In vivo imaging of the rodent eye with swept source/Fourier domain OCT

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Abstract: Swept source/Fourier domain OCT is demonstrated for in vivo imaging of the rodent eye. Using commercial swept laser technology, we developed a prototype OCT imaging system for small animal ocular imaging operating in the 1050 nm wavelength range at an axial scan rate of 100 kHz with ~6 µm axial resolution. The high imaging speed enables volumetric imaging with high axial scan densities, measuring high flow velocities in vessels, and repeated volumetric imaging over time. The 1050 nm wavelength light provides increased penetration into tissue compared to standard commercial OCT systems at 850 nm. The long imaging range enables multiple operating modes for imaging the retina, posterior eye, as well as anterior eye and full eye length. A registration algorithm using orthogonally scanned OCT volumetric data sets which can correct motion on a per A-scan basis is applied to compensate motion and merge motion corrected volumetric data for enhanced OCT image quality. Ultrahigh speed swept source OCT is a promising technique for imaging the rodent eye, proving comprehensive information on the cornea, anterior segment, lens, vitreous, posterior segment, retina and choroid.

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1. Introduction

Rat and mouse models are essential for advancing our knowledge of eye development, evolution, physiology, disease, and therapeutics. Rodent models have contributed to understanding the genetics and mechanisms of diseases such as cataracts, glaucoma, age-related macular degeneration, diabetic retinopathy, retinal degeneration, retinoblastoma, keratitis, and myopia [1]. Enucleation and histology are the gold standard for characterizing morphology and anatomy of the small animal eye, but can only be performed after sacrificing the animal. For disease progression studies using histological examinations, each animal represents only a single time point. Noninvasive in vivo examination of the rodent eye enables monitoring disease progression through its entire course in individual animals, which could provide more accurate monitoring of disease onset, progression, and response to therapies.

Current imaging methods for rodent eyes include fundus photography, fluorescein angiography, slit-lamp imaging, scanning laser ophthalmoscope, magnetic resonance imaging and ultrasound biomicroscopy [2–8]. Most of these methods only reveal two-dimensional information. Magnetic resonance imaging and ultrasound can perform three-dimensional (3-D) imaging, but resolution is limited. Optical coherence tomography (OCT) is a noninvasive imaging modality that can perform high-resolution imaging of tissue morphology in situ and in real time [9]. OCT is analogous to ultrasound except that it measures the echo time delay and magnitude of backreflected or backscattered light. By scanning an optical beam on tissue, two-dimensional cross sectional and three-dimensional volumetric images can be generated. Spectral/Fourier domain OCT (SD-OCT) imaging of the rodent retina was first reported by Srinivasan et al. in 2006 [10]. SD-OCT offers excellent resolution, but has limited imaging speed and imaging range due to spectrometer and camera performance limitations. Current standard commercial OCT systems for small animal imaging are based on 850 nm SD-OCT technology [10–12] and have been used in studies for imaging the retina and limited portions of the anterior segment such as the cornea [13–15]. SD-OCT enables high resolution imaging of the rodent retina using both 850 nm and 1050 nm wavelengths [10–16], but the limited imaging range is insufficient for imaging the whole anterior segment of the rodent eye. Recent advances in swept source/Fourier domain OCT (SS-OCT) enable in vivo ultrahigh speed imaging, offering a promising new technology for rodent eye imaging [17,18]. SS-OCT offers several advantages over SD-OCT, including less sensitivity variation with imaging depth, longer imaging range, higher detection efficiencies and reduced fringe washout. Furthermore, increased penetration into the choroid and optic nerve head with reduced sensitivity to ocular opacities has been reported for OCT imaging in the water absorption window at 1050 nm compared to standard 850 nm wavelengths [19,20].

Standard commercial SD-OCT systems for small animal imaging operate at the 850 nm wavelength range with imaging speeds up to 40,000 axial scans per second, 3-7 µm resolution, and <2.5 mm imaging range in tissue. The systems have interchangeable sample arm optics that enables scanning the small animal retina and anterior eye in different imaging modes. Several studies have been performed using commercial SD-OCT systems [13–15]. However, the limited imaging range of the systems constrain the imaging modes and require retinal, corneal, anterior lens and posterior lens imaging to be performed separately, thereby making it difficult to perform simultaneous imaging of these ocular structures.

