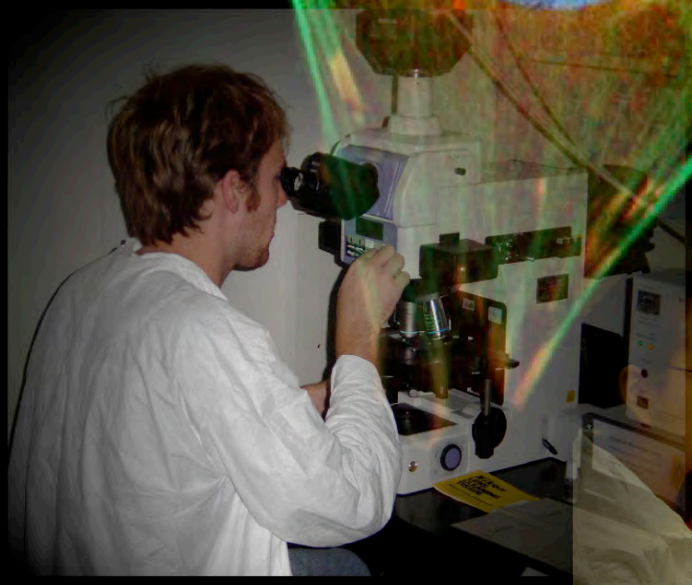
Syllabus

BioS368 Cell Biology Lab



Lehigh University
Dept. Biol. Sciences
Fall '05

Instructor: Prof. M. Falk

BioS 368
Cell Biology Laboratory
Fall 2005
Monday/Wednesday 1:10 – 4:00 pm
Instructor: Prof. Matthias M. Falk

Classroom: C108
Office: D218
Office Phone: 610-758-5896
Office Hours: By appointment
email: MFalk@lehigh.edu

COURSE OBJECTIVE:

Cell Biology is an integrative field that overlaps with many other research areas such as Molecular Biology, Biochemistry, Physiology, Neuroscience, Biophysics, Mathematics, and Bioengineering. Lehigh University offers laboratory courses in all of these areas.

The course will accompany the Cell Biology lecture (BioS 367 & 411) that is taught in parallel in the fall semester. It has been designed to clearly illustrate the theory taught in the lecture ("Structure and Function of Cells") by visualizing cells, subcellular organelles/structures, proteins, and cellular processes. In the course, state of the art fluorescence microscopy techniques including imaging proteins in *living* cells will be applied. The course will have four main chapters: (1) We will learn thoroughly how to culture immortalized cell lines, (2) to stain subcellular structures in fixed and *living* cells using specific probes and antibodies (including double and triple color labeling), (3) to express and observe proteins tagged with auto-fluorescent protein probes (GFP and derivatives, RFPs) in *living* cells, and (4) to interfere with cellular processes using specific drugs. Dependent on time isolation of primary cells and tissues will also be considered.

Experiments are not standard Cell Biology experiments available commercially in kit form, but are based on actual research projects pursued in the instructor's laboratory and have been adapted to the classroom. You will culture your own cells during the entire course and grow cells in dishes and on cover-slips for experimental manipulation and microscopic observation. All course topics are designed to give you hands-on experience in cell biological experimentation.

No exams will be given in this course. Most important is that you are highly motivated! It is imperative that you actively participate in all classes and that you write thorough protocols of all experiments! The protocols should allow you to do these experiments in any future research environment without consulting additional help. To learn to formulate/communicate scientific experiments/results, the aims of an independent research project (designed and conducted by your group) will be formulated in the style of a "research grant proposal". The outcome will be communicated in the format of a scientific publication (a "research paper"), and an oral presentation similar to a short platform presentation on a scientific meeting (one proposal, report, and oral presentation per group).

