Experiment 15: Breadboard Fiber Optic Fluorimeter:  
Calcein Blue Interaction with Pb

FFT Pre-lab to the Fluorescence Experiment

In the fluorescence experiment, as you will find, the chemistry is again complicated. The main point in this particular lab is to give you hands on experience with fluorescence and to provide you with a “transparent” piece of equipment which allows you to look at the “inside” of the black box. You will be asked to observe the mechanics of the monochromator, and of the optical set up, to observe how light is transmitted by fiber optics and to observe the detector.

In addition you will see how the slit width of the monochromator affects the signal.

Finally you will observe the concept of frequency encoding your signal. Frequency encoding is accomplished by putting the source beam on a chopper so that the signal generated is tied to a known frequency, while the background (noise) is on some baseline or variable frequency. What this allows you to do is to recover the signal frequency and remove the noise frequency through the use of a lock-in-amplifier. The way it works is the following.

The source is chopped onto a frequency, $f_{\text{source}}$. The source beam is sent through the fiber optic to the sample cell where it excites a fluorescence signal of the same frequency, $f_{\text{fluorescence}}$ but of a different wavelength. The collection fibers of the fiber bundle collect the fluorescence signal as well as back scattered source light, and all of the light that enters the fiber bundle from the ambient light. The light from the environment is our background and it has its own peculiar frequency, $f_{\text{noise}}$. The fiber bundle passes through a second wavelength discriminator which removes the source wavelength and most, but not all, of the background because some of the background will be at the same wavelength as the fluoresced light. Thus the detector observes the sum of noise and signal at the fluorescing wavelength.

Source: $I_{\text{source}} = I_{\text{source}} = A_{\text{source}} \sin(2\pi f_{\text{source}} t)$
Fluorescence $I_{\text{fluorescence}} = A_{\text{fluor.}} \sin(2\pi f_{\text{source}} t)$
Noise $I_{\text{noise}} = A_{\text{noise}} \sin(2\pi f_{\text{noise}} t)$
Detector $I_{\text{detector}} = A_{\text{fluor.}} \sin(2\pi f_{\text{source}} t) + A_{\text{noise}} \sin(2\pi f_{\text{noise}} t)$

To get only the source a clever trip is pulled. The output of the detector is multiplied by the source frequency:

$$\sin A (\sin A + \sin B) = \frac{1}{2} (1 + \sin 2a) + \frac{1}{2} (\sin(a+b) + \sin(a-b))$$

The part of the signal composed only of source frequency (the fluorescence) is frequency doubled and shifted away from the noise.

At this point a Fast fourier transform can be applied which digitally clips the low frequency component. A fast fourier transform (FFT) is similar to a regular fourier transform but takes some tricks to use up less computational space and time. As a result of the tricks there is a requirement that the
time string of data to be transformed but consist of $2^n$ data points.

To “get a feel” for the whole process I am asking you to (each student) do a series of excel computations to take you through the chopper, summing to noise, lockin step, and the fft, the cutting of the signal by fft and the reconstitution of the signal into a time domain.

Pre-lab Assignment

Open an excel spread sheet. At the top create a base frequency as $f_{\text{source}} = \frac{2\pi}{512}$. We are using 512 as our total data string because $512 = 2^7$. Create a frequency for your noise that is not equal to that of the source, nor should it be some integer multiple. I chose $f_{\text{noise}} = 1.3f_{\text{source}}$.

In column A create a time string from 1 to 512. In column B create the signal associated with the source ($=\sin(2\pi/512)*\text{time in column A}$). In column C create a similar set of data for the noise. In column D sum the source and the noise. In column E multiple column B by column C (this is what the lock in amplifier looks at).

Open the tools pack and go to fourier transform. Specify the 512 data points associated with the detector in column E. Tell it to place the transformation in column F.

