An Interdisciplinary Graduate Laboratory for Biological Physics

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Interdisciplineary graduate education at the boundaries between physics, mathematics, and biology presents unique challenges for students, faculty, universities, and funding agencies. These challenges range from the pedagogical difficulties of bridging enormously diverse backgrounds to the need for separate departments to recognize such teaching efforts. Here we describe a key component of a five-year effort in this area at the University of Arizona, funded by the National Science Foundation – a unique graduate laboratory in biological physics for students from backgrounds as diverse as applied mathematics, biomedical engineering, physics, and genetics. We describe in detail how challenges at the various levels have been addressed, including the conceptual bases for the laboratory experiments and the choice of theoretical background material, the course structure, laboratory infrastructure, and details of experimental setups ranging from neuroscience to optical trapping and bacterial pattern formation. These experiences may serve as a guide for universities and departments considering the creation of such interdisciplinary programs.

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I. INTRODUCTION

It was once said that interdisciplinary work is not possible without the disciplines,[?] and this is certainly true in the exploding field of biological physics. As physics departments move ever faster into this field, hiring faculty, recruiting graduate students, seeking large-scale external funding, and learning to work with life science departments, one recurring issue is how to educate a new generation of graduate students to be well-versed in all the fields that touch on biological physics. As a community we have welcomed the recent appearance of exciting new textbooks in biological physics.[? ? ?] Yet, the experimental side of the curriculum has received far less attention. As a step toward improving this situation, we present here an in-depth description[? ?] of one such effort: a graduate laboratory in biological physics,[?] created at the University of Arizona as part of our IGERT program (Integrative Graduate Education and Research Training)[?] , which has successfully trained a very diverse group of students and may serve as a model for efforts elsewhere. A brief summary of the many issues surrounding the teaching of biological physics, including this laboratory, has also appeared recently.[?]

There has been an ongoing discussion in the physics community about the proper role physics and physicists have in the field called "Biological Physics." Parsegian[?] suggested that physicists should "harness" their hubris and work on problems defined as important by the biology community, and he emphasized the role the physics community has had in formulating molecular theories and developing many experimental methods which have been of great utility. In reply, Austin[?] has argued that physicists have a role beyond that of assistants, and that there are deep questions which physicists can help to frame and to answer. Implicit in this view is the notion that the training of physicists is unique among scientists in its emphasis on adherence to first principles and mathematical precision, belief in the importance of a reductionist thinking, emphasis on the study of simplified models, and breadth of phenomena covered.

Biological physics is often seen as an outgrowth of condensed matter physics, for many in the community began their careers studying problems in statistical physics related to membranes, polymers, and related materials whose biological relevance is clear. Yet, there is another intellectual thread of great importance – applied mathematics – which is less well-known to the physics community, but has played a central in our efforts. Some classic problems in biological physics – the spread of epidemics,[?] the propagation of nerve impulse,[?] and the self-propulsion of microorganisms,[?] for example, involve the study of nonlinear partial differential equations in the reaction-diffusion context or in fluid mechanics, areas which have been the traditional domain of applied mathematics. At the same time, several applied mathematics programs (MIT, the Courant Institute, Arizona, Penn State) have historically had in-house experimental efforts that complement their theoretical research, with a strong biomathematics emphasis. The effort described here grew directly out of an Applied Mathematics laboratory of this type, supported by the Flinn Foundation.[?]

What does all of this discussion imply about the structure of a graduate laboratory course? With the rapid pace of development in experimental methods such as optical trapping, microfluidics, and fluorescence imaging, not to mention those in molecular biology, it would be perfectly reasonable to develop a "methods" course that surveys all these areas. In this regard, it is worthwhile noting the very important report of the National Academy of Sciences, "Bio2010"[?] , which points out the pressing need for life science students to be exposed to

more quantitative approaches. Hopfield[?] has proposed a set of appropriate subjects within the quantitative realm to teach life science students. So, there clearly *is* a need for both theoretical and experimental survey courses.

Yet, in our opinion, such courses would not truly address the deeper intellectual needs of the diverse graduate student body we target. Those needs center around a clear appreciation for the interplay of theory and experiment, indeed, for the scientific method in general. In addition, we aim to develop an *intuitive* application of mathematical and physical principles to biological systems. We therefore took an approach orthogonal to a methods course, more akin to what might be termed *Natural philosophy* in a case-study format. Phrased another way, we argue that for our audience of graduate students the intellectual journey is more important than the destination.

To place in context the syllabus of the course, we first survey the scope of biological physics, organizing the topics by length scales, with a few examples in each:

- *Molecular:* structure and dynamics of proteins; structure, elastic properties, transcription and regulation of nucleic acids; selectivity and gating of ion channels.
- *Subcellular:* polymers such as actin and microtubules; eleasticity and dynamics of membranes, along with phenomena such as vesicle expulsion, pseudopod extension; cell division; signal transduction; cilia and flagella.
- *Cellular:* response of cells to external stimuli – chemotaxis; motility; cell division; metabolism; regulatory networks, bioinformatics.
- *Multicellular:* cell-cell signaling; collective behavior and morphogenesis of populations;
- *Organismal:* neural networks and biologically inspired computation; vision; hearing; cardiac dynamics.
- *Evolutionary:* Darwinian dynamics, in vitro evolution.

Our case-study approach samples phenomena spanning nearly this entire range of length scales, and was guided by our own particular research interests, for development of specialized experiments requires more than a passing acquaintance with the methods. Section **??** summarizes the very diverse set of students who have taken the laboratory course, and the typical strengths and weaknesses they bring to this experience. The basic laboratory infrastructure is described in Sec. **??**, complete with floorplan, outline of general-purpose equipment and the computing environment. A syllabus for the semester-long laboratory course is outlined in Sec. **??**. The specific experiments are described in Sec. **??**. As we detail below, this is a complex laboratory whose creation and use in education has itself been an experiment. It is also very costly, in equipment, space, and faculty time. For these reasons, we point out in Section **??** how the ideas advanced have led to a spinoff on a smaller scale, at the Complex Systems Summer School of the Santa Fe Institute, and could well lead to economonically revised experiments in the advanced undergraduate laboratory sequence. We conclude in Sec. **??** with a frank discussion of the lessons learned, the mistakes made, and prospects for the future. Several detailed appendices provide information about the four primary experiments, including the essentials of the biological protocols, experimental setups and equipment lists.

II. THE STUDENTS

In four years, the course has drawn students from a daunting array of departments; mathematics, applied mathematics, physics, molecular and cellular biology, ecology and evolutionary biology, biomedical engineering, genetics, and even astronomy. All students are required to take the one-semester laboratory course as part of the conditions of support by the IGERT program. Some have taken the course in their first year or two of graduate studies, others significantly later, when deeply involved in thesis work. The strengths and weaknesses the students bring to this program are equally varied. Those from the life sciences are of course very well-versed in biological nomenclature, molecular structure, cellular functions, experimental protocols, and the notion of appropriate controls. Their mathematical preparation is weak compared to the typical physics major, they have likely never been exposed to concepts from fluid mechanics and elasticity theory, and Newtonian force concepts are little-used in the biological curriculum. They also have only passing familiarity with statistical mechanics concepts such entropy, fluctuations, and probability distributions. The profile of physics students is almost precisely the opposite, and the apparent complexity of biological systems seems daunting to them. Strikingly, mathematics students are often the most flexible in what they find interesting, and the most inquisitive of all. Remarkably, though, most have surprisingly little experience with the give and take of theory and experiment. It is not possible for one course to even out the backgrounds above, nor is that a desirable goal. In the next section we outline the course structure developed to take advantage of these disparate strengths.

