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Cell Segmentation and Classification Using Digital Holographic Microscopy Images

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Abstract

In this modern era Digital Holographic Microscopy is very much popular designed for cell imaging. The cellular imaging had developed in growing cell biology. Digital Holographic research (DHM) might be a label-free imaging technique which allows a noticeable illustration of clear cells with imaging cell culture plates. The most advantage is that, it doesn't offer the projected image of the thing however provides 3 dimensional data of the object's optical thickness. Using DHM, machine learning approaches are used for the extraction purpose, variation and calculative blood statistics comparable to the Mean corpuscular Volume (MCV), the Red blood cell (RBC) count, Red blood cell Distribution width (RDW).Segmentation is that the method of partitioning a digital image in to multiple elements. For cell segmentation, first the cell has to be detected. Then the detected cells are used to separate by means of layered segmentation. After the segmentation process, the blood cells are classified by means of K-NN classifier and SVM classifier. This shows the quantitative evidence that it works better than power watershed segmentation.

Index Terms: *Digital holographic microscopy, Red blood cell, Red blood cell count, layered segmentation algorithm, K-NN, SVM, neural network.*

INTRODUCTION

An image can be measured as a matrix of light intensity levels that can be manipulated using MATLAB. Though none of the algorithms developed and which can be used, as of now, in a real time world, they provide some insight into the viability of imaging processing techniques. For image processing, the analysis must be carried out. Image analysis [3] is concerned with the extraction of measurements, data or information from an image by automatic or semiautomatic methods. Image analysis is illustrious from other types of image processing, such as coding, restoration, and enhancement, in that the ultimate product of an image analysis system is usually numerical output rather than a picture.

Red Blood Cell

Red blood corpuscle plays a very important role in your health by carrying contemporary chemical element throughout the body [1]. Red blood cells are spherical with a flattish, indented centre, like doughnuts while not a hole. Your health care supplier will check on the scale, shape, and health of your red blood cells using tests, resembling the entire blood count screening.

Generally in human's body, the RBC's are found to be as versatile and oval concave disks. So as to accommodate most area they have a hemoprotein and there'll be an absence of nucleus and organelles. The fig 1 shows red blood cells that are used in this paper. RBCs are otherwise called as red cell and red blood corpuscles, haematids, erythroidcells or erythrocytes. There are

new erythrocytes that is approximately 2.5 million and they are produced in humans especially in adult.

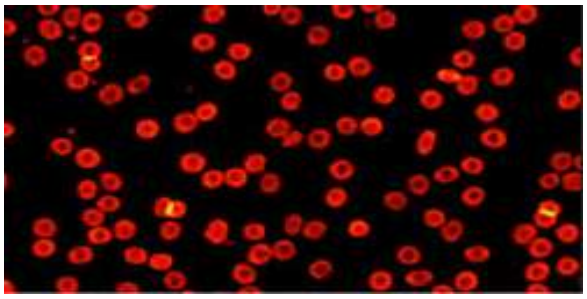

Fig 1. *Red Blood Cells, which has size 49.2 KB and then the dimension is 249x144.*

Red Blood cell count:

The number of RBCs that we have can affect how much oxygen that our tissues receive. Our tissues need oxygen to function effectively. In this paper the blood count for above cell is 92.

Digital Holographic Microscopy

In DHM, we use a numerical reconctruction algorithm which calculates the object of the image clearly. There are lens which is used for traditional microscopy. Then the image is closely related to the microscopy techniques which is mainly used for the information and it is essential for the cost. In such a workflow, accurate cell extraction is a vital step to perform the analysis. Therefore, thorough investigation of cell segmentation inDHMimages is a persistent necessity. Current DHM application studies [7,8] such as simple segmentation tools orutilizegeneric cell segmentation tools that are not designed for DHM and the accuracy of their performance for DHM was not assessed in the literature. One notable exception is the cell segmentation approach. The algorithm uses a sequence [9] of morphological operationsonthephaseimagetogeneratemar kersformaker-controlledwatershed segmentation. The approach is relativelycomplicatedand the main disadvantage is its sensitivity to theparametersof the morphological operators such as the size of the structure element used in every operation. We will overcomethisproblem. In this paper, we present two-stepsegmentationApproach: Cell detection to localize the centers of thecellsand cell segmentation to delineate the boundaries of thecells.Qualitative and quantitative assessment of oursegmentationmethodispresented.Moreo ver, weintroduceacomparisontothe state-of-the art cell segmentation methods.

