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## *Functional imaging of hemodynamic stimulus response in the rat retina with ultrahigh-speed spectral / Fourier domain OCT*

**Massachusetts Institute of Technology**

# Functional imaging of hemodynamic stimulus response in the rat retina with ultrahigh-speed spectral / Fourier domain OCT

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## ABSTRACT

Measuring retinal hemodynamics in response to flicker stimulus is important for investigating pathophysiology in small animal models of diabetic retinopathy, because a reduction in the hyperemic response is thought to be one of the earliest changes in diabetic retinopathy. In this study, we investigated functional imaging of retinal hemodynamics in response to flicker stimulus in the rat retina using an ultrahigh speed spectral / Fourier domain OCT system at 840nm with an axial scan rate of 244kHz. At 244kHz the nominal axial velocity range that could be measured without phase wrapping was +/-37.7mm/s. Pulsatile total retinal arterial blood flow as a function of time was measured using an *en face* Doppler approach where a 200µm × 200µm area centered at the central retinal artery was repeatedly raster scanned at a volume acquisition rate of 55Hz. Three-dimensional capillary imaging was performed using speckle decorrelation which has minimal angle dependency compared to other angiography techniques based on OCT phase information. During OCT imaging, a flicker stimulus could be applied to the retina synchronously by inserting a dichroic mirror in the imaging interface. An acute transient increase in total retinal blood flow could be detected. At the capillary level, an increase in the degree of speckle decorrelation in capillary OCT angiography images could also be observed, which indicates an increase in the velocity of blood at the capillary level. This method promises to be useful for the investigation of small animal models of ocular diseases.

**Keywords:** Optical coherence tomography, neurovascular coupling, hemodynamics, flicker stimulus, Doppler, blood flow, capillary, angiography

## 1. INTRODUCTION

Because many ocular diseases, such as diabetic retinopathy and glaucoma, are associated with alterations in retinal blood flow [1, 2], measuring ocular hemodynamics *in vivo* non-invasively is an important research topic. While the role of basal retinal blood flow as a possible early marker for retinal disease is still under investigation, it is thought that the reduction in functional hyperemia, a rapid transient increase in retinal blood flow in response to flicker stimulus due to neurovascular coupling, can potentially be one of the earliest markers for retinal diseases such as diabetic retinopathy [3]. Therefore, functional imaging of retinal hemodynamics is important for investigating pathophysiology in small animal models of ocular diseases and may also be useful for assessing progression of these diseases in humans.

Optical coherence tomography (OCT) is recently becoming more widely recognized as a useful tool for functional imaging of retinal blood circulation using Doppler and speckle variance techniques as well as for conventional structural imaging of the retina [4, 5]. Ultrahigh-speed OCT is particularly attractive for dynamic imaging of the retina in small animal models because both time-resolved functional and structural information in three-dimensions with micrometer resolution can be measured using a single device, which facilitates large scale pre-clinical and clinical studies.

In this paper, we present a comprehensive study on retinal hemodynamics in response to flicker stimulus in the rat retina with ultrahigh-speed spectral / Fourier domain OCT. Pulsatile total retinal arterial blood flow is measured using an *en face* Doppler OCT technique to avoid the need to measure the Doppler angle, whereas ultrahigh speed OCT

enables high volume acquisition rates necessary for pulsatile total blood flow measurement as a function of time [6, 7]. Wide-field capillary imaging was performed utilizing OCT inter-frame speckle decorrelation which has minimal angle dependency compared to most angiography techniques based on OCT phase information [8].

