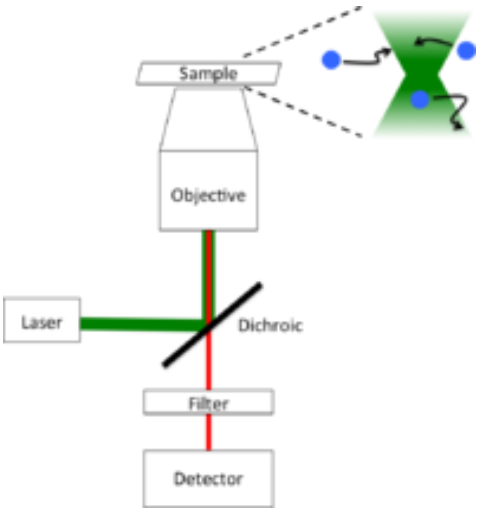
Fluorescence Correlation Spectroscopy

This summer I worked on Fluorescence correlation spectroscopy. It is a technique of analysing the fluctuation in fluorescent intensities due to fluctuation in concentration of molecules in a small region of the solution. This fluctuation in concentration is caused by the Brownian motion of molecules in the solution. These fluctuations provide important physical parameters upon analysis. In my case, we analysed the fluctuations for diffusion constant of a fluorescent protein, Rhodamine6g. We built an apparatus for measuring the fluctuations in intensity of an illuminated rhodamine sample.

Description of apparatus

First, we took a monochromatic laser of wavelength 532nm, which is close to the absorption maxima of rhodamine molecules. We fitted some lenses on the path of the beam to increase its width. The purpose of this is to obtain a sharp focal region in the solution when it is passed through the objective. This wide beam is now allowed to be reflected by a dichroic filter into the objective. A dichroic filter allows certain wavelengths to pass and reflects the others. The incident beam is reflected by the dichroic and emitted rays are completely passed on. Thus, we can separate the emitted and incident waves. Upon passing through the objective of high numerical aperture (NA=1.2), the beam is focussed at a very sharp focal volume in the solution. If we keep the concentration of the protein very low, then there would be only a few particles in the region. Hence any particle moving into the focal region will cause a detectable change in the intensity of emitted light. The emitted light is collected by the same objective, passes through dichroic and it is collected by an Avalanche photodiode(APD) which can count the number of photons entering with a very high precision. We can measure the fluctuation in intensity and hence measure the fluctuation of concentration inside the focal volume.

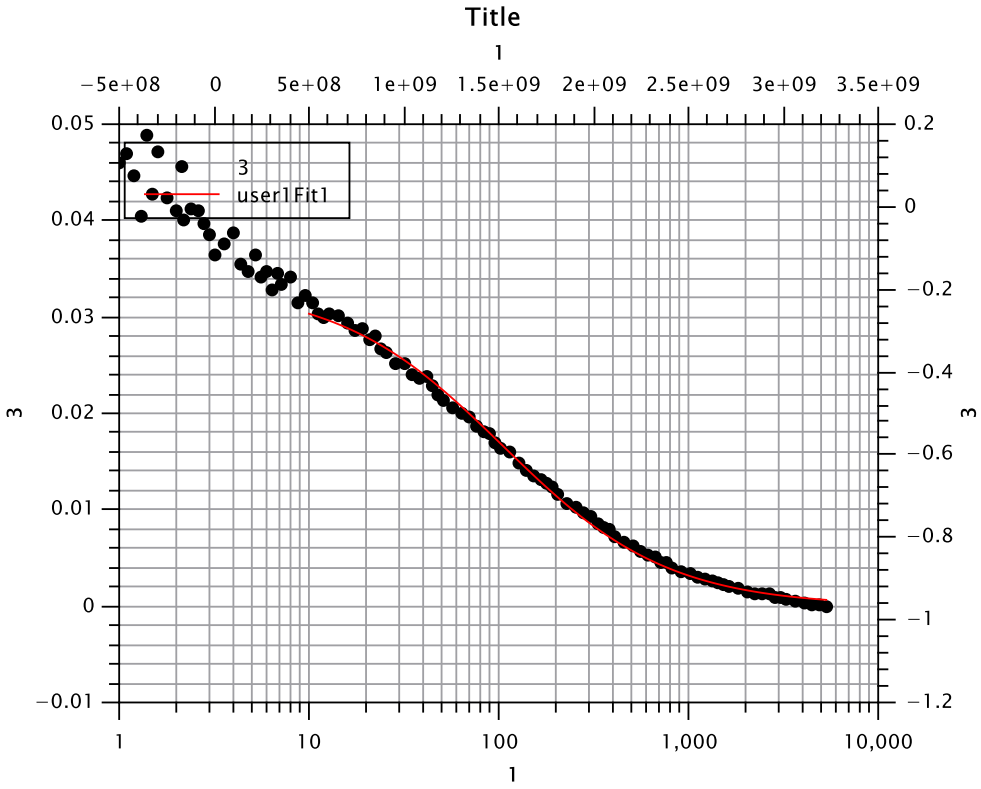


Typical FCS setup

Analysis of Data

The autocorrelation function looks like $G(\tau)=\langle I(t)I(t+\tau)\rangle/\langle I(t)\rangle^2$, where I(t) is the intensity at time t. The output of APD is the value of this function at various τ. For normal diffusion, one can derive the value of G(τ) to be $G(0)/((1+ \tau/ \tau_D)(1+ \tau/a^2 \tau_D)^{1/2})$, where a is w_Z/w_{XY} and is the length/width of the focal volume and τ_D is the characteristic residence time or diffusion time. G(0) gives us the mean number of diffusing particles in the region and is inversely proportional to concentration of the molecules. By plotting G(τ) vs τ and providing a fit to it, we can find 'G(0)', 'τ_D' and 'a' from the data. Once we know τ_D we can find diffusion constant D using $D = w_{xy}^2/4\tau_D$. w_{xy} can be calculated using $w_{xy}^3=V_{eff}/\pi^{3/2}a$. V_{eff} can be known from concentration of the solution and G(0).

Result



Shown above is a plot of one of the measurement sessions and is fitted with the equation $c/(1+x/b)(1+x/a^2b)^{1/2}$. 'c' or G(0) turns out to be 0.03 and 'b' or τ_D turns out to be 75.4µs and 'a^2' turns out to be $3.73*10^4$. Average number of particles in the confocal focus is 33. Concentration of solution was around 100nM and therefore V_{eff} turns out to be $5.48*10^{-13}m^3$. w_{xy} turns out to be 0.11µm and hence D is $4.01*10^{-10}m^2s^{-1}$.