Bespoke Microscopes

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Overview

- Image formation
- Beads and spherical aberration
- Bespoke microscopes in micron
- Bespoke microscope example - DeepSIM

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What is a microscope image

- The microscope produces a magnified, but also distorted, image
- Record the light intensity on a camera.

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Microscopic imaging in mathematical terms.

- Take your sample
- Multiple it at every point by the imaging process in the microscope (convolve the PSF with the object).
- Produce the image.

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The most important things to think about.

Contrast :- What is the difference between what you want to see and everything else?

Resolution :- How small things can you see?

Nothing else

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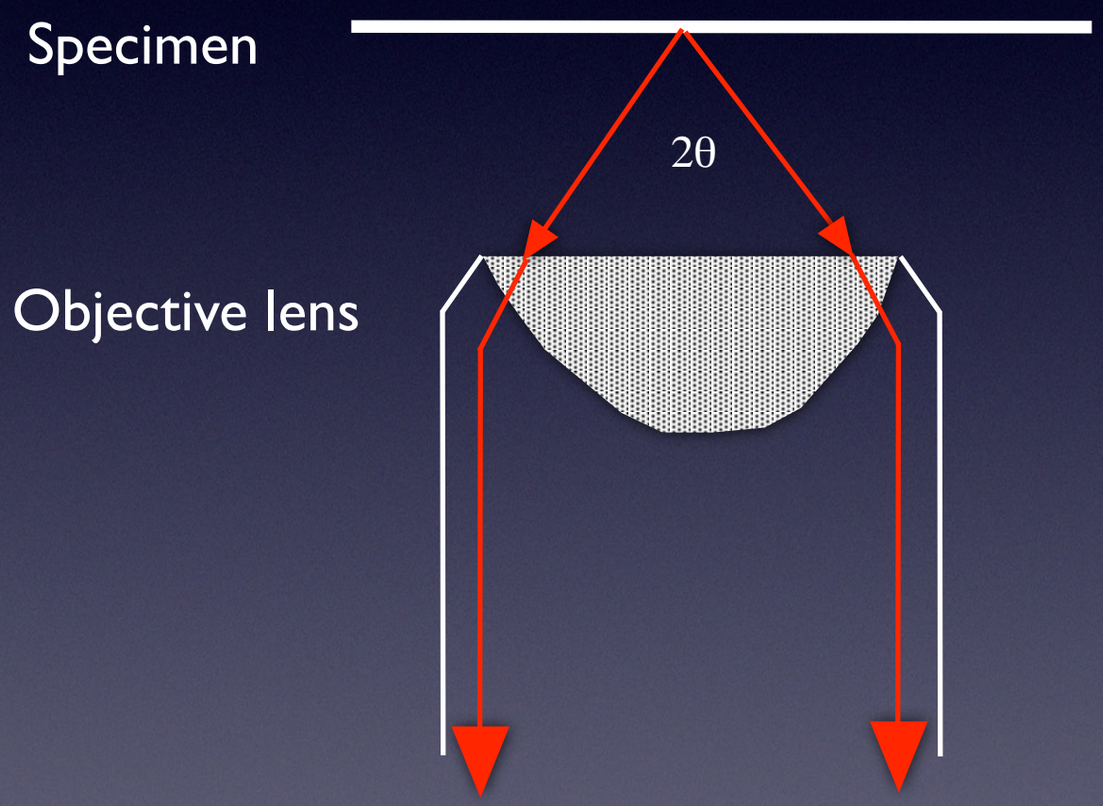
Microscope Resolution

- No lens has perfect resolution, even in theory
- Resolution depends on the <u>angle</u> (θ) of the cone of light that the objective can collect from the specimen.
- Rule of thumb: Resolution limit ~ $\lambda/2$



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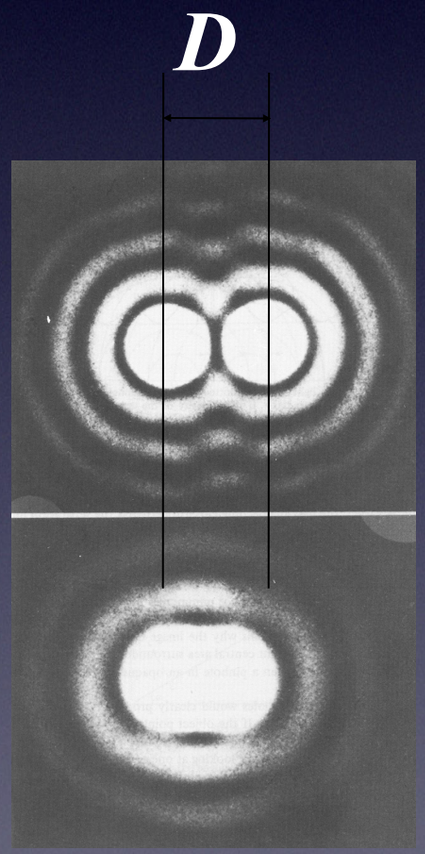
Resolution: A technical definition, the Rayleigh Criterion



D, the distance of two closest points that can be distinguished

$$D=1.22\ \lambda/(NA_{obj}+NA_{cond})$$

Epi-Fluorescence: $NA_{cond} = Na_{obj}$

so $D=1.22\lambda/2NA$

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The Point Spread Function - PSF

- The image of an infinitely small point.
- Limited by resolution
- 3D structure also very important.

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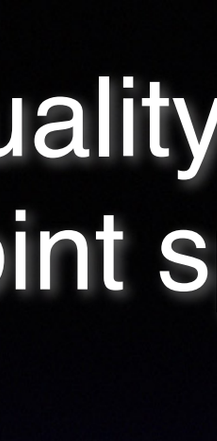
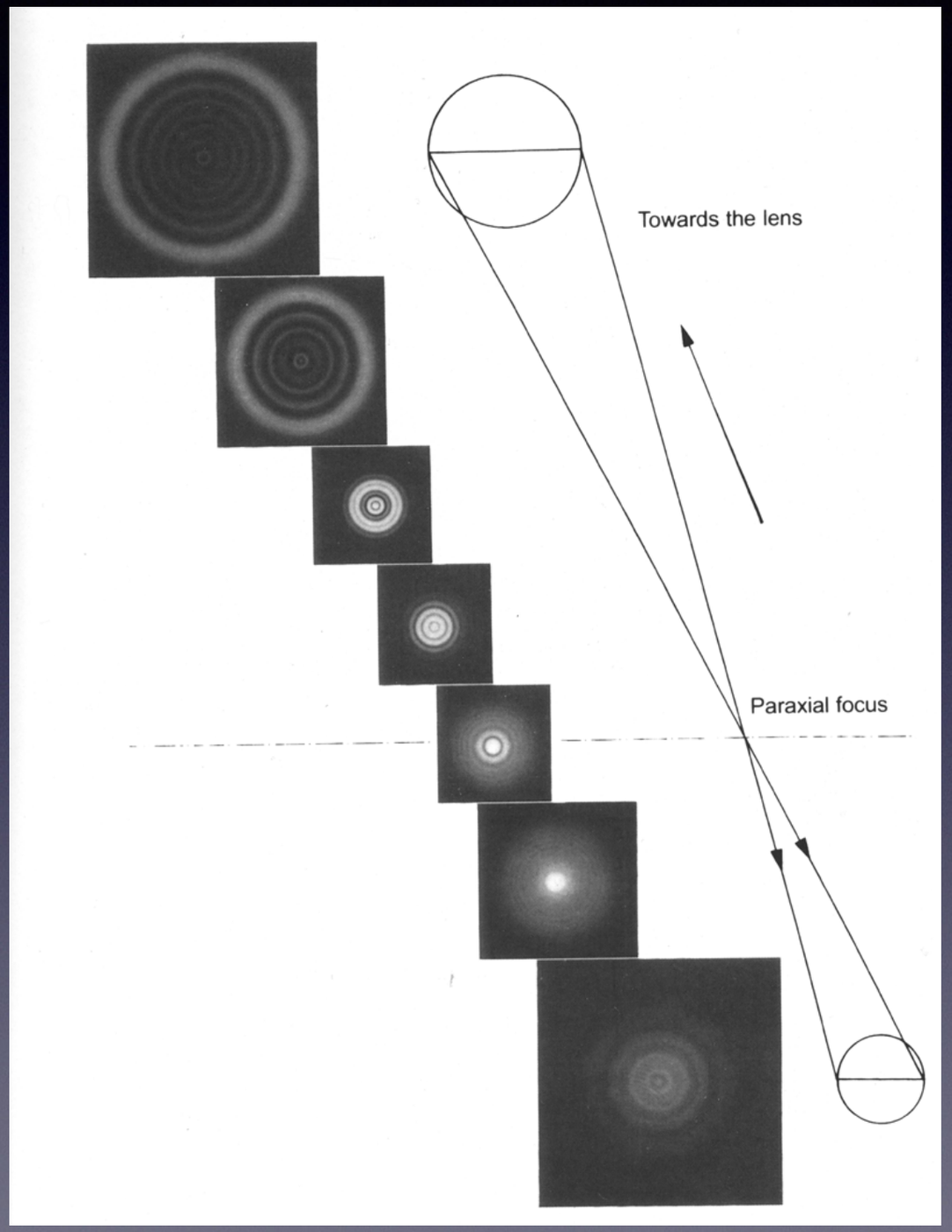
Image quality- the problem of "out-of-focus light" point spread function and airy rings

