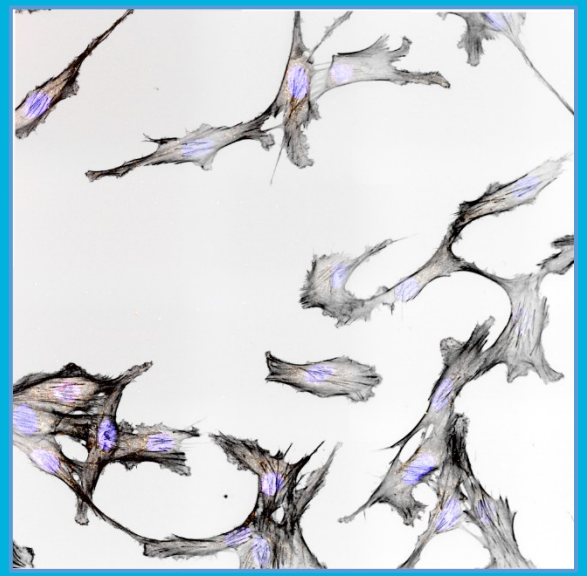
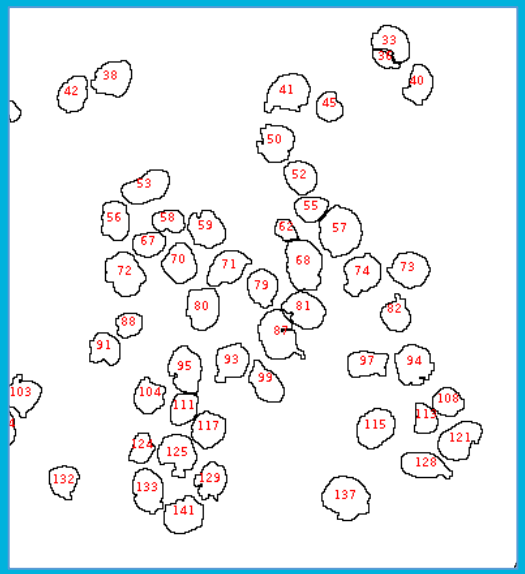
The light microscopy imaging pipeline at Warwick Medical School



Credit: Alex Zwetsloot



Erick Martins Ratamero, Claire Mitchell, Helena Coker

@erickratamero, @WarwickCAMDU

WCPM/CSC, 4th Mar 2019

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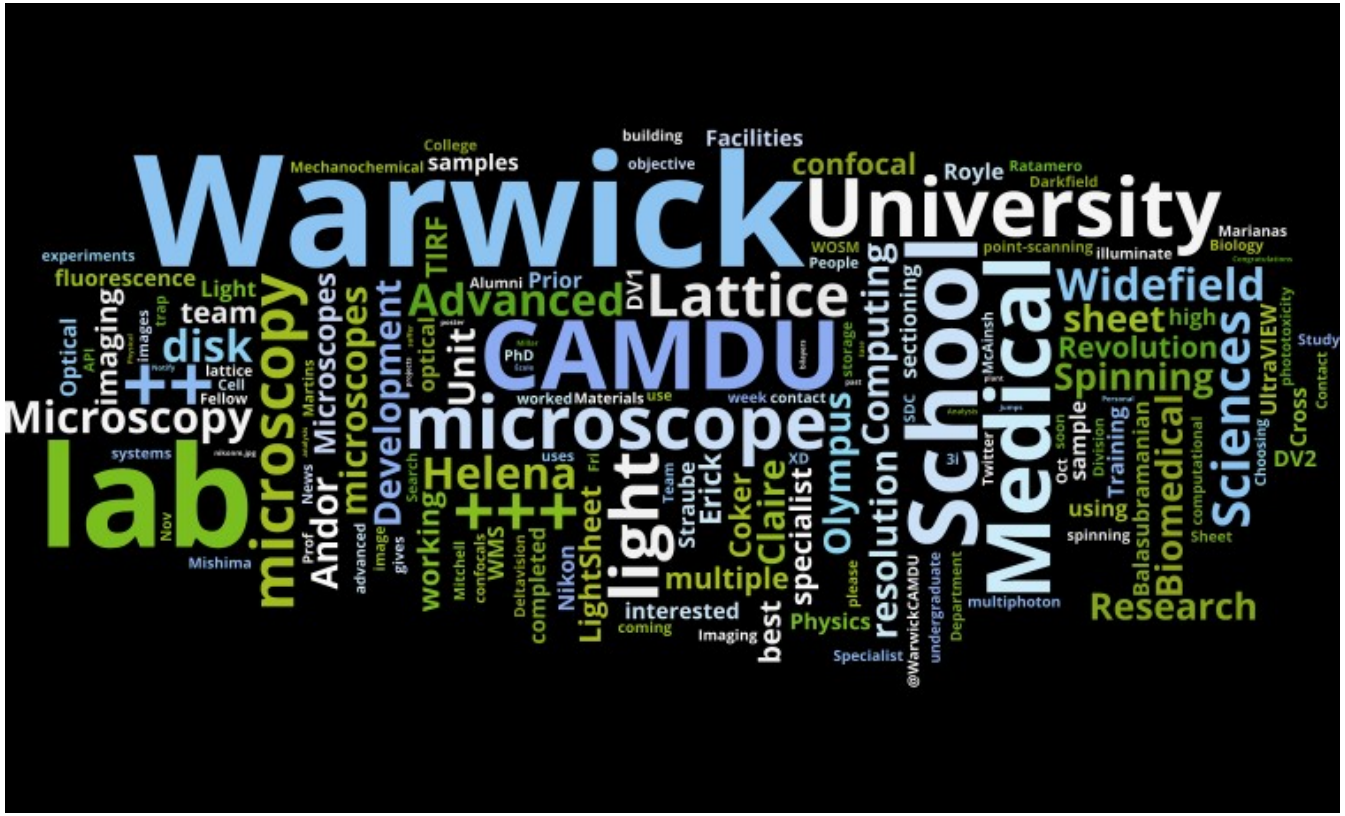
An introduction to CAMDU



Created using Wordle

- Who we are
- Where we are
- Organisation
- Equipment
- Future plans

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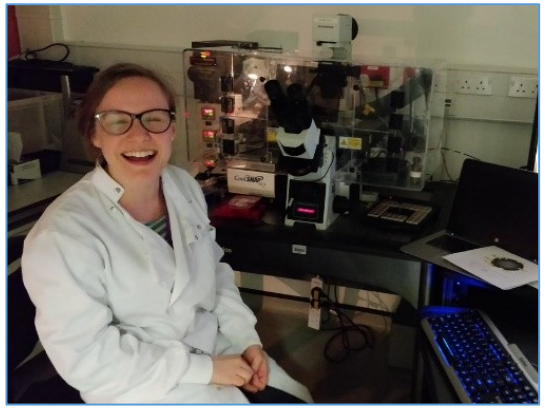
Who are CAMDU?

Erick

Sept 2017

Image Analysis and Data Storage

@erickratamero



Claire

Oct 2017

General microscopy

@DrCMitchell

Helena

July 2018

Lattice light sheet specialist

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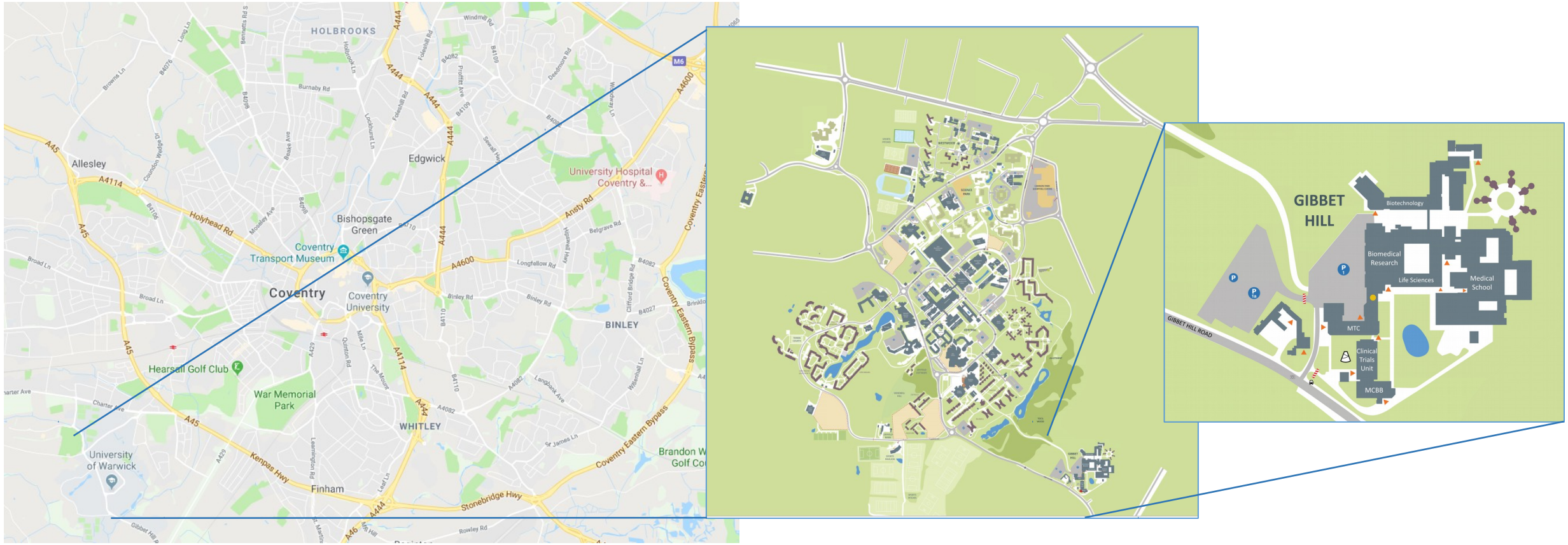
Where we are



