Automated detection in microscopic images using segmentation

Detecção automatizada em imagens microscópicas usando segmentação

Detección automatizada en imágenes microscópicas mediante segmentación

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ABSTRACT
In this paper, we present a segmentation clustering-based approach for automated object detection. This paper deals with the segmentation and classification of blood cells for the purpose of detecting leukemia (abnormal blood cells). After the image acquisition and the preprocessing step, we proceeded to the application of the k-means method. In order to show the interest of the proposed approach, we present the different cancerous regions identified with their characteristics for biomedical diagnostic aid. The proposed method is tested on image dataset and achieves 98% segmentation accuracy. These results show that our approach offers encouraging performance and best automatic leukemia detection. The proposed system is successfully implemented in Matlab, experimental results demonstrate that our approach offers encouraging performance and better quality automatic leukemia detection.

Keywords: segmentation, clustering, classification, image, automatic.
RESUMO
Neste artigo, apresentamos uma abordagem baseada em clustering de segmentação para detecção automatizada de objetos. Este artigo trata da segmentação e classificação de células sanguíneas com a finalidade de detectar leucemia (células sanguíneas anormais). Após a aquisição da imagem e a etapa de pré-processamento, procedeu-se à aplicação do método k-means. Para mostrar o interesse da abordagem proposta, apresentamos as diferentes regiões cancerígenas identificadas com suas características para auxílio ao diagnóstico biomédico. O método proposto é testado em conjunto de dados de imagens e atinge 98% de precisão de segmentação. Esses resultados mostram que nossa abordagem oferece desempenho encorajador e melhor detecção automática de leucemia. O sistema proposto é implementado com sucesso em Matlab, resultados experimentais demonstram que nossa abordagem oferece desempenho encorajador e detecção automática de leucemia de melhor qualidade.

Palavras-chave: segmentação, agrupamento, classificação, imagem, automático.

RESUMEN
En este artículo, presentamos un enfoque basado en la segmentación por clústeres para la detección automatizada de objetos. Este trabajo trata sobre la segmentación y clasificación de las células sanguíneas con el fin de detectar leucemia (células sanguíneas anormales). Después de la adquisición de la imagen y el paso de preprocesamiento, se procedió a la aplicación del método k-means. Con el fin de mostrar el interés del enfoque propuesto, se presentan las diferentes regiones cancerosas identificadas con sus características para la ayuda diagnóstica biomédica. El método propuesto se prueba en el conjunto de datos de imágenes y logra una precisión de segmentación del 98%. Estos resultados muestran que nuestro enfoque ofrece un rendimiento alentador y la mejor detección automática de la leucemia. El sistema propuesto se implementa con éxito en Matlab, los resultados experimentales demuestran que nuestro enfoque ofrece un rendimiento alentador y una detección automática de leucemia de mejor calidad.

Palabras clave: segmentación, clustering, clasificación, imagen, automático.

1 INTRODUCTION

Image processing is involved in a large number of applications. Medical applications are one of the fields in image processing that helps to develop surgery acts, to diagnose disease and therapeutic practice, and so on. The use of microscopic images shows more details challenging to see within the naked eyes and makes it easier to extract useful disease diagnosis information. The disease diagnosis, in some cases of images, is more difficult (information drowned in others, similarity, noise included in image,...). It makes the task difficult for experts. For example, in biomedical image annotation, experienced doctors with knowledge to analyze the images are needed. The annotation of each image consumes a large amount of time where the time factor is crucial in the speed disease diagnosis and treatment of the patient. The automatic annotation process can help reduce the human mistake, so fewer efforts and lower cost can be achieved. Processing image tools,
methodologies, and complex algorithms developed to increase the visibility of images and help to make more manageable all these tasks.

The White blood cells (WBC), also known as leukocytes, are present in the whole human body, in blood as well as in the lymphatic system. These cells consist of nuclei and cytoplasm. Their features can differentiate between a normal and an abnormal cell. They protect the human body from various diseases. They are used to detect different diseases such as leukemia (one of blood cancer results from excessive production of abnormal white blood cells) (Bouzid-daho and al. (2017)), and Danida and al.(2011)). The count and shape, lineage, and maturity level of blood cells could aid in diagnosis of the disease. A correct and speed patient diagnosis is an exhaustive effort and requires extremely trained or qualified experts and professionals.