Advantages of DHM

- ❖ Phase shift images
- ❖ 3-Dimensional information
- ❖ Digital autofocus
- ❖ Optical aberration correction
- ❖ Low cost

Cell Segmentation

Cell segmentation is used to partitioning a image in to several segments. It is mainly used for simulating problem in both complex natures. In cell segmentation manual method is very difficult to segment. So we are going for computerized method.RBC segments the cell from the background which involves their more object. Illumination contradictions and cell obstruction are the main reasons that make cell segmentation challenging [4]**.** Cell classification has extensive interest especially for hospitals and workrooms. Patient's blood cells counting were done physically by curative technologists by watching slide ready with blood sample of the patient under microscope. A labor-intensive count will also give evidence about other cells that are not usually present in marginal blood but might be free in certain virus.

METHODOLOGY

The novelty of our approach is two-fold;
Robust marker generation using cell detection
Layered segmentation
Cell Classification

Block Diagram

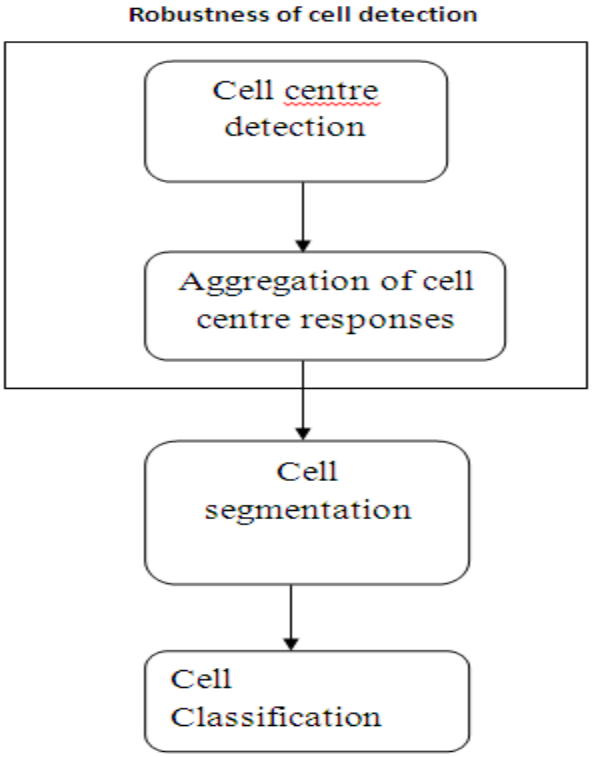

Fig 2. *Block diagram of cell extraction*

Robust marker generation using cell detection

The marker generation problem as an object detection problem where we aim at finding the positions of cell centers. For this purpose, we use a machine learning based approach instead of morphological operations to minimize the sensitivity to parameters' choice. In testing, we compute the probability of each pixel in the image being a centre of a cell; the probability map is threshold to keep only the pixels that are more likely to be a cell centre.

After computing the probability map and applying the threshold, we get a high response inside each cell. However, the response is not necessarily smooth and connected which may lead to false identification of one-cell as multiple cells. Therefore, to aggregate these responses we apply a clustering step on the threshold probability map. The clustering serves two purposes, first, it aggregated the cell responses in a single cell. Second, it brings a larger set of pixels to form as the inner marker for the segmentation. The pixels of each bunch are compound into a single connected constituent that serves as an internal marker for the cell segmentation step. The threshold does not provide a single cell centre but rather a cluster of points in the centre of the cell. Here we are using Otsu threshold method.

Otsu Threshold method

In Otsu's method the two classes [6].

$$t^2k(\mathrm{t}) = \mathrm{k}_0(t)t_0^2(\mathrm{t}) + k_1(\mathrm{t})t_1^2(\mathrm{t}) \quad 2.1$$

Weights $k_{0,1}$ are the probabilities of the two classes separated by a threshold t and $\sigma_{0,1}^2$ are variances of these two classes. The class probability $\omega_{0,1}(t)$ is computed from the **L** histograms:

$$k_0(t) = \sum_{i=0}^{t-1} p(i) \quad 2.2$$

$$k_1(t) = \sum_{i=t}^{L-1} p(i) \quad 2.3$$

Otsu shows that minimizing the intra-class variance is the same as maximizing inter-class variance.

