AUTOMATIC DETECTION OF PHOTOSYSTEMS II IN ELECTRON MICROSCOPE PHOTOGRAPHS

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Abstract

In this paper our algorithm, which helps biologists to examine structure of very small objects, is described. These small objects are many times on the display limit of the best existent microscopes and the quality of the raw images is insufficient. In this case is advantageous to use a digital postprocessing of the images. The digital postprocessing is based on processes of huge amount of the low quality images which depict the same small object and in the specialized software can be estimated one picture of the object in much better quality. Practical problem is to collect the huge amount of source images. Normally, it is collected by hand. It is very boring and it wastes the valuable time of the researchers. Our algorithm collects the source images automatically and so save the time and improve quality of the researchers output.

1. Introduction

In this study we was examined *photosystem I* and *II* structure of red alga *cyanidium caldarium*. The *photosystem I* has approximately round shape and it's diameter vary from 20 to 30 nm. The *photosystem II* is elliptical and has similar size. The size of the photosystems is too small and on the raw microscope images no internal structure is seen (figure 1). Therefore the digital postprocessing had to be used.

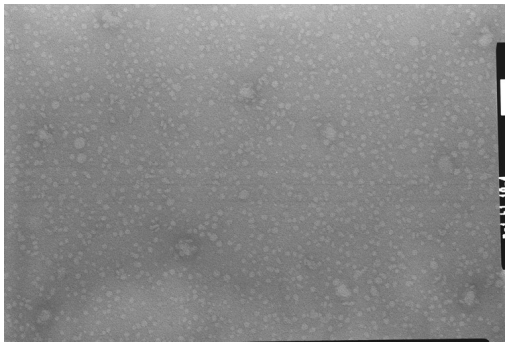


Figure 1: Example of scanned electron microscope photograph

The quality of the postprocessing result is determined by the input quality of the raw images and by the number of images. The number of the input images was estimated about 10000 for sufficient output image quality. It is practically impossible to collect this amount of images by hand therefore an automatic detection algorithm was applied. This algorithm is described in more detail in following text. An ideal result is illustrated in the figure 2 [1].

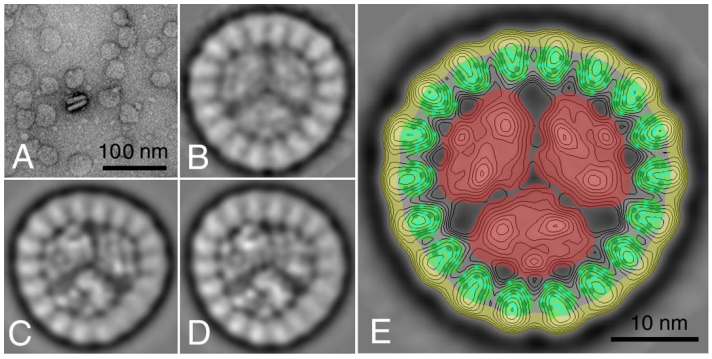


Figure 2: A: source image example, B-D: postprocessed results, E: estimated inner structure

2. Algorithm description

The algorithm consists of five steps and was implemented in *MATLAB 7* with *image processing toolbox.*

2.1. Normalization of the background

First step is normalization of the background. It is the necessary process for following efficient thresholding. Before the normalization a non-image area (negative edges) had to be discarted. The non-image area is easy to detect because it's values are very close to absolute black or white. The normal image values are gray and have a low contrast.

The normalization itself is implemented as a background subtraction. The background is estimated by smoothing of the original image by 2D hamming window. The window length was set heuristically to 151 pixels. Matlab function *filter2* is used for smoothing. A mean of the image is set to neutral gray (value 0.5) after subtraction. An Illustration of normalization effect is depicted in upper part of the figure 3.

2.2. Noise suppression

Second necessary step before thresholding is noise suppression of the input low quality images. The noise level is very high therefore the excessive smoothing is advantageous. Thereafter thresholding and classification work well. It is possible use the same filtration for smoothing like for background normalization, just the window length must be lesser. We use length 13 pixels. The result of denoised image is in bottom part of the figure 3.

