Optical mammography combined with fluorescence imaging: lesion detection using scatterplots

Anaïs Leproux,1,* Marjolein van der Voort,1 Martin B. van der Mark,1 Rik Harbers,1 Stephanie M. W. Y. van de Ven,2 Ton G. van Leeuwen1

1Minimally Invasive Healthcare Group, Philips Research, High Tech Campus 34, 5656AE Eindhoven, the Netherlands.
2Department of Radiology, University Medical Center Utrecht, Heidelberglaan 100, 3508GA Utrecht, the Netherlands.
3Biomedical Engineering and Physics, Academic Medical Center, University of Amsterdam, P.O. Box 227700, 1100 DE Amsterdam, the Netherlands.

*aleproux@uci.edu

Abstract: Using scatterplots of 2 or 3 parameters, diffuse optical tomography and fluorescence imaging are combined to improve detectability of breast lesions. Small or low contrast phantom-lesions that were missed in the optical and fluorescence images were detected in the scatterplots. In patient measurements, all tumors were visible and easily differentiated from artifacts and areolas in the scatterplots. The different rate of intake and wash out of the fluorescent contrast agent in the healthy versus malignant tissues was also observed in the scatterplot: this information can be used to discriminate malignant lesion from normal structures.

©2011 Optical Society of America

OCIS codes: (170.3880) Medical and biological imaging; (170.6280) Spectroscopy, fluorescence, luminescence; (170.6960) Tomography; (170.3830) Mammography.

References and links

1. H. Rinneberg, D. Grosenick, K. T. Moesta, H. Wabnitz, J. Macke, G. Wubbeler, R. Macdonald, and P. Schlag, “Detection and characterization of breast tumours by time-domain scanning optical mammography,” Opto-Electron. Rev. 16(2), 147–162 (2008).

2. D. Grosenick, K. T. Moesta, M. Möller, J. Macke, H. Wabnitz, B. Gebauer, C. Stroszczyński, B. Wassermann, P. M. Schlag, and H. Rinneberg, “Time-domain scanning optical mammography: I. Recording and assessment of mammograms of 154 patients,” Phys. Med. Biol. 50(11), 2429–2449 (2005).

3. S. P. Poplack, T. D. Tosteson, W. A. Wells, B. W. Pogue, P. M. Meaney, A. Hartov, C. A. Kogel, S. K. Soho, J. J. Gibson, and K. D. Paulsen, “Electromagnetic breast imaging: results of a pilot study in women with abnormal mammograms,” Radiology 243(2), 350–359 (2007).

4. A. Corlu, R. Choe, T. Durduran, M. A. Rosen, M. Schweiger, S. R. Arridge, M. D. Schnall, and A. G. Yodh, “Three-dimensional in vivo fluorescence diffuse optical tomography of breast cancer in humans,” Opt. Express 15(11), 6696–6716 (2007).

5. K. Licha, B. Riefke, V. Ntziachristos, A. Becker, B. Chance, and W. Semmler, “Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: synthesis, photophysical properties and spectroscopic in vivo characterization,” Photochem. Photobiol. 72(3), 392–398 (2000).

6. D. J. Hawrysz and E. M. Sevick-Muraca, “Developments toward diagnostic breast cancer imaging using near-infrared optical measurements and fluorescent contrast agents,” Neoplasia 2(5), 388–417 (2000).

7. X. Intes, J. Ripoll, Y. Chen, S. Nioka, A. G. Yodh, and B. Chance, “In vivo continuous-wave optical breast imaging enhanced with Indocyanine Green,” Med. Phys. 30(6), 1039–1047 (2003).

8. A. Hagen, D. Grosenick, R. Macdonald, H. Rinneberg, S. Burock, P. Warnick, A. Poellinger, and P. M. Schlag, “Late-fluorescence mammography assesses tumor capillary permeability and differentiates malignant from benign lesions,” Opt. Express 17(19), 17016–17033 (2009).

9. R. Cubeddu, G. Canti, A. Pifferi, P. Taroni, and G. Valentini, “Fluorescence lifetime imaging of experimental tumors in hematoporphyrin derivative-sensitive mice,” Photochem. Photobiol. 66(2), 229–236 (1997).

