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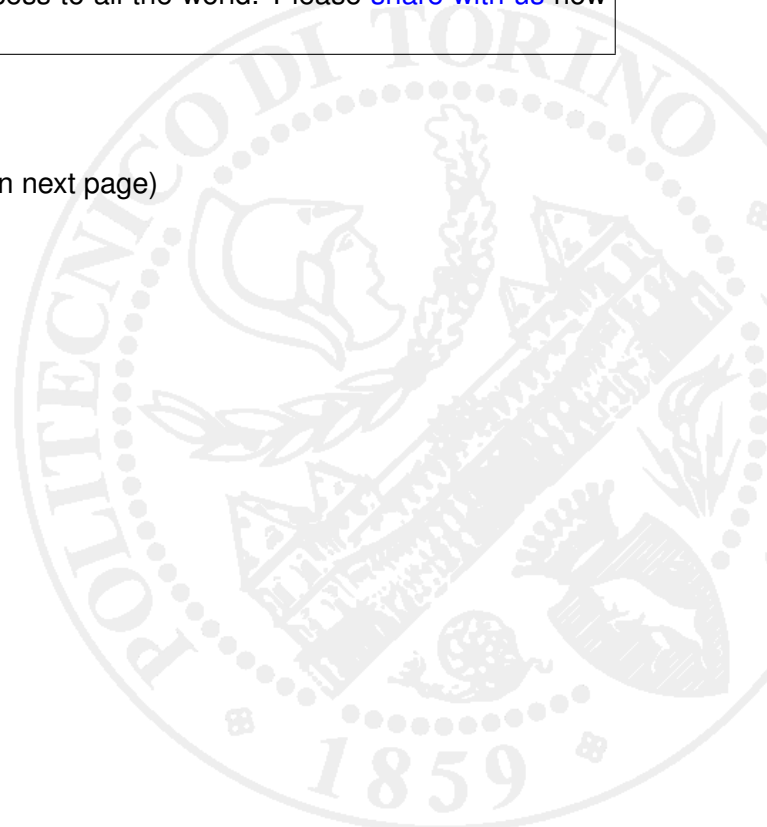
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Measuring the blood cells by means of an image segmentation

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Abstract

The analysis of the blood cells is used for evidencing several diseases in humans and animals. Usually, the analysis is performed manually by means of an optical microscope. However, some approaches, based on an automatic image processing, exist for evidencing and counting the blood cells in the given specimens. Measurements of the cell morphology are possible too. Here we show a method which uses a processing based on an image segmentation obtained by thresholding. After the segmentation, the area of the cells is easily evaluated. Their perimeters are measured using a rose of directions.

Keywords: Image processing, Image segmentation, Counting particles, Cell morphology, Medical Imaging.

Several works had and have as their subject the study of automatic methods for the analysis and counting of the cells in the blood. Some references are given in [1-20]. The reason for the large interest on this subject is quite clear: in medical field, for humans and animals, the analysis of the blood cells is used as an indicator for evidencing several diseases. Usually, the analysis is performed manually by means of an optical microscope. As we can easily see from the titles of the given references, several automatic methods exist for extract information from the specimens, methods which are not only based on microscopic images. However, the large part of the works are concerning automatic approaches based on the image processing.

Besides the counting of particles, from the micrographs that we can obtain by microscopes, some measurements concerning the shape of on cells are possible. Here we discuss an approach based on an image segmentation. In fact, the measurement of the size and size distribution of blood cells is the proper task for the method that we have recently developed and applied [21-28]. To the counting of particles we can add some measurements concerning the cell morphology, measurements that we can obtain by means of a rose of directions. One of the measures is the perimeter of the cell, but it is possible to give several other parameters.

In image processing, a segmentation is a method of partitioning an image into multiple sets of pixels, defined as super-pixels, in order to have a representation which can be simpler than the original one or more useful to the following desired analyses [29]. Specifically, the segmentation is a process of assigning a label to every pixel in an image, such that the pixels having the same label share certain characteristics [29]. As a consequence, the result of a segmentation is a set of "segments", or "super-pixels", or "domains", that are covering the background of the whole image. The segmentation can also give a set of contours, that is of "edges", extracted from the image. In this case, the segmentation gives an "edge detection".

