Supplementary materials

Trans-ocular hypertension assessment

1. Supplemental Methods

1.1 PS-OCT system

Apparatus setup for a single-mode fiber-based polarization-sensitive optical coherence tomography (PS-OCT) is shown in Supplementary Fig. S4. A high-power broadband light source (SLD-371-HP; Superlum, Cork, Ireland) that emits light at a center wavelength of 840 nm with a 50 nm bandwidth coupled through a broadband isolator (OFR) and polarization modulator (NF4102, Newport Corporation, Irvine, CA, US) to control the polarization state in the source arm. In the detection arm, a spectrometer equipped with a single line scan camera (Teledyne e2v, UK, camera including 2048 detectors, 10 μm in 10 μm), a Wollaston prism (to direct polarization components to the line scan camera), a grating and a collimator were used. A 2D galvanometer system (GVS302, Thorlabs, New Jersey, US) was applied to focus the beam onto the retina through an ophthalmic Volk lens (30D) in the sample arm. 80% of the source output light was directed to the reference arm including a mirror, a variable neutral density filter (for attenuation), and the remaining 20% was sent to the sample arm through a single-mode fiber coupler (Gould Fiber Optic, Maryland, USA). The maximum power entering the eye was 600 μW, which is deemed safe for near-infrared light at 840 nm (according to the ANSI standard for safe use of lasers [1]). A pupil camera was added to the system, to help center the scanning beam onto the cornea. A fixation target was provided to the eye that was imaged. Polarization controllers (PC) were used in the setup to fine-tune the polarization state of the light.

Depth-resolved intensity and retardation images were obtained using the described PS-OCT system. Furthermore, equipment (line scan camera, polarization modulator, and galvanometer) used in the PS-OCT system were synchronized with each other using LabVIEW (National instruments, Texas, US) as follow:

Two National Instrument I/O board (NI6733 and NI6110) were equipped to control the polarization modulator and galvanometers with LabVIEW, respectively. A frame grabber (NI PCIe-1433, National Instruments) was also employed to record the camera signal through CameraLink. To generate the polarization states, a waveform was created in LabVIEW and then amplified with a high voltage amplifier and finally sent to the modulator. The camera and polarization modulator were then synchronized in a way that adjacent depth scans (A-lines) were grabbed with different polarization states. The modulator was also synchronized with the galvanometer system such that each cross sectional image (B-scan) was triggered according to the fast and slow scanning axes of the galvanometer.

The axial resolution of the system was 6 μm and the lateral resolution was estimated at 12.9 μm, using trial lenses to correct for the subject's astigmatism [ref your first paper]. In approximately 4 s, 100 cross sectional images (B-scans) were acquired covering 15 by 15 degrees (4.5 mm by 4.5 mm) of the largest blood vessels located within a circle with a diameter of 10˚ centered at the optic nerve head (ONH) [1]. At least two volumetric images (2 × volumetric images: 2 × 100 B-scans × 1000 A-scans × 512 pixels) were recorded from the eyes of each subject to cover the largest blood vessels superior and inferior to the ONH.

1.2 Scanning and imaging protocol

Recruited patients were asked to sign a consent form prior to imaging session. They were then asked to sit in front of the PS-OCT system and look through the ophthalmic lens and focus on the fixation target provided in the sample arm of the PS-OCT system. Three marks along a line were indicated on the fixation target (as shown in Supplementary Fig. S5). The middle mark was used to collect data for system calibration, while the left and right marks were used to take the images of the optic nerve head (ONH) depending on

whether the left or right eye was imaged. First, the subject was asked to focus on the middle mark, while a PS-OCT image was taken from the macula (a single B-scan). This image was used to calibrate the system (linear *k*-space mapping and dispersion compensation) [ref my JOSA A paper]. Then they were asked to focus on either the left mark (when the right eye is imaged) or the right one (when the left eye is imaged). By moving the fixation target, two images from the areas adjacent to the ONH were captured to cover the largest blood vessels of the eye. The imaging procedure can be seen step by step in Supplementary Fig. S5. The images were recorded in approximately 4 s. It took less than a minute prior to each imaging session to focus the light through the pupil onto the retina.

The axial resolution of the PS-OCT system used in this study was 6 μm, based on the full-width half maximum (FWHM) of the coherence function. However, in our previous study we showed that thickness measurements can be performed with a precision of roughly 1 μm [1].

1.3 Determining the edge of a blood vessel wall

To investigate the integrity of the blood vessel walls for classifying hypertensive and healthy subjects, retinal blood vessels are easily accessible with non-invasive optical imaging technology. While blood vessels in the skin are often covered in opaque tissue rendering non-invasive optical imaging ineffective, the largest retinal blood vessels can be found on top of the nerve fiber layer, free from overlying opaque tissue. PS-OCT measurements based on Stokes vectors require a reference measurement inside the tissue that is being quantified; having easy access to the top of the vessel wall facilitates this reference measurement. Secondly, OCT is an imaging modality routinely applied by ophthalmologists and optometrists. PS-OCT and retinal BBI measurements can be provided with the same instruments with some modification.

