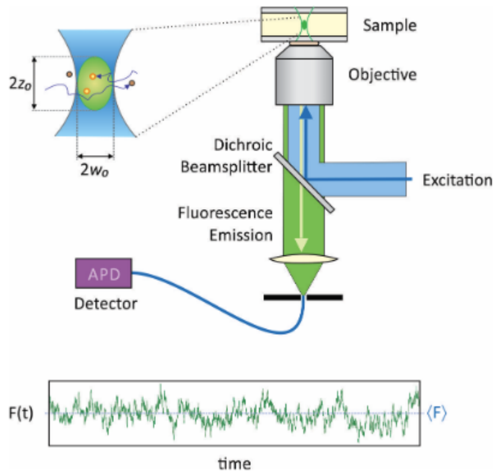
11.3: Fluorescence Correlation Spectroscopy

Fluorescence correlation spectroscopy (FCS) allows one to measure diffusive properties of fluorescent molecules, and is closely related to FRAP. Instead of measuring time-dependent concentration profiles and modeling the kinetics as continuum diffusion, FCS follows the steady state fluctuations in number density of a very dilute fluorescent probe molecule in the small volume observed in a confocal microscope. We measure the fluctuating changes in fluorescence intensity emitted from probe molecules as they diffuse into and out of the focal volume.



- Average concentration of sample: C_0 = <10^{-9} M – 10^{-7} M.
- This corresponds to an average of ~0.1-100 molecules in the focal volume, although this number varies with diffusion into and out of the volume.
- The fluctuating fluorescence trajectory is proportional to the time-dependent number density or concentration:

$$F(t) \propto C(t)$$

- How big are the fluctuations? For a Gaussian random process, we expect $\dfrac{\delta N_{rms}}{N} \sim \dfrac{1}{\sqrt{N}}$
- The observed concentration at any point in time can be expressed as time-dependent fluctuations about an average value: $C(t) = \overline{C} + \delta C(t)$.

To describe the experimental observable, we model the time-dependence of δC(t) from the diffusion equation:

$$\frac{\partial \delta C}{\partial t} = D\nabla^2 \delta C$$

$$\langle \delta C(r,0)\delta C(r',t)\rangle = \frac{C_0}{(4\pi Dt)^{3/2}} e^{-(r-r')^2/4Dt}$$

The concentration fluctuations can be related to the fluorescence intensity fluctuations as

$$F(t) = AW(r)C(r,t)$$

W(r): Spatial optical profile of excitation and detection
A: Other experimental excitation and detection parameters

Calculate FCS correlation function for fluorescence intensity fluctuations. $F(t) = \langle F \rangle - \delta F(t)$

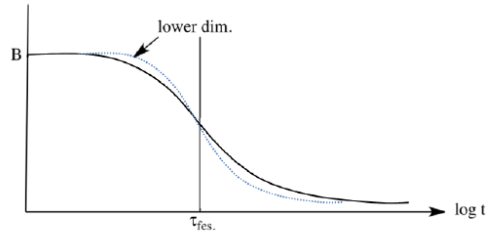
$$G(t) = \frac{\langle \delta F(0)\delta F(t)\rangle}{\langle \delta F\rangle^2}$$

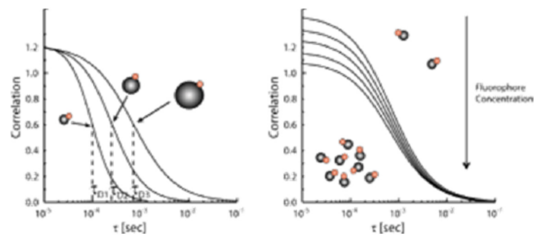
For the case of a Gaussian beam with a waist size w_0:

$$G(t) \sim \frac{B}{1+t/\tau_{FCS}}$$

Where the amplitude is $B = 4\pi A^2 I_0^2 \overline{\delta C_0^2} w_0^2$, and the correlation time is related to the diffusion constant by:

$$\tau_{FCS} = \frac{w_0^2}{4D}$$





Readings

- P. Schwille and E. Haustein, "Fluorescence Correlation Spectroscopy: An Introduction to its Concepts and Applications" in Biophysics Textbook Online.