## 2. METHODOLOGY

### 2.1 OCT system and assessment of Doppler flow velocities

A schematic of the spectral / Fourier domain OCT system used in this experiment is shown in Figure 1[7]. A commercial superluminescence diode (Superlum) and line scan camera (Basler Sprint spL4096-140km) were used to develop an ultrahigh-speed spectral domain OCT system at 840nm with an axial resolution of 5.7μm in tissue. The imaging speed of the system was 244,000 A-scans per second with a camera integration time of 2.8μs. The total imaging range was 1.5mm in tissue. With an incident power of 2.5mW at the cornea, the system sensitivity was measured to be 99dB. The phase stability of the OCT system was measured by inserting a cover slip in the small animal imaging interface in a common path configuration, and was 1.1mrad (standard deviation of phase differences). A non-contact imaging interface with its OCT beam scanning pivot point located at the pupil was used to avoid pressure on the cornea, which may alter intraocular pressure and retinal hemodynamics. Theoretical spot size on the retina calculated with ZEMAX using a standard rat eye model was ~15μm.
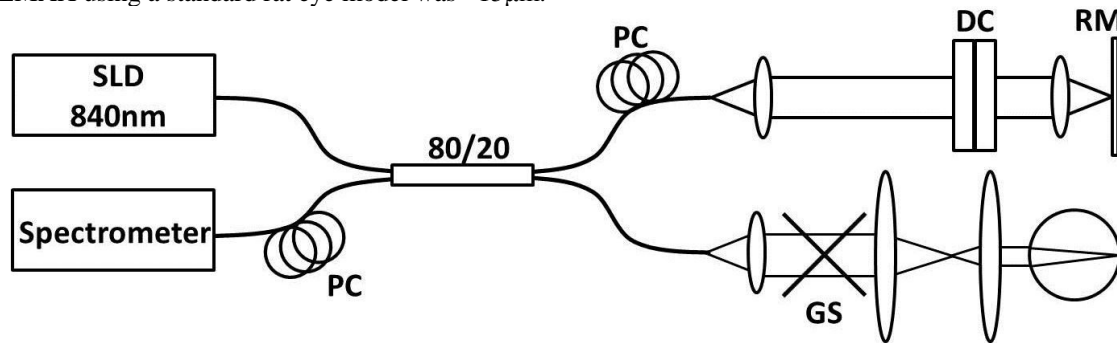


Figure 1. Schematic of the ultrahigh-speed spectral domain OCT imaging system for rats. Non-contact, pivotted scanning configuration was implemented to avoid alteration of intraocular pressure, which may alter retinal blood flow. PC: polarization controller, DC: dispersion compensation glass, RM: reference mirror, GS: galvanometer scanner pair, SLD: superluminescent diode.

Doppler axial flow velocities were calculated according to the following equation [4]:

$$v_z = \frac{\lambda_c \Delta\Phi}{4\pi Tn} \tag{1}$$

where $\lambda_c$ is the light source center wavelength, $T$ is the time elapsing between the A-scans, $\Delta\Phi$ is the phase difference between two A-scans at a given depth position $z$, and $n$ is the index of refraction in tissue.

At an axial scan rate of 244kHz, the nominal axial flow velocity range measurable with Doppler OCT without phase wrapping was ±37.7mm/s in tissue. However, for axial flow velocities up to ~75.4mm/s in tissue, it was possible to unwrap the phase without ambiguity during post processing because only the central retinal artery was used for flow calculation and the direction of flow was known in this case.

### 2.2 Total retinal arterial blood flow measurement using *en face* Doppler OCT

For total retinal blood flow calculation, an algorithm demonstrated by Srinivasan *et al.* in the small animal brain, by Baumann *et al.* in the human retina, and by Jenkins *et al.* in developmental biology specimens was used [6, 9, 10]. Flow is by definition calculated according to the following equation:

$$F = \int_S \mathbf{v} \cdot d\mathbf{A} = \int_S v \cos\theta \, dA \tag{2}$$

where $v$ is the velocity vector of a moving scatterer, and $dA$ is a differential area with the vector perpendicular to the area. In Doppler OCT, this equation can be used to calculate flow through a blood vessel. If we define the plane of integration for flow calculation to be in an *en face* plane, the velocity component perpendicular to a differential area $dA$ is by definition the axial velocity component measured at that differential area. This axial velocity component is readily measured with Doppler OCT as shown in Equation 1 without information on blood vessel angles. This concept is illustrated in Figure 2.
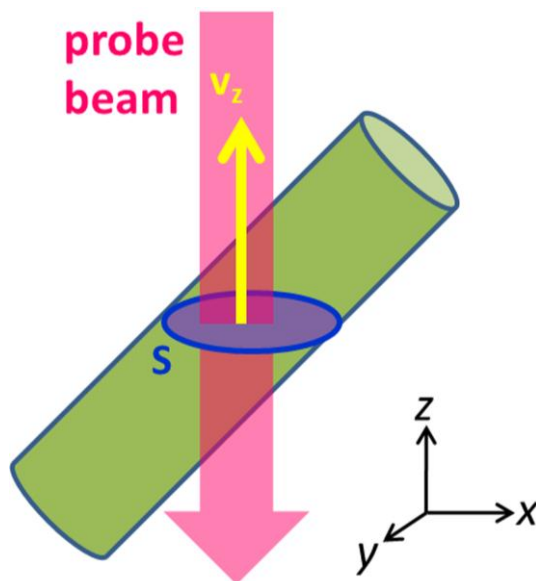


Figure 2. Blood flow through a blood vessel can be measured simply by integrating the axial velocity components over an *en face* cross-section that intercepts the vessel.

