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Abstract

Application of Rényi Entropy-Based 3D Electromagnetic Centroids to Segmentation of Fluorescing Objects in Tissue Sections †

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The understanding of the physico-chemical basis of the intracellular processes requires determination of local concentrations of cell chemical constituents. For that, light microscopy is the irreplaceable method. Using an example of a (auto)fluorescent tissue, we clarify some still ignored aspects of image build-up in the light microscope for maximal extraction of information from the 3D microscopic experiments. We introduce an algorithm based on the Rényi entropy approach, namely on the Point Divergence Gain (see Entropy 20(2), p. 106):

$$PDG_{\alpha}^{l-m} = 1/(1-\alpha)\log_2\{[(n_l-1)^{\alpha} - n_l + (n_m+1)^{\alpha} - n_m]/C_{\alpha} + 1\},$$

where α is the Rényi coefficient; and n_l and n_m are frequencies of occurrence of phenomena (intensity) l in the 1st matrix (digital image) and of phenomena (intensity) m in the 2nd matrix (digital image). The digital images are optical cuts consecutive in a stack obtained along the microscope optical path between which weighted frequencies of occurrences of all phenomena (intensities) in the 1st matrix (digital we exchange a pixel of intensity l for a pixel of intensity m. The term $\mathbf{C}_{\alpha}$ is a sum of α-image).

We removed an image background using $PDG_{50}^{l-m} = 0$ which is an approximation to $PDG_{\infty}^{l-m} = 0$ (analogy to min entropy). Then, we sought voxels (3D pixels) called 3D electromagnetic centroids that corresponded to $PDG_{2}^{l-m} = 0$ (i.e., multifractality approximation to subtraction of two images consecutive in a z-stack). This localized the information about the object independently of the size of this voxel (see Ultramicroscopy 179, pp. 1–14) and gave us cores of the objects' images. At $PDG_{10}^{l-m} = 0$, we obtained extended 3D images of the observed objects called spread functions.

This approach enables us to localize positions of individual fluorophores and their general spectral properties and, consequently, to make approximative conclusions about intracellular biochemistry.