III. LABORATORY INFRASTRUCTURE

The Applied Mathematics Laboratory[?] served as a model for the structure of the IGERT lab. The plan of the single room that houses the laboratory is shown in Figure **??**. It comprises approximately 650 square feet of usable space, divided between the dominant experimental area and a smaller space for presentations and discussions. There are four experimental stations, each consisting of a dedicated 30" × 48" vibration isolation table with a 4" breaboard (Newport VH workstation), with overhead connections to compressed air. Each station has a nearby computer for data acquisition and processing, report presentation, program development, and web access. Since all of the experiments have a strong visual component and at least one video camera mounted

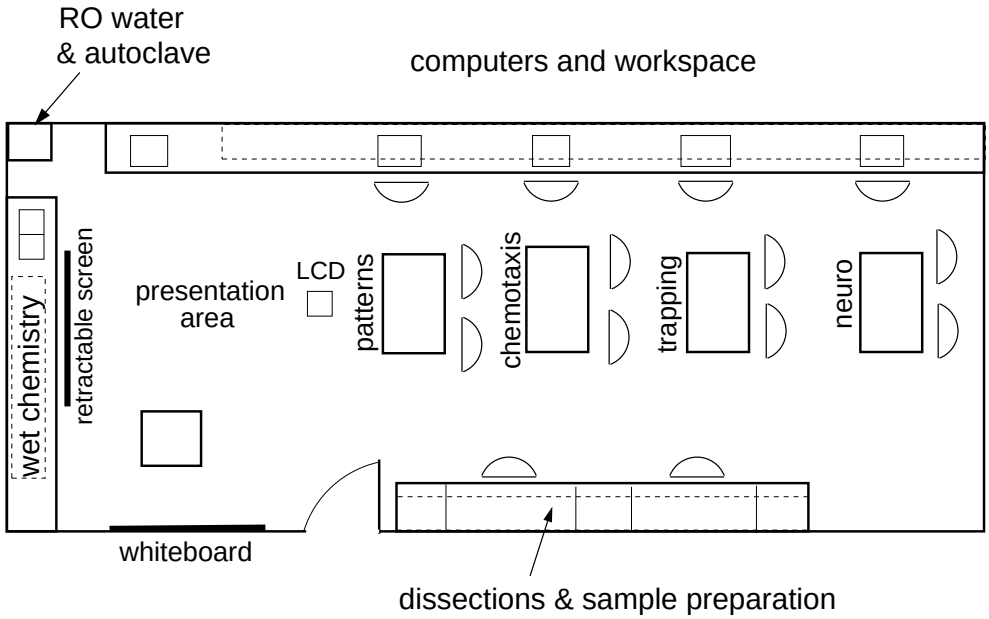

FIG. 1: Layout of the laboratory. Dashed lines indicate upper storage cabinets.

on a microscope or elsewhere, we configured the room so that if the situation were appropriate all of the students could see the images from any particular experiment on a large multi-input monitor (Sony PVM20M4U with Kramer VM84 switcher) in the presentation area. This output of workstation at the front of the lab can also used sent to this monitor, a feature that allows various computer simulations to be demonstrated to the group. A ceiling-mounted LCD projector (Mitsubishi LVP-X70UX) is used for PC-based presentations on a retractable screen at the front of the room, and a nearby whiteboard for class lectures.

The laboratory is essentially self-contained with regard to wet chemistry. A dedicated water purification system (Culligan Maxima, with RO & UV purication and a 75 liter intermediate storage tank) is available along with an autoclave (VWR Accu Sterilizer AS12) for glassware and media. The nearby wet chemistry bench has the typical assortment of equipment, including a pH meter (Corning model 430), microbalance (Mettler-Toledo AV54), hot plate/stirrer, pipetters and a generous collection of glassware, pipette tips, microscope slides and coverslips, etc. An ice machine is available in a nearby research lab.

The dissection and sample preparation bench includes a pipette puller (Sutter Instruments Model P-87) for the electrodes needed to study action potentials, and the dissecting microscope (Nikon SMZ645) and dissecting instruments necessary to isolate and prepare the neurons for study. This bench also houses a supply of electronic components, petri dishes, and reference material.

The experiments generally use Labview (National Instruments) for control of laboratory equipment where necessary. In the course of refining the experiments students have, at times, used Labview to write programs to simulate random walks, to implement particle-tracking algorithms, and to learn about algorithms for image processing. Detailed particle-tracking studies were performed by processing videotaped movie sequences with Nanotrack[?] .

There is no doubt that this laboratory is expensive; the total cost of renovations and equipment was approximately $350,000, divided roughly equally between the two. Our university took a broad approach to this commitment, viewed the laboratory as a long-term investment in the educational infrastructure of the university, particularly of the college of science. The major equipment costs included 3 inverted microscopes, several with DIC and fluorescence capabilities, 3 dissecting microscopes, computers, optical tables, the pipette puller, and electronics, especially for the neurophysiology setup.

FIG. 2: The lab as viewed from the "x" in Figure **??**.

Clearly, the costs would be significantly less without the room renovations. Moreover, it is quite possible to create significantly less expensive versions of these experiments. Section **??** describes such alternatives.

IV. COURSE STRUCTURE

The course consists of 9 hours each week in the laboratory, into two four-hour sessions ($1-5$ p.m.) early in the week for experimental work, and one additional hour on Friday afternoons which serves as a group meeting. At various times during the semester, these Friday sessions serve as a time for the entire class to discuss and solve experimental or conceptual difficulties experienced by the various groups, for student presentations of experimental progress, for discussion of the thesis research of class members, and for presentations by visiting scientists.

We originally thought that students would spend a few weeks each on roughly four experiments during the course of the semester, but the experiments proved sufficiently complex that we concluded instead that it was best to devote the entire semester to a single experiment. A sufficient breadth of experiences was obtained by including several weeks of introductory material for all students at the beginning of the semester and emphasizing cooperative learning throughout the course. With two professors and one research associate (LAN or RR) full-time in the laboratory, and at most 9 students in a given semester, the faculty student ratio is remarkably high (and hard to sustain!).

The introductory material plays a very important role, and follows closely the emerging consensus of subjects outlined briefly elsewhere.[?] Below we present the subjects with the key literature references.

• *Biological fluid dynamics and related subjects.* Most of the phenomena studied in the experiments occur on short enough length scales and long enough time scales that overdamped fluid dynamics is the rule. Indeed, using the typical length scale $L \sim 10^{-6}$ μm of, say, a bacterium, its swimming velocity $U \sim 10^{-5}$ μm/s, and the kinematic viscosity of water $\nu = 10^{-6}$ cm^2/s, the Reynolds number is $Re = UL/\nu \sim 10^{-5}$. This regime is sufficiently foreign to most students that an extended discussion of it is worthwhile. Purcell's classic article[?] is a good starting point, along with later work on chemoreception.[? ?] Many of these ideas really go back to G.I. Taylor[?] . With our mathematical bias, we thought it important to take the students through a reasonably complete derivation of the Navier-Stokes equations and the rescalings which reveal the Reynolds number. Our presentation continues with a discussion of the physics of viscosity, the classic Stokes problems in fluid dynamics,[?] and the Stokes drag on a sphere. We also cover the essentials of diffusion and the competition between advection and diffusion embodied in the Peclet number.

• *Brownian motion.* The significance of thermal fluctuations in the cellular world is generally not appreciated by students in the life sciences, while those from the physical and mathematical side understand stochasticity, but likely have never seen it with their own eyes (!). Using microscopic visualization of spheres fluctuating in water we can readily illustrate the effects of varying particle size on the scale of random motion. A derivation of the Stokes-Einstein relation is presented along with the equipartition theorem. The importance of thermal energy as the typical energy at the molecular level is highlighted by relating the piconewton forces of molecular motors and nanometer molecular displacements through the relation $k_BT \simeq 4$ pN-nm. A brief discussion of polymer statistical mechanics is presented on the basis of random walks.[?]

• *Electrophysiology.* The anatomical and physical considerations that underlie the propagation of action potentials along neurons are the foundation of electrophysiology. The fundamental background material includes a discussion of axon architecture, the capacitive properties of membranes and the conductivity of the axoplasm.[?] The role of ion channels in maintining nonequilibrium concentrations of ions on the two sides of a membrane is discussed along with a derivation of the Nernst equation to explain the characteristic millivolt potential across membranes. This sets the stage for the basic cable model for electrical conduction. The separation of time scales found in the response of Na and K channels is then used to motivate the dynamics of action potentials and eventually the mathematics of traveling-wave solutions. We have found that a historical discussion of the work of Cole and Cole on clamped potentials is very useful.[?] A separate Friday presentation of the anatomy of the human brain (with a real one on hand) is used to place all of this microscopic information in its proper context.

• *Nonlinear dynamics and pattern formation.* Reaction-diffusion systems are ubiquitous in biology and have played an important role in applied mathematics and physics. Although the precise mechanism analyzed in the class work of Turing[?] may not be literally utilized in real biological systems, the competition between diffusion and nonlinearities is central. Once the students have understand the essentials of diffusion as described above, it is straightforward to introduce the simplified FitzHugh-Nagumo model[?] as a convenient context to discuss phase plan analysis, instabilities, and the separation of time scales. The discussion continues with the existence of moving fronts, the formation of spiral waves, and the canonical examples of the Belousov-Zhabotinsky system[?] and pattern formation during the life cycle of the slime mold *Dictyostelium discoideum*.[?]

