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Detection of the Oocyte Orientation for the ICSI Method Automation

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***Abstract*—Automation or even computer assistance of the popular infertility treatment method: ICSI (Intracytoplasmic Sperm Injection) would speed up the whole process and improve the control of the results. This paper introduces a preliminary research for automatic spermatozoon injection into the oocyte cytoplasm. Here, the method for detection a correct orientation of the polar body of the oocyte is presented. Proposed method uses deep learning U-Net architecture for object segmentation. This solution proved to be universal and had no demand for numerous dataset or high-quality images.**

***Index Terms*—image segmentation, ICSI, U-Net, deep learning**

I. INTRODUCTION

The number of couples who have infertility problems increases year by year. According to Eurostat, the fertility rate in the EU countries remains below the stabilising rate necessary for maintaining population size [1]. It is estimated that one in six couples worldwide experience some form of infertility. Very often the only chance for them to have children are artificial methods of fertilization, for example the ICSI method invented over 25 years ago [2].

A. Intracytoplasmic Sperm Injection method

The ICSI procedure involves the injection of a single motile spermatozoon into the oocyte. The rate of the success of the method largely depends on the embryologist's skill level. The embryologist's training lasts up to six months. However, even a well-done manual procedure suffers from low repeatability, inconsistency among operators, and, what is the most important, from their subjective assessment. Automation would not only increase repeatability and enable comparison of the results, but also eliminate subjective evaluation of some process steps, such as the detection of the exact moment of oolemma rupture. The oolemma is not always immediately pierced by simple injection of the needle. Then minimal suction that causes the enter of the ooplasm into the injection pipette needs to be applied [3]. The membrane rupture is indicated by the sudden acceleration of the flow in the pipette after which the aspiration should be immediately stopped and the sperm cell slowly released into the oocyte. The flow acceleration is hard to estimate for the human observer and therefore this step is considered as difficult.

B. Related work

The last 27 years, since the ICSI method was invented, have witnessed substantial engineering efforts to automate at least some of its procedures. Papers [4], [5], [6] describe semi-automatic cell injection. However, procedures challenging for computers, like proper alignment of the micropipette or cell position and orientation, are still performed by human operator.

In 2011 paper [7] introduced the Robotic ICSI system - the system that automatically positions oocyte, tracks and immobilizes sperm. The oocyte injection is semi-automatic

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and demand minimal human involvement.

During ICSI, special attention should be given to the following points [8], [9]:

1) The selection and immobilization of a viable, morphological correct spermatozoa,
2) The correct orientation of the polar body of the oocyte during positioning before injection,
3) Cytoplasm aspiration to ensure oolemma rupture.

In this work, we will focus on the second point. The correct orientation of the oocyte can be recognized by the polar body position. The polar body is a small round-shape structure containing oocyte's genetic information copy. The oocyte is held in 9 o'clock position with optimal suction by the holding pipette. The polar body should be located at the 6 or 12 o'clock position, which avoids damage to the spindle. The injection needle containing the immobilized sperm cell should be introduced at the 3 o'clock position into the equatorial plane of the oocyte only if the holding pipette as well as the oocyte are in perfect focus [8], [9]. Therefore, it is important to determine the position of the oocyte, presented by the relative location of the polar body.

There are numerous intracellular structures detectors in the literature. One of them are circle fitting or Hough transform used in [10], [11]. Authors assumed, that oocyte is always a circle, what is an incorrect assumption especially during oolemma rupture (see Fig. 3). The circular shape is also distorted by the pressure in the holding pipette (see Fig. 2). Method presented in [11] was applied on an oocyte not held by the immobilizing pipette.
Wang et al. proposed polar body detection by applying a complex pipeline of several different algorithms including image opening, Otsu's thresholding, edge detection, filtering, hole filling, inscribed circle detection and ellipse fitting [12]. The pipeline composed of numerous image processing steps, including morphological methods, may suffer from low effectiveness when used on images acquired from different camcorder, with different contrast, scale or light distribution. Furthermore, the proposed solution was evaluated on images with clearly visible polar body without the presence of other round intracellular structures, which are natural in laboratory images. Fig. 2 presents the frame from ICSI procedure. There are other circular structures at the 3 o'clock position, that can be misinterpreted as the polar body by the proposed pipeline. Another method proposed in [13] was based on image texture. However, the impact of lighting condition and the insufficient texture information on the accuracy makes the method insufficient for images acquired with various parameters.
The recent proposition involves Histogram of Oriented Gradient and Support Vector Machine algorithms [14], [15]. However, the learning-based detection demands large set of diverse training data.

