Imaging pipeline for cm-sized tissue slices using mesoscale light sheet microscopy

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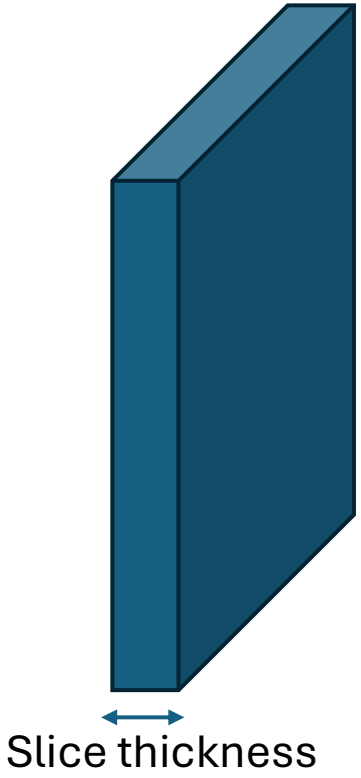
Motivation – Structural imaging of cardiac tissue

- Structural differences in healthy and diseased rabbit hearts
- Imaging tissue slices using protocols such as CLARITY
- Lateral extent of tissue much larger than thickness

Simplify imaging protocol



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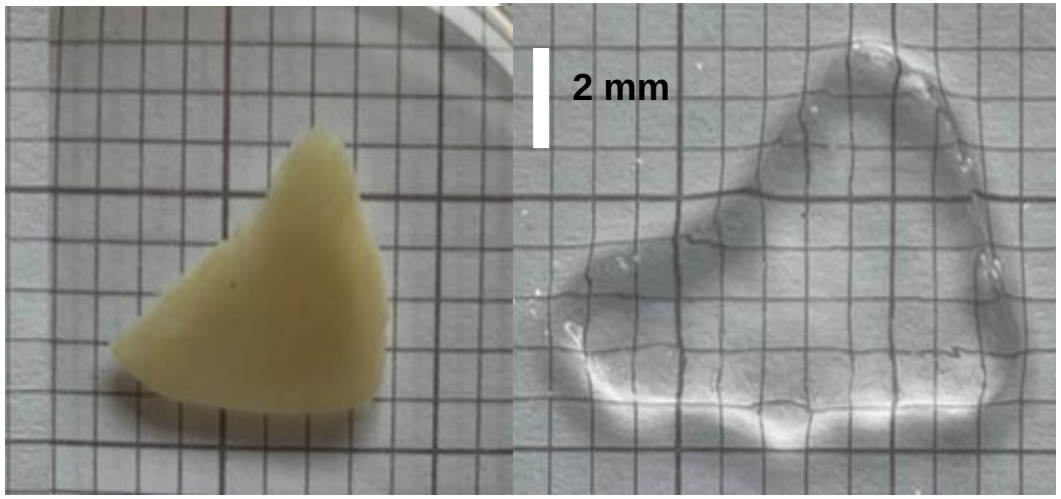
Cardiac tissue – sample preparation

New Zealand white rabbit Left Ventricle → Vibratome 500 µm – 3 mm → Tissue clearing → Fluorescence labelling



- Tissue expansion by 1.6x
- Tissue labelling
  Wheat Germ Agglutinin – Alexa 488

Steven Moreno

Dr. Eline Huethorst

Dr. Erin Boland

Dr. Camilla Olianti

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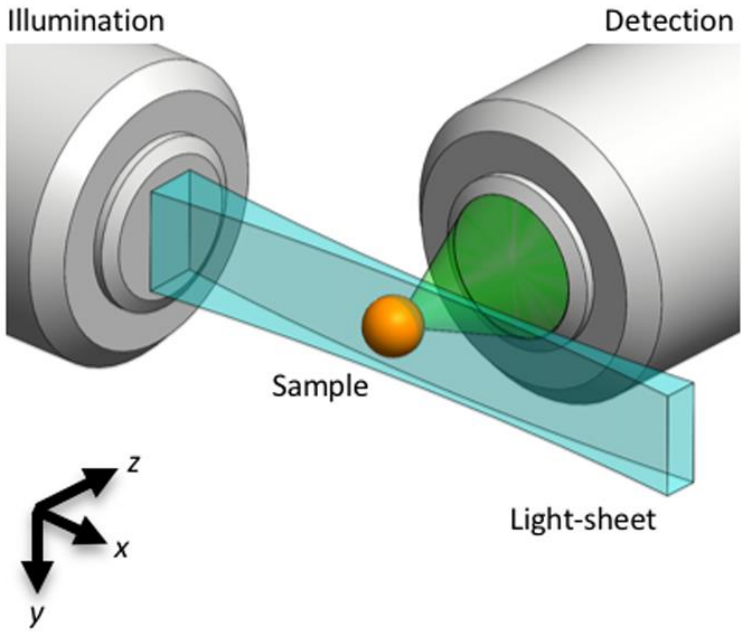
Du *et al*, Experimental and Therapeutic Medicine, 2018
Olianti et al, Progress in Biophysics and Molecular Biology, 2022

Light sheet microscopy



Advantages:

- Widefield imaging
- Reduced photobleaching
- Large samples

Considerations:

