Introduction to Single-molecule Fluorescence Experiments

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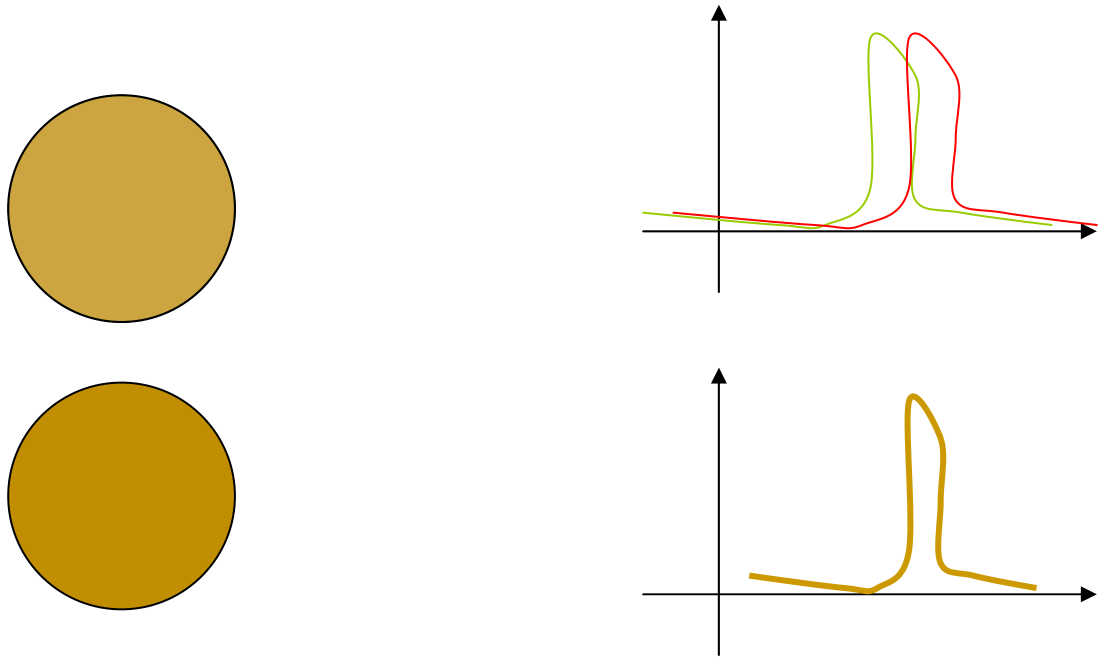
Outline

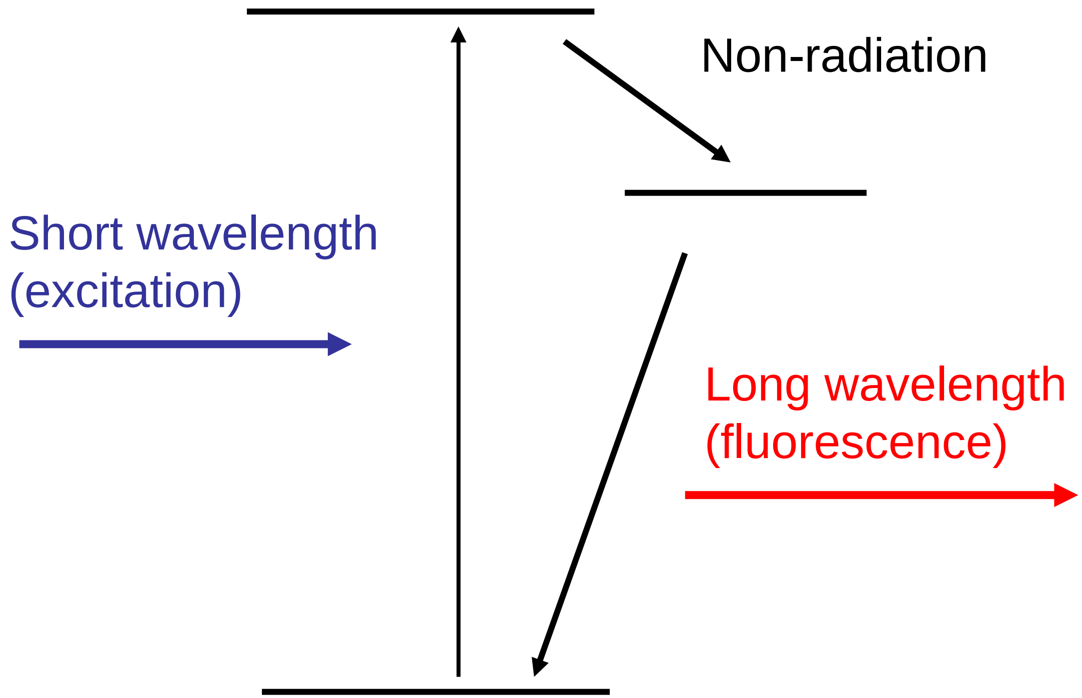
- Why single molecule?
- What is fluorescence?
- Sample preparation
- Experimental setup
- Time trace
- Photoluminescent (PL) spectrum
- Autocorrelation
- Fluorescence lifetime

Why Single molecule?



- The ability to observe phenomena otherwise obscured in ensemble measurements
- Obtain the histogram of molecular properties

What is fluorescence

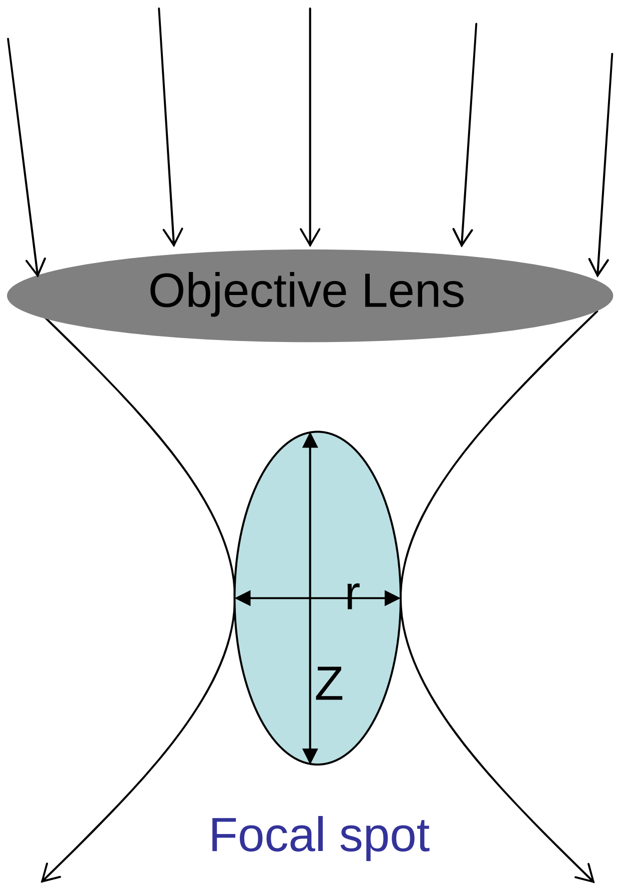


Including:
- Organic dye
- Conjugated polymer
- Quantum dot
- Fluorescent protein
- Defect centers in a diamond
- Mental nano-particles

Sample Preparation

- Dilute..Dilute..Dilute
- Proper solvent
- High quality solvent
- Ultrasonic
- Environment

Dilute..Dilute..Dilute



$$r = 0.5\frac{\lambda_0}{NA_{obj}} \approx 200\text{nm} \qquad Z = \frac{2\lambda_0 n}{(NA_{obj})^2} \approx 800\text{nm}$$

$$\mathrm{V} = \left(\frac{\mathrm{r}}{2}\right)^2 \pi * \mathrm{Z} \approx 2.5\times10^{-2}\,\mu\mathrm{m}^3$$

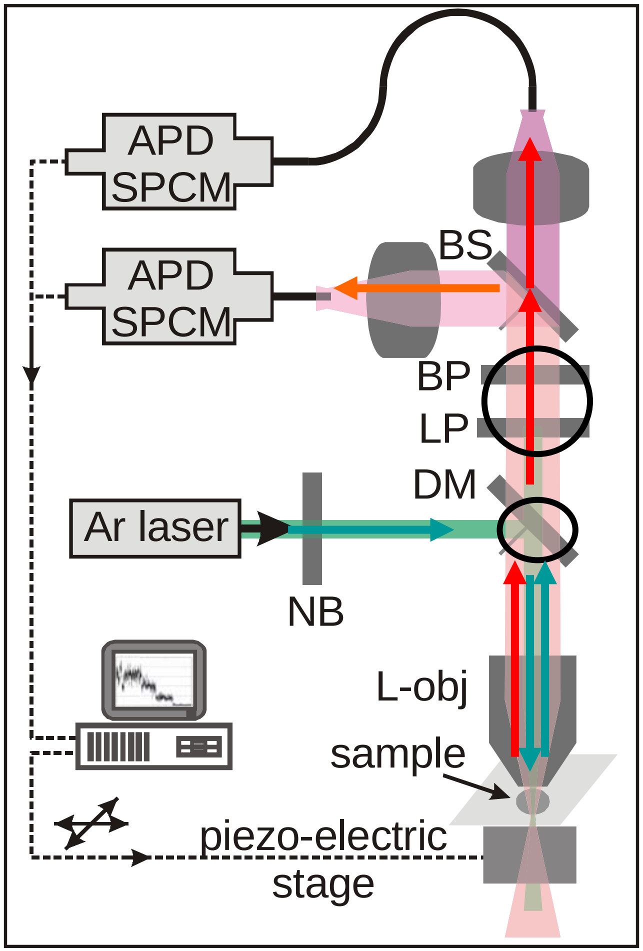
$$1\mathrm{M} = \frac{1\,\text{mole}}{1\,\mathrm{L}} = \frac{6\times10^{23}\,\text{molecule}}{10^{15}\,\mu\mathrm{m}^3} = 6\times10^{8}\,\frac{\text{molecules}}{\mu\mathrm{m}^3}$$

