

Characterization and recognition of cell structures in biomedical images

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Abstract— The study of microtubules by biologists is a very time consuming task. To support the research on microtubules, this paper proposes an automatic analysis algorithm of the microtubules in (EB1) fluorescence images. The proposed algorithm consist of two parts. The first part is a segmentation technique based on mathematical morphology which extracts the microtubules out of a 2D image. After the extraction of the microtubules a tracking algorithm is started to extract information on the dynamics of the microtubules in video.

Keywords— Segmentation, Tracking, Kalman filter, microtubules

I. INTRODUCTION

Microtubules are small tube formed structures witch can be found in every cell. They can be seen as the skeleton of a cell. They are also of major importance for cell division and intercellular transport. To understand the behavior of cell morphology scientist are interested in the dynamic and growing speed of these structures. Gathering quantitative and qualitative measurements of microtubules dynamics is a very time-consuming task. To speedup these observations we have developed an algorithm to analyze the data automatically. This paper is organized as follows: the second section gives a short explanation about the data which will be analyzed; the third section will explain how the microtubules are extracted out of the data; the following section will describe how the microtubules are analyzed during time; in the fifth section we will discuss the results of our algorithm and finally we will make conclusions in the last section.

II. DATA

Microtubules can, in vivo, be bound with fluorescence markers [6]. By measuring this fluorescence the microtubules become visible. The entire microtubules do not need to be marked since we are mainly

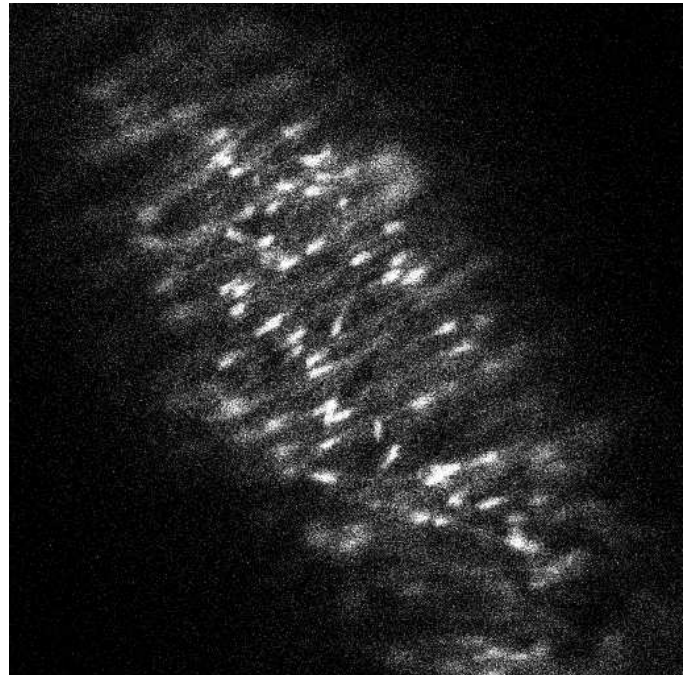


Figure 1: An example of an image where the end of microtubules are bound with a fluorescence marker

interested in the movement of the growing part of the microtubules. By using a special fluorescence marker, EB1, only the ends of a microtubule are bounded. As can be seen in Fig. 1, this results in images where small elliptical shapes, blobs, correspond with the ends of the microtubules.

III. SEGMENTATION

For the extraction of the microtubules a segmentation technique based on morphological reconstruction is used. This technique has already been proved useful for the segmentation of cornea endothelial cells [5]. For a deeper explanation on morphological reconstruction and the morphological operators used by the reconstruction we refer to [1], [4].

A. Morphological reconstruction

Morphological reconstruction starts from two images of the same size: a mask and a marker image. On the marker image morphological dilation is applied. If the pixel value of this dilation is higher than his corresponding mask pixel, its pixel value is replaced by its old value. On the resulting image these two steps of dilatation and replacement are repeated until the resulting image no longer changes. The resulting image is called the morphological reconstruction of an image given a certain mask. In Fig. 2 a 1D example is given. The blue curve is the marker; the black curve is the mask. The red curves are some of the repeated dilations. The green curve is the morphological reconstruction.