C. Background

In this study, we have proposed an algorithm, based on deep learning, that distinguishes polar body from the oocyte, as well as quantifies their surface areas, center of gravity coordinates and circularity ratio. It does not require preprocessing of the microscope image, neither the large training dataset. As a segmentation method we chose U-Net [16]: Fully Convolutional Network dedicated to biomedical image segmentation.
Our decision was preceded by testing of already proposed methods for polar body detection. We were unable to create a pipeline of simple image preprocessing methods, like filtering, thresholding, morphological operations, contour finding or circle fitting, that would be universal for all available recordings. One of the circle fitting weakness are the cases of cell shape change. Small changes occur when the cell is immobilized by the pipette - the oocyte is then more in the shape of ellipse than circle. Larger deformations are present during the pressure of the needle on the cell membrane - the oocyte has non-regular shape in that case (Fig. 3). Another weakness of this approach was the visibility of pipette and other circles of similar size to oocyte, which were often detected instead of the cell itself. The visibility of other small round intracellular structures of oval shape also prevent the correct polar body segmentation. In order to increase the visibility of the oocyte, we have used a variety of filters (sobel, canny, laplace, gabor) and morphological operations (e.g. thresholding or filling) before the fitting, but it did not increase the correctness of the detection.
We have also developed an active contour-based object detection algorithm. However, this approach required a lot of iteration steps, hence was unsuitable for real time working. Nor did it cope with the moment of oolema deflection and a significant change in shape. The active contour method usually fails during the detection of narrowings in the contour. Implementation of Active Contours Model from scikit-image - an image processing library for Python - was used for evaluation.
Finally, we chose U-Net network because it achieved the best results.

D. U-Net

The U-Net network was introduced in 2015 by Ronneberger, Fischer and Brox. It consists of symmetric contracting and expansive path (Fig. 1). The contracting path is composed of multiple convolution and max pooling operations, while in the expansive path the max pooling is replaced by up-convolution. Also features from the contracting path are combined with the upsampled output. Therefore, the architecture is U-shaped. The output is in the form of image where each pixel is labeled. The architecture benefits from Convolutional Neural Networks (CNN) in automatic image features extraction. However, two other features make it an excellent tool for biomedical images segmentation: separation of touching objects and data augmentation. U-Net can easily merge closely placed objects by learning the small separation borders between

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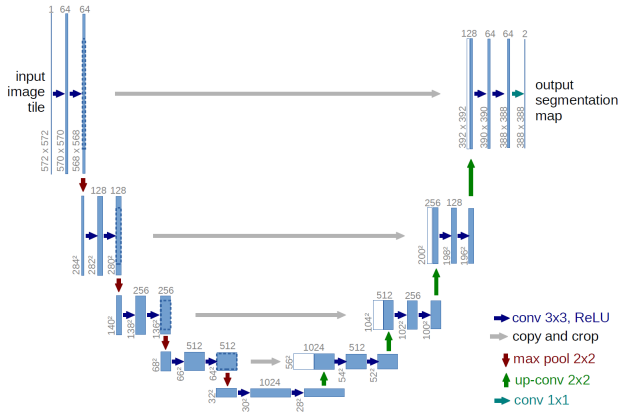

Fig. 1. U-net architecture from [16]. The contracting path is composed of convolution (blue arrow) and max pooling (red arrow) operations. The expansive path consists of convolution and up-convolution (green arrow).Gray arrow means combination of features from the contracting path with the result of up-convolution

touching cells. Random deformations of the input and output segmentation map cause data augmentation, what in turn increases the size of training set. Data augmentation creates simulated images with changes in the observed shapes which are natural under external conditions like pressure of the needle, increase in pressure in the holding pipette, rotation of the cell by the embryologist. In practice, this means less work for experts in annotating the training or acquiring images.
The U-Net architecture proved to outperform standard methods in biomedical image segmentation [17], [18], [19].

