

A Multi Steps Algorithm for Sperm Segmentation in Microscopic Image

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II. MATERIALS AND METHODS

A. Image Acquisition Techniques

The first step of imaging is the preparation of the sperm; therefore, first of all, we have to describe the preparation. The sperm remains alive out of the human body for 45 minutes and, during this period, it has a movement. The first step for preparation of an image capable is to use its morphological studies, is fixing the sperm. Note that sperms have a cyclic movement in their medium; They are fixed by use of staining methods and, for the purpose of homogenization, they are put in an incubator for a period of 30 minutes, But it has been proved that the staining methods have no significant role for determination of sperm specifications, so, in some research centers, such as Isfahan Infertility and Fertility Center, for the purpose of avoidance form quality decrement of the image, they are not stained, Of course, sperms have a three-dimensional movement, in some cases when they are deeper, some image might not show the accurate specifications of the sperm. In some frames of imaging the sperm may produce an image that does not represent its accurate morphological specifications but some other ones may represent its accurate specifications. Therefore, in determination of the accurate morphological specifications of the sperm we can not rely on a unique image. The videos used in this research have been recorded from Isfahan Fertility & Infertility Center or the spermogram laboratory of Royan Institute in Tehran. The sample preparation technique was almost the same in both laboratories: the sample was first put in an incubator for 30 minutes. Once it was homogeneous, a drop of $20\ \mu\text{l}$ of the sample was put on a clean slide, The images were prepared by use of microscopes equipped with camera at $\times 40$ magnification to enable us to study the sperm using uniform samples [7]. To make the videos, three different sets of microscopes and cameras were used with the following specifications:

1. A Nikon trinocular invert microscope with a total magnification of $\times 400$ (objective lens: $\times 40$, eyepieces lens $\times 10$) along with a Sony SSC-DC58AP video camera. The camera was attached to a third eyepiece.
2. The same microscope along with a Motic Moticam 480 digital camera. Unlike the previous camera, this one was attached directly to the eyepiece of the microscope.

Abstract—Nothing that an effective cure for infertility happens when we can find a unique solution, a great deal of study has been done in this field and this is a hot research subject for to days study. So we could analyze the men's seaman and find out about fertility and infertility and from this find a true cure for this, since this will be a non invasive and low risk procedure, it will be greatly welcomed. In this research, the procedure has been based on few Algorithms enhancement and segmentation of images which has been done on the images taken from microscope in different fertility institution and have obtained a suitable result from the computer images which in turn help us to distinguish these sperms from fluids and its surroundings.

Keywords—Computer-Assisted Sperm Analysis (CASA), Sperm identification, Segmentation.

I. INTRODUCTION

HIGH percentage of problems with infertility in couple are from the male and finding ways to resolve this will be helpful to the physicians for a better and faster cure for couples.

In this method the sperm slide is being analyzed by an specialist under microscope which will be much time consuming and with lots of errors, majority of which will be caused by human errors, like grouping by different people with different observations, for this reason the new method of computerizing and classification has been substituted for the researchers, and the first step in this, is distinguishing the correct sperm and the fluid surrounding by computer, because in the process of the segmentation of the image one of the most important step is for distinction and pattern recognition, and the success in the segmentation will guarantee the final result, in this paper we have introduced a unique method for segmentation of sperm based on separation form the surrounding area.

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3. 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.

The size of recorded videos was 288×352 pixels for both cameras. There was a space resolution of 2.8 pixels/ μm for groups (2) and (3).

B. Software for Algorithm Implementation

This algorithm was set and run using MATLAB 6.5.1 software in a windows XP operating system on a 3.00 GHz Pentium IV personal computer.

C. Edge Detection

1. Image Enhancement Method

In the images taken by the microscope there were a lot of excess parts that needs to be eliminate at first, so there is no confusion with the sperms and in turn does not create errors, also we needed to have the image as monotonous as possible for example small particles on the slide, artifacts cause by light source and sampling errors, and similar errors. We also have to note that the liquid is monotonous and other artifacts like blood cells are not present in our samples.

1.1 For improved images we used a median filter with windows of 3×3 to create a monotonous image and eliminate small spots, of course this filter will cause the sharp edges to be disappeared however this will not make much changes to the algorithm of the edges.

1.2 Followed by the step above for the improved images and elimination of the sharp edges and noises and also filling the empty spots and elimination of rice artifact noise we used the following method.