• *Microscopy & micromanipulation.* It is surprising how few students from physics and mathematics are familiar with the workings of a microscope. Those from the life sciences have hands-on experience, but typically very little theoretical background on imaging methods and resolution issues. Given this, we have included in the introductory material a survey of the basic principles of geometric optics, a discussion of diffraction effects and limits, and a survey of the major methods in mi-

TABLE I: Laboratory course syllabus. Describing lab and group-meeting sessions.

Week	Topic
1 (lab)	motivational experiments and background lectures (biological fluid dynamics, diffusion)
1 (group)	lecture and discussion on scientific ethics
2 (lab)	background lectures (Brownian motion, electrophysiology)
2 (group)	anatomy of the human brain
3 (lab)	background lectures(nonlinear dynamics, microscopy, general techniques)
3 (group)	laser safety class (for all students)
4-15 (lab)	lab work on single experiment
4-15 (group)	weekly group meetings to discuss experimental progress
16	oral presentations

croscopy (bright-field, phase contrast and differential interference contrast).[?] Finally, the optical principles underlying laser tweezers are outlined.[?]

• *General techniques.* Several iterations of the course made it clear that students were not generally well-prepared in the analyis of numerical data such as time series and images. We therefore spent one lecture to explore the basic principles of Fourier analysis, Fourier filtering, image acquisition from ccd cameras, FFT analysis of images, and algorithms for particle tracking.[? ?]

V. THE EXPERIMENTS

A. Motivating studies

For several years in the beginning of our efforts grew dissatisfied with the level of comprehension of the material on biological fluid dynamics. We found that an experimental motivating study in the very first lab session served not only to clarify the concepts, but also to bring the students together as a group working to merge theory and experiment. The goal of the experiment is simple; using an assortment of spheres[?] of various materials (delrin, aluminum, brass, stainless steel) of various sizes (1/8" – 1/2"), large graduated cylinders, glycerol, and stopwatches, attempt to verify the Stokes drag law for spheres settling under gravity. Our intent was to have the students measure the time for a sphere to settle a fixed distance under gravity and thence to determine the terminal velocity. Balancing the drag force $F_d = 6\pi\eta R v$ against the buoyant force $F_b = 4\pi R^3 \Delta\rho g/3$ yields

$$v = \frac{2\Delta\rho g R^2}{9\eta} \ . \qquad (1)$$

A verification of the Stokes force law would thus be obtained by a linear relation between v and R^2.

In a typical class of 8 students, each group of two was assigned to perform experiments on the entire range of sizes of spheres of one particular material. The groups had to wrestle with the best way to release the spheres, time their descent, and assign uncertainties. When we first assigned this project we had not done the experiment ourselves prior to the lab session and rather naively thought that a straight line would indeed be obtained. To our surprise and that of the students this was not so. Figure **??**a shows instead the strong curvature seen in all nearly all of the data sets. By far the most interesting aspect of this study was when all the groups convened after acquiring their individual data. Confronted with this curvature and the need to compare their different data sets, a wonderful discussion ensued among the students as to the proper scaling relations that might be applied to collapse the data, and the adoption of a consistent system of units. The results are shown in Fig. **??**b. What causes the curvature in the data? Why is this? The students eventually realized that there are at least two important reasons for the curvature. First, the Reynolds number is not actually negligible, and particularly the larger and denser spheres are still decelerating as they pass through the observation zone. Second, the radius of the graduated cylinders, while large, is not sufficiently large that wall effects[?] are negligible.

The experience of confronting data that does not conform to the simple textbook theory and then seeking explanations for those deviations is one of the most important in the course. In subsequent years we continued with this motivational study, although this time the faculty (but not the students!) knew what to expect.

FIG. 3: Data on Stokes drag.

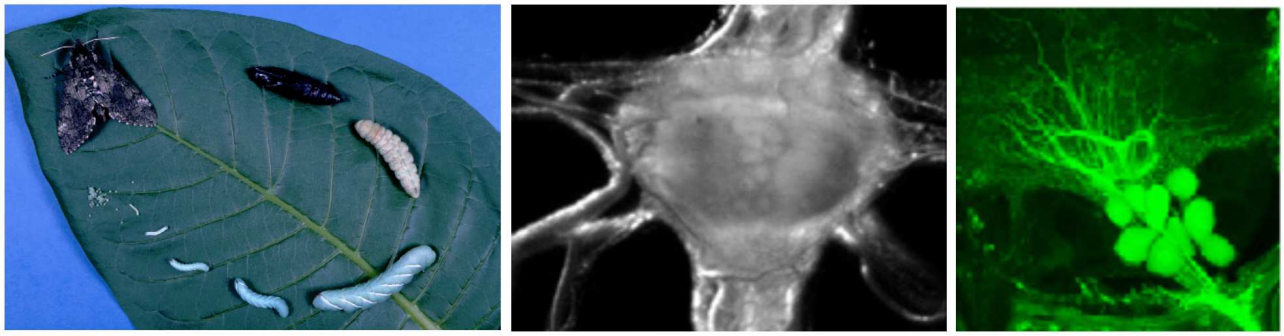

FIG. 4: Manduca sexta.

B. Neurophysiology and Morphology of Manduca sexta neurons.

Mathematical analysis frequently assists the understanding of how microscopic properties combine to produce the macroscopic properties; this is especially well illustrated in analysis of electrical responses in excitable cells. Intra- and extra-cellular recordings of neuronal responses can reveal the effects of ion channels that contribute to the generation and spread of complex neuronal signals. In this module, students will record synaptic- and action-potentials arising spontaneously, or by electrical or pharmacological stimulation. Students will direct single or trains of electrical stimuli to the cell body (intracellularly, resulting action potentials right) or nerve processes (extracellularly, anatomy below) and observe responses. Selected cells will be pharmacologically manipulated by the application of Tetraethylammonium (TEA). These procedures will provide the opportunity to study changes in the passive (membrane), transitional (spike-production) and active (firing rate) properies of recorded cells.

The morphology of recorded cells will be visualized by Lucifer Yellow staining and confocal microscopy imaging.

Images obtained by backfilling peripheral nerves provide an understanding of the overall arragement of clusters of cell bodies, their dendritic arborization and processes (right). Images obtained by intracellularly filling individual cell bodies show their processes in greater detail (left) and allows for more precise measurment of process diameters and segment lengths.[?]

Using both elecrophysiological and morphological data they acquire, students will compare their results to existing mathematical models describing the mechanisms underlying potential generation and spread.[?]

The procedures outlined in the following section assume that the students will use a basic electrophysiological recording rig, as described briefly below. The process of developing recording skills and the resulting data provide a very rich environment for discussion of interdisciplinary research and for data analysis. It was our experience that the rate at which the students acquired the skills they needed to perform the experiments was influenced heavily by the students' native hand-eye coordination skills. Typically, students from a broad range of backgrounds were able to successfully record and analyze physiological and morphological data in meaningful ways after about 70 hours of lab time, which included time for developing hand skills for dissection and microsurgery under a microscope, learning to manipulate the electronic components of the rig, interface with the recording software, and establish extra- and intracellular recordings. While costly and intense, all students came away with a very good understanding of the difficulties and pitfalls of collecting quality data that is suitable for detailed mathematical scrutiny.

Figure xx shows a schematic representation of equipment suitable for electrical and chemical stimulation and recording intra- and extracellular-potentials from large diameter (20-50 um) electrically excitable neurons in the fifth larval instar of Manduca sexta. For details see the Axon Guide for Electrophysiological and Biophysical Laboratory Techniques [1]. For details on morphological procedures see [2]. The insects we used were obtained from a specialized facility at our institution that maintains a colony under carefully controlled temperature (26-27 deg. C), humidity (50-60(17h light/7h dark) conditions. These animals were chosen for their year-round availability and for the simplicity of anesthesia (ice and chilled saline) and dissection.

Preparation. After chilling on ice for 20 minutes, both proximal and distal ends of the anesthetized animals were cut off and the dorsal surface slit lengthwise. The skin was pinned out to a silicone elastomer-coated (Sylgard, [3]) petri dish and, under a dissecting microscope, the fat, gut and connective tissues removed to expose the A4-A6 ganglia. These ganglia and several mm's of the attached nerves were removed under chilled saline and pinned out in a suitable recording chamber. The chamber side walls were formed by cutting a 2-mm thick layer of Sylgard into a box shape and laying it on a Sylgard-coated 50 x 75 mm glass slide. The surface of one of the ganglia, usually A5 in our case, was treated with 3fine dissecting forceps. This procedure requires fine hand-skills, and some students find these slow to develop, particularly if they have not manipulated objects under a microscope.

