Automation of Iron difficiency anemia blue and red cell number calculating by Intictinal villi tissue slide images enhancing and processing

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Abstract

Iron deficiency Anemia is defined as hemoglobin concentration in blood less than 135 g/dl in adult male and less than 11.5 g/dl in females it has many symptoms (weakness , lethargy , palpitation) and less hemoglobin means less carrying oxygen to the body ,the analysis of the Red blood cells that is found in the villi cells in the small intestine will give a lot of useful information about iron deficiency anemia, but the problem that this analysis is still until this century done manually and finding an algorithm to make the process of the slide image analysis automated will make a change in the analysis process . so in This paper we presents a new implemented algorithm that achieves an automated way for the analysis of images taken for intestine villi and this algorithm will count the number blue and the red stained cells blood cells that contain iron in each villi alone and also calculate the percentage of blue cells and red cells in the image and this will give a lot of information that is useful and it starts from the step the follow villi image cropping and determining the ROI that we want to count the number in it and this ROI should be a complete villi or part of it .

Keywords: iron deficiency cell, anemia image processing, microcytic cell counting, villi image analysis

1. Introduction

Small intestine villi cell image analysis is a very important to determine Iron deficiency Anemia, iron deficiency cell image slide that is taken from the villi tissue in the small intestine contain large number of small red and blue spots cells. each cell intensity represent the amount iron in the cell , blue stained cell are the one we want to count its number because they represent the iron deficiency cells , and these images because they contain large numbers of cell spots it took a large amount of time and the process of analysis is slow and it is needed to be analyzed in a very fast automated way and this analysis is very important in discovering the amount of iron deficiency and the best drug to treat it for example adding iron to cells will determine the amount needed by the cells to be saturated with iron . Red blood cells multiply, incorporate iron and produce hemoglobin, the oxygen-carrying component of blood. Often there are multiple reasons why anemia occurs. One reason is inadequate iron absorption in the small intestine [1]

The majority of the body's iron is absorbed by cells lining the first portion of the small intestine, an area known as the duodenum. The tubular small intestine has, long, finger-like projections approximately 1mm in length known as villi that is shown in Figure 1, which improve absorption by increasing the surface area. And that's why analyzing cells in these villi tissue will give us useful information about iron deficiency anemia

Figure 1

2. Materials and Methods:

Small cut part of the Small intestine that contain intestine villi shown in figure 2 which where prepared in the lab by first fixing them using Para formaldehyde to preserve the tissues then they where mounted on paraffin blocks and sliced for a bout approximately 2 mm with a microtome device. And the tissue is now put in a slide then the slides stained for iron using Gomori's Method which contains Hydrochloric Acid and Potassium Ferro cyanide [2]

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Nikon -Eclipse microscope with Plan Flour lenses was used and Spot Insight color camera was took the images for slides and stored to be analyzed using Matlab software that were used to implement the proposed algorithm we used Matlab software to implement the algorithm because Matlab is a high-performance language for education and research It integrates computation, visualization, and programming in an easy-to-use environment where problems and solutions are expressed in familiar mathematical notation and also it has toolboxes for signal processing , neural network, image processing , database … etc , Matlab Image Processing Toolbox is a collection of functions that extend the capability of the Matlab numeric computing environment. The toolbox supports a wide range of image processing operations, such as Image analysis and enhancement, Region of interest operations, Linear filtering and filter design [3]

Figure 2

3. Proposed Algorithm, Discussion and Results

We will start discussion the algorithm from the step that follows preparing the slides and taking the RGB image and store it for the analysis we want to count the number of red and blue cells since each pixel in the RGB image is determined by the combination of the red, green and blue intensities stored in each image that we want to analyze [3].

The first step is converting the selected image that consists of x red spot blood stained cells and B blue spot blood stained cells that is located in the intestine villi as shown in figure 2. also there are the villi edges that surround the cells as shown in Figure 3, Converting the image from RGB images to grayscale as shown in figure 4 by eliminating the hue and saturation information while retaining the Luminance and this can be done first by applying stretching transformation and that's in order to increase the concentration of the image and will make all the image colors used from (0 to 255) as shown in figure 4. [4]

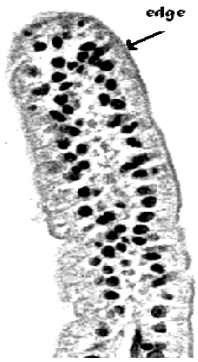

Figure 3

Figure 4

Then applying the grayscale threshold for the stretched image , this step mainly aims to enhance cell spots aims to enhance spots position determination since each pixel in the RGB image is determined by the combination of the red, green, and blue intensities stored in each color plane at the pixel's location with 8 bits for each color, giving a potential of 16 million colors and make it difficult to determine the position of the cell spots using all this combination; so we need to convert it to gray scale image as shown in Figure 5

The gray image can be represented by the function:

f: Df $\longrightarrow$ x …. (1)

Where Df is a real number that represent an ordered set of gray levels, its values is from xmin to ,xmax . f is the gray value of the image at point x = (X , Y), The lighter the gray value of f at point x, the higher the altitude of the corresponding point {x , f } on the surface of the image. So the lower points and the zero values may represent the points the spaces between cells like the background points and the points that contain noise [12]; if the value of the function f = 0 then this point represents the background

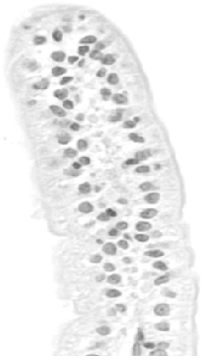

Figure5

The second step is to fined the size of the region of interest (ROI) that we want to count the number of cells in or or the hall image as determined by the analyst, the region of interest here represent a complete villi that contain cells, because in some images we will notice part of villi not a complete one as resulted from the cut of the intestine in preparation time as shown in Figure 6.

