2011 First IEEE International Conference on Healthcare Informatics, Imaging and Systems Biology

Robust Segmentation and Tracking of Generic Shapes of Neuro-Stem Cells

Ishwar Kulkarni[1], Uddipan Mukherjee[1], Chris Sontag[2], Brian Cummings[2], M. Gopi[1]

[1]*Department of Computer Science,*
[2]*Department of Anatomy & Neurobiology*
University of California, Irvine

***Abstract*—Given the time lapse images of human Neuro Stem Cells (hNSC) marked by fluorescent proteins, that are obtained from a confocal laser microscope, we present algorithms to identify, segment, track, and estimate statistical parameters of the cells. The structure of these cells are quite complex and irregular, which makes segmentation and tracking even more challenging. We use a novel combination of Difference of Gaussians and a variant of the Watershed algorithm to segment cells accurately. Our tracking algorithm can identify not only the temporal path of the cells but also events like cell divisions and deaths. Our system is robust, efficient, completely automatic, and removes many drawbacks faced by earlier solutions. We also propose the first geometric algorithm that uses Delaunay triangulation, to find the number of the branches of the cells, which is an important biological feature.**

***Keywords*-Image Processing, Geometry Processing, Biological Imaging, Segmentation, Tracking, Human Neuro Stem Cells;**

I. INTRODUCTION

In cellular biology experiments, studying the cell cultures and their dynamic responses to drugs etc., involve capturing microscopic time lapse images of these live cultures. These images need to be analyzed for understanding the cellular life processes in terms of their metabolism, growth and other biologically significant processes like cell division, deaths, cell lineages and fate. The visual data capturing systems used to take images of the cells produce large amounts of data per experiment, e.g. time lapse images captured for eight experimental conditions at ten minute interval for one week produce two terabytes of uncompressed images (9,000 images). Manually sieving through this large volume of data is tedious and cumbersome. Therefore we propose to build an automatic system to acquire semantic information from the images.

The rest of the paper is organized as follows: In the current section we highlight our main contributions and briefly talk about the imaging system used to capture cell images. In section II we talk about the related work in this field. In Section III we discuss our core algorithm for cell segmentation, in Section IV we discuss our algorithm to identify cell division/death, and in Section V we compute the cell branch statistics. We conclude in Section VI.

A. Main Contributions

In this paper, we propose novel and robust techniques to compute the cell locations (nucleui) and the geometry of the cells. Our method uses a powerful combination of Differences of Gaussians technique and a variant of the Watershed algorithm to robustly segment the cells. Unlike existing techniques (including commercial solutions), our method does not require user intervention, is extremely fast, robust, and reliable. Following this, we propose an efficient algorithm to track the cells over a sequence of time-lapse images. Our tracking algorithm automatically identifies cell divisions (*mitosis*) and cell deaths (*apoptosis*).

The cells, whose images we are considering in this work, are arbitrarily shaped. These cells, which are actually neurons, develop many branches, and the number and length of these branches are of biological importance. Automatically computing the number of branches is very complex and no solution currently exists for this problem. Given the boundary contours of the cell, as computed by our image processing algorithm, we use an elegant and simple Delaunay triangulation based geometric algorithm to separate, count, and measure the branches of each cell.

B. Image Capture System:

The images we are working with are of human Neuro Stem Cells (hNSC), captured by VivaView Incubator Fluorescence Microscope. The system captures images in multi-channel wide-field fluorescence of at least three different wavelengths with exposure of $10ms$. In our experiment we used the images of the cells that are cultured with green fluorescent protein (GFP), illuminated by $488nm$ wavelength laser that corresponds to the fluorescence wavelength of that protein. The protein marked cells show up as bright profile in the image. The cells are between $10 - 20\mu m$ in dimensions with magnification of $20x$ in the images. These images are $16 - bit$ grayscale images of intensity values ranging from $0 - 4096$ (12 bits of actual data). The images are of dimensions 1024×1344 pixels and thus capture an area of $330\mu m \times 433.5\mu m$. Figure 1(Left) shows a typical image captured by this system.

