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OPTOMECHATRONIC SYSTEM FOR AUTOMATED INTRA CYTOPLASMIC SPERM INJECTION*

ASSEN SHULEV, TIHOMIR TIANKOV, DETELINA IGNATOVA,
KOSTADIN KOSTADINOV, ILIA ROUSSEV, DIMITAR TRIFONOV

*Institute of Mechanics, Bulgarian Academy of Science,
Acad. G. Bonchev St., Bl. 4, 1113 Sofia, Bulgaria,*

e-mails: {assen, tihomir, ignatova, kostadinov, ilia, trifonov}@imbm.bas.bg

VALENTIN PENCHEV

Medical Centre – ReproBioMed,

28, Boicho Ognianov St., Ovcha Kupel, Sofia, Bulgaria

e-mail: v.penchev@reprobiomed.eu

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ABSTRACT. This paper presents a complex optomechatronic system for In-Vitro Fertilization (IVF), offering almost complete automation of the Intra Cytoplasmic Sperm Injection (ICSI) procedure. The compound parts and sub-systems, as well as some of the computer vision algorithms, are described below. System capabilities for ICSI have been demonstrated on infertile oocyte cells.

KEY WORDS: Computer vision; Micro-manipulator, In-Vitro Fertilization (IVF), Intra Cytoplasmic Sperm Injection (ICSI).

1. Introduction

Currently, ICSI procedures are widely performed in many in-vitro fertilization clinics in order to overcome some couples' infertility problems. Manual performance of this delicate procedure is time consuming – and has been performed with moderate success so far, due to various subjective reasons. To increase the success rate, a few micro-robotic systems for automated microinjection and controlled penetration of different cells have been proposed recently [1–4].

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* Corresponding author e-mail: assen@imbm.bas.bg

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An important challenge, lying ahead of the modern human embryology, is to inject several egg cells in a short period of time. For this purpose, the egg cells are usually placed in a single Petri dish. Afterwards, each oocyte is fixed individually, taking into account the polar body position, with the help of a pipette, applying gentle suction. Then, a single sperm cell is selected, based on certain criteria, and inserted into the cytoplasm through a tiny injection pipette. This procedure should be repeated for each cell in the dish.

Therefore, a system that could automate the ICSI procedure is supposed to allocate both pipettes, the egg cells, sperm, and to manipulate them in a more precise manner.

In the subsequent pages, we briefly present the main features and parameters of our optomechatronic system for ICSI and the related computer vision algorithms that allow us to speed up and automate the procedure.

2. Optomechatronic System

A complex optomechatronic system comprising of different modules and subsystems is required to automate the ICSI procedure. The main parts of such a system are: a high-quality optical microscope with a digital camera, an XY positioning stage, two micromanipulators, a micro-injection system, a heating module, and a computing system with controllers for all mechatronic components. Figure 1 presents a basic scheme of the entire optomechatronic ICSI system.

2.1. Optical Microscope

ICSI is a quite challenging procedure from imaging point of view due to the big size differences of the observed objects. For example, the sperm cells are about $4\text{ }\mu\text{m}$, while the human egg cells are approximately $120\text{ }\mu\text{m}$ in diameter. Moreover, when several cells have to be injected, a larger area should be observed in order to locate them. Therefore, a microscope with at least two objectives is needed, one with small magnification – for the egg cells localization, and one for the sperm selection – with large magnification, and better resolving power. However, the optical resolution – OR of a microscope, defined by Rayleigh Criterion, is related to the objective's numerical aperture – NA_{obj} , light source wavelength – λ , and the numerical aperture of the illumination system (condenser) – NA_{cond} by the following equation:

$$(1) \quad OR = 1.22\lambda / (NA_{obj} + NA_{cond}).$$

On the other hand, if we want to capture images by digital camera without loss of resolution, then the camera resolution – CR should be equal or

Fig. 1. Scheme of the optomechatronic system for automated ICSI.

smaller than the optical resolution multiplied by the objective magnification:

$$(2) \quad CR = 2p \leq ORm,$$

where: p is the camera pixel size, and m is the objective magnification. The above formulas give a relation between the main parameters of the optical system and allow us to select sufficient objectives, condensers, and a digital camera.

