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Red Blood Cell Classification Through Depth Map and Surface Feature

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***Abstract*—The shape of red blood cell contributes more to clinical diagnosis with respect to blood diseases. In this paper, a compound classification algorithm for red blood cell was proposed, in which the depth map and surface curvature incorporated to deal with different kinds of cell shape. The original image was preprocessed to denoise and the individual 3-D shape of each cell was obtained with shape from shading technique, which was resolved by linear approximation under the partial differential equation IRE. The next procedure was to construct a segmentation algorithm based on surface curvature by fitting smooth multi-scale surface. The number of different shape could be used to classification. It is experimentally shown that the proposed algorithm can segment cells with complex surface shape successfully.**

Keywords-red blood celll ;shape from shading; surface curvature;classification;

I. INTRODUCTION

The erythrocyte deformability plays an important role in the filterability of blood and contributes more to the pathology research of relevant diseases. Traditionally we deal with image segmentation issue using intensity level image. However, the shape of Red Blood Cell provides more information for diagnosing accurately than 2-D gray tone image, which is obtained from Scanning Electron Microscope rather than optical imaging system in our experiment. So it is essential to take the shape into consideration in some real problem. In [1] elaborates the operation principle of Scanning Electron Microscope. SEM produces images that are particularly easy to implement because the brightness in it is a function of the slope of the specimen at that point and form a varied shading image, unlike optical and transmission electron microscope, whose brightness depends on the thickness and optical or electron density instead.

Many of the two-dimensional images have been sections through three-dimensional structures. This is especially true in the various types of microscopy, where either polished flat planes or cut thin sections are needed in order to form the images in the first place. But the specimens thus sampled are three-dimensional and the goal of the microscopist is to understand the three-dimensional structure [2].

Figure 1 shows a typical example of such kind of images we are dealing with. They were captured at 600 times magnification using a scanning electron microscope. There are some characteristics about the images that make our problem significant and challenging. On the one hand, the images represent very good quality with varied shading illuminated by light source. On the other hand, some light grid lines exist in the image, which were added for the sake of manual counting and those shapes of cells take on lots of irregular deformation. Those cause some difficulty in our classification task. We believed that conventional segmentation method based on gray value could not be suitable to this real case. Then a new strategy was proposed to segment according to surface feature, which is obtained by shape from shading, to acquire a 3D height field of cell surface.

The aims of our project are to estimate the distribution of erythrocyte shapes from scanning electron microscope images and classify them automatically. This paper is organized as follows: first the preliminary work including system framework and image preprocessing are introduced in section 2. Section 3 presents the method applied in this project, such as shape from shading technique using linear approximation under the SEM imaging conditions, region growing and curvature calculation, etc. The next section shows experiments and some results corresponding to this approach. In the end , section 5 draws a conclusion.

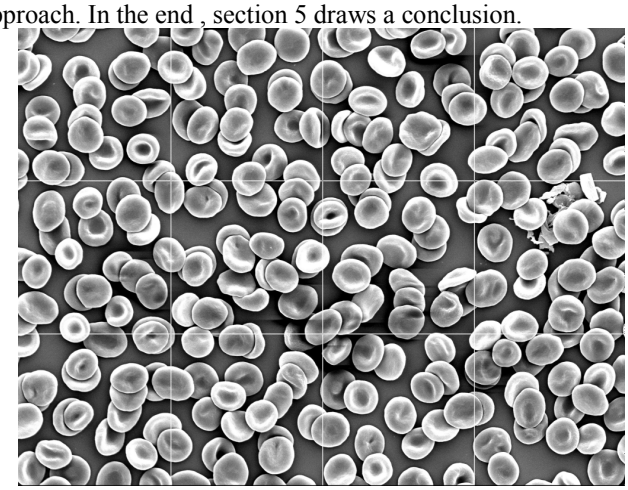

Figure 1. SEM image of Red Blood Cell with varied shading

II. IMAGE PREPROCESSING AND FRAMEWORK

As shown in Figure 1, there are lots of cells with different shapes, which cause a few difficulties to classify them through traditional 2D image processing methods. In terms of the 3D shape information being critical in our case,

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we transformed the 2D issue into 3D reconstruction by means of Shape from Shading technique. Firstly we view the grid lines as noise which we do not want and implement median filtering to remove. The median filter is a smoothing technique that causes minimal edge blurring, which replaces the pixel value at each point in an image with the median of the pixel values in a neighborhood about the point.

