Bespoke Microscopes

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Overview

- Image formation
- Beads and spherical aberration
- Adapting existing systems - Super fast acquisition
- Bespoke microscope example - DeepSIM
- Bespoke microscope design - pro's and cons

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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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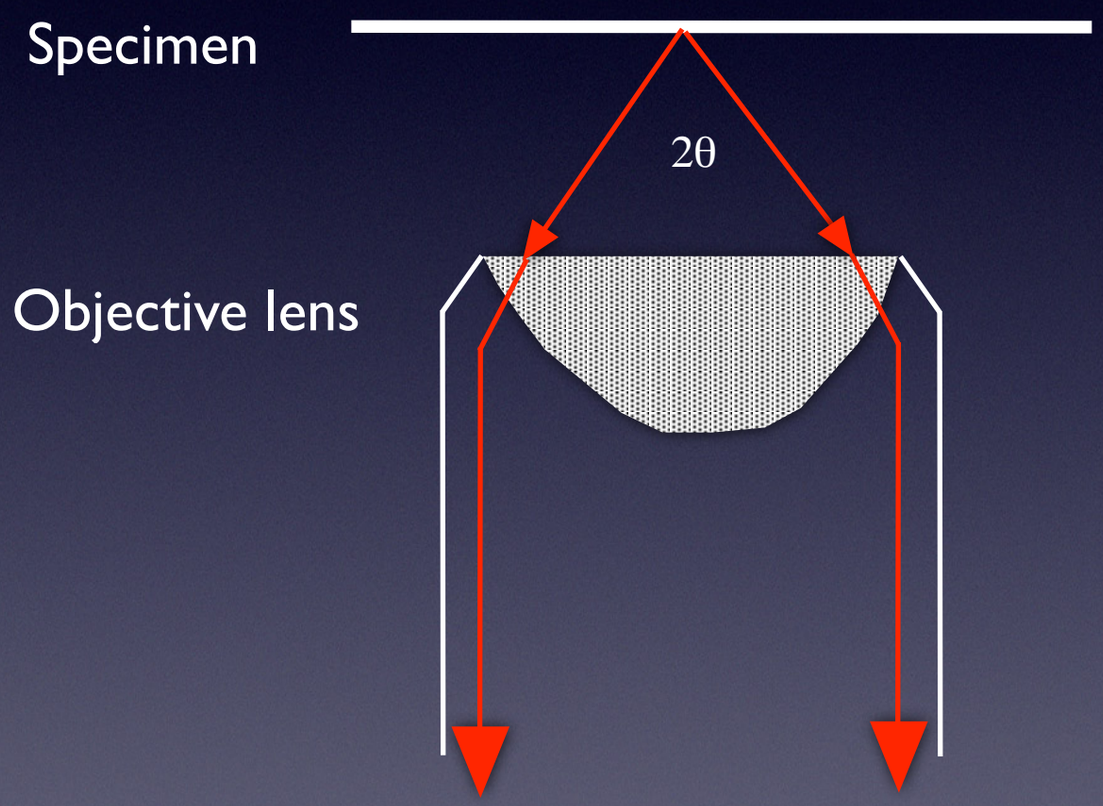
Microscope Resolution

- 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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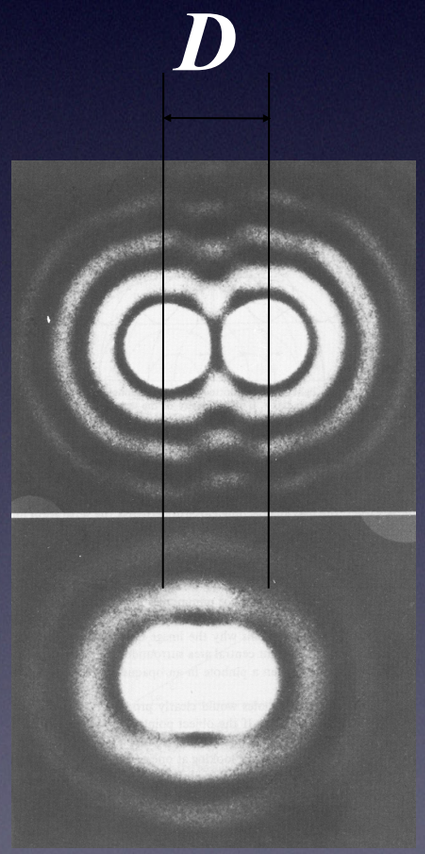
Resolution: A technical definition, the Rayleigh Criterion



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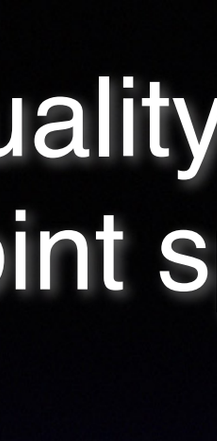
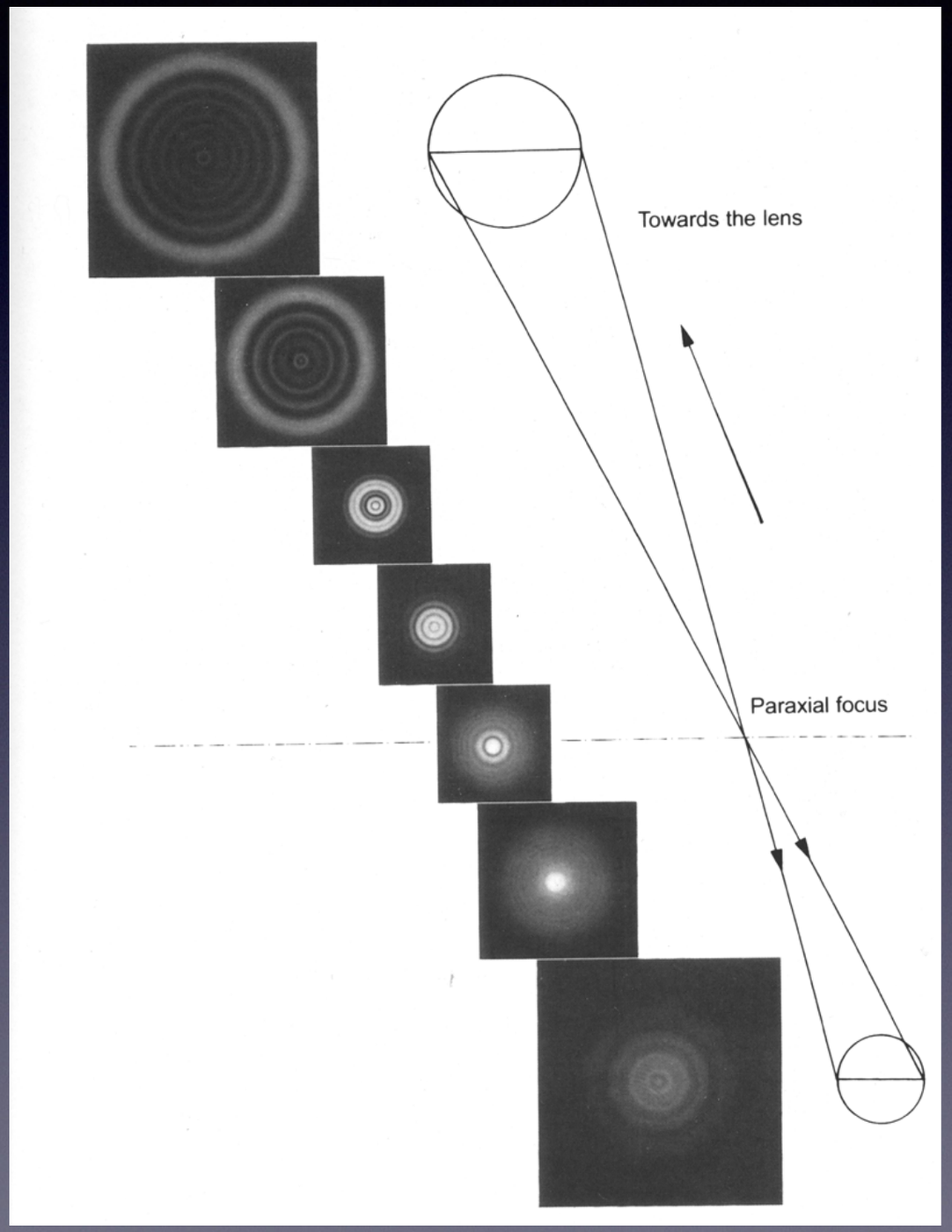
Image quality- the problem of "out-of-focus light" point spread function and airy rings

