Automated Segmentation and Shape Tracking of Fluorescent Cancerous Cells by Morphological Analysis (Wavelet - Otsu Model)

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Abstract --In recent days due to the urbanisation and socialism among people the diseases such as cancers are very common and they need to be treated as early as possible. Reports suggest that 60% of the people are suffering with various traits of cancer. Cancer is caused because of the splitting and merging of infected cells .So in order to detect the vulnerability of the disease we have to study the entire nature of the cells. The main goal of the project is to demonstrate that the proposed tracking scheme is more accurate and significantly faster than the other state-of-the-art tracking by model evolution approaches The crucial tasks are, in particular, segmenting, tracking, and evaluating movement tracks and morphological changes of cells, sub-cellular components, and other particles. The system proposed here not only tracks and segments the cells, it also detects the percentage or vulnerability of the disease by using classes of segmentators. This can be obtained by wavelet otsu model in which the classified levels of cells are found out and they are used for isolating the infected cells. This approach is carried out in rat adipose mesenchymal stem cells, or the carcinogenic cells.

I. INTRODUCTION

In computer vision, image segmentation is the process of partitioning a digital image into multiple segments (sets of pixels, also known as super pixels). The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze. Image segmentation is typically used to locate objects and boundaries (lines, curves, etc.) in images. More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics. The result of image segmentation is a set of segments that collectively cover the entire image. Each of the pixels in a region are similar with respect to some characteristic or computed property, such as color, intensity, or texture.

The applications of any computer vision system require cell tracking to be fast, affordable and, most importantly, precise and robust. Segmentation of the cells is important for allowing different regions of the body to develop differentially for different uses. The process of detecting and tracking biological features such as cancerous cell growth and nuclei is complicated by the fact that they constantly change their shape. Shape changes happen both continuously as the biological features grow and discontinuously as they divide or die. This can be done effectively if they are fluorescent. So fluorescence microscopy technique has to used for segmenting and tracking of cells. The extraction of fluorescence time course data is a major bottleneck in high-throughput live-cell microsc

II. EXISTING METHODOLOGIES

The idea raised for the development of this system for replacing the chanvese model [1] by using morphological operations. The literature survey took in various forums and computer vision research centers and computer vision group of Bristol University in United Kingdom. With the invention of fast cell segmenting and shape tracking methodologies and recent increase of computer power and decrease of man power in the field of medical science especially for tumorogenesis, it has become very common to see a cell tacking and segmenting software that will analyse the disease nature in detail with more ease and accuracy.

A. Chan –Vese model

The Segmentation And Shape Tracking of Fluorescent cells is initiated by using the Chan-Vese model [1]. The methodologies such as Cell tracking, level set and graph cut Optimisation are used efficiently along with the Chan vese model in which the system is used so as to track the multiple cells simultaneously if the number of frames is also maximum. It is a fast and robust approach to tracking the evolving shape of whole fluorescent cells in time-lapse series. It has 2 steps. First, coherence-enhancing diffusion filtering is applied on each frame to reduce the amount of noise and enhance flow-like structures. Second, the cell boundaries are detected by minimizing the Chan-Vese model in the fast level set-like and graph cut frameworks.the major advantage of this method is Ellapsing of frames is reduced , and also multiple tracking is enabled. There are several limitations that we intend to address in future work to improve the overall performance of the proposed tracking scheme. First, a manual separation of cells clustered in the first frame is required to track each of them correctly over time. This complicates the use of the proposed tracking scheme in experiments with high density of tightly packed cells. Furthermore, coherence-enhancing diffusion filtering takes up to about 85% of the total execution

time. Therefore, a different choice of the filtering technique would make the proposed tracking scheme significantly faster and more suitable for high-throughput applications. Last but not least, it might be profitable to integrate the FLS framework with a different approximation of the mean curvature motion to obtain smoother boundaries, or incorporate the object indication function directly to the Kohli-Torr algorithm to improve the overall speed of the GC framework.

