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Analysis of Images of Cells with Neurites

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Abstract

This study is concerned with the segmentation of cytological images and extraction of cellular entities in order to provide quantitative data about the number of cells in culture (statistical tests, morphology, model of evolution, etc). This quantitative supply is useful in biology to evaluate the consequences of the application of active substances on morphological changes and cellular viability.

It is related to the conception of a system dedicated to automatic analysis of cell images, in order to evaluate the effects of drugs on the morphology of neuronal cells. We use a cooperative region/contour segmentation, which gives closed polygonal contours. As the neurites can cross over, the obtained closed polygonal contours can contain several cells. In order to extract each cell contour, a method of entity extraction has been developed. It is based on a vectorial shape descriptor: the bisector network, which is a simplified generalized Vorono diagram.

Keywords: *Cytological images analysis, Region growing, Active contours, Skeletons, Generalized Vorono diagram.*

1. Introduction

During the past twenty years, cell cultures have been rapidly developed in pharmacology and toxicology research. When biologists want to study cell morphology, they do not have access to accurate quantitative data. In this context, the development of an automated system of image analysis seems to be very important for biological research. Several automated systems [1], [2], [3] for analysis of cell's cultures have already been developed, but none of them is able to deal with images of unstained living cells with neurites.

Typical technical problems with this kind of image are (see Figure 2):

- the low separation in average intensity between cells (slightly darker) and background (lighter) as illustrated by the histogram (see Figure1) which is unimodal. So the usual segmentation technique by automatic thresholding cannot be used.

- cells are aggregated, and the separation between cells bodies is often scarcely visible,

- cells emit neurites which can intersect with others, and it is not easy to establish to which cell parts of the detected neurites belong.

The organization of this paper is as follows. Section 2 describes the method of contrast enhancement used to improve the segmentation step. In section 3, we develop the segmentation method and present some results. Finally in section 4, the method to extract the cells from the shapes, obtained by segmentation, is presented.

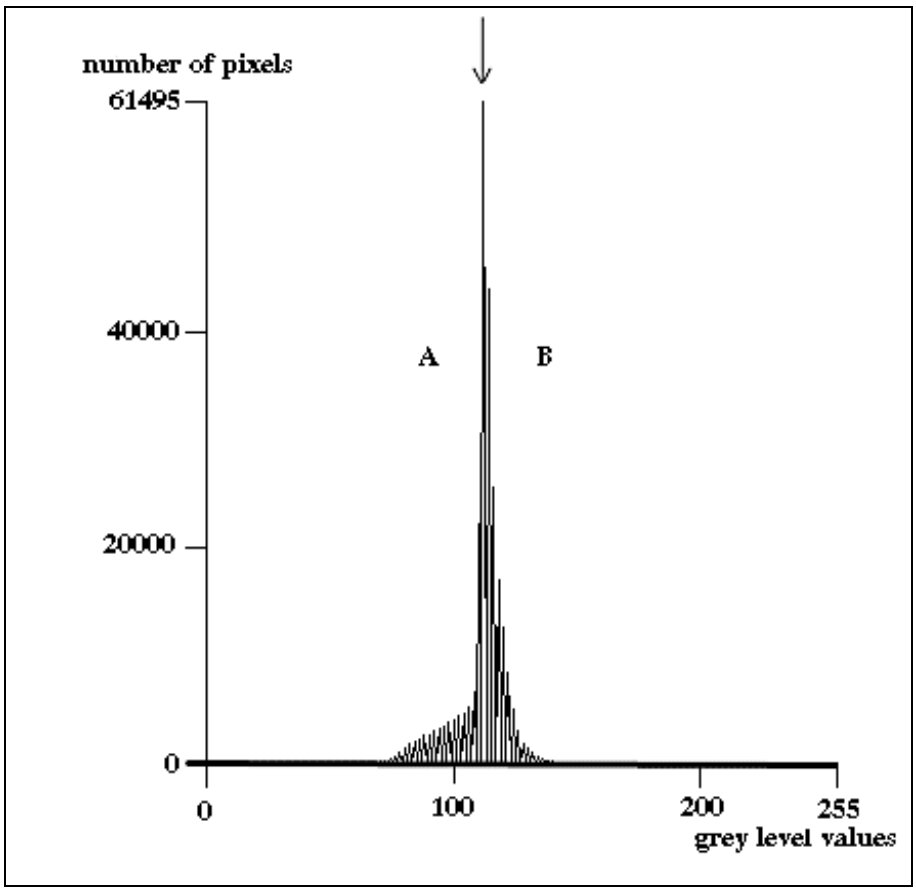

Figure 1. Histogram of the original image

2. Enhancement of the contrast

To enhance the contrast of our images, a polynomial approximation of the 4th degree of a histogram's portion (which includes the cells' pixels and small pixels in the background) (see A Figure 1) is done, and then this approximated curve is stretched on 256 gray levels. This technique provides a great enhancement of the contrast (see Figure 3), but automatic thresholding cannot be used to extract both cell bodies and neurites (whose pixels' values are very close to some pixels' values in the background).