Sample object: a "sub-resolution" fluorescent bead





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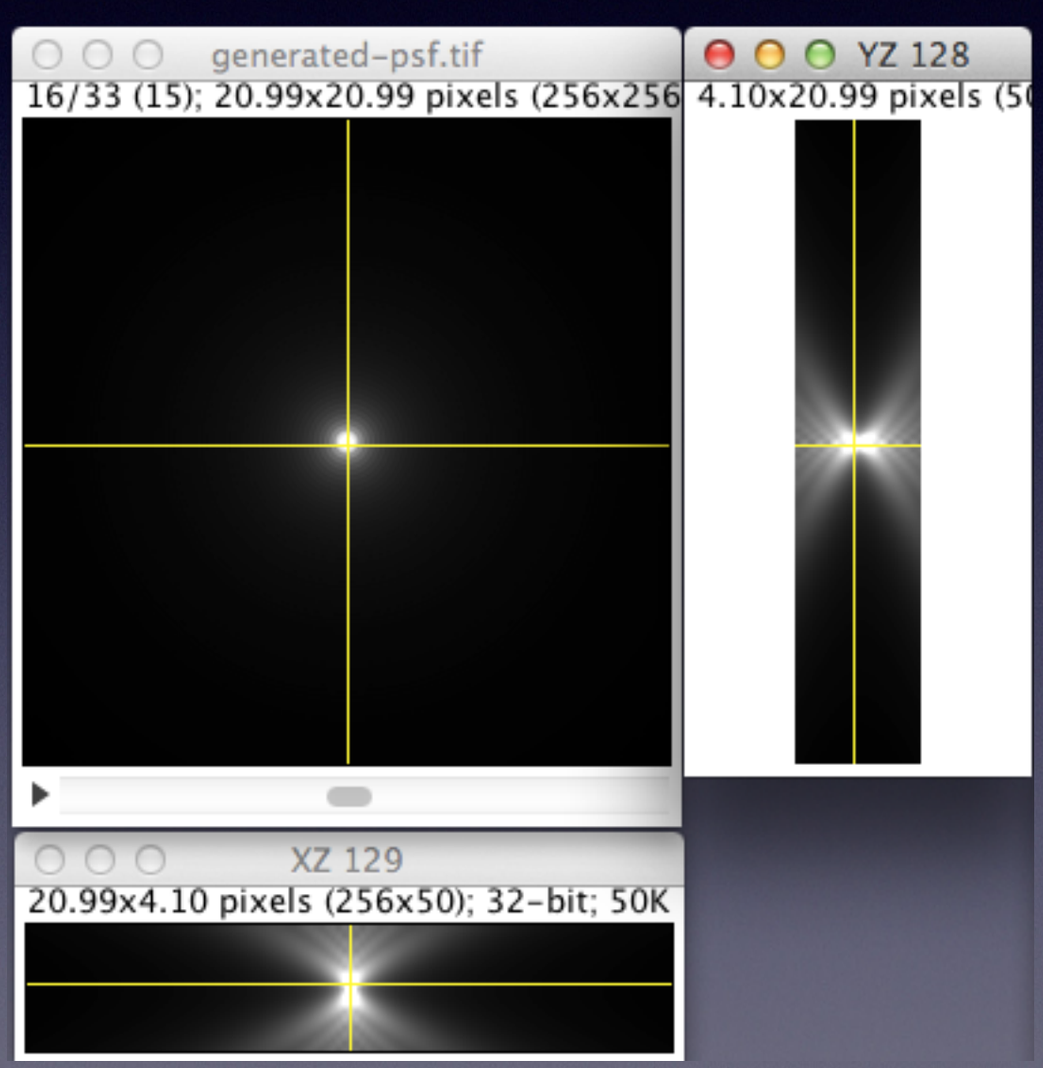
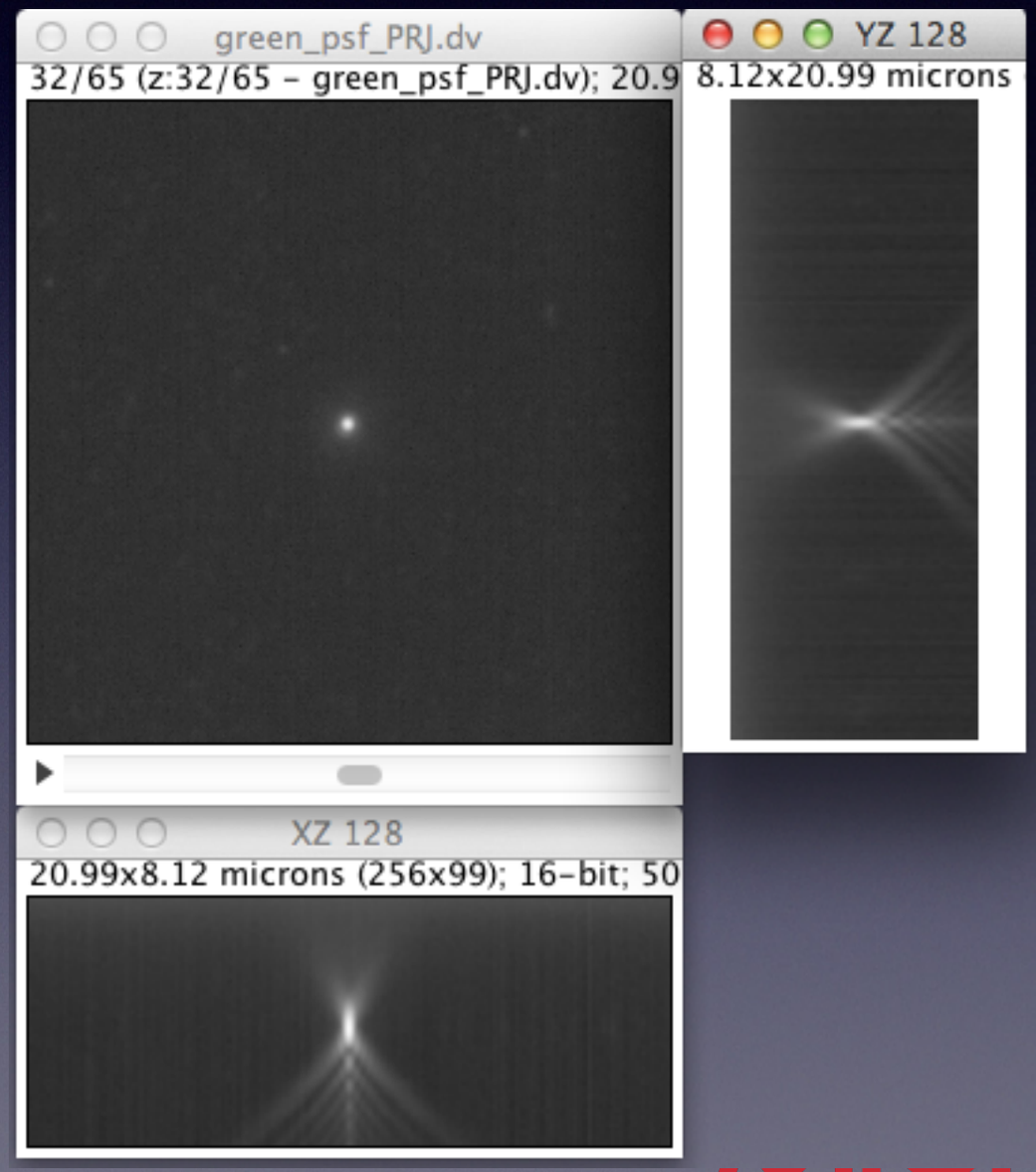
Theoretical and measured PSF Orthogonal views

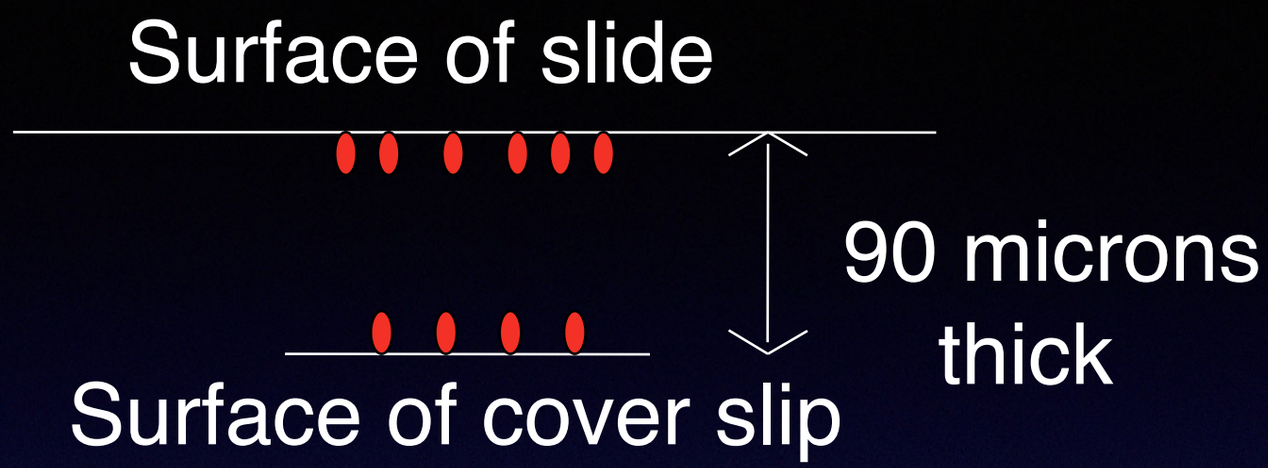


Generated PSF



Real PSF

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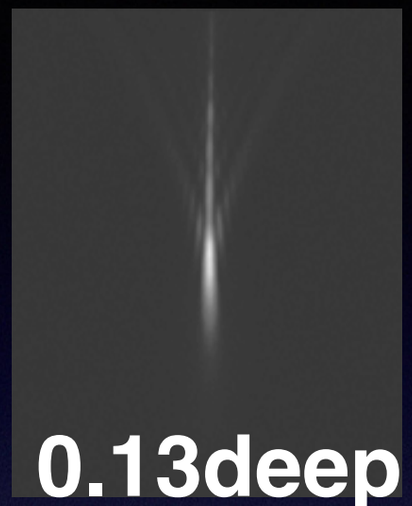
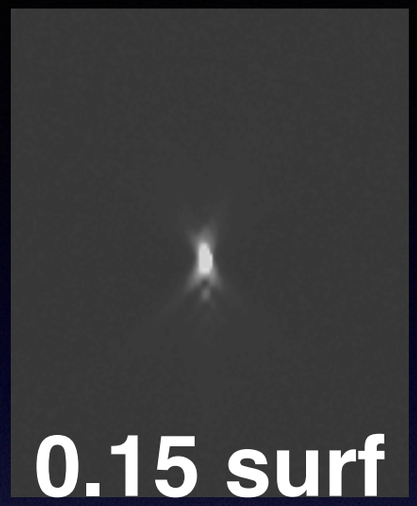
Affects of deep imaging (90μm) and collar settings on spherical aberration and psf of 60X/NA1.2w

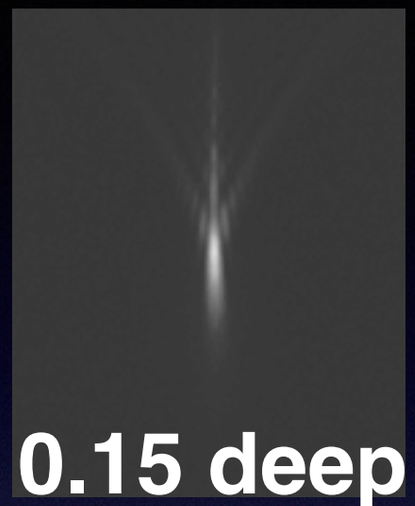
0.13 surf



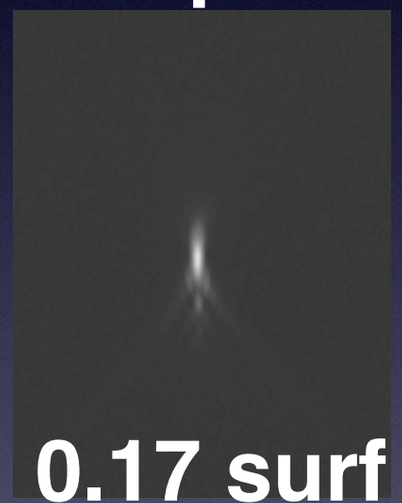
0.13deep



0.15 surf



0.15 deep



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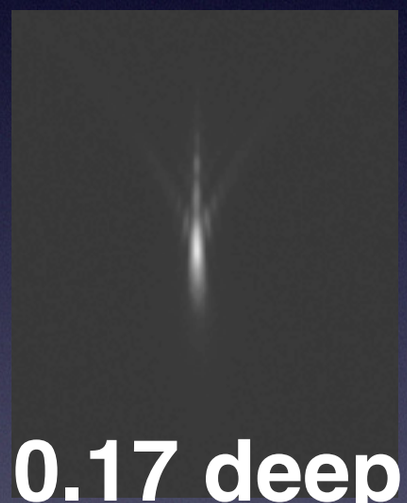
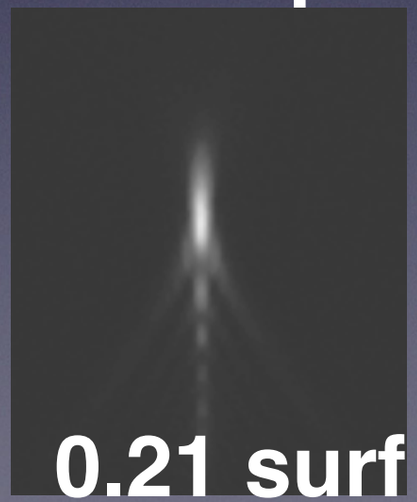


0.17 deep

Data from
Alejandra Clark

0.19 surf

0.19 deep



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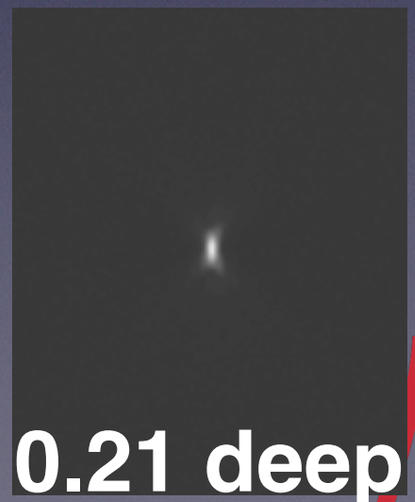
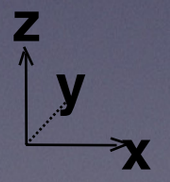