The paper is organized as follows: in section 2 we present the materials and methods. Results are introduced in Section 3 and conclusions in Section 4.

II. Materials and Methods

A. Images

The images used in this paper were acquired from recordings of ICSI procedures by Olympus IX71 microscopes in INVICTA Fertility and Reproductive Center. The procedures were performed by 3 qualified embryologists. A single recording includes short spermatozoons recording, immobilization and spermatozoon collection, transfer of the needle to the oocyte, contingent rotation of the oocyte to the correct position, the needle puncture into the oocyte, interruption of the oolemma, the spermatozoon injection and the needle removal. The lengths of single recording vary significantly from one to two and a half minute and depend largely on the embryologist performing the procedure.

The recordings were made at the resolution of 480 x 272 and 928 x 699 pixels at 19 to 30 fps. The total number of frames acquired from videos was over 67000. However, not all of them contained oocyte, some presented the observation, immobilization and spermatozoon retrieval process.

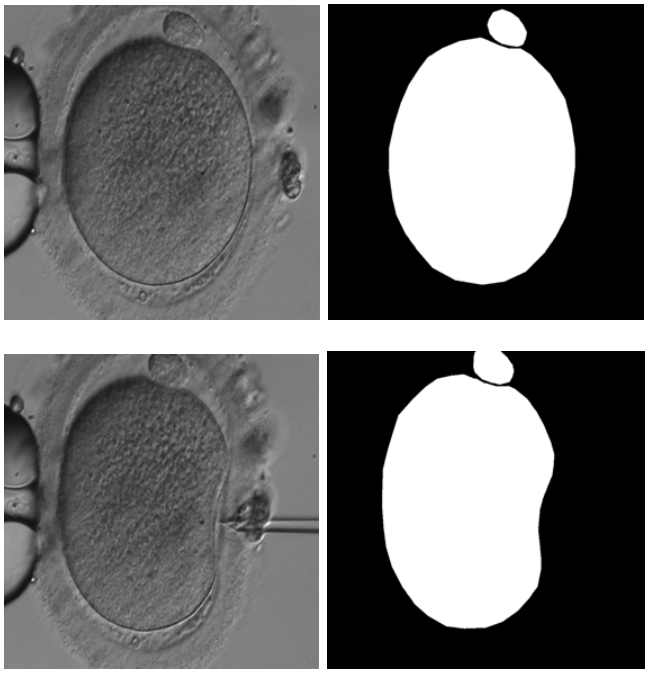

Fig. 2. Input images (on the left) and segmentation maps (on the right) in different stages of ICSI procedure

B. Segmentation

In order to segment the cell and the polar body from the background various computer vision algorithms like edge detection, Hough transform or Active Contours described in papers [11], [10] and [15] have been tested. Low quality and image resolution made it difficult to use the standard object detection methods based on the pixel intensity or its gradient. The changes of the oocyte's shape during the needle insertion prevented the use of the Hough transform. Furthermore, this algorithm neither could be used for the detection of oval-shaped polar body often with hardly visible unambiguous outline. Active contours algorithm in turn was more time-consuming compared to other methods. It also failed in oocyte detection during deflection caused by the introduction of the needle. In order for these algorithms to work well, special attention should be paid to the recording conditions. Probably high fps, resolution or image quality parameters may guarantee the correctness of the detection.

During our research, the only segmentation method successful in detection regardless of the tested image turned out to be the U-Net network [16]. We have trained the U-Net network with 60 images of oocyte in different stages of ICSI procedure. The training images were presenting both oocyte deformed by the needle and not distorted. All the segmentation maps contained an oocyte and a polar body labeled separately (Fig. 2).

