Overview of Physics 173/ BGGN 266 - Prof. David Kleinfeld

Modern Physics/Biophysics Laboratory

This laboratory course consists of "table-top" experiments in biological and solid state physics, with an emphasis on spectroscopy. Students choose from a variety of experiments, which include

1. Fluorescent Microscopy
2. Genetic Transcription
3. Holography
4. Laser Light Scattering
5. Mossbauer Spectroscopy
6. Oocyte Electrophysiology
7. Optical Trapping
8. Chaos in a Model Electrical Circuit
9. Spin Echo NMR
10. Swept Field NMR
11. Visual Transduction in Flies
12. Zeeman Effect
13. Belousov-Zhabotinskii Reaction

- A brief description of some off the more biologically based aspects of the course, along with ideas for future extensions, is given below (pages 1 to 5).
- Summary reports and support material for all projects are found on the web site: http://www-physics.ucsd.edu/neurophysics/ /physics_173_273.html.
- The general philosophy of the course is given in page 5.

Electrophysiology and genetic expression. The electrical dynamics of nerve cells is one of the most basic and rewarding topics in for students to learn in an instructional laboratory. Past approaches to electrophysiology instruction have involved the use of invertebrates, with extensive efforts expended in the preparation of desheathed ganglia. We propose an complementary approach where students use oocytes as the test bed for basic electrophysiology and for the induced expression of individual channels. Oocytes are readily available, commercial equipment exists that allows oocytes to be current or voltage clamped with relative ease, and commercial kits exists that allows genetic material to be readily synthesized.

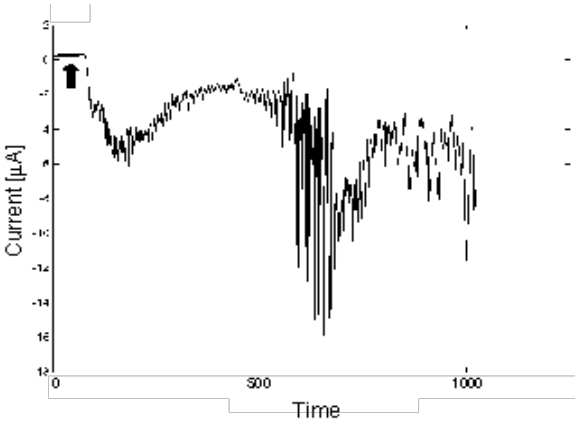

Fig. 1. Oscillations in the membrane conductance in an oocyte. We show the membrane currents of a native xenopus oocyte, observed under voltage clamp, in response to the onset of perfusion with serum (arrow). Data of undergraduate student Brenda Bloodgood.

In their native form, frog oocytes can exhibit inositol 1,4,5-trisphosphate (IP_3) receptor mediated oscillations in their Ca^{2+} currents that can be measured via a Ca^{2+} dependent Cl^- conductance. This oscillation is rapidly induced with bath application of the agonist

lysophosphatidic acid, a component of normal serum. Further, oocytes can transformed with the injection of mRNA so that they express a desired channel, such as the shaker K^+ current. Thus oocytes provide a means for students to learn electrophysiology and modern manipulation techniques. Compared with approaches using invertebrate ganglia, which have the virtue of rich electrophysiological responses across different cell types, the oocyte preparation is technically simpler and adds the possibility of genetic manipulation.

The laboratory sequence includes:

Project #1. Set-up of electrophysiology rig and basic tests of equipment with a model cell. Current and voltage clamp for measurements of passive membrane parameters. Voltage clamp in the presence of serum to determine and characterize oscillatory membrane currents (see Fig. 1). Manipulation of the membrane currents with external ion exchange and buffering of $[Ca^{2+}]_{intracellular}$ via intracellular injection of EGTA and BABTA.

