A Computer Vision Approach to Micro-Nucleus automatic detection and surface measurements

M. Ceccarelli[1,2], D. Grimaldi[3], F. Lamonaca[3], A. Speranza[2,4]
[1]*University of Sannio, Benevento, Italy*
[2]*BIOGEM scarl, Italy*
[3]*University of Calabria, Italy*
[4]*IBB-CNR, Italy*

Abstract-This paper reports a novel method for Nucleus and Micro Nucleuses segmentation. These biological structures are very handy to biologists for relieving structural chromosome aberration. The adopted method consists into a pipeline of advanced computer vision algorithms same of them specifically tailored for the current segmentation problem. Starting from the knowledge of approximate size and shape of Micro Nucleuses it is possible to efficiently segment all the image features by computer vision approach.

I. Introduction

The detection and measurement of Micro Nucleuses (MNs) of human lymphocytes allows to identify structural chromosome aberration. Automated image analysis applied to acquired images of human lymphocytes by flow cytometer can be a reliable tool in order to identify and measure MNs. Pattern matching analysis has been extensively used for detecting MNs, though this procedure may fail due to image alteration which are provoked from incorrect flow cytometer image acquisition. In order to overcome this problematic occurrence a number of image pre-processing algorithms have been suggested in literature in order to improve MNs detection with several degree of success; e.g. punctual, local and global fuzzy procedures [1-2], noise-filter procedure for some well-defined conditions [3-4] and multiple alterations algorithm correction [5-6]. The approach reported here is based on some of the ideas reported in [6] with the aim of integrating into the segmentation procedure both shape and spectral information about the structrures to be detected. This has led to a novel pipeline using region-based segmentation, in particular seeded region growing, where the seed of the regions are computed according to the a voting based scheme, namely the Orientation Macthing (OR) Transform, which has been shown to be effective in other biological imaging fields.

The whole approach consists in firstly mitigate image Gaussian noise and consequently identify the Micro Nucleuses with an orientation matching transform [10] which uses colour as well as shape information; the globular shape and size of nucleus as well as MNs can have a wide range of variation. Orientation matching transform supplies hypothesis of the centres of MN. This information is used as seed for the seeded region growing algorithm which combining spatial information to intensity distribution efficiently segments nucleus and MNs.

II. The proposed approach

In this work an alternative method of MN's segmentation is proposed. The methods is based on a pipeline which provides filtering by non linear diffusion, orientation matching methods, parameter selection and seeded region growing algorithm. Acquired image by flow cytometer may be affected by random alterations due to accidental movements either of lymphocytes or of the acquisition system. Hence, the proposed pipeline aims to mitigate image alteration, to detect MNs and finally to segment and measure it.

A. Non Linear Diffusion

The basic principle of non-linear diffusion filter reported in [7] belongs to the class of iterative geometric scale-space filters first introduced by Perona and Malik [8]. The filter is modelled by a partial differential equation modelling of diffusion process through an inhomogeneous matter. The image gradient, measuring the local homogeneity of the data, is assumed as diffusion coefficient. The approach has been extensively studied in recent years [9] as it can smooth out noise while preserving important features such edges.

B. The Orientation Matching Transform

Then, to the non linear filtered image an orientation matching methods is applied. This method aims to recognize circular object on the basis of both magnitude and direction of the image gradient. It is an extension of the Hough Transform for circles and it has several advantages: it is a correlation-based transform; it does not require a prior edge-detection phase; and it can be applied to circles with a wide range of radii. Finally, it can be tailored to recognize light spots on a dark background and *vice versa* or both. The algorithm returns the centre and the radius of the detected circular object [10-11]. However, MNs cannot be assimilated to circles. Therefore, further processing effort is required in order to segment and measure the MN's surfaces.

C. The Segmentation Step

The segmentation is performed by seeded region growing algorithm (SRG) which inputs are the image intensity and a set of seeds. In this work the seeds, which are individual pixel, are provided by the Cartesian coordinate of the MN's centre, which are determined by the orientation matching methods. The SRG works as following: the region to be segmented is iteratively grown by comparing all unallocated neighbouring pixels to the region. The difference between a pixel's intensity value and the region's mean is used as a measure of similarity. The pixel with the smallest difference measured this way is allocated to the respective region. The process stops when the intensity difference between region mean and new pixel become larger than a certain assigned threshold [12-13]. This is given by analyzing the intensity histogram of the image to be segmented. In Figure 1 is reported a block scheme to resume the procedures above described.

