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Journal

Journal of Biomedical Optics, 1(2)

ISSN

1083-3668

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Publication Date

1996

DOI

10.1117/12.227699

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GROUP REFRACTIVE INDEX MEASUREMENT OF DRY AND HYDRATED TYPE I COLLAGEN FILMS USING OPTICAL LOW-COHERENCE REFLECTOMETRY

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(Paper JBO-042 received Sep. 21, 1995; revised manuscript received Nov. 11, 1995; accepted for publication Nov. 15, 1995)

ABSTRACT

The group refractive index and physical thickness of dry and hydrated type I collagen films are measured using optical low-coherence reflectometry. The average value for the group refractive index of dry and fully hydrated collagen films at $\lambda_0 = 850$ nm is, respectively, 1.53 ± 0.02 , and 1.43 ± 0.02 . The physical thickness of type I collagen films nearly doubles when going from the dry ($56 \mu\text{m}$) to hydrated ($118 \mu\text{m}$) state.

Keywords reflectometry; hydrated type I collagen films; group refractive index.

1 INTRODUCTION

Because the optical properties of human connective tissue are similar to those of collagen when fully hydrated, synthetic type I collagen films are useful phantoms for medical and biological research.¹⁻³ Many spectroscopic investigations of absorption, reflectance, scattering, and transmission properties of human tissue, which primarily contains collagen, have been reported.⁴⁻¹¹ However, few measurement results of the refractive index (n) of collagen are known. Often the refractive index for tissue used in bioengineering studies is an arbitrary value near that of water, or a calculated value based on elemental composition. Reasons for the paucity of measured values in tissue have been noted and discussed by Bolin et al.,¹² who measured the refractive index of various mammalian tissues with a technique using a quartz optical fiber. By substituting the cladding normally used to confine optical radiation in the quartz fiber with the biological material being investigated, measured values of the refractive index at 632.8 nm of several tissues were found to range from 1.38 to 1.41. Although the quartz fiber method can accommodate thicker (opaque) samples, the technique requires homogenization of the tissue and a specialized fiber-tissue geometry. Furthermore, when using this method, the maximum detectable refractive index is limited by n of the quartz fiber.

Optical low-coherence reflectometry (OLCR) is an interferometric technique to characterize optical

properties of various materials and is based on coherent cross-correlation detection of the interference fringe intensity of light backscattered from a sample. This technique was initially introduced for characterizing closely spaced reflections in optical components used in the telecommunications industry.^{13,14} Recently, OLCR has been applied to investigate highly scattering biological tissues.¹⁵⁻¹⁹ For example, the position of static structures within turbid materials can be determined by measuring the interference fringe intensity of backscattered light^{17,19,20} from a test sample. Clivaz et al.¹⁶ determined the refractive index of artery wall at $\lambda_0=1300$ nm by measuring optical reflectivity at various tissue interfaces with a bare fiber. Because this method requires physical contact between the surface of the biological sample and the probe fiber, measured values of the refractive index are dependent upon the interface and can vary. Similar to the quartz fiber method¹² described above, the range of detectable n is limited by the optical fiber refractive index. Sorin et al.²¹ demonstrated a method for simultaneous measurement of group refractive index and physical thickness of an unknown sample with planar surfaces, which can have significant advantages when applied to soft and/or hydrated biological samples. Applying the principles of Sorin's method, we report noncontact and noninvasive tomographic imaging measurements to deduce the group refractive index of collagen. Because evaporation of water from a hydrated biological sample in air results in significant uncertainty in optical

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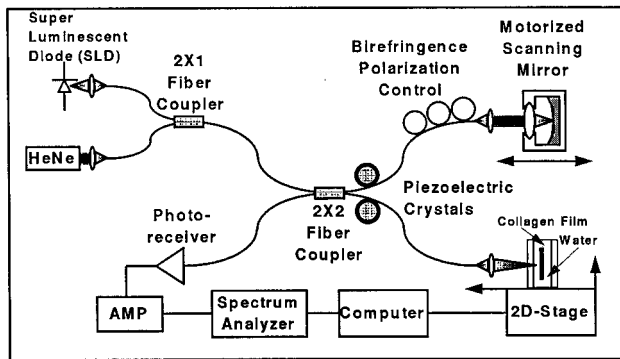


Fig. 1 Schematic of apparatus to measure group refractive index of collagen sample.

measurements, use of a noncontact method allows placement of a collagen test film inside a water-filled cell so that evaporation is eliminated and the sample remains fully hydrated over the duration of a measurement.

