Quantifying optical properties in small animals using MR-guided multispectral time-resolved imaging

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ABSTRACT
We propose the use of Time-Resolved Diffuse Optical Tomography in a multispectral scheme with anatomical constraints supplied by MR imaging to reconstruct functional parameters of the animal model with greater accuracy and resolution. The tomographic imaging system described is capable of acquiring temporal measurements in multiple-views using a gated ICCD camera. A tunable Ti-Sapphire pulsed laser at wavelengths between 700nm - 1000nm is used as the source. Anatomical distribution is determined using MRI in a non-concurrent setting. Time-resolved measurements at multiple wavelengths in the NIR window combined with the anatomical constraints is used to determine a 3D distribution of the functional parameters in vivo. Multispectral spectroscopy measurements on homogenous tissue simulating phantoms are used to demonstrate the accuracy of the system in determining optical parameters in thin tissues. We show that temporal measurements combined with MRI data can be used to accurately quantify optical properties in heterogeneous tissues.

Keywords: Small animal imaging, Time-resolved optical imaging, MR-guided DOT, Multispectral DOT

1. INTRODUCTION
Over the past two decades molecular imaging modalities like Fluorescence Molecular Tomography (FMT) and Bioluminescence Tomography (BLT) have shown promise in longitudinal pre-clinical studies due to their non-invasive nature. However improved quantification of the reconstructed parameters is dependent on the apriori knowledge of optical properties (absorption and scattering coefficients) in vivo. Diffuse Optical Tomography (DOT) is the technique used to determine the optical properties using a continuous intensity source (Continuous wave), intensity modulated source (Frequency domain) or ultra-short laser pulses (Time domain). Time-resolved (TR) optical imaging involves measurement of the temporal spread in the laser pulse upon transmission through tissue. DOT in the time domain (TDOT) uses the temporal information to reconstruct the optical properties of the tissue. Measurements in the TR scheme carry maximum information required for the reconstruction and the efficacy of this technique in optical mammography and brain imaging has been demonstrated.

Reconstruction using time-resolved measurements involves the separation of the temporal pulse spread function (TPSF) into the instrument response function (IRF) and the tissue response function, where the latter carries information on the absorption and scattering properties of the tissue through which the pulse was transmitted. The primary challenge with this approach in small animal imaging is the short diffusion distances which result in narrow TPSF comparable to the IRF, resulting in inaccurate estimation of optical properties. Another approach to DOT explores the reconstruction of parameters which generate the optical properties at a functional level in the tissue, namely total blood volume, oxygen saturation level and water content. This is accomplished by probing the tissue at multiple wavelengths and thereby utilising the contrast in the above parameters in the spectral domain. However DOT remains an ill-conditioned low-resolution problem. The use of anatomical apriori information from Computed Tomography (CT) or Magnetic Resonance (MR) imaging to counter these two drawbacks of the optical imaging modality has been suggested. In this paper we propose a multispectral time-resolved optical imaging system with non-concurrent MR imaging which combines the above three approaches. In Section 2 we give a detailed description of the different components of the system. In Section 3 the characteristics of the system IRF, namely the Full Width Half Maximum
(FWHM) and Peak position \(t_{\text{max}}\), and the imaging resolution are discussed. The characteristics of the detector system are also provided. Section 4 describes three experiments demonstrating the accuracy of the system in estimating optical properties over multiple wavelengths in the NIR range. The improved reconstruction of the tissue properties using this system is also investigated using heterogenous phantoms.

