Optical imaging of the breast: clinical research using an experimental Diffuse Optical Tomography system

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Optical imaging for breast cancer can be performed either by relying on intrinsic breast tissue contrast alone (mapping hemoglobin, water, and lipid content) or with the use of exogenous imaging agents that accumulate at the tumor site, either by targeting cancer-specific molecules or by extravasation due to leaky tumor vasculature.

The light used in optical imaging is commonly monochromatic and in the near-infrared (NIR) range, permitting imaging up to several centimeters deep in soft tissue. Different tissue components have unique scattering and absorption characteristics for each wavelength. The use of a single wavelength gives some diagnostic information on the tissue of interest, e.g. if there is high total blood content, associated with angiogenesis. By combining data from multiple wavelengths, more precise information can be obtained on relative concentrations of oxy- and deoxyhemoglobin, lipid, and water in the tissue (spectroscopic imaging). This may allow for better discrimination between malignant and benign tissue. With the use of exogenous imaging agents, such discrimination may be improved even further, especially with imaging agents specifically targeted to cancer-associated molecular changes (molecular imaging).

Albeit in its infancy, optical breast imaging has some intrinsic advantages over other breast imaging modalities. It uses no ionizing radiation (unlike X-ray mammography), the technology is relatively inexpensive and fast (unlike magnetic resonance imaging (MRI)), and is not user-dependent (unlike ultrasound). Furthermore, currently used breast imaging modalities have limitations in clinical utility (in early detection, diagnosis, or treatment monitoring of breast cancer) [1-4].

Although X-ray mammography reduces mortality significantly, due to earlier cancer detection [5, 6], overall sensitivity is moderate (~75%), resulting in a number of missed breast cancers [7]. In addition, the positive predictive value of X-ray mammography is relatively low and discrimination between benign and malignant lesions with further diagnostic tests is difficult, leading to a high number of unnecessary biopsies [3].

MRI has very high sensitivity (> 95%) and is clinically used as an adjunct to X-ray mammography in high risk patients [8, 9]. However, MRI generally only allows detection and classification of lesions from 5 mm upwards, and has a high false-positive rate [10].

Optical imaging is being pursued as an adjunct to the current imaging modalities due to its potential to provide biophysical and molecular information on breast tissue.

In this overview of previously published data [11-13] we shall summarize our first clinical experiences in the evaluation of an experimental Diffuse Optical Tomography (DOT) system dedicated for breast imaging. We initiated the evaluation under optimal settings in small patient groups. First, we evaluated the potential of optical breast imaging to discriminate between benign and malignant tissue. For this we used only data on intrinsic tissue contrast at four different NIR wavelengths without spectroscopic analysis. In this study we also assessed inter- and intra-observer agreement of DOT image interpretation (study 1)[11].

Optical imaging has some intrinsic advantages over other breast imaging modalities.

Optical imaging has the potential to provide biophysical and molecular information.
Secondly, we investigated the added value of spectroscopic analysis for imaging well-defined benign cysts, by combining all information from the four wavelengths (study 2) [12]. Finally, we evaluated optical breast imaging using escalating doses of a novel fluorescent non-targeted imaging agent (Omocyanine, Bayer Schering Pharma, Berlin, Germany) in patients highly suspected of breast cancer (study 3) [13].

Materials and methods

Patients

A total of 48 patients (mean age 52, range 22 to 85) were recruited for the three studies between October 2006 and September 2007 from the University Medical Center Utrecht and the Diakonessenhuis Utrecht. Each study had different inclusion and exclusion criteria tailored to the research questions. Detailed criteria have been given in previous publications [11-13] but all women had to be diagnosed with a breast lesion on X-ray mammography and/or ultrasound.

Study 1 included women with BI-RADS (Breast Imaging Reporting and Data System) 2-5 lesions. Study 2 comprised women with benign cysts (BIRADS 2) and Study 3 comprised women with highly suspicious lesions (BIRADS 4-5). All study protocols were approved by the institutional ethics committees, and written informed consent was obtained from all patients.

Imaging protocols

Diffuse optical tomography (DOT) scans were performed on an experimental DOT system (Figure 1). The patient was placed in prone position on the scanner bed with one breast suspended in a size-matched cup. The scanning unit of the DOT system consists of a cup with a total of 507 optical fibers mounted on the surface. The light of four continuous-wave solid-state lasers is directed into the cup via 253 source fibers, which are interleaved with 254 detector fibers on all sides of the cup. For each scan, the cup was filled with a matching fluid that has similar optical properties to those of the average breast. This matching fluid provides a stable optical coupling between the fibers and the breast, and eliminates optical short cuts of the diffuse light around the breast.