Sample object: a "sub-resolution" fluorescent bead





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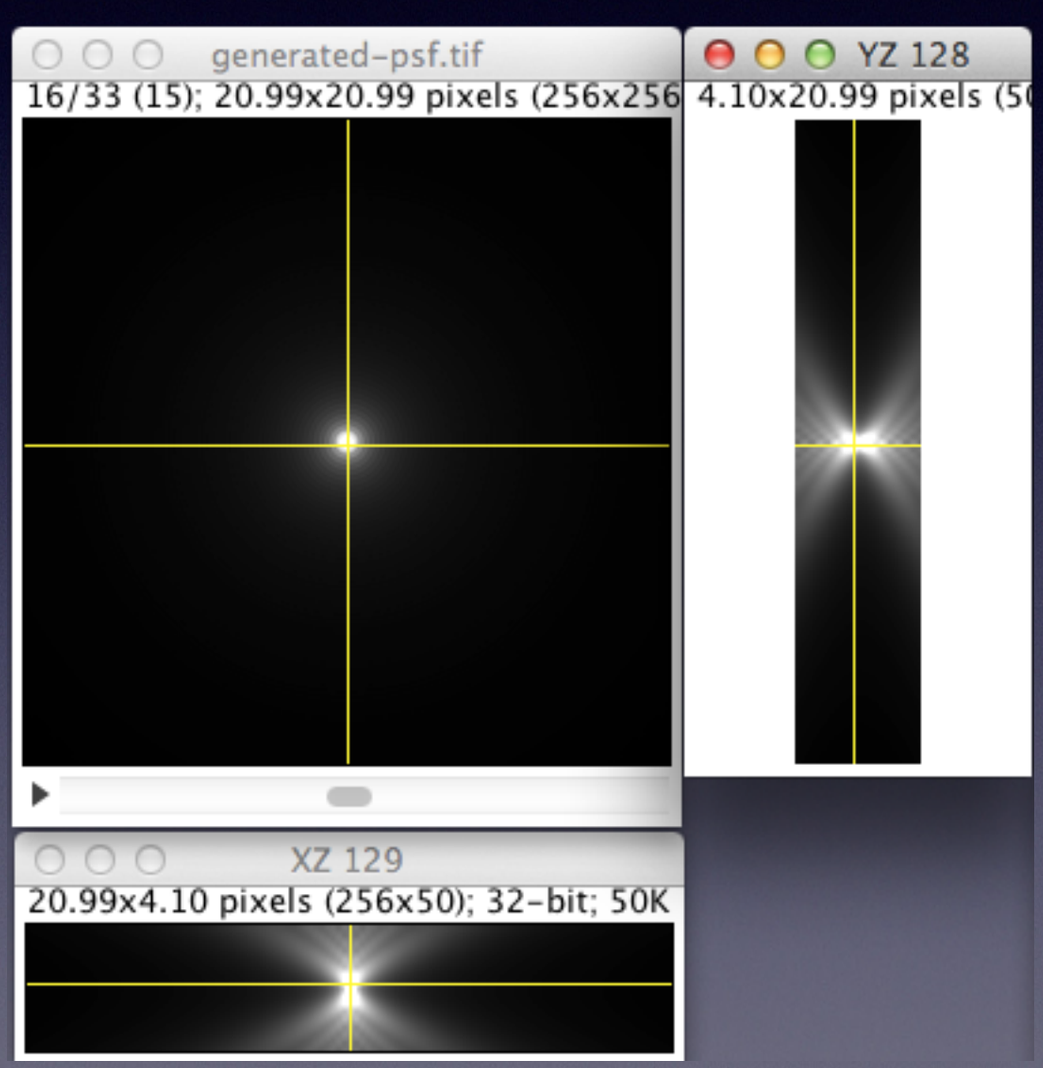
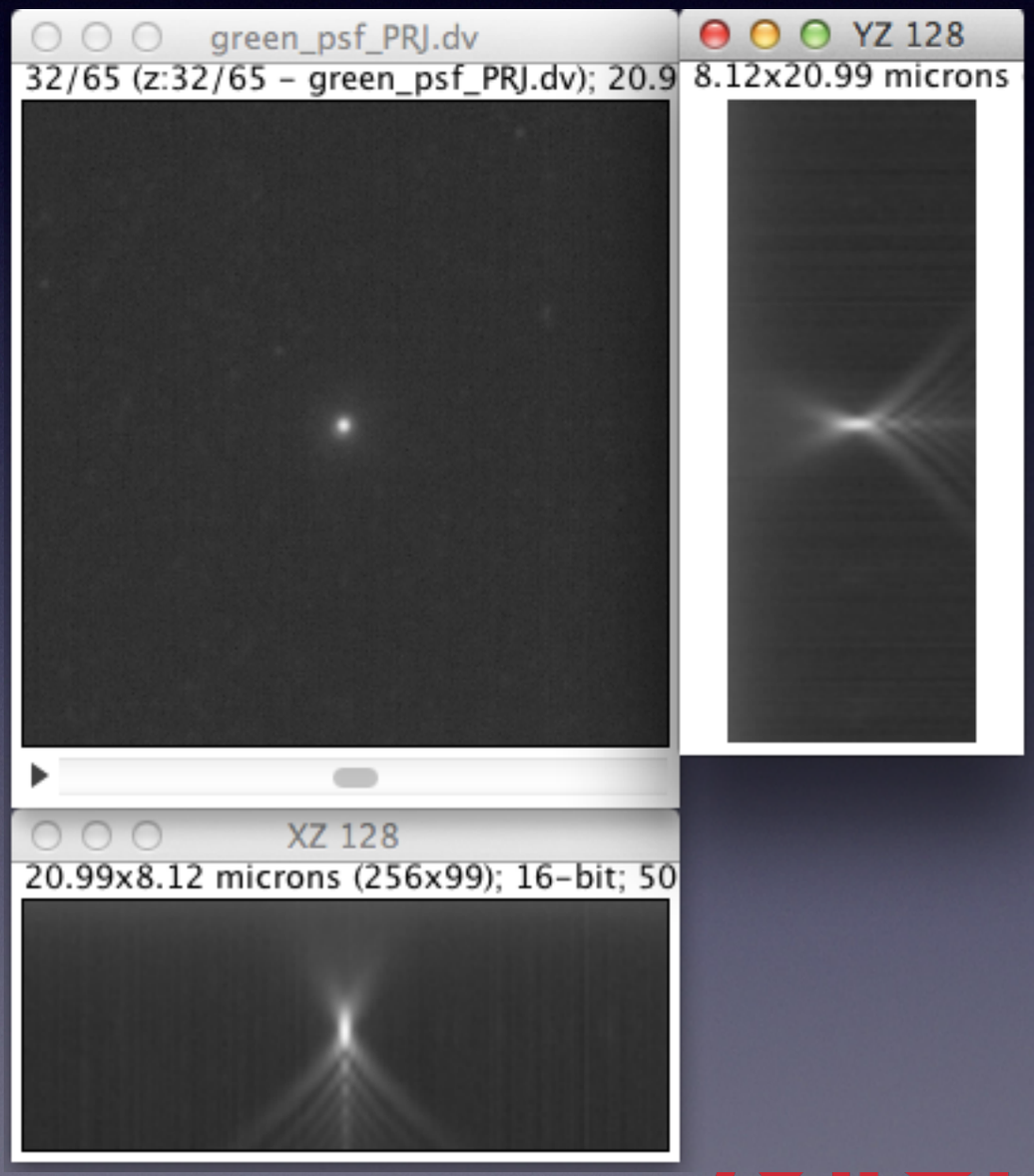
Theoretical and measured PSF Orthogonal views



Generated PSF



Real PSF

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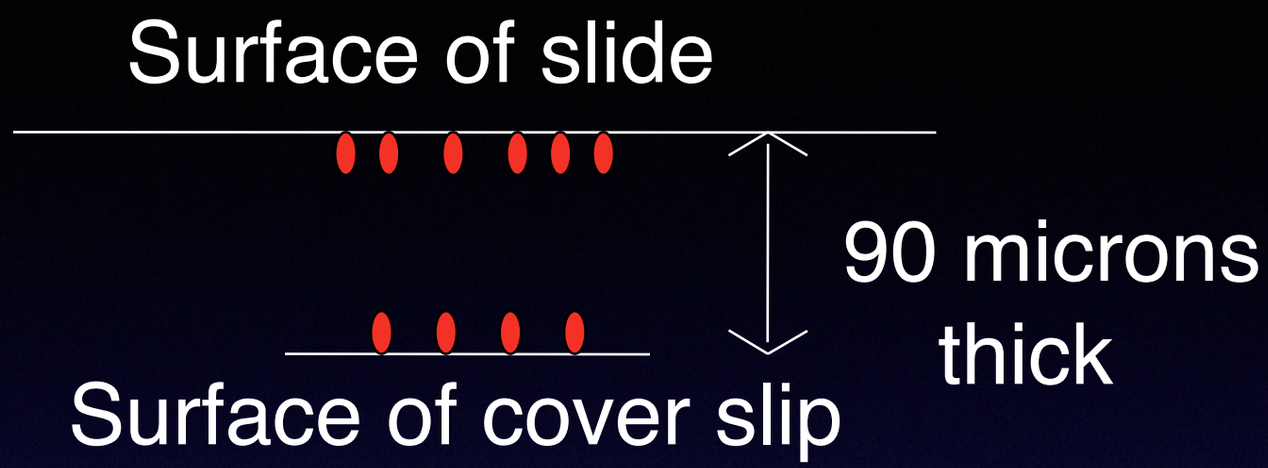
Bead slide



Tetraspeck beads: chromatic registration
DAPI/FITC/Rhodamine/Cy5

Beads (PS Spec): Single fluorochrome
Brighter -better for generating
point spread functions for deconvolution

Inspec Intensity beads: Measure dynamic range

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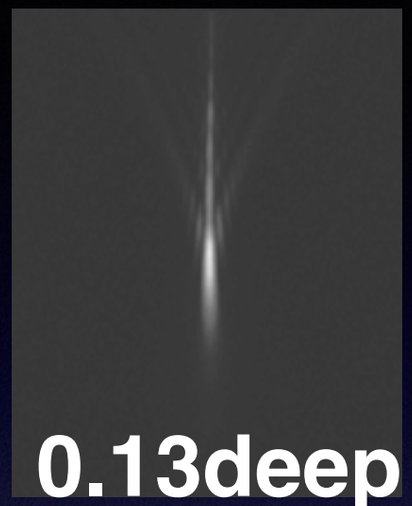
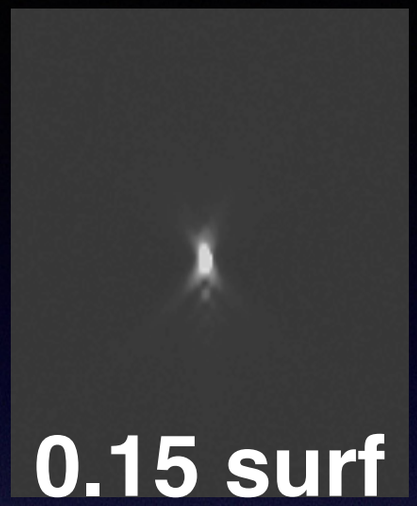
Affects of deep imaging (90μm) and collar settings on spherical aberration and psf of 60X/NA1.2w

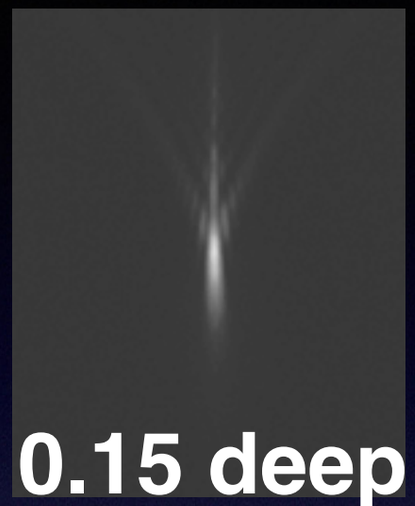
0.13 surf



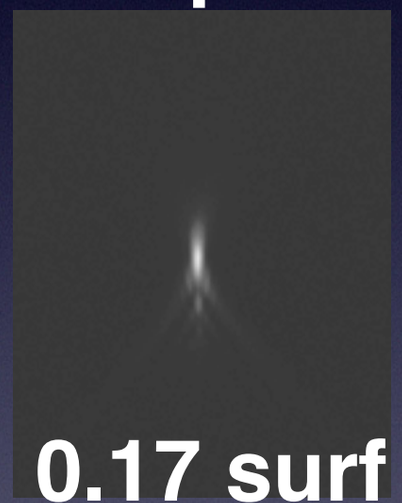
0.13deep



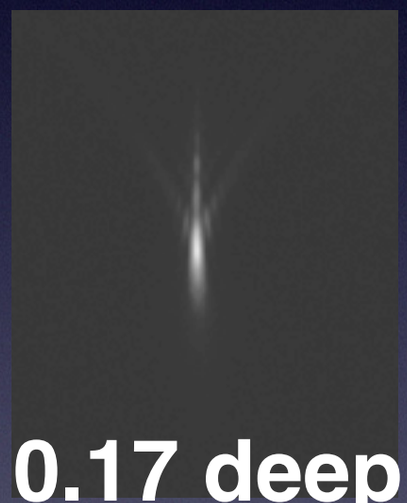
0.15 surf



0.15 deep



0.17 surf

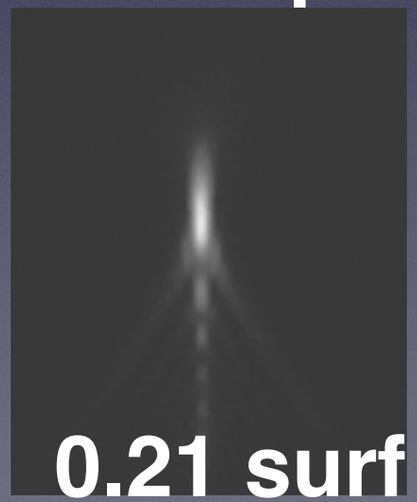


0.17 deep

Data from
Alejandra Clark

0.19 surf

0.19 deep



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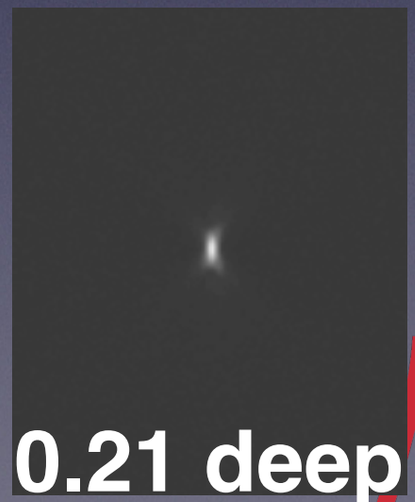
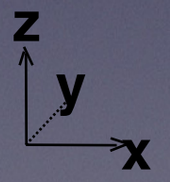


