PHASE CONTRAST IMAGE SEGMENTATION BY WEAK WATERSHED TRANSFORM ASSEMBLY

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ABSTRACT

We present here a method giving a robust segmentation for in vitro cells observed under standard phase-contrast microscopy. We tackle the problem using the watershed transform. Watershed transform is known for its ability to generate closed contours and its extreme sensitivity to image borders. One main drawback of this method is over-segmentation. In order to circumvent this, marked watershed based on the "modified gradient" method has been developed. However, the choice of the watershed mark locations is critical and their inadequacy may cause wrong results. Similarly to randomization and combination procedures used in the machine learning field, the present paper promotes the use of an assembly of marked watershed transforms, in order to increase the segmentation robustness. This results in the definition of candidate segmentations margins (expressed in terms of object border confidence) from which final segmentation can be chosen by means of thresholding.

Index Terms**—** Medical image processing, Image segmentation, Mathematical morphology, Pattern classification, Classifier assembly.

1. INTRODUCTION

The watershed transform is known to give an interesting solution for image segmentation by creating closed contours [2]. It is also well known for its main drawback: over-segmentation, i.e. each of the minima present in the image gives rise to a watershed catchment basin. The marked watershed method uses geodesic reconstruction in order to overcome over-segmentation.

Image segmentation can be seen as a classification problem [11] where pixels are classified in different classes. In particular image binarization corresponds to a two–class problem (object/background). Marked watershed can thus be considered as a supervised classification problem in which labeled marks identify image areas which belong to different image regions to be segmented. The pixels included in a catchment basin generated from a given mark are assigned to the same class. This classification process thus results in a partition of the image into labeled regions (i.e. the segmentation). From this point of view we propose a novel marked watershed segmentation approach based on mixing, randomization, weakening and combination processes, method well known in the machine learning community [1,3,7,8].

Some authors have investigated strategies to combine multiple segmentations (two or more classes) of the same image, generated, for example, by different human experts [11]. More recently, [9] propose to combine partial segmentations resulting from various segmentation techniques applied to a given image. The over-segmented image regions are then merged using similarity criteria. This aims to eliminate bad parts of the individual segmentations.

We describe here a straightforward method that creates an assembly marked watershed transform weakened by randomization (each of them is called a *segmenter*). All these weak segmentations are then combined in order to reveal the intersection of catchment basins that exhibit statistical stablility. This aims to segment one object from the background by identifying robust and relevant basins portions in the sense that they are less dependent on the initial mark positions and stable to small image perturbations.

As illustrated in the present study, the design of robust segmentation methods was motivated by the problem of individually segmenting marker-free living cells observed under standard phase-contrast microscopy *in vitro*. This segmentation step is required to characterize cell phenotype and morphology [5].

Below, we recall some properties of the watershed transform (section 2) and randomization process (section 3). We then explain (section 4) how the watershed assembly is generated and combined. Finally, we illustrate our approaches on a practical segmentation problem and draw conclusions (section 5).

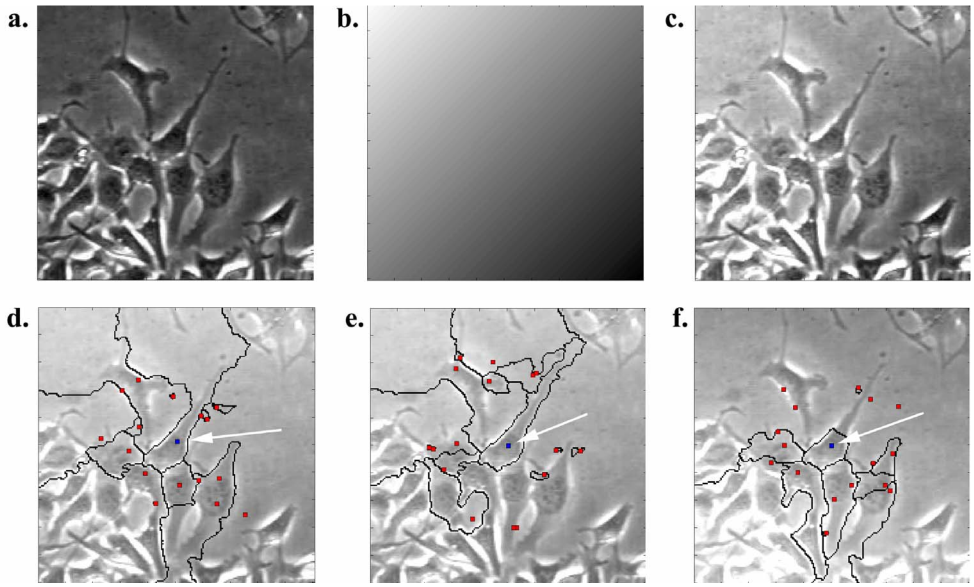

Figure 1: (a.) detail of the original phase contrast image, (b.).random graylevel slope , (c.) random slope added to the original image , (d.)(e.)(f.) watershed basins obtained for three different markers/slope randomizations (arrows designate the object marks), (d.) the central basin contains the solution but also background pixels, (e.) corresponds to a acceptable solution and in (f.) part of the object of interest is missed.