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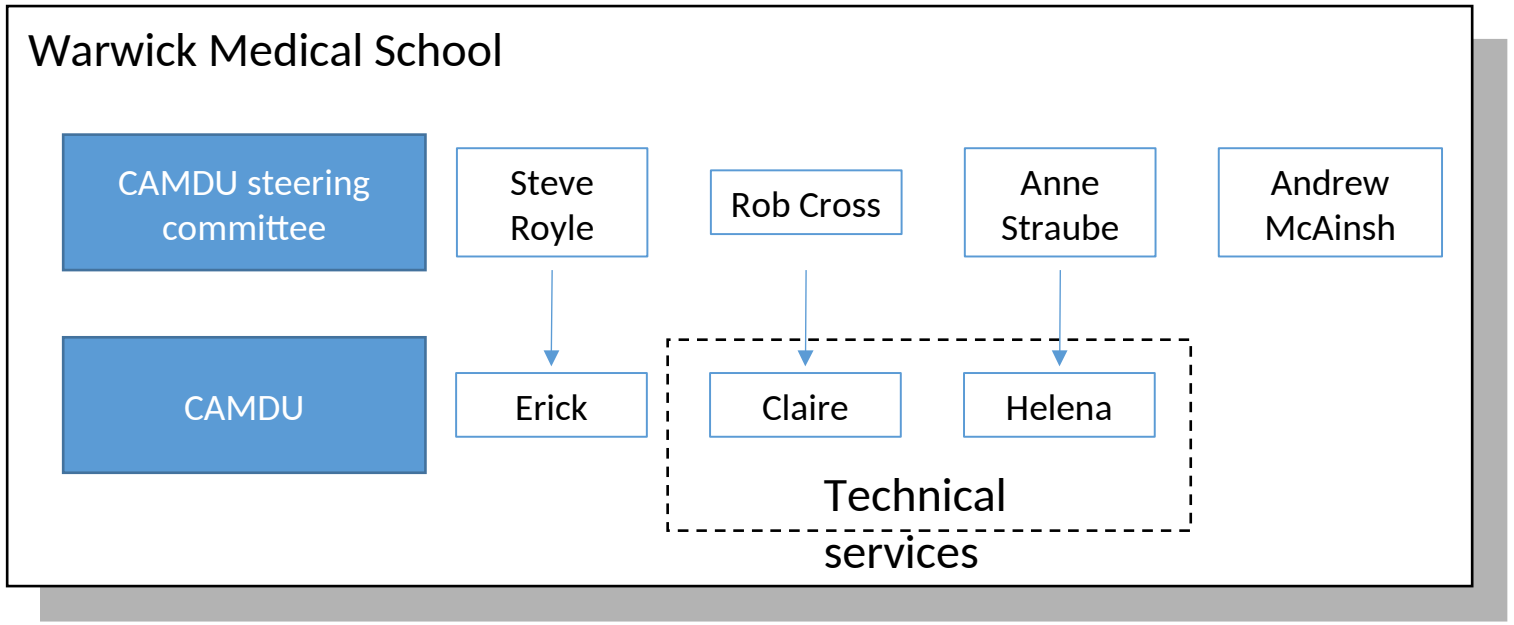
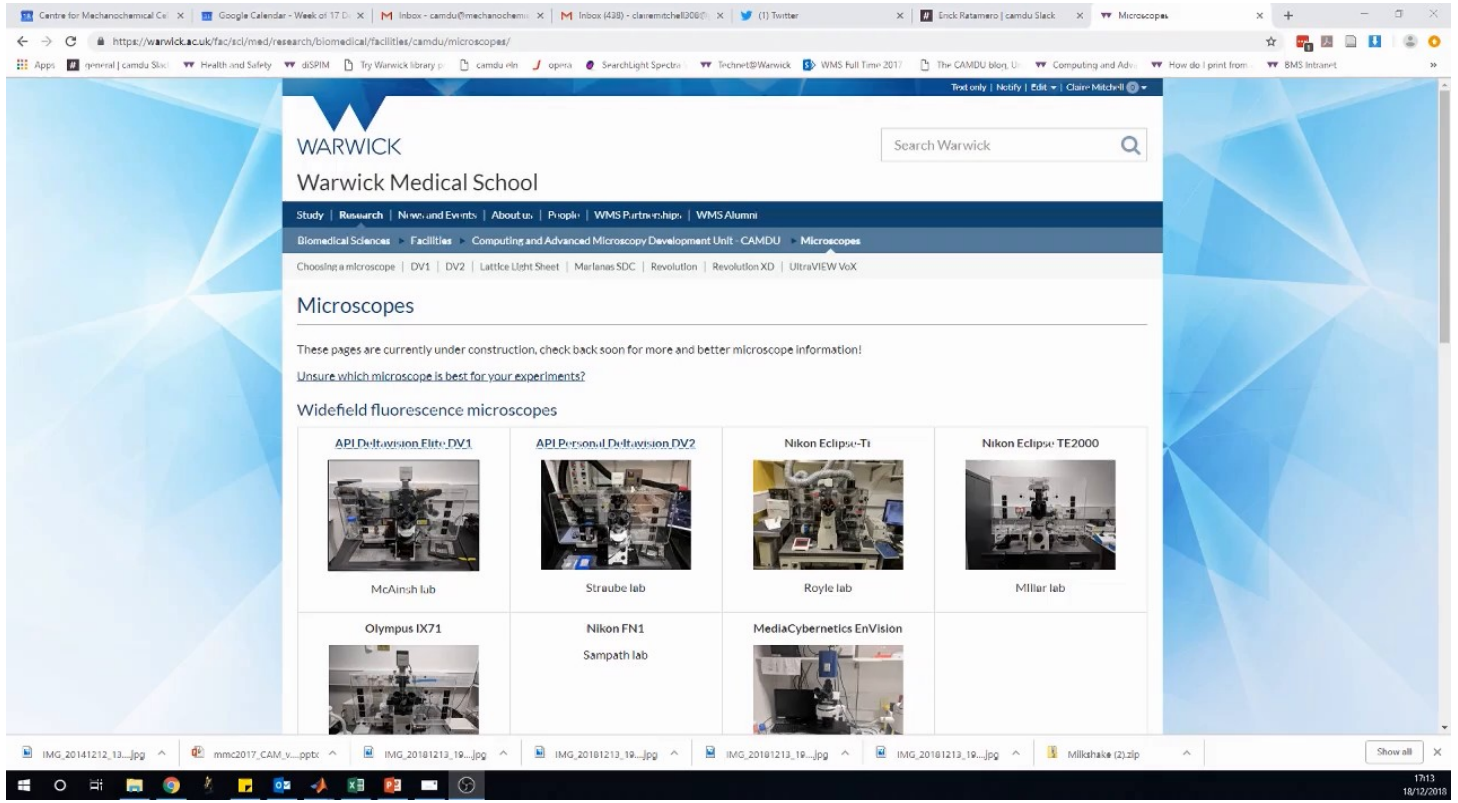
Funding model

- Microscopes brought in through PIs
- Shared use is through goodwill and collaboration
- If service contract covered by division – time made available to other users

Management structure



Equipment



warwick.ac.uk/camdu

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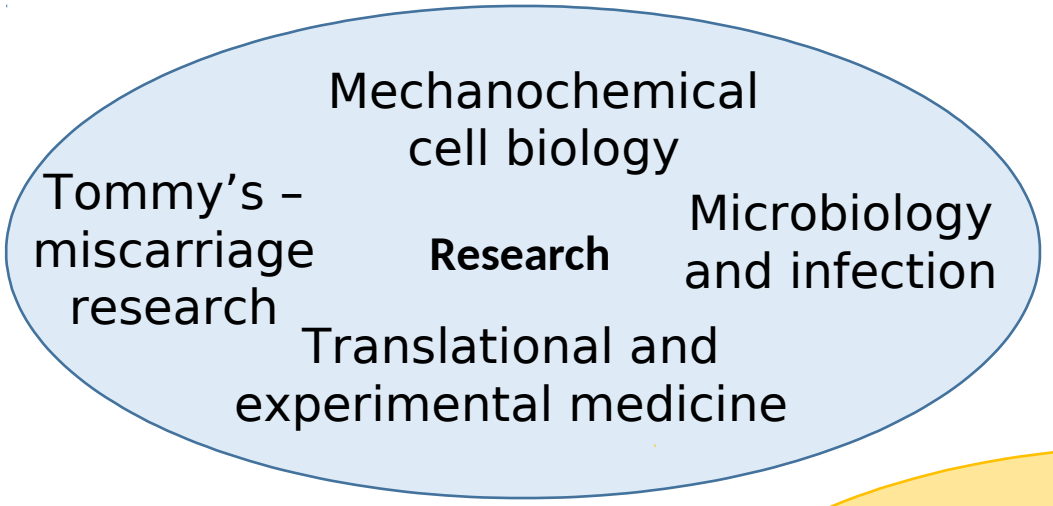
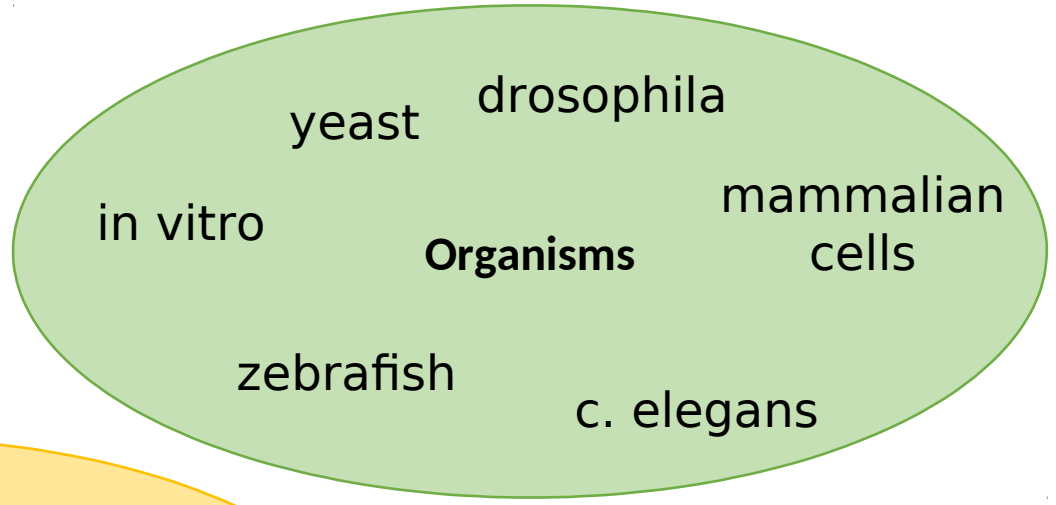
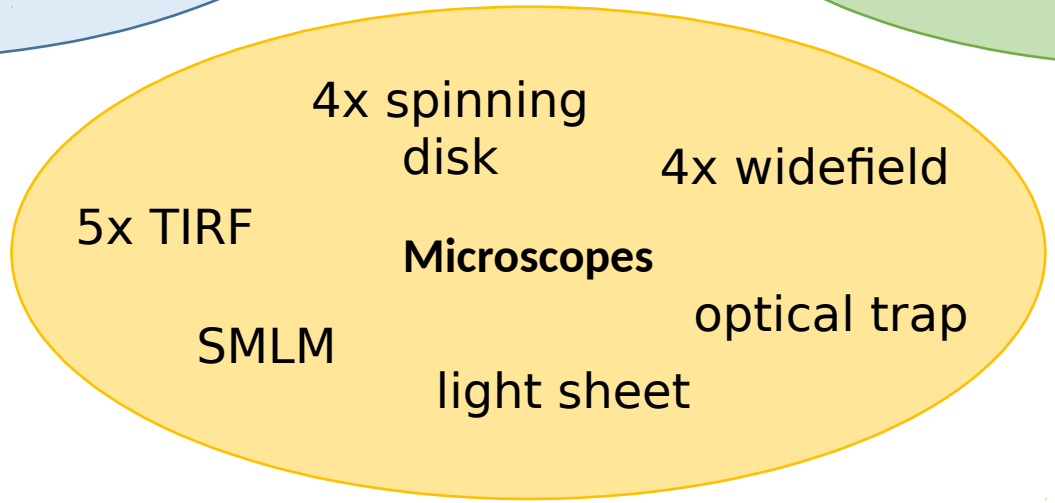