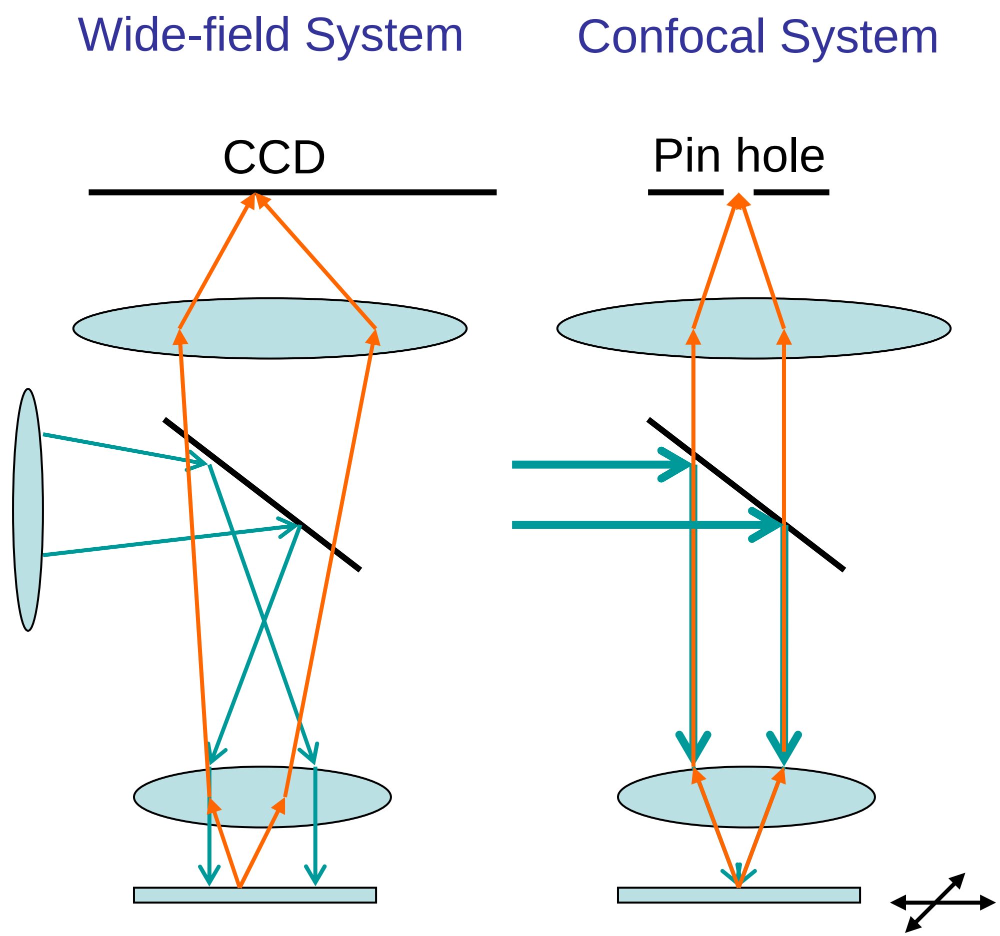
$$\frac{1\,\text{molecule}}{2.5\times10^{-2}\,\mu\mathrm{m}^3} = \frac{1}{6}\,\frac{10^{-23}\,\text{mole}}{2.5\times10^{-2}\times10^{-15}\,\mathrm{L}} = 6.6\times10^{-8}\ \mathrm{M}$$

三聚氰氨($C_3H_6N_6$); 分子量: 81

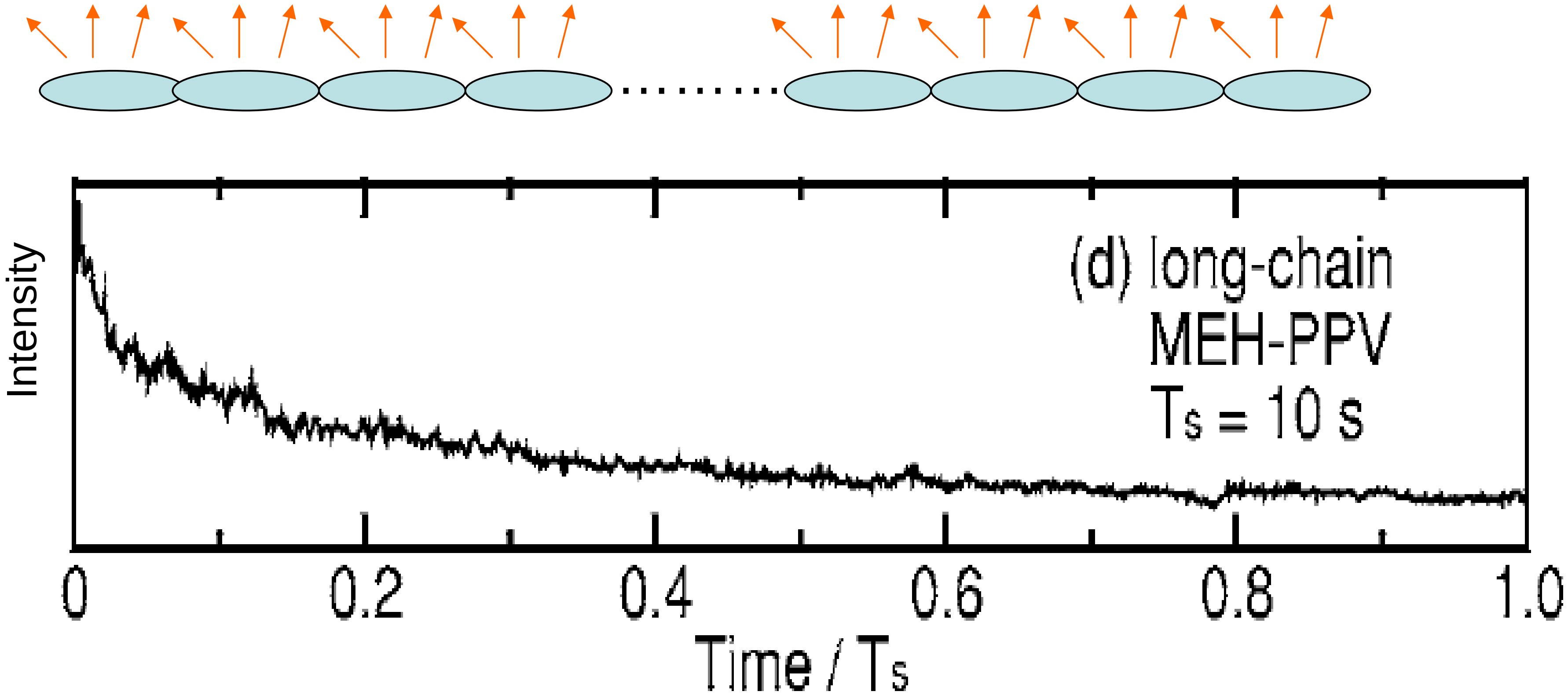
$$1\text{ppm} = \frac{1\text{mg}}{1\text{kg}} = \frac{10^{-3}/81\,\text{mole}}{1\mathrm{L}} = 1.23\times10^{-5}$$

Experimental setup



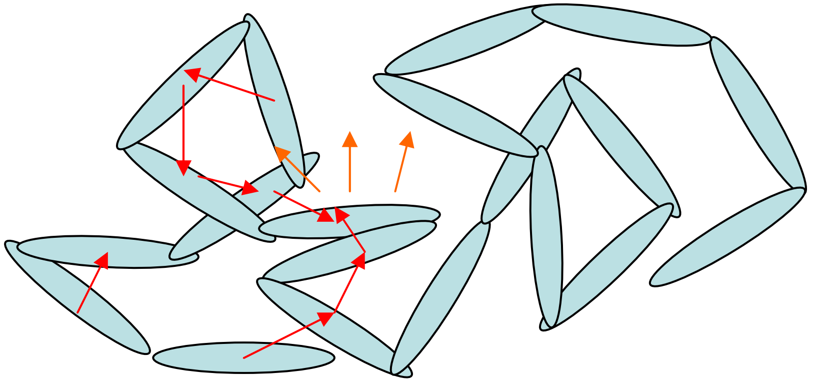


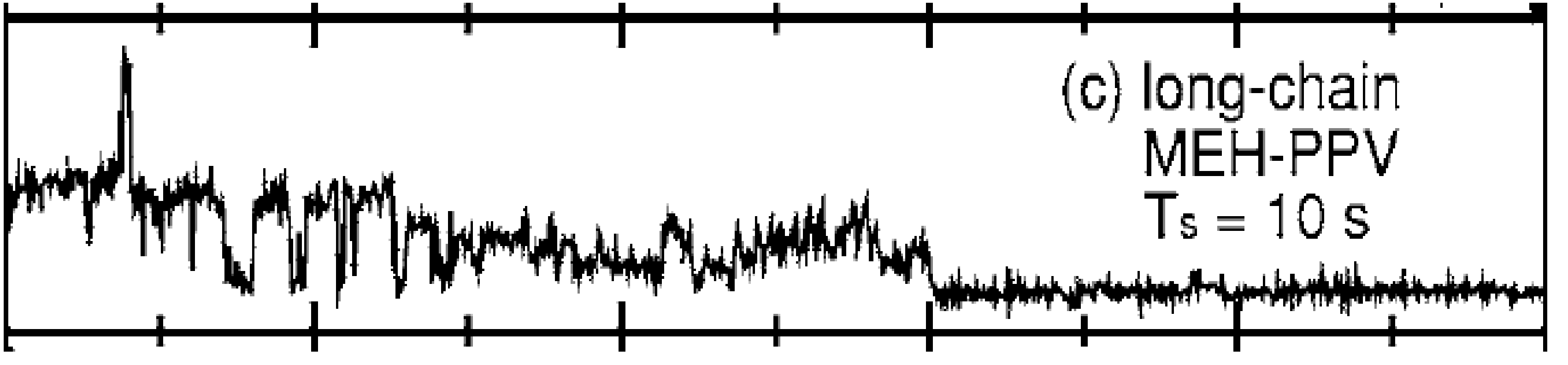
Time trace



- Luminescent intensity degrade with time.
- Chromophores are independent from each other.

