Optical Spectroscopy for Breast Cancer Risk Screening

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Department of Medical Biophysics
University of Toronto

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Abstract

Breast cancer (BC) has the highest mortality rate among women’s cancers worldwide, and incidence rates are rising in low- and middle-income countries (LMIC). Screening programs are well-established in most high-income countries, but there is much debate about screening frequency and the optimal screening ages. In LMIC screening infrastructure is insufficient. Optical spectroscopy (OS) can be used as a pre-screening technique to measure breast composition and predict mammographic density (MBD), a known BC risk factor. The goal of this thesis was to develop and evaluate an OS device which is portable, low cost and requires minimal operator interaction.

The device was based on a research prototype used in previous studies, with two major changes: (1) a 13-laser-wavelength module replaced the broadband light source and (2) the source and detector positions were fixed within rigid holders of different sizes. The wavelengths critical for distinguishing between BC risk groups were selected using a principal components analysis of data from previous studies. Source and detector positions were chosen to match the optically-interrogated volumes of the original device via Monte Carlo simulations of photon
propagation. Two versions were developed – one for women (the Cups device) and one for girls (the LEGACY device).

The Cups device was evaluated in comparison with the research prototype on its ability to predict MBD. For both devices, women with high MBD could be identified from spectra with high sensitivity and specificity and correlation between mammographic percent density (MPD) and OS-predicted MPD was significant, although slightly weaker for the Cups device ($r = 0.62$ vs. $r = 0.74$).

For girls, OS had been used as an objective method for distinguishing between breast development stages. Spectral analysis using only the wavelengths from the LEGACY device showed that the reduced spectral content does not affect the ability to distinguish between development stages.
Acknowledgments

That my PhD remained interesting and engaging all the way through is due in large part to my supervisor, Dr. Lothar Lilge. He has consistently been supportive and has always challenged me to learn and get the most out of my time here. He sets a great example for his students by being eager to learn himself and by always being open to being questioned and arguing things out.

I would also like to acknowledge my committee members – Dr. Anne Martel and Dr. Julia Knight, and past member Dr. Lisa Martin – who have provided useful and constructive advice and have helped keep me on-track.

None of the clinical work would have been possible without the amazing efforts of the clinical coordinators in the Lilge lab – Samantha Dick, Brenda Ornelas and Jennifer Xanthopoulos. I cannot thank them enough for all their help. I would also like to thank the team from the Toronto site of the LEGACY Girls Study, especially Danielle Hanna and Mai-Liis Tammemagi, for their enthusiasm in learning how to use and testing the LEGACY device.

Alan Stummer in the University of Toronto Physics Department was an incredible help in redesigning the electronics and in creating the software for the latest version of the device which is now in use at collaborating institutions. I would also like to thank Robert Morley for his help with the software design.

I would like to thank my lab mates and classmates, who have made the whole experience fun and have encouraged me throughout. In particular I would like to thank Ben Lai for his electronics and optics help early on, Dr. Golnaz Farhat for her support and encouragement, Sarah Forward for her help with data collection, Elena Renzhiglova for her help with simulations, Daniel Huynh for his help with troubleshooting and assembly, as well as Dr. Carl Fisher, Carolyn Niu, Dr. Savo Lazic, Debbie Lo, Dr. Max Loshchenov, Nancy Wu, Duoaud Shah, Dr. Noemi Salazar Hermenegildo, Emma Henderson, Andres Covarrubias, and Yaxal Arenas Heredia for generally being helpful and making the lab an fun and interesting place to be.
I would also like to thank everyone from the machine shop at Princess Margaret Cancer Centre, especially Matt Filleti and Jason Ellis, for all their help with the mechanical design of the devices. I went to the machine shop innumerable times and always received friendly, valuable advice.

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<tr>
<td>3D</td>
<td>Three-Dimensional</td>
</tr>
<tr>
<td>ABUS</td>
<td>Automated Breast Ultrasound</td>
</tr>
<tr>
<td>ADC</td>
<td>Analog-to-Digital Convertor</td>
</tr>
<tr>
<td>AI</td>
<td>Aluminum</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ANSI</td>
<td>American National Standards Institute</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BaSO4</td>
<td>Barium Sulphate</td>
</tr>
<tr>
<td>BC</td>
<td>Breast Cancer</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BPE</td>
<td>Background Parenchymal Enhancement</td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Breast Cancer Genes 1 and 2</td>
</tr>
<tr>
<td>CBE</td>
<td>Clinical Breast Examination</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-Coupled Device</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous-Wave</td>
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<tr>
<td>DAQ</td>
<td>Data Acquisition</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
<td>-------------</td>
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<tr>
<td>HbO2</td>
<td>Oxy-hemoglobin</td>
</tr>
<tr>
<td>HDI</td>
<td>Human Development Index</td>
</tr>
<tr>
<td>HER2</td>
<td>Human Epidermal growth factor Receptor 2</td>
</tr>
<tr>
<td>ID</td>
<td>Identifier</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter-quartile range</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-Red</td>
</tr>
<tr>
<td>LEGACY</td>
<td>Lessons in Epidemiology and Genetics of Adult Cancer from Youth</td>
</tr>
<tr>
<td>LIBRA</td>
<td>Laboratory for Individualized Breast Radiodensity Assessment</td>
</tr>
<tr>
<td>MBD</td>
<td>Mammographic Breast Density</td>
</tr>
<tr>
<td>MC</td>
<td>Monte Carlo</td>
</tr>
<tr>
<td>MPD</td>
<td>Mammographic Percent Density</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>N</td>
<td>Number</td>
</tr>
<tr>
<td>NE</td>
<td>Northeast</td>
</tr>
<tr>
<td>NIR</td>
<td>Near Infra-Red</td>
</tr>
<tr>
<td>OBS</td>
<td>Optical Breast Spectroscopy</td>
</tr>
<tr>
<td>OBSP</td>
<td>Ontario Breast Screening Program</td>
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<tr>
<td>OD</td>
<td>Optical Density</td>
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<td>OR</td>
<td>Odds Ratio</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>OS</td>
<td>Optical Spectroscopy</td>
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<tr>
<td>PC</td>
<td>Principal Component</td>
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<tr>
<td>PCA</td>
<td>Principal Components Analysis</td>
</tr>
<tr>
<td>PC-ABS</td>
<td>Polycarbonate-Acrylonitrile Butadiene Styrene</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed Circuit Board</td>
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<tr>
<td>PLS</td>
<td>Partial Least Squares</td>
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<tr>
<td>ROC</td>
<td>Receiver-Operator Characteristic</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SE</td>
<td>Southeast</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-Nucleotide Polymorphism</td>
</tr>
<tr>
<td>TiBS</td>
<td>Trans-illumination Breast Spectroscopy</td>
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<tr>
<td>TS</td>
<td>Tanner Stage</td>
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<tr>
<td>US</td>
<td>Ultrasound</td>
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1 Introduction

1.1 Breast Cancer

Breast cancer (BC) is the most common cancer and the second most common cause of cancer death among Canadian women, and the leading cause of cancer death among women worldwide. In Canada, 1 in 8 women is expected to be diagnosed with BC in her lifetime. [1, 2] BC is a very heterogeneous disease and BCs are classified first based on the location of origin of the cancer then further classified into molecular subtypes based on the receptor status and grade of the tumour. Almost all BCs are adenocarcinomas (tumours originating in the epithelial cells of glands) and are commonly divided into ductal carcinoma (DC) – cancer originating in the milk ducts; the most common BC for both invasive and in situ cancers – and lobular carcinoma (LC) – cancer originating in the lobules, which are the milk-producing glands. LC is the 2nd most common BC with ≈10% of all invasive BCs being LCs. In Ontario, just over 16% of screen-detected cancers are ductal carcinoma in situ (DCIS). [3] Further differentiation of BCs is based on their hormone receptor (HR) status. Cancers with HR-positive status retain estrogen receptors (ER) and/or progesterone receptors (PR), both of which are normally found in breast tissue cells, and have a better prognosis than HR-negative BCs as their receptors can be targeted by anti-estrogenic drugs. Human epidermal growth factor receptor 2 (HER2) is another marker used to classify BCs. Tumours which overexpress HER2 and are ER-negative (HER2-positive tumours) tend to be more aggressive but targeted therapy (trastuzumab treatment) is available. Triple-negative cancers, which are HR-negative and HER2-negative, tend to be aggressive and have the worst prognosis, with no current targeted treatments available. [4, 5]

1.1.1 Breast Cancer incidence and mortality

Breast cancer incidence and mortality both increase with increasing age. [2, 3] There is large variation globally in BC incidence rates and mortality rates. In general, incidence rates are higher in high-income countries (HIC) while mortality rates are higher in low- and middle-income countries (LMIC). Comparing countries based on Human Development Index (HDI), which includes life expectancy and education indicators as well as per capita income, the incidence rate (age-standardized to the World Standard population) was 78.2 per 100,000 in
countries with very high HDI vs. 32.6 in countries with low HDI while the age-standardized mortality rate was 14.1 per 100,000 in the very high HDI countries vs. 17.0 in low HDI countries. [6] When looking at absolute numbers, because of the larger populations in LMIC, just over 50% of BC cases worldwide are diagnosed in LMIC, and more than 75% of BC deaths occur in LMIC. [1, 7] Furthermore, while in most HIC incidence rates are stable or even decreasing slightly and mortality rates are decreasing, both incidence and mortality rates are increasing in LMIC. [8] In HIC, incidence rates increased during the late 1980s and the 1990s, due mainly to an increase in detection with the implementation of mammographic screening programs but also because of changes in lifestyle risk factors. However, rates have since stabilized. In LMIC the rising incidence rates are mostly attributed to increasing life expectancy and changing reproductive and behavioural patterns. [1, 9] The poor mortality rates in LMIC are due to a number of factors including a lack of screening, lower quality treatment, and/or less access to treatment and screening programs. On average, women in LMIC also present with later stage disease and have a younger age at diagnosis. [7-11] The later stage at diagnosis is likely due to the lack of screening, the younger age at diagnosis can likely be attributed, at least in part, to the skew towards younger ages in the population age distribution of LMICs but may also be due to differences in etiology.

In both the USA and Canada, incidences rates rose in the 1980s but have flattened as screening programs have become well-established. The current Canadian age-standardized incidence rate is approximately 130 per 100,000 (age-standardized to the 2011 Canadian population). [2] In Canada, the mortality rate peaked in 1986 and has fallen significantly from a rate of 41.7 deaths per 100,000 in 1988 to a projected rate of 23.2 deaths per 100,000 in 2017. The decline is attributed to both the use of mammographic screening and improved therapies. [2]

In Ontario, the BC incidence rate increases with age until it peaks around age 70-74 at a rate of about 350 per 100,000, decreasing slightly thereafter. The age-incidence curve shows the characteristic pattern for BC when plotted on a log-log scale. There is a constant, high slope until the late 40s (around the time of menopause) when the slope decreases. The slope decreases again at around 70, flattening out. [3, 12] In Ontario, and in Canada as a whole, the overall age-standardized BC incidence rate has been largely stable since 2004. The mortality
rate in Ontario increases almost exponentially with age, reaching almost 60 per 100,000 at age 60-64 and rising to around 230 per 100,000 after age 85. The overall, age-standardized mortality rate in Ontario has been declining steadily, dropping by almost 30% between 1991 and 2008. [3]

1.1.2 Breast Cancer Risk Factors

There are a number of well-established risk factors for BC, however very few have both high prevalence and large relative risk and few are modifiable. The main established BC risk factors and their estimated relative risks are summarized in Table 1.1. Age is the most influential risk factor with risk more than doubling for every decade of life. [3, 13] Family history and genetics both strongly affect BC risk. Having a first degree relative (mother, sister, daughter) with breast cancer almost doubles BC risk and the risk increases further if there is more than one first degree relative affected. [13] Approximately 5% of BCs are thought to be due to an inherited predisposition. [14] Genetic mutations to the Breast Cancer Genes 1 and 2 (BRCA1/2) cause a substantial increase in risk (a relative risk (RR) of 10-20 compared to the general population), but these mutations are rare, with an estimated prevalence of about 0.1-0.25% in the general population of women. [13, 15-17] However certain ethnic groups have a much higher incidence of BRCA1/2 mutations (e.g. BRCA1/2 mutations are approximately 10 times more common in the Ashkenazi Jewish population). Mutations in several other genes (TP53, PTEN, STK11, CDH1, ATM, PALB2 and CHEK2) are known to increase BC risk, some with similar relative risks to BRCA1/2, but are also very rare. [18] Over 90 single-nucleotide polymorphisms (SNPs) associated with increased BC risk have been identified via genome-wide association studies. The relative risk from each individual SNP is low (odds ratios (OR) of <1.3) but combining multiple SNPs in polygenic risk scores can yield larger relative risks. [18] Ionizing radiation exposure is another established risk factor which can have very high RR, but only at very high levels of exposure and when exposure is at a younger age (<30-40 years old). Normally only women who had radiation treatment for a medical condition such as Hodgkin’s disease would be exposed to levels of radiation that would significantly increase their BC risk.
Reproductive factors are known to affect BC risk. Late age at first pregnancy increases BC risk in the general population, as does nulliparity or low parity. [13] In both the general population and in BRCA1/2 mutation-carriers breastfeeding has a protective effect. [19-22] Early age at menarche and late age at menopause both increase BC risk. [13] Early age at breast development, an increased delay between the start of breast development and menarche, and a shorter time between menarche and regular periods have also been associated with increased BC risk although they have not been studied to the same extent. [23] Exogenous hormone use increases BC risk – both current or recent use of oral contraceptives and use of post-menopausal hormone replacement therapy (HRT), specifically combined estrogen and progesterone therapy, have been found to increase risk. These reproductive risk factors individually have only low to moderate effect on risk (RRs of <2) and, with the exception of breastfeeding and exogenous hormone use, are not modifiable.

A number of anthropometric and lifestyle factors have been investigated as BC risk factors. High body mass index (BMI) increases risk in postmenopausal women, and postmenopausal weight gain is also associated with increased risk. Conversely, high BMI before menopause appears to reduce BC risk. [20, 24] Tall adult height is associated with increased risk, with stronger associations seen in post-menopausal women. [24] Alcohol consumption has been found to increase BC risk while exercise has been found to decrease risk, although the RRs are small. [20, 25] Smoking, diet and other lifestyle variables are also under investigation with respect to BC risk but the results have largely been found to be small or inconclusive.

A personal history of BC increases the risk of subsequent BC diagnosis and certain benign breast conditions, such as atypical hyperplasia also increase risk. [26, 27] Properties of the breast when imaged via mammography are also associated with BC risk. In the 1970s, Wolfe observed that different parenchymal patterns observed with mammography had different associated BC risks. [28] He identified four parenchymal patterns and found a 22-fold increase in BC incidence between the lowest risk “normal breast” parenchymal pattern (no prominent ductal pattern, mainly fat with some fibrous strands) and the highest risk “extremely dense parenchyma” pattern. Subsequent studies did not find as dramatic a difference in risk between the parenchymal groupings but did confirm a difference in risk. Relative risk based on
mammographic parenchymal patterns is estimated to be 2-4 for the denser patterns. [13] Based on Wolfe’s observations, the amount of dense tissue in a mammogram, rather than the parenchymal pattern, was also explored as a risk factor. Mammographic breast density (MBD) has been assessed in a number of ways, both qualitative and quantitative, with quantitative assessment providing the higher relative risk values. [29] Mammographic percent density (MPD), the percent of breast area that is dense on the mammogram, is a well-established, independent BC risk factor and has one of the highest relative risks at 4-6.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2-3 Per 10 years [13]</td>
</tr>
<tr>
<td>BRCA 1/2 mutation</td>
<td>10-20 [13]</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td>≥1.7 Depends on the number of first-degree relatives affected [20]</td>
</tr>
<tr>
<td>Ionizing Radiation Exposure</td>
<td>2-3 Per Gy for exposure before age 40 [13]</td>
</tr>
<tr>
<td>Late age at first pregnancy</td>
<td>1.9 For age &gt;30 years vs. &lt;20 years [20]</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>1.3-1.9 [13, 20]</td>
</tr>
<tr>
<td>Early menarche</td>
<td>1.3 For before age 12 vs. at 16 years [20]</td>
</tr>
<tr>
<td>Late menopause</td>
<td>1.5-2 For age &gt;55 years vs. 45-54 years [13, 20]</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>0.9 Per year of feeding [13]</td>
</tr>
<tr>
<td>Current oral contraceptive use</td>
<td>1.2 [20, 30]</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td>1.35 For use ≥5 years vs. never use [13]</td>
</tr>
<tr>
<td>Personal history of breast cancer</td>
<td>&gt;4 [30]</td>
</tr>
<tr>
<td>Benign breast disease</td>
<td>1.5-4 Depending on the benign disease [20]</td>
</tr>
<tr>
<td>High mammographic density</td>
<td>4-6 [20]</td>
</tr>
<tr>
<td>Overweight (post-menopausal)</td>
<td>1.3-2.5 For BMI&gt;28 vs. healthy BMI [20]</td>
</tr>
</tbody>
</table>
1.1.3 Breast Cancer Risk Models

Breast cancer incidence increases with age, peaking around age 70-74 in Canada. [3] BC does not demonstrate the linear relationship between age and incidence on a log-log scale seen for many cancers, primarily attributed to the fact that BC is a hormone-dependent cancer. Pike and others proposed a “breast tissue aging” model which included different rates of breast tissue aging for different stages of life based on hormone exposure. [12] They proposed that breast tissue aging begins at menarche and continues at an initial high rate, $f_0$, until a first full-term pregnancy (FFTP), when there is a one-time increase in breast tissue age, $b$, and after which breast tissue aging continues at a slower rate, $f_1$, until the peri-menopausal period, during which the rate of aging gradually drops again to a lower rate, $f_2$, after menopause. The Pike model of breast tissue aging matches population incidence vs. age curves well by the appropriate fitting of the rates, $f_0-f_2$, and $b$. Furthermore, the predicted differences in BC incidence between groups based on age at menarche, FFTP and menopause based on their model were also well-matched to actual incidence rates. They estimated that age at menarche, age at FFTP, age at menopause and weight could explain about 85% of the difference in BC incidence rates between different countries. [12] The Pike model, however, is designed to predict population incidence rates for BC based on risk factors and rather than for predicting an individual’s BC risk. In essence, it models the risk for an average woman from that population, using the population average ages for menarche, first pregnancy and menopause, and obtaining population average rates of breast tissue aging in the fitted values for $f_0-f_2$. To modify the model for individual risk assessment it would be necessary to find a method for estimating individual rates of breast tissue aging.

The Gail model is the most commonly used tool for predicting individual BC risk for the general population (women who have not already been identified as being at high risk because of a BRCA mutation or other hereditary syndrome associated with BC, previous BC, or prior radiation therapy to the chest). The original Gail model calculated a woman’s 5-year and lifetime risk of developing BC based on the woman’s age, age at menarche, age at first live birth, number of first-degree relatives with BC, and biopsy history. [31] The model was subsequently modified to include history of atypical hyperplasia and race/ethnicity. The model
has been validated and is the most widely used but has only fair predictive accuracy. [31, 32] In a large study by Rockhill et al., for example, the discriminatory accuracy of the modified Gail model for estimating 5-year risk, as measured by the concordance statistic, was only 0.58 (95% CI = 0.56 to 0.60) [33] Subsequent models have included other risk factors such as MBD, BMI, height and genetic polymorphisms to improve predictive accuracy and, while discriminatory accuracy was improved by the addition of risk factors such as MBD, the discriminatory accuracy of current models is still moderate at best and they are limited to women in or near the breast cancer screening age. [18, 32, 34, 35] BC risk accumulates from an early age, as indicated by the Pike model, and so developing methods to identify women at elevated risk early in life would be beneficial. In particular, it could identify women who would most benefit from lifestyle modifications at an age when these changes could have maximum impact. Interventions aimed at modifiable risk factors such as weight, amount of exercise and alcohol consumption can reduce lifetime risk in women when begun at screening age but have a stronger effect (1-3% greater reduction in lifetime risk per risk factor) if begun early in life. [36]

1.2 Breast Cancer Screening

The goal of BC screening is to detect cancers at an early stage when there are more and better treatment options, survival rates are higher, and the total cost of treatment can be over an order of magnitude lower. The mainstay of BC screening for women not known to be at high risk for BC is mammography and most high-income countries have mammographic screening programs. Where there are specialized screening programs for women with elevated BC risk, they usually include both mammography and magnetic resonance imaging (MRI). Although it is the dominant screening method, there have been a number of debates and controversies around mammographic screening in the past few decades. There is a tenuous overall agreement that mammographic screening does reduce BC mortality. However, there is little agreement on the optimal structure of screening programs. Recommendations on the age to begin screening, screening frequency and the age at which to stop screening vary greatly between different agencies and countries. For example, the U.S. Preventative Services Task
Force recommends that screening before age 50 be at the discretion of the women considering screening and that women between 50 and 74 receive biennial screening. They did not make a screening recommendation for women age 75 and older. [37] In contrast, the American Cancer Society guidelines strongly recommend that regular screening begin at age 45. They recommend that screening be annual from 45-54, biennial for women after age 55 and that women continue screening, regardless of age, so long as they are in overall good health and have a life expectancy of at least 10 years. They also recommend that women age 40-44 have the opportunity to choose to begin annual screening. [38]

The debate over screening centres on a number of factors. Many are concerned about the harms of over-diagnosis, as many of the cancers detected will be in situ cancers, which may be very slow-growing, might never progress to an invasive cancer and in some cases might never have been detected or caused harm without screening. Some believe the harms and risks of over-diagnosis outweigh the mortality reduction benefits of screening, especially in younger age groups (<45 years of age). There are several reasons why screening recommendations vary for different age groups. A primary reason is that incidence increases with age and so more women have to be screened before detecting a cancer when including younger women in the screening program. Younger women also have denser breasts on average and BC detection in dense breasts is more difficult, as cancers can be obscured by dense tissue, although the mean density for women in their 40s is around 28%-38%, which is not considered high density and is only 10-20% higher than the mean density of women in their late 50s or 60s. [39, 40] The radiation exposure from mammography is also of more concern in younger women since radiation sensitivity is inversely related to age. However, the dose from a mammogram is low and for screening programs starting at age 40 the estimated lifetime attributable risk of fatal radiation-induced BC is 11-25 cases per 100 000 screened women. [41, 42] The overall benefit of mammographic screening programs is also difficult to quantify since the widespread implementation of screening has coincided with improvements in treatment, which have also improved mortality rates, and changing lifestyle factors may also have contributed to the change in incidence rates seen after the implementation of mammography programs. Another issue is that existing programs are not optimally exploited. Many women who are invited to
participate in screening programs do not participate or do not participate at the recommended frequency, and since participation decisions can be biased (e.g. women with a first-degree relative who has had BC may be more likely to participate), this complicates the evaluation of the benefits of screening. The Ontario Breast Screening Program (OBSP), which is open to women ages 50-74, had an overall participation rate of 43% in 2010-2011, with an additional 18% who had a mammogram outside the OBSP. The 2009 retention rate (the percentage of eligible women who had an OBSP screening mammogram within 30 months of their previous OBSP mammogram) was quite high at 84.9% overall (74.5% for initial screens, 88.0% for rescreens). [3] Fear of pain or discomfort during the mammogram, anxiety, concerns about radiation, the costs of screening and privacy (particularly concerns about a male technologist performing the exam), accessibility and transportation issues, as well as lack of information about BC risks and the benefits of mammographic screening, have all been identified as barriers to participation. [43]

In LMIC, large-scale mammographic screening programs are less common. There is both insufficient equipment and an insufficient number of trained radiologists to read mammograms for population-wide screening programs. As a result, there is a need to identify women who would benefit most from screening with the available infrastructure.

