A VARIATIONAL METHOD FOR LEUKOCYTE DETECTION

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ABSTRACT

In this paper, we propose a variational method for the detection of leukocytes observed in vivo. An adaptive threshold surface is constructed automatically using boundary information from the image. The surface is created using an objective functional that is minimized via a variational approach. This surface is constrained by an edge field that is also computed with a variational method. Objects extracted from background are pruned according to two geometric criteria. In the experiments, we find the false positive rate of the detector and show that the proposed approach can automatically and accurately identify multiple rolling leukocytes in vivo.

1. INTRODUCTION

Automatic detection of leukocytes is of value as the initialization procedure for an automated leukocyte tracking [1]. Rolling leukocytes are slow moving leukocytes, where the lower velocity is caused by the adhesive force between leukocytes and the endothelium. The automated detection of rolling leukocytes enables the computation of vital parameters in the inflammatory process such as leukocyte flux, rolling leukocytes per unit length, and the rolling leukocyte volume fraction. The focus of this research is targeted toward development of an integrated leukocyte tracking system, which can provide further insight into of the mechanics of leukocytes rolling *in vivo* (within a living subject) and validation of anti-inflammatory drugs [7]. In this paper we are proposing an efficient leukocyte detection solution as an initialization tool for tracking, which will replace the laborious manual initialization.

In the previous studies, for object detection, techniques based on thresholding schemes are commonly used [11]. In those procedures, histogram information from the image is generally used to choose an intensity value that minimizes misclassification of pixels [10]. Most gray level thresholding methods yield acceptable results when the image is uniformly illuminated. A frequent problem is that either the background or the object, even both, may have non-uniform illumination in real imagery.

An example of a non-uniform lighting condition is shown in Fig. 1. In Fig. 1, we see that a bright disk resides on a darker background. The background is a tilted plane. The level lines (or iso-intensity contours [3]) are also shown in Fig. 1. In this case, none of level lines of the image corresponds to the entire boundary of the object. Therefore it is not possible to find a global threshold that successfully segments the gray level image.

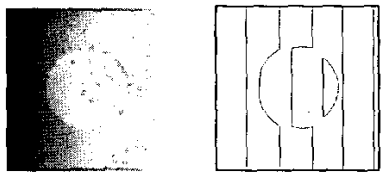

Figure 1. Synthetic image with uneven illumination (left) and level line graph (right)

For our intravital video sequences with poor contrast and an inconsistent background, the general thresholding approaches, or methods based on level lines, may fail to delineate leukocytes from the background correctly. Therefore it is our objective to develop a method to detect leukocytes automatically and accurately from microscopic video sequences in the presence of uniform as well as non-uniform illumination. In this paper we propose a variational method for constructing a threshold surface using boundary information of the image and then detecting the leukocytes using *a priori* information.

2. ALGORITHM

Successful leukocyte detection depends on the construction of the threshold surface. The intuition behind the construction of a threshold surface is shown in Fig. 2. In the detection process, we are most interested in the image intensities at the boundary of the leukocytes. The threshold surface u is obtained by interpolation from these values in conjunction with a smoothness constraint.

A number of different variational approaches [2,5,8] to image segmentation have been developed to date. Chan *et al.* in [4] have proposed a variational scheme for obtaining an adaptive threshold surface. It is a variational version of Yanowitz and Bruckstein's algorithm [13].

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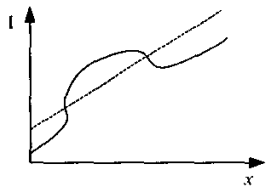

Figure 2. Threshold surface (dotted line) superimposed on a synthetic leukocyte profile.

Our approach is consistent with *Chan et al.*, except that, we notice that pure edge-detection-based algorithms tend to result in less reliable region boundaries. Obviously, the selection of pixel locations used in the interpolation is critical. The final result may be damaged by spurious edge pixels used in the interpolation.

To overcome this limitation, we consider adoption of an optimal boundary function B (where optimality is assessed in terms of minimizing the Mumford-Shah functional [9]). The edge field B is well defined over the entire image domain, approaches unity-value on the boundary and contains small numbers (approaching zero) elsewhere. Note that B is a continuous edge field, instead of a binary edge process. We use B to regularize the process of obtaining an adaptive threshold surface u. The advantage of such an approach is that the interpolation is implemented smoothly during process of minimizing objective functional, and the influence of spurious edges is reduced.

To derive the energy functional, here we assume that such object boundary function B has already been extracted from the image. Given the original image g, the thresholding surface u can be derived by minimizing an objective functional of the form:

$$J(u,B)=\frac{1}{2}\alpha\int_{\Omega}(u-g)^2B^2+\int_{\Omega}\Phi(|\nabla u|) \qquad (1)$$

where $\Omega\subset\Re^2$ denotes the image plane, and Φ is a positive increasing function, $|\nabla u|$ is the modulus of the gradient of $u(x)$, and α is scalar weight that balances the two terms of (1).