The data placed into column F will be complex number representation of the amplitude of the frequency components for 0 ($f_{\text{source}}$), then 1($f_{\text{source}}$), then 2($f_{\text{source}}$) and so on. (An aside: we have deliberately created our source frequency to pass one cycle in 512 second. If you use the FFT in other applications, the fft considers the fundamental period of the frequency to be what happens in the 512 data points you scan). The first point in column F will represent 0 frequency, or DC. Each sequential box will represent the amplitude of frequency 1 (1cycle/512 second), frequency 2 and so forth up to point 256 at which point the numbers display the amplitude of each decreasing frequency.

The data displayed is a complex number. To convert to something you can graph create a column G ($=\text{imabs(columnF)}$).

Create a histogram of column G. Include only the 2$^{nd}$ through the ~ 17 data points (base frequency to frequency multiple 17). You should see a much larger contribution from the 2f component (the frequency double signal) and some smaller components near f= 1 representing the sum and difference frequencies.

To filter the data create column H. Every number in column H should be zero except for the point representing the frequency doubled signal. Copy the complex number in column F over. Note, for some unexplained reason you will find a duplicate of this number at the end of the data string. For example if your frequency doubled contribution occurs three points down column G then it will also occur three points up from the bottom of column G. Copy the complex number from column F over
also.

Now re-create a time string of data by performing the inverse fft on column H. Place the data in column I. This data needs to be truncated. To truncate in column J type =value(column i)

Make a plot which shows the signal, noise, the detector output, and the fft processed data.

**SYNOPSIS**  The interaction of lead with the fluorescent dye, Calcein Blue is monitored by the quenching of calcein blue indicator fluorescence at 445 nm (1, 2).


**SOLUTIONS**

1M NaOAc + 1M HOAc: pH = - 5  
Stock 1000 ppm Pb  
0.01% aqueous solution of Calcein Blue (10 mL) (4-methylumbelliferone-8-methylene-iminodiacetic acid)

4.6 mg CB/10 mL buffer

The molarity of this solution is can be calculated from the molecular weight of the CB (321.29 g/mole)

5.  **Fluorimeter**

To Operate  

a.  *Turn on Power Supply for Lamp (#1).*  
Unlock the output dial.  
*At same time: push in pre-adjust button and dial output dial to 5.4 Amps.*  
Release pre-adjust and lock output dial.  
*Press Lamp start button until lamp fires.*

b.  *Turn on Detector Power Supply (#2).*  
Adjust dial to -1000  *(Do not exceed -110).*

c.  *Turn on Merlin* (lower right-hand side, back).  *Must be turned on after the lamp.*

d.  *Turn on Gateway 486*

e.  *Turn on monitor.*

f.  *To run a fluorescence wavelength scan:*  
   i.  *from DOS*  

   ```
   type cd: runes
   type runespec
   ```
PROCEDURE

1. Make a fresh solution of 10 mL CB in 20 dram vial. Take a full excitation scan with initial instrumental parameters (200-1000 nm).
2. Set the excitation wavelength to 360 nm and scan over the full emission spectrum (300-600 nm).
3. Set the excitation wavelength to 360 nm and the emission wavelength to 450 nm.
4. Add Pb\(^{2+}\) in increments and monitor the emission spectra at each increment. Increments begin with 25 uL, a second 25 uL, 50 uL, 100 uL, then add by 100 uL until a total of 1000 uL have been added. Then add 200 uL increments until a total of 2000 uL have been added. Then add 1000 uL for a total of 3000 uL.

REPORT In addition to methods, materials and results, include the following:

1. Describe the chemistry of the procedure.
3. What might be some serious problems with your method? (I.e. interferences?, background from your soil sample?) I.E., what are the effects of matrix in this method?
4. What would be the estimated time involved in a single measurement?
5. What is the probable structure of the fluorescing compound at intermediate pH values? How does Pb\(^{2+}\) interact with it do reduce the fluorescence? Construct a reasonable chemical argument as to the sensitivity of the method to pH.
6. Would there be any problems with disposal of hazardous materials?
7. How easy would you anticipate it be to instruct a technician on this method?