

Absorption of collagen: effects on the estimate of breast composition and related diagnostic implications

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Abstract. The absorption spectrum of collagen powder is measured between 610 and 1040 nm by time-resolved transmittance spectroscopy. Absorption spectra of breast from healthy volunteers are then interpreted, adding collagen to the other absorbers previously considered (i.e., oxy- and deoxyhemoglobin, water, and lipids). A significant amount of collagen, depending on breast type, is estimated to be present. Adding collagen to the fitting procedure affects remarkably the estimated values of blood content and oxygenation. The quantification of collagen has potential implications for the assessment of breast density and cancer risk. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2699170]

Keywords: absorption spectroscopy; breast; collagen; photon migration; tissue composition; tissue diagnostics.

Paper 06172RR received Jul. 2, 2006; revised manuscript received Sep. 6, 2006; accepted for publication Sep. 6, 2006; published online Mar. 2, 2007.

1 Introduction

Optical techniques are showing increasing potential in medicine and biology, both for characterization and diagnostic purposes. In the last few years, several research groups have suggested and applied optical spectroscopy of breast as a means for the investigation of physiological features, showing that the optical properties are sensitive to parameters such as hormonal status, age, and body mass index.¹⁻⁵ The potential of spectroscopy is also being applied to optical imaging to improve the detection and characterization of breast lesions.^{6,7} The monitoring of neoadjuvant chemotherapy has been recently proposed and showed that optical methods can follow noninvasive changes due to therapy, suggesting a feasible role in the development of personalized therapeutic protocols.⁸ Finally, optical techniques have shown promise for the noninvasive classification of breast density (i.e., parenchymal patterns).⁹

In most cases, the absorption properties are analyzed to derive quantitative information on tissue composition. Some applications, such as muscle oximetry and functional imaging of brain activity, rely on the detection of blood content and oxygenation. Thus, they focus on the two forms of hemoglobin, neglecting other tissue chromophores. More generally, up to four main absorbers are typically involved in this interpretation of tissue absorption data, namely the two forms of hemoglobin, water and lipids.

Collagen is one of the main components of both soft and hard tissues, and in particular of breast tissue. Nevertheless, to our knowledge, neither its optical properties nor its contribution to the optical properties of biological tissues, and breast in particular, have ever been investigated in detail.

By means of a system for time-resolved transmittance spectroscopy, we have measured the absorption properties of

collagen powder from 610 to 1040 nm. The information was then used to account for the presence of collagen among tissue absorbers, when data are interpreted using Beer's law to estimate tissue composition. In particular, we have investigated the contribution of collagen to the absorption spectra of breasts with different parenchymal patterns, and compared results obtained accounting for the presence of collagen and neglecting it.

2 Methods

2.1 Time-Resolved Spectroscopy System

The system is described in detail in Ref. 10. Briefly, a synchronously pumped mode-locked dye (DCM) laser was used as the illumination source from 610 to 695 nm, while an actively mode-locked Ti:sapphire laser provided light in the wavelength range of 700 to 1040 nm. Optical fibers delivered the illumination light to the sample and collected the transmitted light. Time-correlated single photon counting was used for the detection. The time resolution of the system was <120 ps in the red (<700 nm) and <180 ps in the near-infrared (NIR) regions, respectively. Time-resolved data were collected every 5 nm.

2.2 Collagen Measurements

Collagen type 1 powder from bovine Achilles tendon was purchased from Sigma Aldrich and used as obtained. It was placed in a black cylindrical container (10.5 cm diam). Two 1.6-cm-thick samples were measured. The collagen powder was gently pressed, but the resulting samples were not very compact and rather inhomogeneous, principally due to the presence of air gaps. Sample density was 0.196 g/cm³. For each sample, four repeated measurements were performed at each wavelength before tuning the laser to the next wavelength.