The two energy terms in (1) indicate two constraints: the threshold surface u should approximate the input image g near object boundaries, where B is near 1; consequently, u should be as smooth as possible.

Using the divergence theorem, the Euler-Lagrange equation of J is given by:

$$\alpha(u-g)B^2-\nabla\cdot\left(\frac{\Phi'(|\nabla u|)}{|\nabla u|}\nabla u\right)=0 \qquad (2)$$

subject to the conditions

$$u(x,0)=u_0,\quad \text{for } x\in\Omega$$

$$\frac{\partial u}{\partial n}=0,\quad \text{for } x\in\partial\Omega$$

where Δu is the Laplacian of u and $\partial u/\partial n$ denotes the normal derivative to the boundary $\partial\Omega$ of Ω. Gradient descent on J gives:

$$u_t=\alpha(g-u)B^2+\nabla\cdot\left(\frac{\Phi'(|\nabla u|)}{|\nabla u|}\nabla u\right). \qquad (3)$$

Gradient descent may lead to a local minimum. Other methods, such as simulated annealing, can be used to find a global minimum for the objective functional.

For the moment, the problem of finding the optimal boundary function B has not been addressed. In our work, we choose the optimal boundary function proposed in Hewer *et al.* [6]. To accommodate a continuous boundary function B, the Mumford-Shah functional is recast as:

$$J^{MS}(u,B)=\alpha\int_{\Omega}(u-g)^2(1-B)^2+\beta\int_{\Omega}\Phi(|\nabla u|)(1-B)^2+\int_{\Omega}B^2 \qquad (4)$$

The residual term (the difference between successive estimates of u and g in (4)) is expressed as r:

$$r=\alpha(u-g)^2+\beta(\Phi(|\nabla u|)) \qquad (5)$$

Given an approximation image u, the optimal boundary function B can be found explicitly for any nonnegative r:

$$B=\frac{r}{1+r} \qquad (6)$$

and the Mumford-Shah functional is equivalent to the L_1 norm of the optimal boundary functional B:

$$J^{MS}(u)=\int_{\Omega}\frac{r}{1+r} \qquad (7)$$

which can be minimized using the gradient descent method.

3. IMPLEMENTATION

In (1) the function Φ works as a smoothness penalty function. It is shown in [12] that a function Φ that is convex and linear at infinity ensures the existence and uniqueness of a solution of (1) in $BV(\Omega)$ (the space of functions with bounded variation). Here we choose $\Phi(x) = x^2/2$. In this way the descent PDE has the following form:

$$u_t=\alpha(g-u)B^2+\Delta u \qquad (8)$$

The numerical solution adopts the finite difference method to solve the PDE. And, in our experiments, we set $u_0=g$ and $\alpha=1$.

After the generation of the threshold surface and implementation of thresholding, a binary image is created. Then we use two criteria to verify the identification of leukocytes: the area constraint and the isoperimetric ratio [8] constraint. We consider only the connected components that satisfy both constraints.

(1) Area constraint: We first remove objects of undesired area. This operation only preserves regions that are neither too large (twice the expected area or greater) nor too small (less than one half the expected area or smaller), in agreement with area knowledge about the scale of leukocytes.

(2) *Isoperimetric ratio constraints*: Let O be the connected component of interest. Let $a(O)$ denote area and $l(\partial O)$ denotes its boundary length. The isoperimetric ratio of O is defined:

$$i(O) = \frac{l^2(\partial O)}{a(O)} \tag{9}$$

$i(O) = 4\pi$ if and only if O is a disk. The isoperimetric ratio increases as the shape of connect component varies from circularity. We reject the connected components with large isoperimetric ratios. In this way, we remove clutter that has the same scale as the leukocytes.

4. EXPERIMENTAL RESULTS

In this section, we present the results by applying the proposed method to video frames obtained by transillumination of the mouse cremaster.

Fig. 3(a) is a typical sub-image with a poorly illuminated environment obtained from a video microscopic frame showing several rolling leukocytes in a mouse cremaster venule. Figs. 3(b) and (c) display, respectively, the approximation function u and the boundary function B with $\alpha = 1$. Fig. 3(d) shows the threshold surface. Fig. 3(e) is the result directly after thresholding. The final result, applying identification criteria, is shown in Fig. 3(f). A similar example is shown in Fig. 4. The results reveal that shapes of leukocytes are well preserved in the adaptive thresholding and detection process.

Furthermore, the detection process has been tested within 15 experiments. We use same parameters of our algorithm within the 15 experiments. The results are tabulated in Table 1. The total number of leukocytes is 102. As a performance measure, the average false positive rate and the average detection rate are 7.8% and 91.2% respectively. The primary source of error using our method is cell overlap and extreme clutter (muscle striation, for example).

