

**DON'T PANIC****Error analyses (25%):**

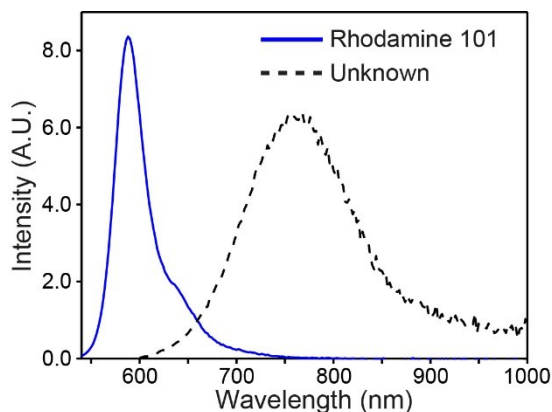
1. An old way to measure the quantum yield (QY) of an unknown chromophore is to compare its integrated emission spectrum to a dye standard. The formula is:

$$QY_{\text{sample}} = QY_{\text{ref}} \times \left( \frac{A_{\text{ref}}}{A_{\text{sample}}} \right) \left( \frac{\text{Int}(F)_{\text{sample}}}{\text{Int}(F)_{\text{ref}}} \right) \left( \frac{n_{\text{sample}}}{n_{\text{ref}}} \right)^2$$

where **ref** refers to a reference dye (here Rhodamine 101,  $QY_{\text{ref}} = 91\%$  in ethanol), **Int(F)** is the baseline corrected integrated fluorescence, **A** is the absorbance at the excitation wavelength, and **n** is the index of refraction of the solvent.

a) Please calculate the QY of an unknown sample in hexane using the following information: **(5 pts)**

$A_{\text{ref}} = 0.04$ ,  $A_{\text{sample}} = 0.1$ ,  $n_{\text{sample}} = 1.375$ ,  
 $n_{\text{ref}} = 1.36$ . As for the integrated emissions, you need to subtract the background fluorescence ( $5.0 \pm 0.01$ ); here are the data:



$\text{Int}(F)_{\text{sample}} = (85 \pm 0.04) - (5.0 \pm 0.01)$ , and  $\text{Int}(F)_{\text{ref}} = (50 \pm 0.03) - (5.0 \pm 0.01)$

b) Now calculate the error in the QY. To do so, simply use the error of the data concerning the integrated fluorescence of the reference and sample, including the baseline correction. Show your work or no points! Hint: formulas are provided, and you have to propagate error more than once! **(15 pts)**

2. The result from question 1b is an error that is very very small (if you did it right!). Do you think the measurement is really that accurate? If not, how would you meaningfully determine the error of the unknown sample's quantum yield? **(5 pts)**

**Fluorescence (65%)****Methods and Instrumentation :**

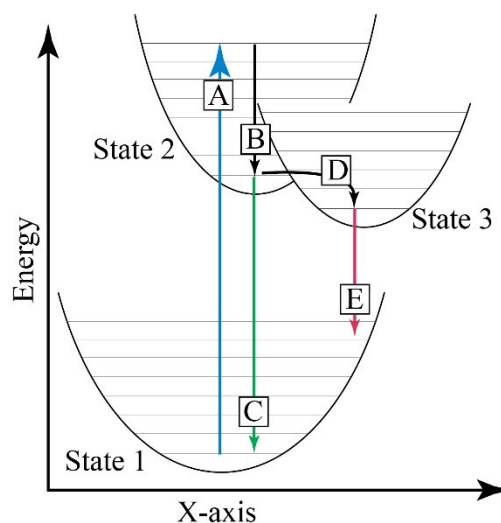
3. Please place the following regimes of the electromagnetic spectrum in order of increasing energy (presently they are in alphabetical order): **(7 pts)**  
**(gamma ray) (infrared) (microwave) (radiowave) (terahertz) (visible) (X-ray)**

4. a) Define quantum yield. (2 pts)  
 b) Define molar extinction. What are the units of molar extinction? (2 pts)  
 c) What kind of values do you expect for the molar extinction of a dye? (2 pts)
5. Which is more sensitive, absorption or fluorescence and why? (5 pts)
6. What is photoluminescence excitation (PLE) spectroscopy? (5 pts)
7. What is an integrating sphere and what do you use it for? (5 pts)
8. Please discuss some of the equipment necessary to do optical single molecule spectroscopy. (5 pts)
9. Define FRET. What is it good for? (5 pts)

