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DESIGN OF A MICROSCOPY IMAGES FUSION AND CLASSIFICATION APPLICATION

José Salgado Patrón, Diego Sendoya-Losada and Johan Julián Molina Mosquera
Department of Electronic Engineering, Faculty of Engineering, Surcolombiana University, Neiva, Huila, Colombia
E-Mail: josesalgadop@usco.edu.co

ABSTRACT

It was made an application for automatic microscopy image fusion and subsequent classification of the cells found there. This application allows the entry of a sequence of images that you want to merge to obtain a large field of view with the characteristics of each of its component images. Similarly, the Myelogram classification can be made, that was the type of sample acquired in this process and which were obtained four sets of patterns. For application development we used Matlab GUI tool for creating a graphical environment which allowed carrying out the process of merging, sorting, editing and managing results.

Keywords: automatic fusion, microscopy, myelogram, patterns.

INTRODUCTION

The automatic fusion of images is a process in which it is sought to obtain a sequence of images of a zone of interest that must be recorded and transformed geometrically for the application of a fusion algorithm that can obtain a composite image where it can be analyzed in a global form. Meanwhile, image classification is the process that allows the identification of characteristics of different regions, for an identification of patterns in terms of similarities and differences.

In the medical work several microscopic images are analyzed in order to generate diagnoses based on what was observed. Unfortunately, there is no digital base of these analyzed images that allow continuous monitoring without having to have the physical plate of the sample, which in turn prevents the possibility of having remote assistance for more advanced diagnostics.

In the field of pathology, it is necessary to have the analysis of the various characteristics in an image stories such as shapes, sizes, quotas, which generate more accurate and opportune diagnostics, as well as carry out a digital record to follow-up each case.

Many studies and works have been carried out in order to generate algorithms that allow the registration of images (González, 2009) and the fusion processes that allow to obtain the best characteristics in the fusion image, both in aerial photography (Santamaría, 2000), as in medical imaging techniques (Larese, 2004). In the image segmentation the tests of different algorithms are carried out that allows to adapt the parameters to obtain a division suitable to the needs of the application (Osorio, 2005), as well as the different types of descriptions to take into account for the region characterization (Domingo, 2006).
The objective is to realize an application that allows to merge images automatically in the image of result to highlight the characteristics of its components and that in turn allows the extraction of characteristics and patterns for the classification of the cells in the image of myelograms.

METHODOLOGY

Collection and selection of information

Information related to image merge and classification work was collected to have a clear idea of the steps to be taken to develop the project, as well as to investigate the medical concepts necessary to be aware of the scope of the project and to seek and select information that provided the sufficient theoretical bases to implement the algorithms of image registration, improvement image, fusion, segmentation and classification. Finally, Matlab was selected as the programming and graphical user interface (GUI) design tool.

Design of the fusion algorithm

In order to merge two images, first the spatial alignment of the images is performed and after the images have been located the fusion of the common region between them is performed. Based on the above idea we developed multiple image fusion algorithm.

First, the implementation of separate image registration algorithms: HARRIS corners detector (Harris, 1988) used to detect points of interest in images, extraction of descriptors of points of interest (Fiot, 2008), matching the points of interest of the images and the algorithm of RANSAC (Capel, 2001) that serves to estimate the geometric transformation matrix between two images. We also implemented three image fusion algorithms: average of images, PCA and Wavelet to select the best of them by measuring the fusing factor on a set of test images. It is important to analyze the need to process the image in gray scale to improve its contrast and highlight its details before passing them through the Harris algorithm in order to obtain better detection results of points of interest.

After observing and understanding each of the image registration and merge algorithms, each of these stages were combined into a single general multi-image fusion algorithm, consisting of the iterative application of simple algorithms (with 2 images) of image registration in gray scale and image merge in color. The proposed algorithm for an input image sequence is:

Let A and B be two juxtaposed input images, G the resulting merged image, $T_{BA}$and $T_{AG}$the transformation matrices that map the points of the image B in the domain of the image A and the points of the image A in the domain of the image G (Lay, 1999). That is:

$A: X_A \in \Omega_A$, $B: X_B \in \Omega_B$, $G: X_B \in \Omega_B$
$T_{BA}: X_B \rightarrow X_A, T(X_B) = X_A$
$T_{AG}: X_A \rightarrow X_G T(X_A) = X_G$

