Bespoke Microscopes

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Overview

- OMX concept and development

- Super fast acquisition

- Bespoke microscope design - pro's and cons

Bespoke Microscopes

Why bother?

Specific applications -better than commercial microscopes

Flexibility

Cost

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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.

How expensive is it?

Building costs

Hardware ~£100-250k

Salaries 1-3 years (~£50-£150)

Total cost ~£150-350k

Commercial Confocal ~ £250k

Commercial OMX system ~£600k

OMX Design Goals

- Extreme stability

- Maximise emission sensitivity

- Double resolution with Structured illumination

- Maximise speed and timing reproducibility

- Extremely flexible hardware and software

Results

- No eyepieces

- Multiple beam paths

- Relatively bad excitation light efficiency

- Exotic dichroics (pass excitation reflect emission)
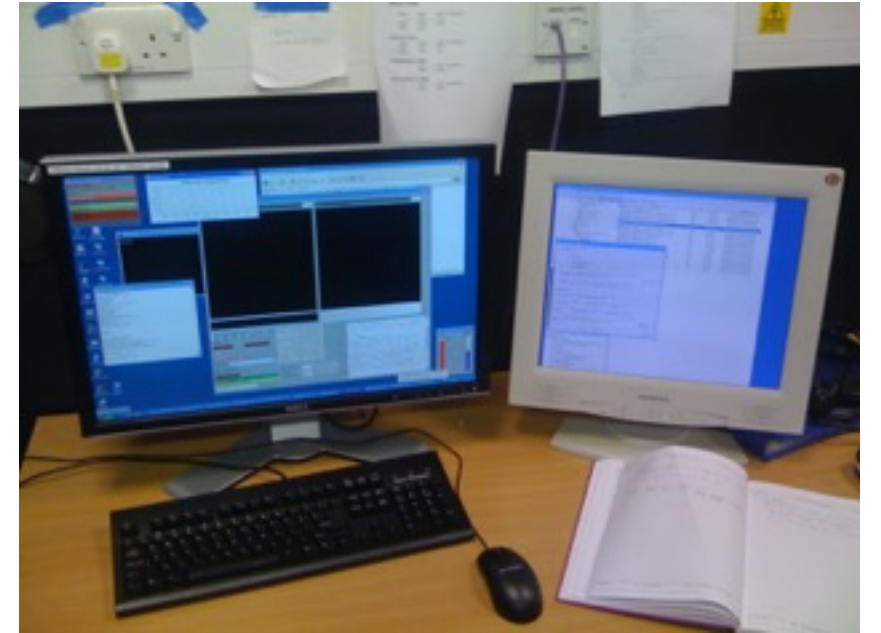
- Complex hardware & software
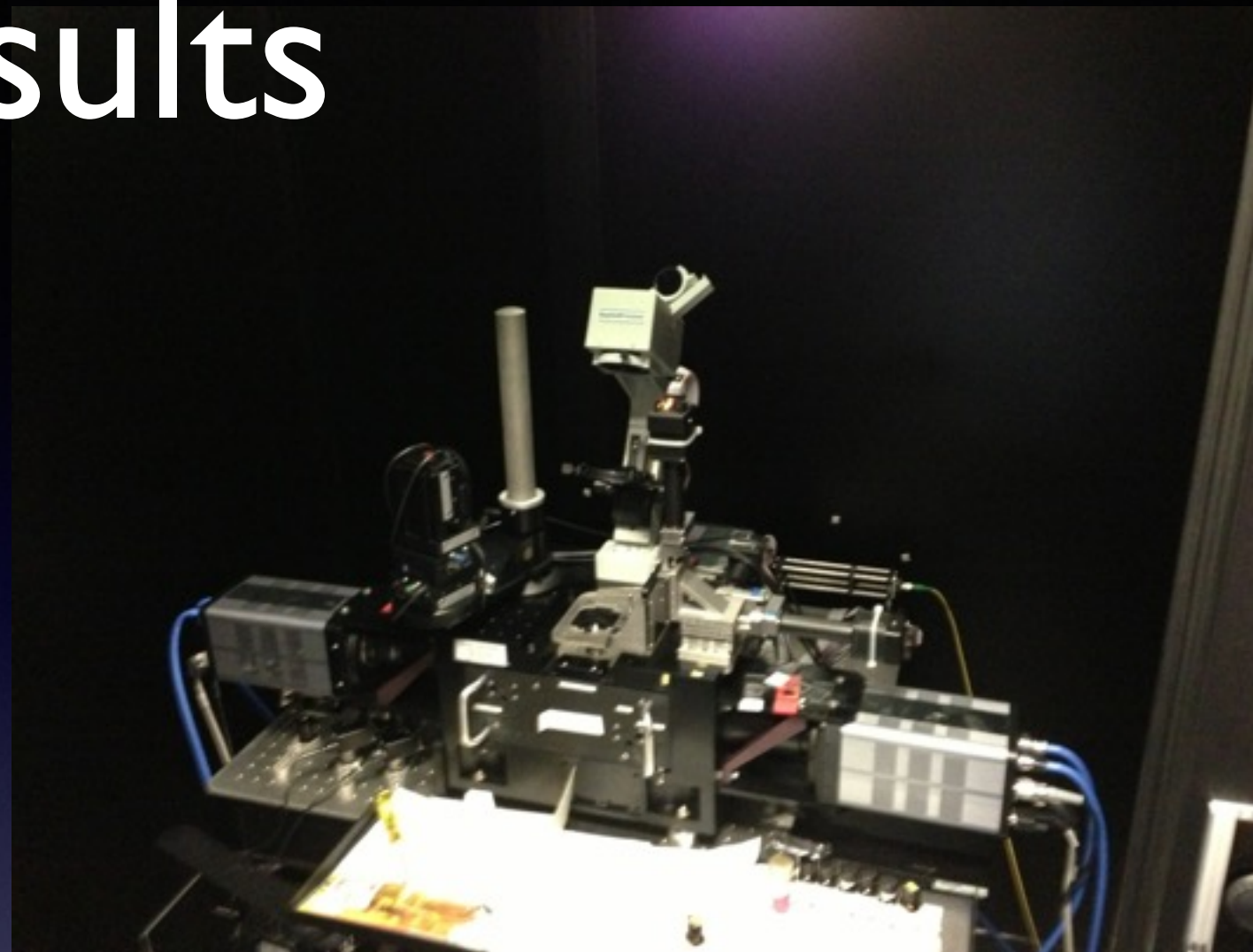
The complete Results
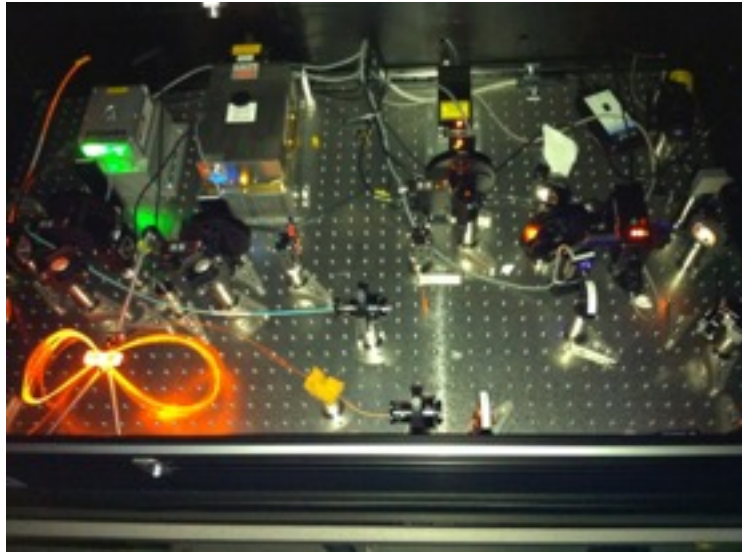
Laser bed

