

Two-photon laser scanning microscopy is a modern method of *in vivo* neurophysiological research, capable of imaging up to hundreds of neurons at once. However, this method produces a large amount of data, difficult to process and analyze manually. This thesis presents Two-Photon Processor, a new toolkit for complex processing of data from two-photon microscope. During the work on this thesis, we designed the SeNeCA segmentation algorithm for detection of neurons in full-frame recording from a two-photon microscope. SeNeCA combines high speed and high quality of segmentation and, according to our evaluation, it currently is the best algorithm for segmentation of neurons in *in vivo* data. Two-Photon Processor is already routinely used in the Institute of Experimental Medicine of the ASCR, Department of Auditory Neuroscience, and it was published in the Journal of Neurophysiology.