Automated Cell Counting Using The Nine Point Circle Rule

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***Abstract*—Numerous procedures in biology and medicine require the counting of cells. The autonomous counting of cells is needed to decrease dependency of person experience and lower error rates. This paper introduces the method of automated cell counting using the nine point circle rule. Using fluorescent microscope and processing images obtained from the proposed method outputs the cell count with low computational needs and low error rate.**

***Keywords*—automated cell counting, nine point circle rule, Feurerbach circle, Euler circle, fluorescent microscope imaging.**

I. Introduction

The count of the cells from a blood test or any other test interests applications on both research and clinical practices. The complete blood count is one of the most common examples where the concentration of red and white blood cells on a complete body is measured to obtain information about the health condition of a person. Similarly, the concentration of bacteria, viruses, and other pathogens in the blood or other bodily fluids can reveal information about the progress of infectious disease and about the degree of success with which the immune system is dealing with the infection. Moreover, the molecular biologists measure the growth rate of microorganisms by cell counting. There are many methods to count cells which of them do not require special equipment so widely applicable but others rely on sophisticated electronic appliances. The counting chamber [10], planting [9], CFU counting [3], coulter counter [8], and the flow cytometry [1] can be some examples of cell counting. Moreover, one of the most current cell counting methods is manually counting some part of the microscope image than multiply with some coefficient. This method is not time-efficient and also has higher error rates relying on a persons experience and knowledge. To solve the time consumption and high error rate, image processing, and computer vision creates a good environment. Computers are easy to use and common everywhere and their speed is increasing rapidly day by day unlike the success rate of manual counting. With algorithms for processing images obtained by a fluorescent microscope, the appropriate results can be achieved with fewer errors than any other method. Also, processing images and counting after processing highly reduces the time consumed for this purpose. The main aim of this paper is to count cells having circular shapes cells correctly from the images obtained by fluorescent microscope.

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Fig. 1. Al-Khazraji Algorithm Results

II. Related Studies

The key point for selection among many different approaches is the practical usability of methods on cells and their level of accuracy. In this section, methods proposed by Al-Khazraji [2] and Samiksha [7] are examined with their usage, results and comments about them.

A. Algorithm by Al-Khazraji

The algorithm created by Al-Khazraji consists of the steps mentioned below:

- Import original image into the algorithm
- Use Otsus thresholding method (graythresh)
- Create a binary image (im2bw)
- Identify the objects (regionprops)
- Label the objects at the centroid
- Determine representative cell nucleus area (trimmean)
- Determine final cell count

The algorithm proposed by Al-Khazraji can be useful in case of no overlapping cells having similar nucleus area. However, it cannot be used for practical usage. Because, the cell overlapping is commonly seen in cell images and if there will be the same smaller or larger cells in this image, these will be omitted while counting.

B. Algorithm by Samiksha

Samiksha created the cell segmentation algorithm using the steps below:

- Import original image into the algorithm
- Use Otsus thresholding method (graythresh)
- Noise removal (bwareaopen)
- Fill the holes (imfill)
- Noise removal (as much as need) (bwareaopen)
- Remove boundary elements (imclearborder)
- Counting algorithm
- Divide image into blocks of equal size, for every block:
- Scan every pixel from the first row to last row
- Label white pixels with according labeling: Use the same label if the neighbor is a white pixel and Use different label if the neighbor is a black pixel

The algorithm proposed by Samiksha can be accepted as the basis of count algorithms and it gives 75-80% accuracy. This algorithm uses a method depending on the approximate width of a cell for overlapping cells after explained steps are applied. However, it is user depended and narrows the range of the use.

Fig. 2. Samiksha Algorithm Results

These two approaches use nearly the same algorithm except their counting methods. The first method underestimates the number of objects. Since it counts a cell if all edges of that cell are connected (there will no space between edges). However, the second method overestimates the number of objects because, the second method traces the exterior boundaries of objects, as well as boundaries of holes inside these objects. Thus, if there are some unfilled holes, it will also count those holes as new cells.

