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Fuzzy Clustering Techniques For Image Segmentation Using Microscopic Images

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ABSTRACT

The bacteria image segmentation of microscope slide imaging is an important and one of the challenging tasks in biomedical image processing. The key contribution of this work is based on algorithm and was executed step by step. The pre-processing technique is adaptive threshold and median filter for noise removal, contrast stretching and morphological operations. This are applied after Fuzzy C-means and Fuzzy K-means algorithms. Fuzzy C-means clustering algorithm used to segment the foreground object and segment the background object using Fuzzy K-means clustering. The Clustering is a major method used for grouping of mathematical and image data in data mining and image processing applications. Clustering makes the job of image recovery easy by finding the images as similar as given in the query image**.** The simulation result shows the PSNR, MAE metrics used comparing resultant of segment images. The comparison between the results of traditional as well as the proposed methods shows that the proposed method yields better results both in visual perception as well as in quantitative analysis.
Keywords : Image Threshold, Median Filter, Contrast Stretching, Histogram Equalizations, Morphological Operations, Fuzzy C-Means Clustering, Fuzzy K-Means Clustering, Peak Signal Noise Ratio, Mean Absolute Error.

I. INTRODUCTION

In computer vision an image segmentation process is a partitioning of an image into those distinct regions. It is based on detect that an image is like line, region, edge. It proves a way to partition an input image into it is constitute parts or objects. Segmentation subdivides an image into it is constitute regions or subjects. The subdivision is carried depends on the problem being solved. Segmentation is one of the difficult tasks in digital image processing. A rugged segmentation procedure brings the process a long way toward successful solution of imaging problems that that require objects to be identified individually. In this paper described about section 2 image segmentation, section 3 microscopic images, section 4 pre-processing techniques on adaptive threshold, median filter, contrast stretching, histogram equalizations, morphological operations, section 5 fuzzy c-means clustering, section 6 fuzzy k-means clustering, section7 result and discussion, section 8 discussed the conclusion.

II. IMAGE SEGMENTATION

This is proves a way to partition an input image into it is constitute parts or objects. Segmentation subdivides an image into it is constitute regions or subjects. The subdivision is carried depends on the problem being solved. Segmentation is one of the difficult tasks in digital image processing. A rugged segmentation procedure brings the process a long way toward successful solution of imaging problems that that require objects to be an identified individually. An image segmentation process is a partitioning of an image into those distinct regions. It is based on detect that an image is like line, region, edge. The segmentation is subdividing the objects because easy to be solved the problem. The partitioning of an image into is based on pixel oriented. The pixel is called as picture element. All pixels must be an assigned to region. An each pixel must belong to a single region only. An each region must be uniform. Any merged pair of adjacent regions must be non-uniform. An each region must be a connected set of pixels. Segmentation is generally the first stage in several attempts to analyze or interpret an image automatically. Segmentation bridges the gap between

- Low-level image processing and
- High-level image processing.

Some kinds of segmentation technique will be found in any an application involving the detection, recognition, measurement of objects in images. The goal of image segmentation is the representation of an image into something that is more meaningful and an easy to analysis. Some boundaries are used for example of lines, curves etc .Because locate to the object of an image.

The result of image segmentations is a set of segments that collectively cover the entire image or a set of counters extracted from the image .Each pixel is based on such as colour, intensity or texture. The simplest method of an image segmentation is thresholding method. This method is based on a clip-level (or a threshold value) to turn a gray-scale image into a binary image. It is called as balanced histogram thresholding[1].

III. MICROSCOPIC IMAGES

Microscope imaging may refer for any imaging technique used in biology. Typical examples include: Bioluminescence imaging, a technique for studying laboratory animals using glowing protein. Calcium imaging, formative the calcium status of a tissue using fluorescent light. Microscopy is the technical pasture of using microscopes to view objects and areas of objects that cannot be seen with the stripped eye (objects that are not within the resolution range of the normal eye). There are three well-known branches of microscopy: optical, electron and scanning search microscopy[2]. Image processing techniques have been widely used in the last decade in medical imaging and the microscopic field received a consistent effort from researchers. Considering the importance of the pathological results for human health and the applications difficulties. The image processing techniques are of special interest because they allow large scale statistical evaluation in addition to classical eye screening evaluation and are used in both sections of the pathology: cytology (the study of cells) and histology (anatomical study of the microscopic structure of tissues). Due to its importance, a great variety of segmentation algorithms have been proposed for a wide range of applications and the publications are widespread in literature: microscopy, biomedical engineering, biomedical imaging, bioinformatics and pattern recognition[3].

