Fluorescent Correlation Spectroscopy Lab for Biophysics course at UNM

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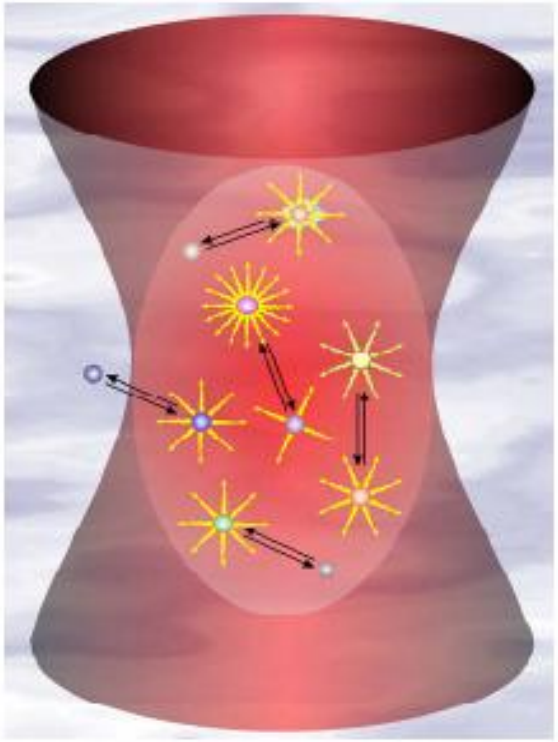
Dr. Thomas' Lab