Mechanochemical cell biology
Tommy's – miscarriage research
Research
Microbiology and infection
Translational and experimental medicine


yeast
drosophila
in vitro
Organisms
mammalian cells
zebrafish
c. elegans


4x spinning disk
4x widefield
5x TIRF
Microscopes
optical trap
SMLM
light sheet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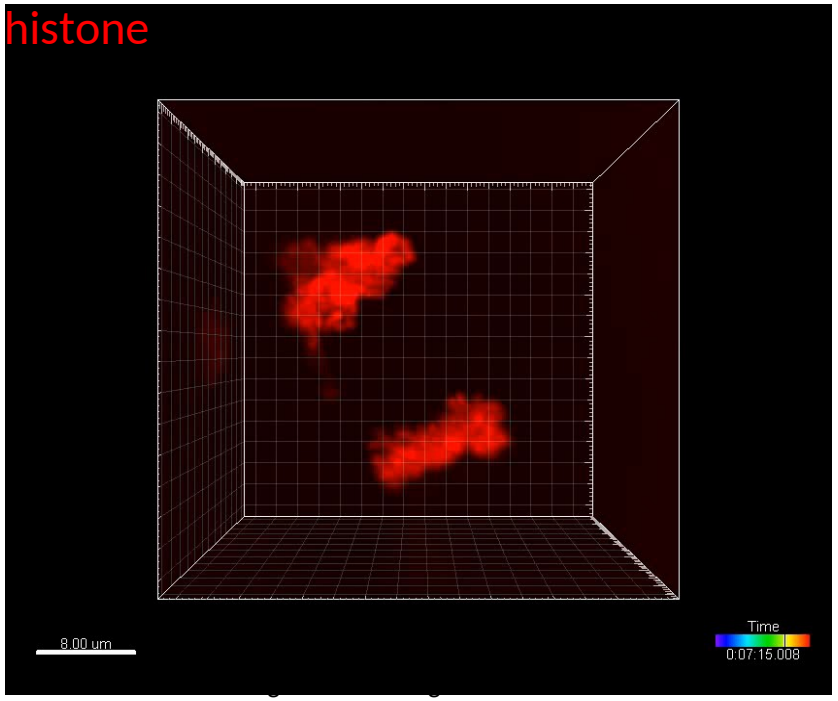
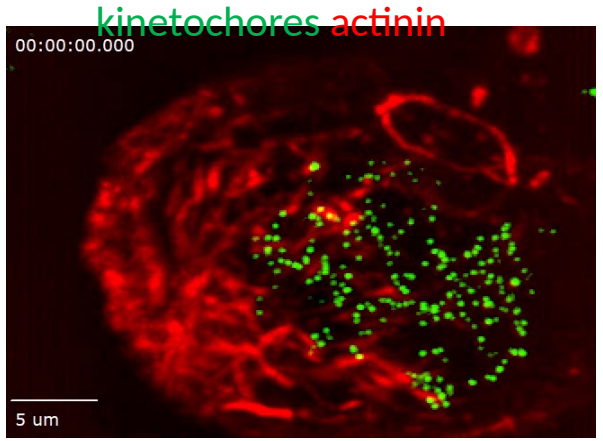
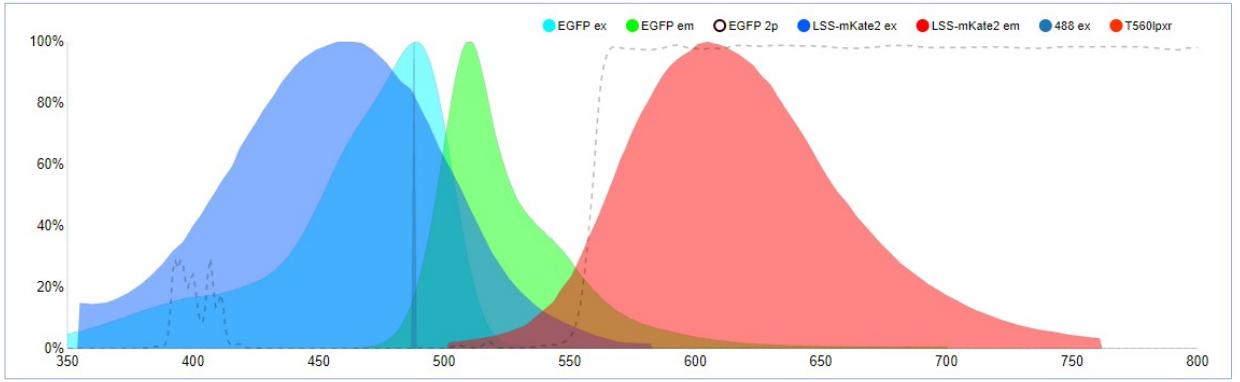
Lattice light sheet



- Dual-camera lattice light sheet from 3i
- Funded through a Wellcome multi-user grant
- Arrived Jan 2018
- Lattice specialist arrived Jun 2018
- **Visitor program available for external users**



simultaneous 2-colour collection on 3i lattice lightsheet



Spectra of eGFP and LSS-mKate2 used for simultaneous excitation on LLS

from fpbase.org

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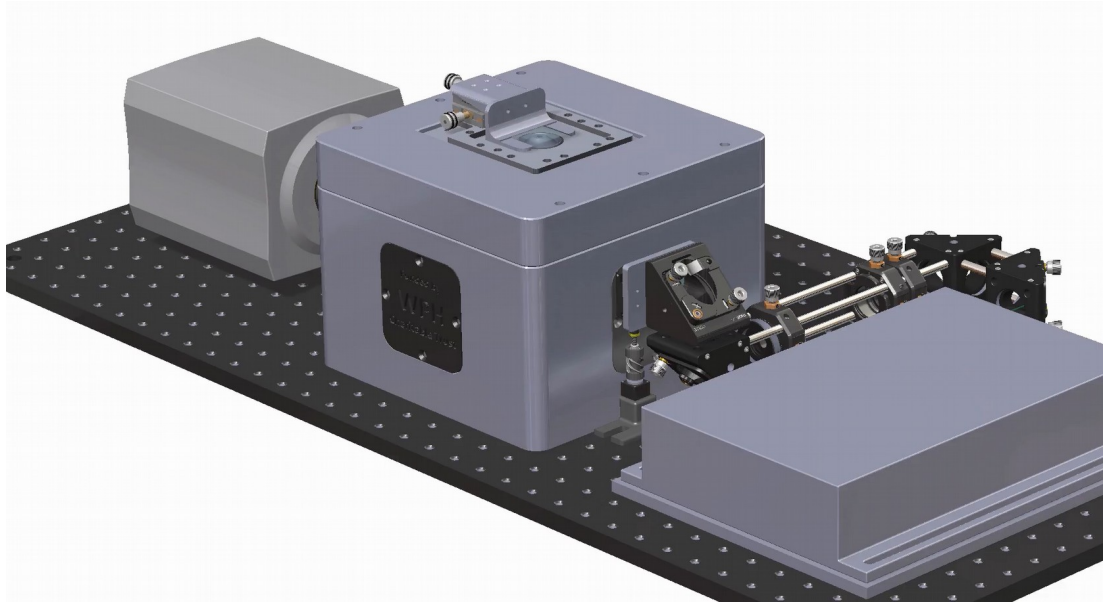
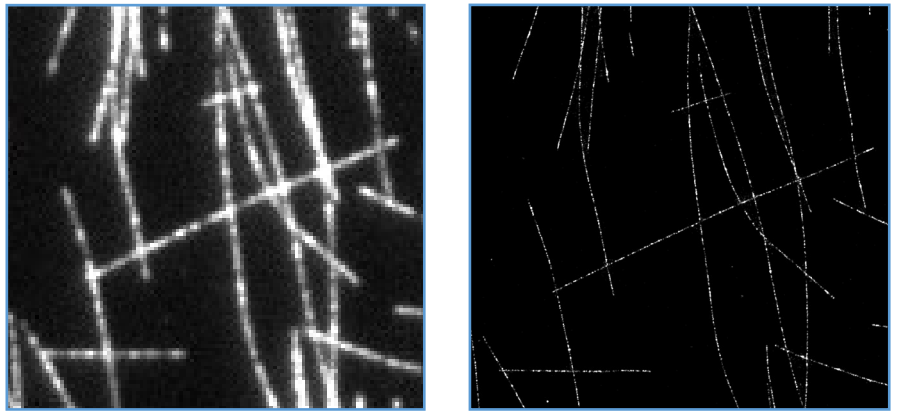
Warwick Open Source Microscope (WOSM)



wosmic.org

- Designed and built by Nick Carter w/ Rob Cross
- Monolithic, highly stable and modular inverted microscope
- Optimised for widefield, TIRF and SMLM
- Custom-designed electronics, browser interface
- Variants in progress:
  - WOSMtrap
  - eduWOSM



