Fiber-based visible and near infrared optical coherence tomography (vnOCT) enables quantitative elastic light scattering spectroscopy in human retina

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Abstract: Elastic light scattering spectroscopy (ELSS) has been proven a powerful method in measuring tissue structures with exquisite nanoscale sensitivity. However, ELSS contrast in the living human retina has been relatively underexplored, primarily due to the lack of imaging tools with a large spectral bandwidth. Here, we report a simple all fiber-based setup to implement dual-channel visible and near infrared (NIR) optical coherence tomography (vnOCT) for human retinal imaging, bridging over a 300nm spectral gap. Remarkably, the fiber components in our vnOCT system support single-mode propagation for both visible and NIR light, both of which maintain excellent interference efficiencies with fringe visibility of 97% and 90%, respectively. The longitudinal chromatic aberration from the eye is corrected by a custom-designed achromatizing lens. The elegant fiber-based design enables simultaneous imaging for both channels and allows comprehensive ELSS analysis on several important anatomical layers, including nerve fiber layer, outer segment of the photoreceptors and retinal pigment epithelium. This vnOCT platform and method of ELSS analysis open new opportunities in understanding structure-function relationship in the human retina and in exploring new biomarkers for retinal diseases.

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1. Introduction

As the structures of neuroretinal cells are fundamentally important for their functions, retinal imaging has been a critical tool in vision science and ophthalmology [1–3]. However, almost all retinal imaging modalities are limited to imaging structures around, or slightly smaller than, 1µm. Thus ultrastructures beyond this limit, such as individual disc in the outer segment (OS) of photoreceptor, neurotubules in the axons of retinal ganglion cells (RGCs), are eluded by direct imaging. Alternatively, elastic light scattering spectroscopy (ELSS) can be used to quantify the statistical nanoscale ultrastructural properties without the need for deterministically resolving the nanoarchitectures [4–8].

Elastic light scattering is the most prominent light-tissue interaction, caused from spatial variation of the refractive index. Given the linear relationship between refractive index and macromolecule mass density [9–11], a proper spectroscopic analysis can reveal the ultrastructural properties down to the length scale of several tens of nanometers [7, 12, 13]. This superb sensitivity has already been leveraged in a broad range of biomedical applications, such as early cancer detection [14–19], characterization of cardiovascular plaques [20], amyloid deposition in cortex [21], and monitoring burn wound healing [22].

Applied in the ex vivo retina, ELSS has been used to characterize the cytoskeleton changes below 300 nm within the nerve fiber layer (NFL) [23, 24]. In addition, studies showed that the sub-cellular structures of photoreceptors [25–27], Müller cells [28], and retinal pigment epithelium (RPE) [29], all naturally designed to facilitate vision formation, are characterized by their particular elastic light scattering properties.

Despite the rich and valuable information, ELSS in human retina in vivo has been largely unexplored. Optical coherence tomography (OCT) is the current standard of care for imaging three dimensional (3D) retinal structures in ophthalmology [30]. As OCT relies on elastic backscattering and uses broadband light sources, it is naturally suited for ELSS using spectroscopic OCT to extract the depth-resolved spectral profiles [31, 32]. However, all commercial OCT devices use near infrared (NIR) light sources either within 800-900 nm or 1000-1100 nm wavelength, which have limited k-space spectral range for a robust and sensitive spectroscopic analysis. Recently, the advance of visible light OCT (vis-OCT) pushed the probing wavelength below 500 nm, and significantly extended the higher k-space spectral limit [33–38]. However, in vis-OCT, the bandwidth is still limited at around 100 nm. A potential solution to the bandwidth limitation is to combine visible and NIR light into a dual-channel setup [36, 39]. By bridging two bands over a large bandwidth gap, the slowly varying spectral contrast can be harnessed by the difference between two channels. However, due to incompatibility of the fiber couplers in each band, an open-space alignment with dichroic mirrors had to be used to combine/split two bands, making the system cumbersome and challenging to implement. Therefore, in spite of the introduction of retinal imaging using dual-channel visible and NIR OCT in 2011 [36], the method has not been applied in the human retina.

