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AN EFFECTIVE MITOSIS RECOGNITION AND SEGMENTATION TOOL FOR STEM CELL EXPLORATION

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ABSTRACT

Stem cells, on regenerative medicine, has enormous potential and impact, lead to the rapidly growing interest for tools to analyze and characterize the behaviors of these cells in- vitro in an automated and high throughput fashion. Measurement of the proliferative behaviors of cells in- vitro is important to many biomedical applications for the measurement of the accurate counting and localization of occurrences of mitosis, or cell division, in a cell culture. In this paper, the performance analysis of clustering for segmenting the mitosis detection is proposed. It is possible to manually identify incidents of mitosis because mitotic cells in culture tend to exhibit intensified surrounding halos under phase contrast illumination. This halo artifact is eliminated by using Diffusion corona filter. Using this method of segmentation precision of 97.1% is obtained which is 1.3% higher when compared with the semi Markov process of segmentation.

Keywords: mitosis, clustering, segmenting, halo artifact, phase contrast microscopy.

INTRODUCTION

Stem cells are distinguished from other cell types by two important characteristics. First, they are capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions. The mitosis division activities of stem cells grown in-vitro are critical tools for monitoring the health and growth rate of a cell population.

Mitosis detection is the process whereby the genetic material of a eukaryotic cell is equally distributed between its descendants through nuclear division, resulting in the birth of daughter cells.

Automated mitosis detection systems are required to localize birth events a birth event is defined in each mitotic event as the time and location at which the two daughter cells first appear and the boundary between the two is clearly observed. Accurate detection of birth events facilitate the quantification of biological metrics, such as the mitotic index and synchrony, allowing biologists to experimentally assess how altering the conditions under which cells are cultured can impact population growth. Precise localization of birth events may aid in the discovery and characterization of novel biological phenomenon that occur at rare frequencies such as a single cell division event giving rise to more than two daughter cells.

Conditional random field (CRF) [1, 2] model for sequence labeling assigns class labels to observations without exploiting hidden states. HCRF method [3] is used to detect mitotic cell regions based on brightness change and it links the regions in consecutive frames into a cell division linkage. Each cell was then validated based on its length .This approach is efficient due to the reduced search space by the cell detection, but the validation scheme is too simple to effectively distinguish actual mitotic events from the other cell. The hidden-state CRF (HCRF) [4] model for sequence classification utilizes hidden states but assigns only a single class label to the entire sequence. Hidden Conditional Random Fields (HCRF) for gesture recognition [5] is trained to examine each of the candidates. This approach does not resort to tracking and adopts explicit candidate detection as well as model-based validation, however, this work un-detects birth events and is limited to mitosis detection hence its results may not be sufficient for accurate quantitative analysis of cell proliferation or cell tracking. Event-detection CRF (EDCRF) model can simultaneously detect mitosis and temporally localize the "birth" of daughter cells. EDCRF is identical to the latent-dynamic conditional random field (LDCRF) [6,7] model for gesture recognition, which is a discriminative graphical model that assigns a class label (gesture type) to each observation (image frame) in the input sequence and incorporates hidden state variables to model the intrinsic dynamics between output labels. The Magnetic resonance imaging (MRI) simulator (Tool) [8] is used for single cell in the image sequence but it cannot be used for different distance measure. The hybrid framework for mitosis detection [9], which combines an image segmentation algorithm based on the principles of phase contrast optics for mitosis candidate extraction [10], and an HCRF model for mitosis sequence classification. HCRF approach [11] to mitosis detection compensates the drawbacks of microscopy image segmentation. Stem cells detection by multiple radius ring filter [12] method is used to detect the cells with 96.5% precision. The main disadvantage is that it is applicable for detecting other structures with variable scales. The Expectation maximization algorithm [13]

detects cell regions but this approach is less efficient in cell colony analysis.

The Automatic detection algorithm [14] of tongue cancer stem cells based on FUZZY pattern improves the low recognition rate. By combining the fuzzy pattern with curvature vector fitting a hybrid approach is done which utilizes the pixel-intensity threshold for candidate extraction. Under the same max-margin framework, a semi-Markovian model [15] was given to address the temporal segmentation of a mitotic event, providing a more detailed timeline of each mitosis. Quantitative evaluation [16] in HSCs (Haematopoietic stem cells) population of time lapse phase contrast microscopy images establishes lineage relationships by tracking the individual cells. There are numerous proposed works in stem cell image segmentation which uses minimization of Chan-vese model in an intelligent custom [17] which combined two frameworks (FLS and GC).