BACTERIA IMAGES ON BACILLUS SUBTILIS

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram positive, catalyse-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus Bacillus, B. subtilis is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. B. subtilis has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. B. subtilis is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies[4].

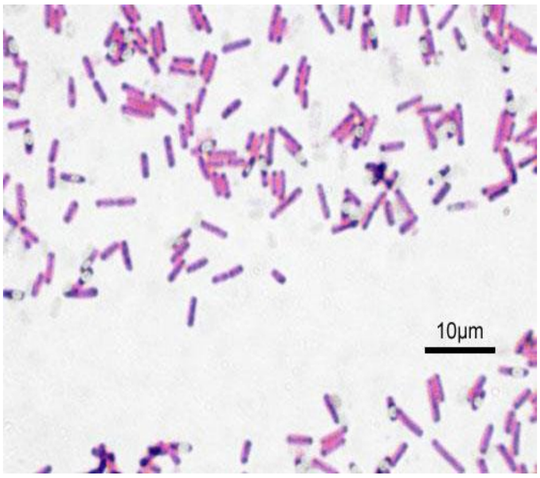


Figure 1. Bacillus subtilis

The Richter Optical U2T trinocular high power laboratory biomedical microscope offers high quality optics at an affordable price point.

An Objective Lenses: Infinity Corrected DIN Plan Achromat 4x, 10x, 40xR, 100xR (oil immersion) objectives. All microscope objectives are parfocalled, parcentered and color-coded. Quad objective turret is ball-bearing mounted for smooth, precise positioning of objectives, features textured rubber knurling for easy grip. Mounted in reverse position to facilitate ease in changing slides.

- **The Eyepieces:** Widefield High Eye point Focusing 10x eyepieces / FN 20mm. Trinocular Siedentopf viewing head with 30° inclined binocular eyepiece with interpupillary adjustment 50mm to 75mm. Vertical trinocular tube for camera integration. Eyepiece can hold a 20mm diameter reticle if the reticle retaining ring is added.
- **Total Magnification:** 40x, 100x, 400x, 1000x.
- **Specimen Stage:** Large 140mm x 135mm stage (5.5" x 5.25"), with professional quality, built-in, low profile, ball-bearing mounted automatic specimen holder with low-position.
  coaxial controls, 78mm (x) by 50mm (y) movement with graduations of 0.1mm. 1.25 N.A. Abbe condenser has rack & pinion focusing and iris diaphragm.
- **Focusing:** Coaxial course and fine focusing controls, coarse has tension adjustment, fine is graduated. Locking position for stage.
- **Illumination:** 3.3v, 3W LED with variable rheostat manage and on/off switch.110~240V power supply.
- **Size & Weight:** "7.25" W x 14.5" D x 16" H, 16 lbs.
- **Warranty:** 5 Year warranty, excluding bulb, cord and fuse [5].

IV. PROPOSED WORK

PREPROCESSING

Threshold

Threshold is a one of the widely methods used for image segmentation. It is useful in discriminating foreground from the background. By selecting an adequate threshold value T, the gray level image can be converted to binary image. The binary image should contain all of the essential information about the position and shape of the objects of interest (foreground). The advantage of obtaining first a binary image is that reduces the complexity of the data and simplifies the process of recognition and classification. The most common way to convert a gray-level image to a binary image is to select a single threshold value (T). Then all the gray level values below this T will be classified as black (0), and those above T will be white (1). Image segmentation is a definition of splitting an image into a set of different regions. Thresholding is a special type of image segmentation. An arbitrary image is segmented into its background and foreground by choosing an optimum threshold value. If the gray-level or brightness of the pixel in an image is greater than the threshold value, then it is a foreground pixel or otherwise it is a background pixel as shown in equation

$$f(i, j)=\begin{cases} 0 \text{ for } g(i,j)\leq T \\ 1 \text{ for } g(i,j) > T \end{cases}$$

Where f (i, j) is the binarized image and (i, j) is the pixel coordinate [6].

Adaptive threshold

Adaptive thresholding classically takes a gray scale or color image as input and, in the simplest implementation, outputs a binary image representing the segmentation. For each pixel in the image, a threshold has to be calculated [7].

