Estimation of Diffusivity of Phycobilisomes on Thylakoid Membrane based on spatio-temporal FRAP images

Štěpán Papáček, Radek Kaňa, Ctirad Matonoha

SXS, Faculty of Fisheries and Protection of Waters, Univ. of South Bohemia, Nové Hrady
Department of Phototrophic Microorganisms, Institute of Microbiology AS CR, Třeboň
Department of Computational Methods, Institute of Computer Science AS CR, Prague 8

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FRAP (Fluorescence Recovery After Photobleaching) technique allows detection of diffusivity of autofluorescence compound like proteins (e.g. phycobiliproteins) and also other non-fluorescence compound that are fluorescently tagged (e.g. green fluorescence proteins - GFP).

This method is based on application of short, intense laser irradiation (the so called bleach) to a small target region (Region Of Interest - ROI) of the cell that causes irreversible loss in fluorescence in this area without any damage in intracellular structures. After the "bleach" (or "bleaching"), the observed recovery in fluorescence in the "bleached area" reflects diffusion of fluorescence compounds from the area outside the bleach.

During a FRAP experiment, a biological sample is briefly exposed to intense laser illumination to bleach a target region of a specified geometry. Assuming (i) local homogeneity, (ii) isotropy, (iii) an unrestricted supply of unbleached particles outside of the target region Ω, the recovery (due to the diffusion characterized by D) of unbleached particle concentration C as a function of spatial coordinate $\vec{r}$ and time t is modelled as follows:

$$\frac{\partial C}{\partial t} - \nabla \cdot (D \nabla C) = R(C) \ , \tag{1}$$

where $R(C)$ is a reaction term.
Initial (IC) and time varying Dirichlet boundary conditions (BC) are:

$$C(t_0) = C_0(\vec{r}, t_0) \text{ in } \Omega, \quad C(t) = g(\vec{r}, t) \text{ in } \partial\Omega \times [t_0, T]. \tag{2}$$

For a linear bleach spot perpendicular to a longer axis, and assuming D is in domain Ω the scalar space-independent diffusion coefficient, we have

$$\frac{\partial C}{\partial t} - D \frac{\partial^2 C}{\partial r^2} = R(C) \ . \tag{3}$$

After introducing the dimensionless variables x, p, τ, y by

$$x := \frac{r}{L}, \; p := \frac{D}{D_0}, \; \tau := \frac{D_0}{L^2}t, \; y := \frac{C}{C_{pre}},$$

where L is the length of our specimen in direction perpendicular to bleach spot, D_0 is a constant with some characteristic value (unit: $\mathrm{m^2 s^{-1}}$), C_{pre} is a pre-bleach concentration of C, and $R(C) = -k_S C$, we have the dimensionless diffusion-reaction equation in one dimension (for $x \in [0, 1]$):

$$\frac{\partial y}{\partial \tau} - p\frac{\partial^2 y}{\partial x^2} = -\frac{k_S L^2}{D_0} y \; . \tag{4}$$

Initial and time varying Dirichlet boundary conditions are

$$y(x, \tau_0) = y_0 \text{ for } x \in [0, 1], \quad y(0, \tau) = g_0(\tau), \; y(1, \tau) = g_1(\tau). \tag{5}$$

We can also assume the flow on the boundary (Neumann BC):

$$\frac{\partial y}{\partial x}(0, \tau) = h_0(\tau), \quad \frac{\partial y}{\partial x}(1, \tau) = h_1(\tau), \quad \tau \geq \tau_0. \tag{6}$$

Based on FRAP experiments, we have a matrix of m pre-bleach and $M+1$ post-bleach experimental values