B. Coupled Active Segmentation

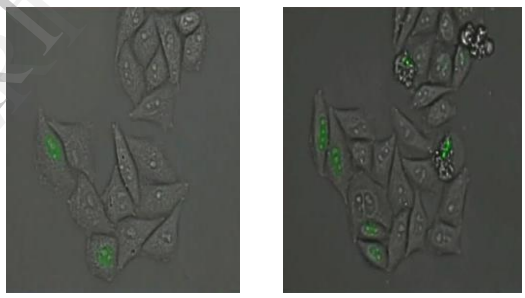
. Segmenting and tracking fluorescent cells in dynamic 3-D microscopy with coupled active surfaces a semi automatic segmentation and tracking method designed to enable quantitative analyses of cellular shape and motion from dynamic three-dimensional microscopy data. The method uses multiple active surfaces with or without edges, coupled by a penalty for overlaps, and a volume conservation constraint that improves outlining of cell/cell boundaries. Cell migrations and deformations play essential roles in biological processes, such as parasite invasion, immune response, embryonic development, and cancer.. Its main advantages are robustness to low signal-to-noise ratios and the ability to handle multiple cells that may touch, divide, enter, or leave the observation volume. We have recently shown that our level-set-based cell segmentation and tracking algorithm is more accurate and robust than other state-of-the-art methods, especially in image sequences with greatly varying object intensity distributions. Using this algorithm as a basis, we have applied a shape-based motion correction algorithm, which to our knowledge has not been used before for this purpose. foci. Based on this, we propose a novel approach to cell-phase identification, which utilizes features from the segmented foci, rather than from the raw (noisy) images. Our system can be potentially used for any biological application requiring combined cell and foci analysis.

III. PROPOSED SCHEME

The proposed system which uses a morphological technique is applied on the fluorescent cells so as to get a clear cut segmented image. The main advantage of the system is it is fully automatic. The frames that are extracted from the video should undergo various preliminary screening techniques. This system eliminates the existing traditional methodologies on segmenting whereas it utilizes some of the techniques used for segmenting traditionally. In this system the cells are segmented and shape tracked based on morphological operations. The morphological operations include the morphological mathematical functions. For this the wavelet otsu paradigm is used in where the image or frame is filtered , segmented and finally they are made to undergo so many stages such as the classes whereas in every classes they should be transformed by means of iterative scannings. . Otsu suggested minimizing the weighted sum of within-class variances of the foreground and background pixels to establish an optimum threshold. The Otsu method gives satisfactory results when the numbers of pixels in each class are close to each other. The Otsu method still remains one of the most referenced thresholding methods. In a similar study thresholding based on isodata clustering. From those scannings, the class differentiation shows the vulnerability of the disease structure in the cell by taking each class result into consideration. This methodology makes use of Thresholding in cells which is entirely new in the domain of medical imaging whereas the cells are fragmented only by means of frequencies and not on threshholding criterians. By calculating the class probability functions the probability, that the spreading of disease while segmentation is easily noted down and can be utilized further. The class means which will be detected is also used for finding effective splitting and spreading stage of infected and uninfected cells.

IV. EXPERIMENTAL RESULTS

An object can be easily detected in an image if the object has sufficient contrast from the background. Fluorescent microscopy allows the direct visualization of molecules at the subcellular level, in both live and fixed cells. .The shape tracking methods can be decomposed in two tracking types the line tracking and the junction tracking. Next the tracking process depends of used tracking elements. These ones are of two types : pixel element and surface element. The experiment is carried out by these methods.. The video that got splitted into frames are allowed to pass through various stages by taking the first frame and the current frame into account.



