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Abstract. Mouse models of ocular diseases provide a powerful resource for exploration of molecular regulation of eye development and pre-clinical studies. Availability of a live high-resolution imaging method for mouse embryonic eyes would significantly enhance longitudinal analyses and high-throughput morphological screening. We demonstrate that optical coherence tomography (OCT) can be used for live embryonic ocular imaging throughout gestation. At all studied stages, the whole eye is within the imaging distance of the system and there is a good optical contrast between the structures. We also performed OCT eye imaging in the embryonic retinoblastoma mouse model Pax6-SV40 T-antigen, which spontaneously forms lens and retinal lesions, and demonstrate that OCT allows us to clearly differentiate between the mutant and wild type phenotypes. These results demonstrate that OCT *in utero* imaging is a potentially useful tool to study embryonic ocular diseases in mouse models. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.8.081410]

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1 Introduction

Embryonic eye development involves a series of highly orchestrated changes. Alterations in this process due to genetic or environmental factors can lead to variety of ocular diseases, such as congenital cataracts, microphthalmia, aniridia, and ocular cancers. To understand the molecular regulation of normal and abnormal mammalian eye development, several mouse mutant lines have been generated, which exhibit embryonic ocular defects similar to those in humans.¹⁻⁴ In addition, a number of large-scale genome-wide screens in the mouse have been initiated, which are focused on functional annotation of the mammalian genome and generation of novel models of human diseases, including models of ocular abnormalities.⁵⁻⁷ The success of these efforts depends on the availability of methods for high-throughput analysis of the mutant phenotypes at embryonic stages.

The majority of studies rely on analyses of histological sections of extracted tissues, which lack dynamic information and require many litters at different time points to deduce phenotypic changes. A live imaging method that allows for *in utero* visualization of embryonic eye development would circumvent the need for sectioning multiple litters and would enable high-throughput analysis for use in large-scale genetic screens. It would also provide dynamic information about eye morphogenesis, allowing studies of the progression of ocular diseases and the prenatal effects of pharmacological and toxicological agents.

Ultrasound biomicroscopy has been successfully used for live imaging of the embryonic eye at different stages of development in the mouse model.^{8,9} However, the resolution of this method is limited to about 30 to 50 μm in axial and 100 μm in transverse directions. Such resolution does not allow reconstruction of the

detailed structure of the capillary network of the hyaloid vasculature and prevents visualization of other ocular abnormalities, which are below this resolution limit, such as small lesions, retinal detachment, hematomas, etc. Currently, there is no method for live imaging of the developing mouse embryonic eye with higher spatial resolution, and such a method is critically needed for embryonic ophthalmology research.

Optical coherence tomography (OCT) is an imaging technique with a resolution of 3 to 12 μm (which is an order of magnitude higher than for ultrasound biomicroscopy) and an imaging depth of a few millimeters in biological tissues.¹⁰⁻¹² Similarly to ultrasound, structural OCT imaging can be combined with Doppler OCT measurement for blood flow and tissue movement analysis.^{13,14} OCT is U.S. Food and Drug Administration approved for use in humans for diagnostic purposes and is widely used in ophthalmology clinics.¹⁵ OCT has successfully been used for ocular imaging in adult mice. However, mouse ocular embryonic imaging with OCT has not been previously performed because mammalian embryos develop *in utero*, and layers of maternal tissue restrict imaging access to the embryo. To address this limitation, we recently have developed protocols for live repetitive imaging of mouse embryos both in culture and *in utero* with OCT.^{14,16,17} The *in utero* imaging procedure involves temporal externalization of the uterine horn through an abdominal incision in an anesthetized female.¹⁶ We have demonstrated that despite its limited imaging depth, OCT is a useful tool to analyze craniofacial features, brain, and limb development throughout gestation.

In this work, we demonstrate that our recently developed imaging approach can be used to study ocular structures at different embryonic stages *in utero*. To analyze the potential of our approach for phenotypic embryonic analysis of ocular abnormalities, we used OCT to visualize eye morphology in

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a Pax6-SV40 T-antigen transgenic mouse line, which spontaneously forms lens and retinal tumors during embryonic development. We demonstrate that OCT imaging permits us to clearly differentiate between the wild type and the mutant embryos, suggesting that it can be useful for phenotypic analysis of mouse embryos with ocular defects.

