FOURIER DOMAIN OPTICAL COHERENCE TOMOGRAPHY AND FLUORESCENT SCANNING LASER OPHTHALMOSCOPY FOR *IN VIVO* RODENT RETINAL IMAGING

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ABSTRACT

In vivo visualization of the internal structures of the retina is critical for clinical diagnosis and monitoring of pathology as well as for medical research investigating the root causes of retinal diseases and developing treatment to improve vision. Rodent models of ocular diseases provide powerful tools for analysis and characterization of disease progression. We present two custom rodent retinal imaging systems: Fourier Domain Optical Coherence Tomography (FD OCT) for non-invasive high resolution cross-section imaging and fluorescent Scanning Laser Ophthalmoscope (fSLO) for information on the molecular content of the retina. The information from these imaging systems is highly complementary, permitting both the retinal structure and function to be investigated non-invasively.

INTRODUCTION

The development of techniques for non-invasive retinal imaging is a rapidly evolving field of ophthalmic research on animal models of human diseases. Non-invasive imaging modalities provide the ability to investigate pathological changes and monitor progression of ocular diseases in order to evaluate therapeutic efficacy [1]. Additionally, these modalities reduce the number of animals sacrificed to histology. The location of the retina at the back of the eye makes non-invasive imaging challenging. The challenge to non-invasive imaging is exacerbated when the eye size is reduced in small animals such as rodents. The small radius of curvature of the rodent eye results in high refractive errors and the introduction of strong aberrations during retinal imaging.

This study will concentrate on the imaging of rodent (mouse, rat) retina. The goal of this project is to provide multimodal instrumentation to complement ophthalmic research investigating pharmaceutical and genetic therapies to various pathologies of the retina. We have constructed a FD OCT prototype and a fluorescent Scanning Laser Ophthalmoscope (fSLO) to provide both structural and molecule contrast images for diagnostic retinal degeneration in rodent.

FD OCT is an optical interferometric imaging technique that facilitates cross sectional imaging with micron-scale resolution approaching that of histology. Rodent retinal layers can be non-invasively observed *in vivo* using FD OCT, appearing as bright and dark alternating bands corresponding to the backscattered intensity of light from the various layers in the sample. However, FD OCT is limited to the detection of coherent light, and thus is insensitive to fluorescence. FD OCT is able to provide three dimensional high resolution images but the image contrast is based on tissue structure, relying on refractive index differences for contrast. Ultimately, this modality does not provide information on the molecular content of the sample. To overcome this constraint, an SLO system was utilized, which is an ophthalmic imaging technique to obtain high resolution *en face* images of the retina, including the retinal vasculature system. Fluorescence detection can readily be incorporated into SLO through the use of dichroic optical elements and highly sensitive photodetectors [2-8].

METHODS

FD OCT system

The FD OCT system utilized spectrometer detection, and a cost-effective, portable continuous Superluminescent Diode (SLD) light source that operates at a central wavelength of 826nm with a FWHM spectral bandwidth of 72nm, corresponding to an axial resolution of ~4 microns (in air). The OCT interferometer was constructed from a 2x2 fiber coupler with a 70/30 splitting ratio to provide 30% of the source light to the sample arm and 70% of the source light to the reference arm. In the reverse direction, 70% of the back-scattered light from the sample was collected and directed to the spectrometer to improve the signal to noise ratio. The reference arm consisted simply of a collimating lens, attenuator, and mirror. The sample arm consisted of a collimating lens followed by a pair of galvanometer mounted mirrors for raster scanning control of the beam. It provided adjustable focusing and increased maneuverability over fixed systems to facilitate coupling of the raster scanned beam into the rodent eye. The calculated spot size given the optical configuration was 12 □m. The overall setup is shown in Figure 1 (a).

The high speed spectrometer was a custom design constructed using a 1200 l/mm transmission diffraction grating to spatially disperse the light

spectrum across an array-type detector. This detector was a 1024 element high speed Gigabit Ethernet (GigE) camera from Dalsa, with 14 μm square pixels. The camera was capable of operating at a maximum line rate of 68kHz, but imaging was usually performed at 20kHz.

