Segmentation of microglia from confocal microscope images combining the Fast Marching Method with Harris Points

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***Abstract*— This study presents a new method to segment thin tree structures, such as extensions of microglia and cardiac or cerebral blood vessels. We are interested in the analysis of biological images acquired with a confocal microscope. The Fast Marching method allows the segmentation of tree structures from a single point chosen by the user when *a priori* information is available about the length of the tree [1]. However, in general, there is no way to stop the propagation automatically. In our case, no *a priori* information about the length of the tree structure to extract is available. We propose here to use characteristic points to define a criterion to stop the propagation. These points can be used also to track the tree structure in image sequences. Numerical results from synthetic and microscopic images are presented.**

I. Introduction

Recent developments in imaging such as fluorescent probes and reporters combined to two photon microscopy brought new field of investigation in neuroscience. It now allows researchers to follow *in vivo* dynamic movements of cells in 3D. Such approaches revealed that microglia, a subtype of glial cells, are particularly motile in the Central Nervous System (CNS). Beside their highly mobile processes, microglia are the major inflammatory effector cells of the brain and consequently are involved in most of CNS diseases. Understanding the logic of microglia motility might at term provide an efficient tool to detect early symptoms of diseases such as Alzheimer or multiple lateral sclerosis. So far attempts have been made to quantified those movements and were restricted to the main branches ([2], [3]). Here we developped an analysis tool that allow us to track dynamic processes from microglia and reconstruct cell dynamics. In our specific case confocal images were composed of a set of 4D (3D+time) image sequences. For each time point, a serie of 23 images perpendicular to the z axis was acquired, thereby covering the three dimensions of the cell. Our aim was first, to segment the microglia extensions in 3D and secondly, to track segmentation changes in time. The main difficulties with this data are: 1.large deformations of the microglia extensions, which correspond to the tree structure, 2.in time, small features belonging to other cells and noise may appear, 3.the data is anisotropic with high resolution in the plane of the slice and lower resolution in the perpendicular direction. Hence, a simple use of the image intensities is insufficient to extract directly the tree structure. Malladi *et al.* [4] used the Level Set methods to extract information from MRI data (which present approximately the same difficulties as the confocal microscope images). The Fast Marching method, introduced by Sethian in [5], and adapted by Cohen *et al.* [1] to extract tree structures, demands less computation time than the Level Set method and works with only one point chosen by the user on the tree. However, this method depends on *a priori* information about the target. In our case no *a priori* information about the tree structure is available. Here, we present an original method to extract tree structures without using any *a priori* information. The method is generic, it can be used to extract any type of tree structure in 2D as well as 3D.

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II. Background

A. Minimal paths

The minimal path theory for the extraction of contours from the image was inspired by the principle of Fermat: the light trajectory minimizes the optical distance between $x_0 = y(0)$ and $x = y(t)$, e.g. it gives the curve y that minimizes the distance

$$\tau(x_0, x) = \int_0^t \frac{\mathrm{d}s}{c\big(y(s)\big)} \tag{1}$$

where propagation speed c is a function depending on the medium of the propagation. In homogenous media the function c is a constant, the trajectories correspond to lines. In a medium with two regions, the function c takes two values: c_1 in the first region and c_2 in the second region . The trajectory, in this case, corresponds usually to two joint segments, each segment belonging to one region. We are interested here in the case of a medium with a continuous velocity c.

In the context of image segmentation Cohen and Kimmel proposed, in [6], a deformable model based on the optical distance (1). The model is formulated as a calculus of variation problem :

$$\text{Min} \int_0^t \big(w + P(y(s))\big)\mathrm{d}s, \tag{2}$$

the minimum is considered in $\big\{y \ : [0,t] \longrightarrow \mathbb{R}^2 : \quad y(0) = x_0, \quad y(t) = x\big\}$. The constant w imposes regularity on the curve. $P > 0$ is a potential computed from the image, it takes lower values near the edges or the features. For instance $P(y(s)) = I(y(s)), \quad P(y(s)) = g(||\nabla I||)$, where I is the image and g is a decreasing function. To compute the solution associated to the source x_0 of this problem, we consider a

Hamiltonian approach: Find the travel time U that solves the eikonal equation

$$||\nabla \mathrm{U}(x)|| \quad = \quad w + P(x) \quad x \in \Omega \tag{3}$$

The ray y is subsequently computed by back-propagation from x by solving the ODE

$$y'(s) = -\nabla \mathrm{U}(y). \tag{4}$$

The only stable schemes that solve the eikonal equation compute a viscosity solution [7]. The first work that uses the viscosity solution is from Vidale [8]. Based on this work Fatemi et al. [9] proposed the first numerical scheme to solve the eikonal equation. To solve eikonal equation through iterations [10], at least $O(mn^2)$ are needed, where n is the total number of grid points and m is the number of iterations that permit an estimation of the solution. In the next section, an algorithm with the complexity $O\big(n\log(n)\big)$ introduced in [5] is presented to solve this problem.

