Vibhav Jha

BE 492 Section B5a

February 19, 2019

Lab 2: Spectroscopy II

**Part A:**

****

**Figure A1:** Background corrected Forward-directed spectra for water and fluorescein.

****

**Figure A2:** Normalized Excitation and Emission spectra for Fluorescein.

The Stokes shift for Fluorescein is 30 nm. Calculated by tracing peak, not through MATLAB script.

Energy has disappeared due to heat from the vibrations or rotations of the molecule. Alternatively, some energy may have been transferred to the lowest energy triplet state instead of from the highest energy singlet state.

From the Bio-Rad website for Fluorescein isothiocyanate1, the excitation peak is at 490 nm and emission peak is at 525 nm. The value of excitation perfectly matches what was found in lab of 490nm, while the emission peak is slightly higher than the value of 520 found in the lab.

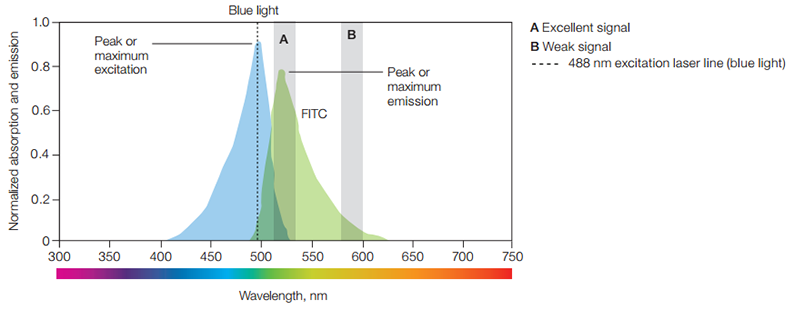


Figure A3. From Bio-Rad flow cytometry/fluorescence web page1.

1. <https://www.bio-rad-antibodies.com/flow-cytometry-fluorescence.html>

%Part A

close all;

%load Data, though importdata may be slower, I tend to find this more robust to use when using .txt, .csv files

partAwaterBG = importdata('PartA\_darkwater\_background.txt');

partAwater = importdata('PartA\_water.txt');

partAfluorescein = importdata('PartA\_fluorescein.txt');

partAfluoresceinBG = importdata('PartA\_fluorescein\_background.txt');

partAfluoresceinSDE = importdata('PartA\_fluoroscein\_side.txt');

%Adjust Background

waterADJ = partAwater(:,2) - partAwaterBG(:,2);

fluorFWadj = partAfluorescein(:,2) - partAfluoresceinBG(:,2);

fluorSDadj = partAfluoresceinSDE(:,2) - partAfluoresceinBG(:,2);

%figureA1

figureA1 = figure('Name', 'Forward Water and Fluorescein');

a1 = plot(partAwater(:,1), waterADJ, 'Marker', '+', 'MarkerIndices', 1:10:2048);

hold on

a2 = plot(partAfluorescein(:,1), fluorFWadj);

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

legend('Water', 'Fluorescein');

title('Background Corrected Spectra of Water and Fluorescein in the Forward Direction');

%figureA2 normalized excitation and emission

figureA2 = figure('Name', 'Fluorescein Excitation and Emission');

excitation\_bl = -(log(abs(partAfluorescein(:,2))./ abs(partAwater(:,2))));

excitation\_normal = excitation\_bl .\* 4500;

a3 = plot(partAfluorescein(:,1), excitation\_normal);

hold on

a4 = plot(partAfluoresceinSDE(:,1), fluorSDadj .\* 0.5);

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

legend('Excitation', 'Emission');

title('Normalized Fluorescein Excitation and Emission');

**Part B:**

The parameters are a = [0.2065 0.7498].

****

**Figure B1:** Plots of Smodel(λ), Sdata(λ) and weighted component spectra a1\*SF(λ) and a2\*SRhodB(λ)

New values are a = [0.2179 0.4389]. The difference in these values could be due to skim milk absorbing more light, thus minimizing the overall contributions of Fluorescein and Rhodamine B. The skim milk is essentially scattering the light and overall blue shifting the spectrum.



**Figure B2:** Plots of Smodel(λ) and weighted components of Fluorescein(a1), Rhodamine B(a2) and water and skim milk (a3).



**Figure B3:** Plots of Smodel(λ) and Sdata(λ) Spectra of mystery solution with 2 drops of skim milk and the calculated model.

Using the least squares method, the vector is re-derived as a = [0.2179 0.4389 0.9840].

Using the pseudo-inverse method, the vector is re-derived as a = [0.2179 0.4389 0.9840].

NB: Some legends and graphical aspects of the plot were done through the property inspector in MATLAB rather than through the script.

