PARAMETRIZATION OF DISCRETE SPHERICITY IN ELECTRON MICROSCOPY IMAGES

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Abstract

Electron microscopy allows us to observe nanostructures of the small objects inside the biological organisms (nuclei, photosysems, membranes). The obtained images are intensity (grayscale) levels, there is no color defined in the electrons beam. The image properties (contrast, bitdepth, dynamic range, resolution, ...) are person-specific, since the experts have an individual imaging-behavior. The background and type or size of other objects vary due to biological reasons.

The aim of work is to discuss the sphericity attribute of several objects for classification purposes. Usually, the very small objects are also of small amount of occupied pixels, therefore the disretization of the object borders come into acount. The classicall Hough transformation is time consuming and valid for well spherical objects. However, in the electron microscopy several types of objects are of the experimentator interrest. Therefore, the detection and classification should be based on parametrization and evaluation of the sphericity attribute. The low time burden sollution could be done be using eliptical parameters, and area to perimeter derived ratio.

The three types of electron microscopy images were tested, of different biological experiments. The parametrization of the classification criteria is automaticaly evaluated via statistical description of the given electron microscopy image.

1 Discretisation effects on the spherical represantition

Digital images allow us to carry on post-processing and analysis of the objects in the observed scene via plenty of semi-automatic software. The accurate objects recognition and further evaluation depends on many attributes, including discriminability (often incorrectly described as resolution [1]), contrast, compactness of the edges, and so on. The detection of the object shape types are complicated by the discrete property of the object values. Especially, with the very small object, the deformation of the exact shapes is well known as so-called pixelation.

The pixelation is nothing else, then the classical aliasing effect, where the discriminability does not fulfill the spatial Shannon-Kotelnikov-Nyquist theorem of sampling frequency, and might leads to existence of the artifacts among real objects. Especially with the real spherical objects, we can obtain squares, crosses, or stairs-like spots.

On the other hand, the proximity or remoteness to the sphericity are important criteria for the classification of the objects, structure identification, and image registration.

Unfortunately, in observation of very small real objects, we are limited by the current technical progress (or funding budget), which is increasing in recent years (two Nobel prices for microscopy in last five years – optical fluorescence in 2012 and electron cryo in 2017). In the focus of the interest are mainly biochemical and biophysical objects, including cell functional organelli, membrane compartments, and metabolic/protein macromolecules. Observation of the intracellular environment helps us to understand properly the its function and behavior.

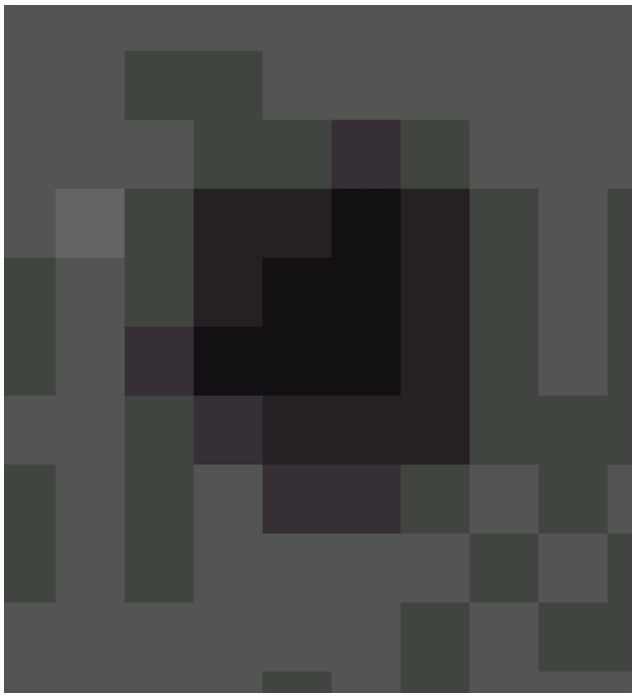

Figure 1: Example of discrete effects – black sphere on gray background.

2 Electron microscopy and datasets

On the appex of the observation of small objects is the electron microscopy, where we are not limited by the point spread function of photons and their energy, but of electrons with much smaller wavelength and therefore also the discriminability. In this article, datasets from three different biological specimens and electron microscopy techniques were tested [3, 4]:

1) Algal photosystems in transmission electron microscopy (TEM) of 20-30 nanometers [nm],

2) Immunolabelling of antibodies gold nanoparticles in TEM, 5-25 [nm],

3) Restriction enzyme in cryo electron microscopy (Cryo-EM) 18-40 [nm].

The images are intensity (grayscale), there is no color defined in the electrons beam. The image properties (contrast, bitdepth, dynamic range, discriminability, ...) are usually operator-specific, since the experts have an individual imaging-behavior.

