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ENHANCED WAVELET OTSU TRACKING METHOD FOR CARCINOMA CELLS

G.Merlin Sheeba*
Department of ETCE, Faculty of Electrical and Electronics, Sathyabama University, Chennai, India.
Email: mersheeb@yahoo.com

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Abstract

In biological processes applications such as immune response, embryonic development, or tumorigenesis the cells have the ability to exert forces as they move in their environment .Recent technological advances in confocal fluorescence microscopy have given researchers the opportunity to investigate these complex processes in vivo .However, they also lead to a tremendous increase in the amount of image data acquired during the studies. Therefore, the analysis of time-lapse experiments relies increasingly on automated image processing techniques. Namely, there is a high demand for fast and a robust method to help biologists to quantitatively analyze time-lapse image data. The aim is to segment and track deformable cells such as amoebae in noisy images from confocal microscopy. Among the tracking by model evolution approaches, the algorithm based on coupled implicit active contours governed by the modified Chan-Vese model, brings long execution times. A different tracking by model evolution approach is proposed making the algorithm as fast as possible and directly applicable to 2D as well as 3D time-lapse series. Enhancement in the proposed scheme is done by using OTSU algorithm which is used to automatically perform histogram based image thresholding (i.e. OTSU background subtraction). Wavelet Transform and Background detection is performed to remove the noise. Finally the frames are constructed back to video and time required for cell tracking by this enhancement method is reduced and more efficient. OTSU 2D histogram method solves this problem by reducing the computation time. The experimental evaluation is performed on 2D and 3D time-lapse series of rat mammary carcinoma cells and human squamous cell carcinoma cells, respectively. Using this proposed method, the problem of its sensitivity to the object size can be overcome. The method will be very helpful for the subsequent processing and improves the success ratio of image segmentation.

Keywords: Chan Vese Model;OTSU method; Carcinoma Cells; 2D Histogram;Coherence Diffusion Filtering;

Introduction:

Understanding the mechanisms of cell motility and their regulation is an important challenge in biomedical research. The cells in biological processes such as embryonic development, immune responses etc. exert force on their environment and alter their shape as they move [1]. As many recent technological advances have bloomed in confocal fluorescence microscopy, researchers are given the opportunity to investigate these complex processes [2], [3].But there is a tremendous increase in the amount of image data acquired during these studies. Therefore, the time-lapse experimental analysis relies increasingly on automated image processing techniques. Namely, there is a high demand for fast and robust methods to help biologists to quantitatively analyze time-lapse image data. The crucial tasks are segmenting, tracking, and evaluating movement tracks and morphological changes of cells, sub cellular components, and other particles [4].

To cope with these difficulties, sophisticated tracking approaches have been proposed during the last decade. Although cell motility and intracellular flows can be analyzed using optic flow or image registration techniques, this paper aims at identifying the boundaries of individual cells in each frame and tracking their evolution over time. Approaches developed for this specific task can broadly be classified as either tracking by detection or tracking by model evolution.

Among the tracking by model evolution approaches, the algorithm based on coupled implicit active contours governed by the modified Chan-Vese model, proposed originally by Dufour et al.[5] and improved by Dzyubachyk et al.[4], is the most excellent approach. But, the representation of each cell by one level set function evolved by numerical solution of a partial differential equation (PDE) brings long execution times, even when the narrow band technique is employed. A different tracking by model evolution approach is proposed aiming to make the algorithm as fast as possible and directly applicable to 2D as well as 3D time-lapse series. Further, to reduce the amount of noise in the acquired image data, the contrast along the cell boundaries is increased. The coherence-enhancing diffusion filtering (CED) succeeds the tracking step.

The technique can be directly applied on cells of various sizes and complex shapes. This framework is used in the proposed tracking scheme and compares their accuracy, execution time, and memory consumption. The cells used her analysis are the 2D time lapse series samples of rat adipose-derived mesenchymal stem cells (ADMSCs) and human lung squamous cell carcinoma cells (H157), respectively.

The proposed tracking scheme is more accurate and but the execution time is very large. So, a enhanced scheme is proposed by using OTSU algorithm which is used to automatically perform histogram based image thresholding (ie OTSU background subtraction). Wavelet Transform and Background detection is performed to remove the noise . Finally the frames are constructed back to video and time required for cell tracking by this enhancement method is reduced and more efficient.

