

Bespoke Microscopes

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Overview

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

What is a microscope image

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

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.

The most important things to think about.

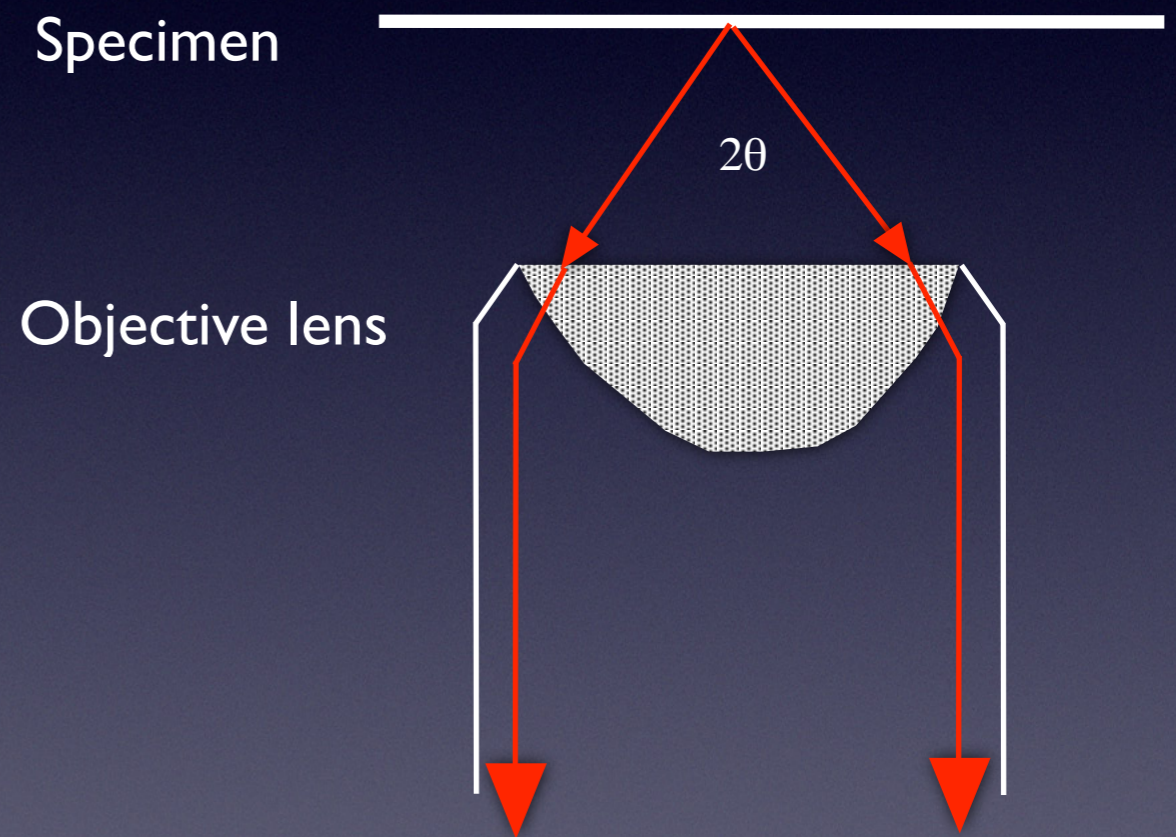
Contrast :- What is the difference between what you want to see and everything else?

Resolution :- How small things can you see?

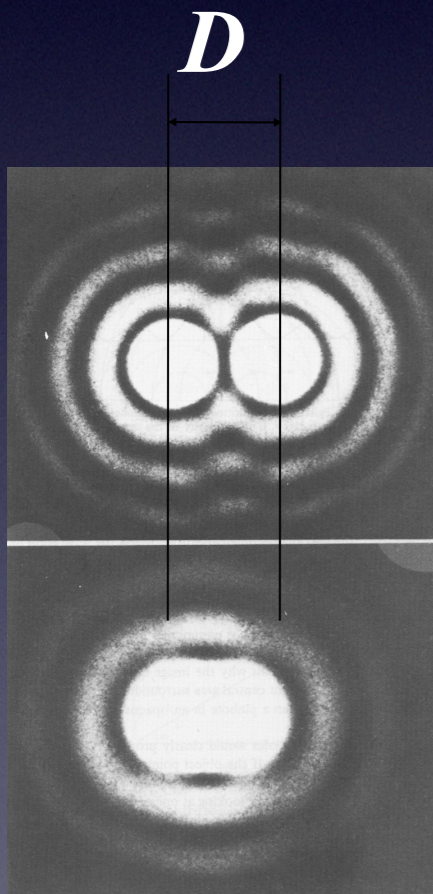
Nothing else

Microscope Resolution

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



Resolution: A technical definition, the Rayleigh Criterion



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

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

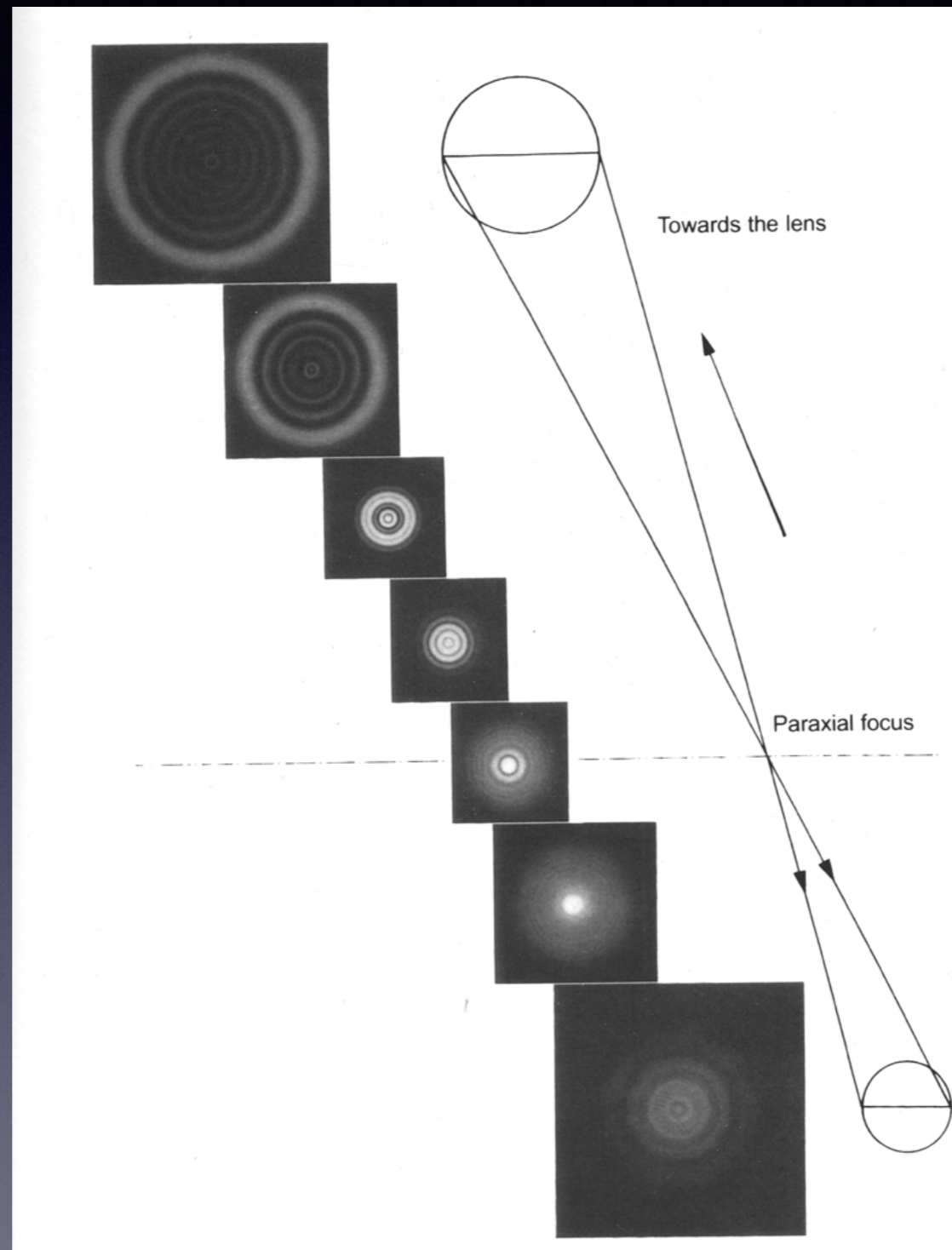
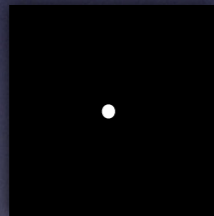
Epi-Fluorescence: $NA_{\text{cond}} = NA_{\text{obj}}$
so $D = 1.22 \lambda / 2NA$

The Point Spread Function - PSF

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

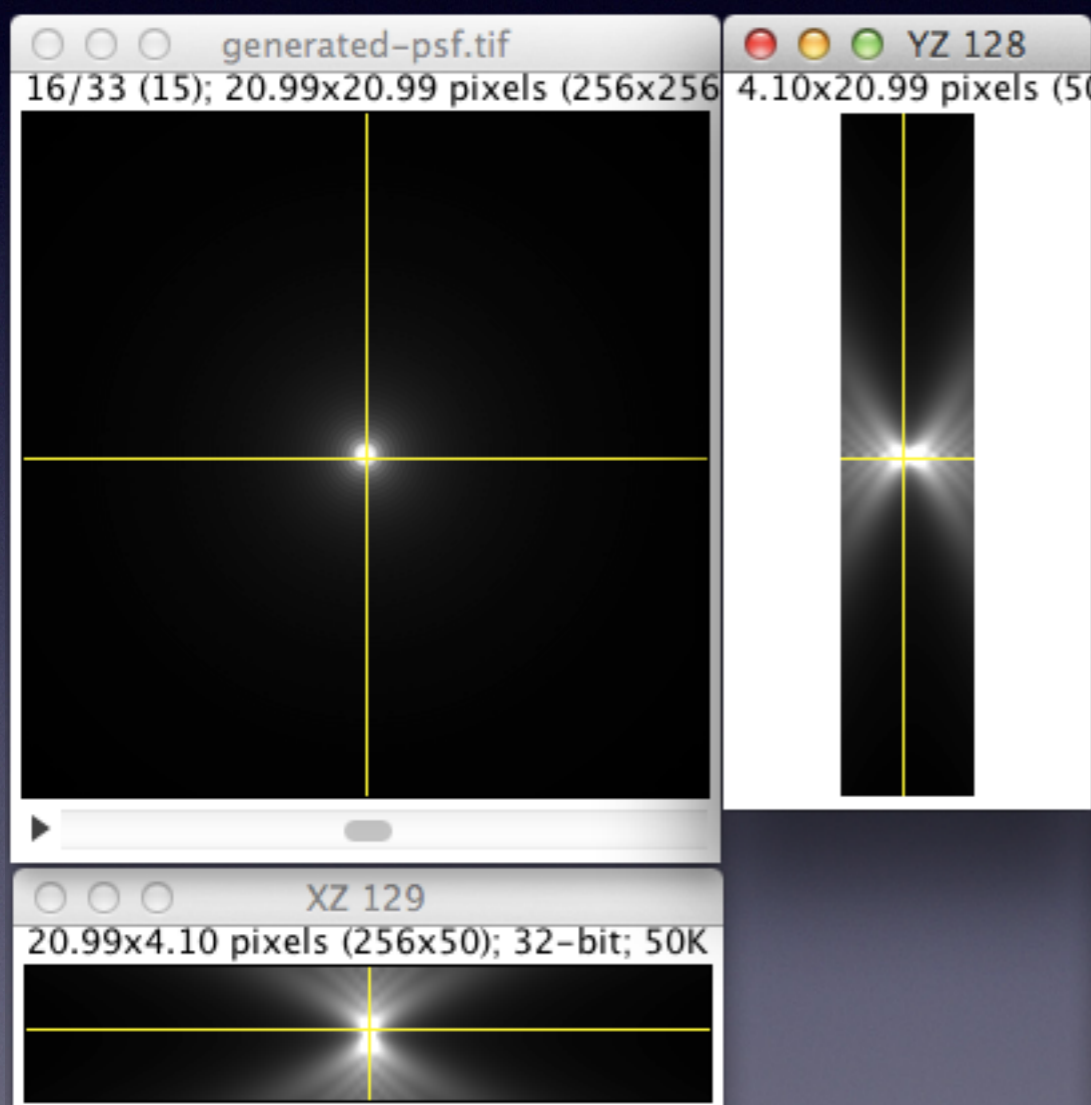
Image quality- the problem of "out-of-focus light" point spread function and airy rings

