Outline ONBI microscopy teaching - Version 7 Ilan Davis/RMP 15 / 10 / 2014

VISION
The overall goal of the two weeks microscopy course is for the students to gain an advanced theoretical and practical understanding of how bright-field and fluorescence microscopy are built, adjusted and used. Throughout the course we will endeavor to make the course fun and engaging by teaching through experience and experimentation. We will try to demystify complex topics, take away fear of challenging topics and inspire the students to think of new experiments and approaches. Every morning will include some theoretical lectures and in the afternoon practical classes, projects, or demonstrations will put what has been covered to practical use. Ample room will be left for discussion and experimentation, especially in the second week, where the emphasis will be on bespoke microscope design and building.

WEEK 1: Principles of light microscope design and fluorescence imaging

Anticipated learning outcomes from week 1:

Day 1:
* Understanding the basic design and use of a bright-field microscope.
* Setting up Kohler illumination correctly for bright-field microscopy.
* Preparing a variety of specimens for different purposes.
* Basic digital acquisition using Micromanager and Raspberry PI & Python.

Day 2:
* Contrast in bright-field: Setting up phase contrast and DIC correctly.
* Understanding the basic design and use of epi- and transmission fluorescence microscope.
* Making a variety of specimens for fluorescence imaging.
* Setting up Kohler illumination correctly for fluorescence microscopy.
* Basic digital acquisition of fluorescent images with Micromanager and Raspberry PI & Python.
* Consolidating the practical experience using basic microscopy kit from Zeiss.

Day 3:
* Basic principles and application of wide-field deconvolution, spinning disc confocal, laser scanning confocal and light sheet fluorescent microscopy.
* Consolidating the practical experience using basic fluorescent microscopy kit.

Day 4:
* Basic principles of super-resolution microscopy methods (3D-SIM, STED, single molecule localization) and F-techniques (FRAP, FLIP, FRET, FLIM, FCS).
* Comparative imaging of live *Drosophila* embryos

Day 5:
* A practical feel for how to use a wide range of advanced research microscopy platforms provided by Micron and the WIMM, including CLSM, TIRF, DSTORM, PALM, 3D-SIM and SPIM.

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SCHEDULE - Week 1: 10th to 14th Nov

Day 1: Mon 10th Nov - Fundamental principles of light microscopy

Health and Safety Until 11:30

1	Ilan (Richard)	11.30-12:00	Welcome to the course.
2	Richard	12.00-12.45	General introduction to light microscopy.
		12:45-1.45	LUNCH BREAK
3	Ian	1.45-2.30	Principles of microscopy and microscope anatomy.

Afternoon practical 2:30 – 5:30
(DTC/Micron prep area, 23 students Groups A and B change over 4:00)

Hands-on demos

- **Practical 1**: Learning to make specimens for bright-field microscopy: onion epidermis, leaf epidermis, Drosophila embryos, *Drosophila* macrophages (**Micron**: Students grp A, B. Richard, Eva, Ana).
- **Practical 2A**: Using microscopes - simple upright, invert (**DTC**: Students grp B, A. Ian, Chris L, Alan, [Ilan]).

Day 2: Tue 11th Nov - Imaging molecules in cells by fluorescence

4	Ian	9.00-9.30	Contrast enhancement (phase contrast and DIC).
5	Ian	9.30-10.00	Fluorescence microscope design.
6	Mark H	10.00-10.45	Fluorescent dyes and proteins.
		10.45-11.15	COFFEE BREAK
7	Antonia	11.15-12.00	Detectors for microscopy (CCDs, APDs and PMTs)
General Discussions		12.00-12.30 (extending over lunch)	
		12.30-1.30	LUNCH BREAK

Afternoon practical 1:30 – 5:30
(Micron Prep Area and Rex Richards DTC, Groups A and B change over 3:30)

* **Practical 2B**: Using microscopes - Kohler Illumination bright field imaging, phase and DIC (**DTC**: Group A, B. Zeiss team [Ian, Ilan, Chris L]).

* **Practical 3:** Hands-on demos of fluorescence imaging (**Micron**: Group B, A. - Richard, Alan, Justin, [Eva]).

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Day 3: Wed 12th Nov - Advanced fluorescence imaging approaches I

8	Richard	9:00-9.30	Live-cell imaging.
9	Alan	9.30-10.30	Confocal, spinning disc and multiphoton imaging.
10	Eva	10.30-11:00	Light-sheet microscopy of cellular dynamics.
		11.00-11.30	COFFEE BREAK
11	Ilan	11.30-12.30	Wide-field deconvolution and bespoke systems.
		12.30-1.30	LUNCH BREAK

Afternoon practical 1:30 – 5:30
(DTC Rex Richards, groups A, B and C change over 2:30, 3:30)

* **Practical 4**: Acquiring PSFs & assessing optical aberrations. Richard/Justin (A,B,C)
* **Practical 5**: Comparing different kinds of objective lenses. Ian/Alan (B,C,A)
* **Practical 6**: Introducing the Raspberry Pi microscope. Ilan/Alex (C,A,B)

Day 4: Thu 13th Nov - Advanced fluorescence imaging approaches II and EM

12 Antonia	9.00-9.30	F* techniques: FRAP, FLIP, FRET, FLIM, FCS.
13 Chris E.	9.30-10.0	STED, FCS.
14 Rainer	10.00-11.00	Principles of localisation microscopy techniques (LM).
	11.00-11.30	COFFEE BREAK
15 Lothar	11.30-12.30	OMX and 3D-SIM.
16 Errin	12.30-1.00	Electron Microscopy.
	1.00-2.00	LUNCH BREAK

Afternoon practical 2:00 – 5:30
(MICRON, Dunn School groups A- D change over 3:45)

Practical 7: Comparison of two imaging techniques:

- Point-scanning confocal vs Z1 light sheet (groups A and B).
- Spinning disc confocal vs DV deconvolution (Groups C and D).

