How do you describe what a molecule does in an excited state?

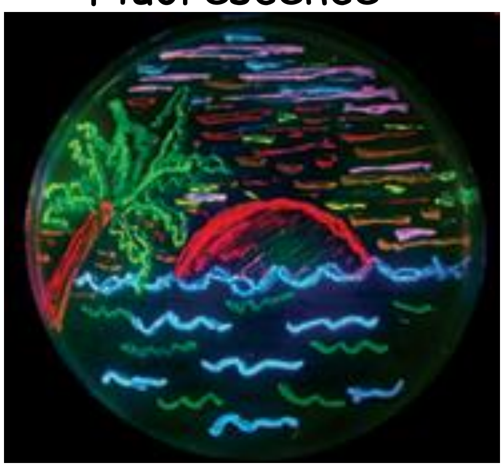
EM Spectrum of molecules

Vibrational Energy → Near - Infrared

Rotational Energy → Infrared

Electronic Energy → Visible and Ultra-Violet

Fluorescence



Roger Tsien Lab

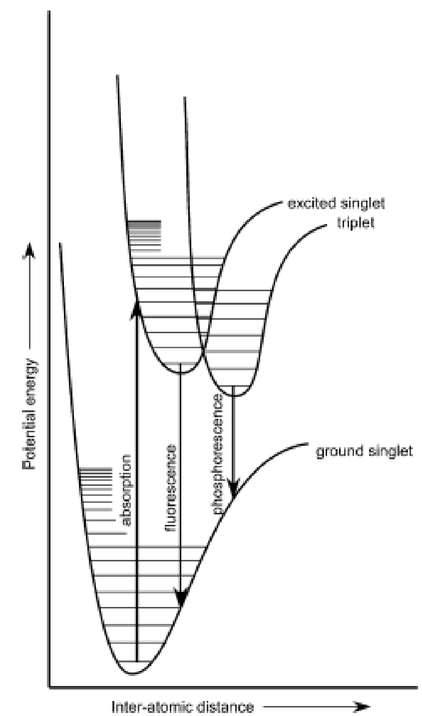


Diagram by Robert Clegg in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

Perrin-Jablonski energy diagram (S0, S1 and S2 transitions)

Alexander Jablonski

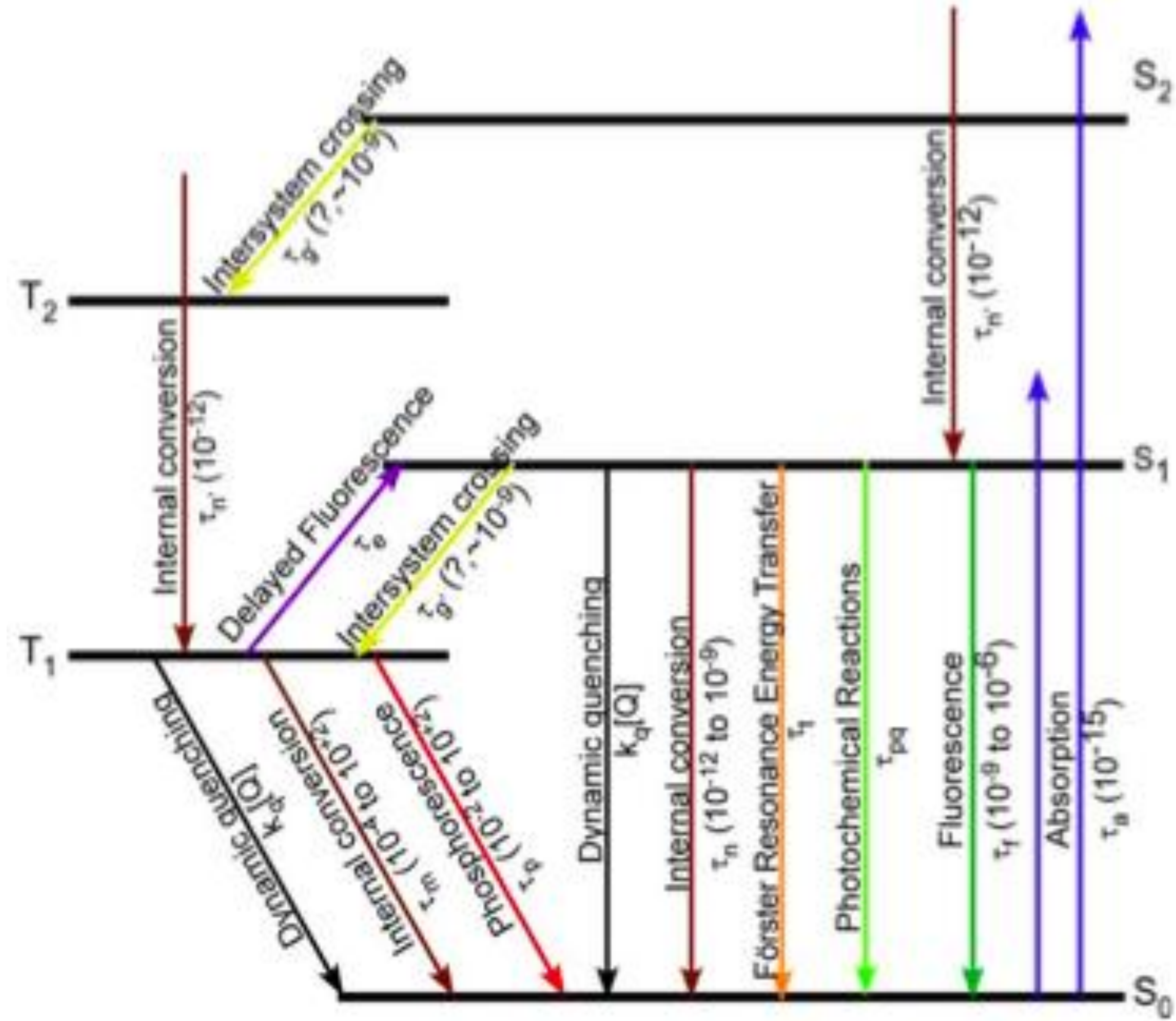
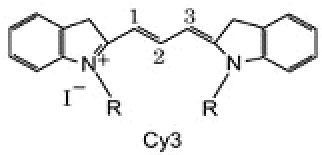
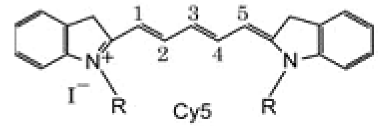


