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A Dual-Modality Optical Coherence Tomography and Selective Plane Illumination Microscopy System for Mouse Embryonic Imaging

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Abstract

Both optical coherence tomography (OCT) and selective plane illumination microscopy (SPIM) are frequently used in mouse embryonic research for high-resolution three-dimensional imaging. Each of these imaging methods provide a unique and independent advantage: SPIM provides morpho-functional information through immunofluorescence and OCT provides a method for whole-embryo 3D imaging. In this study, we have combined rotational imaging OCT and SPIM into a single, dual-modality device to image E9.5 mouse embryos. The results demonstrate that the dual-modality setup is able to provide both anatomical and functional information simultaneously for more comprehensive tissue characterization.

I. INTRODUCTION

Optical coherence tomography (OCT) is a well-established optical imaging technique capable of providing non-invasive, high resolution, three dimensional imaging of turbid biological tissues [1]. OCT has been successfully used in a wide range of biomedical investigations, including developmental biology [2]. Typical OCT has a penetration depth of 1–2 mm in mammalian embryos [3]. Rotational imaging OCT (RIOCT) was developed to improve the imaging depth and it proved to be well-suited for mouse embryonic imaging at later stage of development (beyond E9.5) [4]. However, OCT image contrast is mainly dependent on linear characteristics of tissue, such as scattering, birefringence and refractive index variation. These inherent scattering properties could be very similar for different tissues, which limits OCT from obtaining a more comprehensive sample information. Therefore, there is a need for combining other functional imaging techniques with OCT in order to more accurately characterize different types of tissues and/or tissue function.

In contrast to morphological information obtained by OCT, fluorescence imaging provides functional information derived from location-specific fluorescent marker proteins. Selective Plane Illumination Microscopy (SPIM) is an emerging fluorescence imaging technique that has proven to be especially suitable for developmental studies [5, 6]. The basic principle of

SPIM is to illuminate the sample from the side with a sheet of light around the focal plane of the detection optics which is perpendicular to the illumination axis. Compared to conventional fluorescence microscopy techniques, SPIM can offer highly efficient image acquisition, reduced photo-toxicity and multi-view acquisition.

In this study, we have developed a dual modality imaging setup which combines RIOCT and SPIM for its application in mouse embryonic imaging. The results presented here demonstrate that this dual modality setup could image the sample simultaneously for both scattering structural features and fluorescent features for same region very effectively.

II. MATERIALS AND METHODS

A. Dual modality rotation-image OCT (riOCT)/SPIM setup

The schematic diagram of the dual modality imaging system is illustrated in Figure 1. The system consists of three part: riOCT based on Swept Source OCT subsystem (Thorlabs, SL1325-P16), SPIM subsystem, and the shared sample chamber for both systems. The SS-OCT system used for performing riOCT imaging employs broadband swept-source laser with a central wavelength $\lambda_0 = 1325$ nm and spectral width $\Delta\lambda = 100$ nm. The output power of the laser is 12mW, and the scanning rate over the full wavelength range can reach up to 16 kHz. The lateral resolution of the system was measured to be 8 μ m. The SPIM subsystem was built based as a part of OpenSPIM project, and more details about this subsystem has been described in our previous publication [7]. The excitation light was focused by a cylindrical lens to form a thin light sheet. An objective lens is placed perpendicular to the illumination plane to collect the excited fluorescent light onto the CCD camera. The sample chamber was filled with water for refractive index match, and the sample was imaged through a glass window of the sample chamber

B. Mouse embryo preparation and imaging scheme

Mouse embryos at E9.5 stage collected from *Tg(e-globin-GFP)* mice were deployed in this study for dual modality imaging. The mouse embryos were mounted in the agarose cylinder made from a syringe. A 5mL syringe was used to cast an agar cylinder, and the mouse embryo was mounted inside the agar cylinder. Half of the agar cylinder was pushed out of the syringe and immersed in the water filling the sample chamber.

The syringe was held from above using a micro-positioning stage. The micro-positioning stage could rotate and translate the agarose cylinder for both OCT and SPIM modules. The OCT and SPIM images were taken simultaneously while the sample was rotated at the interval of 90 degrees.

III. RESULTS AND DISCUSSIONS

The procedures for image registration of riOCT images have been described in our previous publication [4] with slight modifications. The OCT images were rescaled to its physical dimensions by correcting for the refractive indices, and then datasets from four angles were registered with each other using the software Amira based on matching best cross sectional images. The registration results are presented in figure 2. Figures 2 (a-d) present the

volumetric 3D OCT images of mouse embryo obtained from different angles, and figures 2(e-f) present the combined 3D riOCT images in coronal and sagittal view.

Figures 3(a-b) show the 3D volumetric riOCT image overlapped with SPIM datasets for mouse embryo. Figures 3(c-d) present selected cross sectional riOCT images overlapped with the corresponding SPIM images. Since the light sheet was illuminated from one side of the sample in the SPIM subsystem, the GFP labeled blood vessels were clearer from the side, but they start to become blurry towards the center of the sample. Double side illumination could further improve the SPIM images.

IV. CONCLUSION

In conclusion, a dual-modality optical imaging system combining riOCT and SPIM has been demonstrated and used for mouse embryonic imaging. This dual-modality system allows for acquisition of two completely distinct and complementary features of the same sample in a single setup. This new setup can be more advantageous over OCT/SPIM carried out using two stand-alone systems in terms of minimizing of datasets registration error by preserving the sample condition during imaging acquisition.

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Figure 1.
Schematic of the dual modality

Figure 2. riOCT 3D images at (a) 0 degree; (b) 180 degree; (c) 90 degree; (d) 270 degree; (e) combined RIOCT image from all angles; (f) combined RIOCT image from all angles from a different view.

Figure 3.

(a) Volumetric 3D RIOCT image; (b) Volumetric RIOCT image overlapped with SPIM images; (c) selected cross sectional OCT image; (d) cross sectional OCT image overlapped with SPIM images.