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AN AUTOMATIC SYSTEM FOR CALCULATING BASIC SEMEN PARAMETERS

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Abstract: Algorithms for examination of the density and selected parameters of movement of sperm cells have been elaborated and implemented. The conducted research is a part of work on a computer system for semen analysis. The system will allow for an increase in the precision of examination thanks to an exact specification of numerical values of the chosen parameters. An additional advantage of the system is an increased time efficiency of examination.

Nowadays, the basic type of examination is an estimated analysis of semen parameters by visual observation of a sample, based on a physician's subjective assessment of an image. Registration of images is impossible in visual analysis.

Keywords: image enhancement, image analysis, semen, sperm cell, cell mobility

1. Introduction

One of the ever more significant problems in the present world is the question of male fertility. Research conducted for many years has shown that the percentage of men with fertility problems has increased. Their semen is "weak", characterized by low density, small fractions of sperm cells with the proper structure and movement. All these make them incapable of natural fertilization.

As a consequence of the development of in vitro fertilization techniques, the assessment of semen quality has become a vital issue. Thanks to these techniques in many cases artificial fertilization is possible despite of the "weakness" of sperm.

As a result of many year of research, World Health Organization standards have been elaborated. They classify the quality of semen on the basis of the value of such coefficients as density, the percentage of sperm cells with the proper structure, the fraction of sperm cells with the proper movement, *etc.* Healthy semen is characterized by the density of 20-250 million sperm cells in 1ml and by the fraction of at least 25%of sperm cells with the proper, *i.e.* linear and progressive, movement [1, 2]. However, the way in which these coefficients can be determined is problematic. L. Witkowski

Currently, the most common method used to find out the above parameters is visual analysis carried out by an andrologist. The analysis is a subjective assessment of a microscopic sample. The precision of such examination could be questionable and it depends on the physician's experience. The possibility of repetition of an examination does not exist. One can neither register images nor create a history of investigated samples. This problem is especially important when a patient changes his andrologist. A new physician has to examine the patient once again and the results of his analysis may vary significantly from those of the previous physician.

The development of computer techniques has brought about an attempt to elaborate such a system that would eliminate the impreciseness of visual analysis. The system would allow us to determine exact values of the coefficients, to register images and create a history of examined samples.

In the 1980's, CASA (Computer Assisted Semen Analysis) systems were created. They were based on signal processors, aimed at fast processing of images. A disadvantage of these systems was their high price, only few clinics could afford them. The requirement of easy access was not fulfilled. Visual examination continued to be predominant.

Thanks to the further development of computers and the decrease of their prices it is now easy to fulfill the postulate of accessibility. The aim of the presented work was to elaborate a computer system for semen examination. It would require a computer synchronized with a microscope. The first stage of the work was to devise an application calculating the density of sperm. The second was to create an application that would determine the parameters of sperm movement.

2. Image analysis

2.1. Acquisition of images

The first step has been to calculate the quality of sperm through acquisition of a sequence of images. For the purpose of registration of alive sperm cells, the semen is not stained. Therefore, the images are not contrasted and it is difficult to extract sperm cells (before analysis one should extract sperm cells and reject any artifacts and other elements of semen that are not sperm cells).

A sequence of images of alive sperm cells is registered by the computer system with the help of a CCD camera and a frame-grabber card.

2.2. Pre-processing

In order to calculate semen coefficients it is necessary to extract sperm cells from the background. To realize the task, operations of image enhancement such as logical, arithmetic, morphologic, neighborhood and point-to-point operations are used. Additional and important help is available in the possibility of detecting movement on two consecutive images by calculating their sum. Further processing of the sum of the images is also available. After the set sequence of operations has been completed the next step is the detection of gravity centers of sperm cells on consecutive images of a sample.

232

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2.3. Trajectories

After the process of pre-processing and extracting the objects (*i.e.* sperm cells), it is possible to determine the trajectories of sperm cells' movements by joining their gravity centers. According to the literature, the maximum speed of a sperm cell is $25 \mu \text{m/s}$ [1].

Taking into consideration the above and that microscope magnification is 20 times and a camera is capable of capturing 12 images per second, one can conclude that the gravity center of a sperm cell can change its location by not more than 20 pixels in a consecutive image. Another important feature of sperm cell movement is the ability to move forward only. In most cases these conditions make it possible to choose accurately the proper next gravity center.

To choose properly between the possible trajectories in the case of a conflict (i.e. more than one gravity center meeting the conditions), additional information can be cosidered: speed. It is known that the speed of a sperm cell cannot change significantly. This allows us to determine accurately and properly the next gravity center for a given trajectory.

2.4. Density

The number of sperm cells (l) is determined on the basis of the number of found trajectories of sperm cell movements, with proper or improper construction, observed in a sequence of 50 images. From the number of sperm cells the density of a sample (ρ_{sample}) is calculated, where the sample is the quantity of ejaculate used to prepare the microscopic sample:

$$\rho_{\text{sample}} = \frac{l}{v_{\text{sample}}} \frac{S}{S_o},\tag{1}$$

where l is the average number of sperm cells within the area of a single image, v_{sample} – precisely measured quantity of the semen used for preparation of a microscopic sample, S – area of a microscopic sample, S_o – area of a single image.

The density of a sample is calculated on the basis of an average value of the number of the sperm cells within the area of a single image, as the number of sperm cells is different in the consecutive images.

