Time domain diffuse optical spectroscopy: *In vivo* quantification of collagen in breast tissue

Paola Taroni[*1], Antonio Pifferi[1,2], Giovanna Quarto[1], Andrea Farina[1],
Francesca Ieva[3], Anna Maria Paganoni[3],
Francesca Abbate[4], Enrico Cassano[4], Rinaldo Cubeddu[1,2]
[1]Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy;
[2]Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche, Piazza Leonardo da Vinci 32, 20133 Milano, Italy;
[3]Dipartimento di Matematica, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy;
[4]European Institute of Oncology, Breast Imaging Unit, Milano, Via Ripamonti 435, 20141 Italy

ABSTRACT

Time-resolved diffuse optical spectroscopy provides non-invasively the optical characterization of highly diffusive media, such as biological tissues. Light pulses are injected into the tissue and the effects of light propagation on re-emitted pulses are interpreted with the diffusion theory to assess simultaneously tissue absorption and reduced scattering coefficients. Performing spectral measurements, information on tissue composition and structure is derived applying the Beer law to the measured absorption and an empiric approximation to Mie theory to the reduced scattering.

The absorption properties of collagen powder were preliminarily measured in the range of 600-1100 nm using a laboratory set-up for broadband time-resolved diffuse optical spectroscopy.

Optical projection images were subsequently acquired in compressed breast geometry on 218 subjects, either healthy or bearing breast lesions, using a portable instrument for optical mammography that operates at 7 wavelengths selected in the range 635-1060 nm. For all subjects, tissue composition was estimated in terms of oxy- and deoxy-hemoglobin, water, lipids, and collagen. Information on tissue microscopic structure was also derived.

Good correlation was obtained between mammographic breast density (a strong risk factor for breast cancer) and an optical index based on collagen content and scattering power (that accounts mostly for tissue collagen). Logistic regression applied to all optically derived parameters showed that subjects at high risk for developing breast cancer for their high breast density can effectively be identified based on collagen content and scattering parameters.

Tissue composition assessed in breast lesions with a perturbative approach indicated that collagen and hemoglobin content are significantly higher in malignant lesions than in benign ones.

Keywords: Collagen, breast cancer, diffuse optics, absorption, scattering, tissue diagnostics

1. INTRODUCTION TO TIME-RESOLVED DIFFUSE OPTICAL SPECTROSCOPY

Time-resolved diffuse optical spectroscopy (TR-DOS) provides non-invasively the optical characterization of highly diffusive media, such as biological tissues. Light pulses (typically shorter than 100 ps) are injected into the medium and the re-emitted pulses are detected either in reflectance or in transmittance geometry (that is either on the same side as the injected pulses or on the opposite side). The effects of light propagation on pulse shape and time delay can then be interpreted with a suitable theoretical model, most often the diffusion approximation to the radiative transport theory to provide a complete optical characterization of the medium, namely to simultaneously assess the absorption and reduced scattering coefficients (μ_a and μ'_s, respectively) of the medium.

As described in the following, when measurements are performed at several wavelengths, information on the medium composition can be derived from the measured absorption, while the scattering properties provide average information on its microscopic structure.

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Over the last years, diffuse optics, and in particular TR-DOS, has been applied for the characterization of different tissue types, both to investigate physiological situations and to diagnose pathological conditions, especially *in vivo*, taking advantage of the full non-invasiveness of the technique. However, most effort has been devoted to two main clinical applications: functional brain imaging and spectroscopy/imaging for breast cancer[1-4].

Breast cancer is a leading causes of death in women[5]. According to estimates of lifetime risk by the U.S. National Cancer Institute, in the U.S. 1 in 8 women will develop breast cancer in their lifetime[6]. Early diagnosis and therapy significantly reduce mortality. Breast screening essentially relies on x-ray mammography. However, false negatives hamper the efficacy of the screening, while false positives lead to unnecessary treatment (typically core biopsy), with involved physical and psychological morbidity and high costs for the health systems. Even small improvements in diagnosis would have huge impact in terms of spared lives and quality of life, and would allow significant reduction in costs for the healthcare systems. Stemming from all these considerations, initially diffuse optical techniques operating in the near infrared (NIR) have been applied for the non-invasive characterization of breast tissue especially in terms of physiologically relevant blood parameters (blood volume, oxygenation level and blood flow), and specifically for breast cancer detection[7]. More recently, the attention has increasingly being directed also toward other potential applications, and in particular to monitoring the effectiveness of neo-adjuvant chemotherapy and assessing breast density and related cancer risk.