$t_b^2(t) = t - t_\omega^2(t) = k_0(l_0 - l_T)^2 + k_1(l_1 - l)^2$
$= k_0(t)k_1(t)[l_0(t) - l_1(t)]^2$ 2.4

Which is expressed in terms of class probabilities ω and class means$l$. While the class mean $l_{0,1,T}(t)$ is,

$l_0(t) = \sum_{i=0}^{n-1} i\frac{p(i)}{k_0}$ 2.5

$l_1(t) = \sum_{i=n}^{R-1} i\frac{p(i)}{k_1}$ 2.6

$l_T = \sum_{i=0}^{R-1} i\,p(i)$ 2.7

The following relations can be easily verified.

$k_0 l_0 + k_1 l_1 = l_T$ 2.8

$k_0 + k_1 = 1$ 2.9

The class probabilities and class means can be computed iteratively.

Algorithm

Compute histogram and probabilities of each intensity level.

1. Set up initial $k_i(0)$ and$k_i(0)$.
2. Step through all possible threshold n=1,.....maximum intensity
3. Update k_i and l_i
4. Compute $t_b^2(t)$
5. Desired threshold corresponds to the maximum t_b^2

The threshold does not provide a single cell centre but rather a cluster of points in the centre of the cell.

Layered segmentation

In image processing, a layered segmentation of a gray scale image is analogous to the notion of a catchment basin of a height map [11. A graph g= $\{\vartheta, \varepsilon\}$ consists of a set of vertices v$\epsilon\vartheta$ and a set of edges e$\epsilon\varepsilon(\vartheta \times \vartheta$.An edge incident to vertices $\vartheta i$and ϑj is denotede_{ij}.In our formulation, each pixel is identified with a node ϑi. A weighted graph is a graph in which every edge e_{ij} is assigned a weight w_{ij}.The seeded segmentation energy was given as

$\min_x \sum_{\varepsilon ij \in \varepsilon} \omega_{ij}^p |x_i - x_j|^q +$
$\sum_{vi} \omega_{Fi}^p \omega_i^p +$
$\sum_{vi} \omega_{Bi}^p |x_i - 1|^q$, 2.10

Such that x(F)=1,x(B)=0,
$s_i = 1$ If$x_i \geq \frac{1}{2}$, 0 if $x_i < \frac{1}{2}$.

Where x_i and x_j are the binary labels associated with vertices ϑ_i and $\vartheta_j$respectively. F and B represent the sets of foreground and background markers. When p→ ∞ and q> 1, this leads [10] to a more general, namely, layered segmentation that yielded better results. Cell segmentation could benefit for the improvement associated with layered segmentation.

ALGORITHM USED FOR CELL SEGMENTATION

1. Image is normalized,
2. Otsu's threshold is applied to obtain I bin,
3. Holes are filled using morphological construction to I bin,
4. Gradient image I grad is computed using Sobel operator.
5. Morphological opening is applied to I bin to obtain I open.
6. Morphological erosion is applied to I open to obtain I erode.
7. Morphological reconstruction is applied with I open as the mask and I erode as a marker to obtain Irec.
8. Compute I sub as I open - Irec.
9. Apply morphological dilation on I erodes to obtain I dilate
10. Obtain the internal markers by combining Isub and Idilate.
11. Apply layered segmentation to separate the red blood cells.

Cell segmentation could benefit for the improvements associated with layered segmentation. Our algorithm is more accurate when compared to other method because it still identified two cells which

are important for an accurate cell count.

For measurable valuation, we assume some of the parameters namely, the Sensitivity, Specificity, Dice and Jacquard similarity index defined as:

Sensitivity [SE] $= \frac{TP}{TP+FN}$

Specificity [SP] $= \frac{TN}{TN+FP}$

Dice similarity index [DSI] $= \frac{2TP}{TP+FP+TN+FN}$

Jacquard similarity index [JSI] $= \frac{TP}{TP+FP+FN}$

Where TP, FP, TN and FN refer to the true positives, false positives, true negatives and false negatives, respectively [5]. The quantitative assessment shows that each component we introduced contributed to improving the segmentation results. However, it is evident that the robust localization of the cells using the cell detection framework played a more crucial role. While the sensitivity improved by ˜10% when using our machine learning marker generation, it only improved ˜2% when using power watershed instead of watershed. Here the accurate cell count is 96.

