PicoQuant's sFCS solution

What is scanning Fluorescence Correlation Spectroscopy (sFCS)?

Scanning Fluorescence Correlation Spectroscopy (sFCS) represents a variant of 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 conventional FCS.

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

Why use sFCS?

Combining temporal fluorescence correlation analysis with fast line scanning offers several advantages:

- **Observe a larger ensemble of species.** Monitoring a larger ensemble thanks to the moving observation volume leads to improved statistics. This also facilitates investigations of species with diffusion times in the millisecond range compared to conventional FCS, which is particularly relevant for studying protein dynamics in various environments such as living cells or membranes.
- **Reduce photobleaching.** Scanning the observation volume across the sample strongly reduces photobleaching and causes less phototoxicity compared to conventional FCS. Our sFCS approach can be combined with the Stimulated Emission Depletion (STED) super-resolution option of our MicroTime 200 platform. Performing STED-sFCS measurements beyond the diffraction limit, which operate in a similar manner as spot variation FCS, allow investigating diffusion properties of fluorophores in membranes.
- **Get the calibration for free.** sFCS provides not only temporal but also long range spatial correlations. The spot size is directly extracted from the collected data and there is no need for additional calibration measurements.
- **Include lifetime information.** A novel key factor in our approach is the combination of sFCS data with lifetime information. The fluorescence lifetime provides an additional contrast, enabling e.g., the unmixing of multiple luminescent species in a sample. In addition, advanced excitation schemes such as Pulsed Interleaved Excitation (PIE) are available for multi-species approaches.

What does PicoQuant's sFCS solution include?

Since sFCS is still a rather new implementation, 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 external third-party software options that are partly available free of charge. In order to also gain access to the fluorescence lifetime information in sFCS analysis, PicoQuant will offer ready-to-use Matlab code developed in collaboration with Jörg Enderlein (Georg August University Göttingen). This code will be available as open source.

Stay tuned for more details, including application examples.