Sample object: a "sub-resolution"
fluorescent bead

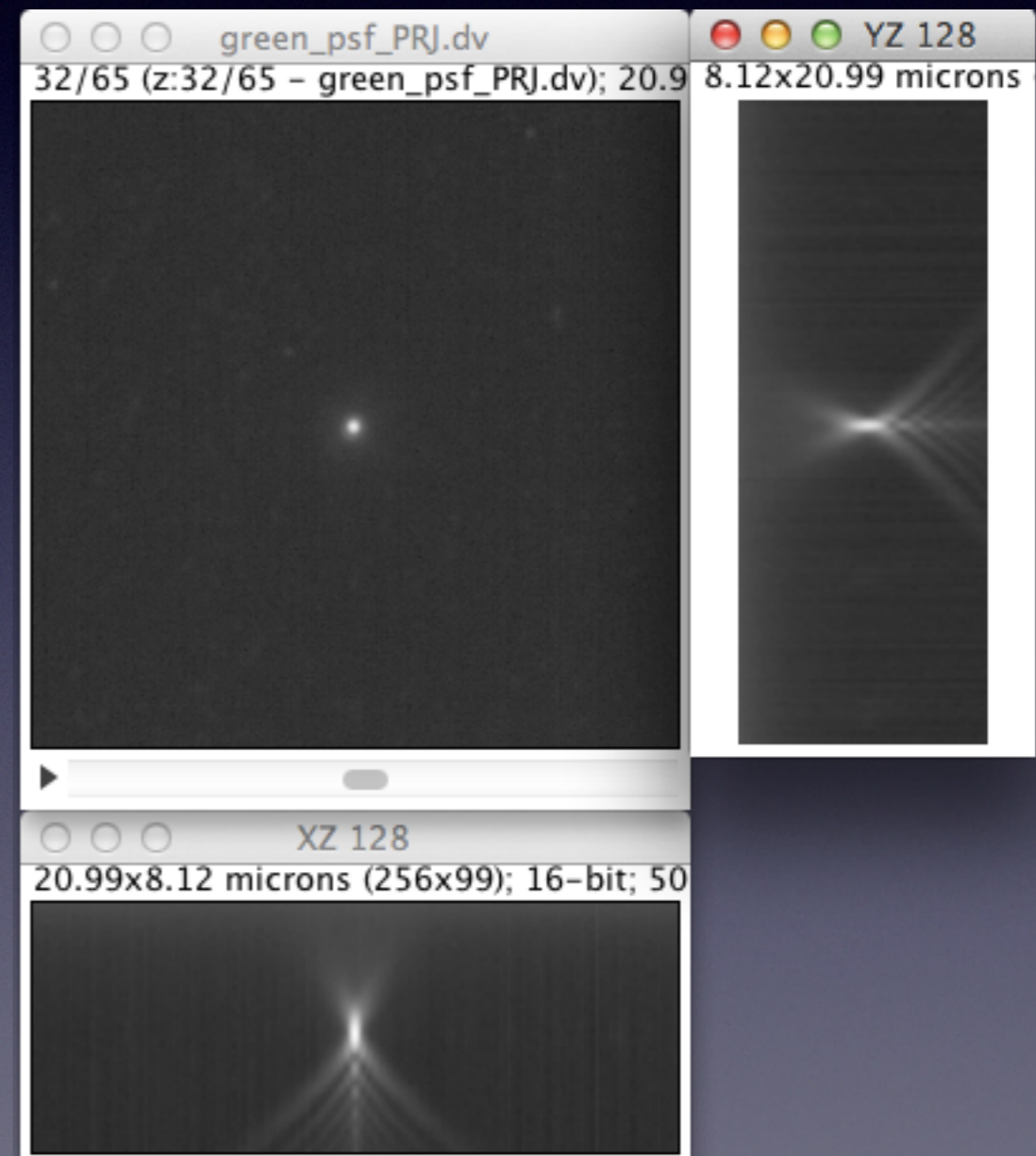


Theoretical and measured PSF

Orthogonal views

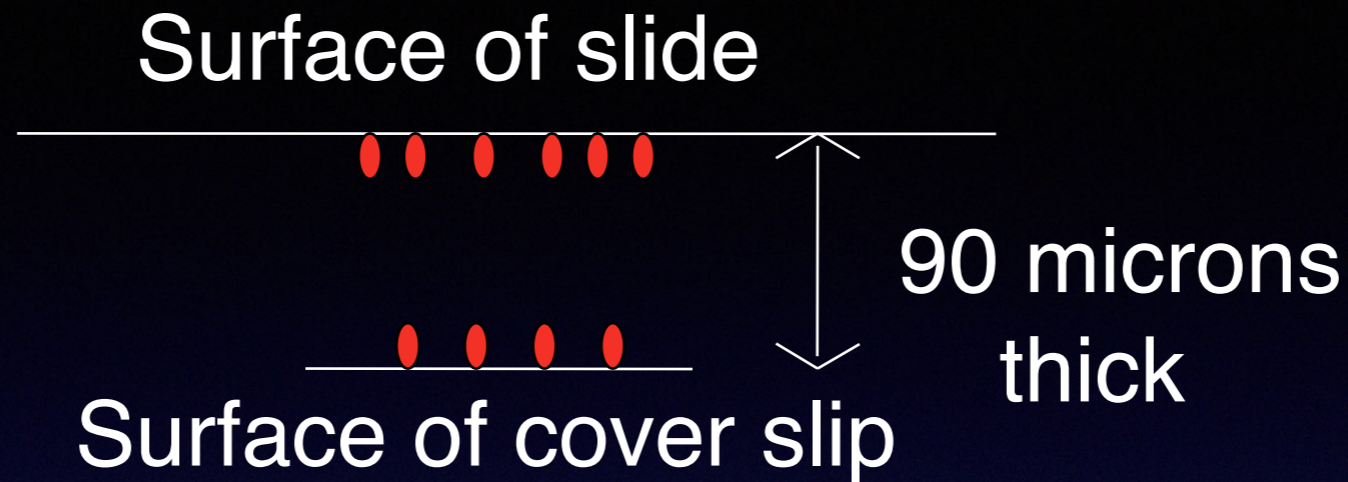


Generated PSF



Real PSF

Bead slide

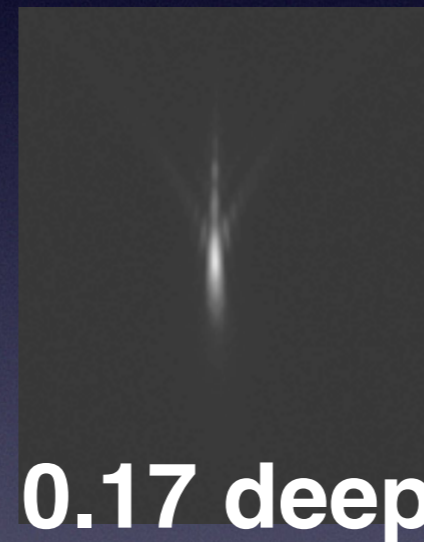
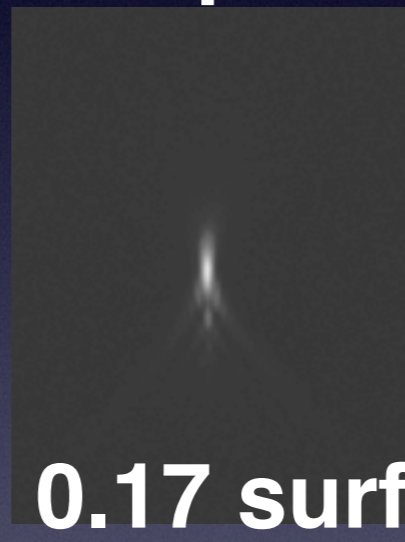
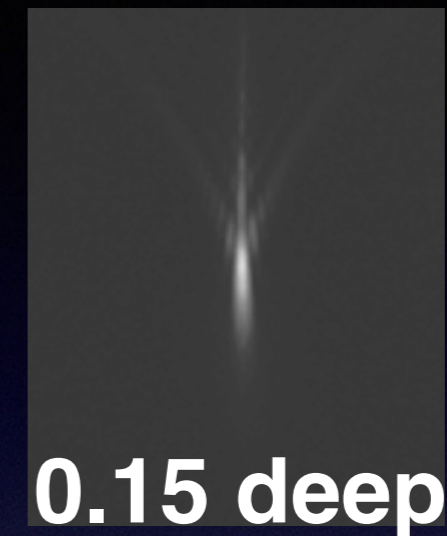
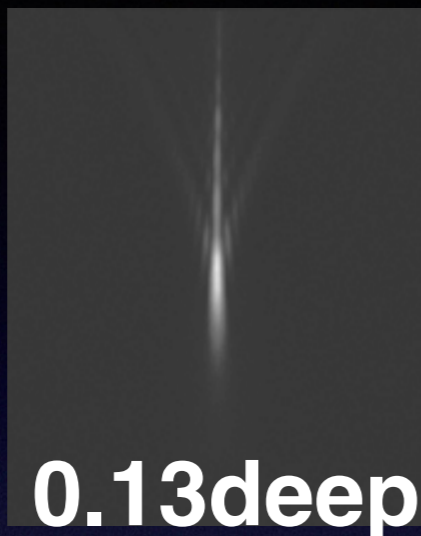
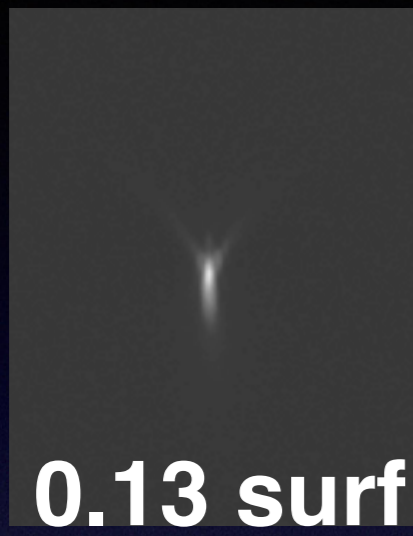


Tetraspeck beads: chromatic registration
DAPI/FITC/Rhodamine/Cy5

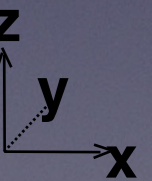
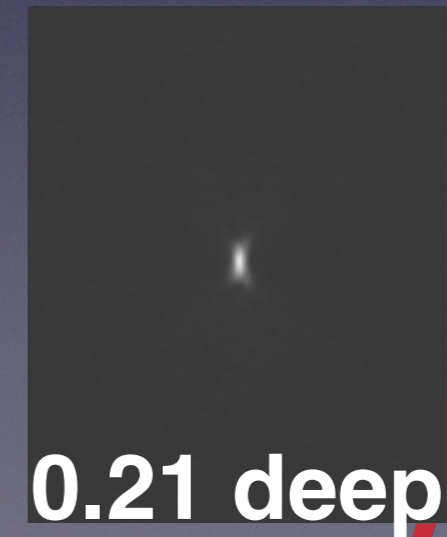
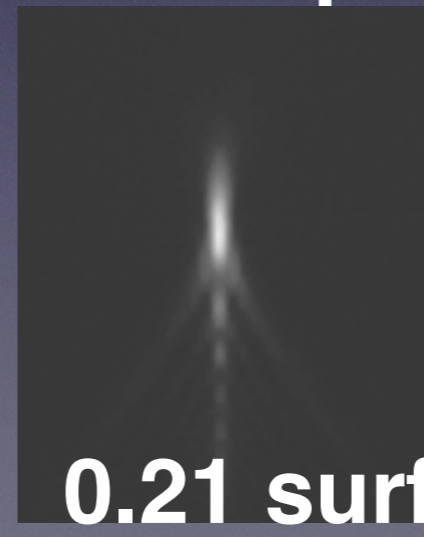
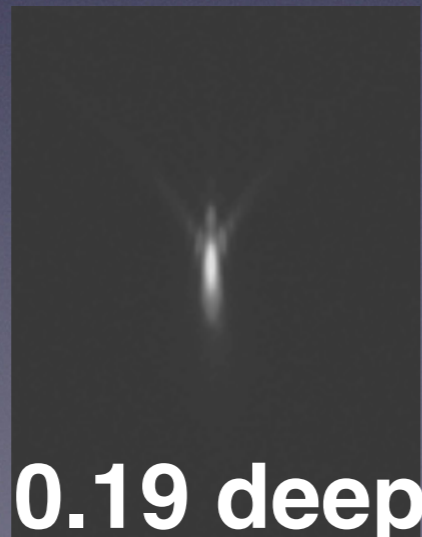
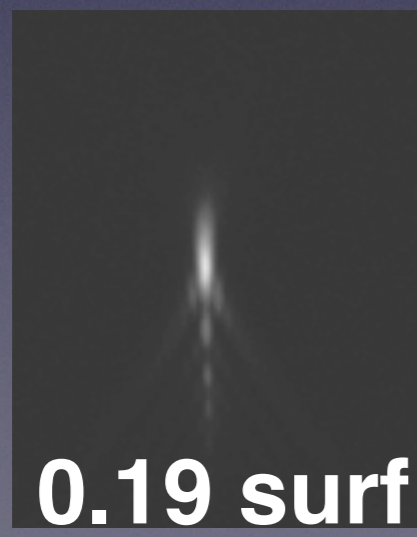
Beads (PS Spec): Single fluorochrome
Brighter -better for generating
point spread functions for deconvolution