2 Materials and Methods

2.1 Imaging Setup

In this study, we used a customized Thorlabs Swept-Source OCT (SS-OCT) system operating at the central wavelength $\lambda_0 = 1325$ nm and spectral width $\Delta\lambda = 100$ with output power $P = 12$ mW. The power on the sample is 4 mW. The light from the swept-source laser (Thorlabs, SL1325-P16) is split between a reference arm and a sample arm as 10% and 90%, respectively. Light that returns from the sample and the reference arm form an interferogram that is detected by a balanced-receiver configuration (Thorlabs, PDB140C) and is digitized using 14-bit ADC. A Mach-Zehnder interferometer (MZI)-based optical clock, which generates an equally-spaced interferogram in the frequency-domain from a small portion of the source output, is used to linearize the data in k-space before application of fast Fourier transform (FFT) algorithms. An OCT intensity in-depth profile (called A-scan) is reconstructed using FFT from a single scan over the operating wavelength range. The A-scan acquisition rate of the system is 16 kHz. By scanning across the sample surface in X and Y directions, 2-D and 3-D data sets are collected. The resolution of the current imaging system was measured to be $8 \mu\text{m}$ in tissue (assuming $n = 1.4$) in both lateral and in-depth directions. More detailed information about the system can be found in Larina et al.¹⁴

2.2 Mouse Manipulations

Experiments were performed using CD-1 wild type mice and Pax6-SV40 T-antigen transgenic mice (an embryonic retinoblastoma model developed in the laboratory of Dr. Overbeek). Ocular structures have been visualized in two litters of CD-1 wild type mice repetitively and in one litter of Pax6-SV40 T-antigen mice at the embryonic day 15.5 (E15.5). In the Pax6-SV40 T-antigen model, the Pax6 promoter directs lens and retina-specific expression of the SV40 early region, which encodes the large T antigen.¹⁸ T antigen induces lens and retinal tumors at embryonic stages by inactivation of the retinoblastoma protein (pRb) family and p53. The retinoblastoma protein (pRb) and p53 are tumor suppressors, which negatively regulate cell proliferation, and mutations in these genes are associated with cancer.¹⁹

All animal manipulation procedures described here were approved by the Animal Care and Use Committee of the University of Houston and the Baylor College of Medicine. Natural matings were set up and the females were checked for vaginal plugs every morning. The presence of a plug was counted as embryonic day 0.5 (E0.5).

Live repetitive embryonic OCT imaging has been performed in CD-1 mice according to the recently developed protocols.¹⁶ The first imaging session was performed at E13.5 and then repeated at E15.5 and E17.5. The pregnant females were anesthetized with isoflurane by inhalation. The mouse body removed using an electric clipper. An incision of 1 to 2 cm

was made in the lower abdomen, and a uterine horn was externalized through the incision to provide imaging access. The uterine horn was covered with transparent plastic wrap to prevent dehydration. To ensure repetitive imaging of the same embryos, each embryo was assigned a number according to its position in the uterus. After the imaging, the incision was closed using surgical sutures. To minimize post-operative discomfort, Carprofen (5 mg/kg) was administered to the mothers every 24 h.

All OCT images have been scaled proportionally in X and Y dimensions; therefore, the scale bars are shown for only one dimension.

3 Results and Discussion

OCT images acquired from the embryonic eye at three different stages of development are shown in Fig. 1. Structural details of the eye such as the eyelids, cornea, lens, anterior chamber, hyaloid cavity, and retina are distinguishable. At E13.5, the lens is relatively small, the cornea has formed, and the eyelids have begun to differentiate [Fig. 1(a)]. By E15.5, the lens is larger in diameter than at E13.5 [Fig. 1(b)]. At this stage, the cornea is separated from the lens by the anterior chamber of eye. Growth of the eyelids is also noticeable at this stage, and the hyaloid vasculature is clearly visible in the vitreous cavity.

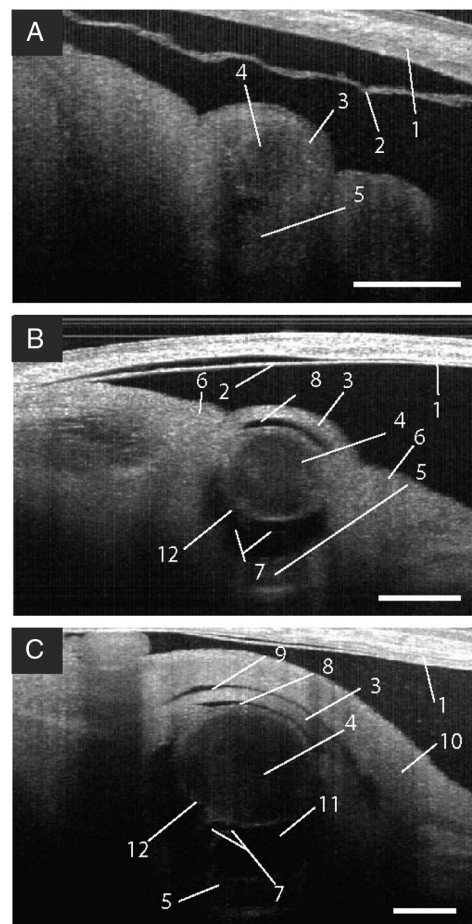


Fig. 1 *In utero* imaging of the embryonic eye at E13.5 (a), E15.5 (b), and E17.5 (c). 1, uterine wall; 2, yolk sac; 3, cornea; 4, lens; 5, retina; 6, differentiating eyelids; 7, vasa hyaloidea propria; 8, anterior chamber of the eye; 9, conjunctival sac; 10, fused eyelids; 11, hyaloid cavity filled with vitreous humour; 12, tunica vasculosa lentis. Scale bars correspond to $500 \mu\text{m}$.

