Lab Report of TCSPC
Influence of Environment on Molecular Process

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1 Basic Principle

Time-correlated single photon counting allows us to measure the time that a molecule stays in its excited state following excitation. The technique works as follows: a laser pulse hits the sample. After the molecules in the sample are excited, they can decay back to the ground state via several pathways, such as internal conversion, fluorescence, and intersystem crossing to the triplet state. The sample goes back to the ground state at a rate at the sum of all the possible decay pathways; the reciprocal of this rate is the excited state lifetime. Even if fluorescence is not the fastest decay pathway, the relaxation is a discrete, probabilistic process. As a result, there is some Poisson probability of detecting fluorescence photons. We can detect these photons using a PMT and build up an exponential decay. Fitting this decay gives us the excited state, or fluorescence, lifetime.
Set the initial fluorescence time as zero, the fluorescence spectra obeys the following formula:

$$I = I_0 e^{-t/\tau} \tag{1}$$

Hence the lifetime is easy to be calculated from spectra data.
Moreover, anistrotropy property of molecule in solution can also be obtained using TCSPC. Anistrotropy r is defined as

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \tag{2}$$

If the molecules obeys totally random distribution, $r = 0.4$. In the spectra of anistrotropy in TCSPC,

$$r(t) = r_0 e^{-t/\theta} \tag{3}$$

where θ represents the rotation correlation time.

$$\theta = \frac{\eta V}{RT} \tag{4}$$

where η represents the viscosity of solution. So after obtaining the value of θ from spectra, the volume of molecule V could be calculated directly.

2 Setup

Reverse mode.
Laser $\longrightarrow$ 2 beams$\Rightarrow$.
Beam 1 $\longrightarrow$ sample $\longrightarrow$ PMT detector $\longrightarrow$ discriminator $\longrightarrow$ stop signal
Beam 2$\longrightarrow$ CFD $\longrightarrow$DELAY$\longrightarrow$start signal
$\Rightarrow$ TAC$\longrightarrow$MCA$\longrightarrow$PC