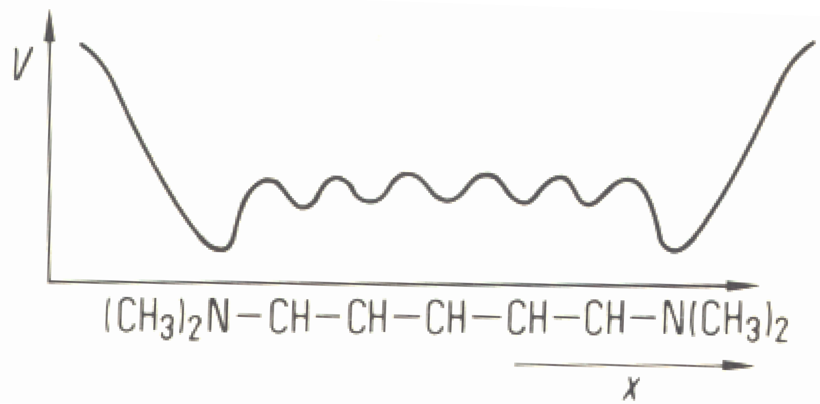
Diagram by Ulai Noomnarm in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

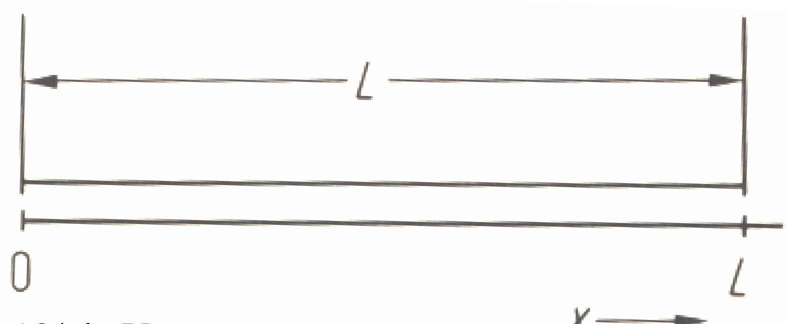
Simple Example: Cy Dyes





Pi electron cloud





Images from Topics in Applied Physics: Dye Lasers Volume 1 Schafer, F.P.

How does a molecule transit through the excited state

Quantum Mechanics: Fermi's Golden Rule

$$\lambda_{if} = \frac{2\pi}{\hbar} \left| M_{if} \right|^2 \rho_f$$

Transition probability (λ_{if})

Matrix element for the interaction (M_{if})

Density of final states (ρ_f)

Transition Dipole ($|M_{if}|^2$)

$$M_{if} = e\left\langle f \middle| \vec{r} \middle| i \right\rangle = e\int \psi_f r \psi_i d\vec{r}$$

$$(\tau)_{\substack{\text{natural}\\ \text{radiative}\\ \text{lifetime}}} = \frac{1}{\lambda_{if}}$$

This QM rate expression is true for any incoherent kinetic process where the system has equilibrated to a quasi-steady-state.

One way (F) to leave the excited state

Take one measurement

$$P(T_o + \Delta t) = 1 - k_F \Delta t$$

k_F = Time-indepdent rate constant for leaving excited state

Definition

$$time = T_o + \Delta t$$

Consider, $T_o = 0$

Take many measurements

$$time = T_o + M\Delta t$$

Then,

$$P(M\Delta t) = (1 - k_F \Delta t)^M = \left(1 - k_F \left(T/M\right)\right)^M$$

