# Supplementary Materials for

## Demystifying the feasibility of in vivo nonresonant Raman spectroscopy of the human retina

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**This PDF file includes:**

Supplementary Text
Figs. S1 to S3

**Supplementary Text**

Multimodal eye model

The multimodal eye model (Fig. S2) consists of an artificial cornea, a water-filled anterior chamber, an adjustable iris diaphragm, a lens, a water-filled vitreous body, an artificial retina and a choroid. The artificial retina has a radius of 12 mm and a total thickness of 500 µm. It consists of three layers with a thickness of 200 µm (layer 1), 150 µm (layer 2) and 150 µm (layer 3). These layers were made of UV-curing resin, Norland Optical Adhesive 61 (NOA61), with a mixture of $TiO_2$ and $Al_2O_3$ distributed in it. The weight concentration of $TiO_2$ and $Al_2O_3$ was chosen such that the layers' reduced scattering coefficient was 1 $mm^{-1}$ at 600 nm excitation wavelength. The vascular structures were made by dissolving 2 mg of Phthalo Blue (Royal Blue, PB15:3) in 1 g of NOA61, which yields an absorption coefficient of 12 $mm^{-1}$ at 635 nm excitation wavelength. Three vessels were embedded in the artificial retina of the multimodal eye model. Two vessels were embedded inside layer 2 with a diameter of 150 µm. The other vessel was embedded inside layer 3 and has a diameter of 250 µm. The choroid has a spherical shape with an inner diameter of 25 mm and a thickness of 5 mm. It was also made of UV-curing resin (NOA61), with a mixture of $TiO_2$ and $Al_2O_3$ distributed in it, resulting in a reduced scattering coefficient of 1 $mm^{-1}$ at 600 nm excitation wavelength.

Multimodal eye model measurement

We demonstrated the imaging capabilities and the performance of the multimodal ophthalmic imaging system by using a dedicated multimodal eye model (Fig. S2), which was mounted in front of the ophthalmic lens, at the same location where the human subject's pupil was located. The fundus IR image is shown in Fig. S3A, which reveals three straight vessels in the 3D-printed retina phantom. The darker profile compared to its surrounding indicates a higher absorption coefficient of the Royal Blue pigment mimicking the absorption of hemoglobin. The visible horizontal stripes are artefacts due to the line field detection scheme. The OCT en face image and the B-Scan image are shown in Fig. S3B and S3C, respectively. The en face image was generated by calculating the maximum intensity projection along the OCT A-Scan. The vessel structure is barely visible on the OCT en face image due to little refractive index differences between the vessel structure and its surrounding. The B-Scan image in Fig. S2B is taken from the position indicated as the yellow-dashed line in Fig. S3A and S3C. The 3 retinal phantom layers can be distinguished from each other as shown by the green arrows. The choroid is indicated by the orange arrow. The magenta arrows are indicating the vessel structure location seen on the B-Scan image. To validate the capability of the RS module, we measured 2 distinct positions, one at the vessel position and the other one at the region without vessel structure. The Raman measurement spots are shown in cyan and red (Fig. S3C) which correspond to the Raman spectra of the background position and the vessel position shown in Fig. S3D, respectively. The outlined measured Raman bands at 679 cm-1, 747 cm-1, 953 cm-1, 1143 cm-1, 1341 cm-1, 1450 cm-1 and 1529 cm-1 reveal the Raman bands of Phthalo Blue (Royal Blue, PB 15:3), similar to the Raman bands found in the ref. (*49*).