To overcome the above longstanding challenges, we herein present a novel fiber-based visible and NIR optical coherence tomography system, vnOCT in short, for human retinal imaging. By implementing custom-made wavelength-division multiplexers (WDM) and a fiber coupler with a newly available Nufern 630HP fiber, the system supports previously unattainable single-mode propagation of both visible and NIR bands. Since the two bands propagate within the same fiber coupler, they inherently share identical sample and reference arms, with a minimum modification to the configurations in any existing OCT device. Empowered by the vnOCT, we further developed methods to quantify the spectral contrast and observed intriguing spectroscopic behaviors of NFL, OS of the photoreceptors and RPE.
The end-to-end design of vnOCT system and ELSS analysis brings a new tool and a novel quantitative marker for a broad range applications in vision science and ophthalmology, including characterization of the NFL ultrastructure in retinal neuropathy, and detection of the photoreceptor and RPE changes in macular degeneration. The use of visible light also permits functional measurements by retinal oximetry [33–38].

2. System setup and human retinal imaging

In this section, we will introduce the hardware setup of the vnOCT system and human retinal imaging. All the experimental procedures were approved by the Boston Medical Center Institutional Review Board. Formal consent was obtained before imaging. Human imaging was performed on undilated eyes.

2.1 System setup

The system schematic is shown in Fig. 1(a). A single supercontinuum laser (SC, SuperK, NKT Photonics) was used to provide a broadband laser output. The light was divided into the visible portion and the NIR portion by a dichroic mirror (DM1), with a cutoff wavelength at...
650nm. The visible portion was polarized by a polarization beam splitter (PBS) and dispersed by a pair of prisms. The visible light was polarized to allow optimizing the interference efficiency by two polarization controllers (PCs). The desired spectrum was selected by a slit aperture and reflected back by a mirror (M). The NIR portion was further separated by another dichroic mirror (DM2) with a cutoff wavelength at 900nm, and filtered through two edge filters to limit the bandwidth from 800nm to 875nm. Then both the visible and NIR portions were combined by a custom-made WDM and sent into an optical fiber coupler (TW670R2A2, Thorlabs). For the sample arm, the light was collimated by an f = 6mm lens (CL), adjusted by an achromatizing lens (AL), steered by a pair of galvanometer mirrors, and relayed to the pupil by a 2:1 telescope. The diameter of the beam on the cornea is about 2mm. For the reference arm, the light was collimated and reflected by a mirror. The dispersion of the light in the sample arm was balanced by several BK7 glass plates (DC) and the intensity of the light was attenuated by a variable ND filter (ND). Alternatively, a water cuvette can be used for dispersion matching [40]. The returning light from the reference and sample arms interfered in the fiber coupler and was split by a second WDM to two spectrometers. Two spectrometers were equipped by two line scanning cameras (spl2048-140km, Basler) with their bandwidth coverages from 535 to 600nm and 780-880nm, respectively.

The mirror in the reference arm was placed on a stepping motorized translation stage (motorized stage 1) to adjust the optical path difference (OPD) between the sample and reference arms, allowing adjustment of the retinal image within the depth of view. As every individual eye has different diffractive power, the collimator lens, the achromatizing lens, the galvanometer mirrors and the first piece of the 2:1 telescope were placed on the second motorized translation stage (motorized stage 2) to adjust the focus on the retina. When adjusting the focus, both stages were moving simultaneously at the same speed to maintain the image position in the depth of view.

