This article can be cited as M. Gamarra, Y. Manjarres, M. Torres Torres, J. Escorcia-Gutierrez and E. Zurek, MC-Kmeans: an Approach to Cell Image Segmentation Using Clustering Algorithms, International Journal of Artificial Intelligence, vol. 19, no. 1, pp. 80-94, 2021.

MC-Kmeans: an Approach to Cell Image Segmentation Using Clustering Algorithms

Margarita Gamarra[1] ; Yesit Manjarres [2] ; Melitsa Torres Torres [2] , Jose Escorcia-Gutierrez[2,3] ; and Eduardo Zurek[4]

[1] Deparment of Computational Science and Electronic, Universidad de la Costa, CUC, Barranquilla (Colombia)
Email: mgamarra3@cuc.edu.co

[2] Faculty of Engineering, Universidad Autónoma del Caribe, Barranquilla (Colombia)
Email: yama140274@yahoo.com; melitsat@gmail.com; jose.escorcia23@gmail.com

[3] Research Center, Escuela Naval de Suboficiales A.R.C. Barranquilla, Barranquilla (Colombia)
Email: jose.escorcia23@gmail.com

[4] Department of System Engineering, Faculty of Engineering, Universidad del Norte, Barranquilla (Colombia)
Email: ezurek@uninorte.edu.co

ABSTRACT

Digital image processing has been a fundamental tool for the diagnostic and treatment of diseases. Several techniques have been used to analyze microscopic images in cell-level processes. Different methods for the segmentation task are recognized for its contribution in the image processing. Nevertheless, not all are useful in the studies at a microscopic level. In most of the biomedical images, cells are visually clustered and this makes that, simple and fast algorithms which are used in the other cases, may fail. This research proposes the development of a segmentation algorithm in HEp-2 cells type, using the marker-controlled watershed and k-means methods. This approach achieves an improvement in the cell segmentation, which allows obtaining effective information in the posterior analysis. We obtained a precision of 82.3% in the performance and in the qualitative analysis the method reached an outstanding performance in comparison with the other segmentation techniques used in the experiments. Finally, we concluded that the algorithm proposed, is suitable for the segmentation of the studied cells.

Keywords: Marker-controlled Watershed, k-means, cell segmentation, digital image processing.

Mathematics Subject Classification: 68U10, 68T20

Computing Classification System: I.2

1. INTRODUCTION

Recent advances allow appreciating inside the human body from a microscopic level to a physiological level. These technologies generate new knowledge about the organ, tissues and cells, which is an advance in biomedical science and engineering.

Similarly, the improvement in image processing algorithms has allowed the development of computer-aided analytical approaches in the cells identification. In some cases, these discoveries have led to the development in medical treatments in humans, which could improve the health of patients. There are different techniques for the segmentation which are applicable in image processing, but not all of them are useful in a microscopic way. In the biological field, it is common to find images where some cells are overlapped, which makes that simple segmentation methods are not pertinent (Carpenter et al. 2006).

The k-means clustering is an algorithm widely used in unsupervised classification processes. The classical k-means algorithm only converges to local minima of the minimum-sum-of-squares. Nevertheless, some optimization strategies could be implemented to achieve the global optimum, as evolutionary optimization (Precup et al. 2016), the p-Median clustering approach (Ushakov, Vasilyev, and Gruzdev 2015) and local search methods (Nino-Ruiz, Ardila, and Capacho 2018). We propose an approach based on k-means clustering in the cell segmentation process, using intensity information to form groups. The parameters of the algorithm were set in a heuristic way.

The main objective is focused on the development of a segmentation algorithm for Hep-2 cells type (Human Epithelial carcinoma strain #2 (Toolan 1954)), in order to extract information of individually interest. This works is part of an extensive research related to virus infection in cells of human bronchial epithelium, using fluorescent microscopy techniques.

2. RELATED WORK

Diverse researches on cells segmentation have been developed, however this task still presents many challenges referring to the right identification and the subsequent cell separation. In this section we expose some of the works related to the cell segmentation process.

The study developed in (Ma, Manuel S Tavares, and Natal Jorge 2009), makes a review of the current segmentation algorithms used for medical images. The algorithms are divided in three categories according to their method: based on the threshold, based on pattern recognition and based on deformable models. Authors exposed the main trend of each category with its main ideas, scope, usability, advantages and disadvantages. For each type considered, typical algorithms are described, some of them applied to the segmentation of the organs and tissues contained in the pelvic cavity.

The proposal given in (Liao et al. 2016), contemplates the automatic segmentation for cells images based on bottle-neck detection and ellipse adjustment. To segment the overlapping cells into microscopic images, it is proposed an automatic method for cell image segmentation based on bottle-

neck detection and ellipse adjustment. First, the cell image is segmented by the threshold method, followed by a polygonal approximation to extract the feature points from the edge of the cell. Second, the candidate split point pairs are obtained by calculating the bottle-neck rate between each pair of feature points, and they are judged by the ellipse adjustment for finding the right split pairs of points. The experimental results show that the proposed method can effectively segment overlapping cells with high precision and less time, which is superior to other methods.

Authors in (Tonti, Di Cataldo, Bottino, et al. 2015), refers to an automated focus of Hep-2 cell segmentation for the indirect immunofluorescence test ANA. The automation of indirect immunofluorescence (IIF) images analysis is of utmost importance for the diagnosis of autoimmune diseases. The proposal is a solution in one of the most challenging steps in the segmentation of the HEp-2 cells, through an adaptive focus marker-controlled watershed, MC-watershed.