- Sample mounting
- Data Handling

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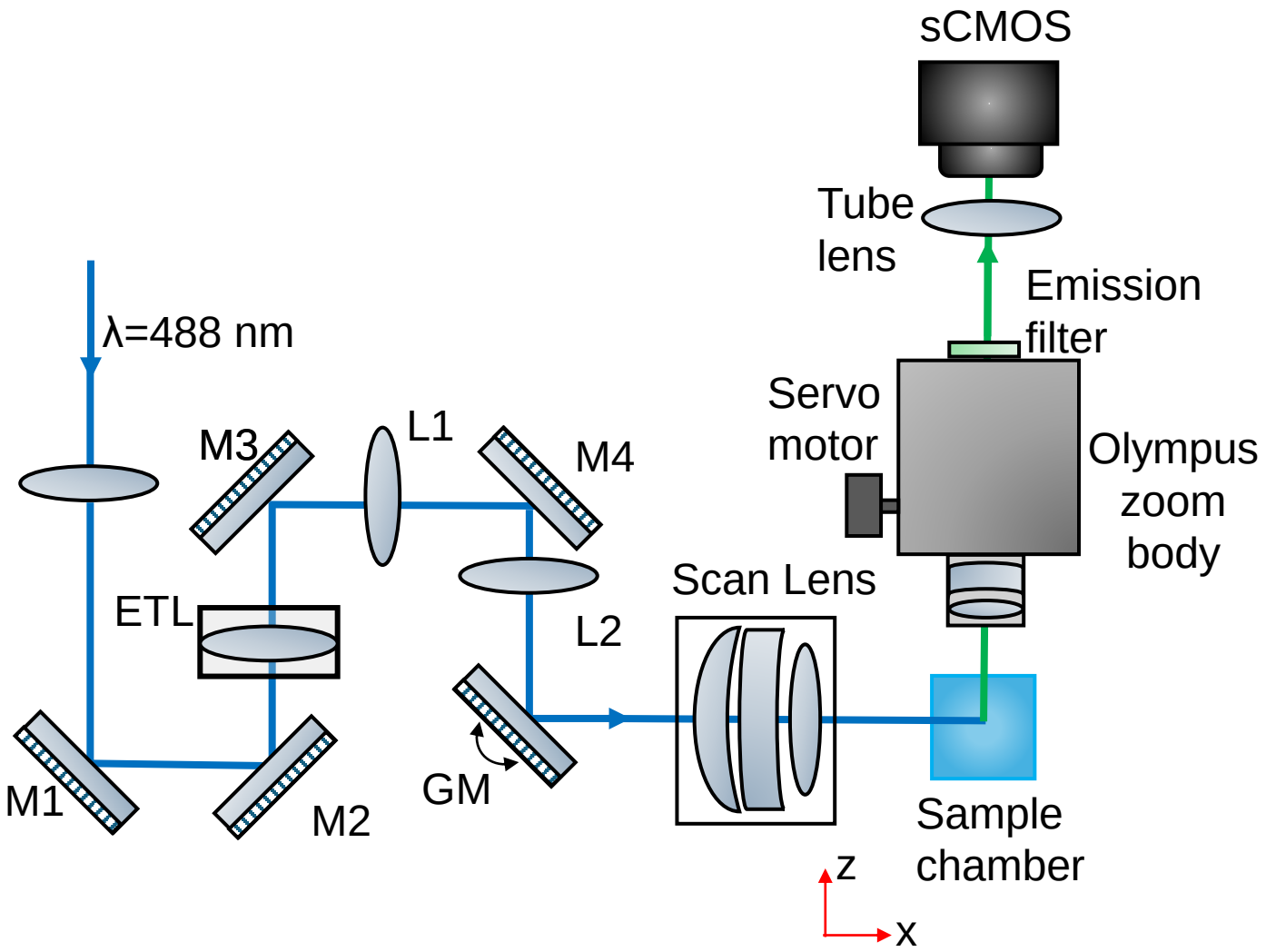
Keomanee-Dizon *et al*, Rev. Sci. Instrum., 2020

Mesoscale Selective Plane Illumination Microscopy (MesoSPIM)



Excitation arm:

- Axially scanned light sheet (ASLM)
- Digitally scanned light sheet
- Excitation NA: 0.1

Detection arm:

- MVX10 Olympus zoom body
- Photometrics Kinetix camera (29.4 mm diagonal FOV)

Magnification	FOV (mm)
2x	14.7
4x	7.35
6.3 x	4.6

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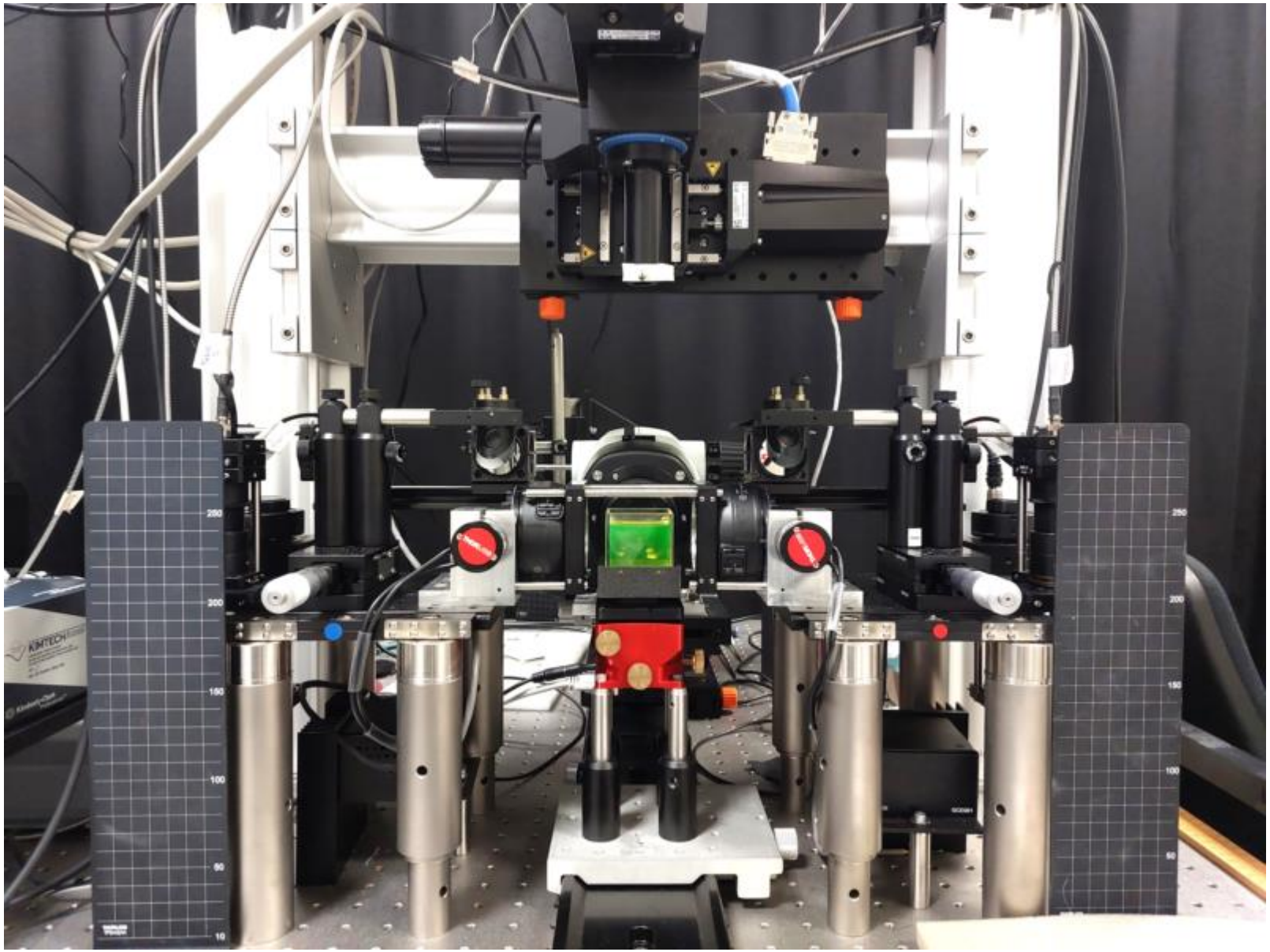
Voigt *et al*, Nat. Methods, 2019

