

# An Integrated Bi-Model Distance Regularization Method for Stem Cell Segmentation



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## Abstract

Image processing is a lively field of research, where engineering and science disciplines cooperate together. The remarkable discovery of the prompted stem cells has been demonstrated in 2D time lapse series with a novel image processing algorithm, developed and tested for accurate segmentation and shape tracking of cells in digital images. CED filtering with a rare blend of Gaussian filtering is being applied by reducing the noise and enhancing the flow like structures with decreased execution time. The cell boundaries can be detected by Chan vese-Re-initialization model. But finding the initial curve in each time creates complexity and time consuming problem. This paper motivated to reduce the problem due to re - initialize and distance regularization. Hence integrated bi-modal distance regularized Chan-veese is proposed and implemented. Moreover this method gives better segmentation when compared with the traditional Watershed segmentation technique. The precision and recall values of the already existing Chan-veese are improved in integrated Bi-model distance regularization. In this case, two or more coupled curves evolve simultaneously to segment images with multiple objects, thereby reducing the computational cost and the level set functions. It also automatically evades the problems of vacuum and overlap. It can also represent boundaries with multiphase topologies.

**Keywords:** Bi-model distance regularization; Chan vese; Coherence enhance diffusion filtering; Gaussian filtering; Segmentation; Stem cell

## Introduction

In stem cell specialization, the morphological features like cell size, shape, and motility [1] play an important role. Due to high demand in automatic methods, probabilistic models are generated for tracking and segmentation of phase contrast microscopy images to identify cell locations by generating cell center for each frame. Threshold based tracking and segmentation [2] are growing to monitor the proliferation and differentiation of stem cells over the lifetime. The cell properties are measured and extracted from a large number of samples for practical approaches. The topological changes in cells are automatically detected in level set [3] functions and they don't require the solution of PDE's. Initialization problems still prevails in level set functions.

The Bi model Chan-veese method affects the curve evaluation during initialization of curves. Multiple objects are simultaneously segmented in multiphase Chan-veese method with two or more coupled curves. Qualitative description [4] of biological processes to identify location, functional status and richness in living cells are crucial in image processing systems. Microscopy images are in demand to biologists to visualize subcellular

components and process in vivo. Fluorescence microscopy [5-7] images have large data set with high dimensionality and give valuable source of information for contemporary biology systems. Biological analysis is indispensable to discover drugs and medicine. The image monitored with confocal microscopy yield a time lapse sequence for better analysis of segmentation. Adaptive Segmentation [6] based on active contour is used to fragment cell areas and in this method scrutinizing the data depended adoption is very challenging. Automated tracking of cell population is important to monitor the living biological specimens. The Bayesian classifiers [8] fragment the mitosis, based on posterior probability, but some mitosis region was missed due to less trailing of samples. To avoid this problem high level object classification is involved. Automated tracking of mitosis detection [9] reduce the effort of human intervention.

The quality of performance is significantly improved by reducing the undetected mitosis candidates. Quantitative evaluation [10] in HSCs (Haematopoietic stem cells) population of time lapse phase contrast microscopy images establishes lineage relationships by tracking the individual cells. Nowadays

regimentation [11] process is popular in recent medical image processing research. The combination of segmentation and registration is called regimentation. It was first introduced by novel taxonomy algorithm and it provides consistency in image guided applications. Normally, automatic methods [12] give more powerful results than a manual process. An interactive cell segmentation based on correction propagation yields best performance to detect uncertain regions [13]. Due to the diversity morphological cell changes, creation of automatic system for segmentation is very challenging in cell analysis. Hence, under diverse conditions different samples are smeared to understand the nature of the data sets [14].

The Chan-veese model was tested out in Multiphase with major directions [15] which was mainly performed to detect the overlapping of objects. Chang-veese model prolonged with re-initialization uses a level set method for segmentation [16]. The Chan-veese model is also extended to Distance regularization [17] which does not obtain the maximum speed of segmentation.

To overcome the distance regularization problem Chan-veese Bi-modal method is accomplished in re-initialization.

