Fluorescence Correlation Spectroscopy (FCS) and its recent modification – dual-focus FCS (2fFCS) are used to study interactions between molecules or changes within a molecule. Both methods can be naturally combined with a laser scanning microscope. In such arrangement the methods allow studying molecular interactions in vivo, and in vitro. Here we used 2fFCS to measure diffusion coefficients of chemically-synthesized dyes (Atto655 and its derivatives, Alexa Fluor® 647 and Cy5) and oligopeptides in buffer solutions. In comparison with the conventional FCS system, 2fFCS allows measuring an absolute value of a diffusion coefficient with better than 1% accuracy. Therefore using this method, we were able to resolve and compare the diffusion behavior of the derivatives of the same dye, here Atto655, and of oligopeptides that differed by two amino acids in length only.

In polymer science the connection between diffusion coefficient, sedimentation constant and molecular weight is described by the Mark–Houwink–Kuhn–Sakurada (MHKS) power-law. Usually this relation is applied to estimate the molecular weight or the shape of long polymer molecules. Here the MHKS power-law was employed to characterize the diffusion behavior of small molecules when their molecular weight is known.

It was found that the diffusion behavior of different derivatives of Atto655 could be described by the Mark–Houwink–Kuhn–Sakurada (MHKS) power law with an exponent of 0.333, which is characteristic of globular molecules. However Cy5 and Alexa Fluor® 647 exhibited slower diffusion than expected for a sphere of the same molecular weight, and their diffusion coefficients were found to be between the limiting values for a sphere and a random coil. The more flexible structure of Cy5 and Alexa Fluor® 647 compared to Atto655 may be accounted for by the different diffusion behavior of those dyes.

The diffusion coefficients of short oligopeptides with amino-acid sequences going from one to eight amino-acids was characterized by an exponent of 0.5 in the MHKS equation, which is typical of a pure random coil polymer. To verify this result, we considered the complex systems (the dye, linker, and oligopeptide) as polymer molecules and modelled the data using various chain models. Intuitively, one would expect that a freely-rotating chain model is giving the best description of such system. Surprisingly, a very simple ideal, freely-joint chain model with a bond length of the peptide bond perfectly described the behavior of our complexes suggesting independent distribution of directions of different bond vectors.