B. Fast Marching method

The idea behind the Fast Marching algorithm is to propagate the wave in only one direction, starting with the smaller values of the action map U and progressing to the larger values using the upwind property of the scheme. Therefore, the Fast Marching method permits only one pass on the image starting from the sources in the downwind direction. Here, the principle of the Fast Marching method is given, for details see [5], [11], [12]. The grid points are partitioned into three dynamic sets: trial points, alive points and far points. The trial points correspond to a dynamic boundary that separates far points and alive points. At each step, the trial point with the minimum value of the action map U is moved to the set of alive points, which are the grid points for which a value U has been computed. The values of alive points do not change. To reduce the computing time, the trial points are stocked in a data structure referred to as min-heap (the construction of this data structure is described in [5], [11]). The complexity to change the value of one element of the min-heap is $O\big(n\big)$. Hence, the total work for Fast Marching is $O\big(n\log(n)\big)$. The Dijkstra algorithm, which is also used to find a minimal path, has the same complexity as the Fast Marching algorithm. However, the Dijkstra algorithm gives a linear approximation and there is no uniqueness result contrary to the Fast Marching algorithm, which converges toward the unique viscosity solution.

C. Detection of points of interest

Characteristic points have been proven successful in solving many vision problems such as tracking [13] or reconstruction [14], and are more efficient in some applications than other geometric primitives such as edges or segments. In this work corner points are used to segment a tree structure. One of the most popular detector of corner points is the Harris detector, which is better or equivalent to the other detectors [15]. The Harris detector [16] is based on the Moravec detector [17], which determines the average changes of image intensity that result from shifting a local window in the image by small variations in various directions. The intensity change E produced by the shift is :

$$E_{p,q} = \sum_{i,j} w_{i,j} |I(i+p, j+q) - I(p,q)|^2 \tag{5}$$

where w specifies the image window. The Moravec detector of corners looks for local maxima of E. The Harris detector computes and compares the eigenvalues of the Taylor expansion of E. By using the Taylor formula with small shift, the variation E can be written as:

$$E_{p,q} = (p,q)M(p,q)' \tag{6}$$

where M is defined by

$$M = \begin{pmatrix} A & C \\ C & B \end{pmatrix},$$

and

$$A = \Big(\frac{\partial I}{\partial x}\Big)^2 \otimes w, \quad B = \Big(\frac{\partial I}{\partial y}\Big)^2 \otimes w, \text{ and } \quad C = \frac{\partial I}{\partial x}\frac{\partial I}{\partial y} \otimes w,$$

where $\otimes$ denotes the discrete convolution.

The eigenvalues λ_1 and λ_2 of M correspond to the principal curvatures of E. The corners are characterized by two large eigenvalues λ_1 and λ_2. Note that it is not necessary to compute λ_1 and λ_2, indeed

$$\mathrm{Tr}(M) \quad = \quad \lambda_1 \lambda_2 = A + B \tag{7}$$

$$\mathrm{Det}(M) \quad = \quad \lambda_1 + \lambda_2 = AB - C^2 \tag{8}$$

The corner measure is defined by

$$R = \mathrm{Det}(M) - k\mathrm{Tr}^2(M), \tag{9}$$

and it is positive in the corner region, negative at the edges and small in the homogenous regions, the parameter k is selected empirically between 0.04 and 0.06.

III. Extraction of tree structures by Fast Marching and characteristic points

The Fast Marching method allows us to extract the minimal path between two points. Here, the aim is to extract a tree with minimal intervention by the user. When *a priori* information about the length of the contour to be extracted is available, Cohen and al. proposed in [1] a method to extract a tree structure from one point selected by the user. In the following sections, methods are proposed for the segmentation of tree structures from only one given point without any other *a priori* information. The methods are based on corner points, which are computed by the Harris detector described in section II-C. A pyramidal approach is used to reduce erroneous detection. For the biological image described in section IV, only the Harris points that appear both in the first pyramid (original image) and in the second pyramid (half resolution of the original image) were used.

Notations:
- x_0 is a starting point located at the root of the tree structure;
- U is the action map.

