

APPLICATION SOFTWARE TO SUPPORT THE DIAGNOSIS OF TUBERCULOSIS USING SPUTUM SAMPLES OF RESPIRATORY SYSTEM (LUNGS).

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ABSTRACT: In the present study the analysis of sputum samples, in order to develop a tool to optimize and streamline the process of counting the resistant acid fast bacilli present in sputum samples is performed. The algorithms developed for segmentation of this bacterium are special in each case; in the pre-processing we use special filters for noise image, that help to remove and improvement of image quality. This is important because that help to avoid quantization errors to be others types of cells around the middle where the bacillus is present. When analyzing the RGB corresponding bacillus color and the environment where it is immersed, is detected that the most important information of the image is in the space R. The software performs the quantification of bacilli in 10 simultaneous images in approximately 15 seconds. It also offers the user the creation of clinical records.

Keywords: Images processing, tuberculosis, quantification, optimizes, lungs, sputum, and bacillus.

INTRODUCTION

Development based on the creation of digital plate forms such as software technologies, revolution of the medical, biological, chemical field among others. In particular, algorithms for information extraction from images are known as image segmentation algorithms that play an important role in a variety of biomedical applications, diagnosis of diseases associated with processing of images.

In the fields of biology and biomedicine images processing is used to visually analyze biological samples and diagnose possible pathologies suffered in different types of patients. An associated case is the analysis of samples of respiratory and non-respiratory - based method of the smear for the diagnosis of tuberculosis, in which a characterization of the bacteria (acid-fast bacillus) is performed, and a quantization is performed the number of these bacteria in the sample from the digital image processing.

Currently the methods for diagnosing tuberculosis, based on manual quantification, which is to count the number of acid-fast present in a patient sample rods.

Traditionally the diagnosis of tuberculosis is made for accounting purposes , which means that the bacteriologist must have count one by one the bacilli present in sputum.

To perform manual quantification using the smear method takes into account some parameters established

for staining used in this method.

Negative: no bacilli seen in 100 fields observed.

- Positive +: less of a bacillus per field on average observed in 100 fields observed.

- Positive ++: observed from 1 to 10 bacilli per field in 50 fields observed average.

- Positive +++: more than 10 bacilli per field average

This project focused on analyzing bacteria present in samples of smear. Mycobacterium tuberculosis (in which the analysis of cell -type acid fast bacilli was required) , allowing their identification , characterization and quantification of automatically by digital processing images, using the MATLAB ® R2010a software providing accurate and reproducible results . Complementarily designed a database that stores and manages the results from quantification.

MATERIALS AND METHODS

In developing this research we choice a number of patients between 18 and 50 years old. Using a methodology of quasi-experimental, since in this study the absolute control and manipulation.

For the development of this research, we use samples with Ziehl Nielsen staining. We choice a total of 200 images, of which 100 were, discarded taking a criteria such as low resolution or poorly prepared staining were analyzed.

For other way was used also matlab to develop the platform and user interface.

To perform the thresholding and segmentation method ,consisting of an algorithm called threshold arithmetic mean deviation using variations in the gray levels which is used has an image; in order to determine the threshold value at which the image segmentation analyzed is performed. It has certain features like image size, intensity, among and others. The segmentation depend on an adjustable value they are a value closer to (0.0), if the pixel image is closer of this value mean that the region have information .

Thresholding convert grayscale images into a new binary image, where the two levels are assigned to pixels that are above and below the threshold algorithm applied in this way to separate objects of interest from background image.

Subsequently segmentation is adjusted to obtain more detail bacilli was achieved segment, by subtracting

matrices each containing images thresholded values.

Once the images are binarized facilitates distinguishing characteristics of each of the structures. To initiate detection of bacilli in the segmented images function creating morphological structuring element that polls or interacts with objects in the segmented image, to extract the shape of objects. After having an adjusted thresholded image, we proceed to perform the detection of bacilli by means of geometric, such as relationships between major and minor axes of the ellipse inside and across the border rectangle enclosing the region features.

EQUATIONS

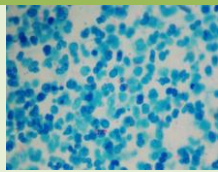
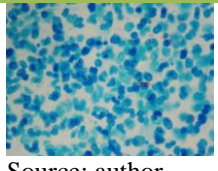
To complete the detection of the bacilli, we make an algorithm called nbacilos, which identifies these structures in the analyzed image was created. This algorithm uses a variable that determines the maximum dimension along the major axis of the ellipse encloses the bladder in order to filter and reduce the error. It also uses a variable secondary dimension that determines the minimum area of the bacilli. This algorithm uses geometric functions as MajorAxisLength referring to the major axis of the ellipse circumscribed, MinorAxisLength which is the minor axis of the ellipse and circumscribed Eccentricity containing a value between 0.0 and 1.0, where 1.0 indicates that the object resembles a straight and 0.0 indicates that the object is circular.

Given the results produced by the tool measures the relationship into tool is: 2 pixel equals 10 microns. With the calculation of the mathematical relationship proceeded to ask the following equations to calculate the area of the circumscribed ellipse.

1. $a = \frac{((Wa(r).MajorAxisLength / 2) * 10)}{2}$
2. $b = \frac{((Wa(r).MinorAxisLength / 2) * 10)}{2}$
3. $A = (a) * (b) * \pi$

Equations 1, 2 and 3 correspond to the calculation of the radii of the major and minor axis. Where to correspond to the radius of the major axis of the ellipse in microns, SV (r). MajorAxisLength contains the calculation of distance in pixels of the major axis. B In equation 2 corresponds to the radius of the minor axis in microns, SV (r). MinorAxisLength contains the calculation value of the distance of the minor axis in pixels and A is the total area of the circumscribed ellipse.

