Optical transillumination spectroscopy to quantify parenchymal tissue density: an indicator for breast cancer risk

1,2 M K SIMICK, MSc and 1,3 L LILGE, PhD

1Department of Medical Biophysics, University of Toronto, 2Sunnybrook and Women's College Health Sciences Centre, Toronto, Ontario, Canada and 3University Health Network, 610 University Avenue, Toronto, Ontario, M6G 2M9 Canada

Abstract. Mammographic screening for early detection of breast cancer has proven valuable in improving breast cancer survival. However, breast cancer incidence is still increasing, and thus preventative oncology needs to receive more attention, with the goal of identifying women with increased risk of developing breast cancer in the future and offering them risk reduction interventions. Mammogram derived parenchymal density pattern has been shown by various authors to provide a high odds ratio for breast cancer. Near-infrared optical transillumination spectroscopy was employed to determine physiological properties of the breast tissue to quantify differences in women with low or high breast cancer risk. Specifically in this study, women who had a recent mammogram underwent examination of their breast tissue by optical transillumination spectroscopy. Areas of adipose and glandular tissues which give rise to mammographic density patterns also have characteristic optical transillumination spectra. Correlation between optical transillumination spectroscopy and mammographic density pattern was established using partial least squares analysis. Results show that predicted tissue density based on optical transillumination spectroscopy correlates with mammographic observed tissue density, with a Spearman Rank correlation coefficient of 0.72. This suggests that optical transillumination spectroscopy may be a promising tool to quantify and monitor changes in breast cancer risk.

Breast cancer is the most commonly occurring cancer in women. The lifetime risk of being diagnosed with breast cancer is approximately 1 in 10 [1], the highest out of all cancers. Breast cancer screening programs have been shown to decrease the mortality rates of women between the ages of 50 and 69 years [2], since cancers are detected at an earlier stage when survival chances are higher [3].

While knowledge of the mechanisms leading to breast cancer is increasing, they are not completely understood. Hence, there is also a strong effort to understand risk factors for the disease [4], based on molecular markers [5], physiological measures [6], family history [7] or lifestyle habits [8]. However, with the exception of genetic markers [9], most known risk factors are available only late in life, such as first degree relatives with breast cancer or high tissue density derived from mammography [10–15]. Therefore, a physical risk assessment tool applicable to women of all ages will enable recruitment of women into risk reduction programs long before aggressive risk reduction interventions, such as chemotherapy or prophylactic mastectomy, may be required. Quantification of a woman’s breast cancer risk using a comfortable and convenient physical assessment tool may also result in higher compliance rates, through more than 6 cm of tissue still have a good signal-to-noise ratio [23, 24], suggesting that non-ionizing optical radiation can be utilized to assess bulk tissue properties, as required for risk assessment. Attenuation is governed by the tissue chromophores and light scattering. The latter is affecting their life style and quality of life less than, for example, chemotherapy [19, 20].

The risk of developing breast cancer can be determined during mammography by the amount of parenchymal densities seen [10–15]. Parenchymal (fibroglandular) tissue in the breast has high X-ray attenuation and hence appears as white cloudy areas in mammograms (Figure 1a,b). The high amount of epithelial tissue within the fibroglandular tissue is considered the source tissue for most carcinomas in the breast.

Physiologically, the presence of increased fibroglandular tissue raises the estimated risk by up to 6 fold [10, 15] after correction for age, and hence represents one of the strongest known risk factors pertaining to the entire female population above the age of 40 years, the onset of screening for breast cancer by mammography in many countries.

Consequently, the relative area of dense tissue within the mammogram is a strong risk factor. Parenchymal density pattern is commonly assessed qualitatively by the radiologist using an ordinal scale, or it can be quantified on an interval scale using computer assisted programs [21]. Changes in the parenchymal density pattern are monitored through the biennial screening program visits and it was shown that they are affected by hormonal and dietary changes [16], but not necessarily by chemoprevention [22].

