Robust Segmentation Of Cancer Affected White Blood Cells Using Modified Level Set Algorithm

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***Abstract* - The cancer cells are multiplicative in nature. Doctors face difficulties in counting the white blood cells (WBCs) at a particular stage due to crowding of cells. This paper proposes the robust segmentation algorithm that can reliably separate touching cells. Segmentation is the main important step in medical image processing. Precisely locating the area of interest in an image, in the presence of inherent uncertainty and ambiguity, is a challenging problem in medical imaging. Hence, one is often faced with a situation that demands proper segmentation. The algorithm is composed of two steps. It begins with a detecting and finding the cells in the region that utilizes level set algorithm. Next, the contour of big cell is obtained using modified level set active contour based on a piecewise smooth function. Finally, the proposed algorithm is compared with several images which aids in applications such as locating the tumours and other pathologies.**

Keywords - Active contour, Level set method, Piecewise smooth function, Heaviside function, Segmentation, Gaussian kernel

I. INTRODUCTION

People affected by Cancer in India are increasing day by day. A status shows that around 25lakh cases have been reported so far and this also predicts that about 0.25% of new cases were reported each year and nearly 0.1% of this population die every year due to this deadly disease. This diagnosis causes emotional trauma and its treatment is too costly to bear [1].

Segmenting individual cells in blood cancer is usually the first step that is required in automatic image analysis. And it is a challenging problem due to the complex nature of the cells. Image segmentation is the process of building a partition of the image into connected regions, such that pixels of the region are homogenous according to some criterion (gray value, motion, etc).

Automatically identifying and multiplying the cells by exploiting the shape and intensity characteristics of the cells was proposed by Dipti Prasad Mukherjee et al. An energy functional dependent upon the gradient magnitude along the cell boundary, the region homogeneity within the cell boundary and spatial overlap of the detected cells is minimized. Bjorn Nilsson at al uses the model based combinational optimization scheme to segment cluster based problems. They concentrate on clustering image not an overlapping region.

Each of these segmentation methods produced good results on regions exhibiting little or no cell crowding; however, they often failed to separate touching cells accurately. The watershed family of algorithms has become one of the most commonly used segmentation methods to address the challenge of touching cells. However, the primary limitation of the watershed approaches is that they often result in over segmentation. Some algorithms such as marker controlled watershed [4], Otsu method [5], rule based strategies [6-8] were developed to address this problem. When the intensity of overlapping regions is brighter (or darker) than the non overlapping regions within individual cells, a set of false seeds will be created in the overlapping regions [9]. This is not surprising because the voting schema in [9], is biased toward the boundary of the object. The edges of overlapping regions contribute to the creation of false seeds within the overlapping regions. The significant improvement of the new algorithm that applies a shifted Gaussian kernel [10] and mean shift onto single-pass voting to generate more accurate and quicker seed detection was proposed later.

The rest of the paper is organized as follows. The introduction to image segmentation and the related methods used in this work is described in section 2 and also we formulated the derivation of level set in this section. The modified level set algorithm is described in section 3. In section 4, we validate our method by various experiments on cancer and normal microscopic blood smear images.

II. IMAGE SEGMENTATION

In image processing and computer vision, partitioning an image into integrated regions is called segmentation [11]. Getting a more meaningful and analyzable image is the main aim of segmentation. Apart from this, locating boundaries is the main objective of segmentation.

More exactly, segmentation deals with assigning an unique label to each pixel in the object of interest such that they show a common characteristics. This reduces the pixel data to region based information.

A. *Need For Segmentation*

In image processing it can be number of pixels with the same intensity in general. Segmentation is to separate the homogeneous area. The analysis of blood slides is a powerful tool in determining the health status of an individual and could detect several diseases. The count and shape, lineage and maturity level of white and red blood cells could aid in the diagnosis of diseases that range from inflammatory to leukemia. Many automated techniques were proposed to overcome the tedious and time consuming task of human experts in counting and classifying white blood cells. Various techniques were used for the segmentation stage including mean shift algorithm, histogram equalization, thresholding, watershed algorithm.

B. *Level Set Method*

The level set method (LSM) is a numerical technique for tracking interfaces and shapes. The advantage of the level set method is that, it doesn't need variable parameters [12]. Also, the level set method easily keeps track of topological changes. When an object of interest merges or splits. The advantage of level set methods are implicit, parameter free method and provides a direct way to estimate the geometric properties of evolving structure and the applications include edge extraction, code tracking and contour tracking.

