

# PicoQuant's sFCS solution

## What is scanning Fluorescence Correlation Spectroscopy (sFCS)?

sFCS represents an evolutionary step in fluorescence correlation spectroscopy (FCS). Instead of recording data from a fixed spot, the observation volume is repeatedly scanned in a defined line over the sample. With this approach, sFCS can overcome several limitations of classical FCS.

Thank to its design, PicoQuant's MicroTime microscopy platform achieves outstanding single molecule sensitivity, making it the best choice ideal for all types of FCS measurements. When equipped with the FLIMbee galvo scanner add-on, the MicroTime 200 is capable of fast linear scan motions with constant speeds. As high linearity with high, constant velocity are key factors in analyzing sFCS data, the FLIMbee galvo scanner is ideally suited for this method.

## Why use sFCS?

By combining fast line scanning with the ability to observe long range spatial correlations, this method provides several advantages:

- **Include lifetime information.** A novel key factor in our approach is the combination of sFCS data with lifetime information. This allows using advanced excitation schemes such as Pulsed Interleaved Excitation (PIE) to reduce spectral crosstalk as well as employing lifetime information as an additional contrast factor, making it possible to unmix multiple luminescent species in a sample.
- **Observe a larger ensemble of species.** Monitoring a larger ensemble thanks to the moving observation volume leads to improved statistics. This strongly facilitates investigations of species with diffusion times in the millisecond range, compared to classical FCS, which is particularly relevant for studying protein dynamics in various environments such as living cells or membranes.
- **Reduce photobleaching.** Our sFCS approach can be combined with the Stimulated Emission Depletion (STED) super-resolution option of our MicroTime 200 platform. A great advantage of such a combination is that the scanning will strongly reduce sample photobleaching compared to conventional STED-FCS. Performing STED-sFCS measurements, which operate in a similar manner as spot variation FCS, allow probing diffusion properties of fluorophores in membranes beyond the diffraction limit.
- Additionally, sFCS provides spatial correlations, allowing directly measuring processes such as drift, flow, etc.

## What does PicoQuant's sFCS solution include?

Since sFCS is still a rather new method, no standardized workflow for data analysis has been established yet. PicoQuant wants to make this novel approach accessible to a wider audience and has thus decided to offer the following:

- **Data acquisition.** A new line scanning mode for sFCS (using the fast x-axis of the FLIMbee scanner) is introduced in the SymPhoTime 64 software. This enables collecting sFCS data using any MicroTime 200 equipped with a FLIMbee scanner.
- **Data analysis.** The acquired data can be analyzed using several external third-party software options that are available free of charge.

Stay tuned for more details, including application examples.