Several methods exist for the image segmentation, as we can appreciate from [29]. Here we use a method based on a thresholding of grey-scale images [21]. By selecting a suitable threshold, the image is converted into a binary (black and white) image. In several cases, this is enough for evidencing the domains among the black or the white pixels. Details of the method of segmentation are given in [21]. Therefore, here we start immediately to show the results of the segmentation of an image of blood cells. We show that we can measure their apparent areas and perimeters.

Chicken blood cells Let us consider the image of chicken red blood cells magnified 1000 times, courtesy John Alan Elson, which is available at <https://commons.wikimedia.org/wiki/File:Chickenrbc1000x.jpg> and here reproduced in the Figure 1. The colour image is converted into a grey-scale image by means of GIMP software. GIMP is also giving us the histogram corresponding to the grey tones. As we can see from the Figure 2, the

histogram has two large peaks. The grey-scale image is converted into a binary (black and white) image, selecting the threshold between these two peaks. The result of thresholding is shown in the same Figure 2.

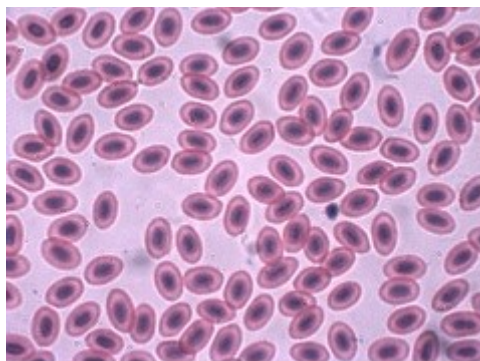


Figure 1: Chicken red blood cells magnified 1000 times, courtesy John Alan Elson.

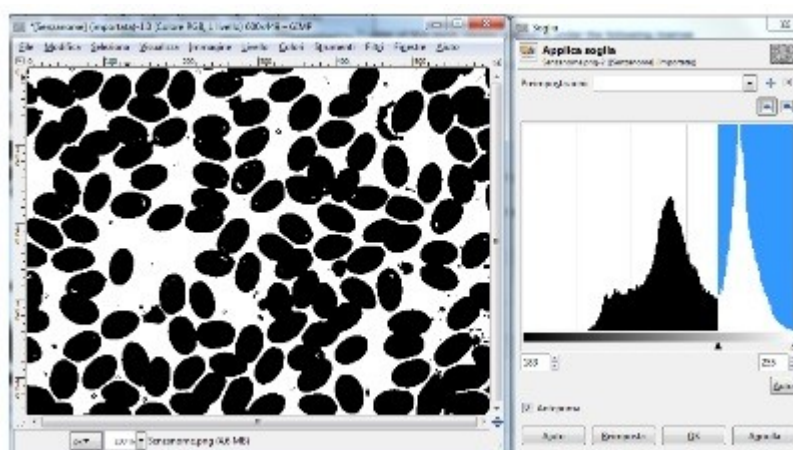


Figure 2: Thresholding of the image in the Figure 1, obtained by selecting the threshold between the two large peaks in the histogram of the corresponding grey-tones.

From the Figure 2, we can see that several cells are attached each others. Therefore, we need to separate them. We can obtain the separation using ImageJ software and its Watershed Separation tool which works on binary images. The result is given in the left panel of the Figure 3. On the right, we can see the segmentation, as obtained by means of the approach proposed in [21], for the analysis of the cellular solids.