Alan (A,B)	Dunn School point-scanning confocal – embryo prep
Antonio/Ana (B,A)	Micron Z1-SPIM – embryo prep
Richard (B,C)	Micron Spinning Disc – embryo prep
Ian (C,B)	Micron DV deconvolution (embryos)

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Day 5: Fri 14th Nov - Demonstrations of imaging systems

Demonstrations of advanced microscopes 9:00 – 3:30 (1hr 45 min rotations)
(MICRON or WIMM: Groups of 3 A-F attend either the 3 WIMM or 3 MICRON rotations 9:00- 10:45 [coffee break] 11:00-12:45 [lunch] 1:45- 3:30)

- Rainer /(Ian or Antonia) - OMX V2 Localisation microsc (Micron)
- Justin/ Lothar - OMX V3 SIM (Micron)
- Richard/Ana - ELITE DV Deconvolution (Micron)

or

- Chris TIRF (WIMM)
- Christian / Leica STED (WIMM)
- Christian / Zeiss 780 FCS / FRAP (WIMM)

3:30 – 4:00 Travelling and preparing presentations

4:00 – 5:30 Discussion session: verbal presentations from groups on what they learnt about "Using different imaging modalities to address biological questions" (Assessed)

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Plan of Week 1 Demos / Practical

Day 1: Mon 10th Nov - Fundamental principles of bright-field microscopy

Afternoon practical 2:30 – 5:30
(DTC / Micron prep area, 18 students Groups A and B change over 4:00)

Hands-on demos

- **Practical 1**: Learning to make specimens for bright-field microscopy: onion epidermis, leaf epidermis, Drosophila embryos, Drosophila macrophages (Micron: Students grp A, B. Richard, Eva).
- **Practical 2**: Using microscopes - simple upright, invert (DTC: Students grp B, A. Ian, Ilan).

Practical 1: Learning to make specimens for bright-field microscopy

Preparing live materials for examination on the light microscope: Onion epidermis, leaf epidermis peels, Drosophila embryos, *Drosophila* macrophages.

Drosophila Embryos – (Jupiter GFP / His RFP)
Drosophila Macrophages – (Jupiter GFP / His RFP)
Onion epidermis – assess cellular architecture, record streaming.
Leaf epidermal peels – guard cells, plastids.

Practical 2A: Using microscopes: simple upright, invert, MicroscoPI

Using the “Practical 2 – Bright field” worksheet, students will learn about the components of a microscope and the light path.

Exploring different Scopes:
Zeiss A1 - simple upright
Olympus IX70 - research inverted scope

Fixed cell test slides
Fixed Drosophila embryos

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Plan of Week 1 Demos / Practical

Day 2: Tue 11th Nov - Imaging molecules in cells by fluorescence

Afternoon practical 1:30 – 5:30
(Micron Prep Area and DTC Rex Richards, Groups A and B change over 3:30)

* **Practical 2B**: Using microscopes - Kohler Illumination bright field imaging, phase and DIC (**DTC**: Group A, B. Ian, Ilan, Chris L, Zeiss team).

* **Practical 3:** Hands-on demos of fluorescence imaging (**Micron**: Group B, A. - Richard, Eva, Ana/Alan/Justin).

Practical 2B: Using microscopes: simple upright, invert, MicroscoPI

Using the "Practical 2 – Bright field" worksheet, students will learn about setting up Koehler illumination and the various techniques for generating contrast in bright field.

Exploring different Scopes:
Zeiss A1 - simple upright
Olympus IX70 - research inverted scope
MicroscoPi – homebuild simple scope

Fixed cell test slides
Fixed Drosophila embryos

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Plan of Week 1 Demos / Practical

Day 3: Wed 12th Nov - Advanced fluorescence imaging approaches I

Afternoon practical 1:30 – 5:30
(Rex Richards, groups A, B and C change over 2:30, 3:30)

* **Practical 4**: Acquiring PSFs & assessing optical aberrations. Richard/Justin (A,B,C)
* **Practical 5**: Comparing different kinds of objective lenses. Ian/Alan (B,C,A)
* **Practical 6**: Introducing the Raspberry Pi microscope. Ilan/Alex (C,A,B)

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Plan of Week 1 Demos / Practical

Day 4: Thu 13th Nov - Advanced fluorescence imaging approaches II and EM

Afternoon practical 2:00 – 5:30
(MICRON, Dunn School groups A- D change over 3:45)

Practical 6: Comparison of two imaging techniques:

- Point-scanning confocal vs Z1 light sheet (groups A and B).
- Spinning disc confocal vs DV deconvolution (Groups C and D).

Alan (A,B)	Dunn School point-scanning confocal – embryo prep
Eva (B,A)	Micron Z1-SPIM – embryo prep
Richard/Riccardo (B,C)	Micron Spinning Disc – embryo prep
Ian (C,B)	Micron DV deconvolution (embryos)

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Plan of Week 1 Demos / Practical

Day 5: Fri 14th Nov - Demonstrations of imaging systems

Demonstrations of advanced microscopes 9:00 – 3:00
(MICRON, WIMM: Groups of 3 A-F attend one of the 6 parallel sessions)

- Rainer/(Ian) - OMX V2 Localisation microscopy (Micron)
- Lothar/Justin - OMX V3 SIM (Micron)
- Richard/Ana- ELITE DV Deconvolution (Micron)
- Chris - Olympus TIRF (WIMM)
- Christian/? – Leica STED (WIMM)
- Christian/? - Zeiss 780 FCS / FRAP (WIMM)