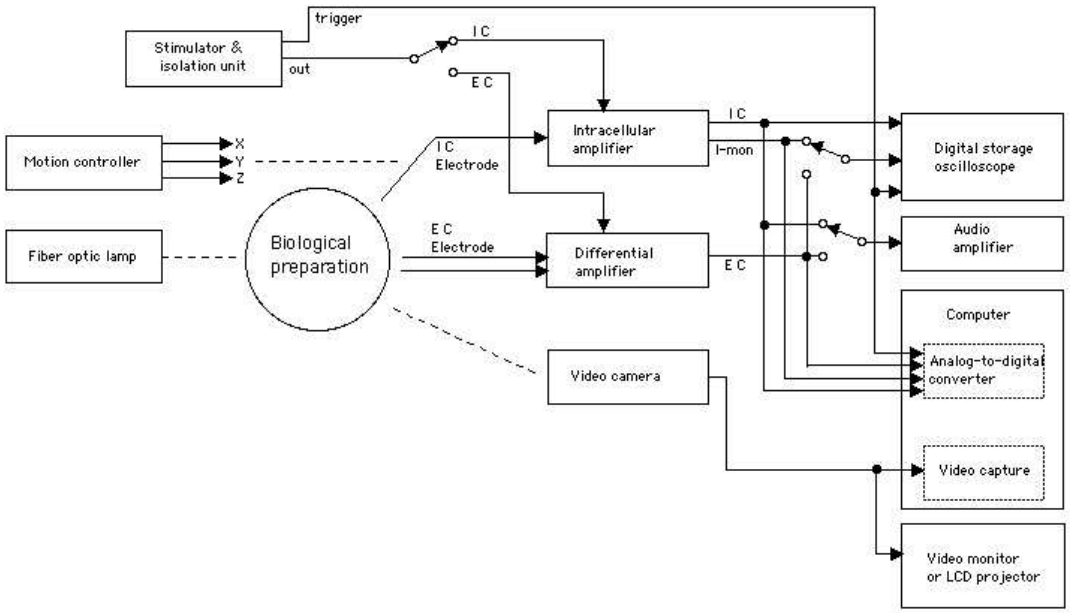

FIG. 5: Layout of neurophysiology experiment.

Recording setup. The chamber was placed on a raised dissecting microscope stage to allow for darkfield illumination [5] of the tissue, which is a critical component of the light pathway in that it can be optimally adjusted to permit excellent simultaneous visualization of individual cell bodies and the electrode tip. Our dissecting microscope has 1-6.3x zoom capability [6] and is fitted with 20x eyepieces and an ordinary B&W video camera [7] from which images can be displayed on the lab's large monitor for demonstration or discussion. Captured frames from the video allow the students to document the location of studied cells, adding that information to the labs "catalog" of recorded neurons. The chamber is perfused with a gravity-fed saline solution (about 1-2 ml/min) either with or without chemical modulators present (see TEA below). Standard stopcock valves permit solution changes during the experiment. A standard mechanical stage allows positioning of the ganglia and another micromanipulator allows optimal positioning of the darkfield objective.

Electrical procedures. A single, silver-silver chloride coiled-wire inserted into the recording chamber forms a ground connection. Extracellular suction electrodes are made from glass pipettes. A silver-silver chloride wire inside is connected to a switch so that it can be connected to an electrical stimulator or differential amplifier to excite or record from peripheral nerves. A precision three-axis microdrive [8] is used to position a glass microelectrode. Electrodes are fabricated [9] with a tip size (approximately 0.5-1 um) that yields a resistance of about 40-120 M-ohms when filled with 3mM KCl (standard experiments) or with 1m K-Acetate (for pharmacological studies that use, for example, the channel blocker tetraethylammonium (TEA).

Neurons are approached by guiding the microdrive, using the microscope/video image to position the tip of the microelectrode near (50 um) the surface of the cell. At this stage the students begin to rely on a conventional oscilloscope (sometimes with the assistance of an audio monitor) to monitor the resting membrane potential (40-70 mV). The cells are actually impaled using the microdrive set to produce brief, high-velocity "jog" motions when the electrode is very near to the cell body. A successful penetration will result in an abrupt change in potential to a steady resting level. Once a stable recording is obtained, students can determine the effect of passing hyperpolarizing and depolarizing current [10, 11] on spike frequency and on passive and transitional properties of the cell, and also can explore the effects of various pharmacological manipulations that alter the behavior of different ion channels.

Recording from an extracellular electrode can add additional value, especially if the students already have determined the nerve through which a cell axon travels by using retrograde labeling techniques (see Morphology below). Spikes propagated along the cell axons from neuronal cell bodies in the ganglion can be monitored with a suction electrode wired to a high-gain (5-20,000x) differential amplifier [12]. If the electrode is wired through a simple switch, it can be connected to an electrical stimulator to elicit back-propagation toward the cell body.

Data acquisition and storage. An A/D converter card in a PC and appropriate software can be used to capture and store data on the PC's hard drive during the course of the experiment (we use in-house developed software [13]). Once saved, additional software can be used to perform detailed analysis. For an in-depth description of

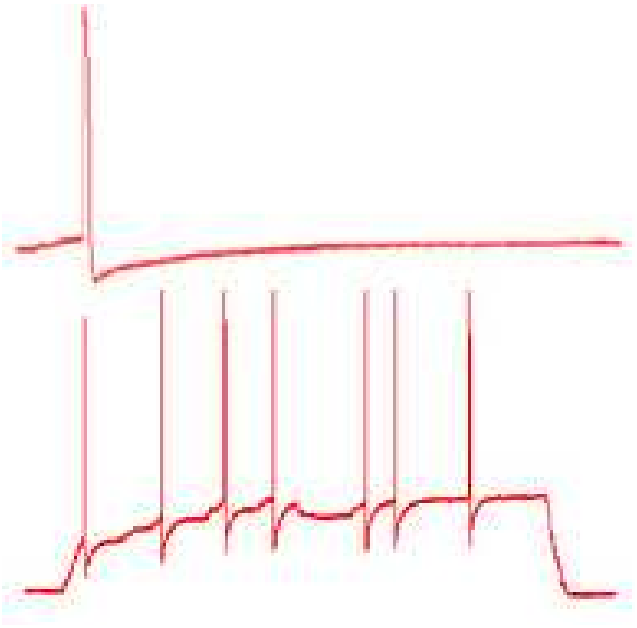

FIG. 6: Action potentials.

the software see [14, 15, 16].

Data analysis. Several interesting basic input-output properties can be studied from the data acquired in these basic experiments. Examples include: passive properties such as cell input resistance, time constant, and resting membrane potential; transitional properties properties including rheobase, threshold to relatively long pulses (0.5-10s at 0.1-5nA), and voltage threshold; and active properties including fine details of the spike-production mechanism such as firing rate (Hz/nA), the effects of various features of the action potential afterhyperpolarization (AHP) on the firing rate and the effects of application of a channel blocker such as tetraethylammonium (TEA).

Morphology. A very useful addition to this module is to have the students use morphological examination of the recorded neurons to ask how differences in the spread of voltage along cell processes can be influenced by morphology. To obtain these dye fills, the ganglia are pinned into the dish as above, a Vaseline well is carefully constructed adjacent to the nerve cord, and the nerve of choice draped across the sidewall of the well and its end dipped into the fluid-filled well. The tissue outside the well is bathed in saline. The nerve is freshly cut, exposed first to distilled water for a few minutes, and then to 10Lucifer Yellow in distilled water (LY, [2]). A pair of conductive silver wires, attached to a power source (1.5VDC battery with a 50K-ohm resistor in series to limit current to 30 uA) are inserted, one into the LY dye bath (made negative) and one into the saline bath (positive). The current is passed for about 20 minutes, before rinsing and preparing the tissue for fixation [2]. The resulting backfill, analyzed by standard confocal microscopy [17], reveals the size and location of cell bodies and the shape of the dendritic arbors within the ganglia.

