Chemistry 590: Physical Chemistry Tools for Spectroscopy and Microscopy in Living Systems

Spring 2019

Location: LSRC B105

TTh 8:30 AM – 9:45 AM

Instructor:	Prof. Kevin Welsher kevin.welsher@duke.edu, FFSC 2213, 660-1509
Office Hours:	Tuesdays 10AM-12PM

Course Description:

The study of dynamics in biological systems requires ever higher precision and information content from live-cell measurements. As the "jack-of-all-trades", physical chemists have been at the forefront of the development of the next generation of tools which push the spatial, temporal and spectral resolution of these measurements. In this course, we will investigate the application of physical chemistry to live-cell measurements.

Prerequisites:

Exposure to the time-independent Schrödinger equation from an undergraduate physical chemistry course such as CHEM 310 or permission of instructor.

Course Goals:

In this course, we aim to develop the physics and chemistry which underlie state-of-the-art live cell microscopy methods. Students will develop models for materials and methods using “paper and pencil” style physics and quantum mechanics, helping to develop a deeper understanding of the critical aspects of different measurement techniques. For models where the mathematics becomes an obstacle, the students will learn to use the software program MATLAB to perform numerical simulation and data visualization. No experience of MATLAB is expected or required.

Course Structure and Grading:

The grading for the course is broken down as follows:

Problem sets (70%): The course content will be broken up into five sections (see **Course Schedule**). For each section, students will be assigned an assignment to be completed covering that section. The assignments will be a mix of traditional problem sets and tasks to be completed in MATLAB.

In-class activities (10%): In many (if not most) classes there will be a small assignment which will require active participation of the student, alone or in small groups. Sometimes this will take the form of a literature discussion. These will be graded in a binary fashion. The sum of all such activities will make up 10% of the final grade

Final Project (20%) The final 20% will come from the final exam, which will be an independent project. This project can take one of two forms:

1. Literature review presentation. The student will present a 20 minute review of a topic which is related to the course material. This can be a deeper look at a topic that was covered in class or another topic of the students choosing. The student will be prepared to answer questions on the presentation from the rest of the class. The presentation will occur on the last week of class.
2. MATLAB based project. The student will complete an in depth calculation or data visualization study on a project of the students choosing (possibly related to your research). The annotated code will be evaluated, along with a write up describing the theory used and the results achieved, not to exceed 5 pages.

Students must select one of these options by March 1st. It is highly encouraged that you discuss any topics you have in mind with me well in advance of this deadline.

Course Schedule

Section 1 (Weeks 1-3): Fluorescence in Biology. This section will look into the use of fluorescence as a label in biology. What makes a molecule well-suited as a fluorescent label? We will develop quantum mechanical models for small organic molecules using simple particle in a box and perturbation theory to understand how radiation interacts with these probes. We will also discuss the now ubiquitous green fluorescent protein, its variants and origin of its unique properties. Finally, we will use time-dependent perturbation theory to investigate how these fluorescent molecules, which can be approximated as two-state systems, behave when exposed to an oscillating perturbation (in this case, optical radiation).

Section 2 (Weeks 4-5): Nanomaterials as Labels. In this section, we will explore the use of nanomaterials as luminescent markers in live cell measurements. We will then model the energies and selection rules of photoexcited carriers ("excitons") in these low dimensional systems. We will also discuss the current state of the art in luminescent nanomaterials, including methods to avoid *in vivo* toxicity (such as functional group engineering) and "blinking" of their photoemission (using alloying of different materials).

Section 3 (Weeks 6-8): Light in Microscopy. Starting with a history of microcopy, this section will cover how light behaves in the focus of a microscope objective. We will discuss the idea of the "Point-Spread Function", the lens as a Fourier transform element and the full 3D description of the electric field at the focus of the microscope objective. In this section we will learn how to manipulate the Fourier plane of a microscope system to achieve custom excitation and emission patterns.

Section 4 (Weeks 9-11): Super-Resolution. In the previous section, we will have arrived at the conclusion that the resolution of a measurement is inherently limited by the wavelength of the light used to interrogate the system. In this section, we will explore the methods that have been used to "break" the diffraction barrier, paying particular attention to the difference between "resolution" and "precision." Topics will include photon counting statistics, STORM, PALM, STED, SIM and other recent super-resolution methods. We will examine the use of photoswitchable probes (in the case of PALM and STORM), the use of saturated stimulated emission (in the case of STED), and the use of complex excitation patterns to surpass the diffraction limit (in the case of SIM and many others).

Section 5 (Weeks 11-14): Non-localization methods. Sections 1-4 operated under the premise that the observer simply requires spatiotemporal information. In this section, we extend our "toolbox" to cover methods which yield more than just position. We discuss the application of Forster Resonance Energy Transfer as a molecular ruler, which at the single molecule level, can act as a real-time readout of molecular dynamics. We also investigate how the rate of fluorescence emission, or fluorescence lifetime, can be a sensitive probe of the local environment. Finally, we will investigate how optical signals such as fluorescence are altered in the presence of metal surfaces, as in metal enhanced fluorescence (MEF) and surface enhanced Raman scattering (SERS), both of which are becoming powerful tools in biological interrogation.