LIVE 4D IMAGING OF EMBRYONIC VERTEBRATE HEART WITH 2-PHOTON LIGHT SHEET MICROSCOPY

Vikas Trivedi1,2, Thai V. Truong2, Le A. Trinh2, Daniel B. Holland2, Scott E. Fraser2

1Division of Biology & Biological Engineering, Caltech, Pasadena CA 91125, USA
2Translational Imaging Center, Univ of Southern California, Los Angeles CA 90089, USA
Email: viktri@caltech.edu

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Developing cardiac tissue in zebrafish is an ideal model system for both genetic and biophysical studies of organogenesis owing to its early development, ease of accessibility, dynamic nature and limited number of constituent cell types. The dynamic nature of the tissue has been the major roadblock preventing fast sub-cellular resolution imaging of the beating organ. The zebrafish heart forms its chambers by 5 days post fertilization (dpf), when it is a dynamic 200um scale structure, moving quasi-periodically in 3D at a few Hertz frequency, with movements >100um. We made straightforward hardware modifications to the conventional Single Plane Illumination Microscopy (SPIM) set up, to create a bidirectional 2-photon illumination SPIM that provides deep and fast optical sectioning [1,2]. This permits us to fully harvest the orthogonal geometry of SPIM to provide an independent signal to accurately decide the spatio-temporal phase of the moving heart for imaging heart in 4D (space + time). We developed a novel image registration technique, utilizing an additional imaging path to define the phase in the cardiac cycle for each SPIM image, resulting in a high-fidelity 4D reconstruction of the heart as it beats, with subcellular resolution, reduced phototoxicity, and minimal post-processing artifacts. Imaging a set of cardiac–specific FlipTrap lines [3] in which full-length Citrine is fused to host proteins, we generate images of the endogenous proteins as illustrated in Figure 1, offering new insights into cardiac physiology and development.

Figure 1: Reconstructions of Tpm4 (cytoplasmic; left) and alpha-catenin (junctional; right) at 84hpf.

REFERENCES
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