NEURON SEGMENTATION IN EXPANDED TETBOW-LABELLED MOUSE BRAIN SAMPLES

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In Expansion Microscopy (ExM) a sample with fluorophores linked to a swellable gel can be expanded homogeneously and isotropically by a factor of 4 to 20 [1, 2]. This enables to resolve neuronal network details at an effective lateral and axial resolution of 90 nm and 400 nm, respectively. Since expanding specimen yields especially voluminous samples, their microscopic analysis without compromises requires an instrument allowing to image rapidly at high resolution, with a long working distance and low phototoxicity. For this purpose we constructed a Light-Sheet Fluorescence Microscope (LSFM), which is designed specifically for imaging expanded mouse brain sections. Compared to a super resolution point-scanning confocal microscope the data acquisition time is reduced by a factor of at least 20, the optical resolution is higher and the working distance greater [3].

The investigation of neuronal networks in the mouse hippocampus requires a labeling method that enables segmentation of individual neurons in a dense cellular environment. We here apply a stochastic multicolor labeling method that uses a tetracycline-operator system (Tetbow) [4] in combination with ExM. Thereby individual neurons can be segmented according to their respective color hue. For the precise analysis of neuronal circuits, it is necessary to segment individual neurons throughout the full image volume. Therefore, we developed a software routine in Python, which allows for an automated masking of single neurons depending on their color hue and position. The program first converts the RGB images of an image stack into HSV color space. For each image a hue-histogram is created and the hue values are clustered with a Gaussian Mixture Model. The clusters define the bounds of hue masks, which are then applied to the images, resulting in a set of binary images containing individual neurons.

We present experimental results from expanded mouse brain tissue labelled with the Tetbow system and automatically segmented neurons within hippocampal subfields.

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