%Part B

close all;

partAwaterBG = importdata('PartA\_darkwater\_background.txt');

partAwater = importdata('PartA\_water.txt');

partAfluoresceinBG = importdata('PartA\_fluorescein\_background.txt');

partAfluoresceinSDE = importdata('PartA\_fluoroscein\_side.txt');

partB3BG = importdata('PartB3\_background.txt');

partB4\_1drop = importdata('PartB4\_1drop.txt');

partB4\_2drop = importdata('PartB4\_2drop.txt');

partB5\_watermilk2drop = importdata('PartB5\_watermilk\_2drop.txt');

partB\_BG = importdata('PartB\_background.txt');

partB\_mystery = importdata('PartB\_mystery.txt');

partB\_Rhod = importdata('PartB\_Rhodamine\_side.txt');

%First Model

purefluorSDE = partAfluoresceinSDE(:,2) - partB\_BG(:,2);

pureRhod = partB\_Rhod(:,2) - partB3BG(:,2);

model1 = @(a)norm(abs(a(1)\*purefluorSDE + a(2)\*pureRhod - partB\_mystery(:,2)))^2;

a = fminsearch(model1, [0,0]);

smodel = a(1)\* purefluorSDE + a(2)\* pureRhod;

%Figure B1

figureB1 = figure('Name', 'Model Plots Smodel Sdata');

b1 = plot(partB\_BG(:,1), smodel);

hold on

b2 = plot(partB\_BG(:,1), partB\_mystery(:,2));

hold on

b3 = plot(partB\_BG(:,1), a(1)\* purefluorSDE);

hold on

b4 = plot(partB\_BG(:,1), a(2)\* pureRhod);

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

%Calculate a1, a2, a3 with milk

model2 = @(b)norm(abs(b(1)\*purefluorSDE + b(2)\*pureRhod + b(3)\*partB5\_watermilk2drop(:,2)-partB\_mystery(:,2)))^2;

b = fminsearch(model2, [0,0,0]);

smodel2 = b(1).\* purefluorSDE + b(2) .\*pureRhod +b(3) .\* partB5\_watermilk2drop(:,2);

%figure B2

figureB2 = figure('Name', 'Model w/ Milk and weighted Fluoroscein, Rhod B');

b5 = plot(partB\_BG(:,1), smodel);

hold on

b6 = plot(partB\_BG(:,1), b(1)\*purefluorSDE);

hold on

b7 = plot(partB\_BG(:,1), b(2)\*pureRhod);

hold on

b8 = plot(partB\_BG(:,1), b(3)\*partB5\_watermilk2drop(:,2));

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

%Figure B3

figureB3 = figure('Name', 'Mystery, Milk, Model');

b9 = plot(partB\_BG(:,1), smodel2);

hold on

b10 = plot(partB\_BG(:,1), partB\_mystery(:,2));

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

legend('S\_m\_o\_d\_e\_l', 'S\_d\_a\_t\_a')

%ReDerivingParameterVector or Matrix calcs

M = [purefluorSDE pureRhod, partB5\_watermilk2drop(:,2)];

A = [b]

B = partB\_mystery(:,2);

lsqrcheck = lsqr(M,B)

pinvcheck = pinv(M)\*B

**Part C:**

****

**Figure C1:** Plots with Mystery solution, Mystery with Methylene Blue, Methylene Blue normalized.

Question C1: For fluorescein the new estimated contribution is 0.1518 while for Rhodamine B it is 0.0995.

Question C2: These greatly differ to the values calculated in part B. This rather large discrepancy could be due to the methylene blue accounting for more absorption than can be handled by the model, however since the value for a3 is so small this cannot be a significant source of error.



**Figure C2:** Mystery Solution with Methylene blue and the least squares regression model. Here the model does not match the mystery solution spectra.

%Part C

close all;

partAwaterBG = importdata('PartA\_darkwater\_background.txt');

partAwater = importdata('PartA\_water.txt');

partAfluoresceinBG = importdata('PartA\_fluorescein\_background.txt');

partAfluoresceinSDE = importdata('PartA\_fluoroscein\_side.txt');

partB3BG = importdata('PartB3\_background.txt');

partB\_BG = importdata('PartB\_background.txt');

partB\_Rhod = importdata('PartB\_Rhodamine\_side.txt');

partC\_BG = importdata('PartC\_background.txt');

partC\_mystery = importdata('PartC\_mystery.txt');

partC\_blustery = importdata('PartC\_mysteryblue.txt');

partC\_FC = importdata('food\_color\_spectrum.txt');

%adjustBackGround

partCmysteryADJ = partC\_mystery(:,2) - partC\_BG(:,2);

partCblusteryADJ = partC\_blustery(:,2) - partC\_BG(:,2);

%Food Color scaling for ease

partCFCscale = partC\_FC(:,2) \* 1700;

%Figure C1

figureC1 = figure('Name', 'Mystery, Blue + Mystery, FC');

c3 = plot(partC\_FC(:,1), partCFCscale);

hold on

c4 = plot(partC\_FC(:,1), partCmysteryADJ);

hold on

c5 = plot(partC\_FC(:,1), partCblusteryADJ);

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

legend('Methylene Blue (Normalized)', 'Mystery Solution', 'Mystery Solution with Methelyne Blue')

%Question Calcs

bluefxn = @(c)norm(abs((exp(-(c(3)\*partCFCscale))) .\*(c(1)\*partAfluoresceinSDE(:,2) + c(2)\*partB\_Rhod(:,2))) - partCblusteryADJ)^2;

c = fminsearch(bluefxn, [0,0,0]);

smodelblufxn = (exp(-(c(3)\*partCFCscale)) .\* (c(1) \* partAfluoresceinSDE(:,2) + c(2)\*partB\_Rhod(:,2)));

%Figure C2

figureC2 = figure('Name', 'Model, Blustery');

c6 = plot(partC\_FC(:,1), smodelblufxn);

hold on

c7 = plot(partC\_FC(:,1), partCblusteryADJ);

xlabel('Wavelength (nm)');

ylabel('Intensity (AU)');

legend('S\_m\_o\_d\_e\_l', 'S\_d\_a\_t\_a')

title('Mystery Solution with Methylene Blue and Least Squares Regression Model');