Graph-based Quantification of Astrocytes

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Abstract. Astroglial cells in the central nervous system (CNS) are able
to change their morphology and shape after different kinds of stimuli.
We have developed a method for the structural description of astrocytes
based on their representation as undirected simple graphs. The underly-
ing image processing chain and the algorithm for the graph construction
are presented and the graph parameters for the quantitative structural
description of the astrocytes are discussed.

1 Introduction

Astroglial cells in the CNS play an important rôle in neuron-glia communication.
They are able to transform into activated astrocytes in response to various kinds
of stimuli. This process is known as astrogliosis, a complex phenomenon and is
characterized by changes in cell morphology (e.g. hypertrophy, fibre growth),
increased immunostaining of intermediate filaments and in some cases by prolif-
eration [1]. Heroin appears one of such exogenous stimuli, inducing changes in
the shape and structure of astrocytes. The main goal of this paper is the presen-
tation of a framework for the quantification of these changes. Characteristic for
the astrocytes is the asterisk-like structure and one of the important structural
parameters appear the length of the glial processes (fibres). While the direct
measurement of the distances between the fibre ends and the astrocyte centre in
the image data would be quite complicated, the description of a single astrocyte
by a simple undirected graph offers an easy access to the structural parameters
of these cells.

The paper is organized as follows: starting from laser scanning images of
astrocytes we develop an image processing chain for the pre-processing of the
images in section 2 and gave an detailed description of the graph construction
in section 3. Based on the graph representation we discuss typical structural
parameters of the graphs for the quantification of the astrocytes in section 4.

Fig. 1. Image processing chain for the graph construction.
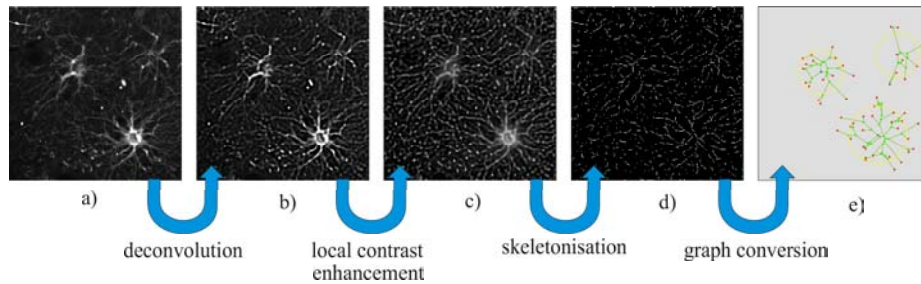

2 Image Pre-Processing

Starting from a carefully taken confocal laser scanning image (Fig. 1a) the first problem to be solved is to enhance the fibres. The main task is the homogenization of the intensity of the astrocytes. This intensity variation has several reasons. The most important reason simply is the obvious fact that the cells are three-dimensional structures and the images show only a two-dimensional projection. Their fibres do not reside only within the focus plane of the microscope, and this causes an intensity variation (Fig. 1a) that should be corrected before the graph is constructed. Therefore, the first step in the pre-processing chain is a double-blind de-convolution [2, 3] (Fig. 1b). A local contrast enhancement is applied onto this image [4] (Fig. 1c). This step increases the noise in the image, but it generates only simple spots or line-like structures that can be easily removed from the final set of graphs. The last step is the binarisation of the image (Fig. 1d) and the construction of the image skeleton [5] (Fig. 1e).

3 Graph Construction

Starting from the binary skeleton image, the skeleton is traced to find the representation as a simple undirected graph $\mathcal{G}(E,V)$ with the edges E and the nodes V. The input image may comprise several astrocytes, and for every simple connected structure a graph $\mathcal{G}_i(E_i, V_i)$ is constructed.

The graph tracing proceeds in three steps. In the first step a endpoint of a fibre is searched, i.e., a pixel that has only one other pixel in its 3×3 neighbourhood. The position of this endpoint is added to the list of nodes V_i and this position is also added to the list of untraced nodes. While only one occupied pixel in the 3×3 neighbourhood is found, the pixel is deleted and the tracing position is set to the new position. When a pixel is found that has more than one pixel in its neighbourhood, a new node is created and the new node is added to the list of untraced nodes, while the edge from the old node to the newly created node is added to the edges E_i. When the tracing has found a pixel that has no occupied neighbours we create a new node, add the corresponding edge