10. S. van de Ven, A. Wietzoff, T. Nielsen, B. Brendel, M. van der Voort, R. Nachabe, M. Van der Mark, M. Van Beek, L. Bakker, L. Fels, S. Elias, P. Luijten, and W. Mali, “A novel fluorescent imaging agent for diffuse optical tomography of the breast: first clinical experience in patients,” Mol. Imaging Biol. 12(3), 343–348 (2010).
11. B. Chance, S. Nioka, J. Zhang, E. F. Conant, E. Hwang, S. Briest, S. G. Orel, M. D. Schnall, and B. J. Czerniecki, “Breast cancer detection based on incremental biochemical and physiological properties of breast cancers: a six-year, two-site study,” Acad. Radiol. 12(8), 925–933 (2005).

12. L. Bakker, M. van der Mark, M. van Beek, and M. van der Voort, "Optical Fluorescence Imaging of Breast Cancer," in Proceedings of IEEE Conference on Biophotonics, Nanophotonics and Metamaterials (Institute of Electrical and Electronics Engineers, New York, 2006), pp. 23-25.

13. E. Scherleitner and B. G. Zagar, “Optical tomography imaging based on higher order Born approximation of diffuse photon density waves,” IEEE Trans. Instrum. Meas. 54(4), 1607–1611 (2005).

14. T. Nielsen, B. Brendel, R. Ziegler, M. van Beek, F. Uhlemann, C. Bontus, and T. Koehler, “Linear image reconstruction for a diffuse optical mammography system in a noncompressed geometry using scattering fluid,” Appl. Opt. 48(10), D1–D13 (2009).

15. R. M. Mann, Y. L. Hoogeveen, J. G. Blickman, and C. Boetes, “MRI compared to conventional diagnostic work-up in the detection and evaluation of invasive lobular carcinoma of the breast: a review of existing literature,” Breast Cancer Res. Treat. 107(1), 1–14 (2008).

1. Introduction

Breast cancer detection using optical imaging methods alone is hampered by a lack of specificity of the intrinsic optical properties and chromophore concentrations in breast tissue [1–3]. Indeed, the endogenous contrast from angiogenesis in tumors is nonspecific to cancer. Therefore, in case of small tumors or tumors in dense breast tissue this contrast is expected to be low. Furthermore, diffuse optical imaging has relatively low spatial resolution (on the order of 5–10 mm). This combination of low contrast and low spatial resolution of the diffuse optical methods results in limited sensitivity and specificity for lesion detection and characterization, especially in case of small lesions and lesions located in dense breast tissue. The use of fluorescent contrast agents may increase the sensitivity and specificity of lesion detection and subsequently could provide a better and earlier diagnosis [4–8]. In the case of blood-pool contrast agent, the intravenously injected fluorescent molecules may preferentially accumulate in diseased tissue, because of firstly, an increased blood content due to tumor angiogenesis and secondly, leaky blood vessels in tumors due to damaged endothelial lining. In addition, the agent may have different decay properties in diseased tissue compared to normal tissue. This pharmacokinetic behavior could be used to localize tumors independently of the concentration of the fluorescent molecule [9].

In a clinical trial performed in 2007, a cyanine-based fluorescent dye (Omocianine) using the Philips Diffuse Optical Tomography (DOT) system dedicated for breast imaging has been evaluated [10]. The fluorescent contrast agent, Omocianine, circulates in the blood stream. Thus, the concentration of the contrast agent in normal tissue can be assumed proportional to the blood concentration. Further, the absorptions of the four wavelengths that were used by the DOT system (690 nm, 730 nm, 780 nm and 850 nm) are sensitive to blood content. Hence, the more blood in breast tissue, the higher the absorption and the more fluorescence is emitted. Since malignant tumors have a higher permeability of their blood vessel walls compared to healthy tissue, the contrast agent tends to accumulate at the tumor location. Therefore, at equal amounts of blood, and hence equal absorption by blood, the fluorescence is higher in tumors than in healthy tissue. The study showed that DOT was feasible and safe for breast cancer visualization in patients, using low doses of Omocianine [10]. However, a serious limitation was the fact that the contrast agent Omocianine is a non-targeted fluorescent dye, causing fluorescent enhancement of normal tissues. In addition, the reconstruction algorithms produced artifacts in the fluorescence and absorption data, which might lead to clinical misinterpretation of the DOT images.

