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## Automated microscopic medical image analysis for human spermatozoa identification: A Survey.

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### ABSTRACT

The various image processing techniques are widely used for extracting the features from microscopic images of sperm cells of different species for more than three decades. This study will explore the low-level process used for improving the quality of an image, middle-level process applied for segmenting the sperm cells and high-level process implemented for the identification of head, mid-piece and tail of sperm cells in the given field of focus. The procedures of an image acquisition and processing of an image is specific to the particular focus of research, hence the various image acquisition methods related to the field of sperm cells are also discussed here. Automatic detection of sperm cells and identification of various parts of spermatozoon in microscopic images are highly important process in the field of assisted method of fertilization. Innovative research in analyzing the morphology of spermatozoa includes segmentation of sperm cells and measuring the size of their head, mid-piece and tail. In this study, we have classified, assessed and conferred several methods for sperm cell segmentation and detection of various parts of spermatozoa. After analyzing these techniques and its limitations, a futuristic view point of morphological analysis of spermatozoa has been explored.

**keywords:** Medical images, Image processing, Spermatozoa, Morphological analysis, k-mean clustering.

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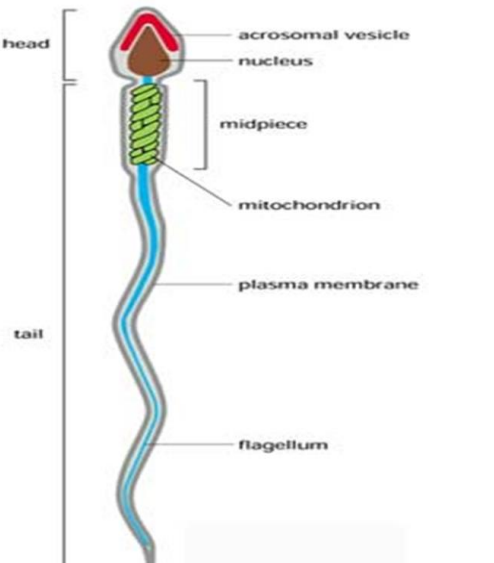
**INTRODUCTION**

Microscopic medical image processing techniques have been implemented to assist the andrologist/urologist to identify the normal sperm cells for assisted method of fertilization. Especially image segmentation is an initial process as well as essential for obtaining the Region of Interest (ROI) from the medical images [1]. Infertility is a clinical problem which affects 15% of couple worldwide [2]. Determining the infertility, the quality examination of semen is based on its characteristics such as viscosity, pH, concentration, motility, vitality and morphology. Even though many systems are available for evaluating the various characteristics of semen, the morphological analysis can be done only by image processing. Analyzing the morphology of a sperm cell using image processing methods can identify the defects in the sperm cell. Morphological defects such as abnormal sperm head, mid-piece and tail in the sperm cell are one of the important male factor infertility which is of 30-40% [2,3]. An abnormal sperm head is strongly associated with low fertilization which reduces the pregnancy rate [4]. The manual investigation of morphological defects of human sperm is always subjective with respect to the investigator, whereas the investigation through image processing techniques is accurate than the former irrespective of the investigator. In view of the fact that the semen test is the initial step for all later steps in infertility treatment [4,5]. In this regard, WHO recommends a guideline for the preparation of sperm smear [6]. Image acquisition is the primary step in microscopic image analysis. So that, many researchers have concentrated well on the acquisition of original images.

This paper gives a relevant study of the various techniques proposed by the researchers for the field of microscopic medical images of human sperm cells. Since, the manual analysis of human sperm cell using microscope is always subjective, will not be guaranteed for the assisted method of fertilization. So the manual evaluation can be reduced by using image processing techniques to detect the normal human sperm cells automatically. Finally, a futuristic perception of indentifying normal sperm cells, total number of normal sperm cells and defective sperm cells from the field of focus has been suggested. The schematic diagram of normal spermatozoon and its guideline value (WHO’s guideline) is shown in Table 1.