More specifically, this implies that total retinal arterial blood flow can be measured by raster scanning an area centered at the central retinal artery, summing the axial velocity components in an appropriate *en face* plane, and multiplying the sum with an area calibration factor. The central retinal artery in the rat retina can be easily accessed by the OCT probe beam, and can be repeatedly and rapidly scanned to achieve a volume acquisition rate high enough for pulsatility characterization. Since this method does not require knowledge of the Doppler angle, total retinal blood flow measurement can be simple and robust.

## 2.3 Capillary imaging with speckle decorrelation

OCT capillary imaging was performed using speckle decorrelation between neighboring B-scans. The equation used for speckle decorrelation calculation is the same as that proposed by Enfield *et al.*[11]. First, multiple B-scans were acquired from the same lateral position on the retina. Before taking the Fourier transform, the OCT spectral interferogram was split into multiple sub-bands by numerical windowing during post-processing to make the lateral and axial resolutions approximately the same as demonstrated by Jia *et al.* and shown in Figures 3(A) and 3(B) [8]. Therefore, each interferogram resulted in multiple depth profiles with isotropic resolution. For each window location in wavelength, speckle decorrelation images were computed between neighboring B-scans acquired from the same lateral position on the retina. The three B-scans resulting from the multiple spectral window positions were treated independently until the speckle decorrelation images were generated, but the final speckle decorrelation images resulting from the same lateral position on the retina were all averaged together to suppress various sources of noise. By scanning different lateral positions in a raster scan pattern, three-dimensional capillary network information could be obtained. By thresholding maximum projection (in depth) speckle decorrelation information and depth-encoding the resulting projection image, three-dimensional information can be displayed in two dimensions as shown in Figure 3(C). Exact speckle decorrelation values are not displayed in this image, but can also be shown in two-dimensions by color-coding the degree of speckle decorrelation instead of vessel depth position, in which case depth information would not be displayed.
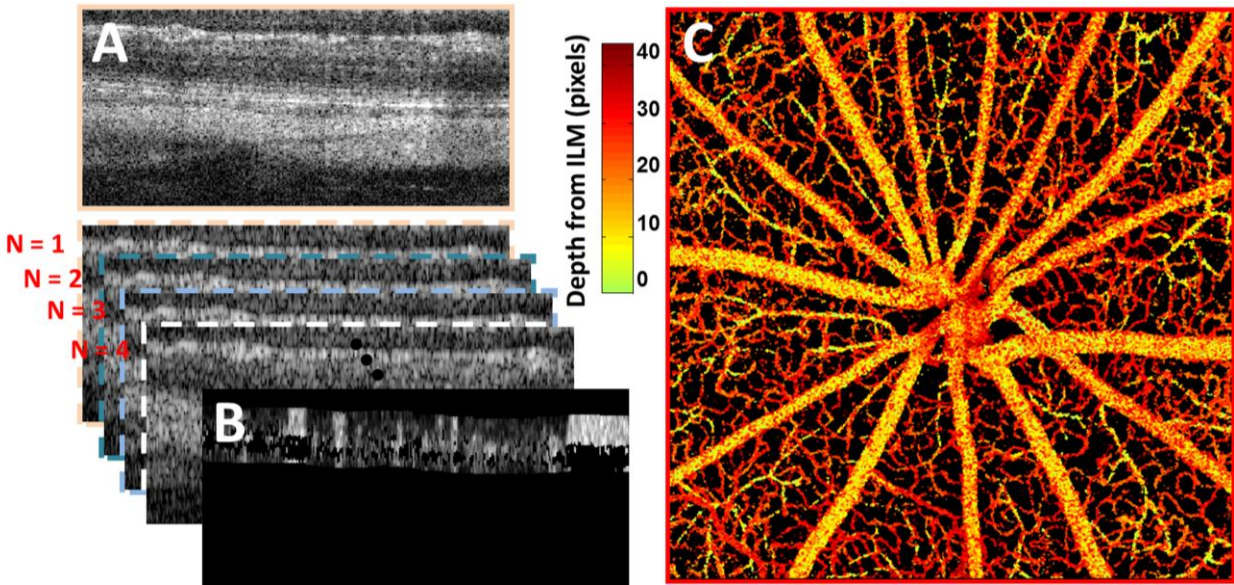
Figure 3. (A) An OCT cross-sectional image with an axial resolution of 5.7μm. (B) OCT cross-sectional images with a numerically adjusted axial resolution of ~15μm, acquired sequentially from a given transverse position on the retina. Comparing two B-scans acquired one after another at a given transverse location can generate a two-dimensional speckle decorrelation capillary image. (C) By performing the operation described in (B) at multiple transverse locations, a depth-encoded capillary image which contains three-dimensional information can be generated. Exact speckle decorrelation values are not displayed in this image, but can also be shown in principle by color-coding the degree of speckle decorrelation instead of vessel depth position.

