WEEK 2: Microscope engineering theory and practice

Predicted learning outcomes week 2:

Day 1:
* Understanding the motivations for bespoke microscope design.
* Grounding in the basic principles of bespoke microscope design.
* Grounding in the basic principles of optics.

Day 2:
* Detectors for particular applications.
* Controlling hardware and user interface software
* Hands on experience.

Day 3:
* Applied super-resolution and diagnosing artefacts.
* Hands on experience.

Day 4:
* Live super-resolution.
* Hands on experience.

Day 5:
* Learning to use advanced microscope systems in Micron, Engineering and Physics.
Small groups 2-3 students, each will spend time on one instrument and then meet in the late afternoon to **report on their experiences of the build projects and the hands on Demos**. **The 10 min talks will be assessed.**

Outline ONBI microscopy teaching - Version 10 **RMP/Ilan Davis 07 / 01 / 2015**

SCHEDULE - Week 2: 12th to 16th Jan

Day 1: Mon 12th Jan - Fundamental principles of optics and bespoke microscope engineering I

1	Ilan/RMP/Ian	9.15-10.00	Introduction to the course. Questionnaire / Recap
2	Ian	10.00-10.45	Optics and bespoke microscope construction.
		10.45-11.15	COFFEE BREAK
Antonia		11.15-12.00	Demo - Setting up a beam path: excitation to detection
		12.30-1.30	LUNCH
3	Ian	1.30-2:15	Introduction to "Hands-On Demos" and build projects

Ian – **laser safety:** **All laser light sources are Class I or II laser pointers <1mW power**

Ian - **Overview of the building projects:**

* Understanding how to approach optical arrangements to build a microscope from scratch. – Excitatation and emission paths.
* Understanding user interface and hardware control using the raspberry Pi and Micromanager systems. Writing and improving an acquisition programme using Python on the RaspberryPI.. Goal: Using the MicroscoPI systems for simple acquisition.

Selection of projects

Afternoon Practical: 45 min "Hands-On Demos" 2:30 – 5:30 (room 40.11 3rd floor)
(Rex Richards DTC in Groups of 2-3, A-D 45 min sessions change over 3:15, 4:00; 4:45: Antonia; Ian; Ilan; Douglas; Christian, Chris, Gil Bub, Martin Booth - assisted by Richard, Alan, Justin)

* Setting up lenses to project images [Antonia / Ian]
* User-interface and hardware control [Ilan/Douglas/Mick and Ian]
* Fibre launch and Collimation, 4F beam: expand and focus [Chris/Christian]
* Simple adaptive optics experiment. [Martin Booth / Xiang Liu]

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Day 2: Tue 13th Jan - Fundamental principles of optics and bespoke microscope engineering II

4	Sebastian	9.15-9.45	Bespoke imaging regimes for advanced imaging modalities.
5	Martin	9.45-10.30	Aberration corrections and challenges of biological imaging.
		10.30-11.00	COFFEE BREAK
6	Gil B.	11.00 11.45	DMDs, time modulated imaging methods.
7	Matt P.	11.45-12.30	Making the most of your detector and the principles of noise.
		12.30-1.30	LUNCH
8	Matt P.	1:30-3:00	Introduction to Micromanager (Including Demo).
		3.00-3.15	COFFEE BREAK

From 3:15 - Extended student lead building projects in groups of 2-3 A-D (days 2-4).
(Rex Richards DTC **room 40.11 3rd floor:** Antonia; Ian; Sebastian; Douglas; Christian, Chris, Gil Bub, Martin Booth - assisted by Richard, Eva, Alan, Chris, Justin)

Build Projects (4 Groups of 2-3 A- D)

- Fluorescence excitation light path diffraction pattern - Antonia/Sebastian/Ian.
- Automated control image capture user interface by Raspberry Pi / PS-3 – Ilan / Douglas / Mick. Micromanager Matt Preston (to be determined).
- Setting up a dual view system on the emission path – Gil Bub.
- Simple adaptive optics – Martin Booth/ Xiang Liu.

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Day 3: Wed 14th Jan - Beyond the diffraction limit

9	Ian	9.30-10.00	Resolution and superresolution (Discussion).
10	Rainer	10.00-10.45	Correlative light-electron microscopy (CLEM).
		10.45-11.15	COFFEE BREAK
~~11~~	~~Achillefs~~	~~11.15-12.00~~	~~Single molecule imaging techniques (PALM, sptPALM).~~ To be incorporated / moved to first week
		12.00-1.00	LUNCH BREAK
12	Chris E	1.00-1.45	STED and STED-FCS (build off first lecture).
Lothar		1.45-2.45	3D-SIM optimization and artifact diagnosis: SIMcheck (Demo).