We have the following notation:
A_{gray} : gray scale image of A
$A_{x,y}$: point if interest of A_{gray}
$A_{descriptors}$: descriptors of $A_{x,y}$

Initial conditions:
- A is equal as the first image of the list, also it is extracted A_{gray}, $A_{x,y}$ and $A_{descriptors}$.
- $G = A$
- According to the previous condition A and G are overlapping and consequently

$$T_{AG} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

Algorithm:
For k = {2,3, ...,n_{image}}
- Make $B = k_{th}$the input image and extract $B_{x,y}$ and $B_{descriptors}$ to register it with A.
- Perform the matching between $B_{descriptors}$and $A_{descriptors}$ and get the matching$B_{x,y}$ and $A_{x,y}$.
- Apply RANSAC with the $B_{x,y}$ and $A_{x,y}$ matching points to obtainT_{BA}.
- Make $B = A$, along with its points of interest and its descriptors, ie. $A = B, A_{x,y} = B_{x,y}$, $A_{descriptors} = B_{descriptors}$
- Calculate the new$T_{AG} = T_{AG} * T_{BA}$.
- Apply fusion algorithm for the common region in images A and G.

End For.

Figure-1 shows a diagram of each of the algorithm variables.

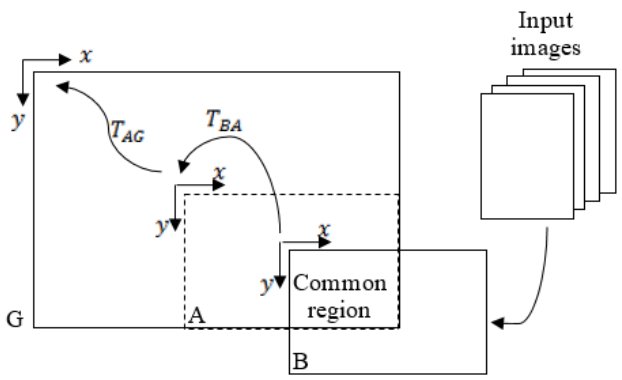


Figure-1. Diagram of the algorithm variables.

Segmentation and classification algorithms design

With the merged image, the next step is the image segmentation in common regions, and the characterization of these regions into parameters that identify them in terms of similarities and differences with the other regions. These regions represent the great variety of blood cells present in the myelograms showing different stages of maturation; For this reason, four groups or patterns to be identified in these samples were selected that allow to be the basis for the learning in the cell recognition (patterns). The types of cells that are we want to find and classify in the samples are shown in Figure-2.

The following are the steps to perform the segmentation algorithm:

- Obtain representative samples of the color to be segmented. Having an input image f is obtained a mask or binary image of the same size in which 0s represent the background and 1s the interactively selected region.
- Rearrange the color pixels in g as I rows, and find the indexes of the rows of the color pixels that are not black. These are the non-background pixels in the mask image.
- Determine the threshold T value.
- Calculate the Euclidean distance according to the parameters obtained, taking into account that the pixels that have a distance less than or equal to the threshold are the points contained within the sphere of radius T and centered according to the coordinates of the region of interest.

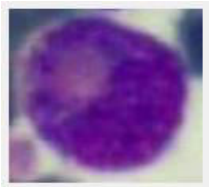
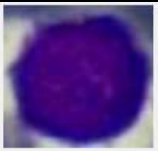
Cells	Sample 1	Sample 2
Myeloid Precursors		
Small T lymphocytes		
Mature lymphocytes		
Nuclear Polymorphs		

Figure-2. Pattern samples.

To define the classification of the different cell types, the following algorithm was developed:

Obtain the value of Moment M2, which is a statistical measure obtained from the histogram of each of

the regions obtained, to make a first division of the cells having:

- For I_{in}, If $M_2 < C_1$, the region is part of the Small Lymphocyte or Nuclear Polymorph (I_1) group.
- For I_{in}, If $M_2 > C_1$, the region is part of the Mature Lymphocytes or Myeloid Precursors (I_2)group.

From the analysis of areas, we have:

- For I_1, If$A < C_2$, Region→ Small Lymphocyte.
- For I_1, If $C_2 < A < C_3$, the region cannot yet be classified (I_3).
- For I_1, If $A > C_3$, Region → Nuclear Polymorph.
- For I_2, If $A < C_4$, Region → Mature Lymphocyte.
- For I_2, If$C_4 < A < C_5$, the region cannot yet be classified (I_4).
- For I_2, If $A > C_5$, Region → Precursor Myeloid.