Based on our previous study [1], and with minor modifications, a similar procedure was adopted to find the edge of the blood vessel and extract the thickness and DPPR/UD of the wall. Supplementary Fig. S6 shows these steps. In short, after finding the proper cross sectional image (B-scan - indicated by a horizontal green line) based on the intensity *en-face* image in step 1, and generating the logarithmic intensity and Doppler flow images (step 2), blood vessels were isolated. Blood vessels can easily be distinguished based on the intensity and flow images as they show high intensity and flow rate (green arrows). Then the intensity and flow images were realigned (step 3) based on the surface of the retina, based on intensity thresholding [2]. In step (4), the blood vessels were isolated (indicated by two vertical red lines). These areas were then flipped by 90˚ (step 5) and used to create the average-intensity (blue) and cumulative retardation (orange) plots (shown in step 6). DPPR/UD values were obtained based on the retardation induced by the birefringent blood vessel walls. Finally, these plots were used to find the blood vessel wall's inner edge. The intensity plot showed a drop in intensity with depth after which point the blood vessel edge ended while the cumulative double-pass phase retardation saw a change in its slope [1]. This point indicated the thickness of the blood vessel wall (thickness = 15 μm). To find DPPR/UD, a least-squares linear fit (green line in step 6) was fit to the retardation plot and the retardation value at the blood vessel wall edge was divided by its thickness (DPPR/UD = 0.64˚/μm). The red highlighted area in step 6 shows the blood vessel wall. There might be some motion artifacts in the images (observed as a shift in *en-face* images), but these artifacts do not impact the DPPR/UD, thickness and BBI measurements as the blood vessels are traced and isolated individually in each cross sectional images (B-scans).

After realigning the intensity cross sectional images (B-scans) with respect to the retinal surface, a reference point on top of the blood vessel wall was selected. This process was most straightforward in blood vessels that are on top of the retinal surface, since the reference point should be positioned inside the blood vessel wall, but not in the retinal nerve fiber layer (RNFL), which has a different DPPR/UD and likely a different fast-axis orientation. In some rare cases, where the top of the blood vessel was embedded inside the RNFL, manual intervention was required to assure that the reference was positioned on top of the vessel wall, and not inside the RNFL tissue.

1.4 Data exclusion criteria

Excluded BBI, DPPR/UD and thickness data were identified as the elements more than three scaled median absolute deviation (MAD) from the median:

$$median - 3 \times scaled\ MAD \leq Included\ data \leq median + 3 \times scaled\ MAD \quad (S1)$$

and the scaled MAD is calculated based on the following equation:

$$scaled\ MAD = 1.4826 \times median\ (|x_i - median\ (x_i)|)\ . \quad (S2)$$

Extracted data were refined prior to analysis according to these exclusion criteria and outlier data were excluded. These data can be seen in Supplementary Fig. S7 and Supplementary Table S4.

The excluded data are identified using the median absolute deviation (MAD) equation, which is a robust method to identify the outliers [3]. While it is more common to use the mean plus minus three standard deviation, this was problematic because both the mean and standard deviation are susceptible to outliers and second, in small sample sizes, the mean and standard deviation are not reliable enough to detect outliers [3]. MAD on the other hand is immune to the sample size and insensitive to the presence of outliers [3].

Furthermore, only BBI data for the hypertensive subjects are excluded (Supplementary Table S4), which could indicate that those data belong to a blood vessel that has been affected strongly by high blood pressure. These values for the arteries are 212 and 315 m. For the veins, excluded BBIs are 203, 211, and 316 m. The corresponding DPPR/UD and thickness values for these excluded BBIs are also listed in the table. Note that all of these data points are at the high end of the BBI range. Including these data would not affect the thresholds that determine discrimination between healthy subjects and hypertensives, which occurs at the lower end. Also, when a single data point was excluded, the corresponding data points for other quantities were also excluded automatically. For instance, for the excluded BBIs, the corresponding thickness and DPPR/UD values were eliminated as well.

A similar conclusion can be made for excluded DPPR/UD and thickness data, since the corresponding BBI values for these data points do not affect the threshold that was used to distinguish between healthy subjects and hypertensives. For instance, for excluded DPPR/UD data points from hypertensive arteries, the corresponding BBI values are all at the higher end of the BBI range. For DPPR/UD excluded data points from normotensive arteries, corresponding BBI values are inside the healthy region and do not have any impact on the thresholds. Likewise, for excluded thickness values, corresponding BBIs are again inside the healthy BBI region.

2. Supplemental References

1. H. Afsharan, M. J. Hackmann, Q. Wang, F. Navaeipour, S. V. K. Jayasree, R. J. Zawadzki, D. Silva, C. Joo, and B. Cense, "Polarization properties of retinal blood vessel walls measured with polarization sensitive optical coherence tomography," Biomed. Opt. Express **12**, 4340-4362 (2021).
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3. C. Leys, C. Ley, O. Klein, P. Bernard, and L. Licata, "Detecting outliers: Do not use standard deviation around the mean, use absolute deviation around the median," Journal of Experimental Social Psychology **49**, 764-766 (2013).
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3. Supplemental Tables

Table S1. Average DPPR/UD, thickness and BBI values of blood vessels of normotensive and hypertensive subjects. Hypertensive subjects have thicker blood vessel walls with lower DPPR/UD and show larger BBI values compared to normotensive subjects. To classify arteries and veins as hypertensive or normotensive, BBI measurements can be solely used, while DPPR/UD and thickness measurements should be considered together. SD: standard deviation.

