Lab 2: Spectroscopy: Measuring Absorption, Scattering, and Fluorescence

GAIA Statement: I **did** use a form of generative AI during the completion of this assignment. If I did, I understand that I must also turn in a completed copy of the course's GAIA Disclosure Worksheet for this assignment.

Part 1: Experimental Setup

[Nothing required]

Part 2: Acquiring and Saving Data in OceanView

[Nothing required]

Part 3: Light Scattering

	Filename	Integration Time (ms)	
Forward water	Lab2_part3_1_water_Scattering_USB2G582531_16-10-01- 093.txt	100	
Forward water dark	Lab2_part3_2_water_Scattering_Dark_USB2G582531_16- 10-27-090.txt	100	
Forward milk	Lab2_part3_3_Milk_Forward_USB2G582531_16-12-33- 767.txt	167	
Forward milk dark	Lab2_part3_4_Milk_Forward_Dark_USB2G582531_16-13- 06-937.txt	167	
Side milk	Lab2_part3_5_Milk_Side_USB2G582531_16-18-14-045.txt	6000	
Side water	Lab2_part3_6_water_SideScatter_USB2G582531_16-19-15- 476.txt	6000	

Part 4: Absorption

	Filename	Integration Time (ms)
Water forward	Lab2_part4_1_water_Forward_USB2G582531_16-36-08-559.txt	67
Water forward dark	Lab2_part4_2_water_Forward_Dark_USB2G582531_16-36-49- 992.txt	67
Phenol red long	Lab2_part4_3_Phenol_Forward_USB2G582531_16-37-26- 349.txt	67
Phenol red long dark	Lab2_part4_5_Phenol_Forward_Long_Dark_USB2G582531_16- 38-34-673.txt	67
Phenol red short	Lab2_part4_4_Phenol_Forward_Short_USB2G582531_16-38- 03-941.txt	67

BE493		Lab 1 Worksheet
Phenol red short dark	Lab2_part4_6_Phenol_Forward_short_Dark_USB2G582531_16- 39-05-268.txt	67
One drop vinegar	Lab2_part4_10_Phenol_Forward_Vinegar_1_USB2G582531_16- 47-56-080.txt	67
Two drops vinegar	Lab2_part4_11_Phenol_Forward_Vinegar_2_USB2G582531_16- 49-03-169.txt	67
Three drops vinegar	Lab2_part4_12_Phenol_Forward_Vinegar_3_USB2G582531_16- 50-13-139.txt	67
One drop bleach	Lab2_part4_7_Phenol_Forward_Bleach_1_USB2G582531_16- 42-05-680.txt	67
Two drops bleach	Lab2_part4_8_Phenol_Forward_Bleach_2_USB2G582531_16- 43-32-251.txt	67
Three drops bleach	Lab2_part4_9_Phenol_Forward_Bleach_3_USB2G582531_16- 44-30-559.txt	67

Long dimension cuvette: <u>1.29 cm</u>

Short dimension cuvette: 0.59 cm

Part 5: Absorption and Fluorescence

	Filename	Integration Time (ms)
Water forward	Lab2_part5_1_water_short_forward_USB2G575791_17-35-04-037.txt	40
Fluorescein forward	Lab2_part5_2_fluorescin_short_illumination_USB2G575791_17-21-33- 921.txt	40
Dark forward	Lab2_part5_3_fluorescin_short_dark_USB2G575791_17-19-25-161.txt	40
Fluorescein side	Lab2_part5_4_fluorescin_short_illumination_sidescatter_USB2G575791_17- 26-02-789.txt	1000
Water background side	Lab2_part5_5_water_short_illumination_sidescatter_USB2G575791_17-27- 04-220.txt	1000

Part 6: Spectral Unmixing: Fluorescence

	Filename	Integration Time (ms)
Rhodamine B side	Lab2_Part6_1_RhodamineB_side_short_USB2G575791_17-37-36-543.txt	1000
Water background side (Rhodamine B)	Lab2_Part6_2_RhodamineB_water_background_USB2G575791_17-38-37- 975.txt	1000
Mystery solution side	Lab2_Part6_3_Mystery_side_short_USB2G575791_17-42-31-429.txt	1200
Water background side (Mystery)	Lab2_Part6_4_Mystery_side_short_water_background_USB2G575791_17- 40-53-140.txt	1200



1) Include Figure 1 that you generated in Step 4.