The work developed in (Dimopoulos et al. 2014) corresponds to the precise cellular segmentation in microscopy images using membrane patterns. The method accurately segments images of various cell types grown in dense crops acquired with different microscopy techniques. In quantitative benchmarks and comparisons with established methods on synthetic and real images, significantly improved segmentation performance is demonstrated despite the irregularity of cell shape, cell-to-cell variability, and cell noise in the image.

Despite the several methods proposed about cell segmentation, there are still factors to improve, such as the indicators of correct segmentation. In this way, the proposed method achieves high precision in the cell separation process, based on marker-controlled defined with the k-means algorithm.

2. MATERIALS AND METHODS

2.1. Dataset

Image processing requires high-tech equipment to perform the acquisition in different visual fields, from the microscopic level to thermal or resonance images, to appreciate inside the human body (Pandit and Shah 2011). Computational tools are required in the analysis of the data and images. The images used in this study were acquired from the virology laboratory of the Universidad del Norte, which is specialized in the study of this kind of cell based on fluorescent microscopy.

The HEp-2 cell line is a particular type of immortalized neoplastic crops cells which are used in scientific research and as a substrate in commercial tests for antinuclear antibodies.

The microscope used (ZEISS Axio Observer) contains a 10x objective or a 20x objective and has two channels, a red one and a green one. In the red channel is observed the entire cells groups in a microscopic perspective, which are registered and saved in the database. In this project, four images were used for the development and validation of algorithms, which are presented in Fig. 1.

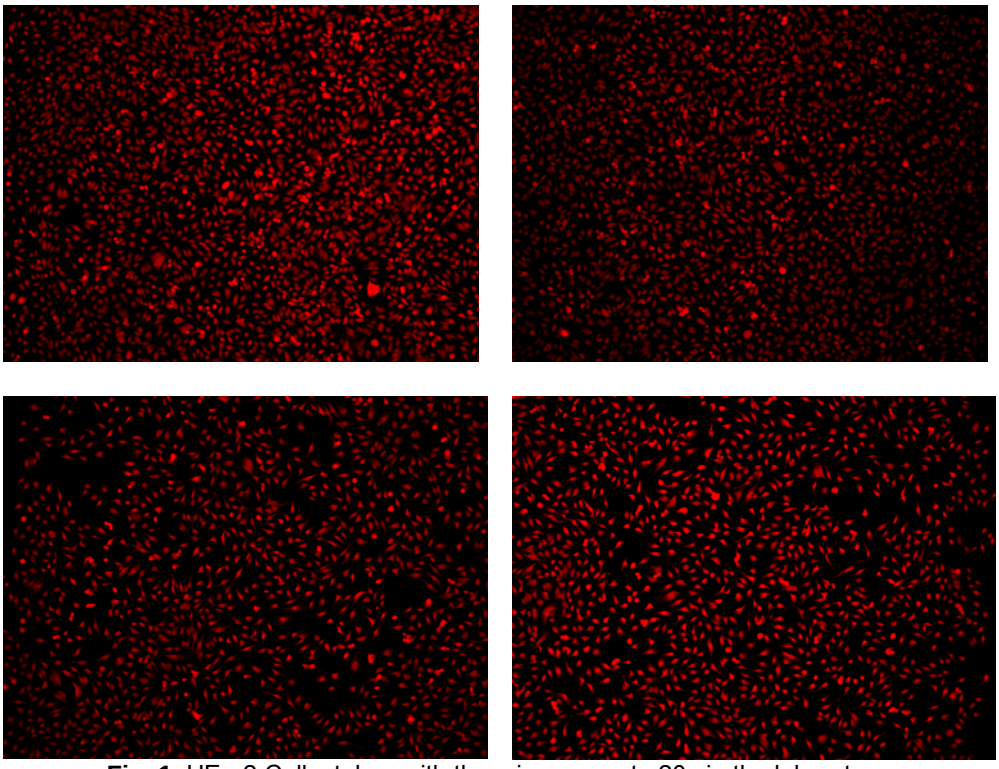

Fig. 1. HEp-2 Cells, taken with the microscope to 20x in the laboratory.

As a measuring tool, specialized software for studying cell images were used, such as CellProfiler and the Matlab image processing toolbox (Petrou 2010) (Gonzalez, Woods, and Eddins 2009), which allowed to evaluate each of the existing segmentation techniques, the algorithm designed and its validation as an final result. The source code and the test dataset are available from the corresponding author upon request.

2.2. Proposed approach

In the segmentation process, it is necessary to separate touching cells, with the aim to identify individually the features of each cell. The main objective of this work is to design a HEp-2 cell segmentation algorithm, using k-means to identify foreground and background markers, and evaluate their performance compared to other current techniques.

The proposed segmentation method, MC-kmeans (Marker-Controlled with k-means), is a new approach which is based on a clustering algorithm. The scheme of the process is presented in Fig. 2. This technique improves the processes to get cell and background markers in the image and finally to apply the watershed transform to separate the elements.

The principal stages of the developed method are illustrated in Fig. 2. The initial stage is the preliminary processing of the original input image in preparation for the subsequent steps. The process is performed to obtain the target (cell) and the background markers. To achieve this distinction, the image pixels are classified and categorized in assigned colours based on the configuration of the classifying algorithm, in this case K-means. In the watershed transform stage

(Huang et al. 2008) (Salman and Salman 2004) (Koyuncu et al. 2012), the gradient operator is applied and computed with the final results of the cell and background marker processes previously aforementioned (He, Zheng, and Sun 2016). The proposed method is explained step by step, taking into account the diagram in Fig. 2 and its representative stages.

