Building Bespoke Microscopes & Image Storage

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Bespoke Microscopes

- Why bother?

➡Specific applications

➡Flexibility

➡Cost

Bespoke Microscopes

- Why **NOT** to

➡Cost

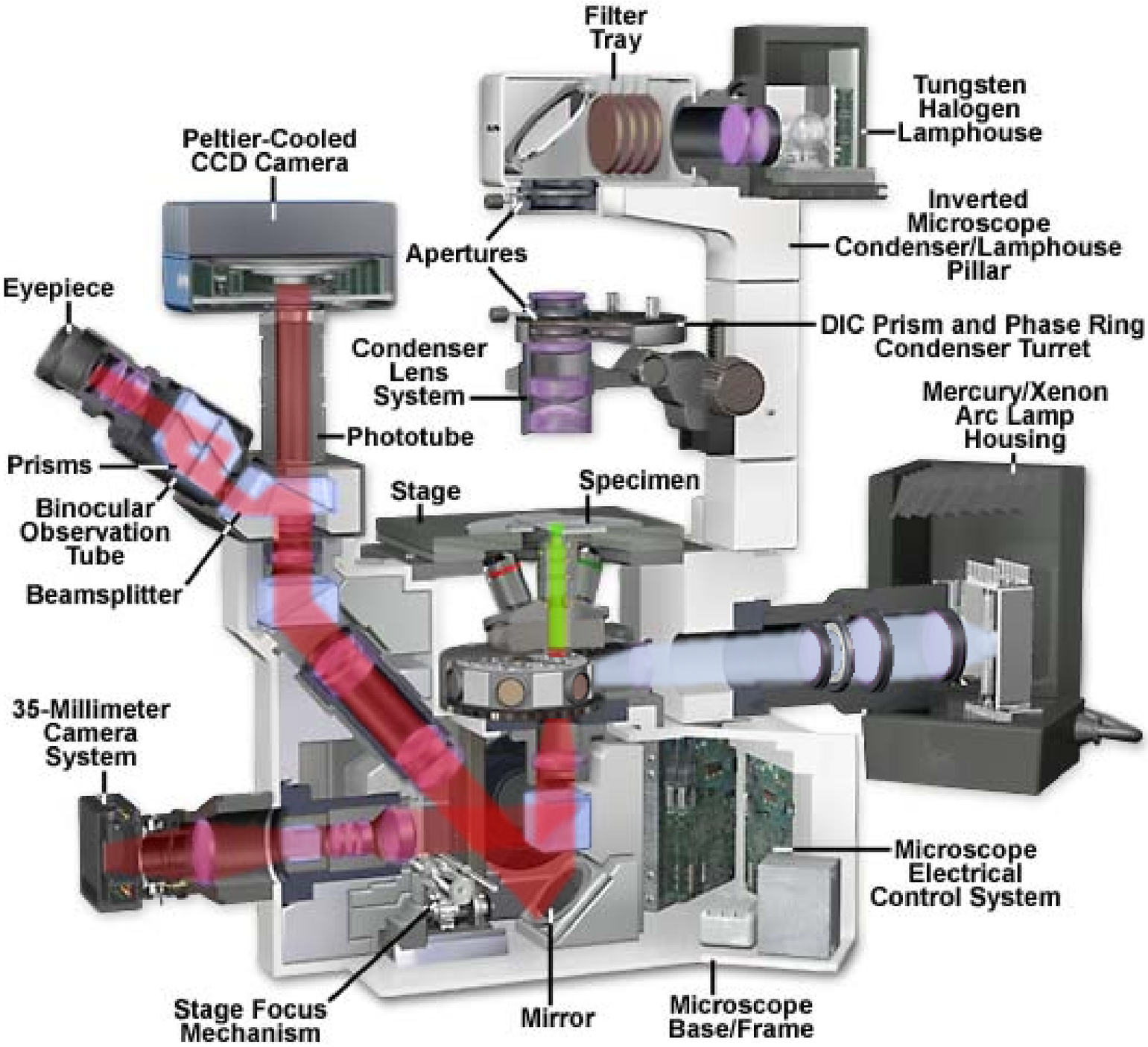
➡Time

➡Usability

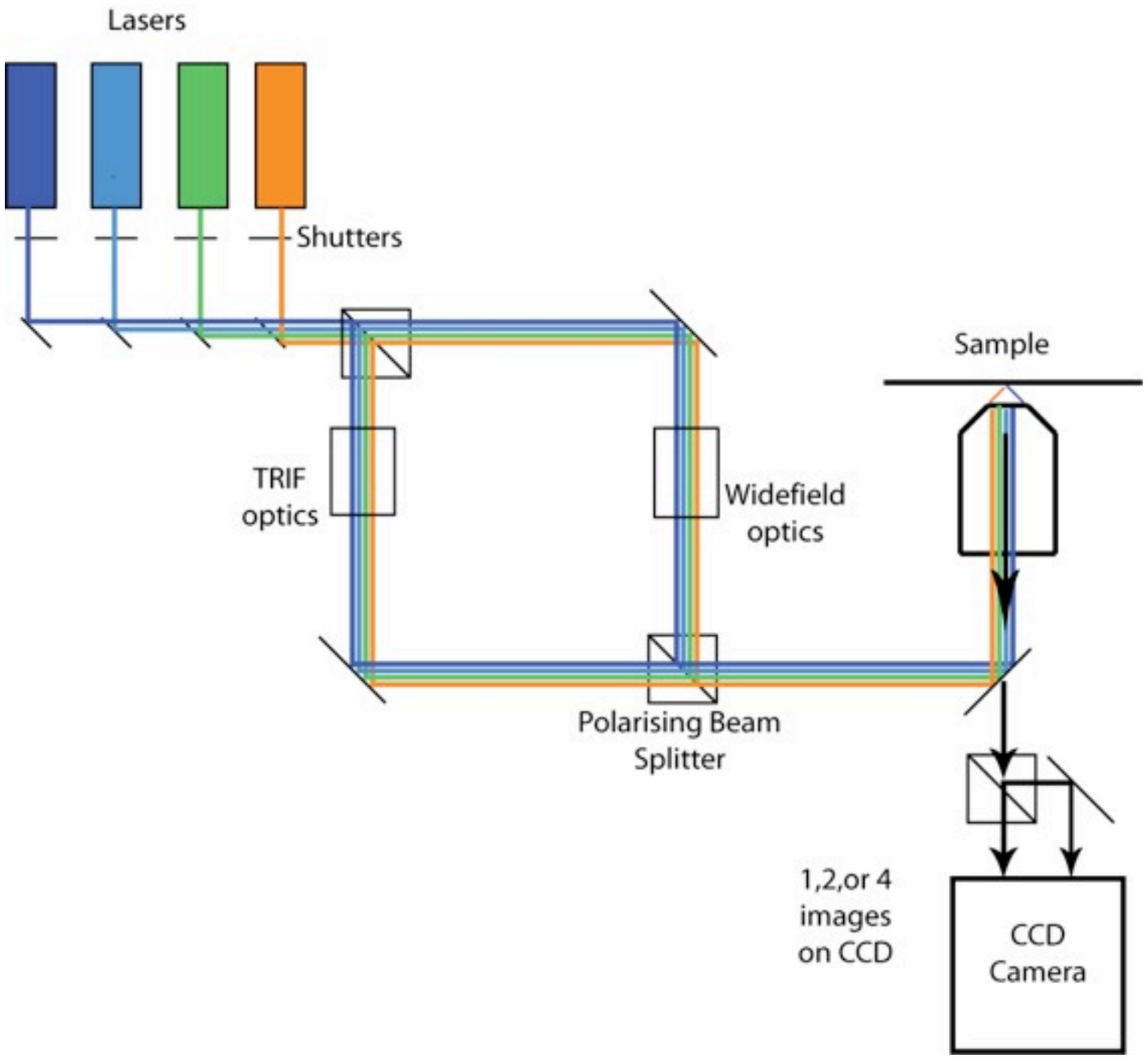
Example Bespoke Microscope

- TIRF - Slimfield Setup
- Built in the biochemistry department in collaboration with Mark Leake (physics).