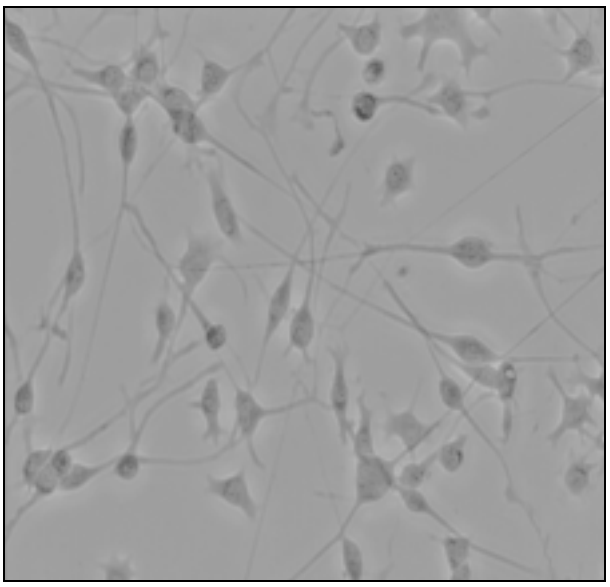

Figure 2. Original image of differentiated N1-E115

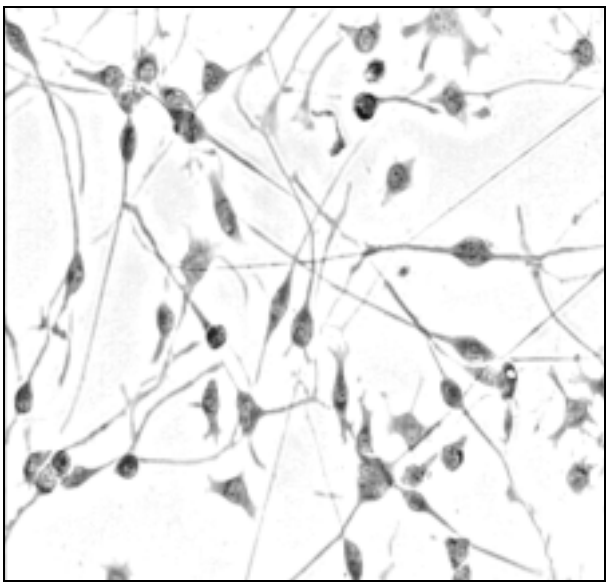

Figure 3. Enhanced image

3. Segmentation

This low level processing stage is essential in the analysis and interpretation of images. Image interpretation tasks, such as quantitative analysis, registration and labeling, require objects to be reduced to a compact, geometric representation of their shape. As the first results obtained by edge detection and edge closing [4] were not satisfactory, especially for the cell bodies, we have chosen a region growing technique, derived from the approach of active contour models, which gives a closed polygonal contour [5].

Active contour models [6] have achieved considerable success in the 80's/90's. These models simulate elastic material which can dynamically conform to object shapes in response to internal forces, external image forces, and user-provided constraints. Although these models are very interesting, they are not without limitations: they are sensitive to their initial conditions; their internal energy constraints can limit their geometric flexibility and prevent the model from representing long tube-like shapes with significant protrusions or bifurcations; most of them are parametric, so the topology of the structure of interest must be known in advance.

Djeziri's model [5] is not based on a calculation of energy minimizing curves as for the active contour models, but is based on a geometrical algorithm simulating a liquid which spills on a plane surface and stops when it meets contours of objects.

3.1. Geometrical Algorithm

We can represent the evolution of a liquid which spills by a curve expanding in the space. In the case of a "normally"(regular rigidity and elasticity in all points of the curve) structure the authors accept the hypothesis that between two close enough instants, the points belonging to the curve C^k have moved on the normal to C^k (see Figure 4).

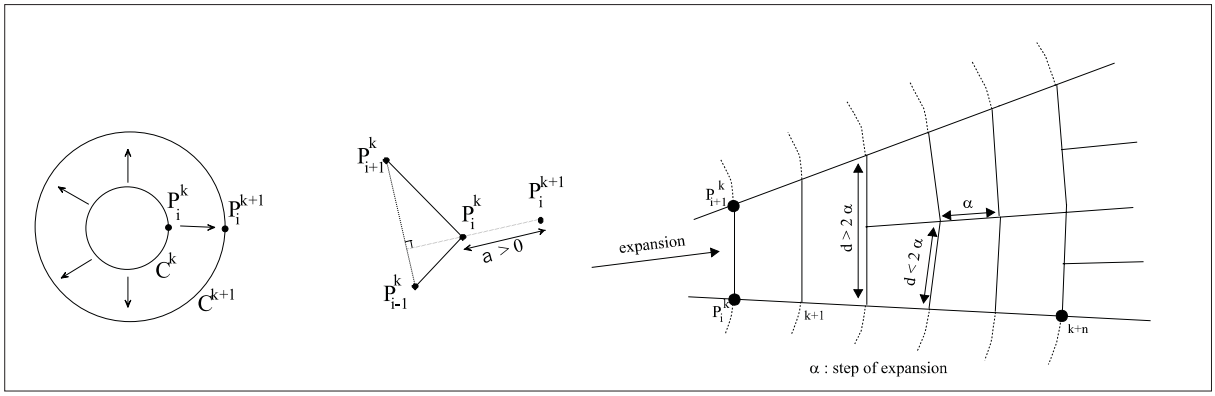

Figure 4. Expansion of a curve according to the geometrical algorithm

The process is initiated by placing a simple convex form (e.g. triangle), in each object or the background. Then the curve is subjected to repeated expansions, with the addition of points when distance between two neighboring points is more than 2α (with α: expansion step). The region growing is stopped when the criterion of expansion (based on the mean of gray level values of triangle's vertices) is no longer verified for all the curve's points.

The management of intersections between edges of the curve (see Figure 5) allows the technique to simultaneously detect objects, or significant protrusions or bifurcations.

