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Authors
Cerussi, AE
Bevilacqua, F
Shah, N
et al.

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The Effects of water and lipids on NIR optical breast measurements

Albert E. Cerussi[1*], Frederic Bevilacqua[1], Natasha Shah[1], Dorota Jakubowski[1], Andrew. J. Berger[2], Ryan Lanning[1], and Bruce J. Tromberg[1]

[1]Beckman Laser Institute and Medical Clinic; University of California, Irvine

[2]Institute of Optics; University of Rochester, Rochester, NY

ABSTRACT

Near infrared (NIR) diffuse optical spectroscopy and imaging may enhance existing technologies for breast cancer screening, diagnosis, and treatment. NIR spectroscopy yields quantitative functional information that cannot be obtained with other non-invasive radiological techniques. In this study we focused upon the origins of this contrast in healthy breast, especially from water and lipids.

Non-invasive NIR measurements were performed on the breasts of 30 healthy women using a seven-wavelength frequency-domain photon migration probe. Subjects included pre- and post-menopausal women between the ages of 18 and 64. A diffusive model of light transport quantified oxygenated and deoxygenated hemoglobin, water, and lipid by their absorption signatures. Changes in the measured light-scattering spectra were quantified by a "scatter power" parameter.

Substantial quantitative differences were observed in both absorption and scattering spectra of breast as a function of age. These physiological changes were consistent with long-term hormone-dependent transformations that occur in breast. Instrument response was not adversely affected by subject age or menopausal status. The impact of neglecting water and lipids upon optical measurements in the breast is to artificially ascribe extra absorption. The effect can be significant, causing 20-30% errors in hemoglobin concentration and 5% in hemoglobin saturation. These errors cold dull the inherent contrast between normal and tumor tissue and thus affect optical mammography.

Keywords: optical tissue spectroscopy, breast, photon migration, blood, water, lipids, tissue scattering

1. INTRODUCTION

1.1 The Role of optics in the breast

Diagnostic methods currently in use, such as mammography, magnetic resonance imaging, and ultrasound offer excellent anatomical lesion-detection capabilities, but are generally unable to provide quantitative information regarding tissue function and composition. Positron emission tomography shows great promise in evaluating the metabolic demands of tissue, but requires exogenous radionuclides and is insensitive to tissue hemodynamics. However, near-infrared (NIR) optical spectroscopy is intrinsically sensitive to the principal components of breast: blood, water, and adipose. Preliminary studies suggest that the fractional contribution of each to NIR signals depends strongly on factors such as age[12], menopausal status[3], and the progression of disease[4]. Thus, NIR optical spectroscopy provides an opportunity for revealing physiological information that is unobtainable by other non-invasive techniques.

* acerussi@bli.uci.edu; phone (949)824-4104; fax (949)824-8413; http://www.bli.uci.edu; Beckman Laser Institute and Medical Clinic, University of California at Irvine, 1002 Health Sciences Road, East, Irvine, CA 92612

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1.2 Optical absorption in breast

NIR absorption in breast is mostly due to blood, water, and lipids. Although other chromophores such as cytochrome and myoglobin absorb NIR light, they do not exist in high enough concentrations to have a dramatic effect on the overall absorption in breast. Figure 1 provides the molar extinction coefficients of hemoglobin[5] in reduced (Hb-R) and oxidized (Hb-O_2) forms, water[6], and lipids.[7] The absorption of a chromophore (μ_a, mm^{-1}) depends both the molar extinction coefficient (ε, $cm^2 M^{-1}$) and the concentration (c, $M\ L^{-1}$), since $\mu_a = 2.303\varepsilon\, c$.

NIR measurements confirm that blood volume constitutes only a few percent of tissue volume (μm concentrations), whereas water is typically in the molar concentration range.[8] Lipids are also very prominent, particularly in breast. Although water and lipids are weak absorbers compared to hemoglobin, their overwhelmingly vast presence in breast tissue translates into enough absorption to compete with hemoglobin. In addition, the balance of water and lipids in the breast depends upon a number of factors, such as hormonal status and age.[9]

1.3 Contributions from the parenchyma

The contributions of water and lipids in breast are often overlooked or assumed known in optical measurements. Reasons for this generally center around instrumentation considerations; many instruments used in the study of the optics of breast tissue employ wavelengths out to 850 nm, which is typically the point where photomultiplier tubes face a dramatic drop in sensitivity. Although water absorption, and to a lesser extent lipid absorption, have less absorption than hemoglobin below 850 nm, some absorption does exist. There is also some overlap between lipids and Hb-R, since both have absorption peaks near 750 nm.

