Fluorescence Correlation Spectroscopy (FCS) relies on the sensitive detection of fluorescence fluctuations, created by single fluorescent molecules moving in and out of a small observation volume (~1 femtoliter). By calculating time correlations of the fluorescence intensity traces, one can extract the average number of molecules within the observation volume and their diffusion coefficient. In the last two decades FCS has developed into a widely used technique in the life sciences it has been used to study a wide range of biological question in vitro and in vivo.

FCS was invented four decades ago. Two obstacles initially prevented its widespread use. First, technological requirements for single molecule sensitivity and for its commercialization were met only in the 1990ies with advances in computer technology, lasers and detectors. Second, although its experimental implementation and application is straightforward, the interpretation of FCS measurements has been more challenging because of the conceptual basis, which is rooted in its statistical data treatment.

In this tutorial we will discuss the basic principles of FCS [1,2] and related techniques and discuss the concepts behind signal fluctuations and correlations. We will discuss data fitting and the different parameters that are extracted in FCS and limitations on their determination. In the second part we will discuss FCS measurements in live cells and tissues and give an overview of different FCS implementations, in particular cross-correlation spectroscopy [3] and imaging approaches to FCS.

The tutorial will take place in two parts:

Part 1:
- Basic principles of correlations
- The calculation of autocorrelation functions in time
- The experimental implementation of FCS
- Data fitting and parameter extraction

Part 2:
- Fluorescence Cross-correlation Spectroscopy
- Measurements in solutions
- Measurements in cells and tissues
- New development in FCS and related techniques