

# **TUTORIAL - FLUORESCENCE CORRELATION SPECTROSCOPY: FROM BASIC THEORY TO APPLICATIONS**

**Thorsten Wohland**

**Departments of Biological Sciences and Chemistry and Centre for Bioimaging Sciences,  
National University of Singapore, 14 Science Drive 4, 117557 Singapore,  
[twohland@nus.edu.sg](mailto:twohland@nus.edu.sg)**

Fluorescence Correlation Spectroscopy (FCS) is a widely used single-molecule sensitive technique to measure the dynamics of molecular processes. Any molecular process creates fluctuations in an observable parameter from which the dynamics and the nature of the process can be inferred. In FCS the observable parameter is the fluorescence signal from a small, typically femtoliter-sized, observation volume. Analysis of the fluorescence fluctuations from this volume allows determining the characteristics of molecular processes ranging from molecular blinking events and photophysics, to chemical reactions, and especially molecular movement.

In this tutorial we will discuss the basic principles of FCS and the concepts behind signal fluctuations and correlations [1]. After explaining the structure of the experimental data and its fitting we will investigate FCS limitations and how they can be solved by various FCS modalities. We will then concentrate on multi-wavelength approaches as used in Fluorescence Cross-Correlation Spectroscopy (FCCS), an especially powerful tool to quantitatively measure molecular interactions [2]. Finally, we will introduce multiplexed approaches in which FCS curves can be recorded in whole images [3]. In all parts of the tutorial we will concentrate on the basics concepts behind the FCS modalities and emphasize biological applications.

[1] X. Shi and T. Wohland, *Nanoscopy*, Chapter 6: “Fluorescence Correlation Spectroscopy” (CRC Press, Taylor and Francis Group, 2010)

[2] Foo, Y.H.; Naredi-Rainer, N.; Lamb, D.C.; Ahmed, S.; Wohland, T. Factors Affecting the Quantification of Biomolecular Interactions by Fluorescence Cross-Correlation Spectroscopy, *Biophys J*, 102 (2012) 1174-1183.

[3] Bag, N.; Wohland, T. Imaging Fluorescence Fluctuation Spectroscopy: New Tools for Quantitative Bioimaging. *Annu. Rev. Phys. Chem.* 65 (2014) 225–48.