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INTERDISCIPLINARY TOPICS

SINGLE SPERMATOZOON HEAD TRACKING FOR ROBOTIC INTRACYTOPLASMIC SPERM INJECTION (ICSI)

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ABSTRACT: In this paper, a new approach for autonomous single spermatozoon tracking has been proposed. During the presented approach, there is no need to track low contrast objects (spermatozoon tail). The most important part is the motion trajectory tracking of the spermatozoon head. The effective sperm head tracking has been realized using image processing and sperm head shape recognition (as a sphere). Without following the sperm tails - moving with very high dynamics, the software application is faster working. The tracking algorithm has been realized in a square Sperm Head Region of Interest (SHROI), with a size of 100×100 pixels. The developed software application is compatible with almost all digital cameras available on the market.

KEY WORDS: *in-vitro* fertilization (IVF), spermatozoa, image processing, object recognition, single spermatozoon head tracking, intracytoplasmic sperm injection (ICSI) in oocyte

1 INTRODUCTION

The assisted reproductive technologies (ARTs) have been developed over the past 40 years. The ARTs are particularly helpful in combating male infertility. ARTs include intrauterine insemination, *in-vitro* fertilization (IVF) and ICSI.

The male and female infertility is a growing global health problem affecting people of reproductive age all over the world. As a disease of male and/or female reproductive system, the infertility is defined by the failure to achieve a pregnancy worldwide. According to the World Health Organization, 8–10% of the couples worldwide require IVF treatment to conceive [1]. It has an impact on their families and communities.

The fertility rate has almost halved in the past 50 years globally. The average fertility rate was measured as 2.5 from 2005 to 2010. It had been recorded as 4.97

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during 1950. The forecasting data for future is even worse (2.3 around 2050 and to 1.99 around 2100). The mobility rate of spermatozoa in humans also decreases from 60% to 30% between 1970 and 2010 [1, 2].

Infertility is commonly caused by serious problems in the male and female reproductive systems and their endocrine regulation.

IVF is a type of assisted reproductive technology. It involves retrieving oocytes from a woman's ovaries and fertilizing them with spermatozoa [2]. The intracytoplasmic spermatozoon injection (commercially most familiar as ICSI) is the leading noninvasive treatment of infertility used especially for significant male infertility cases. ICSI is a reproductive assisting procedure in which a single spermatozoon is injected directly into an oocyte. ICSI procedure accounts for over 70% of IVF treatments globally [2]. This procedure also gives the embryologist the confidence to work within the cytoplasm of the oocyte, which is not necessary during the conventional IVF work. This manipulation has opened the door to a wide field of researches on the oocyte [3]. During ICSI, inverted microscopes, micro-injectors and micro-manipulators are used by well-trained specialists [4, 5].

One of the main aspects of ICSI is the visual tracking of microscopical objects – in particular of motile spermatozoon. The spermatozoon motion is related with the mithochondria situated in the midpiece (the part connecting the sperm head and the sperm tail). There is very important to keep the midpiece in safe because it is not clear if the mithochondria are playing a role during the fertilization [6, 7].

ICSI is more than an oocyte injection. Before the oocyte to be injected, a spermatozoon must be tracked, immobilized and aspirated in a glass micro-needle, for injection. Because of the small size and extremely fast moving spermatozoon, the sperm head and sperm tail visualization is rather difficult in the conditions of the optical microscopy.

Tracking biological objects with low contrast features has been one of the leading scientific tasks during the last two decades in robotics and automation societies. One of the main difficulties is that the information about the edges of the object has often been lost or could not be extracted. Chan et Vese developed an algorithm for detection of objects based only on their contours without edge information [8]. This method requires a lot of iterations before achieving the final solution. Therefore, this method isn't suitable for real time applications. The Kalman filter is optimal for tracking objects with low contrast that show a linear and Gaussian temporal dynamics [9]. Since the spermatozoa motion has a complex non-linear and non-Gaussian time dynamics, the Kalman filter appears to be inappropriate for spermatozoon tracking.