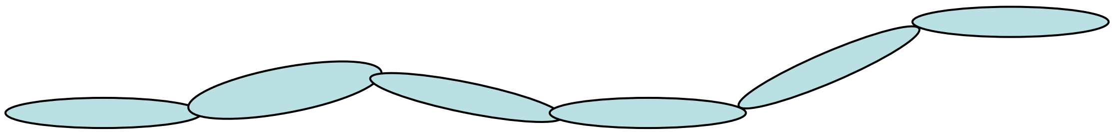
Time trace

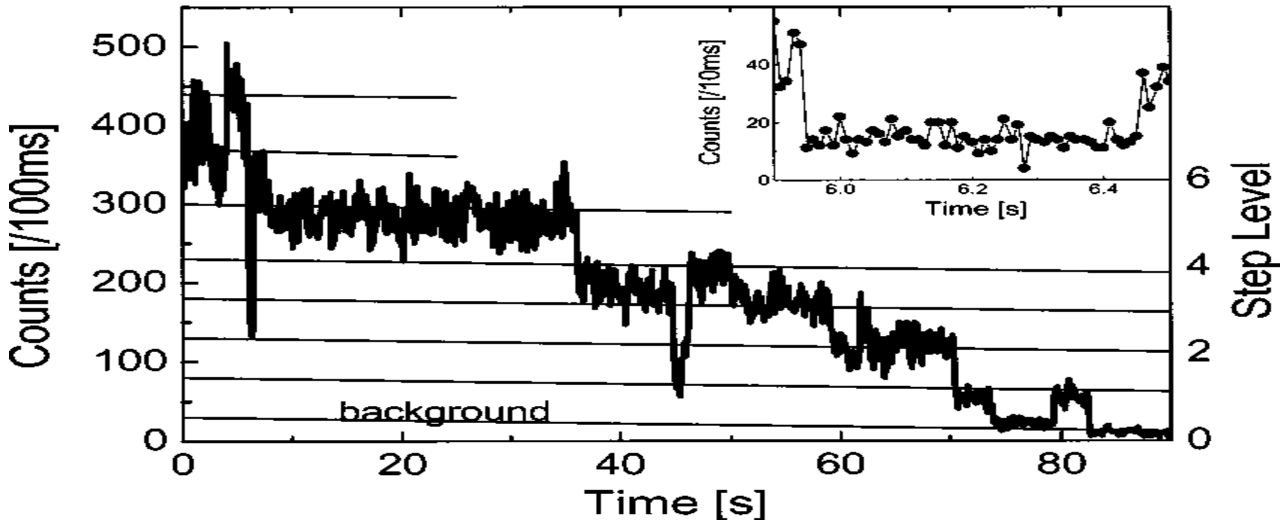




- Blinking Behavior.
- Energy of chromophores transfer to lower energy site to emit.

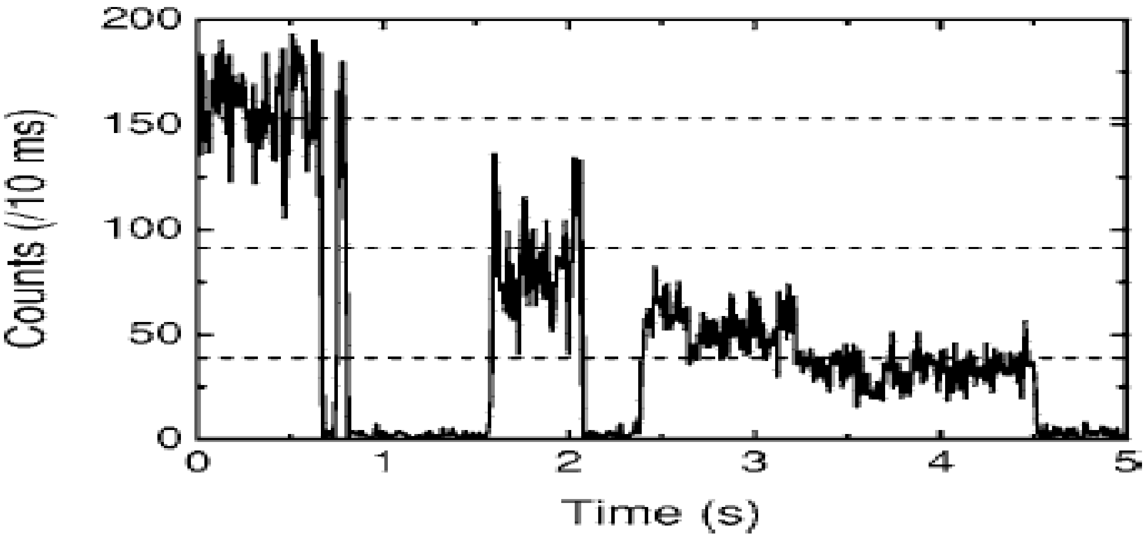
Time trace





- Changes in intensity are abrupt.
- Decrease in emission occurs whenever an chromophore is either permanently or temporarily disabled.
- There are 7 chromophores in the polymer.

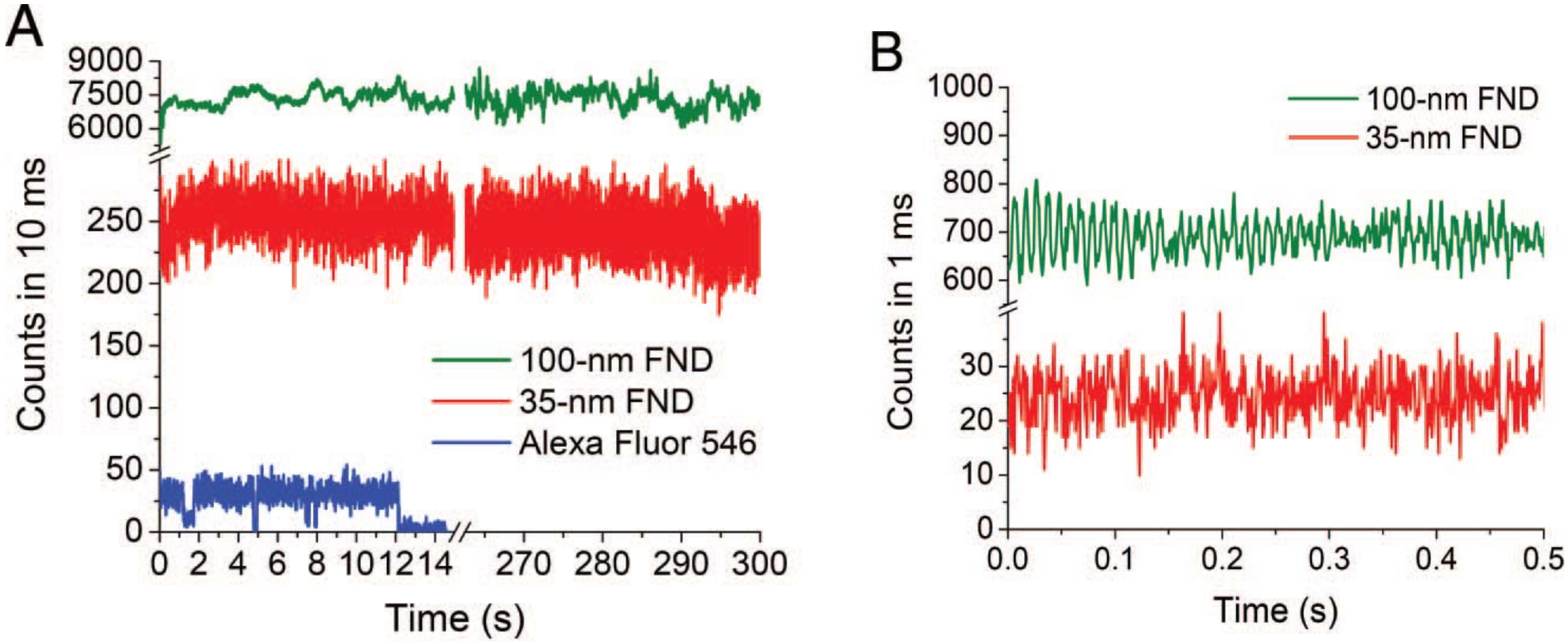
Time trace



•Three discrete intensity levels can be recognized indicating the presence of three active absorbing chromophores
•The intensity always drops to or very close to the background level before rebounding to emit at a different intensity.
•This "on/off" blinking behavior is a significant feature of single emitter.