• First we applied a Closing algorithm by use of a disc element [1].

$$A \bullet B = (A \oplus B) \ominus B \quad (1)$$

• *showClosing*, \oplus *showDilation*, \ominus *showEroton*

These narrow and long dents will be connected and will fill all the small cavities in the images and also fill all the small holes that are in the form of artifacts notices.

• Then we used a special filter for the resulted images

• And, finally, applied opening algorithm with the same structuring element on the images with the preliminary size (the size of image are changed as a result of closing). In this step we reach the enhance image and we can better find edge of the objects.

$$A \circ B = (A \ominus B) \oplus B \quad (2)$$

• *showOpening*, \oplus *showDilation*, \ominus *showEroton*

This action will create a flattened curvature, and if there are narrow spots out of the objects, it will be eliminated [4].

2. Sperm Identification Method

The detection threshold method was used for determination of object edges. After completion of the said steps, we applied the threshold method on border illumination levels histogram; a simple way for separation the objects from the background is the selection of a known threshold (T) to separate two phases from each other. The semen image should be divided into a foreground image and 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 image contains some particles with a homogeneous intensity and a background with a different intensity level. Such an image can be divided into parts through simple threshold:

$$g(x, y) = \begin{cases} 1 & \longrightarrow f(x, y) > T \\ 0 & \longrightarrow f(x, y) \leq T \end{cases} \quad (3)$$



Fig. 1 Image of a head of special sperm after implementation of enhancement and threshold algorithm on the initial image

After implementation of the above-mentioned algorithm, all the background is appeared in a single color and the illumination level of the object is appeared in another color. This leads to an edge formation for the object; as shown in the above figure. Sometimes, a single threshold is used for all the images; but, for the purpose of easy access to the edge of the object that is dependent on the existing illumination levels, a known threshold selection found to be appropriate for the concerned image. Now we have found the edges of our image or have done the segmentation.

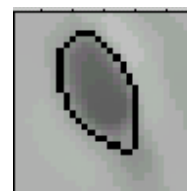


Fig. 2 Image of a head of special sperm after segmentation

D. Recognition of all the Sperms in One Image

By the above threshold method, we can find not only one image but also all the images needed, because the chosen threshold for one sperm can be used for all the sperms, since the light spectrum and focus will be similar for all of them and that means, we will be able to do this for all the edges and the procedure is as follows.

At first the operator will click on one chosen sperm; our software has a square that is bigger than the sperm; this square has both the tail object and the separation threshold. And from this we will obtain a Histogram image, and will impose this on the threshold image and at the end all the objects will be obtained with the same light surface. This is a good method because the sperm images will be all in the same surface and with the same light source.

III. RESULTS

In evaluation of the suggested method for sperm head segmentation, we can use two methods:

Quantitative and qualitative, from quantitative point of view we can compare results with an expert operator, for example the result was compared with the findings from the algorithm and the segmentation. It was found out that from 30 images around 84% of the result was very much similar under special condition, like bad lighting and low artifact noises. if we use the algorithm for distinguishing the images, we can look at this quantitatively and note the following factors.

Total error means the number of objects or background did not separate in good condition divided by total points should be separate.

Precision means the number of objects or background separated in good condition divided by total points should be separate.

Two factors in image processing are specificity and sensitivity which define as: In False Positive (FP) background defined as an object by mistake and in True Positive, Our software defines correctly the object as in object [9].

Sensitivity is as follows based on above mentioned definition

$$SE = \frac{TP}{FN + TP} \quad (4)$$

Specificity define as:

$$SP = \frac{TN}{TN + FP} \quad (5)$$

The results shown in table bellow are based on calculating some random pictures.

TABLE I
 THE RESULTS FOR RANDOM IMAGES

Error	0.16
Precision	0.84
Sensitivity	0.88

The repeatability criterion is as follows:

Repeatability = 82%

For 25 Sperm with 2 repeat for each one

IV. DISCUSSION AND CONCLUSION

In this research we have taken pictures by microscope for segmentation of sperm. So the instrument we used in this research and the way of our usage and the quality of pictures which we have used as an input data are important processing and the final results.

In this project, we use three sets of cameras and microscopes, available in this research centers. For having better result we need to have advanced instrument, so we applied several ways of image enhancement to improve our pictures. These steps will remove sperm tail and middle part of it which was not clear earlier, but show the head of it better than before. However it is better to have advance instruments for better picture in addition, to use better image enhancement algorithm. And final goal in this research is to reach a complete algorithm to identify all part of sperm including tail, middle and head of it separately.

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