Compare the locations of the milk-forward and milk-side spectra in relation to the water-only spectrum. What causes this phenomenon to occur?
 The particles in milk are bigger than the molecules in water, so the milk forward and side spectra are shifted to

The particles in milk are bigger than the molecules in water, so the milk forward and side spectra are shifted to the right and to the left respectively within approximately the same range of wavelengths as the water forward direction. The milk forward spectra is shifted to the right because longer wavelengths pass through the cuvette and is more similar to the water forward spectra. The milk side scattering spectra is more deviated from the water forward spectra because this is where the difference in particle sizes are more obvious as light is scattered from bigger particles in the milk cuvette.

- Report the weighted-average wavelength for background corrected forward water, forward milk, and side milk spectra that you calculated in Step 5.
 Weighted Average= 788.6435 nm
- 4) Briefly <u>EXPLAIN</u> why the transmitted light (forward milk) is red shifted and side scattered light (side milk) is blue shifted in Figure 1.

The forward milk is red shifted (long wavelengths) because longer wavelengths pass through forward transmission where as shorter wavelengths are scattered through the sides which explains why side milk is blue shifted (shorter wavelengths).

Part 8: Analyzing your absorption data

1) Include Figure 2 that you generated in Step 2.



2) In what wavelength range does this absorption dip appear for Phenol Red? Does this dip in the spectrum explain its red appearance?

Between around 450-630 nm. Yes, this dip could be explained by its red appearance as.

3) Include Figure 3 that you generated in Step 3.



4) Include the ratios that you calculated in **Step 4**. How do they compare to what you would expect based on the lengths of the long and short pathlengths that you measured? And how does it compare to the ratio of those pathlengths?

Intensity ratio =1.4512

Pathlength ratio = 2.0508

An equal pathlength ratio is expected however there is a bit of a difference between the two which could be explained by noise and background light that was not accounted for.

5) Include Figure 4 that you generated in Step 5.



6) At what wavelengths (round to the nearest nm) are the main peaks in the different Phenol Red absorption spectra in Figure 4? What colors do these correspond to, and how do they explain the different appearances of Phenol Red?

Phenol Red with 10 uL vinegar: 479 nm, Blue Phenol Red with 20 uL vinegar: 487 nm, Blue-Green Phenol Red with 30 uL vinegar:495 nm, Green Phenol Red: 509 nm Green Phenol Red with 10 uL Bleach: 527 nm ,Dark Green Phenol Red with 20 uL Bleach: 578 nm, yellow Phenol Red with 30 uL Bleach:592 nm ,Orange

Vinegar decreases the PH of Phenol red where as Bleach increases the PH of phenol red. As the PH increases from 6.8 to 8.0, the solution gradually becomes a darker red color as well as seen in figure 4. However, the respective peak wavelength colors don't match the color of the different solutions because more than just the red light wavelength were able to pass through the phenol red solutions because it was diluted and the particles of phenol red aren't that much bigger than those in water.

7) In this section, multiple dark measurements were acquired. Were all of these measurements necessary or would one have been enough as correction for all the measurements in this part of the lab? **Explain** your reasoning.

Yes they were all necessary because different integration times were used throughout the experiment and so its associated background/dark measurement at the same integration time was required to correct the data set that was needed.

Part 9: Analyzing your fluorescence data



1) Include Figure 5 that you generated in Step 2.

2) Include Figure 6 that you generated in Step 4.



- Report the measured Stokes shift to the nearest nm. Stokes Shift= 27 nm
- Compare your measured results for the Fluorescein excitation and emission spectra with results found in the literature. Include the comparison literature spectra, citing the source you used. Are your results similar? Explain your reasoning.

The spectra found in literature was 28 nm and my results are very similar (27nm).

Yumpu.com. (n.d.). *Fluorescence and Fluorescence Applications - Integrated DNA* ... Yumpu.com. Retrieved February 22, 2024, from https://www.yumpu.com/en/document/read/49749324/fluorescence-and-fluorescence-applications-integrated-dna-

5) Essentially all fluorescent molecules in solvent exhibit a Stokes shift, meaning that the energy absorbed by the molecules is greater than the energy re-released by the molecules. This seems to violate the principle of energy conservation (which, of course, can't happen). **Explain** where you think the energy balance has disappeared to.

