A pipeline of clearing, mounting, and mesoscale imaging of sliced cardiac tissue structure

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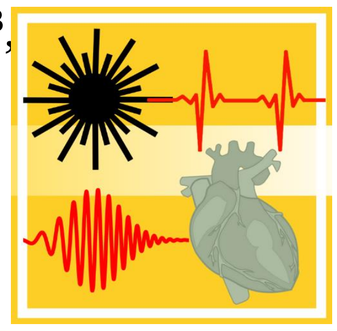


1. Introduction to Optical System

- Observation of axially deep cellular tissues limited by the scattering of light [2].
- Optical clearing renders mesoscale tissues (mm^3 to cm^3) transparent, amenable to optical imaging.
- Light-sheet microscopy (LSM) permits isotropic resolution, large field of views, high throughputs of examined tissues [2].
- Preparation, clearing, staining, and mounting of the delicate cardiac tissue slices difficult to repeat.
- Reliable imaging results requires careful, reproducible protocols to mitigate tissue changes in vitro compared to in vivo.

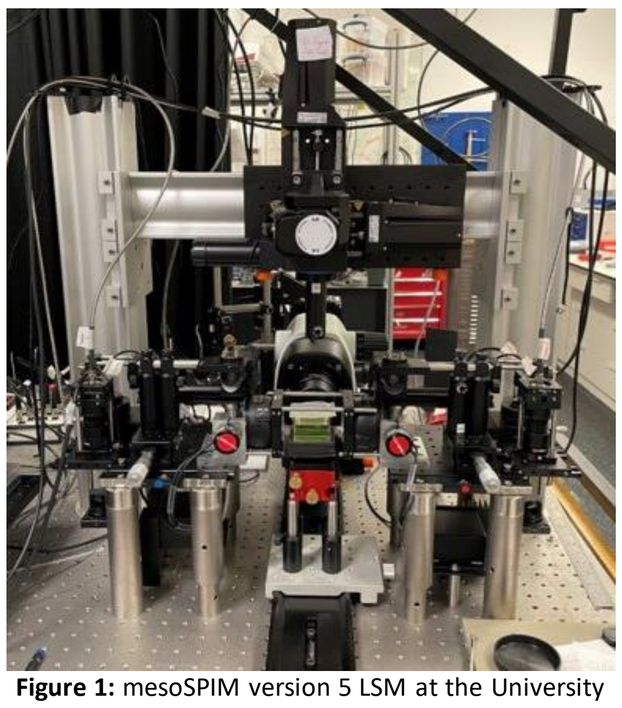
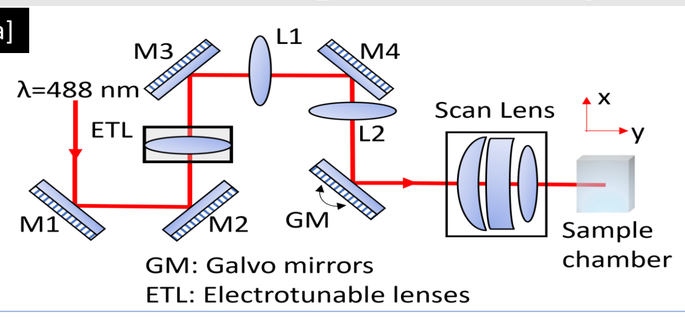


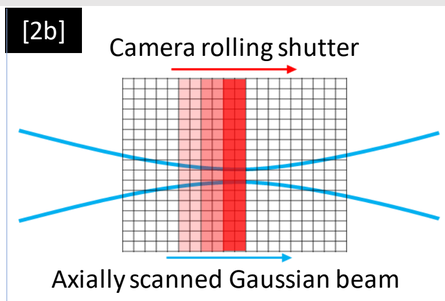
Figure 1: mesoSPIM version 5 LSM at the University of Glasgow, Glasgow, United Kingdom.

2. Optical Pathways of mesoSPIM

[2a]



[2b]



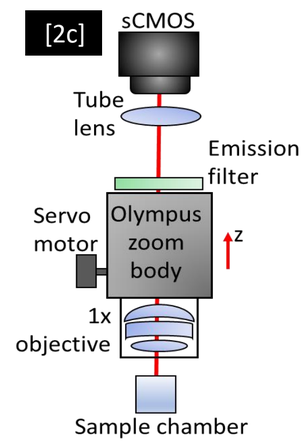
mesoSPIM Excitation Arm (Figure 2a):

- Focus of the Gaussian beam shifted across sample using ETLs using Axially Swept Light-sheet Microscopy mode (ASLM, **figure 2b**) [2].
- ETL sweep synchronised with camera Photometrics Kinetix sCMOS camera (diagonal = 29.4 mm) rolling shutter feature.

mesoSPIM Detection Arm (Figure 2c):