Tissue absorbs light of the near infrared region (600–1200 nm) only weakly. Hence, spectroscopy measurements through more than 6 cm of tissue still have a good signal-to-noise ratio [23, 24], suggesting that non-ionizing optical radiation can be utilized to assess bulk tissue properties, as required for risk assessment. Attenuation is governed by the tissue chromophores and light scattering. The latter is...
proportional to the size and density of scattering particles, such as cells and their intracellular organelles. Variations in tissue, and hence its scattering particle density, lead to differences in optical path length and, therefore, greater overall wavelength dependent attenuation [25]. Dense cellular tissue volumes, such as ductal and fibroglandular tissues, will result in increased scattering and conversely in increased attenuation.

In a previous study of optical transillumination spectroscopy for risk assessment, we demonstrated that classification of breast density in women on an ordinal scale as having either low, or high, parenchymal density is feasible, with a sensitivity and specificity of approximately 0.9 [26, 27]. Classification is predominantly based on relative differences in light scattering and the concentrations of tissue components including water, lipids and various forms of haemoglobin.

The aim of the present study is to test the feasibility of quantifying breast tissue density as an in vivo marker for breast cancer risk using an interval scale, which is a continuation of previous data analysis [26–28]. Quantifying the tissue density on an interval scale, and thus the breast cancer risk, with high accuracy has importance for monitoring the breast cancer risk in individual women as they pass through puberty and menopause, or during risk reduction intervention. The latter may provide women participating in risk reduction intervention with feedback about the efficacy of this intervention.

Optical transillumination spectroscopy is not an imaging technique, in contrast to mammography, where spatially resolved X-ray attenuation information is obtained. However, for risk association, only the relative area of X-ray dense tissue within the mammogram has been shown to correlate as predictor to breast cancer risk [10–15], and hence volumetric optical spectroscopy may provide comparable information.

The study design is that of a cross-sectional study comprising pre- and post-menopausal women. Previous studies [23, 29] have shown that the menstrual cycle can induce changes in cellular proliferation and the relative water and blood content of breast tissue, which in turn
influences the spectral transmission properties. Hence, a subgroup of pre-menopausal women was recruited for repeat measurements to determine the influence of the menstrual cycle on the predictive ability of transillumination spectroscopy as a density assessment tool.

**Methods**

**Instrumentation**

Previous publications [26, 27] describe the instrument, shown in Figure 2, in detail. Briefly, the light source is based on a 20 W halogen lamp with the emission limited to wavelength in the 550–1150 nm range. The output of the lamp (Welch Allyn, Buffalo, NY) is directed via a liquid light guide (Kaiser Electronics, San Jose, CA) to the top surface of the breast resting on a black support plate. The total power delivered to the tissue is \(<250\) mW. Transmitted light is collected by a 7 mm diameter optical fibre bundle mounted in the support plate and aligned co-axially with the liquid light guide. A liquid nitrogen cooled 2D charge coupled device (CCD) (F-125; Photometrics, NJ) coupled to a holographic transmission spectrophotometers (15.7 lines mm\(^{-2}\); Kaiser, Carlsbad, CA) is the primary photo detector. To obtain spectra independent of the spectral transfer function of the optical setup and to account for daily variations in the source emission, all spectra were corrected using a standardization spectrum from a sample comprising of ultrahigh density polyurethane (Gigahertz, Munich, Germany). Ratio spectra were then divided by the interoptode distance, thus expressing the spectra in units of absorbance per centimetre of tissue. No further manipulation of the spectra prior to further analysis (see below) was required.

**Volunteer measurements**

Volunteers were recruited from the Marvelle Koffler Breast Imaging Centre at Mount Sinai Hospital in Toronto, after they had completed a standard screening mammogram and no radiologically suspicious lesions were noted. Exclusion criteria included prior fine needle aspiration, core biopsies or any other type of breast surgery including breast reduction or augmentation, and any type of tattoo. Transillumination spectroscopy measurements were taken in a dark room with the volunteer seated comfortably in an upright position with the breasts resting on a horizontal support (Figure 2). The liquid light guide was placed against the top surface of the breast with minimum pressure, launching the photons towards the inferior portion of the breast. Collection time per spectra was less than 1 min per location and the total time for collection of all 8 positions was approximately 15 min, including re-positioning of the optodes. The four quadrants measured per breast (centre, distal, medial and lateral) are indicated in Figure 2.