Once the spermatozoon is immobilized, it should be aspirated and injected in the cytoplasm of the oocyte. A lot of scientific research has been done for cell injection automation using robotic and automation approaches [5, 10]. The development of

micro and nanorobotics also has found its place in the field of automation of complex micro and nano operations as sperm cell remote control, transportation, positioning and orientation [11–13]. Most of these systems utilize in a direct manner the architecture of the conventional manual operations. They succeeded to automate some particular steps of the complex ICSI procedure. However, there is no information about a successful commercial application of a developed robotic or semi-automated ICSI system that is used in IVF medical centers. It certifies that these systems are still under development and additional steps (like pipette alignment with respect to the oocyte, cell detection, 3D cell orientation according to the polar body, precise sperm cell aspiration, single spermatozoon selection and tracking), could not be realized without a skilled human-operator. The robotic research in this field is based on precise motion control of the micro-robots, development of various computer vision algorithms (for localization and visual tracking of cells) and micro-pipettes, vision servo-control of micro-pipettes or integration of visual and haptic interfaces [14].

The manipulation of the suspension cells attracts the interest of the specialists in the field of robotics and cell biology. Because of the motile nature of the cells in the suspension, spermatozoa are utilized for development of bio-hybrid micro-robots for assembly [11, 15] and transport of drug delivery [16]. In biology, the motile cell needs to be manipulated and immobilized for biological and biochemical analysis. The spermatozoa immobilization is an important step during the analyses in the field of biology and in clinical ICSI where one spermatozoon has to be immobilized before its aspiration into the glass micro-pipette for oocyte injection.

Several techniques have been developed for manipulation of motile spermatozoa using motorized micro-manipulators [17–19], optical tweezers [20], opto-electronic tweezers [21], lithographic based patterning [22,23], fluidic flow [24], magnetic field [25,26], electrical field [27], and acoustic field [28]. Hence, most of these techniques were developed for cell trapping and therefore they could not be applied for tasks related to a permanent immobilization of motile spermatozoa.

The immobilization of the sperm-cells is performed conventionally by well-trained embryologists. The sperm-tail has been tapped to the surface of the Petri-dish using a micro-pipette. Because of the fast spermatozoa motion and their small size and low visualization, the manual operation has stringent skill requirements. Therefore, the success rates vary significantly between the operators. Sun and Nelson have tried to automate the spermatozoa tracking using computer vision and motion control approaches [10]. The authors have developed a specific rotational module that could orient the spermatozoa regarding the motion axis of the immobilization micro-pipette. In this way it was easier to tap the sperm tail without damaging its head, but some perturbations from the needle tip that is in close proximity to the sperm tail, might disturb the process. This approach can only be useful when slow moving spermatozoa are concerned [23].

In this article, we present an approach for spermatozoa tracking, where there is no need to track fast moving sperm tails with low contrast. All we need to know is the trajectory of the spermatozoon motion. Obtaining and discussing this information, we suppose where is the spermatozoon tail and its orientation. The sperm head is kept close to the center of the Sperm Head Region of Interest (SHROI), and during the spermatozoon immobilization there is no need to track the spermatozoon. Therefore, there is no perturbation from the tip needle during the spermatozoon immobilization procedure. The SHROI is enlarged a little bit, applying this approach for faster spermatozoa too.

2 System Setup

Our robotic ICSI system (Fig.1) consists of a standard inverted microscope (Carl Zeiss Axio Observer Z1), equipped with a motorized XY positioning stage (120×80 cm^2 working range and accuracy of 1 μm). The maximal speed of motion of the positioning stage is up to 120 mm/sec. The resolution is 1 μm during PC control. A CCD color camera (Jenoptik ProgRes SpeedXT core 5, full sensor resolution 2576×1932 pixels, pixel size – 3.4 μm$\times$3.4 μm, frame rate – 13 fps, A/D conversion – 12 bit) is connected to the microscope as a visual feedback. Spermatozoa are visualized using 20$\times$ and 40$\times$ microscope objectives. Two micro-manipulators (Sensapex) are used for realization of complex operations with oocytes and spermatozoa. The aspiration and release of the oocytes and spermatozoa have been made with two manual micro-injectors that are automated in our lab using suitable step-motors. The heating module (accuracy of 0.1°C saving the viability of the biological objects) has been integrated inside the positioning stage.

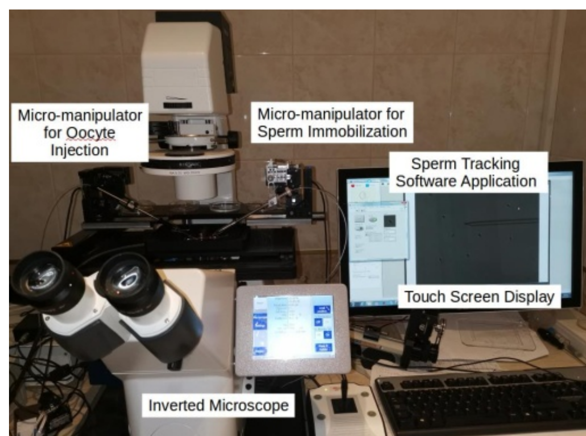

Fig. 1: Experimental setup for robotic ICSI and sperm cell tracking.

