for absorptive targets involves Green’s Functions G , while that for scattering targets involves $\partial G / \partial z \approx -\kappa G$, where $\kappa = \sqrt{\mu_a / D}$ in CW. This relationship with G provides basis for detection and localization of target(s), whether contrast is due to absorption, scattering, or both. We are using transillumination geometry, which is one of

the approaches used by other researchers, and adequate signal for *in vivo* breast imaging is obtained [1, 4].

In this chapter, we presented results when the approaches were used to detect and obtain three-dimensional location information of the targets. We have demonstrated, while developing OTICA [11] that a back-projection formalism can be further implemented to get a cross-section image of the target [11], or the retrieved target locations can be fed into other DOI methods as *a priori* information to get three-dimensional tomographic images. Since the approaches are suited for small targets, these hold promise for detecting and locating breast tumors in early stages of growth, which is crucially important for effective treatment. Further work involving *ex vivo* (model) and *in vivo* imaging of cancerous breast will be needed to establish the full potential of these approaches.

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Chapter 5

Near-infrared optical imaging for detection of tumors in a “realistic model breast”

5.1. Introduction

Time reversal optical tomography (TROT) [1] and nonnegative matrix factorization (NMF) based optical tomography (NMF-OT) have been developed in previous chapters as fast diffusive optical imaging methods to detect and localize target(s) embedded in highly scattering turbid media, such as tumor(s) in human breast. The efficacy of both methods has been evaluated using simulated data and experimental data. We have since tested the methods on *ex vivo* biological tissues with embedded cancerous breast tissues as targets. This chapter presents the results of our study on a “realistic model breast” assembled using *ex vivo* breast tissues with two pieces of human breast tumors of different sizes embedded. A multi-source, multiple wavelength probing and multi-detector signal acquisition scheme was realized to acquire data for analysis using TROT [2, 4] as well as NMF-OT [3] and optical imaging using independent component analysis (O-TICA) [5], two of the decomposition methods presented in Chapter 4. We also investigated the model breast using magnetic resonance imaging (MRI) to serve as a point of reference for optical measurement.

5.2. Formalism

The formalism of TROT has been detailed in Chapter 2 and Chapter 3, while those for NMF-OT and O-TICA have been presented in Chapter 4. These formalisms have been used to analyze the data on the *ex vivo* samples mentioned above. All three approaches use the same data acquired

using a multi-source illumination and multi-detector signal acquisition scheme. For small absorptive and scattering targets, the contribution due to the targets is a perturbation in the light intensities measured on the boundary of the sample. This perturbation data - $I - I_0$, where I and I_0 are the transmitted light intensity distributions on the sample boundary with and without target embedded, is the starting point for all three methods.

In the TROT formalism, the response matrix K is constructed using the perturbation data, $I - I_0$, and describes the transport of light from multiple sources through the embedded target(s) to the array of detectors.

For multi-wavelength TROT, datasets collected using different wavelengths are put together in the same data matrix K . The elements of K are $K_{ij}^\lambda = -\Delta I^\lambda(\mathbf{r}_d^{(i)}, \mathbf{r}_s^{(j)})$ corresponding to i^{th} detector and j^{th} source using light source of wavelength λ . The response matrix is constructed as

$$K = \begin{bmatrix} K^{\lambda_1} & & \\ & K^{\lambda_2} & \\ & & \ddots \end{bmatrix} \quad (1)$$

The time reversal (TR) matrix T is then constructed by multiplying the response matrix by its transpose for continuous-wave (CW) illumination, *i.e.* $T_{DSSD} = KK^T$ (DSSD scheme), where K^T is the transpose matrix of K . By solving an eigenvalue equation of TR matrix T_{DSSD} , eigenvalues and eigenvectors of T_{DSSD} are calculated. The eigenvectors with leading non-zero eigenvalues form the signal subspace, and correspond to the targets, while the eigenvectors with near-zero eigenvalues form the noise subspace which is complementary and orthogonal to the signal subspace. The signal and noise subspaces are separated using an L -curve method [1-12]. For multi-wavelengths data, the eigenvectors in the signal and noise subspaces include those for different wavelengths. A MUSIC pseudo-spectrum P for DSSD scheme is then calculated using Green's functions for slab geometry

$$P(\mathbf{X}_p) = \sum_{\lambda} \frac{\langle \mathbf{g}_p^{\lambda}, \mathbf{g}_p^{\lambda} \rangle}{\left| \langle \mathbf{g}_p^{\lambda}, \mathbf{g}_p^{\lambda} \rangle - \sum_{m=1}^M |\langle \mathbf{u}_m, \mathbf{g}_p^{\lambda} \rangle|^2 \right|}. \quad (2)$$

where $\mathbf{g}_p^{\lambda_k}$ is Green's function vector ($\mathbf{F}$) at voxel p for k^{th} wavelength λ_k , and $\mathbf{u}_m$ ($1 \leq m \leq M$) is the m^{th} leading eigenvector. The locations of the targets are determined using the poles in the pseudo spectrum [1, 4]. As explained in Chapter 2, there are four types of $\mathbf{F}$ s corresponding to each voxel p , one for absorption and three for scattering. For a single wavelength, the $\mathbf{F}$ associated with the detector plane for an absorptive target at $\mathbf{r}$ is formed as $g_d(\mathbf{r}, \lambda) = G^d(\mathbf{r}_i, \mathbf{r}, \lambda)$, $i = 1, \dots, N_d$, which describes light propagation from target position $\mathbf{r}$ to detectors located at $\mathbf{r}_i$; while for each scattering target, three $\mathbf{F}$ s are constructed as, $\alpha g_d, \alpha_x, \alpha_y, \alpha_z$, respectively [4]. For multiple wavelengths, the $\mathbf{F}$ associated with the detector plane is constructed as

$$g_d^{\lambda_1} = \begin{pmatrix} g_d(\mathbf{r}, \lambda_1) \\ \vdots \end{pmatrix}, \quad g_d^{\lambda_2} = \begin{pmatrix} g_d(\mathbf{r}, \lambda_2) \\ \vdots \end{pmatrix}, \quad (3)$$

Three dimensional tomographic pseudo images of sample are generated using the pseudo spectrum. The target locations are determined using the poles associated with specific $\mathbf{F}$ s, which is then used to characterize the targets in principle. For example, if the overall peak shows up in the pseudo spectrum calculated using the $\mathbf{F}$ for an absorptive target, the target is considered to be an absorptive target (absorptive contrast dominates), otherwise, a scattering target. In this study, we mainly focus on locating the targets. We use the maxima in the pseudo spectrum to determine the target position. The full-width-at-half-maximum (FWHM) of the poles is used to estimate dimension of the target(s).

The NMF and ICA analysis were carried using the multi-wavelength datasets, separately. The target locations were retrieved using each dataset. The average value of the retrieved positions was used to determine the position of a target. As discussed in Chapter 4, NCIDs and

ICIDs were obtained using NMF and ICA. The target locations were estimated by fitting the NCIDs and ICIDs, particularly on the detector plane, to the Green's functions that describe light propagation from targets to the detectors. The NCIDs were then back-projected to the z plane of the target positions to generate cross-section images. The size of a target was then estimated using the FWHM in the cross-section images.

5.3. Experimental Methods and Materials

5.3.1. Experiments

Two sets of experiments were carried out on the realistic model breast. First set involved NIR imaging measurements using 780 nm, 808 nm and 850 nm probe beams. The second set was corroborating MRI measurements.

5.3.1.1. NIR Imaging Experiments

The experimental arrangement is shown schematically in Fig. 5.1. The model breast was assembled using two pieces of normal *ex vivo* female human breast tissues and two pieces of cancerous tissues provided by National Disease Research Interchange (NDRI) under an Internal Review Board approval at the City College of New York. The normal breast tissue specimens weighed 11 grams and 12 grams and consisted primarily of adipose tissue, while each tumor (infiltrating ductal carcinoma) pieces weighed approximately 1 gram. Two incisions 1 cm apart were made in the mid-plane (along the z -axis, which was the shortest dimension) of normal tissue pieces and a small amount of tissue was removed from the core region to make two small pouches. The tumor pieces were inserted into these pouches, and the incisions were closed by moderate compression of the tissue-tumor composite from all directions. The sample was placed inside a cylindrical transparent plastic container with a movable end face of diameter 11 mm, which was moved to slightly compress the tissue along the z -axis and hold it in place. The

resulting slab sample filled over of the lateral dimension of the cylinder and had a uniform thickness of 4 mm. It was treated as a single entity in the subsequent imaging experiments.

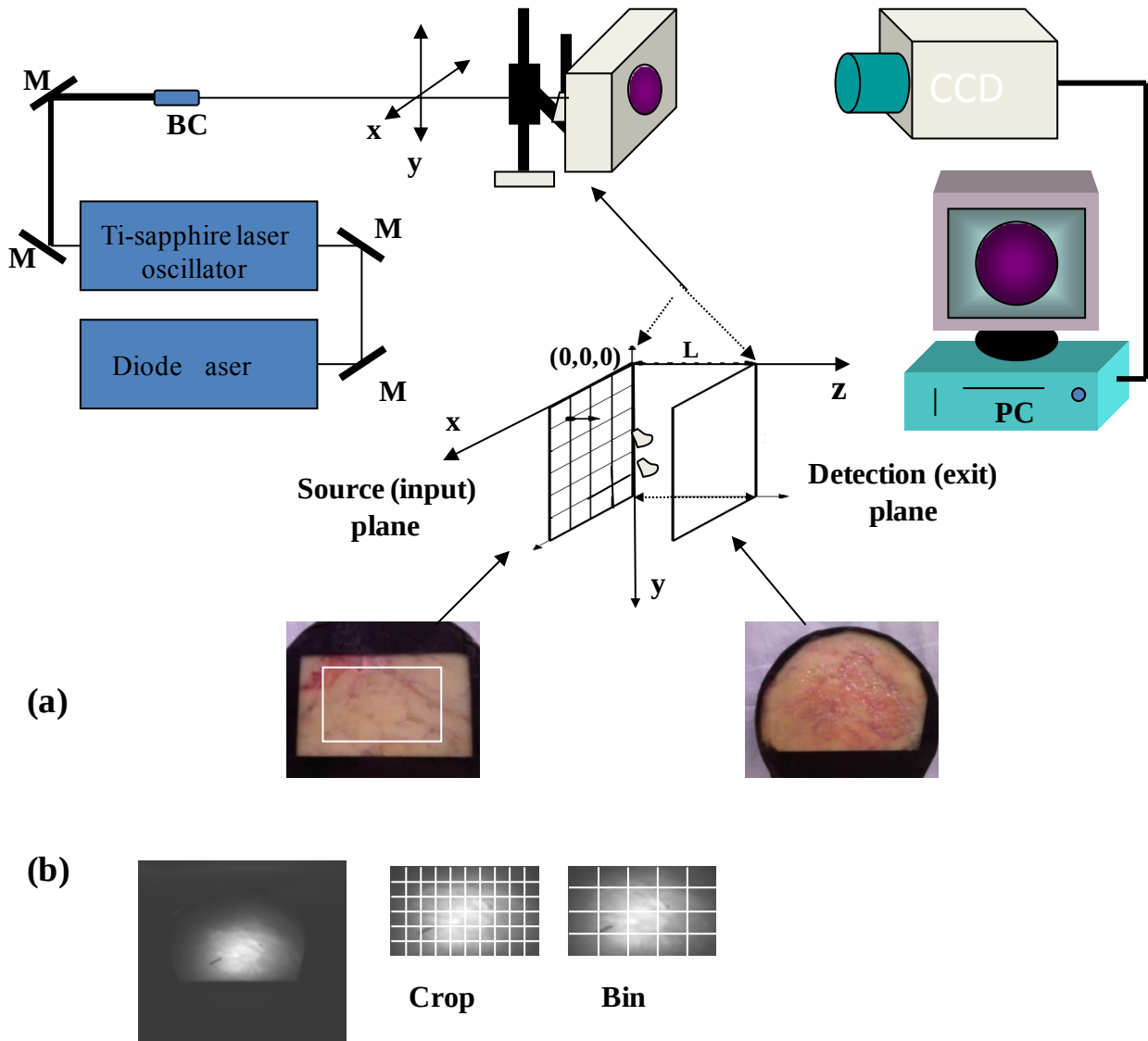


Fig. 1.1. (a) Schematic diagram of the experimental arrangement. (Key: C = beam collimator, CCD = charge coupled device, M = mirror.) Inset below shows the expanded view of the source (input) plane with the scanning grid points. (b) Photographs of the source plane (left) showing the scanned area, and detection plane (right) of the model breast sample. (c) (Upper pane) A typical raw CCD image of the detection plane, and how it is cropped and binned for analysis.

The nominal dimension of the tumor piece located at the left side of the sample (left tumor') was 1 mm 1 mm 1 mm and that located on the right side (right tumor') was 1 mm 1 mm 1 mm. The positions of the tumor pieces within the sample, and the distance between the pieces were known approximately, as those were placed in position as discussed above. The axial orientation of the plastic container and sample within it was preserved for magnetic resonance imaging (MRI) experiments following the optical measurements.

The optical imaging experiments were carried out using the 690-nm, 780-nm, and 850-nm near-infrared (NIR) beams from a continuous wave (CW) Ti:Sapphire laser. The average beam power was maintained at 1 mW for every wavelength. The light beam at any of these wavelengths was collimated to a 1-mm spot onto the entrance face (henceforth referred to as the source plane') of the slab sample. Multiple source illumination was realized in practice by step scanning the slab sample along the horizontal (x) and vertical (y) directions across the laser beam in an x-y array of grid points using a computer controlled translation stage. A camera lens collected the diffusely transmitted light on the opposite face of the sample (henceforth referred to as the detection plane') and projected it onto the sensing element of the cooled 16-bit, 1280 1280 charged couple device (CCD) camera. Each illuminated pixel of the 1280 1280 pixels of the CCD camera could be regarded as a detector. For illumination of every scanned point on the source plane, the CCD camera recorded the diffusely transmitted intensity pattern on the detection plane. A 2.5 mm 2.5 mm area of the source plane was scanned in a 256 256 array of x-y grid points with a step size of 2.5 mm, while the CCD camera imaged the entire detection plane.

The average of all 256 256 images was used to assess the average optical properties of the ex vivo model breast. The radial profile of the transmitted light intensity of the average image was

fitted to the predictions of a diffusion model of light propagation inside a slab. Assuming a typical value of 1 mm^{-1} for the reduced scattering coefficient for breast tissues, the average absorption coefficient, μ_a of the entire model breast was found to be 0.01 mm^{-1} , 0.02 mm^{-1} and 0.03 mm^{-1} for wavelengths of 650 nm , 700 nm and 750 nm , respectively. While the entire detection plane was imaged, each raw image was cropped to retain the region within 10 mm 10 mm for carrying out image reconstruction. The size of 1 pixel in the raw image was 1 mm 1 mm . The raw images were binned by merging 10×10 pixels into one to enhance the signal-to-noise ratio. All the binned images corresponding to probing of the grid points in sequence were then stacked. The difference between each individual image and the average image was calculated, which is the perturbation due to tumors in the measured light intensity distribution on the sample exit surface. The response data matrix K was then constructed for subsequently analysis.

5.3.1.2. MRI experiment

For MRI experiments, the breast model sample in the plastic container was taken to Memorial Sloan-Kettering Cancer Center (MSKCC) small animal MRI facility. The facility currently utilizes a 4.7-T 30-cm bore magnet imaging/spectroscopy system (Bruker BioSpin) operating at 200 MHz for 1-D (standard) imaging experiments. The tissue container was fixed inside the radio-frequency coil (RFC) and placed inside the bore magnet. MR images of the sample were recorded in 2-mm slice thick sagittal slices.

5.3.2. Analysis & Results

5.3.2.1. TROT analysis

In the TROT analysis, the TR matrix $T_{DSSD} = KK^T$ was calculated. Eigenvalues and eigenvectors of the T were found by solving the eigenvalue equation of T . Signal and noise subspaces were

separated with a proper threshold level. The pseudo spectrum was then calculated. The positions of the targets were determined using the poles in the pseudo spectrum.

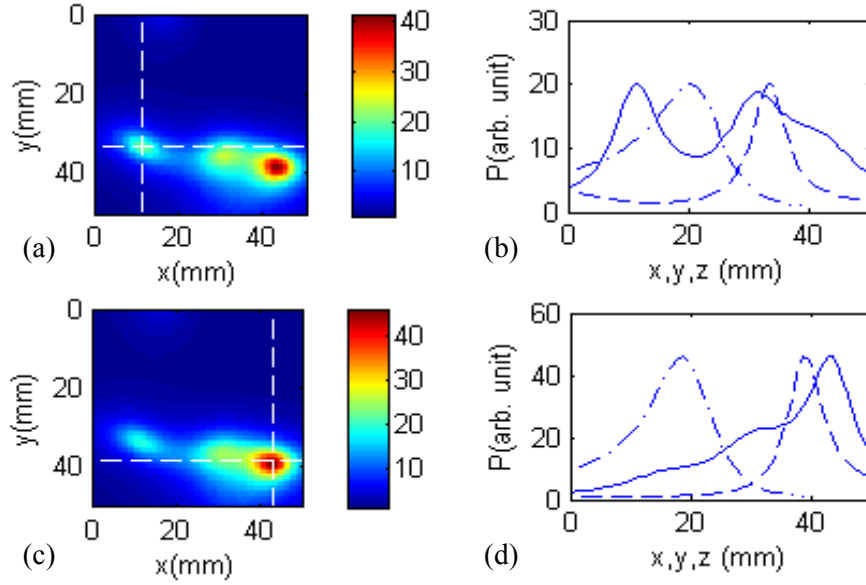


Fig. 2. (a) and (c) are TROT-generated pseudo images at the $z = 2$ mm and 1 mm for the left and right tumors, respectively, using 10 nm; (b) and (d) are profiles of the pseudo image through the target along x(-), y(-) and z(-) directions.

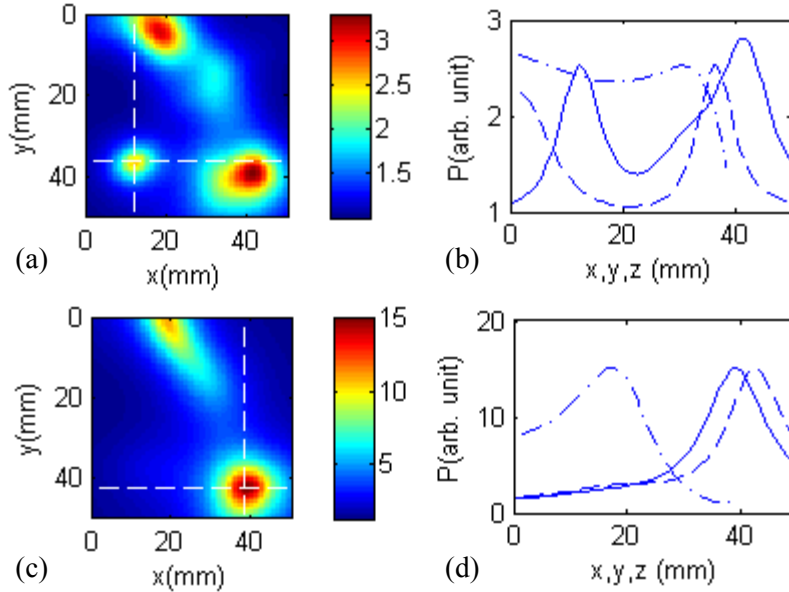


Fig. 3. (a) and (c) are TROT-generated pseudo images for the left and right tumors using 10 nm; (b) and (d) are profiles of the pseudo image through the target along x(-), y(-) and z(-) directions.

TROT was used to analyze the dataset for each individual wavelength first. Then all three datasets were put together as one multi-wavelength dataset and analyzed accordingly. The TROT-generated pseudo image at the z-coordinates of the positions of the tumors for wavelength λ_1 nm is shown in Fig. 2(a) and 2(c). Profiles through the tumor position in the x, y and z directions are plotted in Fig. 2(b) and 2(d). Similar images were obtained for wavelengths of λ_2 nm and λ_3 nm in Figs. 3 and 4.

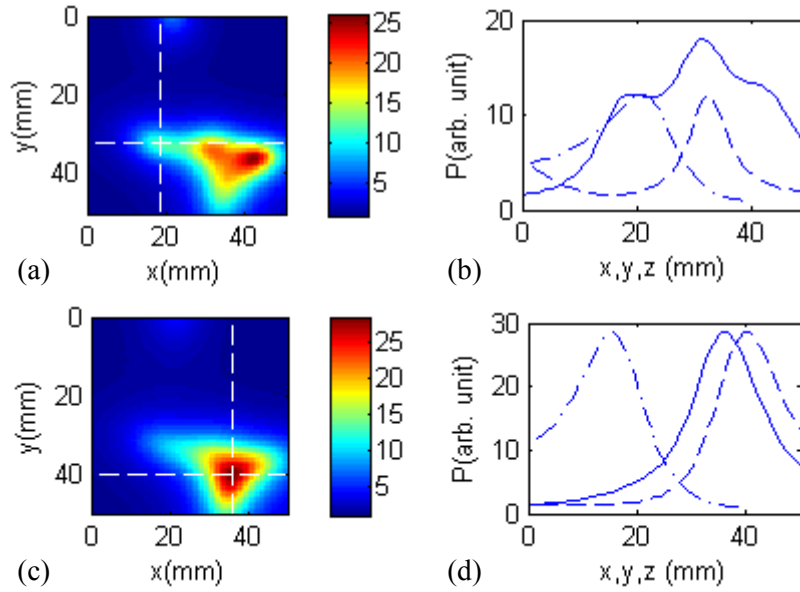


Fig. 4. (a) and (c) are TROT-generated pseudo images for the left and right tumors using λ_1 nm; (b) and (d) are profiles of the pseudo image through the target along x(-), y(--), and z(-) directions.

For λ_2 -nm measurements, a black tape was placed on the exit surface of the sample for testing. Even though there was a local maximum position located at (12.5, 12.5) mm, the global maximum position along the axial direction at the same lateral position is located on the detector plane (exit surface) of the sample, which is the same as the known location of the tape, as shown in Fig. 5. The target shown in Fig. 5(a) could be an artifact due to the tape, or a combination effect due to the tape and the left tumor. Since the left tumor is relatively smaller, as shown in the MRI images, and located at about the same location of the tape, it is hard to detect

and localize it accurately. The target shown on the top of the reconstructed image using $\lambda = 633$ nm is another artifact probably due to the boundary.

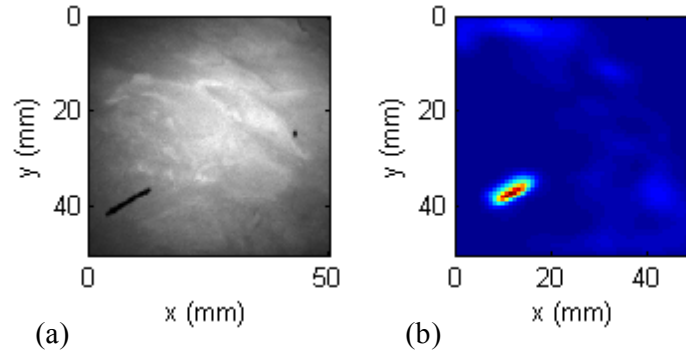


Fig. 3.10. (a) The raw image which shows the tape placed on the exit surface of the sample. (b) TROT-generated pseudo image at $z = 10$ mm which is the neighbor plane of the detector plane using $\lambda = 633$ nm.

When all three wavelengths were combined, similar images were also obtained and shown in Fig. 3.11.

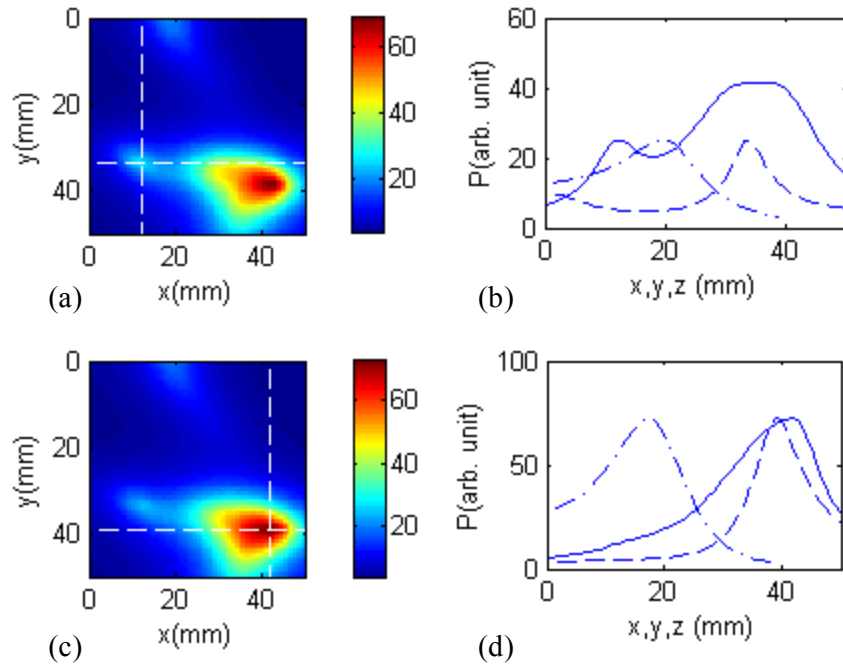


Fig. 3.11. (a) and (c) are TROT-generated pseudo images for the left and right tumors using all three wavelengths; (b) and (d) are pseudo value profiles through the target along x(-), y(--), and z(-) directions.

The peaks in the pseudo spectrum (pseudo images) were used to represent the locations of the tumors (targets). The locations of the tumors found respectively for the three wavelengths were listed in Table .1.

Table .1. Coordinates (x, y, z) of the tumors retrieved by TROT

Wavelength (nm)	Left Tumor position (x, y, z) (mm)	Right Tumor position (x, y, z) (mm)
	11. , .1, 2 .	4 .4, . , 1 .
	12. , .2, .	. , 42. , 1 .
	1 . , 2. , 2 .	.2, 4 .2, 1 .
Combined	12. , . , 1 .	41. , .4, 1 .

FW M of the peaks in the multi-wavelength pseudo spectrum was used to estimate the dimensions of the tumors. The lateral dimensions of the left tumor were estimated to be .2 mm . mm, and the right tumor, 1 . mm . mm. These estimated dimensions of both tumors are comparable to the known dimensions of the tumors.

5.3.2.2. NMF-OT and OPTICA analysis

The experimental data from Section . .1 were then analyzed using NMF-OT and OPTICA. The NCIDs and ICIDs for the -nm illumination of are shown in Fig. . and Fig. . , respectively.

The profiles in the NCIDs and ICIDs were fitted to Green's functions to retrieve the target positions. The Green's function fits for the profiles in the x direction through the maxima in the intensity distributions are shown in Figs. . (b) and . (d) for NCIDs, and Figs. . (b) and . (d) for ICIDs, respectively. Similar fits for the profiles in the y direction were obtained (not shown

here). Figs. . (e) and . (f) show the NCIDs for the left and right targets, respectively on the source plane. Similar images for ICIDs were obtained (not shown here). The source plane was relatively small and had much lower resolution. Therefore, only the NCIDs and ICIDs on the detector plane were used for further analysis.

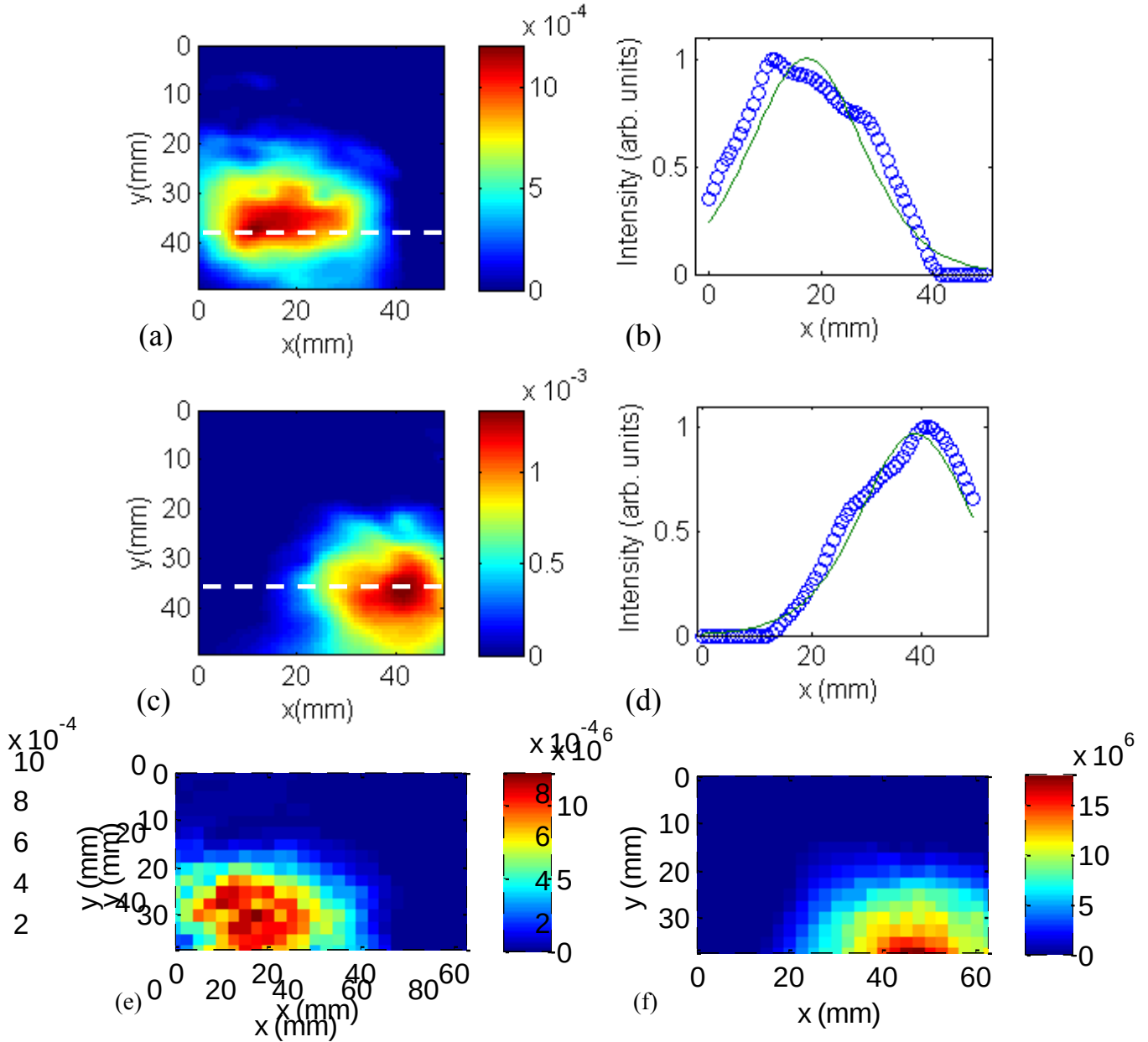


Fig. . . (a) and (c) are NCIDs of the left and right targets, respectively on the detector plane, for $\lambda = 1.55$ nm measurements. (b) and (d) are Green's function fits of the profiles through the white lines. (e) and (f) are the corresponding NCIDs for the left and right targets, respectively on the source plane.

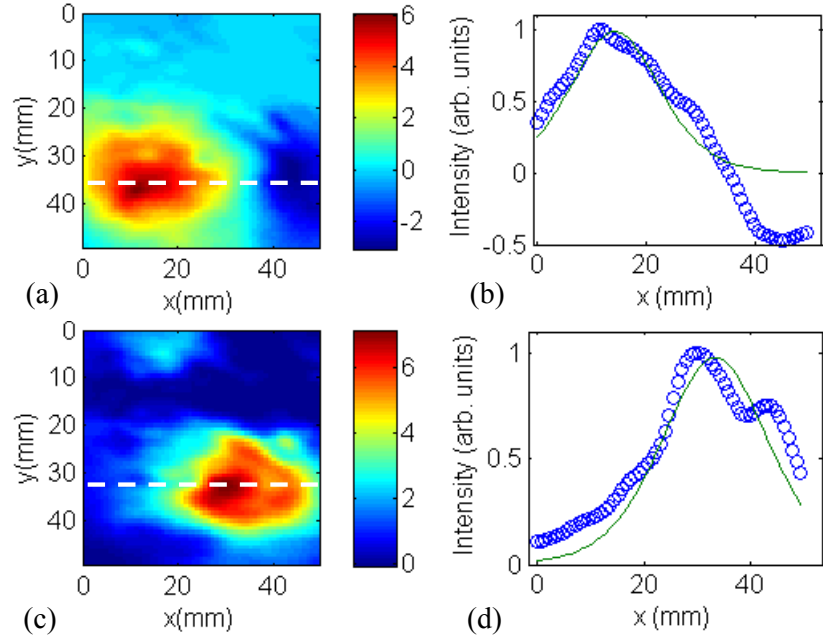


Fig. 11. (a) and (c) are ICIDs of the left and right targets respectively on the detector plane, for 100-nm measurements. (b) and (d) are Green's function fits of the profiles through the white lines.

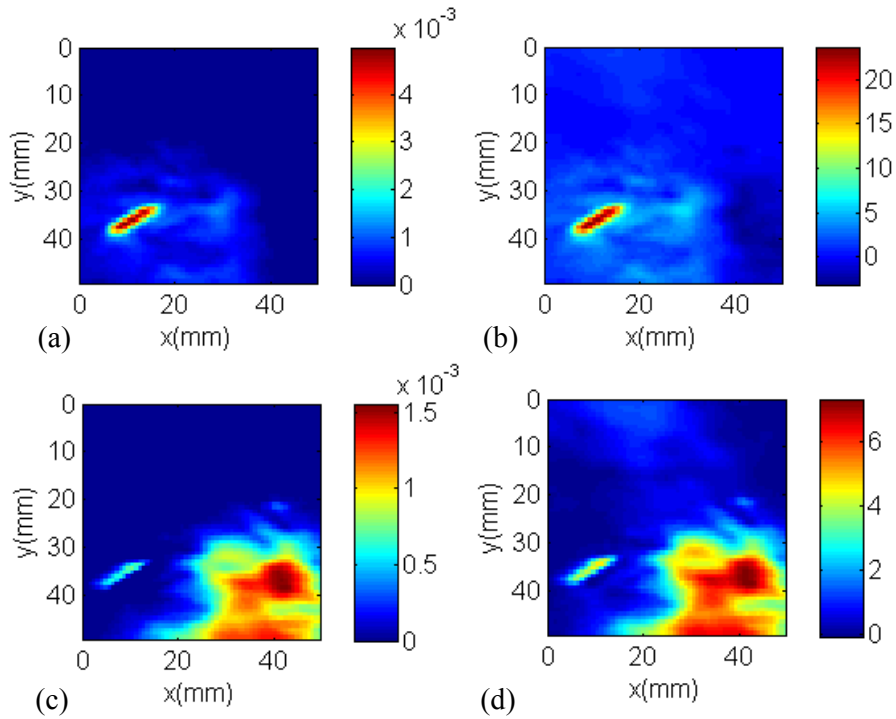


Fig. 12. (a) and (c) are NCIDs of the left and right targets, respectively on the detector plane, for 100-nm measurements, and (b) and (d) are the corresponding ICIDs.

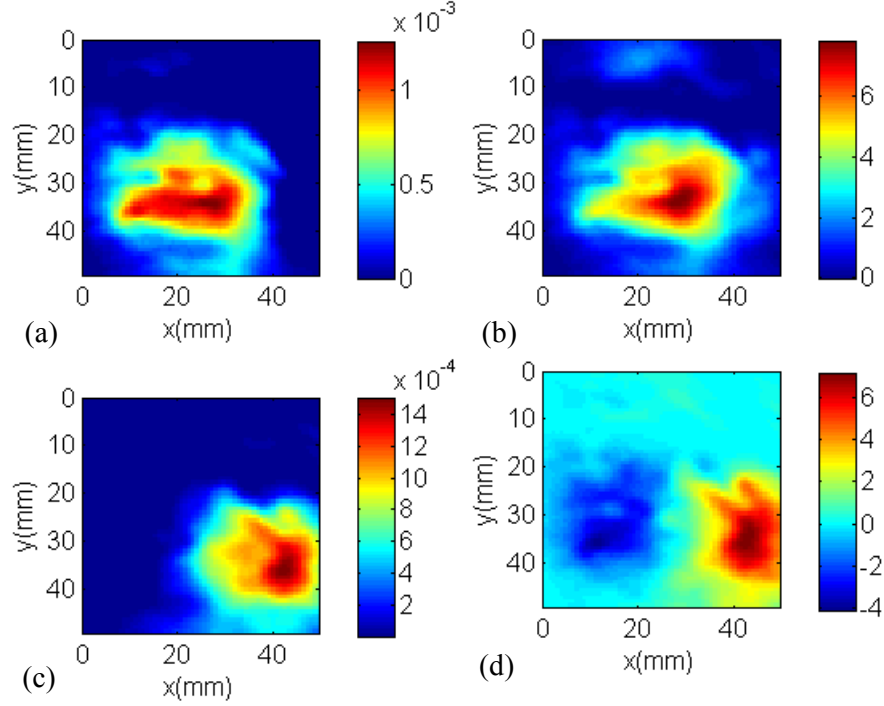


Fig. 1. (a) and (c) are NCIDs of the left and right targets, respectively on the detector plane, for 1064-nm measurements, and (b) and (d) are the corresponding ICIDs.

NCIDs and ICIDs on the detector plane for illumination of 1064 nm and 1550 nm are shown in Fig. 1 and Fig. 1, respectively. Similar NCIDs and ICIDs on the source plane were also obtained (not shown here).

Table 2. Target positions retrieved by NMF-OT and O-TICA

Illumination wavelength (nm)	Left Target position (x, y, z) mm		Right Target position (x, y, z) mm	
	NMF-OT	O-TICA	NMF-OT	O-TICA
1064	14.5, 14.5, 14.5	14.5, 14.5, 21.5	14.5, 14.5, 14.5	14.5, 14.5, 14.5
1550	N/A	N/A	14.5, 14.5, 21.2	14.5, 14.5, 21.4
1064	14.5, 14.5, 14.5	14.5, 14.5, 21.5	14.5, 14.5, 14.5	14.5, 14.5, 14.5
Average	14.5, 14.5, 14.5	14.5, 14.5, 21.5	14.5, 14.5, 14.5	14.5, 14.5, 14.5

As shown in Fig. . , due to the black tape which has approximately same lateral position as the left target on the exit surface of the sample, the left target (small tumor piece) was not obtained.

The target positions were also obtained for nm and nm illumination using reen's function fit. The retrieved positions are shown in Table .2.

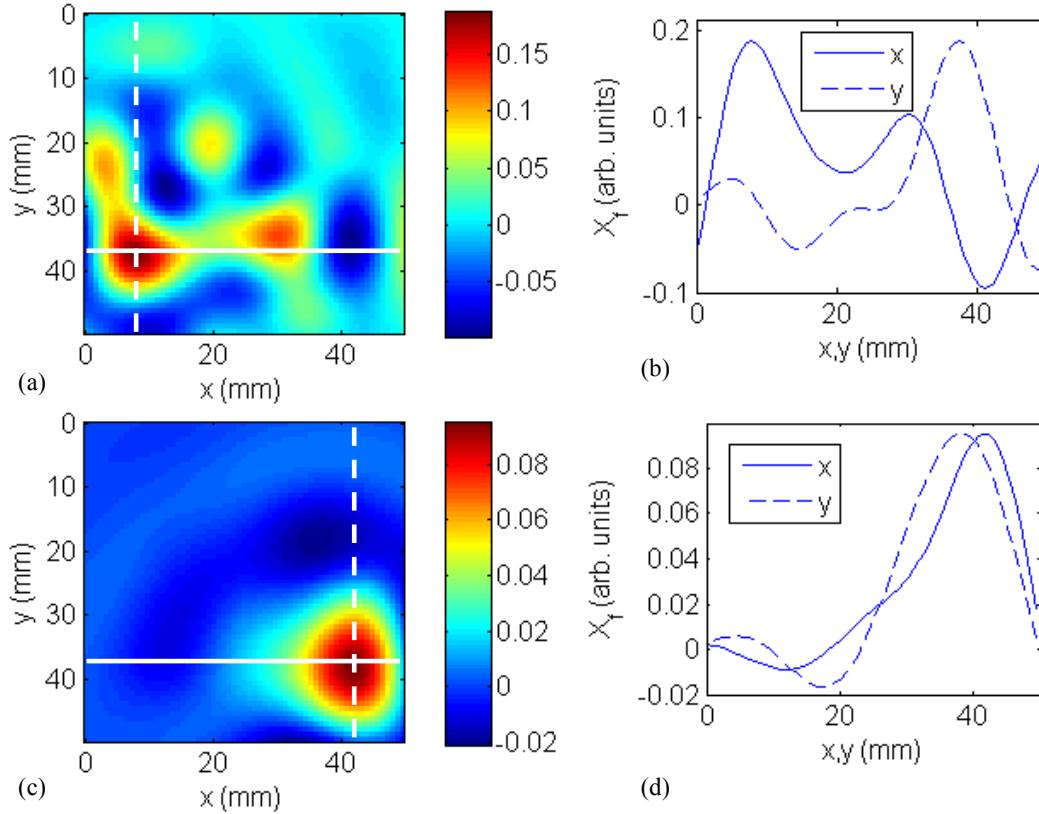


Fig. .11. (a) and (c) are cross-section images of the left and right targets, respectively generated using backprojection of the NCIDs; (b) and (d) are the profiles through the maximum in the images, whose FW Ms are used to estimate the target dimensions.

The lateral distance between the two targets is found to be 21. mm and 2 . mm using NMF-OT and O TICA respectively. These retrieved values of the distance are about 1cm less than that detected by MRI. It is probably because the tissue heterogeneity around the embedded tumors, particularly the left tumor, caused the NCIDs and ICIDs to spread out significantly.

When the weighted center of the optical property was used to describe the target location, it led to difficulty in accurate localization of the target.

The cross-section images of the targets were generated using the backprojection of NCIDs for $\lambda = 633$ -nm measurements, and shown in Fig. .11. Similar cross-section images were generated using measurements with other wavelengths. The profiles through the maxima in the cross-section images are also shown in Fig. .11, FWHMs of which are used to estimate the lateral dimension of the targets. Similar cross-section images of the targets and the profiles in the images were also generated using backprojection of ICIDs, and shown in Fig. .12. The estimated values are shown in Table . .

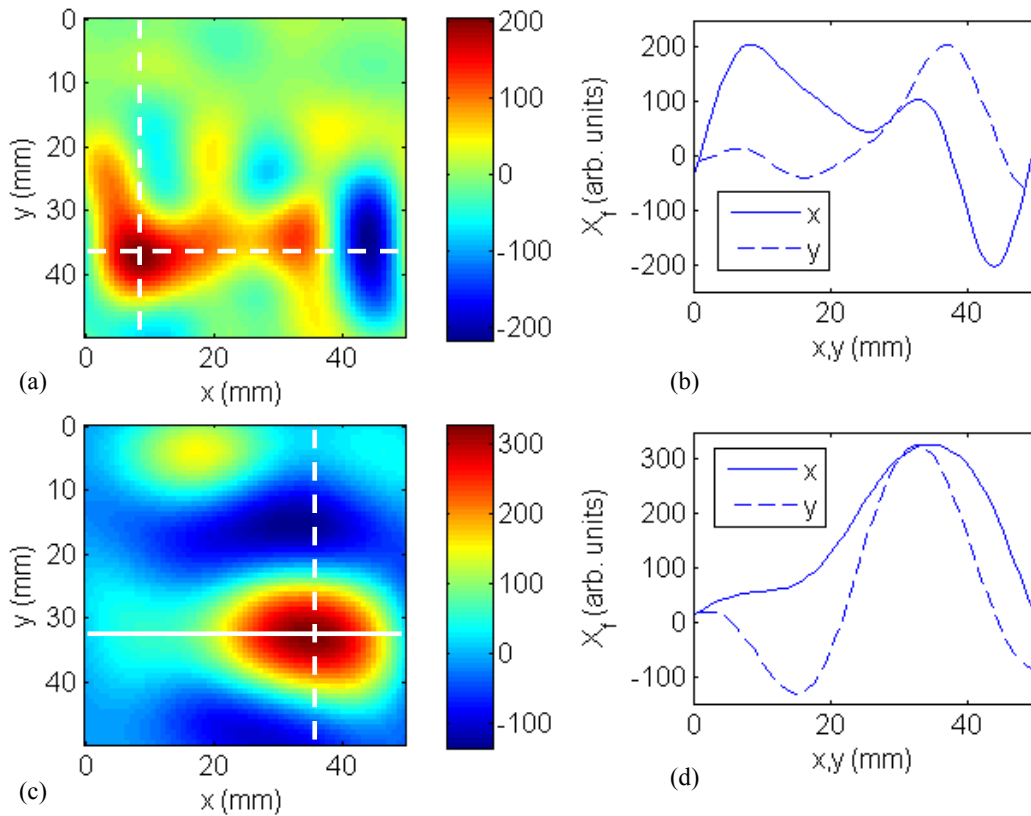


Fig. .12. (a) and (c) are cross-section images of the left and right targets, respectively generated using backprojection of the ICIDs; (b) and (d) are the profiles through the maximum in the images, whose FWHMs are used to estimate the target dimensions.

Table . . . estimated positions and dimensions of the targets using cross-section images

Method	Left Target		Right Target	
	Position (x, y) mm	Dimensions (x, y) mm	Position (x, y) mm	Dimensions (x, y) mm
NMF-OT	4, .	11.2, .	41. , .	14. , 1 .
O TICA	4, .	12. , 11.2	4. , .1	22. , 14.

The estimated target dimension using the FW Ms of the profiles of the cross-section images generated using NCIDs and ICIDs are approximately up to twice as big as the actual target dimensions. The dimensions estimated using NMF-OT are smaller than those using O TICA. This happens since the cross-section image is highly sensitive to the background heterogeneity, and flexibility exists in the regularization process.

For this dataset, even though the modified L -curve is used, the regularization parameter is from around the corner. If lower regularization is used, the target cannot be distinguished from artifacts in the cross-section image. Therefore, the maxima in the cross-section images can also be used to estimate the target positions, since it is not shifted due to regularization. The lateral distance between the two targets is estimated to be .4 mm and 2 . mm, for NMF-OT and O TICA respectively, which are close to the actual distance (mm), and better than those estimated by Green's function fit. This could be due to the higher resolution in the cross-section image than the component intensity distributions.

5.3.2.3. MRI Results

The MRI images of the two targets were recorded for 2-mm-thick slices, and placed around the mid-plane in two sagittal slices that are 4 mm apart. The MRI images of the sample through the known z positions of the two tumors are shown in Fig. .1 .

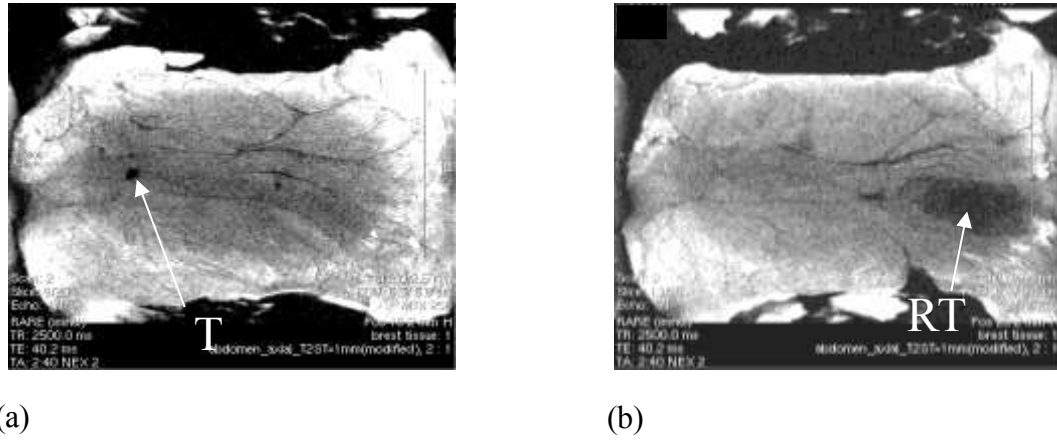


Fig. .1 . Magnetic resonance images of the left tumor and the right tumor pieces (T: left tumor, RT: right tumor)

5.4. Discussion

The efficacy of TROT and NMF-OT for detection and localization of tumors was tested and compared to MRI and O TICA. In realistic conditions, the perturbations in the measured light intensities due to absorptive and scattering inhomogeneities are very similar and difficult to distinguish, which was also reported by other groups [1, 14]. The situation may be improved using frequency-domain measurements. The results from the experiments showed that TROT and NMF-OT could detect, locate and estimate the dimension of targets (breast tumors) inside biological tissue (normal tissue). The locations of the targets retrieved by NMF-OT are comparable to that retrieved by O TICA. However, the locations retrieved by NMF-OT and O TICA using Green's function fit are not as accurate as those retrieved by TROT, particularly

when significant background heterogeneity is present around the targets. This need be verified by further experiments.

The dimensions of the targets estimated by NMF-OT are comparable to O TICA. In the experiment, the NMF-OT and O TICA-estimated target dimensions are less accurate than the TROT-estimated values, which is probably also due to the influence of the background heterogeneity. Tikhonov regularization using a modified *L*-curve method is used in inverse Fourier transform algorithm for NMF-OT and O TICA. However, this method is highly sensitive to the background heterogeneity (signal-to-background ratio), since the regularization parameter is selected by comparing the target to the rest part of the cross-section image which is sensitive to the boundary condition, noise and background heterogeneity. For this dataset, the cross-section images generated using backprojection algorithm used regularization parameter around the “corner” of the *L*-curve, since higher-resolution images cannot be generated using lower regularization. Then the cross-section images were also used to locate the targets, which resulted in improved accuracy in the localization. This may be due to higher resolution in the cross-section image than the component intensity distributions.

The experiments showed the multi-wavelength data may help to improve the localization of targets. The MRI images of the sample displayed in Fig. 1 were obtained to serve as reference for testing the validity of the TROT, NMF-OT and O TICA measurements. The MRI measurements corroborate the assessments of TROT, NMF-OT and O TICA in terms of the number of targets, the distance separating them, and their depth (*z*-position). TROT and NMF-OT-retrieved positions are within 1 mm from the mid-plane (*z* = 2 mm). O TICA-retrieved positions are within 2 mm from the mid-plane. This is a good agreement between the results

from all the methods. Similarly, all the approaches provide comparable estimates for overall distance between the tumor pieces.

In TROT analysis, when each individual wavelength was used, the two targets were clearly detected and accurately localized when 635 nm was used; while the image of left tumor was hardly separated from the right tumor when 785 nm was used. For 635-785-nm illumination, when a black tape was placed on the exit surface of the sample around the same lateral position of the left tumor, the left tumor was not accurately localized in the reconstructed image. This mimics a dynamic condition in *in-vivo* experiments where instability may be present. When all three wavelengths were combined, two tumors were detected with accurate positions retrieved. The black tape as a “random false target” was filtered out and not shown in images anymore. At the same time, the artifact was suppressed by the TROT algorithm automatically, and not shown as a prominent “false” target. The left tumor was shown to be much smaller and difficult to detect than the right tumor. The distance between the centers of the left and right tumors was found to be 1.5 mm. The result is comparable to that from MRI images.

TROT is a fast approach for DOT with no iterations of forward model involved. *A priori* information of the targets was not used for the approach. Even though, the slab geometry, diffusion model and CW measurements were used in this study, TROT is applicable to different geometries, different forward models, and different measurement modes such as frequency-domain and time-domain. TROT has a potential to be used in real-time breast cancer detection and imaging.

In NMF-OT and O-TICA analysis, while the three wavelengths that we used were not targeted for obtaining diagnostic molecular spectroscopic information, we observed some instructive and clarifying advantages of employing light of different wavelengths. In addition to

the two tumor pieces shown in Fig. . Fig. .1 , another NCID and ICID (not shown in any figure) appeared prominently in both NMF-OT and O TICA analysis conducted using all three wavelengths. However, strength of two of the NCIDs and ICIDs corresponding to tumor pieces decreased monotonically with increasing wavelength of the probing light, but no such wavelength dependence was observed in the strength of the third independent component. We tentatively ascribe this feature to an embedded water pocket within the sample. Multi-wavelength measurements helped sort out this artifact. The monotonic decrease in strength of the tumor pieces with increasing wavelength is consistent with the wavelength dependence of light scattering by tissue and tumor constituents.

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Chapter 6

Fluorescence imaging using time reversal optical tomography and non-negative matrix factorization

6.1. Introduction

Near-infrared (NIR) fluorescence imaging is emerging as a promising modality for early detection of cancer for a variety of reasons [1-1]. Fluorescence signals are generally noninvasive, and have the intrinsic potential to provide molecular information about biological tissues and changes in significant physiological parameters associated with the onset and progression of disease. Fluorescence imaging is characterized by higher detection sensitivity and specificity, as well as, higher target-to-background ratio and spatial resolution than absorption and scattering contrast-based optical imaging approaches. The problem of limited tissue depth penetration may be considerably resolved by operating in the NIR spectral range of 700-900 nm. Development of target-specific exogenous contrast agents holds the promise for tumor detection and characterization with high specificity. Much recent effort has gone into the development of fluorescent contrast agents, such as, dyes [2], nanoparticles [3], and molecular beacons [4]. Concomitant developments have taken place in imaging instrumentation and numerical algorithms for image reconstruction [5-1]. These advances have culminated in contrast-enhanced fluorescence tomography approaches that are used more often for *in vivo* studies on animal models [6, 7], than for human subjects [8, 11] or realistic phantoms [9, 10, 11].

In this chapter, we report on two fluorescence tomography approaches. One is the extension of time reversal optical tomography (TROT) [14] to fluorescent targets embedded in a turbid

medium, where, the contrast is based on the difference in fluorescence between the target and the intervening medium. We refer to it as *fluorescence TROT*. We build on the TROT formalism introduced in Chapter 2 for locating small absorptive and scattering targets. TROT combines the methodology of time reversal (TR) imaging [1] with multiple signal classification (MSC), [1] a subspace based processing methodology to locate unknown targets from measurements using multiple probes. We demonstrate that with a nominal modification of experimental arrangement fluorescence TROT and transillumination TROT measurements can be carried out in consecutive runs providing complementary approaches to target detection and localization. [14]

The other approach is based on non-negative matrix factorization (NMF) [1], which is the extension of the NMF decomposition approach introduced in Chapter 4 to fluorescent targets. The NMF based fluorescence tomography approach treats detecting and locating targets within a turbid medium (such as, tumors inside human breast) as a blind source separation (BSS) problem [1], and seeks a solution based on non-negative matrix factorization (NMF) [1], [15]. BSS is a general problem in information theory that involves retrieval of “component” signals from measured signals. The measured signals are weighted mixtures of the component signals contributed by the targets (“blind sources”) and may be expressed as:

$$X = AS, \quad (4.1)$$

where rows of X represent the measured mixed signals, rows of S represent the component signals, and A is the mixing matrix.

Fig. 4.1 schematically illustrates the measurement scheme of the proposed fluorescence tomography approach for a slab sample that consists of fluorescent targets embedded in a highly scattering turbid medium. The scheme uses multi-source illumination of a part of the sample (“*source plane*”) by excitation light of wavelength λ_x and multi-detector acquisition of the induced fluorescence signals of wavelength λ_m that emerge from the opposite face (“*detector plane*”). The excitation light beam incident

at a point on the source plane diffusely transmits through the sample and induces the embedded targets to fluoresce. Fluorescence from the targets diffusely transmits through the sample and gives rise to a two-dimensional (2-D) spatial intensity distribution of fluorescence signal on the detector plane, which is recorded by a multiple-detector arrangement. Similar signals are recorded for excitation beam incident on other points on the source plane. We realize the multi-source illumination scheme by step-scanning the sample across the excitation light beam in a two-dimensional (2-D) array of grid points, and implement the multi-detector signal acquisition scheme by imaging the detector plane onto the sensing element of a charge coupled device (CCD) camera with every pixel acting as a detector. Every recorded fluorescence signal is a weighted mixture of component signals from the fluorescent targets. Such a multi-source probing and multi-detector signal acquisition culminates in a robust data set for retrieving target information.

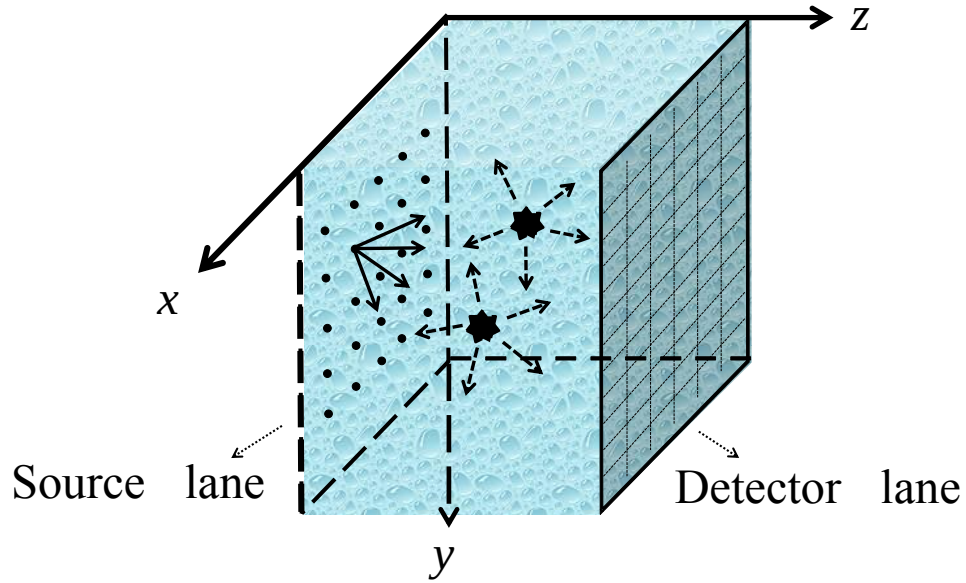


Fig. .1. Multi-detector acquisition of fluorescence (wavelength, λ_m) intensity distribution on the detector plane following multi-source illumination (wavelength, λ_s) of the source plane of the sample.

The task of retrieving target information from these mixtures is a SS problem and here we adapt NMF for the task. The non-negativity constraint makes NMF particularly suited for fluorescence tomography because in principle fluorescence signal appears on a dark background, and in common practice measured on a weak background, and hence is expected to be intrinsically positive.

We test the efficacy of the *fluorescence* TROT and the NMF-based fluorescence tomography using experimental data on a “human breast-simulating phantom”, that is, a sample whose size and key optical properties are similar to the average values of those parameters for a typical human breast.

6.2. Theoretical formalism

6.2.1. Fluorescence TROT

Fluorescence TROT uses a multi-source illumination and multi-detector signal acquisition scheme to acquire multiple angular views of the sample that consists of a target (or, targets) embedded in a turbid medium, such as, a tumor in human breast. excitation beam of light sequentially illuminates an array of N_s source points on the source plane of the sample realizing multiple-source probing. Once any of these N_s points (“excitation sources”) is illuminated the diffusely propagating excitation beam of wavelength λ_x induces the targets (“fluorescent sources”) embedded in the medium to fluoresce. The fluorescent signal at the opposite boundary (“detector plane”) is recorded by N_d detectors. The focus is to detect the target(s) and retrieve three-dimensional ($-D$) location information.

light propagation in a highly scattering medium with embedded fluorescent targets excited by an external light source is approximately described by coupled diffusion equations at the excitation wavelength (λ_x) and emission wavelength (λ_m) [2, 21] :

$$-\nabla \cdot [D_x(\mathbf{r}, \omega) \nabla \phi_x(\mathbf{r}, \omega)] + [\mu_{a_x}(\mathbf{r}, \omega) - i\omega/c] \phi_x(\mathbf{r}, \omega) = \delta(\mathbf{r} - \mathbf{r}_s), \quad (6.2a)$$

$$-\nabla \cdot [D_m(\mathbf{r}, \omega) \nabla \phi_m(\mathbf{r}, \omega)] + [\mu_{a_m}(\mathbf{r}, \omega) - i\omega/c] \phi_m(\mathbf{r}, \omega) = \phi_x(\mathbf{r}, \omega) \gamma(\mathbf{r}) / [1 - i\omega\tau(\mathbf{r})], \quad (.2b)$$

where the subscripts x and m denote parameters pertaining to excitation wavelength and emission wavelength, respectively; ω is the modulation angular frequency; $\phi_x(\mathbf{r}, \omega)$ and $\phi_m(\mathbf{r}, \omega)$ are photon densities at position $\mathbf{r}$; μ_{a_x} and μ_{a_m} are absorption coefficients of the medium; $D_x(\mathbf{r}, \omega)$ and $D_m(\mathbf{r}, \omega)$ are the diffusion coefficients of the medium; $\gamma(\mathbf{r}) = \eta\mu_{a_f}(\mathbf{r})$ is the fluorescent yield of the fluorophore in the target; and η , μ_{a_f} and $\tau(\mathbf{r})$ are the quantum yield, absorption coefficient at the excitation wavelength, and fluorescence lifetime of the fluorophore, respectively.