From sample density the number of sperm cells (L) in a semen sample can be calculated:

$$L = \rho_{\text{sample}} V_{\text{ejaculate}}.$$
 (2)

2.5. The parameters of movement [1-3]

Another important parameter of semen quality are sperm cell movements. According to WHO standards, to define the quality of a sperm cell's movement (index i) one has to calculate the following parameters of its trajectory:

- VSL_i - straight-line velocity (see Figure 1):

$$VSL_i = \frac{\sqrt{(x_M - x_1)^2 + (y_M - y_1)^2}}{(M - 1)\Delta t},$$
(3)

where (x_1, y_1) is the location of the gravity center of a sperm cell on the first image, (x_M, y_M) – location of the gravity center of the sperm cell on the final (M) image, Δt – difference of time between the subsequent images;

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234 L. Witkowski

Figure 1. Illustrated parameters of the sperm cell movement

- VCL_i - curvilinear velocity (see Figure 1):

$$VCL_{i} = \frac{\sum_{j=1}^{M} \sqrt{(x_{j+1} - x_{j})^{2} + (y_{j+1} - y_{j})^{2}}}{(M-1)\Delta t};$$
(4)

- VAP_i - average path velocity (see Figure 1):

$$VAP_{i} = \frac{\sum_{j=1}^{M-1} \sqrt{(\bar{x}_{j+1} - \bar{x}_{j})^{2} + (\bar{y}_{j+1} - \bar{y}_{j})^{2}}}{(M-1)\Delta t},$$
(5)

where $\bar{x}_k = \frac{1}{5} \sum_{i=k-2}^{k+2} x_i$ and $\bar{y}_k = \frac{1}{5} \sum_{i=k-2}^{k+2} y_i$, respectively;

- STR_i - straightness, or deviation of the averaged route in relation to the straight line:

$$STR_i = \frac{VSL_i}{VAP_i};\tag{6}$$

- LIN_i – linearity, or deviation of the trajectory in relation to the straight line:

$$LIN_i = \frac{VSL_i}{VCL_i}.$$
(7)

3. Implementation of semen analysis algorithms

An application has been prepared that realizes the following tasks:

- 1. acquisition of a sequence of images,
- 2. enhancement of images so that objects can be extracted from the background,
- 3. finding trajectories of sperm cells'movements by joining the gravity centers of the extracted objects,
- 4. calculation of the parameters of movement for each trajectory,
- 5. calculation of sample density.

3.1. Acquisition of images

Thanks to the co-operation with the 1st Obstetric-Gynecological Clinic of Professor Bablok, Warsaw, Poland, it was possible to access samples of semen of patients examined in the clinic. To elaborate and test the algorithms, a sequence of images of semen of 2 patients was registered. It consisted of 50 consecutive monochromatic images of 400×400 pixels and 256 levels of gray. The time of a sequence is around 4 seconds (50 images at 12Hz), Figure 2.



235

An Automatic System for Calculating Basic Semen Parameters



Figure 2. Subsequent images of a registered sequence $(\times 20)$

3.2. Pre-processing [4-6]

The following set of operations realizes the first task, i.e. enhancement of images:

- median filtering,
- multiplying and scaling,
- left shift (scaling),
- blur,
- negation,
- max filtering (giving the maximum value from the neighborhood).

These operations are performed on two consecutive images. Then the images are subtracted one from the other and the following operations are processed:

- square,
- dilation,
- closing,
- $\bullet\,$ erosion,
- $\bullet\,$ thresholding,
- dilation,
- $\bullet\,$ erosion.

3.3. Trajectories

The developed application extracted objects on the basis of a threshold operation. Then the gravity centers were found for all the extracted objects and junctions were generated between the found centers of gravity and the traced trajectories, Figure 3.



Figure 3. Trajectories

A problem of inappropriate sperm cell movement was encountered. In two cases only the first condition (max. 20 pixel replacement) was fulfilled. The second (forward movement) was not. To solve the problem, several separate trajectories were found in the first iteration. Then, in the second step, these pieces were joined into a single trajectory of bad sperm cell movement.

The second step did not influence the trajectories of the well-built sperm cells.

3.4. Density

The application calculated the density of the semen on the basis of the quantity of trajectories in 50 images. This is only a pilot evaluation of the semen. In the future, the density of a sample should be evaluated on the basis of a greater number of images of the same patient.

3.5. The parameters of movement

According to WHO standards, a fraction of at least 25% sperm cells with the proper movement classifies a patient as healthy [1, 2].

In the examination of our first patient only 17% of all trajectories were improper. It confirmed that the patient was healthy. In the second case a fraction of 37% improper trajectories was found and the patient was also classified as healthy.

4. Conclusion

The implemented application has allowed for an exact determination of the parameters of sperm cell movement. However, further research on clinical material is required to verify it. Further research is also needed to calculate sperm density in order to ascertain the sufficient size of a sequence of images to determine semen density with a satisfactory precision.

It seems that the application performs its tasks: it analyzes samples and provides a physician with substantial data for a more objective diagnosis, less reliant on his previous practical experience. It offers an easy way of handling and accessibility of the most important components: a computer and a microscope would allow for a broad application of the system in many medical centers.

The application was developed in Pascal. Borland tools (Delphi version 5) and an Intel Image Processing Library (version 2.5) were used [6]. The application is compatible with Windows NT 4.0/2000. The tests were performed on PC with a Pentium III 450MHz processor.

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