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LETTER

Real-time *in vivo* imaging of adult Zebrafish brain using optical coherence tomography

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We report noninvasive imaging of the brain of adult Zebrafish (*Danio rerio*) using real time optical coherence tomography (OCT) capable of acquiring cross sectional 2D OCT images @ 8 frames/sec. Anatomic features such as telencephalon, tectum opticum, eminentia Granularis and cerebellum were clearly resolved in the OCT images. A 3D model of Zebrafish brain was reconstructed, for the first time to our knowledge, using these 2D OCT images.

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1. Introduction

Zebrafish (*Danio rerio*) is a popular model system to understand a variety of human biological processes [1–3]. As vertebrates, Zebrafish provides a good model for humans and is extensively used in medical research. These studies often require measurements of the morphological and physiological parameters of Zebrafish. Therefore, development of noninvasive imaging techniques for this purpose is of considerable interest. The only report to the best of our knowledge that studied in-vivo anatomical structures of adult Zebrafish is by the use of high-resolution magnetic resonance microscopy by Kabli et al. [4]. However, the resolution of the technique was ~78 μm and required a relatively long imaging time of about 8 minutes. Optical coherence tomography (OCT) can provide non-invasive cross-sectional images in real time with spatial resolutions down to few micrometers [5–7]. Recent advances in OCT enable rapid image acquisition speeds and 3D volumetric information. There have been increasing applications of 3D-OCT for imaging of biological microstructures such as human retina [8], human skin [9] and other developmental model systems [10]. The depth of OCT imaging is limited by scattering of the medium that destroys the coherence of the probe beam. Thus while for non-scattering ocular structures, OCT can be used to image the entire structure [11], imaging of scattering tissue like brain is usually limited to a few mm. OCT has therefore been used to image excised or exposed brain tissue

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[12–15]. OCT for in-vivo deep brain probing has also been investigated in rat brain [16]. Non-invasive in-vivo OCT imaging of adult brain however, has not been possible so far. In this report, we demonstrate what we believe for the first time, non-invasive real-time in-vivo optical imaging of Zebrafish brain using OCT. Two-dimensional cross-sectional images of brain were acquired at near video rate and a 3D model of Zebrafish brain was reconstructed.

2. Materials and methods

The adult Zebrafish (*Danio rerio*) was procured from local aquarium suppliers. For imaging, the fish was anaesthetized using clove oil following the protocols described previously [11]. Briefly, clove oil was dissolved in ethanol (1 : 10) and then mixed in 1 litre of water in a glass tank, such that the clove oil concentration is about ~75–100 ppm. The fish lost physical movement within 10 min. When the fish showed no response to physical stimulus, it was transferred to a petri dish for imaging. After imaging, the fish was observed to revive within ~15 minutes after transfer to a freshwater tank.

Figure 1 shows a schematic of the single mode fiber based real time OCT setup. The real-time OCT system, which is similar to the one developed by Rollins et al. [17], utilized a high power broadband superluminescent diode (SLD) optical source (Dense Light, Singapore). The source has center wavelength 1310 nm with full-width at half-maximum (FWHM) 43 nm and 18 mW optical power in single mode fiber. The light emitted by the source was fed into the input port of a three-port optical circulator. The output of the second port is connected to a fiber-optic 50 : 50 coupler for splitting the light into reference and sample arms. The third port is used to redirect the light coming back towards the source to the detector. The reference arm consists of a Fourier domain based rapid scanning optical delay line (RSOD) using a resonant mirror with scanning frequency of 2 kHz. The RSOD comprised of a 2.5 cm wide grating having 600 lines/mm (pitch = 1.67 μm) and a lens of 50 mm focal length. The maximum angular excursion of the resonant scanning mirror was set to affect a usable group delay scan of about 3 mm. Polarization controllers were used in the reference arm to optimize the interference signal. In the sample arm, a stationary assembly of relay lenses was used to deliver the light onto the sample. Lateral scanning was done using a single-axis galvanometer-driven mirror, which was used to change the angle of incidence of the probe beam on the relay lens pair. The frequency of lateral scanning was fixed at 8 Hz. The light reflected back from both these arms interferes at the detector.