978-0-7695-4407-6/11 $26.00 © 2011 IEEE
DOI 10.1109/HISB.2011.40

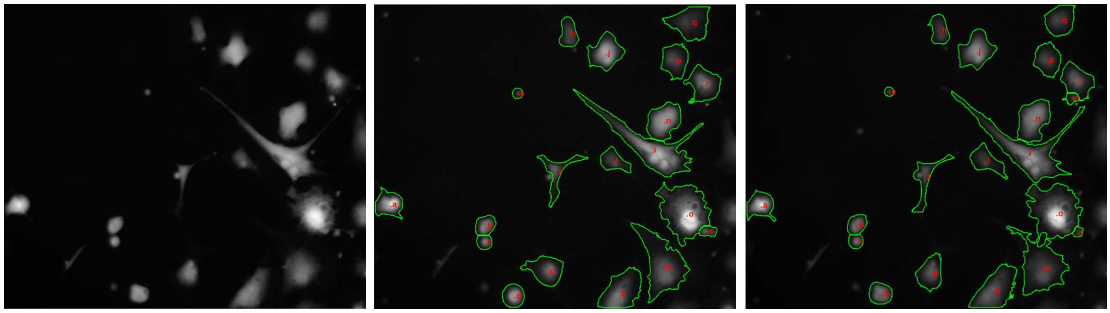

Figure 1. Left: Typical image with contrast enhanced. Center and Right: Segmentation with our method.

II. RELATED WORK

The problem of cell segmentation has been worked on for many years, but segmentation and, in particular, cell segmentation is still an open problem. This is largely because cells are dynamic entities that vary widely in appearance and exhibit varied types of behavior. Segmentation of these images needs to capture such appearance and behavior correctly in identification of a cell. Although cells extend to large areas, parts of the cell away from its nucleus get drastically dimmer in the captured image due to the absence of the fluorescent protein in those areas. As the cells lack sharp and contrasting boundaries, segmentation techniques which use edge detection, such as those proposed in [1] do not produce accurate results.

Many other more advanced techniques for cell image segmentation have been suggested in the literature. They can be categorized into types based on their approach. Many techniques use the level set segmentation method. The method used by [2], uses a zero-set of implicit energy functional, that traces a smooth boundary around cells. But, using a functional that has arbitrary complexity as required by our images is infeasible, both in terms of accuracy of its segmentation and the time it takes to solve the functional.

Another popular approach to cell segmentation is the Active Contour Model, proposed in [3]. These models take a combination of cell features such as cell boundary, internal pixel values etc. and minimize an energy to *wrap* a contour around the cell. These methods have the advantage that they always converge to a solution, and do not require an implicit parametric boundary definition. But in general they require external initialization before the minimization can be started. This method has been used successfully to segment time lapse images in [4], but as the method relies on spatio-temporal tracking, it can be unsuitable in some instances.

Model based segmentation approach is another important class of segmentation technique that identify cells in images and image sequences based on certain well defined assumptions about spatial or temporal attributes of cells and their motion. Methods such as [5] use size and shape based contours to identify cells. Methods such as the one proposed in [6] use motion patterns on leukocytes in images of blood streams to identify cells.

Graph cut methods of image segmentation consider pixels of an image as nodes of a weighted graph. The weights of the edges are defined in terms of similarity between the pixels they connect. The nodes also share an edge to two pseudo-nodes designated as foreground and background. The weight is usually a metric in a feature space consisting of pixel features such as Histogram of Oriented Gradients, etc. The min-cut problem is solved as a convex optimization problem. These methods involve user initialization for designating the foreground and background nodes. This method is used in [7] successfully. [8] proposes a method of repeated min-cut solving to separate the foreground pixels and is popularly known as *soft scissors*. Additionally these model solutions require solving a rich feature space for correct attribute matching at the pixel level. This computation is generally very expensive.

The watershed approach is a commonly used segmentation technique and is the one we use as a step in our segmentation procedure. The problem with watershed generally comes in the form of under and over segmentation of a region, which is to be avoided if statistically correct segmentation is to be achieved. Our method is largely inspired from [9] where the authors proposed a method of segmentation based on *Ultimate Eroded Point*. The method uses two erosion structures one for coarse and other for fine erosion successively. This type of method makes two assumptions: firstly the shape of structure for erosion needs to capture the shape of the cells, and secondly, the topography of the cell image (or the height field) needs to be smooth enough for erosion to a single point to produce a seed. Methods that track cells by shape matching are predominant, these methods use shape descriptors (proposed in [10]). They represent shapes of cells as a number of binned histograms for each point on the outline of the shape and perform graph matching. This method is computationally expensive and also not suitable for highly concave shapes. The images we are segmenting (Figure 1) have pluripotent cells. Therefore the shape and size of the cells varies widely among cells and between the images of the same cell as time progresses. As a