In this study, the goal is to increase the lesion visibility in DOT by combining fluorescence and optical absorption data at the voxel level in one single graph, a scatterplot. This concept was introduced by Chance et al. in optical imaging of breast cancer [11]. They plotted the mean percentage of oxygen desaturation of blood versus the mean blood volume of their patients into a graph. Due to angiogenesis and high metabolism of cancers, data from breasts with cancer were found at high blood volume and high percentage of oxygen desaturation of blood, corresponding to the upper right portion of the graph. Data from cancer
free breasts accumulated in the lower left portion of the graph. Using this concept, we hypothesize that a scatterplot of the fluorescence versus the absorption will improve the separation between malignant and normal tissue within one breast. In this scatterplot, a parameter space is shown in which each dot corresponds to a single voxel in the breast. It should be emphasized that the parameter space does not show an image of the breast. Instead, the dimensions of the space correspond to physical parameters, fluorescence and absorption at a given wavelength. Then, the “grey level” of a voxel in the fluorescence image and the “grey level” of the same voxel in the absorption image together are the coordinates of the corresponding dot in the scatterplot.

In addition, we aim to improve the specificity and sensitivity of the DOT procedure even further by adding a third dimension to the scatterplot, such as the absorption image of another specific wavelength or total hemoglobin concentration. The fluorescence and absorption datasets are reconstructed with 2 different algorithms. Therefore, the reconstruction artifacts in the absorption and fluorescence images are unrelated. Thus, in the fluorescence-absorption scatterplot, we hypothesize that the signal from cancerous tissue will be enhanced, while the signals from artifacts will remain unchanged.

The aim of this paper is to validate a method for breast cancer imaging using fluorescence and optical absorption tomography combined in a scatterplot. The article is organized as follows: the DOT system, the phantom experiments and clinical measurements, and the scatterplot are described first. In the Results section, phantom measurements are used to validate the method under controlled circumstances. Phantom-lesions with a volume down to 0.9 ml and a dye concentration ratio between lesion and background down to 2 are studied. Next, the increase of lesion visibility and the discrimination of structures from tumors in 5 patients, 6 lesions, are investigated. Due to the physiology of breasts, more anatomical structures are visible than in the phantoms. The pharmacokinetics of the cancerous and healthy tissues are compared in scatterplots. Artifacts are discriminated from the lesions in the scatterplots. In conclusion, scatterplots provide a better way to combine fluorescence and absorption data, leading to improved lesion detection and better discrimination between malignant and other structures.

2. Materials and methods

2.1. Instrument

The Philips DOT system used in this study combines a transmission mode and a fluorescence mode [10,12]. During a measurement, the patient, who received an intravenous injection of contrast agent, is lying in a prone position on the system bed with her breast suspended in a cup. A total of 507 optical fibers, 253 source and 254 detectors, were mounted alternatively on the surface of this measurement cup. The breast was illuminated sequentially from all sides with continuous wave light from solid-state lasers at four different near-infrared wavelengths (690, 730, 780, and 850 nm). The light emanating from the breast is detected simultaneously from all sides for each source position. To obtain optical coupling between fibers and breast and to prevent optical shortcuts around the breast, the cup is filled with a matching fluid, whose optical properties (absorption and scattering) are similar to the average optical properties of breasts.

In transmission mode, data are collected for the four wavelengths. In fluorescence mode, the fluorophore is excited at 730 nm and the fluorescent emission is collected by filtering out the excitation light in the detection path. The duration of the measurement is 9 minutes per breast; 4 minutes in total for the 4 transmission scans, and 5 minutes for the fluorescence scan. The detected signals are reconstructed into three-dimensional absorption images, one image per wavelength, and into a three-dimensional image of the distribution of the fluorescent contrast agent. Both the reconstructions of the absorption and fluorescence images use algorithms based on first-order perturbation theory [13,14].
2.2. Fluorescent contrast agent

Omocianine was injected intravenously. Immediately after injection, the agent circulates in the blood vessels and can then be found mainly in the blood. Over time, it is progressively washed out of the body by renal clearance. Since blood vessel walls in malignant tumors have a higher permeability compared to the blood vessels in healthy tissue, the contrast agent tends to permeate through the leaky vessel walls and to accumulate at the tumor location. The results of the clinical study showed lesion-to-normal contrast ranging from 1.8 to 2.8 [10], with the contrast calculated by dividing the mean value in the lesion by the mean value of the background excluding the areola.

The absorption and emission wavelength bands of Omocianine are presented in Fig. 1. The wavelength 730 nm is used as excitation wavelength for the Omocianine.