As $\Delta t \to 0,$ and $M \to \infty$

$$P(T) = \exp(-k_f T)$$

Probability of being in the excited state

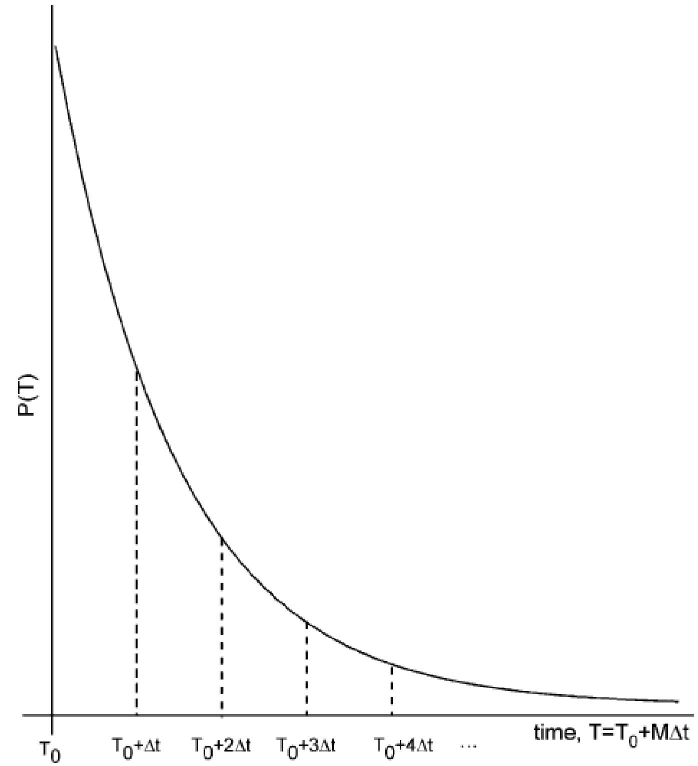


Diagram by Robert Clegg in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

Two ways to leave the excited state (F,t)

$$P(M\Delta t = T) = \left(1-(k_F+k_t)\Delta t\right)^M = \left(1-(k_F+k_t)\left(T/M\right)\right)^M$$

As $\Delta t \to 0,$ and $M \to \infty$

$$P(M\Delta t = T) = \exp\left(-(k_F+k_T)T\right)$$

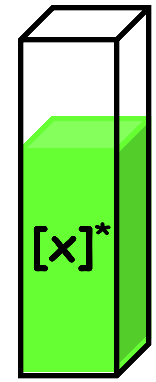
In general

$$\tau = \sum_i \frac{1}{k_i}$$

Decay constant is very sensitive to the environment!

Measuring the Depletion of the excited state

$$\left[\# x^{*}\right] = \left[\# x_{o}^{*}\right] e^{-\left(k_F + k_t\right)t}$$



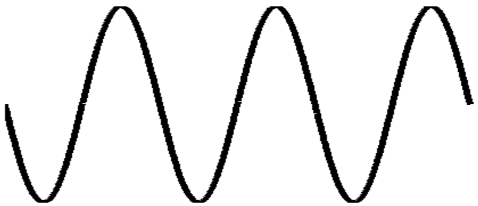
$$\left[\# x^{*}\right]\left(k_F\right)$$ = Intensity that you measure

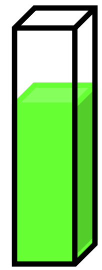
Intensity measured is proportional to the # of molecules in the excited state!

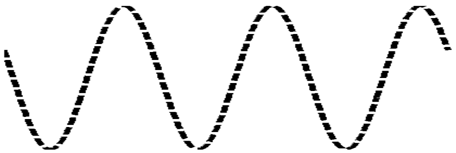
What sorts of instruments can you find that measure lifetimes?

$$E(t) = E_o + E_\omega \cos\left(\omega_E t + \varphi_E\right)$$

$$F(t) = F_o + F_\omega \cos\left(\omega_E t + \varphi_E - \varphi\right)$$







$$\tan\left(\varphi\right) = \omega_E \tau_\varphi$$

$$M = \frac{F_\omega / F_o}{E_\omega / E_o} = \frac{1}{\sqrt{1 + \left(\omega \tau_{Mod}\right)^2}}$$

What do you need?

-Intensity modulators

-Synchronization

Samples Described by Multiple Lifetimes

$$I(t) = \sum_i a_i e^{-t/\tau_i}$$

Some Examples:

- -ECFP (Enhanced Cyan Fluorescent Protein (FP))
- -mCherry
- -Teal FP

$$F(t) = E_o \sum_i a_i \tau_i + E_\omega \sum_i \frac{a_i \tau_i}{\sqrt{1 + (\omega_E \tau_i)^2}} \cos(\omega_E t - (\varphi_i - \varphi_E))$$