The blood pathologies identification is a problem among hematologists, especially the detection of cancerous cells. Although blood cancer remains a relatively rare form, statistics indicate about 9000 people per year are affected [ref 3 (2018)]. Risk factors can be defined, but the overall prognosis is relatively unfavorable in 20% of patients, especially children and the elderly, as they are more likely to present other health problems. This complicates the treatment of leukemia Wang and al. (2016). Most patients with Acute Myeloblastic Leukemia in Joshi and al. (2018) survive no more than 1 year, and only 20 % are still alive 10 years after their illness. They are considered as cured.

Classification and segmentation of these cells are the essential and complex steps of the analysis and diagnosis of the disease. Several segmentation techniques of images have been proposed in literature, Pharm and al. (2000), Soltane (2012), and Abu Osman and al. (2008), generally based on edge detection, region growing, adaptive filtering, mathematical morphology, watershed clustering, and semantic segmentation. The choice of universal and performant method is difficult (Soltane (2012), and Yasodha and al. (2013). Each approach has its advantages and disadvantages. The parameters are generally chosen empirically according to the characteristics of the image. However, one can derive more reasonable properties that one seeks to obtain in a segmentation algorithm or classification (Micha and Deshmukh (2015)). Two essential criteria; The stability where the segmentation obtained must not vary significantly when the acquisition conditions vary slightly (noise, illumination,...). The regularity where the regions obtained must be simple to handle to classify (sufficient size, regular shape,...).

In this paper, we proposed a new simple and automatic method adapted to detect WBCs in a microscopic image and segment their component nucleus to count the cell Numbers and define their form. In our approach, we apply the k-means method to segment
microscopic medical images representing the blood cells, (Aimi and al. (2013) and Bouziddaho et al.(2017)). This method has been used in many applications for its simplicity of implementation and can provide a good approximation of the segmentation (Purohit et al. (2013)). Nevertheless, it suffers from a fault in introducing the spatial discontinuities strong enough to the class borders. Regularization methods used to strengthen the connectedness and thus reduce the number of related components of each class. On the other hand, it needs an empirical choice of its parameters. The performance of our system consists of others in the automatic choice of the cluster value and the centroids initialization.

The paper is organized as follows. Section 2 deals with the literature review; Section 3 presents the methodology and the proposed algorithm. Results are discussed in section 4, and the last section presents the conclusion and future.

2 LITERATURE REVIEW

Abdul Nazeer and al. (2009) proposed an improved algorithm to increase the accuracy and efficiency of the k-means clustering algorithm. Madhu Pathakota and al.(2010) proposed to improve the k-means clustering algorithm with a new method to find the initial centroid is presented; these offers an efficient way to assign data points to clusters reducing the time-complexity. Pallavi and Purohit (2013) and Ritesh Joshi (2013) introduced an approach to clustering using k-means. The approach generates the cluster center by reducing the mean square error in the last stage by reducing the execution time, Aleta and al. (2017); and Mohamed and Far (2012). In Abu Osman an al. (2008), authors reviewed some of the general segmentation methods that have found application in classification in biomedical image and especially in blood cell image processing.

Alan Jose and al. (2014) suggest a segmentation method for brain tumors combining the k-means and Fuzzy C-Means techniques. Scotti and al. (2006) proposed a Fuzzy k-means segmentation method to diagnose leukemia. First, they convert the input image into grayscale using L * a * b color space. They use Histogram threshold to enhance the image, and finally, segment nuclei and cytoplasms by forming the clusters of blood cells using Fuzzy k-means.

