
AFRL-AFOSR-VA-TR-2019-0031

# Molecular imaging of human performance biomarkers at cellular resolution in vivo

**Adam de la Zerda**
**LELAND STANFORD JUNIOR UNIVERSITY**

**02/01/2019**
**Final Report**



Air Force Research Laboratory
AF Office Of Scientific Research (AFOSR)/ RTB2
Arlington, Virginia 22203
Air Force Materiel Command

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 31-1-2019 | Final Report | Nov 2014 - Oct 2018 |

**4. TITLE AND SUBTITLE**

Molecular imaging of human performance biomarkers at cellular resolution in vivo

**5a. CONTRACT NUMBER**

FA9950-15-1-0007

**5b. GRANT NUMBER**

BAA-AFOSR-2013-005

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Adam de la Zerda

**5d. PROJECT NUMBER**

1177101

**5e. TASK NUMBER**

100

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Leland Stanford Junior University, The Stanford University
450 Serra Mall
Stanford, CA 94305-2004

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

Air Force Office of Science and Research
875 Randolph Street
Suite 325 Room 3112
Arlington, VA 22203-1954

**10. SPONSOR/MONITOR'S ACRONYM(S)**

AFOSR

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION/AVAILABILITY STATEMENT**

Approved for public release; distribution unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Previously we described a novel imaging technique developed in our group, Speckle-Modulating Optical Coherence Tomography, (SM-OCT), which dramatically improves the quality of optical coherence tomography images by reducing speckle noise. We have extensively explored the use of SM-OCT for brain imaging, presenting some of the highest resolution images of brain structures taken over a wide field of view of many millimeters. Some of the smaller brain structures are not visible at all in regular OCT images due to speckle noise. In living mice, we find that SM-OCT allows for visualization of distinct cortical layers, as well as individual white matter fascicles. In freshly resected human brain tissue, SM-OCT resolves the first three cortical layers as well as individual myelinated axonal processes, primarily in layers one and three. Finally, our experiments with contrast agents indicate that SM-OCT is highly complementary with our molecular imaging platform MOZART, enabling improved sensitivity of nanoparticle detection. By leveraging these techniques, we demonstrate near real-time tracking of leukocyte migration and distribution within the brain of a living mouse. We believe the resolution and sensitivity of SM-OCT will allow it to be used as a versatile clinical and scientific tool for non-invasive, serial imaging of various neurologic processes.

**15. SUBJECT TERMS**

Photoacoustic imaging, Molecular imaging

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| U | U | U | UU | 6 | 19b. TELEPHONE NUMBER *(Include area code)* |

STANFORD
SCHOOL OF MEDICINE
*Stanford University Medical Center*

Final Annual Performance Report
Nov 1, 2017 – Oct 31, 2018

**Principal Investigator**: Adam de la Zerda, PhD

**Institution**: Stanford University, 450 Serra Mall, Stanford Ca 94305-2004

**Award No**: FA9550-15-1-0007

**Government Program Manager**: Dr. Patrick Bradshaw,
patrick.bradshaw.3@us.af.mil

**Air Force Grant Progress Report 10-31-18**
**Adam de la Zerda, PhD.**

***Objective for Year 4***

***Status of Effort***

In the previous year, we had developed a modification to existing optical coherence tomography (OCT) imaging systems that allows for dramatically improved OCT image quality by reducing speckle noise. We called this technique Speckle-Modulating OCT, or SM-OCT.

Since that time, we have discovered numerous applications for SM-OCT, particularly for imaging in the brain, an effort guided by a resident neurosurgeon in our lab. We performed extensive imaging experiments on the brains of live mice, and ex vivo human samples. The SM-OCT images collected represent the highest resolution imaging of brain structures over a field of view of millimeters, with only endogenous contrast. Further, we have demonstrated how SM-OCT can enable improved molecular contrast for in-vivo imaging, allowing us to capture the dynamics of targeted leukocytes inside of a living mouse's brain.

Based on these findings, we believe SM-OCT lends itself to a myriad of scientific and clinical uses. For example, SM-OCT could allow for serial in vivo imaging in murine models of neurologic disease such as Alzheimer's disease, epilepsy, and stroke. A major advantage of serial imaging is that it allows for the disease process within a single mouse to be tracked over time, in response to treatments, and across different stages of disease. The resolution provided by SM-OCT may yield new insights into neurologic disease processes, a limitation of current studies that rely on MRI or PET, which have inherent resolution limitations.