0.21 deep



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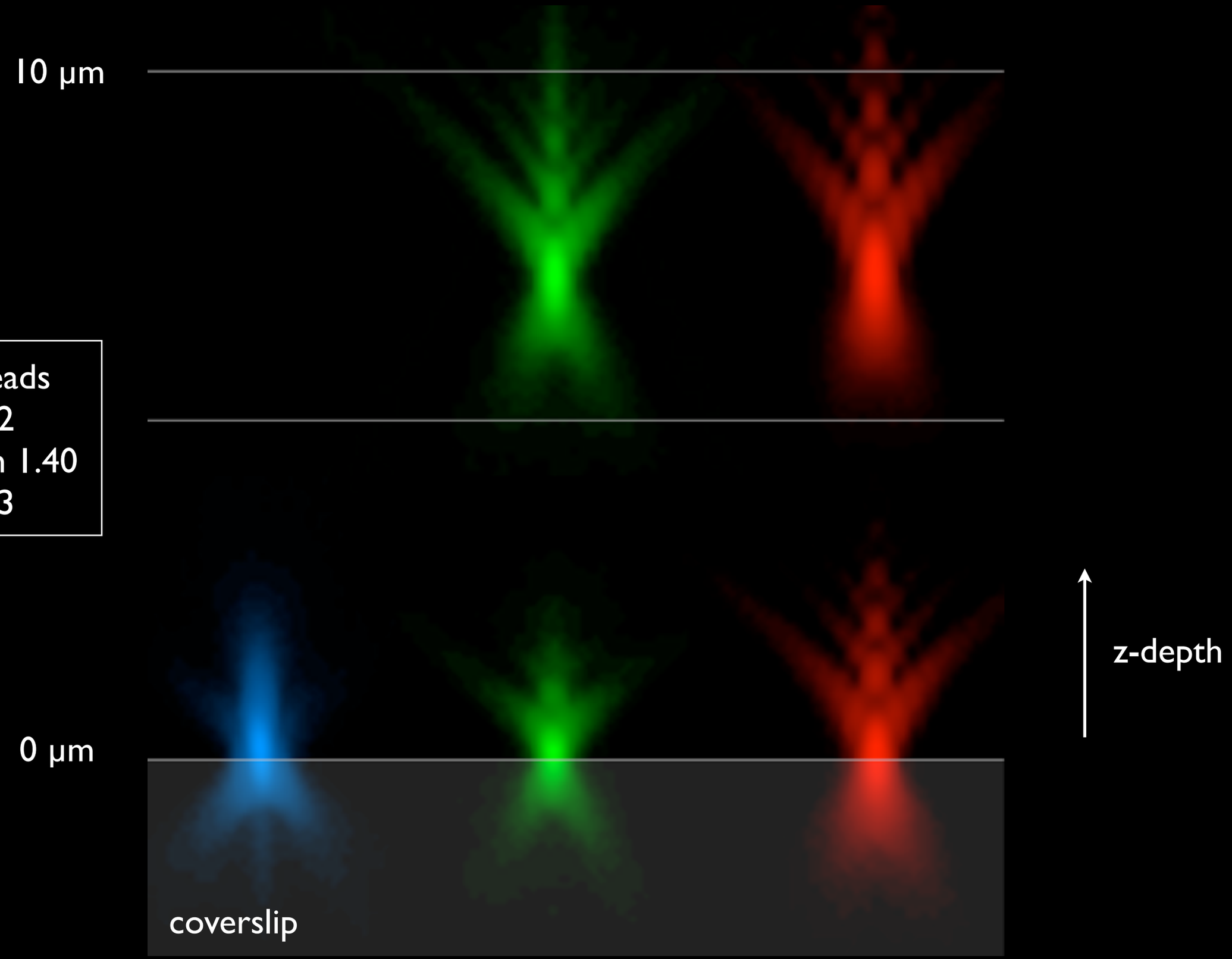
Spherical aberration dependent on wavelength, depth, RI



Adapting Existing Systems - Super Fast Acquisition (FastZ)

- Ramp the Z position instead of stepping it
- Take images as fast as possible during ramp
- Delay between stacks to allow stage to return to initial position

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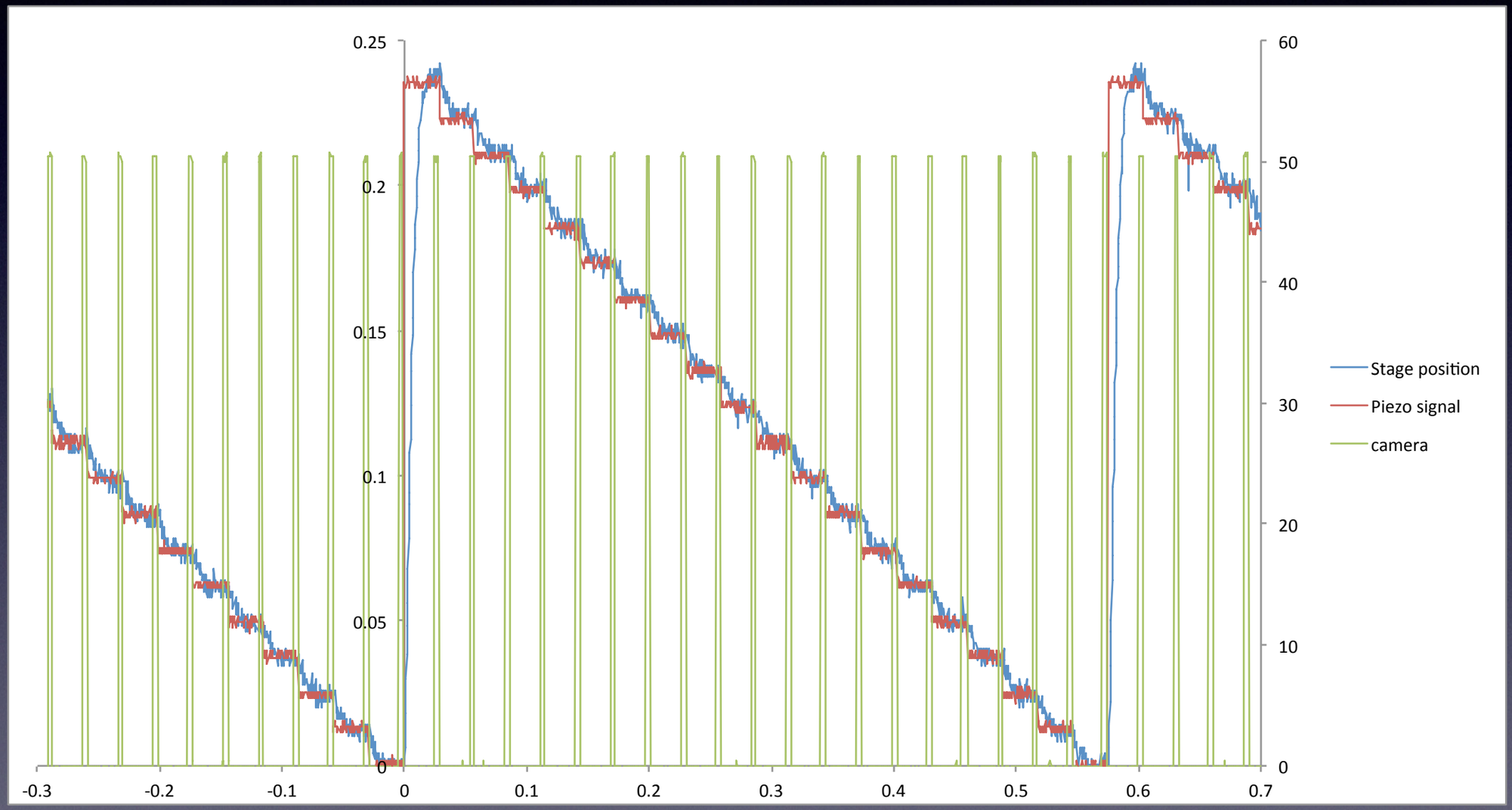
Covential widefield Z stack



20 Z planes as fast as possible

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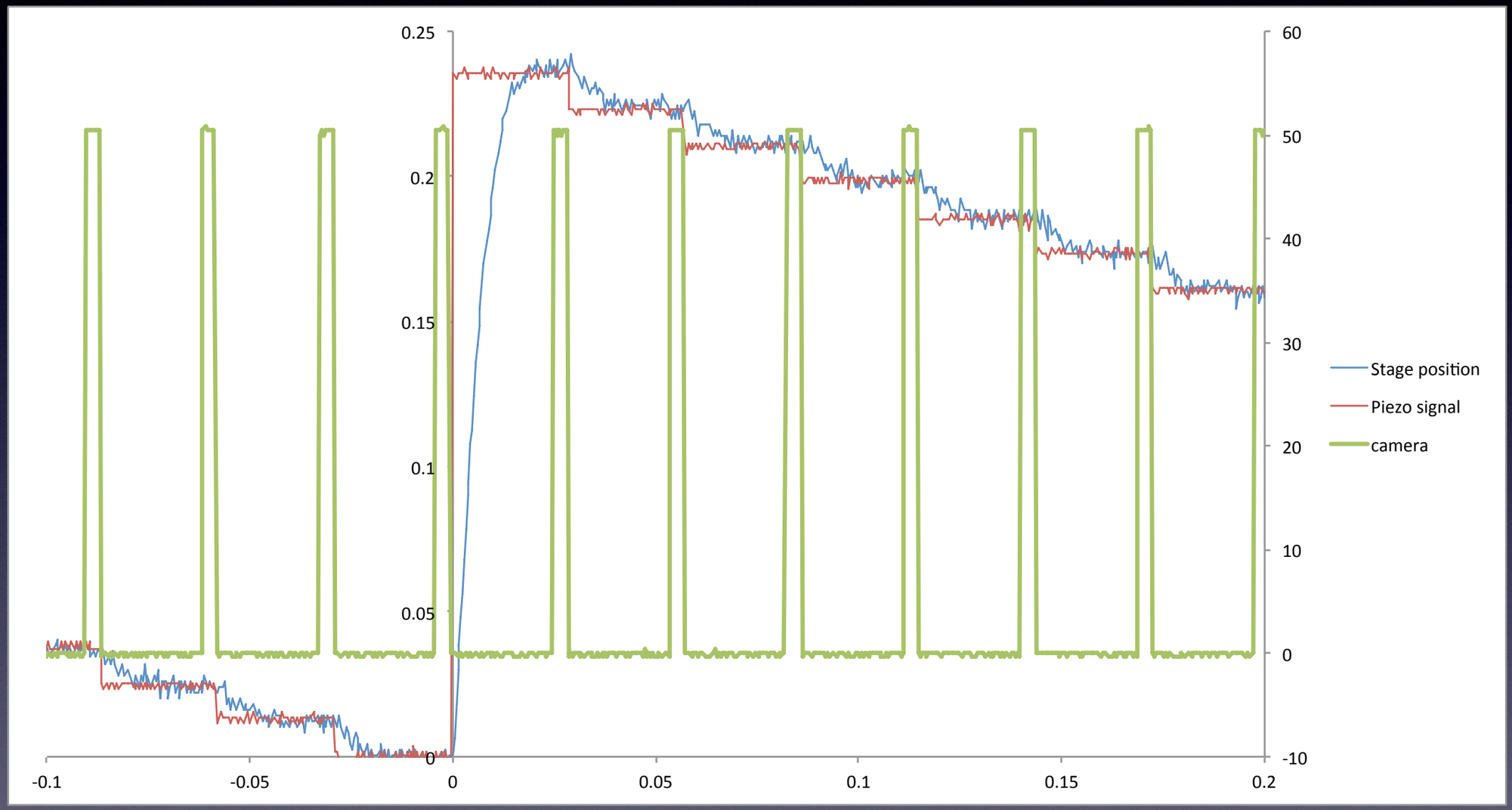
Covention al Z stack