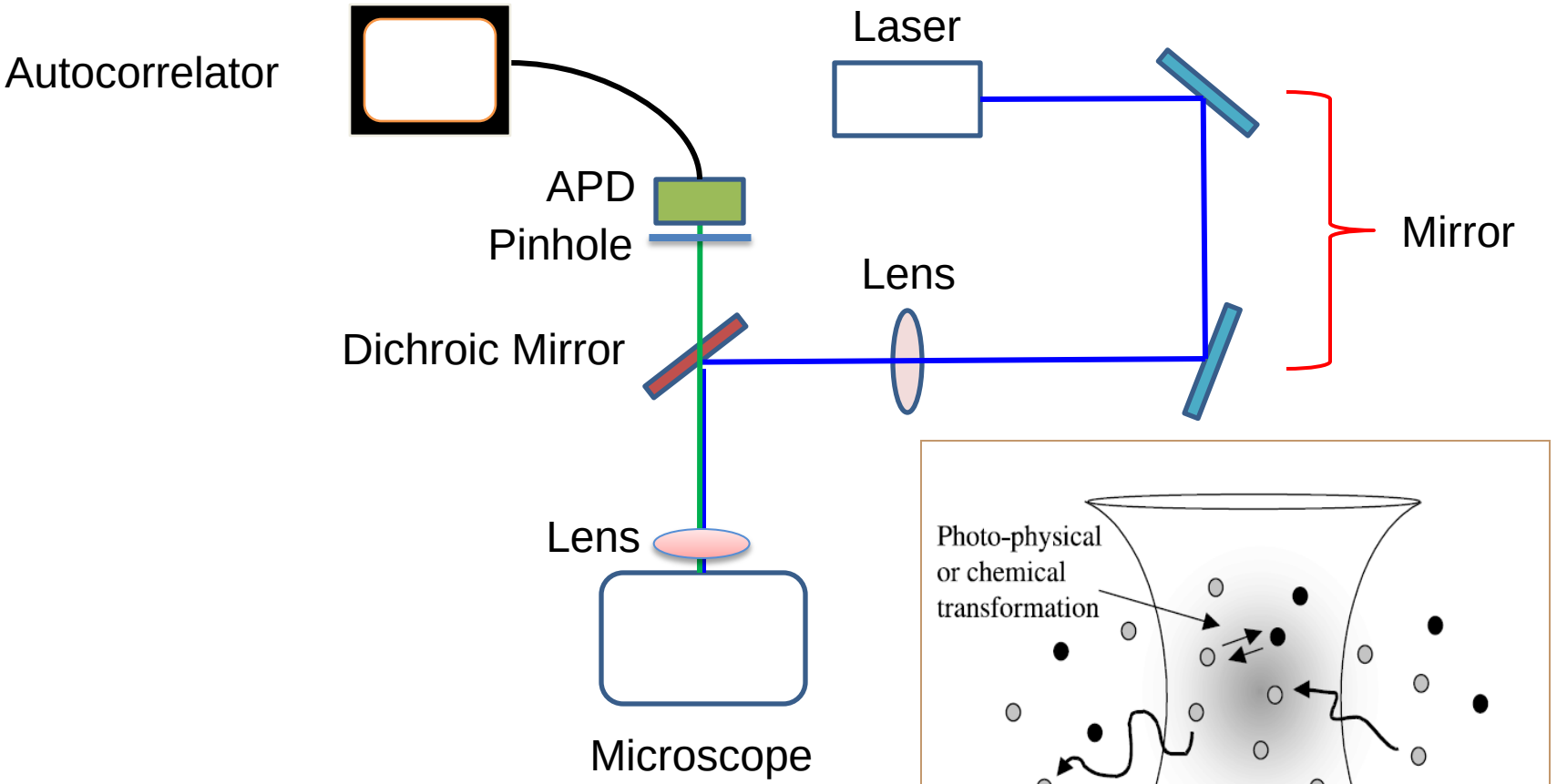
Dr. Lidke's Lab

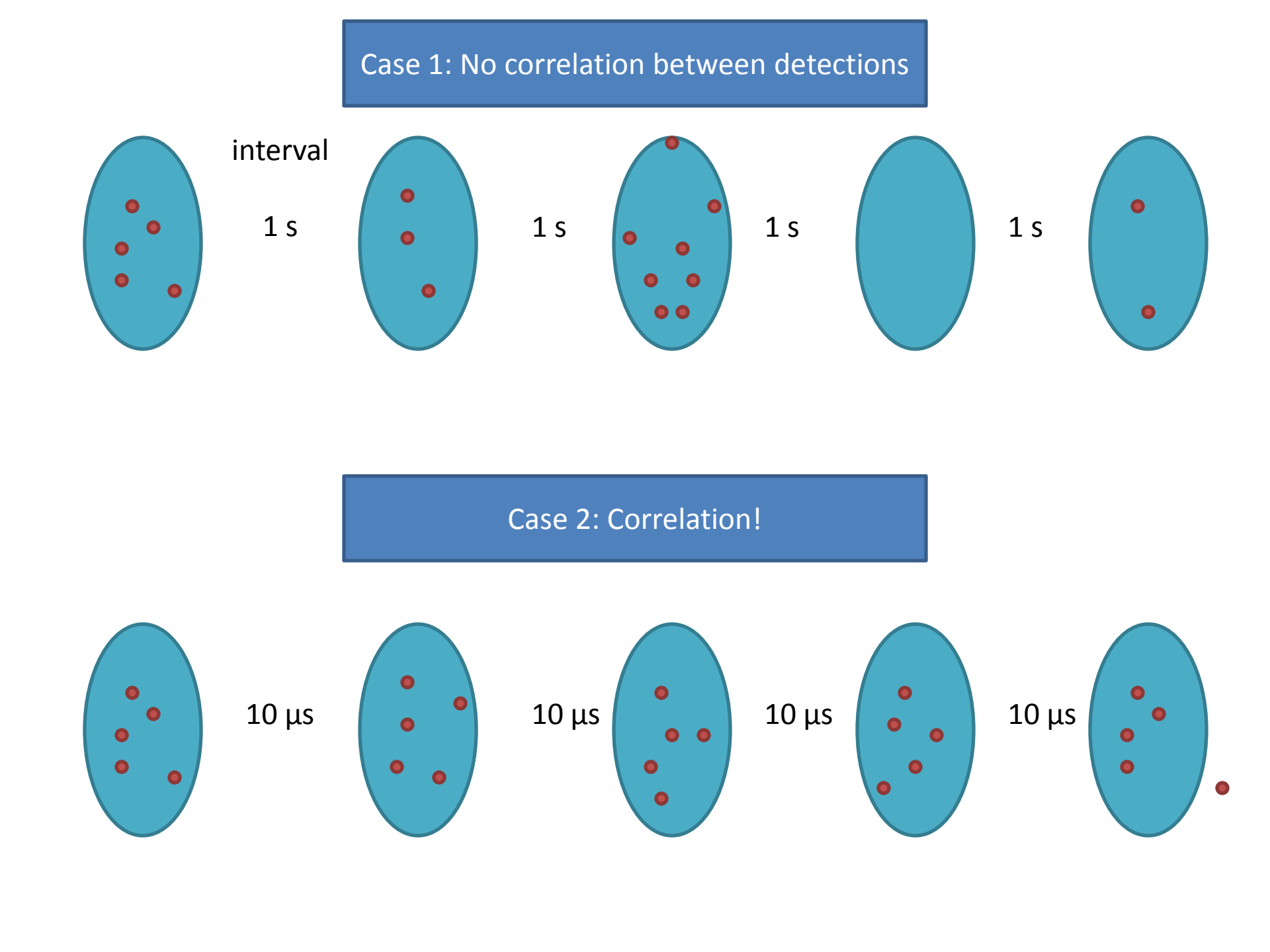
What is FCS

- Fluorescence correlation spectroscopy (FCS)
- It uses statistical fluctuations in the fluorescence intensity of a small illuminated sample volume to obtain information about the processes that provoke these fluctuations.