This paper is presented as follows: section 2 shows the techniques discussed in the literature review for the identification of sperm cells and its parts. Our own perception for the research in human sperm cell morphology is discussed in section 3. Section: 4 show the conclusion.

**Table 1. Schematic Diagram of Spermatozoon**

Schematic diagram of sperm cell [20]	WHO’s guideline for normal sperm [6]													
	<table border="1"> <thead> <tr> <th colspan="2" data-bbox="767 1357 1359 1424">World Health Organization’s guideline for Normal Sperm (5<sup>th</sup> Edition)</th> </tr> </thead> <tbody> <tr> <td data-bbox="767 1424 1102 1469">Volume:</td> <td data-bbox="1102 1424 1359 1469">≥ 1.5ml</td> </tr> <tr> <td data-bbox="767 1469 1102 1547">Total Sperm (millions in ejaculate):</td> <td data-bbox="1102 1469 1359 1547">≥ 39 million is normal</td> </tr> <tr> <td data-bbox="767 1547 1102 1615">Concentration / Count (millions per ml):</td> <td data-bbox="1102 1547 1359 1615">≥ 15 million is normal</td> </tr> <tr> <td data-bbox="767 1615 1102 1659">Motility:</td> <td data-bbox="1102 1615 1359 1659">≥ 40% is normal</td> </tr> <tr> <td data-bbox="767 1659 1102 1704">Morphology</td> <td data-bbox="1102 1659 1359 1704">≥ 30%</td> </tr> </tbody> </table>		World Health Organization’s guideline for Normal Sperm (5 <sup>th</sup> Edition)		Volume:	≥ 1.5ml	Total Sperm (millions in ejaculate):	≥ 39 million is normal	Concentration / Count (millions per ml):	≥ 15 million is normal	Motility:	≥ 40% is normal	Morphology	≥ 30%
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**Literature review**

The assisted method of fertilization requires computer based morphological analysis of human sperm cells for analyzing the male pattern infertility and improving the success ratio in fertilization. The role of computer for the evaluation of various semen characteristics in different species [7-9] are reported four decades ago [10]. The image processing techniques on microscopic medical images of human spermatozoa can be simplified as image pre-processing, sperm segmentation, feature selection and sperm parts identification, which are elaborated in the following divisions.

**Image preprocessing**

Many of the researchers have concentrated on improving the quality of an image by following various pre-processing approaches like gold-standard staining procedure, noise removal, color space transformation, image enhancement, multi resolution processing and image morphology processing. The researchers have transformed the input image from one mode of color space to another mode of color space i.e. RGB to gray scale [13] or RGB to L\*A\*B depends on the process. An image enhancement is one of the important pre-processing methods, which helps improving the quality of an image. The authors [14] used raw semen sample (without adding any staining materials) for acquiring the images and implemented two-stage enhancement technique to remove non-related parts (pixels) in the image using opening and closing filters.

The median filter is also used for reducing the noises in the images. The authors [15] identified the teratozoospermia using four stages of image processing. In the first stage, the RGB colored images were changed into Gray level then the noises were reduced using median filter. The second stage was edge detections; third stage involves segmentation of various parts of sperm and fourth stage concluded with statistical measurement of normal and abnormal spermatozoa. A fully automatic method was proposed [16] for identification of acrosome, nucleus, mid-piece and tail of sperm. An improved-hybrid method was applied to remove noise and segmented the sperm cells from the original image. To analyze the morphology of spermatozoa, Cellular Nanoscale Network based proposed system was introduced by the authors [17]. The authors [18] have applied improved two-stage framework to segment sperm's head. The list of methods covered in the pre-processing stage were used for several applications is illustrated in Table 2.

