Supplemental Document

Biomedical Optics EXPRESS

High-speed, phase contrast retinal and blood flow imaging using an adaptive optics partially confocal multi-line ophthalmoscope: supplement

SOOHYUN LEE,[1,*] STACEY S. CHOI,[1,2] RATHEESH K. MELEPPAT,[3,4] ROBERT J. ZAWADZKI,[3,4] AND NATHAN DOBLE[1,2]

[1] *College of Optometry, The Ohio State University, 338 West 10th Avenue, Columbus, Ohio 43210, USA*
[2] *Department of Ophthalmology and Visual Sciences, Havener Eye Institute, The Ohio State University, 915 Olentangy River Road, Suite 5000, Ohio 43212, USA*
[3] *UC Davis Eye Center, Department of Ophthalmology and Vision Science, University of California, Davis, 4860 Y Street, Suite 2400, Sacramento, California 95817, USA*
[4] *UC Davis EyePod Small Animal Ocular Imaging Laboratory, Department of Cell Biology and Human Anatomy, University of California, Davis, 4320 Tupper Hall, Davis, California 95616, USA*
[*] *lee.10225@osu.edu*

Supplement DOI: https://doi.org/10.6084/m9.figshare.25148327

Parent Article DOI: https://doi.org/10.1364/BOE.507449

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1. THE OPTIMAL LINE SEPARATION

To determine the optimal line separation, retinal images were acquired for three different line separations, 20 (10 ADD), 40 (20 ADD), and 60 (30 ADD) DMD mirror elements. As expected the crosstalk between adjacent lines decreases as the line separation increases, but this comes at the expense of requiring more sub-frames to build a single composite frame. Thus, 40 DMD mirror elements separation (20 ADD, 85 μm at the retina) was employed as this reduced crosstalk while providing a high overall composite frame rate.

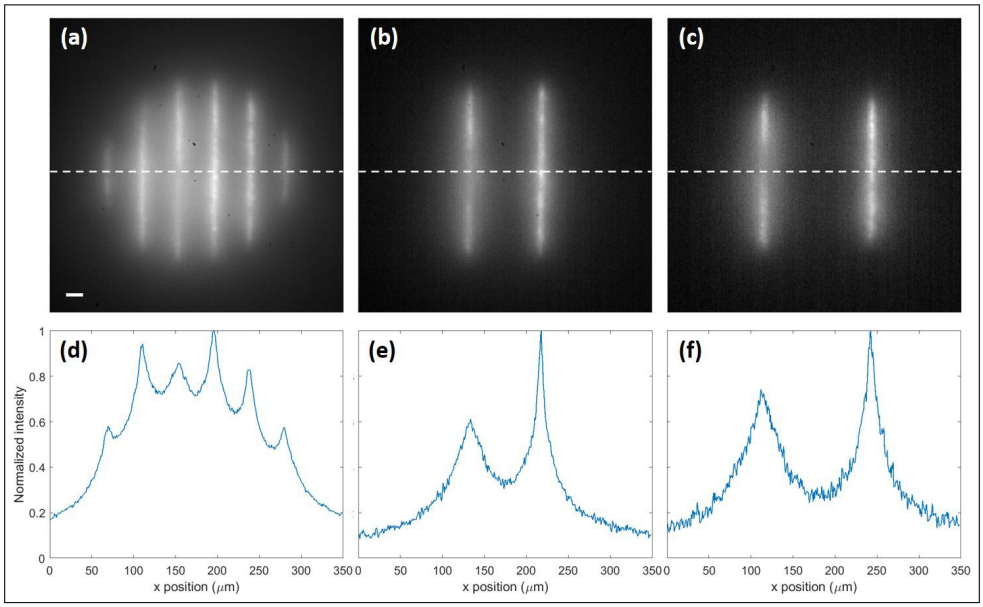

Fig. S1. Average of photoreceptor sub-frames with line separations of (a) 20, (b) 40, and (c) 60 DMD mirror elements. (d)-(f) Corresponding normalized cross-sectional plots indicated by white dotted lines in (a)-(c). The lower magnitude left-hand peak in (e) and (f) is due to a blood vessel. The scale bar is 20 µm.

2. NORMALIZED POWER SPECTRA OF PHOTORECEPTOR IMAGES

Normalized power spectrum show similar shapes for the confocal photoreceptor images captured under vertical and horizontal line illumination. The cone spacing estimated from spatial frequency peak positions in the normalized radial power spectrum were 2.9, 6.9, and 9.7 μm at the fovea, 3° TR and 6° TR, respectively. Normalized power spectra of corresponding phase contrast images have peaks around frequencies matched to the cone spacing.