MesoSPIM



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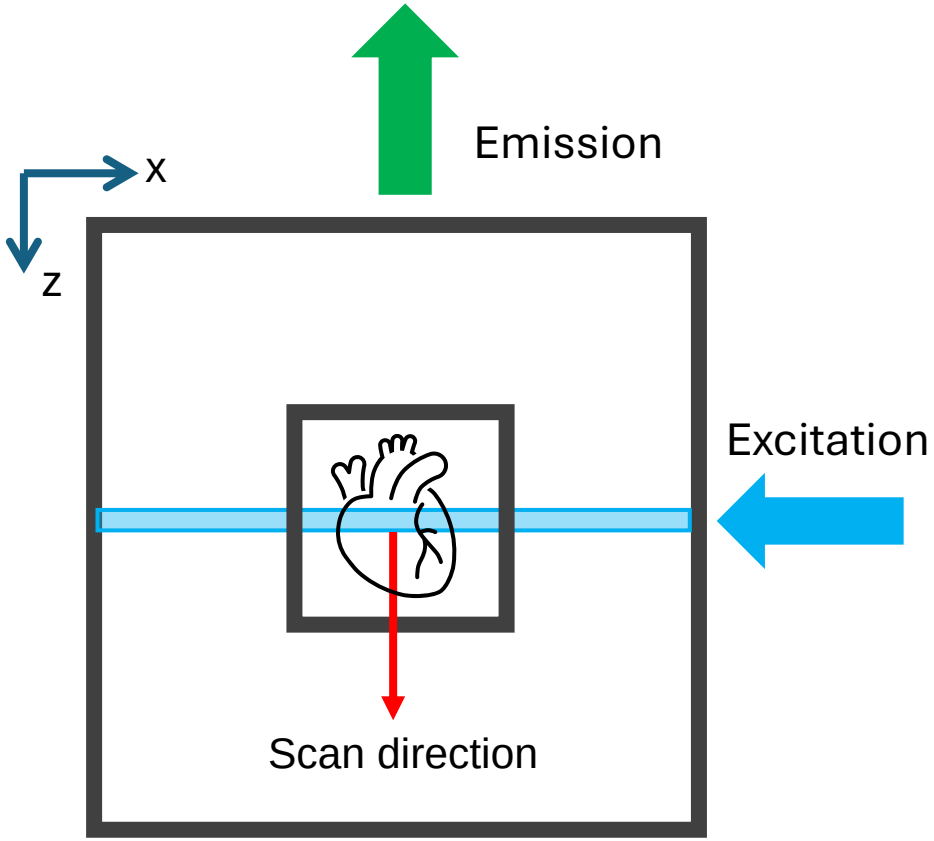
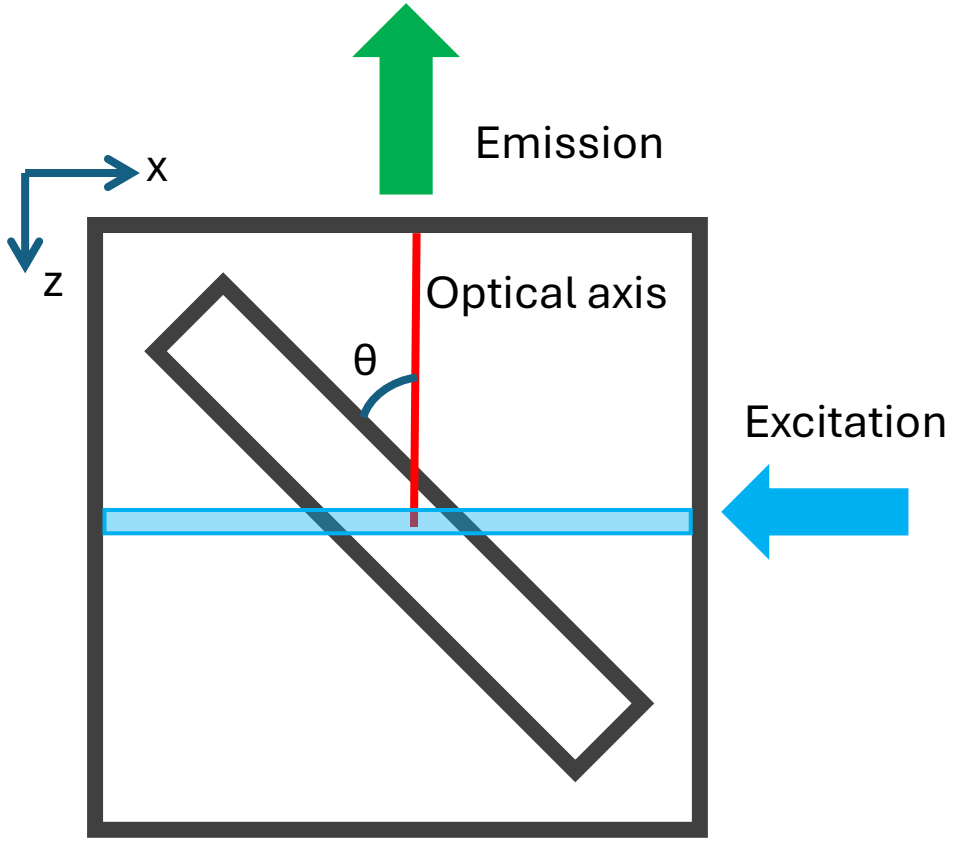
Tissue slice imaging – sample mounting