Conventional microscope



TIRF microscope with split polarisations - schematic

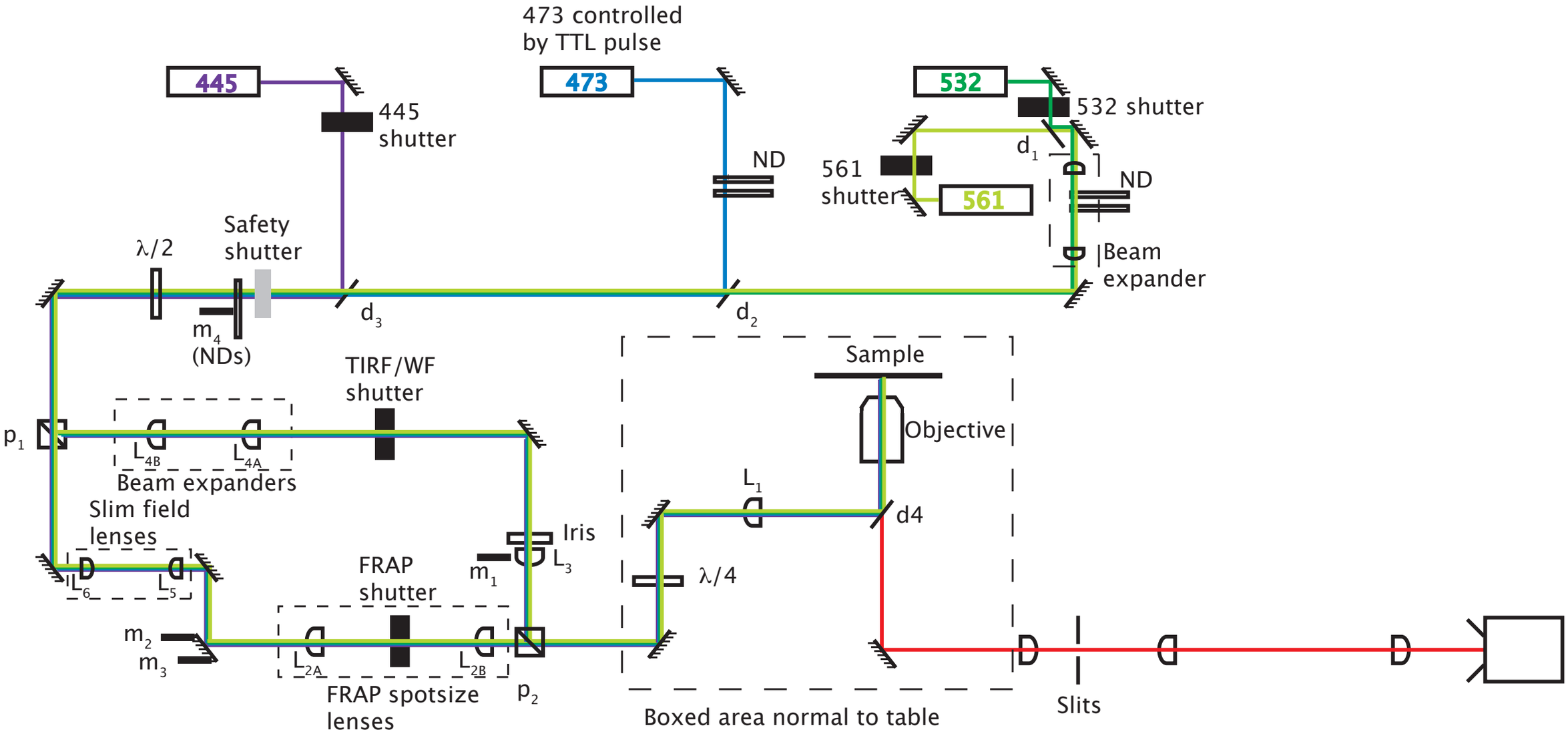


Setup for either

1. TIRF and Widefield
2. Polarisation imaging

Up to 4 images from dual colour in both polarisations

Updated Schematic



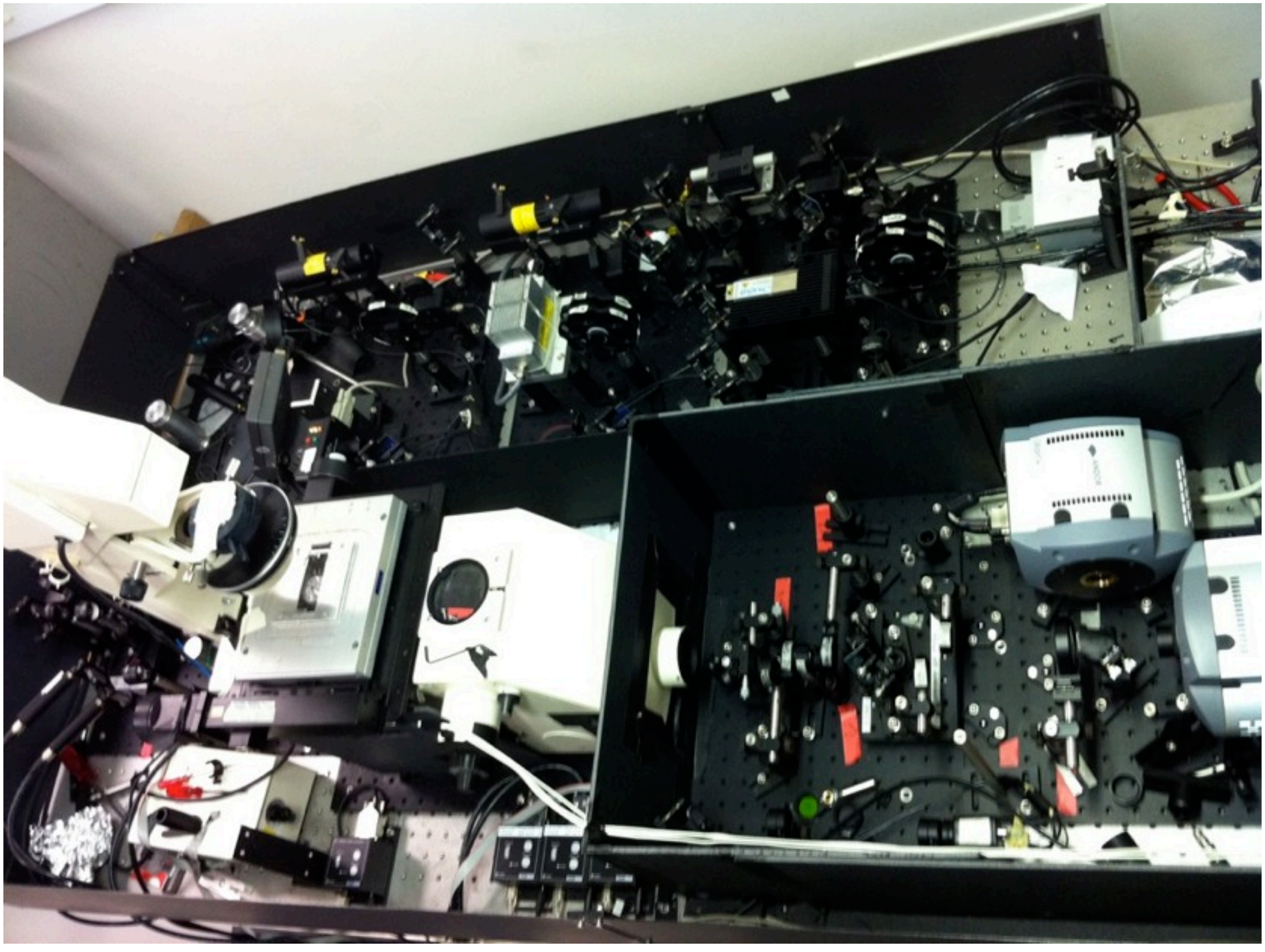
Lenses		Motors	Distances	Dichroics
L_1	f=250 mm	m_1 = TIRF angle	L_1–L_{2A} = 250 + 75	d_1 =
L_{2A}	f=75 mm	m_2 = FRAP spot X	etc....	
L_{2B}	f=200 mm	m_3 = FRAP spot Y		
L_3	f=75?? mm	m_4 = ND wheel		
L_{4A}	f=100?? mm			
L_{4B}	f=200?? mm			
L_5	f=150?? mm			
L_6	f=40?? mm			

