mesoSPIM in cardiac research: vibratome slices, infarction models, and spheroid detection

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Abstract: The open-hardware design of the mesoSPIM light-sheet microscope has transformed access to mesoscale imaging of cleared tissues. At the University of Glasgow, we apply this platform to cardiac tissue samples across three case studies. First, we demonstrate a mechanical de-skewing protocol enabling accurate imaging of laterally extended tissue slices. Second, we present preliminary results from large CUBIC-cleared ventricular sections, used to quantify adhesions in two rabbit models of myocardial infarction. Finally, we evaluate the performance of a Random Forest classifier trained to detect cardiac spheroids embedded within rabbit myocardium. Together, these studies highlight the versatility of mesoSPIM for cardiac research and its continued impact a decade after its inception.

1) Mechanical de-skewing enables high-resolution imaging of thin tissue slices

- Introducing a lateral step during the z-scan mechanically de-skews image stacks of diagonally mounted, laterally extended tissue sections
- We observe an improvement in axial resolution quantified with sub-resolution sized beads and a reduction in computational overhead of volume reconstruction [Moreno et al- manuscript in prep]
- These findings expand the capabilities of the mesoSPIM

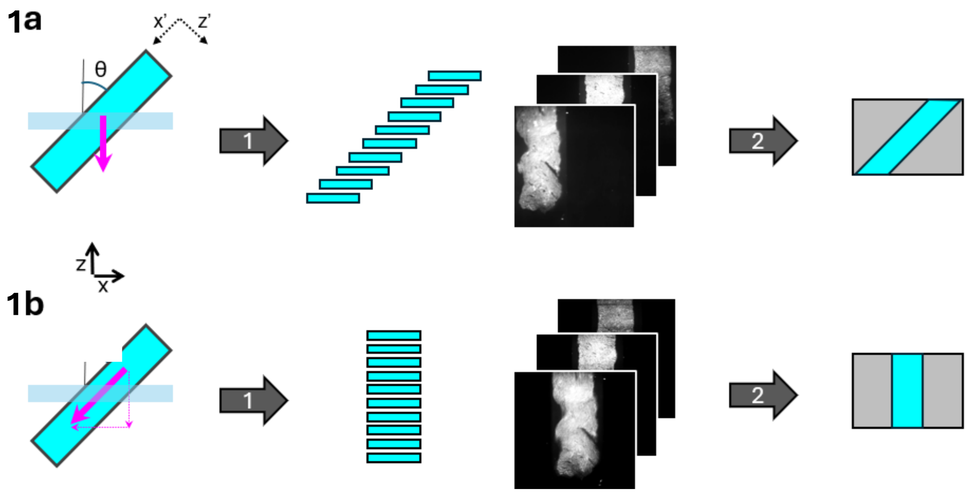
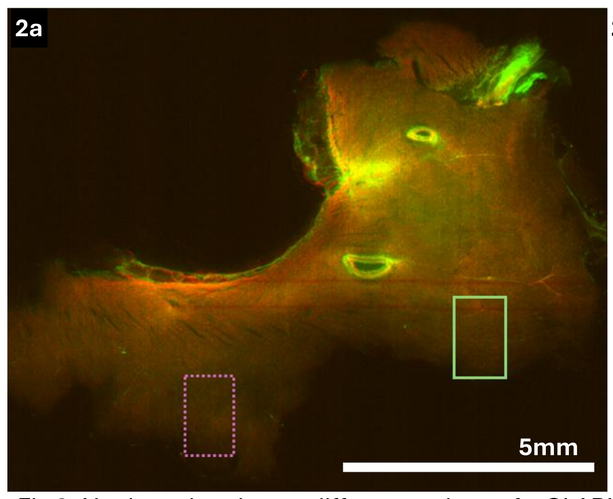


Fig 1: Additional lateral movement (b) with respect to a conventional z-stack (a) keeps the sample in the centre of the FoV



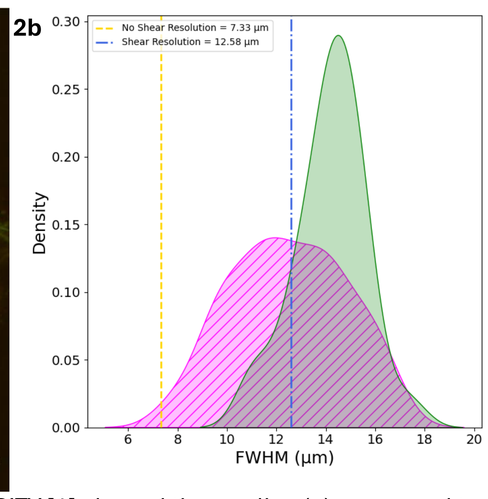


Fig 2: Nuclear sizes in two different regions of a CLARITY [1] cleared tissue slice (a) compared to the resolution of the Shear and No-shear Imaging protocols (b)

