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Diffuse optical characterization of collagen absorption from 500 to 1700 nm

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Abstract. Reduction in scattering, high absorption, and spectral features of tissue constituents above 1000 nm could help in gaining higher spatial resolution, penetration depth, and specificity for *in vivo* studies, opening possibilities of near-infrared diffuse optics in tissue diagnosis. We present the characterization of collagen absorption over a broadband range (500 to 1700 nm) and compare it with spectra presented in the literature. Measurements were performed using a time-domain diffuse optical technique. The spectrum was extracted by carefully accounting for various spectral distortion effects, due to sample and system properties. The contribution of several tissue constituents (water, lipid, collagen, oxy, and deoxy-hemoglobin) to the absorption properties of a collagen-rich *in vivo* bone location, such as radius distal in the 500- to 1700-nm wavelength region, is also discussed, suggesting bone diagnostics as a potential area of interest. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.22.1.015006]

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1 Introduction

In recent years, diffuse optical spectroscopy (DOS) is advancing as a promising tool for noninvasive quantification of tissue constituents in pathological diagnostics.¹⁻⁴ Traditionally, *in vivo* diffuse optical measurements have been performed, at most, in the 600- to 1100-nm window.⁵⁻⁷ This is due to high hemoglobin absorption below 600 nm and dominant water absorption combined with lack of suitable detection technology above 1100 nm. Furthermore, studies are most often performed below 900 nm, where more conventional and cheaper detectors are available. However, recent investigations emphasize the advantage of high penetration depth and enhanced constituent specificity in the short wave infrared (SWIR) 1100- to 1700-nm range.⁸⁻¹⁰ Importantly, the lack of systematic characterization of tissue constituents in the SWIR range prevents its widespread use for *in vivo* applications.

The structural protein collagen is an abundant key tissue constituent in soft and hard tissues. Works by Taroni et al.¹¹ and Provenzano et al.¹² showed that collagen density in breast tissues correlates with breast density and accordingly with the risk of breast cancer. Few studies on the noninvasive characterization of collagen in bone tissues^{13,14} revealed its future prospects in osteology to complement existing technologies, such as dual-energy x-ray absorptiometry (DXA), for bone pathological diagnosis. Enhanced contrast of collagen absorption was reported using photoacoustics at SWIR wavelengths as compared to the near-infrared region.^{15,16} For collagen, the

high absorption in the SWIR region⁹ can be exploited to investigate its dynamics of growth in studies related to tissue culture or engineering.^{17,18}

To our knowledge, the first characterization of collagen absorption in the 600- to 1100-nm range was reported by Taroni et al.¹⁹ in 2009 then followed by Nachabé et al.⁸ However, little information is available on the absolute absorption spectrum of collagen in the SWIR, despite all the interesting features of collagen in that range and the related potential diagnostic applications mentioned above. High scattering, fluorescence in the visible range, and flake-like nature of commercially available collagen are of hindrance to its characterization by direct spectroscopic approaches (e.g., commercial spectrophotometer). Though a recent work has partially solved the problem of scattering using an integrating sphere over the 500- to 1600-nm range,⁸ in the absence of rigorous validation *per se* this approach does not guarantee the effective elimination of distortions caused by scattering. Moreover, fluorescence in the visible region cannot be eliminated in this way. Photoacoustics may be a viable option but it requires tunable and powerful pulsed lasers over the SWIR spectral range, as well as a proper approach to quantitation. In this scenario, time-domain diffuse optical techniques can be an effective solution to characterize the optical properties of collagen, since their natural disentanglement of absorption from reduced scattering enables the assessment of a distortion-free absorption spectrum.

Aim of the current work is to present the absorption spectrum of collagen over a broadband range (500 to 1700 nm). A hybrid time-resolved DOS system (TRS) was developed by combining a clinical TRS system that works in the 500- to 1350-nm range²⁰ with a TRS system specifically designed to work in the SWIR