***Year 4 Accomplishments***

***SM-OCT for in-vivo murine neuroimaging***
While regular OCT imaging penetrates brain tissue to a depth of 1.5mm and can resolve the gross structure of the hippocampus and corpus callosum, speckle noise precludes the resolution of further structural detail (Fig. 1b, 2a, 2d, 3a, and 3c). SM-OCT significantly reduces speckle noise, revealing the fine anatomical structure of the brain including cortical layers (Fig. 1c) and white matter fascicles (Fig. 2h and 2i). We obtained these results by outfitting live mice with glass cranial windows, and then imaging them with both OCT and SM-OCT.

Mammalian cortex has four to six distinct layers that are differentiated both histologically and functionally. SM-OCT show distinct variability between the signal intensity among the different cortical layers, which can be explained by differences in the density of cells and neuritic processes between layers. Small individual white matter fascicles of the cingulum bundle, corpus callosum, and alveolus of the hippocampus are clearly visualized in vivo for the first time with optical imaging using SM-OCT (Fig. 2b, 2e-h, Fig. 3b, 3d. These fascicles are approximately 40-80 um in diameter (Fig 2f-g), and cannot be resolved using OCT (Fig 3a, 3c). To our knowledge, these structures have not been directly visualized in situ in live animals prior to this study.

Closer examination of coronal plane SM-OCT images reveals even smaller structures, whose size (6-18 um) and location are consistent with individual myelinated axons or very small fascicles consisting of a small number of myelinated axons (Fig. 2h). These structures can also be seen coursing from the medial cortex into the cingulum bundle in the axial plane. These structures also cannot be seen with OCT due to speckle noise (Fig. 3c). As a wide-field in vivo neuroimaging platform, SM-OCT can resolve a wide range of tissue features from cortical layers to small white matter fascicles up to 1.5 mm deep within tissue using only endogenous contrast.

***Tracking leukocytes in an in-vivo mouse model***
In a separate set of experiments performed on mouse, in-vivo, we were able to specifically label leukocytes in-vivo with large gold nanorod contrast agents (LGNRs). The combination of SM-OCT with our previously described molecular imaging platform, MOZART, allowed us to resolve small numbers of LGNR's that had been

ingested by individual leukocytes. After labeling, we re-injected the labeled cells and demonstrated near real-time tracking of leukocyte migration and distribution within the brain in full 3d. This allowed us to glean insight into leukocyte movement patterns, such as the distribution of migration speeds, and the total migration distance of individual cells. In future studies, the method described herein may also provide the necessary means to characterize leukocyte response to various therapeutic regimens. The results of this work have been submitted for publication and are currently under review.

***SM-OCT for ex-vivo human brain imaging***

Given the promising results of SM-OCT in murine neuroimaging, we investigated its performance for directly imaging human brain tissue. A fresh, unfixed section of the inferior temporal gyrus from a temporal lobectomy was taken directly from the operating room to our laboratory. As with in-vivo murine imaging, OCT was able to image human brain tissue to a depth of greater than 1.5 mm. However, speckle noise in the OCT image prevents the resolution of cortical layers or axonal processes (Fig. 4a). SM-OCT clearly resolves the first three cortical layers as well as individual myelinated axonal processes, primarily in layers one and three (Fig. 4b, 4d, and 4e). Axons exist in all cortical layers. Since SM-OCT is dependent on reflected light, axons oriented perpendicular to the incident beam, such as those in layers one and three, provide significantly greater signal, allowing them to be resolved very clearly.

