Cell Segmentation from Cellular Image

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***Abstract*-To understand the cell movement and cell behavior into different parts of organs in human or animal body, it is necessary to study the cells in culture medium. Fluorescence microscopy is an emerging tool for acquiring this cellular images. The large number of cellular images produced by fluorescence microscopy is unmanageable for human to analyze them manually. Thus, cellular image segmentation is a primary requirement for higher level analysis of medical diagnosis and research. In this paper, a fully automatic method for segmentation of cells from fluorescent microscopy images is proposed. The method first mark the probable foreground and background seeds planted on the image. Then it applies a popular segmentation method, namely watershed segmentation, based on these seeds. The result is further refined based on the gradient value along the initial segmented lines and then again performing watershed transform on the distance transformed image of the previously founded result. The experimental results shows that this approach for segmenting cell images is both fast and robust.**

I. Introduction

In biology, a common problem is the segmentation of cells for counting and feature extraction purposes. Large scale quantification of the dynamical behavior of cell populations in a variety of experimental systems would provide important capabilities for many areas of cell biology. In addition, such quantitative cellular data would be useful in many theoretical contexts.Segmentation is the partitioning of a scene into different meaningful regions, by identifying regions of an image that have common properties while separating regions that are dissimilar [1]. It is often the first, most vital, and most difficult step in an image analysis task. The result of the segmentation usually determines eventual success of the final analysis. For this reason, many segmentation techniques have been developed by researchers worldwide, and there exist almost as many segmentation methods as there are segmentation problems.Cell image segmentation is a necessary first step of many automated biomedical image processing procedures. There certainly has been much research in the area. Automatic segmentation of cell nuclei from fluorescence microscopic cellular images allows the study of individual cell nuclei within their natural tissue context. Compared with manual methods based on drawing the outlines of the nuclei with a mouse, automatic methods need far less interaction, and the result is more objective and easily reproduced. Automation also increases the amount of data that can be processed. Once the objects of interest have been delineated, a large number of descriptive features can be extracted from the objects [2].

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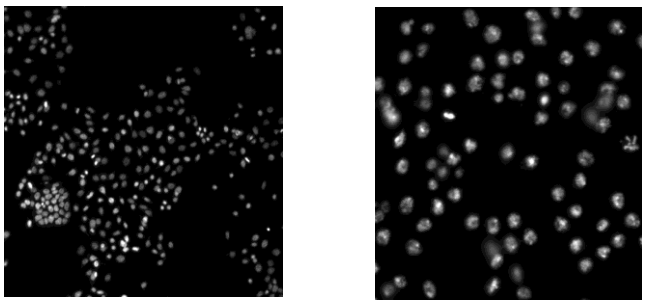

(a) (b)

Fig. 1. Two fluorescent microscopic cellular images (a) human colon cancer cells and (b) drosophila's cells

Cell segmentation is one of the most challenging problems due to both the complex nature of the cells and problems inherent to video microscopy. Segmentation of cells from cellular image is quite difficult for the following reasons:-

1. since the cells are unstained, as the stain would be harmful to the living cells. The contrast is thus quite low.
2. The cell images are also acquired with auto-focus, which sometimes yields a poorly focused image.
3. In tissue culture environment, cells are non-rigid , irregularly shaped bodies. The cells external environment influences their shapes, which in turn affects their locomotory behavior and ultimately how they function.
4. Cells that naturally migrate within organisms can take on a variety of different sizes and shapes, and can migrate at different rates, depending on their current functional state. As the cell changes shape during locomotion, the contrast between the cell boundary and the background varies continually.
5. In addition, equipment related factors which contribute to the quality of the image, such as uneven illumination and electronic or optical noise, also play an important role in the effective segmentation of a digital image.