result, spatio-temporal model based approaches do not work on these images. Cells have a coarse (non-smooth) outline, therefore energy minimization with or without parametric representation become unsuitable. Graph cut methods (such as 'Markov Random Fields' implementations) require user initialization, and thus cannot be employed in cases where a large number of images need segmenting. The GFP-images are essentially single channel and lack features which help in segmentation technique applied to segment rich, multi-channel real-world images. The model we propose in this paper identifies cell centers robustly using Difference of Gaussian Enhancement followed by maxima finding, and segments the image using watershed to give accurate results.

III. PROPOSED METHOD FOR SEGMENTATION

A. Cell Segmentation

Segmentation is the process of dividing the image into cell regions and background. We carry out the segmentation in three important steps:

1) cell extent estimation (demarcating the core region of the cell)
2) robust cell center marking (identifying one point of intensity maximum)
3) final segmentation of the image based on identified cell centers

The output of each step is used in the next step. Robustness of detection of cell centers is an important criterion for determining the quality of segmentation.

As it can be observed, our images have regions of high intensity/brightness that contain cells (figure 1). However one can also see that, not only cell centers but also the branches have a local maxima of intensity. Finally, there is a large variation of intensity locally within the core of the cell which might result in multiple local maxima being found within the core of the cells. All these reasons make the cell extent/center estimation difficult.

We find cell centers in step one and two. To robustly identify the core of the cell, first we identify the regions that are certain to be cells. To robustly find the cell cores with high confidence (at the same time exclude the branches) we need to perform a contrast enhancement that will give consistent high response within the core of cell and low response to regions outside. Contrast enhancement by techniques such as histogram adjustment is prone to exaggerating the local variations and is thus not suitable for our purpose. Therefore we convolve the cell image with the Difference of Gaussians (DoG) kernel as the contrast enhancement technique. As shown in Figure 2, the DoG filter, which is also called the Mexican Hat Kernel due to its shape, is a band pass filter. This method is computationally inexpensive and is easy to implement.

Difference of Gaussians is applied thus: convolve the original image with a Gaussian kernel with large sigma and a

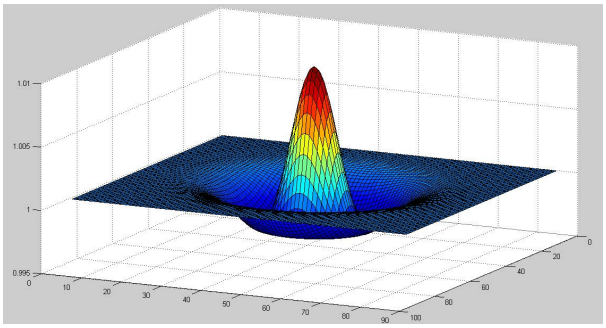

Figure 2. Surface of a 2 dimensional Difference of Gaussians kernel

Gaussian kernel with small sigma, then subtract latter from the former. The size of the Gaussians when large enough makes the enhanced image invariant to variations within the cell, which are basically high frequency components in the image. Convolution with DoG filter gives a large response from edge to edge as required (see Figure 3). In our experiments, we found that the sigma values of 12 and 2 for the two Gaussian kernels give good results.

Once we have high response regions that mark the core of the cells, we can find points of highest gradient. Or in effect perform an edge detection operation to clearly find core regions. The edge detection step also picks out some regions that have high response close to the boundary of the region we seek. For this we perform a morphological dilation operation followed by erosion operation. This step connects the possibly unconnected islands. Lastly, we perform a morphological open operation with large radius in order to remove speckle noise found to have high gradients.

In conclusion, in step one of cell segmentation, we robustly find regions that form the core of the cell. However as multiple cells can be marked as a single region, we follow it with further levels of refinement in the next step of segmentation.

B. Cell Center Marking

When two cells are very close together (either due to their motion or because they are recently born sibling cells), the entire region containing the two cells has a large response under the Difference of Gaussian filter (Figure 5). We need to identify such regions and their centers.