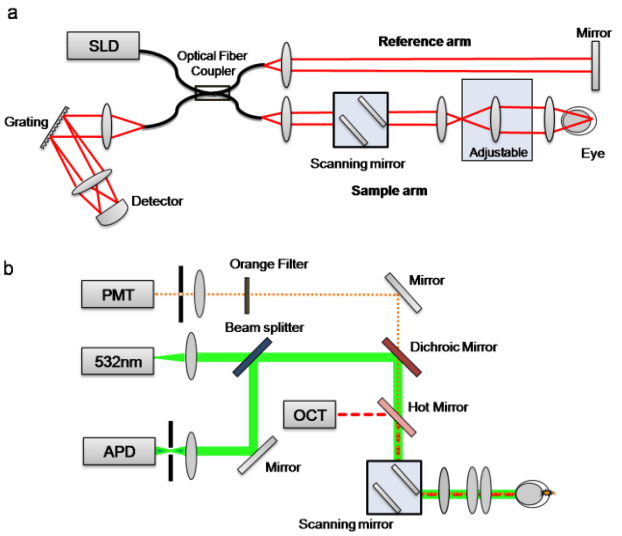

Figure 1: Schematic diagram of (a) the FDOCT interferometric setup used to image the mouse retina and (b) the SLO optical system: green represents SLO beam and orange is the fluorescence. Integration of OCT with SLO can be accomplished using a hot mirror.

Image acquisition and display was performed in real time using a custom multi-threaded software package written in C++, which displayed two dimensional B-scans (depth resolved cross sectional images). Processing performed in real time consisted of resampling of the interferometric data, fast Fourier transform (FFT), dispersion compensation, and contrast and brightness adjustments.

SLO/fSLO system

Using the scanning laser ophthalmoscope technique, a narrow band beam of laser was focused on a single spot on the fundus. The intensity of the back reflected light from the spot corresponded to the brightness of a pixel representing a physical location on the display. A two dimensional image of the sample surface was obtained by raster scanning the spot over the retina and synchronously detecting the back reflected intensities.

A frequency doubled neodymium-doped yttrium aluminum garnet laser which produces green light at a wavelength of 532 nm was used as the light source for the SLO system. This source was also used as the excitation source for fluorescent retinal imaging. For retinal imaging, light must to traverse the vitreous humour prior to reaching the retina. The wavelengths commonly used for SLO imaging are in the visible and near infrared portions of the spectrum. The longer wavelengths are used for deeper tissue imaging because of lower scattering in the tissue.

The schematic of the SLO system topology is shown in Figure 1 (b). A dichroic mirror was used to reflect the excitation wavelength (green light) and to pass the longer wavelength of the fluorescent emission. To obtain higher contrast the SLO, confocal pinholes were integrated into the system prior to the detectors for both fluorescence and regular SLO.

A silicon avalanche photo diode, S5343 from Hamamatsu was used to detect the SLO signal light. A custom designed peripheral circuit was designated to amplify the signal from the APD. For fluorescent detection, the signal from the fluorescence light is much weaker than the excitation intensity, which requires a highly sensitive detector. Here, a Hamamatsu Photo Multiplier Tube (PMT) module H5784 series was used.

A NI-PCI 625 analog to digital converter (ADC) was used to digitize 500 KSPS (kilo-samples per second in 8 differential channels. The frame rate was nominally 2 fps (800 samples per scan, 500 scans per frame), and displayed in real time using the same custom software we developed.

Animal handling

All mouse imaging experiments were performed under protocols compliant to the Canadian Council on Animal Care, and with the approval of the University Animal Care Committee at SFU. The mice and rats were anesthetized (ketamine, xylazine, acepromazine injected intraperitonealy: 50/5/1 mg/kg) prior to imaging in order to minimize sample motion. Some motion was evident, however, due to animal respiration and was observed during image acquisition.

Prior to imaging, the pupils were dilated using a topical solution (atropine sulphate 1%), and natural tear drops were routinely applied to both eyes to maintain homeostatic moisture content.

RESULTS

OCT Results

A representative FD OCT rat retinal B-scan image is compared to a histological section in Figure 2. The *en face* fundus image, shown in Figure 3 (a) was constructed by summing the values of each A-scan, mimicking the operation of SLO [9]. The optic nerve head (ONH) cross sectional image presented in Figure

3 (b), was enhanced by averaging 8 adjacent frames extracted from the position in the fundus image represented by the red solid line in Figure 3 (a).