It is important to note that speckle decorrelation value should increase with blood flow velocity, regardless of vessel orientation. In an ideal situation, speckle decorrelation value should not depend on the vessel orientation because the lateral and axial resolutions were made approximately equal during post-processing. In other words, if blood velocity increases by the same amount starting from the same baseline speed in two capillary branches with different orientations, speckle decorrelation values within those capillary branches should also increase by a similar amount at least within a certain range.

## 2.4  Animal protocol

Animal procedures were performed under an approved protocol by the Committee on Animal Care (CAC) at MIT. For all experiments involving animals, male Sprague Dawley rats weighing between 250 and 500g were used. Immediately before OCT imaging, animals were anesthetized and dilated. The animals were then imaged with ultrahigh speed OCT. During imaging, the animals were closely monitored to check for signs of distress.

## 3.  RESULTS AND DISCUSSION

To date, more than thirty male rats weighing between 250g and 500g were anesthetized were imaged with ultrahigh speed OCT for functional hemodynamics studies. By repeatedly scanning $150 \times 25$ A-scans per volume over a transverse area of 200μm $\times$ 200μm centered at the central retinal artery, a volume acquisition rate of 55Hz can be achieved, which is fast enough for dynamic total retinal blood flow imaging.

A diffuse flicker stimulus was applied to the rat retina during OCT imaging using a dichroic mirror as shown in Figure 4(A), and therefore, potential error from realigning the animal between measurements could be avoided. Figure 4(B) shows a representative functional hyperemic response to a continuous flicker stimulus started at t = 2s. An acute transient increase in total retinal blood flow can be clearly observed.
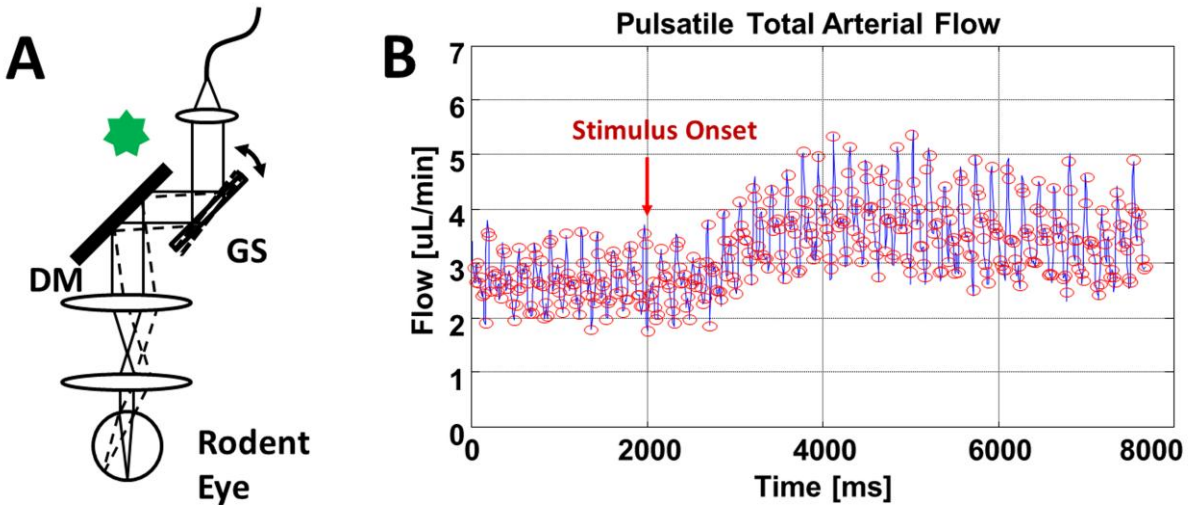
Figure 4. (A) OCT small animal imaging interface for flicker stimulus application. GS: galvanometer scanner, DM: dichroic mirror. (B) An acute transient increase in pulsatile total arterial retinal blood flow in response to continuous flicker stimulus. Flicker stimulus was applied starting from t = 2s and lasted for 15 seconds.