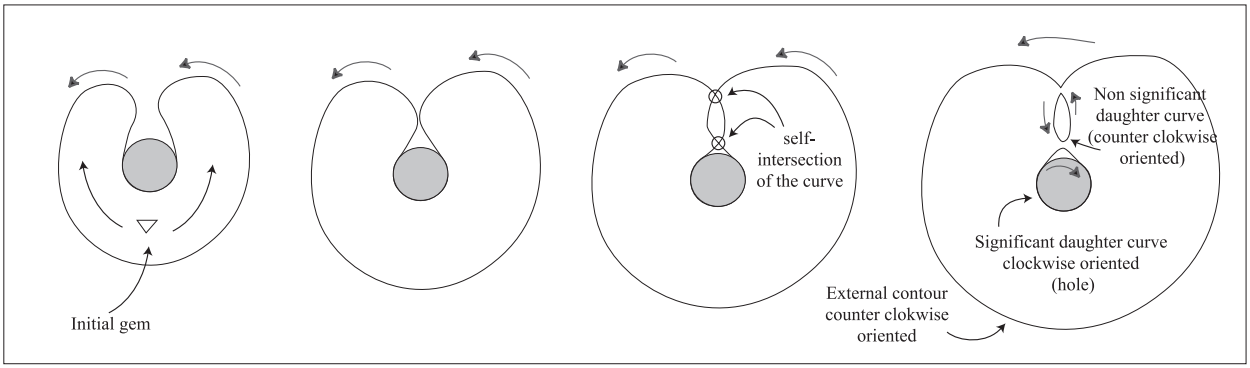

Figure 5. The management of intersections between portions of the curve induces the production of internal and external contours

3.2. Modifications

- Initialisation: The initial curve is placed automatically inside each cell. Two successive erosions of the thresholded enhanced image are done, in order to isolate small zones, called "gems", centered in the cell bodies. The coordinates of these gems will be stored with the segmented shapes.

- Transformed points calculation: The approximation of the points deplacement by a deplacement on the normal to the curve is no longer verified in the case of deformed curves (protrusions, bifurcations etc.). We can also observe intersections between two consecutive curves C^k and C^{k+1}, so that the expansion is no longer regular (see Figure 6). We have chosen to use bisectors rather than normals to eliminate these problems (see Figure 7).

- Criterion of expansion: based on the mean of gray level values of the "gem's" pixels, where is placed on the initial curve.

- Intersections management: To decrease the time spent to manage intersections, we have cut the curve in active (nonlocked points) and nonactive (locked points) chains. Furthermore, all the scanned space is marked. So we only have to search intersections between active chains whose bounding boxes overlap.

- Integration of edge information: to segment accurately all the cells' neurites, we have to integrate information issued from an edge detector (image of the gradient obtained by a Deriche operator) [4].

3.3. Results

This method has been tested on 40 images, and gives good results (see Figure 8). As for the region growing method, a cleaning of the image (all the neurites, whose cell are not in the image, are eliminated) and a good detection of cell bodies can be observed. The integration of edge information allows an accurate segmentation of the neurites, which was not possible without edge information (see Figure 9). In fact, in Figure 9, we can observe that few neurites are detected and that the curve has expanded the background.

4. Cells Extraction

The final step consists of splitting the shapes obtained by the segmentation algorithm in order to extract each cellular entity. In fact, each shape contains one external contour and eventually several internal contours, which are holes related to the external contour (see Figure10.A). Furthermore, each shape can contain several cells because of intersecting neurites (see Figure10.B). The goal of our process is to individualize all the real cells in each shape, i.e. each cell body and its potential neurites (see Figure10.C).

