Biology 3333/5433 - Advanced Microscopy Techniques, Dr. Sheffield (2017 – Tentative)

Tuesday and Thursday, 12:30-1:50, BLS 423 - and scheduled labs in BLS 348

Recommended Textbooks:
Murphy, Douglas and Davidson, Michael, Fundamentals of Light Microscopy and Electronic Imaging, 2nd Edition. Wiley-Blackwell, 2013. Note, it is the Second Edition that is recommended, not the first.

A useful guide to ImageJ can be found here, and is included on your flash drive:
http://blogs.qub.ac.uk/ccbg/files/2014/05/2014-05_Analyzing_fluorescence_microscopy_images.pdf

The purpose of this course is to investigate the variety of image transforms that are possible using the light and electron microscope. The first half of the course, approximately, will cover basic principles of optics - the nature of light, diffraction, refraction, etc., the nature of lenses, and the design of the light microscope. We will discuss phase contrast, dark field, interference contrast, and modulation contrast, as well as polarization and fluorescence microscopy. We will also discuss the use of different labeling procedures to obtain more information about samples. We will then consider several scanning microscope systems, including the scanning confocal microscope and the scanning electron microscope. These systems will be demonstrated in laboratory experiences.
The second half will be devoted to digital transformation of images. We will discuss the nature of digital images, the concepts of LUTs and transforming filters, as well as image mathematics. We will also consider the application of conventional image processing programs to microscopic samples.
Each student will be also expected to select a microscopy project. Time will be divided between laboratory experiences with the different microscopes, and class discussion of the projects in process. Examples of projects by students in the past can be found on the course server, more about that as the class proceeds.
Much of the resource material will be on the flash drive that is distributed in class, although I will expect you to use additional resources when appropriate.
Grading will be based on two exams, and the results of the project. The results of the project will be presented orally at the regularly scheduled Final Exam time (Thursday 5/4 from 10:30 am-12:30 pm (and possibly more). You will be expected to return the flash drive, with your collected images, and the final presentation, at the end of the class.
The fine print:

First class: Tuesday, Jan. 17
Last day to drop (tuition refund available): Monday, January 30
Spring recess: No classes are held during the week March 13-19.
Last day to withdraw (no refund): Wednesday, March 22.
Last Class: Thursday, April 27.

Biology 333/433 Advanced Microscopy Techniques, Tentative Syllabus, Spring, 2015

Date	**Topic**	**Reference**
Jan 17	Introduction, properties of light. Resolution	**General Reference:**\primer\index.html : **General Properties of Light:**\primer\lightandcolor\index.html **Resolution:**\www.microscopyU.com\articles\formulas\formulasresolution.html
Jan 19	Refraction, Image formation by convex lenses	**Refraction:**\primer\lightandcolor\refraction.html **Convex Lenses:**\primer\lightandcolor\lensesintro.html
Jan 24	Convex lenses, continued, Lens Aberrations - Spherical, chromatic, etc	**Lenses:**\primer\java\lenses\converginglenses\index.html **Aberrations:**\primer\anatomy\aberrationhome.html
Jan 26	aberrations redux, the enlargement system of a microscope, microscope objectives. Kohler Illumination, Inverted microscope	**Overall Structure of the Microscope:** \primer\anatomy\components.html **Fixed Tube Length:** \primer\java\components\fixedtubelength\index.html **Infinity Tube Length:** \primer\java\components\infinitymicroscope\index.html **Objectives:**\primer\anatomy\objectives.html \primer\anatomy\specifications.html **Role of immersion oil:** \primer\java\microscopy\immersion\index.html **Condenser Illumination:** \primer\java\components\condenser\index.html **Koehler Illumination:**\primer\anatomy\kohler.html
Lab 1. (Jan 31-Feb 2)	Lab Exercise on elements of the light microscope - room 348	Handouts, "Diffkit" program on course disk.
Jan 31	Oculars, More on conjugate planes, the objective back focal plane.	**Oculars**: \primer\anatomy\oculars.html **Image Formation:**\primer\anatomy\image.html