We perform a low pass filter on the original image to smooth out local variations, which produces a local maxima only at the center of the cell. Low pass filtering is done with a Gaussian kernel having standard deviation 7. In the smoothed image, we find local maxima only within the regions found in step one of segmentation. This is necessary because if all the local maxima were to be found, various points on branches of the cell would also be identified. The maxima found in this step account for only cell centers, because maxima on branches are removed by the DoG filter

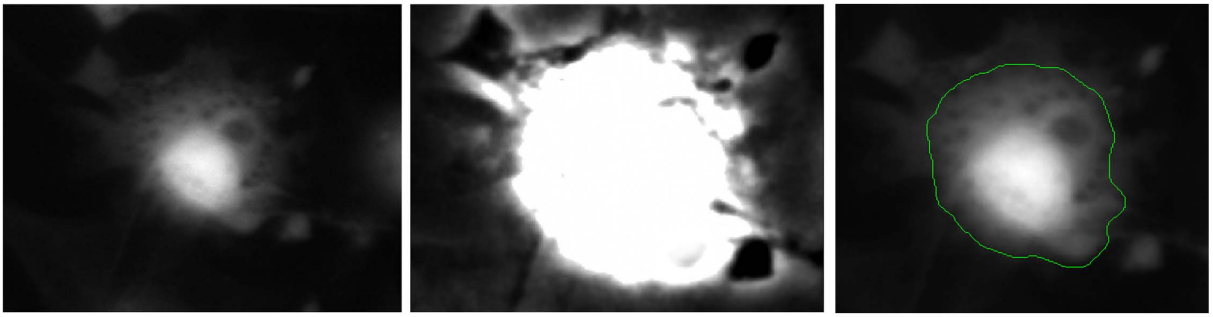

Figure 3. Left: Original image, Center: DoG enhancement, Right: Edges detected on DoG (followed by median filter) overlayed on original. Note that some parts of the cell are left out.

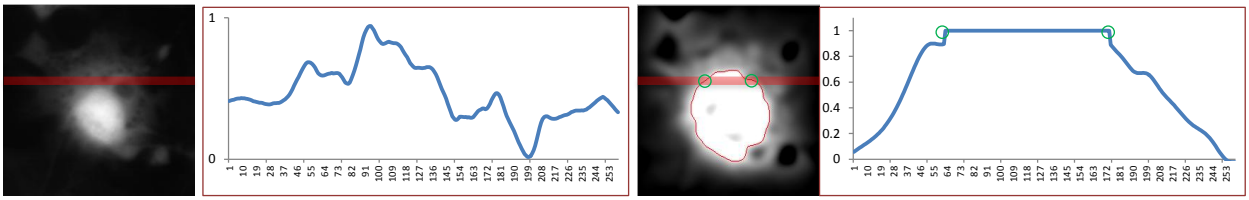

Figure 4. (Left to right) A cell with large local variations, plot of intensities in pixels marked with red line in previous image, DoG filtered image, with same band of pixels and plot of pixels (clamped to range [0,1])

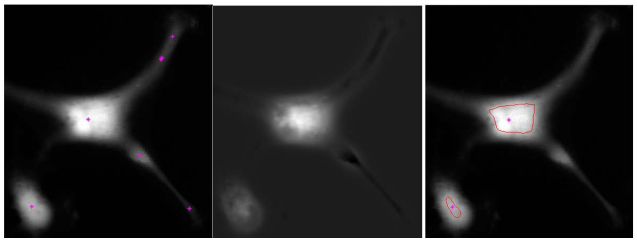

Figure 5. Left: Multiple maxima are found on a cell, Center: The DoG filtered image, Right: Edges found on DoG filtered image (after cleaning). Notice only one maxima remains, which is the cell center

and each cell has its own maxima in the region it belongs to (Figure 5).

With cell centers correctly identified, accurate cell boundaries (the region encompassed by whole cell, including branches) need to be identified and regions with multiple cells need to be split. For this we use Watershed segmentation. Watershed segmentation is a widely used image segmentation technique proposed in [11]. Watershed considers the height-field of the image as ridges and basins, and finds the lines that determine the tips of those ridges. More formally, the Watershed algorithm returns a relation $W : P \times N$, where P is a pixel and N is the integer label for the basin that the pixel belongs to. Most implementations of the watershed algorithm return the boundaries where the mapping changes from one integer to another.