0.21 deep



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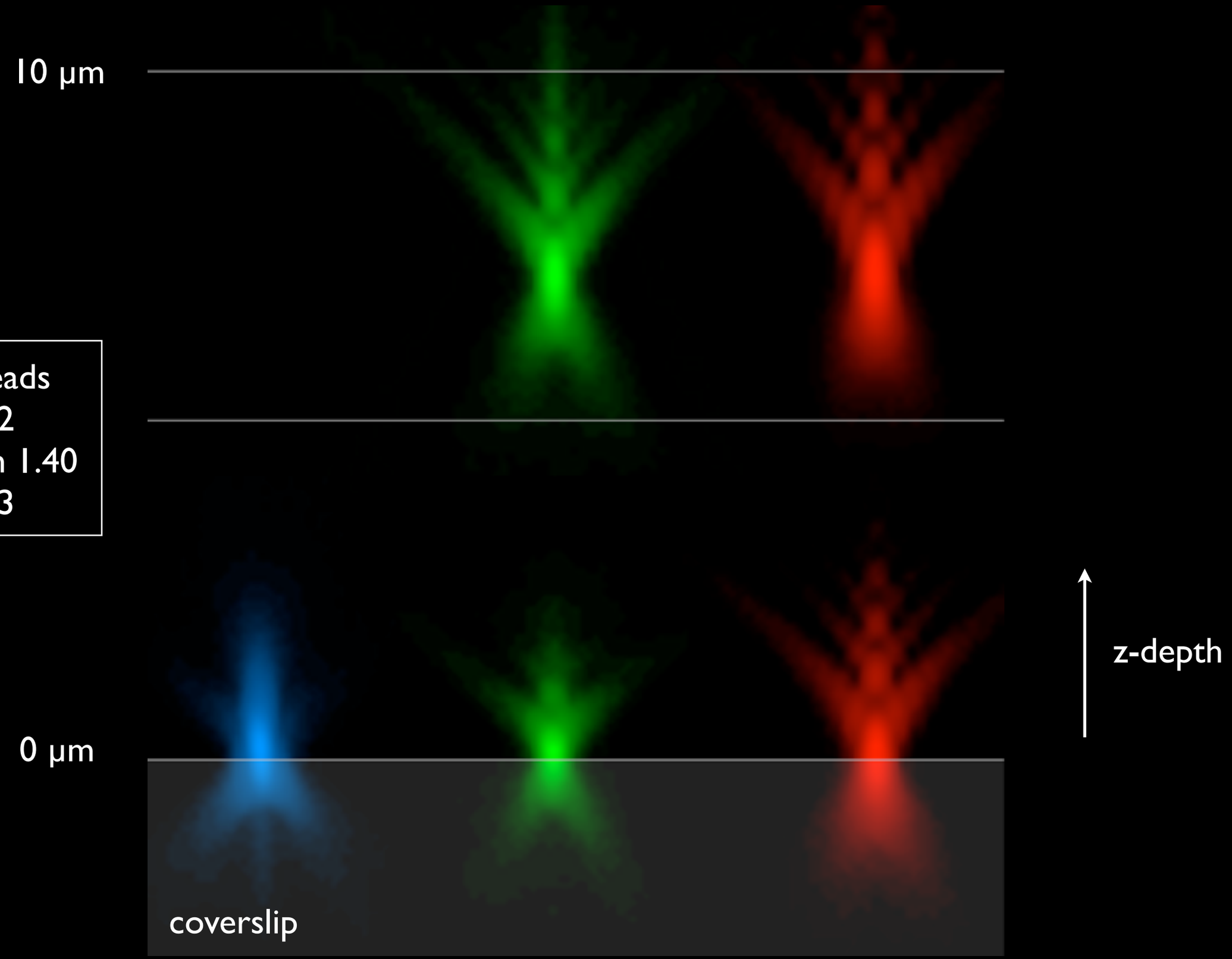
Spherical aberration dependent on wavelength, depth, RI



Bespoke systems in Micron

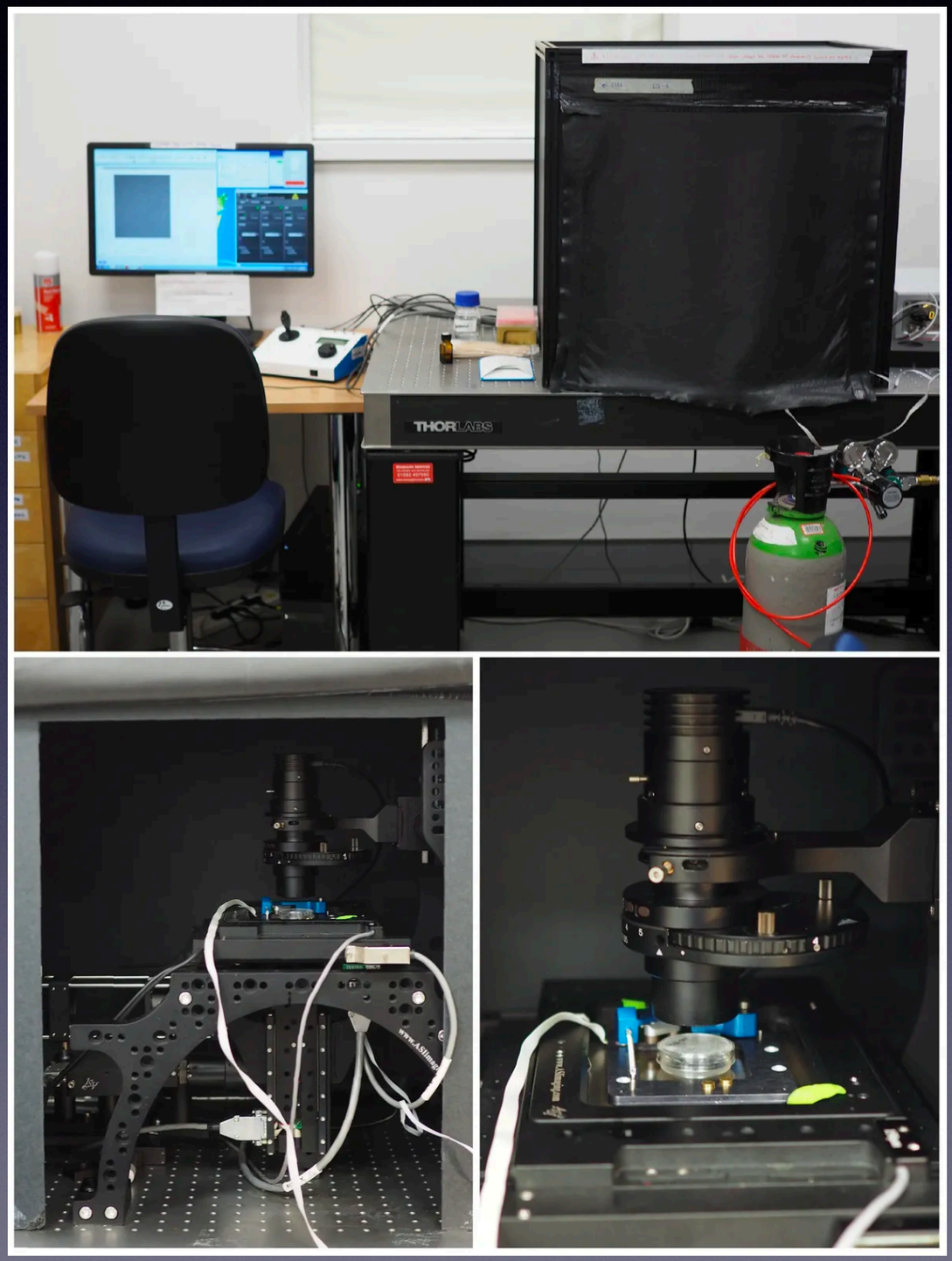
User systems

- Palm/TIRF system - now within facility
- CryoSIM (at Diamond) - A user available facility at Beamline 24 for correlative imaging.

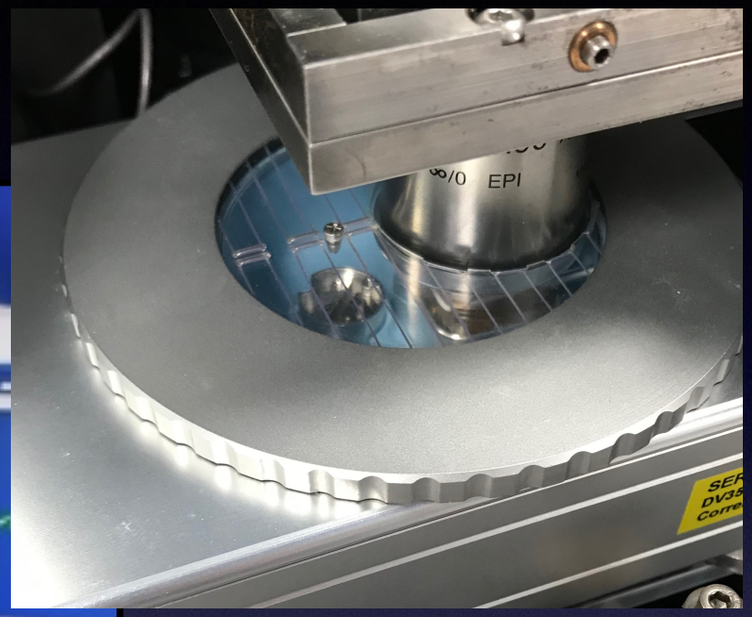
Systems in development

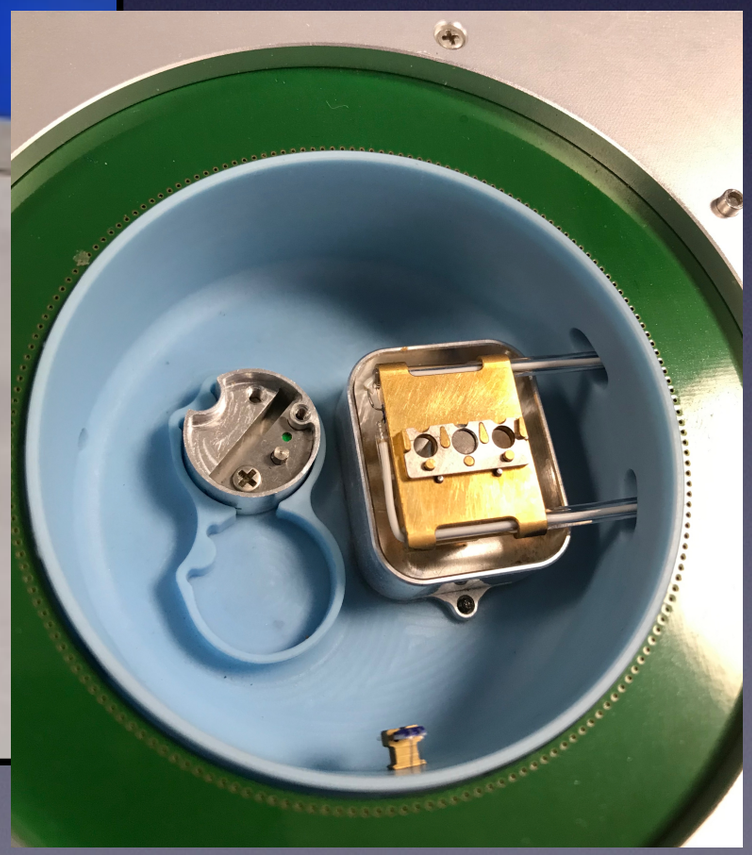
- DeepSIM - upright SIM with AO and remote focus
- 4PI - super high resolution imaging
- CryoSIM II - add AO to CryoSIM setup
- Aurox Clarity AO system - add AO to a novel fast confocal system