This study demonstrates OCT imaging in the rodent eye using a 1050 nm wavelength SS-OCT prototype system at 100,000 axial scans per second with ~6 µm axial resolution and >5.3 mm imaging range. In comparison to the human eye, the rodent eye has a large lens filling up most of the vitreous cavity, making it challenging to image the anterior segment. The OCT system described here is especially suited for rodent eye imaging because of the long imaging
range which can accommodate typical rodent eye lengths of ~6 mm in rats and ~3 mm in mice. OCT imaging protocols using high-definition, high transverse pixel density three-dimensional imaging with dense raster scanning are demonstrated. Long imaging ranges and improved sensitivity roll-off enable comprehensive imaging of the anterior eye along with sections of the posterior lens surface and retina simultaneously. Long wavelength OCT systems at 1050 nm improve visualization of deeper tissue structure compared to 850 nm where standard commercial OCT systems operate. The high speed and deep imaging penetration is ideal for Doppler OCT of posterior eye blood vessels. High speed also enables four-dimensional (4-D) time resolved volumetric imaging of dynamic responses of the eye.

2. Methods

2.1. Ultrahigh speed swept source/Fourier domain system

An ultrahigh speed swept source/Fourier domain OCT instrument was built for small animal imaging (Fig. 1A). A short external cavity, tunable light source (Axsun Technologies, Inc.) centered at 1044 nm which had a 3-dB bandwidth of 103 nm and a 10-dB bandwidth of 111 nm was used (Fig. 1B). The axial scan rate of the OCT system was 100 kHz, set by the sweep rate of the laser. Light from the laser was split into a single pass reference arm and a pre-objective scanning sample arm where the galvanometric scanners are placed in the back focal plane of the objective lenses. Returning light from the reference and sample arms was combined in a second (50:50) fiber coupler and the interferometric signal was detected using a low distortion 330 MHz dual balanced photodetector receiver (prototype; Thorlabs, Inc.). The signal from the photodetector was digitized by a high-speed 8 bit analog-to-digital converter at 1 GS/s (ATS9870; Alazar Technologies, Inc.). Different scan lenses in the sample arm were used to provide different transverse spot sizes and depths of focus. A long working distance infrared microscope objective (M Plan NIR 5X; Mitutoyo Corp.) was used for retinal imaging. The transverse spot size was ~12 µm full width at half maximum (FWHM) measured in air with a beam profiling camera and the incident power was 1.6 mW. For anterior eye and full eye length imaging as well as for posterior eye imaging, a 75 mm focal length achromatic lens was used. The transverse spot size was ~21 µm FWHM in air and the incident power was 2.5 mW. Unlike human eye imaging, a telecentric scanning interface was used for both anterior segment and retinal imaging of the rodent eye. A #1.5 coverslip and index matching gel (Goniosol) was used over the corneal during the retinal and posterior eye imaging procedures. An LED light stimulus was placed adjacent to the sample arm interface without blocking the OCT beam. The measured axial resolution of the system was 6 µm in tissue. The measured sensitivity of the system was 102 dB with a Nyquist limited depth range of 9.1 mm in tissue where the −6-dB roll-off depth was at 2.5 mm in tissue and the −20-dB roll-off depth was at 5.3 mm in tissue. The sensitivity roll-off was limited by the bandwidth of the balanced photodetector and finite light source coherence length (the roll-off from the coherence length itself introduces a 6 dB sensitivity drop at 6 mm in air).