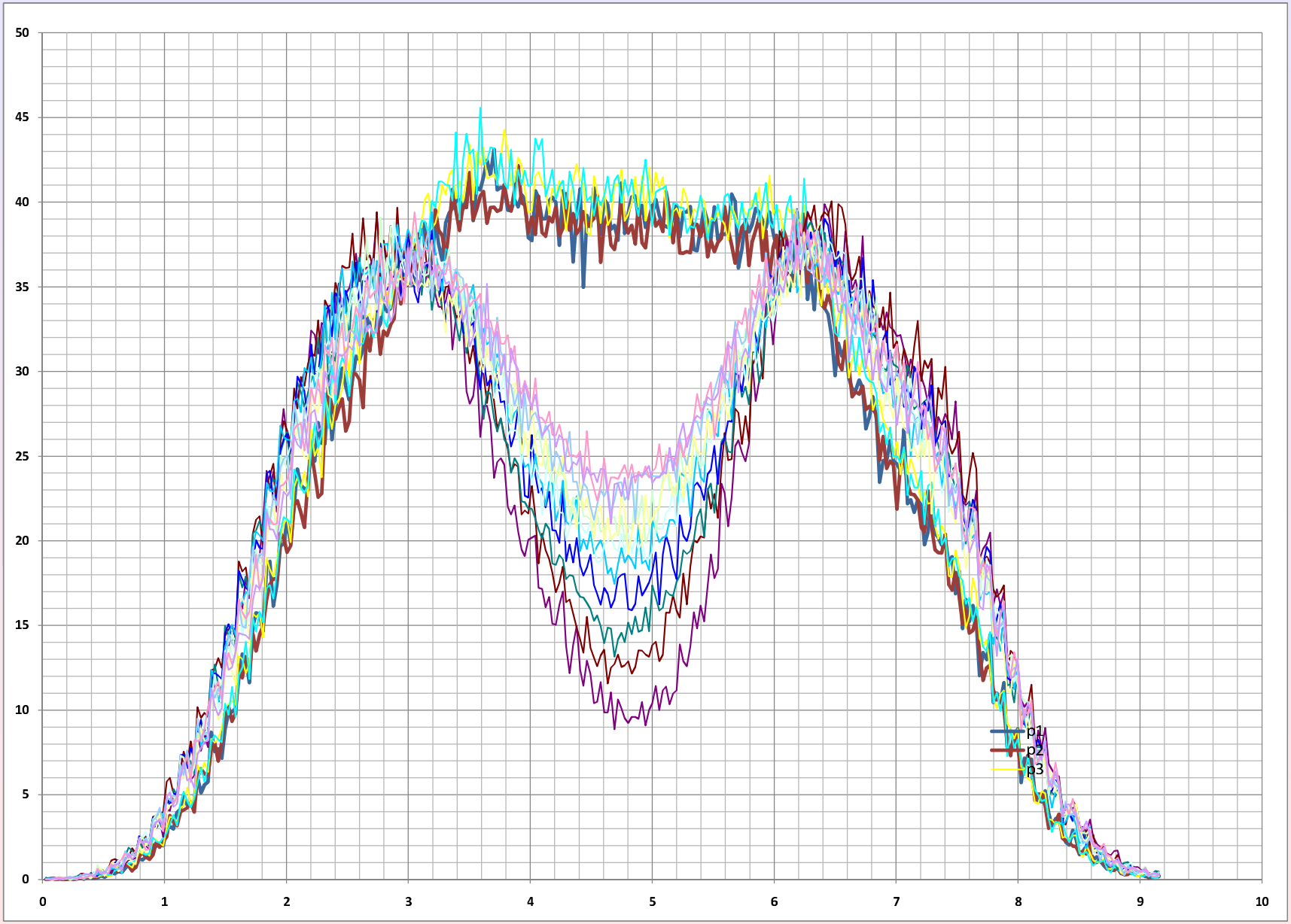
$$y_{exp}(x_i, \tau_j),\ i = 0 \dots N,\ j = -m \dots M,$$

with $x_0 = 0$ and $x_N = 1$, where

- $y_{exp}(x_i, \tau_0),\ i = 0 \dots N$, is a vector for the IC
- $y_{exp}(0, \tau_j),\ j = 1 \dots M$, is a vector of the left BC
- $y_{exp}(1, \tau_j),\ j = 1 \dots M$, is a vector of the right BC

Recall that the re-scaled space steplength $\Delta h = \frac{1}{N+1}$ ($H = \frac{L}{N+1}$).
Let the time interval between two measurements be T (in seconds).
The re-scaled dimensionless time interval is then $\tau_M = T\frac{D_0}{L^2}$.
The re-scaled time steplength $\Delta\tau$ we further define as $\Delta\tau := \frac{\tau_M}{\kappa_t}$, where the value of $\kappa_t \in \mathbb{N}$ concerns the numerical scheme.