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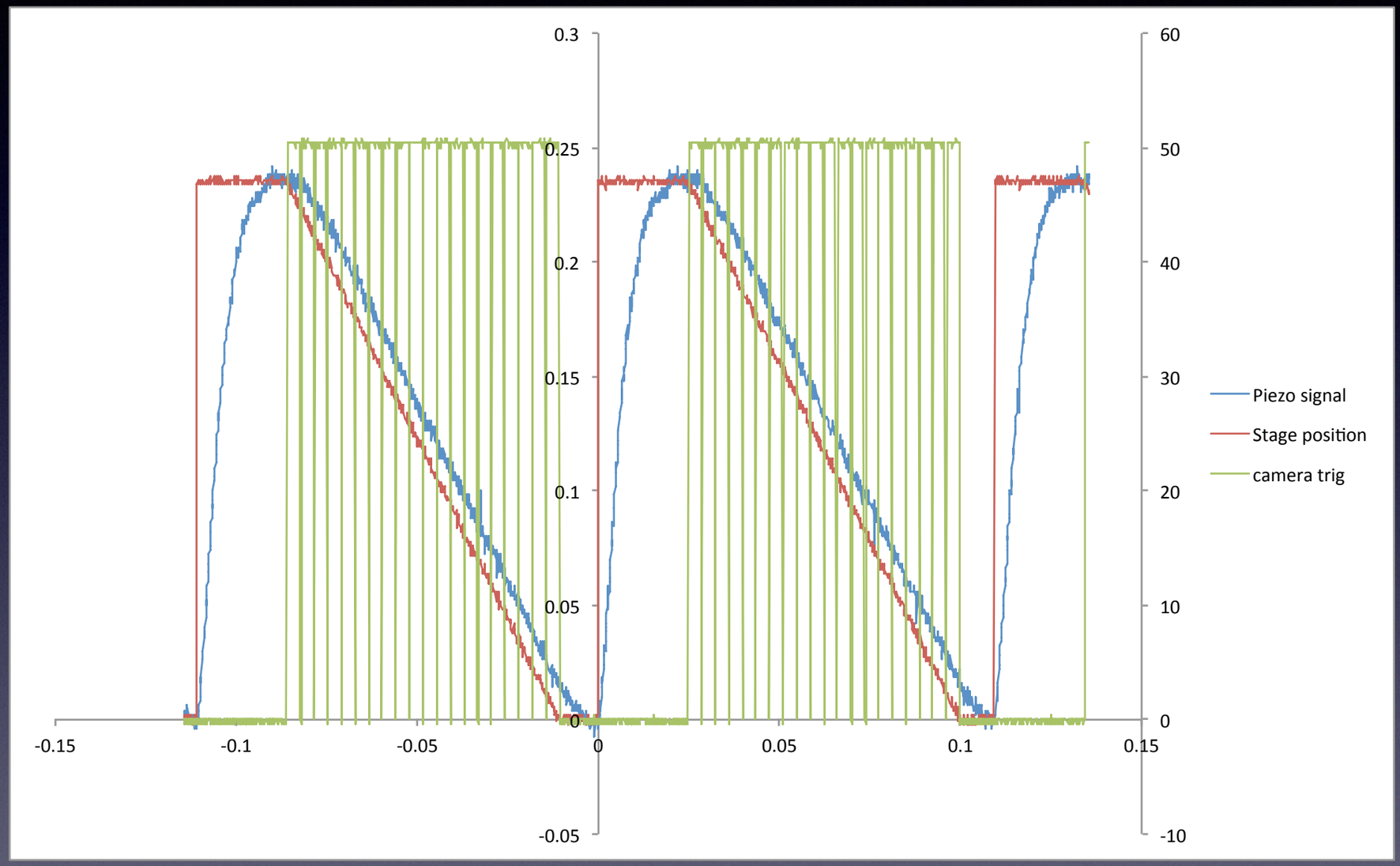
Ramp Z stack



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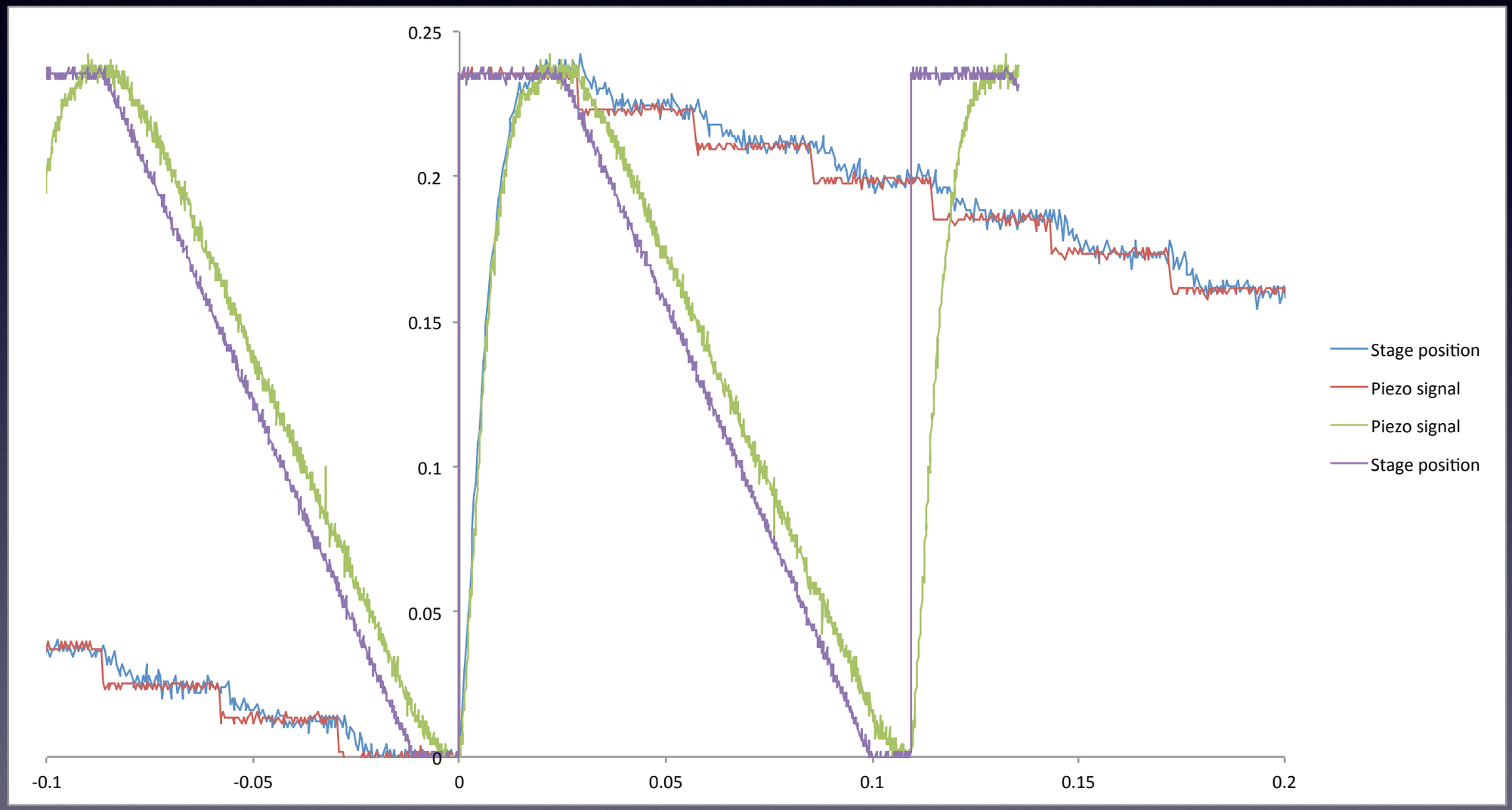
Comparison: FastZ to normal



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Speed increases

- Depends on stack height, image size, exposure time.
- Test sample, 512x512 pixel images, 1 ms exposure 20 Z slices of 200 nm.
- Conventional cycle time = 575 ms
- FastZ cycle time = 109 ms

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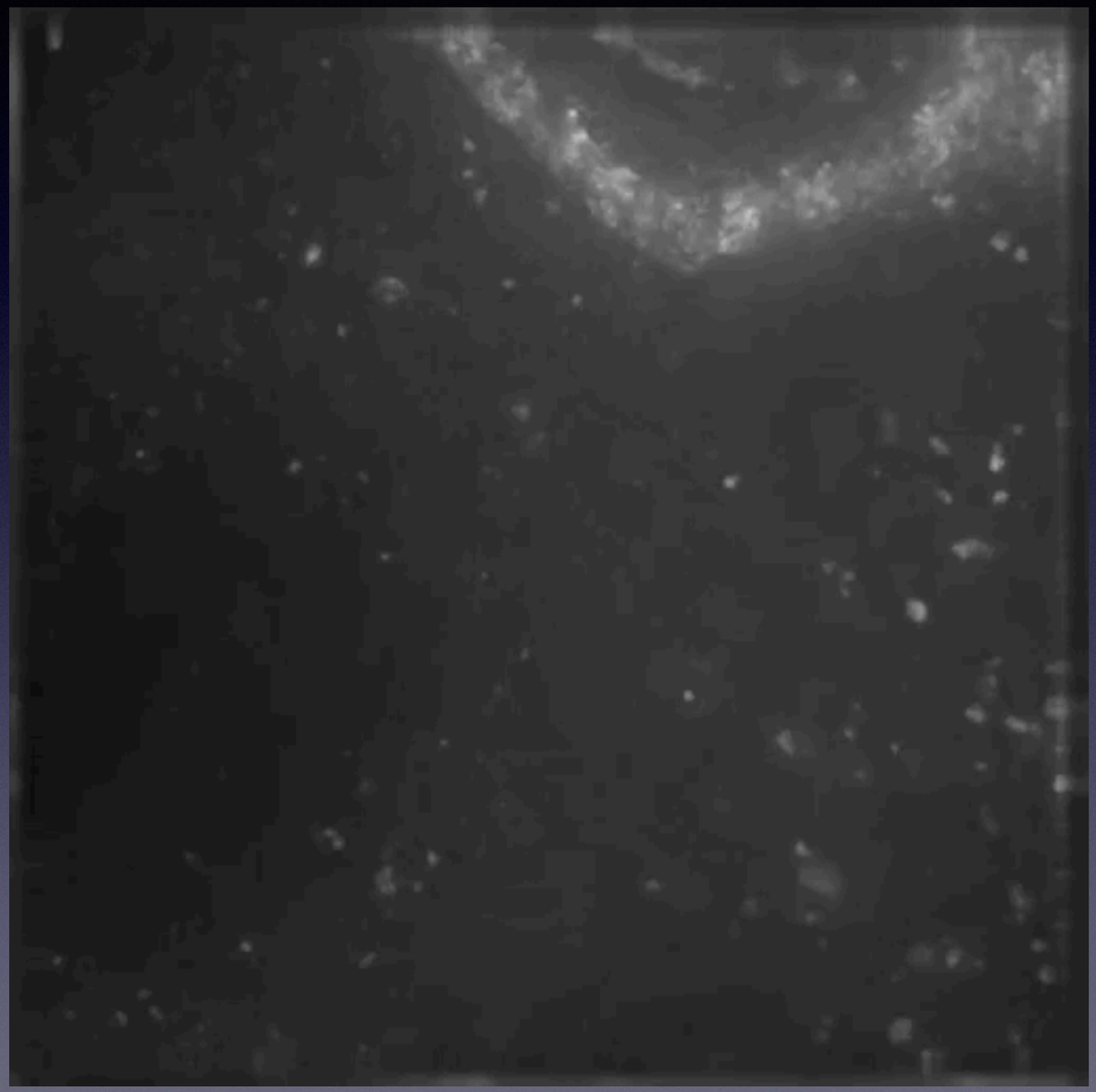
FastZ - Results