Median filter

The Median filter is a nonlinear digital filtering technique, frequently used to remove noise. Median filtering is very widely used in digital image processing because an under certain conditions, it preserves edges whilst removing noise. The main idea of the median filter is to run through the signal entry by entry, replacing each entry with the median of neighbouring entries. Note that if the window has an odd number of entries, the median is simple to define: that is just the middle value after all the entries in the window are sorted numerically. For an even number of entries, there is more than one possible median. The median filter is a robust filter. Median filters are widely used as smoothers for an image processing, as well as in signal process and time series processing. An image containing salt-and-pepper noise will have dark pixels in bright regions and bright pixels in dark regions. This type of noise can be caused by dead pixels, an Analog to digital converter errors, bit errors in transmission, etc. This can be eliminated in large part by using dark frame subtraction and by the interpolating around dark/bright pixels. Noise is a disturbance which causes fluctuations in the pixel values. If the image is corrupted by an impulse noise, morphological operations are used in removing such noise. Impulse noise is caused by sudden disturbance in the image signal. The morphological opening followed by a closing operation can remove the noise [8].

To demonstrate, using a window size of three with one entry immediately preceding and following each entry, a median filter will be applied to the following simple 1D signal: x = [2 80 6 3] So, the median filtered output signal y will be: y[1] = Median[2 2 80] = 2 y[2] = Median[2 80 6] = Median[2 6 80] = 6 y[3] = Median[80 6 3] = Median[3 6 80] = 6 y[4] = Median[6 3 3] = Median[3 3 6] = 3 i.e. y = [2 6 6 3].

Contrast stretching

Contrast stretching (cs) is the difference in luminance or colour that makes an object (or its representation in an image or display) distinguishable. In visual observation of the real world, contrast is determined by the colour and brightness of an object [9]. The human visible system is more sensitive to contrast an absolute luminance; we can perceive the world similarly regardless of the large change an illumination over the day or from place to place. The maximum contrast of an image the contrast ratio or dynamic range.

HISTOGRAM EQUALIZATION

Histogram equalization is a spatial domain process that produces output an image with uniform distribution of pixel intensity means that histogram of an output image is packed down and extended systematically. Histogram is defined as the statistical prospect distribution of every gray level in a digital image. The Histogram Equalization (HE) is a very popular technique for contrast enhancement of images Contrast of images is resolute by this dynamic range, which is defined the same as the ratio between the brightest and the darkest pixel intensities. The histogram provides information for the contrast, overall amount distribution of an image. Suppose input image f(x, y) composed of discrete gray levels in the dynamic range [0, L-1] then the transformation function C (rk) is defined as

$$S_{K=}C\ (r_k) = \sum_{i=0}^{k} p(r_i) = \sum_{i=0}^{k} \frac{n_i}{n}$$

Where $0 \leq sk \leq 1$ and k = 0, 1, 2, …, L-1. In an equation (1), n_i represents the number of pixels having gray level r_i n is the total number of pixels in the input image, and $P(r_i)$ represents as the Probability Density Function (PDF) of the input gray level r_i . Based on the PDF, and the Cumulative Density Function (CDF) is defined as C (rk) [10]. This mapping in (1) is called Histogram Equalization (HE) or Histogram Linearization. Here s_k can easily be mapped to the dynamic range of [0, L-1] multiplying it by (L-1).

MORPHOLOGICAL OPERATIONS

Erosion

In mathematical morphology, erosion is important an operation. The aim of erosion operators is to shrinks the foreground and enlarges background. Erosion is used to make an object smaller and by removing is outer layer of pixels. After applying the erosion operator on the image, the image becomes darker. This operator takes an image and structuring element as inputs and thins the object.

Dilation

Dilation operator can be applied to binary and grey scale images. An objective of this operator is to enlarge the foreground and shrinks background. That gradually increases the boundaries of the region, while the small holes present in an image become smaller. It increases the brightness of the object.

Opening

An opening operation is combination of dilation and erosion operations. If A and are two sets of pixels, then in the opening, first erode A by B then dilate the result by B. Opening is the unification of all B objects entirely contained in A . Use of this operator is smoothing the edges, breaking the narrow joints or separates the objects and thinning the protrusions that are present in an image.

Closing

Closing operation is a dilation operation followed by an erosion operation. Closing is the group of points, which the intersection of object B around them with object A is not empty [11]. Closing is useful for smoothing sections of contours, eliminates the small holes and fills gaps in contours.

