Biological Light and Electron Microscopy: Principles and Practice

CBS 732

Instructor and Course Coordinator:

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Goals and Objectives:

This course is designed for students to learn about the basic light and electron optical methods that may be utilized for cytological evaluation of biological samples so that they can help define structure-function relationships within cells, thus being able to relate biochemical, molecular, physiological, and visual information to better understand cellular behavior.

Students will be able to work with preparative techniques for **scanning electron microscopy, transmission electron microscopy, and light microscopy, and confocal scanning laser microscopy**. Production of **digital images by light microscopy as well as limited use of Adobe Photoshop** to prepare images for **Powerpoint** presentations and publication in printed form will be examined. Finally, methods for **morphometric analysis, utilizing Image-ProPlus** will be explored. Go to our website (**www.cvm.ncsu.edu/research/laelom**) to see examples of images and techniques that will be covered in the course.

Students completing this course will have learned basic principles for fixing and embedding biological materials for electron microscopy. They will be able to evaluate the suitability of a given preparative regimen for their research. They will have gained familiarity with enzymatic cytochemistry, non-enzymatic cytochemistry, and immunocytochemistry. They will have learned the purpose and procedures involved in cryopreservation of biological samples. Students will produce negative stain preparations of particulate samples utilizing film-coated grids which they have prepared

Students will have learned how to operate a transmission electron microscope, a scanning electron microscope, several ultramicrotomes, a vacuum evaporator, a critical point dryer, and a sputter coater.

Darkroom techniques and digital imaging techniques that will be learned will include negative development, print making, design and assembly of materials for publication, Powerpoint presentations, and poster design.

Students will be introduced to the principles of light microscopy utilizing different optical systems and will have the opportunity to have hands-on experience with the Olympus VANOX photomicroscope as well as the Nikon C-1 confocal laser scanning microscope in conjunction with a Nikon Eclipse 2000E motorized inverted microscope. They will also be instructed concerning the applications and capabilities of morphometry, digitizing/archiving programs, and telemedicine capabilities available today.

Students will be made aware of the availability and uses for high voltage electron microscopy, intermediate voltage electron microscopy, electron tomography, field emission scanning electron microscopy, vacuum systems, electron optics, low vacuum scanning electron microscopy, environmental scanning electron microscopy, various light microscopy optical systems, and confocal microscopy.

Textbook and Course Material:

Suggested Textbooks:

Dykstra, M.J., and Reuss, L.E. 2003. Biological Electron Microscopy: Theory, Techniques, and Troubleshooting. 2nd Edition. Kluwer Academic/Plenum Publishers, New York. 534 p.

-or-

Bozzola, J.J., and Russell, L.D. 1999. Electron Microscopy: Principles and Techniques for Biologists. 2nd Edition. Jones and Bartlett Publishers, Boston. 670 p.

Student Manual (provided by instructor at no cost):

Dykstra, M.J. 2004. Electron microscopy in Veterinary Medicine. 84 p.

Course Organization:

The course is designed to be a five (5) hour course. The lecture and laboratory topics are listed in the course syllabus below. The course lectures hours will have some of the lecture sessions devoted to manuscript reviews to evaluate scientific approaches used, applicability of techniques, clarity of description of materials and methods used (for reproducibility), and quality of images created. The laboratories will be hands-on applications of the techniques discussed in lectures.

This a five (5) credit graduate level course. There will be three 50-minute lectures a week and two 2-hour laboratories a week. The **Special Projects** labs are designed for students to finish their technical work that leads to the production of the **laboratory hand-in** that constitutes half of the grade for the course. The course objectives will be presented and covered through a combination of lectures, guided discussions, manuscript reviews, and laboratory exercises.

Review of Manuscripts: Discussions of published literature will be used to highlight concepts considered during lectures.

Personal Laboratory Project as Part of Laboratory Hand-in: Before the third week of class, each student will discuss a potential personal project with the instructor, which shall consist of two tissue or cell samples to be compared in some fashion, utilizing transmission electron microscopy techniques and ultramicrotomy. The results of this project, along with the other laboratory excercises, will be written up in scientific style, presented orally to the rest of the class, and the final write-up will be due at the time of the final exam.

Grading:

The grading scale will be A to F. The grade will be comprised of:

1. Two exams (25%)
2. A final exam (25%)
3. A Laboratory Hand-in (50%)

Miscellaneous:

This course is open to graduate students, staff, and faculty with a biology background. Permission of instructor is required so that students can be apprised of the effort required before they commit themselves.

Attendance at all classes is expected, but justified absences can be made up by students working more in the laboratory to catch up, on their own schedule. The instructor will make every effort to help students who have unavoidable absences catch up through personal instruction or referral to reading material that covers the topics missed.

In this course all laboratory hand-in material is due at the time of the final exam.

It is expected that both the students and instructor will abide by the University policy on academic integrity found in the Code of Student Conduct (http://www.ncsu.edu/policies/student_services/student_discipline/POL1.35.1.php.

