

Topological analysis

The topological analysis allows the contribution of metrical features and their evolution to be studied quantitatively. Segments of cerebellar folia must be selected for the analysis (Figure 2.13) and photographed each day of culture. Using the GUI, the metrical features relevant to the slice topology were directly extracted (Figure 2.14). The variables chosen in this analysis are relevant to study the migration of the cells during the days of culture in a quantitative way. This kind of analysis is important in the study of Purkinje cells from animal models of ASD as some studies show that all migration is defective in ASD.

In the GUI, after a pre-processing step in which images of slices were converted to binary images, some quantitative topological features were computed automatically:

- Mean fluorescence intensity (F): The fluorescence intensity is a measure of the ability of the Purkinje neurons to express GFP in time. It is computed as the mean pixel value of the image converted in grayscale levels.
- Distance (D): The distance between two opposite layers of cells within a segment. The maximum external distance between the two layers was considered.
- Length (L): Two measurements related to the lengths of the two layers of the segment. In order to compute this and the following measures, it was necessary to separate pixels belonging to the two layers. To do that two pixels between the layers must be selected and a line through the two points is computed.
- Average width (W_{av}): Two measurements related to the mean width of the two layers of the segment;
- Min width (W_{min}): Two measurements related to the minimum width of the two layers of the segment;
- Max width (W_{max}): Two measurements related to the maximum width of the two layers of the segment;
- Linear fit error (E): For each layer the width of the layer for each row of the image was computed. For each of the measurements of width found the mean point was then considered. In order to evaluate the degree of the alignment all the mean points were then approximated with a line and the least square error of the fit was computed.