STORM imaging of microtubules using the WOSM

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Future plans

Long-term:

- WMS is growing
- New building w/ 300 researchers

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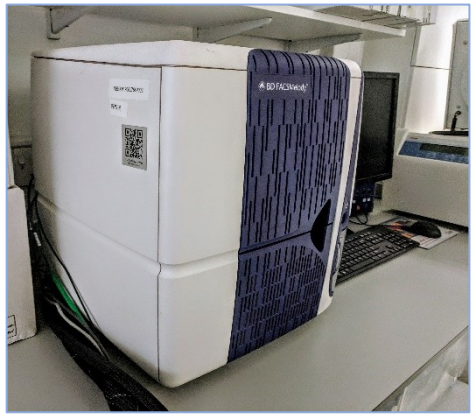
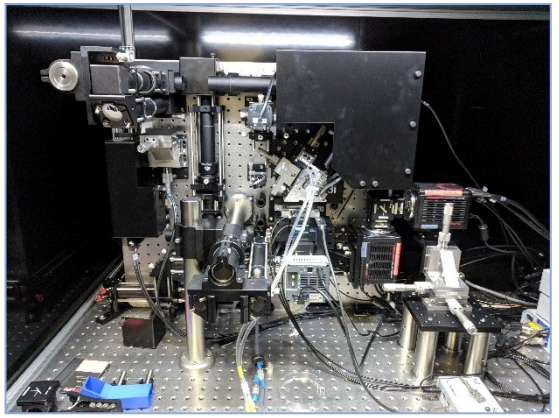
Short-term:

- Introduce robust procedures
- Encourage external users
- Expand into flow cytometry and sorting



Ernie the cell sorter – coming to WMS early 2019



The Lattice light sheet – available to external users through the visitor program

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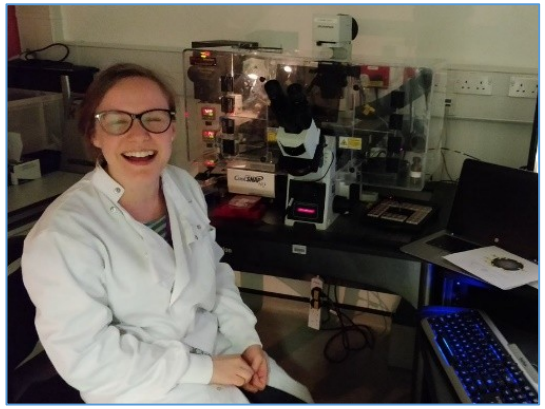
Who am I?

Erick

Sept 2017

Image Analysis and Data Storage

@erickratamero



Claire

Oct 2017

General microscopy

@DrCMitchell

Helena

July 2018

Lattice light sheet specialist

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Background:

- Anything computational
- M.Sc. in Complex Systems
- Ph.D. in Analytical Science (but actually Computational Biophysics)

Current responsibilities:

- Anything that happens post-acquisition
- Data storage/management, image analysis…
- Establishing and maintaining systems
- Writing custom-built tools and advising on existing tools for image analysis
- Anything vaguely computer-y

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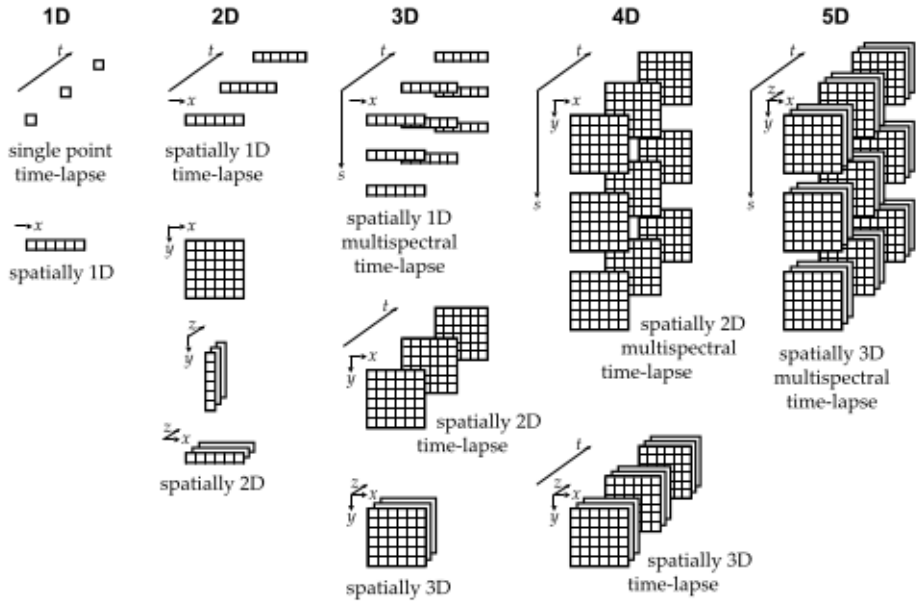
A primer on (bio)image analysis



From Meijering, Erik, and Gert van Cappellen. "Biological Image Analysis Primer." Erasmus MC, Rotterdam (2006).

Some of the tasks

Image Formation
object in → image out

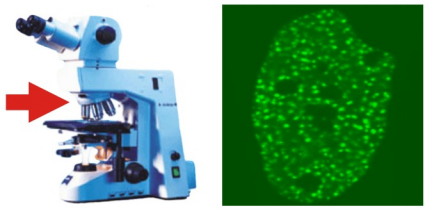


Image Processing
image in → image out

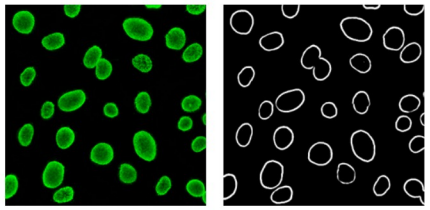
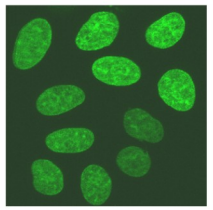


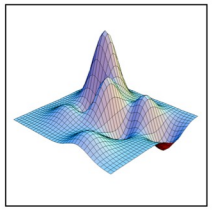
Image Analysis
image in → features out



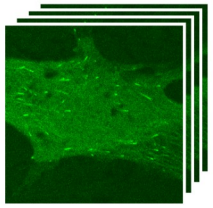
Obj	Area	Perim
1	324.2	98.5
2	406.7	140.3
3	487.1	159.2
4	226.3	67.8
5	531.8	187.6
6	649.5	203.1
7	582.6	196.4
8	498.0	162.9
9	543.2	195.1

Computer Graphics
numbers in → image out

X	Y	I
-3.54	-2.32	0.50
-2.78	-1.90	0.12
-1.15	0.42	3.09
0.45	1.65	5.89
1.83	2.18	7.72
2.98	3.33	2.07
4.21	3.96	-4.58
5.62	4.54	-11.45
7.16	5.02	-3.63

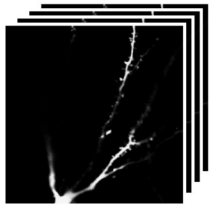


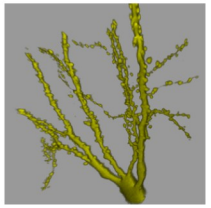
Computer Vision
image in → interpretation out



The series shows microtubule growth in a live neuron. The average speed of the distal ends is comparable in the cell body, dendrites, axons, and growth cones.

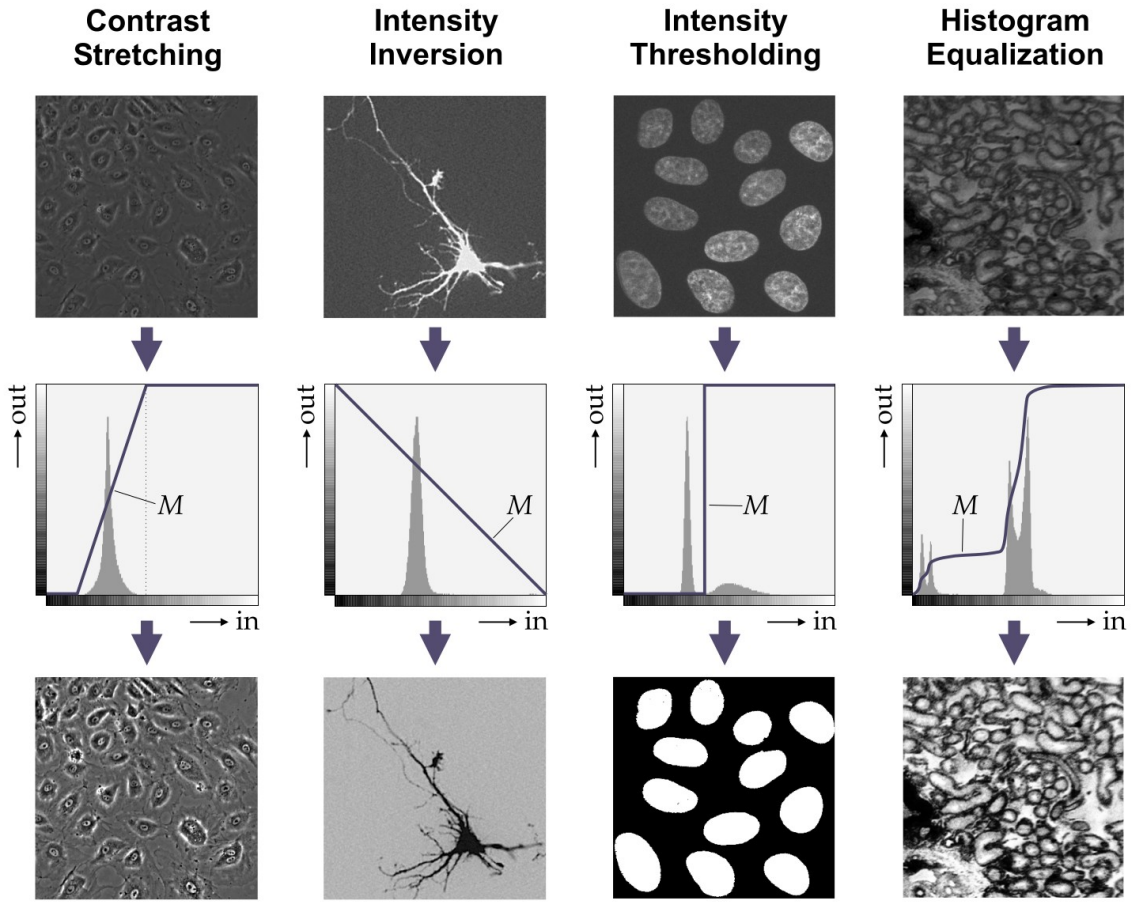
Visualization
image in → representation out





