CH332 Lab: Components and Operation of a time-Resolved Fluorimeter

In this experiment you will measure with the decay kinetics of the fluorescence quinine sulfate and Pt-porphyrin complexes, and the physical processes that effect the florescence lifetime. You will also work with light sources, sample compartments, monochrometers, detectors, and data acquisition systems as part of the laboratory exercise.

Background Reading:

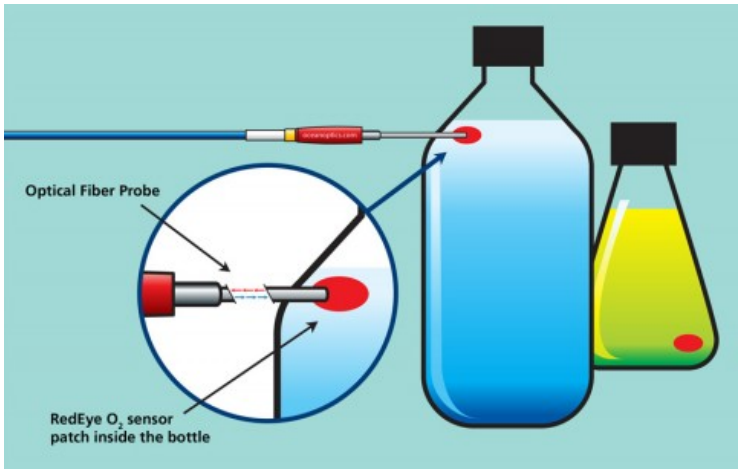
Gutow, Jonathan H. J. Halide (Cl-) Quenching of Quinine Sulfate Fluorescence: A Time-Resolved Fluorescence Experiment for Physical Chemistry. Chem. Educ. 2005 82 302.)

Paul Hartmann and Marc J. P. Leiner. Luminescence Quenching Behavior of an Oxygen Sensor Based on a Ru(l1) Complex Dissolved in Polystyrene. Anal Chem. 1995, 67, 88-93

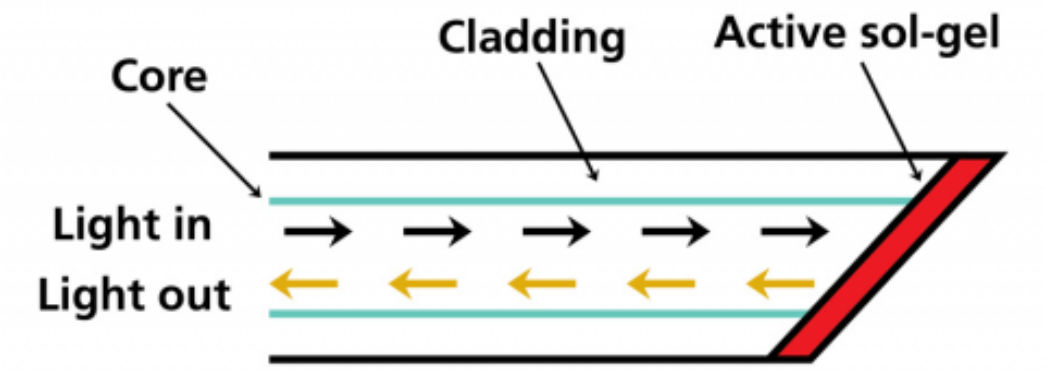
Ocean Optics Technical Note: How Does an Ocean Optics Oxygen Sensor Work?
(https://oceanoptics.com/measurementtechnique/oxygen-sensing/)

Ocean Optics oxygen sensors are based on fluorescence of an indicator material that has been integrated into a matrix. The sensor matrix is then coated on a probe tip, a slide, the wall of a cuvette, or formed into an adhesive patch. We use a matrix with good thermal and mechanical stability, superior chemical compatibility, ease of production and rapid response.
We use two types of indicator materials that fluoresce – ruthenium and Pt-porphyrin complexes. Each works well for a specific range of applications like general laboratory use, high-sensitivity applications or hydrocarbon-rich sample environments. What they have in common is that both indicator materials are highly sensitive to partial pressure of oxygen.