A standard clinical ICSI micropipette has been used for immobilization of the spermatozoa. The micropipette tip is parallel to the substrate of the Petri-dish during the spermatozoon tail tapping. The workstation used in this experimental setup has eight cores Xeon processor at 3 GHz and 16 GB RAM. The live image speed in combination with the high resolution facilitates a precise focusing and easy positioning of specimens without interlaces effect in a more efficient way. This is a clear advantage when analyzing moving objects such as spermatozoa.

3 Algorithm for Autonomous Spermatozoon Tracking for Robotic ICSI

An algorithm for autonomous spermatozoa tracking for robotic ICSI has been developed (Fig. 2).

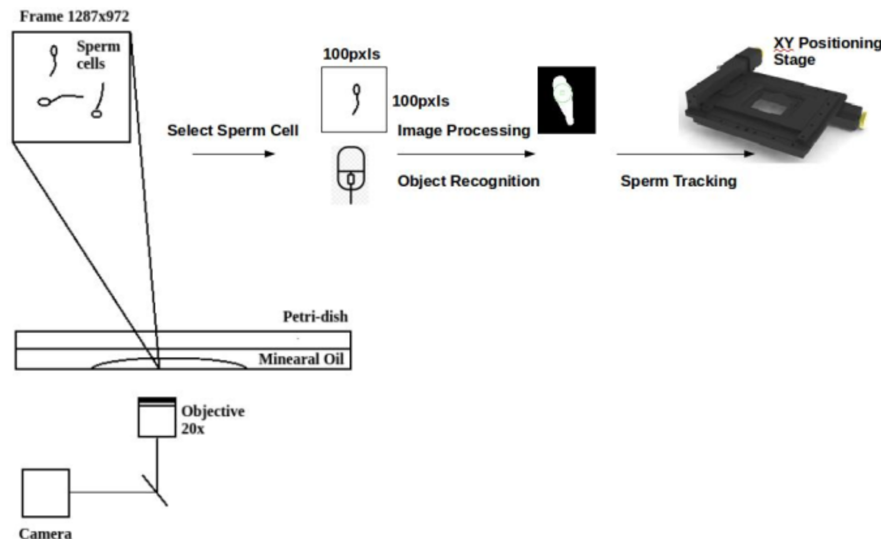

Fig. 2: Algorithm for autonomous spermatozoon tracking for robotic ICSI.

A small drop with spermatozoa has been deposited in a specially developed Petri-dish, placed on XY motorized scan stage. The embryologist observes the motion of the spermatozoa under an inverted microscope with a CCD camera and optical magnification 20×.

At the initial stage, the operator selects a suitable spermatozoon by clicking a computer mouse in proximity to the sperm head. Then a square SHROI of 100×100 pixels is created.

The center point of the SHROI is the mouse clicking point. Using image processing and object recognition methods, the spermatozoon is detected. A circle is set

into the recognized spermatozoon head. The center of the circle is the new center point of the SHROI. The XY microscope scan stage keeps the spermatozoon head close to the SHROI center point. The spermatozoon head must not leave the SHROI boundaries. During the tracking procedure, the spermatozoon is immobilized and prepared for injection into the cytoplasm of the oocyte, thus realizing a standard ICSI manipulation.

4 Spermatozoon Head Motion Trajectory Areas

Different spermatozoon positions – regarding the SHROI are presented below. Two areas are defined in the SHROI (Fig. 3a).