V. OVERVIEW OF FUZZY C-MEANS

Fuzzy c-means (FCM) is a method of clustering which allows one piece of data to belong to two or more clusters. Fuzzy C-means (FCM) is one of the most commonly used to fuzzy clustering techniques for different degree estimation problems. It is provides a method that the solves how to group data points that populate some multidimensional space into a specific number of different clusters which must be the known a priori. FCM employs fuzzy partitioning such that a data point can belong to be several groups with the degree of membership matrix U is constructed of an element that have value between 0 and 1. The aim of FCM is to find the cluster centres that minimize a dissimilarity function. After each iteration membership and cluster centres are updated according to the formula:

$$V_j = \left(\sum_{i=1}^{n} (\mu_{ij})^m x_i\right) / \left(\sum_{i=1}^{n} (\mu_{ij})^m\right), \forall_j = 1, 2, 3, \ldots\ldots c$$

Where,'n' is the number of data points.
'v_j' represents the j^{th} cluster center.
'm' is the fuzziness index $m \in [1,\infty]$.
'c' represents the number of cluster center.
'μ_{ij}' represents the membership of i^{th} data to j^{th} cluster center.
'd_{ij}' represents the Euclidean distance between i^{th} data and j^{th} cluster center.
Main objective of fuzzy c-means algorithm is to minimize:

$$J(U, V) = \sum_{i=1}^{n} \sum_{j=1}^{c} (\mu_{ij})^m \quad \|x_i - v_j\|^2$$

Where, '$\|x_i-v_j\|$' is the Euclidean distance between i^{th} data and j^{th} cluster center.

FUZZY C-MEANS CLUSTERING ALGORITHM

This algorithm works by assigning membership to the each data point corresponding to each cluster center on the basis of distance between the cluster center and data point. More data is near to the cluster center more is its membership towards the particular cluster center. Clearly, summation of membership of an each data point should be equal to one [12].

Let $X=\{x_1,x_2,x_3,\ldots\ldots,x_n\}$ be the set of data points and $V=\{v_1,v_2,v_3,\ldots\ldots v_c\}$ be the set of centers.
Step1: Randomly select 'c' cluster centers.
Step2: Calculate the fuzzy membership 'μ_{ij}' using:

$$\mu_{ij} = 1 / \sum_{k=1}^{c} (d_{ij} / d_{ik})^{(2/m-1)}$$

Step 3: Compute the fuzzy centers 'v_j' using:

$$V_j = \left(\sum_{i=1}^{n} (\mu_{ij})^m x_i\right) / \left(\sum_{i=1}^{n} (\mu_{ij})^m\right), \forall_j = 1, 2, 3, \ldots\ldots c$$

Step 4: Repeat step2 and step3 until the minimum 'J' value is achieved or
$\|U^{(k+1)} - U^{(k)}\| < \beta$.
Where, 'i' is the iteration step. 'β' is the termination criterion between [0, 1]. '$U = (\mu_{ij})_{n*c}$' is the fuzzy membership matrix. 'J' is the objective function.

Advantages of FCM algorithm

The FCM algorithm yields better quality then K-means algorithm. Unlike k-means where data point must exclusively belong to the one cluster center here data point is assigned membership to an each cluster center as a result of which data point may belong to more then one cluster center.

Disadvantages

The FCM algorithm assigns a priori specification of the number of clusters, with the lower value of β we get the better result but at the expense of more number of iteration. A Euclidean distance measures can unequally.