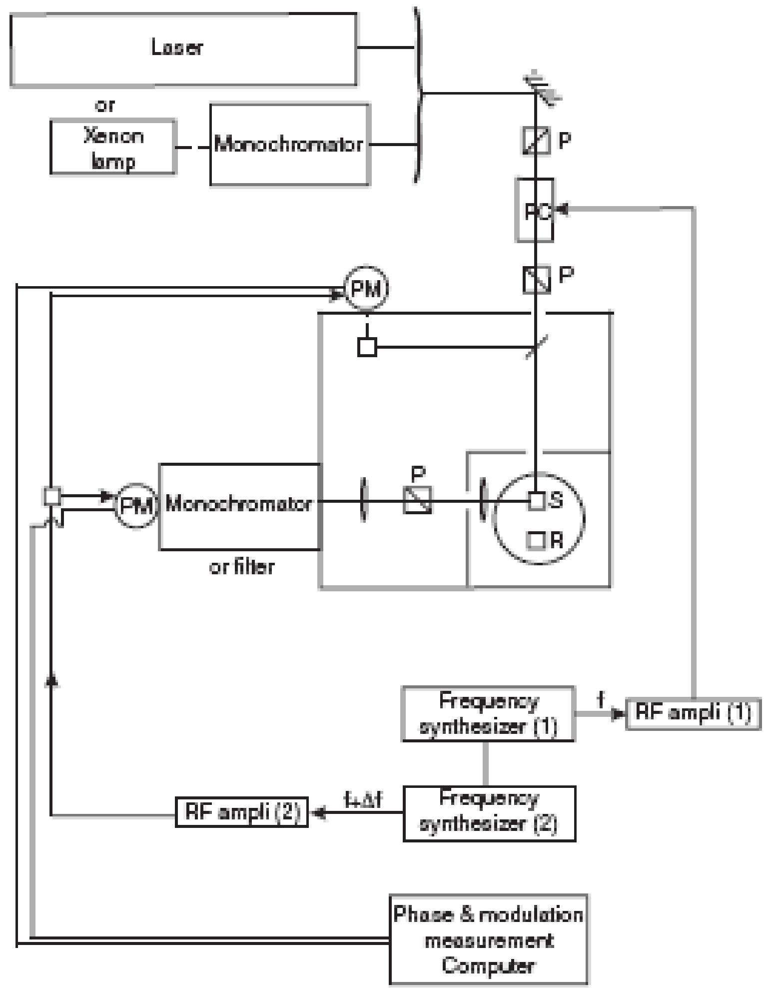
You still can only measure

$$\left(M, \varphi\right)$$

$$\frac{F(t)}{F_o} = 1 + \frac{E_\omega}{E_o} \sum_i \frac{\alpha_i}{\sqrt{1 + (\omega_E \tau_i)^2}} \cos(\omega_E t - (\varphi_i - \varphi_E))$$

$$\frac{F(t)}{F_o} = 1 + \frac{E_\omega}{E_o} M \cos(\omega_E t - (\varphi_i - \varphi))$$

Mixing is used in commercial instruments



$$[G(t)\bullet F(t)] = DC + \boxed{\text{Terms with frequencies } (\omega_E, \omega_G, \omega_E + \omega_G)} + \frac{G_\omega E_\omega}{2} M\left(\cos((\omega_G - \omega_E)t + \varphi_G - \varphi_E + \varphi)\right)$$

Images from Molecular Fluorescence by Bernard Valeur

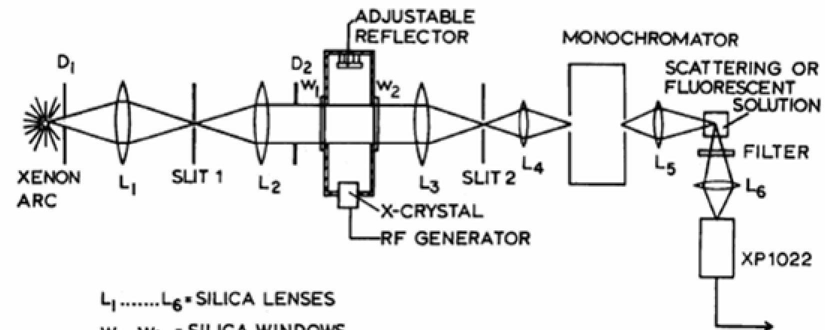
AOMs – Intensity Modulator

MEASUREMENTS OF SUBNANOSECOND FLUORESCENCE LIFETIMES WITH A CROSS-CORRELATION PHASE FLUOROMETER*

Richard D. Spencer and Gregorio Weber
Department of Chemistry and Chemical Engineering
University of Illinois
Urbana, Ill.

Annals of the New York Academy of Sciences Vol. 158 pp 361-376, 1969

CROSS CORRELATION FLUOROMETER OPTICS



L_1.......L_6 = SILICA LENSES
W_1, W_2 = SILICA WINDOWS
D_1, D_2 = DIAPHRAGM

FIGURE 4. Plan of the optics of fluorometer.

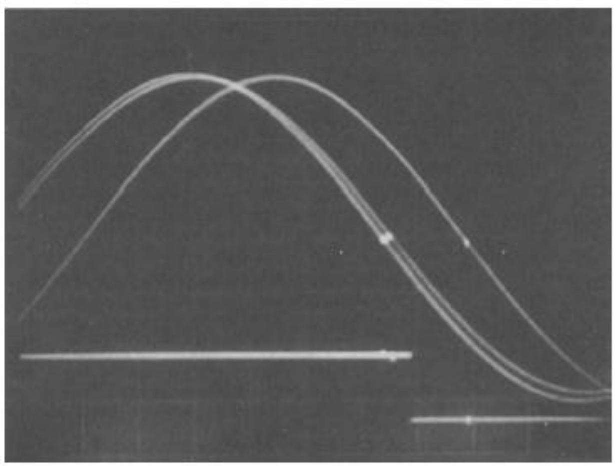


FIGURE 7. The figure shows the cross-correlation photocurrents from a scattering solution (first from left), a solution of NADH in phosphate buffer, pH 7.0, 17° C (second from left), and a solution of fluorescein (1 μgm/ml) in 0.01 M NaOH (right).

