RECTANGULAR FRAP WITH MAXIMUM ENTROPY METHOD FOR MEASURING CONTINUOUS DISTRIBUTIONS OF DIFFUSION COEFFICIENTS

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Fluorescence recovery after photo bleaching (FRAP) has been used extensively to study the mobility on a micrometer scale of molecules in terms of a diffusion coefficient [1]. Recently, a new pixel based FRAP method relying on the rectangular photo bleaching region (rFRAP) was developed to accurately measure the ensemble average diffusion coefficient over a wide range of experimental conditions [2]. The method has the advantage of fast and straightforward quantitative diffusion measurements due to a closed-form expression of the tempo-spatial recovery process. In order to extend rFRAP for the measurement of continuous distributions of diffusion coefficients, we have implemented Maximum Entropy analysis in this framework. Maximum Entropy ensures that features in the resulting distribution (such as local extrema) are statistically warranted by the data.

To test the intrinsic capabilities of this approach, rFRAP experiments were done for a polydisperse sample obtained by mixing 20 kDalton (FD20, 60% sucrose) and 2000 kDalton FITC-dextran (FD2000, 60% sucrose) at a mole ratio 450:1. Figure 1 shows the normalized frequency-distributions of diffusion coefficients obtained by rFRAP with Maximum Entropy analysis. The peak positions (3.72 µm²/s for FD20, 0.37µm²/s for FD2000) are in good agreement with the average values of 4.56 µm²/s and 0.31 µm²/s as determined from separate solutions of FD20 and FD2000 with single-component rFRAP[4]. These results show that rFRAP with Maximum Entropy analysis has good potential for measuring continuous distributions of diffusion coefficients of polydisperse samples. As a next step, the performance of the approach will be tested on simulated experiments of samples with multiple diffusion coefficients to systematically evaluate the effect of different experimental parameters (noise level, size of bleach area, frame rate, etc.) on the diffusion distribution. Afterwards, the method will be applied to estimate the aggregation of proteins and the degradation of macromolecules like DNA.

References