The key component of our vnOCT system is the cascade of two WMDs and the fiber coupler as shown in Fig. 1(b). The 90/10 fiber coupler is designed at the center wavelength of 670nm with ± 50nm per the manufacturer. Although both visible and NIR bands are outside the specified wavelength range, the fiber coupler still maintains excellent performance, with the split ratio of ~90/10 and 95/5 for visible and NIR channels, respectively (Fig. 1(c)). A CCD spectrometer (CCS200, Thorlabs) coupled with an integrating sphere (IS200, Thorlabs) was used to characterize the spectral splitting ratio. Since we have excessive power to spare, 5% of the power is sent to sample arm for imaging. The optical power on cornea was 0.2mW and 0.4mW, in the visible and NIR channel respectively.

Using the same fiber type (Nufern 630HP), we custom-made a 560nm/830nm WDM. We tested the wavelength-splitting performance by sending a mixed light (530-600nm and 800-900nm) into the single port of the WMD. The spectra from the visible and NIR ports are shown in Fig. 1(d). The splitting efficiencies were >90% for the visible channel and >87% for NIR channel.

After permanently splicing two WDMs and the fiber coupler, we evaluated the interference efficiency by mirror reflections from the reference and sample arms. The power was carefully adjusted to be balanced. The resulting interferograms from two spectrometers are shown in Fig. 1(e). The interference efficiency is remarkably high with fringe visibility of 97% and 90% for visible and NIR channel, suggesting that all the fiber components support the single-mode propagation and interference.

2.2 System roll-off characterization

We then characterized the roll-off performance for the two channels. A mirror reflection provided the sample signal. To mimic the weak signal from retina, a neutral density filter (OD = 2) was placed before the mirror. After the filter, the power was attenuated to 2 µW and 3.8 µW for visible and NIR channels, respectively. The exposure time of the camera was 17 µs, for an A-line rate of 50 kHz.
The roll-off curves for both channels are shown in Fig. 2. The signal to noise ratio (SNR) is estimated to be around 87dB and 90dB for visible and NIR channels, taking into account the 40dB attenuation of the neutral density filter. The roll-off rate was about 6dB/mm for both channels within the first 2mm depth. The nonlinear characteristics of the roll-off is more obvious beyond 2 mm in NIR than in visible channel, due to a larger imaging depth range.

2.3 Achromatizing lens design

Human eyes have significant chromatic aberration due to the dispersion of the water refractive index. It has been shown that by adding an achromatizing lens after the collimator, the longitudinal chromatic aberration (LCA) can be corrected [41–44]. Using the same strategy, we designed a triplet achromatizing lens to allow simultaneous focus for both visible and NIR channels.

Fig. 3. The design of the achromatizing lens (AL) for vnOCT system. (a) Photograph and schematic design of the lens (dimension in millimeters). The glass materials are H-ZF88 for the center piece, and S-FPL53 for the two end pieces. (b) The chromatic focal shift in the sample arm with and without the achromatizing lens. (c-d) The spot diagrams at different viewing angles for 550nm and 900nm, without and with the achromatizing lens. The principle ray is 900nm. Bar is 75 μm in (c) and 37.5 μm in (d).
The design of the achromatizing lens is shown in Fig. 3(a), using S-FPL53 for the two ends and H-ZF88 for the center (Ross Optical Industries Inc.). A Zemax human eye model was downloaded from ZEMAX, LLC website [45] and all the other optics in the sample arm were simulated to calculate the LCA in human retina from 500 to 900nm. The chromatic focal shifts with and without the achromatizing lens are shown in Fig. 3(b). The focal shift between the two bands can be effectively reduced to <30μm using AL. The optical design performance can also be evaluated by the spot diagram as shown in Fig. 3(c), 3(d). When the achromatizing lens is applied, both the 550nm and 900nm rays can reach diffraction limited focus size.