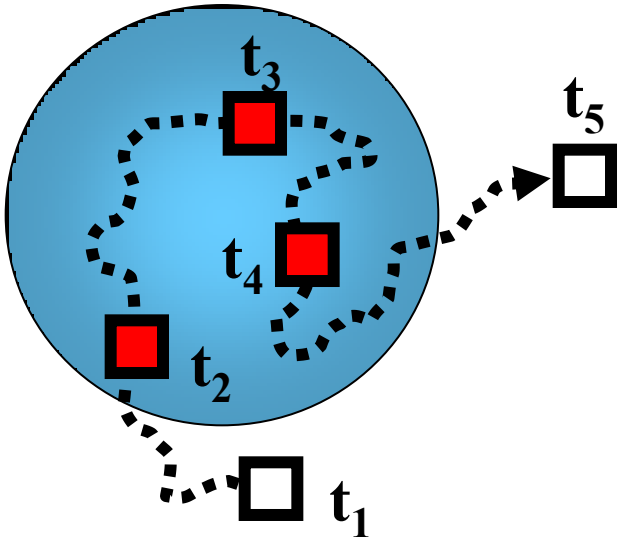


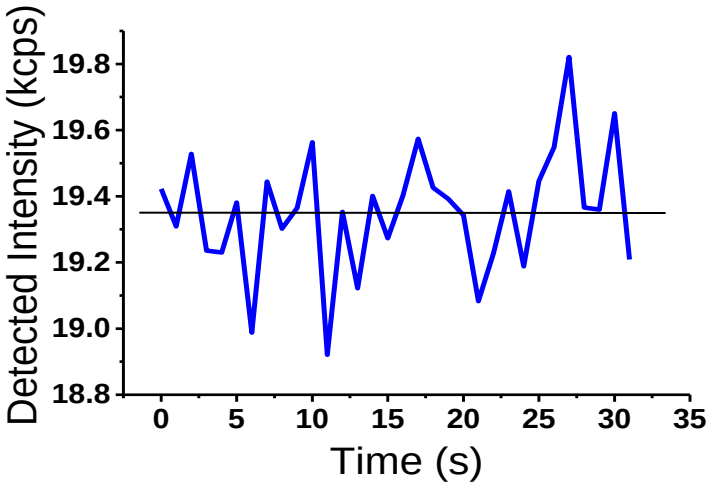
FCS Setup


Autocorrelator
Laser
APD
Pinhole
Mirror
Lens
Dichroic Mirror
Lens
Microscope
Photo-physical
or chemical
transformation
Diffusion out
Diffusion in


Case 1: No correlation between detections
interval
1 s
1 s
1 s
1 s
Case 2: Correlation!
10 µs
10 µs
10 µs
10 µs

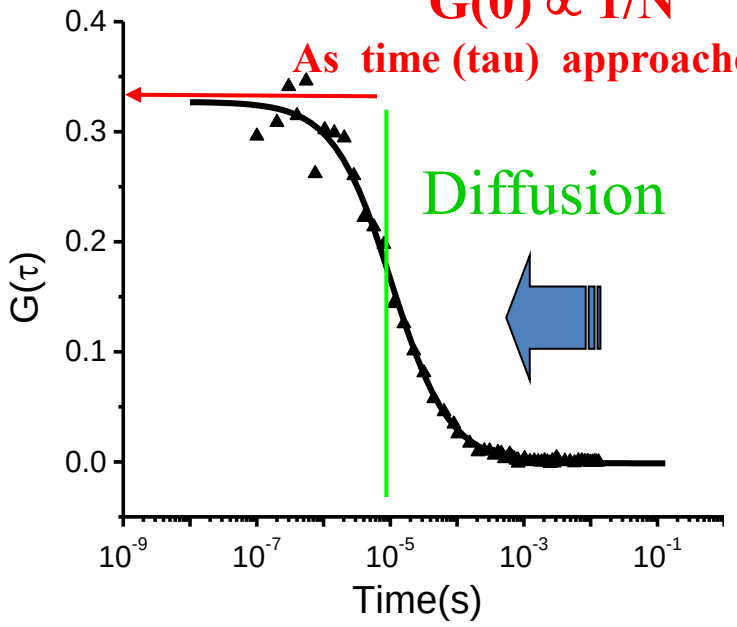
The Autocorrelation Function





G(0) ∝ 1/N

As time (tau) approaches 0



$$G(\tau) = \frac{\langle \delta F(t) \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2}$$

From Enrico Gratton.

FCS theory

G(0) ∝ 1/N ??

Poisson statistics

How to get diffusion coefficient?

$$G(t) = \frac{1}{\bar{N}} \left(1 + \frac{t}{\tau_D}\right)^{-1} \left(1 + \frac{t}{\omega^2 \tau_D}\right)^{-1/2}$$

And derivations…

- Measure local concentration and diffusion coefficient.

$$c = \frac{N}{V_{eff}} \qquad D = \frac{w_{xy}^2}{4\tau_D}$$

- Reaction rate

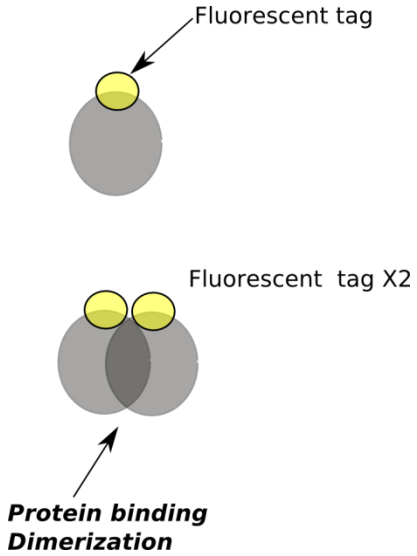
$$\mathrm{EtBr} + \mathrm{DNA} \underset{k_{\mathrm{debinding}}}{\overset{k_{\mathrm{binding}}}{\rightleftharpoons}} \mathrm{EtBr} \bullet \mathrm{DNA}$$

Magde et al (1972)

- Protein conformation changes

$$\frac{< F(t) >}{N}$$

Average fluorescent intensity per complex



Lidke et al (2009) ERK Nuclear Translocation Is Dimerization-independent but Controlled by the Rate of Phosphorylation

