FLUORESCENT HETEROGENEITIES IN TURBID MEDIA: LIMITS FOR DETECTION WITH DUAL-INTERFERING SOURCES EXCITATION

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Abstract- **A quantitative comparison between the single source and the dual-interfering sources configurations for the detection of fluorescent heterogeneities embedded in a piecewise highly scattering homogeneous fluorescent background was carried out. The study is based upon simulations using analytical solutions of the frequency domain diffuse photon density waves and practical signal-to-noise ratio considerations. Results show that the dual-interfering sources outperformed single source techniques for the detection of heterogeneities in terms of fluorophore concentration and lifetime contrast. To detect the same inhomogeneity, less concentration and lifetime contrast are required with dual-interfering sources.**

Keywords- **Phased-array imaging systems; Photon migration; Light propagation in tissues; Photon density waves; Fluorescence.**

I. INTRODUCTION

Diffuse Optical Spectroscopy (DOS) and Diffuse Optical Tomography (DOT), may have a major role in breast cancer research and detection in the future years [1,2]. Even if these techniques are limited to several millimeters in scale [3], their ability to probe hypoxia and angiogenesis, which are two correlates of breast malignancy, place them as good candidates to complement already existing modalities such as MRI.

Already, first clinical *in-vivo* results have been reported [4,5]. They have demonstrated the ability of the techniques to quantify blood concentration and fractional saturation of tissue hemoglobin with oxygen. However, this could be done reliably only for significant tumor masses.

To improve prognosis, early detection of tumors is desirable [6]. Intrinsic parameters in the early stage of tumor growth seldom lead to enough contrast between healthy and diseased tissues. To overcome this difficulty, the use of optical contrast agents is sought. Especially, new generation of extrinsic compounds are engineered to target molecular and physiological functions of the tumors [7]. Moreover, these contrast agents fluoresces in the NIR window allowing probing deep tissue. Therefore, cancers could be detected at their molecular onset before anatomic changes become apparent, according to an appropriate engineered fluorescent probe.

Another solution to improve the ability of NIR diffuse optical techniques for detection of early tumors is to use phase and amplitude cancellation techniques [8,9,10]. In these configurations, two rf-modulated out-of-phase sources with the same strength illuminate the media. The two scalar diffuse photon density waves propagate and add incoherently to create an interfering-like pattern. The modulated amplitude is characterized by a null-plane at mid-distance for the two sources and the phase has a sharp 180° transition across this plane. These two features are specific to phased array systems. The presence of an absorptive inhomogeneity modifies this pattern, in effect pulling the null-line and the phase transition line towards the object. This configuration has been suggested to be more sensitive to the presence of an absorber than single-source configurations [11]. Experimental studies and clinical trials using the configuration have proven valuable for detection of tumors [12] and physiological monitoring [13].

In this present work we focus on a quantitative study of dual-interfering sources excitation in fluorescent mode. Analytical solutions of Fluorescent Diffuse Photon Density Waves (FDPDW) in the frequency domain were used to calculate the fluorescent amplitude and phase. The set-up configurations and the optical values used matched the typical case of breast optical mammography with the use of Indocyanine Green (ICG).

An extensive comparison between single and dual source configurations was carried out. More precisely we compared single and dual-source configurations detection limit dependence upon fluorophore concentration and lifetime contrast between the inhomogeneity and the background.

II. METHODS

A. Analytical solutions

Distribution of contrast agents in the tissue can be mediated by several factors, including leaky vasculature, angiogenesis and hypermetabolism. In many cases, contrast agents are still present in the healthy tissue when measurements are performed. For instance, ICG uptake has been shown to be different in various lines of tumors

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Abstract

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[14,15]. Thus, to model such distribution of contrast agents, we simulate a fluorescent heterogeneity embedded in a piecewise homogeneous fluorescent background.