![Normalized absorption, solid line, and emission, dotted line, spectra of Omocianine dissolved in human serum. The 730 nm wavelength used as excitation is indicated by the vertical line.](image)

2.3. Phantom

We used 3 different double-cone shaped phantom-lesions, with a diameter of 20, 15 and 10 mm and corresponding volumes of 2.1, 0.9 and 0.3 ml. According to the Susan G. Komen Foundation, the usual lesion-size detected with mammography ranges from 12 to 37.5 mm. Our phantom-lesions are in the lower range of the usual detection size of the mammogram. During a measurement, the phantom-lesion is suspended by a thin thread, invisible for DOT, at about half way down the cup and slightly decentered. To obtain contrast and thus mimic the specific extravasation of the dye in the tumor, higher concentration of contrast agent is used in the phantom-lesion than in the background. This set-up mimics a homogeneous breast held in the measurement cup with a single lesion in which the contrast agent has accumulated.

The 3 phantom-lesion sizes can have different dye concentration and phantom-lesion-to-background concentration ratios, presented in Table 1. The highest concentration ratio, 5, is used for validation purposes as this ratio will enable clear lesion visibility in the images. The other ratios, 2.5 and 2, are in the range of the typical lesion-to-normal contrasts of 1.8 to 2.8 seen clinically with this scanner [10].

| Table 1. Contrast agent concentrations (nM) in phantom-lesion and background for various lesion-to-background contrasts |
|--------------------------------------------------|
| Lesion-to-background concentration ratio | 5 | 5 | 5 | 2.5 | 2 |
| In lesion | 50 | 25 | 10 | 25 | 10 |
| In background | 10 | 5 | 2 | 10 | 5 |
2.4. Patients

We investigated the data of 5 patients previously scanned with our optical fluorescence tomography system during a clinical trial. The study protocol was approved by the ethics committee, and written informed consent was obtained from all patients. A total of 6 lesions, 1 invasive lobular carcinoma and 5 invasive ductal carcinomas, were confirmed pathologically with core biopsy. Patient 4 had bilateral lesions. Patients’ information is shown in Table 2. Since the goals of the clinical study included the assessment of the diagnostic efficacy and target dose of the fluorescent contrast agent Omocianine, different doses of drug were administered to different patient groups.

In the 5 patients, baseline optical images were acquired for both breasts. Then Omocianine was injected intravenously in the patient. After injection, optical images were acquired up to 24 hours, with 8 and 5 imaging time points for the ipsilateral and contralateral breasts, respectively. The ipsilateral breast was scanned immediately, 30 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 8 hours, and 24 hours after the dye injection. The contralateral breast was scanned 1 hour, 2 hours, 4 hours, 8 hours and 24 hours after dye injection.

Table 2. Patients’ information

| Patient | Age | Contrast agent dose (mg/kg) | Lesion type | Largest lesion size on MRI (mm) |
|---------|-----|----------------------------|-------------|--------------------------------|
| 1       | 81  | 0.01                       | ILC         | 29                             |
| 2       | 59  | 0.01                       | IDC         | 18                             |
| 3       | 74  | 0.02                       | IDC         | 24                             |
| 4*      | 40  | 0.02                       | IDC and IDC| 74 and 10                      |
| 5       | 55  | 0.02                       | IDC         | 34                             |

*IDC – Invasive Ductal Carcinoma; ILC – Invasive Lobular Carcinoma
*Bilateral lesions

2.5. Determination of total hemoglobin concentration

For the spectral analysis, it was assumed that the total absorption of the probed sample is a linear combination of the different amount of absorption by the 4 main chromophores present in the breast: deoxy-hemoglobin (Hb), oxy-hemoglobin (HbO2), water and lipid. Their known absorption spectra allowed us to derive their concentrations from the 4 wavelengths. Then, the total hemoglobin concentration was determined by adding the calculated concentrations of oxy-hemoglobin and deoxy-hemoglobin.

2.6. Scatterplots

In scatterplots, a parameter space composed of 3-D images of the same object is plotted in a graph. Each dot in the scatterplot will thus correspond to one voxel in the image. The dimensions of the parameter space are physical parameters; in this study, the fluorescence is first plotted as a function of the absorption at 690 nm. The accumulation of contrast agent at the tumor location likely increases the fluorescence signal in tumors, even in case of poor lesion-to-normal contrast in absorption due to angiogenesis. Hence, lesions are expected to correlate with high fluorescence, corresponding to the upper portion of the scatterplot of fluorescence versus absorption. The scatterplot can then be extended with additional information, for instance in the form of a color-scale. For this, the total hemoglobin concentration will be used in the scatterplot of patient measurements. Indeed, the main absorber in tumors is hemoglobin. In case of phantom measurements, hemoglobin is absent, and the increase in absorption is due to the higher concentration of contrast agent in the phantom-lesion as compared to the background. Figure 1 shows that the absorption peak of the fluorescent contrast agent is located around 760 nm. Of our available laser wavelengths, 780 nm is the wavelength that gives the highest absorption value of Omocianine. Hence, the reconstructed absorption at 780 nm is used as third parameter in scatterplots of phantom measurements. Following this approach, the third dimension in the scatterplots is then related.
to the most prominent absorber of the lesion, both for the patient and phantom measurements. For the use of 3 parameters, the scatterplot will be named 3-Parameter (3-P) scatterplot.

