

Cell nuclei segmentation in noisy images using morphological watersheds

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ABSTRACT

A major problem in image processing and analysis is the segmentation of its components. Many computer vision tasks process image regions after segmentation, and the minimization of errors is then crucial for a good automatic inspection system. This paper presents an applied work on automatic segmentation of cell nuclei in digital noisy images. One of the major problems when using morphological watersheds is oversegmentation. By using an efficient homotopy image modification module, we prevent oversegmentation. This module utilizes diverse operations, such as sequential filters, distance transforms, opening by reconstruction, top hat, etc., some in parallel, some in cascade form, leading to a new set of internal and external cell nuclei markers. Very good results have been obtained and the proposed technique should facilitate better analysis of visual perception of cell nuclei for human and computer vision. All steps are presented, as well as the associated images. Implementations were done in the Khoros system using the MMach toolbox.

Keywords: Image segmentation; Watersheds; Mathematical morphology; Medical image processing; Computer vision.

1. INTRODUCTION

A major problem in image analysis and computer vision is the segmentation of the scene components. Image segmentation consists of partitioning an image into meaningful, non-intersecting, regions of interest. These regions are homogeneous with respect to one or more signal or structural property such as brightness, color, texture, context etc. Many computer vision tasks process image regions^{1,2} after segmentation for further analysis or classification. Usually, operations such as feature extraction or region measurements are performed on labeled regions. Therefore, the minimization of errors is then crucial for a good automatic visual inspection system.

Usually, segmentation techniques rely on two broad categories: contour-based methods and region-based methods³. The first approach look for the local gray level discontinuities in the image and the second seeks parts of the image which are homogeneous in some measurable property such as gray levels, contrast or texture. Following Silver⁴, contour-based methods, e.g. “snakes”⁵ or elliptical Fourier series⁶, attempt to trace the edges of regions by following a maximum gradient around the object until they reach the starting point. The edge and all adjacent pixels are included in the region. Two common ways of obtaining the edge information are calculating approximations of the first derivative, where large values are indicative of edges, or the second derivative, where zero-crossings occur at edges.

Conversely, region-based methods incorporate the notion of connectivity that pixels are likely to be part of the same distinct region if they are connected and above a threshold value. Connectivity within regions can be defined^{4,5}:

Definition 1. x_i in a region R is connected to x_j if and only if there is a sequence of $\{x_i, \dots, x_j\}$ such that x_k and x_{k+1} are connected and all the nodes are in R .

Definition 2. R is a connected region if the set of points x in R has the property that every pair of nodes is connected.

The set of regions (R_k) is known as a partition when the entire image is segmented ($\bigcup_{k=1}^m R_k$). Each region is generally a homogeneous area satisfying some criteria, $H(R_k) = \text{true}$, where H is a boolean function, and $H(R_i \cap R_j) = \emptyset$, $i \neq j$. Therefore, a region can be defined as a set of pixels in which there is a path between any pair of its pixels, and all the pixels in the path are also members of the set.

Generally image segmentation system follows the next rules⁷: 1) regions of an image segmentation should be uniform and homogeneous with respect to some characteristics; 2) interior regions should be simple and without any small holes. 3) Adjacent regions of a segmentation should have significantly different values with respect to the characteristics on which they are uniform; and 4) Boundaries of each segment should be simple, not ragged, and must be spatially accurate.

Silver⁴ describes also pixel-based methods that rest entirely upon the value of the pixel. A threshold value determines whether a pixel is part of an "object" or not. The concept of "neighborhood" is not included in this definition. However, the simplest (and earliest) approach when dealing with gray level images is choosing a threshold (or more values, e.g., n) and converting it to a bilevel (or $n+1$ -level) image. The problem is the selection of the proper threshold value: pixels below this value will be classified as, e.g., black, and those above it will be, e.g., white. The threshold values, that also can be global or local adaptive values, can be determined using statistical techniques from the pixel values found in the image, where in the simplest case we use the image histogram. However, the segmentation using simple thresholding technique usually yields poor results, especially when the image contains noise.

It is remarkable that there is not a universal method for the segmentation process. Some existing systems attempt to use a set of global constraints combined with heuristic rules to further define regions. There is a certain ambiguity in the segmentation process. We have to define distance measures and pixel connectivity. Connectivity between pixels is an important concept used in establishing boundaries of objects and regions in an image. Adjacency between pixels must be defined in terms of gray level similarities and neighborhood. A universal method should take into account context or local characteristics of the image as well as global image information. In 2D, objects can be 4-connected (pixels along the axes), or 8-connected (including diagonals), and so forth.

