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TLM-Tracker: software for cell segmentation, tracking and lineage analysis in time-lapse microscopy movies

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ABSTRACT

Motivation: Time-lapse imaging in combination with fluorescence microscopy techniques enable the investigation of gene regulatory circuits and uncovered phenomena like culture heterogeneity. In this context, computational image processing for the analysis of single cell behaviour plays an increasing role in systems biology and mathematical modelling approaches. Consequently, we developed a software package with graphical user interface for the analysis of single bacterial cell behaviour.

Results: A new software called TLM-Tracker allows for the flexible and user-friendly interpretation for the segmentation, tracking and lineage analysis of microbial cells in time-lapse movies.

Availability: The software package, including manual, tutorial video and examples, is available as Matlab code or executable binaries at http://www.tlmtracker.tu-bs.de.

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1 INTRODUCTION

The discovery of phenotypic variations in clonal microbial populations led to an increased interest in the investigation of individual cell behaviour. It was shown that culture heterogeneity can be caused by stochastic events at the level of gene expression. These lead to transient phenotypes up to stable co-existing populations. Documented examples for bacteria include persistance, competence and sporulation (Dubnau and Losick, 2006). However, traditional methods to analyze the dynamics of gene expression in bacterial communities and cultures including transcriptome and proteome analyses only capture the average of temporally overlapping expression levels. Deeper insights into the variability of certain gene regulatory circuits can be achieved by microscopical visualization of cells expressing appropriate fluorescence reporters (Meyer and Dworkin, 2007). Hereby, the complex dynamics of individual cells over time can be visualized using time-lapse movies. Successive frames in time-lapse movies offer the possibility to both temporal and spatial analyses of gene expression including proliferation, differentiation and migration of cells (Locke and Elowitz, 2009). Rapid advances in live cell fluorescence microscopy and developments in image processing allow the computational *in vivo* tracking and analysis of single cells (Wu *et al.*, 2008). Commonly used image processing algorithms allow the preprocessing of all frames to optimize size, brightness and contrast for the identification of background and border regions. In the next step, called segmentation, cells are recognized as meaningful parts that are separated from the background. This spatial separation is followed by cell tracking which is the temporal recognition of cells. In this procedure, cells are connected over time from frame to frame. During the whole process, features such as fluorescence intensity, cell dimensions, growth rate and migration are measured. These data represent important parameters for mathematical modelling approaches in systems biology. Several sophisticated approaches to this analysis were realized in both commercial and open-source software tools (Hand *et al.*, 2009; Young *et al.*, 2012). However, a program that combines sophisticated image processing and analysis with an intuitive application and a high degree of automatization is still missing.

To close this gap, we developed a new dialogue based interactive software package for the analysis of time-lapse movies called Time-Lapse Movie Tracker (TLM-Tracker). The tool includes many image preprocessing options and combines them with well-established segmentation and tracking algorithms. TLM-Tracker allows both full automatization of the analysis and manual intervention at every image processing step. We applied our software in a case study of a green fluorescent protein (GFP) producing *Bacillus megaterium* strain.

2 DESCRIPTION

TLM-Tracker was implemented as open-source software using the Matlab image processing toolbox. It can be run as Matlab application or as stand-alone program on Windows and Unix platforms. The software features a user-friendly dialogue based graphical user interface that enables user interaction at every processing step (Fig. 1). Additionally, customized processes can be defined and stored to enable the automatization of image segmentation functions. The program was designed for the joint analysis of simultaneously generated brightfield and fluorescence image data. Various image and movie formats including ZVI (Zeiss AxioVision format) are supported. After loading the images the main workflow is divided into four major tasks: preprocessing, segmentation, tracking and data export.

During the preprocessing, all frames can be cropped, the brightness can be adjusted to one common level and images

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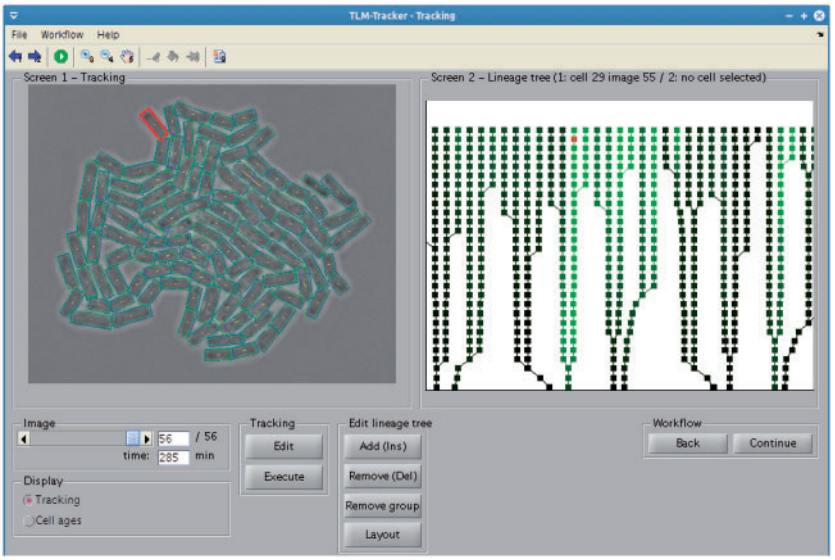

Fig. 1. Screenshot of TLM-Tracker after the segmentation and tracking process of a growing green fluorescent protein (GFP) producing *B. megaterium* microcolony. The left screen shows the recognized cells of one specific frame of the time-lapse movie. In the right screen, the derived lineage tree including GFP levels is shown.

can be removed if they are not suitable for further processing. Segmentation is the process of partitioning a frame into multiple segments to detect cells and separate them from the background. TLM-Tracker provides multiple alternative algorithms for segmentation, namely threshold-based algorithms, watershed transformation and level-set methods that can be applied for each frame individually. After the segmentation process, several quantitative properties of each cell are determined, such as dimension, position, orientation, cell pole age and area of the cell. Moreover, the mean, maximum and standard deviation of the fluorescence intensity of the cell are calculated. In two alternative algorithms, cells are tracked by searching of overlapping cell areas in the proceeding frames of the movie. After the cell lineage is determined, a correction of photobleaching in the fluorescence images is possible. Finally, derived results can be visualized as lineage tree and exported as CSV or XLS formatted file for further analysis.

Details about segmentation/tracking methods and their application are given in the online manual of TLM-Tracker (http://tlmtracker.tu-bs.de/index.php/Manual).

3 RESULTS

The software package was applied on a growing microcolony of a GFP producing *B. megaterium* strain. GFP production is driven by a xylose inducible promoter (Stammen *et al.*, 2010). Cell division events and the induction of GFP were analyzed using the watershed segmentation method in combination with tracking by overlapping cell areas. The workflow was performed in a completely automated way using predefined processes. This example clearly documents that the program is capable of dealing satisfactory with segmentation and tracking (Fig. 1). Despite TLM-Tracker was developed for the analysis of bacteria, it may be used for a variety of other cells.

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