Table 1: Channel time correspondance data

channels	238	388	687	990	1291	1595
time/ns	-6	-4	0	4	8	12

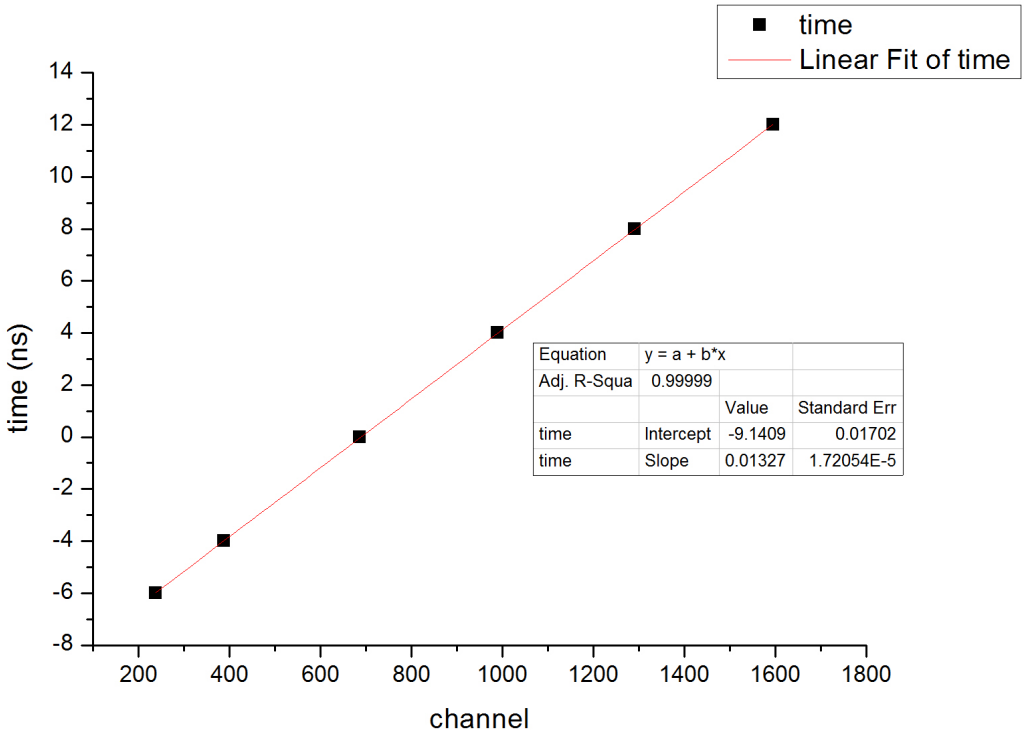


Figure 1: Linear fitting of channels and time

3 Channel Time Correspondance

Firstly, we do experiment on the channel time correspondance. In original data, x axis represents channels, which has a linear correspondace towards time.
According to data in Table 1, we could get the linear equation about channeal and time: $y = -9.1409 + 0.0132x$.
So we could substitute channel with time in the following data.

4 Lifetime of D149

With the data of liquid solution and solid state, the relative results could be obtained by convolution. Here is the result we obtained:
Here, time was in the scale of channel. If we multiple the data with the slope 0.0132, lifetime comes into ns scale, shown in Table 2:

```
liquid sample
Passes: 100, Iterations: 6985, Chi2=1.19333
T0=26.6175        A0=1              (66.7 %, 52295.9)
T1=8.14844        A1=0.499184       (33.3 %, 26105.3)
Full ampl=38403.8 (*52295.9)
Background=0.347717
Shift=1.62345

solid sample
Passes: 100, Iterations: 6773, Chi2=1.15075
T0=163.411        A0=1              (58.5 %, 6186.45)
T1=47.0638        A1=0.708327       (41.5 %, 4382.03)
Full ampl=8029.45 (*6186.45)
Background=36.3429
Shift=-0.5196
```

Figure 2: Results with convolution

Table 2: Life time of D146

States	Liquid	Solid
time/channel	26.62	163.41
time/ns	0.35	2.16

5 Anistrotropy of Rhodamine 6G

When the data of intensity-time of Rhodamine 6G in different direction (horizontal and vertical) are collected, anisotropy-time relations are obtained after treating them with Equation(1).
The original data are shown in Figure(3).
Here can we observe that only after $t > 0$, the molecule started to obey equation(1). But after a long time, the fluctuation of signal is too large. Hence, I choose the time period $[0, 15]$ns to do curve fitting with equation(1).
From the fitting results in figure(4), we list the rotation correlation time θ in Table 3.

6 Questions

1. Imagine two compounds with similar fluorescence quantum yields (e.g. $\Phi_{fl} = 1$) but very different absorption coefficients (ε). Now assume two samples of these with similar absorption (at a given wavelength). What would be the difference between their time-resolved fluorescence spectra? Consider the total fluorescence and how the fluorescence would be distributed over time.

Since the quantum yields are the same, when 2 samples have similar absorption, the exponential fluorescence spectra have similar integral area. According to

$$A = \log(\frac{I_0}{I}) = \varepsilon cl \tag{5}$$

the larger absorption coefficients ε means smaller life time τ. To make sure the same integral area, the intial value of I becomes larger and the spectra decreases faster.

