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Automatic 3D detection and quantification of co-localization

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Optical microscopy is often used to locate where the interaction of two proteins occurs *in vivo*, based on the degree of co-localization of two fluorescently tagged proteins. This qualitative approach has at least two fundamental problems. First, it neglects the fact that some of the overlapping regions occur randomly. The second problem is the bias in deciding on the cutoff intensity values for each stain that determine the specific signal and thus overlap regions.

We present a method to automatically detect and quantify co-localization of fluorescence-tagged molecules in 3D microscopic images. This method is an extension of previous Pearson correlation approaches [1]. It is automatic, which removes the typical bias carried by visual evaluation and allows fast analysis of large stacks of images. It is intensity and background independent, making it robust for repeating measurements. First, the algorithm computes the probability that co-localization is not the result of random overlap between both stains. If the probability indicates presence of non-random co-localization, the percent amount of co-localization for each stain is computed as the total co-localized intensity divided by the total intensity in the region of interest. Finally, an image is returned, where each pixel has its intensity proportional to its estimated overall co-localization contribution.

The method was successfully tested on 3D synthetic images (see Fig. 1) and on biological negative and positive controls (see Fig. 2). The positive controls were obtained from cells stained with the same primary antibody and two different secondary antibodies. Negative controls were obtained from cells stained against two different biological compartments, i.e. lysosome and mitochondria. The analysis was done in a blind manner. All controls showed very good agreement with expected results. In addition, we showed with simulations that our program remained accurate in situations where performance of the typical 2D histogram analysis [2] or the cross correlation approach [3-6] was unsatisfactory. [7]

References

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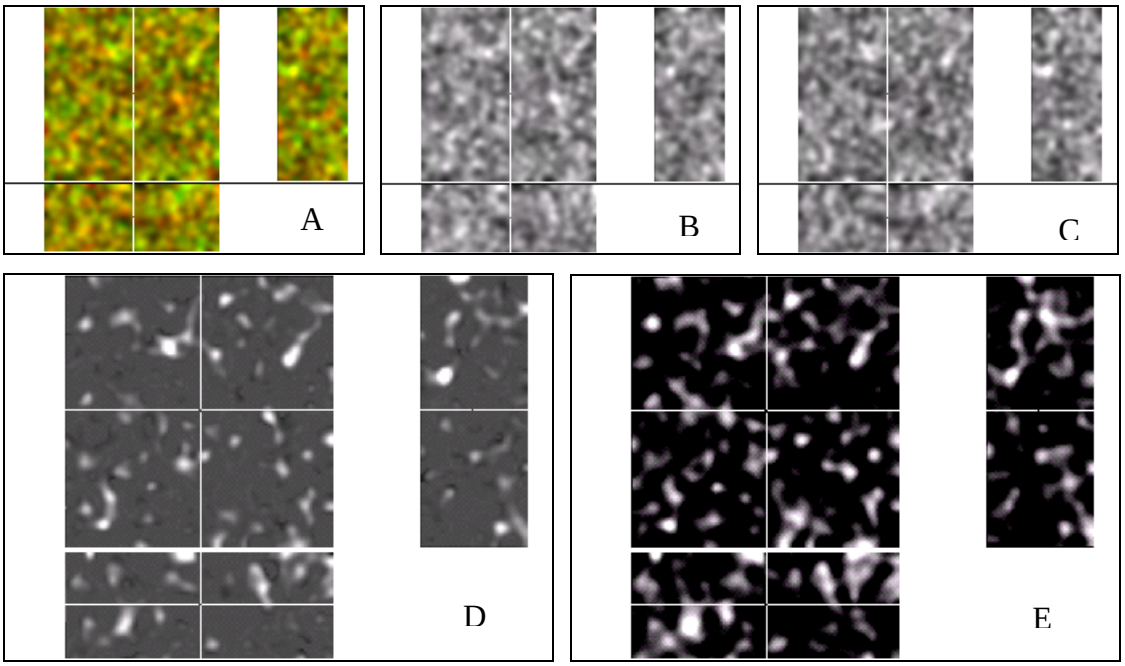

FIG. 1. Panels A, B, C show a section view of a 100x100x40 3D simulated image (overlay, red and green channels respectively). The simulated red and green channels had both 65% co-localization (Panel description: XY plane = top-left view, ZY plane = top-right view, XZ plane = bottom view. Section indicated by cross hair). The probability for non-random co-localization was found to be 100% and the detected amount of co-localization, shown on panel D, was 70%. 5% of the detection was random overlap. Panel E shows the simulated co-localization image, which correlates very well with panel D.

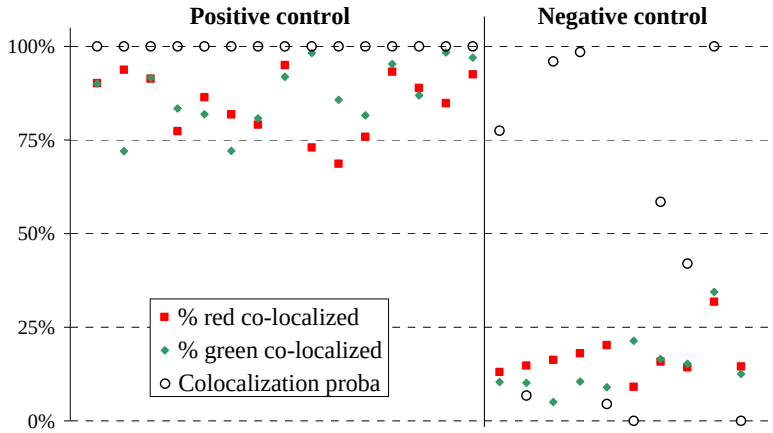

FIG. 2. Biological test. Positive controls were cells stained with a primary antibody against granzyme A, and two different secondary antibodies. Negative controls were cells stained against two proteins that localize in different regions of the cells, i.e. the mitochondria and the lysosome. Each set of three points at the same vertical position on the graph represents a different cell. For the positive control, the probability of non-random co-localization was always 1 and the amount of co-localization was around 85%. On the other hand, the negative controls only showed random co-localization around 15% (probability was much less than 1 for these groups).

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