# Mesoscale light sheet microscopy to study differences in cardiac structure post myocardial infarction

Sharika Mohanan1, Steven M. Moreno1, Eline Huethorst2, Erin Boland2, Callie Lorimer1, Camilla Olianti3, Leonardo Sacconi4, Godfrey Smith2, Caroline Müllenbroich1

1 School of Physics and Astronomy, University of Glasgow, UK

2 School of Cardiovascular and Metabolic Health, University of Glasgow, UK

## 3 European Laboratory for Non-Linear Spectroscopy, Florence, Italy

4 Institute of Clinical Physiology (IFC) - CNR, Florence, Italy

Tissue scattering limits the depth at which a sample can be imaged using optical methods. This effect can be mitigated by rendering the tissue transparent using optical clearing techniques that allow for imaging of cubic cm-sized samples. Light sheet microscopy is especially suited to image these samples as it can provide isotropic resolution across a large field of view whilst imaging at a high speed. By employing mesoscale light-sheet microscopy and optical tissue clearing protocols, we have imaged tissue slices excised from the left ventricle of the heart to assess structural remodeling in a rabbit model due to myocardial infarction.

We have applied an optimized hydrogel-based clearing protocol (CLARITY [1]) on cardiac tissue slices from New Zealand white rabbit as it affords high levels of tissue transparency and structural preservation [2]. The tissues are sliced with varying thickness from 400 - 2000 µm using a vibratome. As the application of the CLARITY protocol results in tissue expansion, the specimens are placed in 3D printed spacers to ensure warping-free, isotropic expansion in the slice plane. Once the tissues are optically clear, the samples are stained with Wheat Germ Agglutinin– Alexa Fluor 488 labelling cell membranes. Once stained, the tissues are placed in a custom-made 3D-printed sample holder and imaged with a custom-built light sheet microscope (mesoSPIM, Mesoscale Selective Plane Illumination Microscopy, [3]). The mesoSPIM is designed to accommodate cleared samples to perform structural imaging studies over a large field of view (14 mm). Furthermore, zoom control on the Olympus MVX10 macroscope affords high-resolution imaging for a zoomed-in region of interest.

Here, we present a detailed characterization of our mesoSPIM including spatial resolution across the large field of view. We present the protocol for clearing, staining, and mounting of rabbit cardiac tissue slices, subsequent post processing to de-skew the image stack and first preliminary data for quantitative assessment of cardiac tissue structure at the cellular level. We believe that this methodology will be useful to obtain quantifiable information of 3-dimensional tissue remodeling on the cellular scale in rabbit hearts that have scarred post myocardial infarction.

References:

[1] Chung, Kwanghun, and Karl Deisseroth. "CLARITY for mapping the nervous system." *Nature methods* 10.6 (2013): 508-513.

[2] Olianti, Camilla, et al. "Optical clearing in cardiac imaging: A comparative study." *Progress in Biophysics and Molecular Biology* 168 (2022): 10-17.

[3] Voigt, Fabian F., et al. "The mesoSPIM initiative: open-source light-sheet microscopes for imaging cleared tissue." *Nature methods* 16.11 (2019): 1105-1108.