Image Segmentation Methods for Identifying Submerged Particles of Low Contrast Images

MAYAR A. A. SHAFAEY[1], MOHAMMED A.-M. SALEM[1,2], DOAA A.-K. HEGAZY[1], and MOHAMED I. ROUSHDY[1]
[1]Faculty of Computer and Information Sciences, Ain Shams University, Cairo, Egypt
[2]Faculty of Media Engineering and Technology, German University in Cairo, Egypt
mayar.al.mohamed@fcis.asu.edu.eg, mohammed.salem@guc.edu.eg , doaa.hegazy@cis.asu.edu.eg, mroushdy@cis.asu.edu.eg

Abstract: - The lack of automated concepts is considered as a major reason for barriers for investments into biotechnological processes that serve bulk chemicals and can provide a substantial part towards a more sustainable economy. Hence, this paper presents a method for enhancing the automation of submerged microalgae particles recognition, counting, and classifying using a microscopic device for in-situ imaging. The proposed method includes *image de-noising* using Anisotropic Diffusion technique; *image normalization* by Contrast Limited Histogram Equalization (CLAHE) method, *image enhancement* by morphological operations, a *region of interest (ROI) extraction*, and *image segmentation*. Furthermore, the ROI are classified into biological algae cells and others (i.e. grain stones) based on ROI's size and texture. This method is applied on different datasets as *synthetic* and *real microscopic* images of microalgae. The experimental results proved that the microalgae particles can be quantified and classified correctly with accuracy reaches up to 99% and 100% for the segmentation and classification processes, *respectively*, compared to reference values.

Key-Words: - Active Contour, Anisotropic Diffusion, CLAHE, Fuzzy C-mean, Watershed Transform, Microalgae Particles, Biomedical Imaging.

1 Introduction

Nowadays, many developments allow the replacement of fish oil ingredients with the products yield from a biotechnological process of algae. These products are used for the ω-3 production, which are used in protection against diabetes, heart, and cancer diseases [1]. The microalgae *Crypthecodinium cohnii* is identified as a good producer of fatty acids [2]. During its life cycle, two forms of *C. cohnii* are observed; motile swimming and non-motile (cysts) cells. Both forms can be differentiated by the size. The small cell (motile) identified as a fresh offspring and the large ones (cysts) represent an adult cell [3].

Through the production phase, the optimal mode is how to determine the switch between those two forms. This is because, during the growth, the cell's pressure must be high enough so that cells are producing accelerated amounts of fatty acids, but must also not be too high so that cell disruption and division occurs [4].

The problem that is addressed in this paper is how to develop an optical detection method to track the morphological changes of the cells automatically because fully manual observation makes the process very boring and time consuming. The images of *C. cohnii* are acquired by probe microscope. The microscopic images may suffer from tricky problem as the objects in the underwater images were not obviously observable due to low contrast and sprinkling of light and the large noise founded in the environment, which makes challenges to this study [5]. Thus, the main objective of this paper is to visually differentiate between the small (non-active) cells and cyst (active) ones in order to follow and observe morphological changes for active cells. To achieve this goal, the microscopic images are segmented and analyzed to extract pivotal feature like cells' size. Then, the cells' texture is measured for accurate classification.

The effective publications for biological image analysis were investigated in a wide variety of articles. The similar investigates were carried out on yeast cells [6], animal cells [7] [8], water-oil emulsions [9], bubble drops [10], bacterium cells [11] [12], or crystal drops [13]. Definitely, the proposed work will follow those approaches taking into consideration the different features of our images and how to process the connected and overlapped particles.

2 Proposed Method

Based on previous literature, we are realized that the image processing and analysis play a major and

important role in in-line observation of microorganisms using a microscopic tool. The proposed method is divided into two major steps; Image Segmentation and Objects Classification.

2.1 Segmentation Methodologies

Image segmentation refers to divide an image into foreground and background using wide different techniques to make the image more simple and meaningful [14].

