

Blebs Optimization in Human Embryonic Stem Cells Videos

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ABSTRACT: The main aim of this paper is to segment the bleb from the human embryonic stem cells. The health of the human embryonic stem cells (hESC) is determined by the characteristics of the blebs – important biological indicator. With the nature of blebbing, the dynamic and apoptotic blebs can be identified. Region based active contour method is used for segmentation of bleb in human embryonic stem cells. To improve the segmentation accuracy, bio-inspired method is adopted. Two types of bleb formation occur: extraction and retraction. The full bleb formation occurs between the stage of extraction and retraction known as intermediate bleb. Bleb changes their size and image properties dynamically in both process and between frames. Thence, adaptive parameters are needed for each segmentation method. To enhance the segmentation results optimization is carried out using cuckoo search algorithm, which provides accurate and quick analysis.

KEYWORDS: Active Contour, Bleb, Cuckoo Search, Extraction, Human Embryonic Stem Cells, Intermediate Bleb, Retraction.

I. INTRODUCTION

Embryonic stem cells are deduced from the inner cell mass (ICMs) of blastocyst, the sperm in the oocyte can be divided into number of totipotent cells which cannot be differentiated. These cells when enter into the blastocyst it can be differentiated into number of specialized cells. Embryonic stem cells are the pluripotent cells that can be derived from the inner cell of mammalian blastocyst. The specialized cells can be further differentiated to produce more number of stem cells. The word “ES cells” was used to differentiate these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells. Embryonic stem (ES) cells have been recognized from several mammalian species, including mouse, monkey, and human. It is well established that various signaling pathways, including phosphoinositide 3-kinase (PI3K)/Akt and Wnt/ β -catenin signaling in the maintenance of ES cell pluripotency, and is known about the signaling pathways, involved in the derivation ES cells from ICMs.

Embryonic stem (ES) cells go through protracted proliferation while remaining composed for multilineage differentiation. A sole network of transcription factors may illustrate self-renewal and simultaneously suppress differentiation. First, the generation of mature lineage from ES cells in culture delivers access to populations of initial precursors that are difficult, if not impossible, to access in vivo. Evaluates of these mutations in vivo are habitually tricky by the early death of the embryo in utero. Second, the developmental potential of ES cells carrying targeted mutations of genes vital for embryonic development can be formed in culture.

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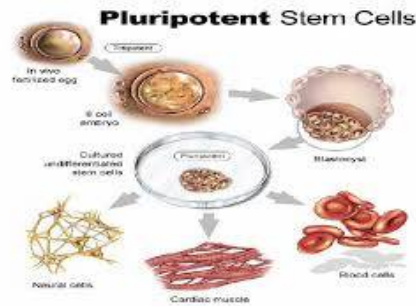


Fig 1. Embryonic Stem cells

Moreover, under well-defined conditions, embryonic stem cells are adept of propagating themselves indefinitely. This permits embryonic stem cells to be hired as beneficial tools for both research and regenerative medicine, as they can yield vast numbers of themselves for continual research or clinical use. For the reason, of their manipulability and hypothetically boundless capacity for self-renewal, ES cell therapies have been suggested for regenerative medicine and tissue replacement after injury or disease.

II. RELATED WORKS

Bio-inspired optimization metric is used to segment the bleb region, where low performances due to over-segmentation are reduced. The bleb modifies their size and image properties; hence using constant parameter provides poor efficiency. To improve bleb are detection accuracy, adaptive parameters are needed for each segmentation [2]. A simple coarse segmentation algorithm combines with Range filter and Marching Square Methods (RFMS) is used to achieve the fine segmentation of cells from the background with the level set method for the images from the datasets. The accurate classification of cells into normal versus apoptotic can be computed by support vector machines (SVM) classifier based on the segmentation results and morphological features [3]. The bio-driven algorithm uses the spatial information to enhance the connectivity of the local pixels to their corresponding region, which shows the little fault in comparison between the intensity distribution of the existing method and the ground-truth intensity distribution. Pre-filtering of the image data can significantly advance the performances [5]. The automatic detection method integrated the concept of spatial information and intensity distribution of data for cell region detection. It has capable to divide the image data into toe Gaussian distribution; intensity distribution of foreground and background data. It allows fast convergence to supreme absolute differences of foreground and background SNR [6].