-limitations in available modulation frequencies

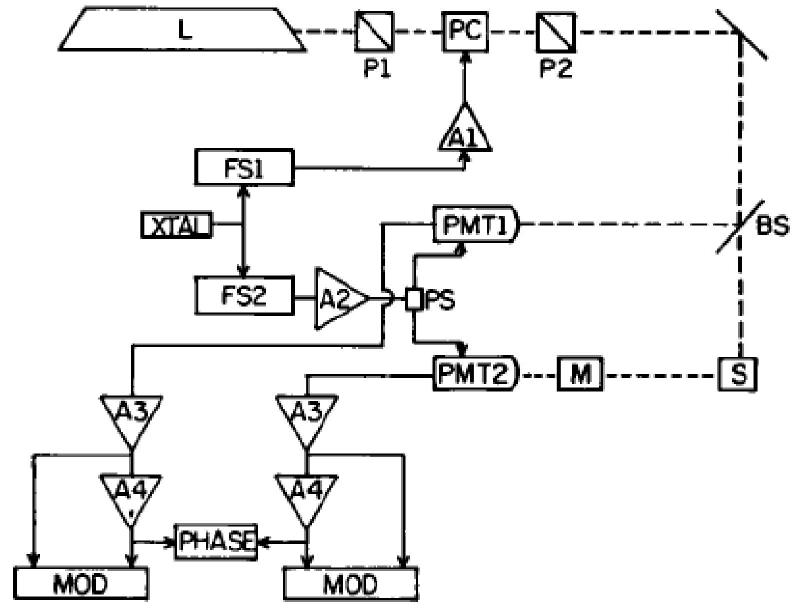
-variations in the intensity modulation caused by temperature

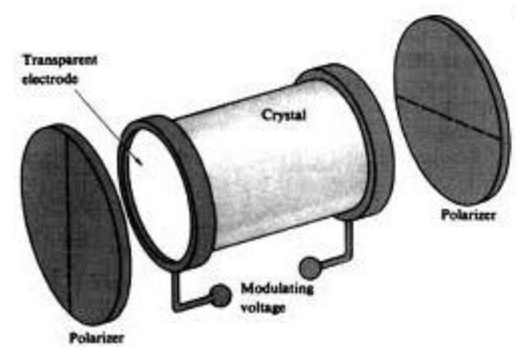
Pockels Cell

A CONTINUOUSLY VARIABLE FREQUENCY CROSS-CORRELATION PHASE FLUOROMETER WITH PICOSECOND RESOLUTION

E. GRATTON AND M. LIMKEMAN
Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Biophysical Journal Vol. 44 (1983) pp 315-324



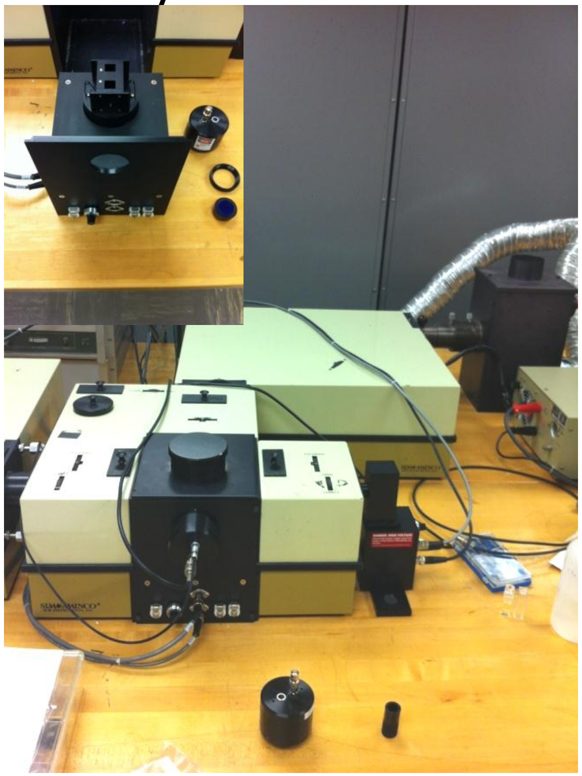


Hecht Optics

Figure 8.57 A Pockels cell.

Directly Modulated Diode

System in ESB



Laser Diodes -> (405nm,436nm,473nm,635nm,690nm,780nm,830nm)

LEDs -> (280nm,300nm,335nm,345nm,460nm,500nm,520nm)