The proposed technique which is applied in this research requires a strong pre-processing method while it is applied to general types of images with (high/low) brightness and contrast. Low-contrast images are hard for segmentation process. After the pre-processing steps, different segmentation algorithms are employed to segment the interested objects. The following subsections will provide the detailed description of the method and discuss its advantages. However, the following figure, Fig 1, summarizes up the proposed approach which presented in this paper.

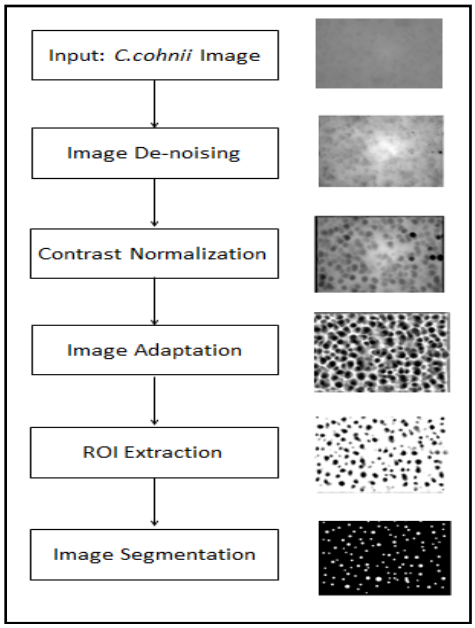

Fig. 1 Block diagram of proposed approach.

2.1.1 Anisotropic Diffusion Based Image De-noising

In image processing and computer vision, anisotropic diffusion, also known as *Perona–Malik* diffusion, is a technique aims to reducing image noise without blurring edges, or removing important parts of the image content, edges, lines or other details that are significant for the understanding of the image [15].

Anisotropic diffusion is an iterative procedure where a relatively simple set of calculations are used to measure each successive image in the family and this process is continued until a sufficient degree of smoothing is achieved.

2.1.2 Contrast Normalization and Enhancement

The conventional morphological operators like *erosion* and *dilation* are used for the normalization of image contrast. The process of contrast normalization, presented here, is composed of five consecutive operations, listed as: *morphological bottom-hat, Gaussian filter, background estimation, average filter*, and *morphological erosion*.

This normalization step is performed three consecutive times to be ready for next step, *Image Adaptation*. As input image quality, while there is no apparent contrast change between objects and background, it is suitable to utilize the *contrast limited adaptive histogram equalization* (CLAHE) method for contrast improvement [16].

2.1.3 ROI Extraction

The general aim is to extract the *region of interest* (*ROI*) from the adapted image. This process could be achieved by subtracting the *integral filtered image* from a de-noised one. *Integral Filter* method filters an image, given its integral image, and a filter kernel. The primary reason for using an integral image is to improve the execution speed for computing box filters [17]. Hence, it is suitable to apply the integral filter, *box filter*, on achieved integral image for a de-blurring purpose. After that, the process of subtracting the *integral filtered image* from a de-noised one is done for *ROI* extraction.

2.1.4 Image Segmentation

The techniques which are used in this approach are: *Otsu's* based Thresholding, *Circular Hough Transform (CHT), Watershed Transform, Active Contour method*, and. *Fuzzy C-Mean.* The aim is to evaluate the output result of each technique and compare between the accuracy values as described in later. The first two techniques, *Otsu's* based thresholding and *CHT* are widely described in many researches [18].

Another method is *Watershed transformation* (WT) which belongs to region based segmentation [19]. The main reason for applying this transformation is to separate the touched and connected cells. However, the (WT) technique is suffering from the over-segmentation issue. So, we tend to use other segmentation methods.

Alternatively, an *active contour* method is applied [20]. *Chan and Vese* proposed multi-phase active contour model which increases the amount of

subsets that active contours can locate simultaneously. This model is used in this work.