Palm/TIRF

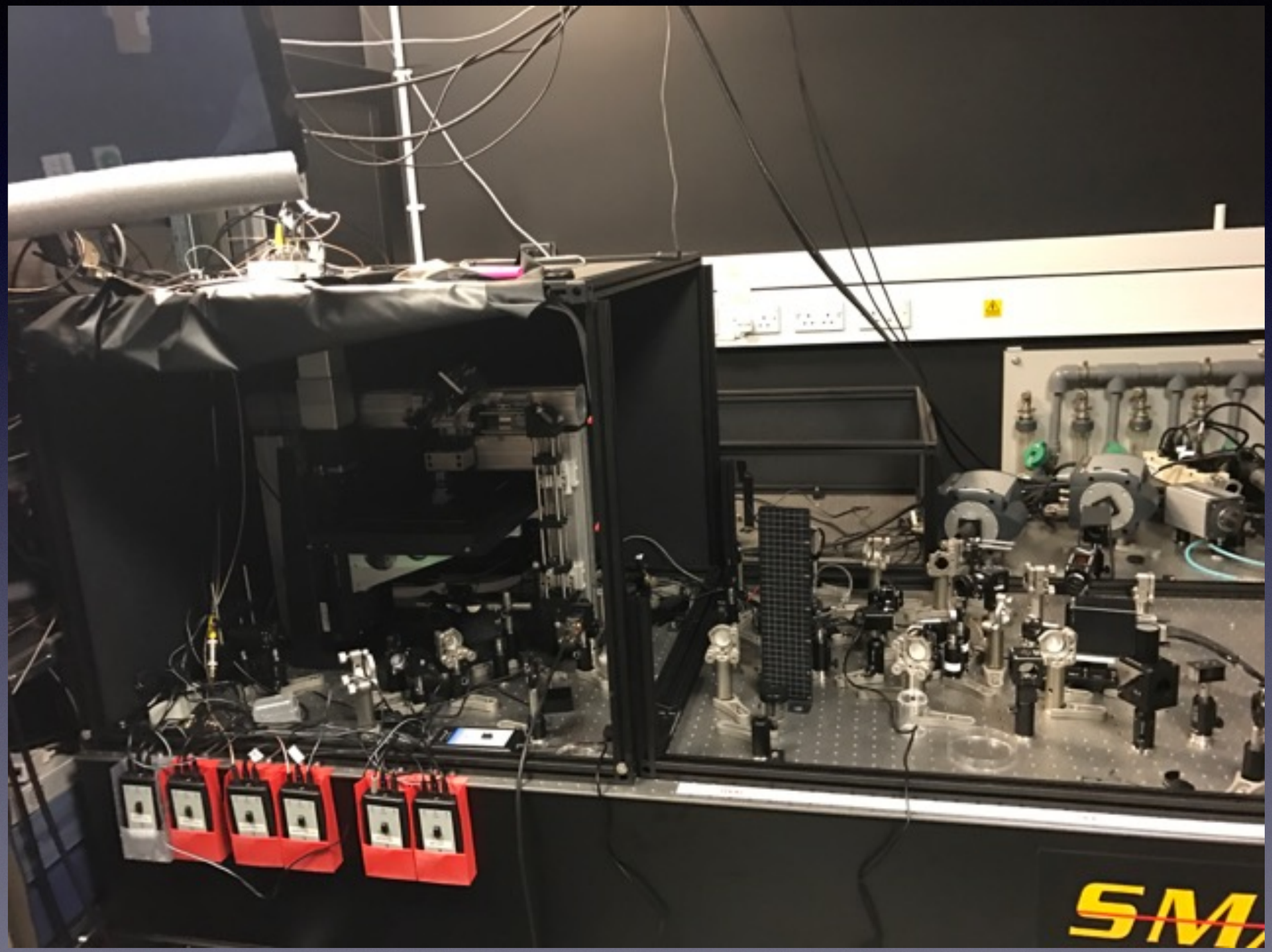


CryoSIM

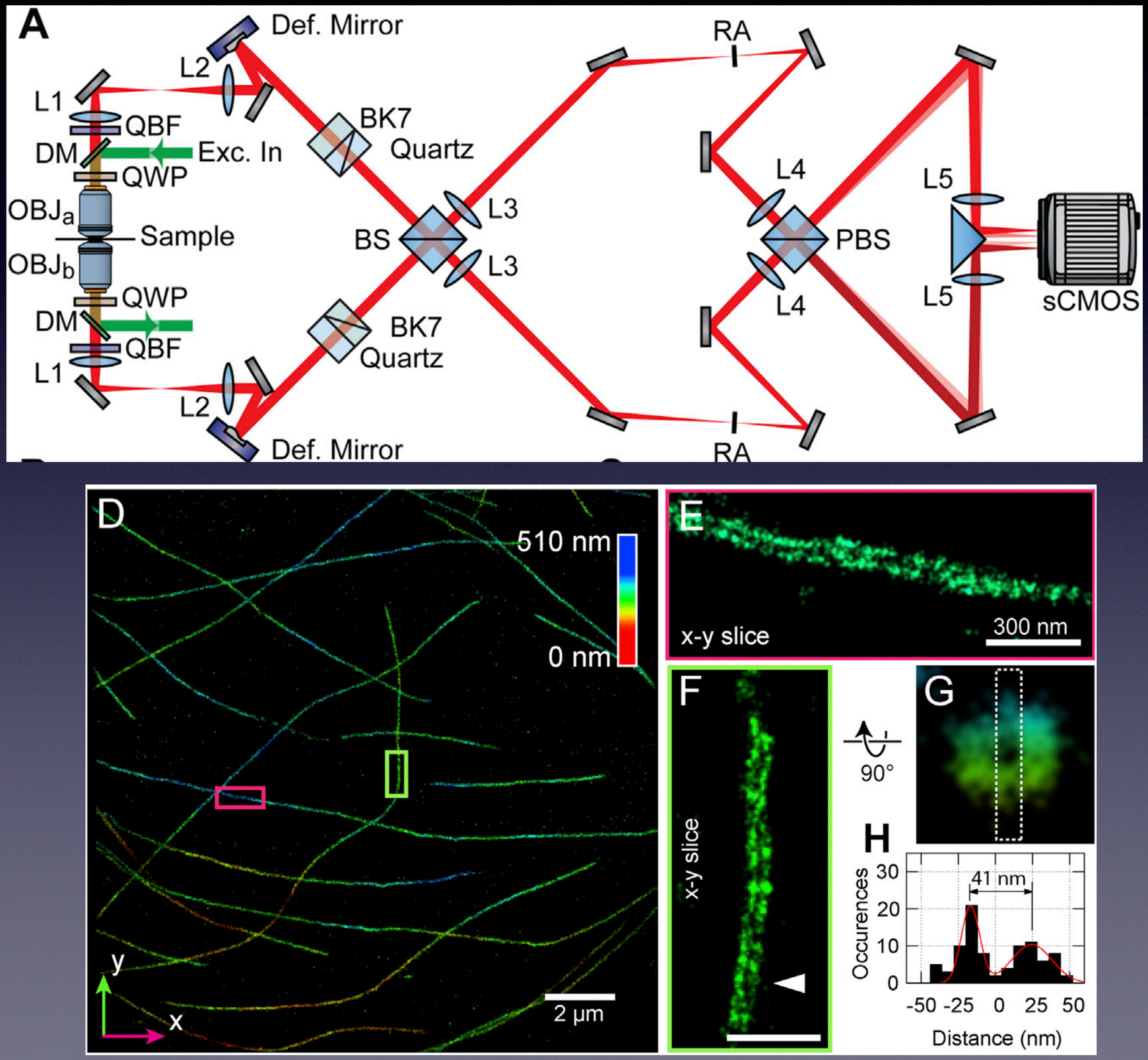




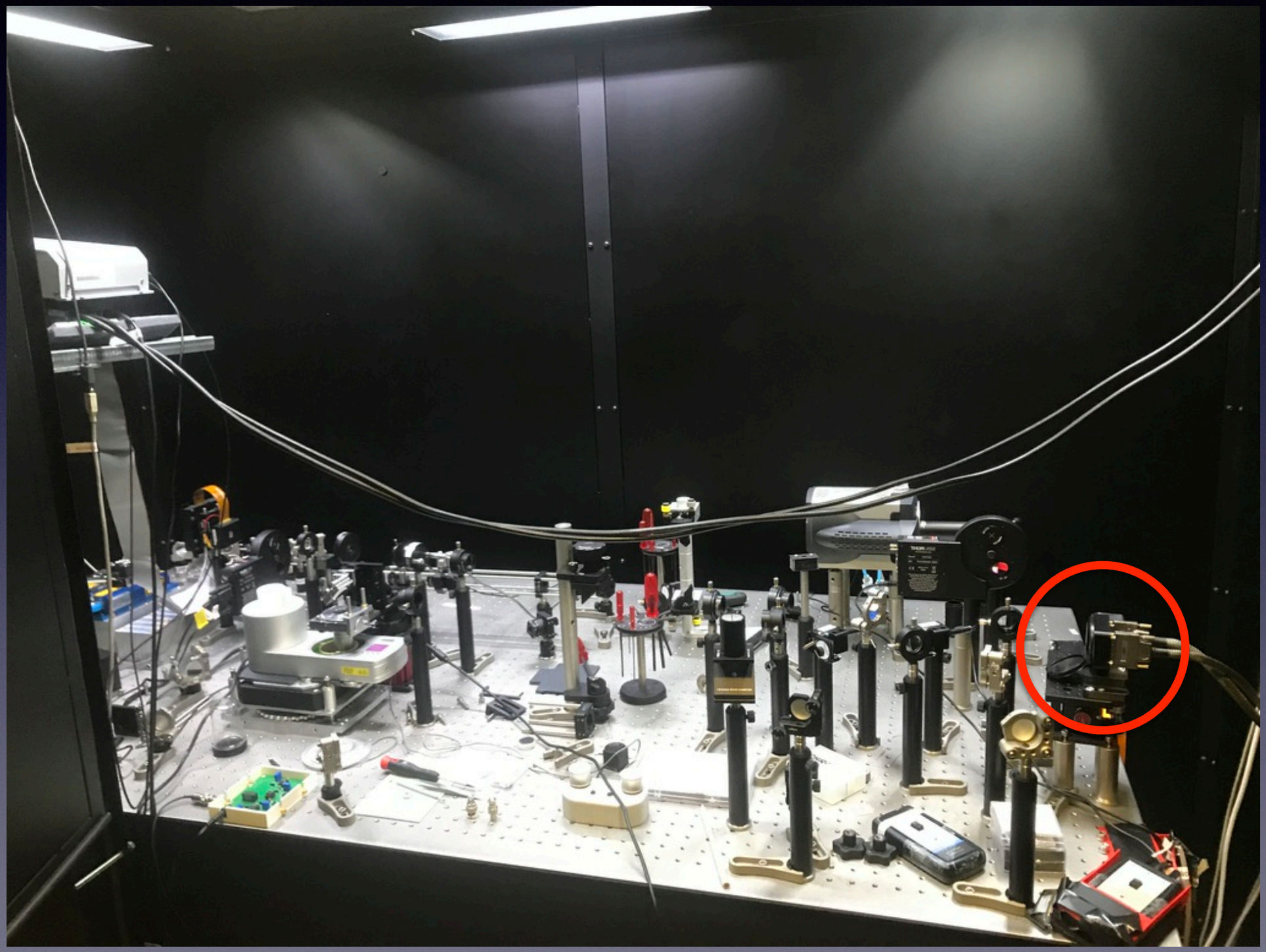
DeepSIM



4Pi microscope



CryoSIM II



Aurox Clarity AO

Justification for Bespoke Systems

- Often necessary for specific specialised problems.
- Easily optimised for several parameters, speed, sensitivity etc...
- Can provide extremely flexible systems

BUT think hard as it is likely to be harder, longer and more expensive than at first thought.

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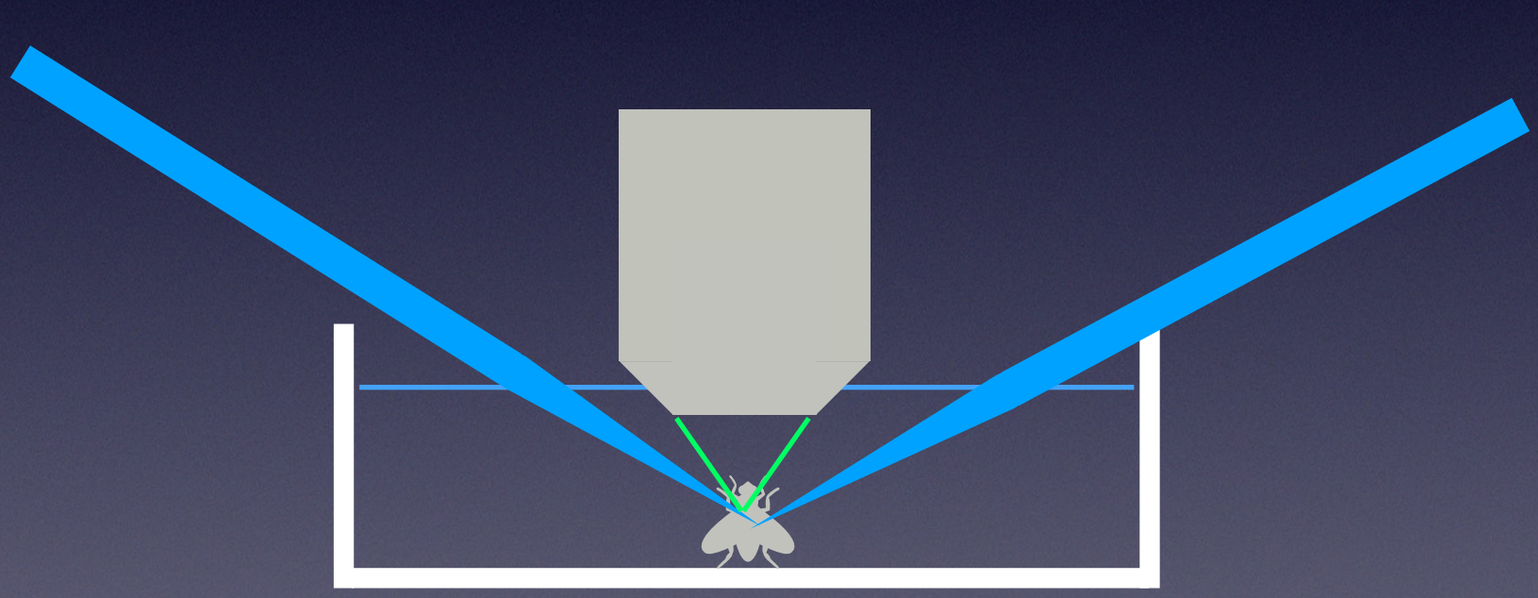
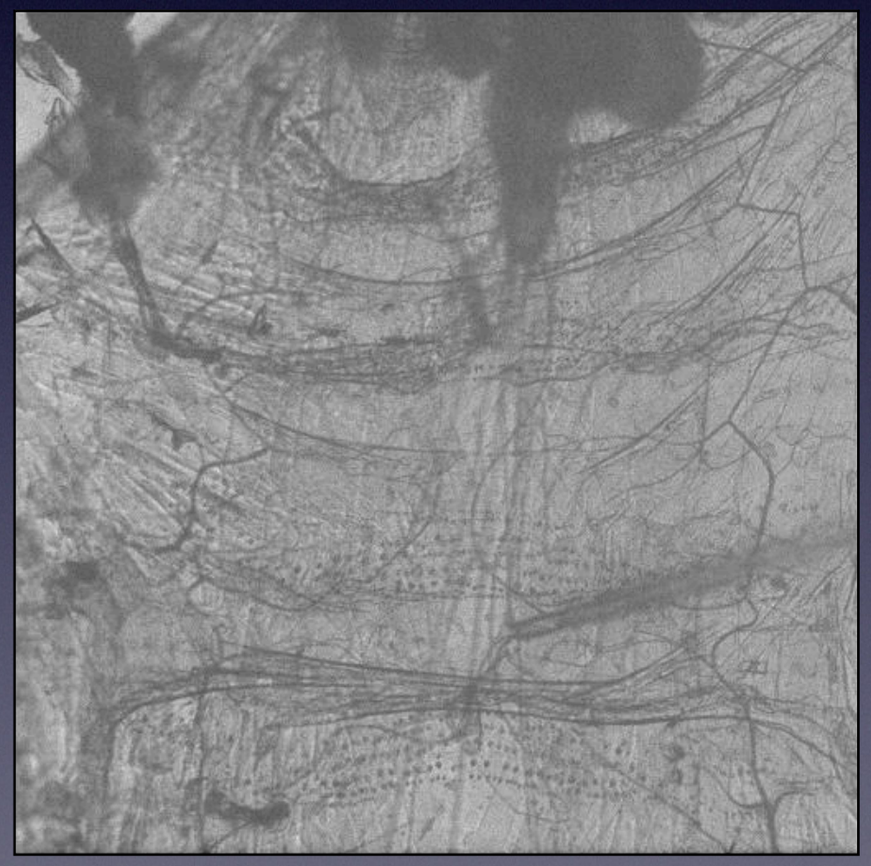
Bespoke Microscope Example - DeepSIM