Time trace

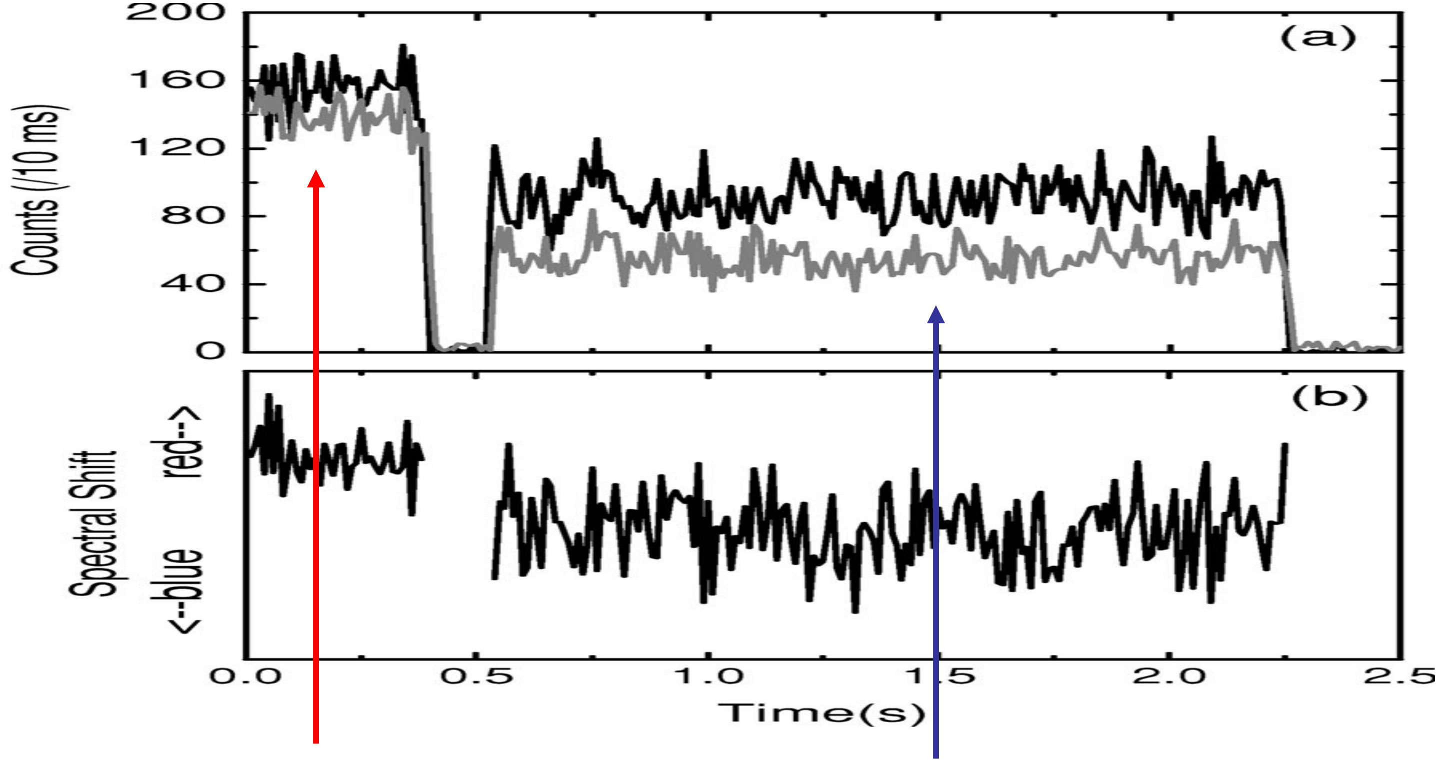
FND : Fluorescent nano-diamond



- Excellent photostability was observed for nano-diamonds.
- No sign of fluorescence blinking was detected within the time resolution of 1 ms
- Single dye molecules such as Alexa Fluor 546 covalently linked to dsDNA photobleached within 12 s

Time trace

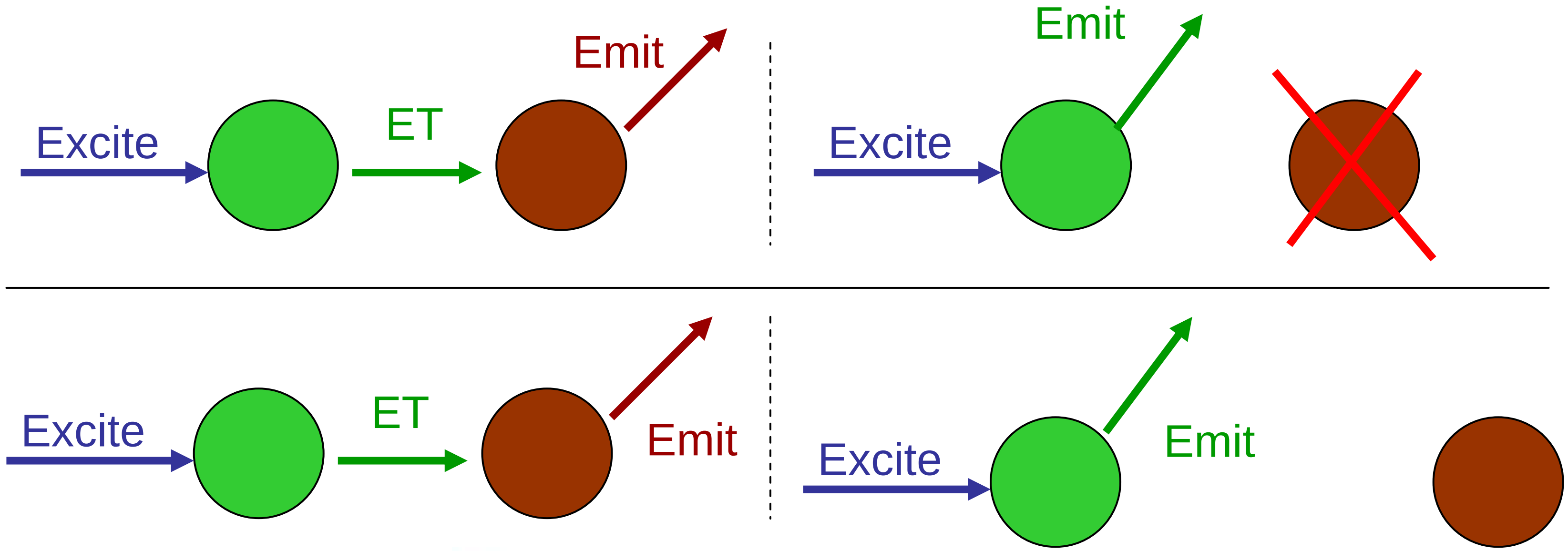
$$S = \frac{I_{red} - \gamma I_{blue}}{I_{red} + \gamma I_{blue}}$$



Lower-energy emitter

Higher-energy emitter

Time trace


Excite
ET
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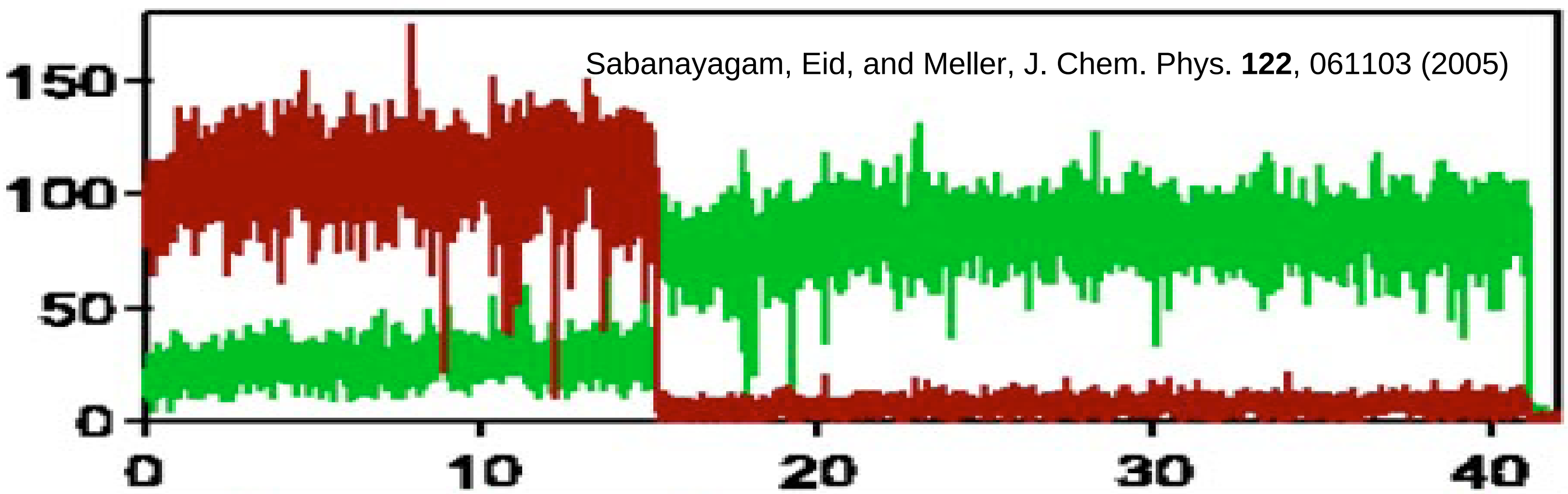
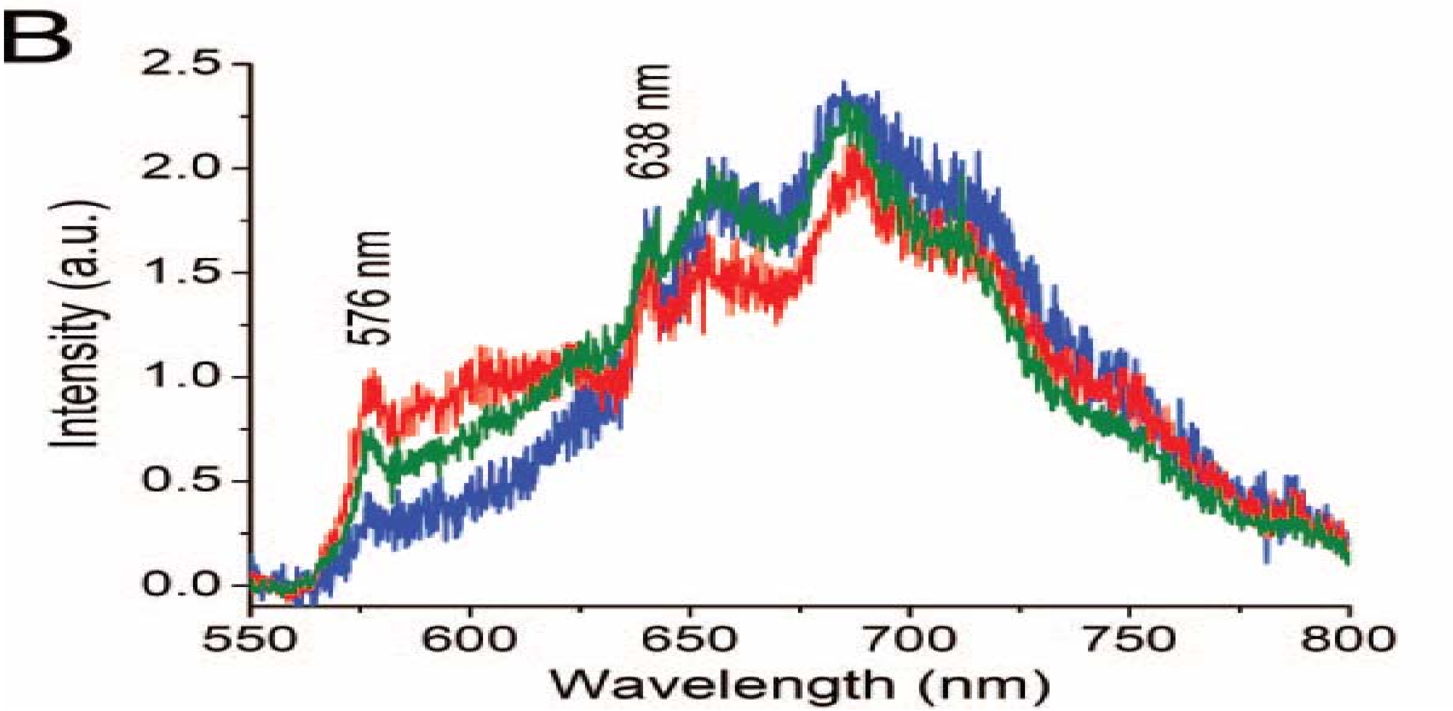
Emit


