

Automated spatial and temporal image analysis of bacterial cell growth

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Abstract

The state-of-the-art technology using flow-chamber microscopy imaging enables us to gain insight into the arcana of bacterial cell growth. However, a large number of high resolution developmental image data sets are produced that need to be properly processed and analyzed. The mathematical challenge lies in the automated image analysis, extraction of cell size profiles and determination of the time to cell division. Detailed noise analysis was carried out to correctly filter out the noise without losing important image information. A novel occluding convex image segmentation is developed which outperforms the existing algorithms in the literature. Next cell size parameters are identified via inertia equivalent ellipse fitting. Finally, individual cell division times are computed using k-means clustering. The information about individual cell division time distributions is of great value, as it has not been available before on such a large scale. Therefore this type of automation plays a key role in the new era of cell growth modelling.

1 Introduction

Uppsala University developed a novel flow-chamber technology (Elfving et al., 2004) that was designed to observe the distribution of the lag- and division times of hundreds of cells. The cells were fixed to the surface of a flow chamber slide due to the desired environmental conditions and then monitored with a dark-field microscope equipped with a 4x objective. The light intensity signal was read by an image analysis program at regular intervals (5 mins), which made it possible to estimate and record the time when the daughter cell was washed away from the anchored mother cell by the flow of the media. This technique combined with an intelligent image analysis pro-

vides microbiologists with information about individual cell growth at a scale never seen before.

Moreover, the effect of environmental conditions (temperature, pH, water activity) can be studied in situ with this method.

We have developed various algorithms to analyze flow chamber images. Due to the nature of these images ‘Vincent Dome Transformation’ (Vincent, 1993) was applied to filter out background noise and subsequently a specific ‘region growing’ technique (Gonzalez and Woods, 2002) was used to segment the image. After filtering and segmenting we determined the x and y -coordinates and the size parameters of the cells. Since on average 5 % of the cells were overlapping we devised a new algorithm based on convex hull to separate them. Based on spatial closeness between subsequent images we are able to follow the evolution of every single cell in time even if their location and orientation changes slightly in the course of time. From these cell size profiles our algorithm automatically determined the time to divisions.

In the next section we briefly explain the image analysis techniques developed to identify cells. Our novel method for noisy overlapping convex image separation is briefly introduced. In Section 3 we sketch our method for automatic division time determination from cell size profiles. Finally we summarize our conclusions in Section 4.

2 Image analysis

Microscopy flow-chamber images are produced to provide an insight into individual cell growth. This state-of-the-art technology combined with suitable unsupervised image analysis techniques could provide vast amount of information about individual cell division.

Flow chamber experiments were set up and

carried out at the Institute of Food Research, Norwich, UK. *Escherichia coli* strains were prepared for inoculation. Images were obtained in every 5 minutes in order to achieve high resolution. First we applied image stretching and ‘power-law’ transformation (Gonzalez and Woods, 2002). The background noise was filtered out using Vincent Dome Transformation (Vincent, 1993). The advantage of this approach is that it is insensitive to sporadic background noise and local intensity peaks. For cell determination a region growing algorithm was developed with two thickening criteria. The first criterium was related to pixel intensity and the other to gradient. To implement the gradient correctly ‘Laplacian of a Gaussian’ filter (Gonzalez and Woods, 2002) proved to be the most suitable. The size of the filter was chosen to be 5, and the standard deviation 2. To subdue the sporadic noise introduced by the Laplacian filter median filtering was applied.

Next the cores of the cells are selected and iteratively swelled. A neighbouring pixel is attached to the core if (1) its intensity is higher than a specific threshold and (2) its median-filtered Laplacian is zero. The threshold was chosen such that the total area of the resulting image should not exceed more than 1.5 times that of the seeds’ total area. Post-processing filtered out cells that are (1) touching the edge of the screen, (2) being small and flagged the ones being too concave.

Since *E coli* cells are ellipsoid shaped, we approximated their two-dimensional projection with inertial equivalent ellipses.