2 EXPERIMENTAL

Continuous near-infrared light ($\lambda_0 = 850$ nm, $\Delta\lambda_0 = 25$ nm) emitted by a superluminescent diode (SLD) (EG&G, Vaudreuil, Canada) is coupled into a fiber optic Michelson interferometer and split into two beams by a 2×2 fiber coupler (Figure 1). The SLD power in the input fiber of the interferometer is set at 1 mW. Optical power in the reference arm of the interferometer is attenuated to $2 \mu\text{W}$ to reduce intensity fluctuations²² and thus achieve a higher signal-to-noise ratio.²³ Light from an HeNe laser ($\lambda_0 = 632.8$ nm) is coupled into the interferometer using a 2×1 fiber coupler and serves as an aiming beam for the probe arm. The optical phase of SLD light in both probe and reference arms of the interferometer is modulated (1000 Hz) by stretching optical fiber wrapped around piezoelectric cylinders which are expanded by a serrrodyne (i.e., ramp) voltage wave form. Prior to each sample measurement, the polarizations of probe and reference beams are matched using stress birefringence to optimize fringe contrast.

A microlens terminating the probe arm focuses light ($\text{NA} = 0.22$) within the collagen to a $5\text{-}\mu\text{m}$ diameter spot size. Light backscattered from collagen recombines with that reflected from the reference mirror in the 2×2 fiber coupler. The two beams interfere and give fringes only when their optical pathlength difference is less than or equal to the coherence length in the sample ($\lambda^2 / \Delta\lambda \sim 20 \mu\text{m}$) of SLD source light. Since the spectral emission profile of SLD source light yields rapid phase decorrelation of the beams for pathlength differences greater than the coherence length, high spatial resolution ($\sim 20 \mu\text{m}$) is achieved.²² Backscattered light from the collagen at a user-specified position can be detected by either scanning the reference mirror of

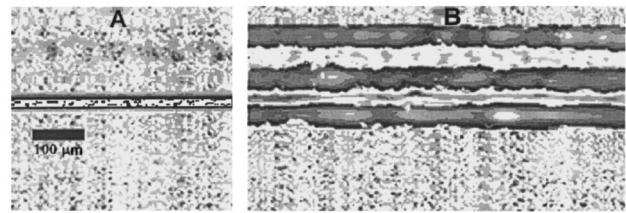


Fig. 2 Tomographic images of dry collagen film. The reference image (A) represents the interface of an air-glass substrate without collagen film. Image when a dry collagen film is placed on the glass substrate (B). The upper and middle horizontal lines represent the front (air-collagen) and back (collagen-air) surfaces of the collagen film. The bottom line is the displaced image of the glass substrate.

the interferometer or translating the probe arm. A silicon photovoltaic detector (New Focus 2001, Sunnyvale, CA) in combination with a spectrum analyzer (Hewlett Packard 8560E, Palo Alto, CA) is used to measure the optical interference fringe intensity. The probe arm is mounted on a high-precision motorized X-Y stage (Newport, PM 500-K, Irvine, CA) and scanned parallel (lateral direction) to the collagen film surface. A scanning scheme in which a small axial increment (2 and $10 \mu\text{m}$ for dry and hydrated collagen films, respectively) is made perpendicular to the film after each lateral scan allows construction of a two-dimensional image. Although random thermomechanical movements (micrometers) in the reference and target arms produce corresponding amplitude and phase variations, fluctuation of the measured interference fringe intensity is insignificant. Because biological samples have a turbid and varying structure and the detection volume of OLCR is small ($\sim 300 \mu\text{m}^3$),¹⁶ useful information cannot be deduced from a single point measurement. The imaging technique allows noncontact measurement of optical and physical properties of a sample to yield reliable measurement values. Data are collected for both dry and fully hydrated type I collagen films (F1310, Colla-Tech, Plainsboro, NJ), which are measured, respectively, in air and in a sealed water-filled glass cell. Collagen is hydrated using double-distilled water; no proteoglycans or glycosaminoglycans normally present in connective tissue are added.

3 RESULTS AND DISCUSSION

Tomographic images of an air-glass interface obtained using OLCR are shown without [Figure 2(A)] and with [Figure 2(B)] a dry collagen film. Spacing between the two upper horizontal lines, which represent reflections from front and back surfaces of the collagen film, gives the optical path length through the dry collagen film at $\lambda_0 = 850$ nm. The bottom line is the displaced image of the glass substrate caused by the additional optical path length between collagen and air. Two typical or-

