DETECTION OF CELL NUCLEI ON SEQUENCES OF CONFOCAL MICROSCOPY IMAGES

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ABSTRACT

This paper presents a method to perform automated detection of cell nuclei. The method starts with a filtering operation aiming at enhancing the image areas that may correspond to nuclei. The enhanced image local maxima can be associated with the potential locations of cell nuclei, but the filter responses also give additional positional information that can be related with nucleus borders, thus allowing the approximation of each nucleus contour by an ellipse. Afterwards, an analysis of the detected areas allows their validation or removal, as well as the adjustment of the final contours in order to facilitate the splitting of some nuclei that were overlapped because they belong to different cell layers.

Index Terms— cell nuclei segmentation, confocal microscopy, image analysis, convergence index filters.

1. INTRODUCTION

This paper describes an automatic method to select, in time-lapse confocal microscopy images of the Arabidopsis thaliana root meristem, the areas that may be associated with the cell nuclei. Some of the reasons for using this particular plant in development biology studies are its short life-cycle, which reduces the time needed to study of several generations, the plant small size, which allows easier capture of images during its development, and, finally, the small genome that facilitates the study of mutations. Some cell nuclei characteristics such as size or shape, as well as the distance between cells, can be used, for instance, to verify the progress of each individual cell cycle, or to determine the areas that show more division activity.

In this work, the image is first processed using a convergence index filter [1] in order to reduce noise and enhance the objects that possess a rounded shape similar to a cell nucleus. Afterwards, the possible locations of the cell nuclei are found from the determination of local maxima, and finally the areas that may correspond to nuclei are delineated and validated.

2. METHODOLOGY

The proposed methodology for cell nuclei detection can be divided into three main steps: image enhancement, detection of cell nuclei candidates and nuclei selection. These steps are described in the following subsections.

2.1. Image enhancement

Image enhancement filters are often used to reduce image degradation due to noise or distortions introduced during the capture process, although these filters can also be used to highlight only the objects that possess a certain type of characteristics. Since the objects of interest in this study usually show a rounded shape, a filter from the convergence index filter family [1] was selected to perform this enhancement task.

Convergence index (CI) filters are based on the maximization the convergence index at each image point of spatial coordinates \((x, y)\), as defined by equation (1),

\[
C(x, y) = \frac{1}{M} \sum_{(k,l)\in R} \cos \theta_i (k,l),
\]

where \(M\) is the number of points that belong to the filter support region \(R\), \(\theta_i\) is the angle between the gradient vector calculated for point \((k,l)\) and the direction of the line that connects points \((x,y)\) and \((k,l)\).

The main difference between the distinct members of the CI family is the definition of the filter support region. For practical reasons, this region is formed by a set of radial lines that emerge from the point where the filter result is being calculated. Four filters were evaluated during the development of this work: the coin filter (CF), the iris filter (IF), the adaptive ring filter (ARF) and a recently proposed filter, the sliding band filter (SBF) [2]. The CF uses a circle with variable radius as support region, and the IR maximizes the convergence index by adapting the radius value on each direction. The adaptive ring filter (ARF) uses a ring shaped region with fixed width and varying radius.
Finally, the SBF combines the basic ideas of the IF and ARF by defining a support region formed by a band of points of fixed width, whose position is changed in each direction to allow the maximization of the convergence index at each point, as defined by equation (2).

$$SBF(x, y) = \frac{1}{N} \sum_{n=1}^{N} \max_{R_{mn}} \left( \frac{1}{d} \sum_{m=n}^{d} \cos \theta_{im} \right),$$ (2)

In equation (2), $N$ is the number of lines of the filter support region that irradiate from $(x, y)$, $d$ is the band width, $n$ is the position of the band in a line that varies from $R_{min}$ to $R_{max}$, and $\theta_{im}$ is the angle between the gradient vector and the direction that is currently being analyzed. After the analysis of the results produced by each of these four filters, we concluded that the SBF yields much better enhancement results for these particular images.

2.2. Detection of cell nuclei candidates

After the application of the enhancement filter, cell nuclei can be associated with the highest image intensities; as a consequence, the detection of image local maxima was selected to identify potential nucleus centers. Since the number of local maxima obtained is normally too high, a threshold for the value of the local maxima is required to reduce the number of points; this threshold value is calculated based on the average of the detected local maxima. Another problem, which exists on the images where the cell walls are still visible on the nuclei image, is the detection of some local maxima that belong to cell walls. As each nuclei image has a corresponding image with the cell walls, a phase symmetry filter [3] is applied in order to detect the presence of well defined cell walls, as well as the non-uniform intensity among nuclei and inside each nucleus, as well as the distance between nuclei. These problems mentioned before, such as the non-uniform intensity among nuclei and inside each nucleus, as well as the detection of some local maxima that belong to cell walls. As each nuclei image has a corresponding image with the cell walls, a phase symmetry filter [3] is applied in order to locate the cell wall points and eliminate all local maxima associated with those points.

2.3. Nuclei selection

In each direction of the SBF region of support, the position of the band $(n)$ that maximizes the convergence index response gives an indication of the nucleus border localization in this particular direction. For each local maximum, an ellipse is adapted to the obtained set of border points, thus forming an initial approximation of the cell nucleus contour. Some of these ellipses have a large overlapping area, either because they correspond to nuclei belonging to distinct layers, or the filtering process resulted in an uneven sliding of the filter band in some directions. To overcome these problems, a procedure was developed for selecting the areas with the highest probability of being nuclei, which allowed also the final reduction of overlapping regions. This procedure starts with the ordering of the local maxima that correspond to overlaid areas. Afterwards, from the highest maximum to the lowest one, the regions that present an overlap above 50% (using as reference the smallest area) are replaced by a circle with diameter equal to the length of the minor axis of the ellipse, and the common area is once again calculated. If the overlap is still higher than 50%, the point with lowest intensity is discarded.

3. RESULTS

Our method was tested using 15 distinct images from different sequences. The images are 512×512 pixels in size and are represented using 256 gray levels.

Figure 1 illustrates the results of the proposed methodology. The original image (left) shows some of the problems mentioned before, such as the non-uniform intensity among nuclei and inside each nucleus, as well as the presence of well defined cell walls. The middle image represents the output of the enhancement phase with the local maxima kept after applying the procedure described in section 2.2 shown in blue. The right image presents the final result produced by the complete algorithm. The average number of cell nuclei per image was around 150, and the achieved detection rate was always above 80%.

4. CONCLUSIONS

The method described on this paper is completely automatic. The results seem to be quite satisfactory to delimit the areas where cell nuclei can be found. The approximation of nucleus borders by ellipses allows the calculation of several nuclei characteristics such as size, eccentricity and distance between nuclei. These characteristics will be used as starting point for future developments in order to automate the detection of cell division processes.

5. REFERENCES