2. THE WATERSHED TRANSFORM

Let us recall here some important aspects of the watershed transform. First introduced by Beucher et al. [2], the watershed transform exploits an analogy with the immersion of the image landscape. Briefly, the image space is considered as a "terrain" where high graylevel values correspond to hils and low graylevel values to valleys. Usually the watershed transform is applied on the gradient image of the objects of interest. In the image space, this corresponds to low valleys (object and background) separated by high-altitude passes (objects borders). Similarly to the water flood in nature, the watershed transform is able to delineate the borders of the catchment basins present in the image "terrain". The watershed transform thus segments an image into several zones rising from each local minimum.

[10] presents a review of several definitions of the watershed transform and the associated algorithms.

Over-segmented image partition occurs because the watershed transform starts from local minima present in the image and needs some pre-processing. In order to tackle the over-segmentation issue, marked watershed was developed. It consists in filling up the non-marked minima before applying the watershed transform. Hence only the marked minima give rise to a label (a connected component) in the resulting segmentation (Fig. 1 (d.)-(f.) illustrates results of the marked watershed transform).

Finding correct marks thus becomes the actual challenge in the marked watershed transfom. A number of classical processing techniques, like filters, image threshold or morphological operators ("erodé ultime"), can be used to build useful marks. Sometimes a priori knowledge on approximate object positions is available, such as in image sequence analysis in which the objects isolated in the previous frame of the sequence can be used as a marker for the following frame [6].

3. RANDOMIZATION PROCESS

The first underlying idea is that segmentation can be considered as a classification process. In the case of marked watershed, one catchment basin is created for each mark (i.e. a set of connected pixels) and the resulting basin set defines a partition of the image into labeled regions. The present study focuses on two-class problems where an object of interest has to be identified from its background. This object is designated by a mark that is put on or close to the object location (the *object mark*).

The second main idea relies on randomization and combination processes. This is motivated by the numerous theoretical and experimental studies which show that a combination of several diverging classifiers (also called "multiple classifier system" or "ensemble approach") is an effective technique for reducing prediction errors [7,12]. The key of this improvement relies greatly on the degree of decorrelation of the errors of the component classifiers. One approach to create error diversity is to manipulate input data in order to train the component classifiers with different training sets (weakened classifiers). More generaly, these diversified training sets need to vary in elements (e.g. input features and/or training cases) for which the used classification method is known to be unstable.

4. WEAK WATERSHED TRANSFORM ASSEMBLY

We play on two aspects for which marked watershed segmentation is unstable: i. mark positions and ii. image perturbations. Randomized mark positions and image perturbations generate weakened segmentations. As it has been demonstrated in the case of classification problems [1,3,8], we expect that the consensus of these weakened segmenters will be more likely to converge toward a better solution that the one that can be expected from one single (optimized) segmentation process.

4.1. Marker randomization

Adding noise to the mark positions results in variable image segmentation created by the watershed basins, as illustrated in figure 1 (d.-f.). Two mark types are used, one mark for the object of interest (the *object mark*) and several marks for the background basins surrounding the object. The central marker position is "weakened" by a random noise (radial gaussian distance). The background marks are randomly set around the central position at a random distance (gaussian ring). These markers then yield a marker image used by the marked watershed algorithm.

4.2. Image perturbation

Recall the landscape analogy, image perturbation can lead to the displacement of the watersheds dam. The chosen image perturbation consists in adding a constant slope with a random orientation to the gray level image. The value of the added slope is arbitrarily set between [-60,+60] (the graylevels are constrained to the 8 bit pixel values [0,255]). As result, some weak gradient levels present in the original image can be reinforced, whereas others are smoothened.

Figure 1 (b.) shows an example of the perturbing gradient and its application (c.) to the original image (a).