Assuming fluorescent targets are localized, that is, the j^{th} target is contained in volume V_j centered at $\mathbf{r}_j$, the fluorescence signal under illumination by a point source of unit power at $\mathbf{r}_s$ is given by,

$$U_m(\mathbf{r}_d, \mathbf{r}_s, \omega) = \sum_j G_m(\mathbf{r}_d, \mathbf{r}_j, \omega) f_j(\omega) G_x(\mathbf{r}_j, \mathbf{r}_s, \omega), \quad (.)$$

where $G_x(\mathbf{r}_j, \mathbf{r}_s, \omega)$ is a Green's function that describes the propagation of the excitation light of wavelength λ_x from the source at $\mathbf{r}_s$ to the j^{th} target contained in volume V_j centered at $\mathbf{r}_j$; $G_m(\mathbf{r}_d, \mathbf{r}_j, \omega)$ is a Green's function that describes the propagation of the fluorescence of wavelength λ_m from j^{th} target to the detector at $\mathbf{r}_d$;

$$f_j(\omega) = \gamma(\mathbf{r}_j) c_m V_j / [1 - i\omega\tau(\mathbf{r}_j)], \quad (.4)$$

is the fluorescence strength of the j^{th} target, where γ is the fluorescence yield, c_m is the speed of light in the medium, and τ is the fluorescence lifetime..

The fluorescence signal can be re-written in a matrix form as

$$X = \sum_j g_d(\mathbf{r}, \omega) f_j(\omega) g_s^T(\mathbf{r}, \omega), \quad (.)$$

where $g_s(\mathbf{r}, \omega) = G_x(\mathbf{r}, \mathbf{r}_s, \omega)^T$ and $g_d(\mathbf{r}, \omega) = G_m(\mathbf{r}_d, \mathbf{r}, \omega)^T$, (the superscript T denotes transpose) are Green's function vectors. X describes the diffuse propagation of the excitation light beam of wavelength, λ_x , from the sources through the medium to the targets, and then the propagation of fluorescence of wavelength, λ_m from the targets to the detectors. It also holds that X^T describes the virtual process of fluorescent light propagation from the positions of detectors to the targets, and then the propagation of the excitation light of wavelength λ_x , from the positions of the targets to the sources. A time reversal matrix $T_{SDDS} = X^\dagger X$, $T_{DSSD} = (X^T)^\dagger X^T = X^* X^T$ in frequency domain is then constructed, where the superscript † denotes hermitian conjugate, or $T_{SDDS} = X^T X$, $(T_{DSSD} = X X^T)$ when using the continuous wave (CW) illumination, i.e. ω is real. T_{SDDS} and T_{DSSD} describe two virtual time-reversed light propagations between the sources and detectors through targets, as described in Section 2.2.1. In Section 2.2.2, the light propagating between sources and targets and the light between targets and detectors have the same wavelengths, while here they are at different wavelengths, namely, one at excitation wavelengths, and the other at emission wavelengths. T_{DSSD} and T_{SDDS} have eigenvectors u_k , $k = 1, \dots, N_d$ and v_l , $l = 1, \dots, N_s$, respectively, with a common set of eigenvalues μ_j , $j = 1, \dots, \min(N_s, N_d)$, where N_s and N_d are numbers of sources and detectors, respectively [14]. If the fluorescent targets are well resolved, the eigenvalues are proportional to squared fluorescence strengths of the targets,

$$\mu_j = |f_j|^2 \|g_d(\mathbf{r}_j, \omega)\|^2 \|g_s(\mathbf{r}_j, \omega)\|^2; \quad (2.20)$$

otherwise, they are linear combinations of the fluorescence strengths, similar to what is the case for absorptive and scattering targets. [14]

The eigenvectors are separated into signal and noise subspaces using an L -curve method with an eigenvalue threshold ε . 22 More details of the L -curve method has been provided in Section

.2. The locations of targets are poles in the M SIC pseudo spectrum 14

$$P_d(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| \mathbf{u}_j^T \frac{\mathbf{g}_d(\mathbf{X}_p, \omega)}{\|\mathbf{g}_d(\mathbf{X}_p, \omega)\|} \right|^2, \quad (. a)$$

associated with the detector plane, where $\mathbf{X}_p$ runs over positions of all voxels in the sample. A similar pseudo spectrum for the source plane $P_s(\mathbf{X}_p, \omega)$,

$$P_s(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| \mathbf{v}_j^T \frac{\mathbf{g}_s(\mathbf{X}_p, \omega)}{\|\mathbf{g}_s(\mathbf{X}_p, \omega)\|} \right|^2, \quad (. b)$$

or for both detector and source planes,

$$P(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left(\left| \mathbf{u}_j^T \frac{\mathbf{g}_d(\mathbf{X}_p, \omega)}{\|\mathbf{g}_d(\mathbf{X}_p, \omega)\|} \right|^2 + \left| \mathbf{v}_j^T \frac{\mathbf{g}_s(\mathbf{X}_p, \omega)}{\|\mathbf{g}_s(\mathbf{X}_p, \omega)\|} \right|^2 \right), \quad (. c)$$

may also be used to retrieve the target position.

From eq. (.), the fluorescence strengths of the targets may then be retrieved by unmixing the data matrix using Green's functions associated with the retrieved positions of the targets, as introduced in Chapter ,

$$\text{diag}(f_1, f_2, \dots) = \mathbf{g}_d(\mathbf{r}_1, \omega), \mathbf{g}_d(\mathbf{r}_2, \omega), \dots^{-1} \mathbf{X} \mathbf{g}_s(\mathbf{r}_1, \omega), \mathbf{g}_s(\mathbf{r}_2, \omega), \dots^T^{-1}, \quad (.)$$

where the data matrix could be rank-reduced data matrix as introduced in Chapter .

In this work, we used the pseudo spectrum, P_d associated with the detector plane because: (a) the images naturally reside in the co-ordinate system based on the field of view of the charge-coupled device (CCD) camera (*detectors*) making data analysis easier; and (b) the use of many more detectors than sources in our experimental arrangement provides a more robust data set for

superior noise-resistant and artifact-tolerant reconstruction in the detector plane than the source plane. We set $\omega = 0$ as a CW laser beam was used in the experiment.

6.2.2. NMF-based Fluorescence Tomography

The formalism considers this fluorescence signal to be a weighted mixture of signals arriving from the embedded targets. A different signal is obtained when another source point is illuminated. The fluorescence signal measured by the detector at $\mathbf{r}_d$ for illumination of the source point at $\mathbf{r}_s$ may be expressed as the following form of eq. (4.1):

$$x(\mathbf{r}_d, \mathbf{r}_s) = \sum_j a_j(\mathbf{r}_d) s_j(\mathbf{r}_s), \quad (4.2)$$

where $a_j(\mathbf{r}_d)$ is the mixing vector and $a_j(\mathbf{r}_d) s_j(\mathbf{r}_s)$ represents the contribution of the j^{th} target to the signal. The sum is over all the targets (“fluorescent sources”). NMF retrieves $s_j(\mathbf{r}_s) = s_j(\mathbf{r}_{s1}), s_j(\mathbf{r}_{s2}), \dots, s_j(\mathbf{r}_{sN_s})$ and $a_j(\mathbf{r}_d) = a_j(\mathbf{r}_{d1}), a_j(\mathbf{r}_{d2}), \dots, a_j(\mathbf{r}_{dN_d})^T$ assuming those to be non-negative, where superscript $(^T)$ denotes transpose. Commonly used NMF algorithms include the multiplicative update method [1] and alternating least squares (ALS) method [24, 25]. We implement the ALS method that uses alternating least squares steps to estimate A (or S), and use that estimate to optimize S (or A), and keep repeating the alternative steps until the desired optimization is obtained. Non-negativity is ensured by setting any negative element of A or S equal to 0. We used an available NMF toolbox [26] to carry out the computation.

Comparing eq. (4.2) and eq. (4.1) we find,

$$s_j(\mathbf{r}_s) = \alpha_j G_x(\mathbf{r}_j, \mathbf{r}_s, \omega), \quad (4.3a)$$

and

$$a_j(\mathbf{r}_d) = \beta_j G_m(\mathbf{r}_d, \mathbf{r}_j, \omega), \quad (4.3b)$$

where α_j, β_j are scaling factors. One refers to $s_j(\mathbf{r}_s)$ and $a_j(\mathbf{r}_d)$ as non-negative component intensity distributions (NCIDs) on the source plane and detector plane, respectively, since those are proportional to the corresponding light intensity distributions and from reciprocity $G_x(\mathbf{r}_j, \mathbf{r}_s, \omega)$ $G_x(\mathbf{r}_s, \mathbf{r}_j, \omega)$. Since we used a slab sample in the experiment, the Green's functions to be used in above equations are those for slab geometry in the diffusion approximation assuming a uniform background medium, as detailed elsewhere [2, 22].

The task of retrieving the locations of the targets involves fitting of the NCIDs to the Green's functions, and we use the following least square fitting for the j^{th} target

$$\arg \min_{\alpha_j, \beta_j, \mathbf{r}_j} \sum_{\mathbf{r}_s} \alpha_j^{-1} s_j(\mathbf{r}_s) - G_x(\mathbf{r}_j, \mathbf{r}_s, \omega)^2 + \sum_{\mathbf{r}_d} \beta_j^{-1} a_j(\mathbf{r}_d) - G_m(\mathbf{r}_d, \mathbf{r}_j, \omega)^2. \quad (.1)$$

The fitting using eq. (.1) provides optimal estimates of the two scaling factors α_j and β_j and the location $\mathbf{r}_j$ of the j^{th} target. The fluorescence strength then is

$$f_j = \alpha_j \beta_j. \quad (.11)$$

Another important consideration is the size of the targets. A back projection of $U_{m_j}(\mathbf{r}_d, \mathbf{r}_s, \omega)$ from the detection plane onto the “target plane” ($z = z_j$ plane) provides an estimate of the target size [1]. The fluorescence signal due to the j^{th} target can be approximated by [1]

$$U_{m_j}(\mathbf{r}_d, \mathbf{r}_s, \omega) = \int_{z=z_j} G_m(\boldsymbol{\rho}_d - \boldsymbol{\rho}, \omega) \chi_j(\boldsymbol{\rho}) G_x(\boldsymbol{\rho} - \boldsymbol{\rho}_s, \omega) d\boldsymbol{\rho}, \quad (.12)$$

where $\boldsymbol{\rho}_s$ and $\boldsymbol{\rho}_d$ are the lateral coordinates of the source and the detector, and the integration is over the $z = z_j$ plane. In the Fourier space $\chi_j(\mathbf{q})$ follows from eq. (.12) as,

$$\chi_j(\mathbf{q}) = \frac{U_{m_j}(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s, \omega)}{G_m(\mathbf{q} - \mathbf{q}_s, \omega) G_x(\mathbf{q}_s, \omega)}, \quad (.1)$$

where $\mathbf{q}$ and $\mathbf{q}_s$ are the spatial frequencies on the x - y plane and $*$ denotes complex conjugate. The inverse Fourier transform of $\chi_j(\mathbf{q})$ provides the cross-section image of the j^{th} target at the $z = z_j$ plane.

This NMF-based optical imaging approach may be realized with slab, cylindrical and other geometries; and time-resolved, frequency domain and continuous wave (CW) measurement schemes. Data collected in backscattering mode may be used as well. In this article, we focus on localization of targets with CW (ω) measurements in forward propagation mode.

6.3. Experimental Materials and Methods

The experimental arrangement for fluorescence imaging of targets in a turbid medium is shown schematically in Fig. 2. It used a multi-source sample excitation and multi-detector fluorescence signal acquisition scheme to acquire multiple angular views of the sample. The fluorescence light intensities were measured on the boundary of the medium by a two-dimensional detector array when an external point source (laser beam) scanned the other side of the medium.

The sample consisted of a 2 mm × 2 mm × 1 mm transparent plastic container filled with Intralipid-2 (scatter, product 202) in water suspension in water as a slab of scattering medium. Separate experiments were carried out with one and two 4.2-mm-diameter 1-mm cylindrical glass tubes filled with a solution of Indo-cyanine green (ICG) dye (Sigma-Aldrich, product I2500) as fluorescent targets. In both cases, the target(s) was (were) embedded in the mid-plane ($z = 0.5$ mm) of the sample cell.

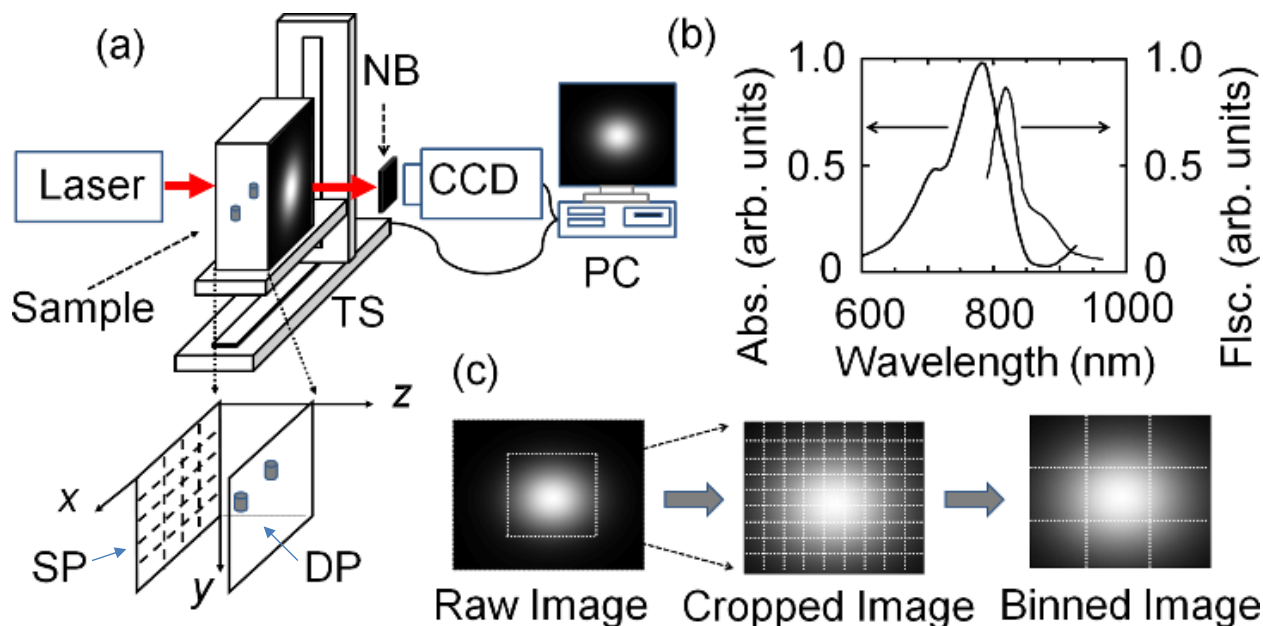


Fig. 2. (a) A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium. Key: NB narrow band pass filter (10 nm), TS translational stage, CCD charge coupled device, PC personal computer, SP source plane, DP detector plane. Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam, and a typical raw image is shown in the PC monitor. (b) The absorption and fluorescence spectra of IC in water. (c) A typical raw image detected by the CCD camera is cropped and binned.

The concentration of Intralipid-2 was adjusted to provide a transport mean free path l_t of 1.5 mm at λ_x 690 nm, and 1.0 mm at λ_m 800 nm. More details about Intralipid suspension have been presented in Section 2.1. We chose the optical properties and the thickness (10 mm) of the sample cell to emulate average values of those parameters for a compressed human breast and the size of the target to resemble that of small tumors. The IC concentration in the targets was 1 M which provided an absorption coefficient of 0.2 mm^{-1} at 690 nm and 0.2 . The absorption coefficient of IC was measured in aqueous solution. The absorption spectrum, shown in Fig. 2(a), spans 600 - 1000 nm wavelength range with a peak at 800 nm. The fluorescence spectrum for 690-nm excitation is shown in Fig. 2(b). It spans the 600 - 1000 nm wavelength range with a peak at 850 nm.

nm wavelength range and peaks around 1 nm. The quantum yield of IC is 0.1. The Intralipid-2 concentration in the target was same as that of the background to ensure that both had the same scattering characteristics. The targets were placed in the mid-plane of the sample cell and their known locations appear in Table 1. The targets were treated as fluorescent targets for the measurements using emitted light in a narrow band around nm, and as absorptive targets for transillumination measurements using light at the excitation wavelength of nm.

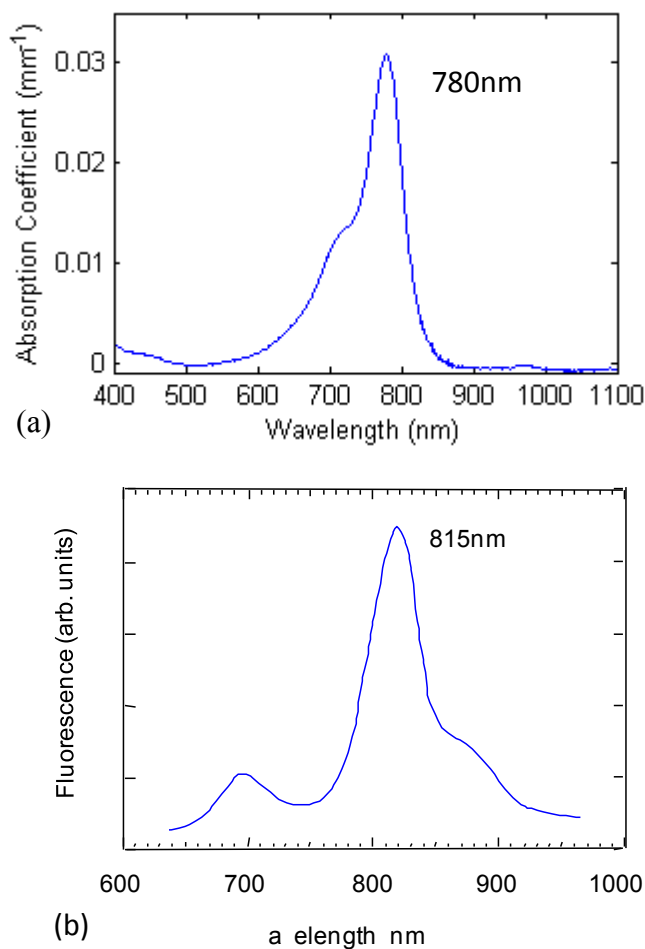


Fig. 1. Room-temperature (a) absorption spectrum, and (b) fluorescence spectrum of 1 μM IC aqueous solution.

A computerized translation stage (Aerotech Model ATS 211-M-4 with index 11 controller) scanned the sample across a 10-mm, 10-mW, CW diode-laser (Covant Technology, Model I1C1) beam in a two-dimensional (x-y) array of 11 × 11 grid points, with a step size of 1 mm to realize the multi-source probing scheme. We refer to the 20-mm × 20-mm sample surface that faced the laser beam as the *source plane*, and the opposite face as the *detector plane*. The forward-propagating fluorescence (or, transmitted) signal was collected by imaging the detector plane onto the sensing element of a 1280 × 1280 pixels cooled CCD camera (Photometrics C11440) using a 100-mm focal-length camera lens. Each illuminated 240-μm pixel of the CCD camera served as a detector. For fluorescence imaging, the signal was passed through a narrow-band interference filter centered at 680 nm (FWHM 10 nm, 90% transmission) to effectively block the scattered 405-nm pump light. The integration time is 0.5 seconds. An interfaced personal computer (C) controlled the sample scanning, as well as, data acquisition and storage operations. The C recorded the raw images for each scan position, and stored those for subsequent analysis. Each illuminated pixel of the CCD camera could be regarded as a detector. A typical image, which is a 2-D intensity distribution, is shown in the left frame of Fig. 2(c). The same arrangement then acquired another set of images with the narrow band filter removed. The camera integration time is 2.0 seconds. Since the diffusely transmitted 680-nm signal was 10 times stronger than the fluorescence signal, we considered this later set of images to be transillumination images, and used those in two different ways. First, analysis of these transillumination images provided estimate of the average value of $\kappa_x = \sqrt{\mu_{a_x}/D_x}$ for the excitation light. The values of these optical parameters of Intralipid-2 suspension in water happened to be very close for the excitation beam and fluorescence light. Second, the images provided a complementary set of raw data for obtaining information about the targets based on

their absorption contrast. The experimental arrangement thus enables correlated imaging and retrieval of target location using both fluorescence and transmission measurements.

6.4. Analysis

The transmission images were analyzed to estimate the average value of $\kappa = \sqrt{\mu_a \mu_s}$ (where μ_a and μ_s are the absorption and reduced scattering coefficients at nm, respectively). The values of these optical parameters of Intralipid-2 suspension in water happened to be very close for excitation and fluorescence wavelengths.

From each fluorescence image, a region of interest was cropped out and then every pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The fluorescence data was normalized [21, 4] using $U/I = \phi/I$ to remove the contribution of unaccounted factors (such as, light-source strength, collection geometry, attenuation in the optical filters and components etc.), where U and I were the computed and measured fluorescence signals with targets inside the background medium, respectively; and ϕ and I_0 were the computed and measured transmitted signals at the excitation wavelength through the background medium “without” targets inside. As it is not practical to remove the target in real life situations, I_0 was estimated as an average of all images acquired at different scan positions [14]. The data matrix, $X = x(\mathbf{r}_d, \mathbf{r}_s)$ was then constructed using the normalized fluorescence signals for all scan positions. One column of X corresponds to signal accumulated for one scan position.

For comparison, the transmission data were also analyzed using both approaches as detailed in Chapter 2 [14, 1]. In that case, the targets were treated to be absorptive, and the contrast was mainly due to higher absorption of the excitation beam by the target(s). It should be noted that

absorption measurement involves changes in the intensity of the excitation beam, and consequently the TROT and NMF analysis used the difference images between the raw transmission images and a reference image for the background medium. The background image was an average of images acquired at all scan positions.

As was done for the fluorescence signal, the transmission data were also normalized using $\Delta\phi/\Delta I = \phi/I - 1$, where I is the measured perturbation in I_0 due to the presence of targets; and $\Delta\phi$ is the computed perturbation in ϕ . Since the targets were more absorptive than the background, $\Delta\phi$ was intrinsically negative, so we used $-\Delta\phi$ for constructing the data matrix to satisfy the non-negativity constraint of NMF [1].

Then the data matrix generated using either fluorescence or transmission data is then used for the further analysis using TROT or NMF.

6.4.1. Fluorescence TROT

A TR matrix was generated using the data matrix X . An eigenvalue equation of the TR matrix was then solved. The pseudo spectrum P_d was calculated over all voxels in the sample space using one dominant eigenvector for the one-target experiment, and two dominant eigenvectors for the two-target experiment, using eq. (3.10). The voxel size was 0.5 mm × 0.5 mm × 1 mm. Three-dimensional tomographic pseudo images were generated using the pseudo spectrum. The positions of target(s) were determined using the maxima in the pseudo spectrum. The fluorescence strengths of the targets were calculated by unmixing the data matrix using eq. (3.11).

6.4.2. NMF-based fluorescence tomography

The acquired data were processed using the following steps.

- (i) NMF decomposition of the data matrix using ALS algorithm [24, 25] was carried out. The fluorescence signal due to each target and the mixing vector, $s_j(\mathbf{r}_s)$ and $a_j(\mathbf{r}_d)$ were retrieved, which are NCIDs on the source and detector planes, respectively.
- (ii) NCIDs $s_j(\mathbf{r}_s)$ and $a_j(\mathbf{r}_d)$ were then fitted to Green's functions $G_x(\mathbf{r}_j, \mathbf{r}_s)$, and $G_m(\mathbf{r}_d, \mathbf{r}_j)$, respectively, using χ^2 (Eq. 1) to find the positions of the targets.
- (iii) The transmission images were then analyzed using the NMF formalism and similar steps to those above for the fluorescent case.
- (iv) The NMF-generated NCIDs from both fluorescence and transmission data were used to calculate the cross-section images of the targets using the back-projection algorithm. Because of the diffuse nature of light propagation in the turbid medium, the estimated cross section is expected to be considerably larger than the actual target size. The calculation of $\chi_j(\mathbf{q})$ using Eq. (1) employed Tikhonov regularization [26], with a modified L -curve method [22] to determine the optimal regularization parameter. However, this optimization is a tradeoff between obtaining a closer estimate of target cross section and fewer artifacts in the back-projection image. Since the positions of the targets were obtained from the previous steps, any artifacts cropping up in the back-projection process could be readily identified from their positions. So, instead of using the “corner” of the L -curve to find the optimal regularization parameter, we settled for a lower regularization using the criterion that the highest artifact peak reaches half of the target peak to improve the size estimate of the cross-section images. The full width at half maximum (FWHM) of the spatial profile of the cross-section image was used as an estimate of the target size.

6.5. Results

6.5.1. Single target

6.5.1.1. Fluorescence TROT

The three-dimensional pseudo images were generated. The single target was detected, and the position of the target was determined using the peak in the pseudo spectrum and listed in Table .1, with comparison to the actual position. The image at the retrieved z-coordinate of the target position ($z = 1.5$ mm) plotted using the pseudo spectrum is shown in Fig. .4(a). Further calculation showed that when more eigenvectors were used, the pseudo spectrum still clearly detected one single target. The determination of the number of targets is not sensitive to the choice of the threshold in the eigenvalue spectrum.

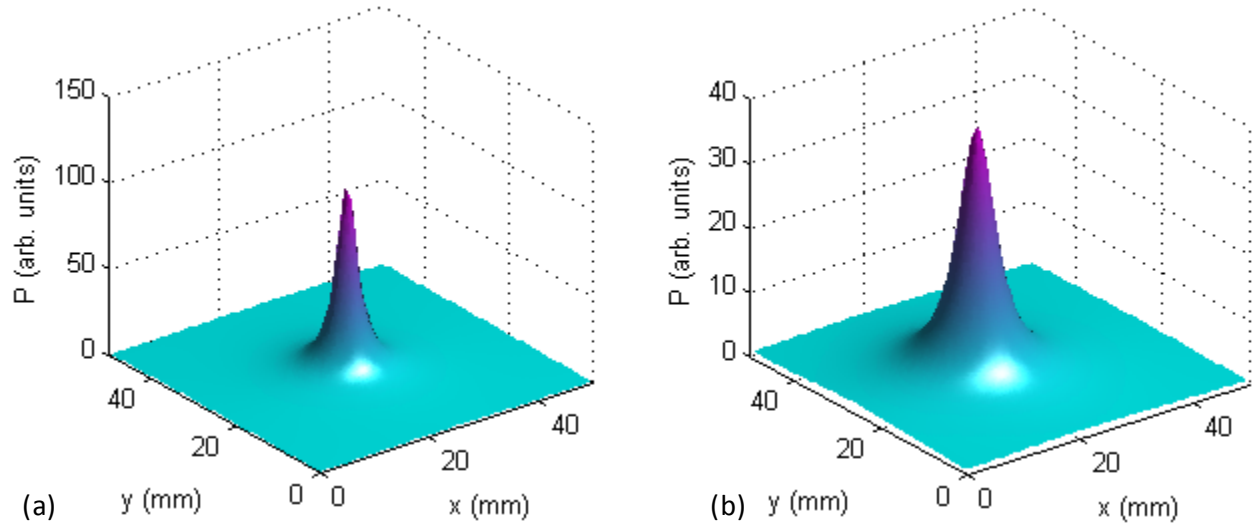


Fig. .4. TROT-reconstructed image at $z = 1.5$ mm using *fluorescence* data is shown in (a), and at $z = 2.5$ mm using transmission data shown in (b).

Following eq. (.4), the actual fluorescence strengths f of the targets was 1.5 mm /ns (i.e. m /s). Using Green's functions corresponding to the retrieved target positions along with eq. ., the fluorescence strengths of the target was directly retrieved to be 1.5 mm /ns, with 0.5% error.

Since the fluorescence signal decayed by a factor of 2. during the experiments for the fluorescence and transmission measurements, the fluorescence strength was corrected using a multiplication factor 2. and retrieved to be $1. \text{ mm /ns}$, with 2.2 error.

Table .1. nown and TROT-retrieved target positions

	nown	Retrieved	
		Fluorescence	Transmission
osition x, y, z (mm)	2 .1, 2 . , .	24.1, 2 . , .	21. , 2 . , 2 .
rror $\Delta x, \Delta y, \Delta z$ (mm)	-	1. , .1, .	. , .1, .
FW M $\delta x, \delta y$ (mm)	-	. , .	. , .

The transmission data was then analyzed for comparison. The target was also detected and its retrieved location is listed in Table .1 for comparison. The image of the target at $z = 2. \text{ mm}$ is shown in Fig. .4(b).

As shown in Table .1, the location of the target retrieved from the fluorescence data is in excellent agreement with the known position, and is consistent with that retrieved using transmission data. The pole of the pseudo image using fluorescence data is sharper than that obtained using transmission data. The FW M of the pole in both x and y directions is $. \text{ mm}$ in the fluorescence-TROT image and $. \text{ mm}$ in the transmission-TROT image.

The absorption strength of the target was also retrieved using the transmission data similarly. The known value of the absorption strength of the targets is $42. \text{ mm /ns}$. The retrieved value for the target was 4.1 mm /ns , with 11.1 error.

The retrieved fluorescence and absorption strengths of the target are shown in Table .2 with comparison to the known values.

Table .2. TROT retrieved optical strength of the target

Measurement	nown strength	Retrieved strength	rror
Mode	(mm /ns)	(mm /ns)	()
Fluorescence	1 .	1 .	2.2
Transmission	42.	4 .1	11.1

6.5.1.2. NMF-based fluorescence tomography

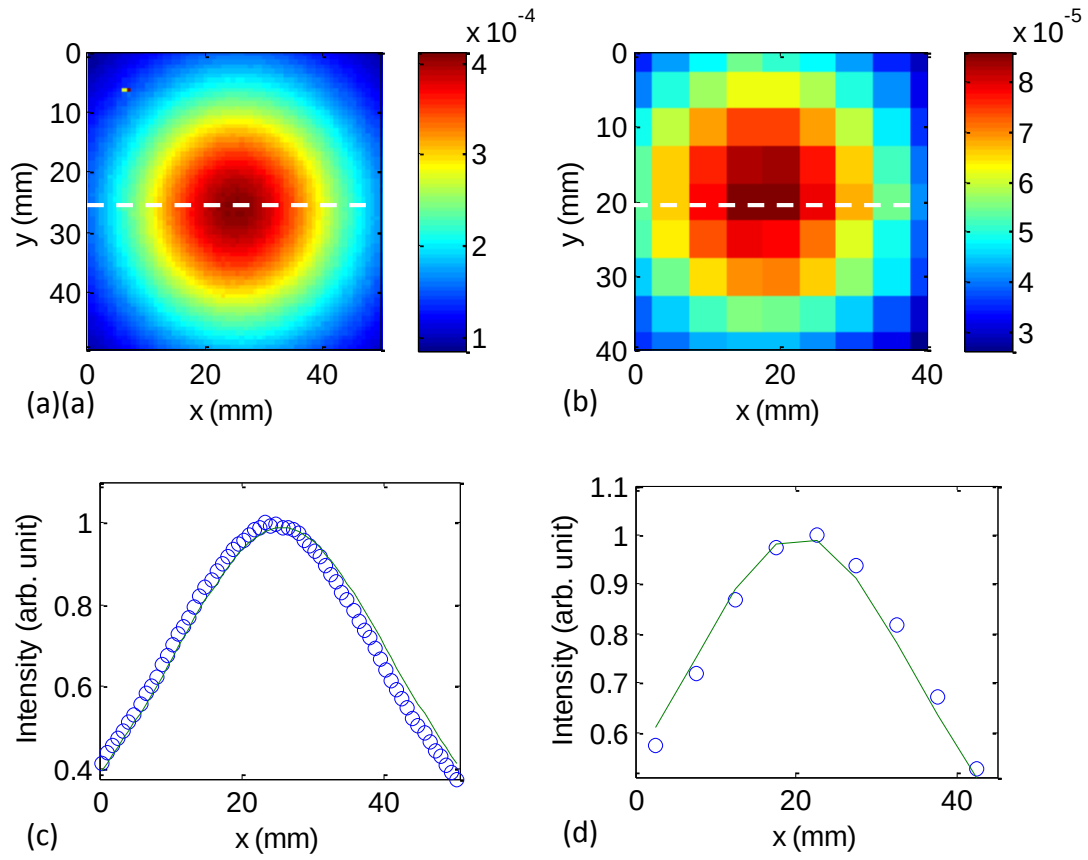


Fig. . . (a) and (b) are NCIDs retrieved from the fluorescence data corresponding to the target on the detector and source planes, respectively; (c) and (d) are least squares fits to the spatial profiles along the white dashed lines in (a) and (b), respectively.

The data matrix was then analyzed using NMF-based approach. The NCIDs of the target on the detector and source planes are shown in Fig. . (a) and . (b). The target position is determined by fitting the NCIDs to Green's functions using eq. (.1). The fitted profiles in the NCIDs through the target in the x direction on the detector and source planes are shown in Fig. . (c) and . (d), respectively. The retrieved position is shown in Table . .

Table . . Known and NMF retrieved target positions

Measurement	Known positions	Retrieved positions	Error
Mode	x, y, z (mm)	x, y, z (mm)	$\Delta x, \Delta y, \Delta z$ (mm)
Fluorescence	2.1, 2.2, 2.3	2.1, 2.4, 2.5	.4, .5, .1
Transmission		22.1, 2.2, 2.3	.1, 1.2, .3

The scaling factors α and β were generated in the least squares fitting using eq. (.1), and the fluorescence strength was estimated using eq. (.11) to be . mm /ns. With the fluorescence decay considered, the corrected fluorescence strength is . mm /ns, with 2. error. The retrieved fluorescence strength is listed in Table .4, with comparison to the known value.

Table .4. Known and NMF-retrieved optical strengths of the targets

Measurement	Known strength	Retrieved strength	Error
Mode	(mm /ns)	(mm /ns)	(%)
Fluorescence	1.1	4.1	2.5
Transmission	.1	.1	.1

A cross-section image at the z-coordinate ($z = 2.2$ mm) of the target position was generated using the back-projection method. The modified *L*-curve method was used with a lower

regularization instead of the regularization at the “corner” of the L -curve, as introduced in Chapter 4. The cross-section image is shown in Fig. . (a). The full-width-at-half-maximum (FW M) of the profile through the maximum in the image is used to estimate the dimension of the target, as shown in Fig. . (b). The edges of in the image shows very intense values due to the boundary effect. Therefore the boundary has been left out in the profiles. The FW M were found to be 1 .4 mm in both x and y directions. The FW M is close to the actual dimension of the target in the y direction, with error. ut the FW M in the x direction is about times of the actual dimension of the target (4.2 mm). Due to the diffusive nature of light transmission, 4.2 mm is considered to be small, and it is hard to detect its boundary. The retrieved FW Ms are shown in Table . .

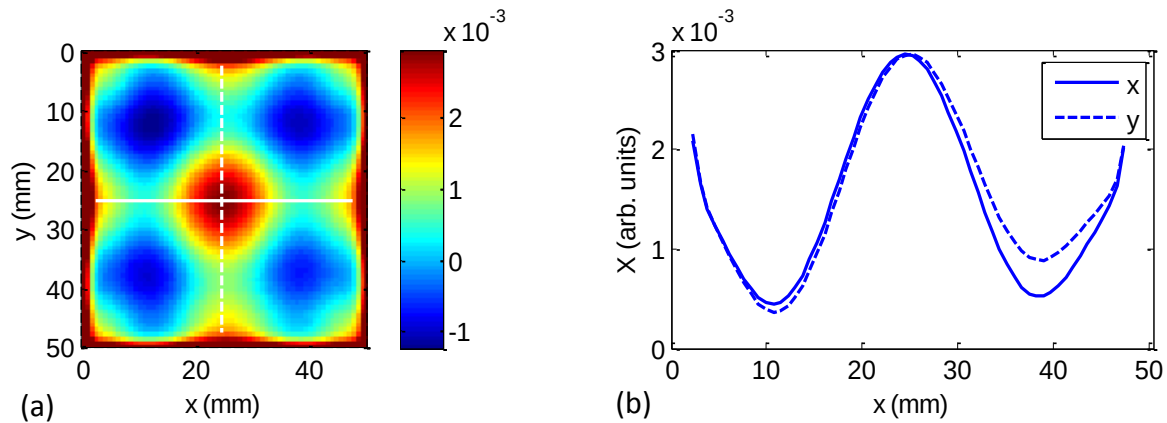


Fig. . . (a) is the cross-section images at the $z = 2.5$ cm plane. (b) is the spatial profiles of the cross-section image along the x and y directions shown by the white lines.

The transmission data were similarly analyzed. The NCIDs and the Green’s function fits of the profiles in the NCIDs are shown in Fig. . . The retrieved target position is also shown in Table . . The absorption strength of the target was estimated from the transmission data to be . mm /ns, with . error. The retrieved absorption strength is also listed in Table .4, with comparison to the known value.

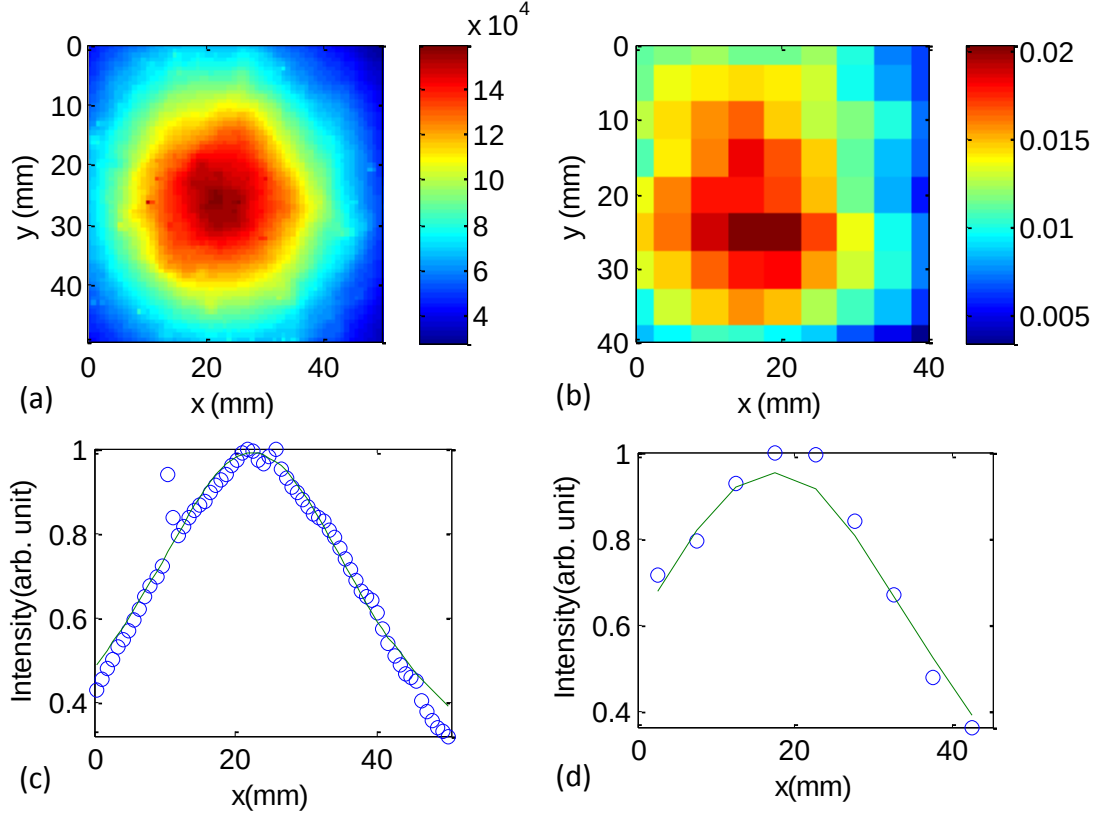


Fig. 11. (a) and (b) are NCIDs retrieved from the transmission data corresponding to the target on the detector and source planes, respectively; (c) and (d) are least squares fits to the spatial profiles along the white dashed lines in (a) and (b), respectively.

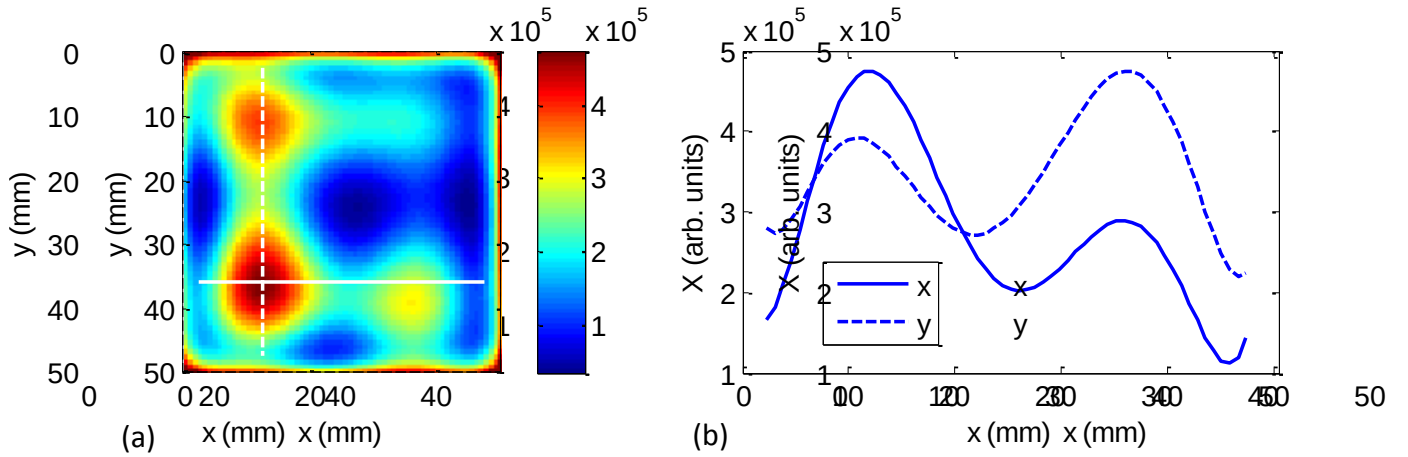


Fig. 12. (a) is the cross-section images at the $z = 0$ cm plane. (b) is the spatial profiles of the cross-section image along the x and y directions shown by the white lines.

Table . . . stimated target size using FW M of the cross-section image of the targets

Measurement Mode	nown size $\delta x, \delta y$ (mm)	Retrieved size $\delta x, \delta y$ (mm)
Fluorescence	4.2, 1	1 .4, 1 .4
Transmission		12. , 1 .

The cross-section image with low regularization was also generated using back-projection, as shown in Fig. . . In the cross-section image, the target position shifted to the left and to the bottom by 1 cm in both x and y directions, which may be due to the noise (distortion) in the NCIDs that corresponds to high spatial frequency. The FW Ms of the profiles through the maximum in the cross-section image were found to be 12. mm and 1 . mm in the x and y directions, respectively, which are similar to those obtained using fluorescence data. The retrieved FW Ms using transmission data are listed in Table . . If the regularization at the “corner” of the -curve is used, the location of the target shown in the cross-section image becomes more accurate (image not shown here). owever the resolution degrades.

6.5.2. Two targets

6.5.2.1. Fluorescence TROT

The TR matrix was generated by multiplying the response matrix by its transpose for our CW probing scheme. The eigenvalue equation of TR matrix was solved.

The first 2 eigenvalues plotted in Fig. . demonstrate that only two eigenvalues are dominant, and consequently those two were included in the signal subspace and separated from the noise subspace.

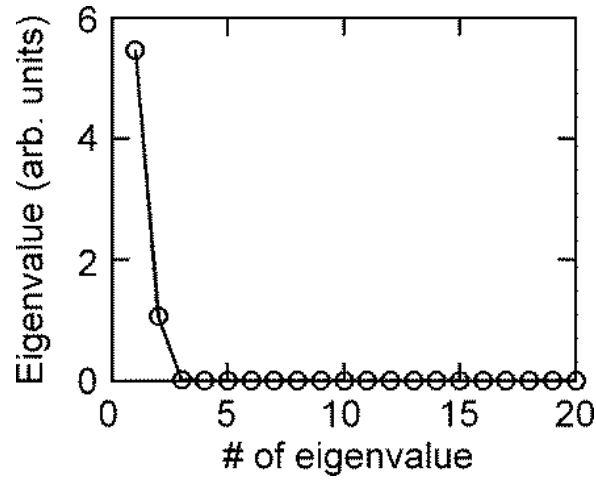


Fig. . . . igenvalue spectrum of TR matrix

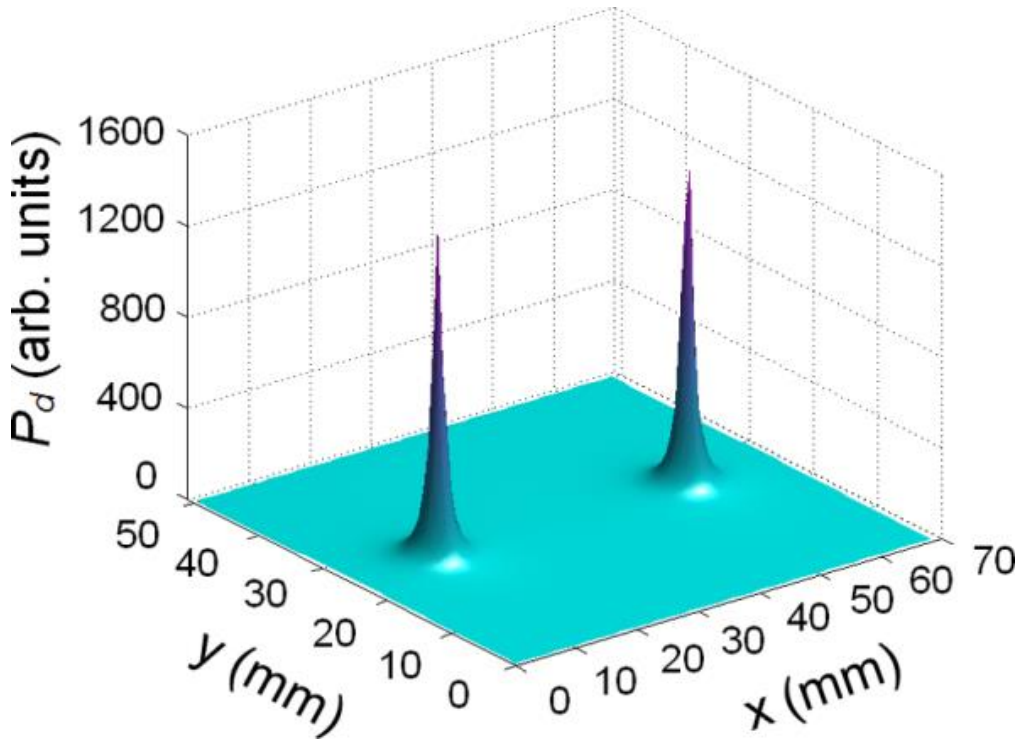


Fig. .1 . TROT-reconstructed pseudo image at $z = 2.5$ mm using *fluorescence* data.

M-SIC algorithm [1] was then used to calculate the pseudo spectrum for all voxels in the $-D$ space of the sample. The voxel size was $.5$ mm $.5$ mm 1 mm. Three-dimensional tomographic pseudo images were generated using the pseudo spectrum. Fig. .1 shows one such tomographic image for $z = 2.5$ mm. The locations of targets taken to correspond to the peaks of

the pseudo images are listed and compared with known positions in Table . . The single point that the method identifies as the location of a target may be considered to be the “center of fluorescence strength” of the target, which may coincide with the geometrical center for a homogeneous target, but would be weighted by the distribution of fluorescence strength for a heterogeneous target.

Table . . Known and retrieved target positions

		Left target	Right target
Known position x, y, z (mm)		14.2, 2.1, 1.1	4.2, 2.1, 1.1
Fluorescence-TROT retrieved	Position x, y, z (mm)	14.4, 24.1, 2.1	4.1, 24.1, 1.1
	Error $\Delta x, \Delta y, \Delta z$ (mm)	0.2, 0.1, 0.0	0.1, 0.0, 0.0
Transmission-TROT retrieved	Position x, y, z (mm)	12.1, 2.2, 1.1	4.1, 2.2, 2.1
	Error $\Delta x, \Delta y, \Delta z$ (mm)	2.1, 2.2, 0.0	0.1, 2.2, 0.0
FWHM $\delta x, \delta y$ (mm) using	Fluorescence-TROT	2.1, 1.1	1.1, 1.1
	Transmission-TROT	0.1, 4.2	0.4, 0.0

The retrieved positions are in good agreement with the known locations of the targets. Further experimentation and simulation reveal that TROT achieves better resolution in the lateral (x, y) directions than in the axial (z) direction, and that the resolution depends on separation between the targets and other experimental parameters. We estimate that under the reported experimental conditions and parameters, the same two targets could be resolved even when the closest lateral (x, y) distance between their surfaces were 2 mm (center-to-center distance of 4 mm) with a noise level of 1%. If the targets had the same lateral position but different z -positions, the separation between them could be determined within 2 mm even with 10% additive random

noise when the separation was 1 mm. As the axial distance between the two targets was reduced to 2 mm, the approach retrieved the separation to be smaller, such as, 1 mm for up to 2 noise, 1.1 mm for 4 noise, and fail to resolve for 4 or higher noise. For a separation of 1 mm the targets could not be resolved even with 2 noise. The axial resolution could be improved if data were acquired in a wider angular view (for example, additional measurements across an adjacent (y-z) side), or using cylindrical geometry.

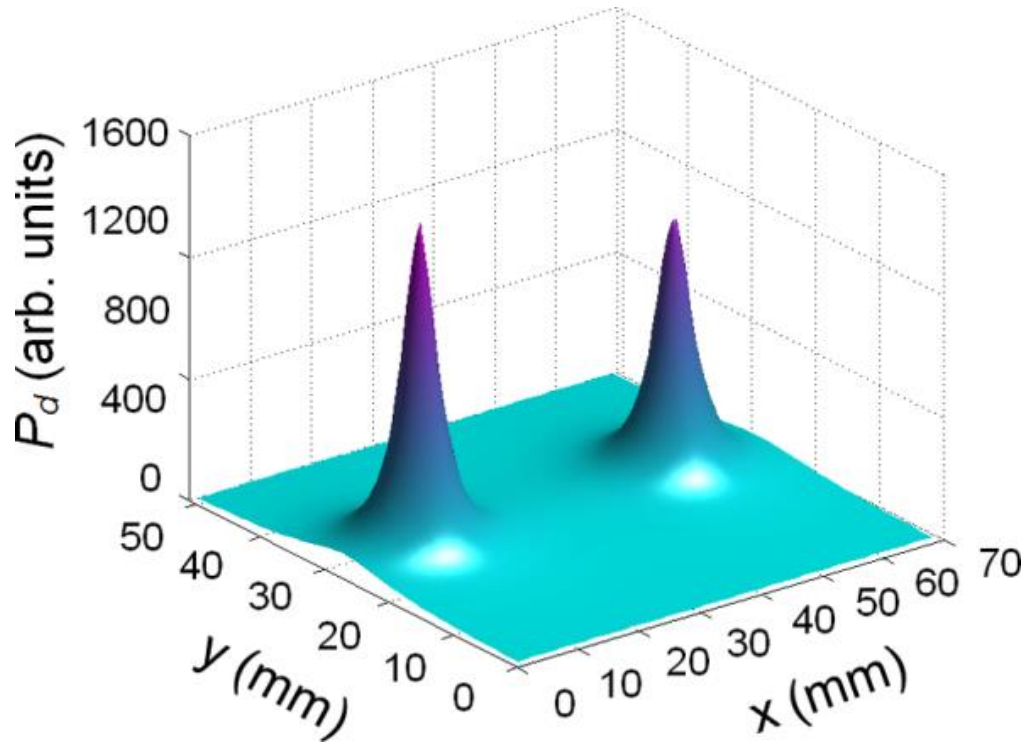


Fig. 11. TROT-reconstructed pseudo image at $z = 2$ mm using transmission data.

Since an excited fluorescent target emits light in all directions, the method can be readily used in back-propagation geometry and is not restricted to transmission mode reported here. We have used CW illumination and rectangular slab sample geometry in experiments reported here, but the approach can be extended to frequency-domain and time-domain measurements and other sample geometries (cylindrical, spherical, and hemispherical) as well.

The TROT reconstructed image at the $z = 2.5$ mm plane using the transmission data is shown in Fig. 11, and the retrieved locations of targets are listed and compared with those obtained from fluorescence data in Table 1.

It follows from Table 1, that the positions of the targets retrieved using fluorescence measurements are in very good agreement (uncertainty ≈ 1 mm) with the known locations, and are quite consistent with those retrieved using transmission measurements. However, the peaks in the reconstructed images using fluorescence data are sharper than those using transmission data. Since only one eigen component was used to reconstruct a target, the targets are treated as point targets, and the pseudo images only carry location information. The FWHMs of the peaks in the fluorescence-TROT and transmission-TROT images are shown also in Table 1. It is shown the FWHM of the peaks in the transmission-TROT images are about 2.5 times of those in the fluorescence-TROT images. The width of the profile of each target reflects on the uncertainty in determination of location of the corresponding target. We tentatively ascribe this better resolution of fluorescence TROT result than that of transmission TROT result to the difference in the background level between fluorescence and transmission signals. Fluorescence signal, in principle, has zero background, while transmission signal involves measurement of changes on a strong background, and such changes for targets in a turbid medium can be rather small. This set of measurements suggests that higher resolution may be achieved with TROT using fluorescence data than that using transmission data, an observation reported by other researchers as well. However, the overall level of fluorescence signal is an important factor in imaging applications, and more work involving different types of samples and target contrast will be needed to obtain a more definitive conclusion.

Following eq. (4), the fluorescence strengths f of the targets were found to be 1.4 mm/ns . Using Green's functions corresponding to the retrieved target positions along with eq. (5), the fluorescence strengths of the targets were retrieved to be 1.4 mm/ns and 1.4 mm/ns , with 2.2 and 11.1 errors for the left and right targets, respectively. The absorption strength of the targets were also retrieved similarly. The retrieved values for the two targets were 42.1 and 22.1 mm/ns , with 2.1 and 2.1 errors, respectively. The retrieved fluorescence and absorption strengths are listed in Table 4.1 with comparison to the known values.

Table 4.1. Retrieved optical strengths of the targets

Target	Measurement Type	Known strength (mm/ns)	Retrieved strength (mm/ns)	Error (%)
Left	Fluorescence	1.4	1.4	2.2
	Transmission	42.1	42.1	2.1
Right	Fluorescence	1.4	1.4	11.1
	Transmission	42.1	22.1	2.1

6.5.2.2. NMF-based fluorescence tomography

Fig. 12(a) and Fig. 12(b) show NCIDs of the left target on the detector and source planes, respectively. Fig. 12(c) and Fig. 12(d) present the corresponding spatial profiles along the white dashed lines. The solid curves in Fig. 12(c) and Fig. 12(d) are the least squares fits of the experimental profiles denoted by circles to Green's functions using eq. (5). Table 4.1 lists the three-dimensional (3-D) locations of the targets extracted from this fitting procedure. Similar NCIDs are obtained for the right target as shown in Figs. 12(e) and 12(f), and the retrieved

target positions are also listed in Table . . The retrieved positions agree within 1mm of the known positions.

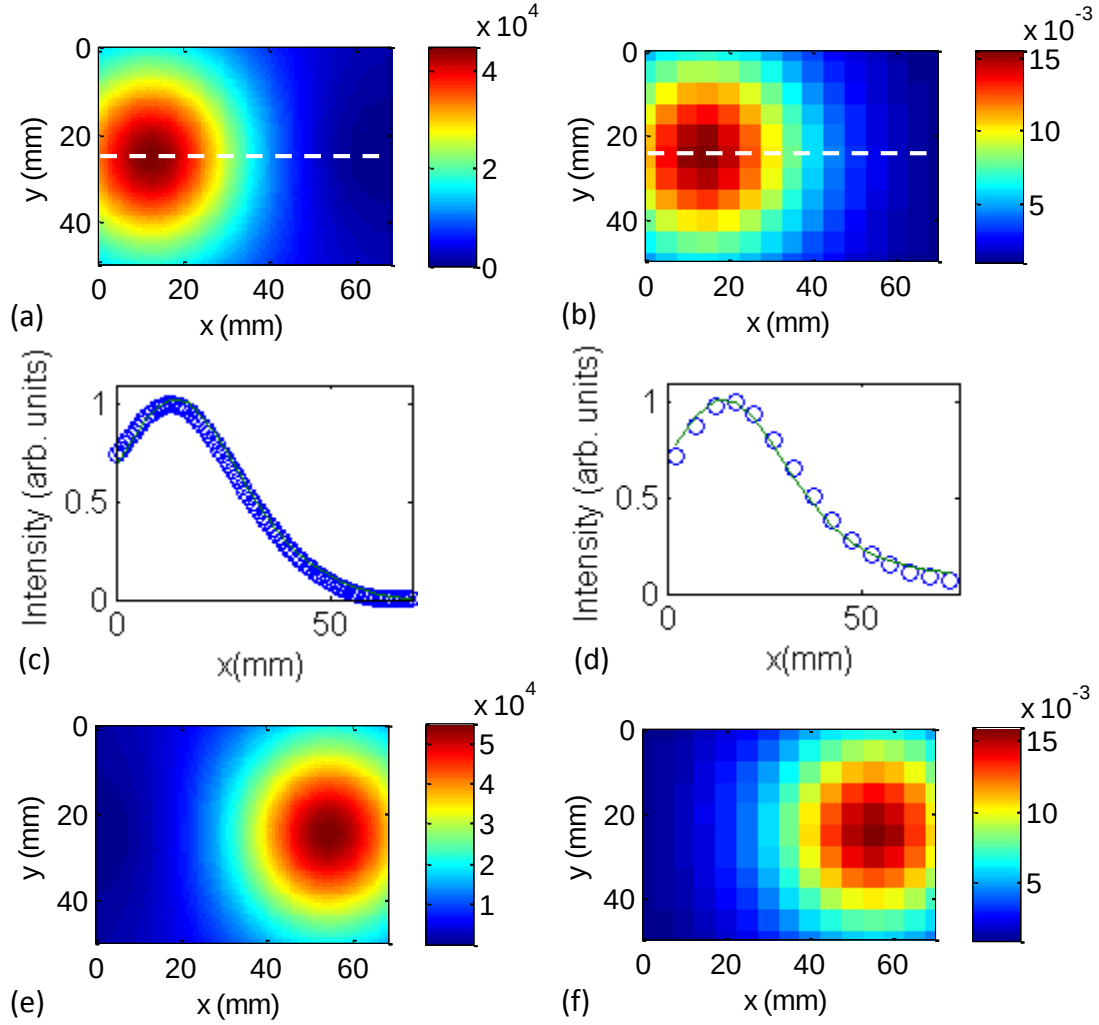


Fig. .12. (a) and (b) are NCIDs corresponding to the left target on the detector plane and on the source plane, respectively; (c) and (d) are least squares fits to the spatial profiles along the white dashed lines in (a) and (b), respectively; (e) and (f) are NCIDs corresponding to the right target on the detector and source planes, respectively.