We use the watershed algorithm for splitting regions into as many cells as there are maxima points (found in step two as described above). As the 'basins, or low lying areas, in the image are demarcated by watershed, the original image is first complemented to make the cells such low lying regions. Due to minor variations in pixel values, over segmentation is a common problem in watershed segmentation. To overcome this, we suppress minima that lie below a certain threshold in the inverted image. From our experiment we see that setting a threshold to a scalar multiple of the mean of the pixels along the core boundary found in step two is a good way of controlling the segmentation. If the particular (biological) experiment's image consists of many cells, we can set this scale factor to be a small value (thus avoiding under segmentation), and a large value if the image is sparsely populated.

Next, we apply the watershed to the image with minima suppressed at a scalar multiple of mean of the pixels sampled from the core. The corresponding regions found at this step (which contain the cells) are matched against cell centers found in step two. Every region (a basin in the watershed algorithm) which contains a cell center defines the entire extent of that cell (Figure 6).

In conclusion, segmentation is done in 3 steps: first we identify regions that contain cells (usually marking the core of each cell), next we find maxima within each region to identify the regions that contain multiple cells, finally, we

apply watershed to segment the image into cells and the segmented cells are identified.

The following table (Table I) summarizes our segmentation results. The first column of the table indicates the spatial density of the cells in the images. A higher density image has more cells in a given area, giving rise to a larger error in cell identification, in the form of over segmented cells. This is because the densely packed cells cause a some false positives for cell centers. We characterize frames with 5 to 15 cells as low density, 15 to 25 as medium density and more than 25 cells per frame as high density frames.

Density	Image Count in Series	Manual Count of Cells (Average)	Segmentation Result (Average)	Under/Over
Medium	10	35.83	36.16	Over
High	25	25	25.32	Over
Low	10	5.5	5.5	(Neither)

Table I
SEGMENTATION RESULTS CARRIED OVER DIFFERENT SETS OF IMAGES

IV. TRACKING

Tracking is the process of associating a cell in one image to another cell in the next image. In implementing tracking, we make the assumption that the changes in cell characteristics are minimal. This assumption holds as the images are always captured at a rate that will always record the changes in the characteristics of the cells. For the purpose of tracking we define an attribute space such that a cell's representation in one image has the smallest Euclidean distance to its counterpart in the next image of the time lapse sequence. This attribute space has four features of cells as the dimensions. These features are chosen so as to capture the cell's spatio-temporal definition as closely as possible. These features are:

1) Pixel-Overlap: the fraction of pixels that lie within two outlines if they are overlaid
2) Cell area: number of pixels forming the cell body
3) Brightness: the cell's total brightness(sum of all pixel values)
4) Distance: Euclidean distance between weighted centroids.

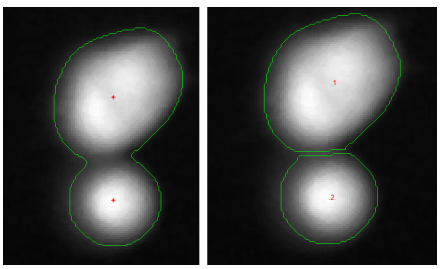

Figure 6. Multiple Maxima within a cell boundary, resolved by in step three (watershed step)

These attributes are weighted by a scalar in accordance to the set up of the experiment. For example, when images are taken at larger intervals, the nearness in Distance and Pixel-Overlap features have a lower weight as the cells will have moved a larger average distance between frames. Similarly, for cells which have large growth rate, the closeness in cell size attribute has lower weight, and so on.

The process of tracking then becomes computing the minima of the following function:

$$\sum_{i \in Image1} \sum_{j \in Attributeset} W_i (X_i - X_j)^2$$

Here, $X_i - X_j$ denote the difference in features between cells in two frames and W_i is the factor scaling this difference. Experiment with images in 10 minute interval has the weights shown in Table II.

Attribute	**Weight**
Cell Size (in pixels)	0.25
Overlap (as ratio)	0.25
Total Brightness (total pixel value)	0.3125
Distance b/w weighted centroids (in pixels)	0.1875

Table II
EXPERIMENT WITH 10 MIN INTERVALS

As it can be seen from section III, robustness of the segmentation is dependent on spatial density of cells, similarly, the accuracy of tracking is directly affected by the temporal density of frames. A larger delay between two consecutive snapshots implies a larger duration in which the cell changes its appearance and location. This leads to larger margin for error. Tables III, IV summarize the tracking results.