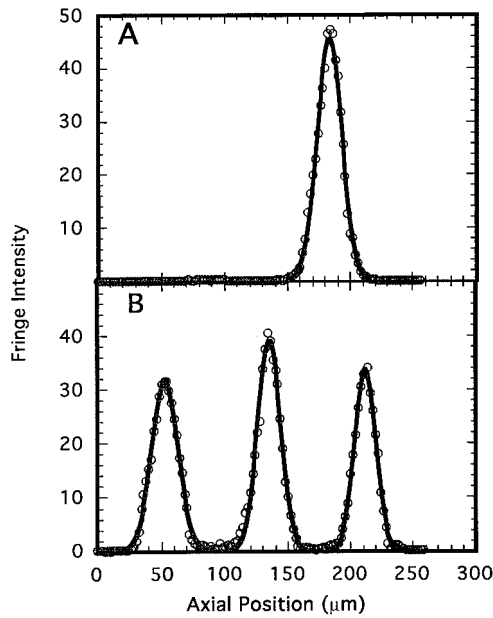


Fig. 3 Typical orthogonal slices taken from images in Figure 2; upper and lower traces taken from Figures 2(A) and 2(B), respectively.

thogonal sliced curves taken from the images (Figure 3, circles) are fitted to Gaussian functions (solid curves); peak position is estimated by computing the maximum. The measurement of group refractive index using a Michelson interferometer with a low coherence light source has been analyzed by Bor et al.²⁴ The group refractive index (n_c) and physical thickness (T) at a given position on the collagen film are related according to

$$\Delta_1 = n_c \cdot T. \quad (1)$$

The apparent displacement of the glass substrate Δ_2 is related to the group refractive index of the collagen (n_c) and the surrounding medium (n_{medium}):

$$\Delta_2 = (n_c - n_{\text{medium}})T. \quad (2)$$

For a dry collagen film in air, $n_{\text{medium}}=1.00$. The group refractive index of collagen is determined by eliminating thickness T in Eqs. (1) and (2):

$$n_c = \frac{\Delta_1}{\Delta_1 - \Delta_2} n_{\text{medium}}. \quad (3)$$

The measured value of the optical path length through the film (Δ_1) is the physical distance the probe arm moves between reflections from front and back surfaces of the collagen. Similarly, the value of apparent displacement (Δ_2) is the measured change in physical position of the probe arm between reflections from the glass substrate without and with the collagen film present. Calculated values of n_c and T (circles) are plotted versus lateral

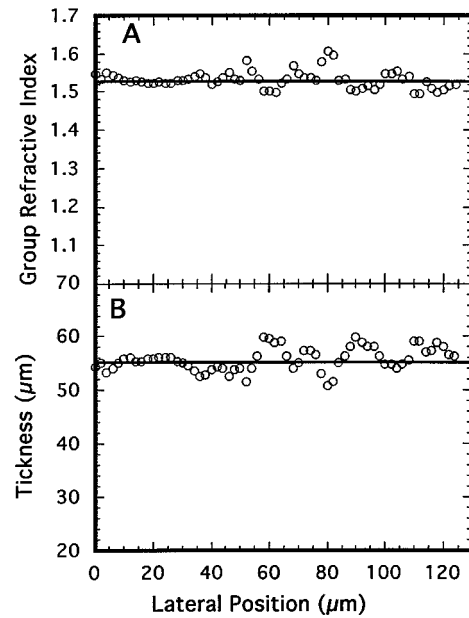


Fig. 4 Plots of group refractive index and physical thickness of dry collagen film versus lateral position. Solid lines represent the average values of group refractive index ($n_c=1.53$) and physical thickness ($56 \mu\text{m}$).

position (Figure 4), where solid lines represent average values. Fluctuations of n_c and T cannot be entirely attributed to measurement error; the correlated variation of the measured values reflects fluctuations within the collagen substrate.

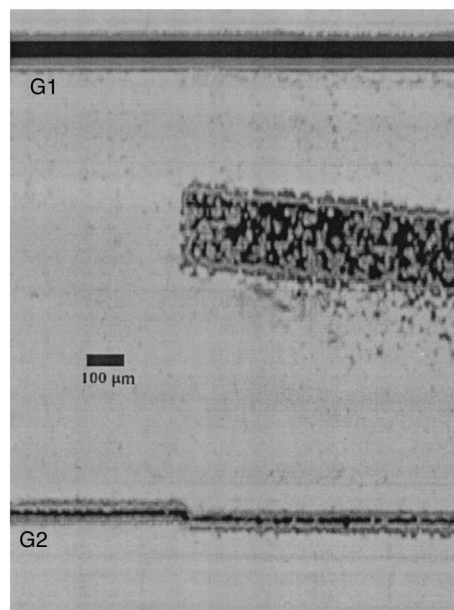


Fig. 5 Tomographic image of a fully hydrated collagen film in a water-filled glass cell. The top (G1) and bottom (G2) lines are reflections from glass–water and water–glass interfaces, respectively. The left portion of the cell is filled with water and serves as a reference. The right portion of G2 is displaced due to greater optical path length through the collagen film.

