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Development of a Multispectral Tissue Characterization System for Optimization of an Implantable Perfusion Status Monitor for Transplanted Liver

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***Abstract*—Optimizing wavelength selection for monitoring perfusion during liver transplant requires an in-depth characterization of liver optical properties. With these, the impact of liver absorption and scattering properties can be investigated to select optimal wavelengths for perfusion monitoring. To accomplish this, we are developing a single integrating-sphere-based technique using a unique spatially resolved diffuse reflectance system for multispectral optical properties determination for thick samples. We report early results using a monochromatic source to measure the optical properties of well characterized tissue phantoms made from polystyrene spheres and Trypan blue. The presented results demonstrate the feasibility of using this unique system to measure optical properties of tissue phantoms. We are currently in the process of implementing an automated Levenberg–Marquardt diffuse-reflectance-profile fitting algorithm to enable near realtime robust computation of sample optical properties. Future work will focus on the incorporation of multispectral capability to provide needed data to facilitate development of more realistic liver tissue phantoms.**

I. Introduction

Post transplantation monitoring of liver tissue viability is conducted via periodic blood tests that quantify liver enzyme levels as a measure of proper liver function. Even utilizing this method, morbidity rates associated with organ failure post transplantation are still high. Additionally, the high costs in time and effort coupled with the scarcity of transplant organs argue for development of an accurate and realtime means for monitoring organ viability. Availability of such capability would afford medical personnel opportunities to make early interventions, e.g., through drug therapy or surgical procedures, to prevent catastrophic outcomes of partial/total organ failure. One measurable critical parameter related to organ tissue health is blood perfusion, which can be used to monitor circulatory system complications responsible for graft morbidity and patient mortality [1,2].

In previous communications, we have presented results on the development of an implantable perfusion monitor that would be attached to a transplanted organ. The sensor would provide timely updates via, wireless telemetry, of perfusion status [3-6]. In principle, the calculated perfusion index (PI) should be independent of the arterial blood oxygen saturation (SaO2) level to prevent changing SaO2 from being erroneously registered as a change in perfusion. This is likely if the PI measurement value is dependent. Fig. 1 is a plot that investigates such dependence for the ratio, R in Eqn. 1, which is used to compute pulse oximetry determined blood oxygen saturation (SpO2) level [7]. It is probable that the normalized ratio

$$R = \frac{\log_{10}\left[(I_{660})_{AC}/(I_{660})_{DC}\right]}{\log_{10}\left[(I_{940})_{AC}/(I_{940})_{DC}\right]}, \qquad (1)$$

is sensitive to SaO2 [3]. Just how much of this dependence (i.e., color coded horizontal shift in the curves among different days) is due to SaO2 versus hematocrit, inter-experimental variations in the variables of probe-to-sample geometry, liver sample hydration, thickness, and the

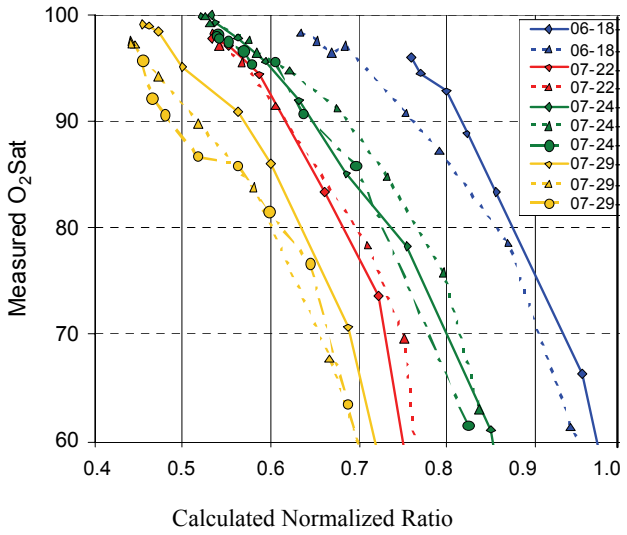

Fig.1. Oxygen saturation of hemoglobin was calculated from sensor data of perfused blood with liver tissue. 107 measurements were obtained while oxygen saturation was varied from 5 to 100% based on measured SaO2 using a commercial blood gas analyzer. Experiments conducted on the same day are grouped by color.

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heterogeneity of liver samples is still undetermined.