(i) first Frame (ii) Current frame

Fig 2. First and the current frames

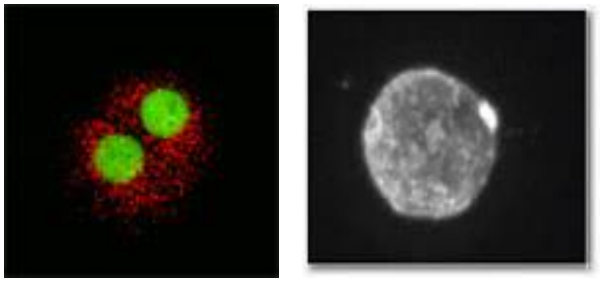
A. Preprocessing

The Preprocessisng of the images or frames obtained by the video are performed with the AHE algorithm. Adaptive histogram equalization (AHE) is a computer image processing technique used to improve contrast in images. It differs from ordinary histogram equalization in the respect that the adaptive method computes several histograms, each corresponding to a distinct section of the image, and uses them to redistribute the lightness values of the image. It is therefore suitable for improving the local contrast of an image and bringing out more detail. However, AHE has a tendency to overamplify noise in relatively homogeneous regions of an image.

Algorithm : *Adaptive histogram Equalisation*

```
for every pixel i in image do
 for every pixel j in last left column do
 Hist[g(j)] = Hist[g(j)]-1;
 end
 for every pixel j in current right column do
```

```
Hist[g(j)] = Hist[g(j)]+1;
End
```

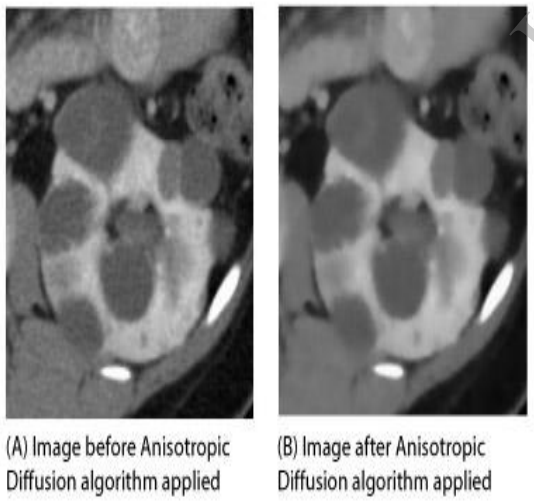


(i) before preprocessing (ii) After Preprocessing

Fig 3. Cells before and after preprocessing

B. Anisotropic diffusion filtering

Anisotropic diffusion resembles the process generates a parameterized family of successively more and more blurred images based on a diffusion process. Each of the resulting images in this family are given as a convolution where the width of the filter increases with the parameter.. A diffusion filter is a translucent photographic filter used for a special effect. When used in front of the camera lens, a diffusion filter softens subjects and generates a dreamy haze. This can also be improvised by smearing petroleum jelly on a UV filter or shooting through a nylon stocking. Diffusion filters may be uniform or may have a clear center area to create a vignette of diffused area around the clear center subject. Anisotropic diffusion is a generalization of this diffusion process: it produces a family of parameterized images, but each resulting image is a combination between the original image and a filter that depends on the local content of the original image.

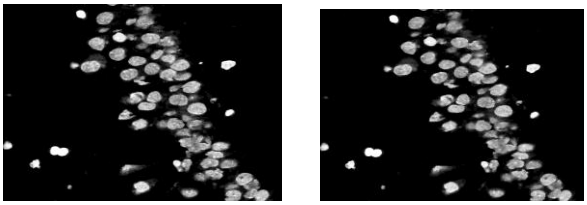


(A) Image before Anisotropic Diffusion algorithm applied (B) Image after Anisotropic Diffusion algorithm applied

Fig 4. Anisotropic diffusion filter when applied to cells

Algorithm : *Anisotropic Diffusion Filtering Equation*

```
function diff_im = anisodiff2D (im, num_iter, delta_t,
kappa, option)

im = double(im); (Convert input image to double.)

diff_im = im; (PDE (partial differential equation) initial
condition)

for t = 1:num_iter (perfoms conventional anisotropic
diffusion (Perona & Malik) upon a gray scale image.)

Finite differences [imfilter (.,.,'conv') can be replaced
conv2 (.,.,'same')]

Fprintf ('\r Iteration %d\n',t);
```



