Saturday: June 10th.

Two-photon Microscope Experiments:

Preliminary Discussion:
Observation of the two-photon volume: One-photon vs. two-photon excitation. This will establish the significance of using two-photon excitation for fluorescence.

Experiments with the Two-Photon Instrument:

Fluorescein Dilution and Binding:

Measure a sample of Fluorescein (~20nM). Perform autocorrelation and analyze to determine the diffusion coefficient and amplitude. You can now estimate the molecular brightness and concentration.

Next we dilute this sample by 2X and observe the effect on the calculated autocorrelation function. What effect will dilution have on the amplitude? How about the diffusion coefficient?

Now we will add ~0.5 nM of IgG (fluorescein antibody used in the fluorometer experiments). From yesterday we know that IgG will cause quenching of the fluorescein. Observe the amplitude and brightness after the addition of the IgG. Any changes in the diffusion coefficient?

Now we will add 5nM of IgG and observe. This time quenching will have a greater effect. What happens when we fit the autocorrelation function?

Cell Measurements:

A powerful application of FCS experiments is inside living cells. With FCS you can observe the motion of enhanced green fluorescent protein (eGFP) diffusing randomly in live cells and quantify it.

We will measure fluorescence of eGFP in the nucleus and analyze the correlation function as we did with fluorescein. What is the most notable difference in the shape of the autocorrelation function for eGFP and fluorescein?

Data Analysis:

For the above experiments we will use software written for IDL (a simple programming language with nice visualization routines). We will read the raw data in and calculate the autocorrelation function. We can then fit this autocorrelation to get quantitative results.