Quantification of Inflammatory Infiltrates in Chagas Disease

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Abstract—**This paper presents a digital image processing software implementation to identify and study models of infection related to Chagas disease caused by Trypanosoma cruzi parasite. The main objective is predicting disease progression to decide the appropriate treatment depending on the tissue damage observed and evaluate the effectiveness of these treatments in diminishing the structural alterations. For this reason, digital images from colored tissue samples are collected, to be able perform the quantification of inflammatory infiltrate present in these samples. This quantification process automation guarantees greater accuracy, objectivity obtaining results and savings in processing times of the samples involved. The software also includes a unique image repository and metadata implementation to provide more efficiency in post-processing activities.**

Keywords— Digital image processing; Chagas image analysis; morphological operation; inflammatory infiltrate.

I. INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is caused by the parasite Trypanosoma cruzi. About 10 million people are infected worldwide, and more than 25 million are at risk of contracting the disease, mainly in Latin America where it is endemic. This disease is potentially fatal and there are two successive phases: the acute phase and the chronic phase. Both phases can be symptom free or life threatening and have different clinical manifestations. In the case of cardiac compromise, it presents an acute myocarditis with histopathological lesions characterized by localized inflammatory reactions. Having this scenario, is necessary identify and study the host infection patterns to predict the disease progression or define an appropriate treatment based on the tissue damage observed.

The identification process requires digital images from conveniently prepared tissue samples in order to get the quantification of the inflammatory infiltrate. Actually, the detection process is executed by each researcher manually using a PC image editor where make estimation about the affected areas, comparing inflammatory tissue sector versus the total sector of the tissue sample, delivering a subjective assessment based on the researcher criteria.

It leads that the quantification process needs to be optimized to get the required accuracy and objectivity for the obtained results; and reduce the processing time per each samples included in the research. For this reason, we proposed a software design and implementation to automate the detection and quantification phases; and one unified metadata model implementation that allows working with the images and the post processing results as a second project iteration.

II. METHODOLOGY

The quantification process of localized inflammatory reactions requires the need of evaluate histopathological lesions present in the acute stage of the disease [1]. On the other hand due to biological polymorphism; for the same lineage, some strains prefer to parasite muscle cells (skeletal or cardiac), others prefer phagocytic mononuclear cells and a third group prefer still other types of tissues (nervous, reproductive system, etc.) [2]. This tissue tropism presented by the different strains implies variations in the lesions produced at the histological level and in the clinical presentation of the disease according to the strain involved in the infection.

Therefore, the identification and study of such lesions in the different infection models (with different parasite populations) and at the level of different host organs may be of interest to predict the disease progression and make decisions in terms of adequate treatment based on the tissue damage observed.

Approximately 30% of infected people develop at some time in their life a variable clinical expression of heart disease [3]. That is why the Chagas Laboratory of the Instituto de Fisiología en la Cátedra de Física Biomédica de la Facultad de Ciencias Médicas de la Universidad Nacional de Córdoba, in search of possible treatments uses Swiss albino mice to analyze muscle tissue in order to execute the quantification process for the inflammatory infiltrate.

Mice were maintained under controlled light conditions (12 hours light/12 hours dark cycle), temperature (23 ° C to 27 ° C) and relative humidity (45% to 65%), and fed with commercial food and water ad Libitum. All procedures have been approved by the Comité Institucional para el Cuidado y Uso de Animales

de Laboratorio de la Facultad de Ciencias Médicas de la Universidad Nacional de Córdoba [4].

The experimental process consists in mice infection with the different strains of Trypanosoma cruzi, followed by protocol activities like evaluation of blood samples at certain dates, ECG controls and parasites counting to finish with the extraction of muscle tissue samples for microscope analysis.

