THE COLOCALIZATION QUESTION IN CONFOCAL MICROSCOPY

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Confocal microscopy allows in depth measurement of fluorescence distributions in biological specimens with 3D sub-micrometric resolution, allowing the identification of the 3D distribution of several proteins and lipid components. One of the obvious question is then to visualize and quantify the colocalizations between fluorescence distributions. To find fluorescence colocalizations, we generally use the implicit notions of 3D resolution and color thresholds, which turn out to be linked to local fluorescence contrast and fluorescence background rejection. However, color coded intensities are not necessarily true indicators of a local coincidence between fluorescence distributions. Searching for "colocalizations" requires 1) appropriate handling of instrument settings during image acquisition, 2) image visualization and restoration tools to provide the best registration and cross talk correction between the different fluorescence data sets, and 3) dedicated software packages, which identify the regions of interest where significant image correlation occurs between fluorescence distributions.

Optimum image acquisition conditions require linear fluorescence measurements, over-sampled pixels in X, Y and Z, and appropriate spectral settings to minimize cross talks between channels. To control the quality of images, we have introduced scatter plots, which display the joined statistical distribution of pixels fluorescence intensities of multicolor images, by analogy to multiparameter classification of cells in flow cytometry. We will present here new developments and main applications of scatter plot analysis such as quality control assessments of fluorescence images. However, since the scatter plot analysis consist of a global analysis of single pixels within whole images, they do not convey unique information on the colocalization between fluorescence distributions on a local scale.

To really identify local similarities, we introduced local image correlation techniques, based on pixel neighborhood analysis, using the notions of local contrast and local background in the images. Our algorithm evaluates the cross-correlation of two color fluorescence distributions within a reference volume rolled on the images, and identifies regions with similar intensity profiles, above a given fluorescence background specified by the user. This process is close to the intuitive perception of colocalized structures identified by virtue of shapes, position, and contrast. As a hallmark of image correlation maps, they are invariant with a change in the PMT detection settings of each instrument. Image correlation map represent therefore a unique tool to compare results obtained under different experimental conditions providing a quantitative assessment of multifluorescence images.

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