In some tissues such as muscle, it is a very reasonable assumption to ignore water and lipid absorption. Hemoglobin concentrations in adult muscle can easily be on the order of 100 μM as determined by deep-tissue measurements in a reflection geometry. Neglecting water in high Hb concentration environments translate into errors less than 5 %percent in StO2 for values above 50%.[10]

However, the breast is a totally different matter. The overall concentrations of Hb are much lower in breast than in muscle, as the overall absorption of breast is far lower than that of muscle.[12-3,11,12] In addition, the amount of lipids will be much more significant in breast than in muscle. To complicate matters further, the amounts of water and lipids in breast can vary wildly with age, hormonal status, and menstruation, making it difficult to pin down a specific value for a given population of women.[9]

After ovulation, blood flow to the breast can increase by up to 50%, there is an increase in breast volume, and parenchymal water content changes by an average of 25% during the latter half cycle.[13,14] One MRI study indicated that menstrual changes in breast did not demonstrate predictable cyclic variations in 3 out of 7 subjects. MRI studies have also observed changes in lipids throughout the menstrual cycle.[14,15]

1.4 Optical cancer detection

Most modern NIR studies in breast measure the concentrations of hemoglobin. Since tumors are expected to have increased metabolism and blood flow relative to normal tissue, hemoglobin provides an endogenous physiological contrast agent for tumor detection and characterization. Important parameters include the total hemoglobin concentration (THC = [Hb-R]+[Hb-O_2]) and the hemoglobin saturation (S_tO_2 = [Hb-O_2]/THC).

The success of native contrast NIR methods in the breast depends upon the ability to distinguish a tumor from normal tissue based upon changes in THC and/or S_tO_2). Errors in μ_a will adversely affect the accuracy and precision of these measurements, and may ultimately result in missed tumors and diagnoses. Although water and lipids absorb far less than hemoglobin, their tissue concentrations are much higher than those of hemoglobin. Tissue is typically about 60% water by mass,[16] and the mammary gland in particular may have 30-73% water[17]. Lipids also

make up about 19% of tissue by mass[16], and approximately 5-56% in the mammary gland[17]. These values will also vary over a wide range of conditions, such as age and menopausal status.

1.5 Scope of this work

The purpose of this study was to investigate the effect of water and lipids upon NIR photon migration measurements in breast. First, we provide measurements of water and lipids in the breasts of 30 healthy volunteers. We will show that there are substantial quantitative differences in the blood, water, and lipid contents of pre- and post-menopausal breasts. Measurements are also presented as a function of subject age, and demonstrate known physiological transformations that occur in the breast as a result of changing ovarian hormone production. We then will demonstrate that neglecting the absorption effects of water and lipids can result in significant errors when trying to determine the hemoglobin concentrations of breast tissue.

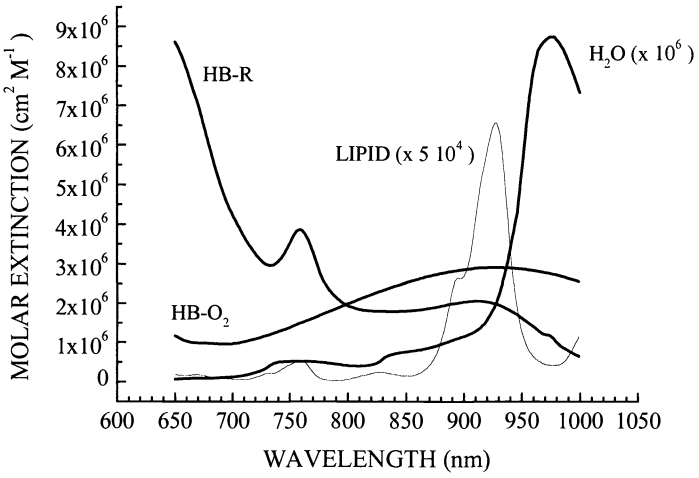

Figure 1 - *Dominant NIR molar extinction coefficients in breast.* The lipid spectrum is shown multiplied by 5 x 10^4, the water spectrum by 10^6.

