Toward noninvasive detection and monitoring of malaria with broadband diffuse optical spectroscopy

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ABSTRACT

Despite numerous advances, malaria continues to kill nearly half a million people globally every year. New analytical methods and diagnostics are critical to understanding how treatments under development affect the lifecycle of malaria parasites. A biomarker that has been gaining interest is the"malaria pigment" hemozoin. This byproduct of hemoglobin digestion by the parasite has a unique spectral signature but is difficult to differentiate from hemoglobin and other tissue chromophores. Hemozoin can be detected in blood samples, but only utilizing approaches that require specialized training and facilities.

Diffuse optical spectroscopy (DOS) is a noninvasive sensing technique that is sensitive to near-infrared absorption and scattering and capable of probing centimeter-deep volumes of tissue in vivo. DOS is relatively low-cost, does not require specialized training and thus potentially suitable for use in low-resource settings. In this work, we assess the potential of DOS to detect and quantify the presence of hemozoin noninvasively and at physiologically relevant levels. We suspended synthetic hemozoin in Intralipid-based tissue-simulating phantoms in order to mimic malaria infection in multiply-scattering tissue. Using a fiber probe that combines frequency-domain and continuous-wave broadband DOS (650-1000 nm), we detected hemozoin concentrations below 250 ng/ml, which corresponds to parasitemia sensitivities comparable to modern rapid diagnostic tests. We used the experimental variability to simulate and estimate the sensitivity of DOS to hemozoin in tissue that includes hemoglobin, water, and lipid under various tissue oxygen saturation levels. The results indicate that with increased precision, it may be possible to detect Hz noninvasively with DOS.

Keywords: global health, diffuse reflectance spectroscopy, frequency domain photon migration, hemozoin

1. INTRODUCTION

Despite significant progress in recent years, low-cost malaria diagnostics with sufficient sensitivity to detect low-level infections are not yet available. In remote areas where malaria presents the largest problem, the most widely used tools are rapid diagnostic tests, similar to blood glucose test kits. These require blood from a finger prick, and are not considered authoritative.[1] A painless test which creates no biohazardous waste and can not only diagnose malaria, but monitor its progression, would be highly desirable.

The "malaria pigment" hemozoin (Hz) has been gaining interest in recent years as a way to track the progression of the disease. Hz is a waste byproduct created through the consumption of red blood cells by the late stages of some malaria parasites, and it has unique optical properties (OPs) which make computer-aided diagnostics possible. Though questions have been raised about the degree of correlation between quantity of Hz present in the bloodstream and severity of infection,[2] the ability to monitor Hz production and elimination in vivo, without repeated blood draws, will provide useful information while monitoring the disease progression and treatment. This approach can also support basic scientific studies of the disease and the development of new diagnostics and treatment approaches.

At the time of this writing, a few optical methods have been investigated as tools to monitor Hz in vivo. A transmission-mode microscope perched on the lip or ear can monitor for the intrinsic birefringence of Hz crystals.[3] Additionally, a photoacoustic approach has been reported with high sensitivity. In this method, a laser heats the Hz crystal, causing the formation of nano-sized bubbles, the collapse of which can be tracked by

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ultrasound.[4] Both of these methods are focused, small volume techniques, but there is room for a system that could probe a larger volume of tissue at low cost. A reflectance-based method might be a useful addition to the growing collection of in vivo Hz detection methods.

2. MATERIALS & METHODS

2.1 Tissue-Simulating Phantoms

To simulate biological tissue, we constructed liquid optical phantoms: mixtures of Intralipid, de-ionized water (DI), and the absorbers under study. To approximate a semi-infinite geometry, we mixed 2L of phantom in an 18cm diameter bucket and continuously stirred at 120rpm. The reduced scattering coefficient, referenced at 660nm, was set between 1mm^{-1} and 1.5mm^{-1}, using a rule of thumb: 1 %v/v Intralipid yields approximately 1mm^{-1} reduced scattering. Absorption of the nigrosin was predicted from its mass extinction coefficient obtained through traditional spectrophotometry. In the first type of phantom, set at 1.2mm^{-1} reduced scattering and 0.005mm^{-1} absorption at 660nm, we added a series of Hz aliquots and measured the OPs after each addition with DOS.