2.2. Animal preparation

Sprague-Dawley rats and C57BL/6 mice were used to demonstrate the imaging capability of the OCT system. Animals were anesthetized intraperitoneally with ketamine (40-80 mg/kg body weight) and xylazine (5-10 mg/kg body weight) for all structural imaging studies. During anterior eye and full eye length imaging, artificial tear drops were applied to prevent cornea dehydration. To perform retinal and posterior eye imaging, eyes were dilated with topical applied tropicamide (1%) drops, and a thin microscope coverslip was placed on the cornea with Hydroxypropyl methylcellulose (Goniosol, 2.5%) to remove corneal refraction and preserve corneal hydration. To perform functional imaging studies, imaging the pupillary reflex, animals were anesthetized intraperitoneally by a cocktail containing ketamine (40 mg/kg body weight), xylazine (3 mg/kg body weight), and acepromazine (1.5 mg/kg body weight).
weight). These studies were in compliance with the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and performed under a protocol approved by the MIT Committee on Animal Care. After anesthetization, the animal was placed in a comfortable mounting tube fixed on a height and tilt adjustable stage pivoted about the animal's eye.

Fig. 1. (A) Schematic of ultrahigh speed swept source/Fourier domain OCT instrument. GS – galvanometric scanners, RR – retroreflector, LS – LED light stimulus, DBP – dual balanced photodetector, A/D – analog-to-digital converter, TRG – trigger signal. (B) Spectrum of the light source. (C) Point spread function showing axial resolution. (D) Sensitivity roll-off.

2.3. OCT image acquisition

OCT cross-sectional previews along with a real-time OCT en face fundus image were used for alignment. For retinal imaging, six raster scans with orthogonal fast scan axis orientation (horizontal and vertical) each consisting of 700 × 700 axial scans requiring an acquisition time of ~5 seconds for each raster (~30 seconds total) were acquired. The six volumetric data sets were then registered using motion correction software and merged. For anterior eye and full eye length imaging, two raster scans with orthogonal fast scan axes (horizontal and vertical) consisting of 500 × 500 axial scans requiring an acquisition time of <3 seconds each were acquired, motion corrected and merged. For posterior eye and Doppler imaging, six orthogonal 500 × 500 axial scan raster scans were acquired, motion corrected, and merged, where each scan was acquired in <3 seconds. For high-speed dynamic volumetric OCT imaging, repeated raster scans with 100 × 100 axial scans were taken in sequence to achieve ~10 volumes per second. The high speed repeated volumes were not motion corrected.

2.4. Doppler OCT

Doppler OCT is a functional extension of OCT which provides velocity and flow information. In Fourier domain OCT, the complex OCT spectral data is processed and the phase information is used for velocity and flow calculations in Doppler OCT [21–25]. A simple phase subtraction of neighboring oversampled OCT axial scans generates quantitative Doppler OCT measurements which was used to visualize the vasculature in eyes:

\[
v(z) = \frac{\lambda}{4\pi n} \int f[\varphi_i(z) - \varphi_j(z)] \, dz.
\]
where $\lambda_0$ is the center wavelength of the light, $n$ is the refractive index, $f$ is the sweep rate, and $\phi_i(z)$ is the phase profile of the $i$th axial scan after Fourier transformation. The maximum detectable velocity before phase wrapping, determined by the imaging speed, is $\pm20$ mm/s in tissue. In theory, the minimum detectable axial velocity is determined by the phase stability of the light source, and was measured to be $1.8$ mrad. In practice, the minimum measurable axial velocity in tissue is limited by the phase decorrelation associated with trigger jitter and scanning the beam [25]. After applying a phase compensation algorithm where bulk motion was calculated and removed using a histogram-based method [23], the standard deviation of phase differences between successive sweeps over a mirror was measured to be $0.11$ rad, corresponding to a minimum measurable axial velocity of $0.7$ mm/s in tissue.

2.5. OCT volumetric registration

Although the imaging was performed with the animals under anesthesia, motion artifacts persist from breathing and heartbeat. Motion during the acquisition of volumetric OCT data distorts the data and is a source of error in quantitative measurements. A motion correction algorithm was recently developed for orthogonally scanned volumetric OCT data [26]. Motion correction is performed by estimating dense displacement fields, which describe the motion of each A-scan, for each input volume and using the time structure of the acquisition process as a constraint. After optimizing a global objective function, the displacement fields are estimated for each volume to correct for motion, then a single volume is constructed by merging each motion corrected volume. Motion corrected volumes do not show visible motion artifacts and a merged registered volume has improved signal quality. The motion corrected, merged volumetric data more accurately represents structure and morphology than individual volumes which can have motion artifacts.