Any student that may have a disability that may interfere with his/her work performance in the course is expected to notify the instructor during the initial conference to obtain permission to take the course. This will allow the College and/or instructor enough time to develop teaching aids or course modifications to accommodate the handicapped student's needs. Any concerns about these issues can be answered by the Student Services Office or instructor prior to the course (http://www.ncsu.edu/provost/offices/affirm_action/dss/).

Before any students are allowed to work in the laboratory, they are trained according to the Safety Plan for the LAELOM that is updated yearly. After the training, they sign certifications to that effect and are continually updated on safety issues during the term of the course.

The only expense to be incurred by students beyond the optional textbook purchases will be a set of jeweler's forceps, at a price from approximately $10-20.

Course Syllabus:

Light and Electron Microscopy: Principles and Practice
CBS-732

Lecture 9:10-10:00 am Monday, Wednesday, and Friday
Lab 2:35-4:25 pm Tuesday and Thursday
Office hours by appointment
Instructor: Dr. Michael J. Dykstra, C-108; 513-6202
Email: Michael_Dykstra@ncsu.edu

Recommended Texts (none are required):

Dykstra, M.J., and Reuss, L.E. 2003. Biological Electron Microscopy: Theory, Techniques, and Troubleshooting. 2nd Edition. Kluwer Academic/Plenum Publishers, New York. 534 p.

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Bozzola, J.J., and Russell, L.D. 1999. Electron Microscopy: Principles and Techniques for Biologists. 2nd Edition. Jones and Bartlett Publishers, Boston. 670 p.

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Lecture #	Topic
1	Course Introduction
2	Principles of Fixation
3	Fixation, Dehydration, Embedding of Tissues
4	Embedding Media, Infiltration, Polymerization
	University Holiday
5	Ultramicrotome design
6	**Review of Published Papers, Preparative Techniques**
7	Ultramicrotomy
8	Section Staining and Historical Review of Electron Microscopy
9	Support Films and TEM
10	Theory of the Electron Microscope
11	Electron Emission, Electron Optics
12	Electron Optics
13	Vacuum Systems
14	Vacuum Systems
15	**Hourly Exam #1**

16	Shadowing and Replicas
17	Negative Staining
18	Capturing Images on Film and Producing Silver-based Prints
19	Photography (Continued)
20	Cryotechniques
	Fall Break
21	Cryotechniques
22	Scanning Electron Microscopy
23	Scanning Electron Microscopy
24	Scanning Electron Microscopy
25	Scanning Electron Microscopy Preparation
26	**Review of Published Papers, Cryotechniques and SEM**
27	Intermediate and High Voltage Electron Microscopy
28	**Hourly Exam #2**
29	Confocal Microscopy
30	Confocal Microscopy
31	Confocal Microscopy
32	Cytochemistry
33	Cytochemistry/Immunocytochemistry
34	Immunocytochemistry
35	Quantum Dots
36	Microanalytical Techniques
37	Microanalytical Techniques/Light Microscopy
38	Light Microscopy
39	Light Microscopy
Thanksgiving Vacation	
40	Telemedicine and Virtual Microscopy
41	**Review of Published Papers, Light and Confocal Scanning Microscopy/Immunocytochemistry or Cytochemistry**
42	Virtual Microscopy/Morphometry/Summation
	Final Exam

Lab Number	**Topic**
1	Orientation to the Laboratory, Assemble Kits
2	Fixation and Tissue Embedment
3	Block Trimming, Glass Knives
4	Glass Knives, Semithin Sectioning
5	Microtomy
6	Microtomy (Ultrathin Sections)
7	Section Staining, Support Films, Section Pick-up
8	Introduction to the Philips/FEICO EM208S/Morgagni
9	Introduction to the EM208S/Morgagni (Continued)
10	Stigmation, Through-Focus Series

11	Vacuum Evaporator Systems and Microtomy
12	Wehnelt Assembly, Column Alignment
13	Negative Staining and Shadowing/Replicas
14	Darkroom Training
	Fall Break
15	SEM Operation in Conventional Secondary Mode
16	SEM, Backscatter Mode, Low Vacuum Mode, Poor-Man's Cryo
17	**Critique**, some Photoshop
18	Light Microscopy/Stereo Pairs
19	Digital Image Production and Adobe Photoshop Use (Through-focus Series; Zeiss Epifluorescence and Cryoultramicrotomy Video)
20	Special Projects
21	Special Projects
22	Zeiss 902 Video and Photoshop Work
23	Special Projects
24	Confocal Demo
25	Special Projects
26	Special Projects
27	Special Projects
	Thanksgiving Vacation
28	Special Projects
29	**Final Critique**

Laboratory Requirements for CBS-732

All materials handed in will be typed

All hand-in materials will be due at the time of the final lecture exam

<u>No incompletes will be given in this course</u>