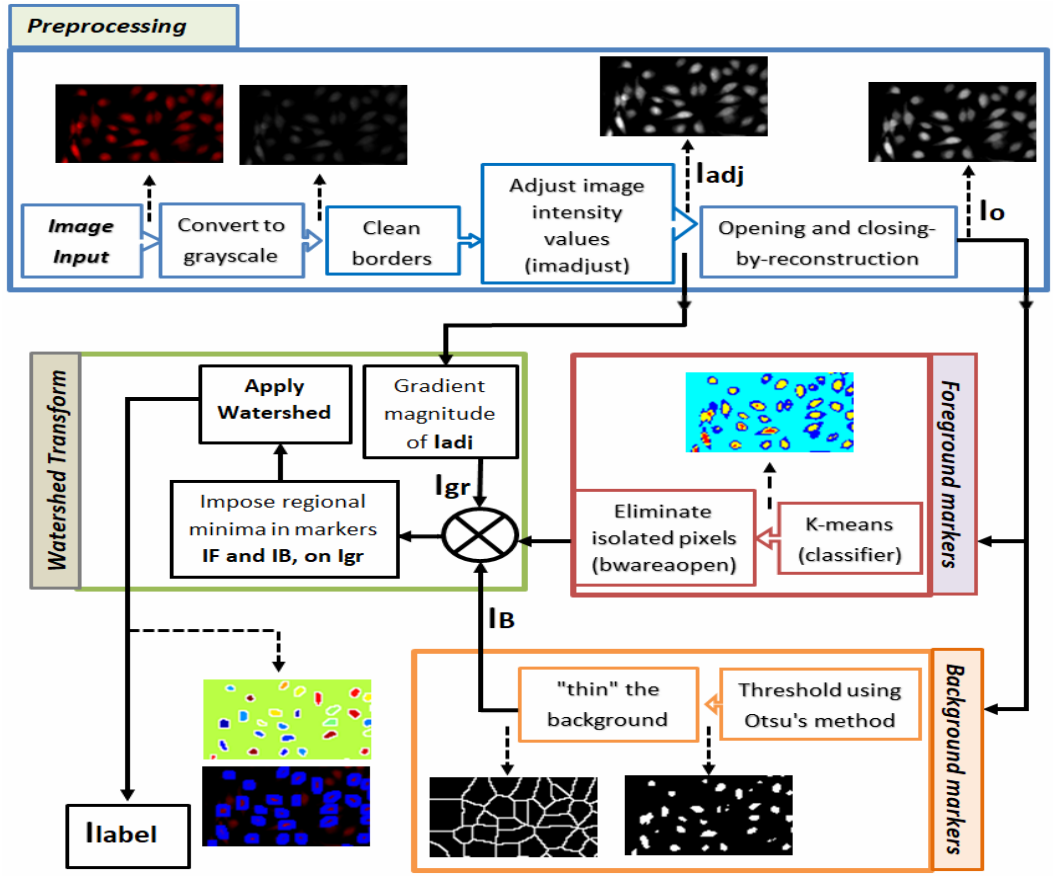

Fig. 2. Diagram of the proposed segmentation method.

2.2.1 First phase: Pre-processing

It is hard to build a filter that can remove all image disturbances and keep intact the target structures, since the filter does not differentiate which structures are relevant for the observer.

With this project, we implemented a process for the HEp-2 cells images segmentation, which different digital pre-processing techniques were applied (Fig. 3).

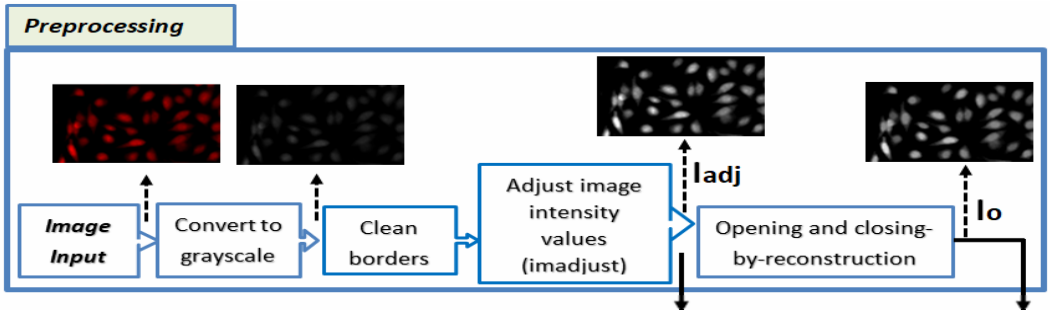

Fig. 3. Preliminary processing of the images.

First, the pre-processing converts the image to grayscale, in order to visualize its intensities and facilitate subsequent phases. Second, the algorithm suppresses the border structures to avoid identification mistakes. Third, a contrast enhancement technique was used, which establishes the differences of intensities. Finally, morphological techniques called “opening by reconstruction” and “closing by reconstruction” were used to clean the image.

It must be noted that the opening is erosion followed by a dilatation, while opening by reconstruction is erosion followed by a morphological reconstruction. The opening or closing based on the reconstruction are more effective than standard opening and closing in the elimination of small stains without affecting the general shapes of the objects (Xing and Yang 2016).

The process continues from the output image of the processing and it is the input to the foreground markers and background markers process (see Fig. 4 and Fig. 5). The marker of cells (foreground) indicates where possibly there is an object, and the background marker sets where there is an object in the background.