		DPPR/UD (˚/µm) ± SD	Thickness (µm) ± SD	BBI (m) ± SD
Arteries	Normotensive	0.55 ± 0.09	15 ± 1	37.4 ± 13.0
	Hypertensive	0.40 ± 0.06	24 ± 3	112.1 ± 25.6
Veins	Normotensive	0.51 ± 0.07	15 ± 2	45.8 ± 11.1
	Hypertensive	0.40 ± 0.06	24 ± 4	114.7 ± 27.9
All blood vessels	Normotensive	0.52 ± 0.08	15 ± 1	41.6 ± 12.7
	Hypertensive	0.40 ± 0.06	24 ± 4	113.4 ± 26.5

Table S2. Calculated pdf-CIs for DPPR/UD, thickness and BBI measurements of arteries and veins of normotensive and hypertensive subjects. Pdf-CIs were calculated based on mean ± 1.96 × SD. The overlap for thickness and BBI is calculated as [upper limit (normotensive pdf-CI) - lower limit (hypertensive pdf-CI)]/ [upper limit (hypertensive pdf-CI) - lower limit (normotensive pdf-CI)] ×100. The overlap for DPPR/UD is [upper limit (hypertensive pdf-CI) - lower limit (normotensive pdf-CI)]/ [upper limit (normotensive pdf-CI) - lower limit (hypertensive pdf-CI)] ×100.

		DPPR/UD (˚/µm)		Thickness (µm)		BBI (m)	
Arteries	Normotensive	0.36 – 0.74	Overlap: 35.6%	12 – 17	Overlap: 5.8%	11.9 – 63.0	Overlap: 0.7%
	Hypertensive	0.28 – 0.52		16 – 29		61.9 – 162.4	
Veins	Normotensive	0.38 – 0.64	Overlap: 42.6%	12 – 18	Overlap: 8.2%	24.0 – 67.6	Overlap: 5.1%
	Hypertensive	0.28 – 0.53		16 – 32		60.1 – 169.4	

Table S3. Detailed characteristics of the study subjects (N = 22). 11 hypertensive subjects and 11 age-matched normotensive subjects were recruited for the study. NTN: normotensive, HTN: hypertensive, BMI: body mass index.

Subjects	HTN/ NTN	Age (y)	Gender	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	NTN	56	M	183	96	28.7
2	NTN	50	M	181	91	27.8
3	NTN	64	M	171	82	28.0
4	NTN	71	F	164	61	22.7
5	NTN	59	M	177	79	25.2
6	NTN	63	M	170	79	27.3
7	NTN	68	F	154	52	21.9
8	NTN	44	F	169	77	27.0
9	NTN	72	M	190	103	28.5
10	NTN	60	M	160	67	26.2
11	NTN	59	F	166	60	22.5
12	HTN	61	M	177	86	27.4
13	HTN	73	M	189	102	28.5
14	HTN	64	M	182	81	24.4
15	HTN	76	M	172	76	25.7
16	HTN	64	M	179	96	30.0
17	HTN	43	M	163	74	27.9
18	HTN	72	M	174	83	27.4
19	HTN	53	M	183	74	23.1
20	HTN	66	M	179	86	26.8
21	HTN	85	M	173	72	24.1
22	HTN	63	M	170	84	29.4

Table S4. Excluded BBI, thickness and DPPR/UD values. These excluded values (also shown in Supplementary Fig. S7) were identified based on being more than three scaled MAD away from the median. Corresponding values are also listed in the table. NTN: normotensive, HTN: hypertensive.

Excluded BBI			
~Excluded BBI (m)	Corresponding DPPR/UD (˚/μm)	Corresponding thickness (μm)	
316	0.26	29	HTN vein
211	0.28	19	HTN vein
203	0.32	19	HTN vein
212	0.37	30	HTN artery
315	0.34	37	HTN artery
Excluded thickness			
Corresponding BBI (m)	Corresponding DPPR/UD (˚/μm)	Excluded thickness (μm)	
25	0.76	20	NTN vein
11	0.84	11	NTN artery
16	0.94	20	NTN artery
12	0.81	11	NTN artery
391	0.26	36	HTN artery
446	0.24	35	HTN artery
282	0.31	37	HTN artery
305	0.29	35	HTN artery
Excluded DPPR/UD			
Corresponding BBI (m)	Excluded DPPR/UD (˚/μm)	Corresponding thickness (μm)	
30	0.72	21	NTN vein
10	0.92	11	NTN artery
25	0.81	22	NTN artery
7	1.00	10	NTN artery