Consider the most general case the following form of curve propagation is

$$C(p,t) = F(k) * N \quad (1)$$

where C is a closed curve propagation
F(k) is a force and N is normal to curve/surface
The level set method represents the curve in the form of an implicit surface is

$$\emptyset(x, y, t) : R^2 x[0, t) \rightarrow R \quad (2)$$

The level set method accounts to representing a closed curve using an auxiliary function ø called the level set function. This is derived from the initial contour according to the following condition

$$C(p, 0) = \{(x,y) : \emptyset(x, y, 0) = 0\} \quad (3)$$

and the level set method manipulates C implicitly, through the function ø. ø is assumed to take positive values inside the region and negative values outside the region which is determined by the curve propagation C.

The level set flow can be re-written in the following form

$$\emptyset_t + H(\emptyset_x, \emptyset_y) = 0 \quad (4)$$

where H is a Hamiltonian.

C. *Mathematics of Level Set Method*

Consider the image I that has N cells. Assume that the image u_o is formed by two regions of approximatively piecewise-constant intensities, of distinct values u_o^i and u_o^o. Assume further that the object to be detected is represented by the region with the value u_o^i. Let denote its boundary by C_o. Then $u_o \approx u_o^i$ we have inside the object [or inside (C_o)], and $u_o \approx u_o^o$ outside the object [or outside (C_o)]. Now let us consider the following "fitting" term or level set energy functional term:

$$F_1(C) + F_2(C) = \int_{inside(C)} |I - c_1|^2 \, dx\, dy + \int_{outside(C)} |I - c_2|^2 \, dx\, dy \quad (5)$$

Where c_1 and c_2 are constants depending on C are the averages of u_o inside C and respectively outside C. The energy term can be written as the sum of two fitting terms. In this simple case, it is obvious that, the boundary of the object, is the minimize of the fitting term

$$\inf_C F_1(C) + F_2(C) \approx 0 \approx F1(Co) + F2(Co) \quad (6)$$

The constants c_1 and c_2 can be denoted as

$$C1 = \int H(x, y)K(x, y)dx\, dy \quad (7)$$

$$C2 = \int (1 - H(x, y))K(x, y)dx\, dy \quad (8)$$

where H(x,y) is Heaviside function which will be discussed in next section and K(x,y) is defined as 2D Gaussian kernel function:

$$K = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{|x^2 + y^2|}{2\sigma^2}\right) \quad (9)$$

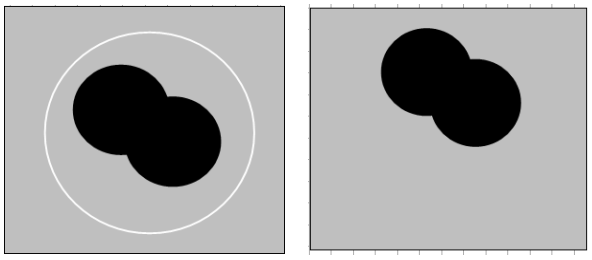
σ is standard deviation.

For instance, if the curve C is outside the object, then $F_1(C) > 0$ and $F_2(C) \approx 0$. If the curve is inside the object, then $F_1(C) \approx 0$ but $F_1(C) > 0$. If the curve is both inside and outside the object, then $F_1(C) > 0$ and $F_2(C) > 0$. Finally, the fitting energy is minimized if $C=C_o$, i.e., if the curve is on the boundary of the object. These basic remarks are illustrated in Figure 1.

In active contour model we will minimize the above fitting term and we will add some regularizing terms, like the length of the curve C, and or the area of the region inside C proposed by M.Kass et al, which is based on the Mumford-Shah model [14]. Therefore, we introduce the energy functional F (c_1, c_2, C), defined by

$$F(c_1, c_2, C) = \mu * \text{length}(C) + v * \text{Area}(\text{inside}(C)) + \lambda_1 \int_{inside(C)} |I - c_1|^2 \, dx\, dy + \lambda_2 \int_{outside(C)} |I - c_2|^2 \, dx\, dy \quad (10)$$