Pre- and post-menopausal women on hormone replacement therapy underwent transillumination spectroscopy in the third week of their menstrual cycle (if present). A group of pre-menopausal women came for four visits, at weekly intervals within a 1 monthly cycle. Table 1 shows the total recruitment of women into these studies. Owing to variations in breast size the interoptode distance was not standardized, so an effort was made to keep the tissue thickness for corresponding quadrants on the left and right breast consistent. In women coming for repeat visits it was also attempted to keep the interoptode distance constant over the four visits, unless discomfort to the volunteer was noted or voiced or no contact between the optodes and the breast was possible.

For women with repeat measurements the menstrual cycle was divided into 7-day intervals and subjects were measured once in each interval. For women with menstrual cycles not equal to 28 days the interval length was modified accordingly.

**Clinical study breakdown**

The available data set included mammograms and spectra from 92 volunteers, with 58 being post menopau-

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Figure 2. (a) Schematic of transmission measurement system. (b) Measurement of the C=centre position in volunteer. The other three measurement positions are denoted as D=distal, M=medial and L=lateral. Note: the minimal point compression of the breast between source optode and support plate.
Low (<25%)[^d]
Medium (25–75%)[^d]
High (>75%)[^d]
Age (years)
BMI (kg m$^{-2}$)
% First degree relative with breast cancer
% HRT current user

[^d]: Classification of global breast tissue density was performed by a radiologist (Dr Roberta Jong) showing representative breast tissue densities for the general population.

BMI, body mass index; HRT, hormone replacement therapy.

Mammographic tissue density collection

Mammography films were digitized using a Lumsys 85 Digital Scanner (Kodak, New York, NY) at 12 bits resolution and a pixel pitch of 260 µm. The images were viewed using Cumulus [21], an interactive density-threshold software, to obtain the area of percent dense tissue and establish the correlation between the mammographic tissue densities on an interval scale with the transillumination spectra. For the mathematical model to become independent of a specific user of the Cumulus program evaluating the mammograms, two different users were used to demonstrate the validity of the mathematical prediction models.

For analysis, either all 8 spectra per volunteer were used for the partial least squares (PLS) training, resulting in $n=736$ observations, or only the centre quadrant measurements from both breasts resulting in $n=184$ observations. The rationale for the latter is the fact that the tissue composition within the four quadrants is inherently different, possibly favouring analysis by breast quadrant.

Partial least squares (PLS) analysis

PLS is an analysis technique optimized for comparison of vector inputs (spectra) with interval target data (% parenchymal density). For a more detailed explanation of PLS, see Haaland and Thomas [30, 31]. The quality of the correlation is determined by plotting the predicted percent parenchymal density as a function of the initial derived percent density and determining the Spearman Rank correlation coefficient, slope and intercept of the fitted regression line.

Results

During the quantification of the global mammography tissue density using the Cumulus program, the intraobserver repeats correlation had a Pearson correlation coefficient $R^2=0.90$ for both readers. The interobserver correlation was lower with a Pearson correlation coefficient $R^2=0.85$ and a slope statistically different from 1 when comparing the results from both readers, suggesting reader bias which would introduce additional uncertainties to the target for PLS training. Hence, independent models were established using the tissue densities (targets) from each reader. The resulting PLS vectors covered on average 85% of the variance of the data set and 90% of the variance in the targets.

PLS performs best when provided with normally distributed target frequency histograms. Here, the target frequency histogram was flat with a higher frequency of mammographic breast tissue density below 10% and above 90% resulting in skewing these densities towards the 50% midpoint. Neither targets nor PLS-predicted densities are normally distributed, requiring regression analysis based on ranked data for correlation analysis. Figure 3 depicts examples of spectra collected from ROIs with less than 10%, 45–50% or more than 90% parenchymal tissue density. For a more detailed explanation of PLS, see Haaland and Thomas [30, 31]. The quality of the correlation is determined by plotting the predicted percent parenchymal density as a function of the initial derived percent density and determining the Spearman Rank correlation coefficient, slope and intercept of the fitted regression line.

