

Sperm Identification Using Elliptic Model and Tail Detection

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Abstract—The conventional assessment of human semen is a highly subjective assessment, with considerable intra- and inter-laboratory variability. Computer-Assisted Sperm Analysis (CASA) systems provide a rapid and automated assessment of the sperm characteristics, together with improved standardization and quality control. However, the outcome of CASA systems is sensitive to the method of experimentation. While conventional CASA systems use digital microscopes with phase-contrast accessories, producing higher contrast images, we have used raw semen samples (no staining materials) and a regular light microscope, with a digital camera directly attached to its eyepiece, to insure cost benefits and simple assembling of the system. However, since the accurate finding of sperms in the semen image is the first step in the examination and analysis of the semen, any error in this step can affect the outcome of the analysis. This article introduces and explains an algorithm for finding sperms in low contrast images: First, an image enhancement algorithm is applied to remove extra particles from the image. Then, the foreground particles (including sperms and round cells) are segmented from the background. Finally, based on certain features and criteria, sperms are separated from other cells.

Keywords— Computer-Assisted Sperm Analysis (CASA), Sperm identification, Tail detection, Elliptic shape model.

I. INTRODUCTION

INFERTILITY is a common clinical problem which causes considerable morbidity, including stress, depression, and sexual dysfunction, in those couples affected [1]. The assessment of the male partner of an infertile couple is based on the conventional criteria of semen analysis.

To assess sperm concentration, the count should be made of complete sperms (head with tail). Defective sperms (pinhead and tailless head) should be counted but recorded separately. Moreover, the ejaculate invariably contains cells other than sperm referred to as 'round cells'. These include leukocytes and immature germ cells. Excessive numbers of leukocytes may be associated with infection and poor sperm quality. But the different types of immature germ cells are usually

indicative of disorders of spermatogenesis. These are often degenerating and difficult to identify [3]. The conventional assessment of human semen is a highly subjective assessment, with considerable intra- and inter-laboratory variability. Therefore, objective analysis of microscopic images of cells has been a major goal of human pathology and cytology. The developments in computerized image analysis of cells have enabled the automation of many functions previously requiring manual calculation. CASA system is an objective analysis technique for semen analysis. However, it was previously not feasible to determine sperm concentration by CASA because of difficulties in distinguishing sperm from particulate debris [3]. Most CASA systems use size criteria for sperm identification. Thus if a piece of debris happens to be in the size range expected for a sperm head, this will be a source of error. The Stromberg-Mika Cell Motion Analyser (SM-CMA) system addresses this problem by requiring an attached tail before a sperm head is identified positively. The SM-CMA correctly recognizes motile spermatozoa, but underestimates the immotile ones [5]. Recently fluorescent DNA stains have been used with CASA to facilitate sperm identification. In this technique, Hoechst dye binds to the DNA in sperm heads, which then fluoresce and are recognized by the computer [6]. For semen analysis in this research, we have used raw semen samples (without any staining materials) and a regular light microscope, with a digital camera directly attached to its eyepiece, to insure cost benefits and simple assembling of the system. One of the drawbacks of this method, however, is that the images lack proper contrast and sharpness. Differentiating sperms from other cells in these images is difficult, especially if the size of debris is the same as that of a sperm head. If we can compensate the disadvantages of these microscopic images, this sperm analysis system can be utilized with all kinds of microscopes in all laboratories.

II. MATERIALS AND METHODS

A. Image Acquisition Techniques

The videos used in this research have been recorded from the semen samples of people who had visited the spermogram laboratory of Royan Institute in Tehran. To make these videos, a ZIESS regular light microscope with a total magnification of $\times 500$ (objective lens: $\times 40$, mid-magnification: $\times 1.25$, eyepiece lens: $\times 10$) along with a Moticam 480 digital camera were used. The camera was

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attached directly to the eyepiece of the microscope. The size of recorded videos was 288×352 pixels. There was a space resolution of 2 pixels/μm. The frame rate of the recorded videos was 30 fps for Moticam 480.

B. Software for Algorithm Implementation

The tracking algorithm was set and run using MATLAB 6.5.1 software in a windows XP operating system on a 2.8 GHz Pentium IV personal computer.

C. Image Enhancement Method

As mentioned in Section 2(A), raw semen images were recorded with regular microscope without any staining materials. To enhance the image quality, the image noise shall be reduced and the extra parts present in the image shall be removed as much as possible before starting the sperm determination step.

A two-step enhancement method was developed in our previous research to remove the extra parts as much as possible [7]. As a result, we have a background image B and a union of the enhanced images $I(:, :, t)$:

$$I(i, j, t) = f(i, j, t) - B(i, j), \quad t = 1, \dots, m \quad (1)$$

In (1), i and j are the coordinates of the point under analysis and $f(i, j, t)$ is the brightness level of these coordinates in the t frame. m shows the number of frames present in the video. To determine sperm concentration, the first frame $I(:, :, 1)$, shortly named I , is used. Theoretically, Image I only includes motile sperms while Image B includes all other cells, immotile sperms, and agglutinated region. In this research, Image B is used to identify completely immotile sperms. Before that is done, however, the image needs to be enhanced to remedy the non-uniform lighting distribution throughout the image B . To enhance Image B , the *closing-opening* filter is used:

$$B_e(i, j) = B(i, j) - B_{co}(i, j) \quad (2)$$

In Relation 2, B_{co} is the results of applying the closing-opening filter on Image B through the structuring element circle [8].

