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Study on Sperm DNA Integrity Detection Using an Image Processing Approach

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ABSTRACT

Human reproductive issues are on the rise across the globe due to change in lifestyle, food habits and deteriorating environmental conditions. Hence, detection and treatment of infertility is gaining impetus. Quality of the sperm plays a crucial role in male infertility. Manual detection of sperm quality is prone to pitfalls. Computer aided sperm analysis is economically distant in developing and under developed countries. Hence, this research is an effort towards developing simple image processing techniques to detect the integrity of sperm DNA. Microscopic images of semen samples from subjects with and without fertility issues were considered. Two image processing techniques, namely, threshold and extended maximum transform and K-means clustering were employed to detect the core and halo parts of the sperm DNA. Based on the difference in the diameters of these parts, the sperm halos were categorized and degraded cells were identified. The sperm cell categorization with K means algorithm provides 96.6% accuracy with the limited number of samples. The results of the study suggest that the proposed method would help in developing simple and economical means of sperm integrity testing.

Keywords: Extended Maximum Transform, Image Processing, K-means clustering, Sperm Integrity, Sperm Morphology Analysis.

1. INTRODUCTION

The World Health Organization defines infertility as the inability to achieve pregnancy after 12 months or more of regular and unprotected sexual intercourse (WHO, 2010). Issues in fertility are categorized as male fertility and female fertility. It is estimated that male infertility constitutes 50% of all known infertility issues. Male infertility is heavily dependent on the quality of semen. In turn, semen quality rests in the quantity and quality of sperm. While sperm quantity is relatively less complex to measure, the quality of semen requires multiple tests to be performed.

Currently routine seminal analyses like assessing sperm morphology, motility, biochemical parameters etc., remain the gold standards to evaluate sperm health (WHO, 1999). However, it has been documented that approximately 15% of the patients with normal seminal parameters are under unexplained male infertility (Agarwal, 2005). Many studies have proven that seminal DNA damage negatively influences the fertilization potential of sperm (Evenson, 1999, Filatov, 1999 & Guzick, 2001). Sperm DNA is found to be highly organized and compact (Evenson, 2002). This ensures that the DNA content is protected through the sperms'journey into the female reproductive tract followed by fertilization of egg. This high level of sperm integrity is found to be essential for maintaining the reproductive potential of sperm (Bungum, 2012 & Fatemeh, 2015). Higher levels of sperm DNA damage has been found to be associated with

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increased failures in artificial reproductive techniques (Agarwal, 2003, Filato, 1999& Sheena, 2013).

There are many factors that led to increased sperm DNA damage, including environmental factors like cigarete smoking, pollution, work environment and other disease conditions like diabetes, cancer etc., (Schulte, 2017). Many methods have been implemented in assessing sperm chromatin integrity like sperm chromatin structure assay, the terminal deoxynucleotidyl transferase-mediateddigoxigenin-dUTP nick-end labeling (TUNEL) technique, comet assay etc., (Sun, 1997 & Sikka, 2016).

2. MATERIAL AND METHODS

2.1 Giemsa Staining and Image Generation

In this study, sperm chromatin dispersion assay was done to determine the sperm DNA damage. The procedure involves chemical denaturation of the sperm cells, followed by deproteination of sperm cell nuclei (Didion, 1985). This results in the appearance of sperm nuclei as a nucleoid, surrounded by extended halo of relaxed DNA loops. Semen samples were taken at a concentration of 5 - 10 X 10^6 cells /ml. 25 ml of the sample was mixed with molten agarose and carefully laid on a precoated slide. The slides were covered with a cover slip and were left at 4^0 C for 5 minutes. The coverslip was then carefully removed and immersed in an acid solution for 7 min. After that, the slides were immersed in the lysing solution, and rinsed with distilled water. The slides were then dehydrated with increasing concentrations of ethanol. After drying, the slides were stained with Giemsa dye, rinsed under water and viewed under a light microscope at 40 X magnification and 100 sperms were counted per slide. Sperms with minimal or no DNA damage appears as large halos and those with DNA damage forms small or no halos (Fernandez, 2005).

2.2 Image Preprocessing

Microscopic images of sperm smear consist of noises due to the stain, various secretions and chromatic debris (Abbiramy, 2010). Hence, pre-processing is done in this study primarily to select the region of interest in the images without the stain. Figure 1 shows the stain appearing in one of the sample images.