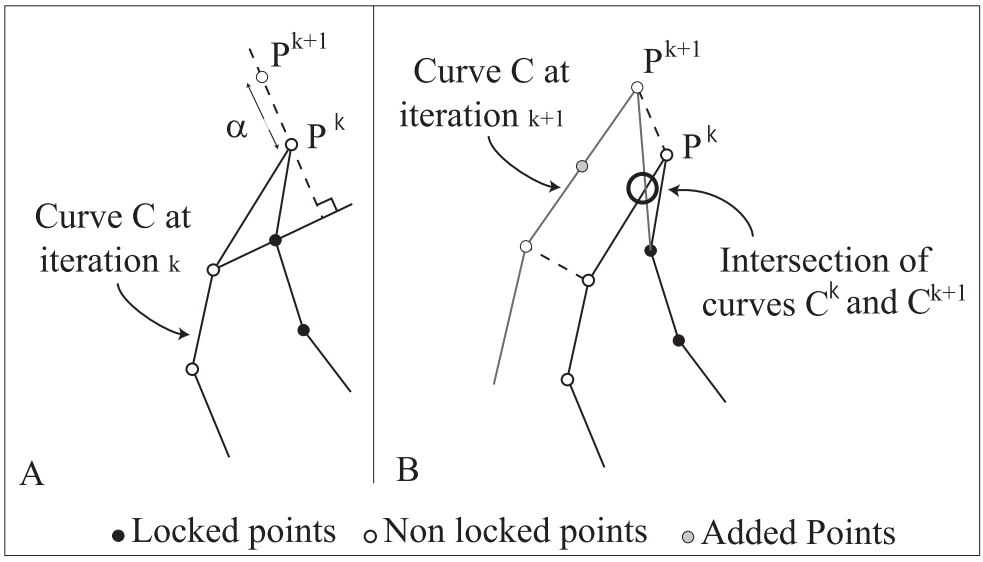

Figure 6. Use of the normal for for transformed points calculation

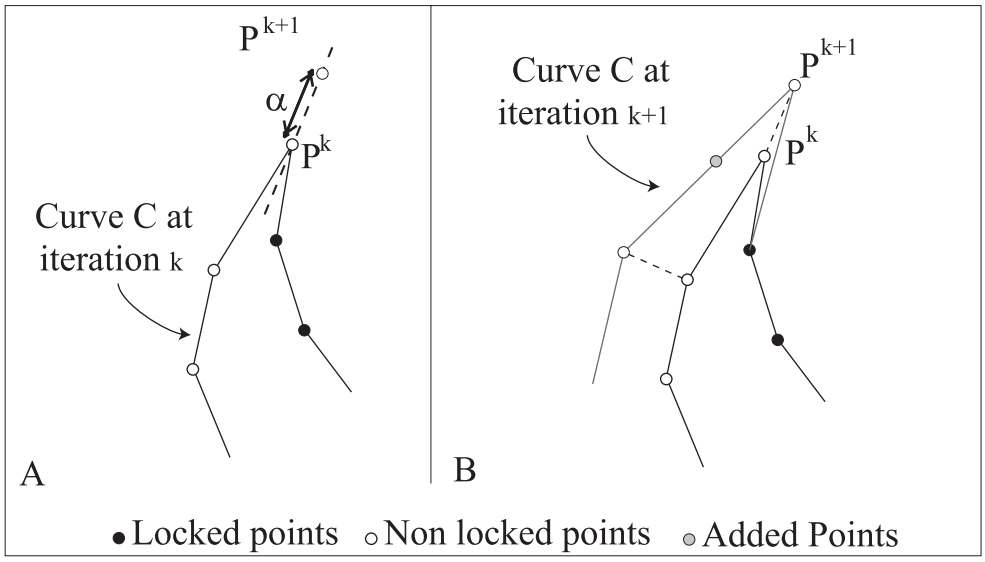

Figure 7. Use of the bisector transformed points calculation

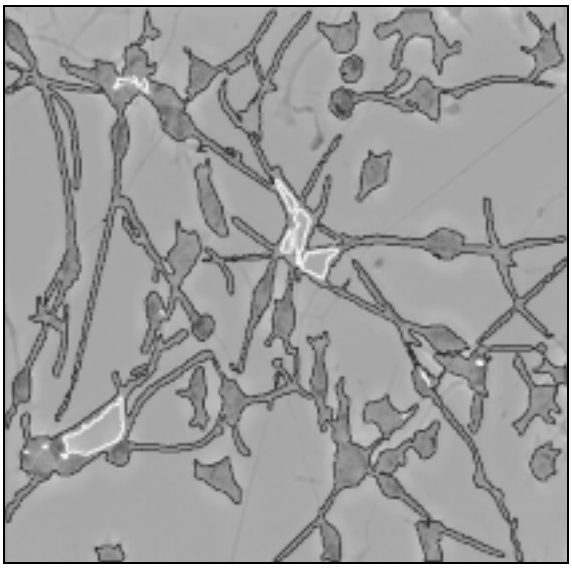

Figure 8. Result of Cooperative Segmentation (α = 1.3)

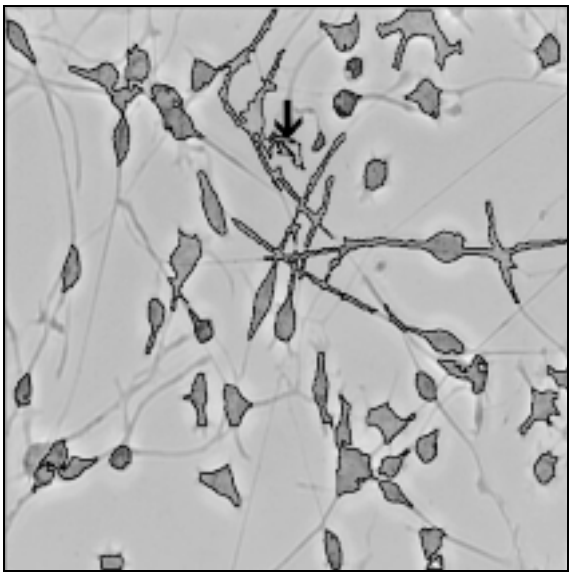

Figure 9. Result of Segmentation without edge information (α = 1.3)

Of course this can seem very ambitious, and in some critical cases it seems utopian to solve the problem in a purely automatic way. However, we have developed an algorithm which allows the automatic individualization of cells in a great number of cases. To solve the most ambiguous cases, we have planned to use selectively human interaction.

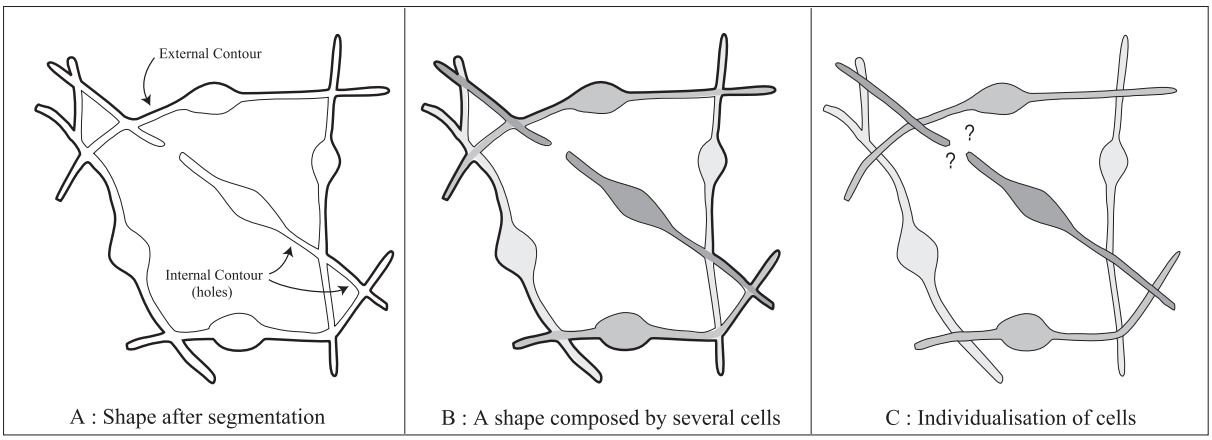

Figure 10. Individualization of each cellular entity by splitting the shape obtained by segmentation

4.1. Algorithm overview

To deal with the problem of cells individualization, the major difficulty is to find where the neurites of two (or several cells) cross over. Instead of using criteria based on shape concavities, which are not robust as the shapes we deal with are not convex, we have chosen a method which involves characteristics of a vectorial shape descriptor: the bisector network. The structure of this skeleton allows the extraction of all the branching areas and allows to find, with other meta-information (e.g. presence of a gem), the branches which have to be connected.