Table 3: Rotation correlation time θ

Liquid	100%EtOH	75%EtOH	50%EtOH
θ/ns	0.372	1.490	6.010

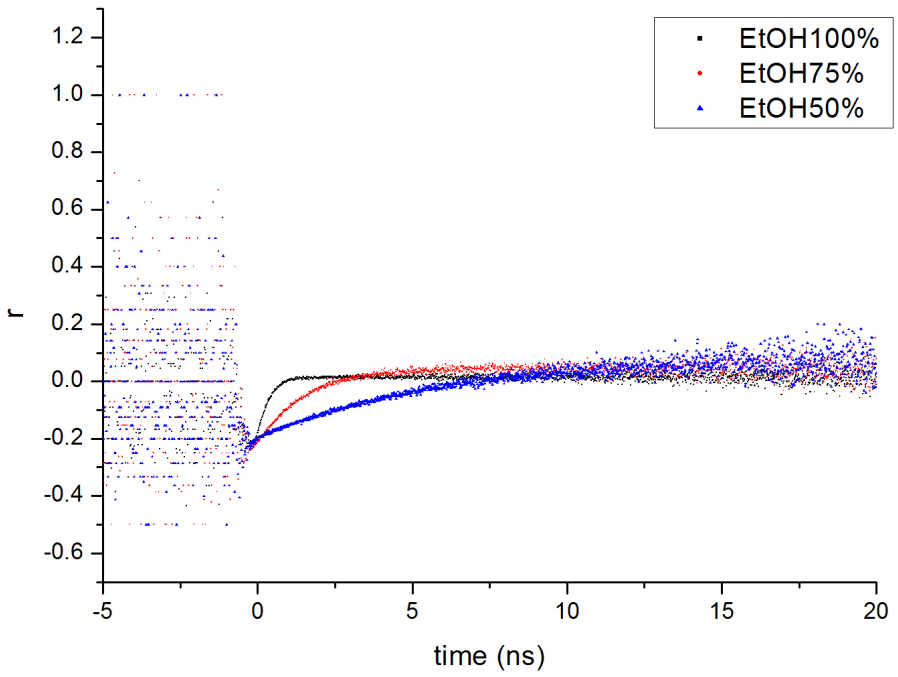
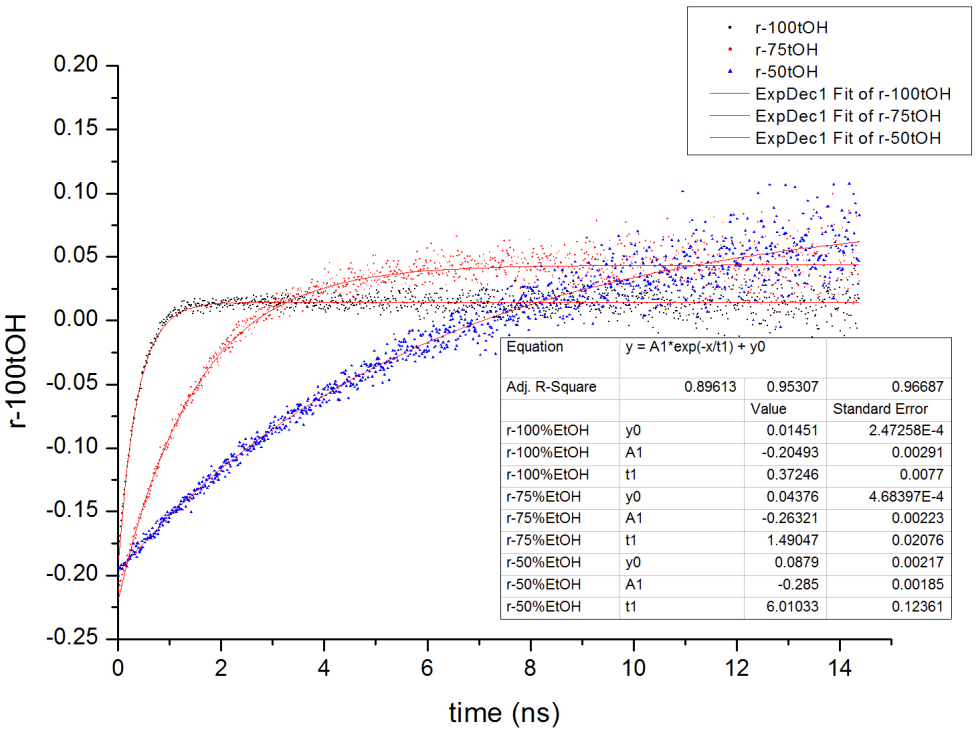


Figure 3: r-time relation of three solutions



Equation	y = A1*exp(-x/t1) + y0		
Adj. R-Square	0.89613	0.95307	0.96687
		Value	Standard Error
r-100%EtOH	y0	0.01451	2.47258E-4
r-100%EtOH	A1	-0.20493	0.00291
r-100%EtOH	t1	0.37246	0.0077
r-75%EtOH	y0	0.04376	4.68397E-4
r-75%EtOH	A1	-0.26321	0.00223
r-75%EtOH	t1	1.49047	0.02076
r-50%EtOH	y0	0.0879	0.00217
r-50%EtOH	A1	-0.285	0.00185
r-50%EtOH	t1	6.01033	0.12361

Figure 4: r-time fitting of three solutions

2. What is the influence of laser intensity fluctuations (say within 20%) on the precision of the measurement in a TCSPC life-time experiment? Compare that with an anisotropy measurement and with fluorescence upconversion and transient absorption spectroscopy.