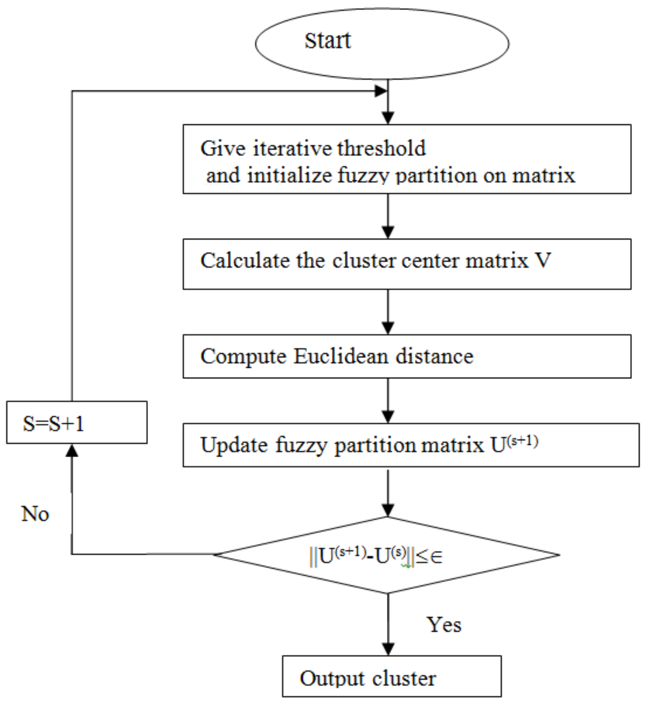


Figure 2. Flow chart on fuzzy c-means

VI. FUZZY K-MEANS CLUSTERING

The Clustering is the process of separating or grouping a given set of patterns into disjoint clusters. This is done such that patterns in the same cluster are like a pattern is belonging to two different clusters are different. The k-means method has been shown to segment the background of object. The K-Means algorithm is the most popular partitioning based clustering technique. It is an unsupervised algorithm which is used in clustering. It chooses the centroid smartly and it compares centroid with the data points based on their intensity and characteristics and finds the distance, the data points which are similar to the centroid are assigned to the cluster having the centroid. New _k'centroids are calculated and thus k-clusters are formed by finding out the data points nearest to the clusters. An each object in the fuzzy clustering has some degree of belongingness to the cluster .So, then an objects that are present on the edge of the cluster are different from the objects that are present in the centroid i.e. objects on edge have lesser degree than the objects in the center. Any object p has assigned a set of coefficients that are present in the k-1th cluster $w_{k-1}(p)$. In a fuzzy c-means, the centroid of a cluster is the mean of all objects present in the cluster, measured by their degree of belonging of points to the cluster [13]:

$$C_{k-1}=\frac{\sum_p W_{k-1}\,(p)^m p}{\sum_p W_{k-1}(p)^m}$$

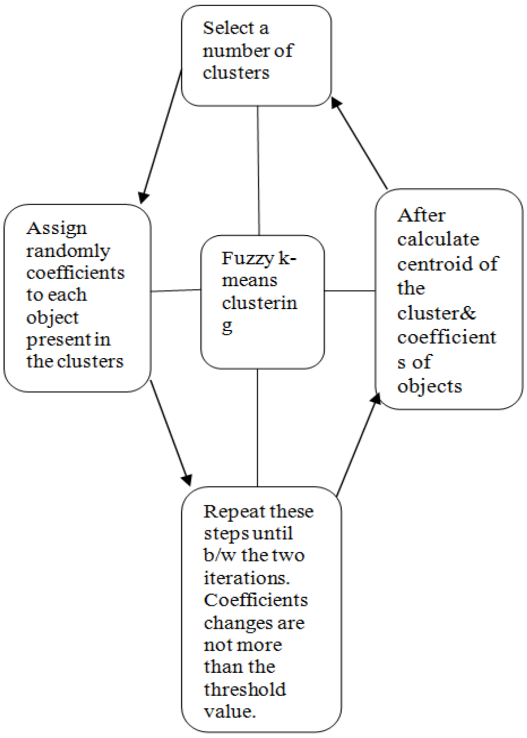


Figure 3. Flow diagram of fuzzy k-means

METRICS

Peak Signal to Noise Ratio (PSNR)

The measure of quality of the image by comparing denoised image with original image. That is an expression used to depict the ratio of maximum possible power of image (signal) with the power of the corrupting noise that affects the quality of its representation. The Peak Signal to Noise Ratio (PSNR) is used to validate the performance of the proposed method. It measures the visual similarity between two images and defined as,