Sabanayagam, Eid, and Meller, J. Chem. Phys. **122**, 061103 (2005)
150
100
50
0
0
10
20
30
40

PL spectrum

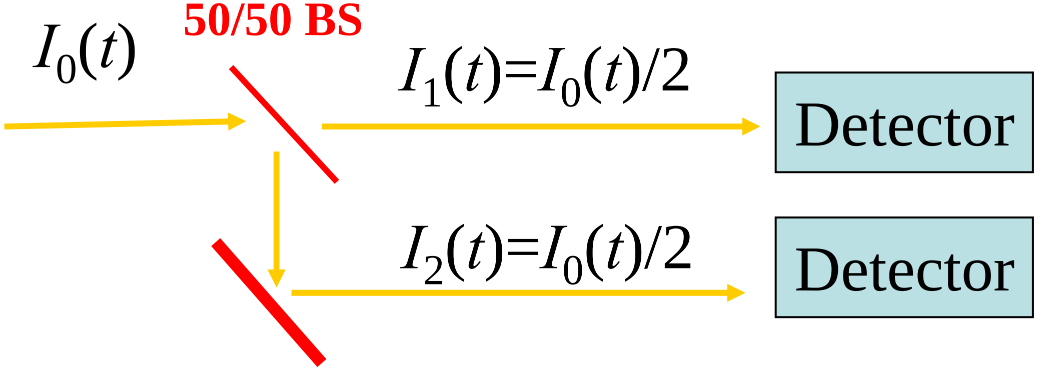


- The fluorescence spectra are highly heterogeneous.
- Two sharp zero-phonon lines (ZPL) of $(N\text{-}V)^-$ to $(N\text{-}V)^0$ can be found.
- These three particles mainly differ in the ratio of $(N\text{-}V)^-$ to $(N\text{-}V)^0$ contents.

Autocorrelation

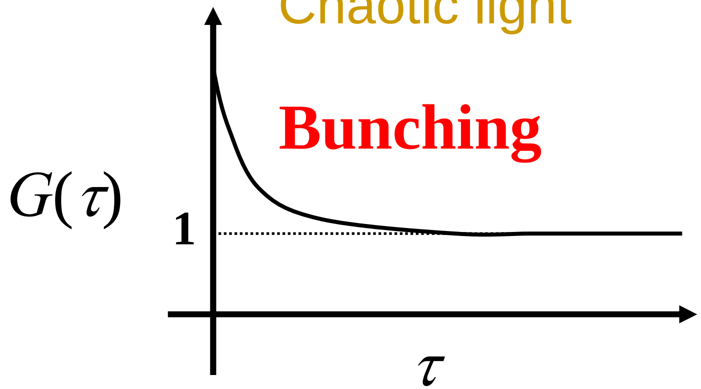
Classical Light Source

(Hanbury-Brown & Twiss experiment)