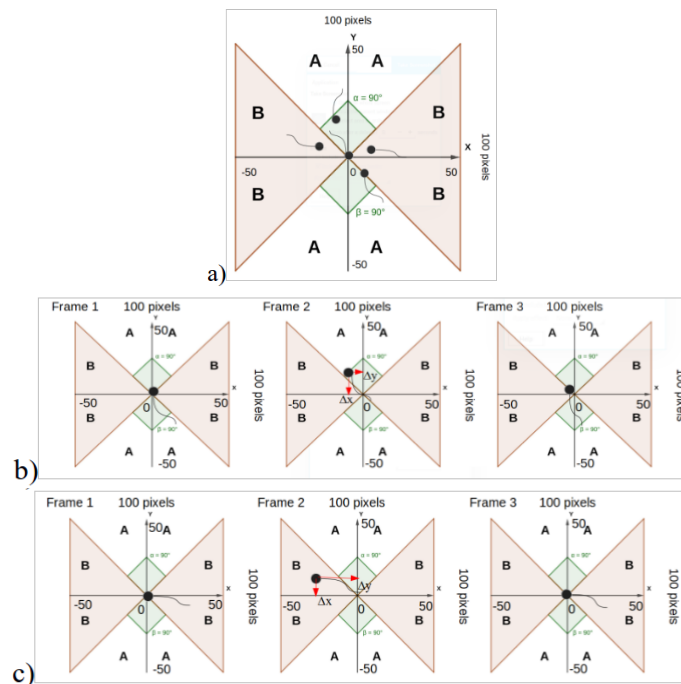

Fig. 3: **a**: Spermatozoon head motion trajectory areas (area A – sperm immobilization area; area B – area where the sperm immobilization is impossible with this approach; SHROI size – 100×100 pixels); **b**: Consecutive frames of the spermatozoon head moving in area A. If $\Delta x < \Delta y$ the sperm head is moving in area A. When the spermatozoon head has been positioned to the center of the SHROI, the sperm tail could be cut and the sperm cell is immobilized; **c**: Consecutive frames of the sperm head moving in area B. If $\Delta x > \Delta y$ the sperm head moves in area B (at this area the sperm cell could not be immobilized).

When the SHROI has been created, the sperm head is close to the center of the SHROI. The time between two consecutive frames is almost 100 ms. During this time, the sperm head is moved a little bit. The new coordinates are evaluated regarding the X–Y coordinate system. If $||x|| \leq ||y||$, then the sperm head motion trajectory is in area A (Fig. 3b).

The sperm tail is almost perpendicular to Y axis. The tip of the glass needle is moving along X axis. In this way it is possible to cut the sperm tail and to immobilize the spermatozoon. If $||x|| \geq ||y||$, then the spermatozoon head motion trajectory is in area B (Fig. 3c). The sperm tail is parallel to the X axis and the tip of the glass needle, making impossible to immobilize the spermatozoon by this approach. The spermatozoon head motion trajectory is defined for 1–2 seconds. During this time, it is possible to define the coordinates of the spermatozoon head and to classify its motion trajectory in area A or area B.

5 Software Application for Single Spermatozoon Tracking

The software application has been developed in LabVIEW. The live streaming and the whole control of the CCD camera have been realized using the software from the manufacturer of the camera. At the beginning, the CCD camera control software has been launched. Using user32.dll library, the software application for single spermatozoon tracking has been moved on top programmatically as a front panel in front of all desktop windows applications launched (Fig. 4).

Fig. 4: Screenshot of the code using user32.dll library launching the sperm tracking software application as a foreground program.

In this way, during the spermatozoon tracking procedure, it is possible to use all the functionalities of both applications. The developed software could be used by any suitable digital camera, without modifications or writing any codes. This approach gives an exceptional flexibility to the software application.

The first step after launching the developed software application is to select the appropriate spermatozoon. The operator-embryologist is clicking with the computer mouse in proximity to the sperm head thus creating a SHROI. The sperm tracking procedure starts automatically. The image processing and object recognition tech-

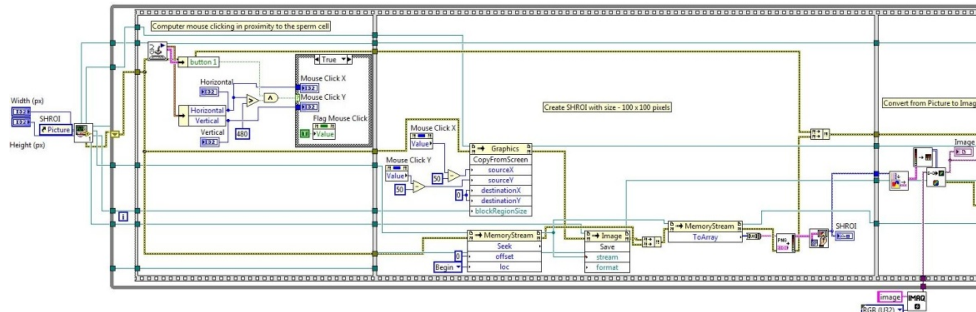

Fig. 5: Screenshot of LabVIEW code creating a SHROI after computer mouse clicking in proximity to the spermatozoon head.

niques are applied to each consecutive image frame. The main goal is to keep the spermatozoon head near the center point of the SHROI (Fig. 5).