Dark/clean room

Control computer

Associated electronics

LMX mapping microscope

Oxford additions

- Leverage a Software Development Kit (FastZ)

- Add STORM path to V2 OMX

- Next Generation OMX (OMXT/DeepSIM/CryoSIM)
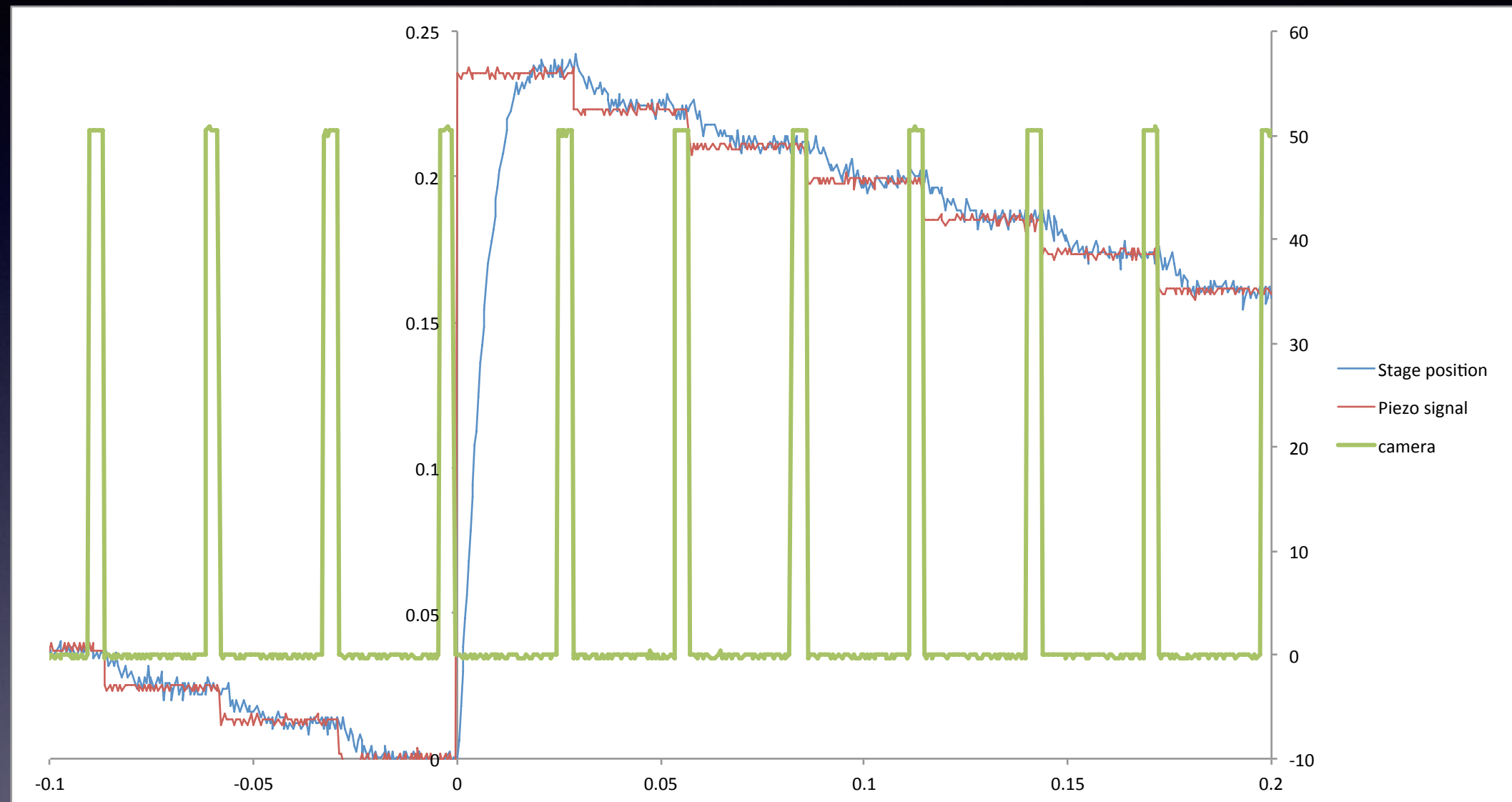
Super Fast Acquisition (FastZ)