2.2.2 Second phase: objective markers

The foreground markers process is developed by the clustering algorithm K-means (Du and Dua 2010)(Zaitoun and Aqel 2015)(Dhanachandra, Manglem, and Chanu 2015). This method treats each observation in the data as an object that has a location in the space. K-means separates the data points into k separated clusters by minimizing the total intra-cluster spread, starting with a randomly chosen set of candidate cluster-representatives or centroids. Each data point is assigned to the cluster corresponding to the closest centroid measured in terms of a distance metric, usually Euclidean, however other metrics has been proposed as weighted distance (T. Zhang and Ma 2017) and divergence-based distance (Chakraborty and Das 2017). The objective function for the K-means clustering algorithm is the squared error function, for N observations in K clusters:

$$\min_{C,\{m_k\}_1^K} \sum_{k=1}^{K} N_k \sum_{C(i)=k} \|x_i - m_k\|^2 \tag{1}$$

$$N_k = \sum_{i=1}^{N} I(C(i) = k) \tag{2}$$

The criterion is minimized by assigning the N observations to the K clusters in such a way that within each cluster the average dissimilarity of the observations from the cluster mean, as defined by the points in that cluster, is minimized. The k-means algorithm can be expressed as:

Algorithm 1. K-means clustering

1. For a given cluster assignment *C*, the total cluster variance, in eq. (1) is minimized with respect to $\{m_1, \ldots, m_K\}$ yielding the means of the currently assigned clusters.
2. Given a current set of means $\{m_1, \ldots, m_K\}$, eq. (1) is minimized by assigning each observation to the closest (current) cluster mean. That is,

$$C(i) = \underset{1 \le k \le K}{\operatorname{argmin}} \|x_i - m_k\|^2 \quad (3)$$

3. Steps 1 and 2 are iterated until the assignments do not change.

In the image process case, the K-means method requires a transformation of the image matrix to a vector, because the algorithm only works with vectors (Borlea et al. 2017). Next, it makes an aggrupation of them, namely it divides them into an "n" observations set. The more groups you assign, the more points or pixels you will mark with specific color (Fig. 4). This process tends to leave some pixels isolated, and they be eliminated. Thus, the "bwareaopen" command in Matlab is used for eliminating all the spots that have fewer pixels than a threshold.

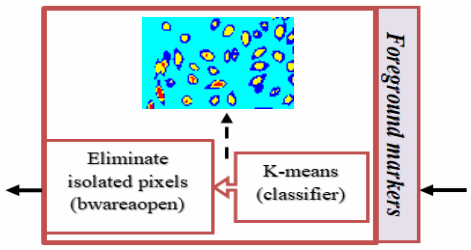

Fig. 4. The K-means process (set algorithm) is applied to the foreground for taking the cells markers.

2.2.3 Third phase: background markers.

A thresholding operation is implemented to define the dark pixels that belong to the background. In this case the Otsu's automatic method is used for binarization. The Otsu's method tries to maximize the variance between the classes or divides zones (Ghaye et al. 2013). The method generates the optimal threshold value and automates the system for finding the threshold in any image which is processed (Creemers et al. 2012). A thinning process (Hsieh et al. 2009) of the background pixels is applied by calculating the "skeleton by zones of influence" to avoid that the background markers be close to the edges of the foreground (Fig. 5).

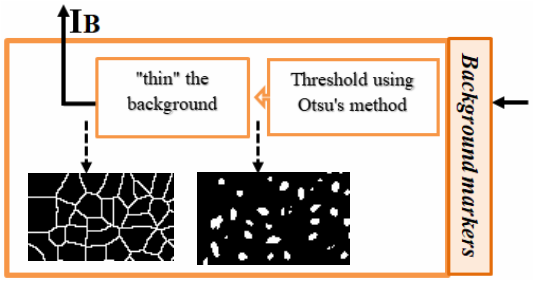

Fig. 5. A thresholding and thinning processes are made to the background and after that the background lines are taken.

2.2.4 Fourth phase: Watershed transform.

After the background and foreground markers are obtained, the labels are used to perform the watershed transform with the image gradient. In this process three images are needed: the image with cells markers, the image with the background markers, which are lines, and the image with the gradient of the adjusted image.

Afterwards, an algorithm is applied to impose a regional minimum in the markers (Fig. 6). The regional minimum correspond to find and mark low intensity pixels surrounded by high intensity. A minimum is imposed on the gradient image using the makers then, the watershed transform is applied. The minimum regional function can be used for modifying an image, so that the minimum regional occurs at certain desired locations at the background and foreground points. The watershed transform calculates the distance between two points and drawing a line in the middle of them.

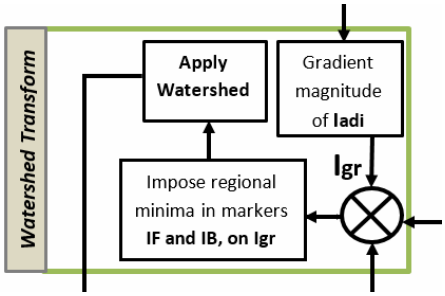

Fig. 6. Watershed transform process.

The result of this stage is an image with individual labelled objects. The contour is superposed in the original image to visualize the segmented cell.

2.3. Performance indicators

The most important performance indicators are shown in detail below. This quantitative evaluation is the first performance validation of the techniques used in the images processing test.

Because there is no a ground truth image for this database, the original input image is converted to a binary image using Otsu thresholding with Matlab 2017b, afterwards, a manual process is achieved in order to individualize each grouped cell for getting the base image. This is the reference that is used

for obtaining the performances indicators and evaluating the segmentation methods involved in the experiment.

MC-kmeans is compared with other two methods: the best configuration set in Cellprofiler and MC-watershed algorithm implemented in Matlab.