4. Supplemental Figures

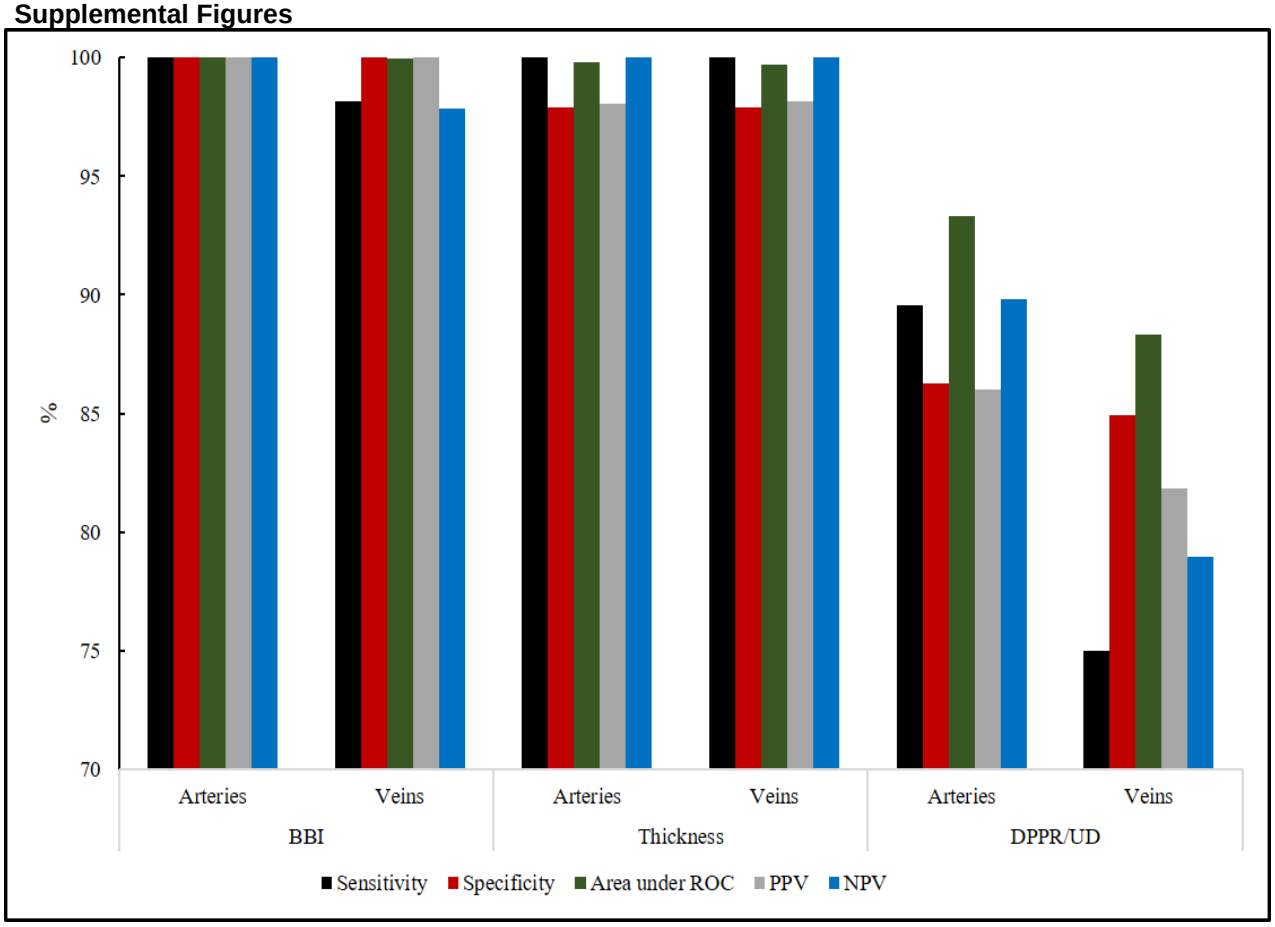

Fig. S1**. Performance of the proposed methods**. Based on ROC curves, BBI shows the highest performance when compared to thickness and/or DPPR/UD measurements. PPV: positive predicted value, NPV: negative predicted value.