CELL CLASSIFICATION

In this paper, we use two classifier. They are

K-NN Classifier

SVM Classifier

K-NN Classifier

Generally K-NN classifier is mainly used for the classification purpose. Because it is simple and very easy to understand but works incredibly well in practice. Also it is astonishingly multipurpose and its applications range from vision to proteins to computational geometry to graphs and so on

It is also a sluggish algorithm. In additional words, there is no clear training phase or it is very negligible.

SVM Classifier

A SVM is a classifier which formally distinct by extrication hyper plane. In extra words, the given branded training data, the algorithm outputs a best hyper plane, which classifies new examples

RESULTS AND DISCUSSION

Input image

The size of the input image is 49.2 KB and then the dimension is 249x144.

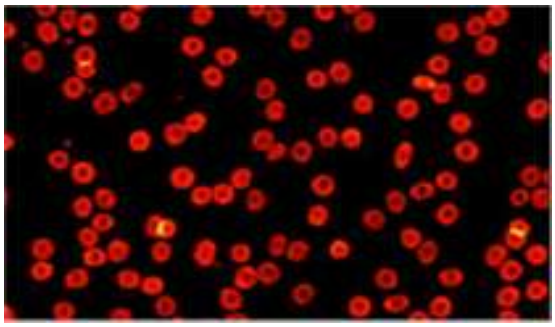

Fig 3. *Input images*

Normalized image

The normalized image obtained from input image is

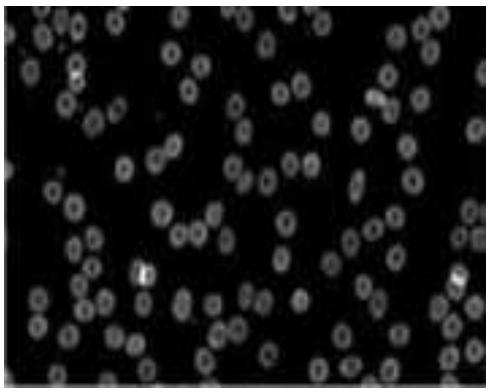

Fig 4. *Normalized image*

Otsu threshold

Otsu threshold is obtained from normalized image, which is applied to obtain I bin.

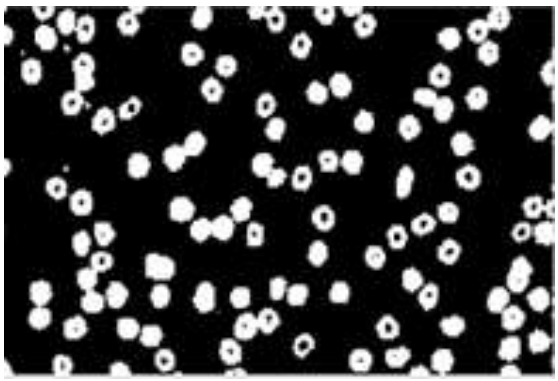

Fig 5. *Otsu thresholds*

Gradient image

Gradient image I grad is computed using sobel operator is

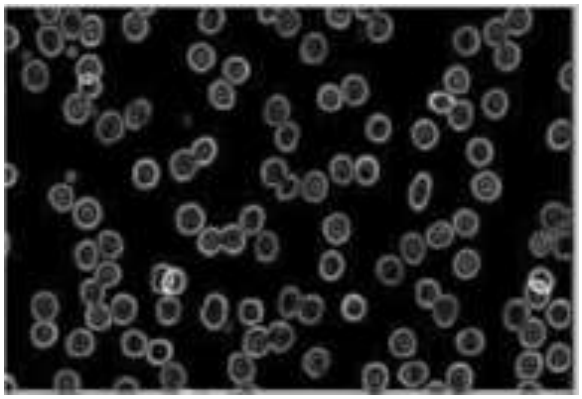

Fig 6. *Gradient images*

MORPHOLOGICAL OPENING

The inaugural serves in processor vision and image processing as a basic workhorse of morphological blare removal.