The NCIDs retrieved from the transmission data are shown in Fig. .1 . Table . further lists the NMF-retrieved target positions using transillumination data for comparison with those obtained from fluorescence data. There are some small differences in the accuracy of retrieved x

and y coordinates (that is in the values of Δx and Δy) for two inclusions. We attribute those to random measurement/ reconstruction error.

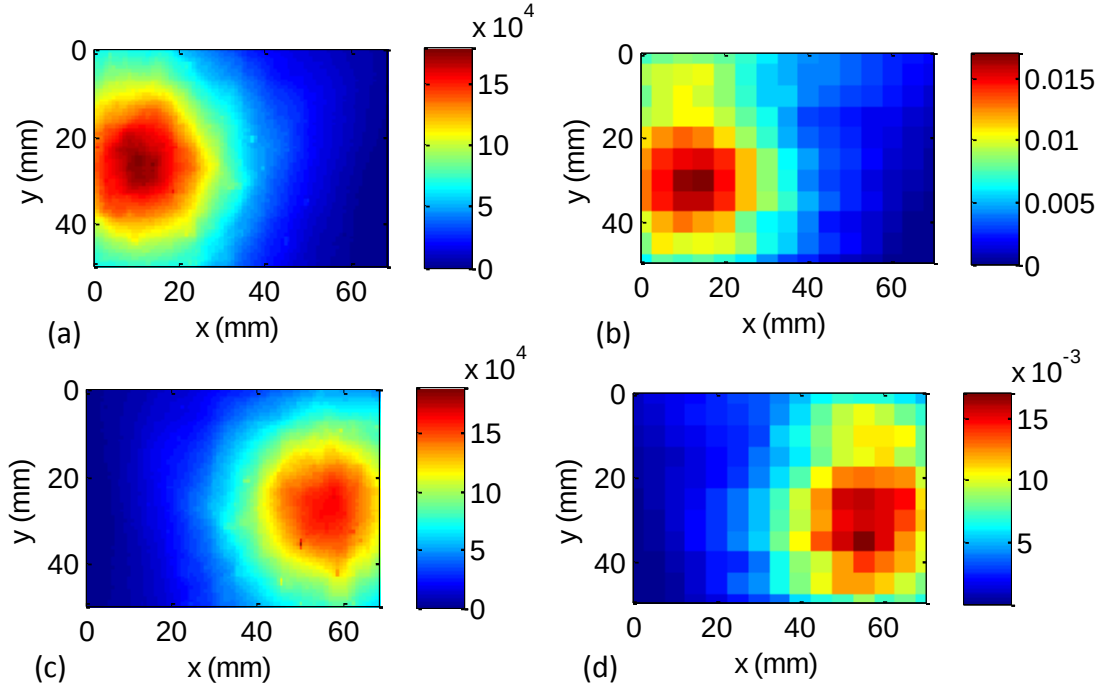


Fig. 1 . (a) and (b) are NCIDs corresponding to the left target on the detector and source planes, respectively; (c) and (d) are NCIDs corresponding to the right target on the detector and source planes, respectively.

NMF-based fluorescence and transillumination approaches both retrieve locations of the targets well. However, as Table 1 shows, errors in position coordinates of the targets retrieved from fluorescence data are significantly smaller than those obtained from transillumination data. We attribute this difference to the role played by the background in the two cases. Ideally, the fluorescence signal is a measurement of emitted light on a dark background, while the transillumination signal is a perturbation on a strong background. In practice, the background is significantly smaller in fluorescence imaging than in transillumination imaging. Other researchers have reported on similar superior performance of fluorescence tomography as well. If the background is substantial (as in the case of fluorescence from contrast agent up taken

by normal tissue, or tissue autofluorescence) the fluorescence tomography may not retain this edge over the transillumination imaging.

Table 3.3. Known and NMF retrieved target positions

Mode	Target	Known positions x, y, z (mm)	Retrieved positions x, y, z (mm)	Error $\Delta x, \Delta y, \Delta z$ (mm)
Fluorescence	Left	14.2, 2.1, 1.1	11.1, 2.1, 1.1	3.1, 0, 0
	Right	4.2, 2.1, 1.1	1.1, 2.1, 2.2	3.1, 0, 1.1
Transmission	Left	14.2, 2.1, 1.1	11.1, 2.1, 1.1	3.1, 0, 0
	Right	4.2, 2.1, 1.1	1.1, 2.1, 2.2	3.1, 0, 1.1

Table 3.4. Retrieved optical strengths of the targets

Target	Measurement Type	Known strength (mm /ns)	Retrieved strength (mm /ns)	Error (%)
Left	Fluorescence	11.1	11.1	14.1
	Transmission	42.1	42.1	0.1
Right	Fluorescence	11.1	14.1	21.1
	Transmission	42.1	4.1	90.1

The scaling factors α and β were generated in the least squares fitting using eq. (3.11), and the fluorescence strengths were estimated using eq. (3.11) to be 11.1 mm /ns and 14.1 mm /ns for the left and right targets, respectively. The uncertainties in the estimated values are 14.1% and 21.1% compared to known values. Similarly, the absorption strength of the targets were

estimated from the transmission data to be 1.5 mm/ns and 2.2 mm/ns for the left and right targets, respectively, which were within 5% of the known value. The retrieved fluorescence and absorption strengths are listed in Table 1, with comparison to known values.

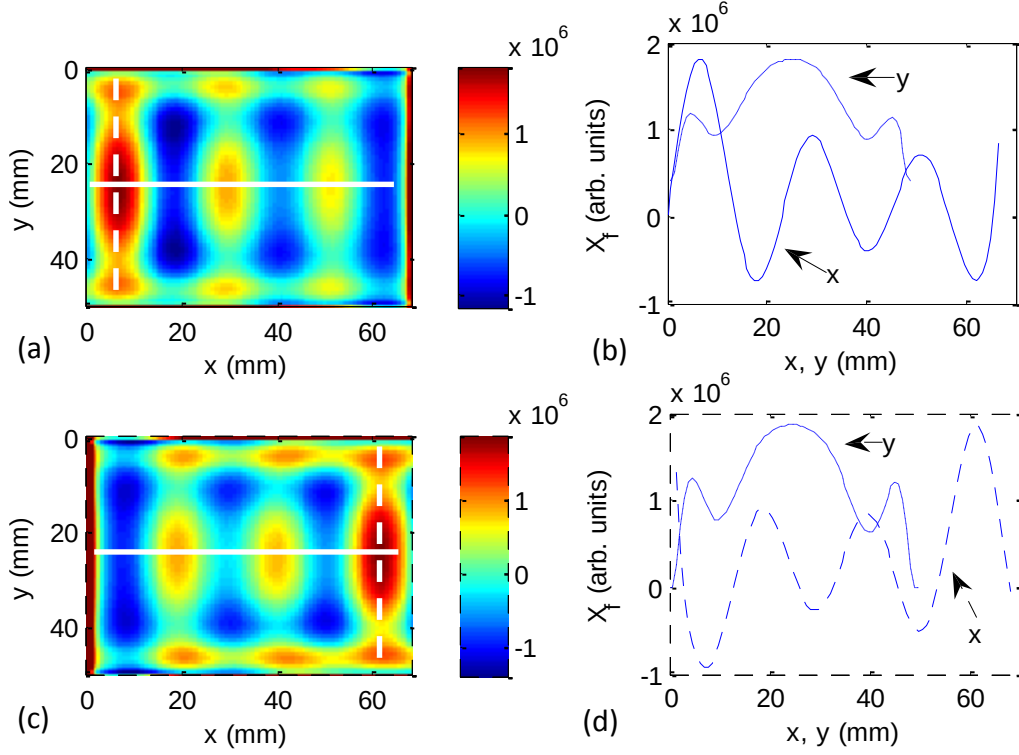


Fig. 14. (a) and (c) are cross sectional images at the $z = 1.5 \text{ cm}$ and 2.2 cm planes, respectively. (b) and (d) are spatial profiles of the cross sectional images along the x and y directions shown by the white lines.

Cross-section images of the targets were generated using the back-projection method. Fig. 14(a) displays a cross-section image of the left target at the $z = 1.5 \text{ mm}$ plane generated from the fluorescence data. The real cross section image of the target is at the left of the frame (confirmed by its location obtained from the previous step), while other two are artifacts whose strength depends in part on the regularization parameter. Similar cross-section image was obtained for the right target. The FWHM of the spatial profiles of the cross-section images were found to be 1.5 mm and 1.1 mm for the left target, and 2.2 mm and 1.4 mm for the right target in x and y directions, respectively. The cross-section images estimate the lateral dimensions of

the targets to be approximately twice their known values. We presume it a consequence of the diffusive nature of light propagation. However, the ratio between the y and x dimensions of the targets estimated to be 2.2 and 2.1 for the left and right targets, respectively, are close to the actual value of 2.4 for both targets. The FWHMs retrieved from fluorescence as the estimate of the sizes of the targets, are listed in Table 1.

For comparison, similar cross section images were generated using the transmission data, as shown in Fig. 1.

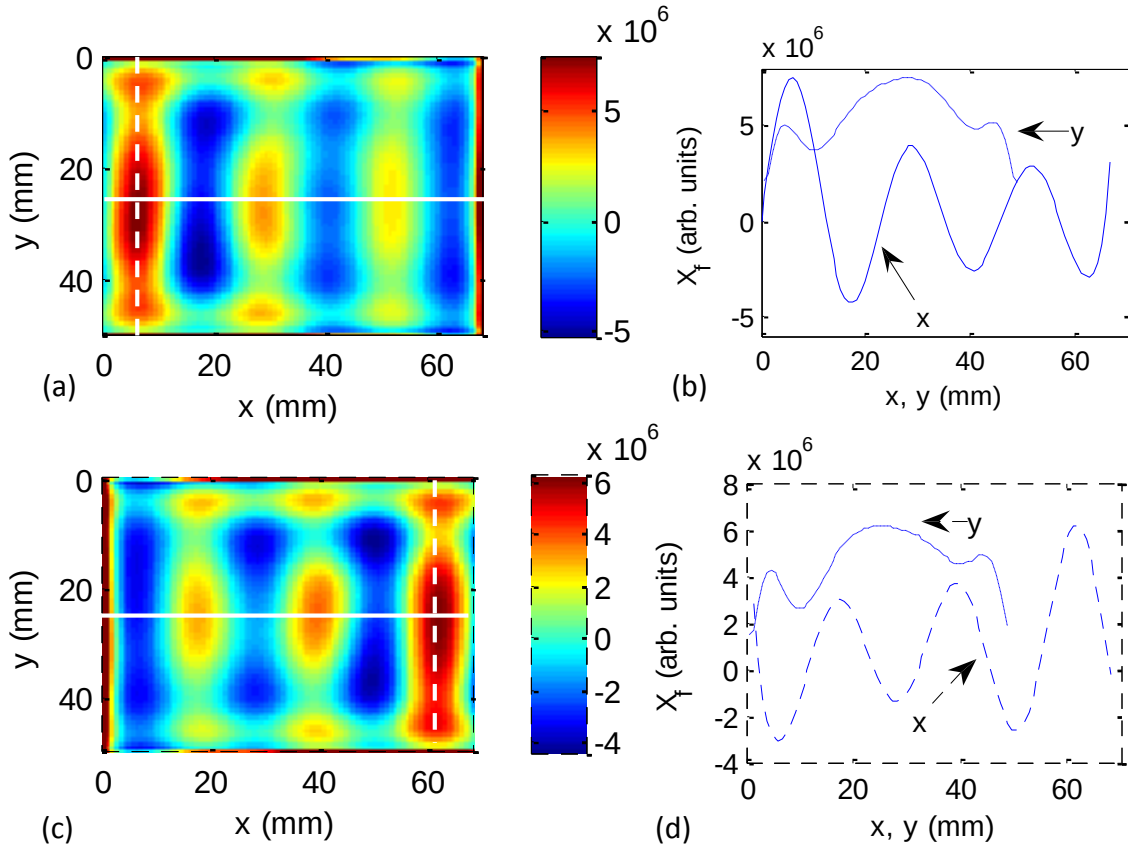


Fig. 1. (a) and (c) are cross sectional images generated at the z -positions of the two targets ($z = 1. \text{ mm}$ and 2.2 mm), respectively. (b) and (d) are spatial profiles of the cross sectional images along the x and y directions shown by the lines.

We found the FW M to be . mm and 1 .4 mm for the left target, and .4 mm and 1 .1 mm for the right target in x and y directions, respectively. The ratio between the y and x dimensions of the targets estimated to be 2.4 and 2. for the left and right targets, respectively. The cross section estimates are comparable for both the fluorescence and transillumination approaches. The FW Ms retrieved from transmission data are also all listed in Table .1 for comparison.

Table .1 . estimated target size using FW M of the cross-section image of the targets

Target	Measurement Type	nown size $\delta x, \delta y$ (mm)	Retrieved size $\delta x, \delta y$ (mm)
eft	Fluorescence	4.2, 1	. , 1 .1
	Transmission		. , 1 .4
Right	Fluorescence		. , 1 .4
	Transmission		.4, 1 .1

6.6. Discussion

Compared to the case with two targets present, when only one target was presented, the cross-section image of the target was almost round, *i.e.* the ratio between the estimated dimensions of the target in the x and y directions was about 1. This distortion in the shape of the image in the single target case is probably due to the noise in the weaker signal of the single target.

Minimum detectable fluorophore concentration and target size are useful considerations for application of the TROT method. While we present data on IC concentration of 1 μM in the target following the common practice , with our current experimental configuration we have tested with IC at various concentrations. Fluorescence signal due to a 1-cm-long and a 2-cm-

long, 4.2-mm-diameter target embedded in the mid-plane of the 100-mm container was measured with various concentrations (2 nM, 1 nM, 1 nM, 2 nM, 1 nM, 1 μ M), respectively. The peak values in the measured signals are shown in Fig. 1 (a). The fluorescence signal due to the 2-cm-long target was approximately twice as much as the 1-cm-long target with IC at the same concentrations. When the IC concentrations was 2 nM, 1 nM and 1 nM, the images of the 1-cm long target are shown in Fig. 1 (b), 1 (c) and 1 (d), respectively. Fig. 1 shows even with 2 -nM IC , the 1-cm-long target could be detected. However, the signal-to-noise ratio was significantly lower than that with 1 -nM IC , as shown in Fig. 1 .

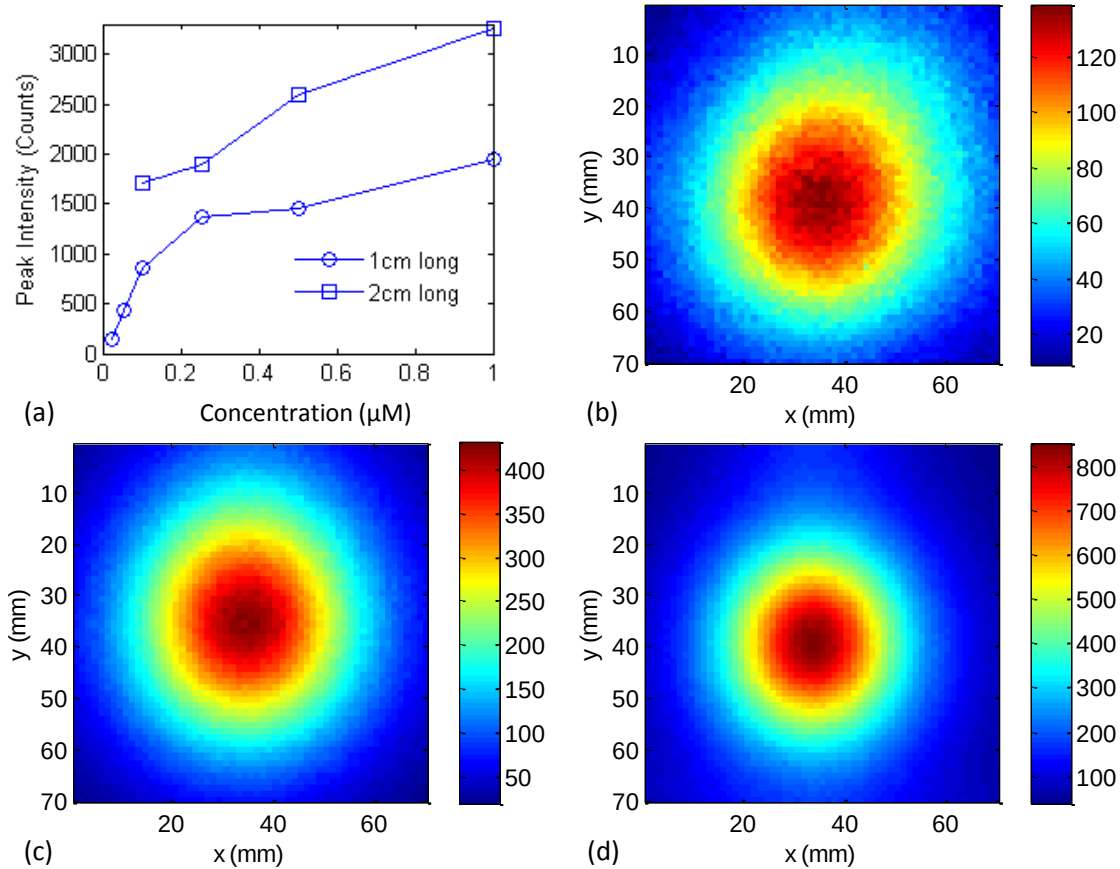


Fig. 1 . (a) The two curves show the peak values in the fluorescence signal using different IC concentrations for the 1-cm-long and 2-cm-long fluorescent targets, respectively; (b), (c) and (d) show the fluorescence signal with IC at concentrations of 2 nM, 1 nM, and 1 nM, respectively.

oth TROT and NMF-OT approaches retrieve locations of targets based on their fluorescence strength, which is a product of the fluorescence yield and the volume of the target.

However, it is impractical to use a point target and make the fluorescence yield arbitrarily large. Based on experimental conditions we anticipate being able to locate targets of size $\sim 4 \text{ cm}$, 4-times smaller than what we used with same IC concentration. In clinical applications involving detecting and locating tumors, these sizes are more than adequate for early detection of cancer.

This set of experiments was done with no background IC uptake. Background fluorescence is seldom zero for *in vivo* experiments and is a common concern for fluorescence DOT. For uniform distribution of fluorophore in the background medium, simulation shows that the present methods would provide target location that is quite accurate in the lateral directions, but is shifted towards the detector plane, and the shift would depend on the target-to-background fluorescence ratio. The fluorescence signal was examined experimentally when there was fluorophore distribution in the background. The peak value in the measured fluorescence light intensity distribution is used to represent the strength of fluorescence. The measured fluorescence signals due to IC in the background at different concentrations and a fitted curve are plotted in Fig. 1 (a). Small sections of the curve in Fig. 1 (a) are plotted in 1 (b)

1 (d). As described by Yuan *et al.* [2], quantum quenching in the fluorescence was observed. Fig. 1 shows the fluorescence signal becomes detectable at 1 nM, and increases linearly within 1 nM. Then it continues to increase nonlinearly and reaches maximum at nM. After that, the fluorescence decreases with concentration. This effect is expected to vary with the scattering and absorption properties of the medium, and the volume, particularly thickness of the medium. For smaller medium with lower absorption and scattering properties, a larger linear signal-increasing range is expected.

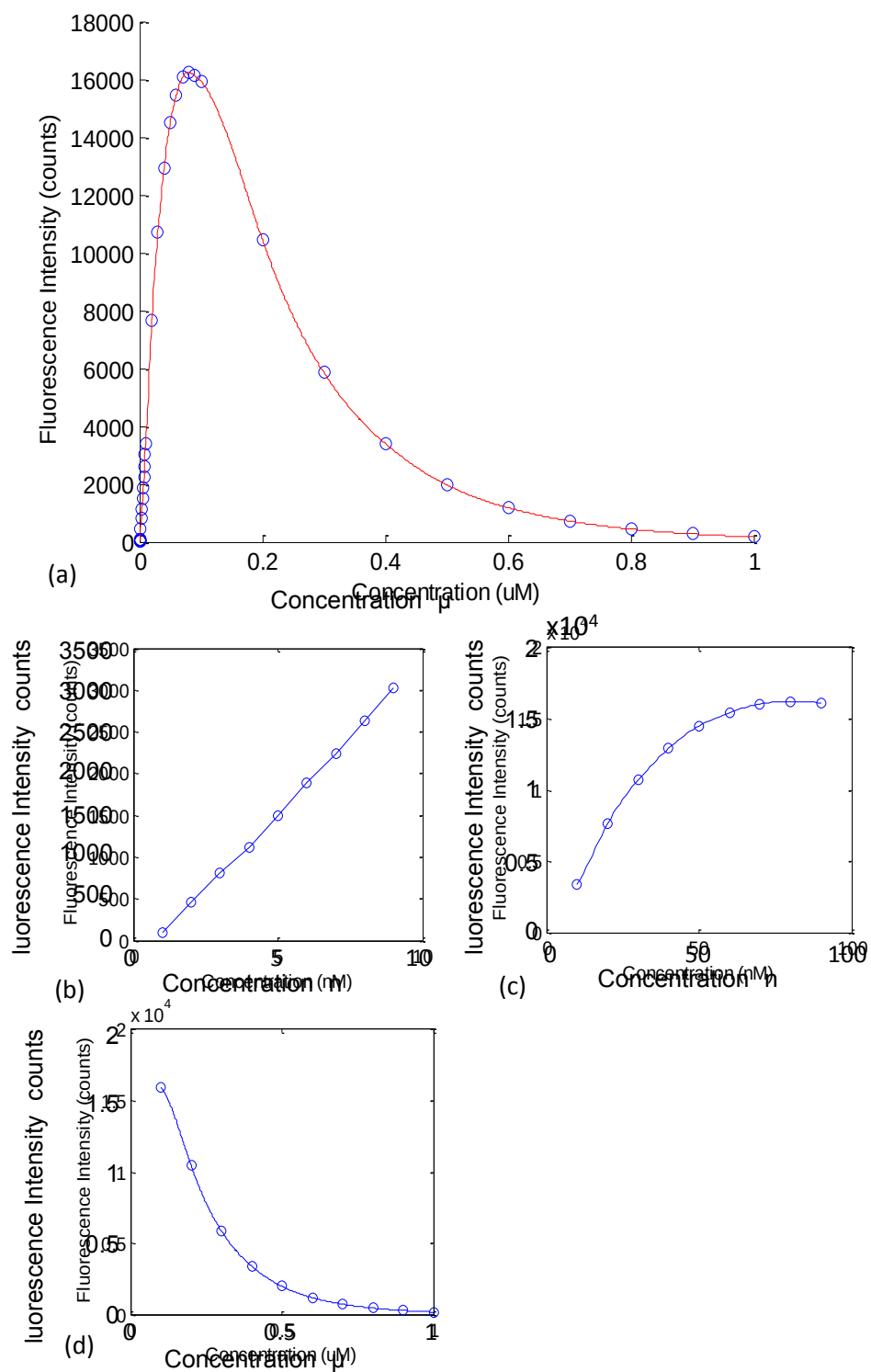


Fig. 1 (a) shows measured fluorescence signal (o) and fitted curve (-) due to the IC in the background medium at different concentrations. (b), (c) and (d) show different sections of the curve in (a).

The results above (Figs. 1 and 1) show that the fluorescence signal due to the background medium in the 2 mm 2 mm mm container has much higher signal before quantum quenching takes place for the same concentration in the background and in the target. For instance, for a concentration of nM, the fluorescence signal from the background is times of that from the target. -nM IC in the background produces similar level of fluorescence signal as -nM IC in the target. The target-to-background fluorescence signal ratio should be higher for a target inside a smaller sample. However, in realistic experimental conditions, the background fluorescence is usually significant and cannot be neglected. Analyzing the data using TROT or NMF directly with background fluorescence neglected will lead to significant error in the results.

The situation may improve if an appropriate background subtraction method may be employed , , . In *in vivo* studies, background fluorescence due to fluorophore uptake by normal tissues may be significantly reduced by adjusting the time delay between administration of fluorophore and the measurements, since the fluorophore gets removed from the normal tissue faster than from the tumor . The method uses the diffusion model of light propagation, which takes into account the effect of background absorption and scattering in the intervening medium.

As explained in Chapter 2 and Chapter 4 respectively, both TROT and NMF are fast reconstruction methods, since they use data matrix with lower dimension than that used in other inverse image reconstruction (IIR) approaches, do not involve iterations of the forward model, and do not attempt to find optical properties for every voxel. The same advantages of these approaches over other IIR methods hold true for fluorescence imaging. The computational complexity of both approaches is less than what it is even for a single iteration of an iterative IIR method. Even though a limited number of acquisition angles were used for the slab geometry,

locations of the fluorescence targets were retrieved by both approaches within 1 mm of known positions for a sample with similar thickness and average optical properties of a typical compressed human breast, which is a significant result.

In summary, *fluorescence* TROT and NMF-based fluorescence tomography approaches have been developed and used to retrieve the target location and relative fluorescence strength of one and two small fluorescent targets embedded in a breast-simulating turbid medium. Locations of targets were retrieved in 3-D with an accuracy of 1 mm under the favorable condition of well-separated targets. Achievable spatial resolution is better for assessment of lateral separation between the targets than for axial separation in the forward propagation mode of signal acquisition using slab geometry. Fluorescence signal appeared to provide better resolution than transillumination signal. The results further suggest the potential of TROT and NMF-based fluorescence tomography for retrieving three-dimensional location information of contrast-enhanced tumors in a human breast at early development stages.

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Chapter 7

Summary and future work

7.1. Summary

In this thesis, the time reversal optical tomography (TROT) and non-negative matrix factorization (NMF) based optical tomography (NMF-OT) were developed and used for imaging through highly scattering turbid media with embedded absorptive, scattering or fluorescent targets. The efficacy of the methods was evaluated using simulations, and experiments using human-breast-simulating phantoms, and *ex vivo* model human breast. The methods were shown to be able to provide three-dimensional location of the targets with high accuracy. The optical characteristics and dimensions of the targets may also be estimated with reasonable accuracy. CW laser and transillumination slab geometry were used in the study. The advantage is that the experimental arrangement is relatively simple, and such an imaging system is easy to build and more affordable. However, more sophisticated experimental arrangements could be developed to improve the imaging efficacy and provides interesting ground for further exploration.

7.2. Future work

The results obtained in the work presented in this thesis suggest further steps for development. Some steps may improve the results of the proposed approaches. Some are necessary to realize the goal of optical mammography ultimately. Our future work includes the following.

(a) Time-resolved and frequency-domain TROT

Time-resolved and frequency-domain measurements may provide more information of the sample and lead to higher resolution and more robust results. Time domain measurements can be

converted to frequency domain using Fourier transform. Phase information in the photon density wave will be obtained. A real “time reversal matrix” can be obtained instead of the one used currently which only involves “round-trip” of light propagation without “time reversal” (phase conjugate).

(b) Cylindrical or semi-spherical geometry

Since the slab geometry provides limited angular view of the sample, the resolution, particularly along the axial direction is not optimized. By using cylindrical or semi-spherical geometry, experimental data in a larger angular view are obtained. This may lead to higher resolution, particularly in the axial direction. The accuracy in the estimate of the shape (size) of an extended target may also be improved.

(c) Multi-wavelength studies using key wavelengths

Key wavelengths which correspond to the spectral features of the key fluorophores in the human breast tissue may be chosen for multi-wavelength measurements. The spectral information of the inhomogeneity may be used for diagnosis, particularly to distinguish cancer from benign growth or other irregularities which behave as an inhomogeneity.

(d) Combining FEM with TROT

The currently proposed TROT approach is mainly used for detection of localized small target such as a tumor. It provides limited information about the structure (heterogeneity) in the target. On the contrary, the widely used finite-element-method (FEM) based approach provides with a distribution of optical property in the sample. However, it is computation intensive. Combining these two methods may provide an optimal solution without aid of traditional imaging modality such as CT or MRI. First, TROT will be used to accurately determine the location, and estimate

the dimension of the target. Then a relatively small region around the target may be chosen for reconstruction using F M. An optical property distribution will be obtained in the region, which uses much less computational time than that for reconstruction of the whole sample.

(e) Animal study

In vivo live animal study can be used to test the proposed approaches in a more realistic condition before it is eventually tested on human body.

(f) *In vivo* study on volunteers

The ultimate usefulness of the approaches may only be assessed by conducting *in vivo* study on volunteers before the approaches are used in clinical trials.

Appendix A

TROT Computer Program

main.m

```
function main

%read_in data for geometry
fid = fopen('geometry.txt', 'r');
ISNX = fscanf(fid, '%f', 1);
ISNY = fscanf(fid, '%f', 1);
IDNX = fscanf(fid, '%f', 1);
IDNY = fscanf(fid, '%f', 1);
MX = fscanf(fid, '%f', 1);
MY = fscanf(fid, '%f', 1);
LL = fscanf(fid, '%f', 1);
fclose(fid);

fid = fopen('input_data.txt', 'r');

%read_in data
n1 = fscanf(fid, '%f', 2); %the reflective index of scattering medium
n2 = fscanf(fid, '%f', 2); %the reflective index of wall (i.e. glass)
n3 = fscanf(fid, '%f', 2); %the reflective index of outside (i.e. air)
l1d = fscanf(fid, '%f', 1); %scattering medium thickness
mua = fscanf(fid, '%f', 1); %absorption coefficient
lt = fscanf(fid, '%f', 1); %transport length
nd = fscanf(fid, '%f', 1); %scattering medium refraction index
omega = fscanf(fid, '%f', 1); %modulation frequency, 0 for cw
z0 = fscanf(fid, '%f', 1); %depth of the center of the first voxel
dz = fscanf(fid, '%f', 1); %z direction division of the scattering medium
zs = fscanf(fid, '%f', 1); %source position
zd = fscanf(fid, '%f', 1); %detector position
delta = fscanf(fid, '%f', 6); %step interval in x and y
rate = fscanf(fid, '%f', 2); %noise level (for simulation)
dmu = fscanf(fid, '%f', 2); %change in mu of the inhomogeneities(abs, sca)
key = fscanf(fid, '%d', 1); %switch for judging the signal from the noise
space, either by readin number r_mm or automatic judging with r_ratio
r_mm = fscanf(fid, '%d', 1); %signal space rank
r_ratio = fscanf(fid, '%f', 1); %cutoff threshold for signal space
key2 = fscanf(fid, '%d', 1); %switch for normalization of the pseudo
spectrum
fclose(fid);

%calculate the extrapolated coefficient from the reflective index
%the galss wall is included
ze = calc_ze(n1, n2, n3);

%exp data
disp('enter exp data');
ryd=zeros(IDNY, IDNX, ISNY, ISNX);
```



```

fid = fopen('signal.txt','r');
ryd(:) = fscanf(fid,'%f');
fclose(fid);

disp('enter trev');
pseudo =
trev1(ryd,key,key2,r_mm,r_ratio,ze,lld,mua,lt,nd,omega,z0,zd,dz,delta);
save trev0.mat pseudo;
[latt_pp] = sort_data(pseudo);
save trev.mat latt_pp;

```

gr.m

```

function s = createhandles
%Call: s = gr;
%s.cgrb(ze,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%s.cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%s.sgrb(ze,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%s.sgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%
%ze=[ze(1),ze(2)]
%omega is modulation frequency, omega = 0 for CW
%
s.cgrb = @cgrb;
s.cgrd = @cgrd;
s.sgrb = @sgrb;
s.sgrd = @sgrd;

%cgrb.r
%This is for diffusion model
%The source position should move 1 lt from real source position
%along incident direction.
%The following formula is little different from Xu.
%In my formula, z=0 is defined from the real boundary
%In Xu's formula z=0 is defined at -ze(1), the extrapolated boundary.

function [rcgrb,icgrb] = cgrb(ze,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%This is background Green's function for absorptive object.
%This is a frequency domain Green's function in real space.
%This Green's function is for a semi-infinite medium
%omegas: the modulated Frequency, Unit: GHz.
%nd: the refractive index in the medium
%c: the light speed in medium, Unit: mm/nsec

ccgrb = zeros(size(x_xs));
rcgrb = ccgrb;
icgrb = ccgrb;

kext=ze(1)*lt;
dd=1/3*lt;
c=2.998*100/nd;
kapa=complex(mua/dd, -2*pi*omega/(dd*c));
kapa=sqrt(kapa);
rr=x_xs.^2+y_ys.^2;

```

```

r1=sqrt(rr+(z-zs)^2);
r2=sqrt(rr+(z+zs+2*kext)^2);
ccgrb=exp(-kapa*r1)./r1 - exp(-kapa*r2)./r2;
rcgrb=real(ccgrb)/(4*pi*dd);
icgrb=imag(ccgrb)/(4*pi*dd);
return

function [rcgrd,icgrd] = cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%function rcgrd = cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%This is background Green's function for absorptive object.
%This is a frequency domain Green's function in the real space
%This Green's function for a slab (thick: lld) medium
%omegas: the modulated Frequency, Unit: GHz.
%nd: the refractive index in the medium
%c: the light speed in medium, Unit: mm/nsec

ccgrd = zeros(size(x_xs));
rcgrd = ccgrd;
icgrd = ccgrd;

kext=(ze(1)+ze(2))*lt;
dd=1/3*lt;
c=2.998*100/nd;
kapa=complex(mua/dd, -2*pi*omega/(dd*c));
kapa=sqrt(kapa);
rr=(x_xs).^2+(y_ys).^2;
for m=-4:4
    r1=sqrt(rr+(z-zs+2*m*(lld+kext))^2);
    r2=sqrt(rr+(z+zs+2*ze(1)*lt-2*m*(lld+kext))^2);
    ccgrd=ccgrd+exp(-kapa*r1)./r1 - exp(-kapa*r2)./r2;
end
rcgrd = real(ccgrd)/(4*pi*dd);
icgrd = imag(ccgrd)/(4*pi*dd);
return

function [rsgrb,isgrb] = sgrb(ze,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%This is background Green's function for scattering object.
%This is a frequency domain Green's function in real space.
%This Green's function is for a semi-infinite medium.
%omegas: the modulated Frequency, Unit: GHz.
%nd: the refractive index in the medium
%c: the light speed in medium, Unit: mm/nsec

rsgrb = zeros([3 size(x_xs)]);
isgrb = rsgrb;
rsgrb1 = zeros(size(x_xs));
rsgrb2 = rsgrb1;
rsgrb3 = rsgrb1;
isgrb1 = zeros(size(x_xs));
isgrb2 = isgrb1;
isgrb3 = isgrb1;

kext=ze(1)*lt;

```

```

dd=1/3*lt;
c=2.998*100/nd;
kapa=complex(mua/dd, -2*pi*omega/(dd*c));
kapa=sqrt(kapa);
rr=x_xs.^2+y_ys.^2;
z1=z-zs;
r1=sqrt(rr+z1*z1);
r1_3=r1.*r1.*r1;
z2=z+zs+2*kext;
r2=sqrt(rr+z2*z2);
r2_3=r2.*r2.*r2;
tt1=(kapa*r1+1).*exp(-kapa*r1)./r1_3;
tt2=(kapa*r2+1).*exp(-kapa*r2)./r2_3;

rsgrb1=x_xs.*real(tt1-tt2)/(4*pi*dd);
rsgrb(1,:) = rsgrb1(:);
rsgrb2 = y_ys.*real(tt1-tt2)/(4*pi*dd);
rsgrb(2,:) = rsgrb2(:);
rsgrb3=(z1*real(tt1)-z2*real(tt2))/(4*pi*dd);
rsgrb(3,:) = rsgrb3(:);
isgrb1=x_xs.*imag(tt1-tt2)/(4.*pi*dd);
isgrb(1,:) = isgrb1(:);
isgrb2=y_ys.*imag(tt1-tt2)/(4.*pi*dd);
isgrb(2,:) = isgrb2(:);
isgrb3=(z1*imag(tt1)-z2*imag(tt2))/(4.*pi*dd);
isgrb(3,:) = isgrb3(:);
return

function [rsgrd,isgrd] = sgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs)
%This is background Green's function for scattering object.
%This is a frequency domain Green's function in the real space
%This Green's function for a slab (thick: lld) medium
%omegas: the modulated Frequency, Unit: GHz.
%nd: the refractive index in the medium
%c: the light speed in medium, Unit: mm/nsec

csgrd = zeros([3 size(x_xs)]);
isgrd = csgrd;
rsgrd = csgrd;
csgrd1 = zeros(size(x_xs));
csgrd2 = csgrd1;
csgrd3 = csgrd1;

kext=(ze(1)+ze(2))*lt;
dd=1/3*lt;
c=2.998*100/nd;
kapa=complex(mua/dd, -2*pi*omega/(dd*c));
kapa=sqrt(kapa);
rr=x_xs.^2+y_ys.^2;

for m=-4:4
    z1=z-zs+2*m*(lld+kext);
    r1=sqrt(rr+z1*z1);
    r1_3=r1.*r1.*r1;
    z2=z+zs+2*ze(1)*lt-2*m*(lld+kext);

```

```

    r2=sqrt(rr+z2*z2);
    r2_3=r2.*r2.*r2;
    tt1=(kapa*r1+1).*exp(-kapa*r1)./r1_3;
    tt2=(kapa*r2+1).*exp(-kapa*r2)./r2_3;
    csgrd1=csgrd1+(tt1-tt2).*x_xs;
    csgrd(1,:) = csgrd1(:);
    csgrd2=csgrd2+(tt1-tt2).*y_ys;
    csgrd(2,:) = csgrd2(:);
    csgrd3=csgrd3+(z1*tt1-z2*tt2);
    csgrd(3,:) = csgrd3(:);
end
for i=1:3
    rsgrd(i,:)= real(csgrd(i,:))/(4*pi*dd);
    isgrd(i,:)= imag(csgrd(i,:))/(4*pi*dd);
end

```

trev1.m

```

function pseudo =
trev(ryd,key,key2,r_mm,r_ratio,ze,lld,mua,lt,nd,omega,z0,zd,dz,delta)

%read_in data for geometry
fid = fopen('geometry.txt', 'r');
ISNX = fscanf(fid,'%f',1);
ISNY = fscanf(fid,'%f',1);
IDNX = fscanf(fid,'%f',1);
IDNY = fscanf(fid,'%f',1);
MX = fscanf(fid,'%f',1);
MY = fscanf(fid,'%f',1);
LL = fscanf(fid,'%f',1);
fclose(fid);

fprintf('%s\n','enter T-matrix');
ryd = reshape(ryd,IDNX*IDNY,ISNX*ISNY);

%calculate the eigen value and eigen vector of T-matrix
fprintf('%s\n','enter eigen');

[u,s]=svd(ryd);
E = diag(s).^2;

fid = fopen('eigen.txt','w');
fprintf(fid,'%s \r\n', 'eigen value = ');
for i=1:IDNX*IDNY
    fprintf(fid,' %1.15E \r\n',E(i));
end
fclose(fid);

%determine mm: the signal space from the noise space
if(key == 1)
    mm=r_mm; %judged the signal space by readin number
else
    mm=1;
    for k=1:IDNX*IDNY

```

```

        if (E(k) > E(1)*r_ratio)
            %eigen value is large, as signal
            mm=k;
        end
    end
end
fprintf('mm_estimated = %d \n',mm);

%determine the pseudo-spectrum
fprintf('%s\n','enter pseudo-spectrum');

pseudo=zeros(4,LL,MY,MX);

x_xd = zeros(IDNY,IDNX);
y_yd = zeros(IDNY,IDNX);
ii = 1:IDNX;
jj = 1:IDNY;

rgd = zeros([4 size(x_xd)]);
gp = zeros(1,4);
gpu = zeros(mm,4);
gu = gp;

grhandles = gr;
for i=1:MX
    for j=1:MY
        for l=1:LL
            x=delta(2)*(i-1);
            y=delta(2)*(j-1);
            z=z0+dz*(l-1);

            x_xd=ones(IDNY,1)*(delta(3)*(ii-1)-x);
            y_yd=(delta(3)*(jj-1)-y) '*ones(1,IDNX);

            %calculate for absorption
            rcgrd = zeros(size(x_xd));
            icgrd = rcgrd;
            [rcgrd,icgrd] =
grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
            rgd(1,:)=rcgrd(:);

            %calculate for scattering
            rsgrd = zeros([3 size(x_xd)]);
            isgrd = rsgrd;
            [rsgrd,isgrd] =
grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
            rgd(2:4,:) = rsgrd(:,:);

            gp = sum(rgd(:,:)'.^2,1);
            gpu = u(:,1:mm) '*rgd(:,:)';
            gu = sum(gpu.^2,1);

            if(key2==1)
                pseudo(:,l,j,i)=gp./abs(gp-gu);
            else

```

```

        pseudo(:,1,j,i)=1./abs(gp-gu);
    end
end
end
end

```

cal_ze.m

```

function ze = calcze(n1,n2,n3)

for j=1:2
    R1=0;
    R2=0;
    for i=1:1001
        cosi=(double(i)-0.99999)/1000.0002;
        sini=sqrt(1-cosi*cosi);
        if(abs(n1(j)-n2(j)) < 1e-06)
            r12=0;
        else
            sinr=sini*n1(j)/n2(j);
            if(sinr > 0.999999999)
                re_par=1;
                re_pen=1;
            else
                cosr=sqrt(1-sinr*sinr);
                re_par=(n2(j)*n2(j)*cosi-
n1(j)*n2(j)*cosr)/(n2(j)*n2(j)*cosi+n1(j)*n2(j)*cosr);
                re_pen=(n1(j)*cosi-n2(j)*cosr)/(n1(j)*cosi+n2(j)*cosr);
            end
            r12=0.5*(re_par*re_par+re_pen*re_pen);
        end
        if(abs(n2(j)-n3(j)) < 1e-06)
            r23=0;
        else
            if(n3(j)-n2(j)*sinr < 1e-06)
                re_par=1;
                re_pen=1;
            else
                cosr=sqrt(1.-sinr*sinr);
                temp=sqrt(n3(j)*n3(j)-n2(j)*n2(j)*sinr*sinr);
                re_par=(n3(j)*n3(j)*cosr-
n2(j)*temp)/(n3(j)*n3(j)*cosr+n2(j)*temp);
                re_pen=(n2(j)*cosr-temp)/(n2(j)*cosr+temp);
            end
            r23=0.5*(re_par*re_par+re_pen*re_pen);
        end
        RR=(r12+r23-2*r12*r23)/(1-r12*r23);
        if(i==1 || i==1001)
            R1=R1+0.5*2.*cosi*RR;
            R2=R2+0.5*3*cosi*cosi*RR;
        else
            R1=R1+2*cosi*RR;

```

```

        R2=R2+3*cosi*cosi*RR;
    end
end
ze(j)=2*(1+R2/1000)/3/(1-R1/1000);
end

```

sort.m

```

function latt_pp = sort_data(pseudo)

DIM = size(pseudo);
MX = DIM(4);
MY = DIM(3);
LL = DIM(2);

% sorting the pseudo-spectrum
fprintf('enter sort pseudo-spectrum\n');

xx = [1:LL]; yy = [1:MY]; zz = [1:MX];

xx2 = xx'*ones(1,MY);
xx3 = ones(LL,MY,MX);
xx3(:) = xx2(:)*ones(1,MX);

yy2 = ones(LL,1)* yy;
yy3 = ones(LL,MY,MX);
yy3(:) = yy2(:)*ones(1,MX);

zz2 = ones(LL,MY);
zz3 = ones(LL,MY,MX);
zz3(:) = zz2(:)*zz;

latt_pp = ones(LL*MY*MX*4,5);
latt_pp = [[zz3(:) yy3(:) xx3(:) ones(LL*MY*MX,1) pseudo(1,:)'];[zz3(:)
yy3(:) xx3(:) 2*ones(LL*MY*MX,1) pseudo(2,:)'];[zz3(:) yy3(:) xx3(:)
3*ones(LL*MY*MX,1) pseudo(3,:)'];[zz3(:) yy3(:) xx3(:) 4*ones(LL*MY*MX,1)
pseudo(4,:)']];

latt_pp = sortrows(latt_pp,-5);
latt_pp = latt_pp';

fid = fopen('pseudo.txt','w');
fprintf(fid,'%4s %4s %4s %4s %s \r\n\r\n','x','y','z','A/S','Pseudo');
fprintf(fid,'%4d %4d %4d %4d %1.15E \r\n',latt_pp);

fclose(fid);

```

Appendix B

PCA and NMF based computer programs

pca1.m

```
function [A S E] = pca(n,type)

%n: objects numbers

fid = fopen('input_data.txt', 'r');
prm = fscanf(fid, '%f', 21); %the reflective index of scattering medium
delta = prm(16:21);
fclose(fid);

fid = fopen('geometry.txt', 'r');
ISNX = fscanf(fid, '%f', 1);
ISNY = fscanf(fid, '%f', 1);
IDNX = fscanf(fid, '%f', 1);
IDNY = fscanf(fid, '%f', 1);
MX = fscanf(fid, '%f', 1);
MY = fscanf(fid, '%f', 1);
LL = fscanf(fid, '%f', 1);
fclose(fid);

disp('enter exp data');
ryd=zeros(IDNY,IDNX,ISNY,ISNX);
fid = fopen('signal.txt', 'r');
ryd(:) = fscanf(fid, '%f')';
fclose(fid);

x = reshape(ryd,IDNY*IDNX,ISNY*ISNX);
if type==1
    x(x<0)=0;
end

M = ISNY*ISNX;
N = IDNY*IDNX;

MM = mean(x,2);
Y=x-repmat(MM,1,M);

[A,D] = svd(Y,0);
E = dot(D',D');
E = E/N;

A = A(:,1:n); E = E(1:n);
S = pinv(A)*x;

for ii = 1:n
    if max(A(:,ii))*max(S(ii,:))<min(A(:,ii))*min(S(ii,:))
        A(:,ii)=-A(:,ii);S(ii,:)=-S(ii,:);
    end
end
```



```

end
figure; set(gcf,'position',[100 300 500 200]);

a1 = A(:,ii);
s1 = S(ii,:);
x1 = a1*s1;

a11 = reshape(a1,IDNY,IDNX);
s11 = reshape(s1,ISNY,ISNX);
x11 = reshape(x1,IDNY,IDNX,ISNY,ISNX);

for nsy = 1:ISNY
    for nsx = 1:ISNX
        x12 = squeeze(x11(:,:,nsy,nsx));

        [ym, jm] = max(x12);
        [yM, im] = max(ym);
        jm = jm(im);
        %xi(ii) = im;
        %yj(ii) = jm;
        jmn(nsy,nsx) = jm;
        imn(nsy,nsx) = im;
    end
end

im = mean(imn(:));
jm = mean(jmn(:));

fprintf('The component intensity is peaked at i = %d and j
= %d\n',int16(im),int16(jm));
subplot(1,2,1);
imagesc(a11);
colormap(gray);
title('Image on detector plane');
xlabel('x(mm)');
ylabel('y(mm)');
set(get(gca,'children'),'xdata',delta(5)*[0 IDNX-1]);
set(get(gca,'children'),'ydata',delta(6)*[0 IDNY-1]);
axis equal;
set(gca,'xlim',delta(5)*[0 IDNX-1]);
set(gca,'ylim',delta(6)*[0 IDNY-1]);
set(gca,'fontsize',12);
set(get(gca,'title'),'fontsize',12);

subplot(1,2,2);
imagesc(s11);
colormap(gray);
title('Image on source plane');
xlabel('x(mm)');
ylabel('y(mm)');
set(get(gca,'children'),'xdata',delta(1)*[0.5 ISNX-0.5]);
set(get(gca,'children'),'ydata',delta(2)*[0.5 ISNY-0.5]);
axis equal;
set(gca,'xlim',delta(1)*[0.5 ISNX-0.5]);
set(gca,'ylim',delta(2)*[0.5 ISNY-0.5]);

```

```

        set(gca,'fontsize',12);
        set(get(gca,'title'),'fontsize',12);

end

```

fitpos.m

```

function [pars,fitdata,resnorm] = fitpos(a,s,type,dx,dy)
% [pars,resnorm] = fitpos(a,s) %fit position of an object(source)
% find the location of the target r and scaling factors by fitting a and s;
%
% a is a certain column of the mixing matrix A
% s is a certain row of component in source matrix B
% type: 0/'abs' - absorption 1/'sca' - scattering
% dx, dy: coordinates mapping - coordinates of the origin of source plane in
the
%   detector plane,  $x - dx = x'$ ,  $y - dy = y'$ , where x,y are detector plane
%   coordinates, x',y' are source plane coordinates
% by: Binlin Wu 06/2008
% CCNY-CUNY

global dx0 dy0
dx0 = dx; dy0 = dy;

if type==0 || isequal(type,'abs')
    kkey = 0; % for absorption
elseif type ==1 || isequal(type,'sca')
    kkey = 1; % for scattering
else
    disp('fitpos(a,s,type) where type is 0/'abs' or 1/'sca'');
    return
end

%%read in geometry
fid = fopen('geometry.txt', 'r');
ISNX = fscanf(fid,'%f',1);
ISNY = fscanf(fid,'%f',1);
IDNX = fscanf(fid,'%f',1);
IDNY = fscanf(fid,'%f',1);
MX = fscanf(fid,'%f',1);
MY = fscanf(fid,'%f',1);
%LL = fscanf(fid,'%f',1);
fclose(fid);

%%read in parameters
fid = fopen('input_data.txt', 'r');

%read in data
n1 = fscanf(fid,'%f',2);           %the reflective index of scattering medium
n2 = fscanf(fid,'%f',2);           %the reflective index of wall (i.e. glass)
n3 = fscanf(fid,'%f',2);           %the reflective index of outside (i.e. air)
lld = fscanf(fid,'%f',1);          %scattering mediu thickness

```

```

mua = fscanf(fid,'%f',1);      %absorption coefficient
lt = fscanf(fid,'%f',1);      %transport length
nd = fscanf(fid,'%f',1);      %scattering medium refraction index
omega = fscanf(fid,'%f',1);    %modulation frequency, 0 for cw
z0 = fscanf(fid,'%f',1);      %depth of the center of the first voxel
dz = fscanf(fid,'%f',1);      %z direction division of the scattering medium
zs = fscanf(fid,'%f',1);      %source position
zd = fscanf(fid,'%f',1);      %detector position
delta = fscanf(fid,'%f',6);    %step interval in x and y
rate = fscanf(fid,'%f',2);     %noise level
dmu = fscanf(fid,'%f',2);     %change in mu of the inhomogeneities
key = fscanf(fid,'%d',1);      %switch for judging the signal from the noise
space, either by readin number r_mm or automatic judging with r_ratio
r_mm = fscanf(fid,'%d',1);     %signal space rank
r_ratio = fscanf(fid,'%f',1);  %cutoff threshold for signal space
key2 = fscanf(fid,'%d',1);     %switch for normalization of the pseudo
spectrum

fclose(fid);

%calculate extrapolation distance
ze = calc_ze(n1,n2,n3);

%find maxima
x = a*s;
a = reshape(a,IDNY,IDNX);
s = reshape(s,ISNY,ISNX);
x1 = reshape(x,IDNY,IDNX,ISNY,ISNX);
for nsy = 1:ISNY
    for nsx = 1:ISNX
        x2 = squeeze(x1(:, :, nsy, nsx));
        [ym, jm] = max(x2);
        [yM, im] = max(ym);
        jm = jm(im);
        jmn(nsy, nsx) = jm;
        imn(nsy, nsx) = im;
    end
end

% im = mean(imn(:));
% jm = mean(jmn(:));

x12 = squeeze(x1(:, :, ceil(ISNY/2), ceil(ISNX/2)));
[ym, jm] = max(x12);
[yM, im] = max(ym);
jm = jm(im);

x=(im-1)*delta(3);
y=(jm-1)*delta(4);

[ym, jma] = max(a);
[yM, ima] = max(ym);
jma = jma(ima);
xma=(ima-1)*delta(5);
yma=(jma-1)*delta(6);

```

```

[ym, jms] = max(s);
[yM, ims] = max(ym);
jms = jms(ims);
xms=(ims-1)*delta(1);
yms=(jms-1)*delta(2);

%%%%% first normalize a and s %%%%%
gamma1 = 1/max(s(:));
gamma2 = 1/max(a(:));
s = s*gamma1;
a = a*gamma2;

%%%%% generate positions %%%%%%%%%
x_xs = zeros(ISNY,ISNX);
y_ys = zeros(ISNY,ISNX);
nsx = 1:ISNX;
nsy = 1:ISNY;

x_xd = zeros(IDNY,IDNX);
y_yd = zeros(IDNY,IDNX);
ndx = 1:IDNX;
ndy = 1:IDNY;

%2d positions
x_xs = ones(ISNY,1)*(delta(1)*(nsx-1)-xms+dx0) + srcX;
y_ys = (delta(2)*(nsy-1)-yms+dy0)'*ones(1,ISNX) + srcY;

x_xd = ones(IDNY,1)*(delta(5)*(ndx-1)-xma) + dtrX;
y_yd = (delta(6)*(ndy-1)-yma)'*ones(1,IDNX) + dtrY;

%%%%%%%%% initialization %%%%%%%%%%%%%

%kkey = 0; %0: abs; 1: sca

% set z initial value in the middle of the volume
l=fix(lld/dz/2);
z = z0+(l-1)*dz;

% calculate alpha_inv, beta_inv, b1 and b2 initial values
grhandles = gr;
if (kkey == 0)
    rcgrd = zeros(size(x_xs));
    icgrd = rcgrd;
    rbb = rcgrd;
    ibb = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rbb = rcgrd;
    alpha =
sign(mean(s(:)))*sign(mean(rcgrd(:)))*max(abs(s(:)))/max(abs(rcgrd(:)));
    b1 = std(s(:) - alpha*rcgrd(:));
else
    rsgrd = zeros([3 size(x_xs)]);
    isgrd = rsgrd;

```

```

    rsb = rsgrd;
    isb = rsgrd;
    [rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rsb = rsgrd;
    alpha =
sign(mean(s(:))*sign(mean(rsgrd(3,:))*max(abs(s(:)))/max(abs(rsgrd(3,:))));
    b1 = std(s(:) - alpha*rsgrd(3,:));
end

%calculate green's functions G(rm,rd)

if(kkey == 0)
    rcgrd = zeros(size(x_xd));
    icgrd = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
    beta =
sign(mean(a(:))*sign(mean(rcgrd(:))*max(abs(a(:)))/max(abs(rcgrd(:))));
    b2 = std(a(:) - beta*rcgrd(:));
else
    rsgrd = zeros([3 size(x_xd)]);
    isgrd = rsgrd;
    [rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
    beta = -
sign(mean(a(:))*sign(mean(rsgrd(3,:))*max(abs(a(:)))/max(abs(rsgrd(3,:))));
    b2 = std(a(:) - beta*rsgrd(3,:));
end

pars0=[x y z alpha beta b1 b2];

options = optimset('MaxFunEvals',1e4,'MaxIter',1e4,'TolX',1e-7);
err =
@(pars)fitpos_err(pars,s,a,ze,lld,mua,lt,nd,omega,zs,zd,delta,kkey,xms,yms,xm
a,yma);
[pars,resnorm] = fminsearch(err,pars0,options);

x = pars(1);
y = pars(2);
z = pars(3);
alpha = pars(4);
beta = pars(5);
b1 = pars(6);
b2 = pars(7);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% plot %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%% generate positions %%%%%%%%%
x_xs = zeros(ISNY,ISNX);
y_ys = zeros(ISNY,ISNX);
nsx = 1:ISNX;
nsy = 1:ISNY;

x_xd = zeros(IDNY,IDNX);
y_yd = zeros(IDNY,IDNX);
ndx = 1:IDNX;

```

```

ndy = 1:IDNY;

%2d positions
x_xs = ones(ISNY,1)*(delta(1)*(nsx-1)-x+dx0);
y_ys = (delta(2)*(nsy-1)-y+dy0)'*ones(1,ISNX);

x_xd = ones(IDNY,1)*(delta(5)*(ndx-1)-x);
y_yd = (delta(6)*(ndy-1)-y)'*ones(1,IDNX);

grhandles = gr;
%calculate green functions G(rm,rs)

if (kkey == 0)
    rcgrd = zeros(size(x_xs));
    icgrd = rcgrd;
    rbb = rcgrd;
    ibb = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rbb = rcgrd;
else
    rsgrd = zeros([3 size(x_xs)]);
    isgrd = rsgrd;
    rsb = rsgrd;
    isb = rsgrd;
    [rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rsb = rsgrd;
end

%calculate green functions G(rm,rd)
if(kkey == 0)
    rcgrd = zeros(size(x_xd));
    icgrd = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);

    subplot(2,2,1);
    plot(delta(1)*(nsx-1),s(jms,1:ISNX),'o',delta(1)*(nsx-
1),alpha*rbb(jms,1:ISNX)+b1);
    title('Fitting image on source plane along x through peak');
    set(gca,'fontsize',12);
    set(get(gca,'title'),'fontsize',12);
    xlabel('x (mm)');
    ylabel('Intensity (arb. unit)');
    set(get(gca,'xlabel'),'fontsize',12);
    xlim([0 delta(1)*ISNX]);
    ylim([min([s(:); alpha*rbb(:)+b1]) max([s(:); alpha*rbb(:)+b1])]);

    subplot(2,2,2);
    plot(delta(2)*(nsy-1),s(1:ISNY,ims),'o',delta(2)*(nsy-
1),alpha*rbb(1:ISNY,ims)+b1);
    title('Fitting image on source plane along y through peak');
    set(gca,'fontsize',12);
    set(get(gca,'title'),'fontsize',12);
    xlabel('y (mm)');
    ylabel('Intensity (arb. unit)');

```

```

set(get(gca,'ylabel'),'fontsize',12);
xlim([0 delta(2)*ISNY]);
ylim([min([s(:); alpha*rbb(:)+b1]) max([s(:); alpha*rbb(:)+b1])]);

subplot(2,2,3);
plot(delta(5)*(ndx-1),a(jma,1:IDNX),'o',delta(5)*(ndx-
1),beta*rcgrd(jma,1:IDNX)+b2);
title('Fitting image on detector plane along x through peak');
set(gca,'fontsize',12);
set(get(gca,'title'),'fontsize',12);
xlabel('x(mm)');
ylabel('Intensity(arb. unit)');set(get(gca,'xlabel'),'fontsize',12);
xlim([0 delta(5)*IDNX]);
ylim([min([a(:); beta*rcgrd(:)+b2]) max([a(:); beta*rcgrd(:)+b2])]);

subplot(2,2,4);
plot(delta(6)*(ndy-1),a(1:IDNY,ima),'o',delta(6)*(ndy-
1),beta*rcgrd(1:IDNY,ima)+b2);
title('Fitting image on detector plane along y through peak');
set(gca,'fontsize',12);
set(get(gca,'title'),'fontsize',12);
xlabel('y(mm)');
ylabel('Intensity(arb. unit)');set(get(gca,'ylabel'),'fontsize',12);
xlim([0 delta(6)*IDNY]);
ylim([min([a(:); beta*rcgrd(:)+b2]);max([a(:); beta*rcgrd(:)+b2])]);
else
rsgrd = zeros([3 size(x_xd)]);
isgrd = rsgrd;
[rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);

subplot(2,2,1);
plot(delta(1)*(nsx-1),s(jms,1:ISNX),'o',delta(1)*(nsx-
1),alpha*squeeze(rsb(3,jms,1:ISNX)+b1));
title('Fitting image on source plane along x through peak'); %2d
set(gca,'fontsize',12);
set(get(gca,'title'),'fontsize',12);
xlabel('x(mm)');
ylabel('Intensity(arb. unit)');
set(get(gca,'xlabel'),'fontsize',12);
xlim([0 delta(1)*ISNX]);
ylim([min([s(:)' alpha*rsb(3,:)+b1]) max([s(:)'
alpha*rsb(3,:)+b1])]); %2d

subplot(2,2,2);
plot(delta(2)*(nsy-1),s(1:ISNY,ims),'o',delta(2)*(nsy-
1),alpha*rsb(3,1:ISNY,ims)+b1);
title('Fitting image on source plane along y through peak'); %2d
set(gca,'fontsize',12);
set(get(gca,'title'),'fontsize',12);
ylabel('y(mm)');
ylabel('Intensity(arb. unit)');
set(get(gca,'ylabel'),'fontsize',12);
xlim([0 delta(2)*ISNY]);
ylim([min([s(:)' alpha*rsb(3,:)+b1]) max([s(:)' alpha*rsb(3,:)+b1])]);

subplot(2,2,3);