- 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

Coventional widefield Z stack
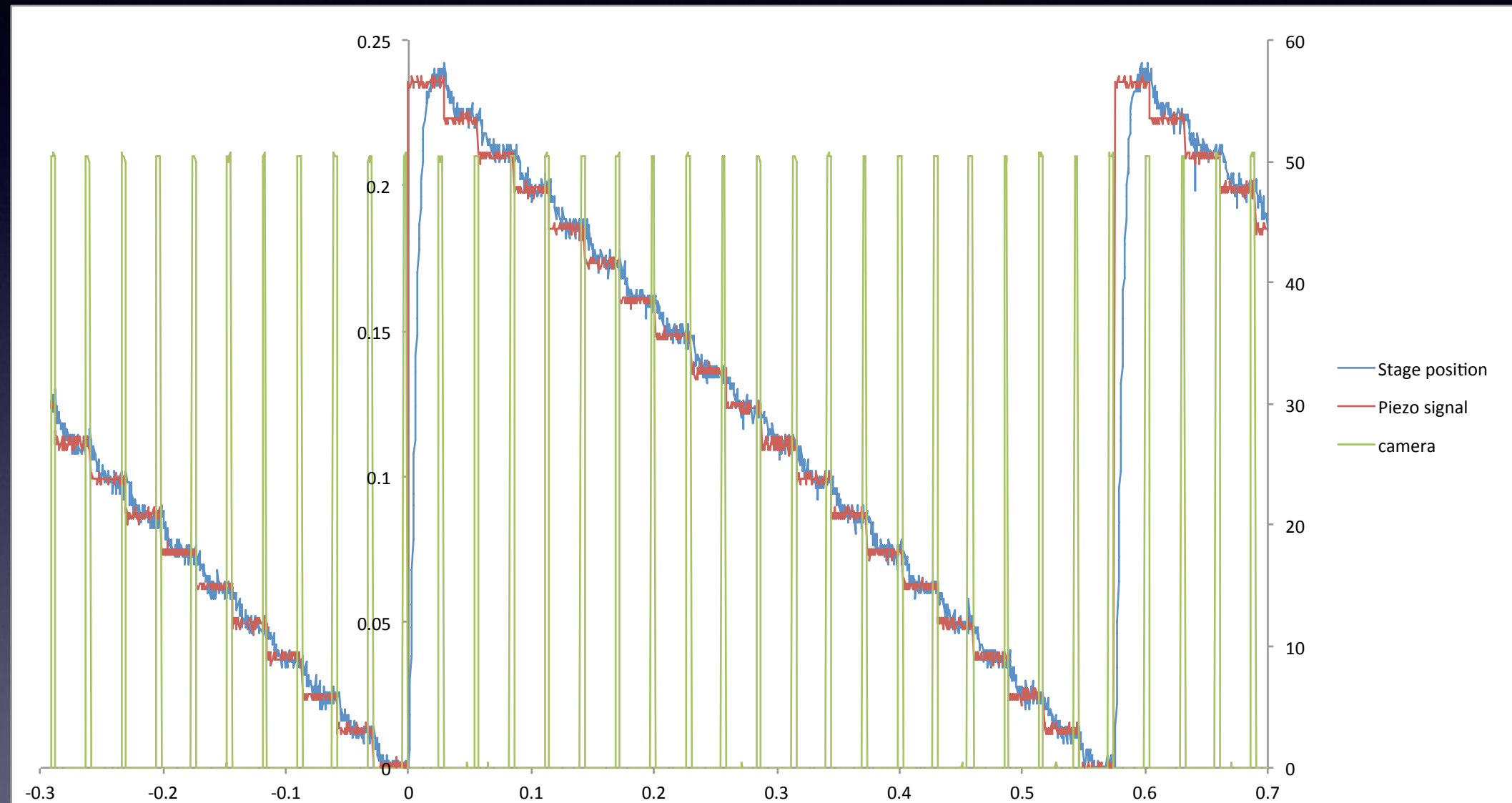
