Study of dynamic processes of nanostructures by means of new generation confocal spectroscopy

LAMICS

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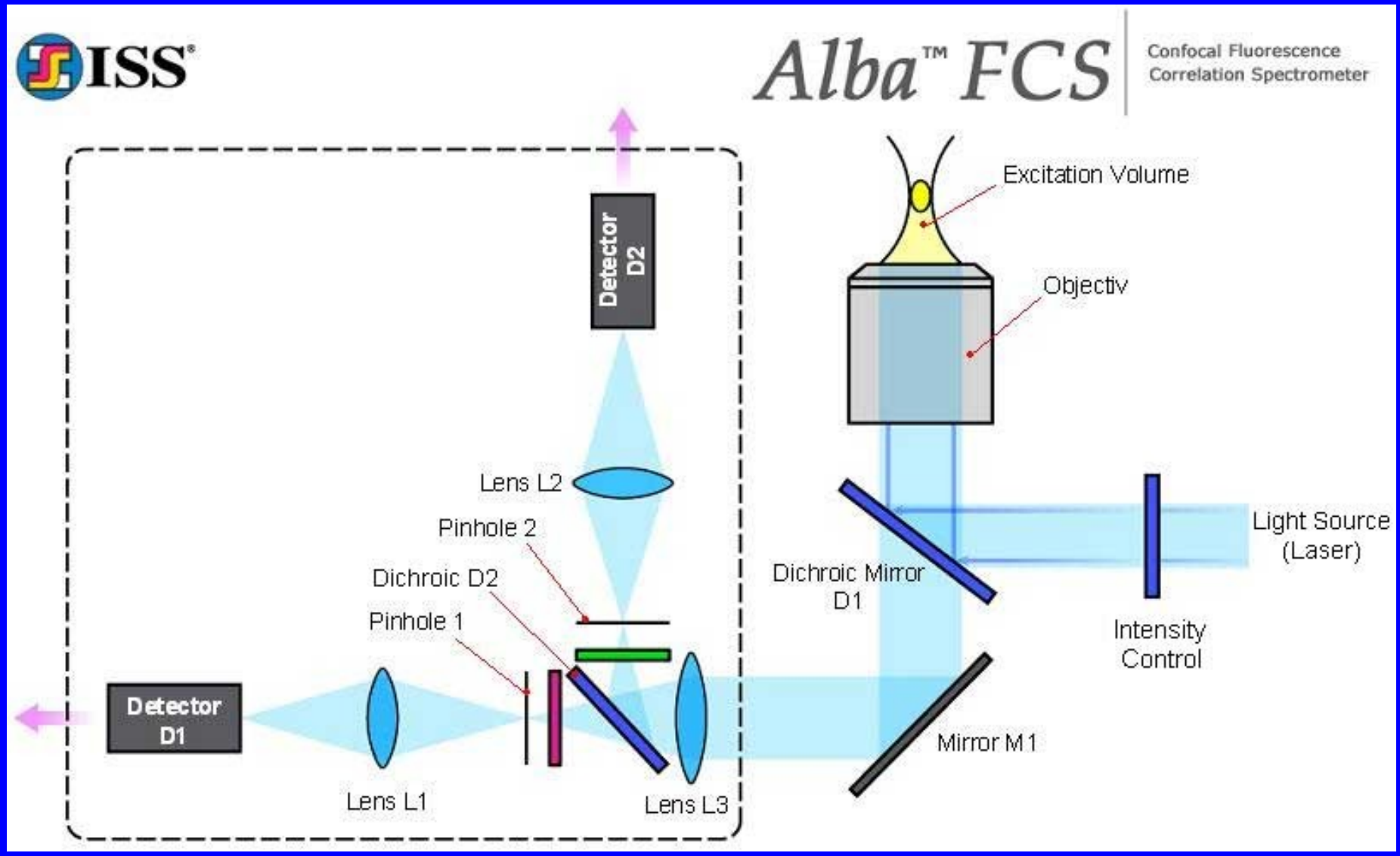
nast
Nanoscience
Nanotechnology
Innovative Instrumentation

Alba FCS™ Fluorescence Correlation Spectrometer

Alba FCS™ is a dual channel spectrometer that combines a confocal scanning microscope with fluorescence correlation spectroscopy (FCS) and is optimized for both single-photon and multi-photon excitation.