For life-time experiment, 20% laser intensity flucturations do not influence the result becasue only 1% intensity is measured for detection rather than the maximum value of the pulse.
For anisotropy measurement, it influences the experiment results because we should do summation and subtraction on different intensity values in different measure direction(vertical or horizontal). In fluorescence upconversion, it does't matter because average intensity are detected.

3. Discuss the different lifetimes that were obtained during the measurement for the different systems, taking into account the environments.

From the results in Table 2, the lifetime of D149 in solid state(2.16ns) is larger than that in liquid solution(0.35), which arises from the solid state restriction of the motion of D149.

4. Estimate the volume of rhodamine 6G in three different ways: a) use the (estimated) density and molecular mass and calculate the volume, b) estimate the diameter of the molecule by assuming standard bond lengths and calculate the corresponding volume if it was a sphere (how realistic is that model?), c) calculate the volume based on the time-resolved data in ethanol. Compare the three ways and comment on which you consider most reliable.

a)

$$V = \frac{m}{\rho} = \frac{479.02 g/mol}{1.26 g/cm^3 \times 6.623 \times 10^{23}/mol} = 0.574 nm^3$$

b) Diameter is about 12 C-C bonds length, which is 0.154 nm.

$$V = \frac{4\pi r^3}{3} = \frac{4 \times 3.14 \times 0.077^3}{4} = 1.91 \times 10^{-3} nm^3$$

c) In 100% EtOH, $\theta = 0.372 ns$. From equation(1),

$$V = \frac{\theta RT}{\eta} = \frac{0.372 \times 10^{-9} \times 8.314 \times 298.15}{1.074 \times 10^{-3} \times 6.023 \times 10^{23}} m^3 = 1.426 nm^3 \tag{6}$$

The second calculation is obviously wrong because molecules stay at a certain distance where their potential is zero in equilibrium. If we only take the distance of bond lengths into account, molecules must be at a great repulsion since they are so close to each other. Hence this result is smaller than other approximation results.

5. Based on the SPC data, calculate the rotational correlation time of rhodamine 6G in ethanol and ethanol/glycerol. Also, calculate the viscosity of the mixtures and compare to the calculated viscosity based on a simple linear interpolation between those of the two pure solvents.

Rotational correlation time is list in Table 3.
If we calculation viscosity from equation(1), we should determine the volume of molecule as $1.426 nm^3$ that I mentioned in question 4(c). The results of viscosity are shown in table 4. The difference of viscosity that calculated from different ways are extremely large when glycerol is added to the solution, which reveals the non-ideal property of ethanol/glycerol solution —we can not deals them as ideal solution and the viscosity of mixed solution can not be added simply.

6. Given a new molecule with completely unknown photophysics/-chemistry, suggest a good strategy on how to learn about possible processes that the molecule can undergo. Suggest possible techniques (flashphotolysis, SPC, pump-probe, upconversion, streak camera, ...), why you use those and what you can learn from these and what not (strengths and weaknesses). Keep in mind that practical aspects can be quite important

Table 4: Different viscosity

Liquid	100%EtOH	75%EtOH	50%EtOH
θ from TCSPC/ns	0.372	1.490	6.010
Viscosity	1.074	4.302	17.34
Linear viscosity calculation(298K)	1.074	234.3	467.5

in many experiments.

Given a new molecule, UV-vis and fluorescence emission spectra, which is extremely cheap and convenient, should be taken into account firstly. After these steps, we clearly know that if this molecule undergoes the $S_0 \rightarrow S_1$ or other transition at common wavelength range of light. And several state-to-state transition wavelength peaks are also obtained.
After pump-probe, different photophysical/-chemical process are shown. Then we obtain its lifetime of corresponding excited states with TCSPC measurement. Streak camera and upconversion are performed later.