- Live fluorescence imaging
- Simultaneous electro-physiology
- Rapid Z stacks, with minimal sample disruption
- Deeper imaging utilising Adaptive Optics (AO)

Live imaging

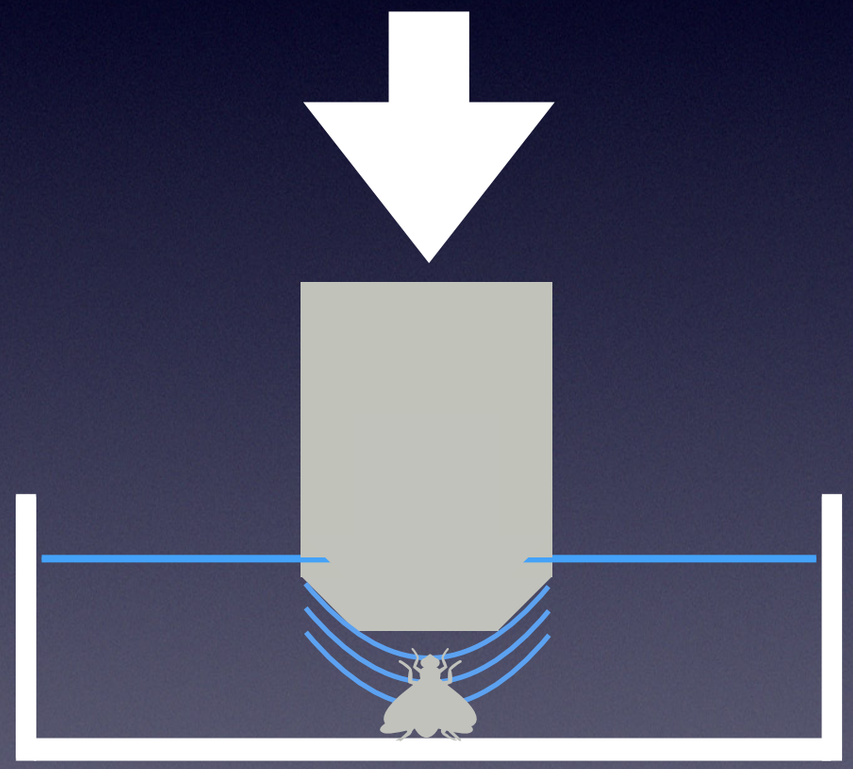


Upright microscope

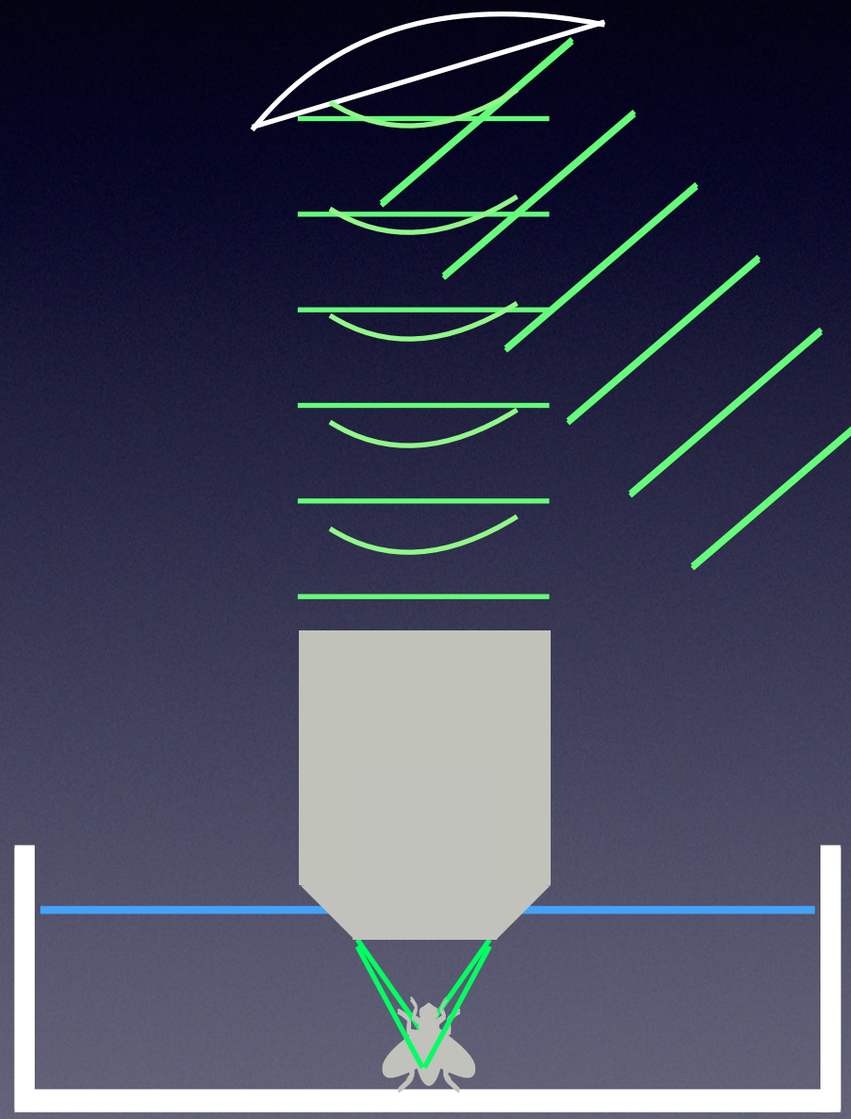


Fast imaging

Fast Z movement issues



AO - Remote Focus



AO - Aberration correction

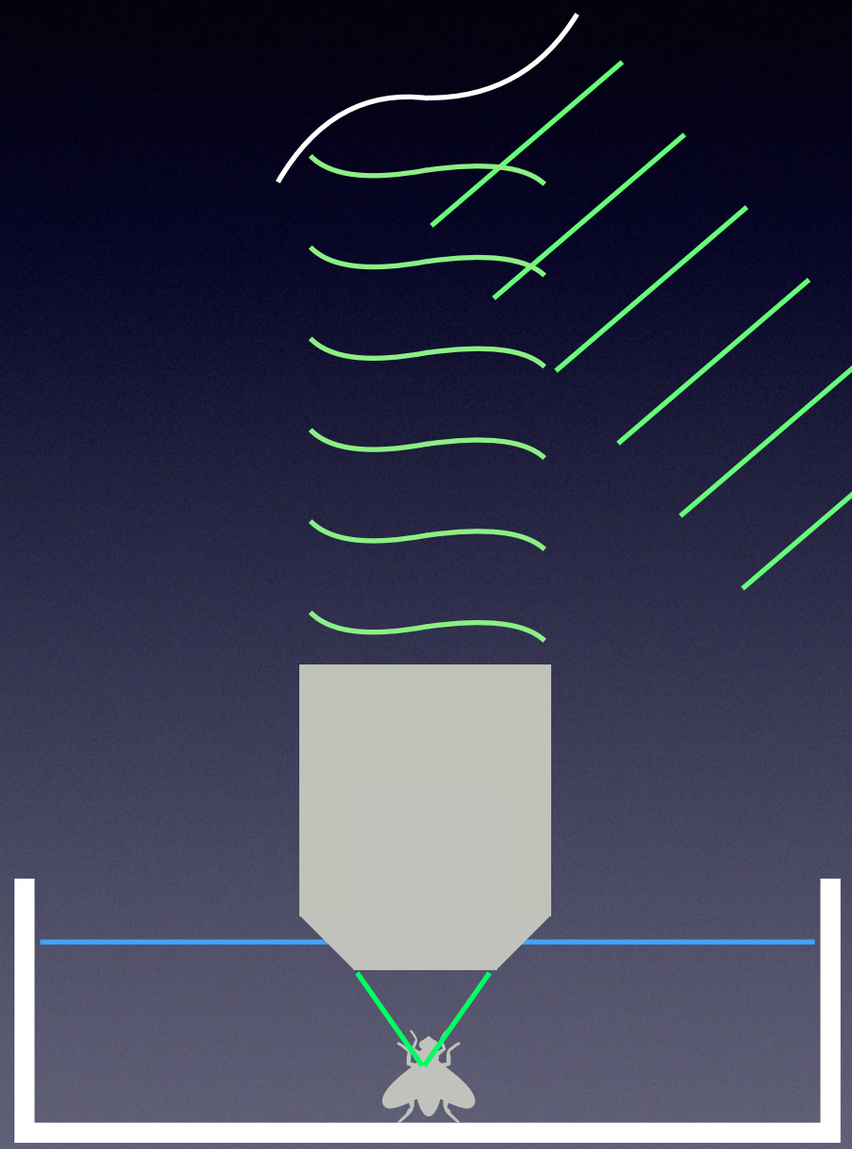
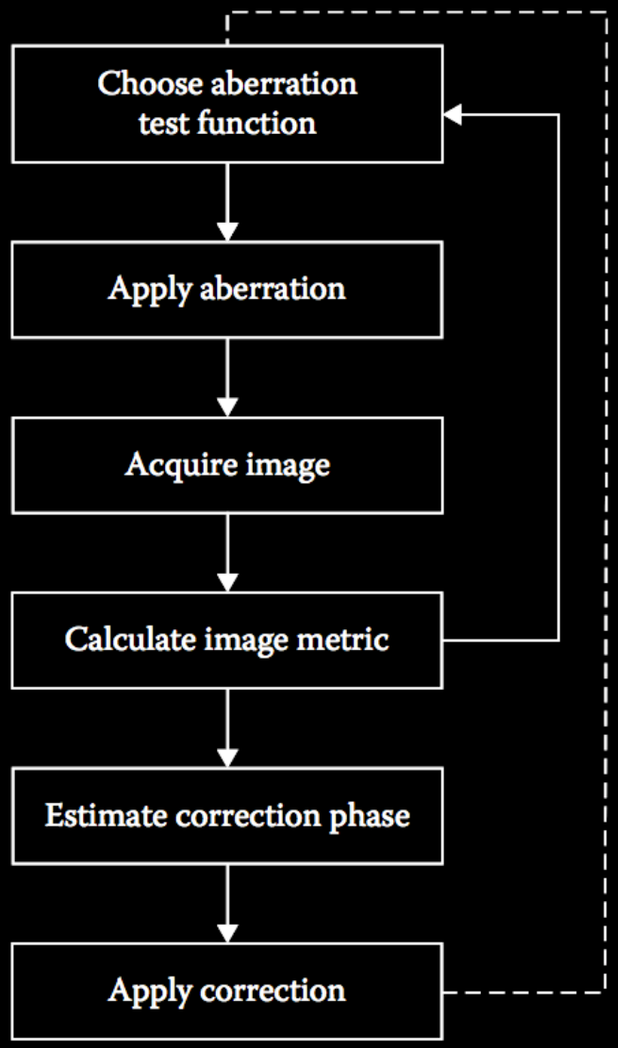
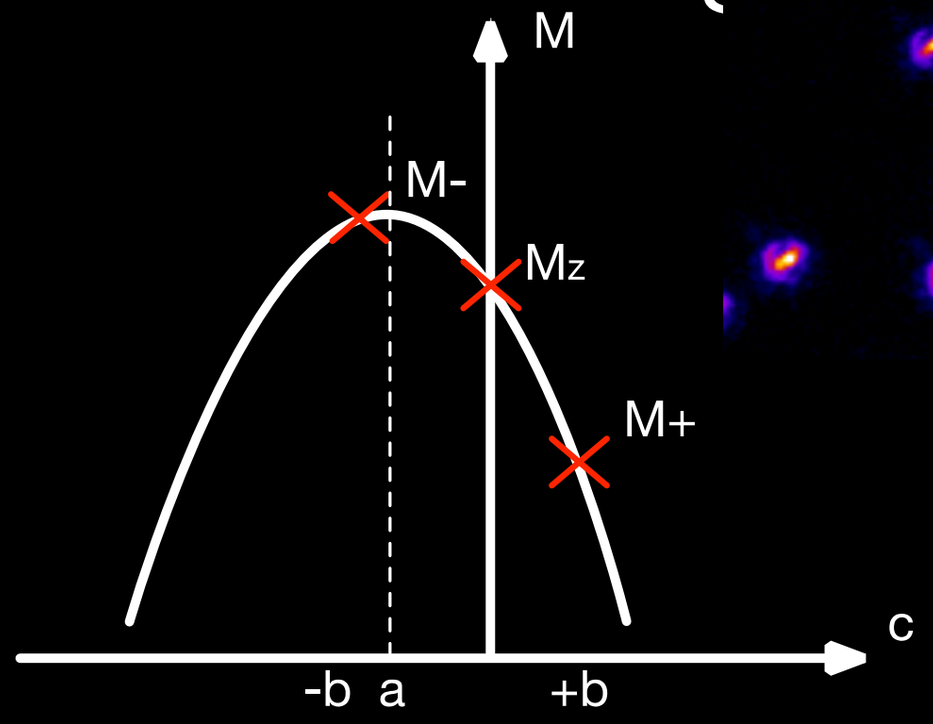


