Absorption and Fluorescence Spectra

In this experiment you will learn how to obtain absorption and fluorescence emission spectra of a particular biomolecule (chlorophyll a) dispersed in a liquid solution (water) using a conventional spectrophotometer and a custom-built fluorescence spectrometer.

Experiment:

Solution Preparation

You will use spinach leaves to obtain chlorophyll a molecules dispersed in water solution for this experiment. Chop a few leaves (2-4) into small pieces and place those pieces into a beaker with about 100 ml of de-ionized water. Take to the microwave for about 1-2 minutes. Your solution should look greenish as chlorophyll a has diffused to the water solution.

Absorption Spectrum

Data Collection: With a small plastic pipette, almost completely fill (up to about 3-5 mm from the top) a quartz cuvette with some of chlorophyll a solution. Avoid putting pieces of leaves into the cuvette. Take to the Cary 300 spectrophotometer. Your GTA will show you how to initialize this instrument and how to collect absorbance data with it. Make sure to collect data for a range from 900 nm – 190 nm (usually spectrophotometers run high to low wavelengths). Set the spectral resolution bandwidth to 2 nm. Use wavelength steps of 1 nm. Select the data collection mode to absorbance. Remember to initialize the instrument with signals for baseline and zero correction.
Absorbance is defined by:

\[ A(\lambda) = -\log_{10}\left(\frac{I - I_d}{I_0 - I_d}\right) \]

where \( I_0 \) is the baseline signal (the baseline signal collected by the spectrophotometer), \( I_d \) is the dark signal (the zero signal collected by the spectrophotometer), and \( I \) is the signal when the sample is present. When you measure a sample after the proper initialization with the baseline and zero signals, the output data of the instrument is already the absorbance data as described by the equation above.

Once you have finished collecting data for your sample, save it to a device you can retrieve later for analysis.

**Fluorescence Emission Spectrum**

**Data Collection:** With a small plastic pipette, almost completely fill (up to about 3-5 mm from the top) a plastic cuvette with some of chlorophyll \( a \) solution. Avoid putting pieces of leaves into the cuvette. Take the cuvette to the experimental setup and place it into the cuvette holder. For the light excitation of the fluorescence emission, you will a laser beam with a wavelength of 445 nm. You will use an orthogonal configuration between the excitation and fluorescence emission to prevent large amounts of excitation light reaching the detector. You will use an optical fiber to collect the fluorescence emission and transfer it to a spectrometer. At the spectrometer, the collected light is dispersed by a diffraction grating. Then a CCD array will be used to collect the light intensity for each wavelength over a broad spectral range. Set the CCD device to collect data centered at about 650 nm. Use a diffraction grating with 150 grooves per mm. Explore different integration times and alignments
of the collecting fiber to improve your signal. Block your excitation laser and collect a trace of the background signal. If you observe significant amounts of background light, search for its origin and try to eliminate. Once you have a configuration where the background signal is negligible, you can collect the data for your sample.

Once you finished collecting your fluorescence emission of chlorophyll a, save the data to a device you can retrieve the information.

**Analysis:**

Plot the absorbance and fluorescence emission spectra you have obtained for chlorophyll a. The absorbance has no units as one can notice from its definition. The fluorescence intensity should be plotted of light intensity, however as we have calibrated our camera for this experiment, you plot your data in terms of arbitrary units, where the relevant information relies on the relative intensity at each wavelength.

The absorption of molecules is usually characterized by a quantity called molar absorptivity $\epsilon(\lambda)$, which is the absorbance per unit length (cm) light propagation and per molar (M) concentration of the sample (1 M = 1 mol/L). The absorbance of molecules is governed by Beer’s law: $A(\lambda) = \epsilon(\lambda)lc$, where $l$ is the length of light propagation, $c$ is molecule concentration in the solution and $\epsilon$ is the molar absorptivity of the molecules of interest. Search the literature for the molar absorptivity of the chlorophyll a molecule dissolved in water solution at the spectral range you have collected data. Take one particular wavelength (for instance, the wavelength of an absorbance peak in the visible region) to calculate the molar concentration of
the chlorophyll solution you have prepared. The pathlength of the cuvette is a standard 1-cm long.

Questions to consider:

1) Do you see any correlation between your absorption and emission spectra?

2) What wavelength can you use for the excitation of the chlorophyll a molecule? Do you expect your spectra to change if you use a different wavelength for the excitation process?