ISS SLM Phoenix Upgrade

Original System

New Upgrades

PMT Housing

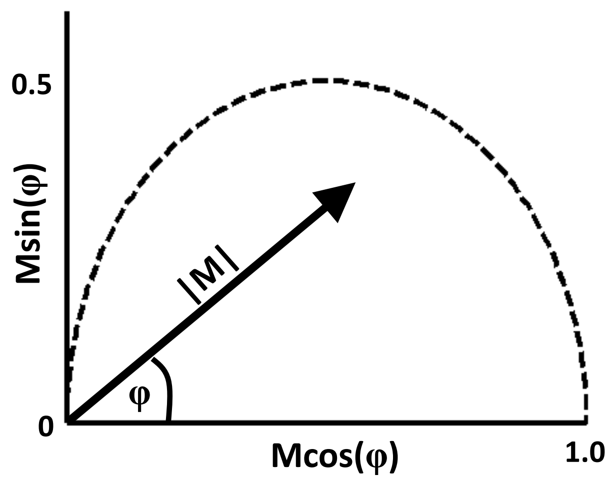
New Acquisition Box

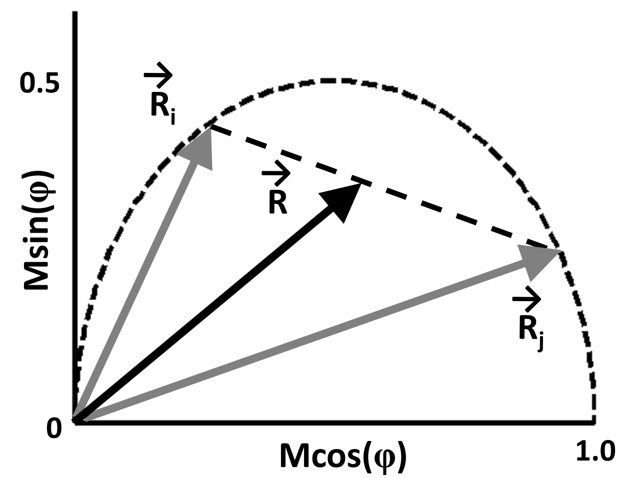
Stepper Motor Controller

-photon counting

-Measures nanosecond lifetime

The Polar Plot





Vectors on the Polar Plot

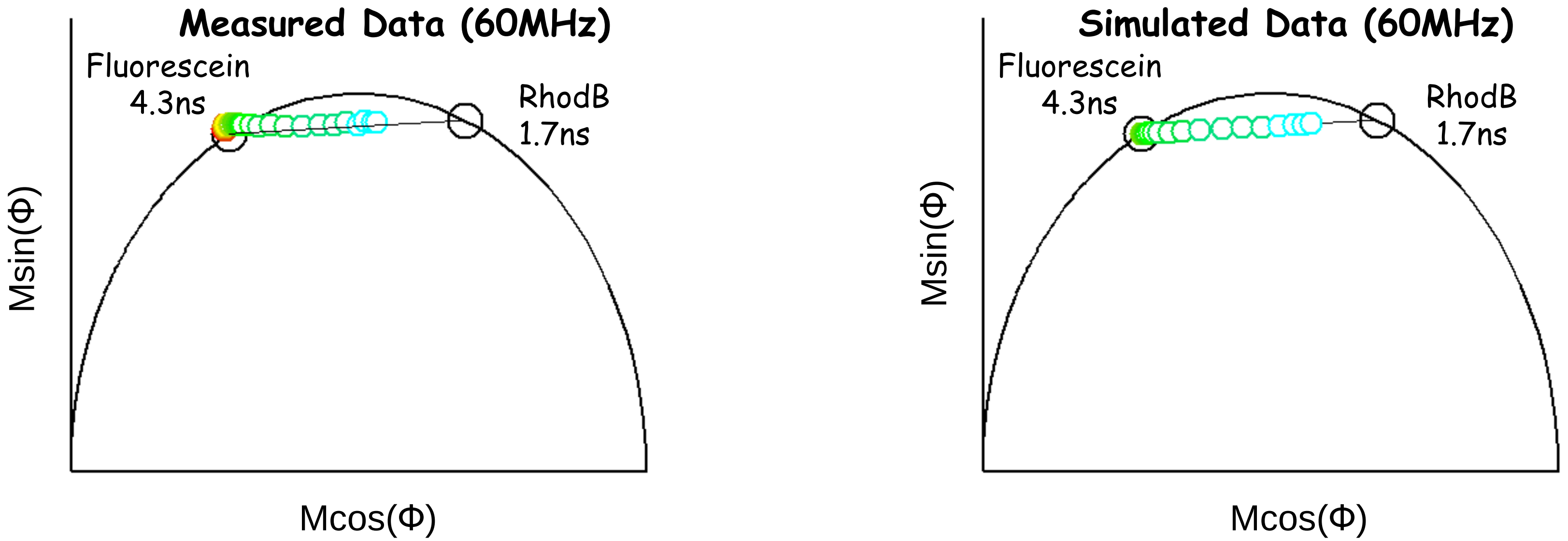
$$\vec{R} = M\cos(\varphi)\hat{x} + M\sin(\varphi)\hat{y}$$

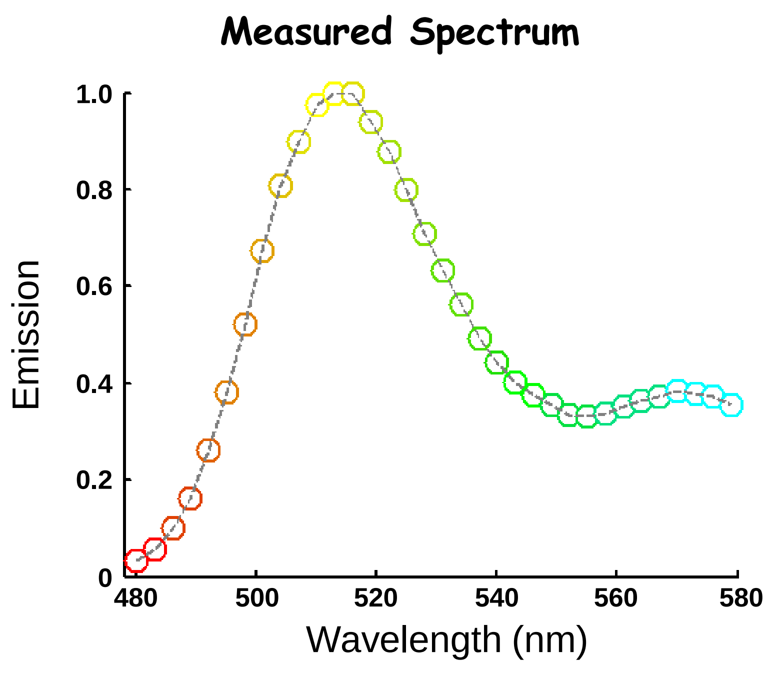
$$\vec{R} = \alpha_i \vec{R}_i + \alpha_j \vec{R}_j$$

$$\vec{R} = \left(\alpha_i M_i \cos(\varphi_i) + \alpha_j M_j \cos(\varphi_j)\right)\hat{x} + \left(\alpha_i M_i \sin(\varphi_i) + \alpha_j M_j \sin(\varphi_j)\right)\hat{y}$$