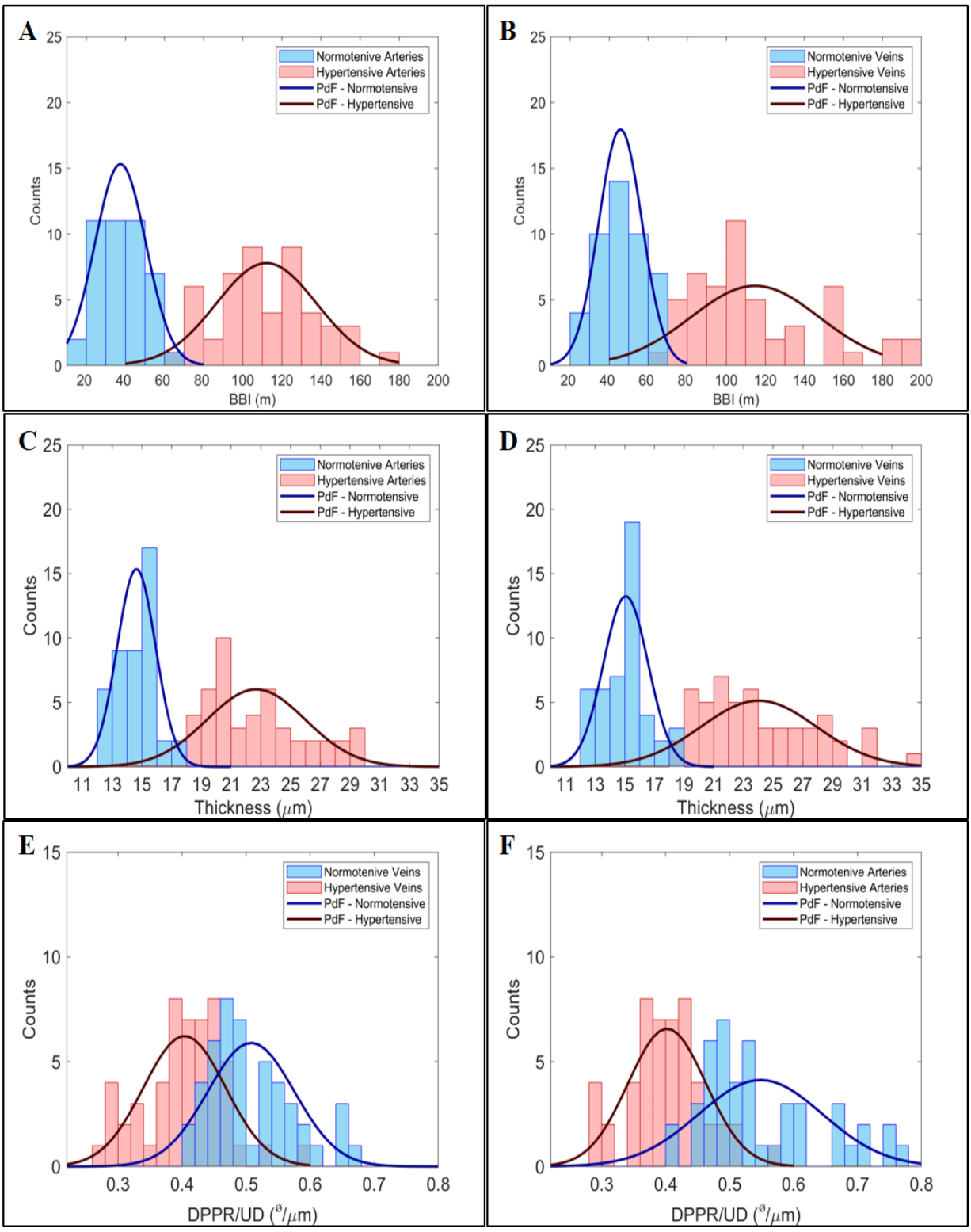

Fig. S2. **Probability density functions used to calculate pdf-CIs.** (A, B) BBI, (C, D) thickness and (E, F) DPPR/UD histograms and respected probability density functions of arteries (left panels) and veins (right panels) of hypertensive (red) and normotensive (blue) subjects. MATLAB was used to generate the pdf curves. Pdf: Probability density functions.

