The light microscopy imaging pipeline at Warwick Medical School

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An introduction to CAMDU

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

![Management structure diagram]

- Warwick Medical School
  - CAMDU steering committee
    - Steve Royle
    - Rob Cross
    - Anne Straube
    - Andrew McAinsh
  - CAMDU
    - Erick
    - Claire
    - Helena
  - Technical services
Mechanochemical cell biology
Microbiology and infection
Translational and experimental medicine

Research

Organisms
yeast
drosophila
mammalian cells
in vitro
zebrafish
c. elegans

Microscopes
4x spinning disk
4x widefield
5x TIRF
SMLM
optical trap
light sheet
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**
Warwick Open Source Microscope (WOSM)

- 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
Future plans

Long-term:
- WMS is growing
- New building w/ 300 researchers

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

[Images of building, equipment, and website link]
Who am I?

Erick
Sept 2017
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Helena
July 2018
Lattice light sheet specialist
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
A primer on (bio)image analysis

Some of the tasks

- **Image Formation**: object in → image out
- **Image Processing**: image in → image out
- **Image Analysis**: image in → features out
- **Computer Graphics**: numbers in → image out
- **Computer Vision**: image in → interpretation out
- **Visualization**: image in → representation 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.

Mapping components

Convolution filtering

If we have time: typical workflows
So what are we doing?
OMERO

From openmicroscopy.org
From openmicroscopy.org
If we have time: live demo!
Results

Tried ranges of 3-5% Ablation laser.

Cells either popped or NTs 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 NPF-BFP vessels and this could be used for some high speed imaging of these alone if necessary.

For IM3 - no transfectants found.

For EMT6-3xGFP, cells quickly regained fluorescence or blew up.

For CAM5AP2, I tried ablating both where there was no signal (to see if I could generate new minus ends that recruit CAM5AP2) and bleaching CAM5AP2 spots that I can see. Nothing seemed to happen.

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

In this example, a vesicle approaches the ablated region from the nucleus and goes backwards and forwards a couple of times.
If we have time: quick tour
Creating workflows

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<td>cells with 2 foci</td>
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<tr>
<td>percentage of cells with 1 foci</td>
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<td>0.5276</td>
</tr>
<tr>
<td>percentage of cells with 2 foci</td>
<td>0</td>
<td>0.006135</td>
</tr>
</tbody>
</table>
Writing new components
Advanced problems

From https://svi.nl/Deconvolution

From https://imagej.net/Deconvolution

From https://imagej.net/Deconvolution
Advanced problems

Advanced problems

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: \(~1\text{TB an hour at maximum capacity}\)
- Challenges on storing, transferring, processing
  - Deskew/deconvolution
  - GPU processing helps
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Ernie the cell sorter – coming to WMS early 2019

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