GCD 8920/BIOL 5950: Quantitative Fluorescence Microscopy

Fluorescence microscopy is an indispensable technique in the Biological Sciences that is used for studying proteins and cells in their physiological state. While fluorescence microscopy is commonly employed for observing molecules or disease progression, its real strength lies in the ability to reveal quantitative information. The main goal of this course is to introduce the fundamental principles of fluorescence, fluorescence microscopy, and fluorescence quantification to extract maximal information from fluorescence experiments. The course will use ImageJ tutorials along with lectures to provide hands-on expertise in image analysis and interpretation. Students will gain both a broad understanding of fluorescence microscopy techniques. Graduate students will gain confidence in quantitative analysis of their own image data. Undergraduate students who proceed to graduate school, medical school, or employment in the biotech industry will find that quantitative microscopy skills will provide them an essential competitive edge.

Instructor: Sivaraj Sivaramakrishnan
Email: sivaraj@umn.edu

Department: Genetics, Cell Biology & Development

Course Prerequisites: For BIOL 5950 - BIOL 4004 or GCD 3033

Credits: 3

Meeting Time & Place:
Lecture: One 50 minute lecture per week
Laboratory: One 3-hr laboratory per week

Course Objectives:
In everyday research, students have to make decisions about experiments involving fluorescence. Upon completion of the course the students will be able to make informed decisions on the following:

- What is the best fluorescent protein/filter/microscope for a particular experiment?
- What is an image and how best to display it?
- What are the measurable quantities in an image, and how to measure them?

In addition the students should also be able to read existing literature with a good grasp of the microscopy methods employed, how to image data were quantitatively analyzed, and whether the data and approaches support the conclusions being drawn by the authors of the study.

Course Material:
There is no required text or course packet to buy for this course. You **will need** a laptop computer or equivalent device (Mac/PC) to download and run ImageJ, which will be the software for majority of the data analysis. All materials will be posted on the course Moodle site. These postings will include handouts of PowerPoint lecture slides, tutorial data sets, homework assignments, and exams. No handouts will be provided in class. You are responsible for downloading all lecture related materials from the website and bringing them with you to class. This includes tutorial data sets and plugins/macros for ImageJ.

Class Attendance:

Attendance for both the lecture and tutorial is mandatory. There will be sign-in sheets for lecture. You must sign in **before** class starts in order to receive credit for attending. To make up for an absence you must:

1. Submit your reasons for missing the class in writing to the course director (Sivaraj Sivaramakrishnan, sivaraj@umn.edu)
2. If it is determined that you have a good reason for missing class (illness, family emergency etc.) you will be allowed to make up for the absence by reviewing the class presentation and completing the tutorial assigned in class.

If you do not have a legitimate reason for missing class, or if you fail to submit an assigned tutorial, you will be given an 'unexcused absence'. If you have more than two unexcused absences you will automatically fail the course.

Required work: (Note differences in GCD 8920 and BIOL 5950)
In addition to attending class and actively participating in the tutorial session, students will be responsible for turning in homework assignments, once a week, based on the material covered in each lecture.

Fluorescence Microscopy & Image Acquisition – **(GCD 8920 and BIOL 5950)** Assignments are due before start of class on Tuesday. Hard-copy printout is required.

Mid-term – **(GCD 8920 only)** Mid-term will cover the Fluorescence Microscopy & Image Acquisition Module and will be a written take-home exam. Students cannot interact with each other or share answers/information.

Quantitative Analysis – **(GCD 8920 and BIOL 5950)** Students will work in groups to present their data analysis assignment during the first hour of class.

Final exam – **(GCD 8920)** The final exam is project based and requires the analysis of an original data set derived from the literature. The students are required to use their combined knowledge of fluorescence microscopy and quantitative image analysis to quantitatively process the image data and test a specific question being proposed by the authors. Students are required to hand in their exam assignments within a week of the posting on Moodle. This is followed by in-class presentations of the data analysis. Students are encouraged to work in groups to tackle the specific assignment and the group is allowed to submit a combined report and present their findings to the class. Following the in-class presentation, the instructor will present the original research article and the findings of the authors who generated the original data set.

(BIOL 5950) Students will have a take-home exam that will cover material presented in both the first and second half of the class. The first component will test the student's understanding of information on Fluorescence Microscopy and Image Acquisition as covered in the homework assignments. The second component will require students to re-analyze data from one of three of the Quantitative Analysis components to address specific questions.

Grading Policy:
The course is initially offered as Pass/Fail. To pass the students must score at least 75/100 points.

Points (GCD 8920)
Class participation including lecture and tutorial – 25 points
Homework assignments – 25 points
Mid-term exam – 25 points
Final exam – 25 points

Points (BIOL 5950)
Class participation including lecture and tutorial – 40 points
Homework assignments – 35 points
Final exam – 25 points

COURSE SCHEDULE
(Introduction; Fluorescence Microscopy & Image Acquisition; Quantitative Analysis; Guest Lectures)