Multiple species

$$G(t) = \frac{1}{\left(\sum Q_k \bar{N}_k\right)^2} \sum Q_j^2 \bar{N}_j \left(1 + \frac{t}{\tau_{Dj}}\right)^{-1} \left(1 + \frac{t}{\omega^2 \tau_{Dj}}\right)^{-1/2}.$$

With chemical reactions $A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftarrows}} B$.

$$G(t) = \frac{1}{\bar{N}} \left(1 + \frac{t}{\tau_D}\right)^{-1} \left(1 + \frac{t}{\omega^2 \tau_D}\right)^{-1/2} \left(1 + K \exp\left(-\frac{t}{\tau}\right)\right)$$

With triplet state correction

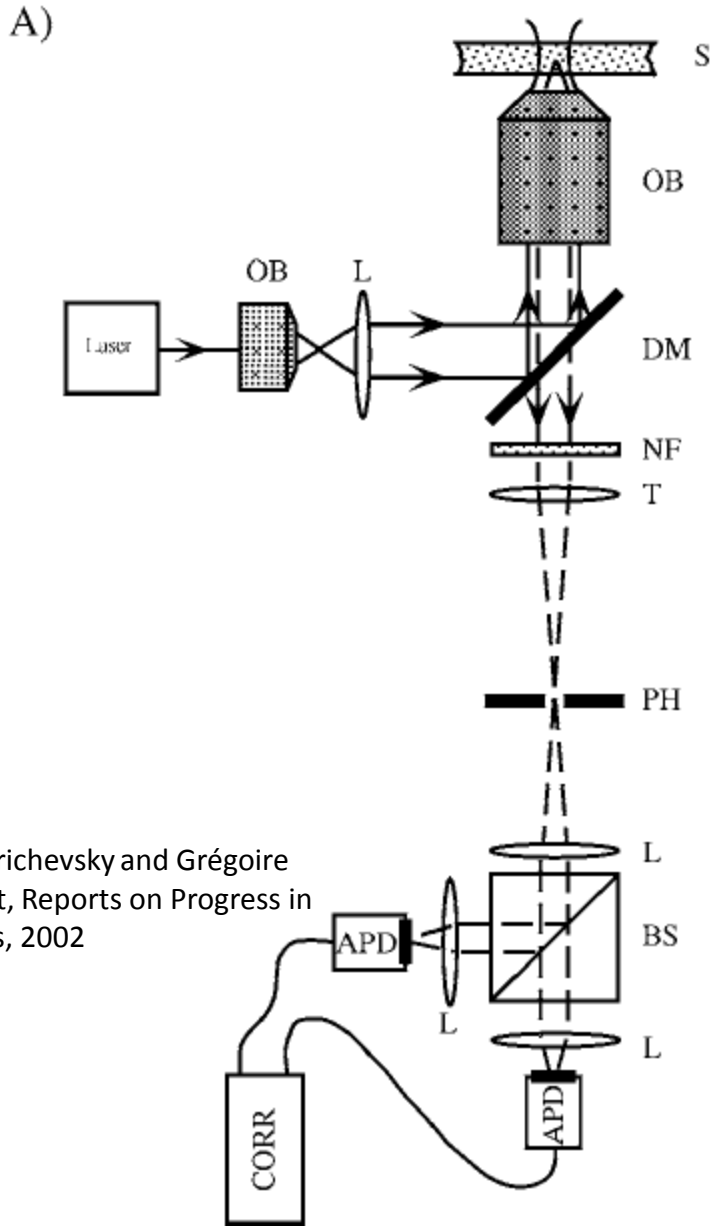
$$G(t) = \frac{1}{\bar{N}} \left(1 + \frac{t}{\tau_D}\right)^{-1} \left(1 + \frac{t}{\omega^2 \tau_D}\right)^{-1/2} \left(1 + \frac{p}{1-p} \exp\left(-\frac{t}{\tau}\right)\right)$$

With directional flow

$$G(\tau) = \frac{1}{N} \cdot \frac{e^{-\frac{1}{\left(1+\frac{\tau}{\tau_{diff}}\right)} \cdot \left(\left(\frac{\tau}{\tau_{flow}}\right)^2 + 1 - 2 \cdot \frac{\tau}{\tau_{flow}} \cdot \cos\left(\varphi \cdot \frac{\pi}{180^\circ}\right)\right)}}{\left(1 + \frac{\tau}{\tau_{diff}}\right) \cdot \sqrt{1 + \left(\frac{r_0}{z_0}\right)^2 \cdot \frac{\tau}{\tau_{diff}}}}$$