III. HUMAN EMBRYONIC STEM CELLS

As stated earlier, human embryonic germ (EG) cells share many of the characteristics of human ES cells, but differ in significant ways. Human EG cells are derived from the primordial germ cells, which occur in a specific part of the embryo/fetus called the gonadal ridge, and which normally develop into mature gametes (eggs and sperm). Gearhart and his collaborators devised methods for growing pluripotent cells derived from human EG cells. The process requires the generation of embryoid bodies from EG cells, which consists of an unpredictable mix of partially differentiated cell types [10]. The embryoid body-derived cells resulting from this process have high proliferative capacity and gene expression patterns that are representative of multiple cell lineages. This suggests that the embryoid body-derived cells are progenitor or precursor cells for a variety of differentiated cell types [11]. The characterization of stem cell cultures has two main purposes: monitoring the genomic integrity of the cells and tracking the expression of proteins associated with pluripotency. Genomic analysis is necessary to ensure stem cells maintained in culture have not undergone chromosomal changes through chromosomal loss or duplication, or changes in their epigenetic profiles. Proteomic analysis ensures that the cells are expressing the factors necessary to maintain pluripotency. Confirmation of the differentiated state by analysing key genetic and protein markers ensures identification and propagation of the

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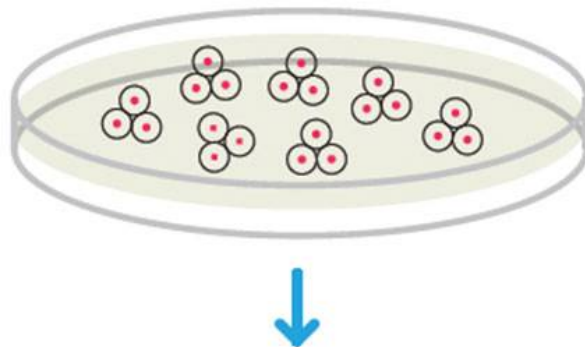
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correct cell type. This section provides an overview of different analysis methods for stem cells including karyotyping, single-nucleotide polymorphism (SNP) analysis, epigenetic profiling, flow cytometry and immunocytochemistry, RT-qPCR, digital PCR, western blotting, biomarker analysis, and teratoma formation. A variety of factors affect stem cell cultures.

Factors
Media composition
Cell density
Feeder cell type/density
Growth factors/additives
Feeder free culture
Passage method
Number of passages
Freezing and thawing protocols
Microbial contamination



Possible changes
Chromosomal
Phenotype/morphology
Differentiation
Pluripotency loss
Epigenetic changes
Tumorigenesis
Loss of self-renewal ability

Stem cell cultures must be periodically assessed for changes in morphology, karyotype, and ability to differentiate. Adaptation to culture conditions and maintenance in culture of any cell type will change the population. As cells divide and are passaged, the composition of the population changes due to selective pressures on the cells. Additionally, cells that grow and divide more rapidly will become dominant, gradually changing the composition of stem cell populations in culture. Mosaic populations often develop, with several distinct populations evolving. Therefore, keeping stocks of early passages of cells and continual monitoring are important. A number of techniques are used for genetic characterization of stem cells: karyotyping, fluorescence in situ hybridization (FISH), comparative genomic hybridization, single nucleotide polymorphism (SNP) analysis, and epigenetic profiling. Some types of stem cells, ES cell pluripotency particularly embryonic stem cells (ESCs) [13] and induced pluripotent stem cells (iPSCs), are more prone to being genetically unstable and should be observed frequently for chromosomal changes. Stem cells express both unique and specific combinations of transcription factors, cell surface proteins, and cytoplasmic proteins. Techniques used for stem cell analysis and characterization include flow cytometry, array-based analysis of the transcriptome, immunocytochemistry, western blots, and biomarker analysis. Different classes and types of stem cells are characterized by different combinations of markers. In addition to confirming that stem cell lines are stable, markers

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can be used for screening during the reprogramming of somatic (adult) cells into induced pluripotent stem cells (iPSCs) or to follow the progression of stem cell differentiation [7,5,3].