The performance indicators are obtained by comparing the resultants images in the segmentation process with the reference image or ground truth (J. Zhang, Wang, and Shi 2009). We used four basic cardinalities of the confusion matrix: true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN). Based on this values, the following performance indicator are obtained (Tonti, Di Cataldo, Macii, et al. 2015) (Vivona et al. 2017) (Li and Shen 2018):

1. Precision: PPV =TP/((TP + FP))
2. Recall: TPR=TP/(TP+FN)
3. Specificity: TNR=TN/(TN+FP)
4. Negative predictive value: NPV =TN/((TN + FN))
5. Accuracy: (TP+TN)/(TP+FN+TN+FP
6. F-index=2(PPV*TPR)/(PPV+TPR)

where:

TP = number of the right pixels or cells correctly assigned to the foreground.

TN = number of the right cells or pixels correctly recognized as foreground.

FN = numbers of the foreground cells and pixels marked by the system as background.

FP = number of the false pixels and cells marked by the system as foreground.

Finally, we compared our MC-kmeans with existing techniques and software used in the cell images segmentation, through these performance indicators, whose values are presented in percentage. Precision and recall are normally considered together, and their values are considered significant (Percannella, Soda, and Vento 2012) (Divya, Subramaniam, and Nanjundaswamy 2016). It must be highlighted that the high precision values and low recall values generate a poor segmentation, and conversely, it represents an excessive segmentation (Tonti, Di Cataldo, Bottino, et al. 2015). A suitable balance between this two metrics is manifested in high values in the F-Index. Nevertheless, these performance indicators are not always an absolute reflection of the efficiency of a method. Since the indicators based on the confusion matrix are not able to discern the performance between the different techniques, we applied an evaluation for visual inspection as additional assessment instrument, normally used in these cases.

On the one hand, we considered the classical method of least squares without variables selection and on the other hand, the *stepwise* selection method of variables is used. These methods were adopted, because they are among the most used methods, and are available in almost all statistical software.

3. RESULTS AND DISCUSSION

In this section the experimental results are presented using the dataset shown in Fig.1. The same data set was manipulated and improved in an appropriate program, in order to be used as base line

(ground truth). Ground truth image was used in this work as reference for comparing and evaluating of each algorithm in the experiment.

In the experiment, the proposal segmentation method, MC-kmeans, was compared with two known approaches more: (1) marker-controlled watershed algorithm with regional maximum in Matlab software, and (2) a method with fixed parameters corresponding to the Cellprofiler medical image evaluation software. Finally, the results of the performance indicators of the experiments are shown of a quantitatively way, while the qualitative assessment of their behaviour is established by visual inspection of the resulting images.

One of the parameters of the K-means algorithm is the number of K-groups in which each of the image pixels are classified and each observation belongs to the group whose average value is closer. Thus, an initial experiment was completed for determining the number of groups that generates a higher performance of the algorithm.

Table 1 presents the performance tests of the proposed method using different groups, based on the quantitative assessment using the K-means algorithm.

Table 1 shows that the highest value in the parameter F-Index is obtained with the configuration of k = 4 groups, being the best segmentation strategy for the proposed method of this work. This experiment was necessary and significant for this work, since it supported the selection of the best configuration for the proposed algorithm.

Table 1: Performance of K-means using different groups

Indicators	3 groups	4 groups	5 groups
accuracy	83.6%	83.6%	82.9%
F-index	64.3%	65.4%	64.1%
NPV	83.3%	84.4%	83.9%
precision	85.3%	82.3%	80.9%
recall	52.7%	56.5%	55.1%
specificity	96.0%	94.6%	94.1%

The performance of our method was compared with CellProfiler and marker-controlled Watershed with maximum regional. Table 2 shows the performance indicators in percentage of the three studied algorithms. A proper behavior between precision and recall generates high F-index values. In our method, there is a slight deficiency in the identification of the cells which causes a low recall, but the identified cells were correctly segmented, which is verified with high precision, compared to the values of the other methods. On the other hand, the accuracy indicator provides comparable values with the other two methods, allowing a suitable segmentation and automatic counting in a cell image.

Through a variance analysis test ANOVA, the performance of the three algorithms was compared. This statistical analysis uses the variances to determine whether the means of the results of the three segmentation methods are different from each other (Gamarra et al. 2019).

Table 2: Performance indicators for the three methods

Indicators	MC-Kmeans	MC-watershed	Cellprofiler
accuracy	83.6%	85.2%	83.9%
F-index	65.4%	71.2%	77.3%
NPV	84.4%	86.4%	97.0%
precision	82.3%	81.6%	66.2%
recall	56.5%	63.8%	93.3%
specificity	94.6%	93.6%	79.4%

The analysis is made by comparing the variance between the group averages and the variance into the groups as a way of determining whether the groups are all part of a larger population or separate populations with different characteristics (Demšar 2006). Table 3 shows the F statistic and the P-value or significance, which is the probability of obtaining F values, or higher, under the null hypothesis of means equality. If the p-value associated with the F statistic is less than 0.05, it is rejected the null hypothesis and accept the alternative, which means that the average values of the three methods are different.

Table3: ANOVA test for the three segmentation algorithms.

Parameter	F	Probability (P-value)	F critical value
Accuracy	0,2635	0,7740	4,25649
f-index	2,8588	0,1093	4,25649
precision	8,9616	0,0072	4,25649
recall	13,4255	0,0019	4,25649

Thanks to the ANOVA test made to the four criteria in question (see Table 3), it was statistically possible to determine that the accuracy indicator and the F-index have a p-value higher than 0.05, this means that there are no differences between the three algorithms for that factor. On the other hand, the null hypothesis, which affirms that the variances of the other two remaining parameters are not equal, is accepted with a confidence level of 95%.