The general algorithm has the following structure:

For each shape S (an external contour and eventually several holes):

Build the Bisector Network of S

Extract two arrays of typical points: extremal and branching points

Match each gem of the initial image with a point of the BisectorNetwork of S

For each branching point:

Build the branching area related to the current branching point

Deal with the branching area with the help of biologic specificrules in order to perform the eventual splits and merges

Remove from the branching array the points related to the currentbranching area

Each step will be detailed in the next sections.

4.2. Skeleton building

The Bisector Network [7] is a vectorial skeleton with the same characteristics as the Generalized Voronoï Diagram [8]. Its major advantage is that it is only composed of straight line segments, which are some particular angular bisectors between the edges of the shape, though the Generalized Voronoï Diagram can be comprised of parabolic portions when the shape is not convex. The bisector network (BN) divides the interior of the shape in cells called BN-cells, each BN-cell is related with one edge of the shape.

Moreover, a bisector is shared by exactly two BN-cells, and is defined by the angular bisector between the two edges related to the two BN-cells.

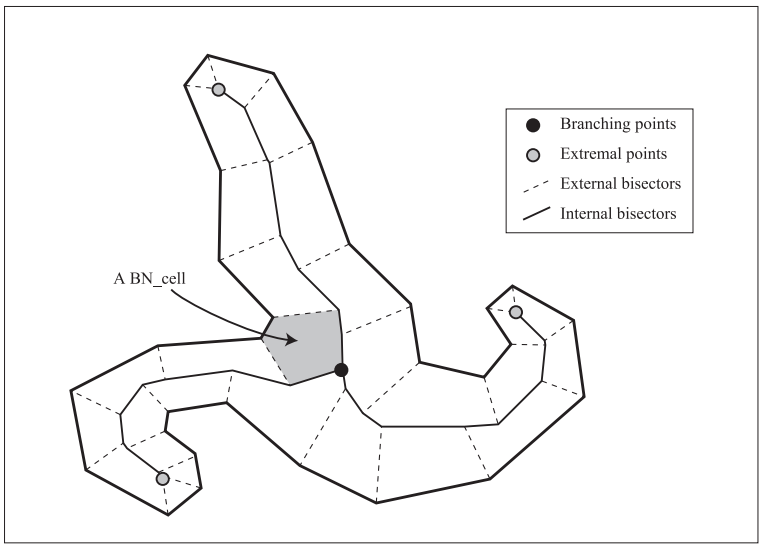

Figure 11. The bisector network and its typical points

The Bisector Network (see Figure 1.) is comprised of external bisectors which have a common point with the shape edges and internal bisectors which have no point in common with shape edges. Therefore, if we take into account only the internal bisectors, we can obtain the skeleton of the shape (dark lines on Figure 11.). Moreover the junction point between two external bisectors defines a topologic extremal point, and the junction point between three internal bisectors defines a topologic branching point. Using the properties described above we can have direct access to the edges sharing the bisectors related to the typical points (see Figure 11).

All these characteristics allow us to extract easily from the shape skeleton the information we need, to identify the typical points of the shape (extremal and branching points), as well as the shape edges, which can belong either to the external or internal (hole(s)) contours, related to these points. We use the algorithm described in [7] to build the Bisector Network. This algorithm has a complexity in O(n2), but the author shows that in practical cases it runs with a complexity in O(nlogn).

4.3. Extraction step

After the bisector network building, and the detection of all extremal and branching points, we match gems, detected during the segmentation, and nodes of the Bisector network. This can be done in O(n) by testing each gem with the various BN-cells of the shape. Once the BN-cell containing the gem has been found, we check all the nodes of the contiguous BN-cells to determine the point of the bisector network, which is the farthest point from the edges related to these BN-cells.

Now, a set of extremal points, a set of branching points and a set of gems are available. The next step consists of finding for each branching point all the neighboring BN-cells involved in the branching area. It is important to note that not all the branching areas are valid. A valid branching area is a list of BN-cells which comprises at least one branching point, and all these BN-cells can be visited by crossing shared bisectors without visiting each BN-cell more than once. If, at any time during the BN-cell scanning, we cannot find another BN-cell with at least one branching point, we stop the process and the set of BN-cells found is

classified as a nonvalid branching area. A branching area is composed of a set of connected BN-cells a set of N branching points and a set of N+2 branches.

Several kinds of branching areas can be encountered. The most obvious case is a simple fork which is composed of only three branches. Even, in this simple case, many solutions can exist. To find the best solution, in addition to initial cytological rules, we include some meta-information about the presence of gems in each branch. Therefore, we can decide if the structure:

-is not a cell (see Figure12.A),

-is a simple fork in a cell neurite (see Figure12.B),

-has to be split in two cells (see Figure12.C); in this case, we look at the angle between each branch (B_1, B_2) related to its cell body and the neurite branch (B_3), and we split the cell branch which makes the lower angle,

-has to be split in three cells (see Figure12.D).