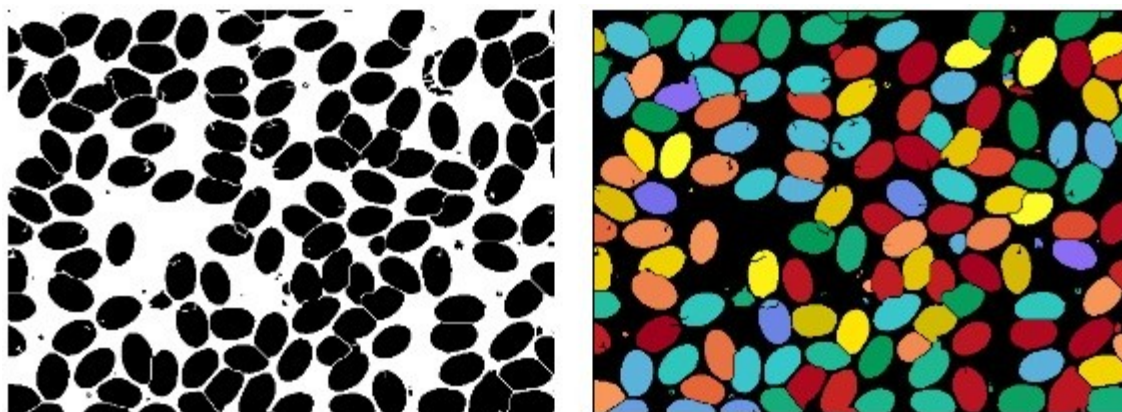


Figure 3: On the left, the binary image after the separation of the cells. On the right, the segmentation obtained as explained in [21].

In the Figure 3, we can see that each of the cells has a different colour. It means that each cell, that is, each

super-pixel in the image is labelled by a different label. Of course, we can determine the area of each cell, simply counting the pixels having the same label. Once we have obtained the areas of all the cells, we can give the size distribution of the sample, as we did in the previous articles [21-28]. The size-distribution is proposed in the Figure 4, where we see the number of super-pixels having areas (in pixels), included in intervals of 200 pixels.

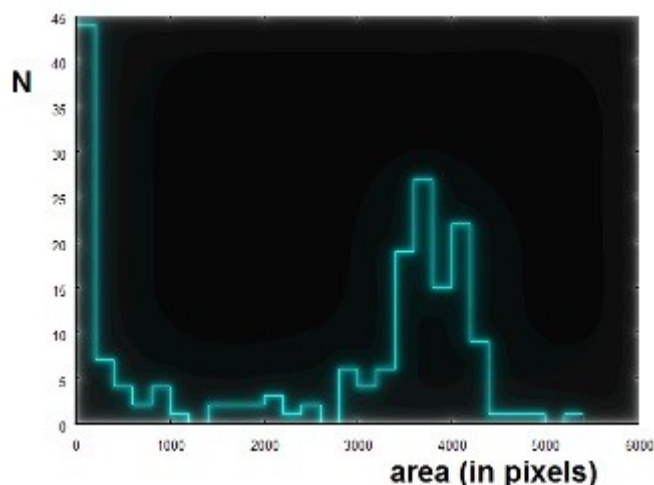


Figure 4: Distribution of the areas of the cells.

After, we can determine the position (in pixels) of the “centre of mass” of each cell. From this centre, we can determine the distance to the cell contour. Here we use a star of 16 directions - in the same approach proposed in [30-32] - to determine 16 distances of the cell edge from the centre of mass. Using the distances given according to this rose of directions, the perimeter of the cell can be easily calculated applying the Carnot theorem. In the Figure 5, we see the upper/left corner of the segmented image, with the black dots which are showing the positions of the centres of mass. On the right, we see the corresponding output of a file giving for each cell the position of the centre of mass, the area (in pixels) of the cell and the 16 distances (in pixels) from the centre of mass to the edge of the cell. The insert in the figure is showing in detail one of the three domains and the rose of directions.

