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Jalba, Andrei C.; Roerdink, Jos B.T.M.

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Automatic segmentation of diatom images

Andrei C. Jalba, Jos B. T. M. Roerdink

Institute of Mathematics and Computing Science
University of Groningen, P.O. Box 800
9700 AV, Groningen, The Netherlands
{andrei,roe}@cs.rug.nl
<http://www.cs.rug.nl>

Abstract. In this paper we present a segmentation technique based on tools from mathematical morphology which can be successfully used for automatic segmentation of diatom images. The proposed method belongs to the class of hybrid segmentation techniques, and is based on the morphological watershed from markers. The novelty of this method is the computation and selection of markers. A new connected operator is used to simplify the input image and to produce candidate marker regions. A further step which selects among these regions is carried out in order to produce the final markers as a label image, and a watershed process initiated from this image is applied on the gradient of the input image. In a post-processing step, the contours of the diatoms present in the resulting image (given as watershed lines) are extracted.

1 Introduction

Segmentation is one of the critical aspects in many image analysis and computer vision tasks, because effective segmentation usually dictates successful analysis. Image segmentation is the process in which an image is divided in its constituent parts, and ideally it should be computationally efficient and correspond well with the physical objects represented in the image. This implies that segmentation should produce a complete partitioning of the image such that object contours are closed and precisely localized.

There are four main approaches [1, 9] for the segmentation of grey-scale images: threshold techniques, boundary-based methods, region-based methods, and hybrid techniques which combine both boundary and region criteria. In this paper, we mention only hybrid techniques, while for a complete review on image segmentation we refer to [9].

Two important representatives in the class of hybrid techniques are morphological watershed segmentation [7, 10] and seeded region growing [1]. The watershed method regards an image as a landscape where intensity values correspond to elevations, and is generally applied to the gradient of the image. Advantages of watershed segmentation are that it (generally) leads to closed boundaries of the image regions and it can describe edge junctions [8]. As pointed out in [6], the seeds in the seeded region growing method play the same role as the markers

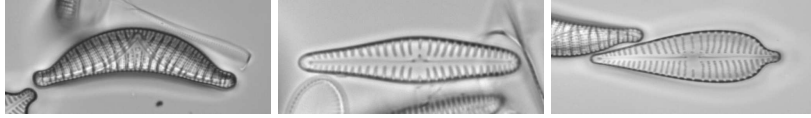


Fig. 1. Some examples of diatom shells.

used in watershed segmentation. Although both methods have the advantages that they are fast and parameter free, the most critical part is the selection of seeds.

Our overall goal in this work is to develop a framework for automatic segmentation of high-magnification, grey-scale diatom images. Diatoms are microscopic, single-celled algae, whose sensitivity to environmental parameters means that they can be used to monitor changes in the environment, or have forensic applications. All these applications require counting and identification of different species present in the sample of interest. However, prior to automatic identification, reliable segmentation should be performed. Therefore, in this paper we propose a hybrid segmentation method which can be successfully used for automatic segmentation of diatom images. The goal is to extract the outline (encoded as chain-codes) of each diatom shell present in the input image (see Fig. 1 for some examples). The typical size of these images is between 600 . . . 900 pixels for the horizontal dimension and 200 . . . 400 pixels for the vertical dimension. Ideally, each image contains a single diatom shell, but as can be seen in the figure, diatoms may lay on top of each other, may not be in proper focus, or can be very close to each other. Moreover, dust specks and background texture may be visible in some images.

2 A hybrid segmentation technique

The main steps of the proposed method are shown in Fig. 2. The processing flow of the input image branches in two paths according to the desired output. One of the paths ends with the selection of markers which produces the label image, while the other ends with the computation of the gradient-magnitude image. Both resulting images are then used in the watershed-segmentation step.

The novelty of the technique is the computation and selection of markers. A new connected operator is used to simplify the input image and to produce candidate marker regions. The final marker regions are selected based on the area of each candidate region, after some morphological processing is performed.

All main steps of the method are described in the next sections, beginning with the steps which yield the label image.