2.6. Light stimulation

For the pupil response experiments, a white light LED stimulus with $\sim800$ cd/cm$^2$ luminance was used. The LED light source was placed adjacent to the OCT beam without blocking the OCT scan. Two stimulus protocols were used: a continuous $>5$ second stimulus and a short $\sim1$ second flash stimulus. The stimulus was activated during OCT data acquisition of repeated raster scans.

3. Results

3.1. 3-D retinal imaging in unpigmented rat eye and pigmented mouse eye

Three-dimensional volumetric OCT imaging of the unpigmented Sprague-Dawley rat retina and pigmented C57BL/6 mouse retina is demonstrated in Fig. 2 and Fig. 3, respectively. The animals were anesthetized so that measurement time is not limited by blinking or motion, as is the case for human ophthalmic imaging. A coverslip was placed on the cornea to remove the refractive power of the air-corneal interface, focusing and scanning the OCT beam directly on the retina through the weaker refraction from the lens. Six orthogonally scanned $700 \times 700$ axial scan data sets were registered and merged.

Scans were performed over a $1.2 \times 1.2$ mm$^2$ area of the mouse eye and a $2.4 \times 2.4$ mm$^2$ area of the rat eye. Each data set is acquired in $\sim5$ seconds. An OCT fundus view is generated by axially summing the merged OCT data set (Fig. 2A and Fig. 3A). Cross-sectional images from the merged data set enable visualization of major retinal layers including the inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor inner segment and outer segment (IS/OS) junction, retinal pigment epithelium (RPE), choroid (CH), and sclera (SC) in both the Sprague-Dawley rat and C57BL/6 mouse eyes (Fig. 2B and Fig. 3B).

In addition, the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), and external limiting membrane (ELM) are visible in the Sprague-Dawley rat (Fig. 2B). The smaller size
Fig. 2. Imaging of the unpigmented Sprague-Dawley rat retina. Registered and merged OCT data set generated from 6 orthogonally scanned OCT data sets. (700 × 700 axial scans over a 2.4 mm × 2.4 mm region) (A) OCT fundus view. (B) Retinal layers visualized in the cropped, enlarged OCT image. (C) 3-D rendering. (D, E, F) OCT images in the X direction. (G, H, I) OCT images in the Y direction.

Fig. 3. Imaging of the pigmented C57BL/6 mouse retina. Registered and merged data set generated from 6 orthogonally scanned OCT data sets. (700 × 700 axial scans over a 1.2 mm × 1.2 mm region) (A) OCT fundus view. (B) Retinal layers shown in the cropped, enlarged OCT image (C) 3-D rendering. (D, E, F) OCT images in the X direction. (G, H, I) OCT images in the Y direction.
of the mouse retina limited the visibility of smaller features. Isotropic transverse sampling of the retina allows for volumetric rendering that shows the structural details of the retina in 3-D (Fig. 2C and Fig. 3C). Since the 3-D OCT volumetric data set is motion corrected, we can extract cross-sectional images in any position and direction without motion artifacts (Figs. 2D–2I and Figs. 3D–3I). Notice that neither cross-sectional images visualized in the horizontal and vertical direction nor the 3-D volumetric rendering exhibit motion artifacts.

3.2. Anterior eye and full eye length imaging in rat and mouse eyes

The long imaging range of SS-OCT also enabled 3-D anterior eye and full eye length OCT imaging in the rat eye and mouse eye using sample arm optics with a larger transverse spot size and longer depth of field. Anterior eye and full eye length imaging provides 3-D information on the anterior chamber and crystalline lens structure as well as biometric information on the full eye length. The animal eye is placed directly in front of the scan lens under anesthesia and after dilation. Two orthogonally scanned 500 × 500 axial scan data sets were registered and merged.