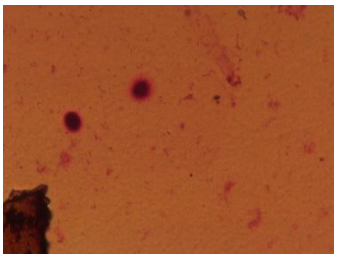


Figure 1. Microscopic sperm cell image with appearance of stain.

The ROI selection was done based on the histogram of the image. Wherever the stain occupied a considerable area in the image, a manual ROI selection was performed. Even though a manual ROI selection is not preferred in image processing methods so as to preserve the resolution of the images, the results of this study has not shown any considerable change due to the variation in ROI areas.

This work considered images from a database maintained by an infertility centre. The samples were of random subjects who consulted the centre in the past few years. The sperm cell images vary from healthy to fragmented DNA and also both. In sperm morphology analysis, a halo is observed around the core of sperm that is a consequence of chromatin released from proteins. The presence and absence of halo can indicate the fertility status. Further, image processing techniques can differentiate between small halo, big halo and medium halo and thus improve the diagnostic accuracy (Tandara, 2014). Figure 2 represents samples of sperm DNA cells with varied halo levels.

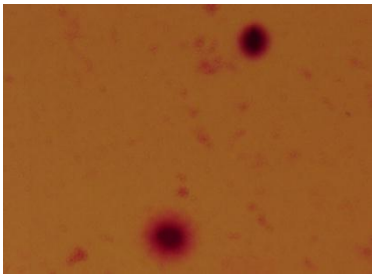


Figure 2. Image showing the variations in sperm halo.

2.3 Segmentation of Sperm DNA

In this study, two image processing techniques are proposed and their results were compared. Literature provides a number of methods for segmentation of colour images (Aimi, 2013&Fatemah, 2015). Figure 3 summarizes the overall approach followed in this work.

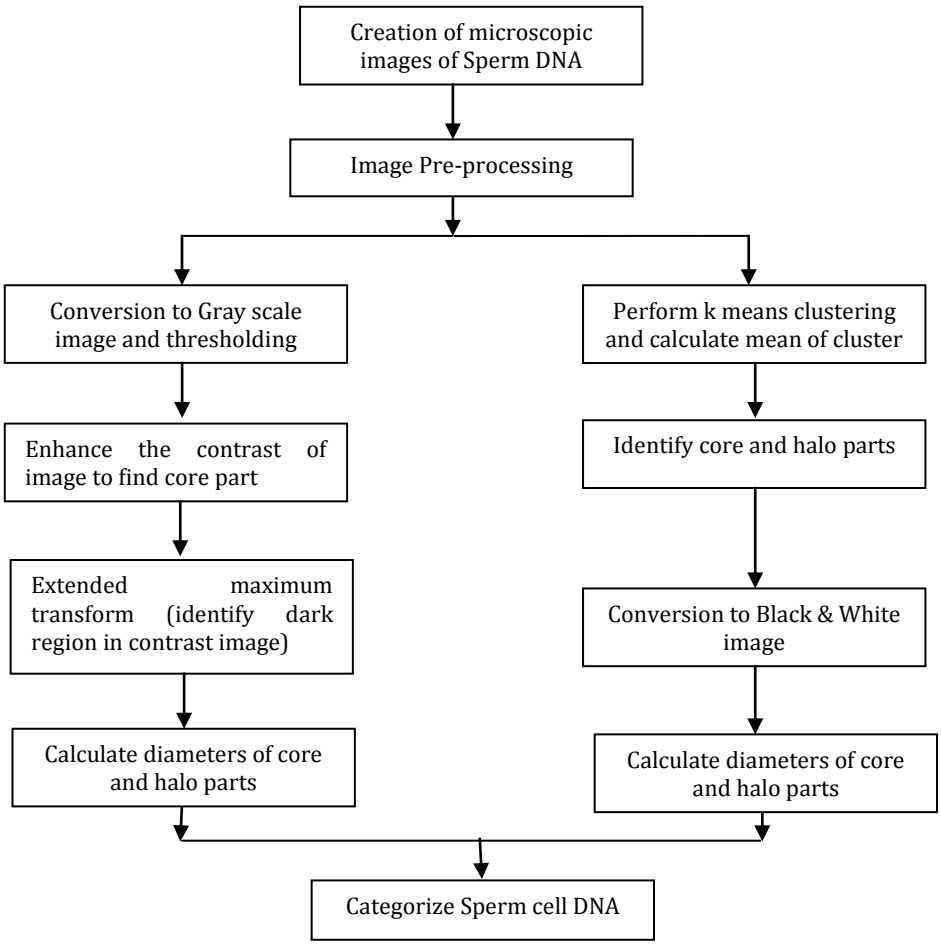


Figure 3. Proposed methodology for sperm cell integrity detection.