B. H-domes segmentation

The morphological reconstruction operator can easily be extended for segmentation. The mask is the image that needs to be segmented. From the grey value of every pixel from this image a certain term h is subtracted. This adapted image is used as marker in the reconstruction. The regional maxima can be extracted by subtracting the reconstructed image from the original. These regional maxima mainly correspond to the blobs we are looking for. There are however maxima coming from noise. To eliminate these maxima, a threshold is applied. The correct value for this threshold is image-dependent. Three different methods to choose the threshold were tested:

- A threshold value based on the number of non-black pixels in the image.
- A threshold value based on the number of non-black pixels in a small region. With this method a different threshold value is calculated for every pixel.
- A threshold value based on the contrast in the image.

IV. TRACKING

The use of tracking algorithms on fluorescence images has already been studied in [2], [3]. In this paper another tracking technique is tested: the Kalman filter. For every blob found by the segmentation algorithm, a Kalman filter is started. The Kalman filter is a prediction-correction method: a prediction is made and then updated by measured data. In this section we will first explain the models which are used by the Kalman filter. Secondly the prediction step will be explained. In the third subsection,

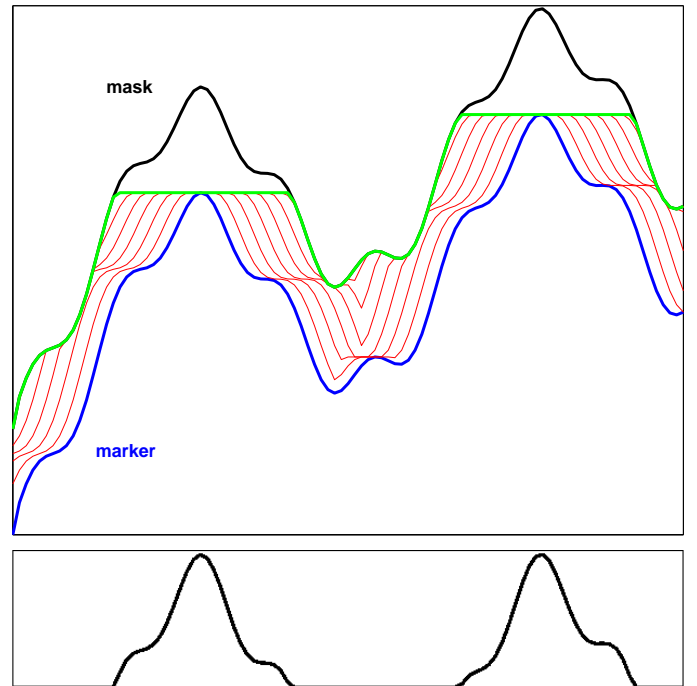


Figure 2: An example of morphological reconstruction used for segmentation. On top the red curves represent repeated dilations from the marker within the mask. The green curve is the morphological reconstruction. Underneath the difference of the mask and the reconstruction is visible. It is on this curve a threshold is applied to get the final segmentation.

several methods to measure the state of a blob at a certain time will be discussed. We conclude with the idea of what the Kalman filter actually does. In this section we immediately apply the Kalman filter for the purpose of tracking microtubules. To get a more general view on the Kalman filter we refer to [7].

A. Models

For every blob detected we initiate a state:

$$\mathbf{s}_0 = \begin{bmatrix} x \\ v_x \\ y \\ v_y \end{bmatrix}$$

Where (x, y) are the coordinates of the blob and (v_x, v_y) is its velocity.