An increase in blood flow speed at the capillary level could also be observed. Figures 5(A) and (B) show capillary images of the same retina acquired before and during flicker stimulus, respectively, with less than 30 seconds time interval between the two image acquisitions without animal realignment. An increase in capillary visibility as well as dilation of larger retinal vessels can be observed. Some of the capillaries become more visible in Figure 5(B) compared to Figure 5(A) because the degree of speckle decorrelation between neighboring OCT B-scans increases with the speed of moving particles, an effect that cannot be detected by simple intensity projection imaging or fluorescein angiography. A detailed quantitation of hemodynamic flicker stimulus response at the capillary level is currently under investigation.
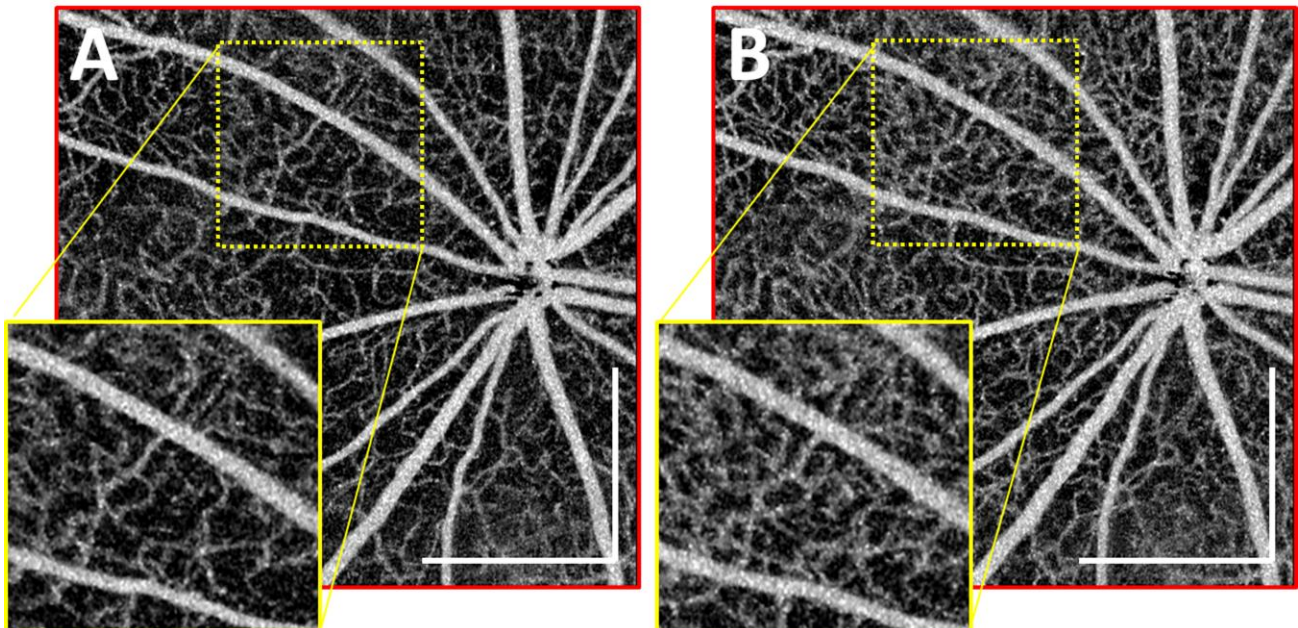


Figure 5. (A) An example of capillary image generated using speckle decorrelation before flicker stimulus. The image was acquired from the same retina as in Figure 4, but at a different time point. (B) A capillary image acquired during flicker stimulus. The image was acquired from the same retina shown in (A) without realigning the animal with less than 30 seconds time interval. Scale bars: 500μm in all images.

# 4. CONCLUSION

These results demonstrate the potential of ultrahigh-speed OCT for investigation of dynamic functional imaging of neurovascular coupling in the retina in response to flicker stimulus as well as conventional three-dimensional structural imaging. This method should be useful for investigation of small animal models of ocular diseases. More detailed quantitation of dynamic responses of the capillary network to flicker stimulus and functional imaging of retinal hemodynamics in small animal models of diabetes are currently under investigation.

# 5. ACKNOWLEDGMENTS

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