A. Histopathological Study

Samples of tissues (heart, skeletal muscle, liver, spleen, adipose tissue, lung, kidneys, brain and intestine) from infected mice are taken, which are fixed in buffered formalin (10%) and paraffin embedded. The 5-micron (μm) thick sections are stained with hematoxylin-eosin. A total of 3 cuts from each organ of each mice (for n = 3 statistical analysis per group) are analyzed under a microscope with 4x, 10x, 20x and 40x objectives. Heart, skeletal muscle, liver, spleen, adipose tissue, lung, kidneys, brain and intestine sections of uninfected mice (n = 3) are taken as controls. The images are also analyzed in search of the presence of inflammatory infiltrates and nests of amastigotes.

B. Inflammatory Infiltrates Quantification Process

To start the quantification process, digital photographs are taken with a 4x magnification in the AxioVision software (ver 4.0) [5] to start the analysis process. For the estimation, the total cut area of each of the tissues is taken as 100% and the percentage of the tissue covered by infiltrates is quantified. The selection process for each affected areas is done manually Fig. 1.

Due to this, the results are based on the subjective assessment performed by the researcher who operates the software by including or excluding image sectors with tissue under analysis.

The values related to the inflammatory percentage for each image are entered manually in Excel spreadsheets where intermediate calculations are performed to obtain the information related to the totals per sample group. This data is again entered manually in statistical analysis software, Infostat [6], to get the results which are used for the final conclusions.
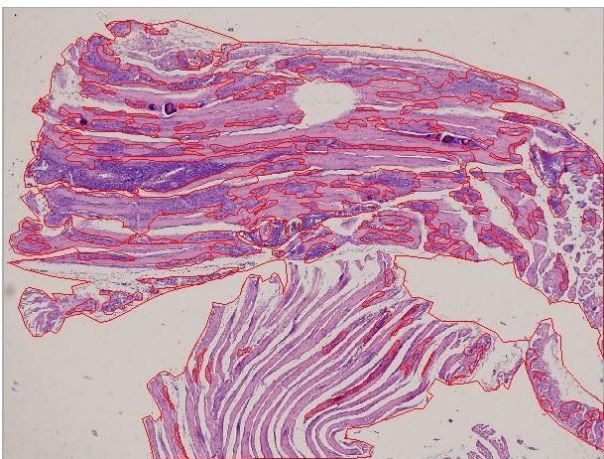

Fig. 1. Manual image processing

Based on the current state of the art for the work done in the laboratory; arises the need to implement a software that supports the image processing phases, which would allow obtaining results with the accuracy required and time savings in the sample images processing compared to the currently manual execution. This proposal will lead to a more efficient evaluation of the treatments tested, always thinking about improving the future patient's treatments.

C. Objectives

The following specific objectives are proposed:

- Automate the quantification process of the inflammatory infiltrate through the use of image processing techniques.

- Using Matlab and Simulink [7] as software tools for the image processing algorithms implementation.

III. DIGITAL IMAGE PROCESSING

With the use of Matlab [8] and its Image Processing Toolbox [9] we executed histogram analysis, geometric transformations and morphological filters; in order to create an algorithm that best suited to identify health tissue versus infected tissue; excluding image sectors without tissue. The flow diagram related to this algorithm is presented in the Fig 2.
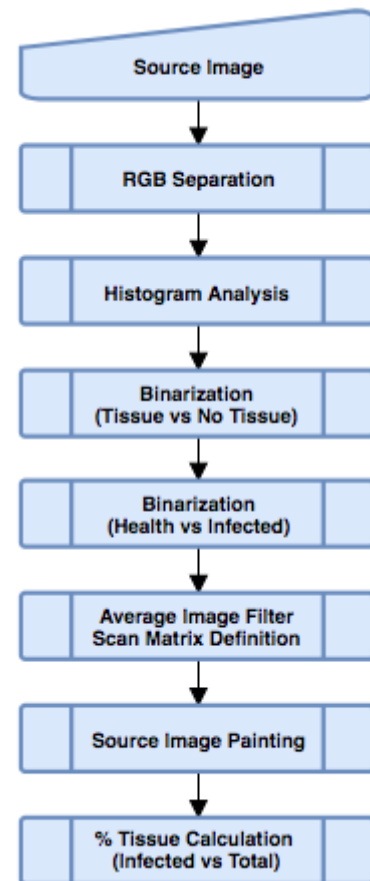

