Prediction of sensitivity thresholds in optoacoustic tomography

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ABSTRACT

Since optoacoustic tomography is considered a high-resolution modality, determination of the absolute detection limit, as it relates to the sensitivity of biomarker detection is not straightforward. This is due to the fact that experimental determination of the sensitivity as a function of target size remains difficult since no established technique exists so far to reproducibly create very small targets containing well-defined concentrations of markers. We combine theoretical analysis with imaging results for large amounts of the marker and place the measured value on the appropriate parameter-dependent signal intensity curve. A performance estimate of the particular experimental system and the expected signal-to-noise-ratio for smaller amounts of markers can then be made.

Keywords: Bio-markers, optoacoustics, molecular contrast agents, small animal imaging

I. INTRODUCTION

Over the past years biomedical research has witnessed significant advances in molecular biology and imaging technologies. These advances have led to dramatic shifts in both disease monitoring and treatment planning approaches. The traditional detection of pathological abnormalities relied upon defining gross anatomy and structure, whose alterations at the anatomical-morphological level are relatively late manifestations of underlying molecular changes. In contrast, recent advances have herald the ability to detect disease at the molecular level instead of the anatomical realm, giving great promise to early detection and increased survival rates of serious aliments. These new imaging technologies often use molecular probes or markers that bind specifically to molecular targets and allow for the non-invasive visualization and quantization of biological processes such as gene expression, apoptosis, or angiogenesis at the molecular level within intact living organisms. All these hold a great promise to enable early diagnosis, provide improved classification of stage and severity of disease, an objective assessment of treatment efficacy, deliver a reliable prognosis, and speed up the development of new therapies.

Growing in importance are optical investigations since they offer various optical contrast mechanisms, low cost, and the use of non-ionizing radiation. Yet, optical methods are plagued by the multiple scattering of light in tissue which reduces resolution and penetration depths [1]. Therefore, the new addition to the molecular imaging arsenal, the Multispectral optoacoustic tomography (MSOT) is a modality that has the potential to mitigate these caveats. Recently, MSOT was showcased as being capable of resolving molecular probes in tissues with ultrasonic resolution [2], owning towards its applicability to visualize molecular signatures in-vivo and longitudinally. Nevertheless, while the feasibility of different optoacoustic imaging implementations has been showcased and hypothetical assumptions have been reported in the literature predicting generally the potential sensitivity of the method for detection of contrast agents and bio-markers, little is known on the sensitivity performance from a theoretical and systematic stand-point. For this reason, we investigated herein the theoretically predicted sensitivity of the method over wide range of imaging-related parameters and provided the necessary experimental reference measurements. Optoacoustic signals were simulated emanating from a target bio-marker, represented by an absorbing sphere, which was embedded in tissue-mimicking scattering and absorbing phantoms. By accounting for diffuse light distribution and ultrasound dispersion as it occurs in tissues, we removed system dependent characteristics to yield a better understanding of performance and physical limitations of target detection using optoacoustics.

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A range of clinically relevant bio-marker concentrations was examined, covering four orders of magnitude in target radii, embedded in different tissue dimensions. We find the optoacoustic detection limits as they are constrained by the interplay of light penetration and ultrasonic frequency-dependent attenuation (dispersion) and further observe a non-linear performance in the detection limit, which invalidates simplistic linear predictions of optoacoustic sensitivity typically assumed in the literature. We experimentally verify and reference our theoretical findings in tissue-mimicking phantoms, using a newly developed multispectral optoacoustic tomography (MSOT) system.

II. NUMERICAL SIMULATION

In order to represent detection of realistic molecular target in typical whole-body small animal imaging configuration, we first simulated optoacoustic signal detected from a 2 mm diameter target containing various concentrations of a Cy5.5 (Molecular Probes, Inc) molecular contrast agent at increasing depths. The target was embedded in a 2 cm diameter turbid cylindrical phantom with reduced scattering coefficient coefficients of $\mu_s' = 9$ cm^{-1} and absorption $\mu_a = 0.15$ cm^{-1}. In realistic imaging scenarios, the light fluence cannot usually be assumed constant throughout the imaged volume, therefore we modeled light distribution by solving light diffusion equation for given geometries. For more accurate modeling, we have also considered frequency-dependent ultrasonic attenuation (dispersion) [3].