The dynamics of a microtubule are modeled so that the state at time t can be predicted by a linear combination of the values of the state at time $t - 1$. This assumption would be too strict so a Gaussian white

noise is assumed on this model, e.g.

$$\mathbf{s}_t = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 \end{bmatrix} \mathbf{s}_{t-1} + \mathbf{w}_t$$

Where

$$p(w_t) \sim N(0, Q)$$

Next to the microtubule dynamics model there also is a model for the measurements m :

$$\mathbf{m}_t = \mathbf{B}\mathbf{s}_t + \mathbf{u}_t$$

Where \mathbf{B} represents the relation between the state and the measurement and where u_t is the noise on the measurements which is assumed

$$p(u_t) \sim N(0, R)$$

For the purpose of tracking microtubules \mathbf{B} is considered the identity matrix.

B. Prediction

The goal of our tracking algorithm is to know the true state at a given time t . Unfortunately both the dynamics and measurement models are affected by noise. If we assume that there is no noise on the dynamics model, a prediction of the state can be made:

$$\hat{\mathbf{s}}_t = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 \end{bmatrix} \tilde{\mathbf{s}}_{t-1} \quad (1)$$

where $\tilde{\mathbf{s}}_{t-1}$ is the state which is assumed to be correct for time $t - 1$.

C. Measurement

For the measurements, another segmentation technique is used. By using the prediction, a region of interest (ROI) is selected where the blob is expected. Not only the place, but the expected shape of the blob is known as well. This shape is used as a mask for linear filtering the ROI. The centre of mass of the filtered ROI is used to extract the coordinates of the blob. Two other variations of this measurement are tested:

- In the mask for the linear filter every background pixel is set to -1 to penalize bigger blobs in the ROI.
- To avoid that the measurement is an average of multiple blobs in the ROI, The filtered ROI is tested if it contains several blobs. If so, the blob closest to the prediction is chosen and all other blobs are set to zero.

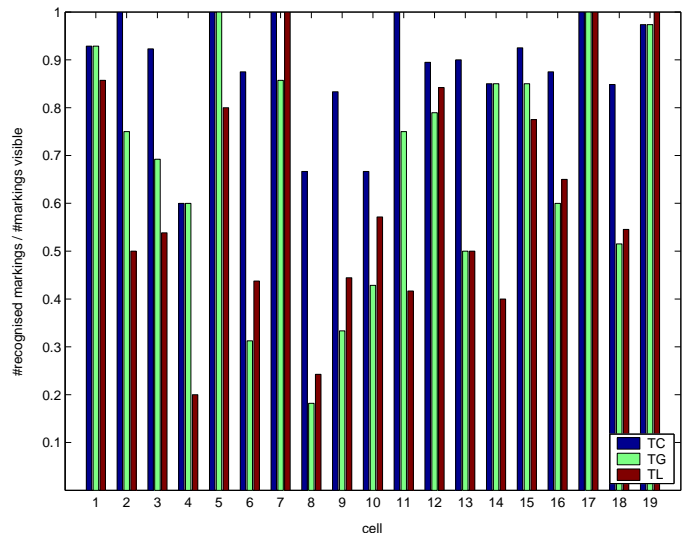


Figure 3: The number of recognised markers normalized by the number of markers visible for every tested cell. The blue represents the threshold value based on the contrast of the image. Red is based on the intensity values in a local neighborhood and green is based on the intensity values in the global image.

D. Filtering

From the prediction and the measurement, the Kalman filter calculates the most probable state given Q and R . This is:

$$\tilde{\mathbf{s}}_t = \hat{\mathbf{s}}_t + K(\mathbf{m}_t - \mathbf{H}\hat{\mathbf{s}}_t) \quad (2)$$

Where K minimizes $E[\mathbf{s}_t - \tilde{\mathbf{s}}_t]$. For an introduction to the Kalman filter we refer to [7].

V. RESULTS

A. Segmentation

To evaluate the segmentation techniques, 19 cells were manually segmented. A total of 436 blobs were found. In Fig. 3 the percentage of recognized blobs is shown for every cell for all three segmentation techniques. The technique based on the contrast measurement gives the best performance for all cells. This technique recognized an average of 88% blobs a cell.