The scans were performed over a 2.6 × 2.6 mm² area of the mouse eye and a 7 × 7 mm² area of the rat eye. Figure 4A is an OCT en face view of a Sprague-Dawley rat eye. The Y-shaped shadow in the center of the eye is the lens suture pattern on the anterior crystalline lens which can be visualized when an en face cross section at appropriate depth is extracted from the volume (Fig. 4D). Cross sectional images in Fig. 4B and Fig. 4C reveal detailed structures of the cornea, iris, and lens, as well as visible signal from the vitreous and retina. A thin liquid film from the eye drops is visible on the cornea of the rat eye. In addition to lens sutures, the nucleus is visible in the cross sectional images.

Figure 4E is an OCT en face view of a C57BL/6 mouse eye. This particular mouse eye exhibits lens opacities which can be visualized by summing en face pixels near the posterior lens surface, as shown in Fig. 4H. Cross sectional images in Fig. 4F and Fig. 4G reveal...
detailed structures of the cornea, iris, lens, vitreous and retina. The 3-D data set illustrates the larger lens volume compared to vitreous volume in the rat and mouse eyes. The irises and pupils are dilated. Ocular biometry measurements of the eyes can be performed after refraction correcting the OCT images in the axial direction.

### 3.3. 3-D posterior eye imaging in unpigmented rat eye

In order to better image the posterior eye of the unpigmented Sprague-Dawley rat, the focus was advanced towards the posterior eye and a coverslip was placed on the eye to minimize corneal refraction. Although the larger transverse spot size decreases the transverse resolution compared to the retinal imaging mode described in section 3.1, the longer focal depth and long imaging range enables visualization of the posterior lens, vitreous and retina. Figure 5 is a posterior eye data set generated from registering and merging six orthogonally scanned volumes of 500 × 500 A-scans each. The scans were performed over a 2.6 × 2.6 mm² area of the rat eye. The data set shows the posterior lens surface, where the Y-shaped lens sutures can be visualized in an inverted orientation as seen on the en face image in Fig. 5B. The hyaloid vessel as well as floaters can be seen in the vitreous. Retinal layers can be clearly visualized in cross-sectional images (Figs. 5C–5D). The deep penetration of 1050 nm wavelength light allows visualization of tissue structure in the choroid and sclera. The fundus image shows that major retinal blood vessels radiate from the center of the optic disk.

Fig. 5. Posterior eye imaging of the unpigmented Sprague-Dawley rat including retina, vitreous, and posterior lens. (A) OCT en face view of the registered and merged data set from six orthogonally scanned 500 × 500 axial scan volumes. Imaging of the rat posterior eye was performed over a 2.6 mm × 2.6 mm area. (B) En face cross section showing inverted Y-shaped posterior lens sutures. (C, D) Cross-sections from the registered data set showing the retina, hyaloid vessel and posterior part of the crystalline lens. The red line indicates the depth position of the en face cross section. (E) 3-D rendering of the data set (Media 1).

### 3.4. 3-D Doppler imaging in unpigmented rat eye

Doppler OCT imaging of the Sprague-Dawley rat was performed using the phase difference of neighboring axial scans in the six orthogonally scanned 500 x 500 axial scan data sets acquired in the posterior eye imaging mode described in section 3.3. The larger spot size decreases the number of samples required to obtain Doppler OCT information over the same scan area compared to the retinal imaging mode in section 3.1. The Doppler OCT data sets were merged using motion correction information (displacement fields) from the registered intensity images (Fig. 6). Intensity and Doppler OCT cross-sections present complementary information on the structure and function of the retina. When the Doppler image is overlaid on its structural counterpart (Figs. 6B–6D), the combined images may provide insight into the
relationship between structural and functional changes of the retina in pathology and disease progression. A 3-D rendering of the vasculature in the retina and choroid is shown in Fig. 6E. Two types of vessels can be distinguished in the retina by looking at the direction of blood flow. When blood moves towards the OCT beam, the Doppler shift is positive, as indicated with the red (warm) color. The retinal arteries are oriented in this direction and are hence visualized in red. On the other hand, blood returning from retinal tissue produces negative Doppler shifts. Consequently, retinal veins are visualized in a blue (cold) color. In the rat retina, arterial and venous vascular systems can overlap one another. Since Doppler OCT can only measure flow velocities in the axial direction, the vessels appear to be discontinuous or disconnected when they are perpendicular to the OCT beam. In addition to retinal vessels and the central retinal artery, some choroidal vessels, as well as long posterior ciliary arteries are also visible owing to the deep penetration of 1050 nm wavelength light.