For such a configuration, we consider an intensity-modulated point light source that excites the fluorophores. When the excited fluorophores radiatively relax, fluorescent photons are generated that propagate diffusely through the turbid medium. The emissions from all the fluorophores in the medium collectively add to create a FDPDW. Xingde *et al.* [16,17] derived analytical solutions to model this phenomenon. For the case of a single source excitation, they have the mathematical form:

$$\Phi^{\text{flr}}_{\text{hetero}}(r_s, r_d, \omega, a) = \Phi^{\text{flr}}_{\text{homo}}(r_s, r_d, \omega) + \Phi^{\text{flr}}_{\text{sc}}(r_s, r_d, \omega, a)$$
$$= \frac{\varepsilon q_1 \eta_1 N_1}{1 - i\omega\tau_1} F_1(r_s, r_d, \omega) + \frac{\varepsilon q_2 \eta_2 N_2}{1 - i\omega\tau_2} F_2(r_s, r_d, \omega, a) \qquad (1)$$

where $\Phi^{\text{flr}}_{\text{homo}}(r_s, r_d, \omega)$ is the homogeneous FDPDW, and $\Phi^{\text{flr}}_{\text{sc}}(r_s, r_d, \omega, a)$ the scattered FDPDW. Here r_s and r_d are the source and the detector positions, respectively, a is the radius of the inhomogeneity, and ω is the angular source modulation frequency. N_1 and τ_1 are the fluorophore concentration and lifetime respectively, in the homogeneous background medium (*i.e.*, outside the spherical inhomogeneity); N_2 and τ_2 are the concentration and lifetime inside the inhomogeneity (*cf.* **Fig. 1**). ε is the fluorophore extinction coefficient at the excitation wavelength λ_{ex} and q_1 (q_2) is the quenching factor outside (inside) the object. η_1 (η_2) is the fluorescence quantum yield outside (inside) the object. For simplicity, we set $\eta_1 = \eta_2 = 10\%$ in this discussion. Expression of $F_1(r_s, r_d, \omega)$ and $F_2(r_s, r_d, \omega, a)$ could be found in ref 16.

To model dual-interfering sources we use the addition theorem, *i.e.* we compute independently the field for two different positions of the sources with a 180° phase difference. The summation of the two fields gives us the value of the field in the case of a dual-interfering excitation.

B. Models

The medium simulated is presented in **Fig. 1**. It's an infinite long slab with a finite width of 5cm. A single spherical inhomogeneity is embedded in the center of the slab. To model the planar boundary conditions, extrapolated zero boundary conditions with image sources were applied [18].

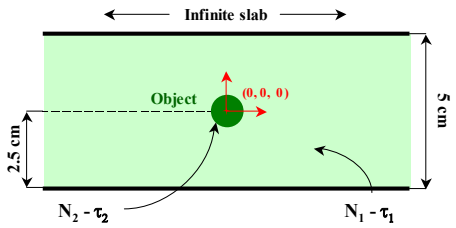

Fig. 1: Geometrical set-up simulated. The background (inhomogeneity) medium is characterized by a fluorophore concentration N_1 (N_2) and a lifetime τ_1 (τ_2).

Values of the chromophore optical properties at the excitation wavelength (λ_{ex}) and the emission wavelength (λ_{em}) are presented in **Table 1**. These values were used for all the simulations herein.

Table 1: Chromophore optical properties at λ_{ex} and λ_{em} and source modulation frequency. Units in (cm^{-1}).

Background				Inhomogeneity			
λ_{ex}		λ_{em}		λ_{ex}		λ_{em}	
μ^c_{a1}	μ'_{s1}	μ^c_{a1f}	μ'_{s1f}	μ^c_{a2}	μ'_{s2}	μ^c_{a2f}	μ'_{s2f}
0.02	8.0	0.025	8.0	0.0205	8.0	0.0256	8.0

The optical properties related to the homogeneous medium are indicated by a subscript of 1; optical properties related to the object are indicated by a subscript of 2; optical values related to the fluorescent DPDW's are indicated with an extra subscript of f. We use a superscript of c to indicate chromophore related parameters.

In the case of single source, the object was located in front of the source and the detectors. In the case of the phased array system, the sources were separated by 2cm and modulated at 200MHz. The detector was placed 0.25cm off the null line, closer to the source in front of the object. The object in the dual-interfering configuration was placed in front of a source.

The characteristics of the chromophore simulated are presented in **Table 2**.

Table 2: Quantum yield η, background fluorophore concentration N_1, lifetime τ_1 and excitation coefficients ε and ε_f.

η	N_1	τ_1	ε (at λ_{ex})	ε_f (at λ_{ex})
10%	0.1 μM	1.0 ns	0.5×10^5 $cm^{-1}M^{-1}$	0.05×10^5 $cm^{-1}M^{-1}$

These parameters were chosen to be closer to those of ICG [19]. It is important to note that the background concentration is relatively low. This enables us to avoid problems such as auto-quenching that can occur at higher concentrations.