2. SYSTEM DESCRIPTION

2.1 Source
Time-resolved imaging in the multispectral scheme was carried out using the system depicted in Figure 1. The source used in this system is a tunable Ti-Sapphire laser (Mai Tai HP, Newport-Spectra Physics, Mountainview CA) having a pulse width of 100 fs and a repetition rate of 80 MHz. The laser can be tuned from 690 nm - 1020 nm and has a peak power output of 3W at 800 nm. A computer-controlled power control for tunable lasers (Application Note # 30, Newport-Spectra Physics, Mountainview CA) is used in conjunction with the source to stabilise and control the output power. This system can be used to maintain the power in the 3 mW - 200 mW range over all wavelengths. The beam exiting the power control is injected into a 50 micron multimode fiber having a numerical aperture of 0.2 using a 10x objective lens and directed to the imaging stage. The fiber is connected to the stage through an achromatic collimating lens (74-ACR, Ocean optics, Dunedin, FL). The lens focusses the beam onto a galvanometer-based XY scanner (XLR8 QS7, Nutfield Technology, Windham, NH) used to position the source on the imaging stage.

2.2 Detector
An ultrafast gated ICCD camera (Picostar HR, LaVision GmbH, Germany) is used as the detector. A reference beam from the laser is passed through an optical trigger (OCF-401, Becker & Hickl GmbH, Berlin, Germany) to generate a pulse train which is used to synchronise the intensifier shutter with the laser pulses. The pulse train is further conditioned by a trigger delay unit to achieve 1 ps temporal resolution. The intensifier control receives the pulse train and controls the shutter of the ICCD. The camera has a minimum of 200 ps gatewidth and an integration time of \(~250\) ms per gate.

The detector system also comprises of a CCD coupled with a structured illumination laser (SNF-599L, Stocker, Quebec, Canada) used to extract the surface topography of the subject being imaged. The two components of the detector system can be rotated about the subject (\(-90^\circ\) to \(+90^\circ\)) to obtain multiview measurements.
3. SYSTEM CHARACTERISATION

In this section we look at the temporal characteristics of the source. The characteristics of the ICCD and the features of the imaging and scan area on the stage are also discussed.

3.1 Source characteristics

- The FWHM of the IRF was measured for different wavelengths over the NIR window over four separate experiments. As shown in Figure 2a the FWHM was found to be $\sim 100$ ps and increased to a maximum width of 140 ps beyond 850 nm. All measurements were made at 10 ps temporal resolution.

- The power control system is used to maintain a high signal-to-noise ratio for varying tissue thickness and also to correct for reduced sensitivity of the detector at wavelengths higher than 850 nm. Figure 2b shows the variation in IRF FWHM and drift for different power settings. The measurements were made at 900 nm at 10 ps temporal resolution.

- The drift of the source was measured to be $\sim 5$ ps/h over a period of 2 hours with measurements at 120 s intervals at a 5 ps temporal resolution. A jitter of 5 ps was also observed.

Figure 2. (a) Variation in IRF FWHM with wavelength. (b) Variation in IRF FWHM and $t_{\text{max}}$ with output power.

Figure 3. (a) Image area and scan area shown on a 2 mm grid. 121 sources at 3mm separation are shown in red. (b) Spatial profile of source spot.
• Figure 3a shows the imaging and scan area (8 cm x 6.5 cm) on the 12 cm x 12 cm stage.

• The source has a gaussian distribution as shown in Figure 3b. The 1/e^2 radius of the source is 0.5 mm. The images are binned post-acquisition to obtain 1 x 1 mm detectors.

• The XY-scanner can position the source beam in the scan area within 1 mm accuracy. This is shown in the source locations shown for 121 sources at 3 mm separation in Figure 3a.

### 3.2 Detector characteristics

![Graph showing spectral sensitivity and spatial sensitivity](image)

(a) (b)

Figure 4. (a) Spectral sensitivity of the ICCD. (b) Spatial sensitivity of the CCD (Provided by manufacturer).

• The quantum efficiency of the detector falls below 10% for wavelengths longer than 850 nm as shown in Figure 4a. As mentioned above, this effect can be countered by using higher source power. Figure 4b shows the sensitivity of the CCD and it can be seen that the imaging area shown in Figure 3a has uniform sensitivity.

• The ICCD has a minimum gate width of 200 ps and minimum gate increment (temporal resolution) of 1 ps. The IRF FWHM increases with higher gate width and gate increments while the SNR of the measured TPSF decreases. The CCD is therefore operated at minimum gate width to improve accuracy in small animal studies where tissue thickness ranges from 1.5 cm to 2.5 cm resulting in narrow TPSF. The gate increment employed depends on the temporal window being imaged and is limited by a maximum of 225 gates per TPSF acquired.