![Fig. 6. Doppler OCT imaging in a unpigmented Sprague-Dawley rat retina. Doppler OCT analysis was performed using posterior eye OCT data (6 orthogonally scanned 500 × 500 axial scan OCT data sets over a 2.6 mm × 2.6 mm region). The 6 Doppler OCT volumes were merged using the displacement fields from registered structural OCT data. (A) OCT fundus image. (B, C, D) OCT color Doppler images with blood flow information overlaid on structural images. (E) 3-D Doppler OCT angiography (Media 2).](image)

3.5. 4-D imaging of pupillary response in rat and mouse eyes

High speed OCT imaging enables time resolved volumetric 4-D imaging of the dynamic responses of the eye to stimulus. A Sprague-Dawley rat and a C57BL/6 mouse were sedated lightly to preserve pupillary responses. Figures 7 and 8 are demonstrations of 4-D imaging of the pupillary response in the rat and mouse eye. OCT volumes of 100 × 100 axial scans were acquired at ~10 volumes per second for 5 seconds. Scans were performed over a 7 × 7 mm² area of the rat eye and a 3.5 × 3.5 mm² area of the mouse eye. All volumes were scanned with a horizontal fast scan axis and motion correction was not performed. A continuous stimulus and a short ~1 second flash stimulus were applied to both the rat and mouse eyes. 3-D dynamics can be visualized and quantitative measurements of the pupil area can be obtained. Despite the high volume rate of the acquisition, motion is visible in the data sets predominantly due to breathing and heartbeat. As more light enters the eye, the iris responds by rapid contraction, which leads to the decrease in pupil diameter. When the stimulus is turned off, the iris muscles slowly relax. The pupil area from each acquired volume was measured.
4. Discussion and conclusion

Structural and Doppler angiographic imaging of the rodent eye was demonstrated using a novel ultrahigh speed swept source/Fourier domain OCT instrument. The swept source OCT was based on a recently developed instrument for high speed ophthalmic OCT imaging at long wavelengths [17]. The long imaging range and high imaging speed of SS-OCT, along with the deep penetration into tissue of 1050 nm wavelength light enables 3-D volumetric imaging of the retina and posterior eye, as well as the anterior eye and full eye length.
registration algorithm was applied to remove motion artifacts and merge multiple data sets for enhanced visualization.

OCT has been widely used for in vivo retinal imaging of rodent models. This manuscript presents the first motion corrected 3-D OCT images in rat and mouse eyes using SS-OCT. Although current 1050 nm wavelength SS-OCT technology has limited resolution compared to 850 nm SD-OCT, the improved tissue penetration provides additional information in the choroid and sclera. Motion correction enables large volumes to be acquired without motion artifacts and merging improves image quality. Although visibility of features is poorer in the mouse compared with the rat, major retinal layers including the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor inner segment and outer segment (IS/OS) junction, retinal pigment epithelium (RPE), choroid (CH), and sclera (SC) can be resolved. Doppler OCT provides motion contrast highlighting the retinal vasculature and enables differentiating arteries and veins. Quantitative information of blood flow velocity can also be obtained. No obvious artifacts due to pulsatility remain in the motion corrected and merged Doppler OCT data which was obtained from multiple orthogonally scanned volumes. The deep penetration into tissue allowed Doppler OCT imaging of the long posterior ciliary arteries.

