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

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EXAMINATION OF THE DENSITY OF SEMEN AND ANALYSIS OF SPERM CELL MOVEMENT.

The algorithms for examination of the density and chosen parameters of movement of sperm cell were elaborated and implemented. The conducted research is a part of a work on a computer system for analysis of semen. The system will allow for an increase of the preciseness of examination thanks to the exact specification of numerical values of chosen parameters. Additional advantage of the system is a shorter time of the examination. Nowadays, the basic type of examination is an estimated analysis of parameters of semen done by visual observation of a sample. This type of examination is based on a subjective assessment of an image by a physician. Moreover the registration of images in visual analysis is not possible.

1. INTRODUCTION

One of the more and more significant problems in the present world becomes the question of fertility of men. Researches conducted since many years show that the percentage of men having problems with fertility has been increasing. Their semen is "weak", characterized by low density, small fraction of sperm cells with proper structure and movement. All these make them unable for natural fertilization.

As a consequence of the development of the techniques of in vitro fertilization, the assessment of the quality of semen becomes vital. In many cases thanks to these techniques artificial fertilization is possible despite of "weak" sperm.

As a result of long year researches, World Health Organization standards have been elaborated. They classify the quality of semen on the basis of the value of such coefficients as: density, percentage of sperm cells with proper structure, fraction of sperm cell with proper movement etc. Healthy semen is characterized by the density of 20 – 250 millions sperm cells in 1 ml. as well as by the fraction of at least 25% of sperm cells with proper i.e. linear and progressive movement. Problematic is, however, the way in which these coefficients can be determined.

Currently the most common method used to find out the above parameters is visual analysis carried out by andrologist. The analysis is a subjective assessment of a microscope sample. The preciseness of such examination could be questionable and it depends on the experience of doctor. The possibility of repetition of the examination does not exist. One cannot either register images or create history of investigated samples. This problem is especially important in the case when a

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patient changes his andrologist. A new physician has to examine his patient once again and the results of his analysis may vary significantly from the ones done by the previous physician.

The development of the computer techniques caused an attempt for the elaboration of such system that would eliminate the impreciseness of visual analysis. This system would allow for determination of exact value of the coefficients, for registration of images and creation of the history of examined samples.

In 80' CASA (Computer Assisted Semen Analysis) systems were created. They were based on signal processors – aimed at fast processing of images. Disadvantage of these systems was their high price – only few clinics could afford it. The requirement of easy access was not fulfilled. Still the main analysis was visual examination.

Thanks to the further development of computers and the decrease of their prices it is easy to fulfill the postulate of accessibility. Therefore I decided to elaborate a computer system for examination of semen. It would require a computer synchronized with a microscope. The first stage of the work was to prepare an application enumerating the density of sperm. The second step was creation of an application that would determine parameters of sperm movements.

2. MATERIALS

Thanks to the co-operation with the 1st Obstetric-Gynecological Clinic of professor Bablok in Warszawa it was possible to have an access to samples of semen of patients examined in the clinic. To elaborate and test algorithms a sequence of images of semen of a healthy patient was registered. It consists of 50 subsequent monochromatic images of size 400 X 400 pixels and 256 levels of gray. The time of a sequence is around 4 seconds (50 images at 12 Hz).

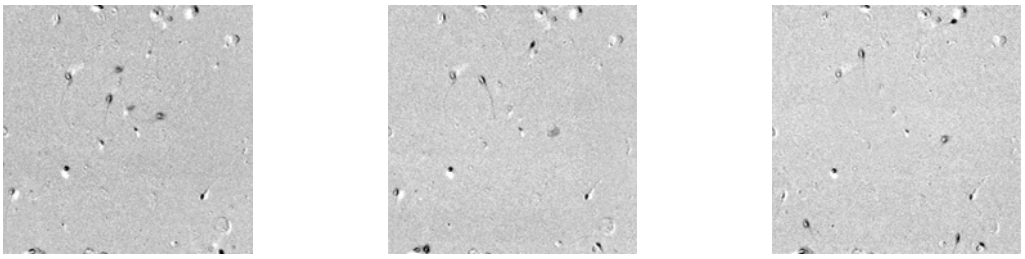

Fig.1. Subsequent images of a registered sequence (x20)

The sample is a sequence of alive semen images. On account of registration of alive sperm cells the semen is not stained. Therefore, the images are not contrast and it is difficult to extract sperm cells (one should extract sperm cells and reject any artifacts and other elements of semen that are not sperm cells before the analysis).

3. IMAGE ANALYSIS

3.1. PRE-PROCESSING

In order to calculate semen coefficients it is necessary to extract sperm cells from the background. To realize the task one uses operations of image enhancement like: logical, arithmetic, morphologic, neighborhood, point to point. Additional and important help is a possibility of detection of movement on the two following images by calculation of their sum. Also available is further processing of the sum of the images. After the set sequence of operations has been done next step is the detection of a gravity center of sperm cells on consecutive images of the sample.

