International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Comparison of Different Image Segmentation Algorithms to Evaluate Healing Progression

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ABSTRACT :

Chronic wound management has become a global healthcare challenge. To study healing progression, traditional assessment methods are widely reliant on manual perception that often causes ambiguities and inadequacies. Morphological alteration of epithelial rete-pegs is an important aspect for monitoring healing progression which is associated with epithelial maturation and disrupted with injuries or any pathological intervention. At present there is little information available for computational analysis of rete-pegs integrity. Though several algorithms exist for analysis of the histological features, selection of suitable computational methods for segmenting the epithelium rete pegs is a difficult task. To understand the healing progression in terms of epithelial maturity different established segmentation methods have been applied on histo-pathological images at different phases of healing process. To select appropriate segmentation method, performances of different methods are compared in terms of nonlinear objective assessments (energy, entropy), processing speed and performance matrices [specificity, sensitivity, accuracy, Peak signal to Noise Ratio (PSNR) and Mean Square Error (MSE)]. Present study demonstrated that amongst six different segmentation methods k-means with thresholding is suitable for segmentation of the desire epithelial rete-pegs from histo-pathological images at different healing stages. Nonlinear objective assessment parameters are significantly improved in k-means with thresholding approach in comparison to other methods. Further performance of k-means with thresholding segmentation is better compared to other algorithms as it has high PSNR value and low MSE value and notable accuracy value distinguish it from other algorithms.

Keywords: Epithelial Rete Pegs, segmentation methods, K-Means with thresholding, Non-linear objective assessment, Accuracy, PSNR, MSE

Introduction

Wound healing is a complex and multi-factorial biological process. Management and treatment of chronic wound healing contribute a larger share in annual healthcare burden. Therein identification of healing stages becomes an important step in assessment of healing progression. However, wound evaluation mostly depends on visual perception and often results in ambiguous outcomes [1]. In this respect, computer aided image segmentation can profoundly be accurate in wound assessment [2]. For evaluating healing progression histological changes are still an essential aspect. Appearance and morphology of different epithelial features are important aspects for evaluating epithelial maturation and appropriate re-epithelialization phase [3]. Healing process can be categorized into three stages based on their epithelial features. In the initial stage of wound healing, flat epithelial surface indicates epithelial immaturity. With healing progressing rete-pegs began to re-appear and epithelial undulation as well as gradual reformation of rete-pegs has been observed after appropriate therapeutic interventions. Further, the rete-pegs become more matured in terms of length, width and periodical appearance of rete-pegs indicating the restoration of normal epithelial structures and completion of re-epithelialisation [4]. Hence, segmentation of epithelial rete-pegs with suitable image processing method would possibly help in more precise assessment of healing progression. Although image segmentation methods are well studied, there is no universal method that can be applied on any image type [5-7].

Different segmentation algorithms have been proposed for computational analysis of image features in the last few decades [8-12]. Various research groups have applied different methods for standardization of automatic segmentation of epithelial features to improve diagnostics approach as well as to monitor therapeutic progression. Report by Jadav et al [13] analyzed histological features like length of rete- ridges, their radius of curvature, conical papillae, epithelial cell count, cell population density in different zones of epithelial, cell density gradient, variation of epithelium thickness in different cancer model using segmentation algorithm like region growing technique, optimal thresholding and thinning algorithms to extract the target features. Kumar *et al* [14] have developed an interactive tool for accurate assessment of the healing status of the wound, where KNN is used for all types of wounds and Fuzzy is applied for diabetic and venous ulcers. In another study conducted by the same authors, healing characterization was performed through developing an efficient wound healing classifier that segment the wound images and classified them according to the wound types [15]. Briskillal *et al* [16] employed color image segmentation for analyzing dermatological ulcers. Different color image classifiers have been used to classify the segmented image, and the classification rate is also calculated to estimate their performance. Texture characteristics used for detection of healing process by Loizou *et al* [2] in which they performed deep wound characterization which is otherwise difficult to characterize because of its small surface area where color-based detection can lead to erroneous results. Aaron *et al* [17] have made a comparative study between segmentation algorithms and thresholding algorithms. Comparative study of two segmentation algorithms namely "watershed segmentation algorithm" and "k-means clustering segmentation algorithm" have been used to evaluate the efficiency of detection of brain tumor. K-means clustering is an algorithm to classify or to group the objects based on attributes or features into k number of groups. In K-means clustering, numbers of clusters are generally determined in advance [18-20]. In thresholding only intensity value is considered so there is no certainty that the pixels identified by the thresholding process are contiguous.