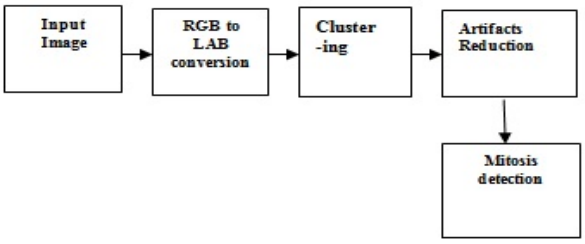
This method ,max-margin semi- Markov model (MM-SMM), segments each mitosis event into four consecutive stages, such as Interphase, start of mitosis, formation of daughter cell and separation of daughter cells, and the analysis is made on each stage based on the markovian process. However, this method is not widespread in biological applications since the mitosis event cannot be analyzed in stable conditions.

Segmentation of an image is done by threshold, clustering, compression based method and edge detection. Under which clustering reduces the error by grouping the image part, by which the blurring can be eliminated. In this proposed work clustering is used for mitosis detection segmentation. Clustering, a method for segmentation is done by modified fuzzy C means method. This increases the mitosis detection segmentation precision with high PSNR value

CLUSTERING BASED MITOSIS DETECTION SEGMENTATION APPROACH:

This proposed model for mitosis detection and segmentation is performed by the following steps described by the flow chart. Un sharp masking (USM) is an image manipulation technique, often available in digital image processing software.

For mathematical operations RGB image is converted into LAB image. A *Lab* color space is a color-opponent space with dimension *L* for lightness *a* and *b* for the color-opponent dimensions, based on nonlinearly compressed CIE XYZ color space coordinates. *Lab* color is designed to approximate human vision. It aspires to perceptual uniformity, and its *L* component closely matches human perception of lightness. Convert the image to L*a*b* color space using make c form and apply c form.



Figur-1. Mitosis detection flow diagram.

The Algorithm Modified fuzzy c-means (FCM) is a method of clustering which allows one piece of data to belong to two or more clusters. It is based on minimization of the following objective function:

Step-1: Initialize $U=[u_{ij}]$ matrix, $U^{(0)}$

Step-2:
$$j_m = \sum_{i=1}^{N} \sum_{j=1}^{C} u_{ij}^{m} \left\| x_i - c_j \right\|^2 , 1 \le m < \infty \quad (1)$$

where m is any real number greater than 1, u_{ij} is the degree of membership of x_i in the cluster j, x_i is the i^{th} of d-dimensional measured data, c_j is the d-dimension center of the cluster, and ||*|| is any norm expressing the similarity between any measured data and the center. Modified fuzzy partitioning is carried out through an iterative optimization of the objective function shown in step-2, with the update of membership u_{ij} and the cluster centers c_j expressed by:

Step-3:
$$u_{ij} = \frac{1}{\sum_{k=1}^{C} \left(\frac{\left\| x_i - c_j \right\|}{\left\| x_i - c_k \right\|} \right)^{\frac{2}{m-1}}} \quad (2)$$

$$c_j = \frac{\sum_{i=1}^{N} u_{ij}^{m} \cdot x_i}{\sum_{i=1}^{N} u_{ij}^{m}} \quad (3)$$

This iteration will stop when $\max_{ij} \left\{ \left| u_{ij}^{(k+1)} - u_{ij}^{(k)} \right| \right\} < £$, where £ is a termination criterion between 0 and 1, whereas k are the iteration steps. This procedure converges to a local minimum or a saddle point of J_m.. The above formulas are referred [18].

Phase contrast microscopes convert the minute phase shifts caused by transparent specimens to the illuminating light source into variations in light amplitudes that can be observed by naked eyes or captured by cameras. Due to the optical principle and the inherent imperfections of the conversion process, phase

contrast images contain artifacts such as *halos* (local contrast reversal surrounding the specimen) and *shade-off*. The Diffusion corona filter is used to reduce artifacts present in the image.

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. Objects and boundaries (lines, curves, etc.). Of the mitosis detected cell in images are located. Visual characteristics of the mitosis detected cell are obtained.

PERFORMANCE ANALYSIS AND RESULTS:

First initialize the **weight index** for clustering the image. In order to simplify the mathematical process the M x N Matrix is converted into M x 1 Matrix. Bay's Rule determines the posteriori probability of the Minima pixel value. Maxima image pixel value is obtained from the length of the matrix and the histogram for the matrix is found. The pixels intensity value and the location of the pixel are estimated by comparing the histogram of each pixel with the help of centre point. Hence by using bay's rule maxima and minima pixel value is estimated efficiently with the help of centre point and the mean value is calculated. Finally image segmentation is performed by taking the absolute values of the entire image. The Original stem cell Input image shown in Figure-2.