3.2. TRAJECTORIES

After the process of pre-processing and extracting of objects (sperm cells) it is possible to search trajectories of sperm cells' movements by joining their gravity centers. Some knowledge of semen movement is known from the literature. It is known that the maximum speed of a sperm cell is 25 μm/s.

It can be concluded from this data, knowing that the microscope magnification was 20 times and camera was capable of capturing 12 images per second that the gravity center of a sperm cell can change its location maximally by 20 pixels on a single consecutive image. Another important feature of sperm cell movement is the ability to move only forward. Those conditions in most cases make possible to choose accurately the next proper gravity center.

In the case of conflict (more than one gravity center meet previous conditions) to choose properly between possible trajectories one can take into consideration additional information: speed. It is known that the speed of a sperm cell cannot change significantly. This allows for an accurate and appropriate determination of the next gravity center for a given trajectory.

3.3. DENSITY

The number of sperm cells (l) is determined on the basis of the number of found trajectories of sperm cell movements, that had either proper or improper run, observed in a sequence of 50 images. On the basis of the number of sperm cells one calculates the density of the sample (ρ_{sample}), where under the sample one understands the quantity of ejaculate used to prepare the microscope sample:

$$\rho_{sample} = \frac{l}{v_{sample}} * \frac{S}{S_o}$$

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

The density of the sample is calculated on the basis of 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.

On the basis of the density of the sample one can calculate the number of sperm cells (L) in the sample of semen.

$$L = \rho_{samlpe} * V_{ejaculate}$$

3.4. THE PARAMETERS OF MOVEMENT.

Another important parameter evaluating the quality of the semen is sperm cells movements. According to WHO standards to define the quality of sperm cell movement (index i) one has to calculate the following parameters of its trajectory:

VSL_i – straight-line velocity:

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

where: (x_1, y_1) – 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

VCL_i – curvilinear velocity:

$$VCL_i = \frac{\sum_{j=1}^{M} \sqrt{(x_{j+1} - x_j)^2 + (y_{j+1} - y_j)^2}}{(M-1)\Delta t}$$

VAP_i – average path velocity:

$$VAP_i = \frac{\sum_{j=1}^{M-1} \sqrt{(\bar{x}_{j+1} - \bar{x}_j)^2 + (\bar{y}_{j+1} - \bar{y}_j)^2}}{(M-1)\Delta t}$$

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: deviation of the averaged route in relation to the straight line:

$$STR_i = \frac{VSL_i}{VAP_i}$$

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

$$LIN_i = \frac{VSL_i}{VCL_i}$$

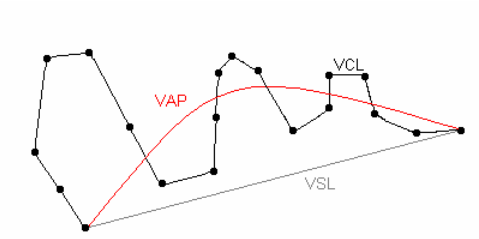

Fig.2. Illustrated parameters of the sperm cell movement

4. IMPLEMENTATION OF SEMEN ANALYSIS ALGORITHMS.

Worker out application realizes the following tasks:

1. enhancement of images in a such way that one could extract objects from the background
2. ranging out trajectory of the movement of sperm cell by joining gravity centers of extracted objects
3. calculation of the parameters of movement for each trajectory
4. calculation of the density of a sample

4.1. PRE-PROCESSING

The following set of operations realizes the first of tasks, that is: enhancement of images:

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

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

- square
- dilation
- closing
- erosion
- thresholding
- dilation
- erosion

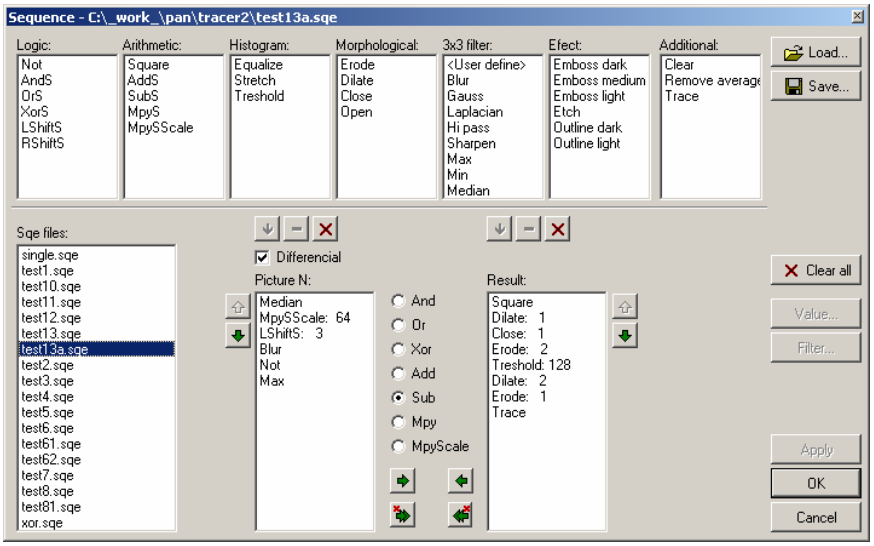

Fig.3. Window for defining of the sequence of operations

4.2. TRAJECTORIES

The worked out application extracted objects on basis of threshold operation. Then the gravity centers were found for all the extracted objects and generated junctions between found centers of gravity traced trajectories.

There were 12 trajectories in the sample: 10 of them of proper run and 2 of a bad run.

A problem of inappropriate sperm cell movement was met. In two cases only the first condition (max. 20 pixel replacement) was fulfilled. The second (forward movement) was not. To solve the problem in the first processing several separate trajectories were found. After that in the second step those pieces were joined into a single trajectory of bad sperm cell run.

The second step did not make any influence on trajectories of well build sperm cells.

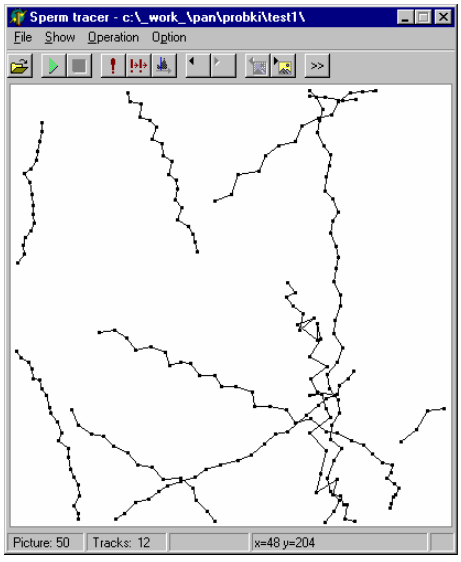

Fig.4. Trajectories

4.3. DENSITY

The application calculated the density of the semen on the basis of 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 bigger amount of images of the same patient.

4.4. THE PARAMETERS OF MOVEMENT.

The calculated values of movement confirmed a prior observation of trajectories. 10 of them were proper and 2 were wrong.

The table below presents the result of measurements of all the parameters of cells movements for 12 trajectories. Those marked indicated improper trajectories.

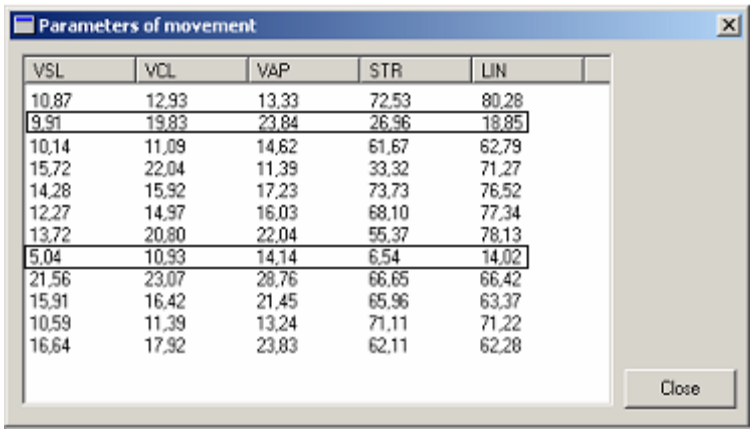

Fig.5. Trajectories

According to WHO standards fraction of at least 25% of proper movement sperm cells classifies patient as healthy.

In the examination of our patient only 17 % of all trajectories were improper. It confirmed the patient was healthy.

5. CONCLUSION

The implemented application allowed for an exact determination of the parameters of sperm cells movement. However, further research on clinical material is required to verify appropriateness of the implemented. Further researches are needed also to calculate the density of sperm in order to answer the question how big should be the sequence of images to determine density of semen with an assumed precision.

It seems that the worked out application performs its tasks: it analyzes sample, provides physician with substantial data, thanks to which he can issue more objective diagnosis. A long year experience for a physician would not be necessary. Easy way of handling and accessibility of the

most important components: computer and microscope would allow a broad application of the system in many medical centers in the future.

The application was developed in Pascal language. The tools of the Borland firm (Delphi version 5) and library: Intel Image Processing Library version 2.5 was used. The application is compatible with Windows NT 4.0/2000. The tests were performed on computer PC with a processor Pentium III 450 MHz.

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