This paper discusses the application of mathematical morphology to segment cytological images. Some features such as form and the number of cell nuclei on a pathology image can assist pathologists in diagnosis of diseases, such as breast cancer. According to Hasegawa et al.⁸, currently, most pathologists make a diagnosis based on a rough estimation of the number of nuclei on pathology images. Because of the rough estimation, the diagnosis is not objective. An automatic system that could be able to recognize and count several kinds of nuclei would assist pathologists to make a consistent, objective and fast diagnosis.

The morphological operator (transform) watershed uses regional minima as seeds for segmenting regions. It can be seen as a hybrid technique, that combine both boundaries and region growing approaches (This transformation could be considered as a topographic region growing method). When we have a noisy image, many regional minima occur, and direct application of watersheds leads to image oversegmentation. This paper describes the steps for an application of segmentation of cell nuclei in digital noisy images using using an efficient homotopy image modification module. Diverse operators were used, such as sequential filters, distance transforms, opening by reconstruction, top hat, morphological gradients, skeleton by thinning, and others. Some operations were performed in parallel, some in cascade form, leading to a new set of internal and external cell nuclei markers. After homotopy modification, very good segmentation results were obtained by the watershed operator, and the proposed technique should facilitate better analysis and visual perception of cell images for human and computer vision. Implementations were done in the Khoros system using the MMach (Morphological MACHine) toolbox⁹.

The structure of the paper is as follows: Section 2 gives a brief introduction to mathematical morphology. Section 3 is dedicated to the image segmentation approach using watersheds. Section 4 presents information about the image processing applications environment: the Khoros system. Section 5 shows project steps and some results, and section 6 provides conclusions and final remarks. This paper does not aim to be a review on morphological techniques of image segmentation. The main purpose is to present an applied work using MMach and Khoros system. The notation used in this paper follows the same used in Barrera et al.⁹.

2. MATHEMATICAL MORPHOLOGY

Originally developed by Matheron ¹⁰ and Serra ¹¹, Mathematical Morphology (MM) is an image processing technique with its roots in the analysis of the properties of materials and material textures. Following Barrera et al. ⁹, MM is a general theory that studies the decompositions of operators between complete lattices in terms of some families of simple operators: dilations, erosions, anti-dilations and anti-erosions. It provides a set of powerful tools for texture analysis and has been used in a number of image processing applications since its development in the late 1960s. Unlike classical linear processing, MM belongs to the nonlinear branch of image and signal processing and has been used in many applications of image analysis. Examples include medical image applications, materials science, nonlinear statistics, geometrical probability, topology and various algebraic systems such as lattice and group theories. Although originally developed over binary images, morphological paradigms are being extended into various domains even beyond images such as graphs and symmetry groups. As this paper does not aim to review basic concepts of MM we suggest more general MM readings ¹⁰⁻¹³.

3. IMAGE SEGMENTATION USING WATERSHEDS

The watershed algorithm was introduced for the purpose of segmentation by Lantuéjoul and Beucher ¹⁶ and is one of the most powerful tools used for image segmentation. In MM, it is usual to consider that an image is a topographical surface. This is done by considering the gray level as an altitude. According to Najman and Schmitt ¹⁷, places of sharp changes in the intensity make a good set in which one can search for contour lines. It is then rather straightforward to estimate the variation from the gradient of the image. For the purpose of segmentation, we then look for the crest lines of the gradient image. A way to characterize these lines is to apply the watershed algorithm to the modulus of the gradient image. The idea of the watershed algorithm ^{16,17} is to associate an influence zone to each of the regional minima of an image (connected plateau from which it is impossible to reach a point of lower gray level by an always descending path). We then define the watershed as the boundaries of these influence zones. The watershed of a surface of the image has several useful properties ⁷: The watershed lines form closed and connected regions; The intersection of these regions is null; Union of these regions and the watershed lines separating them makes the whole surface; Each region contains a single regional extrema (usually minimum) as a single point or region) and each regional extrema contains a single catchment basin (watershed basin).