During imaging, the breast was sequentially illuminated with continuous wave near-infrared light from all source positions. Light emanating from the breast was detected in parallel for each source position. Images were obtained at four discrete wavelengths (690, 730, 780, and 850 nm). Each breast was scanned separately. During each scan the system was operated in two modes: transmission and fluorescence mode (the last used for the study with Omocyanine).

The transmission measurements were intended to acquire information on the optical absorption and scattering properties of the breast at four wavelengths. The fluorescence measurements were performed with excitation at one wavelength (730 nm), and the emitted fluorescent signal was detected at a different wavelength (> 750 nm) while the laser light was blocked by filters in the detection path. The duration of the examination was approximately one minute per wavelength in the transmission mode, and five minutes for the fluorescence mode, making a total of nine minutes per breast.

After optical data acquisition, three-dimensional absorption images were reconstructed at each of four wavelengths (based on the Rytov-approximation [14]), as well as three-dimensional fluorescence images for the studies with Omocyanine (based on the Born approximation [15]). In the spectroscopic study of patients with cysts, optical information from the four wavelengths was combined to convert the absorption coefficients into hemoglobin, oxy-hemoglobin, water, and lipid concentrations [12]. This information was used to generate three-dimensional enhanced-water maps, with high signal intensity for high water concentration, and three-dimensional enhanced-blood maps, with high signal intensity for high blood concentration (and low signal intensity in case of blood-depletion).
MRI of the breast was performed on a 3.0T clinical MR system (3.0T Achieva, Philips Healthcare, Best, the Netherlands) using a dedicated four-element SENSE compatible phased-array bilateral breast coil (MRI devices, Würzburg, Germany). Fat-suppressed T1-weighted, T2-weighted, and dynamic T1-weighted images were acquired according to routine clinical protocols. MRI was used as a benchmark for the optical image analysis, because both MRI and DOT are tomographic imaging techniques that provide three-dimensional data and in which patients are positioned in a similar way. In addition, MRI gives high-resolution anatomical information with excellent soft tissue contrast, which made it our method of choice to use for comparison with optical imaging and to derive lesion location and size.

**Data analysis and study-specific methodology**

**Study 1. Discrimination of malignant and benign lesions**

Per lesion, a region of interest (ROI) was drawn on the absorption image for all three wavelengths at the site of the lesion, derived from the axial MRI slice that showed the lesion at its maximum diameter. For comparison, an identical ROI was drawn at an exactly mirrored location on the image of the contralateral breast, where no lesion was detected by MRI.

The visibility of the lesions on DOT was assessed both quantitatively and qualitatively. Quantitative contrast values were obtained by dividing the mean absorption coefficient within an ROI by the mean absorption of the background on that specific tomographic slice, including the rest of the breast but excluding the lesion. Furthermore, all images were anonymized, placed in random order, and independently scored by two readers separately, blinded for other examinations as well as pathology results. These two readers allotted qualitative contrast scores for every ROI, on a scale from -4 to +4, where: 0 = no visibility; 1 = slight heterogeneity; 2 = moderate contrast, but less than other structures on the image; 3 = contrast comparable to that of other structures; 4 = major contrast. A minus sign was used for signals lower than the background (less absorption), and a plus sign for higher signals (more absorption).

Images were scored again after three months in a second independent reading by the two investigators. Intra- and interobserver agreements were calculated using kappa statistics and intraclass correlation coefficients. Discriminatory values for presence of malignancy were determined by Receiver Operating Characteristic (ROC) analyses. Cancer detection rates were calculated using a qualitative score of ≥2 as a cut-off.

**Study 2. Spectroscopic analysis of benign cysts**

The visibility of the cysts on DOT was assessed in a qualitative manner both for the absorption images and for the spectroscopic reconstruction images. When the values at the lesion site (derived from MRI) were lower than those of the surrounding tissue the cyst was considered to be visible (visibility score < -2). The physiological enhanced-water and enhanced-blood maps were evaluated and compared to MRI data in the axial plane. Maximum diameters of the cysts were estimated on the physiological maps from the full width at half maximum of the signal intensity through the center of the cyst region, and compared to the maximum diameters measured on the MR images. The Bland Altman method was used to measure the agreement of lesion size between the MRI and DOT measurements [16]. The Pearson correlation coefficient was calculated to estimate the correlation between the two methods.

**Study 3. Dose-escalation of Omocyanine, a novel fluorescent imaging agent**

The study protocol comprised three periods:

- a screening period to verify inclusion criteria
- an imaging period, during which the study agent was administered in a single intravenous injection (0.01, 0.02, 0.05, or 0.1 mg/kg bodyweight) and optical images were acquired at five different time points (up to 24 hours after injection)
- a follow-up period of one week to monitor adverse events.