III. Preprocessing of Proposed Algorithm

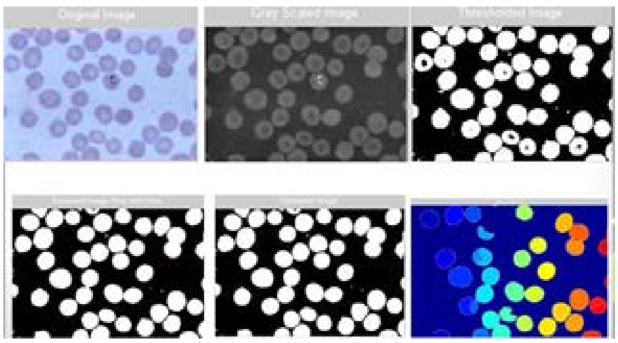

Fig. 3. Preprocessing methods used in the proposed paper

This paper uses similar preprocessing methods with the algorithm of Samiksha as shown in Fig. 3. The preprocessing before the nine-point circle rule for cell segmentation is described below:

- Import RGB image into the algorithm
- Check the type of the image (RGB or Grayscale), if RGB convert image to Grayscale Image
- Use Otsus thresholding method (graythresh)
- Create binary image (im2bw)
- Erosion with disk (imerode, strel(disk,1))
- Fill the objects with holes (imfill(,holes))
- Denoise (bwareopen)
- The nine-point circle rule for cell segmentation

IV. Nine Point Circle Rule for Cell Segmentation

This paper introduces automated cell counting using the nine-point circle rule. This rule is described as follows:

In every triangle, three midpoints of the side, the three base points(feet) of the altitudes and the midpoints of the three segments from the orthocenter to the vertices lie on a circle which is called the nine-point circle rule [5]. The nine-point circle is also known as the Feuerbach Circle.

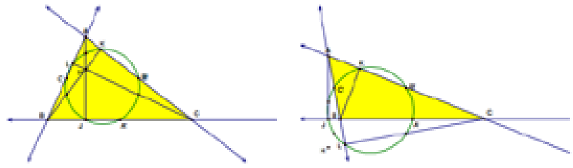

Fig. 4. Nine Point Circle Rule

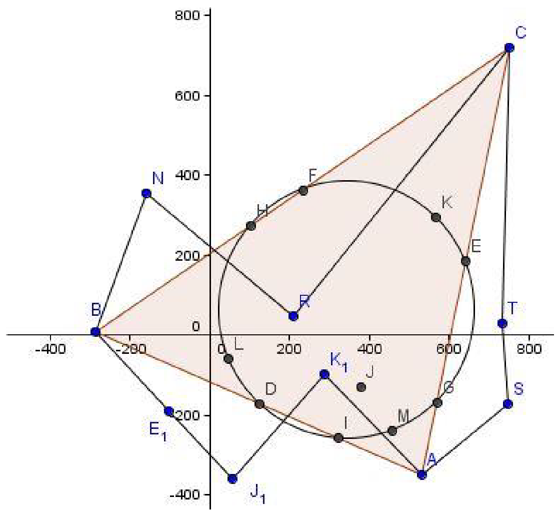

Fig. 5. Nine Point Circle Rule Example

The nine-point circle rule can be explained as follows in Figure 5. First, arbitrarily select three points from the cell for example A, B, C. Then find nine points which are three midpoints D, E, F, three base points(feet) of the altitudes G, H, I and the midpoints of three segments from the orthocenter to the vertices K, L, M Also, it can be seen that J is the orthocenter. Finally, the points inside the cell should be counted. For the shape BJK_1AE_1STC, the six points D, E, G, K, L, M are inside the cell and the other three points are outside of the cell. The procedure should be repeated until the average is an appropriate result.