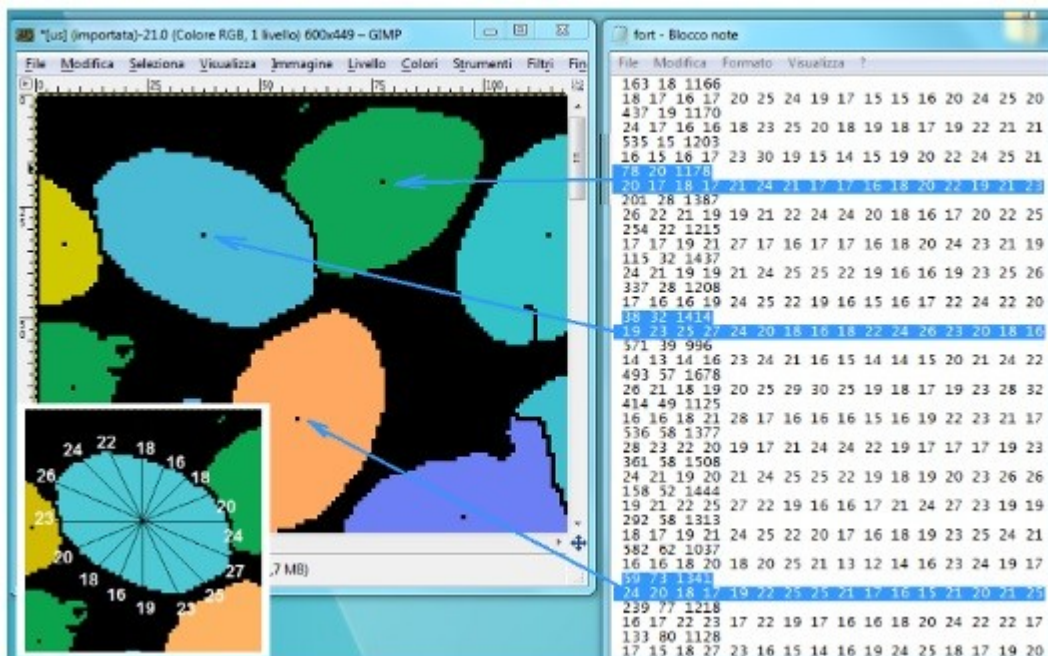


Figure 5.

As previously told, once we have the rose of the 16 distances, it is possible to calculate the perimeter of the cell. It is enough to use the Carnot theorem for each of the triangles having two distances as sides and the angle of $22\frac{1}{2}$ degrees between them. For instance, for the cell with centre (78,20) and area 1178, the perimeter is of 118 pixels. For the cell at (38,32), it is of 132 pixels and for the cell at (59,73), of 125 pixels. Of course, several other parameters can be calculated from the measures obtained by means of the rose of directions.

Human blood

Let us consider the image of human blood cells, courtesy Bobjgalindo, available at https://commons.wikimedia.org/wiki/File:Monocyte_40x.JPG and here reproduced in the Figure 6 (left panel). Again, the colour image is converted into a grey-scale image and then the binary image obtained manually by means of GIMP (Figure 6, central panel). As we can see, the white cell is not well defined in the binary image. For this reason, in this preliminary work, we modified manually the image to be used for segmentation. The modified image is given in the right panel of the Figure 6 (we have also applied the ImageJ Watershed tool to separate the cells).

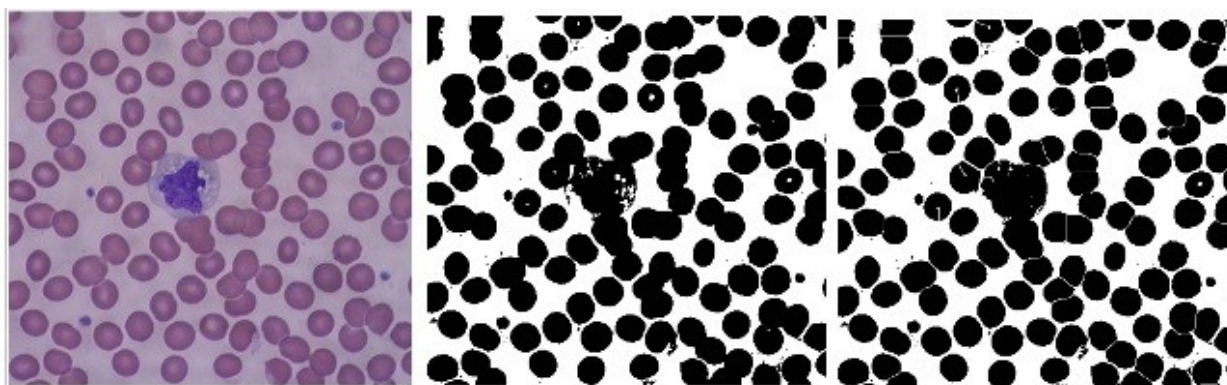


Figure 6: Human blood cells magnified 40 times, courtesy Bobjgalindo.

