Automated cell quantification allows inference of protein half-life from single cell time-lapse microscopy

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***Abstract*—In the last few years, single cell time-lapse fluorescence microscopy has emerged as a key technology in the toolbox of experimental life science [1], [2], [3]. Cell-tracking and segmentation of fluorescently tagged proteins allows to combine the information of cellular progeny with time resolved protein dynamics [4], [5], [6]. Whenever quantitative data on the intensity of the fluorescent signal is required, a careful image processing pipeline has to be applied to account for uneven illumination, background signal, varying illumination strength or photobleaching [7], [8].**

Here, we present a custom software tool which integrates these methods in a semi-automatic approach and allows to conveniently acquire high-quality expression data from tracked single cell time-lapse microscopy data. As an application, we present a new method to estimate the protein decay rate of fluorescently tagged proteins in cells without the need of any further chemicals. For this purpose we actively bleach cells with different known amounts of excitation. The resulting bleached single cell time courses can be fitted to a simple bleaching model. Using the linear behavior of bleaching we are able to infer kinetic protein parameters which are essential for further quantitative models. To show the reliability of our method we compare the results to well-established methods like cycloheximide treatment. Our method can be applied to every kind of fluorescence time-lapse experiment without interfering with the actual experimental goal.

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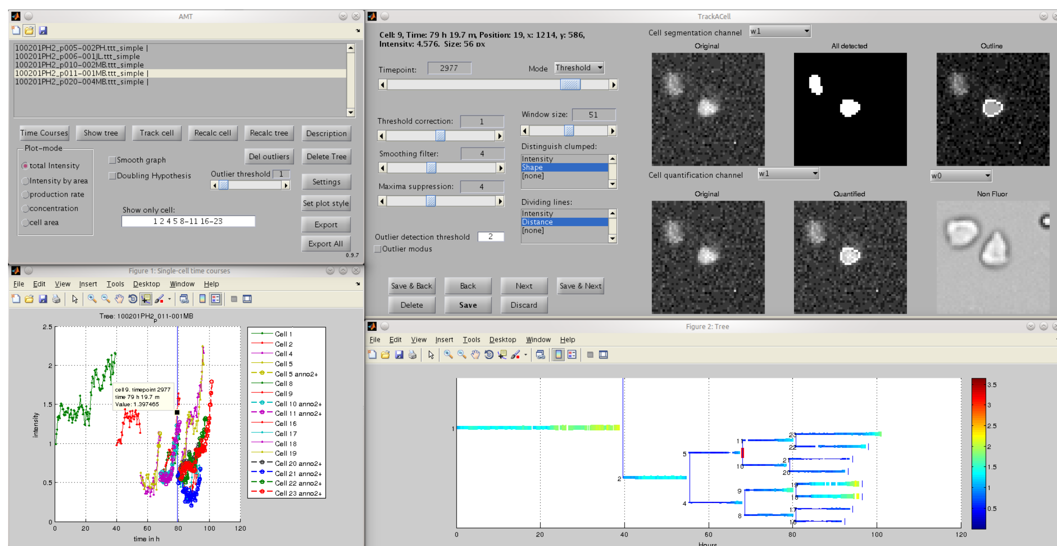

Fig. 1. Screenshot of our quantification tool. After data import (top left), the tool normalizes each image of the movie as described in [8], segments every tracked cellular signal automatically which results in raw single-cell time courses (bottom left). The tool highlights segmentation problems and allows to manually adjust every segmentation parameter (top right) to acquire high-quality expression data together with the cellular progeny (bottom right).