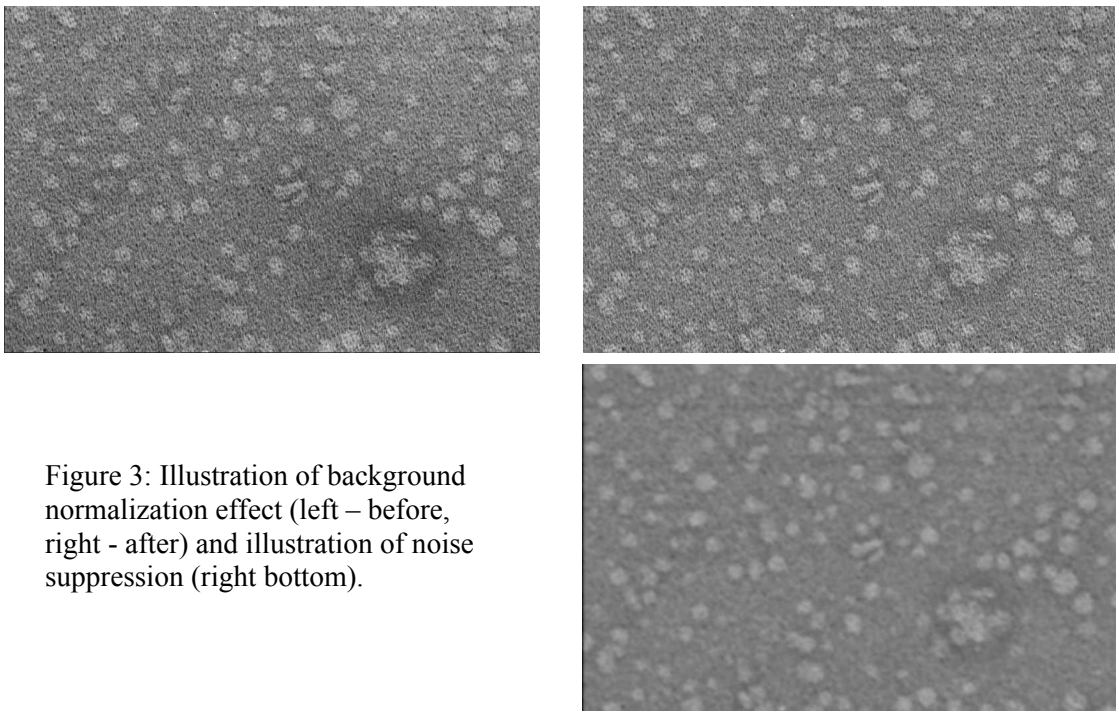


Figure 3: Illustration of background normalization effect (left – before, right - after) and illustration of noise suppression (right bottom).

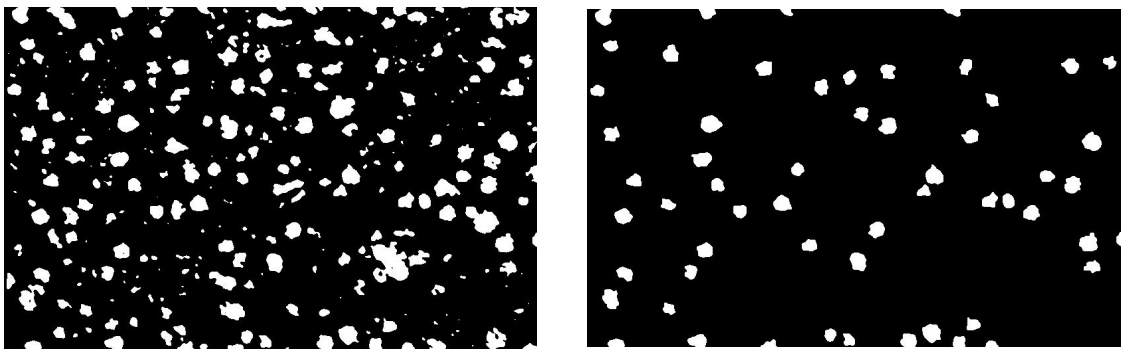


Figure 4: Thresholded image (left) and finally chosen *photosystems I* (right).

2.3. Thresholding

After preprocessing steps, the image is ready to thresholding. Since density of the objects is very consistent in all measurements, we use relative thresholding method. The threshold was set with a view to get 14% of image area as the objects.