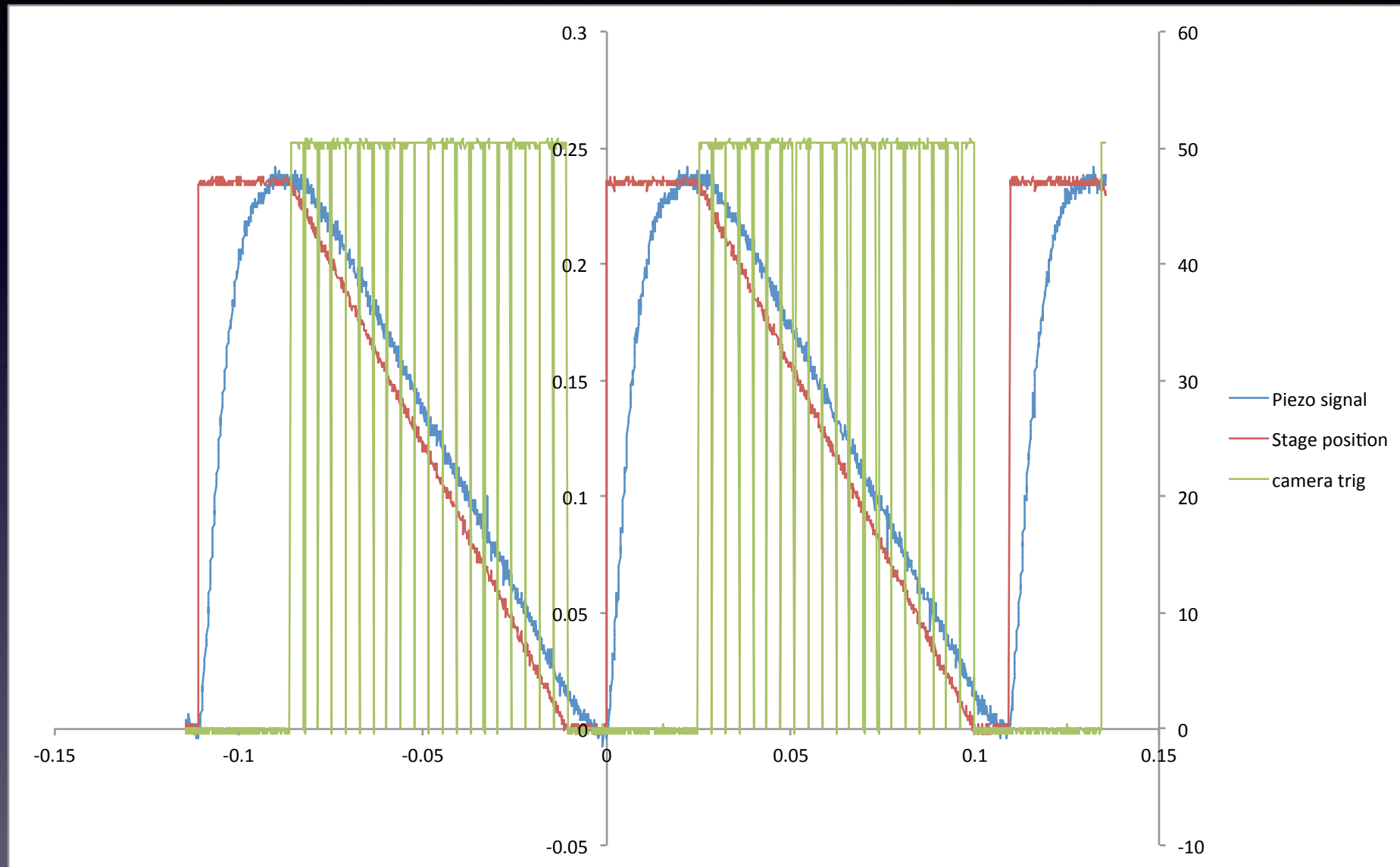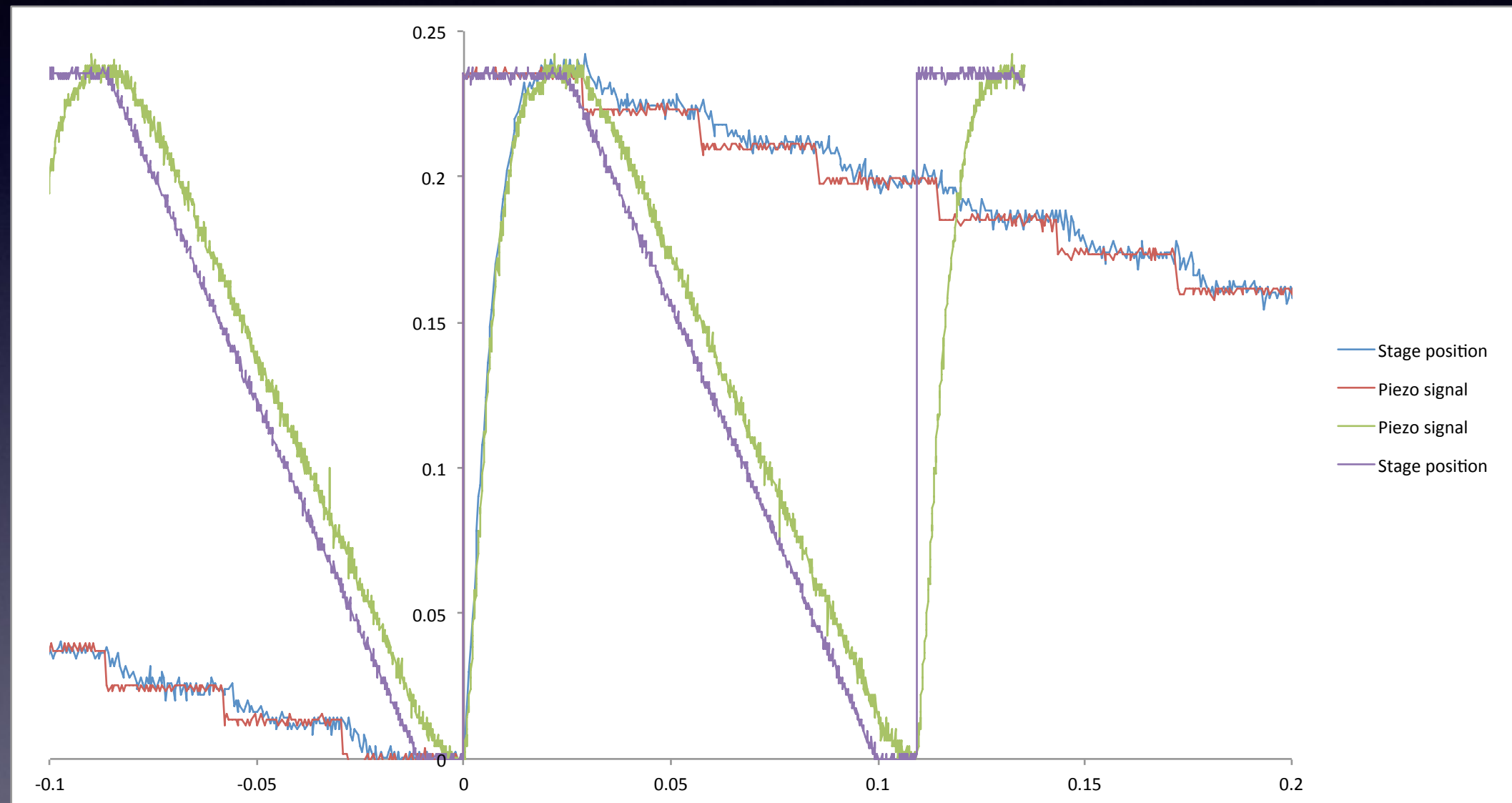20 Z planes as fast as possible