• The MCP gain voltage can be varied from 260 V to 890 V. However, IRF broadening was observed for gain voltages greater than 600 V.

### 4. SYSTEM VALIDATION RESULTS

In this section we demonstrate the accuracy of this system in two related schemes. First, we show the range of optical properties found in small animals that can be estimated accurately by the previously described system using time-resolved spectroscopy over multiple wavelengths. Second, the accuracy of the system in 3D reconstruction of optical properties in an heterogenous model is described.
4.1 Accuracy limits in estimating absolute optical properties

4.1.1 Materials and Methods

A homogenous liquid phantom was used to simulate the range of optical properties $\mu_a$ and $\mu'_s$ found in small animals. The phantom was placed in a polycarbonate tank of dimensions (8 cm x 4 cm x 2 cm) to replicate small animal dimensions. The phantom was made with a mixture of distilled water, Intralipid 20% (Sigma-Aldrich, St. Louis, MO) as scattering agent and Red India Ink (Sanford Design Higgins) as the absorber. The ink was calibrated prior to the experiment using a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL). The scattering properties of Intralipid were estimated using the relation based on Mie-theory. A single source at 700 nm was used for excitation and the TPSF was measured using $\sim 250$ detectors over $\sim 1$ cm radius at 1 mm separation. As detailed in Section 3.2, the gate width was set to 200 ps with 20 ps gate resolution. Measurements were acquired over 3 ns time window. The properties were estimated by fitting the measured data to the diffusion approximation solution in transmittance using a CG-based least-squares solver.

4.1.2 Results

Figure 5 shows the results for absorption coefficients ranging from 0.04 cm$^{-1}$ to 0.5 cm$^{-1}$. Figure 6 shows results for scattering coefficient estimation for values in the 7 cm$^{-1}$ to 20 cm$^{-1}$ range. Table 1 and Table 2 give the relative estimation errors for select values estimated in this experiment. It can be seen that while absorption coefficient can be estimated within 5% error to a value of 0.3 cm$^{-1}$, the estimation error increases to more than 20% for higher values of $\mu_a$. The scattering coefficient can be estimated accurately over the entire range. The error in absorption estimation can be attributed to competing effect of the IRF and TPSF at high absorption values where the TPSF is considerably narrow.

<table>
<thead>
<tr>
<th>Expected $\mu_a$ (cm$^{-1}$)</th>
<th>$\mu_a$ Estimation Error (%)</th>
<th>$\mu'_s$ Estimation Error (%)</th>
</tr>
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<tbody>
<tr>
<td>0.0365</td>
<td>2.2168</td>
<td>14.3430</td>
</tr>
<tr>
<td>0.1077</td>
<td>1.7664</td>
<td>9.6280</td>
</tr>
<tr>
<td>0.3077</td>
<td>3.7116</td>
<td>3.5632</td>
</tr>
<tr>
<td>0.4896</td>
<td>25.1159</td>
<td>25.91</td>
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<table>
<thead>
<tr>
<th>Expected $\mu'_s$ (cm$^{-1}$)</th>
<th>$\mu_a$ Estimation Error (%)</th>
<th>$\mu'_s$ Estimation Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1531</td>
<td>4.6205</td>
<td>2.5854</td>
</tr>
<tr>
<td>12.3598</td>
<td>4.7542</td>
<td>4.2288</td>
</tr>
<tr>
<td>17.3288</td>
<td>-2.0101</td>
<td>-3.4990</td>
</tr>
<tr>
<td>19.7291</td>
<td>3.0915</td>
<td>2.1063</td>
</tr>
</tbody>
</table>

4.2 Estimating absolute optical properties over multiple wavelengths

4.2.1 Materials and Methods

A calibrated solid polyurethane phantom provided by ART (Montreal, Canada) was used for this experiment. 6 wavelengths spanning the NIR window (700nm - 850 nm) were selected for excitation. A single source-detector pair was used to measure the TPSF and estimation was done by the algorithm described above.
Figure 5. (a) Estimation of $\mu_a$ for increasing concentration of India Ink. (b) Estimation of $\mu'_s$ for increasing concentration of India Ink.

