

## **Efficient 3D segmentation of crowded neuronal cell nuclei in confocal stacks**

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Even though the molecular mechanisms of gene-expression in neurons are well described in the literature, little is known about the relationship between these processes and the architecture of the neuronal cell nucleus. Accordingly, we wanted to study cell nuclei in neurons of the hippocampus, the brain region involved in both learning and epilepsy, using confocal microscopic immunocytochemistry. To do this, however, one needs to quantify properly fluorescent signals in three-dimensional stacks of confocal images. An essential prerequisite to any quantification is a segmentation of the neuronal nuclei which are typically tightly packed within the cell layer, and frequently appear to overlap, due to limitations in microscope resolution. Therefore, we established an algorithm based on continuous boundary tracing criterion aiming to reconstruct nucleus surface and to separate adjacent nuclei. The rough position of nuclei is determined by the harmonic analysis and serves as an input to trace the boundary. Whilst subsequent slices are being analyzed a certain set of conditions is checked in order to determine whether the recognized outline is acceptable, needs an iterative retracing or becomes discarded. The algorithm does not use a rigid threshold what makes it robust against variations in image intensity and poor contrast. In the aftermath the reconstructed surface is used to identify objects in the interior of the nucleus and to study their morphology and spatial arrangement. Our program is an efficient segmentation tool for crowded and overlapping objects in 3D space. It allows us to study quantitatively the architecture of the neuronal nucleus using confocal-microscopic approach.