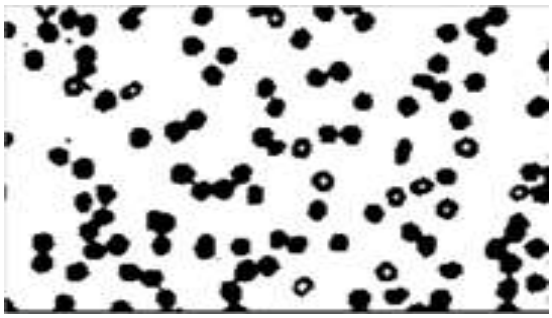

Fig 7. *Morphological Opening*

Morphological erosion

Morphological erosion is used to remove the pixels from the object depends on the size and shape of the structuring element used to *process* the *image*.

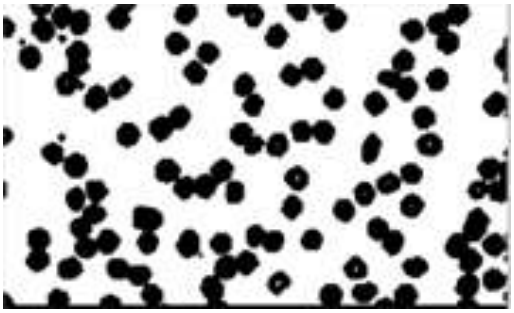

Fig 8. *Morphological erosion*

Morphological dilation

Morphological dilation is used to add the pixels from the object be contingent on the size and shape of the structuring element used to *process* the *image*.

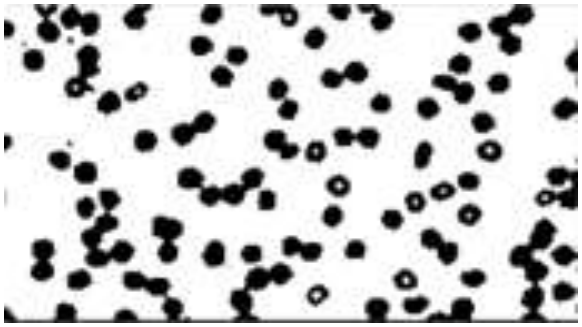

Fig 9. *Morphological dilation*

Output image

The output image is

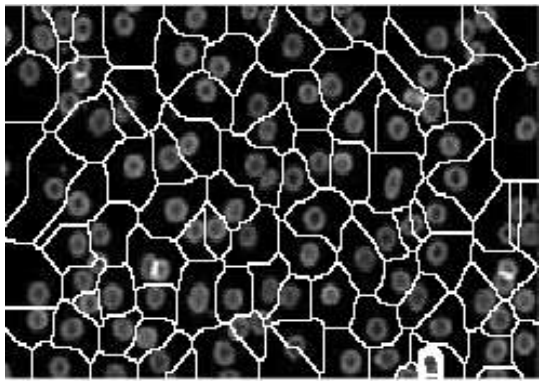

Fig 10. *Output image*

Quantitative Assessment Table

Table 1. *Quantitative Assessments.*

SE	88.13	89.35	96.43	97.8398.00
SP	65.35	68.36	77.20	78.33 87.04
DSI	81.50	82.58	83.60	84.91 94.23
JSI	53.27	60.35	66.32	76.27 85.96

Classification Table

Table 2. *Classification table*

	K-NN	SVM
Accuracy	50	70
Specificity	1	40
Sensitivity	0	1
PPV	NaN	62
NPV	50	1

CONCLUSION AND FUTURE WORK

Image segmentation is a modest and effective technique. The healthy indicator generation presented in the paper output achieves the morphological workflow for marker generation that is usually used to pledge layered segmentation. We introduced the layered segmentation to the cell segmentation problem and presented quantitative evidence that it works better than power watershed. The paper presented a quantitative assessment of the prospect approach to the common methods used for cell segmentation. In the paper we have a tendency to arrange to add post process that identifies the overlapping cells and formulates a superimposed segmentation algorithmic rule to separate properly the overlapping cells.

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