COURSE SYLLABUS
CSB222 / BIOS 152: Biological Light Microscopy
Fall 2017

Instructor Information

Instructor:	Mary Teruel, Assistant Professor, Dept. of Chemical and Systems Biology
Office Location:	CCSR 3155B
Email:	teruel1@stanford.edu
Office Hours:	Fridays 11AM-12PM or by appointment

Instructor:	Jon Mulholland, Director of the Stanford Cell Sciences Imaging Facility (CSIF)
Office Location:	Beckman B050A
Email:	jwm@stanford.edu
Office Hours:	by appointment

Instructor:	Andrew Olson, Director of the Stanford Neuroscience Microscopy Service (NMS)
Office Location:	Lokey Stem Cell Building, Suite G0901
Email:	aolson6@stanford.edu
Office Hours:	by appointment

Teaching Assistant:	Michael Zhao, PhD student in Chemical and Systems Biology
Email:	mlzhao13@stanford.edu

Imaging Facility Staff: Cedric Espenel, PhD, Shriram-CSIF
espenel@stanford.edu

Kitty Lee, Beckman-CSIF
microscopy@stanford.edu

Course Identification

Dates:	6 weeks from Mon. Sept. 25 – Wed. Nov. 3, 2017
Lecture Times:	Monday and Wednesdays: 1:30–2:50PM
Lab:	Fridays 1:30-4:30PM
Prerequisites:	Some college physics

Course Description/Overview

This intensive laboratory and discussion course will provide participants with the theoretical and practical knowledge to utilize emerging imaging technologies based on light microscopy. Topics include microscope optics, resolution limits, Köhler illumination, confocal microscopy, fluorescence, two-photon, TIRF, FRET, photobleaching, super-resolution (SIM, STED, STORM/PALM), and live-cell imaging. Applications include using fluorescent probes to analyze subcellular localization and live cell-translocation dynamics. We will be using a “flipped” classroom for the course in that students will watch iBiology lectures before class, and class time will be used for engaging in extensive discussion. Lab portion involves extensive in-class use of microscopes in the CSIF and NMS core microscopy facilities.

Course Learning Goals

The learning goals for this class are for students to learn how to operate wide-field and confocal scanning microscopes, acquire and analyze data, and interpret and report results.

By the end of this course,

- Students should be able to undertake a basic investigation of fluorescently-labeled cells and tissue samples using either wide-field or confocal scanning microscopy, be able to optimize conditions to obtain properly Nyquist-sampled images, and to carry out basic image analysis of the images using ImageJ.
- Students should understand the concepts of numerical aperture, resolution and detection limits, and should know how to obtain point-spread-functions to verify their objectives and instrumentation are working correctly.
- Students should understand the basic physics of fluorescence excitation and emission, and the basic chemistry of fluorescent probes, reporters and proteins, and should be able to choose correct illumination and detection combinations to do multi-fluorescence imaging.
- Students should be familiar with advanced microscopy approaches including two-photon microscopy, super-resolution microscopy, and deconvolution.

Course Resources

Course Website

Recommended Course Text

- **Fundamentals of Light Microscopy and Electronic Imaging** by Douglas B. Murphy and Michael W. Davidson
- **(Virtual) Texts:**
  - http://www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-course.html
  - http://micro.magnet.fsu.edu/primer/index.html

Grading Scheme

Grading System

Grades will be assessed as per the General University Grading System
https://studentaffairs.stanford.edu/registrar/students/grades-definition

Grading Policy

Participation in class	30%
Lab reports/presentations	40%
Lab exercises/HW:	10%
Final Project report/presentation:	20%
Total	100%

Reading and Video Assignments
Reading and video assignments will be posted on the course website. These assignments should be completed prior to the lecture.

Labs
There will be 5 labs and 4 groups (4 students/gp) for each lab.

For lab #1, the groups will use the following microscopes:

- 2 groups in the Lokey Stem Cell (suite G0901): Andrew Olson, Michael Zhao; OMX, Zeiss Axioimager
- 1 group in the Beckman-CSIF (B050): Jon Mulholland, OMX
- 1 group in Shriram-CSIF (B023): Cedric Espenel - Zeiss Axioimager

For labs #2-4, the groups will use the following microscopes:

- 2 groups in the Lokey Stem Cell: Andrew Olson, Michael Zhao Zeiss; LSM710, LSM510
- 1 group in the Beckman-CSIF (B050): Jon Mulholland, Kitty Lee - Zeiss LSM 880
- 1 group in Shriram-CSIF (B023): Cedric Espenel - Zeiss LSM 780

For lab #5, there will be a choice of the following microscopes:

- Structured illumination/deconvolution (3-color, both NMS and CSIF have scopes we can use);
- STED (CSIF has 2-color system, but need to be careful with choosing fluorophores and sample; CSIF has sample slide);
- Airy scan (CSIF has this 2 of these, are add-ons to the confocal, 1.7X increase in resolution, can subsample PSF, close to SIM, can possibly do thicker samples);
- TIRF (NMS and CSIF both have these systems),
- FLIM (CSIF has system);
- two-photon (NMS has 2: a tissue-slice rig and an in vivo rig; CSIF has 2 systems)

Assignments for first class

Readings: 39 Steps by Pawley
(http://labs.pbrc.cellbiology/documents/39steps.pdf)

iBiology Videos: Lenses and image formation (Fletcher, 37 min)
https://www.ibiology.org/ibioeducation/taking-courses/lenses-image-formation.html

optional:
What is Light? (Bo Huang, 24 min),
https://www.ibiology.org/ibioeducation/taking-courses/what-is-light.html

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Course Schedule

Week 1

Mon, Sept. 25

Topic: Orientation / Light, lens, and image formation
Lab (in classroom): Work with lenses and ray boxes

iBiology Videos (for Wed class discussion and lab): Microscope imaging and Kohler (Vale, 23 min).
https://www.ibiology.org/ibioeducation/taking-courses/microscope-imaging-and-koehler-illumination.html

iBiology Videos – the 3 videos listed below are required viewing by Monday April 10th and will be the basis of Monday's class discussion, they will also assist you in understanding Friday's psf lab; we list them here so you can start viewing them now if you want:
Diffraction (Lichtman, 26 min), resolution (Lichtman, 21 min), Abbe Diffraction (Thorn, 21 min).
https://www.ibiology.org/ibioeducation/taking-courses/diffraction.html
https://www.ibiology.org/ibioeducation/taking-courses/resolution.html
https://www.ibiology.org/ibioeducation/taking-courses/abbe-diffraction-demonstration.html

Wed, Sept. 27

Topic: The compound microscope
Lab (in classroom): Build an optical bench microscope

For Friday Lab:
Hand-out description and goals for lab 1.

Reading: Nat Protoc. 2011 Nov 10;6(12):1929-41. Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control.
http://www.nature.com/nprot/journal/v6/n12/full/nprot.2011.407.html

iBiology Videos: psf (Lichtman, 29 min), Measuring a Point Spread Function (Nico Stuurman, 16 min)
https://www.ibiology.org/ibioeducation/taking-courses/point-spread-function.html
https://www.ibiology.org/ibioeducation/taking-courses/measuring-a-point-spread-function.html

Fri, Sept. 29

Lab #1: Widefield microscope PSF collection (sub-resolution fluorescent bead z-stack) & Kohler illumination
All groups will use widefield microscopes

iBiology Videos (for Monday discussion): Diffraction (Lichtman, 26 min), resolution (Lichtman, 21 min), Abbe Diffraction (Thorn, 21 min).
https://www.ibiology.org/ibioeducation/taking-courses/diffraction.html
https://www.ibiology.org/ibioeducation/taking-courses/resolution.html
https://www.ibiology.org/ibioeducation/taking-courses/abbe-diffraction-demonstration.html

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Week 2

Mon, Oct. 2

Topic: Diffraction, psf and resolution
Discuss: Interference, diffraction, Airy disk and image formation: the diffraction limit.

iBiology Videos (for Wed discussion): Optical sectioning and confocal (Thorn, 25 min),
https://www.ibiology.org/ibioeducation/taking-courses/optical-sectioning-and-confocal-microscopy.html

Optional:
Deconvolution (Agard, 39 min)
https://www.ibiology.org/ibioeducation/taking-courses/deconvolution-microscopy.html

Wed, Oct. 4

Presentation of Lab 1 (PSF)
Topic: Optical sectioning with confocal, deconvolution
Discuss: Setting up the scanning confocal, deconvolution scopes. Optimizing for Z series.

iBiology Video: Abbe diffraction experiment (Evenett, 42 min)
https://vimeo.com/2150123

Fri, Oct. 6

Lab #2: confocal training, z-stack

iBiology Video (for Monday discussion): Cameras and detectors I: how do they work (Stuurman,22 min), and II: specifications and performance (Stuurman, 37 min), Resolution in Microscopy (Lichtman, 39 min).

https://www.ibiology.org/ibioeducation/taking-courses/cameras-and-detectors-i-how-do-they-work.html
https://www.ibiology.org/ibioeducation/taking-courses/cameras-and-detectors-ii-specifications-and-performance.html
https://www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course/resolution-in-microscopy.html