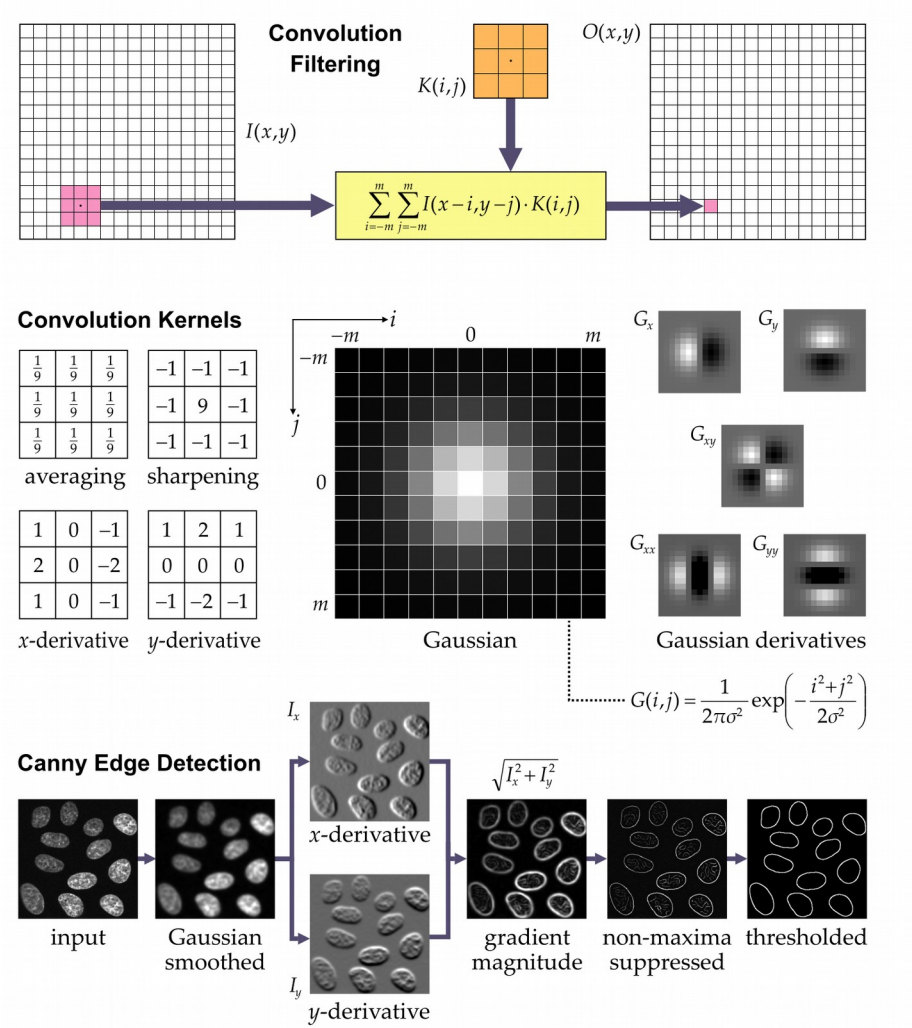
From Meijering, Erik, and Gert van Cappellen. "Biological Image Analysis Primer." Erasmus MC, Rotterdam (2006).

Mapping components



From Meijering, Erik, and Gert van Cappellen. "Biological Image Analysis Primer." Erasmus MC, Rotterdam (2006).

Convolution filtering



From Meijering, Erik, and Gert van Cappellen. "Biological Image Analysis Primer." Erasmus MC, Rotterdam (2006).

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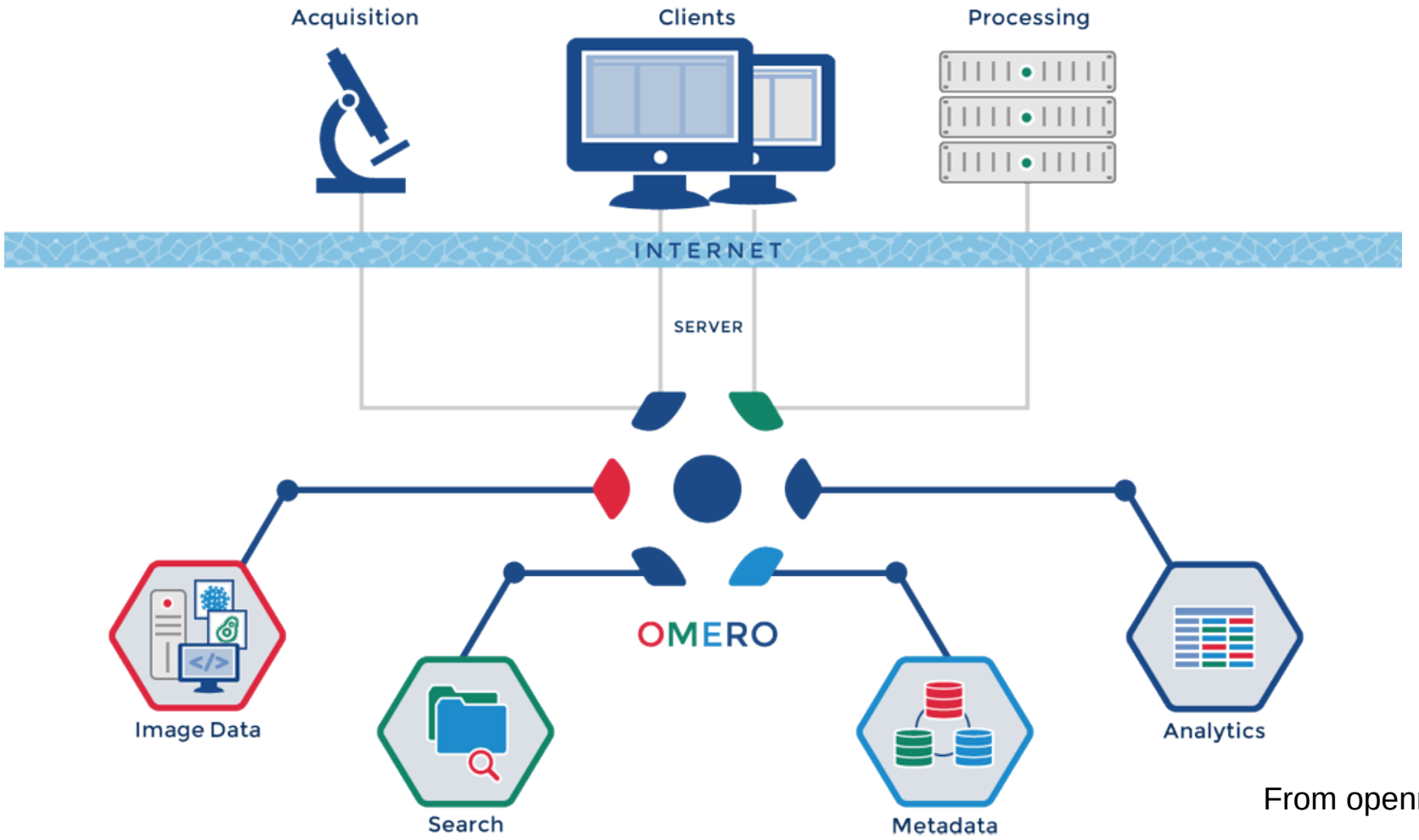
If we have time: typical workflows

So what are we doing?