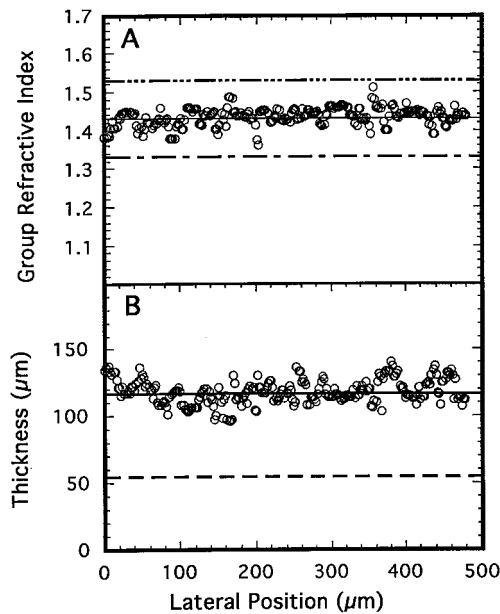


Fig. 6 Plots of group refractive index and physical thickness of hydrated collagen film versus lateral position. Solid lines represent average values. The dashed lines (--- and ---) in A give the average group refractive index of dry collagen ($n_c=1.53$) and water ($n_{\text{water}}=1.340$) at $\lambda_0=850$ nm. The dashed line (---) in B gives the average physical thickness ($56 \mu\text{m}$) of dry collagen film.

A tomographic image (Figure 5) is used to estimate the group refractive index of a hydrated type I collagen film, which is positioned in a sealed water-filled glass cell. Top and bottommost features in the image (G1 and G2) represent reflections from glass-water and water-glass interfaces, respectively. In the left portion of the image, only water is present and is used as a reference between water-glass interfaces. In the right portion of the image, the lower water-glass substrate interface (G_2) below the hydrated collagen film is displaced (Δ_2) due to increased optical path length (D_1). Values of n_c and T of the hydrated collagen film are obtained (Figure 6) from Eqs. (1) and (2) using as group index of water, $n_{\text{medium}} = n_{\text{water}} = 1.340$ ($\lambda_0 = 850$ nm, $T = 25$ °C).^{25,26} Because the magnitude of optical dispersion ($\lambda dn/d\lambda$) in water can be significant (0.01), an accurate value of the refractive index of collagen cannot be deduced from the given measurement. Solid lines represent the average values of n_c and T over a range of lateral positions. The dashed lines [Figure 6(A)] show average values of the group refractive index of dry collagen (top) and water (bottom). The average group refractive index of a fully hydrated collagen film ($n_c=1.43$) is approximately equal to the average value of dry collagen ($n_c=1.53$) and water ($n_{\text{water}}=1.340$), suggesting that the dry collagen film has absorbed a quantity of water equivalent to the original volume when fully hydrated. This is consistent with the data presented in Figure 6(B), where the physical thickness is

nearly doubled between dry ($56 \mu\text{m}$) and hydrated ($118 \mu\text{m}$) states.

In addition to measuring changes in group refractive index and physical thickness, tomographic images indicate that optical scattering increases in collagen upon hydration. When hydrated, collagen fibers within the film straighten, become better aligned, and open interfiber spaces which become filled with water. With hydration, the spatial frequency distribution of refractive index variations gives rise to increased scattering of visible and near-infrared light. As is evident in the hydrated tomographic image (Figure 5), similar collagenous structures appear in the region below the back side of the film; notably, however, similar structures are not observed on the front side of the film. Because the two surfaces of the film are identical, we suspect the appearance of these structures may be due to photons undergoing multiple scattering events in the collagen before being backscattered into the probe fiber. Experiments are under way in our laboratory to investigate the effects of multiple scattering on recorded tomographic images.

4 CONCLUSIONS

The group refractive index ($\lambda_0=850$ nm) of dry and hydrated type I collagen films has been measured using optical low-coherence reflectometry. The average value for the group refractive index of dry and fully hydrated collagen films at $\lambda_0 = 850$ nm is, respectively, 1.53 ± 0.02 and 1.43 ± 0.02 . The physical thickness of type I collagen films nearly doubles when going from the dry ($56 \mu\text{m}$) to hydrated ($118 \mu\text{m}$) state.

Acknowledgments

The authors wish to thank Wayne Sorin and Gerald Lucassen for helpful discussions, and Yagang Liu for technical assistance. This project is supported through an academic equipment grant from Hewlett-Packard Laboratories, a postdoctoral fellowship (XJW), and independent research grants (JSN, TEM) from the National Institutes of Health. Institute support from the Department of Energy, Office of Naval Research, National Institutes of Health, and Beckman Laser Institute Endowment is also gratefully acknowledged.

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