**Photophysics:**

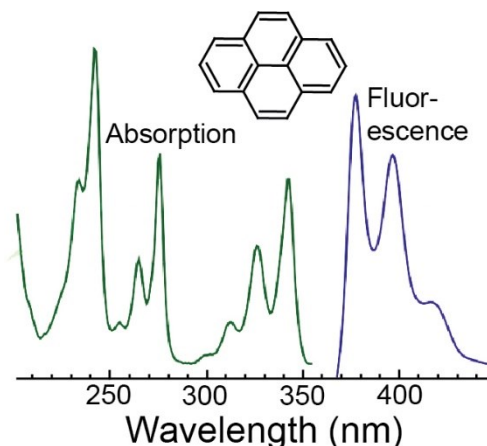
10. Here is a Jablonski Diagram:

- a) Can you identify processes A→E? Hint: not shown is internal conversion, and only radiative decay processes to the ground state are depicted.  
 b) Describe states 1, 2 & 3.  
 c) What does the x-axis represent?  
 (9 pts)



11. What is a typical lifetime for fluorescence? For phosphorescence? (4 pts)

12. Pyrene is a well-known organic chromophore, its structure is shown here, including the absorption and emission spectra. Why does the absorption and emission of pyrene look this way, i.e. with the sharp peaks superimposed over broader ones? (5 pts)



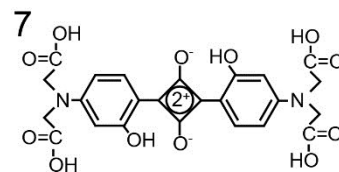
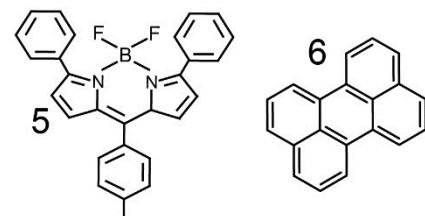
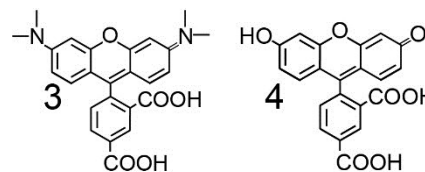
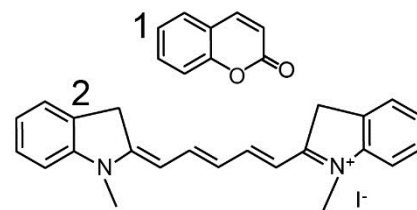
**Dyes:**

**13. Match.** Please correctly label 1-2-3 etc. to the following:

- a) Bodipy
- b) Coumarin
- c) Cy.5
- d) Fluorescein
- e) perylene
- f) Rhodamine
- g) squaraine

- h) Two of the dyes are in the xanthene family. Which two?
- i) Which dye is in the cyanine family?

**(9 pts)**



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**Safety (10%, 2 pts each)**

14. Which concentrated acid is worse: HCl or H<sub>2</sub>SO<sub>4</sub> and why?

15. Which acid is worse, HF or HCl and why?

16. What should you do if an Illinois OSHA inspector is knocking on your lab door and wants to enter the lab?

17. Other than freezing your skin, what's a major hazard of liquid nitrogen?

18. What is an MSDS?

## EQUATIONS

$$\text{Var} = \frac{1}{N} \sum_{i=1}^N (x_i - \bar{x})^2 \quad \sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \bar{x})^2} = \sqrt{\text{Var}}$$

Function	Error of function output
$f(x, y) = x \pm y$	$\sigma_f = \sqrt{\sigma_x^2 + \sigma_y^2}$
$f(x, y) = x \cdot y$	$\sigma_f = (x \cdot y) \sqrt{\frac{\sigma_x^2}{x^2} + \frac{\sigma_y^2}{y^2}}$
$f(x, y) = \frac{x}{y}$	$\sigma_f = \left(\frac{x}{y}\right) \sqrt{\frac{\sigma_x^2}{x^2} + \frac{\sigma_y^2}{y^2}}$
$f(x) = \frac{x}{c}$ , c has no error.	$\sigma_f = \frac{ \sigma_x }{ c }$
$f(x) = c \cdot x$ , c has no error.	$\sigma_f =  c  \cdot  \sigma_x $