

# SEM Image Analysis of Megaloblastic Anemia

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**Abstract:** Megaloblastic anemias are a heterogeneous group of disorders that have common blood abnormalities and symptoms. Its characteristics consist of large oval erythrocytes, hyper segmented neutrophils and large abnormal platelets. In this paper, a new approach is adopted for the diagnosis of megaloblastic anemia in blood smear. Different SEM images of megaloblastic anemia are compared with two level set segmentation models for the diagnosis. The image segmentation result of level set method is subject to appropriate initial contour and optimal configuration of controlling parameters. These models can detect objects whose boundaries are not defined by gradient and local regions. This paper describes an autonomous approach for deciding initial contour of level set and hence help in diagnosis.

**Keywords:** Megaloblastic anemia, Image processing, Level set segmentation

## 1. Introduction

Megaloblastic anemia has been recognized as a clinical entity for over a century. This results from the inhibition of DNA synthesis during RBC production [1]. The defect in red blood cell DNA synthesis is most often due to the deficiency of vitamin B12 and/or folic acid. These vitamins are essential for DNA biosynthesis. Megaloblastic anemia leads to morbidity if unrecognized or misdiagnosed [2]. In Megaloblastic anemia, red blood cell disorder is caused by incomplete formation of the red blood cell resulting in large numbers of immature and incompletely developed cells. These cells are underdeveloped, and they also have a short life expectancy. Megaloblastic anemia can be identified by cells having hyper segmented neutrophils (those exhibiting five or more nuclear lobes or segments) [3].

Megaloblastic anemia can be diagnosed using different image processing techniques. One of the best techniques that can be used is the level set segmentation. Hyper segmented neutrophils can be identified by level set segmentation and can find the number of segmented portion to confirm whether it is megaloblastic anemia or not.

The level set method can be used to address efficiently the problem of curve or surface propagation in an implicit manner. The central idea is to represent the evolving contour using a signed function, where its zero level corresponds to the actual contour [4]. Then according to the motion equation of the contour, one can easily derive a similar flow for the implicit surface that when applied to the zero-level will reflect the propagation of the contour [5, 6]. The level set method encodes numerous advantages: it is implicit, parameter free, provides a direct way to estimate the geometric properties of the evolving structure, can change the topology and is intrinsic. One can conclude that it is a very convenient framework to address numerous applications of computer vision and medical image analysis [3]. So, research into various level set data structures has led to very efficient implementations of this method.

## 2. Mathematical Expressions

### 2.1 Manual contour in classical level set

Level set method has become a well-established tool in the field of image processing. In two spatial dimensions, the technique extends to three dimensions with implicit interface definition. Let of  $I$  be a given  $n$ -dimensional image and let  $\Omega$  be a bounded open subset of  $I$ . In the level-set form, the evolving interface  $C$  is represented as the zero level-set of a sign distance function  $\phi$  of  $n+1$  dimension that satisfies [7]:

$$\begin{aligned} \phi(x) &> 0, \forall x \in \Omega_{in} \\ \phi(x) &< 0, \forall x \in \Omega_{out} \\ \phi(x) &= 0, \forall x \in C \end{aligned} \quad (1)$$

Where in  $\Omega_{in}$  the region of inside in bounded  $C$ , and the region out  $\Omega_{out}$  is defined as  $\Omega \setminus \Omega_{in}$ .

### 2.2 Active Contours without Edges – Chan-Vese (C-V) Model

A well-known and classical level set model is C-V model that uses constant intensity energy, and it's one of models combining with our initialization method. In [8], Chan and Vese defined the energy functional  $F(C, u, v)$ , as follow:

$$F(C, u, v) = \mu \text{Length}(C) + \lambda_1 \int_{\text{inside}(C)} |I - u|^2 dx dy + \lambda_2 \int_{\text{inside}(C)} |I - v|^2 dx dy \quad (2)$$

This energy models the foreground and background as constant intensities represented by their means,  $u$  inside  $C$  and  $v$  outside  $C$ . However, they can be quite different from

the original data if the intensities in either inside  $C$  or outside  $C$  are not homogeneous. Therefore, the C-V model generally fails to segment images with intensity inhomogeneity [9].

Keeping  $u$  and  $v$  fixed, and minimizing  $\phi$  ( $\phi$  denotes the signed distance function, also called SDF), they deduce the associated Euler-Lagrange equation for  $\phi$  as follows:

$$\frac{d\phi}{dt} = \delta_{\epsilon}(\phi) \left[ \mu \text{div} \left( \frac{\nabla \phi}{|\nabla \phi|} \right) - \lambda_1 (I - u)^2 + \lambda_2 (I - v)^2 \right] \quad (3)$$

Where  $\delta$  denotes one-dimensional Dirac measure.