Laboratory Sessions:

Lab sessions will be divided into two main units. In the first unit you will learn the experimental techniques mentioned above (see below for details). In the second unit you and your lab group will design an independent lab experiment. Below, I have suggested some projects that build on the techniques learned in the course, however, you also can choose and design your own project. Availability of equipment and reagents, however, has to be considered. You will have 4-6 weeks to carry out your research projects. Again, your motivation is what counts most; unexpected results are tolerable as long as you try to explain the outcome of your experiments.

EXPERIMENTAL PROCEDURES AND TENTATIVE SCHEDULE:

First course day: Monday, Aug. 29, 2005

Cell Culture: (about 3 weeks, Aug. 29 - Sept. 14)
Intro into cell culture: Sterile techniques, medium (components and pH indicators), cell culture flasks and dishes, incubators, measuring CO_2 concentration using a Fyrite
To start a culture from frozen stocks, counting, splitting, and seeding cells
Viability testing
Cryo-preserving cells
Potential contamination with bacteria/yeast/fungi; testing for Mycoplasma contamination
Coating cover-glasses with different cell-adhesion substrates (e.g. uncoated, BSA, collagen, fibronectin, poly-L-lysine, laminin5) and its influence on cell growth, morphology and motility
Growing cells on glass coverslips for microscopic observation

Cell types that will be grown in the course:
HeLa (Human cervix carcinoma, not contact inhibited, not polarized, negative for gap junctions), COS7 (African green monkey kidney cells, immortalized, contact inhibited, not polarized, expressing endogenous GJs), or similar
and/or MDCK (canine kidney epithelium cells, polarized, positive for tight-, gap- and adherens junctions)

Introduction into fluorescence microscopy techniques using high-end upright and inverted fluorescence microscopes

Specific subcellular compartment stains for fixed and living cells (single, double, and triple stains): (about 2 weeks, Sept. 19 – Sept. 28)
Specific for nucleic acids (nucleus), endoplasmic reticulum (ER), Golgi apparatus, Mitochondria, acidic compartments (Lysosomes), actin filaments

Indirect immunofluorescence techniques using specific monoclonal and polyclonal antibodies (single, double, and triple stains): (about 2 weeks, Oct. 3 - Oct. 12)
ABs specific for microtubules, intermediate filaments, actin binding proteins (myosins, vinculine), tight-, adherens- and gap junctions, secretory and endocytic machinery components

Pacing Break: Monday, Oct. 10 (no course)

Transfection and expression of auto-fluorescent protein tagged proteins: (about 2 weeks, Oct. 17 – Oct. 31)
Connexin43-GFP/connexin43-DsRed (gap junctions)
N-cadherin-GFP (adherense junctions)
Tubulin-YFP (microtubules)
α-actinin-GFP (actin cytoskeleton)
rab9-YFP (Late Endosomes)
Myosin-VI-GFP (endocytic vesicles)

Additional Experiments and Independent Research Project Suggestions (Nov. 2 - Dec. 5):
-- Direct cell-to-cell communication via gap junctions measured by scrape-loading dye transfer
-- Extracellular calcium and N-cadherin mutants and their influence on adherense junctions
-- Gene silencing via RNAi technology
-- Specific drugs and their influence on subcellular components and cellular processes (mitosis, secretion, endocytosis, etc.):
Actin – Cytochalasin D, Latrunculin A, etc.
Microtubules – Taxol, Nocodazole, etc.
Golgi – Brefeldin A
Clathrin mediated endocytosis – high levels of extra-cellular sucrose, clathrin knock-downs
Etc.

Thanksgiving Break, Wed. Nov. 23 (no course)

Last Day, Wed. Dec. 7: Oral Presentations of Independent Research Projects

Laboratory attire: Please wear appropriate laboratory clothing; sandals and "Flip-Flops" are not appropriate (see attached EH&S Memo). Laboratory coats will be supplied and have to be worn. Gloves and protective eyeglasses are also provided and are recommended when performing sterile techniques, or handling corrosives.