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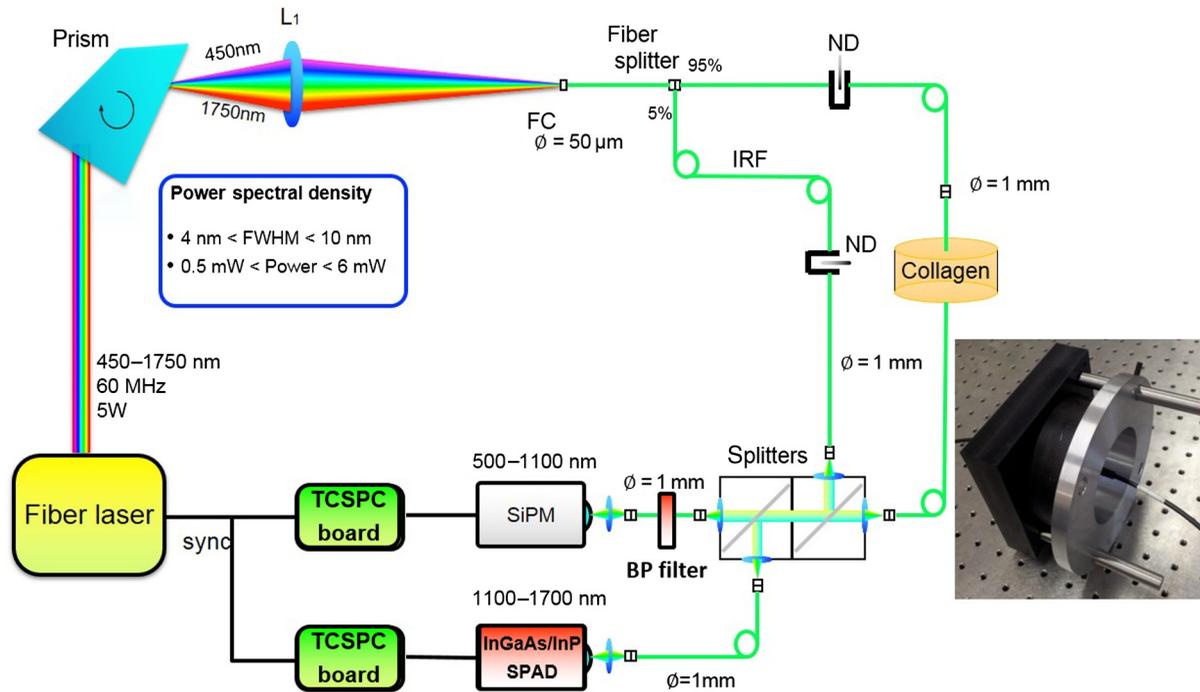


Fig. 1 Schematic of experimental setup with SiPM (500 to 1100 nm) and InGaAs/InP SPAD (1100 to 1700 nm). The inset on the right shows the customized transmittance probe.

region¹⁰ (1100 to 1700 nm), in order to effectively cover continuously the whole range from 500 to 1700 nm. The simulation of *in vivo* measurements is presented for an area of the human body where bone is close to the surface (radius distal), which is rich in collagen, to better appreciate in different spectral regions the contributions of collagen as compared to the other key tissue absorbers.

2 Materials and Methods

2.1 Time-Domain Diffuse Optical Spectrometer

Figure 1 depicts the schematic of the system. A broadband (450 to 1750 nm) supercontinuum fiber laser along with a Pellin Broca prism allows wavelength selective coupling of the 60-MHz laser pulses into a 50- μm core fiber. The maximum power ranges between 0.5 and 6 mW, depending on the selected wavelength. Due to prism dispersion, the bandwidth at the selected wavelength varies between 4 and 10 nm. A strategy for the elimination of temporal drift and system fluctuations was implemented by the acquisition of a reference signal. To effectively cover the desired broadband range (500 to 1700 nm), two detectors were employed: (i) at 500 to 1100 nm, a silicon photomultiplier (SiPM, Excelitas Technologies, C30742-11-050-T1) with home-made front-end electronics²¹ that guarantees a narrow temporal response [about 100 ps full-width at half maximum (FWHM)] and a good detection efficiency (peak of 40% at 500 nm and 10% at 800 nm) and (ii) at 1100 to 1700 nm, an InGaAs/InP SPAD detection module,²² with a good temporal resolution (<200 ps FWHM), good detection efficiency (peak of 42% at 1100 nm and still 15% at 1600 nm), and moderate dark count rate and afterpulsing (15 kcps and 5%, respectively, when operated in gated mode at $T = 230$ K). A detailed description on various aspects of the system can be found in Refs. 10, 20, and 23.

2.2 Sample and Measurement Protocol

Type I collagen from bovine achilles tendon (C9879) was purchased from Sigma-Aldrich. Collagen flakes were placed in a customized cylinder of a wide diameter (105 mm), which minimizes contamination of the absorption spectrum arising from photons reflected at the cylinder boundaries. The sample was tightly packed (density = 0.206 g/cm³) to minimize air gap between the flakes. Measurements were performed in transmittance geometry, with 6.8 mm as sample thickness. The wavelength was tuned in 5-nm steps over the range 500 to 1700 nm. Data were collected four times for each wavelength. In the visible range (500 to 650 nm), to eliminate fluorescence, bandpass filters (BP filters) of 10-nm bandwidth were placed in front of the detector at each measured wavelength.

