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

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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 $\mu$m) to hydrated (118 $\mu$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 light17,19,20 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 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 light17,19,20 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
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 optical properties of the sample are measured. The optical pathlength difference is less than or equal to the coherence length in the sample (c). Use of a long coherence length beam for the probe arm. The optical phase of the interference fringe is significantly constant (200 kHz) across the entire sample. A microlens terminating the probe arm focuses light on a specific point of the sample. Two typical images of dry and hydrated collagen films are shown. A microlens terminating the probe arm focuses light on a specific point of the sample. Two typical images of dry and hydrated collagen films are shown.

3 RESULTS AND DISCUSSION

A microlens terminating the probe arm focuses light on a specific point of the sample. Two typical images of dry and hydrated collagen films are shown. A microlens terminating the probe arm focuses light on a specific point of the sample. Two typical images of dry and hydrated collagen films are shown.
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.\textsuperscript{24} The group refractive index ($n_c$) and physical thickness ($T$) at a given position on the collagen film are related according to

$$\Delta_1 = n_c \cdot T.$$  \hspace{1cm} (1)

The apparent displacement of the glass substrate $\Delta_2$ is related to the group refractive index of the collagen ($n_c$) and the surrounding medium ($n_{\text{medium}}$):

$$\Delta_2 = (n_c - n_{\text{medium}}) T.$$  \hspace{1cm} (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):

$$n_c = \frac{\Delta_1}{\Delta_1 - \Delta_2} n_{\text{medium}}.$$  \hspace{1cm} (3)

The measured value of the optical path length through the film ($\Delta_1$) is the physical distance the probe arm moves between reflections from front and back surfaces of the collagen. Similarly, the value of apparent displacement ($\Delta_2$) 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 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.

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

**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 \text{ \mu m}].

**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.
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 (G2) below the hydrated collagen film is displaced (Δd) due to increased optical path length (D1). Values of n, and T of the hydrated collagen film are obtained (Figure 6) from Eqs. (1) and (2) using as group index of water, n_water = 1.340 (λ=850 nm, T=25 °C). Because the magnitude of optical dispersion (∆n/∆λ) 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, 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 = 1.43) is approximately equal to the average value of dry collagen (n = 1.53) and water (n_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 μm) and hydrated (118 μm) 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 (λ=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 λ = 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 μm) to hydrated (118 μm) state.

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