Broadband diffuse optical characterization of elastin for biomedical applications

[a*]Sanathana Konugolu Venkata Sekar, [b]Joo Sin Beh, [c]Andrea Farina, [a]Alberto Dalla Mora, [a,c]Antonio Pifferi, [a,c]Paola Taroni

[a]Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133, Milano, Italy
[b] Department of Health Science and Technology, Aalborg University, Aalborg, Denmark
[c]Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche, Piazza Leonardo da Vinci 32, 20133 Milano, Italy

Abstract: Elastin is a key structural protein of dynamic connective tissues widely found in the extracellular matrix of skin, arteries, lungs and ligaments. It is responsible for a range of diseases related to aging of biological tissues. The optical characterization of elastin can open new opportunities for its investigation in biomedical studies. In this work, we present the absorption spectra of elastin using a broadband (550-1350 nm) diffuse optical spectrometer. Distortions caused by fluorescence and finite bandwidth of the laser source on estimated absorption were effectively accounted for in measurements and data analysis and compensated. A comprehensive summary and comparison between collagen and elastin is presented, highlighting distinct features for its accurate quantification in biological applications.

Keywords: time-resolved spectroscopy, biomedical optics, elastin, absorption, scattering, diffuse optics, collagen, near infrared spectroscopy.

* E-mail: sanathana.konugolu@polimi.it

1 Introduction

In recent times, various optical methods have been successfully implemented for the diagnosis of physiological and pathological conditions of human tissues, e.g., from diffuse optics to Raman or fluorescence techniques [1][2][3][4][5][6]. The increasing interest of biomedical optics in human studies triggered the need of rigorous optical characterization of biological materials. Elastin a key tissue constituent of dynamic connective tissues is responsible for the elasticity in tissues such as skin, arteries, ligaments and lungs [7]. Recent studies revealed the relation of dermal skin aging to the ratio of collagen to elastin content [8]. In the field of breast characterization and tumor detection, different optical methods opened new possibilities for the non-invasive *in vivo* diagnosis of breast cancer [9][10][11]. As reaffirmed by various techniques in literature (namely magnetic resonance elastography [12][13], static elastography [14], supersonic shear wave imaging [15]), elasticity of breast tissue correlates with breast cancer. However, all these techniques assess breast based on its mechanical properties while ignoring the chemical composition of tissues. Interestingly, the elastic properties of breast are expected to depend on major constituents of connecting tissues, namely collagen and elastin present in human breast [16], where the former gives the rigidity, the latter is responsible for the elasticity of the tissue.

Work of Taroni et al., first characterized the collagen spectrum over 600-1100 nm [17] which was later extended to 500-1700 nm [18], and applied it to quantify the collagen in breast tissue by means of diffuse optical spectroscopy [19]. However, the lack of elastin quantification leaves

a void in the effectiveness of breast tissue diagnosis through diffuse optics. The characterization of elastin in human breast can open up new opportunities for the physiological and pathological assessment of human breast. Also, the knowledge of elastin spectrum can impact other fields like the near-infrared laser bonding and wound healing [20] to find optimal laser wavelength for welding tissues, in diagnosis and treatment of aortic diseases where collagen and elastin dynamics plays a crucial role [21].

Unfortunately, the elastin absorption spectrum present in literature [22][23] lack broadband range and rigorous characterization. In particular, the high scattering nature of commercially available elastin powder causes problems for its characterization using conventional spectrophotometers. A Previous attempt to characterize it in the 0.8-2.3 μm range led to a distorted elastin spectrum due to high scattering [23]. Characterization of elastin performed measuring eye lens [22] over the limited range of 900-1300 nm too suffered from inherent scattering and high water absorption, leading to distortion of elastin peaks. Methods based on integrating sphere can be an option, but fail to account for the fluorescence of elastin in the visible region (550-700 nm). Time domain diffuse optical spectroscopy (TD-DOS) can be an effective method, as it can naturally disentangle absorption from scattering.

The aim of this work is to provide the broadband (550-1350 nm) characterization of elastin absorption, as a first step towards the *in vivo* quantification in biological tissues using optical techniques, and diffuse optical spectroscopy. A broadband (500-1350 nm) clinical TD-DOS system designed specifically to have high responsivity in the short wave near infrared (SWIR) region was exploited for this purpose. A comprehensive comparison between elastin and collagen spectra is presented, commenting distinct and similar features characterizing them.

