A FRAP simulator to quantify influences of experimental setup and model simplifications

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1. Introduction

Since biological experiments are often expensive and the number of different conditions and time points is limited, simulations of such intracellular processes constitute an essential tool. To evaluate the effects of a bleaching experiment in a cellular environment, we established a new approach allowing simulations of spatio-temporal dynamics in real cell geometries. These bleaching simulations were used to quantitatively determine the effects of several experimental and analytical aspects. Therefore, the parameters were sampled following the *Morris* approach [2]. Using these parameter sets simulations of bleaching experiments were performed. In order to provide a quantitative measure on the importance of factors influencing the outcome of such an experiment, the *Elementary Effects* of individual parameters were determined [3] .

2. Initial condition

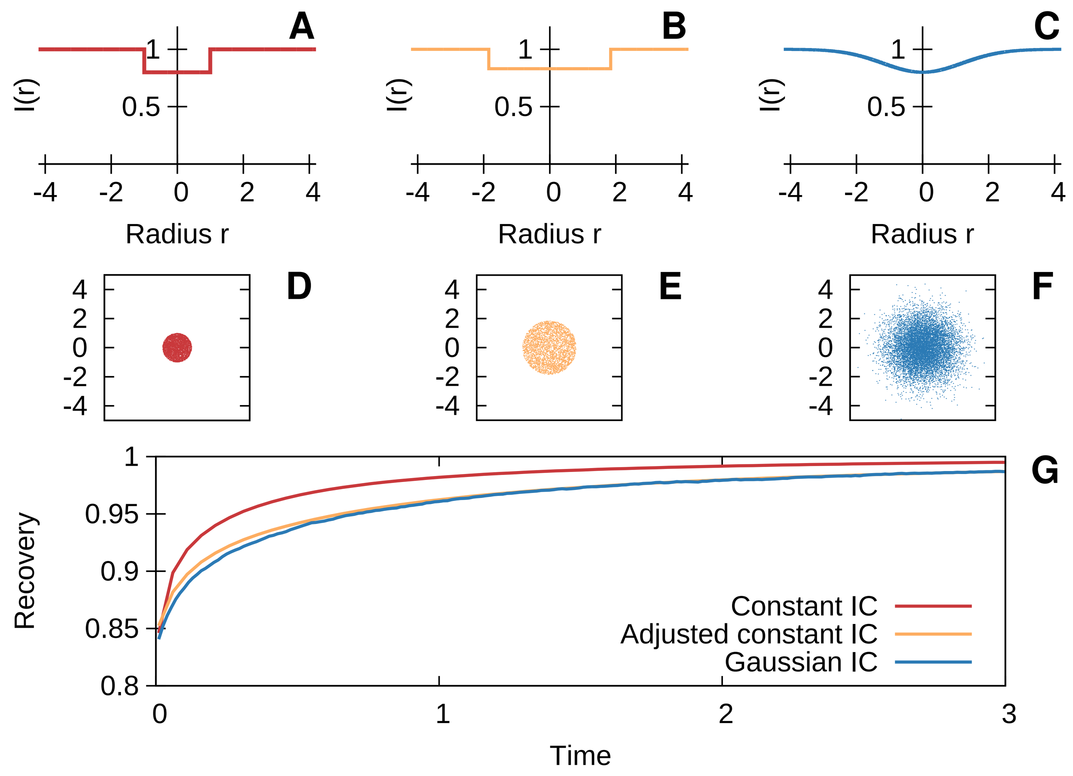

Fig. 1: Different types of initial conditions (IC) within bleaching spot. (A, D) constant IC, (B, E) adjusted constant IC, (C, F) *Gaussian* IC, and (G) FRAP recovery curves.