Incorrect swapping of labels for a pair of cells by the system is termed 'mismatch'. Mismatches generally occur when two cells are very close together in the attribute space describes in table II). A cell is termed 'missed' if it has a different label from its self in the previous frame. Cells with longer trails or span of frames are prone to higher levels of mismatch. This is because the longer the cells stay in field of view, the more susceptible the system to make false labeling.

The probability of mismatch scales with the duration of cell's existence in the Field of View of the camera, giving rise to larger mismatch error for such cells. Frames in these experiments have an approximate 30 cells, with a trail of 40 frames (the longest trail) less than 3.25% error rate is recorded in our system, (Table IV).

Another point to note here is that in the case of 40 minutes interval, we cannot find cells that last long enough in the FOV to capture their entire tracks, this causes better average-mismatch ratios in table IV.

Cell's trail Length in Frames	(total) Number of Mismatched cells pairs	Average missed cells
0-5	(Missed all, debris)	NA
5-10	None	NA
10-15	None	NA
15-20	4	1.25
20-25	7	1.75
25-30	6	1.2
30-50	39	1.95

Table III
TRACKING RESULTS: TIME DELAY 20 MIN

Cell's trail Length in Frames	(total) Number of Mismatched cells pairs	Average missed cells
0-5	0	NA
5-10	None	NA
10-15	4	0.8
15-20	3	0.6
20-25	4	1.25
25-30	6	1.2

Table IV
TRACKING RESULTS: TIME DELAY 40 MIN

V. STATISTICS COLLECTION AND EVENT DETECTION

A. Statistics Collection

Various measurements about the cells make up important experimental data for the biologists. The number of branches also forms an important part of the cell description. Therefore we propose a novel use of a geometric algorithm to estimate number of branches described in section V-C.

Once the correspondence between the cells is found across the sequence of images, various temporal characteristics such as cell velocity, rate of deformation etc. can be found. Important biological events (such as Mitosis and death) have a point of inflection in statistical measure such as size, shape and number of branches. Identification of such events is discussed in next section.

B. Event Detection

Cell Split or Mitosis is said to have occurred when a cell in an image captured at time t is invariably mapped to two different cells in the image at time $t + \Delta t$, and each of the new cells have a large distance on the cell-area dimension (described in previous section). Also, it will be noticed that the cell's total intensity just before mitosis equals the sum of cell intensities of the daughter cells. This is true because the brightness of the cell, as described in section I is proportional to GFP protein content of the cell, and when the cell undergoes mitosis the protein content is divided among its daughter cells. Figure 7 shows the process of mitosis of a cell. *Cell Death:* When a cell suffers apoptosis in a culture, it loses its branches and floats away in the medium,

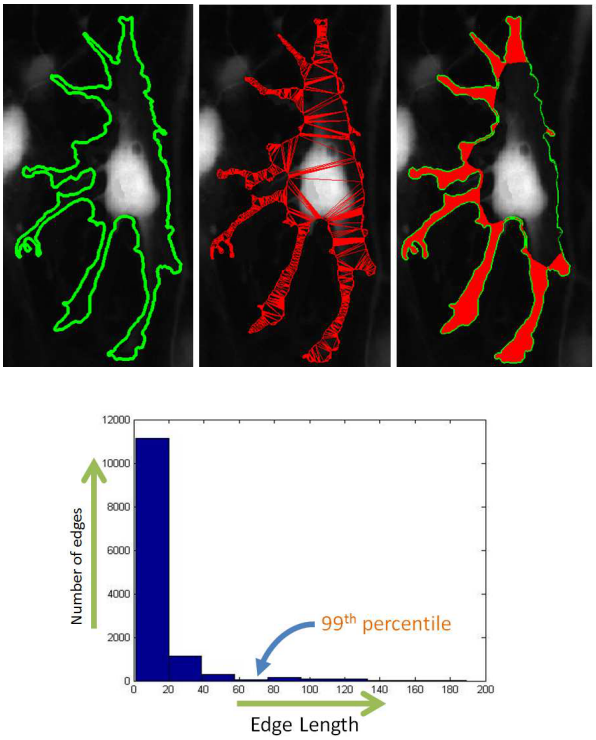

Figure 9. Top: Left to right, cell outline, constrained delaunay triangulation, tringles with edges less than 99th percentile, Bottom: histogram of edge sizes

this floating is generally similar to Brownian motion and has much higher velocity than the firmly rooted live cells. Thus when a cell dies, the cell tracking algorithm usually returns no match. The best match will have a large distance to the cell in previous image (Figure 8). Statistically, we term this event as cell death. Thus, a cell death is also characterized by its swift mobility – when its speed is far greater than the average speed of the other cells.