Numerous techniques have been proposed to compute the watershed. A good introduction to watersheds can be seen in Vincent and Soille ^{18,21,22}. They introduced a fast and flexible algorithm for computing watersheds in digital images based upon an immersion process analogy in which the flooding of the water in the picture is efficiently simulated by a queue of pixels. Other key points of Vincent and Soille's method is that their algorithm can be adapted to any kind of digital grid and also generalises to n-dimensional images and graphs. A hybrid segmentation process using both watersheds and relaxation labelling was proposed by Hansen and Higgins ¹⁹. They attempt to match the speed of watersheds with the noise resistance associated with relaxation methods. The watershed algorithm was initially used to subdivide an image into catchment basins, effectively clustering pixels together based on their spatial proximity and intensity homogeneity. According to Perry ²⁰, their work is also unusual in that interactively defined region cues are used as an aid to the segmentation. In the system the user selects part of a homogeneous object from which means and regional variances are calculated, leading to a more accurate segmentation. In this way performance is much better than if a standard homogeneity function were used.

The oversegmentation problem

Since the gradient operator has the property of enhancing gray-level transitions, the watershed line of the gradient gives theoretically a good segmentation operator. The problem of applying the watershed transformation directly on the input image is that oversegmentation may occur due to intrinsic noisy nature of gray-scale images. The oversegmentation produced by the coarse application of the watershed is due to the fact that each regional minimum gives rise to a catchment basin. However, all the catchment basins do not have the same importance ¹⁷. There are important ones, but some of them are induced by the noise, others are minor structures in the image.

Some papers describe a region-region linkage type growing process which is then employed to improve the over-segmentation by merging adjacent regions². In some cases, the oversegmented image serves as input to a module that uses graph theory ²³. A better approach is to prevent oversegmentation by using an efficient homotopy

image modification module ²⁴. A regularization process, called modification of the gradient homotopy is used to successfully solve this intrinsic problem ²⁵. The regularization process requires the selection of a set of markers which in some cases can be a non-trivial task, but simpler than the original segmentation problem. The process impose markers as the regional minima of the gradient and then suppressing all the other minima by way of a morphological reconstruction operation. Hence, in our case, for each cell nucleus within the image, one must provide an internal marker and also a background or external marker. Some systems allow manual selection of these markers. In our case, the markers were obtained automatically (see section 5). Usually, the most complex part is how to choose (or derive an method to obtain) the markers.

Summarizing, the general approach for a segmentation using watershed would be ¹⁷: 1) Find the markers, i.e., one connected component for each object and one connected component for the background; 2) Compute the image on which the watershed will be constructed (gradient image); 3) Impose the minima by efficient homotopy image modification (or gray-scale geodesic reconstruction); and 4) Compute the watershed.

4. THE KHOROS SYSTEM: IMAGE PROCESSING APPLICATIONS ENVIRONMENT

The Khoros system is an open and general environment for Image Processing and Visualization that has become very popular. A key point of Khoros system ^{26, 31} is its flexibility: it supports several standard data formats and runs on UNIX based architectures, has tools to users develop their own programs and uses a visual programming language, *cantata*. Some objectives of the Khoros Project ²⁷⁻²⁹ are to build a complete application development environment that redefines the software engineering process to include all members of the work group, from the application end-user to the infrastructure programmer, in the productive creation of software. The Khoros environment include ²⁷ a broad set of Application Programming Interfaces (API) that can be used as a source of reusable code for visual program development and a visual software development tools that can be used to quickly prototype new software and maintain developed software. It also supports four different levels of user interaction, from end user to expert programmer. Khoros is accepted as a preferred environment by a large (over 45) consortium of government agencies and industry.

Khoros is distributed via the Internet as Free Access Software. Source code and binaries are available throughout the world via File Transfer Protocol, e.g., <ftp://khoros.unm.edu> or <http://www.khoral.com>; it can be used by any organization, but it can not distributed for profit without a license. A Khoros digital image processing (DIP) course ³⁰ using Khoros can also be freely accessed via world wide web at <http://www.unicamp.br/DIP>. Diverse areas of applications have been using successfully the Khoros. Applications range areas such as medical imaging, process control, remote sensing, virtual reality, etc.

MMach: a Morphological Machine toolbox

The MMach, a Mathematical Morphology Toolbox for the Khoros system, is a fast and comprehensive mathematical morphology toolbox for the Khoros system ^{9, 26}. It complement Khoros with a set of MM operators and is able to deal with 1- and 2-D gray-scale and binary images, whereas specialized algorithms are chosen automatically according to the input data. The Toolbox is composed by four groups of programs: tools, basic (elementary) image operations and operators, first (operators that use only once each basic operator), second (operators that use more than once each basic operator), and third level image operators (that use an a priori undefined number of basic operators). For example, first level operators include opening and closing, conditional (dilation, erosion, thickening, thinning), morphologic gradient and so on. A specific problem can be solved by the right composition of primitive operators, which can also be built by combining other (simpler) operators.