Exogenous contrast agents provide two kinds of contrasts for tumor detection. The first one is related to the relative concentration of fluorophore in the tumor versus the background that can result from angiogenesis and high permeability of the capillary bed. The other one is the fluorophore lifetime that can be environmentally sensitive (PH, PO_2…). Therefore, we investigated these two contrasts.

B. Criteria

The criteria that we use to determine the detection limits are based on the signal-to-noise analysis. In the case of single source, the noise model was considering the fractional amplitude noise level of 1% and the phase random noise of 0.5°. Thus, the heterogeneity was detectable when the

relative amplitude and phase of the FDPDW, were above the noise threshold ($\left|\Phi^{flr}_{hetro}/\Phi^{flr}_{homo}\right|-1>1\%$ and $\left|\mathrm{Arg}(\Phi^{flr}_{hetro})-\mathrm{Arg}(\Phi^{flr}_{homo})\right|>0.5°$).

The noise model used for the dual-interfering sources configuration is detailed elsewhere [20]. With this model, a level of noise was estimated for the homogeneous measurement. The case of the optical parameters of **Table 1** and fluorochrome characteristics of **Table 2** lead to a noise of 1.5% in amplitude and 0.7° in phase. Thus the criteria employed in the phased array configuration were: $\left|\Phi^{flr}_{hetro}/\Phi^{flr}_{homo}\right|-1>1.5\%$ and $\left|\mathrm{Arg}\left(\Phi^{flr}_{hetro}\right)-\mathrm{Arg}\left(\Phi^{flr}_{homo}\right)\right|>0.7°$.

III. Results and Discussion

A. Relative concentration and lifetime variation contrast

Using the signal-to-noise criteria described above, fractional amplitude perturbation and relative phase change contours for different inhomogeneity radii over a broad range of fluorophore concentration and lifetime contrast are presented in **Fig. 2**.

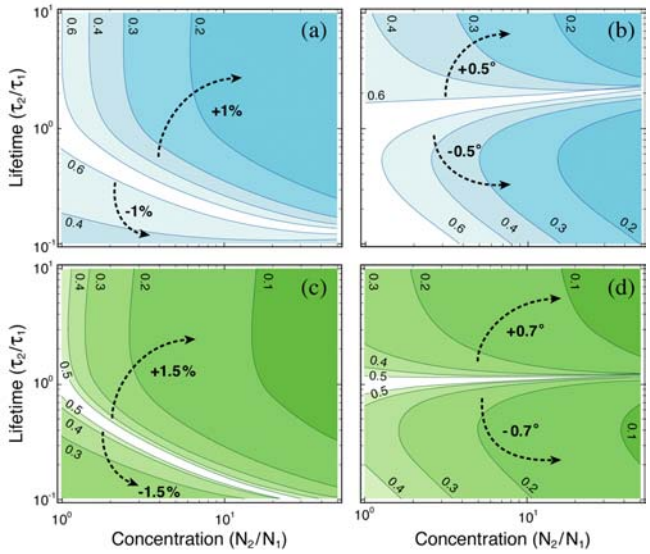

Fig. 2: Contour plot of the (a)-(c) fractional amplitude and (b)-(d) phase difference for single source system ((a)-(b)) and phase array system ((c)-(d)). The legends on the iso-contours correspond to the radius of the inhomogeneity. The filled areas correspond to the parameter space where the perturbation was higher than the criteria used.

First, we can note that in both single and dual sources cases, the increase of concentration and lifetime contrast induced an increase in the perturbation, both in amplitude and phase. Thus, the higher the concentration contrast and the higher the lifetime contrast, the smaller the detectable object is.

The increase of fluorophore concentration induces two opposites effects. The first one is the increase in fluorescent photons due to the higher number of fluorophores excited. Second is the decrease of photon fluence due to the increase in absorption both at the excitation and fluorescent wavelength. In all the simulations herein, the second effect is small as long as the absolute concentrations are low. This is depicted in the fractional amplitude contours (**Fig. 2** (a)-(c)). The higher the concentration, the higher the perturbation is. So, for a given lifetime τ_2, as the fluorophore concentration N_2 increases, the object size required to produce the same amount of amplitude and phase perturbation decreases, and therefore a smaller object can be detected.

In a similar manner, the increase in the fluorophore lifetime leads to two opposite effects on the FDPDW amplitude. On one hand, the fluorescent fluence increases with longer lifetimes due to less quenching ($q_2 \propto \tau_2$). On the other hand, the fluorescent fluence decreases with longer lifetimes due to greater demodulation ($\propto 1/(1-i\omega\tau_2)$). For a given fluorophore concentration N_2, the fluorescent amplitude will increase until the two effects described above cancel and the fluorescent fluence reaches a saturation state.