Me31B-GFP Drosophila oocyte
25-slices, 8 stacks/s - 200 frames/s

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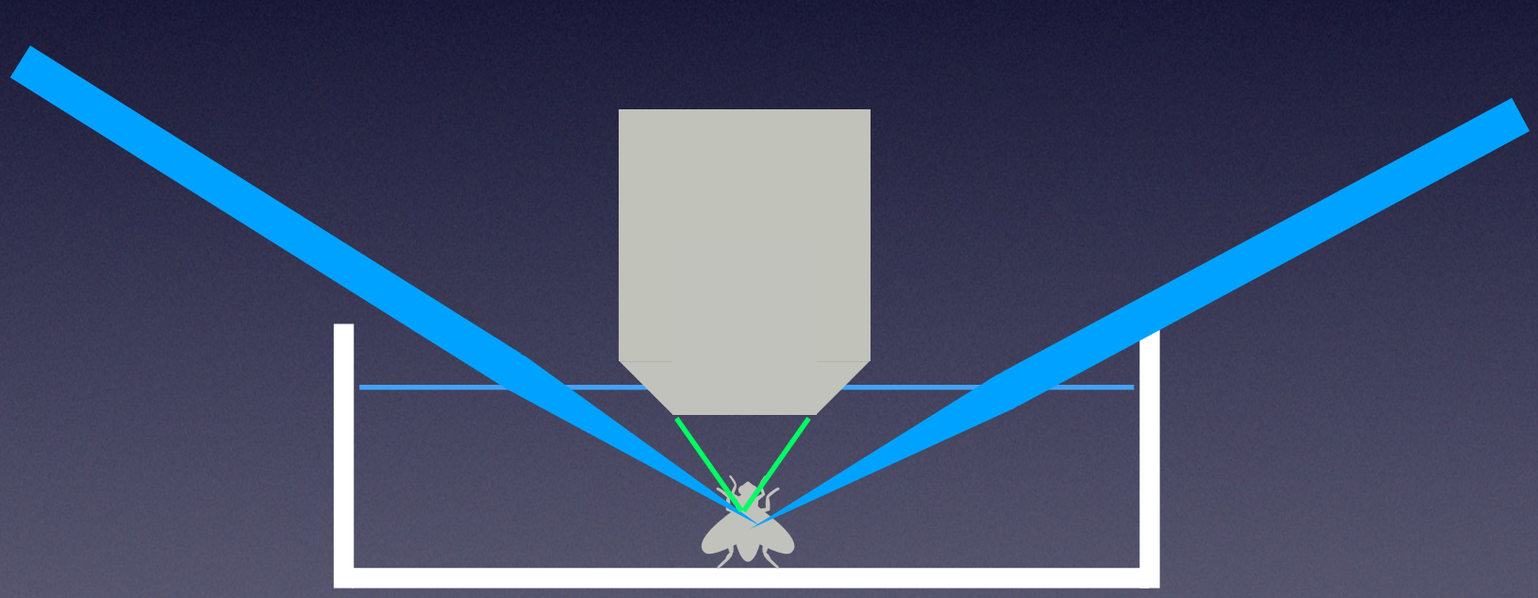
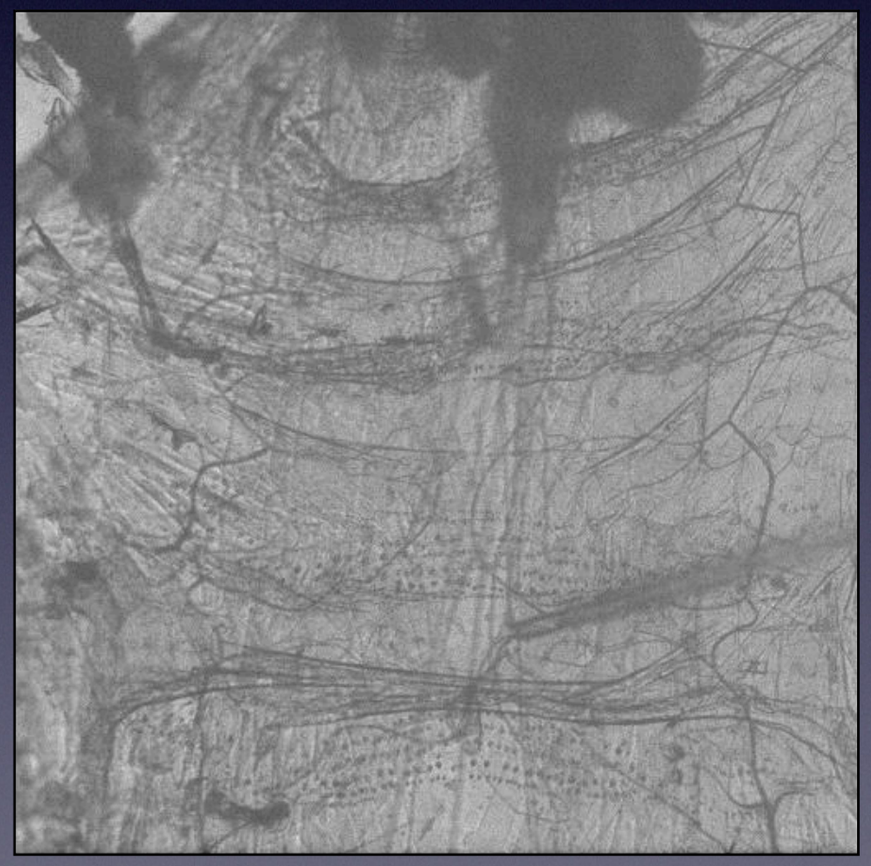
Bespoke Microscope Example - DeepSIM