Tirf setup

Ian Dobbie 4/1/2009

The real system

The real system II



Features of system

- Multi-colour illumination for widefield, TIRF or slimfield
  - 440, 473, 532, 561
- Dual camera - 512x512 and 128x128
- Simultaneous 2 or 3 colour imaging
- Dual polarisation on excitation and emission
- Dual excitation path for simultaneous photobleaching and imaging.

Advantages of TIRF Slimfield system

- TIRF - slimfield - widefield - FRAP
- More sensitive than commercial system.
- Speed
- Massively more flexible than commercial system.

Disadvantages of TIRF Slimfield system

- Custom written control software.
- Complication.
- Massively more flexible than commercial system.

TIRF-Slimfield system
How expensive was it?

- Building costs ~ £100k (hardware)
- Time ~1 person year
- Total cost £150-200k
- Commercial TIRF system ~£150-200k

Should you build a bespoke system?

Yes!

and NO

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.

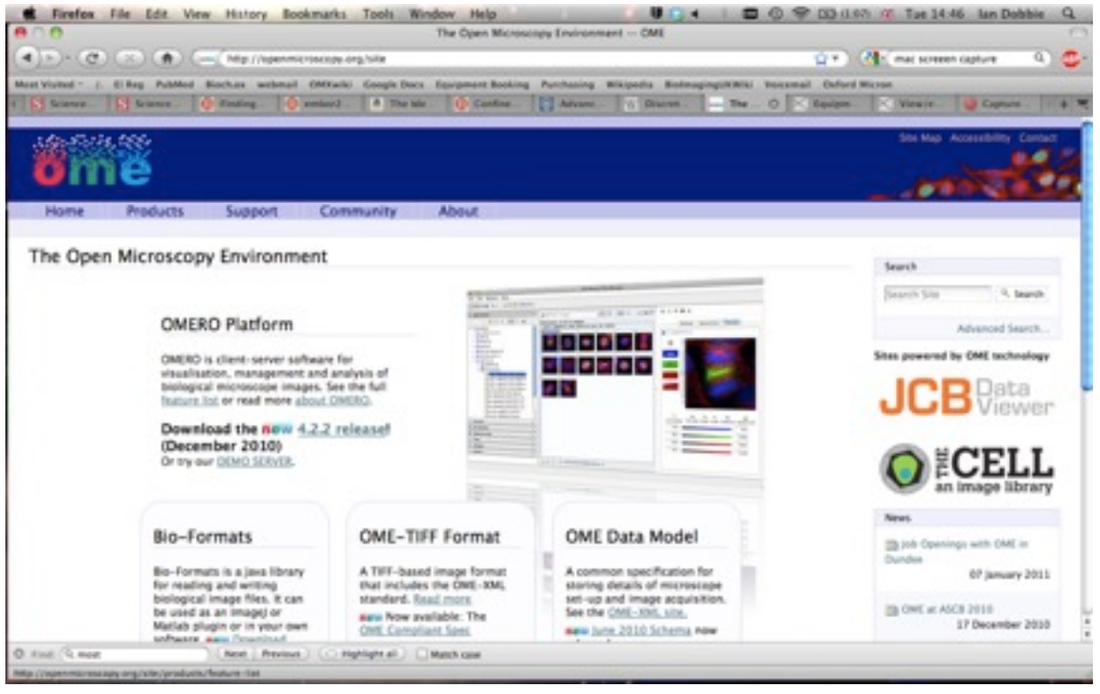
Image Storage

- A major issue especially for 3D, super-resolution and time lapse images
- Where to keep all that data?
- How to organise the data?
- How do you find that data from 3 years ago?