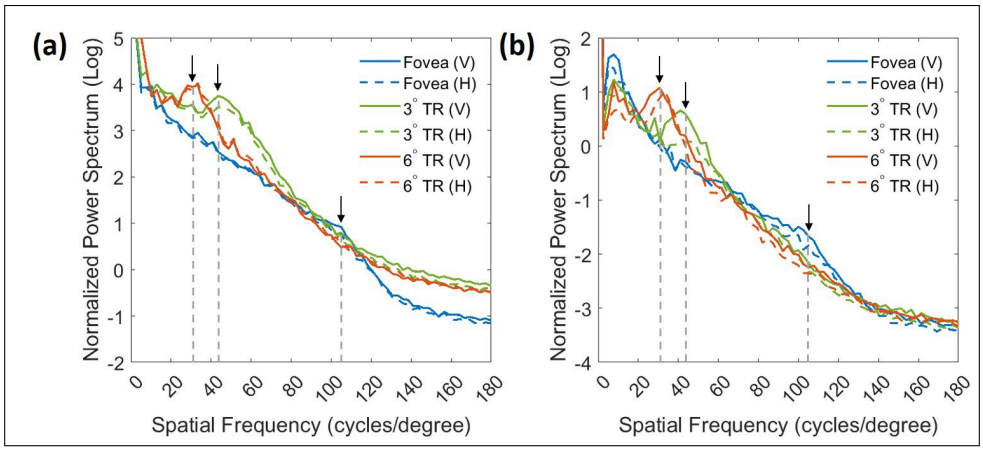

Fig. S2. Normalized radial power spectrum of (a) confocal photoreceptor images and (b) phase contrast images. The arrows indicate peak frequencies at 30.8, 43.6, 105 cycles/degree used for estimating the cone spacing (assumes 1° equals 300 μm on the retina). V: vertical line illumination, H: horizontal line illumination.

3. AVERAGE FLOW VELOCITIES AND VESSEL LUMEN DIAMETERS

The measured velocity range and fitted line were reasonably matched with that of control vessels having diameter of <40 μm in [1].

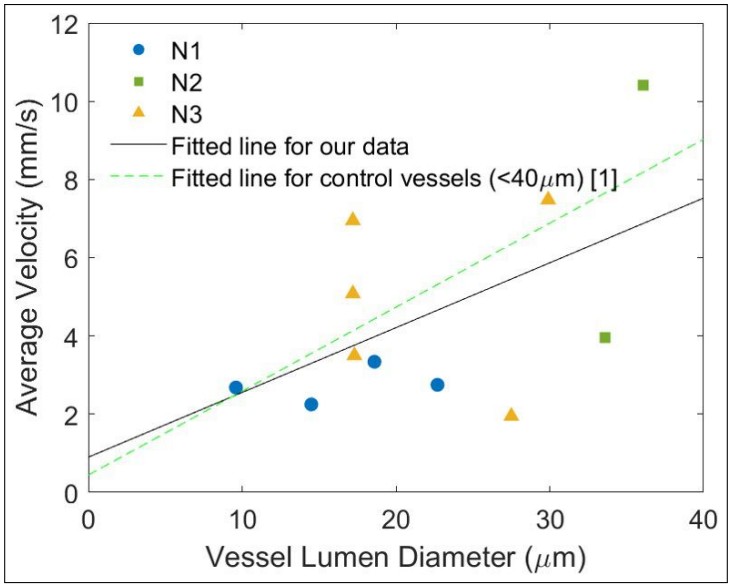

Fig. S3. Scatter plot of blood flow velocity as a function of vessel lumen diameter. Fitted line for our data and control vessels having lumen diameter <40 μm reported in [1].

4. VELOCITY MEASUREMENT USING CONFOCAL AND PHASE CONTAST IMAGES

To examine the role of contrast on velocity measurement, the kymographs of the retinal capillary C1 (Fig. 9(d)) obtained from the confocal and phase contrast images are presented with measured velocities, and it is clear that the signal from the phase contrast approach is less noisy.

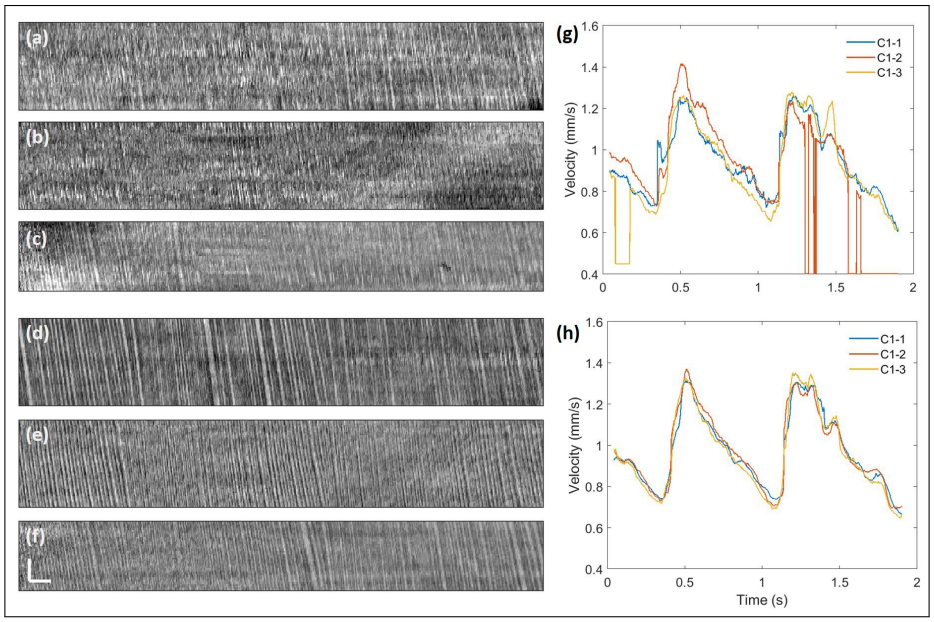

Fig. S4. Kymographs of different segments of a capillary, (a) C1-1, (b) C1-2, and (c) C1-3 obtained from confocal images and (d)-(f) corresponding kymographs from phase contrast images. Blood flow velocities of a capillary measured using (g) confocal and (h) phase contrast images. The horizontal scale bar is 80 ms, and the vertical scale bar is 10 µm.

REFERENCES

1. C. M. A. Palochak, H. E. Lee, J. Song, *et al.*, "Retinal blood velocity and flow in early diabetes and diabetic retinopathy using adaptive optics scanning laser ophthalmoscopy," J. Clin. Medicine **8** (2019).