There are numerous proposed works in stem cell image segmentation which uses minimization of Chan-veese model in an intelligent custom [18] which combined two frameworks (FLS and GC). To overcome all the former drawbacks in this paper produces an experimental analysis of Chan-veese re-initialization model and motivated to integrate the Bimodal Chan-veese method and Distance regularization to improve the proficiency of segmentation process.

**Methodology**

INPUT: Time lapse series image of stem cells. In naturally slow process method, continuous projection of the frames gives an accelerated view of the process. In the above said method 8 samples are taken, with the period of 24 hours and each set is applied to the system for segmentation process followed by filtering process (Figure 1).

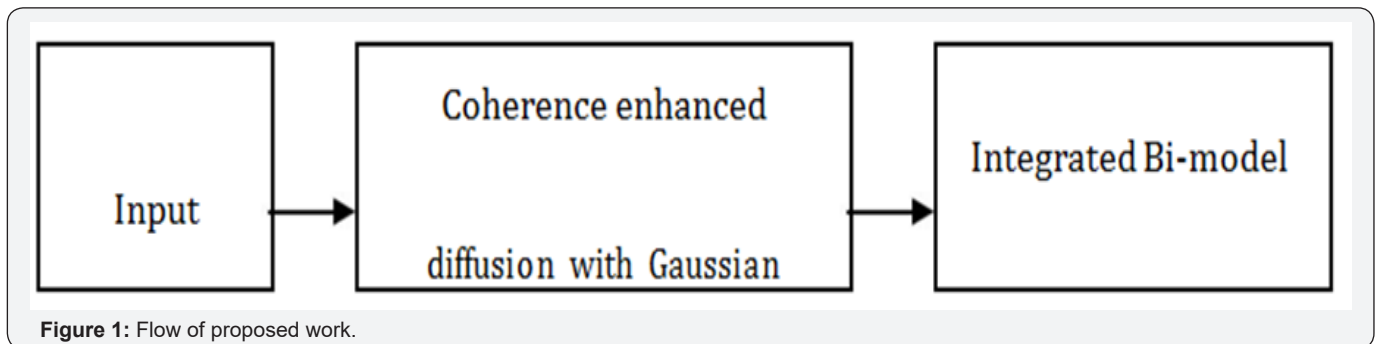


Figure 1: Flow of proposed work.

**Coherence-enhancing diffusion filtering**

In the paper [18], filter for enhancing coherent structures in vector-valued images have been presented based on two ideas: a generalized structure tensor for vector images, and anisotropic nonlinear diffusion filtering with a diffusion tensor. It shows that its main distinction from other color diffusion models lies in an additional integrated scale which gives a semi local average over the preferred orientation. This integration leads to significantly improved smoothing orientations, which is of importance for the enhancement of one-dimensional structures and it clarifies the role of the necessary parameters and proposed heuristics for their selection. Examples have been presented which shows that coherence-enhancing color diffusion is very robust under noise and of potential interest in various application areas. It is governed by the equation 1, 2, 3. From the paper [18].

$$\partial_t u - \text{div}(D \cdot \nabla u) = 0 \text{ in } I \times \Omega \tag{1}$$

$$\partial_n(u) = 0 \text{ in } I \times \Omega \tag{2}$$

$$U(x, 0) = f(x) \tag{3}$$

Where  $\Omega \in R^d$  is the image domain

$I=[0,T]$  is the potentially in bound time interval. The essential ingredient in this equation is the diffusion tensor  $D$  of the scaled image  $u$  which steers the diffusion process. It amplifies diffusion along flow-like structures, and hinders diffusion perpendicular to those patterns whose function is given by the equation 4.

$$S_p(u(x)) = G_p(\nabla u(x) \cdot (\nabla u(x))^T) = (G_p * (\partial x_i(x) \partial x_j u(x))) \tag{4}$$

For  $i, j=1,2,\dots,d$ ,

Here,  $G_p$ \* Gaussian standard deviation of  $p$

However, more general averaging procedures can be used. If  $\nabla u \neq 0$  the matrix  $(\nabla u \cdot (\nabla u(x))^T)$

Has rank one, the eigenvector  $\nabla u(x)$  belongs to the only non-zero eigenvalue  $|\nabla u(x)|^2$ . The eigen values represent the contrast in the directions of the eigen spaces. The averaging process then creates a matrix with full rank which contains valuable directional information.