IV. BLEBBING OF HUMAN EMBRYONIC STEM CELLS

Blebs are bulge like protrusion that seems and vanishes from the surface of the cells [12]. Bleb formation consists of two processes: 1) expansion; 2) retraction [12], [14]. Bleb expands sporadically; they grow to the size of $\sim 2\mu\text{m}$ over $\sim 30\text{s}$; while cell body size decrease, bleb size rises. During retraction, bleb shrinks over $\sim 120\text{s}$; while bleb size reduces, cell body size increases. Complete retraction takes place in the healthy bleb, partial retraction or retraction occurs slowly leads to cell death. In between expansion and retraction, there is a full bleb formation at the end of the expansion stage is known as an intermediate bleb. The intermediate stage has the maximum size of bleb [2], [12], [14]. Inhibition of Rho-associated coiled-coil kinase (ROCK) activity enhances the clonal growth of hES cells without harming their capability to form teratomas [16]. The difficulty in recovering the hES cells from cryopreserved stock can be significantly minimized [15]. Blebbing is related to the signaling pathway which is useful for the biologist to determine that the inhibitors can alter the behavior of bleb through the Rho-Rock pathway [2].

V. ACTIVE CONTOUR

Active contours or snakes are used broadly for image processing and segmentation applications, mainly to trace object boundaries. It can attain the segmentation results near to the object contours, which can be appropriately used for analysis and recognition of the object shape. The active contours exploit numerous types of previous knowledge, such as intensity distribution information of the image, information about the boundary shape, and texture information, to attain accurate results for object boundaries in image analysis. It can be characterized as region-based models or edge-based models. Edge-based segmentation seems for breaks in the image intensity. Region-based segmentation seems for homogeneity within a sub-region; under desired property, e.g. intensity, color, and texture. Instinctive interpretation of images is a very tough problem in computer vision. Numerous ways are developed in past decade to enhance the segmentation performance in computer vision. Our approach is based in the active contour algorithm with the region based segmentation.

The following energy functional for calculating the snake energy:

$$E_{\text{snake}} = E_{\text{internal}} + E_{\text{external}} + E_{\text{constraint}}$$

Snake energy (E_{snake}) contains of three terms. E_{int} denotes the internal energy of the snake while E_{img} represents the image forces, E_{con} represents external constraint forces.

Internal Energy (E_{int}) hinges on the inherent properties of the curve and is the addition of elastic energy and bending energy, which is given in equation

$$E_{\text{int}} = E_{\text{elastic}} + E_{\text{bending}}$$

$$E_{\text{int}} = \int \frac{1}{2} (\alpha |v_s|^2 + \beta |v_{ss}|^2) ds$$

Where $\alpha(s)$ and $\beta(s)$ are user defined weights.

External energy (E_{ext}) of the contour is derived from the image.

$$E_{\text{ext}} = \int E_{\text{image}}(v(s)) ds$$

Image energy is function of the features of the image.

$$E_{\text{image}} = w_{\text{line}} E_{\text{line}} + w_{\text{edge}} E_{\text{edge}} + w_{\text{term}} E_{\text{term}}$$

$w_{\text{line}}, w_{\text{edge}}, w_{\text{term}}$ are the weight of salient features.

VI. CUCKOO SEARCH ALGORITHM

Cuckoo search is an optimization algorithm, which is inspired from brooding parasitism of cuckoo species in nature with of Lévy flight behaviour. It is used to resolve structural optimization problem. Two important features as follows: 1) Intensification, 2) Diversification. Intensification expects to examine about current best solution and choose

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the best applicants or results. Diversification makes define that the algorithm can discover the search space more effectively, by randomization.