$F_1(C)>0, F_2(C)\approx 0$, Fitting>0 $F_1(C)\approx 0, F_2(C)>0$, Fitting>0



$F_1(C)>0, F_2(C)>0$, Fitting>0 $F_1(C)\approx 0, F_2(C)\approx 0$, Fitting$=0$

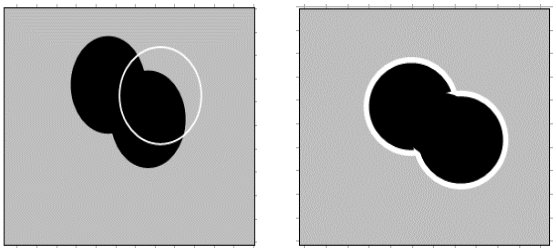


Figure1. Boundary condition

$\mu \geq 0$, $v \geq 0$, $\lambda_1, \lambda_2 > 0$ are fixed parameters. In numerical calculations, $\lambda_1 = \lambda_2 = 1$ and $v = 0$. If this value is small enough, then it segments smaller objects otherwise it segments larger objects in the cell region.
Therefore, consider the minimization problem is

$$\inf_{c_1, c_2, C} F(c_1, c_2, C) \quad (11)$$

In the isoperimetric inequality the length is comparable with area is given by

$$\text{Area (inside(C))} \leq c^* \text{ length (C)} \quad (12)$$

where c is a constant.

D. Relation with the Mumford - Shah Function

The Mumford–Shah functional [15] for segmentation is

$$F^{MS}(u, C) = \mu * \text{length}(C) + \lambda \int_{\Omega} |I - u(x,y)|^2 \, dxdy + \int_{\Omega/C} |\nabla u(x,y)|^2 \, dxdy \quad (13)$$

where μ and λ are positive parameters. A reduced form of this problem is simply the restriction of F^{MS} to piecewise constant functions u, i.e., u = constant c_i on each connected component of Ω/C. Therefore, as it was pointed out by D. Mumford and J. Shah, c_i = average (u_o) on each connected component. The reduced case is called the minimal partition problem.
The active contour model with $v=0$ and $\lambda_1 = \lambda_2 = \lambda$ is a particular case of the minimal partition problem, in which we look for the best approximation u of u_o, as a function taking only two values, namely

$$u = \begin{cases} \text{average}(u_o) \text{ inside } C \\ \text{average}(u_o) \text{ outside } C \end{cases} \quad (14)$$

and with one edge C, represented by the snake or the active contour.

E. Level Set Formulation

In the level set method [15-16], the closed curve C, inside(C) and outside(C) which is represented by the zero level set of a Lipschitz function as in figure 2.

$$C = \partial\omega = \{(x,y) \in \Omega : (x,y) = 0\}$$
$$\text{inside}(C) = \omega = \{(x,y) \in \Omega : (x,y) > 0\} \quad (15)$$
$$\text{outside}(C) = \Omega / \bar{\omega} = \{(x,y) \in \Omega : (x,y) < 0\}$$

For the level set formulation of our variational active contour model, we replace the unknown variable C by the unknown variable ø.

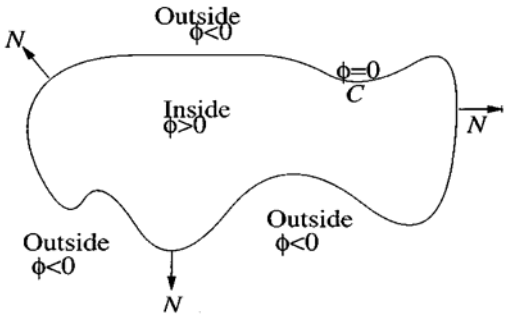


Figure 2. Lipschitz Function

Using the Heaviside function H, and the one-dimensional Dirac measure δ_o, and defined, respectively, by

$$H(z) = \begin{cases} 1, & z \geq \epsilon \\ 0, & z \leq -\epsilon \\ \frac{1}{2}\left[1 + \frac{z}{\epsilon} + \frac{1}{\pi}\sin\left(\frac{\pi z}{\epsilon}\right)\right], & |z| \leq \epsilon \end{cases} \quad (16)$$

Differentiate Heaviside function with respect to z, we get

$$\frac{dH}{dz} = \lim_{\Delta z \to 0} \frac{\Delta H}{\Delta z} \quad (17)$$

where $\Delta H/\Delta z$ is the slope of the closed curve. H should be a flat line in regions, therefore slope becomes zero. Then dH/dz is zero for $z > \epsilon$ and $z < \epsilon$.
If $z = \epsilon$, $z_- = -a/2$, $z_+ = +a/2$, then slope gives

$$\frac{dH}{dz} = \lim_{a \to 0} \frac{1}{a} \quad (18)$$

$$\frac{dH}{dz} = \infty \quad (19)$$

The delta function,

$$\delta(z) = \frac{dH}{dz} = \begin{cases} 0, & z < \epsilon \\ \infty, & z = \epsilon \\ 0, & z > \epsilon \end{cases} \quad (20)$$

