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ADAPTATIVE MORPHOLOGICAL SEGMENTATION : APPLICATION TO HISTOLOGICAL AND CYTOLOGICAL IMAGES

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ABSTRACT

Medical image segmentation is a complex problem by reason of the wide range of image classes and aims to reach. It is usually achieved through the use of procedures which are solely fitted to one class and which integrate, in an opaque way, several kinds of knowledge. So a general strategy allowing to process some resolutely different microscopic image classes with the same approach is presented here. It deals with a region growing operator drawn from the mathematical morphology tool that is the watershed transformation and combines image features in an adaptive way.

Key words: adaptative segmentation, color segmentation, mathematical morphology, medical imaging.

INTRODUCTION

To segment a particular medical image class, an expert of the application usually implements a strategy dealing with a series of image processing tools. After a more or less long test-assessment stage, his strategy becomes reliable for the current case but may be unsuited if the analysis aim or the image class are changed. Indeed, to choose his method, the expert implicitly makes use of many kinds of knowledge whose precise justification (what comes from image classes, processing tools or aims to reach) is embedded in the procedure.

Our work deals with adaptative segmentation and consists in following a resolutely different methodology. We focus our mind on a segmentation process taking into account the analysis objective and which gradually uses, in an explicit way, a priori knowledge about both image processing tools (parameters, behaviour with each class, swiftness,...) and image features (intensity, homogeneity, size, shape,...). The image processing tools we consider are essentially provided by Mathematical Morphology (Serra, 1982). Both kinds of knowledge are first integrated in an extraction step of object and background markers depending on constraints imposed by the aim to reach. Then, only the intrinsic features of image classes are linked in a segmentation step based on an extension of the watershed transformation (Vincent & Soille, 1991) that we propose here. We result in a Generalized REgion Growing Operator (**GREGO**) with optimal scanning which can be adapted to a large class of algorithms thanks to its implementation dealing with ordered queues. To show its adaptiveness, we use it with two cases provided by cytological and histological microscopic images of breast cancers.

MATERIAL

In the cytological image class (fig. 1), isolated nuclei are prepared by thick paraffin section disaggregation and Feulgen staining as described in Herlin et al (1992). Images are made up of 512 x 512 pixels with 256 gray levels. They are observed under ordinary light through a BH2 Olympus microscope with a 125 X magnification providing a 0.12 μm^2 pixel size. They are recorded by a Sony CCD camera. The final goal for analysing this image class is to build the ploïdy curve of a tumor by computing the Integral Optical Density (**IOD**) of the DNA proportional staining. Segmentation constraints are to detect all the objects lying in the image (including debris) with a reliable border localization, without splitting overlapped nuclei on which **IOD** measurement should be mis-valued.

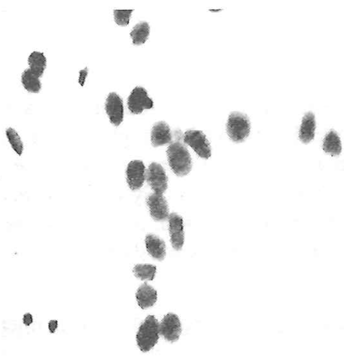

Fig. 1. Image of isolated nuclei of breast cancer.

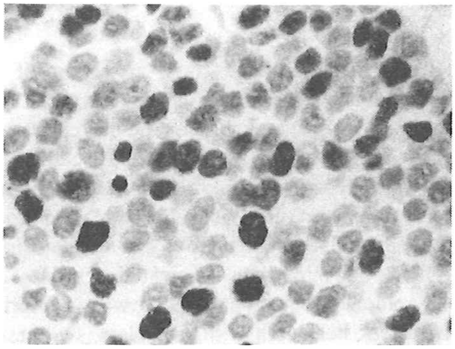

Fig. 2. Image of histological section of breast cancer (normally in true color).

In the histological image class (fig. 2), images are provided by 5μm paraffin sections observed in ordinary light with a 132 X magnification. Images are recorded by a 3CCD color camera (JVC KY-F30) issuing three 768 x 576 video frames, each one with 256 gray levels. Their analysis allows to quantify some proteins (estrogen or progesterone receptors, proliferation markers such as BrdU, Ki 67, PCNA) by revealing a staining bound to a specific associated antibody. Here, the tumoral tissue structure is preserved and objects to segment are nuclear profiles. Sections were double-stained. First, nuclei were stained blue with hematoxylin. Then an immunohistochemical technique (diaminobenzidine-peroxydase) was used to identify positive locations (brown). The final goal for analysing this image class is to compute the immunostaining ratio which is defined as the positive nuclear profile area divided by the entire nuclear profile area. Constraints are not as severe as for the first class since a light oversegmentation is not a real problem (it must be added that the thickness of sections makes uneasy a reliable localization of overlapped nuclei by any pathologist).

The **GREGO** operator has been written in standard C and implemented on a SUN Classic workstation as a submenu of the image processing toolbox NOESIS-Visilog 4 (Domaine technologique de Saclay, 91892 ORSAY FRANCE).