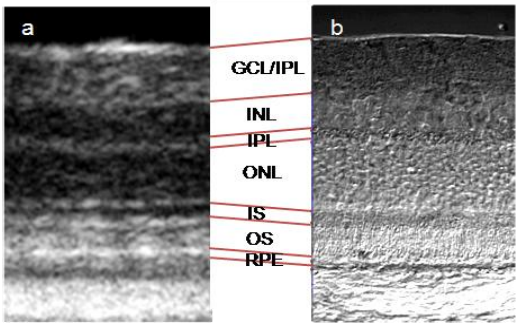

Figure 2: Comparison of the wild type mouse retinal layers observed in (a) FD OCT depth scan and (b) a histological section. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium.

A software package called Amira was used to reconstruct volumetric images of the data set. This software facilitated visualization of the cross sectional planes and en face profiles as presented in Figure 3 (c) and (b).

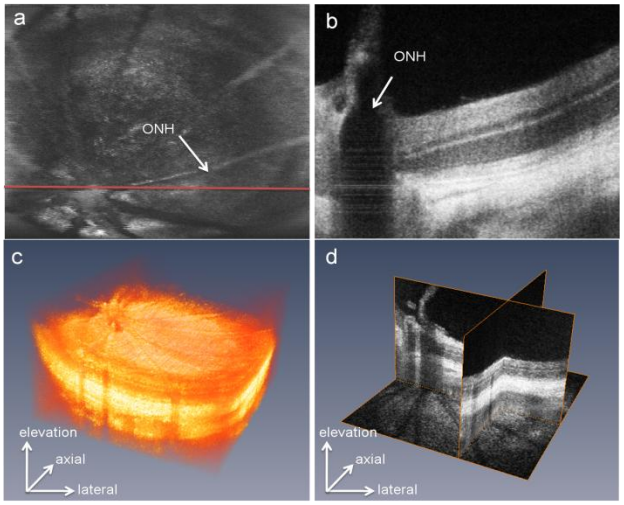

Figure 3: (a) *Enface* fundus image of a rat eye with ONH (b) A cross sectional image extracted from the position of the red line (c) Volumetric image of the rat retina generated using Amira software (d) Image planes in all three dimensions.

SLO-Fluorescence Results

The mouse retina fundus images acquired from both pigmented and albino mice using the SLO system were presented in Figure 4. Optical imaging of the retinal blood vessels in albino mice is significantly easier that in the pigmented wild type counterparts. The RPE is a highly pigmented layer which absorbs light significantly. In the albino mice, the RPE is not pigmented, so light is less absorbed and providing a brighter, higher contrast signal. Higher magnification SLO images are shown in Figure 4 (c) and (d). Image sharpness and visibility of small features such as small vessels, micro capillaries and nerve fibres are indications of high resolution acquisitions. These features are pointed out with red arrows on higher magnification images in Figure 4 (c). For pigmented mice, without the strong back reflection from the choroid layer, the retinal images have a higher contrast for the superficial layers like the retinal nerve fiber layer which could not be observed in albino mice. The nerve bundles were discernible pointed out with red arrows in Figure 4 (d). However, small capillaries of inner retina could not be seen in the SLO images of the pigmented mice due to the strong absorption of RPE and absence of choroid reflection.

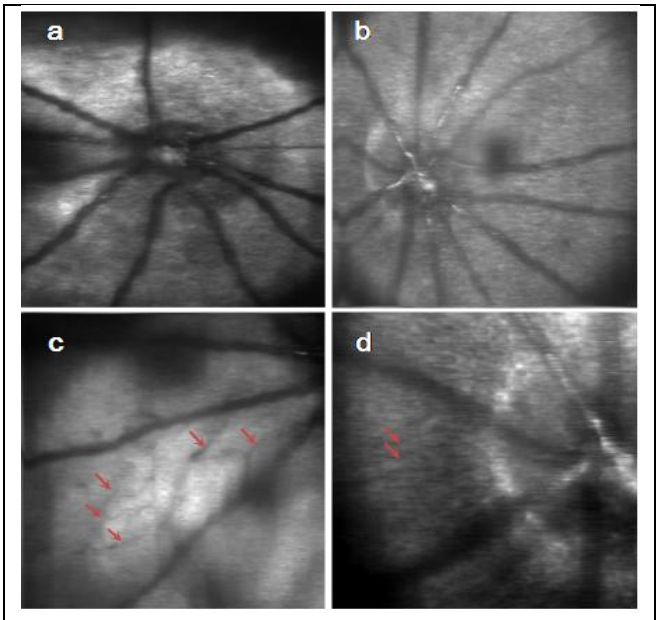

Figure 4: (a) SLO image from a wild type albino mouse (b) SLO image from a wild type pigmented mouse, and zoom in SLO images acquired (c) from a wild type albino mouse and (d) from a wild type pigmented mouse.