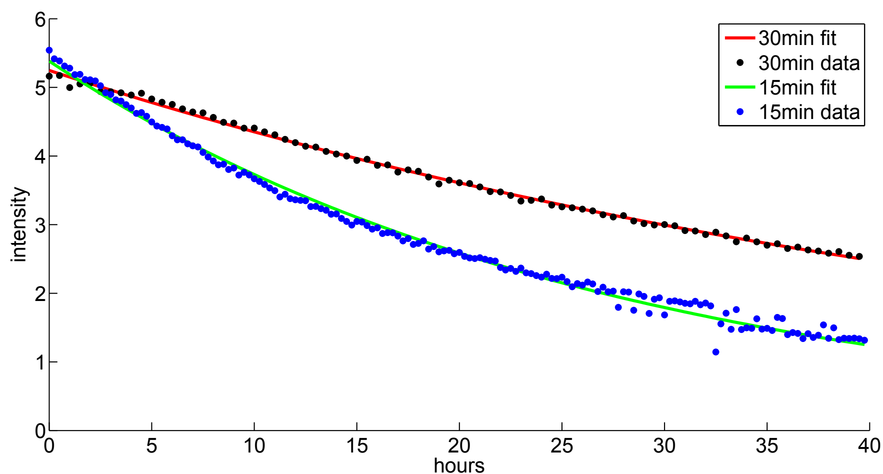

Fig. 2. Bleaching linearity. We analyze time courses of bleached beads which have been imaged every $t_1 = 15$ (black dots) and $t_2 = 30$ (blue dots) minutes. We perform a least square fit of an exponential decay $P(t) = P_0 \cdot e^{-\beta t}$ to the mean bead intensity $m(t)$ in both time courses, resulting in the bleaching parameters β_i (red and green line). In the case that the bleaching rate is directly proportional to the exposure intervals t_i, we expect a bleaching rate ratio of $\beta_1 : \beta_2 = 2 : 1$. The fitted rates $\beta_1 = 0.0367$ and $\beta_2 = 0.0187$ perfectly fulfill our assumption having a ratio of $1.96 : 1$. In the following we assume that bleaching scales linearly with the exposure intervals. Nevertheless, we need further experiments to confirm this assumption and measure the linear regime.

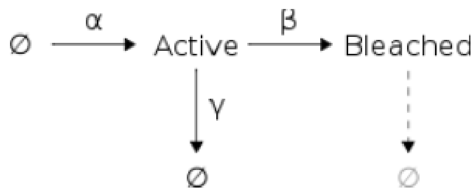

Fig. 3. Bleaching model. The process of fluorescent protein expression and decay can be simplified by the following model: an active protein is produced, decays and bleaches to an inactive state with the rates α, γ and β, respectively. The dynamics of the number of active proteins $P(t)$ can be described by a simple ODE: $\dot{P} = \alpha - P \cdot (\beta + \gamma)$. The further kinetics of the bleached proteins do not matter since bleached proteins can not be reverted to an active state nor do they emit any fluorescence. In this case we have to deal with two exponential decays which can not be distinguished by single time courses. We solve this indeterminacy by taking different experiments with known relations between the bleaching parameters β_i.

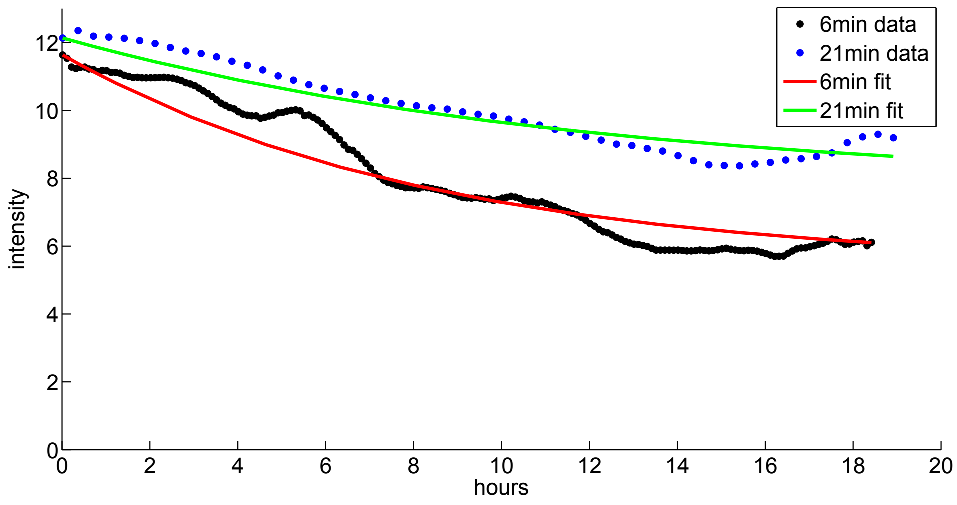

Fig. 4. We here assume that in each experiment the rates α and γ remain constant using the same cell types and conditions. Furthermore, we assume that the protein production does not change within one single-cell time course. In our experimental setup we acquired two different movies in which the bleaching rates β_1 and β_2 however differ. In the first movie, cells with YFP tagged proteins are imaged every 6 minutes (black dots), in the second movie every 21 minutes a fluorescence image has been taken (blue dots). Due to the linearity of the bleaching process, we know the relation $\beta_1 : \beta_2 = 1 : 3.5$. As described in the previous Fig. 2, we fit the mean of about 30 single-cell time courses from time-lapse movies according to the solution of the above ODE, $P(t) = \frac{\alpha}{b} + e^{-b_i t} \cdot (P_0 - \frac{\alpha}{b_i})$ with $b_i = \beta_i + \gamma$. We globally optimize the parameters for both experiments by forcing α to be the same and receive two independent decay parameters b_1 (red line) and b_2 (green line). By including the known ratio $x = 3.5$ between the two experiments we can solve the indeterminacy and infer the protein decay rate: $\gamma = \frac{b_2 - x \cdot b_1}{1-x}$. The resulting half-life of 9.9 h^{-1} is in accordance with a second experiment where the cells have been treated by cycloheximide.