METHODS

Segmentation principle in the field of mathematical morphology

The segmentation principle in the field of mathematical morphology consists of two main steps (Beucher, 1990) :

Step 1 Image simplification and feature extraction by means of marker seeking procedures taking into account some knowledge about the objects, the image class and the processing tools.

Serra (1982) showed that this step is of great significance because watershed transformation results in a strong oversegmentation when applied on a lightly smoothed image. To get rid of this problem, one can use some image simplification techniques dealing with features such as topography, contrast differences, object size or shape, etc. These techniques may be classified in several groups according to their properties :

- ➤ Searching for connex components satisfying one or many homogeneity criteria in an image :
  - component with levels equal to or lower than a limit (thresholding).
  - gray level difference between a point and its neighborhood lower than a fixed value.
- ➤ Searching for topographical attributes in a gray level image :
  - regional extrema or derived features such as r-h-extrema (Lantuejoul and Maisonneuve, 1982).
  - top-hat transformation.
  - regional maxima of a distance transformation.
- ➤ Searching for particular attributes in a binary image :
  - ultimate erosion, skeleton.
- ➤ Searching for contrasted regions :
  - by means of alternate sequential filters or centre of families of filters (Serra, 1988).
  - by means of residual analysis (this method consists in producing a binary image of pixels with negative value provided by the algebraic difference of a low-smoothed image and a high-smoothed image).

The adaptative part of this first step may be invoked at three main levels :

- choosing filtering techniques to apply on the original image by minimizing a cost function including criteria bound to the object frontier localization and/or the signal/noise ratio.

- choosing the family of algorithm for the extraction step according to the required accuracy.
- tuning the intrinsic parameters of the image processing tools concerned thanks to the object description supplied by a manual segmentation.

The quality of the marker extraction process is finally evaluated by comparing the number of well-positioned markers with the number of objects to analyse.

Step 2 Image segmentation by computing the watershed lines of a "potential function" whose crest lines are supposed matching the object frontiers (the most frequently used function is usually a gradient modulus). There are many efficient algorithms which can achieve this merely mechanical step (Vincent & Soille 1991, Beucher & Meyer 1992). The basic principle is to flood the catchment basins of the potential function (which may be seen as a topographic surface) starting from its regional minima, while dams are erected in order to prevent the merging of neighbouring basins.

Here, the adaptative part of the step depends on only one topographical criterion: the potential function. For example, this drawback constrains to reduce a color image into a single object-representative component (usually a gradient image of luminance) before applying the watershed transformation. Moreover, the information provided by the potential function is local to the points being in process. It may be inadequate for objects with low contrasted frontiers for which the process of flooding should involve a "leakage" of concerned basins.

The new alternative we propose is to implement a region growing algorithm dealing with multiple local and global information (Belhomme et al., 1995).

Adaptative region growing technique by integration of multiple informations

The segmentation process we have implemented derives from the watershed algorithm based on ordered queues proposed by Beucher and Meyer (1992), but differs by the way it manages queues and priorities. Here, the queues are not removed as soon as they become empty during the flooding stage of catchment basins, thus allowing the arrival of new points with higher priority, whatever their level is. Above all, these priorities are not read into a single image, as in the watershed process, but they come from the combination of several local informations (features of a point such as its gray or color levels, its gradient modulus, etc), and global statistical parameters iteratively computed for each region during the growing process (area, mean gray or color levels, standard deviations, perimeter, etc). This new implementation is decomposed in two steps :

Initialization

The points that belong to the neighborhood of object markers and background markers are stacked in the highest priority queue. Statistical parameters are also computed for each marker and stored in an array called '*stat_basin[]*'.

Growing

The queues are scanned in ascending order to search for the first point to unstack. This point takes the same label as the region in its neighborhood whose "feature distance" is the smallest one, then all of its unprocessed neighbors are stacked with a priority $\varphi(p)$ depending on multiple information $\varphi_i(p)$.

The function $\varphi(p)$ may be expressed as a linear expression :

$$\varphi(p)=\sum_{i=1}^{n}\alpha_i.\varphi_i(p) \qquad \varphi(p)\in[0;N] \qquad \sum_{i=1}^{n}\alpha_i=1$$

where:

$\varphi_i(p)$ is a criterion. It can correspond to the value of any image at the location of p (local information), or to a mathematical expression involving features of p and of its neighborhood (global information).

α_i is the weight given to $\varphi_i(p)$ in the linear combination.

n is the number of examined criteria.

N is the maximal number of queues.

To build the watershed of an image I, one simply has to take :

$$n=1,\ \alpha_1=1,\ \varphi_1(p)=I(p)$$

To take into account other information, one has to choose some new criteria and new weights.

The region growing process can now be adapted to the problem to solve by choosing multiple effective criteria as well as their weights.

To evaluate the final result of the automatic segmentation, one can compare it with an interactive one. In that case, an ideal set of manually placed markers must be used before starting the growing process.