OMERO



From openmicroscopy.org

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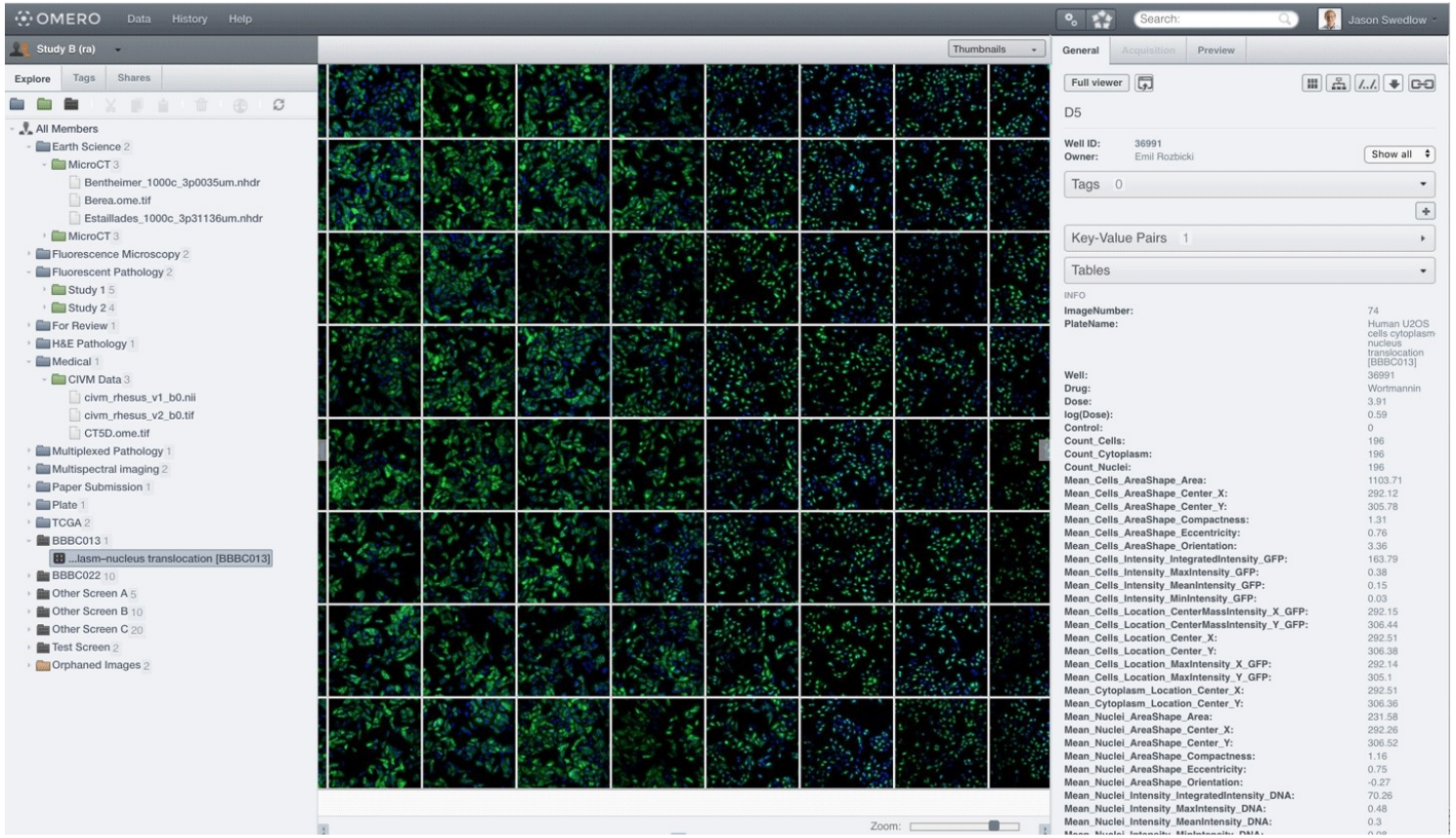
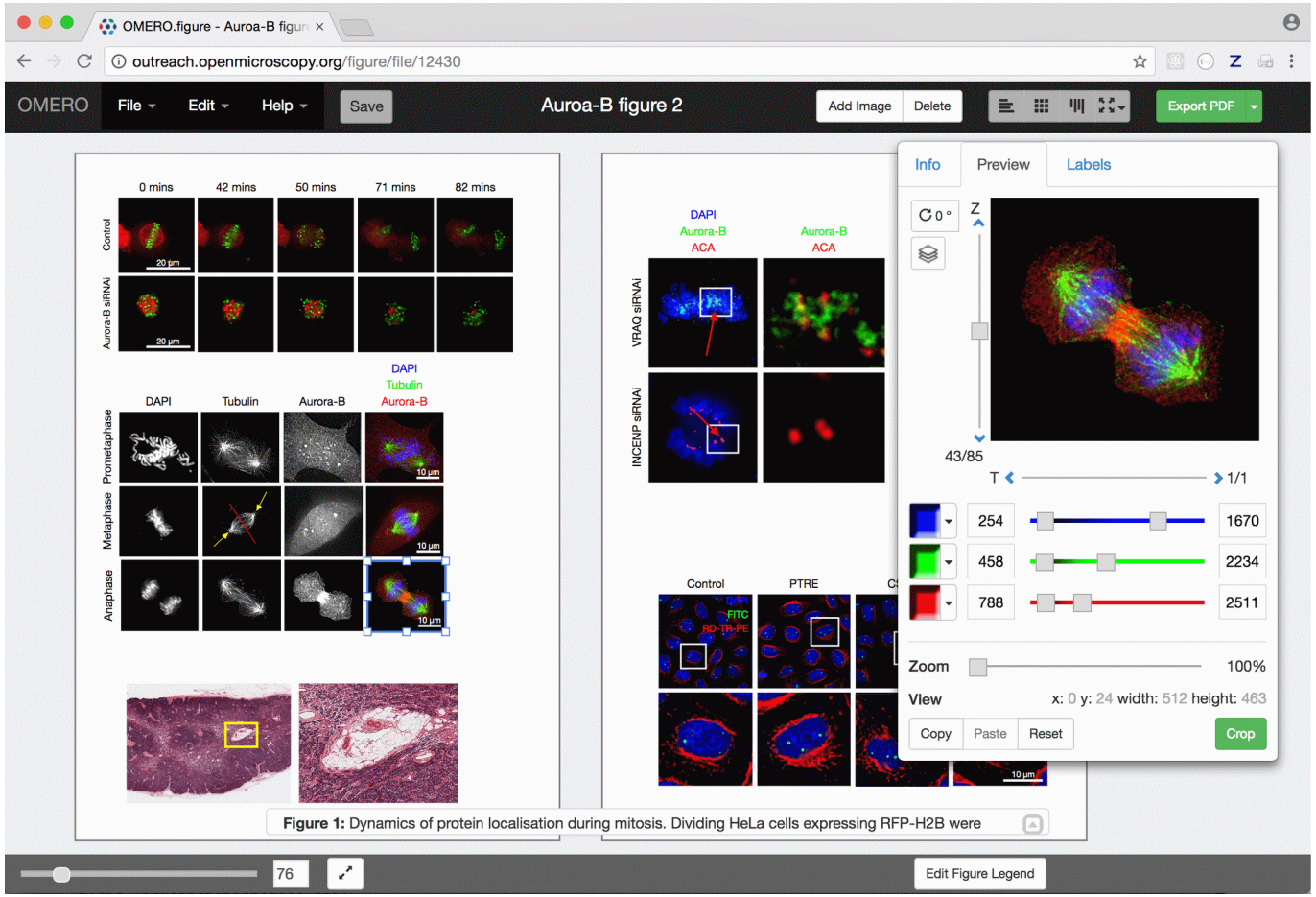
OMERO



From openmicroscopy.org

OMERO



From openmicroscopy.org

If we have time: live demo!

Wordpress

Results

Tried ranges of 3-15% Ablate! laser.

Cells either popped or MTs were just bleached.

Transfection rate was not great and the wide field->eyepiece on the 3i system was very low intensity which meant it was nearly impossible to search many fields of view.

Microscope has really nice signal-to-noise on the NPY-RFP vesicles and this could be used for some high speed imaging of those alone if necessary.

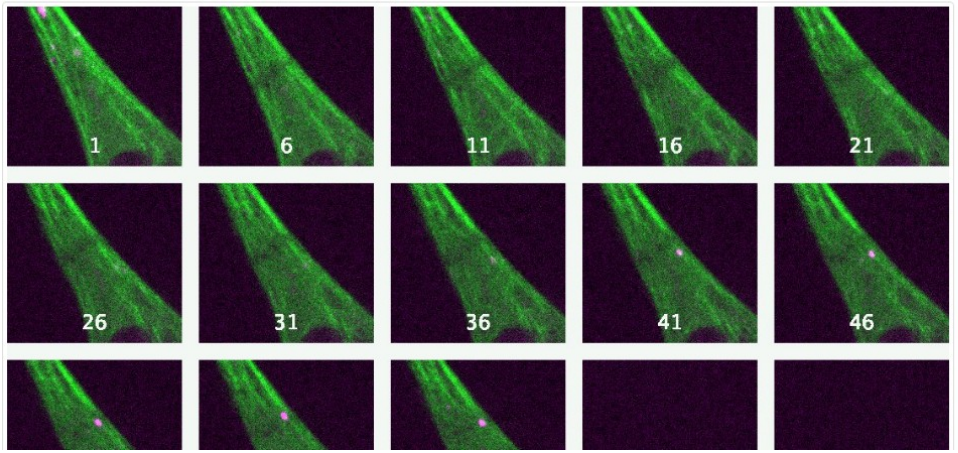
For EB3 – no transfectants found.

For EMTB-3xGFP, cells quickly regained fluorescence or blew up.

For CAMSAP2, I tried ablating both where there was no signal (to see if I could generate new minus ends that recruit CAMSAP2) and bleaching CAMSAP2 spots that I can see. Nothing seemed to happen.

For GFP-tub, there was probably a little bit of ablation going on but there was also a lot of cells blowing up.

In this example, a vesicle approaches the ablated region from the nucleus and goes backwards and forwards a couple of times.



Alex on JB001 Ni-NTA and Size Exclusion Purification of MDV US3

astraube on JB001 Ni-NTA and Size Exclusion Purification of MDV US3

Alexander Zwetsloot on JB001 Ni-NTA and Size Exclusion Purification of MDV US3

ARCHIVES

February 2019
January 2019
December 2018
November 2018
October 2018
September 2018
August 2018
July 2018
June 2018
May 2018
April 2018
March 2018
February 2018
January 2018
December 2017
November 2017