<b>Table 2. List of methods in Pre-processing</b>		
Pre-processing Category	Method used	Comments
Color image processing	Color space transformation	Transformed input image from RGB to gray scale [13-17]
		RGB to L*A*B [18]
Image enhancement	Contract adjusting and opening-closing filters	Improved image quality for better visual interpretation [14].
Image filter	Median filter	Reduced the noises in the image [15].
Noise Removal	Improved hybrid method (4 <sup>th</sup> order partial equation at first stage, Median filter at 2 <sup>nd</sup> stage)	Noise removed from sperm image (R –Red component) [16]
	Relaxed Median filter	Preserved image information (sharp corners & thin lines)

**Sperm segmentation**

The author [10] implemented threshold based segmentation to segment the sperm cells from the background. The threshold value was calculated by differentiating the staining intensity value of background and sperm head, which required high contrast staining. The adaptive thresholding method was implemented based on the regularized intensity value of input image [11]. Connection with this method of staining, the strategic Hough transformation was used for the detection of sperm head [12] based on the elliptical shape with 5 parameters.

Table 3. Various types of Segmentation	
Sperm segmentation	Comments
Thresholding	Separated the sperm cells from the environment, threshold value calculation based on staining intensity [10].
Adaptive thresholding	Thresholding based on Regularized intensity value [11].
Bayes' criteria –thresholding & Otsu method	Rectangular boundary identified by changing the pixels to binary (0's, 1's) form ; objects partitioned (first stage) [12].
Otsu method	Two stages procedure implemented based on histogram of the image [13]. Otsu with intra-class variance considered; Two-step thresholding algorithm - Simple thresholding used: partitioned sperm particles. Finally, Sperms were identified [14].
Sobel operator	Applied for detecting the edges to get foreground and background of an image [15].
Watershed segmentation	Carried out for segmenting the normal and abnormal spermatozoa from the field of focus [15]
Simple threshold, Rotating calipers algorithm & entropy based EM algorithm and Markov-random field model	Created a mask includes acrosome, nucleus and midpiece, segmented the various parts [16].
Otsu's Thresholding	Segmented the objects from background based on class-differences [17]
Morphological process – CNN based dilation and erosion	Extracted image components based on Cellular-nanoscale network [17]
k-means clustering	Segmenting the foreground (sperm) as one cluster and background as another cluster [18].
Morphological process – erosion, opening	Used for removing the sperm cells, which were in border, morphological opening was applied for segmenting the head portion in the sperm [18].

Hough transformation is used for detecting ellipse, line and circle like shapes. In the original image, first, the rectangular region was identified by converting the pixel into binary form using Bayes' criteria thresholding, second, 3 stages of hough transformation was implemented to identify the sperm head, and finally, the morphological attributes (width, length, perimeter and area) of the sperm head were calculated using elliptical parameters directly. The authors of this work stated that this method gave objective criterion for the process of classifying the sperm's morphological shapes. A two-stage procedure for segmenting sperm head and mid\_piece was developed [13]. In the first stage, Otsu method was used for extracting the objects and classified them using histogram analysis.

At the same time, unwanted portions were removed based on their size, which was helpful for detecting the sperm's head and tail (wrapped in bounding box). In the 2<sup>nd</sup> stage, nth-fusion method was handled for partitioning the head and mid\_piece. The nth level thresholding was applied then intersection with 'n' special growing masks was included. The authors [14] have applied two step thresholding-algorithms to segment foreground from the background, using Otsu's method. The watershed segmentation was carried out by the authors [15], based on marker-controlled connected component. The rotating calipers algorithm

was applied by the authors [16] for fixing best-fitted rectangle on each region of the sperm image. The segmentation of acrosome, nucleus and midpiece of the sperm was based on entropy based EM and markov random field model. The k-mean is a cluster based algorithm used for segmenting [18] the sperms and background. The morphological process (erosion) was applied for removing the sperms which are touching the border. The head was detected and segmented from the sperm using morphological opening method. The list of various segmentation methods are displayed in the Table 3.