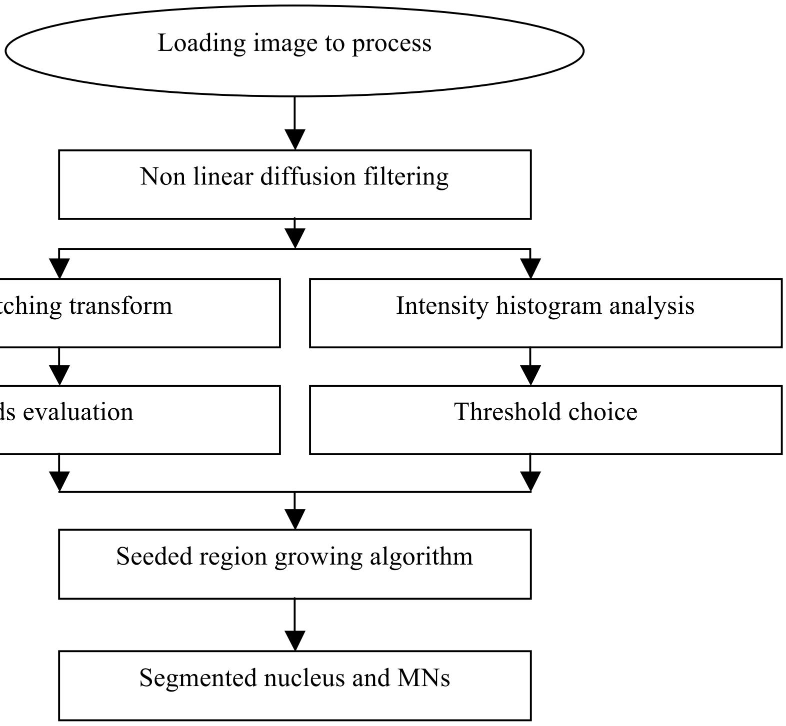

Figure 1. block scheme resuming the pipeline used to segment nucleus and MNs.

III. Preliminary Results

A non linear filtering algorithm is applied to flow cytometer image in order to mitigate Gaussian noise, see Figure 2 a-b. Non linear filtering preserves image futures: nucleus and MNs.

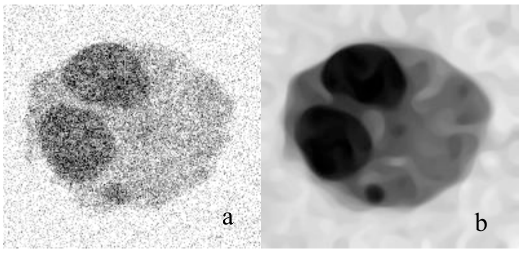

Figure 2. a: flow cytometer image; b: image after non linear filtering

To segment nucleus and MNs by (SRG) the algorithm seeded region growing [12] two parameter needs to be determined: threshold and seeds. Intensity histogram of the non-linear filtered image, Figure 2b, is evaluated aiming to define intensity modal distribution. This information provides the range of intensity within which each either nucleus or MN is confined. The intensity histogram can be opportunely assimilated to a multi Gaussian distribution. Hence, by an Expectation Maximization (EM) algorithm can be estimated the maximum likelihood of the parameters (modes m and sigma s) of a Gaussian mixture model with a certain numbers of components. The algorithm, choosing random starting points, evaluate the maximum likelihood for a number of Gaussian mixture with 1, 2, 3, 4, etc. components. The parameters of the Gaussian mixture, with a certain number of components, having the maximum likelihood are adopted. With respect to above mentioned Figure, the intensity histogram has a three-modal distribution with mode values of 0.045, 0.33 and 0.81 respectively, see Figure 3.