1.3 Mammographic Breast Density

Mammographic breast density (MBD) is breast tissue which appears light (radiopaque) in the mammogram. The source of contrast in a mammogram which determines MBD is the difference in the radiological density of fat vs. epithelium and stroma. Fat is radiolucent and appears dark on a mammogram while epithelium and stroma are more radiopaque and appear light. Figure 1.1 shows an example of a mammogram with low MBD and one with high MBD. It is well established that higher MBD is associated with an increased BC risk. [44]

Besides being one of the strongest breast cancer risk factors, with a relative risk of 4-6 for MBD ≥75% vs. <10%, MBD has a number of attributes which make it a particularly useful metric for BC. High MBD has a high prevalence, especially compared to some of the other risk factors with large relative risks. In the Ontario Breast Screening Program, for example, approximately 10% of
women were classed as having MBD ≥75%, usually used as the highest-risk category. [3] MBD is a significant risk factor for BC overall and also for individual BC subtypes. [45, 46] MBD is also one of the few modifiable risk factors. MBD has been shown to change in response to Tamoxifen use, and the magnitude of the change was related to whether the Tamoxifen therapy corresponded with a reduction in BC risk. MBD may therefore be useful as a metric for the effectiveness of Tamoxifen therapy. [47-49] MBD has also been shown to increase in women who are using hormone replacement therapy (HRT). [50, 51] MBD changes over a woman’s lifetime, even without external interventions. MBD decreases with age, with a significant drop at menopause. [51] MBD also has utility as a prognostic marker. MBD is associated with risk of invasive recurrence following an initial diagnosis of BC. [52, 53]

MBD is often expressed as a percent or categorized into percent ranges, although qualitative categories and the area that appears radio-dense are also used. Percent density has the strongest relationship with BC risk and risk appears to increase continuously and monotonically with increasing density. [54] The American College of Radiology developed a system to standardize reporting for mammograms called the Breast Imaging Reporting and Data System (BI-RADS). In addition to the assessment categories, which indicate the likelihood of the presence of disease, there are composition categories which are used to report mammographic density. The categories are A. almost entirely fatty, B. scattered areas of fibroglandular density, C. heterogeneously dense, which may obscure small masses, and D. extremely dense, which lowers the sensitivity of mammography. The categories were chosen based on whether the breast characteristics are likely to obscure tumours in the mammogram and affect the ability of the radiologist to detect the presence of cancer. The BI-RADS categories relate to screening and the detection of cancer, rather than risk assessment. Thus percent density is not included in the current BI-RADS scores despite the fact that percent density is more strongly associated with BC risk than categorical variables. [55] BI-RADS categories do still show a significant relationship with risk of future BC but it is weaker than that for MPD since the risk of future BC due to high MBD and the risk of a current BC being obscured and undetected due to high MBD are independent. [54, 56]
The standard software for quantifying MPD is Cumulus. It is a semi-automated thresholding method that was developed for use with digitized screen-film mammograms, but has also been validated for digital mammograms. [57-59] The Cumulus program requires the user to select a grayscale level to distinguish the breast edge from the background, manually draw a line indicating the location of the pectoral muscle to mark the posterior edge of the breast, and to select the threshold level indicating dense tissue. The pixels inside the selected edges of the breast are totalled to give the breast area, then the number of pixels above the dense tissue threshold is counted to give the dense area. The percent density is calculated as the dense area divided by the breast area multiplied by 100. While MPD determined using Cumulus does show a correlation with BC risk, there is inter- (and to a lesser extent intra-) reader variability that is not large enough to affect broad classification into low- or high-risk categories but would need to be accounted for to accurately follow the change in MPD in an individual over time. [54, 59]

Fully automated programs have been developed to remove errors due to inter-operator variability, including the Laboratory for Breast Radiodensity Assessment (LIBRA) software tool and an Image-J based tool, both of which are essentially fully-automated versions of Cumulus. [60, 61] They are both publicly available and have been validated against Cumulus and as BC risk factors. [62, 63] Fully-automated programs which yield volumetric measures of density rather than the area-based percent density calculated using Cumulus have also been developed with the idea that, since a mammogram is a 2-dimensional image of a 3-dimensional organ, volumetric measurements should more truly reflect the breast composition and should have a stronger correlation with BC risk [64-66]. To date, the volumetric measurements have shown a similar but not significantly stronger relationship with BC risk than the traditional MPD measurements. [54, 58, 62, 67]
Figure 1.1 Mammogram of a breast with (A) low density and (B) high density.
1.4 Complementary and Alternative Techniques for Screening and Density Measurement

Although mammography is the most widely used method for BC screening, it is by no means optimal. There are many techniques which are used or are under investigation as alternatives to mammography or as complementary to mammography. MRI, ultrasound (US) imaging and clinical breast examination are the most widely used techniques for breast cancer screening after mammography. Some of these alternate techniques, including MRI and US, can also be used to measure breast density.

1.4.1 Magnetic Resonance Imaging

One of the main benefits of MRI is that breast density does not reduce the contrast of the image. [68] Thus, MRI screening for BC is particularly useful in younger women and women with dense breasts. It has also been shown to be useful for women with BRCA mutations, in whom mammography is less sensitive than in the general population. [69] In these populations, MRI has higher sensitivity than mammography but similar specificity. [68] MRI does not expose women to ionizing radiation, which is an important consideration in screening younger women. MRI does require the use of a contrast agent (gadolinium-based); however, it is very well-tolerated, although not recommended for patients with very poor renal function. The measurements are also long and require that the patient remain still in a confined space, which can be difficult, especially for patients with claustrophobia. The main drawback of MRI is that it is a much more expensive technique and so is usually only recommended for women with very elevated risk of BC (women with BRCA mutations, other high-risk genetic mutations, strong family history of BC or women exposed to high levels of radiation to the chest at a young age). [70] MRI can also be used to assess breast composition and determine density. The volumes of water and fat in the breast can be extracted from the images and the percent water content in the breast shows a strong correlation with MPD. [71] Background parenchymal enhancement (BPE) – the increase in signal intensity of normal fibroglandular breast tissue after the administration of a contrast agent – is another MRI parameter which has been associated with
BC risk. Studies in women at high risk of BC have found that increased BPE is associated with increased risk of BC and that this risk is independent of the risk associated with MBD. [72, 73]

1.4.2 Ultrasound

Ultrasound (US) imaging has many advantages as a tool for BC screening, including low cost and no ionizing radiation, but is not used as a first-line screening tool as it has poor specificity for general screening. The main role for US in screening is as a tool for evaluating abnormal regions identified in mammograms, for which its specificity and sensitivity are good, and in image guidance for biopsy. [74] It is also used in women who have dense breasts but are not at high enough risk of BC to be recommended for MRI screening and for pregnant and breastfeeding women, for whom mammography and MRI would not be recommended because of ionizing radiation exposure risk for the former and cost and the use of a contrast agent for the latter. Another drawback of conventional US for BC screening is that it is operator-dependent and it requires that radiologist interpret the images at the time of the visit. US screening therefore requires considerable time from the radiologist and also suffers from inter-operator variability. Automated breast ultrasound (ABUS) systems have been developed to remove the operator-dependence and to allow for image interpretation by a radiologist at a later time. Modern ABUS systems show comparable performance to hand-held US and ABUS in conjunction with mammography appears to improve the sensitivity of screening in dense-breasted women. ABUS is FDA-approved in the United States for screening women with dense breasts. [75-77]

Breast density can also be measured via ultrasound. Percent density measured using US is a volumetric measurement of density. The source of contrast is the increased sound speed in dense breast tissue. Correlations of US-based percent density with MPD are strong ($r^2$ values of 0.6-0.7). [78, 79]

1.4.3 Clinical breast examination

Clinical breast examination (CBE) is a physical and visual examination of the breast performed by a trained clinician. The clinician checks for lumps, hardening or thickening of the breast,
changes in skin colour, temperature or texture, rashes and overall changes in the breasts. CBE was part of the standard of care for BC screening for many years but based on more recent studies there is little evidence that CBE improves sensitivity or specificity when used in conjunction with mammographic screening. Both the Canadian Task Force on Preventive Health Care and the U.S. Preventive Services Task Force do not recommend that CBE be performed in women who are participating in mammographic screening as there is insufficient evidence of benefit. [37, 80, 81] In recent studies of women at high risk for BC, CBE alone showed significantly lower sensitivity compared to mammography, US or MRI, although specificity is very good. [68, 82]

1.4.4 Mechanical imaging and elastography

Mechanical imaging and elastography rely on the increased stiffness of tumour tissue to identify cancers, similar to the idea behind the palpation during a clinical breast exam. [83] Various methods, both qualitative and quantitative, are used to measure the mechanical properties of breast tissue. In mechanical imaging, the tissue is deformed and the resulting pressure at the surface of the tissue is measured and mapped using pressure sensors. [84] With ultrasound elastography, the tissue is compressed and the resulting deformation is measured using US. [83] These techniques have not been studied as an independent screening method but rather as a complementary technique to mammography to improve the discrimination between benign and malignant lesions in women who have been identified as needing follow-up after an initial screening mammography. [84-86]

1.4.5 Dual-Energy X-ray Absorptiometry

Dual-energy x-ray absorptiometry (DXA) is a technique which can be used to assess volumetric breast density by determining the percent volume of fibroglandular tissue. It is a modified version of a technique used to measure bone density. DXA uses two different x-ray energies, both at higher x-ray tube voltages than mammography but with approximately a 10 times lower x-ray dose overall, and uses the differential attenuation at the two energies to determine breast composition. Although DXA does use ionizing radiation, the much lower dose makes it safe for use even in young girls. DXA does not require compression of the breast, making it
more comfortable for women and also more likely to be reproducible compared to mammography. The equipment is also widely available as it is commonly used for bone density measurements. [87, 88] A study comparing percent fibroglandular volume determined by DXA and MPD in 101 women found a correlation of 0.76 between the two measurement types. [89] DXA composition measures showed high correlations between the left and right breasts, similar to or slightly higher than for mammographic measures. [89, 90] DXA-determined density has not yet been evaluated as a BC risk factor.

1.4.6 Thermography

Thermography uses infra-red (IR) cameras to measure the surface temperature of the breasts. The underlying rationale behind the technique is that tumour tissue often has elevated temperature compared to healthy tissue due to vasodilation, increased (and aberrant) vascularisation due to high tumour metabolism, and inflammatory cell recruitment. [86] Thermography does not use ionizing radiation, does not require breast compression and can be relatively inexpensive. Thermography was intensely researched in the 1970s but was found to have poor sensitivity. With the development of better cameras for IR imaging, thermography has again become the focus of ongoing investigations. Thermography has a number of drawbacks, however, which make it an unlikely candidate as an independent screening technique. Thermography images the surface temperature of the breast so deep-seated tumours and “cold” tumours which do not have high metabolism may be missed. Inflammation due to non-cancerous lesions may also show up in a thermogram, and normal vasculature close to the breast surface may increase temperature locally, leading to false positives. The airflow and local heating from equipment and lighting in the study room must be carefully controlled to avoid artefacts. Most studies assessing thermography have evaluated it as a diagnostic rather than a screening technique, however it has not shown adequate sensitivity and specificity to be used in either capacity – either as a screening tool or as an adjunct to mammography. [70, 86, 91-93] One study which assessed thermography as a screening tool, also evaluated it as a risk assessment technique but found no relationship between an abnormal thermogram and the 5-year risk of developing BC. [91]
1.4.7 Electrical Impedance Measurements

The measurements of the electrical impedance (EI) properties of malignant and benign lesions and normal tissue have been made using both invasive and surface electrode designs and a variety of electrode configurations. Differences between malignant lesions and other tissues have been found consistently, with malignant lesions having lower impedance, but the source of the change in impedance (either a change in resistance or reactance) varied between studies. [94] To date, studies have not focused on BC screening in the general population but rather on pre-selected populations such as women identified for biopsy. Thus, the technology is not yet recommended for use as a general screening tool. [70, 86] However, after a clinical trial showing its utility as an adjunct to mammography, an electrical impedance mapping device, the TS2000, was granted US FDA’s pre-market approval in 1999 to be used to provide additional information before biopsy recommendation. [94]

1.5 Optical Breast Spectroscopy

Optical techniques have been used for measuring breast composition and density, detecting BC and monitoring the breast during neoadjuvant chemotherapy or HRT. Breast tissue has relatively low absorption in the red and near infra-red (NIR) range, and transmitted light can be detected though several centimetres of tissue. Scattering is the dominant tissue interaction for light in this wavelength range, making imaging difficult, and although reconstruction and scanning techniques are used to create images, the resolution is poor compared to mammography. The main advantages of optical techniques are that the optical contrast provides both compositional and functional information about the tissue and that they do not use ionizing radiation and so are safe for repeated use on women of any age and even during pregnancy and breastfeeding. Optical techniques can also often be implemented at comparably low cost.

1.5.1 Optical contrast in tissue

Optical contrast depends on both the absorption and scattering properties of the tissue. Although tissue has relatively low absorption in the red and NIR, there are a number of tissue components (chromophores) which have significant absorption in this range. The main
chromophores usually included in the analysis of breast tissue are oxyhemoglobin and deoxyhemoglobin, water, lipid and collagen. The hemoglobins dominate absorption in the visible part of the spectrum, while water and lipid have significant, distinct peaks in the 900-1000nm range. **Figure 1.2** shows the absorption spectra of these five chromophores. The hemoglobin contrast provides functional information about the tissue and all five chromophores provide information about the tissue composition. In addition, all five chromophores are of interest in relation to BC risk or detection. Abnormal vasculature is a hallmark of tumours, so hemoglobin concentration and saturation are of interest in detecting and monitoring tumours. The relative amounts of water and collagen vs. lipid in the breast reflect the amount dense tissue and are therefore should relate to BC risk. Collagen is of particular interest as there is evidence that increased levels of collagen promote tumour formation and tumour invasion. [95, 96] Scattering is the dominant interaction in breast tissue, and there is contrast due to the different scattering properties of the various tissue components. Scattering depends on the size and density of the scatterers in the tissue and distinct differences in scattering properties between benign and malignant tissues and between low- and high-density breast tissues have been observed. [97, 98] Scattering is wavelength-dependent, but the variation is smooth and featureless. Scattering as a function of wavelength is often modeled using an empirical power law model (*Equation 1.1*), where the reduced scattering coefficient, $\mu'_s$, depends on wavelength, $\lambda$, the scattering amplitude, $A$ (which is the reduced scattering coefficient at the reference wavelength, $\lambda_0$), and the scatter power, $b$. [97, 99]

$$\mu'_s = A \left(\frac{\lambda}{\lambda_0}\right)^{-b} \quad \text{Equation 1.1}$$

Optical attenuation through tissue depends on both scattering and absorption. Decomposing the attenuation spectra into scattering and absorption spectra is usually achieved by using frequency modulated signals (100s of MHz range) or using pulsed lasers (ps pulses) and time-correlated detection. [98-101] For continuous-wave (CW) systems, the absorption and scattering spectra can be estimated using solutions of the light diffusion equation or Monte Carlo simulation with chi-square fitting routines when information about the source-detector
positions is available. Fitting uses the known spectra of the tissue chromophores and the
power-law model for the scattering spectrum to match experimental spectra from breast
tissues. Fitting constraints which restrict fitted values to biologically reasonable ranges can be
added to improve fit convergence. [102]
Figure 1.2 Absorption spectra of (A) oxyhemoglobin (red dashed line) and deoxyhemoglobin (solid purple line) and (B) lipid (green dashed line), water (blue dotted line), and collagen (solid grey line).
1.5.2 Previous Optical Breast Spectroscopy Studies

Past studies using optical spectroscopy (OS) to assess the breast have focused on three main areas – detecting BC or identifying tumour tissue, monitoring women during neoadjuvant chemotherapy for BC, and correlating breast optical properties with BC risk.

Using optical methods for the detection of BC as an alternative or adjunct to mammography was the original area of research for OS with respect to breast oncology. Optical differences between healthy and cancerous breast tissue have consistently been found, with higher total hemoglobin concentration, higher water content, lower lipid content and lower Hb saturation being observed in most tumour tissue. However, despite most studies with modern OS systems having been performed on pre-selected populations (enriched for women with identified tumours or women undergoing biopsy), the sensitivity and specificity values attained have not been high enough to consider OS as an alternative to mammography. [103-108]

The use of OS as a means of assessing tumour response to neoadjuvant chemotherapy early in the treatment has been under investigation for more than 10 years. The types of OS devices used and the classification of patients by their response to the treatment (whether only a pathological complete response or a good but incomplete pathological response was considered as a positive result) have varied from study to study, making it difficult to compare their results between studies. Nevertheless, overall the results have indicated the OS may be useful in evaluating neoadjuvant chemotherapy response. [108-111] A decrease in hemoglobin content (both oxy- and deoxyhemoglobin), a decrease in water content and an increase in lipid content were seen in those who responded (either completely or almost completely) to treatment while no significant change was seen for those who did not respond.

The source of contrast which underlies the quantification of MBD is the difference in x-ray attenuation between adipose tissue versus fibroglandular tissue. Water, lipid and collagen all have optical contrast in the red and NIR, and so the possibility of estimating breast density using OS has been explored. Also, since breast density is a bulk tissue property, its quantification is not as much impacted by the high light scattering in tissue compared to imaging applications, where the light scattering severely impacts and limits image formation
and resolution. Prior to the start of and during my thesis, several studies were carried out using an OS system (called the Trans-illumination Breast Spectroscopy (TiBS) device) based on CW light and a trans-illumination geometry investigating optical properties of the breast in comparison with MBD and other risk factors. Given the difficulty of separating the attenuation due to scattering and absorption from CW spectral measurements, for most of these studies, principal components analysis (PCA) was used to reduce the attenuation spectra to a few principal component (PC) scores describing the spectral shape. In a study of 292 pre- and post-menopausal women with normal mammograms recruited from the general screening population, significant differences in PC scores were found between women with high and low MBD. Using a multivariate logistic regression model, women with ≥75% MBD (the population at highest risk) could be identified with good sensitivity and specificity resulting in an area under (AUC) the receiver-operator characteristic (ROC) curve of 0.922. [112] In another study using a subset of the same population, the attenuation spectra were used in a partial least-squares regression model to predict mammographic percent density (MPD) measured using Cumulus density-thresholding software. There was a strong correlation between MPD predicted from the OS spectra, and the MPD determined using Cumulus. The $R^2$ value for the validation set for the final model was 0.78. [113] A chromophore fitting analysis based on a constrained light diffusion model was performed to estimate the percentages of lipid, water, collagen and oxy- and deoxyhemoglobin from the attenuation spectra. As was expected, lipid content was inversely correlated with MPD and water content was positively correlated with MPD for both pre- and post-menopausal women. [102] Other groups have since found associations between breast chromophore concentrations determined by time-resolved OS and BI-RADS density categories and between chromophore concentrations determine by a combined frequency-domain and CW OS device and breast density determined by MRI. [98, 114, 115]

The use of OS for BC risk assessment has been explored beyond the measurement of breast density via OS. OS has been used to monitor women during the course of BC risk-changing events or treatments and to compare women with different BC risks based on other risk factors. In this lab, the TiBS device was used to monitor two groups of pre-menopausal women aged 25-45 over a period of >2 years – a group of 27 women undergoing a first full-term
pregnancy (an event which changes BC risk) and a group 188 of nulliparous women. Both
groups were monitored for an average of 3 years with OS measurements performed every 3
months. For both groups, significant changes in several PC scores were seen over a 2-year
period, with larger temporal changes seen in the pregnancy group. For the pregnancy group,
the dominant changes were in the period 6-9 months post-partum, likely corresponding with
the cessation of or a reduction in breastfeeding. For the nulliparous group, the changes in PC
scores were smaller and more gradual and correspond to changes in breast composition due to
breast tissue aging, including glandular atrophy. [116] Another study using the TiBS device
compared optical spectra between three groups of pre-menopausal women who have different
BC risks based on reproductive factors. The groups comprised 140 nulliparous women aged 18–
21, 115 nulliparous women aged 31-40 and 36 parous women aged 31-40. There were
significant differences in PC scores between both the two age groups of nulliparous women and
between the parous and nulliparous women aged 31-40, indicating that OS with the CW TiBS
device is sensitive to changes in breast composition associated with both aging and parity. [117]
A third study using a modified version of the TiBS device, which was designed for use on girls
and which measures diffuse reflected rather than transmitted light, looked at the possibility of
using OS as a method of safely, non-invasively and objectively assessing breast development
stage during puberty. The timing of breast development during puberty is associated with BC
risk and is currently determined either by a physician, self- or maternal assessment, with the
latter two methods being less reliable. [118] This was a preliminary analysis of a small ongoing
study, with only 102 girls in total and only 16 in the first developmental stage and 6 in the final
stage. Nevertheless, distinct and significant changes in PC scores were found between different
stages of breast development, indicating that OS may be useful for assessing breast
development stage. In addition to breast development staging, a BC risk score was calculated
for each girl based on detailed family history data. The BC risk scores were found to be
significantly associated with 3 of the OS PC scores. [119]

1.5.3 Trans-illumination Breast Spectroscopy (TiBS) Device

The TiBS device used in our lab has been described in detail previously. [120, 121] It uses a
broad-band 150W halogen projector lamp (Ushio Inc., Tokyo, Japan) coupled to a 5mm
diameter optical fibre bundle as the light source. The detector fibre bundle is composed of 140 fused silica fibers with 200μm core diameter and a numerical aperture of 0.36 arranged in a spot to line configuration (P&P Optica, Kitchener, Canada). The spot diameter at the light collection end is 3mm. The distal, line end of the detector fibre bundle acts as an entrance slit and connects to an imaging spectrometer containing a volume phase holographic grating (P&P Optica, Kitchener, ON) which projects onto either a thermoelectrically cooled, 128x1044 pixel, back-thinned CCD detector (Hamamatsu Corp. NJ) or a 256 × 1440 pixel CCD (Photometrics, NJ, USA). Spectra are averaged over the short axis of the CCD to increase the dynamic range. The source and detector bundles are held coaxially facing each other connected via a caliper quantifying their separation (see Figure 1.3). The fibre bundle movement is restricted to a track in a breast support platform. The source fibre bundle moves with the detector fibre bundle and its height is adjusted to be at the surface of the breast with only mild compression. The height is adjusted and the source-detector separation is recorded. All spectra are corrected for the dark signal and a reference standard (a 3.5cm thick block of white, ultrahigh-density polyurethane, Gigahertz Optics, Munich, Germany) to quantify the daily system throughput.

Attenuation spectra are measured at four positions per breast for each participant – two along the midline of the breast, one 2cm from the chest wall and the other approximately 2cm back from the nipple, and one each on the lateral and medial sides approximately 2cm from the breast edge and >2cm from the chest wall. Because of the high scattering of breast tissue, most of the breast volume is sampled by these four measurements as verified via photon propagation models (see chapter 2 below).

The device is required to have a large dynamic range as there is considerable inter-person variation in both breast optical properties and breast size. There is also a large variation in attenuation across the wavelength range being measured. A large dynamic range for the TiBS device is achieved via a 12-bit analog-to-digital converter (ADC) for the CCD, illuminating multiple rows of the CCD (∼140) per wavelength, allowing for a variation of a factor of ∼10 in overall light source intensity by adjustment of an iris at the distal end of the light source fibre, and by varying the integration time for the CCD by a factor of 40. However, the dynamic range is limited by the non-uniformity of the light source, essentially a black body emitter, which has a
variation of about a factor of 20 across the wavelength range. Overall, the dynamic range of the TiBS device is approximately 7 orders of magnitude.

Although the TiBS device has shown great promise as a risk assessment tool, the results achieved to date have been the result of single-institution studies. To validate OS-determined properties as independent risk factors large-scale, long-term, multi-institutional trials are required. There are a number of drawbacks to the TiBS device that make it unsuitable for such trials. In its current form, the TiBS device is very operator-dependent. The fibre positioning, amount of compression from the source fibre bundle, selection of integration time, recording of the source-detector fibre bundle distance and the setting for the light source iris are all performed by the operator and are therefore susceptible to inter-operator variability and operator error. In a longitudinal study of women with repeat measurements every 6 months, we found a difference of 1-1.5 cm in average recorded source-detector distance when there was a change in operators. Inter-operator comparison on test subjects indicated that this was due to differences in positioning and the amount of compression applied and to flexibility in the fibre holder. Measurement of the dark signal and reference standard before and after each participant visit is required as both the light bulb signal and the dark signal are temperature dependent and vary during the course of a day. The dark signal is especially variable when the device is first powered up as an equilibrium between room heating from the light source and cooling by the thermoelectric element must be achieved for the signal to become stable. Typically after approximately 30 minutes the signal stabilizes and only a slow but steady change over the course of hours is observed.

To compare results between devices and institutions, the spectra obtained must be device-independent. Spectra from the TiBS device are corrected relative to particular reference standards, which in themselves are difficult to maintain. Because of the large variation in optical properties and optical pathlengths between women, the dynamic range of the device is set such that unattenuated light from the source fibre bundle is beyond the detectable range and saturates the spectrometer. Absolute calibration of each TiBS device is therefore not possible. For multiple copies of the TiBS device to be used effectively in multi-centre trials, a version which can be absolutely calibrated should be developed, as ideally reference standards
would be used as a quality control tool for monitoring the device over time rather than as a calibration method.

The TiBS device is a CW device and so separation of absorption and scattering information is difficult, and the lack of standardized source-detector positions limits the type of modelling that is feasible. To date, only a diffusion model, which is less computationally intensive than Monte Carlo modelling, has been applied when attempting to separate the contributions of absorption and scattering to the TiBS attenuation spectra. However, Monte Carlo modelling could allow for more accurate correction for boundary effects.

Another drawback of the TiBS device is that it is not truly portable. The fragility of the spectrometer, the bulkiness of the apparatus for fibre positioning and the requirement for cooling fans allow for a device that can be safely moved within an institution but is difficult to ship and cannot be used as a mobile device for in-home visits, which was a requirement of one collaborating institution.
Figure 1.3 Measurement platform of the TiBS Device showing the optical fibre bundles, adjustable caliper, and the dark block and white reference standard.
1.6 Rationale

Optical spectroscopy measurements using the TiBS device demonstrated that CW OS might be a useful tool for assessing BC risk and providing information which may help personalize BC screening programs or design more cost-effective screening programs for low- and middle-income countries. The utility of the OS measurements in this capacity needs to be validated in large-scale, multi-centre trials for which the current TiBS device is not well-suited. The goal of this research has been to develop and validate a modified version of the TiBS device which is suitable for large-scale, multi-centre trials. The modified device, called the ‘Cups device’, has photodiode detectors at fixed positions in rigid cups which fit over the breast. Light source modules fit into the cups and contain lasers with wavelengths in the red-NIR range which are cycled on and off in sequence, providing the spectral resolution. Four sizes of cup are made to fit a range of breast sizes.

1.7 Hypothesis

The current TiBS device can be modified to a simple, portable, lower cost technique suitable for population-wide screening by reducing the spectral content and by implementing parallel spatial detection at fixed positions, without significant loss of clinically relevant information.

1.8 Specific Aims

1. To select a small number of specific wavelengths for the light source lasers and several fixed positions for the light source and detectors that will provide a measurement of the bulk optical properties of the breast equivalent to the measurement made with the original TiBS device.

