# Lecture M2 - Bespoke Microscopes

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# Overview

- Image formation and airy rings
- Beads and spherical aberration
- Super fast acquisition
- 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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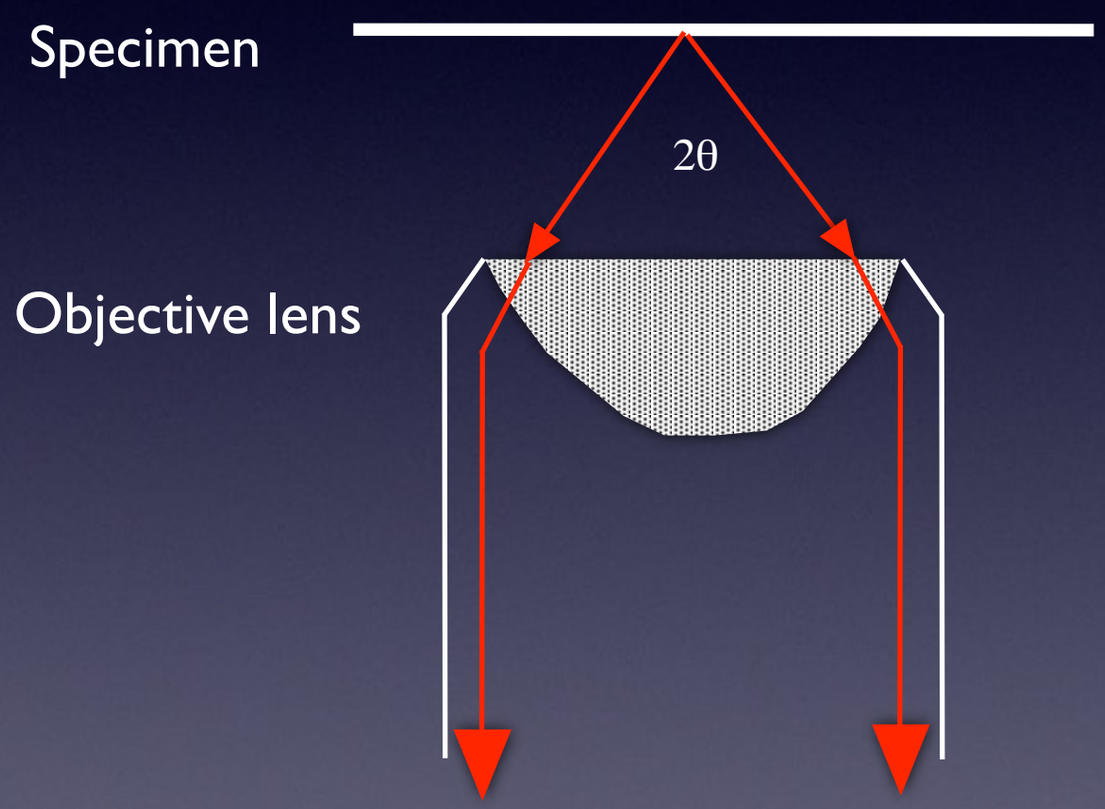
# Microscope Resolution

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

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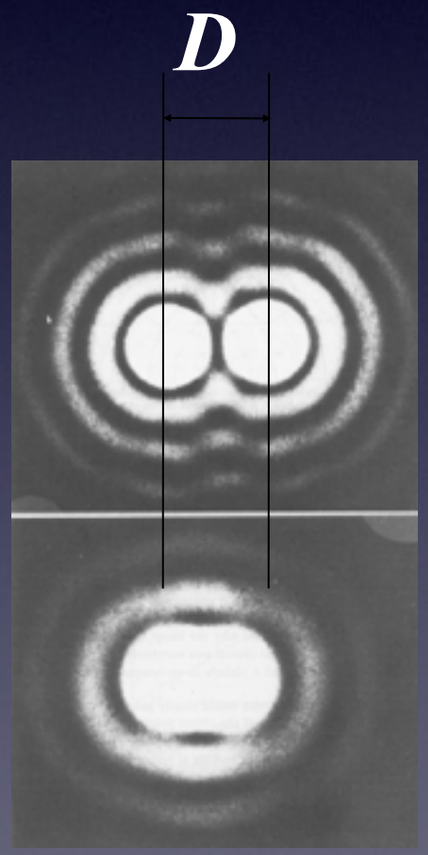
# Resolution: A technical definition, the Rayleigh Criterion




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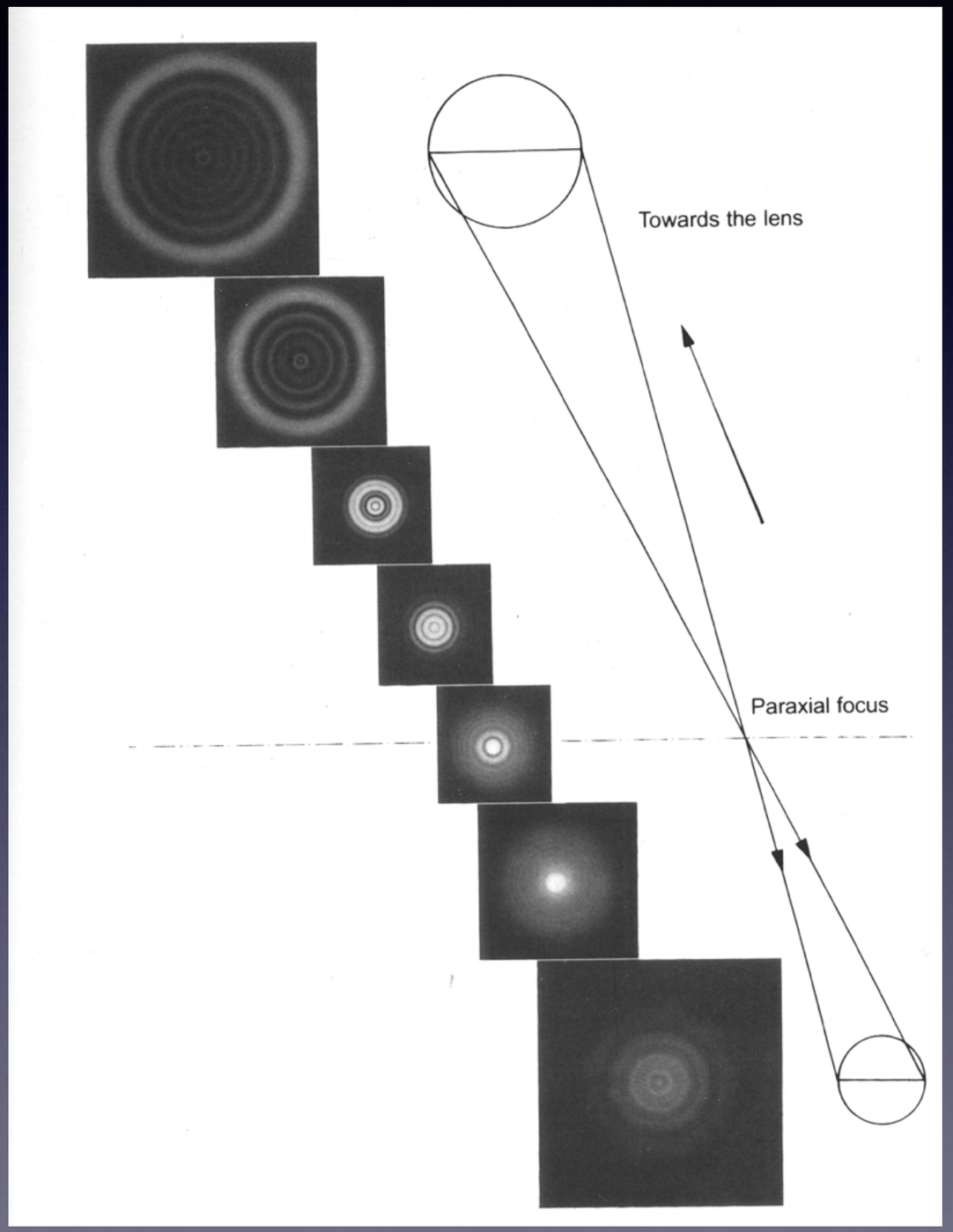
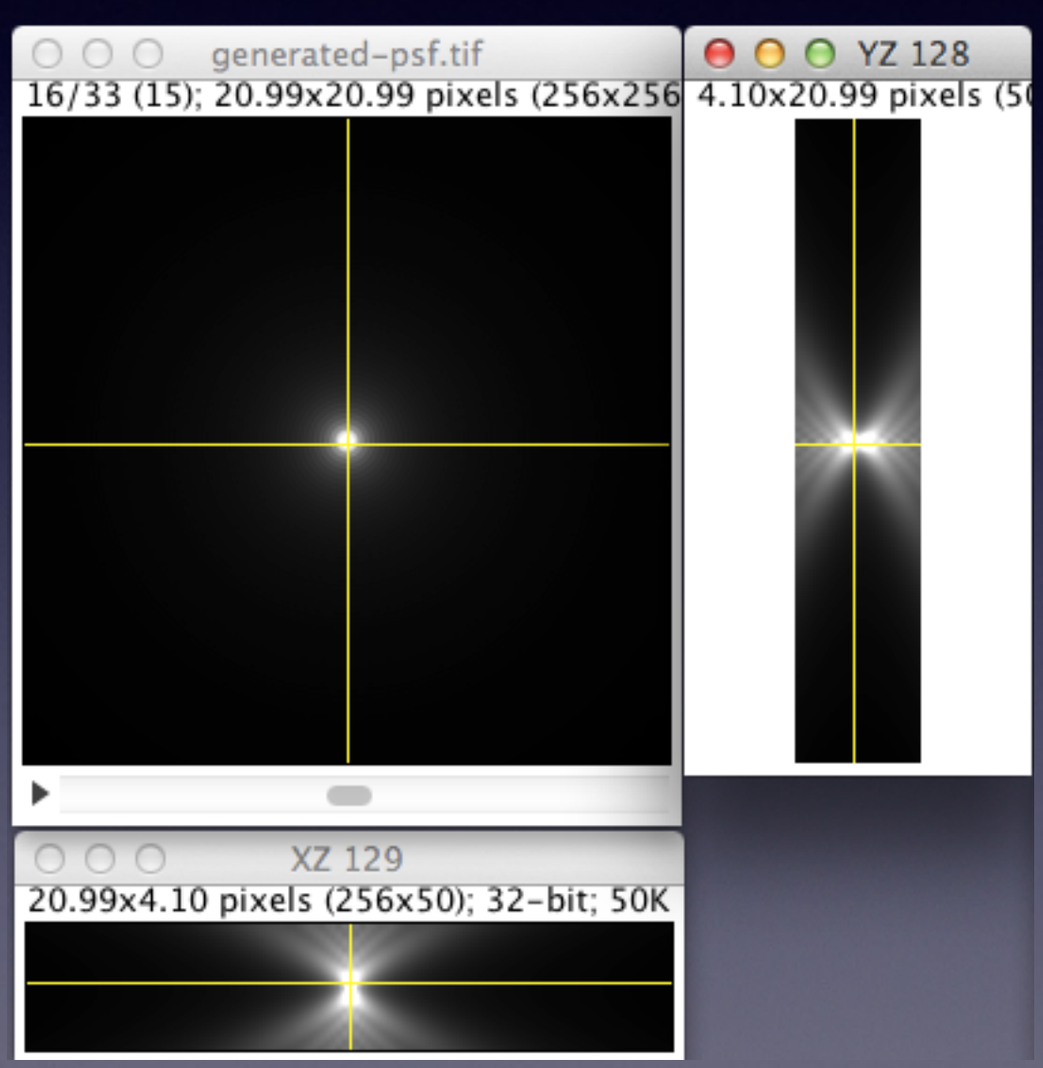
# Image quality- the problem of "out-of-focus light" point spread function and airy rings