RESULTS

Cytological images

A test has been realized with a series of ten images including 245 nuclei. The results are compared with those obtained by software previously developed in our laboratory (Masson, 1992), in which the segmentation step is performed by contour detection and linkage (this method sometimes involves the removal of the most textured nuclei, especially the pathologically abnormal nuclei). The segmentation accuracy is of course bound to the good quality of extracted markers. By combining an automatic thresholding (Sahoo et al., 1988) to detect the image background with the residual analysis, we obtain a score of 97% of well-marked objects for only 3% of over-segmented ones. This allows the analysis of 19% more nuclei than the reference software. Both smoothings used in the residual analysis are made up of square-box filters with sizes 6x6 and 20x20 (these values correspond to the smallest and median nuclear radius). The segmentation of a cytological image I is then processed with two criteria :

$\varphi_1(p)=I(p)$ and $\varphi_2(p)=\left|\bar{I}_R-I(p)\right|$ where $\bar{I}_R$ is the mean of the gray tones of I inside R.

The weights we have retained are : α_1=0.2 and α_2=0.8.

This combination leads to a distance of 3.5% between the frontiers of the new segmentation and those of the reference and a scattering between the quantitations of **IOD** of about 1.5%.

Histological images

A test has been realized with a series of ten images. Due to the great complexity of this class, the segmentation reference is obtained from an interactive drawing of some objects that a pathologist can distinguish. The number of nuclear profiles is thus unknown. The extraction of markers is performed in two steps. First, a residual analysis is achieved onto the luminance component yielding a rough binary image (the sizes of smoothing filters are the same as for the previous image class). Then, the regional maxima of a distance transformation computed onto the binary image are extracted to provide a set of object markers; the closest ones are combined by means of two dilations.

The segmentation of the original color image I is processed with six criteria :

$\varphi_1(p)$ The gradient modulus allows to detect contours of well-contrasted objects. With a color image I one disposes of many components to produce the gradient image. We have decided to apply Deriche's operator onto luminance image I_L so :

$$\varphi_1(p) = |grad(I_L)|$$

$\varphi_2(p)$ On uniform background areas or lightly textured nuclear profiles, the values of gradient are small. In order that the region growing process increases priorities of both uniform areas and lower gradient values than the mean of a neighboring region R, we have considered the following criterion which can be negative :

$$\varphi_2(p) = \varphi_1(p) - \overline{\varphi_1(R)}$$

$\varphi_{3,4,5}(p)$ This class deals with RGB color images. In order to care about color difference between p and one of its neighboring region R, we have considered the criteria :

$$\varphi_{3,4,5}(p) = \left| I_{r,g,b}(p) - \overline{I_{r,g,b}(R)} \right| \qquad r,g,b \equiv red,\ green,\ blue$$

$\varphi_6(p)$ In order to increase priorities of nuclear profile regions to the detriment of image background regions, we used the luminance component I_L where objects cover dark values and background covers light ones so :

$$\varphi_2(p) = I_L$$

The best weight combination allowing to extract uniform regions in difficult zones while preserving main object edges appeared to be :

$\alpha_1 = 0.4$; $\alpha_2 = 0.1$; $\alpha_3 = \alpha_4 = \alpha_5 = 0.1$; $\alpha_6 = 0.2$

DISCUSSION

This implementation of a watershed line extension, based on the use of ordered queues and a computation of priorities performed by a linear combination of local and global information, leads to a generalized region growing algorithm with optimal scanning. It can be adapted to several image classes such as monochromatic and color images. Thanks to an appropriated function which gives the neighbors of any point, it can be also fitted to multiple grids (2D, 3D or graphs). The ability to make co-operating many criteria is of great interest for segmenting images with high information content but relatively poor quality. This is very useful in the field of biomedical imaging where the large variety of situations does not fit in with a mono-criterion segmentation, and where manual intervention of an expert can sometimes be take into account (in that case it could be incorporated as a new criterion).

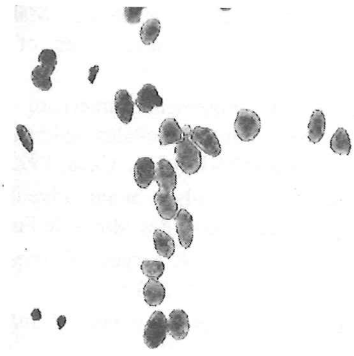

Fig. 3. Segmentation of figure 1.

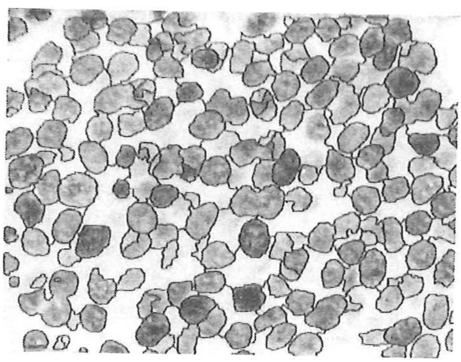

Fig. 4. Segmentation of figure 2.

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