Fig. 2. Image processing algorithm flow diagram

For our digital image processing we use tiff format images; Fig. 3, obtained from the photographic system mounted on the Olympus IX81 microscope.
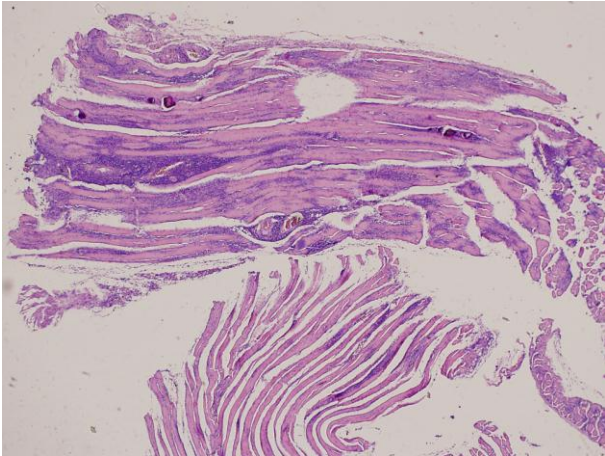

Fig. 3. Source image in tiff format obtained with the microscope

Before start with the processing operations, it is necessary to establish that represent each color for the original image; where the white/light gray sector means no-tissue, light violet means health-tissue and dark violet are related to infected-tissue.

Since digital processing is performed on grayscale images, it is necessary to separate the RGB image into the three primary color layers. The result will be three two-dimensional arrays, associated with red, green and blue respectively. In these arrays the luminance value is maintained and the tone/saturation information of the original image is eliminated.

To identity each tissue type presented in the digital image, the umbralization method is executed for image segmentation [10]. This method requires a histogram analysis [11] per each primary color distribution, to identify the thresholds that allow grouping image pixels with similar characteristics. In the resulting histogram the existence of a valley between two peaks will indicate the threshold value that can be used to apply the binarization process.
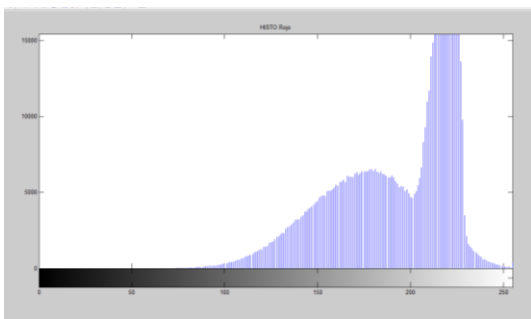

Fig. 4. Red layer histogram for tissue vs no-tissue identification

To identify in the digital image the tissue and no-tissue sectors we analyze the histogram related to the red layer, Fig. 4. Following the same objective; we use green layer histogram to separate health tissue and infected tissue, Fig. 5.
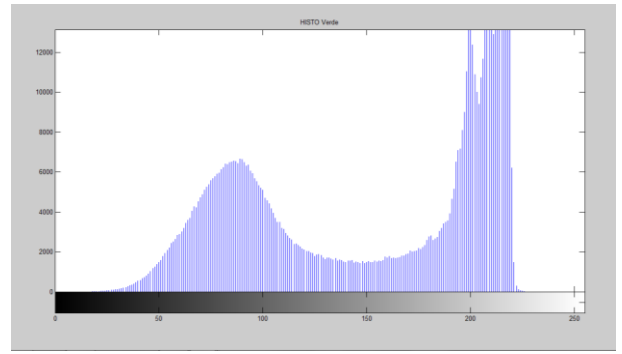

Fig. 5. Green layer histogram for health tissue vs infected tissue identification

Having the histogram analysis done, we have three selection threshold reference values where the minimum and maximum are the result for processing different digital image groups. These values are presented in the Table I.

