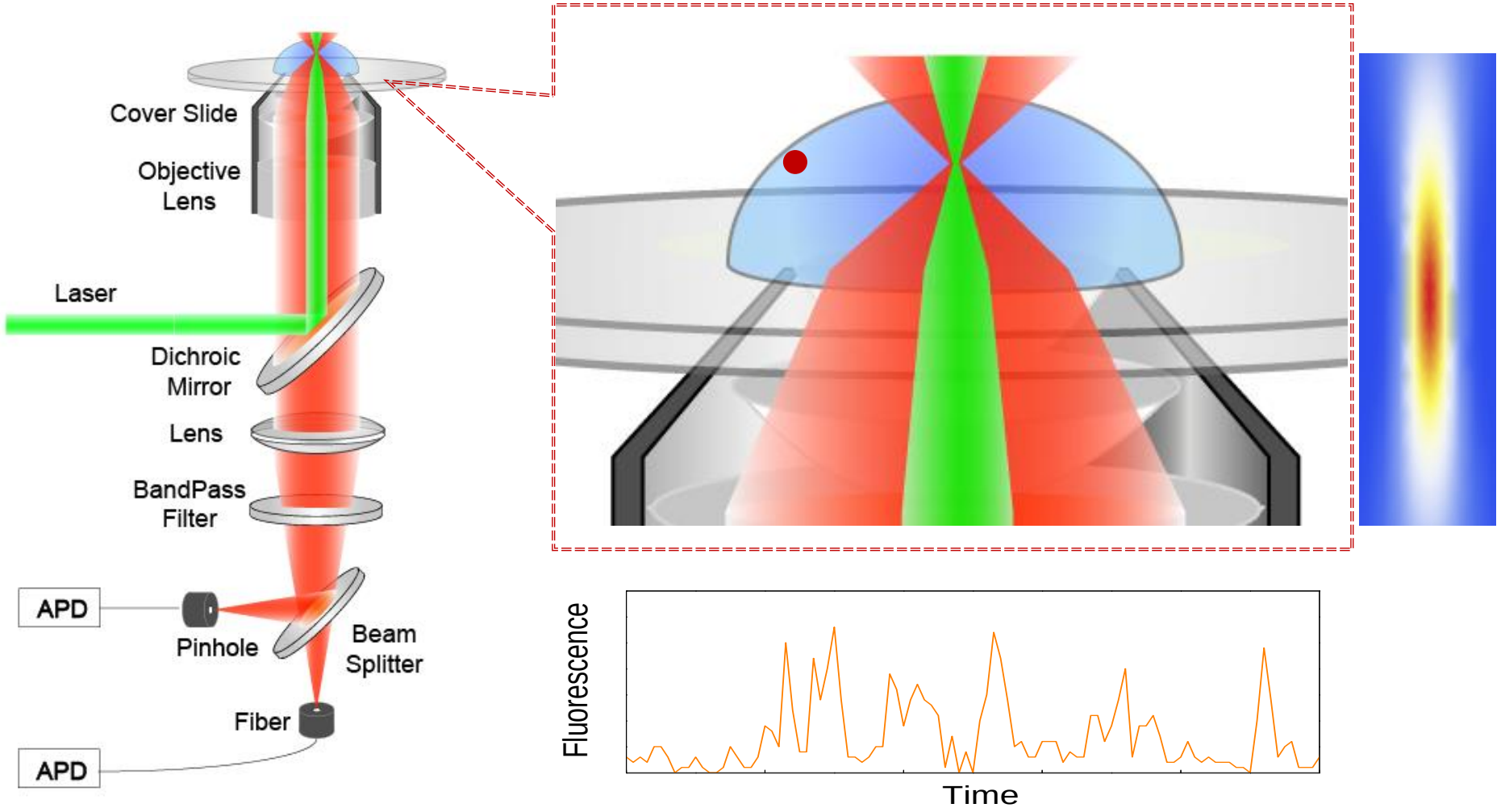
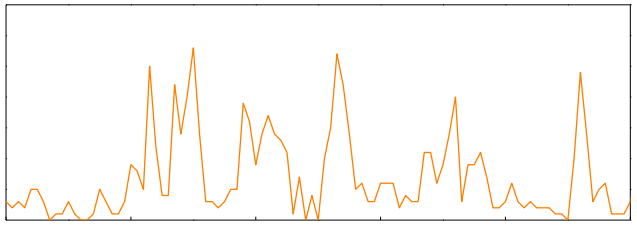
Confocal Microscopy & Low Concentration Dye



1nM concentration dye is exist in 1μm^3 (1fL) Detection Volume

One Molecule at a time

Fluorescence Fluctuation in the Detection Volume



$$\delta F(t) = A\int_V W(r)\cdot\delta C(r,t)\cdot dV$$

A : absorption coefficient, quantum yield …
W(r) : Excitation Beam profile & Confocal Coupling Function
δC(r,t) : Fluorophore concentration change in Detection Volume

