**TIME TABLE**

 **Advanced Microscopy Course 11th-15th Nov 2019**

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| --- | --- | --- | --- |
| **Day 1** | **Time** | **Lecture** |  |
| **11-Nov** | 9.30-9.45 | 0-Welcome to the course | Ian & Nadia |
|  | 9.50-10.35 | 1-General introduction to light microscopy | Ilan Davis |
|  | 10.35-10.55 | **Break** |  |
|  | 11.00-11.44 | 2-Principles of microscopy and microscope anatomy | Ian Dobbie |
|  | 11.50-12.30 | Questions / Discussion session | Ian Dobbie & others |
|  | 12.30-1.30 | **Lunch** |  |
|  | 1.35-2.20 | 3-Cameras for Imaging | Louis Keal |
|  | 2.30-5:00 | Olympus Microscopes (viewing / demo) | Olympus |
|  |  |  |  |  |  |
| **Day 2** | 9.30-10.30 | 4-Contrast enhancement (phase contrast and DIC) | Ian Dobbie |
| **12-Nov** | 10.35-11.20 | 5-Basics of fluorescence microscopy | Nadia Halidi |
|  | 11.20-11.45 | **Break** |  |
|  | 11.45-12.30 | 6-Fluorescent dyes and proteins | Mark Howarth |
|  | 12.30-1.30 | **Lunch**  |  |
|  | 1.30-2.30 | 7-Basic image processing / Image & data management | David Pinto |
|  | 2.30-5.00 | Olympus Microscopes (viewing / demo)  | Olympus |
|  |  |  |  |  |  |
| **Day 3** | 9.30-10.15 | 8-Increasing contrast and resolution using optical sectioning | Alan Wainman |
| **13-Nov** | 10.20-11.05 | 9-Live cell imaging | Ilan Davis |
|  | 11.05-11.30 | **Break** |  |
|  | 11.30-12.30 | 10-Imaging at the molecular level: F-techniques | Chris L. |
|  | 12.30-1.30 | **Lunch** |  |
|  | 1.30-2.15 | 11-Advanced image analysis (part 1) | David Pinto |
|  | 2:15-3:00 | 12-Advanced image analysis (part 2) | David Pinto |
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| **Day 4** | 9.30-10.15 | 13-Imaging Biology in context in organs & Tissues | Matthew Stower |
| **14-Nov** | 10.20-11.05 | 14-Super resolution and SIM | Lothar Schermelleh |
|  | 11.05-11.30 | **Break** |  |
|  | 11.30-12.30 | 15-Nanometer resolution by localisation microscopy | Jarno Makela |
|  | 12.30-1.30 | **Lunch** |  |
|  | 1.30-2.15 | 16-STED | Chris L./Silvia Galiani |
|  | 2.15-3.00 | 17-Bespoke microscope design | Ian Dobbie |
|  | 3.00-3.45 | Conclusion  |  |
|  | 4:00-6:30 | **Beer & Pizza** |  |
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**TIME TABLE**

 **Advanced Microscopy Course Optional 15th Nov 2019**

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|  |  | **Optional Sessions**  |  |
| **Day 5****15-Nov** | 9.30-10.15 | EM DAY (See separate schedule) | Errin Johnson et al |
|  |  | **OR** |  |
|  | 10.00-12.00 | Demonstrations of equipment (Morning rotations) | Micron Team |
|  | 12:00-2.00 | **Break** |  |
|  | 2.00-4:00 | Demonstrations of equipment (Afternoon rotations) | Micron Team |
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Micron Course 2019 November 11th to 15th Lectures Bullet-Point listings:

**Day 1: Principles of light microscopy (Monday 11th)**

**0 Welcome to the course / Announcements**

**1     Ilan Davis General introduction to light microscopy – what is important?**

* *Outline the goals of the microscopy course*
* *What is microscopy?*
* *What is important for good microscopy? The fundamental principles / PSF*
* *How to approach which technique to use*

**2     Ian     Principles of microscopy and microscope**

 **anatomy**

* *Basic understanding of refraction and diffraction, and properties of lenses*
* *Understanding of two different sets of conjugate planes, especially importance of objective back-focal plane*
* *Understanding of factors affecting image resolution*

