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A novel image segmentation algorithm with applications on confocal microscopy analysis



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Palabras clave: segmentation; cytometry; image analysis; watershed

RESUMEN

Motivation: Developing cells change their gene expression profiles dynamically upon induction by proper triggers, typically diffusible morphogens that are spatially distributed (1). These changes impact cell cycle and apoptosis regulators differentially, eventually determining the final structure and size of the mature organs (2). A quantitative model that links gene regulation and tissue growth must be provided with precise experimental data at cell resolution level in order to proceed to its validation, which in some cases is essential for model screening (i.e. reverse ingineering methods). Image analysis from laser confocal microscopy (LCM) has already been used to address modelling problems in developmental tissues such as these (3). However current methods for LCM segmentation rely upon watershed algorithms that show variable efficiency, relatively high parametrization and oversegmentation problems that are critical on very aggregated objects (4). Here we present a different segmentation method based on the maximum complementary n-ball set (MCnB set) concept. The segmentation algorithm takes a full MCnB set as a starting graph representation of the whole stack, which is later contracted using a parallel implementation approach.

Results: We assayed the performance by segmenting a randomly generated set of spheres with different resolutions, signal aggregation levels and densities, and compared to the results delivered by a common segmentation free software, (i.e. Vaa3D), which is based on watersheds (5). We also applied this comparison on DAPI stained samples from Drosophila eyeantenna imaginal discs. The results indicate that the mean square displacement of detected spheres centroids is higher in the 3D watershed implementation results than when our method is applied. The same results are obtained when the number of sets or their size are checked instead.

Conclusions: The results indicate that our method is adequate enough for image segmentation in three dimensions. It makes no assumptions on what the shape or signal features of the objects are, and does not require any calibration since it can proceed with no specific user parameters. Moreover it beats at least one segmentation method that has already been set up for counting and segmentation. Since the shape of the voxel aggregates is not critical, we sugget that further implementations could be potentially applied in higher dimension samples with interesting applications in developmental biology (i.e. 4D 'movies' segmentation). However one major drawback is that at least one operation runs with a O(n^2) time complexity, which is time (and memory) consuming for very big images.

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