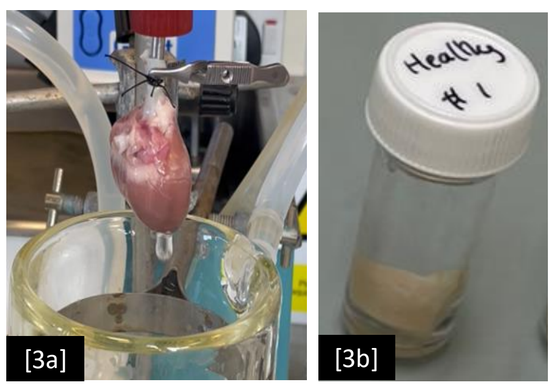
- MVX-10 Olympus zoom body changes the FOV from 20.8 mm (1×) to 3.3 mm (6.3×) [2].
- MVXPLAP01 Olympus Objective focuses onto illuminated sample with 0.65 NA, 65mm WD

[2c]



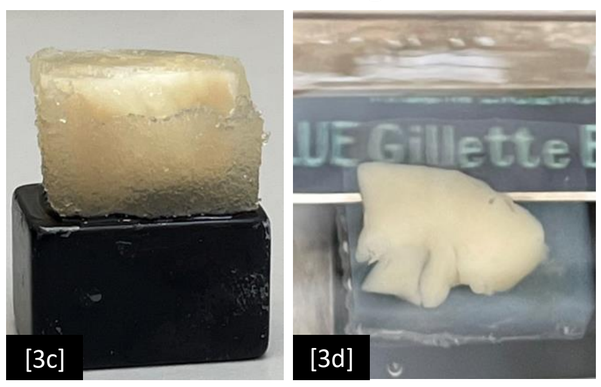
3. Cardiac Tissue Fixation and Slicing

Tissue Extraction and Fixation



- Cardiac samples Langendorff perfused, expelling remaining blood (**Figure 3a**)
- Cardiac Fixation done using Paraformaldehyde (PFA), stored then in Phosphate Buffered Saline (PBS) (**Figure 3b**) [1]

Tissue Slicing



- Fixated tissue is embedded in agar block
- Block glued onto base platform of Vibratome tissue slicer (Figure 3c)
- Tissue in agar sliced at desired thickness (400-3000µm) using metal or ceramic blade (**Figure 3d**)
- Agar edges removed; slices returned to PBS storage.

4. Tissue Clearing Protocols: CLARITY

CLARITY Protocol [1,3]
(Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging Tissue hYdrogel)

1. Washed in PBS to remove non-tissue molecules (PFA, blood, bacterium, etc.)
2. Wash in Hydrogel solution (PFA and hydrogel monomers), ensuring homogeneity.
3. Degassed in N_2, polymerizes monomers into hydrogel mesh.
4. Washed clearing solution (detergent with weak acid) to expel lipids.
5. Saturated clearing solution changed out every 2-3 days (for up to 6-7 months)

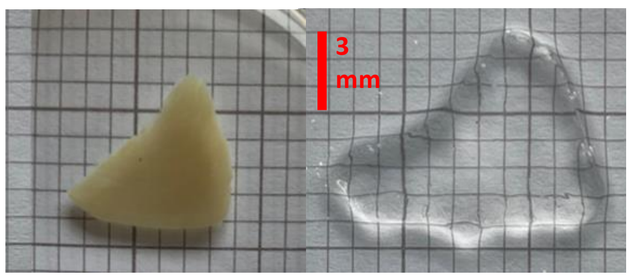


Figure 4a: 400µm Cardiac Tissue Sample Before and After CLARITY Tissue Clearing Protocol (±20 weeks)

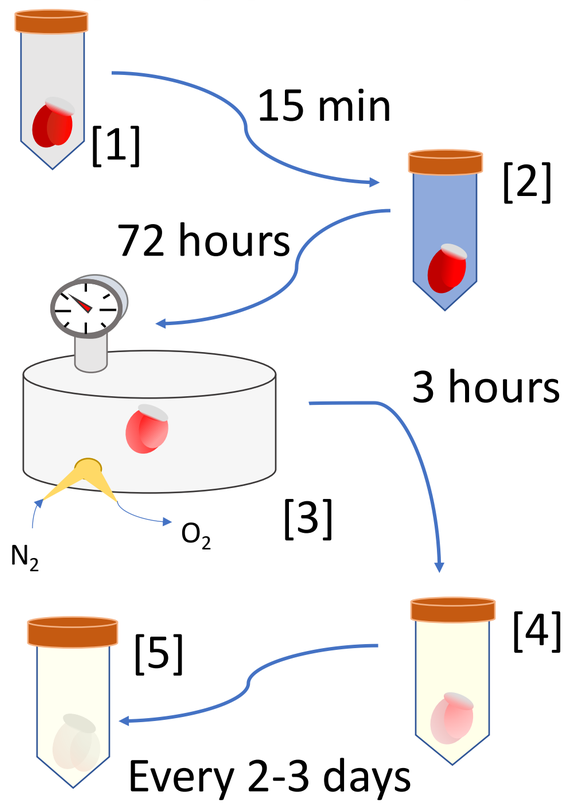
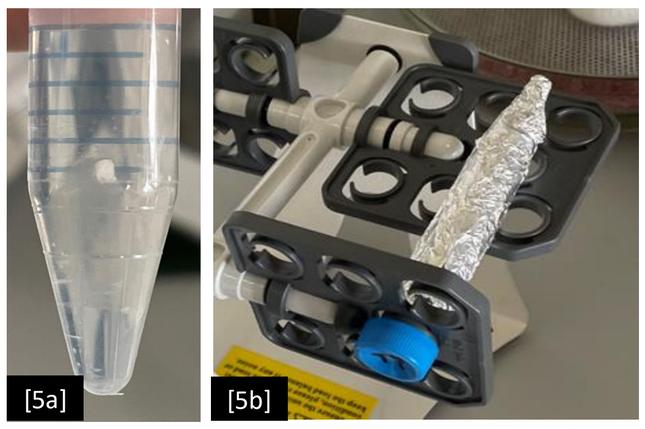