In our system, the optical microscope has four different objectives: $5\times$, $10\times$, $20\times$, and $40\times$, mounted on a computer-controlled motorized revolver. Focusing mechanism and illumination are also controlled by the computing system. The parameters of the Couple Charged Device (CCD) camera are as follows: 2580×1944 pixels (5.0 Mpix), pixel size – $3.4 \mu\text{m} \times 3.4 \mu\text{m}$, frame rate – 13 fps, A/D conversion – 12 bit, dynamic range – 61 dB. The camera noise is a major parameter that affects directly the computer vision precision. Therefore, knowing the camera noise level [5] allows to define the system control accuracy.

2.2. Positioning stage

Before being injected, each oocyte cell from the Petri dish should be placed inside the microscope's field of view. Therefore, a positioning stage with working range larger than the Petri dish diameter is preferable. In the current system, an XY positioning stage with $13 \times 10 \text{ cm}^2$ working range and accuracy of $1 \text{ }\mu\text{m}$ has been used. It is driven by two computer-controlled step-motors with integrated feedback sensors.

2.3. Micro-manipulators

Fine positioning, of the immobilising pipette close to the egg cell is realized by a fast and precise holding micro-manipulator with 3 DoF. It is driven by 3 piezoelectric slip-stick actuators, providing 7 nm resolution, working space of $2 \times 2 \times 2 \text{ cm}^3$, and speed of 5 mm/s. The injection micromanipulator is the same but with mirror symmetry relative to the Z axis.

2.4. Injection system

The injection system realizes oocytes immobilization, sperm cells suction, and insertion into the cells' cytoplasm by applying different pressures to the fluid into the holding and injection pipettes. It consists of two separate micro-syringes driven by computer-controlled piezoelectric actuators. Each syringe is connected to the corresponding pipette through a tiny plastic tube. In this way, a precise pressure control could be achieved, allowing gentle cell manipulations.

2.5. Heating module

A vital parameter for the living cells is the surrounding temperature. Therefore, a heating module maintaining a constant temperature proper for the cells' evolution has been integrated inside the positioning stage. It is digitally controlled with an accuracy of $0.1 \text{ }^\circ\text{C}$.

2.6. Computing system with controllers

Each of the above mentioned components has its own protocol and controller for communication. Most of these controllers have been integrated into the computing system. All data from all sensors of the optomechatronic system should be acquired and processed simultaneously. Since the proposed ICSI automation relies on computer vision algorithms to control the translational stage and both micro-manipulators, the computing system should be powerful enough to process all captured images in real time. It is worth mentioning the fact, that the camera frame rate defines how many times per second we can inspect the system. This parameter, along with the computational power and algorithm efficiency define the computer vision control speed. In case, we

Fig. 2. Subsequent pictures of the pipette point detection procedure.

have efficient numerical algorithms and a fast computer sub-system, the vision control is limited only by the camera frame rate.

Here, we use a workstation with eight cores Xeon processor at 3 GHz, with 25 MB cash, and 16 GB RAM.

3. Computer vision

A key role in this approach to automated ICSI plays the computer vision. It relies on the image information captured by the camera, and image processing algorithms to extract information about the objects and their position. Main goals of the computer vision algorithms are to detect, focus, and track both pipette tips in the image space, to establish a correspondence between the manipulators' sensors and the image space, to detect the egg and sperm cells.

3.1. Pipette tips detection

When mounting the holding and injection pipettes on both micromanipulators a small variances of their tips' position may appear. Therefore, the computer vision has to detect both tips in the image space. For this purpose, we remove the Petrie dish and place an objective with small magnification allowing to observe larger areas. It is preferable to use a contrast technique that presents the transparent pipette as bright object on a dark background. Then, the holding pipette is positioned at the expected center. If the pipette is not in the image space then a zigzag searching procedure should be performed by the holding micromanipulator. Subsequent frames of the pipette during its detection are shown in Fig. 2.

