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**ID-8-P-3228 Algorithm for extraction of diffracting objects’ PSF from z-stack of bright-field microscopy images of living cells**

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We bring a segmentation algorithm for diffracting objects observed by bright-field microscopy (Fig. 1), where a binary mask for extraction of the studied objects is created by analysis of changes of information between consequent images in the z-stack. This may be in fact done by simple level-by-level intensity comparison, but the information analysis brings on top of that image point classification. Here we report information remaining unchanged at 0th digital level of green channel of two consequent PDG-subtracted images (inputs 1). In these PDG-images, borders of the objects are highlighted by subtracting of two following images in z-stack of the cell based on Renyi information entropy concept – Point Divergence Gain (PDG):

\[
\text{PDG}_{\alpha,x,y} = \frac{1}{1-\alpha} \left( \ln \sum_{i=1}^{n} p_{i,x,y}^{\alpha} - \ln \sum_{i=1}^{n} p_{i,(x+1,y+1)}^{\alpha} \right),
\]

where \(p_{i,x,y}\) is the probability density function of the intensity with the examined point of coordinates \(x, y\) in the previous image number \(l\), \(p_{i,(x+1,y+1)}\) is the probability of the occurrence of the given intensity in the image where the examined point of coordinates \(x, y\) was replaced by the point at the same location in the following image number \(l + 1\), \(\alpha = 4.0\) is a dimensionless coefficient, and \(n\) is a number of intensity levels (\(n = 256\) for an 8-bit image). For each PDG-subtracted image, two images were obtained – with positive and negative change of PDG\(_{\alpha,x,y}\) value. Thus, information of each original image is saved in four parallel PDG-subtracted sub-images. In green channel of PDG-images, we can observe diffracting objects. In the blue channel we observe mainly the fluorescence and in the red channel the contribution of near infra-red absorption is observed. At the 0th PDG level of each channel, large objects with the minimal change of the point spread function between z levels are visible. Another intensity levels corresponds to diffraction response of less observed objects, objects surroundings as well as other parts of the point spread function belonging to large organelles.

After creation of the binary mask from PDG-images, small objects (pixels) are removed by morphological operation opening (ROI). Fluorescent organelles and Airy discs, which are also projected at 0th intensity level of the green channel of PDG-images due to no change of information in two following microscopy images, were removed by elimination of the intensities higher than the intensity mode of the background (input 3) from the image of the cell alone (input 2).

Finally, the diffracting objects were segmented (output) by application of the binary mask to the image of the cell with deleted fluorescent objects and Airy discs. The remaining points in the segmented RGB image where removed by combination of morphological operation erosion and dilation.

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Fig. 1: Scheme of image processing for segmentation of diffracting objects from a bright-field microscopic image of cells.