Imaging multicellular specimens with real-time optimized tiling light sheet selective plane illumination microscopy

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Cells live in three-dimensional environments. A more accurate understanding of cell behaviors and cell-cell interactions can be obtained by studying cells in their native, multicellular environments. Despite the progress made in selective plane illumination microscopy, high-resolution 3D live imaging of multicellular specimens remains challenging. Tiling light sheet selective plane illumination microscopy (TLS-SPIM) with real-time light sheet optimization was developed to respond to the challenge [1]. It improves the 3D imaging ability of SPIM in resolving complex structures and optimizes SPIM live imaging performance by using a real-time adjustable tiling light sheet and creating a flexible compromise between the spatial and temporal resolution.

In TLS-SPIM, instead of using a large light sheet to illuminate the entire FOV simultaneously, a small but thin light sheet is tiled quickly within the image plane to illuminate the whole FOV, while only the fluorescence signal generated at center of each tiled light sheet is used in the final image. As a result, both high spatial resolution and good optical sectioning capability are maintained within a FOV that is much larger than the light sheet itself, by which multicellular specimens could be imaged by TLS-SPIM with either higher spatial resolution, better optical sectioning capability or both compared to regular SPIM. Furthermore, TLS-SPIM enables the implementation, tiling and real-time optimization of the latest SPIM light sheets, including the Gaussian, Bessel and Lattice light sheet.

We demonstrate the 3D imaging ability of TLS-SPIM in resolving complex structures, verify its 3D live imaging performance, and compare TLS-SPIM with regular SPIM techniques by imaging cellular and subcellular behaviors in live *C. elegans* and zebrafish embryos. We also show how TLS-SPIM can facilitate cell biology research in multicellular specimens by studying left-right symmetry breaking behavior of *C. elegans* embryos. In addition, we combine TLS-SPIM with the multiview configuration, and examine its 3D imaging ability on large multicellular specimen by imaging live zebrafish embryos and cleared mouse brains.

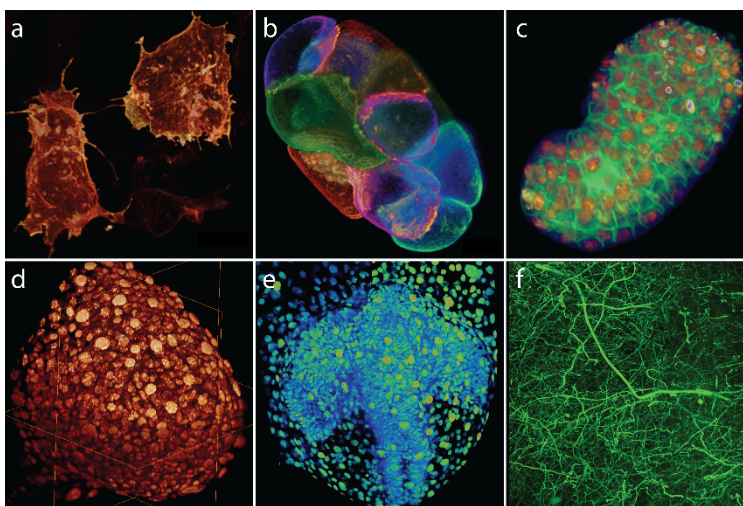

Figure 1. 3D renderings of (a) two live mesoderm cells in a zebrafish embryo, (b) an early stage *C. elegans* embryo (OD95) with all cell segmented, (c) a late stage *C. elegans* embryo (OD95), (d) the tail bud of a zebrafish embryo, (e) the head of a zebrafish embryo, and (f) a region of interest within a clear mouse brain, imaged by TLS-SPIM.

1. Q. Fu, B.L. Martin, D.Q. Matus and L. Gao, *Nat. Commun.*, (2016) 7:11088