C-V method is not based on an edge-function to stop the evolving curve on the desired boundary, and it doesn't need to smooth the initial image, even if it is very noisy, the locations of boundaries are very well detected and preserved [8]. Although C-V model is no specific requirement for the location of initial contour, it is often computationally expensive and not used to obtain desirable segmentation when initial contour is improper. In summary, C-V model has some disadvantages. Firstly, it is easily influenced by initial position, for improper initial position will mostly affect the convergence effect and time. Secondly, when the finite difference is used to solve the Euler-Lagrange equation of functional of C-V model, the time step must be very small due to CFL (Courant-Friedrichs-Lewy) condition. In addition, after updating level set function several times, it is necessary to reinitialize Sign Distance Function (SDF) to ensure stability of calculation. In order to handle these problems, an autonomous approach for deciding initial contour of level set. The initial contour attained by our method is very close to the real contour [10].

### 2.3 Localizing region-based active contours – Lankton model

In [6], Lankton proposed local region-based framework for guiding active contours. The framework describes foreground and background in terms of smaller local regions instead of global statistics. In Lankton model, they let  $B(x, y)$  to mask local regions, and there is an important spatial variable  $y$ . They used  $x$  and  $y$  as independent spatial variables each representing a single point in  $\Omega$ , where  $x$  is the point of zero level set,  $y$  is defined in the following characteristic function in terms of a radius parameter  $r$ :

$$B(x, y) = \begin{cases} 1, & |x - y| < r \\ 0, & \text{otherwise} \end{cases} \quad (4)$$

This function will be 1 when the point is within a ball of radius  $r$  centered at  $x$  and 0 otherwise. They defined the energy functional in terms of a generic force function  $F$  as follows:

$$E(\phi) = \int_{\Omega} \delta\phi(x) \int_{\Omega} B(x, y) \cdot F(I(y), \phi(y)) dy dx + \lambda \int_{\Omega} \delta\phi(x) |\nabla\phi(x)| dx \quad (5)$$

In the paper of [6], Lankton introduced some specific forms of  $F$  that can be inserted into  $E(\phi)$ , Chan-Vese energy [8], Yezzi energy [11].

In the localized version, the inside mean  $u$  and outside mean  $v$  in C-V model are respectively replaced by local means  $u_x$  and  $v_x$ . Local region-based method proposed by Lankton can effectively solve the over-segmentation phenomenon, but it brings some other problems at the same time. The manual initialization is uncertain. Someone may think about magnifying the variable radius  $r$  to handle this problem, but it is also invalid. Because at this time, the efficiency of algorithm is similar to the global C-V method and even needs more time cost for increasing the step of local extraction. In

addition, it is likely to make mistakes for the radii of adjacent point may intersect each other. What's more important with the radius becoming larger, the number of iterations and amount of calculation increase more quickly. One effective solution is to find an initial contour close to the object edge [10, 12]. In this paper, to achieve this goal, we propose an autonomous approach for deciding initial contour of level set.

### 3. Results and Discussion

In order to demonstrate the strengths and limitations of the proposed method, part of image segmentation process and results on various images are compared with different level set methods under autonomous initialization. In this section, we present the comparison results of C-V level set segmentation by autonomous initialization and then make a comparison between Lankton level set segmentation by autonomous initialization. Experiment results confirm the effectiveness of autonomous approach for deciding initial contour. All algorithms are implemented with the help of a software framework Creaseg with Matlab R2009a (Math Works) in a Windows XP system (Microsoft). The samples are collected from different patients who is having megaloblastic anemia. The results show that Chan-Vese and Lankton models can easily detect the hyper-segmented neutrophils in blood samples.

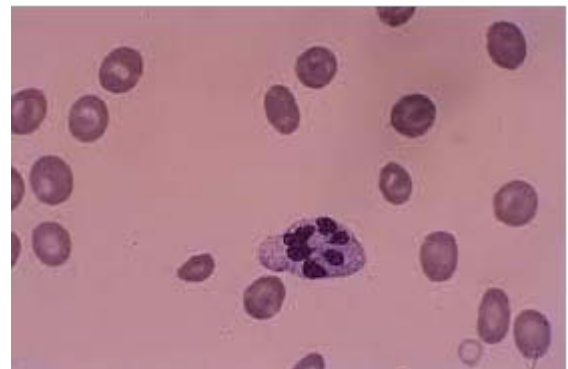


Figure 1: First SEM Image of megaloblastic anemia blood sample



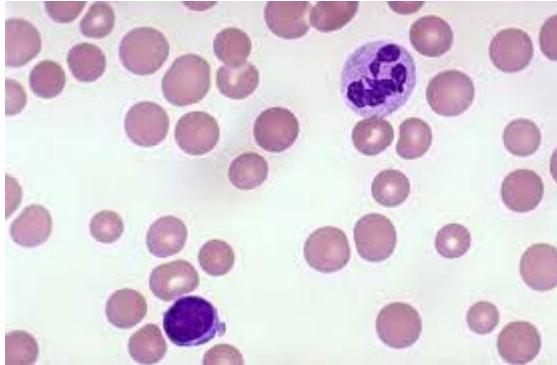
Figure 2: First blood sample which is segmented by C-V model