Based on above we proposed that combination of K-means and thresholding method may provide a novel approach for addressing the limitations of individual methodologies. Epithelial rete pegs can be isolated more accurately using k-means segmentation compared to other used algorithms. But thresholding process is necessary to overcome the segmentation of additional part besides region of interest. Present study compares performance of six different segmentation methods (Thresholding, Otsu, Watershed, Marker Controlled Watershed, K-Means, K-Means with Thresholding) for processing of epithelial rete-pegs and proposes a novel method for combination of K means and thresholding process for successful segmentation of rete pegs at different healing stages.

Materials and Methods

1.1. Clinical study: Patients (n=5) of either gender (age = 40-60 years) with non-healing wounds non-responding to conventional topical antibiotics were included under informed written consent. Ethical clearance was obtained from institutional ethical committee according to Helsinki declaration. Subsequently, honey based occlusive dressing (i.e., honey-soaked gauge followed by a layer of dry cotton tied with crepe elastic bandage) was applied on LLW [19, 20]. Redressing was performed with an interval of 24 h for initial 7-8 d and the interval further increased to 48–72 h [21, 22].

2.1.1 Processing of collected biopsies: Incisional biopsies from wound edge were collected from patients under local anaesthesia (Xylocaine) at different time points. Biopsies fixed with 10% phosphate buffered formalin and processed for 4 µm thick paraffin sections on poly-L-lysine (Cat.No. P 8920 Sigma-Aldrich) coated slides and stained with H & E for histo-pathological intervention.

2.1.2 Microscopic studies: Stained slides were observed by Nikon inverted fluorescence microscope (Nikon eclipse Tί, Japan) at 20x magnification. Field of view for image was 690 x 515 μm^2 and pixel resolution was 0.17 µm.

1.2. Image Processing: The methodology comprises 1) Input Image 2) Image Preprocessing 3) Image Segmentation (4) Required output. The block diagram of image processing technique is listed in Fig 1