The energy function can be minimized by iteratively employing the gradient descent method.

$$\frac{\delta E}{\delta \emptyset} = \lim_{\varepsilon \to 0} \frac{d}{d\varepsilon}\left(\frac{1}{2}\int_{\Omega} |I - c_1 H_\varepsilon(\emptyset) - c_2(I - H_\varepsilon(\emptyset))|^2\right)$$
$$\lim_{\varepsilon \to 0}\left(-\int_{\Omega}[I - c_1 H_\varepsilon(\emptyset) - c_2(1 - H_\varepsilon(\emptyset))](c_1 - c_2)\delta_\varepsilon(\emptyset)\eta dx\right)$$
$$= -\int_{\Omega}[I - c_1 H_\varepsilon(\emptyset) - c_2(1 - H_\varepsilon(\emptyset))](c_1 - c_2)\delta_\varepsilon(\emptyset)\eta dx \quad (21)$$

By this method, all the edges are detected. But some of the smaller cells are not detected. To detect those smaller areas, Euler Lagrange equation is employed. This equation acts locally to separate smaller objects. Finally, all the edges are detected by level set formulation.

III.MODIFIED LEVEL SET ALGORITHM

We modified the level set algorithm by incorporating the active contour into level set. We have applied the level set only within the region. This is achieved by the convolution of piecewise smooth function (active contour) and Heaviside function (level set). We incorporate this modification in step 4 which will discuss in later section.

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F. Active Contour

Active contour model [17], delineates the area of interest outline from a noisy image. The objective is energy function minimization ie. Energy function of the present contour is represented (breakup into) as it is a summarization of external and internal energy. This model is also known as snake model.

Internal energy is defined within the contour itself to maintain the contour smooth and external energy is computed from the image data to move the contour toward an object boundary. When the external and internal energy becomes equal, the energy attains equilibrium and contour stabilizes.

Internal Energy of the snake is

$$E_{internal}=E_{cont}+E_{curv} \quad (22)$$

where E_{cont} is the energy of the snake contour

E_{curve} is the energy of the spline curvature.

$$E_{internal} = (\alpha(s)\,|v_s(s)|^2 + \beta(s)\,|v_{ss}|^2)\,/2$$

$$= (\alpha(s)\|\frac{d\mathbf{v}}{ds}(s)\|^2 + \beta(s)\,\|\frac{d^2\mathbf{v}}{ds^2}\|^2)\,/\,2 \quad (23)$$

The first-order term makes the snake act like a membrane and second-order term makes it act like a thin plate. Large values of α(s) will increase the internal energy of the snake as it stretches more and more, whereas small values of α(s) will make the energy function insensitive to the amount of stretch. Similarly, large values of β(s) will increase the internal energy of the snake as it develops more curves, whereas small values of β(s) will make the energy function insensitive to curves in the snake. Smaller values of both α(s) and β(s) will place fewer constraints on the size and shape of the snake.

The 2D active uses the point distribution model to minimize the shape range to a well known domain as a result of training. The advantages over classical techniques are such that snakes are autonomous and self-adapting in their search for a minimal energy state. They can be easily manipulated using external image forces. They can be made sensitive to image scale by incorporating Gaussian smoothing in the image energy function. They can be used to track dynamic objects in temporal as well as the spatial dimensions [17].

This model is used to segment white blood cell areas by embedding the local image information. The energy functional area is given by

$$E(C, f_1, f_2) = \lambda_1 \int_{\Omega} \int_{inside(C)} K_\sigma(x-y)|I - f_1|^2 dy\,dx + \lambda_1 \int_{\Omega} \int_{outside(C)} K_\sigma(x-y)|I - f_2|^2 dy\,dx \quad (24)$$

Where λ_1 and $\lambda_2 > 0$ are fixed parameters, K_σ is Gaussian kernel with standard deviation σ, f_1 and f_2 are two smooth functions that approximate the local image intensities inside and outside of contour C respectively.

$$f1(x) = \frac{K_\sigma * [H_\epsilon(\varnothing) I(x)]}{K_\sigma * H_\epsilon(\varnothing)} \quad (25)$$

$$f2(x) = \frac{K_\sigma * [(1-H_\epsilon(\varnothing)) I(x)]}{K_\sigma * (1-H_\epsilon(\varnothing))} \quad (26)$$

The standard deviation σ plays an important role in practical applications and this value varies for several images.