This implementation is based on the semi-implicit scheme stabilized by an additive operator splitting. 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.

**Gaussian filtering**

As CED filtering is slower in its filtering process and combination of CED along with Gaussian filter will not only increase the speed of filtering but also produces an efficient output compared to the conventional filters. Gaussian filtering [19] is used to remove noise by introducing blurriness in the image. It incorporates gaussian function for processing 1-dimentional image is

$$G(x) = 1 / \sqrt{2\pi\sigma^2} e^{-x^2/2\sigma^2} \tag{6}$$

Where

G(x)-Gaussian function

σ - standard deviation of gaussian distribution.

For processing 2D- image

$$G(x) = 1 / \sqrt{2\pi\sigma^2} e^{-x^2+y^2/2\sigma^2} \tag{7}$$

Where

X- Distance from the origin in the horizontal axis.

y- Distance from the origin in the vertical axis. σ is the standard deviation of the distribution.

**Filtering involves convolution of an integral transform**

$$I = \int \exp(-x^2) dx = \sqrt{\pi} \tag{8}$$

Where Gaussian function is non zero as  $x \in [-\infty, \infty]$  and can require infinite window length. The gaussian blur to an image is same as convolving the image with a gaussian function, which has the effect of reducing the image high frequency components since the gaussian blur is a low pass filter. This filter removes gaussian noise and gives more weightage to the pixels near the edges. As the standard deviation (σ) of the gaussian distribution increases then the degree of smoothing becomes more intensive. Gaussian function gives the probability distribution of data and it is a smoothing operators. It is a smoothing function and it is never equal to zero. This filter works by using 2D- Distribution as a point spread function which is achieved by controlling the 2D gaussian function with the image. This filter is a non-uniform low pass filter which cannot preserve image brightness. It allows fast computation by giving co-efficient values nearer to zero at the edge of the mask.

**Segmentation**

Integrative Bi-model Distance Regularization

In order to control the smoothness of the zero level set and further avoid the occurrence of small isolated regions in the final segmentation, regularization term is added as a length penalty term L(C) which is defined related to the length of the evolving curve C. Let C be a smooth closed planar curve C (p): [0,1]→Ω

parameterized by parameter  $p \in [0, 1]$ . The length functional can be written as

$$L(C) = \oint dp \tag{9}$$

through replacing the curve C by the level set function, L(C) can be reformulated as

$$L(\phi = 0) = \int_{\Omega} y \nabla(dx\phi(d/y, x)) / y = \int_{\Omega} y \delta(\phi(dx\nabla dy)(d, x)) H \tag{10}$$

Where, H (z) is Heaviside function and δ (z) Dirac delta function. The use of length penalty term implies that the evolving curve C which minimizes the overall energy functional should be as short as possible. It imposes a penalty on the length of the curve that separates the two phases of image, i.e., foreground and background, on which the energy functional will make a transition from one of its values, c1 (d1), to the other, c2 (d2). In many situations, the level set function will develop shocks, very sharp and or flat shape during the evolution, which in turn makes further computation highly inaccurate in numerical approximations. To avoid these problems, it is necessary to reshape the level set function to a more useful form, while keeping the zero location unchanged. A common numerical scheme is to initialize the function φ.

(X, t = 0) as a signed distance function before the evolution, and then re-initialize the function φ(X, t) to be a signed distance function periodically during the evolution, which can be written as

$$\phi(X, t) = dist(X, C_t) \quad \text{if X is inside C,} \tag{11}$$

$$X \in C_t \tag{12}$$

$$-dist(X, C_t), \text{ if X is outside } C_t$$

Where, dist(X, Ct) is the shortest Euclidean distance of X to the points on the evolving curve Ct at time t. It is crucial to keep the evolving level set function as an approximate signed distance function during the evolution, especially in the neighborhood around the zero level set.