C. Counting branches using Constrained Delaunay Triangulation

Starting with the contour of the cell, we perform Constrained Delaunay Triangulation on the boundary. Specifically, we perform a Delaunay triangulation on the boundary points of the cell and remove those triangles having edges outside the cell contour. If the ordering (clockwise or counter clockwise) of cell boundary points are known, the triangles formed by the Delaunay Triangulation can easily be partitioned into two groups those lying within the cell, and those lying outside. Once we obtain all the triangles within the cell, we observe that the triangle edges near the branches of the cell are considerably smaller in length than the triangles near the central region of the cell. As shown in Figure 9 edges that are longer than the 99th percentile (based on edge lengths) are usually the edges that form

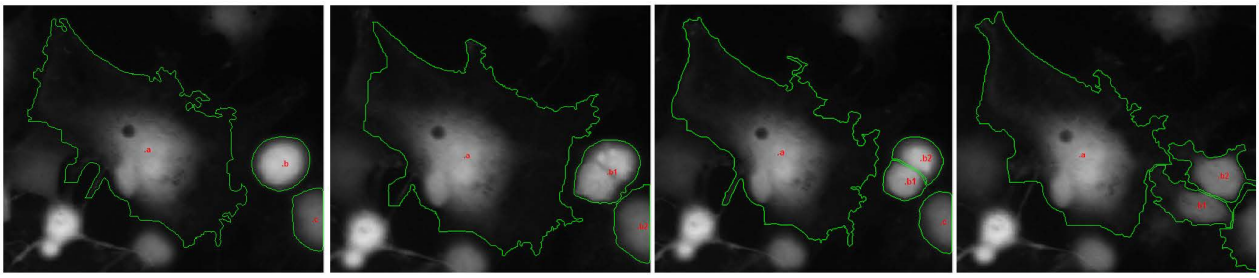

Figure 7. Sequence showing mitosis. L to R: The Cell, Onset of mitosis, Split completes(mitosis identified), (18 frames later) Cells Separate

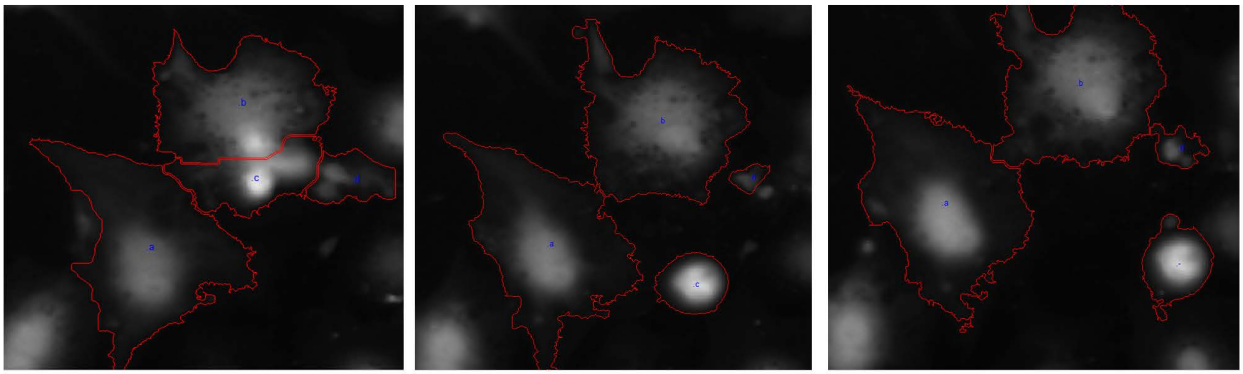

Figure 8. Cell Death, the cell marked as C moved fast compared to other cells, and in image at right, goes unidentified (marked '-')

Detect Cell Branches
Input: The cell boundary points in order
Output: An estimate of the number of cell branches
Algorithm:

- Perform a Delaunay Triangulation on the boundary points to obtain a set of triangles, T.
- For each triangle formed by vertices (i, j, k) in the set T, where i, j, and k are in the same order as in the cell boundary, if 'j' is a concave vertex, remove the triangle. A new smaller set of triangles T' is formed.
- For each triangle in T', if each edge is less than the 99th percentile of edge lengths, retain the triangle; otherwise remove the triangle from T'. Thus, T' is reduced in size.
- Determine the number of connected components of the triangles in T'. This gives a measure of the number of branches.