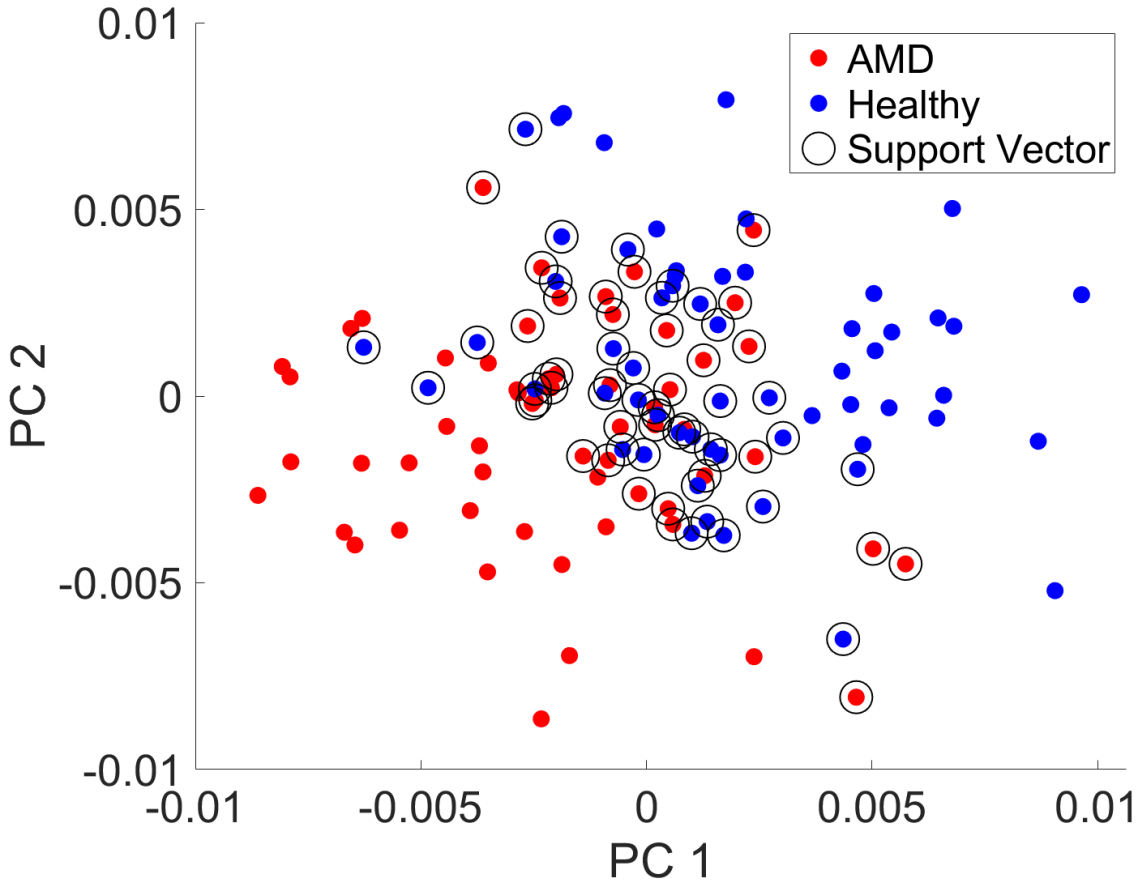


**Fig. S1. PCA-SVM result from single measurement (no Raman signal averaging for each data point)**

60 AMD and 60 healthy Raman spectra were analyzed using principal component analysis and support vector machine with 5-fold cross validation, resulting in 70.83% of accuracy.

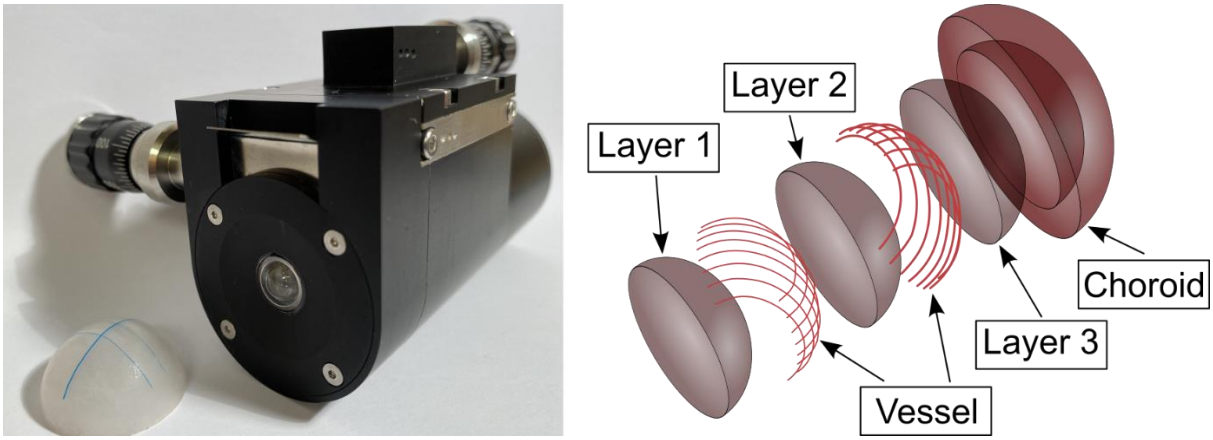


**Fig. S2. Multimodal eye model**

Photo and illustration of the multimodal eye model and the retina phantom. The inner retina phantom consists of 3 layers with vessels between each layer. 3 vessel lines are present in the actual retina phantom.