Initialization:
- detect the Harris points present in the image;
- initialize the front propagation, by setting $\mathrm{U}(x_0) = 0$.

Loop: proceed according to the Fast Marching algorithm II-B, updating action maps and min-heap data.
Segmentation: Extract the paths between all the Harris points and the root.

Fig. 1. Algorithm for the propagation from the root.

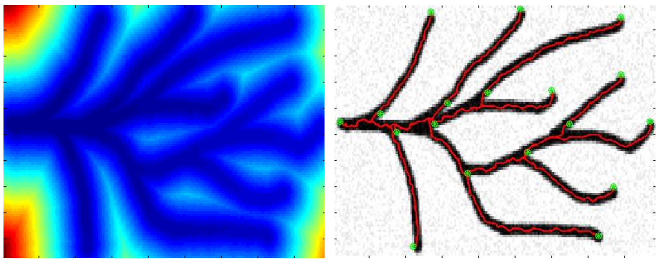

Fig. 2. Segmentation results obtained with algorithm 1. Left panel: the action map; right panel : the extracted tree, the green circles correspond to the Harris points and the red lines trace the paths from the Harris points to the root of the tree.

A. *Propagation from the root*

Within the image, a set of Harris points are detected. A method is proposed that uses the Harris points to extract a whole tree structure from an image. Among the Harris points some points correspond to the root of the tree. We defined here the root as a point selected by the user from the Harris points. Note that some Harris points correspond to the real root of the tree. From the root, a front is propagated with the Fast Marching method. For each Harris point that is not a root, a path to the root is extracted by back-propagation. The algorithm is given in the figure 1. Figure 2 shows that the algorithm is able to extract a tree structure correctly from a noisy synthetic image.

B. *Propagation from the Harris points*

To limit the propagation to the set of pixels necessary for the extraction of the tree structure, we propose a method based on the partial front propagation [18], [1]. The first idea is to propagate the fronts simultaneously and separately from the root and each Harris point. One of the Harris points is considered as the root of the propagation. The propagation is stopped when the fronts emanated from the Harris point and the root, respectively, meet (see figure 3). The segmentation of the tree structure is obtained by extracting minimal paths between the root and the meeting points and a minimal path between each Harris point and its associated meeting point. The first meeting point is referred to as a saddle point. The separate propagation allows a parallelization of the algorithm, which reduces the computing time considerably when there is a large number of Harris points.

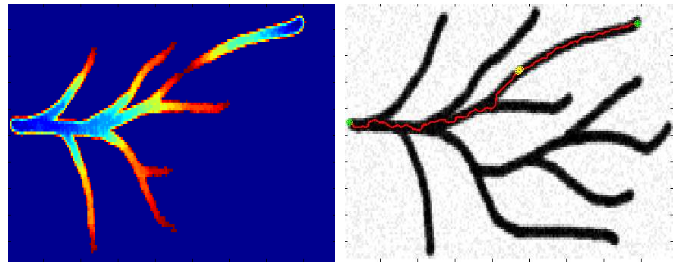

Fig. 3. Segmentation results obtained by partial propagation. Left panel: the partial action map obtained by propagation from two Harris points. Right panel: the extracted contour, the red lines trace the paths from the Harris points (green circles) to collide point (yellow circle).

The second idea is to propagate the fronts simultaneously from the Harris points in the same Fast Marching process. However, in this case it is difficult to find a stop criterion for the partial propagation that permits the correct extractraction of the tree, although it is possible to compute the action map on the whole of the image and to compute the saddle points for the extraction of the paths.

C. *Extraction of long structures*

When the structure to segment is long, the minimal path extracted by the Fast Marching method can correspond to a short cut which is not necessarily a contour present in the image. To overcome this problem, the method of keypoints is used [18], [19]. During the propagation an Euclidean action map can be computed. After the onset of the propagation, the first point that permits to travel a given Euclidean distance is considered as a keypoint, the geodesic and Euclidean action maps of the keypoint is seted at zero and the distribution continues with the Euclidean distance. However, for this approach, *a priori* information about the length of the tree structure that one wants to extract is necessary. To overcome this problem, the Harris points are used, as described in the previous section, to extract the tree without using any *a priori* information. The method computes automatically the sufficient number of keypoints to cover the tree. The keypoints are separated by a given distance, which depends on the application. The method consists of computing the geodesic distance between the last keypoint detected and each Harris point. The starting point of the propagation is the root of the tree, which is chosen by the user from the Harris points or another point. Progressively, the keypoints are detected based on the parameter of Euclidean distance λ and the points are removed from the set of Harris points when the geodesic distance between the last keypoint and the Harris points is larger than the fixed parameter μ. The process of propagation is reiterated until the set of Harris points is empty. Figure 4 shows this algorithm. The algorithm permitted a correct segmentation of a tree structure from a noisy synthetic image, see figure 5.