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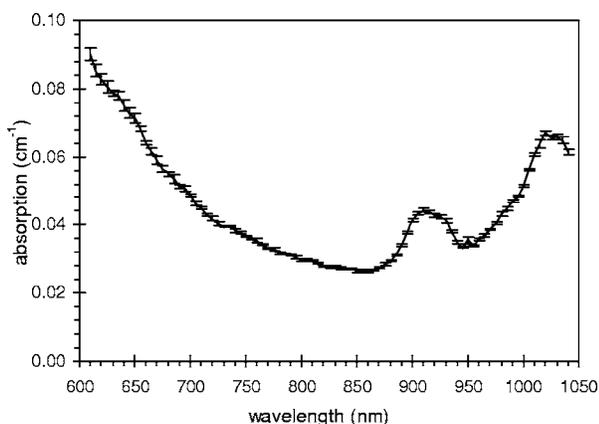


Fig. 1 Absorption spectrum of collagen powder with density of 0.196 g/cm^3 .

2.3 *In Vivo* Breast Measurements

Details on the measurement protocol are reported in Ref. 10. Six healthy volunteers were analyzed. They were sitting in an upright position. The breast was placed between parallel plates, and mild compression was applied. Measurements were performed in transmittance geometry in the upper outer quadrant.

2.4 Data Analysis

The values of absorption and scattering coefficients (μ_a and μ_s' , respectively) were estimated at each wavelength by fitting the experimental time distributions (obtained both from collagen and *in vivo* from breast) to an analytical solution of the diffusion approximation¹¹ with the extrapolated boundary condition¹² for a homogeneous infinite slab. Data points corresponding to a very low number of counts ($<10^3$) were not considered for further analysis and are not shown in the figures.

Based on Beer's law, the absorption spectra of breast were then best fitted in the range 615 to 1040 nm to reference spectra of lipids,¹³ water, oxy- (HbO₂) and deoxy-hemoglobin (Hb),¹⁴ and to the spectrum of collagen to determine the content of each constituent in breast tissue. For comparison, the best fit to four components, neglecting the contribution of collagen, was also performed.

3 Results and Discussion

The absorption spectrum of collagen powder is displayed in Fig. 1. Two peaks are observed around 915 and 1020 nm, respectively. Furthermore, the absorption increases progressively moving toward short wavelengths, suggesting the presence of another intense absorption peak out of the observation range. Absorption values that are always higher than 0.026 cm^{-1} and the presence of specific spectral features indicate that the contribution of collagen is likely to affect the absorption properties of breast tissue in the entire red-NIR wavelength range. The scattering spectrum was also obtained from time-resolved transmittance measurements. It is rather flat, with scattering values around 40 to 45 cm^{-1} . However, due to the different structure of collagen powder and collagen in physiologic conditions, the information on the scattering

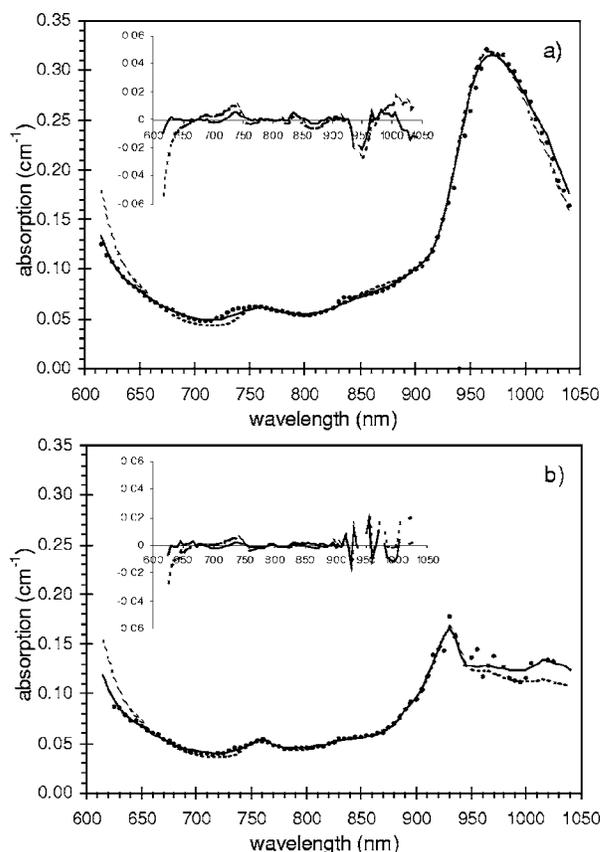


Fig. 2 Absorption spectra of: (a) a dense breast and (b) an adipose breast. Symbols refer to experimental data and lines to best fits, neglecting (fit 1, gray dashed line) and including (fit 2, black solid line) collagen content. The insets show the residuals (gray dashed line for fit 1 and black solid line for fit 2).

