# GAINING PRACTICAL EXPERIENCE WITH PHYSICS-BASED APPROACHES TO THE MICRO- AND NANOSCALE WORLD OF BIOLOGY

BY NANCY R. FORDE

In the last few years, new textbooks focussed on Biophysics have been developed, making it easier for departments to introduce Biophysics lecture courses for their undergraduate students. With a few notable exceptions (e.g., [2] and [3]), however, there are few text-based resources available that describe practical approaches to exploring the concepts, such as computational or laboratory-based activities. Some individual examples of modules can also be found in the *American Journal of Physics* [4, 5] or in other sources [6, 7].

In this *Education Corner* article, we present highlights of pedagogical activities that tie in with the theme of this issue "Nanoscale Approaches to Biological Systems". They are organized approximately by topic, and have been developed by faculty across Canada for application in their teaching to different target audiences. Stand-alone practical courses in Biophysics (such as offered at McMaster University and Simon Fraser University) are the exception, but many offer computational or experimental biophysics modules as part of other courses. The purpose of this article is to present an overview of some available activities, with the aim of providing a useful starting point and resource for others in the Canadian Physics community who are interested in implementing or adapting these modules in their teaching.

Additionally, we hope that the content and contacts within this *Education Corner* article may serve as a starting point for those interested in establishing a "community of practice" in Biological Physics Education in Canada. Contact information for each of the module developers is provided, and we encourage interested readers to connect and help grow this Biological Physics teaching community.

**SUMMARY**

**As the field of Biophysics has grown in popularity, Physics departments have been adapting traditional course offerings to involve more biological content and introducing courses focused on Physics at its intersection with Biology (e.g., ref. [1]).**

## DIFFRACTION

**Experimentally reproducing DNA's "X" diffraction pattern using springs and laser pointers** – Cécile Fradin, McMaster University. fradin@physics.mcmaster.ca

Developed for a second-year Biophysics course; takes two hours for the experiment plus analysis time.

The structure of DNA was famously solved after Rosalind Franklin obtained an X-shaped diffraction pattern from DNA fibers. She immediately recognized (as did Francis Crick when he later gained access to her data) that the X-shape meant that DNA had a helical structure. A very simple experiment, involving small springs and laser pointers, can demonstrate that helices indeed give X-shape diffraction patterns.

This lab is based on a publication by Braun *et al.* [8], in which students use a simple optical set-up to enlarge the beam of a small laser pointer, and obtain a diffraction pattern from small metallic springs. Proper collimation of the laser beam and proper positioning of the spring ensure the observation of a beautiful X-shaped diffraction pattern. Students can use their phone to take a picture of the diffraction pattern and perform a quantitative analysis of its features. They can also use a microscope equipped with a USB camera and calibration slide to take a real space image of the spring.

Beyond explaining the relationship between a helical structure in real space and an X-shaped pattern in reciprocal space, this lab allows the introduction or illustration of some simple diffraction principles, such as the fact that diffraction informs us about the repetitive features of an object, the relationship between the size of a feature of the object and the size of the corresponding peaks in the diffraction pattern, and the relationship between wavelength and resolution. An associated tutorial done in class in parallel with this experimental module introduces a

Nancy R. Forde,
<nforde@sfu.ca>

Department of Physics
Simon Fraser University,
Burnaby, BC

quantitative analysis of DNA X-shaped diffraction patterns that can build on these simple principles.

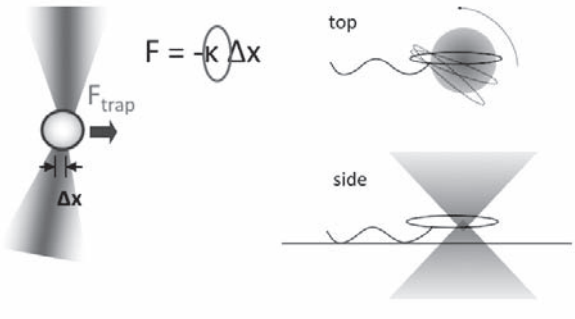


Fig. 1 Optical trapping experiments implemented in the Biological Physics lab course at SFU include using power spectral analysis to calibrate the optical trap stiffness (left) and determining the rotation rate of E. coli flagella as the bacterium rotates through a weak optical trap (right). At the right, the side view shows a snapshot of the bacterium body in the optical trap and its flagellum attached to the surface of the slide. The top view is a schematic illustration of the bacterium periodically moving through the trap as its flagellum rotates the cell body [4].

## NANO- AND MICRO-SCALE MOTION: DIFFUSION AND DIRECTED MOTION

Many modules have been developed, utilizing a wide variety of different approaches, in which students observe and/or simulate diffusive motion and learn how to quantify it. Some of the modules extend to contrast diffusion with biased motion, quantitatively characterizing the different response for each.