In the presence of molecular oxygen, the fluorescence properties exhibited by these materials are altered. The most obvious change is a quenching of the fluorescence, or decrease in fluorescence intensity, as oxygen levels increase. The quenching happens because an excited indicator molecule has come into contact with an oxygen molecule, transferring its excess energy to the oxygen molecule in a non-radiative transfer (a gentle handshake of sorts). The degree of fluorescence quenching depends on the frequency of collisions, and therefore on the oxygen concentration of the sample, as well as its pressure and temperature. An advantage of these probes is the ability to place the fluorescent probe material inside a closed container and measure the fluorescence through the container wall using a fiber optic probe.

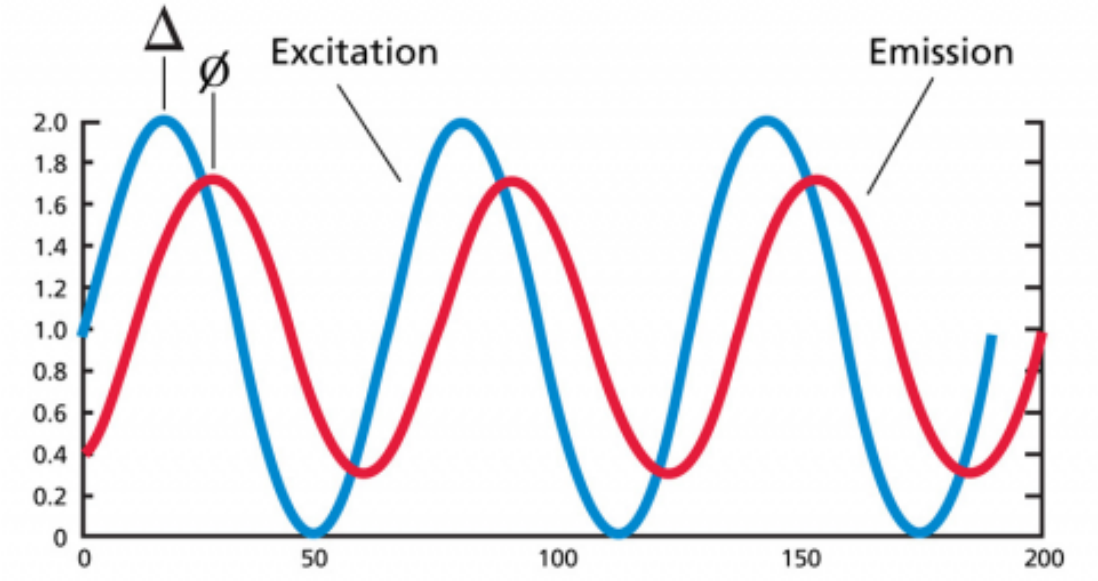


OOI particular oxygen sensors use a blue LED for excitation at 450 nm, transmitted to the sensor material using a fiber optic probe. Fluorescence at visible wavelengths is collected back through the same probe and routed to an avalanche photodiode for detection.



A more subtle effect of fluorescence quenching is that the average fluorescence lifetime of the indicator material decreases as oxygen concentration increases. By pulsing the excitation LED and looking at when maximum fluorescence is emitted relative to those pulses (the phase shift), the average fluorescent lifetime can be determined. This method is called phase-sensitive detection. This is the method used by all OOI NeoFox systems.

Phase sensitive detection is a more accurate method than intensity-based detection, as it is insensitive to electronic and light source drift, scattering within the sample, and intensity variations due to fiber bending and ambient light. It is also unaffected by chemical degradation of the indicator material, and by refractive index changes caused by calibrating in gas prior to measurements in a liquid. Photobleaching is vastly reduced in platinum-based chemistries as compared to ruthenium-based.