The most straight-forward way of implementing the re-initialization operation is to extract the zero level set and then explicitly compute the distance function from it. However, this method is generally time-consuming. To overcome this difficulty, a now widely accepted method has been proposed in order to re-initialize the level set function by solving the following partial difference equation

$$\partial \phi / \partial t = sign(\phi_0)(1 - |\nabla \phi|) \tag{13}$$

Where, φ0 is the function to be re-initialized, and sign (φ0) is the sign function. When the steady state of above equation is reached, φ will be a distance function with the same zero level set as φ0 despite φ0 is a distance function or not. This is commonly known as the standard re-initialization procedure. Another equivalent approach is to solve the following eikonal

equation:  $|\nabla \phi| = 1$

With the boundary condition on  $\phi = 0$  on  $\{\phi_0 = 0\}$  (14)

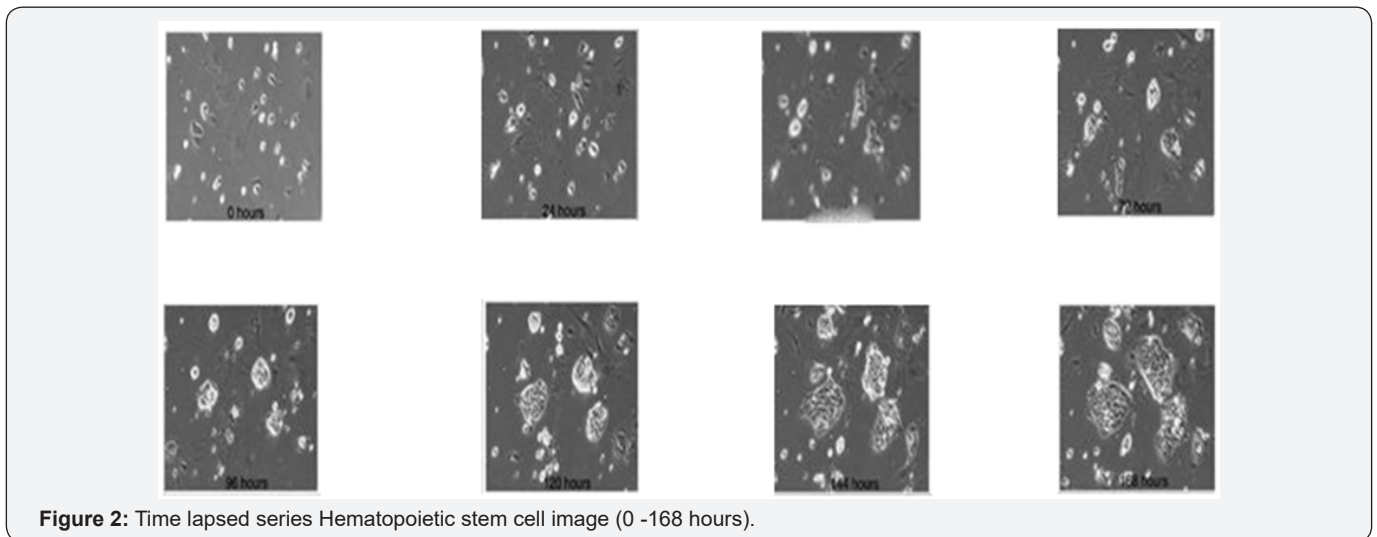


Figure 2: Time lapsed series Hematopoietic stem cell image (0 -168 hours).

**Experimental Analysis and Results**

**Outputs of combined ced and gaussian filters**

Filtering is an important pre-processing in image segmentation. To attain an efficient filtering combination of CED and Gaussian filter is used. The first 2 steps belong to CED and the final steps are trailed by Gaussian filtering process (Figure 2).