Project #2. Preparation of mRNA from cDNA, to be supplied by established research laboratories, using commercially available viral kits. Intracellular injection and expression of mRNA. Voltage clamp of voltage dependent channels, e.g., shaker (A-type) K^+-current (see Fig. 2) or the rapidly inactivating Na^+ current.

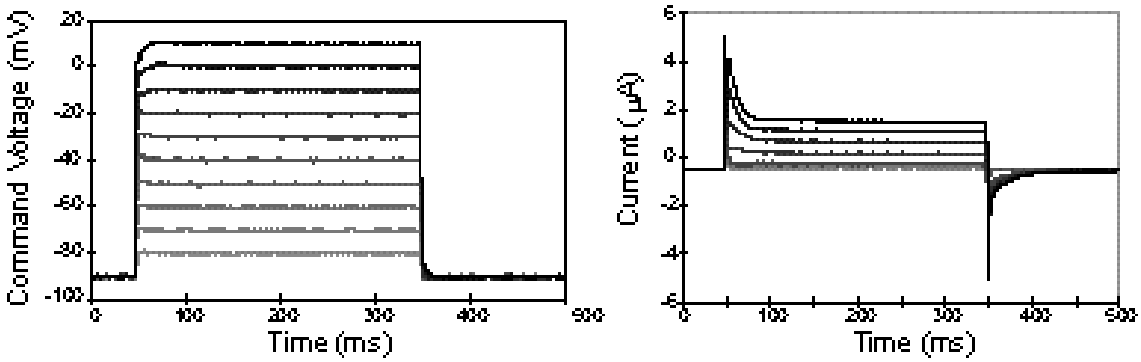

Fig. 2. Voltage-clamp of A-type K^+ current in an oocyte. Voltage clamp records from a xenopus oocyte subsequent to the injection and expression of the mRNA for the shaker A-type K^+ current. Note the onset of the rapidly inactivating outward current that occurs commensurate with the increase in amplitude of the command voltage steps. Data of undergraduate students Jennifer Coates and Gary Tedeschi.

Over time, the electrophysiology sequence will be extended to single channel currents and their statistics. This will cement the connection between noise and thermodynamic equilibrium and ion kinetics.

Biophotonics. This module explores the use of light and optics for imaging cellular function and for spectroscopy and manipulation of bioactive agents. Since much of the instrumentation involved is quite flexible, e.g., optical benches can be reconfigured, we incorporated a substantial degree of flexibility in this module. Some of the experiments depend on a readily available source of cultured neurons, albeit small numbers of plates; these are now a common feature of many research laboratories.

Project #1. Imaging of $[Ca^{2+}]_{intracellular}$ during Ca^{2+}/IP_3 oscillations in oocytes. This project builds on the electrophysiology module. We consider a confocal measurement using a pinhole and a modified conventional microscopes and optical sectioning using a vibrating

grating technique. This project also introduces the modeling of reaction-diffusion systems, as is appropriate for Ca^{2+} waves.

Project #2. Imaging the dynamics of subcellular organization. The availability of neuronal cultures with protein markers, e.g., EGFP-labeled actin, will enable study the dynamics of cellular outgrowth using time-lapse fluorescent microscopy.

Project #3. Optical tweezers for understanding the fluctuation-dissipation theorem, central to any system in equilibrium. This can be addressed by measuring the force of a trap on polystyrene balls in terms of the relaxation of the ball upon a perturbation of its position, versus a measurement of the fluctuations in the position of the ball found from an ensemble of snapshots of the position within the trap (see Fig. 3).

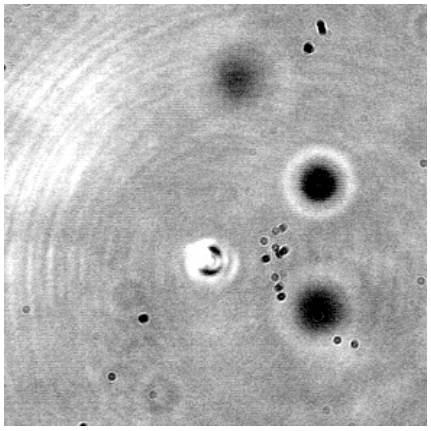

Fig. 3. Optical trapping. A one micron diameter bead that is trapped at the focus of 1.3 NA objective (100 mW, 832 nm wavelength). The field of view is ~25 μm. Out-of-focus particles are also visible. Data of undergraduate student Peter Rickgauer.

