Spermatozoa Segmentation and Morphological Parameter Analysis Based Detection of Teratozoospermia

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ABSTRACT
An important parameter assessed during the semen analysis is the overall morphology, or shape of the sperm. Currently, the morphological analysis of sperm is done manually and is based on visual observation of at least 200 spermatozoa in a microscope followed by a classification stage based on strict criteria. But this method has led to incorrect results due to various factors such as different staining procedures, experience of technicians and human errors. So this paper focuses on morphological classification of spermatozoon either as normal or abnormal using Matlab. The first stage is the image preprocessing stage which involves the conversion of RGB image to a gray scale image and then image noises are removed using median filter. The second stage is the detection and extraction of individual spermatozoon which involves the extraction of sperm objects from images using sobel edge detection algorithm. The third stage segments the spermatozoon into various region of interest such as sperm head, midpiece and tail. The fourth stage involves the statistical measurement of spermatozoon which classifies Spermatozoa as normal or abnormal.

Keywords:
Morphology, Spermatozoon, Spermatogenesis, Segmentation, IVF, IUI, WHO, Semen analysis, DNA, Oocyte, Acrosome, Teratozoospermia.

1. INTRODUCTION
Infertility is commonly defined as the lack of pregnancy following 12 months of unprotected intercourse. Sperms are produced by a highly complex process of spermatogenesis. **Spermatogenesis** is the process by which male spermatogonia develop into mature spermatozoa also known as a sperm cell

The basic test of a man's ability to conceive children is the semen analysis. A **semen analysis** measures certain sperm parameters like sperm count, motility, morphology, volume, fructose level and pH. The current belief is that sperm morphology assessment should be used primarily as a fertility tool. The measurement of the percentage of spermatozoa having an 'ideal' morphology using so-called strict method is the method recommended in the latest edition of the World Health Organization (WHO) laboratory manual for semen analysis [1]. The shape of the sperm is a reflection of proper sperm development in the testicle, or spermatogenesis. Men with a defect in sperm maturation tend to have problems with sperm morphology and may then be at risk for failure of their sperm to fertilize their partner's eggs [2].

Strict morphologic assessment was developed to predict fertilization outcomes during In Vitro Fertilization (IVF) and embryo transfer. A positive correlation was found between the fertilization rate (FR%) and the proportion of the sperm with a normal (oval) head shape ($P < .001$), the sperm exhibiting acrosomal vacuoles ($P < .003$), the sperm with a normal acrosomal size (40% –70%) of total head area ($P < .025$) and the sperm undergoing acrosome reaction after adding human follicular fluid ($P < .001$) [3]. In [4] an algorithm for finding sperms in low contrast images was explained. Then, the foreground particles (including sperms and round cells) are segmented from the background. Finally, sperms are separated from other cells.

The paper [5] has discussed that neural networks was useful for morphological classification of sperm head. The methodology uses a preprocessing scheme in which invariant Fourier descriptors are lumped into "energy" bands. The resulting networks are pruned using *Optimal Brain* Damage. The presence of increased number of morphologically abnormal sperms with impaired motility in males with occupational exposure to high temperature was reported in [6]. The author has discussed the relationship between abnormal sperm morphology and chromosomal content or aberrations in individual spermatozoa [7].

In [11] & [12] a novel method for segmenting objects in microscopic images into its constituent's parts is proposed where the method called n^{th} fusion is the framework of the segmentation algorithm.

2. SPERM MORPHOLOGY
Sperms are microscopic creatures which look like tiny tadpoles swimming about at a frantic pace. Each sperm is composed of neck, midpiece and tail. The sperm head contains the genetic material of the father in its nucleus.

The mid-piece of the sperm contains mitochondria, which provides the energy for sperm motion. The sperm has a long tail in order to propel the head of the sperm, which carries all the DNA information, towards the egg. A healthy human sperm is about 40 to 250 μM long and the anatomy of a sperm is shown in the Fig. 2.1

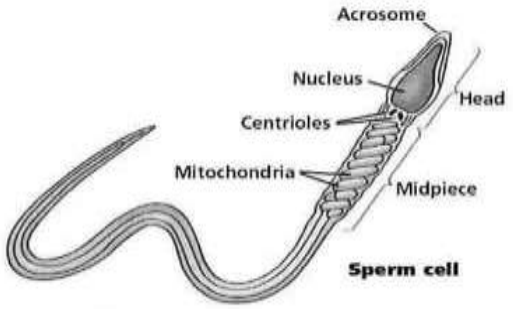


Fig. 2.1 Anatomy of a Sperm

Several different shapes or forms of human sperm have been identified and it falls into one of the following categories

