Tissue Bound Water Studies on Breast Tumors using Diffuse Optical Spectroscopy

So Hyun Chung[1], Albert Cerussi[1], Sean Merritt[2], David Hsiang[3], Rita Mehta[4] and Bruce J. Tromberg[1]

[1]Laser Microbeam and Medical Program, Beckman Laser Institute, University of California, Irvine, Irvine, CA
[2]Masimo corporation, Irvine, CA
[3]Division of Oncological Surgery, Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, Orange, CA
[4]Department of Medicine, University of California Irvine Medical Center, Orange, CA

Abstract

Differences in tissue water state have been measured in normal and malignant breast tissues. Broadband Diffuse Optical Spectroscopy (DOS) has been used to acquire 650-1000 nm absorption spectra of normal and tumor breast tissues from 7 patients *in vivo*. The absolute values of spectral differences between normalized tissue water spectra and pure water spectra were combined and divided by the number of points in the sum to form the bound water index (BWI). In all subjects, the average BWIs of line scan points were significantly lower in tumor tissues ($1.62 \pm 0.27 \times 10^{-3}$) than normal tissues ($3.06 \pm 0.51 \times 10^{-3}$, Wilcoxon Ranked Sum Test $z = 0.003$ and power=0.98). These results imply that the water in tumors behaves more like free water than the water in normal tissue.

Key Words: Bound Water, Diffuse Optical Spectroscopy, Breast Cancer

1. INTRODUCTION

Water is an important chromophore which can indicate physiological and pathological changes in tissue (1,2). Several optical research groups recognize the importance of water and measured increased water fraction in tumor tissues compared to normal tissues (1-5). Even the water concentration has been shown to have a correlation with cancer progress and tumor size (1). Water in tissues is also being measured routinely by MRI groups by observing the intensity of pixels in T2 weighted images. Measurements of water state can provide more details about pathological changes of tissues. For example, cellularity is related to the amount of protein-bound water, which is different from free water. MRI research groups have measured the mobility of diffusing water, and they have observed variations in the Apparent Diffusion Coefficients of water (ADCw) in tumor and normal tissues (6-8). By comparing ADCw to histological data, it has been shown that low ADCw values are correlated with cellularity.

In this study, we measured water state differences in malignant tumor tissues and normal tissues using broadband Diffuse Optical Spectroscopy (DOS). DOS measures absorption and scattering spectra in cm-thick tissues using a combination of frequency domain and steady-state spectroscopies.(1,9) Because of increased binding of water molecules to macromolecules such as protein, the water absorption feature at 980nm undergoes broadening and red shifting. We introduce a Bound Water Index (BWI) that quantifies this spectral shift. In order to validate the BWI we report preliminary results from breast cancer patients.

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2. METHODS

2.1 Instrumentation

The details of the broadband DOS system have been described in other papers (1, 9). The core characteristic of the broadband DOS system is the combination of modulated multi frequency domain (FDPM component) and broadband steady state domain(SS component). For FDPM, the lights from 658, 682, 785, 810, 830 and 850nm laser diodes were amplitude modulated from 50 to 600MHz sweeping 401 frequencies by combining a DC current and an RF modulation current provided by a network analyzer. The laser diodes delivered less than 20mW optical power to the tissue. An avalanche photodiode detector (APD) detected the phase and amplitude of the diffused optical signals after the light's propagating through the tissue. The detected phases and amplitudes were compared to those of the source by the network analyzer which worked as a fast electronic heterodyning digitizer. The SS system is composed of a high intensity tungsten-halogen light source and a high resolution spectrometer (B&W Tek 611). Because the bound water calculation algorithm is based on the absolute wavelengths of the spectrum, automatic and stable calibration of the spectrometer is necessary. The SS system enabled acquisition of a continuous absorption spectrum even in the water spectrum wavelength range (>935nm) longer than the wavelengths of the laser diodes.

We employed a handheld probe as described in the paper of Cerussi et al. to measure breast cancer patients. Optical fibers for the source of the FDPM system, for the source light of the SS system and for the detector of the SS system are secured in the handheld probe. The APD is housed in the handheld probe directly. The distance between sources and detectors of the FDPM and SS systems can be changed by moving an attachment on the probe.