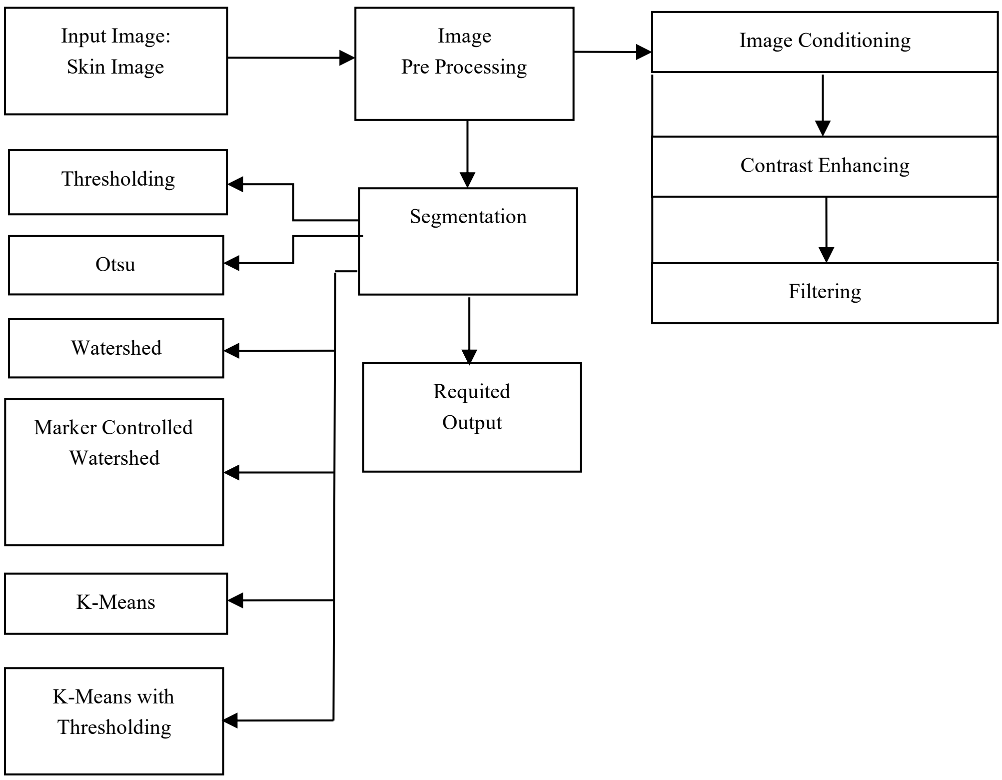


Fig.1. Block Diagram of Image Processing Technique

In present study six segmentation algorithms have been used to evaluate the best performance for segmenting the epithelial rete-pegs. The algorithms which are used (1) Thresholding, (2) Otsu Method, (3) Watershed Segmentation, (4) Marker Controlled Watershed Segmentation, (5) K-Means, (6) K-Means with Thresholding. Total 21 images at different healing stages were analyzed through the above-mentioned segmentation methods. Performance of all these algorithms was compared in respect to their average processing time and nonlinear objective assessments like energy and entropy.

1.2.1. Thresholding: Thresholding is basic and most representational algorithm in image segmentation. In local thresholding process, a threshold value (T) is first set, if the image intensity is less than fixed threshold value T, then each pixel in image has replaced with a black pixel or a white pixel if the image intensity is greater than that constant threshold value.

1.2.2. Otsu Method: Otsu is global thresholding method. It is an automatic threshold selection region-based segmentation method. It works on only gray value of image. Computation of gray level histogram is required before running Otsu method. Then cumulative sums, means and global intensity means are computed then find the threshold that minimizes the weighted within-class variance.

1.2.3. Watershed Algorithm: It is a simple intuitive method; it is fast and can be parallelized. It divides the image into separated regions even if the contrast is poor. In Watershed algorithm firstly computing the gradient magnitude of the input image, and then computing the watershed of the gradient magnitude, finally superimpose the gradient on the original one.

1.2.4. Marker Controlled Watershed Segmentation: In Marker Controlled Watershed Segmentation Algorithm two markers have defined- a) internal and b) external markers. Marker is connected component belonging to an image. A marker can be a single point or a set of connected or disconnected points. Markers are used to modify the gradient image. Markers are placed inside an object of interest; internal markers associate with objects of interest, and external markers associate with the background. After segmentation, the boundaries of the watershed regions are arranged on the desired ridges, thus separating each object from its neighbors.

1.2.5. K-Means Clustering Algorithm: K-Means is one of the popular partitioning algorithms. The idea is to classify the data into k clusters where k is the input parameter specified in advance through iterative relocation technique which converges to local minimum. It consists of two separate phases first phase is to determine k centers at random one for each cluster. Next phase is to determine distance between data points in Dataset and the cluster centers and assign the data point to its nearest cluster. Euclidean distance is generally considered to determine the distance. When all the data points are included in some clusters an initial grouping is done. New centers are then calculated by taking the average of points in the clusters.