Table 1. Demographics of study population

<table>
<thead>
<tr>
<th>Menopause status</th>
<th>Total</th>
<th>Study</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>population (%)</td>
<td>population (%)</td>
</tr>
<tr>
<td>Low (&lt;25%)</td>
<td>5</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Medium (25–75%)</td>
<td>18</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>High (&gt;75%)</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.5±4.2</td>
<td>57.7±6.9</td>
<td>53.5±8.1</td>
<td></td>
</tr>
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<td>24.7±4.6</td>
<td>26.5±5.5</td>
<td>25.9±5.2</td>
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<tr>
<td>15.2</td>
<td>25.9</td>
<td>22.0</td>
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<tr>
<td>0.0</td>
<td>52.6</td>
<td>33.3</td>
<td></td>
</tr>
</tbody>
</table>

[^d]: Classification of global breast tissue density was performed by a radiologist (Dr Roberta Jong) showing representative breast tissue densities for the general population.

BMI, body mass index; HRT, hormone replacement therapy.
Figure 5 by thickness-corrected absorption spectra from the four quadrants of a single volunteer. A very good reproducibility of the spectral shape and intensity is seen between the left and right breast. A closer look at the left–right reproducibility for mammographic and PLS derived tissue density reveals better correlation for PLS derived densities, see Figure 6. This may be indicative of limitations in assessing the position and size of the ROI in the mammograms, and may also reflect some of the intra-reader variability presented above.

A PLS model trained on the density target for the centre position obtained during the third week of the menstrual cycle was used to investigate the change in predicted tissue density as function of time during the menstrual cycle, by applying the PLS vector to the transillumination spectra collected during the remaining 3 weeks of the menstrual cycle. Figure 7 shows the resulting predicted vs global menstrual percent density for all 4 weekly measurements and Table 3 lists the resulting Spearman Rank correlation coefficients, intercepts and slopes for both readers. An analysis of variance for repeated measures showed that for n=25 volunteers the menstrual cycle was not statistically significant, in the prediction of the tissue density by optical transillumination at $p<0.10$ and $p<0.13$ for reader 1 and reader 2, respectively.

**Discussion**

This study extends a former publication correlating optical breast transillumination with density classification, to an analysis using density on an interval scale [32]. Through this analysis we intend to elucidate the possibility of using transillumination spectroscopy for the monitoring of women during puberty, menopause and preventative intervention, with the latter possibly providing individualized feedback about interventional efficacy. Specifically for feedback during preventative treatment [33] or to evaluate the effects of hormone replacement therapy [34], classification of breast tissue density is too coarse to provide clinically relevant information.

Only a small number of mammograms had >75% tissue density when considering the entire breast area, due to the edge effect in the mammograms where the outer regions of the breast do not fill the space between the compression plates, thus tending to show lower attenuation values [32] even when dense tissue is present. This occurs specifically in subjects with small breasts.

The accuracy of tissue density prediction using PLS is limited by the accuracy in determining the target, here the tissue density from the mammograms. The latter is subject to interobserver and intraobserver errors in calculating percent dense tissue from the mammograms as well as positioning and size of the ROIs within the mammogram. The accuracy of PLS training is further restricted by differences in the interrogated tissue volumes between the two techniques. For X-ray mammography this volume consists of a cylindrical volume comprising equally contributing voxels, whereas optical radiation interrogates an ovoid shaped volume between the optodes and a voxel’s contribution to the spectra decreases with increasing radius from the optical axis. Lastly, the small number of spectra available for PLS training limits the generalization of the results, specifically when the training set does not cover the entire variability of the population. While the intrareader repeatability of the global tissue density is adequate, specifically for pre-menopausal women, the left to right breast symmetry of the ROI densities measurement was weak, as seen in Figure 6. Approximately 75–120 pixels contribute to the density measure within each

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**Figure 3.** Sample spectra corresponding to regions of interest with (a) <10% (b) 45–55% and (c) >90% tissue density. All spectra were corrected for tissue thickness and the spectrophotometer transfer function.
ROI and thus the mammography result can vary considerably according to the location of the ROI within the mammogram, minor shifts in the left–right density patterns or breast alignment within the field of view. Additionally, by determining the percent breast density.

