Enhancing FCS through Rapid Scanning and Pattern Matching

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Fluorescence Correlation Spectroscopy (FCS) is an essential tool for understanding the dynamics of complex cellular processes. By applying the approach know as scanning FCS (sFCS), significant improvements can be obtained when studying slow moving molecules as is often the case in cell membranes. In sFCS, the excitation volume is scanned rapidly, which reduces the time required to record a high number of transits.

The shorter residence times lead to lower photon doses experienced by each detected molecule and thus reduce the risk of photobleaching. This is especially important for sensitive fluorophores or when performing Stimulated Emission Depletion (STED) FCS measurements. The additional information gained by recording the focal position during measurement can be used to determine the shape and size of the confocal volume without requiring an additional calibration step. This information can also be used to determine parameters related to flow and other active transport mechanisms.

Using the unique pattern matching analysis method [1], multiple species can be excited with the same laser and later discriminated while ensuring that the excitation volume remains identical for all species. This approach allows for a high degree of separation even for labels with very close emission wavelengths.

[1] T. Niehoerster et al., *Multi-target spectrally resolved fluorescence lifetime imaging microscopy*, Nature Methods, 257-262, 13(3), 2016.