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

Live imaging

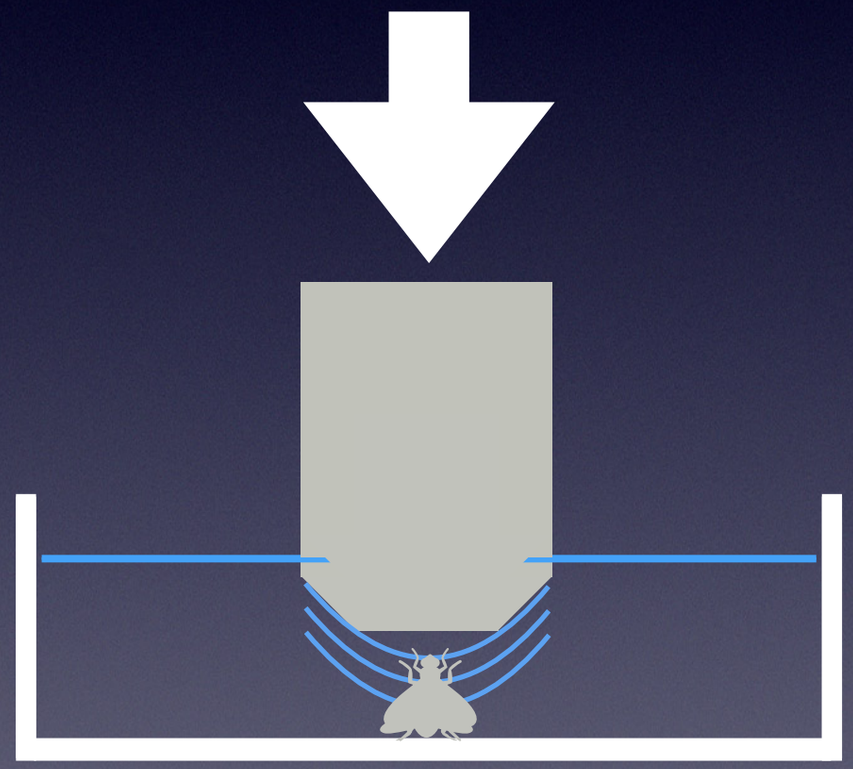


Upright microscope

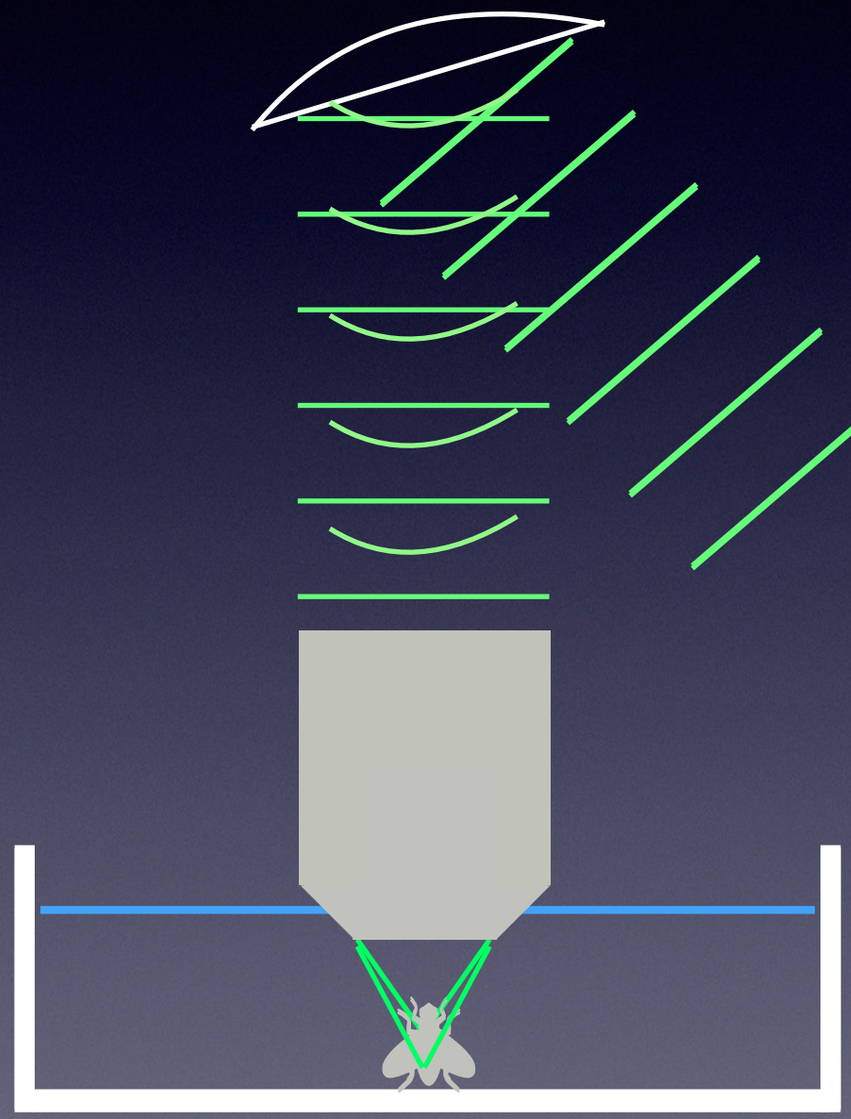


Fast imaging

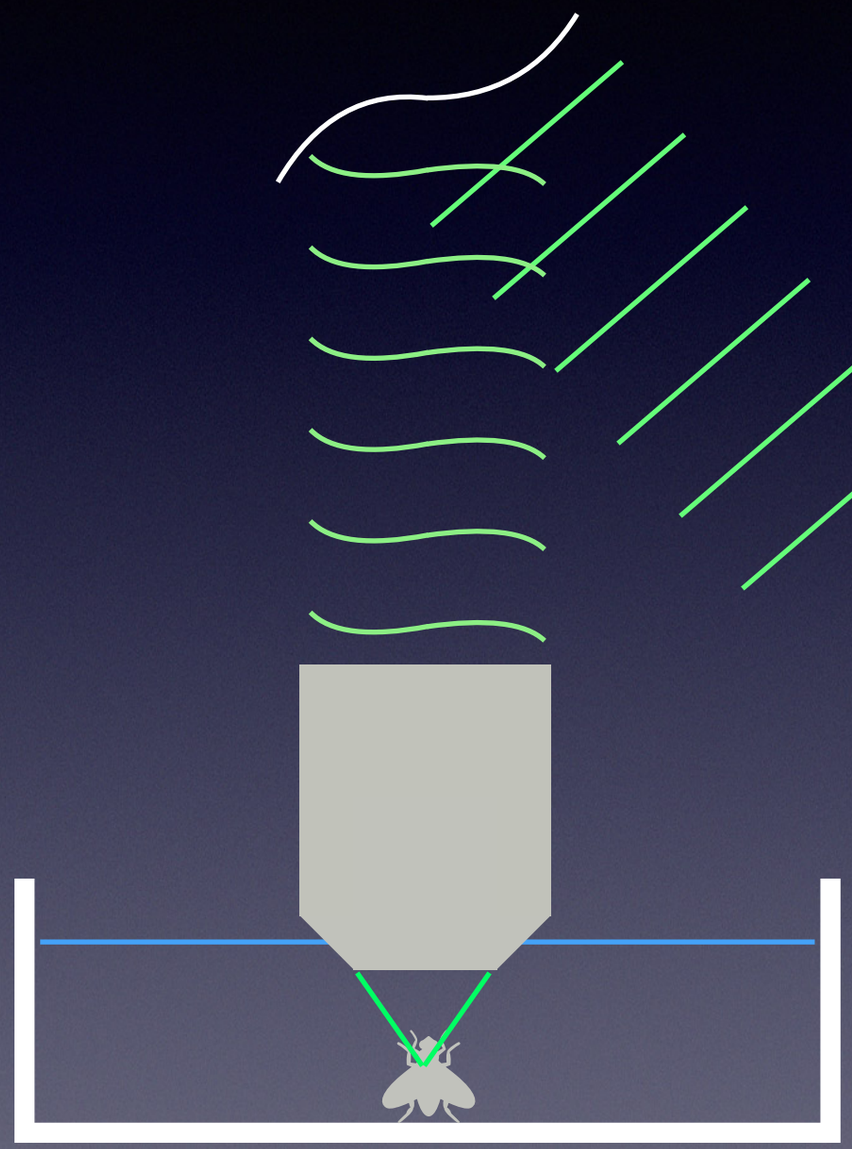
Fast Z movement issues



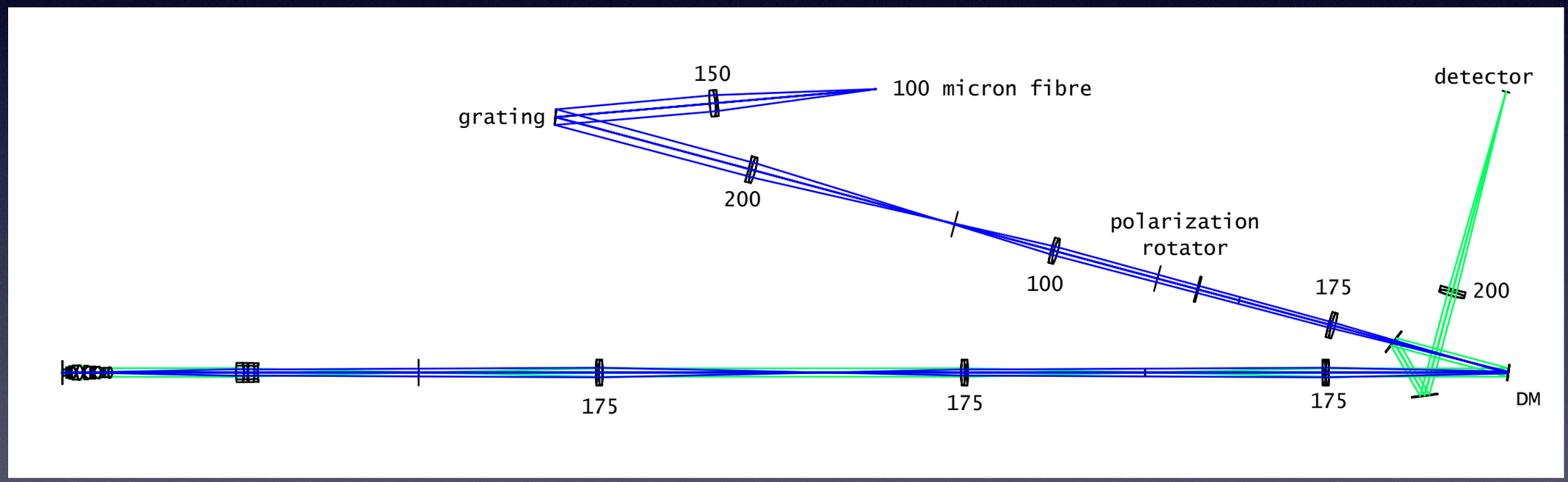
AO - Remote Focus



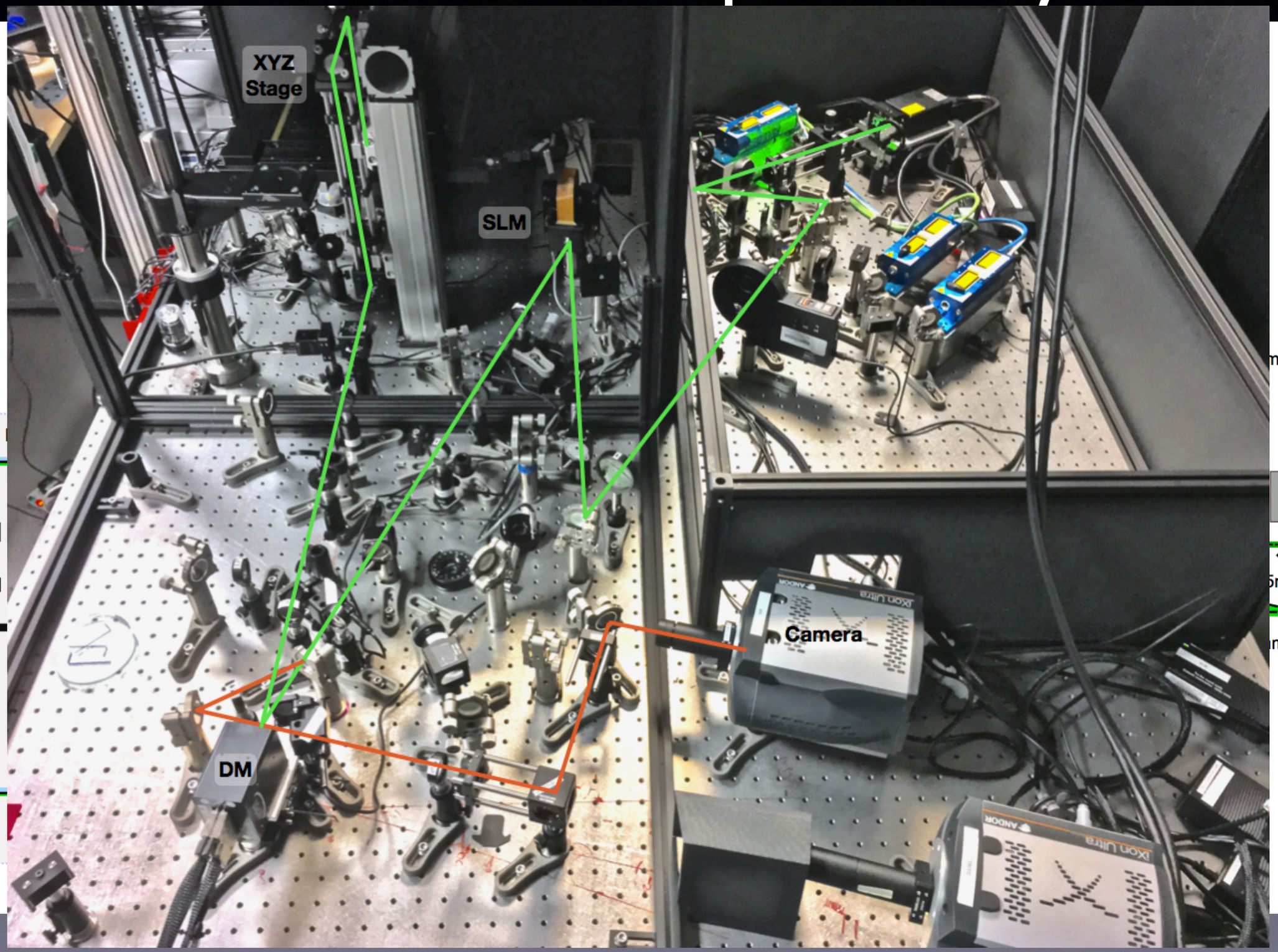
AO - Aberration correction

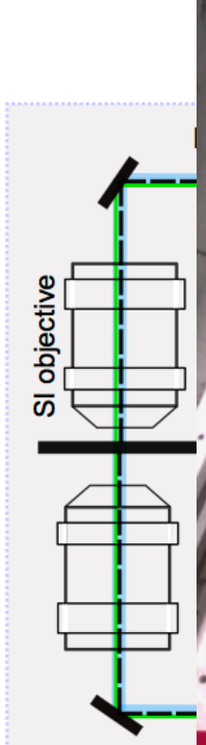


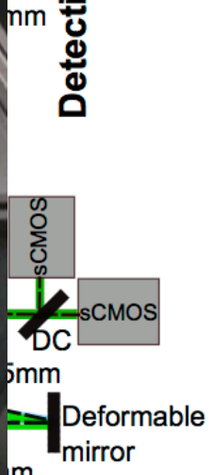
Start with simple design



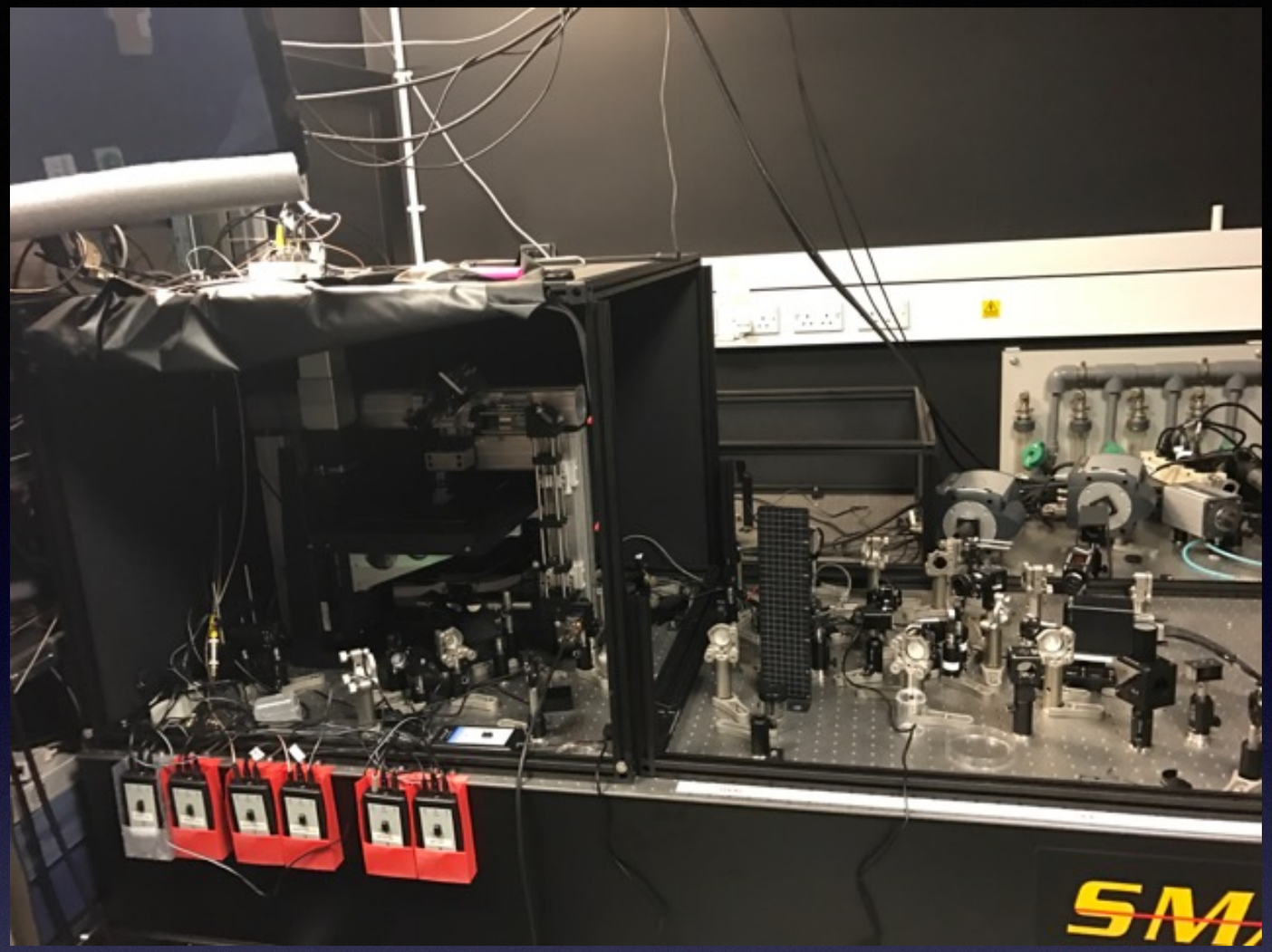
Add complexity





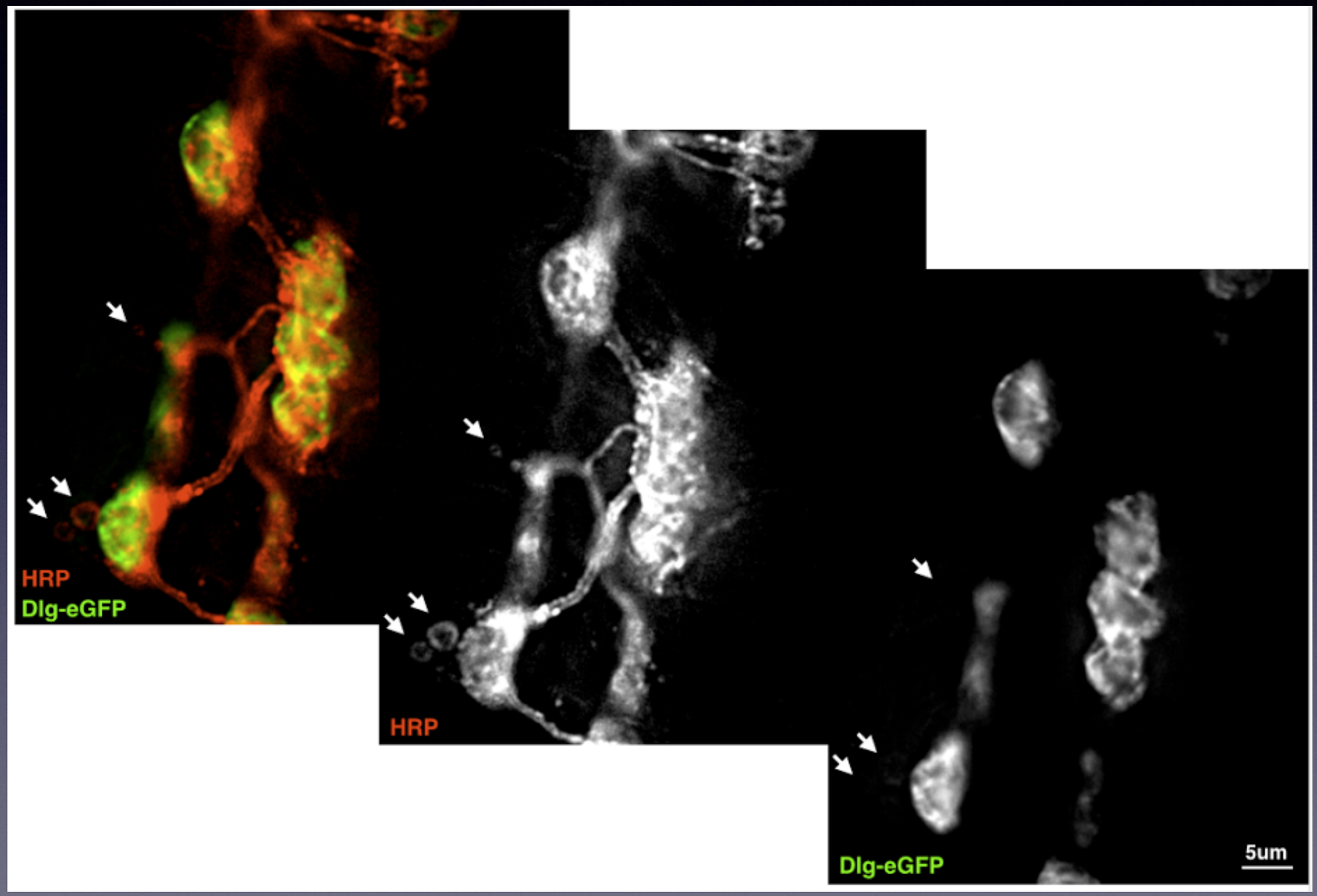


DeepSIM

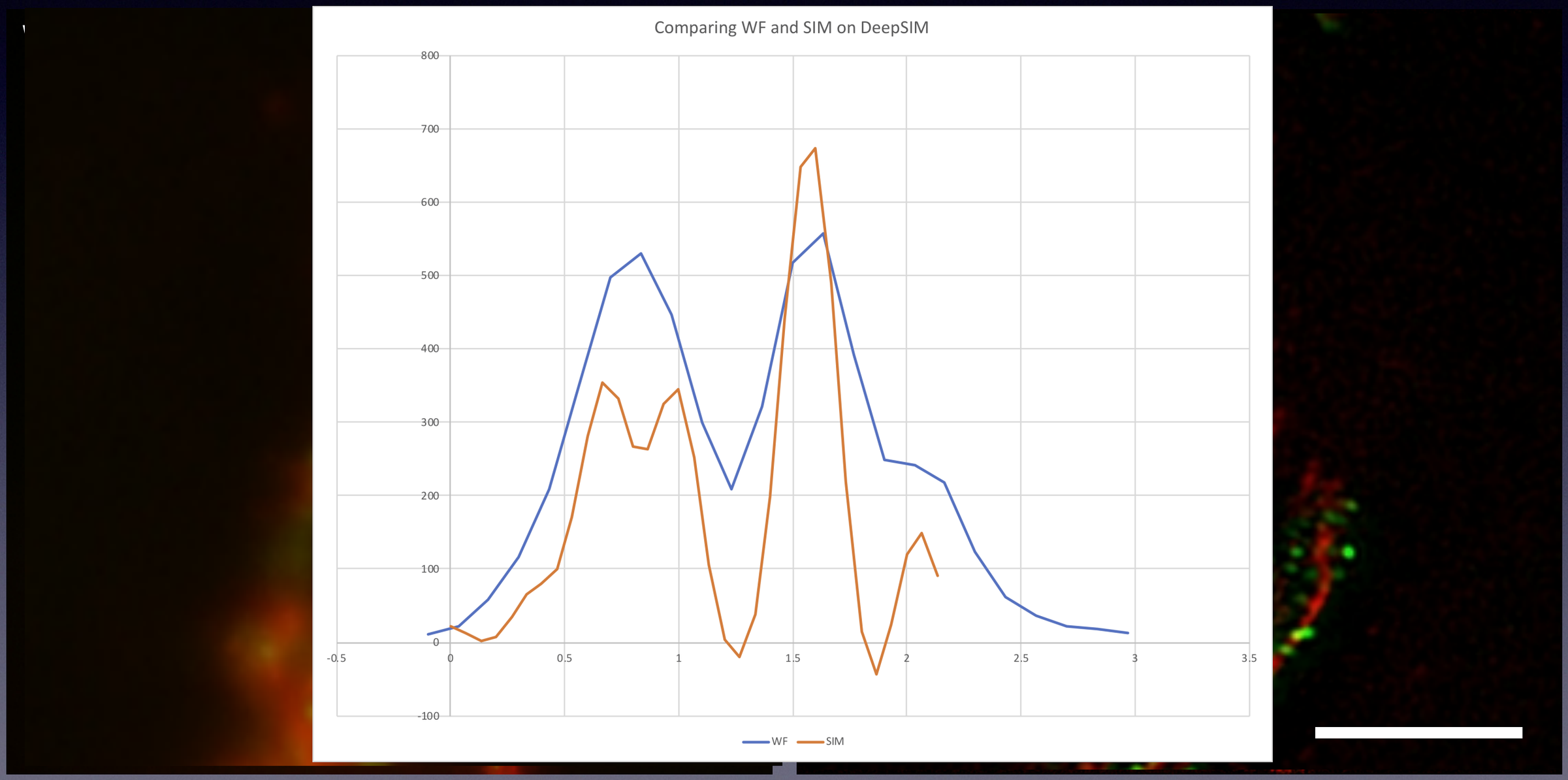


- System built and working for WF and SIM with Adaptive Optics
- Starting real biological experiments