D. Sperm Identification Method

Image I includes some debris and some immotile sperms because of their slow passive movement. Thus Image I should be used for identification of the motile/immotile sperms from other foreground particles. Usually the size of debris is larger than that of the sperms and one may separate the sperms from other cells based on the size criterion. But if the piece of debris is in the size range expected for a sperm head, this can be a source of error. To remedy this, first the foreground image is segmented from the background image by applying a two-step thresholding algorithm. Then, the sperms are distinguished from other foreground particles based on the existence of tail and some other morphological features.

First, the semen image should be divided into a foreground image and a background image. Such an image can be divided into two parts through simple thresholding:

$$I_{w1}(i, j) = 1 \quad \text{if } I(i, j) > T_1 \quad (3)$$

$$= 0 \quad \text{if } I(i, j) < T_1$$

The selection of Threshold T_1 is based on Otsu's method which chooses the threshold to minimize the intra-class variance of the black and white pixels. This method works properly on images that have a bimodal histogram [8]. Our images do not have that due to a large semen background area compared to the cells area, as well as to the gradually (as opposed to sharply) varying intensity of the cells and that of the background. Therefore, the binary image I_{w1} does not have enough precision. Instead of selecting T_1 based on the complete image histogram, we have used Otsu's method on the local image histogram. To do this, we have selected a particle recognized in I_{w1} and applied Otsu's method on a small square box (here a 8x8 pixels-box) around this particle. In this way, the new threshold is obtained:

$$I_{w2}(i, j) = 1 \quad \text{if } I(i, j) > T_2 \quad (4)$$

$$= 0 \quad \text{if } I(i, j) < T_2$$

I_{w2} has more precision than I_{w1} because the variation of intensity through the box is ignorable.

Now, the foreground image contains some distinct particles from amongst which the sperms need to be identified. To do that, the following features have used: (1) area, (2) major axis length to minor axis length ratio (elongation), and (3) tail. For human sperm, the area and the elongation have a maximum and a minimum margin. Thus a particle which is larger or smaller than these margins is not a sperm. However, some pieces of debris are in the size range expected for a sperm head and they can not be separated from the sperms through the area-elongation criteria. Therefore, the last feature (tail) should be used for identification.

After all the large and small particles have been removed, other particles have been examined for the existence of tail. First, the boundaries of the particles should be determined. But as mentioned in Section 1, the images used in this research do not have enough contrast and the boundaries of the particles are vague. Therefore, identification of these boundaries does not have enough precision based on the binary image I_{w2} , and the boundaries can only be estimated. The estimation criterion is to be the maximized correlation coefficient between an elliptic shape and the particle box.

$$M(x_m, y_m, R_a, R_b) = \{x, y \mid \frac{(x - x_m)^2}{R_a^2} + \frac{(y - y_m)^2}{R_b^2} \leq 1\} \quad (5)$$

x_m and y_m are the coordinates of the center of the ellipse and R_a and R_b are axes of the ellipse. During the boundary estimation procedure, these parameters are varied until the maximum correlation is obtained:

$$\text{best fitting} \equiv \max_{x_m, y_m, R_a, R_b} M(x_m, y_m, R_a, R_b) * S_i \quad (6)$$

* is the correlation operator. Based on Relation 6, the best elliptic shape is fitted on the object. Then the boundary of the ellipse is the boundary of the particle and the axes of the ellipse are major and minor axes of the particle. Now, we can

examine the particle for the existence of tail: Often the sperm's tail exits the sperm's head in the direction of its major axis. Thus the major axis of the particle should be examined. To do that, we have examined the image intensity variations throughout the major axis and compared it with intensity variations throughout the minor axis. If the intensity variations throughout the two axes are similar, the tail does not exist. In the contrary, if the intensity sharply varies throughout the minor axis while only slowly varying throughout the major axis, the tail may exist.

III. RESULTS

To count the sperms in the semen, (motile/immotile) sperms must be distinguished from other cells in the semen. First, the enhanced semen image (I) should be divided into a foreground image and a background image. The foreground image is the set of all particles in the image and the background image contains the semen plasma. In the image used in this research, most of the cells have a lower intensity level than the background. Therefore, the segmentation algorithm based on a threshold level is applied. Figure 1 shows the result of the algorithm.

Because of the slow movement of cells other than the motile sperms, Image *I* includes some debris and some immotile sperms from which the sperms must be differentiated. To do so, an ellipse fitting algorithm is applied (Relations 5 and 6). Figure 2(a) shows a sperm while Figure 3(a) shows a non-sperm particle. The accuracy of the algorithm was represented in Table I.