Attendance: Missed Laboratories: As you will be working within a lab group, and individual laboratories require considerable set-up and preparation, you will be expected to attend your normal laboratory session**. Please make all efforts to attend class! If you have to miss a laboratory, please notify me prior to the lab and the reason for absence.**

Academic Honesty. Issues of academic dishonesty will be handled according to the guidelines put forth by the Lehigh Academic Honesty Committee.

Peer Collaboration: Students will work in groups of two to three students. However, ALL students should participate in performing the laboratory procedures. For example, everybody should get a chance to feed, harvest and seed cells during cell culture experiments. Although ideas should be shared, each student should write her/his own experimental protocols.

COURSE EVALUATION:
Written or oral exams will not be conducted in this laboratory course. Performance will be evaluated from your motivation and lab conduct/participation, and the quality of your experiment protocols, independent project proposal, report, and oral presentation.
Students are expected to maintain their cell cultures throughout the course. More careful analysis of stained microscopic slides is possible for interested students. The cell culture room and the microscopy room are available outside class hours and can be used after notification during weekdays. Each student should write her/his own laboratory protocols. Research Proposal, Research Results (scientific paper), and oral presentation can be prepared by the group, however, each student must have a copy of each. Lab protocols are due no later than 2 weeks after the lab. The Independent Research Proposal is due no later than 2 weeks after beginning of independent projects. The Independent Research Report (Research Paper) is due no later than 2 weeks after the end of the experiment.

Your class grades will be based on the following:

Lab Conduct/Participation	50 %
Experiment protocols	25 %
Independent project proposal Independent project talk Independent project report	25 % together
	100 % total

Each protocol, proposal, report, and oral presentation will be graded on a scale from 0 to 10. Points will be added and weighted according to the percentages above. The final grade is the summary of the three categories above. Grade weights will be given according to Lehigh University's Faculty Resource Guide as follows: A (40>37), A- (37>33), B+ (33>30), B (30>27), B- (27>23), C+ (23>20), C (20>17), C- (17>13), D+ (13>10), D- (10>7), F (7-0). (Example: Student A's lab particip.: 8, protocols: 9, independ. project: 10; 8+8+9+10=35; A-)

Laboratory Notebooks and Lab Conduct:

You should write a Lab notebook. A notebook is a diary. It is to be used to recount what happens from day to day during your project. The notebook will help you to remember how you performed the experiments and to write the experiment protocols. You should include dates, experimental goals, alterations made to procedures, calculations, results and analysis of results, manufacturers and concentrations of chemicals/solutions used, etc.

Experiment Protocols:

Experiment protocols are due two weeks from the completion of the experiment. You will lose 10% of your earned grade for every day that a report is late.

The goal of the experiment protocols is to give you experience in writing a detailed protocol of an experiment you performed. This is important to track why experiments may not have worked, to document to a co-worker what you have done, and to summarize materials and methods in a publication. For each lab report, you should prepare a **Materials** list (including manufacturers and concentrations), a **Methods** section (with a detailed step by step protocol of the procedure), a **Results** section, and a short **Discussion** section. In the Results section, you should present the data you obtained in the experiment performed to test your hypothesis. If you add figures, include figure titles and figure legends that summarize the figure/experiment, gives proper units (when necessary), identifies symbols, etc. In the Discussion section, evaluate your experiment. Include a brief discussion of possible reasons for any discrepancies in your data, as well as any suggestions for better ways to design or execute the experiment.

Independent Research Project Proposals (Grant Proposal):

The last 4-6 weeks of the semester will be left open for work on independent research projects. Research proposals for your independent project are due no later than 2 weeks after beginning of the independent project. Your proposals should consist of (1) a short "**Abstract**" summarizing the project and background information (100 - 200 words). (2) A summary of the "**Specific Aims**". (3) An "**Introduction**" explaining the background of what you want to do, and your hypothesis. (4) A "**Preliminary Results**" section if the project is a continuation of an experiment of the course, or if the student has done previous research related to this project. (5) The actual "**Research Proposal**" explaining in detail the experiment and how you want to do the

research including the materials you will need. (6) "Bibliography". This format is the typical format of a fellowship, or research grant proposal.