Coventional Z stack

Ramp Z stack

Comparison:
FastZ to normal

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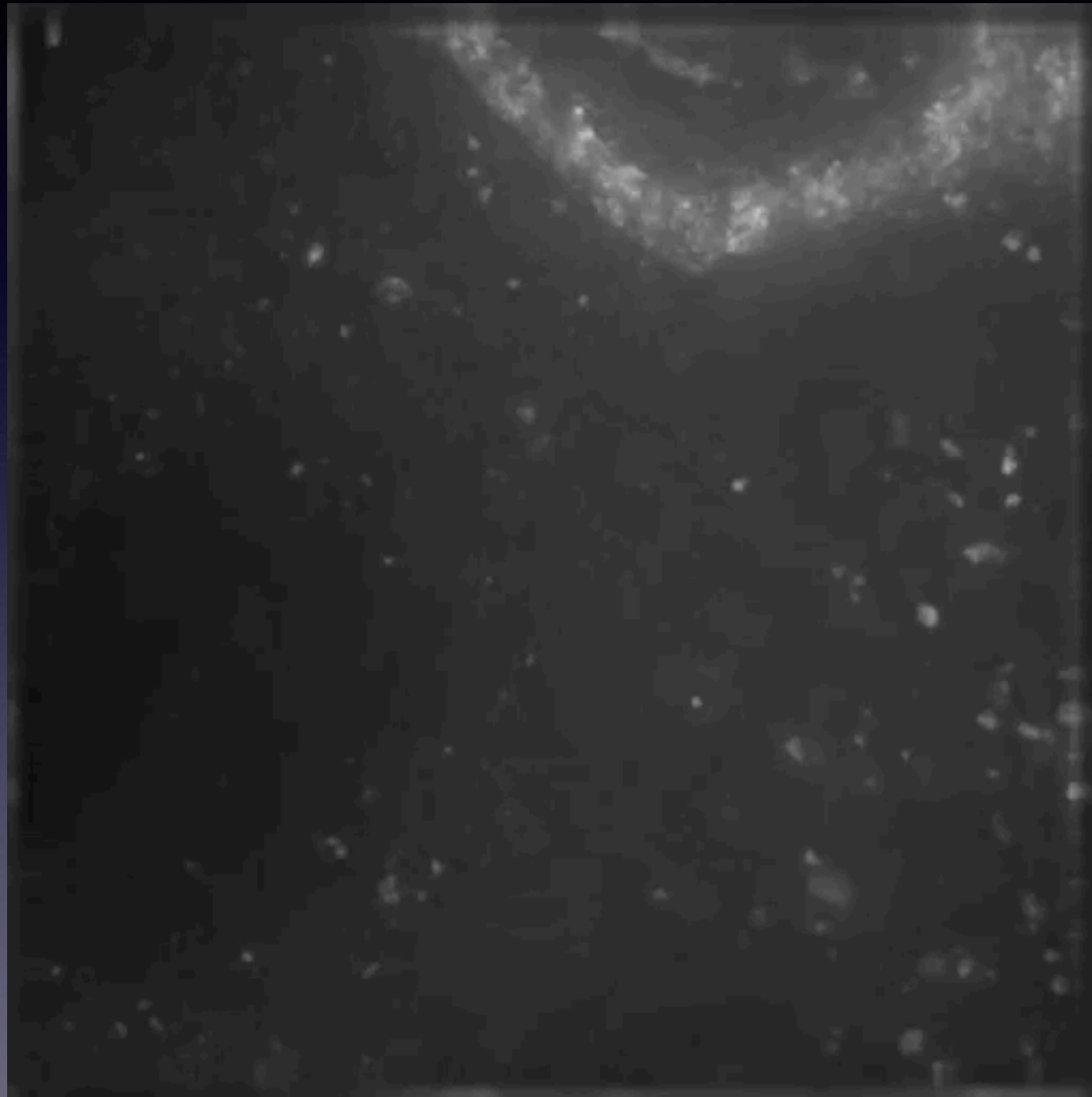
Me31B-GFP Drosophila oocyte
25-slices, 8 stacks/s - 200 frames/s

