Fluorescence and Electron Microscopy: Imaging Cells and Molecules
GS04 1051 - 100 (7704); Monday, 1-5 pm

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TAs:  Hung Ton-That

Chris Evans (FM)
Belkys Sanchez/Julie Chang (TEM)
Dustin Morado (Cryo-EM)

Week 1 (1/09/2017)

Lecture: Principles of Light Microscopy, MSB 1.180  Morano
• Optics (physics and practice)
• Introduction to microscopy: brightfield, darkfield, DIC, fluorescence

MLK Holiday (1/16/17)

Week 2 (1/23/17)

Lecture: Hardware and Equipment, MSB 1.180  Morano
• Understanding objectives, mirrors, lightpaths
• Widefield, confocal, spinning disk
• Cameras
• Automation and computer control

Lab: Preparation of samples and light microscopy, MSB 1.022  Morano and Margolin

Week 3 (1/30/17)

Lecture: Whole Cell Imaging, MSB 1.180  Margolin
• Live cell imaging with fluorescent proteins and stains
• Longitudinal imaging: media support and microfluidics
• Fixed cell imaging: stains, indirect immunofluorescence

Lab: Live cell imaging, time-lapse imaging, & Immunofluorescence, MSB 1.022  Morano and Margolin
Week 4 (2/06/17)
Lecture: Image Post-Processing, MSB 1.180 Margolin
   • Deconvolution techniques and principles
   • Kymographs, time-lapse and overlays
   • Data analysis, quantitation, and management

Lab: Image post-processing, MSB 1.022 Margolin

Week 5 (2/13/17)
Lecture: New Technologies, MSB 1.180 Margolin
   • FRET, two-photon, TIRF
   • Single molecule and super-resolution methods (SIM, PALM, STORM, STED)

Weeks’ Day (2/20/17)

Week 6 (2/27/17)
Lecture: Introduction to Electron Microscopy, MSB 1.180 Ton-That
   • Transmission electron microscopy (TEM)
   • Scanning electron microscopy (SEM)
   • Sample preparation and staining

Lab: Operational principles of TEM, MSB 2.221M Ton-That

Week 7 (3/06/17)
Lab: Specimen Preparation for TEM, MSB 1.022 Ton-That
Immuno-gold labeling of biological samples and negative staining

Spring Break (3/13 – 3/17/17)

Week 8 (3/20/17)
Lab: TEM – Operation and Data Collection, MSB 2.221M Ton-That
Operating a transmission electron microscope and data collecting using previously prepared samples

Week 9 (3/27/17)
TEM: Negative Staining of Proteins from Biological Samples, MSB 2.221M Waxham
TEM Operation and Data Collection using prepared samples

Week 10 (4/03/17)
Lecture: Cryo-electron microscopy, MSB 1.180 Liu
   • Cryo-electron microscopy (cryo-EM)
   • Cryo-electron tomography (cryo-ET)

Lab: Preparation of biological samples for cryo-EM, MSB G.606 Liu
**Week 11 (4/10/17)**

Lecture: Cryo-EM – Operation and Data Collection, MSB 1.180  
Liu  
Operating a cryo-electron microscope and data collecting using previously prepared samples  

Lab: Cryo-EM – Operation and Data Collection, MSB G.606

**Week 12 (4/17/17)**

Lecture: Cryo-EM – Image Processing, MSB 1.180  
Liu  
Basic image analysis: from 2D images to 3D reconstruction  

Lab: Cryo-EM – Image Processing, MSB 2.233

**Week 13 (4/24/17)**

Lecture: Electron Cryomicroscopy, MSB 1.180  
Serysheva  
- Electron Image formation (very basic)  
- Single-particle electron cryomicroscopy (cryo-EM)  

Lab: Specimen preparation for cryo-EM, MSB 6.630

**Week 14 (5/01/17)**

Lecture: Image processing (concept), MSB 1.180  
Serysheva  
- Initial data analysis and *Ab initio* 3D reconstruction  
- Image refinement in 2D and 3D  

Lab: Specimen preparation for cryo-EM (continuation), MSB 6.630

**Grading**  
Pass/Fail  
The total grade is based on attendance (20%), lab participation (50%), and six assignments (5% each).  

**Assignments:** Students will generate a publishable figure with a figure legend from data obtained from each instructor’s sections.  

**Reading Materials**  
Students are encouraged to read reading materials, provided by instructors, before class.