The scatterplot is a technique to compare different parameters from multiple 3-D images of the same measurement. However, the information of the spatial location of the voxels is not preserved in the scatterplot itself. Nevertheless, any areas from the scatterplot can be correlated to voxels in the 3-D images, and vice-versa.

3. Results

3.1. Phantom measurements

In Fig. 2 (a), an example of the typical morphology of the scatterplot of fluorescence versus absorption at 690 nm from a phantom measurement can be seen. The dots located in the upper right portion of the graph and in the form of a tail correspond to the phantom-lesion. As expected, they are located at high fluorescence and high absorption at 690 nm. Figures 2 (b) and (c) show the reconstructed fluorescence and absorption at 690 nm images, respectively, for the same phantom measurement. The arrow highlights that each dot in the scatterplot corresponds to one corresponding voxel in the 3-D images.

![Fig. 2. Measurement of the medium size phantom-lesion, with dye concentrations of 50 and 10 nM for the lesion and background, respectively. (a) Scatterplot of the fluorescence versus the absorption at 690 nm. (b) Fluorescence image (arbitrary units). (c) Absorption image at 690 nm (mm⁻¹). Every dot in the scatterplot, (a), corresponds to a voxel in the fluorescence and absorption images in (b) and (c), respectively.](image)

Figure 3 presents the 3-P scatterplot of the same phantom measurements. As expected, the dots corresponding to the phantom-lesion voxels show high absorption at 780 nm. Similar results were observed for the large size phantom-lesions with any dye concentration and any concentration ratio between lesion and background.

The scatterplots present the signal strength of one parameter per voxel as a function of the signal strength of another parameter measured for the same voxel. Hence, spatial information, and consequently information on lesion size is not present in the scatterplots. However, the location of the lesion in the breast can be determined from the scatterplots by correlating lesion dots in the scatterplot to the voxels in the image.

In case of medium and small size phantom-lesions, similar results were observed except in four measurements. These 4 measurements correspond to 2 situations with both the medium and small phantom-lesions: first, with low lesion-to-background concentration ratio, i.e. 2, then with low dye concentration in lesion and background, i.e. 10 and 2 nM, respectively. In
Fig. 3. 3-P scatterplot of the fluorescence versus the absorption at 690 nm and with the absorption at 780 nm in a form of a color-scale, of the measurement of the medium size phantom-lesion, dye concentrations of 50 and 10 nM for the lesion and background, respectively. The dots corresponding to the lesion-voxels show high absorption at 780 nm.

Fig. 4. Measurement of the small phantom-lesion, with dye concentrations of 10 and 5 nM for the lesion and background, respectively. (a) Scatterplot of the fluorescence versus absorption at 690 nm. (b) Absorption image at 690 nm (mm$^{-1}$). (c) Fluorescence image (arbitrary units). The green voxels in (c) correspond to the suspected area circled with the dashed line in (a). (d) 3-P scatterplot of the fluorescence versus the absorption at 690 nm and with the absorption at 780 nm in a form of a color-scale. The area with voxels correlating to the phantom-lesion position is encircled. (e) Fluorescence image (arbitrary units). The voxels in red show the phantom-lesion position and correspond to the dots circled in (d).

these 4 measurements, the scatterplot did not show a tail shaped extension pointing at high fluorescence and high absorption values. In addition, the lesions were not visible in the
fluorescence image or in the absorption images. Figure 4 shows an example of a lesion missed in the fluorescence versus absorption at 690 nm scatterplot, (a), and in the absorption, (b), and fluorescence, (c), images. An area with high fluorescence signal in the scatterplot and circled with the dashed line in Fig. 4 (a) can be suspected to be the phantom-lesion. The voxels correlating with this area are shown in green in the fluorescence image in Fig. 4 (c): they do not correspond to the pre-defined phantom-lesion position which is half way down the cup and slightly decentered. However, in the 3-P scatterplot in Fig. 4 (d), an area is present with high absorption at 780 nm in the portion of the graph with relatively high fluorescence correlated. Indeed, the voxels correlated with this area in the 3-P scatterplot are located at the pre-defined position of the phantom-lesion in the cup in Fig. 4 (e). All the phantom-lesions were detected using the scatterplots; Table 3 summarizes the scatterplot in which the phantom-lesions were observed.