To perform the last division that allows to characterize the four families in a definitive way, the PCA analysis is performed, for which it is necessary to create an array of image vectors that serves as a basis for comparison of the input images. To create this matrix, selections are taken from the base images for each of the families that have the best characteristics and describe it in the best way. From there the histogram of each of these regions is obtained and the matrix is formed, each histogram being one of the vectors of the matrix.

From the results obtained from the PCA analysis we have that the region is classified in the group whose value is the minimum difference with respect to the reference axes of each pattern, therefore:

- If PCA I_3 →Small Lymphocyte
  → Nuclear Polymorph.
- If PCA I_4 →Mature Lymphocyte
  → Myeloid precursor.

C_1, C_2, C_3, C_4, and C_5 refer to values selected according to the data of the descriptors obtained that provides a division of the patterns into families and subfamilies.

Implementation of the graphical user interface

After the implementation of the respective fusion, segmentation and classification algorithms, a graphical user interface (GUI) was developed that allowed the practical and efficient use of the programs performed. For this, the Matlab GUIDE tool was used, which is a development environment that facilitates the realization of graphical user interfaces.

Within this interface, in addition to the programs mentioned above, other necessary tools such as image editing were added, which allowed the elimination of edges of an image, visualization of algorithms that allowed to see the behavior of the registration algorithms and finally the tool of report generation that allows to generate a file with the final results of cells segmentation and classification present in the myelograms.

RESULTS

Designed application

The image merge window (Figure-3) allows a juxtaposed image sequence to generate a fused image so that it can be subsequently analyzed, and has the option to change the parameters of the Harris and RANSAC algorithms and observe the behavior of each one separately, to be able to improve their behavior according to the type of images we are going to use. Finally, there is an input image editing tool to eliminate dark edges that are on the edges of the image that affect the result of images merging.

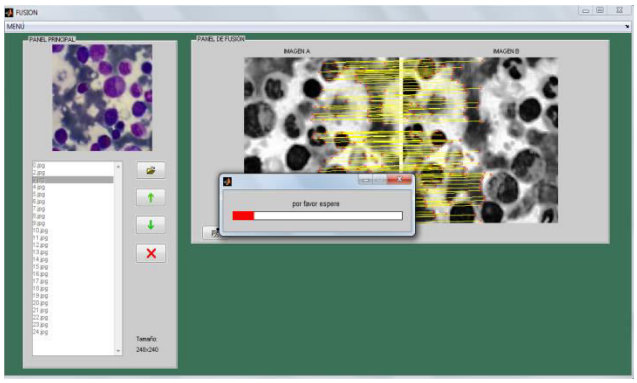


Figure-3. Fusion window.

The segmentation and classification window of Figure-4 allows the user to define Threshold and Seed / Level variables by selecting samples of the image to be processed. This segmented image is divided into regions representing the possible types of cells found there, which are characterized in terms of similarities and differences. When all regions have been characterized, the classification algorithm according to established rules is applied which will identify the types of cells that have been found in the sample. Once the classification is done, a report or record of the parameters and results of the classification is obtained.

Tests performed

A sequence of 11 images was taken with the help of the Motic B3 microscope from a myelogram plate, these images are observed in Figure-5, which were used to test the effectiveness of the multiple image fusion algorithm from which the fused image was obtained as shown in Figure-6.