(i) Anisotropic Diffusion filter with 5 iterations (ii) Anisotropic Diffusion filter with 10 iterations

Fig 5. Diffusion with iterations

C. K- Means Clustering

Image after running *k*-means with $k = 16$. Note that a common technique to improve performance for large mages is to downsample the image, compute the clusters, and then reassign the values to the larger image if necessary. The K-means algorithm is an iterative technique that is used to partition an image into *K* clusters. The basic algorithm is:

1. Pick *K* cluster centers, either randomly or based on some heuristic
2. Assign each pixel in the image to the cluster that minimizes the distance between the pixel and the cluster center
3. Re-compute the cluster centers by averaging all of the pixels in the cluster
4. Repeat steps 2 and 3 until convergence is attained (i.e. no pixels change clusters)

In this case, distance is the squared or absolute difference between a pixel and a cluster center. The difference is typically based on pixel color, intensity, texture, and location, or a weighted combination of these factors. *K*can be selected manually, randomly, or by a heuristic. This algorithm is guaranteed to converge, but it may not return the optimal solution. The quality of the solution depends on the initial set of clusters and the value of *K*. It uses similar idea of combining clustering in RGB space with adaptive

thresholding. At first, thresholding reveals objects on background. Then image is clustered with k-means algorithm to distinguish fluorescent cells from other objects. Correct segmentation is crucial to obtain good quality features measurements and consequently successful diagnosis. The system of malignancy classification was tested on a set of real case medical images with promising results. Clustering is one of the most common automated segmentation techniques used for biomedical image segmentations. This research utilizes an optimized initial centroids for K-Means clustering algorithm for segmenting acute leukemia blood cells images. Experimental results shows better segmentation images using the proposed initialization method of classic K-Means clustering as compared to randomly choose centroids K-Means. This k means clustering algorithm is applied to the first and the current frames taken for our study .

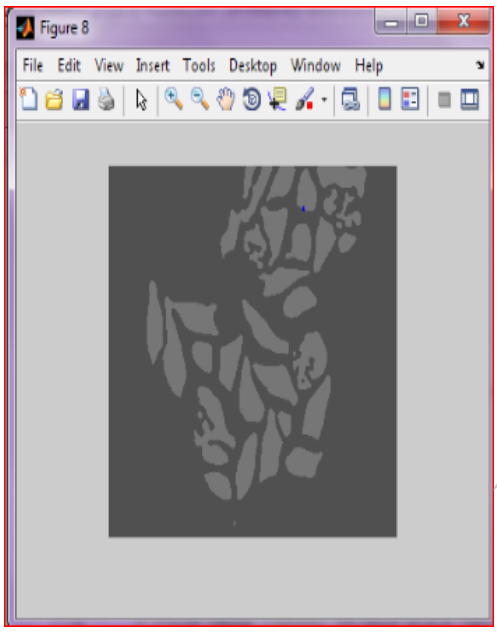


Fig 6. K-means clustering applied on our video

D. *Morphological Analysis*

Morphological analysis is important to study the cellular organization and the physiological state of the cells, and thus it can be commonly used as a qualitative and quantitative measure of various biological assays. Analysis of cell morphology remains increasingly important, as the image analysis aids in the detailed examination of microscopic cells; study of cell behavior, and also provides quantitative measure of its curvature, area, perimeter, eccentricity and additional metrics of nuclear morphology for large populations of cells. Analysis of the cells based on their morphological differences is applied to study the differentiation of stem cells, cancer cells, and in hematology. A wide variety of image analysis software packages have been developed that helps to convert to the microscopic images into more relatively quantitative measurements. In order to control the growth of the infectious cancerous cells during , a fully-automated sampling and analysing system has been developed.. This measuring system combines quantitative and qualitative analysis methods in a digital image processing technique, which offers the possibility of on-line estimation of cell development that approximates real-time. Perturbations within the process are detectable in time and regulative actions can be done without any delay.