2. To validate the equivalency of the modified Cups device and the original TiBS device in a clinical trial.

3. Design a light source and detector configuration for reflectance measurements on girls with the modified device.

An outline of the thesis work is shown in Table 1.2.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Aims</th>
<th>Study Population(s)</th>
<th>Device(s) Used</th>
</tr>
</thead>
</table>
| 2       | • Selection wavelengths for Cups device  
• Selection of number of sources and detectors, and positions of sources and detectors.  
• Theoretical evaluation of the effect of reducing the spectral content on distinguishing between women in different BC risk groups based on mammographic density or age and parity | • Women age 34-77 recruited from the general screening population (292 women)  
• Nulliparous women age 18-21, nulliparous women age 31-40 and parous women age 31-40 (300 women in total) | TiBS |
| 3       | • Evaluation of Cups device vs. TiBS device based on  
  • Identification of women with high mammographic density based on optical spectra  
  • Prediction of mammographic percent density from optical spectra | • Women age 29-73 recruited from the average-risk population (40 women) and the high-risk screening population (20 women) | TiBS, Cups |
| 4       | • Theoretical evaluation of the effect of reducing the spectral content on determining Tanner Stage based on optical spectra  
• Selection of the curve shapes needed to match breasts from TS 1 through TS 5.  
• Evaluation of the modified OS device for girls in comparison with the TiBS device (measuring in diffuse reflectance mode) | • Girls age 10-15, TS 1-5 (102 girls) for the theoretical evaluation of spectral content  
• Girls age 11-17, TS 2-5 (35 girls) for evaluation of the modified device | TiBS, Modified device for girls |
2 Design of a Multi-wavelength, Laser-based “Cups” Optical Spectroscopy Device for Breast Density and Breast Cancer Risk Pre-screening

This chapter describes the development of a laser-based, portable device for measuring breast composition in women. This work has been published in the Journal of Biophotonics and is reused here, with minor modifications, with permission from the publishers. [122]

2.1 Introduction

Mammographic screening for breast cancer has been a hotly debated topic in recent years. The age at which screening should begin and end, the frequency of screening and whether there is an overall benefit to screening, especially considering the problem of over-diagnosis, have all been contested.[123-129] A general consensus that there is a significant reduction in breast cancer mortality with mammographic screening seems to be emerging, however mammographic screening programs are far from optimized and methods of personalizing breast cancer screening programs based on the risk of developing breast cancer are needed. Furthermore, participation rates in some mammography screening programs are below effective target rates.[43, 130] Personalization of the recommendation to participate in screening may change women’s perception of the risk-benefit ratio and may improve screening uptake and retention. Risk information as a modifier to the standard screening program is currently applied only to small subsets of women who have a highly elevated risk of breast cancer such as those with mutations in the BRCA1 and BRCA2 breast cancer susceptibility genes, which confer a greatly increased risk (relative risk ratios of >10) but are only present in a very small percentage of the population (~0.1% of the general population).[13] [15] The Ontario Breast Screening Program, for example, is divided into a general screening program and a high-risk screening program. The high risk screening program, which begins at age 30 rather than 50 and includes annual MRI in addition to digital mammography, is available only to women who have a confirmed genetic mutation known to lead to a strong increase in breast cancer risk, first-degree relatives of such mutation carriers who have not themselves been tested for genetic mutations, women with a family history of breast cancer and an estimated lifetime risk
of greater than 25%, and women who had radiation therapy to the chest before age 30. [14] Outside this relatively small cohort of women with greatly increased risk, there is still considerable variability in risk between women and several risk factors which are applicable across the general population and could be used to personalize and streamline standard screening programs. Assessing a woman’s risk of developing breast cancer is currently based on multiple risk factors such as age, family history, parity, mammographic breast density (MBD), ionizing radiation exposure, age at menarche and menopause, and genetics. [31, 44, 131] The Gail score for breast cancer risk, which takes into account age, age at menarche, age at first live birth, breast biopsy history and number of first degree relatives with breast cancer, is applicable across the standard screening population and shows significant variation within that population. [132] MBD is a strong, independent risk factor for breast cancer with only two other known risk factors – age and mutations such as BRCA1/2 – having higher relative risk (RR) ratios (the RR ratio for MBD is 4-6 for women with high (≥75%) mammographic density vs. with women with low or no density (<10%). [44, 54, 133, 134] Approximately 10% of the screening population over age 50 has MBD ≥75%. [3] MBD information is currently only available after a women has begun mammographic screening and is not often used to modify individual screening protocols. The anatomical structures, comprising predominantly lipid, water and collagen, giving rise to MBD also have identifiable optical absorption spectra. These and additional tissue chromophores of the breast, in particular the hemoglobins, have distinctive absorption spectra in the red/NIR spectral region. As MBD is a global scalar measure of a breast, and no spatial information is retained, the lack of spatial information by the optical trans-illumination is not a concern. Optical breast spectroscopy (OBS) was explored with the intention to provide a measure of bulk breast optical properties which could be used to identify women with high MBD, even before they have a first mammogram. [71, 99, 112] The advantage of OBS being that a possible comparably high odds ratio for breast cancer risk can be established at a comparatively low cost to the healthcare system, without ionizing radiation exposure to the women and with less discomfort during the physical measurement, listed as main reasons for non-compliance in some countries. [43]
Various initial studies have and are being carried out in an academic setting establishing the potential of OBS using a research prototype Trans-illumination Breast Spectroscopy (TiBS) device.[102, 112, 113, 116, 117, 120, 135-137] This device, previously described in detail, has a broadband halogen bulb coupled to a fibre bundle as the light source placed in contact with the skin on the top of the breast and a coaxial opposing fibre bundle leading to a CCD-based spectrometer for the detection. The light source and detector fibre bundles are placed sequentially on four positions on the breast by a study nurse or clinical coordinator. While initial studies have indicated a strong potential for OBS as a risk assessment or pre-screening tool, its further development and translation of the technology into large population-based studies requires a redesign of the TiBS device aimed at making it more robust, portable, cheaper to build, faster in accumulating transmittance data, and less operator dependent. To that end, two main changes to the TiBS device have been implemented: (1) eliminating the spectrophotometer as the most expensive and most fragile component and operating with a lower spectral content using individual laser diodes turned on in sequence and (2) fixing the positions of the light sources and detectors in cups of four standard sizes to fit over the breast, eliminating the variable positioning by the study personnel. This study describes the selection of the laser diode wavelengths based on data from two previous studies correlating OBS spectra with breast cancer risk factors and the selection of the positions of the light sources and detectors based on Monte Carlo simulations to maintain optically interrogated volumes similar to the original TiBS device.

2.2 Material and Methods

2.2.1 Study Populations for Wavelength Selection

Data from two study populations, originally aimed at correlating Optical Breast Spectroscopy (OBS) spectra with mammographic density and parity respectively (two established breast cancer risk factors), were used select the wavelengths and to test the feasibility of a reduced spectral content “Cups” version of the TiBS device.[112, 117] The first study population comprised 300 pre- and post-menopausal women (age range 34-77, mean 50.9 years) with no radiologically suspicious lesions and no previous breast surgery recruited from the regular
screening program at the Marvelle Koffler Breast Centre in Mount Sinai Hospital, Toronto, Ontario. A complete description of the study population has been published previously.[112] The second study compared OBS spectra between 3 groups of pre-menopausal women – young, nulliparous women (age 18-21, group 1), older nulliparous women (age 31-40, group 2), and older parous women (age 31-40, group 3). [117] Table 2.1 gives the summaries of the study population demographics. Both studies were approved by the Ethics Committees for human subject research at both the University Health Network and Mount Sinai Hospital. The mammographic density study was also reviewed and approved by the Institutional Review Board of the University of Toronto.
Table 2.1. Demographics of the MBD* and parity study† populations used in this analysis

<table>
<thead>
<tr>
<th>Menopausal status</th>
<th>Pre</th>
<th>Post</th>
<th>Total</th>
<th>Study Population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt; 25%) MBD</td>
<td>33</td>
<td>70</td>
<td>103</td>
<td>35.3</td>
</tr>
<tr>
<td>Medium (25 - 75%) MBD</td>
<td>78</td>
<td>69</td>
<td>147</td>
<td>50.3</td>
</tr>
<tr>
<td>High (&gt; 75%) MBD</td>
<td>27</td>
<td>15</td>
<td>42</td>
<td>14.4</td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>45.9 ± 4.3</td>
<td>55.4 ± 6.4</td>
<td>50.9 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>25.3 ± 5.9</td>
<td>26.2 ± 4.9</td>
<td>25.8 ± 5.5</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from [21]

<table>
<thead>
<tr>
<th>Age</th>
<th>Nulliparous</th>
<th>Nulliparous</th>
<th>Parous</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-21</td>
<td>63.1±14.7</td>
<td>69.8±17.2</td>
<td>68.3±15.3</td>
</tr>
<tr>
<td>31-40</td>
<td>162.7±7.0</td>
<td>163.0±5.6</td>
<td>162.5±5.5</td>
</tr>
<tr>
<td>Days since last period, mean ± SD(^a)</td>
<td>15.6 ± 10.0</td>
<td>15.7 ± 9.0</td>
<td>14.3 ± 8.8</td>
</tr>
<tr>
<td>Ethnicity, N (%)</td>
<td>European</td>
<td>NE and SE</td>
<td>Asian(^b)</td>
</tr>
<tr>
<td></td>
<td>62 (42.2)</td>
<td>78 (67.8)</td>
<td>46 (31.3)</td>
</tr>
<tr>
<td></td>
<td>27 (18.4)</td>
<td>19 (16.5)</td>
<td>7 (6.1)</td>
</tr>
<tr>
<td>Hormonal Contraceptive Use</td>
<td>12 (8.2)</td>
<td>11 (9.6)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Never [%]</td>
<td>50.3</td>
<td>24.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Ever [%]</td>
<td>49.7</td>
<td>75.7</td>
<td>89.5</td>
</tr>
</tbody>
</table>

\(^a\) Missing for nine women
\(^b\) Northeast and Southeast Asian
†Adapted from [30]
2.2.2 Wavelength Selection

Wavelengths for the laser modules were selected based on the analysis of the spectra from the first study, while the second study served to validate the selection. In the original publication for the first study, a principal components (PC) analysis was performed on the mean-centred spectra to reduce the 436 attenuation values measured in 1nm increments over the wavelength range 625-1060nm to a few PC scores describing the spectral shape. The resulting PC scores (PCₙ) for each individual were calculated from the spectra by multiplying each spectrum (Sᵢ), mean-centred by subtracting the average spectrum for all women (S̅), by the wavelength-dependent weighting factors (pₙλ) which are the eigenvectors of the covariance matrix calculated in the PC analysis (Equation 2.1).

\[ PC_{in} = \sum_{\lambda} (S_{i\lambda} - S_{\lambda}) p_{n\lambda} \]  

Equation 2.1

As the spectral data are highly correlated with respect to wavelength, PC scores for the first 4 principal components (PC1-PC4) captured 99.66% of the variance among the spectra and so only those 4 of the n= 436 PC scores were used in creating a mathematical model to identify women with high (≥75%) mammographic density.[112] In selecting the wavelengths for the modified OBS system, the goal is to reduce the spectral content while minimizing the loss of biologically and clinically relevant information. The laser wavelengths were selected, (from commercially available laser diode wavelengths), using the wavelength-dependent PC weighting factors derived in the original study, based on their proximity to local absolute maxima in the 4 PC weighting vectors as larger deviations from zero indicate that these wavelengths have a larger contribution to the scores used for identification of women with high mammographic density, see below. Wavelengths were chosen to cover all peak regions in these 4 vectors while taking into account the availability of affordable laser diodes of sufficient optical power. For the final design, a total of 13 wavelengths were selected heuristically. To assess the effect of the reduction of the full spectra to only these 13 wavelengths on the necessary spectral information content, a PC analysis was performed on these spectra using only these 13 wavelengths. The correlations between the scores derived from this spectrally reduced PC analysis (PC₁₃λ scores) and the scores derived from the original full spectral range (PCₐₙλ scores)
were calculated. Also, the statistical analyses comparing the 4 spectral PC scores between high density (≥75%) and low density (<75%) tissues carried out in the original publication for this study were repeated using the PC\textsubscript{13λ} scores to determine whether the differences between groups seen for the original study were still significant. Specifically, three analyses were repeated: (1) the Mann-Whitney U test was used to determine if there were significant differences between the PC scores for high density and low density breast tissue for all women and for pre- and post-menopausal women individually, (2) univariate logistic regression was used to determine both the unadjusted odds ratios (OR) of high vs. low MBD over an increase equal to the interquartile range in each PC score and also the area under the curve (AUC) for the receiver operator characteristic (ROC) curves for the probability of having ≥75% MBD, and (3) a multivariate logistic regression for a model including PC3, the interaction between PC4 and menopausal status, BMI (body mass index), and menopausal status was used to obtain an AUC for the ROC curve for the model for the probability of having ≥75% MBD. [112]

As an initial validation of this wavelength selection, the statistical analysis from the second study with data derived from a different population with lower average age was repeated after reducing the spectral data again to these 13 wavelengths and performing a PC analysis on the reduced spectra to determine whether the loss of spectral content would degrade the ability to distinguish between groups. The majority of these pre-menopausal women would have high breast density compared to the older population of the mammographic density study and MBD is not an established risk factor in this age range. The women for this study were grouped based on parity and age, two other established risk breast cancer risk factors. Principal components analysis was performed separately on the 13-wavelength spectra for groups 1 and 2 together (younger and older nulliparous women) and for groups 2 and 3 together (parous and nulliparous older women) as had been done with the full spectra in the original study. Differences between the two groups for both of those analyses for each principal component were assessed using linear regression with the PC scores as the dependent variable and risk group as an independent variable. Height, weight, ethnicity, hormonal contraceptive use and days since last menstrual period were also included as independent variables. Results with the PC\textsubscript{13λ} scores were compared to those from the original study with all wavelengths. [117]
2.2.3 Light Source and Detector Placement

The selection of the number and location of light sources and detectors had the goal of maintaining the optically interrogated breast tissue volume and enabling uniform sampling across that volume, while minimizing the contribution of adjacent tissues (the pectoral muscle/chest wall) to the optical signal since it is not possible to distinguish between the signal from the breast and signal from adjacent tissues. Monte Carlo (MC) simulations were used to model the propagation of light through the breast and adjacent tissue for the original TiBS device and for the geometries for the modified Cups device. A modified version of the tMCimg MC program, recording paths for each photon packet detected, was used for the simulations.[138, 139] A map of the optically interrogated volume was created from these photon paths by incrementing a voxel’s signal for each scattering event occurring in it by the corresponding photon packet’s weight at detection. The photon weight, \( W \), was calculated using the Beer-Lambert law (Equation 2.2) where \( l_i \) is the total distance traveled in each medium (breast tissue, pectoral muscle and the black absorbing platform), \( \mu_{ai} \) is the absorption coefficient of each medium, and \( m \) is the total number of media.

\[
W = e^{-\sum_{i=1}^{m} \mu_{ai} l_i}
\]

Equation 2.2

For the TiBS device, where the breast rests on a black platform, the breast shape was modeled as a quarter-ellipsoid. A 1mm thick highly absorbing layer was added below the tissue to represent the black platform and a 2cm thick layer was added at the back of the breast to represent the pectoral muscle of the chest wall. A voxel size of 1mm\(^3\) was used. Since the shape and size of the volume sampled is highly dependent on scattering, and since scattering varies significantly between women, two values for the scattering coefficient (\( \mu_s \)) of the breast tissue were used for each geometry to bracket the range of scattering values likely across this wavelength range for both pre- and post-menopausal women.[100] Optical properties used for the simulations are listed in Table 2.2. The new Cups design uses a hemispherical breast shape for the 3 largest sizes (B, C and D cups) while a hemi-ellipsoid with a circular base will be used for the smallest breast sizes (A cup). Measurements of breast dimensions and source and detector positions were obtained from a large ongoing study and grouped by self-reported bra cup size. Average values for breast volume and corresponding average source-detector
positions for the TiBS device were calculated for the 4 cup sizes. As the C size was the most common in our previous studies, its volume was used for the initial optode placement selection process in the new design. The Cups device has the light sources and detectors fixed in rigid black cups, representing matched boundary conditions and eliminating the detection of photons having travelled through the skin and sub-cutaneous fat more than twice. The cup volumes for the Cups device correspond to the breast volumes determined for the original TiBS device. The hemi-ellipsoid A had a base diameter equal to the B cup. Considering the matched boundary conditions due to the black absorbing cups surrounding the entire breast shape, the MC simulations were simplified by setting the index of refraction of both the breast and pectoral muscle tissues to 1.0. All other tissue properties were the same as for the simulations of the TiBS device geometries (Table 2.2).
<table>
<thead>
<tr>
<th>Medium/Tissue</th>
<th>Absorption Coefficient, $\mu_a$ [mm$^{-1}$]</th>
<th>Scattering Coefficient, $\mu_s$ [mm$^{-1}$]</th>
<th>Anisotropy factor, $g$</th>
<th>Index of Refraction, $n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original TiBS Device Simulations</td>
<td>Breast</td>
<td>0.004</td>
<td>High scattering: 1.300 Low scattering: 0.500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Black absorbing platform</td>
<td>100</td>
<td>2.000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pectoral Muscle</td>
<td>0.020</td>
<td>0.670</td>
<td>0</td>
</tr>
<tr>
<td>Modified Cups Device Simulations</td>
<td>Breast</td>
<td>0.004</td>
<td>High scattering: 1.300 Low scattering: 0.500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pectoral Muscle</td>
<td>0.020</td>
<td>0.670</td>
<td>0</td>
</tr>
</tbody>
</table>
A grid of potential optode (source and detector) positions with 1cm spacing was projected onto the transverse plane, centered along the midline of the breast, and with the restriction that each position was ≥2cm from the chest wall (base of the hemisphere) and ≥1cm from the center of the nipple (assumed to be at the tip of the hemisphere). Source positions were limited to the positions on the top half of the breast, at least 3cm from the chest wall. In the original TiBS device the minimum distance from the light source to the wall was 2cm (for the central position), chosen to give a low contribution of signal from the chest wall. Since the breast is not flattened on the bottom with the cup design, allowing for larger maximum source-detector distances and correspondingly wider interrogated volumes, and since there would be fewer than 4 source positions, the minimum distance from the chest wall was increased to 3cm. Optode position combinations were evaluated relative to the TiBS system based on 3 metrics derived from the MC simulation data: the percent of the breast volume sampled, (which should be maximized), the sampling uniformity area under the signal-volume curve (AUC$_{S-V}$, which should be minimized to give the most uniform sampling), and the percent of the signal from the pectoral muscle, (also to be minimized since it is not possible to distinguish in the detected signal between photons that have traveled only through the breast and photons which have traveled through the adjacent tissues as well). The percent breast volume sampled was calculated as the fraction of voxels in the breast with signal greater than or equal to 1% of the average voxel over the total number of voxels in the breast volume multiplied by 100. To calculate the sampling uniformity, the voxels of the breast were ordered according to decreasing signal strength and a plot of the total signal fraction vs. fraction of breast volume was generated. The area under the curve was taken as the measure of the signal uniformity, with an ideal value of 0.5 meaning equal contribution by all voxels, while a value of 1 indicates that only a single voxel is sampled (Figure 2.1). The signal from the adjacent tissues was given by the total signal from the voxels in the pectoral muscle slab divided by the total signal from the entire simulation volume.
Figure 2.1. Description of two metrics used to evaluate positions of light sources and detectors from MC simulation data – area under the fraction of total signal vs. fraction of breast volume sampled curve, which measures sampling uniformity, and the total breast volume sampled.
Possible source-detector combinations were simulated to determine the number of sources and detectors needed to achieve a similar volumetric contribution to the optical signal based on the above three metrics as in the TiBS system. Combinations with 1 source and 2-6 detectors, then 2 sources and 2-6 detectors were tested. For a single source and two detectors, all source-detector combinations from the grid of potential positions were tested with the restrictions that the source-detector distances be between 2 and 8 cm (the range of inter-optode distances for the TiBS device). For combinations with 3 or more detectors or for 2 sources, the number of possible source-detector combinations was prohibitively large. To select position combinations for simulation likely to produce the optimal volumetric coverage, the use of the total inter-optode distance as a design guideline was tested with the idea that the further apart the optodes were, the less overlap there would be between their interrogated volumes and the further the detectors were from the sources, the larger their interrogated volumes would be. The total inter-optode distance is the sum of all the inter-optode distances – source-detector, source-source and detector-detector – for a given optode combination. The total inter-optode distance showed good correlation with the percent of breast volume sampled and good inverse correlation with the AUC_{S.V}, (the metric of sampling uniformity), for the single source simulations, where all potential optode combinations from the optode grid were simulated, Figure 2.2, indicating that optode combinations with high total inter-optode distances are most likely to give the best volumetric sampling parameters. Based on this, the total inter-optode distance was calculated for all source-detector combinations from the optode grid for the simulations and the 100 source-detector position combinations with the highest total inter-optode distances were selected for MC simulation for each number of sources and detectors (i.e. 100 position combinations for 2 sources, 3 detectors and 100 combinations for 2 sources, 4 detectors, etc.).
Figure 2.2. The area under the signal-volume curve (AUC) vs. the total inter-optode distance for single-source optode combinations (A) and the percent of the breast volume sampled vs. total inter-optode distance (B). Optode combinations with high values for the total inter-optode distance have better volumetric sampling parameters.
2.2.4 Optical Power and Laser Safety

The light from the all selected laser diodes must be coupled into the tissue via a single aperture to obtain a well-defined source position essential for modelling light propagation to the different detectors. In order to have high optical power without exceeding the maximum permissible exposure for skin, a large aperture (7mm diameter) is used as in the original TiBS device. To obtain coupling with repeatable throughput for all wavelengths, the 13 laser diodes were embedded in the walls of an aluminum cavity with a single 7mm diameter circular aperture. The walls of the cavity were painted with a 3:2 Barium Sulphate:Water-based white paint mix, (BaSO4 – 243353, Sigma-Aldrich; Paint: Opaque White 5212, Airbrush Colors, Createx), to create a diffuse reflective surface generating a uniform light source with Lambertian re-emission from the cavity wall. To optimize the shape of the cavity, commercial ray tracing simulations (ASAP, Breault Research Organization, Inc., Tucson, AZ, http://www.breault.com/software/about-asap ) were employed to maximize the number of photons coupled into tissue via the aperture. Geometries tested included a sphere with a slice cut off at the bottom, a sphere with a short hollow cylindrical connection and a hemisphere sitting on top of a cone with cone half angles of 15, 30, 40, 45, 60 and 75 degrees. All geometries had a 7mm diameter aperture. In the simulations the apertures of the laser diode emitters were not considered as they were all kept at 1mm². Each simulation traced 1000 rays sufficient to determine the photon fraction coupled into the tissue. Optical properties for Al and BaSO₄ were all taken from the program’s built-in libraries. The laser diodes were placed in these laser modules in a manner that they did not directly illuminate the aperture to comply with eye safety regulations. Since only diffuse reflections of the laser emission can be viewed, the lasers are considered embedded for laser safety purposes. Laser diode powers were measured at the laser module aperture and adjusted, via hardware and software limits to the maximum currents supplied by the laser driver, so as not to exceed the maximum permissible exposures for the eye and skin at those wavelengths calculated based on the ANSI Z136.3 (1995) standards. For the specific maximum irradiance values at the aperture please consult Appendix A.
2.3  Results

2.3.1 Laser Wavelength Selection

The 13 wavelengths heuristically selected for the laser modules were 655nm, 685nm, 735nm, 760nm, 785nm, 808nm, 830nm, 880nm, 905nm, 915nm, 940nm, 980nm, and 1050nm. These wavelengths cover all absolute maxima regions of the 4 PC weighting vectors from the mammographic density study spectra (Figure 2.3A) and equally cover the absorption features of the 5 dominant chromophores in breast tissue, (lipid, water, collagen, hemoglobin and deoxyhemoglobin) (Figure 2.3B). The PC scores calculated from the PC analysis on the spectra reduced to only these 13 wavelengths resulted in scores (PC$_{13\lambda}$) which were strongly correlated with those from the PC analysis using the full spectral content (PC$_{All\lambda}$) with resulting correlation coefficients of $r = 0.9992$, 0.9698, 0.9740, 0.9419 for PCs 1-4 respectively, (Figure 2.4). The results of the Mann-Whitney U-Test and univariate logistic regression comparing PC scores between $\geq$75% density and $<$75% density breast tissues are shown in Table 2.3 and 2.4 for both the PC$_{13\lambda}$ and the PC$_{All\lambda}$ scores. Significant differences between groups were observed for the same PCs for both the PC$_{13\lambda}$ analysis and the PC$_{All\lambda}$ analysis. The area under the curve (AUC) values for the receiver operator characteristic (ROC) curves predicting the outcome of density $\geq$75% derived from the logistic regressions were similar when calculated for the PC$_{13\lambda}$ scores as with the PC$_{All\lambda}$ scores. The odds ratios (OR) for the logistic regressions were significantly different from 1 for PC1 and PC3 for the PC$_{13\lambda}$ scores but the OR for PC4 was significantly different from 1 only for post-menopausal women for the PC$_{13\lambda}$ scores, not for all women as it was for the PC$_{All\lambda}$ scores. However, the final multivariate model developed from the original study included the interaction between PC4 and menopausal status, (as well as PC3, BMI and menopausal status), and the AUC (with 95% confidence intervals) for the final model was 0.910 (0.866-0.954) for the PC$_{13\lambda}$ scores vs. 0.922 (0.876-0.968) for the PC$_{All\lambda}$ scores. The 95% confidence intervals for the AUC were calculated using a non-parametric method based on the Wilcoxon statistic. [140] Given that the reduction in spectral content to 13 wavelengths did not significantly affect the final model for identifying women with $\geq$75% MBD, the effect of further reducing the spectral content was explored. When the spectral content was reduced to 12 wavelengths by removing any one of the 13 selected wavelengths and the PC analysis repeated,
the AUC values for the final model remained high (ranging from 0.839 to 0.921) and were significantly different from the AUC based on the PC$^{All\lambda}$ scores only when either of the two highest wavelengths (980nm or 1050nm) were removed (Figure 2.5).