2.4 Human retinal imaging by vnOCT

The imaging protocol is modified from a previous publication [34]. There are two operating modes: alignment and acquisition. In the alignment mode, a 128 x 8 raster scanning pattern is continuously performed and one B-scan is calculated and displayed in real time for both channels. Once the focusing and the adjustment of the image position are optimized, the system switches to the acquisition mode to perform a raster scan and record the data simultaneously from two channels. The acquisition waveforms are preloaded in the analog output card to eliminate any time delay when switching modes. Two sampling densities were used in this paper: 512x512, and 4096x16 pixels in x and y directions. The total viewing angle was either 20° x 20° or 30° x 30° at the pupil, calculated from the scanning angle of the galvanometer mirrors and the angle magnification by the 2:1 telescope. Unless otherwise specified, the A-line rate is 50 kHz with an exposure time of 17μs. A software is written in LabVIEW to control the imaging system. The system operation is demonstrated in Visualization 1. The OCT images are generated following the standard steps, including spectral normalization, resampling in k-space, DC removal, digital dispersion compensation, and Fourier transform.
The maximum permissive exposure (MPE) for visible light OCT has been previously calculated based on the ANSI standard, at the conservative level of ~2mW [34, 37]. According to the formula by Delori et al. [46], the MPE for the NIR OCT is about two times higher than the visible light channel at about 4 mW. In our system, the power level on the cornea is 0.2 mW and 0.4 mW for visible and NIR channels, respectively. The laser safety condition for vnOCT is regulated by

\[
\Phi_{\text{vis}}/\text{MPE}_{\text{vis}} + \Phi_{\text{NIR}}/\text{MPE}_{\text{NIR}} \leq I_1
\]

where \( \Phi \) is the optical power on the cornea. In our system, the left side of the equation is about 0.2, well within the safety exposure limit.

Representative results of in vivo human retinal imaging over a 30°x30° angle of view by vnOCT are shown in Fig. 4. In each row, the en face projections of the visible and NIR channels, and one cross-sectional B-scan from each channel were plotted. In each column, the scale was kept constant for comparison. The system allows simultaneous acquisition of both visible and NIR channels, and they are inherently registered on the transverse plane. Without the achromatizing lens, the focus can only be optimized for one channel at a time, while the SNR of the other channel drops significantly due to longitudinal focal shift, as shown in Fig. 4(a)-4(d) and in Fig. 4(e)-4(h). As a comparison, with the achromatizing lens, both channels can be focused at the same time with the optimized SNR. The benefit of correcting LCA is easily appreciated in Visualization 2.

3. ELSS in human retina

We now introduce the methods and results for in vivo ELSS analysis in the human retina enabled by vnOCT. The flow chart of data processing is shown in Fig. 5. The input is the absolute value of OCT images after Fourier transform from two channels, as \( I_{\text{vis}} \) and \( I_{\text{NIR}} \). The output is a parameter that we call VN ratio, defined by the intensity ratio between the visible and NIR channels. All the following processing is performed in the linear intensity scale.

![Flow chart of the data processing method for the ELSS analysis by vnOCT.](image)

**3.1 Image registration and segmentation**

Images from two channels are inherently registered in the transverse plane, but registration in the depth dimension is required to match the two 3D data sets.

The systematic roll-offs of each channel were first compensated for according to the system characterization as shown in Fig. 2. The physical axial dimensions were calculated for
two channels, assuming a 1.33 refractive index. NIR images were then interpolated to the same axial scale as that of the visible channel. Cross correlation was performed to calculate the relative offset in the depth direction between two channels, which was then compensated for by shifting NIR images. After the image registration, the background noise was calculated by the average intensity above the retina and subtracted.

In order to conduct ELSS analysis in different anatomical layers, we performed a layer segmentation to identify the NFL, the inner and outer segment junction (IS/OS), and the Bruch’s membrane (BM). First, a B-scan image, \((I_{vis} + I_{NIR})/2\), was smoothed by a moving average filter, and an intensity threshold was calculated based on Otsu’s method [47]. The image was then binarized using the threshold, allowing detection of the retinal surface. After flattening the surface of the retina, the intensity threshold was updated within the inner retina to again binarize the flattened image. The lower boundary of NFL can then be detected and referenced to the retinal surface. The same procedure was performed to update the intensity threshold within the outer retina on the flattened image. After the third binarization, the bright band from IS/OS and BM is highlighted. The upper and lower boundaries of the bright band can then be detected to mark the IS/OS and BM. The step-wise process is detailed in Appendix Fig. 10.

