MIZUTAMA

Mizutama is a Delphi 2.0 application designed to count cells in ordinary microscopic photographs (maximum 3,600 pixels allowed, in JPG or JPEG format) of blood smears. It uses the thresholding method to transform original photographs into grayscale trinary images, where pixels are categorized as positive, gray, or null, according to a specified threshold criterion. Mizutama searches photographs for spots (typically the nucleus of the cell) that may be surrounded by a lighter halo (the cytoplasm). Mizutama identifies these elements according to the parameters specified and counts their number. The search and identification is based on five parameters that must be specified in the program:

1. Contrast (button "Contraste"): this determines how dark a pixel must be in order to be considered "positive". It could have any positive value (but ideally should be between 0.3 and 1.5), allowing the software to be used with photographs varying in the degree of staining or illumination. Moreover, if different cell types vary in saturation, the Contrast value can be adjusted to facilitate their discrimination.

2. Gray Threshold (button "Umb. Gris"): this determines how dark a pixel must be in order to be considered "gray". Values range between 0 (completely white) and 1 (as dark as positive pixels). With these two parameters, Mizutama classifies the pixels according to their light intensity (I). For I < Contrast, the pixel is considered "positive"; for Contrast < I < Gray Threshold, the pixel is considered "gray". For I > Gray Threshold, the pixel is considered null.

3. Diameter (button "Diámetro"): this sets the size of the circle equivalent to the cell (i.e. the size of the cell in the photograph). This should be a positive value based on the size of the cell to be counted and the microscope magnification used. With this parameter, the software scans the image searching for positive pixels, and when a positive pixel is found, a circle of the size previously set is superimposed over the pixel. The next two parameters determine whether or not the spot found can be considered the cell type being sought.

4. Fraction (button "Fracción"): this indicates the proportion of positive pixels that must be found inside the circle in order to be considered a nucleus (i.e. the relative size of the nucleus). The value 1 indicates that half of the pixels inside a circle correspond to positive values (i.e. the nucleus surface area is half the cell surface area).

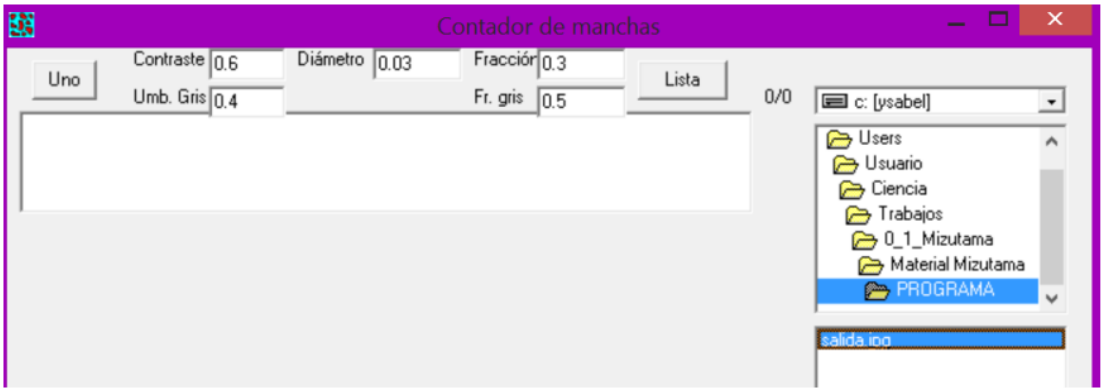
5. Gray Fraction (button "Fr. Gris"): This indicates the minimal proportion of gray pixels that must be found inside the specified diameter in order to be considered a halo, and therefore to count the spot as a cell (i.e. the relative size of the cytoplasm).

With these latter two parameters, we determine the relative size of the nucleus and cytoplasm of the cell. Therefore, the software can distinguish cells that differ in size or in the relation of nucleus surface area vs. cytoplasm surface area.

When the software is run, we can select only one photograph (in the button "Uno") or analyze a set of photographs stored in a folder (by using the button "Lista"). In both cases, an explorer window enables us to select the file or folder we wish to analyze. Once the file to analyze is selected, the software identifies a cell according to the

procedure described above. Next, it shows a grayscale image of the photograph that classifies the pixels (black, gray, or white), highlighting the identified spots. In red, the software surrounds the spots that match the parameters that were set, identifying the cells being searched for. The software also uses green circles to indicate any spots that fulfill the parameters for the nucleus (Fraction), but not for the cytoplasm (Gray Fraction); these spots may represent a cell type we do not wish to count (e.g. isolated thrombocytes). Yellow circles highlight spots that do not fulfill all the parameters necessary to be considered a cell (e.g. staining spots or ruptured cells), leaving the evaluation to visual inspection.

Then, Mizutama counts the number of red circles in the image and gives the values in the upper bar of the window or, when in batch mode, in a txt file. For each photograph, the output includes the values set for Contrast, Diameter and Fraction (first three columns from the left), the number of positive pixels in the photograph (fourth column), the number of correct cells counted (fifth column), and information on the photograph, its size in pixels (sixth and seventh columns) and its title (last column).



Screenshot showing the console of Mizutama. See the boxes where the parameters should be introduced (Contrast, Gray Threshold, Diameter, Fraction, and Gray Fraction). See also the button “Uno” for treating photographs one to one, and the button “Lista” for treating simultaneously the photographs in a folder selected in the explorer (in the box on the right).

CAUTION:

The results are recorded in a file with the name of the folder and the .txt extension next to the executable. The file is overwritten without asking, so you have to be careful if there are folders with the same name.

Do not use zones on the edges of the sample as well as areas that due to problems of staining or fixation that did not allow the correct identification of cell types. Use monolayer zones.

Photographs should not exceed 3600 pixels.

Sometimes it can give problems if the size of the photographs exceeds 1Mb.

The file "salida.jpg" that is generated when analyzing photos one to one is overwritten.