In order to isolate and better understand the current R and potential PI dependence on SaO2, we have found it necessary to pursue a more controlled liver tissue phantom study for which only SaO2 will be varied. A tissue phantom platform provides a means for elimination of aforementioned experimental variables that existed in the earlier Fig.1 study. Furthermore, a multispectral implementation will permit investigating the adequacy of 660 and 940nm for liver blood perfusion measurement. These wavelengths were selected based on literature reports and need to be confirmed experimentally; insufficiency would necessitate wavelength selection optimization [8]. Ideally, we will end up with two wavelengths—on opposite sides of the 805nm oxy/deoxy-hemoglobin isobestic point—that greatly penetrate and interrogate equal volumes of liver tissue while maintaining sensitivity to blood absorption.

The first step to accomplishing this is to accurately characterize the optical properties of liver tissue. Utilizing this data, we can then construct a suitable realistic tissue phantom that approximates both the circulatory structure and function of native liver. An x-ray tomographic image, as presented in Fig. 2, can be used to reveal both liver anatomy and blood perfusion characteristics to guide phantom design and construction. This paper presents our efforts towards accomplishing the first objective, namely, developing a means to accurately measure liver tissue optical properties. For this, we introduce a single integrating sphere diffuse reflectance based approach for characterizing thick tissue samples, thus obviating the need for tissue freezing and microtome tissue sectioning.

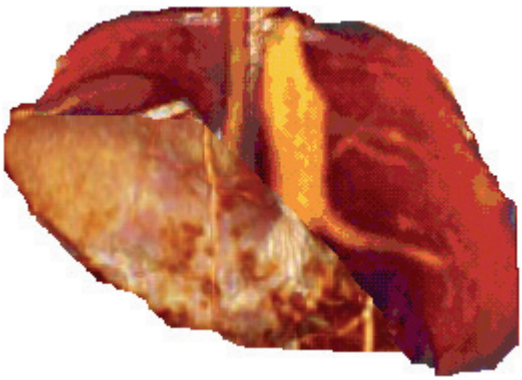

Fig. 2. An x-ray computed tomography reconstruction of mouse liver, taken using a blood pool contrast agent that reveals tissue gross anatomy and large vasculature.

II. Methodology

Tissue optical properties (TOP) of absorption and scattering coefficient are sufficient parameters to describe/model tissue light propagation in the photon diffusion limit. In the case of multispectral optical properties determination, there is a fundamental lack of uniqueness in the determination due to current unavailability of wavelength dependent tissue scattering anisotropy values. Therefore, regardless of the model applied—whether diffusion or transport theory, analytical, Monte Carlo simulations, or hybrid techniques—reasonable assumptions are made to match experimentally determined light fluence values [9,10]. A resulting consequence is a lack of standards for validating TOP determination. This coupled with variations in measure-ment systems for the determination makes it challenging to ascertain "true optical properties" for various tissues based on literature reports. This difficulty is further evidenced by the lack of commercially-available TOP systems.

One challenge in developing a TOP determination system is achieving a means for acquiring repeatable results to enable suitable comparisons of results between multiple samples and experiments, and between different systems and protocols. An integrating sphere is designed to collect all inputted light and to register fluence without regard to original input beam source direction and polarization; therefore, it is a realistic approximation of a system standard for collecting/measuring tissue sample optical properties. For this reason, it is the platform for our development of a multispectral optical properties determination system for characterizing thick optically dense tissue samples, e.g. liver. The novelty of our system is the introduction of a unique light occluding slider device to eliminate the need for tissue thickness measurement. In principle, this enables utilization of full thickness tissue sample for measurement to provide values more representative of the whole (totality of) tissue rather than just that of a thinly sliced specimen. Once validated, the system will enable us to accurately match native liver TOP for necessary controlled phantom studies. We also envision that such a system will lead to reproducible and more standardize measurement of TOP.

A. System Components

An optical properties system comprising the following components: (1) 8 inch integrating sphere (Sphere Optics), (2) an apertured 635nm, 4mW output power laser (Power Technology Inc.), (3) a custom developed, linearly translatable light occlusion slider device system, and (4) Dell Pentium IV workstation with LabVIEW® 8.2, and were utilized for the development of a tissue optical properties characterization system.