3.2 Data processing method for ELSS analysis

In order to eliminate any systematic variations between the visible and NIR channels, we used the circulating blood as the spectral reference. Blood optical properties have been well studied and characterized. The depth-dependent spectrum \(I(\lambda, z)\) within blood vessels can be expressed by

\[
I(\lambda, z) = I_0(\lambda)R(\lambda)\exp[-2\mu(\lambda)z],
\]

where \(I_0\) is the light source spectrum, \(R\) is the reflectance, and \(\mu\) is the attenuation coefficient given by

\[
\mu = \mu_s a(g) + \mu_a,
\]

where \(\mu_s\) and \(\mu_a\) are the scattering and absorption coefficients of whole blood, respectively. The scaling factor \(a(g)\) takes into account the forward scattered photons that, by nature of their forward direction, do not contribute to signal loss with depth. The expression of \(a(g)\) is given by [48]:

\[
a(g) = 1 - \exp[-(1-g)^{0.651} \frac{1555}{0.1555}],
\]

where \(g\) is the anisotropy factor.

After the normalization by the source spectrum, an integration over a thickness of \(z_l\) results in

\[
\Sigma \frac{z}{R} = \int_0^z \frac{I}{I_0} dz = \frac{R(\lambda)}{2\mu(\lambda)}[1 - \exp(-2\mu(\lambda)z)].
\]

We approximate \(R(\lambda)\) to be a constant \(R\) over the spectrum [49]. The spectra of \(\mu_s, \mu_a\) and \(g\) are readily provided by the literature [50]. Therefore, \(\Sigma z/R\) can be theoretically predicted. Figure 6(a) shows the spectrum of \(\Sigma z/R\) after the integration of 50\(\mu\)m thickness of blood. Despite the spectral differences in the visible light range, oxygenated and deoxygenated blood have similar VN ratios by averaging the spectrum within each band, with a rounded mean value of 0.6 (0.58 and 0.63, respectively). Therefore, when we scale either visible or NIR channel such that the blood VN ratio is equal to 0.6, VN ratio from other retinal tissues can reflect the actual spectroscopic feature instead of an arbitrary quantity.
The processing for ELSS analysis is further illustrated in Fig. 6(b)-6(d). We should note that the intensity of OCT images are squared prior to the ELSS analysis, as indicated in Fig. 5. Figure 6(b) shows an example of squared B-scan images from visible and NIR channels, with one vessel location labeled. Over the vessel, averaged A-lines are plotted in Fig. 6(c). Depth is measured from the retinal surface. We scaled the NIR channel such that the VN ratio from top 50µm thickness of blood was equal to 0.6, as mentioned above. The same scaling factor was then applied to the entire NIR image. Figure 6(d) is an example of a B-scan image color-encoded by VN ratio. It is apparent that the deeper parts of the vessels have a low VN ratio, due to the greater light absorption of blood in the visible light range. VN ratio is also small in the choroid, as little light can penetrate RPE in the visible channel. The plexiform and nuclear layers appear to have higher VN ratios.

3.3 ELSS analysis on nerve fiber layer (NFL)

The NFL is composed of axon bundles of retinal ganglion cells that relay signals through the optic nerve to the brain. Therefore, ELSS analysis in the NFL is of great interest, as structural damage of axons is a key pathological event for a variety of optic neuropathies such as glaucoma.
We first examined the peripapillary region since all the nerve fibers merge at the optic disc and extend to brain. Figure 7(a), 7(b) show the representative \textit{en face} projections from the visible and NIR channels, with a 20° x 20° viewing angle centered at the optic disc. The cross sectional B-scan images along the dashed lines are shown in Fig. 7(c), 7(d), with the upper and lower boundaries of the NFL marked. Aided by the boundary detection, the \textit{en face} map of the NFL thickness can be plotted as in Fig. 7(e). The major blood vessels create artifacts on the thickness map due to the low signal underneath the vessels. We summarized the thickness data within the shaded area in Fig. 7(e), and plotted against the radial angle in Fig. 7(f). The averaged thickness ranges from about 75µm to 180 µm, thicker in the superior and inferior quadrants and thinner in the nasal and temporal quadrants, as expected from a normal eye.

