Error analysis of three methods for the parameter estimation problem based on spatio-temporal FRAP measurement

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#### Abstract

Since the 1980's, with first commercial confocal microscopes (CLSM), Fluorescence Recovery after Photobleaching (FRAP) technique is very useful in studies of protein dynamics in live cells. FRAP is based on measurement of the fluorescence intensity of either fluorescently tagged or autofluorescent molecules in a region of interest (ROI) in response to a change provided by an external stimulus, so-called bleach. Earlier work has derived analytical result for some geometries of ROI and bleach spot, and for some additional assumptions, turning the quantification of the transport and binding rate parameters into the *curve fitting problem*. Nevertheless, the underlying process of redistribution of fluorescent molecules can be modelled and solve numerically and the parameter estimation turns into an optimization problem, by minimizing the disparity between simulated and experimentally measured data. We follow this framework, however there are unexpected pitfalls residing mainly in the ill-posedness of our problem, see e.g. [1].

Based on experimental data different parameter estimation methods provide different results. Performing the error analysis, we assess the **parameter uncertainty**, i.e. we provide the parameter standard errors (based on previously determined parameter sensitivities) for each respective method.

#### Real data from spatio-temporal FRAP measurement



Time series of *Fluorescence intensity* (averaged along the shorter axis, in arb. units) vs. *Position* along the longer axis  $[\mu m]$ . Experimental data from FRAP experiment with unicellular red algae *Porphyridium cruentum* describing the fl. particle mobility (due to the diffusion) on the membrane.



3D plot of synthetic data: *Fluorescence intensity* y(x, t) vs. *Position* and *time*. Fluorescence is normalized,  $y(x, t) \in [0, 10]$ . Spatial coordinate  $x \in [0, 1]$ , space step is 0.1. Time  $t \in [0, 2]$ , time step is 0.1.

Assuming local homogeneity, isotropy (diffusion coefficient *D* within the ROI - domain  $\Omega$  is space-invariant), an unrestricted supply of unbleached particles outside of ROI, the following diffusion equation describes the unbleached particle concentration y(r, t):  $\frac{\partial y}{\partial t} - \nabla \cdot (D\nabla y) = 0$ . Furthermore, for the special geometry residing in one-dimensional simplification getting *y* as a function of dimensionless spatial coordinate *x*, time  $\tau$ , and re-scaled diffusion coefficient *p*:

$$\frac{\partial y}{\partial \tau} - p \frac{\partial^2 y}{\partial x^2} = 0 , \qquad (1)$$

where  $x := \frac{r}{L}$ , L is a characteristic length,  $\tau := \frac{t}{T}$ , T is a constant with some characteristic value, and  $p := D\frac{T}{L^2}$ . The initial condition (IC) and Dirichlet boundary conditions (BC) are:

$$y(x, \tau_0) = f(x), \quad x \in [0, 1],$$
 (2)

$$y(0,\tau) = g_0(\tau), \quad y(1,\tau) = g_1(\tau), \quad \tau \ge \tau_0.$$
 (3)

Denoting by  $p = (p_1, \ldots, p_m)$  the parameter vector, the inverse problem can be formulated as a system of non-linear equations:

$$F(p) = z^{\delta}, \quad F = G \circ S.$$
 (4)

Here,  $F = G \circ S$  represents the parameter-to-output map, defined as the concatenation of the IBVP solution operator S onto the solution vector y of the underlying system (1)-(3), i.e.  $S(p) = y_{i,j}$  and the observation operator G that evaluates y on certain space-points  $i \in \{1, \ldots, n\}$  and time-points  $j \in \{1, \ldots, m\}$  where the experimental observations (also referred to as the model output) are taken, i.e.  $G(y_{i,j}) = z(\tau_j)$ . Due to noisy data, model imperfections, and ill-posedness of our problem, system (4) is replaced by a nonlinear least squares regularization problem

$$\| z^{\delta} - F(p) \|^{2} + \alpha \| p - p_{0} \|^{2} \rightarrow \min_{p, p_{0} > 0}$$

$$(5)$$

where the positive regularization parameter  $\alpha$  enforces stable dependency of  $p_{\alpha}^{\delta}$  (the solution to (5)) on the noisy data  $z^{\delta}$  and  $p_0$  represents an a-priory guess subjected to the minimization.