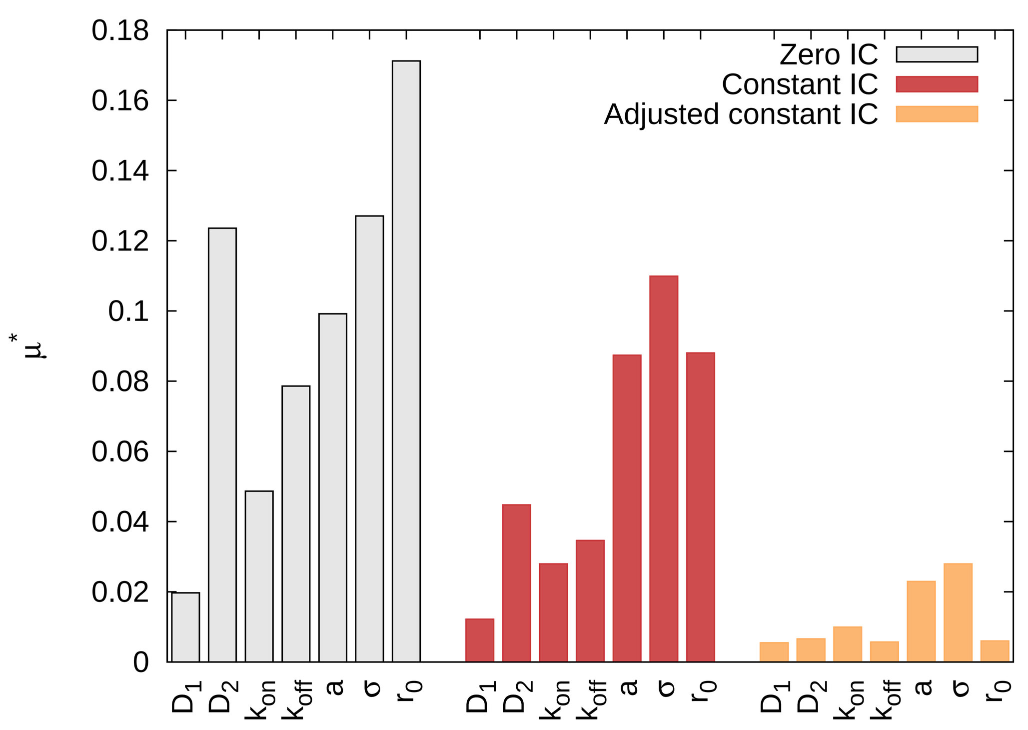

Fig. 2: Influence of different parameters on error between *Gaussian* IC vs. simplified ICs: *Elementary Effects* of reaction-diffusion parameters (D_1, D_2, k_{on}, k_{off}) and the experimental setup (depth a and variance σ of *Gaussian* profile and radius of spot r_0).

3. Boundary condition

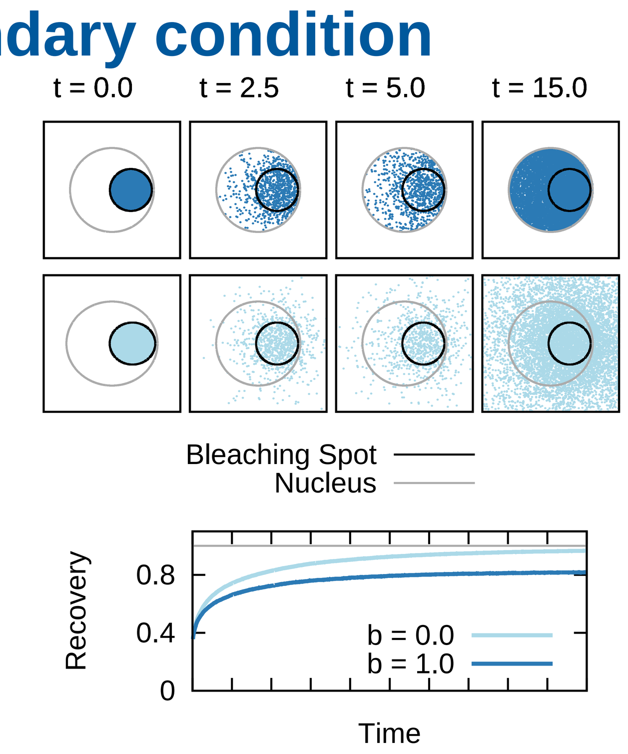

Fig. 3: Different types of boundary conditions (BC) of the bleached compartment. Distribution of bleached particles over time with (Top) no flow BC ($b = 0.0$) , and (Bottom) unhindered flow BC ($b = 1.0$) and corresponding recovery curves.