2) Study of the epicardium in two rabbit models of myocardial infarction

- Using CUBIC [2], we cleared and stained large sections of rabbit left ventricles
- We used Wheat-Germ Agglutinin (WGA) to stain tissue membranes and Propidium Iodide (PI) for nuclei
- We aim to compare the epicardium by two methods of inducing myocardial infarction: either through thoracotomy [3] or a percutaneous protocol entering through the carotid artery to create an occlusion in the heart using a catheter tip [3]

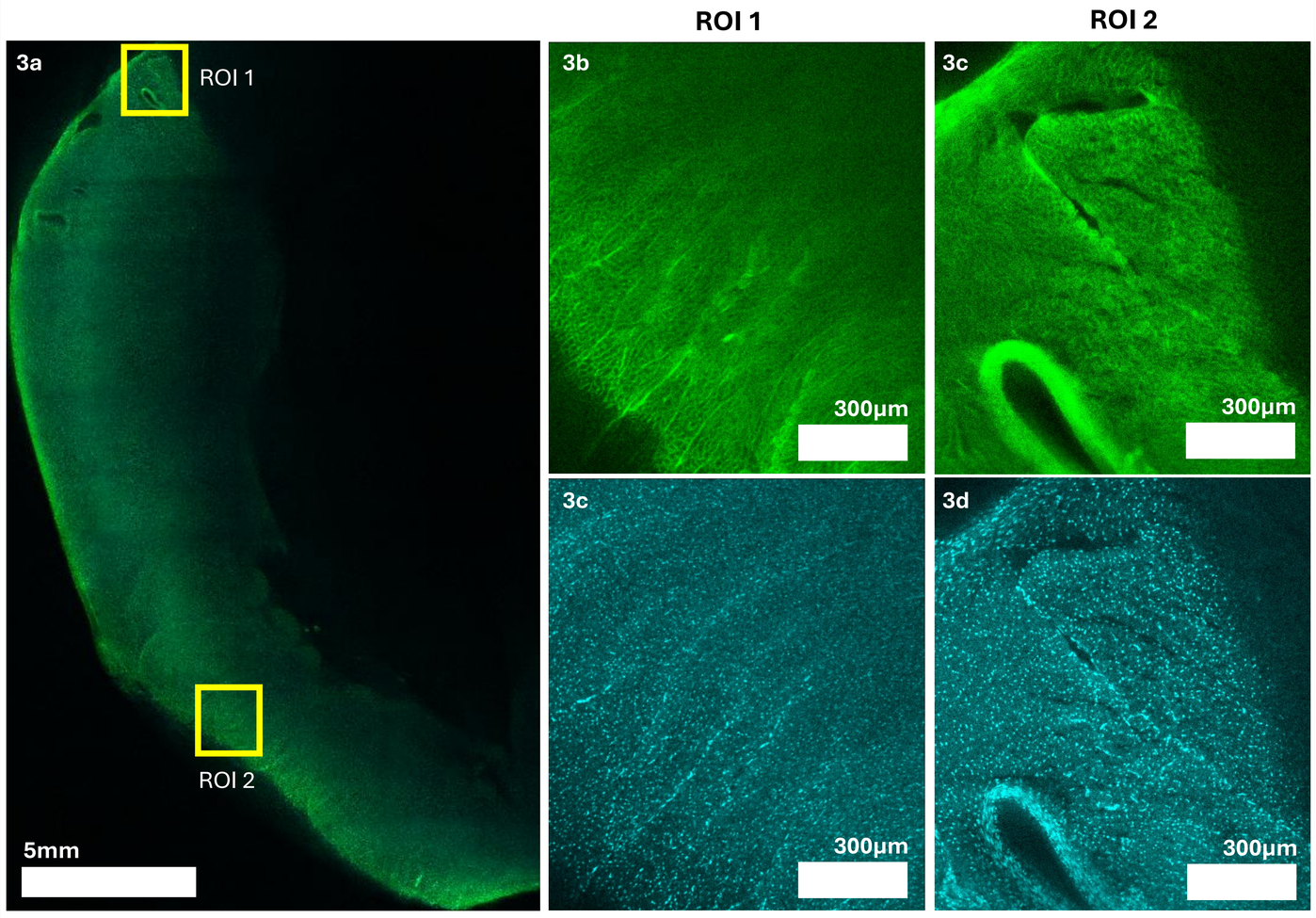


Fig 3: A non-MI, control sample (thoracotomy sham). 3b,c: ROIs of the WGA channel. 3c,d: ROIs in the PI channel.