Our Solution - OMERO

- OMERO is a image storage database optimised for microscopy data
- It has a server, where data is stored and several clients that access the data in different ways.

http://openmicroscopy.org/



More reasons for using OMERO

- Allows storage of images from multiple instruments in a single store.
- Metadata is extracted and all images can be labelled with multiple arbitrary tags.
- System is setup so labs can share images, but other users cannot alter your images, just look at them.
- Ensures storage of images for the long term , even when lab members leave.
- You can access your data from anywhere.

Reasons for using OMERO

- Image data is stored on departmental file store: Doesn't count against your quota
- Data is stored in multiple locations and automatically archived to tape, you wont loose it.
- OMERO automatically extracts meta-data and indexes it, allowing rapid searching.

OMERO Clients

Clients are written in Java and run on Mac's, Windows and Linux.

- OMERO.importer - imports images into the database
- OMERO.insight - for everything else.
- OMERO ImageJ plugin - load files from OMERO directly into ImageJ.

OMERO demo

Future plans for OMERO

- Plug image analysis routines into OMERO
- Data will be analysed on the server, so large data sets, or complex analysis can be done.
- Analysis parameters and results will be linked with the original data.

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Advanced Bioimaging Unit

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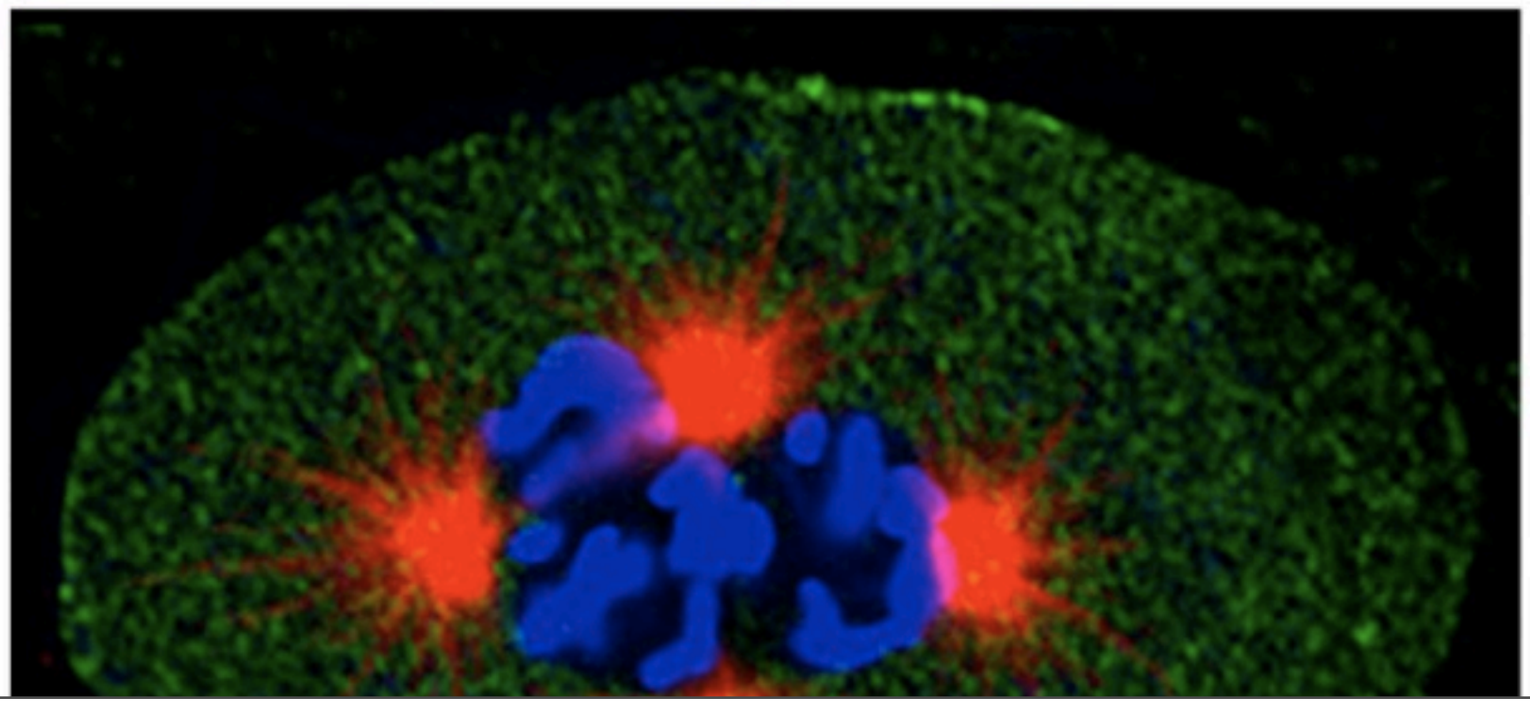
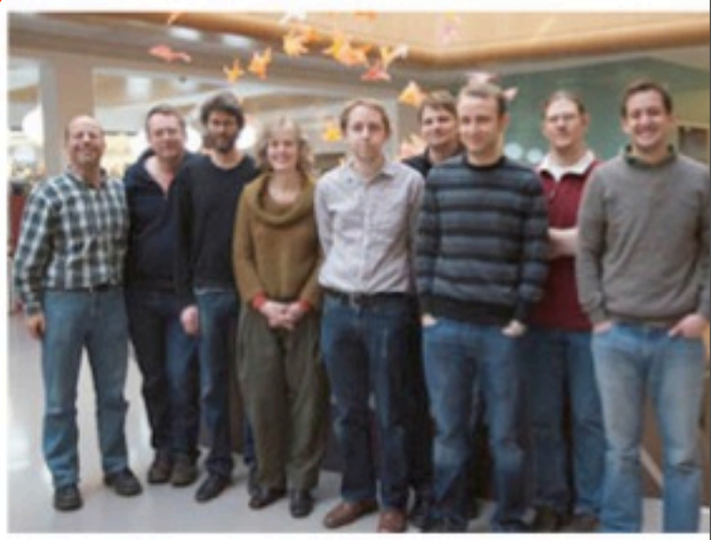