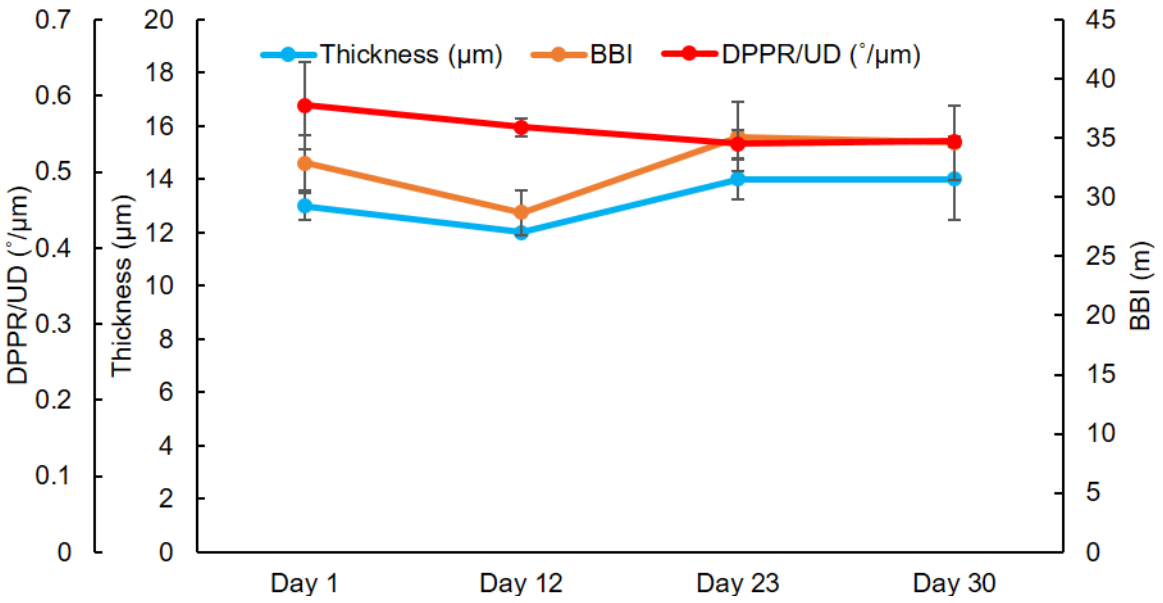

Fig. S3. **Repeatability measurements showing averaged values for thickness, DPPR/UD and BBI.** Data were recorded from the right eye of a healthy subject over four adjacent cross-sectional images (B-scans) during four visits. Error bars indicate standard errors. Based on ANOVA analysis, the repeatability for DPPR/UD, thickness and BBI measurements were calculated as 13%, 12% and 10%, respectively.

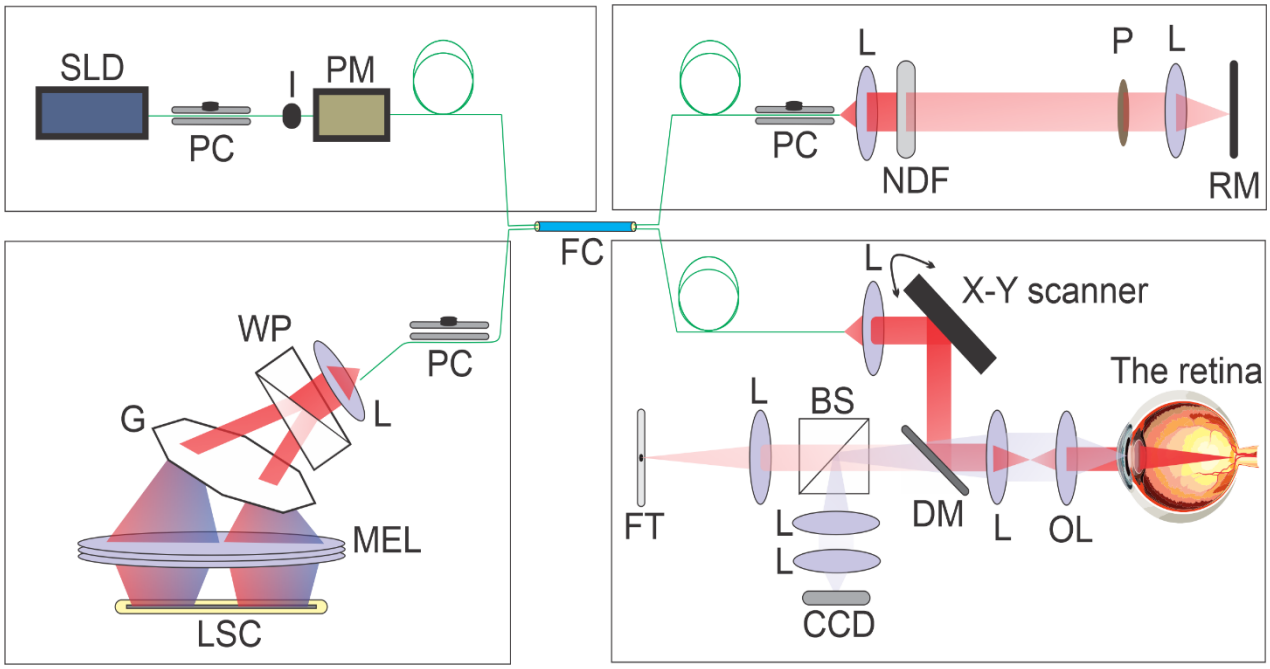

Fig. S4. **Schematic of the PS-OCT system.** Light from a broadband light source (SLD) coupled with an isolator (I) was polarization modulated through a polarization modulator (PM). The polarized light was distributed into the sample and reference arms through a single-mode 80/20 fiber couple (FC). In the reference arm, the light traveled through a neutral density filter (NDF) and reflected back from the reference mirror (RM). In the sample arm, the retina was scanned with the use of an X-Y galvanometer scanning system, a dichroic mirror (DM) and an ophthalmic lens (OL). A pupil camera (CCD) was also housed into the sample arm through a beam splitter (BS) to center the beam onto the pupil. A fixation target (FT) was added to stabilize the eye. A polarization sensitive spectrometer including a collimator, a calcite Wollaston prism (WP), a transmission grating (G), a photographic multi-element lens (MEL) and a line-scan camera (LSC) were used in the detection arm to detect the interference fringes. Polarization controllers (PC) were also employed in the source, reference and detection arms to fine tune the polarization of the light. L: lens, P: polarizer.

