

# COMPUTER-ENHANCED IMAGING FLUORESCENCE FLUCTUATION SPECTROSCOPY

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**KEY WORDS:** quantitative bioimaging, fluorescence correlation spectroscopy, number and brightness analysis, machine learning, deep learning

Camera-based fluorescence techniques have a temporal resolution below milliseconds and a spatial resolution beyond the optical diffraction limit. These measurements of high spatiotemporal resolution nevertheless require substantial measurement time of seconds if not minutes to obtain a sufficiently high signal-to-noise ratio. This leads to data sets of tens of thousands of frames that easily reach gigabyte size with a large number of evaluation possibilities.

These sets of data pose two essential challenges. The first is the time needed to evaluate the data in all its possibilities in a time frame that is acceptable for a researcher. Ideally the data is evaluated in seconds or less to allow the researcher full control over the experiment. This first challenge can be met nowadays by parallel computing and in particular with Graphic Processing Units (GPU), that can speed up calculations several orders of magnitude. The second challenge is the sheer amount of data collected. If 100×100 pixels per frame are collected with a time resolution of 1 millisecond and a total measurement time of 1 minute, and if each pixel is to be evaluated individually, this leads to 10,000 functions, each calculated from 60,000 fluorescence values, that need to be evaluated every minute. This is beyond the capability of humans and we therefore need automated evaluation strategies. This second challenge is met by machine learning approaches that can help classify and evaluate data in a timely manner.

Here, we demonstrate the application of GPU based evaluations to considerably speed up the calculation and evaluation of imaging fluorescence correlation spectroscopy (imaging FCS) and Number and Brightness (N&B) data to assess the dynamics of live biological systems in real time. To address the second question of the automated evaluation of data we introduce a machine learning approach that not only correctly evaluates FCS data but improves on the data fitting compared to traditional non-linear least-square fitting. The two approaches together, accelerated computational evaluation via GPUs and deep learning-based data evaluation bring us a step closer to a generalize quantitative bioimaging approach in which data is recorded at an optimal spatiotemporal resolution which then can be evaluated in a plethora of different ways to investigate the dynamics and organization of biological systems in vivo.