Table 1. Images select.

CLASSIFICATION OF SELECTED IMAGES	IMAGE	characteristic
Clear and good image Lighting	 Source: author	Digitized under 100X magnification optical images. Bacillus typical structures are observed
Image with increased definition of features suitable for	 Source: author	Images scanned under optical magnification of 100X. A panoramic view of the cross section of the sample is observed, which allows a more accurate quantification

RESULTS

To perform the correct quantification of bacilli present in the images first start by pre-processing of images which allowed correcting the degradation in images acquired by using a digital camera. The problem in the images is associated with a low or insufficient dynamic range, as well as poor color reproduction.

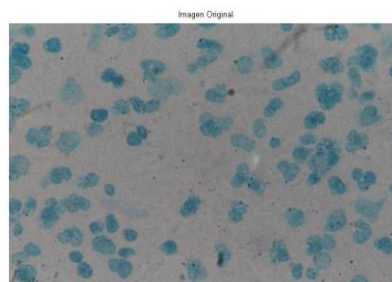


Figure 1. Original image.

Seeking correct variations present in images in terms of color reproduction levels an arrangement thereof is performed by imadjust function that adjusts the intensity values in the image in its color map from assigning a numerical range between 0 and 1. Applying this series of vectors able to increase the contrast of the final image and thus highlight the structures of bacilli.



Figure 2. Adjusted image

Once masks intensity adjustment applied by `imadjust` function, the transformed images are converted to grayscale and a new modification is done in shades of gray with `imadjust`.

After pre-processing, is continuous with the digital image processing for analysis bacterial load present in them.

To perform the thresholding segmentation method consisting of an algorithm called threshold arithmetic, that mean use variations in the gray levels, In order to determine the threshold value at which the image segmentation analyzed is performed.

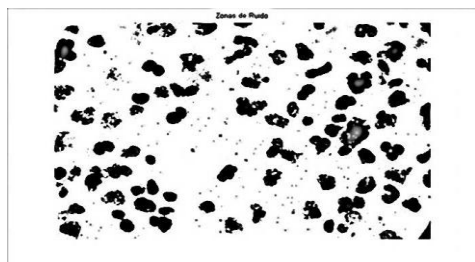


Figure 3. Thresholding

Subsequently segmentation is adjusted to obtain more detail bacilli was achieved segment, by subtracting matrices each containing images threshold values.

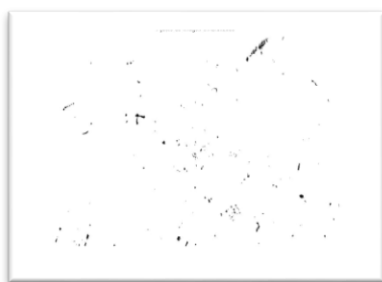


Figure 5. bacilli detected

To finish off the bacilli detection, an algorithm called `nbacilos` which identifies these structures in the analyzed image was created. This algorithm uses a coordinate variable determining the maximum length of the major axis of the ellipse encloses the bladder in order to filter and reduce the error.

It also uses a variable secondary dimension that determines the minimum area of the bacilli. This algorithm uses geometric functions as `MajorAxisLength` referring to the major axis of the ellipse circumscribed,

`MinorAxisLength` which is the minor axis of the ellipse and `circumscribed Eccentricity` containing a value between 0.0 and 1.0, where 1.0 indicates that the object resembles a straight and 0.0 indicates that the object is circular.

Design data record. We designed a way to save the product analysis data count of bacilli. This data record is developed by MATLAB®, the data are displayed on a table in the GUI application and then this information can be exported into Microsoft Excel format for advanced use of the data collected by the software.

These data can be viewed in Microsoft Excel with the same attributes and entities that are displayed in the interface software. Microsoft Excel files containing the data analysis can be opened from the interface using the menu in the Open / File option analysis. This data record contains a table where you can see, the name of the image and the number of bacilli was quantified.

For selecting the colors of the interface is taken into account the fuchsia color refers to the color of the bacilli on the stain used and because it is a neutral gray and passive color, also representing the combination of the ends of the scale colors.



Figure 7. User interface.

The software correctly identified 92% of the bacilli and 8% not quantified due to the quality of staining and image definition. This percentage was obtained by making a parallel between manual and quantification made by the program.



Figure 6. Data record.

Design of the user interface. It was designed to be presented in a visually dynamic way and easy operation,

you can export the data analysis performed counting

DISCUSSION

There are only few jobs in the world and in Colombia related to this project, but do not focus on the detection of bacilli if not calculating areas and perimeters of cells. For example in Colombia at the Andes University some student make software in 2012, where they can make a calculation of bacterial areas, as well as Boston University developed a software performing a count perimeters of cells. Other Work with relationships was one make in California University, where some students made software that calculated the cells area of lepra sputum. But don't exit a work like this because, this software is unique because it focuses primarily on the cuatificacion bacillus by bacillus, and also helps to make the diagnosis of tuberculosis much faster.

CONCLUSION

- We make a final test with help of bacteriologist that counted 578 bacilli versus 532 counts for the software.
- This software is first in his class, don't exist one tool like that: This is important because we contribute to development of tools for the diagnosis of pathologies.
- This software make a tuberculosis diagnosis faster because normally is make manual by the bacteriologist and this result to slower.
 - Data logging software is developed for easy handling and dynamic, are designed with the possibility of an advanced manipulation of data in another application outside of MATLAB®, and Microsoft Excel for editing and interaction with the data provided by the software.
- The software performs the quantification of bacilli in 10 simultaneous images in approximately 15 seconds, with an accuracy of 92%.

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