We can detect the exact position of those lines in the image by projection, which leads to a handily approach, i.e., filling locally with a median filtering, 7x7 structure element predefined.

The major process of the whole approach is described as follows :

STEP 1: Guided boundary contour tracing[3].

STEP 2: 3-D height field reconstruction using Shape from Shading Technique

STEP 3: Cell extraction individually based on seed region growing.

STEP 4: Computing surface feature, namely mean curvature accompanied with Gaussian curvature.

STEP 5: Segmentation through multi-scale surface fitting.

STEP 6: Classification by counting numbers of different surface types.

III. METHODS

A. Tracing contour

Vromen and McCane[4] proposed a model based contour tracing approach to the problem of automatically segmenting red blood cells in a Scanning Electron Microscope image. Because we don't care about what exact number of red blood cells contained in a whole image, only those on the top-level we want to deal with, assumed that the distribution of overlapped or obscured cells is identical to the overall distribution. After implemented tracing boundary contour, each cell's boundary points and center point can be drawn out, which can be used to extract all pixels in each of the cells through region growing with 4 neighbors relationship.

Each trace is initiated by choosing an initial direction perpendicular to the gradient vector at its starting point, as shown in Figure 2.

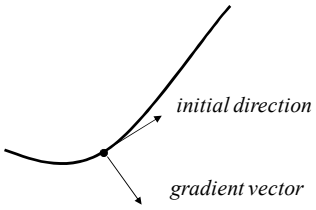


Figure 2. Schematic of tracing contour

After initialization, each iteration of the algorithm evaluates all possible directions using a probability distribution derived from gradient data around the focus point and the most likely direction based on the history of the trace so far, regarding only the directions with angles in a certain window around the predicted direction.

B. Image Transformation

When the sampled values in an image array represent light intensity at each point, knowledge of the gray level image formation process and an appropriate set of constraints can be used to recover the shape of 3D scene[5]. Image Irradiance Equation (IRE) indicates the relationship between reflection function and image irradiance. The recovered shape can be represented by depth map Z, normal (nx,ny,nz),or surface gradient (p,q). The radiance of surface patch is depend on gradient, light source location and reflectance property. The gray level of a pixel in the image is determined by light direction and normal vector. Assumed Lambertian model, the reflection function under the condition of SEM imaging can be formulated by[6][7]:

$$R(p,q)=\frac{\sqrt{1+p^2+q^2}\sqrt{1+p_s^2+q_s^2}}{1+pp_s+qq_s}$$

where $p=\frac{\partial Z}{\partial x}$ and $q=\frac{\partial Z}{\partial y}$, $(p_s,q_s,1)$ is the illumination direction, and Z is depth map of (x,y) . In our experiment, we set $p_s=q_s=0$.

By approximating the p and q discretely, we get

$$p=\frac{\partial Z}{\partial x}=Z(x,y)-Z(x-1,y) \quad q=\frac{\partial Z}{\partial y}=Z(x,y)-Z(x,y-1)$$

Through Taylor Expansion and Jacobi iteration[8], we can obtain the iterative equation of depth map Z.

$$Z^n(x,y)=Z^{n-1}(x,y)+\frac{f\left(Z^{n-1}(x,y)\right)\cdot(1+pp_s+qq_s)^2\cdot\sqrt{1+p^2+q^2}}{\left((1+pp_s+qq_s)(p+q)-(1+p^2+q^2)(p_s+q_s)\right)\cdot\sqrt{1+p_s{}^2+q_s{}^2}}$$

After tracing contour, each cell's boundary points and center point can be traced, which can be used to extract all pixels in each cell through region growing with 4 neighbor relationship. The 3-D height field corresponding to each pixel can be obtained by using shape from shading.