For the second study with the younger, pre-menopausal population, the PC$^{13\lambda}$ scores were again strongly correlated with those from the PC$^{All\lambda}$ analysis, yielding correlation coefficients for PCs 1-4 of $r = 0.9999, 0.9877, 0.9888, 0.9298$ for the PC analysis of the younger and older nulliparous women and $r = 0.9999, 0.9872, 0.9904, 0.9294$ for the PC analysis of the older parous and nulliparous women. When comparing scores between groups, significant differences between the same pairings of groups based on age and parity were observed with PC$^{13\lambda}$ scores as with the PC$^{All\lambda}$ scores ($p \leq 0.05$ was considered significant) as previously reported. [117] Specifically, comparing older (31-40) vs. younger (18-21) nulliparous premenopausal women, linear regression coefficient ($\beta$) estimates for PC 3 and 4 were significantly different from zero between the two groups for both the PC$^{13\lambda}$ and the PC$^{All\lambda}$ analysis. Comparing parous vs. nulliparous older women (31-40), $\beta$ estimates for PC 1 and 3 were significantly different from zero between the groups for both analyses.
Figure 2.3A. The 13 wavelengths selected for the laser modules for the TiBS Cups Device (black x) shown against the weighting vectors (PC1-4) from the PC analysis of the mammographic density study spectra. Lines are PC1 – solid blue, PC2 – dotted green, PC3 – red dash-dot, PC4 dashed turquoise. B. The normalized absorption spectra for the 5 dominant chromophores in breast tissue. [21, 35-40] Lines are collagen – solid dark gray, lipid – dotted green, water – blue dash-dot, deoxyhemoglobin – dashed purple, and hemoglobin – solid red.
Figure 2.4. Scores for PCs 1-4 from the PC$_{13\lambda}$ analysis vs. the PC$_{All\lambda}$ analysis with corresponding correlation coefficient (r) values.
Table 2.3. Median, interquartile range (IQR) and results of Mann-Whitney U-test for women with high MBD (≥ 75%) vs. women with low MBD (< 75%) for PC scores 1 to 4 for all women and by menopausal status for both the full wavelength PC analysis (PC_{Allλ}) [21] and for the 13 wavelength analysis (PC_{13λ}).

<table>
<thead>
<tr>
<th></th>
<th>All women</th>
<th></th>
<th>Pre-menopausal</th>
<th></th>
<th>Post-menopausal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;75% n = 250</td>
<td>≥75% n = 42</td>
<td>p-value</td>
<td>&lt;75% n = 250</td>
<td>≥75% n = 42</td>
<td>p-value</td>
<td>&lt;75% n = 250</td>
</tr>
<tr>
<td>PC_{Allλ} 1</td>
<td>0.91 (4.36)</td>
<td>-1.26 (4.72)</td>
<td>&lt;0.001</td>
<td>0.01 (4.73)</td>
<td>-1.35 (6.06)</td>
<td>0.01</td>
<td>1.48 (3.67)</td>
</tr>
<tr>
<td>PC_{Allλ} 2</td>
<td>0.09 (0.55)</td>
<td>0.02 (0.87)</td>
<td>0.57</td>
<td>-0.09 (0.56)</td>
<td>-0.003 (0.80)</td>
<td>0.68</td>
<td>0.19 (0.40)</td>
</tr>
<tr>
<td>PC_{Allλ} 3</td>
<td>0.08 (0.29)</td>
<td>-0.31 (0.46)</td>
<td>&lt;0.001</td>
<td>0.10 (0.27)</td>
<td>-0.32 (0.47)</td>
<td>&lt;0.001</td>
<td>0.03 (0.30)</td>
</tr>
<tr>
<td>PC_{Allλ} 4</td>
<td>-0.02 (0.24)</td>
<td>-0.09 (0.19)</td>
<td>0.001</td>
<td>-0.08 (0.19)</td>
<td>-0.12 (0.31)</td>
<td>0.45</td>
<td>0.05 (0.21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC_{13λ} 1</td>
<td>0.15 (0.71)</td>
<td>-0.20 (0.77)</td>
<td>&lt;0.001</td>
<td>0.01 (0.78)</td>
<td>-0.20 (0.95)</td>
<td>0.02</td>
<td>0.24 (0.58)</td>
</tr>
<tr>
<td>PC_{13λ} 2</td>
<td>0.02 (0.09)</td>
<td>0.00 (0.14)</td>
<td>0.05</td>
<td>-0.01 (0.10)</td>
<td>0.00 (0.13)</td>
<td>0.64</td>
<td>0.04 (0.07)</td>
</tr>
<tr>
<td>PC_{13λ} 3</td>
<td>-0.01 (0.06)</td>
<td>0.05 (0.09)</td>
<td>&lt;0.001</td>
<td>-0.02 (0.06)</td>
<td>0.06 (0.10)</td>
<td>&lt;0.001</td>
<td>-0.001 (0.05)</td>
</tr>
<tr>
<td>PC_{13λ} 4</td>
<td>-0.01 (0.03)</td>
<td>-0.01 (0.03)</td>
<td>0.02</td>
<td>-0.01 (0.03)</td>
<td>-0.01 (0.05)</td>
<td>0.81</td>
<td>0.01 (0.03)</td>
</tr>
</tbody>
</table>
Table 2.4. Results of univariate logistic regression analysis for each PC score (1 to 4) from the PC analysis using all wavelengths (PC\textsubscript{Allλ}) [21] and using only 13 wavelengths (PC\textsubscript{13λ}) for all women (pre- and post-menopausal, n = 292) with mammographic density as the outcome (≥75% dense tissue vs. <75% dense tissue).

<table>
<thead>
<tr>
<th>PC</th>
<th>OR\textsuperscript{a)}</th>
<th>95% CI\textsuperscript{a)}</th>
<th>IQR</th>
<th>p-value</th>
<th>AUC\textsuperscript{b)}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC\textsubscript{Allλ}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>0.43</td>
<td>0.29</td>
<td>0.64</td>
<td>4.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC2</td>
<td>0.81</td>
<td>0.54</td>
<td>1.22</td>
<td>0.59</td>
<td>0.32</td>
</tr>
<tr>
<td>PC3</td>
<td>0.25</td>
<td>0.16</td>
<td>0.38</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC4</td>
<td>0.59</td>
<td>0.38</td>
<td>0.93</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>PC\textsubscript{13λ}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>0.46</td>
<td>0.31</td>
<td>0.67</td>
<td>0.702</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC2</td>
<td>0.60</td>
<td>0.39</td>
<td>0.90</td>
<td>0.105</td>
<td>0.01</td>
</tr>
<tr>
<td>PC3</td>
<td>0.27</td>
<td>0.40</td>
<td>0.18</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC4</td>
<td>0.81</td>
<td>0.57</td>
<td>1.16</td>
<td>0.031</td>
<td>0.24</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Odds ratio (OR) and 95% confidence interval (CI) for OR calculated over an interquartile range (IQR) of the respective density score; \textsuperscript{b)} Area under the curve (AUC) for the receiver-operator characteristic curve derived from the individual probabilities of having density ≥75% calculated from the univariate logistic models.
Figure 2.5. AUC values (with 95% confidence intervals) for the ROC curves predicting the outcome of density ≥75% derived from the final logistic regression model with 12 wavelengths (each of the 13 wavelengths from the final design individually removed). For reference, the solid lines show the AUC values for all wavelengths from the original broadband TiBS device (red) and for the 13 wavelengths from the Cups device (blue) with the corresponding dashed lines indicating 95% confidence intervals.
2.3.2 Light Source and Detector Placement

The average breast volumes for the A, B, C and D bra sizes calculated from participant measurements from the ongoing study were $159 \text{ cm}^3$, $260 \text{ cm}^3$, $469 \text{ cm}^3$ and $687 \text{ cm}^3$ respectively. Sampling characteristics of the TiBS device were calculated from the MC simulations of the original geometry and results for all four breast sizes are summarized in Table 2.5. The percent of the breast volume sampled was generally higher for the lower scattering breast tissue simulations and ranged from 72% to 92%. The metric of sampling uniformity, $(AUC_{SV} - \text{the area under the curve for the fraction of total signal vs. the fraction of breast volume contributing to the signal})$, was similar for both high and low scattering simulations and ranged from 0.82 to 0.88. The percent of the total signal from the pectoral muscle was higher in the low scattering than the high scattering case and ranged from 0.04% to 0.31%.

The C size with the low scattering breast optical properties was used for determining the number and initial positioning of sources and detectors for the Cups design of the OBS device. Sampling metrics were initially calculated for a single source and multiple detectors however it was not possible to achieve adequate values for the sampling uniformity $AUC_{SV}$ metric with a single light source as the area around the source is over-sampled. With two sources it was possible to achieve values for all three metrics close to or improving on the values obtained for the simulations of the TiBS geometry with 6 detectors. Since the same cup must be used for both breasts, the additional constraint that the sources and detectors have symmetry about the longitudinal axis was added before selecting the final optode positions. Figure 2.6A shows the average C size optode positions for the simulations of the original TiBS device geometry. Figure 2.6B shows the grid of potential optode positions for the C Cup used to select the number of sources and detectors. The final optode positions for the C cup are shown in Figure 2.6C. Figure 2.6D shows the final source and detector positions and the paths of detected light in the low-scattering breast for the C cup for a single emitting source. The optode positions for the B and D cups were the same as the C cup, scaled down and up respectively based on the relative cup volumes. Positions for the A cup were the same as the C cup scaled down based on relative volume except in the transverse plane where the spacing was compressed as the hemisphere.
was flattened to an ellipse. Average source-detector distances were larger for the Cups geometry than for the TiBS geometry (4.9, 5.0, 6.3 and 7.2 cm vs. 3.9, 4.6, 5.8 and 6.8 cm for the A-D sizes respectively). MC simulations with the final optode positions for the Cups device were performed for all four sizes to confirm that the sampling metrics for the corresponding sizes with the TiBS device were sufficiently matched with the final Cups positions. Values for the three sample metrics for the final optode positions are shown in Table 2.5. All three sample metrics are closely matched or improved upon for the B and C sizes. For the D size, sampling uniformity and the percent of the breast volume were improved with the Cups design for low scattering but worse by approximately the same amount in the high scattering case. For the A size sampling uniformity and volume sampled are improved with the Cups geometry but the signal from the pectoral muscle is also significantly increased. This was expected as the average source-detector distances were larger for the Cups geometry and the flattened ellipsoid shape of the A cup did not allow for the detectors to be placed far from the chest wall and, while significantly increased, the signal from the pectoral muscle remains low (around 2.8%).
Table 2.5. Sampling metrics calculated from MC simulations for the original OBS device geometry (modeled as a $\frac{1}{4}$ ellipsoid) and the corresponding values for the new Cup geometry (modeled as a hemisphere). Metrics were calculated from simulations using both high and low scattering properties for the breast tissue.

<table>
<thead>
<tr>
<th></th>
<th>Sampling Uniformity Metric ( \text{AUC}_{5-V} )</th>
<th>% of Breast Volume Sampled</th>
<th>% of Total Signal from Pectoral Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low ( \mu_s )</td>
<td>High ( \mu_s )</td>
<td>Low ( \mu_s )</td>
</tr>
<tr>
<td>Original Geometry (( \frac{1}{4} ) ellipsoid)</td>
<td>A</td>
<td>0.847</td>
<td>0.847</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.821</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.826</td>
<td>0.866</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.883</td>
<td>0.882</td>
</tr>
<tr>
<td>New Cup Geometry (hemisphere)</td>
<td>A</td>
<td>0.783</td>
<td>0.784</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.831</td>
<td>0.860</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.832</td>
<td>0.870</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.846</td>
<td>0.915</td>
</tr>
</tbody>
</table>
Figure 2.6. Optode positions for the MC simulations of the C size breast. A. Light source (red dots) and detector (blue dots) positions for the simulations of the original TiBS device geometry. B. Potential light source (red crosses) and detector (blue dots) positions for the selection of the number of optodes for the Cups design. C. Light source (red dots) and detector (blue dots) positions on the C cup for the final Cups design. All axes labeled in cm. D. Maximum intensity projection into the coronal plane on a logarithmic intensity scale showing the paths of detected light in the low-scattering breast for the C cup for a single emitting source.
2.3.3 Laser Module Design

The ray tracing simulations indicated that a conical shape for the base of the laser module cavity would maximize the light coupled into the tissue and that coupling would decrease approximately linearly with the internal surface area of the cavity, with no strong deviation from linearity dependent on the angle of the cone. Therefore for the final design the internal surface area of the cavity was minimized, restricted by the geometrical constraints of the laser diodes and the positioning of the laser modules on the cups, to a final value of 1770 mm$^2$.

**Figure 2.7** shows a cross-sectional view of the laser module cavity. Coupling efficiency of the assembled laser modules was measured and was found to be relatively high, with an average of 42% (range 14-65%) of the emitted laser power detected at the laser module aperture over all wavelengths. For lasers with a power at the aperture above the maximum permissible exposure for skin and/or eye the laser output was hardware and software limited to a safe level.
Figure 2.7 Cross-sectional view of the laser module cavity, cut along the midline of the cavity. The interior diameter of the top hemisphere of the cavity is 25mm.
2.4 Conclusion

Although the choice of 13 wavelengths for the initial spectral reduction was essentially heuristic, they adequately maintained the predictive ability of the original broadband measurements both in identifying women with high MBD in the regular mammographic screening population and in differentiating between groups of pre-menopausal women with different breast cancer risks (younger vs. older women who had never been pregnant and parous vs. nulliparous older women). Further reduction of the spectral content was explored and only two of the 13 wavelengths had a significant impact on the AUC values for the ROC curves predicting the outcome of density ≥75% derived from the final logistic regression model when individually removed, indicating that it may be possible to use 12 or even fewer wavelengths while maintaining the MBD information content of the spectra (Figure 2.5). However, since there may be additional information relating to breast cancer risk in the optical spectra beyond that which correlates to MBD, all 13 wavelengths were included in the final design. Relative amounts of water, lipid and collagen are most important for determining MBD and the optical spectra of these tissue components have their main features in the 900-1050nm range however there may be important information about breast cancer risk or precancerous changes in the breast from the amounts of oxy- and deoxyhemoglobin, which are the dominant chromophores in the 600-800nm spectral region. Data analysis from OBS studies of women at high risk for developing breast cancer and of women who had a breast tissue biopsy are still ongoing and while OBS is still being developed as a breast cancer risk assessment tool it is practical to over-sample in the wavelength domain for the first prototype of the Cups OBS device.

The MC simulations of light propagation through breast tissue for the two device geometries indicate that the average optical sampling parameters for the original TiBS device can be matched in the Cups device with 2 light sources and 6 detectors per cup. A simple and robust design for the laser modules was devised, which allowed coupling of light from all 13 laser diodes to a single light source aperture. Relatively high light through-puts to the aperture were obtained (14-65%) and coupling efficiency may also be improved by optimization of the technique for creating the aperture in the diffusely reflective coating on the glass surface of the
laser diode cans. Currently the laser diode surfaces are painted over along with the aluminum cavity and a small (~1mm diameter) area of the paint in the centre of the glass cover over the emitting area is manually removed using a needle after each coat of paint. The aluminum cavity of the modules acts as a sufficient heat-sink for the laser diodes as the lasers are only on for short intervals (~10ms per laser) so no active cooling was necessary.

The implementation of the OBS device as a low-cost, portable device that does not rely on a trained operator for positioning should give more repeatable measurements and allow for easier implementation of large-scale studies in different populations. A first prototype of the Cups OBS device has been built and a trial comparing measurements with the original TiBS device to measurements with the Cups device is currently underway. The study population includes pre- and post-menopausal women and has women at average risk and at high risk for breast cancer based on the Gail score for breast cancer risk. [31] Correlation between the spectra from the two devices as well as the ability to predict mammographic density from the OBS scores will be evaluated. If there is a strong correlation between the spectra with the two devices, indicating that the average breast optical properties measured by the two devices are similar, and if the ability to predict mammographic density from OBS scores is not significantly decreased with the 13 wavelength Cups system for the in vivo data, then further reduction of the spectral content can be explored.
3 Optical Assessment of Mammographic Breast Density by a 12-Wavelength versus a Continuous-Spectrum Optical Spectroscopy Device

This chapter describes the evaluation of the laser-based, portable optical spectroscopy device in comparison with the continuous-spectrum optical spectroscopy device from which it was developed. The two devices were compared on their ability to predict mammographic breast density. This work has been published in the Journal of Biophotonics and is reused here, with minor modifications, with permission from the publishers. [141]

3.1 Introduction

Mammography is the gold standard technology for breast cancer (BC) screening and most high-income countries have mammographic screening programs. The specific guidelines for mammographic screening, however, are controversial with different organizations having conflicting recommendations, particularly for the age of screening onset and the screening frequency. [37, 127, 128, 142, 143] Most screening programs are standard for the whole female population and entrance is based on age, except in the case of the presence of strong risk factors which only apply to a small percentage of the population such as the BRCA 1/2 genetic mutations or radiation to the chest at a young age. [3, 13, 15, 132] While age is the strongest risk factor for BC, tailoring screening based on other risk factors in addition to age may allow for a more efficient use of the mammography infrastructure, particularly in economically depressed areas as well as in middle- and low-income countries. Ways to personalize screening programs using additional risk information are under investigation. [18, 27, 144] Risk factors used for the personalization of screening should ideally be applicable population-wide, easy to obtain, objective and independent of age. Mammographic breast density (MBD) is one of the strongest BC risk factors, and it is applicable population-wide.[44, 54, 133, 145, 146] MBD also represents a modifiable risk factor, changing with aging, in response to reproductive history, (e.g. pregnancy or menopause), or in response to interventions such as hormone treatment with Tamoxifen. Therefore MBD information may be useful both in assessing risk and in evaluating response to interventions. [47-49, 51, 147, 148] However, MBD as a risk factor is
only available once a woman has had her first mammogram and, thus, already entered screening. The source of contrast in MBD is the differential x-ray attenuation by adipose versus glandular and connective tissue and thus is an objective, quantifiable measure. The tissue structures giving rise to MBD also have different light scattering and absorption properties throughout the red and NIR optical spectrum. Thus, optical breast spectroscopy (OBS) has been explored as a method for determining MBD without using mammography. Studies using a spectrometer-based trans-illumination breast spectroscopy (TiBS) device demonstrated that women with high MBD could be identified with good sensitivity and specificity, percent mammographic density (MPD) can be predicted from optical spectra, and changes in breast composition in response to BC risk-modifying interventions can be detected over time [112, 113, 116, 137]. Since OBS poses no radiation risk and is safe for frequent use at any age, it is a promising tool both for pre-screening and personalizing screening programs (both age at entry and screening frequency) by identifying women at higher risk, and for monitoring the response to risk-modifying interventions. This could be especially helpful for high-risk younger women, for whom interventions could be most effective but for whom mammography is least effective and poses the highest risk. OBS also has the potential to be a low-cost technology and provide clinically relevant information without the need for interpretation by a radiologist, making it particularly useful in low- or middle-income countries where there is reduced BC screening infrastructure and an insufficient number of radiologists. However, the TiBS device used in past studies, described in detail previously, has various drawbacks which make it unsuitable for use in large-scale, multi-center trials and ultimately clinically. [120, 135, 136, 149] The key elements of the TiBS device are a broad-band halogen bulb as a source and a high-throughput CCD-based spectrometer detector, which require a complicated instrument calibration. The TiBS device also requires a well-trained operator who selects and records various instrument and participant parameters to enable the derivation of quantitative transmission spectra. This creates a risk of data-recording errors and inter-operator variability affecting data interpretation and analysis. Additionally, the lack of standardized source and detector positions makes simulations of light propagation difficult as a large number of possible geometries need to be modeled. Lastly, the spectrometer is expensive and also large and fragile, so the device is not portable.
A redesign of the TiBS device was undertaken with the idea of creating a device that is portable, could run off batteries, is lower cost, has standardized source, detector, and tissue geometries, and requires minimal operator interaction and training. Hence, a new version of the device – called the ‘Cups’ device – was developed. The goal of this study was to evaluate the modified Cups device in comparison with the original, spectrometer-based TiBS device. The TiBS and Cups devices were compared on their ability to identify women with high MBD and to predict mammographic percent density (MPD) based on the optical attenuation spectra.

3.2 Materials and Methods

3.2.1 Study population

Participants were recruited from within an ongoing study at Princess Margaret Cancer Centre designed to monitor breast composition changes over time using the original TiBS device. That study comprised women at high risk for BC – recruited from local high-risk screening programs, which are part of the Ontario Breast Screening Program and have standardized entry requirements – and women with low or average risk based on Gail score (Gail score < 1.1 at study entry). [3] Participants were invited to participate in the current study as an extension of their participation. Inclusion criteria were no prior BC, no bilateral breast biopsy or fine needle aspiration within 1 year of the study’s start, no bilateral mastectomy, lumpectomy or cosmetic breast alteration (reduction or augmentation), no previous or current chemotherapy or prevention therapy, at least 3 years since last pregnancy at the start of the study, and ability to provide informed consent. This study was approved by the Research Ethics Board of the University Health Network. Of the 375 participants in the ongoing TiBS study, 271 had expressed interest in additional OBS studies and were contacted to participate here. Of these, 160 did not respond, 2 did not qualify, 34 declined, and 75 agreed to participate. Of the 75 who agreed to participate, 69 were successfully scheduled and completed one or two visits. Of the 69 participants, 60 had a recent mammogram available and underwent optical measurements with both devices and were included in the analysis here. One woman had 3 previous
lumpectomies of the left breast, and so measurements were only conducted on her right breast. Therefore 119 breasts were included in the final analysis below.

The participants’ neck, waist and hip circumferences, weight and height were measured by the study coordinator. Body mass index (BMI) was calculated from the participants’ weight and height [kg m^{-2}]. Information about the study population is summarized in Table 3.1. Study visits were carried out from May through September 2015.
### Table 3.1. Description of study population

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD (range)</td>
<td>56.3 ± 7.4 (29-73)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg m$^2$), mean ± SD (range)</td>
<td>25.5 ± 5.5 (17.6-48.2)</td>
<td></td>
</tr>
<tr>
<td>Menopausal status</td>
<td>21 Pre</td>
<td>39 Post</td>
</tr>
<tr>
<td>Risk Group</td>
<td>40 Control</td>
<td>20 High Risk</td>
</tr>
<tr>
<td>Mammographic Density</td>
<td>8 &gt;50%</td>
<td>19 &gt;25%,≤50%</td>
</tr>
</tbody>
</table>
3.2.2 Design of Cups device

The Cups device has 4 cup sizes, 2 laser modules which insert into the selected cup, and 6 detectors per cup (one of which was encased in a removable housing and was moved between cups for this prototype device). The device, depicted in Figure 3.1, contains silicon photodiodes with a 33.6 mm² area and built-in preamplifier (S8746-01, Hamamatsu Corp, Hamamatsu, Japan). The light source wavelengths, source and detector positions and cup sizes selection has been described previously and was based on data from previous studies using the spectrometer-based TiBS device. [112, 117, 122] The source-detector distances for each cup are listed in Table 3.2, and an interior view of a cup showing the positions of the sources and detectors is shown in Figure 3.2. Each laser module contained 12 diode lasers at 658, 682, 733, 755, 806, 829, 877, 895, 908, 940, 980, and 1052 nm respectively. While the original wavelength selection also included a 785 nm laser diode, the diode was unreliable and hence not included in this study. Lasers, activated in sequence, were driven by an LDTC 2/2 E laser driver (Wavelength Electronics Inc., Bozeman, MT, USA). An internal photodetector in each laser module (FDS100, Thorlabs Inc., Newton, NJ, USA) monitored the output of the lasers. Data acquisition from all detectors was via an NI USB-6251 DAQ card (National Instruments Corp, Austin, TX, USA), which also controlled the laser driver, the switching circuitry for the lasers and the power supplies. The cups were designed using Solidworks (Dassault Systèmes SolidWorks Corporation, Waltham, MA) and 3D-printed in black PC-ABS.
Figure 3.1. A. Schematic of the Cups device, which comprises a cup with 6 built-in detectors which fits over the breast, 2 laser modules which insert into the cups, the instrument box, which includes power supplies, the laser switching relays, and the data acquisition card, and the computer. B. Photo of the first prototype of the Cups device used for this study which shows 1. one of the four sizes of cup, 2. the laser modules, 3. the instrument box, and 4. the computer.
Table 3.2. Source-detector distances and radii for the four cups.

