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Methods in eye research

Method for single illumination source combined optical coherence tomography and fluorescence imaging of fluorescently labeled ocular structures in transgenic mice

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ABSTRACT

In vivo imaging permits longitudinal study of ocular disease processes in the same animal over time. Two different *in vivo* optical imaging modalities – optical coherence tomography (OCT) and fluorescence – provide important structural and cellular data respectively about disease processes. In this Methods in Eye Research article, we describe and demonstrate the combination of these two modalities producing a truly simultaneous OCT and fluorescence imaging system for imaging of fluorescently labeled animal models. This system uses only a single light source to illuminate both modalities, and both share the same field of view. This allows simultaneous acquisition of OCT and fluorescence images, and the benefits of both techniques are realized without incurring increased costs in variability, light exposure, time, and post-processing effort as would occur when the modalities are used separately. We then utilized this system to demonstrate multi-modal imaging in a progression of samples exhibiting both fluorescence and OCT scattering beginning with resolution targets, *ex vivo* thy1-YFP labeled neurons in mouse eyes, and finally an *in vivo* longitudinal time course of GFP labeled myeloid cells in a mouse model of ocular allergy.

1. Introduction

Non-destructive *in vivo* imaging permits the longitudinal study of disease processes in the same organism over time. Observing longitudinal changes in the same organism is necessary for understanding the evolution of existing disease processes or for observing chronological changes in age dependent diseases. Two important non-destructive *in vivo* ophthalmic imaging techniques are fluorescence imaging and optical coherence tomography (OCT). Fluorescence imaging is used throughout the biological sciences to selectively image specific processes or cell populations – such as retinal ganglion cells (RGCs) – that can then be used to longitudinally study the associated disease processes (Higashide et al., 2006; Leung et al., 2008; Walsh and Quigley, 2008; Chauhan et al., 2012). OCT is a separate non-destructive, *in vivo* imaging modality used in both the basic science and clinical settings. OCT produces label-free, high resolution cross-sectional images to allow detection of changes in ocular microanatomy as a result of disease (Schuman et al., 1995; Bowd et al., 2000; Budenz et al., 2007; Konstantopoulos et al., 2011). These two modalities provide complementary types of pathophysiological information – cellular and microanatomical data.

The most straightforward way to gain the benefits of both fluorescence and OCT imaging techniques is to use both modalities one right after the other (Chauhan et al., 2012; Nakano et al., 2011). This incurs costs such as longer imaging sessions, more animal handling and anesthesia, prolonged light exposure for the animal, and additional post-processing time to register and correlate the fluorescence and OCT images together. In our experience, faster imaging promotes animal health. More recently, in an effort to obtain the advantages of both imaging modalities in a single apparatus and imaging session, a confocal scanning laser ophthalmoscope and OCT system were combined and used to correlate fluorescently labeled RGC loss with retinal anatomical changes over 2 weeks in a N-methyl-D-aspartate induced mouse model of RGC

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death (Nakano et al., 2011). However, this system utilized two independent systems with two separate light sources and two differing fields of view acquired simultaneously. Therefore, light exposure was still increased and post processing time was still required for correlation of both imaging modalities.

To achieve truly simultaneous OCT and fluorescence imaging, we have developed a dual modality system that efficiently uses a single illumination source and a single set of optics to obtain identical fields of view for both modalities. Our OCT-fluorescence system uses wavelengths centered at 482 nm for the use of imaging transgenic, fluorescent protein expressing mice instead of the infrared light source typical in current commercial OCT systems. A similar system was previously used in the limited application of autofluorescence imaging of lipofuscin within mouse eyes (Jiang et al., 2014). The use of shorter wavelength light provides two advantages: 1) higher resolution for a given numerical aperture (confocal lateral resolution) and source bandwidth (OCT axial resolution) and 2) the ability to excite common fluorophores such as green and yellow fluorescent proteins (GFP and YFP). This means that as blue light is scanned across the sample, both OCT and fluorescent images are *simultaneously acquired in the exact same location* and are thus automatically spatially registered. This significantly decreases animal handling, anesthesia exposure, and image-processing time because both images are acquired simultaneously. Here we present materials and methods for others to build their own systems to image transgenic mice exhibiting green and yellow fluorescent proteins offering significantly more targets of application.

2. Materials and supplies

We developed this OCT-fluorescence system utilizing only off-the-shelf parts (Fig. 1). We designed the system to image samples with cells expressing GFP and YFP which have their respective excitation bands at approximately 488 nm. Individual filters may be swapped out to image other fluorophores.