To remove obstacles of cable length and source strength variability of the FDPM system, a tissue-simulating phantom with known optical property has been used as described in the paper of Cerussi et al. For the SS system, an integrating sphere has been used to eliminate wavelength dependent artifacts from the system.

2.2 Spectral Processing

Reduced scattering coefficient (μ_s') and absorption coefficient (μ_a) have been measured and separated by FDPM theory. The details of this theory have been described in other literature (1, 9, 10). The volume fraction of major chromophores (ctHHb, $ctHbO_2$, ctH_2O and lipid concentration) have been calculated as described in Cerussi et al. but there were some differences in the employed molar extinction values. For water, the molar extinction coefficients were obtained by our group by measuring distilled water in a spectrophotometer at various temperatures. Those water spectra of various temperatures have been used in the post-processing step to cancel out temperature effect. The employed molar extinction values of lipid were obtained by van Veen et al (11).

2.3 Post-processing for bound water measurements

The obtained μ_a values were post-processed to measure different states of water. In order to get only water spectrum, spectra of oxy- and deoxy- hemoglobin and lipid were subtracted from the original absorption spectrum under the assumption that only oxy- and deoxy- hemoglobin, lipid and water are major influential chromophores in breast tissues. Then the obtained water spectrum was compared to a pure water spectrum of the breast temperature. The difference between the tissue water spectrum and the pure water spectrum was calculated by subtracting pure water spectra from normalized tissue water spectra in the wavelength range from 935nm to 998nm. Then, the absolute values of the differences were combined and divided by the number of points in the sum to form Bound Water Index (BWI).

2.4 *In-vivo* breast measurements

Line scans were performed to measure *in-vivo* malignant breast tumors. There were 7 patients (age: 48.3±7.4) and all of them were measured by the same spectrometer (B&W Tek 611) which performed the auto calibration. The methods used for characterizing the optical and physiological properties of line

scans were the same as those employed by Cerussi et al. Identical arbitrary DOS parameters were employed to characterize physiological properties of tumor and normal tissues. In order to test statistical significance, the two-tailed Wilcoxon/Kruskal-Wallis Rank Sums test was employed.

3. RESULTS

Tissue absorption spectra were acquired from 7 subjects who had malignant breast tumors. The measurements were performed contralaterally so that spectra could be acquired from both normal tissues and tumor tissues from the same subject. In Figure 1, we can observe water peak changes in normal and tumor tissues compared to pure water peak after normalization. As explained in the methods section, BWI was acquired by calculating the differences between the pure water spectra and the tissue water spectra. BWIs calculated on every line scan point were compared to Tissue Optical Index (TOI: ctH2O x ctHHb / Lipid) and ultra sound reports of each patient. BWIs were lower in tumor areas than in normal tissue areas in all subjects (Fig.2) which is contrary to the trend of TOI. Two arbitrary parameters were employed to compare BWI of normal tissues and tumor tissues. In normal tissues, BWIs of all line scan points were averaged to represent Navg. In tumor tissues, the minimum three points of BWI were averaged to form Tpeak. The BWI was 3.06±0.51 in Navg and 1.76±0.30 in Tpeak from the 7 subjects. To test the statistical significance of this result, the two-tailed Wilcoxon/Kruskal-Wallis Rank Sums test has been performed between average values of all line scan points of normal and tumor tissues. We arrived at a z-value of 0.003 with power 0.98, which indicates that the BWIs of normal and tumor data sets were significantly different from each other.