The Extended Maxima Transform method has been used in morphology analysis of round shaped objects in images (Yibo, 2015). In this transform, the original image is converted into its gray scale equivalent. The algorithm then extracts the local maxima. A threshold, h, determines the maxima that are retained if found above the threshold. Maxima below the threshold are naturally suppressed. Maxima are found as connected components of pixels with a constant intensity value. The external boundaries of the maxima should have a lower intensity level.

The Extended Maximum Transform uses mathematical morphology to extract regional maxima from the source greyscale image to the target image. The regional or local maxima are group of pixels with same intensity value and lower pixel value. To perform this transform, the regional maximum is calculated. The extended maximum transformation suppresses all regional maxima whose value is below or equal to threshold and finally forms binary image. Alternatively, extended maximum transform is obtained from regional minima of the corresponding H-maxima transformation. In this H-maxima transformation is obtained by dilation of greyscale image (f) from (f-h) where h is threshold followed by reconstruction.

$$\text{H MAX h(f)} = \text{R}_{\text{f}}^{\delta}(\text{f} - \text{h}) \quad (1)$$

The extended maximum transformation using H-maximum transformation is given as

$$\text{E MAX h(f)} = \text{R MAX[H MAX h(f)]} \quad (2)$$

The contrast of the greyscale image is increased by using contrast-limited adaptive histogram equalization (Zuiderveld, 1994). The contrast enhanced image is shown in figure 4. In this work, the threshold value was assigned with 145 and connectivity was selected as 8 based on observations on comparison with the ground truth. Figure 5 represents the segmented core parts and their overlaying on the original image.

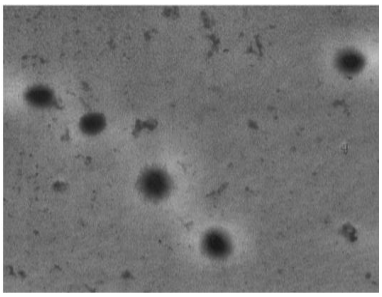
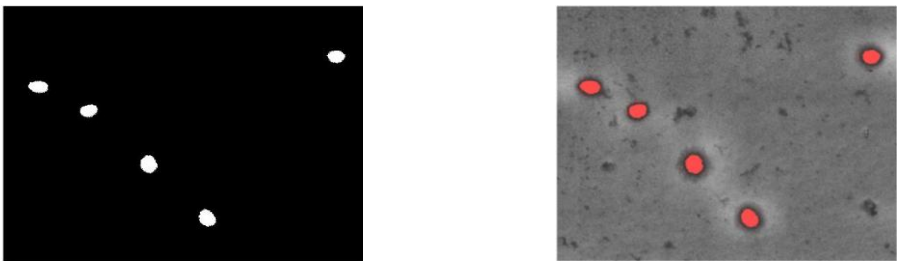


Figure 4. Contrast enhanced image during extended transform.



(a) **(b)**

Figure 5. (a) Segmented sperm DNA core using extended maximum transform and (b) core superimposed on the contrasted image.

K-means clustering is a popular, yet simple method of clustering based on unsupervised learning. In a related work on sperm morphology analysis (Chang, 2014 & Halkiotis, 2007), K-means clustering was employed to separate sperm cell images from its background. This algorithm divides the given images into a number of clusters (Shehroz, 2004 & Nameirakpam, 2015). Then, centroids are measured for each cluster. Using Euclidean distance parameter, every data point in the image is reassigned to the cluster which has its nearest centroid.

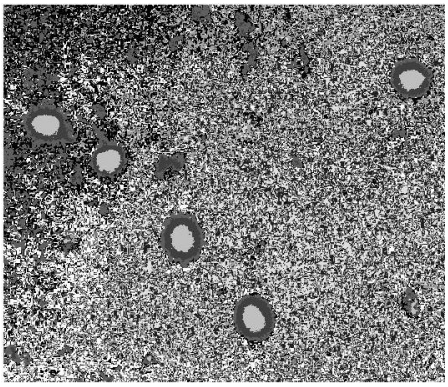


Figure 6. Segmentation of core and halo by K means.

2.4 Detection of Core and Halo

The DNA integrity of sperm cell was assessed based on the area of halo region. Initially, areas with certain threshold value (core - 2200 pixels, halo - 5000 pixels) were selected and the halo part was filled. Table 1 lists the threshold pixel values based on the observation.