2.1 Preprocessing

In the pre-processing step, a non-linear method for contrast enhancement [3] is applied to the input image. The basic idea of this algorithm is to perform a sliding

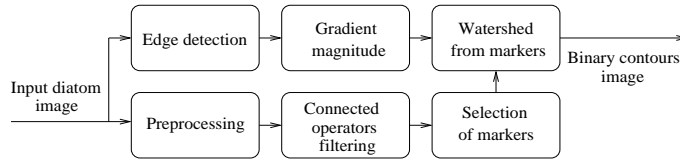


Fig. 2. Hybrid segmentation method diagram.

downhill on the gradient-squared surface until a local minimum is reached. Then all pixels along this path are given the image intensity of this local minimum. The squared image gradient is computed using a Sobel operator [4].

The reasoning for using this method for contrast enhancement is that it performs a (re)quantization of the grey levels in the input image, controlled by the gradient-squared function. Since spurious pixels are linked to homogeneous regions, areas of these regions increase, and in turn, this provides an increased robustness of the operator in Eq. (1). However, the technique is not used on the path which leads to the gradient-magnitude image, since it may introduce false edges which hampers the evolution of the watershed. Nevertheless the method shows desirable results especially for low contrast images, and can be used for marker extraction, even though it may introduce false edges. The regions associated with the false edges can be eliminated either by the subsequent filtering step, or during the selection of marker regions. If some regions still survive, they can be neglected when the contours are extracted (see Sect. 3), due the property of the watershed to allow for T-junctions.

2.2 Connected operator filtering

It is common to represent a grey-scale image by its level components (connected components, in the binary case). Let R be the domain of a grey-scale image f . A *flat zone* L_h at level h of grey-scale image f is a connected component of the level set $X_h(f) = \{p \in R | f(p) = h\}$. A *regional maximum* M_h at level h is a flat zone which has only strictly lower neighbours, and a *peak component* P_h at level h is a connected component of the threshold set $T_h(f) = \{p \in R | f(p) \geq h\}$. At each level h there may exist several such components, indexed as L_h^i, P_h^j, M_h^k , with i, j, k from three index sets.

Max-trees were introduced by Salembier *et al.* [11] as a versatile data structure for anti-extensive connected set operators. A max-tree is a rooted tree, in which each of the nodes C_h^k at grey-level h corresponds to a peak component P_h^k . However, C_h^k contains only those pixels in P_h^k which have grey level h . In other words, it is the union of all flat zones $L_h^j \subseteq P_h^k$. An example of a 1-D signal, its peak components and its max-tree representation is shown in Fig. 3.

Once the max-tree has been built, it can be used for processing of the input image, since the tree is its representation. For tasks of filtering, this is a three-step process: construction of the max-tree, criterion assessment and decision, and image restitution. The filtering step analyzes each node C_h^k by evaluating a

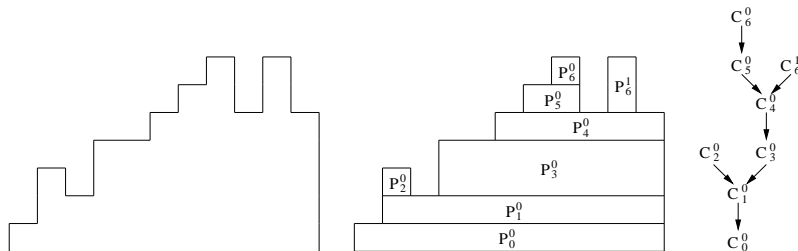


Fig. 3. The max-tree structure. *Left:* a 1-D signal; *center:* peak components P_h^k of the signal; *right:* its corresponding max-tree.

specific criterion $T(C_h^k)$ and takes a decision on the elimination or preservation of the node. The last step, called here restitution, transforms the filtered max-tree into an output image.

Let us assume that components with large areas compared with the area of their parent component are important and are to be preserved by the filtering process. Let $A_{C_m^n}$ be the area of the component associated with the node C_m^n . We propose to use the *relative percentage difference* as a measure of closeness between the area of the component and the sum of areas of its child components (corresponding to child nodes $C_{h_i}^k$), *i.e.*

$$d_A = 100 \times \frac{|\sum_i A_{C_{h_i}^k} - A_{C_m^n}|}{A_{C_m^n}} \quad (\%), \quad (1)$$

with $h_i > m$, and k, n from two index sets. Starting with the root node, d_A is recursively computed according to Eq. (1). If for a given node C_h^k this value is larger than a threshold λ then *all* its child nodes are marked as deleted. In a subsequent step, all marked nodes are merged with their nearest preserved ancestors; in our implementation $\lambda = 1\%$.