The association of Khoros environment with MMach allows an excellent environment for algorithm development and prototyping of new operators. MMach is composed ²⁵ of a platform independent MMachLib which runs on Unix and Windows 32 bits and a Khoros dependent user interface KMMach. The MMach, now in its forth version, is freely distributed at <http://www.dca.fee.unicamp.br/projects/khoros/mmach/tutor/mmach.html>. An updated reference to MMach is Barrera et al. ²⁶.

5. PROJECT STEPS AND SOME RESULTS

As written in the section 3, the approach for preventing against watershed oversegmentation was: given a image, find the markers (internal and external) and use them to modify the homotopy of the gradient image. In the resulting image, we apply the watershed transform.

A practical example of these concepts is presented below. Figure 1 shows a 512 x 400 8 bits/pixels image in which we pretend to segment the nuclei (the darkest regions). If we apply the watershed directly on the input image, we obtain a oversegmented image whose regions contours are showed in the binary image presented in figure 2.

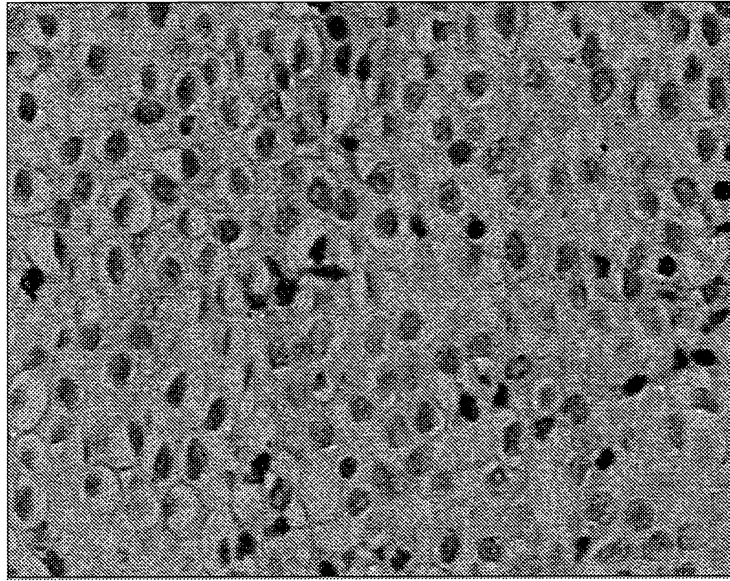


Figure 1: original cell image.

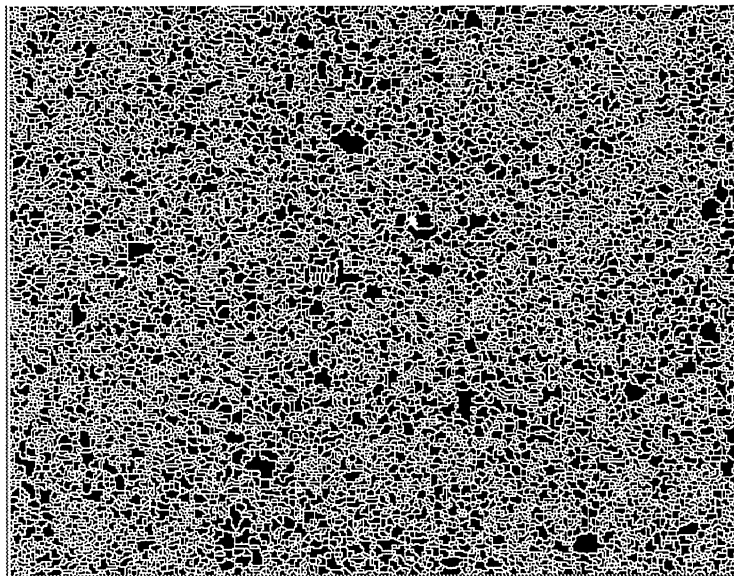


Figure 2: oversegmented image due the direct application of the watershed transform on the input image.

The following steps describe the sequence of operations that were adopted for segmenting the image using watershed and the modification of homotopy module. Implementations and results were performed using

Khoros v2.1 and MMach v1.3 running on Sun Workstations UltraSparc and Sparc 20 at the LCA – Laboratory of Computation and Automation of the UNICAMP. The major steps are presented hereafter and the workspaces and associated images can be obtained via www at <http://www.dca.fee.unicamp.br/~costa/spie97>.