Step 1: The essential gradient is the diffusion tensor D of the scalar image which steers the diffusion process.

Step 2: Produces diffusion along flow-like structures, and hinders diffusion perpendicular to those patterns.

Step 3: Discrete approximations to the Gaussian function.

Step 4: Convolution of the 2D Gaussian distribution function with the image.

Figure 2 shows that the input image of Hematopoietic stem cell image with 0-168 hours, in which 0-hour normal stem cells are presented. And after 24-hours due to mitosis proliferation process daughter cells are created. The cell growth are improved in each 24-hours. At 168-hours or in one week the total cell colony is captured.

Figure 3 shows that CED with Gaussian filtered output. In the case of CED the processing time will be more. Hence to reduce the processing time and to improve the quality of the image Gaussian is added by blurring the background pixels. So that the foreground pixels is enhanced in each set of the image.

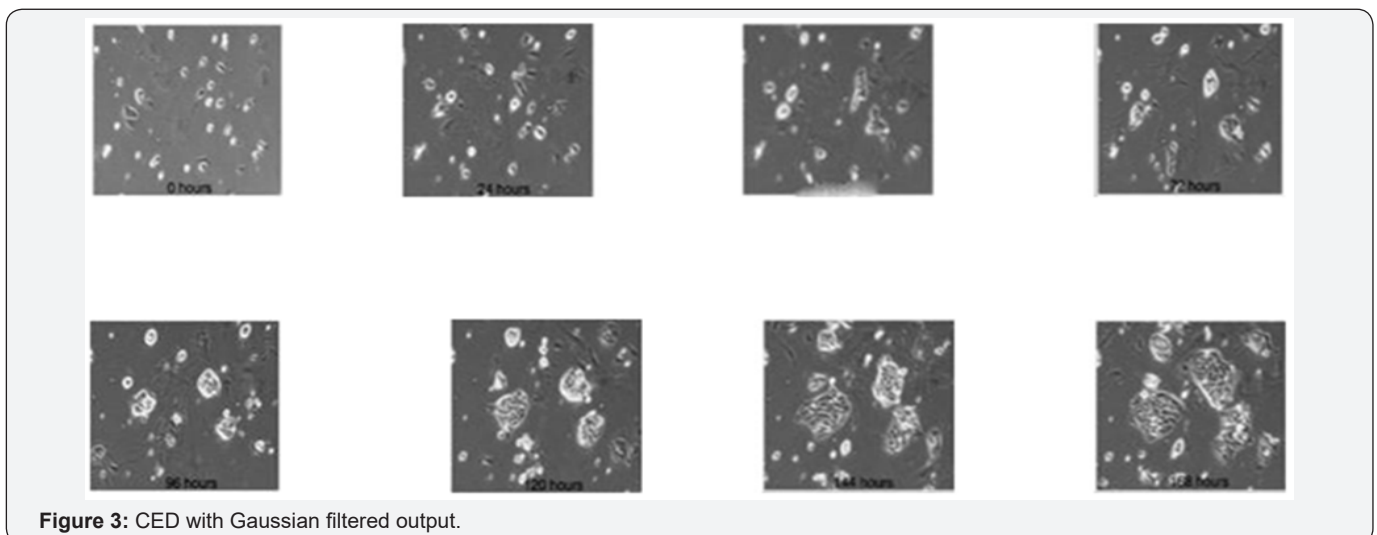


Figure 3: CED with Gaussian filtered output.

**Experimental results of watershed segmentation**

The watershed algorithm solves the problem of [19] over-segmentation in medical images, in this method the original

image is transformed into a morphological gradient image and then the image is treated with a filter to get the reference image with less noise. Then the noise free image is segmented by using the watershed algorithm (Figure 4).