Algorithm *: Wavelet - Otsu Morphological Analysis*

```
function otsu(histogram, total) {
   var sum = 0;
   for (var i = 1; i < 256; ++i)
      sum += i * histogram[i];
      sumB += i * histogram[i];
      mB = sumB / wB;
      mF = (sum - sumB) / wF;
      between = wB * wF * Math.pow(mB - mF, 2);
      if (between > max) {
         max = between;
         threshold = i;
      }
   return threshold;
}
```

V. CONCLUSION

In this paper, we have presented a fast and robust approach to tracking whole fluorescent cells in time-lapse series. The proposed tracking scheme combines CED filtering with diffusion. It allows simultaneous tracking of multiple cells over time by applying a topological prior that exploits the object indication function. The experimental evaluation was performed on 2-D and 3-D time-lapse series of rat adipose-derived mesenchymal stem cells and human squamous cell carcinoma cells, respectively. It clearly verified the improved accuracy up to about 9% . Thus, they could be preferred in studies focused on local morphological changes in the cell shape, in which as-accurate-as-possible cell boundaries are the most crucial task. There are several limitations that we intend to address infuture work to improve the overall performance of the proposed tracking scheme. A manual separation of cells clustered in the first frame is required to track each of them correctly in the previous methodologies and that is corrected here. This complicates the use of the proposed tracking scheme in experiments with high density of tightly packed cells.

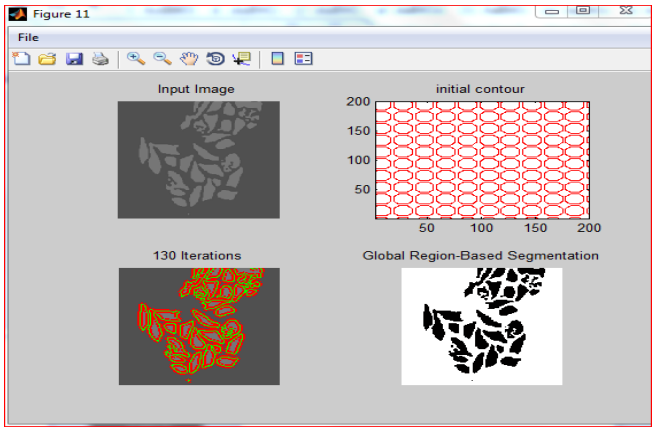


Fig 7. Input image when segmented through various phases

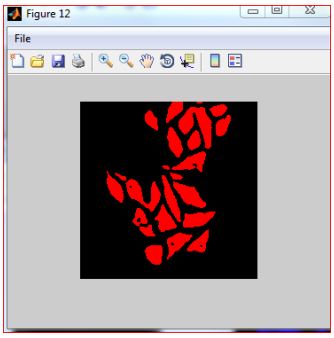


Fig 8. Initial frame

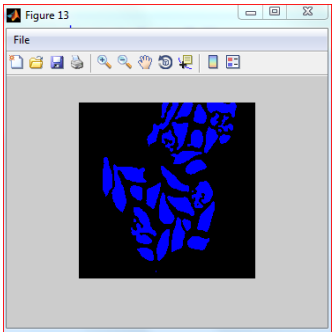


Fig 9. Current frame

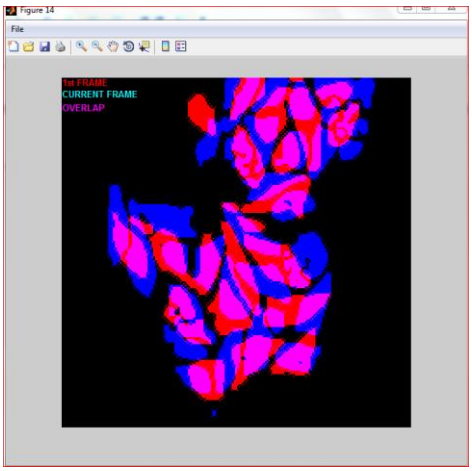


Fig 10. Overlapped frames with clear separation of cells

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