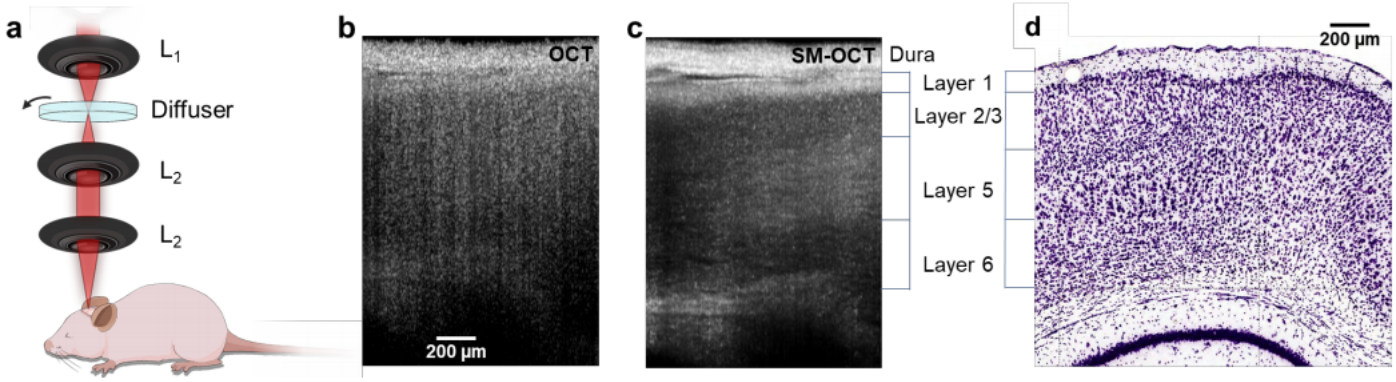


**Figure 1: SM-OCT imaging of mouse brain in vivo reveals cortical layers.** a) The sample arm of the SM-OCT system, L1 is the main lens of the OCT, the diffuser is rotated in the focal plane, which is relayed by two lenses, L2, in a 4f configuration. b) OCT B-scan of mouse cortex. c) SM-OCT B-scan of mouse cortex, showing the cortical layers, which are revealed by removing the speckle noise. d) Histology of mouse brain (image credit: Allen Institute), showing the corresponding cortical layers to SM-OCT

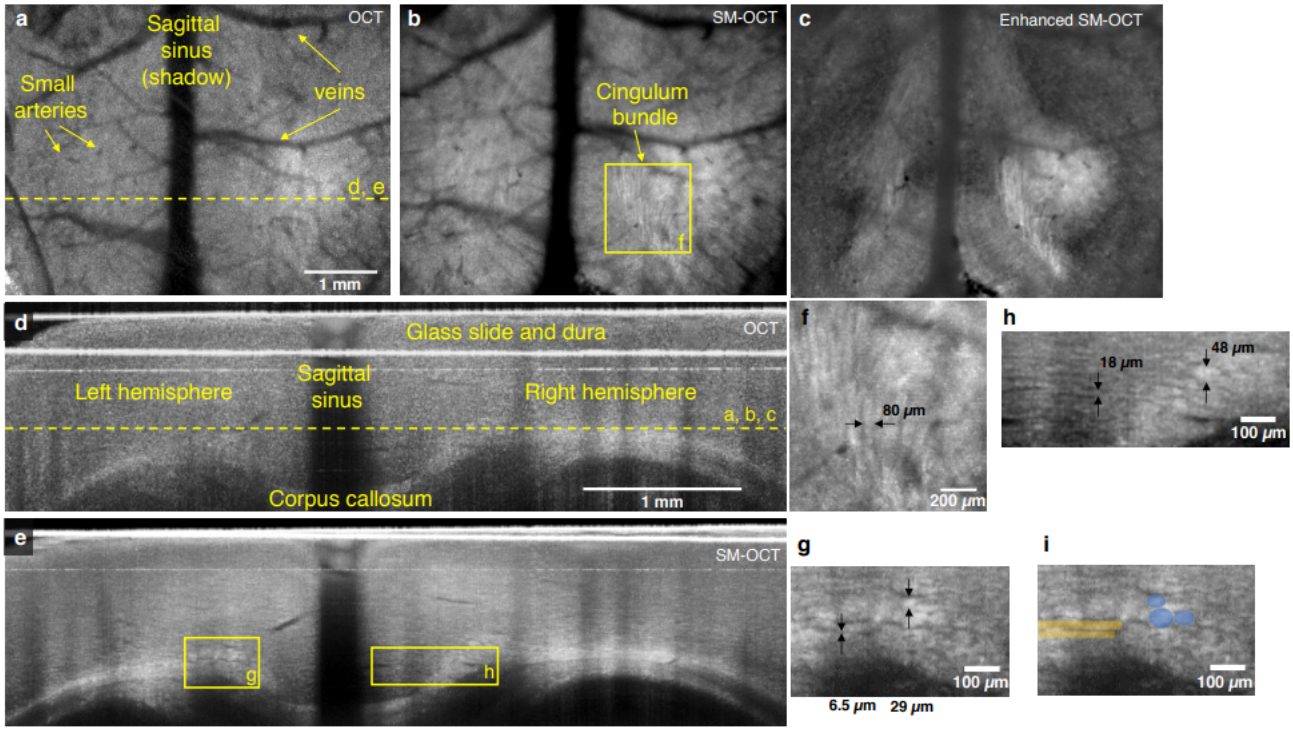


**Figure 2: SM-OCT reveals white matter fascicles in mice in vivo.** a) OCT axial view of mouse cortex, depth is shown as yellow dashed line in (d). b) SM-OCT image of the region showed in (a). The removal of speckle reveals white matter structures, including the cingulum bundle. c) The white matter structures shown by SM-OCT can be enhanced by image processing. d) OCT coronal view of mouse cortex, the location is shown as yellow dashed line in (a). SM-OCT image of region shown in (d), revealing the white matter structures in high-resolution. f) A close-up axial view of the cingulum bundle. g, h) close-up coronal views of white matter structures of various sizes including the tracts of the cingulum bundle and very small unnamed fascicles. i) close-up coronal view with manual segmentation of several fibers of cingulum bundle (purple) and very small unnamed fascicle (yellow).