The steps of K-means algorithm are simply described as follows

1. Input: The number of clusters k has taken as input and N objects to be cluster {x1, x2…, xn};
2. Output: k clusters and the sum of dissimilarity between each object and its nearest cluster center is the smallest.
   - k objects are selected arbitrarily as initial cluster centers (m1, m2..., mk);
   - Calculate the distance between each object xi and each cluster center, then each object is assigned to the nearest cluster, formula for computing distance as:

$$d(x_i, m_j) = \sqrt{\sum_{j=1}^{d} (x_{i1} - m_{j1})}$$

i=1, 2,…N; j=1, 2,…k; and $d(x_i, m_j)$ which denotes the distance between data i and cluster j

- As the new cluster centers the mean of objects in each cluster is Calculated,

$$m_i = \frac{1}{N_i} \sum_{j=1}^{N_i} x_{ij}$$

i=l, 2 . . . k; N_i is the number of samples of current cluster i.

K- Means algorithm segmented the desire epithelial rete pegs portion as well as it also contains some unwanted information which is not our region of interest. So, thresholding methods are used to remove that unwanted information.

1.3. Calculation of Elapsed time: Achieving a high performance in image processing system is the principal goal. How much time it takes for a segmentation method to generate the output is called elapsed time. Elapsed time for each sample was calculated for individual algorithms and then the average values were taken.

1.4. Nonlinear Objective assessment: To estimate the quality of the segmented images using different algorithms following different nonlinear objective assessment parameters used for evaluations are energy and entropy.

1.5. Determination of Energy: Energy: The gray level energy indicates how the gray levels are distributed. It is expressed as,

$$E(X) = ?_{i=1}^{X} P(X)$$

where

E(X) - refers the gray levels energy with 256 bins

P(X) - represents the probability distribution function

1.5.1. Determination of Entropy: Entropy: Unpredictability or information content is measured by entropy. It is a statistical measure of randomness. It is also used to characterize the texture of the input image. Entropy is formulated as

$$H(X) = -\sum_{i=1}^{K} p\,(i)\log_2 p(i)$$

Where,

H(X) - represents the entropy

P(i)- represents the probability distribution function

1.5.2. Determination of Sensitivity, Specificity and Accuracy: Accuracy: It is the proportion of true results (both true positives and true negatives) in the population.

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

Sensitivity - It is the proportion of positives measured as such

$$\text{Sensitivity} = \frac{\text{TP}}{\text{Tp} + \text{FN}}$$

Specificity - It is the proportion of actual negatives which are correctly identified as such

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

Determination of MSE (Mean Square Error) and PSNR (Peak Signal to Noise Ratio)

MSE - It measures the average of the square of the difference between the segmented image and the original image.

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(I\_seg_i - I\_orig_i\right)^2$$

PSNR- It represents region homogeneity of the final partitioning. The higher value of PSNR represents the better segmentation result.

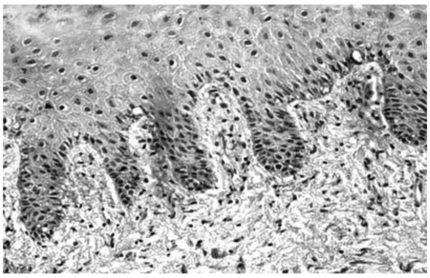
$$PSNR = 10\log_{10}\frac{\max intensity^2}{MSE}$$