**Feature selection and sperm parts identification**

The authors [12] have implemented the strategic Hough transformation method to detect the sperm part(head) as well as measured the size of sperm head with the help of elliptical parameters. The authors [13] used 2 growing mask for separating head and acrosome. The segmentation of head is based on nth-fusion, used 2 level thresholding (Otsu and fixed threshold). After segmenting the head, the acrosome and mid-piece were identified and partitioned based on intersection of segmented head. They concluded that their algorithm detected 95 spermatozoa out of 216. The authors [14] have identified the tail of the sperm using elliptic model.

**Table 4. Feature selection procedures**

Identification of Sperm parts	Comments
Strategic Hough Transformation	Sperm head was detected using this method and measured the size of sperm head using elliptical parameters [12].
nth-fusion 2 stage segmentation	First stage: used Otsu method – enhanced bottom part of sperm – head was segmented Second stage: fixed threshold method – acrosome and mid-piece were partitioned [13]
Maximum correlation-coefficient	Ellipse-fitting algorithm was used; area, elongation and tail were taken to find maximized correlation_coefficient between elliptic and partition box, Finally Tail was detected [14].
Euclidean distance transform	Various sperm parts detected - Head area, perimeter, head length, head width, midpiece length, tail length, orientation and Eccentricity and Equvidiameter was calculated based on Euclidean distance among the pixels [15].
SSIM	Structural Similarity Index was calculated for all pixels- initial pixel placed at end of midpiece and find next pixel based on highest SSIM – this method is based on local entropy estimation [16].
Dice coefficient & Hausdorff distance	Applied this evaluation metric to ensure the extracted features [18]

The estimation criteria used for identifying tail was maximized-correlation-coefficient. The Euclidean distance was calculated by the authors [15], to measure the head area and other parts of sperm cells. The features were selected based on the Structural Similarity index of every pixel [16]. Finally, the authors have achieved 96.829 % of accuracy in the process of identifying sperm parts. The evaluation metric used by the authors [18] were Dice coefficient and Hausdorff distance and concluded that their system was able to identify the sperm cells in 98 % of accuracy. The methods used for feature extraction is given in Table 4.

**Possible approaches for future research**

The various image-processing techniques are widely used for processing the images related to various domains of research. In this section the low-level, middle-level and high-level image processing techniques are suggested for the futuristic research on sperm cells is given in Table 5.

**Table 5. Futuristic methods**

Category	Name of the method	Benefits
Low-level	Color transformation – (from RGB to Gray level or LAB)	Origin for feature extraction
	Image filtering and enhancement – contrast stretching, smoothing	Improving the quality of the image
	Image Restoration – noise removal filters (mean, median, average etc.,)	Removing artifacts
	Morphology processing – opening, closing, dilation and erosion	Complete the discontinuity of the object boundaries
Middle-level	Threshold based, edge based, region based, cluster based and entropy based segmentation	Segment the Region of Interest from the background
High-level	Neural network, Genetic algorithm, optimization techniques etc., can be applied for image processing	Object identification and feature extraction

**CONCLUSION**

In this survey, it has been made clearly to explore the various methods of low-level, mid-level and high-level processing applied for the identification of human spermatozoa and its morphological structure. The cluster-based segmentations are well suited for microscopic medical image segmentation [19].