In the second type of phantom, Incomplete Cell Media (ICM) was used in the place of DI water. The ICM contains Gibco RPMI 1640 with L-glutamine, Corning HEPES (5.94 g/L), and Calbiochem hypoxanthine (0.5g/L). ICM was used to maintain pH and osmolality suitable for erythrocytes in the phantom. Scattering was targeted to 1.5mm^{-1} and 20mL filtered human erythrocytes were added, for a simulated blood volume fraction of 2%, at 50% hematocrit. The temperature of this phantom was raised to physiologic temperature and allowed to equilibrate with the surrounding air for an hour and a half to achieve full oxygen saturation of the erythrocytes. Deoxygenation was achieved by slowly adding Fleischmann's Active Dry Yeast.

2.2 Hemozoin

5mg synthetic Hz crystals (Invivogen, San Diego, CA) arrived in a sealed vial. We injected 1mL DI water into the vial and dispersed the crystals with a probe-type sonicator (Branson SFX 550 with 1/2" bio horn) set at 30% amplitude for 60s continuous operation. The vial and probe were placed in a beaker of water that functioned as an ultrasonic bath. For the hemozoin aliquot series, we rinsed the vial repeatedly with DI water and recovered the contents, for a stock solution of 5mg Hz crystals dispersed in 10mL DI water.

2.3 Diffuse Optical Spectroscopy

The phantoms were characterized using a DOS technique that is a combination of near-infrared frequency domain photon migration (FDPM) and broadband continuous-wave near-infrared spectroscopy. This quantitative broadband DOS approach estimates the OPs (absorption and reduced scattering coefficients) of turbid media from 650-1000nm, and was first described by Bevilacqua et al.[5] Briefly, FDPM involves measuring the attenuation and phase of propagating photon density waves in the sample produced by amplitude-modulated laser light, and fitting this data to a time-dependent model of light propagation in turbid media.[6] We modulate the laser diode sources (660, 688, 781, 806, 828, and 849nm) between 50 and 600 MHz with an average power of 20-30mW, and utilize a fiber-coupled avalanche photodiode to collect the multiply scattered light at fixed or multiple source-detector separations (multi-distance). The sources are controlled via a laser diode controller (ILX Lightwave LDC-3908), and RF power is sourced and measured by a network analyzer (Agilent 8753ES) and RF switch. Each laser diode and the APD module have thermo-electric coolers to maintain their temperature and mitigate instrument drift. Two custom fiber bundles terminated with 1/4" diameter stainless steel ferrules (Leoni, Inc) transport light to and from the phantom. The model we employ is the P1 approximation to the radiative transport equation of photons in a homogeneous medium in a semi-infinite reflectance geometry.[7–9]

To recover the OPs continuously across the broadband spectrum, we applied reflectance theory, matched to the FDPM results, as described by Bevilacqua 2000.[5] According to Mie Theory, a typical distribution of scatterers results in a scattering spectrum that is well-approximated by an inverse power function. We thus fit this function to the FDPM-measured reduced scattering coefficients in order to estimate the broadband reduced scattering spectrum. We then measured the broadband reflectance of the sample using a white light source (Ocean Optics HL-2000-FHSA) and spectrometer (Avantes SensLine HS2048XL-EVO), and, after calibration, apply reflectance theory to extract the broadband absorption spectrum from the reflectance and scattering spectra. This approach has been used extensively, particularly in clinical studies of breast cancer treatment.[10]

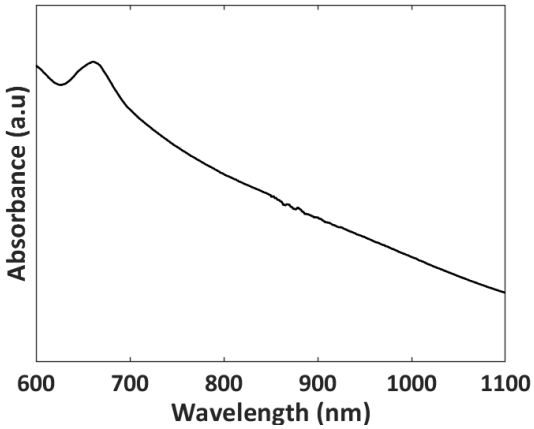

Figure 1. Optical attenuation spectrum of synthetic Hz crystals, measured with spectrophotometry.

2.4 Multi-Distance Measurements

FDPM measurements typically require calibration to account for the modulation frequency dependent instrument response function. In order to determine and remove the system response, we acquired FDPM data at multiple source detector separations before each experiment, similar to the technique described by Zhigang Sun et. al.[11] Here we simultaneously fit the difference in phase and amplitude at each source-detector separation to the previously described P1 approximation. We also confirmed the validity of the approximation by comparing the results to a Monte Carlo model of light propagation.[12] To limit fitting errors caused by large differences in magnitude between variables, both theory and data were normalized before a least-squares regression was performed. For these trials, we placed the detector fiber on a translating stage (Thorlabs LTS300) and performed a measurement at evenly spaced 1mm intervals from 10 to 28mm. After accounting for small (but significant) differences in the positions of the individual source and detector fibers within the bundles, we performed the model fits for a range of separations and frequency ranges and chose a set of parameters with maximum signal to noise. The multi-distance measurement was used to calibrate subsequent measurements in the phantom.