WORK FLOW OF PROPOSED METHOD

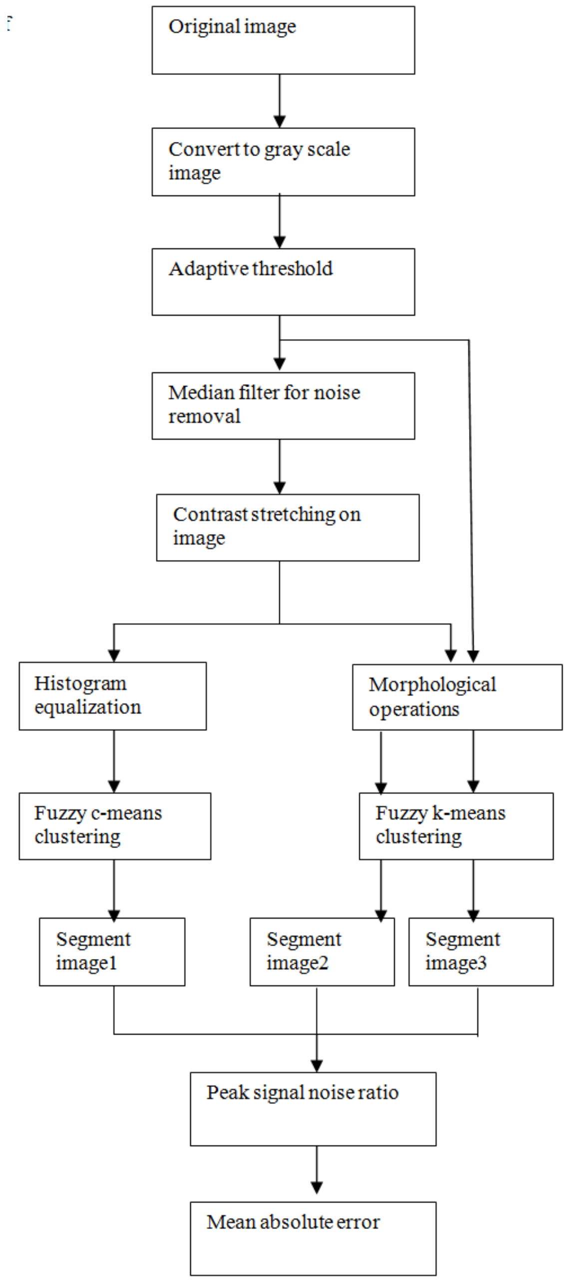


Figure 4. Work flow of proposed method diagram

PSNR=10 log10 (M/MSE)

The Peak Signal to Noise Ratio (PSNR) is used to validate the performance of the original image and an output image.

Mean Absolute Error (MAE)

It is absolute error between the original image and the de-noised image. It represents an average value of introduced deviation per pixel with respect to original image [14].

MAE = sum (sum (abs (error))) / sum (sum (origImg));

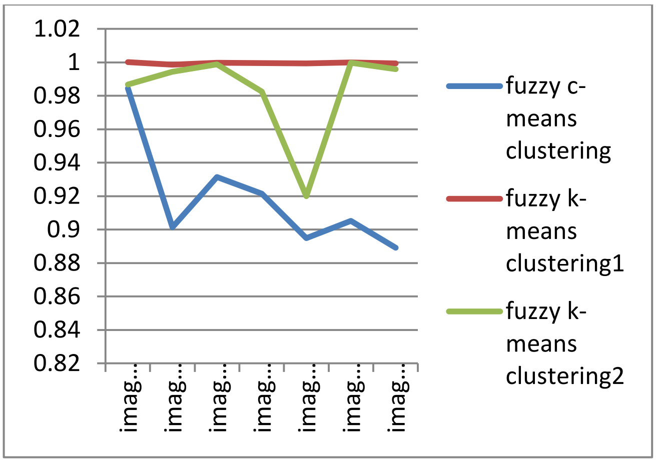


Figure 5. Comparison Of PSNR

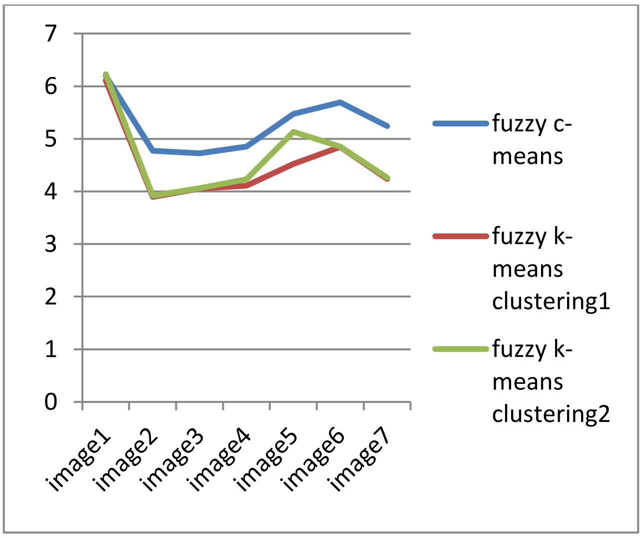


Figure 6. Comparison of MAE

Table 1. PSNR Values of The Results Of Proposed Methods

Images	Segment image1	Segment image2	Segment image3
Image1	0.9844	1.0001	0.9867
Image2	0.9013	0.9986	0.9944
Image3	0.9315	0.9997	0.9988
Image4	0.9215	0.9996	0.9826
Image5	0.8948	0.9994	0.9200
Image6	0.9053	1.0000	0.9997
Image7	0.8892	0.9994	0.9960

This table show that an evaluate metrics on PSNR and then calculate the original image with segment the fuzzy clustering images.