Another method which is used for segmentation purpose is the *Fuzzy C-Means* (FCM) [21]. The FCM clustering algorithm is a segmentation method that has been widely used for microscopic image segmentation. FCM algorithm classifies the image by grouping similar data points in the feature space into clusters. In our case, clusters are identified by similarity intensity measurement. This clustering is achieved by iteratively minimizing a cost function that is dependent on the distance of the pixels to the cluster centers in the feature domain:

$$J = \sum_{j=1}^{N} \sum_{i=1}^{C} u_{ij}^{m} \left\| x_j - v_i \right\|^2 \quad (1)$$

where; u_{ij} represents the membership of pixel x_j in the i^{th} cluster, v_i is the i^{th} cluster center, and m is a constant to control the fuzziness of the resulting partition.

Membership grades are assigned to each of the data points. These membership grades indicate the degree to which data points belong to each cluster. Thus, points on the edge of a cluster, with lower membership grades, may be in the cluster to a lesser degree than points in the center of the cluster. This is also a way to segment the cells those touched in their borders.

2.2 ROI Classification

In this approach, the classification process is based on two parameters, *cells size* and *cells texture*. Simply, the area of the cell is determined from its radius. As mentioned before, the large size of particles identifies the active cells and smaller one represents the non-active ones.

Indeed, the object is recognized by computing connected component labeling operation after applying the segmentation approach. After that, relevant parameters of cell size could be obtained. *Area*, *perimeter*, *diameter*, and *radius* of each cell could be calculated simultaneously. Also, we could rely on *cell texture* [22] as a powerful way for accurate classification to ensure the effectiveness of this process.

3 Results and Discussions

3.1 Datasets

There are two types of datasets are used to evaluate the proposed method presented in this paper. The first dataset is a *Synthetic image*, an image made by a computer program. The advantage of these images that having a ground truth for all cells since containing the location (center) of each cell and its radius. The presented methods are applied on different synthetic images; all have the same dimensions, 1024 x 1024, and almost equal number of cells. The purpose of that is testing the reliability of the proposed method.

The second dataset is the real images of *C. cohnii* in a liquid environment. The presented method is applied on different images (high contrast) with dimension 2272x1704, and on different low contrast images with 688x550 dimensions. The benefit of these images is making a challenge for introducing a high accuracy of the method proposed.

The decision to verify the correctness of low/high contrast images is based on measuring the *contrast* using texture properties of the whole image. If the contrast value ranging between 0 and 12, then the image is a low contrast, while if the contrast value ≥ 18, then it is classified as a high contrast one.

3.2 Experimental Results

The following figures represent the output of each step in the experiment as to the steps mentioned in section 2. Fig. 2 represents the initial *C.cohnii* image. Fig. 3 shows the effect of the de-noising process with *anisotropic diffusion* filtering.

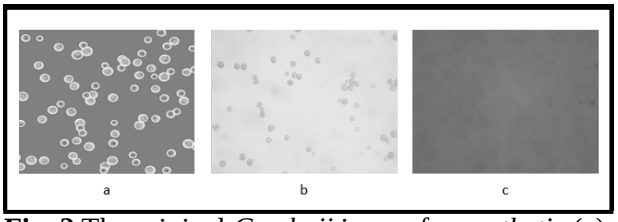

Fig. 2 The original *C.cohnii* image for *synthetic* (a), *high contrast* (b), *low contrast* (c) images.

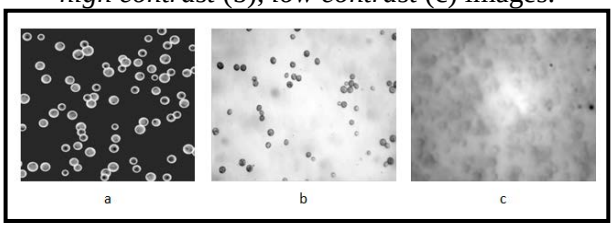

Fig. 3 The de-noised image.

Fig. 4 shows the repetition of the pre-processing steps as the second step in the proposed method. The morphological *bot-hat* operation is applied firstly by *disk-shaped* with size 1 for synthetic and high contrast images, and size 15 for low contrast image. The following step is applying the *Gaussian* filter using σ = 2.5. The filtered image is subtracted from the original one and then filtering the result using *average* filter by mask window [3 x 3] for synthetic and high contrast images, and [9 x 9] for low contrast image, then it is eroded by *disk-shaped* and these five steps are repeated twice.