properties cannot be exploited for the interpretation of *in vivo* data. It should also be considered that collagen measurements were performed at 20°C , and we cannot exclude an influence of the higher *in vivo* temperature on the optical properties. Moreover, data were acquired from bovine collagen type 1 purchased as a lyophilized powder, the only collagen type that could be obtained in reasonable amounts to perform reliable time-resolved transmittance measurements. Collagen type 1 is of interest because it is abundant in breast and has already been investigated for its potential correlation with cancer development, but both origin and preparation methods might influence its optical properties.

Examples of absorption spectra of breast measured from two volunteers with different breast patterns are shown in Fig. 2. As is evident, main intersubject differences are observed at wavelengths longer than 900 nm. They are typically attributed to the different content of water and lipids, as both of them show strong absorption in that wavelength range. The differences observed are in agreement with the classification of breast density based on x-ray mammography. Actually, the breasts in Figs. 2(a) and 2(b) (subjects 1 and 5, see Table 1 in the following) were classified as dominated by prominent linear and nodular densities and as dominated by adipose tissue, respectively. At long wavelengths, in Fig. 2(b), notwithstanding the lower absorption values than in Fig. 2(a), the noise

Table 1 Content of breast tissue as estimated by fitting only on water, lipid, Hb, and HbO₂ (fit a) or including also collagen powder (fit b).

Subject	1		2		3		4		5		6	
	<i>a</i>	<i>b</i>										
Water (%)	59.7	57.6	52.6	50.0	34.6	30.4	20.9	19.5	14.9	14.9	12.2	12.3
Water (mg/cm ³)	597	576	526	500	346	304	209	195	149	149	122	123
Lipid (%)	7.4	5	15.3	9.5	32.9	29.7	65.6	62.4	71.2	66.6	73.3	67.7
Lipid (mg/cm ³)	67	46	139	87	300	270	597	568	648	606	667	616
Collagen (%)	-	8.5	-	8.5	-	6.3	-	4.8	-	6.1	-	0
Collagen (mg/cm ³)	-	110	-	111	-	82	-	62	-	80	-	1
tHb (μM)	22.7	14.2	16.4	8.1	28.3	22.9	23.0	18.3	20.6	14.6	14.1	14.3
SO ₂ (%)	65.6	77.9	60.4	81.0	76.0	87.1	77.5	86.8	69.4	81.6	79.7	80.1

level is higher due to the bigger breast thickness, which led to a significantly higher light attenuation. The tail of the visible absorption of hemoglobin accounts for increasing breast absorption at short wavelengths, and typically smaller intersubject variations characterize that spectral range.

To quantify the contribution of collagen and its effect on the quality of the fit and on the estimate of constituent concentrations, breast absorption data were interpreted using Beer's law either restricting the fitting parameters to Hb, HbO₂, water, and lipids, or including also collagen. The contribution of collagen is not highly apparent from the fitted absorption spectra of breast, as other absorbers show sharper spectral features that dominate the line shape. However, as estimated from the residuals (shown in the insets of Fig. 2), its presence allows a better fit at short wavelengths, below 800 nm, especially on the tail of hemoglobin absorption, which is clearly overestimated in the absence of collagen, but also around 740 nm, where water shows a secondary absorption peak. Some improvement is observed even above 950 nm, in the region of the main water absorption.