Image based correction strategy



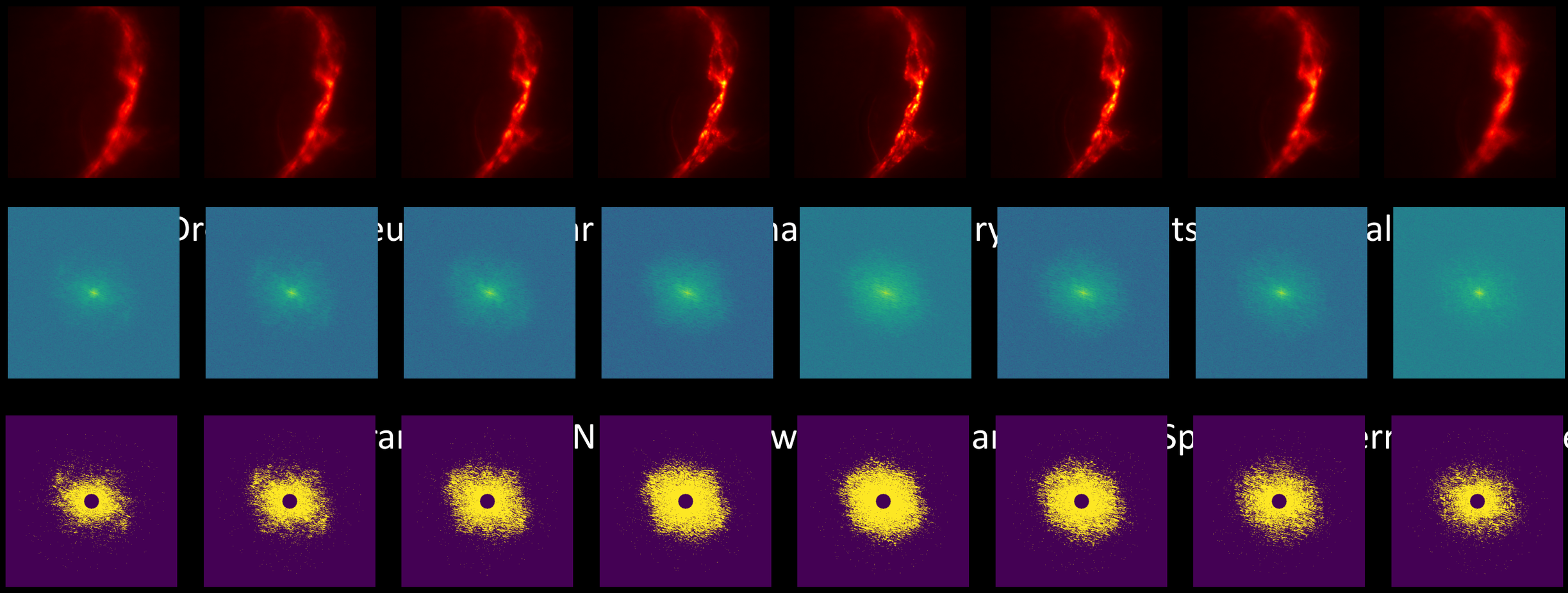


$$a = -\frac{b(M_+ - M_-)}{2M_+ - 4M_z + 2M_-}$$

At least three measurements are necessary for quadratic maximization

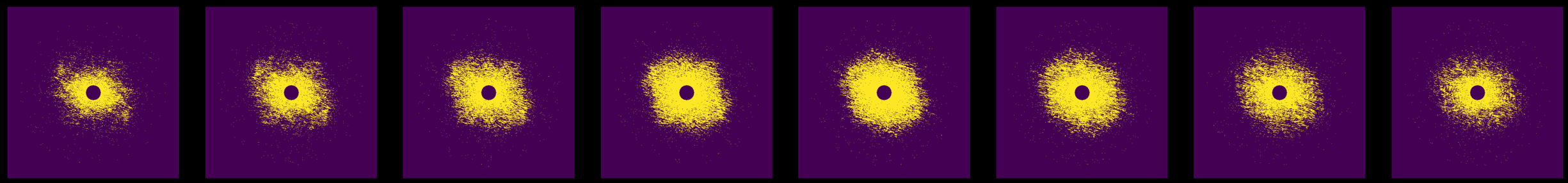
Image based correction strategy : Fourier metric

-2. Amount of Spherical aberration applied 2.0

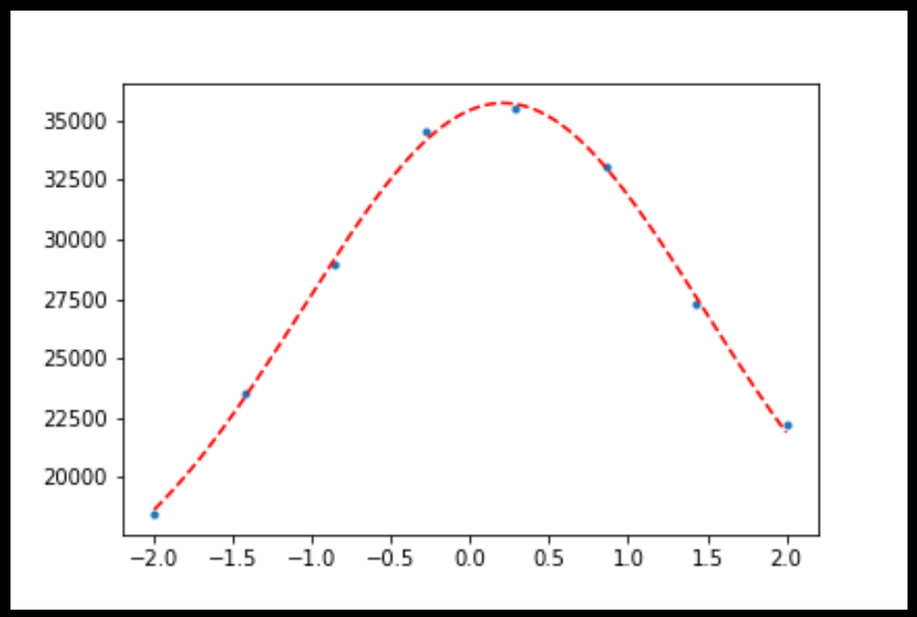


Noise masks of Fourier transforms with varying amounts of Spherical aberration applied

Sensorless correction: Fourier Metric

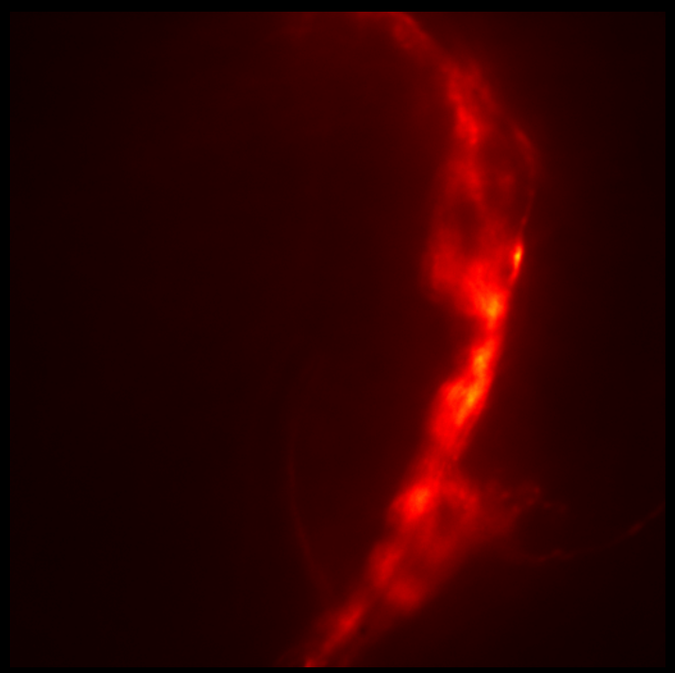


Noise masks of Fourier transforms with varying amounts of Spherical aberration applied