**Experimentally distinguishing Brownian from directed motion at the microscale** – Nancy Forde and David Boal, Simon Fraser University. nforde@sfu.ca

Developed for a fourth-year Biological Physics laboratory course and easily adapted for lower levels; takes 2 × 3 hour lab periods.

Surrounded by a fluid in thermal equilibrium, all cells move in response to random interactions with their environment according to Brownian motion. Some cells are also capable of self-propulsion, perhaps by swimming as driven by their flagella or cilia, or by pushing their way past other cells, changing shape as needed. Bacteria may swim in search of food sources, while the macrophages of our bodies may hunt down and swallow invading cells that could be a threat to our health. Such motion can be studied quantitatively using an optical microscope and a CCD camera.

In this module, students learn to operate a microscope in brightfield mode and capture images of diffusing particles and of swimming bacteria. By tracking their positions over time, students distinguish between random diffusion ($\langle r^2 \rangle \propto t$) and directed motion ($\langle r^2 \rangle \propto t^2$). Through knowledge of the Stokes-Einstein relation, students can also compare their experimental estimate of bead size with that provided by the manufacturer.

A related experimental module has been developed by Cecile Fradin at McMaster University, which involves reproducing the Perrin tracking experiment that quantitatively validated Einstein's predictions of random motion.

**Simulating fluorescence images of diffusing molecules and performing image correlation analysis** – Albert Kamanzi, Simon Sehayek and Sabrina Leslie, McGill University. sabrina.leslie@mcgill.ca

Developed for 3rd year students, but can be adapted for lower levels; takes about 7 hours of lectures, plus 3 hours of tutorials for helping students; plus 10-20 hours for completion.

Image correlation analysis is a powerful technique that is used to measure the diffusion coefficients and interaction strengths of molecules in solution. This module begins by instructing students in basic programming skills using Matlab. The students simulate random walks, and gradually convert these simulations to create artificial fluorescence images of diffusing molecules. It introduces technical aspects of fluorescence imaging, such as the finite point-spread function associated with the diffraction limit. Ultimately, the module guides students to perform correlation analysis on their simulated images, from which they can extract biophysical information such as diffusion coefficients, and furthermore understand the impact of measurement settings, such as finite exposure time and noise, on results.

Specific tasks in the modules are the following:

1. Generating symmetric and asymmetric random walks by using Matlab's normally distributed random number function. Using plotting tools to map out trajectories in space and time.
2. Using scatter plots and histograms to analyse the distribution of the end displacements, over several random walks.
3. Estimating the number of particles found in a box of given volume. Finding the one-dimensional expression for the time-correlation function. Finding the characteristic diffusion coefficient from the correlation function.
4. Computing and plotting the correlation function of the generated data, as a function of simulated experimental systematics such as finite exposure and noise, and comparing results to the theoretical expression of the same function.

**Fluorescence Imaging and Diffusion** – Carl Hansen, University of British Columbia. chansen@phas.ubc.ca

Developed for a second-year Physics lab course; performed in stages from prescribed to open-ended over six weeks of 3-hour lab periods.

Students assemble a basic fluorescence microscope and use it to observe both diffusion and chemotaxis. After first assembling a fluorescence microscope from components, students use it to explore diffusion in a concentration gradient of dye molecules created in a microfluidic device. They then explore diffusion of fluorescent beads by tracking the position of beads over time, and finally explore diffusion of *E. coli* bacteria.

**Fluorescence correlation spectroscopy to investigate DNA diffusion** – John Bechhoefer and Nancy Forde, Simon Fraser University. johnb@sfu.ca, nforde@sfu.ca

Developed for a fourth-year Biological Physics laboratory course; takes $3 \times 3$ hour lab periods plus analysis time.

Fluorescence correlation spectroscopy measures the intensity fluctuations of a fluorescently labeled object as it diffuses (and, possibly, drifts) through the focal region of a tightly focused laser beam. It allows single-molecule measurements of diffusion and drift velocity. In advanced applications, one can resolve populations of different classes of objects and changes in properties induced, for example, by ligand binding, and even the motion of single dye molecules.

Recent developments in optics, lasers, photon-detection, counters, and software make do-it-yourself instruments feasible for undergraduate and graduate laboratory courses. In the module developed at SFU, students start from basic exercises that utilize a chopper wheel to temporally modulate laser signal, to develop intuition for the meaning of a correlation function. They then progress to measurements of sizes of fluorescent beads and DNA molecules.