There are many algorithms used for cell segmentation, and some of them segmented an image based on the object while some can segment automatically. Now-a-days, no one can point out which the optimal solution is due to different constraints. A model-based contour tracing approach used to the problem of automatically segmenting a Scanning Electron Microscope(SEM) image of cells [3]. This method forces the contours to be smooth by using a model-based approach, such as matching ellipses to edge data. It define gradient vector for each pixel in the gradient map image. It traces the contours of the cell by using history-based

prediction & data- based prediction. Finally it use culling algorithm for reducing noise contours or noise within cell. However, there are some non-elliptic cells, which can not be detected correctly and efficiently using this method.Another popular method is to use thresholding , based on histogram characteristics of the pixel intensities of the image[4]. In order to obtain a satisfactory segmentation result by thresholding, a uniform background is required. Many background correction techniques exist[5], but they may not always result in an image suitable for further analysis by thresholding. So, one have to use thresholding technique in a different way. The [6] developed a multistage segmentation strategy, using two image features associated with cell regions, namely, intensity level and local variation of intensity. The first step applies a global threshold to the local variation of intensity. This step segments a region of the image, consisting of a cell and the nearby surrounding background, from the distant background. The region of the image that is segmented is referred to as the approximate region. It is then further segmented by applying a global intensity threshold to the approximate region. At this stage, the cell has been segmented from the background. Smoothing and filling schemes are implemented to obtain a cell boundary representation. The problem in this approach is, it assumes that the gray levels of the object and background are normally distributed but in reality, this may not happened because- (1) The transition between object and background may be diffuse, making an optimal threshold level difficult to find. (2) The image background intensity is often uneven due to auto fluorescence from the tissue and fluorescence from out-of-focus objects. A popular region growing method, which has proved to be very useful in many areas of image segmentation and analysis, is the so-called watershed algorithm[7]. If the intensity of the image is interpreted as elevation in a landscape, the watershed algorithm will split the image into regions similar to the drainage regions of this landscape. The watershed borders will be built at the crests in the image. In a gradient magnitude image, water will start to rise from minima representing areas of low gradient, i.e. the interior of the objects and the background, and the watershed borders will be built at the maxima of the gradient magnitude. However, if watershed segmentation is applied directly to the gradient magnitude image, it will almost always result in over-segmentation, owing to the intensity variations within both objects and background. So, one have to apply watershed algorithm in a different way. The [8] combine watershed algorithm with thresholding technique for segmentation efficiency. The segmentation of the image is implemented in three levels. Initial automatic segmentation is taking place at first level, where a fuzzy threshold is performed on the image and then a fuzzy gray weighted distance transform is applied. Then it uses the extended h-maxima transform[9] to find suitable seed points for the watershed algorithm. Segmentation on poorly focused images is in second level where it use a fast geometric active contour model based on the level set algorithm. However, The problems of segmenting clustered objects and choosing a suitable threshold level for objects with unsharp edges will remain. It also use complex geometric computation.Edge-based segmentation techniques, which try to connect local maxima of the gradient image, often run into problems when trying to produce closed curves. That is why region-based methods, such as region growing or watershed, that group similar pixels are often used. Another group of methods that do not have the problem of being required to produce closed curves are methods related to snakes or active shape models. From a rough marking of the border or a seed inside the object of interest a curve expands until it finds a strong edge. The function describing the expansion consists of different energy terms attracting the curve to edges. The approach with expanding curves has been used for cell nuclei segmentation[10]. The problems with this method are defining suitable energy terms and, again, the problem of constructing automatic seeding methods, which are restricted to one unique seed per nucleus.There doesn't exist any segmentation method that will alone produce a satisfactory result on images of fluorescence-stained nuclei in tissue if (1) the nuclei are clustered, (2) the image background is variable and (3) there are intensity variations within the nuclei. Thus our proposed segmentation method give careful attention for the above facts to get efficient and correct segmentation result. We add to this a new method that automatically extracts cells from microscopic imagery, and does so in four phases. Phase 1 applies some morphological operations on the cellular image to identify and mark foreground and background seeds of objects with an overall accuracy of >97%. Phase 2 of the method uses a well known segmentation algorithm, called Watershed Algorithm, on this seeds of objects to identify cells, quickly but results some over-segmentation. In phase 3, we refine the previous result by using edge strength of the cells. In the final phase, we further refine the result based on the shape of the cells. The method takes only four input parameters and takes less than 1 minute to operate on a microscopic image.