Inspeck Intensity beads: Measure dynamic range

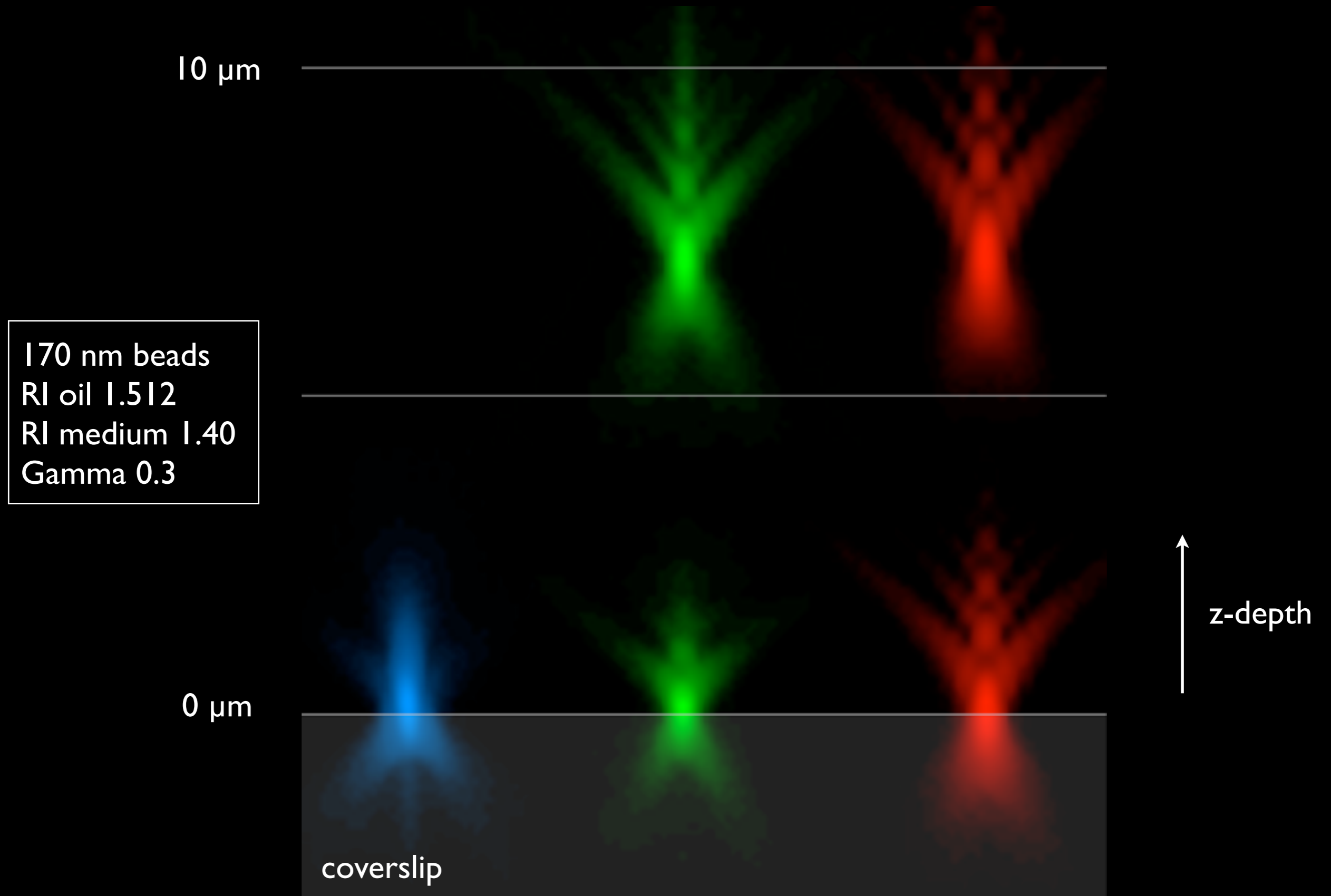
Affects of deep imaging ($90\mu\text{m}$) and collar settings on spherical aberration and psf of 60X/NA1.2w



Data from
Alejandra Clark



Spherical aberration dependent on wavelength, depth, RI



Bespoke systems in Micron

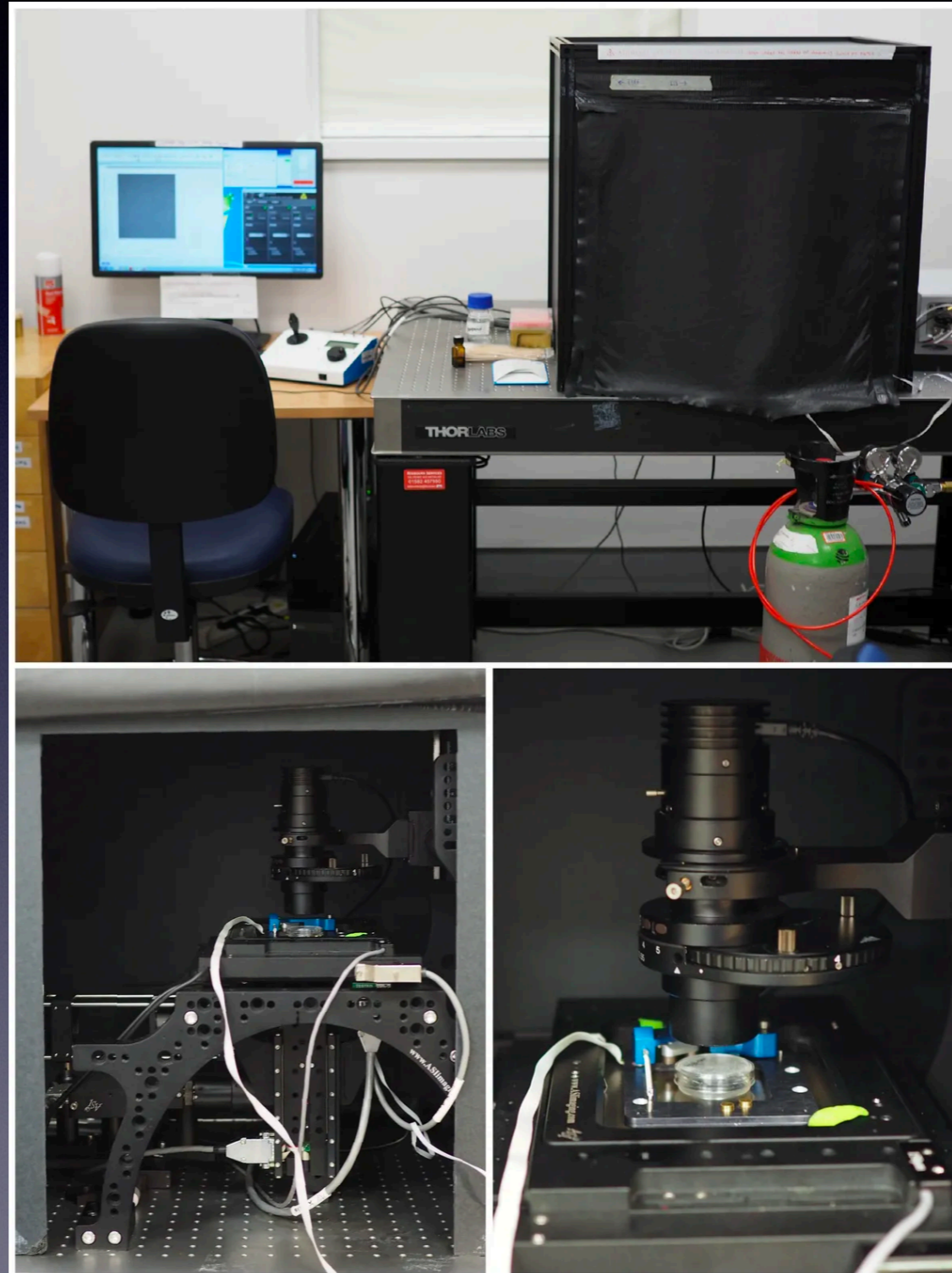
User systems

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

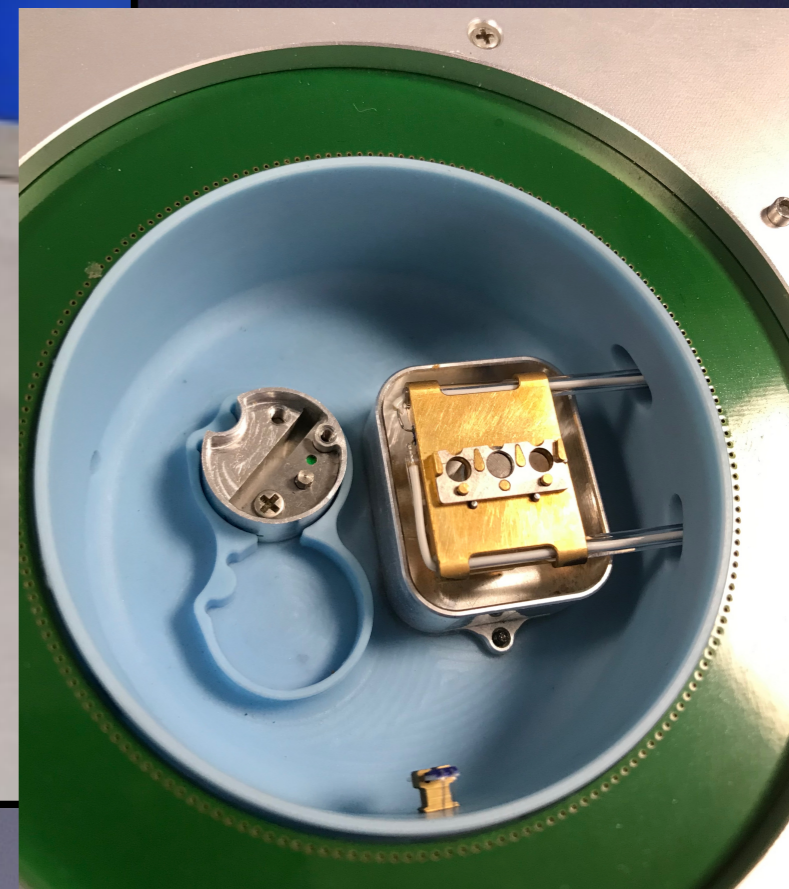
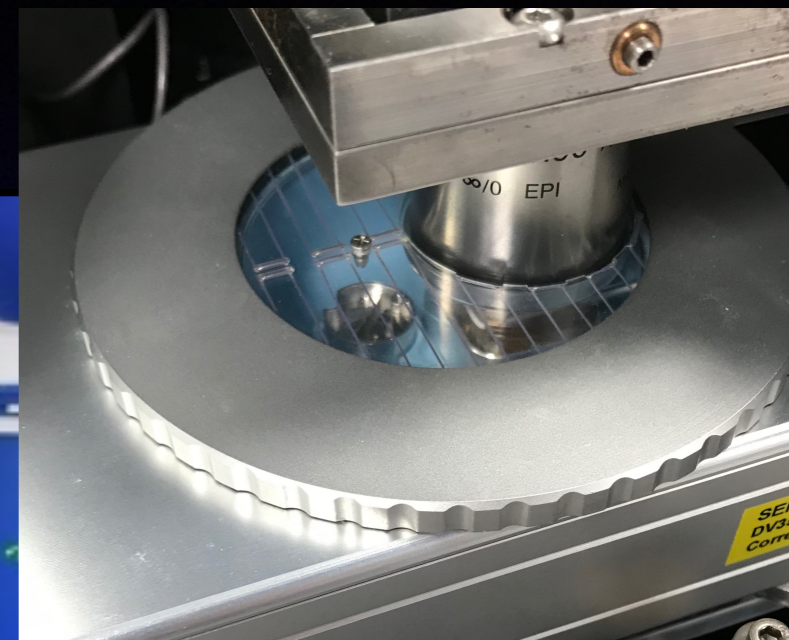
Systems in development