Putzu and al. (2013) use the SVM (Support Vector Machine) classifier to detect leukemia. They proposed to use a large number of variables for separating the classes of WBCs and the SVM (Support Vector Machine) as a classifier. In Nazlibilek and al. (2014), an automatic approach for counting the white blood cells and their sizes is evaluated for easy detection. The method classifies four types of WBCs as lymphocyte, monocyte,
basophil, and neutrophil. It adapts the threshold and converts the grayscale image to a binary image, using Otsu’s method. A PCA algorithm is used to extract features from the image. Yan Li and al. (2016), combine the RGB color space based single-threshold technique and HSV color space based single-threshold technique to construct a dual-threshold. Binarisation and mathematical morphology as erosion are done. Median filtering is used, at the last stage, to denoise and remove incomplete WBC. Their results show that a dual-threshold is outperformed than a single-threshold; it achieves a WBC segmentation accuracy of 97.85%.

Five years ago, Olaf Ronnebergeran and al. (2015) developed a prevalent method, an end-to-end encoder-decoder network for semantic segmentation called U-Net. Their method is based on a network and training strategy that relies on the strong use of data augmentation. The method consists of a contracting path to capture context and a symmetric expanding path to enable precise localization. The method uses very few images and outperforms for the segmentation of neuronal structures in electron microscopic stacks.

Xin Zhenga and al. (2018) proposed a cell segmentation approach based on self-supervised learning, using unsupervised initial segmentation and supervised segmentation refinement. First, they extract the overall foreground region from the cell image by the K-means clustering method and then generate a coarse WBC region by touching-cell splitting. The method uses the first module's segmentation results as automatic labels to train a Support Vector Machine (SVM) classifier. Sara Colantonio and al. (2007) proposed an automated method for the segmentation of lymphatic cell nuclei. The method follows a two-step, firstly, images are clustered to localize the cells (nuclei) and, then, to refine the segmentation, an Artificial Neural Network (ANN) is applied. Liqun Lin and al. (2018) proposed an improved algorithm based on feature weight adaptive K-means clustering to extract the complex leukocytes. The approach initializes the clustering center chosen according to the histogram distribution of a cell image. The adherent complex WBCs are separated using a watershed algorithm. Finally, a classification based on CNN (convolutional neural network) is used. The method achieves 95.81% segmentation accuracy.

3 PROPOSED METHODOLOGY

In this section, we present our approach (figure 1). It contains three essential steps: Preprocessing, segmentation, and postprocessing to count the abnormal blood cells.
3.1 DATASETS

Images of the dataset are captured with an optical laboratory microscope coupled with a Canon PowerShot G5 camera. All images are in JPG format, 24 bits of color images with 1712x1712 pixels resolution. The ALL_IDB1 version 1.0 can be used both for testing the segmentation capability of algorithms, the classification systems, and image preprocessing methods. The dataset is composed of 108 images. The images contain about 39000 blood elements, where expert oncologists have labeled the lymphocytes. The images acquisition uses different magnifications of the microscope ranging from 300 to 500 (Ref 28 (2018)).

Figure 1 - Block diagram of proposed system

3.2 PREPROCESSING STEP

During the acquisition step, external conditions can intervene to impair the acquired image quality, such: the lighting conditions, rate of spurious signals, sampling resolution of the optical microscope, and the calibration of the cameras. Some bio-images obtained by the optical microscope are of low quality. In one case, it is desired to extract the most important information for later analysis. In other cases, we want to alter the image to make
specific effects and highlight the image areas.

3.2.1 Image Resizing

Initial RGB images are 1712x1712x3 pixels size. We have reduced them to 256x256x3 pixels to have the same size and easy handling by our proposed system. An example of this step is illustrated in figure2.

3.2.2 Filtering

Filtering is necessary to improve the image to have a better segmentation. A good filter is one to eliminate noise without smoothing edges. The median filter has shown that it is one of the rare filters that can denoise an image of impulse and Gaussian noise without smoothing edge, (Padmavathi and al. (2009).

The median filtering of a pixel P, on a neighborhood V(P) of size (M×N), orders the values of the pixels of V(P) in ascending order and assigns in the output the median value on this neighborhood to the pixel P (non-linear operation). Studies have shown that a large window size (MxN) eliminates noise better but can blur the edge and distortion of edge corners (figure3). The choice of adequate size is essential in the process of smoothing.
The marginal median filter (MMF) is identical to the usual median filter, except that it is used for color images (RGB). For each channel, Red, Green, and Blue, respectively, the median filter is applied, and the Filtered RGB image is reconstructed (Li, Y et al. (2006)). The disadvantage of MMF, it shows edge distortion (figure 3), as well as a color change in some cases.