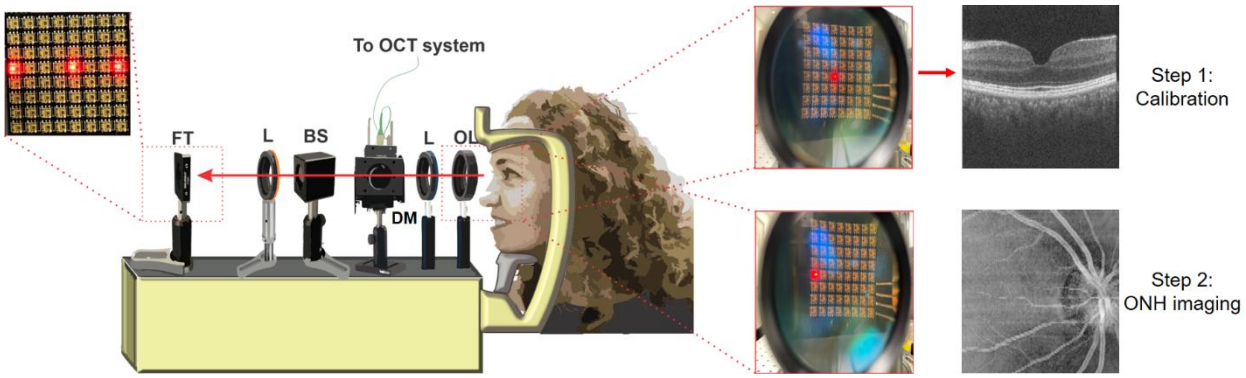

Fig. S5. **Scanning protocol**. The subjects were asked to focus on the fixation target. In step 1, they looked at the red light in the middle to perform a calibration procedure (step 1). A single cross sectional image was taken in this step from the fovea of the subject. Then (step 2) they were asked to look at the light on the left or right hand-side of the fixation target (depending on the imaged eye), while a volumetric scan was recorded from the area around the optic nerve head. This scan was used to extract polarization information of the blood vessels. FT: fixation target, L: lens, BS: beam splitter, DM: dichroic mirror, OL: ophthalmic lens.