5. CONCLUSION

We have presented an approach to identify rolling leukocytes *in vivo*. The scheme is based on minimizing a variational function from which a thresholding surface is constructed. Connected components of foreground in the thresholded binary image are identified as leukocytes in this process. We use a verification criterion, which incorporates geometric constraints, to obtain reliable results. Experimental results reveal the effectiveness of the proposed method.

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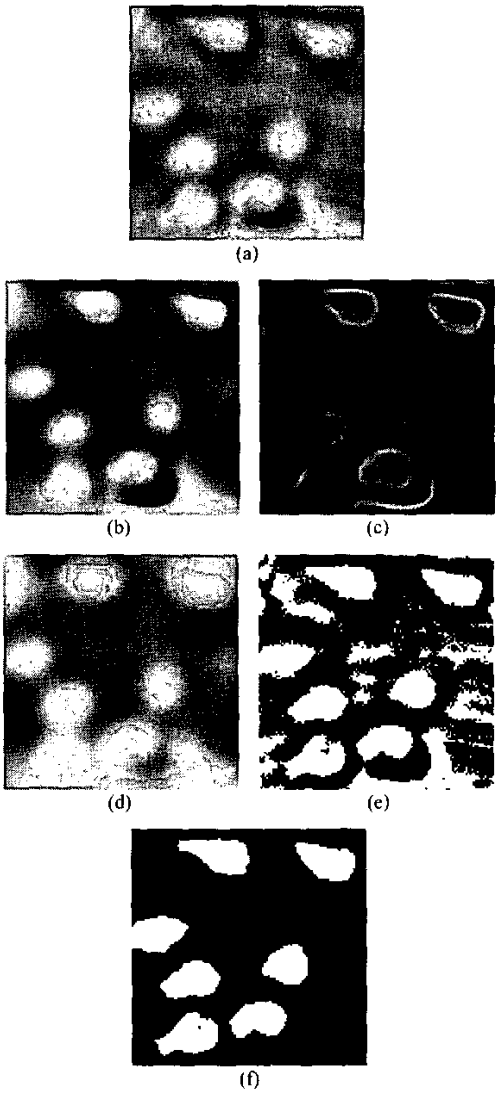

Figure 3. (a) Leukocytes, (b) the approximation image, (c) the boundary function, (d) the threshold surface image, (e) the result after thresholding, and (f) the final detection result.

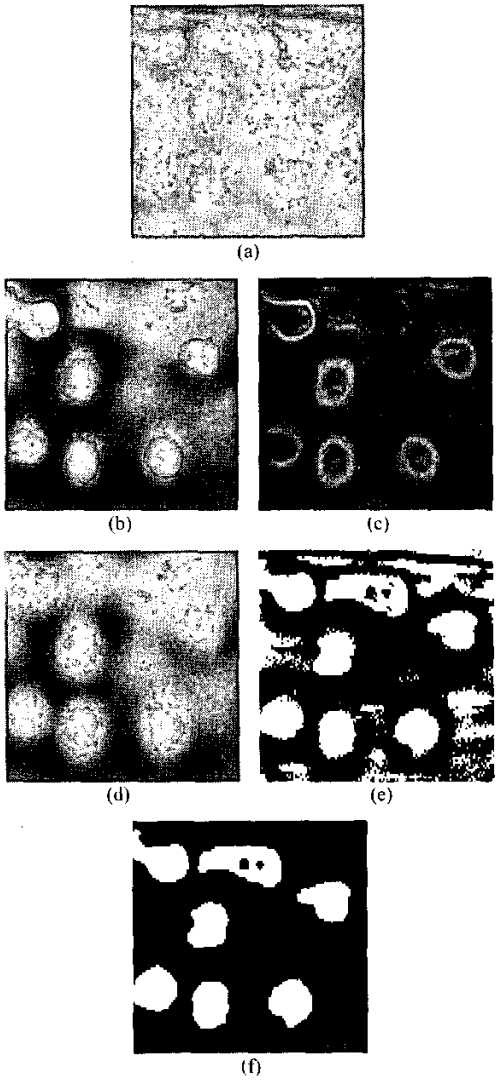

Figure 4. (a) Leukocytes, (b) the approximation image, (c) the boundary function, (d) the threshold surface image, (e) the result after thresholding, and (f) the final detection result.

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Table 1. Detector performance for 15 experiments.

	No. of leukocytes	No. detected	No. missed	No. of false positives
EXP. 1	7	7	0	0
EXP. 2	8	7	1	0
EXP. 3	8	6	2	1
EXP. 4	5	5	0	1
EXP. 5	6	6	0	0
EXP. 6	6	5	1	1
EXP. 7	7	6	1	0
EXP. 8	6	5	1	1
EXP. 9	5	5	0	1
EXP. 10	7	7	0	1
EXP. 11	7	7	0	0
EXP. 12	7	7	0	1
EXP. 13	6	6	0	0
EXP. 14	8	7	1	0
EXP. 15	9	7	2	1
Total	102	93	9	8