![Fig. 7. Elastic light scattering spectroscopic analysis at the peripapillary region from a healthy male subject aged 31. (a-b) The representative 20°x20° \textit{en face} projection of the peripapillary region. Signal form the labeled vessels (white arrows) were averaged to serve as the \textit{in vivo} spectral reference. After scaling the NIR channel, the VN ratio from the labeled vessels are 0.60 ± 0.04 (mean ± sem). (c-d) The cross-sectional B-scan images from visible and NIR channels at the dashed lines in panel (a) and (b), with the boundary of neural fiber layer (NFL) demarcated. (e-f) The \textit{en face} maps for the NFL thickness, and VN ratio averaged from the superficial 50µm of tissue. (g-h) The NFL thickness and VN ratio with respect to the radial angle within the shaded circular region. A 7x7 moving average was performed on the VN ratio map. The high VN ratio around the rim of optic nerve head (indicated by the white arrow) is an artefact due to the failure of the segmentation. T, S, N, I stands for temporal, superior, nasal, and inferior. The blue dots are individual data points, and the red curves are the average value over 15 degrees.

The VN ratio averaged from the superficial 50µm of the NFL was calculated and presented in a similar way in Fig. 7(g) and 7(h). The VN ratio is more or less uniform across the peripapillary region. When the data were plotted with respect to the radial angle within the shaded area (Fig. 7(h)), there was no apparent angle-dependence. One interesting observation is that the VN ratio from NFL is generally smaller than one, indicating a less backscattering coefficient in the visible than NIR channel.
We then examined the NFL at the macular region. Figure 8(a) and 8(b) display the representative en face projections of visible and NIR channels centered at the fovea over a 30°×30° viewing angle. The same image segmentation method was applied for the NFL and the NFL thickness map is shown in Fig. 8(c). The map of VN ratio from the top 50μm thickness is plotted in Fig. 8(d). A higher VN ratio is observed within fovea, and the temporal side of the retina. This finding is due to the thin NFL in those regions, where the superficial 50μm thickness included the ganglion cell layer. The angular dependence of VN ratio within the shaded area in Fig. 8(d) is plotted in Fig. 8(e). Again, the VN ratio of NFL in superior, nasal and inferior is significantly lower than one, consistent with our observations at the peripapillary region.

3.4 ELSS analysis in outer retina

We further performed ELSS analysis in the outer retina. The photoreceptors and RPE are of particular interest as they are often affected in diseases such as macular degeneration and inherited retinal degeneration. Thanks to the three-dimensional capability of OCT, the OS of the photoreceptors and RPE can be clearly isolated, as shown in Fig. 9(a). The OS was selected ~30 μm below the boundary of IS/OS, and the RPE was ~20um above the boundary of BM.

Figure 9(b), 9(c) show the en face VN ratio maps for the OS and RPE, respectively. In OS, the values of VN ratio are generally around one; while in RPE, the values are well below one, presumably due the stronger absorption of melanin in the visible light range. The data from the shaded area in Fig. 9(b) is summarized in Fig. 9(d)-9(g). When the data is plotted against the radial angle, we observed an angle-dependent variation of VN ratio in both OS and RPE, as shown in Fig. 9(d), 9(e). When we looked at the changes along the radial distance from fovea, a reduction of VN ratio is consistently observed in both OS and RPE (Fig. 9(f), 9(g)). This finding in OS coincides with the distribution of cone photoreceptors that are enriched in fovea and become sparse towards the perifoveal regions.

