SM2

Mesoscale light sheet microscopy for imaging cm-sized cleared tissue slices

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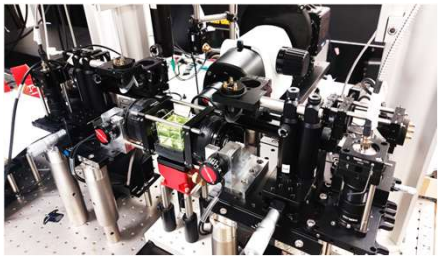
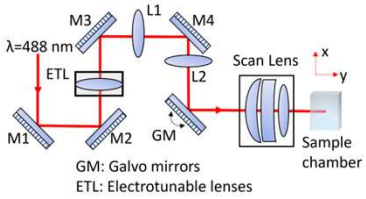
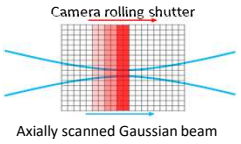
1. Introduction

- The mesoSPIM (Mesoscale Selective Plane Illumination Microscope) is an open-source hardware light-sheet system [1].
- Built to image large cleared samples.
- Digitally scanned light-sheet with dual excitation arms and orthogonal detection arm.
- We characterise the mesoSPIM across its large field of view (FOV) and apply it to image cm-sized cleared rabbit tissue slices.



2. ASLM and zoom



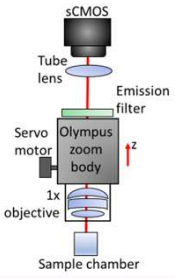


Excitation Arm (above):

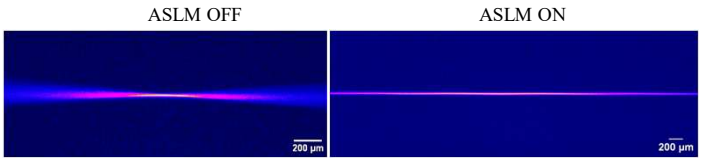
- Gaussian focus shifted across sample using ETL in Axially Swept Light-sheet Microscopy mode (ASLM) [2].
- Sweep of the ETL synchronised with the rolling shutter of the Photometrics Kinetix sCMOS camera (diagonal = 29.4 mm).

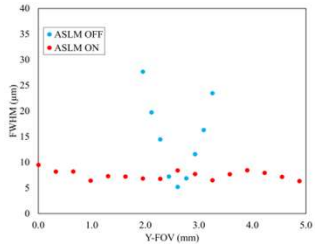
Detection Arm (right):

- MVX-10 Olympus zoom body changes the FOV from 20.8 mm (1×) to 3.3 mm (6.3×).



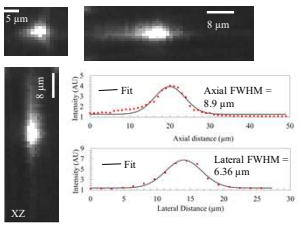
3. Gaussian beam characterisation

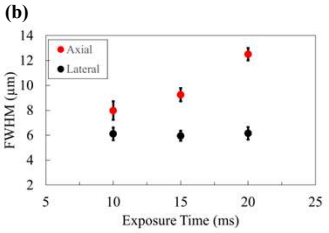




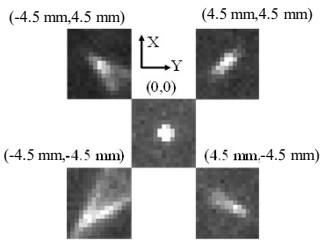
- 'ASLM OFF' mode shows the Gaussian focus. 'ASLM ON' mode extends optical sectioning across a large lateral (x-y) FOV.
- Increasing exposure time decreases axial (z) resolution as each row averages fluorescence signal corresponding to wider parts of the illumination beam
- The mean 'ASLM ON' FWHM beam waist diameter is 7.5 µm at 20ms exposure time.
- Confocal parameter increases from 750 µm to 5 mm at 4× magnification in 'ASLM ON' mode

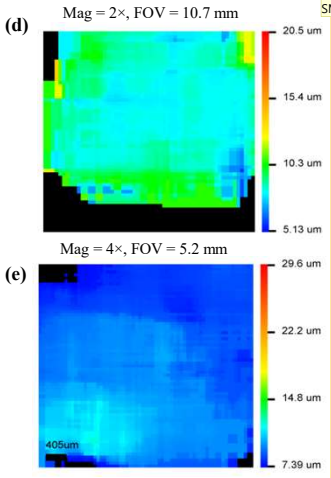
4. Aberrations across FOV





- FWHM calculated using 1 µm diameter fluorescent beads in refractive index matched agarose gel using PSFj software [3].
- Representative bead imaged at 20 ms exposure time at 4× magnification (a).
- Increase in exposure time shows decrease in axial resolution (b).

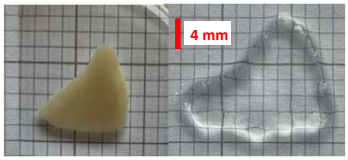


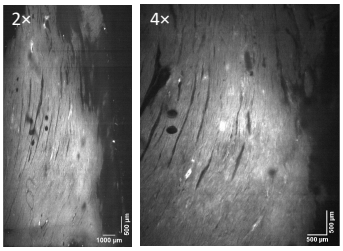


- Imaged at 2× magnification (c), the numbers indicate the (x,y) distance from the optical axis.
- We observe increasing amounts of field dependant coma at edges of the FOV.
- The heatmaps (d,e) show the variation of the axial FWHM across the lateral FOV.
- Black regions indicate areas where beads are rejected from analysis due to aberrations.

5. Imaging of cleared tissue slices

- 500 µm tissue slices were excised from the left ventricle of the rabbit heart.
- Tissue clearing performed using hydrogel tissue transformation-based CLARITY protocol [4,5].
- CLARITY creates highly transparent tissue however with low structural integrity in the heart.
- We observe tissue expansion by × ~1.7.





- Cleared tissue mounted between two quartz slides immersed in refractive index-matching solution (EasyIndex).
- Sample angled at 45° with respect to detection axis.
- Tissue imaged in autofluorescene mode.
- Raw dataset deskewed to obtain XY projection.

6. Future work

- Improving the imaging quality of the system by using objectives that are aberration free across the entire 10.7 mm FOV at 2× magnification.
- Implement fluorescence staining on cleared tissue using Wheat Germ Agglutinin to demarcate myocardial cells.
- Assess and quantify structural differences in healthy vs. diseased heart tissue.

REFERENCES

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FUNDING

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CAIRN RESEARCH

Slide 1

SM1 Cant change colormap values as it is automatically set on psfj.
Sharika Mohanan,
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SM2 Placeholder image. Will change to correct image tomorrow.
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