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OMX Advances-examples

CryoSIM (Diamond)
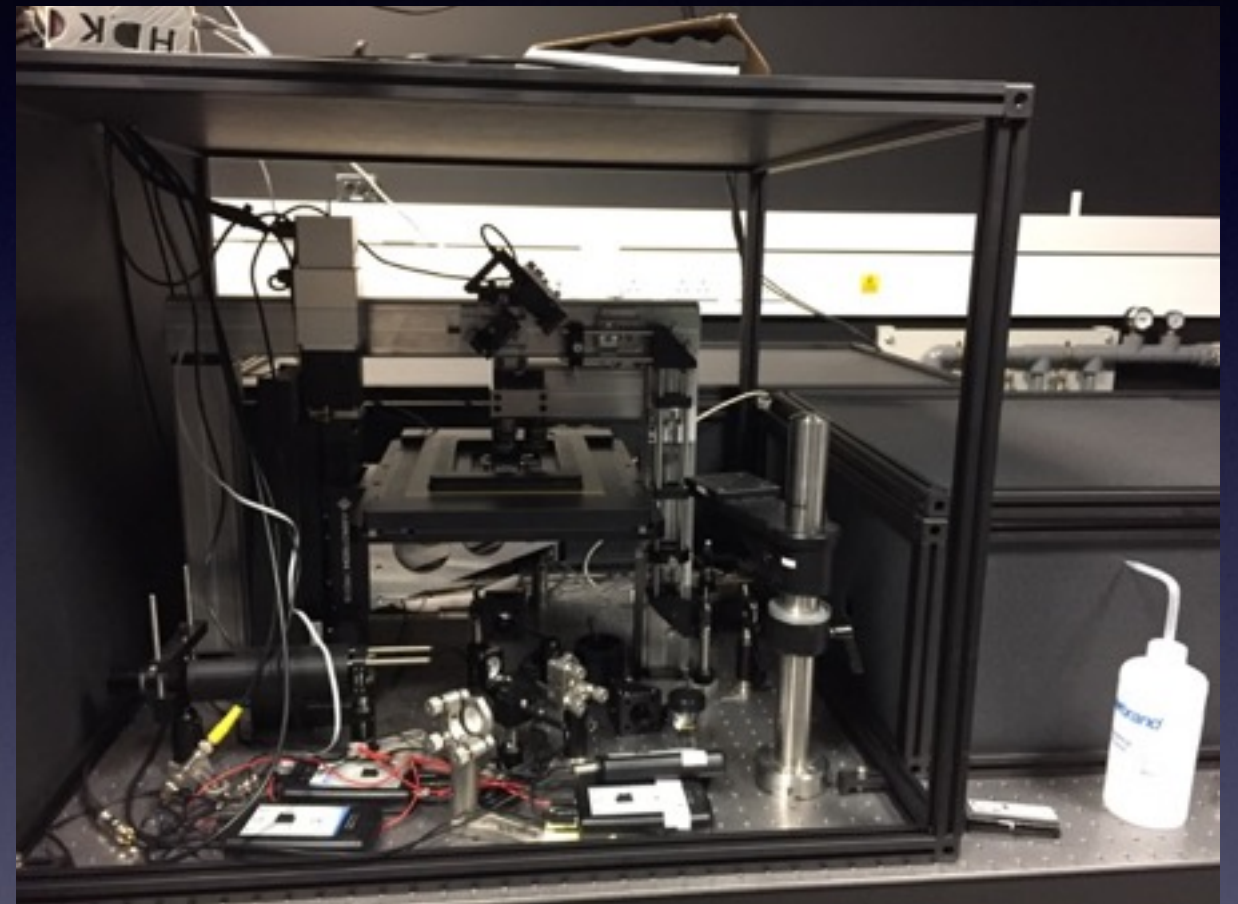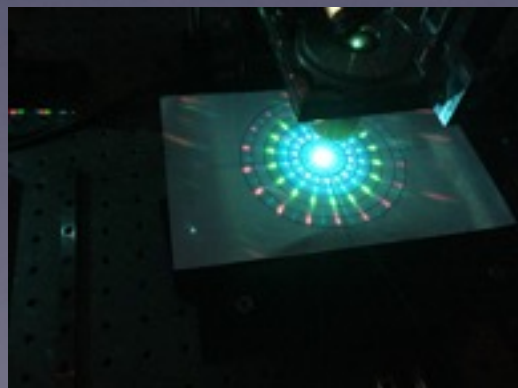
DeepSIM

DeepSIM Aims

- Upright SIM system for simultaneous super-res neuro-physiology

- Fast for live cell imaging

- Flexible for multiple imaging modes and equipment control

- Adaptive optics to enable fast Z steps with dipping lenses and improve Z depth in super-resolution

Locally developed control software - Cockpit

Why re-invent the wheel (or micromanager)

- Timing - hardware based (3 options)