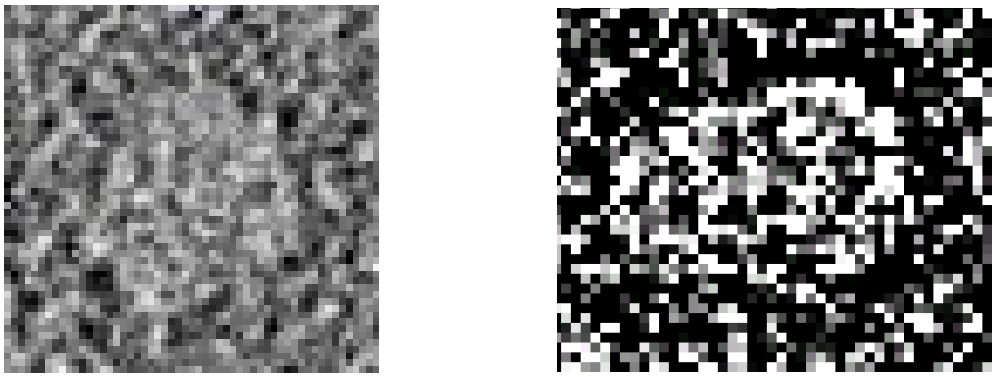

Figure 2: Photosystem on the left, enzyme on the right.

3 Methods

The sets of input images were separated into training and testing subsets. On the training images filtration, edge detections, segmentation, and region of interest (ROI, the objects) parametrization were carried out, compared, and tuned. The sucessfull approach was evaluated using the testing images. The Hough transformation [5] is not recommended for this task, the objects are too small and not perfectly round. The computational burden is the typical dissadvantage of the Hough method. ROI parametrization is the main subtask, while the specification of the criterion function determines the overall results of the detection algorithm. The processing of the images was performed using Matlab Image processing toolbox and Python with OpenCV, on Intel Core2 Duo CPU E8400, 3GHz, 4GB RAM, using double precision.

4 Results and conclusion

There was adopted a ratio between radii computed from area and perimeter [4] in extension to the eccentricities and elliptical axes, which suffer with error propagation, since it depend on the estimation of object orientation . Thus:

$$ra = \mathrm{sqrt}(Area/\ pi),$$

while

$$rp = perimeter\ /\ (2*pi),$$

and the ratio is

$$ap = ra\ /\ rp.$$

The ideal sphere in a vacuum will has the ratio equals 1. Therefore the distance from the unity represents also the distance from the sphericity. Due to discrete representation, the observed deviation in sphericity ratio for presumably spherical ROIs was of 0.2 both directions. Therefore, the shape analysis of very small objects is unable to distinguish small real deviations of the sphericity, since the pixelation deformed image representation of objects. The additional parametrization of intensity, convexity, and inertia has to be taken into account.

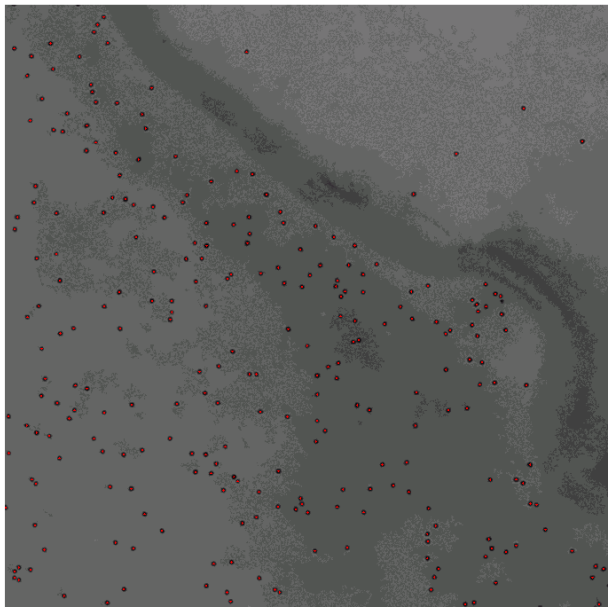

Figure 3: Example of results for gold nanoparticles TEM detection. Detected particles are labeled by red marks.

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References

[1] Urban, J., Císař, P., Pautsina, A., Soukup, J., Bárta, A. Discrete representation and morphology. *Technical Computing Prague 2013*, 322.

[2] Von Byern, J., Dorrer, V., Merritt, D. J., Chandler, P., Stringer, I., Marchetti-Deschmann, M., McNaughton, A., Cyran, N., Thiel, K., Noeske, M., Grunwald, I. (2016) Characterization of the fishing lines in titiwai (Arachnocampa luminosa Skuse, 1890) from New Zealand and Australia, PLoS ONE, Volume 11(12), e0162687.

[3] Urbanova P, Detection of gold nanoparticles in transmission electron microscopy images , SVK FAV 2017, Pilsen.

[4] Vanek J., Urban J., Gardian Z.: Automated detection of photosystems II in electron microscope photographs, Technical Computing Prague, p.102, 2006.

[5] Duda, R. O., and P. E. Hart. Use of the Hough transformation to detect lines and curves in pictures. Communications of the ACM 15.1 (1972): 11-15.

[6] Rychtáriková, R., Náhlík, T., Shi, K., Malakhova, D., Macháček, P., Smaha, R., Urban, J., Štys, D. (2017). Super-resolved 3-D imaging of live cells' organelles from bright-field photon transmission micrographs. Ultramicroscopy, 179, 1-14.

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