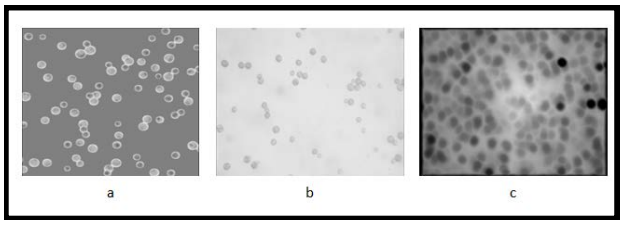

Fig. 4 The contrast normalization and Enhancement.

As observed in the previous images, the foreground objects are improved in vision. The dark regions indicate the interested objects which need to extract it. For more clarification, the *CLAHE* method is applied with window [21 x 21] and clip limit = 0.0001, 0.001, and 1 for synthetic, high, and low contrast images, *respectively*. See Fig. 5.

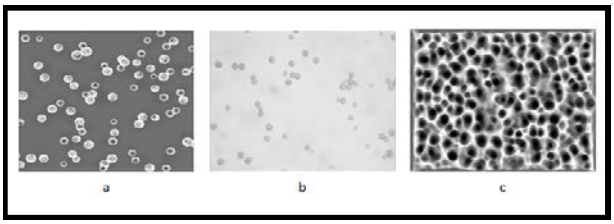

Fig. 5 The images after adaptation**.**

After that, the integral image is computed and the integral filter is applied using [1 1 11 11] mask window for synthetic, high contrast images, and [3 3 7 7] for low contrast image. That is done for de-blurring the adapted image. Furthermore, for extracting the dark objects out of surrounding shadows, the filtered integral image is subtracted from the de-noised image, see Fig. 6.

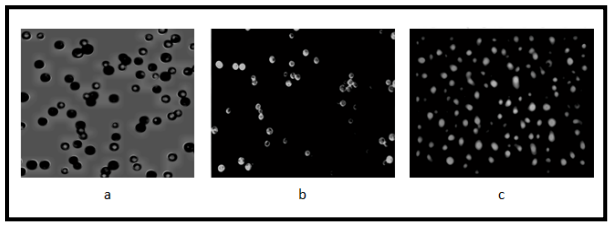

Fig. 6 Foreground objects by subtracting the integral filtered image from the original one.

For image segmentation, the *Otsu's* thresholding is used and the result is shown in Fig. 7. Alternatively, *CHT* is used by radius range [12 – 150], [5 - 50], and [5 – 20] for synthetic, high contrast, low contrast images, respectively, see Fig. 8. Also, the distance transform *chessboard* along with *watershed* is used to segment the objects, see Fig. 9. Alternatively, *active contour* technique is applied using the *chan-Vese* method as in Fig. 10. Alternatively, the *Fuzzy C-Mean* is applied by using 2, 3, and 4 classes for synthetic, high contrast, low contrast images, *respectively*, as in Fig. 11.

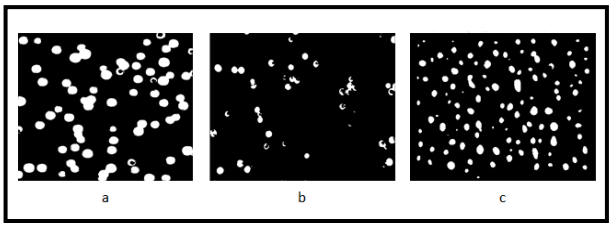

Fig. 7 Image segmentation using *otsu's* method.

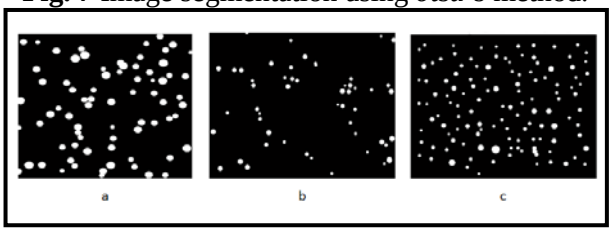

Fig. 8 Image segmentation using *CHT* method.