<table>
<thead>
<tr>
<th>Cup*</th>
<th>Source-Detector Distances (cm)†</th>
<th>Radius (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a  b  c  d  e  f</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.1 3.2 4.7 5.7 6.7 7.1</td>
<td>5.0/3.0‡</td>
</tr>
<tr>
<td>2</td>
<td>2.1 3.3 4.7 5.9 6.8 7.3</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>2.9 4.2 5.9 7.3 8.3 8.9</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>3.9 5.1 6.8 8.3 9.4 10.0</td>
<td>6.9</td>
</tr>
</tbody>
</table>

* cups are numbered from smallest to largest
† source-detector distances are labeled as shown in Figure 3.2
‡ all cups are hemispherical except the smallest cup, which is flattened along the transverse plane
Figure 3.2. View of the interior of a cup with sources shown as red circles and detectors as blue squares. Source-detector distances are labeled alphabetically from shortest to longest. The corresponding distances for each cup are listed in Table 3.2.
3.2.3 Optical Measurements

Participants were scheduled for two visits, and 43 of the 60 participants completed both. At each visit, the breast optical properties were measured using both OBS devices – the original spectrometer-based TiBS device and the prototype Cups device with fixed positions for the detectors and laser modules. For both devices, measurements were made in the dark with the participant seated in an upright position.

The TiBS device and its measurement protocol have been previously described in detail. [113, 120, 121, 136] Instrument throughput was validated using a transmission reference (3.5 cm thick white ultra-high-density polyurethane, Gigahertz Optics, Munich, Germany) and measurement of the dark signal as a function of CCD integration time before and after the participant measurements. As in all previous studies with the TiBS device, the optical properties were measured at 4 positions on each breast at least 2cm from the chest wall and 2cm from the breast edge – two along the midline from the chest wall to the nipple and one each on the lateral and medial sides. The source-detector distance at each position was recorded. TiBS attenuation spectra were calculated according to Equation 3.1. Participant ($S_p$) and reference ($S_{ref}$) spectra were corrected for the time-matched dark signal ($S_{dark}$) and then divided by their corresponding integration times ($t$). The negative log of the resulting spectrum divided by the corrected reference spectrum gives the attenuation spectrum relative to the reference. Adding the known attenuation spectrum of the reference, $A_{ref}$, and then correcting for the source-detector distance, $d$, provides an absolute attenuation spectrum, $A_{i\lambda}$, in units of optical density per cm (OD/cm).

$$A_{i\lambda} = \left(-\log\left(\frac{(S_{p,\lambda}-S_{dark,p,\lambda})/t_p}{(S_{ref,\lambda}-S_{dark,ref,\lambda})/t_{ref}}\right) + A_{ref,\lambda}\right)/d$$ \hspace{1cm} \text{Equation 3.1}

Usable spectra for each breast (up to 4) were averaged, excluding those with insufficient signal or with CCD errors, to give a single spectrum per breast.

For the Cups device measurements, women select the size of cup giving a snug fit, so the breast surface is in contact with all detectors. A silicone hemisphere (P4 platinum-cure silicone, Eager Plastics, Chicago, IL), doped with titanium dioxide as a scattering agent and black India ink as an
absorbing agent, served as an instrument throughput reference through which spectra were collected before and after each participant visit. The participant placed the cup over the breast and held it in place for approximately 30 seconds while the 12 lasers in each of the two laser modules were cycled on and off in sequence. The laser driver current was adjusted to just over the lasing threshold, according to the manufacturer’s specifications, followed by a high current, (either the maximum laser current or the current yielding the maximum permissible power based on laser safety standards, whichever was lower). Then the current was ramped up from zero to the high power value in increments of 10%. Between each current setting, the lasers were turned off to collect dark readings from the detectors. The attenuation spectra for all source-detector pairs in the Cup device are calculated according to Equation 3.2. For each wavelength, the detector voltage \( V_{\text{det}} \) and the laser module detector voltage \( V_{\text{lmdet}} \) are determined at the maximum laser power not saturating either the detector or the reference detector in the laser module. The corresponding dark voltages \( V_{\text{dark, det}} \) and \( V_{\text{dark, lmdet}} \) are subtracted, and the negative log of the ratio of the two detector voltages is obtained. To account for the voltage to optical power conversion, the wavelength- and laser module-dependent ratio value \( R_{\text{lmdet}} \) is applied. This gives the true attenuation spectrum which is then divided by the source-detector distance resulting in the units of OD/cm.

\[
A_{\lambda} = \left( -\log\left(\frac{V_{\text{det},\lambda} - V_{\text{dark, det}}}{V_{\text{lmdet},\lambda} - V_{\text{dark, lmdet}}}\right) + R_{\text{lmdet, \lambda}} \right)/d
\]

Equation 3.2

The \( R_{\text{lmdet}} \) value includes both the voltage to optical power conversion ratio for the detectors, which is wavelength-dependent but the same for all detectors, and the ratio of the voltage detected at the internal reference detector in the laser module to the optical power at the aperture of the laser module, which depends on the laser, the wavelength-dependent sensitivity of the reference detector and the optical throughput of the laser module cavity at that wavelength. The \( R_{\text{lmdet}} \) values were empirically determined, via a rearrangement of Equation 3.2, by calculating the absolute attenuation spectra of the silicone references for the Cups device \( (A_{\text{ref}}) \) using the TiBS device as per Equation 3.1 and then subtracting the corresponding voltage-based attenuation spectra measured by the cups device (Equation 3.3).
The reference spectra for the Cups device were stable over time, (for 80% of the detector-wavelength combinations the slope of the attenuation value over time was not significantly different from zero and for the remaining 20% of values the drift was small – millivolts per day or smaller while maximum signal was 10V – and showed no consistent trends across wavelengths or detectors), so average \( R_{\text{det}} \) values from the entire study period were used in the data analysis.

For the Cups device, up to 12 spectra were collected per breast. Spectra with insufficient signal or detector saturation were discarded, and the remaining spectra for each breast averaged to give a single spectrum per breast.

### 3.2.4 Quantification of MBD

Recent digital mammograms were collected for the 60 participants analyzed here. Percent mammographic breast density (MPD) was calculated from the cranio-caudal view mammograms using two different density thresholding programs – Cumulus, which is semi-automated, and LIBRA which is fully-automated and reader-independent. [57, 60, 62, 150] MPD, as determined by LIBRA, has been validated against Cumulus-determined MPD, the current “gold standard” for thresholding programs, and has been shown to be an independent BC risk factor with odds ratios similar to other fully-automated thresholding programs. [58, 62] For the Cumulus reads, two readers – one experienced (LL) and one novice (EJW) – did 2 repeat reads of all mammograms, with each repeat broken into 3 batches. MPD values were averaged across both readers and all repeats. For participants for whom the OBS measurements were approximately midway between two mammograms, their MPD values were the average of both mammogram dates. Intra-reader, inter-reader, and inter-program (LIBRA vs. Cumulus) correlations were calculated to evaluate the quality of the MPD measurements, acting here as the gold standard.

\[
R_{\text{ldet},\lambda} = A_{\text{ref},\lambda} d + \log\left(\frac{V_{\text{ref},\lambda} - V_{\text{dark},\text{ref}}}{V_{\text{im,ref},\lambda} - V_{\text{dark,im,ref}}}\right)
\]

Equation 3.3
3.2.5 Correlation between spectra

At each of the Cup device wavelengths, the correlation between the corresponding attenuation values of the TiBS and Cups spectra over all measurements was calculated to evaluate the similarity between the spectra obtained using the two devices.

3.2.6 Principal Components Analysis (PCA)

A principal components analysis was executed to reduce the dimensionality of the spectral data prior to logistic regression to predict high mammographic density. PCA is a commonly used data analysis tool for spectral analysis and has been employed in past analyses of the TiBS device OBS data. [112, 120, 122, 135, 137] PCA was performed separately on the spectra from the TiBS and the Cups devices, using the average distance-corrected spectrum for each breast. Before implementing PCA, the mean spectra was calculated and subtracted from all spectra, so the derived principal components (PCs) are mutually orthogonal and therefore give independent principal component scores.

3.2.7 Logistic Regression to Identify Women with High Mammographic Density

Using the derived PC scores, univariate and multivariate logistic regression was performed to assess the ability to identify the women with high mammographic density. For the logistic regression analysis, mammographic breast density (MBD) was treated as a dichotomous variable, identifying women with high density (≥50% MBD) versus women with low density (<50% MBD). Age, menopausal status, and BMI were considered as potential confounding variables, and correlation analysis was executed between the PC scores, MPD, BMI and age to determine the relationship between the variables. Correlations were also evaluated as a function of menopausal status (post-menopausal vs. pre-menopausal). The calculated probabilities from the univariate and multivariate analyses were used to generate receiver operator characteristic (ROC) curves and derive the area under the curve (AUC) values to evaluate how well each model predicted high MBD.

The robustness of identifying women with high MBD using optical attenuation spectra was assessed by using PC scores calculated from the TiBS and Cups spectra using the PC load vectors
derived from spectra from the previous study comparing TiBS PC scores and MBD. [112] To generate those load vectors, two different PCAs were performed on the previous study data with the wavelength ranges limited to that of the current version of the TiBS device and the 12 wavelengths of the Cups device. The multivariate logistic regression analysis was then repeated using the best final models from the previous study, which included PC3, PC4, menopausal status and BMI. ROC curves were generated and AUC values were calculated to evaluate how well the model predicted high MBD.

3.2.8 Partial Least Squares (PLS) Regression

PLS regression was employed to evaluate the ability of the spectra from each device to predict mammographic density on an interval scale. MPD values determined by the density thresholding programs act as ‘gold standard’ and the targets for this analysis. The left and right breasts were treated as separate events, and the average spectrum was used for each breast. Spectra were randomly divided into training and validation subsets by participant ID so that both spectra from each participant were included in the same subset, as there is a correlation between the left and right breast spectra in most women. This avoids bias from validating with spectra which may be correlated to the training spectra. 75% (n_t = 45) of the women were included in the training set and 25% (n_v = 15) in the validation set. The correlation between the PLS regression-predicted MPD values and the ‘gold standard’, mammogram-based MPD values was calculated. The PLS regression was repeated with different random subsets to provide an estimate of the correlation strength variability.

3.3 Results

3.3.1 Optical Measurements

Participant reaction to the Cups device was positive with all participants indicating it was equally or more comfortable compared to the original TiBS device. Measurement times were shorter with the modified Cups device than the original TiBS device. Based on the spectrometer integration times, the mean data collection time for the TiBS device for all four positions on a single breast was 151 sec (minimum 25 sec, maximum 300 sec). For the Cups measurements, the spectra collection time was fixed at 12 sec per breast. Some participants indicated that it
was difficult to feel whether the breast was in contact with all detectors and the source apertures of the cups as there was an insufficient sensation on the breast surface. Templates cups, which were the same shape as the device cups but with holes at the source and detector locations, were made to allow women to try on the different sizes and visually confirm the size is providing best optode contact.

For the shorter source-detector distances (< 4.5 cm) the detector signal often saturated (~90% saturated), even at the lowest laser powers, and for the longest distances (>9 cm) there were many instances of insufficient signal (~80% had insufficient signal). Thus, of the 12 potential spectra from all the source-detector pairs, only some (mean n = 5, range 1-9) were averaged to give the final breast spectrum for a single measurement. For the TiBS device, the mean number of spectra averaged was 3.8, (range 2-4).

3.3.2 Quantification of MPD

Inter-read correlations (and 95% confidence intervals) for MPD derived using Cumulus were $r=0.79$ (0.71-0.85) for the trained reader (LL) and $r = 0.76$ (0.67-0.83) for the novice reader (EJW). The inter-reader correlation was $r=0.83$ (0.77-0.88) for the two readers average reads. When comparing the MPD values between the two thresholding software platforms, the correlation coefficient was $r=0.84$ (0.78-0.89). There was a strong correlation between the left and right breast density of each participant with both software platforms – $r=0.87$ (0.79-0.92) for Cumulus and $r=0.91$ (0.85-0.94) for LIBRA. All correlations were significant with $p<0.001$. MPD was significantly inversely correlated with age and with BMI when derived using Cumulus or LIBRA software ($r = -0.26$, $p = 0.005$ and $r = -0.25$, $p = 0.006$ respectively for age and $r = -0.51$, $p < 0.001$ and $r = -0.55$, $p < 0.001$ respectively for BMI).

MPD values were not normally distributed. The median (and range) for the MPD values calculated with Cumulus and with LIBRA software were 18.2 % (1.5-58.4 %) and 21.4 % (1.6-66 %) respectively. The MPD and MBD values calculated with LIBRA were used exclusively in further analyses since LIBRA is completely reader-independent.
### 3.3.3 Correlation between spectra

The correlations between the average TiBS and Cups attenuation spectra for each participant can be executed only at the Cups wavelengths. The correlations at these wavelengths were significant (p<0.001) with an average correlation coefficient $r = 0.66$ (range $r = 0.51$-$0.77$). However, the linear regression slopes of the of the Cups attenuation values vs. the TiBS attenuation values were all significantly less than 1 for all wavelengths, with an average slope of $m = 0.62$ (range $m = 0.48$-$0.74$). **Figure 3.3** shows typical attenuation spectra for two breasts measured with both devices.

### 3.3.4 Principal Components Analysis (PCA)

Here, as for past analyses, four principal components capture most of the variance in the spectra and each spectrum’s PC scores ($t_{in}$) were calculated from the 4 load vectors ($p_n$) of the 4 derived PCs. These first 4 PCs captured 99.06% (94.13, 2.77, 1.52, and 0.63%), and 99.82% (95.96, 2.28, 1.22, and 0.36%) of the variance for the Cups and the TiBS device spectra respectively. **Figure 3.4** shows the PC eigenvalues (which are proportional to the variance captured by each PC) for both analyses. Each mean-centred spectrum, $A_{mi}$, can be expressed as a sum of the PC scores multiplied by their corresponding load vectors plus a residual error, $\epsilon$.

$$A_{mi} = t_{i1}p_1 + t_{i2}p_2 + t_{i3}p_3 + t_{i4}p_4 + \epsilon$$

**Equation 3.4**

Principal component loads from TiBS device were similar to those from the previous study comparing TiBS spectra with mammographic density (**Figure 3.5**).

There was a significant correlation between the scores for PC 1, 3 and 4 and MPD for the PC scores derived from the TiBS device spectra ($r = -0.729$, $p < 0.001$; $r = -0.297$, $p = 0.001$; $r = -0.261$, $p = 0.004$ respectively). (**Table 3.3**) When separated by menopausal status (post-menopausal vs. pre-menopausal), there were significant correlations between the TiBS PC scores and MPD for PC 1 and 3 for the post-menopausal women ($r = -0.711$, $p < 0.001$ and $r = -0.580$, $p < 0.001$ respectively). For the pre-menopausal women there were significant correlations between the TiBS PC scores and MPD for PC 1, 2 and 4 ($r = -0.616$, $p < 0.001$; $r = -0.381$, $p = 0.041$; $r = -0.461$, $p = 0.012$ respectively).
Figure 3.3. Typical left (blue) and right breast (red) spectra measured with the TiBS (solid lines) and the Cups device (triangles). A. Average breast spectra for a participant with low (~5%) MBD. B. Average breast spectra for a participant with high (~56%) MBD.
Figure 3.4. PC eigenvalues from A. the Cups spectra PCA and B. the TiBS spectra PCA. The y-axis is broken to show more clearly the eigenvalues for PC>1 since in both cases PC1 has an eigenvalue more than 30 times greater than the PC2 value. For both eigenvalue plots, there is a clear elbow in the curve above PC4.
Figure 3.5. Principal component loads from the PCA of spectra measured using the TiBS device (solid black lines), PC loads from the PCA of the Cups device spectra (blue xs), and PC loads from the PCA of spectra from a previous study comparing TiBS spectra with mammographic density (dotted red lines). A. PC 1 load, B. PC 2 load, C. PC 3 load, and D. PC 4 load.
Table 3.3. Correlation coefficients, r, with 95% confidence intervals (CI) between PC scores and MPD for all women and by menopausal status.

<table>
<thead>
<tr>
<th>PC Score</th>
<th>All Women</th>
<th>Pre-menopausal Women</th>
<th>Post-menopausal Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r 95% CI</td>
<td>r 95% CI</td>
<td>r 95% CI</td>
</tr>
<tr>
<td>TIBS PC1</td>
<td>-0.729</td>
<td>-0.804 -0.633 &lt;0.001</td>
<td>-0.616 -0.802 -0.323 &lt;0.001</td>
</tr>
<tr>
<td>TIBS PC2</td>
<td>-0.164</td>
<td>-0.334 0.017 0.075</td>
<td>-0.381 -0.656 -0.017 0.041</td>
</tr>
<tr>
<td>TIBS PC3</td>
<td>-0.297</td>
<td>-0.453 -0.123 0.001</td>
<td>-0.154 -0.493 0.225 0.425</td>
</tr>
<tr>
<td>TIBS PC4</td>
<td>-0.261</td>
<td>-0.422 -0.085 0.004</td>
<td>-0.461 -0.708 -0.114 0.012</td>
</tr>
<tr>
<td>Cups PC1</td>
<td>-0.486</td>
<td>-0.612 -0.335 &lt;0.001</td>
<td>-0.311 -0.608 0.063 0.101</td>
</tr>
<tr>
<td>Cups PC2</td>
<td>-0.404</td>
<td>-0.545 -0.242 &lt;0.001</td>
<td>-0.497 -0.730 -0.159 0.006</td>
</tr>
<tr>
<td>Cups PC3</td>
<td>0.191</td>
<td>0.011 0.359 0.038</td>
<td>0.360 -0.008 0.642 0.055</td>
</tr>
<tr>
<td>Cups PC4</td>
<td>0.022</td>
<td>-0.159 0.201 0.814</td>
<td>0.088 -0.287 0.441 0.648</td>
</tr>
</tbody>
</table>
For the PC scores derived from the Cups spectra PCA, there was a significant correlation between the scores for PC 1, 2 and 3 and MPD ($r = -0.486$, $p < 0.001$; $r = -0.404$, $p < 0.001$; $r = 0.191$, $p = 0.038$ respectively). When separated by menopausal status (post-menopausal vs. pre-menopausal), there were significant correlations between the Cups PC scores and MPD for PC 1, 2 and 3 for the post-menopausal women ($r = -0.404$, $p < 0.001$; $r = -0.343$, $p = 0.002$; $r = 0.250$, $p = 0.027$ respectively). For the pre-menopausal women there were significant correlations between the Cups PC scores and MPD for PC2 and 3 ($r = -0.497$, $p = 0.006$; $r = 0.360$, $p = 0.055$ respectively).

Different PCs being associated with MBD in pre- and post-menopausal women was seen previously and is unsurprising as there are multiple optical chromophores, which can be separately distinguished, which contribute to the "dense" signal in an OBS measurement and since the primary sources of the density are different in the two populations. In pre-menopausal women, milk glands and milk ducts are strong contributors to density, whereas, in post-menopausal women, who have at least begun the process of lobular involution, connective tissue is likely the dominant source of density.

### 3.3.5 Logistic Regression to Identify Women with High Mammographic Density

Since none of the women had MPD $\geq 75\%$, a threshold of MPD $\geq 50\%$ for high mammographic density was used. There were 13 individual breasts out of 119 breasts measured which had a MPD $\geq 50\%$. Therefore 11% of breasts were classified as having high MBD.

The results of the univariate logistic regression are summarized in **Table 3.4**. It shows the odds ratios (OR) with 95% confidence intervals (CI) for the odds ratios for the PC scores for both the TiBS and Cups spectra, age, BMI and menopausal status (pre- vs. post-menopausal). For the PC scores, age, and BMI the OR and associated 95% CI are given over the inter-quartile (IQR) range of that variable. The OR represent the odds of $\geq 50\%$ dense tissue vs. the odds of $< 50\%$ dense tissue for an increase in the respective variable over the IQR (from the first quartile to the third). The $p$-value for the logistic regression and the area under the curve (AUC) of the receiver operator characteristic curve (ROC curve) are also given. The OR were significantly different from 1 for all four of the PC scores generated from the PCA of the TiBS spectra. For the Cups
spectra PC scores, the OR were significantly different from 1 for PC 1-3 scores. The OR were significantly different from 1 for BMI and menopausal status but not for age.

The final multivariate logistic regression model for identifying breasts with high (≥ 50%) MBD using the PC scores from the TiBS spectra included the scores for all four PCs, BMI and menopausal status. The AUC (and 95% CI) for the final model was 0.97 (0.94-1.00), p < 0.001. The final model for the PC scores from the Cups spectra included the scores for the first three PCs, BMI and menopausal status since the scores from PC 4 were not significantly associated with high mammographic density either independently or when adjusted for other covariates. The AUC (and 95% CI) for the final model was 0.89 (0.83-0.95), p < 0.001.

When using the PC load vectors derived from the previous density study spectra and the corresponding final multivariate models from that study, the AUC (and 95% CI) for the ROC curve calculated from the TiBS spectra was 0.92 (0.87-0.97), p < 0.001, and 0.87 (0.79-0.95), p < 0.001, for the Cups spectra.
Table 3.4. Results of univariate logistic regression analysis for each PC score from both the TiBS and the Cups spectra PCAs, age and body mass index (BMI) (n = 119).

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>OR - 95% confidence intervals</th>
<th>IQR</th>
<th>p</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lower</td>
<td>upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TiBS PC1 Score</td>
<td>0.05</td>
<td>0.01</td>
<td>0.21</td>
<td>9.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TiBS PC2 Score</td>
<td>0.60</td>
<td>0.37</td>
<td>0.98</td>
<td>1.06</td>
<td>0.042</td>
</tr>
<tr>
<td>TiBS PC3 Score</td>
<td>0.45</td>
<td>0.23</td>
<td>0.90</td>
<td>0.840</td>
<td>0.024</td>
</tr>
<tr>
<td>TiBS PC4 Score</td>
<td>0.22</td>
<td>0.07</td>
<td>0.69</td>
<td>0.698</td>
<td>0.009</td>
</tr>
<tr>
<td>Cups PC1 Score</td>
<td>0.31</td>
<td>0.14</td>
<td>0.66</td>
<td>1.14</td>
<td>0.003</td>
</tr>
<tr>
<td>Cups PC2 Score</td>
<td>0.26</td>
<td>0.12</td>
<td>0.67</td>
<td>0.195</td>
<td>0.004</td>
</tr>
<tr>
<td>Cups PC3 Score</td>
<td>1.62</td>
<td>1.04</td>
<td>2.52</td>
<td>0.0965</td>
<td>0.031</td>
</tr>
<tr>
<td>Cups PC4 Score</td>
<td>0.95</td>
<td>0.58</td>
<td>1.55</td>
<td>0.0640</td>
<td>0.035</td>
</tr>
<tr>
<td>Age</td>
<td>0.58</td>
<td>0.26</td>
<td>1.28</td>
<td>11.0</td>
<td>0.175</td>
</tr>
<tr>
<td>BMI</td>
<td>0.07</td>
<td>0.01</td>
<td>0.33</td>
<td>4.92</td>
<td>0.001</td>
</tr>
<tr>
<td>Menopausal Status</td>
<td>0.28</td>
<td>0.09</td>
<td>0.93</td>
<td>-</td>
<td>0.037</td>
</tr>
</tbody>
</table>
3.3.6 Partial Least Square (PLS) Regression

Correlation coefficients with 95% confidence intervals for the association between MPD read using LIBRA, and MPD predicted using PLS regression of the TiBS or Cups spectra for both the training and validation sets are displayed in Table 3.5. The LIBRA MPD and the PLS-predicted MPD values were significantly correlated for all data sets. The correlation was stronger for the MPD values predicted from the TiBS spectra ($r = 0.90$ for the training set, $r = 0.83$ for the validation set) than for the MPD values predicted from the Cups spectra ($r = 0.75$ for the training set, $r = 0.62$ for the validation set). The results of the linear regression analysis of the PLS-predicted MPD vs. the LIBRA MPD showed that the slopes were not significantly different from 1 over all repeats of the PLS-regression for both devices and both the training and validation sets. PLS regression using the Cumulus MPD values as the targets gave similar results, which are summarized in Table 3.6.
Table 3.5. Average correlations between MPD read using LIBRA and MPD predicted using PLS regression of TiBS or Cups spectra. Values are the averages for 30 random repeat assignments of the spectra into training and validation sets. n = 89 or 90 spectra (45 women) for the training sets, n = 29 or 30 spectra (15 women) for the validation sets.

<table>
<thead>
<tr>
<th>Spectra</th>
<th>Data Set</th>
<th>r</th>
<th>r - 95% confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>lower</td>
<td>upper</td>
</tr>
<tr>
<td>TiBS</td>
<td>training</td>
<td>0.90</td>
<td>0.85</td>
<td>0.93</td>
</tr>
<tr>
<td>TiBS</td>
<td>validation</td>
<td>0.83</td>
<td>0.67</td>
<td>0.92</td>
</tr>
<tr>
<td>Cups</td>
<td>training</td>
<td>0.75</td>
<td>0.65</td>
<td>0.83</td>
</tr>
<tr>
<td>Cups</td>
<td>validation</td>
<td>0.62</td>
<td>0.34</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 3.6. Average correlations between MPD read using Cumulus and MPD predicted using PLS regression of TiBS or Cups spectra. Values are the averages for 30 random repeat assignments of the spectra into training and validation sets. n = 89 or 90 spectra (45 women) for the training sets, n = 29 or 30 spectra (15 women) for the validation sets.