I. Related Works

M. Maska et.al.[6] have used the technique of coherence-enhancing diffusion filtering to reduce the amount of noise on each frame .The enhanced cell boundaries are detected by minimizing the Chan-Vese model. J. Weickert et.al.[7] has addressed the problem by presenting a multiscale method in which a nonlinear diffusion filter is steered by the so-called interest operator. An m-dimensional formulation is performed and its well-posedness and scale-space properties are analyzed. An efficient scheme is presented which uses a stabilization by a semi-implicit additive operator splitting (AOS), and the scale-space behavior of this method is illustrated by applying it to both 2-D and 3-D images.Dufour et.al.[5] describes a fully automatic segmentation and tracking method for analyzing the cellular shapes and motion taken from dynamic three-dimensional microscopy data. Active surfaces with and without edges are used in this method along with a volume constraint also. They have validated the results with real biological data and a quantitative validation based on synthetic images is performed.

Jun Zhang et.al.[8] have given a detailed analysis of Otsu method .Otsu method behaves well in segmenting 2D images of low SNR than one dimensional (1D).As the number of pixels in each class are close to each other only satisfactory results are obtained. The authors had used 2D histogram projection to correct the Otsu threshold. The extrema of the projected histogram is experimentally evaluated using an algorithm based on wavelet transform. Alexandre Dufour et.al.[9] presents a fully automated technique for segmenting and tracking cells in 3-D. The main advantage of this method is the ability to handle touching cells, dividing cells and strong robustness to image noise. Significant room for improvement remains to remove several important restrictions. First, since the method still relies on the existence of an image background, it is ill adapted to tight cell clusters such as those occurring in tissue. This could be addressed by staining both nuclei and membranes and using an additional active surface to outline the cell boundaries coupled to active surfaces targeting the nuclei. Inclusion of additional image constraints such as motion is also likely to improve outlining of intercellular boundaries. Second, the a priori constraints on surface and volume are less suited to outline cell boundaries with high curvature such as filopodia.

II. Materials and Methods

A. Chan-vese model

The Chan-Vese algorithm is an example of a geometric active contour model. Such models begin with a contour in the image plane defining an initial segmentation. The goal is to evolve the contour in such a way that it stops on the boundaries of the foreground region. There are different ways to define the evolution equation, like the contour might move with a velocity which depends on the local curvature at a given point or the image gradient at that point. The Chan-Vese algorithm evolves this contour through a Fast Level Set(FLS) method.

The Chan-Vese model proposed by Mumford and Shah is formulated as

$$E_{cv}(C, c1, c2) = \mu|C| + \lambda 1 \int_{\Omega 1} (uo(x) - c1)\,2dx + \lambda 2 \int_{\Omega 2} (uo(x) - c2)2\ dx \rightarrow (1)$$

Where c1 and c2 denote the unknown average intensity levels inside contours Ω 1and Ω 2, respectively, and μ,λ1, and λ2 are positive, user-defined weights. The optimal segmentation (C; c1; c2) corresponds to a global minimum of the above equation. The minimization is usually accomplished in three different frameworks: explicit active contours ,FLC and Graph Cuts(GC). The algorithm is slow, especially when dealing with large images.

B. *Proposed Tracking scheme*

The tracking scheme applies the coherence enhancing diffusion filter [7] on each frame first to reduce the amount of noise.Next the cell boundaries are detected by minimizing the Chan-Vese model. The flow diagram of the proposed tracking scheme is shown in Fig. 1.

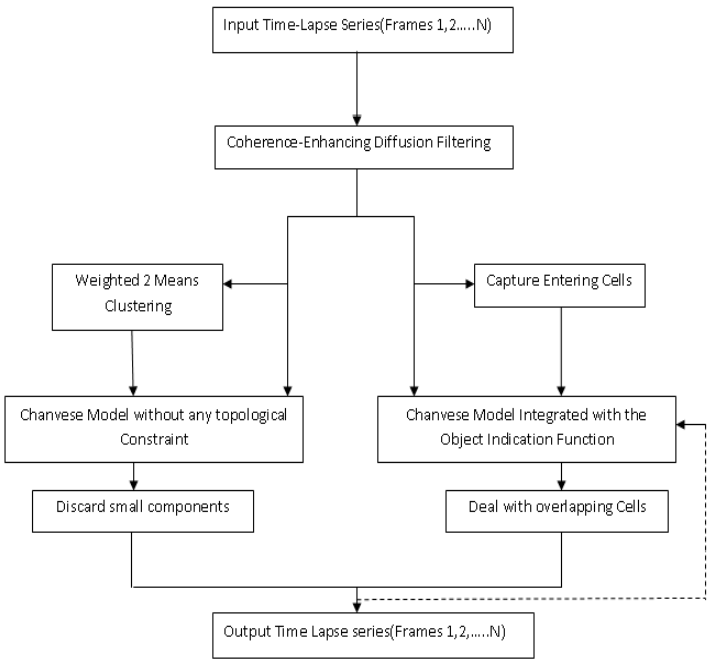

Fig. 1. Flow Diagram of Tracking Scheme.

