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


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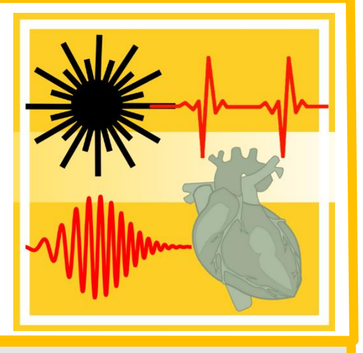


## 1. Introduction to Optical System

- 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 damage/alteration in vitro compared to in vivo.

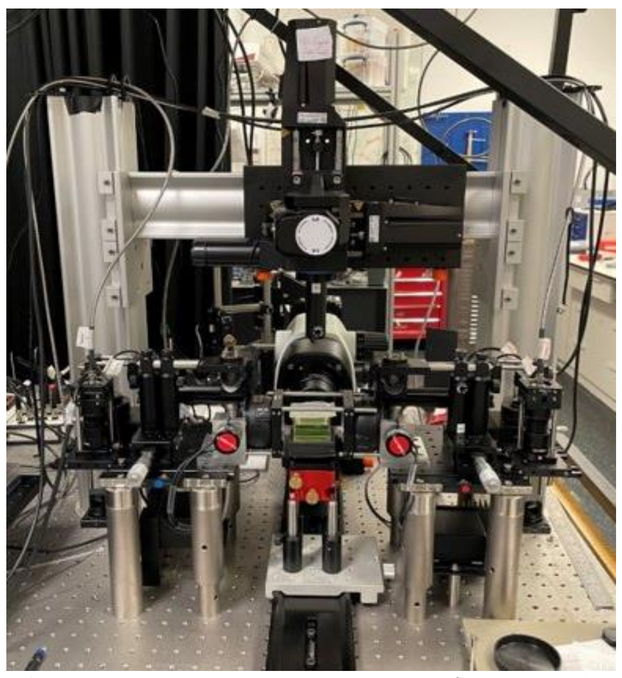
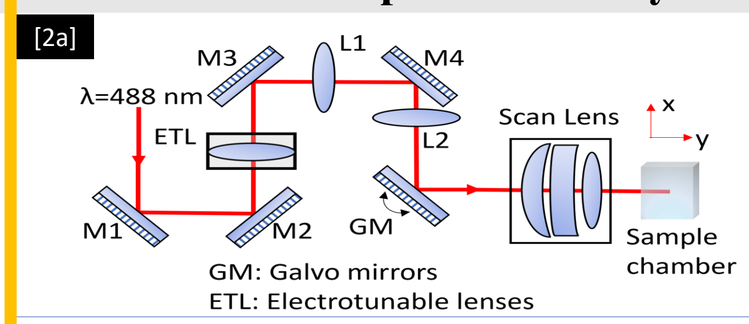
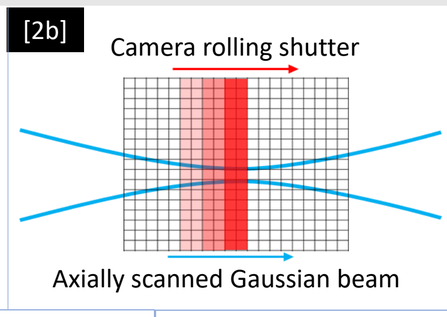


**Figure 1:** mesoSPIM version 5 LSM at the University of Glasgow, Glasgow, United Kingdom.

## 2. Optical Pathways of mesoSPIM



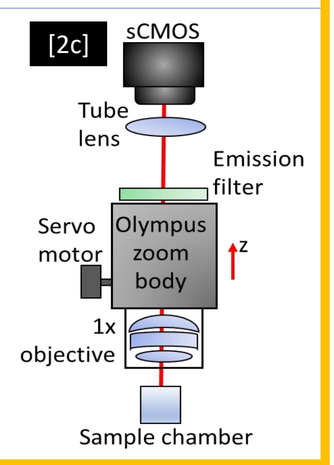




**mesoSPIM Excitation Arm (Figure 2a):**

- 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.

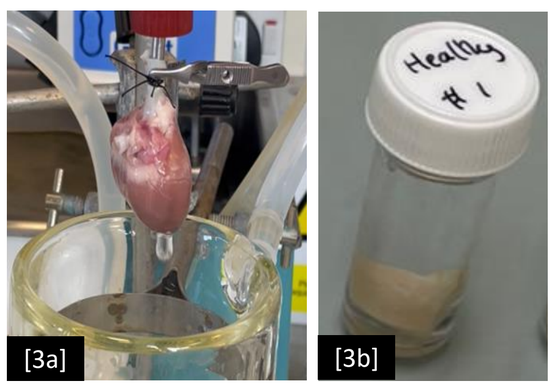
**mesoSPIM Detection Arm (Figure 2c):**

- 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



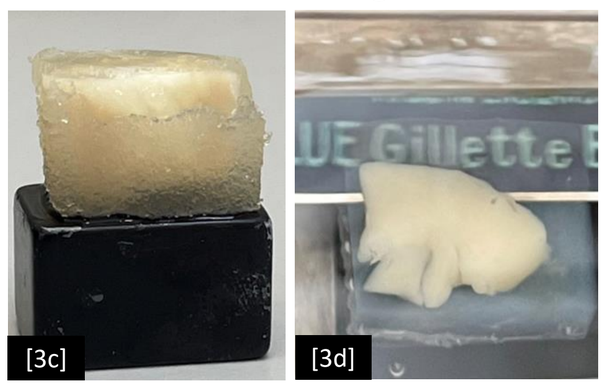

## 3. Cardiac Tissue Fixation and Slicing

### Tissue Extraction and Fixation




- Extracted cardiac samples undergo Langendorff perfusion, expelling remaining blood in the organ (**Figure 3a**)
- Cardiac Fixation done using Paraformaldehyde (PFA), stored 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) (**Figure 3d**)
- Excess agar 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 (paraformaldehyde and hydrogel monomers), ensuring homogeneity.
3. Degassing in $N_2$ polymerizes monomers into a hydrogel mesh.
4. Wash in clearing solution (detergent with weak acid) to expel lipids.
5. Changing out of clearing solution every couple of days (for up to 6 months)