- 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

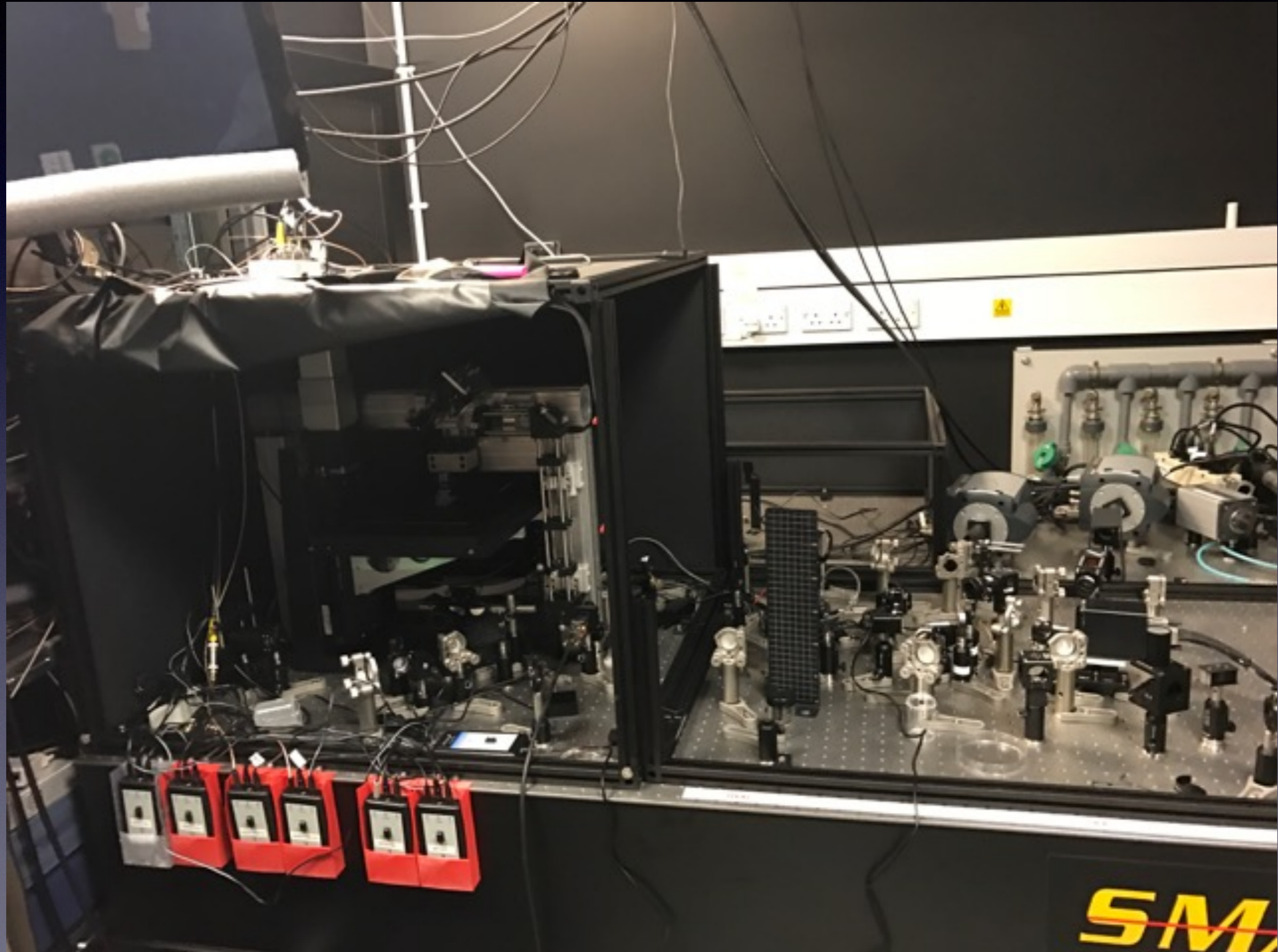
Palm/TIRF



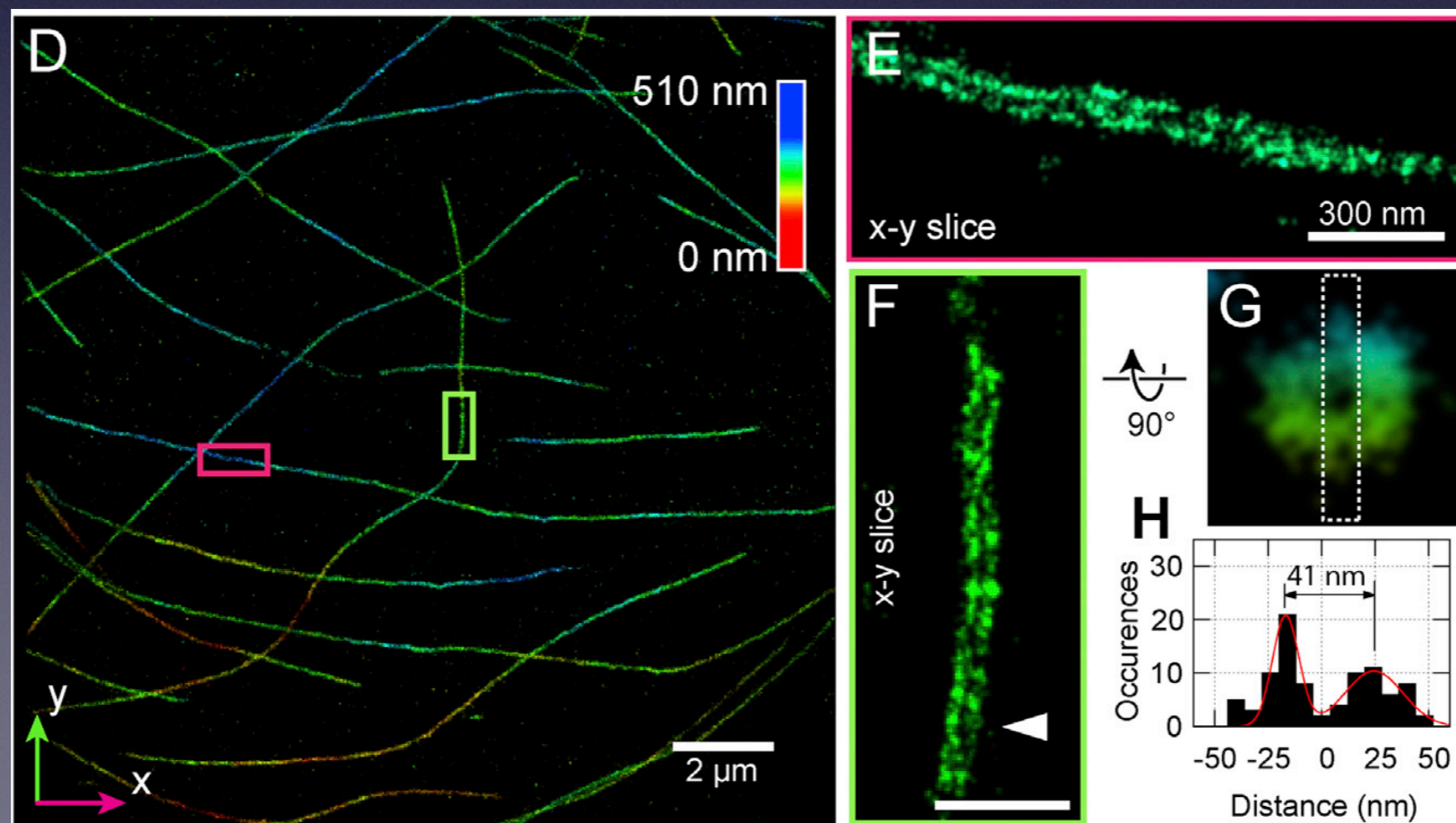
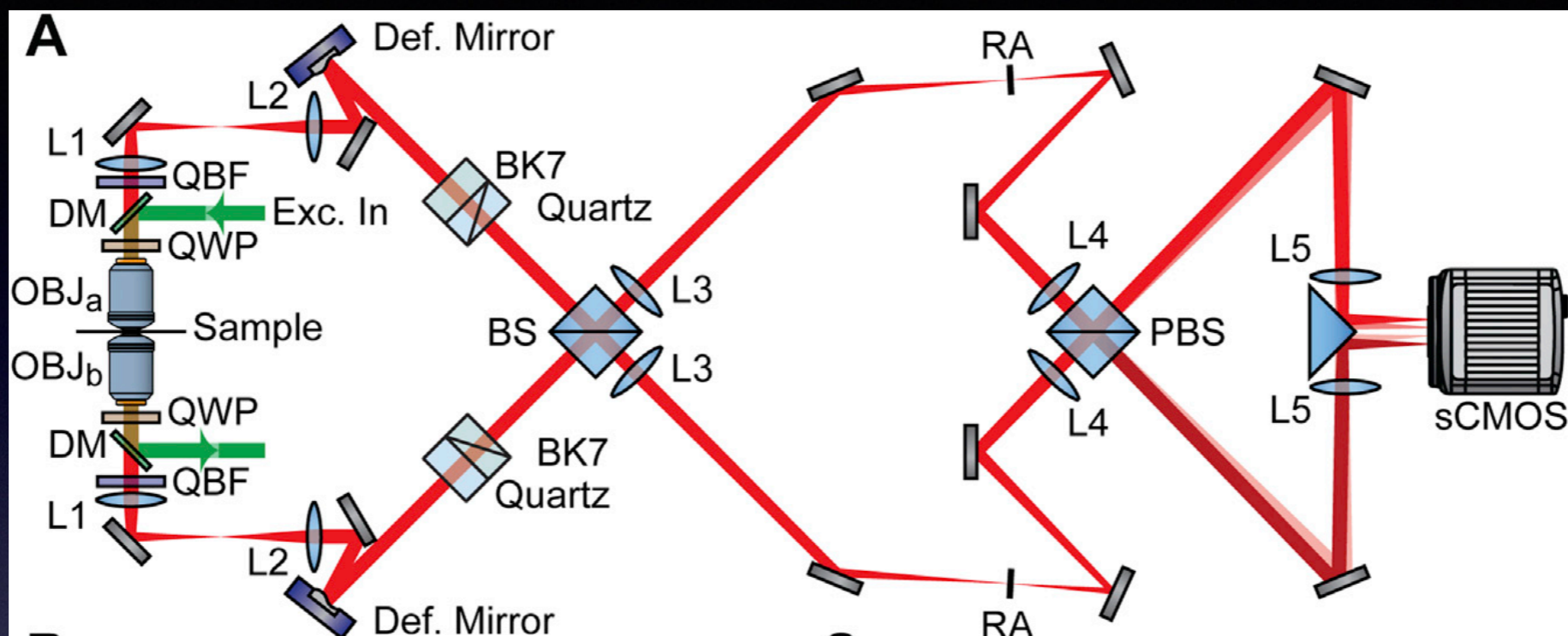
CryoSIM