B. Final Stage

Initially, using a collimated high intensity white light source input, the system was configured for single integrating sphere multispectral determination of optical properties [11]. In this configuration, the absorption coefficient, μ_a, and the reduced scattering coefficient, μ'_s, of thin optically dense tissue phantoms were determined for multiple wavelengths using Prahl's Inverse Adding-Doubling algorithm (IAD) [12]. This was accomplished by measuring diffuse reflectance, R, and transmittance, T, and then using the IAD algorithm for determining the albedo, a, and optical thickness, τ, which combined with the

knowledge of the actual sample thickness was used to yield μ_a and μ_s.

Measuring the optical properties of optically dense liver tissue using this approach would entail the preparation of very thin microtome slices and an accurate determination of slice thickness. Microtome slicing first requires freezing the tissue. This leads to measurement artifacts due to inherent tissue structural changes that occur during the freezing and thawing processes. Furthermore, the non-trivial but necessary challenge of accurately measuring slice thickness of unfrozen samples is burdensome. This is necessitated by the significant change in sample thickness when the frozen slice is made and the unfrozen state that is characterized because of the high water content of tissues. Additionally, it is challenging to simultaneously acquire sufficient diffuse reflectance for multiple wavelengths of a multispectral system. All of these combined with the introduced variability from having to move the sample for each of the required measurements, clearly argues for an alternative. Therefore, our approach has been to develop a means for multispectral assessment of optical properties of thick samples—i.e., without the requirement for microtome slicing—also one that is void of sample thickness measurement and sample movement. Fig. 3 depicts the system implementation for the measurement.

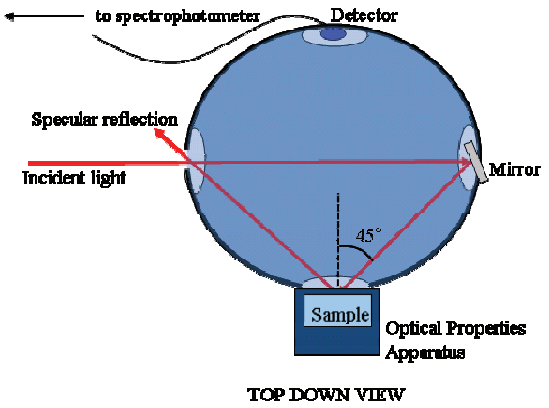

Fig. 3. System setup. Sample is mounted outisde a side port of the integrating sphere in an optically transparent sample holder.

C. Conceptual Basis

We implement a modified version of diffuse reflectometry [13-15] to determine sample optical properties. Essentially, a linear translatable fixed width light occlusion slider (LOS) is used to block light remittance, R, from a spatial position, x, on the sample surface thereby generating a spatially resolved diffuse reflectance map that can be used to determine tissue optical properties (see Fig. 4). The diffuse intensity profile for each spatial position on the tissue surface, $R_d(x)$, is determined by subtracting the value of diffuse reflectance with the LOS at that specific position, $R_{occlusion}(x)$, from the total diffuse reflectance of the sample, i.e., without the LOS occluding any portion of the sample surface, R_{total}.

$$R_d(x) = R_{total} - R_{occlusion}(x), \tag{2}$$

Once the diffuse reflectance profile, given by Eqn 2, is determined, the offset, Δx, of the position of maximum intensity from the point of light input is used to determine the optical properties. This is defined as,

$$\Delta x = \frac{\sin(\theta_t)}{\mu'_s + 0.35\mu_a}, \tag{3}$$

where θ_t is the angle of refraction of the input beam into the tissue sample. This profile is determined using the processes outlined in the aforementioned references [12-14]. Note that for this method the only sample thickness dependence is the diffusion theory requirement that the sample is semi-infinite in thickness. This can easily be accomplished without an actual sample thickness measurement, merely by ensuring that the sample is sufficiently thick so as to prevent light transmittance.

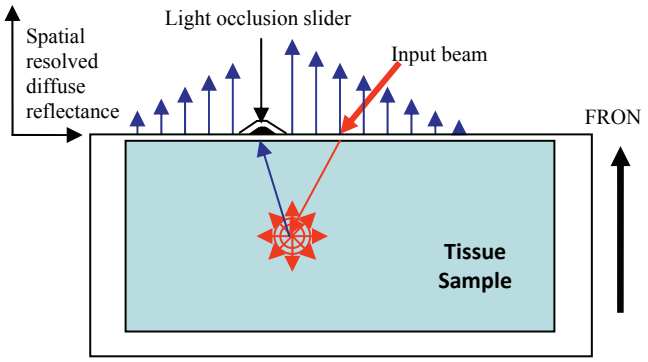

Fig. 4. Exploded view of spatial-acquisition apparatus device of Fig. 3 that enables collection of diffuse reflectance map of tissue for optical properties determination. The light occlusion slider moves along the front surface of the sample to enable the generation of a diffuse reflectance map. Note that the sample is fixated during the whole characterization process.