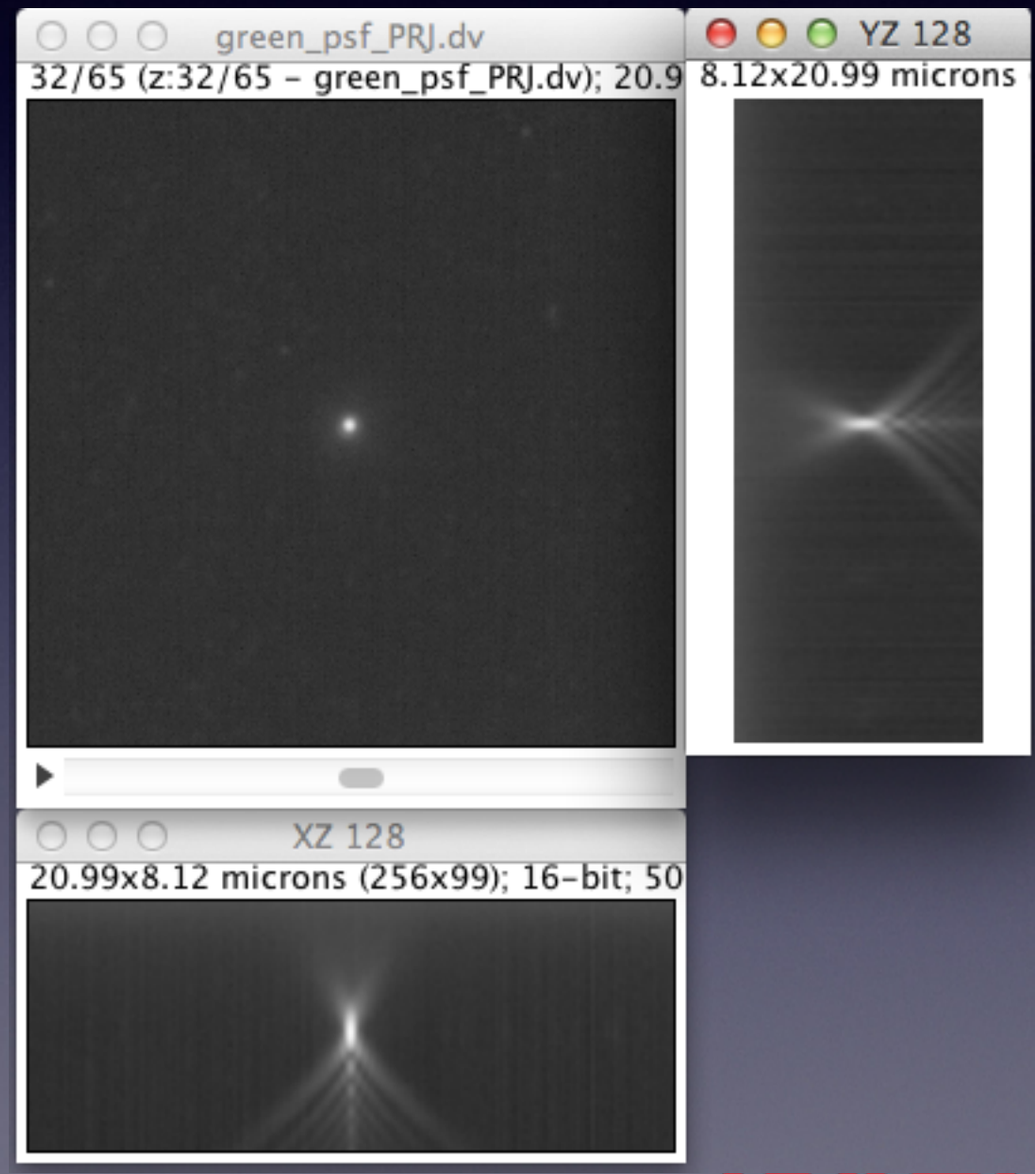
Sample object: a "sub-resolution" fluorescent bead



# Theoretical and measured PSF Orthogonal views

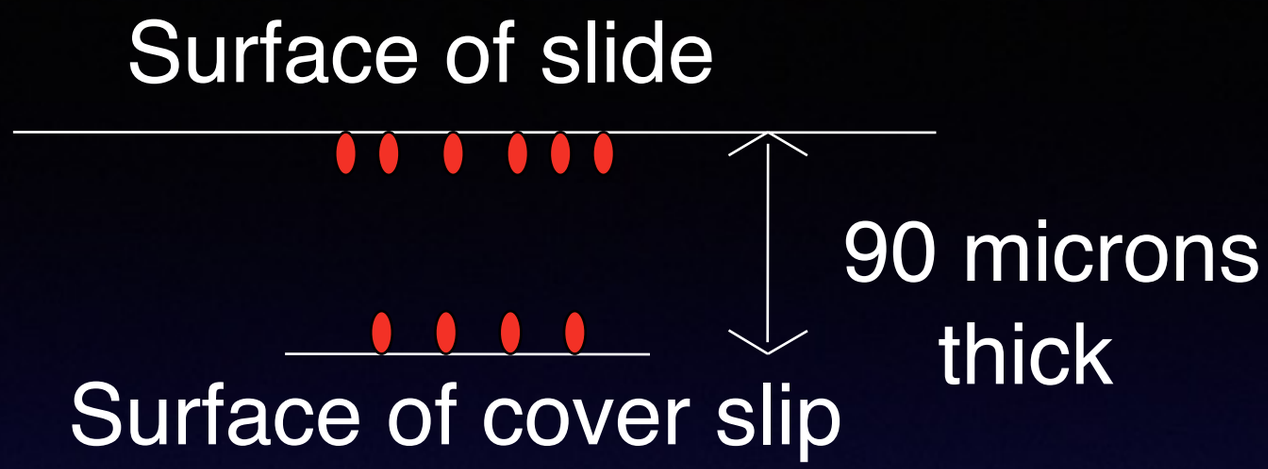



Generated PSF




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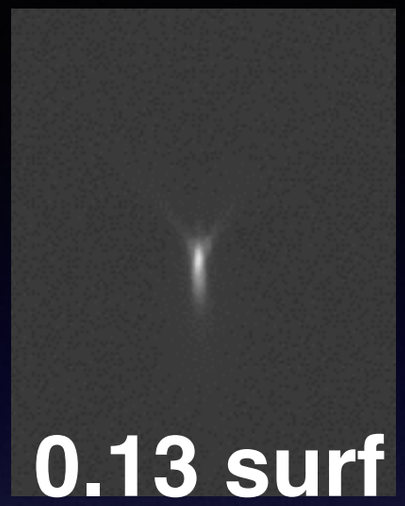
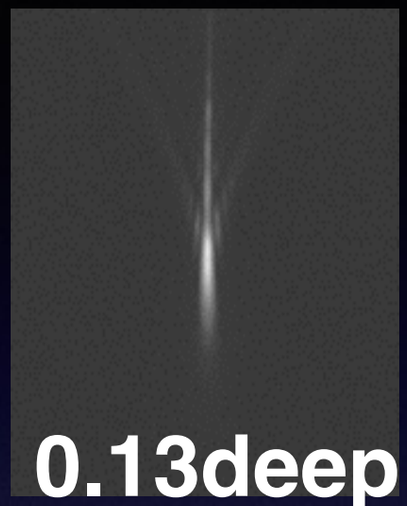
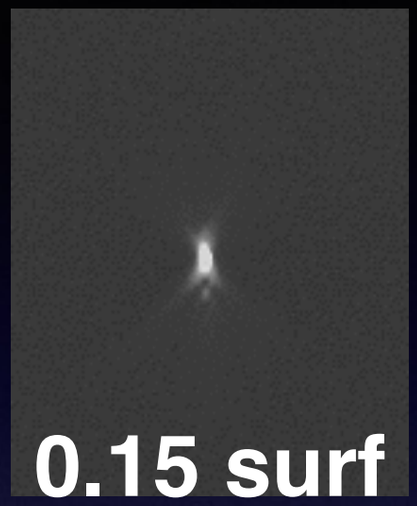
# Affects of deep imaging (90μm) and collar settings on spherical aberration and psf of 60X/NA1.2w