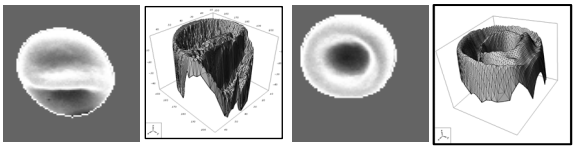


Figure 3. Original cells and their 3D shapes

A map from intensity image to range image has been established after height field reconstruction, in which the value of each pixel denotes the distance or depth to physical surfaces from a known reference surface. Figure 3 shows two kinds of cells with different shape accompanied by 3D height field. We can extract useful surface features from depth map to further each cell's segmentation.

C. Curvature Calculation

There are 8 different types of surface altogether, namely peak, pit, ridge, valley, flat, minimal surface, saddle ridge, saddle valley. The surface type of each data point on a scene object can be designated by the signs of mean curvature and Gaussian curvature uniquely. Both of these two curvatures can be calculated by local convolution[9]. Each data point in

a given $N \times N$ window is associated with a 2-dimensional position (u,v) from the set $U \times U$,

$U = \{-(N-1)/2,...,-1,0,1,...,(N-1)/2\}$

where N is odd.

Space curved surface $G \subset R^3$ can be parameterized as:

$$G = \left\{ g(u,v) = \begin{bmatrix} g_1(u,v) \\ g_2(u,v) \\ g_3(u,v) \end{bmatrix} : \begin{matrix} u_{\min} < u < u_{\max} \\ v_{\min} < v < v_{\max} \end{matrix} \right\}$$

The partial derivative estimate images are computed via the appropriate 2-D image convolutions (denoted *):

$$g_u = D_u * S * g \qquad g_v = D_v * S * g$$
$$g_{uu} = D_{uu} * S * g \qquad g_{vv} = D_{vv} * S * g$$
$$g_{uv} = D_{uv} * S * g$$

where $[S] = \vec{s}\vec{s}^T$ is a 7×7 binomial smoothing window.

$$\vec{s} = \frac{1}{64}[1 \;\; 6 \;\; 15 \;\; 20 \;\; 15 \;\; 6 \;\; 1]^T$$

And

$$[D_u] = \vec{d}_0\vec{d}_1^{\,T} \qquad [D_v] = \vec{d}_1\vec{d}_0^{\,T}$$
$$[D_{uu}] = \vec{d}_0\vec{d}_2^{\,T} \qquad [D_{vv}] = \vec{d}_2\vec{d}_0^{\,T}$$
$$[D_{uv}] = \vec{d}_1\vec{d}_1^{\,T}$$

where

$$\vec{d}_0 = \frac{1}{7}[1 \;\; 1 \;\; 1 \;\; 1 \;\; 1 \;\; 1 \;\; 1]^T$$
$$\vec{d}_1 = \frac{1}{28}[-3 \;\; -2 \;\; -1 \;\; 0 \;\; 1 \;\; 2 \;\; 3]^T$$
$$\vec{d}_2 = \frac{1}{84}[5 \;\; 0 \;\; -3 \;\; -4 \;\; -3 \;\; 0 \;\; 5]^T$$

The mean curvature and Gaussian curvature can be calculated by partial derivatives as follows:

$$H = \frac{(1+g_v^{\,2})g_{uu} + (1+g_u^{\,2})g_{vv} - 2g_u g_v g_{uv}}{2\left(\sqrt{1+g_u^{\,2}+g_v^{\,2}}\right)^3}$$

$$K = \frac{g_{uu}g_{vv} - g_{uv}^{\,2}}{(1+g_u^{\,2}+g_v^{\,2})^2}$$

IV. EXPERIMENTS

A. Segmentation Through multi-scale surface fitting[5]

The segmentation procedure is divided into two different parts. Firstly we compute the surface type label image

$T = 1 + 3(1 + 3\,\mathrm{sgn}_{\varepsilon_H}(H)) + (1 - \mathrm{sgn}_{\varepsilon_K}(K))$.

where T denotes the surface type ranging from 0 to 9.