Feb 2	Contrast: Special microscopy techniques -manipulating the light within the microscope. phase contrast, darkfield, Hoffman modulation	**Contrast:**\www.microscopyU.com\articles\formulas\specimencontrast.html **Phase Objects:** \www.microscopyU.com\tutorials\java\phasecontrast\phasespecimens\index.html \primer\java\contrast\rounded\index.html \primer\java\darkfield\cardioid\index.html **The Phase Microscope** \www.microscopyU.com\articles\phasecontrast\phasemicroscopy.html **Alignment:** \www.microscopyU.com\tutorials\java\phasecontrast\microscopealignment\index.html **Hoffman:**\primer\techniques\hoffmanindex.html
Lab 2 (Feb 7-9)	Lab exercise on Phase Contrast microscopy - room 348	
Feb 7	DIC (Differential Interference Contrast), Polarization microscopy, Fluorescence microscopy.	**Polarization** : \primer\java\polarizedlight\filters\index.html \primer\techniques\polarized\polarizedhome.html **DIC**: \primer\techniques\dic\dicoverview.html \primer\java\dic\lightpaths\index.html \primer\techniques\dic\dicintro.html
Feb 9	Fluorescence Microscopy 1	**Comparison of Phase and DIC** \primer\techniques\dic\dicphasecomparison.html **Fluorescence:** /primer/techniques/fluorescence/introduction.html /primer/java/jablonski/index.html /primer/techniques/fluorescence/excitation.html **The microscope:** /primer/techniques/fluorescence/reflectlightpaths.html /primer/java/microscopy/fluorescence/index.html

Lab 3 (Feb 14-16)	Lab exercise on Fluorescence microscopy and DIC - room 348.	
Feb 14	Fluorescence Microscopy 2	
Feb 16	Scanning confocal microscopes 1	/www.microscopyU.com/articles/confocal/index.html /Confocal/theory/index.html **Basic Info** **http**://www.physics.emory.edu/~weeks/confocal **Minsky Paper: Check Blackboard** **Multiphoton** /primer/java/multiphoton/excitationbleaching/index.html
Lab 4 (Feb 21-23)	More on Fluorescence Microscopy	
Feb 21	Confocal Microscopes II	
Feb 23	Superresolution I – STED, SIM	
Lab 5 (Feb 28-Mar 2)	Critical Focus, Image characteristics	
Feb 28	**Exam 1**	\
March 2	Capturing Images - Photography	primer\photomicrography\index.html
Lab 6 (March 7-9)	Capturing images.	
March 7	Digital Imaging - capture, spatial resolution, bit depth, image histograms. Brightness/contrast, file	**Intro\primer\digitalimaging\digitalimagebasics.html** **Sampling Frequency - spatial resolution**

	types. Note that we will use an image processing program in class for parts of this section of the course. We will use ImageJ, which will be installed on the laptop computers. You should also install the program at home. Be aware of the changes that will be mentioned during class. The web site for ImageJ is:http://rsb.info.nih.gov/ij/	\primer\java\digitalimaging\processing\samplefrequency\index.html \primer\java\digitalimaging\processing\spatialresolution\index.html **Bit Depth** \primer\java\digitalimaging\processing\bitdepth\index.html **Image Histograms** \primer\java\digitalimaging\processing\histogramstretching\index.html
March 9	Image manipulations 1.,.,LUTs, pseudocolor, image math, Background subtraction	\primer\java\digitalimaging\processing\backgroundsubtraction\index.html
Lab (March 21-23)	Image manipulation with captured images.	background subtraction.
March 21	Image manipulations 2. Convolutions, unsharp mask, Fourier analysis	\primer\digitalimaging\imageprocessingintro.html **Kernels** \primer\java\digitalimaging\processing\convolutionkernels\index.html **Fourier Transformation** \primer\java\digitalimaging\processing\fouriertransform\index.html
March 23	Plugins Deconvolution	See lab manual pp33-33
Lab (March 28-30)	Instruction on Confocal Microscope. Image manipulation with captured images.	2 people on confocal, others work on background subtraction.
Mar 28	Image Manipulations 3. Stacks, 3-D imaging., confocal revisited	

Mar 30	Image manipulations 4. More on Stacks, Time lapse, Labels, calibration, scale bar	
Lab (Apr 4-Apr 6)	Instruction on Confocal Microscope -	2 people, others work on Lab #7 in Manual
Apr 4	Colocalization -	
April 6	Quantitation of Fluorescence-Quantitation of Measurements	
Lab (April 11-13)	Work on Projects	Lab Exercise #9
April 11	superresolution: STED, SIM	
April 13	PALM, STORM	
Lab (April 18-20)	Instruction on Confocal Microscope (if needed)	Work on Projects
April 18	Light Sheet Microscopy	
April 20	Introduction to Scanning Electron Microscopy	
Open Lab (April 25-27	Work on Projects	
April 25	Review	
April 27	**Exam 2**	
Open Lab	Work on Projects.	
May 4	10:30-12:30 Class presentations, Pizza Party	

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