** need to have all computer workstations booted in windows before Fiji / Simcheck sent **

3.00-3.15 COFFEE BREAK

From 3:00: extended student lead building projects continued

Antonia; Ian; Mick; Douglas; David Pinto, Christian, Chris, Gil Bub, Martin Booth (assisted by Richard, Alan, Justin)

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Day 4: Thu 15th Jan - The principles of live cell imaging: No free lunch

13	Josh T.	9.00-9.30	Experimental manipulation on a custom microscope.
14	Mark H.	9.30-10.15	Labeling tricks.
15	Chris L.	10.15-11.00	Viability comparison SPIM/Multiphoton/Confocal/Wide-field.
		11.00-11.30	COFFEE BREAK
"All"		**11.30-12.30**	**Discussion of build projects**
		12.30-1.00	LUNCH BREAK
~~16~~	~~Lothar~~	~~1.00-1.45~~	~~Live super-resolution~~ – to be removed / incorporated into Wed Lecture
		1.45-2.00	COFFEE BREAK

From 2:00: Extended student lead building projects continued
Antonia; Ian; Mick; Douglas; David Pinto; Christian, Chris, Gil Bub, Martin Booth (assisted by Richard, Alan, Justin)

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Day 5: Fri 16th Jan - Hands on advanced microscopy

Friday: "Hands-On Demos" – lab based, 9.00 - 4.00
(Biochemistry-Micron; Physics: groups A-D of 2-3 each attend a single station)

Projects:

- Structured illumination OMX-V2 / OMX-V3 (Micron) Ian/Justin/Sebastian.
- Driving OMX with pithon (Micron) Ilan/Mick/Douglas
- DMD device imaging Gil Bub
- Adaptive Optics (Physics)- Martin Booth / Xiang Liu

Schedule:

9.00 - 9.15 - Assemble at the DTC 2nd Floor room 03 to be taken to individual labs.

9.30 – 11.30 – Hands on Demo session

11.00-11.30 COFFEE BREAK

11.30 – 12.30 – Optional extension of the Demo session if required

12.30-1.30 LUNCH BREAK

13.30 – 16.00 – Data analysis and preparing presentations

16.00 – 16.30 Break / travelling time – assemble at DTC

16.30 – 18.00 – Presentations from each group (**10 min each, 5 min discussion**). **ASSESSED**

Please note that the Assessed Presentations by the students will be based upon the Tuesday to Thursday Build Projects and the Friday Demo. It will be a 10 min presentation, should involve all people from the group (two or three) and can be in any format, powerpoint, verbal, diagrams on whiteboards….

18.00-20.00 Beer and pizza (free dinner)

Build Projects (4 Groups of 2-3 A- D)

1) Fluorescence excitation light path diffraction pattern - Antonia/RMP (Sebastian/Ian).
2) Automated control / image capture user interface by Raspberry Pi / PS-3 – Ilan / Douglas / Mick. Micromanager - Matt Preston (to be determined).
3) ~~Dual channel imaging: setting up a dual view emission path system~~ – Gil Bub/Mick Phillips.

** Revised 2A – Driving motors with Arduinos**

4) A simple adaptive optics configuration – Martin Booth/ Xiang Liu.

Project 1: Fluorescence excitation light path diffraction pattern
Supervisors: Antonia/RMP (Sebastian/Ian).

Goals: To build a simple excitation path and get familiar with the basic principles of optics, polarisation and the generation of patterned illumination as used in Structured Illumination Microscopy (SIM).

Skill Set: Optics; alignment; polarization; structured illumination, super-resolution**.**

Day-to-day:

Tuesday: Setting up the optics for specimen excitation by expanded laser beam. Separating and re-combining red/green laser light (laser pointers). Using simple optics to steer multiple beams into the IX-70 microscope body.

Wednesday: Finalising the beam paths and examining fluorescent samples (beads). If there is enough time we will investigate applying a polarizer and different laser pointers to understand the basics of polarization and its significance.

Thursday: Using the optical setup to apply structured illumination to a sample. Integration of a diffraction grating into the beam path.

Friday Demo: The DV-OMX-V2 and 3 commercial 3D SIM imaging systems

Comparing the optical setups used to produced structured illumination in the V2 and V3 SIM systems.

Using a high-end commercial microscope to apply structured illumination and to achieve "super-resolved" images

Presentation Goals:

- Outline the important features of a fluorescence excitation path.
- Explain the application of structured illumination in super-resolution imaging and how this can be achieved.
- Compare the optical solutions used in the V2 and V3 systems highlighting the benefits and drawbacks of each approach.