Neoadjuvant chemotherapy is a standard treatment for locally advanced inoperable breast cancer, but it is increasingly being used also for patients with operable cancer. In the latter case, it aims at reducing the tumor size, thus improving surgical options, or even at achieving pathologic complete response. Currently, no standardized modalities and timing for the assessment of an early pathologic response have been established. Optical techniques are showing good promise for monitoring the effectiveness of neoadjuvant chemotherapy and even predicting the therapeutic response[8-10]. Actually, it seems possible to predict the outcome of the neoadjuvant chemotherapy just after the first day of treatment, or even before the start, based on characteristic features of breast tissue that provide indication on its responsiveness to treatment[11-13]. If confirmed, prediction of response might allow avoiding unnecessary drug morbidity and delays in surgery in non-responders patients (namely, approximately 50% of treated patients).

Breast density (i.e. fibrogladular tissue fraction) is a strong independent risk factor for breast cancer, often referred to as the strongest risk factor beside age and gender[14]. Nowadays, it is estimated from the visual inspection or computer analysis of mammographic images. Thus, its knowledge implies the use of ionizing radiation. On the other end, the availability of a non-invasive means for its assessment would allow the early identification of high-risk subjects that could undergo dedicated screening and preventive paths. Breast density depends on the relative amounts of fibrogladular and adipose tissue. Thus, diffuse optical techniques, which are sensitive to tissue composition, could be effectively applied for its non-invasive estimate. In fact, continuous-wave transmittance data collected over a broad spectral range (550-1300 nm) interpreted using principal component analysis allowed the researchers to achieve very good correlation with mammographic features, leading to quantitative estimate of mammographic density by optical means[15].

Up to now most of the diffuse optical studies of breast tissue have focused on blood parameters (total blood volume and oxygen saturation), as hemoglobin absorption can effectively be assessed performing measurements in the NIR range 650-800 nm. Indeed, efficient detectors have long been available for that spectral range, allowing the development of reasonably simple and cheap instruments suitable for clinical use. Now, measurements are becoming more feasible even at longer wavelength (up to > 1 μm). This has favored a more complete investigation of tissue composition that include also lipid and/or water content[16-19].

Collagen is involved in the onset and progression of breast cancer[20]. Powerful information on collagen content and spatial distribution in tissue can effectively be obtained using techniques such as multiphoton microscopy, but they are not suitable for *in vivo* investigation of breast tissue (*e.g.*, Ref. [21] and references therein). The availability of a non-invasive means for collagen assessment could thus prove useful for cancer diagnosis, for monitoring and perhaps predicting the outcome of neoadjuvant chemotherapy, and also for the estimate of cancer risk. Thus, over the last years we have worked for the optical characterization of collagen in the NIR range, and we have included collagen among breast tissue constituents estimated *in vivo* by means of diffuse optical spectroscopy.

2. INSTRUMENTATION FOR TR-DOS

Two different set-ups were developed and used for time domain diffuse optical spectroscopy and imaging studies reported here.

Laboratory set-up for broadband TR-DOS

The system is fully automated for what concerns both data acquisition and analysis.

An actively mode-locked Ti:Sapphire laser provides picosecond pulses from 900 to 1100 nm[22]. To cover shorter wavelengths (600-900 nm), a supercontinuum fiber laser is used. Wavelength selection is achieved through the computer controlled rotation of a Pellin-Broca prism, so that the selected wavelength can be extracted from the dispersed light by coupling it into a 50 μm-fiber optic, placed distal to the prism[23].

Optical fibers deliver the illumination light to the sample and collect the diffusely transmitted or reflected light. Time-correlated single photon counting is used for the detection of the re-emitted pulses.

The overall time resolution of the system varies between 70 and 160 ps, depending on wavelength. For the present study, time-resolved data were collected every 5 nm, with acquisition time of 1 s per wavelength with the supercontinuum fiber laser and 4 s per wavelength with the Ti:Sapphire laser.

Sample. Collagen Type I powder of bovine origin (bovine Achilles tendon) was measured.

Optical mammograph[24]

The instrument is designed to collect projection images in compressed breast geometry, similar to convention x-ray mammography.

Time-resolved transmittance measurements are performed at seven wavelengths (635, 685, 785, 905, 930, 975, 1060 nm) using picosecond pulsed diode lasers. Pulses at the seven wavelengths are time-multiplexed and coupled to a single injection optical fiber.