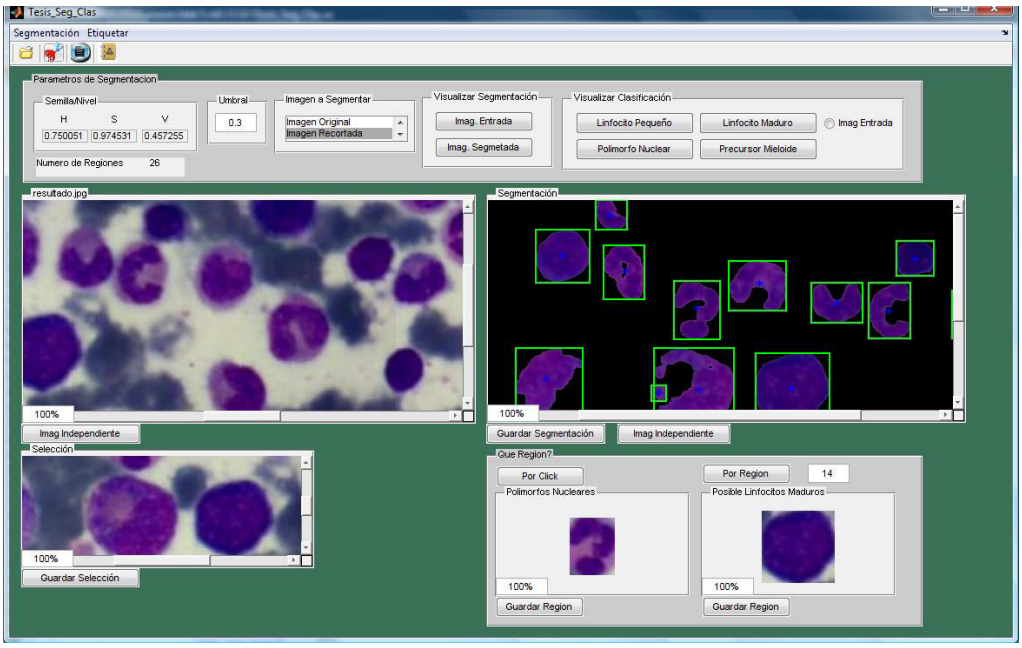


Figure-4. Segmentation and classification window.

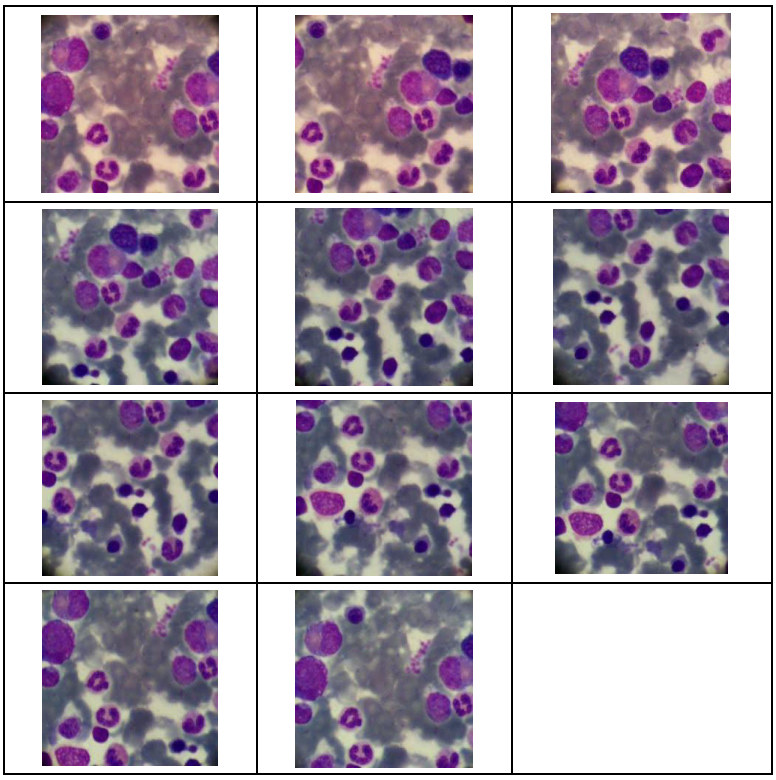


Figure-5. Image sequence.