It has to be mentioned that previously published fitted spectra of breast tissue¹⁰ were obtained considering also a wavelength-independent offset as a fitting parameter, in addition to the four main tissue absorbers (Hb, HbO₂, water, and lipids). This was done to account for the presence of collagen and eventual other absorbers of unknown optical properties. The presence of the offset is the reason for the difference between the fitted spectra published previously¹⁰ and those reported here (fit 1 in Fig. 2). If the fitting procedure is performed with both collagen and offset (together with Hb, HbO₂, water, and lipids), the results are similar to those obtained with collagen only, but the fit becomes more unstable, probably due to the exceedingly high number of variable parameters.

Tissue composition estimated from transmittance data with and without collagen is reported in Table 1 for all volunteers. The content of collagen varies among subjects. Less adipose breasts, identified by a low lipid percentage and a correspondingly high water percentage, seem to be characterized by

higher collagen concentrations, 110 to 111 mg/cm³ against 60 to 80 mg/cm³. In one case of a markedly adipose breast, the estimated amount of collagen was negligible.

Considering collagen among the fitting parameters causes only a small reduction (≤6% of breast volume) in the estimated percentage content of water and lipids. On the contrary, considerably lower values are obtained for total hemoglobin content (tHb), with an average reduction of approximately 30%. The decrease is more marked for Hb than for HbO₂, leading to significantly higher oxygen saturation (SO₂) values. Even though data from a low number of subjects is available, both changes seem to be more marked for glandular breasts than for adipose ones.

The concentrations of water, lipids, and collagen in breast tissue were determined based on densities of 1.00, 0.91, and 1.30 g/cm³, respectively.¹⁵ Collagen might have different organization and structure in different types of tissue, and the density value considered here was derived from a study on bone. Nevertheless, it is worth noting that this affects only the quantification of collagen content and has no effects on the estimate of other tissue constituents. Further, it does not affect the intersubject collagen grading.

We have shown how accounting for the absorption properties of collagen can affect the estimate of blood parameters. Just a few subjects have been analyzed up to now and the observation needs to be confirmed, but it seems that the effect on blood volume and oxygenation is more or less marked, depending on tissue composition. This could be relevant not only for breast tissue, and consequently, at least in principle, for breast cancer detection, but also for other applications of photon migration that involve the estimate of tissue composition or the quantification of specific constituents.

The noninvasive quantification of collagen content in breast could also have a more direct application, as it could be of interest for the classification of breast density. The concept of increased breast density is often associated with "more glandular" tissue, thus implying a higher percentage of epithelial tissue.¹⁶ However, the major tissue fraction is stroma, not epithelial tissue. Thus, changes in mammographic density

may reflect stromal changes, and collagen is a major constituent of stroma.¹⁷ It should be noted that the quantification of collagen content based on optical measurements would provide a direct assessment of breast density, as compared to the indirect estimate obtained from the analysis of x-ray mammograms.

Furthermore, alterations of stromal architecture and composition are a well-known aspect of both benign and malignant pathologies, and may play an initial role in breast carcinogenesis.¹⁸ More specifically, collagen seems to be related to cancer development. High mammographic density is a recognized risk factor for breast cancer.^{19,20} Recently, it was observed that collagen in high density breasts is different from that found in low-density breasts.²¹ Thus, the potential relevance of the noninvasive estimate of collagen content for breast cancer detection deserves to be better explored.

4 Conclusion

For the first time to our knowledge, we measure the absorption properties of collagen in the red and near-infrared spectral range, and apply our results to estimate the composition of breast tissue from time-resolved transmittance measurements performed *in vivo*.

When examining the outcomes of our analysis, a few elements should be taken into account. First, just a few subjects are considered, while a wider set of data is needed to derive reliable information on the correlation between collagen content and breast density. Second, possible correlation with other parameters, such as age, needs to be investigated. Finally, time-resolved data are interpreted with the diffusion model for homogenous media, while it is well known that biological tissues, and breast in particular, are heterogeneous media.

However, we do show that significant amounts of collagen are present in breast tissue, the content of collagen can be estimated noninvasively by time-resolved spectroscopy, and it seems to correlate with breast type, with dense breasts being characterized by higher collagen content.

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