The compressed breast is raster-scanned continuously, moving the illumination fiber and a collection fiber bundle (on the opposite side of the compression unit) in tandem, and recording transmittance data every millimeter. The distal end of the bundle is bifurcated, and coupled to separate detectors for wavelengths <800 nm and >800 nm, respectively. Two PC boards for time-correlated single photon counting record the time distributions of the transmitted photons at the seven wavelengths.

The acquisition time depends on the overall area of the compressed breast, and is typically of the order of 5 min. Optical images are routinely acquired from both breasts in cranio-caudal and medio-lateral oblique (45°) views.

Patient study. The Institutional Review Board at the European Institute of Oncology approved a clinical study with a twofold aim: i) the non-invasive assessment of breast density by optical means, and ii) the optical characterization of malignant and benign lesions. 218 subjects enrolled. Ninety-five subjects had a malignant lesion and 27 had a benign lesion. Written informed consent was obtained from all the participants.

3. DATA ANALYSIS

Absorption and reduced scattering coefficients (μ_a and μ'_s, respectively) at each wavelength λ are generally estimated by fitting the time distribution of the re-emitted pulses to an analytical solution of the diffusion approximation (with the extrapolated boundary condition) for a semi-infinite medium (reflectance) or an infinite homogeneous slab (transmittance)[25,26].

The Beer law is used to relate the absorption properties to the concentrations of the main tissue constituents (*e.g.*, oxy- and deoxy-hemoglobin, water, lipids, and collagen):

$$\mu_a(\lambda) = \sum_i c_i \varepsilon_i(\lambda) \quad (1)$$

where $\varepsilon_i(\lambda)$ is extinction coefficient and c_i is the concentration of the i-th constituent at the wavelength λ.

From oxy- and deoxyhemoglobin (HbO_2 and Hb, respectively), it is then easy to calculate more widely used physiological parameters, like total hemoglobin concentration $tHb = Hb + HbO_2$ and oxygen saturation $SO_2 = HbO_2/ tHb$.

The scattering properties can be modeled through a simple empiric approximation to Mie theory to provide information on the microscopic structure of the medium[27,28]:

$$\mu'_s(\lambda) = a\left(\frac{\lambda}{\lambda_o}\right)^{-b} \quad (2)$$

where λ_o = 600 nm. a is the scattering coefficient $\mu'_s(\lambda_o)$ and b is the slope of the scattering spectrum. They are known as scattering amplitude and power, respectively. The scattering amplitude is directly related to the concentration of scattering centers (cells, nuclei, and organelles), while the scattering power is inversely related to the size of the scattering centers.

Instead of estimating the optical properties μ_a and μ'_s and subsequently applying Eqs. 1 and 2, a spectrally constrained global fitting procedure can also be exploited, where free parameters are the concentrations of oxy- and deoxy-hemoglobin, water, lipids, and collagen, together with the scattering amplitude a and power b. The latter method is especially convenient when measurements are performed at a limited number of wavelengths (*e.g.*, in the case of the optical mammograph), as it provides increased robustness[29].

For the assessment of breast density, tissue composition and scattering parameters were averaged over the four images (cranio-caudal and oblique views for both breasts) acquired from a patient, thus yielding average information on the breast tissue of that subject. For the discrimination between malignant and benign breast lesions, a perturbative approach based on the high-order calculation of the pathlength of photons inside the lesion was applied to estimate tissue composition in the lesion with respect to the average composition of that breast[30].

4. RESULTS AND DISCUSSION

Absorption properties of collagen

The knowledge of the absorption properties of collagen is needed to estimate collagen content in breast tissue. However, in the NIR spectral range they were not available from the literature. Therefore, as a preliminary step, we performed the optical characterization of collagen from 600 to 1100 nm[31]. The absorption properties of collagen Type I of bovine origin in the NIR range are shown in Figure 1, together with the absorption of the other major tissue constituents and NIR absorbers: *Hb*, HbO_2, lipids, and water.