The energy difference between excitation and emission could be explained by heat loss.

Part 10: Analyzing your unmixing data

1) Include Figure 7 that you generated in Step 2.



- Report your measured peak emission wavelengths for Fluorescein and Rhodamine. Fluorescein- 517 nm Rhodamine B- 582 nm
- 3) Include Figure 8 that you generated in Step 3.



- 4) Report the weighting coefficients for Fluorescein and Rhodamine, a_1 and a_2 calculated in Part 10, Step 3. $a_1 = 0.2707$, $a_2 = 0.5679$
- 5) Report the weighting coefficients for Fluorescein and Rhodamine, a_1 and a_2 calculated in Part 10, Step 4 using the backslash operator.

 $a_1 = 0.8622$, $a_2 = 1.0088$

- 6) Report the peak fluorescence intensities in S_{data}(λ) at the peak emission wavelengths of Fluorescein and Rhodamine, respectively (use the measured values from Part 10, step 5).
 Max Fluorescein intensity = 0.9929;
 Max Rhodamine B intensity = 0.9835;
- 7) <u>COMPARE</u> a_1 and a_2 computed with the back slash operator (Part 10, step 4) to a_1 and a_2 , respectively, computed with fminsearch(Part 10, step 3). How similar or different are they? Provide potential reason(s) for why they are similar or different.

They are very different because fminsearch performed least squares minimization on the data so the function found the best fitting solution to the linear equations when there is no exact solution whereas the inverse matrix method gives the exact solution since the values are known.

8) How similar or different are the a_1/a_2 ratios calculated using the three methods? Provide potential reason(s) for why they are similar or different.

Ratio of peaks = 1.0096

a1/a2 ratio using fminsearch = 0.4767

a1/a2 ratio using backslash operator = 0.8547

They are all very different. The a1/a2 ratios are different because two different methods were used to find these ratios and the least squares minimization method found the best solution to linear equations that had no solution and the backslash operator found the exact ratio without minimizing the errors caused due to noise hence the negative value. The ratio of the peak intensities are 1 because the data sets were normalized such that their peaks intensities were equal to 1.

9) Discuss the advantages of and disadvantages of each type of fitting method and when each type can/should be used.

Least squares minimization is a good method to use when there are many unknown equations as well as when there is noise present because the method reduces the error as much as possible caused by this noise however it only provides an approximate solution. The back slash operator method (or the inverse matrix method) is a good method to use for small data sets to get an exact value however, when used with noisy data, the results might be distorted and inaccurate.

how to make one subplot bigger than the other on matlab

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You can create subplots of different sizes in MATLAB using the subplot function along with the Position property to specify the size and position of each subplot. Here's a general approach:

matlabCopy code

In this example, the second subplot will appear larger than the first one because we've increased its height and decreased its distance from the bottom. Adjust the values in pos(2) and pos(4) according to your preference to achieve the desired size difference between the subplots. Also, replace x1, y1, x2, and y2 with your actual data and plotting commands for each subplot.

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how to get a newline in the xlabel of matlab

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You can insert a newline character in the **xlabel** of MATLAB using the **sprintf** function to create a string with a newline character and then pass it to the **xlabel** function. Here's an example:

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In this example, **sprintf** is used to create a string with two lines separated by **\n**, which represents a newline character. Then, the resulting string is passed to **xlabel** to set the label of the x-axis with two lines of text. You can adjust the text content as needed for your specific use case.



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how to increase width of a subplot

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You can adjust the width of a subplot by changing its position within the figure. The position of a subplot is defined by a four-element vector [left, bottom, width, height], where (left, bottom) specifies the position of the subplot's lower-left corner, and (width, height) specify the width and height of the subplot, respectively. Here's an example of how you can increase the width of a subplot:

matlabCopy code

In this example, the width of the second subplot is increased by multiplying its current width by 1.5. You can adjust the multiplication factor according to your preference to achieve the desired width for the subplot.

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how to print symbols on the y axis of a plot matlab

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To print symbols on the y-axis of a plot in MATLAB, you can use the yticks and yticklabels functions. Here's an example of how to achieve this:

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In this example, ytick_locations defines the positions of the y-axis ticks, and ytick_labels specifies the symbols you want to print at those positions. Then, yticks and yticklabels are used to set the tick locations and labels, respectively, for the y-axis. You can adjust the ytick_locations and ytick_labels arrays according to your specific symbols and positions.