- Truly open source

- Multi-modal and usability

Command Prompt - cockpit.bat

```
C:\>cd cockpit
C:\cockpit>cd ian
C:\cockpit\ian>cd cockpit
C:\cockpit\ian\cockpit>cockpit.bat
C:\cockpit\ian\cockpit>python cockpit.pyw
C:\cockpit\ian\cockpit>cockpit.bat
C:\cockpit\ian\cockpit>python cockpit.pyw
C:\cockpit\ian\cockpit>python cockpit.pyw
C:\cockpit\ian\cockpit>cockpit.bat
C:\cockpit\ian\cockpit>python cockpit.pyw
Unhandled exception in thread started by
Unhandled exception in thread started by
C:\cockpit\ian\cockpit>cockpit.bat
C:\cockpit\ian\cockpit>python cockpit.pyw
```
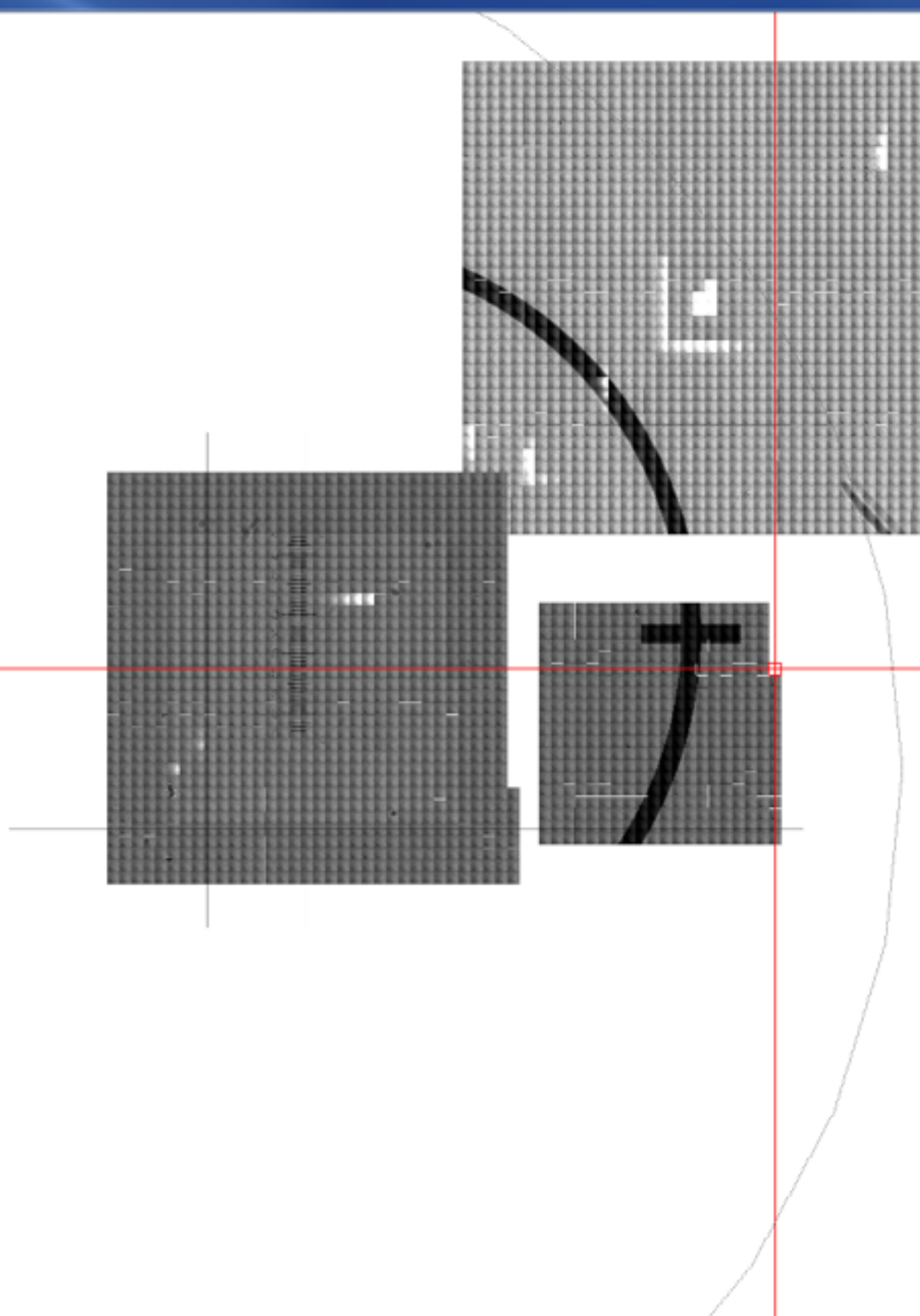
Camera views   (401, 185) 1293

GFP (East)   No camera

Objectives
10x   60xwater

Cockpit program (currently logged in as ian)

| 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:
Mode: not set	Mode: Conv 3MHz	piDIO	OFF	conventional
		DSP TTL	step	
EM Gain 0	EM Gain: 0		angle: 0 phase: 2 wavel: 488	SI
exp. trigger: clean, no FT	exp. trigger: clean, no FT			

Illumination controls:

405nm power	488nm power	561nm power	647nm power		
SET: 74.9mW OUT: 0.0mW	SET: 60.0mW OUT: 0.0mW	SET: 120.0mW OUT: 0.0mW	SET: 35.0mW OUT: 0.0mW		
405nm	488nm	561nm	647nm	ambient	Trans
100ms	100ms	100ms	100ms	100ms	100ms

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 |

Macro Stage XY   Macro Stage Z   Experiment Histogram

X: 372760   step 000um
Y: -706220   step 000um

Z 211300 10000   step 200um

Save top	3010.0	Go to top
z-height (µm):	10.00	Go to center
Save bottom	3000.0	Go to bottom

Set safeties | Switch control | Recenter   Set safeties | Touch down

PatchClamp
cockpit.bat
dome5u
dome5u.RDP
domedsp
domedsp....
domesim
domesim....
dome-tran...
(dome5u)
Laser-Cont...

1319

Snap a single image and import it into the mosaic.

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 -
SOFTWARE!

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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

Holographic microscope
Irwin Said and Richard Berry, Micron Oxford

Software options

- Lab view

- Micromanager

- DIY: SDKs - C++, Python, Visual basic

Lab view example

Micromanager

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

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