Additional morphological data can be obtained from injecting a dye solution, such as Lucifer Yellow (4-5directly into the cell body. After filling the electrode tip with Lucifer, the electrode is backfilled with LiCl (for LY-lithium salt) or K-acetate (for LY-potassium salt). Dye-filled electrodes have a somewhat higher resistance (80-160 M-ohms) and consequently are slightly more noisy, but still can be used to obtain much of the same physiological data as discussed above. The cell is filled using a 50duty cycle, depolarizing current for 5-30 min depending on the amount of current passed, then processed as described for the nerve-fill staining procedure above.

C. Optical tweezers, Kramers rate theory

The instrument described is a so-called "dual-beam optical trap" in which light from the laser light source is split based on its polarization state to generate two non-interfering optical tweezers in the specimen plane of the microscope. Such a design represents only a modest modification compared to the simplest setup with just a single optical tweezers but enables a larger set of potential experiments that can be done in the lab. During the first year of the course, two weeks were dedicated to the construction of the optical tweezers by the students under full-time guidance of the instructor. This proved very successful, even for students lacking any experience with optics, optical alignment, etc., and proved useful in recognizing any problems and their possible cause during late experimentation. We chose not to repeat this exercise in the interest of time, but it is an option that may be considered depending on the specific educational goals or the students involved in the experiment. After construction, the optical tweezers are calibrated and used to investigate Kramers rate processes or to record the motion of kinesin motor molecules.

A review[?] about the principles of optical tweezers, construction and calibration is advisable. Useful information about the optical components used in construction can be found in the tutorial sections of the manufacturers catalogues (e.g. Newport, Melles Griot). In our instrument, a polarized, near-infrared laser (Crystalaser, IRCL-700-1064, wavelength 1064 nm) is coupled into an inverted microscope (Nikon, Diaphot 200) equipped with an oil-immersion objective (NA=1.4) that focuses the laser light into the specimen plane to form the optical tweezers. The laser power used for the optical tweezers is determined by the polarization state, which is controlled by a half-wave plate ($\lambda/2$), in combination with a polarizing beam splitter (PBS). Unwanted light is directed onto a beam dump constructed of a stack of razor blades, where it is absorbed and dissipated. A beam expander then increases the beam diameter ($1/e^2$) to fill, or slightly overfill the entrance pupil of the objective to guarantee effective three-dimensional trapping of silica and polystyrene beads, respectively. Note that it may be

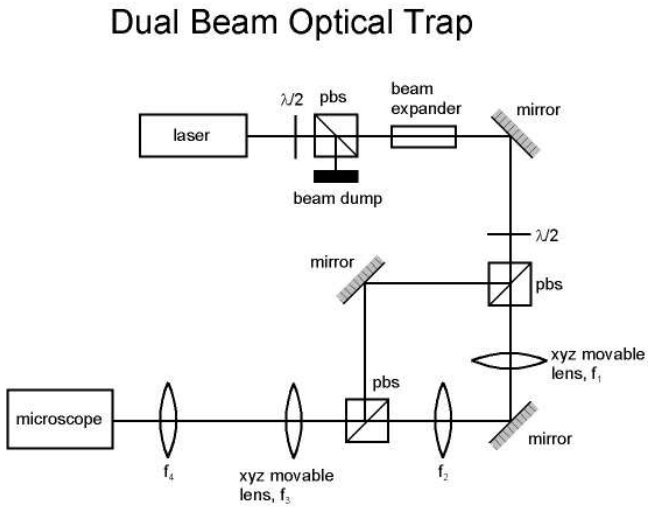

FIG. 7: Layout of optical trapping setup.

advisable to position the beam expander in front of the power controller to lower the laser intensity (W/m^2) at the half wave plate when working with higher powered lasers. After being redirected by a dielectric mirror (high reflectance 1064 nm) the laser beam is split by a second PBS based on its polarization state, which is controlled by a half wave plate. Horizontally (p) polarized light will be transmitted whereas vertically (s) polarized light is deflected ninety degrees. Transmitted light is focused by a movable lens f_1 and collimated by a lens f_2 arranged to form a 1 : 1 telescope (focal distances f_1=f_2, distance between the two lenses equals f_1+f_2=2f_1, typical focal length is $\sim$ 100 mm), enabling positioning of the horizontally polarized trap in the specimen plane. After recombination of the two optical paths by another PBS the laser beams traverse a second 1 : 1 telescope (f_3=f_4) in which movable lens f_3 ensures simultaneously movement of both traps. The movable lenses f_1 and f_3 are mounted on three-axis translation stages for proper lateral positioning and focusing of the optical traps. To ensure that the laser beam is aimed through the entrance pupil of the objective independent of the position of f_1 and f_3, these lenses need to be imaged onto the entrance pupil, which is achieved by the lenses f_2 and f_4. As a result, in this set up, the distance between f_2 and f_3 should equal 2f_2, while between f_4 and the objective entrance pupil it should equal 2f_4. The choice of the focal length of f_4 is determined by the physical limitations of mounting this lens as close to the microscope as possible. These telecentric lens systems causes the laser beam to rotate around the center of entrance pupil rather than to move over the pupil, and guarantee that the strength of the trap does not depend upon the location of the trap in the specimen. The laser beam enters the microscope through the epi-fluorescence illumination port, which enables the use of the fluorescence filter cube holder to mount a dichroic mirror transparent for visible light but reflecting the near infra-red laser light up into the objective. It goes without saying that correct alignment of the optical components will be essential for successful operation of the optical tweezers. Resources on the construction of optical traps, including tips for alignment are available.[?]

The microscope is equipped with a video camera (Dage-MTI CCD100) and controller (Dage-MTI RC100) connected to a VCR and Linux PC with a framegrabber for image acquisition and analysis using ISEE image processing software (http://www.iseeimaging.com/). The microscope is operated in differential interference contrast mode mainly for imaging of individual microtubules required for kinesin experiments. Conveniently, this also produces very high contrast images of microscopic beads used for other experiments, such as calibration of the tweezers. After construction of the trap, and/or after familiarizing the students with high numerical, oil-immersion microscopy, they proceed to calibrate the video imaging system by recording and analyzing images of a 10?m-spaced specimen grid. This familiarizes them with the equipment and provides them with the pixel dimensions expressed in nanometers (typically in our set up, 1 pixel: 50nm x 50nm) needed for all of their subsequent experiments. Since it has been well established that the restoring force of the trap increases linearly with the displacement from the center of the trap (up to 200nm at 1064 nm wavelength) the trap strength can be readily characterized by a spring constant or stiffness. One of the first experiments the students, equipped with an optical power meter, do is to determine the trap stiffness as a function of the optical power. Questions arise about where to measure the optical power and how to determine the stiffness of a "spring" this small? At this point to answer the second question we (re)introduce the Boltzmann distribution and derive the equipartition theorem. The probability density of finding a bead trapped at a position x from the center in the potential U(x) of the tweezers is governed by the Boltzmann distribution:

$$p(x) = C \exp(-U(x)/k_B T) \ ,$$

where C is a proportionality constant, k_B is the Boltzmann constant and T is the temperature. The potential $U(x)$ is then readily calculated once the position of a bead in the trap is sampled many times and plotted in a histogram, which is proportional to p(x). Subsequent plotting of U(x) will indicate that for small x (up to 200 nm) the potential is parabolic $U(x) \propto x^2$ and that a spring constant k can be assigned to the optical trap. Calculation of the mean squared position using p(x) and will then lead to the equipartition theorem relating the trap stiffness and thermal energy. With 4.1 pNnm at room temperature the trap stiffness and potential can be readily calculated once the mean squared displacement has been determine using the Nanotrack video particle tracking module in the ISEE software. It is straightforward to write down the Langevin equation for a microscopic bead held in an optical trap in water and to show that it is identical to the ordinary differential equation describing a simple low-pass RC filter with

noise presented at the input. In particular student with a background in electrical engineering may now suggest other means of determining trap stiffness by for example calculation of the power spectrum, cross correlation, or step response of a bead in held in a trap. However, in most cases these other methods cannot be used in our video-based data acquisition set up as whereas the sampling rate is only 60 Hz (NTSC) or 50 Hz (PAL), too slow with respect to the typical time response of a bead in a trap of 1-100 ms.