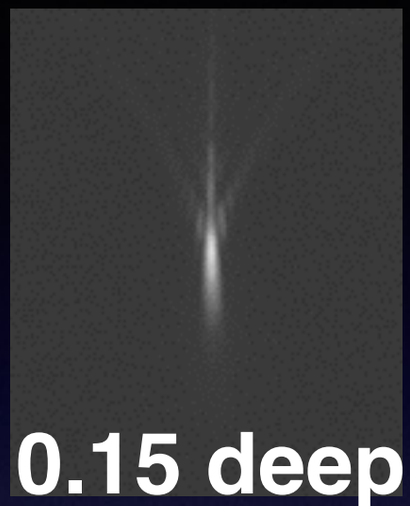
0.13 surf



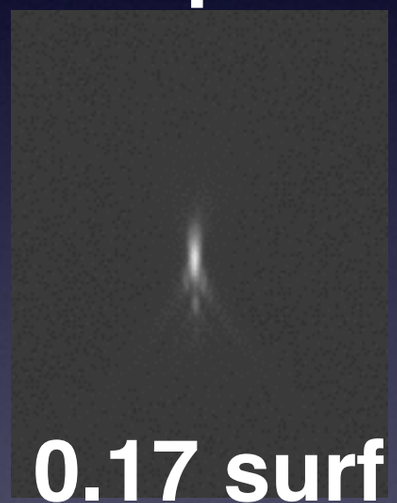
0.13deep



0.15 surf



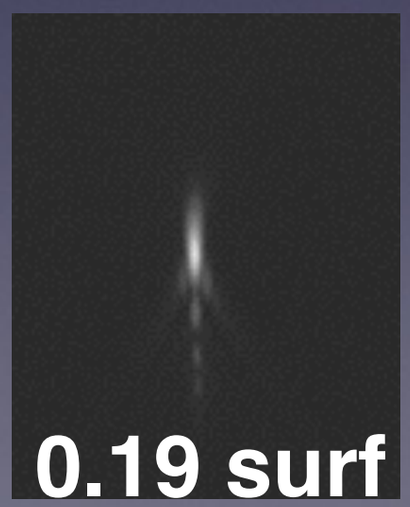
0.15 deep



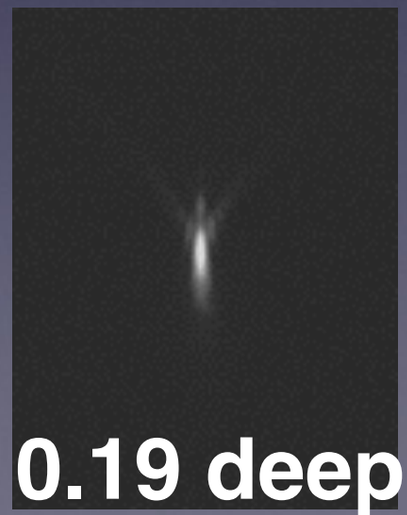
0.17 surf

0.17 deep

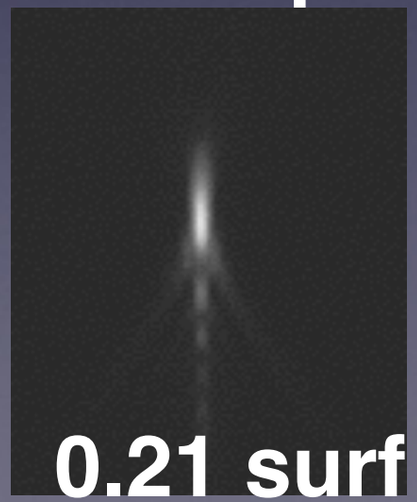
Data from
Alejandra Clark



0.19 surf

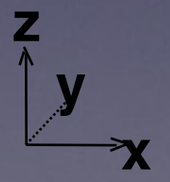


0.19 deep



0.21 surf

0.21 deep

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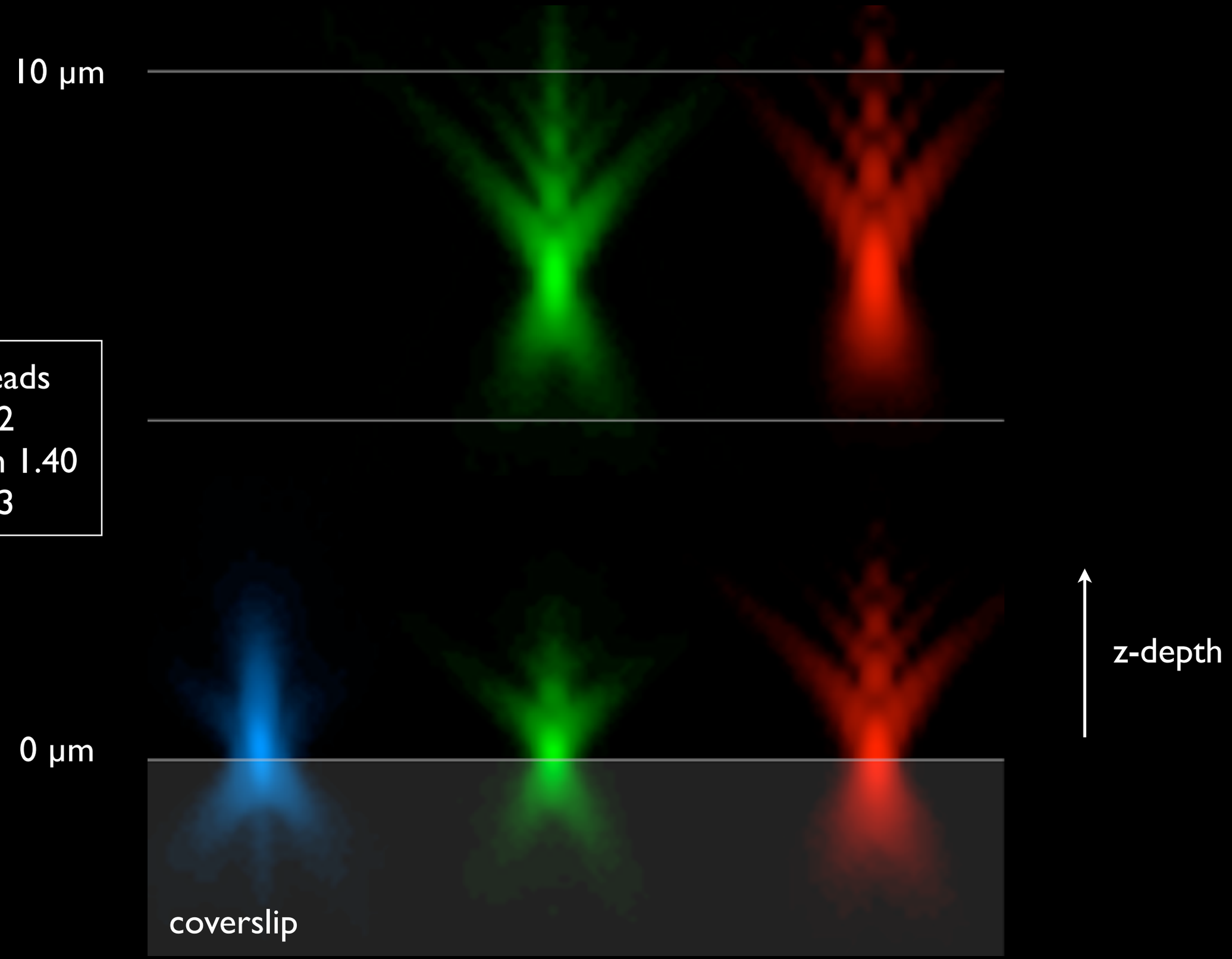
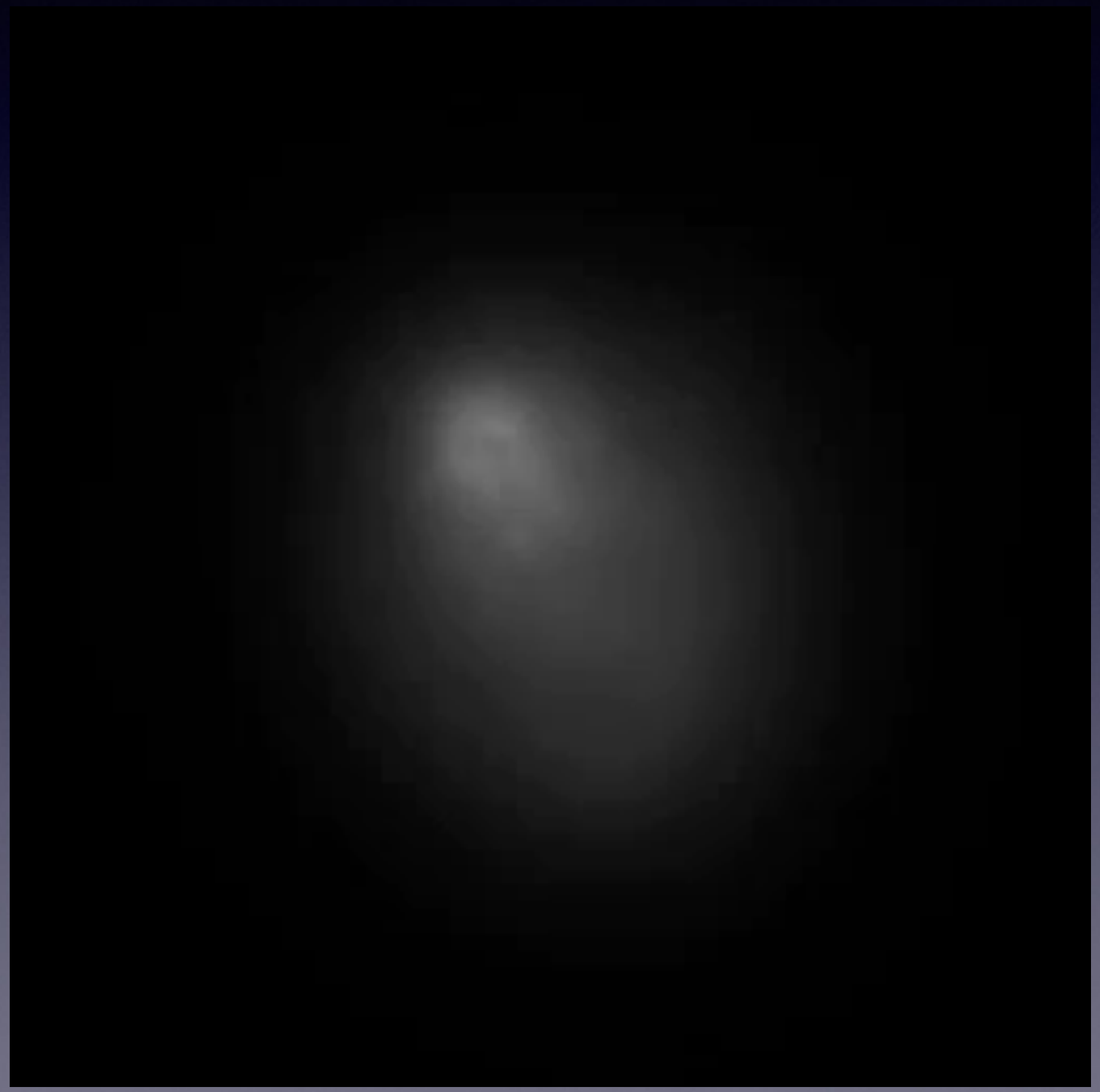
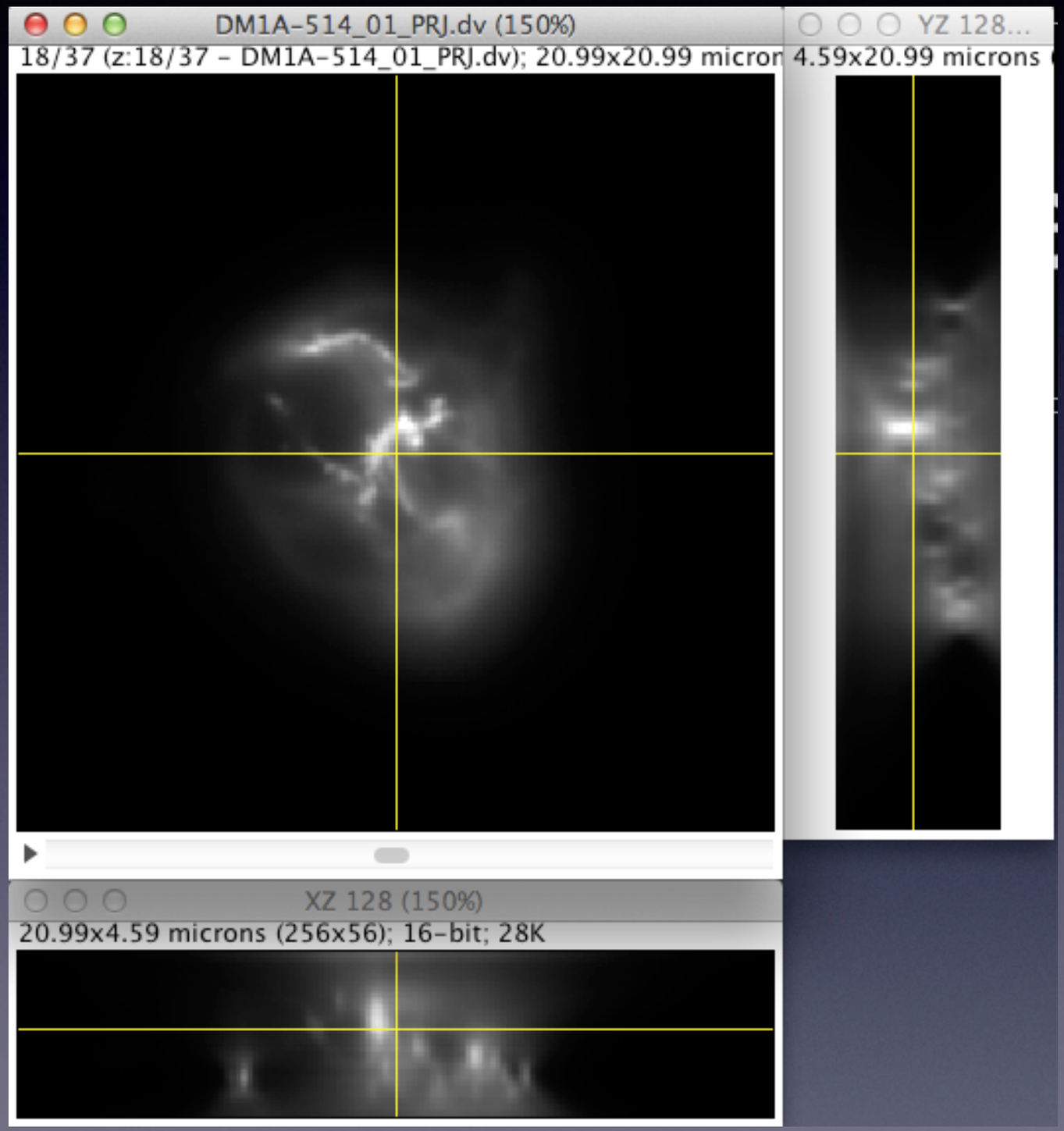
# Spherical aberration dependent on wavelength, depth, RI



# Conventional Epi-Fluorescence Image



# Orthogonal views

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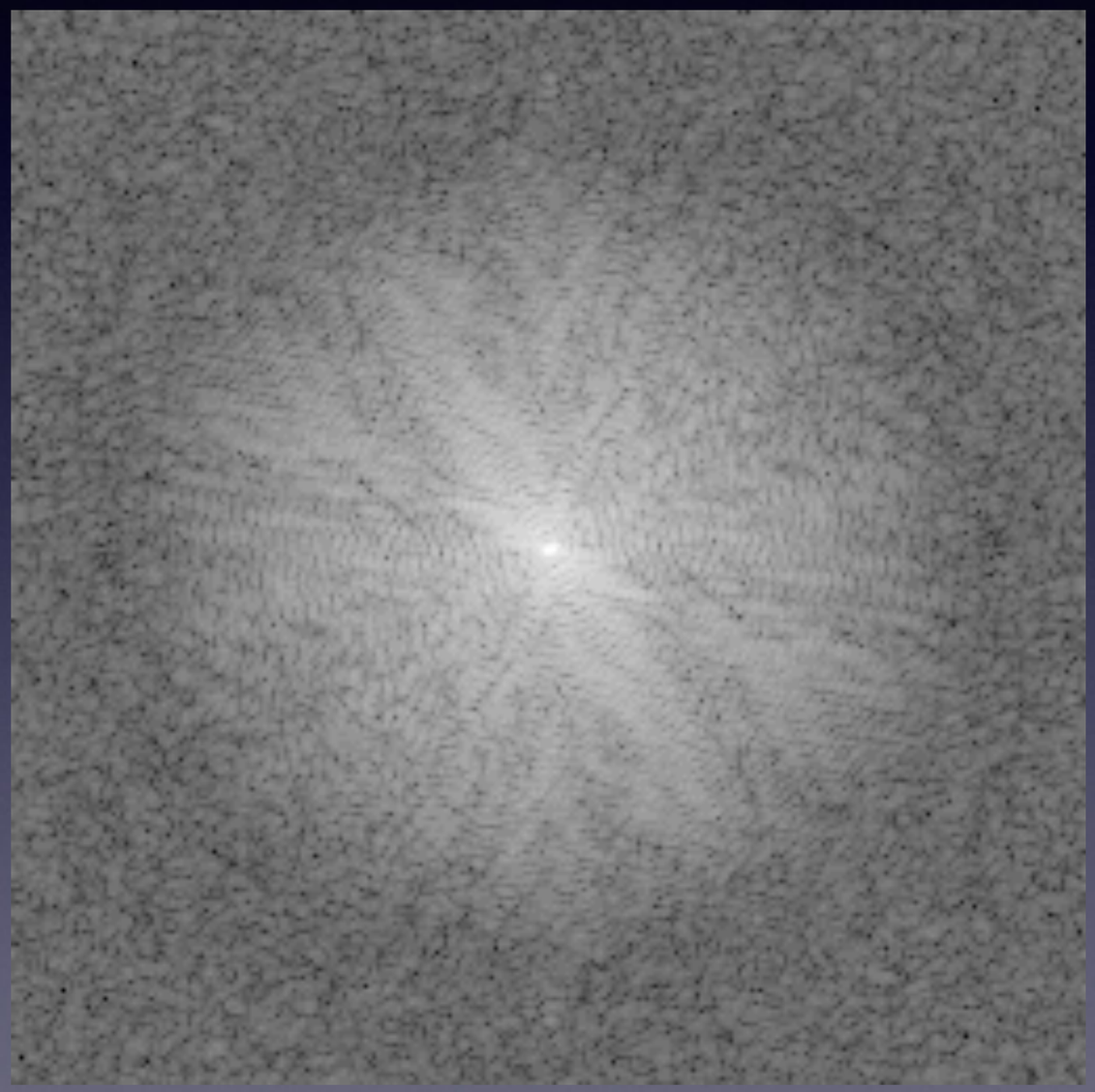
# Fourier Transform



FFT of a single slice (Z plane) of image stack

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# How a DIC prism effects fluorescence imaging