D. *Summary of the methods proposed for the segmentation of tree structures*

In sections III-A, III-B and III-C three different methods were proposed for the segmentation of tree structures. All

Notations:

- x_0 is a starting point located at the root of the tree structure;
- U is the action map and L is the Euclidean; distance map;
- λ and μ are counters.

Initialization:

- detect the Harris points present in the image;
- initialize the front propagation, by setting $\mathrm{U}(x_0) = 0$, and $\mathrm{L}(x_0) = 0$;
- λ and μ parameters are fixed by the user.

Loop: While the set of the Harris points is non-empty

- while the set of the trial points is non-empty, do
  - find x_{min}, the trial point with the smallest U value;
  - if $\mathrm{L}(x_{min}) \geq \lambda$, then
    - x_{min} is defined as the new keypoint;
    - set $\mathrm{U}(x_{min}) = 0,\ \mathrm{L}(x_{min}) = 0$.
  - else, do
    - tag x_{min} as alive and proceed according to the Fast Marching algorithm II-B, by examining its neighbors, updating action maps and min-heap data.
- if the geodesic distance between the last keypoint and the Harris points is larger than μ, move those points from the set of Harris points.

Segmentation: Extract the shortest paths from the saddle points to the keypoints and Harris points.

Fig. 4. Algorithm fot the segmentation of long structures, see section III-C.

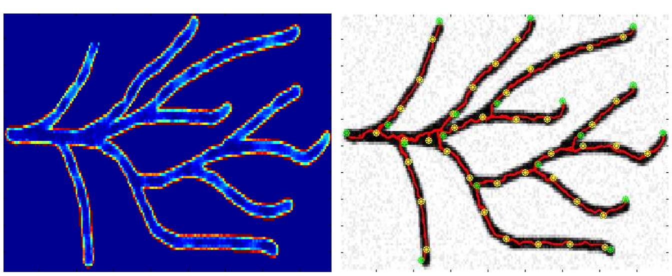

Fig. 5. Segmentation results obtained by using the algorithm 4. Left panel: action map; right panel: the extrated tree structure, the green circles are the Harris points, the yellow circles are the keypoints and the red lines trace the paths from the Harris points to the root.

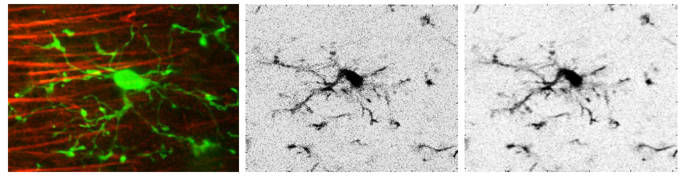

Fig. 6. confocal microscope images of a microglia. Left panel: a projection of 23 slices. The green structures correspond to the microglia and neuronal extensions are red. Center panel: a single image from a series of images perpendicular to the z axis, showing the microglia. Right panel: a second pyramid of the image shown in the center panel (half of the resolution of the original image). This image was used to compute the Harris points.

three methods use corner points to guide the segmentation process. In the first method described in section III-A, the action map which is used to extract the tree structure by back-propagation is computed from the wole image starting at the root. Conversley, the algorithm proposed in section III-B restricts the propagation to the set of pixels necessary for the extraction of the tree, thereby reducing the computing time. However, when the tree structure one wants to segment has long extensions, the minimal path extracted with the Fast Marching method sometimes corresponds to a short cut and not to a real contour present in the image. To covercome this problem, a method based on keypoints is proposed in section III-C.

IV. Segmentation of microglia extensions from confocal microscope images

In this study, segmentation was restricted to 2D, e.g. only one time point and only one image from the image series acquired at this time was considered. The microglia images contained much noise. Therefore, a pyramidal approach was used to detect the Harris points in the image. Only the Harris points that appeared in both the first pyramid (original image, see figure 6, center panel) and in the second pyramid (see figure, 6 right panel, half the resolution of the original image) were used. Some of the erroneous detections were eliminated in this way. Figure 7 shows the segmentation results with algorithm 1. The center of the cell, which corresponds in to the root of the propagation was chosen manually in the first image. The segmentation results are satisfying . However, some parts of the tree were not present in the studied image, but could be found in the other 22 images of the image series. Hence, some segments of the tree extracted in the 2D segmentation do not correspond to a real contour present. An extension of the proposed methods to the 3D segmentation should solve this problem.