Each image processing has been followed by the scan stage movement along X and Y axes. At the beginning the coordinates have been taken from the mouse clicking operation. The data has been converted from pixels to centimeters because of the specific functionality of the used positioning stage. The sperm head has been displaced regarding the center point of SHROI and the new data has been stored for the next frame (Fig. 6).

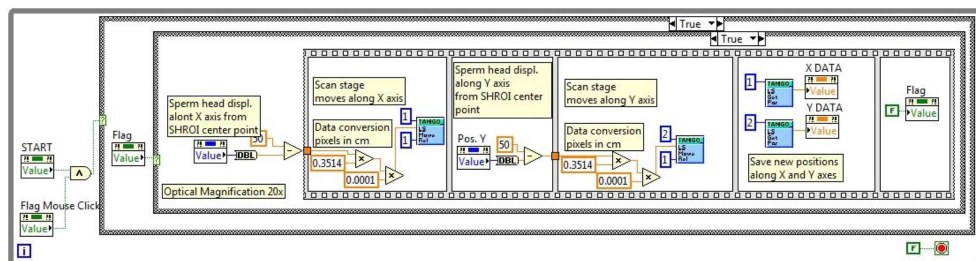

Fig. 6: Screenshot of LabVIEW code receiving the coordinates of the sperm head during its movement. Converting the displacement along X and Y axes from pixels to micrometers and send this information to the microscope scan stage for keeping the spermatozoon head to the center of the SHROI.

The front panel of the software application has been represented in Fig. 7. The software interface is giving the possibility to start the application. Using the IMAQ Image display control in LabVIEW the operator can track the sperm head realizing the algorithm presented in Fig. 8. The size of the SHROI could be changed by the edit labels Height (px) and Width (px). The edit label “Nb Sperm” counts the number

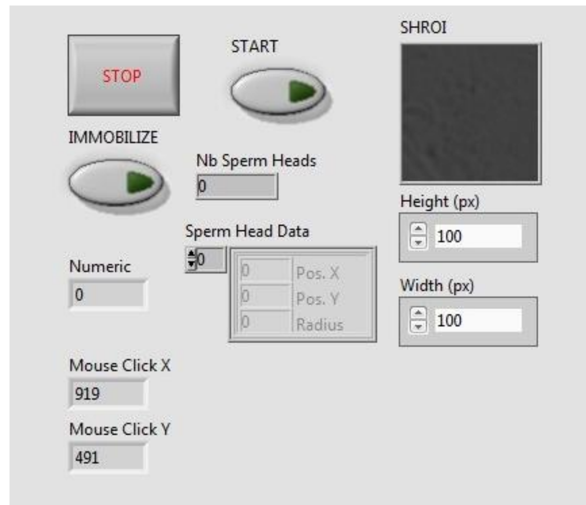

Fig. 7: Front panel of the software application for spermatozoon tracking.

of spermatozoon heads entering the SHROI. All the time the operator can check the radius of the spermatozoon head and the center point coordinates of the spermatozoon head along X and Y axes.

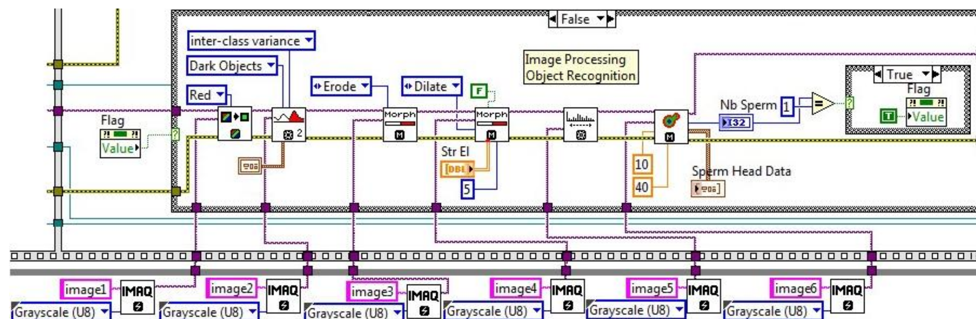

Fig. 8: Screenshot LabVIEW code performing image processing and spermatozoon tracking.