Suggested Revisions:
** need to get quad dichroic and filters – to allow recombining of the split red/green / need sections of stiff white card.

** need alternative laser sources, three colour
** need one more 90x30 purchased and for the excitn path demo need 3 plates and a 1 cm stand base plate for the microscope
** alignment laser rig
** 2x half wave plates
** Alignment targets printed onto acetates

** Fluorescent plastic slides, bead slide

Project 2A/B: Automated control of stage movement and image capture on a 3D printed Raspberry Pi based microscope with a PS3 Controller.

Supervisors: Ilan / Douglas / Ian / Mick / David.

Project description: Modifying and developing functionality in Python on our Raspberry PI (RPI) based automated 3D-printed microscopy (MicroscoPi). The setup has a "mobile phone" type CMOS camera for HD still and video capture and stepper motor focusing and stage movement, using hobbyist equipment that is highly affordable.

Goals: In the project you will be tasked with modifying a stepper motor controller for automated focusing of a 160mm microscope using a raspberry PI (or an arduino as an alternative). Identify problems associated with the inaccuracy of the motor, including skipping steps and lack of feedback control. Propose and if possible begin to implement a method to overcome the inaccuracy. Furthermore, time permitting, you will design and implement a stitching algorithm initially in 1D to make a tiled larger image from individual overlapping image, eventually taking into account motion errors.

Skill Set: Python programming; Image analysis (in Python); integration of hardware and software; control of hardware components (motors).

Day-to-day supervision:

Ilan Davis / Ian Dobbie for Hardware. Douglas Russell for Software.

Advice from David Pinto on Image analysis algorithms.

Friday Demo: Driving OMX with python (Micron) Ilan/Mick/Douglas

Presentation Goals:

Describe the existing system and characterize its errors and limitations

Describe the characteristics of a system you would aspire to build finally using similar hardware

Describe the goals of the project achieved and how they were achieved

Describe a vision for how you would build a better system in the future with improved performance including the kind of budgets required.

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Suggested Revisions:
** Better defined revision of project 3 as 2A!
** Alternative motor driver board
** All code to be put on GitHub for next year

Project 3: Splitting fluorescence to two sensors

Supervisors: Gil Bub/ Alex Corbett

Goals: To build an optical component that splits light to independent sensors for two color fluorescence microscopy.

Skill Set: Optics; alignment; understanding aberations; image capture

Day-to-day:

Tuesday: Capturing fluorescent images through the side port; setting up an optical telescope; changing magnification for one sensor.

Wednesday: Splitting the light with a dichroic mirror; Integrating a second sensor with different magnification.

Thursday: Estimating performance characteristics of the optical system; synchronising image capture with two cameras; simple spatial filters in infinity space.

Friday Demo: Commercial dual view solutions

Presentation Goals:

- Understanding aberations and distortions for simple lenses
- Explain magnification, field of view and vignetting in your system.
- Compare the optical solutions used in the commercial system, price vs performance comparison.

Suggested Revisions:
** Better defined revision of project 3 as 2A!
** Incorporate a Monday demo that reflects project 3 (2A) better.

Project 4: Simple adaptive optics for microscopy

Supervisors: Xiang Liu, Martin Booth.

Goals: To build a simple adaptive optical system, in the form of an auto-focussing microscope using an electrically tunable lens. To be able to program a control system using image information to determine the optimum focal position. To understand the principles of adaptive optical microscopy.

Skill Set: Optics; alignment; simple programming; feedback control systems.

Day-to-day:

Tuesday: Setting up the optics for illumination, magnification and imaging onto a camera. Grabbing of images using the computer program provided.

Wednesday: Implement focus control using the tunable lens via the computer program provided. Test the use of an image quality metric to indicate location of focal plane. Adapt the program to search for the optimum focal position.

Thursday: Implement automated focal tracking. Investigate further properties of the adaptive optical system (optional, should time permit).

Friday demo: Adaptive optics aberration correction in widefield fluorescence microscopy

Using a custom built fluorescence microscope incorporating a deformable mirror to correct for specimen-induced aberrations. Understand how the concepts used earlier in the week can be extended to more complex adaptive optics systems.

Presentation Goals:

- Outline the important features of the autofocus microscope.
- Explain the principles of image-based feedback in an adaptive optical microscope and how this can be applied to the autofocussing system.
- Explain how these concepts are extended in order to compensate complex aberrations in a full adaptive optics microscope.