2 Materials and Methods

2.1 Time domain diffuse optical spectrometer

The schematic layout of the system is shown in Fig. 1. A broadband (450-1750 nm) supercontinuum fiber pulsed laser operating at 60 MHz repetition rate was used as the light source. Wavelength selective coupling of laser pulses into 50 μm fiber was achieved by a Pellin Broca prism. Depending on the selected wavelength, the maximum power ranges between 0.5 mW and 6 mW. The bandwidth at the selected wavelength varies between 4 nm and 10 nm. A reference signal was acquired to account for the temporal drift of the system. The desired broadband range (550–1350 nm) was effectively covered by employing two detectors: i) at 500-940 nm, a Silicon Photomultiplier (SiPM, Excelitas Technologies, C30742-11-050-T1) with home-made front-end electronics (temporal resolution <120 ps full width at half maximum, FWHM) [24], and ii) at 940-1350 nm, an Indium gallium arsenide (InGaAs) photomultiplier tube (PMT) (Hamamatsu mod.H10330A-45) with a good temporal resolution (< 340 ps FWHM). The TD-DOS system was rigorously tested for its accuracy, linearity, stability and repeatability. In particular, the MEDPHOT protocol [25] was applied to validate the system performance over the entire spectral range (550-1350 nm). An absorption linearity with < 3% error was observed for absorption values up to $\mu_a = 1$ cm^{-1} and for reduced scattering $\mu'_s \geq 5$cm^{-1} (which includes

the entire range of optical properties spanned by the elastin characterization). For comprehensive spectral validation, a good agreement of accuracy was observed by measuring the well-known water spectrum in Intralipid® solution [26]. The stability of the system was well within 1%, and day to day reproducibility of < 4 % was observed. Furthermore, system has been well validated in various clinical [27][28] and phantom [29][30] studies. A detailed description on various aspects of the system can be found elsewhere [26][31].

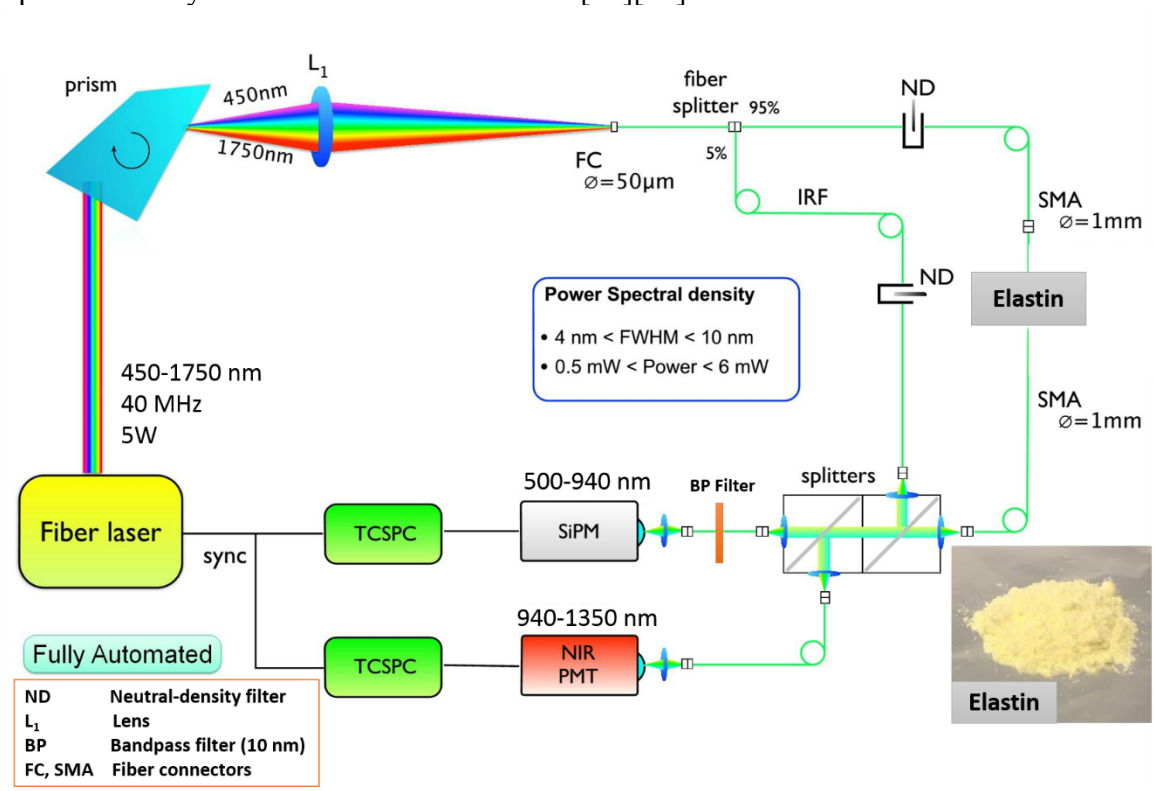

Fig. 1 Schematic layout of the diffuse optical spectrometer with SiPM (500–940 nm) and InGaAs NIR PMT (940–1350 nm) detectors. Lower right corner image of elastin powder.