Fig. 2. A graph structure of normal astrocytes (left) and the graph of stimulated astrocytes under the influence of heroin (right).
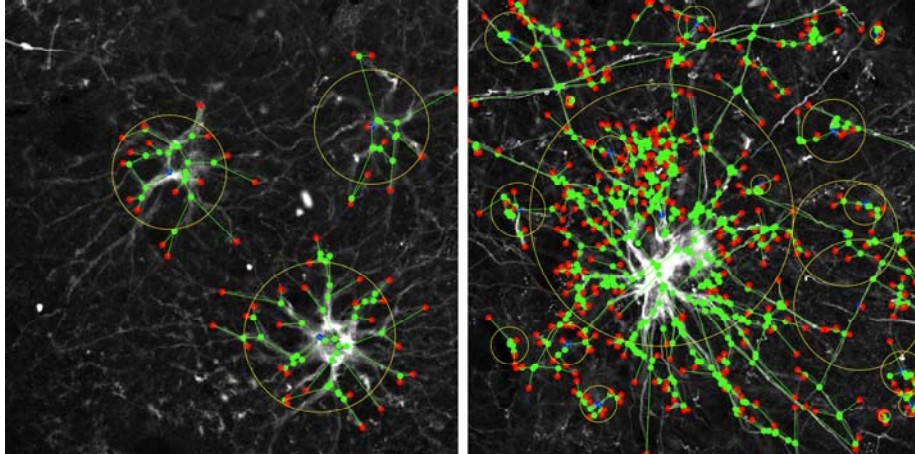
and restart the tracing with one of the untraced nodes. The construction of the graph terminates when no more untraced nodes do exist. We will restart the search for an endpoint and construct a new graph until no endpoints are found.

Since the first step will only find structures in the skeleton that have at least a single endpoint, in a second scan we search for a pixel that is part of a closed-loop like structure with at least two neighbours in its 3×3 neighbourhood. When such a point is found we create a new graph and add this point to the list of untraced nodes. The tracing proceeds as in the first step.

The last step is applied to all graphs. The tracing process described above can create loops (an edge that links a node with itself) in the graph and parallel lines (two edges one that link node i with node j and a second one that link node j with node i). We remove possible loops by replacing the edge with the loop $i \rightarrow i$ by three edges $i \rightarrow i_1$, $i_1 \rightarrow i_2$ and $i_2 \rightarrow i$ by creating two new nodes i_1 and i_2. The parallel edges $i \rightarrow j$ and $j \rightarrow i$ are removed by replacing one of the edges, say $j \rightarrow i$, with the two edges $j \rightarrow j_1$ and $j_1 \rightarrow i$ and the creation of the new node j_1. Finally we remove the direction information from the graph.

4   Evaluation of Graph Parameters

From the procedure outlined so far, we get a set of graphs. We neglect all graphs with less than 16 nodes for the future evaluation. This removes all graphs that represent noise or short pieces of fibres from other astrocytes form different depths in the volume.

The graph structure makes it easy to identify the ends of the fibres as nodes in the graph that are only connected by a single edge with the other nodes (red dots in Fig. 1e). We assume, that the centre of the astrocyte is the node closest to the mean value of all nodes that belong to the graph. One of the most

Fig. 3. Distributions of different distance measures for normal (green, front row) and heroin exposed astrocytes (red, back row).
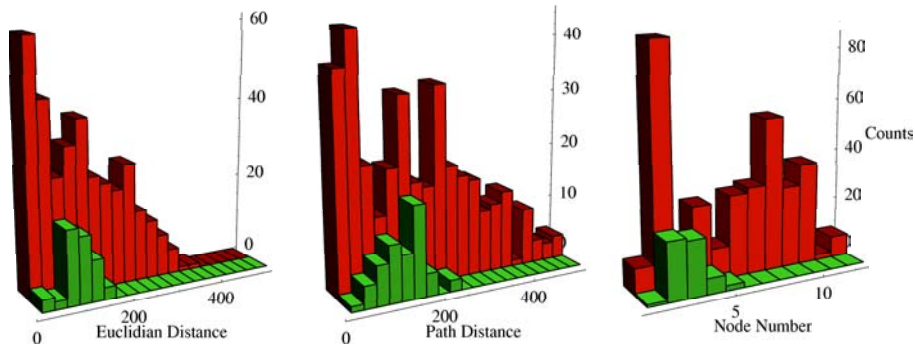
interesting parameters of the cells is the distance from the fibre ends to the centre. The computation of this distance can be done in three different ways:

1. Euclidian distance between the fibre ends and the centre
2. shortest path in the graph from the ends to the centre [6], using the length of the path in the skeleton as weight
3. shortest path in the graph from the ends to the centre using a single weight for every edge.

The last choice for the path length simply counts the number of nodes along the shortest path and is scaling-independent for images over a wide range of microscopic magnifications.

Fig. 2 shows two typical results of the conversion of the images into graphs. The red dots mark fibre ends, green dots ordinary graph nodes and the yellow circles mark the averaged radius of the respective cell. Fig. 3 shows the distribution for the three distance measures. All above mentioned distance measures exhibit a clear difference in the length distribution between normal astrocytes and heroin exposed ones. While the Euclidian distance distribution (Fig. 3, left) is only wider, the distribution along the path (Fig. 3, middle) shows a second maximum and the node count along the path (Fig. 3, right) allows for a good separation of the two maxima.

5 Conclusions

The evaluation of parameters for the graphs created from the astrocyte images is a powerful tool for the quantitative description of the respective cell structure. Other parameters of the graph, like the density of the graph, the averaged edge number per node and like the angular distribution of the branchings may be used as well for the description. The value of these parameters for the description of the pathological changes of the astrocytes under heroin exposure are currently under investigation.

References

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