2.3 Selection of markers

In this subsection a simple method is presented which selects the final markers from candidate marker regions. The selection of markers is the most critical part of the segmentation based on watershed from markers. As the number of markers does not change during the watershed evolution, a marker region lost during marker selection cannot be recovered later. Therefore, special care must be taken during the marker selection process, for which we propose the following procedure.

- Compute the morphological gradient (the difference between dilated and eroded images), and label with zero all pixel positions where its value is greater than zero.
- Do a connected component labeling (with labels greater than zero) of all regions which are not assigned a value of zero;

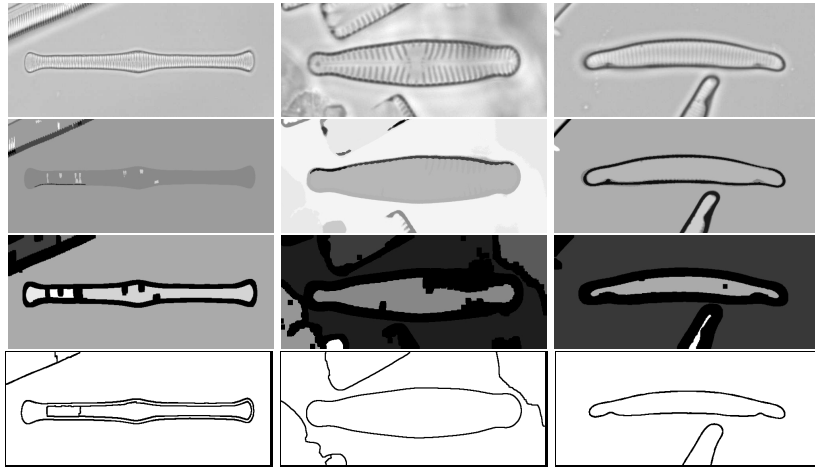


Fig. 4. Hybrid segmentation technique. Examples. *First row:* diatom images; *second row:* filtered images; *third row:* label images; *fourth row:* resulting binary images.

- Regions with small areas are not considered marker regions, *i.e.* they are marked with a zero label.

At the end of this procedure, all marker regions are given a unique label greater than zero, and all other regions, with uncertain region membership, are marked with zero. The decision about their region membership is made by the watershed transform by growing basins from marker regions under the control of the magnitudes of edges.

Notice that an initially connected region may be split into more than one during this process, and assigned different labels. Fortunately this problem can be corrected during the post-processing step (see Sect. 3), due to the property of the watershed to allow for T-junctions.

The size of the structuring element for both dilation and erosion was set to 13×13 , and the threshold on area, for small region removal, was set to 100 pixels.

2.4 Gradient magnitude computation

The gradient of the image along with a certain degree of smoothing is obtained by convolving the initial image with a derivative Gaussian filter. In our implementation the width of the kernel σ was set to 4.0.

2.5 Watershed from markers

Our implementation of the watershed from markers is based on the Image Foresting Transform (IFT) [5]. An advantage of the IFT compared to the classical watershed is that it guarantees the optimality of the solution, as long as the

cost path is a non-decreasing function of the arc weights. Also, similarly to the watershed from markers using an ordered queue, the IF^T does not need a change of homotopy (a minima imposition operator which changes the homotopy of the image in such a way that the desired markers are the only regional minima of the image [7]).

All regions in the label image whose pixel values are greater than zero provide the marker regions from which the watershed segmentation is initiated; the watershed propagation is done on the gradient-magnitude image (see Sect. 2.4). As a final step, the watershed lines are drawn in black on a white background, in order to provide a binary image.

2.6 Examples

Some example results produced using the proposed hybrid segmentation technique are shown in Fig. 4. The input diatom images are shown in the first row of the image. The resulting images, after connected operators filtering (see Sect. 2.2), are shown in the second row. The label images (see Sect. 2.3) and the resulting binary images are shown in the third and fourth rows, respectively. Although the large region corresponding to the central diatom, present in the first image, is split by the marker selection procedure, this is not a problem since all extracted contours are flood-filled in the post-processing step (see Sect. 3). The T-junctions produced by the watershed lines can be observed in the first two cases.

