Mechanical Analysis and Experimental Research on Adjusting Technique for Cellular Position and Attitude in Microinjection

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Abstract. In microinjection, Cell position and attitude adjusting is the key step, which greatly affects the experimental result. However, based on laser, electric field, and magnetic field etc., the techniques have many disadvantages such as high damage, complex adjusting process, and low precision and efficiency. On micro scale, this paper analyses demand and principle of cell position and attitude adjusting and then proposes an automatic adjusting method. With end-actuator of holding micropipette, the cell position and attitude adjusting technique makes adjusting motion decompose into rotary motion of two orthogonal planes. Therefore, cell position and attitude adjusting system is developed. According to attitude angle of cell reference point calculated by image retrieval system, position and attitude of cell is adjusted. With the above technique, shrimp egg position and attitude adjusting is achieved. The result shows that the technique simplifies adjusting processing and improves the stability of processing. Additionally, the adjusted cell position and attitude meets the demand of automatic positing in cell microinjection.

Introduction

Cell microinjection is a typical biological micromanipulation technique which needs cell position and attitude to be adjusted. So whether the adjusting technique is appropriate or not directly affects the efficiency and success rate of experiments. Currently, manual cell micromanipulation is limited by the proficiency and fatigue of operators. Therefore, it has low test efficiency and precision, poor repeatability, and unstable success rate. Improving the automation of cell manipulation and injection can promote the development of cell engineering.

Existing adjusting technique of cell position and attitude can be classified as non-contact and contact manipulation. Contact method normally brings some mechanical damage to cells. Non-contact manipulation mainly includes laser trapping[1], electro-rotation[2], magnetic rotation[3], ultrasound control[4], fluidic control[5]and the combination of these methods. Because they cannot provide a stable support for the microinjection and cannot provide accurate positioning accuracy, they are still used in the research phase.

In order to achieve accurate positioning and simple operation of cell position and attitude adjusting, we propose an adjustment method with mechanical motion control. Due to small cell size, small spatial displacement of cell position and attitude adjustment, large angular displacement and complex control parameter, this method with borosilicate glass holding micropipette fixing cell, makes adjusting motion decompose into rotary motion of two orthogonal planes. Based on the adjustment method, we design a cell position and attitude adjustment system. Through shrimp

position and attitude adjustment experiments, the method is verified its accuracy, efficiency and applicability.

Analysis of cell position and attitude adjusting principle

The cell position and attitude adjusting criterion

In cell microinjection, the oocyte was reduced to small spheres model, shown in Figure 1.

In ICSI (intracytoplasmic sperm injection), Black[6] et al. indicated that when oocytes were injected with the first polar body positioned at 7 and 11 o'clock and the injection pipette is situated with the bevel facing 6 o'clock and is inserted into the oocyte at 3 o'clock, for clock compass, the survival and fertilization rates were the highest. So in this paper we use 7 and 11 o'clock position as adjustment target locations and the holding micropipette is end-actuator. Under the control of the micro-positioning stage and pressure controller, positioning control of cell 3D pose parameters can be achieved by rotational movement in two orthogonal planes that are the first and second plane.
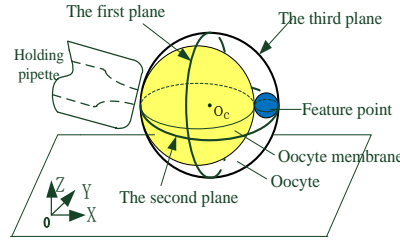

Fig.1: Model and characteristic plane of oocyte

Determination of cell rotation attitude angle

In ICSI, the identification of cell attitude is completed relied on feature point position, through the coordinates of the points on the surface of a two dimensional projection imaging into space coordinates of measured points, can be obtained by the rotation angle of cells in two orthogonal planes.

As shown in Figure 2, the model is perspective projection model. World coordinate system O_w-$X_w Y_w Z_w$ is established, and target point P is (X_w, Y_w, Z_w). Camera coordinate system O_c-$X_c Y_c Z_c$ with the center at camera optical center O_c and the optical axis as Z axis is established. (X_c, Y_c, Z_c) is camera coordinate of the same point. In addition, the image plane is parallel to $X_c Y_c$ plane, and the center of image plane $O_1(u_0, v_0)$ is the intersection point of the optical axis and the image plane, and f is the focal length of microscope. In the ideal perspective projection model, image coordinates of the point P is (x, y)(mm). And image coordinates of the point P is (u, v) what unit is pixel.
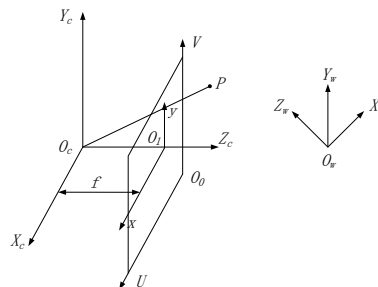