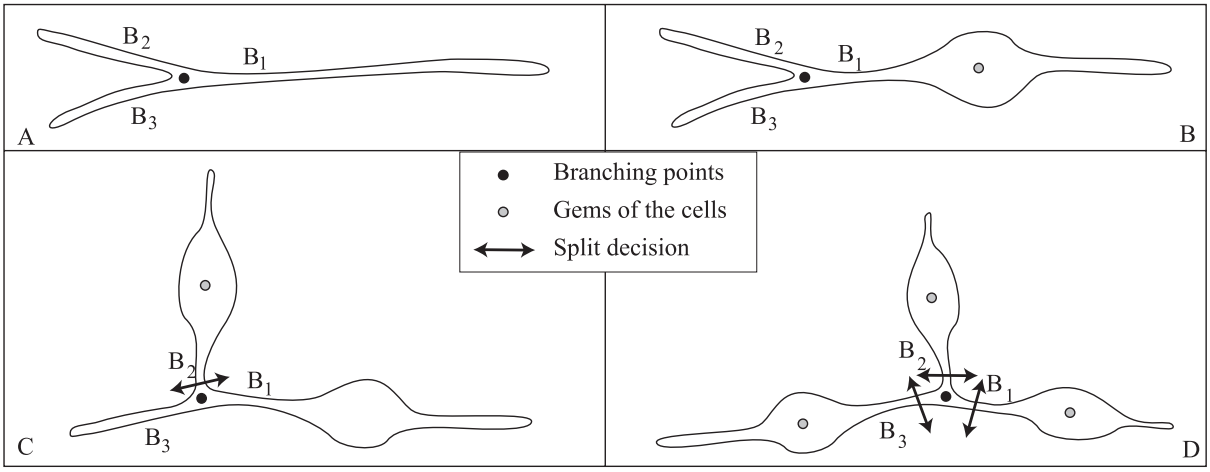

Figure 12. Different cases where the branching area is a fork

To allow the handling of the maximum number of different cases, we have implemented a common procedure for all the other cases of branching (number of branches greater than 3). The basic idea of our method is to cut the different branches according to their relative position. For example, in a branching area with four branches, numbered consecutively from 1 to 4, we connect the opposite pairs of branches, i.e. B_1 with B_3 and B_2 with B_4 (see Figure13A).

This method can easily be extended to any even number n of branches. In this case, we only have to connect the branches B_1 with B_6, B_2 with B_7, that is to say in a general way the branches $i (i <= n/2)$ and $i + n/2$ (see Figure13B). The process of an odd number of branches is developed below.

As the developed algorithm works with an even number of branches, in the case of an odd number of branches, we have to group two branches into one to reduce the problem to two branching areas: one with an even number of branches, and one fork (which will be processed later). To choose the two branches which need to be merged, we scan each consecutive pair of branches and take into account the three following properties, which are listed in order of priority:

- at least one of the two selected branches is not related to a gem,
- the selected pair of branches shares (if possible) an external branching point. By external branching point, we mean a branching point connected with only one other branching point in the branching area (see Figure 14).

- the angle between the two selected branches has to be lower than the angle between the branches of the other pairs of branches which are candidates (see Figure 14)