Experimental values



We construct an objective function J representing the disparity between the experimental and simulated time-varying concentration profiles, and then within a suitable method we look for such a value p minimizing J:

$$J_j = \sum_{i=1}^{N} \left[y_{exp}(x_i, \tau_j) - y_{sim}(x_i, \tau_j) \right]^2 + \alpha \left(p - p_{est} \right)^2, \quad j = 1 \ldots M \qquad (7)$$

where $y_{sim}(x_i, \tau_j)$ are the simulated values resulting from the solution of PDE (4) with the initial and boundary conditions (5), $\alpha \geq 0$ is a regularization parameter, and p_{est} is an estimation of our result (we deal with the **ill-posed problem**). For the sake of clarity, we further neglect the reaction term, i.e. we put $k_S = 0$ in (4).

We have used a suitable optimization method from the UFO system which generates a sequence of iterates $p^{(0)}, p^{(1)}, \ldots, p^*$.
Recall that we can also take both sums for i and j in (7) together:

$$\min J = \sum_{j=1 \ldots M} J_j$$

Problem (4)-(5) for simulated data $y(x_i, \tau_j) \equiv y_{sim}(x_i, \tau_j)$ was solved numerically using the finite difference scheme for uniformly distributed nodes with the space steplength $\Delta h = 1/(\kappa_s N)$ and the variable time steplength $\Delta\tau$:

1. The explicit scheme of order $\Delta\tau + \Delta h^2$:

$$y_{i,j} = \beta y_{i-1,j-1} + (1 - 2\beta) y_{i,j-1} + \beta y_{i+1,j-1}$$

2. The Crank-Nicholson implicit scheme of order $\Delta\tau^2 + \Delta h^2$:

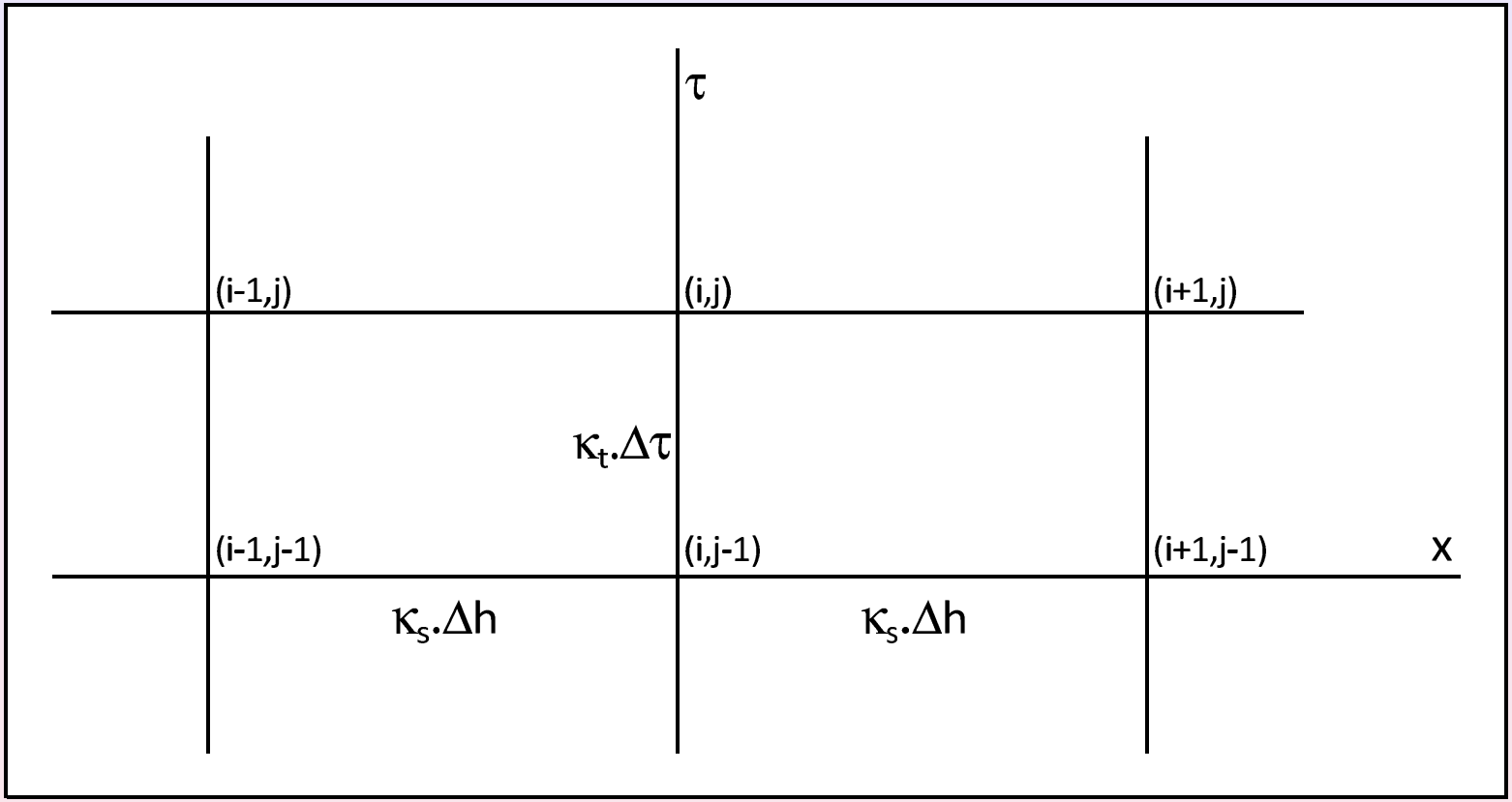
$$-\frac{\beta}{2} y_{i-1,j} + (1+\beta) y_{i,j} - \frac{\beta}{2} y_{i+1,j} = \frac{\beta}{2} y_{i-1,j-1} + (1-\beta) y_{i,j-1} + \frac{\beta}{2} y_{i+1,j-1}$$

Here $\beta := \frac{\Delta\tau}{\Delta h^2}\, p$ and $y_{i,j} \equiv y(x_i, \tau_j)$ are the computed values in nodes.
Recall that for the explicit scheme $\beta \leq 1/2$ must hold.
In order to get from the $(j-1)$-th time row to the j-th, we need to perform at least

$$\kappa_t = \frac{T D_0}{L^2 \Delta\tau}$$

substeps.

The nodes



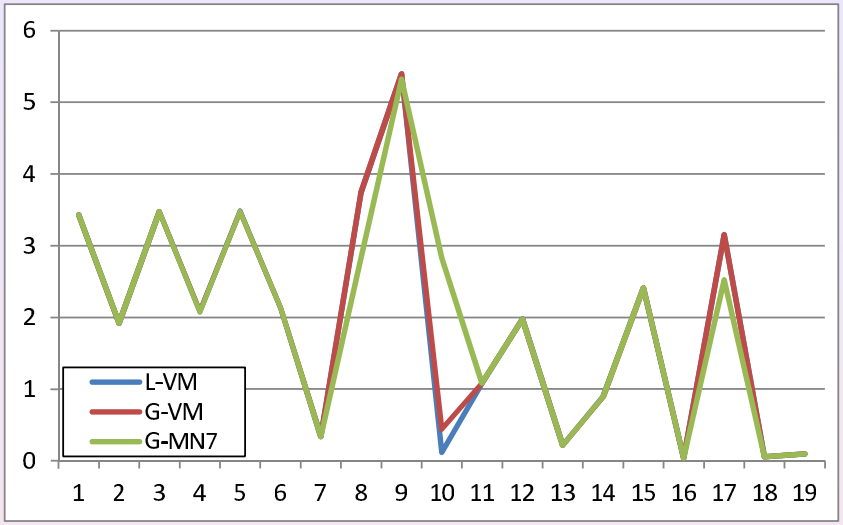
From the biological point of view, we take into account pre-bleach data. In this case, we compute the average value of them and post-bleach data are divided by this average pre-bleach. We have „new“ experimental values y_{exp}.