CATEGORIES

Cake
Cell Culture
Cloning

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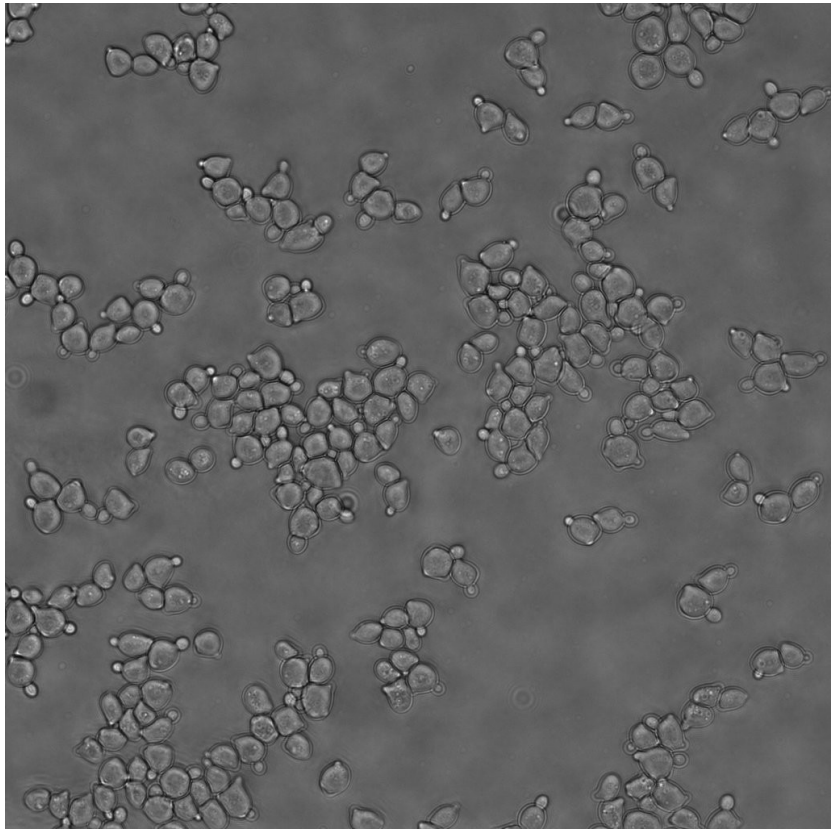
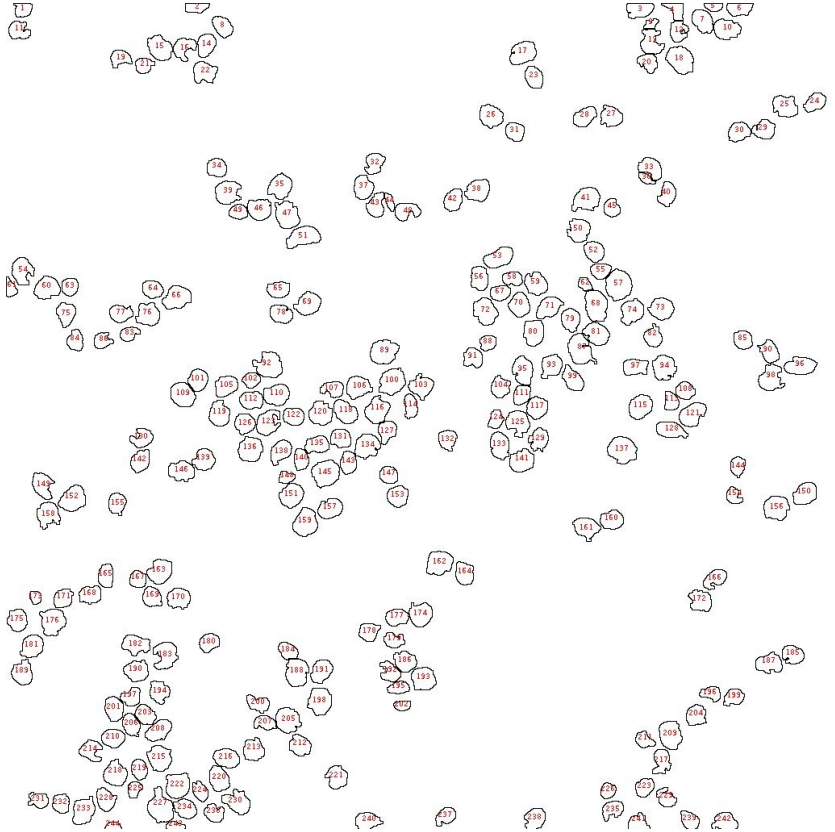
If we have time: quick tour

Creating workflows





	A1.tif	A2.tif	A
total cells	244	163	
cells with 0 foci	57	77	
cells with 1 foci	188	86	
cells with 2 foci	0	1	
percentage of cells with 0 foci	0.234	0.4724	
percentage of cells with 1 foci	0.771	0.5276	
percentage of cells with 2 foci	0	0.006135	

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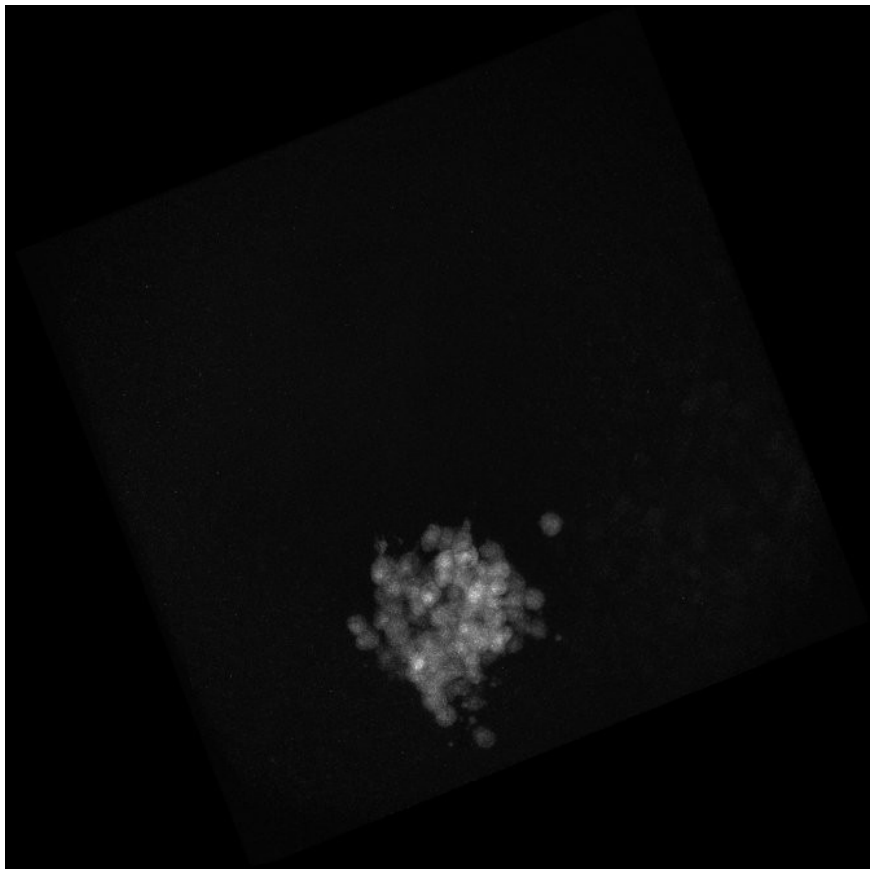
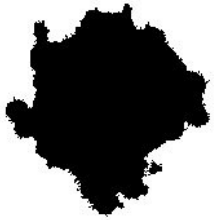
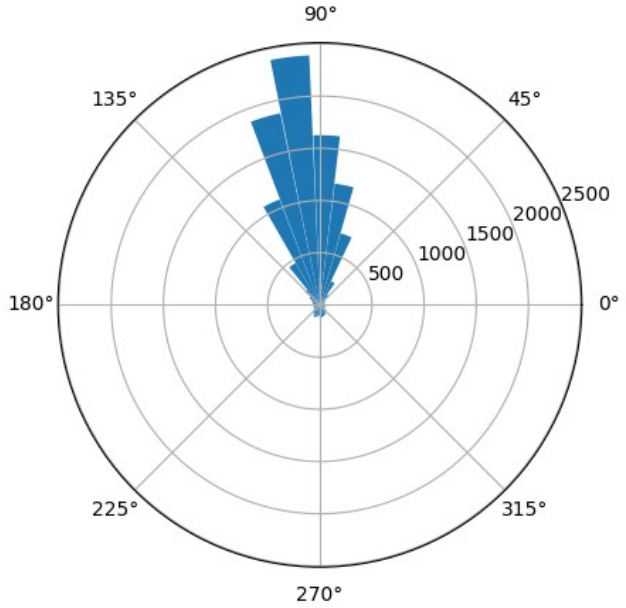
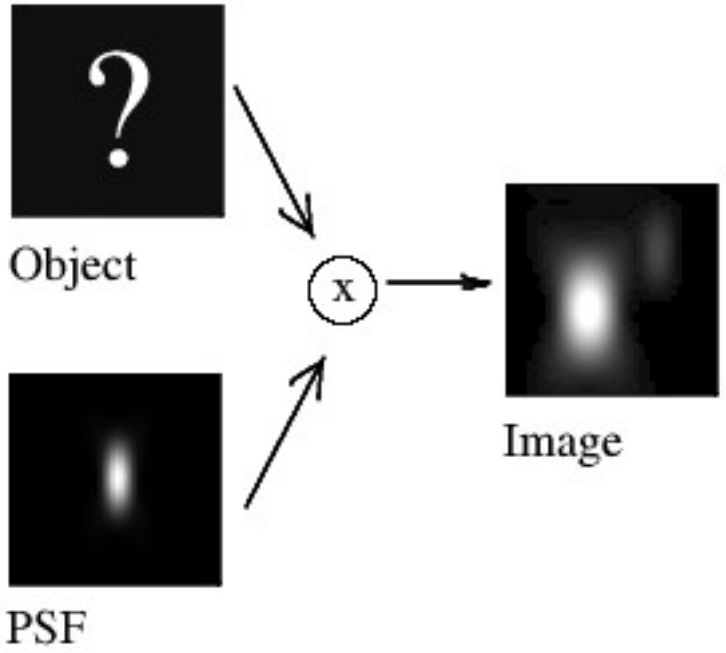
Writing new components