A note on data-acquisition. The camera used in our instrument has automatic gain control (AGC) which can be turned off if required. To the surprise of most students, the stiffness determined at constant laser power seems to depend on the brightness of the microscope illumination. Surely, the incoherent light coming from the tungsten or mercury-arc light source, cast onto the sample by the microscope condenser does not contribute to the trap stiffness. So, what is going on? When operating in AGC mode the camera uses a shutter to control light levels on the CCD chip. As a result at low light levels this shutter tends to remain open for 1/60 second, effectively averaging the motion of the bead over this time frame, thus reducing and overestimating the trap stiffness. Only at high light levels, when the shutter time is 1/10,000 is the estimate of the trap stiffness accurate. We have used this effect to make students aware of the danger of systematic errors that may occur when they do not understand in detail how a measurement is done. Turning off the AGC, or better, buying a camera with a manually controllable shutter, resolves the issue. Students are now prepared to study noise-activated escape over an energy barrier as a proposed model for reaction rate kinetics by Kramers (Kramers, H., Physica 7, 284 (1940), for a review: Hnggi, P., Talkner, P. Borkovec, M. Rev. Mod. Phys. 62, 251 (1990); Simon, A. Libchaber, A., Phys Rev Lett. 68, 3375 (1992)). Two optical traps are positioned closely together so that the trap potentials overlap partially. The trap stiffnesses are adjusted by the power controller and the half-wave plate splitting the laser beam, and are set to make the potentials rather shallow (weak traps). The distance between the traps then is adjusted until back and forth hopping of a bead between the two traps is observed. Images of the beads are recorded and positions tracked using the Nanotrack module in the ISEE imaging software. As with stiffness calibration, the potential, a double-well shape, is calculated from the histogram of bead positions. The mean time before escape over the barrier has been calculated by Kramers,

$$\tau_K = \frac{2\pi\alpha}{\sqrt{k_b k_u}} \exp\left(-\frac{\Delta E}{k_B T}\right) ,$$

where kb and ku are the stiffnesses associated with the curvature at the bottom of the well and the top of the barrier, respectively. ?E is the height of the barrier, and ? is the viscous drag coefficient. The Kramers time calculated from the measured potential profile can be directly compared with the mean residence time of a bead in each of the trap as determined from a histogram of residence times dtermined from the bead position vs. time graph. As there is only a single rate-limiting transition (speaking in reaction kinetics language) in jumping over the barrier once, this histogram should have a single-exponential decay. However, if we were to plot the time to hop from one trap into the other and back, the barrier has to be surmounted twice, and the histogram of these times should be a double exponential, i.e. the convolution of two exponentials, associated with it a reduced

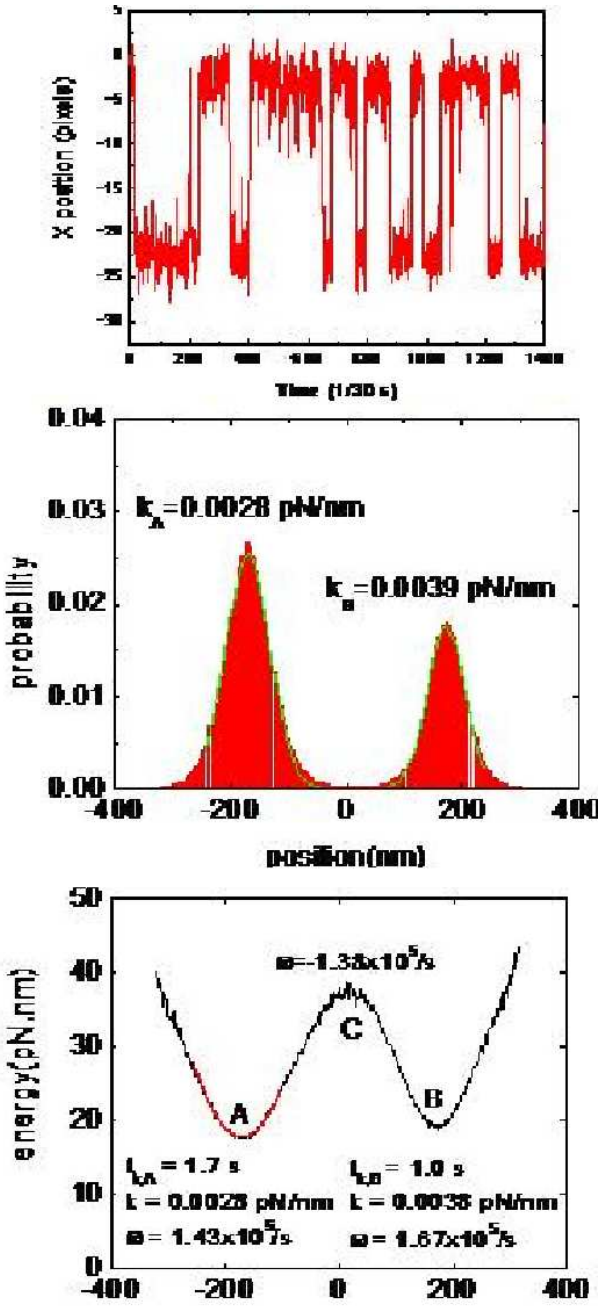

FIG. 8: Kramers problem.

variance when compared with the single exponential case (if the potentials are chosen such that the Kramers time for both transitions are approximately equal). This provides an excellent means to introduce the student to the idea of variance or randomness analysis to determine the number of rate-limiting transitions in a reaction (Svoboda, K., Mitra, P.P. Block, S.M., PNAS USA 91, 11782 (1994); Schnitzer, M.J Block, S.M. Cold Spring Harb Symp Quant Biol. 60, 793 (1995))

D. Kinesin motility

Kinesin is a molecular motor that converts the energy derived from adenosine triphosphate (ATP) hydrolysis into mechanical work and moves along polymeric tubes of the protein tubulin. These tubes, called microtubules, are found in virtually all eucaryotic cells and provide lines of transport and communication between different levels in the cell. Thus kinesin functions as an intracellular transport vehicle, binding to organelles or supply vesicles and carrying them down microtubules to all parts of the cell. While the details of how chemical energy stored in ATP is released as mechanical energy in kinesin stepping remain unknown, it is clear that kinesin hydrolyses one ATP molecule for each 8 nm step forward. In this module, students investigate kinesin velocity, measured in steps taken per second, as a function of ATP concentration. Although the kinesin motor is far too small to be observed with standard microscopy techniques, the activity of kinesin motors is still possible to observe thanks to the fact that the kinesin tail binds well to materials like glass. Individual, or collections of motors, can be made to carry a small glass bead (diameter 1 um) which can be easily observed using differential interference contrast (DIC) microscopy (right). Knowledge of the techniques of laser tweezing, microtubule polymerization and the preparation of a kinesin motility assay are required before measurements commence. Data are captured onto VHS tape via CCD camera from DIC microscopy observations. The frame-by-frame position of transported beads is analyzed with computer software.[?]

Motility of kinesin molecules may be recorded as the gliding of microtubules over a microscope cover glass coated with kinesin molecules (gliding assay) or as the motion of kinesin-coated beads along surface-immobilized microtubules. In the IGERT lab we have chosen the more complicated bead assay in which students deposit kinesin-coated beads onto microtubules imaged with the video-enhanced differential interference contrast microscope, and track bead motion using the Nanotrack module in ISEE. In many cases a gliding assay may provide similar data, while not requiring optical tweezers. Best of all, a kit containing all required proteins and buffers complete with detailed instructions for a gliding assay is commercially available from Cytoskelton, Inc. (www.cytoskeleton.com , Video Enhanced DIC Microscopy Based Motility Kit, Cat. BK028). This kit,

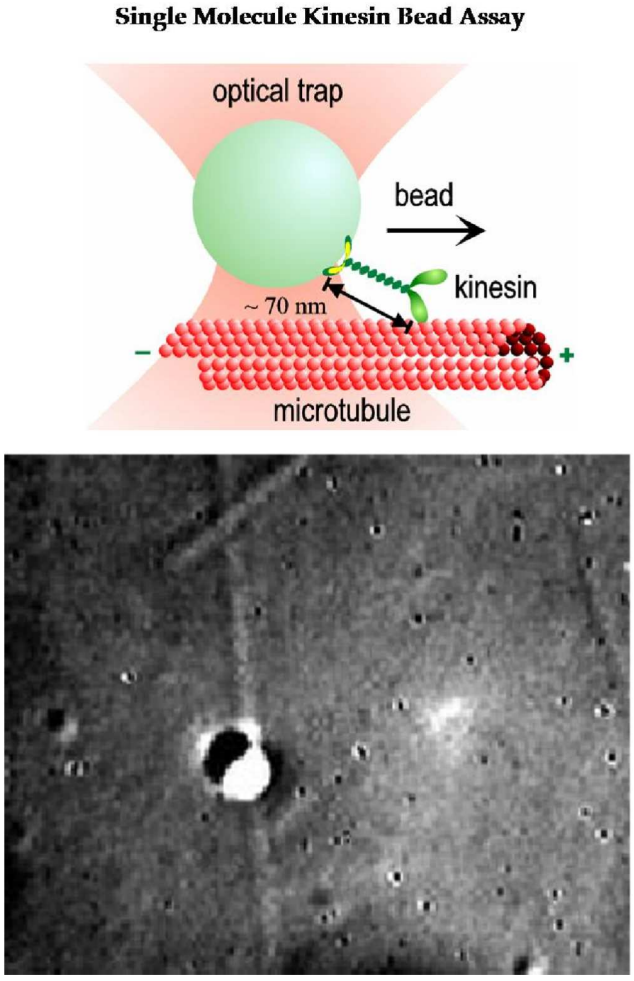

FIG. 9: Optical trapping and kinesin. (a) Motility assay. (b) Image of bead and microtubule.