Separate ROIs were drawn on a single optical image slice at the lesion location derived from the MRI scan for all fluorescent images over time (1, 2, 4, 8, 24 hours after injection). For comparison, a similar ROI was drawn for each time point at the mirror image lesion location of the contralateral breast, where no lesion was found on MRI. The mean fluorescence intensity was determined for all the ROIs. To calculate the lesion-to-background ratio, this value was divided by the mean fluorescence of the background, which included the rest of the breast on that slice except for the lesion and the nipple (that showed very high fluorescence intensity). The same was done for the mirror image ROI to compare the values in the ipsilateral and contralateral breast. Absorption images obtained before contrast administration were also assessed.
Figure 2. Study 1. Example of ROI on DOT image compared to MRI. The MRI image on the right shows a BI-RADS 5 lesion in the right breast; the DOT image shown on the right had a visibility score of +4. Final diagnosis was invasive ductal carcinoma [11].

Discriminatory values for presence of malignancy were determined by Receiver Operating Characteristic (ROC) analyses. Areas under the ROC curves (AUC) ranged from 0.92 to 0.95 for quantitative scores, and from 0.97 to 0.99 for qualitative scores (with both observers having exactly the same AUC). Cancer detection rates for the four wavelengths (690, 730, 780, and 850 nm, respectively) were 70%, 80%, 80%, and 70% for Observer 1; and 60%, 70%, 70%, and 60% for Observer 2, using a qualitative score.
Table 1. Study 1. Median (range) of quantitative absorption score according to lesion presence and lesion type [11].

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Malignant (n=10)</th>
<th>Fibroadenoma (n=2)</th>
<th>Cyst (n=6)</th>
<th>No lesion (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>690</td>
<td>3.03 (1.91 to 3.40)</td>
<td>1.75 (1.36 to 2.14)</td>
<td>0.23 (0.10 to 0.73)</td>
<td>1.39 (0.32 to 3.21)</td>
</tr>
<tr>
<td>730</td>
<td>2.94 (1.74 to 6.31)</td>
<td>1.50 (1.01 to 1.98)</td>
<td>0.17 (0.07 to 0.75)</td>
<td>1.34 (0.29 to 3.18)</td>
</tr>
<tr>
<td>780</td>
<td>2.57 (1.54 to 3.22)</td>
<td>1.36 (1.13 to 1.58)</td>
<td>0.13 (0.08 to 0.82)</td>
<td>1.29 (0.72 to 2.58)</td>
</tr>
<tr>
<td>850</td>
<td>2.15 (1.27 to 2.55)</td>
<td>1.30 (1.24 to 1.35)</td>
<td>0.13 (0.08 to 0.89)</td>
<td>1.16 (0.71 to 2.34)</td>
</tr>
</tbody>
</table>

Table 2. Study 1. Median (range) qualitative visibility score according to lesion presence and lesion type [11].

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Malignant (n=10)</th>
<th>Fibroadenoma (n=2)</th>
<th>Cyst (n=6)</th>
<th>No lesion (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>690</td>
<td>2 (0 to 4)</td>
<td>1 (0 to 2)</td>
<td>-4 (-4 to -1)</td>
<td>0 (-2 to 2)</td>
</tr>
<tr>
<td>730</td>
<td>2 (0 to 4)</td>
<td>1 (1 to 1)</td>
<td>-4 (-4 to 0)</td>
<td>0 ( 0 to 2)</td>
</tr>
<tr>
<td>780</td>
<td>2 (0 to 4)</td>
<td>0 (0 to 0)</td>
<td>-4 (-4 to -1)</td>
<td>0 (-1 to 1)</td>
</tr>
<tr>
<td>850</td>
<td>2 (0 to 4)</td>
<td>0 (0 to 0)</td>
<td>-4 (-4 to 0)</td>
<td>0 (-1 to 1)</td>
</tr>
</tbody>
</table>

Qualitative visibility was scored on a scale from -4 to +4, where: 0 = no visibility; 1 = slight heterogeneity; 2 = moderate contrast, but less that other structures; 3 = contrast comparable to other structures; 4 = major contrast; a minus sign was used for signals lower, and a plus sign for signals higher than the background.

Table 3. Study 3. Overview of results per patient for optical imaging with imaging agent [13].