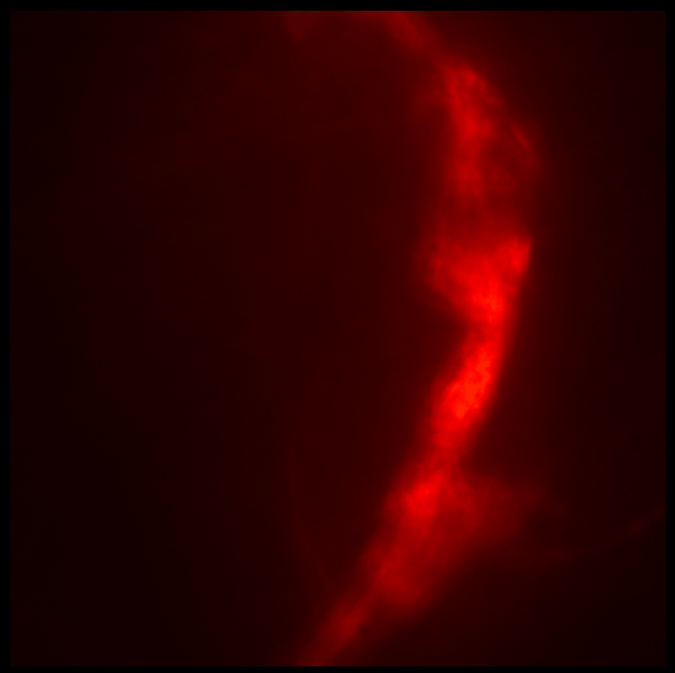


Spherical aberration amplitude fitting

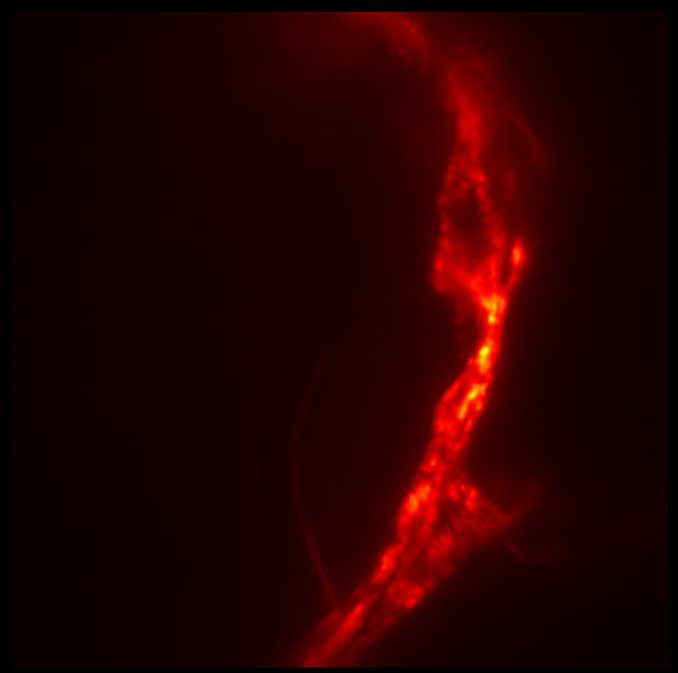
Sensorless correction: Fourier Metric



NMJ before correction

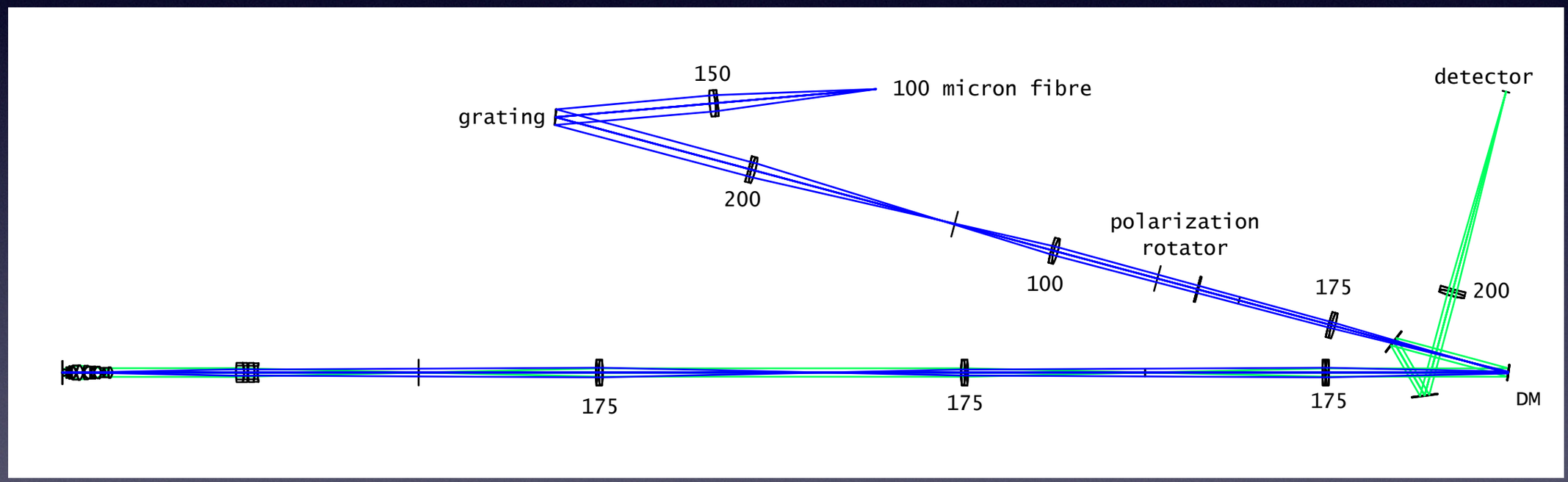


Sensorless correction routine on NMJ data

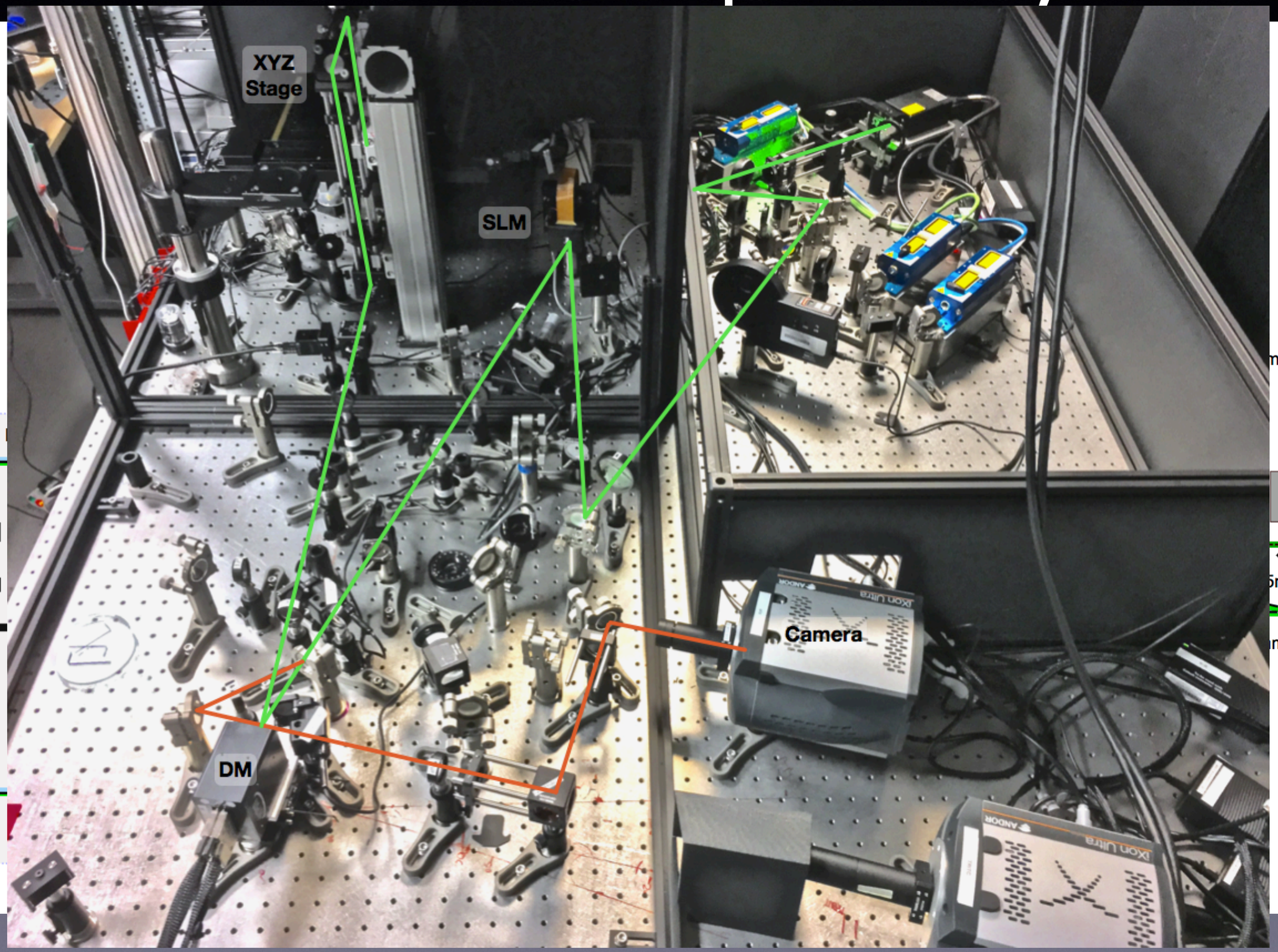


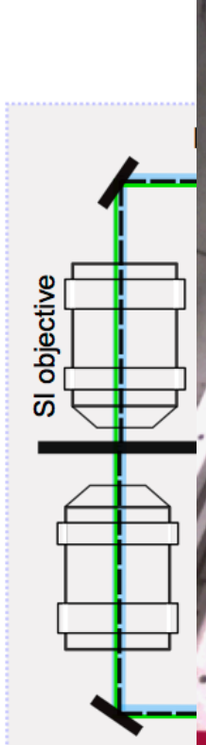
NMJ after correction

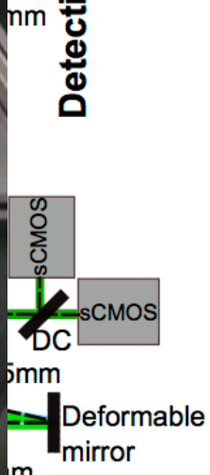
Start with simple design



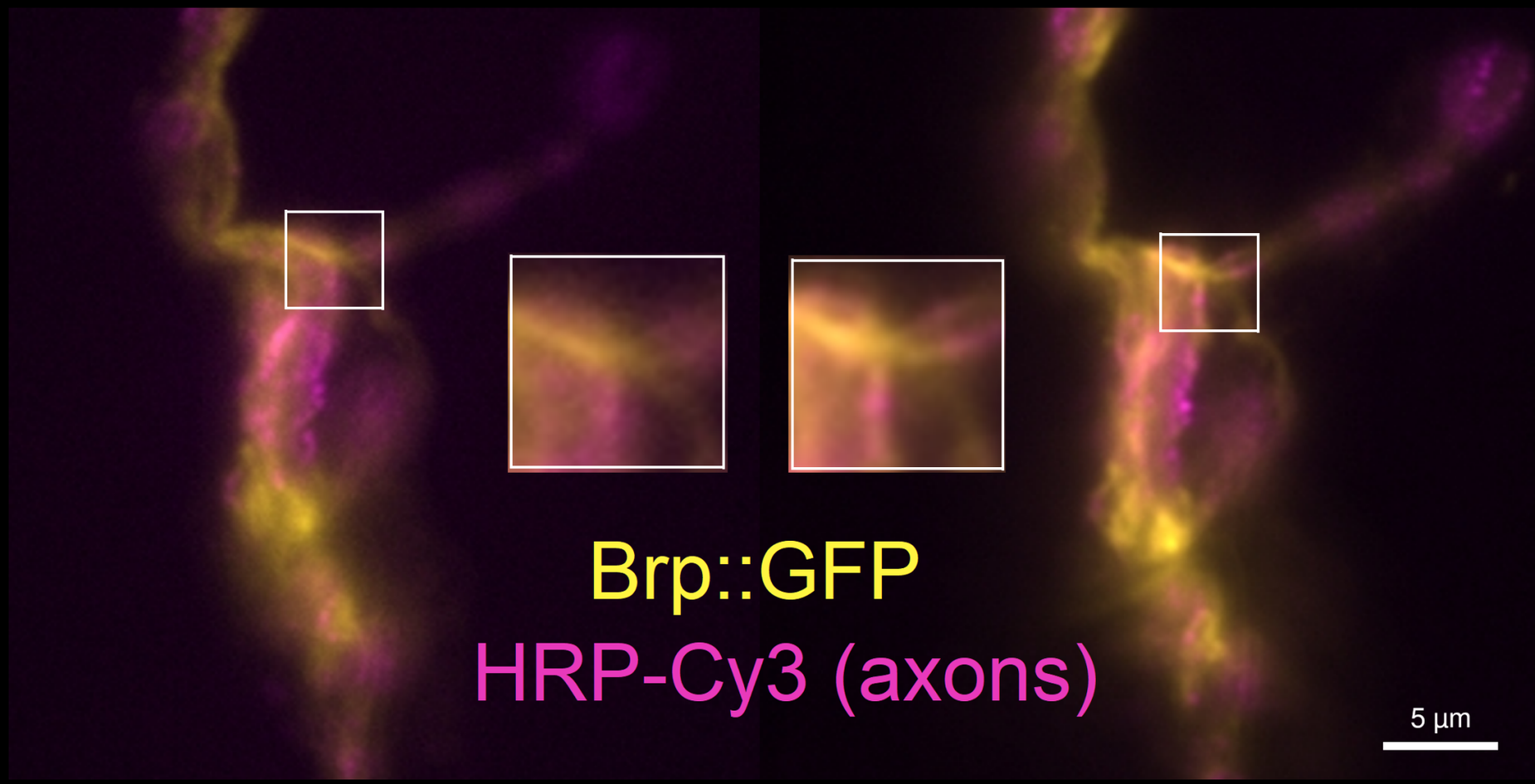
Add complexity







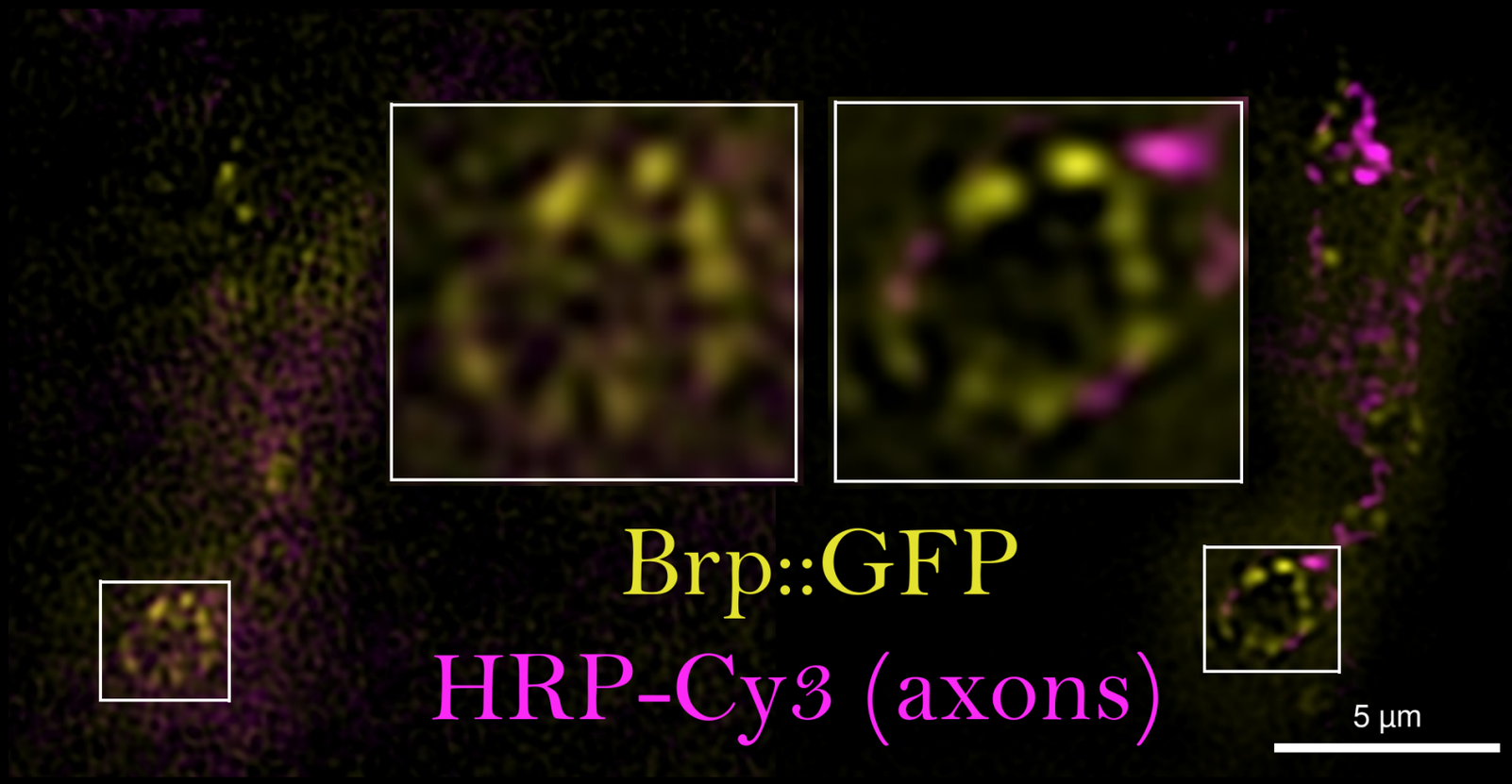
Drosophila Neuro-muscular Junction: Pseudo-widefield



Pseudo-widefield without AO correction

Pseudo-widefield with AO correction

Drosophila Neuro-muscular Junction: 3D SIM reconstruction



3D SIM reconstruction without AO correction

3D SIM reconstruction with AO correction

Control software

Python - Microscope

- Python low level control of hardware
- Exports devices with a standard API
- Can control system entirely from python

Cockpit

- GUI built onto of microscope
- Allows easy control of even complex microscopes
- Intuitive control and sample navigation

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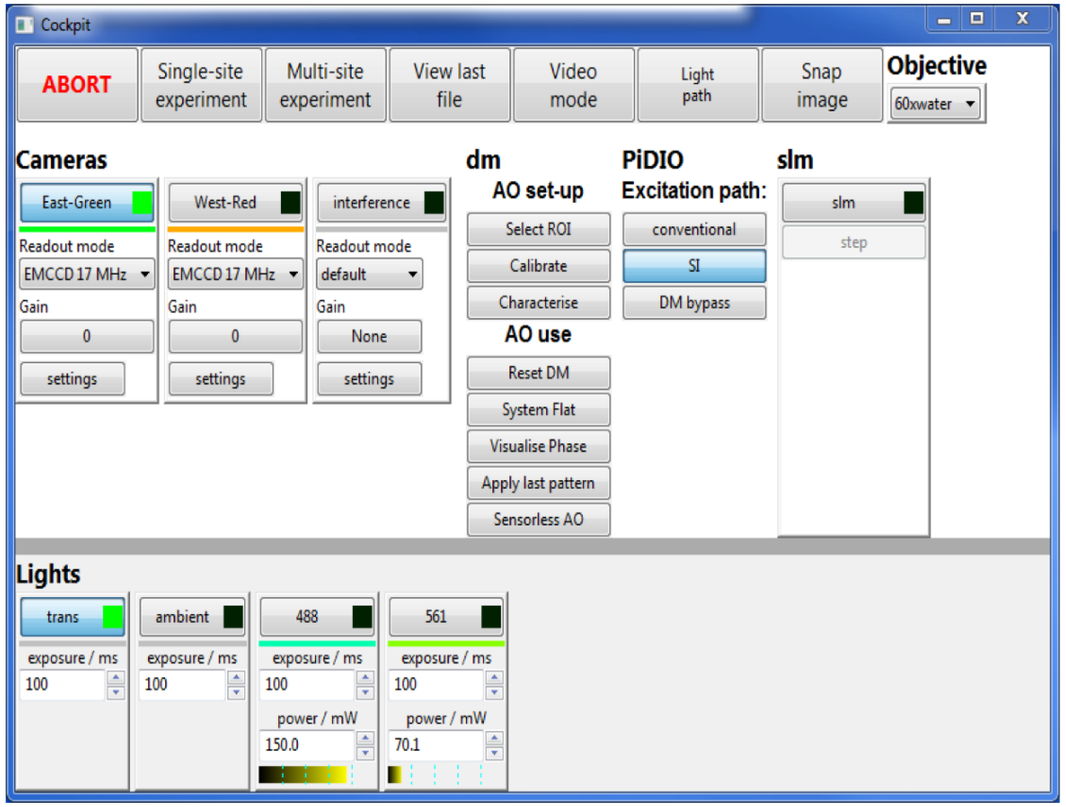
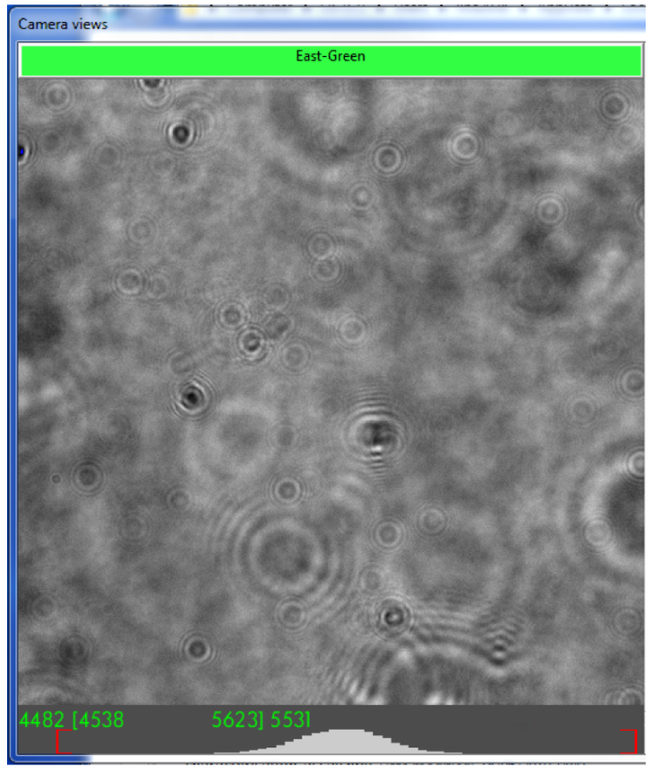
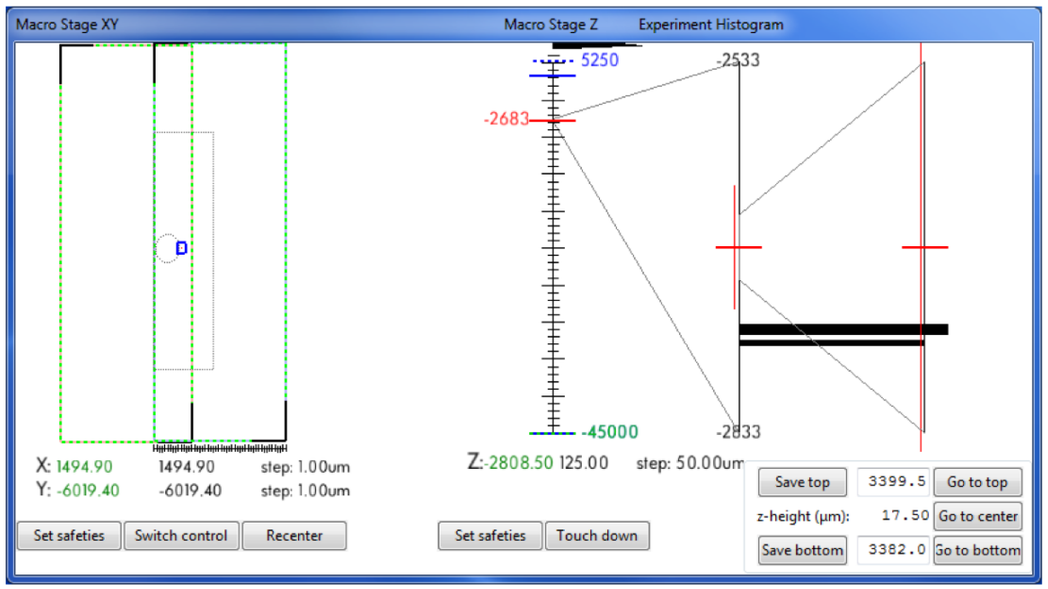
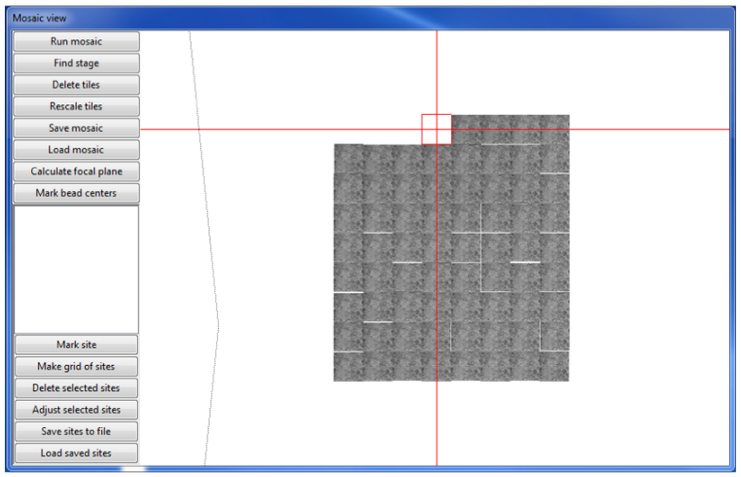

Cockpit
ABORT
Single-site experiment
Multi-site experiment
View last file
Video mode
Light path
Snap image
Objective
60xwater
Cameras
East-Green
West-Red
interference
Readout mode
EMCCD 17 MHz
EMCCD 17 MHz
default
Gain
0
0
None
settings
settings
settings
dm
AO set-up
Select ROI
Calibrate
Characterise
AO use
Reset DM
System Flat