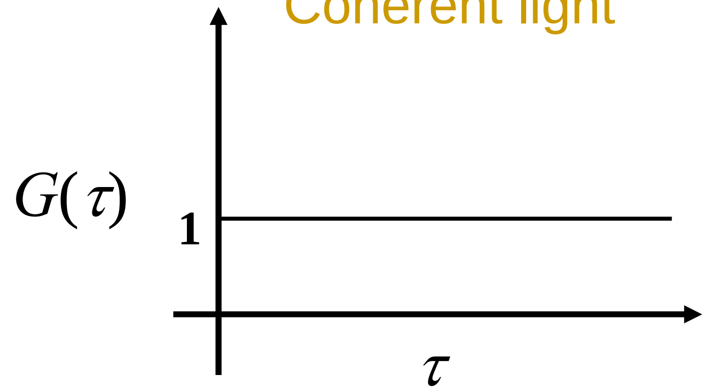


$$G(\tau) = \frac{< I_1(t) I_2(t-\tau) >}{< I_1(t) >< I_2(t) >}$$

Chaotic light

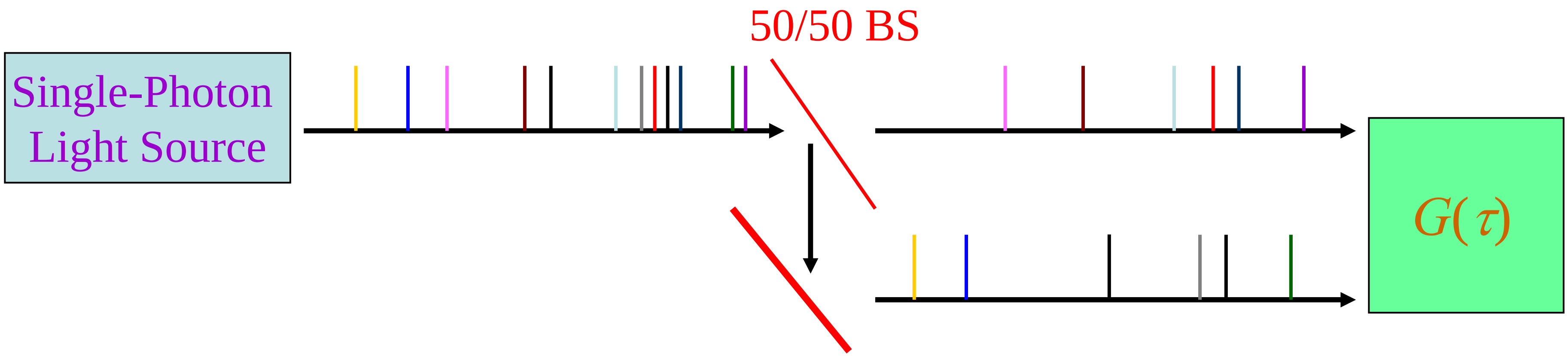


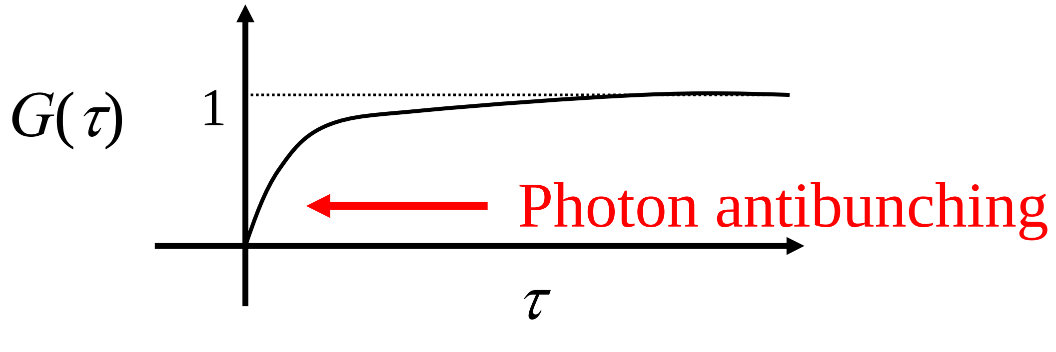
Coherent light



Autocorrelation

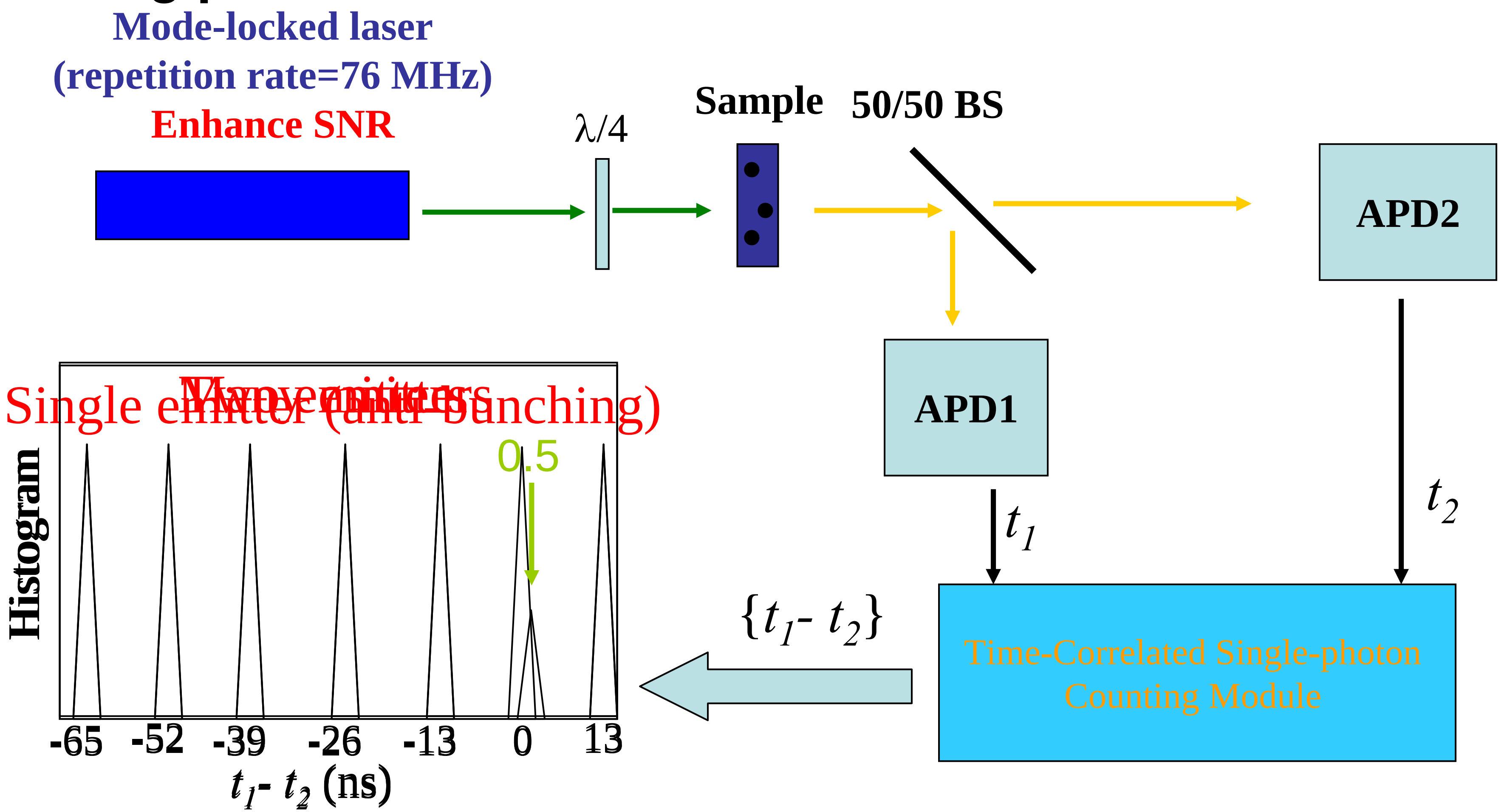
Non-classical Light Source



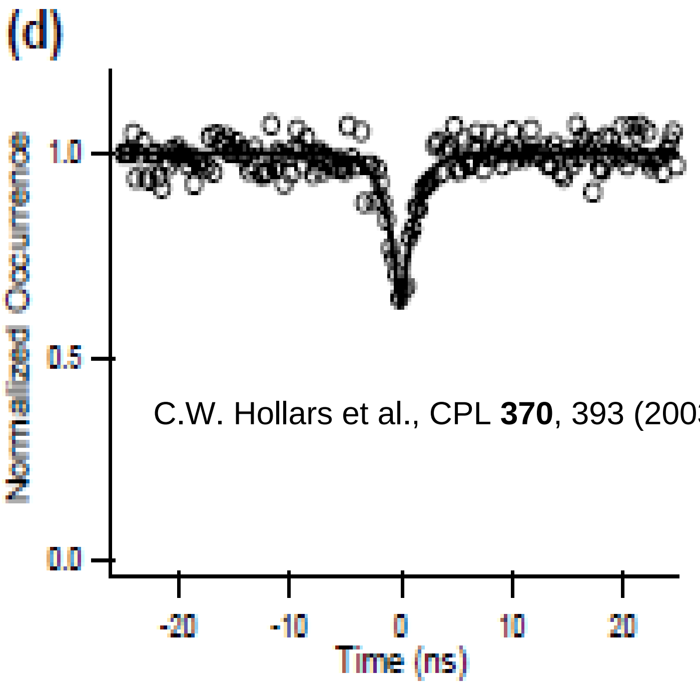


Autocorrelation

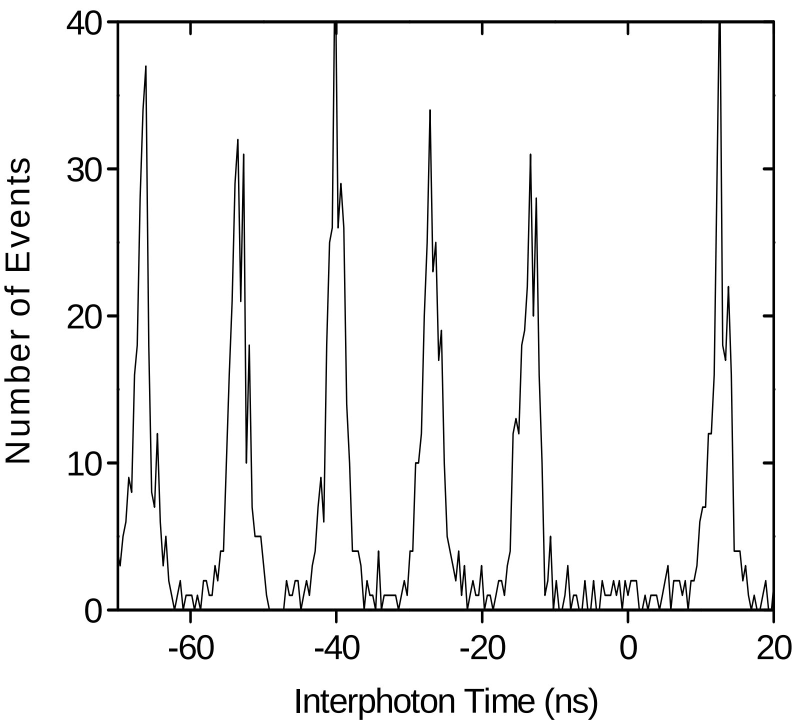
Using pulse laser



Autocorrelation



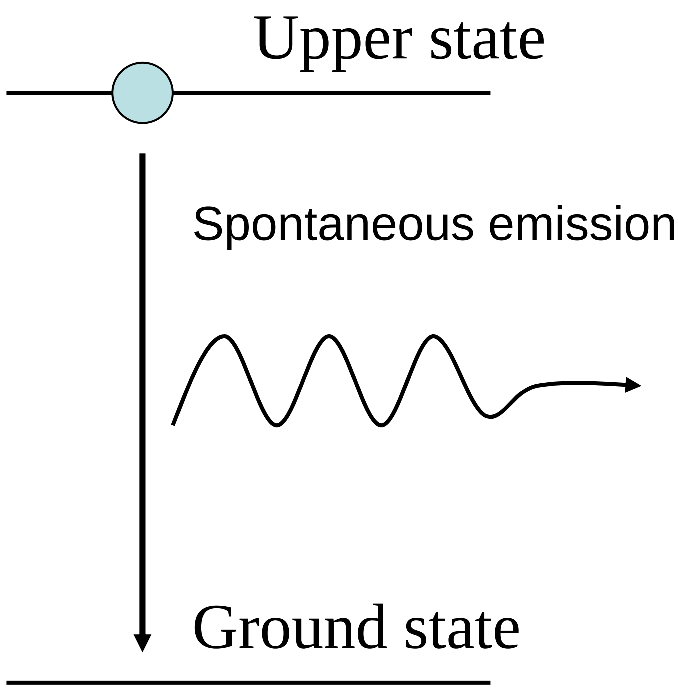
C.W. Hollars et al., CPL **370**, 393 (2003).