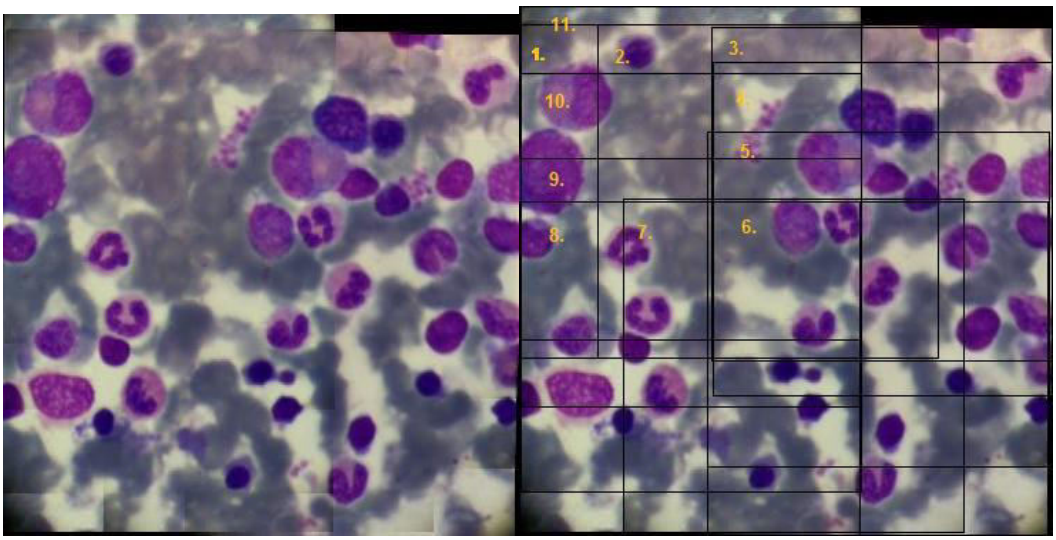


Figure-6. Fused image.

In the classification stage we aim to identify the four cell types according to their maturation state that are present in these sample classes. Taking a partial sample of a merged image, the segmentation and classification process is performed. See Figures 7 to 10.

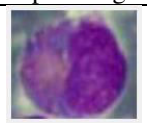
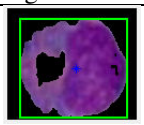
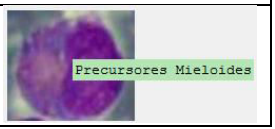
H	S	V	Threshold
0.78831	0.67475	0.54666	0.3
Input image	Segmentation		Classification
			

Figure-7. Segmentation and Classification Parameters - Myeloid Precursors.

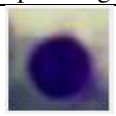
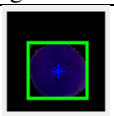
H	S	V	Threshold
0.70862	0.99640	0.32941	0.3
Input image	Segmentation		Classification
			

Figure-8. Segmentation and classification parameters - Small T lymphocytes

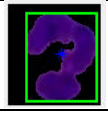
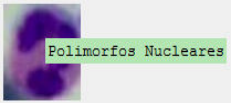
H	S	V	Threshold
0.75023	0.98736	0.45882	0.3
Input image	Segmentation		Classification
			

Figure-9. Segmentation and classification parameters - Nuclear Polymorphs

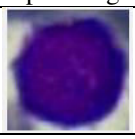
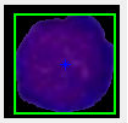
H	S	V	Threshold
0.75097	0.98557	0.46117	0.3
Input image	Segmentation		Classification
			

Figure-10. Segmentation and Classification Parameters - Mature Lymphocytes.

CONCLUSIONS

It was observed that the image fusion algorithm with PCA is very effective when it comes to fusing images that have been taken under similar lighting conditions and capture sensors.

When performing the image registration, the sequence must be passed through areas that present an optimum amount of detail (edges and corners), for this reason it was verified that the registration algorithm behaves very well for other types of images such as aerial shots of cities or places because of its myriad details.

The combination of different descriptors such as the area, texture and histogram of an image makes it possible to create rules that allow an adequate classification of the patterns that are sought to recognize.

To classify the different types of cells found in myelograms it is necessary to segment the image by taking the samples individually according to each family to obtain the proper descriptors of each of them and are correctly classified.

RECOMMENDATIONS

Have a computer for the development of the application that has a higher processing speed so that the results can be reached in real time.

The images taken for the fusion must have a juxtaposition of details of not less than 50% and must be shots in which the edges do not have dark areas.

For the classification of the regions, a more robust network could be implemented to create an apprenticeship of the characteristics of each one of the patterns and that allow adapting to the cases in which the acquired knowledge is the necessary one for the classification.

Due to the complexity of the implemented algorithms the execution time of them is high; therefore, it is recommended to program with a lower level language like C++ to improve the execution time.

The algorithms that are part of the registry such as Harris and RANSAC have configuration parameters that change according to the type of images to be used, so when changing the type of image, it is recommended to review these algorithms with the observation tool algorithms to ensure they work properly.

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