2.2 Sample preparation, measurement protocol and data analysis

Elastin extracted from bovine neck ligament was purchased from Sigma Aldrich (part no. E1625, lot no. SLBG4446V). As shown in the inset (bottom right) of Fig. 1, elastin is an ivory colored granular powder. The elastin sample was placed in a cylindrical container, which was homebuilt using the Thorlabs 1-inch cage system. The density of elastin in the cylinder was around 0.333 g/cm^3. Measurements were carried out in collinear transmittance geometry with a source-detector separation d = 4 mm. Time-resolved transmittance curves (4 repeated measures with acquisition time of 1 s each) were acquired at each wavelength over 550-1350 nm at 5-nm step size. The fluorescence of elastin in the visible range (550-650 nm) was effectively eliminated by appropriate use of bandpass filters of 10 nm bandwidth placed in front of the visible range detector.

Absorption and scattering spectra of elastin were retrieved by fitting temporal curves acquired at each wavelength to an analytical solution of the diffusion equation [32] with extrapolated boundary conditions for an infinite slab [33]. The fitting range (from 80% of the rising edge to 1% of the falling edge of the temporal curves) was chosen for residual minimization, and effectively utilizes the entire acquisition window for optical properties estimation.
Further data analysis was carried out to understand the effect of the laser finite bandwidth and of elastin fluorescence on the estimate of elastin absorption spectra. To this purpose, the analysis was performed for different fitting ranges (80-50%, 80-10%, 80-1%). In general, a large source bandwidth distorts the recovered absorption values. This occurs because of contamination caused to the tail of the temporal curves by less absorbed photons in the source bandwidth, and the effect is enhanced by sharp spectral variations absorption properties [34]. In this work, a bandwidth test was inevitable due to the combination of sharp variation of elastin absorption around 1100 nm (SWIR region) and large spectral bandwidth of the system in that region. In the visible region, the presence of fluorescence leads to underestimated absorption at long fitting range (80% to 1% of temporal curve). Analysis was carried out to test the effectiveness of fluorescence elimination by bandpass filters.