Table 2. MAE Values Of The Results Of Proposed Methods

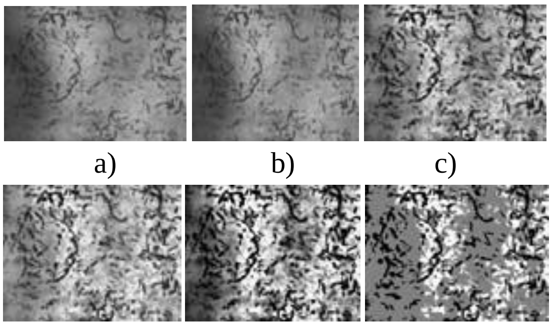
Images	Segment image1	Segment image2	Segment image3
Image1	6.1959	6.1128	6.2268
Image2	4.7762	3.8937	3.9213
Image3	4.7228	4.0547	4.0606
Image4	4.8530	4.1123	4.2291
Image5	5.4742	4.5234	5.1333
Image6	5.6925	4.8490	4.8508
Image7	5.2406	4.2391	4.2633

This table show that an evaluate metrics on MAE and then calculate the original image with segment the fuzzy clustering image.

VII. RESULT AND DISSCUSSION

The results have been simulated in MATLAB. The images have been taken from the biological database and the segmentation is done by fuzzy c means and k-means which classify the image into clusters. It is used to evaluate the efficiency of the clusters based on each iteration and the computational time required to simulate the image **.**

Pre-processing techniques on image

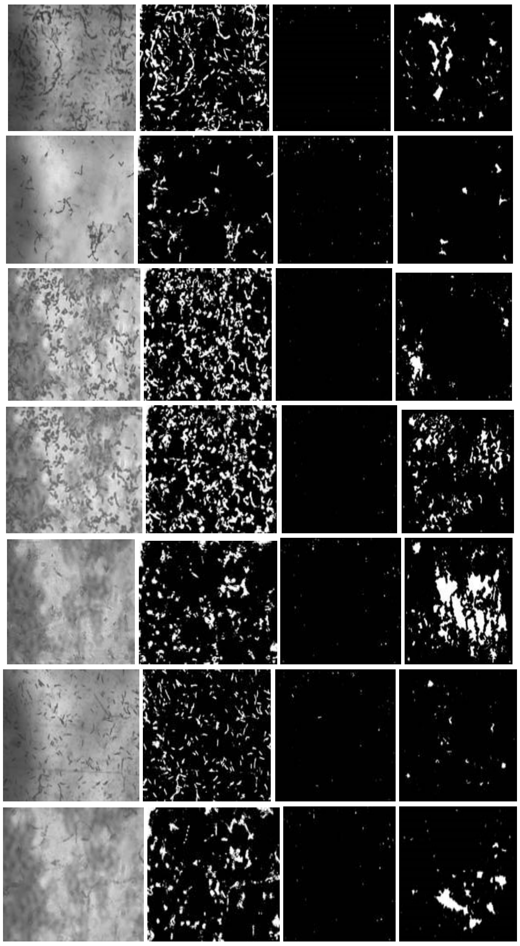


a) b) c)

d) e) f)

Figure 7. Pre-processing images on a) original image b) median filter noise removal c) adaptive threshold d) contrast image e) histogram equalizations f) morphological operations.

PROPOSED METHOD ON IMAGE



a) b) c) d)

Figure 8. a) Original image b) foreground image c) dotted image d) background image

VIII. CONCLUSION

Here we discussed about an Image segmentation is often used to distinguish the foreground from the background. In pre-processing used threshold, median filter, contrast stretching, morphological operation applied in Fuzzy clustering method. Segment the microscopic image to get three segment output. Compare the segment image peak signal noise ratio and mean absolute error, get best metrics output on proposed Fuzzy clustering method. From the results the proposed method yields better results both in visual perception as well as in quantitative estimation values.

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