Each probe has a slightly different response to oxygen concentration, so prior to use it must be calibrated using known levels of oxygen. Once the phase shift is measured, it can be related to the oxygen concentration or partial pressure using the Stern-Volmer equation. If pressure and temperature cannot be controlled and kept constant from calibration to measurement, they must also be factored into the calculations, and a multi-point calibration must be used.

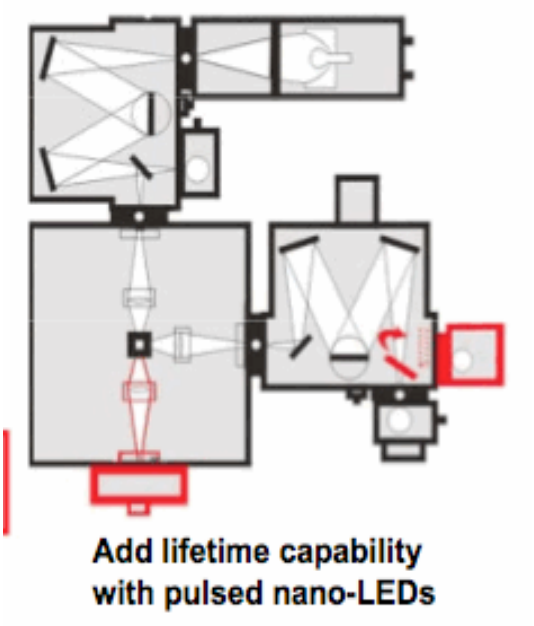
It is important to note that this type of sensor requires oxygen to diffuse into the sensor material, so the sensor tip or patch must be kept in direct contact with the sample. However, since the indicator material is trapped in a matrix, it is immobilized and protected from exposure to the sample, making these sensors unusually chemically inert and robust when used with an overcoat. Notably, they have minimal response to environmental changes in pH, salinity and ionic strength.

Instrument Details:

Please note that we will be using the **Quanta Master Instrument from PTI** with a pulsed nano-LED as an excitation source instead of a laser.

The optical diagram of our instrument is shown here. Your lab instructor will show you how to turn on the instrument. The instrument is controlled using Felix32 software running on the Acer PC. Details on each acquisition technique are provided below.

You should first take an emission scan of the quinine sulfate solution and then measure the fluorescence decay of three solutions at different quencher concentrations (chloride is a good quencher of quinine sulfate).



1) Measure the **excitation and emission spectra** of QS. Excitation or Emission Scan Tutorial, Text Directions (scroll to Emission).

2) Measure the **fluorescence decay** rate constant of a scattering solution (scattering reference) and the QS. The scattering sample is a dilute solution of coffee creamer. Please refer to the instructional video - Timebase. You will also find it helpful to read about Time Resolved Basics. Data analysis is described on page 144 of the manual and online (Decay Rate Calculations).

3) Compare the results obtained with the PTI and NEOFoxy instrument to the fluorescent decay times reported in the literature.

4) Carefully draw and document each of the optical components of both instruments in your laboratory notebook

Questions:

1. What is the value of kq? What does kq refer to? Is the quenching of OOI FOSPOR sensing patch static or dynamic? Explain. Are you confident in your data? (include stats)

2. Compare and contrast the two fluorescence methods used in this lab (scans vs lifetime). Be specific regarding the information obtained in each experiment and differences in instrument operation.

3. How could this instrument be used to analyze two different compounds with similar emission and excitation spectra but very different fluorescence lifetimes?

4. Why is it possible to use a 310 nm pulsed diode to excite the sample when the maximum excitation wavelength is 450 nm?

5. How would the results of this experiment change if you ran the entire experiment at 5 degrees C?

(Motivated by the article: Gutow, Jonathan H. J. Halide (Cl-) Quenching of Quinine Sulfate Fluorescence: A Time-Resolved Fluorescence Experiment for Physical Chemistry, Chem. Educ. 2005 82 302. and the new OOI Oxygen Optodes.