TECHNICAL LAYOUT



Excitation Modality

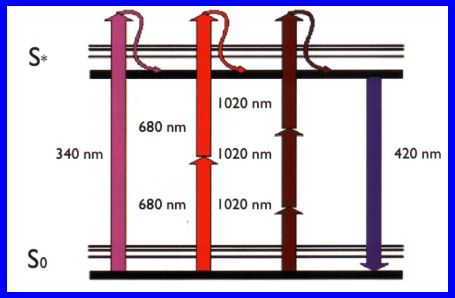
Single photon excitation (using an argon ion, krypton ion, or dye-laser); or Multi-photon excitation (using a Ti:Sapphire laser).

LIGHT SOURCES

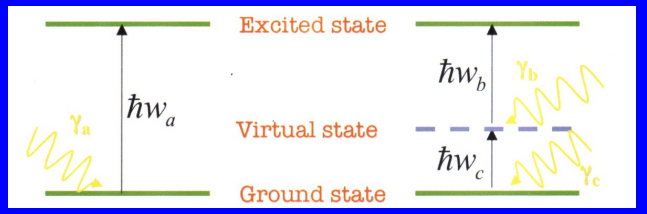
• Up to five **SINGLE PHOTON** lasers housed in a laserlauncher with computer-controlled beam expander, laser intensity and shutters. Wavelengths: 405, 457, 488, 514, 635 nm

• **MULTI-PHOTON** excitation with computer-controlled beam expander, laser intensity and shutters.

Two (or more) photons excitation: rate energy transfer

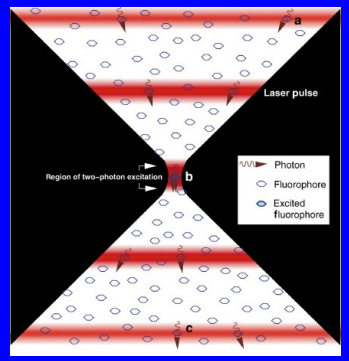


$\lambda ex_n = n\,\lambda ex$



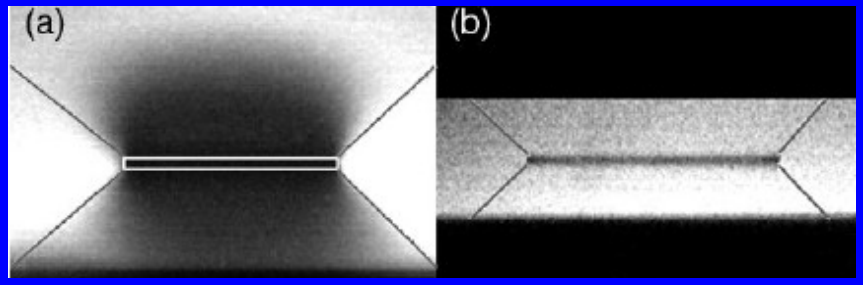
photons co-localization

- spatially ⟶ by focusing
- in time ⟶ by pulses of mode-locked laser



ADVANTAGES:

- low excitation probability ($\propto I^2$) $\Longrightarrow$ excitation volume ≡ larger focalization region ($\leq 1\ \mu m$)
- cromophore out of focal plane are not excited $\Longrightarrow$ reduced photobleaching and photodamage
- the excitation light penetrates through the sample to the plane of focus $\Longrightarrow$ increased sample penetration reduction background



two photons 3D exc.is that of an ideal confocal microscopy

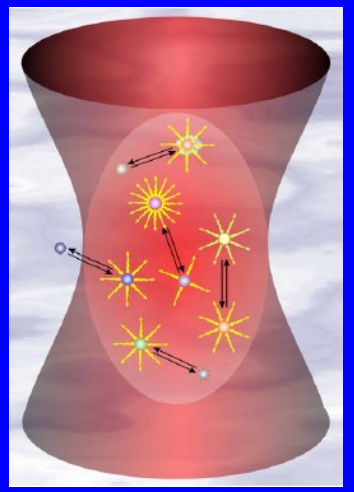
FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

fluorescence emission fluctuations measurement around average value

HIGH DILUTION
High background fluorescence

VOLUME OBSERVATION REDUCTION
Sample fluorescence observation

Single molecule fluorescence



- *Confocal Microscopy* $\rightarrow V_{exc} = 1\mu m^3$
- *Two photon excitation* $\rightarrow$ *maximum photons excitation focalized* $\rightarrow$ *increased* $\lambda \Rightarrow$ *scattering reduction*

possibility to obtain small excitation volume

Measure of a fluctuation signal requires statistical methods to be analyzed

The FCS utilizes:

- **Autocorrelation function**: describes the time dipendent decay of fluctuations intensity of fluorescence signal compared to equilibrium value

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

G(τ) si directly obtained from FCS experiments → $D=K_BT/6\pi\eta R$

G(τ) si directly obtained from FCS experiments → G(0)= 1/<N>

- **Photon Counting Histogram:** describes probability distribution of photons recorded at each sampling time

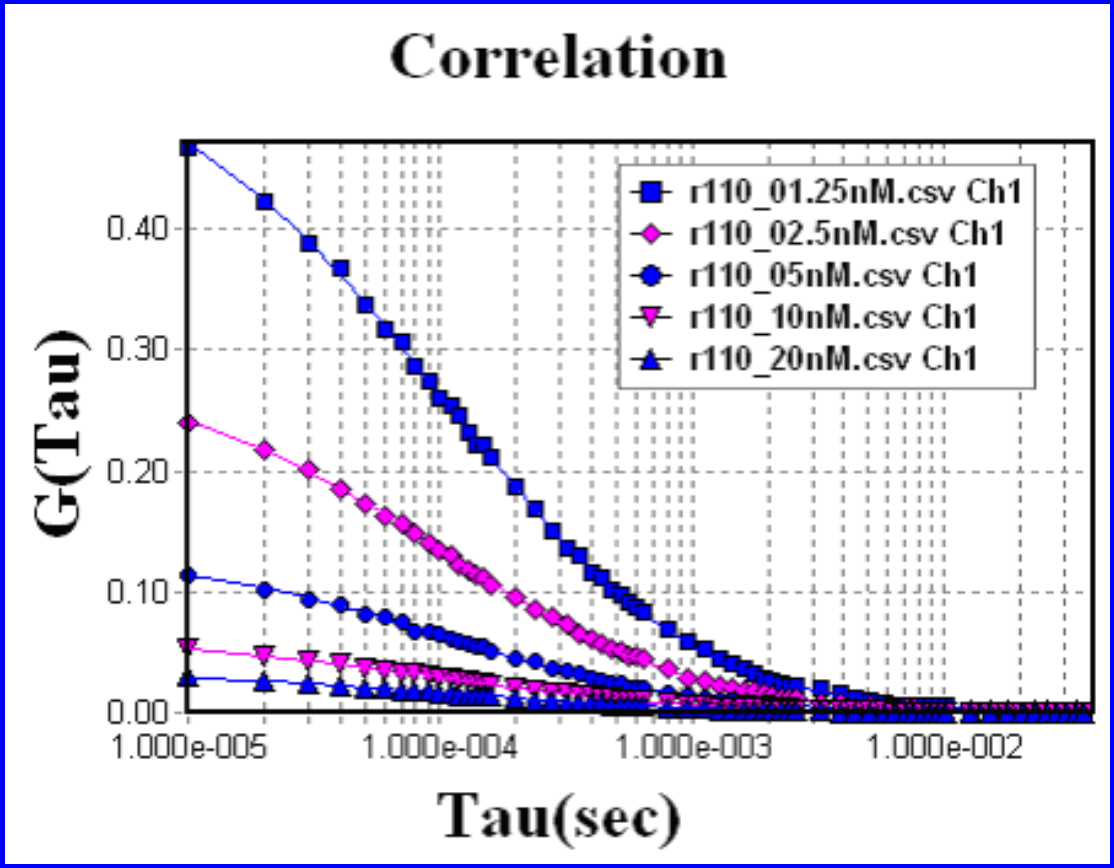
$$P(N) = \frac{\overline{N}^N \cdot \exp(-\overline{N})}{N!}$$