- **Normal forms**

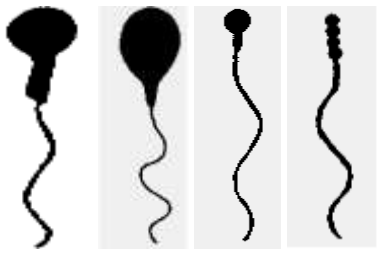
Normal sperm have oval head shapes, an intact "mid" section and an uncoiled, single tail as shown in **Fig. 2.2 (a).**

- **Abnormal heads**

Some of the sperm head abnormalities are enlarged round head (**Fig. 2.2 (b)),** small head (**Fig. 2.2 (c)) ,** pinhead (**Fig. 2.2 (d)),** double head (**Fig. 2.2 (e))** and an absence of identifiable head are all observed in semen analysis. Tapered sperm head and constricted head have been seen also. Overall abnormalities in appearance may be termed "amorphous" changes.

- **Abnormal tails**

Broken tails or less than half of the normal length should be categorized abnormal. Coiled sperm tail is also sometimes seen as in (**Fig. 2.2 (f))**. Double, triple and quadruple tails are also sometimes seen (**Fig. 2.2 (g))** and are considered as abnormal.



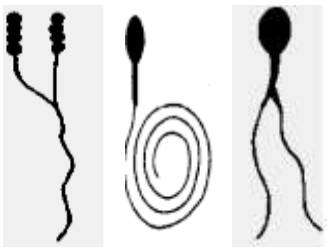


Fig. 2. 2 From Top left to right (a) (b) (c) (d) (e) (f) (g)

There are two methods for performing sperm morphological evaluations during semen analysis. They are

1. Crude estimation of the percentage of normal sperm specimen
2. Kruger "strict" sperm morphological evaluation.

The World Health Organization says good quality semen should contain 60 percent normal sperm morphology. The strict and WHO morphology score which predicts the sperm's potential for fertilization is shown in Table 2.1. [14]. But most of the labs use "strict" criteria for judging sperm normality.

Table 2.1 Strict and WHO III Morphological analysis reference values

Parameter	Percentage of Normal forms	Fertilizing capability
Strict morphology	> 14%	Excellent
	4-14%	Decreased
	0-3%	Impaired Fertility or Infertility
WHO III Morphology	>30%	Excellent
	15-30	Decreased
	<15%	Impaired Fertility

3. MATERIALS AND METHODS

3.1 Image Preprocessing

This stage is concerned with analyzing the microscopic image and to examine the format of the image. If the image is in RGB form, it is transformed to a gray scale image. The grayscale image is then filtered using median filter to remove the noise. The median filter is a nonlinear digital filtering technique, which is often used to remove noise. Matlab 6.5 provides a specialized implementation of the 2D-median filter:

K= medfilt2(J);

which uses a 3 X 3 neigborhood to compute the median and pads the border of the input with 0s. Applying a 3 X 3 median filter produces an ouput as shown in the Fig. 3.1

3.2 Detection and extraction of individual spermatozoon

Stage 1: Edges of sperm objects are extracted using Sobel edge detection algorithm. Edge detection is the most common approach for detecting meaningful discontinuities in intensity values. Such discontinuities are detected by using first and second-order derivatives. The first-order derivative of choice in image processing is the gradient. The gradient at the center point in a neighborhood is computed as

$$g = [G^2x + G^2{}_y]^{1/2}$$

$$G^2x = [(z_7 + 2z_8 + z_9) - (z_1 + 2z_2 + z_3)]^2$$

$$G^2y = [(z_3 + 2z_6 + z_9) - (z_1 + 2z_4 + z_7)]^2$$

Stage 2: The image is then smoothened to reduce the number of connected components using conv2.

C = conv2(A,B)

which computes the two-dimensional convolution of matrices A and B.

Stage 3: The number of connected components in a binary image is calculated using bwlabel.

[L, num] = bwlabel (f, conn)

where f is an input binary image and conn specifies the desired connectivity (either 4 or 8). Output L is called a label matrix and num gives the total number of connected components found.

Stage 4: Any connected components can be extracted using find().

[row, col] = find(X, L==1)

and then the extracted spermatozoon is stored in an array which is then displayed.