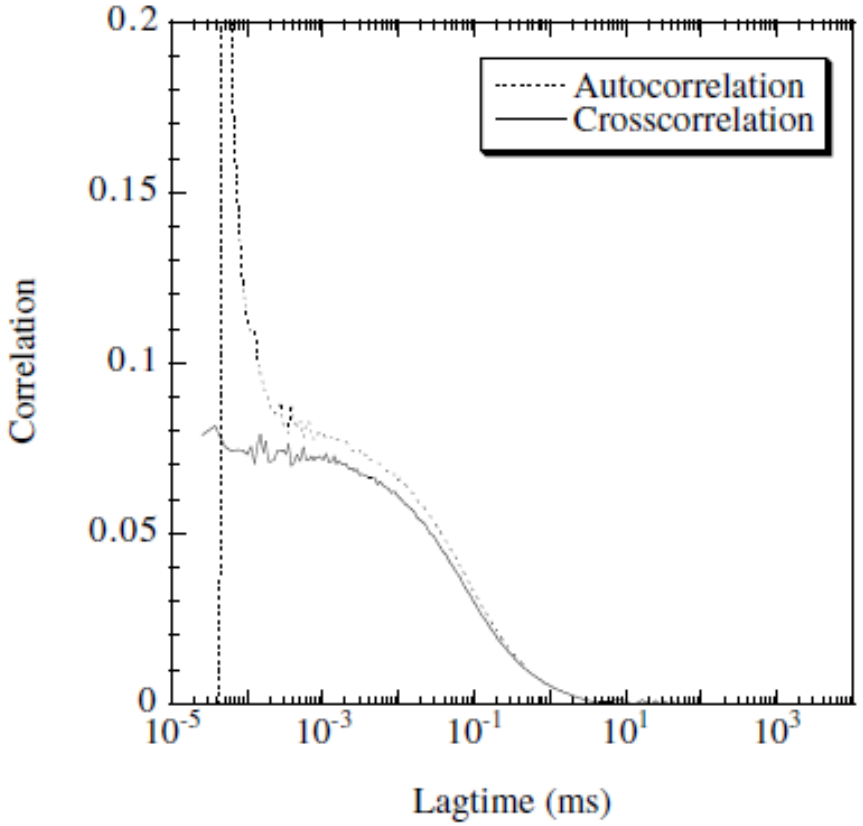
Oleg Krichevsky and Grégoire Bonnet, Reports on Progress in Physics, 2002

Cross correlation for ‘after pulsing’ effect for photon detecters.



Oleg Krichevsky and Grégoire Bonnet, Reports on Progress in Physics, 2002

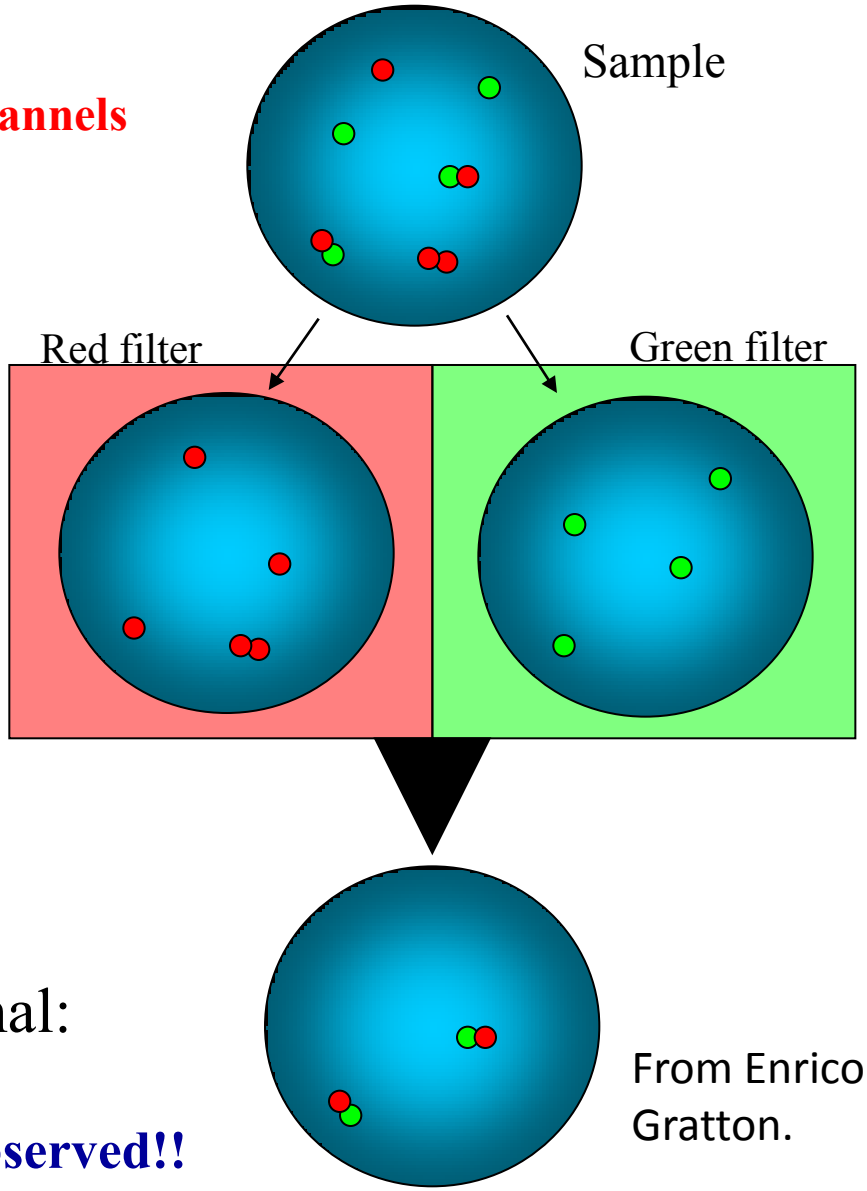
$$G(\tau) = \frac{\langle \delta F_1(t) \delta F_2(t+\tau) \rangle}{\langle F(t) \rangle^2}$$