<table>
<thead>
<tr>
<th>Spectra</th>
<th>Data Set</th>
<th>r</th>
<th>r - 95% confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>lower</td>
<td>upper</td>
</tr>
<tr>
<td>TiBS</td>
<td>training</td>
<td>0.88</td>
<td>0.83</td>
<td>0.92</td>
</tr>
<tr>
<td>TiBS</td>
<td>validation</td>
<td>0.74</td>
<td>0.53</td>
<td>0.87</td>
</tr>
<tr>
<td>Cups</td>
<td>training</td>
<td>0.75</td>
<td>0.64</td>
<td>0.83</td>
</tr>
<tr>
<td>Cups</td>
<td>validation</td>
<td>0.62</td>
<td>0.35</td>
<td>0.80</td>
</tr>
</tbody>
</table>
3.4 Discussion

MBD is a well-established, strong BC risk factor and the ability to identify women with high MBD and to predict MPD from optical spectra has been previously demonstrated for the TiBS device. [112, 113, 120, 135-137] Quantifying MPD, a modifiable risk factor, can become an important tool in future BC prevention research. [47, 51, 146, 147, 151, 152]

The ‘TiBS’ optical spectroscopy device, which has a broadband light source and a CCD spectrometer detector, has been under investigation by our group as a BC risk assessment and optical pre-screening tool. A modified ‘Cups’ version of the optical breast spectroscopy device was developed with the goal of making the device more portable, more robust and less operator-dependent while reducing data collection time. The purpose of this study was to evaluate the modified Cups version in comparison to the original TiBS research prototype device. The devices were compared on their ability to identify women with high mammographic breast density and to predict MPD from optical spectra.

Measurements with the Cups device were >12 times faster than with the TiBS device, even without considering the repositioning time between TiBS measurements.

On average, only 5 of the 12 source-detector pair spectra were usable for each breast measurement with the Cups device, usually due to detector saturation. Several approaches have been implemented to reduce saturation. Template cups were made to assist women in selecting the cup size which provided the best contact with the source and detector apertures, thus eliminating signal from the light that did not travel through the breast. For the TiBS device, the maximum attainable dynamic range is determined by the dynamic range of the analog-to-digital convertor (ADC) for the CCD spectrometer (for this TiBS device the ADC was 12-bit per pixel), the number of rows illuminated per wavelength (approximately 140), the integration time for the measurement (ranging from 100 to 4000ms), the intensity variation across the wavelength range of the light source (approximately a factor of 20), and the iris setting for the light source (adjusted between two settings varying in overall intensity by a factor of 10). Both integration time and iris setting were selected by the operator. The overall dynamic range for the TiBS device was about 7 orders of magnitude. For the Cups device, the dynamic range of the
device is determined by the dynamic range of the ADC for the detectors (the ADC for this version of the Cups device was 16-bit) and the optical power range of the laser diode modules. The algorithm for setting the laser power in the Cups device resulted in 11 power settings for each laser. While this should have resulted in a sufficient dynamic range, by relying on the threshold currents from the manufacturers’ specifications, some of these minimum laser driving currents did not exceed the laser emission threshold. As a result, some detectors saturated at the lowest powers above the actual lasing threshold and the smallest difference between the minimum and maximum laser power for any wavelength was a factor of 8.5. This led to an overall maximum attainable dynamic range of between 5 and 6 orders of magnitude, which is lower than for the TiBS device. An improved algorithm that actively searches for the optical power resulting in the highest signal without saturation for each detector at each wavelength has been implemented. The individual lasing threshold currents for each laser diode are also empirically determined. The active algorithm increases measurement times but the total spectral data collection time per breast (~40 sec) remains low compared to the TiBS device (~150 sec per breast). More tailored selection of the photodetector amplifier gain is required, particularly for the short source-detector distances in the small cups, to adjust detector sensitivity, so the full dynamic range of each detector is exploited. This has been implemented in the next generation device.

The average spectrum for each breast was used in this analysis since not all source-detector pairs gave usable data, with a variant number between breasts. Averaging over all positions on the breast will mask the heterogeneities that are present in some breasts. Also, depending on the coverage of the breast, intra-breast heterogeneities may be covered differently or not captured for some breasts and MBD may be over- or under-estimated if a smaller percentage of the breast volume is interrogated. Furthermore, since different volumes of the breast are interrogated for the different source-detector pairs, an optimal algorithm for predicting MPD from the optical spectra should likely include weighting to account for the different sampling volumes. Finally, the degree of asymmetry between the left and right breasts may be useful as a BC risk factor, and this could be more accurately assessed if the spectra are compared section-by-section between the two breasts. [106, 153] Including all positions separately in the
analysis when they are available, (when the previously-mentioned saturation issues have been adequately addressed), should improve the ability to predict MPD and BC risk.

The attenuation coefficients at the 12 Cups wavelengths were significantly correlated with those obtained by the TiBS device, with correlation coefficients ranging from 0.51 to 0.77. This is encouraging, considering the various sources of noise in the two devices, but not necessarily a required feature as it is the correlation between the Cups spectra and MBD/MPD that are of clinical interest. The slopes of the fits of attenuation coefficients from the Cups device vs. those from the TiBS device were significantly below one and, correspondingly, the overall amplitudes of the Cups spectra were lower than the TiBS spectra. This is likely a systematic effect due to the optical throughput calibration of the Cups device, which needs to be addressed if analyses quantifying the amount of each chromophore are desired. However, since the spectral shapes are retained here, as indicated by the strong correlation between attenuation values from the two devices, the amplitude difference does not affect the conclusions of this analysis.

Principal component loads for the spectra from the TiBS device closely matched those from a previous study comparing TiBS spectra with mammographic density (Figure 3.5). [112] The correlations between the PC loads for those two studies were strong and significant with correlation coefficients ranging from 0.865 to 0.956 (p < 0.001) (Table 3.7). Thus, despite the fact that fewer women participated in this study and no women were identified with very high MBD (≥75%), the spectral variances in the populations were similar. The load vectors for the Cups device were similar to the TiBS loads for PC 1-3; the correlations between the load vectors were strong and significant for PC 1 and 2 and approached significance for PC 3 (Table 3.7). However, as the spectra themselves were not perfectly correlated and since the Cups spectra contain fewer data points, the principal components were not expected to be the same.
Table 3.7. Correlations between PC load spectra for the TiBS and Cups devices and a previous study using a TiBS device correlating TiBS PC scores with mammographic density (TiBS Density).[112]

<table>
<thead>
<tr>
<th>Load Spectra</th>
<th>r (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiBS PC 1 vs. TiBS Density PC 1</td>
<td>0.956 (0.947, 0.963)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TiBS PC 2 vs. TiBS Density PC 2</td>
<td>0.924 (0.937, 0.909)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TiBS PC 3 vs. TiBS Density PC 3</td>
<td>0.960 (0.952, 0.967)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TiBS PC 4 vs. TiBS Density PC 4</td>
<td>0.865 (0.839, 0.887)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cups PC 1 vs. TiBS Density PC 1</td>
<td>0.807 (0.434, 0.944)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cups PC 2 vs. TiBS Density PC 2</td>
<td>0.664 (0.146, 0.896)</td>
<td>0.018</td>
</tr>
<tr>
<td>Cups PC 3 vs. TiBS Density PC 3</td>
<td>-0.556 (-0.857, 0.026)</td>
<td>0.060</td>
</tr>
<tr>
<td>Cups PC 4 vs. TiBS Density PC 4</td>
<td>0.251 (-0.378, 0.721)</td>
<td>0.432</td>
</tr>
<tr>
<td>Cups PC 1 vs. TiBS PC 1</td>
<td>0.791 (0.397, 0.939)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cups PC 2 vs. TiBS PC 2</td>
<td>0.789 (0.938, 0.394)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cups PC 3 vs. TiBS PC 3</td>
<td>-0.727 (-0.918, -0.264)</td>
<td>0.007</td>
</tr>
<tr>
<td>Cups PC 4 vs. TiBS PC 4</td>
<td>0.366 (-0.263, 0.777)</td>
<td>0.241</td>
</tr>
</tbody>
</table>
Both devices resulted in significant correlations between the PC scores and MBD. For the TiBS device-derived PC scores, PC 1, 3 and 4 scores were significantly associated with MBD as in the previous study when analyzing for all women (pre- and post-menopausal). [137] Not seen in the previous MBD study was that PC 2 scores were significantly correlated with MBD in pre-menopausal women. Also, in the previous study PC 4 was only significantly associated with MBD in the post-menopausal group whereas in this study PC 4 was significantly associated with MBD in pre-menopausal women. Since PC 4 only accounts for a small proportion of the spectral variance in the study populations (0.36% for the TiBS spectra, 0.35 % for the mammographic density study), this difference is unsurprising given the large difference in the population sample sizes and the correlation between the PC load vectors of the two studies was indeed weakest for PC 4 ($r = 0.865$).

For the Cups device PC scores, PC 1-3 scores were all significantly correlated with MBD when looking at the entire population. PC 2 and 3 scores also were significantly correlated with MBD when separated by menopausal status for both the pre- and post-menopausal groups. Cups PC 4 scores were not correlated with MBD for the entire study population or when separated by menopausal status.

Correlations between the load spectra and the spectra of known absorbers in the tissue were calculated to evaluate which tissue components contributed most to each of the PC loads (Table 3.8). As in all previous OS studies, PC 1 was negative and relatively flat across the entire spectrum, for both the PC derived from the TiBS and the Cups spectra, indicating it represents the overall attenuation. [112, 117, 119]. However, in both cases, it is also strongly and significantly inversely associated with the water absorption spectrum (Table 3.8). For both devices, the correlation between PC 1 scores and MPD was negative, indicating that higher density breasts attenuate light more overall and also contain more water. For the TiBS device, the PC 3 load spectrum was dominated by an inverse correlation with collagen, a positive correlation with HbO$_2$ and a negative correlation with Hb and also showed a significant positive correlation with lipid and negative correlation with water. The TiBS PC 4 load spectrum was dominated by inverse correlations with collagen, lipid, and Hb. Both the PC 3 and 4 scores were inversely associated with MPD, indicating that denser breasts have more collagen and tend
towards lower blood oxygen saturation, as anticipated. [112, 114, 154] For the Cups device, the PC 2 load spectrum was positively correlated with Lipid and HbO₂ and negatively correlated with Hb. The Cups PC 3 load spectrum was positively associated with water content. Cups PC 2 scores were negatively correlated, and PC 3 scores were positively correlated with MPD, confirming that, as expected, dense breasts have less lipid, lower blood oxygen saturation, and more water content.

In the previous studies comparing TiBS spectra with MBD, density was assessed in two ways. [112, 113] Classification of MBD by an expert radiologist on an ordinal scale into low (< 25%), medium (25% to <75%) or high (≥75% dense tissue area) categories was used in the logistic regression analysis. When assessing density on an interval scale, the percent mammographic density was determined using Cumulus software. There was a strong trend for the radiologist to assign higher MBD values than readers using the Cumulus software, resulting in a mean Cumulus-determined percent density of 55.9% (±10.6%) for women categorized as having ≥ 75% density (Figure 3.6). [112, 113] This justifies the use of a ≥ 50% LIBRA-determined density measure as the high MBD category.
Table 3.8. Correlations between TiBS and Cups PC load spectra and chromophore spectra

<table>
<thead>
<tr>
<th></th>
<th>Deoxy-hemoglobin</th>
<th>Oxy-hemoglobin</th>
<th>Lipid</th>
<th>Water</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (95% CI)</td>
<td>p</td>
<td>r (95% CI)</td>
<td>p</td>
<td>r (95% CI)</td>
</tr>
<tr>
<td>TiBS PC1</td>
<td>-0.27 (-0.36, -0.18)</td>
<td>0.000</td>
<td>-0.23 (-0.31, -0.14)</td>
<td>0.000</td>
<td>-0.04 (-0.14, 0.05)</td>
</tr>
<tr>
<td>TiBS PC2</td>
<td>0.45 (0.38, 0.52)</td>
<td>0.000</td>
<td>-0.70 (-0.75, -0.65)</td>
<td>0.000</td>
<td>-0.63 (-0.68, -0.57)</td>
</tr>
<tr>
<td>TiBS PC3</td>
<td>-0.47 (-0.54, -0.39)</td>
<td>0.000</td>
<td>0.49 (0.41, 0.55)</td>
<td>0.000</td>
<td>0.42 (0.34, 0.50)</td>
</tr>
<tr>
<td>TiBS PC4</td>
<td>-0.41 (-0.49, -0.33)</td>
<td>0.000</td>
<td>0.17 (0.08, 0.26)</td>
<td>0.000</td>
<td>-0.57 (-0.63, -0.51)</td>
</tr>
<tr>
<td>Cups PC1</td>
<td>0.19 (-0.43, 0.69)</td>
<td>0.58</td>
<td>-0.40 (-0.79, 0.23)</td>
<td>0.20</td>
<td>-0.49 (-0.83, 0.12)</td>
</tr>
<tr>
<td>Cups PC2</td>
<td>-0.63 (-0.88, -0.08)</td>
<td>0.03</td>
<td>0.74 (0.29, 0.92)</td>
<td>0.01</td>
<td>0.90 (0.67, 0.97)</td>
</tr>
<tr>
<td>Cups PC3</td>
<td>-0.01 (-0.58, 0.56)</td>
<td>0.97</td>
<td>0.00 (-0.58, 0.57)</td>
<td>0.99</td>
<td>-0.02 (-0.59, 0.56)</td>
</tr>
<tr>
<td>Cups PC4</td>
<td>-0.33 (-0.76, 0.30)</td>
<td>0.30</td>
<td>0.43 (-0.19, 0.80)</td>
<td>0.17</td>
<td>0.03 (-0.56, 0.59)</td>
</tr>
</tbody>
</table>
Figure 3.6. Box plot of percent mammographic density measured using Cumulus grouped by mammographic density category as assessed by an expert radiologist. Data is from previous studies comparing mammographic density and TiBS spectra [112, 113].
All 4 principal components showed significant odds ratios in the univariate logistic regression analysis of the TiBS spectra PC scores. Although the associations were not significant for PC 2 and 3 after individually adjusting for BMI, menopausal status, and age, (the odds ratios were no longer significantly different from 1 after adjusting for BMI), the final model predicting high mammographic density for this study included all four PC scores as well as BMI and menopausal status. The AUC for the resultant ROC curve for TiBS PC scores to identify high MBD was very high at 0.971 (0.938-1.000).

The final multivariate model for identifying high MBD from the Cups PC scores included PC 1-3 scores, BMI and menopausal status, all of which had odds ratios significantly different from 1 in the univariate analysis, although none of the three PC scores reached significance individually after accounting for BMI, age and menopausal status (p = 0.18, 0.23 and 0.19 for PC 1 to PC 3 respectively). The AUC for the ROC curve for the final multivariate logistic regression model for predicting high MBD from the Cups spectra PC scores was 0.890 (0.826-0.954), which was not significantly different (p = 0.24) from the AUC for the final model for the TiBS spectra PC scores, (Figure 3.7). The reduction in spectral content for the new Cups device, therefore, does not appear to significantly reduce the ability to identify women with high MBD, despite the sub-optimal dynamic range of the first prototype of the Cups device used in this study.
Figure 3.7. Receiver-operator characteristic (ROC) curves for the final multivariate logistic regression models identifying breasts with ≥ 50% MBD using (A) the TiBS PC scores and (B) the Cups PC scores. n = 119 breasts. Black curves are based on the model with PC scores calculated using the loads from the PCA of the data from this study, red curves are based on the model with PC scores calculated using the loads from the previously published study comparing TiBS spectra with MBD [112].
The PC loads and multivariate regression model from the previous study identifying women with high MBD-based on optical spectra were also applied to the TiBS and Cups spectra. The resulting AUC values were 0.922 (0.871-0.973) for the TiBS device spectra and 0.872 (0.794-0.950) for the Cups device spectra. The AUC values for the two devices were not significantly different (p = 0.55) and neither value was significantly different from the AUC for the final multivariate logistic regression model for the previous TiBS study PC scores, reported at 0.922 (0.876-0.968), (p = 1.00 for the TiBS device, p = 0.48 for the Cups device). [112] The extremely close replication of the previous studies results in a new population, even with a different version of the TiBS device, indicates that the model for identifying women with high MBD developed from the previous study is robust. However, for the attained sensitivity and specificity to be confirmed as clinically relevant, the association between high MBD identified via OBS measurements and higher BC risk needs to be further established in clinical trials.

MPD predicted by PLS regression of optical spectra was significantly correlated with MPD determined using LIBRA or Cumulus for spectra measured using both the TiBS and the Cups devices. However, the correlation coefficient was significantly higher for the MPD values predicted using the TiBS spectra. The correlation coefficients for the TiBS spectra values for both the training and validation sets were similar to those found in the previous study using TiBS spectra to predict MPD measured using Cumulus. [113] For both the TiBS and Cups data sets, the magnitude of the average residuals (LIBRA MPD minus PLS-predicted MPD) was greater for the higher LIBRA MPD values, and the average residuals were larger overall for the PLS-predicted MPD from the Cups data, (Figure 3.8). The larger-magnitude residuals at higher MPD were seen previously and are most likely because there were fewer breasts with high MPD on which to train the PLS algorithm. [113] When looking at all repeat PLS-regressions, the slopes from the regression analysis were not significantly different from 1 for the predicted MPD values derived from either the TiBS or the Cups spectra, indicating that there is no significant bias in the PLS-predicted MPD. However, when the average PLS-predicted MPD over all repeat PLS analyses is plotted against the LIBRA MPD, there appears to be a significant bias in the predicted PLS values for both the TiBS and the Cups spectra, with predicted MPD over-estimated for lower MPD values and under-estimated for values over ~20%. This could be due
to boundary effects as the lower density breasts tend to scatter light less, leading to less optical confinement and a larger area sampled, which in turn could lead to a larger contamination of the signal by adjacent tissues such as the pectoral muscle [122].

Given the high AUC values obtained for the PCA scores-based logistic regression models, they are likely better suited to identify women at higher risk based on their MBD. Nevertheless, the PLS-predicted MPD values could also be used to classify women into high- vs low/medium-density categories. Kappa statistic values were calculated to assess the quality of the inter-rater MPD prediction agreement between LIBRA and OS. While the agreement between LIBRA and TiBS OS classifications is good (K=0.74), it is moderate (K=0.42) for the Cups OS spectra-based values using an MPD of 50% as the high-density threshold for LIBRA and 46% as the threshold for both OS classifications. However, the intended use of the PLS-predicted MPD values is as a tool for tracking longitudinal changes in breast composition and breast density in response to risk-changing events or interventions to be validated in future studies.
Figure 3.8. Average PLS regression-predicted MBD vs. MBD read using LIBRA for all breasts ($n = 119$) for (A) PLS regression of TiBS device spectra and (B) PLS regression of Cups device spectra. (C) and (D) show the residuals (LIBRA MBD minus PLS regression-predicted MBD) for the TiBS and Cups spectral data respectively.
3.5 Conclusions

The modifications made for the Cups version of the device were successful in decreasing the measurement time and reduced the amount of operator interaction required, as well as the amount of data that is manually recorded by the operator. The Cups device is also smaller and portable. These changes were necessary to make the device suitable for large-scale, multi-center trials. Despite the modifications, including a significant decrease in the spectral content, the ability to identify women with high MBD, the most clinically relevant group, was not significantly reduced. The PLS regression results demonstrated an ability to predict MBD on an interval scale with the Cups 12 wavelength version based on the strong and significant correlation with MPD determined by the LIBRA thresholding software ($r = 0.66$, $p < 0.001$). Hence the 12 wavelength Cups device can report MBD on a categorical and interval scale. The latter, in particular, could have utility in BC prevention trials or therapies, as it is a quantitative, dynamic and modifiable risk factor, and this needs to be evaluated in future longitudinal interventional studies.

The Cups device used in this study was not optimized to fully exploit the dynamic range possible and so further improvements to the device should improve the quality of the spectra obtained and may improve the ability to predict MPD from the spectra. Several changes have been implemented for subsequent versions of the Cups device including changing the amplifier gain and active area on some detectors, using template cups to help select the appropriate cup size and using an active algorithm which cycles through different laser powers based on the signal at each detector.

The results for the TiBS version of the OS device, both for identifying women with high MBD and predicting MPD from optical spectra using PLS regression, closely replicate previous results with that style of OS device, indicating the TiBS spectra are a robust predictor of MPD and women with high MBD. Other metrics, which may be identified from the OS spectra and may be useful for BC risk assessment or pre-screening, such as the rate of change of the spectra, hemoglobin content and/or oxygenation and left-right symmetry, are also under investigation.
4 Optical Spectroscopy Measurements in Pre-teen and Teenaged Girls

4.1 Introduction

This chapter describes the development of a laser-based, portable device for measuring breast composition in pre-teen and teenaged girls during puberty.

Breast cancer (BC) risk assessment is generally not performed for women without a strong family history or known genetic mutation. Tools have been developed to assess BC risk in the general population but they are not applicable until they are in their 40s or 50s. There is increasing evidence, however, that BC risk accumulates from an early age and that events early in puberty have a significant effect on BC risk. [155] Early menarche is long established as a risk factor and there is evidence that early thelarche (the start of pubertal breast development) and a longer time between thelarche and menarche also increase BC risk. [23] Modifiable factors affecting BC risk, such as diet, exercise, and alcohol consumption, also have a cumulative effect over time, with their strongest impact in youth and early adulthood. Interventions aiming to reduce risk by addressing these modifiable factors have a stronger effect if begun early. [36] Tracking breast development and measuring breast composition during puberty may allow for early estimation of BC risk, enabling the identification of girls who would most benefit from early interventions targeting modifiable factors and could provide information about the progress and effectiveness of these interventions.

Optical spectroscopy (OS) is a technique that can be used to assess the composition of breast tissue that is safe to use repeatedly at any age. It is, therefore, a promising technique for monitoring breast development and evaluating the associations between breast composition and timing of development and BC risk. Preliminary evaluation of OS in this role has been carried out as part of the Lessons in Epidemiology and Genetics of Adult Cancer from Youth (LEGACY) Girls Study. [156] The LEGACY study is a prospective cohort of 1040 girls enrolled at ages 6-13 years. It was designed to assess the relationships between early life exposures and factors associated with breast cancer risk, such as pubertal timing and breast tissue characteristics, in a cohort which includes both girls with a family history of BC and girls with no
family history at an approximately 1:1 ratio (and is thereby enriched in BC family history positive girls relative to the general population). The study also investigates psychosocial well-being and health behaviours in relation to BC family history. Girls were recruited at 5 different sites, 4 in the US and 1 in Canada. Pubertal timing was assessed using Tanner stages (TS), which separately evaluate changes in the breast and pubic hair. Breast TS range from 1 to 5. TS 1 is no breast development, TS 2 is when breast buds first appear, TS 3 is when the areola and breast are larger than buds but the areola is not raised away from the breast, TS 4 is when the contour of the areola and nipple forms a separate mound beyond the contour of the breast, and TS 5 is the mature breast with only the nipple protruding beyond the contour of the breast. [157] TS is usually assessed by a clinician using both palpation and visual assessment but can also be self-assessed or assessed by a parent based on explanatory drawings and descriptions, although this is less reliable than the clinician assessment [118]. A subset of the participants from the Canadian site of the LEGACY study participated in a pilot study to evaluate the utility of optical spectroscopy (OS) for measuring breast composition and determining breast TS. In that study, OS measurements were made on 105 girls. Age, BMI, parent-assessed TS, and a breast cancer risk score (based on family history information) were also recorded. Complete data sets were available for 102 girls and so 102 were included in the final analysis. Transmission measurements are not feasible for girls as there is insufficient breast tissue. The TiBS device was therefore modified to allow optical assessment using diffuse light reflectance for these girls. Measurements were made at two source-detector fibre bundle distances – 15mm and 30mm – however, there were significant problems with detector saturation at the 15mm distance so only the 30mm was used in the analysis. A PCA was performed on the optical spectra and the associations between the resulting PC scores and breast TS were evaluated. Significant differences were seen in the PC scores with changing TS, although the relationships were not linear but instead followed distinct patterns. [119] The transition from TS 1 to 2 was marked by a significant increase in PC3. Early (TS 1-2) vs. late TS (TS 3-5) could be distinguished based on a decrease in PC2 as well as increases in PC 6 and 7, age and BMI. PC2 values continued to decrease with TS and the final stages (TS4-5) could be distinguished from TS1-3 based on PC2 in conjunction with age and BMI.
After successful implementation of OS measurements at one site, the OS component of the LEGACY study was expanded to all sites. A dedicated LEGACY OS device was developed for this purpose. As one site planned to perform in-home visits, portability was a particularly important criterion for the device. The design of the dedicated LEGACY OS device incorporated the main principles of the Cups device – fixed position PIN photodiode detectors and a light source module with 13 wavelengths of laser diode turned on in sequence – but with the mechanical design changed for quantification of the diffuse reflectance.

4.2 Distinguishing early and late Tanner Stage with reduced wavelength spectra

The pilot study using the TiBS device to measure tissue optical properties showed that PC scores, which are essentially variables describing the spectral shape, were sufficient to distinguish girls at different Tanner stages. To determine whether the reduction in the spectral content of the dedicated LEGACY OS system would affect the ability to distinguish between TS based on optical spectra, the TiBS spectra from the pilot study were reduced to only the 10 wavelengths used in the analysis with the final dedicated LEGACY OS system. Although the light source module for the dedicated LEGACY OS system had 13 different wavelengths of laser, as for the Cups device, only 10 wavelengths (658, 682, 735, 761, 784, 806, 879, 911, 940, and 980nm) resulted in usable signal levels for all measurements and were used in the final analysis. PCA was performed on the reduced spectra and the analysis looking at differences in PC scores between TS groups were repeated. The full spectral content pilot study showed that it is possible to distinguish between TS 2 and TS1 based on PC3, early (TS 1-2) vs. late (TS 3-5) Tanner stage can be distinguished based on PC 2, 6 and 7 and TS 4-5 can be distinguished from the earlier Tanner stages (TS 1-3) based on PC2. [119]

The PCs of the reduced-wavelength PCA (PCA_{10λ}) do not necessarily correspond with the PCs of the full-wavelength PCA on a one-to-one basis, so an analysis of variance (ANOVA) was performed to determine which PC_{10λ} predicted breast TS. TS5 was excluded from this analysis as only 6 participants were at TS5. The results of the ANOVA are summarized in Table 4.1. PC_{10λ} 2, 3, 5 and 8 all showed significant associations with breast TS (p < 0.01 for all four). PC 2 and 3
were also significantly associated with breast TS for the all-wavelength analysis. However, the trends for both were the inverse for the 10-wavelength analysis than for the all-wavelength analysis. Looking at the load vectors for PC2 and PC3, the vectors do appear to have a similar shape, although inverted, when comparing between the two PCAs. PC 5 and 8 were not associated with breast TS for the all-wavelength analysis and the load vectors for PC\textsubscript{10λ} 5 and 8 do not appear to directly correspond to any of the load vectors from the all-wavelength analysis.