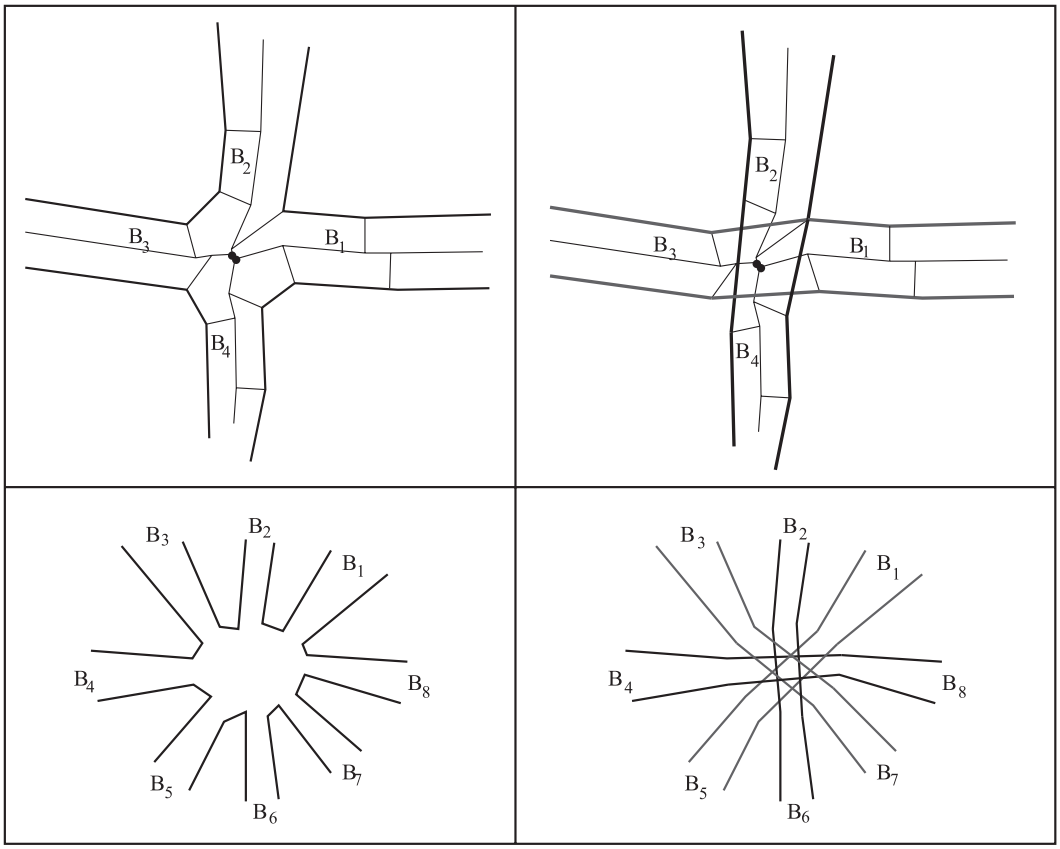

Figure 13. How to deal with branching areas with an even number of branches

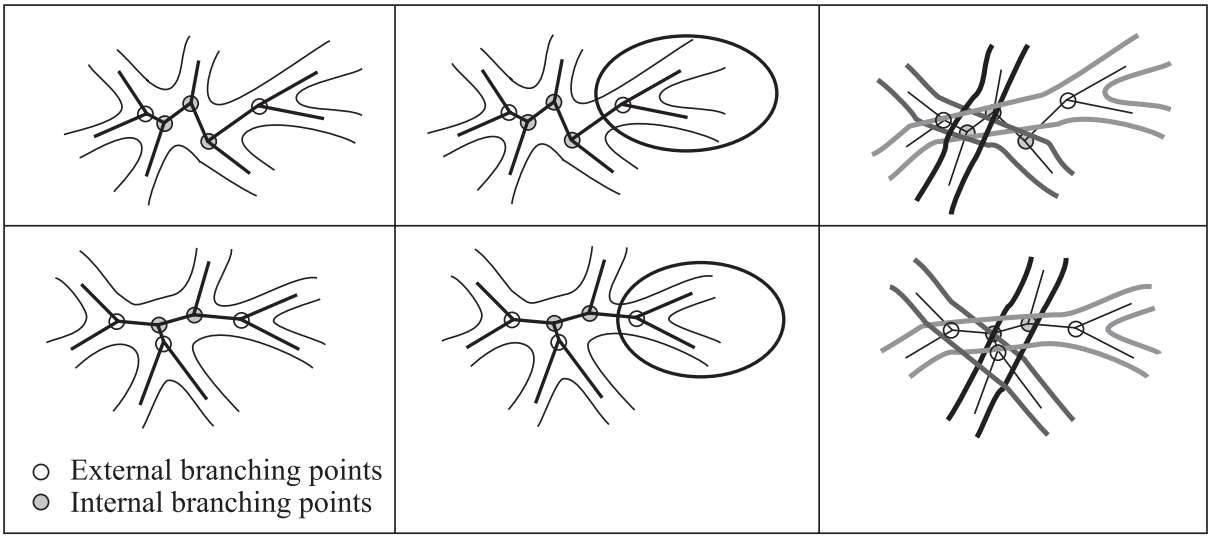

Figure 14. How to deal with branching areas with an odd number of branches

The only possibility of not finding a good pair of branches, is in cases where each branch is related to a gem. But, the solution is obvious and we cut all the branches in order to build as many cells as branches.

In all the cases, the most simple branching areas (according to the number of branching points) are processed first, and those which are the closest to gems, in order to eliminate rapidly the obvious cases. This allows us to remove the maximum indecisions for the processing of other branching areas.

The result of the algorithm applied to the primitives, obtained by segmentation of the original image, is shown in Figure 15. Figure 15.A shows the bisector network of the shape and the typical points, and Figure 15.B shows the result of the algorithm. A great number of branching areas (about 80 % of the branching areas which are not located in aggregated cell bodies) are correctly processed. But we can see that in ambiguous cases it seems necessary to use more information to obtain better results.