2. MATERIALS AND METHODS

2.1 Patient measurements

All volunteers enrolled in this study competently provided informed consent for participating in one of two trials (#95-563 and #99-2183) under the guidelines of the Institutional Review Board of the University of California at Irvine. The 30 volunteer ages ranged between 18 and 64. Seventeen of the volunteers were pre-menopausal (average age 30 +/- 10). Seven were post-menopausal (average age 56 +/- 2). The remaining six (average age 56 +/- 5) were women taking some form of hormone replacement therapy (HRT); three of these six women classified themselves as peri-menopausal. None of the women had any known cancerous lesions or other known forms of breast disease.

Each volunteer rested in a supine position during the measurement. The instrument probe, which is slightly larger than an ultrasound probe, was the only item placed in contact with the volunteer. All measurements were performed in a reflection-style geometry, using a source-detector separation of 22 mm. The probe was placed on the breast with minimal pressure using only the force of gravity; no compression was used. In this configuration, we estimate that the light sampled approximately 1 cm below the skin.

2.2 Instrumentation

The specific details of our 1 GHz frequency-domain photon migration (FDPM) instrument have been described in detail elsewhere,[18] but the relevant technical information is mentioned here. The instrument employs multiple diode lasers that provide visible and NIR light at seven wavelengths (672, 800, 806, 852, 896, 913, and 978 nm). A hand-held probe has been designed to house an avalanche photodiode that records the modulated diffuse light signals after propagation through the tissue. This probe has a plastic attachment on the casing to position a source optical fiber a fixed distance from the avalanche photodiode. The optical power launched into the tissue ranged from 5-25 mW for each wavelength. A sweep over all seven wavelengths ranged from approximately 35 to 60 s. The system was wheeled into a medical clinic for the each measurement. Instrumental artifacts were removed by calibrating on a tissue-simulating phantom with known absorption and scattering properties.

The instrument records phase and amplitude as a function of source modulation frequency from the diffuse reflectance of detected photons. Tissue absorption and reduced scattering (μ_s') were determined from simultaneous fitting of phase and amplitude to a light-diffusion model (the P_1 Approximation to the Boltzmann Transport Equation).[19,20] When the optical properties μ_s' and μ_a are recovered for the seven wavelengths, the spectral-dependence of the absorption was combined with known values of molecular extinction coefficients (see Figure 1) to calculate physiologically-relevant parameters.

For each measurement we determined four hemoglobin parameters: [Hb-R], [Hb-O_2], *THC*, and S_tO_2, as well as [H_2O] and the lipid mass density. These values were measured by performing a weighted least squares fit of the measured μ_a values, and fitting them to the known molar extinction coefficients provided in Figure 1. We assumed that these extinction values are not significantly different from those present in actual tissues.

The scattering properties of the tissue also yield important physiological information. NIR scattering in tissue has the following dependence: $\mu_s' = A\,\lambda^{-SP}$, where A is a constant, λ is the wavelength (nm), and the exponent SP is the scatter power. Scatter power is related to the scattering center size (d) compared to the optical wavelength. As an example, scatter power is 4 in the case of Rayleigh scattering ($d<<\lambda$) and is ~1 for large Mie-like scatterers ($d \sim\lambda$).

3. RESULTS

3.1 Premenopausal and postmenopausal examples

Figure 2 presents a typical series of measurements of seven absorption and scattering coefficients. The points represent an average of several measurements in the center of the left upper outer quadrants of two volunteers; a 32-year old pre-menopausal woman (squares) and a 54-year-old post-menopausal woman (triangles). Error bars show the standard deviation of repeated measurements. There are vast absorption and scattering differences between pre- and post-menopausal breast tissue. The solid lines of Figure 2a represent a weighted-least-squares fit of the seven absorption coefficients using published extinction coefficients for Hb-R, Hb-O_2, H_2O, and lipids (see Figure 1). Lines between the measured points have been interpolated. The solid lines of Figure 2b represent a fit of the scattering coefficients to the equation $\mu_s' = A\,\lambda^{-SP}$. Error bars again represent the standard deviation of repeated measurements.