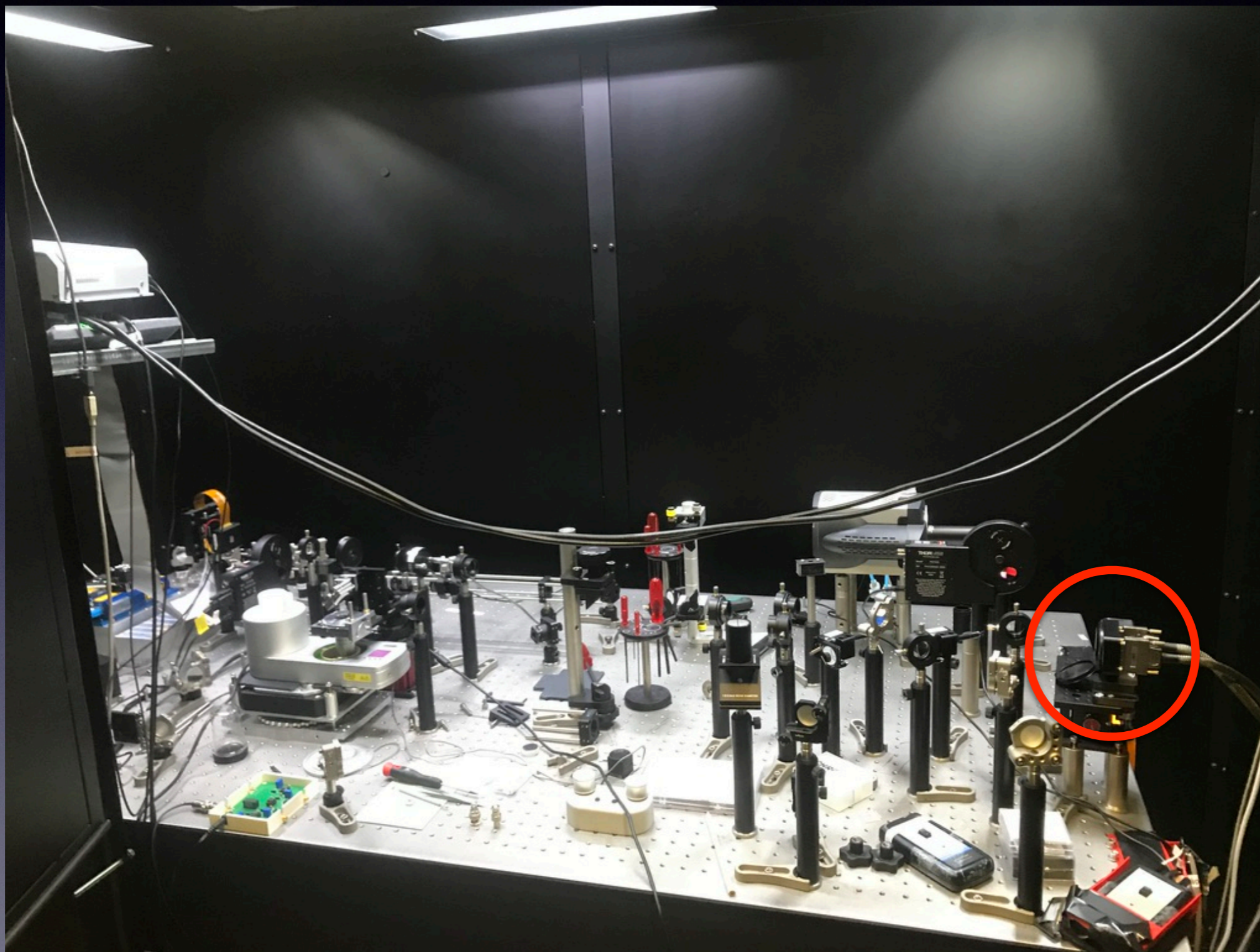
DeepSIM



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

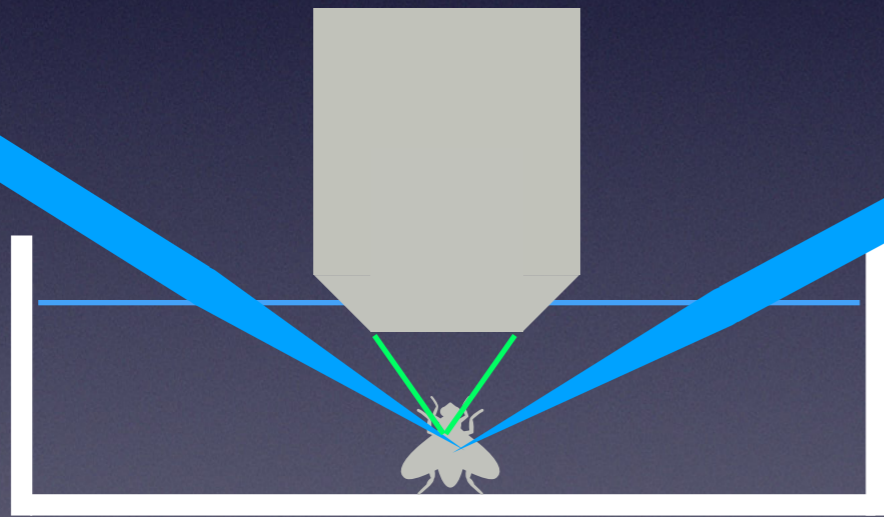
BUT think hard as it is likely to be harder, longer and more expensive than at first thought.