2 channel live imaging



DeepSIM AO images



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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Example of Bespoke Microscopes

DeepSIM microscope
Designed and built by John Sedat, Antonia Göhler, Mick Phillips, Ian Dobbie and Mantas Zurauskas, Micron Oxford

Live PALM microscope
Designed and built by Stephan Uphoff and Achillefs Kapanidis, Micron Oxford

WOSM
Designed and built by Nick Carter and Rob Cross, Warwick University

Openspim
Designed and built by Pavel Tamacek and his team at Dresden MPI

CryoSIM microscope
Designed and built by Mick Phillips and Ian Dobbie at Diamond Light Source

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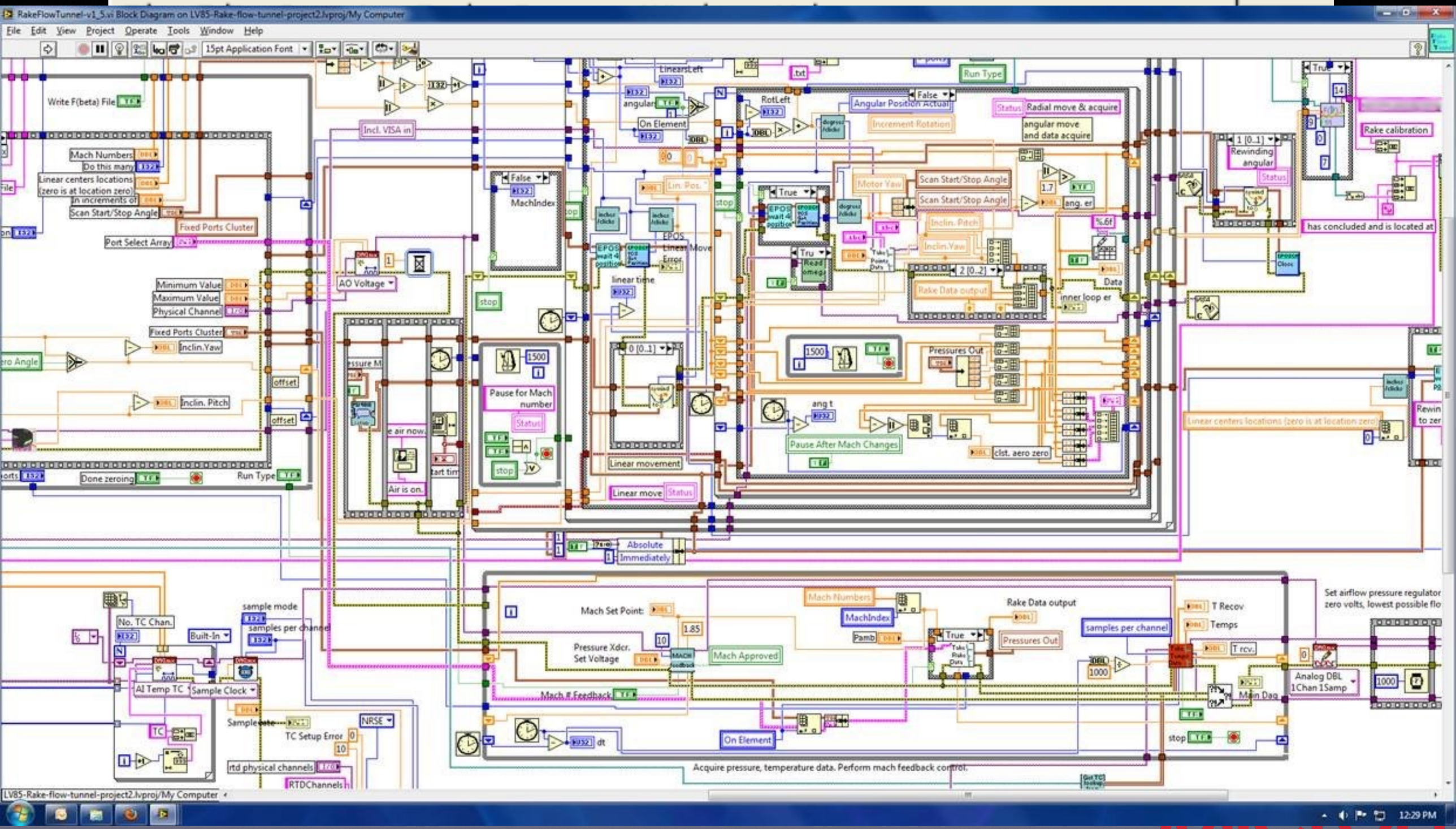
Software options