**3   Louis Keal Cameras for Imaging**

* *Different camera technologies and how they work*

**Day 2: Generating Contrast (Tuesday 12th)**

**4     Ian   Contrast enhancement (phase and DIC)**

* *Reiteration of how images are formed – stressing conjugate planes*
* *The limits of bright field*
* *Dark field microscopy*
* *Phase Contrast microscopy*
* *Differential Interference Contrast microscopy*

**5 Nadia Understanding and applying fluorescence microscopy**

* *Why Fluorescence? Contrast*
* *What is fluorescence? very simplified Jablonski diagram*
* *Basic principle and components of a fluorescence microscope*
* *Fluorescent light sources*
* *PSF, OTF and aberrations*
* *Fixation for light microscopy*

**6 Mark Howarth Fluorescent dyes and proteins**

* *Energy levels in fluorescence; Jablonski diagram*
* *Key characteristics of a fluorophore: Stokes shift, quantum yield, photostability*
* *Organic and inorganic fluorophores*
* *Antibody targeting and how to label protein with dye; direct / indirect labelling*
* *Site-specific protein labeling methods (SNAP-tag etc.)*
* *Labeling different organelles*
* *Different fluorescent proteins- advantages and concerns*

**7 David Pinto Basic image processing & Image and data management**

* *What is a digital image?*
* *What makes a good image?*
* *Options and likely current candidates for data storage after acquisition*
* *Pitfalls/Advantages of many approaches including annotation, de-duplication, backup, sharing and searching for data*
* *OMERO approach to solving the above*

**Day 3: Imaging approaches for molecules and cells (Wed 13th)**

**8 Alan Increasing contrast and resolution using optical sectioning**

* *History*
* *Point scanning (galvo mirrors)*
* *PMT detectors (Including GaAsP detectors)*
* *Setting offset and gain*
* *Bleed through and sequential scanning*
* *Spectral unmixing*
* *Spinning Disc Microscopes*
* *Multi-photon Microscopes*
* *Comparison of Scanning Confocal vs. Spinning Disc vs. Wide field*

**9   Ilan Live-cell imaging**

* *Why live? Comparison of live cell imaging and fixed cell studies*
* *Requirements of live imaging: experimental design and choice of equipment*
* *Optimisation: dyes and stains; filter sets; adaptive optics*
* *Choice of imaging techniques and sample preparation*
* *Image processing and analysis*

**10    Chris L. Imaging at the molecular level: F-techniques**

* *Fluorescence techniques for molecular dynamics; FRAP, FLIP, FLAP, FCS*
* *Techniques to measure molecular interactions such as FRET and FCCS*

**11/12 David Pinto Advanced image analysis Parts 1 & 2**

* *Brief recap of image characteristics, image processing concepts & image restoration*
* *Design of experiments to achieve a specific measurement goal*
* *Quick summary of available image processing & analysis software*
* *Filtering images to enhance features of interest*
* *Image segmentation*
* *Colocalisation statistics*
* *3D visualisation & analysis*
* *Automation of processing and analysis tasks*
* *Using MATLAB to build a custom analysis tool (particle tracker)*

**Day 4: Beyond Conventional Imaging (Thursday 14th)**

**13 Matthew Stower Imaging biology in context in organs and tissues**

* *Problems of imaging deep*
* *Approaches*
* *Using light-sheet imaging to study development*

**14 Lothar Super-resolution and SIM**

* *Principles of structured illumination microscopy (SIM)*
* *Linear SIM methods*
* *Biological application of 3D-SIM: nuclear organisation, live cell imaging*

**15 Jarno Makela Nanometer resolution by localisation microscopy**

* *The principle of localisation microscopy; imaging individual molecules*
* *Variants of LM: PALM, FPALM, STORM, dSTORM, GSDIM*
* *Applications of localisation microscopy; pros and cons*

**16 Silvia/Chris STimulated Emission Depletion microscopy – true**

 **optical super resolution**

* *STED, RESOLFT, STORM/PALM (Differences, Commonalities).*
* *An example of STED-FCS (membrane organization of lipids/proteins)*

**17    Ian Bespoke Microscope Design**

* *Custom microscope design (includes subsequently commercialized examples)*

 **\*\*\* Conclusions &Beer and Pizza \*\*\***