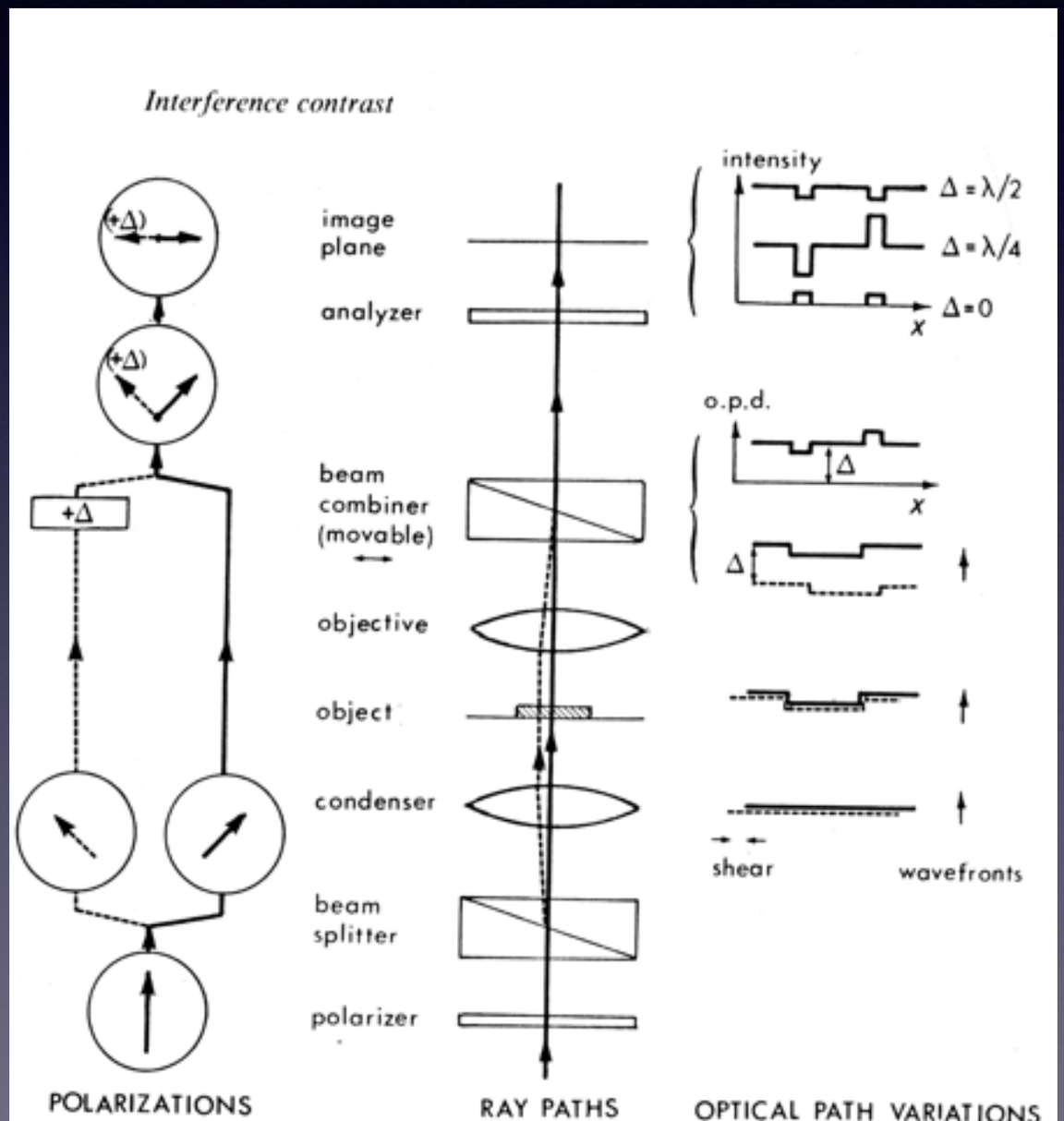


Fig. 38. The optical system for interference contrast.

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# With/without DIC prism

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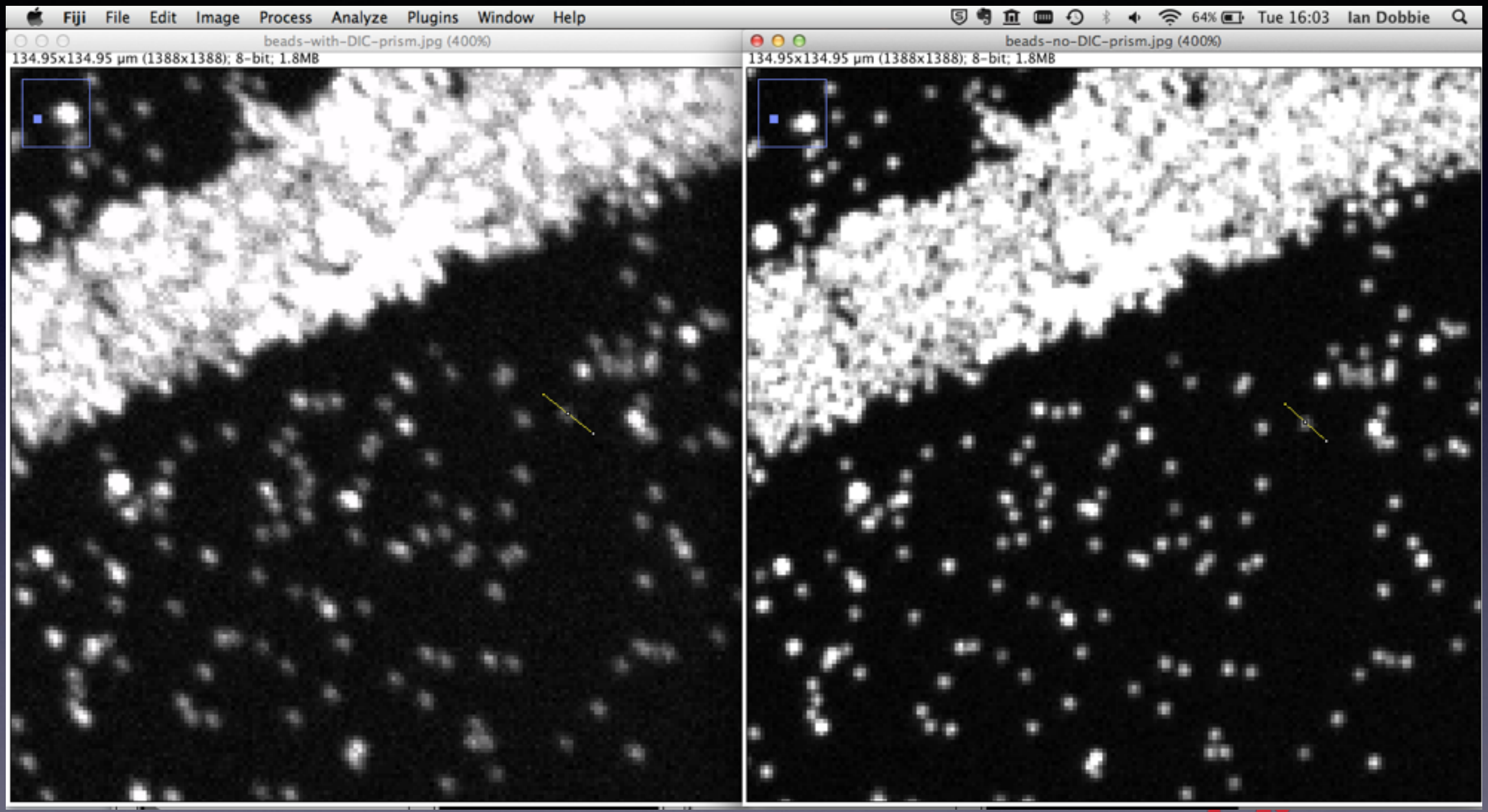
# With/without DIC prism

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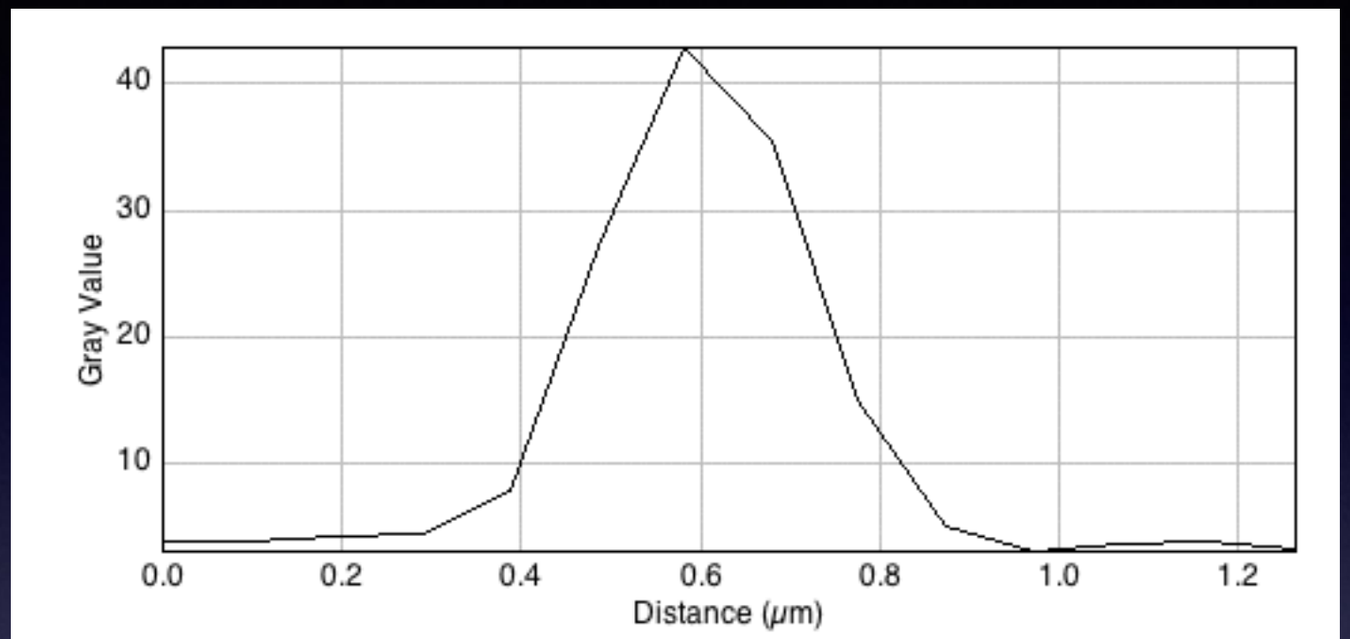
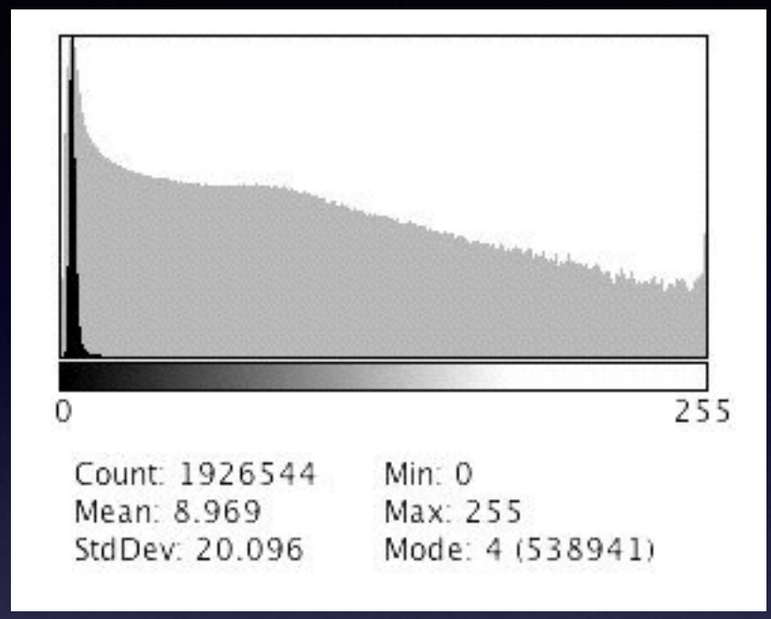
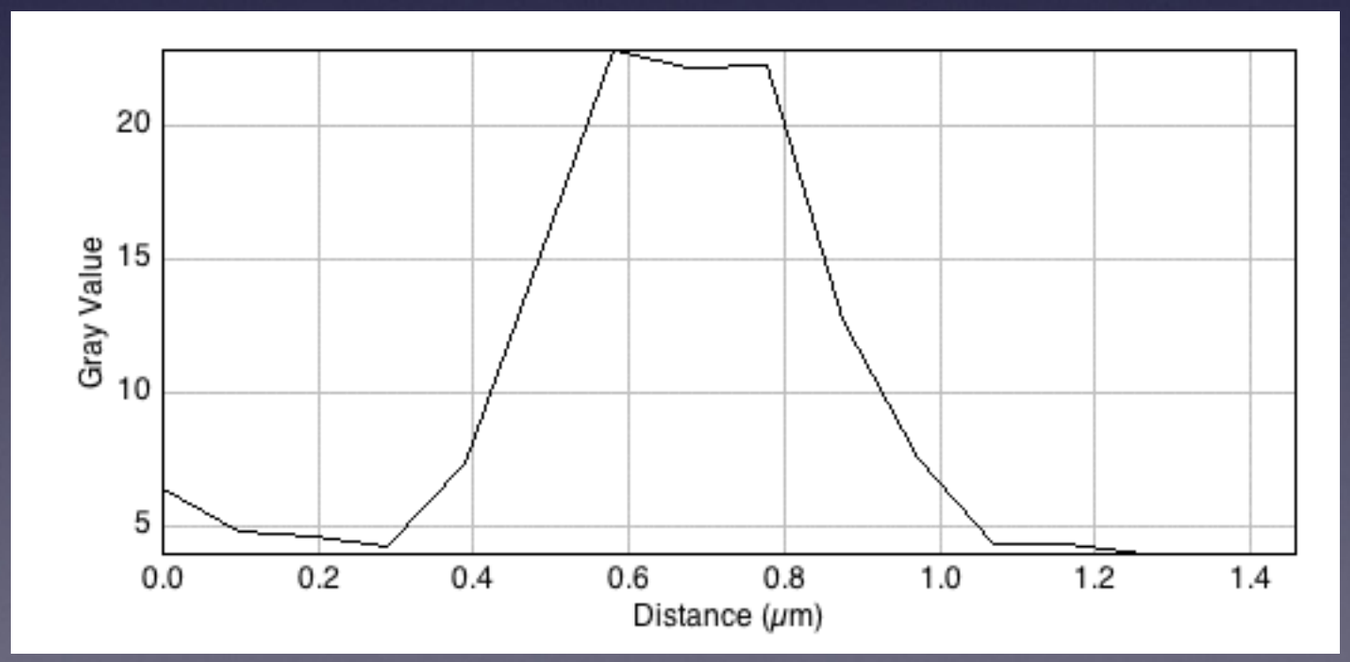
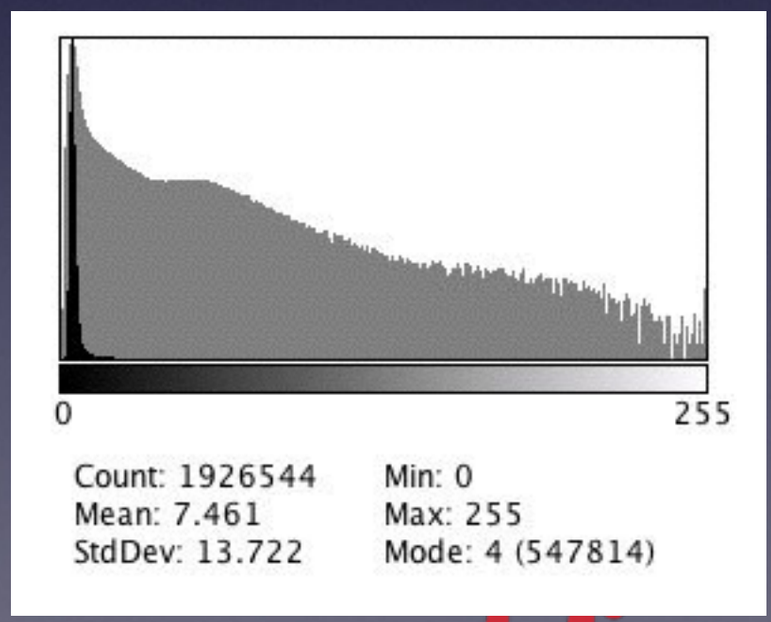
# Line scans and histograms