Visualise Phase
Apply last pattern
Sensorless AO
PiDIO
Excitation path:
conventional
SI
DM bypass
slm
slm
step
Lights
trans
ambient
488
561
exposure / ms
100
100
100
100
power / mW
150.0
70.1


Camera views
East-Green
4482 [4538
5623] 5531


Macro Stage XY
Macro Stage Z
Experiment Histogram
5250
-2683
-45000
-2533
-2833
X: 1494.90
1494.90
step: 1.00um
Y: -6019.40
-6019.40
step: 1.00um
Z:-2808.50 125.00
step: 50.00um
Set safeties
Switch control
Recenter
Set safeties
Touch down
Save top
3399.5
Go to top
z-height (µm):
17.50
Go to center
Save bottom
3382.0
Go to bottom


Mosaic view
Run mosaic
Find stage
Delete tiles
Rescale tiles
Save mosaic
Load mosaic
Calculate focal plane
Mark bead centers
Mark site
Make grid of sites
Delete selected sites
Adjust selected sites
Save sites to file
Load saved sites

Bespoke Microscopes

Why bother?

Specific applications -better than commercial microscopes

Flexibility

Cost

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Bespoke Microscopes

Why NOT to bother?

- Salary of physicist/engineer required
- Long building time required (it's hard)
- Not supported by a company
  (repairs are costly and lengthy)
- Not always easy to use by biologists

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How expensive is it?

Building costs

Hardware ~£100-250k

Salaries 1-3 years (~£50-£150)

Total cost ~£150-350k

Commercial OMX system ~£400k

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Summary

- Recap on image formation
- Fluorescent beads showing aberrations
- Examples of bespoke development
- Bespoke microscope building projects pro's and cons.

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