The result of this stage is shown in Figure 4(a). Then find all connected components of each surface type label image and sort it to get histogram distribution. For surface fitting purpose, seed region is extracted through erosion (contraction) operation: for each ON pixel in the input binary image, test each of the eight neighbors of that pixel in the input image. If any neighbor is OFF, turn that pixel OFF in the input binary image. Pixels that are OFF in the input binary image remain OFF in the output binary image.

Iterative variable order multi-scale surface fitting is performed from the lowest order (planar), if it is OK using

(a) surface type imge (b) flat (c) pit (d) valley

Figure 4. Three kinds of surface type after thresholdi ng

RMS error and region test, then goto region growing step, otherwise increase the order and fit again until order is greater than 4. The region growing consists of finding new region involving compatible connected neighboring pixels. In our experiment, we set the RMS fit error $\varepsilon = w_1 . \sigma_{img}$, where σ_{img} means noise variance, and

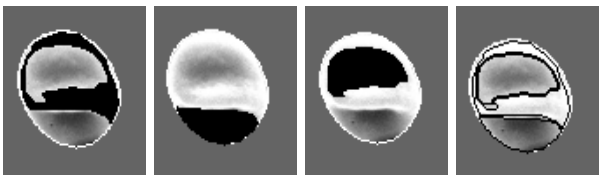
$$\hat{z}(p) = \hat{f}(m^k, \vec{a}_l, x(p), y(p))$$

is compared with $z(p) = \tilde{g}(x(p), y(p))$ to see if the pixel p is compatible with the approximating surface function. If the magnitude of the difference between the function value and the digital surface value is less than the allowed tolerance value, denoted $w_0 \cdot \varepsilon_l^k$, then the pixel p is added to the set of compatible pixels, denoted $C(m_k, \vec{a}_l^k, \varepsilon_l^k)$, which are compatible with the surface fit to the region $\hat{R}_l^k$. Otherwise, the pixel is incompatible and discarded. The result of this process is the compatible pixel list:

$$C(m^k, \vec{a}_l^k, \varepsilon_l^k) = \{p \in I : |\hat{z}(p) - z(p)| \le w_0 \varepsilon_l^k\}$$

we chose $w_1 = 4.5$ and $w_0 = 8$ experimentally.

All of the cells only contain three different surface type provided the thresholded mean curvature and Gaussian curvature: flat, pit, and valley respectively. As shown in Figure 5, the cell is segmented into three isolated parts perfectly, which are obtained through fitting based on surface type.



(a) flat region (b)pit region1 (c) pit region2 (d)segmentation

Figure 5. Segmentation through multi-scale surface fittingUnits

B. Classification

We proposed a simple criterion for red blood cell classification with regard to the counting number of different surface shapes involved. For example , the cell in Fig.5 includes 1 flat, plus 2 pits. Those cells with the same surface type number should be classified into one class. The classification result is shown in TABLE I.

V. CONCLUSION

Microscopic analysis of SEM image aims to classify the red blood cell. These objects often have huge variable shape and overlap heavily. Classification on these images is very challenging.

In this work, we developed a new strategy to extract surface features based on shape from shading and curvature computation. We believe that our features could be improved by histogram equalization to remove the side effect of varied shading. During the sample preparation, one thing that we have to pay more attention to is that the immediate fixation of the blood upon drawing is important because erythrocytes which are not immediately fixed tend to change their shape. Although some deformation exists during manual operation, the distribution of erythrocyte shapes from SEM images can be estimated correctly.

TABLE I. CELLS NUMBER IN EACH KIND OF SURFACE TYPE

Type	Flats	Pits	Valley	Cells Number
1	1	1	0	63
2	1	2	0	15
3	2	1	0	11
4	0	1	0	7
5	3	4	0	18
6	2	2	1	3
7	3	1	0	23
8	2	2	0	4
9	1	1	1	2
10	2	1	1	1
11	1	2	1	2

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