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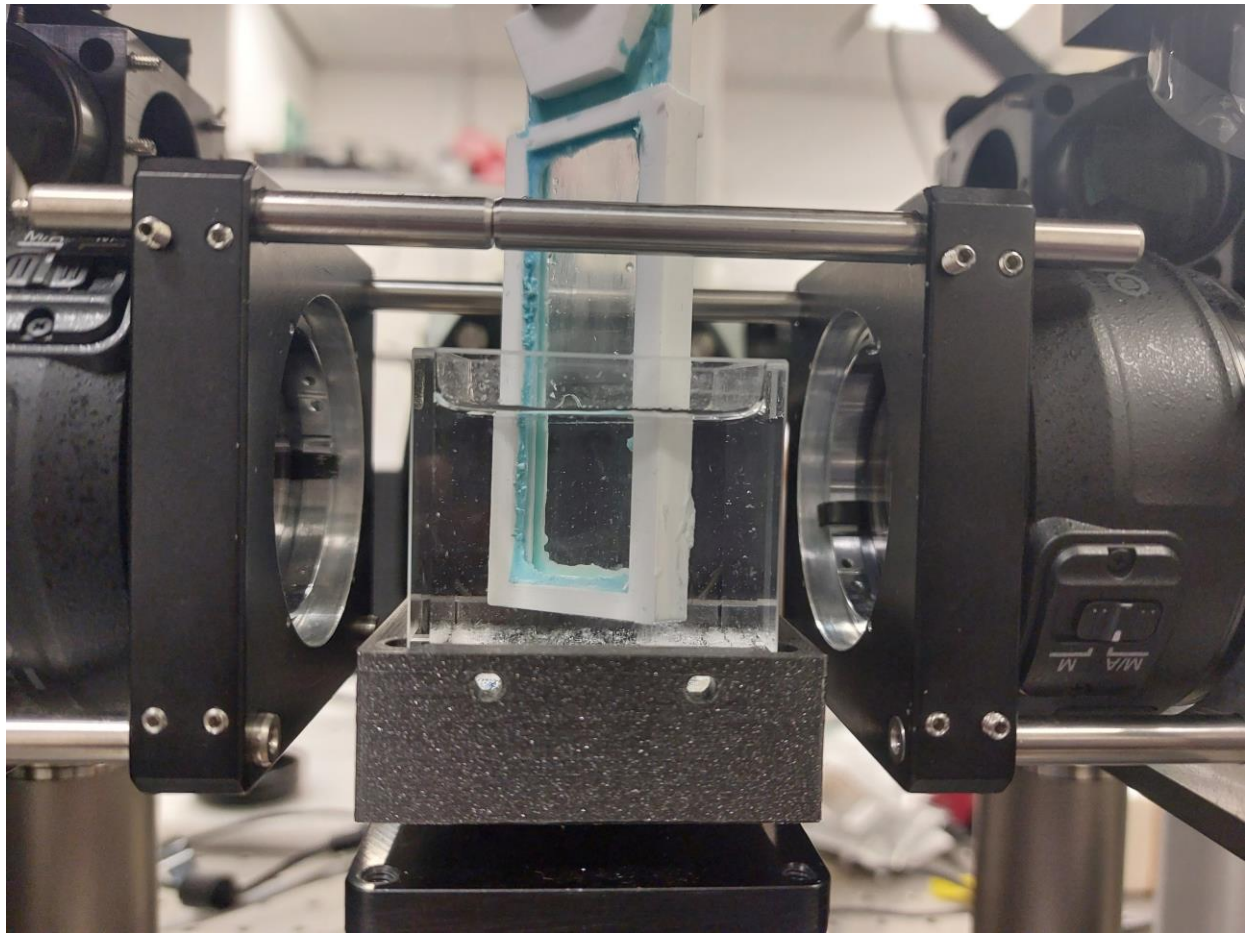
Tissue slice imaging – sample mounting

Steven Moreno



- Mounted on a x,y,z,θ stage
- Tissue placed between two quartz slide “sandwich”
- Outer and inner “sandwich” cuvette filled with refractive index matching solution