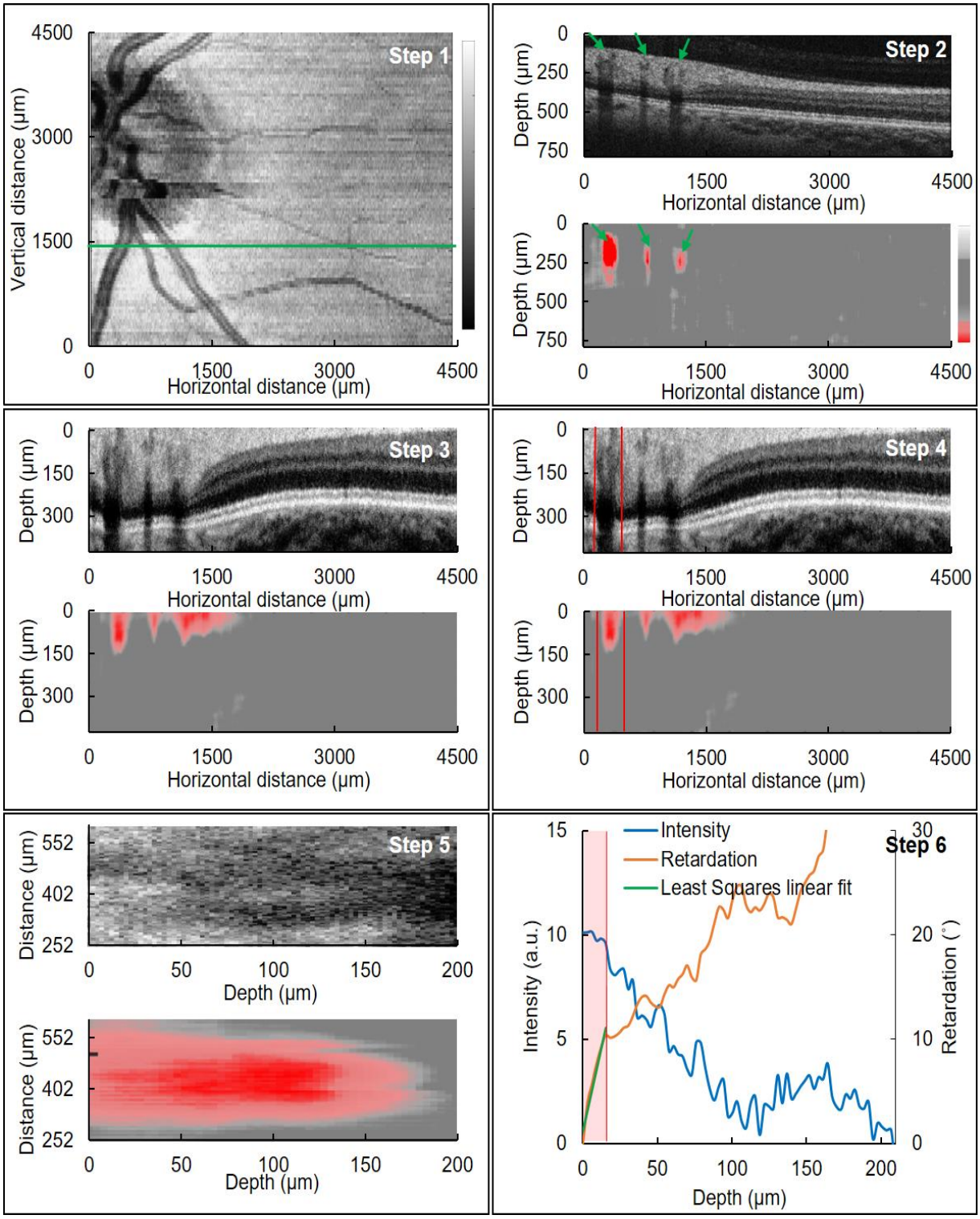

Fig. S6. **Determining the blood vessel wall edge.** Step 1: *En-face* intensity image (right eye of a healthy female subject). Step 2: Logarithmic intensity and Doppler flow images (indicated by a green line in step 1) showing three blood vessels marked with green arrows. Step 3: Realigned logarithmic intensity and Doppler flow images with respect to the retinal surface. Step 4: Isolated blood vessel inside the two vertical red lines. Step 5: 90˚ flipped intensity and flow images. Step 6: Averaged-intensity (blue) and cumulative retardation

(orange) plots and a least-squares linear fit (green) used to find the DPPR/UD. At the interface between blood vessel wall and the blood, the intensity experienced a decrease and the rate of change in retardation decreased. Shaded red area indicates the blood vessel wall thickness equal to 15 μm. The DPPR/UD of the wall is 0.65˚/μm. Intensity color-bar (a): darker areas represent a lower intensity. Flow color-bar: -4.7×10^{-4} to $+4.7 \times 10^{-4}$ (a.u.). Images were generated in MATLAB.

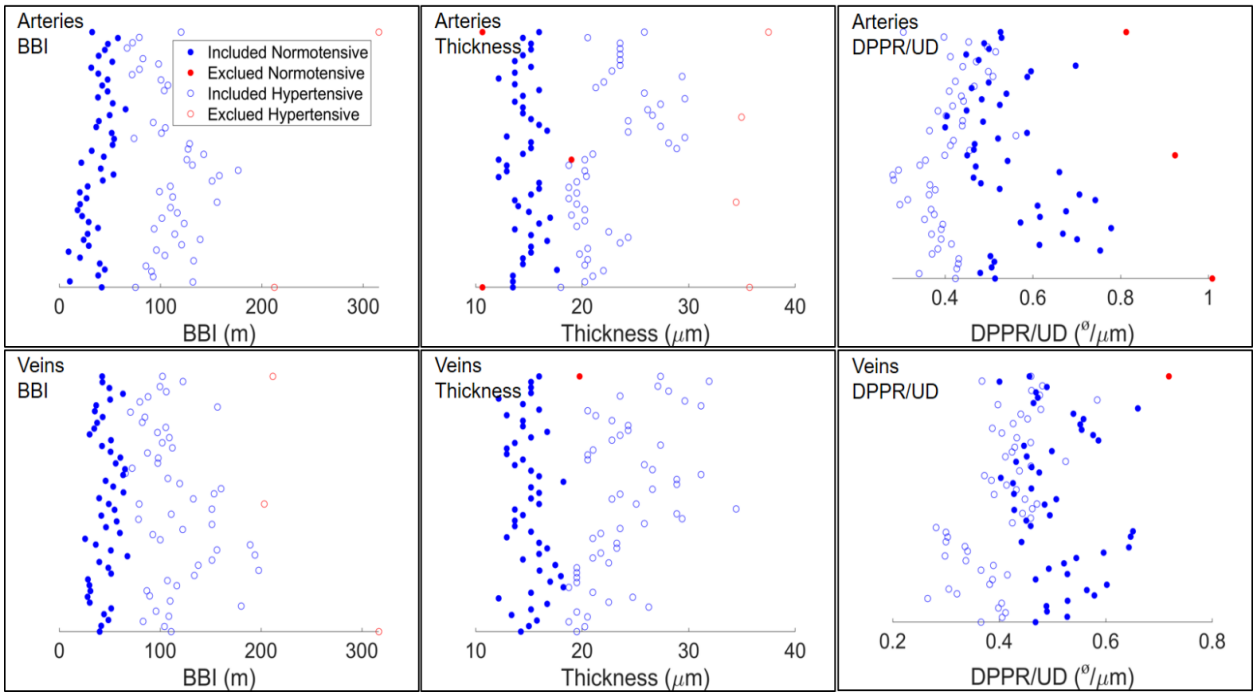

Fig. S7. **Excluded and included BBI, thickness and DPPR/UD data**. Outlier data were excluded according to the exclusion criteria. Any data-points more than three scaled MAD from the median were excluded from the study. Filled and unfilled circles represent normotensive hypertensive data-points, respectively. Blue and red indicate included and excluded data-points, respectively. In total, 5 BBI data-points, 4 DPPR/UD data-points and 8 thickness data-points were excluded from the study. Plots were generated using MATLAB.