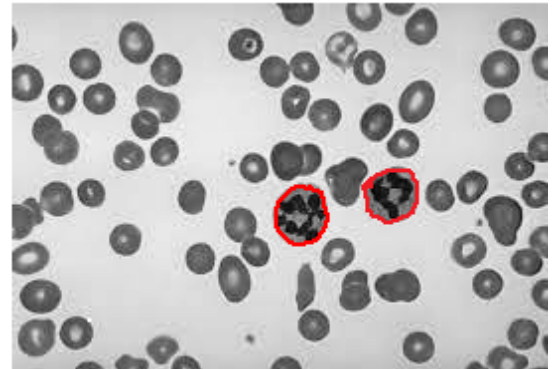
**Figure 3:** First blood sample which is segmented by Lankton model



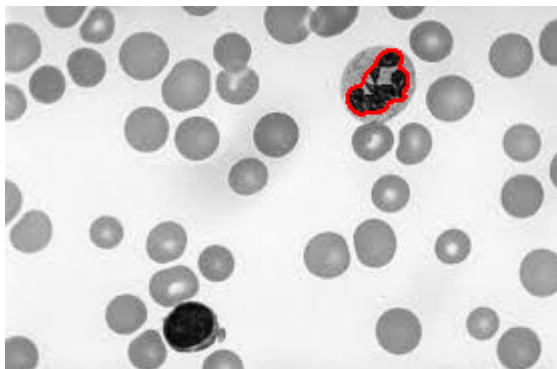
**Figure 7:** Third SEM Image of megaloblastic anemia blood sample



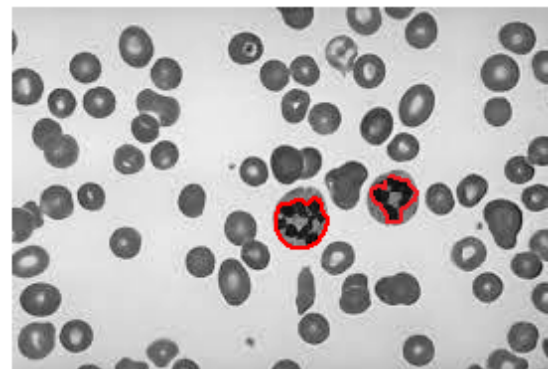
**Figure 4:** Second SEM Image of megaloblastic anemia blood sample



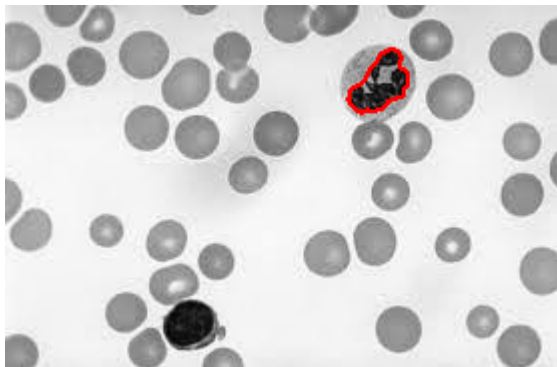
**Figure 8:** Third blood sample which is segmented by C-V model



**Figure 5:** Second blood sample which is segmented by C-V model



**Figure 9:** Third blood sample which is segmented by Lankton model



**Figure 6:** Second blood sample which is segmented by Lankton model

In all the SEM blood sample images there is the presence of neutrophils but has to know whether it is megaloblastic anemia by determining the characteristics of this type of anemia. Chan-Vese and Lankton models determine the images in two different ways. Chan-Vese identifies the hyper-segmentation of neutrophils by marking the neutrophils inside the red blood cells. Lankton models can also segment the neutrophils by marking around the neutrophils by which it determines the number of hyper-segmented neutrophils.

In most cases, the level set segmentation methods with autonomous initialization method not only largely reduces manual intervention, but also improves the result of segmentation and reduces calculation cost during evolution process. The result that is obtained identifies the megaloblastic anemia cells in the blood smear. The hyper



segmented neutrophils are segmented by different models of level set segmentation. Thus diagnosis has become more convenient. A considerable challenge in image segmentation in real world images is intensity inhomogeneity. The existing image segmentation algorithms rely on region based and it typically features similarity of image intensities in the region of interest. Images that detect the megaloblastic anemia is somehow be more proper to detect the fully hyper segmented neutrophils. The present work merely focused on deciding the initial contour of neutrophils to determine whether the blood sample image is megaloblastic or not.

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