4. Discussion

In this paper, we present fiber-based visible and NIR optical coherence tomography for human retinal imaging. The unique contrast between the two bands enables quantitative
measurement of ELSS properties in various anatomical layers, including NFL, OS and RPE. We developed a rigorous data processing method to eliminate systematic variations within the visible and NIR channels, and provided robust quantification of VN ratio. This simple and elegant platform offers new opportunities for ELSS analysis for a broad range of applications.

The successful implementation of our fiber-based vnOCT relies on the same fiber type for both WDM and fiber coupler. The designation of 560nm and 830nm for WDM allows high efficiency of combining and splitting visible and NIR bands, and the permanent fusion of WDM and fiber coupler eliminates the connection loss. The excellent performance of this fiber-architecture, as demonstrated in the paper, is instrumental for the ELSS analysis by vnOCT on retina.

In an attempt to measure the actual spectroscopic features, we used the blood signal as the in vivo reference to calibrate our VN ratio on other retinal tissues. The major retinal vessels are located on the surface of the retina, naturally preventing the complication of light attenuation. And fortunately, the VN ratio from blood is relatively independent of oxygenation, where the values are 0.58 and 0.63 from a 50μm oxygenated and deoxygenated blood, respectively (Fig. 6(a)). This offers a reliable spectral reference. We note that the integration of the signal over a 50μm slab on blood vessels would include a thin layer of tissue, roughly ~10-20μm thick. Since the scattering and absorption coefficients of blood are almost 10 times higher than other retinal tissues [51], the attenuation is predominantly caused by blood and we neglect the impact from tissues above. This assumption would allow a simple calibration method without overcomplicating the image processing. In practical clinical applications, it would allow standardizing the imaging protocol to perform the spectral scaling for individual patients, to ensure the reliability of VN ratio measurements. We should also note that our method relies on the modeling described in Section 3.2. We

![Fig. 9](image_url) Elastic light scattering spectroscopic analysis at the macular region on the outer retina. (a) The zoomed-in B-scan images at the outer retina from two channels. The depth range of outer segment (OS) of photoreceptors, and RPE were labeled. (b-c) The en face maps of VN ratio from OS and RPE. A 7x7 moving average was performed on the VN ratio maps. (d-e) The angular dependence of VN ratio from OS and RPE within the shaded area in (b). (f-g) The dependence of VN ratio on the radial distance from the foveal center, r, within the shaded area in (b).
didn’t consider other complicated factors, such as blood flow and blood cell orientations, which may or may not affect the VN ratio measurements.

To remove the impact of optical aberration in calculating VN ratio, we corrected the longitudinal chromatic aberration by using an achromatizing lens. There are moderate transverse chromatic aberration when the viewing angle >10° (Fig. 3(d)), and we used a moving averaging to minimize the effect. There might be other high-order optical aberrations that could affect the accuracy of VN ratio measurement, which remains to be investigated. In addition, the localized VN ratio calculation relies on image segmentation of various layers. In this paper, we used a simple image segmentation by thresholding. There are more advanced image segmentation methods developed by others [52–54], which can be later adopted in vnOCT to provide better segmentation accuracy.

There are several intriguing observations of the ELSS signatures in human retina by our vnOCT system. First, the VN ratio in NFL is smaller than one in both the peripapillary and macular region, suggesting that the NFL produces less backscattering in the visible light range than in NIR. This finding is somewhat surprising as the scattering coefficient typically is lower at a longer wavelength in soft tissues. However, OCT detects the backscattered light which in principle can be stronger in longer wavelengths [32, 55]. We also noted that our measurements differ from previous spectral characterization of nerve fiber bundles in rats, where the backscattering spectrum decays along the wavelength [24, 56]. Difference in the species and the experimental condition (i.e. *in vivo* in human retina vs. *ex vivo* rat retina) may account for this discrepancy. While our measurements require further validation, the low VN ratio from NFL would perhaps be naturally advantageous as it may facilitate transmission of visible light to the deeper photoreceptors with less light being scattered back.