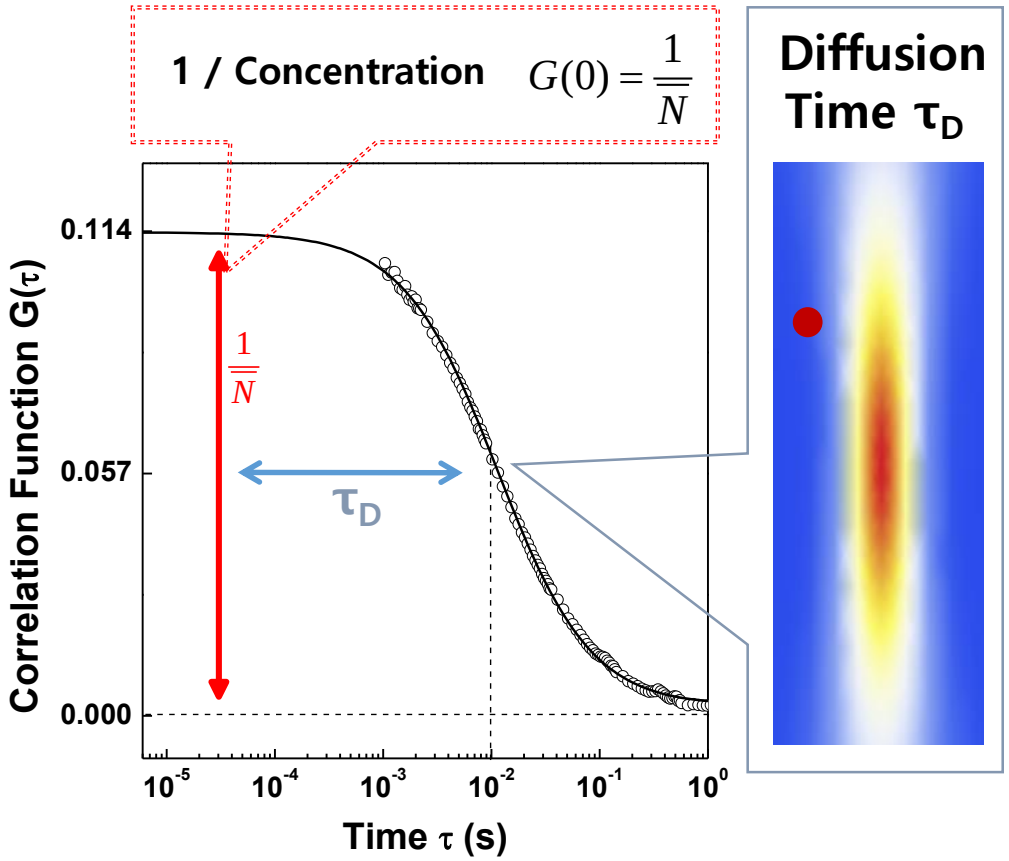
Correlation Function (in Gaussian beam geometry)

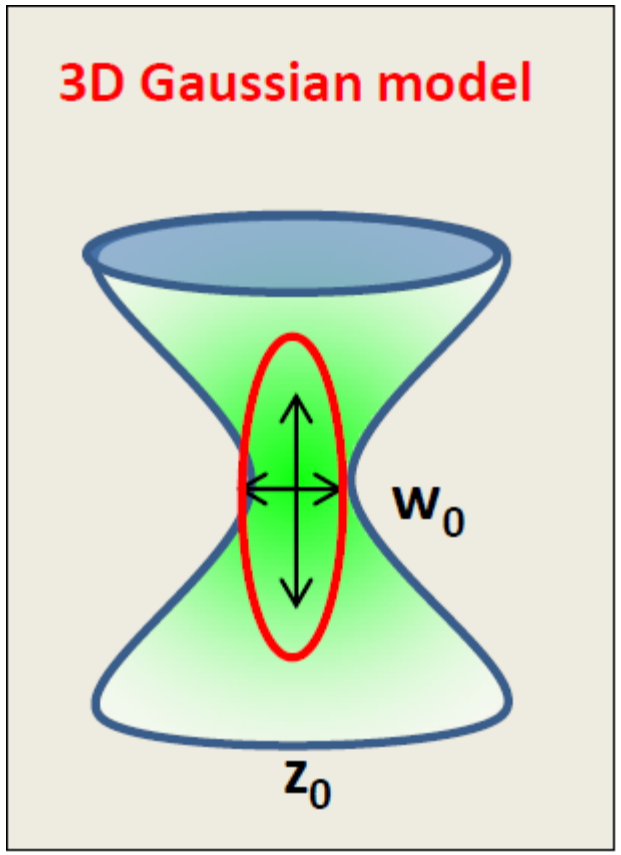
$$G(\tau) = \frac{\langle \delta F(t) \cdot \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2}$$

$$G(\tau) = \frac{1}{N} \frac{1}{1+\frac{\tau}{\tau_d}} \frac{1}{\sqrt{1+\left(\frac{r_0^2}{z_0^2}\right)^2 \frac{\tau}{\tau_d}}}$$