| Table 3. Summary of the lesion visibility in scatterplot of phantom measurements per lesion size |
|--------------------------------------------------|
|  | “Lesion-to-background” concentration ratio |
|  | 5 | 5 | 5 | 2.5 | 22 |
| Lesion | 50 | 25 | 10 | 25 | 10 |
| Background | 10 | 5 | 2 | 10 | 5 |
| Concentrations of contrast agent (nM) | |
| Lesion visibility in scatterplot per lesion size | |
| Large | 2-P | 2-P | 2-P | 2-P | 2-P |
| Medium | 2-P | 2-P | 3-P* | 2-P | 3-P* |
| Small | 2-P | 2-P | 3-P* | 2-P | 3-P* |

|  | 2-P – lesion visible in the scatterplot of fluorescence and absorption at 690 nm; 3-P – lesion visible only in the scatterplot of fluorescence, absorption at 690 nm and absorption at 780 nm; * the phantom-lesion is not visible in the fluorescence and absorption images. |

In Fig. 5 (a), sparse dots around the main shape of the scatterplot are selected and highlighted in red. The corresponding voxels in the fluorescence image are shown in red in Fig. 5 (b). These voxels are artifacts that were caused by the reconstruction algorithm and occurred at the fiber positions. It can be observed in the scatterplot in Fig. 5 (a) that the appearance of the fiber artifacts is very distinct from the appearance of the rest of the scatterplot: the dots do not seem to accumulate together unlike the lesion dots.

In Fig. 6, the scatterplots of the ipsilateral, (a), and contralateral, (b), breasts of patient 3 are presented. The corresponding fluorescence images of both breasts are shown in Figs. 6 (c) and

3.2 Patient measurements

In Fig. 6, the scatterplots of the ipsilateral, (a), and contralateral, (b), breasts of patient 3 are presented. The corresponding fluorescence images of both breasts are shown in Figs. 6 (c) and
The red and green arrows are pointing at the lesion and areola, respectively. The fluorescence data used in the scatterplots was acquired 8 hours after the injection of the contrast agent. The lesion is observed in an extension of the scatterplot: the dots corresponding to the lesion voxels are highlighted in red. Compared to phantom measurements a new feature, highlighted in green and located at high fluorescence and high absorption at 690 nm, appeared in the scatterplot: the areola. Without the extension due to the areola, the scatterplot morphology is close to the morphology of scatterplots observed in phantoms. In patient 4 with bilateral lesions, a scatterplot similar to the scatterplot of Fig. 6 (a) was observed for both breasts.

Figure 7 presents the scatterplots of the ipsilateral and contralateral breasts of the same patient with fluorescence data acquired at different time points after the injection of contrast agent: 1 hour 30 minutes, 4 hours, 8 hours and 24 hours after injection. It can be observed that at 1 hour 30 minutes, in Fig. 7 (a), the dye has not yet accumulated in the tumor of the ipsilateral breast. Then, at 4 hours after dye injection, in Fig. 7 (c), the lesion was visible in the scatterplot of the ipsilateral breast. The fluorescence signal from the areola was still increasing compared to the 1 hour and half measurement. At 8 hours after dye injection, in Fig. 7 (e), in the ipsilateral breast, the fluorescence signal from the lesion was similar to the 4 hours post injection measurement, while the fluorescence signal from the areola was already decreased. In the contralateral breast, the fluorescence signal in the areola seems to increase until 8 hours after dye injection, in Figs. 7 (b), (d) and (f). Finally, 24 hours after injection, in Figs. 7 (g) and (h), the contrast agent was considerably washed out from both breasts. No structure similar to the lesion is observed in the scatterplots of the contralateral breast. From the scatterplots at the different time points, the specific pharmacokinetics of the lesion can clearly be observed. Note in Figs. 7 (c) and (e) that the tail shaped extension corresponding to the lesion points at lower absorption values as compared to normal tissue. Out of the 5
Fig. 7. Patient 1. Scatterplots at different time points for the ipsilateral, middle column, and contralateral, right column, breasts. The fluorescence data of these scatterplots were acquired at the time after injection of contrast agent indicated in the left column. The lesion dots are encircled with the solid line and the areola dots with the dashed line.

patients, this patient stands alone by having this type of scatterplot morphology in the ipsilateral breast.