2.3 Data Analysis

The absorption coefficient of collagen at each wavelength was extracted by fitting the measured temporal curves to a solution of the diffusion equation²⁴ with extrapolated boundary conditions²⁵ for an infinite slab model. The fitting range for residual minimization between theoretical and measured curves extended from 80% of the rising edge to 1% of the falling edge of the temporal curves, effectively using the entire time window for residual minimization.

At long wavelengths (1100 to 1700 nm), a fitting procedure based on Monte Carlo (MC) simulations was used to extract the absorption spectrum.^{10,26} In particular, a library of temporal point spread functions (TPSFs) was created at different scattering values and null absorption. Later, the absorption was added by multiplying the TPSF by the Lambert-Beer exponential term $\exp(-\mu_a vt)$, where μ_a is the absorption coefficient, v is the light speed in the medium, and t is the photon time-of-flight. A linear interpolation was used to obtain the TPSF for scattering values different from the simulated TPSFs scattering.¹⁰

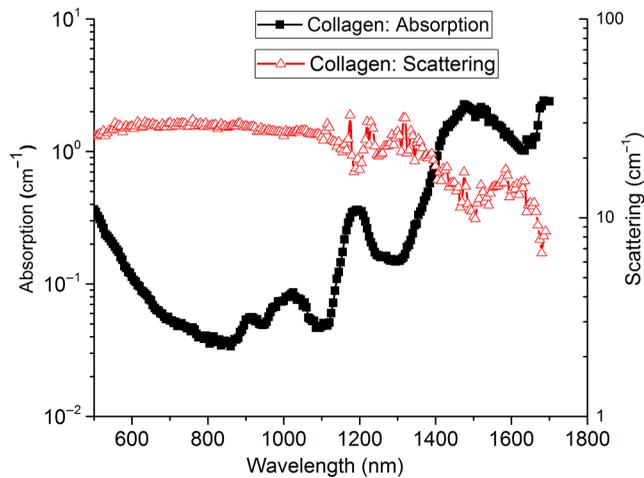


Fig. 2 Absorption (squares) and reduced scattering (triangles) spectrum of type I collagen.

Apart from the above mentioned routinely used fitting range, analysis was also performed for different fitting ranges (80% to 50%, 80% to 10%, and 80% to 1%) to understand the effect of the laser bandwidth on the estimated absorption. As reported in the literature,²⁷ large source bandwidth distorts the recovered absorption values. This is due to the contamination of the tail part of the temporal profile by less absorbed photons in the source bandwidth, which happens under the conditions of sharp absorption variation. In our case, a bandwidth test is needed in the SWIR region, where our system has a large bandwidth (5 to 10 nm) and collagen shows sharp absorption variations. In the visible region, in the presence of fluorescence, we expect an absorption underestimation for extended fitting ranges, such as 80% to 1%, due to increased late fluorescence photons. Therefore, we tested for the effect of fluorescence and took actions to eliminate it (as described above and discussed in the following).

3 Results and Discussion

The absorption and reduced scattering spectra of collagen measured by our system are shown in Fig. 2 (black and red lines, respectively). As expected, the absorption in the SWIR region is higher than in the visible region. Major peaks of collagen are observed, such as the second overtone of CH_2 stretching¹⁶ around 1200 nm, the peak around 1500 nm corresponding to a combination of CH_2 stretching and nonstretching modes,¹⁵ and the shoulder around 1700 nm due to the first overtone of CH_3 stretching.¹⁶ Comparing peak intensities, a factor of 5 is seen between the 1200- and 1000-nm peaks, whereas the shoulder at 1700 nm and the peak around 1500 nm are found to be 25 and 38 times stronger than the absorption peaks at 1000 and 930 nm, respectively.

To provide the best possible assessment of collagen optical properties, care was taken both in the measurement setup and data analysis.

Residual collagen fluorescence was observed, which elongates the tail of experimental time-resolved curves especially at short wavelengths, thereby distorting the estimated absorption spectrum. Therefore, the undesired contribution of fluorescence was effectively suppressed by means of filters in the 500- to 650-nm region. As discussed in Sec. 2.3, we also cross checked

this in the analysis process, which confirmed the absence of significant fluorescence contributions to the collected data.

Moreover, the possible effect of the finite sample size on the estimated absorption coefficient was investigated. Specifically, tests for boundary influence were performed by fitting experimental data with a brick model²⁸ and comparing the results with what was obtained with the infinite slab model. The tests proved that no boundary effects were present, as a result of the small source-detector distance (6.8 mm) and large diameter of the cylindrical sample holder (105 mm).