We implemented an inertia equivalent ellipse fitting algorithm also for fuzzy sets. First greyscale image pixel intensities are converted into probability values by a monotone increasing function, since the higher the pixel intensity is the more probable it is to belong to a cell. Second-order central moments (μ_{ij}) are computed according to

$$\mu_{ij} = \sum_x \sum_y (x-\bar{x})^i (y-\bar{y})^j p(x, y), \quad i, j = 0, 1, \dots$$

where $p(x, y)$ is the probability value of pixel (x, y) . The parameters of the inertia equivalent ellipse are: major axis (α), minor axis (β), orientation (θ), and they can be obtained from (Visen

et al., 2001).

$$\alpha, \beta = 2 \left(\frac{\mu_{20} + \mu_{02} \pm \sqrt{(\mu_{20} - \mu_{02})^2 + 4\mu_{11}^2}}{\mu_{00}} \right)^{1/2}$$

$$\theta = \begin{cases} \frac{1}{2} \left[\pi + \tan^{-1} \left(\frac{2\mu_{11}}{\mu_{20} - \mu_{02}} \right) \right] & : \begin{cases} \mu_{11} < 0 \\ \mu_{20} < \mu_{02} \end{cases} \\ \frac{1}{2} \tan^{-1} \left(\frac{2\mu_{11}}{\mu_{20} - \mu_{02}} \right) & : \text{otherwise.} \end{cases}$$

Thousands of cells are inoculated in a slide, and hence cell occlusion is inevitably present. This problem can affect up to 10% of the cells and careless analysis of overlaps often lead to erroneous results. For this reason it is essential to identify cell overlaps and devise automatic cell segmentation algorithm.

Occluding image segmentation has been highly motivated by overlapping chromosome segmentation (Charters and Graham, 2002; Vossepoel, 1989; Lerner et al., 1998). The majority of the methods in the literature use smoothed curvature, *k-curvature*, as a measure of concavity (e.g. (Visen et al., 2001; Vossepoel, 1989; Lerner et al., 1998)).

Touching cells can be detected by convexity analysis. To this end we defined the *convexity measure* (CM) of a set (I) as the maximum of the distances between the *boundary points of the set* (bI) and the closest *boundary point of the convex hull* (bcI):

$$\text{CM}(I) = \max\{d(z, bcI) | z \in bI\}$$

where $d(z_1, z_2)$ is the usual Euclidean distance of two objects. We consider every object for overlapping segmentation, which has a CM greater than 2.

Next our convex hull based algorithm segments the suspicious objects in two steps. First, it converts the binary image (I) into intensity image (bwI) by the following transformation.

$$bwI(x, y) = d((x, y), bcI) - d((x, y), bI) .$$

Second, the algorithm finds a ‘ridge’ in the intensity image which cuts the image into two. Each ‘ridge’ contains pixels which are local intensity maxima in the direction perpendicular to the tangent of the ridge.

Our novel convex hull based algorithm outperformed the curvature based ones (Visen et al.,

2001; Vossepoel, 1989; Lerner et al., 1998), producing most significant advantage in case of (1) acute angle occlusion, (2) multiple object overlapping and (3) images with large noise.

Having correctly identified cells in an image, we then keep a record for each cell of the centroid of its pixels, and the minor and major axis lengths. The final task involves determination of cell division times.

3 Determination of cell division

To follow how a cell evolves in time one has to compare the location of the cells from one time point to another. The closest object in the image at time $t + 1$ to the centroid of a cell at time t has to be the centroid of the same cell at time $t + 1$, and vice versa. Furthermore, the distance is not allowed to be greater than the sum of the two major axes of the cell at times t and $t + 1$.

Once we are able to follow in time each cell, time course cell size profiles can be plotted ($c(t)$) to identify cell division. Various noise filtering techniques were built into our algorithm to repair cell growth profiles to concord with biological rules in case we had sufficient statistical evidence of corrupted cell size measurement.

Finally, k-means clustering was applied to $c'(t)$ to single out probable cell division times. These candidates were screened further based on the ratio of cell size before and after division. An original profile is shown in Figure 1 with the automated selection of first and second division times. Note how our method successfully ignored the first noise peak.

4 Conclusions

A framework for automated image analysis of bacterial cell growth was presented. Our algorithms provide a fully unsupervised analysis for flow-chamber microscopy images. The analysis includes noise filtering, cell recognition, occluding image segmentation and cell division time determination.

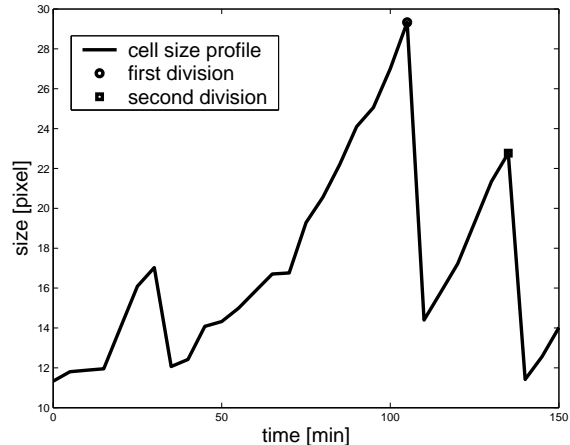


Figure 1: Noisy cell size profile.

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