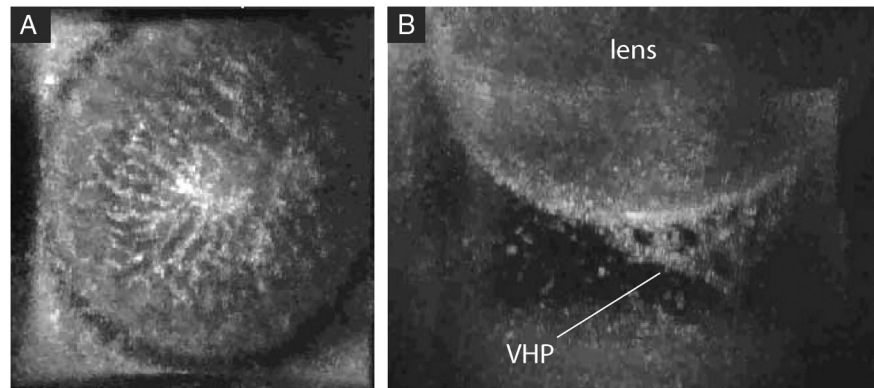


Fig. 2 Live *in utero* OCT imaging of the hyaloid vasculature at E16.5. (a) 3-D reconstruction of the tunica vasculosa lentis, a vascular network over the posterior aspect of the lens. (b) 3-D reconstruction of the vasa hyaloidea propria (VHP), a capillary network in the vitreous.