The VMF is similar to the mean filter, which smooth data by taking the mean within a windowed subset. Instead of finding the mean for every windowed subset, the VMF finds the median vector. The median vector is the median member $a_{m}$, according to the minimum-distance definition (2), is the member whose distance to all other members in the set is smallest (Benjamin et al. (2017)).

Given a set of vectors:

$$S_i = \{a_{i-j}, a_{i-j+1}, ..., a_{i+j-1}, a_{i+j}\}$$  \hspace{1cm} (1)

Where:

Figure 2 - RGB image blood cell: a) image size 1712x1712 pixels, b) image size 256x256

Source: The authors, 2024
j is the window half-width, the median vector is defined as:

$$a_{m_i} = \arg \min_{a_{m_i} \in S_i} \sum_{k=i-j}^{k=i+j} ||a_m - a_k||_L$$ (2)

The VMF possesses three important properties: closure, edge preservation, and iteration invariance. These properties allow preserving discontinuities while attenuating noise. Its disadvantage is the Computational time for a large data set can be expensive. We tested both filters on our images. The results obtained are similar. Contents of the circular contours of the cells, no distortion of contours observed. A small advantage for the MMF processing time. We opt for the MMF content filter because of its simplicity and results.

Our algorithm divides the RGB image into its three channels, red, green, and blue. The median filter is applied on each R, G, and B band separately. The color image is recomposed by the three images filtered. It is denoised without loss of edges.

3.3 SEGMENTATION

The segmentation is the most delicate phase in the reconstruction process. The overall performance of the system depends mostly on it (Soltane (1999)). In our medical context, the regions correspond to the different blood cytological structures constituting the different regions of interest. The automatic determination of the number of regions with the same characteristics (clusters) is a real problem (Talengaonkar and al. (2014) and Bouzid-Daho and al. (2019)). Often, the parameter K (clusters) is supposed to be known as a priori information about the anatomical structures under investigation. These values are arbitrary, and they can obviously be chosen empirically and modified. Our approach aims to define this parameter automatically by analyzing the blood cell image characteristics.

As the three components (red, green, and blue) are highly correlated, it is difficult to extract the pertinent information. Image is transformed from RGB space to L*a*b* space.
3.3.1 Conversion step RGB to L*a*b

The L*a*b* space consists of a luminosity 'L*' or brightness layer, chromaticity layer 'a*' indicating where the color falls along the red-green axis, and chromaticity layer 'b*' indicating the color falls along the blue-yellow axis. For our blood cells, the colored information is in the chromaticity layers ‘a’ and ‘b’. The measure the difference between two colors using the Euclidean distance metrics (Neelima and al. (2017)).

3.3.2 Implementation k-means

The algorithm of mobile centers (k-means) for the automatic clustering of a set of data \((x_1, \ldots, x_n)\) k-means minimizes the criterion of error (distortion) depending on the centers of the classes

\[
\psi = (\mu_1, \ldots, \mu_k) \text{ and the classes } z = (z_1, \ldots, z_n): (z, \psi)
\]

It is the Euclidean distance between total each data \(x_i\) and the center \(\mu_{z_i}\), the closest to the meaning of the Euclidean distance:

\[
\|x_i - \mu_k\|^2 = d(x_i, \mu_k) = \sqrt{\sum_{j=1}^{d} (x_{ij} - \mu_{kj})^2}
\]

In the expression of the criterion \(I\), \(z_k\) is the binary variable, it’s value= 1 if the class of \(x_i\) is \(k\) and 0 otherwise (Salvaraichal et al. (2014) and Bouzid-daho and al. (2018)). The main steps of k-means algorithm are:

- **Initialization**: the algorithm initializes the centers of the classes \((\mu_1^{(0)}, \ldots, \mu_k^{(0)})\) and gives the no departure (for example, choosing randomly to centers that "virtual," or k data among the data to treat). It is therefore used to start to the iteration \(t = 0\) with initial values for the model parameters \((\mu_1^{(0)}, \ldots, \mu_k^{(0)})\).