2.5 Simulations of Hz in Tissue

To develop an understanding of the limits of the system, we simulated tissue absorption curves with various levels of Hz. Simulated noise was added by measuring the variability of broadband OPs from 30 fully oxygenated and 30 fully deoxygenated liquid erythrocyte phantom measurements. Three standard deviations provided the maximum error we can anticipate in a single broadband reflectance measurement under similar acquisition conditions. Linear interpolation was used to predict the error at intermediate oxygen saturations.

3. RESULTS & DISCUSSION

Fig. 1 shows the Hz attenuation spectrum as measured with traditional spectrophotometry. This attenuation curve, a combination of absorption and scattering, possesses the characteristic power-law decay typical of distributions of scattering particles. The signature of the Hz extinction spectrum in this plot is a peak near 660nm.

We performed a multi-distance, multi-frequency data acquisition for a series of increasing Hz concentrations in a semi-infinite Intralipid-based tissue-simulating phantom, from 0 to 2.5 ug/ml with a background absorption of 0.005 mm-1 at 660nm. The broadband absorption spectra were recovered for each concentration via multi-distance analysis, and are shown in Fig. 2. We observed increasing absorption in the region of the expected Hz absorption feature with increasing Hz concentration. Fig. 3 shows the recovered absorption coefficient as a function of the actual Hz concentration in the phantoms. The data show a linear dependence on concentration. Below approximately 250ng/ml, the absorption change falls below the noise level of the recovered values. Following the analysis of Newman et al, if we assume 0.06 µg/mL represents 0.002% parasitemia, this figure corresponds to roughly 0.008% parasitemia.[13]

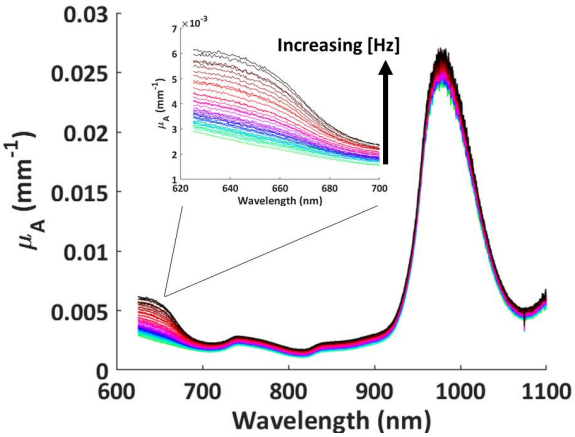

Figure 2. Recovered absorption spectra at a series of Hz concentrations from 25 to 2250 ng/ml.

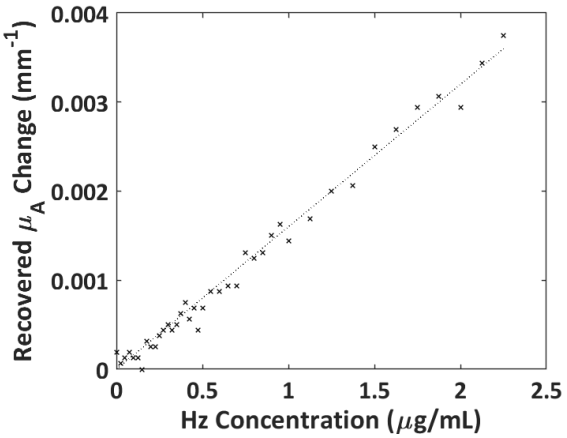

Figure 3. Hz absorption change (635 nm) versus concentration of crystals in phantom, extracted from broadband absorption spectra.

We performed a linear least-squares fit to the recovered absorption data to estimate the Hz extinction spectrum (Fig. 4). We observed that the Hz absorption peak was unexpectedly blue-shifted approximately 20nm compared to spectrophotometry, and are currently investigating the origin of this effect.