Table 2. Summary of partial least squares results following training by either 184 centre spectra for two readers extracting tissue density from mammograms. Predicted values restricted between 0 and 100%.

<table>
<thead>
<tr>
<th></th>
<th>Spearman coefficient</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reader 1 Training set</td>
<td>0.879</td>
<td>0.74 (0.67–0.81)</td>
<td>12 (8–16)</td>
</tr>
<tr>
<td>Validation set</td>
<td>0.664</td>
<td>0.66 (0.44–0.89)</td>
<td>20 (8–31)</td>
</tr>
<tr>
<td>Reader 2 Training set</td>
<td>0.885</td>
<td>0.77 (0.70–0.83)</td>
<td>10 (6–14)</td>
</tr>
<tr>
<td>Validation set</td>
<td>0.719</td>
<td>0.74 (0.52–0.97)</td>
<td>14 (2–25)</td>
</tr>
</tbody>
</table>

CI, confidence interval.

Figure 4. Partial least squares regression predicted vs actual tissue density for (a, b) Reader 1 and (c, d) Reader 2. (a, c) Results for the PLS training and (b, d) for the validation spectra. Regression line and 95% confidence intervals are indicated.

Figure 5. Thickness-corrected transillumination spectra from a single volunteer. Shown are the bilateral four quadrant measurements, indicating a high degree of symmetry between the breast tissue in the respective bilateral quadrants.
tissue density using a threshold value, areas with moderate density will not contribute to the mammographic density, but will contribute to the optical transillumination spectra.

While the recruitment of women for this study approximated the population incidence for low, medium and high tissue densities, as shown in Table 1, the ROI density histogram shows a rather flat distribution (not shown). PLS or similar techniques such as principal component analysis (PCA) perform better with normally distributed data; the predicted tissue density distribution was noticeably skewed against the target distribution. This flat distribution of the targets and the density results required the use of non-parametric correlation analysis. Examining the regression lines from the PLS results shown in Table 2 indicates slopes from 0.66 to 0.77 instead of the anticipated slope of 1, and the intercepts are all significantly larger than 0. In combination, they result in an overestimate of the lower densities and equivalently to an underestimate of the predicted breast tissue densities for high densities when compared with the mammogram results and to enlarged confidence intervals. The confidence intervals of the validation sets are always larger than the training set, indicating that the current training set is insufficiently capturing the population variability. Reducing the interreader and intrareader variability when extracting mammographic densities will not only provide improved targets for PLS training but also allow training on a dataset using both readers.

Despite the above shortcomings, the accuracy of the percent density predictions from the PLS data demonstrates that the optical transillumination spectroscopy technique predicts percent density to within 25% for the training data sets and < 35% for the validation data sets, based on the respective 95% confidence intervals. This suggests that optical transillumination spectroscopy is capable of predicting the actual parenchymal tissue density on an interval scale and hence may be an adequate technique for quantification of breast cancer risk. It is noteworthy that no stratification of the volunteers according to age, menopausal status, ethnic background or parity was executed prior to PLS training as the limited available number of volunteers does not permit this.

PLS analysis is not unique and similar correlation coefficients were obtained when using principal component regression (PCR, data not shown), although PLS handles non normally distributed data better and resulted in correlation slopes closer to 1.0 when compared with PCR. Restricting the predicted data set artificially within 0% and 100% did not affect the slopes significantly when compared with non restricted data (data not shown). The PLS derived result b-vectors are zero at the lipid and water peaks, and typically show local extremes at the absorption inflection points or midpoints between strong absorbers, such as at 955 nm between the lipid and water absorption, indicating that the relative proportions of water and lipids are important in the differentiation of breast densities. Similar maxima at inflection points in the lower wavelength range are due to the spectral characteristics of haemoglobin in both dominant forms as Hb and HbO2.