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Imaging agent dose (mg/kg)</th>
<th>Lesion type</th>
<th>Lesion diameter on MRI (mm)</th>
<th>Detected by DOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>0.01</td>
<td>IDC</td>
<td>25</td>
<td>No *</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>0.01</td>
<td>ILC</td>
<td>29</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>0.01</td>
<td>IDC</td>
<td>18</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>0.02</td>
<td>IDC</td>
<td>24</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>0.02</td>
<td>IDC</td>
<td>74</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>0.02</td>
<td>IDC</td>
<td>34</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>0.05</td>
<td>IDC</td>
<td>14</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>0.05</td>
<td>FA</td>
<td>15</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>62</td>
<td>0.05</td>
<td>IDC</td>
<td>14</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>51</td>
<td>0.1</td>
<td>ILC</td>
<td>51</td>
<td>No *</td>
</tr>
<tr>
<td>11</td>
<td>55</td>
<td>0.1</td>
<td>IDC</td>
<td>13</td>
<td>No</td>
</tr>
</tbody>
</table>

IDC – Invasive Ductal Carcinoma; ILC – Invasive Lobular Carcinoma; FA – Fibroadenoma; * in these cases the lesion was located close to the patient’s chest wall and physically too far above the upper optical fibers in the cup to be measured by the current DOT system.
Figure 3. Study 2. Example of the MRI compared to the enhanced-water and enhanced–blood maps of the optical data sets. The cyst shows a high signal intensity on the T2 weighted MR image and the enhanced-water map (high water content) and a low signal on the enhanced-blood map (low blood content) [12].

Figure 4. Study 3. Example of the fluorescence images in a patient with invasive lobular carcinoma in the left breast (diameter 2.5 cm); optical images obtained 1, 2, 4, 8, and 24 hours after fluorescent imaging agent injection. Contralateral and ipsilateral breast are shown, the lesion is indicated by the green arrows, and the nipple is encircled. Some reconstruction artifacts are visible in the top middle of the images (blue arrows). MRI is shown for comparison. The gray scale of the optical images is adjusted individually for each image to range from 0 to 5 times the average background value. In this way the varying contrast between lesion and background in the ipsilateral breast is visible [13].

Based on the absorption images only, 6/20 (30%) benign cysts were clearly distinguishable with DOT, showing marked lower absorption compared to surrounding tissue. When the spectroscopically derived physiological enhanced-water and enhanced-blood maps were evaluated and compared to the MRI data, 13/20 (65%) benign cysts were evident on DOT. The enhanced-water and enhanced-blood maps showed high water content and low total hemoglobin content at the position of these cysts (Figure 3).

Detected lesions after spectroscopic analysis had maximum diameters of 15 mm and larger. Two cysts with diameters of 8 and 10 mm were not visible with DOT. Five cysts that were located close to the chest wall were not detected because they were outside the field of view of the current system. There was good agreement between optical size measurements and MRI (Pearson correlation coefficient 0.7; p<0.01). DOT overestimated the lesion size on average by 2.5 mm (mean difference MRI-DOT, -2.5 mm; 95% confidence interval, -6.4 to 1.3).

Study 3. Dose-escalation of Omocyanine, a novel fluorescent imaging agent
Following initial evaluations of the system without imaging agent, we tested its ability for optical imaging with contrast agent using a novel fluorescent imaging agent in a patient group highly suspected of breast cancer [13]. Eleven women (mean age 54, range 23 to 81) diagnosed with a 1 – 5 cm BI-RADS 4/5 breast lesion on mammography were included in the study.

Histopathology results showed invasive ductal carcinoma in eight patients (median lesion diameter 21 mm), invasive lobular carcinoma in two patients (median lesion diameter 40 mm), and a benign fibroadenoma (lesion diameter 15 mm) in one patient (Table 3).

In the lowest dose group (0.01 mg/kg), lesions were detected in two patients on the fluorescence DOT images. In the second dose group (0.02 mg/kg), all three lesions were detected by DOT. No lesions were detected in the two highest dose groups (0.05 and 0.1 mg/kg). In total, five of the ten malignant lesions (50%) were visualized by DOT using the fluorescent imaging agent. The locations of lesions detected with DOT showed excellent agreement with MRI. Lesion location on the optical images was reproducible over time (Figure 4). Optimal lesion-to-background signals were obtained after eight hours, ranging from 1.8 to 2.8 for the detected lesions. Non-specific fluorescent

of ≥ 2 (at least moderate contrast) as a cut-off. Between 0% and 22% false positive results were found per wavelength for both observers.

Intra- and inter-observer agreements for the qualitative scores were excellent. The intra-observer agreement was assessed with three months between the readings, and the intraclass correlation coefficient was 0.978 for Observer 1 and 0.987 for Observer 2, whereas the kappa statistic was 0.88 for both observers (combining the data from all wavelengths). With regard to interobserver agreement, the intraclass correlation coefficients varied between 0.96 and 0.98 over the four wavelengths and the kappa statistics varied between 0.77 and 0.95.