There are also some interesting observations in the macular region. First, the VN ratio for OS is higher at the fovea than peri- and parafovea region, which coincides with the distribution of color-sensitive cone photoreceptors (Fig. 9(b)). This suggested that there may be a relationship between high VN ratio in OS and concentration of cones. Second, the VN ratio for RPE is significantly smaller than one, indicating lower reflectance in the visible light range than NIR. At first glance, this suggests that low VN ratio in RPE might be related to melanin content due to its stronger absorption to visible than NIR light. However, we observed higher VN ratio for RPE at the fovea, seemingly conflicting the higher melanin concentration and higher RPE density at fovea [57]. Thus, the VN ratio for RPE is likely also affected by other contributors.

The interpretation of ELSS measurements and above observations on VN ratio can resort to computational modeling. Various ELSS models have been proposed to shed light in understanding the biophysical functions of retinal ultrastructures. The nanoscale fiber bundle model has been used to interpret the backscattering spectrum from NFL of rodent retina [24, 58]. A continuous refractive index fluctuation model has been also proposed to interpret the NFL’s ultra-structural properties [59]. Finite domain time difference (FDTD) model and a layered scattering model have been used to simulate the exact wave propagation in OS and photoreceptors [26, 60]. In RPE, a T-matrix model has been used to predict the full metrics of optical properties of melanosomes, which is hypothesized to play important roles in age-related macular degeneration [29]. The combination of the computational models and experimental measures by vnOCT can be a powerful approach to reveal the structure-function relation in human retina.

Last but not least, we should note the fiber-based architecture for vnOCT can be extended to all other OCT systems, not limited in retinal imaging. We also note that the use of visible light OCT will enable retinal oximetry in human eyes, as previously demonstrated.

### 5. Conclusion

In conclusion, we present a novel fiber-based visible and near infrared optical coherence tomography system (vnOCT), enabling quantitative and comprehensive elastic light scattering
spectroscopy in the human retina. The novel system design and methodology for ELSS analysis reveal intriguing spectroscopic behavior in NFL, OS and RPE. The innovation may generate significant impacts for both clinical and basic science studies by providing better understanding and quantification of the ultrastructures in living human retina.

Appendix

Image segmentation

The step-wise method for the image segmentation is detailed in Fig. 10 and Table 1.

Step 1: After the image registration, the average image from two channels \(\frac{I_{vis} + I_{NIR}}{2}\) was used as input. A 5x10 moving average was applied to smooth the image. A threshold was determined by Otsu’s method from the whole image, based on which the image was binarized. The first non-zero points were then identified, and smoothed as the retinal surface.

Step 2: The image intensity was projected along the depth, and normalized across the lateral direction. Then the image was flattened by the retinal surface. The Otsu’s method was used within the inner retina to update the threshold for a second binarization. The bottom edge was detected by the first zero points beyond the first 20 pixels. After smoothing the edge, the bottom boundary of nerve fiber layer (NFL) was detected.

Step 3: The Otsu’s method was used within the outer retina to update the threshold for a third binarization. The first non-zero points from top and bottom sides over the bright band in outer retina were detected. After smoothing the two edges, the IS/OS and Bruch’s membrane layers were identified, respectively.

![Fig. 10. (a) Step-wise processing method for image segmentation for the edges for nerve fiber layer, IS/OS, and Bruch’s membrane. (b) Example of the image segmentation on a representative B-scan image.](image)

| Component | Part No.       | Manufacturer          |
|-----------|----------------|-----------------------|
| CL        | 49-948-INK     | Edmund optics         |
| Telescope | Part 1: 49-363 and 49-360 Part 2: 49-357 and 49-360 | Edmund optics         |
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