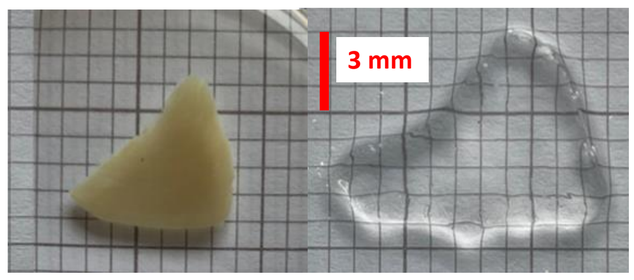



**Figure 4a**: 400µm Cardiac Tissue Sample Before and After CLARITY Tissue Clearing Protocol (±20 weeks)

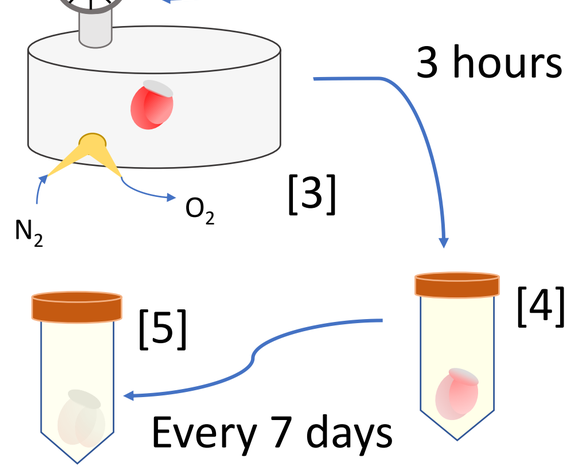
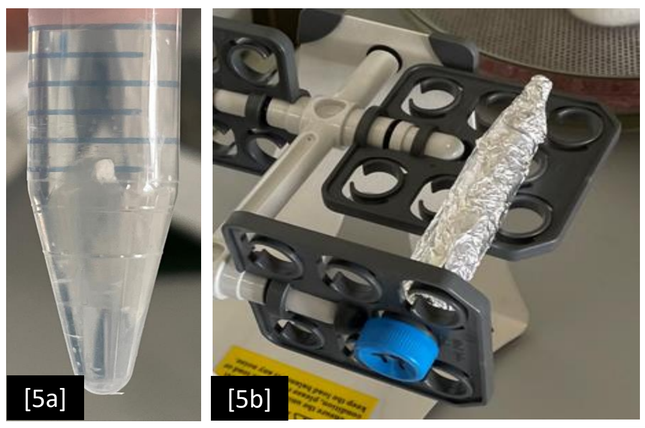



**Figure 4b**: CLARITY Tissue Clearing Protocol Steps and Timeline. [1,3]

## 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 Phosphate Buffered Saline, Triton-X100 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)
- Sample angled at 45° from excitation/detection paths
- Angled position prevent frame from blocking optical pathways

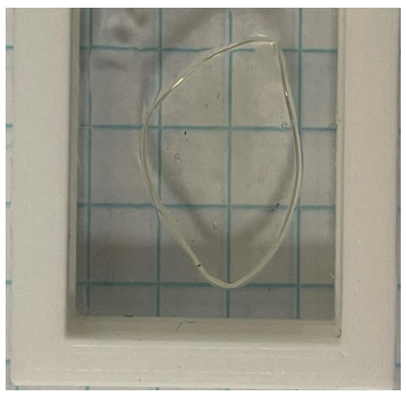


**Figure 5c**: 1000µm Cardiac Tissue Sample in custom mount before immersion in RI matching solution, imaging in mesoSPIM

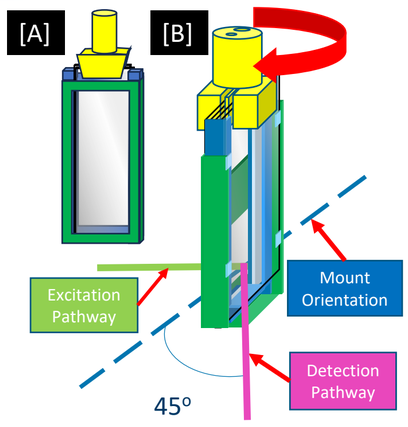



**Figure 5d:** 3D model of mount at 0° [A] and 45° Orientation [B].

## 6. Imaging of Cleared Tissue Slices

- Z-stacks of stained samples recorded and processed
- Sliced with thickness of 500um, 1mm, 3mm produced high resolution images
- De-skewing program applied to obtain images stacks at 0°





**Figure 6a:** De-skewed Image of 500µm Healthy Cardiac Tissue, x6.3 mag, WGA-Fluorescein 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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