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Diagnosis of Iron Deficiency Anemia Using Image Processing Techniques

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Abstract: This study aims to implement an image processing algorithm for detection of both red and blue stained blood cells to help in diagnosis of iron deficiency anemia in a more effective and efficient way. Our approach allows us to obtain the exact number of each stained blood cells. The algorithm also calculates the percentage of blue and red stained cells in the given specimen image sample. This information is vital in detecting the disease and determining its severity. Although the algorithm is designed in such a manner to provide flexibility regarding the selection of either a particular region or a whole given image sample, the calculations are done for the desired region only. Images of villi cells taken from the small intestine of humans were used.

Keywords: Cell counting, iron deficiency anemia, villi cells

INTRODUCTION

Iron deficiency anemiais a common type of anemiacaused by insufficient dietary intake and absorption ofiron and/or iron loss from intestinal bleeding, menstruation and parasitic infection. The most significant cause of iron-deficiency anemia isparasitic worms:hookworms,whipworms androundworms (Brady, 2007).

Ironis present in allcellsin thehuman body and has several vital functions. One of these functions is carrying oxygen from thelungs to the tissuesin the form ofhemoglobin. Too little iron can interfere with this vital function and lead tomorbidityanddeath (Umbreit, 2005).

Red blood cells contain iron and are not formed when iron is deficient (Brady, 2007).

Iron deficiency causes approximately half of all anemia cases worldwide and affects women more often than men. Children andpre-menopausalwomen are the groups most prone to the disease. World estimates of iron deficiency occurrence are somewhat vague, but the true number probably exceeds one billion persons (Calis *et al*., 2008).

Anemia can be diagnosis by performing full blood count to check the level of hemoglobin in the blood (number of each of the different types of blood cell), the size of these red cells and the amount of hemoglobin in each red cell and by testing the blood under a microscope to check the size and shape of the red blood cells and to assess the different white cells that are present. Assessing Serum iron, iron binding capacity and ferritin levels will also help in the diagnosis. Determining the disease severity can be judged by the exact count of red blood cells. Thus by counting the exact number of iron deficient cells in a given region of tissue, we can early detect Iron deficiency anemia (Pagana and Timothy, 1998; Gordon *et al*., 1992).

The cells lining the small intestines are the region for absorption of major amount of iron in the body. The inner walls of small intestine have minute protrusions (1 mm) throughout its surface. These are known as villi. By increasing the surface area these tend to enhance the absorption of large amount of iron. Intestinal villi are one ofthe primary tissues used for such studies. Being the earliest to be affected it can serve our purpose to the best extent. A single slide of villi tissues contain a large number of stained cells. The intensity of each cell and its closeness to red represents the amount of iron in it. On mere counting of these cells the disease condition can be diagnosed. Presence of large numbers of cells make the counting a very tedious process and often expose results to errors. Thus it would be more advantageous if such process can be carried out by machines rather than humans. Automating such process not only saves time but also diminishes errors. This results in an easier and more accurate diagnosis (Ruifrok, 1997; Nancy, 2000).

Motivation: Iron deficiency anemia has prevailed since very early times. At certain times it has proved to be an epidemic in various parts of the world. The primary reason behind this fact has been improper and late diagnosis (Teresa *et al*., 2005). For this reason taking up an approach for faster and better identification of the disease condition seemed very plausible. Also this method will be inexpensive and as such be readily available to all masses of the society.

Objectives: To implement an image processing algorithm able to help in proper diagnosis and early detection of Iron Deficiency Anemia we first need to investigate the anemia relevant affects and mode of action on villi cells of small intestine. The major affect was found to be a variation in the intensity of cells. Due to iron deficiency

