

Biology 542 – Tentative Syllabus -2011  
Light Microscopy for Biology and the Biomedical Sciences

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Class: Tues, Thurs: 3:00-4:15, Old Wilson 202

Microscopy Book: Fundamentals of Light Microscopy and Electronic Imaging, Douglas Murphy, Wiley-Liss, 2001 (pages to read in book) (Can order from Amazon.com)

Handouts in Class: TBA

Grades: 3 Take-Home Exams, Presentations

### TENTATIVE SCHEDULE

- Jan. 11 The Origins and Evolution of Light Microscopy for Biology  
13 Geometrical optics and basic imaging light paths (15-50)  
18 Basic illuminating light paths, conjugate image planes (1-13)  
20 Microscope assembly, alignment, identification of major components, identification of conjugate image planes, measure NA, (29-42)  
25 Wave optics of image formation, resolution, diffraction, Objective Point Spread Function, Diatoms as resolution test specimens (61-116)  
27 Dark field and Phase Contrast Microscopy
- Feb. 1 Polarized light interaction with matter (117-133)  
3 Polarization, DIC microscopy (135-175)  
8 (Lab Demo) Polarization and DIC microscopy

#### Take Home Exam I

- 10 Basic Fluorescence, Fluorescence Microscopy, fluorophores, filters, light sources (177-203)  
15 Practical aspects of objectives, condensers, ... (50-60)  
17 Alignment for Epi-fluorescence, Practical Aspects Optics
- 22 Intro. Video and Digital Cameras, digital images, digital image acquisition, microscope control: A Multi-Mode Digital Imaging Microscope (MetaMorph and Image J; 236-238; 260-267; Image J Instructions)  
24 Getting quantitative information from photon counting to digital images and digital image processing (259-281)
- Mar 1 Point Scanning Confocal and Multi-photon Microscopy: Basic concepts and practical aspects (205-231)  
3 Intro. Super-Resolution Microscopy: SHREC, SIM, PALM, STORM, Take-Home Exam II, to be Returned at End of Break
- Mar. 14 Introduction to fluorescent probes, their fundamental properties and how they can be used as bio-sensors

	17	GFP and its derivatives
	22	Applications of Multi-wavelength GFP, YFP, CFP and DIC imaging in budding yeast (K. Bloom)
	24	3-D Image De-Convolution
	29	Advanced Fluorescence Methods: FRAP, FLIP, FRET, TIRF
	31	Examples of Single Molecule Imaging of protein function with TIRF
Apr.	5	Examples of FRET BioSensors: phosphorylation, tension .....
	7	Cell based screening for identifying functional proteins
	12	Presentation Journal Articles
	19	Presentation Journal Articles
	21	Presentation Journal Articles
	26	Presentation of Journal Articles
Take Home Exam III		

Example Listing of Biomedical Microscopy Methods (Assume Digital Imaging, Processing and Recording, as well as Computer Automation of Microscope Controls (shutters, filter wheels, cameras, stage translation, temperature, etc)

1. Histochemical Bright Field, Transmitted Light
2. Dark Field, Transmitted Light
3. Phase Contrast, Transmitted light
4. Polarization Microscopy, Transmitted Light
5. Fluorescent Molecular Labels, Wide-field Epi-Illumination
6. Confocal Epi-Fluorescence Microscopy: remove out-of focus fluorescence
7. Multiphoton Epi-Fluorescence Microscopy: image deep into specimens
8. Deconvolution Epi-fluorescence Microscopy: Remove out of focus fluorescence
9. Immunofluorescence, all Epi-Illumination methods
10. Fluorescent Molecular Analogs Using fluorescent Dies or Genetically Encoded Fluorophores (e.g. GFP), all Epi-Illumination
11. Fluorescence Recovery After Photobleaching (FRAP): molecular turnover, dissociation rate constants
12. Fluorescence Lifetime Imaging after Photobleaching (FLIP): like FRAP
13. Fluorescence Photoactivation: like FRAP
14. Fluorescence Speckle Microscopy: Measure cytoskeletal assembly and motility dynamics; dynamics of molecular turnover
15. Fluorescence Imaging at One nm Accuracy (Fiona): measure molecular movements
16. Single (Speckle) fluorophore(s) High-Resolution Co-Localization (SHREC): Use two color fluorescent labels to measure molecular separations to <10 nm accuracy
17. Fluorescent Resonance Energy Transfer (FRET): two color technique measures label separations between 1-10 nm; bases for many Fluorescent Biosensors (e.g. Kinase activity, activation of G proteins, receptor-ligand binding).
18. Molecular Fluorescent BioSensors (e.g. Fura-2 measures Ca<sup>2+</sup>; membrane voltage sensors, pH sensors)
19. Super-Resolution Microscopy Methods: Beats Abbe Diffraction Limit (e.g. I-PALM, STORM, SIM)
20. Laser Optical Traps: Manipulation and Force Measurements
21. Laser Ablation: Spatially Resolved Molecular Damage(e.g. Chromophore Assisted Laser Ablation (CALI)