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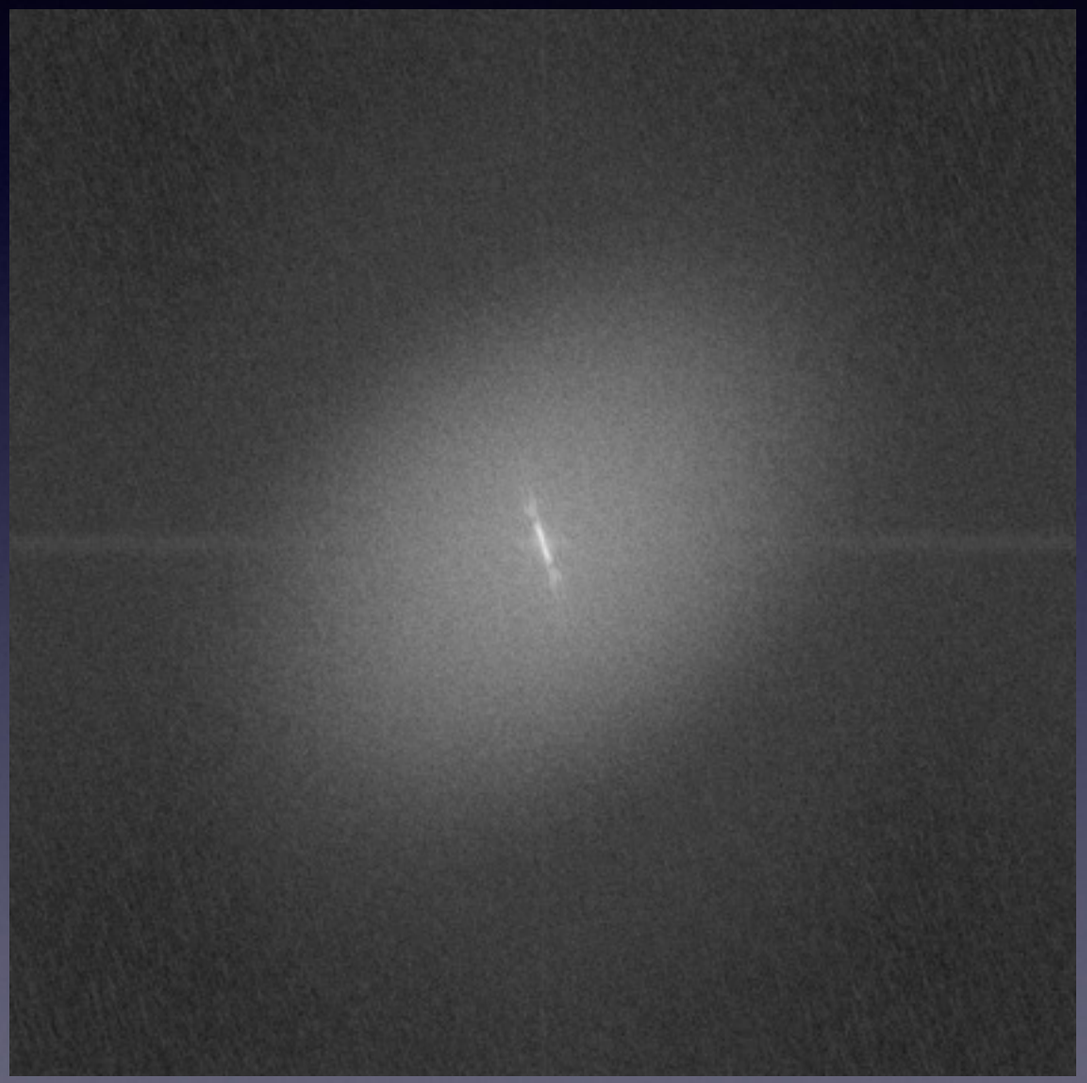
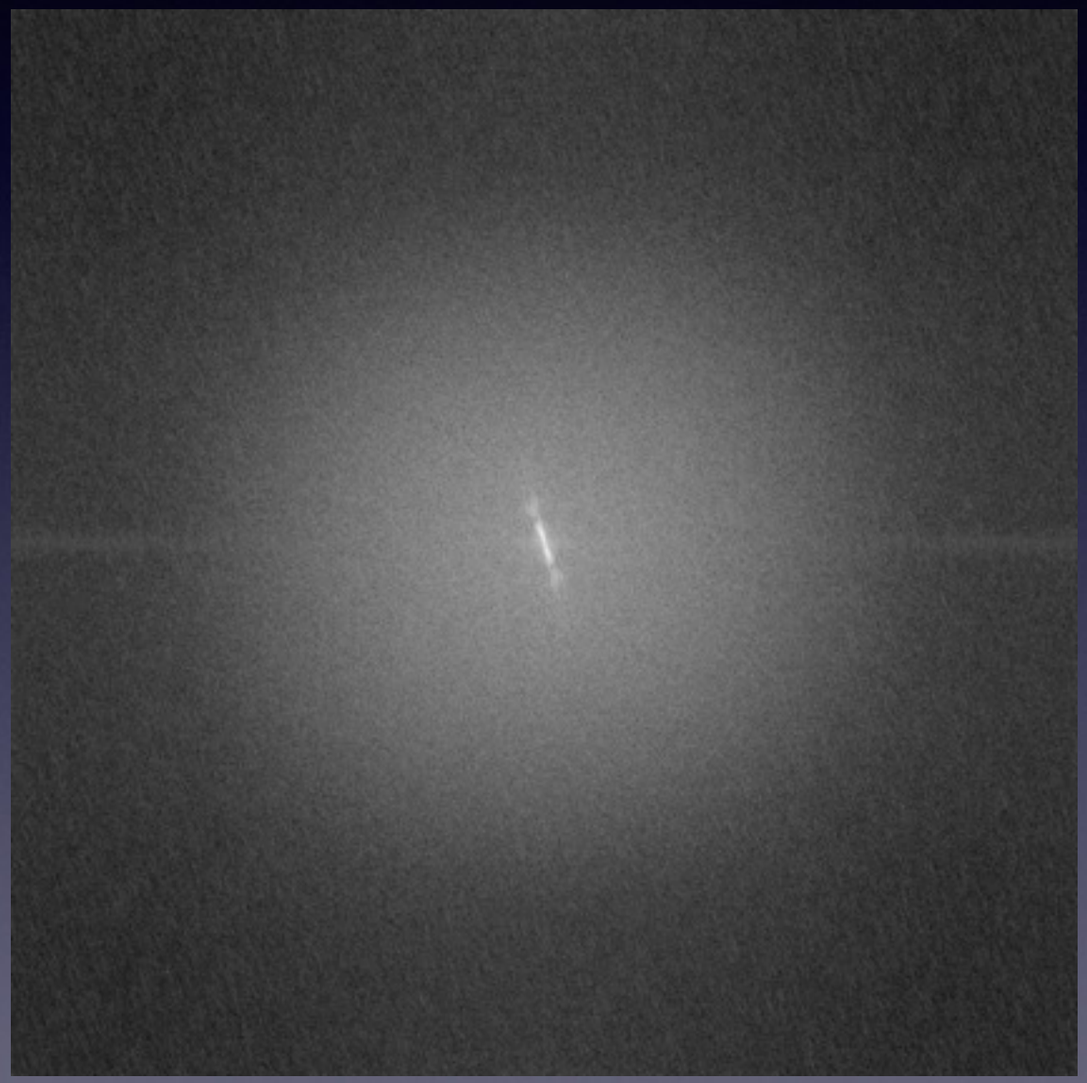
# FFTs with/without DIC prism





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# 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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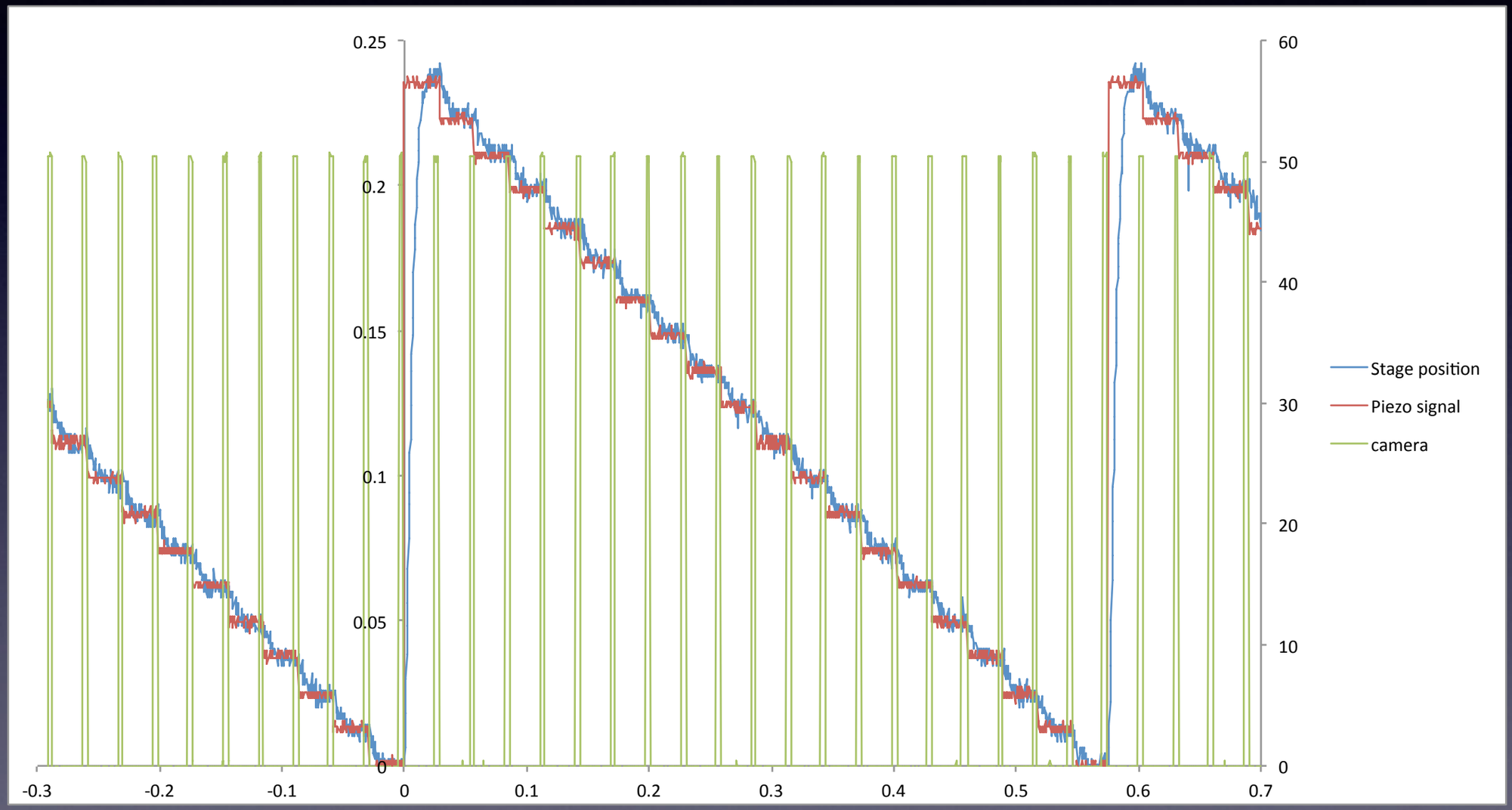
# Covential widefield Z stack