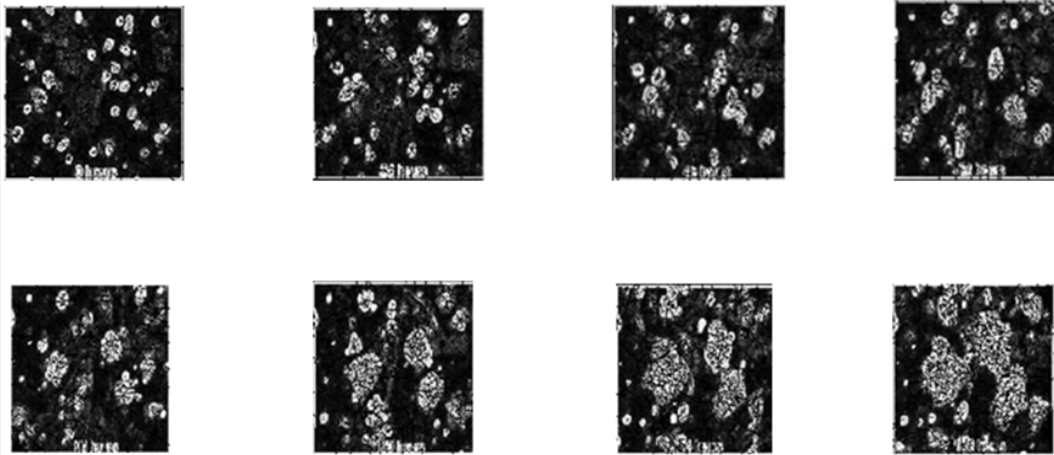


Figure 4: Watershed segmented output.

- Step 1: Read in the Image and Convert it to Gray scale.
- Step 2: Use the Gradient Magnitude as the Segmentation Function.
- Step 3: Mark the Foreground Objects.
- Step 4: Compute Background Markers.
- Step 5: Compute the Watershed Transform of the Segmentation Function.

Step 6: Obtain the final output.

This algorithm has two major drawbacks. This technique enhances the segmentation by removing a lot of non-significant contours as shown in Figure 5. By applying watershed method again, the segmentation may be either better or even worse. The second drawback is even more serious. When applying successive Waterfalls transforms over segmentation takes place without the knowledge of number of iterations.

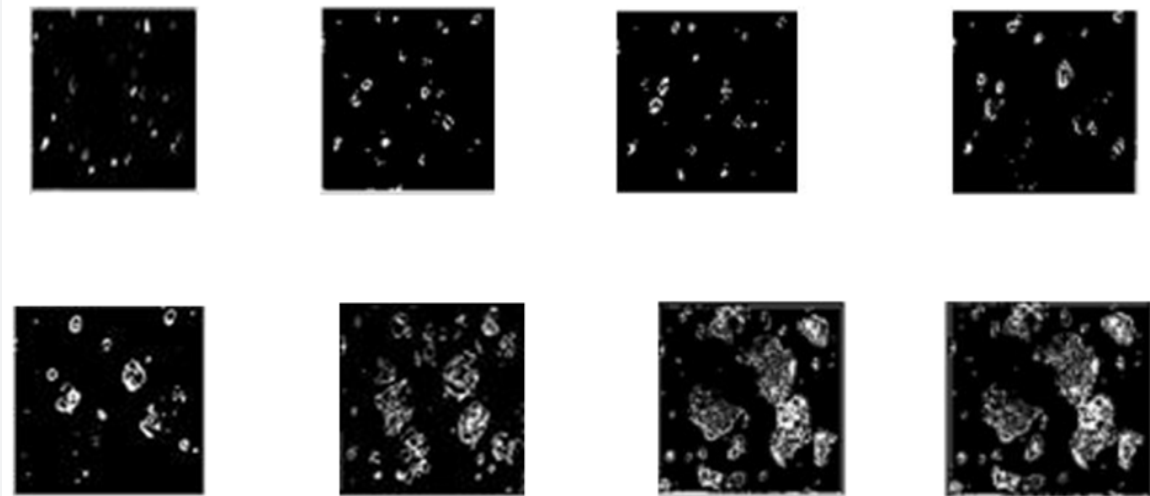


Figure 5: Integrated Bi-model distance regularization segmented output.