Fig. 3.1 also shows the sample image with abnormal spermatozoa and an extracted abnormal spermatozoon. Fig. 3.2 shows the sample image with normal spermatozoa and an extracted normal spermatozoon.

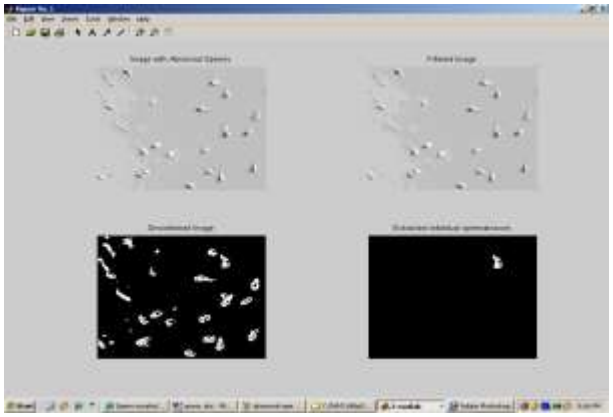


Fig. 3.1 Image with abnormal spermatozoa and an extracted abnormal spermatozoon.

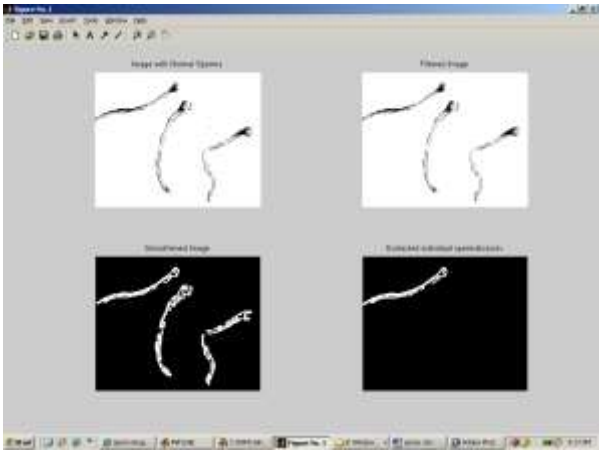


Fig. 3.2 Image with normal spermatozoa and an extracted normal spermatozoon

3.3 Image Segmentation

The main purpose of the segmentation stage is to subdivide a spermatozoon into various constituent parts such as head, mid-piece and tail. Segmentation is done using the Marker-Controlled Watershed Segmentation. A marker is a connected component belonging to an image. Internal markers and external markers are then computed transform and it follows the following basic procedure:

Step 1: Read the color image and convert it to grayscale.
Step 2: Use the gradient magnitude as the segmentation function.
Step 3: Compute internal markers which are inside each of the objects of interest.
Step 4: Compute external markers which are contained within the background. These are pixels that are not part of any object.
Step 5: Use internal and external markers to modify the gradient image by a procedure called minima imposition. It modifies a gray-scale image so that regional minima occur only in marked locations. Other pixels are pushed up as necessary to remove all other regional minima.
Step 6: Finally, compute the watershed transform of the marker-modified gradient image and the results are shown in Fig. 3.3.1, Fig 3.3.2 & Fig. 3.3.3