```

```

    plot(delta(5)*(ndx-1),a(jma,1:IDNX),'o',delta(5)*(ndx-
1),beta*squeeze(rsgrd(3,jma,1:IDNX)+b2)');
    title('Fitting image on detector plane along x through peak'); %2d
    set(gca,'fontsize',12);
    set(get(gca,'title'),'fontsize',12);
    xlabel('x(mm)');
    ylabel('Intensity(arb. unit)');
    set(get(gca,'xlabel'),'fontsize',12);
    xlim([0 delta(5)*IDNX]);
    ylim([min([a(:)' beta*rsgrd(3,:)+b2]) max([a(:)' beta*rsgrd(3,:)+b2])]);

    subplot(2,2,4);
    plot(delta(6)*(ndy-1),a(1:IDNY,ima),'o',delta(6)*(ndy-
1),beta*rsgrd(3,1:IDNY,ima)+b2);
    title('Fitting image on detector plane along y through peak'); %2d
    set(gca,'fontsize',12); set(get(gca,'title'),'fontsize',12);
    ylabel('y(mm)');
    ylabel('Intensity(arb. unit)');set(get(gca,'ylabel'),'fontsize',12);
    xlim([0 delta(6)*IDNY]);
    ylim([min([a(:)' beta*rsgrd(3,:)+b2]) max([a(:)'
beta*squeeze(rsgrd(3,:)+b2)'])]); %2d
end

```

```

%%%%%% print results %%%%%%%%%%

```

```

alpha = pars(4)/gamma1;
beta = pars(5)/gamma2;
strength = alpha*beta;
fprintf('\nObject location: x = %fmm; y = %fmm; z = %fmm\r\n',x,y,z);
fprintf('Scaling factors: alpha = %1.14f; beta = %1.14f; strength
= %1.14f\r\n',alpha,beta,strength);
fprintf('Intercept/Background: b1 = %1.14f; b2 = %1.14f\r\n',b1,b2);

```

```

if type == 0
    fitdata = rcgrd;
else
    fitdata = rsgrd;
end

```

```

return

```

```

function err =
fitpos_err(pars,s,a,ze,lld,mua,lt,nd,omega,zs,zd,delta,kkey,xms,yms,xma,yma)
global dx0 dy0

```

```

%%read in geometry
fid = fopen('geometry.txt','r');
ISNX = fscanf(fid,'%f',1);
ISNY = fscanf(fid,'%f',1);
IDNX = fscanf(fid,'%f',1);
IDNY = fscanf(fid,'%f',1);
MX = fscanf(fid,'%f',1);
MY = fscanf(fid,'%f',1);
LL = fscanf(fid,'%f',1);

```



```

fclose(fid);
grhandles = gr;

x = pars(1);
y = pars(2);
z = pars(3);
alpha = pars(4);
beta = pars(5);
b1 = pars(6);
b2 = pars(7);

%%%% generate positions %%%%%%%%%%
x_xs = zeros(ISNY,ISNX); %only a horizontal line
y_ys = zeros(ISNY,ISNX); %only a vertical line
nsx = 1:ISNX;
nsy = 1:ISNY;

x_xd = zeros(IDNY,IDNX); %only a horizontal line
y_yd = zeros(IDNY,IDNX); %only a vertical line
ndx = 1:IDNX;
ndy = 1:IDNY;

x_xs = ones(ISNY,1)*(delta(1)*(nsx-1)-x+dx0);
y_ys = (delta(2)*(nsy-1)-y+dy0)'*ones(1,ISNX);

x_xd = ones(IDNY,1)*(delta(5)*(ndx-1)-x);
y_yd = (delta(6)*(ndy-1)-y)'*ones(1,IDNX);

%calculate green functions G(rs,rm)
if (kkey == 0)
    rcgrd = zeros(size(x_xs));
    icgrd = rcgrd;
    rbb = rcgrd;
    ibb = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rbb = rcgrd;
else
    rsgrd = zeros([3 size(x_xs)]);
    isgrd = rsgrd;
    rsb = rsgrd;
    isb = rsgrd;
    [rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xs,y_ys,z,zs);
    rsb = rsgrd;
end

%calculate green functions G(rm,rd)
if(kkey == 0)
    rcgrd = zeros(size(x_xd));
    icgrd = rcgrd;
    [rcgrd,icgrd] = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
    err11 = (s(:) - alpha*rbb(:) - b1).^2;
    err12 = (a(:) - beta*rcgrd(:) - b2).^2;
    err =
    sum(err11(:))/length(s(:))/var(s(:))+sum(err12(:))/length(a(:))/var(a(:));

```

```

else
    rsgrd = zeros([3 size(x_xd)]);
    isgrd = rsgrd;
    [rsgrd,isgrd] = grhandles.sgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);

    errl1 = (s(:) - alpha*rsb(3,:)' - b1).^2;
    errl2 = (a(:) - beta*rsgrd(3,:)' - b2).^2;
    err =
sum(errl1(:))/length(s(:))/var(s(:))+sum(errl2(:))/length(a(:))/var(a(:));
end

```

backprojection.m

```

function backproj(A,z,lambda)
% A is the NCID/ICID/PCID
% z is the z-position of the target
% lambda is the optimal regularization number chosen from the L-curve

close all
% ----- initialization -----
fid = fopen('geometry.txt', 'r');
ISNX = fscanf(fid,'%f',1);
ISNY = fscanf(fid,'%f',1);
IDNX = fscanf(fid,'%f',1);
IDNY = fscanf(fid,'%f',1);
MX = fscanf(fid,'%f',1);
MY = fscanf(fid,'%f',1);
fclose(fid);

fid = fopen('input_data.txt', 'r');
n1 = fscanf(fid,'%f',2); %the reflective index of scattering medium
n2 = fscanf(fid,'%f',2); %the reflective index of wall (i.e. glass)
n3 = fscanf(fid,'%f',2); %the reflective index of outside (i.e. air)
lld = fscanf(fid,'%f',1); %scattering mediu thickness
mua = fscanf(fid,'%f',1); %absorption coefficient
lt = fscanf(fid,'%f',1); %transport length
nd = fscanf(fid,'%f',1); %scattering medium refraction index
omega = fscanf(fid,'%f',1); %modulation frequency, 0 for cw
z0 = fscanf(fid,'%f',1); %depth of the center of the first voxel
dz = fscanf(fid,'%f',1); %z direction division of the scattering medium
zs = fscanf(fid,'%f',1); %source position
zd = fscanf(fid,'%f',1); %detector position
delta = fscanf(fid,'%f',6); %step interval in x and y
rate = fscanf(fid,'%f',2); %noise level
dmu = fscanf(fid,'%f',2); %change in mu of the inhomogeneities
key = fscanf(fid,'%d',1); %switch for judging the signal from the noise
space, either by readin number r_mm or automatic judging with r_ratio
r_mm = fscanf(fid,'%d',1); %signal space rank
r_ratio = fscanf(fid,'%f',1); %cutoff threshold for signal space
key2 = fscanf(fid,'%d',1); %switch for normalization of the pseudo
spectrum
fclose(fid);

```

```

ze = calc_ze(n1,n2,n3);
grhandles = gr;

%% ----- Initialization -----
x_xd = ones(IDNY*2-1,1)*(-IDNX+1:IDNX-1)*delta(5);
y_yd = (-IDNY+1:IDNY-1)'*delta(6)*ones(1,IDNX*2-1);
gd = grhandles.cgrd(ze,lld,mua,lt,nd,omega,x_xd,y_yd,z,zd);
gd1 = [gd zeros(2*IDNY-1,2*IDNX-2);zeros(2*IDNY-2,2*IDNX-1+2*IDNX-2)];
gd1f = fft2(gd1);

A1 = zeros(4*IDNY-3,4*IDNX-3);
A1(IDNY:2*IDNY-1,IDNX:2*IDNX-1)=reshape(A(:,1),IDNY,IDNX);
A1f = fft2(A1);

%% -- Tikhonov regularization on Ax = b, check in Fourier domain START
% (conj(A)A+lambda) x = conj(A)b => x = conj(A)b/(conj(A)A+lambda)
k = 1;
h = waitbar(0,'Please wait...');
clear Alf_recon X1f

for lambda = 1:100
X1f(:, :) = conj(gd1f).*(A1f./(conj(gd1f).*(gd1f +
min(min(real(conj(gd1f).*(gd1f))))*10^lambda));
Alf_recon(:, :) = X1f(:, :).*(gd1f);

resNorm(k) = log(sqrt(squeeze(sum(sum(abs((Alf_recon(:, :)-A1f).^2))))));
solNorm(k) = log(sqrt(squeeze(sum(sum(abs(X1f(:, :).^2))))));

waitbar((lambda)/100,h);
k = k + 1;
end
close(h)

% plot L-curve
figure(1)
plot(resNorm,solNorm)
set(gca,'fontsize',12); set(get(gca,'title'),'fontsize',12);
xlabel('Residue norm (arb. units)')
ylabel('Solution norm (arb. units)')

%% choose corner from L-curve
%lambda = 25
X1f(:, :) = conj(gd1f).*(A1f./(conj(gd1f).*(gd1f +
min(min(real(conj(gd1f).*(gd1f))))*10^lambda));
X1_recon = ifft2(X1f(:, :));

%% -- plot cross section image --
X1s = X1_recon(1:IDNY,1:IDNX);
% X1s([1 end], :) = 0; X1s(:, [1 end]) = 0;
figure(2)

[yy,xx]=find(X1s==max(X1s(:)));
set(gcf,'position',[100 300 900 250]);

subplot(1,2,2);

```

```

plot((0:IDNX-1)*delta(5),X1s(yy,:), 'linewidth',2);
hold on
plot((0:IDNY-1)*delta(6),X1s(:,xx), '--', 'linewidth',2);
xlim([0 IDNX*delta(5)])
set(gca, 'fontsize',12);
xlabel('x (mm)');
ylabel('X (arb. units)');
legend('x','y')

subplot(1,2,1);
imagesc(X1s, 'xdata', delta(5)*[0:IDNX-1], 'ydata', delta(6)*[0:IDNY-1]);
colorbar
axis equal tight;
hold on
plot((0:IDNX-1)*delta(5),ones(1,IDNX)*(yy-1)*delta(5), 'w', 'LineWidth',2)
plot(ones(1,IDNY)*(xx-1)*delta(6), (0:IDNY-1)*delta(6), 'w--', 'LineWidth',2)
set(gca, 'fontsize',12);
set(get(gca, 'title'), 'fontsize',12);
set(get(gca, 'xlabel'), 'FontSize',12);
set(get(gca, 'ylabel'), 'FontSize',12);
xlabel('x (mm)');
ylabel('y (mm)');

%% find FWHM in the cross section image

[xm,xM]=searchMin(X1s(yy,:),xx);
[ym,yM]=searchMin(X1s(:,xx),yy);
if X1s(yy,xm) < 0
x1 = interp1(X1s(yy,xm:xx),xm:xx,(X1s(yy,xx))/2);
else
x1 = interp1(X1s(yy,xm:xx),xm:xx,(X1s(yy,xx)+X1s(yy,xm))/2);
end

if X1s(yy,xM) < 0
x2 = interp1(X1s(yy,xx:xM),xx:xM,(X1s(yy,xx))/2);
else
x2 = interp1(X1s(yy,xx:xM),xx:xM,(X1s(yy,xx)+X1s(yy,xM))/2);
end

if X1s(ym,xx) < 0
y1 = interp1(X1s(ym:yy,xx),ym:yy,(X1s(yy,xx))/2);
else
y1 = interp1(X1s(ym:yy,xx),ym:yy,(X1s(yy,xx)+X1s(ym,xx))/2);
end

if X1s(yM,xx) < 0
y2 = interp1(X1s(yy:yM,xx),yy:yM,(X1s(yy,xx))/2);
else
y2 = interp1(X1s(yy:yM,xx),yy:yM,(X1s(yy,xx)+X1s(yM,xx))/2);
end

dX = (x2-x1)*delta(5)

```

```
dY = (y2-y1)*delta(6)
```

```
function [xm,xM]=searchMin(y,x0)
D = length(y);
x = x0;
while 1
    if x - 1 < 1
        xm = x;
        break
    end
    if y(x-1)>=y(x)
        xm = x;
        break
    end
    x = x-1;
end

x = x0;
while 1
    if x+1 > D
        xM = x;
        break
    end
    if y(x+1)>=y(x)
        xM = x;
        break
    end
    x = x+1;
end
return
```

Appendix C

List of Patents, Publications and Presentations

List of Patents

Binlin Wu, Wei Cai, and Swapan Ghosh, “Time reversal optical tomography,” U.S.

Patent Provisional Application Serial No. 61/111,112.

List of Publications

Journal Articles

1. **Binlin Wu**, W. Cai, S. Ghosh, “Three-dimensional optical fluorescence imaging using time reversal optical tomography,” *Appl. Phys. Lett.* **101**, 211101 (2012).
2. **Binlin Wu**, W. Cai, S. Ghosh, “Time Reversal Optical Tomography,” *Optics and Photonics News (OPN)*, Vol. 2, Optics in 2012 (Issue 12), pp. 41-41 (2012). (Selected by the editors for publication in the *Virtual Journal for Biomedical Optics (VJBO)*, Volume 2, Issue 1, Topic “Diffuse Imaging, Tissue Optics, and Scattering”.
<http://www.opticsinfobase.org/vjbo/abstract.cfm?uri=opn-2-12-41>).
3. **Binlin Wu**, M. Alrubaiee, W. Cai, M. Xu and S. Ghosh, “Diffuse optical imaging using decomposition methods,” *Int. J. Opt.* **2012**, 1-4 (2012).
4. **Binlin Wu**, W. Cai, M. Alrubaiee, M. Xu and S. Ghosh, “Time reversal optical tomography: locating targets in a highly scattering turbid medium,” *Opt. Express* **19**, 21111-21121 (2011). (Selected by the editors, Andrew Dunn and Anthony Durkin, for publication in the *Virtual Journal for Biomedical Optics (VJBO)*, Volume 2, Issue 11, Topic “Diffuse Imaging, Tissue Optics, and Scattering”.
<http://www.opticsinfobase.org/vjbo/abstract.cfm?uri=oe-19-22-21111>).

1. **Binlin Wu** and S. J. Rayen, "Fluorescence tomography of targets in a turbid medium using nonnegative matrix factorization", submitted to Phys. Rev. (2011).

Conference Proceedings

1. **Binlin Wu**, W. Cai, S. J. Rayen, "Time reversal optical tomography locates fluorescent targets in a turbid medium," in Multimodal Biomedical Imaging III. Proceedings of SPIE, vol. 794, pp. 794N-1 - 794N-4 (2011).
2. **Binlin Wu**, W. Cai, M. Xu and S. J. Rayen, "Time-reversal optical tomography: detecting and locating extended targets in a turbid medium", in Multimodal Biomedical Imaging I. Proceedings of SPIE, vol. 721, pp. 721-1 - 721-4 (2012).
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List of Presentations

1. **Binlin Wu**, W. Cai, S. J. Rayen, "Time reversal optical tomography locates fluorescent targets in a turbid Medium," paper 794-1 presented at the SPIE's International Symposium on Multimodal Biomedical Imaging III, IOS'11 / Photonics West, 29-January, San Francisco, California.
2. **Binlin Wu**, W. Cai, M. Xu, and S. J. Rayen, "Time-reversal optical tomography: detecting and locating extended targets in a turbid medium," paper 721-1 presented at the SPIE's

International Symposium on Multimodal Biomedical Imaging II, IOS'12/ Photonics West, 21-22 January, San Francisco, California.

3. **Binlin Wu**, W. Cai, M. Alrubaiee, M. Xu, and S. S. Jayen, "Time reversal optical tomography for breast tumor detection", paper C 114-2 presented at the 11th Annual Meeting of the Department of Defense (DOD) Breast Cancer Research Program (BCRP), 21-22 August, Orlando, Florida.
4. M. Alrubaiee, **Binlin Wu**, M. Xu, W. Cai, S. S. Jayen, "Multi-wavelength diffusive optical tomography using independent component analysis and time reversal algorithms", paper 2-1 presented at the SPIE's International Symposium on Diffuse Optical Imaging III, European Conferences on Biomedical Optics (ECBO) 2011, 22-23 May, Munich, Germany.
5. **Binlin Wu**, W. Cai, M. Alrubaiee, M. Xu and S. S. Jayen, "Three-dimensional time-reversal optical tomography", paper 2-1 presented at the SPIE's International Symposium on Multimodal Biomedical Imaging I, IOS'11/ Photonics West, 22-23 January, San Francisco, California.
6. **Binlin Wu**, M. Alrubaiee, W. Cai, M. Xu, and S. S. Jayen, "Optical imaging of objects in turbid media using principal component analysis and time reversal matrix methods", paper TuC1 presented at Frontier in Optics (FiO), OSA's Annual Meeting on Computational Optical Sensing and Imaging, October 11, 2011, San Jose, California.
7. **Binlin Wu**, S. S. Jayen, and M. Xu, "Native excitation and emission matrix fluorescence spectroscopy for quantification of tissue native fluorophores and cancer diagnosis," submitted to Therapeutics and Diagnostics in Biophotonics, SPIE Photonics West (2014).

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Time reversal optical tomography locates fluorescent targets in a turbid medium

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ABSTRACT

A fluorescence optical tomography approach that extends time reversal optical tomography (TROT) to locate fluorescent targets embedded in a turbid medium is introduced. It uses a multi-source illumination and multi-detector signal acquisition scheme, along with TR matrix formalism, and multiple signal classification (MUSIC) to construct pseudo-image of the targets. The samples consisted of a single or two small tubes filled with water solution of Indocyanine Green (ICG) dye as targets embedded in a 250 mm $\times$ 250 mm $\times$ 60 mm rectangular cell filled with Intralipid-20% suspension as the scattering medium. The ICG concentration was 1 μ M, and the Intralipid-20% concentration was adjusted to provide $\sim$ 1-mm transport length for both excitation wavelength of 790 nm and fluorescence wavelength around 825 nm. The data matrix was constructed using the diffusely transmitted fluorescence signals for all scan positions, and the TR matrix was constructed by multiplying data matrix with its transpose. A pseudo spectrum was calculated using the signal subspace of the TR matrix. Tomographic images were generated using the pseudo spectrum. The peaks in the pseudo images provided locations of the target(s) with sub-millimeter accuracy. Concurrent transmission TROT measurements corroborated fluorescence-TROT findings. The results demonstrate that TROT is a fast approach that can be used to obtain accurate three-dimensional position information of fluorescence targets embedded deep inside a highly scattering medium, such as, a contrast-enhanced tumor in a human breast.

Keywords: Times reversal, optical tomography, fluorescence, indocyanine green (ICG), near infrared (NIR) imaging, turbid media, breast cancer

1. INTRODUCTION

Near-infrared (NIR) fluorescence imaging is attracting attention as a promising modality for noninvasive detection of cancer because of its potential for providing molecular information about biological tissues and changes in physiological parameters relevant to the onset and progression of cancer[1-13]. Other attractive features of fluorescence imaging include: higher detection sensitivity and specificity, as well as, higher target-to-background ratio and spatial resolution than absorption and scattering contrast-based optical imaging approaches. The problems of limited tissue depth penetration and auto-fluorescence may be reduced by operating in the 700 – 1000 nm NIR spectral range. Advances in the synthesis of fluorescent contrast agents, such as, dyes,[2, 7] nanoparticles,[3] and molecular beacons,[4] and concomitant developments in imaging instrumentation and numerical algorithms for image reconstruction[5-13] have resulted in contrast-enhanced fluorescence tomography approaches that are used more often for *in vivo* studies on animal models[5, 7, 9] than for human subjects[6, 11] or realistic phantoms.[8, 10, 11]

In this manuscript, we report on the development of *fluorescence* TROT, which extends time reversal optical tomography (TROT)[14] to fluoresce-based contrast. TROT combines the methodology of time reversal (TR) imaging[15] with multiple signal classification (MUSIC),[16] a subspace based processing methodology to locate unknown targets from measurements using multiple probes. TROT was shown to be a fast and accurate approach to locate both absorptive and scattering targets in turbid media.[14]

2. THEORETICAL FORMALISM

We are developing fluorescence TROT for slab samples with embedded targets (a female human breast compressed between two optically transparent plates is a decent approximation). The experimental arrangement uses a multi-source illumination and multi-detector signal acquisition scheme to acquire multiple angular views of the sample. The input surface (source plane) of the slab sample is scanned across an external point light source (laser beam). The fluorescent target(s) embedded in the highly scattering medium are excited by diffusely propagating light of wavelength λ_x . A

fraction of the forward propagating fluorescence signal emitted by the targets at wavelength λ_m , is detected on the other side of the sample by a two-dimensional detector array. For small fluorescent targets at $\mathbf{r}_j$ with volume V_j , the fluorescence signal from the targets illuminated by a point source of unit power at $\mathbf{r}_s$ is given by[17, 18]

$$K = \sum_j g_d(\mathbf{r}_j, \omega) f_j(\omega) g_s^T(\mathbf{r}_j, \omega), \quad (1)$$

where $g_s(\mathbf{r}, \omega) = \{G_x(\mathbf{r}, \mathbf{r}_s, \omega)\}^T$ and $g_d(\mathbf{r}, \omega) = \{G_m(\mathbf{r}_d, \mathbf{r}, \omega)\}^T$, (the superscript T denotes transpose); $G_x(\mathbf{r}, \mathbf{r}_s, \omega)$ is a Green's function that describes the propagation of excitation light at excitation wavelength λ_x from the source at $\mathbf{r}_s$ to the target at $\mathbf{r}$; $G_m(\mathbf{r}_d, \mathbf{r}, \omega)$ is a Green's function that describes the propagation of the fluorescent light at emission wavelength λ_m from the target at $\mathbf{r}$ to the detector at $\mathbf{r}_d$; ω is the modulation angular frequency of the light; $f_j(\omega)$ is the fluorescence strength of the j^{th} target, given by

$$f_j(\omega) = \gamma(\mathbf{r}_j) c_m V_j / [1 - i\omega\tau(\mathbf{r}_j)], \quad (2)$$

γ is the fluorescence yield, c_m is the speed of light in the medium, and τ is the fluorescence lifetime. K describes that the diffuse propagation of excitation light of wavelength, λ_x from the sources through the medium to illuminate the targets, and then the propagation of emitted fluorescence of wavelength, λ_m from the targets to the detectors. In this study, continuous wave (CW) illumination is used, *i.e.* $\omega = 0$. A time reversal matrix $T_{SDDS} = K^T K$ ($T_{DSSD} = K K^T$) is constructed. A set of eigenvectors $\{u_k, k = 1, \dots, N_d\}$ and $\{v_l, l = 1, \dots, N_s\}$ are calculated for T_{DSSD} and T_{SDDS} , respectively, with common eigenvalues $\{\mu_j, j = 1, \dots, \min(N_s, N_d)\}$, where N_s and N_d are numbers of sources and detectors, respectively.[14] The eigenvalues $\mu_j = |f_j|^2 \|g_d(\mathbf{r}_j, \omega)\|^2 \|g_s(\mathbf{r}_j, \omega)\|^2$ are proportional to squared fluorescence strengths of the targets if the targets are well resolved; otherwise, they are linear combinations of individual fluorescence strengths of different targets.[14]

By using an L -curve method with an eigenvalue threshold ε [19], eigenvectors are separated into signal and noise subspaces. A pseudo spectrum associated with detector plane is calculated using multiple signal classification (MUSIC) for a test target position $\mathbf{X}_p$ in the sample volume[14]

$$P_d(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| u_j^T \frac{g_d(\mathbf{X}_p, \omega)}{\|g_d(\mathbf{X}_p, \omega)\|} \right|^2. \quad (3)$$

The locations of targets are retrieved to be the poles of the pseudo spectrum. A similar pseudo spectrum for the source plane,

$$P_s(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left| v_j^T \frac{g_s(\mathbf{X}_p, \omega)}{\|g_s(\mathbf{X}_p, \omega)\|} \right|^2. \quad (4)$$

or for both source and detector planes

$$P(\mathbf{X}_p, \omega) = 1 / \sum_{\mu_j < \varepsilon} \left(\left| u_j^T \frac{g_d(\mathbf{X}_p, \omega)}{\|g_d(\mathbf{X}_p, \omega)\|} \right|^2 + \left| v_j^T \frac{g_s(\mathbf{X}_p, \omega)}{\|g_s(\mathbf{X}_p, \omega)\|} \right|^2 \right). \quad (5)$$

may also be used to retrieve the target position.

3. EXPERIMENTAL MATERIALS, METHODS, AND DATA PROCESSING

The sample was a 250 mm $\times$ 250 mm $\times$ 60 mm rectangular transparent plastic cell filled with Intralipid-20% (Baxter) suspension in distilled water with one or two fluorescent targets embedded inside. The thickness of the sample was comparable to that of a typical compressed breast. The concentration of Intralipid-20% was adjusted[20] to provide a transport mean free path l_t of ~ 1 mm, which happens to be about the same for both excitation and emission wavelengths, and was similar to the average value of l_t for human breast tissue at these wavelengths. Each fluorescent target was a 4.2 mm

inner-diameter 10-mm long cylindrical glass tube filled with solution of ICG dye (Sigma-Aldrich) water. The dye solution in the targets was prepared by dissolving ICG at a concentration of 1 μM in the Intralipid-20% suspension of same concentration as the background medium to ensure that the targets had the same scattering coefficient as the background medium, but a higher absorption coefficient of 0.027 mm^{-1} . The water solution of ICG absorbs light over the 600 – 900 nm range with peak at 780 and fluoresces in the 790 – 966 nm range with peak at around 825 nm in the NIR enabling deeper penetration of light in tissues. It has been approved by Food and Drug Administration (FDA) for biomedical applications.[21]

Separate experiments were carried out with one target and two targets. In both cases, the target(s) was (were) embedded in the mid-plane ($z = 30 \text{ mm}$) of the sample cell. The exact locations of the targets are given in Table I and II. A multi-source sample excitation and multi-detector fluorescence signal acquisition scheme was used to acquire multiple angular views of the sample. The experimental arrangement is shown schematically in Fig. 1.

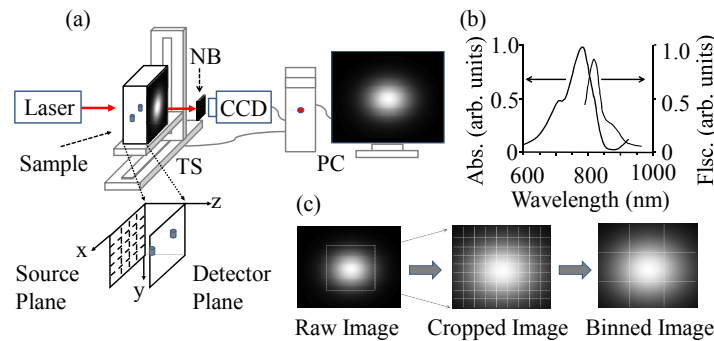


Fig.1. (a) A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium. [Key: NB = narrow band pass filter, TS = translational stage, CCD = charge coupled device, PC = personal computer] Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam, and a typical raw image is shown in the PC monitor. (b) The absorption and fluorescence spectra of ICG in water. (c) A typical raw image detected by the CCD camera is cropped and binned.

The entrance face of the slab sample (*source plane*) was illuminated by a 100-mW 790-nm diode laser beam. The multi-source illumination scheme was realized by scanning the sample across the laser beam in a two-dimensional x - y array of grid points using a computer-controlled translation stage. For the single target a 9×9 array and for two targets a 15×11 array of grid points with a step size of 5 mm were scanned. A cooled CCD camera equipped with a 60-mm focal-length camera lens collected and sensed a fraction of the forward propagating fluorescence signal from the opposite face of the sample (*detection plane*) through a narrow-band (full-width-at-half-maximum (FWHM) bandwidth of 10 nm) interference filter with 50% peak transmission at 830 nm. The CCD camera had 1024×1024 pixels with a pixel size of $24 \mu\text{m}$, and was considered as multi-detector, since each illuminated pixel could be considered as an individual detector. The NB filter was chosen to select a substantial fraction of the ICG fluorescence around the peak emission wavelength and block the transmitted 790 nm pump light. The scanning and data acquisition processes were controlled by a personal computer (PC). Raw images were recorded by the PC for each scan position, and stored for subsequent analysis. A typical image, which is a 2-D intensity distribution, is shown in the left frame of Fig. 1(c).

At every scan position, an image was also acquired with the NB filter removed. In this case, the recorded images were essentially transillumination images since the fluorescence signal was negligible compared to the much more intense transmission signal (ratio of transmission signal to fluorescence signal ~ 1500). Thus correlated imaging and retrieval of target location using both fluorescence and transmission measurements were enabled in this experiment. The transmission images were analyzed to estimate the average value of the optical property of the medium $\kappa = (3\mu_a\mu_s')^{1/2}$, where μ_a and μ_s' are the absorption and reduced scattering coefficients at 790 nm, respectively. In fact, the values of these optical parameters of Intralipid-20% suspension in water happened to be very close for excitation and fluorescence wavelengths.

From each fluorescence image, a region of interest was cropped out and every 5×5 pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The response data matrix was constructed using the transmitted fluorescence light intensity distribution in the processed images. The TR matrix was generated by multiplying the response matrix by its transpose for our CW probing scheme. The eigenvalue equation of TR matrix was solved. Then the signal and

noise subspaces were separated. MUSIC pseudo spectrum was calculated and target locations were determined using the poles in the pseudo spectrum.

For comparison, the transmission data were also analyzed using TROT as detailed in Ref. 14. In this case, the target was absorptive, and the contrast was mainly due to higher absorption of the excitation beam by the target. It should be noted that absorption measurement involves changes in the intensity of the excitation beam, and consequently the TROT analysis used the difference images between the raw transmission images and a reference image for the background medium. The reference image may be estimated as the average of the images acquired at all scan positions.

4. RESULTS

4.1. Single Target

For the first experiment where one target was present, the TR matrix was constructed using the fluorescence data, and an eigenvalue equation was then solved. The eigenvector with the dominant eigenvalue was used to calculate the pseudo spectrum for all voxels in the 3-*D* space of the sample. The voxel size was 0.77 mm × 0.77 mm × 1 mm. Three-dimensional tomographic pseudo images were generated using the pseudo spectrum. The single target was detected, and the position of the target was determined using the peak in the pseudo spectrum and listed in Table I, with comparison to the actual position. The image at the retrieved *z*-coordinate of the target position (*z* = 30.5 mm) plotted using the pseudo spectrum is shown in Fig. 2(a). Further calculation showed that when more eigenvectors were used, the pseudo spectrum still clearly detects one single target. The determination of the number of targets is not sensitive to the choice of the threshold in the eigenvalue spectrum.

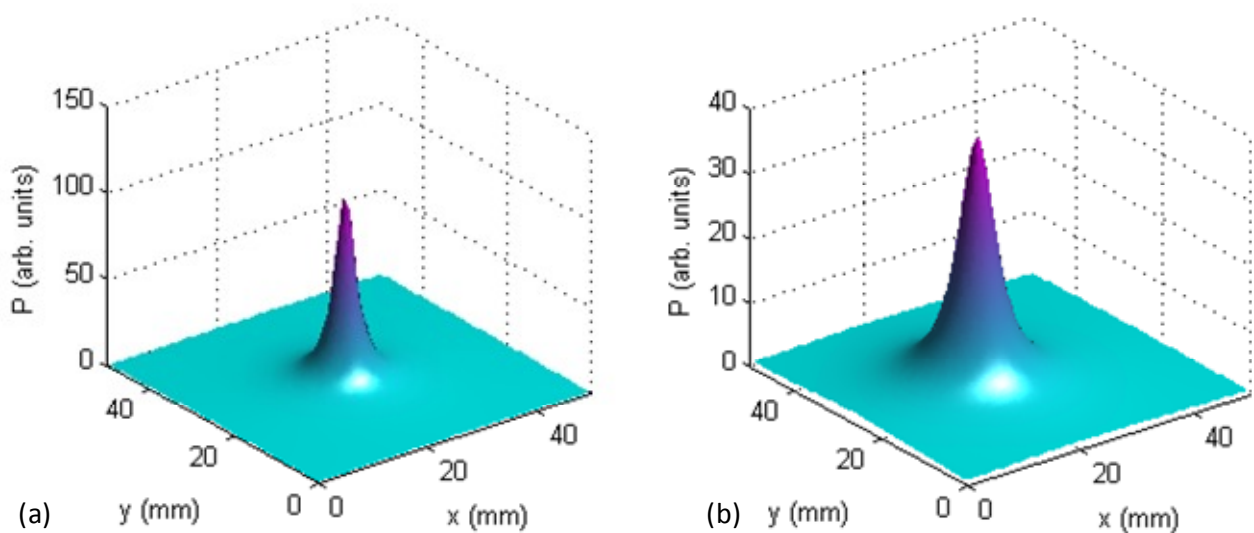


Fig. 2. TROT-reconstructed image at *z* = 30.5 mm using *fluorescence* data is shown in (a), and at *z* = 29.5 mm using *transmission* data shown in (b).

Table I. Known and retrieved target positions.

	Known	Retrieved	
		Fluorescence	Transmission
Position [<i>x</i> , <i>y</i> , <i>z</i> (mm)]	25.1, 25.7, 30.0	24.1, 25.6, 30.5	21.8, 25.6, 29.5
Error [Δx , Δy , Δz (mm)]	-	1.0, 0.1, 0.5	3.3, 0.1, 0.5
FWHM [δx , δy (mm)]	-	3.8, 3.8	6.9, 6.9

The transmission data was then analyzed for comparison. The target was also detected and its retrieved location is listed in Table I for comparison. The image of the target at $z = 29.5$ mm is shown in Fig. 2(b).

As shown in Table I, the location of target retrieved from the fluorescence data is in excellent agreement with the known position, and is consistent with that retrieved using transmission data. The pole of the pseudo image using fluorescence data is sharper than that obtained using transmission data. The FWHM of the pole in both x and y directions is 3.8 mm in the fluorescence-TROT image and 6.9 mm in the transmission-TROT image.

4.2. Two targets

Similar processing and analysis of data were carried out for the second experiment with two targets. Both targets were detected. TROT-reconstructed images at the $z = 29.5$ mm plane using the fluorescence and transmission data are shown in Fig. 3(a) and Fig. 3(b), respectively. The retrieved locations of targets are listed and compared with known positions in Table II.

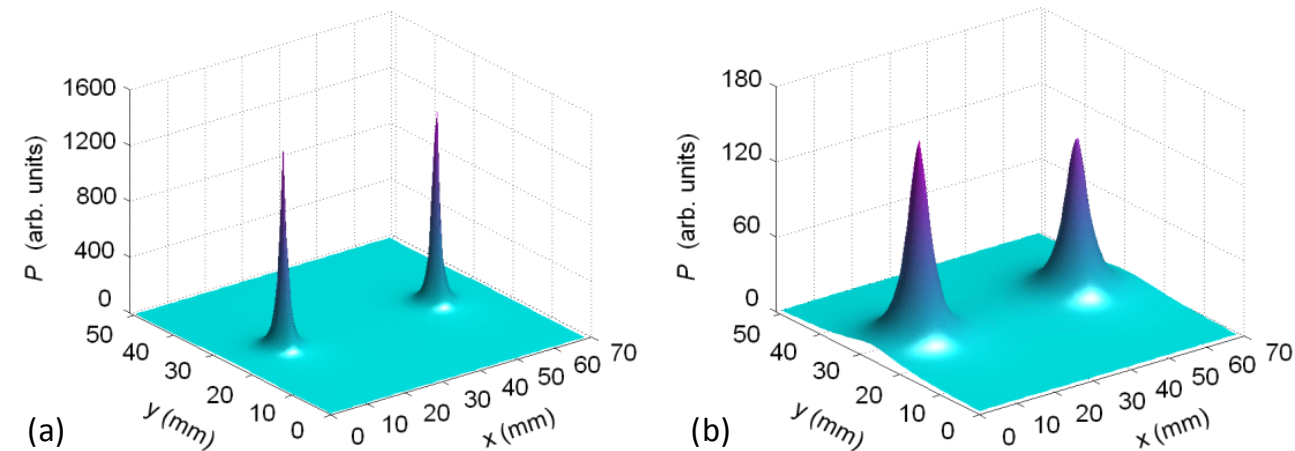


Fig. 3. TROT-reconstructed images at $z = 29.5$ mm using *fluorescence* and *transmission* data are shown in (a) and (b), respectively.

Table II. Known and retrieved target positions.

		Left target	Right target
Known Position $[x, y, z \text{ (mm)}]$		14.2, 25.0, 30.0	54.2, 25.0, 30.0
Fluorescence-TROT retrieved	Position $[x, y, z \text{ (mm)}]$	13.4, 24.9, 29.5	53.9, 24.1, 30.5
	Error $[\Delta x, \Delta y, \Delta z \text{ (mm)}]$	0.8, 0.1, 0.5	0.3, 0.9, 0.5
Transmission-TROT retrieved	Position $[x, y, z \text{ (mm)}]$	12.6, 27.2, 30.5	53.9, 27.2, 29.5
	Error $[\Delta x, \Delta y, \Delta z \text{ (mm)}]$	1.6, 2.2, 0.5	0.3, 2.2, 0.5
FWHM $[\delta x, \delta y \text{ (mm)}]$ using	Fluorescence-TROT	2.0, 1.5	1.7, 1.8
	Transmission-TROT	5.7, 4.2	6.4, 5.0

As in the case of the single target, the position coordinates of both the targets are retrieved within 1 mm of the known coordinates using fluorescence data. The locations retrieved using transmission data are consistent with those retrieved using fluorescence data, but the poles are wider. The FWHM of the spatial profiles of poles corresponding to the left

target and the right target in the x and y directions for both fluorescence and transmission measurements are presented and compared in Table II.

We then carried out a simulation-based investigation to assess the ability of the approach to resolve the two targets for different separations between them and different noise levels. A random additive Gaussian noise in the intensity distribution was used.

We found that the two targets can be resolved with a 6-mm center-to-center separation, or 2-mm edge-to-edge separation, with 10% noise; but not with 20% noise. Peaks in the pseudo image corresponding to the two targets get closer when the noise level is higher. The center-to-center separation is retrieved to be 4 mm with 0 noise, 3 mm for 5% noise and 2 mm for 10% noise. With 40-mm lateral separation, the targets could be resolved even with 50% noise.

The resolvability degrades if the targets have the same lateral (x , y) position but are separated in the axial (z) direction. With 5% noise, 40-mm separation between targets is exactly retrieved, 30 mm is retrieved to be 29 mm, 20 mm is retrieved to be 18 mm, 18 mm is retrieved to be 6 mm, 17 mm or less cannot be resolved. We ascribe this limitation to the slab geometry and transmission mode of signal acquisition which results in limited angular views for the finite size of the source and detector planes used in the experiment. The resolution may improve if one uses a larger angular view and/or other sample geometries, such as cylindrical or semi-spherical, to acquire data.

5. DISCUSSION

We have developed the *fluorescence* TROT approach and used it to retrieve the locations of a single and two small fluorescent targets embedded in a breast-simulating turbid medium with the thickness comparable to that of a realistic compressed breast. The location of targets was retrieved with an accuracy of ~ 1 mm in all three dimensions.

Since a single eigenvector in the signal subspace was used for each target, the targets were treated as point objects, and the pseudo spectrum only carried location information of the targets. The FWHM width of a pole in the pseudo spectrum reflects on the uncertainty in the determination of the location of the corresponding target. Fluorescence signal appeared to provide a better resolution than transillumination signal under our experimental conditions. Similar results have been reported by other researchers as well.[6, 8] We tentatively ascribe this to the difference in the background level between fluorescence and transmission signals. In this study, fluorescence signal had zero background because there was no fluorophore in the background medium, while transmission signal involved measurement of changes on a strong background, and such changes for targets in a turbid medium could be rather small. If the background fluorescence signal is significantly high, particularly in *in vivo* experiments because of auto-fluorescence, or fluorophore uptake by the background medium, the fluorescence-based approach may not retain this edge. A proper method has to be employed to obtain the fluorescence signal due to targets, which may be treated as perturbation in the background fluorescence signal [8, 22]. More work involving different types of samples and target contrast will be needed to obtain a more definitive conclusion.

Compared to other model-based inverse image reconstruction (IIR) methods, TROT is faster as discussed in Ref. 14. The data matrix used in TROT has a lower dimension than those used in other approaches. TROT does not involve iteration of forward model, and attempts to locate the targets without retrieving the optical property in every voxel. Fluorescence TROT has the same advantage over other IIR methods for fluorescent targets.

In this study, CW measurements and a limited acquisition angle for the slab geometry were used. TROT can be used in frequency domain and other geometries such as cylindrical or semi-spherical with larger angular view, and may lead to even better results, particularly for the resolution in the axial direction.

The results further suggest the potential of TROT for retrieving three-dimensional location information of contrast-enhanced tumors in a human breast at early development stages.

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Three-dimensional localization of fluorescent targets in turbid media using time reversal optical tomography

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An optical tomography approach for locating fluorescent targets embedded inside a turbid medium is introduced. It uses multi-source probing and multi-detector signal acquisition to collect diffuse fluorescence signal, and time reversal matrix formalism with subspace based signal processing for image reconstruction. It could provide three-dimensional position co-ordinates of two small fluorescent targets embedded in Intralipid-20% suspension of thickness ~ 60 times the transport mean free path with an accuracy of ~ 1 mm. Fast reconstruction and high spatial resolution make the approach potentially suited for detecting and locating contrast-enhanced breast tumor at early stages of growth. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4771997>]

Near-infrared (NIR) fluorescence imaging is emerging as a promising modality for early detection of cancer for a variety of reasons.^{1–13} Fluorescence imaging is generally noninvasive, and has the intrinsic potential to provide molecular information about biological tissues and changes in significant physiological parameters associated with the onset and progression of disease. Fluorescence imaging is characterized by higher detection sensitivity and specificity, as well as higher target-to-background ratio and spatial resolution than absorption and scattering contrast-based optical imaging approaches. The problem of limited tissue depth penetration may be considerably resolved by operating in the NIR spectral range of 700–1000 nm. Development of target-specific exogenous contrast agents holds the promise for tumor detection and characterization with high specificity. Much recent effort has gone into the development of fluorescent contrast agents, such as, dyes,^{2,7} nanoparticles,³ and molecular beacons.⁴ Concomitant developments have taken place in imaging instrumentation and numerical algorithms for image reconstruction.^{5–13} These advances have culminated in contrast-enhanced fluorescence tomography approaches that are used more often for *in vivo* studies on animal models^{5,7,9} than for human subjects^{6,11} or realistic phantoms.^{8,10,11}

In this letter, we report on an alternative fluorescence tomography approach, which extends time reversal optical tomography (TROT)¹⁴ for fluorescent contrast and refer to it as *fluorescence TROT*. TROT combines the methodology of time reversal (TR) imaging¹⁵ with multiple signal classification (MUSIC),¹⁶ a subspace based processing methodology to locate unknown targets from measurements using multiple probes. TROT was shown to be a fast and accurate approach to locate both absorptive and scattering targets in turbid media.¹⁴

Fluorescence TROT uses a multi-source illumination and multi-detector signal acquisition scheme to acquire multiple angular views of the sample that consists of a target (or,

targets) embedded in a turbid medium, such as, a tumor in human breast. The fluorescence light intensities are measured on the boundary of the medium by a two-dimensional detector array when an external point source (laser beam) scans on the other side of the medium. The focus is to detect the target(s) and retrieve three-dimensional (3-D) location information.

The theoretical formalism for fluorescence TROT considers fluorescent target(s) embedded in a highly scattering medium being excited by diffusely propagating light of wavelength λ_x , and detection of fluorescence emitted by the targets at wavelength λ_m , as a coupled diffuse transmission problem.^{17,18} Assuming fluorescent targets are localized, that is, the j th target is contained in volume V_j centered at $\mathbf{r}_j$, the fluorescence signal under illumination by a point source of unit power at $\mathbf{r}_s$ is given by

$$K = \sum_j g_d(\mathbf{r}_j, \omega) f_j(\omega) g_s^T(\mathbf{r}_j, \omega), \quad (1)$$

where $g_s(\mathbf{r}, \omega) = \{G_x(\mathbf{r}, \mathbf{r}_s, \omega)\}^T$ and $g_d(\mathbf{r}, \omega) = \{G_m(\mathbf{r}_d, \mathbf{r}, \omega)\}^T$, (the superscript T denotes transpose) are Green's function vectors; $G_x(\mathbf{r}, \mathbf{r}_s, \omega)$ is a Green's function that describes light propagation at excitation wavelength λ_x from the source at $\mathbf{r}_s$ to the target at $\mathbf{r}$; $G_m(\mathbf{r}_d, \mathbf{r}, \omega)$ is a Green's function that describes the propagation of fluorescence light at emission wavelength λ_m from the target at $\mathbf{r}$ to the detector at $\mathbf{r}_d$; ω is the modulation angular frequency of the light; $f_j(\omega)$ represents the fluorescence strength of the j th target, given by,

$$f_j(\omega) = \gamma(\mathbf{r}_j) c_m V_j / [1 - i\omega\tau(\mathbf{r}_j)], \quad (2)$$

where γ is the fluorescence yield, c_m is the speed of light in the medium, and τ is the fluorescence lifetime. K describes the diffuse propagation of the excitation light of wavelength, λ_x , from the sources through the medium to the targets, and then the propagation of fluorescence of wavelength, λ_m from the targets to the detectors. K^T describes the virtual process of fluorescent light propagation from the

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positions of detectors to the targets, and then the propagation of the excitation light of wavelength λ_x , from the positions of the targets to the sources. A time reversal matrix $T_{SDS} = K^\dagger K$ [$T_{DSS} = (K^T)^\dagger K^T = K^* K^T$] in frequency domain is then constructed, where the superscript $\dagger$ denotes Hermitian conjugate, or $T_{SDS} = K^T K$ ($T_{DSS} = K K^T$) when using the continuous wave (CW) illumination, i.e., $\omega = 0$. T_{DSS} and T_{SDS} have eigenvectors $\{u_k, k = 1, \dots, N_d\}$ and $\{v_l, l = 1, \dots, N_s\}$, respectively, with a common set of eigenvalues $\{\mu_j, j = 1, \dots, \min(N_s, N_d)\}$, where N_s and N_d are numbers of sources and detectors, respectively.¹⁴ If the fluorescent targets are well resolved, the eigenvalues are, $\mu_j = |f_j|^2 \|g_d(\mathbf{r}_j, \omega)\|^2 \|g_s(\mathbf{r}_j, \omega)\|^2$; otherwise, they are linear combinations of the fluorescence strengths, similar to what is the case for absorptive and scattering targets.¹⁴

The eigenvectors are separated into signal and noise subspaces using an L -curve method with an eigenvalue threshold ε .¹⁹ The locations of targets are poles of the MUSIC pseudo spectrum¹⁴

$$P_d(\mathbf{X}_p, \omega) = \frac{\|g_d(\mathbf{X}_p, \omega)\|^2}{\left\| \sum_{\mu_j > \varepsilon} u_j^T g_d(\mathbf{X}_p, \omega) \right\|^2} \quad (3)$$

associated with the detector plane; $\mathbf{X}_p$ spans probable target locations. A similar pseudo spectrum for the source plane $P_s(\mathbf{X}_p, \omega)$, or the product of the two, $P(\mathbf{X}_p, \omega) = P_s(\mathbf{X}_p, \omega) P_d(\mathbf{X}_p, \omega)$, may also be used to retrieve the target position. In this work, we used the pseudo spectrum, P_d associated with the detector plane because: (a) the images naturally reside in the co-ordinate system based on the field of view of the charge-coupled device (CCD) camera (*detectors*) making data analysis easier; and (b) the use of many more detectors than sources in our experimental arrangement provides a more robust data set for superior noise-resistant and artifact-tolerant reconstruction in the detector plane than the source plane. We set $\omega = 0$ as a CW laser beam was used in the experiment.

The experimental arrangement, shown schematically in Fig. 1, used a multi-source sample excitation and multi-

detector fluorescence signal acquisition scheme to acquire multiple angular views of the sample. The fluorescence light intensities were measured on the boundary of the medium by a two-dimensional detector array when an external point source (laser beam) scanned the other side of the medium. The sample was a 250 mm $\times$ 250 mm $\times$ 60 mm rectangular transparent plastic cells filled with Intralipid-20% (Baxter) suspension in distilled water with two fluorescent targets embedded inside. The concentration of Intralipid-20% (background scattering medium) was adjusted²⁰ to provide a transport mean free path l_t of ~ 0.99 mm at 790 nm (excitation wavelength), and 1.05 mm at 830 nm (emission wavelength), which were similar to the average value of l_t for human breast tissue. The sample cell thickness of 60 mm was comparable to the thickness of a typical compressed breast. The fluorescent targets were two 10-mm long, 4.2 mm inner-diameter cylindrical glass tubes filled with indocyanine green (ICG) dye (Sigma-Aldrich) solution. The dye solution in the targets was prepared by dissolving ICG at a concentration of 1 μ M in the Intralipid-20% suspension of same concentration as the background medium to ensure that the targets had the same scattering coefficient as the background medium, but a higher absorption coefficient of 0.027 mm⁻¹. ICG is chosen because it absorbs and fluoresces in the NIR enabling deeper penetration of light into tissues, and has been approved by Food and Drug Administration (FDA) for biomedical application.²¹ The two targets were embedded in the mid-plane ($z = 30$ mm) of the sample cell. The exact locations are given in Table I.

A 100-mW 790-nm diode laser beam was used to illuminate a 250 mm $\times$ 250 mm entrance face of the slab sample (*source plane*). The multi-source illumination scheme was realized by scanning the sample across the laser beam in a 15 $\times$ 11 two-dimensional x - y array of grid points with a step size of 5 mm using a computer-controlled translation stage. A cooled CCD camera equipped with a 60-mm focal-length camera lens collected and sensed a fraction of the fluorescence signal from the opposite face of the sample (*detector plane*) through a narrow-band (NB) interference filter centered at 830 nm (FWHM 10 nm, 50% transmission). The narrow-band filter was chosen to select a substantial fraction of the ICG fluorescence around the peak emission wavelength and block the scattered 790 nm pump light. The CCD camera had 1024 $\times$ 1024 pixels with a pixel size of 24 μ m. The scanning and data acquisition processes were controlled by a personal computer (PC). Each illuminated pixel of the CCD camera could be regarded as a detector. Raw images were recorded by the PC for each scan position, and stored for subsequent analysis. A typical image, which is a 2-D intensity distribution, is shown in the left frame of Fig. 1(c).

For every scan position, images were also acquired by removing the NB filter that was used to block the pump beam. With the NB filter removed, the recorded images were essentially transillumination images since the fluorescence signal was negligible compared to the much more intense transmission signal (ratio of transmission signal to fluorescence signal ~ 1500). The experimental arrangement thus enables correlated imaging and retrieval of target location using both fluorescence and transmission measurements. The transmission images were analyzed to estimate the average

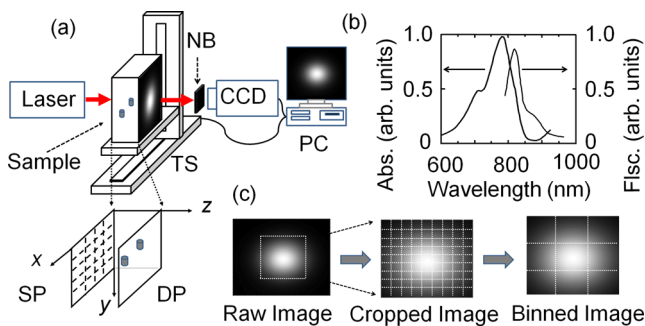


FIG. 1. (a) A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium [Key: NB = narrow band pass filter (830 nm), TS = translational stage, CCD = charge coupled device, PC = personal computer, SP = source plane, DP = detector plane]. Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam. A typical raw image is shown in the PC monitor. (b) The absorption and fluorescence spectra of ICG in water. (c) A typical raw image detected by the CCD camera is cropped and binned.

TABLE I. Known and retrieved target positions.

Target	Known positions [x, y, z (mm)]	Retrieved positions [x, y, z (mm)]		Error [Δx , Δy , Δz (mm)]	
		Fluorescence	Transmission	Fluorescence	Transmission
Left	14.2, 25.0, 30.0	13.4, 24.9, 29.5	12.6, 27.2, 30.5	0.8, 0.1, 0.5	1.6, 2.2, 0.5
Right	54.2, 25.0, 30.0	53.9, 24.1, 30.5	53.9, 27.2, 29.5	0.3, 0.9, 0.5	0.3, 2.2, 0.5

value of $\kappa = \sqrt{3\mu_a\mu_s'}$ (where μ_a and μ_s' are the absorption and reduced scattering coefficients at 790 nm, respectively). The values of these optical parameters of Intralipid-20% suspension in water happened to be very close for excitation and fluorescence wavelengths.

From each fluorescence image, a region of interest was cropped out and then every 5×5 pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The response data matrix was constructed using the transmitted fluorescence light intensity distribution in these processed images. The TR matrix was generated by multiplying the response matrix by its transpose for our CW probing scheme. The eigenvalue equation of TR matrix was solved.

The first 20 eigenvalues plotted in Fig. 2 demonstrate that only two eigenvalues are dominant, and consequently those two were included in the signal subspace and separated from the noise subspace.

MUSIC algorithm¹⁶ was then used to calculate the pseudo spectrum for all voxels in the 3-D space of the sample. The voxel size was $0.77 \text{ mm} \times 0.77 \text{ mm} \times 1 \text{ mm}$. Three-dimensional tomographic pseudo images were generated using the pseudo spectrum. Fig. 3 shows one such tomographic image for $z = 29.5 \text{ mm}$. The locations of targets taken to correspond to the peaks of the pseudo images are listed and compared with known positions in Table I. The single point that the method identifies as the location of a target may be considered to be the “center of fluorescence strength” of the target, which may coincide with the geometrical center for a homogeneous target, but would be weighted by the distribution of fluorescence strength for a heterogeneous target.

The retrieved positions are in good agreement with the known locations of the targets. Further experimentation and simulation reveal that TROT achieves better resolution in the lateral (x, y) directions than in the axial (z) direction, and that the resolution depends on separation between the targets

and other experimental parameters. We estimate that under the reported experimental conditions and parameters, the same two targets could be resolved even when the closest lateral (x, y) distance between their surfaces was 2 mm (center-to-center distance of 6 mm) with a noise level of $\sim 10\%$. If the targets had the same lateral position but different z -positions, the separation between them could be determined within 2 mm even with 50% additive random noise when the separation was 30 mm. As the axial distance between the two targets was reduced to 20 mm, the approach retrieved the separation to be smaller than 20 mm, such as, 18 mm for up to 20% noise, 11 mm for 40% noise, and failed to resolve for 45% or higher noise. For an axial separation of 17 mm, the targets could not be resolved even with 0 noise. The axial resolution could be improved if the data were acquired in a wider angular view (for example, additional measurements across an adjacent (y - z) side), or using cylindrical geometry.

Since an excited fluorescent target emits light in all directions, the method can be readily used in back-propagation geometry and is not restricted to transmission mode reported here. We have used CW illumination and rectangular slab sample geometry in experiments reported here, but the approach can be extended to frequency-domain and time-domain measurements and other sample geometries (cylindrical, spherical, and hemispherical) as well.

For comparison, the transmission data were also analyzed using TROT as detailed in Ref. 14. In that case, the targets were absorptive, and the contrast was mainly due to higher absorption of the excitation beam by the target(s). It should be noted that absorption measurement involves changes in the intensity of the excitation beam, and consequently the TROT analysis used the difference images between the raw transmission images and a reference image for the background medium. The background image was an average of images acquired at all scan positions. The TROT

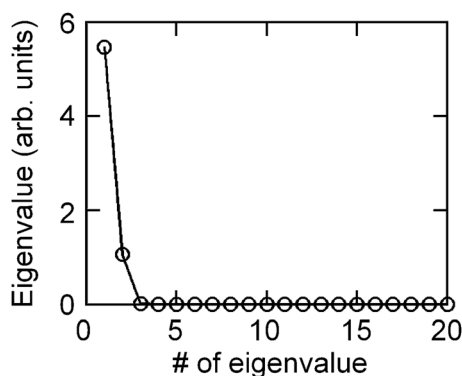
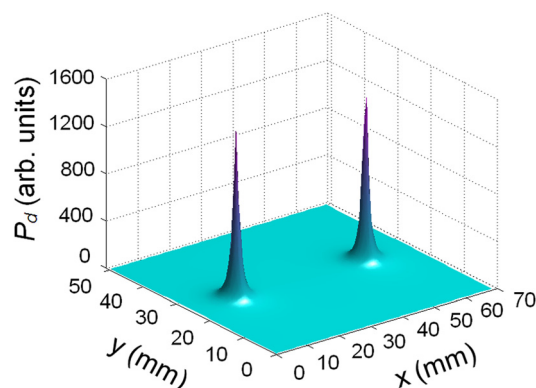


FIG. 2. Eigenvalue spectrum of TR matrix.

FIG. 3. TROT-reconstructed pseudo image at $z = 29.5 \text{ mm}$ using fluorescence data.

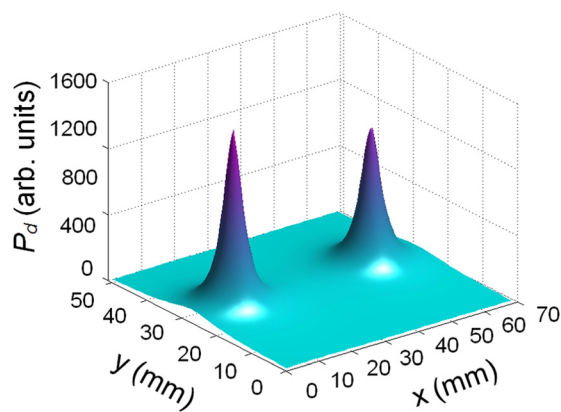


FIG. 4. TROT-reconstructed pseudo image at $z = 29.5$ mm using transmission data.

reconstructed image at the $z = 29.5$ mm plane using the transmission data is shown in Fig. 4, and the retrieved locations of targets are listed and compared with those obtained from the fluorescence data in Table I.

It follows from Table I that the positions of the targets retrieved using fluorescence measurements are in very good agreement (uncertainty < 1 mm) with the known locations, and are quite consistent with those retrieved using transmission measurements. However, the peaks in the reconstructed images using fluorescence data are sharper than those using transmission data. Since only one eigen component was used to reconstruct a target, the targets were treated as point targets, and the pseudo images only carried location information. The FWHM of the peaks in the transmission-TROT images is about 2.8 times of those in the fluorescence-TROT images. The width of the profile of each target reflects on the uncertainty in the determination of location of the corresponding target. We tentatively ascribe this better resolution of fluorescence TROT result than that of transmission TROT result to the difference in the background level between fluorescence and transmission signals. Fluorescence signal, in principle, has zero background, while transmission signal involves measurement of changes on a strong background, and such changes for targets in a turbid medium can be rather small. This set of measurements suggests that higher resolution may be achieved with TROT using fluorescence data than that using transmission data, an observation reported by other researchers as well.^{6,8} However, the overall level of fluorescence signal is an important factor in imaging applications, and more work involving different types of samples and target contrast will be needed to obtain a more definitive conclusion.