TABLE I
COMPARISON BETWEEN TWO SEGMENTATION ALGORITHMS

	Vague-boundary particles	Clear-boundary particles
segmentation algorithm in [9]	20	2
The present algorithm	8	2

(from 100 analyzed particles, 50 have vague boundaries)
(the numbers describe the wrongly segmented particles)

Through the fitting algorithm, the large and the small axes of the ellipse are determined. These axes are equal to the major and the minor axes of the particle. Because the exit point of the tail from the sperm's head is in the direction of the major axis, we have considered the intensity profile throughout the major axis extension and compared it with the minor axis profile. As seen in Figures 2(c), the major axis profile varies gradually in the tail region while the minor axis profile varies sharply. In contrast, the variations of the major and the minor axes profiles are sharper and less uniform in the case of non-sperm particles. This is one of the features used for the identification of sperms from other cells in the semen.

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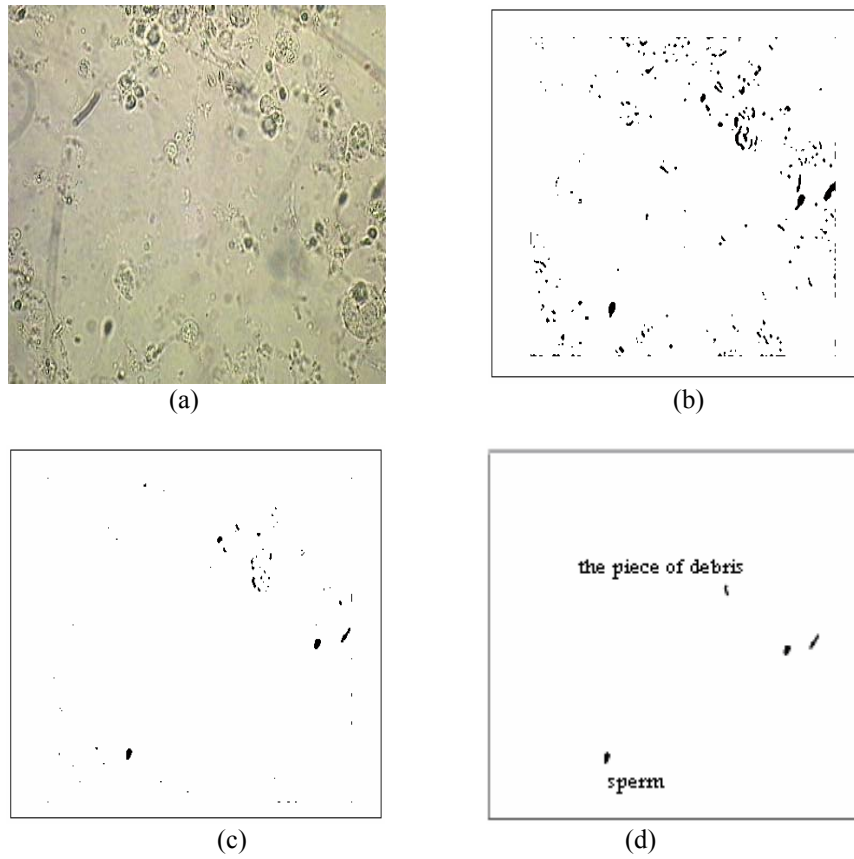


Fig. 2
 (a) Original image (b) First-step segmentation (c) Second-step segmentation
 (d) After removing out-of-size particles

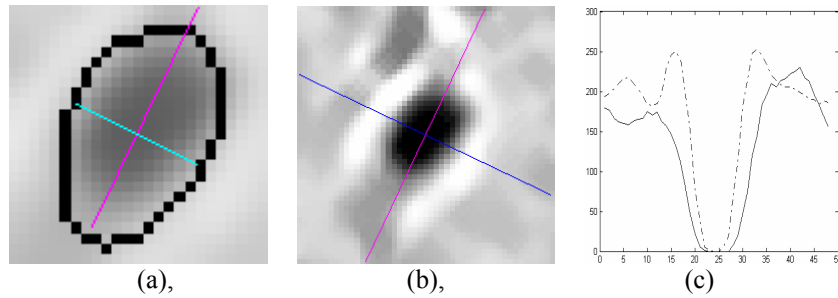


Fig. 3
 Sperm modeling: (a) Ellipse fitting (b) Tail finding procedure (c) Major and minor axes profiles

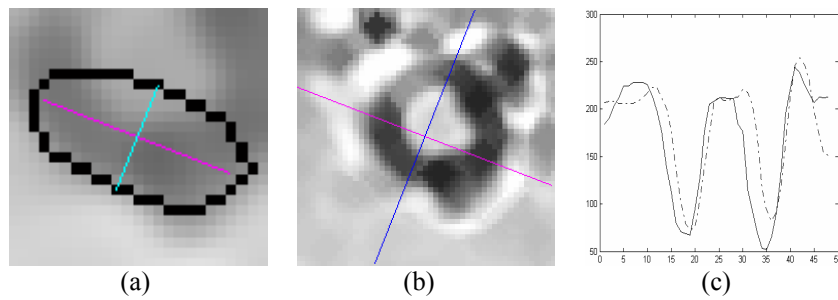


Fig. 4
 Non-sperm modeling (a) Ellipse fitting (b) Tail finding procedure (c) Major and minor axes profiles