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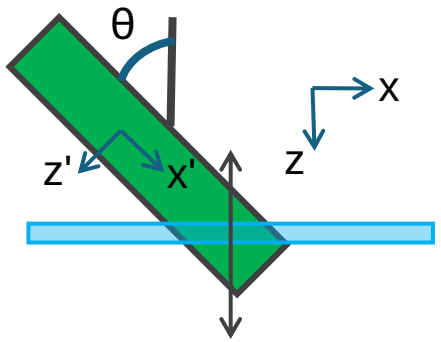
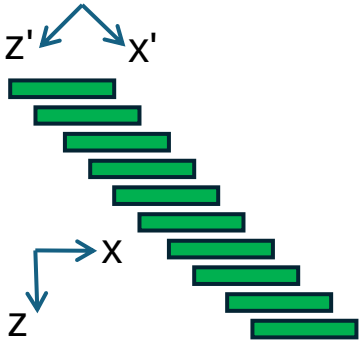
Tissue slice imaging



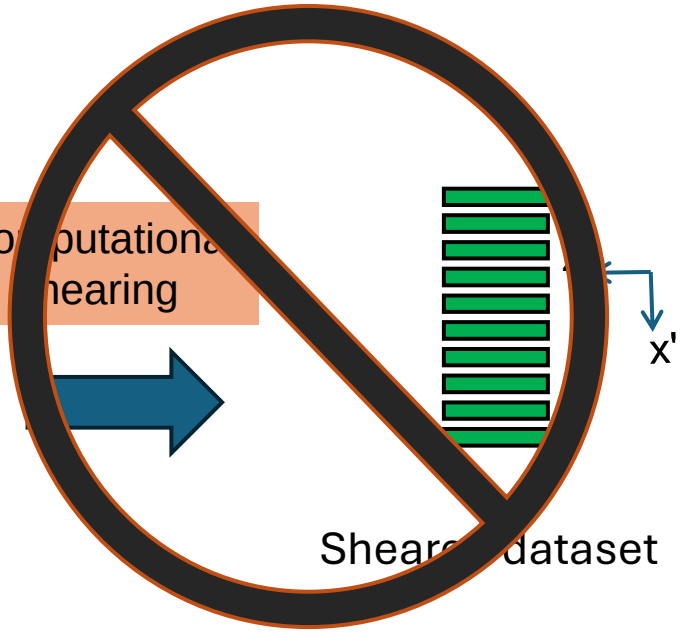
Scan direction

z scan



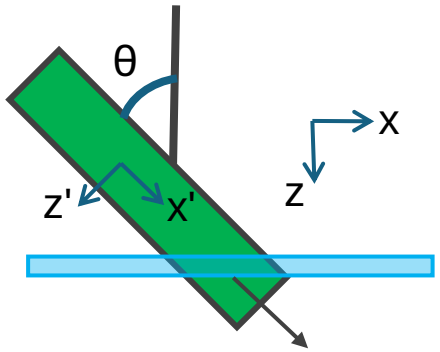
Raw image stack

co putationa
hearing



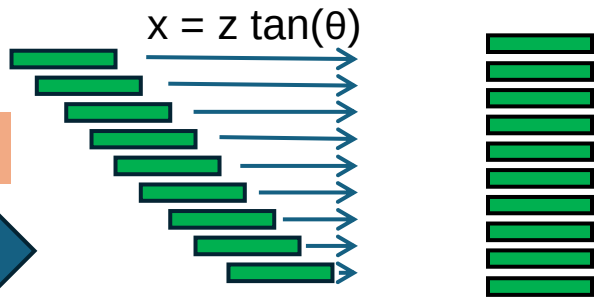
Shear dataset

Oblique Scanning



Scan direction

x' scan



Raw image stack

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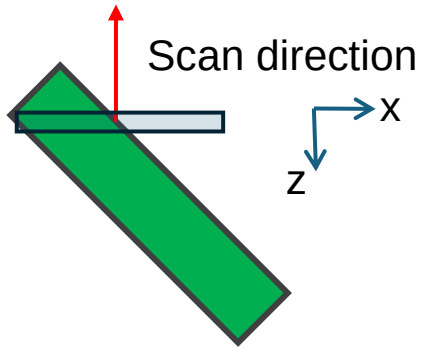
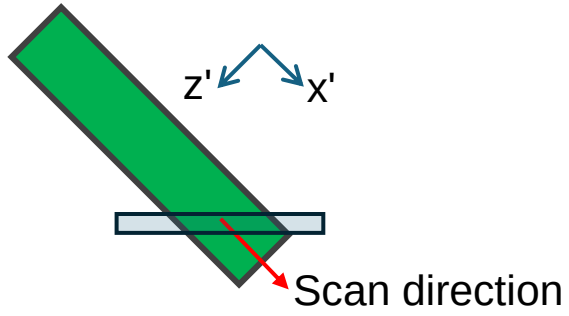
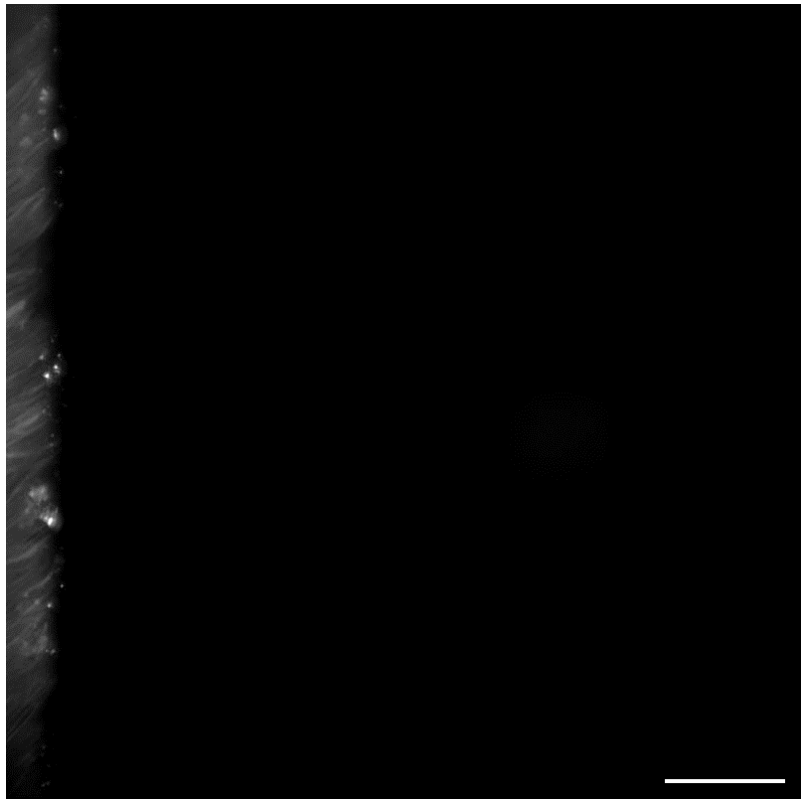
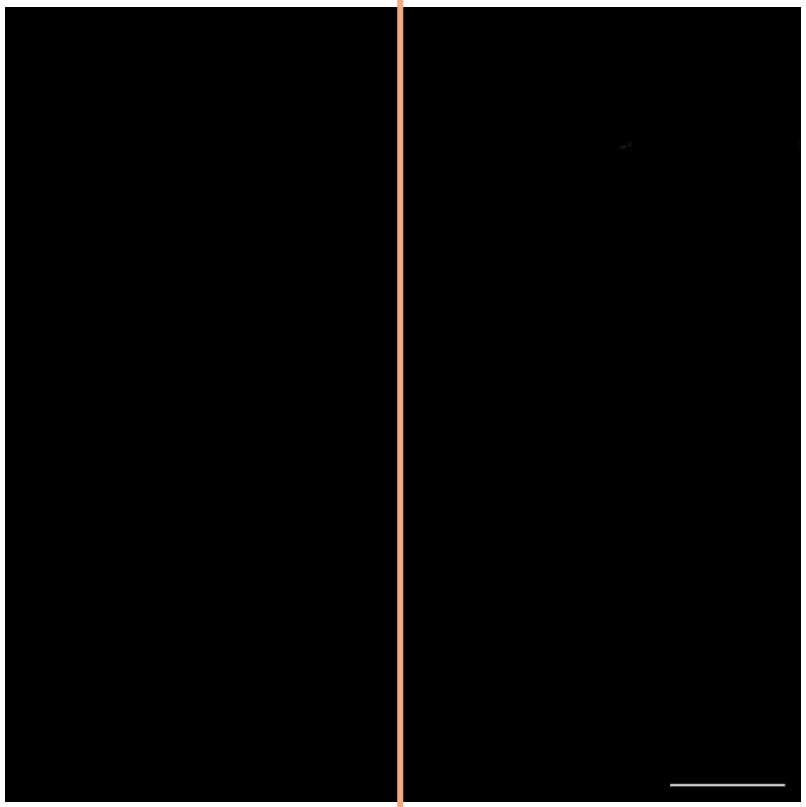
Tissue slice imaging – oblique scanning



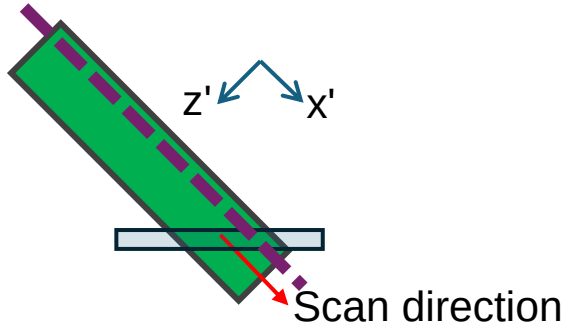


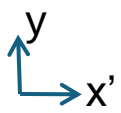