Notation: let Z be the set of integers, and let E be a rectangle of Z^2 , representing a subset of the square grid, and let K be an interval $[0, k]$ of Z , with $k > 0$. A gray-scale image is any function from E to K . Then, for a $x \in E$, we can represent an image as $f(x)$.

Application of Watershed with homotopy modification steps:

1. After opening the original image, $f(x)$, we performed the negation over f , resulting in $f_1(x)$. The main reason was to change dark pixels for bright ones and vice-versa.

$$f_1(x) = (-f)(x) = k - f(x)$$

2. Using $f_1(x, y)$ as conditioning image, and an Euclidean disk of radius 2 (B), we performed the Top Hat by reconstruction using the frame of the image as marker (h). The main reason was to minimize effects from non uniform illumination over the image. This will make easier a posterior use of a threshold. Let $f_n(x, y)$ be the resulting image of the step n .

$$f_2(x) = f_1(x) - \bigcup (d_{B, f_1(x)}^n(h): n = 1, \dots)$$

where $B \subset Z^2$, $e f \in K^E$. and $\delta_{B, f_1(x, y)}^n$ is the n -conditional dilation by B given $f_1(x)$.

3. This step implements a threshold (value chosen was 5) over $f_2(x)$. Then the output of this step is a binary image $f_3(x)$.

4. To reduce isolated points (that we could see as noise) we applied sequential filters over $f_3(x)$. These were four filters of the type opening-closing ($\varphi_{nB} \cdot \gamma_{nB}(f)$), where $n = 2, 3, 4, 5$. Although isolated points were reduced, the resulting image ($f_4(x)$) still shows many cell nuclei connected.

5. Let A and B be two infinite sequences[31] of n subsets in Z^2 , respectively, with elements $A_i \in B_i$ such that $A_i \subset B_i$. This fifth step employed the *skeleton by thinning* (σ_{AB}) over the $f_4(x)$, which was obtained using the distance transform. The operator Σ_{AB} from K^E to K^E , is given by the following infinite successive compositions

$$\Sigma_{AB} = \sigma_{A, B, F} \circ \sigma_{A, B, F} \circ \sigma_{A, B, F} \circ \dots$$

The employed structural elements were

$$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 1 & 1 & 1 \end{bmatrix} \text{ and } \begin{bmatrix} 1 & 1 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

6. On the distance transformed image $f_4(x)$ the negation was applied and subsequently the watershed operator. A threshold was performed, resulting in a binary image where the white pixels correspond to the watershed lines.

7. A dilation of $f_5(x)$ with structural element with diameter three was performed. The objective was to increase line thickness. Then we performed the union between the resulting image of the dilation with the negation of the resulting image from skeleton by thinning (step 5). The negation of the result are the internal and external markers of the cell nuclei.

8. The morphological gradient was applied over the original image $f(x)$. A structural element (Euclidean disk) with radius 10 was used.

9. The modification of the homotopy of the gradient image $f_8(x)$ is then performed using a disk structural element with radius 2 and using the new minima of the image resulting from step 7.

10. Once the modification of homotopy was performed, the watershed transform can be applied. The figure shows, for purpose of visualization only, the union of the watershed lines (white) with the original image. Once again, these lines were obtained by performing a threshold on the resulting image of the watershed (labeled image).

The following pictures show some of the above steps. Note, in comparing figure 2 with figure 13, the great reduction in the number of connected regions.

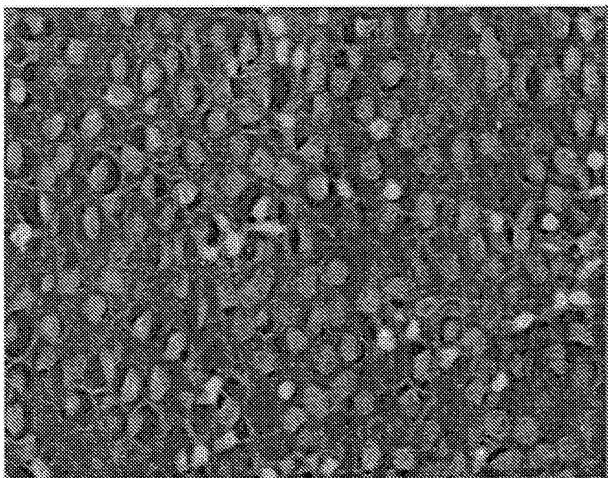


Figure 3: $f_1(x)$ – negation over $f(x)$, the original image.