Figure 8 presents the 3-P scatterplots, including total hemoglobin concentration, of the ipsilateral breasts of patients 2 and 5. The fluorescence data used in these scatterplots were acquired 8 hours after the injection of the contrast agent. For these patients, 2 and 5, the signal from the areola was dominant over the signal from the lesion. However, the lesions were visualized in the 3-P scatterplot.
Fig. 8. 3-P scatterplots of the fluorescence at 8 hours after dye injection versus the absorption at 690 nm and with the total hemoglobin concentration (arbitrary units) as colorscale. (a) Ipsilateral breast of Patient 2. (b) Ipsilateral breast of Patient 5. The solid and dashed lines highlight the dots corresponding to the lesion and areola voxels, respectively.

Figure 9 (a) shows an example of false positive structures in the fluorescence image, highlighted with the red and green squares. These structures were not located in a suspicious area of the scatterplot, as can be seen in red and green in Fig. 9 (b); therefore, they can immediately be discriminated from the tumors in the scatterplot.

Fig. 9. Patient 2. (a) Fluorescence image. Structures with high fluorescence are highlighted with the red and green squares. (b) Scatterplot of the fluorescence at 8 hours after dye injection versus the absorption at 690 nm. The dots corresponding to the voxels highlighted in a) are shown in red and green.

4. Discussion

Phantom measurements show that, first, 3-P scatterplots allow us to detect lesions that were not visible in the fluorescence and absorption images. Second, reconstruction artifacts are easily identifiable in the scatterplots. In patients, the lesions as well as artifacts and areola can be identified in specific areas of the scatterplots. The lesions could be discriminated from healthy tissue in scatterplots as a function of time.

4.1. Phantom measurements

In the 15 phantom measurements, all phantom-lesions were detected in the 2-P or 3-P scatterplots, while only 11 lesions were detected in the fluorescence images, see Table 3. The 4 phantom-lesions visible only in the scatterplots correspond to a low ratio of dye concentration in the phantom-lesion and background, small phantom-lesion or low dye concentration. This suggests that scatterplots improve lesion detection in phantom measurements.
Reconstruction artifacts in the fluorescence image located at the rim of the cup -typically at the fiber positions- can limit the clinical interpretation of fluorescence images. However, these artifacts are seen as sparse dots and are very distinct from the dense area of dots corresponding to phantom-lesions in scatterplots. Therefore, they are easily identified in the scatterplots.

4.2. Patient measurements

The 6 investigated patient lesions were all identified in the 2-P or 3-P scatterplots, as shown in Table 3. In patients 1, 2, 3 and 5, we have observed in the scatterplot that the area corresponding to the lesion of the ipsilateral breast does not exist in the scatterplot of the contralateral breast. This confirms the absence of malignancy in the contralateral breast. Patient 4 has bilateral lesions and both lesions were seen in the scatterplots of the measurements of both breasts.

In the scatterplots of all the 5 patients, the maximum absorption value at 690 nm was observed in the areola and the maximum fluorescence value in both the lesion and areola. Plotting the scatterplots for patient 1 over time, we have observed that the temporal variations of the dots corresponding to the lesion-voxels did not correlate with the temporal variations of the dots corresponding with healthy breast tissue, Fig. 7. The fluorescence contrast agent was retained in the tumor location and was thus eliminated from the tumor after the healthy tissue. Therefore, using the specific wash out rate of lesions in scatterplots can help discriminating cancer from healthy tissue.

In the fluorescence images, many structures were visible, as seen for instance in Fig. 9. The areola is sometimes located close to the cup wall and the fiber artifacts can be misinterpreted as the areola in the fluorescence image. However, in the phantom measurements, we have seen that the fiber artifacts at the rim of the cup were easily discriminated from the lesion in the scatterplot. In the absorption images, other types of tissue structures can be expected in patient measurements due to the assumption in the reconstruction algorithm that the scattering is constant over the breast volume. Indeed, the variations of scattering present in breasts are compensated in the reconstructed absorption images. The artifact structures in the fluorescence and absorption images perturb the lesion visualization and therefore the clinical interpretation of the images. Previous research showed that 5 of the 6 investigated lesions were visible in the fluorescence images [10]. However, without the information from the MRI and the pathologic examination, these lesions may have been missed, because of the high number of non-malignant structures in the fluorescence images. Here, we showed that these non-malignant structures were clearly separated from the lesion area in the scatterplot.