Figure 6. (a) Estimation of $\mu_a$ for increasing concentration of Intralipid-20%. (b) Estimation of $\mu'_s$ for increasing concentration of Intralipid-20%.

Figure 7. (a) Estimation of $\mu_a$ for increasing concentration of Intralipid-20%. (b) Estimation of $\mu'_s$ for increasing concentration of Intralipid-20%.
4.2.2 Results

Figure 7 shows the estimation results for the above mentioned wavelengths. The $\mu_a$ values were estimated for all wavelengths within 10% error while higher error values were seen for $\mu'_s$ values. Table 3 gives the error for select wavelengths.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>$\mu_a$ Estimation Error (%)</th>
<th>$\mu'_s$ Estimation Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>710</td>
<td>5.7366</td>
<td>-20.4028</td>
</tr>
<tr>
<td>740</td>
<td>-6.0851</td>
<td>-14.8474</td>
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<tr>
<td>800</td>
<td>0.0235</td>
<td>-2.1405</td>
</tr>
<tr>
<td>820</td>
<td>5.3538</td>
<td>5.8349</td>
</tr>
</tbody>
</table>

Figure 8. (a) 3D volume distribution determined using MRI. (b) Reconstructed optical properties. Each image shows a 1mm slice in the X-Y plane.

4.3 MR-guided DOT

4.3.1 Materials and Methods

In this experiment we investigate the capability of this system to accurately determine the perturbations in optical properties in an heterogenous medium. A polycarbonate tank (8cm x 4cm x 2cm) containing a mixture of water, Intralipid-20% and India ink with two suspended inclusions made of agarose was used as the phantom. The concentrations of ink and Intralipid-20% were calculated such that the background had $\mu_a = 0.06cm^{-1}$, $\mu'_s = 6cm^{-1}$ and the inclusions had $\mu_a = 0.6cm^{-1}$, $\mu'_s = 4.58cm^{-1}$.

The apriori spatial information was determined using the Bruker 7T Pharmascan $\mu$MRI. The MR images were acquired using T1-weighted contrast with a spatial resolution of 0.5 mm$^3$.

The optical measurements were carried out at 700 nm using 30 sources at 3 mm separation and 56 detectors at 2 mm separation over an image/scan area of 1.4cm x 1.6cm.

The reconstruction was done using a perturbative Monte carlo approach$^{12}$ with spatial constraints imposed using a Bayesian approach$^8$ and the CW value (integrated photon count over all gates) extracted from the temporal data was used as datatype to reconstruct the absorption coefficients.
4.3.2 Results

Figure 8a shows the location and dimensions of the two inclusions as obtained from the MRI images. This information was used as spatial apriori information while solving the inverse problem to obtain the reconstruction shown in Figure 8b. The absorption coefficient of the inclusion had a peak value of 0.5276 cm\(^{-1}\) with a mean value of 0.368 cm\(^{-1}\) and a variance of 0.0045 cm\(^{-1}\). The background voxels had a peak value of 0.09 cm\(^{-1}\) with a mean value of 0.01 cm\(^{-1}\) and variance 0.0002 cm\(^{-1}\).

5. CONCLUSION

We present a time-resolved optical imaging system capable of multispectral acquisitions. The system has successfully estimated optical properties in spectroscopic experiments on homogenous phantoms similar to murine models for comparable optical properties. We have also shown the preliminary results of MR-guided DOT experiment for heterogeneous phantom with a contrast in the absorption coefficient. Future work will focus on extending the MR-guided DOT study into the multispectral domain to accurately estimate functional tissue parameters in small animals. The efficacy of this system in Fluorescence Diffuse Optical Tomography (fDOT) using apriori optical properties will also be studied.

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REFERENCES