# A Yeast Segmentation and Tracking Algorithm

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Accurate segmentation and tracking of individual cells from time lapse live microscopy is essential to understand single cell behavior and signaling. Here we introduce a fully automated segmentation and tracking algorithm for budding yeast that can accurately segment and track individual yeast cells for at least 60h. The algorithm does not require any specific biomarkers and can segment cells with arbitrary morphologies (e.g. sporulating, and pheromone treated cells), with high efficiency. In addition, the algorithm is largely independent of the specific imaging method (bright field / phase), objective or image resolution.

*Keywords* — Segmentation, Yeast, Watershed, Morphological Image Processing

#### I. INTRODUCTION

RECENT technological advances enabled us to follow gene expression and protein dynamics in single cells using time-lapse microscopy. To generate such data for some given cell, two requirements have to be met: First, cell boundaries have to be identified (segmentation), and second, the cell has to be tracked through time (tracking).

Here, we introduce a yeast segmentation and tracking algorithm [1], which improves the accuracy and speed of our previously published method [2]. In particular, we drastically improve its performance with respect to yeast cells with irregular boundaries such as spores and pheromone treated cells. In addition, we introduce a novel automatized seeding step, which replaces the semi-automatic seeding of the previous method and enables us to have a fully automatic segmentation algorithm.

#### **II. RESULTS**

#### A. Seeding

Our algorithm segments images backwards in time, i.e. it starts from the last time point and goes to the first and uses the segmented image of the previous image to track and segment the subsequent image. The first step in this process, called seeding, involves providing the algorithm the segmentation of the last image, the seed. This step was a bottleneck in the previous version of our algorithm since it was only semi-automated.

To resolve this bottleneck, we developed a fully automated seeding step that (1) pre-processes the image using morphological image analysis and applies watershed transform to the processed image, and then (2) fine-tunes the cell boundaries and automatically corrects under- and over-segmentation.

## B. Segmentation and Tracking

In addition to automatizing seeding, we introduced the following improvements to our previously published algorithm [2]:

## Parallelization

The new algorithm is fully parallelizable, which significantly decreases the run-time.

## Cell Intersections

Imaging artifacts may create the impression that two cells are overlapping, which results in contiguous cells claiming the same area. Instead of discarding the pixels that appear to be overlapping, in this algorithm we accurately distribute them between cells.

## Using existing fluorescent channels

Live cell imaging applications often have fluorescent channels in addition to the phase or the bright field image, which harbor information about the cell location that the previous algorithm did not exploit. In this algorithm, we improve segmentation accuracy by using these arbitrary fluorescent channels to generate composite images, which have higher contrast between cell and non-cell pixels. <u>Applications</u>

Using our algorithm, we segment and track time lapse movies of (1) cycling cells imaged with 40X objective and (2) 63X objective, (3) of sporulating cells imaged with 40X objective, (4) pheromone treated cells imaged with 63X objective, and (5) bright field images of cycling cells imaged with 40X objective.

## **III. CONCLUSION**

Here we introduce a fully automated and parallelizable algorithm that can segment yeast cells with arbitrary morphologies and imaging conditions.

#### **IV. METHODS**

The algorithm is implemented in MATLAB and is available upon request.

#### REFERENCES

- [1] NE Temamogullari, A Doncic. An improved algorithm for automated yeast segmentation and tracking with a novel seeding step, in preparation
- [2] A. Doncic, U. Eser, O. Atay, J. M. Skotheim, An algorithm to automate yeast segmentation and tracking. *PLoS One* 8, e57970 (2013).

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