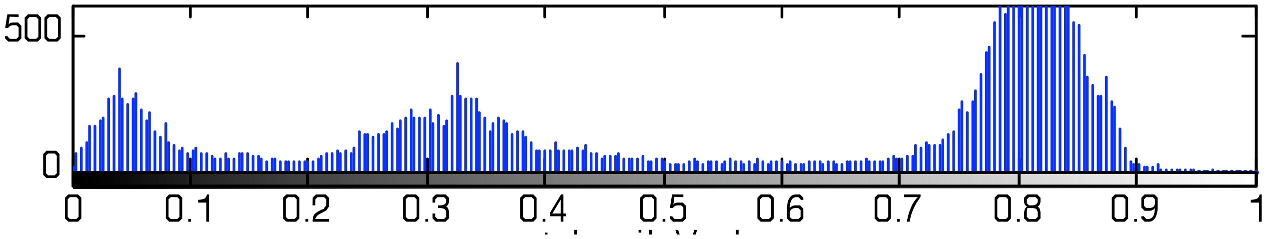

Figure 3. intensity histogram of Fig. 2b

Starting from each mode and determined sigma, it is possible to select an intensity range (± 3s), a certain threshold for the SRG algorithm, inside which all the pixel's intensity of interest (99.73%) are contained. Therefore, choosing a range of ±0.08, ±0.30 and ±0.19 for the mode of 0.045, 0.33 and 0.81 respectively, it is possible to segment all image components providing the corresponding seeds. The outcome is depicted in Figure 4.

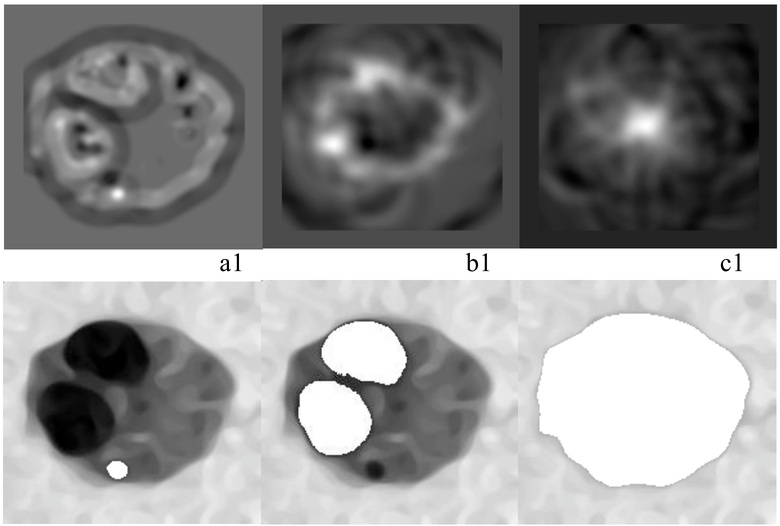
a1 b1 c1
a2 b2 c2

Figure 4. a1, b1, c1: filtered image after orientation matching transform with circle range 5-10, 25-45 and 65-85 respectively. a2: MN segmented image with threshold 0.08 and seed as determined in image a1. b2: MNs segmented image with threshold 0.08 and seeds as determined in image b2. c2: Nucleus segmentation with threshold 0.30 and seed as in c1.

Seeds are provided by applying to non linear filtered image the orientation matching transform. This procedure reveals the centre of each circular object as a spot of higher intensity with respect to the image background. Figure 4 (a1), the centre of the smallest MN is identified. Appling a threshold of 0.08 and giving as seed the Cartesian coordinate of the spot to the SRG algorithm the MN can be segmented: Figure 4 (a2). Similarly, providing as seeds the two spots identified in Figure 4 (b1) by orientation matching transform and applying the same threshold i.e. 0.08, the left MNs are segmented: Figure 4 (b2). Finally, in order to segment the entire nucleus a new seed as well as a new threshold i.e. 0.30 needs to be applied to SRG; the picked-up seed is the nucleus centre as depicted in Figure 4 (c1). The outcome segmentation is shown in Figure 4 (c2).

IV.Conclusion

So far the proposed pipeline has been successful ad reliable in segmenting image features such as nucleus as well as MNs, however, more work needs to be done on the front of automatic threshold selection of SRG algorithm. The threshold selection is done on the base of intensity histogram modality which can differ from image to another. Therefore, future works will aim to include in the proposed pipeline and automatic intensity histogram modality detection and on this base to select appropriate threshold level for SRG algorithm segmentation.

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