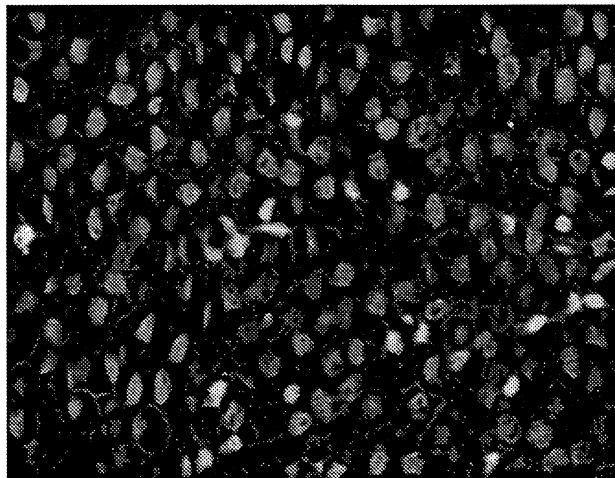


Figure 4: $f_2(x)$ – Top Hat by reconstruction over $f_1(x)$.

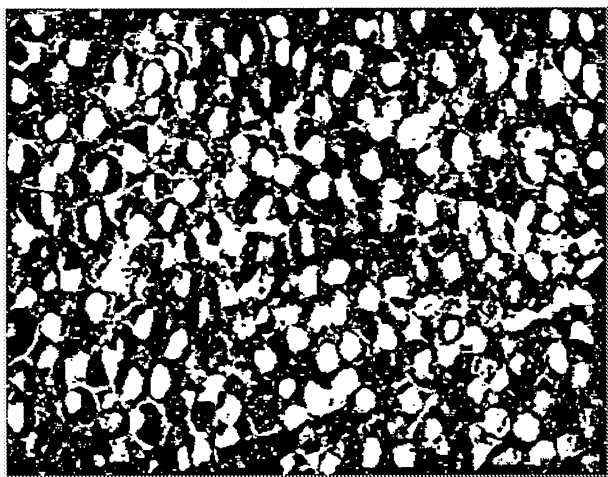


Figure 5: $f_3(x)$ – threshold over $f_2(x)$

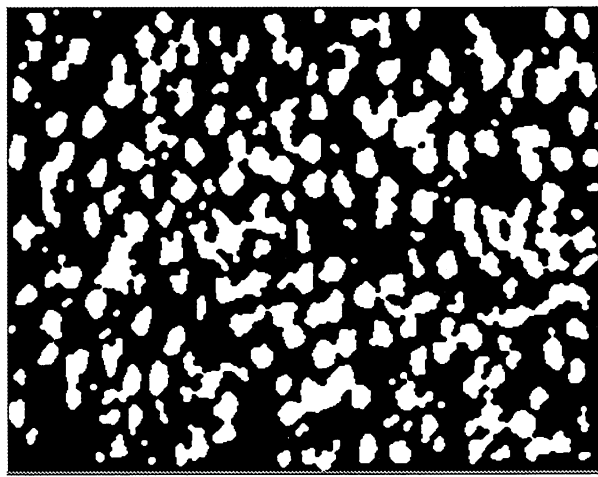


Figure 6: $f_4(x)$ - sequential filters over $f_3(x)$.

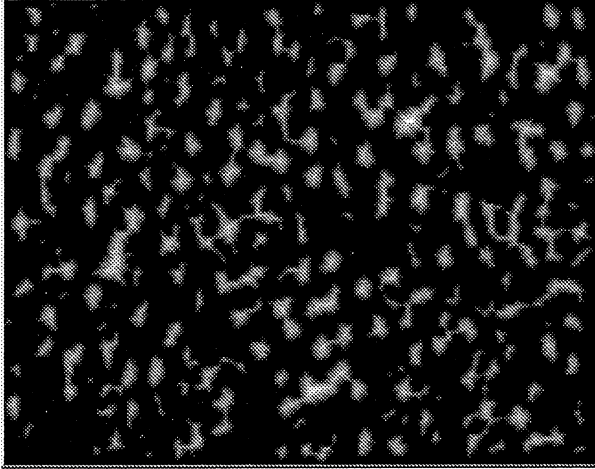


Figure 7: After application of distance transform in $f_4(x)$.