Background fluorescence is seldom zero for *in vivo* experiments and is a common concern for fluorescence diffuse optical tomography (DOT).⁶ For uniform distribution of fluorophore in the background medium, simulation shows that the present method would provide target location that is quite accurate in the lateral directions, but is shifted towards the detector plane in the axial direction, and the shift would depend on the target-to-background fluorescence ratio. The situation may improve if an appropriate background subtraction is employed.²² In *in vivo* studies, background fluorescence due to fluorophore uptake by normal tissues may be significantly

reduced by adjusting the time delay between administration of fluorophore and the measurements, since the fluorophore gets removed from the normal tissue faster than from the tumor.⁶ The method uses the diffusion model of light propagation, which takes into account the effect of background absorption and scattering in the intervening medium.

TROT is a fast reconstruction method, since it uses data matrix with lower dimension than that used in other inverse image reconstruction (IIR) approaches, does not involve iterations of the forward model, and does not attempt to find optical properties for every voxel. The same advantages of TROT over other IIR methods hold true for fluorescence imaging. As detailed elsewhere,¹⁴ computational complexity of TROT is less than what it is even for a single iteration of other iterative IIR methods. Even though a limited number of acquisition angles were used for the slab geometry, locations of the fluorescent targets were retrieved within ~ 1 mm of known positions for a sample with similar thickness and average optical properties of a typical compressed human breast, which is a significant result.

From the transmission data, $\kappa = \sqrt{3\mu_a\mu_s'}$ of the background medium was found to be $\sim 0.06 \text{ mm}^{-1}$. The fluorescence data were used to compare the fluorescence strengths of the targets. The fluorescence strengths of the targets in Eq. (1) can be unmixed from the data matrix using the pseudo inverse of the matrices generated by Green's functions associated with the retrieved target locations. Instead of using the whole data matrix, a reconstructed rank-reduced data matrix²³ can be used so that the deleterious effect of noise is significantly reduced when assessing optical properties. The approach is detailed in Ref. 23, and Eq. (4) therein was used in this calculation. The fluorescence strengths were estimated to be 0.17 and 0.20 for the left and right targets, respectively. Since the fluorescence data contain an unknown scale factor associated with collection geometry, filter coupling, and signal attenuation,¹⁸ the retrieved fluorescence strengths are in arbitrary units. In this experiment, the two targets were designed to have identical optical properties, and retrieved relative fluorescence strengths are consistent with the actual condition. Further experiments using targets with different optical properties are needed to verify the efficacy of property retrieval.

Minimum detectable fluorophore concentration and target size are useful considerations for application of the TROT method. While we present data on ICG concentration of $1 \mu\text{M}$ in the target following the common practice,^{6,8} with our current experimental configuration we can detect target ICG concentration down to 50 nM . The method retrieves locations of targets based on their fluorescence strength, which is a product of the fluorescence yield and the volume of the target. However, it is impractical to use a point target and make the fluorescence yield arbitrarily large. Based on experimental conditions, we anticipate being able to locate targets of size 0.04 cm^3 , 4-times smaller than what we used with same ICG concentration. In clinical applications involving detecting and locating tumors, these sizes are more than adequate for early detection of cancer.

In summary, *fluorescence* TROT approach has been developed and used to retrieve the target location and relative fluorescence strength of two small fluorescent targets

embedded in a breast-simulating turbid medium. Locations of targets were retrieved in 3-*D* with an accuracy of ~ 1 mm under the favorable condition of well-separated targets. Achievable spatial resolution is better for assessment of lateral separation between the targets than for axial separation in the forward propagation mode of signal acquisition using slab geometry. Fluorescence signal appeared to provide better resolution than transillumination signal. The results further suggest the potential of TROT for retrieving three-dimensional location information of contrast-enhanced tumors in a human breast at early development stages.

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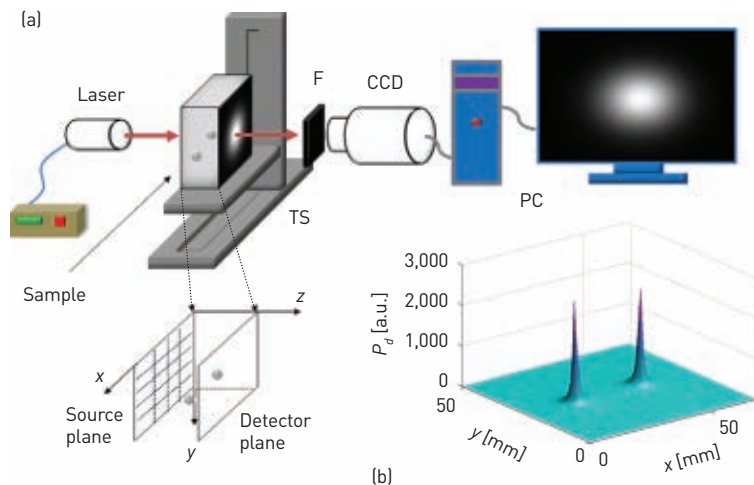
Time Reversal Optical Tomography

There has been a surge of interest in diffuse optical tomography (DOT) that uses near-infrared light to detect, localize and diagnose maladies such as breast cancer and brain injury.¹ Scattering and light attenuation limit the resolution and accuracy of DOT methods that use small differences in optical properties to distinguish lesions from normal tissue. Researchers need a DOT approach that can, for example, quickly reconstruct images to detect and map tumors at early growth stages and determine if they are malignant or benign.²

Time reversal optical tomography (TROT) extends the use of TR imaging and a subspace-based method of multiple signal classification (MUSIC) from acoustic and radar imaging to optical imaging.^{3,4,5} TROT uses a multisource illumination and multi-detector signal acquisition scheme to acquire multiple angular views of the sample.

The perturbation in light intensity distribution due to the targets is extracted from the data and organized in a matrix K . The leading eigenvalues of the TR matrix, $T = K^t K$, correspond to the targets whose locations are determined using MUSIC, along with Green's functions for light propagation in the sample.

We first tested the efficacy of TROT using a 60-mm-thick slab of Intralipid-20 percent suspension in water and 9-mm diameter glass spheres as absorptive or scattering targets. We filled the glass spheres with ink dissolved in the suspension to provide absorptive targets, and with a higher concentration of Intralipid to provide scattering targets. We chose the optical properties and size of the sample and targets to emulate average values for breast tissue and small



(a) F = signal transmitting narrow-band filter; TS = translation stage; CCD = charge coupled device; and PC = computer. Continuous wave 790-nm diode laser light illuminates the front of the sample cell. Diffusely transmitted light from the opposite face is collected by a camera lens through F and sensed by a CCD camera. The sample cell is step-scanned across the laser beam in a 2-D x-y array of grid points using the computer-controlled TS. (b) A TROT-generated pseudo image of two absorptive targets at $z = 30.5$ mm plane when the targets are separated by 27.6 mm.

tumors. We found that TROT could retrieve the location of a single target with millimeter accuracy and resolve two targets when their adjacent surfaces were only 4-mm apart.

Another experiment involved a realistic breast model composed of *ex vivo* breast tissue with two pieces of embedded tumors; TROT accurately located the positions of both the tumors. We have extended TROT for locating fluorescent targets.

TROT is non-iterative and faster than other iterative DOT approaches. It is particularly suited for detecting point-like targets. **OPN**

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Time-reversal optical tomography: detecting and locating extended targets in a turbid medium

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ABSTRACT

Time Reversal Optical Tomography (TROT) is developed to locate extended target(s) in a highly scattering turbid medium, and estimate their optical strength and size. The approach uses Diffusion Approximation of Radiative Transfer Equation for light propagation along with Time Reversal (TR) Multiple Signal Classification (MUSIC) scheme for signal and noise subspaces for assessment of target location. A MUSIC pseudo spectrum is calculated using the eigenvectors of the TR matrix T , whose poles provide target locations. Based on the pseudo spectrum contours, retrieval of target size is modeled as an optimization problem, using a “local contour” method. The eigenvalues of T are related to optical strengths of targets.

The efficacy of TROT to obtain location, size, and optical strength of one absorptive target, one scattering target, and two absorptive targets, all for different noise levels was tested using simulated data. Target locations were always accurately determined. Error in optical strength estimates was small even at 20% noise level. Target size and shape were more sensitive to noise. Results from simulated data demonstrate high potential for application of TROT in practical biomedical imaging applications.

Keywords: Diffuse optical imaging, time reversal, optical tomography, biomedical imaging, breast cancer, Multiple Signal Classification (MUSIC), near-infrared imaging

1. INTRODUCTION

Time Reversal Optical Tomography (TROT) has been developed [1] to detect and locate small targets embedded in highly scattering turbid media. The method was based on Diffusion Approximation of the Radiative Transfer Equation (RTE) to describe light propagation in a highly scattering turbid medium, and a Time Reversal (TR) Multiple-Signal-Classification (MUSIC) algorithm developed by other groups in acoustics and radar applications [2-7].

In this paper, we extend the TROT approach further to detect, locate and retrieve size and optical property information of extended targets embedded in a turbid medium. We test the formalism so developed using simulated data for a single absorptive target, a single scattering target, and two absorptive targets assuming different noise levels. This paper is organized as follows. Section 2 outlines the TROT formalism for extended targets. In section 3, simulated data, TROT analysis and results are presented. Section 5 serves as discussion and summary.

2. FORMALISM

TROT formalism for locating small (point-like) targets has been detailed in our earlier publication [1], and may be used to locate extended targets as well. Here we present a brief overview of the formalism for completeness, and outline how the size and optical strength of an extended target may be estimated. The propagation of a near-infrared (NIR) beam of light through a highly scattering turbid medium with embedded targets, whose optical properties are different from that of the intervening medium, may be approximated to be the diffuse transmission of light through background medium of uniform optical characteristics, with targets as perturbations. In the first order Born approximation, the perturbation in

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the light intensity distribution due to the presence of the targets (inhomogeneities in optical properties) can be expressed using a data matrix [1] of the form:

$$K \approx \left\{ \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{X}_m) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j) \right\} = \sum_{m=1}^M g_d(\mathbf{X}_m) \tau_m g_s^T(\mathbf{X}_m), \quad (1a)$$

for absorptive targets, and

$$K \approx \sum_{m=1}^M \sum_{\alpha \in \{x, y, z\}} \partial_\alpha g_d(\mathbf{X}_m) \tau_m \partial_\alpha g_s^T(\mathbf{X}_m), \quad (1b)$$

for scattering targets, where $g_s(\mathbf{r}) = \{G^s(\mathbf{r}, \mathbf{r}_j), j = 1, \dots, N_s\}$ and $g_d(\mathbf{r}) = \{G^d(\mathbf{r}_i, \mathbf{r}), i = 1, \dots, N_d\}$ are the Green's function vectors (GFVs) associated with the source and detector planes, respectively; the superscript T denotes transpose; $G^s(\mathbf{r}, \mathbf{r}_s)$ and $G^d(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagations in the background medium from a source at $\mathbf{r}_s$ to a target (inhomogeneity) at $\mathbf{r}$ and from the target to a detector at $\mathbf{r}_d$, respectively; $\tau_m = \delta\mu_a(\mathbf{X}_m)c\delta V_m$ ($\tau_m = \delta D(\mathbf{X}_m)c\delta V_m$) is the absorptive (scattering) optical strength of the m^{th} absorptive (scattering) target at $\mathbf{X}_m$ with volume δV_m ; $\delta\mu_a$ (δD) is the difference in the absorption (diffusion) coefficients between the target and the background medium; N_s , N_d and M are the numbers of sources, detectors and targets, respectively. It is assumed the number of targets is less than the number of sources and detectors, $M < \min(N_d, N_s)$; c is the light speed in the medium. A time reversal matrix is constructed as $T_{SDDS} = K^T K$ [$T_{DSSD} = (K^T)^T K^T = K^* K^T$] in frequency domain, and $T_{SDDS} = K^T K$ ($T_{DSSD} = K K^T$) in the continuous wave illumination. T_{DSSD} and T_{SDDS} have a common set of eigenvalues $\{\lambda_j, j = 1, \dots, \min(N_s, N_d)\}$, and different sets of eigenvectors $\{u_i, i = 1, \dots, N_d\}$ and $\{v_j, j = 1, \dots, N_s\}$, respectively. The eigenvectors are separated into signal and noise subspaces using an L -curve method [8] with an eigenvalue threshold ε . For absorptive targets, the locations are determined using the MUSIC pseudo spectrum [1]

$$P_s(\mathbf{X}_p) = \left\| g_s(\mathbf{X}_p) \right\|^2 / \left\| g_s(\mathbf{X}_p) \right\|^2 - \sum_{\lambda_j > \varepsilon} |v_j^T g_s(\mathbf{X}_p)|^2, \quad (2a)$$

associated with the source plane or a similar form for the detector plane $P_d(\mathbf{X}_p)$, or the product of these two,

$$P(\mathbf{X}_p) = P_s(\mathbf{X}_p) P_d(\mathbf{X}_p), \quad (2b)$$

where $\mathbf{X}_p$ is a test target position in the sample volume. Since the eigenvalues and eigenvectors of T_{SDDS} and T_{DSSD} can be connected using singular value decomposition (SVD), *i.e.*

$$K \approx \bar{U} \tilde{\Sigma} \bar{V}^T, \quad (3)$$

where $\bar{V} = \{v_j\}$ and $\bar{U} = \{u_i\}$, corresponding to $\tilde{\Sigma} = \text{diag}\{\sqrt{\lambda_j} > \varepsilon\}$, are matrices for the signal subspaces. By comparing Eq. (3) and Eq. (1), the target optical property can be retrieved by transforming the eigenvalue matrix $\tilde{\Sigma}$ via

$$\Gamma \approx (G^d)^{-1} \bar{U} \tilde{\Sigma} \bar{V}^T ((G^s)^T)^{-1}, \quad (4)$$

where $\Gamma = \text{diag}\{\tau_m, m = 1, \dots, M\}$; $G^s = \{g_s(\mathbf{r}_m)\}$, $G^d = \{g_d(\mathbf{r}_m)\}$ are matrices including GFVs associated with the retrieved target positions. For scattering targets, the GFVs g_d and g_s in Eqs. (2) and (4) are replaced by $\partial_\alpha g_d$ and $\partial_\alpha g_s$, $\alpha = x, y, z$, respectively.

An optimal contour (a surface Ω when plotted in 3D) in the pseudo spectrum in logarithmic scale is selected to be the boundary of the target(s), via [4]

$$\Omega = \arg \min_{\Omega} \|K - K_{cal}(\Omega)\|^2, \quad (5)$$

where K is normalized data matrix obtained from known target surface in simulation (from experimental measurements for real targets) and $K_{cal}(\Omega)$ is normalized data matrix calculated from the contour of the pseudo spectrum in logarithmic scale. The Green's functions used in the calculation are those for the intervening medium.

3. SIMULATIONS AND ANALYSIS

The sample was taken to be a 40-mm thick uniform scattering slab with lateral dimension of $80 \text{ mm} \times 80 \text{ mm}$. Its absorption and diffusion coefficients were assumed to be $\mu_a = 0.003 \text{ mm}^{-1}$ and $D = 1/3 \text{ mm}$ (transport mean free path, $l_t = 1 \text{ mm}$), respectively, which are similar to the average value of those parameters for human breast tissue. The index of refraction n of the medium was taken to be 1.33. The speed of light is $2.998 \times 10^8 \text{ m/s}$, or 299.8 mm/ns in vacuum, and 225.4 mm/ns in the medium. Three simulated datasets were generated with 10-mm diameter spherical targets embedded. In the first dataset, an absorptive target was centered at $(40, 40, 20) \text{ mm}$. In the second dataset, two absorptive targets were located at $(20, 40, 20) \text{ mm}$ and $(60, 40, 20) \text{ mm}$, respectively. In the third dataset, a scattering target was centered at $(40, 40, 20) \text{ mm}$. The absorption coefficient of all the absorptive targets was set to be higher than the background with $\Delta\mu_a = 0.001 \text{ mm}^{-1}$, while the diffusion coefficient was taken to be the same as that of background. The diffusion coefficient of the scattering target was set to be lower than the background (higher scattering coefficient) with $\Delta D = -0.1 \text{ mm}$ ($l_t = 0.7 \text{ mm}$), while the absorption coefficient was taken to be the same as that of the background. The volume of all targets was 515 mm^3 when the sample volume is discretized into $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$ voxels in the forward model. The optical strength of the absorptive targets was $\Delta\mu_a c \Delta V = 116.08 \text{ mm}^3/\text{ns}$; while the optical strengths of the scattering target was $\Delta D c \Delta V = -11608.1 \text{ mm}^5/\text{ns}$. The incident CW beam step scanned the sample at 41×41 grid points covering an $80 \times 80 \text{ mm}^2$ area, with a step size of 2 mm . Light on the opposite side was recorded at 41×41 grid points covering the same area. Additive Gaussian noise of different noise levels was added to the simulated data. The data matrix K was then obtained using Eq. (1) directly, and analyzed using TROT. The results are shown below. For simplicity, when the reconstruction result for one target is displayed, a smaller volume of $40 \text{ mm} \times 40 \text{ mm} \times 40 \text{ mm}$ around the center is selected. Due to the distortion in the retrieved target shape, target volume will be used to describe the target size.

3.1 One absorptive or scattering target

The first 20 eigenvalues of the TR matrix for one absorptive target with 20% added noise are plotted in logarithmic scale and shown in Fig. 1(a). The dimension of the signal subspace is determined to be 3. Using Eq. (2), the pseudo spectrum was calculated. Both of axial and sagittal views of the target using the pseudo spectrum are plotted in logarithmic scale in Figs. 1(b) and 1(c).

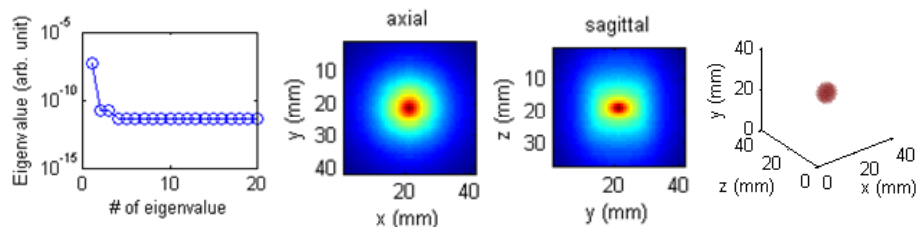


Fig. 1. (a) Eigenvalues plotted in logarithmic scale; (b) and (c) are the axial and sagittal views of the target using the pseudo spectrum in logarithmic scale; (d) is the retrieved target image

The center of the target was accurately determined to be $(40, 40, 20) \text{ mm}$. Using Eq. (4), the optical strength was found to be $112.4 \text{ mm}^3/\text{ns}$ when all 3 eigenvalues and eigenvectors in the signal subspace were used, as shown in Table 1. We also found that if only the first eigenvalue and eigenvector in the signal subspace were used, the optical strength was found to be $112.4 \text{ mm}^3/\text{ns}$ as well. From this observation, we conclude that the property information of the extended target is mainly contained in the first eigenvalue, *i.e.* the number of the eigenvalues/eigenvectors used is the same as the number of targets.

Estimation of the target volume begins with choosing the optimal contour. Starting from the maximum of the pseudo spectrum, successive contour levels are selected. When a contour which is a surface Ω plotted in $3D$, is selected, the volume enclosed inside Ω is assumed to be the target volume, and then the data matrix is calculated and compared with the working (simulated or experimental) data. When the next bigger volume is selected, only the signal generated by the extra volume dV is calculated. By using this “local contour” method [6], the optimal contour is found using Eq. (5). The reconstructed $3D$ image of the absorptive target is displayed in Fig. 1(d). The retrieved target volume is shown in Table 1 and compared with the known volume.

Similar simulated data was generated and the subsequent analysis was conducted when no noise, 5% noise, and 10% noise was added separately. Similar images were obtained (not shown). The target location and optical strength of the

target was accurately retrieved in all cases. The size in all cases was also retrieved. The retrieved target optical strength and size are all shown in Table 1, and compared to the known values.

Similar simulations with one scattering target were carried out. The eigenvalues and eigenvectors of the TR matrix, and the pseudo spectrum were calculated. The target location of the scattering targets was accurately found to be (40, 40, 20) mm at all noise levels (0%, 5%, 10% and 20%). Axial and sagittal images of the scattering target were obtained using the pseudo spectrum as was done for the absorptive target. The scattering optical strength of the target was retrieved within 5.4% error and the size of targets was found within 30% error for all noise levels.

Further simulations show the optical strength can be retrieved even if the noise level is further increased or the target size and location vary; however, the error in the retrieved target size may increase dramatically if the target size further increases. The accuracy of the retrieved target size and optical property seems not to well correlate with the noise level.

Table 1. Optical strength and size of an absorptive target and a scattering target (10-mm diameter).

Noise Level (%)	Absorptive Target				Scattering Target			
	Optical Strength		Size		Optical Strength		Size	
	Retrieved (mm ³ /ns)	Error (%)	Retrieved (mm ³)	Error (%)	Retrieved (mm ⁵ /ns)	Error (%)	Retrieved (mm ³)	Error (%)
0	112.4	3.2	478	7.0	-10985.4	5.4	413	19.8
5	112.4	3.2	484	6.0	-10985.8	5.4	535	3.9
10	112.4	3.2	370	28.2	-10985.2	5.4	665	29.1
20	112.4	3.2	385	25.2	-10987.2	5.3	576	11.8

* Known values: volume: 515 mm³, absorptive optical strength: 116.08 mm³/ns, scattering optical strength: -11608.1 mm⁵/ns

3.2 Two absorptive targets

Next we considered the case of two spherical absorptive targets (diameter 10 mm) embedded in the medium, with a center-to-center separation of 40 mm. Different added noise levels: 0%, 5%, 10%, 20%, and 100% were considered. The eigenvalues and eigenvectors of the TR matrix, and the pseudo spectra were calculated. The target locations of two targets were accurately found to be (20, 40, 20) mm and (60, 40, 20) mm at all noise levels. Typical axial and sagittal images of the targets generated using the pseudo spectra for a noise level of 20% are displayed in Fig. 2. Similar images were obtained for other noise levels. The optical strength and size of both targets were found. The optical strength of each target was calculated within 3.3% error at all noise levels. The size of targets was found within 13.4% error when no noise was present, and varied with noise added. The retrieved optical strength and size of the targets are listed in Table 2 for different noise levels. Since both targets have the same optical property and size in the simulated data and the retrieved data, only one set of values is shown in the table.

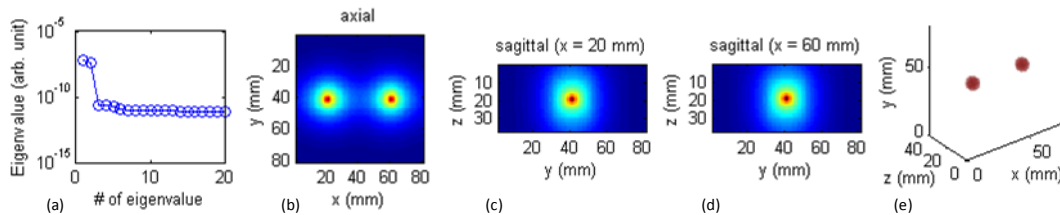


Fig. 2. (a) Eigenvalues plotted in logarithmic scale; (b), (c) and (d) are the axial and sagittal views of the targets using pseudo spectrum in logarithmic scale; (e) is the retrieved target image. The added noise level was 20%.

Further simulations (not shown here) indicate that retrieved target size was more accurate when small targets were involved. Simulations with one target at different locations and two targets with different separations were also tested for absorptive and scattering targets. The target locations were accurately retrieved in all of these cases. The retrieved optical strength in all cases was much less sensitive to the target location and size than the retrieved target size. As expected, it was more challenging to retrieve the optical strength and size of scattering targets than absorptive targets.

Further simulations also showed closer distance between two targets made target size retrieval more difficult, because of the cross talk between the two targets showing up in the contour of the pseudo spectrum (logarithmic scale).

Table 2. Optical strength and size of two absorptive targets (10-mm diameter).

Noise Level (%)	Optical Strength		Size	
	Retrieved (mm ³ /ns)	Error (%)	Retrieved (mm ³)	Error (%)
0	112.3	3.3	584	13.4
5	112.3	3.3	295	42.7
10	112.3	3.3	368	28.5
20	112.3	3.3	487	5.4
100	112.2	3.3	663	28.7

* Known values: volume: 515 mm³, optical strength: 116.08 mm³/ns

4. SUMMARY AND DISCUSSION

Time reversal optical tomography (TROT) was further developed to deal with extended targets. The center position of the target(s) is determined rather accurately for both absorptive and scattering targets. It is found that the optical strength (absorption or scattering) can be retrieved for different target size, target location and noise level. However, it is much more challenging to retrieve the target size. The retrieved target size is determined by the details of the pseudo spectrum. There seems to be a lack of well defined correlation between the noise level and the size and optical strength of the targets, which needs to be understood. The efficacy of the approach will be further tested using experimental data.

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Research Article

Diffuse Optical Imaging Using Decomposition Methods

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Diffuse optical imaging (DOI) for detecting and locating targets in a highly scattering turbid medium is treated as a blind source separation (BSS) problem. Three matrix decomposition methods, independent component analysis (ICA), principal component analysis (PCA), and nonnegative matrix factorization (NMF) were used to study the DOI problem. The efficacy of resulting approaches was evaluated and compared using simulated and experimental data. Samples used in the experiments included Intralipid-10% or Intralipid-20% suspension in water as the medium with absorptive or scattering targets embedded.

1. Introduction

Diffuse optical imaging (DOI) for detection and retrieval of location information of targets in a highly scattering turbid medium may be treated as a blind source separation (BSS) problem [1, 2]. Various matrix decomposition methods, such as, independent component analysis (ICA) [3], principal component analysis (PCA) [4], and nonnegative matrix factorization (NMF) [5, 6] have been developed for solving the BSS problem and retrieving desired information.

Xu et al. adapted ICA of information theory to develop optical tomography using independent component analysis (OPTICA) and demonstrated its application for diffuse imaging of absorptive, scattering, and fluorescent targets [7–11]. ICA assumes the signals from different targets to be *independent* of each other and optimizes a relevant measure of independence to obtain the ICs associated with different targets. The position coordinates of targets in three dimensions are determined from the individual components separately.

PCA assumes that the PCs contributing to the signal are *uncorrelated* and explain the most variance in the signal. PCA has been widely used in various applications, such as spectroscopy [12], face recognition [13], and neuroimaging [14]. NMF seeks to factorize a matrix into two nonnegative matrices (component signals and weights) and requires the

contributions to signal and the weights of the components to be *non-negative*. It does not imply any relationship between the components. NMF has also been widely used in biological analysis [15] and spectral analysis [16].

The objective of this study is to test and compare the efficacy of these algorithms when used to solve the DOI problem. Results are presented and compared using simulated data and experimental data using absorptive and scattering targets embedded in model scattering media. Our interest in solving the DOI problem derives from the need for a noninvasive modality for detecting, locating, and diagnosing breast tumors in early stages of growth.

The remainder of the paper is organized as follows. In Section 2, the formalisms of the three methods are introduced. Section 3 evaluates the resulting imaging approaches using simulated data. The approaches are further examined in Section 4 for experimental data acquired using absorptive and scattering targets embedded in model scattering media. Section 5 summarizes and discusses the results.

2. Formalism

2.1. Blind Source Separation Problem. Blind source separation (BSS), also known as blind signal separation, is a general problem in information theory that seeks to separate different individual signals from the measured signals, which

are weighted mixtures of those individual signals. Assuming M individual signals, $s_j(t)$, $j = 1, \dots, M$, are linearly mixed instantaneously, the BSS problem is modeled as follows. The dimension of $s_j(t)$ is N_s , the number of sampling times. In this study, t will be replaced by spatial positions of the excitation light sources. A total of N_d detectors sense N_d different mixtures of $s_j(t)$. The mixture measured by the i th detector can be presented as $x_i(t) = \sum_{j=1}^M a_{ij}s_j(t)$, or $X = AS$, in a matrix notation, where $A \in R^{N_d \times M}$ is a mixing or weighting matrix, $S \in R^{M \times N_s}$, $X \in R^{N_d \times N_s}$, and $M < \min(N_s, N_d)$. The objective of BSS is to retrieve the signals $s_j(t)$ and their weights, a_{ij} . ICA, PCA, and NMF are statistical analysis methods used to solve the BSS problem.

2.2. Diffuse Optical Imaging Problem. In DOI, one measures the signal at the sample boundary, which includes a weighted mixture of contributions from embedded targets. One uses the diffusion approximation [17–19] of the radiative transfer equation [20, 21] as the forward model to describe light propagation in a highly scattering turbid medium. The perturbation in the light intensity distribution measured on the boundary of the sample due to the presence of the targets (which are localized inhomogeneities in the optical properties within the sample volume) may be written, in the first-order Born approximation, as [22, 23]

$$\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = - \int G(\mathbf{r}_d, \mathbf{r}) \delta\mu_a(\mathbf{r}) c G(\mathbf{r}, \mathbf{r}_s) d^3\mathbf{r} - \int \delta D(\mathbf{r}) c \nabla_{\mathbf{r}} G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_{\mathbf{r}} G(\mathbf{r}, \mathbf{r}_s) d^3\mathbf{r}, \quad (1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagation from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ and δD are the differences in absorption coefficient and diffusion coefficient between the targets and the background medium, respectively; and c is the light speed in the medium.

A multisource illumination and multidetector signal acquisition scheme is used to acquire light transmitted through a scattering medium. For small absorptive targets, a perturbation data matrix is constructed using $-\Delta\phi$ for all sources. The elements of the data matrix pertaining to absorptive targets represented by the first term in (1) may be written in a discrete form as

$$X_{ij} = \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{r}_m) \tau_m G^s(\mathbf{r}_m, \mathbf{r}_j) \quad (2)$$

($i = 1, 2, \dots, N_d; j = 1, 2, \dots, N_s$),

where $\mathbf{r}_i$, $\mathbf{r}_j$, and $\mathbf{r}_m$ are the locations of the i th detector, j th source and m th target, respectively; N_s , N_d , and M are the numbers of sources, detectors, and targets, respectively; $\tau_m = \delta\mu_a(\mathbf{r}_m) c \delta V_m$ is the optical absorption strength of the m th target of volume δV_m ; $G^s(\mathbf{r}_m, \mathbf{r}_j)$ and $G^d(\mathbf{r}_i, \mathbf{r}_m)$ are the Green's functions that describe light propagation from j th source to m th target and from m th target to i th detector,

respectively. The number of targets is assumed to be less than that of sources and detectors, $M < \min(N_d, N_s)$.

The m th target may be considered to be a virtual source of strength $\tau_m G^s(\mathbf{r}_m, \mathbf{r}_j)$ excited by the real light source located at $\mathbf{r}_j$. The data matrix $X = \{X_{ij}\}$ may be considered to be a set of combinations of light signals from all virtual sources mixed by a mixing matrix $\{G^d(\mathbf{r}_i, \mathbf{r}_m)\}$. Therefore, this problem can be treated as a BSS problem.

As the second term in (1) suggests, each scattering target is represented by three colocated virtual sources of strength: $\tau_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j)$, where $\partial_p = \partial/\partial p$, ($p = x, y, z$), and $\tau_m = \delta D(\mathbf{r}_m) c \delta V_m$, is the optical scattering strength of the m th target [8]. The mixing matrices become $\{\partial_p G^d(\mathbf{r}_i, \mathbf{r}_m)\}$ for the three virtual sources generated by the m th target. The elements of the data matrix for scattering targets may be written as

$$X_{ij} = \sum_{m=1}^M \sum_{p=\{x,y,z\}} \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m) \tau_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j). \quad (3)$$

Since one absorptive target is represented by one centrosymmetric virtual source, while three virtual sources (one centrosymmetric and two dumb-bell shaped) represent one scattering target [7, 8], the number and patterns of virtual sources may be used, in favorable situations, to identify the target as absorptive or scattering in nature. In this paper, only small targets are considered since all three algorithms are suited for small targets, and early detection, when the tumors are more amenable to treatment, is of practical interest.

2.3. DOI as a BSS Problem. The data matrix for the DOI problem may be written as

$$X = AS = \sum_{m=1}^M A_{im} S_{mj}, \quad (4)$$

where $A \in R^{N_d \times M}$, $S \in R^{M \times N_s}$, and $X \in R^{N_d \times N_s}$. For absorptive targets,

$$A_{im} = \beta_m G^d(\mathbf{r}_i, \mathbf{r}_m), \quad S_{mj} = \alpha_m G^s(\mathbf{r}_m, \mathbf{r}_j), \quad (5a)$$

while for scattering targets,

$$A_{im} = \beta_m \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m), \quad S_{mj} = \alpha_m \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j). \quad (5b)$$

$\{S_{mj}\}$ ($j = 1, 2, \dots, N_s$) and $\{A_{im}\}$ ($i = 1, 2, \dots, N_d$) are two-dimensional intensity distributions on the source and detector planes, respectively. Source and detector planes are the boundaries of the sample through which light enters and exits the sample volume, respectively. The scaling factors β_m and α_m are related to the target optical strength, $\tau_m = \alpha_m \beta_m$.

The location of the target and the scaling factors can be retrieved using a least squares fitting via

$$\underset{\alpha_m, \beta_m, \mathbf{r}_m}{\operatorname{argmin}} \left\{ \sum_j \left[\alpha_m^{-1} S_{mj} - G^s(\mathbf{r}_m, \mathbf{r}_j) \right]^2 + \sum_i \left[\beta_m^{-1} A_{im} - G^d(\mathbf{r}_i, \mathbf{r}_m) \right]^2 \right\}, \quad \text{or} \quad (6a)$$

$$\underset{\alpha_m, \beta_m, \mathbf{r}_m}{\operatorname{argmin}} \left\{ \sum_p \left\{ \sum_j \left[\alpha_m^{-1} S_{mj} - \partial_p G^s(\mathbf{r}_m, \mathbf{r}_j) \right]^2 + \sum_i \left[\beta_m^{-1} A_{im} - \partial_p G^d(\mathbf{r}_i, \mathbf{r}_m) \right]^2 \right\} \right\}, \quad (6b)$$

for absorptive and scattering targets, respectively. However, when a scattering target is embedded deep in a turbid medium, only the $\tau_m \partial_z G^s(\mathbf{r}_m, \mathbf{r}_j)$ virtual source remains significant. So, only $p = z$ may be used for fitting in (6b) [8].

2.3.1. ICA. OPTICA assumes that the virtual sources are *independent* of each other [8]. So, they can be retrieved through an iterative process which seeks to maximize the independence among the components. In practice, the independent components are found by maximizing some measure of non-Gaussianity, such as kurtosis (the fourth-order cumulant), of the unmixed components. A Matlab program for ICA was adopted from <http://scn.ucsd.edu/eeglab/>. The location of the target can be retrieved by fitting the independent component intensity distributions (ICIDs) to Green's functions or derivatives of Green's functions using (6a) and (6b).

2.3.2. PCA. PCA assumes that the virtual sources are uncorrelated so that the correlation (covariance) between them is ideally zero and minimal in practice. The covariance matrix of S , $\operatorname{cov}(S)$ should be diagonal. The general process of PCA is as follows. The data matrix $X = AS + \mathcal{N}$, where $\mathcal{N}$ is random noise added to the data, A and S the same as defined in (4). When S is mean centered, elements of the mean-centered matrix S' are defined as

$$S'_{mj} = S_{mj} - \frac{1}{N_s} \sum_{j=1}^{N_s} S_{mj}. \quad (7a)$$

Similarly,

$$X'_{ij} = X_{ij} - \frac{1}{N_s} \sum_{j=1}^{N_s} X_{ij}. \quad (7b)$$

PCA looks for a matrix P that decomposes X into virtual sources, $S = PX$. It also holds that $S' = PX'$, since P is just a rotation matrix which does not change the center of the data.

$$\operatorname{cov}(S) = S' S'^T = (PX')(PX')^T = PX' X'^T P^T = \Lambda, \quad (8)$$

where $\Lambda = \operatorname{diag}\{\lambda_1, \lambda_2, \dots\}$. The eigenvalues λ_m are variances in the covariance matrix. Therefore, $X' X'^T P^T = P^T \Lambda$, where P^T is orthonormal. PCA is realized by eigenvalue decomposition (EVD) of the covariance matrix of X . The eigenvectors with leading eigenvalues (largest variances) are selected to be the PCs using the L -curve [24].

Since $X = P^T S \approx AS$, A is determined as a matrix including only PCs. S is calculated as $S \approx (A^T A)^{-1} A^T X$. Rows of S and columns of A represent principal component intensity distributions (PCIDs) on the source plane and detector plane, respectively and are proportional to the images of the virtual sources projected on the source and detector planes. The target positions are determined using (6a) and (6b).

2.3.3. NMF. NMF is a group of multivariate analysis algorithms that factorize a matrix X into A , and $S : X = AS$, A and S are nonnegative [6]. Unlike ICA and PCA, NMF does not imply any relationship between the retrieved components; instead, it just enforces non-negativity constraints on A and S . There are various algorithms developed to solve NMF, such as the multiplicative update method [5] and alternating least squares method [25, 26].

In the multiplicative update implementation of NMF, A and S can be found by minimizing the square of Euclidean distance $\|X - AS\|^2$ as the cost function, where $A \geq 0$ and $S \geq 0$, using the multiplicative update rule:

$$A_{ik} \leftarrow A_{ik} \frac{(XS^T)_{ik}}{(ASS^T)_{ik}}, \quad (9a)$$

$$S_{kj} \leftarrow S_{kj} \frac{(A^T X)_{kj}}{(A^T AS)_{kj}}. \quad (9b)$$

The alternating least squares implementation of NMF uses alternate least squares steps to estimate A (or S), and use that estimate to optimize S (or A), repeating the alternative steps until the desired optimization is obtained. Nonnegativity is ensured by setting any negative element of A or S equal to 0.

An NMF toolbox was obtained from <http://cogsys.imm.dtu.dk/toolbox/> to perform NMF computation. A built-in command *nnmf* is also available in Matlab (R2011a).

NMF algorithm requires that the non-negativity assumption must hold in the problem. In particular, for absorptive targets, when X is constructed with $-\Delta\phi$, τ_m should be positive, that is, the targets should be more absorbing than the background. If the targets have weaker attenuation properties than the background, X needs to be constructed with $+\Delta\phi$ instead. For scattering targets, X should be treated similarly to keep its elements positive.

When NMF is applied to a scattering target, only the centrosymmetric component shows up properly, since the other two components have dumb-bell shape which includes negative values [8]. So without any prior knowledge or some other experimental means to assess if the target is absorptive or scattering, NMF may not distinguish between the two possibilities.

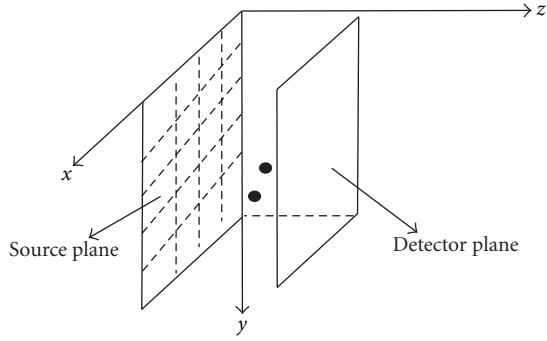


FIGURE 1: Light intensity distribution on the detector plane is recorded when a point source scans on the source plane.

The decomposition methods can be applied with different sample geometries such as slab and cylindrical geometries, and different measurement domains such as time-resolved domain, frequency domain, and continuous wave (CW). In this paper, Green's functions for slab geometry [23] with CW measurement were used for simulation and experiments.

3. Simulation

The sample was considered to be a 40 mm thick uniform scattering slab with lateral dimension of 80 mm $\times$ 80 mm, as shown in Figure 1. Its absorption and diffusion coefficients were taken to be $\mu_a = 0.003 \text{ mm}^{-1}$ and $D = 1/3 \text{ mm}$ (transport mean free path, $l_t = 1 \text{ mm}$), respectively, which are similar to the average value of those parameters for human breast tissue. An absorptive and a scattering point targets were placed at (50, 60, 15) mm and (30, 30, 25) mm, respectively. The index of refraction n of the medium was taken to be 1.33. The speed of light is $2.998 \times 10^8 \text{ m/s}$ or 299.8 mm/ns in vacuum, and 225.4 mm/ns in the medium. The absorption coefficient of the absorptive target was set to be higher than the background with $\Delta\mu_a = 0.001 \text{ mm}^{-1}$, while the diffusion coefficient was taken to be the same as that of background. The diffusion coefficient of the scattering target was set to be lower than the background (higher scattering coefficient) with $\Delta D = -0.1 \text{ mm}$ ($l_t = 0.7 \text{ mm}$), while the absorption coefficient was taken to be the same as that of the background. The volumes of both targets are set to be 8 mm^3 . The optical strengths of the absorptive and scattering targets were $\Delta\mu_a c \Delta V = 1.803 \text{ mm}^3/\text{ns}$ and $\Delta D c \Delta V = -180.3 \text{ mm}^5/\text{ns}$, respectively. The incident CW beam step scanned the sample at 21×21 grid points covering an $80 \times 80 \text{ mm}^2$ area, with a step size of 4 mm. Light on the opposite side was recorded at 41×41 grid points covering the same area. Multiplicative Gaussian noise of 5% was added to the simulated data. The data matrix X was then obtained using (2) and (3) directly and analyzed using the three different algorithms.

3.1. ICA Analysis. One independent component for the absorptive target and three independent components for the scattering target were retrieved by ICA. The independent component intensity distributions (ICIDs) on the detector

plane are shown in Figures 2(a), 2(c), 2(d), and 2(e). Similar ICIDs were obtained on the source plane. Figure 2(g) shows the centrosymmetric ICID for the scattering target, and Figure 2(i) shows the ICID for the absorptive target, on the source plane.

The components in either the detector plane or the source plane can, in principle, be used to extract position and optical strength of the target(s). However, in our experimental arrangement signal is collected by a 1024×1024 pixels CCD camera, while the source plane is scanned in an x - y array of points, which is much smaller than the number of pixels in the CCD camera. Consequently, the resolution in the detector plane is much better, and the data set more robust than the source side. So, we used the images on the detector plane for retrieving target information using experimental data. While it would not matter in simulation, to be consistent with experimental situations, we employed detector plane images when using simulated data as well for all three algorithms. Table 1 lists the locations and strengths of the absorptive and scattering targets retrieved by fitting the spatial intensity profile of the centrosymmetric components on the detector plane to Green's functions and derivatives of Green's functions using (6a) and (6b), respectively, as shown in Figures 2(b) and 2(f). Figures 2(h) and 2(j) show the corresponding fits to the profiles on the source plane.

3.2. PCA Analysis. Eigenvalue equation of the covariance matrix of X was solved. The eigenvalues found by PCA were sorted in a descending order. Figure 3 shows a plot of leading 20 eigenvalues on a logarithmic scale.

First four leading eigenvalues were selected for PCs. The corresponding PCIDs were calculated. The PCIDs on the detector plane are shown in Figure 4. Similar images for PCIDs on the source plane were obtained. The scattering target has one centrosymmetric (Figure 4(a)) component and two dumb-bell shaped (Figures 4(c) and 4(d)) components, while the absorptive target has only one component (Figure 4(e)).

Figures 4(b) and 4(f) show fits to the spatial intensity profile of the centrosymmetric component of the scattering target and that of the absorptive target, respectively, to retrieve the locations of the two targets. The locations and optical strengths of the targets retrieved by PCA are also shown in Table 1.

3.3. NMF Analysis. The mixing matrix and virtual sources were retrieved from the data matrix X using NMF as explained in Section 2.3.3. As in the other two approaches, only one component is extracted for the absorptive target. Since NMF has a non-negativity constraint, only the centrosymmetric component for the scattering target is obtained. Nonnegative component intensity distributions (NCIDs) on detector planes are shown in Figure 5. Similar images for NCIDs on source plane were also obtained using the virtual sources in S . The results are also shown in Table 1.

3.4. Results and Discussion. The positions and optical strengths of the targets retrieved by ICA, PCA, and NMF

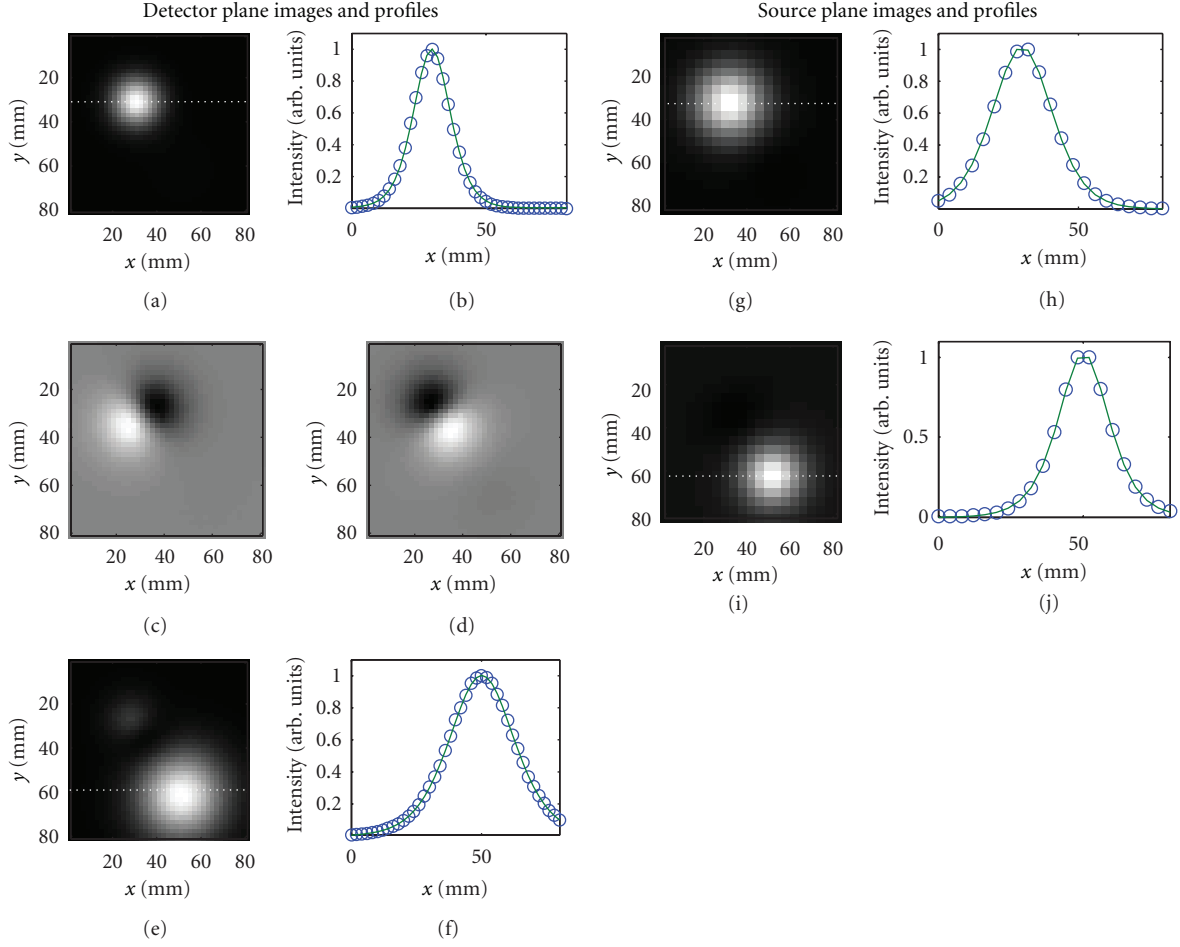


FIGURE 2: ICA-extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component; (c) and (d) dumb-bell shaped components of the scattering target; (e) the absorptive target. Similar intensity distribution on the source plane of: (g) the centrosymmetric component of the scattering target and (i) the absorptive target for comparison. Fits to the spatial intensity profile on the detector plane along the white dashed line (shown in figures) of the centrosymmetric component of the scattering target is shown in (b), and that of the absorptive target is shown in (f). Corresponding fits to spatial profiles on the source plane are displayed in (h) and (j), respectively.

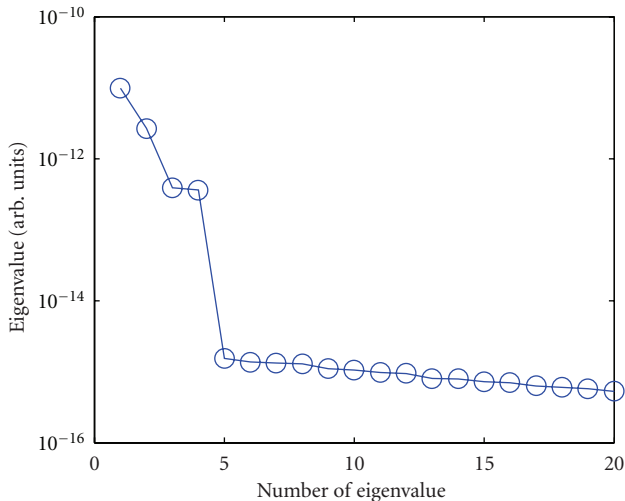


FIGURE 3: A logarithmic plot of the first 20 PCA eigenvalues.

algorithms are shown in Table 1, and compared to the known values. The retrieved results using all three algorithms from this simulated data are in excellent agreement with the known values.

4. Experiments

4.1. Experimental Materials and Methods. In this Section, the algorithms are evaluated using experimental data for absorptive and scattering targets embedded in model scattering media whose absorption and scattering properties are adjusted to mimic the average values of those parameters for human breast tissues. Two different experiments were carried out with two different samples. The first sample used a 250 mm × 250 mm × 50 mm transparent plastic container filled with Intralipid 10% suspension in water as the background medium. The concentration of Intralipid

TABLE 1: Positions and optical strengths retrieved using simulated data and ICA, PCA, and NMF algorithms.

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)	Known strength*	Fitted strength*	Error (%)
Sca.	(30, 30, 25)	ICA	(29.9, 30.0, 25.1)	(0.1, 0, 0.1)	-180.3	-179.9	0.22
		PCA	(30.0, 30.0, 25.0)	(0, 0, 0)	-180.3	-180.1	0.11
		NMF	(30.0, 30.0, 25.0)	(0, 0, 0)	-180.3	-178.5	1
Abs.	(50, 60, 15)	ICA	(50.1, 60.2, 15.0)	(0.1, 0.2, 0)	1.803	1.826	1.28
		PCA	(50.1, 60.1, 14.9)	(0.1, 0.1, 0.1)	1.803	1.812	0.5
		NMF	(50.1, 60.1, 15.0)	(0.1, 0.1, 0)	1.803	1.803	0

*The unit for absorption strength of the target is mm^3/ns and for scattering strength is mm^5/ns .

10% was adjusted to provide [27, 28] an absorption coefficient of $\mu_a \sim 0.003 \text{ mm}^{-1}$, and a transport mean-free path $l_t \sim 1.43 \text{ mm}$ at 785 nm. The second sample used a similar container with dimension of $250 \text{ mm} \times 250 \text{ mm} \times 60 \text{ mm}$ filled with Intralipid 20% suspension in water. The concentration of Intralipid 20% was adjusted to provide [27, 28] $\mu_a \sim 0.003 \text{ mm}^{-1}$, and $l_t \sim 1 \text{ mm}$ at 785 nm. These optical parameters of the medium were selected to be similar to the average values of those parameters for human breast tissue. The thickness of the samples was also comparable to that of a typical compressed female human breast.

In the first experiment, two absorptive targets were embedded in the medium. The targets were $\sim 10\text{-mm}$ diameter glass spheres filled Indocyanine green (ICG) dye dissolved in Intralipid-20% suspension in water to obtain an absorption coefficient $\mu_a = 1.15 \text{ mm}^{-1}$ at 785 nm, and to match the background scattering coefficient of 1.97 mm^{-1} . The targets were placed at (57.2, 18.1, 20.0) mm and (19.9, 48.1, 25.0) mm, respectively.

In the second experiment, two scattering targets were embedded, which were also $\sim 10 \text{ mm}$ diameter glass spheres, filled with Intralipid-20% suspension in water. The transport mean free path, l_t was adjusted to be 0.25 mm, with scattering coefficient $\mu_s \approx 11 \text{ mm}^{-1}$, and absorption coefficient μ_a same as that of the background medium. The targets were placed in the middle plane ($z = 30 \text{ mm}$) in the container with a lateral distance of 40 mm from each other (center to center).

The experimental setup is schematically shown in Figure 6. A 10 mW 785 nm diode laser beam was used to illuminate the first sample, while a 100 mW 785 nm diode laser beam was used for the second sample. The input surface (source plane) of the samples was scanned across the laser beam in an x - y array of grid points to realize the multi-source interrogation of the samples. The transmitted light from the exit surface (detector plane) was recorded by a 1024 pixel $\times$ 1024 pixel (pixel size = $24 \mu\text{m}$) CCD camera (Photometrics CH350) equipped with a 60 mm focal-length camera lens. Each pixel of the CCD camera can be considered to be a detector implementing the multidetector signal acquisition arrangement. A set of 16 bit 1024 pixel $\times$ 1024 pixel images were acquired. The two samples were scanned in an array of 11×12 and 11×15 grid points, respectively, with a step size of 5 mm in both cases. The processes of scanning and data acquisition were controlled by a personal computer. At all scan positions, raw transillumination images of the samples were recorded by the computer for further analysis.

4.2. Analysis and Results. A region of interest (ROI) was cropped out from each image. Then, every 5×5 pixels in each cropped image were binned to one pixel to enhance signal-to-noise ratio. A background image was generated by calculating an average image for all scan positions to approximate the transillumination image without target(s) embedded.

This averaging method for generating background image is suitable for small targets used in our experiments, as the ratio of the volume of the sample to that of the target was quite high ($\sim 500:1$). For *in vivo* imaging of tumors in early stages of growth, the breast-to-tumor volume ratio will be similarly high, and the averaging method will be applicable. Alternative approaches for generating a background image include using image of (a) a phantom that has the same average optical properties as the sample [29]; (b) the healthy contralateral breast for breast imaging [30]; (c) the sample obtained using light of wavelength for which the target(s) and the background have identical optical properties [31]. Still another approach is to compute the background using an appropriate forward model [32]. A more detailed discussion of this important issue appears in one of our earlier publications [33].

The background image was also cropped and binned corresponding to the ROI for each scan position. Perturbation in the light intensity distribution, $\Delta\phi$ due to targets in each image was found by subtracting the background image from the image. The data matrix X was then constructed using the light intensity perturbations at all scan positions. ICA, PCA, and NMF decomposition algorithms were performed on the data matrix separately. Results are shown and discussed below.

4.2.1. Absorptive Targets. The images on the detector plane obtained using the ICA, PCA, and NMF algorithms are shown in Figures 7, 8, and 9, respectively. Similar images on the source plane were also obtained using all three algorithms. The right side of each figure shows the corresponding spatial intensity profile. Locations of the targets are extracted from fits to these spatial intensity profiles, as described in Section 2.3 using (6a) and (6b). The results are presented in Table 2. In Figure 7, images on the source plane are shown in (e) and (g), and Green's function fits to their spatial profiles are shown in (f) and (h) for comparison.

It follows from the comparison of the results in Table 2 that the positions retrieved by all three algorithms are in good agreement with the known positions. The errors

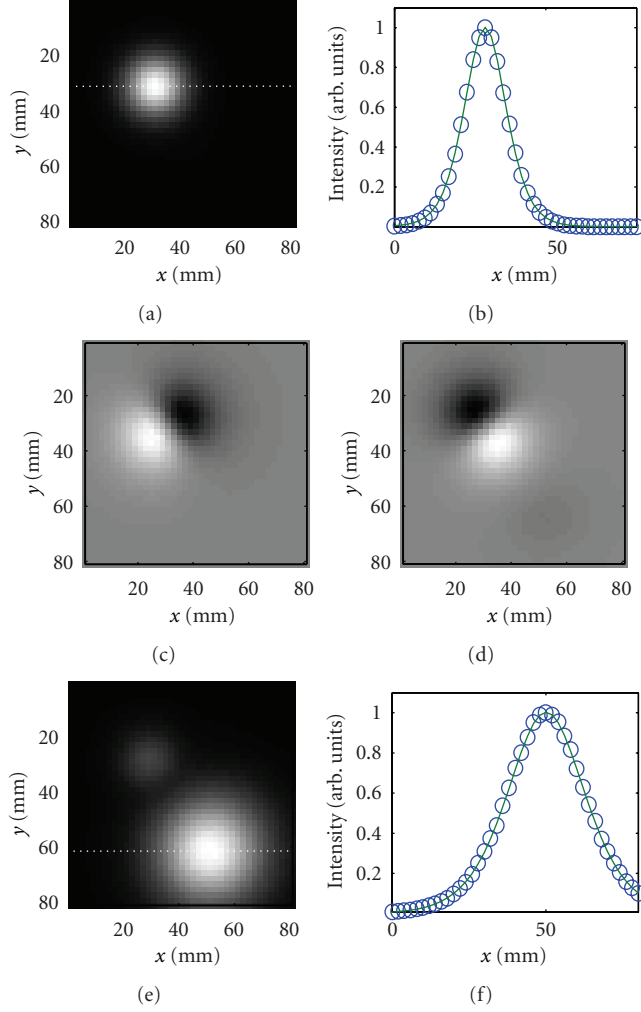


FIGURE 4: PCA-extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component; and (c) and (d) dumb-bell shaped components of the scattering target; (e) the absorptive target. Green's function fits to the spatial intensity profiles along the dashed line (shown in figures) of the (b) centrosymmetric component of the scattering target and (f) absorptive target, respectively, to retrieve the locations of the two targets.

in the retrieved locations (x, y, z) of the two targets were within 1.7 mm. The PCIDs were not totally separated. Some “residue” was observed in one PCID from the other. ICA and NMF separated two components from this dataset more clearly.

4.2.2. Scattering Targets. The “images” corresponding to the centrosymmetric components of the virtual sources (targets) on the detector plane obtained using the ICA, PCA, and NMF algorithms are shown in Figures 10, 11, and 12, respectively. Similar images on the source plane were also obtained. The right side of each figure shows the corresponding spatial intensity profile. Locations of the targets are extracted from fits to these spatial intensity

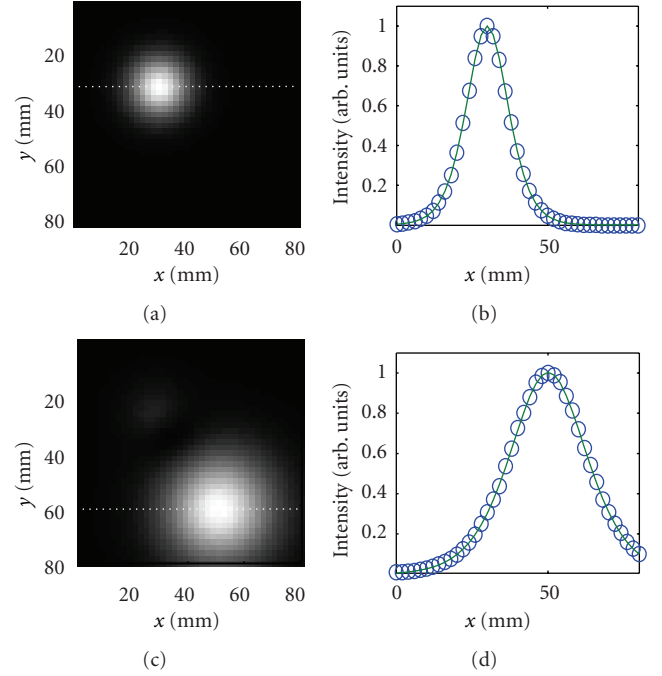


FIGURE 5: NMF-extracted two-dimensional intensity distribution on the detector plane of: (a) the centrosymmetric component of the scattering target; (c) the absorptive target. Fits to the corresponding spatial intensity profiles along the dashed line (shown in figures) are shown in (b) and (d), respectively.