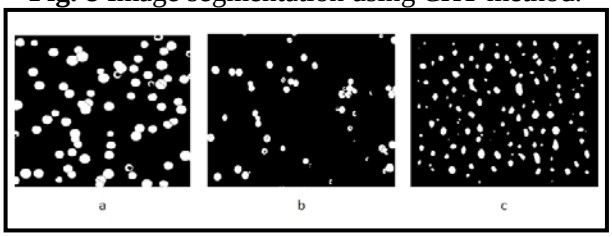

Fig. 9 Image segmentation using *Watershed*.

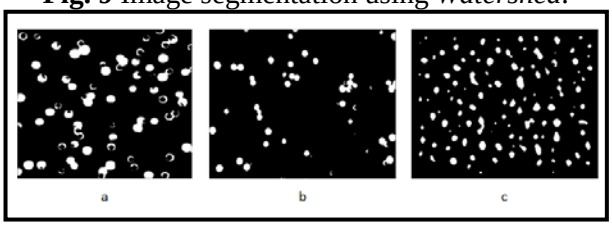

Fig. 10 Image segmentation using *Active Contour.*

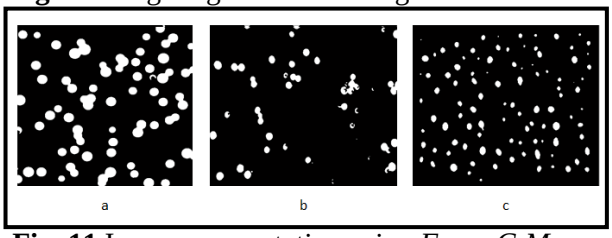

Fig. 11 Image segmentation using *Fuzzy C-Mean.*

Finally, the images are post-processed by some *morphological operations* and such filters as *median filter* to preserve the circular shape of objects.

After that, the area of each particle is computed separately and gets the localization centers easily. Whatever the segmentation method which is applied or the datasets which are used, the correct localization of the cells would help the classification operation for achieving an accurate result. Actually, the size parameter is the most important factor in differentiating between the cells. As aforementioned, the small objects are identified as non-active and other cells are active/biological cells.

Based on the area histogram computation, for *synthetic* images, an active cell has radius >= 27. And for high contrast real images, the cells with radius >= 29 are classified as active ones. It happens

by chance to be a group of cells have a radius 30 or more but aren't biological cells. So, we will tend to use more parameter for accurate classification.

The *Energy Texture* parameter is measured for accurate classification. In *real* images, the objects whose have energy >= 0.12 are classified as active objects, otherwise, are classified as non-active ones. Hence, the classification process is depending on *radius* parameter >= 29 and *energy* parameter >= 0.12, see Fig. 12.

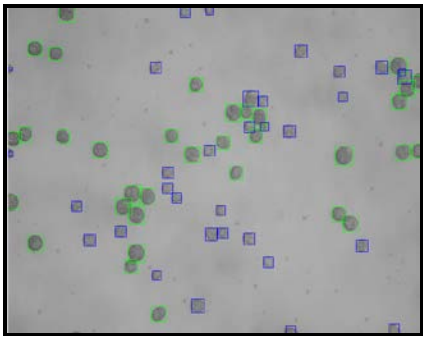

Fig. 12 The classification for *real* images. The green squares represent the active objects and blue ones represent the non-active cells.

Truth be told, the merge between these two parameters, *cell's size*, and *energy* texture analysis, could classify the objects correctly based on segmentation process. All images which are passed this method could classify correctly with percentage reaches up 100 %.

3.3 Discussions

For a quick look, we observed that the *Fuzzy C-Mean* method has been able to segment all objects correctly for low contrast images. The objects' shadow may effect on the size and center of the object. The fuzzy c-mean has been able to get rid of this issue. Conversely, the *CHT* method has been a preferred one for synthetic and high contrast images. It kept the objects in uniform circular shape and separated the connected objects as in Fig.8.

