MEASURING MOLECULAR DYNAMICS BY FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING (FRAP) AND SINGLE PARTICLE TRACKING (SPT)

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Obtaining quantitative information on the dynamics of molecules and particles in a variety of (bio)materials is of fundamental in many areas, ranging from cell biology to material sciences. Several complementary advanced fluorescence microscopy techniques are available to that end. In this tutorial lecture we will focus on two of them, fluorescence recovery after photobleaching (FRAP) and single particle tracking (SPT), with emphasis on practical guidelines on how to apply them.

In a FRAP experiment the fluorescent molecules are photobleached in a user-defined region of the sample by a high-power laser beam [1]. After photobleaching, the fluorescence inside the bleached area will recover due to the inward diffusion of fluorescent molecules from the surrounding unbleached areas. By analysing the kinetics of the fluorescence recovery, information on diffusion and binding can be obtained. In this tutorial we will go through the most important aspects of quantitative FRAP measurements with a focus on FRAP methods that are particularly robust and convenient to use.

While FRAP gives information on the mobility of a large ensemble of molecules, single particle tracking (SPT) is about visualising and analysing the movement of individual fluorescently labelled molecules or nanoparticles [2]. In this second tutorial, we will briefly go over the required instrumentation before moving on how to localize single particles in an SPT movie. It will be explained how this set of coordinates can be used to calculate the particle trajectories, which can be further analysed to obtain quantitative information on the particle's mobility.

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