20 Z planes as fast as possible

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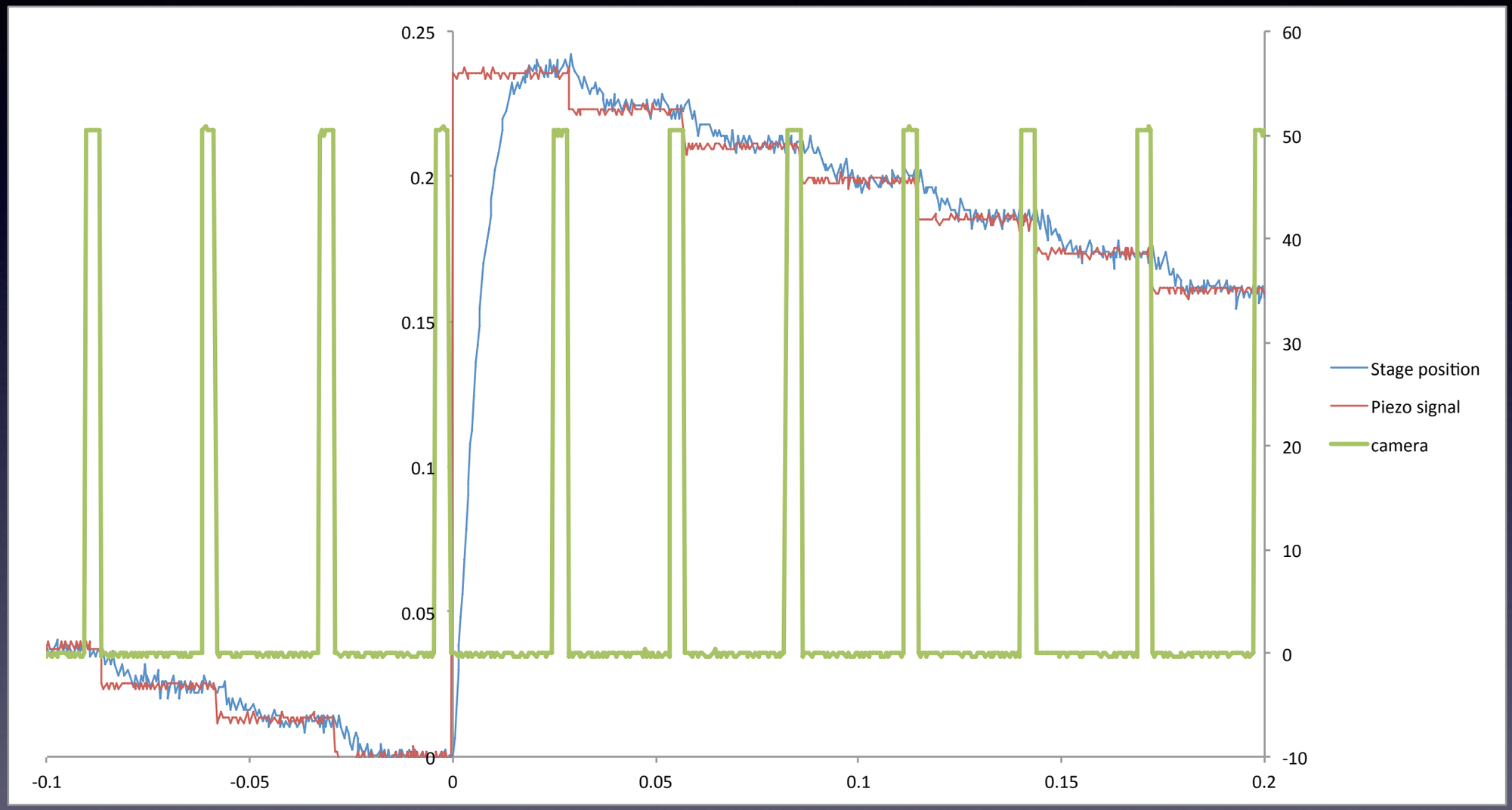
# Covential Z stack

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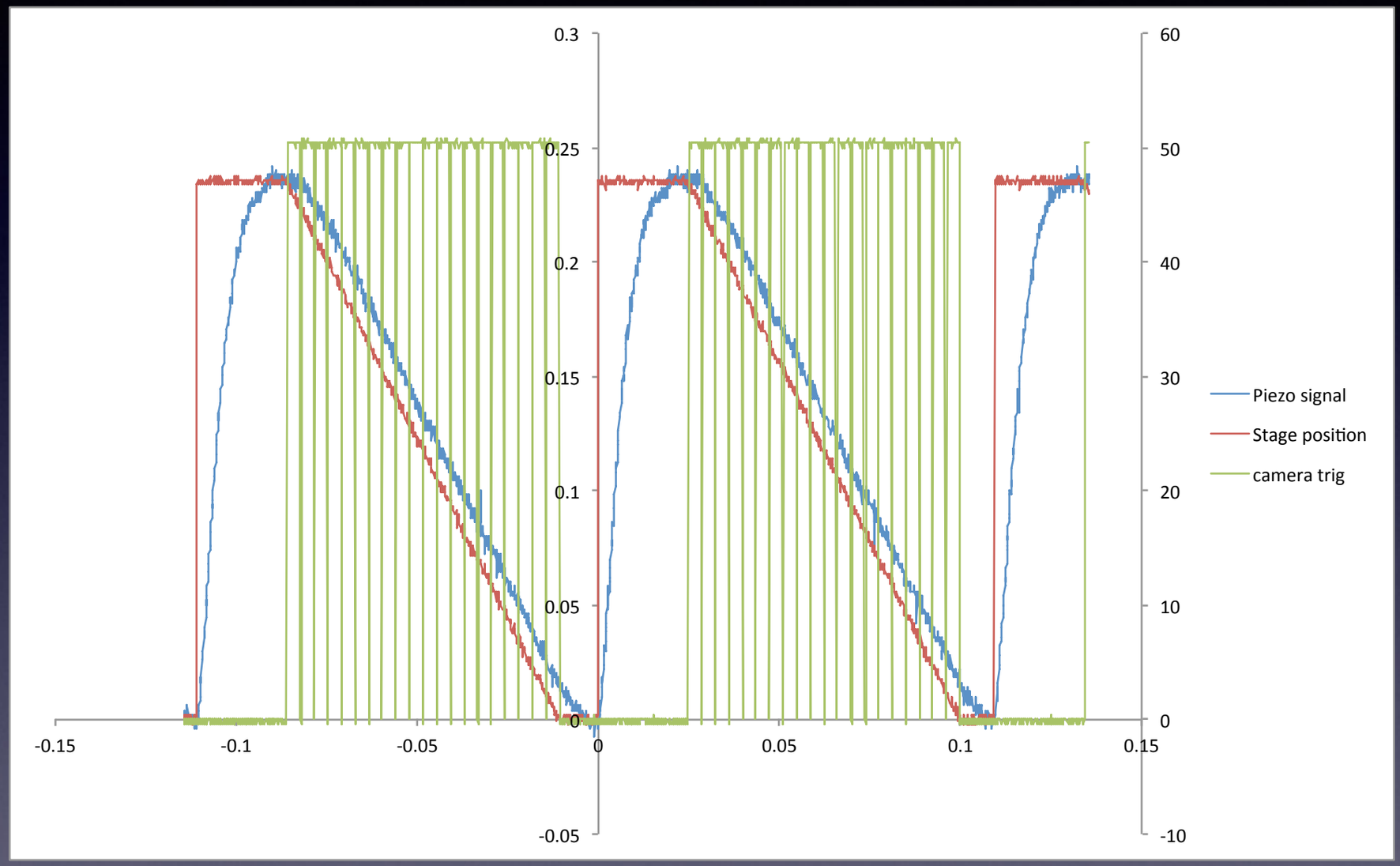
# Ramp Z stack

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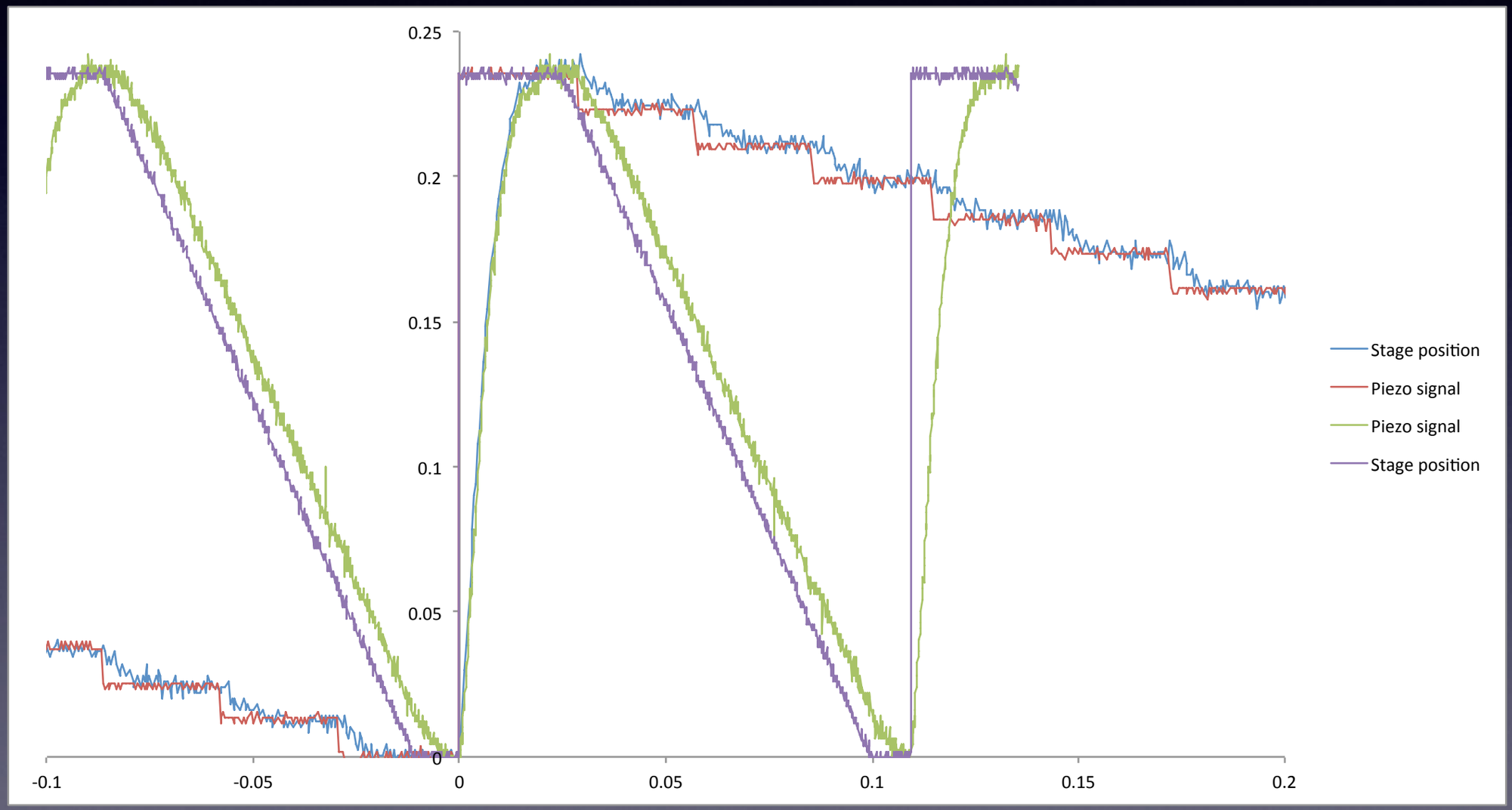
# Comparison: FastZ to normal



# 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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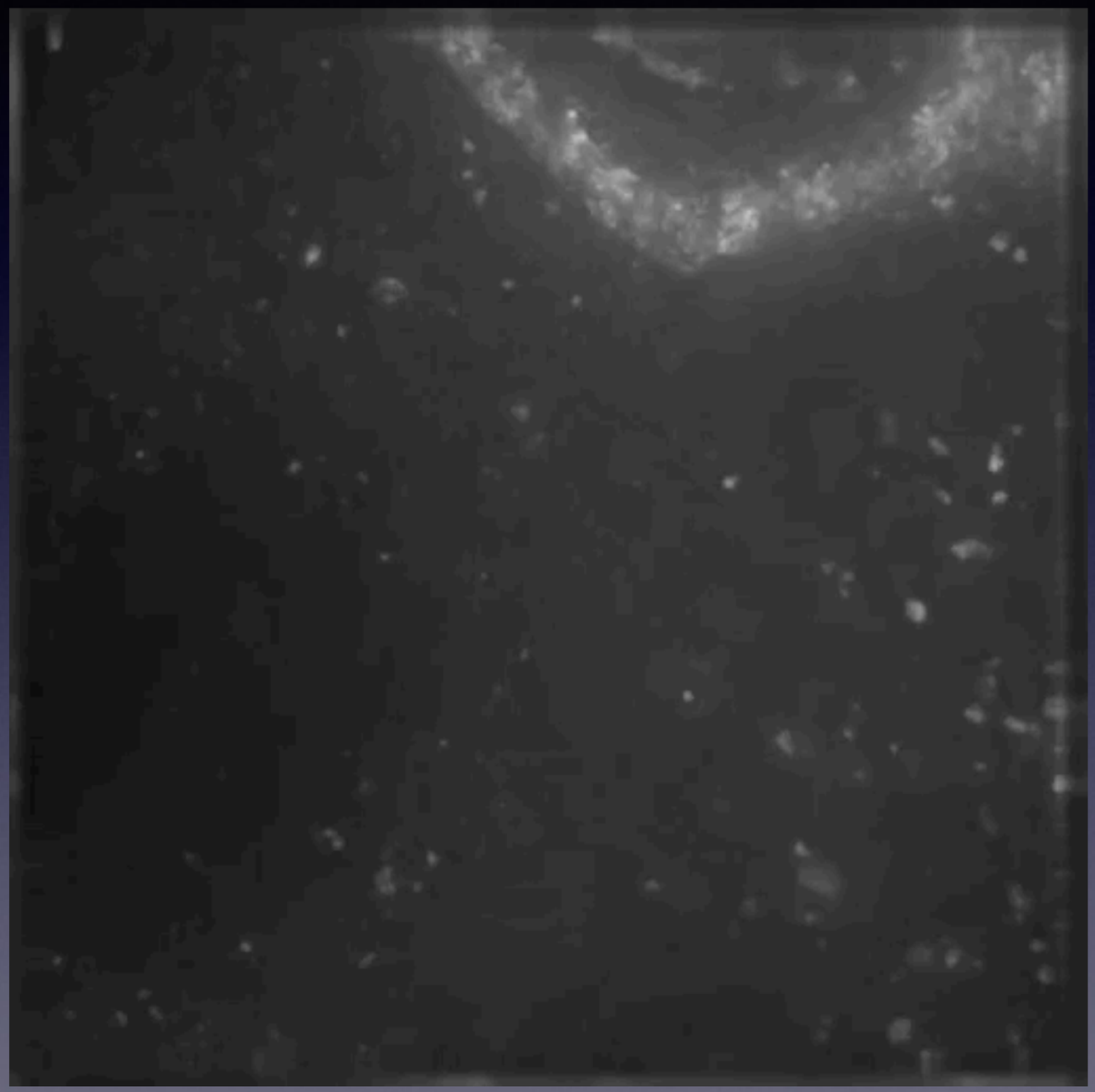
# FastZ - Results