III. Results and Discussion

Table 1: Quantification results of optical properties characterization for the samples presented in Fig. 5.

Sample	Optical Properties	Δx	μ'_s	μ_a
(8,0.4)	Measured	0.068	7.64	0.42
	Theoretical	0.065	8.00	0.40
	% Error	4.5	4.5	5.8
(8,0.6)	Measured	0.084	6.12	0.63
	Theoretical	0.065	8.00	0.60
	% Error	29.4	23.5	5.4
(10,0.6)	Measured	0.068	7.69	0.45
	Theoretical	0.052	10.00	0.60
	% Error	30.1	23.1	25.7

To test the system, three tissue phantom samples were made using mixtures of polystyrene spheres (scatterers) and

Trypan blue dye (absorber). Preliminary results presented in Table 1 and Fig. 5 were collected using a monochromatic 633nm diode laser input source. In the plots, even though the left sides of the diffuse reflectance data demonstrate a deviance from the theoretical cone-shaped profile, the fitted models still converge to provide reasonable Δx values. The errors of the experimentally determined Δx, μ_a, and μ'_s are presented in Table 1. These are expected to decrease with system optimization, which entails better system alignment and elimination of multiple scatter from the glass surfaces of the sample holder. We are currently working on automated implementation of a Levenberg-Marquardt algorithm to fit the experimental data points in LabVIEW® 8.2 to provide near realtime characterization results.

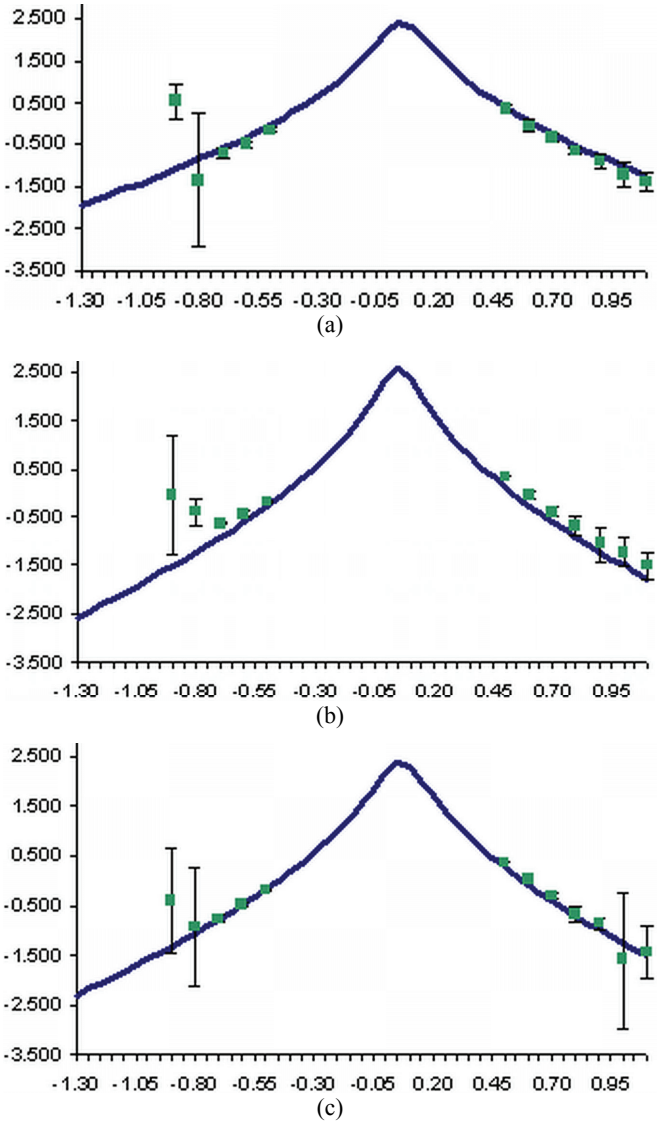

Fig. 5. (a)-(c) Plots of experimental diffuse reflectance values (squares) plotted with fitted diffuse reflectance profile (solid line) for three tissue phantoms of optical properties [μ_s,μ_a]: [8,0.4], [8,0.6], and [10,0.6] respectively. The vertical axes are diffuse reflectance in arbitrary units [AU] and the horizontal axes are position x [cm] with respect to input-beam-incidence. Standard deviations bars for 5 repetitions are included.