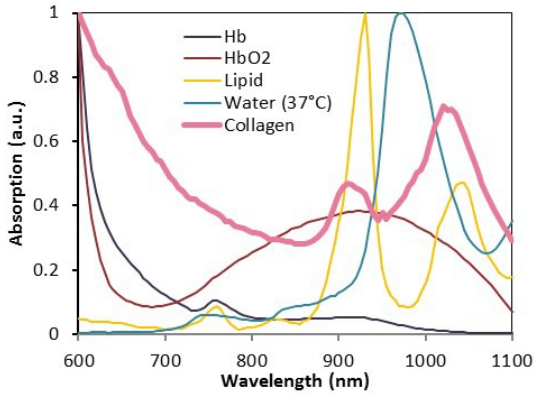

Figure 1. Normalized absorption spectra of Hb, HbO2, lipids, water, and collagen.

As evident from the Figure 1, the absorption peaks of collagen are spectrally close to those of lipids and water. However, overall the spectral shape is different enough to allow the quantification of collagen from Eq. 1 when tissue absorption is measured *in vivo*.

Breast density and cancer risk

Intersubject differences in breast tissue composition (currently quantified by mammographic breast density) can be marked, and depend on a wide range of parameters, including hereditary factors, age, body mass index (BMI), and use of oral contraceptives. In clinical practice, the most common classification of breast density consists in 4 qualitative categories, known as BI-RADS categories and defined as follows[32]: 1 – almost entirely fat; 2 – scattered fibroglandular densities; 3 – heterogeneously dense; and 4 – extremely dense. Collagen is a key constituent of stroma and, more generally of fibroglandular tissue. Thus, breasts belonging to different BI-RADS categories are expected to be characterized by apparent differences in collagen content. This is in line with what obtained measuring breast tissue composition with the optical mammograph on a population of 147 subjects: on average the estimated amount of collagen increases upon increasing BI-RADS category, going from clearly adipose to markedly fibrous breasts, as shown in Figure 2(a). The positive correlation between collagen content and BI-RADS categories suggests that optical measurements could provide an effective means for the assessment of breast density, alternative to presently used x-ray mammography.

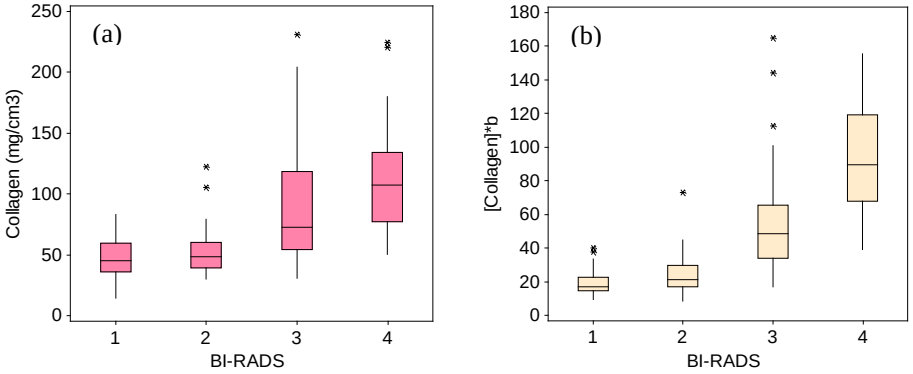

Figure 2. Collagen content (a) and collagen index *CI* (b) as a function of BI-RADS category.

The scattering slope *b* depends strongly on collagen content in tissue, determining very steep scattering spectra for collagen rich-tissues, such as tendon[33]. Moreover, it might also be sensitive to collagen microstructure in tissue, as it is determined by the size of the scattering centers (interfaces at microscopic level) that are present in tissue. Thus, to better account for collagen contribution to breast density, a collagen index *CI* was introduced as:

$$CI = [Collagen] \times b \tag{3}$$

As shown in Figure 2(b), the collagen index improves the correlation with BI-RADS categories. Subsequent BI-RADS categories are characterized by significantly different *CI* values ($p < 0.02$ between category 1 and 2, and $p < 0.0001$ between category 2 and 3, and between category 3 and 4, based on the Mann-Whitney U-test), further supporting the potential of optical measurements for non-invasive assessment of breast density.

Identifying subjects that are at high risk for developing breast cancer because of their high breast density is an important goal. If they were identified early in life, dedicated diagnostic paths could be designed for them, including the use of diagnostic techniques, like MRI, that are not suited for screening. Moreover, the potential use of preventive means (*e.g.*, drugs, changes in diet and lifestyle) could be considered. Deeming BI-RADS categories as the golden standard for breast density assessment, we identified high-risk subjects with subjects in BI-RADS category 4. Logistic regression was applied to optically derived information on tissue composition (blood parameters, lipid, water and collagen) and structure (scattering parameters *a* and *b*) to identify high-risk subjects (BI-RADS category 4). The best logistic regression model turned out to depend only on collagen content and scattering parameters, which in turn depend linearly (*a*) and non-linearly (*b*) on collagen[34]. Thus, collagen proves to be a key tissue constituent for the estimate of density-related risk. The agreement between optical and mammographic identification is promising, with a simple kappa of 0.84, significantly higher than obtained for inter-rater agreement of radiologists assessing the same mammographic images.