3 Results and Discussion

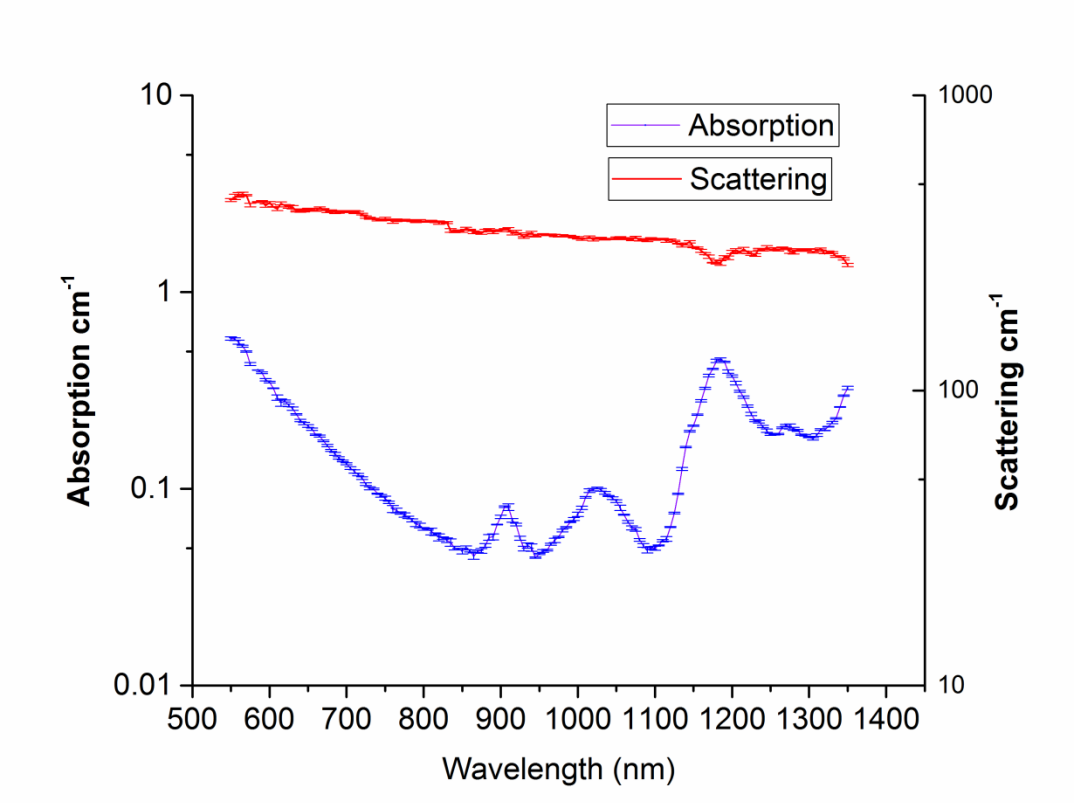

Fig. 2: Absorption (blue line with standard deviation bars) and reduced scattering (red line with standard deviation bars) spectra of elastin (0.333 g/cm^3) as measured by the diffuse optical spectrometer. Narrow error bars show negligible error in measuring the absorption and scattering properties of elastin.

The absorption and reduced scattering spectra of elastin are plotted in Fig. 2. In total, 4 peaks (910 nm, 1025 nm, 1185 nm, 1275 nm) are visible in the elastin absorption spectrum over the measured range (550-1350 nm). Among all the peaks, the most intense peak is located at 1185 nm, whereas low absorption is seen in the visible-NIR region (800-1100 nm) which also contains two local minima at 910 and 1025 nm. Furthermore, below 859 nm, the absorption increases progressively and markedly upon decreasing wavelength down to 550 nm. The peaks at 910 and 1025 nm are well resolved and enhanced as compared to what reported in the literature [22], where the scattering contribution was neglected and contamination from water present in the eye lens cannot be ruled out.

The scattering, as expected for powder samples, is high, with values ranging from 460 to 280 cm^{-1} over the 550-1350 nm range, to elucidate the highly scattering nature of elastin sample. This explains the high distortion observed in literature spectra measured using a conventional spectrophotometer, which did not account for the high scattering of elastin [23]. The high sample scattering eliminated the boundary effects due to the finite size of the sample cylinder, as proved by comparing the results shown here (infinite slab model, which does not account for the finite sample size) to those obtained with a brick model [35]. Furthermore, as discussed in Section 2.2, analysis was carried out to at different fitting ranges (80% to 50%, 80% to 10%, and 80% to 1%). From the scattering spectrum (Fig. 2), the distortion caused by the finite laser bandwidth [34] is slightly seen as a small dip around 1200 nm, which is due to the sharp absorption change around the 1185 nm peak. However, negligible distortion was observed upon changing fitting range, which assures the minimal effect of laser bandwidth on the estimated elastin absorption spectrum. Also it is worth noting that, to prevent fluctuations in the scattering spectrum from affecting the absorption spectrum, in the entire spectral range the scattering spectrum was averaged over 4 repeated measurements and the resulting scattering values were input as fixed values to the fitting procedure, which was then performed a second time having only absorption set as free parameter [36][37].

The fluorescence in the visible range was eliminated using 10 nm bandwidth bandpass filters, analysis was carried out as mentioned in Section 2.2 to test the effectiveness of bandpass filtering. No measurable variation in absorption was observed in the visible range upon changing the fitting range of temporal curves. This reassures the effectiveness of bandpass filters in eliminating fluorescence, as the result of the increased contribution from fluorescence photons at the tail of the temporal curves would cause a decrease in the estimated absorption.

Though the absorption spectrum of elastin shows many peaks and features, for its effective *in vivo* quantification we need to understand its unique features with respect to other tissue constituents. In particular, the spectrum of elastin is found to be similar to that of collagen, as somehow expected since both of them are structural proteins. Further, most often both elastin and collagen are present together in tissues. To avoid coupling between these proteins, the right choice of region should be figured out for their coupling-free effective quantification. From Fig. 3, all features seem to be similar for both spectra. However, on careful notice we can observe that the peak at 1275 nm is unique to the elastin spectrum. This peak can be an ideal point to distinguish between elastin and collagen. Furthermore, elastin has a steeper rise in absorption around 600-800 nm as compared to collagen, which can be a further feature to distinguish between them. However, careful attention is needed in *in vivo* studies at blood rich regions (blood vessels, hearth valves) to avoid coupling with hemoglobin, which has high absorption in the same region. Interestingly, the peaks of elastin at 915 and 1185 nm are intense and sharper as compared to the corresponding peaks of collagen, suggesting these points can also contribute to

their distinction. In brief, though both collagen and elastin exhibit similar absorption trends, on careful analysis we found three potential regions to disentangle the two contributions, which re-emphasis the potential of our study to characterize elastin for human *in vivo* diagnostic studies.