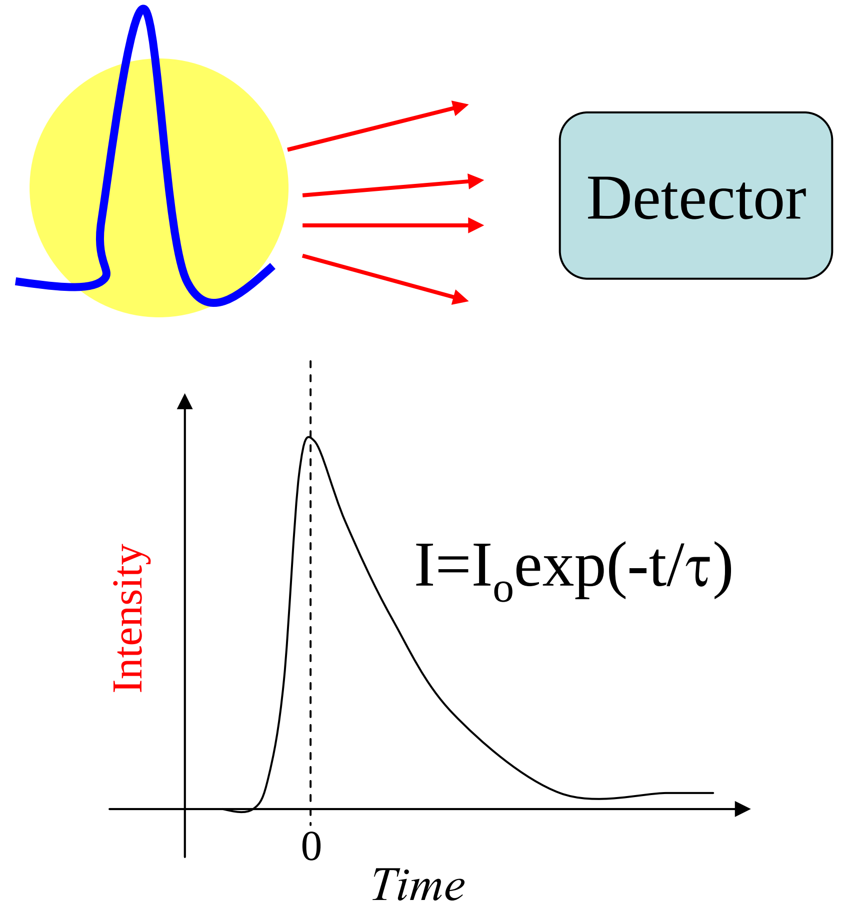


- On average only two to three discrete emitter sites

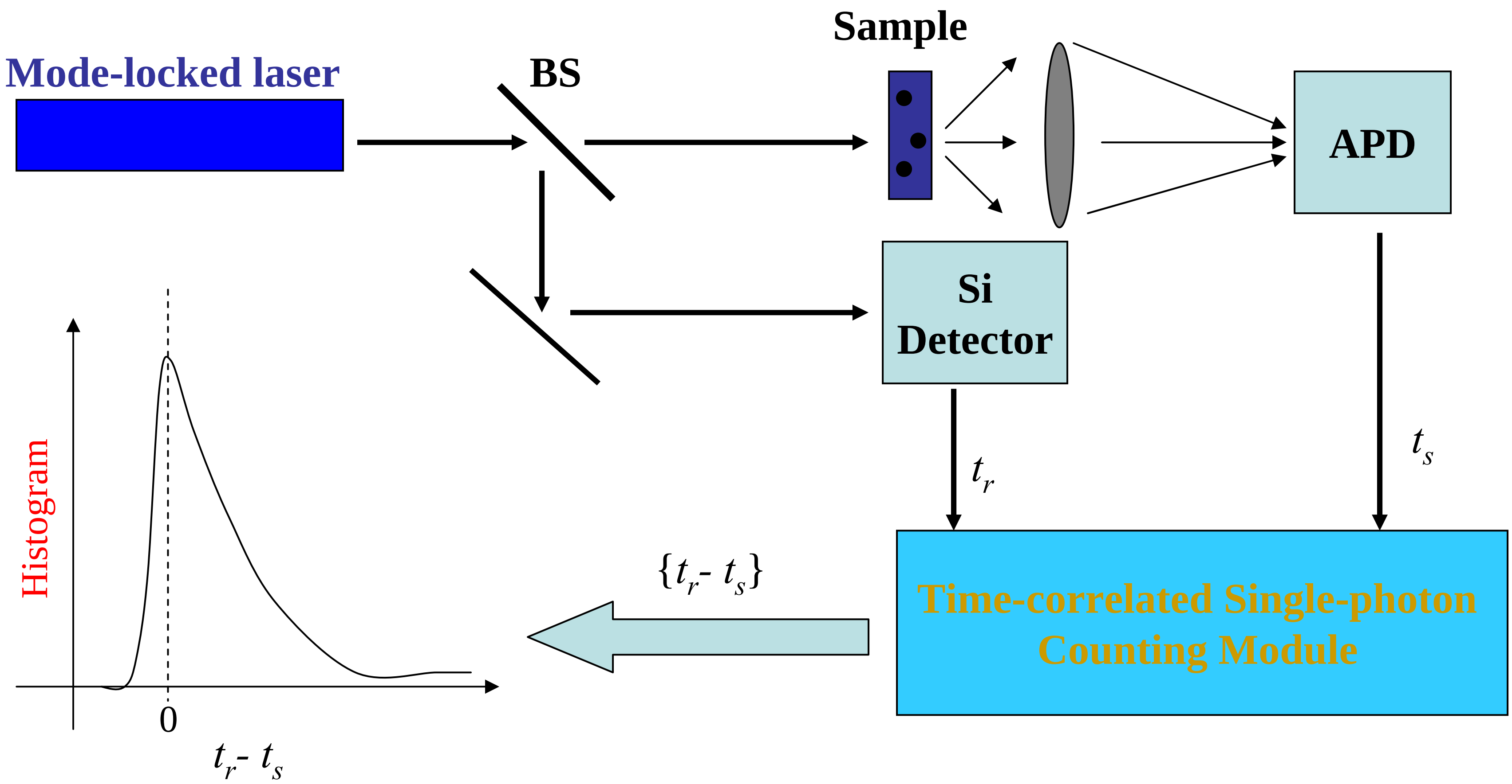
- Single-photon light source

Fluorescence lifetime





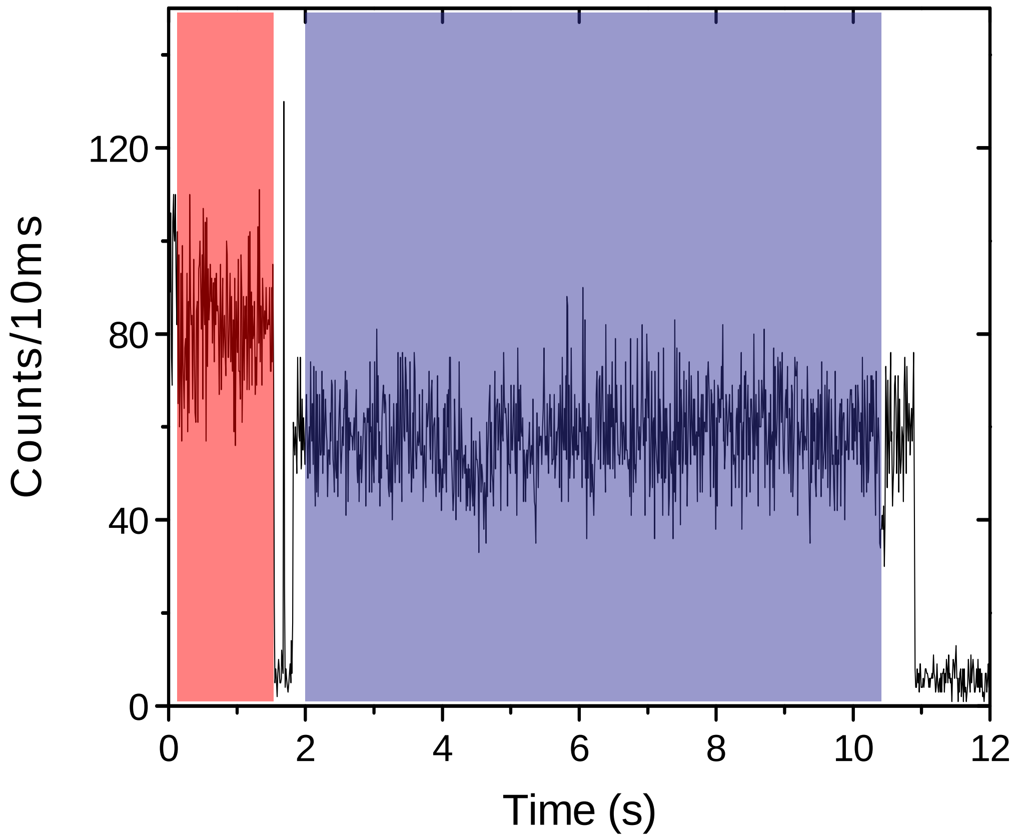
Fluorescence lifetime

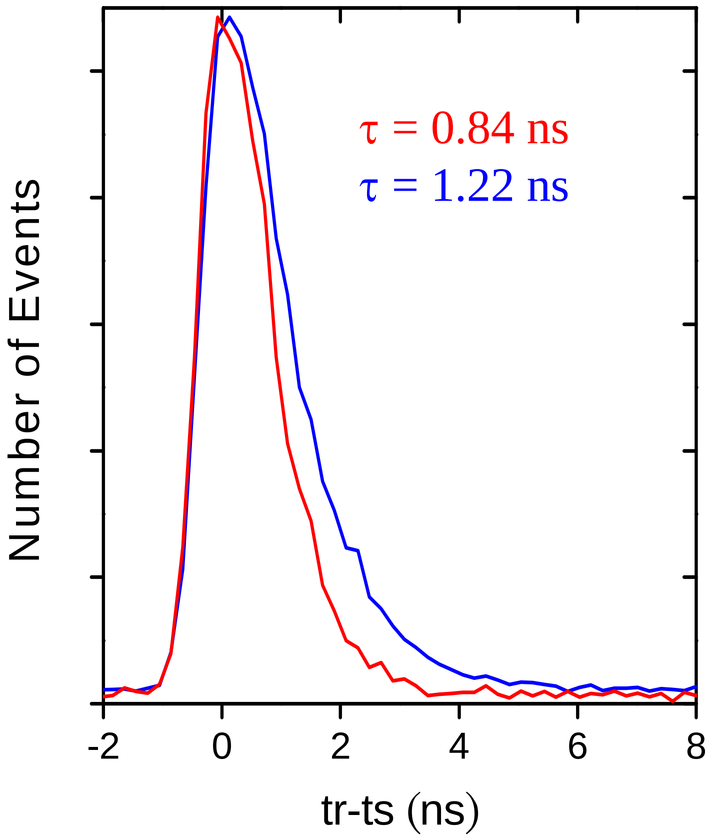


Fluorescence lifetime

$$k_r = A \propto |\mu_N|^2 \rightarrow |\mu_N|^2 = \frac{2|\mu|^2}{N+1}\left[\cot\left(\frac{\pi}{2N+2}\right)\right]^2 \rightarrow E = E_0 + 2\beta\cos\left(\frac{\pi}{N+1}\right)$$