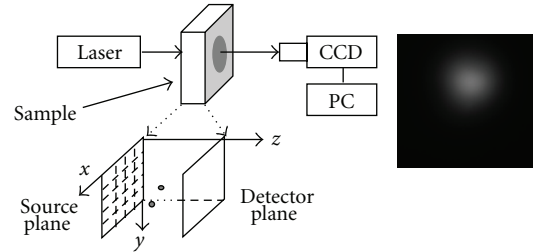


FIGURE 6: A schematic diagram of the experimental arrangement used for imaging objects embedded in a turbid medium. The inset at the bottom shows the 2D array in the input plane that was scanned across the incident laser beam; the inset to the right shows a typical raw image recorded by the CCD. (CCD: charge coupled device, and PC: personal computer).

profiles, as described in Section 2.3 using (6a) and (6b). The results are presented in Table 3.

Both targets were detected by all three algorithms. The target locations retrieved by three algorithms are shown in Table 3 and compared with known locations. Overall, all three algorithms detect and locate the scattering and the absorptive targets with good accuracy, the maximum deviation of any one coordinate from the known value being ~ 3 mm. Since the maximum difference between the known and retrieved position coordinates was larger for the scattering targets, we calculated the squared correlation coefficient γ to assess the fitting quality. NMF retrieves

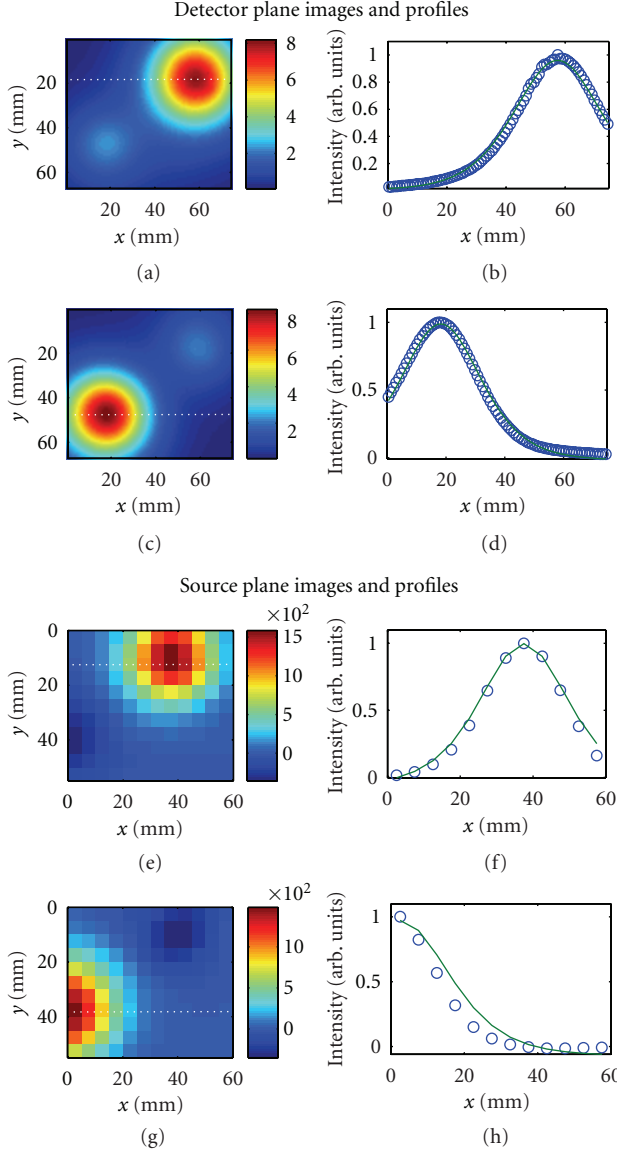


FIGURE 7: ICA-generated ICIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed lines are shown in (b) and (d). ICIDs on the source plane are shown in (e) and (g); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (f) and (h).

the position coordinates better (within 0.5 mm) for the scattering Target 2 than done by ICA and PCA (deviation from known values being between 2-3 mm). NMF retrieved the position coordinates for Target 1 with 3.0 mm error in z direction, which is not as good as that done by ICA and PCA. But γ is 0.783 and 0.778 in the fittings for ICA and PCA, respectively, as compared to 0.993 for NMF, indicating that the quality of the fitting is better for NMF. The quality of fitting is presumably affected by the efficacy of decomposition. The decomposed NCIDs by NMF were more "clean" than those decomposed by ICA and PCA. We ascribe the observed higher errors in ICA and PCA estimates of the

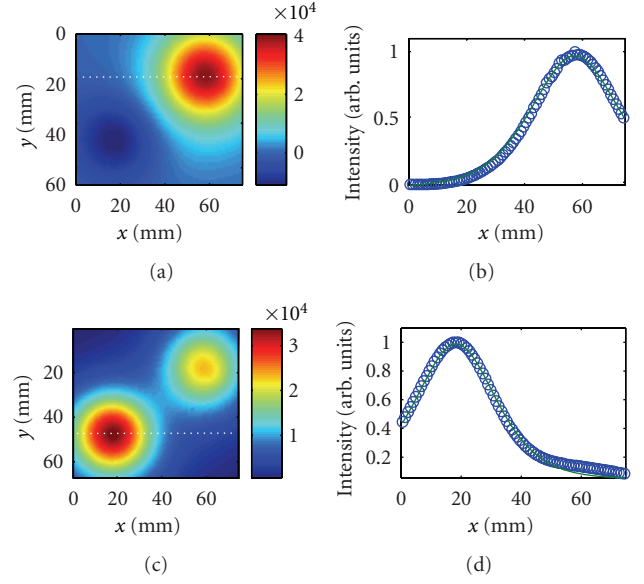


FIGURE 8: PCIDs on the detector plane are shown in (a) and (c); and corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

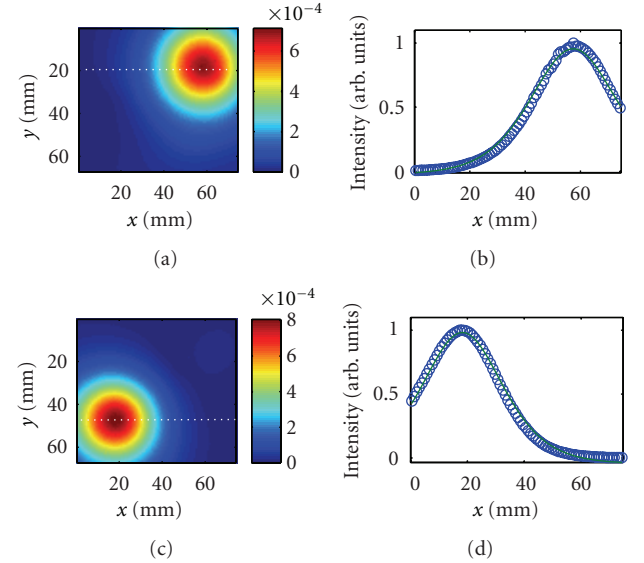


FIGURE 9: NCIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

position coordinates of the scattering Target 2 than the NMF estimates to the interference from the other virtual source (corresponding to Target 1) in ICA (Figure 10(c)) and PCA (Figure 11(c)) images. It is commonly believed that errors in locating a scattering target are higher than that for locating an absorptive target, and the results of this study conform to that notion.

TABLE 2: Known positions versus retrieved positions of the absorptive targets using ICA, PCA, and NMF algorithms.

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)
1	(57.2, 18.1, 20)	ICA	(57.4, 18.2, 21.5)	(0.2, 0.1, 1.5)
		PCA	(57.4, 18.2, 20.6)	(0.2, 0.1, 0.6)
		NMF	(57.4, 18.2, 19.5)	(0.2, 0.1, 0.5)
2	(19.9, 48.1, 25)	ICA	(18.2, 46.7, 24.7)	(1.7, 1.4, 0.3)
		PCA	(18.2, 47.6, 25.9)	(1.7, 0.5, 0.9)
		NMF	(18.2, 47.6, 23.3)	(1.7, 0.5, 1.7)

TABLE 3: Known positions versus retrieved positions of the scattering targets using ICA, PCA, and NMF algorithms.

Target	Known position (mm)	Algorithm	Fitted position (mm)	Error (mm)
1	(13.0, 28.0, 30.0)	ICA	(12.6, 28.7, 29.1)	(0.4, 0.7, 0.9)
		PCA	(12.6, 28.7, 28.6)	(0.4, 0.7, 1.4)
		NMF	(12.0, 28.5, 33.0)	(1.0, 0.5, 3.0)
2	(53.3, 28.5, 30.0)	ICA	(51.0, 31.8, 26.8)	(2.3, 3.3, 3.2)
		PCA	(50.9, 31.8, 26.7)	(2.4, 3.3, 3.3)
		NMF	(53.3, 28.0, 30.3)	(0.0, 0.5, 0.3)

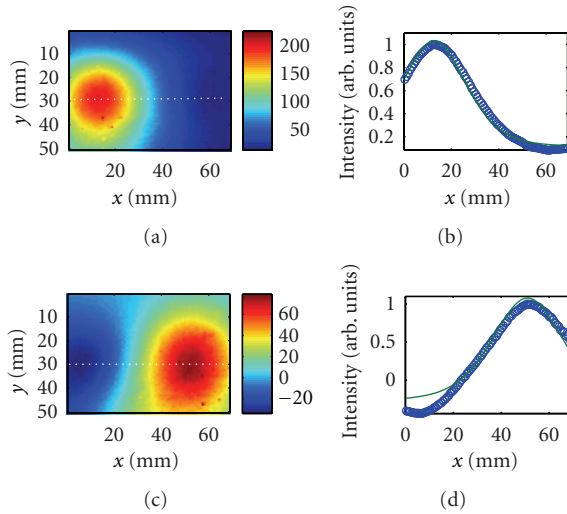


FIGURE 10: ICA-generated ICIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

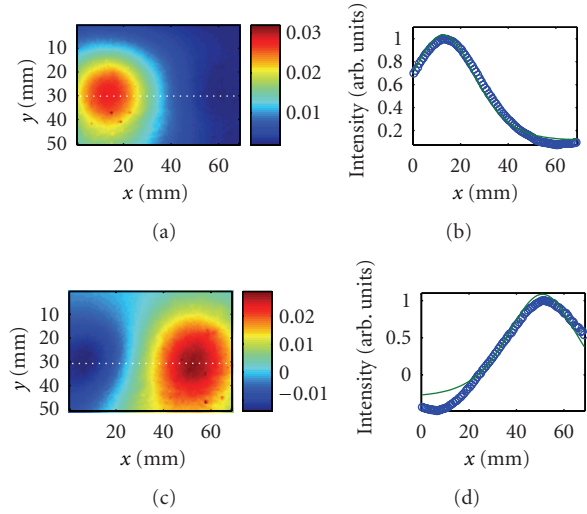


FIGURE 11: PCIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

5. Summary and Discussion

Diffusive optical imaging was modeled as a BSS problem. ICA, PCA, and NMF were used to decompose the data matrix and locate the targets embedded in a highly scattering turbid medium. Only the components corresponding to the targets were extracted from a large dataset for target detection and localization.

It may be instructive to compare the objectives, scope, and computational complexity of these decomposition methods with model-based reconstruction methods. Decomposition methods obtain the 3D locations of targets (the number of targets is generally small). Based on the retrieved locations, the methods may then be further

extended to retrieve size and optical property information of the targets [9]. The common practice of model-based inverse reconstruction methods is to discretize the sample volume into $N \times N \times N$ voxels and estimate absorption and/or scattering coefficient in each voxel iteratively. Voxels with significantly different optical properties than the surrounding are regions of interest and may be identified as targets. While estimating the optical properties, the forward model is solved repeatedly to calculate the intensity of the multiply scattered light on the sample boundary. The difference between the intensity of the multiply scattered light predicted by the forward model and the experimental measurements is minimized by seeking an optimal set of the optical properties of every voxel in the sample volume. The number of variables

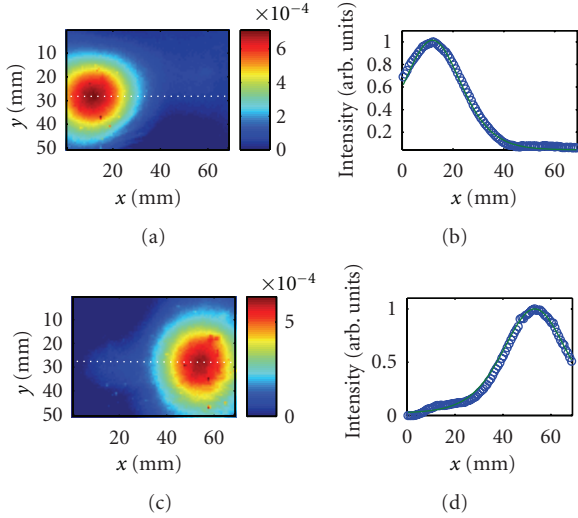


FIGURE 12: NCIDs on the detector plane are shown in (a) and (c); corresponding Green's function fits to the horizontal spatial profiles through the dashed line are shown in (b) and (d).

is thus, on the order of N^3 . To determine location(s) of target(s) in three dimensions, the decomposition methods process the data matrix to retrieve the main components (A and S). Here, A and S are two-dimensional matrices with the number of unknowns on the order of N^2 . The number of unknowns is, hence, reduced N times in the decomposition methods compared to the model-based approaches, which leads to a substantial saving in the computational time when N is large. No repeated solution of the forward model is involved in decomposition methods. Consequently, decomposition methods are considerably faster.

A comparison of the computational complexity of these two types of approaches may shed further light on their relative computation economy. For a model-based iterative reconstruction method, an equation of the form $b = Wx$ is solved to find the targets, where W is a weight matrix of size $N_d N_s \times N_v$, N_d , N_s , and N_v are the numbers of detectors, sources and voxels, respectively, b is an $N_d N_s \times 1$ vector describing the perturbation in the detected light intensity due to the presence of targets, and x is the perturbation in the optical properties from the background values with dimension of $N_v \times 1$. The computational complexity is typically $O(N_d N_s N_v^2)$ for a single iteration. For the decomposition approach, b is written as a 2D matrix X with dimension $N_d \times N_s$. To decompose matrix X , the computational complexity per iteration is typically of order $O(N_d N_k)$ for ICA [34], and $O(N_d N_s N_k)$ for NMF [16], where N_k is the number of components that relates to the number of targets and is usually a small number. For PCA using SVD, the complexity is $O(N_s^2 N_k)$ [34]. The computational complexity of the intrinsic iterative process involved in the matrix decomposition algorithms is much lower than that in the model-based inverse reconstruction methods.

All three matrix decomposition methods presented in this manuscript can potentially be used in *in-vivo* real-time breast cancer imaging. The three algorithms have

different assumptions, which may lead to different favored conditions. In this study, the algorithms were evaluated using simulative and experimental data using model scattering media and absorptive and scattering targets. The (x, y, z) positions of the targets were retrieved with good accuracy. The decomposition provided by ICA is "cleaner" than that of the PCA. PCA did not clearly separate the two absorptive targets used in the first experiment. NMF decomposition seems to provide residue-free "cleaner" images than the other two methods in this study. However, since NMF is based on nonnegativity assumption, the results might deteriorate when such a non-negativity assumption does not hold well. While continuous wave measurements were used in the work presented in this paper, the approaches could be used with frequency domain and time domain measurements as well.

The work presented here focuses on detecting and locating small targets, which derive impetus from the need to detect tumors in early stages of growth when those are more amenable to treatment. All three methods are applicable for extended targets as well and are expected to provide the "center of optical strength" as the location of the target.

All three approaches are applicable for both scattering and absorbing targets and may be used in clinical setting. The contrast between a tumor and surrounding normal tissue can be due to differences in absorption, scattering, or both absorption and scattering properties and may depend significantly on the wavelength of light used. However, *a priori* knowledge of the optical characteristics (absorptive or scattering) is not crucial. As has been shown in (2) and (3), the expression for elements of the data matrix for absorptive targets involves Green's Functions G , while that for scattering targets involves $\partial G / \partial z \approx -\kappa G$, where $\kappa = \sqrt{\mu_a / D}$ in CW [9]. This relationship with G provides basis for detection and localization of target(s), whether contrast is due to absorption, scattering, or both. We are using transillumination geometry, which is one of the approaches used by other researchers, and adequate signal for *in vivo* breast imaging is obtained [29, 35–38].

In this paper, we presented results when the approaches were used to detect and obtain three-dimensional location information of the targets. We have demonstrated, while developing OPTICA that a backprojection formalism can be further implemented to get a cross-section image of the target [11], or the retrieved target locations can be fed into other DOI methods as *a priori* information to get three-dimensional tomographic images. Since the approaches are suited for small targets, this hold promise for detecting and locating breast tumors in early stages of growth, which is crucially important for effective treatment. Further work involving *ex vivo* (model) and *in vivo* imaging of cancerous breast will be needed to establish the full potential of these approaches.

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Time reversal optical tomography: locating targets in a highly scattering turbid medium

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Abstract: A time reversal optical tomography (TROT) method for near-infrared (NIR) diffuse optical imaging of targets embedded in a highly scattering turbid medium is presented. TROT combines the basic symmetry of time reversal invariance and subspace-based signal processing for retrieval of target location. The efficacy of TROT is tested using simulated data and data obtained from NIR imaging experiments on absorptive and scattering targets embedded in Intralipid-20% suspension in water, as turbid medium. The results demonstrate the potential of TROT for detecting and locating small targets in a turbid medium, such as, breast tumors in early stages of growth.

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OCIS codes: (110.0113) Imaging through turbid media; (110.6955) Tomographic imaging; (110.3080) Infrared imaging; (170.3880) Medical and biological imaging; (170.3010) Image reconstruction techniques; (170.3830) Mammography; (170.0170) Medical optics and biotechnology; (170.6510) Spectroscopy, tissue diagnostics.

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1. Introduction

Optical imaging of targets embedded in a highly scattering turbid medium, such as, a tumor in a breast, is a challenging problem because light is strongly absorbed and scattered by the medium leading to poor signal-to-noise ratio, as well as, loss of phase coherence and polarization. As a consequence distinct, sharp image of the targets may not be formed directly. Various frequency-domain, time-resolved, and steady-state inverse image reconstruction (IIR) [1–5] approaches are being pursued to form tomographic images using diffusively scattered light measured at the sample boundary. IIR is an ill-posed problem and the development of reliable and fast approaches remains a formidable task. Recent IIR algorithms, such as Newton-Raphson-Marquardt algorithms [6] and direct linear inversion of 3-D matrices [7], are time consuming. The iterative methods [7,8] may not ensure that the obtained result arrives at a "global minimum" or converges to a "local minimum". Still the potential for developing non-invasive imaging approaches with diagnostic ability motivates the ongoing diffuse optical tomography (DOT) research using NIR light.

Many applications require rather accurate determination of location of target(s) in three dimensions. For example, a recent study involving 35,319 patients underscores the influence of primary tumor location on breast cancer prognosis [9], and makes it imperative that DOT for breast cancer detection be able to obtain three-dimensional (3-D) location of the tumor. While two-dimensional (2-D) IIR approaches may provide only lateral positions, 3-D IIR approaches attempt to retrieve all three position coordinates of the target(s). Various frequency-domain, time-domain, and steady-state DOT approaches have addressed the target localization problem with different measures of success [1–8]. Several groups have paid particular attention to retrieving target location. Kepshire *et al.* developed a subsurface DOT approach to obtain location information of absorbing and fluorescent targets, but observed the sensitivity to vary nonlinearly with depth [10]. Mohajerani *et al.* reported a fluorescent tomography method for locating fluorescent targets embedded in a heterogeneous medium using partitioning of the fluorophore distribution into an object subspace and a background

subspace [11]. Godavarty *et al.* developed another fluorescent tomography approach that used a hemispherical breast phantom, near-infrared light-induced fluorescence from a contrast agent, and finite element method-based reconstruction algorithms to obtain target information up to a depth of 2 cm from breast phantom surface [12]. Zhao *et al.* introduced a layer-based sigmoid adjustment method to improve depth resolution of DOT and achieved positioning error within 3 mm for depths from 10 to 30 mm [13]. Optical tomography using independent component analysis (OPTICA) approach developed by Xu *et al.* uses multi-source probing and multi-detector signal acquisition scheme and a numerical algorithm based on independent component analysis of information theory to obtain 3-D position information of absorbing, scattering and fluorescent targets embedded in highly scattering turbid media, and “model breast” assembled using *ex vivo* human breast tissue [14–17]. Co-registration approaches that use another modality, such as, ultrasound, magnetic resonance imaging, and x-ray mammography for locating suspect areas and DOT for obtaining images have also been introduced [18–21].

In this article we report on the development of a time reversal optical tomography (TROT) [22–24] approach for NIR optical imaging of target(s) in a turbid medium, and present initial results of its efficacy using both simulated and experimental data.

Time reversal (TR) invariance, the basic symmetry that commonly holds in microscopic physics, forms the basis for macroscopic TR imaging. TR imaging using the so-called “time-reversal mirrors” (TRMs) has been used as an experimental tool in acoustics with practical applications in medicine, underwater imaging, and nondestructive testing [25–28]. The theoretical and numerical techniques involved in time reversal have been used for applications involving both acoustic waves and electromagnetic waves (radar) [28–33].

Devaney and associates developed a theoretical framework for a TR imaging method with Multiple Signal Classification (MUSIC) for finding the location of scattering targets whose size is smaller than the wavelength of acoustic waves or electromagnetic waves (radar) used for probing the homogeneous or inhomogeneous background medium in which the targets were embedded [34,35]. While their initial focus was on *back-propagation geometry* that used coincident acoustic or electromagnetic transceiver array for interrogating the targets, they later extended the formalism to *transmission geometry* where sources and detectors were distinct and separated [36]. They also generalized the theory which was based on distorted wave Born approximation (DWBA) to account for multiple scattering between the targets [37]. In its basic form TR-MUSIC found target location from knowledge of the response matrix K , which was constructed from multi-static data collected by the transceiver array [34,35]. TR-MUSIC provided higher spatial resolution than the conventional TR imaging, especially in the case where targets were not well resolved [34,35,38].

We are adapting and extending the TR-MUSIC approach to the optical domain, *i.e.* to diffusive optical imaging for detecting and locating targets embedded in a turbid medium. In this paper, TROT is studied in details using both simulated data and data from transillumination NIR imaging experiments in slab geometry. A TR matrix is obtained by multiplying the response matrix formed using experimental or simulated data to its conjugate matrix. The leading non-zero eigenvalues of the Hermitian TR matrix determine the signal subspace due to presence of the targets. The signal subspace is separated from the noise subspace using an L -curve method [5,39,40]. The vector subspace method, MUSIC, along with Green’s functions calculated from an appropriate forward model for light propagation through the turbid medium is then used to determine the locations of the targets. The MUSIC algorithm judges if the calculated Green’s function vector corresponding to a location in the sample is mapped into the signal subspace or the noise subspace.

Several salient features make TROT attractive and potentially more promising than other IIR methods. First the size of the TR matrix is much smaller than those used in other IIR approaches, which makes solution of the eigenvalue problem easier and faster. Second, to determine locations of targets, TR-MUSIC approach runs the program over all voxels only once, and there is no need to carry out an iterative procedure done by other inverse approaches. Other IIR approaches seek to determine the absorption and scattering parameters

at all voxels into which the sample is divided. The process is iterative, computationally intensive, and leads to a solution of the inverse problem that is not unique because the problem is ill-posed, even when there is no noise. In contrast, TROT seeks to determine the locations of the targets first and thereafter retrieve other information, such as, the size and optical properties of the limited number of targets in the medium, which requires significantly less computation time. The focus of this paper is on finding the locations of targets.

Our result using simulated data shows that without the presence of noise TROT determines the locations of the embedded targets accurately with high resolution. TROT exhibits promise to locate targets both in simulations and experiments even when substantial noise is present. Images of small targets obtained by this approach are sharper than that obtained by other IIR approaches.

This paper is organized as follows. In section 2, the formalism of the TROT approach is presented. In section 3, the numerical algorithm of TROT is described. In section 4, the efficacy of the formalism is tested using simulated data. Section 5 presents the results when the formalism is applied to experimental data using Intralipid-20% suspension in water as the highly scattering turbid medium. Section 6 discusses the results.

2. Formalism

2.1 Diffusion approximation, perturbation method and response matrix

The starting point for the TROT formalism is the diffusion approximation [41–43] of the radiative transfer equation (RTE) [44,45]. The perturbation in the light intensity distribution due to small inhomogeneities (targets) embedded in a homogeneous medium, to the first order Born approximation, can be written as [46,47]

$$\Delta\phi(\mathbf{r}_d, \mathbf{r}_s) = -\int G(\mathbf{r}_d, \mathbf{r})\delta\mu_a(\mathbf{r})cG(\mathbf{r}, \mathbf{r}_s)d^3\mathbf{r} - \int \delta D(\mathbf{r})c\nabla_r G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_r G(\mathbf{r}, \mathbf{r}_s)d^3\mathbf{r}, \quad (1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a point-like source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green's functions that describe light propagations from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ is the difference in absorption coefficient and δD is the difference in diffusion coefficient between the targets and the background medium; and c is the light speed in the medium.

A multi-source interrogation and multi-detector signal acquisition scheme is used to acquire transillumination data, from which the difference in the light intensity distribution due to the targets, $\Delta\phi = \phi - \phi_0$, is found, where ϕ is the light intensity distribution measured on the sample boundary with targets embedded in the scattering medium and ϕ_0 is ideally the light intensity distribution without the targets, which in practice is approximated by an “average” over all the multi-source measurements. A response matrix K is constructed with $-\Delta\phi$, to describe the transport of light from different sources through the embedded objects to the array of detectors [22,36].

For small, point-like absorptive targets, the matrix elements can be rewritten in a discrete form as:

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{X}_m) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), i=1,2,\dots,N_d; j=1,2,\dots,N_s, \quad (2)$$

where $\tau_m = \delta\mu_a(\mathbf{X}_m)c\delta V_m$ is the optical absorption strength of the m^{th} target, δV_m is the volume of m^{th} target, $\mathbf{r}_i$, $\mathbf{r}_j$ and $\mathbf{X}_m$ are locations of the i^{th} detector, j^{th} source and m^{th} target, respectively. Due to the reciprocity of light propagation in the medium, $G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r}', \mathbf{r})$. Thus,

$$K_{ij} = \sum_{m=1}^M G^d(\mathbf{X}_m, \mathbf{r}_i) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j), \quad (3)$$

and

$$K = \{K_{ij}\} = \sum_{m=1}^M g_d(\mathbf{X}_m) \tau_m g_s^T(\mathbf{X}_m), \quad (4)$$

where $g_s(\mathbf{r})$ and $g_d(\mathbf{r})$ are Green's function vectors (GFVs) associated with the source array and detector array, respectively. GFVs are defined as

$$g_s(\mathbf{r}) = [G^s(\mathbf{r}_1, \mathbf{r}), G^s(\mathbf{r}_2, \mathbf{r}), \dots, G^s(\mathbf{r}_{N_s}, \mathbf{r})]^T, \quad (5a)$$

$$g_d(\mathbf{r}) = [G^d(\mathbf{r}_1, \mathbf{r}), G^d(\mathbf{r}_2, \mathbf{r}), \dots, G^d(\mathbf{r}_{N_d}, \mathbf{r})]^T, \quad (5b)$$

where the superscript T denotes transpose; and N_s , N_d and M are the numbers of sources, detectors and targets, respectively. It is assumed the number of targets is less than the number of sources and detectors, $M < \min(N_d, N_s)$. It also holds that $K^T = \{K_{ji}\}$ describes light propagation from the positions of detectors through the medium and targets to sources.

For a homogeneous background medium, the rank R of matrix K , is equal to the dimension of the source array vector space $\mathcal{G}_s$ spanned by $g_s(\mathbf{r}_m)$, and also equal to the dimension of the detector array vector space $\mathcal{G}_d$ spanned by $g_d(\mathbf{r}_m)$, where $\mathcal{G}_s \subseteq C^{N_s}$ and $\mathcal{G}_d \subseteq C^{N_d}$. For absorptive targets, R is equal to the number of targets M .

Similar forms of the response matrix and GFVs can be obtained for scattering targets. As the dot product in the second term of Eq. (1) implies, each scattering target is represented by three components coexisting at one location. The elements of the K matrix for L scattering target may be written as

$$\begin{aligned} K_{ij} &= \sum_{l=1}^L \tau_l \nabla_r G^d(\mathbf{r}_i, \mathbf{X}_l) \cdot \nabla_r G^s(\mathbf{X}_l, \mathbf{r}_j) \\ &= \sum_{l=1}^L \tau_l \sum_{\alpha=\{x,y,z\}} \partial_\alpha G^d(\mathbf{r}_i, \mathbf{X}_l) \partial_\alpha G^s(\mathbf{X}_l, \mathbf{r}_j), \end{aligned} \quad (6)$$

where $\tau_l = \delta D(\mathbf{X}_l) c \delta V_l$ is the optical scattering strength of the l^{th} target. The K matrix for scattering targets can be written in a manner similar to that for absorptive targets:

$$K = \sum_{l=1}^L \sum_{\alpha=\{x,y,z\}} \partial_\alpha g_d(\mathbf{X}_l) \tau_l \partial_\alpha g_s^T(\mathbf{X}_l). \quad (7)$$

The Green's function for a slab geometry is [16,47]

$$G(\mathbf{r}, \mathbf{r}') = G(\mathbf{r}', \mathbf{r}) = \frac{1}{4\pi D} \sum_{k=-\infty}^{\infty} \left(\frac{e^{-\kappa r_k^+}}{r_k^+} - \frac{e^{-\kappa r_k^-}}{r_k^-} \right), \quad (8a)$$

$$r_k^\pm = \left[(x - x')^2 + (y - y')^2 + (z \mp z' + 2kd)^2 \right]^{1/2}, \quad (8b)$$

where $\kappa = [(\mu_a - i\omega/c)/D]^{1/2}$ in frequency domain with amplitude modulation frequency ω , and $k = 0, \pm 1, \pm 2, \dots$. The extrapolated boundaries of the slab are located at $z = 0$ and

$z = d = L + 2z_e$, respectively, where L is the physical thickness of the slab and the extrapolation length z_e is determined from the boundary condition of the slab [48,49].

Under ideal conditions, when all three scattering components of each of the L scattering targets are well-resolved, the rank of K contributed by L scattering targets is $3L$. In practice, four components (one for absorption and three for scattering) are calculated for each target, since the targets may have both scattering and absorptive characteristics, or the exact nature may not be known *a priori*. The dominant characteristic is used to label the target as absorptive or scattering in nature.

2.2 Point Spread Functions

If light emitted by a source of unit power at target position $\mathbf{X}$ propagates in the sample medium, the signal measured by the detector array at the sample boundary is $G^d(\mathbf{r}, \mathbf{X})$. The signal is then “time-reversed” and back-propagated with the Green’s function of the background medium. TR operation is phase conjugation in Fourier domain [28,50]. So the signal evaluated at $\mathbf{r}$ is [34]

$$\begin{aligned} H_d(\mathbf{r}, \mathbf{X}) &= \sum_{i=1}^{N_d} G^d(\mathbf{r}, \mathbf{r}_i) G^{d*}(\mathbf{r}_i, \mathbf{X}) = g_d^T(\mathbf{r}) g_d^*(\mathbf{X}) \\ &= g_d^\dagger(\mathbf{X}) g_d(\mathbf{r}) = \langle g_d(\mathbf{X}), g_d(\mathbf{r}) \rangle, \end{aligned} \quad (9)$$

where $*$ denotes phase conjugate, $\dagger$ denotes adjoint, and $\langle \cdot \rangle$ denotes inner product. $H_d(\mathbf{r}, \mathbf{X})$ is the detector array point spread function (PSF). A source array PSF can be similarly formed as

$$H_s(\mathbf{r}, \mathbf{X}) = g_s(\mathbf{X})^\dagger g_s(\mathbf{r}) = \langle g_s(\mathbf{X}), g_s(\mathbf{r}) \rangle. \quad (10)$$

Due to the time reversal assumption, $H_d(\mathbf{r}, \mathbf{X})$ peaks at $\mathbf{r} = \mathbf{X}$, so it can be considered as an image of the source at $\mathbf{X}$ formed by the TR detector array. PSF vanishes when $\mathbf{r}$ is far away from $\mathbf{X}$. A similar interpretation can be used for $H_s(\mathbf{r}, \mathbf{X})$.

2.3 Time reversal and MUSIC

The TR matrix may be constructed to represent light propagation from sources to detectors and back denoted by T_{SDDS} , or to represent light propagation from detector positions to source positions and back denoted by T_{DSSD} , a consequence of the reciprocity of light propagation [29,34,38,50,51]. For frequency-domain data, $T_{SDDS} = K^\dagger K$, and $T_{DSSD} = (K^T)^\dagger K^T = K K^T$, where response data matrix K is formed using modulated intensities, instead of the field with phase information used in the conventional TR. For CW measurements, $T_{SDDS} = K^\dagger K$, and $T_{DSSD} = K K^T$ (K is real and only includes intensity values).

Since T_{SDDS} and T_{DSSD} are Hermitian ($T^\dagger = T$), they have complete sets of orthonormal eigenvectors $\mathbf{v}_j$ ($j = 1, \dots, N_s$) and $\mathbf{u}_i$ ($i = 1, \dots, N_d$), with a common set of non-negative real eigenvalues. For $M < \min(N_s, N_d)$ absorptive targets without the presence of noise, the rank of T_{SDDS} and T_{DSSD} is M . The eigenvalues $\lambda_j > 0$, when $j = 1, \dots, M$, and $\lambda_j \approx 0$, when $j = M + 1, \dots, N_s$ for T_{SDDS} and $j = M + 1, \dots, N_d$ for T_{DSSD} . The eigen system $\{\mathbf{v}_j, \mathbf{u}_j, \lambda_j > 0\}$, $j = 1, \dots, M$, is related to the targets. The TR matrix T_{SDDS} can be written as [22,34]

$$T_{SDDS} = \sum_{m=1}^M \sum_{m'=1}^M \tau_m^* \tau_{m'} \langle g_d(\mathbf{X}_m), g_d(\mathbf{X}_{m'}) \rangle g_s^*(\mathbf{X}_m) g_s^T(\mathbf{X}_{m'}). \quad (11)$$

Subsequent formalism may be different depending on whether the targets are “well resolved” or “poorly resolved.”

2.3.1 Well-resolved targets

If the m^{th} and m'^{th} targets ($m \neq m'$) are well resolved, defined by the conditions: $H_s(\mathbf{X}_m, \mathbf{X}_{m'}) \approx 0$ and $H_d(\mathbf{X}_m, \mathbf{X}_{m'}) \approx 0$, i.e. the GFVs at $\mathbf{X}_m$ and $\mathbf{X}_{m'}$ are orthogonal, $\langle g_d(\mathbf{X}_m), g_d(\mathbf{X}_{m'}) \rangle = H_d(\mathbf{X}_{m'}, \mathbf{X}_m) = \|g_d(\mathbf{X}_m)\|^2 \delta_{mm'}$. So we have

$$T_{SDDS} = \sum_{m=1}^M |\tau_m|^2 \|g_d(\mathbf{X}_m)\|^2 g_s^*(\mathbf{X}_m) g_s^T(\mathbf{X}_m), \quad (12)$$

where $\|\cdot\|$ denotes $L2$ norm [52]. The eigenvectors of T_{SDDS} are proportional to the phase conjugate of the GFVs associated with the M targets [22,34], i.e.

$$T_{SDDS} g_s^*(\mathbf{X}_m) = |\tau_m|^2 \|g_d(\mathbf{X}_m)\|^2 \|g_s(\mathbf{X}_m)\|^2 g_s^*(\mathbf{X}_m). \quad (13)$$

The eigenvectors are

$$\mathbf{v}_j = \frac{g_s^*(\mathbf{X}_j)}{\|g_s(\mathbf{X}_j)\|}, \quad (14)$$

with eigenvalues $\lambda_j = |\tau_j|^2 \|g_d(\mathbf{X}_j)\|^2 \|g_s(\mathbf{X}_j)\|^2$, $j = 1, \dots, M$. Thus T_{SDDS} is a projection operator that projects a vector onto the conjugate of the source array vector space $\mathcal{G}_s$. The j^{th} non-zero eigenvalue λ_j is directly related to the optical strength τ_j of the j^{th} target. Similar equations can be derived for T_{DSSD} , which is a projection operator for the conjugate of the detector array vector space $\mathcal{G}_d$. The eigenvectors of T_{DSSD} are

$$\mathbf{u}_j = \frac{g_d^*(\mathbf{X}_j)}{\|g_d(\mathbf{X}_j)\|}, \quad (15)$$

$j = 1, \dots, M$, with the same eigenvalues as T_{SDDS} .

Therefore, for well-resolved targets, the target locations can be determined by the inner product [22,34,36,51]

$$\begin{aligned} \psi_j^s &= \langle \mathbf{v}_j^*, g_s(\mathbf{X}_p) \rangle = \mathbf{v}_j^T g_s(\mathbf{X}_p) = \frac{g_s^\dagger(\mathbf{X}_j)}{\|g_s(\mathbf{X}_j)\|} g_s(\mathbf{X}_p) \\ &= \frac{1}{\|g_s(\mathbf{X}_j)\|} H_s(\mathbf{X}_p, \mathbf{X}_j), \end{aligned} \quad (16a)$$

or

$$\begin{aligned} \psi_j^d &= \langle \mathbf{u}_j^*, g_d(\mathbf{X}_p) \rangle = \mathbf{u}_j^T g_d(\mathbf{X}_p) = \frac{g_d^\dagger(\mathbf{X}_j)}{\|g_d(\mathbf{X}_j)\|} g_d(\mathbf{X}_p) \\ &= \frac{1}{\|g_d(\mathbf{X}_j)\|} H_d(\mathbf{X}_p, \mathbf{X}_j), \end{aligned} \quad (16b)$$

where $\mathbf{X}_p$ is a test target position, which is the position of any voxel in the sample space. ψ_j^s and ψ_j^d peak when $\mathbf{X}_p$ is the position of the j^{th} target. In the classical TR imaging

[25,29,38,50], for ideally resolved targets, each eigenvector of the TR operator can be used to focus on one particular target. Here ψ_j^s and ψ_j^d represent focusing of signals from the source and detector planes on to the target position, respectively. Use of the eigenvectors v_j and u_j , $j=1, \dots, M$, ensures that the j^{th} target is sorted out. When T_{SDDS} and source array vector space (T_{DSSD} and detector array vector space) are used, we call the scheme SDDS (DSSD). Both source and detector arrays can be considered simultaneously to locate the target by calculating

$$\psi_j = \psi_j^d \psi_j^{s*} = \frac{1}{\|g_s(\mathbf{X}_j)\| \cdot \|g_d(\mathbf{X}_j)\|} H_d(\mathbf{X}_p, \mathbf{X}_j) H_s^*(\mathbf{X}_j, \mathbf{X}_p), \quad (17)$$

$j=1, \dots, M$, which is computationally equivalent to a process that light emitted from a virtual source of unit power at a test target position $\mathbf{X}_p$, propagates to the TR source array and back to a true target position $\mathbf{X}_j$; then it is re-emitted and further propagates to the TR detector array and back to the original position $\mathbf{X}_p$. ψ_j peaks when the test target position $\mathbf{X}_p$ coincides with the true target position $\mathbf{X}_j$ associated with the j^{th} eigenvector.

2.3.2 Poorly-resolved Targets and MUSIC

When the targets are too close to each other or the sources and/or detectors are significantly sparse, the targets are considered to be poorly resolved and the GFVs at $\mathbf{X}_m$ and $\mathbf{X}_m$ are not orthogonal. In such cases, the eigenvectors v_j and u_j do not correspond one-to-one with the GFVs associated with target positions $\mathbf{X}_m$ ($j, m=1, \dots, M$). The image resolution degrades because of contributions from multiple targets. To solve this problem, the subspace-based method, MUSIC was implemented with TR [34,36,51]. MUSIC algorithm is based on the idea that although the vectors characterizing the targets are no longer orthogonal with each other, they are all located in the signal subspace, which is orthogonal to the noise subspace.

The orthonormal sets $\{v_j^*\}$ ($j=1, \dots, N_s$) and $\{u_j^*\}$ ($j=1, \dots, N_d$) span the spaces C^{N_s} and C^{N_d} associated with the source and detector arrays, respectively. While $\{v_j^*\}$ and $\{u_j^*\}$, with $\lambda_j > 0$, form the signal subspaces on the source and detector arrays, $\mathcal{S}^s = \{v_j^*\}$ and $\mathcal{S}^d = \{u_j^*\}$ ($j=1, \dots, M$), respectively; $\{v_j^*\}$ and $\{u_j^*\}$, with $\lambda_j \approx 0$, form the noise subspaces, $\mathcal{N}^s = \{v_j^*\}$ ($j=M+1, \dots, N_s$) and $\mathcal{N}^d = \{u_j^*\}$ ($j=M+1, \dots, N_d$), respectively. Thus $C^{N_s} = \mathcal{S}^s \oplus \mathcal{N}^s$ and $C^{N_d} = \mathcal{S}^d \oplus \mathcal{N}^d$ [36,51]. Since the dimensions of the signal subspaces $\mathcal{S}^s$ and $\mathcal{S}^d$ and of the GFV spaces $\mathcal{G}_s$ and $\mathcal{G}_d$ are all equal to M , $\mathcal{G}_s \equiv \mathcal{S}^s$ and $\mathcal{G}_d \equiv \mathcal{S}^d$ [51]. The GFVs, $g_s(\mathbf{X}_m)$ and $g_d(\mathbf{X}_m)$, $m=1, \dots, M$, are linear combinations of v_j^* and u_j^* , $j=1, \dots, M$, respectively. Therefore, $g_s(\mathbf{X}_m) \in \mathcal{S}^s$ and $g_d(\mathbf{X}_m) \in \mathcal{S}^d$, $m=1, \dots, M$, associated with m^{th} target are orthogonal to $v_j^* \in \mathcal{N}^s$ ($j=M+1, \dots, N_s$) and $u_j^* \in \mathcal{N}^d$ ($j=M+1, \dots, N_d$), respectively:

$$\langle v_j^*, g_s(\mathbf{X}_m) \rangle = v_j^T g_s(\mathbf{X}_m) \approx 0, \quad j=M+1, \dots, N_s, \quad (18a)$$

$$\langle u_j^*, g_d(\mathbf{X}_m) \rangle = u_j^T g_d(\mathbf{X}_m) \approx 0, \quad j=M+1, \dots, N_d. \quad (18b)$$

The locations of targets can be determined by calculating the following squared sum of inner products:

$$Q_s(\mathbf{X}_p) = \sum_{j=M+1}^{N_s} |v_j^T g_s(\mathbf{X}_p)|^2, \quad (19a)$$

$$Q_d(\mathbf{X}_p) = \sum_{j=M+1}^{N_d} \left| \mathbf{u}_j^T \mathbf{g}_d(\mathbf{X}_p) \right|^2. \quad (19b)$$

$Q_s(\mathbf{X}_p)$ and $Q_d(\mathbf{X}_p)$ vanish when the test target position $\mathbf{X}_p$ is a true target position. Similar to Eq. (17), $Q = Q_s Q_d$ can be calculated with both source and detector arrays considered simultaneously. An alternative approach to accentuate a target position is to plot a pseudo spectrum defined as

$$P_s(\mathbf{X}_p) = \left\| \mathbf{g}_s(\mathbf{X}_p) \right\|^2 / \left| Q_s(\mathbf{X}_p) \right| \quad (20a)$$

associated with the source array, or

$$P_d(\mathbf{X}_p) = \left\| \mathbf{g}_d(\mathbf{X}_p) \right\|^2 / \left| Q_d(\mathbf{X}_p) \right| \quad (20b)$$

associated with the detector array, or

$$P(\mathbf{X}_p) = P_s(\mathbf{X}_p) P_d(\mathbf{X}_p) \quad (20c)$$

associated with both source and detector arrays [22,34,36,51], where $\left\| \mathbf{g}_s(\mathbf{X}_p) \right\|^2$ and $\left\| \mathbf{g}_d(\mathbf{X}_p) \right\|^2$ are used for normalization. The poles of the pseudo spectrum correspond to target locations. These MUSIC pseudo spectra can also be used to locate well-resolved targets.

Since the dimension of the signal subspace is generally much smaller than that of the noise subspace, it is preferred that in Eq. (19) and Eq. (20), the signal subspace is used rather than the noise subspace for ease of computation. Using the properties of the projection operators associated with the source and detector arrays [22,34,36,51], $Q_s(\mathbf{X}_p)$ and $Q_d(\mathbf{X}_p)$ can be calculated as

$$Q_s(\mathbf{X}_p) = \left\| \mathbf{g}_s(\mathbf{X}_p) \right\|^2 - \sum_{j=1}^M \left| \mathbf{v}_j^T \mathbf{g}_s(\mathbf{X}_p) \right|^2, \quad (21a)$$

$$Q_d(\mathbf{X}_p) = \left\| \mathbf{g}_d(\mathbf{X}_p) \right\|^2 - \sum_{j=1}^M \left| \mathbf{u}_j^T \mathbf{g}_d(\mathbf{X}_p) \right|^2. \quad (21b)$$

When the targets are embedded in a non-uniform medium, or when there is significant noise present, the noise or false targets contribute significantly to the eigenvalues. The near-zero and non-zero eigenvalues are not as well separated as when there are no noise. In this case, the rank of the TR matrix is larger than the number of targets M . The TR matrix may even be full rank. However, as long as M is less than $\min(N_s, N_d)$ and eigenvalues contributed by the noise and false targets are smaller than those contributed by the real targets with a threshold ϵ , the target positions can be obtained using a pseudo spectrum [36,51] associated with the source array,

$$P_s(\mathbf{X}_p) = \left\| \mathbf{g}_s(\mathbf{X}_p) \right\|^2 / \left| Q_s(\mathbf{X}_p)_{\lambda_j \leq \epsilon} \right|, \quad (22)$$

where $Q_s(\mathbf{X}_p)_{\lambda_j \leq \epsilon} = \left\| \mathbf{g}_s(\mathbf{X}_p) \right\|^2 - \sum_{\lambda_j > \epsilon} \left| \mathbf{v}_j^T \mathbf{g}_s(\mathbf{X}_p) \right|^2$. Pseudo spectra associated with the detector

array or with both source and detector arrays can also be obtained similarly. In practice, the threshold is selected to separate the signal and noise subspaces using a method similar to L -curve regularization [39].

When scattering targets are concerned, the GFVs $\partial_\alpha g$ ($\alpha = x, y, z$), associated with the test target position $\mathbf{X}_p$ will be used to calculate the pseudo spectrum. For a target with both absorption and scattering properties at the wavelength of probing light, one GFV corresponding to absorption constructed as g and three GFVs corresponding to scattering target constructed with $\partial_\alpha g$, ($\alpha = x, y, z$), are used to calculate the pseudo spectrum over every voxel. Ideally, for an absorptive and scattering target four pseudo-values will be obtained for every target position. If the dominant value corresponds to the absorptive (any of the scattering) GFV the target will be identified as absorptive (scattering) in nature.

3. Algorithm

Implementation of TROT to locate targets embedded in a highly scattering turbid medium involves the steps outlined below. For simplicity, the sizes of source array and detector array are assumed to be the same, i.e., $N_d = N_s = N$.

- (a) A response matrix K with size $N \times N$ is constructed using experimental data (or estimated data in simulation). Data consist of the perturbations in the light intensity distribution due to the targets, $\Delta\phi = \phi - \phi_0$, where ϕ is the light intensity distribution measured on the sample boundary with targets embedded in the scattering medium and ϕ_0 is ideally the light intensity distribution without the targets. In practice, ϕ_0 is approximated by an “average” over all the multi-source measurements, while in simulation it can be estimated without such approximation.
- (b) A detector array TR matrix, $T_{DSSD} = KK^T$ with size $N \times N$ for CW measurements is constructed. All the eigenvalues and the eigenvectors of the T_{DSSD} matrix are computed. The eigenvectors are orthogonal to each other. It is to be noted that in this procedure we only deal with a matrix of dimension N , not a matrix of dimension of $N \times N$ as done in traditional inverse procedures.
- (c) The non-zero eigenvalues of T_{DSSD} belonging to the signal subspace are separated from the near-zero eigenvalues belonging to the noise subspace using the L -curve method [5,39,40].
- (d) MUSIC approach [34,36,51] is next used to determine the locations of the targets as follows. (i) The 3- D medium is divided into a certain number of voxels. A detector array GFV, $g_d(\mathbf{X}_p)$, associated with an absorptive test target position $\mathbf{X}_p$ at p^{th} voxel is calculated using Diffusion Approximation of RTE. Other proper forward models could be used as well. In order to check if $g_d(\mathbf{X}_p)$ is located in the signal subspace or in the noise subspace, a pseudo spectrum associated with the detector array is computed using Eq. (20b), where M is the dimension of the signal subspace found in step (c). If $g_d(\mathbf{X}_p)$ is located in the signal subspace, the corresponding pseudo value $P(\mathbf{X}_p)$ in Eq. (20b) will become a maximum. (ii) Pseudo spectra are also calculated using the other three GFVs, $\partial_\alpha g_d(\mathbf{X}_p)$, ($\alpha = x, y, z$) for scattering property. (iii) All pseudo values are put together and sorted in a descending order. Since the leading pseudo values at $\mathbf{X}_p$ are associated with targets and specific GFVs, the positions of the embedded targets and their nature (absorptive or scattering) are determined. The pseudo spectrum in the whole sample space can be used to plot pseudo tomographic images.

In this approach, only a single run is needed for calculating the pseudo spectrum and no iterative procedure is involved, which makes it faster and computationally less intensive than the traditional IIR approaches. Similar procedure can be used for application of TROT when

$N_d \neq N_s$. The pseudo spectrum associated with either the source array, or the detector array, or both source and detector arrays, as outlined in Eq. (20) can be used to obtain target positions.

It is instructive to compare the computational complexity of TROT formalism with that of typical iterative methods. For a typical iterative method, an equation $b = Wx$ is solved to find the inhomogeneities (targets), where W is a weight matrix with size $N_d N_s \times N$, N is the number of voxels, b is an $N_d N_s \times 1$ vector describing the perturbation in the detected light intensity due to the presence of inhomogeneities, and x is the perturbation or variation in the optical properties from the background values with dimension of $N \times 1$. The computational complexity is typically $O(N_d N_s N^2)$ for a single iteration. The computational complexity of TROT is much smaller than that for even one iteration of an iterative method. For the SDDS scheme, the complexity for TROT is $O(N_d N_s^2)$ if $N_d N_s > NN_k$, and $O(N_s NN_k)$ otherwise, where N_k is the dimension of the signal subspace. In the DSSD scheme, the complexity is $O(N_s N_d^2)$ if $N_d N_s > NN_k$, and $O(N_d NN_k)$ otherwise. TROT does not involve any iteration.

In the following sections, TROT will be tested using simulation and experimental data.

4. Testing TROT with simulated data

To test the efficacy of the TROT approach, we first consider a rather challenging task of detecting and locating six targets embedded in a simulated sample which is a 40-mm thick uniform scattering slab. Its absorption and diffusion coefficients are $\mu_a = 1/300 \text{ mm}^{-1}$ and $D = 1/3 \text{ mm}$, respectively. The incident CW beam was step-scanned in an x - y array of 41×41 grid points with a step size of 2 mm on the input plane covering an $80 \text{ mm} \times 80 \text{ mm}$ area. Light transmitted from the opposite side (output plane) was recorded at 41×41 grid points covering the same area. No random noise was added.

The six ($M = 6$) point-like absorptive targets, with absorption coefficient difference of $\Delta\mu_a = 0.01 \text{ mm}^{-1}$ from the background, were placed at A (24 mm, 26 mm, 9 mm), B (38 mm, 38 mm, 15 mm), C (38 mm, 38 mm, 21 mm), D (40 mm, 38 mm, 21 mm), E (44 mm, 42 mm, 21 mm) and F (30 mm, 30 mm, 31 mm), respectively. The origin (0 mm, 0 mm, 0 mm) was located at the upper-left corner of the input boundary (source plane) of the sample. The medium was divided into $40 \times 40 \times 20$ voxels, with each voxel of size $2 \text{ mm} \times 2 \text{ mm} \times 2 \text{ mm}$. As can be seen from the assigned coordinates, targets C and D are located at two adjacent voxels, and are close to target E , and these three targets are located in the same z layer. Consequently, targets C and D are expected to be very difficult to resolve, and hard to distinguish from target E . Target B and C have the same lateral position x and y , and different depths. Target A is close to the source plane, while F is close to the detector plane.

Using the Diffusion Approximation of the RTE as the model for light propagation in slab geometry, signals arising from light propagation from the source array to the detector array through medium *with* and *without* the targets were calculated. The difference between the two sets, which is the perturbation due to the targets, was used as the “simulated data”. The size of the K matrix is $N \times N = 1681 \times 1681$. The TR matrix $T = KK^T$ was constructed. Then, 1681 eigenvalues and 1681 eigenvectors of T were found.

The first seven (7) computed eigenvalues in a descending order of magnitude are listed in the first column of Table 1. The leading twenty eigenvalues are plotted in Fig. 1(a) on a logarithmic scale. The first six (6) eigenvalues are at least 10 orders-of-magnitude higher than the 7-th and other smaller eigenvalues. Hence, the dimension of the signal subspace and the number of targets are determined to be six. The pseudo spectrum (consisting of $40 \times 40 \times 20 \times 4$ elements) was calculated using the M eigenvectors in the signal subspace. The values of elements in the pseudo spectrum were sorted in a descending order. The seven leading pseudo values are listed in Table 1 with the corresponding positions of voxels. The six peaks are found to be associated with the GFVs for absorptive targets. Namely, the corresponding six targets are identified to be absorptive targets.

All six large pseudo-values are located at the exact known target locations and their values are approximately 9 orders-of-magnitude larger than those at their neighborhood locations. A

2-D slice of the pseudo spectrum on $z = 21$ mm plane is shown in Fig. 1(b), showing the locations of the three difficult targets.

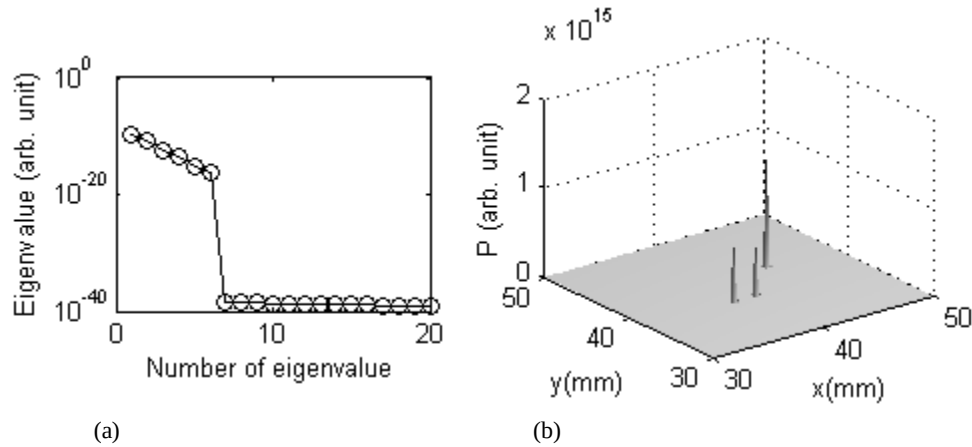


Fig. 1. (a) A plot of first twenty (20) eigenvalues on logarithmic scale. (b) 2-D slice of the pseudo spectrum on $z = 21$ mm plane showing the location of the three difficult targets described in the text. Similar 2-D slices were also obtained for $z = 9$ -mm, 15-mm, and 31-mm planes (not shown).

Table 1. Eigenvalues, pseudo spectrum and the corresponding positions

Leading Eigenvalues	Poles of Pseudo Spectrum	Retrieved Position (x, y, z) mm	Known Position (x, y, z) mm
2.6697E-010	1.5911E + 015	(44, 42, 21)	(44, 42, 21)
1.1722E-011	8.6376E + 014	(38, 38, 15)	(38, 38, 15)
4.0081E-013	7.9559E + 014	(38, 38, 21)	(38, 38, 21)
3.6676E-014	7.2328E + 014	(40, 38, 21)	(40, 38, 21)
5.2629E-016	6.3010E + 014	(24, 26, 9)	(24, 26, 9)
6.4837E-017	2.1159E + 014	(30, 30, 31)	(30, 30, 31)
2.8337E-039	2.4353E + 005	(38, 38, 19)	
...	...		

With the highly encouraging result from simulation even for a considerably challenging task, we proceeded to test the approach for the realistic situation of detecting and locating targets from experimental data.

5. Testing TROT using Experimental Data

5.1 Experimental materials and methods

Three different experiments with three different samples were carried out to test the efficacy of the TROT approach in detecting and locating targets in a turbid medium. All three samples used a 250 mm $\times$ 250 mm $\times$ 60 mm transparent plastic container filled with Intralipid-20% suspension in water as the background medium. The concentration of Intralipid-20% was adjusted to provide an estimated [53,54] absorption coefficient $\mu_a \sim 0.003 \text{ mm}^{-1}$ at 790 nm, and a transport mean free path $l_t \sim 1$ mm, which were similar to the average values of those parameters for human breast tissue, while the cell thickness of 60 mm was comparable to thickness of a typical compressed breast.

In the first experiment, the depth (position along z-axis) of an absorptive target was varied to explore how the accuracy of position estimate depended on depth. The target was a glass sphere of diameter ~ 9 mm filled with ink dissolved in Intralipid-20% suspension in water (μ_s was adjusted to be the same as that of the background medium, while $\mu_a \approx 0.013 \text{ mm}^{-1}$ was about 3 times higher than that of background medium).

In the second experiment, the separation between two absorptive targets was varied to test how close those could be and yet be resolved as separate objects. Both the targets were similar to the target in the first experiment.

In the third experiment, the depth of a scattering target was varied to explore the efficacy of TROT in locating and characterizing a scattering target. The target was a glass sphere of diameter 10 mm filled with Intralipid-20% suspension in water to provide a transport mean free path l_t of 0.25 mm, and a scattering coefficient $\mu_s \approx 11 \text{ mm}^{-1}$.

A multi-source interrogation and multi-detector signal acquisition scheme, shown in Fig. 2, was used to acquire data. A 100-mW 790-nm diode laser beam was used to illuminate the samples. A 1024×1024 pixels charge coupled device (CCD) camera equipped with a 60-mm focal-length camera lens was used on the opposite side of the sample to detect the transmitted light on the boundaries of the slab samples (detector plane). The pixel size was $24 \text{ }\mu\text{m}$. The multi-source illumination scheme was realized by scanning the sample across the laser beam in a two-dimensional x - y array of grid points using a computer-controlled translation stage. The first and third samples were scanned across the laser beam in an array of 9×9 grid points, and the second sample was scanned in an array of 15×11 grid points, with a step size of 5 mm in all cases. The scanning and data acquisition processes were controlled by a personal computer (PC). Raw transillumination images of the sample were recorded by the PC for each scan position, and stored for subsequent analysis. A typical image, which is a 2-D intensity distribution, is shown in the right side of Fig. 2.

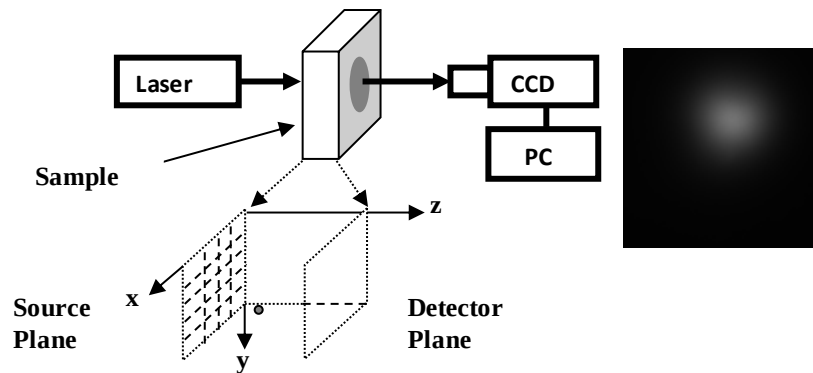


Fig. 2. A schematic diagram of the experimental arrangement for imaging objects embedded in a turbid medium. (Key: CCD = charge coupled device, PC = personal computer) Inset (below) shows the 2-D array in the input plane that was scanned across the incident laser beam, and inset (right) shows a typical raw image.