TABLE I. TISSUE SELECTION THRESHOLD REFERENCE VALUES

Type	Minimum	Maximum
Non-tissue (Image sector without tissue)	200	255
Health tissue	160	200
Infected tissue	80	160

a. The numbers represent the luminance range for tissue selection

The luminance values related to the histogram valleys defined for our analysis (red and green layers) will be the thresholds used as input for the binarization process execution, Fig.6. As a result we will obtain two images whose pixels will have two possible values of luminance, 1 for those that are greater than the threshold in the original image and 0 for the smaller ones.

```
133
134        % separo tejido por Binarizacion
135 -      imagen_B1_binarizada = inputGray < umbralTejido;
136 -      imagen_B2_binarizada = inputGray <umbralInflamatorioAlto;
137
```

Fig. 6. Binarization process implemented using MatLab

As a result of the binarization process, a first image is obtained where tissue is separated from non-tissue (Imagen_B1_Binarizada) Fig.7. This image includes individual non-tissue pixels in the areas of health tissue and individual pixels of health tissue in the non-tissue area; for this reason a homogenization of each area is required, applying a digital filtering procedure.
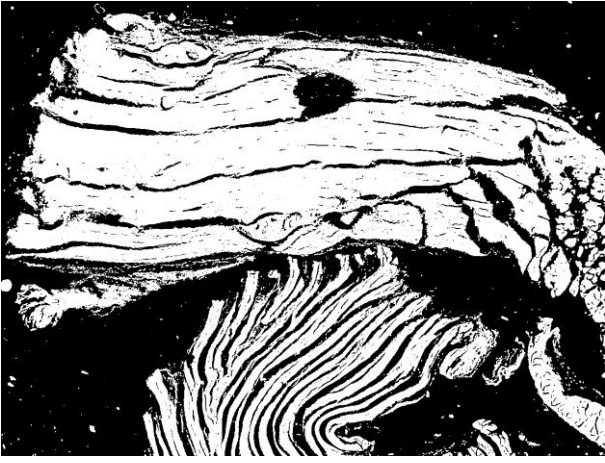
Fig. 7. Binarized image B1. Tissue versus Non-Tissue separation process.

After evaluating the different image filtering options available, the average type was chosen [12]. The filter function requires two input parameters, first the digital image, and second the dimension of the scan matrix, a user-defined value, Fig. 8.

```
150        % filtrado promedio
151
152 -      I3 = imfilter(imagen_B1_binarizada,fspecial('average',[tam tam]));
153 -      I4 = imfilter(imagen_B2_binarizada,fspecial('average',[tam tam]));
154
```

Fig. 8. Average filter process with binarized images using MatLab

The filtering procedure is also applied to the binarized image related to health tissue / infected tissue (imagen_B2_binarizada), Fig. 9.

Fig. 9. Binarized image B2 with average filter implementation.

In the next processing stage, the tissue/no-tissue binarized image (imagen_B1_binarizada) is scanned, in order to modify the pixels color of the original image. For this process the original image is masked with the binarized image to modify the pixels colors that compose the first one. Therefore the pixels representing the non-tissue areas are painted in gray color; whose luminance value is 0 in the binarized image, and with yellow the remaining pixels.

Using the intermediate image from the previous process; a new masking process is applied using the health tissue/infected tissue binarized image (imagen_B2_binarizada) to paint with fuchsia color the pixels that indicate existence of infected tissue; it means luminance value equal to 1 in this second binarized image; Fig 10.
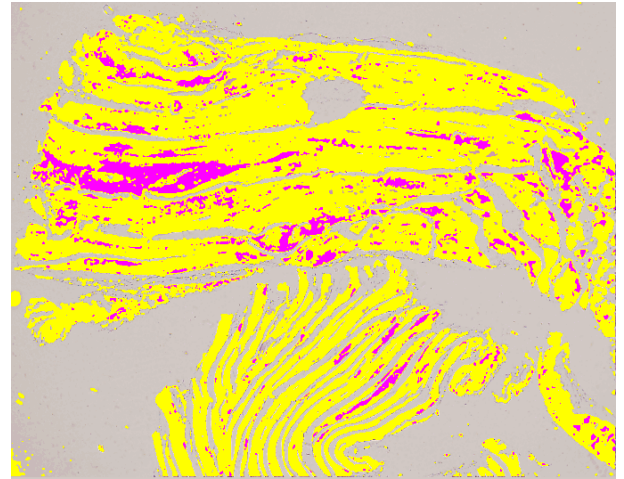