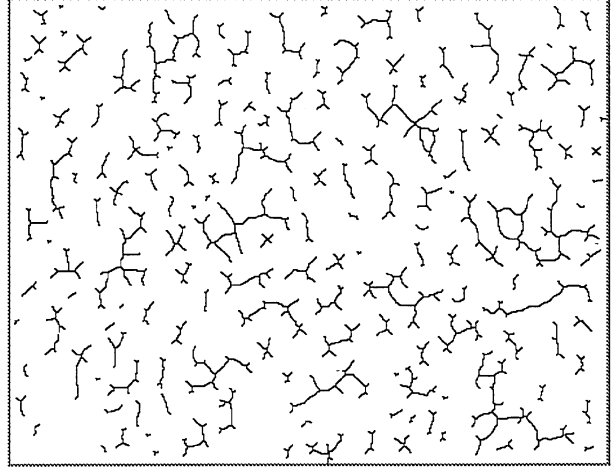


Figure 8: image after skeleton by thinning – step 5: $f_5(x)$.

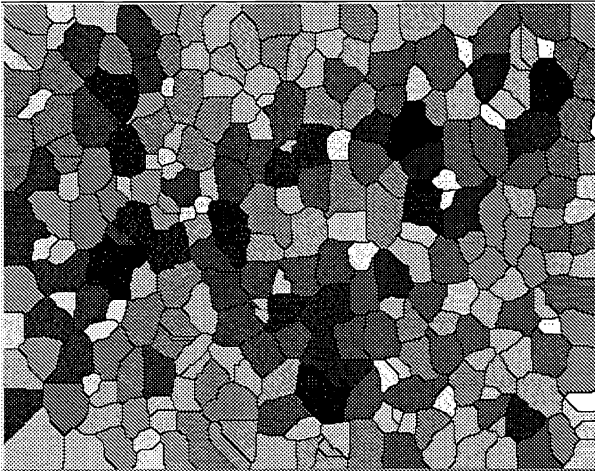


Figure 9: Watershed applied over the image $f_4(x)$.

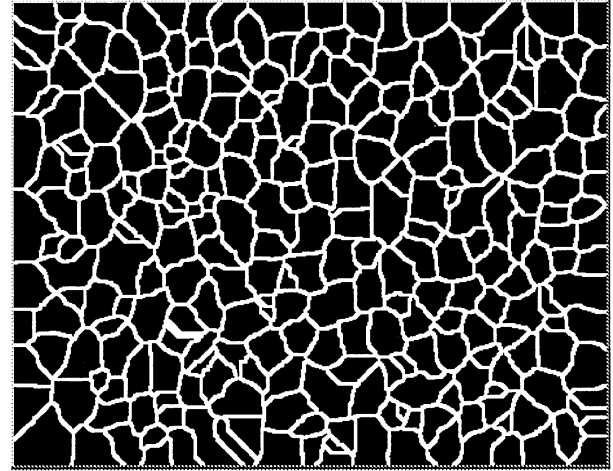


Figure 10: Dilation of $f_6(x)$ with structural element with diameter 3

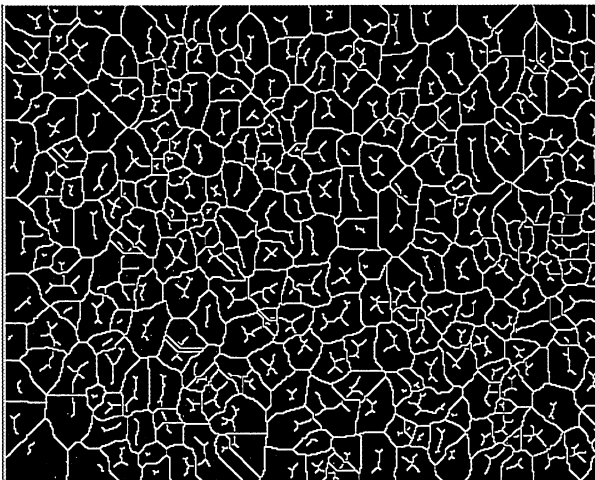


Figure 11: Internal and external markers of the cell nuclei.
Result from step 7.

