Large scale high sensitivity optical diffraction tomography of zebrafish

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3D optical imaging is used in many areas of biology and even medicine as is evidenced by the multitude of 3D imaging modalities that have been developed. One of these techniques is optical diffraction tomography (ODT). Currently, ODT is applied to homogeneous large samples [1] or small inhomogeneous biological tissues in the tens of microns size range such as cells [2]. This is caused by the fact that on the one hand ODT relies on the refractive index (RI) differences inside the sample to be sufficiently large to create image contrast, but on the other hand requires that RI differences are sufficiently small in order to prevent a change in direction of the rays passing through the object.

In this work we demonstrate to our knowledge for the first time that high contrast and high resolution 3D refractive imaging of large scale biological samples is possible. The sample is a 3 day old optically cleared zebrafish embryo (wild type) embedded in agarose. We use a large image sensor and phase shifting digital holography to make full use of the spatial frequency bandwidth of the system. We theoretically demonstrate that with these parameters we come close to the optimum field of view over resolution ratio. We perform high sensitivity RI detection through off-axis sample placement [3] combined with numerical focus tracking during rotation and acquisition of a large number (1440) of projections. The combination of these two noise reduction methods is essential to strongly suppress the noise and make large scale ODT possible. We minimize scattering and refraction by using an optimized passive optical clearing procedure. Virtual cross-sections of the zebrafish are shown in Fig. 1(a-c) where the RI difference is quantitatively visualized on a linear gray scale. The RI distribution of the reconstructed background (optically cleared agarose) is depicted in Fig. 1(d) and found to have a standard deviation of $8 \cdot 10^{-5}$ indicating the low noise in the reconstructed images. The total imaged volume is $5.5 \times 5.5 \times 4.1$ mm^3 with a 4 micrometer spatial resolution.

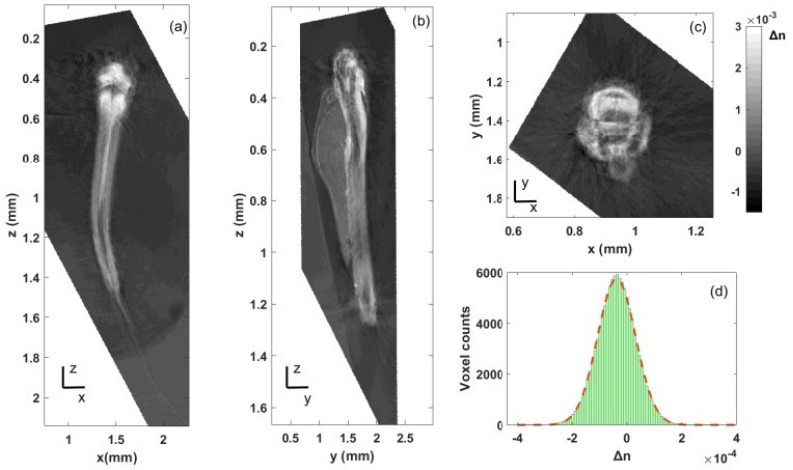

Fig. 1. (a-c) Cross-sections of ODT reconstructed refractive index contrast of an optically cleared 3 day old zebrafish larva. (d) RI distribution of agarose background.

References

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