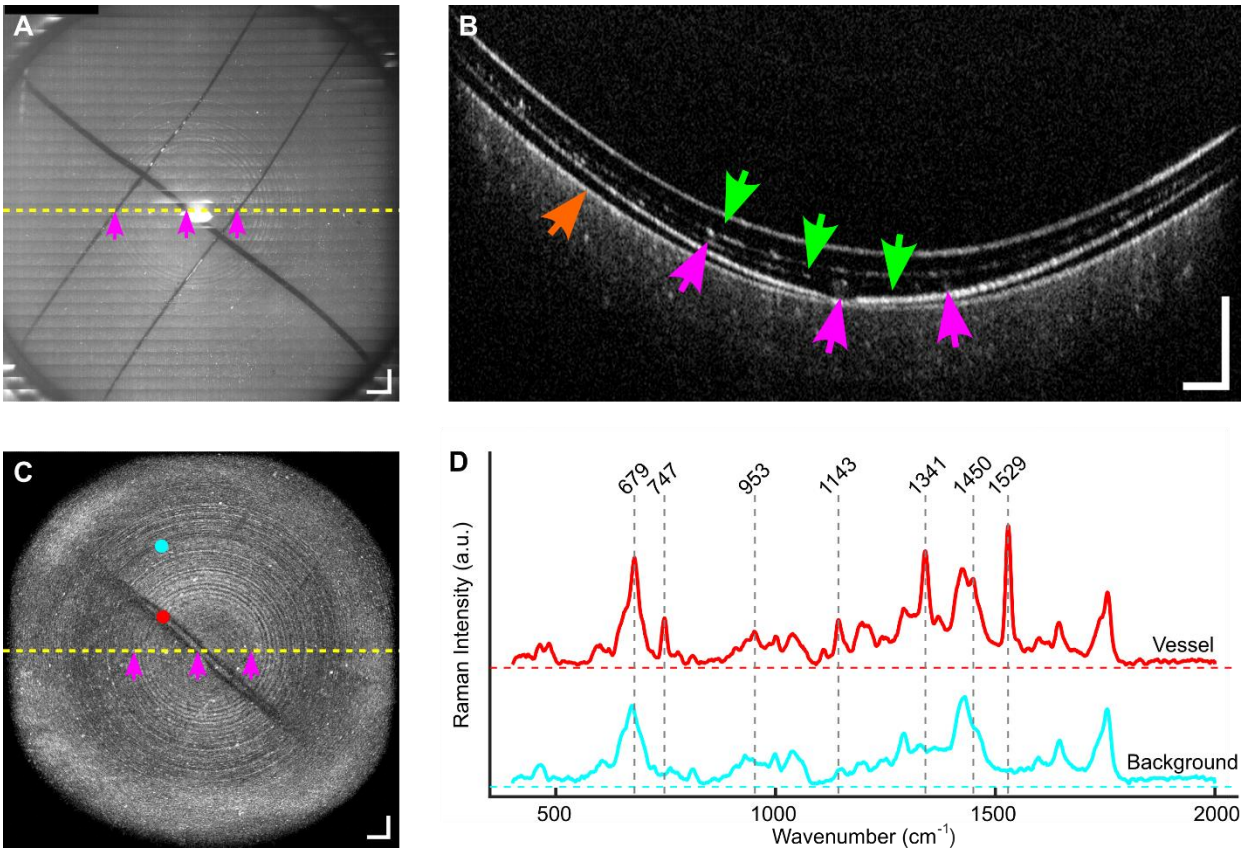


**Fig. S3. Multimodal eye model measurement.**
(A) Image acquired by the fundus IR system. (B) OCT B-Scan slice at the position indicated as the yellow dashed line in (A) and (C). The magenta arrows indicate the 3 vessels as shown in (A) and (C). The green arrows and the orange arrow indicate layer 1 to layer 3 and the choroid, respectively, as illustrated in Fig. S1. (C) en face projection of the acquired OCT volume. (D) The Raman spectra at the background and the vessel location, indicated as cyan and red point in (C), respectively. The indicated wavenumbers show the Raman signals of the vascular structure, which corresponds to the Raman spectrum contribution of the blue pigment. Scale bar: 1 mm.