- **Affectation step (Classification)**: Each data is assigned to the class of the center of which it is the nearest \(\forall: i = 1, \ldots, n\)
Centers registration step: the Centre $\mu$ of each class $k$ is recalculated as the arithmetic average of all the data apartment in this class (following the step of previous assignment): \[ \forall k = 1 \ldots k \]

\[
\mu_k^{(t+1)} = \frac{\sum_{i=1}^{n} x_i^{(t)} z_{ik}^{(t)}}{\sum_{i=1}^{n} z_{ik}^{(t)}}
\]  

(t) Current iteration.

The convergence is reached if the relative value to the level of distortion $j$ (1) becomes less than a small threshold prefixed, or if the maximum number of iterations prefixed is reached.

In order to find the optimal number of clusters for a k-means, it is recommended to choose it, based on the context of the image. This is a subjective method if you know a specific number of groups in the image. To determine the cluster number automatically, the Elbow method, can be the solution using the within cluster sums squares (Dhendra Marutho et al. (2018)). The location of a knee in the plot is considered as an indicator of the appropriate number of clusters. This means that adding another cluster does not improve much better the partition. Figure 4 shows the curve of Elbow method. It looks at the total within-cluster sum of square (WSSC) as a function of the number of clusters. The analysis of the graph shows a curvature ranging from 2 to 5 clusters. The result is identical for all the blood cell images analyzed. We deduce that there is a maximum of 5 clusters in the images. This method seems to suggest 3 or 4 clusters.

Figure 4 - Elbow method to determine the K optimal clusters number

Source: The authors, 2024
3.4 POSTPROCESSING STEP

In the biomedical field, a good classification essentially depends on a fair characterization. The hematologist is the best classifier of the data (cancerous region, for example). The extraction of the relevant parameters in the cancerous areas makes it possible to establish the right diagnosis. Nevertheless, we can extract attributes from mathematical approaches (morphological operator) to make the necessary classification for these abnormal blood cells.

This step consists of counting the set of abnormal blood cells detected after the segmentation phase, realizing this operation based practically on a set of mathematical morphology elements.

**Figure 5 - Developed K-means algorithm**

```
Begin
1. Input image
2. Read image
3. Resizing
4. While (segmentation is failed)
   4.1 Decompose RGB Image in 3 Channels
   4.2 Filtering
   4.3 k-means implementation
   For
   4.3.1 Automatic choice of the initial position of “k” clusters.
   4.3.2 Reassign them to a cluster along a distance minimization criterion (usually as a measure of Euclidean distance).
   4.3.3 Once all placed objects, recalculate the “k” centroid
   4.3.4 Reiterate steps 2 and 3 until no reassignment is left
For
End wile
5. Blood cells segmented
```

Source: The authors, 2024

4 EXPERIMENTAL RESULTS AND DISCUSSION

We have implemented our algorithms (see Figure 5) using MATLAB language (R2020a) and tested on a common PC Pentium © Dual-Core CPU 2.20 GHz with 4 GB RAM. Before applying the k-means algorithm, it is necessary to perform preprocessing step that consists of resizing and filtering the RGB images so that our proposed diagnostic aid system can count cancerous blood cells.

Figure 6 shows the results of the marginal median filter (MMF). For each channel, Red, Green, and Blue, respectively, the median filter is applied. The filtered RGB image is reconstructed.

We have tested several windows sizes to the marginal median filter. Experience
shown that a large size, can cause blurring and edge corners distortion and a small size, can leave isolated points and noise, as shown in the K-means segmentation in figure 10. R system, we chose to use a 5x5 window size.

Figure 7 - VMF filtering results

![Image of VMF filtering results](source: The authors, 2024)

Figure 6 - MMF filtering results: Mask size 5x5

![Image of MMF filtering results](source: The authors, 2024)

Figure 7 shows the results of the vectorial median filter (VMF). The VMF preserves discontinuities while attenuating noise. Its disadvantage is the Computational time can be expensive. We have tested both those filters on our images. Results were similar. The advantage is that the blood cells’ are circular edges, so no distortion of the edge was observed. We opt to use the MMF filter for our system because of its simplicity and good results obtained.