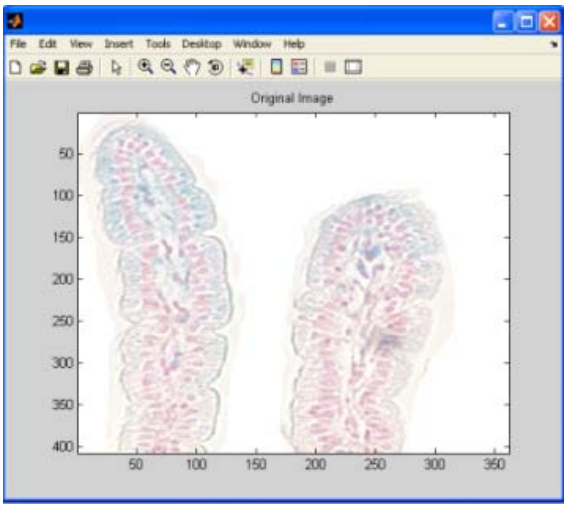

Fig. 1: Villi image in RGB format

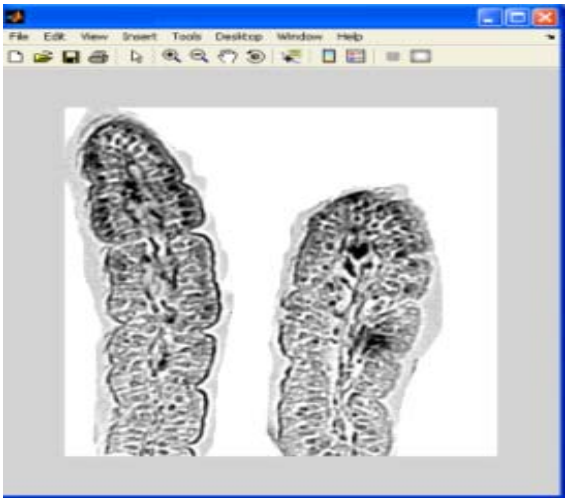

Fig. 2: Villi image of RGB into grayscale

cells lost their red color to varying degrees. This was the main focus for designing the algorithm.

The primary objective is to study the affected images to find abnormalities and then design an algorithm to identify these abnormalities.

MATERIALS AND METHODS

Intestine villi tissues were collected from anemic patients and treated with par formaldehyde to preserve the tissue. The tissues were then mounted on paraffin blocks and thin slices of 2 mm were cut using microtome device. Slides were prepared from these tissues and stained with iron using Gomori's method. This method includes treatment with hydrochloric acid and potassium ferrocyanide (Luna, 1968; Chard, 1990).

The microstructure of tissue was magnified using Nikon-eclipse microscope with plan four lenses. Spot insight color camera took the images of the slides for storage and further processing. Matlab software was used for further processing tasks. The selection of Matlab was due to its high-performance nature and widespread use throughout the world for all scientific study and research purposes (Rafael *et al*., 2009).

PROPOSED ALGORITHM

The digital villi images were taken and stored as discussed previously. The images were taken in RGB format where each pixel is a combination of red, green and blue. There were 'r' number of red cells and 'b' number of blue cells as shown in Fig. 1.

The very first transformation was to convert this RGB image into grayscale. The villi image has edges as shown in Fig. 2. The hue and saturation information was eliminated while the luminance of the image was retained.

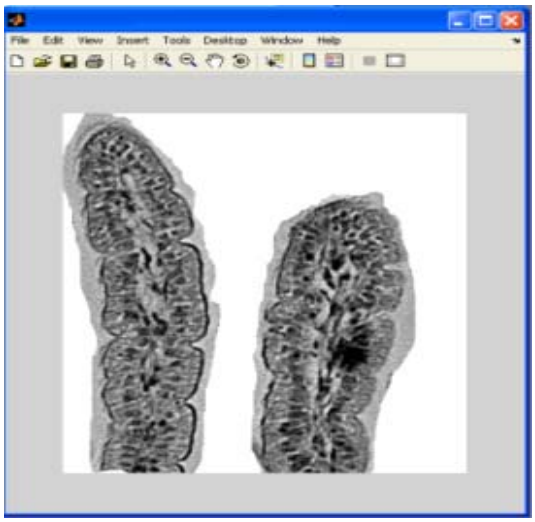

Fig. 3: Villi image in stretching transformation

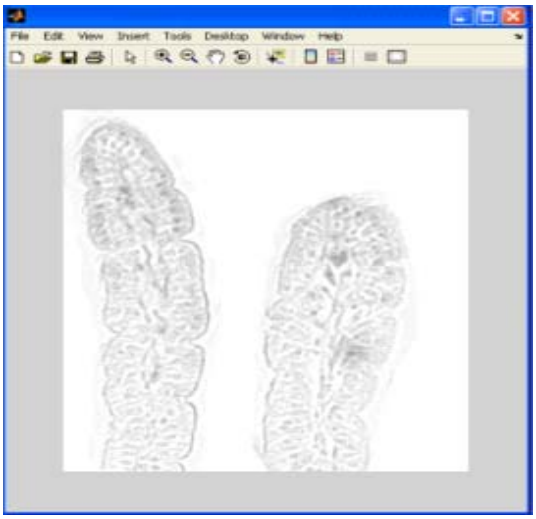

Fig. 3: Villi image grayscale show the position of each cell spot

Stretching transformation was introduced and that is in order to increase the concentration of the image as shown in Fig. 3 (Rafael *et al*., 2007).

Grayscale threshold is applied to the stretched image in order to enhance cell spots. Each pixel in an RGB image is determined by the combination of red, green and blue intensities stored at in each color plane at the pixel's location (Young *et al*., 2009). Each color of a pixel is represented by 8 bits giving a potential of 16 million colors. This huge number of combination makes it a tedious task to determine the position of each cell spot. Conversion to grayscale image solves all of these problems. The converted grayscale image is shown in Fig. 4.