Project #4. Optical tweezers for the manipulation of subcellular organelles. One means that we tested involved the manipulation of neuronal processes in cultured hippocampal cells that engulfed polystyrene balls.

Over time, and with the continued drop in price of lasers and optical components, it will be possible to set up a scanning system for optical sectioning of cells. This represents a natural evolution of the optical tweezers, whose realization includes a slow scan system.

Spectroscopic analysis. This module spans the use of nonionizing radiation, both light and radio waves, for the analysis of macromolecules and small (<0.1 μm) assemblies. This module serves to illustrate basic optical concepts, magnetic resonance concepts, and the interaction of radiation and matter.

Project #1. Correlation spectroscopy, using coherent light scattering, to determine the diffusion constant and thus size of macromolecular structures. A model system can utilize 100 to 500 Å diameter beads (see Fig. 4). A second system is to measure the size distribution of a suspensions of organelles, such as synaptosomes.

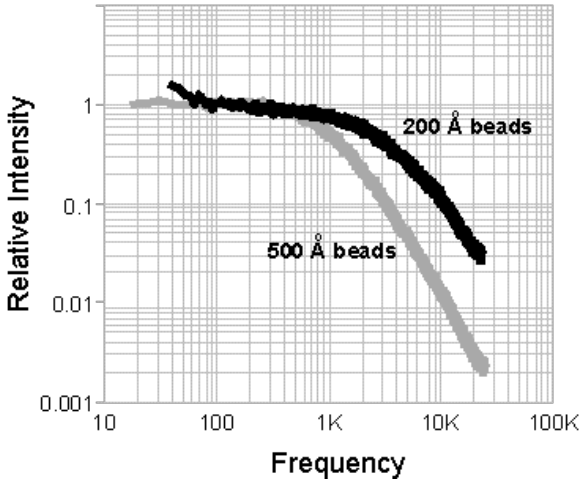

Fig. 4. Coherent light scattering from diffusive particles. The intensity of He-Ne laser light that was coherently scattered from a solution with latex beads in solution is plotted as a function of spectral frequency of the fluctuations in the intensity. Results for two different samples are shown. The break frequency for the Lorentzian spectrum is expected to be inversely proportional to the bead diameter, as shown. The break for 500 Å beads is 650 Hz, as compared to the calculated value of 940 Hz. Data of undergraduate student David Cupp.

Project #2. Nuclear magnetic resonance on solid and liquid samples for the chemical identification of molecules. This utilizes both conventional proton NMR and pulsed (spin echo) NMR to identify molecules and their interaction with the solvent. Small magnets are available for this purpose.

An extension of these projects is to pursue low resolution imaging. The two possibilities are time-of-flight optical tomography and NMR imaging, at least with phantoms or systems involving static versus flowing electrolytes.

Computational neuroscience. This module incorporates a series of experiments that are related to algorithmic aspects of neuroscience. In particular, visually controlled flight control in the fly provides a superb means for students to explore computational issues that, as for the case of oocytes, involves a relatively simple and robust preparation. This module also serves to introduce fundamental concepts from linear systems analysis, statistics, and control theory.

Project #1. Encoding of visual flight information by a wide-field sensory neuron, H1, in the anchored blowfly. This experiment utilizes recording from wide-field neuron H1 in response to a LabView™ controlled rotating world of local design (see Fig. 5). The experiment lends itself to the study of linear response, adaptation and the related concepts of receptive fields and optimal response.