Results and Discussion

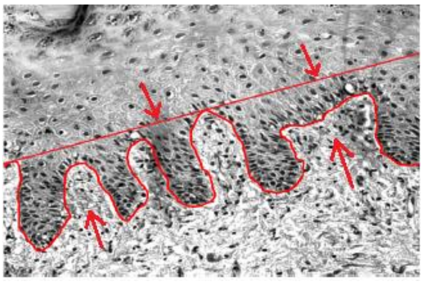
Table 1 shows the input image with ground truth and output of different segmentation methods. Thresholding is the simplest segmentation method that only considers the intensity value, means pixels are partitioned depending on their intensity value; no relationships exist between the pixels. Thus, there is no guarantee that the pixels identified by the thresholding process are contiguous. Also, extraneous pixels that are not part of the target region are easily included as pixels intensity doesn't represent the normal intensity in the region [21]. Moreover, selection of appropriate threshold value is also difficult. Thus, Otsu is used for this purpose. This method requires computing a gray level histogram before running and considers pixel's gray-level information instead of spatial neighborhood information of any pixel, so it is difficult to obtain satisfactory segmentation result [23]. This algorithm also fails to accomplish due to the global distribution of the target and background which are widely varied. It has been found that this algorithm never works on those images when the two classes are very unequal. Since, the segmentation by watershed has been used which combines the two main approaches - frontier and the region approach and is very powerful technique for rapid detection of both edges and regions thus watershed segmentation is one of the commonly used approaches. The application of watershed algorithm is greatly influenced by the noise and other local irregularities in the image [24]. But the major problem of the watershed transformation is over-segmentation due to the presence of many local minima that means many segmented regions [24] [25] [26]. To decrease the effect of severe over segmentation, marker-controlled watershed transformations have been applied on study images. This method is based on the concept of markers and is mostly used to modify the gradient image. This algorithm expressed the boundaries as ridges. As it initially marks the regions followed by segmentation, it is very hard to optimize the marking regions. Thus, this algorithm is unable to segment the epithelial rete pegs properly. K–means algorithm has been considered for segmentation of rete pegs portions. It has been found that K-means gives best results when data sets are distinct or well separated from each other and it does not produce the same result each run since the resulting clusters depend on the initial random assignments. So, it is very difficult to compare the quality of the cluster produce. Another disadvantage of this algorithm has been observed is prior specification of the number of cluster centers.

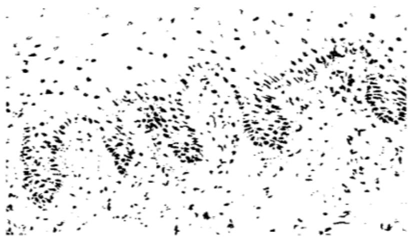
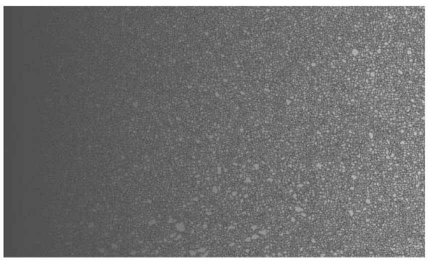
Table 1 Comparison of Different algorithms

Input Image



Ground Truth



Serial No.	Algorithms Used	Disadvantage	Segmented Outputs
1	Thresholding	Only the intensity value is considered. There is no guarantee that the pixels identified by the thresholding process are contiguous.	
2.	Otsu	It does not take into account pixel's spatial neighborhood information but only consider the pixel's gray-level information, so it is difficult to obtain satisfactory segmentation result.	
3.	Watershed	Over Segmentation	

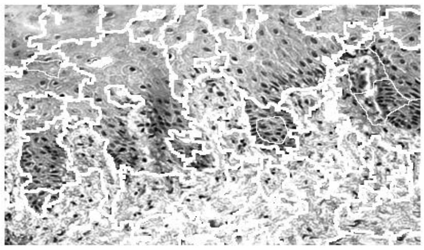
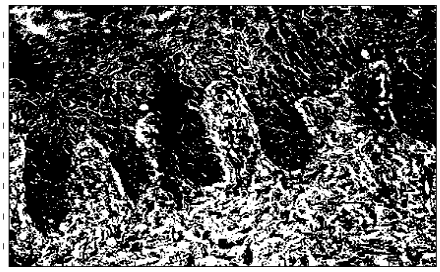
4.	Marker Controlled Watershed Segmentation Algorithm	Epithelial rete pegs are not properly segmented.	
6.	K-Means	Need to specify the number of clusters in advance. The algorithm doesn't have guarantee for optimal solution because its performance depends on an initial centroid.	

