AUTOMATED RECOGNITION AND QUANTIFICATION OF CONTACTS BETWEEN NEURONS IN CONFOCAL LASER SCANNING IMAGES

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Presynaptic axon terminals involved in synaptic connectivity in the central nervous system usually possess dimensions in the 0.5µm-1.0µm range. By contrast, post-synaptic structures range between small dendritic spines or spinules and large or very large structures like the stems of dendrites and perikarya. At the sites of the synapses the pre- and postsynaptic structures are in close contact. As the diameter of the contact zone is a fraction of that of the presynaptic terminal, the anatomical visualization of such a contact requires a resolution higher than that offered by routine microscopy. Our approach to visualize neuronal connectivity is to label pre-and postsynaptic structures with different fluorochromes, e.g. Alexa Fluor 488 and Alexa Fluor 594. Next we scan at high magnification with multichannel confocal laser scanning microscopy volumes of tissue containing presumed connectivity. The follow-up consists of deconvolution of the images followed by computer assisted 3D object- and contact recognition. All 3D object recognition procedures require setting a threshold for isodensity mapping for each channel. We compared a subjective, operator-mediated manual threshold setting procedure with an 'objective' processing method of the same datasets through software (plugins and scripts in ImageJ and SCIL_Image). Furthermore, Abbe-type refraction causes the edges of biological objects to appear always blurry, even after deconvolution. When we 3D reconstruct tightly apposed small biological objects each tagged with a different fluorochrome, a certain degree of overlap of voxels between channels is inherent. We call this overlap of contacts of two 3D rendered structures their 'footprint', and we calculated with our automated method the numbers of contacts between fibers and post-contact neurons at different footprints. We found a high correlation between the manual procedure and the automated method that uses 'objective' thresholding and 'footprinting'. Automated 3D object recognition is much faster than manual processing and allows the investigation of many more samples within a given time frame. A very important advantage is that 'objective' thresholding reduces human bias and thus improves standardization and reproducibility of observations by independent researchers.