with modifications should also be useful in setting up a bead assay if so desired. A recent paper describes the motion of carboxylated polystyrene beads coated with recombinant kinesins obtained from Cytoskleton, and contains useful information for assembling a bead assay (Romagnoli, S., Cai, G. Cresti, M. In vitro assays demonstrate that pollen tube organelles use kinesin-related motor proteins to move along microtubules. Plant Cell. 15, 251-69 (2003)).

Performing the gliding or bead assay requires good command of the Video Enhanced DIC microscope to image individual microtubules. Often students confuse detectability and resolution, and incorrectly claim that individual microtubules with a diameter of 25nm, much smaller than the resolution limit of the optical microscope, cannot be visualized using an optical microscope. This misconception is readily corrected once individual microtubules are made visible on a video monitor.

[?]

E. Chemotaxis and Effective Brownian Motion

One of the areas of biology in which applied mathematics has made a number of important contributions concerns the swimming of microorganisms. In the world of Stokes flow relevant to motile bacteria, where motion is primarily geometrical rather than dynamical in origin, self-propulsion by rotating helical flagella or waving elastic flagella is a central subject. The experiments in this module are designed to introduce students to the elementary properties of viscous flows as they relate primarily to self-locomotion. Following the classic work of Taylor, and later Purcell, the essential features of slender-body hydrodynamics will be revealed through the gravity-driven settling dynamics of rods, helices, and the like.

? The chemotactic response of individual cells to external chemical gradients is a phenomenon which involves not only complex biochemical pathways but also the physics of diffusion and propulsion. As classic experimental work on E. coli (right) has revealed, regulation of the flagellar motors leads to swimming motion that involves "runs" and "tumbles." As a consequence, cells execute a random (or biased random) walk. In this experimental module students will track the motion of individual bacterial cells to determine the statistics of runs and tumbles, and verify that the long-time behavior of such a system is diffusive. This will allow the biology students to see the connection between microscopic behavior and macroscopic descriptions (such as a diffusion equation), and gives the students from physics and mathematics backgrounds the opportunity to understand the biology behind a diffusive contribution to a partial differential equation governing bacterial pattern formation.?

F. Pattern formation

Reaction-diffusion phenomena are ubiquitous in biology, from the propagation of electrical impulses in the heart to population dynamics on the scale of kilometers.

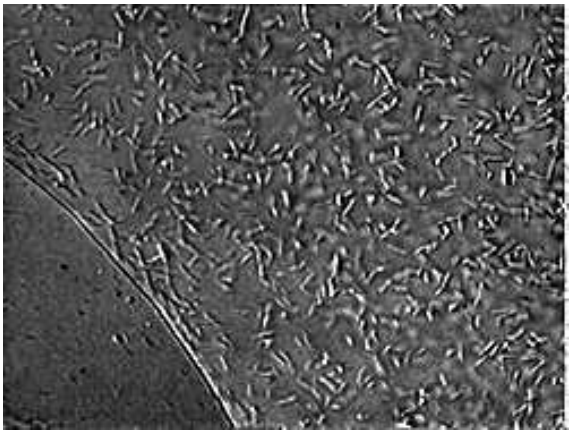

FIG. 10: Bacteria with fluorescent microspheres.

In this module, two examples of systems that constitute excitable media and display spiral waves will be studied: populations of the amoebae Dictyostelium discoideum and the Beluosov Zhabotinski (BZ) reaction.

A much-studied organism within developmental biology and also more recently within the physics community interested in pattern formation, populations of Dictyostelium form spectacular rotating spiral waves of cyclic AMP as a prelude toward aggregation into multicellular structures in response to starvation. These waves can be visualized by dark-field techniques (left) through their effect on cell shape and hence light scattering, and varying simple experimental control parameters results in an important competition between spirals and targets controlled by pacemaker cells.

In the BZ reaction (right), a purely non-living system, analogous patterns form through classical activator-inhibitor dynamics. In both cases, the primary experimental quantity of interest is the dispersion relation for the spirals, in comparison with theoretical results.

1. *Bioconvection*

Bioconvection: The influence of individual cellular swimming on large-scale pattern formation will be examined in the context of bioconvection, in which the upward swimming of cells in a thin layer of fluid leads to an unstable density stratification and overturning flows. This phenomenon will serve as well to introduce students to the principles of hydrodynamic stability theory. Large numbers of the bacterium Bacillus subtilus (left) in a layer of water organize into distinctive, quasi-periodic patterns, not unlike those seen in thermal convection or other pattern-forming systems. This self-organization is a result of the interplay of the tendency of the bacteria to swim up towards oxygen in the upper layer and of

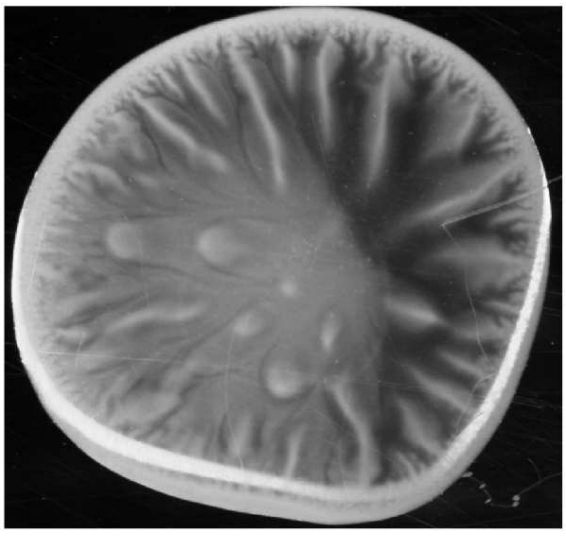

FIG. 11: Bioconvection in a sessile drop.

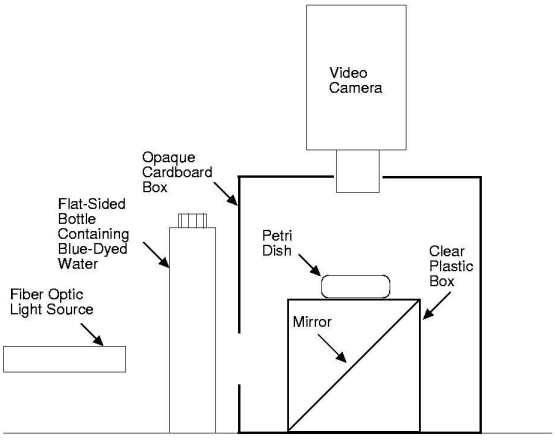

FIG. 12: Dark-field setup.

gravity acting on the bacteria, which are less bouoyant than water. This results in convection rolls, with plumes of bacteria rising to the oxygen-rich layer of water, and then descending in plumes alongside the rising ones.[?]

2. *The Belousov-Zhabotinsky reaction*

background[?]

$$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-} \quad (2)$$
$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (3)$$
$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (4)$$
$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (5)$$
$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (6)$$

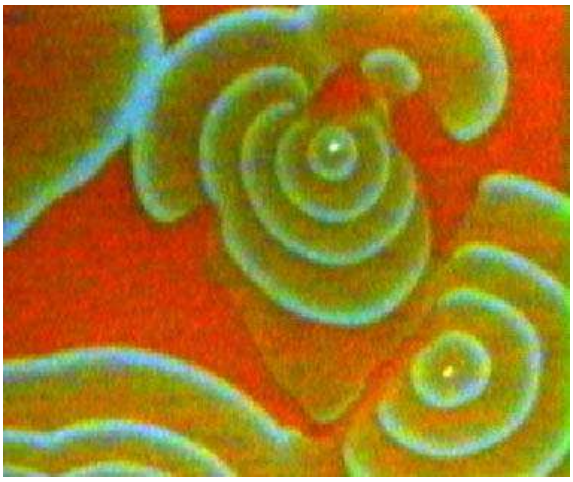

FIG. 13: The BZ reaction.