Bespoke Microscope

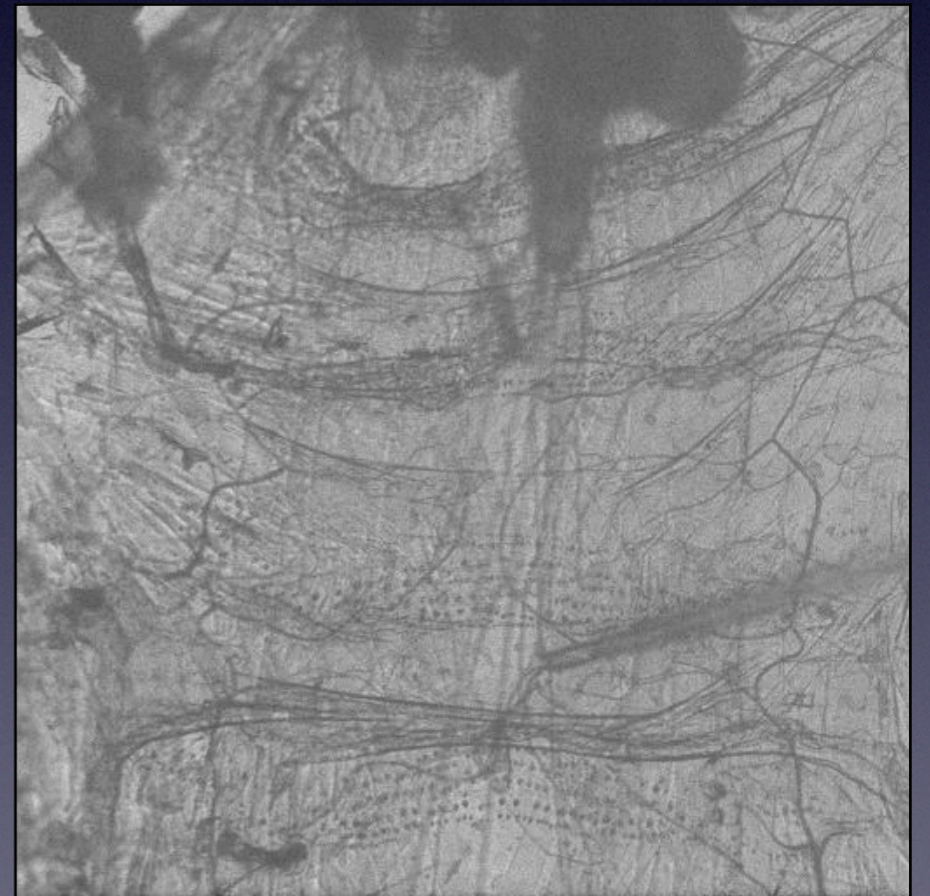
Example - DeepSIM

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

Live imaging

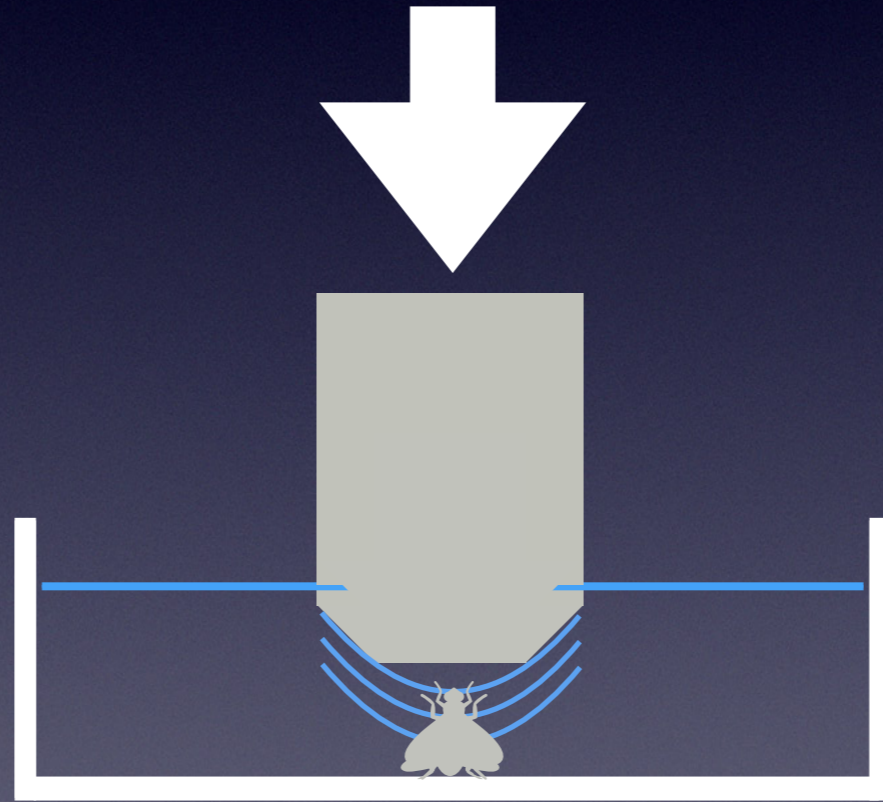


Upright microscope

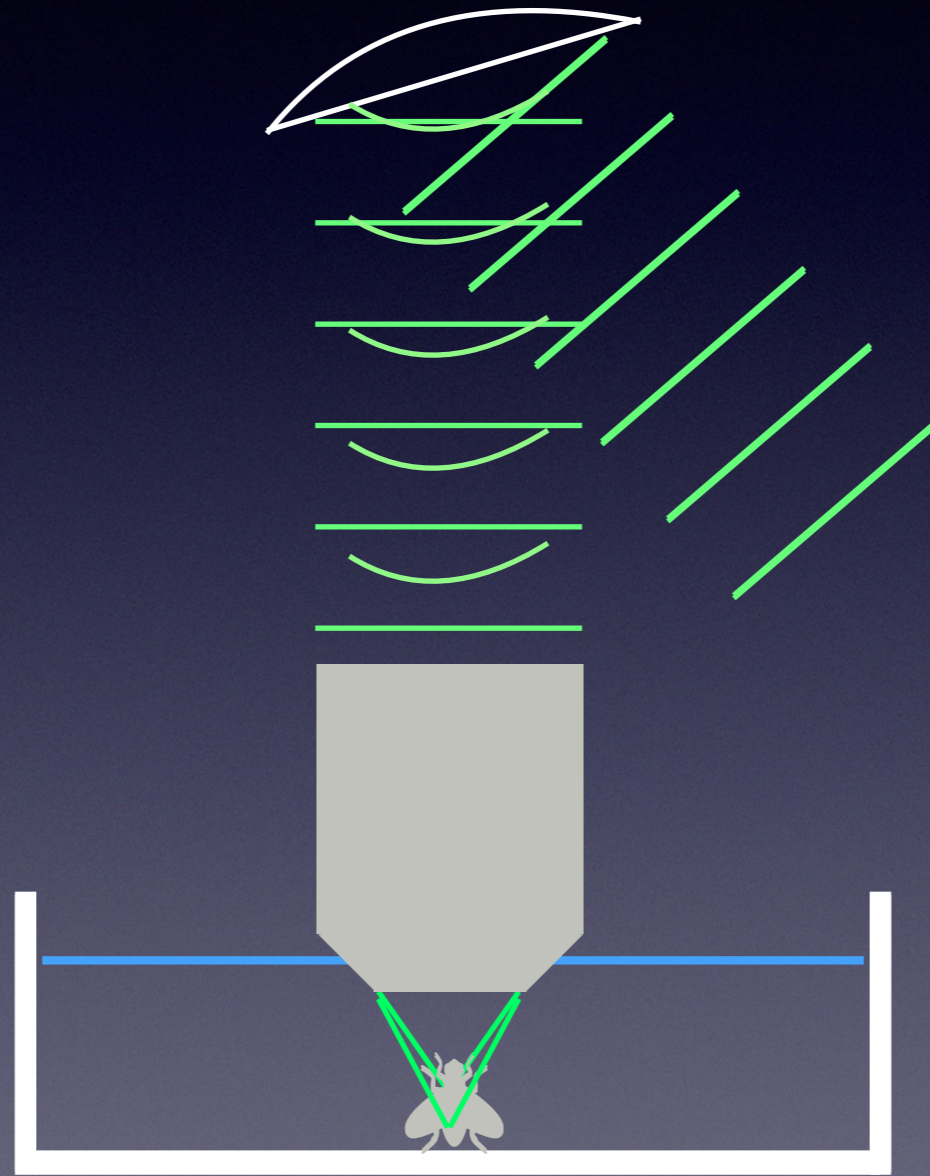


Fast imaging

Fast Z movement issues



AO - Remote Focus



AO - Aberration correction

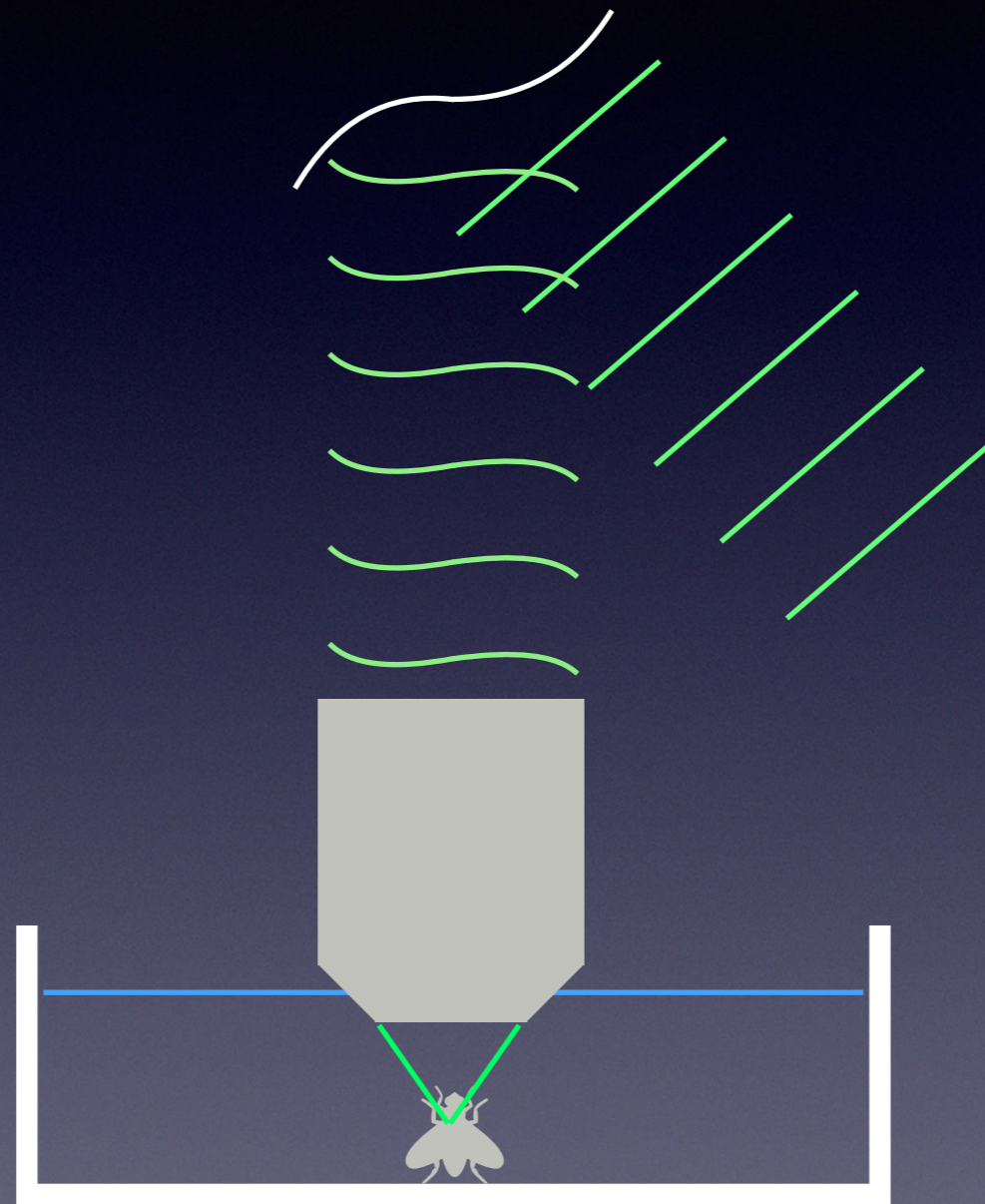
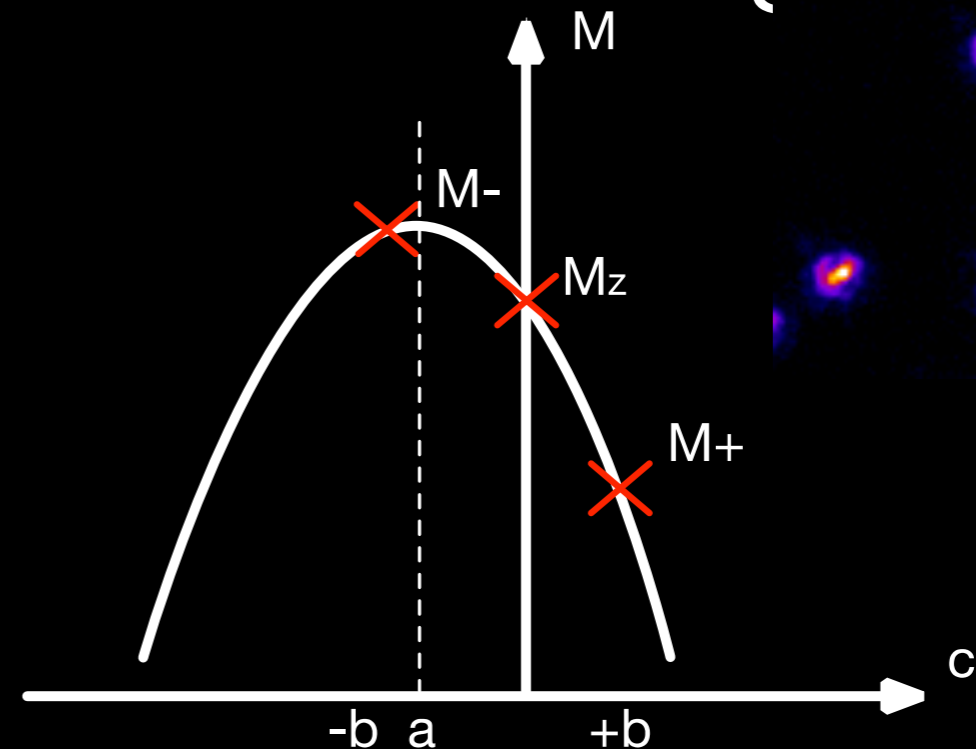
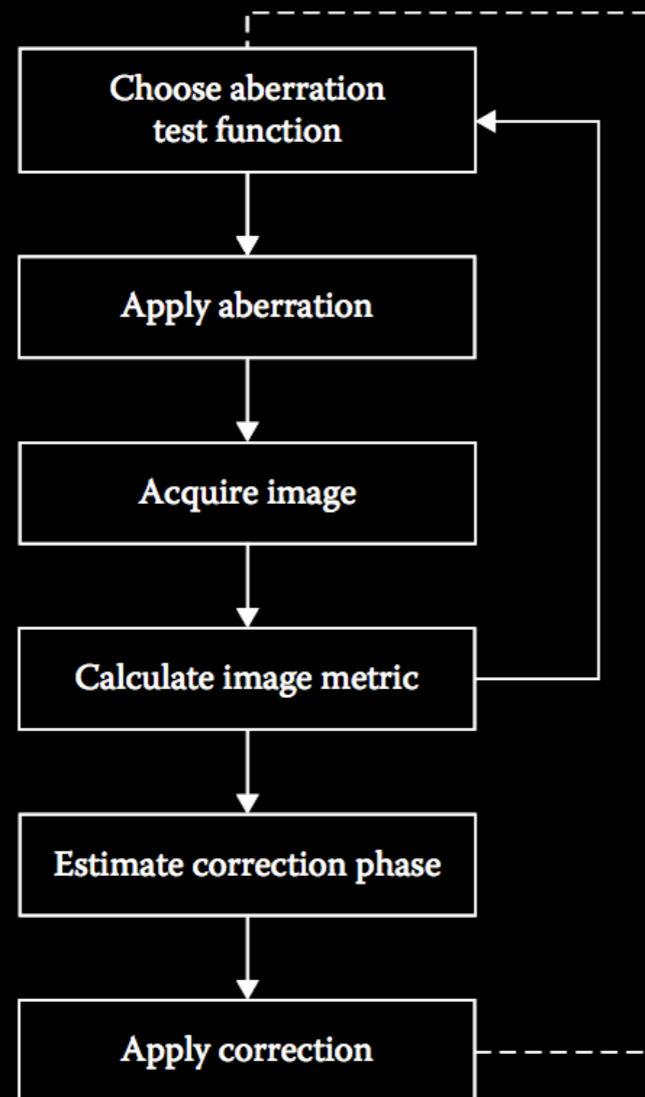


Image based correction strategy



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

At least three measurements are necessary for quadratic maximization

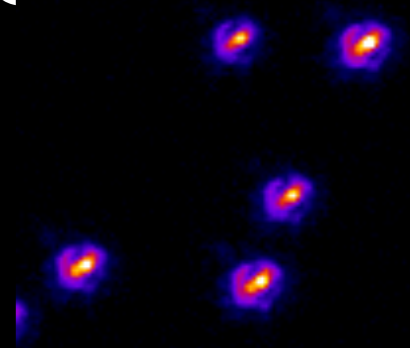
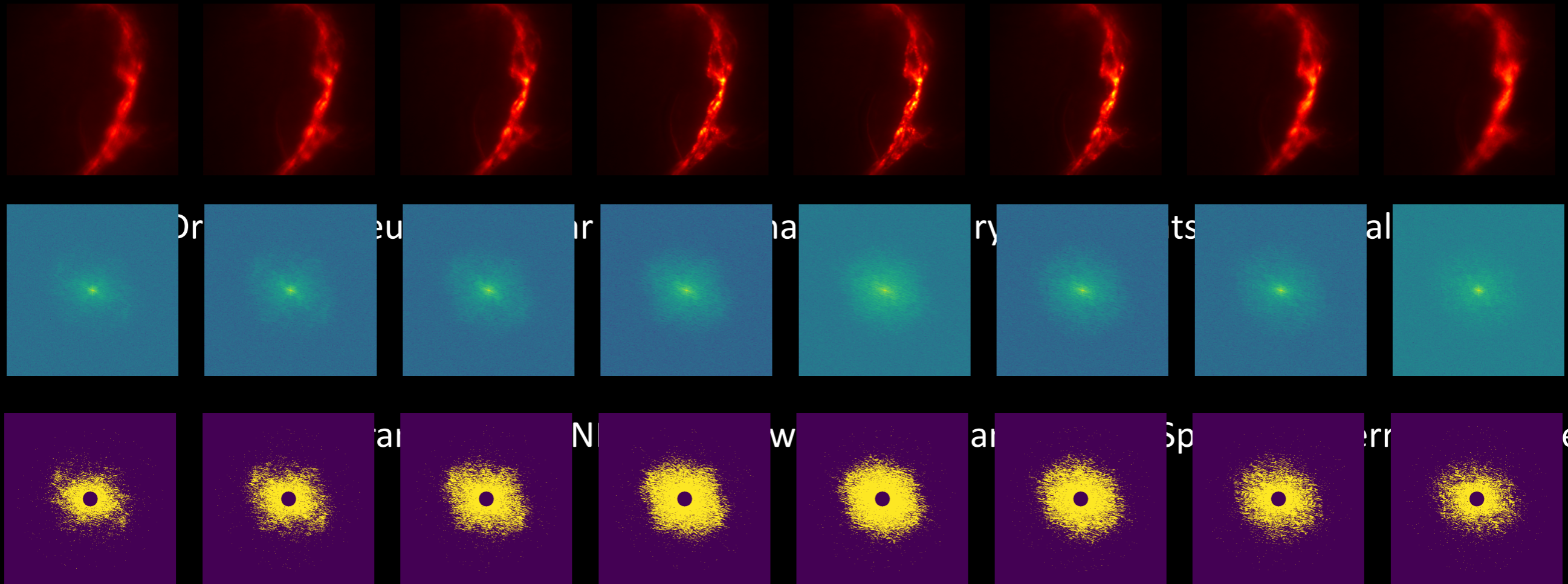


Image based correction strategy : Fourier metric

Amount of Spherical aberration applied

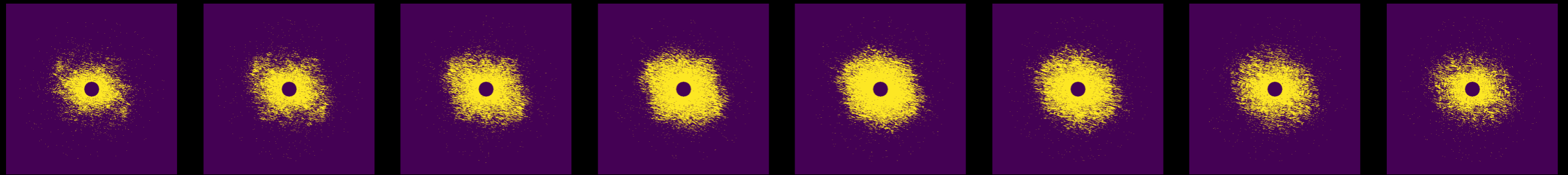
-2.