→ Brightness

→ $\overline{N}$

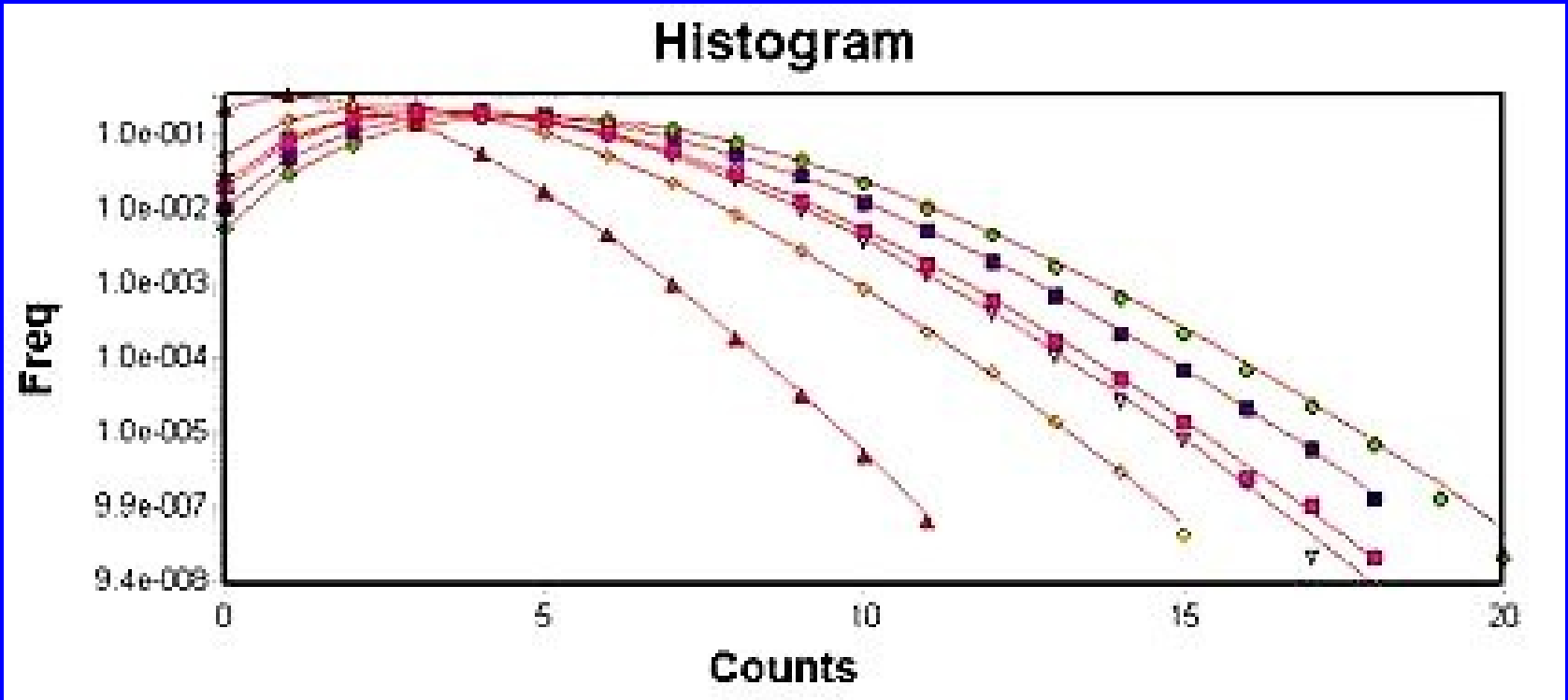
ALBA FCS™ Measurement Examples

Alba FCS™ provides two fundamental and complementary features to obtain information about the molecular dynamics of the sample under observation: the autocorrelation function and the photon counting histogram (PCH).



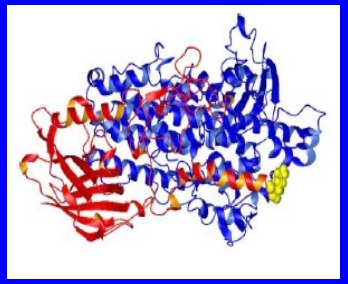
Auto-correlation curves and 3D-Gaussian fits for a concentration series of 1.25, 2.5, 5.0, 10 and 20 nM Rhodamine 110 in water.