2.1. Materials for light source

- Supercontinuum laser emitting desired excitation wavelengths – SuperK EXB-6 or EXW-12, NKT Photonics A/S
- Broadband dielectric mirrors for visible wavelengths – BB1-E02, Thorlabs, Inc.
- Neutral density filter – NDC-50C-4M, Thorlabs, Inc.
- 482 nm 25 nm bandpass filter – FF01-481/25-25, Semrock, Inc.
- Objective – RMS10X–PF, Thorlabs, Inc.
- 2 × 2 fused fiber coupler - FC488-50B-APC or FC488-90B-APC, Thorlabs, Inc.

2.2. Materials for OCT and fluorescence system

- Reflective fiber collimator – RC02APC-P01, Thorlabs, Inc.
- Fluorescence collection fiber collimation lens – AC254-075-A, Thorlabs, Inc.
- Dichroic mirror – MD498, Thorlabs Inc.
- 525 nm 39 nm bandpass filter – MF525-39, Thorlabs, Inc.
- 10 mm X–Y galvonometer scanning mirror pair – GVS012, Thorlabs, Inc.
- Telecentric imaging lens – V40LC, Volk Optical, Inc.
- Posterior segment imaging diopter control – 2× #63–707, Edmund Optics, Inc.
- Posterior segment imaging optics – 1× #47–711, 1× #47–700, 1× #63–706, Edmund Optics, Inc.

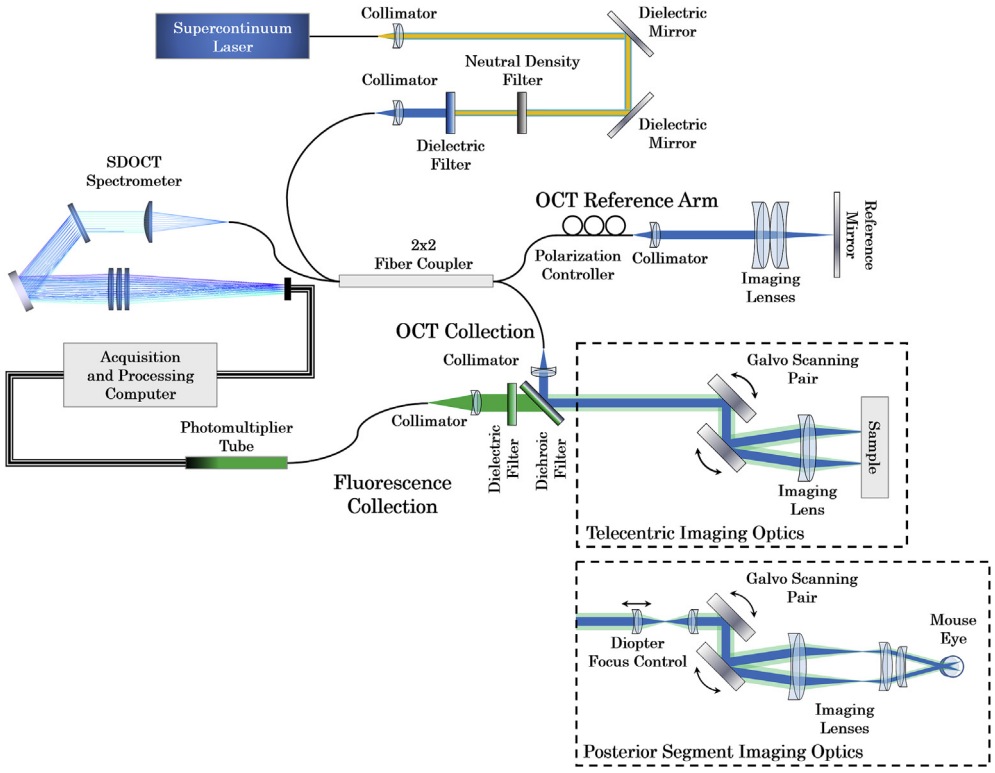

Fig. 1. OCT-fluorescence imaging system schematic. Illumination and backscattered light is in blue. Fluorescence emission is shown in green. Telecentric imaging optics were used for imaging phantoms, *ex vivo* murine anterior segment and retina, and *in vivo* murine anterior segment. Posterior Segment imaging optics were used for imaging *in vivo* murine retina. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)