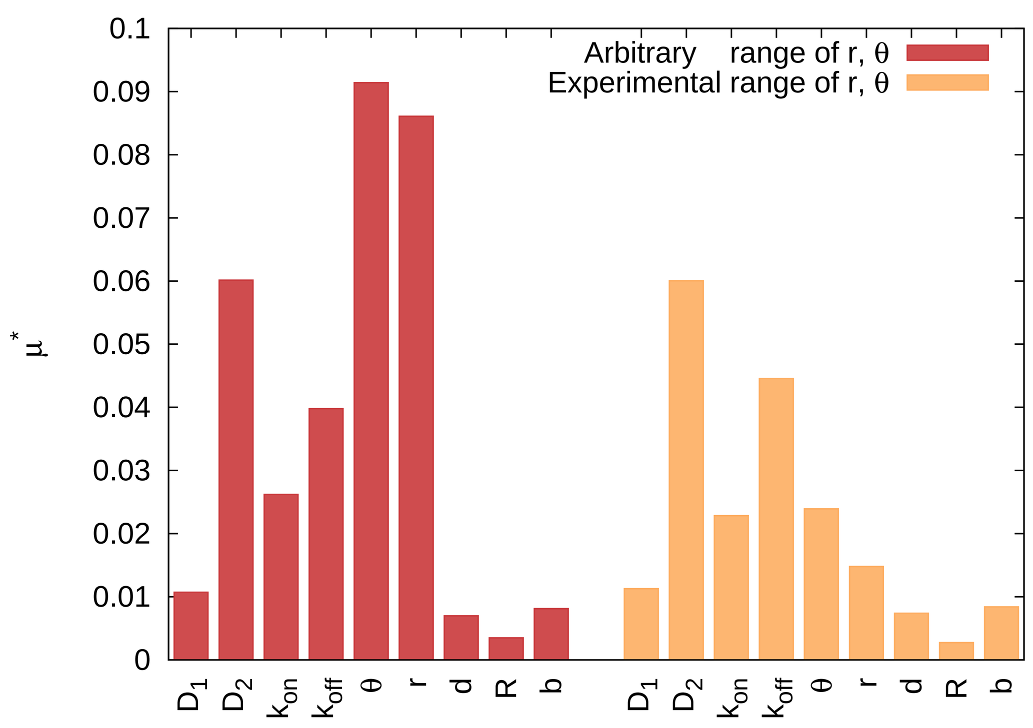

Fig. 4: *Elementary Effects* of reaction-diffusion parameters (D_1, D_2, k_{on}, k_{off}) and experimental setup (depth θ and adjusted radius r of bleaching spot, distance between center compartment and center spot d, radius R of circular compartment and probability allowing particles crossing the membrane b).