In the Figure 7, the result of the segmentation is proposed.

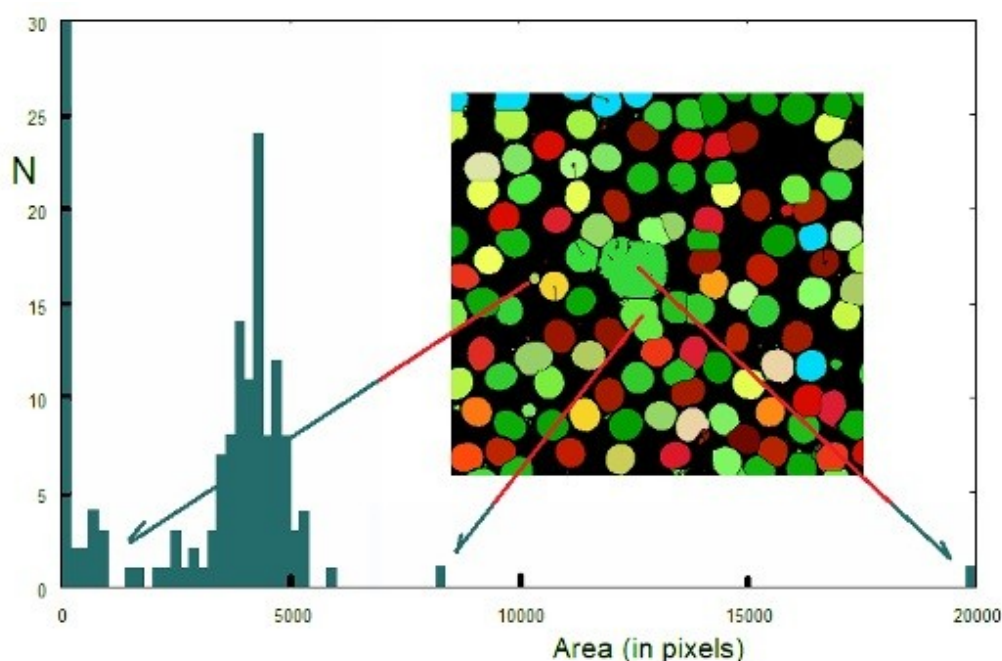


Figure 7. Segmentation and size distribution. Note that the white cell has a quite different size. In a case, ImageJ Watershed was not able to separate the cells.

Of course we can measure the size and the perimeter of the cells too. In this preliminary work, in the case of the human blood, we needed to act manually to adjust the shape of the white cell. Further work is necessary to determine an automatic manner of improving the visibility of the white cells. A possible approach could be based on the use of the image entropy, in particular of the generalized entropies of Kaniadakis and Tsallis, and the related bi-level and three-level segmentations [33-35]. These entropies contain an entropic index, which can be used to enhance the different cell morphologies. An example of thresholding using generalized entropies is given in the Figure 8. As in the Ref.35, the best entropic index is chosen in order to have the largest number of edge pixels, that is, of pixels defining the edges of the cells. The result is quite interesting, because it is evidencing the white cells.