Taking into account the results of the ANOVA test and with the similar values for the three tested methods, it is not possible to define clearly which of them was the most efficient. These indicators (F-Index, precision and recall) do not always give an idea of how good the segmentation is. This is because these metrics are based on the comparison of the segmented image against the ground truth image, which contain clustered cells too.

Therefore, in these cases where it is not possible to demonstrate that one algorithm is better than the other using performance indicators, it is recommended to implement a visual inspection assessment. In this way, we proceed to the visual identification of the obtained results from the three methods.

In Fig. 7.1), the image is the ground truth, result of the binarization and manual adaptation made by an expert. Based on this image, all performance indicators were obtained. This process was used to demonstrate the performance and efficiency of the experiments.

Fig. 7.2) shows the result of the segmentation process of the Marker-Controlled method with maximum Regional. We can observe that almost all the cells (red elements) were identified by the algorithm, but it is possible to appreciate noise and excessive non-separated cells, indicating that the process made by the regional maxima was deficient.

Fig. 7.3) shows the image after the segmentation with Cellprofiller. Likewise, it illustrates failures in the segmentation process with non-identified cells and clustered cells, indicating poor processing.

Fig. 7.4) shows the result of the proposed method, and the objects detected by the MC-Kmeans algorithm are observed. Although there are cells that were not identified, a correct separation between the detected cells is evident. Therefore we can state that their performance was better than the other two methods in the separation process of clustered element.

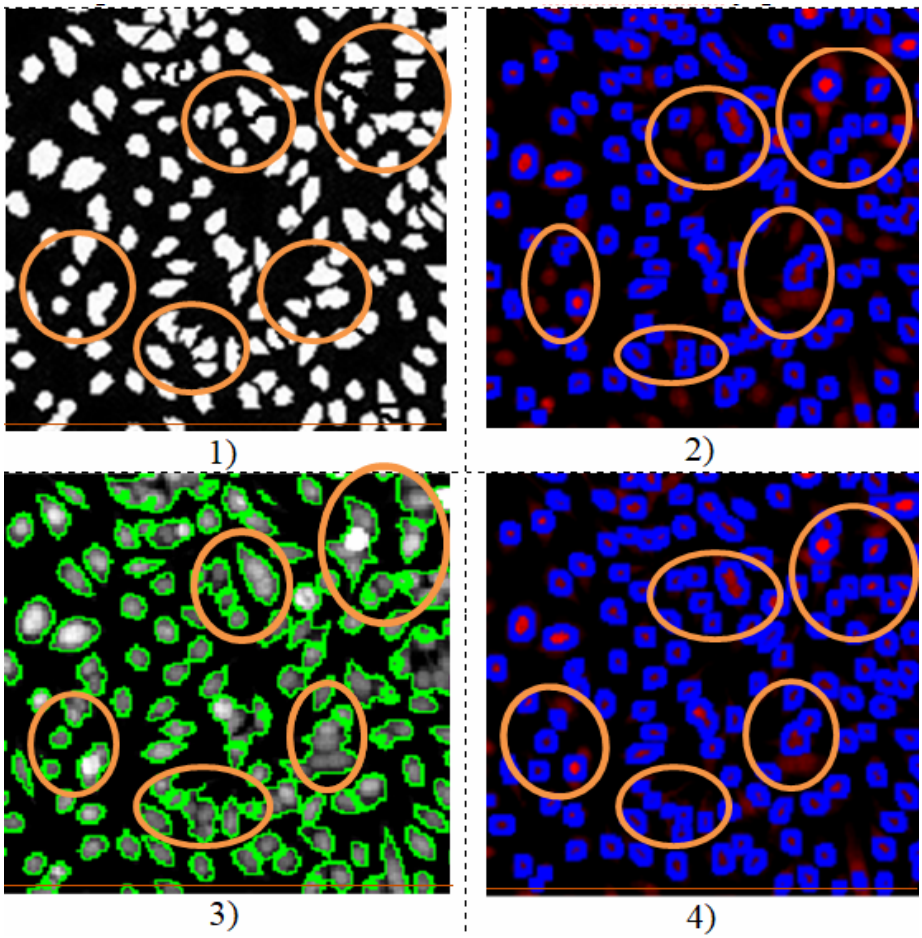

Fig. 7. HEp-2 cells images from the segmentation algorithms. 1) Ground Truth, 2) MC-watershed 3) Cellfrofiler and 4) our method, MC-KMeans.

4. CONCLUSIONS

In this work, we presented a new approach of marker-controller based on K-means algorithm for the cell segmentation in fluorescence microscopy images. The proposed approach includes a preprocessing step of the original input image. The process to mark the cells is supported by the k-

means algorithm and the Otsu thresholding is used to mark the background. These coarse segmented images were the input to apply the watershed transform to separate the clustered cells.

A quantitative and qualitative evaluation was completed to provide an adequate validation of the method. Satisfactory results were generated with the assessment by visual inspection. This demonstrated that the MC-kmeans is a suitable approach for cell separation. The algorithm allowed a proper characterization cell-by-cell for more complex subsequent studies of this type of specimen. Thanks to the manually adjustment made and applied to the Ground-Truth image, it was possible to validate the results and obtain the performance of the proposed algorithm.

The qualitative assessment demonstrated satisfactory results in terms of cell segmentation, compared with their counterparts, the Cellprofiler and marker-controlled with maximum regional.