3) Random forest classification to detect injected spheroids in host rabbit myocardium

- Human-induced Pluripotent Stem Cell derived cardiomyocyte (hi-PSC) cardiomyocyte spheroids were injected into the LV of a rabbit heart for an electrical coupling experiment for regenerative medicine
- After functional studies, the samples are CUBIC cleared, stained, and imaged to determine the morphology of the injection site and the retention of spheroids in the host myocardium
- We apply a random forest (RF) classification [4] to segment the spheroids inside the host.
- The RF classification uses 100 trees with no depth limit, training the classifier to completion (2-5 minutes)

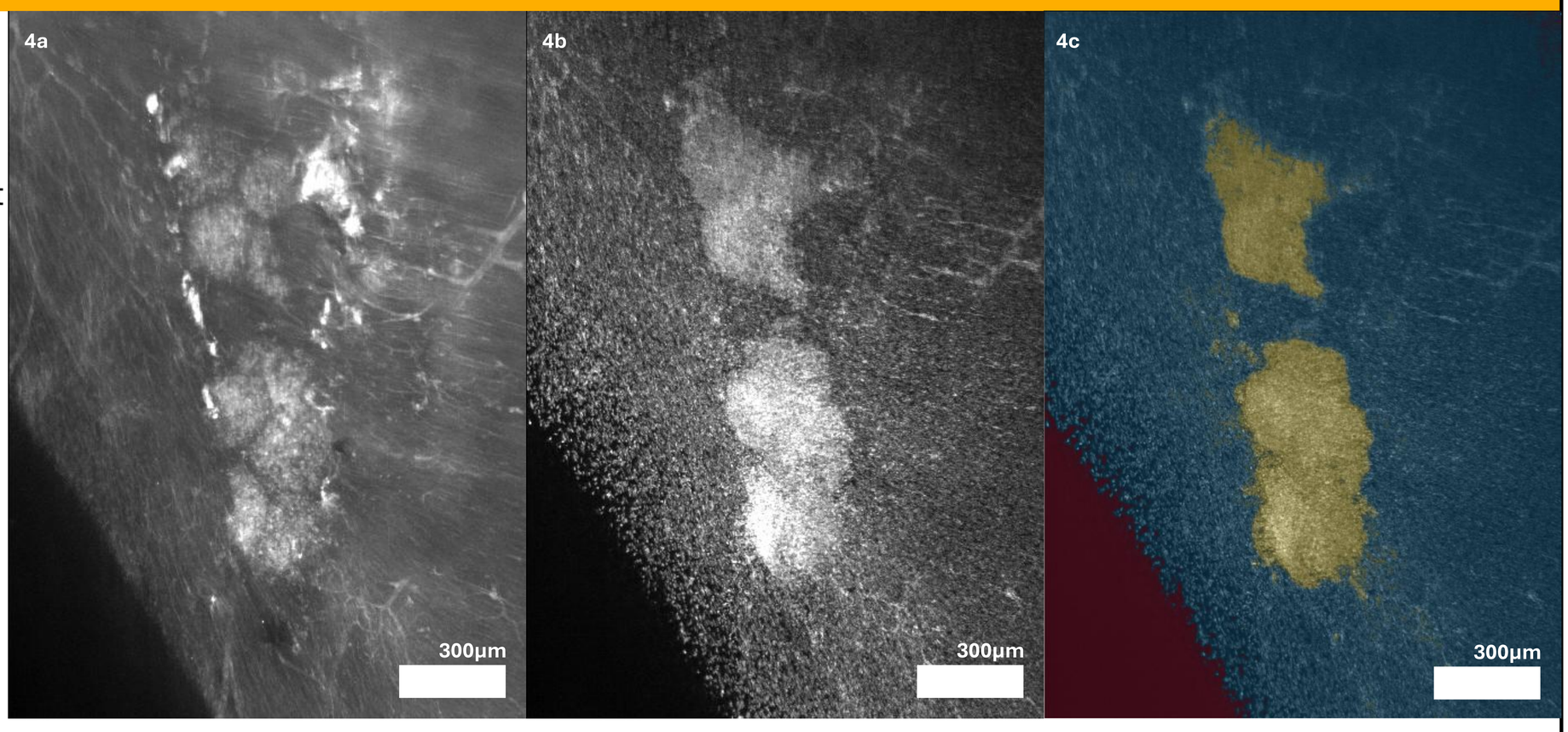


Fig 4a: Membrane Stain – WGA 647, 4b) nuclear staining – SYTOX 488, 4c) Ilastik [4] Random forest classification of Spheroid pixels (yellow). Blue labels non-spheroid tissue. Classifier used 100 trees and no depth limit, ensuring training until completion.

References

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British Heart Foundation
Funding a healthier future
MEDICAL RESEARCH SCOTLAND
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