Fig. 2 shows the different patterns of epithelial rete pegs at different time points, segmented images using k-means and thresholding approach and the epithelial mask of those segmented images. From Table 1 it has been observed that individual k-means and thresholding is unable to segment the desired rete pegs portions. Using thresholding resulted in the loss of additional region and k- means include the extraneous unwanted region. But their combination which has been represented in Fig 2 segmented the desire epithelial rete pegs portion properly.

Fig. 2(a) depicted the image of wound bed at 0 day exhibiting almost flat epithelial surface indicating epithelial immaturity. Histo-pathological observation after 20 days of healing showed the appearance of rete-pegs and epithelial undulation (Fig. 2d) and gradual reformation of rete-pegs in this stage indicating healing progression. H and E image after 35 days of healing showed appearance of mature rete-pegs (Fig. 2g) in terms of length, width and periodical appearance. In fact, in this stage the pattern of rete pegs is more alike to the normal epithelial structure.

The algorithms are evaluated using the following metrices,

(i) Processing Time, (ii) Energy, (iii) Entropy, (iv) Specificity, (v)Sensitivity, (vi)Accuracy, (vii) PSNR, (viii) MSE

Original Image | **K-Means with Thresholded Image** | **Segmented Epithelial Mask**

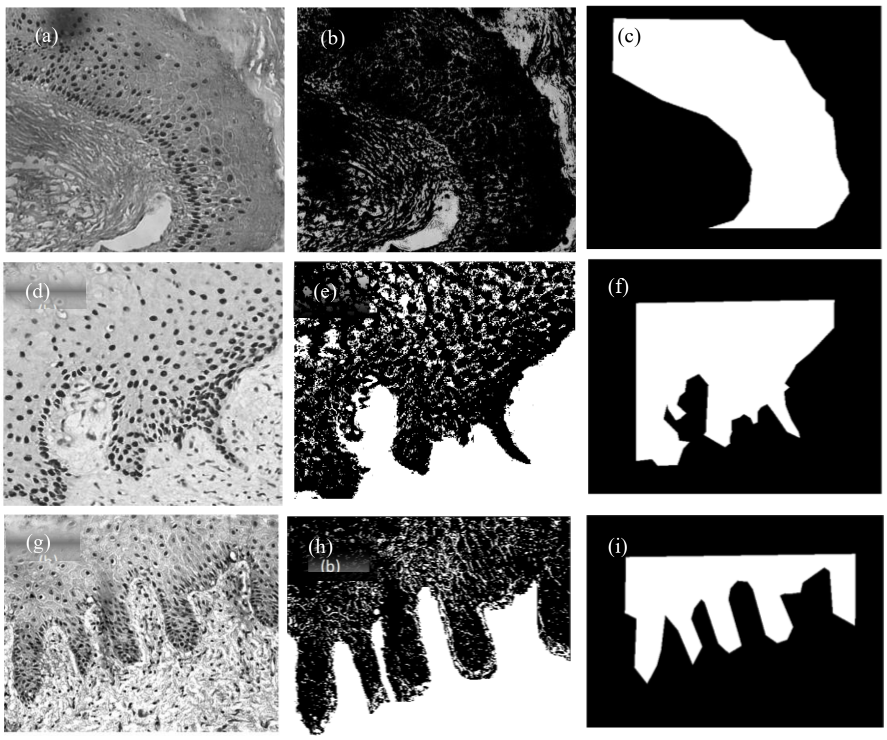


Fig.2. Original Image, K-Means with Threshold Image and their segmented epithelial mask