Figure 4b: CLARITY Tissue Clearing Protocol Steps and Timeline. [1,3]

5. Staining and Mounting of Cleared Samples



Tissue Staining

- Slice cardiac samples stained using Wheat Germ Agglutin Conjugates (WGA-Fluorescein, Alexa Flour 488) (**Figure 5a**)
- Washed in 1x PBS/Triton-X100 solution, PBS/3% PFA solution [1]
- Concealed from light throughout staining to prevent photobleaching (**Figure 5b**)

Tissue Mounting

- Cleared tissue mounted between two quartz slides
- Immersed in Refractive Index matching solution (RI = 1.45) 24 hrs prior.
- Sample angled at 45° from excitation/detection paths
- Angle prevents mount frame from blocking optical pathways

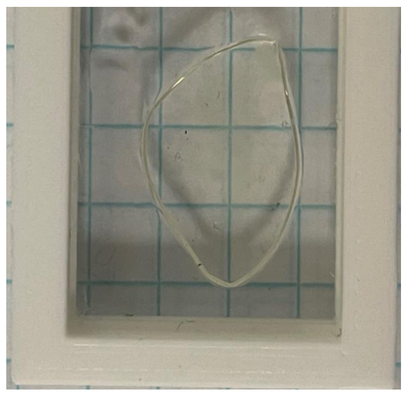


Figure 5c: 1000µm Cardiac Tissue Sample in custom mount before immersion in RI matching solution, imaging in mesoSPIM

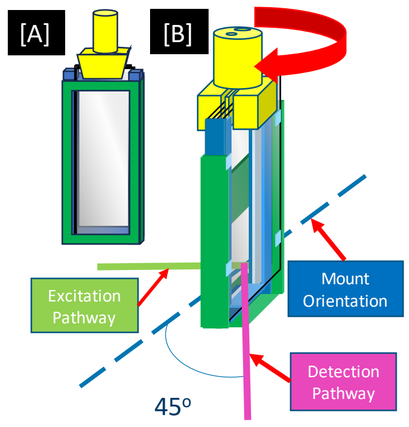
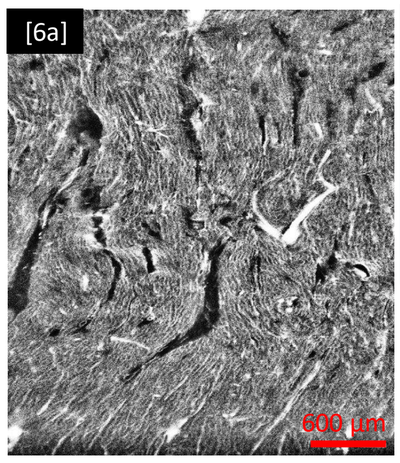


Figure 5d: 3D model of mount at 0° [A] and 45° Orientation [B].

6. Imaging of Cleared Tissue Slices

- Z-stacks of stained samples recorded and processed
- Sliced with thickness of 500um - 3mm produced high resolution images
- Deskewing program applied to obtain images stacks at 0°



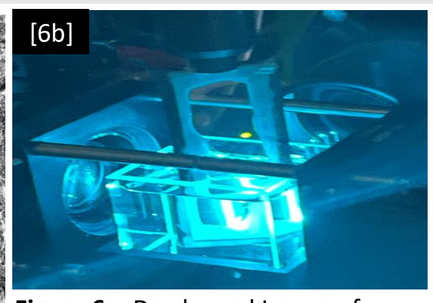


Figure 6a: De-skewed Image of 500µm Healthy Cardiac Tissue, x2 mag, WGA-Alexa 488 Staining
Figure 6b: Mounted Slice Imaged in mesoSPIM

7. Future work

- Modify existing Detection Pathway using new latest open-source design to mitigate aberrations, vignetting associated with expanded FOV
- Implement staining on samples from diseased hearts to assess structural changes relative to healthy hearts
- Proceed with quantitative, structural analysis of cardiac tissue for images obtained.
- Examine clearing results from alternative clearing protocols (SHIELD, CUBIC-L/RA), assessing image quality relative to CLARITY

REFERENCES

[1] Olianti, Camilla, et al. *Progress in Biophysics and Molecular Biology* **168** (2022): 10-17.
[2] Voigt, Fabian F., et al. *Nature methods* **16.11** (2019): 1105-1108.
[3] Tomer *et al*, *Nature protocols* **9.7**, (2014): 1682-1697.

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