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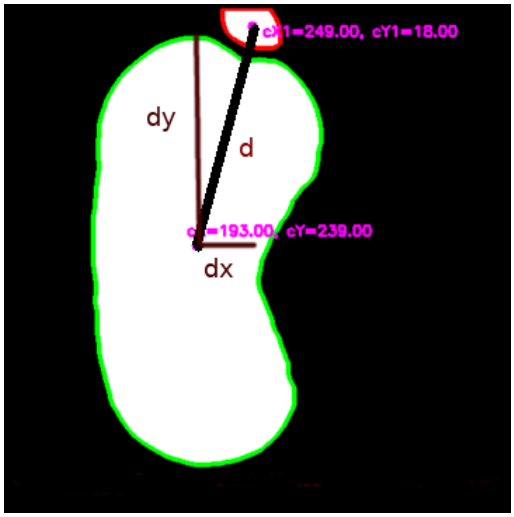

Fig. 3. Result of the object segmentation, labeling and center of gravity calculation. Green border marks the oocyte, while red border - the polar body. The c_x with c_y and c_{x1} with c_{y1} are the centers of gravity of oocyte and polar body respectively. The black line d is an euclidean vector pointing from the center of gravity of the oocyte to the polar body with x- and y-components marked as d_x and d_y.

C. Polar body position

The proper objects detection and separation of the polar body from the oocyte enabled further analysis. The next step was object labeling by the Connected Component algorithm. Then we calculated the shape and position factors of the labeled objects: the center of gravity, area, perimeter and the circularity ratio described by (1).

$$f_{circ} = \frac{2\sqrt{\pi A}}{P} \tag{1}$$

where:
A - the area of the object,
P - the perimeter of the object.

The circularity of a circle is 1, and less than one otherwise. The value of circularity ratio can be used to monitor the degree of the needle pressure on the oolema before the rupture.

However, the most important parameters from the oocyte orientation point of view are the centers of gravity presented in Fig. 3. The algorithm for recognition, whether the oocyte is properly oriented, that is whether the polar body is located at the 6 or 12 o'clock position, counts the Euclidean vector $\overrightarrow{d}$ pointing from the center of gravity of the oocyte (with coordinates c_x, c_y) to the polar body (c_{x1}, c_{y1}). In the next step the ratio of vector components along x- and y-axis $\frac{d_x}{d_y}$ is calculated and the following conditions are checked:

1) if $\|\frac{d_x}{d_y}\| < 0.5$, the polar body is in polar location,

   a) if $d_y > 0$, the polar body is in 6 o'clock position,

   b) if $d_y < 0$, the polar body is in 12 o'clock position,

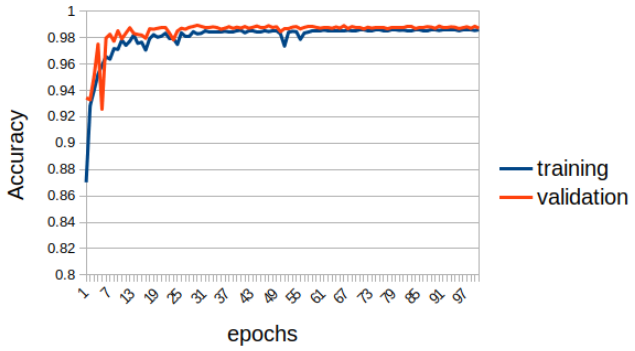

Fig. 4. Training and validation accuracy analysis over 100 epochs

III. RESULTS

In this study we trained U-Net model over 100 epochs with batch size equal 1, using gray-scale input images. The results of performance analysis in the form of training and validation accuracy as well as training loss, are presented in Fig. 4 and Fig. 5 respectively. Accuracy for both training and validation has exceeded the value of 0.985. The loss function used was the binary cross entropy. The exemplary results of image prediction for testing database by the trained network are shown in Fig. 6. The oocyte as well as the polar body are correctly segmented with clearly visible border between them. The oocyte is properly detected throughout the entire ICSI procedure, regardless of its shape. Only sometimes at the point of deflection caused by the needle, the edges of the detected object lose their smoothness (Fig. 6).
In order to quantify evaluation of segmentation predictions, we have counted Intersection over Union (IoU) parameter for all testing images. The IoU metric measures the ratio of the number of pixels common between the target and prediction masks to the total number of pixels in both masks. The average IoU value for testing images was 0.982. Differences in the intensity of pixels for target and prediction masks in most cases result from the place of deflection of the oolema by the needle. Thresholding with a properly selected threshold will increase the value of IoU parameter.