Micron Oxford is located within the Department of Biochemistry and the Dunn School of Pathology in the South Parks Road science area at the University of Oxford, and is funded by a strategic award from the Wellcome Trust. We are a collaborative, multidisciplinary bioimaging unit working with biomedical researchers in the Oxford area and beyond to apply advanced cellular imaging techniques to address key questions in biology. We are focusing on the development and use of single molecule methods, light sheet and super-resolution microscopy.





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Wiki

We have a Micron wiki, which is constantly evolving and being updated with useful information. Internal users can register and edit the wiki (external users will soon be be able to view a snapshot of the most useful information).

FAQs

Q. Who can use the Micron facilities, and what are the charges?

Access is straighforward for researchers belonging to Biochemistry or Pathology, and relatively easy to arrange for those in other Oxford departments. External users are welcome to enquire about working collaboratively. Charges are usually around £20 per. hour for internal users.

Protocols

- Fluorescent RNA in situ

Other bioimaging resources

- Nikon MicroscopyU: confocal microscopy
- Olympus Microscopy Resource Center

Micron Advanced Microscopy Course

We hold an annual advanced microscopy course f Wellcome Trust postgraduate students (and other interested research staff).

This years Lecture course will be held on the 12th-14th March 2012. Register here.

2012 lecture presentations

can be downloaded below:-

Lecture01 -

General Introduction to light Microscopy

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