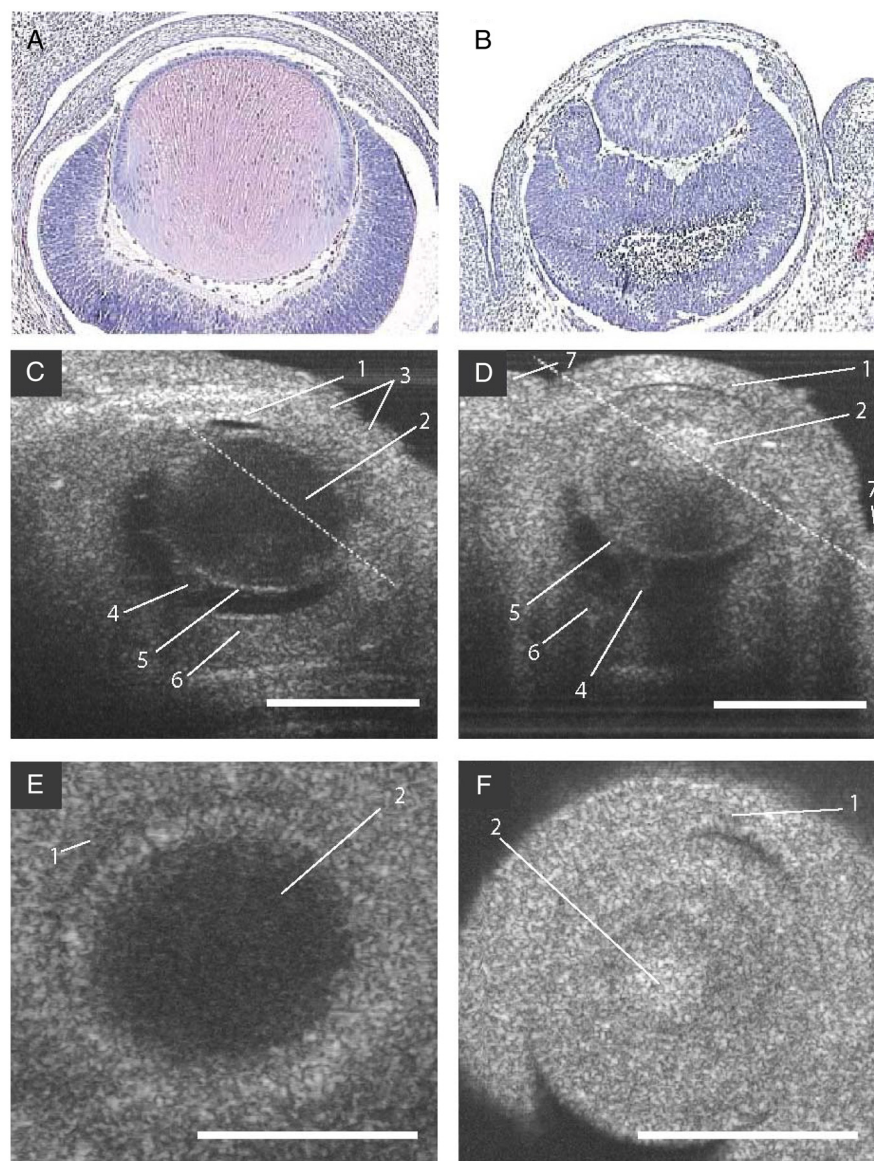


Fig. 3 OCT imaging of the embryonic eye in Pax6-SV40 T-antigen embryos at E15.5. (a, b) Representative H&E staining images of the eye in the E15.5 wild type and the Pax6-SV40 T-antigen embryo, respectively. (c, d) 2-D OCT scans through the eye the wild type and the Pax6-SV40 T-antigen embryo, respectively. (e, f) Corresponding views through the lens in the wild type and the Pax6-SV40 T-antigen embryo, respectively. The positions of the cross-sectional planes are labeled by the lines in panels (c, d). 1, cornea; 2, lens; 3, fused eyelids; 4, vasa hyaloidea propria; 5, tunica vasculosa lentis; 6, retina; 7, differentiating eyelids. Scale bars correspond to 500 μm .

At E17.5 [Fig. 1(c)], all structures are significantly larger, and the upper and lower eyelids are fused together to form the conjunctival sac. At all stages shown, the whole eye is within the imaging distance of the system and there is a good optical contrast between the structures. It is interesting to note that the elongated fiber cells in the central and posterior regions of the normal lens are nearly transparent, while the epithelial cells at the anterior of the lens are refractive.

Proper vascularization of the eye is highly important for normal growth and maturation. The primary intraocular vascular network, the hyaloid vasculature, extends from the optic nerve, branches through the vitreous cavity forming the vasa hyaloidea propria, and wraps around the lens forming the tunica vasculosa lentis.²⁰ Later in development (in humans before birth, while in mice during postnatal stages), the hyaloid vasculature regresses to insure optical transparency for proper vision. Figure 2 shows 3-D reconstructions of the hyaloid vasculature, a capillary network of the embryonic eye, acquired *in utero* at E16.5 with OCT. Tunica vasculosa lentis, a vascular network over the posterior aspect of the lens [Fig. 2(a)], and vasa hyaloidea propria, a capillary network in the vitreous [Fig. 2(b)] are clearly visible. This result demonstrates that OCT is suitable for visualization of the hyaloid capillary network and can potentially be used to assess vascular remodeling during embryonic development. Failure of the hyaloid vasculature to regress in human patients is known as persistent hyperplastic primary vitreous (PHPV).²¹ It results in retinal detachment, cataracts, glaucoma and visual impairment. The described OCT imaging approach might be useful in studying this disorder in mouse models.

To test if OCT can be used to visualize ocular abnormalities during embryonic development, we imaged embryonic retinoblastoma mouse model Pax6-SV40 T-antigen, which spontaneously forms lens and retinal tumors *in utero* [Fig. 3(a) and 3(b)]. In this model, the Pax6 promoter drives expression of the SV40 early region in the lens¹⁸ with leaky expression in the retina. The SV40 early region encodes the large T antigen, which inactivates the retinoblastoma protein (pRb) family and p53. The transgenic lens fiber cells lose their phenotype of terminal differentiation and proliferate as tumor cells. The retinoblasts become disorganized shortly after the onset of T antigen expression. Until now, structural characterization of this model was performed by histological analysis of extracted tissues, which lacks dynamic information. This model can potentially benefit from live *in utero* analysis. Figure 3(c) to 3(f) shows OCT images of the eye in a transgenic embryo and its wild-type littermate. The lens of the wild-type embryo looks dark on the image because it is transparent and has low light scattering properties. The lens of the transgenic embryo is highly scattering and looks notably brighter due to forming lesions. This result demonstrates that OCT allows unambiguously differentiating between the phenotypes. In future experiments, OCT will be used to characterize the time course of tumor progression in correlation with neovascularization in the Pax6-SV40 T-antigen mice as well as other models.

There is a clear advantage of the described OCT ocular embryonic imaging in terms of spatial resolution compared to the ultrasound biomicroscopy.^{8,9} OCT can also be potentially utilized to guide microinjections and micromanipulations in ocular tissues. The described method can also be used to

study the effects of toxicological agents on neovascularization and eye development.

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