Fluorescence Recovery after Photobleaching (FRAP) is a versatile technique to determine diffusion coefficients of suitably labelled species in fields like pharmaceutical research, biophysics or macromolecular chemistry [1]. Basically, a FRAP-experiment is realized by means of bleaching a certain area of the sample by a short and intense laser irradiation. Afterwards, the temporal recovery of fluorescence intensity in the bleached region due to the diffusion of unbleached molecules from the surroundings into this region is monitored by excitation with a highly attenuated beam. The diffusion coefficient can be deduced from the rate of recovery after suitable calibration. This procedure forms the basis for a variety of "classical" approaches to the evaluation of FRAP-experiments [1].

However, when FRAP-experiments are performed on a confocal laser scanning microscope (CLSM), the recovery process can be followed with high spatial resolution besides temporal resolution. Utilizing this aspect for a systematic analysis of FRAP data enables us to estimate the diffusion coefficient and the dimensionality of the diffusion process without the need for calibration [2].

This presentation shows the fundamentals of the latter approach. One- and two-dimensional diffusion processes (starting from a plane or a line source of the diffusing bleached species, respectively) can be readily realized on a CLSM equipped with an objective lens with low numerical aperture and accordant bleaching of a line- or a spot-pattern into the confocal plane. The fact that in reality the bleaching pulse is neither infinitely sharp (Dirac-shaped) nor infinitely short is taken into account by a proper shift of the experimental time scale. A number of experiments performed by using this method shows that the diffusion coefficient and the expected dimensionality are obtained with high accuracy and consistency. Furthermore, using the temporal and spatial FRAP-information enables us to estimate multiple diffusion coefficients in experiments where more than one diffusing species is involved. This approach is extended to deduce the distribution of diffusion coefficients from one multi-component FRAP-experiment without any need for previous calibration.