To handle touching and dividing cells over time, both FLC and GC frameworks are integrated with a topological prior that exploits the object indication function [8]. The coherence enhancing diffusion filter is a robust method based on nonlinear anisotropic diffusion equation. This filter method enhances flow like structures with reduction of noise. The continuous diffusion process is discretized using finite difference schemes and solved iteratively. The implementation is based on the semi-implicit scheme stabilized by an additive operator splitting [7].This scheme can be easily extended into higher dimensions and allows the use of longer time steps compared to explicit schemes, which results in a lower number of iterations and faster computation.

1) First Frame Segmentation

Since the number of cells in the first frame is not known in advance, the cell boundaries are detected using the Chan-Vese model without any topological constraint. Both frameworks are initialized using a fully automated weighted 2-means clustering [10] that corresponds to the minimization of Ecv without the regularization term. The FLS framework takes the final contour provided by the clustering, whereas graph cuts start with the corresponding foreground and background statistics. Once a steady state is reached, small components enclosing foreign particles such as dust are discarded from the final binary mask. The remaining ones are established as the cells to be tracked.

2) Object Indication Function

One of the main aims of every tracking algorithm is to keep the identity of each tracked target over time. Since the FLS and GC frameworks are both topologically flexible, a simple binary separation of the image domain might yield undesired results. It is necessary to preserve the cell identities hence the isolated cells in one frame could merge in the next frame, so the contours must be prevented from merging. To construct a cell lineage tree, the information between mother and daughter cells relation needs to be maintained.

To fulfill these requirements, the FLS and GC frameworks have been integrated with a topological prior exploiting the object indication function ψ: $\Omega \rightarrow \{0, 1, 2,\ldots.\}$ [11] that assigns the zero label to the background grid points and a positive label to the foreground ones. It assumes that the image domain Ω is divided into a possibly disconnected background region $\psi 0$ and M possibly disconnected disjoint foreground objects $\psi 0$, $\psi 1$,.. ψM. Each foreground object ψi can split into several regions that can merge again later in time. The object indication function allows each foreground object to adapt freely to topological changes of the corresponding cell [11]. The object indication function is integrated into the static maximum flow algorithm [12] by maintaining four search trees instead of the original two.

The Kohli-Torr algorithm [13] to analyze the first frame for which no topological prior is involved, whereas the modified Boykov-Kolmogorov algorithm [12] is used for the others.

3) Segmented and Tracked Image Input data

All experimental images were acquired using spinning-disk confocal microscopes. A summary of the cell types, image data properties is given below; Sample frames from each of the datasets are shown in Fig 2. Mouse Mammary Carcinoma Cells Imaged on CV1000 Confocal microscopy stack projected with Magnification: 60X1.3NA, Pinhole size: 25um and Timelapse: 10ms and No. of frames :480.

The procedures are repeated and the cells are tracked using Chanvese model for first and current frame as shown in fig 3and 4.

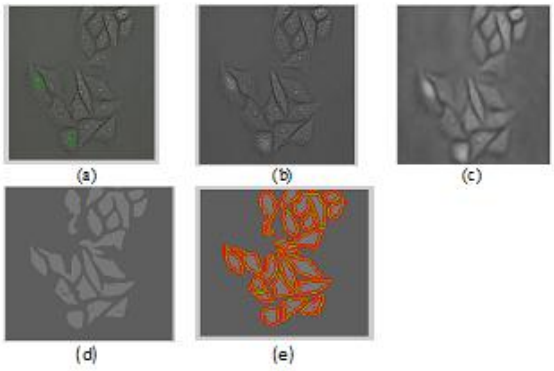

Fig. 2. Time-lapse datasets of Carcinoma cells (a) first Frame,(b) Contrast Enhanced output,(c) CED output,(d) clustered output,(e) Segemented and Tracked Cells.