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

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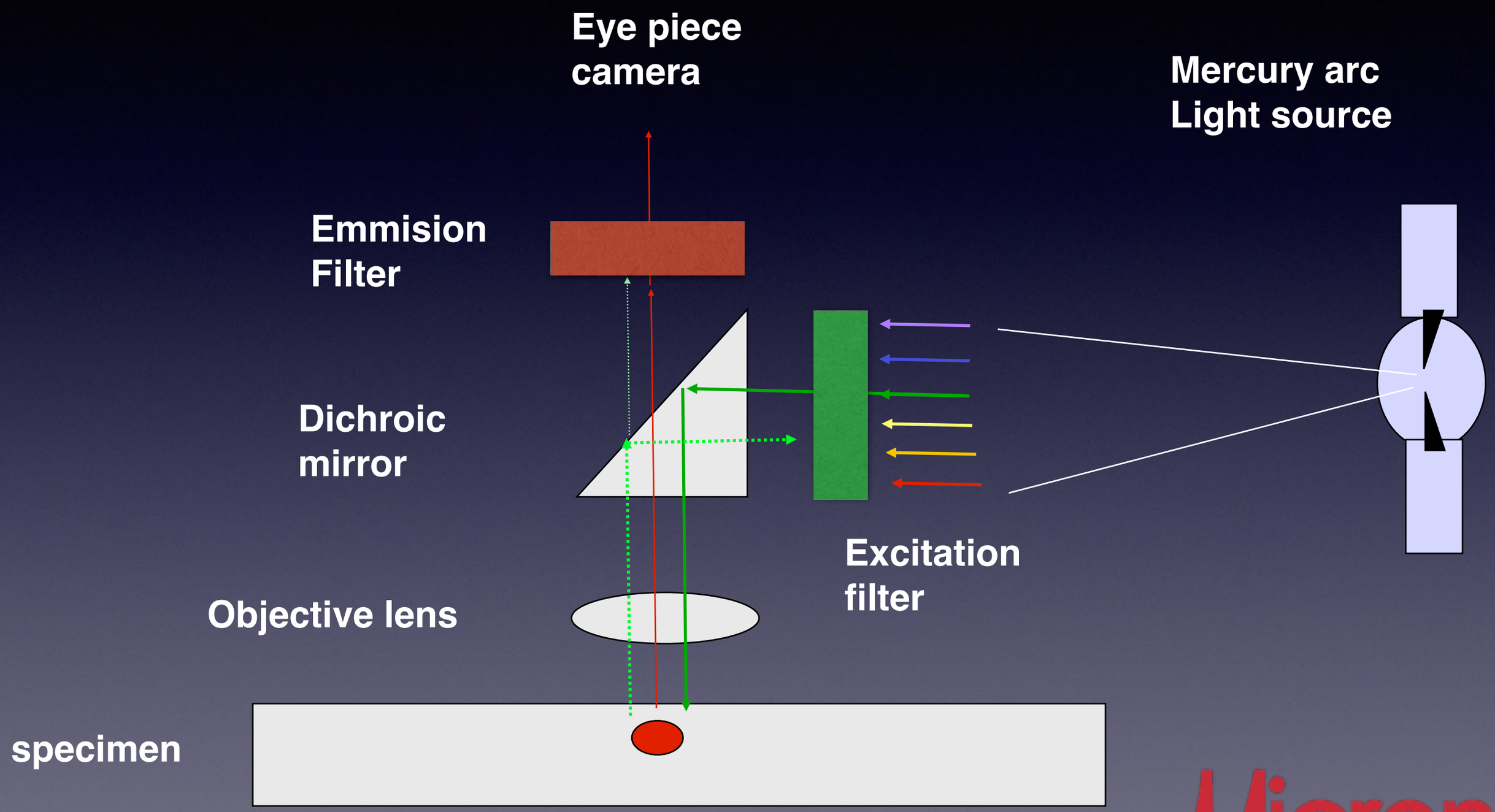
# Reminder

## How do fluorescence microscopes work ?

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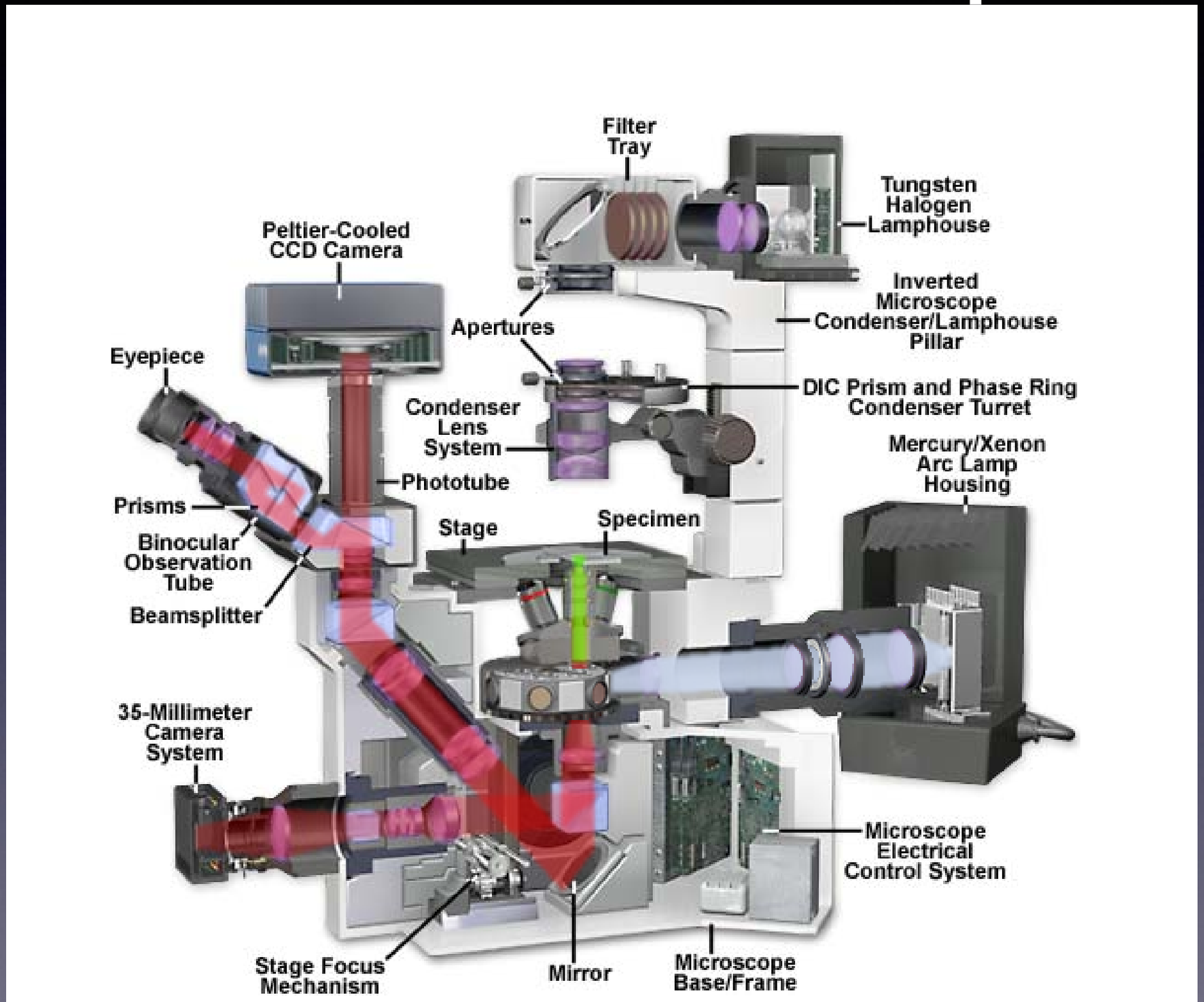
# Problem: the design of all conventional microscope stands

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# How can we improve the basic design of widefield microscopes?

## By dispensing with the normal microscope stand and building your own microscope from optical components on a breadboard

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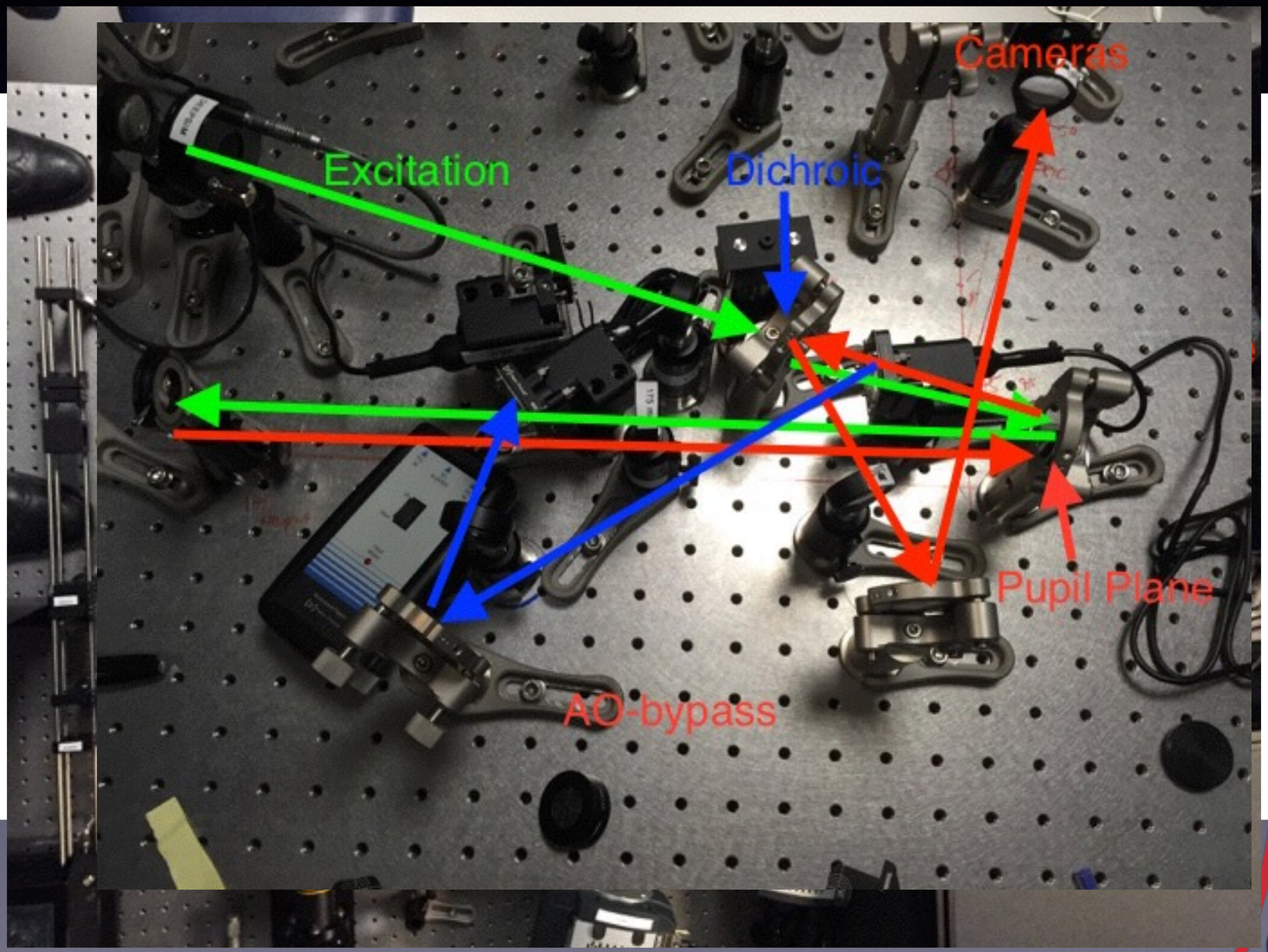
# The solution -build your own bespoke microscope