Table 1 Threshold levels in K-means clustering method

Cell Assessment	Diameter difference (pixels)
Degraded cell	Less than zero
Small halo	10 to 70
Medium halo	70 to 100
Large halo	Greater than 100

The diameters of core and halo part were obtained by taking the average of major axis length and minor axis length. Table 2 represents the diameters obtained by Extended Maximum Transform (EMT) and K means clustering.

3. RESULTS AND DISCUSSION

This study was aimed to assess the damage of sperm cell DNA through Image Processing techniques. Microscopic images were generated from the stained sperm samples. It was observed that the staining procedure highlighted the sperm cells primarily in shades of pink and purple (Fernandez, 2005). Hence, to assess the DNA damage, two algorithms were proposed. Initially, the core and halo parts were detected using threshold and extended maximum transform. However, the detected core and halo part did not match with the ground truth dimensions of the original image. In the case of degraded cells where halo was absent, the observed threshold misinterpreted halo. Figure 6 shows the degraded cell being detected as a halo part.

Table 2 Diameter of core and halo obtained through EMT and K means clustering

Image Index No.	Extended Maximum Transform			K means clustering		
	Halo diameter (pixels)	Core diameter (pixels)	Diameter difference (pixels)	Halo diameter (pixels)	Core diameter (pixels)	Diameter difference (pixels)
1	241.19	156.83	84.35	254.19	116.18	138.00
	188.08	136.94	51.13	184.11	124.37	59.74
2	198.24	108.82	89.42	209.57	113.78	95.78
3	295.05	269.86	25.19	307.30	269.30	37.99
	352.03	312.61	39.42	381.65	282.46	99.18
	291.31	268.79	25.51	303.22	263.79	39.43
4	179.78	144.60	35.18	186.01	133.14	52.87
	174.33	137.69	36.63	178.59	120.99	57.59
	152.90	129.56	23.33	155.02	118.64	36.38
	173.53	136.00	37.52	182.30	117.92	64.37
5	183.21	94.29	88.92	195.45	111.80	83.65
	156.92	90.37	66.54	163.83	112.37	51.46
	196.39	104.23	92.16	200.82	121.30	79.52
6	189.96	174.60	15.35	202.64	117.79	84.85
	188.80	167.63	21.17	207.85	120.24	87.60
	180.87	167.54	13.32	195.19	116.73	78.45
7	151.97	117.20	34.77	165.94	125.14	40.79
	169.31	116.77	52.54	193.66	112.42	81.24
8	166.32	110.20	56.12	185.44	116.86	68.58
	145.86	105.25	40.60	152.22	113.23	38.98
	174.78	121.19	53.58	192.21	119.57	72.63
	174.95	115.60	59.35	186.99	115.64	72.21
	164.62	104.76	59.85	170.53	107.59	62.93
9	117.31	39.89	77.15	0	113.31	-113.31
	173.15	87.56	85.59	191.97	104.89	87.07
	183.26	93.61	89.65	188.73	116.43	72.29
10	178.39	116.19	62.20	177.39	104.94	72.45
	72.39	62.35	10.04	0	73.67	-73.67
	173.60	116.46	57.14	175.19	111.17	64.02

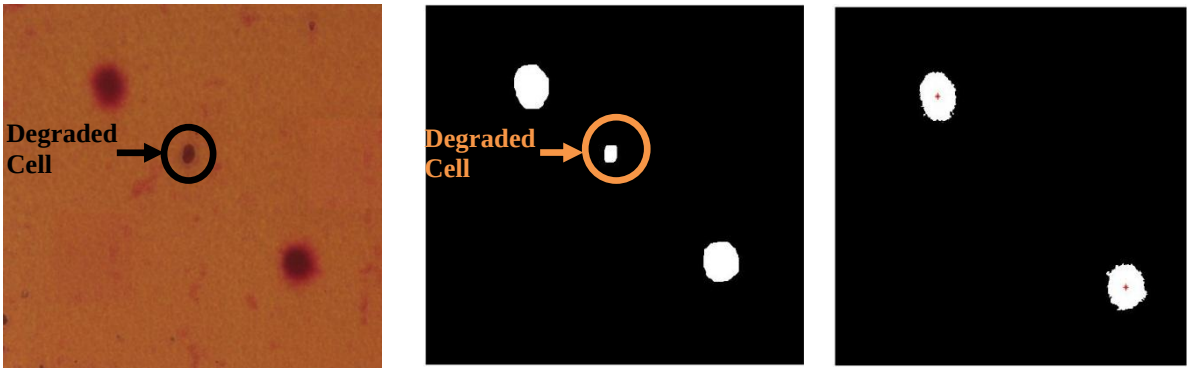


Figure 6. (a) Microscopic image with degraded cell (b) Threshold image with detected halo in degraded cell(c) K means image without halo in degraded cell.