Also, the *watershed transform* has been separated the connected objects but it had a drawback of an over-segmentation. Sometimes, it divides a perfect object to multiple ones and counts them as more than one object. Finally, the *Otsu's* method and *active contours* had unsatisfied results. The thresholding process couldn't detect all the objects, conversely, the active contour has been segmented all the objects i.e. true objects, shadows, or artifacts. Also, the connected objects problem wasn't resolved by those two latter methods. There is no doubt that the combination of the segmentation methods may improve the segmentation results.

In concise words, the proposed method has a proud accuracy value for in-line identification of micro-organisms compared to other methods that previously introduced, for example in [23], the authors reached about 93% accuracy using edge detection and ANN techniques for images classification. In [24], the authors applied *Top-hat, Wiener filter, edge detection,* and *Back Propagation* to reach 98% accuracy. Also, in [25], the *edge detection,* and *texture measurements* were applied to get 97.7% accuracy. However, our proposed method reaches up 99%. The accuracy value is computed by manually compare the centers and radius of the segmented cells with the ground truth values.

4 Conclusion

In this work, a method for in-line identification of micro-organisms has been presented and applied for all given datasets and achieved high accuracy. This method has been included: *image de-noising* via anisotropic diffusion technique , *image normalization* by CLAHE method, *image enhancement* using morphological operations, *region of interest extraction* using integral filter, and *active contour method,* or, *fuzzy c-mean* alternatively, *Otsu's thresholding, circular Hough transform,* or, *watershed transform* as segmentation step, and cells classification using their *size*, and *energy texture* parameters.

There is no doubt that a parallelization on a multi-core PC would decrease those calculation times dramatically. Therefore, the in-line process of observation and control is possible.

References:

[1] JP. Wynn, P. Behrens, A. Sundararajan, J. Hansen, and K. Apt, Production of Single Cell Oils by Dinoflagellates. *In: Single Cell Oils*, AOCS, 2005.

[2] ES. Martin, Docosahexaenoic acid production by the marine alga crypthecodinium cohnii. *Applied microbiology and biotechnology*, Netherlands: Delft University Press, 2003.

[3] U. Michal, E. Malte, and S. Eberhard, A Crypthecodinium cohnii-like dinoflagellate feeding myzocytotically on the unicellular red alga porphyridium sp, *European Journal of Phycology*, Vol. 32, No. 2, 1997, pp. 133– 140.

[4] D. Prabowo, O. Hiraishi, and S. Suda, Diversity of crypthecodinium spp (dinophyceae) from okinawa prefecture, *Marine Science and Technology*, Vol. 21, 2013, pp. 181– 191.

[5] Bazila, and Dr. AH. Mir, Segmentation of Microscopic Images of Living Cells: A Study, *International Journal of Signal Processing, Image Processing and Pattern Recognition*, Vol. 7, No. 2, 2014, pp. 327–338.

[6] C. Bittner, G. Wehnert, T. Scheper, In situ microscopy for on-line determination, of biomass, *Biotechnology and Bioengineering*, Vol. 60, No. 1, 1998, pp. 24–25.

[7] B. Plasier, D. Lloyd, GC. Paul, CR. Thomas, M. Al-Rubeai, Automatic image analysis for quantification of apoptosis in animal cell culture by annexin-V affinity assay, *Journal of Immunological Methods*, Vol. 229, No. 1, 1999, pp. 81–95.

[8] F. Wartelle, P. Dhulster, JS. Guez, JPh. Cassar, and H. Suhr, Real time in situ microscopy for animal cell-concentration monitoring during high density culture in bioreactor. *Journal of Biotechnology*, Vol. 111, No. 3, 2004, pp. 335–343.

[9] S. Inta, K. Ralf, and M. Gerald, Automated image analysis as a control tool for multiple emulsions, *Food Hydrocolloids*, Vol. 19, No. 3, 2005, pp. 617 – 624.