Univariate multinomial logistic regression (including all TSs but with TS4 and TS5 grouped together) was used for the comparison of TS2 vs TS1. As in the full-wavelength analysis, PC3 from the reduced-wavelength PCA significantly distinguished between TS 2 and TS 1 (p = 0.002). In a multivariate multinomial logistic regression model including age, BMI, PC\textsubscript{10λ} 2, 3 and 8, PC\textsubscript{10λ} 3 was still able to distinguish TS2 from TS1 (p = 0.003).

Binary logistic regression was used when predicting late vs. early breast TS. When classifying late TS as TS3-TS5, univariate binary logistic regression showed that PC\textsubscript{10λ} 2, 5 and 8 significantly distinguished early from late breast TS (p < 0.01 for all three components). The final multivariate model included age, BMI, PC\textsubscript{10λ} 2, 5 and 8 (p = 0.12, 0.04, 0.002, 0.05 and 0.03 respectively). This final model had an AUC for the ROC curve of 0.93 (95% confidence interval of 0.88-0.98, p <0.001) which is not significantly different from the AUC for the corresponding multivariate model from the original all-wavelength PCA analysis from the LEGACY pilot study which was 0.94 (0.88-0.99, p<0.001). [119]

The binary logistic regression was also repeated with the late and early breast TS groups defined as TS4-TS5 and TS1-TS3 respectively. The univariate binary logistic regression showed that PC\textsubscript{10λ} 2 and 8 were significantly able to distinguish between these two TS groups. For the all-wavelength PCA, only PC2 had significantly distinguished between the groups. [119] The final multivariate model included age, BMI, PC\textsubscript{10λ} 2 and 8 (p = 0.02, 0.007, 0.08 and 0.28 respectively). The multivariate model for the reduced-wavelength PCA had an AUC for the ROC curve of 0.90 (95% confidence interval of 0.84-0.96, p <0.001) which is not significantly different
from the AUC for the corresponding multivariate model from the original all-wavelength PCA analysis from the LEGACY pilot study which was 0.90 (0.83-0.96, p<0.001).

The above results indicate that the reduced wavelength content of the spectra from the dedicated LEGACY OS device should not significantly affect the ability to distinguish between breast Tanner stage groups based on OS data.
Table 4.1 Association between breast Tanner stage and OS principal component scores from 10-wavelength TiBS spectra of the LEGACY OS pilot study.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Breast Tanner Stage</th>
<th>Mean 1</th>
<th>Mean 2</th>
<th>Mean 3</th>
<th>Mean 4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC10.1</td>
<td>0.19</td>
<td>-0.01</td>
<td>0.11</td>
<td>-0.37</td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>PC10.2</td>
<td>-0.48</td>
<td>-0.28</td>
<td>0.27</td>
<td>0.44</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>PC10.3</td>
<td>0.49</td>
<td>-0.19</td>
<td>-0.09</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>PC10.4</td>
<td>0.06</td>
<td>-0.01</td>
<td>0.08</td>
<td>-0.23</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>PC10.5</td>
<td>-0.41</td>
<td>-0.23</td>
<td>0.24</td>
<td>0.38</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>PC10.6</td>
<td>0.07</td>
<td>0.10</td>
<td>-0.07</td>
<td>-0.05</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>PC10.7</td>
<td>0.07</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.15</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>PC10.8</td>
<td>-0.44</td>
<td>-0.29</td>
<td>0.17</td>
<td>0.29</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
4.3 Design of the LEGACY optical spectroscopy device

The dedicated LEGACY optical spectroscopy (OS) device consists of a 13-wavelength laser module light source with an internal light-monitoring photodiode (FDS100, Thorlabs Inc., Newton, NJ, USA) and 6 silicon photodiode detectors with built-in pre-amplifiers (S8745-01 and S8746-01, Hamamatsu Corp., Hamamatsu, Japan). A low-noise laser driver supplies the laser currents (WLD3343, Wavelength Electronics, Inc., Bozeman, MT, USA) and a USB data acquisition (DAQ) card (USB-1408FS-Plus, Measurement Computing Corp., Norton, MA, USA) controls the laser driver and laser switching and records the detector signals. Software controlling the device was written in LabVIEW 2012 (National Instruments Corp, Austin, TX, USA). The 6 detectors are mounted on a 15cm long flexible circuit board at different distances (1, 2, 3.5, 5, and 7.5cm) from the laser module aperture (Figure 4.1A). The flexible printed circuit board (PCB) was designed to be centred on the nipple with the laser module aperture 2.5cm from the centre. A silicone strip fits over the circuit board around the light source aperture and detectors to create a flat surface level with the detector faces. The PCB and silicone are covered with black heat shrink, and holes are cut in the heat shrink using tissue biopsy punches to uncover the sensing areas of the detectors (Figure 4.1B). The flexible PCB strip is bent to fit into a curved frame which is placed over the participant’s breast. Several frames with different levels of curvature are used.

To determine what frame shapes would be necessary, study coordinators measured breast curvatures using a bendable, shape-holding ruler for 56 girls participating in the LEGACY girls study at 3 of the sites. The ruler was bent to the shape of the breast going horizontally across the breast starting from the sternum, going over the nipple, and ending at the edge of the chest. The ruler was then traced onto a template sheet and the position of the nipple was marked. Age, self-reported Tanner Stage (TS) and TS evaluated by the mother were also recorded on the template. The template sheets were then scanned and printed on transparency paper so that the curves could be overlaid, compared and grouped. When grouped by similar curve shapes, 6 representative curves covered the variation across the study
population. One representative curve from each of the 6 groupings was scanned and saved as an image file. These 6 standard curves represented pre-teen and teenaged girls from age 10 to 14 and covered all TS 1-5. The coordinates of the curves and the coordinates of the nipple were extracted from the image files in Matlab and fitted to a third-degree polynomial. Frames were designed in Solidworks (Dassault Systèmes S.A., Waltham, MA, USA) to fit the flexible PCB so that the curvature of the frame and the detector surface of the PCB strip at the breast matched the fitted curves. The frames were 3D-printed in black PC-ABS (Figure 4.2).

The flexible PCB has a hole for the laser module aperture cone to fit through and the PCB is rigidly connected to the laser module housing at that point. A flexible ribbon cable connects the PCB to the internal circuit board in the laser module housing. The laser module housing and PCB slide into the different frame shapes while connected. The laser module, as for the adult OS Cups device, had 13 laser diodes arranged in an aluminum hemisphere, which fit over a cone-shaped base. The interior surface of the module was painted with a 2:3 white paint and BaSO₄ mixture (BaSO₄ – 243353, Sigma-Aldrich; Paint – Opaque White 5212, Airbrush Colors, Createx) to create a diffusely reflecting surface. A 1mm hole in the bottom cone, perpendicular to the laser module aperture, led to the internal reference detector for the laser module. The measured laser diode centre wavelengths for the device used at the Toronto site of the LEGACY study are 658, 682, 735, 761, 784, 806, 830, 879, 897, 911, 940, 980, and 1048nm.
Figure 4.1 A Printed circuit board layout showing the positions of the detectors and the laser module. B Detector strip mounted in the flat calibration frame.
Figure 4.2 The six frame shapes used for the dedicated LEGACY device. Each frame has an orientation arrow and is labelled with the frame number.
4.4 Device comparison study population

To evaluate the dedicated LEGACY OS device and compare it with the TiBS device, a small comparison study was carried out. LEGACY participants who had completed a LEGACY OS measurement with the TiBS device during the pilot study for LEGACY OS or whose sister had completed an OS measurement were invited to complete measurements with both the dedicated LEGACY OS device and the LEGACY version of the TiBS device during one of their follow-up visits. 35 girls participated and completed measurements with both devices. Of those 35 girls, only 16 had usable measurements with both devices as there were problems with failure of the detector electrical connections in the dedicated LEGACY device due to the high mechanical stresses on the PCB as it was moved from frame to frame. Age, BMI, TS, age of menarche and BC family history information for all 35 participants and for the 16 who had usable measurements with both devices is listed in Table 4.2. Both self- and parent-reported TS were recorded for most girls. In the pilot study, parent-reported TS was used in the final analysis. For this study, self-reported TS may be more appropriate as this population is older, with the youngest participant being 11.3 years at the time of the measurements. Self-reported TS has been found to be more accurate after age 11. [118] Self- and parent-reported TS ranged from 2 to 5 with a mean TS of 3.5 for the former and 3.7 for the latter. No girls were in TS 1. The average TS in this comparison study was much higher than in the pilot study, where 46% of the girls were TS 1 or 2. This was due to the fact the girls were almost exclusively recruited from the pilot study (only 5 of the 35 girls were not included in the pilot study). For the girls who participated in both studies, the average time between their measurement for the pilot study and their measurement for this study was 2.5 years and the average change in TS was 1.2.
Table 4.2 Participant information for the girls who completed LEGACY measurements with both OS devices.

<table>
<thead>
<tr>
<th></th>
<th>Girls with usable measurements with both devices (n=16)</th>
<th>Girls with usable measurements with TiBS device only (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean ± SD (range)</strong></td>
<td>14.8±1.8 (11.3-17.6)</td>
<td>14.6 ± 1.8 (11.3-17.6)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 to &lt;12</td>
<td>1 (6.2)</td>
<td>2 (5.7)</td>
</tr>
<tr>
<td>12 to &lt;14</td>
<td>5 (31.2)</td>
<td>12 (34.3)</td>
</tr>
<tr>
<td>14 to &lt;16</td>
<td>6 (37.5)</td>
<td>12 (34.3)</td>
</tr>
<tr>
<td>≥16</td>
<td>4 (25.0)</td>
<td>9 (25.7)</td>
</tr>
<tr>
<td><strong>BMI, mean ± SD (range)</strong></td>
<td>20.0±2.6 (16.1-24.5)</td>
<td>20.4±2.8 (15.0-25.6)</td>
</tr>
<tr>
<td><strong>BMI (kgm^-2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 to &lt;20</td>
<td>10 (62.5)</td>
<td>17 (48.6)</td>
</tr>
<tr>
<td>20 to &lt;25</td>
<td>6 (37.5)</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td>≥25</td>
<td>0 (0)</td>
<td>2 (5.7)</td>
</tr>
<tr>
<td><strong>Age at Menarche, mean ± SD (range)</strong></td>
<td>12.6±0.9 (11-14)</td>
<td>12.4±1.1 (10-14)</td>
</tr>
<tr>
<td><strong>Age at Menarche (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menarchal</td>
<td>3 (18.8)</td>
<td>9 (25.7)</td>
</tr>
<tr>
<td>&lt;12</td>
<td>1 (6.2)</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>12 to &lt;14</td>
<td>10 (62.5)</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td>≥14</td>
<td>2 (12.5)</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td><strong>Breast Tanner Stage (self-evaluated)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (12.5)</td>
<td>5 (14.7)</td>
</tr>
<tr>
<td>3</td>
<td>8 (50.0)</td>
<td>11.5‡ (33.8)</td>
</tr>
<tr>
<td>4</td>
<td>3 (18.7)</td>
<td>11.5‡ (33.8)</td>
</tr>
<tr>
<td>5</td>
<td>3 (18.7)</td>
<td>6 (17.6)</td>
</tr>
<tr>
<td><strong>Breast Tanner Stage (parent-evaluated)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (13.3)</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>3</td>
<td>6 (40.0)</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>4</td>
<td>4 (26.7)</td>
<td>14 (43.8)</td>
</tr>
<tr>
<td>5</td>
<td>3 (20.0)</td>
<td>6 (18.8)</td>
</tr>
<tr>
<td><strong>Positive BC Family History, number (%)</strong></td>
<td>8 (50.0)</td>
<td>22 (62.9)</td>
</tr>
</tbody>
</table>

* Mean age calculated using only ages of girls who had reached menarche (n = 13 for girls who had usable measurements with both devices and n = 26 for girls who only had usable measurements with the TiBS device)

‡ Self-evaluated breast TS was not recorded for one girl and parent-evaluated breast Tanner stage was not recorded for 3 girls, one of whom had usable measurements with both devices. For all girls, at least one breast TS value was recorded – either self- or parent-evaluated.

‡ One girl self-evaluated her breast TS as being either 3 or 4 so she is counted as 0.5 in both categories
4.5 OS measurements on girls with the TiBS device

The TiBS device and the protocol for OS measurements on girls using that device has been described previously [119]. Two slightly flexible, black urethane holders hold the light source and detector fibre bundles flush with each other at the breast surface at a fixed distance apart (Figure 4.3A-C), with source-detector distances of 15mm and 30mm. For the 15mm distance, two sheets of a 0.3OD neutral density gel filter paper (LEE Filters, Burbank, CA, USA) were glued onto the holder, covering its entire surface, extending the spectrometer’s dynamic range and thus avoiding saturation.

LEGACY measurements with the TiBS device are done in a darkened room with the participant lying on her back. A thin, black, flexible, circular template is placed over the breast to guide placement of the fibre bundle holders. Four measurements are performed on each breast with both of the fibre bundle holders. The four positions are the upper centre, the medial side, the lower centre and the lateral side of the breast (Figure 4.4). Two study coordinators participate in taking the measurements. One places the fibre bundle holder in the different positions of the template and holds it in place. The second study coordinator controls the software, selecting the integration time for the spectrometer at each position and the iris setting for the light source and recording the participant and measurement information (participant ID, the holder being used, position, iris setting, etc.).

Instrument throughput was validated as for adult TiBS measurements using a transmission reference (3.5 cm thick white ultra-high-density polyurethane, Gigahertz Optics, Munich, Germany) and measurement of the dark signal as a function of CCD integration time before and after the participant measurements. These reference measurements were done in transmission rather than in reflectance as it was difficult to get both fibre bundles in good contact with the rigid polyurethane reference block while in the LEGACY holders. Measurements of the polyurethane reference block with the 0.3OD filter paper were made to quantify the attenuation of the filter paper across the full red/NIR range of the spectrometer.
Figure 4.3 Semi-flexible fibre bundle holders for reflectance measurements on girls with the TiBS device. A. 30mm and 15mm inter-optode distance holders. B. Tissue contact surface of the 30mm inter-optode distance holders. C. 15mm inter-optode distance holder showing the gel filter paper at the tissue contact surface.
Figure 4.4 Templates for positioning the fibre bundle holders. The small centre hole is positioned over the nipple and the template is oriented so that the number 1 position is in the superior position and position number 2 is placed medially.
4.6 Measurements with the LEGACY OS Device

For each measurement, the laser diodes are cycled on and off in sequence. Each laser is turned on for 1000ms, first at a low current, (the lasing threshold specified by the manufacturer), and then a high current. High current is either the maximum current specified by the manufacturer or the highest current which produced an optical power output at the laser module aperture just below the maximum permissible exposure for eyes, according to the ANSI Standards for a bright, non-collimated light source. In between each different current setting, the lasers were turned off for 100ms to record the dark signal from the detectors. The voltage output from the detector preamplifiers was acquired at a sample rate of 1000Hz.

Before and after each participant measurement with the LEGACY OS device, reference standard measurements are made. For these measurements, the PCB strip is mounted in a flat frame which fits over a black PC-ABS holder containing a reference standard of silicone doped with titanium dioxide as a scattering agent, and red ink and black India ink as absorbers. Two reference standards with different concentrations of the absorbing and scattering agents, one weakly attenuating and one strongly attenuating, are used.

Participant measurements are performed in a darkened room with the girl seated in an upright position. The girl tries the different frames and selects the frame curvature which best fits her breasts. The flexible PCB and laser module is then mounted into the selected frame by the study coordinator. The girl holds the frame against her breast in four different positions per breast and the study coordinator controls the software to record the data. The first position is with the long axis of the frame oriented vertically and the orientation arrow (see figure 4.2) on the frame pointed up. The frame is then rotated in 90° increments for the remaining three measurements so that the frame is next horizontal and the orientation arrow is pointed medially, then vertical with the arrow down, and finally horizontal with the arrow pointing laterally.
4.7 Calculation of Attenuation Spectra

4.7.1 LEGACY Spectra measured with the TiBS Device

The attenuation spectra using the TiBS device are calculated according to Equation 4.1, essentially the same for the LEGACY participants as for the adults. All spectra are first smoothed using a 7 point boxcar smoothing. An integration-time-matched dark spectrum ($S_{dark}$) is subtracted from each participant spectrum ($S_p$) and reference spectrum ($S_{ref}$), then all spectra are divided by their integration times ($t$). A cubic spline interpolation is applied to give spectra in 1nm increments between 610nm and 1050nm (the original wavelength sampling of the spectrometer is in 0.46nm increments from 582.41nm to 1055.88nm). The participant spectra are then divided by the average reference spectrum for that visit, matched for light source iris setting. Participant spectra measured with the 15mm interoptode spacing are corrected for the transmission of the 0.3OD filter. Finally, taking the negative log of this participant-reference ratio spectrum, adding the attenuation spectrum of the reference ($A_{ref}$), and dividing by the source-detector distance, $d$, gives the attenuation spectrum ($A$) in units of optical density per cm ($ODcm^{-1}$).

$$A_{i,\lambda} = -\log\left(\frac{(S_{p,\lambda} - S_{dark,p,\lambda})/t_p}{(S_{ref,\lambda} - S_{dark,ref,\lambda})/t_{ref}}\right) + A_{ref,\lambda}/d$$  \hspace{1cm} \text{Equation 4.1}

Spectra with CCD errors or with signal below zero after correction for the dark spectrum were discarded.

4.7.2 LEGACY spectra measured with the LEGACY OS device

The average voltages at all laser powers and for all dark sections were calculated as a function of wavelength for the 6 detectors in contact with the tissue and the laser module monitoring detector. As the detectors have high-impedance amplifiers, they have long rise- and fall-times (>30ms). The detector voltages also have a significant 60Hz noise. To account for these factors, the central 700ms of each laser-on section (42 cycles at 60Hz) was used to average the signals, and the final 50ms of each dark section (3 cycles at 60Hz) were averaged to provide the dark signal. For each wavelength, there is a high and low laser power signal value for each detector.
The high laser power signal value was used unless either the detector or the laser module monitoring detector was saturated, in which case the low power signal was used. If the selected signal value was not significantly greater than the dark signal, or if both measurements at that wavelength were saturated then the signal was recorded as zero. The attenuation spectra were calculated using Equation 4.2. The dark signal, $V_{\text{dark}}$, was subtracted from the selected detector signal from the participant measurement, $V_p$, and the corresponding signal from the laser module monitoring detector, $V_{\text{lm}}$. Taking the negative log of the dark-corrected detector signal divided by the corrected laser module monitoring signal and adding the wavelength-, detector-, and laser module-dependent voltage to optical power conversion factor, $R_{\text{det},\lambda}$, gives the optical attenuation spectrum. Finally, dividing by the physical source-detector distance, $d$, gives the attenuation spectrum, $A$, in units of ODcm$^{-1}$.

$$A_{i,\lambda} = \left( -\log\left( \frac{V_{p,\lambda} - V_{\text{dark},p}}{V_{\text{lm},p,\lambda} - V_{\text{dark,lm},p}} \right) + R_{\text{det},\lambda} \right) / d$$

Equation 4.2

The voltage to optical power conversion factor, $R_{\text{det},\lambda}$, is calculated according to Equation 4.3 using the detector signal values from measurements of the reference standards, $V_{\text{ref}}$, and the optical attenuation spectra of the reference standards, $A_{\text{ref}}$, as measured using the TiBS device.

$$R_{\text{det},\lambda} = \log\left( \frac{V_{\text{ref,}\lambda} - V_{\text{dark,ref}}}{V_{\text{lm,ref,}\lambda} - V_{\text{dark,lm,ref}}} \right) + A_{\text{ref,}\lambda}$$

Equation 4.3

The values of $R_{\text{det},\lambda}$ were not constant throughout the study as the detector PCB had to be replaced part-way through due to failure from high mechanical stress. The values of $R_{\text{det},\lambda}$ were therefore calculated for each visit based on the visit’s standard reference measurements.

4.8 Results

4.8.1 OS Spectra with the dedicated LEGACY device

Measurements were made with both devices on 35 girls. Analysis of the spectra, however, showed that with the new dedicated LEGACY OS device only 16 of the girls had usable spectra from the detectors at the two inter-optode distances (20mm and 35mm) which bracket the
inter-optode distance used for the TiBS device (30mm) and thus were useful for comparison. **Figure 4.5** shows some typical spectra obtained with the two devices.

Spectra from the two devices were compared on an individual basis to determine whether they were correlated. Although the two devices are not expected to provide identical attenuation spectra, as the measurement locations were not identical and they have different inter-optode distances, and thus sample different tissue volumes, there is overlap in the interrogated volumes, and thus some correlation between the spectra is expected. For each of the 16 girls who had measurements with both devices, the attenuation values from the TiBS spectra for each position at the 10 wavelengths of the dedicated LEGACY device were plotted vs. the corresponding attenuation values from the corresponding LEGACY device spectra. A linear regression was performed and the slope, intercept, and correlation coefficient were calculated. For the 35mm interoptode distance LEGACY spectra there was a significant correlation (p<0.05) between the TiBS and LEGACY device spectra for 14 of the 16 girls (mean $R^2 = 0.43$, range 0.11-0.74). For 6 of the 16 the slope from the linear regression was not significantly different from 1. One of the 16 girls did not have 20mm interoptode distance LEGACY spectra. For the 20mm interoptode distance LEGACY spectra, there was a significant correlation between the TiBS and LEGACY device spectra for 14 of the 15 girls (mean $R^2 = 0.33$, range 0.11-0.56) and for 6 of the 15 the slope from the linear regression was not significantly different from 1. For the 35mm interoptode distance LEGACY spectra, there was a significant inverse correlation between the strength of the correlation between the LEGACY and TiBS device spectra and the range of attenuation values for each LEGACY spectrum ($R^2 = 0.56$, $p < 0.01$).
Figure 4.5 Typical spectra for two girls obtained with the two devices. The blue line is the average spectrum obtained with the TiBS device for the 30mm inter-optode distance. The black x symbols are the average spectrum for the 20mm inter-optode distance with the dedicated LEGACY device, and the red circles are the average spectrum for the 35mm inter-optode distance with the dedicated LEGACY device. For A, the $R^2$ value for the correlation between the LEGACY device spectra and the TiBS device spectra for that girl is 0.45 for the 20mm LEGACY interoptode distance and 0.61 for the 35mm distance. For B, the $R^2$ value is 0.49 for the 20mm distance and 0.007 for the 35mm distance.
4.8.2 Distinguishing between Tanner stages based on optical spectra

Spectra were obtained for 35 girls with the TiBS device. The principal component loads calculated in the pilot study were used to derive the PC scores from the TiBS spectra. The ability to distinguish between Tanner stages based on PC scores, seen in the pilot study, was tested to determine whether the associations were also seen with this second population. Since no girls were at TS 1, the ability to distinguish between TS 1 and 2, which was seen in the pilot study, could not be assessed. In the pilot study early (TS 1 and 2) vs. late TS (TS 3-5) could be distinguished based on PC 2, 6 and 7 for the full-wavelength TiBS Spectra. Univariate binary logistic regression was performed for age, BMI, and all 8 PC scores using TS groupings based on both self- and parent-reported TS. For self-reported TS groupings (n = 34), only PC2 significantly distinguished between early and late TS (p = 0.03). For parent-reported TS groupings (n = 32), none of the variables significantly distinguished between early and late TS in the univariate analysis, however, PC2 approached significance (p = 0.08). Multivariate binary logistic regression was also performed, using the final model from the pilot study which included age, BMI, and PCs 2, 6 and 7 and a ROC curve generated for the model. The multivariate models were significantly able to distinguish between early and late TS for both self- and parent-reported TS. For TS groupings based on self-reported TS the AUC for the ROC for the final model was 0.81 (p = 0.03, 95% CI of 0.58-1.00). For TS groupings based on parent-reported TS the AUC was 0.97 (p = 0.001, 95% CI of 0.91-1.00).

The univariate and multivariate logistic regression was also repeated with the TS groups defined as TS2-3 for early and TS4-5 for late TS. With this grouping, age and BMI were both significantly able to distinguish between the groups but PC2, which was significant in the pilot study, was not able to distinguish between the groups for either self- or parent-reported TS. The final multivariate model used in the pilot study, which included age, BMI, and PC2, was significantly able to distinguish between the groups with an AUC of 0.83 for the ROC (p = 0.001, 95% CI of 0.68-0.97) for self-reported TS and an AUC of 0.81 (p = 0.004, 95% CI of 0.67-0.96) for parent-reported TS, however, this was entirely due to the contributions of age and BMI as a multivariate model including only age and BMI gave equivalent results – AUC of 0.83 (p = 0.001,
95% CI of 0.68-0.98) for self-reported TS and AUC of 0.81 (p = 0.004, 95% CI of 0.65-0.96) for parent-reported TS.