Even if the same trends could be found in both techniques (single and dual sources), there are quantitative differences. For instance, let us consider the detection of a 0.2cm radius inhomogeneity. In the case without lifetime contrast (which is relevant to ICG as long as this dye does not appear to exhibit lifetime sensitivity related to tissue state [14]), the concentration contrast needed falls from 8.5 in single source configuration, to 3 in the case of the dual-interfering sources when amplitude is used. Thus, the threshold of detection is improved roughly by three in this case. When phase changes are considered in this specific case ($\tau_1=\tau_2=1\mathrm{ns}$), no obvious enhancement in detection threshold is achieved. On the other hand, if the lifetime contrast increases, even if the amplitude enhancement is not improved, the phase sensitivity is highly enhanced. For the 0.2cm radius heterogeneity, and for the lifetime contrast of 5, the detection threshold in term of concentration contrast falls from 20 to 2. As stated above, this enhancement also decreases with the increase in the heterogeneity radius.

B. Modulation frequency

A crucial point in the detectability of the phased array system is the configuration of the pair sources. This configuration could be modified in different way such as the separation between the sources, the modulation frequency, the relative amplitude and the relative phase.

The modulation frequency of the sources is an important factor as it has been shown that optimal modulation frequency can de derived for the single source system when phase detection is concerned in fluorescent mode. An example of the dependence of perturbation upon the modulation frequency for both single source and dual source system are presented in **Fig. 3**.

From **Fig. 3** one can see that both techniques follow the same trends in terms of frequency dependence. The contrast, in the case of phase array technique is quantitatively more important. But as stated by Xingde *et al.* [16], a simple estimate of the maximum perturbation can

de done using $\omega\tau_2 \approx 1$. This rule is important to optimize the system for a given lifetime contrast. Inadequate modulation frequency can tremendously hamper the ability to detect the inhomogeneity in both configurations

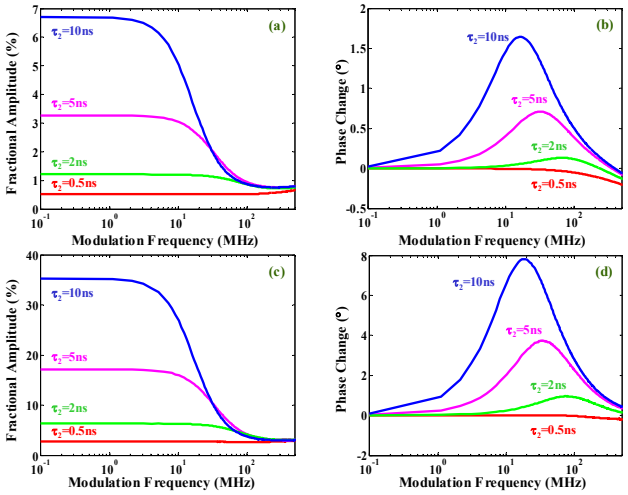

Fig. 3: (a)-(c) fractional amplitude and (b)-(d) phase difference for single source system ((a)-(b)) and phase array system ((c)-(d)). The legends on the plots correspond to lifetime contrast. The inhomogenity radius is set to 0.2cm with a concentration contrast of 5 (N_2=0.5μM).

VI. Conclusion and Perspectives

A study based on simulations using analytical solutions for fluorescent diffuse photon density waves in heterogeneous turbid media has been presented in this paper. The detection limit of dual-interfering sources compared with single source configuration was investigated. It was shown that dual-interfering sources were outperforming the single source technique for the spherical object detection. The detection threshold enhancement was up to 3 times for configuration relevant to ICG if amplitude was considered. Moreover, if a lifetime contrast was induced, this enhancement was even more appreciable. Also, the optimal modulation frequency was found to follow the rule of thumb proposed for single source, *e.g.* $\omega\tau_2 \approx 1$. These results are promising when considering the new generation of contrasts agents that are tumor specific.

However, these results are dependent upon the configurations investigated. Different parameters are involved in the sensitivity of phase cancellation technique. Modulation frequency and source separation have a fundamental impact as long as the technique is based on destructive summation. Thus, the impact of these parameters should be investigated simultaneously to find the best configuration. Finally, the quantification of chromophore concentration and lifetime contrasts could be achieved with proper analytical or numerical models to perform fluorescent DOT with dual-interfering sources [21].

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