MCB 2225: CELL BIOLOGY LABORATORY
Spring 2016 TLS 253

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Lab web site: http://homepages.uconn.edu/~mb2225vc/MCB_2225/Course_overview__h1_MCB2225,_Cell_Biology_Lab__h1_.html

Course Description

This course is designed to give students an opportunity to investigate various cellular process using modern techniques in a research setting. The laboratory is equipped with 7 computer controlled fluorescence microscope workstations with digital cameras for acquiring time lapse images of living cells. Each workstation will accommodate a pair of students who will collaboratively perform experiments designed to investigate different aspects of cellular function and behavior. Students will also make use of instruments in the Confocal Microscopy and Flow Cytometry Facilities. Each experiment will focus on measuring the effect of mutations or pharmacological perturbations on cell function. A focus of the course is using digital image processing for visualization and quantification of cellular processes. Students will learn specific techniques, but be given flexibility to design their own experiments utilizing those techniques. Since numerous experiments do not fit easily into a 3 hour lab period, students will also have unrestricted access to the laboratory in order to continue experiments on their own schedule. Each student will also conduct an independent research project of their own design during the last month of the semester. The course allows students to master a range of specific techniques that could be used in undergraduate research at UConn and provides a solid base for graduate school or employment in the Biotechnology industry.

Course Evaluation

1. **Midterm (15%)**: There will be a midterm exam designed to reinforce learning of important concepts. The exam will be an open book take home, but will focus on topics discussed in lectures associated with the labs.

2. **Web Site and Lab Notebook (20%):** You and your partner will together maintain a web site where you post your data and analysis for all the world to see. This will be continuously evaluated by the TA's providing feedback for improvement. In addition, you will each keep a laboratory notebook where you record the protocol for each experiment and any information or data relevant to the experiment.

3. **Lab reports (20%)**: You will turn in and be graded for 2 lab writeups during the first half of the semester. These reports should be a mixture of the actual prelab experimental design, notes written while carrying it out, data collected and then graphed or analyzed as appropriate, and then a more formal discussion of the results and conclusions. **Most of the work of the report is the notebook and should be done while preparing for and performing the experiment. The notebook and report are for the most part the same thing. You should not need to go back and rewrite your experimental design section. This can and should be handwritten and rough.** You should write down all observations whether your experiments work or not. More importantly, write everything down including the intricate details you observe in every experiment. In most cases, small things are critical to the success of your

experiments. Late labs reports will have a deduction of 5 points/day or 25 points/ week.

4. **Independent study (40%)**: For the last half of the course, you will be doing an independent study using the skills you have learned. You will discuss an outline of your planned experiments with Dr. Knecht before Spring Break. This will help shape the foundation of your study and give direction to your research. What's important is how you logically design your experiments to answer relevant scientific questions.

5. **Pre-lab quizzes (5%)** You should read the lab writeup before coming to class on days when we are starting a new lab project. Come to class with questions to be discussed. There will be short quiz on the basic questions being addressed before lecture begins.

Missed Class and Makeups:

It is extremely important that you show up for each class and perform each laboratory. All the techniques build upon each other for the duration of the semester, so you cannot easily miss a class without making up the material. We expect individual responsibility for doing your job as any employer would in the real world.

1. If you know in advance you cannot be in class and have a good reason (illness, interview, sporting event), let the TA's or Dr. Knecht know in advance. In this situation, arrangements will be made to make up the lab either alone or together with your lab partner.
2. If you simply do not show up for class or lab, and have no good reason, you will not be able to make up the lab and will get no credit for that lab or anything associated with that lab (website grade, lab report grade, etc). If you miss two labs in this way, you will fail the course.

Dictyostelium web site

http://dictybase.org/

General Microscopy web sites

http://micro.magnet.fsu.edu
http://www.microscopyu.com

Date	Day	Lecture/ Experiment
1/19	Tue	Lecture: Introduction to the course Dictyostelium discoideum, Pipet calibration, Benchtop microscopes, Cell Trituration, Cell Settling, hemocytometer, sterile technique **Experiment 1:** Calibrate pipettes **Experiment 2**: Start cells to measure growth rate
1/21	Thu	Lecture: Cell culture and maintenance, iWeb, Excel **Laboratory**: Culture maintenance, iWeb **Experiment 3: Compare growth of wild-type and myosin II mutant cells. Collect data at least twice per day until Tuesday.** **Handouts for next lab: Microscopy**
1/26	Tue	Snow day Lecture: Intro to Micro-manager and Fiji Software **Laboratory**: Plot growth data, add data to iWeb, Hands on microscopes- try objectives, camera, micromanager, small movie of cells moving in a dish **Experiment 4: Trial Cell Motility**
1/28	Thu	Lecture: Microscopes, Cameras, digital imaging, quicktime **Laboratory**: Set up Koehler illumination, calibrate objectives **Experiment 5: Time lapse video capture of wild type and mutant cells moving and dividing on a surface.**
2/02	Tue	Lecture: Quantitative imaging time-lapse, stacks **Laboratory:** set up motility, image shaking cells
2/04	Thu	Lecture: Fluorescence **Experiment 6:** quantify motility
2/9	Tue	Lecture: Chemotaxis, quicktime **Experiment 7**: transfection
2/11	Thu	Lecture: Vectors and transformation of cells Laboratory:. Folate chemotaxis: Compare speed and directionality of NC4A2 vs Myosin null mutants. Continue folate chemotaxis analysis Electroporation using GFP and GFP-ABD vectors
2/16	Tue	**Experiment 8**: analysis of chemotaxis and imaging transfected cells Compare multicellular development of myosin mutant and wild-type. If possible- do chimeric aggregation with fluorescent cells (A) Over agar development using NC4A2 and Myo-cells (B) Under agar development using NC4A2 and Myo-cells (C) Growth and development of NC4A2 and myoII- on SM plates with Ka.

2/18	Thu	Continue development
2/23	Tue	Lecture: Fixation and fixatives **Experiment 9:** Immunostaining of cells to visualize F-actin and microtubules Start cells for nuclear staining on Thursday
2/25	Thu	Lecture: Confocal Microscopy **Experiment 10:** Image nuclei of NC4A2 and HK321 grown in suspension using nuclear staining with Propidium iodide
3/1	Tue	Lecture: Confocal Microscopy **Experiment 11**: Imaging slides in confocal microscope
3/3	Thurs	Lecture: 3D image Processing Experiment 11 (cont) Work with Fiji to process data from confocal microscope
3/8	Tue	Lecture: Endocytosis: Microscopy and Flow Cytometry Rhodamine dextran uptake and exocytosis in NC4A2 and HK321 cells. Quantify fluorescent vesicles in cells.
3/10	Thu	Endocytosis Part 2: Flow Cytometry Measure fluid uptake and exocytosis of cells by flow cytometry
	adhesion?	

3/13 -3/19: SPRING BREAK

3/21 Tues

After Break- Independent Projects