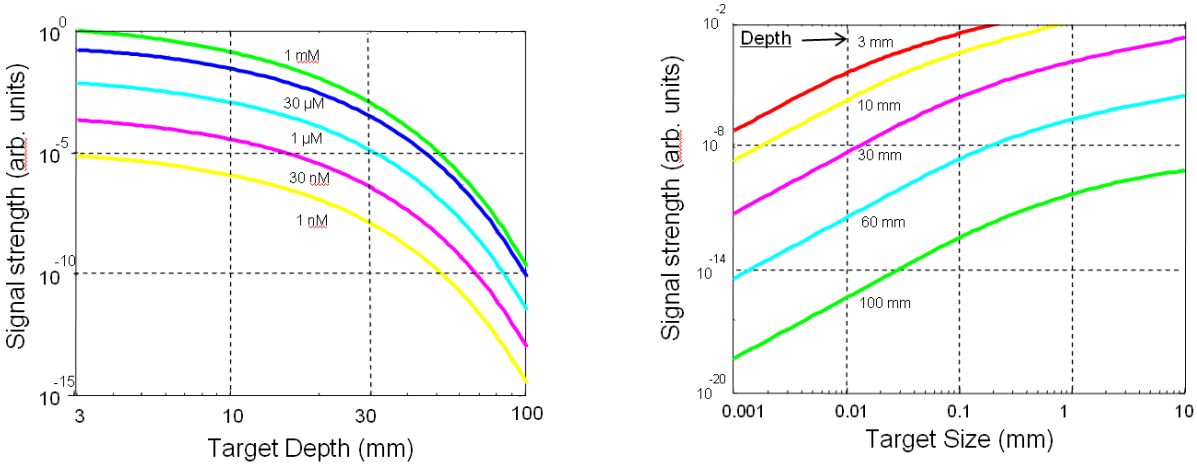

Figure 1. Simulated intensity of optoacoustic response from a small spherical target containing various concentrations of a Cy5.5 molecular contrast agent. The target was embedded in a 2 cm diameter turbid cylindrical phantom with reduced scattering coefficient coefficients of $\mu_s' = 9$ cm^{-1} and absorption $\mu_a = 0.15$ cm^{-1}. Detected optoacoustic signal variations are shown (a) for constant target size and varying concentration and depth; (b) for constant concentration and varying target depth and size.

As observed in Fig. 1(a), signals originating from contrast agents retain a linear dependence on concentration [4]. Conversely, varying depth scales non-linearly affect the detected signal intensity because signal amplitudes are affected by the attenuation of both light and ultrasonic waves as they propagate through tissue. Fig. 1(b) further emphasizes the dominate role ultrasonic attenuation plays by presenting the detection of pressure wave magnitudes from increasing sized targets containing 1 µM concentration of the dye ($\mu_a = 0.24$ cm^{-1}) at various depths. As seen in Fig. 1(b), as target radius decreases, the frequency content of the acoustic signals increases, resulting in the ultrasonic attenuation of signals to play a more dramatic role in signal detection [4].

III. EXPERIMENTAL VALIDATION

Phantoms were prepared in order to obtain physical measurements which could be compared against the simulated data. Tissue-mimicking cylindrical phantoms were created (cast to simulate optical parameters of tissue, $\mu_s' \approx 10$ cm^{-1} and $\mu_a \approx$

0.15 cm^{-1} at 670nm) were prepared. Insertions containing 5 μM of Cy5.5 near-infrared fluorescent dye (excitation peak 675 nm, emission peak 694 nm) suspended in a matching liquid, were introduced into the phantom. Optoacoustic signals were generated and recorded using the setup shown in Fig. 2.

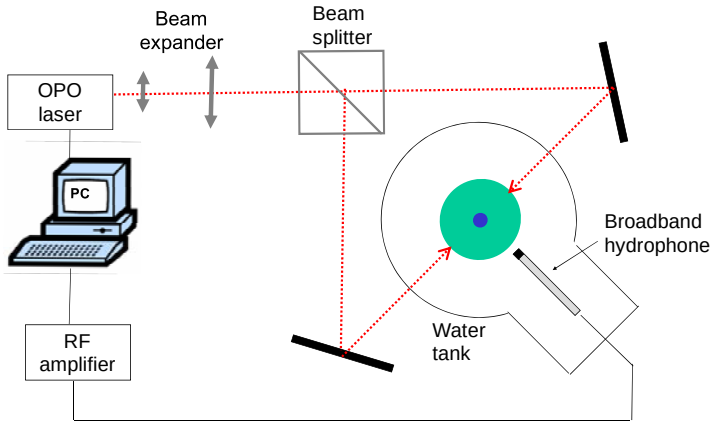

Figure 2. Schematics of the multispectral optoacoustic tomography (MSOT) system used for the experimental part.

Experimental validation was performed to determine the soundness of our optoacoustic signal detection theory, and the findings can be seen in Fig. 3. As evident, the experimental values are in substantial agreement with the simulated curves seeing as the acquired data points exhibit the same order of magnitude decay for signal attenuation in both conditions studied. Furthermore, we measured the system noise floor (dashed line in Fig. 3) that allowed us to predict detection limits of molecular probe detection. Those were found to be at concentrations of around several hundreds of nanomolars or equivalent amounts of several hundreds of femtomoles for experimental parameters used in this study [4].