There are different methods to find the pipette tip - some of them make use of Laplace's differential operator [6], or calculate image differences taken before and after a small manipulator's movement [7]. In Fig. 2 – to the right, a snake scanning for intensity increase in the captured image is performed, depicted by a yellow line. This approach requires prior information about the pipette tip's orientation. The first intensity peak, larger than the camera noise

level, means that the pipette tip is in a near surrounding. A small sub-image around this intensity peak is chosen, and a focusing procedure in this sub-image has to be performed. Once the pipette tip is focused, the snake scanning procedure is repeated again to mark its position with certainty. The same procedure should be performed for the injection pipette.

3.2. Pipette point focusing

Most focusing algorithms measure the sharpness relying on gradients [8], spectrum analysis [9], image correlation [10], and image variance [11]. We use the image variance method, since its computational efficiency and robustness against noise has been previously noted [12]. The variance sharpness measure can be defined as:

$$(3) \quad V = \frac{1}{MN} \sum_{x=1}^N \sum_{y=1}^M (t(x, y) - \bar{t})^2,$$

where: $t(x, y)$ is an sub-image, $\bar{t}$ is the mean value of this sub-image, and M, N are the image dimensions in pixels. The sub-image values $t(x, y)$ might be considered proportional to the light intensity in the image plane. Actually, only the pipette tips should be focused, and that is why the variance sharpness is calculated over a small sub-image surrounding the pipette point, depicted with a starlet in Fig. 2 – to the right.

Accurate focusing is a very important task, in view of the fact that it could be used to control the height of the pipette. Changing the pipette height leads to variance sharpness change. Best focus corresponds to the highest variance sharpness measure. We have verified experimentally that the smallest change of the pipette height, that can be detected with the proposed focusing algorithm, is $15 \mu\text{m}$, when microscope objective $5\times$ is used, and less than $1 \mu\text{m}$ when $40\times$ is used. Therefore, we can control the pipettes' height with a very high accuracy. In Fig. 3, the focusing procedure for the holding pipette is presented. The focusing step for the first 6 frames is 1 mm, while for the last frame is $15 \mu\text{m}$.

An objective with small field of view depth, corresponding to highest optical magnification, is used in order to align both pipettes in one plane. The pipettes aligned according to their height are shown in Fig. 4. In order to demonstrate the alignment precision, the injection pipette is inserted into the holding one.

3.3. Pipettes tracking

Small rectangular sub-image surroundings are chosen once the pipette's tips are detected and focused. They are presented with rectangles in Fig. 4 –

Fig. 3. The holding pipette's tip focusing procedure, sharpness variance – V vs. number of focusing steps

Fig. 4. Pipette tips focusing, height alignment, and tips sub-image selection

to the right. These sub-images are used as samples for the numerical algorithm which searches the entire image, finding the best mech. In this way, the pipette tips can be tracked in the image space. The computer tracking routine is based on the cross-correlation [13, 14] between the captured image and each sub-image sample. Normalized cross correlation between an image and one of the sub-image samples can be described as:

$$(4) \quad C(u, v) = \frac{\sum_{x,y} [f(x, y) - \bar{f}_{u,v}][t(x - u, y - v) - \bar{t}]}{\sqrt{\sum_{x,y} [f(x, y) - \bar{f}_{u,v}]^2 \sum_{x,y} [t(x - u, y - v) - \bar{t}]^2}},$$

where: $f(x, y)$ is the image, $\bar{t}$ is the mean of the corresponding sub-image sample, $\bar{f}_{u,v}$ is the mean of $f(x, y)$ in the region under the sub-image sample.