Scale bar = 500 µm



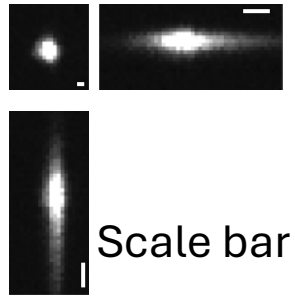
Tissue slice imaging – oblique scanning





Scale bar = 500 µm
FOV: 9000 x 3000 x 300 µm

Tissue slice imaging – oblique scanning


Scale bar = 5 μm

Computational time for shearing

- Lateral shift algorithm in MATLAB
- **Data size: 11 GB**
  3200x3200x500 pixels
  Time: 312 ± 79 s

Spatial resolution in sheared images

	xy FWHM, μm	z FWHM, μm
3D z-scan	**5.07 ± 0.76**	**6.51 ± 0.46 μm**
Sheared slice scan	**7.22 ± 0.43**	**6.27 ± 1.08 μm**

Mechanically Sheared Axially Swept Light-Sheet Microscopy

JINLONG LIN[1,2], DUSHYANT MEHRA[1,2], ZACH MARIN[1,2], XIAODING WANG[1,2], HAZEL M. BORGES[1,2], QIONGHUA SHEN[1,2], SEWERYN GAŁECKI[1,2,3], JOHN HAUG[1,2], AND KEVIN M. DEAN[1,2,*].