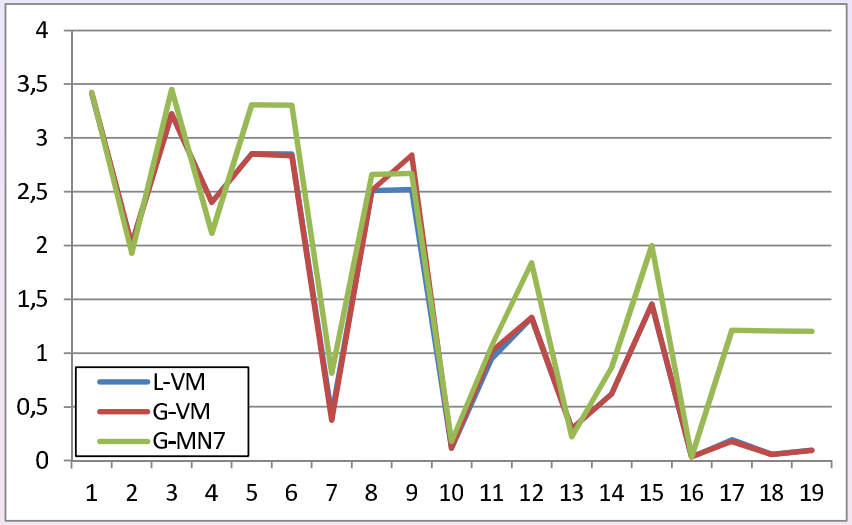
Because of the **ill-posedness of the problem**, we apply the regularization technique based on minimization of function (7) taking $\alpha > 0$.

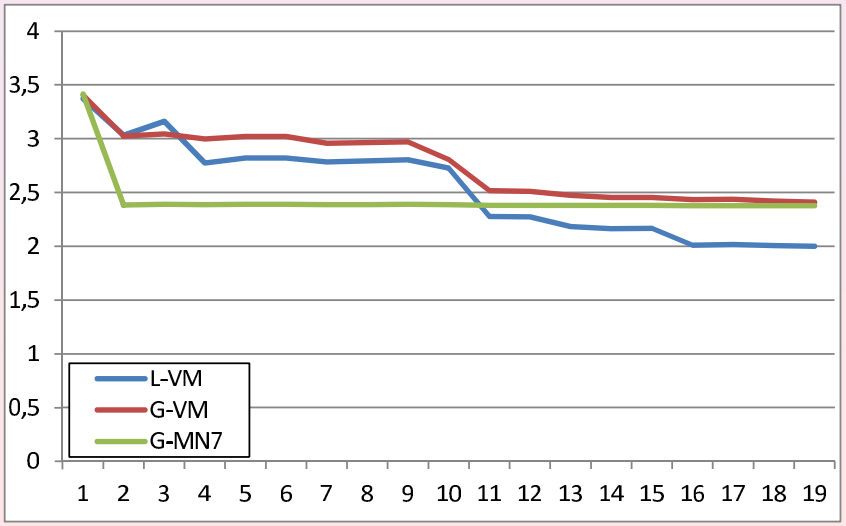
As we are interested in the „hollow“ in the experimental data, we can only work with data x_A to x_B for $A > 0$ and $B < N$ instead of x_0 to x_N.