In the above equation, the regularized parameter of Heaviside function H and dirac function ∂ are as follows:

$$H_\epsilon(z) = \frac{1}{2}\left[1 + \frac{2}{\pi}\arctan\left(\frac{z}{\epsilon}\right)\right] \quad (27)$$

$$\partial_\epsilon(z) = \frac{1}{\pi}\frac{\epsilon}{\epsilon^2+z^2} \quad (28)$$

The flow of level set using ACM is as follows:

STEP 1: Initialize the parameters: Є, σ, σ_{phi}.

STEP 2: Find distance between the center and radius.

STEP 3: Apply Heaviside function and Delta function.

STEP 4: Apply piecewise smooth function to energy functional terms.

STEP 5: Start the iteration value.

STEP 6: Obtain the convolution of distance and K-Phi.

STEP 7: Update the iteration value in order to segment the interested region.

IV. RESULTS AND DISCUSSION

Microscopic blood smear images and the cancer affected blood images were taken to validate the algorithm. Normal blood cells and cancer affected blood cells were segmented individually using modified level set algorithm and the parameters were calculated and listed in Table 1.

The figure 3 shows that input images which contains Red Blood Cells (RBC)&WBC. Image C&D is affected by cancer and remaining cells normal blood cells. Cancer cells are cells that grow and divide at an unregulated, quickened pace. However, more recent research has that the failure to recognize cancer cells is caused by the lack of particular co-stimulated molecules

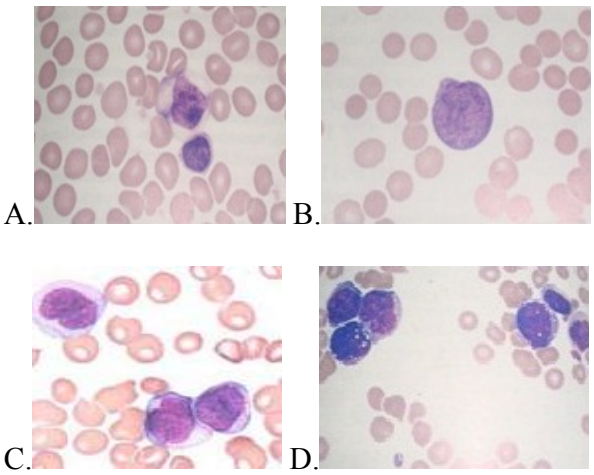


Figure 3. Input Images

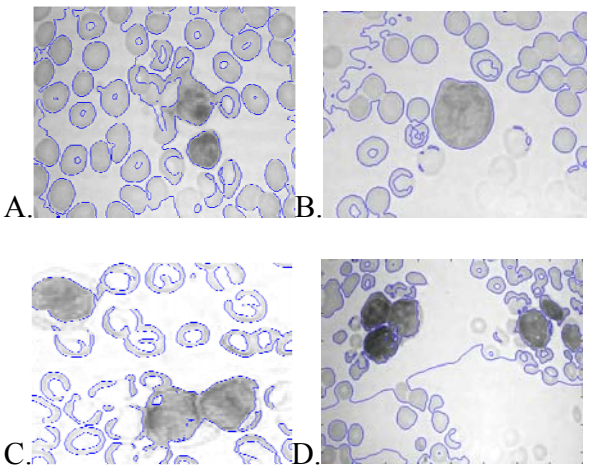


Figure 4. Level set algorithm result without adding active contour

That aid in the way antigens react with lymphocytes(WBC). WBC is thought to use a dual receptor system when they determine whether or not to kill human cells.

The figure 4 shows that the existing algorithm results in segmenting both RBC and WBC. For image segmentation, we have to find the radius of the cell region. Figure 5. show the radius for various input images