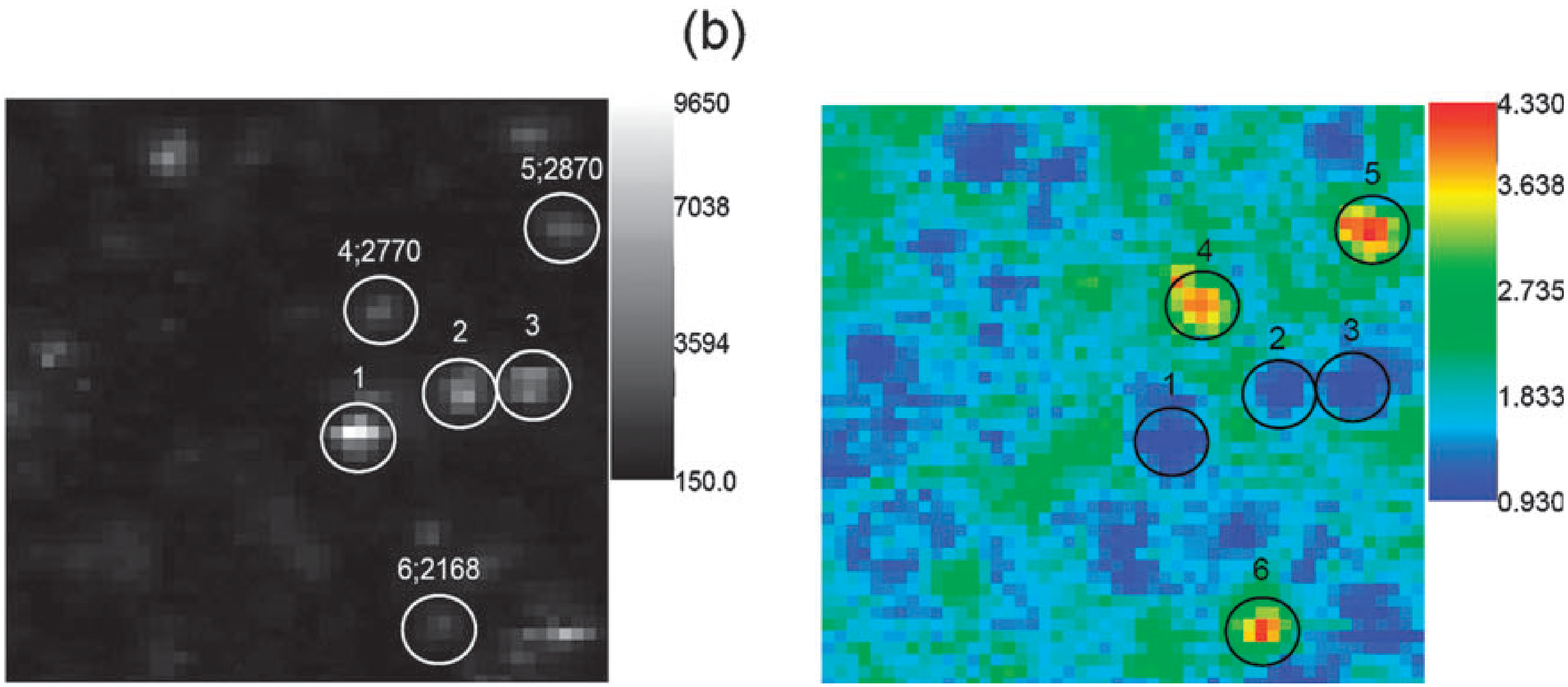
Larger τ (smaller k) → larger energy



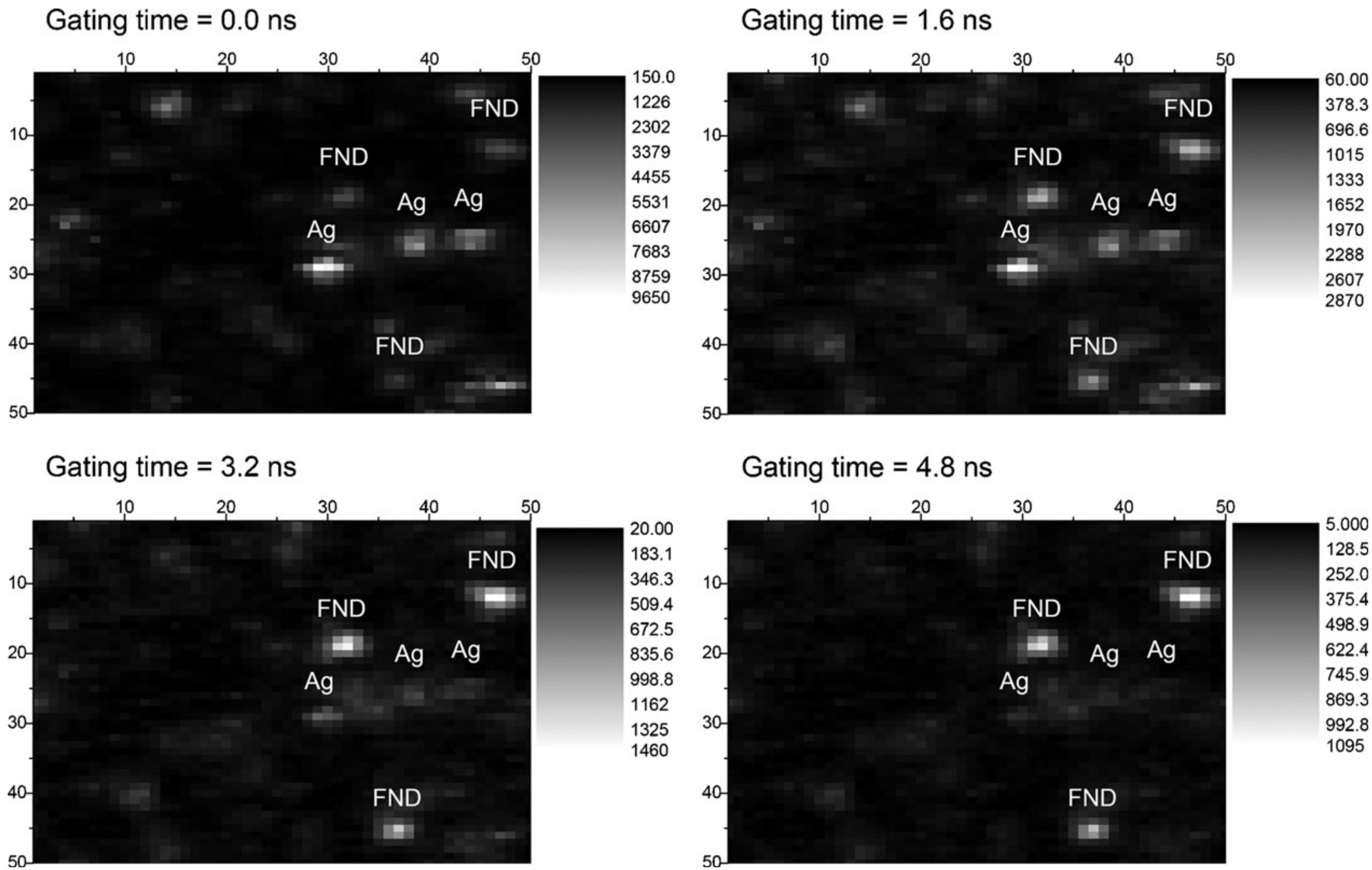


Fluorescence lifetime image microscope (FLIM)

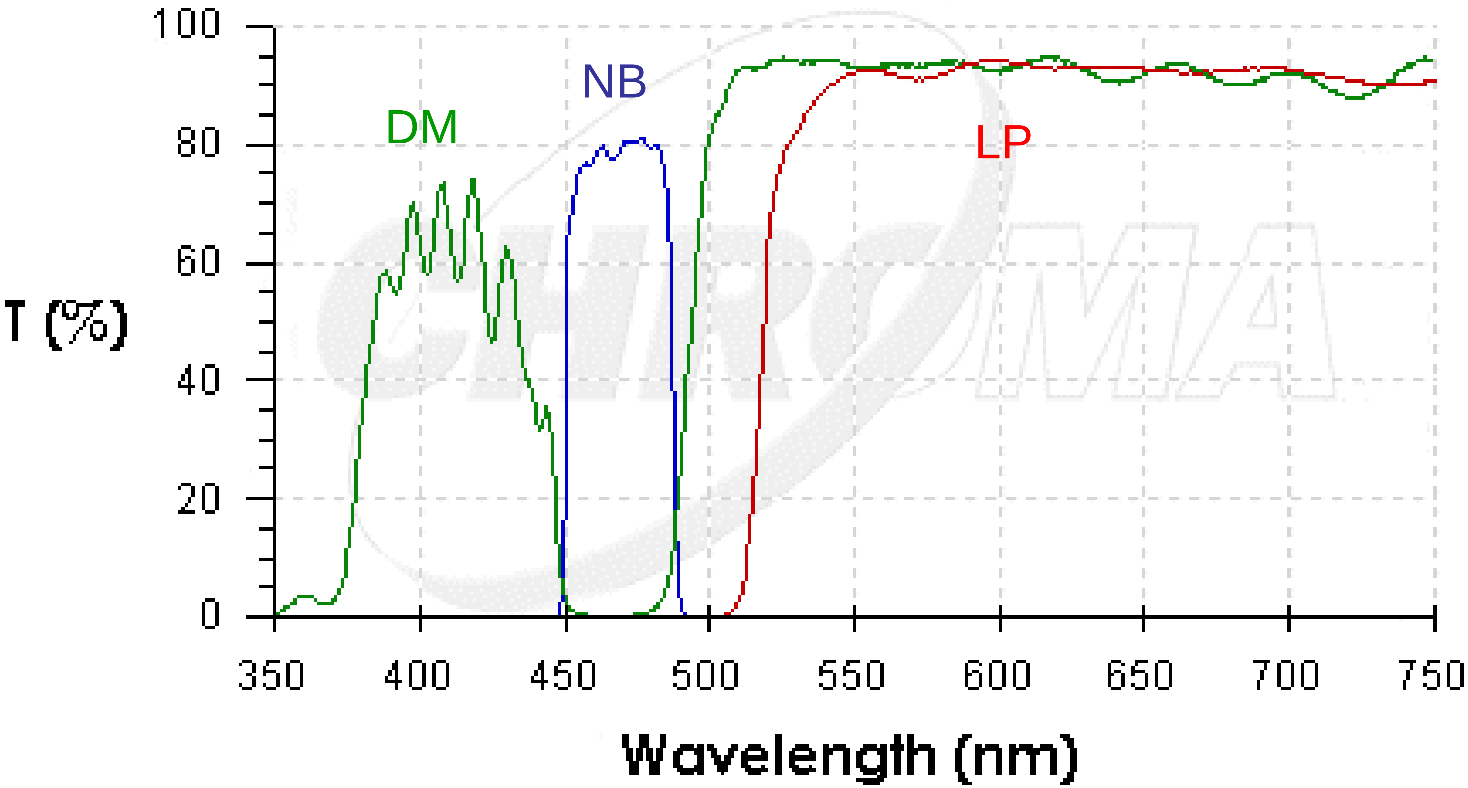
FND on a silver-island film



Time gating measurement



Thank you for your attention



100
80
60
40
20
0
T (%)
DM
NB
LP
350
400
450
500
550
600
650
700
750
Wavelength (nm)

back