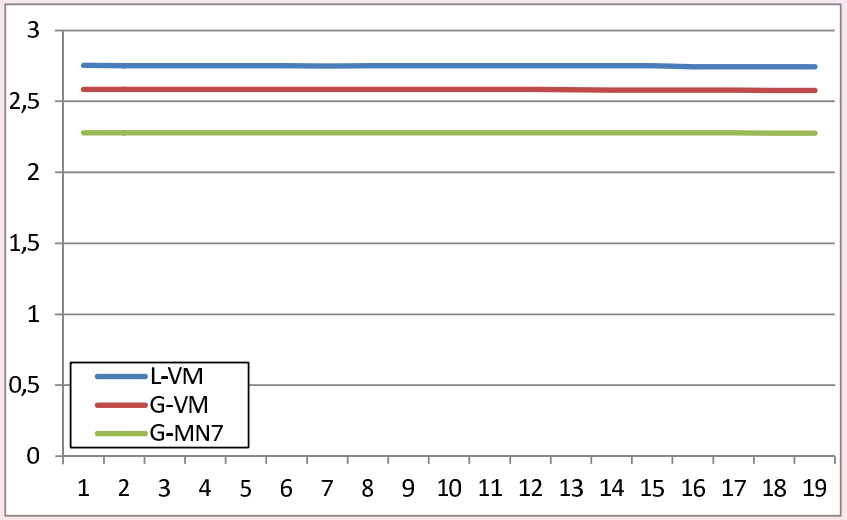
When considering each j-th time column separately, see (7), we use hot starts when approaching the next time column, so the initial $p^{(0)}$ is equal to p^* from the previous time column.

$$\alpha = 0,\ \alpha = 0.01,\ \alpha = 0.1,\ \alpha = 1$$









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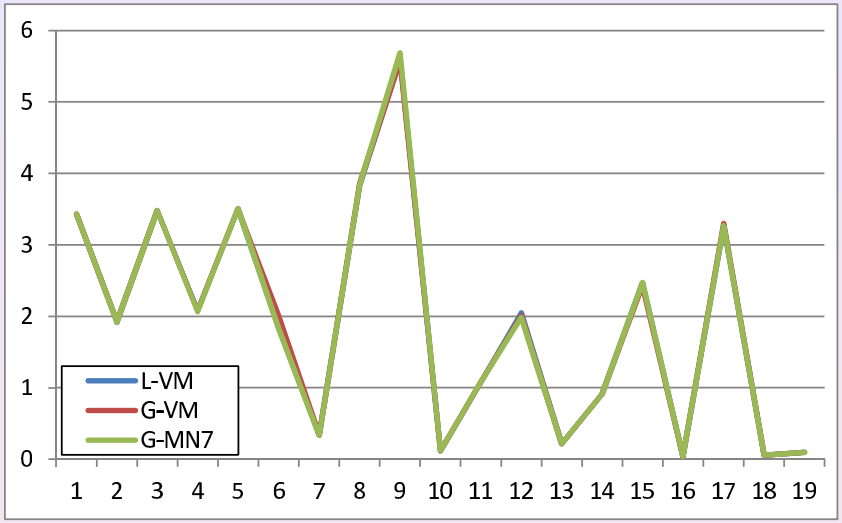