The disadvantage of threshold and extended maximum transform was overcome by K - means clustering technique. It was found that red component of RGB value was crucial for automated determination of core and halo part. By measuring the difference between core and halo diameter, the sperm cells were classified as small (SH), medium (MH), large halo (LH) or without halo (DE). Table 3 lists the results of the present study.

Table 3 Comparative results of Sperm Cell categorization

Image	Extended maximum transform					K means clustering				
	SH	MH	LH	DE	Total	SH	MH	LH	DE	Total
1	1	0	1	0	2	1	0	1	0	2
2	0	1	0	0	1	0	1	0	0	1
3	3	0	0	0	3	2	1	0	0	3
4	4	0	0	0	4	4	0	0	0	4
5	1	2	0	0	3	1	2	0	0	3
6	2	1	0	0	3	0	3	0	0	3
7	2	0	0	0	2	1	1	0	0	2
8	4	1	0	0	5	3	2	0	0	5
9	0	3	0	0	3	0	2	0	1	3
10	3	0	0	0	3	1	1	0	1	3
Total	**20**	**8**	**1**	**0**	**29**	**13**	**12**	**1**	**2**	**29**

4. EVALUATION METRICS FOR THE PROPOSED METHODOLOGIES

The proposed algorithm is evaluated by using true positive rate, false positive rate and precision. The positive rate, false rate and can be calculated using the formulae given in (3), (4) and (5).

$$\text{True-positive rate} = \frac{TP}{TP + FN} \tag{3}$$

$$\text{False-positive rate} = \frac{FP}{FP + TN} \tag{4}$$

$$\text{Precision} = \frac{TP}{TP + FP} \tag{5}$$

Where TP (True positive) is the number of correctly detected cells, TN (True negative) is the number of correctly detected non cells, FP (False positive) is the number of wrongly detected non cells, and FN (False Negative) is the number of wrongly non detected cells.

Table 4. provides the comparative results provide by K means clustering and Ground truth. With the data provided in the table, we can find true positive, true negative, false positive and false negative.

Table 4 Comparative Results of K means Vs Ground truth

Image	K means Clustering					Results Provided by Experts (Ground truth)				
	SH	MH	LH	DE	Total	SH	MH	LH	DE	Total
1	1	0	1	0	2	1	0	1	0	2
2	0	1	0	0	1	0	1	0	0	1
3	2	1	0	0	3	2	0	1	0	3
4	4	0	0	0	4	4	0	0	0	4
5	1	2	0	0	3	1	2	0	0	3
6	0	3	0	0	3	0	3	0	0	3
7	1	1	0	0	2	1	1	0	0	2
8	3	2	0	0	5	3	2	0	0	5
9	0	2	0	1	3	0	2	0	1	3
10	1	1	0	1	3	1	1	0	1	3
Total cells	**13**	**13**	**1**	**2**	**29**	**13**	**12**	**2**	**2**	**29**

Thus, true positive and false positive is given as 28 and 1 whereas true negative and false negative is predicted as 0 from the Table 4. However in case of extended maximum transform, true positive and false positive is given as 22 and 7 whereas true negative and false negative is predicted as 0 from the Table 3.

Table 5 provides the accuracy, true positive rate and false positive rate for our proposed algorithms. It was found that for the specimen images considered in this work, K means clustering delivered 96.6% accuracy in the detection. But extended maximum transform delivered only 75.9% accuracy. Hence, halo and core detection using K means algorithm is found to be suitable for this research work.

Table 5 Comparison of accuracy for the two proposed methods

Statistical Parameters	Proposed methods	
	K means clustering	Extended maximum transform
Accuracy	96.6%	75.9%
True positive rate	100%	100%
False positive rate	100%	100%

5. CONCLUSION

In this work, a cost effective sperm chromatin dispersion test using Giemsa stain procedure was followed. An image processing algorithm based on threshold and extended maximum Transformation and K-means clustering technique was developed to assess the quality of sperm cells by identifying the core and halo parts of the cells. Despite, the results of the proposed system matches the ground truth by 96.6% with only one false positive; the study is in its infancy stage due to limited set of images. The future scope of this study is to aggregate large number of real time samples consisting of varying sperm cell structures and defects and thus to build a robust sperm quality assessment system.

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