While we have scanned the sample and kept the source fixed in the experiments reported here, a more clinically relevant approach would be to scan the source and fix the sample. In the experimental arrangement, the source scanning may be accomplished by: (a) delivering the beam using an optical fiber, and translating the delivery end of the fiber in an x - y array using a computer-controlled translation stage; or (b) raster scanning the laser beam using two orthogonal (x - y) galvanometers. The main change in the processing of data would involve alignment of the images so that laser beam positions are overlapped before averaging to generate the background image.

5.2 Data Processing and Analysis

From each image, a region of interest was cropped out and then every 5×5 pixels in the cropped image were binned to one pixel to enhance the signal-to-noise ratio. The background image was generated by taking an average of the original images for all scan positions, which is a reasonable approximation since for most of the scan positions the target(s) is (are) not along the direction of the incident beam. Then the background image was also cropped and binned corresponding to the region of interest for each scan position. Perturbation in the light

intensity distribution $\Delta\phi$ due to targets in each image was found by subtracting the background image from each individual image. The response matrix was constructed using the light intensity perturbations, $-\Delta\phi$. The TR matrix was generated by multiplying the response matrix by its transpose for our continuous-wave (CW) probing scheme. The eigenvalue equation was solved and the signal subspace was selected and separated from the noise subspace. MUSIC was then used to calculate the pseudo spectrum for all voxels in the 3-D space of the sample. For each voxel, four pseudo values, one for absorption and three for scattering as described in Algorithm step (d), were calculated. The voxel size was $0.77 \text{ mm} \times 0.77 \text{ mm} \times 1 \text{ mm}$. By sorting the pseudo spectrum in a descending order, the target(s) were located.

The voxel size to be used in reconstruction and its relation to the target size is an important consideration. In general, smaller voxels provide reconstruction of higher resolution at the cost of increased computational time. Finer details of an extended target may be obtained using smaller voxels. Decreasing the voxel size indefinitely may not improve resolution because of the diffusive nature of light propagation in the turbid medium. However, the computation time increases dramatically, which has been observed by other researchers [55]. The optimal voxel size for a given reconstruction problem will depend on factors, such as, target size, experimental geometry, and noise level.

Since the signal used in image reconstruction is taken to be the difference between the image recorded for a scan position and the background image, estimation of the background image is an important issue. This is a common problem for every diffuse optical imaging modality using perturbation method, and needs further elaboration. We accumulated data in the transillumination slab geometry, and generated the background image by averaging images for all scan positions after proper alignment with respect to the incident source position. This averaging method for generating background image worked well for small targets that we used in our experiments, as the ratio of sample volume to target volume was quite high ($\sim 500:1$). This volume ratio for breast and a tumor in early stages of growth will also be substantially high for the averaging method to be applicable. Assuming a scenario where the volume ratio is substantially smaller than in above examples, a modified approach would be to select recorded images which were minimally affected by embedded targets for averaging [56]. As long as the targets only occupy a limited volume within the host medium, a clean background image can be generated in this fashion. It should also be noted that while estimation of target optical properties, such as absorption coefficient and scattering coefficient, are sensitively dependent on background image estimation, estimation of target positions are not so sensitive.

Several alternative ways of generating background image are suggested in the literature. One experimental approach is to record image using a phantom that has the same average optical properties as the sample, such as human breast [57]. Along the same line, image of the healthy contralateral breast taken under the same experimental conditions as that of the suspect breast may be used as background image for breast imaging [58]. Some authors have suggested acquiring data at a wavelength for which the target(s) and the background have identical optical properties for assessing the background, *e.g.*, measurement using 805-nm light for which hemoglobin and oxyhemoglobin have the same absorption coefficient may serve as background to image hemoglobin oxygenation [59]. Still another approach is to compute the background using an appropriate forward model [18]. Any of these approaches may be employed for generating the background image for use with the TROT formalism presented here.

The geometries commonly used in DOT include slab, cylindrical, hemispherical, and semi-infinite; and different source-detector combinations have been used to record images in these geometries. As long as multiple source-detector combinations provide multiple angular views of the sample the TROT formalism can be adapted to obtain target location for these geometries. TR imaging and TR-MUSIC was originally developed for reflection (backscattering) geometry that used coincident transceiver array to detect the return signal

[28–30,34,35]. With requisite modification in the experimental arrangement TROT would be suited for use in the reflection geometry.

5.3 Results

5.3.1 Single absorptive target at different depths

In the first experiment, only one target was used, the lateral (x, y) position of the target was kept the same at (25.5 mm, 24.7 mm), while seven different depths (position along z -axis) of 15 mm, 20 mm, 25 mm, 30 mm, 35 mm, 40 mm and 45 mm were used. The eigenvalue spectrum plotted on logarithmic scale for the target at $z = 30$ mm is shown in Fig. 3. Similar eigenvalue spectra were obtained for other cases.

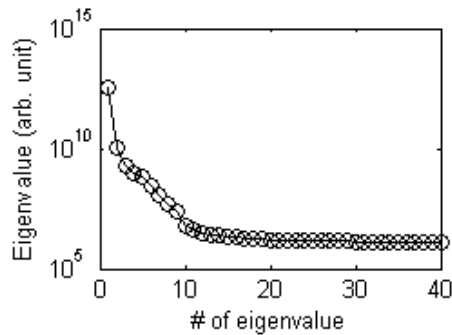


Fig. 3. A semi-log plot of eigenvalue spectrum with first 40 leading eigenvalues for the target at $z = 30$ mm.

Both SDDS and DSSD pseudo spectra were calculated using Eq. (20). The target was identified as an absorptive target. In the DSSD pseudo spectrum, the absorptive pseudo value at the peak position is ~ 41 times of the scattering pseudo value associated with $\partial_z g_d$, and even larger than those associated with $\partial_x g_d$, and $\partial_y g_d$, as shown in Table 2. Similarly in the SDDS scheme, the absorptive pseudo value at the peak position is ~ 33 times of the scattering pseudo value with $\partial_z g_s$, and much larger than the other two.

Table 2. Pseudo values associated with absorptive and scattering components at the peak position

Scheme with GFV (g)	Absorptive pseudo value	Scattering pseudo value		
		$\partial_x g$	$\partial_y g$	$\partial_z g$
DSSD (g_d)	1305.0	1.0	1.0	31.7
SDDS (g_s)	2729.3	14.0	1.1	81.6

Three-dimensional tomographic images were generated using the whole absorption pseudo spectrum for all voxel positions in the sample. The left pane of Fig. 4(a) shows a tomographic image for the target at $z = 30$ mm. The spatial profiles in the x, y and z directions, shown in the right pane of Fig. 4(a) were used to assess the target location. Similar images were generated for other depths. The retrieved target positions are compared with known positions in Table 3.

As is evident from Table 3, when DSSD scheme was used, the TROT-assessed lateral positions (x, y) were within 0.6 mm of the known values, which is an excellent agreement. The accuracy of the z -position was found to be optimal when the target was located in the middle plane of the sample, and deteriorated when the target was closer to the source plane or the detection plane. When using SDDS scheme, the TROT-assessed lateral positions were also within 0.6 mm of the known positions, except for $z = 40$ mm and 45 mm, where the error in y direction was 1.2 mm and 2 mm, respectively. However, remarkable improvement in the accuracy of the z -position estimation was observed, the error Δz being within 0.5 mm for all cases except for $z = 35$ mm, where the error was 1.5 mm. We ascribe the superior

performance of the scheme using T_{SDDS} , to the much larger size of the detector array (1024×1024) than that of the source array (9×9) used in the experimental arrangement.

Table 3. Positions of one target located at different depths

Known Positions x, y, z (mm)	DSSD Scheme		SDDS Scheme	
	Retrieved Positions x, y, z (mm)	Error $\Delta x, \Delta y, \Delta z$ (mm)	Retrieved Positions x, y, z (mm)	Error $\Delta x, \Delta y, \Delta z$ (mm)
25.5, 24.7, 15	24.9, 24.4, 17.5	0.6, 0.3, 2.5	24.9, 25.2, 15.5	0.6, 0.5, 0.5
25.5, 24.7, 20	25.7, 24.4, 21.5	0.2, 0.3, 1.5	24.9, 25.2, 20.5	0.6, 0.5, 0.5
25.5, 24.7, 25	25.7, 24.4, 26.5	0.2, 0.3, 1.5	25.7, 24.4, 24.5	0.2, 0.3, 0.5
25.5, 24.7, 30	25.7, 24.4, 30.5	0.2, 0.3, 0.5	25.7, 25.2, 29.5	0.2, 0.5, 0.5
25.5, 24.7, 35	25.7, 25.2, 33.5	0.2, 0.5, 1.5	24.9, 24.4, 36.5	0.6, 0.3, 1.5
25.5, 24.7, 40	24.9, 25.2, 36.5	0.6, 0.5, 3.5	24.9, 25.9, 40.5	0.6, 1.2, 0.5
25.5, 24.7, 45	24.9, 25.2, 39.5	0.6, 0.5, 5.5	24.9, 26.7, 45.5	0.6, 2.0, 0.5

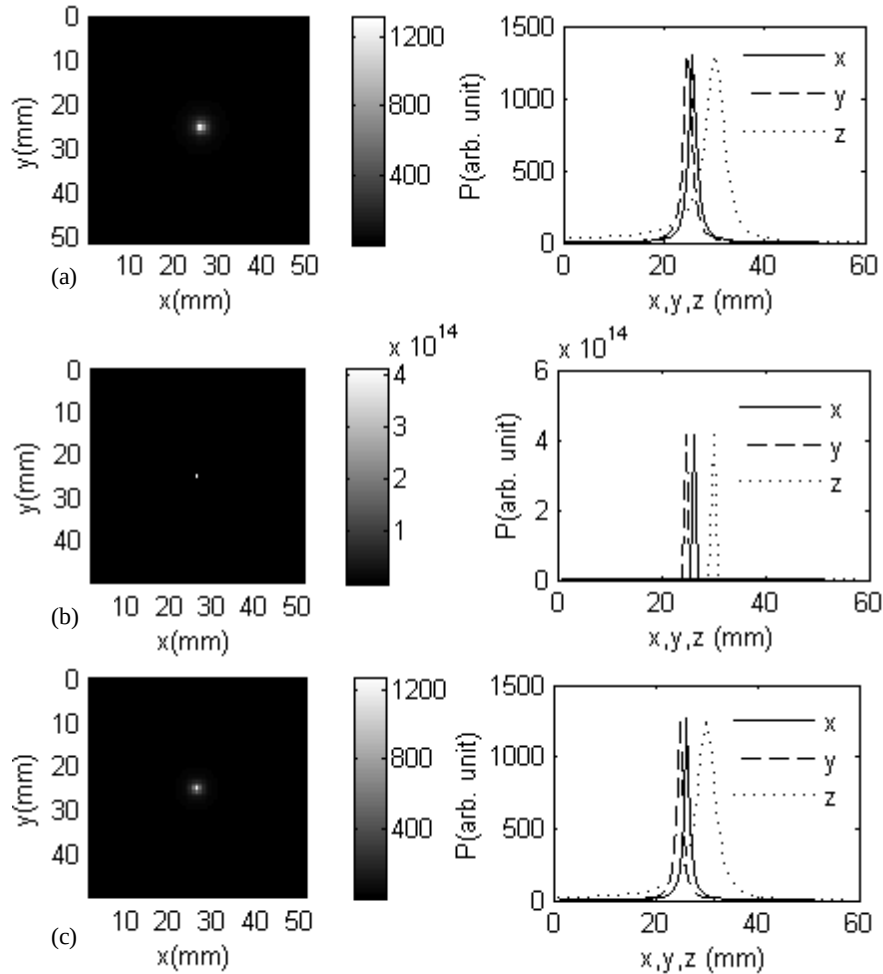


Fig. 4. Pseudo image of the target (left pane) and corresponding spatial intensity profiles (right pane) when the target is located at $z = 30$ mm: (a) experimental data; (b) simulation without any added noise; and (c) simulation with 20% Gaussian noise added. The pseudo values are calculated using Eq. (20).

It should be noted that the choice of either DSSD or SDDS scheme depends on experimental parameters, such as, the number and density of sources and detectors, and does not depend on the characteristics of the background medium. When more detectors than

sources are used and inter-detector spacing is small, SDDS would provide better resolution than DSSD, and vice versa. However, due to the diffusive nature of light propagation in the turbid medium, increasing the numbers and decreasing the spacing of the sources/detectors beyond a limit may not always improve the results.

While the target position could be obtained from the experimental data, it was observed that the difference between the smaller eigenvalues in the signal subspace and the larger eigenvalues in the noise subspace were not as pronounced as observed in the simulation in Section 4. To assess the effect of noise and to what extent noise may be present in the experimental data; we generated simulated data mimicking the experimental conditions, and added different noise levels. The lateral positions were (25.5 mm, 24.7 mm) and all seven z -positions (depth) of 15 mm, 20 mm, 25 mm, 30 mm, 35 mm, 40 mm, and 45 mm were tested. Typical pseudo images generated for $z = 30$ mm without and with 20% Gaussian multiplicative noise to compare with the experimental result are shown in Fig. 4(b) and Fig. 4(c), respectively. Simulated data provided the known position coordinates.

The simulated spatial profiles with zero added noise are much sharper than the profiles obtained from experimental data, or from simulated data with 20% added Gaussian noise. Broadening of spatial profile is an indication of the uncertainty in determination of position coordinates. Results from simulation show that uncertainty in position determination increases with added noise, and that experimental data behave in a way similar to simulated data with added noise.

5.3.2 Resolving two absorptive targets

In the second experiment using two targets the depth (z) and height (y) were kept same ($z = 30$ mm, $y = 26.0$ mm), while three different center-to-center separations, Δx of ~ 12.6 mm, 17.6 mm, and 27.6 mm, between them along the x -axis were considered. A cross-sectional pseudo image of the targets when separated by a center-to-center distance of 27.6 mm, generated using the pseudo spectrum is shown in the left pane of Fig. 5(a). Figure 5(b) shows a similar image for the separation 12.6 mm (separation between nearest edges ~ 4 mm). A similar image for the separation 17.6 mm was also obtained (not shown in the figure). The profiles in the x , y and z directions through the right target are shown in the right panes of Fig. 5(a)–Fig. 5(c). These profiles were used to assess locations of the targets, and the separation between the two targets. In all cases, the targets were determined to be absorptive, because peaks occurred in the pseudo spectrum with the GFVs corresponding to absorption property.

The known and retrieved positions from the experiments and separations Δx between the two targets appear in Table 4. In all the cases, the two targets were resolved, even when their center-to-center separation was 12.6 mm apart, nearest sides separated by only ~ 4 mm. For all retrieved positions, the maximum error in the lateral positions is 3.0 mm, and the maximum error in the axial positions is 1.5 mm. The errors in the lateral positions increase as the targets get closer. We ascribe this increase in error in the lateral position to the crosstalk between the two targets, the peak due to one target being affected by the other. The shift in the peaks is also affected by noise. When the two targets are very close or significant noise is present, the two peaks merge, so that the two targets are not resolved. This behavior was confirmed in simulations.

The results were compared with simulated data using similar conditions. For the more challenging case of two targets located at $z = 30$ mm and separated by 12.6 mm, exact target locations were found when no noise was added. With 10% noise present, the positions of the two targets were found to be (39.0 mm, 24.8 mm, 29.0 mm) and (30.0 mm, 24.8 mm, 29.0 mm) with target separation 9.0 mm, compared to 12.6 mm (known) and 6.9 mm retrieved from experiment. The pseudo image and the corresponding profiles through the right target are shown in Fig. 5(c). Similar images were also obtained for the left target. The retrieved separation between the two targets in simulation with 10% noise was smaller than the actual separation. But the error was less than the experimental result. However, when 20% noise was added, the two peaks merged (not shown here).

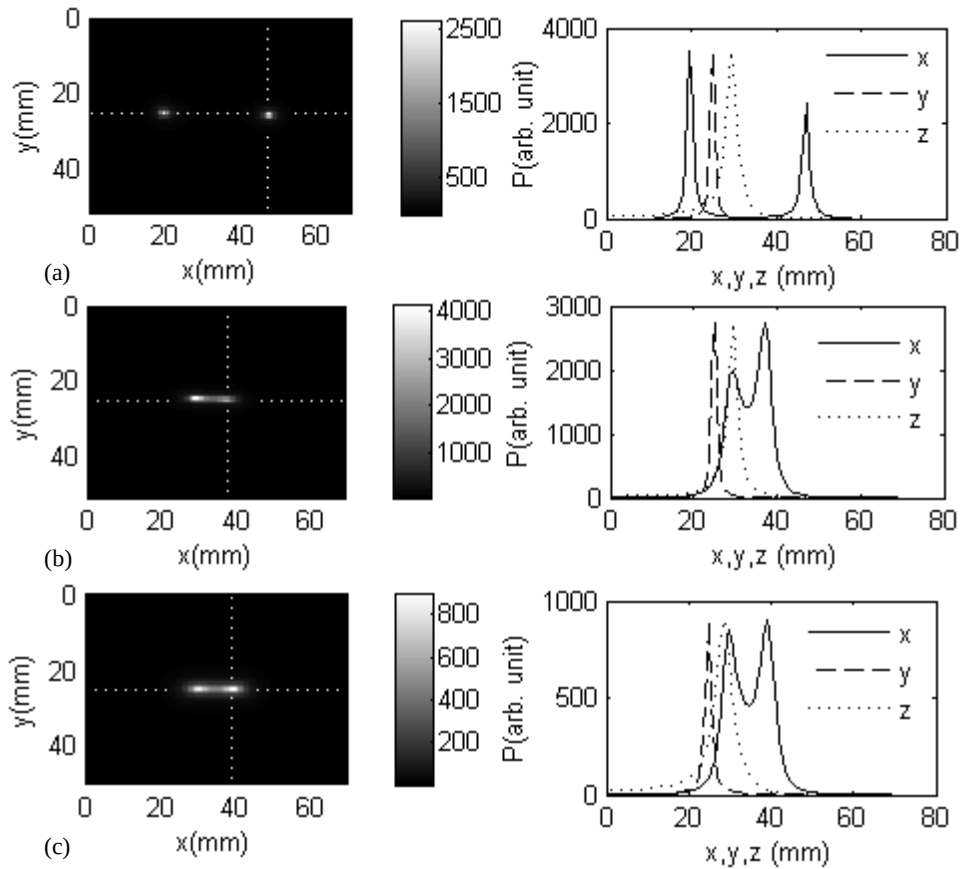


Fig. 5. (a) (Experiment): TROT generated cross-section pseudo image when the targets are separated by 27.6 mm is shown in the left pane and pseudo-value profiles through the right target along x , y and z directions are shown in the right pane. (b) (Experiment): TROT generated cross-section pseudo image when the targets are separated by 12.6 mm is shown in the left pane and the corresponding spatial profiles through the right target along x , y and z directions are shown in the right pane. (c) (Simulation): TROT generated cross-section pseudo image when two targets are separated by 12.6 mm is shown in the left pane and the corresponding pseudo-value profiles are plot in the right pane. In simulation 10% Gaussian noise is added for comparison with the experimental results. P is pseudo value calculated using Eq. (20).

Table 4. Positions of two targets separated with different distances

Known Separation [Δx (mm)]	Target #	Known Position [x, y, z (mm)]	Retrieved Position [x, y, z (mm)]	Error (mm)	Retrieved Separation [Δx (mm)]
12.6	1	27.6, 26.0, 30	30.3, 24.4, 28.5	2.7, 1.6, 1.5	6.9
	2	40.2, 26.0, 30	37.2, 25.2, 29.5	3.0, 0.8, 0.5	
17.6	1	25.1, 26.0, 30	26.4, 24.4, 28.5	1.3, 1.6, 1.5	14.6
	2	42.7, 26.0, 30	41.0, 25.2, 29.5	1.7, 0.8, 0.5	
27.6	1	20.1, 26.0, 30	19.5, 25.2, 29.5	0.6, 0.8, 0.5	27.6
	2	47.7, 26.0, 30	47.1, 25.2, 30.5	0.6, 0.8, 0.5	

The limits on the size of targets, separation between the targets, and the target-to-background contrast ratio that are needed to detect and locate the targets depend on noise level, experimental parameters (such as, number and concentration of sources and detectors), and ultimately on the diffuse nature of light propagation in the turbid medium. Coordinated experimental work and numerical modeling will be needed to assess those limits.

5.3.3 Single scattering target at different depths

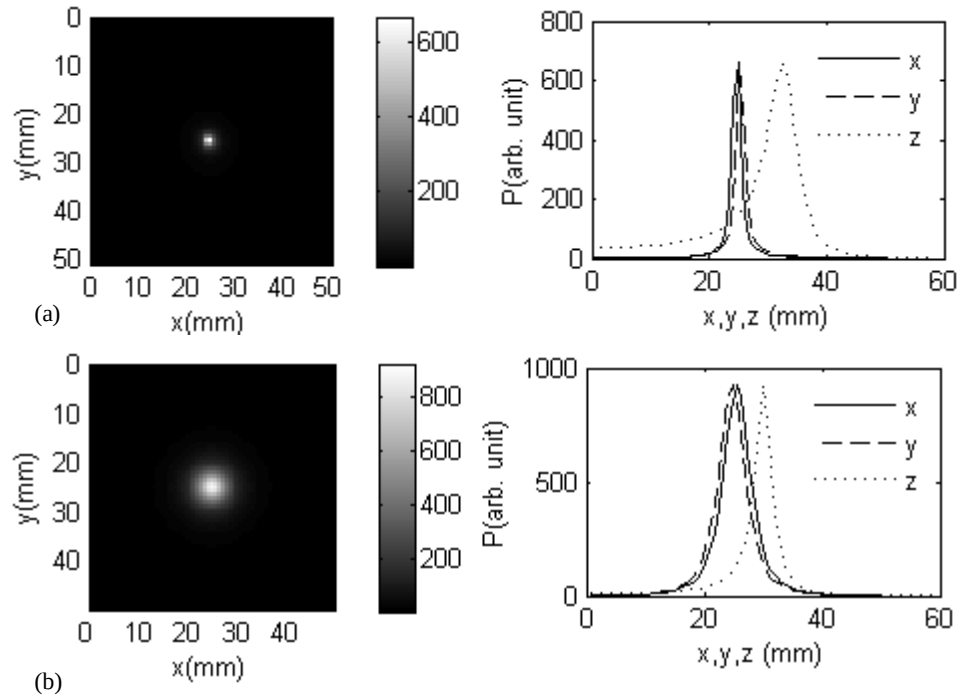


Fig. 6. Pseudo image of the target (left pane) and corresponding spatial intensity profiles (right pane) when the target is located at $z = 30$ mm: (a) experimental data; (b) simulation with 20% Gaussian noise added. P is pseudo value calculated using Eq. (20).

The experiment involving the third sample is the same as the first one except that the target was scattering in nature. The scattering target was a 10-mm diameter glass sphere filled with Intralipid-20% suspension in water, whose concentration was adjusted to provide $l_t = \sim 0.25$ mm, $\mu_s = 11.3$ mm⁻¹. The same scanning and data acquisition scheme was used as for the absorptive targets and the following z -positions of the target were used: 15 mm, 20 mm, 25 mm, 30 mm, 35 mm, 40 mm, and 45 mm. DSSD scheme was used to calculate the pseudo spectrum. A cross-section pseudo image and the corresponding spatial profiles are displayed in Fig. 6(a) when $z = 30$ mm. It is compared to the simulation results with 20% Gaussian noise (Fig. 6(b)). The lateral (x, y) spatial profiles of the pseudo image generated using simulated data are considerably wider, while the axial (z) spatial profile is narrower than those obtained using experimental data, and the peak values from the two cases are of the same order. The retrieved target positions are listed in Table 5. SDDS scheme was also used and provided with similar results.

Table 5. Positions of one scattering target located at different depths

Known Positions [x, y, z (mm)]	Retrieved Positions [x, y, z (mm)]	Error [$\Delta x, \Delta y, \Delta z$ (mm)]
25.7, 24.5, 15	24.9, 25.9, 18.5	0.8, 1.4, 3.5
25.7, 24.5, 20	27.2, 26.7, 20.5	1.5, 2.2, 0.5
25.7, 24.5, 25	25.7, 26.7, 23.5	0.0, 2.2, 1.5
25.7, 24.5, 30	24.9, 25.2, 32.5	0.8, 0.7, 2.5
25.7, 24.5, 35	24.9, 25.2, 36.5	0.8, 0.7, 1.5
25.7, 24.5, 40	24.9, 25.9, 41.5	0.8, 1.4, 1.5
25.7, 24.5, 45	24.9, 25.9, 45.5	0.8, 1.4, 0.5

In Fig. 5, and more prominently in Fig. 6, the image resolution seems better for experimental data than simulated data. Since the peak values and bandwidth of lines (the

poles) in the pseudo spectrum depend strongly on the noise, this difference in image resolution is presumably due to lower noise level in the experiments than that used in simulations.

A comparison of experimental results for scattering and absorptive targets validate the common notion that it is more challenging to locate and image scattering targets than absorptive targets in a highly scattering medium. Also the lateral (x , y) positions are determined with higher accuracy than the axial (z) position. Overall the TROT-retrieved target positions are in good agreement with the known positions.

6. Discussion

The article presents the development of time reversal imaging approach with subspace classification, MUSIC in the optical domain. The results from experiment and simulation show that TROT is a faster and less computation intensive approach for detecting small targets in highly scattering turbid media and determining their locations in 3-D than other inverse image reconstruction techniques. While the dominant features in the pseudo spectrum are related to the square of the difference between the absorption (scattering) coefficient of the targets and that of the background, the approach does not directly determine these parameters. It is common for IIR approaches to estimate the optical properties of every voxel in the sample and identify target(s) from differences of these properties between the sample and the target(s), which is a considerably computation intensive undertaking. On the contrary, TROT identifies the targets as poles of the pseudo spectrum and focuses on determining their positions, which do not require as much computation time. Other IIR approaches involve iteration, while TROT is non-iterative. In TROT the data dimension is lower compared to other IIR approaches, which enables analysis and utilization of very large data sets. These two features together make TROT faster. Fast image reconstruction algorithms are of particular interest.

It was observed that lateral (x , y) positions are better determined than the depth (z). Also the spatial profile is more spread out along z compared to that along x , y . We ascribe this difference to fewer data along z -direction compared to those along x - y planes. Addition of another set of data with light incident and signal collected perpendicular to the z -direction is expected to further improve resolution in this dimension. Even without that addition, TROT determines the target position well.

While we have used slab geometry and CW illumination, the TROT approach may be used for other geometries (such as, cylindrical, and spherical), different types of illumination (e.g. frequency domain and pulsed) and different models for light propagation through the medium. Application and adaption of the TROT formalism to inhomogeneous media and extended targets may require careful consideration of several factors. In a non-uniform, inhomogeneous medium, structures other than the desired targets may appear as “false targets” and may interfere with identification of “real targets”. However, as long as the contributions to the signal by any false target is smaller than that made by real targets, TROT with MUSIC will be useful in detecting and locating targets, by choosing a proper threshold to separate the signal and noise subspaces. What is even more important, expected wavelength dependence of the target spectroscopic properties could be used to assess the difference between the real and false targets in experiments using multi-wavelength interrogation of the sample.

The TROT formalism presented in this article is particularly suited for point-like targets requiring fewer eigenvectors in the signal subspace to construct a pseudo spectrum. However, for extended finite-size targets, the formalism needs to be modified and much more eigenvectors may be needed to calculate the pseudo spectrum [40,60,61]. These interesting problems for further study are currently being pursued.

Acknowledgements

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Three dimensional time reversal optical tomography

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ABSTRACT

Time reversal optical tomography (TROT) approach is used to detect and locate absorptive targets embedded in a highly scattering turbid medium to assess its potential in breast cancer detection. TROT experimental arrangement uses multi-source probing and multi-detector signal acquisition and Multiple-Signal-Classification (MUSIC) algorithm for target location retrieval. Light transport from multiple sources through the intervening medium with embedded targets to the detectors is represented by a response matrix constructed using experimental data. A TR matrix is formed by multiplying the response matrix by its transpose. The eigenvectors with leading non-zero eigenvalues of the TR matrix correspond to embedded objects.

The approach was used to: (a) obtain the location and spatial resolution of an absorptive target as a function of its axial position between the source and detector planes; and (b) study variation in spatial resolution of two targets at the same axial position but different lateral positions. The target(s) were glass sphere(s) of diameter ~ 9 mm filled with ink (absorber) embedded in a 60 mm-thick slab of Intralipid-20% suspension in water with an absorption coefficient $\mu_a \sim 0.003 \text{ mm}^{-1}$ and a transport mean free path $l_t \sim 1$ mm at 790 nm, which emulate the average values of those parameters for human breast tissue. The spatial resolution and accuracy of target location depended on axial position, and target contrast relative to the background. Both the targets could be resolved and located even when they were only 4-mm apart. The TROT approach is fast, accurate, and has the potential to be useful in breast cancer detection and localization.

Keywords: Time reversal, MUSIC, diffusive optical imaging, optical tomography, biomedical imaging, breast cancer imaging, scattering medium, diffusion approximation

1. INTRODUCTION

Optical imaging of targets embedded in turbid media, such as a tumor in a breast, has attracted much attention in the last two decades. When a beam of light propagates through a highly scattering medium, photons are scattered and diffused into a broad area; phase coherence and polarization of light deteriorate; short pulses broaden; and consequently sharp images of the targets cannot be formed directly. Various algorithms have been developed to perform image reconstruction¹⁻⁴. Inverse image reconstruction (IIR) is an ill-posed problem and search of reliable and fast approaches is an important and formidable task for optical imaging of human tissue such as breast. Recent inverse algorithms, such as Newton-Raphson-Marquart algorithms⁵ and direct linear inversion of 3-D matrices⁶, are time consuming. The iterative methods^{6,7} may not ensure that the obtained result arrives at a “global minimum” or converge to a “local minimum”.

Time reversal optical tomography (TROT)^{9,10} was introduced as a reconstruction method for imaging targets in turbid media non-invasively using light, following the development of TR imaging using multistatic radar signal⁸. Compared to other inverse methods which usually require iterations⁴, TROT is fast since there is no iteration involved. The signals and noise are separated into orthogonal subspaces. A method similar to L-curve regularization is used to select the signal subspace. Then a pseudo spectrum is calculated directly for all voxels in the sample using the vector subspace method, MULTiple SIGNAL Classification (MUSIC)⁸⁻¹⁰. Tomographic pseudo images can be generated using pseudo values. Locations and characteristics of targets are determined by the global maximum (or local maximum in low signal-to-noise (SNR) cases) components in the pseudo spectrum.

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This paper is organized as follows. In section 2, the formalism of the TROT approach is presented. In section 3, the experimental arrangement, materials and parameters are described. In section 4, the TROT analysis on the experimental data and results are presented. Section 5 serves as discussion and summary.

2. FORMALISM

Using diffusion approximation of the radiative transfer equation (RTE), the scattered light due to small weak inhomogeneities (targets) embedded in a homogeneous medium to the first order Born approximation can be written as⁴

$$\phi_{sca}(\mathbf{r}_d, \mathbf{r}_s) = - \int G(\mathbf{r}_d, \mathbf{r}) \delta\mu_a(\mathbf{r}) c G(\mathbf{r}, \mathbf{r}_s) d^3\mathbf{r} - \int \delta D(\mathbf{r}) c \nabla_r G(\mathbf{r}_d, \mathbf{r}) \cdot \nabla_r G(\mathbf{r}, \mathbf{r}_s) d^3\mathbf{r} , \quad (1)$$

where $\mathbf{r}_s$, $\mathbf{r}_d$, and $\mathbf{r}$ are the positions of a point-like source of unit power, detector and target, respectively; $G(\mathbf{r}, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r})$ are the Green functions that describe light propagations from the source to the target and from the target to the detector, respectively; $\delta\mu_a$ and δD describe the differences of absorption and scattering properties between the targets and the background medium, respectively; and c is the light speed in the medium. For an absorptive target, a matrix form of the response matrix K is constructed as $\{K_{ij}\} = \{\sum_{m=1}^M G^d(\mathbf{r}_i, \mathbf{X}_m) \tau_m G^s(\mathbf{X}_m, \mathbf{r}_j)\}$, ($i = 1, 2, \dots, N_d$; $j = 1, 2, \dots, N_s$), where $\mathbf{r}_i$, $\mathbf{r}_j$ and $\mathbf{X}_m$ are locations of the i^{th} detector, j^{th} source and m^{th} target, respectively; N_s , N_d and M are the numbers of sources, detectors and targets, respectively. It is assumed the number of targets is less than the number of sources and detectors, $M < \min(N_d, N_s)$.

A multi-source interrogation and multi-detector acquisition scheme is used to acquire multistatic transillumination data, from which the scattered light due to the targets is found, $\phi_{sca} = \phi - \phi_0$, where ϕ is the light intensity measured on the boundary with targets embedded in the scattering medium and ϕ_0 is the background image without targets embedded, which can be approximated by an “average” of all acquired images. Thus, the response matrix K is constructed with perturbations of spatial intensity distributions on the boundary.¹¹

A time reversal matrix T is then constructed as $T = KK^\dagger$ (or $T = KK^T$ for continuous wave (CW)), which is similar to the time reversal matrix used in the time reversal by Devaney⁸⁻¹¹. Eigenvalues λ_j and eigenvectors u_j of T are found, where $j = 1, 2, \dots, \min(N_d, N_s)$. The leading eigenvalues correspond to the targets. The vector subspace method, MUSIC is used to determine the locations of hidden objects. A Green's function vector on the detector array $g_d(\mathbf{X}_p)$ associated with a test target position $\mathbf{X}_p$ at p^{th} voxel is calculated using diffusion model, where $g_d(\mathbf{X}_p) = [G^d(\mathbf{r}_1, \mathbf{X}_p), G^d(\mathbf{r}_2, \mathbf{X}_p), \dots, G^d(\mathbf{r}_{N_d}, \mathbf{X}_p)]^T$ and the Green's functions $G^d(\mathbf{r}_j, \mathbf{X}_p)$, $j = 1, 2, \dots, N_d$, describe light propagation from the test target position $\mathbf{X}_p$ to detectors at $\mathbf{r}_j$. Then the eigenvectors u_j corresponding to the leading eigenvalues are used to calculate the MUSIC type pseudo spectrum using the following formula^{8,9}:

$$P(\mathbf{X}_p) = \frac{|g_d(\mathbf{X}_p)|^2}{|g_d(\mathbf{X}_p)|^2 - \sum_{\lambda_j \leq \epsilon} |u_j^\dagger g_d(\mathbf{X}_p)|^2}, \quad (2)$$

where ϵ is the threshold determined using an L-curve method to separate the signal and noise subspaces. When both absorption and scattering properties are considered¹², one Green's function vector associated with absorption property and constructed with Green's functions G^d and three Green's function vectors associated with scattering property constructed with $\partial G^d/\partial x$, $\partial G^d/\partial y$ and $\partial G^d/\partial z$, respectively are used to calculate the pseudo spectrum at each voxel. Hence, four pseudo values are obtained at each voxel, one for absorption, and three for scattering.

A maximum value of $P(\mathbf{X}_p)$ is obtained when $\mathbf{X}_p$ is the position of one of the hidden objects. By sorting pseudo values $P(\mathbf{X}_p)$, the positions of the embedded objects are determined. At the same time, absorptive and scattering objects are distinguished according to the type of the Green's function vector $g_d(\mathbf{X}_p)$ associated with maximum pseudo value.

In this MUSIC procedure, only a single run for calculating the pseudo spectrum over voxels is required, without iterative procedure used in the traditional inverse approach.

3. EXPERIMENTAL METHODS AND MATERIALS

Two different samples were used to test the efficacy of the TROT approach in two different experiments. Both samples used a 250 mm × 250 mm × 60 mm transparent plastic container filled with Intralipid-20% suspension in water. The concentration of Intralipid-20% was adjusted to provide an absorption coefficient $\mu_a \sim 0.003 \text{ mm}^{-1}$ at 790 nm, and a transport mean free path $l_t \sim 1 \text{ mm}$, which were similar to values of those parameters for human breast tissue. In the first sample, the depth (position along z -axis) of an absorptive target was varied to explore how the accuracy of position estimate depended on depth. In the second sample, the separation between two absorptive targets was varied to test how close those could be and yet be resolved as separate objects. The target(s) were glass sphere(s) of diameter 8-9 mm filled with ink dissolved in Intralipid suspension in water (μ_s was adjusted to be the same as that of the background medium, $\mu_a = 0.013 \text{ mm}^{-1}$ which was about 3 times higher than that of background medium).

A multi-source interrogation and multi-detector acquisition scheme was used to acquire data. A 100-mW 790-nm diode laser beam was used to illuminate the samples and scan the source plane with a 5 mm step-size. A charge coupled device (CCD) camera was used on the other side of the sample to detect the transmitted light on the boundaries of the slab samples (detector plane). The first sample was scanned with the laser beam in an array of 9×9 grid points, and the second sample was scanned in an array of 11×15 grid points. The scanning and acquisition process was controlled by a computer (PC). A schematic diagram of the experimental setup is shown in Fig. 1.

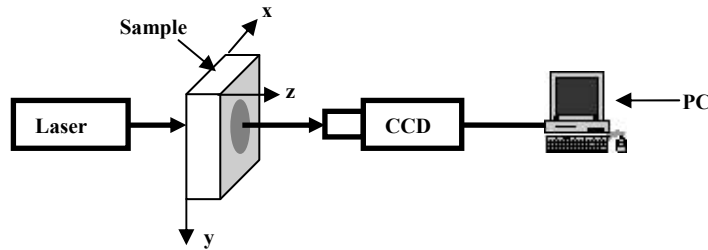


Fig. 1. A schematic diagram of the experimental arrangement used for imaging objects embedded in a turbid medium.

4. ANALYSIS AND RESULTS

One whole dataset for each experiment is of order 10^8 source-detector pairs, considering each pixel in a CCD camera as a detector. From each image, a region of interest was cropped out and then every 5×5 pixels in the cropped image were binned to one pixel. Light intensity perturbation due to targets in each image was found by subtracting the background image from each individual image. The response matrix was constructed using the light intensity perturbations. The TR matrix was generated by multiplying the response matrix by its adjoint matrix (transpose for continuous-wave (CW) illumination). The eigenvalue equation was solved and signal subspace was selected and separated from the noise subspace. MUSIC was then used to calculate the pseudo spectrum for every voxel in the 3D space of the sample. For each voxel, one absorption and three scattering components were calculated. The voxel size was 0.77 mm × 0.77 mm × 1 mm. By sorting the pseudo spectrum, the object(s) were located.

In the first experiment using only one target, the lateral (x, y) position of the target was kept the same at (25.5 mm, 24.7 mm), while five different depths (position along z -axis) of 15 mm, 20 mm, 25 mm, 30 mm, 35 mm, 40 mm and 45 mm were used. A cross-sectional pseudo image was generated using the pseudo spectrum for all voxel positions in the sample. The spatial profiles in the x, y and z directions of the images through the targets (a typical image and profiles for the two-target case are shown in Fig. 2) are used to assess target location. The retrieved target positions are compared with known positions in Table 1.

Table 1. Positions of one target located at different depths

Known Positions [x, y, z (mm)]	Retrieved Positions [x, y, z (mm)]	Error (mm)
25.5, 24.7, 15	24.9, 24.4, 17.5	0.6, 0.3, 2.5
25.5, 24.7, 20	25.7, 24.4, 21.5	0.2, 0.3, 1.5
25.5, 24.7, 25	25.7, 24.4, 26.5	0.2, 0.3, 1.5
25.5, 24.7, 30	25.7, 24.4, 30.5	0.2, 0.3, 0.5
25.5, 24.7, 35	25.7, 25.2, 33.5	0.2, 0.5, 1.5
25.5, 24.7, 40	24.9, 25.2, 36.5	0.6, 0.5, 3.5
25.5, 24.7, 45	24.9, 25.2, 39.5	0.6, 0.5, 5.5

The TROT assessed positions were in good agreement with the known positions. The accuracy of the z -position was found to be optimal when the target was located in the middle plane of the sample, and deteriorated when the target was closer to the source plane or the image plane.

In the second experiment using two targets the depth was kept fixed ($z = 30$ mm), while the separation between them separated with distances of ~ 11.8 mm, 16.8 mm, and 26.8 mm, in the x direction. A cross-sectional pseudo image of the targets when separated by a center-to-center distance of 11.8 mm (separation between nearest edges ~ 4 mm), generated using the pseudo spectrum are shown in the left pane of Fig. 2. Similar images for other target separations were also obtained. The profiles in the x , y and z directions through the right target are shown in the right pane of Fig. 2. These profiles were used to assess locations of the targets, and the separation between the two targets. In all cases, the targets were determined to be absorptive, because peaks occurred in the pseudo spectrum with the test Green's function vector corresponding to absorption property.

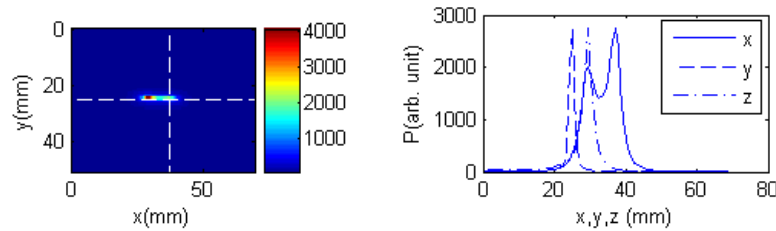


Fig. 2. TROT generated cross-section pseudo image when the targets are separated by 11.8 mm is shown in the left pane and pseudo-value profiles through the target along x , y and z directions are shown in the right pane.

The known and retrieved positions and separations Δx between of the two targets appear in Table 2. In all the cases, the two targets were resolved, even when their center-to-center separation was 11.8 mm apart, nearest sides separated by only ~ 4 mm. For all retrieved positions, the maximum error in the lateral positions is 3.0 mm, and the maximum error in the axial positions is 1.5 mm. The errors in the lateral positions increase as the targets get closer.

Table 2. Positions of two targets separated with different distances

Known Separation [Δx (mm)]	Obj. #	Known Position [x, y, z (mm)]	Retrieved Position [x, y, z (mm)]	Error (mm)	Retrieved Separation [Δx (mm)]
11.8	1	27.6, 26.0, 30	30.3, 24.4, 28.5	2.7, 1.6, 1.5	6.9
	2	40.2, 26.0, 30	37.2, 25.2, 29.5	3.0, 0.8, 0.5	
16.8	1	25.1, 26.0, 30	26.4, 24.4, 28.5	1.3, 1.6, 1.5	14.6
	2	42.7, 26.0, 30	41.0, 25.2, 29.5	1.7, 0.8, 0.5	
26.8	1	20.1, 26.0, 30	19.5, 25.2, 29.5	0.6, 0.8, 0.5	27.6
	2	47.7, 26.0, 30	47.1, 25.2, 30.5	0.6, 0.8, 0.5	

Similar experiments were also done when the target had a higher concentration of ink corresponding to a greater absorption coefficient. In that case, smaller errors occurred in the retrieved position which should be due to higher signal-to-noise ratio.

5. SUMMARY AND DISCUSSIONS

The work presented above demonstrate that TROT is effective in obtaining three-dimensional location information of a single absorptive target at different axial locations, and two absorptive targets separated by different distances along a lateral direction. The known lateral positions of the targets were retrieved within ~ 2 mm for breast tissue simulating samples. The accuracy for assessing the axial position (depth) depended on the target location. High accuracy was obtained for target in the mid-plane, while the accuracy deteriorated as the target was moved towards the source plane or the detector plane. Breast tumors commonly grow further from the breast surface, and the TROT approach is expected to be effective in locating those even at an early stage.

The experiments presented in this article were carried out for samples in slab geometry. The approach can be adapted to cylindrical geometry as well. While the results presented here are for absorptive target(s), our preliminary work show that TROT will be effective for scattering targets as well, and we are in the process of developing it for scattering targets, as well as, for targets that are both scattering and absorptive in nature. Our experiments used continuous-wave light, but the approach would work with frequency domain and time-resolved data as well.

One important difference between the sample used in our experiments and human breast is that in our sample the intervening medium can be treated as uniform, while human breast is not uniform. The pseudo spectrum is calculated using the known Green's function of the background medium. When the medium is non-uniform, Green's function for a uniform medium is used as an approximation.¹³

The approach is fast as no iteration is involved. The code used to perform the computation is written in Matlab. With pre-processed data, a typical time to perform the 3D image reconstruction ($5 \text{ cm} \times 5 \text{ cm} \times 6 \text{ cm}$) with a sub-millimeter resolution using a Pentium 2GHz CPU processor and 2GB memory, is approximately 10 minutes. The computation time can be further reduced by improving our algorithm, and using C/C++ code.

In summary, the TROT approach based on the concept of time reversal and using MUSIC shows promise to locate objects in turbid media, such as a tumor in human breast with useful accuracy.

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Multi-wavelength diffusive optical tomography using Independent Component Analysis and Time Reversal algorithms

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ABSTRACT

Optical imaging using independent component analysis (OPTICA) and time reversal optical tomography (TROT) approaches are used to detect, locate, and obtain cross-section images of two tumor pieces inside a model human breast assembled using *ex vivo* human breast tissues and configured as a semi-cylindrical slab of uniform thickness. The experimental arrangement realized a multi-source probing scheme to illuminate an end face (source plane) of the slab sample using 750 nm, 800 nm and 830 nm beams of laser light. A multi-detector signal acquisition scheme measured transmitted light intensity distribution on the other end face (detection plane). This combined multi-source probing and multi-detector sensing approach culminated in multiple spatial and angular views of the sample necessary for target localization. The perturbations in light intensity distribution in the detection plane were analyzed using both the OPTICA and the TROT approaches to obtain locations of the tumor pieces. A back-projection technique with OPTICA provided cross-section images and estimates of cross section of the targets within the sample. The estimated locations and dimensions of targets are in good agreement with the results of a corroborating magnetic resonance imaging experiment and known values.

Keywords: Medical and biological imaging, Image reconstruction techniques, Light propagation in tissues, Inverse problems, Three-dimensional image processing, Tomography, Breast cancer, Magnetic resonance imaging, Independent component analysis.

1. INTRODUCTION

Optical detection of targets in a turbid medium makes use of the difference in optical properties, such as, scattering coefficient, absorption coefficient, index of refraction, and fluorescence between the targets of interest and the intervening medium [1]. Multiple scattering of light by the turbid medium produces a noise background that blurs the image, and in severe cases makes direct imaging impossible. Inverse image reconstruction approaches that are commonly used to retrieve image information have to deal with the fact that inverse problems are ill posed, and attain different measures of success [2].

We are developing the optical tomography using independent component analysis (OPTICA) [3-5], and time reversal optical tomography (TROT) [6-8] approaches for detecting and obtaining three-dimensional location information of target(s) in highly scattering turbid media in general, and of tumor(s) in human breast, in particular. In this paper we present the results of our study of a “realistic model cancerous breast” formed using *ex vivo* human breast tissues with two pieces of tumors embedded within. We use a multi-source, multi-wavelength probing and multi-detector signal acquisition scheme, and analyze the resulting data using both OPTICA and TROT approaches to obtain images and locations of the tumor pieces. These results are compared with magnetic resonance imaging measurements as reference.

2. EXPERIMENTAL METHODS AND MATERIALS

The experimental arrangement for detecting and locating tumors in the realistic model breast using OPTICA and TROT is shown schematically in Fig. 1. The model breast was assembled using two pieces of normal *ex vivo* female human breast tissues. Two pieces of cancerous tissues were sandwiched at different locations within the two normal pieces. The normal breast tissue specimens weighed 119 grams and 127 grams and consisted primarily of adipose tissue, while each tumor (infiltrating ductal carcinoma) piece weighed approximately 1 gram. The sample was placed inside a cylindrical

transparent plastic container of diameter 110 mm with a movable end, which was moved to slightly compress the tissue along the z-axis and hold it in place. The tumor piece located at the left side of the sample ('left tumor') was of the size of 8 mm × 5 mm × 3 mm and that located on the right side ('right tumor') was of the size 10 mm × 10 mm × 5 mm. The axial orientation of the plastic container and sample within it was preserved for magnetic resonance imaging (MRI) experiments following the optical measurements.

The optical imaging experiments were carried out using a beam of light collimated to a 1-mm spot size. Light of three different wavelengths 750 nm, 800 nm, and 830 nm from a Ti:sapphire laser were used in the measurement. The average beam power was maintained at 10 mW for every wavelength. Multiple source illumination was realized in practice by scanning the sample in a 26 × 16 array of x-y grid points with a step size of 2.5 mm, while the CCD camera imaged the entire detection plane. Each illuminated pixel of the 1024 × 1024 pixels of the CCD camera could be regarded as a detector. For illumination of every scanned point on the source plane, the CCD camera recorded the diffusely transmitted intensity pattern on the detection plane.

For MRI experiments, the breast model sample in the plastic container was taken to Memorial Sloan-Kettering Cancer Center (MSKCC) small animal MRI facility. The facility currently utilizes a 4.7-T 33-cm (Bruker BioSpin). MR images of the sample were recorded in 2.0-mm slice thick sagittal slices.

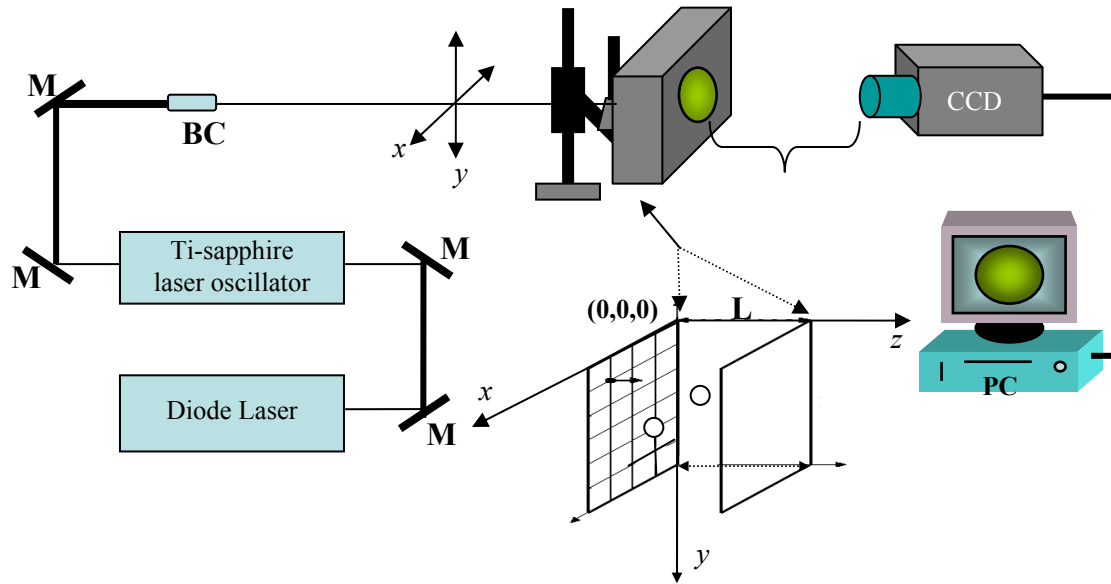


Figure 1. Schematic diagram of the experimental arrangement (M = mirror, BC = beam collimator, CCD = charge-coupled device, PC = personal computer)

3. THEORETICAL FORMALISM

The perturbation of the detected light intensities on the boundaries of the medium, the scattered wave field, due to scattering inhomogeneities is given, in Diffusion Approximation (DA), by [3-5]

$$\begin{aligned}
 -\Delta I(\mathbf{r}_d, \mathbf{r}_s; \lambda) = & \int d^3 r \delta \mu_a(\mathbf{r}, \lambda) c G(\mathbf{r}_d, \mathbf{r}; \lambda) G(\mathbf{r}, \mathbf{r}_s; \lambda) \\
 & + \int d^3 r \delta D(\mathbf{r}, \lambda) c \nabla_r G(\mathbf{r}_d, \mathbf{r}; \lambda) \cdot \nabla_r G(\mathbf{r}, \mathbf{r}_s; \lambda)
 \end{aligned} \tag{1}$$

in the first-order Born approximation assuming that light diffuses inside the medium. In Eq.(1), $\mathbf{r}_s$ and $\mathbf{r}_d$ are the positions of the source and the detector on the boundary, respectively; $\delta \mu_a(\mathbf{r}, \lambda) = \mu_a(\mathbf{r}, \lambda) - \mu_{a0}(\lambda)$ and $\delta D(\mathbf{r}, \lambda) = D(\mathbf{r}, \lambda) - D_0(\lambda)$.

$\lambda) - D_0(\lambda)$ are the differences in absorption coefficient and diffusion coefficient, respectively, between the target at $\mathbf{r}$ and the background; c is the speed of light in the medium; and $G(\mathbf{r}, \mathbf{r}'; \lambda)$ is the Green's function describing light propagation from $\mathbf{r}'$ to $\mathbf{r}$ inside the background turbid medium of absorption and diffusion coefficients $\mu_{a0}(\lambda)$ and $D_0(\lambda)$ where λ is the wavelength of the probing beam. OPTICA Formalism has been detailed elsewhere [3-5]. For TROT algorithm, the experimental data obtained using the multiple-source and multiple-detector arrangement, were as a response matrix K , expressed as[6-7]

$$K = \{K_{l,j}\} = \sum_{m=1}^M G^d(\mathbf{R}_l, \mathbf{X}_m) \tau_m G^s(\mathbf{X}_m, \mathbf{R}_j) \equiv \sum_{m=1}^M g_m^d \tau_m g_m^s \quad (2)$$

$$K = \{K_{j,l}\} = \sum_{m=1}^M G^s(\mathbf{X}_m, \mathbf{R}_j) \tau_m G^d(\mathbf{R}_l, \mathbf{X}_m) = \sum_{m=1}^M g_m^s \tau_m g_m^d$$

where $j = 1, 2, \dots, N$ and $l = 1, 2, \dots, N$ are indices of sources and detectors, respectively; $m = 1, 2, \dots, M$ is the index of the targets with $M < N$; $\mathbf{R}_j$, $\mathbf{R}_l$ and $\mathbf{X}_m$ are the positions of a source, a detector and a target respectively; τ_m is the difference in the optical parameters (absorption and scattering) of the target from that of the background medium (the background medium may be uniform or non-uniform); $G_s(\mathbf{X}_m, \mathbf{R}_j)$ and $G_d(\mathbf{R}_l, \mathbf{X}_m)$ are the Green's functions in the background medium. The vector $g_m^d = [G^d(\mathbf{X}_m, \mathbf{R}_1), G^d(\mathbf{X}_m, \mathbf{R}_2), \dots, G^d(\mathbf{X}_m, \mathbf{R}_N)]$ in Eq. (2) has N components [6-7]. From K a time reversal matrix $T = K^T K$ is constructed, and its eigenvalues and eigenvectors are determined. Leading eigenvalues lead to the targets.

4. RESULTS

4.1 OPTICA

OPTICA-generated independent intensity distributions on the detector plane for the two tumors using 750-nm probing are shown in Figure 2(a). Similar intensity distributions were obtained for probing using light of other two wavelengths as well. The x - y - z locations of the left tumor and the right tumor determined from fitting these ICA profiles to the Green's function for all three wavelengths are shown in Table I. The average of the probed tumor positions from all wavelengths is shown in bold. The cross-section images of the two tumors constructed using Back-projection Fourier transform [5] are shown in Figure 2(b).

Table I. The coordinates (x , y , z) of the left tumor and right tumor

Wavelength (nm)	Left Tumor			Right Tumor		
	y (mm)	x (mm)	z (mm)	y (mm)	x (mm)	z (mm)
750	50.3	54.9	20	26.1	48.7	20.2
800	50.5	55.4	17.9	27.1	49.4	19.5
830	49.6	54.2	17.2	27.1	47.2	21.5
Average	50.1	54.8	18.4	26.8	48.4	20.4

4.2 TROT

The TROT algorithm-generated cross-section image of the tumors for $\lambda = 750$ nm is shown in Figure 3(a). Similar images were obtained using other two wavelengths. The locations were found to be (11.5mm, 33.1mm, 22.5 mm) for the left tumor and (42.6 mm, 38.6 mm, 20.5 mm) for the right tumor. Since the tumors depth location is at the mid plane ~ 20-mm, we compare the horizontal separation between the two tumors and find it to be 35.8-mm using OPTICA and 31.1-mm using TROT. The optical results are in good agreement with the MR images at depth 22.5 mm shown 3(b) and 3(c) and separation of 35 mm.

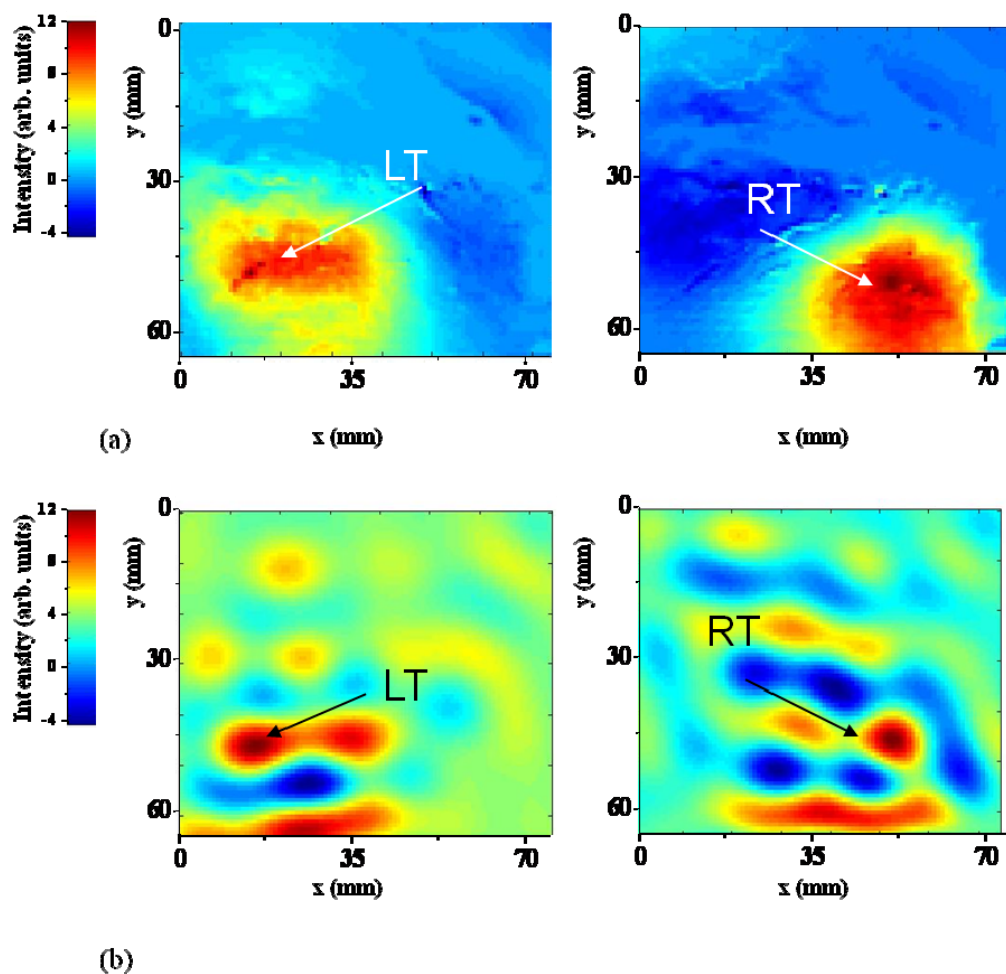


Figure 2. (a) OPTICA generated intensity distributions pertaining to the left tumor (LT) and right tumor (RT) for 750-nm probing. (b) Cross-section images formed by Back-projection Fourier transform.

