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# Tropical – parameter estimation and simulation of reaction-diffusion models based on spatio-temporal microscopy images

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**ABSTRACT**

**Summary:** Tropical is a software for simulation and parameter estimation of reaction-diffusion models. Based on spatio-temporal microscopy images, Tropical estimates reaction and diffusion coefficients for user-defined models. Tropical allows the investigation of systems with an inhomogeneous distribution of molecules, making it well suited for quantitative analyses of microscopy experiments such as fluorescence recovery after photobleaching (FRAP).

**Availability:** Tropical is available free of charge for academic use at http://www.dkfz.de/tbi/projects/modellingAndSimulationOfCelluarSystems/tropical.jsp after signing a material transfer agreement.

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**Supplementary information:** http://www.dkfz.de/tbi/projects/modellingAndSimulationOfCelluarSystems/tropical.jsp

## 1 INTRODUCTION

Fluorescence microscopy techniques like time-lapse microscopy and FRAP have emerged as standard tools for visualizing molecule dynamics in living cells. Quantitative characterization of the motility of molecules requires the fitting of reaction-diffusion models to spatio-temporal data extracted from images (Beaudouin, et al., 2006; Delon, et al., 2006; Sprague, et al., 2004). Computational tools for such quantitative analysis are still missing. Our motivation was thus to develop a program to enable parameter estimation and simulation for complex reaction-diffusion models. Tropicals' main advantages are that a) an inhomogeneous distribution of binding partners can be considered and b) that it directly operates on microscopy images. So far only a few studies have incorporated the inhomogeneous distribution of binding sites (Beaudouin, et al., 2006; Sprague, et al., 2006).

## 2 PROGRAM OVERVIEW

An overview of Tropical is given in Fig. 1. Tropical estimates the specified parameters and runs a simulation with the best estimation result based on the input of a) spatio-temporal microscopy images, b) initial images for all state variables and c) a user-defined model. The model is composed of one ordinary differential equation describing the reaction of each molecule. The diffusion term is added automatically. We applied a normal diffusion term which can be modified as described by Siggia et al. (2000). Binary compartment images serve as masks for the simulation grid. They define the area where parameters are estimated and specify regions for computing a recovery curve. Additionally, an output window provides the intermediate results during runtime. A log file of the complete parameter estimation process and a result file with estimated parameters and numerical control values are also written. Results are also provided in tiff formatted images and a text file. The complete source code is written in C++ and is available for Linux and Windows. The graphical user interface was developed using QT, version 4.1.2 (www.trolltech.com).

### 2.1 Algorithms and libraries

Tiff image I/O is handled by the open source library libtiff (v.3.7.2) (www.remotesensing.org/libtiff/). The model description is compiled from a text file by the open watcom C++ compiler (www.openwatcom.org). Spatial discretization of the partial differential diffusion equations is done by a finite differences approach with Neumann boundary conditions. The underlying grid for this discretization is represented by the pixels of the input images. An optional binning algorithm reduces the resolution and thus accelerates computation. The differential equations are solved by a Runge-Kutta 4$^{th}$ order algorithm with adaptive step size. adapted from Press et al. (2002). For parameter estimation the Levenberg-Marquardt algorithm, published by Press et al. (2002) or a modified version of it, which can lead to faster convergence (see handbook for details), may be used.

### 2.2 Tests and application

To verify Tropicals functionality, we simulated two scenarios with defined parameters using Berkley Madonna (www.berkeleymadonna.com), one diffusion and one reaction-diffusion system. The resulting matrices were transformed into Image Series using ImageJ (http://rsb.info.nih.gov/ij/) which were then used as input for parameter estimation.