VI. EXTENSIONS

Applied Mathematics laboratory, Santa Fe Institute

VII. LESSONS LEARNED AND FUTURE PROSPECTS

lessons that can be applied elsewhere in the curriculum. At the undergraduate level - need to see Brownian motion through a microscope - repeat Einstein's analysis to get Avogadro's number. Surely such experiences are just as important as measuring the speed of light or the lifetime of the muon.

FUTURE CHALLENGES: Sustainability

LAB AS A RESOURCE

Acknowledgments

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M.E. Fisher, private communication.
K.A. Dill and S. Bromberg, *Molecular Driving Forces. Statistical Thermodynamics in Chemistry and Biology* (Taylor & Francis, 2003).
P. Nelson, *Biological Physics: Energy, Information, Life* (W.H. Freeman and Co., 2004).
J. Kondev, *Physical Biology of the Cell*, (publisher, city, 2004).
NSF98-70659 (Principal investigators M. Tabor, R.E. Goldstein, N. Mendelson, L. Tolbert, and T. Secomb) - "Multidisciplinary Training at the Interface of Biology, Mathematics, and Physics."
This article is an expanded version of a talk delivered by R.E.G. at the March 2004 meeting of the American Physical Society, Montreal, Canada.
A description of this laboratory was presented at the NSF-sponsored workshop on *The Role of Theory in Biological Physics and Materials* 15-17 May, 2004, Tempe, AZ. See `biophysics.asu.edu/workshop`.
For general information on the University of Arizona IGERT program, see `w3.arizona.edu/∼bmpi/`.
R.E. Goldstein, P.C. Nelson, and T.R. Powers, "Teaching Biological Physics," *Physics Today* **57**, 000-000 (2004).
V.A. Parsegian, "Harness the Hubris: Useful things physicists could do in biology," *Physics Today* **50**, 14 (1997).
R.H. Austin, "How should physicists, biologists work together? The 'Harness the Hubris' debate continues - Reply," *Physics Today* **50**, 14 (1997).
R.A. Fisher, *Ann. Eugenics* **7**, 355-369 (1937).
A.L. Hodgkin and A.F. Huxley, "A quantitative description of membrane current and its application to conduction and excitation in nerve," *J. Physiology (London)* **117**, 500-544 (1952).
G.I. Taylor
National Research Council, *Bio2010: Transforming Undergraduate Education for Future Research Biologists* (National Academies Press, Washington DC 2003) (available at `http://books.nap.org`).
J.J. Hopfield, "Form follows function," *Physics Today* **55**, 10-11 (2002).
The Flinn Foundation: `www.flinn.org`.
For a description of the applied mathematics laboratory, see `applmath.web.arizona.edu/labs.html`.
Nanotrack is produced by ISee Imaging Systems: `www.iseeimaging.com`.
E.M. Purcell, "Life at low Reynolds-Number," *Am. J. Phys.* **45**, 3-11 (1977).
E.M. Purcell and H.C. Berg, "Physics of Chemoreception," *Biophys. J.* **20**, 193-219 (1977).
H.C. Berg, *Random Walks in Biology* (Princeton University Press, Princeton, N.J., 1983).
G.G. Stokes, "title," *Trans. Cambridge Philos. Soc.* **9**, 8-x (1851).
Doi and Edwards
Nossal and LeCar
Cole *Membranes, Ions, and Impulses*
A.M. Turing, "The chemical basis of morphogenesis," *Phil. Trans. Roy. Soc. London B* **237**, 37-72 (1952).
R. FitzHugh, *Biophys. J.* **1**, 445 (1961); J.S. Nagumo, S. Arimoto, and Y. Yoshizawa, *Proc. IRE* **50**, 2061 (1962).
general reference on BZ reaction
general reference on Dictyostelium
Microscopy intro
tweezer intro
J. Gelles *Nature* **x**, (19xx).
J.C. Crocker and D.G. Grier, "Methods of digital video microscopy for colloidal studies," *J. Coll. Int. Sci.* **179**. 298-310 (1996).
Small Parts Catalog
stokes wall effect
K. Svoboda and S. M. Block, "title," *Annu. Rev. Biophys. Biomol. Struct.* **23**, 247-285 (1994).
"Construction of optical tweezers" by S. Block of Stanford University, `http://www.stanford.edu/group/blocklab/cshl-chap.htmlchapter` or `http://www.cshlpress.com/chap`$_{c}$*ells.tpl*(*Block*, *S.M.Constructio*
ALaboratoryManualVol.II, *Sec.*7, *Eds.SpectorD.L.*, *Goldman*, *R.D.L*
K. Visscher, M.J. Schnitzer and S.M. Block, "Single kinesin molecules studied with a molecular force clamp," *Nature* **400**, 184-189 (1999).
A. Simon and A. Libchaber, "Escape and synchronization of a Brownian particle," *Phys. Rev. Lett.* **68**, 3375-3378 (1992).
X.L. Wu and A. Libchaber, "Particle diffusion in a quasi-two-dimensional bacterial bath," *Phys. Rev. Lett.* **84**, 3017-3020 (2000).
chemotaxis intro
bioconvection intro
bz intro
P.G. Drazin and W.H. Reid, *Hydrodynamic Stability* (Cambridge University press, Cambridge, 1981).
The Axon Guide for Electrophysiology and Biophysics Laboratory Techniques, P/N 2500-102m, Axon Instruments Inc., 1993.
L.A. Oland, G. Orr, G. L.P. Tolbert, "Construction of a Protoglomerular Template by Olfactory Axons Initiates the Formation of Olfactory Glomeruli in the Insect Brain," *J. Neuroscience* **10**, 2096-2112 (1990).
Sylgard 184, Dow Corning, Midland, MI, `<www.dowcorning.com>`
Sigma-Aldrich, St. Louis, MO, `<www.sigmaaldrich.com/>`
Molecular Expressions website: `<micro.magnet.fsu.edu/primer/techniques/darkfieldindex.html>`
Also see Rice University website experimental biosciences: `<www.ruf.rice.edu/ bioslabs/methods/microscopy/dfield.html>`
Nikon SMZ800,Microscopes center website: `<www.microscopescenter.com>`
Video camera, Model PC-23C, Super Circuits, Liberty Hill, TX, ¡www.supercircuits.com¿
Microdrive, Model 860-C2, Newport Corp, Irvine, CA, `<www.newport.com>`
Pipette puller, Model P-87, Sutter Instrument Co, Novato, CA, `<www.sutter.com>`
Electrometer, Model Intra 767, World Precsion Insturments, Inc., Sarasota, Florida, `<www.wpiinc.com/>`
Stimulator, Model 2100, A-M Systems, Carlsborg, WA, `<a-msystems.com/>`
Differential amplifier, Model 1700, A-M Systems, Carlsborg, WA, `<a-msystems.com/>`
LabVIEW, National Instruments, Inc, Austin, TX, `<ni.com>`
R.M. Reinking, Y. Laouris, and D.G. Stuart, "Real-time

control and acquisition of neurophysiological data: Resolution of key problems," In: *Proceedings of National Instruments User Symposium/1989*, edited by L. Anglin, Austin TX: National Instruments. pp. 119-133 (1990).

R.M. Reinking, M.A. Nordstrom, and D.G. Stuart, "Simplifying biomedical data-acquisition and instrument control with a modern computer language." In: Case Studies in Medical Instrument Design, edited by T. Nagle and W.J. Tompkins. New York: Institute of Electrical and Electronic Engineers, pp. 65-81 (1992).

R.B. Gorman, R.M. Reinking, and D.G. Stuart, "LabVIEW programming code for characterization of the biophysical properties of spinal neurons," in *The Official SEAM '95 Conference CD-ROM, edited M. Duncan. Worcester, MA: MacSciTech. (1995).*

Confocal and Two-Photon Microscopy Foundation, Applications, and Advances, edited by A. Diaspro. John Wiley & Sons, Inc. 2002.

S.P. Smith, S.R. Bhalotra, A.L. Brody, B.L. Brown, E.K. Boyda, and M. Prentiss, "Inexpensive optical tweezers for undergraduate laboratories," Am. J. Physics 67, 26-35 (1999).