2.0

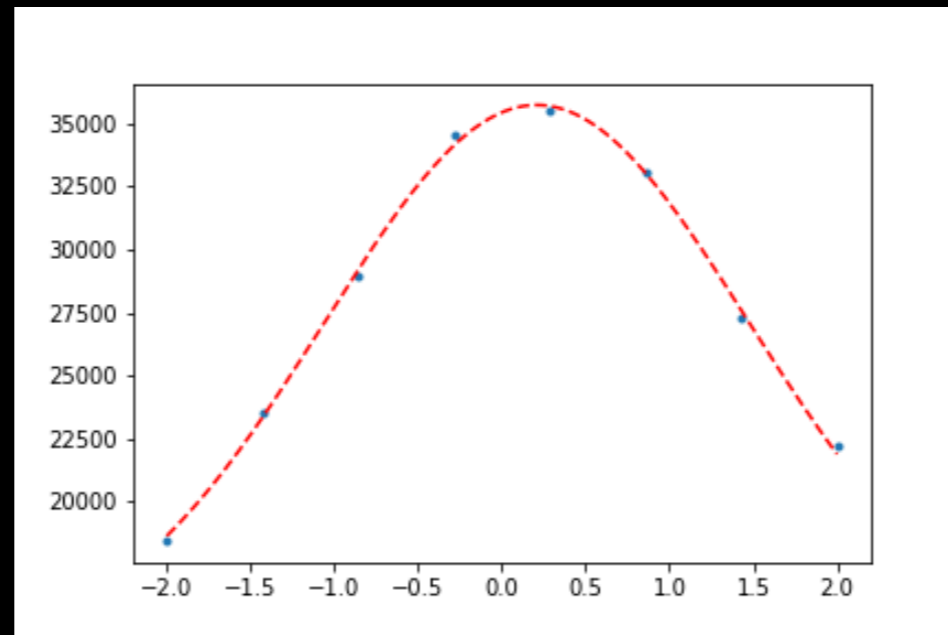


Noise masks of Fourier transforms with varying amounts of Spherical aberration applied

Sensorless correction: Fourier Metric

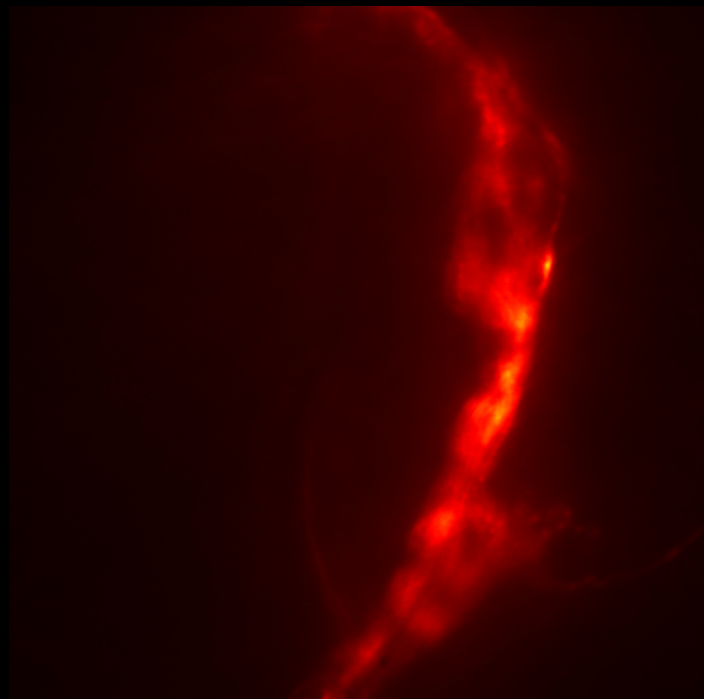


Noise masks of Fourier transforms with varying amounts of Spherical aberration applied

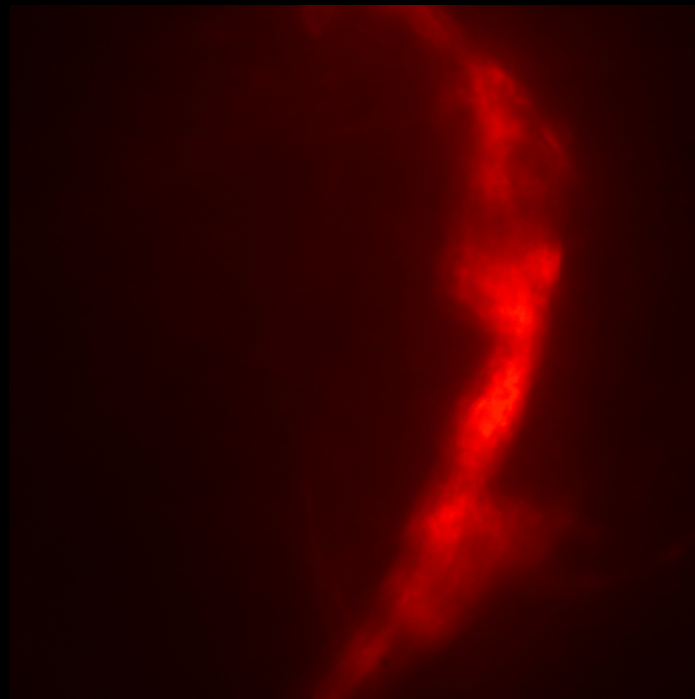


Spherical aberration amplitude fitting

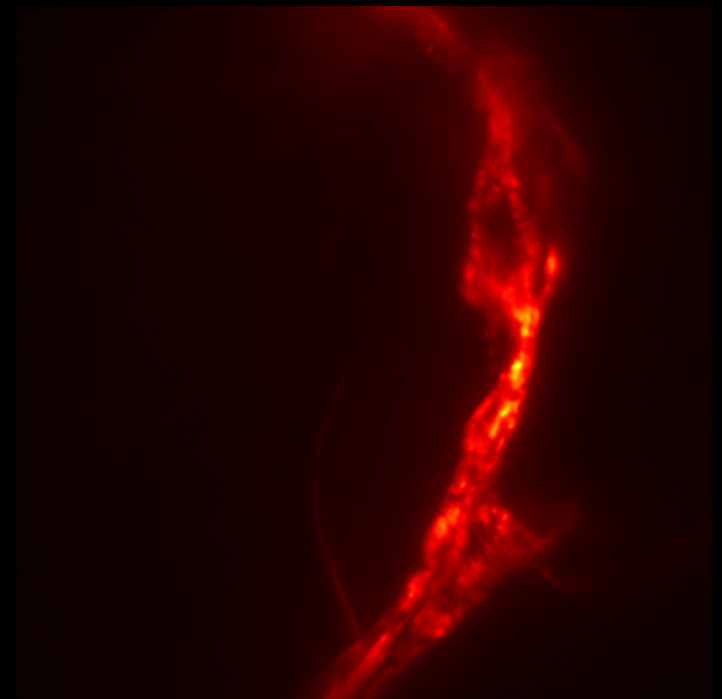
Sensorless correction: Fourier Metric



NMJ before correction

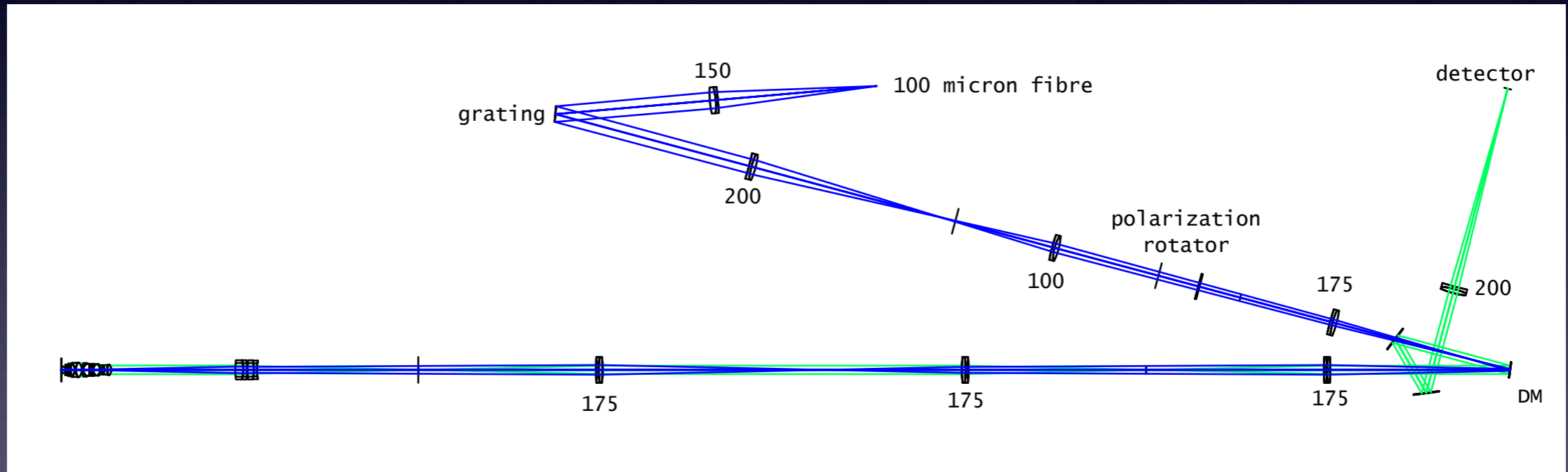


Sensorless correction routine on NMJ data

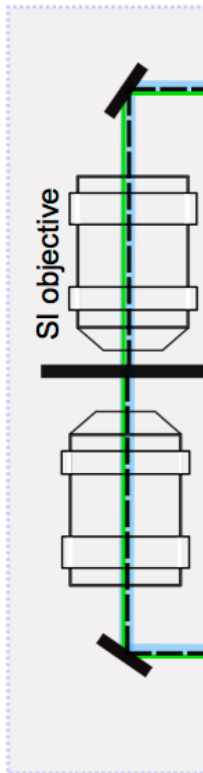
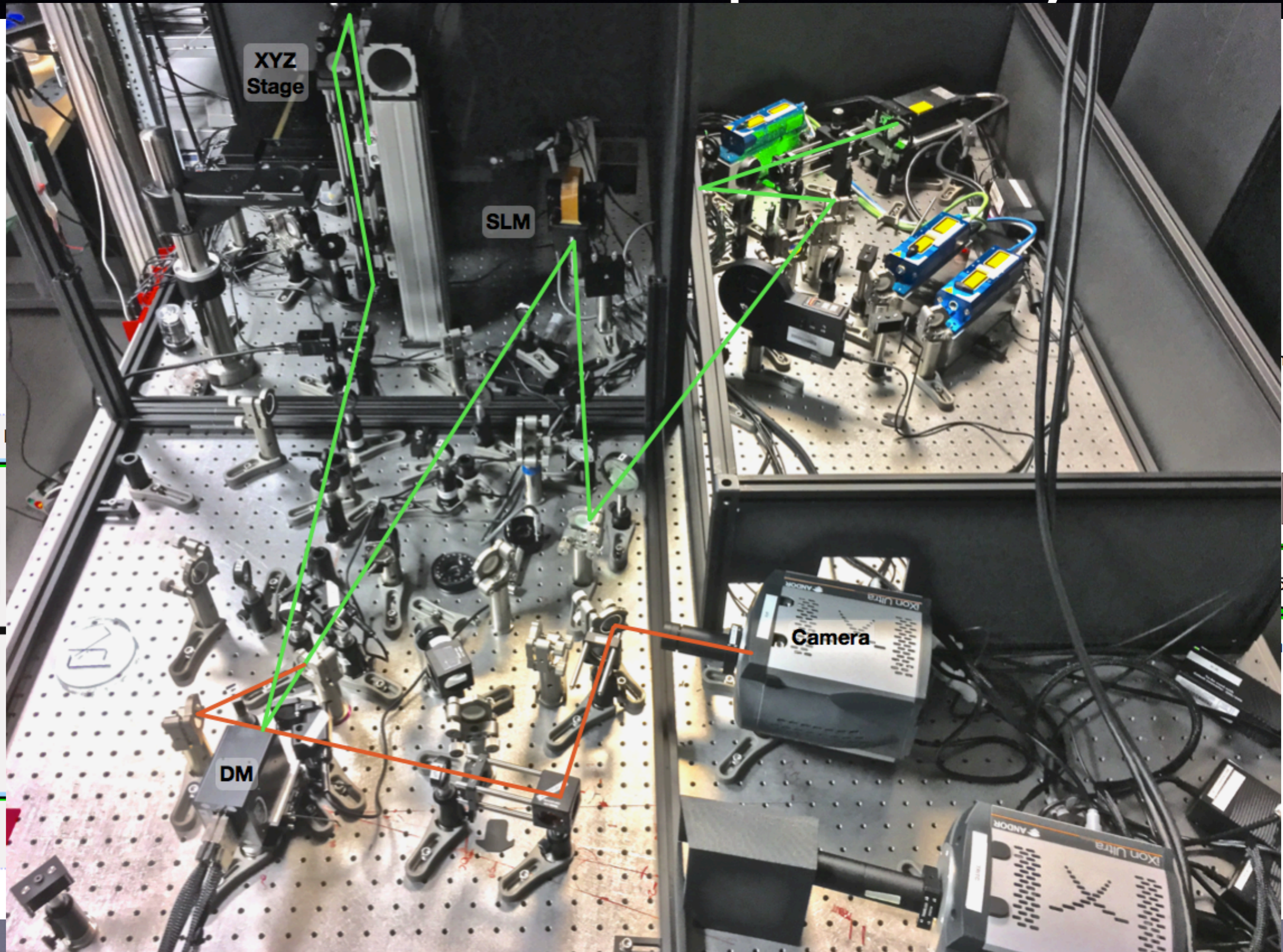


