HIGHLY ACCURATE FRAP FRAMEWORK FOR ESTIMATION OF DIFFUSION COEFFICIENTS AND DIFFUSION AND BINDING KINETICS

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Fluorescence recovery after photobleaching (FRAP) is a powerful technique for estimation of diffusion coefficients and diffusion and binding kinetics. Briefly, using a confocal laser scanning microscope (CLSM), fluorescent particles are irreversibly photobleached in a well-defined region. The recovery of fluorescence within the region is modeled as a temporal (using the so-called recovery curve) or spatio-temporal (using the full set of pixels in the microscopy images) process through which mobility parameters are estimated.
Data analysis approaches in FRAP starts with an analytical or numerical (reaction-)diffusion model for the fluorescent species. The model assumptions vary greatly in terms of the bleach region shape, if more than one bleach frame is allowed, if motion during bleaching is accounted for, and so on. Further, parameter estimation is then performed either by fitting the recovery curve i.e. the average intensity in the bleach region or to the full set of pixels in the microscopy images [1].
In this work, we introduce a new numerical FRAP model based on spectral methods, covering pure diffusion and diffusion and binding (reaction-diffusion) with immobile binding sites, as well as arbitrary bleach region shapes. Both conventional recovery curve-based estimation and pixel-based estimation are supported. Multiple bleach frames, diffusion (and binding) during bleaching, and bleaching during imaging are explicitly accounted for. No other FRAP framework incorporates all these model features and estimation methods. We validate the model by comprehensive comparison to stochastic simulations of particle dynamics (see Figure 1) and find it to be highly accurate. We perform both simulation studies and experiments to compare recovery curve-based estimation and pixel-based estimation in realistic settings and show that pixel-based estimation is the better method [2].

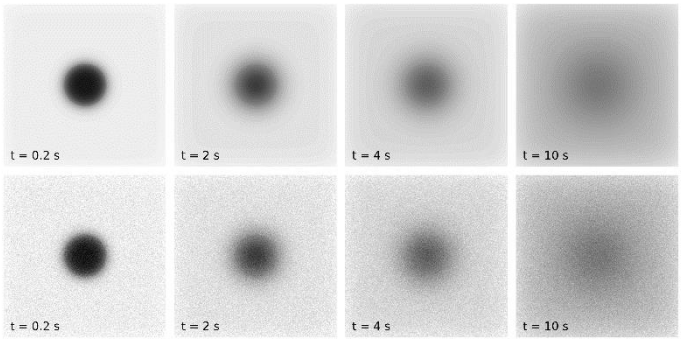

Figure 1: Example of a comparison between numerical solution (top) and stochastic solution (bottom) using 10^9 particles. The times indicated are relative to the time of the last bleach frame.

[1] N Lorén et al. Fluorescence recovery after photobleaching in material and life sciences: putting theory into practice. *Quarterly reviews of biophysics*, **48**, 323-387, 2015.
[2] M Röding et al. A highly accurate pixel-based numerical FRAP model based on spectral methods. *Submitted*.