Living cell with interior is a multifractal system, and lens system of an optical microscope during experiments introduces other multifractal characteristics to the image which is consequently discretized by a camera chip. Because each multifractal (sub)object has its probability of occurrence in the image, we extracted the information content of the z-stack image of the cell by Renyi entropy gain approach – Point Divergence Gain (PDG). By this procedure, we determine, the change of information of the image of the cell by exchange of the particular data point between two following images in z-stack at different values of dimensionless parameter α. Then each component of the multifractal (sub)object have its own generalized spectrum, i.e., each of the PDG-values at each α has its occurrence in the image explained as particular image intensity.

For 3D reconstruction of cell interior we used PDG at α = 4 with the highest amount of the occupied intensity levels, i.e., highest number of separable groups of different information contribution. The 0th intensity level of PDG4,0 represents lowest change of information between two following images of the z-stack. This identifies part of the point spread function which (a) belongs to frequent objects and (b) does not change significantly between two consecutive images in the z-stack. Points at this PDG level were used for creation of a binary mask by which the part of the image at a given z-level belonging to a distinguishable organelle image was sectioned from the original RGB image. The properties of the course of real point spread function (PSF) were examined and a model of the organelle was prepared. In that, we considered that a larger object – organelle – is filled by elementary diffracting objects. Thus, as long as certain elementary objects reside in the focus, the change of the PSF along the z-axis remains similar. Outside this region, the behaviour of the PSF changes. At the same time, in the focus the image is the darkest. We have thus two indices which we may use for determination of the position and shape of the organelle: change in the size of the spot and its coloration.

Then, the focal plane of examined nucleolus of MG63 cell corresponds to the level of the PSF with the lowest mean G-intensity and local area minimum (Fig. 1B). This focal plane for red and green channel is the same. The course of PSF in blue channel is different as we deal with the projection of fluorescent, not diffracting, objects. The nucleolus probably occurs in the region between local area maxima around this local area minimum (Fig. 1C). The other local minima and maxima likely correspond only to light interferences along the build-up of the image projection along the optical system of the microscope.

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Fig. 1: Analysis of PSF of nucleolus of MG63 cell. Course of PSF in the focal region of the cell (A), its analysis (B) and course of PSF in the expected occurrence of the nucleolus (C). The colorbar below the figures shows intensity in each colour channel in Fig. A and C.