ABSTRACT

In this work, we use Spectroscopic Optical Coherence Tomography and Morlet wavelet transformation to deduce hemoglobin oxygen saturation in blood sample. The technique makes use of the different absorption properties of hemoglobin and oxyhemoglobin around 800 nm. In order to test the technique, we combined three superluminescent light emitting diodes together in a Fourier Domain Common Path OCT set-up. The result shows that this technique is promising in differentiating blood oxygenation status.

1. INTRODUCTION

Hemoglobin oxygen saturation is an important medical diagnosis that requires accurate measurement. A widely used device is pulse oximeter [1], which gives real time monitor of arterial blood oxygen saturation non-invasively. Other imaging techniques have also been developed to measure the oxygen saturation with high spatial resolution, such as multi aperture camera [2] and hyperspectral microscopy [3]. All these methods are based on the different absorption properties of hemoglobin (Hb) and oxygenated hemoglobin (HbO2). Being able to provide localized spectra of sample under examine, Optical Coherent Tomography (OCT) has the potential of measuring localized oxygen saturation level [4-7]. A technique called spectroscopic OCT was used to extract spectroscopic information of the sample [8]. In spectroscopic OCT, A-scan signal is segmented with a window function and the windowed signal is Fourier analyzed. The result gives the spectral property of the sample at a specific depth. Applying this technique to blood sample can be used to analyze the localized oxygen saturation level. We used common path Fourier Domain OCT developed in our lab to measure the oxygen saturation level and the result is promising.

2. COMMON PATH OCT

The interferometer and spectrometer design in our FD CP OCT system are described in Ref [9], shown in Fig 1. The reference surface is at the distal end of probe arm and the interference signal of reference and sample light is detected by a spectrometer. To achieve a broad bandwidth source output, multiple SLEDs were combined to serve as broadband source, which centers at 800nm.

3. THEORY AND MORLET WAVELET TRANSFORMATION

To obtain the localized spectrum from the sample, Morlet wavelet transformation was used to analyze the A-scan signal. Morlet wavelet transformation first filters out the signal centering at a depth \( l \) with a Gaussian window and then performs inverse Fourier Transform to the filtered data. The transform can be expressed as following:

\[
 r_i(k,l) = \left| \text{IFT} \left[ i(z+l) \exp \left( -z^2 / L^2 \right) \right] \right|^2
\]

(1)

\( r_i(k,l) \) can be understand as the wavelength dependent path length resolved reflectance. \( k \) is wavevector and \( k = 2\pi / \lambda \). The spatial resolution of this analysis is determined by the width of the Gaussian window \( L \) and the spectral resolution is inversely proportional to \( L \).

Assuming the OCT signal decays exponentially, we thus have...
There is integration in this equation, because the spectral modification is cumulative. \( \alpha_s \), the attenuation coefficient, is the sum of scattering \( \alpha_s \) and absorption coefficients \( \alpha_a \).

\[
\alpha_s = \alpha_s + \alpha_a
\]

Both \( \alpha_s \) and \( \alpha_a \) varies with different wavelength, and \( \alpha_a \) is oxygen saturation dependent.

\[
\alpha_s(\lambda, SO_2) = SO_2 \alpha_{HbO_2}(\lambda) + (1 - SO_2) \alpha_{Hb}(\lambda)
\]

Although it is difficult to quantitatively extract attenuation coefficient of the tissue from OCT measurement and scattering coefficient is much larger than absorption coefficient, we could still use the difference of \( r_s(\lambda, l) \) at different wavelength as an indication of oxygenation status, which is shown below:

\[
\Delta \alpha_s(SO_2) = \alpha_s(SO_2, \lambda_1) - \alpha_s(SO_2, \lambda_2)
\]

\[
= SO_2 \left[ \alpha_{HbO_2}(\lambda_1) - \alpha_{HbO_2}(\lambda_2) - \alpha_{Hb}(\lambda_2) + \alpha_{Hb}(\lambda_1) \right] + \alpha_s(\lambda_2) - \alpha_s(\lambda_1) - \alpha_{Hb}(\lambda_1) - \alpha_{Hb}(\lambda_2)
\]

\[
= aSO_2 + b
\]

Equation (5) shows that the scattering term contributes as a constant background when \( SO_2 \) varies and \( \Delta \alpha_s(SO_2) \) is linearly proportional to \( SO_2 \).