As future work it is necessary to improve the algorithm to identify completely the cells. Although the MC-Kmeans cell segmentation method functioned well in cells separation on fluorescence imaging, more extensive studies are needed to improve the non-identified cell indicators and eliminate the noise. Additionally, the method to select the k value in the K-Means algorithm can be automated, using an iterative method as Expectation-Maximization.

5. REFERENCES

Borlea, Ioan Daniel, Radu Emil Precup, Florin Dragan, and Alexandra Bianca Borlea. 2017. “Centroid Update Approach to K-Means Clustering.” *Advances in Electrical and Computer Engineering* 17 (4): 3–10. https://doi.org/10.4316/AECE.2017.04001.

Carpenter, Anne E, Thouis R Jones, Michael R Lamprecht, Colin Clarke, In Han Kang, Ola Friman, David A Guertin, et al. 2006. “CellProfiler: Image Analysis Software for Identifying and Quantifying Cell Phenotypes.” *Genome Biology* 7 (10): R100. https://doi.org/10.1186/gb-2006-7-10-r100.

Chakraborty, Saptarshi, and Swagatam Das. 2017. “K−Means Clustering with a New Divergence-Based Distance Metric: Convergence and Performance Analysis.” *Pattern Recognition Letters* 100 (December): 67–73. https://doi.org/10.1016/j.patrec.2017.09.025.

Creemers, Carolien, Khadija Guerti, Stig Geerts, Karin Van Cotthem, Alessandro Ledda, and Vincent Spruyt. 2012. “HEp-2 Cell Pattern Segmentation for the Support of Autoimmune Disease Diagnosis,” 1–5. https://doi.org/10.1145/2093698.2093726.

Demšar, Janez. 2006. “Statistical Comparisons of Classifiers over Multiple Data Sets.” *Journal of Machine Learning Research* 7: 1–30. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.141.3142&rep=rep1&type=pdf.

Dhanachandra, Nameirakpam, Khumanthem Manglem, and Yambem Jina Chanu. 2015. “Image Segmentation Using K-Means Clustering Algorithm and Subtractive Clustering Algorithm.” *Procedia Computer Science* 54: 764–71. https://doi.org/10.1016/j.procs.2015.06.090.

Dimopoulos, Sotiris, Christian E Mayer, Fabian Rudolf, and Joerg Stelling. 2014. “Accurate Cell Segmentation in Microscopy Images Using Membrane Patterns.” *Bioinformatics* 30 (18): 2644–51. https://doi.org/10.1093/bioinformatics/btu302.

Divya, B. S., Kamalraj Subramaniam, and H. R. Nanjundaswamy. 2016. “HEp-2 Cell Classification Using Binary Decision Tree Approach.” In *IECBES 2016 - IEEE-EMBS Conference on Biomedical Engineering and Sciences*, 507–12. Institute of Electrical and Electronics Engineers Inc. https://doi.org/10.1109/IECBES.2016.7843503.

Du, Xian, and Sumeet Dua. 2010. “Segmentation of Fluorescence Microscopy Cell Images Using Unsupervised Mining.” *The Open Medical Informatics Journal* 4: 41–49. https://doi.org/10.2174/1874431101004020041.

Gamarra, Margarita, Eduardo Zurek, Hugo Jair Escalante, Leidy Hurtado, and Homero San-Juan-

Vergara. 2019. “Split and Merge Watershed: A Two-Step Method for Cell Segmentation in Fluorescence Microscopy Images.” *Biomedical Signal Processing and Control* 53 (August): 101575. https://doi.org/10.1016/J.BSPC.2019.101575.

Ghaye, Julien, Madhura Avinash Kamat, Linda Corbino-Giunta, Paolo Silacci, Guy Vergères, Giovanni De Micheli, and Sandro Carrara. 2013. “Image Thresholding Techniques for Localization of Sub-Resolution Fluorescent Biomarkers.” *Cytometry Part A*. https://doi.org/10.1002/cyto.a.22345.

Gonzalez, Rafael C, Richard E Woods, and Steven L Eddins. 2009. *Digital Image Processing Using MATLAB, 2nd Ed.* 2nd editio. S.I.: Gatesmark Publishing. http://www.amazon.com/Digital-Image-Processing-Using-MATLAB/dp/0982085400.

He, H. J., C. Zheng, and Da Wen Sun. 2016. “Image Segmentation Techniques.” In *Computer Vision Technology for Food Quality Evaluation: Second Edition*, 45–63. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802232-0.00002-5.

Hsieh, Tsu Yi, Yi Chu Huang, Chia Wei Chung, and Yu Len Huang. 2009. “HEp-2 Cell Classification in Indirect Immunofluorescence Images.” In *ICICS 2009 - Conference Proceedings of the 7th International Conference on Information, Communications and Signal Processing*. https://doi.org/10.1109/ICICS.2009.5397624.

Huang, Yu Len, Chia Wei Chung, Tsu Yi Hsieh, and Yu Lang Jao. 2008. “Outline Detection for the HEp-2 Cell in Indirect Immunofluorescence Images Using Watershed Segmentation.” In *Proceedings - IEEE International Conference on Sensor Networks, Ubiquitous, and Trustworthy Computing*. https://doi.org/10.1109/SUTC.2008.72.

Koyuncu, Can Fahrettin, Salim Arslan, Irem Durmaz, Rengul Cetin-Atalay, and Cigdem Gunduz-Demir. 2012. “Smart Markers for Watershed-Based Cell Segmentation.” Edited by Konradin Metze. *PLoS ONE* 7 (11): e48664. https://doi.org/10.1371/journal.pone.0048664.