Cuckoo Search is a metaheuristic algorithm developed by Yang and Deb. Heuristic is an optimization technique intended for resolving a problem hurriedly when conventional approaches are too slow, or when it cannot find the exact solution. Metaheuristic optimization is a genetic algorithm and heuristic is the application. Cuckoo search algorithm is better than other algorithm such as PSO and GA by solving the problem in a quick manner, resolving larger problem, attaining robust algorithm and a small number of parameters are required for fine tuning. The female cuckoo relies on the other host birds to raise their young. If the eggs of the cuckoo in the host bird nest are revealed, the host bird may throw away or her nest will be abended and raise her own brood somewhere else. In the CS algorithm, each egg in the host bird nest characterizes a solution, and a cuckoo egg signifies a new solution. If a new solution is better than nest available, it will replace the worst one. If there is a large search space, Levy flight are typically supplementary efficient. It is a distance approach could optimize efficient search under definite condition and the undertaking parameter are firmed by the power law. Law of Lévy flight revealed that random-walk style search is better implemented rather than simple random walk.

Three rules of the CS algorithm are defined as follows:

- 1) One egg is laid by each cuckoo at one time and randomly the nest has been chosen to dump their egg.
- 2) High quality of egg can be moved on to the further generation with the better nest.
- 3) The integer of existing host nests is stationed, and the cuckoo eggs are learned with a probability $pa \in (0, 1)$ of the host bird.

VII. EXPERIMENTAL RESULT

The video frames are phase contrast images. Each video was captured using 2 x objectives with 512x512 resolutions. Each video frame is acquired at 0.1 seconds time interval. The input video is shown in figure 2.

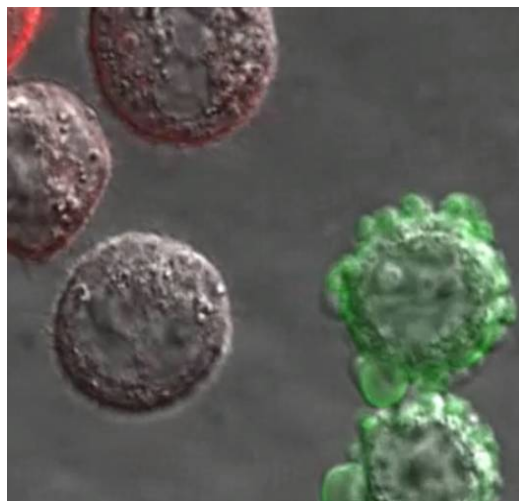


Fig 2. Input stem cells

The command window gives information about the video format; here 25 frames were obtained per interval is shown in figure 3.

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Command Window

videoInfo =

        Audio: 0
        Video: 1
VideoFrameRate: 25
        VideoSize: [512 512]
        VideoFormat: 'RGB '
    
