Quantitative Analysis of Image Quality for Mechanically Deskewed Sample Scans

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Summary

Light-sheet microscopy (LSM) is a powerful technique for imaging large, optically cleared tissue samples at high resolution. Commonly, LSM such as the mesoSPIM [1], employ inner cuvettes to embed large, cubic cm-sized samples in refractive index matched media. We have developed mounting protocols to image laterally extended vibratome-sliced tissue in our mesoSPIM LSM. However, the resulting image stacks suffer from shearing artifacts due to the 45-degree sample orientation between illumination and detection axes. We therefore developed a methodology to mechanically deskew our datasets during image acquisition [2]. Here we present a quantitative assessment of mechanically deskewed and conventionally acquired datasets.

Introduction

To investigate cardiac tissue remodelling in a rabbit model of myocardial infarction, we mount vibratome-sliced ventricular samples with thickness of a few hundred microns on refractive index-matched glass slides within 3D-printed frames. This method of mounting is necessary to prevent the formation of hypoxic cores in the samples. Unlike traditional cuvette mounts aligned with the detection axis, these frames are positioned at 45 degrees between the illumination and detection axes. This setup introduces a shearing effect during z-direction scanning, where the illuminated sample region moves laterally across the field of view. This displacement can lead to image dead space and reduced axial resolution, complicating quantitative analysis of biological structures.

Methods

Using a custom-built mesoSPIM system [1], we imaged fluorescent bead samples using both standard z-scans and a ‘mechanically deskewed’ scanning protocol [2]. The latter introduces synchronized lateral stage movement to maintain the illuminated region at the image centre. We quantitatively assessed image quality in both methods with fluorescent bead samples (1 micron dragon green beads suspended in 1% agarose) and point spread function analysis. For the analysis, a cross-field of view approach was taken where point spread functions were evaluated in the 9 distinct regions of the image in a 3x3 grid segmentation. A custom-written python module was utilised to evaluate image quality using SKimage peak detection and SciPy

curve-fitting tools. The module allowed quick and simple execution of bead detection, segmentation, and curve fitting to find full widths at half maxima – followed by R^2 curve-fit filtering to ensure quality fits.

Results and Discussion

Our results show that mechanically deskewed scanning significantly improves image quality by reducing dead space and improving axial resolution. Compared to standard shearing scans, the deskewed approach offers an improvement in axial resolution by a third. These findings support the adoption of mechanically deskewed scanning in light-sheet microscopy for improved imaging of cardiac tissue samples.

Conclusion

Building upon prior advancements in tissue mounting for mesoSPIM imaging, our mechanically deskewed scanning protocol offers a practical solution for mitigating shearing artifacts. By minimizing lateral displacement, this method enhances image resolution in the axial direction, making it a promising approach for imaging tissue slices with high isotropic resolution while reducing data size and computational overhead. These findings expand the capabilities of mesoSPIM for high-resolution imaging of fragile samples, making it a valuable tool for biomedical research.

References:

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