A. Implementation Details

For arbitrarily selected three points which are the vertices $A(x_1, y_1), B(x_2, y_2)$ and $C(x_3, y_3)$ of arbitrary triangle ABC, the nine points which are the points that obeys the nine point circle rule are found with the following procedure. Firstly, the three midpoints M_{AB} M_{BC} and M_{AC} of the vertices AB, BC, AC are found in Eq. 1, correspondingly.

$$
\begin{aligned}
M_{AB} &= \left(\frac{x_1 + x_2}{2}, \frac{y_1 + y_2}{2}\right) \\
M_{BC} &= \left(\frac{x_2 + x_3}{2}, \frac{y_2 + y_3}{2}\right) \\
M_{AC} &= \left(\frac{x_1 + x_3}{2}, \frac{y_1 + y_3}{2}\right)
\end{aligned} \tag{1}
$$

The first base point(feet) $H_{AB}(H_{AB,x}, H_{AB,y})$ can be found by Eq. (2) as shown in Fig. 6.

$$
\begin{aligned}
H_{AB,x} &= \frac{(y_3 - y_2) * x_3 + (x_3 - x_2) * (\frac{y_1 + (x_3 - x_2) * x_1}{(y_3 - y_2)} y_3)}{y_3 - y_2) + \frac{(x_3 - x_2) * (x_3 - x_2)}{(y_3 - y_2)}} \\
H_{AB,y} &= -\frac{x_3 - x_2}{y_3 - y_2} * H_{1,x} + y_1 + (x_3 - x_2) * \frac{x_1}{(y_3 - y_2)}
\end{aligned} \tag{2}
$$

The second $H_{BC}(H_{BC,x}, H_{BC,y})$ and third base points $H_{AC}(H_{AC,x}, H_{AC,y})$can be found by some manipulations on Eq. (2). $H_{AB,x}$ should be changed $H_{BC,x}$ and $H_{AC,x}$ correspondingly. Likewise, (x_1, y_1) should be changed with (x_2, y_2) and (x_3, y_3) correspondingly.

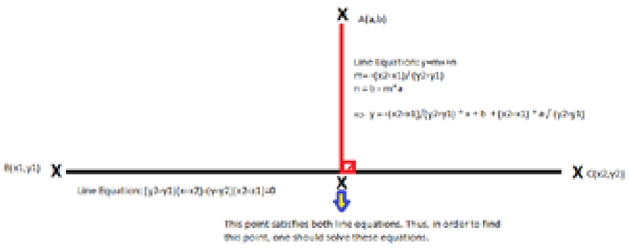

Fig. 6. Finding the base points

To find the midpoints of the three segments from the orthocenter to the vertices, the orthocenter $Q(Q_x, Q_y)$ should be first found as in Eq. (3).

$$
\begin{aligned}
Q_x &= \frac{y_3 - y_1 + \frac{(x_1 - x_2) * x_3}{(y_1 - y_2)} - \frac{(x_3 - x_2) * x_1}{(y_3 - y_2)}}{\frac{x_1 - x_2}{y_1 - y_2} - \frac{x_3 - x_2}{y_3 - y_2}} \\
Q_y &= -\frac{x_3 - x_2}{y_3 - y_2} * x_q + y_1 + \frac{(x_3 - x_2) * x_1}{y_3 - y_2}
\end{aligned} \tag{3}
$$

From the orthocenter, the midpoints of the three segments from the orthocenter to the vertices Q_{AB}, Q_{BC} and Q_{AC} are found in Eq. (4).

$$
\begin{aligned}
Q_{AB} &= \left(\frac{x_1 + Q_x}{2}, \frac{y_1 + Q_y}{2}\right) \\
Q_{BC} &= \left(\frac{x_2 + Q_x}{2}, \frac{y_2 + Q_y}{2}\right) \\
Q_{AC} &= \left(\frac{x_1 + Q_x}{2}, \frac{y_1 + Q_y}{2}\right)
\end{aligned} \tag{4}
$$

B. Practical Usage in Cell Image

The practical application on cell images is one of the important points of this method because of the main aim whose is used in cell images. As mentioned in the comments section, defining the standard to understand whether cells are connected or how many cells are connected in each border is another difficult task. This part of the report will discuss the procedure used for that purpose. However, the procedure for defining standard is a preliminary procedure. Thus, it is highly recommended for future researchers to go further on that topic. The procedure for average detection and standardization is given below:

- Find the size of the image
- Find the boundaries of each segment of the image after processing
- For each segment, create an image from boundaries
- Fill the holes in the image
- For each image created (filled with holes) repeat the nine-point calculation for the selected repeat count (3000 for min. and 5000 for optimal)
- Find the mean value of how many points of special nine points are inside for each image
- Find the total time consumption for each image

C. Standardization Procedure

The algorithm should start with the zero count value to accomplish standardization. For each average value found for each segment the following rule is accepted:

- If average is higher than the circle rate (5.6 optimal), it means that it is not connected so increase count by one.
- If average is lower than the circle rate and higher than the noise rate (5.2 optimal), increase count by two.

After the examination of manually created images, the usefulness of this method is understandable. However, examining the borders on a cell image can improve ones knowledge about that procedure significantly. The procedure to obtain the results from the cell image is given below:

- Process the image with cell counting function
- Use boundaries and bound2im function to obtain images

- Fill the holes with imfill function
- Obtain the results on Matlab
- Process the results on Excel

V. Experimental Results

The main software environment is MATLAB with an image processing toolbox. The image obtained by a fluorescent microscope is processed with various types of functions and counted with the special count function. The input of the algorithm should be entered as RGB or Grayscale image and the output will be the cell count in the image. Moreover, the processed image can also be outputted for further studies. To accomplish these purposes, the cell segmentation algorithm concentrates on the segmentation of the cells having circular shapes by using geometrical rules, especially for circles. The nine-point circle rule used to detect whether the cell is circular or not. Also, it gives a clue about whether the cells are connected or not.

The results on Table I are given for figures in Fig 7.

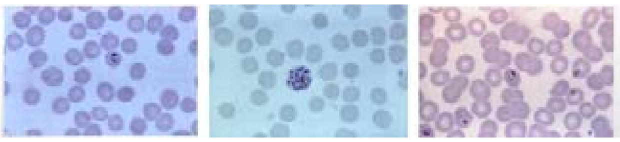

Fig. 7. Cell Images (from left to right Cell 1, Cell 2, Cell 3)

TABLE I
Cell Counts

Cell Number	1	2	3
Exact Cell Count	50	38	64
Algorithm by Al-Khazraji	36	270	287
Algorithm by Samiksha	518	2369	1139
Nine Point Circle Rule Method	42	44	75

Table II gives the count results of images obtained from the MATLAB environment.

TABLE II
Counts of Matlab Images

Image Name	Coins	Eight	Rice
Exact Cell Count	10	4	101
Algorithm by Al-Khazraji	10	1	151
Algorithm by Samiksha	37	263	158
Nine Point Circle Rule Method	10	4	93

Being a new approach in image processing and computer vision for counting cell images, the suggested algorithm gives proper results with a better success rate than the compared algorithms as seen from Table I and Table II.

VI. Conclusion

Automated Cell Counting Using The Nine Point Circle Rule is a practically useful paper because of the following reasons. Firstly, there is a huge need for cell count for medical purposes. Secondly, almost every hospitals and medical centers have computers and the algorithm can run. Thirdly, the usage of this method is very user-friendly because if taking a photo of cells and load into the program will be automatized, the all process will become full-automatic.

This method is significant because the nine-point circle rule is not used for cell segmentation before. Also, the time consumption for this method is highly efficient for manual counting. It lowers the time consumption than other methods like Hough Transform because of less amount of for and while loops in the algorithm. Also, this algorithm allows distinguishing the cells if connected or not. The more circular the shape, the higher the average algorithm gives so, when the cells are not connected, the average will be higher.

To conclude, this paper creates a new approach for the automated cell counting domain. It introduces the mathematical rule for cell segmentation approach. It also gives lower time complexity results than compared methods. Moreover, it is user-friendly.

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