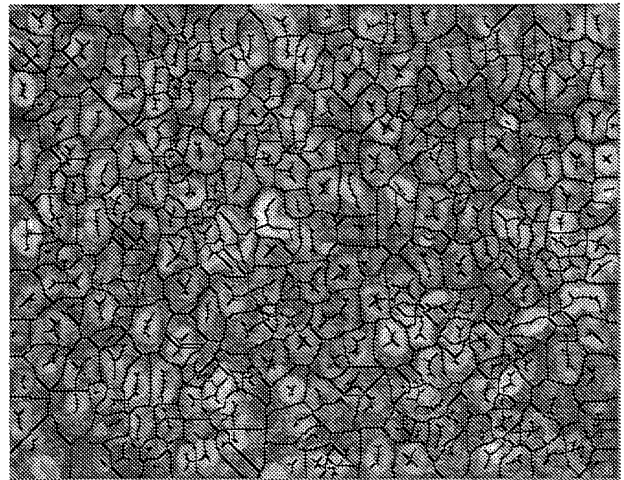


Figure 12: Result from step 9: modification of the homotopy of the gradient image $f_8(x)$ performed using a disk structural element with radius 2 and using the new minima from step 7.

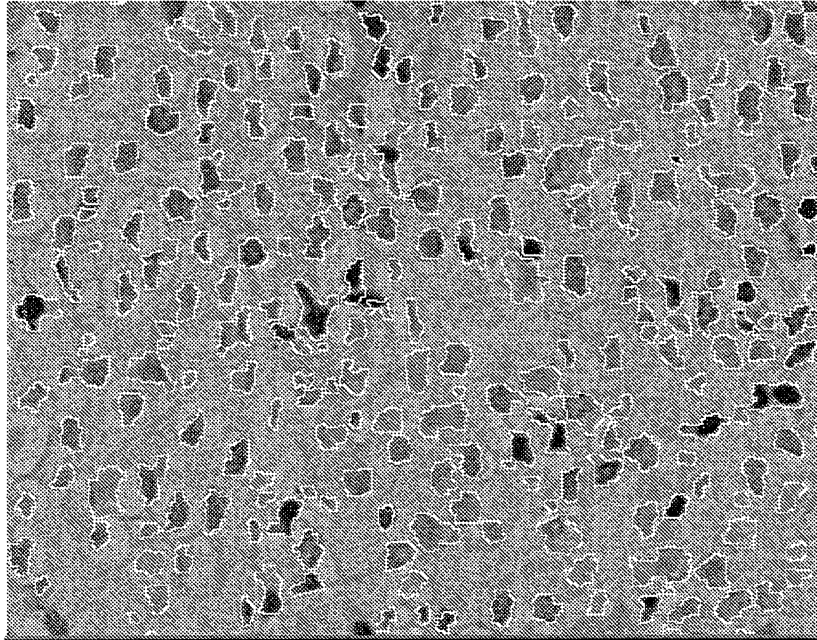


Figure 13: Result from watershed segmentation (superimposed in the original image. The contour were obtained after threshold over the watershed result).

6. CONCLUSIONS AND FINAL REMARKS

This paper discussed the use of morphological watersheds in an important task in image processing and computer vision: the segmentation of regions. Although very powerful, direct application of watershed leads to a poor result: an excess of the segmented regions, which in our case were cell nuclei. Much of the oversegmentation is due to the noise present within the image. One way to solve the oversegmentation is to impose new minima to each catchment basin. This was accomplished by a regularization process, called modification of the gradient image homotopy. The regularization process required the selection of a set of markers which in many cases can be a non-trivial task, but simpler than the original segmentation problem. By imposing internal and external markers to each cell nucleus within the image as the regional minima of the gradient, and then suppressing all the other minima, the watershed was performed successfully. These markers were obtained automatically (see section 5) by a MM module that used diverse operators such as sequential filters, distance transforms, opening by reconstruction, top hat, etc., some in parallel, some in cascade form. Usually, the most complex part is how to choose (or derive an method to obtain) the markers. Once we have a good segmented image, cell measurements, such as area, volume, average intensity and locations, and other statistical features are calculated on the result of watershed segmentation. The algorithm has been successfully applied to the automated image analysis, such as in our case of medical imaging application.

We also discussed briefly about mathematical morphology and about the Khoros system, which was the image processing environment. The paper presented an application in the context of medical images of the MMach, a Mathematical Morphology Toolbox for the Khoros system, mainly developed in Brazil, by the ANIMOMAT Project. Implementations were done in Sun UltraSparc workstations under Unix and X11R5, and are available via *www* and can be freely used for scientific purposes. Future research will be directed to hierarchical MM approaches to segmentation and focusing on the geodesic reconstruction¹⁷ and the novel concept of dynamics of contours.

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