- Lab view
- Micromanager
- DIY: SDKs - C++, Python, Visual basic

Lab view example



Micromanager

http://valelab.ucsf.edu/~MM/MMwiki/

µManager

THE OPEN SOURCE MICROSCOPY SOFTWARE

OVERVIEW · DOWNLOADS · DOCUMENTATION · DEVICES · PROGRAMMING · SUPPORT · EVENTS · CREDITS · LOG IN

welcome to micro-manager!

Micro-Manager Open Source Microscopy Software

µ-Manager

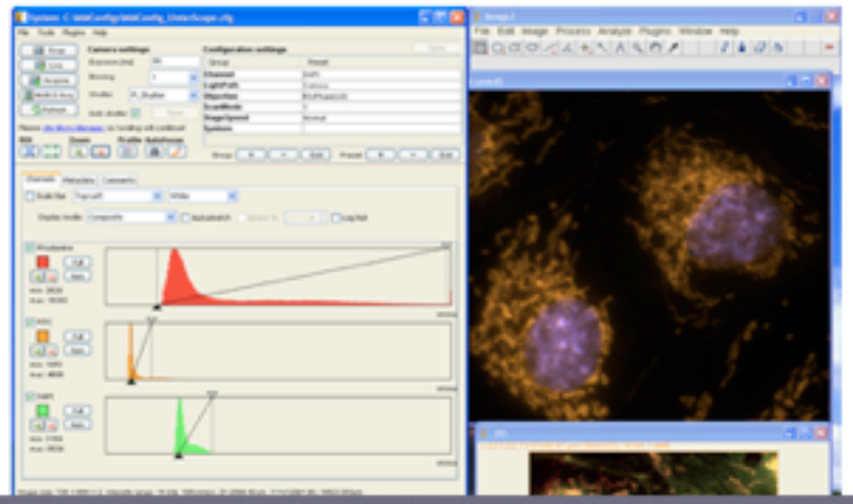
The Open Source Microscopy Software

µManager is a software package for control of automated microscopes. Together with the image processing application ImageJ, µManager provides a comprehensive, freely available, imaging solution.

News

- Micro-Manager Programmer Job Opening!
- ImageJ Conference
- [Open SPIM]
- Micro-Manager 1.4 Released
- Recap of Micro-Manager at 2011 ASCB meeting
- New Getting Started ScreenCast
- [Watch Micro-Manager in Action]
- Support for Nikon and

Download the most recent version (1.4) from our website. Also check out our ScreenCast for a quick tour on getting started.



µManager has a simple and clean user interface, through which it lets you execute common microscope image acquisition strategies such as time-lapses, multi-channel imaging, z-stacks, and combinations thereof. µManager works with microscopes from all four major manufacturers (Leica, Nikon, Olympus and Zeiss), most scientific-grade cameras and

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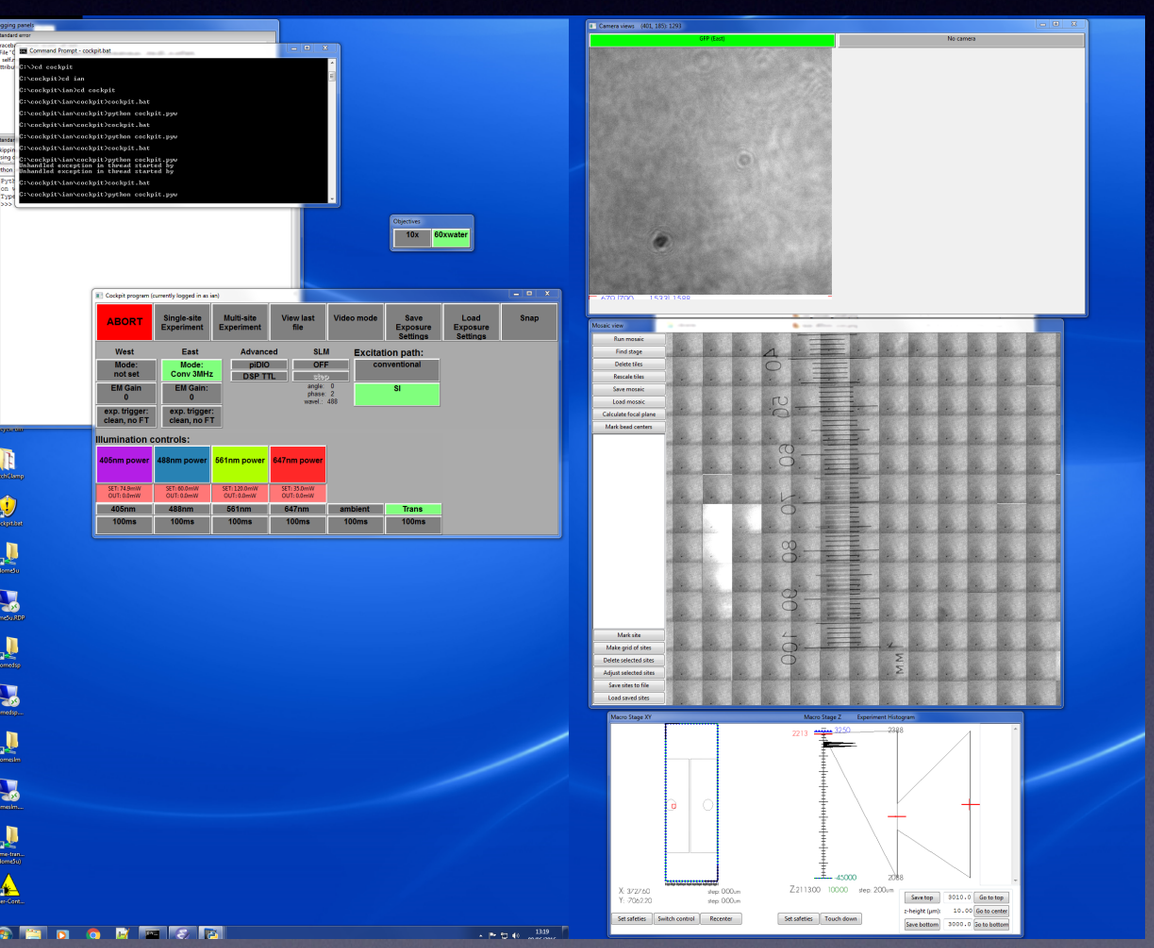
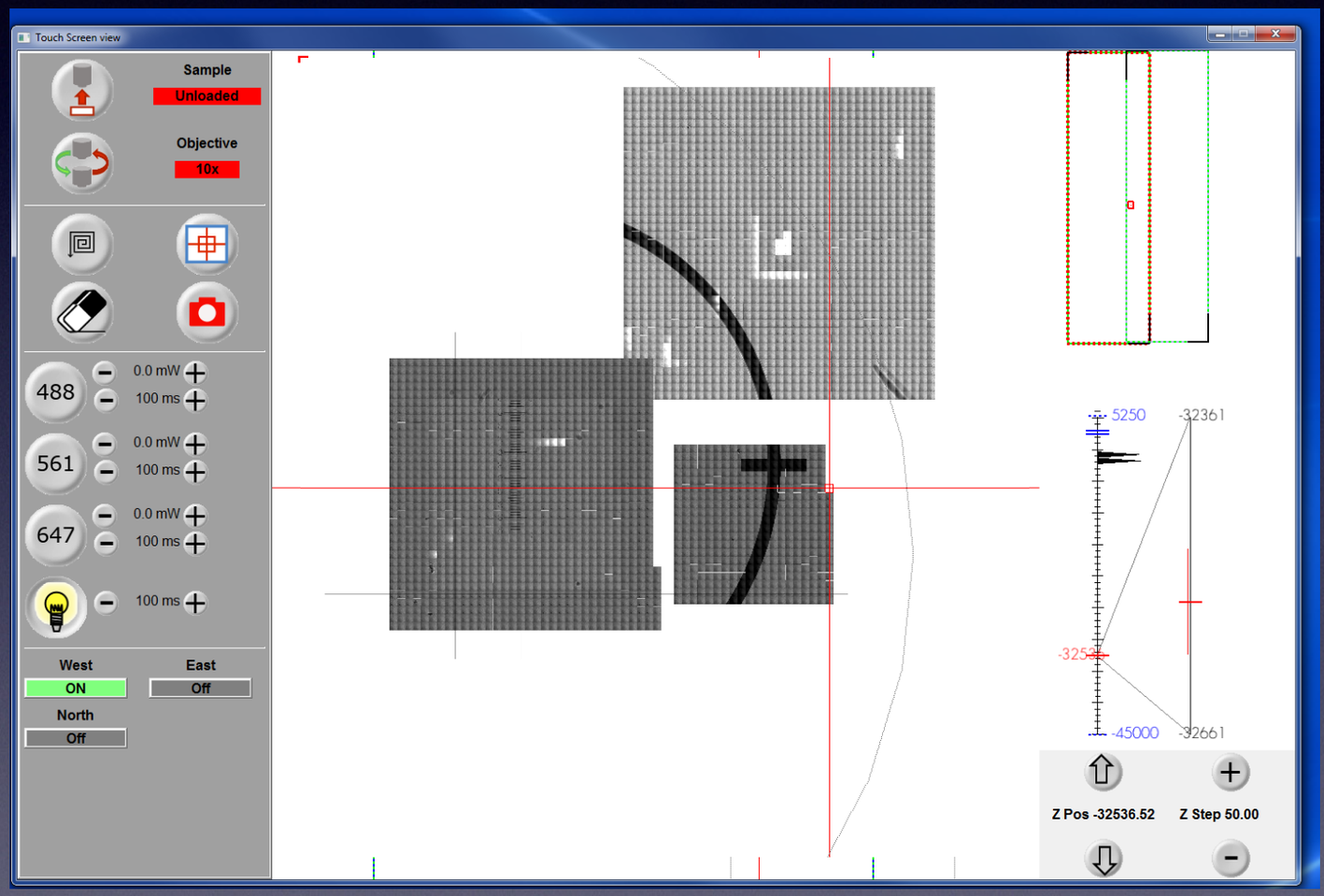
Cockpit & microscope





Some rules of thumb

- Clean and dust free environment
- Oscilloscope and soldering iron - you will need them!
- Good tools and spare parts
- Important to think about user interface
- Important to think about continuity of the project and workflow of experiments
- Important to think about data storage and analysis

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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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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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