These optical spectra provide insight into breast tissue composition. Recovery of the absorption spectra allows calculations of the tissue concentrations of Hb-R, Hb-O_2, H_2O, and lipids. There are higher concentrations of

hemoglobin (i.e., both Hb-R and Hb-O_2) in the pre-menopausal volunteer, as evidenced by the overall higher absorption in the 670 to 850 nm range. There is also more water relative to lipids in the pre-menopausal subject, as revealed by the large water absorption peak at 980 nm. Also significant is the difference in S_tO_2. The pre-menopausal breast has a higher density of highly vascular glandular tissue, compared to the post-menopausal breast, where the glandular tissue begins to atrophy into fatty tissue because of the cessation of ovarian estrogen production. Figure 3 demonstrates the contribution of the blood, water, and lipids to the total absorption spectrum for both the pre- and post-menopausal volunteers. The light scattering intensity and scatter power is significantly lower in the post-menopausal breast. Table 1 provides a summary of the fitted physiological properties for these two subjects.

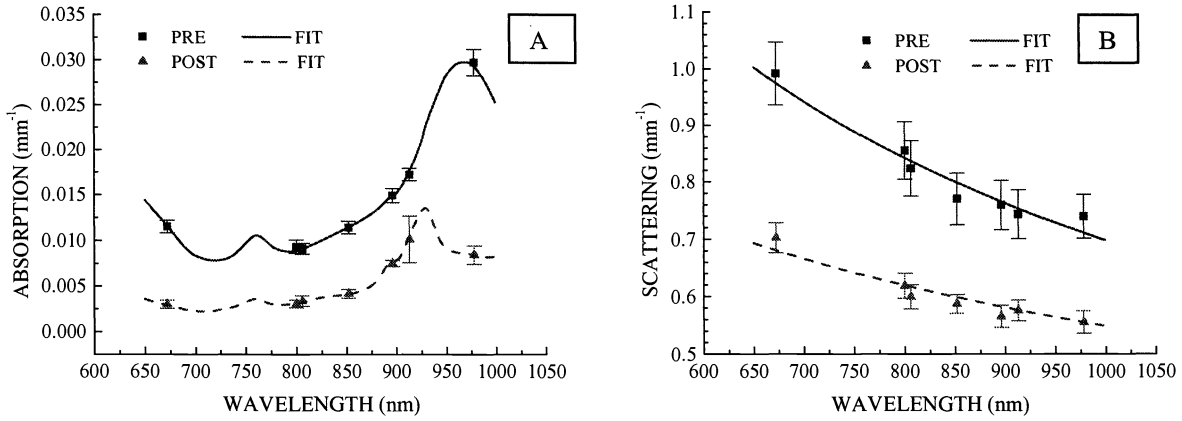

Figure 2 *Spectra of a pre- (squares) and a post-menopausal (triangles) breast.* Points represent the average of several measurements in the center of the left upper outer quadrant. Panel (a) provides the measured absorption values. The lines represent a least-squares fit (extrapolated to all wavelengths) assuming the breast absorption is due to only Hb-R, Hb-O_2, H_2O and lipids. Panel (b) provides the scattering for same volunteers. The lines represent a fit to $\mu_s' = A\,\lambda^{-SP}$. Results of the fits from this measurement are listed in **Table 1**.

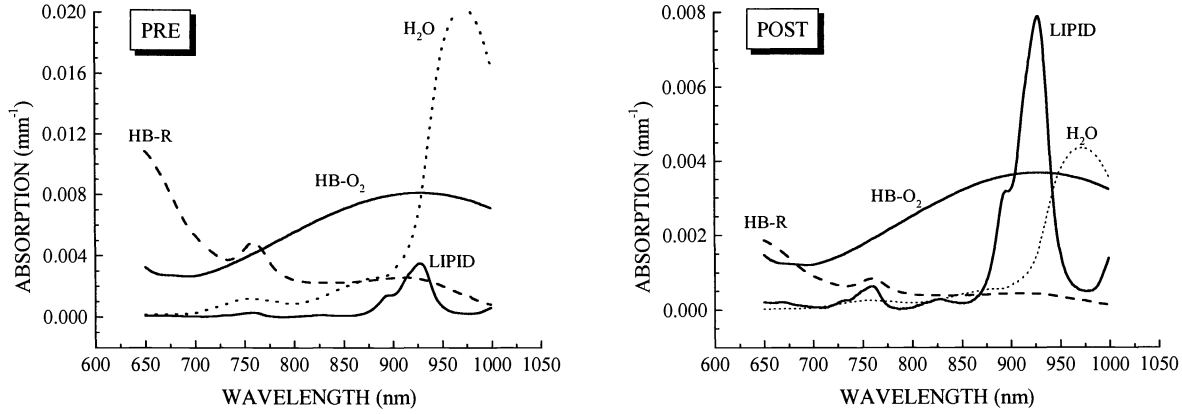

Figure 3 *Breast component spectra contributions to total absorption.* These plots translate the values in Table 1 for the pre-menopausal (PRE) and post-menopausal (POST) spectra into absorption spectra using the molar extinction coefficients provided in Figure 1. The four-component spectra sum to the total absorption spectra provided in Figure 2.