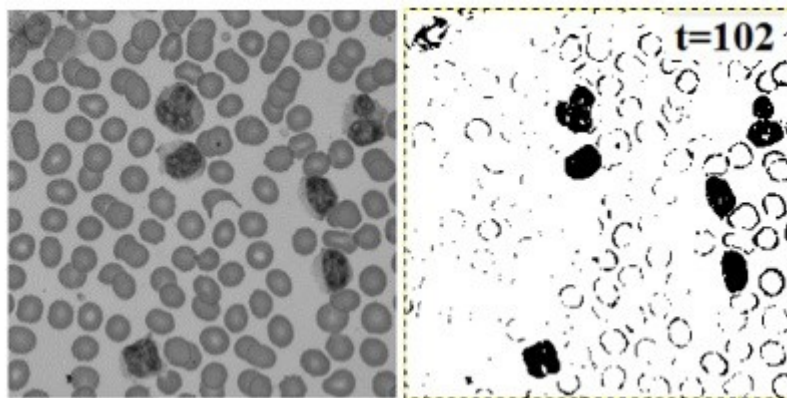


Figure 8: Microscopic image of blood cells (courtesy Wikipedia) on the left. If we assume the “best” bi-level image being that giving the largest number of edge pixels, we have the image on the right (threshold is at the value 102). The result is obtained by using Kaniadakis or Tsallis entropies, and is quite different from that obtained by using the Shannon entropy.

By the segmentation of the Figure 8, right panel, we can measure the morphology of the white cells. Let us conclude showing another interesting result, that we obtained in [34], using a three-level image made of white, black and grey pixels. The results is reproduced in the Figure 9.

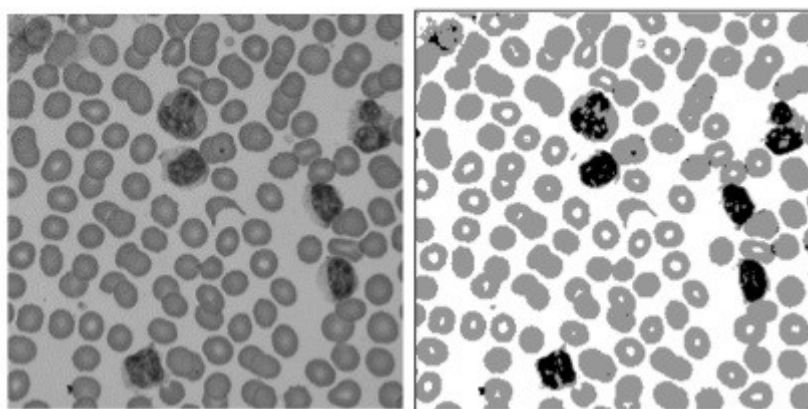


Figure 9: Original (on the left) and three-level (on the right) images. The three-level image is obtained as described in [34].

Using only the black pixels, we have a map as given in the Figure 10 on the left. Using only the grey pixels, we have the map, on the right, in the same figure. On these two maps, the approach for having the cell morphology can be applied, after an ImageJ Watershed separation. A problem still exists for the red cells and it consists in the fact that some domains can have an empty core (see Figures 9 and 10). A method of processing the shape of the cells having an empty core is under development.

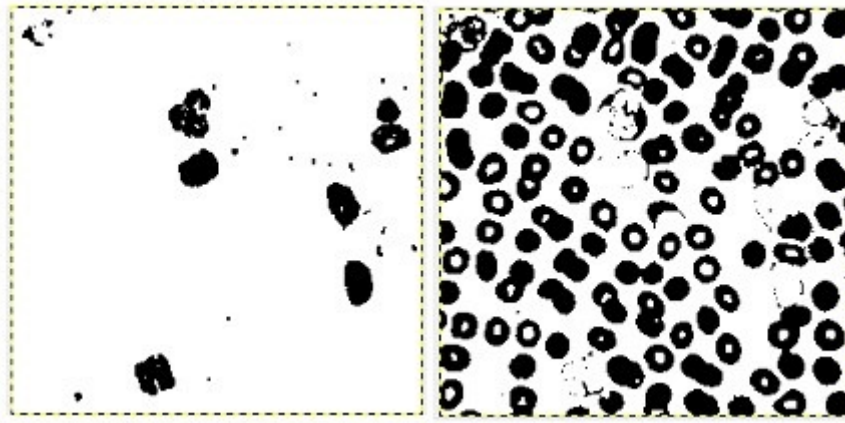


Figure 10: These two maps have been obtained from the three-level image in the Figure 9. On the left, we see the black pixels and, on the right, the grey pixels. To these two maps we can apply the approach previously discussed for the determination of the cell morphology.

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