This two major problems are solved by applying bi- model integrated chan-vease algorithm. This algorithm is parametric and always ends with good segmentation of image as illustrated in the following process.

### Experimental results of integrated bi-modal distance regularization

To overcome the drawback of Chan-vease algorithm due to re-initialization, Integrated bi-modal distance regularized Chan-vease algorithm is implemented. Time series images taken at the regular intervals are given as input. Figure 5 shows the

simulation result of integrated Bi-modal distance regularized Chan-vease algorithm.

### Output parameter

The above tabulation Table 1 gives the comparison results for the existing watershed segmentation algorithm and the proposed integrated Bi-model distance regularization technique. These results show that the proposed technique gives better performance of 61% in terms of PSNR for 168 hours of stem cell segmentation growth. On the other hand from the above results it is evident that the watershed algorithm gives only minor

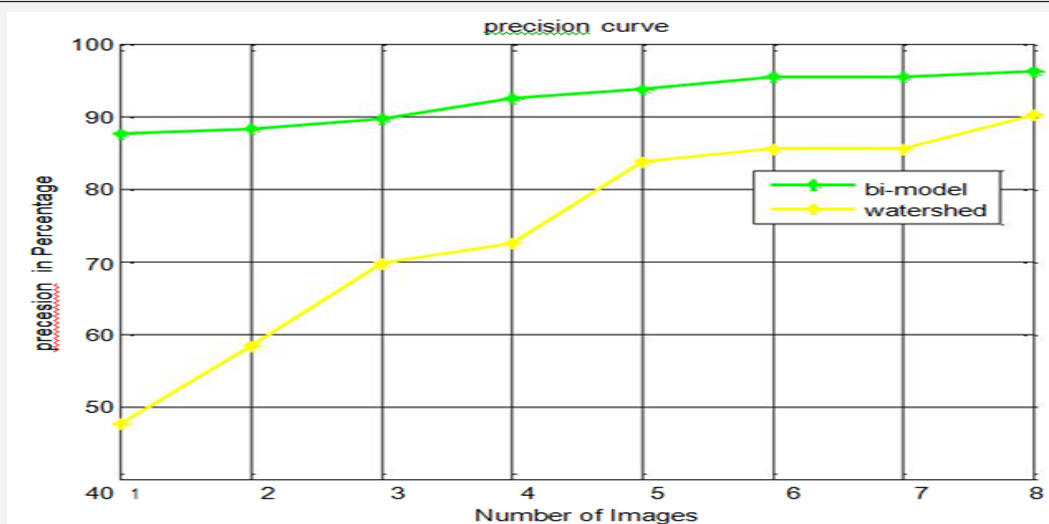
performance of 40%. Thus it is obvious that the integrated bi-model distance regularization technique gives effective results when compared with the existing technique.

The precision and recall values for the already prevailing watershed algorithm are 90.2% and 90.7% respectively. These values are increased by the proposed work. The obtained precision and recall from the newly proposed bi-model distance

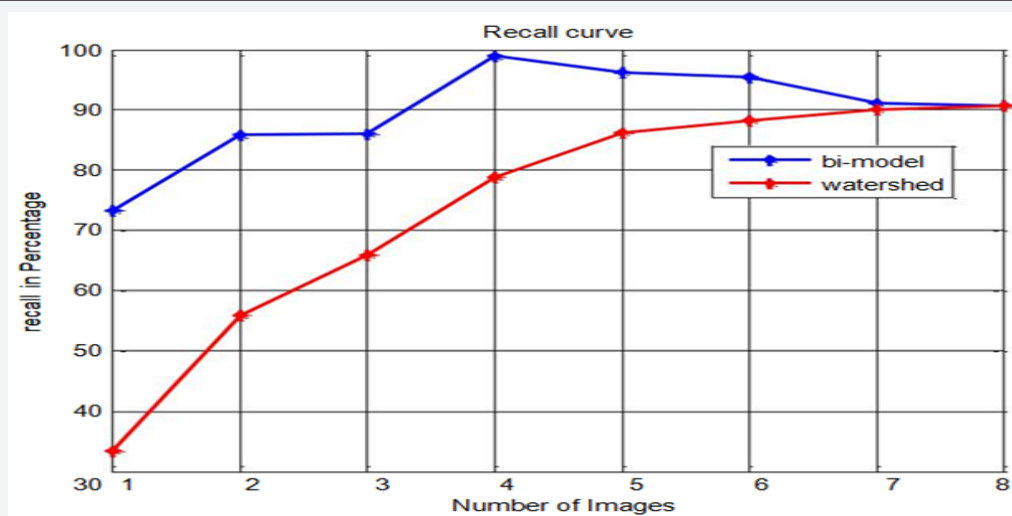
regularization technique are 98.4% and 95.2% respectively. These techniques are performed for different time durations and the matured stem cells are segmented and its PSNR values are tabulated above from 0 to 168 hours. From these results it is clear that the proposed technique gives better performance in terms of PSNR. The integrated bi-model distance regularization technique can be further improved by reducing its execution time.