V. Discussion and conclusion

In this work, methods for the segmentation of tree structures were proposed that do not require interventions or only the selection of one point by the user. No *a priori* information about the tree structure was used, contrary to other methods [1], where the length of the tree structure was given as *a priori*. The main contribution of this work is the use of characteristic points to guide the segmentation process. To our knowledge this the first time that corner points were used as

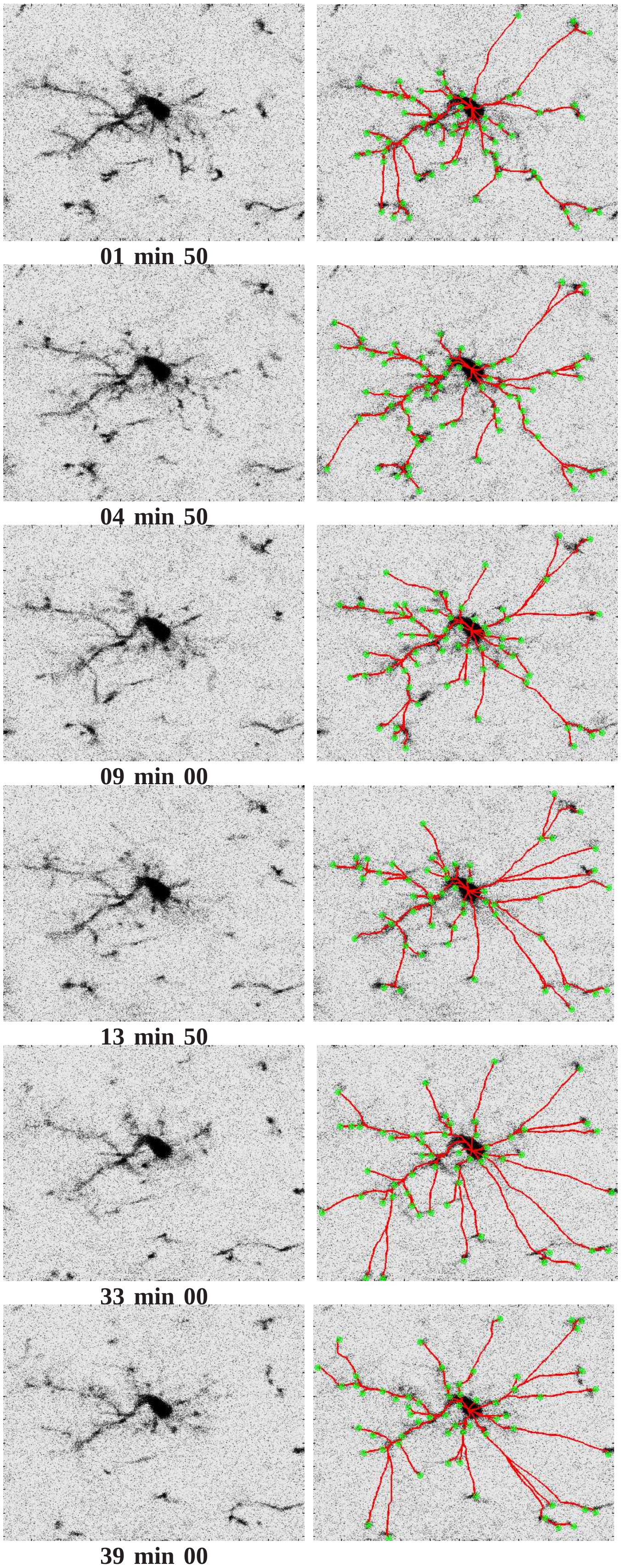

Fig. 7. Segmentation of the microglia from confocal microscope images. Left panel: the microglia images (1 image / 90 seconds), right panel: the extracted tree structure, the green circles are the Harris points, the red lines trace the paths from the Harris point to the root. The root corresponds to the cell center.

a stopping criterion of the Fast Marching propagation. The corner points were used as extremities of the propagation. The numerical results obtained with these methods were very satisfying in terms of rapidity of analysis and coherence with visual aspect of the cells. Although this method needs to be compared to manual segmentation, the present results are encouraging and should lead to a future extension of the method to 3D segmentation. In conclusion, this work shows that the computed corner points can be used to segment and to track tree structures in image sequences.

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