```

Fig 3. Video information in command window

The video is acquired in color format which is not effective for image processing. HSV is very useful in image processing applications. The color information is typically noisy than the HSV information. Henceforth RGB can be altered into HSV, which is shown in figure 4.

Median filter is applied to clean the image from acquisition noise.

$$v(m,n) = \text{median}\{y(m-k,n-l), (k,l) \in W\}$$

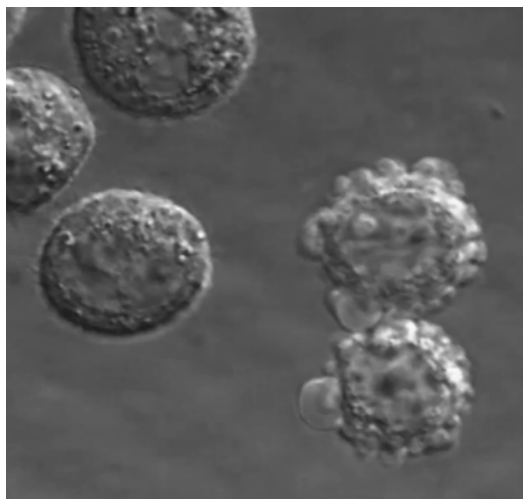


Fig 4. Preprocessed output

Region based active contour is used for extraction of blebs from human embryonic stem cells. The automatic changes in the topology of the image during the progression of the curve can be efficiently deal by this method. The figure 5 displays the thin white line which indicates the area of bleb region.

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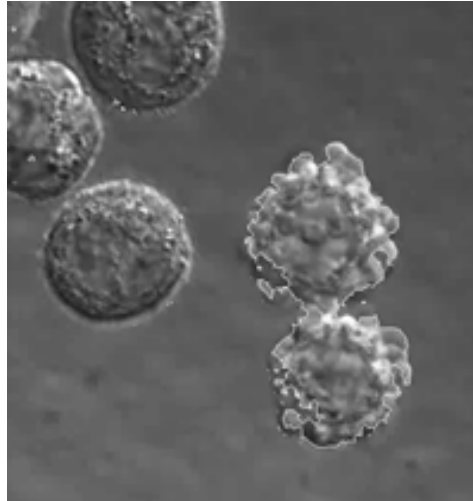


Fig 5. Segmented output

The optimization is a technique which enhances the quality of segmentation result. Cuckoo Search algorithm is applied on the segmentation result to find out the area of bleb region exactly in figure 6.

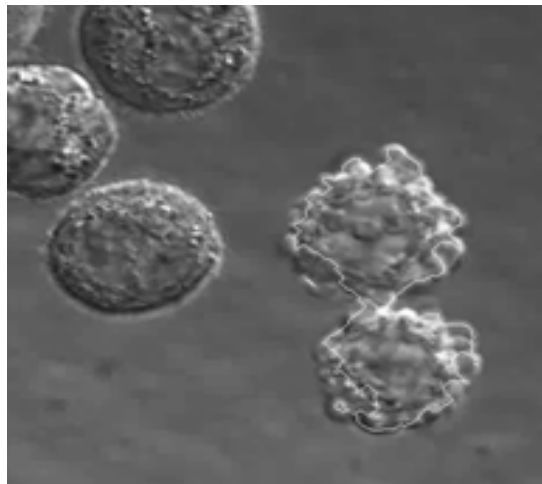


Fig 6. Optimized output

VII. COMPARISON OF RESULTS

For comparison, jaccard similarity and specificity metrics are used [17], [18]. The performance measure of both segmentation and optimization is shown in Table 1.

The Jaccard (JAC) is the measure of the similarity between experimental results and the ground truth.

$$JAC = \frac{TP}{TP+FP+FN}$$

Specificity (SPC) measures the proportion of actual negative which are correctly identified.

$$SPC = \frac{TN}{FP+TN}$$

True Positive (TP): indicates the overlapped region of the detected bleb's binary mask and the bleb ground truth's binary mask.

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True Negative (TN): is the overlapped region of the detected background's binary mask and the background ground-truth's binary mask.

False Positive (FP): is the detected background's binary mask that is falsely identified as a part of the bleb region.

False Negative (FN): is the detected bleb's binary mask that is falsely identifies as part of the background.

TABLE 1

METHOD	SEGMENTATION			
	MIN	MAX	STD	MEAN
JACCARD SIMILARITY	9.57	13.27	12.07	33.80
SPECIFICITY	8.92	16.78	12.17	34.34

METHOD	OPTIMIZATION			
	MIN	MAX	STD	MEAN
JACCARD SIMILARITY	26.4279	67.037	19.288	48.2222
SPECIFICITY	99.536	82.2608	0.2037	76.2048

VIII. CONCLUSION

The bio-optimized segmentation method has reduced the over-segmentation than the other traditional method, which enhances the performance of overall process. The characteristics of bleb such as bleb expansion and retraction are used to indicate health of human embryonic stem cells. The complete retraction occurs in healthy bleb, while in non-healthy bleb: cells do not retract or partially retract or retract them slowly. The area of bleb region can be detected by active contour segmentation. To improve the detection accuracy cuckoo search optimization is implemented. The method also matches the trend of the ground truth experiment closely. To improve the accuracy further, it is necessary to investigate into getting more frames per second to establish inter frame relationship for detecting small tiny bleb regions and it will classified into dynamic and apoptotic blebs.

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