# Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy uses fluctuation in fluorescence intensity to calculate diffusion coefficients.

A small volume of the sample, the confocal volume, is illuminated by a laser. Brownian motion of fluorescent nanoparticles and molecules will cause temporal variations in intensity of the light emitted from the confocal volume. These fluctuations are used to derive an autocorrelation curve, which will be related to the number of fluorophores and their average time for passing through the confocal volume. Since the confocal volume is known, the concentration of fluorescent nanoparticles and their average diffusion coefficients can be derived from the autocorrelation function, and the Stokes relation can be used to calculate their average hydrodynamic diamter.

FCS is extremely sensitive, and can determine low concentrations of fluorescent nanoparticles on sub-nm resolution. Since it measures real-time diffusion in liquid samples, sample pertubations that can occur in other methods due to drying and surface-interactions of the nanoparticles, are minimized in FCS. Another great advantage is that fluorescent nanoparticles can be measured inside living cells.