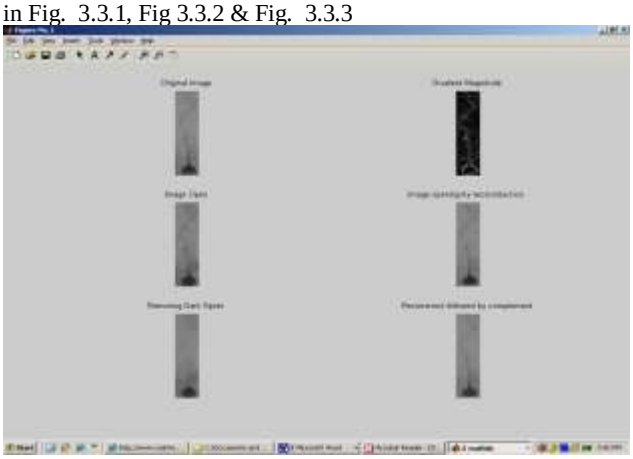


Fig. 3.3.1 Result of the Image Segmentation stage

Fig. 3.3.2 Result of the Image Segmentation stage

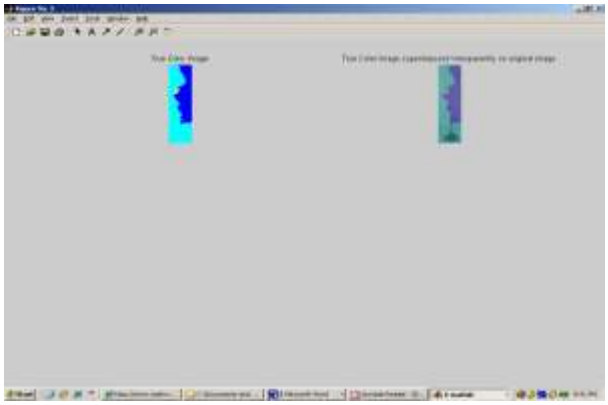


Fig. 3.3.3 Result of the Image Segmentation stage

3.4 FEATURE EXTRACTION

Feature extraction is performed over the image extracted from the previous stage. These features are used to classify

Head Area: It is the number of pixels contained in the segmented head region.
Perimeter: It is the number of pixels in the boundary of the Spermatozoa.
Head Length: Head length (in pixels) is calculated with the major axis. Major axis is defined as a line that contains the center of mass point and has a slope equal to the line defined by the highest value pixel in an Euclidean distance transform.

Head width: Head width (in pixels) is calculated with the minor axis. Minor axis is defined as the line perpendicular to the major axis.
Mid-piece length: It is the major axis of the midpiece of the mid-piece and it is measured in pixels.
Tail Length: It is the major axis of the midpiece of the tail and it is measured in pixels.
Orientation: It is the angle (in degrees) between the neck and tail to the major axis of the head.
Eccentricity: The ratio of the head length to the head width (ie.major axis to the minor axis) is called the eccentricity.
Equvidiameter: It is defined as the Euclidean distance between the two farthest points on the boundary.

4. RESULTS AND ANALYSIS

The Table 4.1 shows the statistical measurements of morphological parameters of spermatozoon obtained based on which the images are classified as Normal or Abnormal spermatozoon.

5. CONCLUSIONS AND FUTURE WORK

Even though the assessment of sperm morphology for either normal spermatozoa or for sperm defects is relatively extensive, the different stages in the analysis of human spermatozoon morphology were presented. This paper has focused on the measurement of parameters like sperm head length, width, area, perimeter, midpiece length and tail length. It is possible to detect the sperm abnormalities such as round heads, pin heads, very large heads, double heads, abnormal midpiece, absent tails and double tails. As abnormal sperm will not be able to fertilize the egg, morphologic assessment discussed in this paper could be helpful to detect the pregnancy outcomes in couples undergoing Intrauterine Insemination / In Vitro Fertilization (IUI/IVF).

As the size and shape of the acrosome is particularly important for sperm binding to the oocyte, this paper could be extended to measure the level of acrosome in the sperm head. 'Excess residual cytoplasm' on sperm produced by imperfect spermatogenesis could also to be measured. The mitochondria in the midpiece supplies the energy for the sperm's activity, it needs to be analysed.

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Table 4.1 Statistical Measurement of Morphological Parameters of Spermatozoon

Input Images	Area	Perimeter	Sperm Head		Eccentricity	Midpiece Length	Tail Length	Orientation	Equiv Diameter	Class
			Length	Width						
Image1	36	46.7032	9.8914	5.3217	0.8429	15.62	67.57	78.5457	6.7703	Normal
Image2	158	191.2335	23.6182	8.6646	0.9303	19.11	66.23	81.9619	13.9116	Abnormal (Large Head)
Image3	1	3.6280	1.1547	1.1547	0.9600	10.23	44.69	0	1.1284	Abnormal (Smaller Head)
Image4	37	25.453	10.1335	5.3444	0.8496	19.12	35.54	88.9338	6.8637	Abnormal (bent midpiece)
Image5	10	11.8454	4.2583	3.2083	0.6575	14	74.31	90	3.5682	Abnormal
Image6	1	3.6280	1.1547	1.1547	0	15.07	44.16	0	1.1284	Abnormal (Pinheaded)
Image7	7	13.4457	5.7735	1.8145	0.9493	15.35	38.77	45	2.9854	Abnormal (Double Tail)
Image8	7	10.1697	3.8791	2.4300	0.7795	15.35	42.27	45	2.9854	Abnormal (Double Headed)

Courtesy: The images used in this paper algorithm were obtained from the Advanced Fertility Center of Chicago, Infertility and In Vitro Fertilization with ICSI Specialists, Gurnee & Crystal Lake, Illinois. Fixed and stained human sperm pictures with high magnification are taken from IVF lab.

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