After the conversion of RGB color space to L* a* b*, we find that our image obtained is to quantify these visual differences in the three planes L, a, and b, which helps
us to apply our method of segmentation by implementing the k-means algorithm. For K=3, figure 8 shows an image has 3 classes: the background color (yellow) represents the cytoplasm, the green color represents the red blood cells, and the blue is the white blood cells. We can easily visually distinguish these colors from each other.

Figure 8 - K-means clustering results with 3 clusters (K=3)

To separate microscopic blood cell image as red cell, Nuclei, cytoplasm, and plasma (background). Our algorithm uses K=4 for K-means clustering. Figure 9 illustrates the case for 4 clusters to separate four different classes from the image blood cells. According to the Elbow method and the optimal number of cluster determined (figure 4) and to detect and segment just the WBC cell, we choose K=3.
4.1 POSTPROCESSING STEP

Figure 11 shows that our algorithm gives a good detection of abnormal blood cells with a percentage of 98% on all the images, but the iteration repetition negatively influenced the computation time. According to figure 4, the first value given by the curve for an optimal cluster is 2. It seems quite logical that this value of $k = 2$ is the most appropriate since we will see in all the images tested that there are essentially two objects: one represents the bottom (plasma and red blood cell), and the other represents abnormal blood cells (region of leukemia). As a result, we have shown in Figure 11. The segmentation result obtained by our system shows that segmented images contain two classes. These classes were colored
according to the membership provided by the k-means algorithm and eliminate all incomplete cells border the image.

![Segmentation by k-means modified](source: The authors, 2024)

4.2 PROPOSED SYSTEM EVALUATION

To verify the proposed algorithm effectiveness, experiments were carried out on the basis of images used (figure 12). The performance of the proposed algorithm is compared with the other variant of k-means existing (without modification). We have measured and quantified the segmentation performance of all the test images with respect to the terrain truth images. We used two criteria: the recognition rate (recall) and accuracy. Then a comparison of these results of our automatic method with those obtained manually, on the same basis of microscopic images.
Figure 12 (a) (c) (e) Original image; (b) (d) (f) Automated leukemia detection

Figure 12 analysis shows our proposed system performs and detect the cells in all microscopic images. Therefore, we can conclude that our method is effective in detecting cancer cells (leukemia). However, these methods will never replace the clinician's eyes, but by providing a faster and more accurate interpretation tool to help clinicians. They will be a robust tool for automated detection of pathology.

Table 1: Proposed method performance for automated leukemia detection

<table>
<thead>
<tr>
<th>Images</th>
<th>Manual count</th>
<th>Auto count</th>
<th>Accuracy</th>
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<td>95.34%</td>
<td>98%</td>
</tr>
<tr>
<td>18</td>
<td>100%</td>
<td>98.66%</td>
<td></td>
</tr>
</tbody>
</table>

Source: The authors, 2024

Table 1 shows the comparison of our automated method and manual method. It summarizes the results obtained by the same basis of microscopic images. Better segmentation results are achieved by using different color spaces to take advantage of the complementary nature of these spaces. This comparison will highlight the insufficiency of the only information of luminance (gray levels) to discriminate the texture and give an interesting rate of recognition.
5 CONCLUSION

In this study, we proposed a diagnostic aid system for abnormal blood cell detection, using clustering based on the k-means approach. The clustering aims to separate blood cells into 3 homogeneous clusters. The results are analyzed by examining the number of cells in each cluster according to their properties: small healthy cells have shown that the higher the average cluster inertia is low over the classification is right and vice versa. We also note that there are healthy cells that belong to the same class of abnormal cells, and this is due to the clustering quality. The experimental results show that the proposed system has a good performance than the other systems developed in the literature. However, the automated visualization is still limited by the scarce diffusion of post-processing software. It is expensive and must be validated by experts. Our future work is focused on the use of other characteristics such as tumor size and form, and development of new image classification algorithms based on Deep Learning using a cloud platform, another project is to develop an end-to-end deep learning approach to another image medicals.
REFERENCES