The contrast agent Omocianine used in this study does not specifically bind to a cancer-associated target. Therefore, enhancement of other normal tissues is also possible, as we have seen for the areola. Glandular tissue is vascularized and would show fluorescence enhancement that may mask signals from tumors. As we have seen in patient 1, whose breasts were mainly composed of fatty tissue, differences in pharmacokinetics are observed between tumors and normal tissue in the scatterplots plotted as a function of time, see Fig. 7. This method implied acquiring multiple images over several hours. This would become a burden for the patients when using Omocianine which has an uptake of several hours. For patient comfort, the method of detecting lesion in the scatterplot as a function of time should be used preferably with a contrast agent with shorter pharmacokinetics. Note also that for known uptake of a contrast agent, this method would in principle require measurements at only 2 time-points; one measurement after the dye injection and the other measurement at the expected maximum contrast in lesion.

Scatterplots as a function of time could in principle be also used to discriminate between cancerous tissue and glandular tissue. For instance, Fig. 10 presents the average fluorescence signal at 4 and 8 hours in the lesion, in red, and in suspected glandular tissue, in blue, in the
contralateral breast for patient 2. The two regions of interest have different dye uptake; the fluorescence signal in the lesion increases from 4 to 8 hours after dye injection, while the fluorescence signal in the suspected glandular tissue decreases. In patient 5, suspected glandular tissue was observed in the contralateral breast. However, we lacked data covering the washing out of the contrast agent, disabling the discrimination of the suspected glandular tissue from the malignancy. No more suspected glandular tissue was observed in the scatterplots of the other patients. In patients with dense breasts, tumors are frequently located in the glandular tissues. Moreover, it is known that mammography does not perform well with dense breasts. Here, our results suggest that we would be able to detect the presence of an abnormality with the scatterplots as a function of time. Besides, with the knowledge of increased lesion visibility in case of low endogenous lesion contrast, as observed in phantom measurement, one can expect that scatterplots would have enhanced sensitivity for lesion detection in dense breasts compared to the separate absorption or fluorescence images. It would be of great interest to perform a study with dense breasts to further investigate the discrimination between glandular tissue and malignancies.

![Fig. 10. Patient 2. Dye uptake at 4 and 8 hours after the injection of the fluorescent contrast agent in lesion, in red, and in suspected glandular tissue, in blue, of the contralateral breast.](image)

Generally, the lesions in the scatterplot were located at relatively high absorption as compared to the background tissue excluded the areola. In addition, they were pointing towards high fluorescence values. However, in patient 1, the lesion was located at high fluorescence but also at rather low absorption and it was pointing towards low absorption values, Fig. 7. One may think that this effect is due to small lesion sizes for which angiogenesis is just starting or to low lesion-to-background absorption contrast. However, the lesion of patient 1 was not the smallest lesion of the study, see Table 2, and this effect was only observed in this patient. In addition, both lesions of patients 1 and 5 were missed in the absorption images, but the lesion of patient 5 was not located at low absorption values in the scatterplot, as shown in Fig. 8 (b). Interestingly, histopathology reported ILC in patient 1 and IDC for the rest of the patients. While IDC is structured as a lump, ILC is usually diffuse and infiltrates to the surrounding connective tissue [15]. The tumor blood vessels in ILC are thus likely not as compacted as in IDC. While the fluorescent dye extravasates at the tumor location independently of the vessels structures, the increase in absorption due to ILC may then not be locally as strong as in the IDC. This could explain why in the scatterplot, the structure of the ILC was seen at equally high fluorescence value as with IDC but at lower absorption values. This would suggest a potential in lesion characterization of the scatterplots.
4.3. Targeted fluorescent contrast agent and scatterplots

Many research groups are now working on the development of targeted contrast agents. With this new type of contrast agents, only cancerous tissue is expected to show fluorescence enhancement. The morphology of scatterplots of patients will then be certainly different: it might become similar to the morphology of the phantom scatterplots presented in this paper. Therefore, the issue of discrimination of healthy structures from malignancy can be overcome. However, problems with reconstruction artifacts and with small lesions or lesions deep inside the breast will remain. Therefore, the use of 3-P scatterplots has good potential for the next generation of contrast agents for breast cancer detection.

4.4. Conclusion

In conclusion, the combination of the scatterplots and the 3-D fluorescence, absorption and total hemoglobin concentration images improves the detection rate of lesions in DOT.

Acknowledgments

This work is supported by a European Commission Marie Curie contract MEST-CT-2004-007832. The authors are grateful to Tim Nielsen for reconstructing the data. The authors also thank Martin van Gemert (Academic Medical Center, Amsterdam, the Netherlands) and Suzanne van den Berg-Dams (Philips Research, Eindhoven, the Netherlands) for correcting the manuscript.