Week	Lecture	Tutorial	Dates (2017)	Homework # Due
1	Introduction to the Course	-	Jan 13	
2	Quantitative Fluorescence Microscopy in the Biomedical Sciences	UIC Tour & Microscope Demo	Jan 18 & 20	
3	Microscope Basics	Kohler Illumination	Jan 25 & 27	
4	Fluorophores	Chroma Spectra/Filter Selector	Feb 1 & Feb 3	1
5	Image formation and point-spread function	Decipher microscope objectives	Feb 8 & 10	2
6	Electronic Imaging: CCD and PMT	Camera demo and specsheets	Feb 15 & 17	3
7	Confocal and 2-photon	Deconvolution	Feb 22 & 24	4
8	Quantitative Analysis	ImageJ	Mar 1 & Mar 3	5
9	Guest Lectures Opto-genetics & TIRF microscopy	Data analysis	Mar 8 & 10	6
	Midterm (GCD 8920)		Due Mar 22	
10	FRAP and photoactivation	Dynamics of the mitotic spindle	Mar 22 & 24	
12	Morphometric analysis	Cellular hypertrophy	Mar 29 & 31	7
14	Super-resolution & particle tracking	Molecular motors	Apr 12 & 14	8
15	Colocalization	Cellular autophagy	Apr 19 & 21	9
16	FRET	Signal transduction	Apr 26 & 28	10
17	**Final Presentation (GCD 8920)** **Final exam (BIOL 5950)**		May 3 & 5 May 3rd	

Student Conduct Code:

The University seeks an environment that promotes academic achievement and integrity, that is protective of free inquiry, and that serves the educational mission of the University. Similarly, the University seeks a community that is free from violence, threats, and intimidation; that is respectful of the rights, opportunities, and welfare of students, faculty, staff, and guests of the University; and that does not threaten the physical or mental health or safety of members of the University community. As a student at the University you are expected adhere to Board of Regents Policy: Student Conduct Code. To review the Student Conduct Code, please see: http://regents.umn.edu/sites/default/files/policies/Student_Conduct_Code.html.

Scholastic Dishonesty:

You are expected to do your own academic work and cite sources as necessary. Failing to do so is scholastic dishonesty. Scholastic dishonesty means plagiarizing; cheating on assignments or examinations; engaging in unauthorized collaboration on academic work; taking, acquiring, or using test materials without faculty permission; submitting false or incomplete records of academic achievement; acting alone or in cooperation with another to falsify records or to obtain dishonestly grades, honors, awards, or professional endorsement; altering, forging, or misusing a University academic record; or fabricating or falsifying data, research procedures, or data analysis. (Student Conduct Code: http://regents.umn.edu/sites/default/files/policies/Student_Conduct_Code.html). If it is determined that a student has cheated, he or she may be given an "F" or an "N" for the course, and may face additional sanctions from the University. For additional information, please see: http://policy.umn.edu/Policies/Education/Education/INSTRUCTORRESP.html.

Makeup Work for Legitimate Absences:

Students will not be penalized for absence during the semester due to unavoidable or legitimate circumstances. Such circumstances include verified illness, participation in intercollegiate athletic events, subpoenas, jury duty, military service, bereavement, and religious observances. Such circumstances do not include voting in local, state, or national elections. For complete information, please see: http://policy.umn.edu/Policies/Education/Education/MAKEUPWORK.html.

Appropriate Student Use of Class Notes and Course Materials:

Taking notes is a means of recording information but more importantly of personally absorbing and integrating the educational experience. However, broadly disseminating class notes beyond the classroom community or accepting compensation for taking and distributing classroom notes undermines instructor interests in their intellectual work product while not substantially furthering instructor and student interests in effective learning. Such actions violate shared norms and standards of the academic community. For additional information, please see: http://policy.umn.edu/Policies/Education/Education/CLASSNOTESSTUDENTS.html.

Sexual Harassment

"Sexual harassment" means unwelcome sexual advances, requests for sexual favors, and/or other verbal or physical conduct of a sexual nature. Such conduct has the purpose or effect of unreasonably interfering with an individual's work or academic performance or creating an intimidating, hostile, or offensive working or academic environment in any University activity or program. Such behavior is not acceptable in the University setting. For additional information, please consult Board of Regents Policy: http://regents.umn.edu/sites/default/files/policies/SexHarassment.html

Equity, Diversity, Equal Opportunity, and Affirmative Action:

The University will provide equal access to and opportunity in its programs and facilities, without regard to race, color, creed, religion, national origin, gender, age, marital status, disability, public assistance status, veteran status, sexual orientation, gender identity, or gender expression. For more information, please consult Board of Regents Policy: http://regents.umn.edu/sites/default/files/policies/Equity_Diversity_EO_AA.html.

Disability Accommodations:

The University is committed to providing quality education to all students regardless of ability. Determining appropriate disability accommodations is a collaborative process. You as a student must register with Disability Services and provide documentation of your disability. The course instructor must provide information regarding a course's content, methods, and essential components. The combination of this information will be used by Disability Services to determine appropriate accommodations for a particular student in a particular course. For more information, please reference Disability Services: http://ds.umn.edu/student-services.html.

Mental Health and Stress Management:

As a student you may experience a range of issues that can cause barriers to learning, such as strained relationships, increased anxiety, alcohol/drug problems, feeling down, difficulty concentrating and/or lack of motivation. These mental health concerns or stressful events may lead to diminished academic performance and may reduce your ability to participate in daily activities. University of Minnesota services are available to assist you. You can learn more about the broad range of confidential mental health services available on campus via the Student Mental Health Website: http://www.mentalhealth.umn.edu.

Academic Freedom and Responsibility:

Academic freedom is a cornerstone of the University. Within the scope and content of the course as defined by the instructor, it includes the freedom to discuss relevant matters in the classroom. Along with this freedom comes responsibility. Students are encouraged to develop the capacity for critical judgment and to engage in a sustained and independent search for truth. Students are free to take reasoned exception to the views offered in any course of study and to reserve judgment about matters of opinion, but they are responsible for learning the content of any course of study for which they are enrolled.