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Week 3

Mon, Oct. 9

Topic: Cameras, detectors, signal-to-noise, Nyquist
Discuss: choice of objectives for max resolution, cameras, and Nyquist sampling

iBiology Videos (for Wed discussion): Intro to fluorescence microscopy (Stuurman, 34 min),
https://www.ibiology.org/ibioeducation/taking-courses/introduction-to-fluorescence-microscopy.html

optional:
Fluorescent probes (Mitchison, 21 min), Light Sources (Nico Stuurman, 7 min)
https://www.ibiology.org/ibioeducation/taking-courses/fluorescent-probes.html
https://www.ibiology.org/ibioeducation/taking-courses/light-sources.html

Wed, Oct. 11

Topic: Fluorescence microscopy
Discuss: Choosing a probe, choosing a light source, choosing a filter set, adjusting for a tradeoff between S/N and bleaching. Controls for antibody staining, bleedthrough, autofluorescence

Fri, Oct. 13

Lab #3: confocal PSF z-stack, and nanoruler resolution target

iBiology Video (for Monday discussion): Fluorescent proteins (Tsien, 35 min), FRET (Bastiens, 36m)
https://www.ibiology.org/ibioeducation/taking-courses/fluorescent-proteins.html
https://www.ibiology.org/ibioeducation/taking-courses/forster-resonance-energy-transfer-fret-microscopy.html

Optional:
Fluorescent protein indicators (Tsien, 42 min), TIRF (Axelrod, 42 min), FRAP/FLIP (Jennifer Lippincott-Schwartz 27 min)
https://www.ibiology.org/ibioeducation/taking-courses/fluorescent-protein-indicators.html
https://www.ibiology.org/ibioeducation/taking-courses/total-internal-reflection-fluorescence-tirf-microscopy.htm
https://www.ibiology.org/ibioeducation/taking-courses/measuring-dynamics-photobleaching-and-photoactivation.html

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Week 4

Mon, Oct. 16

Guest lecture: Michael Lin, Stanford Depts. of Bioengineering and Neurobiology

Topics: GFP, Fluorescent probes

iBiology Video: Live Cell Imaging and Environmental Control (Kurt Thorn, 9 min); Minimizing Damage from Fluorescence (Ron Vale, 9 min), Optimizing Detection of GFP (Roger Tsien, 9 min), FRAP/FLIP (Jennifer Lippincott-Schwartz 27 min)
https://www.ibiology.org/ibioeducation/taking-courses/live-cell-imaging-and-environmental-control.html
https://www.ibiology.org/ibioeducation/taking-courses/minimizing-damage-from-fluorescence.html
https://www.ibiology.org/ibioeducation/taking-courses/optimizing-detection-of-gfp.html
https://www.ibiology.org/ibioeducation/taking-courses/measuring-dynamics-photobleaching-and-photoactivation.html

Wed, Oct. 18 — Topic: Live-cell imaging, FRAP, FRET, cell tracking algorithms

Fri, Oct. 20 — **Lab #4: live cell imaging**
Start work on Advanced Microscopy Projects

iBiology Videos: Super-Resolution: Localization Microscopy (Huang, 27 min),
https://www.ibiology.org/ibioeducation/taking-courses/super-resolution-localization-microscopy.html

Optional:
Super-Resolution: Overview and Stimulated Emission Depletion (STED) Microscopy (Hell, 39 min).
https://www.ibiology.org/ibioeducation/taking-courses/super-resolution-overview-and-stimulated-emission-depletion-sted-microscopy.html

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Week 5

Mon, Oct. 23 — Guest Lecture: Alistair Boettiger, Stanford Dept. of Dev. Bio.
Topic: SIM and STED super-resolution microscopy
Discuss: Sample prep, what you need to get blinking, AiryScan

iBiology Videos: Super-Resolution: Structured Illumination Microscopy (SIM) (David Agard, 25 min)
https://www.ibiology.org/ibioeducation/taking-courses/super-resolution-structured-illumination-microscopy-sim.html

Optional:
Fourier space (Huang, 21 min)
https://www.ibiology.org/ibioeducation/taking-courses/fourier-space.html

Wed, Oct. 25 — Topic: STORM/PALM: Super-Resolution Localization Microscopy

Fri, Oct. 27 — **Lab #5: work on Advanced Microscopy Projects**

Week 6

Mon, Oct. 30 — Lecture on Electron Microscopy
Class Summary/Review

Wed, Nov. 1 — Final Presentations

Fri, Nov. 3 — Final Presentations