Table 1- Measured physiological properties of a pre-menopausal (PRE) and a post-menopausal (POST) breast

	[Hb-R] (μM)	[Hb-O_2] (μM)	S_tO_2 (%)	THC (μM)	[H_2O] (M)	LIPID (Kg L^{-1})	SP
PRE	12.6±0.7	27.9±2.7	68.9 ± 1.3	40.4 ± 2.7	25.1 ± 2.0	0.268 ± 0.050	0.864 ± 0.068
POST	2.36 ± 0.49	12.2 ± 1.6	83.7 ± 2.5	14.4 ± 1.9	5.73 ± 0.44	0.608 ± 0.033	0.555 ± 0.036

3.2 Age-dependent parenchymal components

Figure 4 presents the tissue water concentration (panel (a)) and the lipid mass density (panel (b)) as functions of age. Premenopausal subjects (i.e., age <50) display a variety of values. This spread is the result of inter-subject variations, including, but not limited to, menstrual cycle differences and gynecological age. The same general trend occurs in the THC and scatter power, although not shown here.[1] The THC and to a lesser extent the water seem to increase in pre-menopausal subjects (ages 18 to 39), perhaps reaching a peak value near the age of 30. Between the ages of 40 to 49, the THC appears to level off, while water and scatter power seem to decline. After age 50 (predominantly post-menopausal) there is a general decrease with age in THC, water, and scatter power, and an increase in lipids. Error bars represent the results of repeated measurements. The late decrease in THC and water correlates well with previous histological studies showing both the atrophy of well-vascularized lobular tissue and the increase of the fat-to-collagen ratio after menopause.[9] Compositional analysis data show lower blood and water content for fat versus glandular tissue.[16]

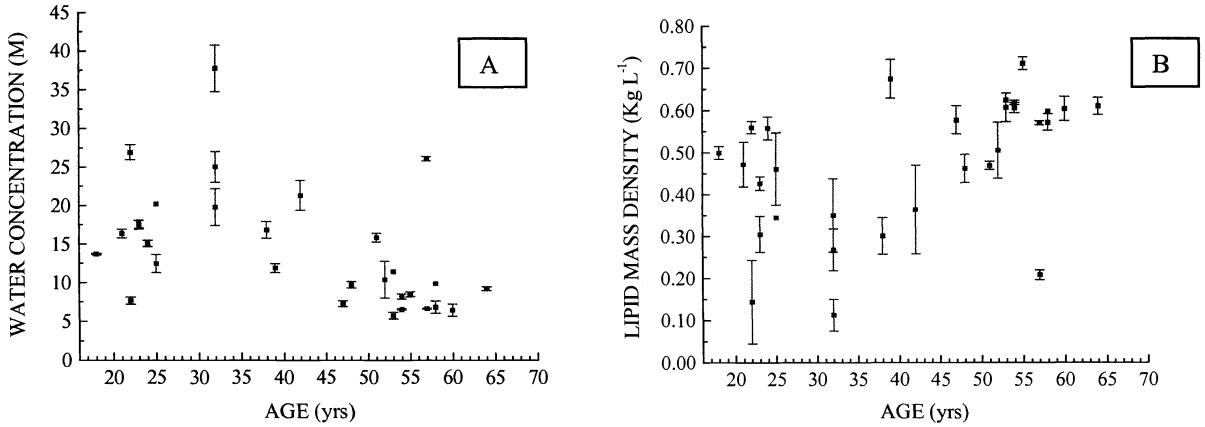

Figure 4 *Age dependent water and lipid values.* Panel (a) displays the water concentration. Pre-menopausal volunteers (< age 50) show considerable variation over age because of intra-subject variations such as menstrual cycle variations and overall hormone production differences. Post-menopausal and peri-menopausal (> age 50) volunteers appear to show a decrease in water content. The situation is essentially reversed for the lipid mass density (panel (b)).