It is worth noting that, for its involvement in cancer development, collagen might also represent an independent risk factor. Thus, the sensitivity to collagen (as provided for example by the collagen index *CI*) might prove useful for the direct estimate of cancer risk, more than achieved x-ray mammography, which is mostly sensitive to water content.

Malignant and benign breast lesions

Higher amount of collagen and presence of different types have been detected in breast cancer as compared to healthy tissue, suggesting that collagen could provide useful information for the discrimination between malignant and benign lesions, and allow prediction of breast cancer recurrence and survival in patients[35].

To investigate whether diffuse optical measurements can detect significant differences in breast lesions, and whether such differences can effectively be exploited to discriminate different lesion types, we have applied a perturbative approach to the time domain diffuse optical data collected with our 7-wavelength optical mammograph[30]. This has yielded preliminary results on tissue composition in 33 malignant and 29 benign breast lesions. On average, all lesions (malignant and benign) contain more blood, water and collagen and less lipid than the healthy tissue in the same breast. When the composition of malignant and benign lesions is compared, the difference is statistically significant only for hemoglobin and collagen content, which are both higher in malignant lesions than in benign ones ($p < 0.01$ and $p < 0.04$, respectively). Higher blood content was expected based on the neo-angiogenesis that often characterizes malignant lesions, and higher collagen content is in agreement with changes in architecture and composition of the extracellular matrix that are typical of cancer development and that can be ascribed especially to collagen[35]. Work is now in progress to analyze the full dataset and to determine how optically derived information can best be exploited for the effective identification of malignant lesions.

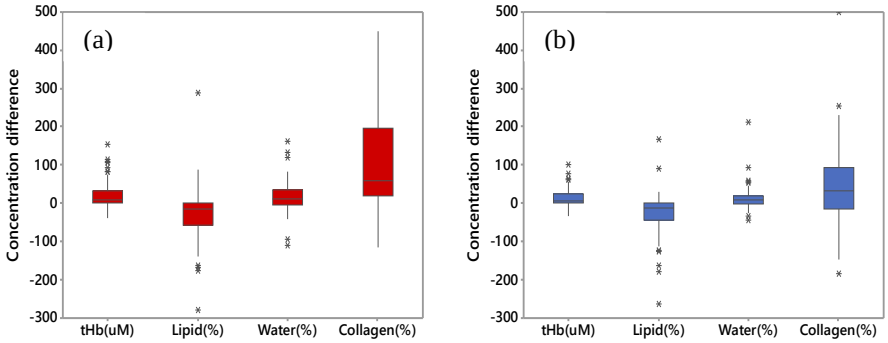

Figure 3. Difference in composition between lesions and average tissue of the same breast for: (a) malignant lesions and (b) benign lesions.

5. CONCLUSION

Time domain diffuse optical spectroscopy is suitable to estimate non-invasively *in vivo* breast tissue composition in terms of oxy- and deoxy-hemoglobin, water, lipids, and collagen, and to provide information on the microscopic structure of breast tissue. This was shown by results obtained in a clinical study performed on 218 subjects.

In particular, collagen proved to be a key tissue constituent for the non-invasive estimate of breast density and for the identification of subjects that are at high risk for developing breast cancer because of their high breast density.

Collagen seems also an essential tissue constituent for the discrimination between malignant and benign breast lesions.

As mentioned above, further data analysis is in progress on the full dataset. Moreover, instrumental development is being carried out to explore the potential of longer wavelengths. An innovative laboratory set up for TR-DOS up to 1700 nm has recently been realized[36]. Work is now ongoing to upgrade an existing portable set-up (600 and 1100 nm)[23], suitable for *in vivo* measurements, and extend its spectral range of operation towards 1700 nm[37]. The optical characterization of collagen is also being extended to 1700 nm[38].

Access to the workstations for the optical characterization of highly scattering media in the 600-1700 nm range can be granted via the LaserLab Europe initiative[39].

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