At long wavelengths, increased absorption posed challenges on the validity of the diffusion equation, as scattering and absorption become comparable. To overcome this problem, the MC technique was used to analyze data ranging from 1100 to 1700 nm.

Finally, as described in Sec. 2.3, tests were performed to understand the effect of the laser bandwidth on our measurements. To this end, we fitted the temporal-resolved curves over different fitting ranges (80% to 50%, 80% to 10%, and 80% to 1%). Since negligible differences were observed upon changing the fitting range, we can exclude possible bandwidth distortion, which are more pronounced for wider fitting ranges on the trailing edge of the photon temporal distribution.²⁷

The instrument performances were rigorously validated elsewhere over the two spectral ranges $R1 = 500$ to 1100 nm and $R2 = 1100$ to 1700 nm.^{10,20} In particular, the accuracy and linearity tests were performed applying the MEDPHOT protocol,²⁹ achieving an agreement within 3% in the $R1$ range up to $\mu_a = 1$ cm^{-1} and for $\mu'_s \geq 5$ cm^{-1} . For the $R2$ range, using MC fitting, good linearity is observed up to $\mu_a = 2.6$ cm^{-1} with $\mu'_s = 8$ cm^{-1} . Furthermore, as a comprehensive spectral validation, very good agreement was obtained on the recovery of the spectrum of water for $R1$ and of lipids for $R2$. The noise in the retrieved absorption (coefficient of variation of the fitted values) is 2% under standard operating conditions (10^6 photon counts, $\mu_a = 0.1$ cm^{-1} , $\mu'_s = 10$ cm^{-1}). The temporal stability over 1 h is 3%, and the day-to-day reproducibility is within 4%.

To further add confidence on our results, we present the scattering spectrum of collagen in Fig. 2. The scattering of collagen ranges between 8 and 29 cm^{-1} , which is well within the validated range of our system. The scattering spectrum is almost flat in the visible range (500 to 1100 nm) while fluctuations are observed in the SWIR range. However, such fluctuations are instrument response function dependent and have minimal effect on the estimate of the absorption properties in the absorption range of 0.1 to 2.6 cm^{-1} ,¹⁰ which is well within the collagen absorption range. Importantly, to further avoid these random fluctuations from affecting the absorption spectrum, we smoothed the scattering spectrum and fixed its value when fitting to estimate the absorption spectrum, as described in detail elsewhere.^{30,31}

We compared our data with collagen absorption spectra reported in the literature,^{8,19,32,33} as displayed in Fig. 3. The main peaks are in good agreement for all the reported studies. Differences corresponding to a multiplying factor among the spectra can be attributed to the difference in density of the collagen samples analyzed. The sample used by Tsai et al.³² and Biggs³³ is gelatin, which is a hydrolyzed form of collagen. Gelatin has translucent optical properties and could raise some challenges for conventional spectrophotometers, which are typically designed to work with transparent samples. This seems to affect the measurement in the form of an offset, possibly more marked at short wavelengths, which would be in agreement

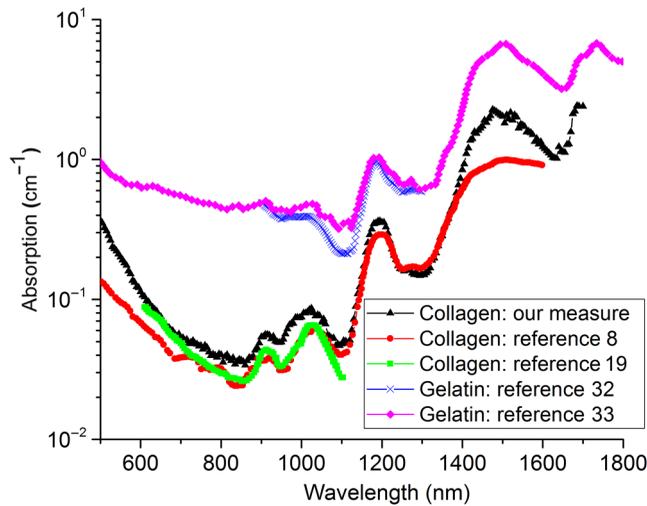


Fig. 3 Spectra of collagen reported in the literature along with our measured spectrum (triangles).

with the contribution of residual scattering. However, the general trend of gelatin matches all other reported spectra. Nachabé et al.⁸ used the same type I collagen as in the present study, but they exploited an integrating sphere on collagen tightly packed in cuvette. The spectrum trend seems to be in line with our spectrum up to 1400 nm. At longer wavelengths, it differs in trend and seems to underestimate the marked absorption around 1500 nm, compared both to our spectrum and Ref. 33. At short wavelengths, the presence of fluorescence can distort the absorption spectrum unless it is eliminated, as in our case.