Finally, using the measured Hz extinction spectrum from the Intralipid phantom experiment, and the measurement variability from the erythrocyte phantom experiment across oxygenation levels, we simulated optical measurements of tissue at different oxygenation levels containing 250, 500, and 1000 ng/mL Hz. This corresponds to 0.01, 0.02, and 0.03% parasitemia, respectively. Fig. 5 shows the simulated mean and uncertainty in the broadband absorption spectra of tissue with and without Hz at 70 and 100% tissue oxygen saturation. We observe that the uncertainty in the absorption spectrum below 700nm increases dramatically as deoxyhemoglobin concentration increases (and tissue oxygen saturation decreases) for our acquisition conditions. This uncertainty, from 0.0026 to 0.0035 mm^{-1}, complicates the ability to identify low levels of Hz in the tissue. Thus, even though we can recover the absorption of low concentrations of Hz in an Intralipid-based phantom, once we include the high absorption of blood at the relevant wavelengths, the anticipated error is greater than the added absorption of the Hz. We note that the measurement variability that we observed with this broadband DOS technique is consistent with that reported in a recent multi-center clinical study utilizing the technology.[14]

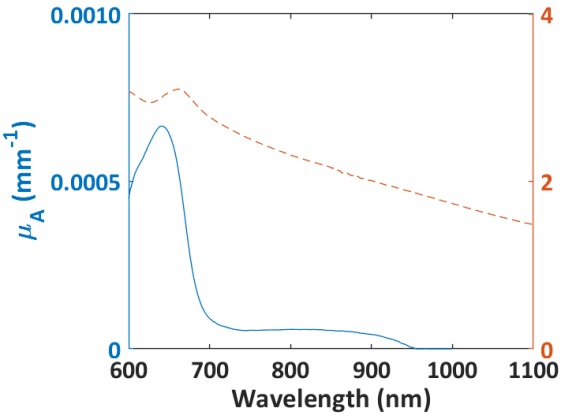

Figure 4. Hz extinction spectrum in Intralipid.

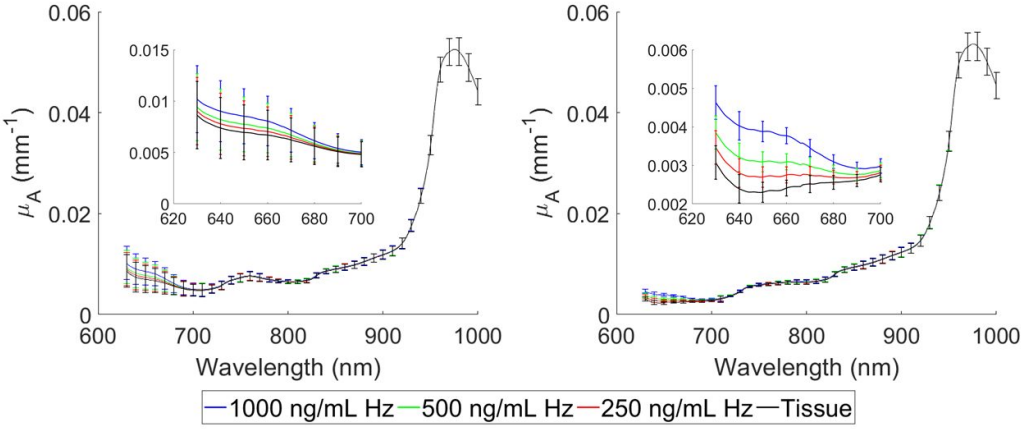

Figure 5. Simulated broadband absorption for tissue at 70% saturation (left) and 100% (right), with 250, 500, and 1000 ng/mL Hz added, and the estimated experimental error.

4. CONCLUSION

These experiments investigated the ability of DOS to detect quantities of homogeneously dispersed Hz crystals. Analysis of this type presents some difficulties, because the solid crystals do not dissolve as a typical chromophore might. For this reason, we cannot recover a molar extinction coefficient for Hz. We still have the shape of the spectrum, however, and we could eventually use this to define a standard Hz absorption unit.

Moving forward, we will need to assess how the size distribution of these crystals compares to what might be found in the tissue of an infected individual. We will also need to improve the signal quality in our absorption measurements. If we could decrease the absorption uncertainty below 700nm to less than $1\text{x}10^{-4}$ mm^{-1}, for 70% saturation, we could recover accurate Hz concentrations of 60 ng/ml in a phantom at normal saturations.

Steps that will improve measurement sensitivity include averaging multiple broadband acquisitions to overcome uncertainty in the operation of our equipment. Additionally, we plan to modify our broadband source to augment the optical power below 700nm where there is significant attenuation due to deoxyhemoglobin and scattering. Once the measurements meet the proposed criteria, we plan to test the equipment in a phantom containing live, malaria-infected erythrocytes.

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