Small Intestine Villi Cell Counting and Quantization Using Digital Image Processing

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Abstract
Research on iron-deficiency anemia is being conducted in the Kling lab at UW-Madison. After rats are feed with a test diet, they are sacrificed and slices of their intestine are prepared and analyzed. The slices are stained for iron, meaning that any red blood cells which contain iron turn blue in color. The researchers then view the sliced intestine under a microscope and count the number of blue cells that are present in each villi of the intestine. This procedure of counting cells is extremely time consuming, subjective, and vulnerable to human error. This paper proposes an algorithm which uses digital image processing to eliminate manual counting of the blue cells. The program contains a graphical user interface and outputs the number of cells, cell area, and the percentage of blue and red cells for a selected area.

Introduction: Background and Motivation
Anemia is a condition in which the blood is not efficient in bringing oxygen to tissue. The ability to carry oxygen to tissue is exceptionally important in infants who are continually developing; however, in premature infants anemia is a growing problem [3]. When the body detects anemia it reacts by producing erythropoietin, which is a hormone that stimulates red blood cell development [1]. The red blood cells then need iron to be healthy and produce hemoglobin, the component of the blood that carries oxygen to the tissues. If iron is unavailable, the cell can not function properly and the individual develops iron-deficiency anemia. This condition can lead to serious health issues such as constant tiredness, heart conditions, and other health complications [4].

A current study is being conducted in the Kling lab at UW-Madison, to look at the affects of diet on iron absorption in the intestine of newborn lab rats. After a certain diet has been administered for a set amount of time, tissues from the lab rats are obtained and put on microscope slides [1]. The slides are stained so iron becomes blue in color, so that iron absorption can be quantized. Currently the number of villi which contain iron and the number of cells within the villi that contain iron are counted manually. This procedure is very time consuming, is vulnerable to human error, and is somewhat subjective. It would be ideal to create a digital imaging tool to automate the counting process and in some way quantify the degree of iron absorption, i.e. find percentage of blue compared to red cells.

A current Kling lab algorithm uses manually set thresholds in the RGB channels for each individual image to find the blue pixels. A statistical algorithm is then implemented to estimate the number of cells which are within the blue area [1]. This system is confusing and is also time consuming. A quicker and less manual solution would be an improvement.

Sharon Blohowiak, a researcher in the Kling lab, suggested the best solution to this problem would be to create an algorithm to count the number of blue cells and also calculate the percentage of blue pixels and red pixels. The percentage detection was implemented due to the fact that some slides contain villi which have regions of blue cells where the cells can not be distinguished from one another visually.

Approach

The main purpose of the algorithm is to separate the blue and red cells from the rest of the image. Once the blue and red cells are separated the area and cell number can be calculated. Various methods were explored to extract the red and blue pixels from the image. The following is an overview of the methods that were tried.

According to the preceding experiments [1], predefined thresholds to each RGB channel does not yield satisfactory results since each image varies in contrast, brightness, and color cast. Moreover, the existing computer counting method requires the user to select regions of the image to evaluate and then manually set thresholds. As a result, the process is time consuming and needs a high performance computer to evaluate the data. Due to this knowledge simple thresholds on the original image were only briefly explored and proved to be a non-efficient solution.

In addition to predefined thresholds, adaptive thresholds for each channel have been tested, but do not provide good results due to a difference in number of cells, cell sizes, and cell areas in each slide image. Owing to the fact that any red pixel must have a red value greater than the blue value and vice versa for a blue pixel, a threshold was created based on each pixel's red and blue value. The inter-channel threshold was tested and resulted in a good discrimination between red and blue regions. Unfortunately, the inter-channel threshold method alone could not single out the blue and red areas from the image (Figure 1). Therefore, image preprocessing is mandatory to reduce the inconsistency among each image.

Image contrast stretching was used to separate the blue and red cells from the rest of the image; however villi edge continued to be a problem in the final image. Edge detection was considered to eliminate the villi edge, but it also eliminated too much of the blue cell area along with the villi edge. On the other hand, background elimination proved to produce a desirable outcome without villi edge which can be processed by inter-channel thresholds efficiently without user input.

Figure 1. The two images display the pixels which are passed (values of 1) for red > blue, and red < blue. The image on the left will select blue pixels and not red, however one can see that other parts of the image are also selected. The image on the right will allow red pixels to pass and not blue. It is obvious that further processing must be done to the images.