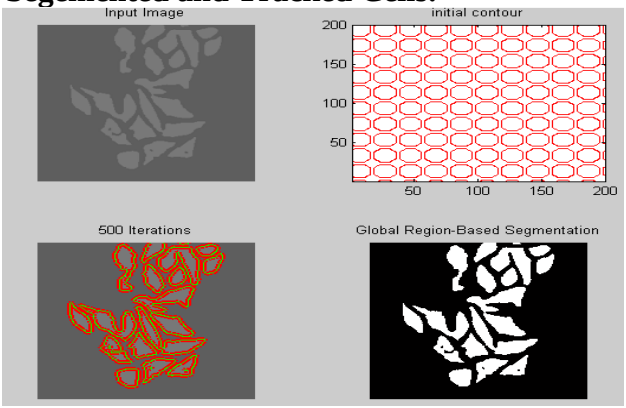

Fig. 3. Results of first frame using Chan Vese Model.

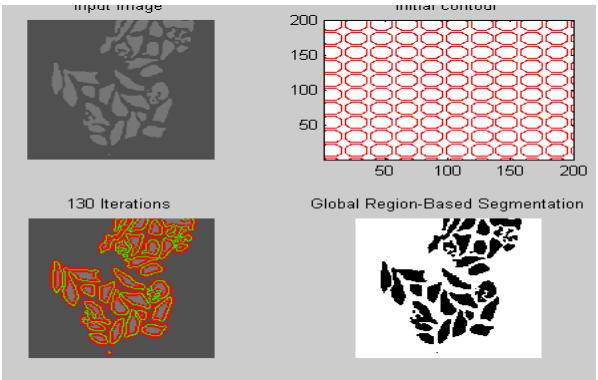

Fig. 4. Results of current frame using Chan Vese Model.

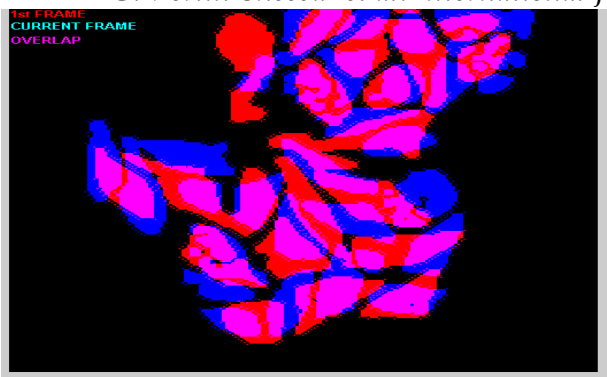

Fig 5.Overlapping of first and second frame.

When video runs on the whole the current and first frames will be overlapped as shown in fig 5 .But the computation time for the execution of Chan Vese model is more due to the CED filter. So enhancement is done by OTSU method.

III. Enhanced Tracking Scheme

C. Wavelet OTSU method

OTSU thresholding technique is one of the global thresholding methods and has been cited as an effective technique. The flow diagram of OTSU method is shown in fig 6 .One drawback according to research is its sensitivity to the object size. If the object proportion is much less than background, the pixels in background will be wrongly classified as object and if the object proportion is much more than background, the pixels in object will be wrongly classified as background. As for our live moving fluorescent cell samples, the object size is much less than background, the wrong classification of pixels by traditional Otsu method will lead to the failure in segmentation[14]. To solve this problem, a histogram analysis based on wavelet transform is proposed to correct the Otsu threshold in this paper.

D. Two Dimensional Histogram Analysis

Otsu method will give the improper results when the object size is very different from background; the 2D histogram projection [15] is used to correct the Otsu threshold. For renal biopsy samples, the object has a high gray level. If we project the 2D histogram in x and y axes, the last peak must be object. So after projection, the valleys corresponding to the last peak in x and y axes are regarded as the auxiliary threshold.

The final threshold (Sfinal, Tfinal) is calculated as

$$(S_{final}, T_{final}) = (\frac{S_{otsu} + S_{hist}}{2}, \frac{T_{otsu} + T_{hist}}{2}) \longrightarrow 2$$

Where (Sotsu,Totsu) is the threshold by Otsu method; (Shist,Thist) is the threshold by histogram analysis.