II. Segmentation Strategy

Our new improved cell segmentation method requires few input parameters and gives stable results. Morphological filtering on the intensity image is used for finding object seeds. Morphological filtering of the gradient magnitude image is used for finding background seeds. Seeded watershed segmentation is then applied to the gradient magnitude image, and region borders are created at the crest lines in the gradient image. More than one seed in an object means that the object will be divided into more than one region, i.e. we will start with over-segmentation. After watershed segmentation, we merge neighbouring objects and only keep those borders that correspond to strong edges. This step will also remove objects with poor contrast. If the nuclei are tightly clustered, no edge is present where they touch, and they will therefore not be separated. Objects found by the first steps of the segmentation process are

further separated on the basis of shape. Shape-based cluster separation using the distance transform is applied to all objects found by the previous steps, but only those separation lines that go through deep enough valleys in the distance map are kept. After this step, we get our desired segmented cellular image. Fig. 2 shows the block diagram of our segmentation method.

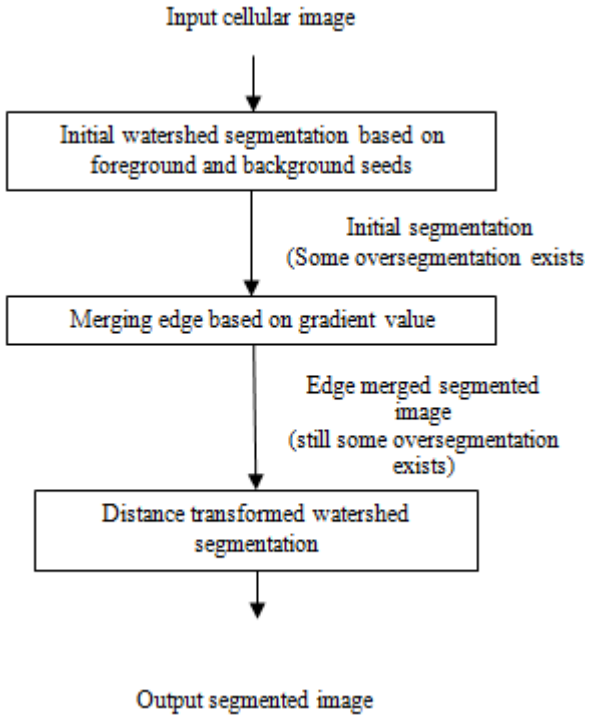

Fig. 2. Block diagram of our new improved cell segmentation strategy

Methodology of our new improved segmentation algorithm is given below:-

1) Firstly, we need to remove noise from the image. Thus, the image of size 512*512 was smoothed by a 3*3 Gauss filter. No other pre-processing was necessary. (Noise is a random unpatterned variation of intensity in the image or it is think of as unwanted pixels in the image that do not part of any foreground/background object)

2) Then we need some seeds that marks probable foreground regions(cells) planted on the image. The images, we consider here, contains bright objects on a darker background. Hence, each object of interest contains at least one local intensity maximum. We define foreground seeds in the original image using the extended h-maxima transform. The extended h-maxima transform filters out the relevant maxima using a contrast criterion. All maxima whose heights are smaller than a given threshold level h are suppressed. The extended h-maxima transformation can be implemented using sorted pixels and searching for local maxima with a given contrast compared with the local neighbourhood.

3) Now we need to define our background seeds. For this purpose we have done the followings:-

i) First, we have find the gradient magnitude of the original image.(Gradient magnitude image is those that have a high intensity value in the edge of a object and low intensity value on the other places of the image.)

ii) Just as the objects can be seeded by extended h-maxima in the original image, the background can be seeded by extended h-minima in the gradient magnitude image, i.e. local minima deeper than a certain depth h .This step will also remove the small components.

