## Learning based interactive cell-counting of fluorescent labeled mouse tooth pulp nociceptors

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Recently, we established reproducible Dil labeling of dental primary afferent neurons (DPANs) in the mouse, which is important for investigating the molecular and functional specialization of DPANs within the trigeminal nociceptive system. To quantify the number of labeled neurons we developed a new ImageJ/Fiji plugin for interactively and semi-automatically detect and count cells in 3D fluorescent images. The datasets to analyze were imaged on a 2-photon microscope as multiple tiles which were subsequently stitched in Fiji. Possible cell candidates were identified as local maxima after an appropriate Difference-of-Gaussian filter. To distinguish true cells from false detections, we implemented an interactive learning environment. The user repetitively marks cell candidates either as correct or false. Each correction triggers the fitting of a Random-Forest-Classifier, based on local features of the user-provided ground truth. The classifier calculates predictions of so far unmarked cell candidates, and the result is displayed to the user. The process of interactive correction, learning and classification is iterated until the predicted outcome matches the user's expectation. Our plugin significantly accelerates cell counting in difficult settings where simple automatic methods such as thresholding fail. It is dedicated, but not limited to counting Dil labeled mouse DPANs in 2-photon images, although preprocessing will in general need to be adapted to the images at hand.



**Figure 1:** 3D-reconstruction of multiple tiles of stacks of a DiL labelled primary afferent neuron network in the mouse, imaged on a 2-photon microscope.