User Manual: A 2D Quantitative Retinal Blood Flow Measurement Method in Humans with Multimodal Adaptive Optics Imaging

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A 2D Quantitative Retinal Blood Flow Measurement Method in Humans with Multimodal Adaptive Optics Imaging

AO imaging system

The multimodal AO system used for the development of the tool was custom-built and has been described in detail elsewhere [1]. In brief, the mAO system has OCT and SLO channels, which can be used simultaneously (slow scan mode) or independently (fast scan mode), where slow or fast scan mode refers to the OCT channel with respect to the 27 Hz AO-SLO frame rate. Superluminescent diodes with center wavelengths of 756 nm (Δλ = 20 nm) and 830 nm (Δλ = 60 nm) were used for AO-SLO and AO-OCT imaging, respectively. AO-SLO imaging was achieved with a resonant scanner operating at a line rate of 13.6 kHz. The axial resolution for the AO-OCT volume is ~3.7 µm (n = 1.38).

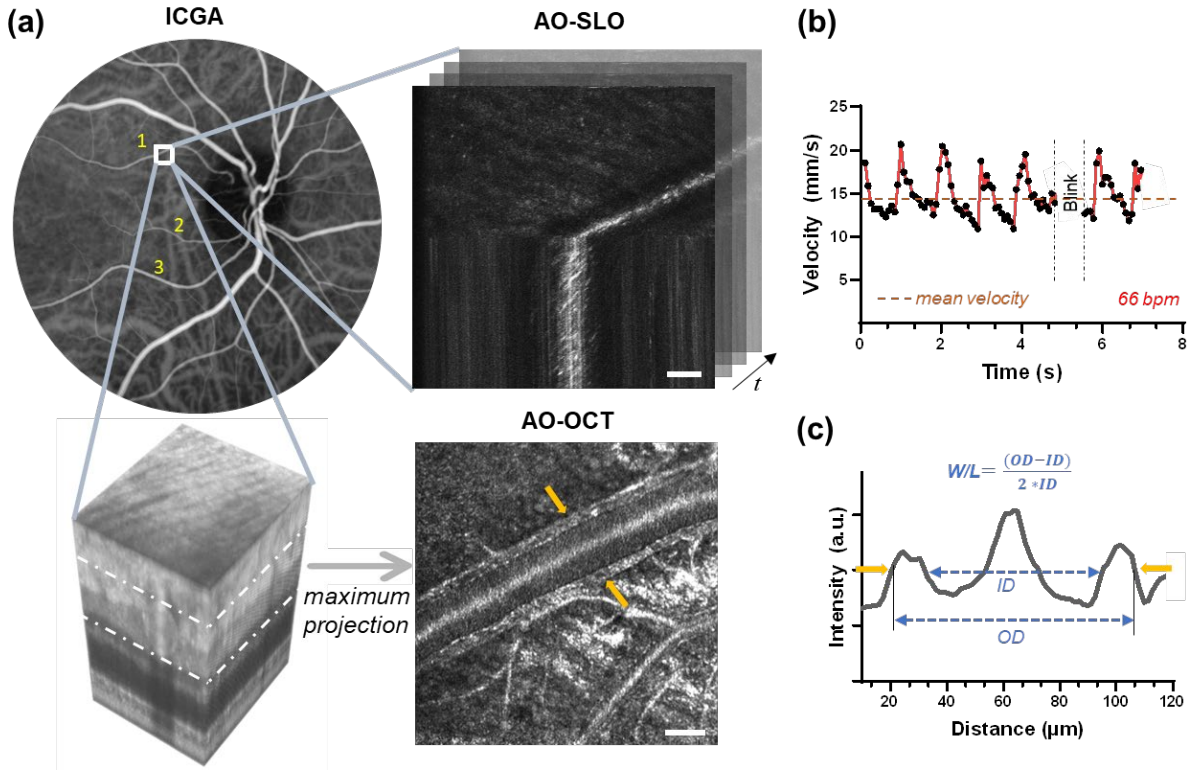

Fig. 1. (a) Representative indocyanine green angiography (ICGA) image from a healthy volunteer showing inner retinal vessels and a highlighted region of interest. Corresponding AO-SLO space-time images reveal streaks originating from moving blood cells, with slope proportional to its absolute velocity. (b) Mean velocity is derived from the pulsatile velocity plot. A maximum *en face* projection from the AO-OCT volume in the inner retina shows clear vessel walls where arrows indicate outer edges. (c) Automated ImageJ macro analysis of the line profile at half max quantified inner diameter (ID), outer diameter (OD) and wall-lumen ratio (W/L). Scale bar = 50 µm

Imaging protocol

Indocyanine green angiograms (ICGA) images collected with a scanning laser ophthalmoscope device (Heidelberg Spectralis) aid in the identification of vessels of interest prior to AO imaging but is not a pre-requisite, as shown in Fig. 1. Particularly vessels that meet the vessel angle criteria of our AO-SLO line scan approach (<45°). The shallow angle requirement ensures discreet streaks on space-time images and a more reliable blood flow velocity analysis, as described by Joseph et al. [2]. Following are important steps:

- The volunteer's pupil should be dilated with 0.5% tropicamide for AO imaging.
- For vessel imaging, the AOSLO channel should be operated in line-scan mode to generate space-time images by holding the slow scan axis galvanometer scanner stationary. In line scan mode, the system acquisition software should allow an interleaved sequence of line-scan and full-scan frames. For this method demonstration, we operated with a 1:1 ratio between line-scan and full-scan frames and collected 200 frames (~7.4 sec video) which span multiple cardiac cycles.
- For moderately sized retinal vessels (25-100 µm in diameter), 0.9 µm/pixel for the AO-SLO mode (27.2 frames/s, 500×500 pixels) was used with a field of view of 1.5°×1.5°.
- System focus was set by the deformable mirror to the inner retinal layer for both modes.
- Switch to AO-OCT channel and collect at least 30 volumes at the same retinal location to generate adequate contrast of retinal vessel walls. It may be possible to measure the vessel wall with a lower number of average volumes so some trial-and-error may be required.
- For moderately sized retinal vessels, a lateral pixel density of 1.5 µm/pixel was used for the AO-OCT fast scan mode (2.3 vol/s, 300×300 pixels) with a field of view of 1.5°×1.5°.
- Both AO-SLO and AO-OCT images should be acquired from the same set of retinal vessels consecutively, depending on the exact device specifications. Most device configurations don't typically allow simultaneous collection of OCT volumes and lines scan SLO images except from completely separate devices.

Analysis protocol

Two key measures for calculating blood flow rate are vessel diameter and flow velocity, both derived from AO-OCT and AO-SLO analysis, as outlined below.

1. *AO-OCT analysis for vessel diameter*

   The acquired AO-OCT volumes were reconstructed, and a custom sub-cellular accuracy 3D registration algorithm was applied to remove eye motion artifacts [3, 4]. The thirty AO-OCT volumes should be averaged to improve SNR and used for vessel morphological analysis. An *en face* AO-OCT image was obtained by maximum intensity projection over ~37 µm (10 pixels) in the inner retina layer (superficial vascular plexus) that contained the vessel of interest. A custom macro, adapted from the VasoMetrics macro used in multiphoton microscopy, in ImageJ (NIH, Bethesda, MD) is made available, which should be used to measure lumen inner diameter (ID) and outer diameter (OD) [5].

2. *AO-SLO analysis for single cell velocity*

A semi-automated MATLAB (MathWorks, Natick, MA) algorithm was developed for AO-SLO image analysis based on the Radon algorithm detailed elsewhere [2, 6], with several enhancements specifically designed for human imaging such as blink removal, intraframe motion detection and two-step registration as reported in Raghavendra et al. [7]. The key parameters that influence the flow velocity determination and hence the mean flow rate includes scanner frequency, FOV, spatio-temporal resolution, vessel orientation, axial length of the eye, registration parameters, radon step-angle, SNR thresholding and binning time window. The MATLAB code is made available at GitHub for complete AO-SLO image analysis [5].

Flow rates should be computed by combining mean velocity ($v_{mean}\ mm/s$) and mean lumen ID (μm) assuming a circular cross-sectional area for the retinal vessels:

$$Q = v_{mean} \frac{\pi \times ID_{mean}^2}{4}$$

where Q is the mean blood flow rate in µL/min. Conservation of flow rates for a retinal vessel branch was performed to validate our mAO RBF quantification method from a healthy volunteer (Fig. 2). The mean flow rate in the parent vessel was 1.95 µL/min and the sum of the measured flow rates in the two daughter branches was 1.99 µL/min, validating the conservation of flow measurement.

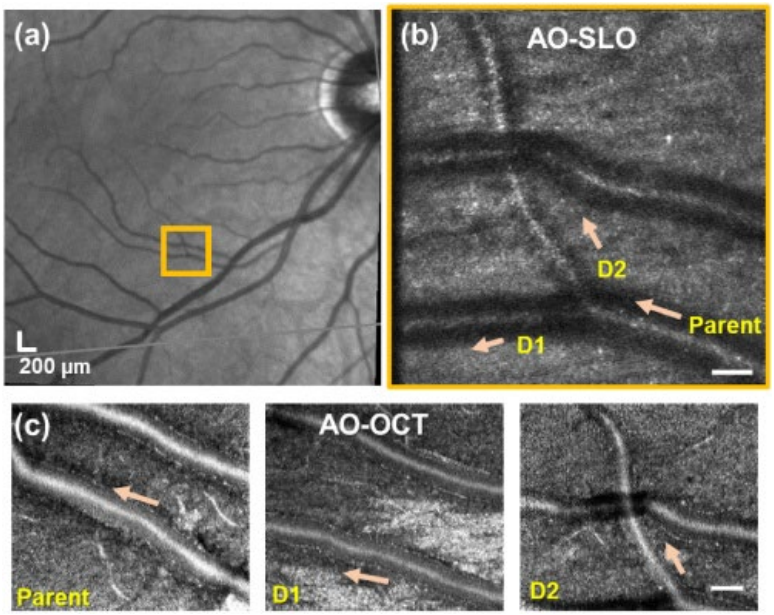

Fig. 2. Conservation of flow with the mAO RBF quantification method. (a) The Spectralis SLO image shows the retinal vessels of a healthy volunteer in the inferior macula region and the target vessel branch location. (b) An AO-SLO image of the target location with a field of view of 1.5°×1.5° identifying the parent and two daughter branches (D1 and D2). (c) Corresponding AO-OCT en face images for each vessel branch enables diameter measurement along the vessel. Scale bars = 50 µm.

References

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