**Table 1:** Output parameter comparison between integrated Bi-model distance regularization & Watershed algorithm.

Image	Precision (Bi-Model)	Precision(Watershed)	Recall (Bi-Model)	Recall (Watershed)	PSNR(Bi-Model)	PSNR (Watershed)
0 Hours	0.8988	47.6	0.7783	33.3	66.83	48.01
24 Hours	0.9058	58.3	0.903	55.8	74.68	50.23
48Hours	0.9192	69.7	0.9047	66	72.61	49.06
72Hours	0.9478	72.5	0.988	78.9	75.75	51.56
96Hours	0.9606	83.5	0.9619	86.2	74.02	50.22
120Hours	0.9777	85.5	0.9529	88.3	66.58	48.14
144Hours	0.9771	85.5	0.9554	90.1	63.61	43.78
168Hours	0.984	90.2	0.9518	90.7	61.1	40.23



**Figure 6:** Precision curve for Integrated Bi-model distance regularization Chan-vee.



**Figure 7:** Recall curve for Integrated Bi-model distance regularization Chan-vee.

The above Figure 6 gives the graphical representation of stem cell segmentation by plotting the precision values from Table 1. In this graph x-axis indicates the number of images taken and processed with the time duration of 24 hours and the y-axis indicates its corresponding precision percentage values. From this graph it is clear that the proposed integrated bimodal distance regularization technique outperforms the existing watershed algorithm. This is because the watershed technique introduces over and under segmentation while processing which in turn degrades its performance thus this degradation is overcome by implementing distance regularization in bidirectional chan-veese technique (Figure 7).

The above precision and recall curve reveals that the integrated bi-model Distance regularization method gives accurate object detection by segmenting in two directions with less level set functions when compared with the exiting watershed and chan-veese re-initialization technique which gives ineffective segmentation with intensity inhomogeneity. In Figure 6 the precision and recall curve is plotted according with true false, true negative and false negative. Hence from Table 1 the maximum segmentation accuracy of 98.4% can be achieved with the Integrated Bi-model distance regularization model.

The Chan-veese model is a piecewise constant approximation to the functional formulation of image segmentation introduced by Mumford and Shah. It has become popular in the image processing community mainly due to its ability to detect objects not necessarily defined by a gradient. Chan-veese algorithm will lead to the unsuccessful segmentation of images with intensity in-homogeneity. The sensitivity to the placement of initial contour and the extraordinary time-consumption if re-initialization step is adopted for maintaining stable curve evolution and ensuring more precise results. Therefore, Bi modal distance regularized Chan-veese algorithm is proposed. Here, the re-initialization is not directly used to keep the level set function as a signed distance function and it is more like a metric which characterizes how close a function  $\phi$  is to assigned distance function. The metric plays a key role in the elimination of re-initialization. This algorithm is also capable of segmenting in two directions. Therefore, there is no possibility of missing any cells as in the case of watershed technique. The accuracy of segmentation is still increased by the usage of integrated bi-modal Distance regularization algorithm.

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