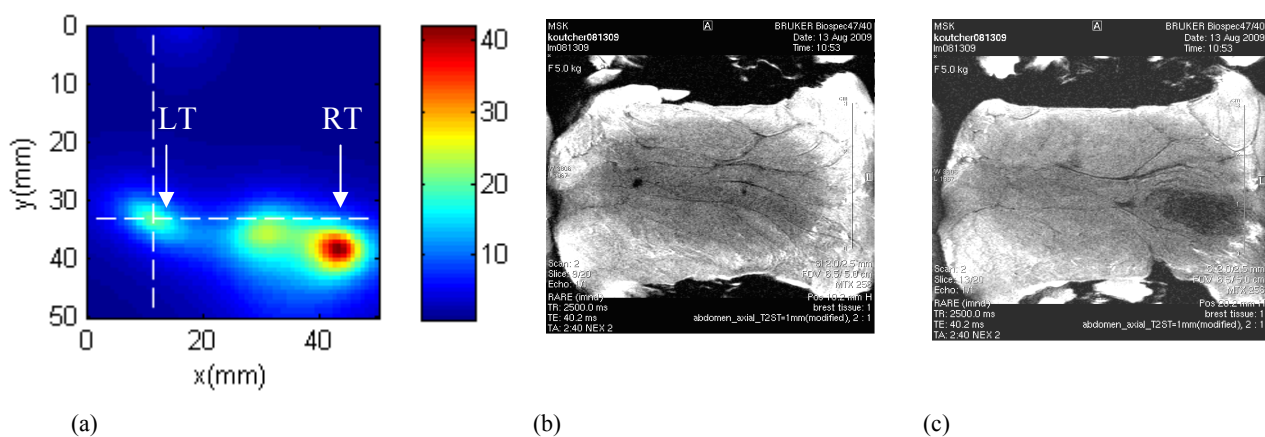


Figure 3. (a) TROT generated cross-section image. (b) Left tumor LT at slice depth $z = 18.5$ -mm, and (c) Right tumor RT at slice depth $z = 22.5$ -mm

5. SUMMARY

The two approaches provided comparable information, and successfully detected and located the two tumors. The use of multi-wavelength method eliminated some artifacts. The implementation time was under 1 minute. Although we have used the DA in this case, the approach can use other light propagation models. It can be used with continuous wave, frequency-domain and time-resolved experimental data. Future work on this approach will be directed towards estimating the optical properties, as well as, size and shape of targets.

ACKNOWLEDGEMENTS

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Optical Diffuse Imaging of an *Ex Vivo* Model Cancerous Human Breast Using Independent Component Analysis

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Abstract—Optical imaging using independent component analysis (OPTICA) has been used for detection, 3-D localization, and cross-section imaging of a tumor inside a model human breast composed of *ex vivo* human breast tissues. OPTICA uses a multisource target illumination and multidetector signal acquisition scheme to obtain multiple spatial and angular views of the sample for target localization. Independent component analysis of the perturbations in the spatial light intensity distribution measured on the sample boundary sorts out the signal originating from individual targets. A back-projection technique estimates the cross-section of each target. The approach correctly provided the positions of a tumor located at the mid-plane and two glandular structures located at different positions within the 33-mm-thick model breast. The reconstructed cross-section images are in good agreement with known dimensions of the structures, and pathological findings.

Index Terms—Breast cancer, diffuse optical imaging, independent component analysis, near infrared (NIR) imaging, optical mammography, optical imaging using independent component analysis (OPTICA).

I. INTRODUCTION

NEAR-INFRARED (NIR) diffuse optical tomography (DOT) is an emerging technology for functional characterization of biological tissues, and has been actively investigated to image lesions in human body organs, such as human breast [1]–[3], brain [4]–[7], and joints [8], [9]. A state-of-the-art DOT illuminates the sample (consisting of targets embedded in a turbid medium) with NIR light, measures the emergent light on the boundary of the turbid medium, and uses an iterative image reconstruction method for repeatedly solving the forward model of light propagation in the medium with an updated estimation of its optical properties to match the detected light intensities.

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This problem of imaging targets in a turbid medium is an ill-posed inverse problem, and *a priori* knowledge about the optical properties of the medium need to be used to obtain a unique solution at a cost of reduced resolution [10]–[13]. Various prior information such as anatomical structures obtained from X-ray or magnetic resonance imaging (MRI) and the absorption spectra of chromophores have been used to improve the imaging quality of the DOT [14]–[16]. The iterative image reconstruction is computation time intensive and reconstruction in 2-D planar sections instead of a 3-D volume is commonly practiced. Noniterative approaches have also been pursued [17]–[19]. Irrespective of these developments, reconstruction of images with adequate spatial resolution and accurate localization and characterization of the targets remain a formidable task.

We have developed an alternative approach for optical imaging using independent component analysis (OPTICA) [18], [20] that uses a multisource sample illumination and multidetector signal acquisition scheme to generate an extensive data set providing a variety of spatial and angular views of the medium. The signals from individual targets within the interrogated medium are then sorted out by using independent component analysis (ICA) based on their statistical independence. ICA is a statistical technique from information theory that is able to recover independent signals from their measured mixtures [21], [22]. ICA has been successfully applied in many biomedical applications, such as electroencephalogram (EEG) [23] and functional magnetic resonance imaging (fMRI) [24], and has been shown to be effective in separating signals from different brain activity centers. In DOT, excess light absorption or scattering by the individual targets embedded in the medium serve as the source of independent signals whose weighted mixture is recorded by a detector on the boundary of the medium. Since an independent component originating from any particular target relates directly to how light propagates from the source to the target and from the target to the detector, the recovered independent components can serve as the starting point for 3-D localization and optical characterization of individual targets in the medium. Such a staged procedure has been shown to significantly improve the sensitivity to small/weak absorptive, scattering and/or fluorescent targets, and can achieve a 3-D localization of the targets with remarkable accuracy and resolution [18], [25], [26].

The independent component is proportional to the strength of the target (the product of the difference in the absorption/scattering coefficient between the target and the background, and the volume of the target) and the convolution of the light propagators from the source to the target and from the

target to the detector. The two light propagators can be deconvoluted in the Fourier space. A 2-D cross-section image of the target is obtained by back projecting the independent component onto the transversal plane at the axial location of the target. Every independent component retrieved by ICA represents the signal from only one target with localization determined from earlier stage of analysis. So, a back projection formalism with little or no regularization can be applied to obtain a cross-section image of the target with improved spatial resolution than what is feasible in a conventional DOT.

We have previously tested the efficacy of OPTICA on samples consisting of absorbing or scattering targets within tissue phantoms and fluorescent targets in *ex vivo* tissue [18], [25], [26]. In this paper, we use OPTICA to investigate a tumor and other structures embedded in a “realistic” model breast assembled using *ex vivo* human breast tissues, as a prelude to *in vivo* breast imaging. The remainder of the paper is organized as follows. Section II presents the theoretical formalism of OPTICA and the back-projection approach for obtaining the cross-section image of a target. Section III describes the experimental arrangement, method, and parameters. Experimental results appear in Section IV. The implications are discussed in Section V.

II. THEORETICAL FORMALISM OF OPTICAL IMAGING USING INDEPENDENT COMPONENT ANALYSIS

The presence of targets (optical inhomogeneities) inside a turbid medium perturbs the spatial intensity distribution of light emergent from the medium under illumination by a probing beam. When illuminated by a point source of unit power, the change in the light intensity distribution on the boundary of the specimen due to absorptive and scattering targets can be written as [27], [28]

$$-\Delta I(\mathbf{r}_d, \mathbf{r}_s) = \int d^3\mathbf{r} \delta\mu_a(\mathbf{r}) cG(\mathbf{r}_d, \mathbf{r}) G(\mathbf{r}, \mathbf{r}_s) + \int d^3\mathbf{r} \delta D(\mathbf{r}) c\nabla_{\mathbf{r}} G(\mathbf{r}_d, \mathbf{r}) \nabla_{\mathbf{r}} G(\mathbf{r}, \mathbf{r}_s) \quad (1)$$

in the first-order Born approximation assuming that light diffuses inside the medium [29]. Here, $\mathbf{r}_s$ and $\mathbf{r}_d$ are the positions of the source and the detector on the boundary, $\delta\mu_a(\mathbf{r}) = \mu_a(\mathbf{r}) - \mu_{a0}$ and $\delta D(\mathbf{r}) = D(\mathbf{r}) - D_0$ are the differences in absorption coefficient and diffusion coefficient, respectively, between the target at $\mathbf{r}$ and the background medium, c is the speed of light in the medium, and $G(\mathbf{r}, \mathbf{r}')$ is the Green's function describing light propagation from $\mathbf{r}'$ to $\mathbf{r}$ inside the medium of absorption coefficient μ_{a0} and diffusion coefficient D_0 .

OPTICA assumes each inhomogeneity within the turbid medium to be a virtual source and expresses the change of the light intensity on the boundary of the specimen as

$$-\Delta I(\mathbf{r}_d, \mathbf{r}_s) = \sum_j a_j(\mathbf{r}_d) s_j(\mathbf{r}_s) \quad (2)$$

where $s_j(\mathbf{r}_s)$ represents the j th target illuminated by the incident wave at $\mathbf{r}_s$ and $a_j(\mathbf{r}_d)$ is the weighting matrix describing the propagation of light from the j th inhomogeneity to the detector at $\mathbf{r}_d$. Each absorptive inhomogeneity contributes one term in

(2), and each scattering inhomogeneity contributes three terms in (2) [18]. The detected change of the light intensity $-\Delta I$ is, hence, a linear mixture of signals where a_j and s_j can now be interpreted as the j th weighting matrix and virtual source, respectively. Owing to the statistical independence between these virtual sources, independent component analysis of $-\Delta I$ will yield a list of independent components and recover both a_j and s_j . Here, a_j and s_j are the independent intensity distribution on the detector and source planes, respectively, for the j th target. The number of the leading independent components gives the number of objects. The location of the j th target is obtained from the analysis of the retrieved independent component (s_j and a_j) that relates directly to the source-to-object and object-to-detector Green's functions $G(\mathbf{r}_j, \mathbf{r}_s)$ and $G(\mathbf{r}_d, \mathbf{r}_j)$ and the optical property of the target where $\mathbf{r}_j$ is the position of the j th object [18], [20], [25], [26].

For the slab geometry investigated here, there are three virtual sources of specific patterns (one centrosymmetric and two dumbbell-shaped) associated with each scattering inhomogeneity, whereas only one centrosymmetric virtual source is associated with each absorptive inhomogeneity. Among the three virtual sources associated with a scattering inhomogeneity, the centrosymmetric virtual source is the strongest and more amenable to detection in a thick turbid medium [25]. The centrosymmetric virtual source and the corresponding weighting matrix are $s_j \propto G(\mathbf{r}_j, \mathbf{r}_s)$ and $a_j \propto G(\mathbf{r}_d, \mathbf{r}_j)$, and $s_j \propto \partial G / \partial z(\mathbf{r}_j, \mathbf{r}_s)$ and $a_j \propto \partial G / \partial z(\mathbf{r}_d, \mathbf{r}_j)$, respectively, for absorptive and scattering inhomogeneities. A simple least square fitting of the centrosymmetric component, such as

$$\min_{\mathbf{r}_j, \alpha_j, \beta_j} \left\{ \sum_{\mathbf{r}_s} [\alpha_j^{-1} s_j(\mathbf{r}_s) - G(\mathbf{r}_j, \mathbf{r}_s)]^2 + \sum_{\mathbf{r}_d} [\beta_j^{-1} a_j(\mathbf{r}_d) - G(\mathbf{r}_d, \mathbf{r}_j)]^2 \right\} \quad (3)$$

for the absorptive object, can be used to yield the 3-D location $\mathbf{r}_j$ and the strength $\alpha_j \beta_j$ of the target. When *a priori* knowledge about the property of the target is not available, (3) can still be used to estimate the 3-D location of the target regardless of the absorption or scattering property of the target. This is due to the fact that $\partial G / \partial z(\mathbf{r}_j, \mathbf{r}_s) \simeq -\kappa G(\mathbf{r}_j, \mathbf{r}_s)$ and $\partial G / \partial z(\mathbf{r}_d, \mathbf{r}_j) \simeq -\kappa G(\mathbf{r}_d, \mathbf{r}_j)$, where $\kappa = \sqrt{(\mu_{a0} - i\omega/c)/D_0}$ is chosen to have a nonnegative real part with ω the modulation frequency of the incident wave.

The signal from the j th target is simply given by $-\Delta I_j = a_j(\mathbf{r}_d) s_j(\mathbf{r}_s)$. On the other hand, the centrosymmetric signal of the j th target can be approximated as a double convolution

$$-\Delta I_j(\mathbf{r}_d, \mathbf{r}_s) = \int G(\boldsymbol{\rho}_d - \boldsymbol{\rho}, z_d, z_j) X_j(\boldsymbol{\rho}) G(\boldsymbol{\rho} - \boldsymbol{\rho}_s, z_j, z_s) d\boldsymbol{\rho} \quad (4)$$

where the integration is over the $z = z_j$ plane, X_j represents the target, and $\boldsymbol{\rho}_d$ and $\boldsymbol{\rho}_s$ are the lateral coordinates of the detector and the source, respectively. The cross-section image of the j th target X_j is a 2-D distribution of the absorption/scattering coefficient of the target on the $z = z_j$ plane. In the Fourier space,

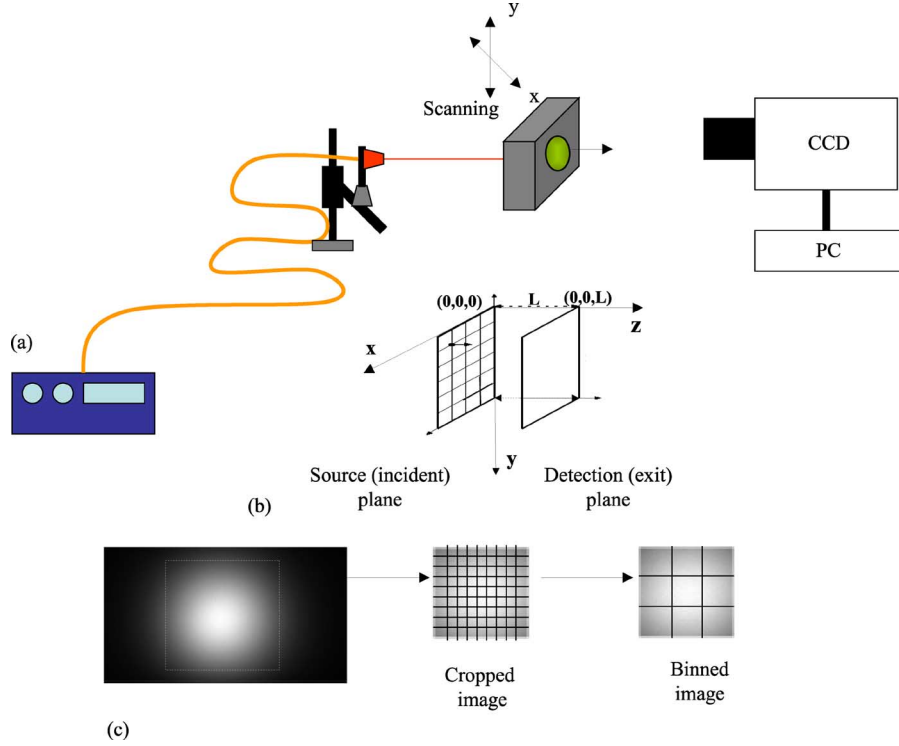


Fig. 1. (a) Schematic diagram of the experimental arrangement. CCD, charge coupled device; PC, personal computer. (b) Expanded view of the sample input (source) plane and exit (detection) plane showing the grid points in the x - y plane. (c) Typical raw CCD image of the detection plane, and how it is cropped and binned for analysis.

the target function X_j can be obtained from (4) as

$$X_j(\mathbf{q}) = -\frac{\Delta I_j(\mathbf{q} - \mathbf{q}_s, \mathbf{q}_s)}{G(\mathbf{q} - \mathbf{q}_s, z_d, z_j)G^*(\mathbf{q}_s, z_j, z_s)} \quad (5)$$

where $\mathbf{q}$ and $\mathbf{q}_s$ are the spatial frequency on the lateral plane and “*” denotes complex conjugate. We choose $\mathbf{q}_s = 0$ in the evaluation of the target function (5) since sources are usually much sparser than detectors in our setup where a charge-coupled device (CCD) camera is used to detect the emergent light intensity on the surface of the medium. The inverse Fourier transforms of $X_j(\mathbf{q})$ yields the high-resolution cross-section image of the j th target due to the high density of detecting pixels of the CCD. The size of the target is estimated by the full-width at half-maximum (FWHM) of the cross-section image X_j .

To sum up, OPTICA first detects and retrieves independent components corresponding to each target embedded inside a turbid medium, then obtains the 3-D location and strength of the target from these independent components, further reconstructs the cross-section image of the target on the transversal plane where the target locates, and finally, the size and the optical property of the target are estimated.

III. EXPERIMENT

The experimental arrangement for detection and localization of the tumor in the *ex vivo* model breast sample is shown in Fig. 1(a). The model breast was a 70 mm × 55 mm × 33 mm slab composed of excised female human breast tissues provided to us by National Disease Research Interchange under an Inter-

nal Review Board approval at the City College of New York. The model breast was assembled using two pieces of *ex vivo* human breast tissues. The larger piece was normal tissue that included mainly adipose tissue and streaks of fibroglandular tissues. The existence of the fibroglandular tissues was not known prior to making the measurements.

The second piece was mainly a tumor (infiltrating ductal carcinoma) with a small amount of normal tissues in the margins with an overall approximate dimension of 8 mm × 5 mm × 3 mm. An incision was made in the mid-plane (along the z -axis, which was the shorter dimension of the tissue) of the normal piece, and some amount of the normal tissue was removed from the central region making a small pouch. The tumor piece was then inserted into the pouch, and the incision was closed by moderate compression of the composite consisting of the normal tissue and the tumor along xyz -directions. The breast tissue slab was contained inside a transparent plastic box. One of the sides of the box could be moved to uniformly compress the tissue along the z -axis and hold it in position. The resulting specimen, a 70 mm × 55 mm × 33 mm slab, was treated as one entity in the subsequent imaging experiment. The position of the tumor within the slab was known since it was placed in the position as discussed earlier. One of the tests of the efficacy of this imaging approach was to see how well the known position is assessed.

A 200 μ m optical fiber delivered a 784 nm, 300 mW continuous-wave beam from a diode laser for sample illumination. The beam was collimated to a 1 mm spot onto the entrance face (henceforth referred to as the “source plane”)

of the slab sample. Multiple source illumination was realized in practice by step scanning the slab sample across the laser beam in an xy array of grid points using a computer-controlled translation stage. The xy array was 22×16 with a step size of 2.0 mm. The signal from the opposite face of the sample (henceforth referred to as the “detection plane”) was collected by a camera lens and projected onto the sensing element of a cooled 16 b, 1024×1024 pixel CCD camera. Although the scanned area is $42 \text{ mm} \times 30 \text{ mm}$ on the source plane, the imaged area of the detection plane was much larger, covering the entire $70 \text{ mm} \times 55 \text{ mm}$ transverse area of the model breast. Each illuminated pixel of the CCD camera could be regarded as a detector. For illumination of every scanned point on the source plane, the CCD camera recorded the diffusely transmitted 2-D intensity pattern on the detection plane. Each image acquisition took 100 ms, and one stepping of the translational stage took 1 s. A total of 352 images were completed within 7 min. The OPTICA reconstruction and cross-section imaging is expected to be completed within 2 min once fully automated.

IV. RESULTS

A typical 2-D raw image of transmitted light intensity distribution on the detector plane for illumination at a typical scanning position is shown in Fig. 1(c). The average of all the 22×16 images was used to obtain the optical property of the slab of breast tissue. The radial profile of the intensity of the transmitted light on the average image was fitted to that predicted by a diffusion model of light propagation inside a slab. The transport mean free path was assumed to be 1 mm, the value for a typical human breast tissue at 785 nm. The reduced scattering coefficient was then 1 mm^{-1} . From the decay of the radial profile of the intensity of the transmitted light, the average absorption coefficient of the entire model breast is found to be $\mu_a = 0.0039 \text{ mm}^{-1}$. Each raw image is first cropped to retain the region within the window of $50.4 \text{ mm} \times 51.3 \text{ mm}$ (out of a total $70 \text{ mm} \times 55 \text{ mm}$ transverse area of the model breast) over which image reconstruction would be performed. The size of 1 pixel in the raw image is $187 \mu\text{m} \times 187 \mu\text{m}$. The raw images are binned by merging 5×5 pixels into one to enhance the SNR, resulting in a total of 352 images of 54×55 pixels each. All the binned images corresponding to illumination of the grid points in sequence were then stacked, and used as input for independent component analysis.

The independent light intensity distributions obtained by OPTICA is displayed in Fig. 2(a). The 3-D location of the targets were obtained from least squares fitting using (3). The fittings of the independent light intensities over lines passing through the maximum value and along the horizontal direction are displayed in Fig. 2(b). The tumor C is found at 14.8 mm from the detection plane and centered at (33.3, 21.5, 18.2) mm. In addition, two glandular sites were identified. The first glandular site A is found to be located at 2.5 mm from the detection plane and centered at (11.2, 22.4, 30.5) mm; the second glandular site B is at 14.6 mm from the detection plane and centered at (21.5, 37.3, 18.4) mm. Comparison of known and 3-D positions ob-

tained from OPTICA for the cancer site and two glandular sites is given in Table I.

The cross-section image of the tumor obtained from a 2-D inverse Fourier transform of (5) is shown in Fig. 3 (left pane). The right pane of Fig. 3 displays the intensity profiles of the cross-section image along the x - and y -directions denoted by the white dashed lines. The FWHM values of the intensity profiles yield estimates of the lateral dimensions of the tumor to be $10.3 \text{ mm} \times 7.4 \text{ mm}$, while the known dimensions are $8 \text{ mm} \times 5 \text{ mm}$. Histological micrograph of the suspect site confirmed tumor. Similar back-projection cross-sectional images and histological micrographs were obtained (not shown here) for the glandular tissues as well and their transverse sizes were estimated from OPTICA. The existence, location, and size of the glandular tissues were not known *a priori*. The glandular structure A near surface is estimated to be 2.7 and 1.6 mm in size along the x - and y -directions from the cross-section image, respectively. The size of the glandular structure B at the midplane is 8.7 and 9.2 mm in size along the x - and y -directions, respectively.

Low regularization was used in generating the cross-section images in Fig. 3 to achieve maximal spatial resolution. The artifacts in the cross-section images can be suppressed with a higher regularization at a cost of lower spatial resolution. Since the target has been localized in the earlier stage of analysis, the target will not be confused with artifacts in the cross-section images and low regularization is beneficial here.

The investigated *ex vivo* breast sample contained minimal amount of blood, and hence, the reconstructed images are for the scattering property of the sample. The change of the reduced scattering coefficient μ'_s for the targets can further be estimated from the reconstructed independent components for the sites A, B and C. The value of $\delta\mu'_s$ is given by the ratio of the strength of the target and its volume. The sites A and B have lower scattering while the site C has enhanced scattering compared to the background (mainly adipose tissue). The values of $\delta\mu'_s$ are ~ 0.2 and $\sim -0.4 \text{ mm}^{-1}$ for the tumor and glandular tissues, respectively. Subsequent pathological analysis confirmed the site C as infiltrating ductal carcinoma, and identified the other two structures as glandular breast tissues.

V. DISCUSSION

The results of the experiments clearly demonstrate that OPTICA can locate the tumor inside the model breast with high accuracy. As can be seen from Table I, the lateral positions of the tumor agree within 0.5 mm, while the axial position agree within ~ 1 mm of the known values. Similar high accuracy in the respective positions of the two pieces of glandular tissues is observed as well. The accuracy of the lateral positions does not depend significantly on the depth of the targets, while that of the axial position shows a weak dependence. For the target located close to the detection plane (glandular site A at a distance of 2.5 mm from the detection plane), the axial position is determined exactly, while for targets in the midplane that are much more challenging to locate, the accuracy is within 1 mm. Given

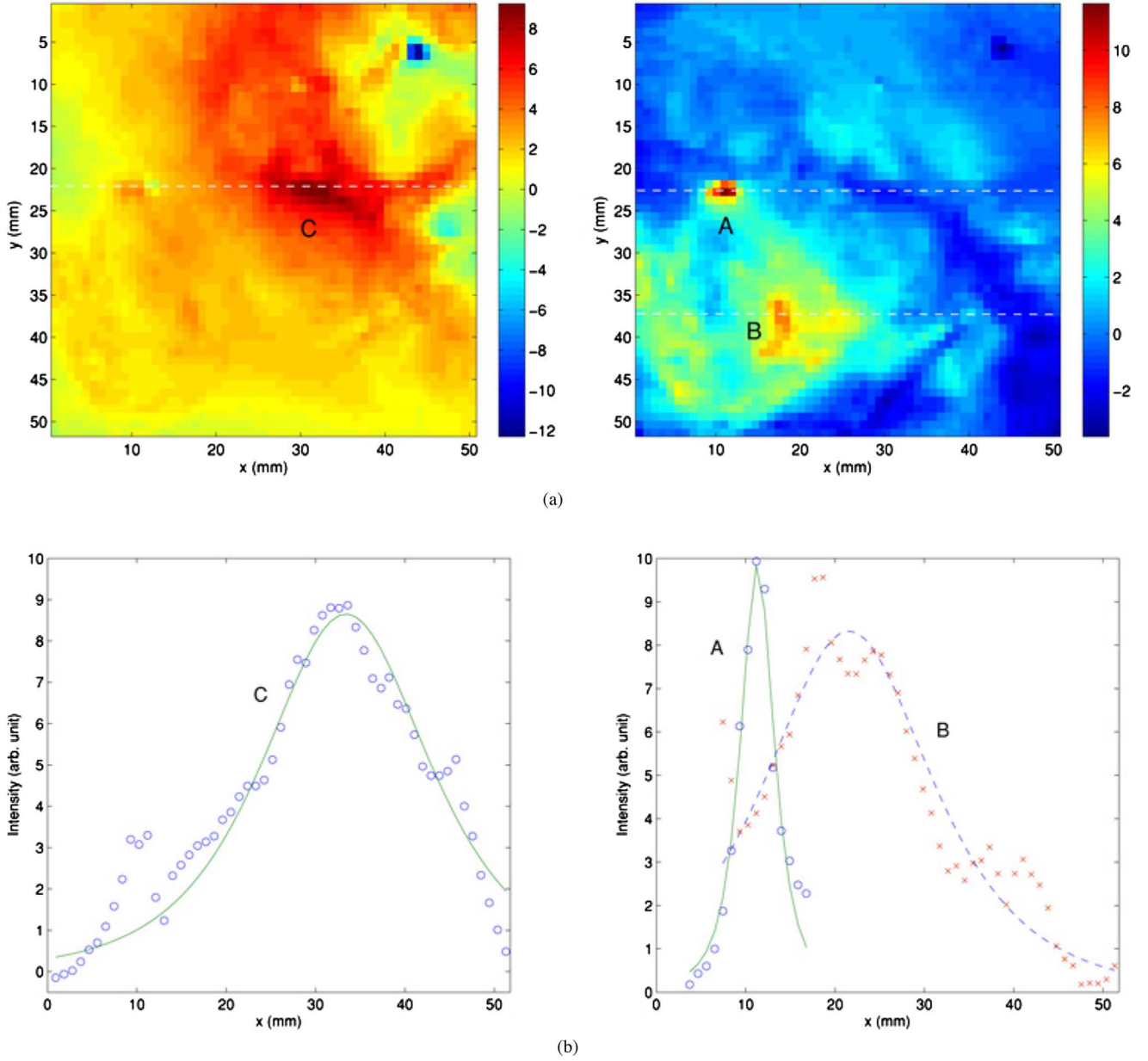


Fig. 2. (a) Independent intensity distribution on the detector plane ($z = 33$ mm) obtained by OPTICA for the tumor C (left pane) and the glandular structures A and B (right pane). (b) Corresponding bottom panes show the Green's function fits (solid lines) to the horizontal spatial profile (denoted by circles and crosses) through the center of the intensity distributions along the dashed lines.

TABLE I
COMPARISON OF KNOWN AND OPTICA ESTIMATED TARGET LOCATIONS

Target	Known Position (x, y, z) (mm)	OPTICA Estimated Position (x, y, z) (mm)
Cancer Site (C)	(33,21,16.9)	(33.3,21.5,18.2)
Glandular Site (A)	(11,22,30.5)	(11.2,22.4,30.5)
Glandular Site (B)	(21,37,17)	(21.5,37.3,18.4)

that light propagation is highly diffusive in breast tissues, this level of accuracy is quite significant.

The back-projection formalism estimates the FWHM values of the lateral dimension of the tumor to be 10.3 and 7.4 mm in size along the x - and y -directions, respectively, whereas the known dimension is 8 mm $\times$ 5 mm. This result is expected due

to diffusion of light in the tissue, and is in line with the results that we obtained in our earlier OPTICA studies [26].

Another important finding was that OPTICA predicted different scattering properties for the adipose tissue (medium), the tumor, and the glandular tissues. The glandular tissues were found to be less scattering than the adipose tissues at the wavelength of interrogation, i.e., 784 nm. The tumor was found to be more scattering. These observations are consistent with the known literature values of scattering properties of different types of tissues [30].

The nature of the inhomogeneity (either absorptive or scattering or mixed) can be discerned by OPTICA with continuous-wave measurement when the SNR is high [20], [25]. When the SNR is not favorable, the recovered independent component

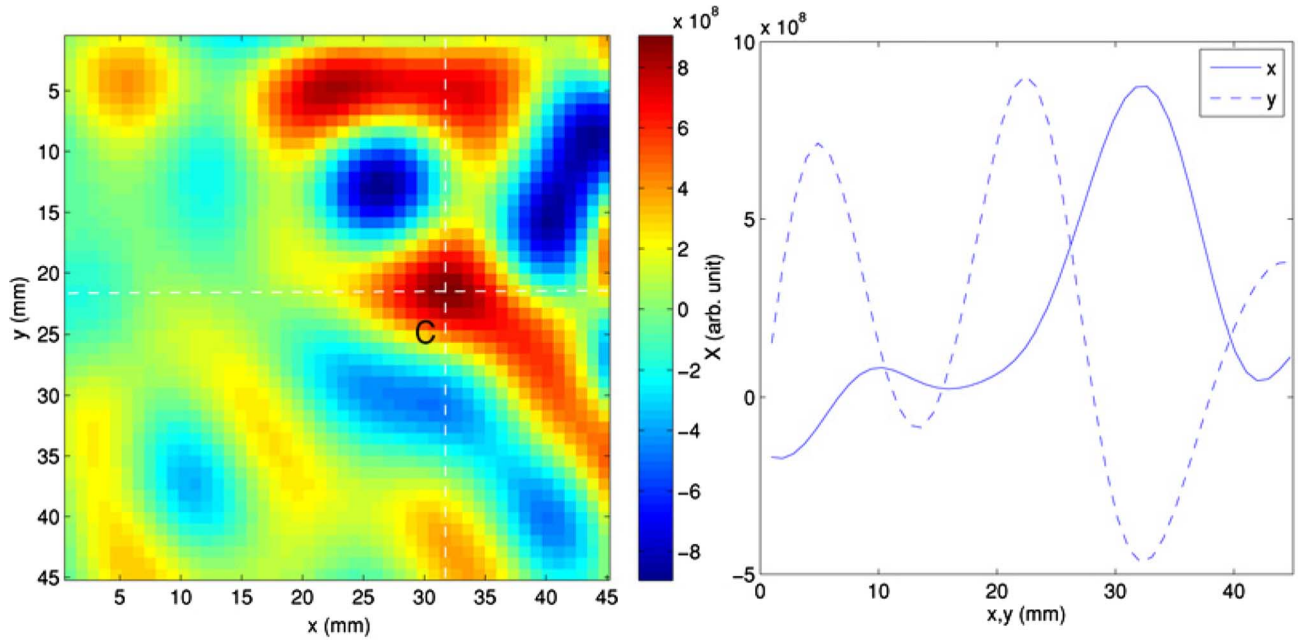


Fig. 3. Cross-section image of the tumor at the $z = 18.2$ mm plane formed by back-projection (left pane). Right pane: Spatial profiles of the cross-section image along the x - and y -directions shown by the white dashed lines (right pane). The FWHM of the cancer site is 10.3 and 7.4 mm along the x - and y -directions, respectively.

will be due to both absorption and scattering perturbations at the site of the inhomogeneity. The strength of the target will be proportional to $\delta\mu_a + \kappa^2\delta D = \delta\mu_a + (\mu_{a0} - i\omega/c)\delta D/D_0$, which provides a way to discriminate between absorption and scattering if measurements of multiple modulation frequencies ω are available. The capability of OPTICA for separating absorption from scattering inhomogeneities can be significantly improved with a time-domain or frequency-domain measurement. Another enabling factor will be carrying out multispectral OPTICA studies for potential diagnostic information.

OPTICA can be used for fluorescent targets as well [26]. The same experimental arrangement may be used, except for the introduction of filters to block the excitation beam and to transmit the fluorescence light. What is even more interesting is that, a beam-splitter and two detectors combination with appropriate filters may be used to simultaneously pursue absorption/scattering OPTICA and fluorescence OPTICA studies of biological samples for obtaining coregistered information from dual probes.

OPTICA is suited to detect small objects. Given its ability to identify low-contrast small objects, the approach is expected to be especially useful for the detection of breast and prostate tumors at their early stages of growth.

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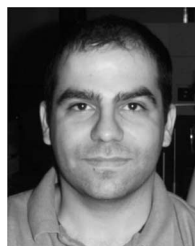
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Near-Infrared Center-of-Intensity Time Gated Imaging for Detection of a Target in a Highly Scattering Turbid Medium

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A near-infrared optical imaging approach for locating a target embedded in a turbid medium is introduced. The target localization is based on an analysis of the spatial variation of the transmitted-light intensity distribution for illumination at different positions on the sample boundary. The approach is used to detect, locate and generate images of absorbing targets embedded inside model scattering media of thickness approximately 50 times the transport mean free path of the medium, as well as, of *ex vivo* biological tissue specimens.

Key words: Near-infrared imaging; Time gated imaging; Turbid medium; Center of intensity; Optical mammography.

Introduction

Optical detection of a target embedded in a highly-scattering turbid medium is a challenging problem with diverse potential applications, such as, imaging of a tumor in human breast, mines in shallow coastal water, and airborne surveillance through cloud or fog cover (1-3). Light multiple scattered by the intervening medium encroaches into the region of geometrical shadow, and obscures the direct transillumination image of the target. A variety of time-resolved, frequency-domain, and continuous-wave methods for direct imaging and inverse reconstruction of images has evolved over the years (4-8). Implementation of many of these approaches is quite involved requiring complex experimental arrangements, sophisticated analytical schemes and time-intensive numerical algorithms for generation of images.

In this article, we introduce a transillumination imaging approach for prompt target detection with potential applicability in biomedical imaging, such as, breast cancer detection. The approach is based on the premise that a target (that is, an optical inhomogeneity) within the turbid medium alters the propagation of light through the medium. Consequently, the spatial distribution of the output light intensity (SDOLI) is different with an embedded target than that without it. The shadow generated by the target alters the SDOLI and enables computation of a two-dimensional (2-D) map of the target.

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Abbreviations: 1-D = One-Dimensional; 2-D = Two-Dimensional; CIC = Center-of-Intensity Curve; CCD = Charge Coupled Device; FWHM = Full Width at Half Maximum; ND = Neutral Density; PC = Personal Computer; PS = Pulse Stretcher; RGA = Regenerative Amplifier; SDOLI = Spatial Distribution of the Output Light Intensity; UGICS = Ultrafast Gated Intensified Camera System.

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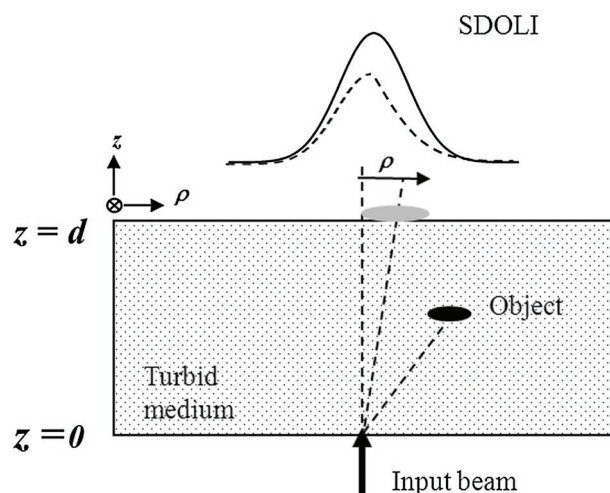


Figure 1: A schematic diagram of the diffuse light transmission through a turbid medium showing the target and the shadow. The inset at the top shows spatial intensity distribution without the target (solid curve) and with the target (dotted curve).

The remainder of the article is organized as follows. First we present the formalism for generating 2-D map of a sample (target embedded in the turbid medium). Experimental methods, materials, and parameters come next. Results for different targets are then presented, and a comparison is made with conventional diaphanography, which is followed by a discussion of the implication of the results.

Formalism

The SDOLI depends on where and how the turbid medium is illuminated, as illustrated schematically in Figure 1. In the slab geometry of Figure 1, a narrow beam of light is incident on the input plane ($z = 0$ plane). A fraction of the beam

energy propagates diffusely in the z -direction, and emerges through the opposite side, the detection plane ($z = d$ plane). The SDOLI at the detection plane is a two-dimensional (2-D) intensity distribution that can be measured by a charge coupled device (CCD) camera. The black ellipse represents a target in the medium, and the grey spot at the detection plane is the shadow of the target. For a uniform scattering medium without the target, the intensity distribution of the diffused light inside the medium is symmetric about the incident direction, as shown by the solid curve of intensity distribution in Figure 1. When there is a target with different optical properties than that of the turbid medium, as shown by the black ellipse, the transmitted light intensity distribution will be altered because of scattering and/or absorption by the target. Consequently, the spatial distribution of the light intensity at the output plane of the medium will be distorted and different with an embedded inhomogeneity than that without it, as shown schematically by the dashed curve. It is possible to locate the target within the highly scattering turbid medium through an analysis of this intensity variation. The analysis starts with a calculation of the center of the SDOLI at the output surface given by

$$M = \frac{\int_{x_1}^{x_2} Ix dx}{\int_{x_1}^{x_2} I dx}, \quad [1]$$

where I is the light intensity recorded by CCD camera and x is the position of each pixel. For a uniform medium illuminated by a point source, the transmitted intensity distribution centers on the incident direction. When the target is located to the right side of the incident direction, as shown in Figure 1, it will introduce a shadow at the right part of the output beam and lead to a decrease and distortion in the output intensity

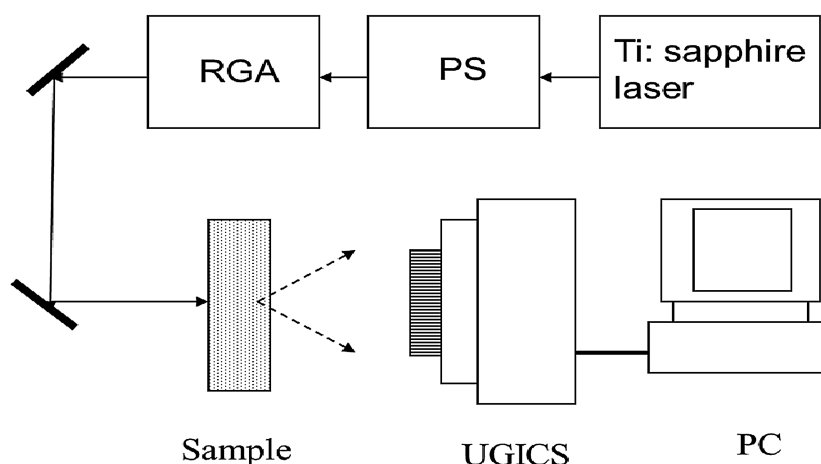


Figure 2: A schematic diagram of the experimental arrangement (Key: PS = pulse stretcher, RGA = regenerative amplifier, UGICS = ultrafast gated intensified camera system).

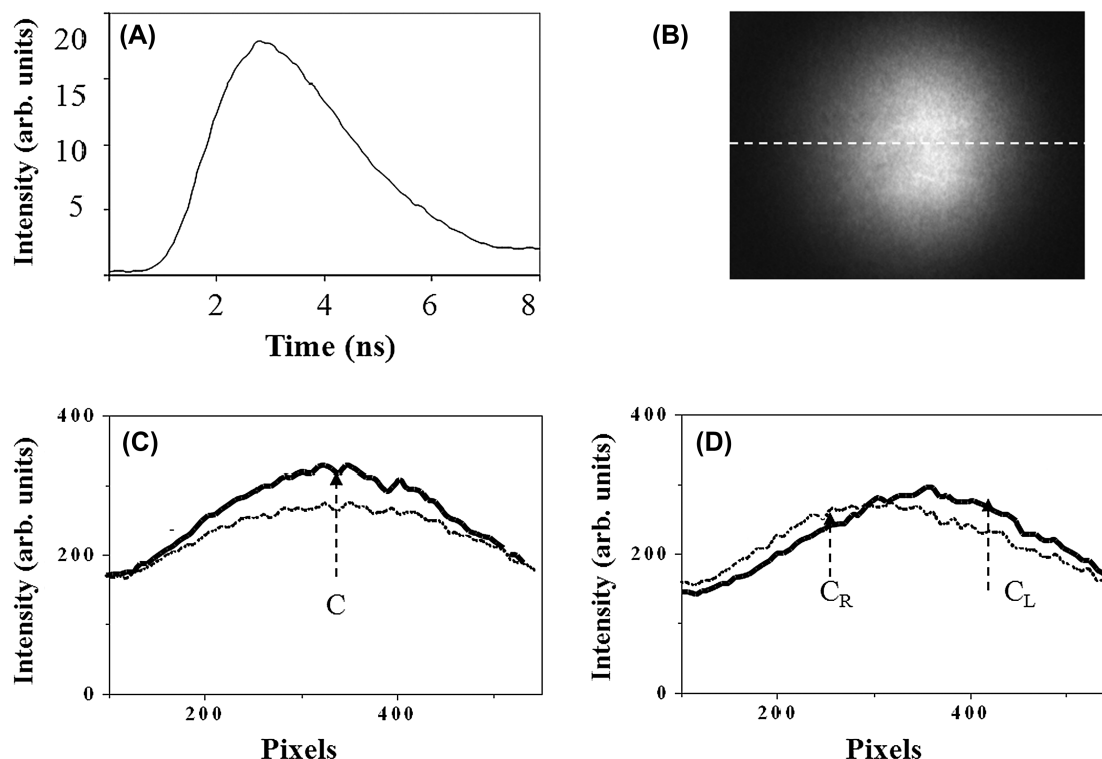


Figure 3: (A) Temporal profile of the transmitted pulse. (B) A typical time-gated image of the (2-D light intensity distribution) of the output plane. (C) Spatial intensity profile without the target (solid line), and with the target in the incident beam path (dashed line). The profiles are obtained by integrating a 3-pixel wide area around a horizontal line through the center of the image, as shown by the white dashed line in (B). The dashed arrow **C** indicates the peak of the intensity distribution. (D) Similar spatial profiles as in (C), except the solid (dotted) curve corresponds to target being located to the left (right) of the incident beam direction.

distribution. This results in a shift of the intensity peak of the output beam to the left of the incident beam direction. Consequently, the center of intensity distribution M will show a left (negative) shift. When the target is to the left of the incident beam, it will introduce a shadow at the left side of the output beam, and M will show a right (positive) movement. When the object is on the axis of the input beam, the center of intensity of the output beam profile would be same as that without target inside, but the peak intensity would be reduced.

The sample is next scanned across the incident laser beam in a linear array of data points for obtaining a one-dimensional (1-D) or in an x -y array to obtain a 2-D image. For each scan position a value of M is obtained. The perturbation in intensity distribution due to the presence of the target is given by $\Delta M = M - M_0$, for every scan position where M_0 ideally is the center of SDOLI when the target is not present. However, it is not practical to take the target out for obtaining M_0 in a real-life situation, a scheme is needed to generate the reference, and depending on application different schemes are used (8-10). We used the average of the intensity profiles recorded for different scanning positions for assessing M_0 , which turned out to be a reasonable approximation for the experimental conditions used in our initial proof-of-principle

investigation involving small targets (target volume $\ll$ sample volume). When the scanning beam is far away from the target, ΔM is close to zero. A center-of-intensity curve (CIC) is then generated by plotting ΔM as a function of scan position (x , say) along a line that passes through the target or its vicinity. A CIC will start from a near-zero value at one end of the scan range far away from the target, reach an extremum

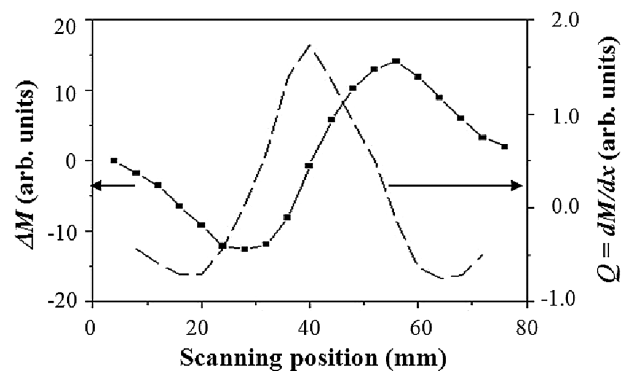


Figure 4: Variation of the center of intensity distribution, ΔM (solid rectangles joined by lines), and its first derivative, Q (dashed line) as a function of the scan position (x).

closer to the sample, pass through zero at the target center and reach the opposite extremum after the sample location and then gradually reach a near-zero value at the other end of the scan. The first derivative of CIC, $d(\Delta M)/dx$ vs. x will peak at the target location.

Experimental Materials and Methods

The experimental arrangement for realizing the approach in practice is shown in Figure 2. The scattering medium was a suspension of Intralipid-10% in water. The concentration of Intralipid-10% suspension was adjusted to provide an estimated reduced scattering coefficient of $\mu_s' \sim 1.168 \text{ mm}^{-1}$ (transport length 0.86 mm) and an absorption coefficient of 0.0021 mm^{-1} at 800 nm (11). The Intralipid-10% suspension was held in a rectangular glass container of dimension $240 \text{ mm} \times 160 \text{ mm} \times 60 \text{ mm}$. A $10 \text{ mm} \times 10 \text{ mm} \times 3 \text{ mm}$ -neutral density filter (absorption coefficient 0.23 mm^{-1} at 800 nm) was placed inside the medium as the target for the first experiment. Subsequent experiments used neutral density (ND) filters of different sizes. In the initial proof-of-the-principle experiments, glass neutral density filters were used as targets because those provided consistently uniform and well calibrated absorption contrast that would not degrade

with time and exposure to laser light. Another experiment using *ex vivo* porcine tissue as the intervening medium and a small piece of porcine liver tissue as the target was then carried out to put the approach to a more realistic test and assess its potential for optical biomedical imaging.

The scattering medium was illuminated by 800-nm, 200-ps, 1 kHz repetition-rate pulses from a Ti: sapphire laser and regenerative amplifier system (12). The amplified pulses were not compressed. The average beam power was 150 mW and the beam spot size was approximately 2 mm. The laser beam was incident along the z -axis into one of the $240 \text{ mm} \times 160 \text{ mm}$ flat faces of the container and the target was placed in the mid-plane, 30 mm from the front surface. The 2-D intensity distribution of light emergent from the opposite end face of the cell (geometric distance of 60 mm) was recorded by an ultrafast gated intensified camera system (UGICS). The UGICS provides an electronic time gate whose full-width-at-half-maximum (FWHM) duration can be set from a minimum of 80 ps to a maximum of 2 ns. The gate position could be varied over a 20 ns range with a minimum step size of 25 ps. In the experiments reported here the gate width was chosen to be 80 ps, and the gate was centered at a time that corresponded to the peak position of the transmitted pulse profile to ensure adequate signal, and reduce the deleterious effects of multiple-scattered, late-arriving light. The sample cell was mounted on a translation stage for lateral scanning. The input beam and the UGICS were not scanned. An 80 mm linear region of the sample was scanned in 4 mm steps providing 21 data points in the first experiment.

Results

Single Target

The first experiment involved a single ND filter target embedded in the Intralipid-10% suspension in water as described above. Figure 3(A) shows the temporal profile of the transmitted pulse without the target inside the medium. The 80-ps time gate was centered at a time that corresponded to the peak position of the transmitted pulse profile. Two-dimensional images were recorded at each scanning position, a typical image being shown in Figure 3(B). Figure 3(C) shows the horizontal intensity profile obtained by integrating a 3-pixel wide area around the white dashed line shown in Figure 3(B). The solid line in Figure 3(C) is a spatial profile without the target inside the medium, while the dotted line is a corresponding spatial profile when the target was on the incident beam path. The solid (dotted) line in Figure 3(D) presents the spatial profile when the target was to the left side (solid curve, C_L) or to the right side (dashed curve, C_R) of the incident beam path.

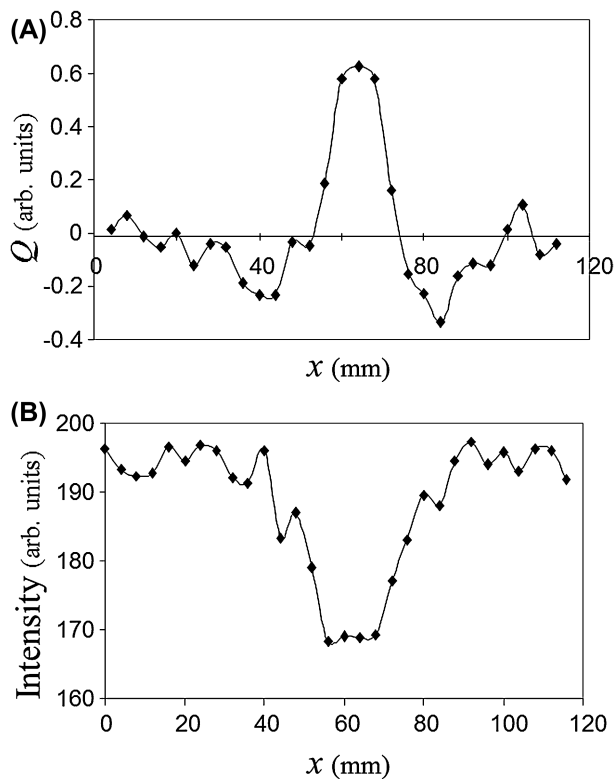


Figure 5: Line image showing variation of (A) Q and (B) transmitted intensity, as a function of position (x) to compare the current imaging approach with a diaphanography-type approach.

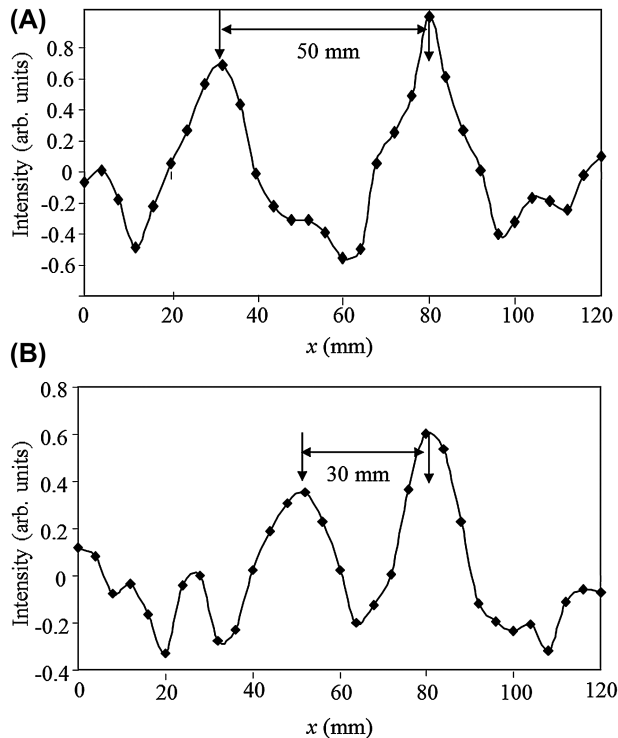


Figure 6: Resolving two targets described in the text when the separation between them is (A) 50 mm and (B) 30 mm.

For a quantitative evaluation of this intensity distortion, the spatial intensity profiles were obtained for all 21 scanning positions, and the corresponding centers of intensity distributions, M_i ($i = 1, 2, \dots, 21$) were calculated using Eq. [1]. In this scheme, the average of the 21 spatial intensity profiles was obtained, its center of intensity was calculated, and taken to be the reference, M_0 . It should be noted that M_0 thus obtained agreed very well with the value calculated using the solid curve in Figure 3(C), the profile without the target embedded in the medium. This close agreement validates the approximation that the average profile is a good representation of the profile without target, and that the approach is applicable for practical situations when it is not realistic to take the target out, provided the target-to-sample volume ratio is small. We have found that this approximation for background works well in other optical imaging applications, as well (13).

The differences, $\Delta M_i = M_i - M_0$, were calculated and plotted as a function of scanning position as shown by the rectangles in Figure 4. ΔM is negative in the left side of this CIC, passes through a minimum, reaches zero, rises to a maximum and tends to zero at the right end of the scan. The curve has inversion symmetry about the zero position. The zero point represents the center position of the embedded target. The dashed curve in Figure 4 shows the first derivative of the center

of intensity $Q = d(\Delta M)/dx$, which peaks at the target position. The FWHM of Q vs. scan position curve in Figure 4 is approximately 12.6 mm, which is close to the known target width of 10 mm.

Comparison with Transillumination Imaging

While the current approach uses the transmission geometry, it is different from transmitted intensity-based approaches, such as, diaphanography that has been used to obtain 2-D images, and many image reconstruction approaches make use of transmitted intensity. A comparison of with a transmitted intensity-based imaging approach would shed light on the efficacy of the current approach. We used a sample similar to that used in the experiment described above, except that the target was a $5 \text{ mm} \times 5 \text{ mm} \times 3 \text{ mm}$ neutral density filter placed into the Intralipid-10% suspension at a distance of 25 mm away from the input surface. The sample was scanned in the horizontal (x) direction in 4-mm steps over a distance of 120 mm, and time-gated 2-D images similar to that in Figure 3(B) were recorded for each scanning position. Figure 5(A) shows a plot of Q vs. x . The corresponding transmitted intensity-based plot, displayed in Figure 5(B), presents the integrated intensity of the respective 2-D images as a function of scan position. The FWHM of the Q -profile is 10 mm, and that of the

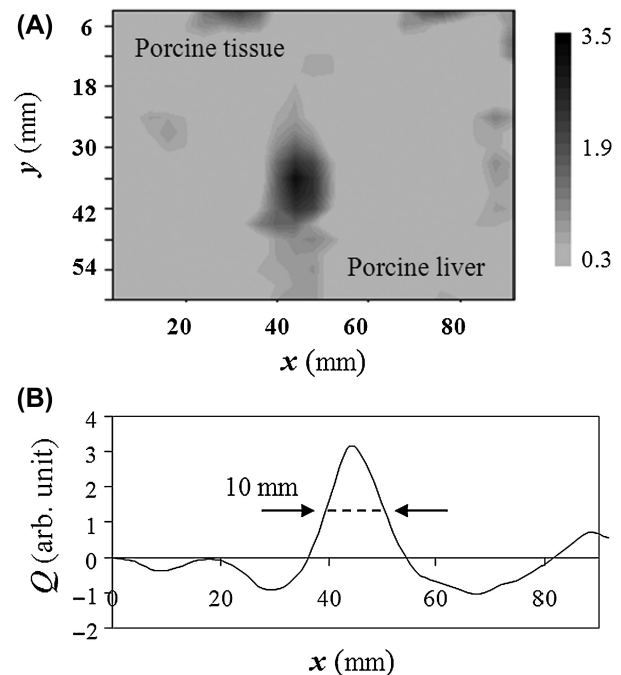


Figure 7: (A) Two-dimensional image (Q in gray scale vs. lateral position) of an approximately $5 \text{ mm} \times 5 \text{ mm} \times 5 \text{ mm}$ piece of porcine liver embedded inside a $150 \text{ mm} \times 90 \text{ mm} \times 50 \text{ mm}$ slab of porcine tissue. (B) Horizontal spatial profile showing Q vs. x along a horizontal line through the image (dark area) center.

transmitted intensity-based profile is 23 mm, both of which should be compared to the known 5-mm lateral dimension of the ND filter target. While the over estimation of the target size is a consequence of the diffuse nature of light propagation through turbid media, the center-of-intensity approach seems to provide a substantially higher spatial resolution than the transmitted intensity-based approach!

Imaging of Two Targets

We next tested the ability of the center-of-intensity approach to image more than one target. We used the same Intralipid-10% suspension as the turbid medium, and two ND filters of dimensions $5\text{ mm} \times 5\text{ mm} \times 3\text{ mm}$ and $5\text{ mm} \times 5\text{ mm} \times 6\text{ mm}$, as two targets. The targets were placed in the same mid-plane ($z = 30\text{ mm}$). Figure 6(A) and Figure 6(B) show the respective Q vs. x plots when center-to-center distance between the two targets was maintained at 50 mm and 30 mm, respectively. As the figures reveal both the targets are well resolved, and the respective center-to-center separations between the two targets are also retrieved within 2 mm from the known values.

Imaging of an ex vivo Tissue Specimen

The potential of this approach for optical biomedical imaging was next explored in an experiment using *ex vivo* biological tissues. The target was an approximately $5\text{ mm} \times 5\text{ mm} \times 5\text{ mm}$ piece of porcine liver tissue embedded inside $150\text{ mm} \times 90\text{ mm} \times 50\text{ mm}$ slab of porcine tissue held in a rectangular plastic cell. The center of the target was at a distance of 25 mm from both the entrance and exit faces of the porcine tissue sample. A $100\text{ mm} \times 60\text{ mm}$ inner region of the sample was scanned across the incident laser beam. The sample cell was scanned with a step size of 4 mm along the x -direction (100 mm segment). The sample was then stepped by 3 mm along the y -direction, and the x -scan was repeated. This scanning pattern was continued until the entire $100\text{ mm} \times 60\text{ mm}$ region was scanned. CIC curves were generated for every scan along the x -direction, and the value of Q was estimated for each scanning position. Plotting of Q vs. position (x, y) generated a 2- D gray-scale image of the target, as shown in Figure 7(A). The dark area with high Q -value represents the porcine liver specimen that absorbed 800-nm light more strongly than the porcine muscle tissue. Figure 7(B) shows the spatial profile of the image obtained by integrating a 3-pixel wide area around a horizontal line through the center of the dark area in Figure 7(A). The FWHM of the profile is $\sim 10\text{ mm}$, which is larger than the target width of $\sim 5\text{ mm}$, but comparable to the resolution that other optical tomography methods obtain (8, 13). Porcine tissue is an inhomogeneous medium and we tentatively attribute the fainter structures in the image of Figure 7(A) to such inhomogeneities.

It should be noted that target width estimation always yielded a larger width than the actual size of the targets used in different experiments reported in this article. This size broadening is a consequence of the diffusive nature of light propagation through a highly scattering medium. In principle, using a shorter gate width may reduce the variation but the estimate will always be somewhat higher. Too short a gate-width will lead to a significant reduction in overall signal level and may compromise the image quality.

Summary

In summary, the paper presents a new approach for detecting, imaging and retrieving 2- D location information of absorbing targets embedded inside a highly-scattering turbid medium. It uses a two-stage reconstruction method that relies on time-gated transmittance measurements and calculation of the center of the spatial distribution of the output light intensity. Shadow generated by a target alters the SDOLI enabling computation of 2- D localization map. It is in this respect that the approach differs from transmitted intensity-based, diaphanography-type optical imaging techniques. In a test-of-concept experiment it provided a better spatial resolution than that provided by a typical transmitted intensity-based method. The method is suitable for detection and 2- D mapping of multiple targets. A 2- D imaging experiment using *ex vivo* porcine tissue demonstrated the potential of this approach for optical biomedical imaging, such as, a tumor in the breast. More work using normal and cancerous human breast tissues will be needed to realize that potential.

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