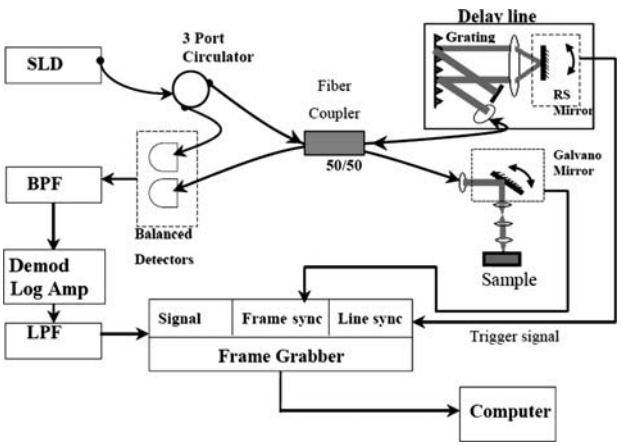

Figure 1 Schematic of the real time OCT setup. SLD– Superluminescent diode; BPF– Band pass filter; LPF– Low pass filter; RS– resonant scanner.

The differential output of the balanced detector was fed into a band pass filter and amplified using a logarithmic amplifier. The output from the logarithmic amplifier was low pass filtered and fed to a frame grabber board. The image acquisition software detects the envelope of the interferometric signal and displays the cross-sectional image on the screen. The image acquisition was done at a rate of 125 A-scans per frame at 8 frames per second. The free space axial and lateral resolutions of the setup were both estimated to be ~20 μm. The signal to noise ratio (SNR) of the setup was measured to be ~96 dB.

3. Results and discussion

The real time OCT setup was used to acquire two-dimensional cross sectional images (XZ plane, as shown in Figure 2) of the brain of anesthetized Zebrafish. About 90 cross-sectional images of the brain were taken by moving the sample in the Y direction in a step of 0.05 mm. A sequence of 20 images were

Figure 2 (online colour at: www.biophotonics-journal.org) Image of adult Zebrafish and imaging geometry.

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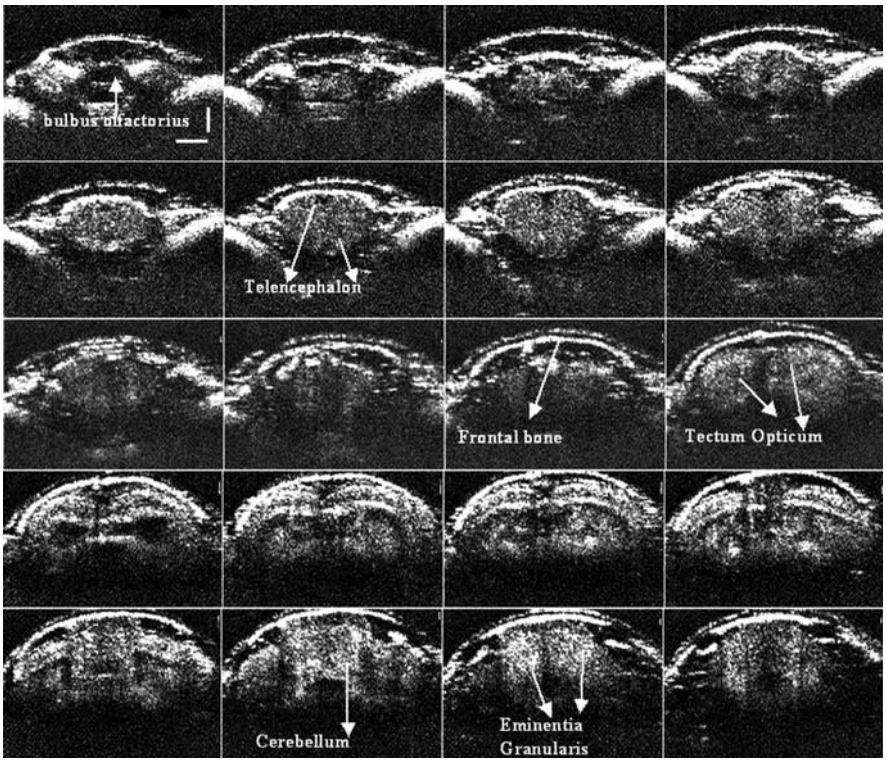