To understand the impact of collagen absorption in a diffuse reflectance measurement on biological tissues, we have calculated the contribution of key tissue constituents on the absorption spectrum of a bone prominence location. The radius distal location was chosen, as it has thin superficial muscle, giving easy access to bone probing in an *in vivo* measurement.¹⁴ Importantly, radius distal is one among the locations considered for bone mineral assessment by DXA scans and is rich of collagen, which can be of interest for diagnosing bone pathologies.³⁴ Figure 4 depicts the calculated broadband (500 to 1700 nm) weighted spectra of major tissue constituents, namely oxy-hemoglobin (HbO_2 , 16 μM), deoxy-hemoglobin (Hb, 6 μM), lipid (750 mg/cm^3), water (300 mg/cm^3), and collagen (120 mg/cm^3) for a simulated *in vivo* measurement on the forearm at radius distal location. The data required for this simulation were derived from *in vivo* measurements reported in Ref. 35. From Fig. 4, collagen dominates the other constituents around 700 nm, where the absorption of water and lipid is minimal, and also Hb and HbO_2 contributions are small. Still, the flat spectral shape of collagen in this region hampers its robust estimate. Local minima of water and local maxima of collagen around 1050 nm make it a suitable spectral window for collagen detection. However, the competing lipid peak and limited penetration depth of light due to high scattering challenge the detection of deep tissue collagen (bone). Finally, around 1700 nm, the absorption of collagen is 5 times stronger than lipid absorption, compared to only 1.5 times around 1100 nm, and water shows a local minimum. These features could make the spectral range around 1700 nm more specific for *in vivo* collagen detection from deep tissues, provided that the decrease in penetration depth and signal due to increased

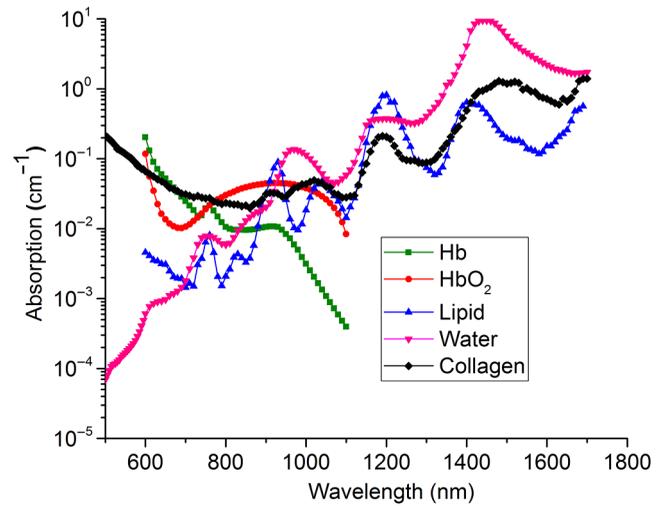


Fig. 4 Simulation of *in vivo* constituent spectra for a measurement performed at radius distal location. Average constituent concentrations are extracted from Ref. 35.

absorption is compensated by the penetration depth and signal gained by decrease in scattering at 1700 nm.³⁶ Apart from this specific example, due to its specificity and high absorption, we predict that the detection of collagen peak at 1700 nm may have potential applications in the intraoperative and *in vitro* study of collagen-rich regions.

4 Conclusion

We have presented the first absorption characterization of collagen flakes in the 500- to 1700-nm range performed using time-domain diffuse optics. Few other spectra, obtained with different techniques and/or on other collagen forms, are reported in the literature. Apart from amplitude differences due to the different form of collagen (gelatin) and density/concentration, the main spectral features in our spectrum are similar to the previously published results. In our measurement and analysis, we paid specific attention to eliminate/suppress fluorescence, boundary effects, bandwidth effects, and absorption coupling with scattering by thorough setup of the experimental apparatus (large sample size, small source–detector separation, and use of BP filters) and use of MC simulations to fit collected data. An insightful simulation of measurements performed at radius distal location was presented, investigating the potential for *in vivo* studies in the 1100- to 1700-nm range. Improving instrument sophistication (e.g., integrated circuit solutions for InGaAs/InP SPAD under development³⁷) will guarantee strong cost and size reduction in the near future, making miniature handheld technologies available,⁵ and we see point of care devices as one of the possible beneficiary to use these long-wavelength spectral features at *in situ* conditions.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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