3.3 Scaling of lipids

Measured lipid and scatter power are presented as functions of body-mass index (BMI) in figures Figure 5a and Figure 5b respectively. The BMI scale is determined by dividing body mass (Kg) by height squared (m), and numbers between 20-25 are considered normal. The histograms represent an average over all BMI values within a single BMI unit, except for the two highest BMI values. There is a general increasing tend in breast lipid mass density with BMI. Overall body mass and breast mass are not sure-fire correlates, however, so that this correlation need not be perfect. Fattier breasts should scatter light less given that the ratio of adipose to glandular tissue will be less. Compared with adipose, collagen and glandular tissue scatter light with a higher intensity and a steeper spectral dependence.[21] Thus, smaller scatter powers are expected in fatty tissue. This interpretation may not be valid given that the amount of collagen in each breast is not known.

3.4 Errors from ignoring water and lipids

Figure 6 provides a simple example of the potential cost for ignoring the effect of water and lipid absorption in the breast. Panel (a) shows the effect upon the THC, whereas panel (b) shows the effect upon the S_tO_2. The same label scheme was used in both panels for two volunteers: a 24 year old pre-menopausal woman (PRE) and a 53 year old post-menopausal woman (POST). The dark (left) bar represents a single measurement made with our system after taking into account all four chromophores and using all seven wavelengths. The light (right) bar represents the same measurement, but using only the first four wavelengths (672, 800, 806, and 849 nm) and fitting for only Hb-R and Hb-O_2. It is clear from this simple example that neglecting the effect of water and lipids has the effect of making it appear that there is more oxygenated blood in the tissue: THC values increase by about 20 and 30% for the pre- and post-menopausal cases, respectively. This is mostly due to a perceived increase in Hb-O_2, since the S_tO_2 value increases by about 5% after neglecting water and lipids.

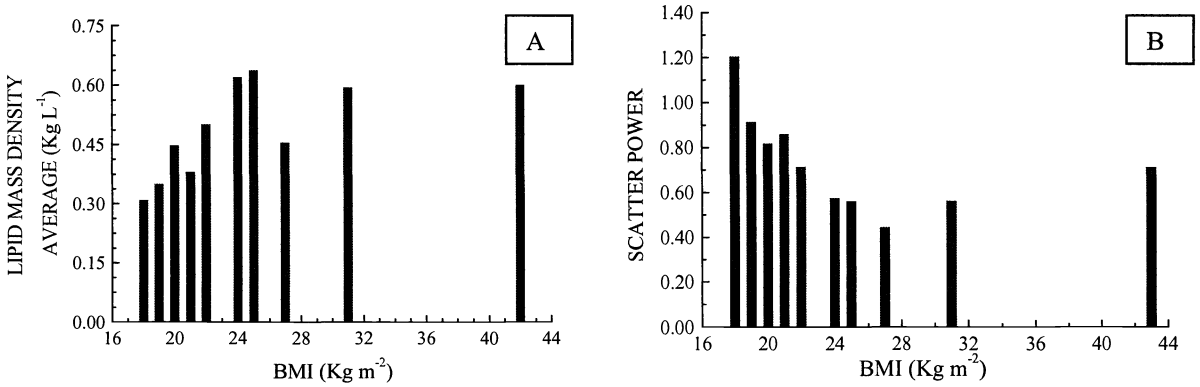

Figure 5 – *Histograms of lipids and scatter power over BMI.* Panel (a) presents the measured lipid mass density as a function of BMI. Values within 1 BMI value were averaged together. Panel (b) presents the same idea but with the scatter power.

4. DISCUSSION

Although it is difficult to directly validate non-invasive *in vivo* measurements, these initial results indicate that NIR spectroscopy is a reasonable quantitative reflection of long-term hormone-controlled breast remodeling. All of the measured physiological quantities match well with the expected transitions that occur in breast over time. Our results provide indirect validation of the general accuracy of NIR breast spectroscopy. It must be stressed that this quantitative physiological information is not obtainable by any other non-invasive radiologic method.

An important feature of NIR methods is the ability to characterize quantitatively the breast tissue of women regardless of age, hormonal status, or mammographic density. Increased mammographic density contributes to a 22% false negative rate as well as a high false positive rate (56.2% cumulative risk after ten exams) in women less than 50 years of age.[22,23] Radiographically dense breast tissue is due to differing amounts of fat, collagen, and water.[24] NIR spectroscopy has unique potential for quantifying the elements of breast tissue that contribute to mammographic density. This observation may be of importance in screening, since it may identify breast tissue at physiological risk for malignant transformation and can be performed easily in e all women.