# Results for synthetic data: The role of regularization parameter $\alpha$ on the solution smoothness



#### Error Analysis: The tool for parameter uncertainty assessment

Our error analysis exploits the properties of sensitivity matrix  $\chi = \frac{\partial z}{\partial p}$ , i.e., the Jacobian matrix of the output, being evaluated at  $p_0$ :

$$\chi_{jk}(p_0) = \frac{\partial z(\tau_j; p)}{\partial p_k} |_{p=p_0}, \quad 1 \le j \le m, \quad 1 \le k \le m.$$
(6)

The statistical model for the observation process is following:

$$z_j^{\delta} = z(\tau_j; p_0) + \varepsilon_j. \tag{7}$$

Assuming  $E[\varepsilon_j] = 0$ ,  $\operatorname{var}(\varepsilon_j) = \sigma_0^2 < \infty$ ,  $\operatorname{cov}(\varepsilon_j, \varepsilon_k) = 0$  whenever  $j \neq k$ , we have  $E[z_j^{\delta}] = z(\tau_j; p_0)$ ,  $\operatorname{var}(z_j^{\delta}) = \sigma_0^2$ . The standard errors of parameters  $p_k$  used to quantify uncertainty in the estimation are

$$SE_{k}(p_{\alpha}^{\delta}) = \hat{\sigma} \sqrt{\left[\chi(p_{\alpha}^{\delta})^{T} \chi(p_{\alpha}^{\delta})\right]_{kk}^{-1}}, \quad 1 \le k \le m.$$
(8)

where  $\hat{\sigma}^2$  is an approximation of  $\sigma_0^2$  and  $\chi^T \chi$  is the Fisher information matrix (FIM).

The propagation of uncertainty from the observation process to the estimated parameter vector is induced by  $\varepsilon = (\varepsilon_1, \dots, \varepsilon_m)^T$  in equation

$$\boldsymbol{\rho} \approx \boldsymbol{\rho}_0 + \left[ \chi(\boldsymbol{\rho}_0)^T \chi(\boldsymbol{\rho}_0) \right]^{-1} \chi(\boldsymbol{\rho}_0)^T \varepsilon.$$
(9)

#### Three FRAP methods: Standard errors and parameter sensitivities

• C. W. Moulineaux *et al.*, Nature (1997), for the infinite domain  $(r \in R)$  and initial Gaussian bleaching profile, obtained the solution y(r, t) of diffusion equation (1) as  $y(r, t) = \frac{y_{0,0}r_0}{\sqrt{r_0^2 + 8Dt}} \exp \frac{-2r^2}{r_0^2 + 8Dt}$ . The time evolution of maximum depth y(0, t), i.e. the single observed data point z(t), and the Fisher information matrix  $FIM = \chi^T \chi$  are given by:

$$\begin{aligned} z_{M}(t) &= \frac{y_{0,0}r_{0}}{\sqrt{r_{0}^{2} + 8Dt}}, \quad FIM_{M} = \sum_{j=1}^{m} \left[\frac{4y_{0,0}r_{0}t_{j}}{(r_{0}^{2} + 8Dt_{j})^{3/2}}\right]^{2} \\ \text{Accordingly to (8): } SE(\hat{D}) &= \sigma_{0}/\sqrt{FIM_{M}}. \end{aligned}$$

- J. Ellenberg *et al.*, J. Cell Biol. (1997) receives excellent results for SE(D̂), however...
- FD approximation of IBVP & Tikhonov regularization based method, see [1], provides the general framework for all kind of IC and BC, and is able to detect the time evolution of parameter  $p_k$  as well.

### PAPÁČEK Š., KAŇA R., AND MATONOHA C.

Estimation of diffusivity of phycobilisomes on thylakoid membrane based on spatio-temporal FRAP images. Mathematical and Computer Modelling (2012), doi:10.1016/j.mcm.2011.12.029.

#### Standard errors and parameter sensitivities for [1] and CWM method



Standard errors and parameter sensitivities  $\chi_k(p_0)$  were calculated numerically by FD approximation of IBVP and using (6).  $SE(\hat{D})$  and *FIM* for CWM method will be discussed personally...