Figure 3 Sequence of Cross-sectional OCT images of adult Zebrafish brain. Scale bar is 0.5 mm and is same for all the images.

displayed in Figure 3 (with a separation of 0.15 mm). The major lobes of the brain such as bulbus olfactorius, telencephalon, tectum opticum, cerebellum, frontal bone and eminentia granularis were clearly distinguishable in these images. The raw images were thresholded for minimizing the speckle noise. Using these images, a three-dimensional model of the Zebrafish brain was constructed in both the axial and sagittal planes (Figure 4) with *AMIRA software.* An animation of the rotating 3D Zebrafish brain is included as data supplement created using ImageJ (rsbweb.nih.gov/ij/). The reconstructed isosurface model of the Zebrafish brain was found to be in close resemblance to the model reported previously [18]. The brain surface studies using non-invasive in vivo imaging modalities help in monitoring the dynamic process of brain maturation with age and can be used for detection of abnormal changes [19]. Also, a measure of mean curvature of the brain surface may serve as an index of normal versus abnormal brain development [20]. It is pertinent to compare the Zebrafish brain cross-sectional images obtained using OCT with the 2-D transverse images posted on the VCCRI fishnet website (www.fishnet.org.au). The fishnet website provides images taken with optical projection tomography for a 17 mm length (adult) fish [21]. These images do not clearly show the structure of bulbus olfactorius (slide no.134) and eminentia granularis (not marked in the fishnet images). The other structures such as telencephalon, frontal bone, tectum opticum and cerebellum are resolved better in OCT images than the fishnet database (slide no. 176, 218, 260). The cavity inside tectum opticum could be clearly visualized in our images compared to the fishnet database (slide no. 302). Due to the presence of a highly scattering layer in the hind portion of the Zebrafish brain the structures beyond the cerebellum such as crista cerebellaris, medulla spinalis, parasphenoid, palatoquardrate etc. were not clearly distinguishable in OCT images. Use of OCT set up with higher sensitivity (~110 dB) and better resolution (~1–3 μm) may help distinguish the anatomical structures more effectively.

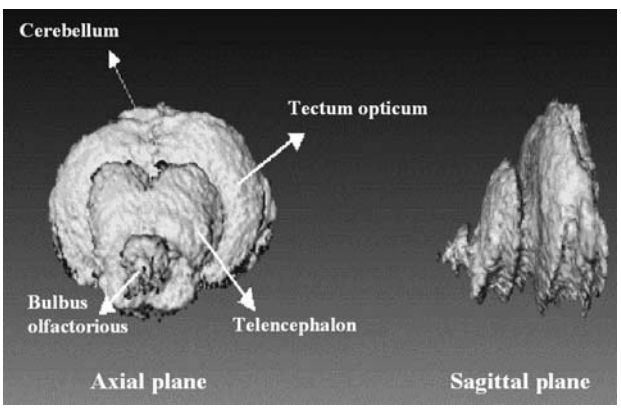

Figure 4 Reconstructed 3D view of the Zebrafish brain in the axial and sagittal planes.

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4. Conclusions

In summary, we have demonstrated the use of OCT for obtaining in-vivo noninvasive real time images of Zebrafish brain. Anatomic features such as telencephalon, tectum opticum, eminentia granularis and cerebellum are resolved in the OCT images. A 3D model of Zebrafish brain was reconstructed for the first time to best of our knowledge using several 2D sections.

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