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i want to label my y-axis as mu

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If you want to label your y-axis as "µ" (the Greek letter mu), you can directly use it in the ylabel function:

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This will label your y-axis as "µ-axis". MATLAB supports the use of Greek letters and other symbols directly in strings like this.

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i want to label my y-axis mu sub a

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To label your y-axis as "µ sub a," you can use the Unicode representation for subscript characters. In MATLAB, you can use LaTeX syntax to achieve this. Here's how you can do it:

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In MATLAB, the LaTeX syntax μ_{a} will produce " μ sub a" as the y-axis label.

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and lambda

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For " λ sub a" labeling, you can similarly use LaTeX syntax:

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This will label your y-axis as "λ sub a".

To smooth data using a 5-point moving average, you can create a moving window of size 5 and compute the average of the values within that window for each data point. Here's how you can do it in MATLAB:

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In this code:

- x and y represent your original data.
- We create an array moving_average to store the smoothed values.
- We iterate through each data point, starting from the third one and ending before the last two.
- For each data point, we compute the average of the values within the 5-point window centered around that point.
- We plot the original data and the smoothed data.

Adjust the loop indices according to your data length and adjust the starting and ending points according to the size of your moving window.



what about the smooth function

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Yes, you can use the smooth function in MATLAB to apply various smoothing techniques, including the moving average. The smooth function offers a more convenient and efficient way to perform smoothing compared to manual implementation. Here's how you can use it to apply a 5-point moving average to your data:

matlabCopy code

In this code:

- We use the smooth function to apply a 5-point moving average to the data stored in the vector \mathbf{y} .
- The windowsize variable specifies the size of the moving window for the smoothing operation.
- The smoothed data is stored in the variable smoothed_data.
- We plot both the original data and the smoothed data for comparison.

Using the smooth function provides a more concise and readable way to perform smoothing operations, especially for larger datasets or when multiple smoothing techniques need to be applied.

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how to make dotted lines on matlab

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You can create dotted lines in MATLAB plots by specifying the line style when plotting. MATLAB provides several line styles, including dotted lines. Here's how you can create a plot with dotted lines:

matlabCopy code

In this code:

- 'b--' specifies a blue dotted line for the plot of y1.
- 'r:' specifies a red dotted line for the plot of y2.
- The '--' and ':' symbols represent the line styles for dotted lines.

You can change the colors and line styles according to your preference. For example, 'k:' would represent a black dotted line, 'g:' would represent a green dotted line, and so on.

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how to remove imaginary numbers in a large vector

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To remove imaginary numbers from a large vector in MATLAB, you can use the **real** function. The **real** function returns the real part of complex numbers and converts real numbers to their own values. Here's how you can use it:

matlabCopy code

In this code:

- randn (1, 100) generates a vector of 100 random real numbers.
- 1i * randn (1, 100) generates a vector of 100 random imaginary numbers.
- large_vector combines the real and imaginary parts to create a complex vector.
- real_vector extracts the real part of large_vector, effectively removing the imaginary parts.

Now, real_vector will contain only the real parts of the elements in large_vector. If there were no imaginary parts in the original vector, using real won't affect the values.



how to increase font size and add a grid to my graphs

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To increase the font size and add a grid to your graphs in MATLAB, you can use the set function to adjust the properties of the current axes. Here's how you can do it:

matlabCopy code

In this code:

- FontSize property is used to set the font size for the xlabel, ylabel, and title.
- grid on adds a grid to the plot.
- set(gca, 'FontSize', 12) adjusts the font size of the tick labels on both axes.

You can adjust the font sizes (e.g., 14 for xlabel, ylabel, 16 for title, and 12 for tick labels) according to your preference.

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how to make font bold

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To make the font bold in MATLAB, you can use the **'FontWeight'** property when setting the font properties for labels and title. Here's how you can make the font bold in your plot:

matlabCopy code

In this code:

- **'FontWeight**', 'bold' is added to specify that the font should be bold for the xlabel, ylabel, and title.
- The font size and other properties remain as shown in the previous example.

Adjust the font sizes and other properties according to your preference.