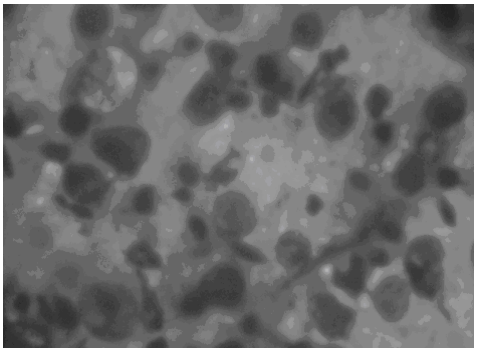


Figure-2. Original stem cell input image with mitosis event captured by phase contrast microscopy.

For mathematical convenience RGB image is converted into LAB image shown in fig 3. A *Lab* color space is a color-opponent space with dimension *L* for lightness and *a* and *b* for the color-opponent dimensions. *Lab* color is designed to approximate human vision. It aspires to perceptual uniformity, and its *L* component closely matches human perception of lightness.

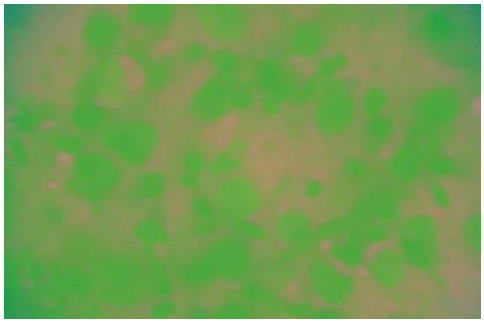


Figure- 3. LAB image.

The lab converted image is sent through the process of clustering by modified fuzzy c means method and the resultant mitosis detected grouped image is shown in Figure-4.

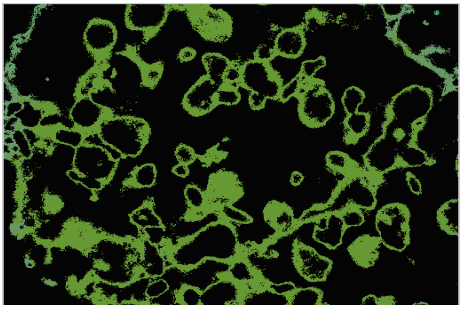


Figure-4. Objects in cluster with mitosis detected grouped image 1.

The clustered output contains three layer images. For simplification the three layer image is converted into two layer image as shown below in fig. 5 with halo artifacts due to phase contrast microscopy illumination.

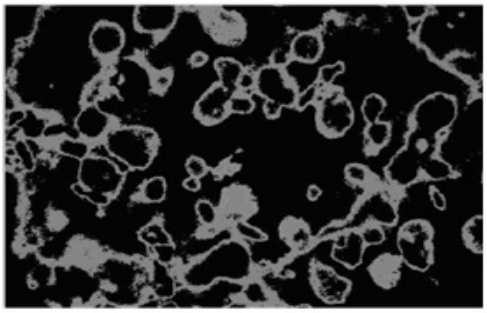


Figure-5. Halo artifact image due to phase contrast microsecopy.

Phase contrast microscopes convert the minute phase shifts caused by transparent specimens to the illuminating light source into variations in light amplitudes that can be observed by naked eyes or captured by cameras. Due to the optical principle and the inherent imperfections of the conversion process, phase contrast

images contain artifacts such as *halos* (local contrast reversal surrounding the specimen) and *shade-off*. Applying Diffusion corona filtering in Figure-5 reduces the artifacts present in the image, given in Figure-6.

Diffusion corona filter creates soft edges without affecting the sharpness and contrast of the image. It is a scale space technique where each and every data is varied based on its local features for restoring the image. The Diffusion equation is,

$$\partial_t u = \text{div}(D.\nabla u) \quad (4)$$

Where

D-Diffusion tensor

∇u- Gradient of the input imageDiffusion tensor is spatially invariant which determines the diffusion direction .Under homogeneous condition diffusion tensor becomes 1. Hence,

$$\partial_t u = \text{div}(\nabla u) = \Delta u = u_{xx} + u_{yy} \quad (5)$$

For high contrast region low diffusion is performed whereas for low contrast region high diffusion is performed. In existing diffusion filters time diffusion direction is fixed. Whereas in diffusion corona filter the diffusion orientation is made adaptive and hence the diffusion direction over an image is varied which in turn preserves and enhance the image features.

$$g(|\nabla u|^2) = 1/(1 + (|\nabla u|^2 / \lambda^2)) \quad (6)$$

Where, g= continuous boundary mapping

λ = Contrast parameter to discriminate noise from edges.