$$\tau_d = \frac{r_0^2}{4D}$$

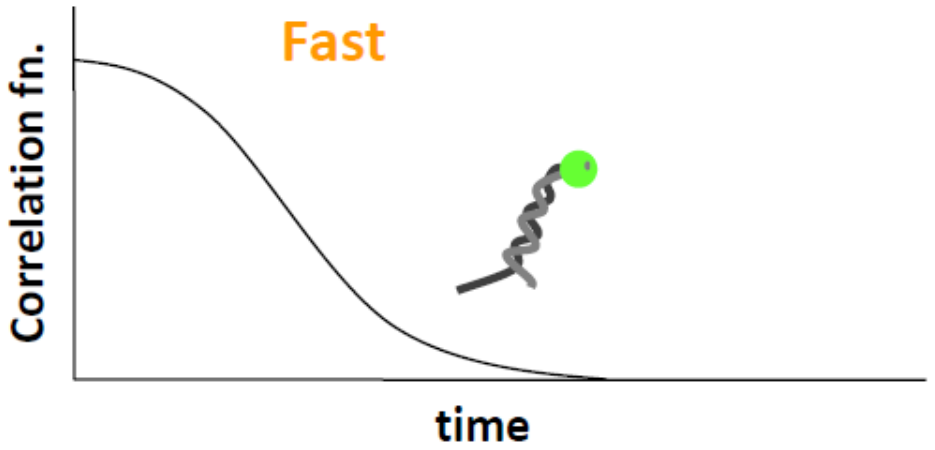
D : Diffusion coefficient
r_0 : transverse length of focal volume
z_0 : longitudinal length of focal volume

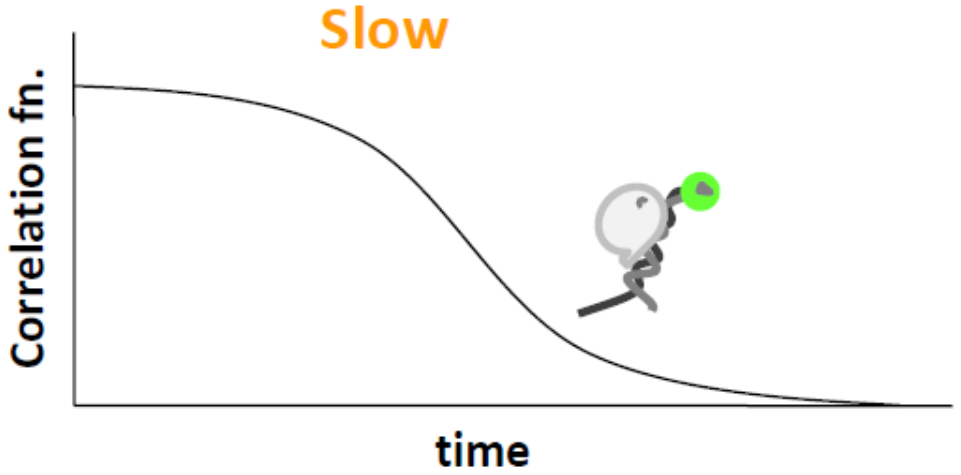




$$V_{eff} = \pi^{2/3} w_0^2 z_0$$

2 species model

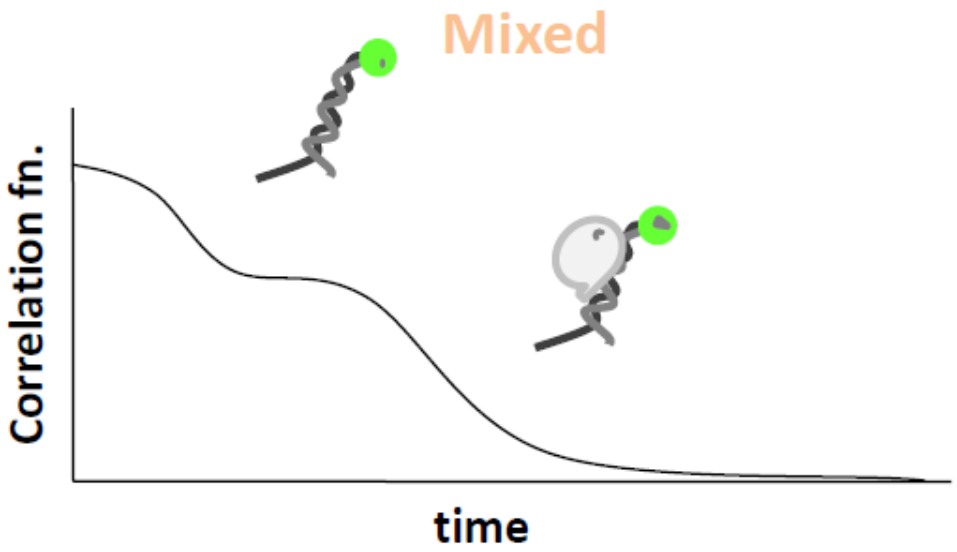




Stokes-Einstein relation

$$D = \frac{k_B \cdot T}{6\pi \cdot \eta \cdot R}$$

R : Particle size
k_B : Boltzmann constant
η : Viscosity
T : Temperature



$$G(\tau) = F_1^2 \cdot G_1(\tau) + F_2^2 \cdot G_2(\tau)$$