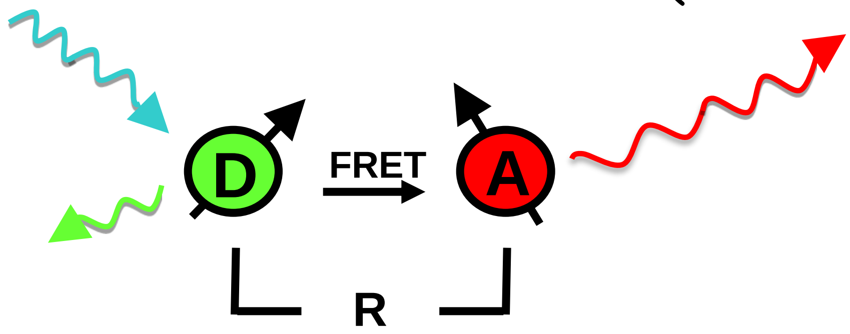
-movement of the vector depends on the emitted intensity of each species *(i)*

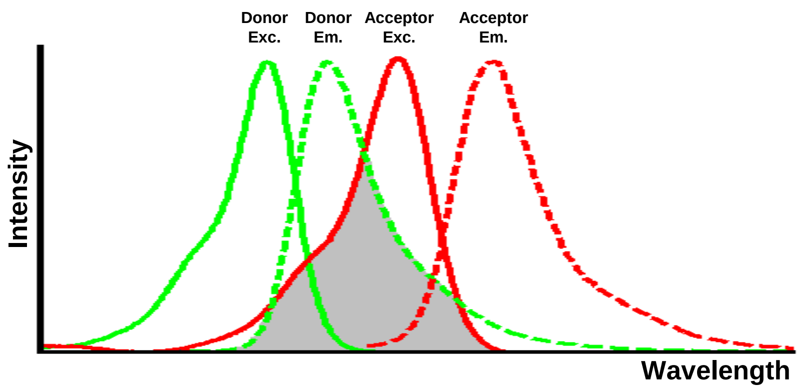
Spectral Analysis on the SLM

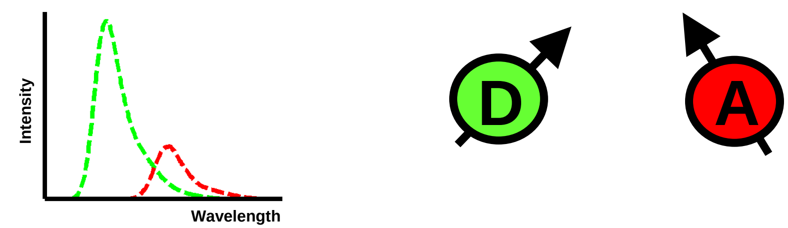




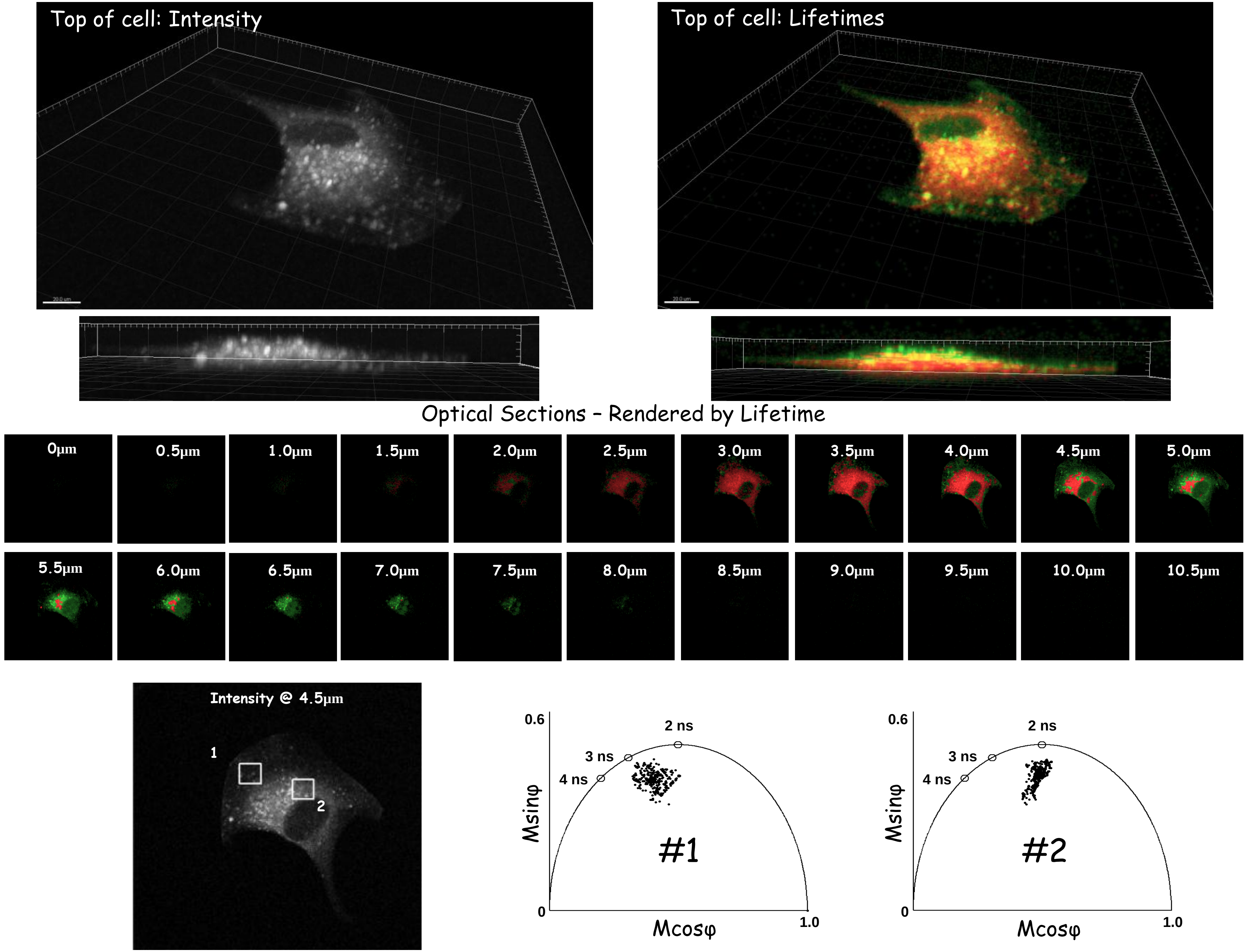
Quantum Yield/FRET





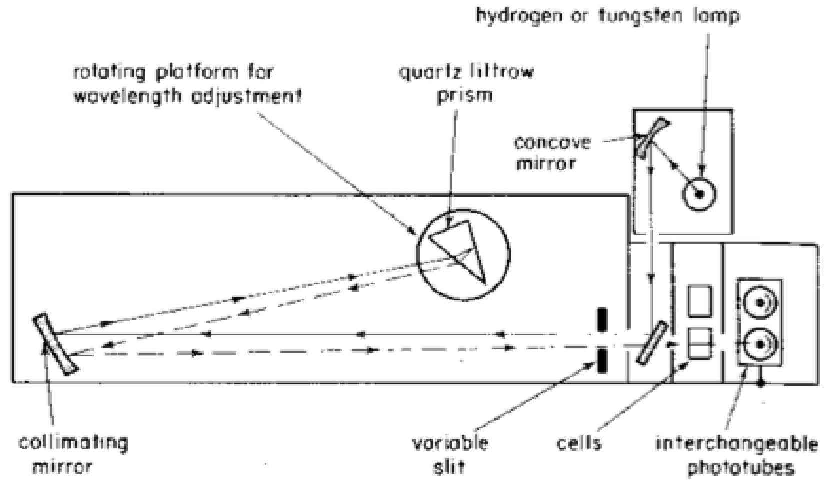


$$\text{Quantum Yield of Energy Transfer} = \frac{\text{Rate of Energy Transfer}}{\text{Total de-excitation rate}} = \frac{\left(\frac{1}{\tau_{DA}}\right) - \left(\frac{1}{\tau_D}\right)}{\left(\frac{1}{\tau_D}\right)} = 1 - \frac{\tau_{DA}}{\tau_D}$$

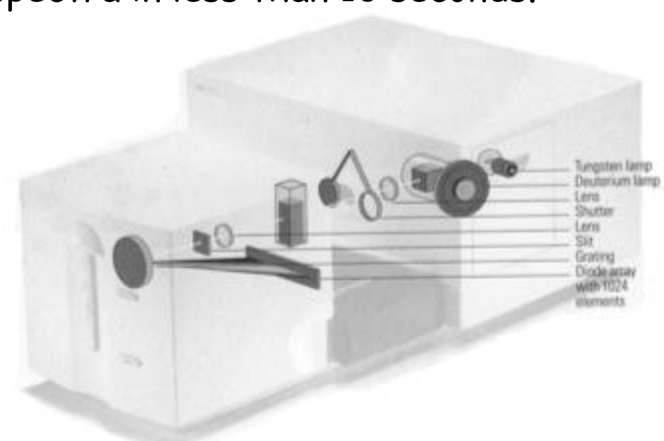

Top of cell: Intensity
Top of cell: Lifetimes