3 Post-processing and contour extraction

First, *all* contours present in the binary image, produced by the segmentation method, are traced using a standard contour following algorithm [4]. Then, all extracted contours, which are necessarily closed, are filled at grey-level zero by a flood-fill algorithm, and all obtained regions are drawn in the same image. In a further post-processing step, an opening with a structuring element of size 3×3 is performed, in order to prune thin structures, which may still be connected to diatom regions, due to debris or fragments of other diatoms. In this way, the union of all diatom and inner-diatom regions is performed and *all* diatom contours can be found by tracing only *one* contour per region. Finally, contours which enclose regions of areas smaller than 4900 pixels are not considered as diatom regions, and are rejected.

The whole tracing process is illustrated in Fig. 5. The initial diatom image and the binary contour image are shown in Fig. 5(a) and (b), respectively. The above procedure for contour extraction yields three contours depicted in Fig. 5(c). The regions obtained after flood-filling the traced contours are shown in Fig. 5(d). Notice that, surviving inner-diatom regions, which were not removed by filtering or by the marker selection procedure (see Sect. 2.2 and 2.3, respectively), are now merged in one large diatom region. After opening (Fig. 5(e)), and removal of small regions, the final contour(s) can be traced (Fig. 5(f)).

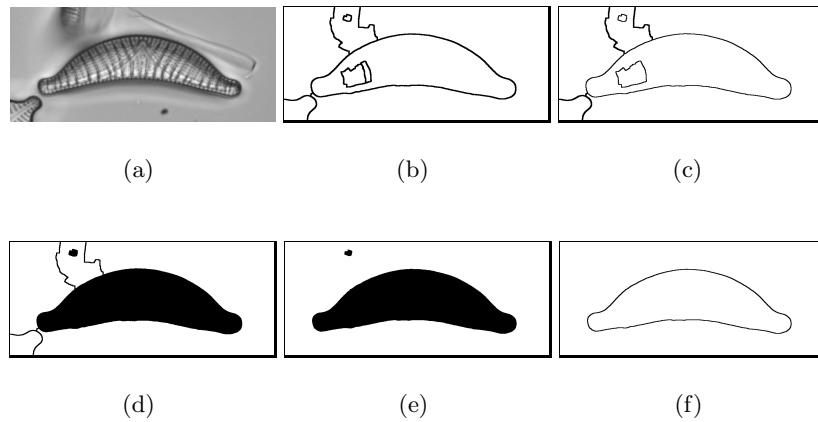


Fig. 5. Contour extraction. Example. (a) initial image; (b) binary image with watershed lines; (c) extracted contours; (d) regions obtained after flood-filling the contours; (e) opened image; (f) the final contour.

4 Experimental results

The proposed segmentation method was used to extract binary contours from a large set of diatom images [2] comprising 808 files. The quality of the extracted contours was evaluated visually.

Unfortunately, because there is no established theory which defines the quality of a segmentation, we had to rely on some heuristic. Therefore, visual estimation of the quality of the contours was guided by the following criteria: (i) the contours should be smooth, (ii) they should correspond well with the perceived diatom outlines, and (iii) they should not enclose debris or diatom fragments. All contours that did not fulfill the above requirements and all images for which no appropriate contours could be extracted were considered errors. The hybrid technique showed very good segmentation results, with only 16 errors, leading to 98% correctly extracted contours.

5 Conclusions

In this paper we developed a framework for automatic segmentation of microscopic diatom images based on watershed segmentation from markers. The novelty of the proposed segmentation technique is the computation and the selection of markers. As the number of markers does not change during the watershed evolution, a marker region lost during marker selection cannot be recovered later. Therefore, we have proposed procedures which (i) compute candidate marker regions based on connected operators filtering, and (ii) select final markers based on the area of each candidate region, after some morphological post-processing is performed.

The proposed segmentation method was applied on a large set of diatom images and the extracted contours were evaluated qualitatively, by visually estimating the quality of the contours.

The technique yielded good results, obtaining 98% correctly extracted contours, with a very good quality of the contours. Therefore, our conclusion is that the segmentation of diatom images can be performed automatically, and with very good results.

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