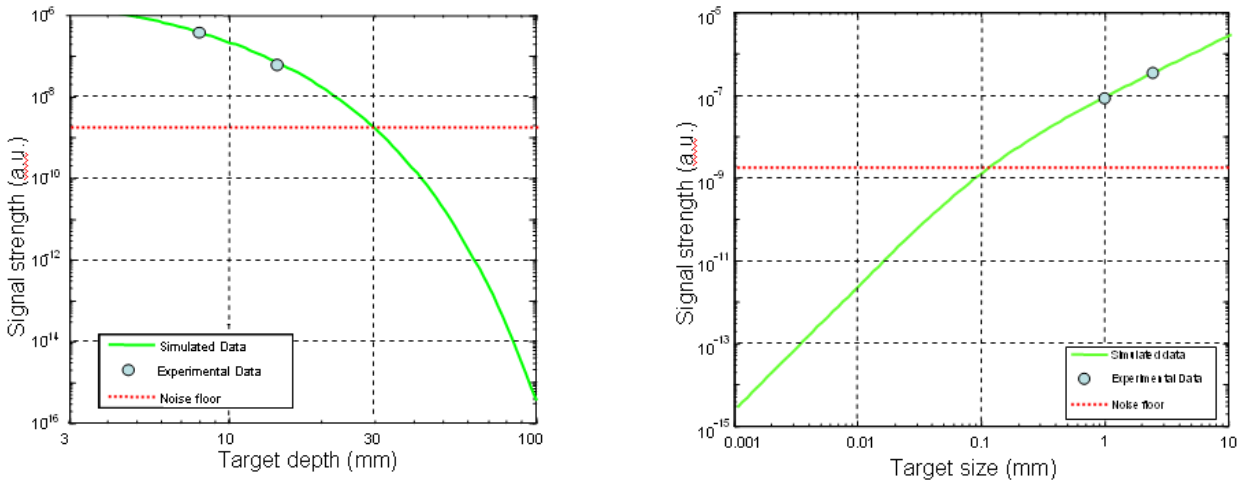

Figure 3. Optoacoustic signal detection phenomena from an experimental point of view.

IV. SENSITIVITY OF FLUORESCENT PROTEIN DETECTION

Using this theoretical and experimental framework, we have further predicted the sensitivity of the MSOT system (Fig. 2) for fluorescent protein detection. For this, tissue-mimicking phantom containing inclusion with DsRed-expressing HeLa cells was prepared (Fig. 4). The MSOT measurements has precisely resolved the location of the cells with contrast to noise ratio (CNR) of 32 (Fig 4d) by using spectral decomposition of images acquired at different wavelengths [2, 5]. Subsequently, since we know the actual concentration of cells, and by assuming a minimum detection limit corresponding to CNR of 5, the minimal detectable number of cells was estimated to be about 10^3 [5].

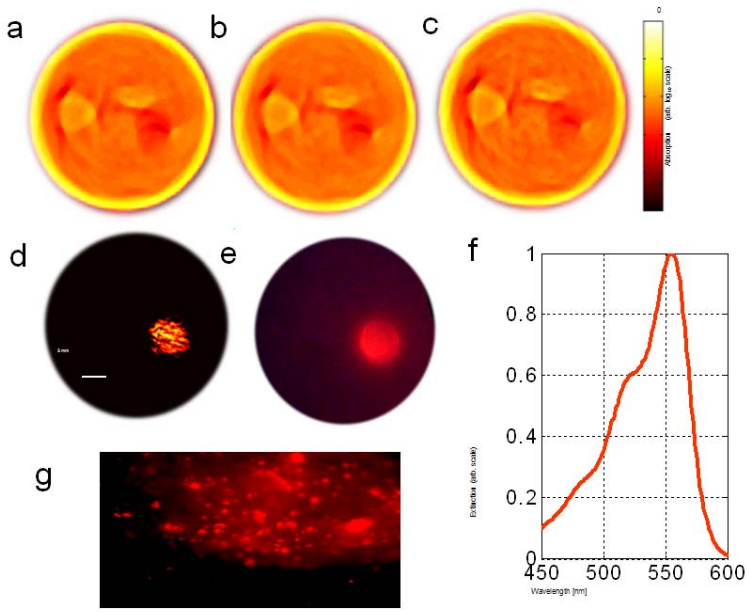

Figure 4. Multi-spectral optoacoustic imaging of tissue-mimicking phantom containing DsRed-expressing HeLa cells. Single-wavelength optoacoustic images of the phantom acquired at (a) 550 nm, (b) 560 nm, and (c) 570 nm; (d) Spectrally resolved (MSOT) image of DsRed distribution in the phantom; (e) Fluorescence image of dissected phantom at approximately the same imaging plane (red color corresponds to the location of fluorescent cells; (f) Extinction spectra of DsRed; (g) Magnified image of phantom at the boundary of area containing DsRed cells.

IV. DISCUSSION AND CONCLUSIONS

In this work we aimed in better understanding of performance and physical limits of molecular imaging using multispectral optoacoustic tomography, Comprehensive simulation of different parameters affecting signal generation was done using appropriately modified optoacoustic equation that incorporates frequency dependent attenuations of light and ultrasound on signals originating from absorbing spheres in tissue-mimicking phantoms. The theoretical framework was used to predict the sensitivity of detection in realistic whole-body small animal imaging scenarios. It was estimated that MSOT can resolve sub-picomole amounts of common far-red and near-infrared fluorescent molecular probes while the sensitivity limits for red-shifted fluorescent proteins is currently estimated to be on the order of 1000 cells with high expression levels.

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