Processing data from Two-Photon microscope

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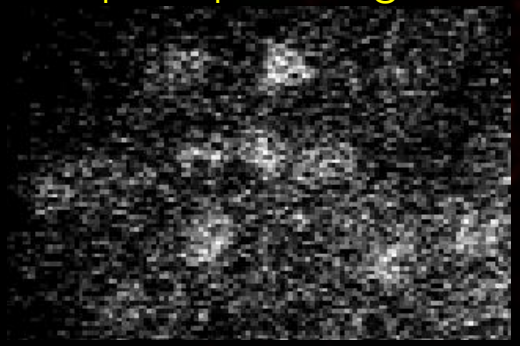
Two-Photon microscopy

- A very recent method of observing the activity of brain *in vivo* (in living animals).
- Is capable of high-quality imaging up to hundreds of neurons at once.
- Typically used to produce video where neurons become brighter when active.
- Is not without problems:
  - Produces large quantity of data.
    →Automated analysis is necessary.
  - Brain moves during measurement; the data are noisy .
    → Current methods tuned for *in vitro* data (e.g., from brain slices) often fail.

Thesis aim

To develop a software toolkit, Two-Photon Processor, for complex processing of *in vivo* data, capable of overcoming the above mentioned problems.

Sample input image



Our work

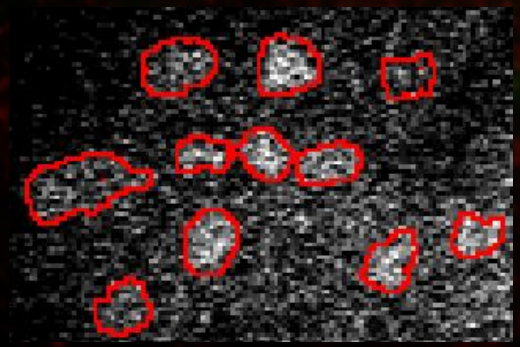
Two-Photon Processor

- Automates the process of obtaining calcium traces (brightness over time) of neurons, while tracking the movement, appearances and disappearances of neurons across tissue and correcting motion artifact caused, e.g., by breathing or heartbeat.
- Supports line-scan mode of microscope and planning of efficient scan path through the observed tissue.
- Using already published algorithms, the calcium traces may be converted into neuronal spike trains.
- Provides means of displaying 3D structure of the brain.

SeNeCA

- A crucial cell segmentation algorithm that we designed; probably the most important part of the thesis. It is used in the process of obtaining calcium traces of neurons.
- Uses a combination of dynamic thresholding and appropriately interrupted watershed.
- Unlike most segmentation algorithms, it can cope with typical problems related with *in vivo* data: high amount of noise, uneven background brightness and 3D movement of tissue over time.

Sample SeNeCA segmentation



Results

Two-Photon processor

- The only existing software toolkit containing the full pipeline which takes video as its input and produces spike trains as its output.
- Some other toolkits can generate calcium traces from video, but even there, Two-Photon Processor is much better due to more powerful segmentation (SeNeCA).

SeNeCA

- The best available algorithm for segmenting both *in vivo*, *in vitro* and *in silico* data.
- Better performance than that of a human annotator (a professional neuroscientist).
- At the same time, SeNeCA is one of the fastest segmentation algorithms available, bested only by the most primitive algorithms, e.g., simple thresholding.
- The high speed of segmentation allows the whole pipeline (reading image from HDD, image denoising, segmentation, tracking, measuring intensities, simplified spike mining) to run in real time (at least 80 FPS in images of 256x256 with circa 50 cells).

Successes

- Published in Journal of Neurophysiology (IF 3.4).
  - *Two-Photon Processor and SeNeCA-A freely available software package to process data from two-photon calcium imaging at speeds down to several ms per frame.*
- Already used in laboratories in USA, UK, Japan, France, Turkey and the Czech Republic.