NMJ after correction

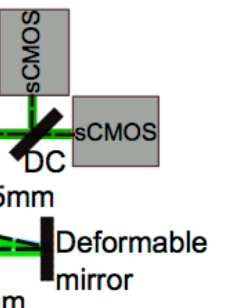
Start with simple design



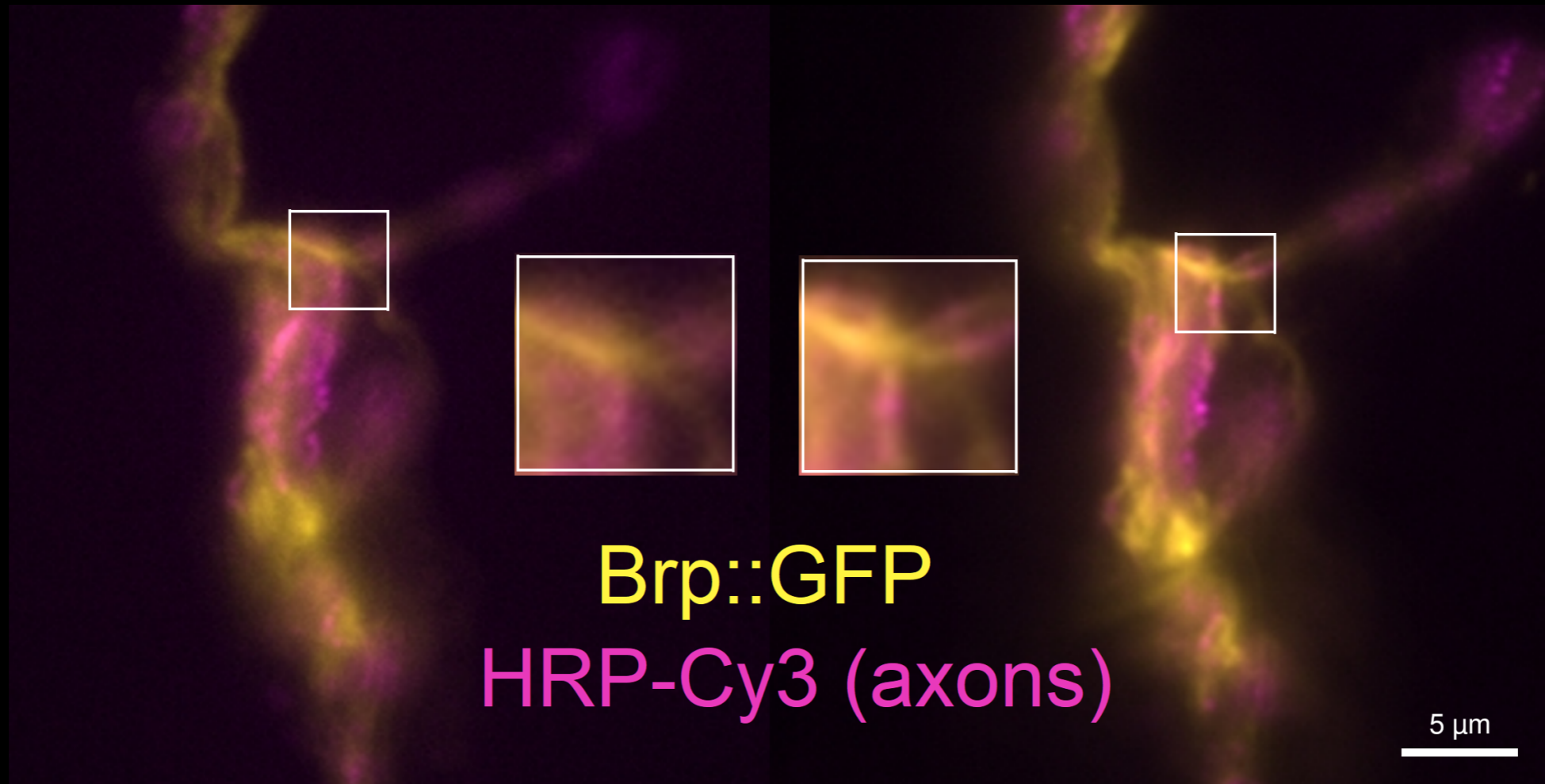
Add complexity



Detection path



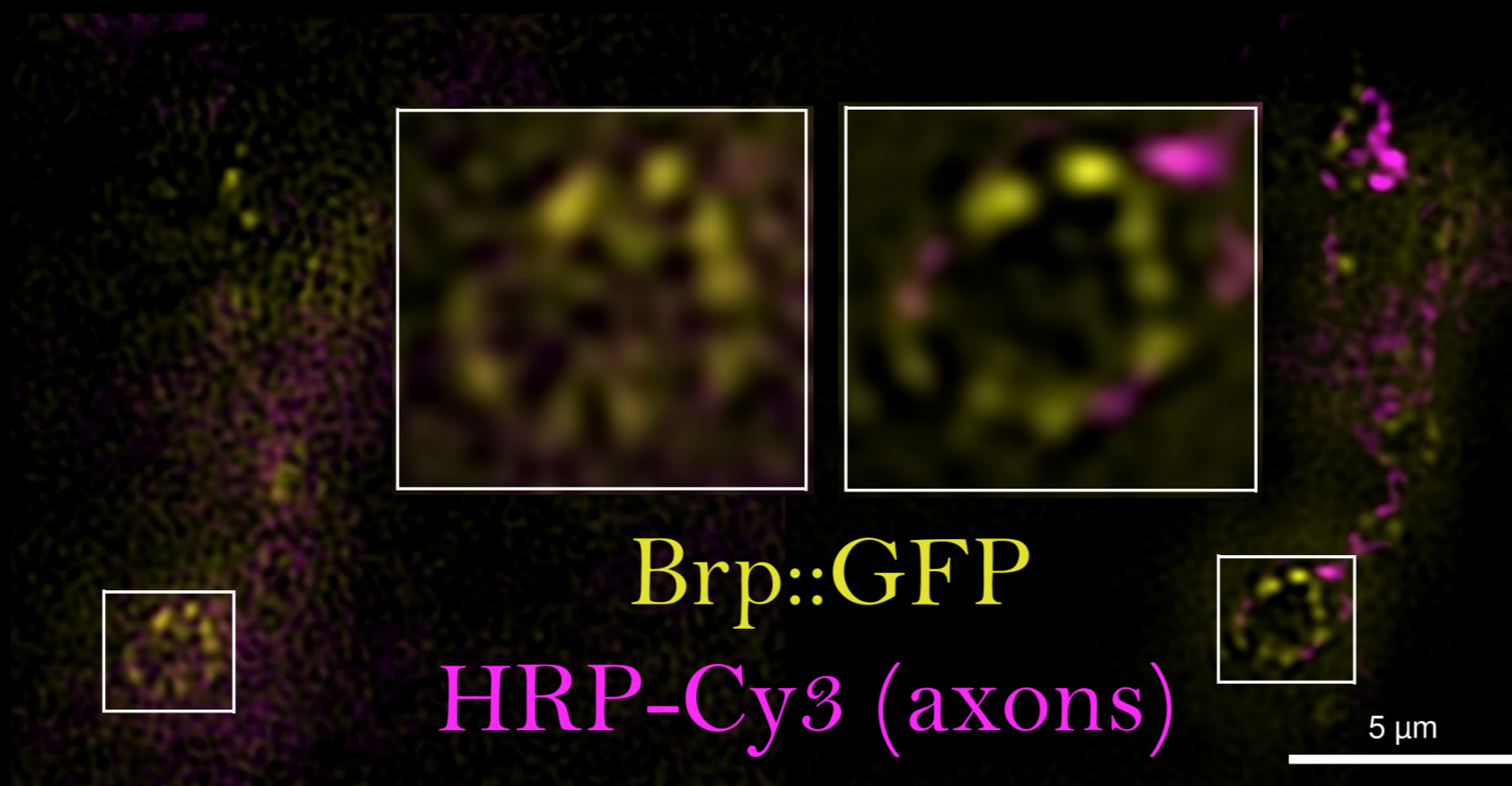
Drosophila Neuro-muscular Junction: Pseudo-widefield



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

Cockpit

ABORT Single-site experiment Multi-site experiment View last file Video mode Light path Snap image Objective 60xwater

Cameras

East-Green <input checked="" type="checkbox"/>	West-Red <input checked="" type="checkbox"/>	interference <input checked="" type="checkbox"/>
Readout mode: EMCCD 17 MHz	Readout mode: EMCCD 17 MHz	Readout mode: default
Gain: 0	Gain: 0	Gain: None
settings	settings	settings

dm AO set-up

Select ROI Calibrate Characterise

PiDIO Excitation path: conventional SI DM bypass

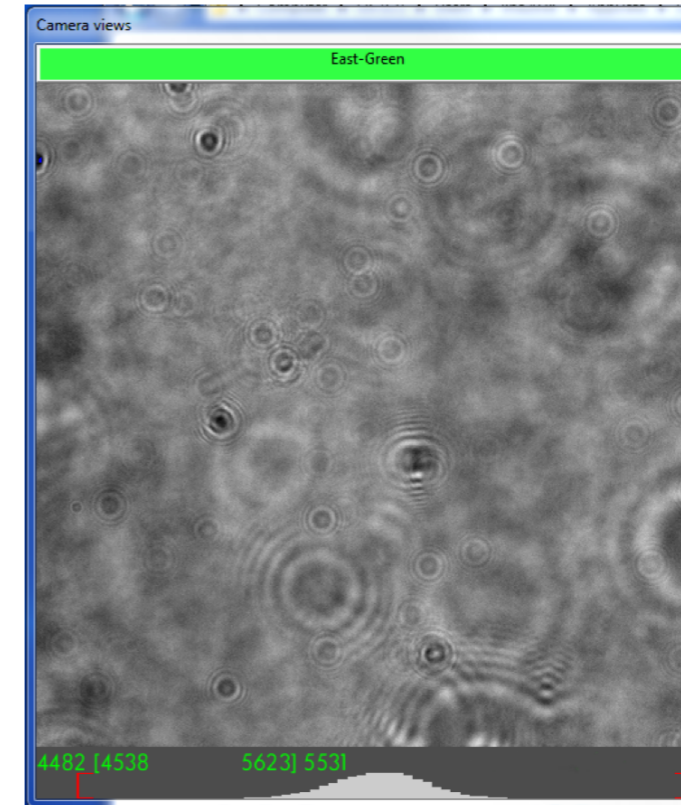
slm slm step

AO use

Reset DM System Flat Visualise Phase Apply last pattern Sensorless AO

Lights

trans <input checked="" type="checkbox"/>	ambient <input checked="" type="checkbox"/>	488 <input checked="" type="checkbox"/>	561 <input checked="" type="checkbox"/>
exposure / ms: 100	exposure / ms: 100	exposure / ms: 100	exposure / ms: 100
		power / mW: 150.0	power / mW: 70.1



Macro Stage XY Macro Stage Z Experiment Histogram

X: 1494.90 1494.90 step: 1.00um
Y: -6019.40 -6019.40 step: 1.00um

Z: -2808.50 125.00 step: 50.00um

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

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

How expensive is it?

Building costs

Hardware ~£100-250k

Salaries 1-3 years (~£50-£150)

Total cost ~£150-350k

Commercial OMX system ~£400k

Summary

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