**REFERENCES**

- [1] Alireza Norouzi, Mohd Shafry Mohd Rahim, Ayman Altameem, Tanzila Saba, Abdolvahab Ehsani Rad, Amjad Rehman and Mueen Uddin, Medical Image Segmentation Methods, Algorithms and Applications, IETE Technical Review, ISSN: 0256-4602 (Print) 0974-5971 (Online) Journal homepage: <http://www.tandfonline.com/loi/titr20>,
- [2] A.Isidori, M.Latini, F.Romanelli , Treatment of male infertility, Contraception 72 (2005) 314-318,
- [3] K.Stouffs, H. Tournaye,J. Van der Elst, I.Liebaers, W.Lissens, Is there a role for the nuclear export factor 2gene in male infertility? Fertile. Sterile. 90 (2008) 1787-1791.
- [4] G.Lo Monte, F. Murisier, I. Piva, M. Germond, R. Marci., Focus on intracytoplasmic morphologically selected sperm injection (IMSI): a mini-review, Asian J.Androl.15(2013), 608-615.
- [5] Henry Carrillo, Jorge Villarreal, Miguel Sotaquira, et al., Spermatozoon Segmentation Towards an Objective Analysis of Human Sperm Morphology, Proceedings of the 5<sup>th</sup> International Symposium on Image and Signal processing and Analysis (2007).
- [6] World Health Organization, WHO laboratory manual for examination of human semen and sperm cervical mucus interation. 4 Ed. Cambridge: Cambridge university press, 1999.
- [7] V. González-Castro, E. Alegre, P. Morala-Argüello, S. Suárez,in: A combined and intelligent new segmentation method for boar semen based on thresholding and watershedtransform, Int. J. Imaging 2 (S09) (2009) 70–80.
- [8] L. Sánchez, N. Petkov, Estimation of boar sperm status using intracellular density distribution in grey level images, in:Similarity-Based Clustering, Springer-Verlag, 2009, pp.169–184.
- [9] E. Alegre, V. González-Castro, R. Alaiz-Rodríguez, M.García-Ordás, in: Texture and moments-based classification of the acrosome integrity of boar spermatozoa images,Comput. Methods Progr. Biomed. 108 (2) (2012) 873–881.
- [10] Moruzzi, J. F., A. J. Wyrobek, B. H. Mayall, and B. L. Gledhill. Quantification and classification of human sperm morphology by computer-assisted image analysis. FertIL Steril. 50:142-152, 1988.
- [11] Kruger, T. F., T. C. du Toit, D. R. Franken, R. Menkveld, and C. J. Lombard. Sperm morphology: assessing the agreement between the manual method and the sperm morphology analyzer IVOS. FertIL Steril. 63:134- 141, 1995
- [12] Kwang suk park, Won jin yi and Jae seung paick, Segmentation of Sperms Using the Strategic Hough Transform, Annals of Biomedical Engineering, Vol. 25, pp. 294-302, 1997.

- [13] H. Carrillo, J. Villarreal, M. Sotaquira, M. Goelkel, R.Gutiérrez, Spermatozoon segmentation Towards an objective analysis of human sperm morphology, in: Proceedings of the 5th International symposium on image and signal processing and Analysis (2007)
- [14] Vahid Reza Nafisi, Mohammad Hasan Moradi, and Mohammad Hosain Nasr- Esfahani, Sperm Identification Using Elliptic Model and Tail Detection, in: World Academy of Science, Engineering and Technology, International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering Vol:1, No:6, 2007.
- [15] V.S. Abbiramy, Dr. V. Shanthi, Spermatozoa segmentation and Morphological Parameter Analysis Based Detection of Teratozoospermia, International Journal of Computer Applications (0975-8887), Volume 3, - No. 7, June 2010.
- [16] Ahmad Bijar, Antonio Peñalver Benavent, Mohammad Mikaeili, Rasoul Khayati, Fully automatic identification and discrimination of sperm's parts in microscopic images of stained human semen smear, J. Biomedical Science and Engineering, 5, 84-395, 2012.
- [17] O. Levent S, avkay\_, Evren Cesury, M"us,tak E. Yalc,in\_ and Vedat Tavs,ano"gluz, Sperm Morphology Analysis with CNN Based Algorithms, 978-1-4799-6007 @ IEEE.
- [18] Violeta Changa,c, Jose M. Saavedraa,b, Victor Casta"nedac, Luis Sarabiad,Nancy Hitschfelda, Steffen H"artel, Gold-standard and improved framework for spermhead segmentation, computer methods and programs in biomedicine117(2014) 225-237.
- [19] Siti Noraini Sulaiman and Nor Ashidi Mat Isa, Adaptive Fuzzy-K-means Clustering Algorithm for Image Segmentation, IEEE Transactions on Consumer Electronics, Vol. 56, No. 4, November 2010.
- [20] <http://www2.nau.edu/gaud/bio301/content/spmtz.htm>