IV. Conclusions

In this paper we have described the development of an integrating sphere-based diffuse reflectometry system for measuring optical properties of thick tissue samples with a unique light occlusion slider. The preliminary data collected on well characterized phantom samples demonstrates the feasibility of the approach. In the future we will be transitioning to a high intensity white light source for multispectral implementation. This will allow for concurrent measurement of multispectral optical properties once the response and limitations of the system for multi-wavelength characterization are determined. With such capability, we will be able to proceed to the development of a liver tissue phantom that matches actual liver in TOP, gross anatomy, and blood perfusion characteristics for the purpose of ascertaining PI dependence on SaO2 and other variables.

References

[1] B.J. Stange, M. Glanemann, N.C. Nuessler, U. Settmacher, T. Steinmüller and P. Neuhaus, "Hepatic artery thrombosis after adult liver transplantation," *Liver Transplant,* vol. 9, no. 6, pp. 612-620, June 2003.

[2] U. Settmacher, B. Stange, R. Haase, M. Heise, T. Steinmuller, W. O. Bechstein, and P. Neuhaus, "Arterial complications after liver transplantation," *Transpl Int,* vol. 13, pp. 372-378, 2000.

[3] M.N. Ericson et al., "Implantable sensor for blood flow monitoring after transplant surgery," *Min Invasiv Ther*, 13(1), pp. 1-8, Apr. 2004.

[4] S. J. Lee et al., "Wavelet and Fast Fourier Transform Analysis of Oximeter-Based Perfusion Data," *IEEE T Bio-Med Eng*, 52, pp. 1350-52, 2005

[5] H. Subramanian et al., "Real time separation of perfusion and oxygenation signals for an implantable sensor using adaptive filtering," *IEEE T Bio-Med Eng*, 52, pp. 2016-23, 2005.

[6] H. Subramanian et al., "An autocorrelation based time domain analysis technique for monitoring perfusion and oxygenation in transplanted organs," *IEEE T Bio-Med Eng,* 52, pp. 1355-58, 2005.

[7] J. G. Webster, ed. *Design of Pulse Oximeters*. Philadelphia, PA: Institute of Physics, 1997.

[8] P.D. Mannheimer, J.R. Cascini, M.E. Fein, and S.L. Nierlich**,** "Wavelength selection for low-saturation pulse oximetry," *IEEE T Bio-Med Eng*, vol. 44, no. 3, pp. 148-158, Mar. 1997.

[9] H. C. van de Hulst and R. Graaff, "Aspects of similarity in tissue optics with strong forward scattering," *Phys Med Biol,* 41**,** pp. 2519-2531, 1996.

[10] G. Yoon, S. A. Prahl, and A. J. Welch, "Accuracies of the diffusion approximation and its similarity relations for laser irradiated biological media," *Appl Opt*, 28, pp. 2250-2255, 1989.

[11] M.S. Allegood and J.S. Baba, "Extension of the Inverse Adding-Doubling Method to the Measurement of Wavelength-Dependent Absorption and Scattering Coefficients of Biological Samples," *J Undergraduate Res*, Vol. VIII, pp. 8-14, Nov.-Dec. 2008.

[12] S.A. Prahl, M.J.C. van Gemert, and A.J. Welch, "Determining the optical properties of turbid media by using the adding-doubling method," *Appl Opt,* 32, pp. 559-568, 1993.

[13] L. Wang and S.L. Jacques, "Use of a laser beam with an oblique angle of incidence to measure the reduced scattering coefficient of a turbid medium," *Appl Optics,* 34, pp. 2362, 1995.

[14] S. Lin, L. Wang, S. L. Jacques, and F. K. Tittel, "Measurement of tissue optical properties by the use of oblique-incidence optical fiber reflectometry," *Appl Optics,* 36, pp. 136-143, 1997.

[15] G. Marquez and L. Wang, "White light oblique incidence reflectometer for measuring absorption and reduced scattering spectra of tissue-like turbid media," *Opt Express,* 1, pp. 454-460, 1997.