2.4. Filtering and clasification

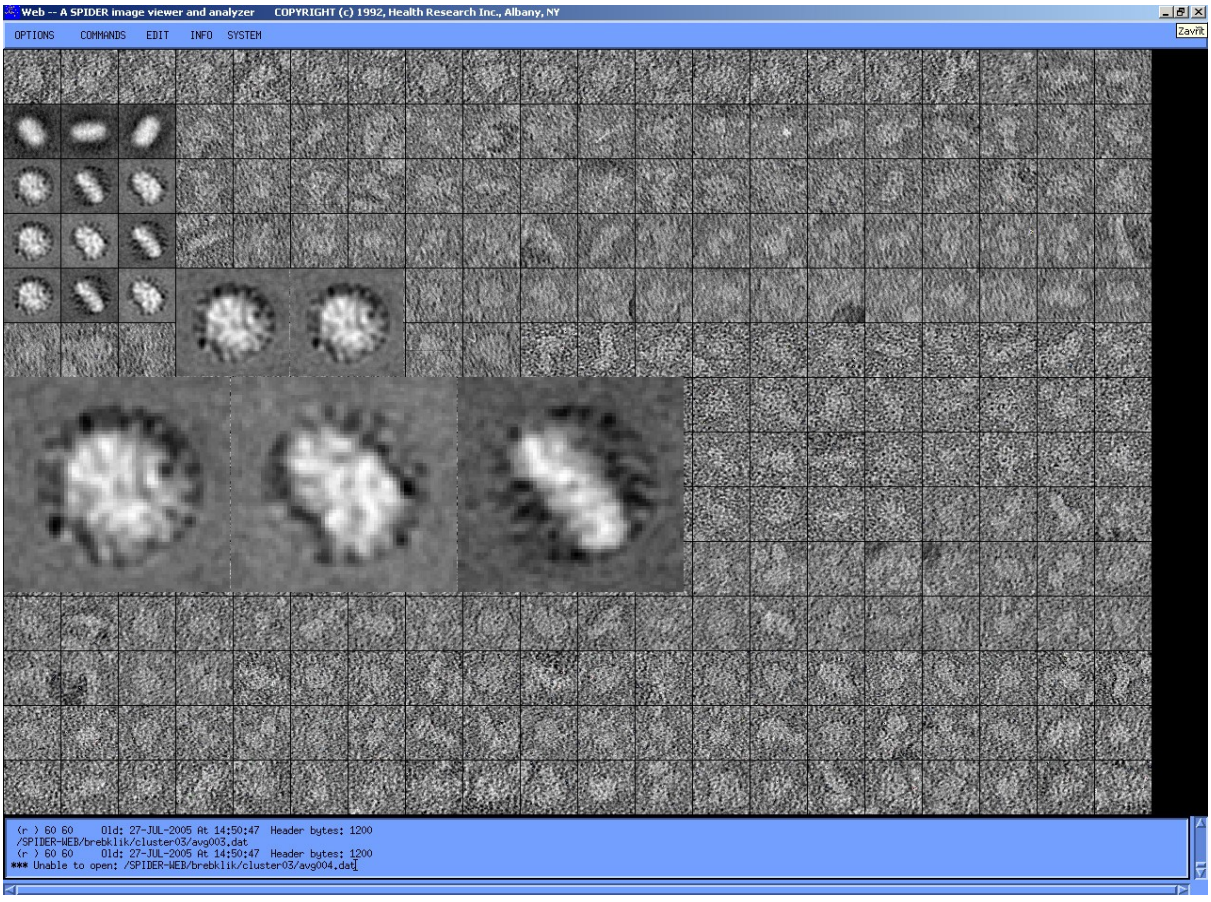
In the thresholded image is lot of mess and only a few object are really the searched photosystems. Most of the objects are very small therefore a morphological opening is applied to the thresholded image. The opening is implemented by the *image processing toolbox* functions *imerode* and *imdilate.* The residual objects are labeled by the function *bwlabel.* Thereafter areas of all labeled objects are computed and filtering by the area is realized.

Finally, the classification by the shape is done. In recent work [2] only the *photosystem I* was searched. There was the shape classification based on computing "round ratio". It is ratio between real perimeter of the object and estimated perimeter computed from the object area for ideal round. Because the *photosystems I* have round shape, only the objects which had round ratio lesser then 1.2 were chosen. The result are illustrated in the figure 4.

The classification of the *photosystems II* is more complicated because the *photosystems II* are elliptically shaped, all the same the similar approach is used. At first, the ideal ellipse is fitted to real object by function *regionprops* and the perimeter ratio between real perimeter and ideal perimeter is calculated in the same way like round ratio mentioned above. At second, the eccentricity is calculated. On the end, the object with perimeter ratio lesser then 1.2 together with eccentricity between 0.35 and 0.7 are chosen.

2.5. Exporting

The chosen objects are finally exported in 60x60 pixels frame to a Spieder&Web format. The Spieder&Web is the specialized software for particle analysis and is able to estimate one quality image from many low quality images. It's work desktop is depicted in figure 5.



3. Results

From our results (figure 6) we can declared thad automatic algorithm produce comparable results with by hand selection of the particles and in addition it is much faster and fully automatic. But in this time the low number of measurement is accomplished and therefore the numbers of selected particles are insufficient to get quality image with clear inner structure of the photosystem. If you are interested more in biological bacground of this work, you can see references [1] and [3].

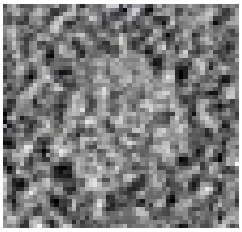
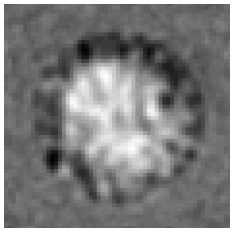
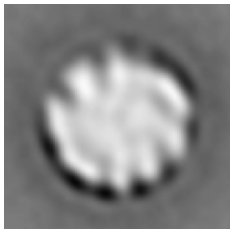
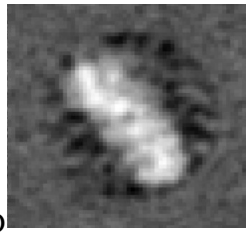
A  B  C  D 

Figure 6: A: photosystem I in raw source image.

B: postprocessed photosystem I from 877 automaticly extracted particles.

C: postprocessed photosystem I from 2 467 particles.

D: postprocessed photosystem II from 1 219 particles.

References

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