The large variability in the mammographic ROI density seen in Figure 6 is possibly a reflection of small differences in placement of the ROIs within the anatomical images of

**Table 3.** Summary of partial least squares (PLS) data for each week of the menstrual cycle for both readers. Minimum and maximum values artificially limited between 0 and 100%

| Reader 1 | Week 1 | 0.890 | 0.90 (0.74–1.05) | 1 (−10–11) |
| Reader 2 | Week 1 | 0.877 | 0.84 (0.70–0.98) | 10 (0–19) |

CI, confidence interval.
the left and right mammograms or shifts within the density patterns. The improvement in the correlation between the left and right breast density seen in the PLS over the mammographic predicted density may suggest that the optical sample volume is larger than the selected ROI.

The time of measurement during a menstrual cycle does not alter the correlation of predicted and measured breast density significantly in this study using a PLS model trained on data collected during the third week. There is a trend towards poorer prediction by the PLS model at the other weeks, but this is not statistically significant even for trend towards poorer prediction by the PLS model at the week during the menstrual cycle is not too surprising as the composition of breast tissue (e.g. blood content, water levels) varies during the cycle [35]. These chromophore concentration changes will affect the tissue optical properties, and in turn will result in variations of the transillumination spectra. However, the error introduced due to the timing within a menstrual cycle is smaller than that due to the determination of density within the ROI. Hence, it is conceivable that optical transillumination as a pre-screening tool can be executed independently of the menstrual cycle.

Summary and conclusions

This study demonstrated that in vivo optical transillumination spectroscopy is a physical examination method capable of predicting breast tissue densities with clinically relevant accuracy and thus a promising tool for breast cancer risk assessment. As a pre-screening method it would be applicable to women outside of the recommended age range for X-ray mammography, allowing identification of those women who will benefit most from a standard X-ray screening, as their mammography risk/benefit ratio is altered in a favourable manner. Additionally, the use of non-ionizing radiation and the lack of plate compression is appealing to women commonly non-compliant with standard screening and thus again can encourage women for whom a standard X-ray will be highly beneficial to join screening programs.

PLS training using the centre quadrant spectra for breast tissue density prediction showed a correlation coefficient of up to 0.72 with the tissue density obtained from mammograms. The differences in the correlation coefficients, slopes and intercepts between the two readers illustrate the inter-reader influence on predicting breast tissue density, which has been documented in previous studies using conventional risk assessment techniques [3].

Kaizer et al [36] used ultrasound to evaluate women with high breast tissue density (low density breasts are weakly echogenic and therefore, not suitable for ultrasound examinations). Of the five tissue density groups that were defined in this study, correlation was highly significant (Kendall’s tau-b=0.736±0.025) when dysplasia (corresponding to DY pattern by Wolfe [12]) was the dominant mammographic pattern examined. In the case of MRI [37], a positive correlation was established between the relative content of water in the tissues and percent breast density (r=0.79). Optical transillumination is a pre-screening tool, as presented here, that showed results which are equivalent or superior to ultrasound and MRI as a tissue density predictor, both showing a correlation of \( r = 0.72 \) [36]. Deriving the tissue density from pre-set mathematical models is an added advantage of transillumination spectroscopy over ultrasound and MRI as no highly trained personnel are required for assessment. This reduces the overall cost to the healthcare system for a risk-assessment technique.

Spectral features associated with tissue density prediction include absorption by water, and lipids, spectral features related to total haemoglobin its oxygen saturation and the change in photon path length due to scattering. Unlike X-ray based diagnostics, optical transillumination does not only exploit the atomic composition of the breast tissue, but rather some of its biomolecular markers. This may hold potential in the use of optical transillumination during risk reduction intervention as complementary information on density can be extracted. Optical transillumination is a painless procedure and its inherent safety will likely result in a high compliance rate. Additionally, as the device does not require special infrastructures, miniaturization may make it available to general physicians and gynaecologists so they can monitor changes in breast cancer risk among their patients.

The results of this and our previous studies [26–28] suggest that a longitudinal cohort study to develop a direct correlation between optical transillumination and breast cancer risk may be warranted.

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