Fig.2: The perspective projection mode

The relationship of the point P between the pixel coordinate and the three-dimensional space

coordinate is given as follow:

$$Z_c \begin{bmatrix} u \\ v \\ 1 \end{bmatrix} = \begin{bmatrix} \frac{1}{dx} & 0 & u_0 \\ 0 & \frac{1}{dy} & v_0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} f & 0 & 0 & 0 \\ 0 & f & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} R & T \\ 0^T & 1 \end{bmatrix} \begin{bmatrix} X_w \\ Y_w \\ Z_w \\ 1 \end{bmatrix} \tag{1}$$

$$= \begin{bmatrix} a_x & 0 & u_0 & 0 \\ 0 & a_y & v_0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} R & T \\ 0^T & 1 \end{bmatrix} \begin{bmatrix} X_w \\ Y_w \\ Z_w \\ 1 \end{bmatrix} = \begin{bmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \end{bmatrix} \begin{bmatrix} X_w \\ Y_w \\ Z_w \\ 1 \end{bmatrix} = MX$$

According to formula (1), the pixel coordinates of the geometric center of the oocyte and the feature points can be determined. However, different paths of the operation will directly affect the range of operating space. When the feature points is in the microscopic view, if cell is first rotated in second plane, rotation angle of Z axis will be increased significantly, which largely expanded the corresponding operation space. Therefore, in consideration of the working space of operating system requirements in the second plane, this paper determines cell first is rotated in first plane, and then in second plane.
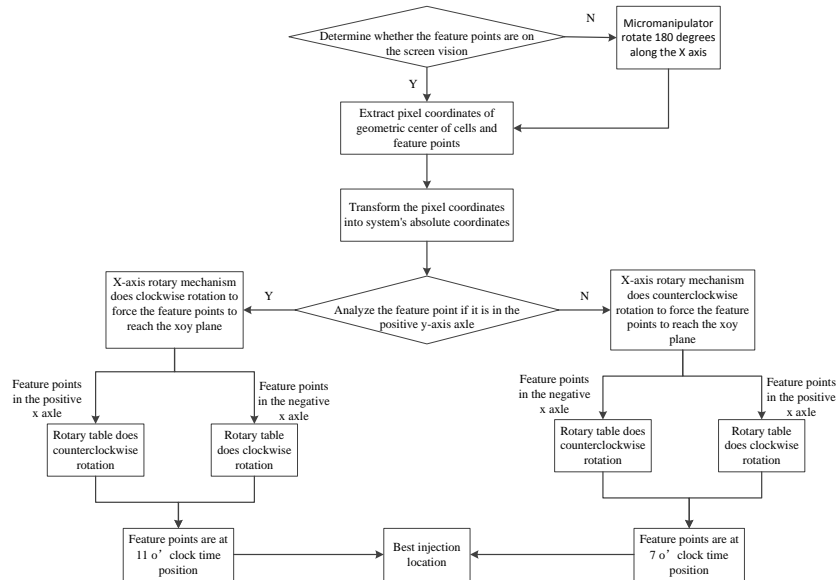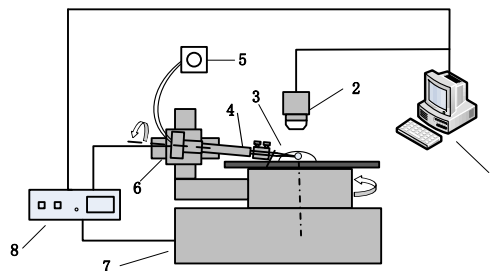

Fig.3: Diagram of cell position and attitude adjustment path planning

According to the analysis of cell rotation angles of the different paths in the second plane, the rotation angles of the oocyte in the first plane and the second plane are calculated. The path planning process for cell position and attitude adjustment is shown in Figure 3.

Design and experiment of cell position and attitude adjustment system

Design of cell position and attitude adjustment system

According to the analysis of the cell position and attitude adjusting principle, we designed the cell position and attitude adjusting system, as shown in Figure 4.

