Title: 3D imaging of engineered heart tissues using inverted selective plane illumination microscopy

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Abstract

The leading cause of mortality globally being cardiovascular diseases, there is an urgent need for advanced heart tissue models that replicate native cardiac structure and function. Engineered heart tissues (EHTs) provide aligned cardiac contractions in 3D, support more mature tissue models, and allow straightforward force measurements. However, imaging EHTs at cellular and subcellular levels presents significant challenges due to photobleaching and limited imaging depth of currently used techniques. To address these challenges, we developed an optimized inverted selective plane illumination microscopy (iSPIM) system for high-resolution, volumetric imaging of EHTs.

The iSPIM system, equipped with a 10x/0.3 numerical aperture (NA) water immersion objective for excitation and a 20x/0.5 NA water immersion objective for detection, was used to acquire optical Z-stacks of fixed EHTs. Three lasers (488 nm, 561 nm, and 638 nm) were used for imaging fluorescence from connexin 43, troponin T, and nuclei, respectively. The resolution of the iSPIM was measured at 1 µm laterally and 9.6 µm axially, and neural networks were implemented for enhancing the axial resolution. Optimized deskewing and deconvolution were performed using Python and Huygens Essential software.

Using the iSPIM system, we achieved high-resolution visualization of key components within EHTs. This system enabled rapid imaging across the entire tissue thickness and, through segmentation, provided detailed insights into cell alignment. Our optimized iSPIM system is a powerful tool for analyzing EHTs, demonstrating its versatility for imaging complex 3D tissue structures. Future adaptations will allow extended imaging periods of live EHTs, enabling high-speed imaging of tissue dynamics.