The gray value in the image can be considered as a function of pixel values as follows:

$$f: D_f \quad x \qquad (1)$$

where, D_f represents a subspace of real numbers representing an ordered set of gray levels ranging between the values from x_{min} to x_{max}.

f is the gray value of the image at any given point x = (X, Y) on the image surface. x represents the spatial co-ordinates of a given point. 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. The lower points and the zero values may represent the spaces between

Fig. 5: Blue plane

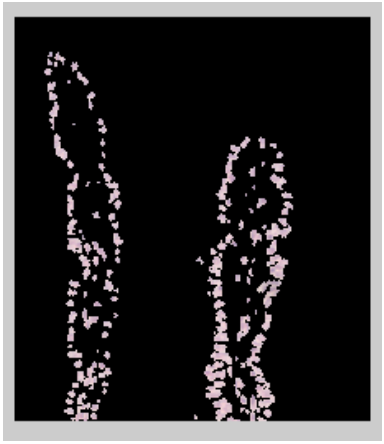

Fig. 6: Red plane

cells (like the background points and the points that contain noise). If the value of the function $f = 0$ then this point represents the background.

This algorithm provides flexibility in the selection of the Region of Interest (ROI); in which the numbers of cells have to be counted. This gives the analyst higher chances of better diagnosis. The ROI can be a complete villi or a specific part of it. This is to compensate for any discrepancies during the slide preparation.

The stretched grayscale image is the converted to binary image for better extraction of the blue and red cells. This is achieved by simple thresholding and new images are created. An appropriate threshold level is selected and then the image is stored as a logical array of '0' (off pixel) and '1' (on pixel). The output binary image has a value of 0 (black) for all pixels with intensity values less than the threshold and 1 (white) for all pixels with intensity greater than the threshold value.

An RGB image consists of three different planes of red, green and blue. For counting the number of cells belonging to each colors these color planes need to be separated. The separated blue and red planes are shown in Fig. 5 and 6.

The noise is removed as the next step and the red and blue cells are separated from the background. The edges can also be a source of error while counting, hence these are also eliminated. Dilation easily dissolves all the edges (Rafael *et al*., 2007).

While separating the red and blue planes many of the respective spots are lost due to improper thresholding. Retrieval of these spots is very necessary for accurate results. Intersecting the images together will reveal the lost spots. The retrieved spots are shown in Fig. 7.

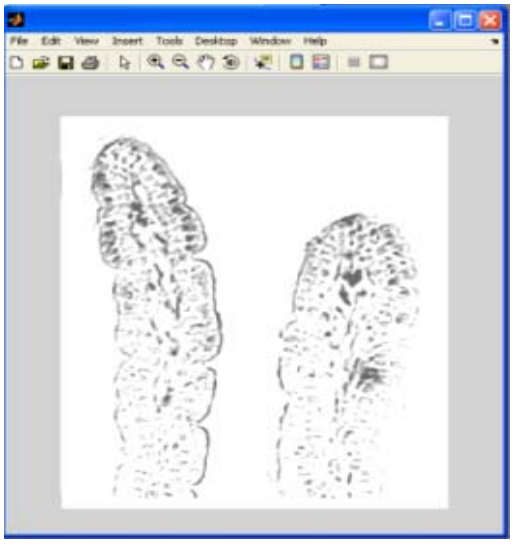

Fig. 6: Seprate the red andblue plane from image and retrive the spot

The final task is to count the number of respective spots present in the ROI. The sub image of each color plane is dilated and spots are detected and counted in the respective sub images. The final dilated sub images showing blue and red cells are shown in Fig. 8 and 9.

All the results generated are displayed in a crisp manner. This enhances the analysis and research.

The number of red cells and the number of blue cells will be displayed as a result of experiment. After that the system will calculate the percentage between them and if there are no blue cells, the symbol N will be displayed. Otherwise, the percentage of anemia is displayed. This categorization of the percentage of blue cells will help the analyst to compare various slides and also to study the effect of certain drugs on the patients.

CONCLUSION AND FUTURE WORK

This study described an improved automated algorithm for detection of iron deficiency anemia in humans. The primary advantage of this method is that once the slides are prepared and properly imaged these are no more needed for analysis (only the images will serve the purpose). Also storing images is considered to be easier and cheaper than storing all the slides (for researchers). Exact number of cells in the precise region of the slides can be determined. This may lead to better diagnosis and management of the disease and thus achieve the research objectives.

This method can also be adopted for other diseases where a visible change in intensity or color can be detected in any type of animal cells. Thus improve health and decrease the percentages of morbidities and mortalities due to various diseases.

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