# Fluorescence and Electron Microscopy: Imaging Cells and Molecules GS04 1051 - 100 (7704); Mondays, 1-2pm (lectures\*), 2-4pm (labs)

Course Directors: Bo Hu (director) William Margolin (co-director)

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TAs: Kara Schoenemann/Davi Goncalves(FM)

Xiangan Liu(EM)

Week 1 (1/06/2020)

Lecture: Principles of Light Microscopy, MSB 1.180 Morano

• Optics (physics and practice)

• Introduction to microscopy: brightfield, darkfield, DIC, fluorescence

Week 2 (1/13/20)

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

**MLK Holiday (1/20/20)** 

Week 3 (1/27/20)

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, & Morano and Margolin

Immunofluorescence, MSB 1.022

## Week 4 (2/03/20)

Lecture: New Technologies, MSB 1.180

Margolin

- FRET, two-photon, TIRF
- Single molecule and super-resolution methods (SIM, PALM, STORM, STED)

Lab: Super-resolution fluorescence microscopy, MSB 4.202

Levental, Margolin

# Week 5 (2/10/20)

**Lecture: Image Post-Processing, MSB 1.180** 

Margolin

- Deconvolution techniques and principles
- Kymographs, time-lapse and overlays
- Data analysis, quantitation, and management

## Presidents' Day (2/17/20)

#### Week 6 (2/24/20)

**Lecture: Introduction to Electron Microscopy, MSB 1.180** 

Tsai

- Transmission electron microscopy (TEM)
- Sample preparation and staining

Lab: Operational principles of TEM, MSB 2.221M

Tsai

#### Week 7 (3/02/20)

Lab: Specimen Preparation for TEM, MSB 1.022

Tsai

Immuno-gold labeling of biological samples and negative staining

#### Week 8 (3/9/20)

Lab: TEM - Operation and Data Collection, MSB 2.221M

Tsai

Operating a transmission electron microscope and data collecting using previously prepared samples

#### Spring Break (3/16 - 3/20/20)

#### Week 9 (3/23/20)

Lecture: Cryo-electron microscopy, MSB 1.180

Hu

- Cryo-electron microscopy (cryo-EM)
- Cryo-electron tomography (cryo-ET)

Lab: Preparation of biological samples for cryo-EM, MSB G.606

Hu

#### Week 10 (3/30/20)

**Lecture: Cryo-EM – Operation and Data Collection, MSB 1.180** 

Hu

Operating a cryo-electron microscope and data collecting using previously prepared samples

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

Week 11 (4/06/20)

Lecture: Cryo-EM – Image Processing, MSB 1.180 Hu

Basic image analysis: from 2D images to 3D reconstruction

Lab: Cryo-EM – Image Processing, MSB 1.180 Hu

Week 12 (4/13/20)

Lecture\* (2 hours): Electron Cryomicroscopy, MSB 1.180 Serysheva

Vitrification of Biological Samples

Lab: Specimen preparation for cryo-EM, MSB 6.630 Serysheva

**Grading** Pass/Fail

The total grade is based on attendance (20%), lab participation (50%), and three assignments (10% each).

<u>Assignments</u>: Students will write Materials & Methods and Results Sections accompanied with a publishable multi-panel figure and a figure legend corresponding to data obtained from each section, i.e. fluorescence microscopy (Margolin and Morano), electron microcopy (Tsai), and cryo-EM (Hu and Serysheva).

## **Reading Materials**

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

Assignment Due Dates: pdf documents sent to Bo.Hu@uth.tmc.edu on the due dates (see below)

2/24/20 Assignment 1
3/23/20 Assignment 2
5/06/20 Assignment 3