First, we tested parameter estimation on a diffusion system with different initial parameters and different numerical

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parameters, such as initial step size or step size tolerance for the ODE solver and number of maximum iterations for the Levenberg-Marquardt algorithm. Second, we tested a two-dimensional reaction-diffusion system similar to a FRAP experiment, with an interaction of one observable diffusing molecule on a non-diffusing invisible binding site. The image series for parameter estimation showed the effective sum of the diffusing and the bound observable molecule. This corresponds to a realistic setting, where free and bound forms of a protein cannot readily be distinguished.

In both test cases highly accurate diffusion and reaction coefficients were achieved. For pure diffusion, results differed by 1.7±1.99% from the expected values. For the reaction-diffusion model, D differed by 1.86±0.85% and $k_{off}$ by 0.14±0.04% from the original D and $k_{off}$ , respectively. Deviations of the parameters were calculated by performing ten parameter estimation runs using different initial parameters. The mean of the ten estimated parameters was compared to the parameters used to generate the original data with Berkeley Madonna.

Adding noise up to 7% standard deviation of the maximum intensity did not strongly affect the accuracy. The estimated parameters deviated 5-10% from the expected values showing that Tropical can cope with typical levels of noise in fluorescence microscopy images. To test Tropical on a realistic example, we estimated the dissociation and diffusion coefficients from a FRAP experiment on the nuclear protein nucleophosmin (B23). B23 diffuses in the nucleoplasm and reversibly binds to unspecified binding sites located inside the nucleoli (Fig. 1 B)(Chen and Huang, 2001). A model of this binding-diffusion system was created which consisted of two state variables (free and bound pool) and two parameters (diffusion coefficient D of the free pool and dissociation rate $k_{off}$ of the binding reaction):

$$\frac{\partial[B23_f]}{\partial t} = D\Delta[B23_f] - k_{off} * \frac{[B23_b]_{ss}}{[B23_f]_{ss}} * [B23_f] + k_{off} * [B23_b]$$

$$\frac{\partial[B23_b]}{\partial t} = k_{off} * \frac{[B23_b]_{ss}}{[B23_f]_{ss}} * [B23_f] - k_{off} * [B23_b]$$

where $[B23_f]$ and $[B23_b]$ denote the intensity of the free and the bound B23, respectively, $k_{off}$ is the dissociation rate, D is the diffusion coefficient, and *ss* denotes the steady-state intensity. Results of the simulation using the estimated parameter set resulting in the best fit of simulated and experimental data are shown in Fig. 1 B and C.

## 3 SUMMARY AND FUTURE DEVELOPMENT

Tropical is an easy to use software for parameter estimation and simulation of reaction-diffusion models using microscopy images and user-defined models. We presently apply this system to a number of proteins for estimating their reaction-diffusion parameters *in vivo*.

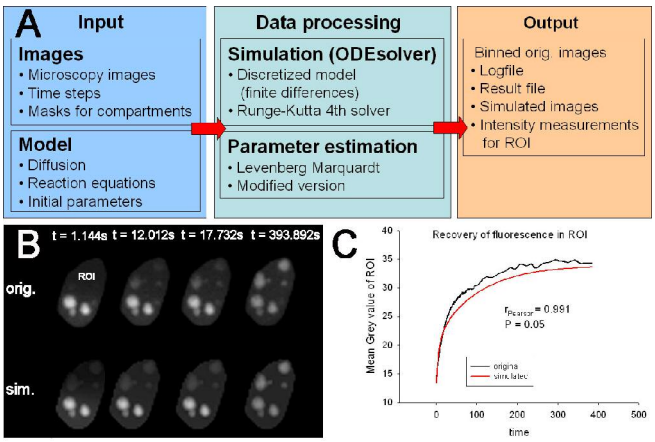


**Fig. 1**. A. The structure of Tropical. B. Four out of the original 149 FRAP images (top) of B23 and corresponding simulated images of B23 (bottom) at selected time points. ROI indicates the region used to plot the recovery curve. C. The fluorescence recovery curve of the B23 model shows a good fit (149 time points, values taken from ROI plotted in B).

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