Study 2. Spectroscopic analysis of benign cysts
A total of 20 benign cysts were included in the study. Maximum lesion sizes ranged from 8 to 40 mm (median size 21 mm).

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enhancement of glandular tissue was clearly visible on all optical fluorescence images, starting after 30 minutes and still evident 24 hours later (Figure 4). The imaging agent signal evidently increased with dose. Higher concentrations were problematic for the current reconstruction algorithm. This algorithm assumed absorption by the imaging agent to be significantly lower than tissue absorption, an assumption that did not hold true for the higher doses. The absorption images obtained with DOT before the contrast administration showed higher attenuation in the lesions than in the surrounding normal parenchyma (mean lesion-to-background ratios 1.4 - 2.6).

No adverse events related to the study agent were observed during this study.

**Discussion**

In our first system evaluation studies we have shown that, based on intrinsic breast tissue contrast alone, the DOT system was able to visualize cysts and elucidate their high water and low total hemoglobin content by spectroscopic analysis, and has the potential to discriminate malignant from benign breast tissue by assessing optical properties of the tissue in a reproducible quantitative and qualitative way. Using a low dose of the fluorescent imaging agent Omocyanine, the DOT system has the potential to safely visualize malignant breast tumors in patients.

During the course of these studies, we encountered several limitations of our experimental DOT system. First, the system was unable to visualize some breast lesions located close to the chest wall. These lesions were most likely physically located too far above the upper optical fibers in the cup to influence the light pathways. Advances in cup geometry are feasible and would result in improved visualization of these lesions.

Secondly, the spatial resolution of DOT is poor, resulting in a lower signal-to-noise ratio and limited detectability for small lesions. Lesion detection seems to be more difficult and size measurement less precise in the center of the cup compared to the edge of the cup, because longer light pathways decrease spatial resolution. This may be a limitation in large breasts with centrally located lesions. Optical data acquisition using slab geometry with slight breast compression could offer a solution to this problem.

Cancer detection rates in our study were between 60% and 80% for each wavelength separately, using a cut-off value of 2 (at least moderate contrast visible compared to surrounding structures).

We have to note that we used knowledge of lesion localization from MRI for the optical data interpretation, which may have resulted in limited false positive findings with consequent overestimation of the ROC analyses. The detection rates and false positive results can probably be improved when combining information of four different wavelengths in one model.

At present, our spectroscopic model works well for cysts but is still being optimized for different lesion types. However, based on current literature, the diagnostic performance of optical imaging without imaging agent is likely inadequate for clinical application [17, 18]. We think that the development of imaging agents that target specific molecular changes associated with breast cancer formation will provide the opportunity for clinical success of optical breast imaging.

With the use of target-specific imaging agents (i.e. molecular imaging), optical imaging could be a valid candidate for the early detection of breast cancer, e.g. in young women with dense breasts who are at increased risk for breast cancer and for whom X-ray mammographic screening has very limited sensitivity due to the tumor-camouflaging projection of this dense glandular tissue [7, 19]. NIR light is likely to be far less hampered by this glandular tissue. Other potential applications of this technique may be the selection of appropriate adjuvant treatment and evaluation of response to such treatment in breast cancer patients.

Important advantages of optical imaging in the molecular imaging arena are that it uses no radioactive components (unlike PET and SPECT), and that its sensitivity for probe detection is very high (possibly in the nanomolar to the 100 picomolar concentration range) as compared with MRI (micromolar to millimolar range).

As of now, however, no targeted optical imaging probe is available for clinical use. This is in contrast to PET or SPECT, where various radioligands are available, for example targeting human epidermal growth factor receptor 2 [HER2], epidermal growth factor receptor [EGFR], and carcinoembryonic antigen [CEA] [20-22]. On the other hand, optical probes are abundantly used in preclinical research settings [23-25].
Critical hurdles in the introduction of such probes for human use include molecular target identification and translation of preclinical evaluated probes to the clinical setting. In the Netherlands, efforts are being made within the Center for Translational Molecular Medicine to develop new optical molecular imaging agents for breast cancer. These agents will be evaluated in an experimental setting using both animal models and the new DOT system, aiming for eventual translation to a clinical setting (MAMMOTH project) [26].

In conclusion, we have described some first steps in the evaluation of a new experimental system for diffuse optical tomography of the breast. Further developments in system design and relevant molecular imaging agents could eventually allow for this technique to be used in routine clinical practice.

References


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