To eliminate the influence of the pseudo valley, use wavelet transform to obtain the smoothed histogram without tiny changes. The boundary detected and segmented first frame image of mammary carcinoma cells is shown in the fig. 7 and fig. 8 using the enhanced wavelet OTSU method. The time required to run a Fluorescent Cells data video using

the Chanvese model will be more to detect the cells movement and few edge information will be lost. So enhancement in the proposed scheme is done by OTSU algorithm which is used to automatically perform histogram based image thresholding (ie OTSU background subtraction), Wavelet Transform and Background detection is performed to remove the noise . Finally the frames are constructed back to video. Thus it reduces computational time and it will be more efficient. The above methods will be applied on video with different cell intensities. This gives clearer and more accurate output of segmented and tracked image

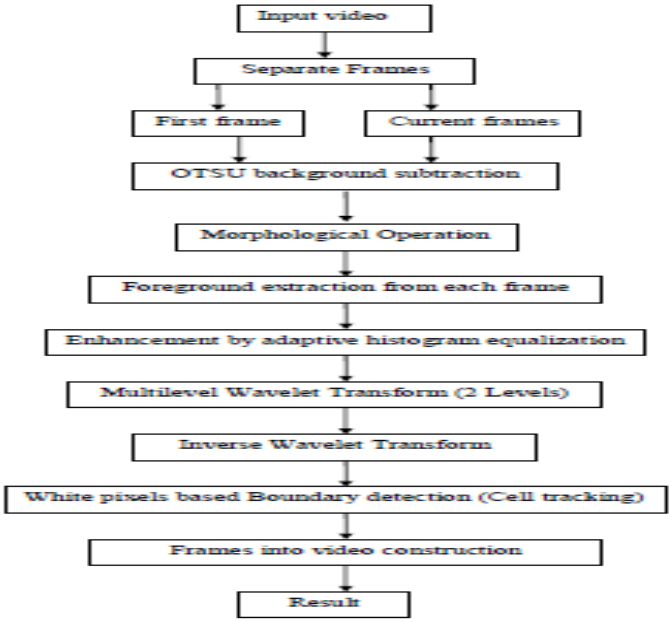

Fig 6.Block Diagram of Wavelet OTSU method.

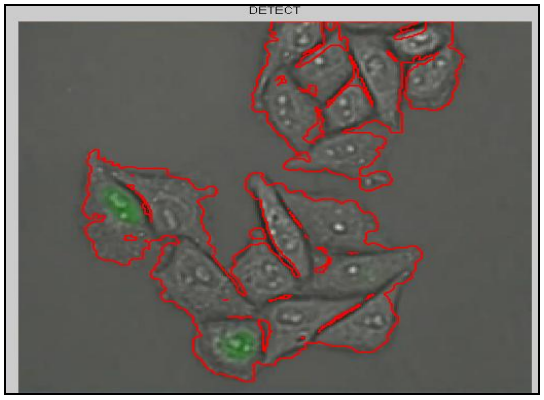

Fig 7.Boundary detected cells using OTSU method based on 2D histogram technique.

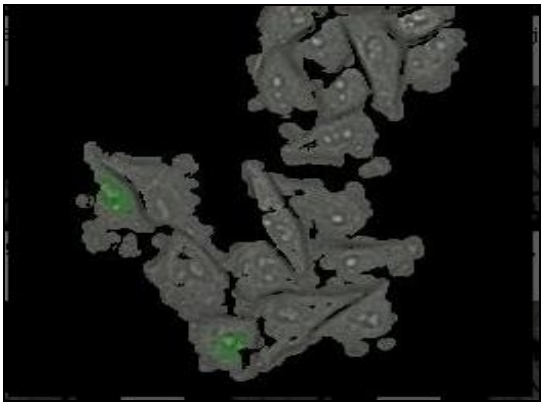

Fig. 8 Segmented Frame using OTSU method based on 2D histogram technique.

IV. Conclusion

The proposed tracking scheme combines CED filtering with the FLS and GC frameworks that minimize the Chan-Vese model. 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 2D and 3D time-lapse series of rat mammary carcinoma cells. But the time required to run a Fluorescent Cells data video using the above model will be more to detect the cells movement and few edge information will be lost. This paper also introduces an OTSU Wavelet 2D histogram projection analysis to solve the problem of computational speed in Chan Vese method. Using this method, the problem of its sensitivity to the object size can be overcome. It is very helpful for the subsequent processing and improves the success ratio of image segmentation.

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Corresponding Author:

G.Merlin Sheeba*,

Email: *mersheeb@yahoo.com*