Project #2. Motor feedback control of visually guided flight in the tethered native fruit fly. This experiment will utilize an optical shadowing technique to measure the wing amplitude and yaw in responds to a changing visual pattern, controlled as above. When This torque-like signal can be fed back to the display to have students closed-loop feedback and control.

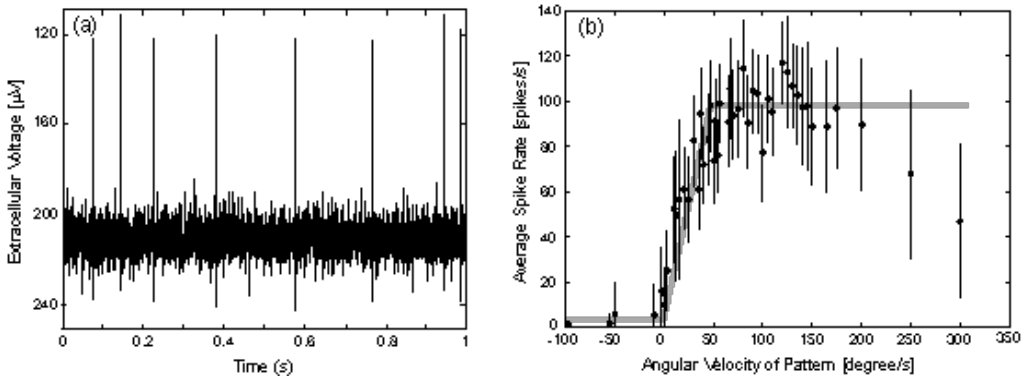

Fig. 5. Spike response from a motion sensitive neuron in fly during simulated flight. Extracellular recording from neuron H1 from an immobilized blowfly in response to a rotating visual stimulus of high contrast vertical stripes along a 270° electrooptic "drum". **(a)** Typical extracellular record of visually induced spiking close to zero angular velocity. **(b)** The spike rate as a function of angular velocity. Each point is an average over 10 s of data. Note the saturating response of the cell. The gray curve is a guide to the eye that passes through the error bars (1□) of all but one point. Data from graduate teaching assistant Evren Tumer.

Overview of Class Philosophy

Laboratory instrumentation. Throughout the laboratory course, we emphasize the need to think critically about what measurements must be performed to answer a given scientific question. The students are asked to perform a feasibility analysis, which includes a sketch of the necessary instrumentation and an estimate of the signal-to-noise ratio for a particular measurement. In as much as possible, we allow students the freedom to reconfigure their experiment in light of their analysis.

Hardware tools. Virtually all of the projects involve a mixture of specialized equipment along with standard components and building blocks, such as microscopes, optical benches, etc. We will try to emphasize the use of building blocks, such as optical bench components or high-level analog electronic elements, as much as possible.

Computational tools. We will emphasize standard control and analysis packages, such as LabView™ for instrumental control and data acquisition and MatLab™ for data acquisition, analysis, and modeling.

A working knowledge of standard tools will provide undergraduate students a high level of skill and self-confidence in the research environment These building blocks provide an economical and readily available means to achieve flexibility within a research setting. They also increase the marketability of students for careers in academic or industrial research.

Presentation and communication skills
The clear and didactic presentation of experimental results and the analysis of these results are of paramount importance to the growth and advancement of a scientist. We nurture these organizational and presentation skills as part of the Advanced Instructional Laboratory.

Group presentations. Each class period is proceeded by a 30 to 45 minute “round up” session of presentations by three different students, plus questions. An emphasis is placed on the didactic reasoning used to solve experimental problems. Brevity and clarity are stressed. Questions on all levels are encouraged largely as a means for the speaker to learn how to explain issues in a clear way in a public forum

Written report. Students are expected to write up the data from at least one module in a form comparable to that in a scientific journal. We emphasize the concept of review. Thus students will be expected to modify and resubmit their reports, as required.