Fig. 10. Final image. Health tissue versus infected tissue identification

The last step in the quantification process is counting the pixels per each color (gray, yellow and fuchsia) to obtain the final percentage values, Fig. 11. The final percentages for the reference image are presented in Table II.

```
215        % Calculo de PORCENTUALES
216 -      pixelTotal = ancho * alto;
217 -      porcentualTejido = (pixelTejido/pixelTotal) * 100;
218 -      porcentualTejidoInflamatorio = (pixelTejidoInflamatorio/pixelTotal) * 100;
219 -      porcentajeFinal = ( porcentualTejidoInflamatorio / porcentualTejido ) * 100;
220
```

Fig. 11. Final percentages calculation using MatLab.

TABLE II. QUANTIFICATION PROCESS - PERCENTAGE RESULTS

Data	Percentage (%)
Image area with tissue (yellow pixels)	49.71
Image area with infected tissue (fuchsia pixels)	3.06
Infected tissue versus health tissue	6.20

IV. RESULTS

At the beginning of the quantification process histograms analysis from different images were done; to get reference values for the thresholds to be used in the segmentation.

Table I shows minimum and maximum threshold values, which can be adjusted by the researcher for each group of images.

The researcher starts from the reference values; if necessary adjust these values and execute the sample image processing until reach an acceptable threshold values and apply them to all the images related to the group under analysis. This adjustment

process is required because the tissue samples are affected by conditions happened during the preparation process (tissue cut, stain and glass mount) or when the digital image is captured (light intensity/color from the microscope).

Following the presented quantification approach, 200 images were subsequently processed and validated for the researchers to determine threshold values for different tissues types. These thresholds are the reference values to be used for future sample images processing. The results are presented in Table III.

TABLE III.   THRESHOLDS BY TISSUE TYPE

Type	Infected Tissue Threshold		Health Tissue Threshold		No Tissue Threshold	
	Range		Range		Range	
Health skeletal muscle	0	114±12	186±5	192±5	196±3	255
Infected skeletal muscle	0	153±15	181±16	198±12	196±3	255
Health heart	0	121±22	187±4	193±5	196±3	255
Infected heart	0	143±15	170±19	188±17	196±3	255

V. CONCLUSIONS

Based on the work being done, we implemented an algorithm for image processing to be executed on the images in order to separate the tissues involved in the evaluation.

During our tests, we identified the need to offer threshold values adjustments based on tissue type; without these feature it is not possible to obtain an acceptable result for the image evaluation due different conditions presented at the sample tissue preparation and digital image capture stages. Although there are no reference images (Gold_Standard), we worked together with the researchers to validate the process and the results obtained.

In our second project iteration related to image processing, we must be able to quantify the tissue infiltrates, having different sectors for the same tissue in more than one digital image capture. Other feature will be replace the first segmentation stage with histogram analysis, by segmentation using the Otsu method [13] to automate the first threshold calculation (tissue / no tissue), so the researcher only need adjust one threshold value (heath tissue / infected tissue).

The second phase for this project involves the implementation of a unified web repository of images and their respective metadata, in order to facilitate their identification and classification by different criteria (virus isolates / strains, infected organs / types of tissues, applied treatments, dates, between others), to optimize the teamwork between the specialists; avoiding the images transfer between workstations and even rework for images already processed.

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