Quantitative analysis of mounting-induced aberrations when imaging cleared tissue slices

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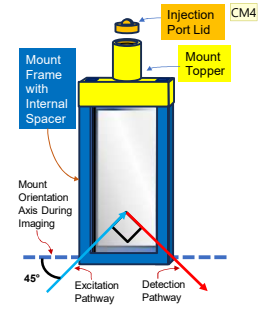
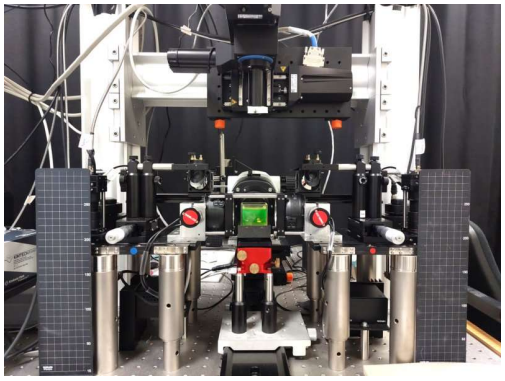
Introduction

- light sheet microscopy (LSM) of cleared tissues [1] is ideally suited to provide cellular resolution over cm-sized fields of view (FOV)
- we apply LSM to observe structural remodelling post myocardial infarction in rabbit hearts
- samples are vibratome sliced hundred microns and subsequently cleared while mounted to maintain structural integrity
- we have observed aberrations introduced by stress placed on the glass
- here we seek to devise a mount that minimised aberrations while maintaining structural integrity of our samples

CM1 CM2



CM4



Mount Design

- sample mounting in RI-matched glass slide sandwiches held in 3D printed frames
- frames act as spacers matched to sample thickness cut in vibratome; this avoids structural warping
- mounts made watertight through epoxy (xy manufacturer)
- 3 mount variation were tested to minimise shear stress on glass
- sample consisted of XYZ beads (manufactuer) mounted in agarose (xy%) and perfused with XY RI matching solution for xy hours

CM3

B1 B2 B4

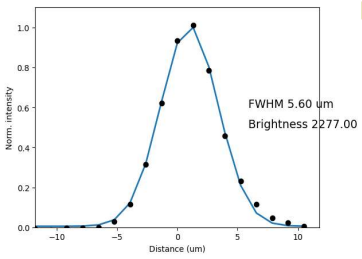
Bead Analysis Code

- modified python script based on the psf python library [2] source code
- script takes in lateral and axial FOVs and pixel sizes along with a *.tiff* image file which gets read in as a numpy array
- skimage local maxima detection finds bead centres while removing any centres that are located within 3µm of each other
- gaussian fitting over each bead centre outputs amplitues and full-width-at-half-maximum (FWHM)
- Results compared with PSFj [3] analysis as control

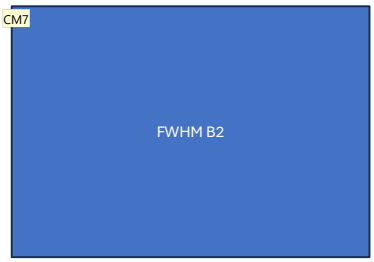
Results

CM9

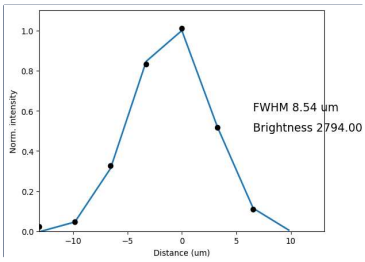
- Given the output full width half maxima of each of the three mounts (below), it was evident that the "Big Window" mount B4 resulted in the highest resolution.
- A PSFj [3] analysis of the samples confirmed this.



CM7



Inner Cuvette (5.6um)



B3 (8.54um)

References

CM5

[1] Voigt *et al. Nat Methods* **16**, 1105–1108 (2019)
[2] Gohlke, C. Point Spread Function calculations for fluorescence microscopy
https://github.com/cgohlke/psf (2024)
[3] Theer, P., Mongis, C. & Knop, M. PSFj: know your fluorescence microscope. *Nat Methods* **11**, 981–982 (2014).
https://doi.org/10.1038/nmeth.3102

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CM6

Slide 1

CM1 Follow narrative from abstract. At no point, was the first bullet point you wrote the first and most important sentence in our abstract.
Caroline Muellenbroich, 2024-11-22T14:25:01.027

CM1 0 Don’t use full sentences, they are bullet points for a reason. Be stingy about every single word typed on your poster and try and minimise them
Caroline Muellenbroich, 2024-11-22T14:25:44.187

CM1 1 Keep editing clean within microsoft! Adjust line spacing rather than returning emtpy lines
Caroline Muellenbroich, 2024-11-22T14:26:20.467

CM2 This is an empty word “introduction”. It is void of meaning and ubiquous on all posters all over the world. Can you replace it with something more meaning ful like you have done for the other headings?
Caroline Muellenbroich, 2024-11-22T14:27:46.338

CM3 Crop more tightly. Add cad model schematic of mounts from Steven’s poster
Caroline Muellenbroich, 2024-11-22T14:30:09.378

CM4 Ask Steven, he might have a better one. One that blows up and shows spacing inbetween
Caroline Muellenbroich, 2024-11-22T14:49:45.821

CM5 Just first author, journal volume year
Caroline Muellenbroich, 2024-11-22T15:04:24.706

CM6 Use new logo.. UKRI EPSRC
Caroline Muellenbroich, 2024-11-22T15:05:07.127

CM7 Use data points for data and reserve lines for fits
Caroline Muellenbroich, 2024-11-22T15:06:29.365

CM8 This sentences is a conclusion not a result infromation
Caroline Muellenbroich, 2024-11-22T15:08:16.173

CM8 0 How many beads did you fit for each window?
Caroline Muellenbroich, 2024-11-22T15:08:33.646

CM8 1 Do you have qualittaive images of the PSFs?
Caroline Muellenbroich, 2024-11-22T15:08:44.933

Slide 1 (Continued)

CM9 Where is that data?

Caroline Muellenbroich,
2024-11-22T15:09:00.293