Two-Color Cross-correlation

The cross-correlation
ONLY if particles are observed in both channels

Each detector observes
particles with a particular color

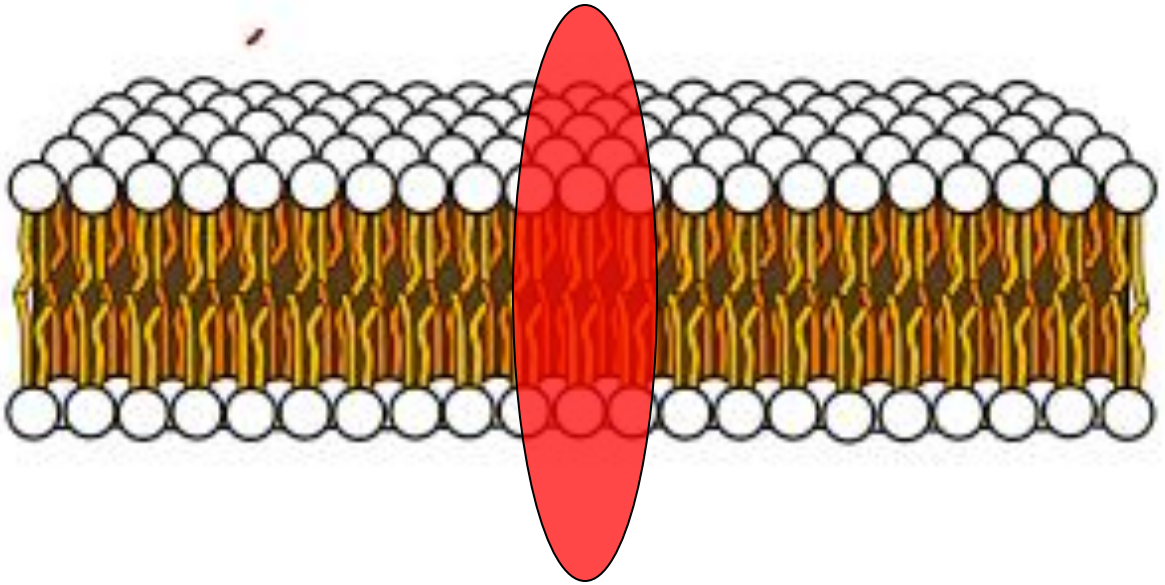


The cross-correlation signal:

Only the green-red molecules are observed!!

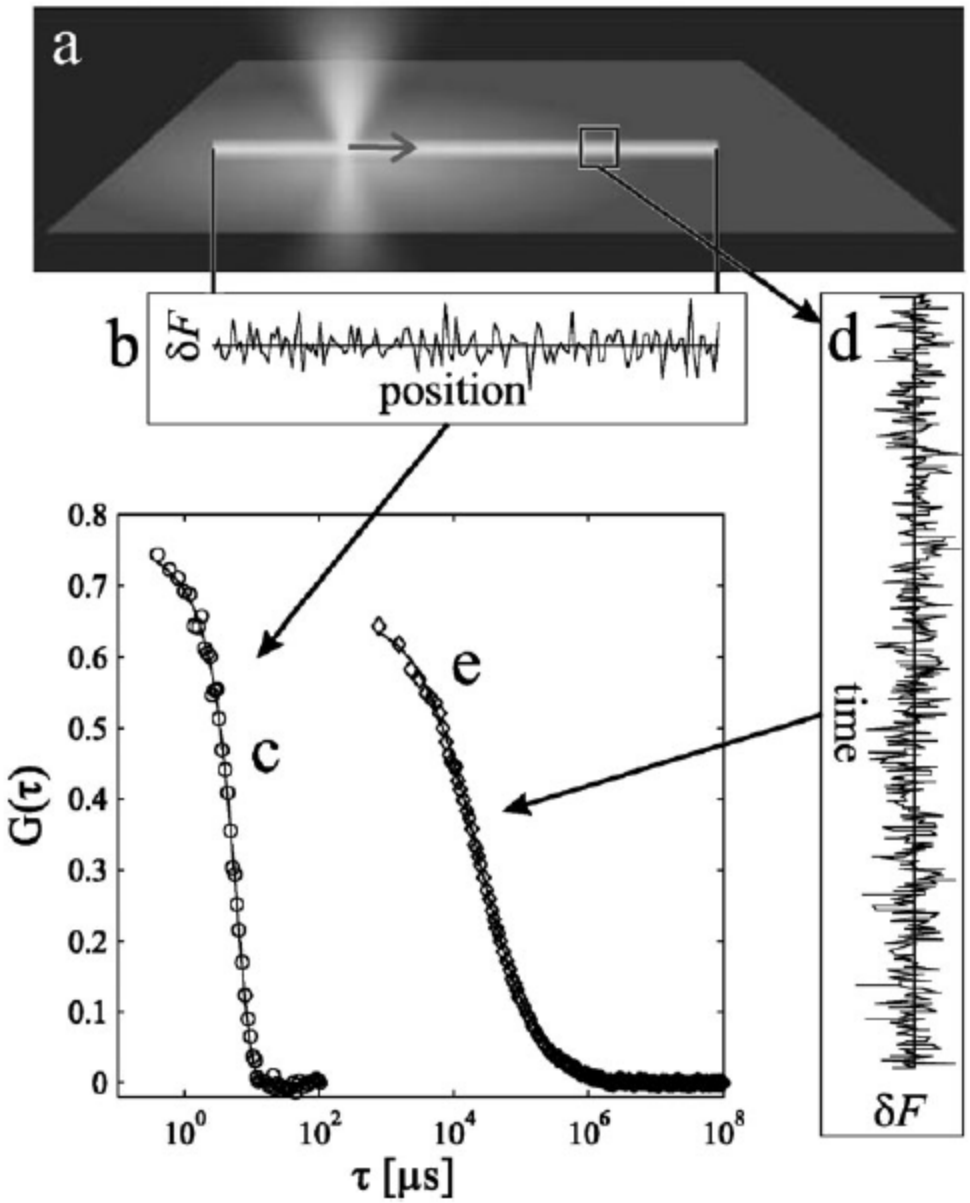
From Enrico Gratton.