4.3. Counter image and consensual segmentation

Figure 1 (d.)-(f.) shows the results obtained for three random applications of the weakened watershed method, we observe the the obtained segmentation are well decorrelated. While a correct segmentation can occur (e.), it is not usually the case. A counter is then allocated to each pixel. Considering a set of weakened segmentations, the counter value of a pixel is incremented each time the pixel belongs to the object marker basin. The result of this procedure is an image exhibiting high values for pixels that are often considered as the object and low values for the background pixels. Figure 2 (a.) illustrates the values of the normalized counter image resulting from 30 randomized (weakened) watershed transforms. In figure 2 (b.) we clearly observe that the object of interest appears on a significantly higher plane. Other regions of the image appear as lower planes. Thresholding the counter image results in a candidate object border (the red contour in figure 2 (b.). corresponds to the 0.5 value of the counter which selects pixels that belong to at least 50% of the randomized object basins). If the threshold value is high, only pixels that are most often inside the object basin are preserved. The counter value can thus be used as a confidence level for the segmented object.

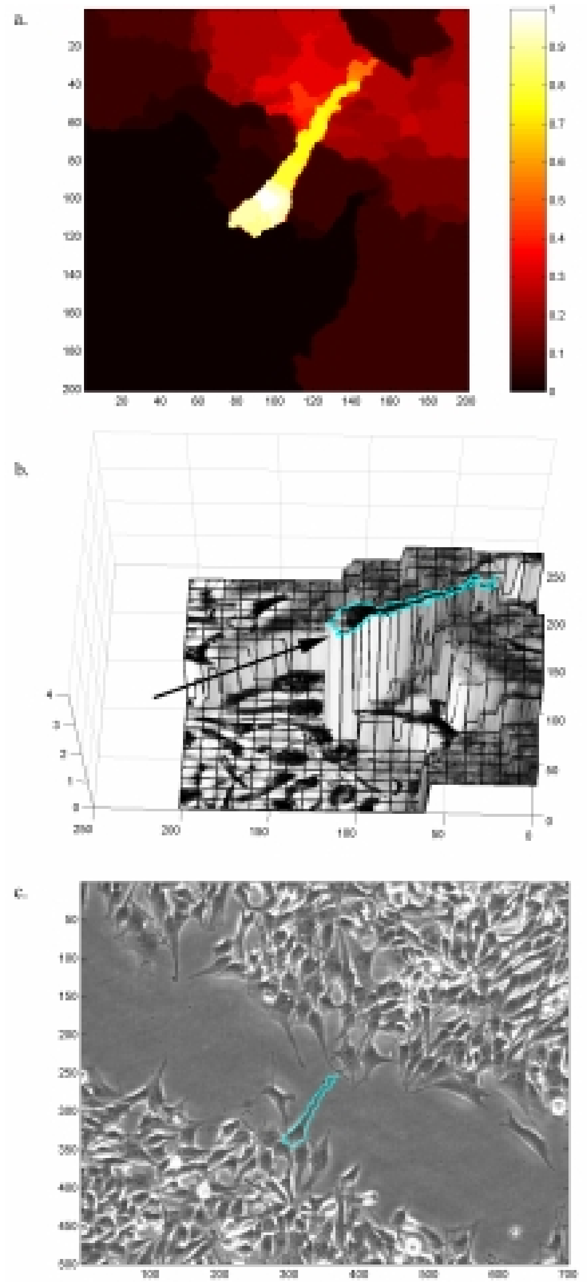

Figure 2 : (a.) normalized counter map obtained from 30 randomized watershed transforms on the object marked in Fig. 1(d.-f.), (b.) synthetic view of the counter map textured with the original image, (c.) final segmentation result.

5. RESULTS AND CONCLUSIONS

As illustrated in figure 2 (c.) the proposed method is able to tackle complex segmentation of phase contrast-images by using a simple and straighforward approach.

Comparing to a single marked watershed transform, the proposed method is more robust. Indeed the assembly of weakened watersheds strongly decreases the impact of the object marker location. Moreover, this approach also provides a confidence level associated with segmentation. By choosing the proportion of weakened watersheds selecting each pixel, it becomes possible to isolate better object pixel candidates from the background in a quasi continuous way.

We observed that the combination of multiple weakened marked watersheds exhibits similar properties to those observed in the supervised classification domain, i.e. that several weakened classifiers (segmenters) can by consensus identify a better solution than the solution obtained by a single optimized one. Robustness to marker position and image quality is also an important aspect that has been observed experimentally (data not shown) and will be studied more in depth.

The proposed method will be applied to fully qualify cell shape in the domain of *in vitro* cell tracking. A tracking method previously developed in this context [4] is able to provide individual cell centroids which will be used to guide the selection and randomization of object and background marks required for our segmentation method. .

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