Development of structured illumination based Digital Scanned Light-sheet Microscope (DSLM) system for 3D tissue imaging

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Abstract: We have developed a ‘3D HiLo’ based structured illumination method in DSLM and further implemented photon re-assignment to improve signal to background with lower image processing artefacts. We are using this microscope to understand the tissue regeneration in early embryonic development of zebrafish.

Introduction:
We have developed a digital scanned light sheet microscope for 3D live tissue imaging. We implemented 3D HiLo based structured illumination for suppression of the background haze due to tissue scattering, limiting the visibility of sample features. We have further implemented Photon re-assignment for effective background rejection for spatially varying tissue scattering of the biological samples and enhance signal to background ratio.

Results:
We compare our 3D reconstruction method with normal DSLM imaging and 3D HiLo based image reconstruction (as shown in fig-1). We refer to our new approach as “3D HiLo,” because it combines HiLo images from two orthogonal directions (excitation objective point of view and detection objective point of view) to reconstruct a 3D tissue image. 3D HiLo images are well background noise subtracted and also have lower image processing artifacts.

We further use a photon reassignment 3D image reconstruction method for tissue specimen subjected to spatially varying scattering observed during light-sheet excitation. Here we estimate the scattering in the specimen and the performance of the proposed image reconstruction method is analyzed for the recovery of the full spectrum of object’s spatial frequencies features under varying scattering. The proposed method utilizes the physical model of the optical system as ‘a priori’ information and maximum likelihood estimation (MLE) is performed using the data recorded under non-structured and structured illumination conditions. This process provides the better utilization of the volumetric photons for the recovery of the true 3D fluorophores features with higher signal-to-noise (SNR) and signal-to-background (SBR) ratios. We have imaged the structures of both nuclei and cell membranes of drosophila and zebrafish embryos using normal and structured illuminated DSLM microscope and recovered images with much better signal to background using photon assignment algorithm.

References