Independent Research Project Report (Oral Presentation):

On the last day of the course the groups will present their independent research project to the other groups in a short talk. The presentation will be about 10-15 minutes long plus time for discussion. The talk should give background information, the hypothesis, experimental results, and some discussion of the results if applicable to allow the other groups to understand what you have done in your independent project and why. The outline of the oral presentation should form the framework for the written report (research paper) described below. This format is the typical format of an oral short presentation on a scientific conference.

Independent Research Project Report (Research Paper):

The formal lab report of your independent project should be written so that a scientist, which is not an expert in the field of work, can understand your objectives and results, and can logically follow your interpretations. It should include six parts, organized, as described below:

1.) The **Abstract** should contain a very brief (a few sentences) summary of the entire experiment. By reading the abstract, the reader should be able to get the gist of what you did and what you found.

2.) The **Introduction** includes a brief literature review of the current knowledge with respect to the technique or the specific system. This should be properly referenced to the Reference section at the end of the report. The most important item in your introduction is a clear statement of your hypothesis. This is best placed at or near the end of the introduction. Note that you will probably use an edited version of the introduction from your research proposal.

3.) The **Materials and Methods** section includes Materials used and the ways in which you performed the experimental techniques.

4.) The **Results** section presents the data you obtained in the experiment performed to test your hypothesis. This section should include figures (pictures), tables, graphs, and other summarized forms of your data, as well as a verbal description of those data. Be sure to include proper units, legends, identification of symbols, graphs and figure titles, etc. The legend should contain enough information to enable understanding of the figures without reference to other parts of the report.

5.) In the **Discussion** section, you will interpret your results more broadly. You should address: your hypothesis, and whether or not the experiment supported the hypothesis, and importantly, you should make reference to how your results relate to information you've found in your lab manual, your Cell Biology textbook and other current literature. You should also include a brief discussion of possible reasons for any discrepancies in your data, as well as any suggestions for better ways to design or execute the experiments.

6.) **References** include published works you used in your report. They should be listed in alphabetical order of the first author's last name, and written in a standard format. Citations should be made in the body of your report by placing the author's last name(s) and the year of publication in parentheses (see scientific papers for examples), immediately following the material derived from that reference work. (Please reference literature with full titles). This format is the typical format of a manuscript reporting novel findings in a scientific journal or periodical.

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Lehigh University
Environmental Health & Safety

MEMORANDUM

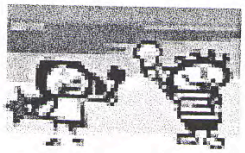
TO: Mailing List Recipients

FROM: Dr. Barbra A. Plohocki
Director, Environmental Health and Safety

RE: Proper Laboratory Attire and Children and Pets in University Buildings

Please keep in mind it is a Pennsylvania state law that eye protection must be worn in ***all*** laboratories. The laboratory supervisor has flexibility in determining the type of eyewear appropriate for the task. The issue of personal protective equipment must be addressed by all laboratory supervisors. In addition, laboratory supervisors should set an example by always wearing safety glasses in the laboratory.

There is also a concern regarding ***"proper laboratory attire"***. In many cases, graduate students wear shorts and sandals when using chemicals and other hazardous materials. This is not appropriate laboratory clothing and should not be allowed by the laboratory supervisor. I strongly encourage all laboratory supervisors to enforce the Chemical Hygiene Plan in regards to appropriate laboratory attire.



It has come to my attention that children and animals have accompanied graduate students and employees to work and have been seen in University laboratories. Under no circumstances, are children or animals permitted in research as well as undergraduate teaching laboratories.

Please call me at X83643 or e-mail me at bap2@lehigh.edu, if you have questions.

BAP:dd

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