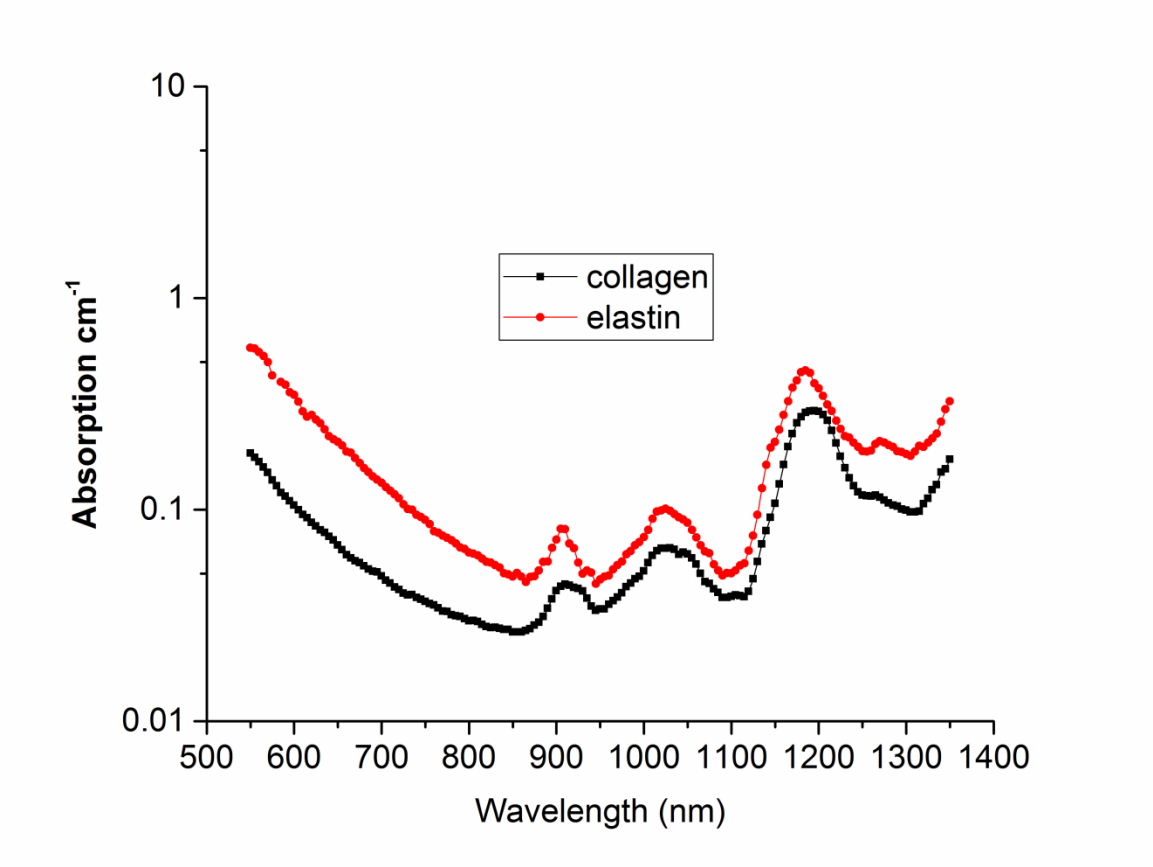

Fig. 3: Comparison between collagen ([18], density = 0.206 g/cm^3) and elastin (density = 0.333 g/cm^3) spectra with elastin exhibiting distinguishable features over the broadband range.

Conclusion

We have presented the absorption and scattering characterization of elastin over a broadband range (550-1350 nm). A well validated TD-DOS spectrometer was used for this purpose. Various effects, like laser bandwidth effect and fluorescence of elastin in the visible range, were carefully removed by effective use of diffusion models and bandpass filters, respectively. Significant absorption changes were observed in elastin spectrum: specifically, 4 peaks were identified in the measured range, with the most intense absorption peak at 1185 nm. In view of the need to effectivly quantify elastin in the presence of collagen, a brief comparison between them was presented, which revealed three important regions for their discrimination. This characterization can lay the ground for the *in vivo* studies aiming at quantifying elastin in biological tissues. With increasing interest of photonics in the field of biomedical studies, we believe that our characterization will open new opportunities in elastin related applications: laser wielding, aortic diseases diagnosis, breast cancer detection can be few areas of interest. Even in case the discrimination of elastin from collagen

proved not easy due to their spectral similarity, nonetheless the knowledge of overall protein (collagen + elastin) content with respect to other constituents (e.g., oxy- and deoxyhemoglobin, water, lipids) is a valuable information.

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