4) These seeds serve as starting points in the watershed algorithm applied to the gradient magnitude image. Watershed segmentation can be understood by interpreting the intensity image as a landscape. A hole is drilled in every minima of the landscape, and the landscape is submerged in water. Water will then start to fill the minima, creating catchment basins. As the water rises, water from neighbouring catchment basins will meet. At every point where two catchment basins meet, a dam, or watershed, is built. These watersheds are the segmentation of the image. Watershed segmentation can be implemented with sorted pixel lists.

5) Now we need to merge the area with weak borders. If too many seeds are created in the seeding step, some objects will have more than one seed. These objects will be oversegmented after the watershed algorithm, because each seed results in one region. However, if two seeds are in the same object, the magnitude of the gradient at the region Boundaries will usually be low. Thus, by comparing the gradient magnitude image and previously founded segmented image, we remove those segmented lines where gradient value is low. So, in this way we remove oversegmentation/under segmentation.

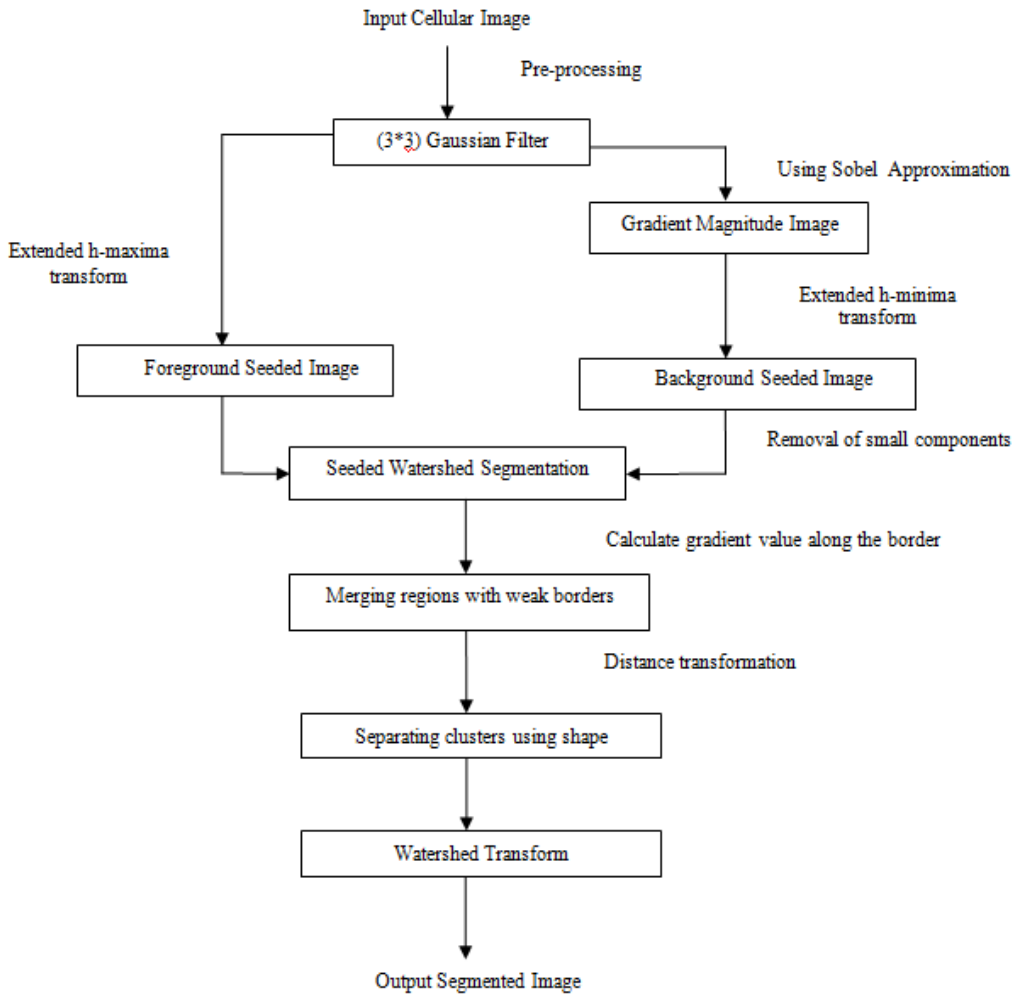

Fig. 3. Flow diagram of our cell segmentation strategy

6) The clustered cells will be separated using shape. To do so, we use the seeded and watershed result image as binary input to distance transformation. The distance transform of a binary image assigns to each object pixel the distance to the closest background pixe

7)Taking the inverse of the distance image, the distance maxima serve as regional minima for watershed segmentation. Now we again apply watershed segmentation in the resulting image. After this step, the resulting segmented image will be appear. Fig. 3 shows the flow diagram of our new improved cell segmentation method.

III. Implementation & testing

The implementation was done in MATLAB 7 software. It has rich image processing toolbox and enough functions

for implementation efficiency. Once the four input parameters h_1, h_2, s, t were set, the experiments needed no human interaction. The speed of the segmentation depends on image size and the number of objects in the image.

