

Exercise number: 3	Title: Measurement of fluorescence excitation and emission spectra and lifetime of rhodamine 6G dye in isopropanol
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Tasks

You are given three samples of rhodamine 6G (R6G) solution in isopropanol designated R6G_A, R6G_B and R6G_C with decreasing concentration of the dye. Absorbance of these solutions at 531 nm is approximately 1.7, 0.4 and 0.1, respectively. Your goal is to measure the emission and excitation spectra of samples under varying conditions (excitation wavelength, optical filters, right angle vs. front face geometry), identify any differences or unexpected features, discuss them with classmates and lecturer and provide explanations.

The final task is to determine the lifetime of R6G_B with use of nanosecond flash lamp and TCSPC technique.

Procedure

1. Measure absorption spectra of given solutions in 1 cm cuvettes. Use cuvette filled with solvent as a reference. (R6G_A, R6G_B, R6G_C)
2. Measure fluorescence emission spectrum of R6G_C in the range from 480 nm to 750 nm, $\lambda_{\text{exc}} = 348$ nm, excitation bandwidth (ExcBW) 5 nm, emission bandwidth (EmBW) 1 nm, 1 nm step and 0.2 s dwell time. (R6G_C_em_348nm)
3. Change λ_{exc} to 298 nm and repeat measurement. (R6G_C_em_298nm)
4. Place 455 nm cut off filter into the emission beam path and repeat previous measurement. (R6G_C_em_298nm_filter455nm)
5. Measure fluorescence excitation spectrum of R6G_C in the range from 200 nm to 580 nm, $\lambda_{\text{em}} = 600$ nm, excitation bandwidth (ExcBW) 1 nm, emission bandwidth (EmBW) 5 nm, 1 nm step, 0.2 s dwell time and 550 nm cut off filter in the emission beam path. (R6G_C_ex_600nm_filter550nm)
6. Measure fluorescence excitation spectrum of R6G_B in the range from 200 nm to 580 nm, $\lambda_{\text{em}} = 600$ nm, excitation bandwidth (ExcBW) 1 nm, emission bandwidth (EmBW) 5 nm, 1 nm step, 0.2 s dwell time and 550 nm cut off filter in the emission beam path. (R6G_B_ex_600nm_filter550nm)
7. Measure fluorescence excitation spectrum of R6G_A in the range from 200 nm to 580 nm, $\lambda_{\text{em}} = 600$ nm, excitation bandwidth (ExcBW) 1 nm, emission bandwidth (EmBW) 3 nm, 1 nm step, 0.2 s dwell time and 550 nm cut off filter in the emission beam path. (R6G_A_ex_600nm_filter550nm)
8. Place 455 nm cut off filter into the emission beam path. Measure fluorescence emission spectrum of R6G_B in the range from 480 nm to 750 nm, $\lambda_{\text{exc}} = 348$ nm, excitation bandwidth (ExcBW) 3 nm, emission bandwidth (EmBW) 1 nm, 1 nm step and 0.2 s dwell time. (R6G_B_em_348nm_filter455nm)

9. Measure fluorescence emission spectrum of R6G_A in the range from 480 nm to 750 nm, $\lambda_{\text{exc}} = 348$ nm, excitation bandwidth (ExcBW) 1 nm, emission bandwidth (EmBW) 1 nm, 1 nm step and 0.2 s dwell time. (R6G_A_em_348nm_filter455nm)
10. Repeat previous measurement in front face geometry. (R6G_A_em_348nm_filter455nm_FF)
11. Place 550 nm cut off filter into the emission beam path. Measure fluorescence excitation spectrum of R6G_A in front face geometry in the range from 200 nm to 580 nm, $\lambda_{\text{em}} = 600$ nm, excitation bandwidth (ExcBW) 1 nm, emission bandwidth (EmBW) 3 nm, 1 nm step and 0.2 s dwell time. (R6G_A_ex_600nm_filter550nm_FF)
12. Measure decay curve of R6G_B with use of nanosecond flash lamp and TCSPC technique; $\lambda_{\text{exc}} = 350$ nm, $\lambda_{\text{em}} = 560$ nm, time window 50 μs . Place scattering solution (LUDOX) into sample holder and measure instrument response function (IRF); $\lambda_{\text{exc}} = \lambda_{\text{em}} = 350$ nm. Compute the lifetime of R6G_B from the decay curve and IRF with use of reconvolution fit.
13. Use measured data to plot following figures:

Figure 1 Absorption spectra of R6G_A, R6G_B and R6G_C

{R6G_A, R6G_B, R6G_C}

Figure 2 Fluorescence emission spectrum of R6G_C excited at 348 nm and emission spectra of R6G_C excited at 298 nm without and with 455 nm cut off filter (all normalized).

{R6G_C_em_348nm, R6G_C_em_298nm, R6G_C_em_298nm_filter455nm}

Figure 3 Fluorescence excitation spectra of R6G_A, R6G_B and R6G_C measured at 600 nm in right angle geometry and fluorescence excitation spectrum of R6G_A in front face geometry (all normalized).

{R6G_A_ex_600nm_filter550nm, R6G_B_ex_600nm_filter550nm, R6G_C_ex_600nm_filter550nm, R6G_A_ex_600nm_filter550nm_FF}

Figure 4 Normalized fluorescence emission spectra of R6G_A, R6G_B and R6G_C measured in right angle geometry and fluorescence emission spectrum of R6G_A measured in front face geometry (all normalized).

{R6G_A_em_348nm_filter455nm, R6G_B_em_348nm_filter455nm, R6G_C_em_298nm_filter455nm, R6G_A_em_348nm_filter455nm_FF}

Questions:

Q: What can you tell about relation between absorption and excitation spectra? How does the concentration of sample affect the shape of excitation spectra and what is the inner filter effect? What is the advantage of front face measurement? (Figures 1 and 3)

A:

Q: Why do the normalized fluorescence emission spectra of R6G_C measured at various excitation wavelengths (Figure 2) differ? What is the cut off filter good for here?

A:

Q: How (and why) do the normalized fluorescence emission spectra of R6G_A, R6G_B and R6G_C (Figure 4) differ? What is the reabsorption? What can be done to avoid reabsorption?

A:

Q: What was the value of measured lifetime of rhodamine 6G in isopropanol (R6G_B)?

A: ... ns