6 Image Processing for Effective Spermatozoon Head Tracking

The image processing is realized using image format 1287×972 pixels. The optical magnification is 20× DIC contrast. At the beginning, Otsu's method has been used to perform automatic image thresholding in its simplest form. The algorithm separates the pixels into two classes, foreground and background. In LabVIEW this method is

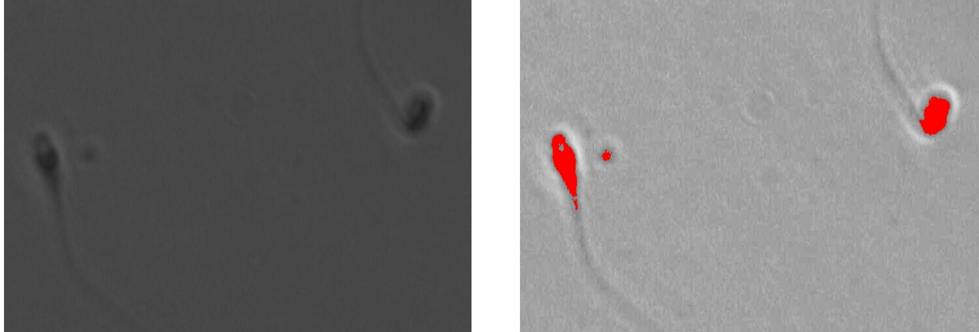

Fig. 9: The image on the right side presents the Otsu's method application to the original image frame (left image). The dark objects are spermatozoon heads.

known as Auto Threshold Inter Variance. The Otsu's method applied in this application has been used for dark objects, such as the spermatozoon heads (Fig. 9).

The basic morphology operations in LabVIEW are applied to binary images to process each pixel based on its neighborhood. Each pixel is set to 1 or 0, depending on its neighborhood information and the operation used. These operations always change the overall size and shape of particles in the image. The basic morphology functions are used for images in which particles have been set to 1 and the background is equal to 0. The primary morphology transformation called Erosion has been used in the developed image processing approach (Fig. 10).

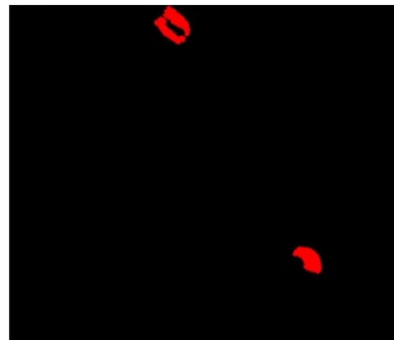

Fig. 10: Application of the primary spermatozoa morphology transformation. Erosion, eliminating smaller pollution particles from the sperm tracking procedure.

Erosion eliminates pixels isolated in the background, and erodes the contour of particles according to the template defined by the structuring element. In this way, particles with smaller size than the sperm heads are eliminated from the background or their size is decreased a little bit more. As a result, the pollution particles are eliminated, thus improving the tracking procedure of the spermatozoa.

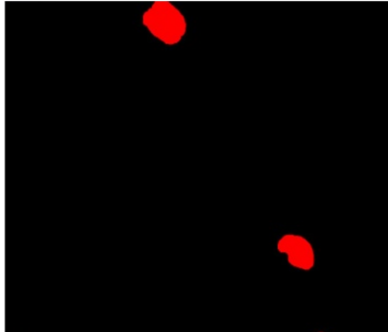

Fig. 11: Application of the primary morphology transformation. Dilation expanding the spermatozoa heads and improving the sperm tracking procedure.

The primary morphology transformation called Dilation (Fig. 11) eliminates extremely small holes isolated in particles and expands the particle contours according to the template defined by the structuring element. This function has the opposite effect of the Erosion because the Dilation is equivalent to erode the background. After the elimination of the extremely small obstacles from the image, then the other objects as sperm heads expand their size, thus improving the spermatozoa tracking operation.

The Lookup Table (LUT) transformations are basic image-processing functions that highlight details in areas containing significant information, at the expense of other areas. LUT is used to improve the contrast and brightness of an image by modifying the dynamic intensity of regions with poor contrast. A LUT transformation converts input gray-level values from the source image into other gray-level values in the transformed image.

Fig. 12: Application of circle detection function for the purposes of spermatozoon head object recognition and sperm tracking.

In the developed approach, Equalize function has been applied. It is a LUT operation that alters the gray-level of pixels so that they become evenly distributed in the defined grayscale range, which is 0 to 255 for an 8-bit image. The Equalize function has been used to increase the contrast in images that do not use all gray levels.

The final step of the proposed approach in this publication is the circle detection function (Fig. 12). The radius size is defined from 15 to 25 pixels. The circle has been inserted into the spermatozoon head. In this way the coordinates of the circle center point will be known for each consecutive image frame, thus tracking the spermatozoon head and keeping it in the center of the SHROI, respectively.