To compare \( r_s(\lambda, l) \) at the wavelength range above and below 800nm, we averaged \( r_s(\lambda, l) \) from 730nm to 780nm and 810nm to 870nm, respectively. The ratio between the two averaged values, defined in (6), was used as an indication of the oxygen saturation level.

\[
R(l) = \frac{\text{mean} \left[ r_s(\lambda, l) \right]_{\lambda_1}}{\text{mean} \left[ r_s(\lambda, l) \right]_{\lambda_2}}
\]

4. RESULT

4.1 ex vivo experiment with blood sample

In this experiment, the probe arm is composed of a 10X objective lens, a 1mm-thick glass cuvette, and a mirror, shown in Fig 2. The cuvette and the mirror are fixed together. By adjusting the position the objective lens vertically, we were able to focus on the lower glass-air interface of the cuvette. Also, light reflected by the mirror gets coupled back to CPOCT system. As a result, we detected interference signal between electrical field \( E_1 \) and \( E_2 \). Under this circumstance, either of the fields can be called reference field and hence the other can be sample field. The interference term in detected intensity is of interest, which is

\[
I_{\text{int}} \propto \exp(-2\alpha_s l)
\]

We took three sets of data while keeping the probe arm setup unchanged. The first set of data (Fig 3a) was taken when the cuvette was filled of water; the second (Fig 3b) and third (Fig 3c) one were taken when we replaced water with whole blood (\( SO_2=100\% \)) and deoxygenated blood (\( SO_2=0\% \)). De-oxygenation was achieved by adding Sodium Dithionite into the blood. The color of blood turned dark once we added the Sodium Dithionite. The interference signals in Fig 3 are analyzed by Morlet wavelet transformation. First, Fourier Transform is performed and then the OCT signal corresponding to the interference between mirror and glass reflection is filtered out. Afterward, inverse Fourier Transform is applied to get the spectra corresponding to the interference signal. The result is shown in Fig 4a. We normalized the spectra obtained when hemoglobin and oxy-hemoglobin is in the cuvette with the one obtained when water is in the cuvette, to eliminate spectral distortion caused by spectral properties of optics fiber, coupler and objective lens, as well as distortion caused by the response of spectrometer.
The result in Fig 4a is consistent with the result in literature shown in Fig 4b, which verifies that the common path OCT setup is able to differentiate the absorption spectra of hemoglobin and oxyhemoglobin with OCT. Gaussian windows with different Full Width Half Maximum (FWHM) are applied to get the absorption spectrum. The result is shown in Fig 5. The cross over behavior of Hb and HbO2 is observable even when we applied a narrow Gaussian window with FWHM of 10μm. The result reveals that we can achieve a very high spatial resolution by our OCT.

We conducted an in vivo experiment with chick embryo as sample. We simply used a fiber cut in right angle as probe. A chick embryo with the inner shell membrane peeled was put inside a container. 5 sets of A-Scans were taken. Afterwards, we lighted an alcohol burner inside the container, wait about 5 minutes to obtain another 5 consecutive sets of A-scans. The incomplete burning of alcohol produces carbon monoxide, which combines with hemoglobin to form Carboxyhemoglobin (COHb), which has a distinctly different absorption spectrum from Hb and HbO2. Since hemoglobin bonds to carbon monoxide preferentially much higher (200:1 or higher) rate compared to oxygen, introduction of even a small amount of carbon monoxide can significantly change the SO2 level in a chicken embryo and thus significantly change the spectral properties of the measured OCT signal.

The result of this experiment is shown in Fig 5. For all the data taken, we processed them with Morlet Wavelet transform and calculated \( R(l) \). Data were divided into two groups. One group of data was taken before the burning of alcohol lamp and the other group of data was taken after. \( R(l) \) corresponding to each group are averaged to give red and blue curves in Fig 6. The mean value of the red curve is larger than the blue curve, because the COHb absorption spectrum is relatively flat compared to the HbO2 absorption spectrum and HbO2 absorbs more light with the longer wavelength. The result of this experiment verifies we are able to differentiate different oxygenation status in vivo.

### 4.3 Experiment with B scan

In this experiment, the probe is also a bare fiber. We scanned the fiber probe laterally above a vessel in a chicken embryo attached to the eggshell to form a 2D image, as shown in Fig 6a. A Gaussian filter is applied to select the Region of Interest (ROI), shown in Fig 6b, which is the surface of the eggshell. Fourier Analyzing each column in the image if Fig 6b, we obtain the image in Fig 6c, which is formed by spectra at different lateral position. The signal intensity in Fig 6b varies significantly. Therefore each spectrum in Fig 6c was normalized to its maximum value. There is remarkable difference between the region underneath the blood vessel and the region under the membrane in Fig 6c. For each spectrum in Fig 6c, we
applied equation (6) to obtain R value at every lateral point and the result is in Fig 6d, where the difference can be clearly seen.

Fig 5 a. OCT image of vessel; b. Region of interest in the OCT image obtained; c. spectra of ROI; d. R value as a function of lateral position

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11. REFERENCES