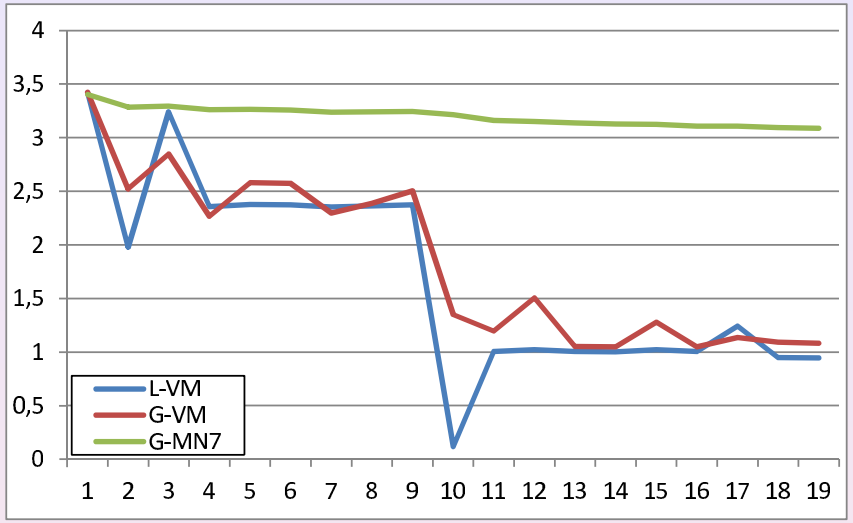

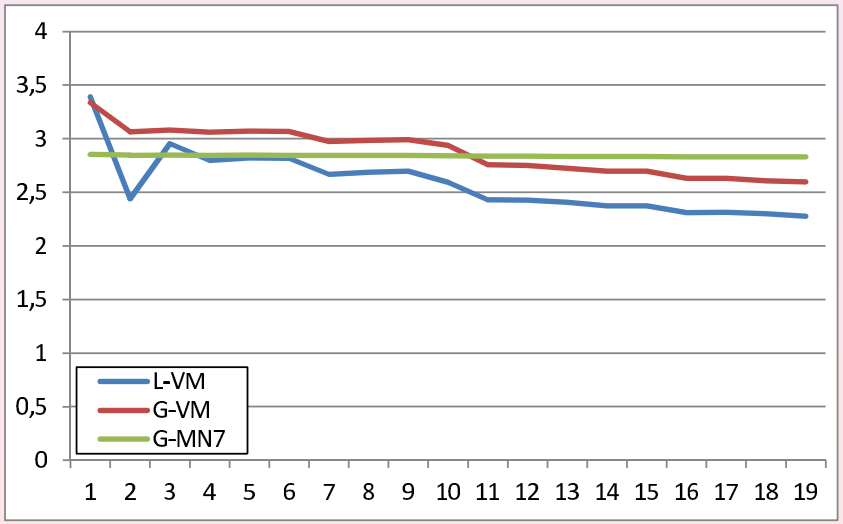

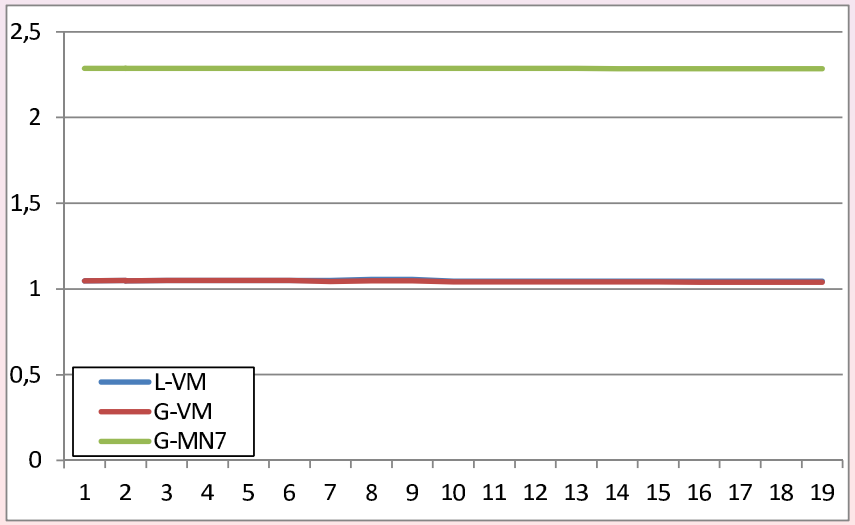

- Our method improves on other models by accounting for experimentally measured post-bleaching fluorescence profiles and time-dependent boundary conditions, and can include also a reaction term to account for the low level bleaching during scanning and the time varying fluorescence signal.
- Finding a biologically reliable optimal solution p is quite a difficult task. We are looking to improve our method either by an adequate implementation of a suitable regularization technique (based on the assessment of the measurement noise) and a more robust optimization procedure.
- For the previously known diffusion coefficient (the synthetic data were simulated by the random walk model) our program computes correct results. Furthemore, we determined the diffusivities for the real FRAP data with the red algae *Porphyridium cruentum*. The range of result $10^{-14}\mathrm{m}^2\mathrm{s}^{-1}$ is in agreement with reference values.