The main advantages of SS-OCT compared to SD-OCT are increased sensitivities, imaging speed, and imaging range. For rodent eye imaging, the high speeds and extended range enable 3-D imaging of the retina, posterior eye, anterior eye and full eye length. Combined with image registration motion correction, we demonstrated 3-D in vivo SS-OCT full eye length volumetric imaging while preserving corneal, iris, lens, and retinal topography in both rats and mice. This promises to allow quantitative measurements tracking the changes in morphogenesis and pathological processes in the same animal eye over time.

SS-OCT at 1050 nm may provide new means for in vivo rodent hyaloid vessel and lens development studies. In our results, we were able to visualize the hyaloid vessel, lens nucleus, lens opacities, and lens suture patterns. Comparing Fig. 4D with Fig. 5B, it can be seen that suture patterns in the anterior and posterior pole of the crystalline lens have opposite orientations. Lens fibers make up the bulk of the lens and form lens suture patterns which produce an upright ‘Y’ pattern anteriorly and an inverted ‘Y’ pattern posteriorly. This is the first time that the lens suture pattern in the rat eye has been visualized using OCT. In the mouse eye, we could observe lens opacities possibly induced by ketamine-xylazine anesthesia (Fig. 4E) suggesting that cataract characterization may be possible [27]. Meanwhile in the mouse full eye length image, the retina remains highly visible despite the lens opacities. This also suggests that the reduced scattering of 1050 nm wavelength light will be useful for OCT retinal imaging even in rodent eyes with cataracts.

For mouse eye imaging and biometry measurements, Wang et al. [18] has described a 28 kHz SS-OCT imaging system with a center wavelength of 1056 nm and 70 nm scanning bandwidth. A subsequent paper demonstrated the ability to measure the heritability of ocular component dimensions in mice. These papers reported the ability to acquire 17.6 μm axial resolution cross-sectional data in air using a fast Dispersion Encoded Full Range (DEFR) algorithm over a depth range of ±5 mm in air with −18-dB roll-off at ±3 mm in air where conjugate artifacts compromise the alignment of the imaging system. By taking advantage of the longer imaging range with −20-dB roll-off at 5.3 mm in tissue, the higher 6 μm axial resolution in tissue, the 3.6 times faster 100 kHz imaging speed, and registration-based motion correction algorithm, our results provided motion-corrected 3-D OCT data of the full eye length in not only the shorter mouse eye, but also the longer rat eye. Three-dimensional motion corrected data can be refraction corrected and processed for more accurate biometry measurements unaffected by alignment errors or motion artifacts when acquiring only a single cross-sectional OCT image. Although the limited detector bandwidth along with the limited coherence length of the swept source laser used in this paper affected signal in the rat retina
during full eye length imaging (Fig. 4B Fig. 4C), next generation swept lasers with ultralong coherence lengths will have virtually no signal roll-off within the range needed for rodent full eye length imaging [28].

We also performed the first demonstration of 4-D time resolved volumetric OCT imaging of the mouse and rat full eye length. 4-D OCT dynamic imaging could provide spatial-temporal information of 3-D volumetric datasets for functional imaging studies. A simple demonstration of pupillometry is shown in this paper. In addition to pupil area, 4-D OCT provides information of the iris structure. The contraction of the iris was visualized in 3-D. Other 4-D OCT applications include imaging of structural and blood flow responses to intraocular pressure [29,30] and the dynamics of neurovascular function in the eye [31,32].

To conclude, SS-OCT is a powerful imaging technique providing comprehensive 3-D information on the rodent eye including motion corrected posterior eye, anterior eye and full eye length imaging, retinal imaging with Doppler OCT angiography, as well as 4-D dynamic imaging of functional responses of the eye. While standard excisional biopsy and histology require enucleating the eye, OCT can perform repeated, noninvasive in situ imaging and quantitative measurements of the rodent retina. Therefore, SS-OCT technology for rodent eye imaging is a potentially useful tool for in vivo imaging of disease phenotypes such as corneal opacity, uveitis, keratitis, glaucoma, cataract, retinoblastoma, retinal degeneration, retinal vascular disease and myopia.

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