Robust Segmentation in Low SNR Fluorescence Microscopy by Homological Persistence: An Application to Shape Analysis of Neuroblasts in Development

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Biological introduction and goals

In the adult mammalian brain, neuronal precursor cells are continuously produced in the subventricular zone and evolve during their migration along the rostral migratory stream to integrate the olfactory bulb, where they further differentiate.
In the case of some neurodegenerative diseases (e.g. Parkinson's disease or multiple sclerosis), redirecting newborn neuroblasts toward lesion sites is a very promising therapeutic strategy.
During the last years, several factors involved in migration and differentiation of neuroblasts have been identified, but a lot of them remain unknown, for example the role of ProteinX investigated here.

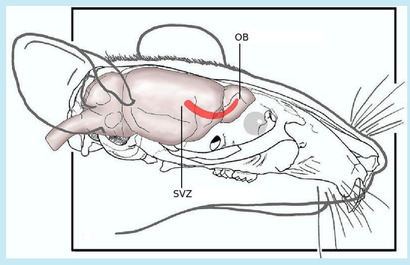

Figure 1: Head of a mouse showing the location of the rostral migratory stream (RMS) (in red), along which newly generated neuroblasts migrate from the subventricular zone (SVZ) into the olfactory bulb (OB), retouched from [1].

Retroviruses carrying a transgene for ProteinX are injected into the subventricular zone of the mouse. **At different stages along the rostral migratory stream, the changes are monitored by fluorescence microscopy: the transfected neuroblasts are identified by green fluorescent protein expression, whereas cell nuclei are stained in red.**

In order to gain further insight into the alterations in neuroblast development due to ProteinX, a **comparative shape analysis** is needed, which relies on robust **segmentation** of neuroblasts as a first step. The latter is a particular challenge due to very low SNR (signal-to-noise ratio) and the intricate elongated shapes of neuroblasts. Standard shape priors based on small surface areas or curvatures are not suitable for this application and higher-level priors cannot be built due to a missing database of typical shapes. Consequently, state-of-the art variational or probabilistic methods lead to severe segmentation errors with respect to shape and topology and it becomes attractive to investigate alternative approaches based on **persistent homology**.
Since each neuroblast should contain exactly one nucleus, a correct identification of the nuclei would enable a further test on the correctness of the neuroblast segmentation. Unfortunately, the segmentation of the low SNR red channel picture is even more difficult as the green channel one.

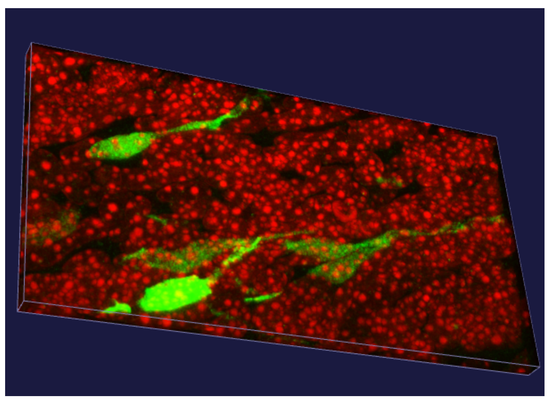

Figure 2: Neuroblast overexpressing ProteinX in the middle part of the rostral migratory stream of the mouse, sagittal section, $12\,\mu m$.

Union-find algorithm

After edge preserving preprocessing with a Bregman-Total-Variation method, we apply an **union-find algorithm**, constructing a forest where each tree is a connected component of the 3D green channel picture:

- At the beginning, all voxels having maximal green intensity are selected and the connected components they define are listed.
- The lower intensity voxels are added gradually and the list of the connected components is actualised with the possible birth of a new component or the fusion of two objects.

The green intensity value can therefore be interpreted as the time component of our filtration.

To avoid considering noise components, objects under a given size or a given life time are rejected. The limit life time is estimated from the standard deviation of the intensities.