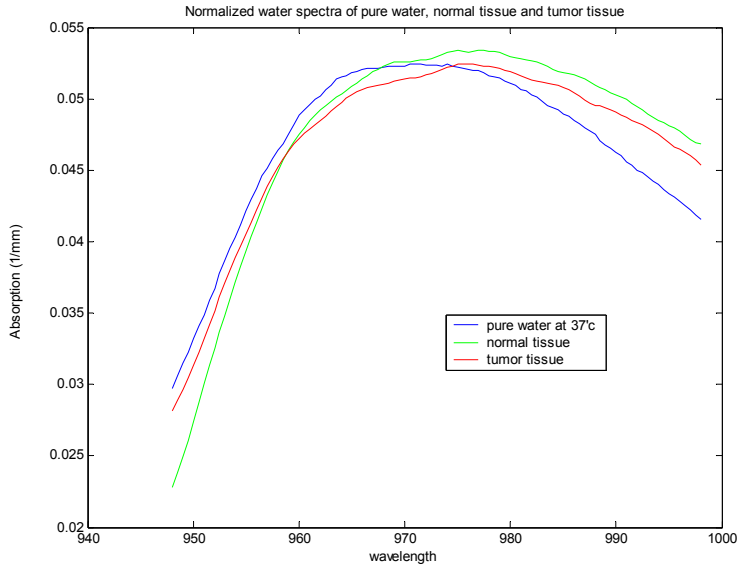

Fig. 1 Normalized water spectra in range of 935 to 998nm of pure water at 37’c (blue), normal breast tissue (green) and tumor tissue (red)

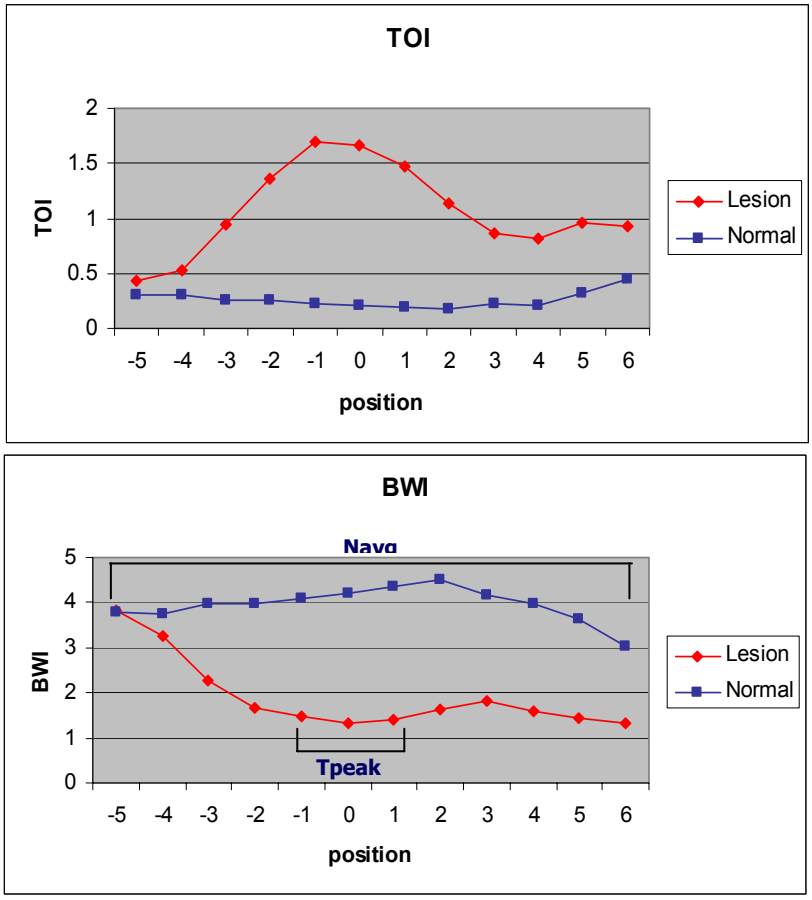

Fig. 2 Tissue Optical Index (TOI) and Bound Water Index (BWI) of line scanned breast tissues from one of the subjects. This subject had tumor from approximately -3 to 3.

4. DISCUSSION and CONCLUSIONS

Tissue bound water has been measured in breast cancer tissues using broadband DOS. BWI of normal tissues and malignant tumor tissues differed with statistical significance. Lower BWI in tumor tissues means that there is more free water in tumor tissues than normal tissues. This is likely due to tumor tissue swelling and edema. Future work will include increasing the number of patients, developing a tissue bound water phantom, comparing optical measurements to gold standard methods such as NMR and electrical impedance tomography.

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