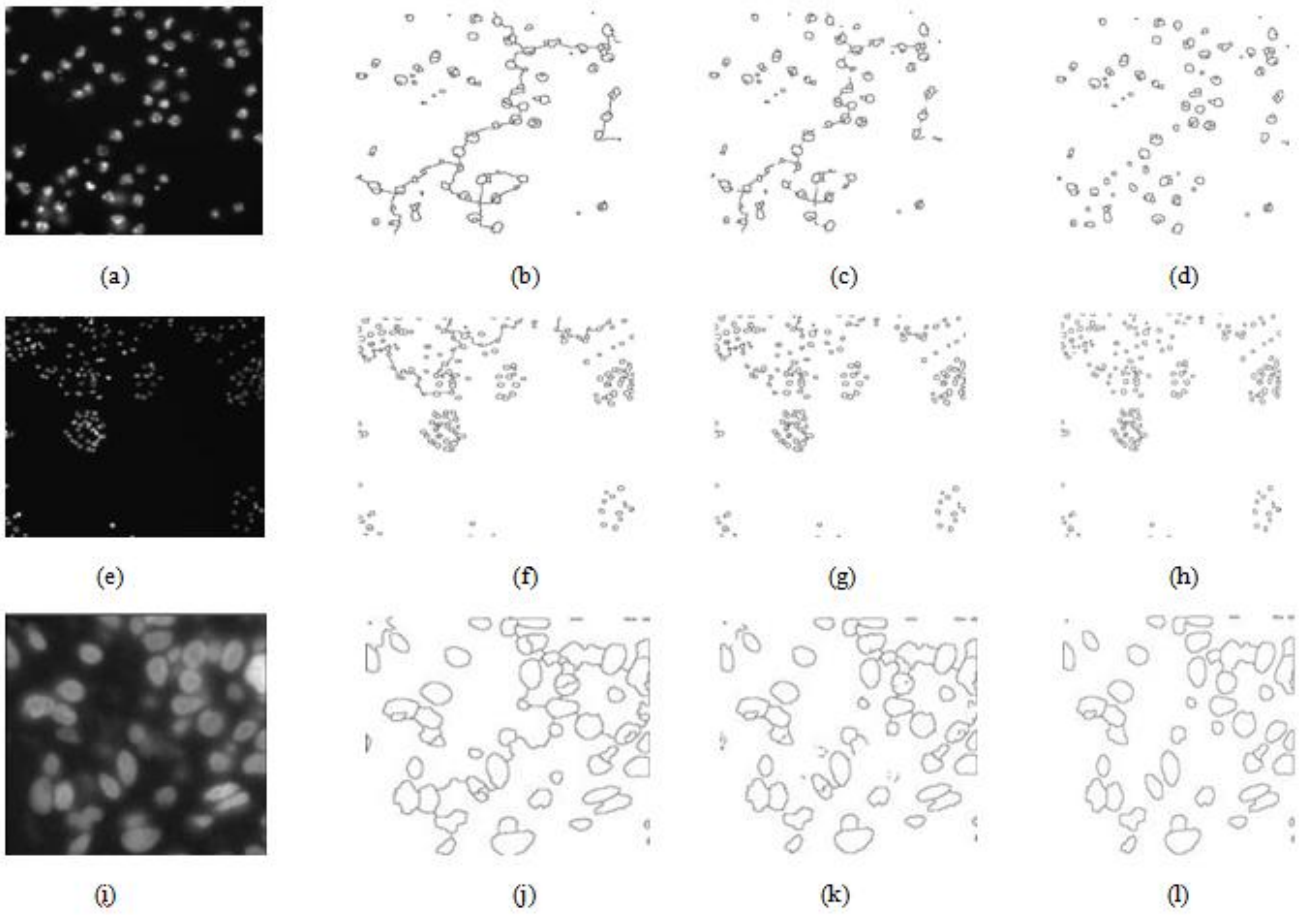

Fig. 4. The three-stage cell segmentation method: (a),(e),(i) are three fluorescent microscopic cellular images of Drosophila, Human colon cancer and slice of tumor cells, respectively. (b),(f),(j) are the results after initial seeded watershed segmentation. (c),(g),(k) are the results after merging edge based on gradient value. (d),(h),(l) are the results after final segmentation stage. Parameters are: (1) For Drosophila cells, h_1=7, h_2=9,s=13, t =11 (2) For Human colon cancer cells, h_1=7, h_2=7,s=11, t =17 (3) For Tumor cells, h_1=7, h_2=9,s=13, t =16 .

IV. Experimental Results

Fig. 4 shows the results after each segmentation stages applied on three different cellular images. In the first stage of the algorithm, initially watershed segmentation is applied on the image based on foreground and background seeds, three parameters are specified – foreground seeds height (h_1), background seeds height (h_2) and structured element size(s). A low $h_1$will result in many seeds, often more than one seed per object. A high h_1 will result in fewer seeds, and some objects may not get a seed at all. Owing to a subsequent merging step based on gradient magnitude, we use a rather low h_1 value to ensure that each object gets at least one seed. The previous step results some oversegmented line. These oversegmented lines are seen in those place where the gradient value of the gradient magnitude image is low. In the next stage , we choose a threshold value(t) for merging edge.An optimum value is chosen so that the resulting oversegmented line is removed.The second stage also results some undesirable segmented line due to the shape of the cells. To remove these undesirable lines, we perform a distance transformed watershed segmentation which requires no parameters.

V. Conclusion

We have presented a three stage segmentation approach for fluorescent microscopic cellular images of live and unstained cells. The basic idea behind successful segmentation is to efficiently restrict the parameter, especially the foreground seeds height(h_1) and merging threshold(t). Very little pre-processing is needed, even if the background variation in the image is large. The input parameters are at present manually set for a test image, and the same parameters are thereafter used for fully automatic segmentation of images created under the same imaging conditions. As only four input parameters are required, this can be done quickly.Methods for automatic parameter approximation are subjects for future work. When a new type of specimen is imaged, adjustment of input parameters will only be necessary if image dynamics or nuclear size changes. The segmentation method can be useful for many different segmentation tasks where a simple foreground/background threshold is not sufficient. Further processing, such as removal of nuclei that are damaged or under-segmented, by a size threshold, or more advanced statistical methods, may improve the result. Automatic

segmentation is not only faster than manual segmentation, it is also observer-independent and reproducible.The segmentation can be used by medical clients to validate the effectiveness of strain imaging in locating and distinguishing benign and malignant cells. Once the interested cell region and background region are defined, quantitative measures such as contrast or area can be calculated.

VI. Acknowledgment

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VII. References

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