[10] G. Enrique, CL. Patricia, B. Teresa, MC. Soledad, T. Blanca, V. Leticia, and C. Gabriel, Development of advanced image analysis techniques for the in situ characterization of multiphase dispersions occurring in bioreactors, *Journal of Biotechnology*, Vol. 116, No. 3, 2005, pp. 261– 270.

[11] GRC. Garofano, CG. Venancio, CAT. Suazo, and PIF. Almeida, Application of the wavelet image analysis technique to monitor cell concentration in bioprocesses, *Brazilian Journal of Chemical Engineering*, Vol. 22, No. 4, 2005, pp. 573 – 583.

[12] JC. Eun, YO. Jung, YC. Hyun, WY. Jong, Production of exopolysaccharides by submerged mycelial culture of a mushroom Tremella fuciformis, *Journal of Biotechnology*, Vol. 127, No. 1, 2006, pp. 129–140.

[13] B. Presles, J. Debayle, A. Rivoire, J. Pinoli, G. Fevotte, In situ particle size measurements during crystallization processes using image analysis, *In: XII Congrès de la Société Française de Génie des Procédés Pour relever les défis industriels du XXI siècle A la croisée des Sciences et des Cultures, France: Societe Fancaise de Genie des Procedes*, 2009.

[14] Mohammed A-Megeed Salem, Medical Image Segmentation: Multiresolution- based Algorithms, V*DM Verlag Dr. Müller*, ISBN 978-3-639-38067-5 2011.

[15] R. Motiur, R. Shohel, I. Aminul, R. Masudur, HT. Mehedi, A New Filtering Technique for denoising Speckle Noise from Medical Images Based on Adaptive and Anisotropic Diffusion Filter, *International Journal of Research in Computer and Communication Technology*, Vol. 2, No. 9, 2013, pp. 689-693.

[16] HD. Cheng, and XJ. Shi, A simple and effective histogram equalization approach to image enhancement, *Digital Signal Processing*, Vol. 14, No. 2, 2004, pp. 158-170.

[17] E. Shoaib, FC. Adrian, R. Naveed, and DM. Klaus, Integral Images: Efficient Algorithms for Their Computation and Storage in Resource-Constrained Embedded Vision Systems, *Sensors*, Vol. 15, No. 7, 2015, pp. 16804-16830.

[18] Mohammed A.-M. Salem, Alaa Atef, Alaa Salah, Marwa Shams, Recent Survey in Medical Image Segmentation, *Handbook of Research on Machine Learning Innovations and Trends*, IGI Global, Chapter 19, Volum I, pp 424-464, 2017, ISBN: 9781522522300.

[19] GK. Hassana, and B. Zou, Region-Based Segmentation versus Edge Detection, *In: Fifth International Conference on Intelligent Information Hiding and Multimedia Signal Processing*, 2009, pp. 1217-1221.

[20] PL. Cheolha, *Robust Image Segmentation using Active Contours: Level Set Approaches*, North Carolina State University, 2005.

[21] HAJ. Farah, I. Waidah, A. Rosalina, H. Rosline, Image Segmentation Using A Hybrid Clustering Technique and Mean Shift for Automated Detection Acute Leukaemia Blood Cells Images, *Journal of Theoretical and Applied Information Technology*, Vol.76, No.1, 2015, pp. 89-96.

[22] M. Andrzej, and S. Michal, Texture Analysis Methods - A Review, *Technical University of Lodz, Institute of Electronics, COST B11 report, Brussels*, 1998, pp. 1-33.

[23] AA. Mogeeb, M. Hayat, M. Sorraya, M. Pozi, S. Aishah, A preliminary study on automated freshwater algae recognition and classification system, *BMC Bioinformatics*, Vol. 13, No. 17, 2012, pp. 1-13.

[24] S. Natchimuthu, P. Chinnaraj, S. Parthasarathy, K. Senthil, Automatic Identification of Algal Community from Microscopic Images, *Bioinformatics and Biology Insights*, Vol. 7, 2013, pp. 327-334.

[25] P. Sansoen, W. Pakaket, S. Nuttha, Automated Microalgae Image Classification, *Procedia Computer Science*, V. 29, 2014, pp.1981-1992.