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[2]*Cecil H. and Ida Green Center for Systems Biology, UT Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390.UT Southwestern Medical Center, Dallas, TX 75390, USA.*
[3]*Department of Systems Biology and Engineering, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland.*
**kevin.dean@utsouthwestern.edu*

Lin *et al*, Bioarxiv, 2024

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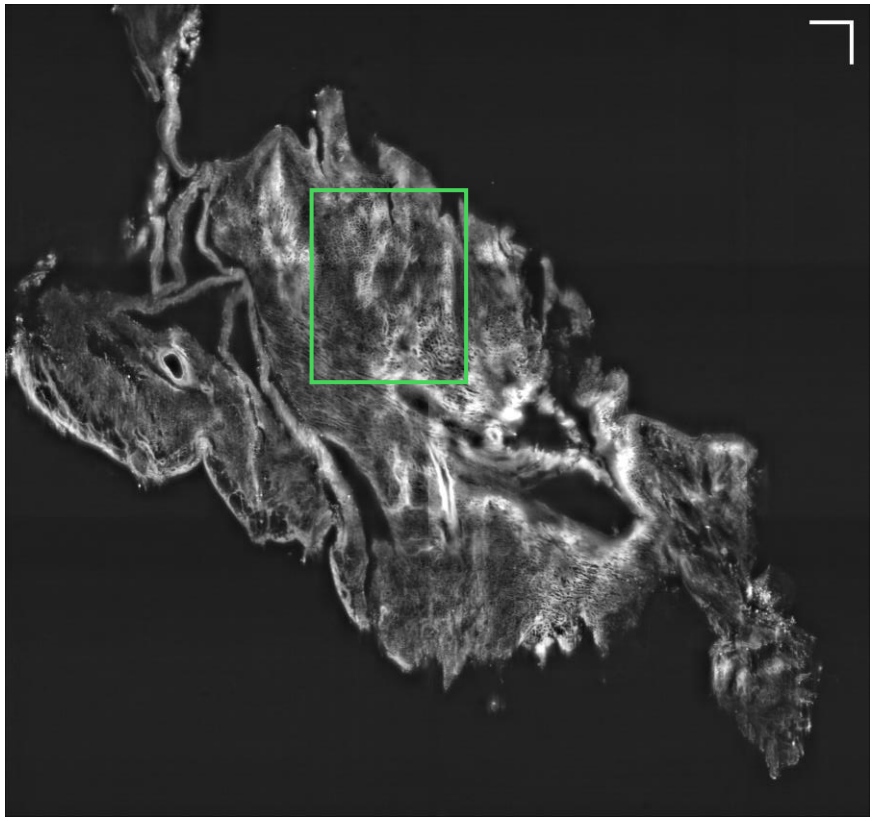
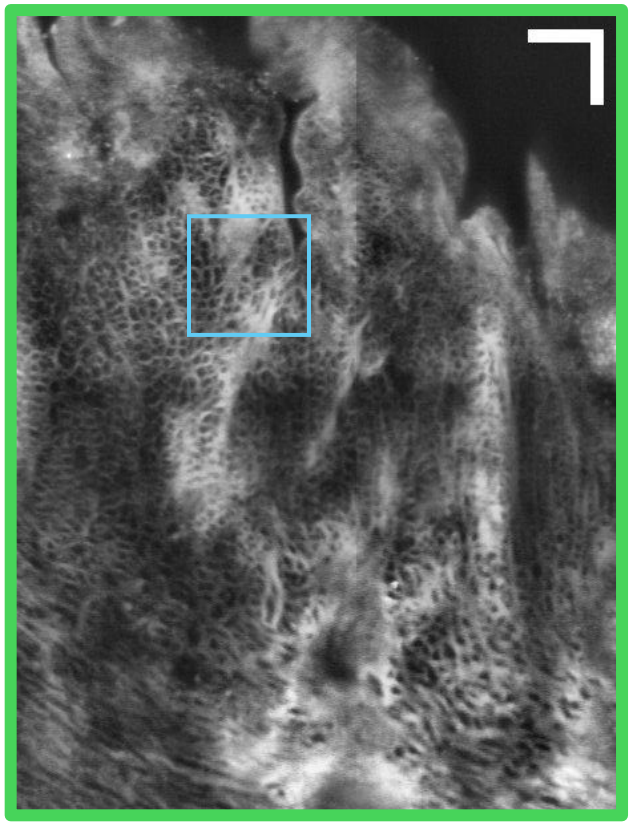
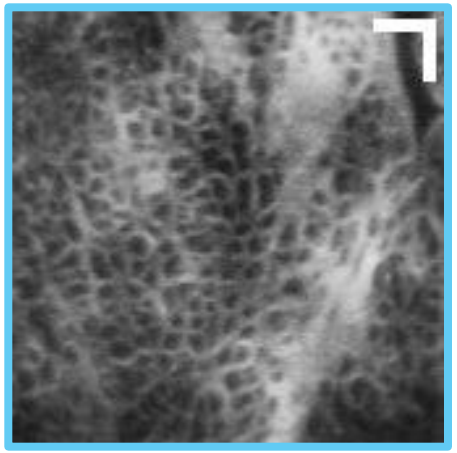
Cardiac Structural Imaging



Scale bar = 500 µm



Scale bar = 250 µm



Scale bar = 25 µm

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Conclusion

Advantages of oblique scanning:

- Reduced computational overhead
- Reduced tiling
- Maximum lateral extent (x') of tissue is determined by the working distance of objective
- No extra hardware or optics required for implementation

Acknowledgements

University of Glasgow

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Dr. Eline Huethorst
Dr. Erin Boland