7 Experimental Setup

The experiments are realized with the developed software application and the robotic ICSI system. Usually the speed of the spermatozoon is around 25 μm/sec. Using the approach described in this publication, it is impossible to track such fast moving biological micro-objects. As a result, the software application could be applied successfully for spermatozoa using polyvenilpyrrolidone (PVP). The PVP is a soluble polymer (in water) that is decreasing the motility of the sperm cells. Thus, it is possible to select a suitable spermatozoon clicking with the computer mouse. An optical magnification 20$\times$ and DIC prism are used during the experiments. The resolution of the CCD camera is 1287$\times$972 pixels. The size of the SHROI is 100$\times$100 pixels. This size gives the opportunity to track faster spermatozoa. After each consecutive image frame the spermatozoon head has been positioned in the proximity of the center point of the SHROI. The positioning procedure has been realized automatically from the microscope scanning stage. The spermatozoa crossing the boundaries of the SHROI and leaving immediately have not been detected. The biggest drawback of this approach is the possibility of other spermatozoa to enter the SHROI and to disturb the tracking operation. This software application can only be used for image tracking of spermatozoa heads that are situated in area A (Fig. 3b). The sperm cells in area B (Fig. 3c) could not be immobilized without an additional orientation. This tracking procedure takes up to 5 seconds. During this time, the trajectory of motion has been evaluated and the software application makes the decision for immobilization of the spermatozoa.

The front panel of the developed software application is always on the top of the computer display. The control application of the CCD camera is working as background during the sperm tracking procedure. The computer mouse clicking operation is active only when the mouse cursor is situated in the image frame.

The foreground and background parts of the software approach are presented in Fig. 13.

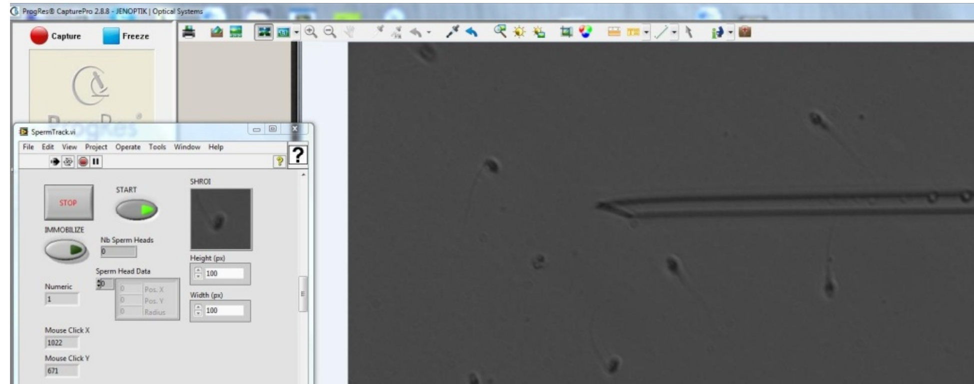

Fig. 13: User interface of the software application for sperm cell tracking.

The operator selects a desired spermatozoon using the computer mouse (Fig. 14). The mouse cursor has to be in proximity to the sperm head. During the creation of the SHROI the mouse cursor coordinates are at the center point of the SHROI.

Fig. 14: Screenshot of the mouse cursor situated near the spermatozoon head.

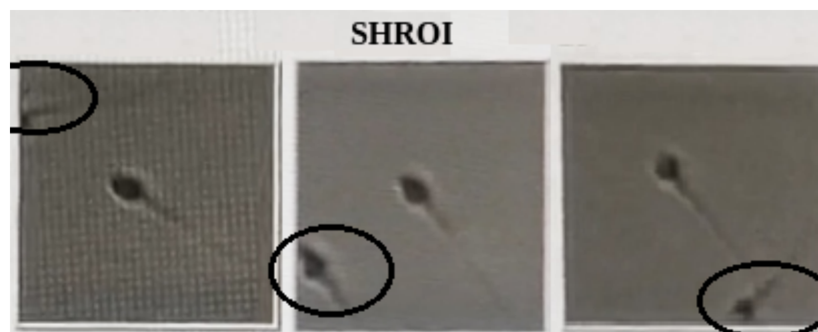

Fig. 15: Screenshots with additional spermatozoa entering the SHROI (near the boundaries) without disturbing the spermatozoon tracking procedures.

During the spermatozoon tracking, the software application can be disturbed when another spermatozoa are entering the SHROI. If the spermatozoa are close to the boundaries of the SHROI, there is no interference during the single spermatozoon tracking (Fig. 15).