When looking at only the spectra from the 16 girls who had measurements with both devices, there were only 2 girls at TS 2, which was insufficient to perform the logistic regression. There were 8 girls with self-reported TS 3 and 6 girls with parent-reported TS 3, which is sufficient to perform the logistic regression analyses, however, because there was no significant difference between the TS1-3 and TS4-5 groups based on optical spectra for the full 35 girls, there would be no significant difference in the subgroup.

4.9 Discussion

Re-analysis with reduced wavelength content of the spectra from the pilot study indicates that the 13 wavelengths used in the dedicated LEGACY OS device should be more than sufficient to distinguish between Tanner stages based on the optical spectra. Furthermore, the multivariate binary logistic regression model for the TiBS spectra used to distinguish between early (TS 1-2) and late (TS 3-5) in the pilot study was also able to distinguish between those groups in the device comparison study. This was the only model that could be repeated with the device comparison study given the small study size and the dearth of participants in TS 1 and 2, but the replication of the previous results strengthens the argument that OS may be a useful tool for objectively evaluating breast TS. One drawback of this analysis, and that of the pilot study, is that self- and/or parent-evaluated TSs were used as the gold standards rather than clinician-evaluated TS. Although the results of the analysis for the comparison study were significant and showed the same trend regardless of whether self- or parent-evaluated TS was used, 9 out of the 31 girls whose TS was evaluated both ways had a different TS depending on the method of evaluation. Clinician-evaluated TS would provide a more reliable standard to use to establish how OS spectra change with breast development.

Spectra were obtained for only 16 girls using the dedicated LEGACY OS device, which was insufficient to repeat the analyses from the pilot study. For some spectra (e.g. figure 4.5A) the spectra from the dedicated device closely matched the average spectrum from the TiBS device, however for others (e.g. figure 4.5B) there was a significant offset between the spectra as well
as differences in spectral shape. There were several issues with the first prototype of the
dedicated LEGACY OS device that need to be addressed to improve the reliability of the spectra.
One issue was that the controlling software only set the laser power to two values – a low one
just above the manufacturer-specified lasing threshold and one at maximum power (either the
manufacturer-specified maximum for that laser diode or the maximum based on laser safety,
whichever was lower). This was not sufficient to provide the dynamic range necessary to
measure all tissues with all detectors at all wavelengths. In some cases, there was detector
saturation for both laser powers, or there was saturation for the maximum power but
insufficient power at the minimum power to yield a signal significantly above zero. There is
considerable variability in the lasing threshold around the manufacturer-specified average
value and for some lasers the minimum used may not have been above the lasing threshold or
may have been significantly above the threshold, yielding relatively high power. Another issue
with the prototype device was that there are significant stresses on the flexible circuit board
when it is held in the curved frames and as it is being moved from frame to frame. The stresses
on the board caused the connections to fail over time, leading to a floating, nonsense signal
from disconnected detectors. Dedicated detectors mounted rigidly in each of 6 frames would
eliminate the failures due to mechanical stresses on the components. Less sensitive detectors
can be used for all but the detectors furthest from the light source to minimize the associated
cost increase since detector saturation, rather than low signal, was the more common problem.
In fact, reducing the sensitivity of the detectors should improve the performance of the device
as it will allow the lasers to be run at higher currents rather than near threshold current where
the current-power relationship may not be linear. The flexible circuit board in the rigid frames
also made it difficult to ensure consistent contact between the detectors and the tissue and
especially between the detectors and the surface of the reference standards. The inverse
correlation between the range of attenuation values for the 35mm interopertode distance
LEGACY device spectra and the R^2 value for the correlation between the LEGACY and TiBS
spectra likely reflects the effect of inconsistent detector contact with the LEGACY device on
spectral repeatability. Rigidly mounting the detectors in the frames will also improve the ability
to gauge contact between the detectors and the reference standards during reference
measurements. Changes in contact between the detector and the reference standard will affect
the optical coupling and likely account for some the offset seen between the TiBS spectra and the dedicated device spectra. An alternative method of addressing the issue of contact and optical coupling would be to use a reference standard which is never in contact with the detectors, such as a diffusely reflecting surface. Finally, as discussed in the previous chapter for the Cups device, the algorithm controlling the laser powers should be modified to an active algorithm which changes the laser power and checks the signal from each of the detectors to ensure that a non-zero, un-saturated signal is obtained for each detector at each wavelength.
Conclusions and Future Work

The goal of this thesis was to develop and test an OS device suitable for large-scale, multi-centre trials by modifying the TiBS OS device to make it portable, low-cost and simple to use. Two main modifications were made to achieve this – the broadband light source and spectrometer were replaced with 13-wavelength laser modules and photodiode detectors, and the light sources and detectors were placed a fixed positions in either four different sizes of breast cup or in strips with differing curvatures for the developing breast. The thirteen wavelengths which were used to distinguish between women with different breast composition were identified from the PC loads obtained from a previous study. MC simulations of light propagation through breast tissue were used to determine the minimum number of fixed-position sources and detectors which would match the breast volume sampled with the original TiBS device, while maintaining the sampling uniformity and the minimal signal contribution from surrounding tissues. 6 detectors and 2 light source modules were found to sufficiently match the sampling properties of the TiBS device. The modifications to the OS device were successful in creating a low-cost instrument that can easily be made portable. Two versions of the device were designed, built and tested, one for use on women (the Cups device) and one for use on pre-teen and teenaged girls (the LEGACY device). A clinical comparison between the prototype Cups device and the TiBS device found that there was no significant deterioration in the ability to identify women with high MBD (the high-risk group) between the two devices, although the ability to predict MPD on an interval scale was slightly reduced for the Cups device versus the original, full-spectrum TiBS device. This difference is likely related to electronic and mechanical design issues, rather than to the wavelength reduction or the change in optode number and position. Preliminary analyses for both modified devices (for adult women and for girls during puberty) indicate that the reduced spectral content is highly unlikely to negatively affect the ability to obtain clinically relevant breast composition information relating to breast development or breast cancer risk. In fact, for both devices, significant associations were seen between optical spectra and the parameters of interest when the final spectra used in the analyses had fewer than 13 wavelengths. It is likely that the spectral content can be further reduced, but while the relationships between optical spectra and breast composition, BC risk
and BC detection are still being explored, it is prudent to keep enough wavelengths to capture all distinct features of the five main chromophores, as well as the dominant features of the PC load vectors from previous studies.

Both devices require refinements which have been or are being implemented. While the prototype Cups device discussed in this thesis was no more portable than the TiBS device, an improved version has since been developed with professionally redesigned electronics that allow for a much smaller, readily portable device. The improved electronics have also significantly reduced the 60Hz noise of the prototype design. The dedicated LEGACY device was required to be portable even for the first prototype as one of the LEGACY sites planned to perform measurements during home visits. This was achieved by selecting a smaller DAQ card, smaller power supplies and a compact housing. The redesigned electronics for the latest version of the Cups device, however, are considerably more compact and could easily be adapted for the LEGACY device. In addition, the most recent design has reduced power consumption, which further improves portability.

The software has been updated for subsequent versions of the Cups device and now includes an active algorithm which, for each detector at each wavelength, finds the highest laser power that does not saturate that detector. The active algorithm is a simple linear search which starts at the midpoint in the driving current range (threshold to maximum current) and increases or decreases in increments of 6% of the range depending on the detected signal level in the detector of choice. This algorithm is not optimal, however, and refinements allowing finer incremental changes in laser power would increase dynamic range – a key requirement to enable measurement of all wavelengths at all positions. More sophisticated algorithms, such as a binary search algorithm, could achieve this without significantly increasing overall measurement times. In fact, if measurement time becomes an issue (it has not been a problem to date, with most women finding the measurements relatively quick and average times being more than an order of magnitude shorter than with the original TiBS device), a custom-designed variation on a binary search algorithm which considers the signal level at all detectors simultaneously could be developed. A similar algorithm should also be implemented for the LEGACY device.
For the studies described here, absolute spectra for the Cups and LEGACY devices were obtained by calibrating the device relative to a reference standard. This method is not ideal, especially as there were some issues in consistently obtaining contact between the detectors and the reference standards for the LEGACY device. A more robust method would be to determine the wavelength-dependent voltage to optical power conversion factors separately for the light source and the detectors by simultaneous measurement with the laser module and device detectors and a calibrated optical power meter. For the internal detectors in the laser modules, this can be a straightforward measurement with the calibrated optical power meter placed at the aperture of the laser module and recording optical power as the internal detector records voltage. The main concern is ensuring the spot size at the aperture fits within the detection area of the optical power meter. As the aperture of the laser module integrating cavity is open, the throughput of the module will likely change over time due to loss of the diffuse reflection resulting in a decrease in the cavity’s multiplication factor, and so regular recalibration (annual or semi-annual) will be necessary. For the main detectors, it is important to calibrate using simultaneous measurements with the optical power meter as there is variation in the output of the laser module with repeated use. A possible, relatively simple set-up for calibration is to couple the laser module to the input port of an integrating sphere with two output ports – one for the detector being calibrated and one for the calibrated optical power meter. The areas of the two output ports should be the same and smaller than the detection area both of the cup detector and the calibrated power meter. The reference standards would then be used to track changes in the instrument over time between calibrations and to assess inter-operator variability.

The interchangeable, flexible detector strip used in the LEGACY device was developed primarily as a cost-saving measure, to allow measurement of several breast curvatures with a single set of detectors. The strains on the detector strip when mounting and unmounting it from the frames led to a high failure rate for the flexible PCB in the detector strip. In addition, the flexibility of the strip and the necessity of allowing sufficient space around the strip to allow it to be easily mounted in and unmounted from the frames led to difficulty in sensing the amount of contact between the detectors and the reference standard surface, which in turn led to
variability in the reference standard measurements. Switching to a separate set of fixed, rigidly mounted detectors for each frame shape would eliminate the strain-related failure of the detector PCBs and would make the reference standard measurements more reliable. This can be achieved without leading to high device costs as the sensitivity of the detectors was well beyond what was necessary and lower cost, less sensitive photodiodes are available that can be used for all detectors except the one farthest from the source. In fact, as saturation was a more common problem than low signal for both the LEGACY and the Cups devices, less sensitive detectors for the closer positions will improve the dynamic range. Lower cost, less sensitive detectors have also replaced the original detectors in most positions of the Cups device. For the Cups device, photodiodes with a 6mm$^2$ detecting area and a built-in 500MΩ gain resistor on the preamplifier (ODA-6W-500M, Opto Diode Corp., Camarillo, CA) are now used for all detectors that do not have a source-detector distance greater than 7.5cm. These detectors are roughly $\frac{1}{3}$ to $\frac{1}{4}$ the price and about 11 times less sensitive than the detectors used in the first prototype of the Cups device and in the two positions farthest from the light source in the LEGACY device (33.6mm$^2$ detecting area, built-in 1GΩ gain resistor on the preamplifier) and are approximately $\frac{1}{2}$ - $\frac{1}{3}$ of the price and are roughly half as sensitive compared to the detectors used for the 4 positions closest to the light source in the LEGACY device (5.8mm$^2$ detecting area, built-in 1GΩ gain resistor on the preamplifier). Photodiodes with even lower sensitivity should be sourced for the LEGACY device detectors closest to the light source (10mm source-detector distance).

The software designed for the prototype Cups and LEGACY devices used in this thesis did not provide feedback to the user on the quality of the measurements obtained. Real-time quality assurance incorporated into the program would lead to fewer measurement sets being discarded due to detector saturation associated with poor contact between the optodes and the tissue or reference standards. It would also immediately alert users to component failures in the device, which was a common issue with the current mechanical set-up of the LEGACY device. The Cups and LEGACY devices were designed with the goal of using them in large, multi-centre trials. To be able to make valid comparisons between data taken at different institutions, centralized data collection and analysis systems will need to be devised, as well as quality
assurance and calibration protocols which include inter-device and inter-institution comparisons. [158, 159]

There are 5 source-detector distances per frame in the dedicated LEGACY device; however, only two distances were considered in this thesis as the goal of the study described here was to compare the dedicated LEGACY system with the reflectance version of the TiBS device, for which only one source-detector distance was used. Different source-detector distances will probe different tissue depths so using multiple distances will add depth-dependent breast composition information, which could both improve the ability to distinguish between Tanner Stages based on optical spectra and also further characterize the changes in breast composition that take place during puberty. Future analysis of measurements with the dedicated LEGACY device from the 5 sites of the LEGACY cohort should provide this information.

The fixed positions of the light sources and detectors in the Cups device will reduce the complexity of the system when modelling light propagation in tissue, allowing for more time-consuming simulations as fewer parameters will have to be varied. Given the fixed number of geometries to simulate and the availability of highly efficient Monte Carlo programs, it may be possible to simulate the range of likely breast optical properties (determined based on different possible concentrations of the 5 dominant chromophores and scattering parameters observed in OS studies with time- or frequency-dependent information). These simulations could then be used to create a look-up table of wavelength-dependent signal levels at each of the detectors in a cup or frame. Chromophore concentrations could then be estimated based on a best fit to the look-up table values of the detector signals obtained. Obtaining chromophore concentrations is more difficult with a CW device than with devices that include time- or frequency-dependent information, however simulation of an accurate model geometry, without simplification of the model boundaries, and simultaneous consideration of multiple, source-detector distances and arrangements should improve the accuracy of decoupling the absorption and scattering contributions.

One of the advantages of optical techniques for measuring breast composition as it relates to breast density and BC risk is that it can provide contrast between two of the components that
attenuate x-rays and thus give rise to mammographic density – water and collagen – while most other techniques assessing density either measure only one component (e.g. the amount of water measured by MRI) or do not have contrast between different components of dense tissue (e.g. tissue is dichotomized into fatty or dense tissue for mammography). Previous OS studies have found both water and collagen concentrations to be correlated with MBD but separate analysis of their relationship with BC risk may lead to better risk prediction. [154, 160] Collagen is of particular interest as studies have indicated a role for collagen in BC tumour initiation, invasion and metastasis. [95, 96] To date, OS studies have only shown correlations between optically-derived parameters (such as water and lipid concentrations) and mammographic measures of breast density, but have not directly correlated to BC risk. These studies are difficult to do for OS measurements as there is no large pool of women with previous OS measurements who could be studied retrospectively. The situation was different when mammographic density was established as risk factor, when a very large amount of retrospective data was already available.

We currently estimate that to establish OS measurements as a risk factor using a prospective study, more than 2000 women would be required based on the following considerations. The average 5-year risk for women of screening age (50-74 years in Ontario) in the general screening population is 2%. Assuming a relative risk of 4 for the high risk group vs. the low risk group for OS measurements (based on the fact that mammographic density can be predicted from OS measurements and the relative risk for >75% MBD vs. <10% MBD is 4-6), then a sample size of 204 per group would be required for a confidence level of 0.95 and a power of 0.80. However, the population frequency of high (>75%) MBD is only 10% so to obtain a high MBD population of 204 women, just over 2000 women would be needed. As the women would have to be followed for 5 years to check their BC status, the number would need to be even higher to account for loss to follow-up and other sources of attrition. Furthermore, considering that the largest benefit of OS would be for women in the 35 to 50 years age bracket where the breast cancer incidence is much lower (approximately 0.75%), the size of a prospective cohort must be even larger. Assuming the same power, confidence level, relative risk, and population frequency of high MBD, then a minimum total of 5670 women would be required before even
considering the loss to follow-up, etc. It is also important to ensure that the study population has a relatively uniform distribution of ages across the age range being studied, and that the familial BC risk, menopausal status and ethnicity of the study population are representative of the population as a whole, as these are confounding factors which will affect the ability to accurately determine the relative risk associated with OS parameters.

Consent was obtained to follow women who participated in studies with the TiBS device through the Ontario Cancer Registry to check for BC incidence. However not all of these women were from the average-risk, general screening population. A total of approximately 800 women are currently in our database, insufficient to complete prospective studies with appropriate statistical power. To overcome these limitations, two large population surveillance studies are planned in Pakistan and Uzbekistan, which will evaluate the Cups device as a pre-screening device. These studies should create an optically screened population which will be large enough to be used for retrospective risk analysis, although it will be critical to ensure that the population evaluated with OS is representative of the general population in terms of age, familial BC risk, menopausal status and ethnicity.

If the OS devices are to be used to monitor changes in breast composition over time, with the goal of correlating composition or rate of composition changes to risk, then it will be necessary to determine the measurement repeatability and the precision with which the OS parameters can be determined. The magnitude of the measurement variability must be small compared to the overall population variability for any associated relative risk between different sub-populations to be meaningful. Tissue-mimicking phantom studies using phantoms with a range of optical properties spanning the range of absorption and scattering properties of the breast can be used to establish the variability associated with repeated measurements, different operators and different instruments.

The main advantages of the CW device for OS measurements are that CW measurements can be implemented at relatively low cost in a robust and portable form, allowing measurements to be made in a variety of environments rather than limiting studies to hospitals or research institutes. However, there are interesting applications of optical techniques which are not as
simple or portable but may provide additional information. As already discussed, OS techniques which include time- or frequency-dependent information can accurately separate the contributions of scattering and absorption from the attenuation spectra. However the optics and electronics required for these systems are less portable and significantly more expensive. Structured-light-based diffuse OS is another new technique being explored which may be useful for obtaining volumetric/depth dependent breast composition information. [161] This technique uses different spatial patterns of light to illuminate the tissue and measures the intensity and spatial patterns of the transmitted or diffusely reflected light to obtain spatially resolved composition information.

The importance of MBD as a risk factor and determinant of the effectiveness of mammography as a screening tool is highlighted by the recent implementation of mandatory reporting laws for mammographic density. In the USA, more than 20 states have breast density notification laws. [162] OS provides a low-cost, portable method of determining MBD, without the need for radiologist interpretation, which could be used on women before they reach the general screening age. Thus, density information could be used to both inform the decision on when to start screening and also the type of screening that may be most appropriate, (for example US screening may be recommended for younger women with very dense breasts). The utility of OS for measuring MBD and breast composition, however, may be most pronounced in low- and middle-income countries where the breast screening infrastructure is inadequate. The additional BC risk information provided by determining MBD can help in identifying the population which will most benefit from the limited screening resources. In addition, there is evidence that composition information determined by OS, particularly local variations in composition and asymmetries between the left and right breasts, can be used to identify women with BC or with lesions which require further investigation. [107, 163, 164] The portability of the final version Cups device developed in this thesis is especially advantageous for use in low- and middle-income countries as there is a larger rural population which does not have ready access to screening facilities.

The OS devices developed in this thesis are low-cost, portable, straight-forward to use and require little operator training or operator intervention. Although further improvements to the
devices to optimize the signal-to-noise ratio as well as the dynamic range of the data acquisition are an ongoing project, the first prototype of the Cups device was successful in predicting mammographic density and identifying women with high MBD. The current versions of the Cups device are suitable for trials assessing OS as a breast cancer pre-screening tool and to validate OS-determined breast density as a BC risk factor. Cups devices have been built for studies being carried out in the USA, Chile, Australia and Uzbekistan, and additional studies with the Cups device are planned for Pakistan and Mexico.
References


M. Simick, "Near infrared transillumination spectroscopy of breast tissue for correlation with mammographic density" in *Medical Biophysics*, University of Toronto, Toronto (2002).


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# Appendix A

Maximal permissible exposure for the eye and skin according to ANSI 136.3 standards (1995).

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Maximum Permissible Exposure - Eye [mW]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Maximum Laser power at the Eye [mW]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Maximum Permissible Exposure - Skin [mW]&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Maximum Laser Power Measured at the Aperture [mW]</th>
</tr>
</thead>
<tbody>
<tr>
<td>655</td>
<td>0.70</td>
<td>0.14</td>
<td>77</td>
<td>5</td>
</tr>
<tr>
<td>685</td>
<td>0.70</td>
<td>0.29</td>
<td>77</td>
<td>10</td>
</tr>
<tr>
<td>735</td>
<td>4.11</td>
<td>0.10</td>
<td>88</td>
<td>3</td>
</tr>
<tr>
<td>760</td>
<td>4.61</td>
<td>0.09</td>
<td>101</td>
<td>3</td>
</tr>
<tr>
<td>785</td>
<td>5.18</td>
<td>1.64</td>
<td>114</td>
<td>58</td>
</tr>
<tr>
<td>808</td>
<td>5.76</td>
<td>2.68</td>
<td>127</td>
<td>94</td>
</tr>
<tr>
<td>830</td>
<td>6.37</td>
<td>0.48</td>
<td>140</td>
<td>17</td>
</tr>
<tr>
<td>880</td>
<td>8.02</td>
<td>0.15</td>
<td>176</td>
<td>5</td>
</tr>
<tr>
<td>905</td>
<td>9.00</td>
<td>0.40</td>
<td>197</td>
<td>14</td>
</tr>
<tr>
<td>915</td>
<td>9.42</td>
<td>1.51</td>
<td>207</td>
<td>53</td>
</tr>
<tr>
<td>940</td>
<td>10.57</td>
<td>1.16</td>
<td>232</td>
<td>41</td>
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<tr>
<td>980</td>
<td>12.71</td>
<td>1.42</td>
<td>279</td>
<td>50</td>
</tr>
<tr>
<td>1050</td>
<td>17.54</td>
<td>0.67</td>
<td>386</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated based on the following formulas:

- $400 \leq \lambda < 700$nm: $\text{Power} [\text{W}] = (7 \times 10^{-4} \times t^{0.75}) / t$
- $700 \leq \lambda < 1050$nm: $\text{Power} [\text{W}] = (7 \times 10^{-4} \times t^{0.75} \times 10^{0.002(\lambda-700nm)}) \times 5 / t$

using an exposure time, $t$, of 1s. Maximum permissible exposures are for the power to the dilated pupil at a distance of 13cm from the light source.

<sup>b</sup> Calculated at 13cm from the laser opening aperture over an area equal to that of the dilated pupil (diameter = 7mm).

<sup>c</sup> Calculated using the laser opening aperture of diameter 7mm for the area calculation and calculating power density based on the following formulas:

- $400 \leq \lambda < 700$nm: Power Density $[\text{W/cm}^2] = 0.2$
- $700 \leq \lambda < 1400$nm: Power Density $[\text{W/cm}^2] = 0.2 \times 10^{0.002(\lambda-700nm)}$
Appendix B

List of Publications and Presentations Arising From the Thesis

Refereed Publications:


EJW and LL designed the device. EJW built the device with help from Benjamin Lai and Daniel Huynh. Device software was written by EJW. The study design was developed by EJW and LL. Participant recruitment and data collection was performed by three clinical coordinators (Brenda Ornelas, Samantha Dick and Sarah Forward). EJW performed the instrument calibration and spectral analysis. A study coordinator retrieved the mammograms and prepared batches for reading (Jennifer Xanthopoulos). EJW and LL measured the mammographic densities using Cumulus. EJW performed the statistical analysis with input from LL. The outline for the manuscript was developed by EJW and LL. The manuscript was written by EJW and edited by LL.


EJW designed the optical spectroscopy device and maintained instrument calibration and converted transmission measurements in effective attenuation data for further analysis, and participated in acquisition of the data and writing the manuscript. LL designed the optical spectroscopy device and maintained instrument calibration and converted transmission measurements in effective attenuation data for further analysis, participated in the design and acquisition of the data, conceptualized the analyses, directed the data analysis and interpretation, and participated in writing the manuscript. MBT conceptualized the design of the overall parent study and participated in the assembly of the data, conceptualized the analyses, directed the data analysis and interpretation, and participated in writing the manuscript. DP conceptualized the design of the analyses, analyzed the data and participated in interpretation and in writing the manuscript. GG participated in the design of the overall parent study, acquisition of the data, and writing the manuscript. DH and MT participated in design, acquisition of data, and writing the manuscript. AB, SBB, MD, and EMJ conceptualized the design of the overall parent study and participated in interpretation of the data and writing the manuscript.
JAK conceptualized the design of the overall parent study and participated in the acquisition of the data, analysis and interpretation, and writing the manuscript. ILA conceptualized the design of the study and the analyses presented, participated in the acquisition of the data, data analysis and interpretation, and writing the manuscript.


EJW and LL designed the study. EJW performed the spectral analysis for wavelength selection. EJW performed the statistical analyses with input from JAK and LL. EJW and LL developed the manuscript outline. EJW wrote the manuscript, and LL and JAK edited it.


KMB, JAK, EJW and LL conceived and designed the experiments. KMB, EJW and LL performed the experiments. KMB and EJW analyzed the data. KMB, JAK, EJW and LL contributed reagents/materials/analysis tools. KMB and EJW wrote the manuscript.

**Conference Proceedings:**


EJW and LL designed the experiments. EJW performed the analysis. EJW wrote the manuscript and LL edited it.

**Conference Presentations:**


Copyright Acknowledgements

Chapter 2 has been previously published in the Journal of Biophotonics, (E. J. Walter, J. A. Knight, and L. Lilge, "A multi-wavelength, laser-based optical spectroscopy device for breast density and breast cancer risk pre-screening," Journal of Biophotonics 10(4), 565–576, 2017). Minor modifications and formatting changes have been made for this thesis. The article is used here with permission of the publisher.

Chapter 3 has also been previously published in the Journal of Biophotonics and only minor modifications and formatting changes have been made for this thesis, (E. J. Walter, and L. Lilge, "Optical assessment of mammographic breast density by a 12-wavelength versus a continuous-spectrum optical spectroscopy device," Journal of Biophotonics, 2017). The article is used here with permission of the publisher.