**Simulating the chemotactic motility of *E. coli* bacteria** – Cécile Fradin & Paul Higgs, McMaster University. fradin@physics.mcmaster.ca, higgsp@mcmaster.ca

Developed for a second-year Biophysics course; takes about 4 hours of class time, plus 10-20 hours for completion, depending on programming experience.

*E. coli* are micron-sized bacteria subject to thermal noise. To swim in this environment, they utilize rotation of their flagella (corkscrew-shaped tails). Switching between counterclockwise and clockwise rotation of the flagella controls whether the bacteria swim in a directed fashion or "tumble" randomly.

In this module, students learn some simple techniques for computer simulation that can be applied to problems in physics and biology. Students practice writing their own programs and think about the way a complex real-world problem can be turned into a set of rules that is simple enough for a computational model. These exercises make use of the Netlogo programming environment, which can be downloaded for free and is simple to install on a personal computer. The examples relate to the swimming dynamics of bacteria:

1. Swimming Bacteria - trajectories of bacteria subject to thermal noise
2. Chemotactic Bacteria - switching between swimming and tumbling motion can lead to chemotaxis
3. Foraging strategies - evolution of a chemotactic response is an effective evolutionary strategy in a patchy environment

An experimental module to determine the frequency of *E. coli* flagellar rotation using optical trapping of genetically modified *E. coli*. [4] has been implemented at Simon Fraser University.

## MOTION IN EXTERNAL FIELDS

**Non-linear Electrophoresis** – Andre Marziali, University of British Columbia. andre@phas.ubc.ca

Developed for a second-year Physics lab course; performed in stages from prescribed to open-ended over multiple 3-hour lab periods

DNA mobility in a gel is both length and field dependent – in particular, the velocity of long DNA strands is non-linear with electric field $\boldsymbol{E}$. Students are tasked with designing and carrying out an experiment to accurately measure the non-linearity of velocity vs. $\boldsymbol{E}$. for short and long DNA fragments. After completing this, students choose from a variety of experimental questions, and are required to design an experiment that addresses the question of their choice. One example question is to design an experiment that demonstrates the "IZIFE" (Interface Zero Integrated Field Electrophoresis) effect that exploits the non-linearity of DNA velocity with field previously measured to concentrate DNA at a gel-buffer interface. A second example is to explore biased reptation of large molecules at high electric field strengths, where the velocities are expected to be proportional to field squared.

Students explore the research process, including instrumentation development, experiment design, data analysis, and innovation. The lab is intended to mirror a research lab experience in contrast to the traditional undergraduate lab format.

A related experimental module has been developed by Nancy Forde at Simon Fraser University, and involves students preparing DNA from *E. coli*, and investigating how its electrophoretic mobility is influenced by the DNA topology (e.g., supercoiled, relaxed circular or linear) and length.

## DYNAMICS IN LIVING ORGANISMS MODELLED WITH DIFFERENTIAL EQUATIONS

**Mathematically modelling molecular dynamics in living organisms** – Teresa Zulueta-Coarasa and Rodrigo Fernandez-Gonzalez, University of Toronto. rodrigo.fernandez.gonzalez@utoronto.ca

Developed for a third year Engineering Science laboratory course, and easily adapted to lower levels; takes $3 \times 3$ hour lab periods.

The advent of new microscopy modalities, together with advances in genome editing and computer engineering, is providing a highly detailed view of the molecular interactions and dynamics that govern cell behaviour within living animals. However, as we generate and collect massive amounts of new data, our ability to integrate and interpret these data decreases. Computer models are excellent tools to explore and manipulate complex biological systems in a rapid and inexpensive way, and formulate hypothesis about the molecular underpinnings that control system behaviour for further experimental testing.

In this module, students learn how to build mathematical models of molecular dynamics using Matlab. The module is divided into three sections:

1. Introduction to Matlab and the tools that it provides to implement and solve systems of ordinary and partial differential equations. Students are asked to solve simple systems such as the Lorenz Strange Attractor, used to model chaotic systems, or the one-dimensional heat equation, which describes temperature changes over time.
2. Students implement a system of ordinary differential equations to model a signalling network responsible for the wave patterns that travel through colonies of the myxobacterium *Myxococcus xanthus*. *Myxococcus* glide back and forth in an oscillatory pattern governed by a biochemical clock. Students are asked to use their model to reproduce published experimental results, and to extend the model to formulate a novel biological hypothesis about the molecular mechanisms that regulate the wave patterns.
3. Students implement a model based on partial differential equations to investigate dorsal-ventral patterning in embryos of the fruit fly *Drosophila melanogaster*. The dorsal-ventral patterning system specifies cell fates along the back-to-front axis of the animal. As above, students are asked to validate their model by reproducing experimental results, and to extend the model to provide new biological insight.

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