Table I presents the results of shape parameter calculation. The first and the last cell in the table are characterized by the shape most resembling circle and their circularity ratios are close to 1. The least round shape is the third one having the smallest circularity ratio equal 0.73.

The circularity ratio quantifies the shape of the oocyte and can be used to detect the needle penetration degree.

IV. CONCLUSIONS

The solution presented in this paper is a first step in the development of the ICSI method automation. The proposed method of cell and polar body segmentation is universal. There are no demands for the quality of images, regular shape,

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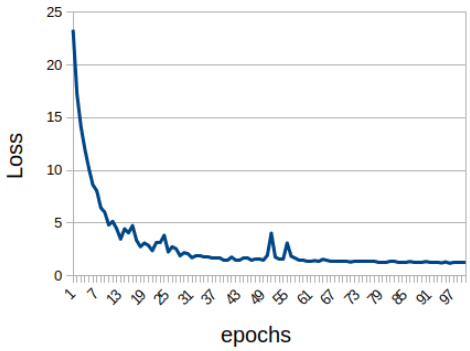


Fig. 5. Training loss analysis over 100 epochs

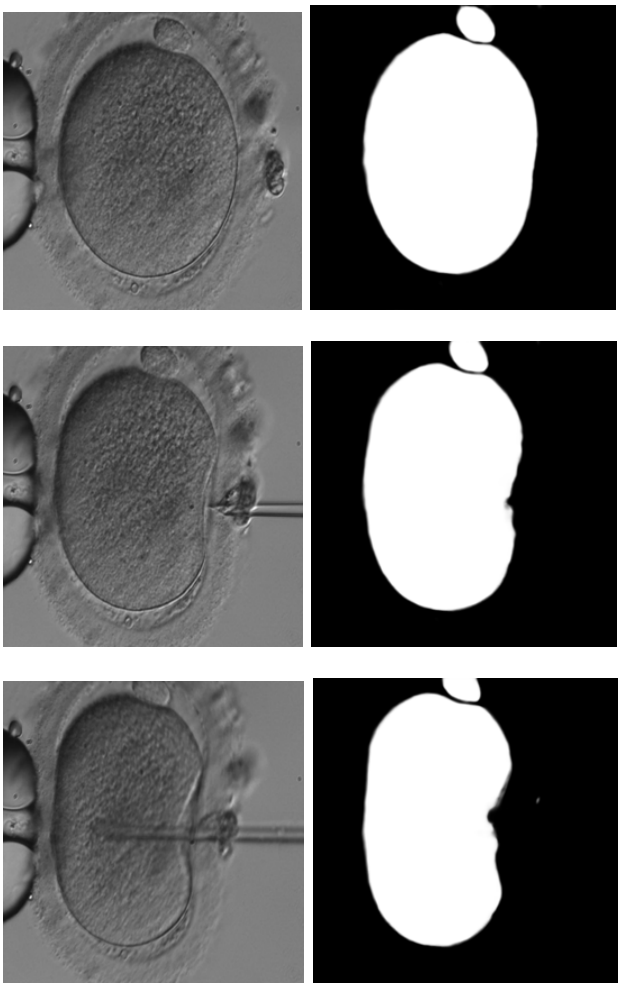
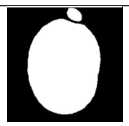
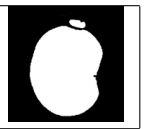


Fig. 6. Input test image (on the left) and the results of image prediction by the trained network (on the right)

TABLE I
CIRCULARITY RATIO

				
f_{circ}	0.92	0.82	0.73	0.88

the continuity nor high contrast of the objects' edges. That is why it is more versatile than earlier proposed solutions. The correct detection of the oocyte's polar body enables easy oocyte's orientation monitoring. System also allows to control the position of the needle while piercing the membrane, regardless of whether it is done automatically or manually by the embryologist.

When the correct positioning of the cell and the control of the needle penetration are ensured, the next step of the ICSI procedure - detection of oolemma piercing - can be automated. In the further part of the work, we intend to automatically monitor the velocity of cell fluids inside the needle, the changes of which determine rupture of hardly visible oolema.

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