

ANNB395 - Special topics in neuroscience - Techniques in optical microscopy

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This team-taught course will serve as an introduction to many of the optical microscopic techniques available at the University of Vermont College of Medicine. The course shall be organized with lectures, literature discussions and demos. Students will also be required to complete a research proposal. Topics shall include general light microscopy and contrast techniques, epifluorescence, confocal, multi-photon confocal and post-processing (deconvolution) techniques. Applications of these techniques (FRET, ion imaging, second-harmonic generation imaging, FRAP, TIRF, FLIM etc.) will also be discussed as time permits.

This course meets twice a week (Tuesdays and Wednesdays) 3:30-5 in Given Hall B. Sessions with papers will include student presentations/discussion of 2 papers ( $\frac{1}{2}$  h each) and  $\frac{1}{2}$  h demo time.

Date	Topic 1	Instructor	Date	Topic 2	Instructor
9/7	Introduction + light properties Diffraction/ refraction	Locknar	9/8	Basic Scope/ Kohler, resolution, aberrations	Taatjes
9/14	Phase, Hoffman, DIC	Taatjes	9/15	Kohler paper + demo/lab	Taatjes
9/21	Dye chemistry- absorbance, emission, excitation, bleaching	Locknar	9/22	Fluorescence illumination- lamps, lasers, filters, monochromators, epi-scope	Fiekers
9/28	Fluorescence detectors- CCD, PMT, noise	Taatjes	9/29	Sample preparation techniques Immunohistochemistry, live cells, fixation methods, transfection	Fiekers
10/5	Epifluorescence Papers + demo	Fiekers	10/6	Confocal + spinning disks	Taatjes
10/12	Confocal papers + demo	Taatjes	10/13	TIRF	Warshaw
10/19	TIRF papers + demo	Warshaw	10/20	Multiphoton confocal- Including SHG	Locknar
10/26	Multiphoton papers + demo	Locknar	10/27	Post-processing deconvolution	Locknar
11/2	Deconvolution papers + demo	Locknar	11/3	Calcium/ ion imaging Ratiometric, non-ratiometric	Fiekers
11/9	Ion imaging papers + demo	Fiekers	11/10	FRET	Fiekers
11/16	FRET papers + demo	Fiekers	11/17	Spectral Detection	Taatjes
11/23	Spectral papers + demo	Taatjes	11/24	THANKSGIVING	
11/30	FLIM	Locknar	12/1	FLIM papers + demo	Locknar
12/7	FRAP/ FLIP	Locknar	12/8	FRAP papers + demo	Locknar

RESEARCH PROPOSALS DUE 12/10/04.

Grading:

50% of the grade will be based on class preparedness and participation

50% will be based on the research proposal

## Discussion sections:

Students will present papers of their choosing on the current topic. **Please limit your paper choices to topics that have been discussed already and have your selection ready one week before your scheduled date.** That will give us all a chance to photocopy and read the paper. This is a techniques-based course, so the presentations should focus mainly on the methods used and should reflect on the following questions:

1. What technique was used?
2. Was the technique well-suited to the question they were asking?
3. Was the technique well-suited to their sample type?
4. Were the proper controls done?

## Research Proposal:

Each student will prepare an original research proposal that uses one or more of the techniques presented in this course. It will be **no more than 10 pages long** (main body double spaced) including figures (not including references). The topic may (or may not) be related to the student's research. The three main instructors will read the proposal and an average grade will be assigned. Please include the following sections in your proposal:

### AIMS (~1 pg)

- Include 1 or 2 very specific and focused aims

### BACKGROUND (~3 pgs)

- Show a good appreciation of the literature. Try to survey the field uniformly.

### PRELIMINARY RESULTS (~1 pg)

- If your proposal is based on your research, you may have preliminary data to report here. However, your specific aims should be an **extension** of that research and should not be a project that is already completed.

### EXPERIMENT DESIGN & METHODS (~5 pgs)

- Be specific about the techniques you plan to employ
- Divide into: "specific objectives"; "rationale"; "research plan"; "expected significance".
- List possible pitfalls and how you plan to deal with them. This is very important.