Brain Cell Culture and Imaging: A Laboratory Course
Neuroscience 625
Fall 2013

Cellular imaging provides powerful and versatile tools to improve our understanding of the molecular basis of neuronal development and function. Rapid advances in both microscope technology and molecular tools has greatly expanded and deepened what research scientists can now view within living and fixed cells. Providing students an opportunity to experience, both conceptually and practically, the power of modern cellular imaging techniques is fundamental to the education of students in the biological sciences. This laboratory course for senior undergraduate and graduate students will provide hands-on laboratory training in neuronal cell culture, live and fixed neuron labeling techniques, several forms of microscopy to visualize both living and fixed neurons in culture, as well as image analysis methods.

Credits: 4
Time: **Tuesday/Thursday 1-5 pm** and other hours as required by experiments
Location: **Main Lab – 354 Bardeen**, **Lectures – 341 Bardeen**
Instructors: Erik Dent (office – 262-4672, cell – 698-3314)
Tim Gomez (office – 263-4554, cell – 215- 6287)
Tim's Lab: 265-3590 (Grad students - Miguel, Pat, Bob, Undergrads- Kelly, Kevin, Kate)

If we didn't make it clear when you signed up for the class, let us reiterate. This is an independent study class that requires quite a bit of time outside of the usual Tues/Thurs time block (especially in Oct. – Dec.). We are dependent on having viable frog embryos to do the experiments and the timing cannot always be determined well ahead of time. Many times will not be on Tues/Thurs, but we don't expect more than 8hrs lab time/week on average.

Evaluation
Students will be evaluated in several ways:
- Laboratory experimental performance (30% of grade)
- Proposal, Journal Club, in class discussion (20% of grade)
- Final Group Presentation: creation and presentation of scientific presentation in Powerpoint outlining project and results (20% of grade)
- A short (2 pg.) "Future Studies" paper written independently and turned in at end of course (10% of grade)
- Study questions (10% of grade)
- Quizzes (10% of grade)

Reading List
- Lab manual and hand-outs
- Assigned reviews and primary articles
- Primary articles related to your group topic

WEEK 1

September 3 – Introduction to Cell Culture - 341 Bardeen

Handouts (on Learn@UW)

- Syllabus and Lab Manual
- 4 review papers (Neurotrophins/ Cell Signaling, Alzheimer's/Cytoskeleton, Endocytosis/F-BAR proteins, Retinotectal/Calcium signaling)
- Read/familiarize yourselves with all the reviews to prepare for the lectures on Sept. 5th.

Lecture

Erik

Introduction/Expectations

- Introductions of students and instructors
- General course overview/goals
- Expectations of students and teams
- Timing of experiments and working as teams
- Mammalian culture options
- Review of the syllabus and lab manual

Tim

30-min overview on

- Overview of Xenopus development/staging
- type of nerve cell cultures
- general principles of cell cultures
- basic skills to characterize cell cultures (cell morphology, immunohistochemical staining, immunoblotting)
- Specifics on *Xenopus* culture
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Lab **– 354 Bardeen**

- Lab tour
- Watch Xenopus dissection video (?)

September 5 – Intro to research topics – 341 Bardeen

Lecture (3 hr)

- Lectures covering 4 project topics/review papers
- Discuss the formation of teams and projects (4 groups of 3 students – **Preferably teams will be assembled today**)

Handouts/posted

- 2-4 primary papers on team 1 research topic (2 papers for discussion)
- All students read both discussion papers for following class
- Explain journal club format

WEEK 2

September 10 – Research topic #1 and basic lab exercises for *Xenopus* neuronal cultures

Lecture/Journal Club (2 hr) - **341 Bardeen**

- Discussion of two primary papers (Team 1 – Neurotrophin Signaling)
- Team 1 will be responsible for presenting two papers
- Study questions on these papers handed out (due following class)

Lab (2 hr)

Practical exposure to *Xenopus* culture

- Culture media and substrata, semi-sterile technique
- Examples of live neurons in culture
- Demonstrate spinal cord explant cultures from stage 22 embryos
- Demonstrate retinal explant cultures from stage 28 embryos
- Student's practice dejelly/devitelline embyros
- Student's practice dissections

Handouts

- 2-4 primary papers on team 2 research topic (2 of them for discussion)
- All students read the discussion papers for following class

September 12 – Research topic #2 and basic lab exercises for *Xenopus* neuronal cultures

Lecture/Journal Club (2 hr) - **341 Bardeen**

- Discussion of two primary papers (Team 2 - Endocytosis/F-BAR proteins)
- Team 2 will be responsible for presenting two papers
- Study questions on these papers handed out (due following class)
- Hand in study question set 1

Lab (2 hr)

More specifics on *Xenopus* culture

- Embryo micro-injection of proteins, small molecules, Dextrans, DNA, mRNA.
- Over-view of available protein biosensors and mutant protein constructs
- *In vitro* fertilization of eggs
- cysteine de-jellying of 2-4 cell stage embryos
- Demonstrate targeted blastomere injection
- Students practice injecting fluorescent dextrans