The strength of diffusion at the edges can also be changed and controlled by using eigenvalues .Thus diffusion corona filter is a fast and stable filter which gives high performance with flexibility. It can be observed that two regions with almost constant grey value evolve which are separated by a fairly steep edge. Edge enhancement is caused by the fact that, due to the rapidly decreasing diffusivity, smoothing within each region is strongly preferred to diffusion between the two adjacent regions. The edge location remains stable over a very long time interval. This indicates that, in practice, the determination of a suitable stopping time is not a critical problem. After the process of contrast enhancement is concluded, the steepness of edges decreases very slowly until the gradient reaches a value where no backward diffusion is possible anymore. Then the image converges quickly towards a constant image.

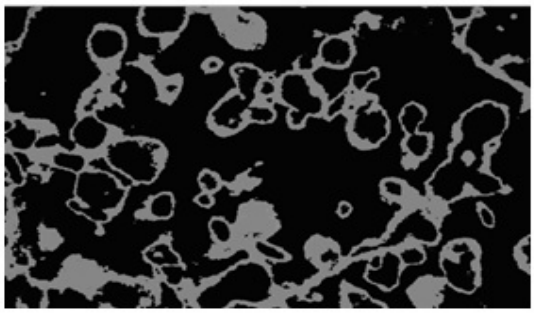


Figure-6. Halo artifact reduction using diffusion corona filtering.

By Calculating posteriori probability using baye's rule and then estimate intensity pixels by comparing the histogram of each pixels. The mitosis is identified by calculating the location and absolute centroid point of intensity pixels. Finally the identified mitosis region is segmented. The segmented mitotic region is shown below in Figure-7.

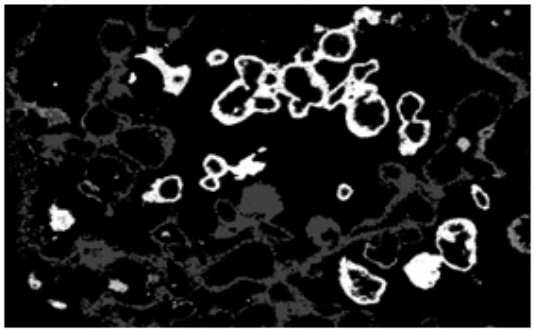


Figure-7. Enhanced mitotic region.

SENSITIVITY AND SPECIFICITY

$$Sensitivity = \frac{TP}{TP + FN} \quad (7)$$

$$\text{Precision} = SS * \left(\frac{TP}{TP + FP} \right) \quad (8)$$

$$\text{Recall} = SX * \left(\frac{TP}{TP + FN} \right) \quad (9)$$

$$PSNR = 20 * \log_{10} \left(\left(\frac{255^2}{mse} \right) \right) \quad (10)$$

Where- TP- True Positive, FN- False negative, FP- False Positive and MSE- Mean square error. By using above formula the obtained parameters are listed below:

Table-1. Result analysis obtained for different parameters.

Parameter	Value
Precision	97.11%
Recall	97%
PSNR	15.11
Sensitivity	50
Specificity	75

When compared HCRF and SMM method, the FCM method achieves higher precision and recall. Since SMM is limited because of its inability to accommodate long-range dependencies CRF is limited in that it cannot capture intermediate structures using hidden-state variables. The results are shown in Table-2. Result shows that the precisions of proposed method are significantly and consistently better than of SMM.

Table-2. Accuracy comparison of mitosis detection. Segmentation.

Method	Precision (%)	Recall (%)
FCM	97.1	97.0
MM-HCRF+MM-SMM	95.8	88.1
MM-HCRF	82.8	92.2
EDCRF	91.3	87.0
CRF	90.5	75.3

CONCLUSIONS

The identification of healthy and unhealthy stem cells is very important in biomedical applications. The segmentation of mitosis detection is achieved by modified fuzzy C-means algorithm in which the mitosis content image is grouped in cluster 1. The halo artifact introduced in this process is reduced by using Diffusion corona filter. The precision obtained by this algorithm is 97.11%. The comparison results obtained reveals that the proposed work gives better performance when compared with MM-HCRF+MM-SMM models.

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