The sharpest peak location of the normalized cross-correlation presents the pipette tip position. A bi-parabolic interpolation of the cross-correlation

Fig. 5. Oocyte cell detection based on Hough Transform for magnifications $5\times$, $10\times$, $20\times$, $40\times$

peak could be performed [15], for sub-pixel detection if needed.

It can be established a one-to-one correspondence between the image and the manipulator's working spaces after we have a reliable approach to track the tips of both pipettes. For this purpose, each micro-manipulator moves in small steps covering the entire field of view. For each step, the manipulators' positioning sensors are read, and the corresponding positions of the pipette tips in the image space are computed. This process is usually called image space calibration and is performed for both micro-manipulators, the positioning stage, and for all objectives of the microscope.

3.4. Cells detection

In this section, we shall discuss the egg cell detection algorithms, highlighting a few possibilities. Oocyte cells are spherical in shape, and appear circular in the image. Therefore, a Hough circle detection [16] – as one of the most common and reliable methods could be applied to detect the cells. A complete cell detection algorithm, where the coarse detection eliminates the unwanted parts and the fine detection, precisely tracks the wanted cell using Bayesian estimation, is described in [17]. Another approach is to compare the captured images with an existing database of oocyte cells. This technique utilizes image-processing algorithms for translation, rotation, and rescaling [18]. Its main advantages are that the oocyte polar body could be detected, and the cell could be classified. In Fig. 5, oocyte detection based on Hough Transform is presented for different optical magnifications. The algorithm detects the center of the cell and its radius.

3.5. Sperm cells tracking

One of the most important tasks of ICSI is to select a sufficient sperm cell. Different computer-assisted methods for sperm analyses based on multi-object tracking, motility, and energy estimation have been developed [19, 20]. Here, we use a common technique, for sperm speed measurement, known as Particle Image Velocimetry (PIV). It utilizes Digital Image Correlation (DIC)

Fig. 6. Subsequent images of sperm cells taken at 45 fps – left, and right – displacement measured by DIC

techniques to compute the velocity of particles in a given fluid. For illustration in Fig. 6 – to the left and in the middle, two subsequent frames of sperm cells are shown. The estimated speed is proportional to the distance of each sperm cell divided by the time capture interval. Figure 6 – to the right, shows the difference between these two frames and the arrows depict the measured sperm velocity. In practice, the developed computer vision algorithms detect and allocate several high-speed sperm cells with linear trajectory and allow the embryologist to select interactively one of them.

4. ICSI application

The proposed optomechatronic system has been experimentally tested on infertile oocyte cells. The performed successive steps are: initial localization of both pipettes in the image space, focusing of their tips, image space calibration of both micro-manipulators for all objectives, image space calibration of the translational stage for all objectives. Then, a Petrie dish is placed and scanned for egg cells using Hough Transform. Coordinates of each cell are estimated and recorded. Each oocyte is placed in the field of view, the holding pipette is positioned next to it and gentle suction is applied to fix the cell. Next, the fastest sperm cells are detected and one of them is interactively chosen. Positioning stage centers and compensates the movement of this sperm cell placing it close to the injection pipette. Then a precise suction is applied and the sperm cell enters the pipette – Fig. 7.

The injection pipette is placed next to Oocyte's membrane and the sperm is injected inside – Fig. 8. A gentle suction is applied, Fig. 8 – middle, before the cell penetration. These procedures are performed for all egg cells into the dish.

Fig. 7. Sperm cell positioning near the injection pipette and its suction

Fig. 8. Three steps of the automated sperm injection of an oocyte cell

5. Conclusion

In this paper, we have briefly presented an optomechatronic system for automated ICSI. Its main components and their parameters, along with the computer vision algorithms have been described. The System performance has been verified and demonstrated realizing automated ICSI. The procedure takes less than forty seconds per cell. Some boring technical details have been omitted here in order to keep consistency, while other innovative elements have not been discussed at all. The reason for this is that a patent application procedure is under way and the information is still confidential. In the following paper, we shall reveal some of its details that both simplify and speed up the ICSI procedure.

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