Table 2 Evaluation of algorithms at different healing stages

0_ Day			**15_ Days**			**35_ Days**		
Sensitivity (%)	**Specificity (%)**	**Accuracy (%)**	**Sensitivity (%)**	**Specificity (%)**	**Accuracy (%)**	**Sensitivity (%)**	**Specificity (%)**	**Accuracy (%)**
68.15	**61.90**	**63.5**	**70.85**	**60.86**	**65**	**65**	**58.33**	**67.5**
63.15	**61.90**	**63.5**	**61.11**	**63.63**	**65**	**65**	**75**	**70**
50	**40**	**47.5**	**51**	**54**	**52.5**	**61.53**	**50**	**57.5**
70	**70**	**70**	**70**	**75**	**72**	**80**	**73.68**	**75.60**
72.4	**73.6**	**74**	**71.54**	**74.64**	**76**	**76.2**	**78.4**	**79**
84.21	**76.19**	**80**	**81.81**	**84.21**	**85**	**90**	**87.5**	**87.5**

The database of 21 images has been processed to test and evaluate the performance of all the algorithms. Table 2 shows the Performance of different algorithms in terms of specificity and sensitivity quantifying their performance related to false positive and false negative instances at different stages of wound based on their ground truth. The accuracy calculated with the number of correct and incorrect classification in each possible value of the images classified. The following four relative metrics are calculated when comparing the segmented output of the epithelial rete pegs with the ground truth those were determined by biopsy:

TP: the number of true positives. Predicts the epithelial rete pegs correctly.

TN: the number of true negatives. Predicts the epithelial surfaces which were not marked as rete pegs that were also not classified as rete pegs.

FN: the number of false negatives. Predicts epithelial rete pegs as flat surface wrongly.

FP: the number of false positives. Predicts epithelial rete pegs as flat surface wrongly.

Table 3 Evaluation of algorithms at different healing stages using Energy & Entropy

	0_ Day		**15_ Days**		**35_ Days**		
Methods	**Energy**	**Entropy**	**Energy**	**Entropy**	**Energy**	**Entropy**	**Elapsed Time**
Threshold	**0.62**	**0.92**	**1.68**	**1.64**	**2.37**	**3.64**	**62.78**
Otsu	**0.46**	**0.98**	**1.25**	**1.96**	**2.16**	**3.09**	**74.46**
Watershed	**0.09**	**1.98**	**1.01**	**2.96**	**1.42**	**4.64**	**50.66**
Marker Controlled Watershed	**0.29**	**1.46**	**1.22**	**2.42**	**1.96**	**4.11**	**82.66**
K-Means	**0.77**	**0.86**	**2.26**	**0.58**	**4.64**	**1.69**	**56.76**
K-Means with Thresholding	**0.96**	**0.06**	**2.64**	**0.43**	**5.84**	**2.62**	**30.26**

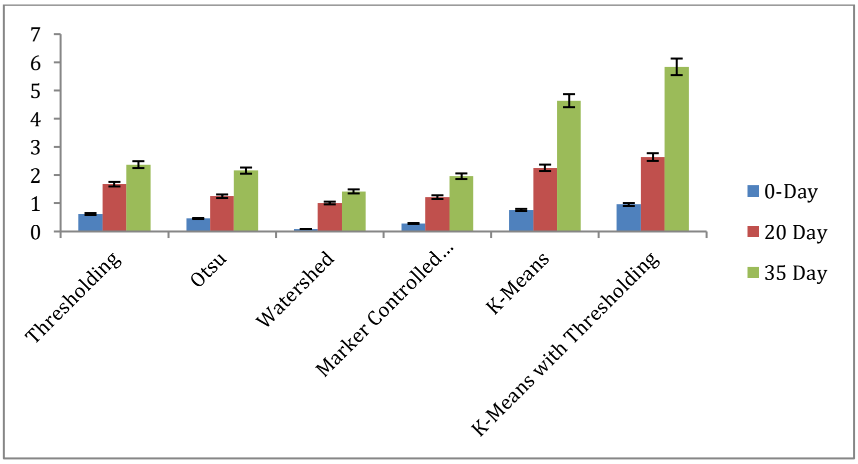


Fig.3. Comparison graph of different algorithms based on Energy

Fig.3. shows that k-means with thresholding has the high energy value compared to other algorithms from day 0 to day 35 that indicates the healing progression.