Difficulty for FCS on cell membrane

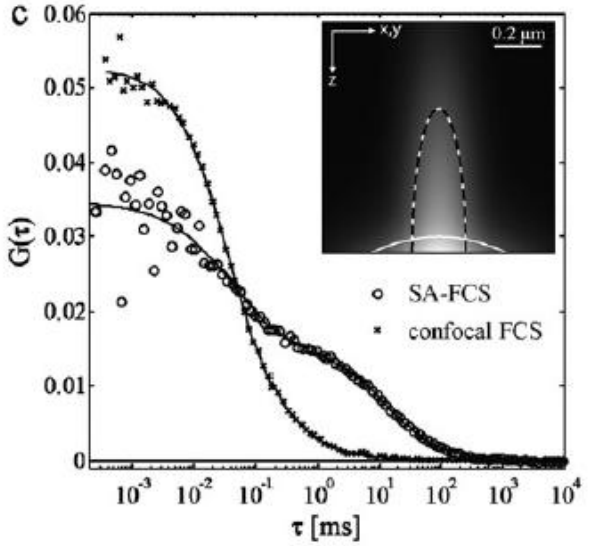


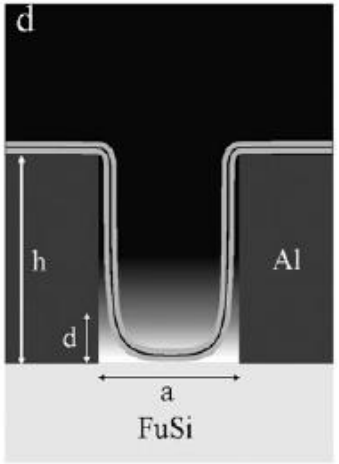
1. Very few fluorescent proteins within the focal volume.
   →Low signal to noise ratio
2. Slow diffusion coefficient on membrane make photo bleach the main problem for FCS in membrane.
3. 2-D diffusion model for lipid membrane can never reaches a steady state.

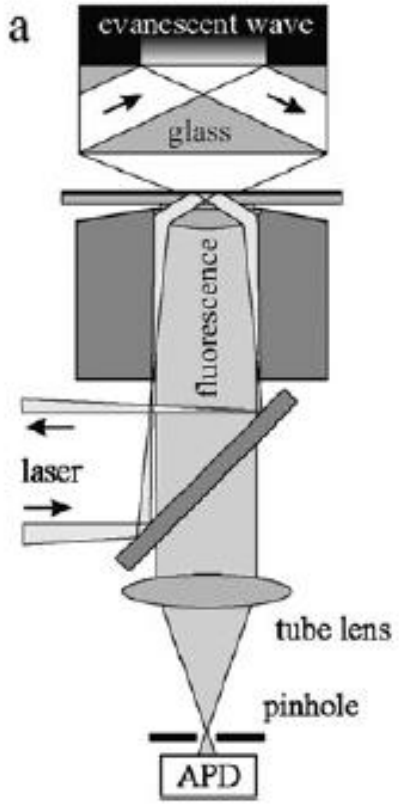
Scanning FCS for membranes

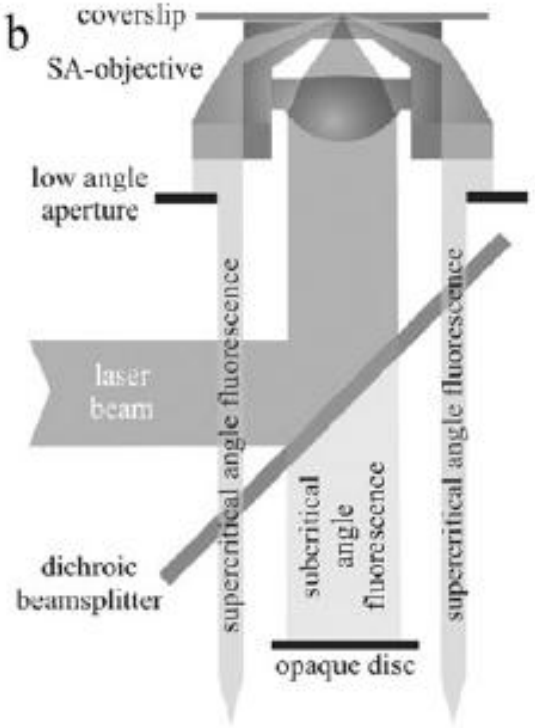


Jonas Ries ,Petra Schwille. Phys. Chem. Chem. Phys.,2008.