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Dr. Leonardo Sacconi

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Dr. Camilla Olianti

British Heart Foundation

Funding a healthier future
MEDICAL RESEARCH SCOTLAND

SUPA

RMS

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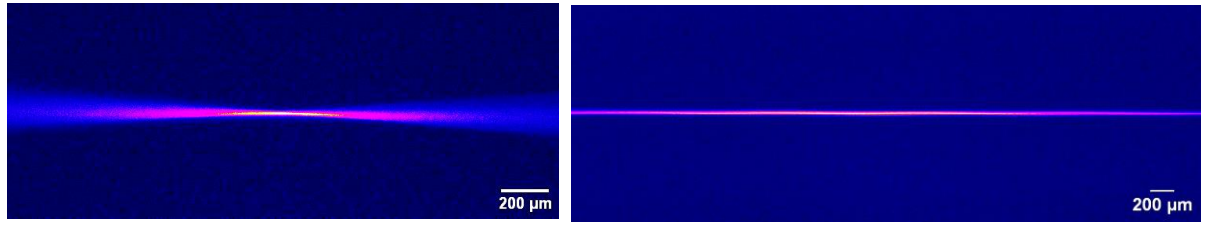
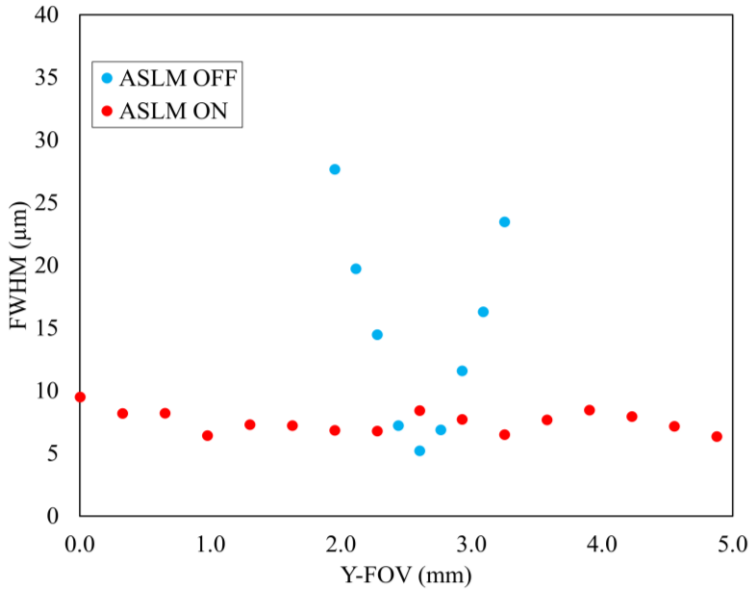
MesoSPIM characterisation

Field of View

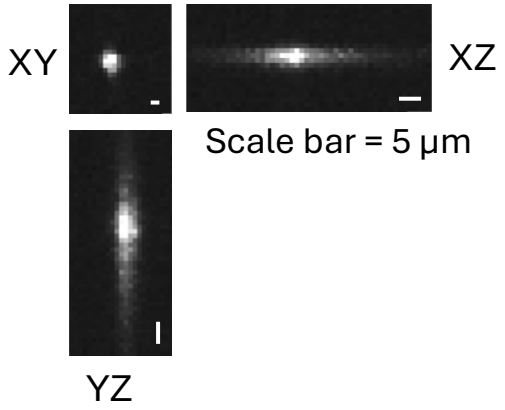
ASLM Off

ASLM On



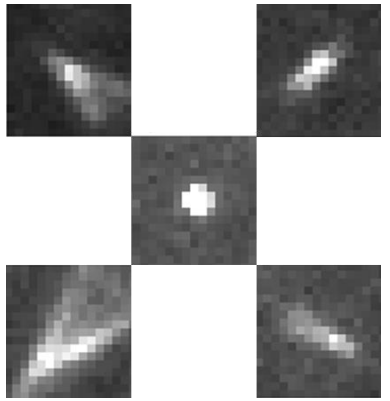


Resolution



Scale bar = 5 µm

Lateral resolution: **5.07 ± 0.76 µm**
Axial resolution: **6.51 ± 0.46 µm**
Mean of 70 beads across FOV



At 2x
10.4 mm FOV

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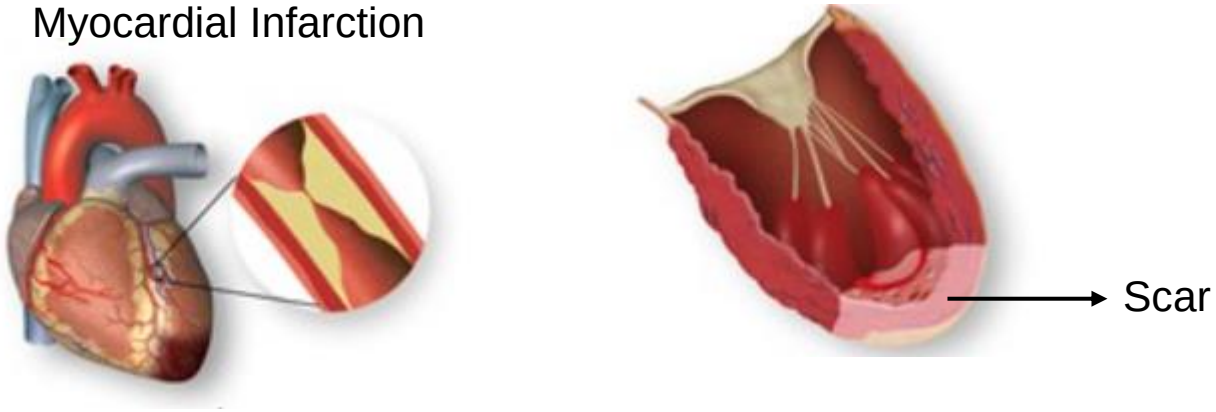
Cardiac tissue – sample preparation

New Zealand white rabbit Left Ventricle → Vibratome 500 µm – 3 mm → CLARITY → Fluorescence staining

Myocardial Infarction



Riddell *et al*, Cardiovascular research, 2020

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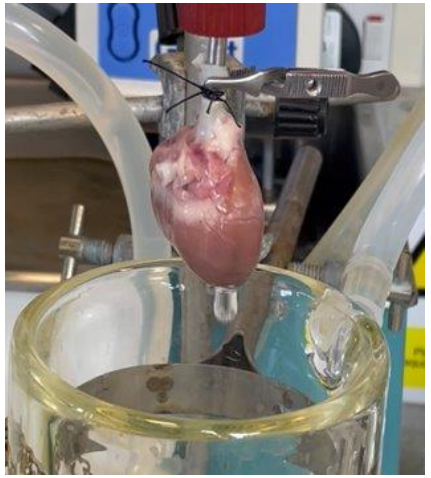

- Langendorff perfusion
- Tissue fixation in PFA
- Embedded in agar

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