Optical Sections – Rendered by Lifetime
0μm
0.5μm
1.0μm
1.5μm
2.0μm
2.5μm
3.0μm
3.5μm
4.0μm
4.5μm
5.0μm
5.5μm
6.0μm
6.5μm
7.0μm
7.5μm
8.0μm
8.5μm
9.0μm
9.5μm
10.0μm
10.5μm
Intensity @ 4.5μm
1
2
0.6
2 ns
3 ns
4 ns
Msinφ
#1
0
Mcosφ
1.0
0.6
2 ns
3 ns
4 ns
Msinφ
#2
0
Mcosφ
1.0

Measuring Absorbance

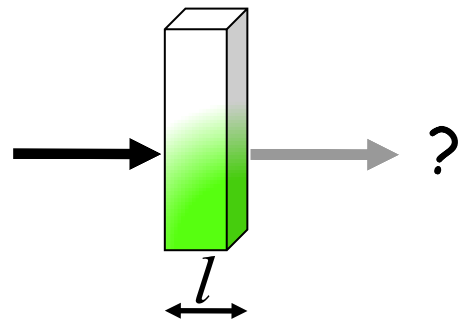
One of the very first commercially available instrument that measures absorbance was the Beckman DU



Machine nowadays that utilizes diffraction grating and diode array detector can acquire an absorbance spectra in less than 10 seconds.



Absorption (S_0-S_1)



Beer-Lambert's Law

$$\log(I_0) - \log(I) = \varepsilon c l$$

Extinction coefficient: Concentration