The ability to acquire fluorescence data in combination with SLO is demonstrated through retinal angiography in Figure 5. The fluorophore used was Alexafluor555 conjugated to bovine serum albumin which was injected to the mouse via the lateral or dorsal caudal veins. The blood vessel pattern observed in the fluorescent SLO (fSLO) image corresponds to the dark absorbing blood vessels in the regular reflectance SLO image. The overlay of the fluorescence image on the SLO image indicated the source of fluorescence correlates to the blood vessels.

DISCUSSION

We demonstrated high resolution OCT, and SLO images which provided complementary information. The drawback of FD OCT is that alignment on the fundus (a 2D surface) is very challenging. For rodent imaging, locating the ONH is critical for post processing and analyzing, but is difficult to find especially in darkly pigmented. With the blood vessel pattern shown in fundus image, we can align the incident beam to image the right location on the retina. However, FD OCT requires approximately 10 s to acquire a fundus image. In comparison the SLO system only requires approximately 0.5 s to generate a comparative image.

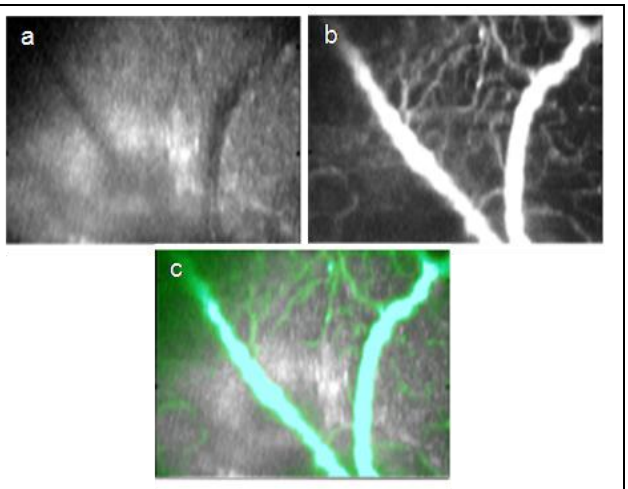

Figure 5: (a) image acquired from the SLO channel (b) image acquired simultaneously from the fluorescent channel (c) the overlay of the fluorescence image on the SLO image.

The SLO fundus image contains only the information from the surface of the retina. Although some level of depth sectioning may be obtained from the confocal detection, the calculated axial resolution is on the order of the thickness of the whole retina. With FD OCT, detailed depth profile of each layer can be observed. Thus, we approach a multimodal system by complementing SLO with FD OCT.

Given that both FD OCT and SLO are raster scanning techniques, integration of these two imaging modalities is a natural pairing. The system design shown in Figure 1 (b) can be combined by using heat control mirrors to separate out the light source from different system (FD OCT in the near infra red and SLO in the visible) according to wavelengths. By using a single pair of scanning mirrors, and overlaying the FD OCT with the SLO beam, perfect alignment of the two systems can be attained. Images acquired with each system will be located at the same position on the retina. This permits rapid alignment of the field of view with the SLO system, followed by the high resolution imaging with the FD OCT system with slower acquisition speed.

The challenges for this multimodal system will be the broad band coated optics which are coated for both light sources, and possible differences in the focal requirements. A particular constraint of FD OCT imaging is that the DOF of the sample arm beam must be long enough to contain the entire sample. For the mouse images shown here, the SLO system had a calculated depth of focus (DOF) of ~220µm and the *en face* images. It was similar to the DOF of FD OCT system designed for mouse imaging, suggesting that integration of the systems will be relatively straight forward. The mouse retina images acquired from both systems have high resolution and good quality, suggesting that integration is straightforward. The DOF of the FD OCT system for rat retina imaging is significantly longer, suggesting that there will be additional challenges to match the optical systems.

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