The algorithm can be divided in **three steps** of union-find modules:

Step I: First union-find run to the zero intensity

Although trivially delivering one only final connected component, this first union-find run is very useful: birth and death of each of the components can be read on the **persistence barcode**, allowing the placing of the **stop time** after all birth events.

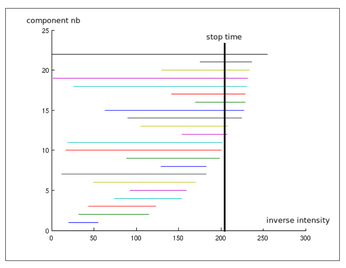

Figure 3: Persistence barcode

Step II: Second union-find run to the defined stop time

This **first segmentation** is inadequate for some of the components: locally, the adding of voxels should have stopped earlier, at a time when some other components possibly did not exist yet.

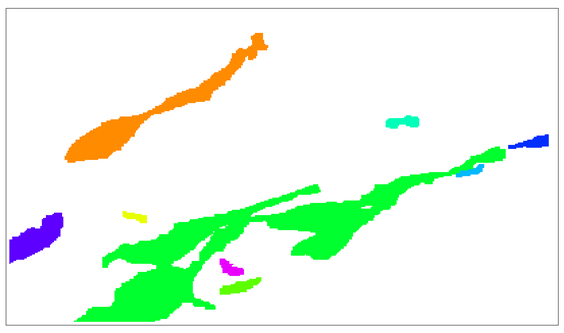

Figure 4: Connected components after Step II, in projection.

Step III: An union find for each component (local rewinds)

For each of the components obtained at the end of the second run, we examine its history by constructing a **local** forest.

In particular, a late fusion, where two objects are separated from each other by low intensity voxels, can be detected and we can **rewind** locally to a time before the fusion.

We can also get locally to the point before the fusion if the final object exhibits several nuclei. The components without nucleus should be rejected as noise.

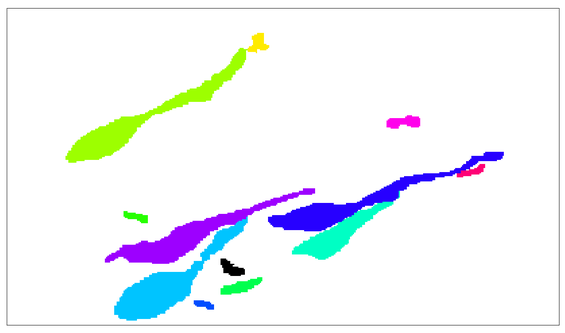

Figure 5: Connected components after Step III, in projection. The components touching the boundary have been removed.

Future Work

The work is still in progress.

Because a precise segmentation of the numerous red nuclei seems impossible, we will have to find an indirect way to decide if a component contains zero, one or several nuclei. In particular, because the components may be thickened by adding neighbouring lower intensity voxels, it can be difficult to distinguish neighbouring nuclei from included ones. This could be achieved by defining a signed distance function and levelsets on each component.

In a further step,

- the surface of every neuroblast will be smoothed (again using the levelsets) allowing a more precise estimation of its volume and its surface;
- various shape factors will be considered;
- as a further shape characterisation, the branches will be be counted;
- an orientation tensor will be drawn.

A differential analysis at the different places of the migratory stream will then be carried.

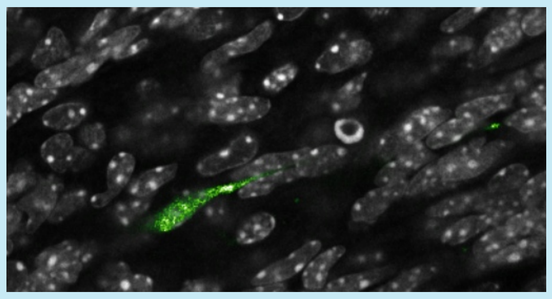

Figure 6: Grayscale nuclei and green neuroblasts (slice of the sample).

References

[1] Lennington et al. (2003). Reproductive Biology and Endocrinology

[2] H. Edelsbrunner, J.L. Harer (2010). *Computational topology: an introduction*, American Mathematical Society.

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