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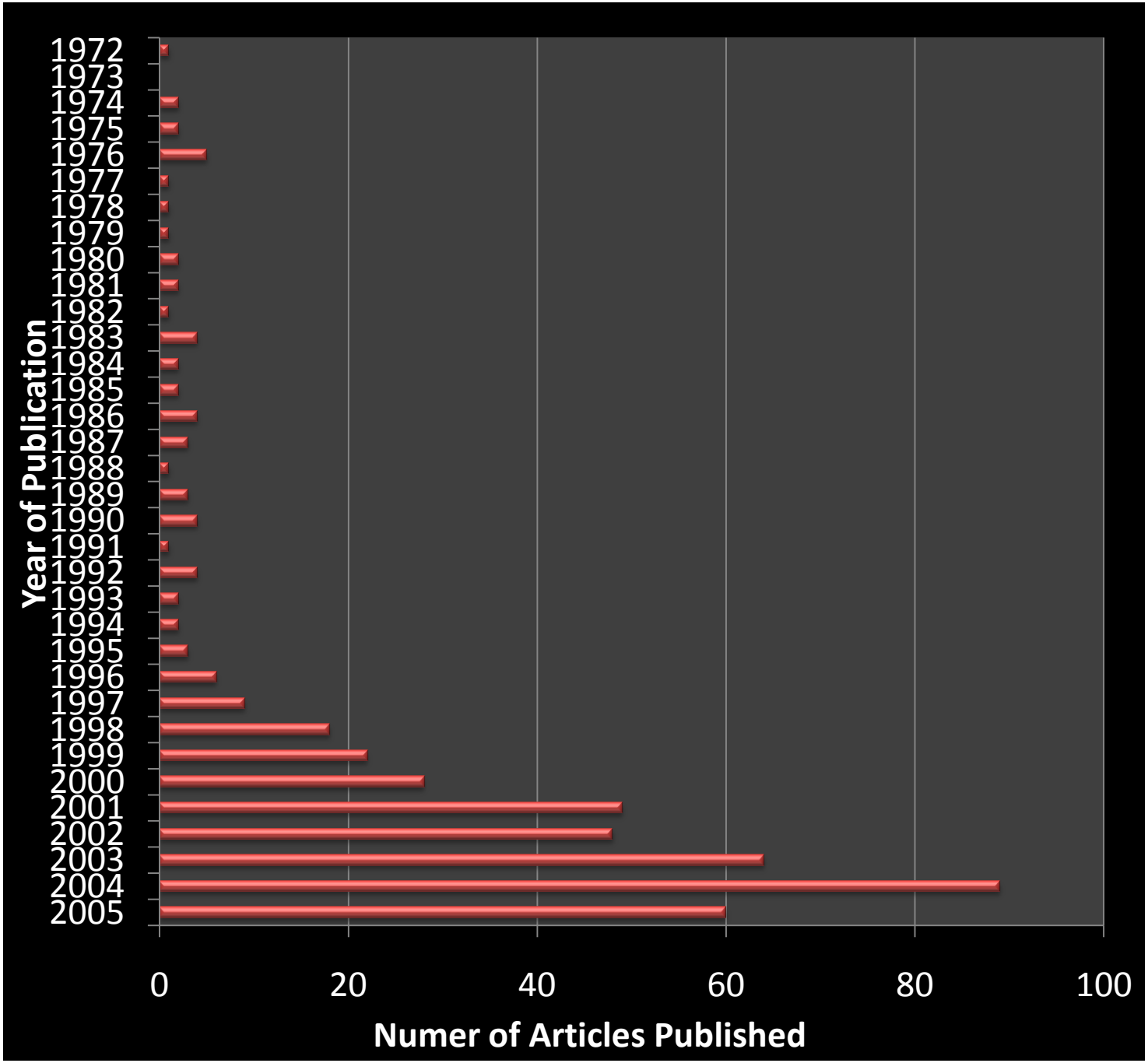

From Enrico Gratton.

Good review papers on FCS

- “Fluorescence correlation spectroscopy an introduction to its concepts and applications” by P Schwille
- “Fluorescence correlation spectroscopy: the technique and its applications” by Oleg Krichevsky and Gregoire Bonnet

These review papers can be obtained from google search

Fitting function for our Lab

$$G(\tau) = \frac{1}{N} \frac{1}{(1+\tau/\tau_D)} \frac{1}{\left(1+\frac{w_{xy}}{w_z}\tau/\tau_D\right)^{1/2}} + 1$$

w_{xy} and w_z is as given in FCS lab class.

Processing FCS data from the autocorrelator.

- Data files end in '.sin' can be opened in in notepad which will include autocorrelation function (ACF) , histogram and also intensity history.
- Use only the correlation function section in the file will be enough. Under that section, the first column is correlation time (lag time τ) and 2nd column is your correlation values G(τ).
- Plot G(τ) vs τ in matlab with x axis (τ axis) in log scale and you will be able see the correlation function raises at around 10-100 ms. ACF from 1μs to 30s is the range of data where the fitting should be focused on
- Fit G(τ) with FCS formula in the range of 1 μs to 30s in order to obtain diffusion time.