8 Discussions

The proposed approach for single spermatozoon head tracking could be applied mainly for ICSI assisted procedure. One of the most important advantages of this approach is its simple structure that makes it extremely fast during the tracking of highly dynamic biological micro-objects for a short period of time (approximately up to 5 seconds) in 2D space.

The robotic system for clinical ICSI applications developed [23] also is realizing a visual automated tracking of motile spermatozoa similar to our approach. The researchers have adapted the probabilistic data association filter by adding spermatozoon head orientation into the state variables for robust tracking of the spermatozoon [29]. In this way they assumed that the spermatozoa shape is a centroid. The orientation of the spermatozoon has been calculated using the angle between the major axis of the spermatozoon head and the horizontal axis of the image frame.

The proposed approach also is tracking the spermatozoon head but the spermatozoon shape is assumed to be a sphere. In this way the tracking of the spermatozoon head orientation has been avoided and the image recognition procedure has been realized faster. The scientists [23] made an estimation of the spermatozoon tail positioning according to the orientation of the moving centroid.

In our approach we also estimate the spermatozoon tail positioning but regarding the direction motion of the spermatozoon head.

The researchers [23] classified spermatozoa in 4 quadrants based on their head orientations. With a specially developed rotational stage, the researchers performed a visual serving to orient the target spermatozoon in the appropriate quadrant for immobilization. This additional reorientation slow down the whole tracking procedure and increase the positioning error.

Our approach classified spermatozoa on the same way as in [23]. We didn't use a rotational stage for additional orientation of the spermatozoon according to the immobilization glass tip needle. A small suction from the glass needle has been applied, thus changing the spermatozoon orientation. The other possibility is to select a new spermatozoon for visual tracking and immobilization.

Spermatozoa swim in three dimensions (3D), to find oocytes. The spermatozoa motility has been mostly studied in two dimensions. Corkidi and Dorszon [30] have described a new method allowing 3D tracking and analysis of the trajectories of multiple free swimming spermatozoa. A piezo-electric device was displacing a large

focal distance objective mounted on a microscope to acquire image stacks spanning a depth of 100 μm. In this way the spermatozoa have been visually tracked in depth.

At this stage, our approach didn't track the spermatozoa in depth. Our optical system has an autofocus module but this procedure will slow down the visual tracking. In future we intend to use a piezo-electric device as an actuation module that will help to increase the speed for image acquisition along Z axis.

In the literature there is information about the development of innovative microrobots (spermbots) [12]. These small microtubes or helicoidal springs have been manufactured using 3D nanoprinting technologies. Flexible polymers with electroconductive coatings have been used. They have been controlled by an external magnetic field. They have a great potential for IVF applications as they could displace motile and immotile spermatozoa. The technology at this stage of development is extremely expensive. The availability of a low magnetic field could not still convince the scientists that this approach is sufficiently biocompatible.

Semen analysis is currently performed using visual assessment (manual observation) and computer aided sperm analysis (CASA) systems. CASA systems are very expensive and they cannot be used in the everyday laboratory practice. CASA systems provide multiple analyses automatically by using image and video processing concepts with machine learning ideas. Thus, they could not be applied for a real time tracking of single spermatozoon in ICSI procedures.

Various algorithms have been developed and involved in CASA systems as phase contrast based computerized spermatozoa motility analysis, hybrid generative-discriminative tracker for single spermatozoon tracking, Gaussian and Laplacian of Gaussian filters for a contrast increase, joint probabilistic data association filters, etc. Nearly all commercial CASA devices use the nearest neighbor tracking algorithm to find the trajectory of sperm and nearest global neighbor [14, 31]. A novel cheap approach for analyzing a spermatozoa motility by a smartphone based device, has been also proposed by [1].

In fact, all CASA systems could be used mainly for preliminary analyses of the motility and morphology of the spermatozoa [32, 33], before their use for ICSI procedures.

9 Conclusions

A new approach for automation spermatozoa tracking has been proposed. The software application has been developed using LabVIEW, image processing and object recognition functions. A SHROI has been created and the sperm head has been kept close to the middle point of the SHROI (for 5 seconds). According to the motion trajectory of the spermatozoon, the software application is sending a signal to the robotic system for starting the immobilization procedure with a micro-pipette cutting

the spermatozoon tail. The huge advantage of this approach is that there is no need from a sperm tail tracking. Hence, the software application is running faster and it is suitable for integration in real medical procedures. The developed software application for spermatozoa tracking is extremely flexible because it could be combined with the commercial software of almost all digital cameras available on the market.

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