1. Upper computer 2. Digital microscope 3. Holding micropipette 4. X axis rotary mechanism 5. Negative pressrue

controller 6. Micro-move platform 7. Z axis rotary bench 8. Motor driver

Fig.4: Diagram of cell position and attitude adjust system

Image detection system is composed of digital microscope and computer. It real-time monitors the cell position and attitude adjusting process and figures the cellar location information, and the principle is shown in Figure 5. Upper computer fetches image feature information and communicates with control circuit by calling MATLAB program. By fetching the microscopic image and its feature information, along with the transformation from position information (i.e. pixel coordinates) to system coordinates, the system can control the stepper motor moving forward, reverse and make angle rotation, finally automatically complete the regulation of cell position and attitude. As shown in Figure 6, the cell position and attitude adjustment system works as follows.
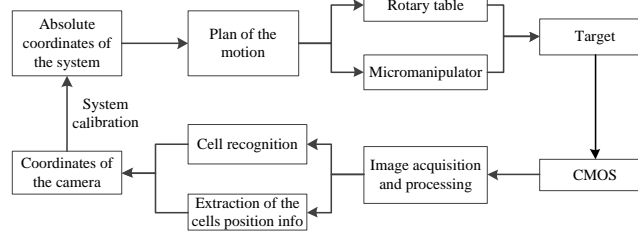
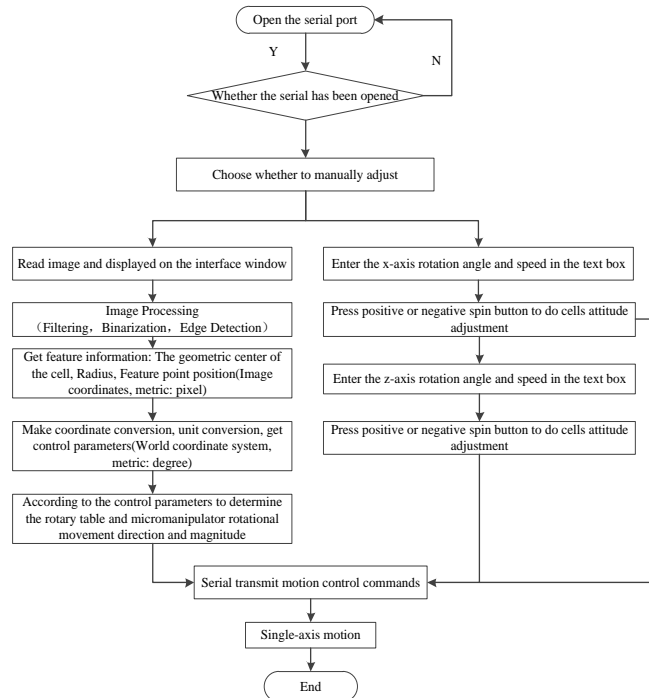Fig.5: Principle diagram of cell position and attitude adjust control system

Fig.6: Flow chart of cell position and attitude adjustment control system

Shrimp egg position and attitude adjustment experiment

The holding micropipette used by the experiment, which has a smooth and flat section, is made by 350μm bore glass capillary with special processes, like interception and wrought. In the experiment, we use mature shrimp eggs as oocytes, and set the magnification of digital microscope as 65. Cell micromanipulation bench is shown as Figure 4.

In the experiment, we treat the black mark on the shrimp eggs as the reference object, and adjust it to the 11 o'clock position, the adjustment process is shown in Figure 8.

As shown in Figure 7(a), firstly we fix the shrimp eggs in the front of the holding micropipette, through the negative pressure inside the holding micropipette, which is generated by pressure regulator. After the fixing process, computer will acquire the microscopic image, and handle on it.

Then the computer will get the coordinates of centroid position of the cells, which is (775, 707), and the position coordinates of the reference object, which is (762, 695). According to formula (1), upper-computer calculates the rotation angles of the shrimp eggs inside the first plane and the second plane are 76 ° and 15.2 °. Then the upper-computer will launch commands, and control the auto-rotary mechanism and rotation bench, finally adjust the cells to 11 o'clock position, which is the best injection status. The process above is shown as Figure 7(b), (c), (d). Keep the holding micropipette fixed during the best injection status of the cells, and the microinjection instrument controls the feed movement of the needle to accomplish the injection, which is shown as Figure 7(e), (f).
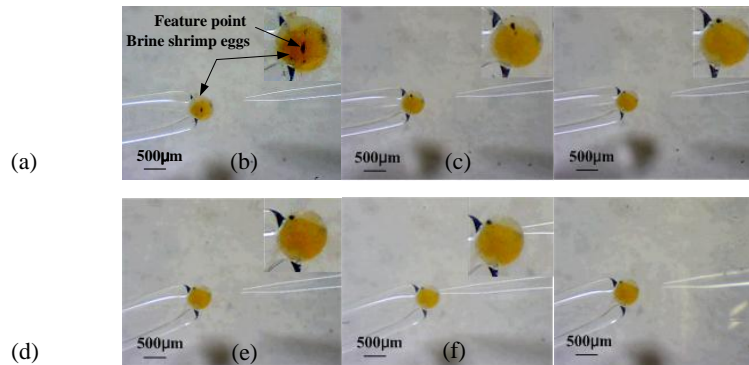
(a) Fixing shrimp eggs and getting their position information (b) The rotation process of shrimp eggs in the first plane (c) The shrimp egg is rotated to the second plane (d) The shrimp egg is rotated to 11o'clock position (e) The injection micropipette of the microinjection (f) Opposite to process

Fig. 7. Adjusting and microinjection process

Conclusions

i) Based on the analysis of cell position and attitude adjusting requirements and regulation mechanism on micro scale, and automatic adjusting method is proposed. With end-actuator of holding micropipette, the technique makes adjusting motion decompose into rotary motion of two orthogonal planes. And the adjusting system is designed;

ii) According to the automatic cell position and attitude adjustment method, to build a cell microinjection system and the system of regulation and microinjection experiments study pose shrimp egg cell, in the course of the experiment, cell regulation process is simple, high stability, cell position and attitude adjusting rear can meet the automatic positioning requirements of cell microinjection technique in cell.

Acknowledgements

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