Knowledge of the "normal" values of NIR chromophores will play a role in evaluating the usefulness of optical methods in detecting and characterizing lesions in the breast. Several investigators have reported a 2-4 fold THC contrast between normal and tumor structures. In vivo tumor S_tO_2 values are also typically lower than normal tissue.[4,25] Neglecting the effects of water and lipids further complicates efforts for accurate quantitative diagnosis. The effect of falsely ascribing lipid and water absorption to hemoglobin will inflate measured values of Hb-0_2, and thus inflate measurements of *THC* and S_tO_2. This change in contrast will decrease the probability for success of non-invasive optical lesion characterization. For every woman the effect of water and lipids will be different so that the error in measured hemoglobin levels will be different, and potentially unpredictable. Worst still, the effect will be more sever for patients with lower *THC* levels, i.e., older women.

Detailed studies of normal tissue are essential for determining the sensitivity required of optical instrumentation for detecting lesions in women of varying age and hormonal status. As baseline levels are characterized, data on an individual's absorption and scattering variations could provide important insight into disease appearance and progression. When applied to patients receiving chemotherapy and/or HRT, this information could also be used to generate feedback that would permit customized treatment planning based upon individual physiologic response.

One potential problem with our analysis is the absorption overlap between lipids and water. Seven wavelengths may not provide enough spectral resolution to quantify these two chromophores without some form of crosstalk. We have shown that the information provided by water and lipid absorption parallels that obtained from measuring the scatter power.[1] Thus, although some form of crosstalk between water and lipids may exist, we do not expect this to affect the spirit of our findings.

It is fortunate that two of the diodes, the 913 and the 978 lie very close to the absorption peaks of lipids and water, respectively. In order to increase our sensitivity to these and other chromophores, we have implemented a steady-state measurement in order to fill in all wavelengths between 650 to 1000 nm.[26] By accurately measuring the wavelength-dependent scattering over the wavelength region of interest, we may calculate the scattering at NIR wavelengths by assuming that $\mu_s' = A\,\lambda^{-SP}$. Thus, a broadband reflectance spectrum may be corrected for scattering by using the results of our FDPM measurements.

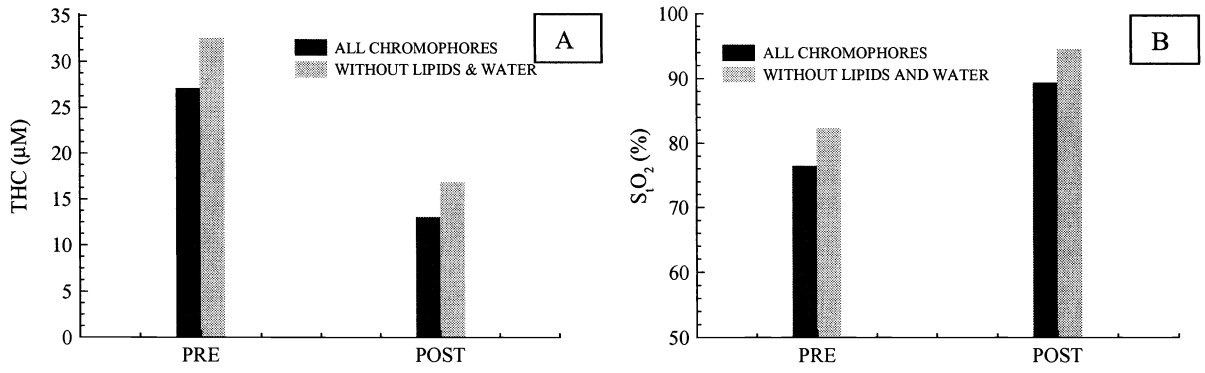

Figure 6 –*Effect of neglecting water and lipid absorption.* This is a simple example demonstrating the potential effects of neglecting water and lipids in NIR tissue measurements. See text for details.

5. CONCLUSIONS

We have demonstrated that our FDPM measurements provide a reasonable functional picture of the breast in a sample of 30 healthy women. By quantifying the concentrations of water and lipids, we have provided a more complete picture of the breast. The water and lipid levels in normal breast vary significantly with age and hormonal status, particularly between pre- and post-menopausal subjects. Ignoring water and lipid absorption can produce artificially high THC and $S_tO_2$values of perhaps 20-30% and 5%, respectively. These errors are systematic, and different for each patient. Although not discussed in this paper, characterization of water and lipids may also be important in their own right for characterizing breast disease.

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