PCH Measurement Examples



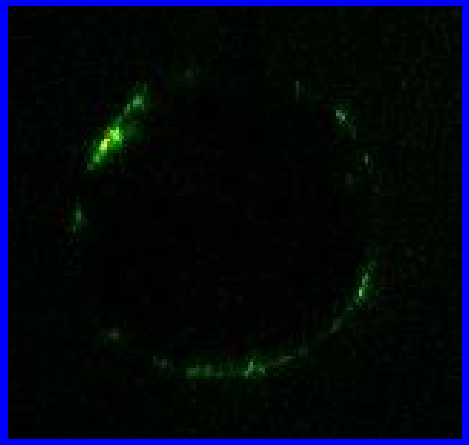
In the measurement shown above, solutions of different concentrations of Coumarin and Rhodamine110 were prepared and exposed to 2-photon excitation at 780-nm. A global PCH analysis was then performed on the data. From these data, the following parameters can be determined: brightness and number of molecules in the excitation volume.

Investigation of **LOX-15** structural changes involved in membrane binding

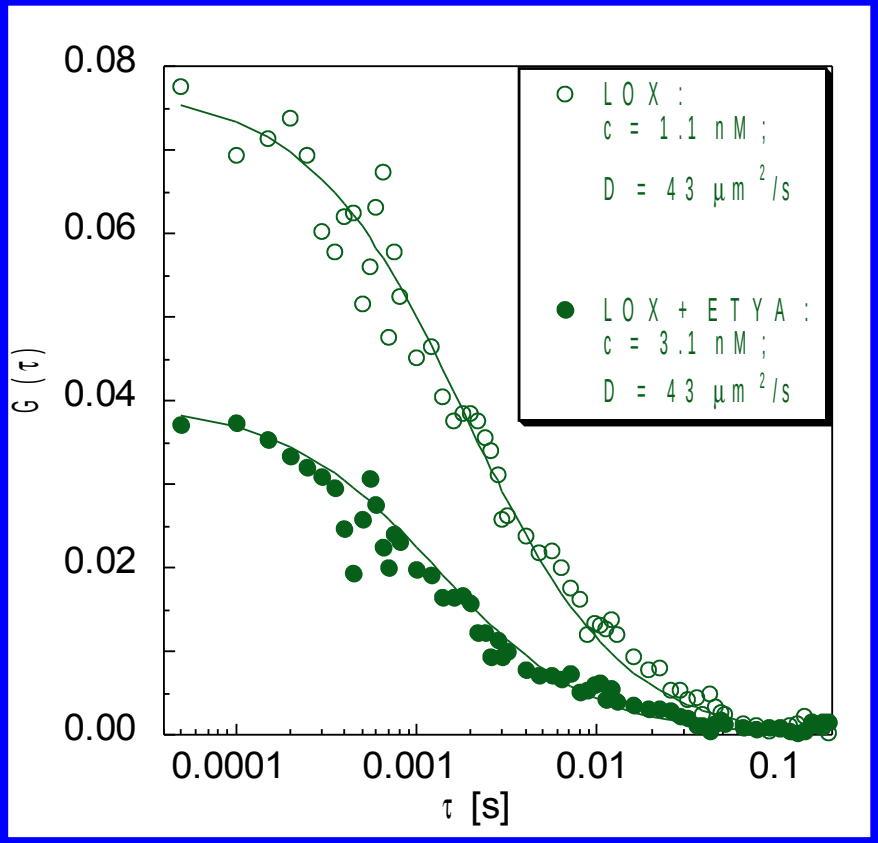


LOX-15 soybean

ETHYA



Imaging of ALEXA labeled Lox bind to GUV



FCS measurement outside the GUV volume

Alba FCS™, Spectroscopy on Single Molecules

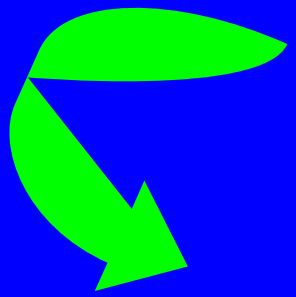
MEASUREMENTS

Imaging

Fluorescence Correlation Spectroscopy

Photon Counting Histogram (PCH)

Scanning FCS



- Single molecule observation
- Low damage radiation “in vivo”
- Observation of localized kynetics phenomena