Figure 10. Steps in finding branch size

the region near the center. These edges are removed and the remaining triangles correspond to the branches in the cell. The number of connected components of these triangles gives us an estimate of the number of branches present in the cell. Figure 10 explains the entire process of detecting the number of branches as explained above.

One biologically important statistical pattern that can be observed is that when a cell is about to undergo mitosis, its circularity increases (the shape gets closer to that of a circle), it loses its branches and its protein content aggregates. These features can be used to predict mitosis, or in other words candidate cells for possible mitosis in near future can be identified and reported. This higher level semantic can be helpful to see 'rate of mitosis', etc.

VI. Conclusion

In this paper we have presented a novel solution to cell segmentation circumventing the inherent challenges introduced by images of stem cells. In summary, the segmentation and tracking process is divided into four steps: Cell core region identification, cell center identification via maxima finding, final segmentation using watershed and tracking using attribute matching across images. We show the statistics collection and event detection which is an immediate consequence of tracking. Biologically important events such as mitosis and death are successfully identified. We also present a geometric algorithm to count the branches in a cell which is an important statistical measure for a cell.

References

[1] D. Anoraganingrum, "Cell segmentation with median filter and mathematical morphology operation," in *Image Analysis and Processing, 1999. Proceedings. International Conference on*, 1999.

[2] D. Mukherjee, N. Ray, and S. Acton, "Level set analysis for leukocyte detection and tracking," *Image Processing, IEEE Transactions on*, vol. 13, no. 4, pp. 562 –572, 2004.

[3] M. Kass, A. Witkin, and D. Terzopoulos, "Snakes: Active contour models," *INTERNATIONAL JOURNAL OF COMPUTER VISION*, vol. 1, no. 4, pp. 321–331, 1988.

[4] F. Bunyak, K. Palaniappan, V. Chagin, and M. Cardoso, "Cell segmentation in time-lapse fluorescence microscopy with temporally varying sub-cellular fusion protein patterns," in *Engineering in Medicine and Biology Society, 2009. EMBC 2009. Annual International Conference of the IEEE*, 2009, pp. 1424 –1428.

[5] N. Ray, S. Acton, and K. Ley, "Tracking leukocytes in vivo with shape and size constrained active contours," *Medical Imaging, IEEE Transactions on*, vol. 21, no. 10, pp. 1222–1235, 2003.

[6] Y. Sato, J. Chen, R. Zoroofi, N. Harada, S. Tamura, and T. Shiga, "Automatic extraction and measurement of leukocyte motion in microvessels using spatiotemporal image analysis," *Biomedical Engineering, IEEE Transactions on*, vol. 44, no. 4, pp. 225 –236, 1997.

[7] K. Held, E. Kops, B. Krause, W. Wells III, R. Kikinis, and H. Muller-Gartner, "Markov random field segmentation of brain MR images," *Medical Imaging, IEEE Transactions on*, vol. 16, no. 6, pp. 878–886, 2002.

[8] J. Wang, M. Agrawala, and M. F. Cohen, "Soft scissors: an interactive tool for realtime high quality matting," *ACM Trans. Graph.*, vol. 26, July 2007. [Online]. Available: http://doi.acm.org/10.1145/1276377.1276389

[9] X. Yang, H. Li, and X. Zhou, "Nuclei segmentation using marker-controlled watershed, tracking using mean-shift, and kalman filter in time-lapse microscopy," *Circuits and Systems I: Regular Papers, IEEE Transactions on*, vol. 53, no. 11, pp. 2405 –2414, 2006.

[10] S. Belongie, J. Malik, and J. Puzicha, "Shape matching and object recognition using shape contexts," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, pp. 509–522, 2002.

[11] L. Najman and M. Schmitt, "Watershed of a continuous function," *Signal Processing*, vol. 38, no. 1, pp. 99–112, 1994.