G. *Modified Level Set Algorithm*

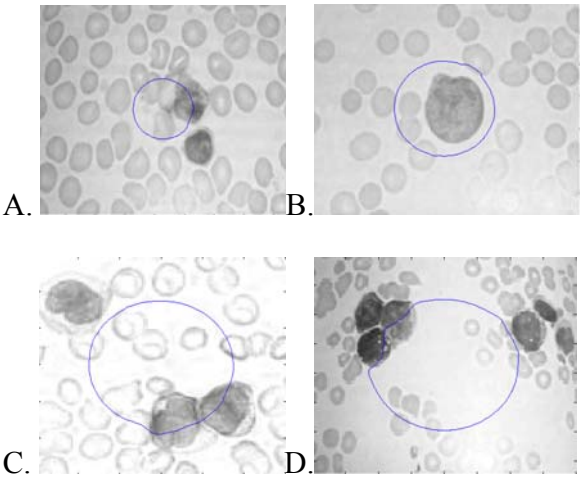
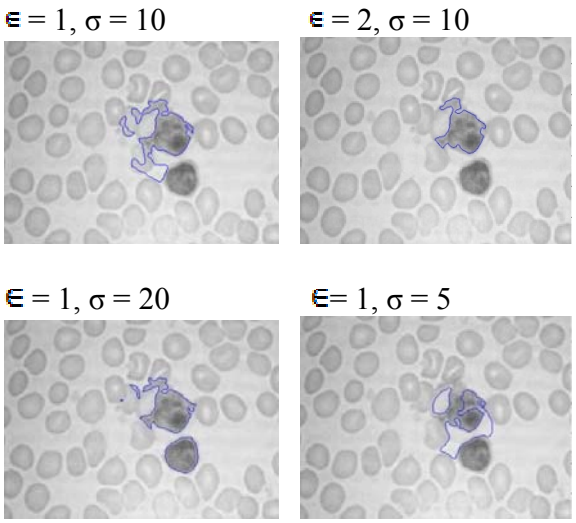


Figure 5. Radius of the images

After finding the radius, it starts segmenting the white blood cells. The parameters to obtain the optimum result are as follows in Table 2. This parameter will be tuned properly for various images as shown in figure 6.

H. *Parameter Validation*



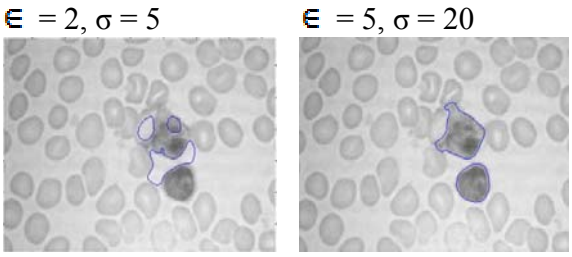


Figure 6. Parameter Validation for our method

Here, for different values of ∈(epsilon) and σ(sigma) segmentation is done to normal images. From that we can examine that segmentation occurs best for ∈=5 and σ=20. So we fix these values for the remaining segmentation evaluation.

TABLE I PARAMETER VALIDATION FOR INPUT IMAGES

Parameter	Image A	Image B	Image C	Image D
σ_{phi}	1.3	0.5	1	1.4
∈	5	5	5	5
Radius, r	30	25	40	45
σ	20	20	20	20

The segmented result for the constant values of ∈, σ is as follows in figure 7.

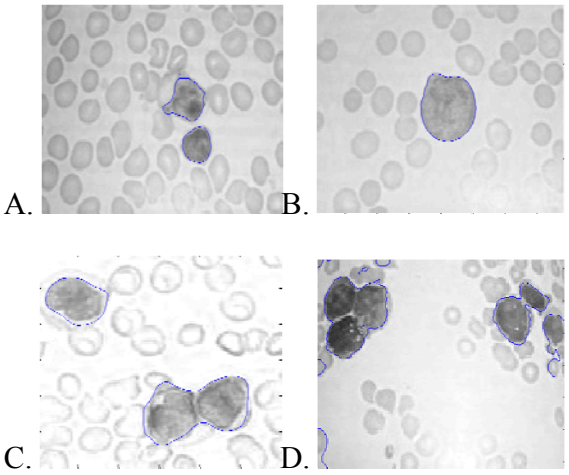


Figure 7. Segmented Result for our method

The parameters will vary for different images. The parameter σ_{phi} plays an important role in segmentation and it must be tuned very properly.

TABLE II VALUES FOR VARIOUS BLOODS SMEAR IMAGES

Measure	*Image A*	*Image B*	*Image C*	*Image D*
Iteration	3300	2400	4000	4000
Mean	35.2128	29.1133	1.3009	1.6395
Variance	241.3513	193.9551	9.1203	8.3557
Output Value	57.5403	51.1224	10.2471	10.5306
Heaviside Function	0.9724	0.9938	8.8555	0.8589

V.CONCLUSION

Thus the level set using active contour algorithm was evaluated and tested for various blood cells. This algorithm gives the segmentation result of white blood cells. This algorithm begins with detecting the cells in the region and by using those regions, white blood cells alone segmented.

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