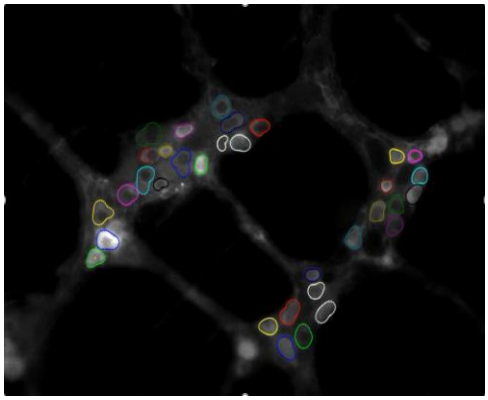
Automatic cell tracking in Ca2+ imaging recordings of the enteric nervous system using B-Spline Explicit Active Surfaces

Youcef Kazwiny, Joao Pedrosa, Werend Boesmans, Jan D'hooge, Pieter Vanden Berghe
KU Leuven
Herestraat 49, Leuven 3000, Belgium
Youcef.kazwiny@kuleuven.be

Ca^{2+} imaging is an exceptionally valuable optical microscopy technique to record neuronal activity and investigate neuronal circuitry in complex networks like the enteric nervous system (ENS), which controls intestinal function. Ca^{2+} imaging analysis, however, faces several challenges. Image quality limitations such as low SNR and out-of-focus frames plague recordings produced with this technique These limitations, which are often unavoidable because of biological reasons, greatly limit the applicability of common segmentation techniques.

Practically, manual delineation such as marking spatially static regions of interest (ROIs) is still the prominent method used to extract the average pixel-intensities and derive Ca^{2+} signals from recordings. This procedure is time-consuming and often not applicable. For instance, if cells move or tissues deform, no static ROI can contain all cell-belonging pixels in every frame of the recording. Specific segmentation techniques have been developed for Ca^{2+} imaging but require frame alignment [1]. Standard registration techniques struggle against some contraction related tissue movement and the rapid temporal intensity changes of cell-belonging pixels. Therefore, there is a need for accurate cell tracking that allows to track cells in moving frames and is insensitive to large intensity changes in consecutive recording frames. We propose a novel method based on a B-Spline Explicit Active Surfaces (BEAS) framework [2] that delineates neuronal cell bodies and tracks them during tissue movement. In this algorithm, semi-automatic cell detection is initiated in a brief static scene based on a combination of morphological operations including distinguished features of neurons expressing a genetically-encoded Ca^{2+} indicator. Every cell is then represented by an active contour whose development depends on local energy terms at every node of the contour and global energy terms such as curvature, size, and size differential between frames. The contours are coupled using additional penalties that represent cells competing for space throughout the recording (Figure).

We exploit the distinct dark appearance of nuclei specific to popular Ca^{2+} sensors by creating a coupled two-layer segmentation mode that tracks a nucleus' boundaries as well as the cytoplasmic contour and optimizes the two layers together to obtain a stable delineation throughout recordings. In addition to the tracking algorithm, we incorporate a robust technique for tracking manual ROIs throughout the most challenging recordings by extrapolating the geometric transformation representing movement of the cell contours that were tracked successfully. Compared to manual delineation and other segmentation methods the proposed algorithm can track cells during relatively large tissue deformations and high-intensity changes such as during neuronal firing events. Our analysis package represents a significant improvement to available Ca^{2+} imaging analysis workflows for challenging recordings.



[1] Murayama et al. "Detecting cells using non-negative matrix factorization on calcium imaging data " *Neural Networks* (2014)

[2] Barbosa, et al. "B-spline explicit active tracking of surfaces (BEATS): Application to real-time 3D segmentation and tracking of the left ventricle in 3D echocardiography" *IUS* (2012)