4. Position of Bleaching Spot

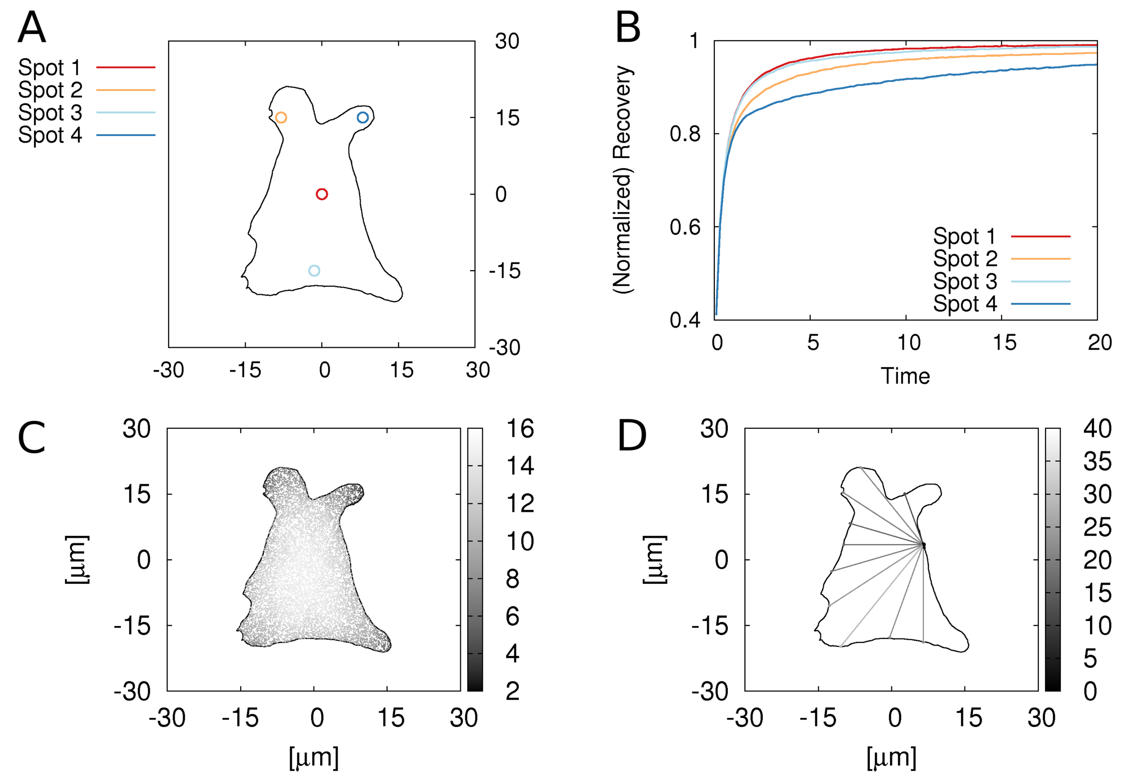

Fig. 5: A FRAP experiment simulated at different positions within a cytoplasmic geometry (A) yields diverse recovery curves (B). A distance measure of the bleaching spot center to the geometry's boundary is applied such that positions in "branches" are overall closer to the membrane than centrical positions (C). The distance d_M equals the mean length of 60 line segments form the bleaching spot center to the geometry boundary (D).

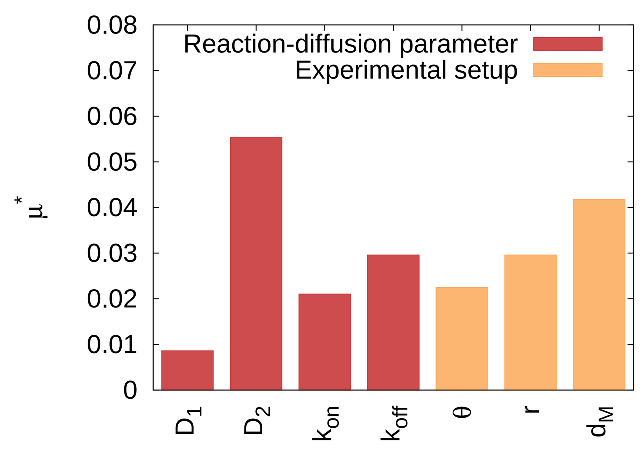

Fig. 6: *Elementary Effects* of reaction-diffusion parameters (D_1, D_2, k_{on}, k_{off}) and experimental setup (depth θ and adjusted radius r of bleaching spot as well as median distance d_M of bleaching spot center to compartment's boundary) in real geometry.

5. Results

A main influencing factor is the **initial condition** chosen for analyzing the data of the bleaching experiment. The *Elementary Effects* reveal that the realistic *Gaussian* initial condition can be simplified reliably using an adjusted constant initial condition (IC) (Fig. 1). This adjusted constant IC facilitates data analysis, since only in this simplified case an analytical solution is available [1]. Another investigated aspect is the **boundary condition** used, i.e. the flow condition across the membrane of the bleached compartment (nucleus, cytoplasm). Our analysis shows that the effect of the boundary condition chosen is negligible. In addition, the influence of the **position** of the region of interest (ROI, region where the bleaching is performed) within the cellular environment was investigated. In this case simulations were performed in a real cell geometry using parameter ranges deduced from real FRAP experiments. In this scenario the parameters of the experimental setup were as sensitive as the reaction-diffusion parameters (Fig. 6). Particularly, locations which allow a diffusion only in one main direction have a significant impact on the experimental results. Consequently, the positioning of the ROI has to be chosen carefully to obtain comparable results in bleaching experiments.

This is the first study determining the main influencing factors in performing and analyzing FRAP experiments quantitatively using simulations.

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