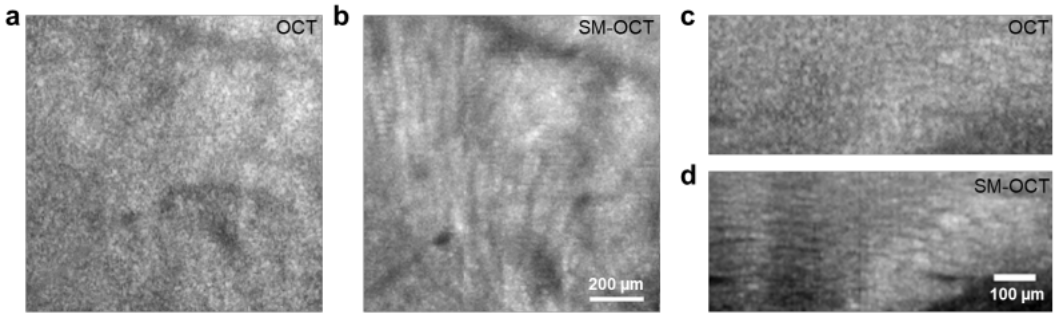


**Figure 3: Comparison between SM-OCT and OCT images of mouse white-matter structures in vivo.** a, b) OCT and SM-OCT (respectively) axial close up views (en-face) of the cingulum bundle. The structures of the fascicles are revealed through speckle removal. c, d) OCT and SM-OCT (respectively) coronal close up views (B-scans) of the white matter structures, revealed in high-resolution when using SM-OCT.

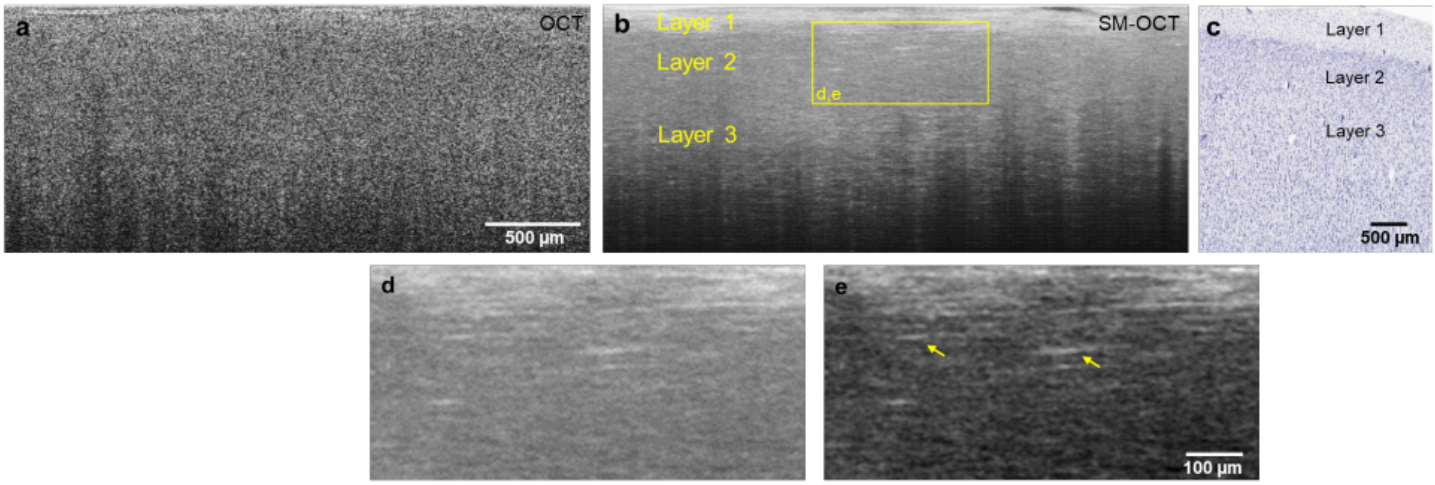


**Figure 4: SM-OCT of ex vivo human brain sample reveals cortical layers and axons.** a, b) OCT and SM-OCT B-scans of cortex. The SM-OCT image reveals cortical layers and myelinated axonal projections. c) Corresponding histology (image credit: Allen Institute 57). d, e) a close-up view of the myelinated axons shown in b. The contrast in e is enhanced to highlight the myelinated axons

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**Personnel Supported: Updated for 2018**

List professional personnel (Faculty, Post-Docs, Graduate Students, etc.) supported by and/or associated with the research effort.

| Name | Project Role | Grant Support |
|---|---|---|
| Adam de la Zerda | Co-PI | 5% |
| Peng Si | Post-doc | 100% |