Handouts

- 2-4 primary papers on team 3 research topic (2 of them for discussion)
- All students read the discussion papers for following class

WEEK 3

September 17 – Research topic #3 and basic lab exercises for *Xenopus* neuronal cultures

Lecture/Journal Club (2 hr) **- 341 Bardeen**

- Discussion of two primary papers (Team 3 – Retinotectal/Calcium signaling)
- Team 3 will be responsible for presenting two papers
- Study questions on these papers handed out (due following class)
- Hand in study question set 2

Lab (2 hr)

Handling live cell cultures

- Cell culture mounting for live imaging
- Immunocytochemistry

Handouts

- 2-4 primary papers on team 4 research topic (2 of them for discussion)
- All students read the discussion papers for following class

- For next week students will be given background reading on microscopy
- (visit Nikon http://www.microscopyu.com/ and Olympus websites http://www.olympusmicro.com/primer/index.html)

September 19 – Research topic #4 and basic lab exercises for *Xenopus* neuronal cultures

Lecture/Journal Club (2 hr) *****In Lab – 354 Bardeen*****

- Discussion of two primary papers (Team 4 – Alzheimer's/cytoskeleton)
- Team 4 will be responsible for presenting two papers
- Study questions on these papers handed out (due following class)
- Hand in study question set 3

Lab - nerve cell cultures (*Xenopus* spinal cord and retina)

- Students practice de-jellying, injecting and dissecting embryos and plating neurons

WEEK 4

September 24 – Microscopy lab/Discuss Potential Projects (Teams 1&2) -

Lecture – Basic microscopy skills and imaging software **– 341 Bardeen**

- Collection of images, stacks
- Image manipulations for presentation
- Image measurements
- Student's image live cells on microscopes (Phase, DIC, fluorescence). Microscope comparison

Discussion of project ideas (Teams 1&2)
- 30 minutes with instructors for each group

Lab – Dissections/imaging (Teams 3&4)

September 26 – Discuss Potential Projects (Teams 3&4)

Quiz
**Quiz on cell culturing and microscopy (30 minutes) – short answer

Discussion (Teams 3&4)
- 30 minutes with instructors for each group

Lab – Dissections/imaging

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WEEK 5

October 1 – Practice lab techniques/discuss projects within groups/medline search– ERIK TRAVELING

Hand in study question set 4

Lab - nerve cell cultures (*Xenopus* spinal cord and retina)

- Students practice de-jellying, injecting and dissecting embryos and plating neurons
- Teams discuss possible project directions
- Additional medline searches for ideas

Each group will meet with instructors the following week to discuss possible experiments

October 3 – Dissection/Live cell microscopy – ERIK TRAVELING
Lab – Students will work independently on their research projects

October 8 [Week 6] – Presentation of Project Outline (Teams 1-4)

Presentations **- 341 Bardeen**

- 4 teams present ideas for their independent research projects
- Format: Present hypothesis and explain what experiments will test this hypothesis (flow diagram or outline)

Lab – Students will work independently on their research projects

October 10 [Week 6] – Dissection/Live cell microscopy
Lab – Students will work independently on their research projects

October 15 [Week 7] – Dissection/Live cell microscopy
Lab – Students will work independently on their research projects

October 17 [Week 7] – Dissection/Live cell microscopy
Lab – Students will work independently on their research projects

October 22 [Week 8] – Dissection/Live cell microscopy
Lab – Students will work independently on their research projects

October 24 [Week 8] – Independent Lab Work
Lab – Students will work independently on their research projects

October 29 [Week 9] – Progress Report and Lab Work
Discussion – Students will present their progress in 15-20 min sessions to Tim and Erik

October 31 [Week 9] – Independent Lab Work
Lab – Students will work independently on their research projects

November 5 [Week 10] – Independent Lab Work
Lab – Students will work independently on their research projects

November 7 [Week 10] – Independent Lab Work
Lab – Students will work independently on their research projects

November 12 [Week 11] – Independent Lab Work (ERIK AND TIM AT SFN MEETING)
Lab – Students will work independently on their research projects

November 14 [Week 11] – Independent Lab Work
Lab – Students will work independently on their research projects

November 19 [Week 12] – Progress Report and Lab Work
Discussion – Students will present their progress in 15-20 min sessions to Tim and Erik

November 21 [Week 12] – Independent Lab Work
Lab – Students will work independently on their research projects

November 26 [Week 13] – Independent Lab Work
Lab – Students will work independently on their research projects

November 28 [Week 13] – Thanksgiving Holiday (NO CLASS)

December 3 [Week 14] – Independent Lab Work
Lab – Students will work independently on their research projects

December 5 [Week 14] – Independent Lab Work
Lab – Students will work independently on their research projects

December 10, 12 [Week 15] – Last Day of Class (PRESENTATIONS!)

(341 Bardeen)

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· Each team will present their research project a Powerpoint Format (30-45 min.)

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· Each person turns in 2-page future study paper (written independently) regarding future experiments that you would conduct if you had another semester to work on your project