Figure 6

the next step is applied to the original stretched image it to binary image not the grayscale image that is resulted in the previous step and this step include Creating an new image by using threshold. Computing an appropriate threshold to use it to convert the intensity image to binary and in binary image, each pixel assumes one of only two discrete values. Essentially, these two values correspond to on and off. A binary image is stored as a logical array of 0's (off pixels) and 1's (on pixels). The output binary image has values of 0 (black) for all pixels in the input image with luminance less than level and 1 (white) for all other pixels which here these pixels represent the red blood cells in the villi and the edge of the villi which will be removed in the next steps [4].

Since the RGB image consist of green and blue and red and the green represent both red and blue colors we will isolate each color plane alone and this will give 2 new images as show in figure 7 and figure 8

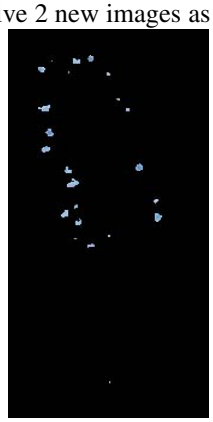

Figure 7

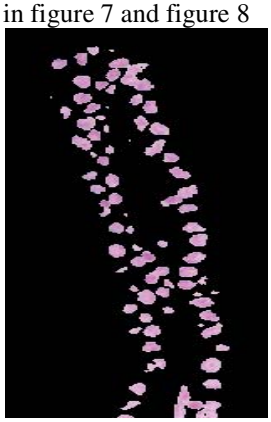

Figure 8

The fourth step of the proposed algorithm is to removing the noise from the image and isolate the red spots and the blue spots from the background after that we remove the edges that have resulted from the first step because these edge will give errors while continuing to the next steps of the algorithm and will make the process of counting the spots give wrong results and to do this dilation was applied to the image so that it will remove the edges [4, 6].

The fifth step is to retrieving the red or blue spot cells that where eliminated by the isolation of the red, blue and background new produced sub images by intersecting the images together and this will make some of the lost spots appear as shown in figure 9 , figure 10.

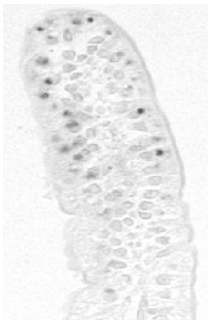

Figure 9

Figure 10

The next step is to determine the number of blue and red spot cells that is found in the ROI in the binary image after removing the noise from the image, and this is done first by locating the place where the spots are found and this is done first by applying dilation as shown in Figure 11, Figure 12 to the blue sub image that is resulted from the third step and then to the red plane sub image after that the spots number is calculated [5]

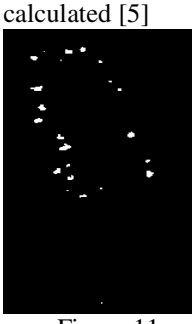

Figure 11

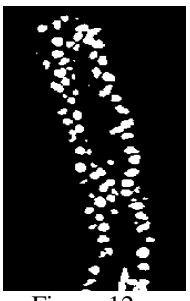

Figure 12

The final step is displaying the results that is needed in a fast and clear way for analysis and caparison studied and for continued researches the results will be displayed for the analyst showing the number of red cells , the number of blue cells and the percentage

between them and if there is no blue cells it will display the symbol A which mean that there is no iron anemia in the studied tissue , if the results shows the symbol B this mean that 10% or less of the studied image are blue and have anemia with this percent and the same for the reset of percents the implemented algorithm will display the symbol C if the percent is below 25 and higher than 10 and E if the percent below 50 and above 25 and F if the percent below 75 and above 50 and G if the percent is below 100 and above 75% and we choose this way so that it will the analyst can determine the state of blue cells he has in the studied villi and compare it to other villi that he has and easier to get the comparison with other slides and if he have done certain tests and he want to see the improvement of the results this way will give him a clear goal of what he wants if he reached the studied slide gave result A this mean that the there is no anemia and so on for the rests of symbols [7].

The proposed algorithm depend on the automation of the counting process of blue and red cells in the studied image and this algorithm is better than previous manual counting methods that is used till now in laboratories It is very time consuming, subjective, and give error. While the proposed automated implemented algorithm give the analyst a better control over the counting process and he can choose any part of the image or the whole image to study it and to count the cell spots in it in a very fast way that take some seconds.

4. Conclusions

This paper described a new automated algorithm for determining spot number of red cells and the number of blue cells that represent the iron deficiency in the cell and also calculate the ratio between them in order to determine the percentage of the presence of blue cells compared to the hall number of cells it improves cell number determination by making it automated, also because it is very fast method that require seconds for the analyzing the image and get the result

Iron deficiency cell image analysis is a very interesting research area that's why our future work intended to pursue the research for developing a new algorithm for automation all types of Iron deficiency image that is done manually in laboratory and take a lot of time.

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