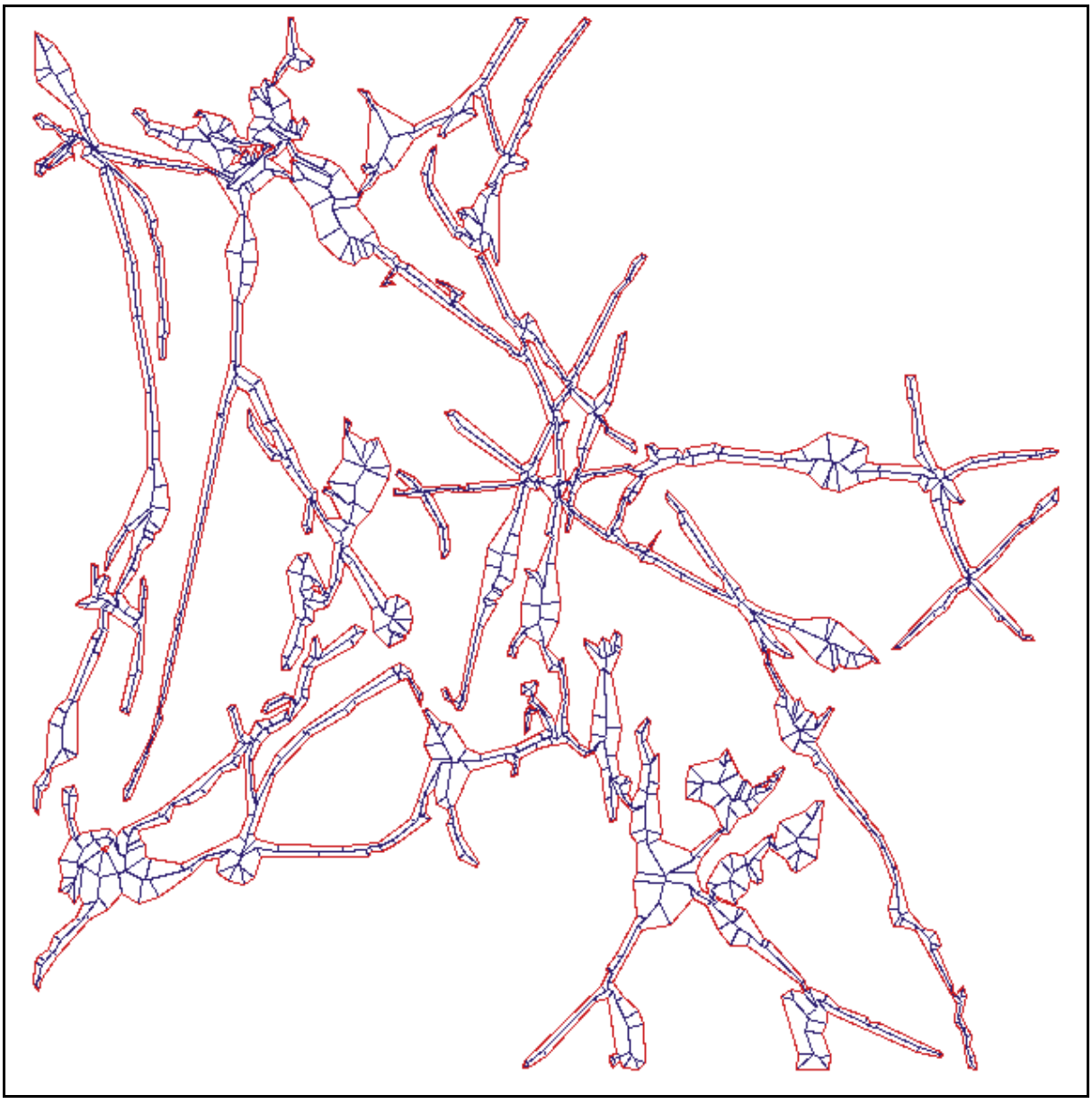

Figure 15A. Bisector network of the shape

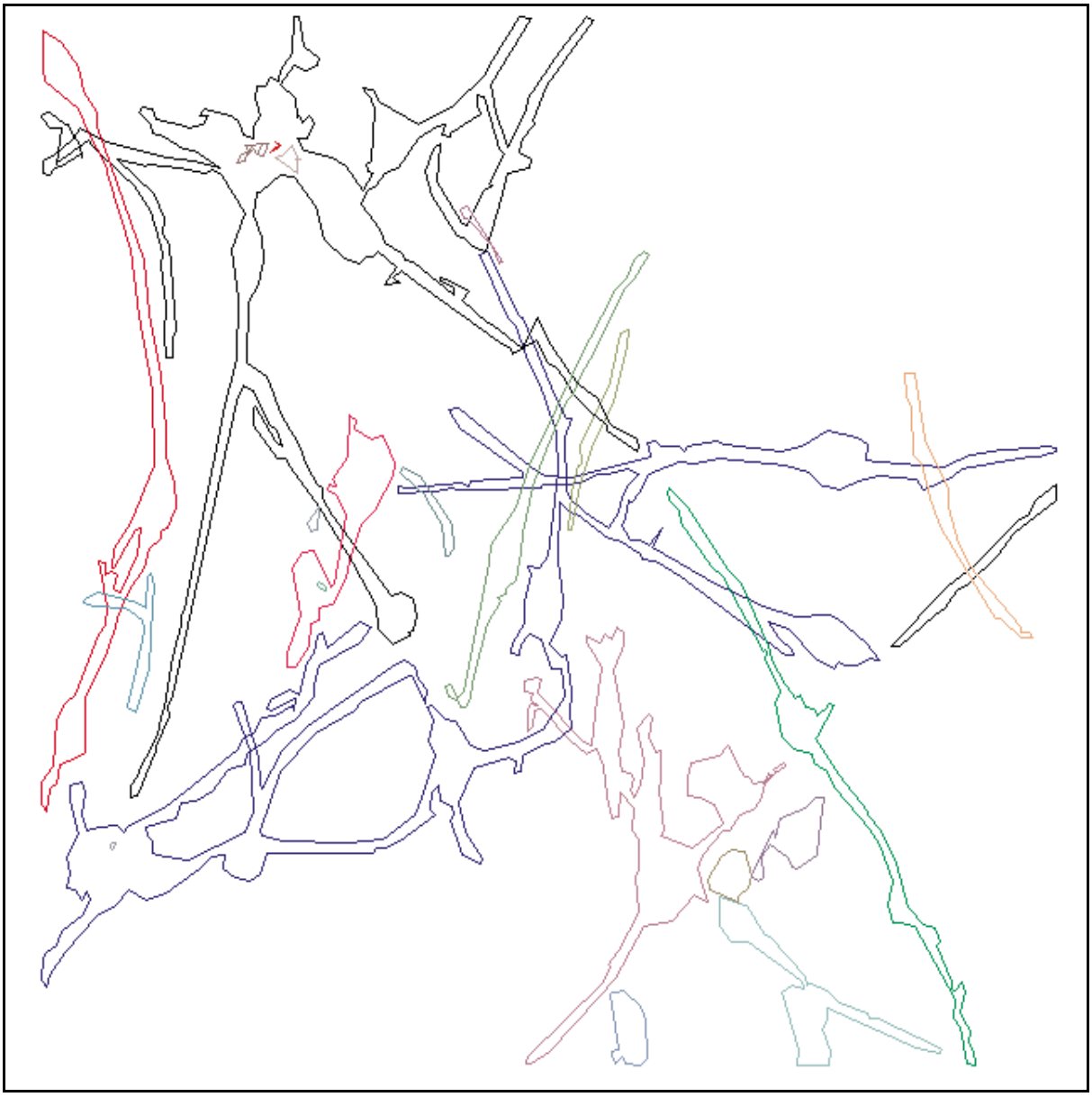

Figure 15B. Result of the cell extraction algorithm

5. Conclusion

We have presented a method to segment and extract living and unstained cells with neurites. This system is a great contribution for biological imaging as, to our knowledge, no system is able to process unstained living cells with neurites.

The method of segmentation integrates edge and region information to give the best results. Our method of cell entity extraction gives encouraging results on the processed images.

However, it seems necessary to improve the branching algorithm by taking into account other cytological rules (e.g two branches each related to a gem cannot be connected). At present, we are working on these types of improvements in order to increase the reliability of our method. Furthermore, to process a

larger type of cell image, we are developing a method to separate aggregated cells bodies in order to obtain more accurate results. On the other hand, a rapid validation by the user, with possible back-tracking, seems to be very useful in case of ambiguous branching areas. These improvements will allow us to extract each cellular entity in a very accurate way, so as to obtain quantitative data with a minimal rate of errors.

The biological validation of these results, as well as validation of results obtained with other types of cell cultures, will allow the introduction of this method to more intelligent dedicated systems in cytological image analysis.

In order to show the genericity of the entity extraction, we are looking for applications where intersections have to be processed like arterial networks in medical imaging or roads networks in cartography.

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