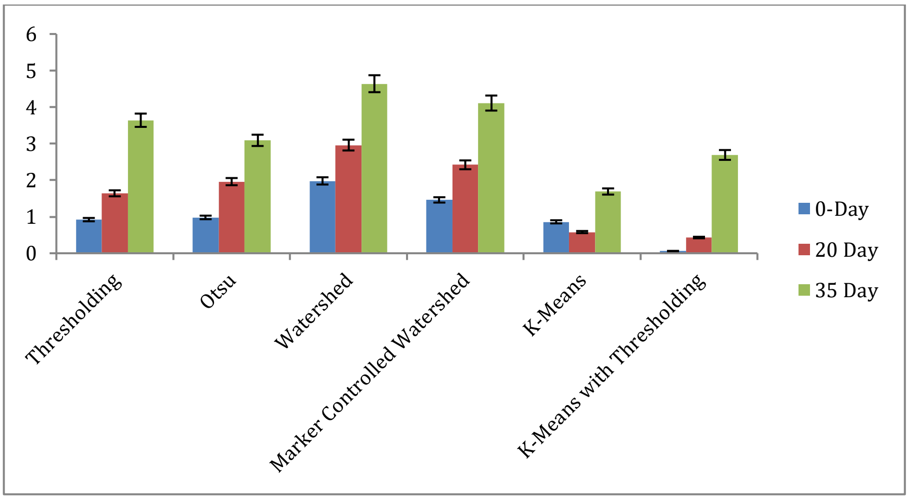


Fig.4. Comparison graph of different algorithms based on Entropy

Fig.4 depicts the comparison of different algorithms based on entropy. The k-means with threshold method gives smaller entropy value compared to others segmentation algorithms.

Both Fig.3 and Fig.4 present the range and standard deviation (SD) which are used for describing error bars because they show how the data are spread at 0, 20 and 35 days for different algorithms.

Table 4 Evaluation table for algorithms using PSNR and MSE

Methods	PSNR	MSE
Thresholding	**56.86**	**123.41**
Otsu Method	**33.26**	**64.59**
Watershed	**28.21**	**124.41**
Marker Controlled Watershed	**68.48**	**54.48**
K-means clustering	**64.09**	**36.21**
K-means with threshold method	**22.25**	**205.81**

Table 4 shows the PSNR and MSE values of different algorithms. The average values of the different healing stages have been taken. Image quality measurement plays an important role in various image processing applications. There is an inverse relationship between PSNR and MSE. Hence higher PSNR value indicates the better image quality. Greater the accuracy value more efficient the segmentation process. From this comparative study it is observed that K-means with threshold method is an efficient method compared to other algorithms as its PSNR value is high and MSE value is low and significant accuracy level has been achieved also.

Both PSNR and MSE have been improved wound bed at 35 days successively compared to 0 day and 20 days that establishes the fact of progression towards heal. As inverse relation exist between PSNR and MSE that means high PSNR value and low MSE value gives the better result and this graph also presents that, throughout different healing stages.

Conclusion

Segmentation of target features plays key role in image processing. Present study compares and evaluates the effectiveness and accuracy of different segmentation algorithms used to segment out epithelial features from histo pathological images at different stages of healing. The data revealed that combination of k-means with thresholding approach achieving overall more accurate segmentation result in comparison to other algorithms. Different non-linear objective assessment parameters have been used for the quantitative measures, sensitivity, specificity, accuracy, peak signal to noise ratio (PSNR), mean square error (MSE) to evaluate different segmentation techniques. Comparative studies illustrated that the k-means with threshold approach is an efficient method not only for segmenting the epithelial rete pegs parts but also evaluates the healing progression in respect of PSNR and MSE. After the evaluation the overall results present that k-means with threshold method gives the better result in terms of elapsed time, energy, entropy, PSNR, MSE and accuracy in case of histo pathological images.

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