B. Tracking

To evaluate the tracking algorithms blobs were manually tracked in 21 cells. There are two important criteria to evaluate the algorithms:

- The number of blobs correctly tracked until the end. Because of the presence of multiple objects, the measurements of the Kalman filter not always correspond to the tracked blob. There is a risk of

Table I: Test results for the Kalman filter depending on what measurement method was used. Methods based on *correlation* (A), *correlation with background penalization* (B), *probabilistic correlation* (C) and a combination of two earlier mentioned methods (D) where tested.

	A	B	C	D
# detected blobs	914	838	1071	918
median error (pixels)	3.6843	3.5022	3.2773	2.9971
mean error (pixels)	6.1977	4.8293	4.9925	4.3727
median loss/cell (%)	70	36.36	18.18	0
mean loss/cell (%)	55.82	36.27	30.96	18.89
mean loss (%)	62.82	45.30	35.04	20.51

tracking the wrong blob. The number of blobs who are mistaken by the measurement, and therefore wrongly tracked is called loss rate

- If a blob is correctly tracked, the distance between the filtered state and the true state is a second evaluation criterion. Both criteria work as a trade-off. We can reduce the loss rate by assuming that the noise on the measurement is higher, resulting in a greater belief of the Kalman prediction. This results in a greater distance between the true state and the filtered state.

B.1 Loss

The loss rate is very depended on what measurement method is chosen as can be seen in Table I. The method which combines penalization of the background and choosing the blob closest to the prediction, is capable of losing no blobs with more than half the tested cells.

B.2 Error

The measurement method based on blob prediction and background penalization does not only perform best on the criteria of loss, both also on the average distance between the true and filtered state. In Fig. 4 we see the normalized histogram of the error. We only consider the errors smaller than 35 pixels. Results that have a bigger error are considered in the category of lost blobs.

VI. DISCUSSION AND CONCLUSION

The Kalman filter works in general very well for the purpose of microtubules tracking. There are still cells where the filter does not perform as good. There are two important difficulties which result in loss:

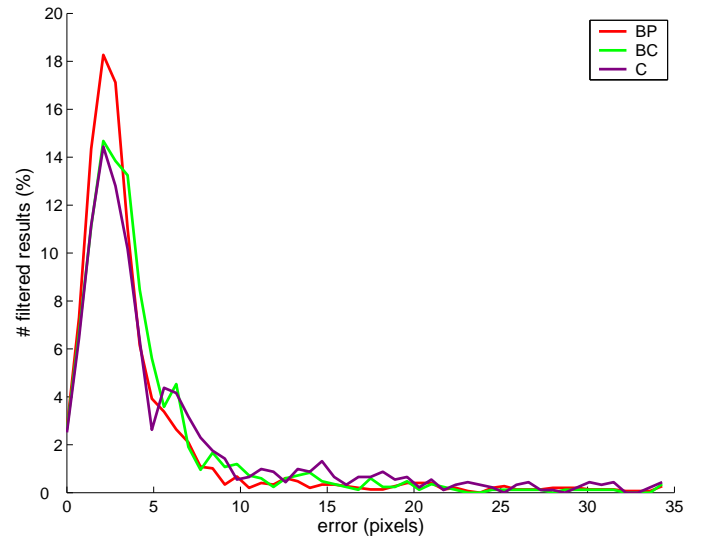


Figure 4: A normalized histogram on the errors for the different measurement methods. The red curve combines the prediction with the background penalization. The green curve penalizes the background and the purple curve is the correlation.

- In cells which have too much fluorescence, it is very difficult to decide whether a blob represents a microtubule or not.
- The appearing, disappearing and reappearing of a blob makes it very difficult to decide when a certain blob does no longer need to be tracked. The risk of loss by tracking the wrong blob is also much higher if blobs temporary disappear.

The results for cells which give poor results could probably be improved using post processing techniques. More research has to be done on these cells to get a proper idea of the overall performance of the proposed algorithm.

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