# Bespoke Microscopes

**Why bother?**

**Specific applications -better than commercial microscopes**

**Flexibility**

**Cost**

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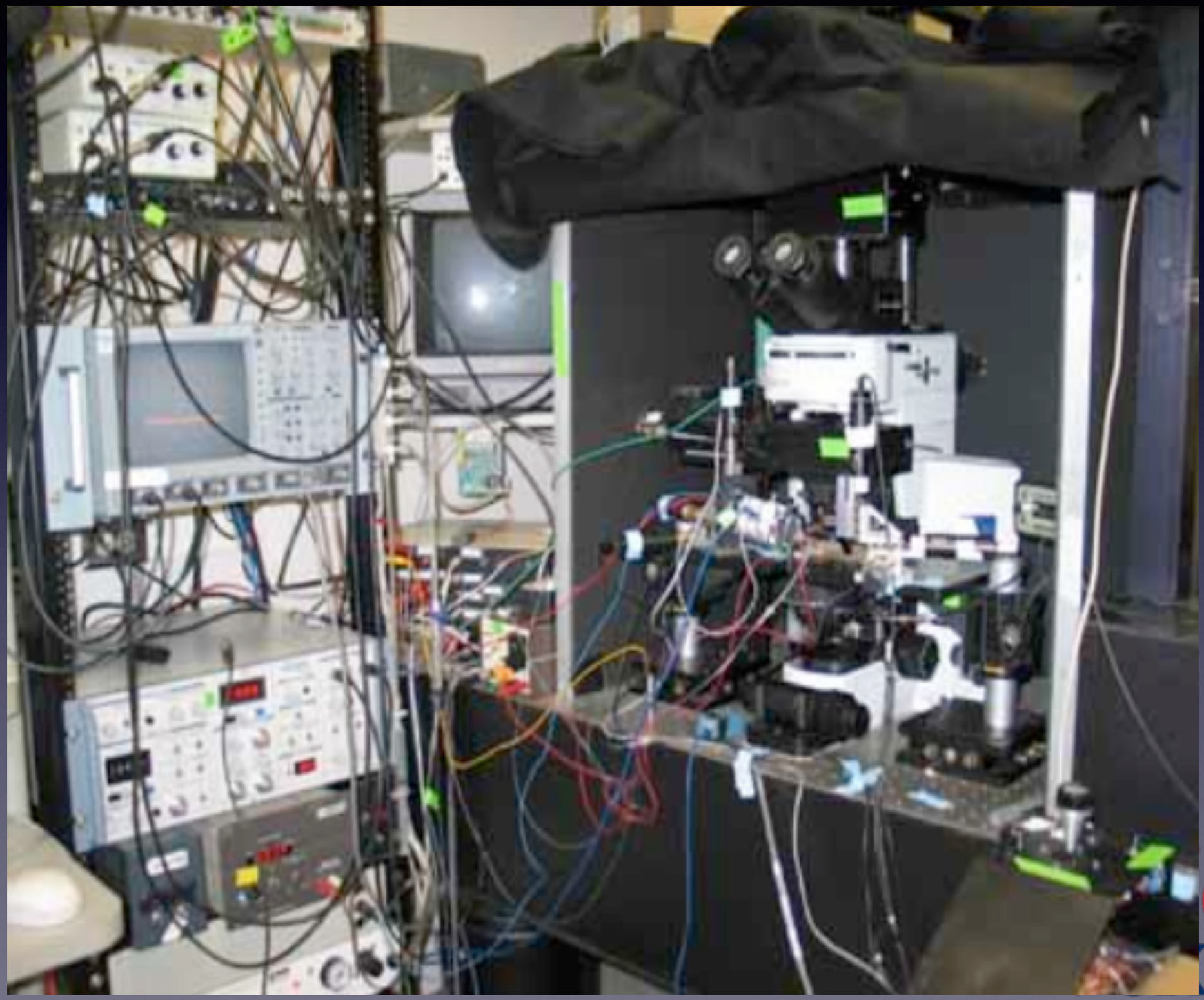
# Popular bespoke microscope

## Multiphoton for neuroscience work



# 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

**OMX-T microscope**
**Designed and built by John Sedat and Dave Agard, UCSF**

**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**

**DeepSIM**
**Antonia Göhler,Mick Phillips, Mantas Zurauskas, Micron Oxford**

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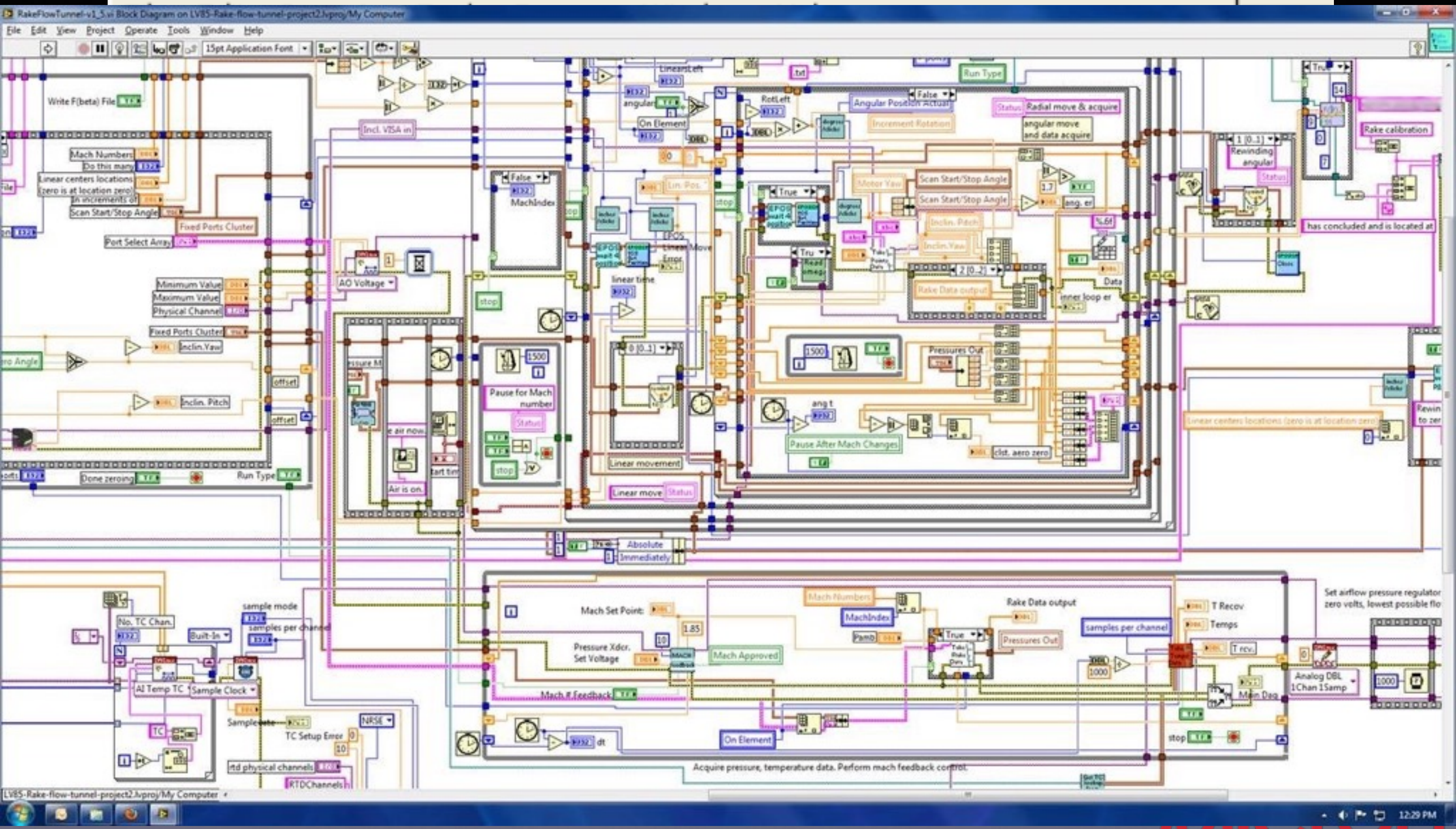
# Software options

- LabView
- Micromanager(semi open source java)
- Cockpit (open source python code)
- 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

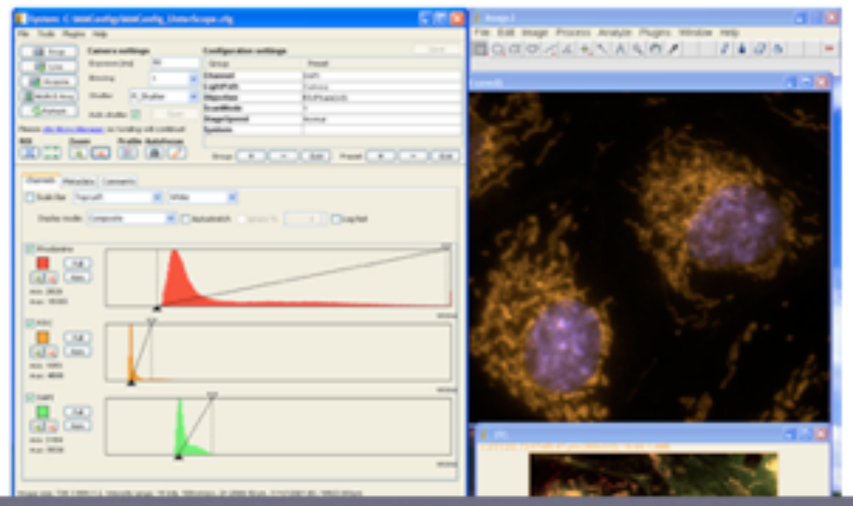
The Open Source Microscopy Software

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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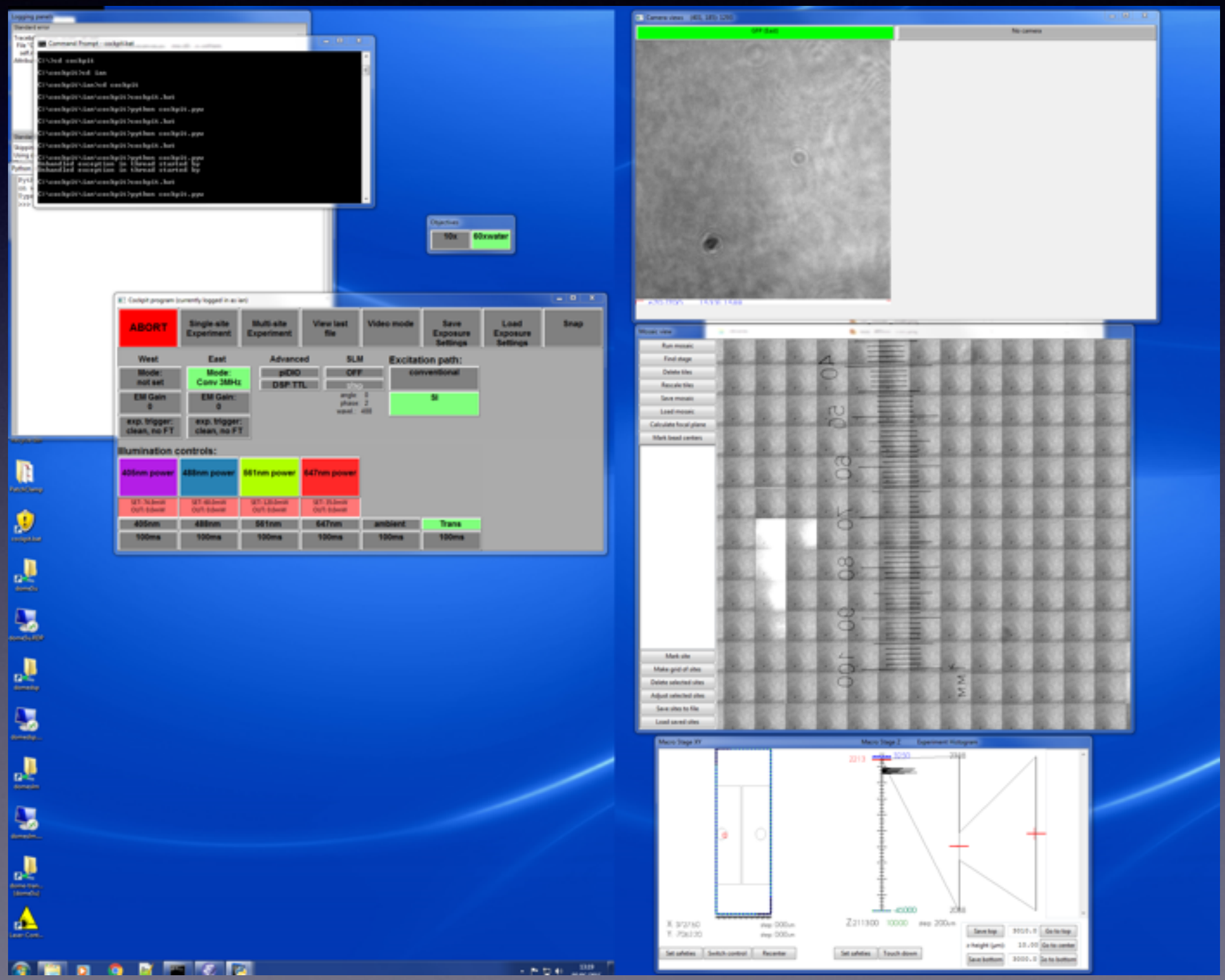
# Cockpit


ABORT
Single-site Experiment
Multi-site Experiment
View last file
Video mode
Save Exposure Settings
Load Exposure Settings
Snap
West
East
Advanced
SLM
Excitation path:
Illumination controls:
405nm power
488nm power
561nm power
647nm power
ambient
Trans

# 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 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-£150k)**

**Total cost ~£150-400k**

**Commercial  OMX system ~£400k**

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# Summary

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