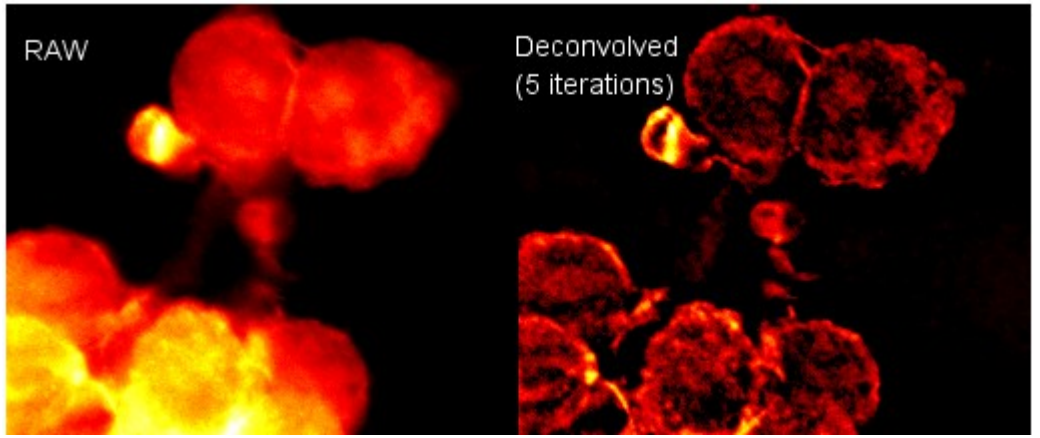




Advanced problems

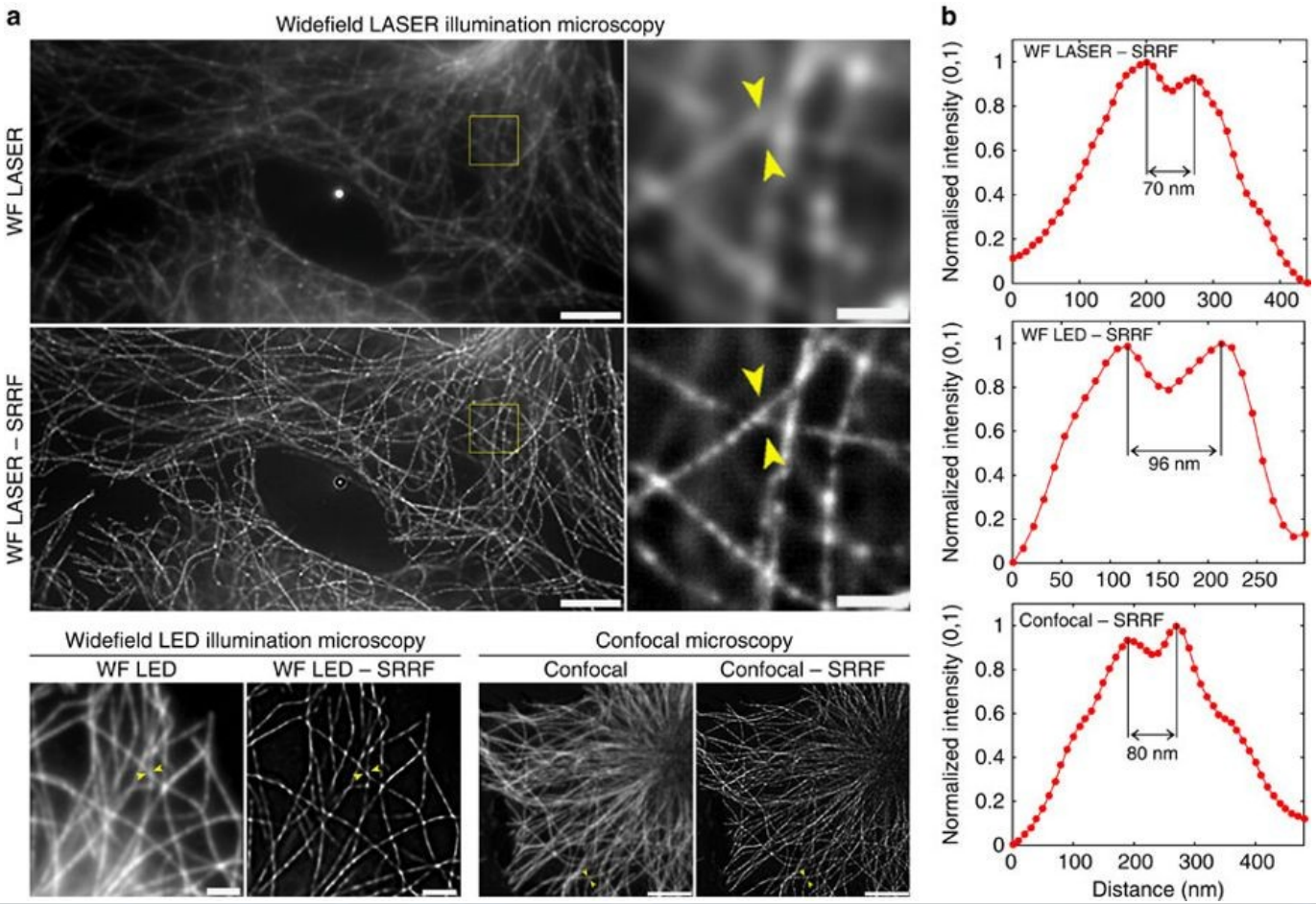


From https://svi.nl/Deconvolution



From https://imagej.net/Deconvolution

Advanced problems



Gustafsson, Nils, et al. "Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations." Nature communications 7 (2016): 12471.

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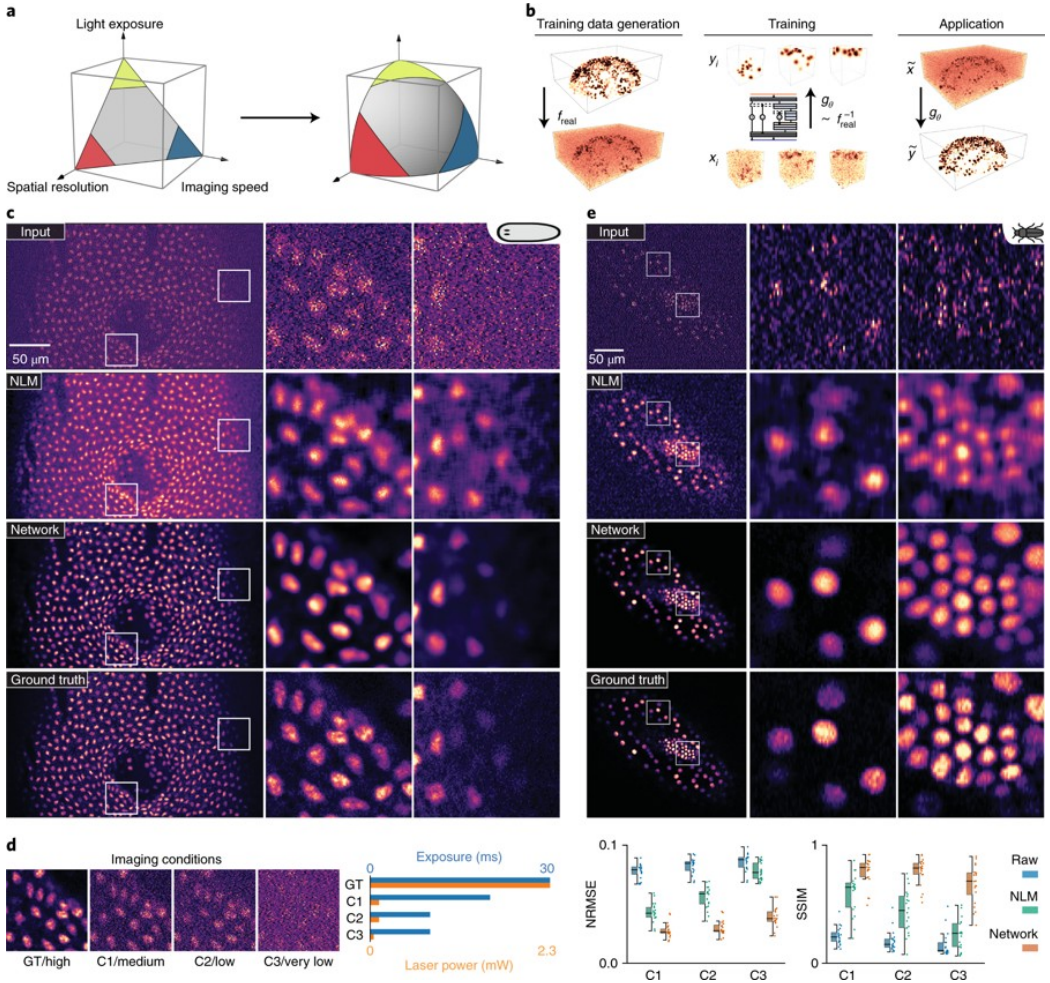
Advanced problems



Weigert, Martin, et al. "Content-aware image restoration: pushing the limits of fluorescence microscopy." Nature methods 15.12 (2018): 1090.

Challenges

- Adoption in general
  - In particular: moving people from “folder structure” to “data management
- Dealing with legacy equipment
- Starting a culture of automated work in Biology
- Implementing FAIR principles on everything we do
- Finding time to try new things

Challenges – computational resources

- All workstations, nothing else
  - Not a problem for most day-to-day tasks
- LLSM data: ~1TB an hour at maximum capacity
- Challenges on storing, transferring, processing
  - Deskew/deconvolution
  - GPU processing helps

Future plans

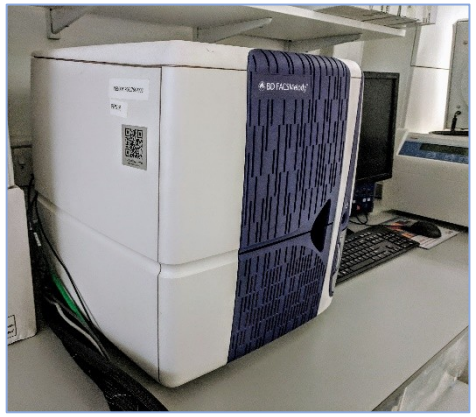
Long-term:

- WMS is growing
- New building w/ 300 researchers

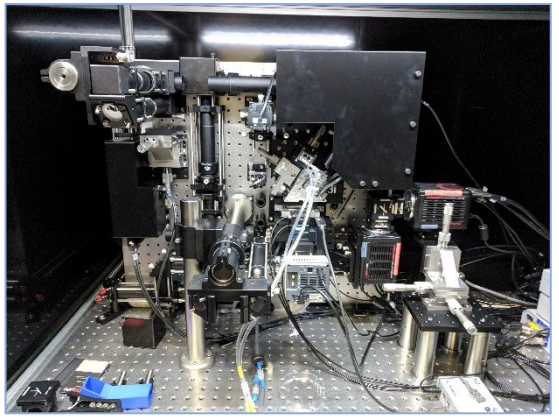
warwick.ac.uk/services/estates/developments/ibrb

Short-term:

- Introduce robust procedures
- Encourage external users
- Expand into flow cytometry and sorting



Ernie the cell sorter – coming to WMS early 2019



The Lattice light sheet – available to external users through the visitor program

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My future plans

Long-term:

- WMS is growing!
- We need to establish computational work that can scale
- More training, more infrastructure

Short-term:

- Increase adoption of solutions that are already in place
- Expand into ML techniques
- Make sure all new starters go through training