Li, Yuexiang, and Linlin Shen. 2018. “CC-GAN: A Robust Transfer-Learning Framework for HEp-2 Specimen Image Segmentation.” *IEEE Access*. https://doi.org/10.1109/ACCESS.2018.2808938.

Liao, Miao, Yu Qian Zhao, Xiang Hua Li, Pei Shan Dai, Xiao Wen Xu, Jun Kai Zhang, and Bei Ji Zou. 2016. “Automatic Segmentation for Cell Images Based on Bottleneck Detection and Ellipse Fitting.” *Neurocomputing* 173: 615–22. https://doi.org/10.1016/j.neucom.2015.08.006.

Ma, Zhen, João R Manuel S Tavares, and R M Natal Jorge. 2009. “A REVIEW ON THE CURRENT SEGMENTATION ALGORITHMS FOR MEDICAL IMAGES.” In *1st International Conference on Imaging Theory and Applications (IMAGAPP)*, 135–40.

Nino-Ruiz, Elias D., Carlos Ardila, and Rafael Capacho. 2018. “Local Search Methods for the Solution of Implicit Inverse Problems.” *Soft Computing* 22 (14): 4819–32. https://doi.org/10.1007/s00500-017-2670-z.

Pandit, Hardik, and D. M Shah. 2011. “Application of Digital Image Processing and Analysis in Healthcare Based on Medical Palmistry.” *International Journal of Computer Application*, 56–59.

Percannella, G., P. Soda, and M. Vento. 2012. “A Classification-Based Approach to Segment HEp-2 Cells.” In *Proceedings - IEEE Symposium on Computer-Based Medical Systems*, 1–5. IEEE. https://doi.org/10.1109/CBMS.2012.6266311.

Petrou, Maria Petrou and Costas. 2010. *Image Processing: The Fundamentals*. *United Kingdom: John Wiley & Sons*. 2 ed.

Precup, Radu-Emil, Radu-Codrut David, Emil M. Petriu, Alexandra-Iulia Szedlak-Stinean, and Claudia-Adina Bojan-Dragos. 2016. “Grey Wolf Optimizer-based Approach to the Tuning of PI-Fuzzy Controllers with a Reduced Process Parametric Sensitivity.” *IFAC-PapersOnLine* 49 (5): 55–60. https://doi.org/10.1016/j.ifacol.2016.07.089.

Salman, Nassir, and Nassir Salman. 2004. “Image Segmentation Based on Watershed and Edge Detection Techniques.” http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.167.1162.

Tonti, Simone, Santa Di Cataldo, Andrea Bottino, and Elisa Ficarra. 2015. “An Automated Approach to the Segmentation of HEp-2 Cells for the Indirect Immunofluorescence ANA Test.” *Computerized Medical Imaging and Graphics : The Official Journal of the Computerized Medical Imaging Society* 40 (March): 62–69. https://doi.org/10.1016/j.compmedimag.2014.12.005.

Tonti, Simone, Santa Di Cataldo, Enrico Macii, and Elisa Ficarra. 2015. “Unsupervised HEp-2 Mitosis Recognition in Indirect Immunofluorescence Imaging.” In *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*. Vol. 2015-Novem. https://doi.org/10.1109/EMBC.2015.7320282.

Toolan, Helene Wallace. 1954. “Transplantable Human Neoplasms Maintained in Cortisone-Treated Laboratory Animals: H.S. #1; H.Ep. #1; H.Ep. #2; H.Ep. #3; and H.Emb.Rh. #1.” *Cancer Research* 14 (9): 660–66, Consultado el 15 de marzo de 2014.

Ushakov, Anton V., Igor L. Vasilyev, and Tatyana V. Gruzdev. 2015. “A Computational Comparison of

the P-Median Clustering and k-Means." *International Journal of Artificial Intelligence*^{TM} 13 (1): 229–42. http://www.ceser.in/ceserp/index.php/ijai/article/view/3550.

Vivona, Letizia, Donato Cascio, Salvatore Bruno, Alessandro Fauci, Vincenzo Taormina, Amel Benammar Elgaaied, Yousr Gorgi, et al. 2017. "Unsupervised Clustering Method for Pattern Recognition in IIF Images." In *IPAS 2016 - 2nd International Image Processing, Applications and Systems Conference*. Institute of Electrical and Electronics Engineers Inc. https://doi.org/10.1109/IPAS.2016.7880124.

Xing, Fuyong, and Lin Yang. 2016. "Robust Nucleus/Cell Detection and Segmentation in Digital Pathology and Microscopy Images: A Comprehensive Review." *IEEE Reviews in Biomedical Engineering* 9: 234–63. https://doi.org/10.1109/RBME.2016.2515127.

Zaitoun, Nida M., and Musbah J. Aqel. 2015. "Survey on Image Segmentation Techniques." *Procedia Computer Science* 65 (Iccmit): 797–806. https://doi.org/10.1016/j.procs.2015.09.027.

Zhang, Junhua, Yuanyuan Wang, and Xinling Shi. 2009. "An Improved Graph Cut Segmentation Method for Cervical Lymph Nodes on Sonograms and Its Relationship with Node's Shape Assessment." *Computerized Medical Imaging and Graphics* 33 (8): 602–7. https://doi.org/10.1016/j.compmedimag.2009.06.002.

Zhang, Tengfei, and Fumin Ma. 2017. "Improved Rough K-Means Clustering Algorithm Based on Weighted Distance Measure with Gaussian Function." *International Journal of Computer Mathematics* 94 (4): 663–75. https://doi.org/10.1080/00207160.2015.1124099.