A Real-Time Oocyte Polar Body Detection Method

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Abstract. Based on the extensive application requirements of cell micromanipulation in bioengineering, this paper focuses on the intracytoplasmic sperm injection technology of assisted reproductive technology in the field of obstetrics and gynecology, and develops algorithms to automatically and accurately identify the position of the polar body of the oocyte, laying a foundation for the realization of sperm and oocyte automated injection. This paper proposes an algorithm that can accurately locate polar body of oocyte; this method uses a lightweight U-Net network to semantically segment oocyte images and extract their edges, and then search for polar body based on image edges. This method has been verified on 524 pictures of mice oocyte, which is real-time and robust.

1. Introduction

Cell micromanipulation currently is an indispensable part of bioengineering. It is widely used in assisted reproductive medicine [1], cell characteristic analysis [2-3], protein analysis [4], immunological research [5] and drug analysis [6].

However, cell micromanipulation requires high precision, besides operator training is difficult, and manual operation is laborious, which easily causes operator fatigue. In addition, manual microinjection has poor repeatability and low efficiency. Therefore, realizing the automation of cell micromanipulation has become the focus of bioengineering technology research. At present, automated microinjection technology has been extensively researched and developed [7-10].

Visual feedback plays a key role in automated microscopic cell micromanipulation. To take oocyte micromanipulation as an example: in Intracytoplasmic sperm injection (ICSI), the polar body of the oocyte must be avoided to ensure the zygote can develop well [11-12]. In polar body biopsy, a complete polar body must be obtained for genetic analysis [13]. Therefore, it is necessary to accurately locate the oocyte polar body through visual feedback.

At present, many polar body detection methods can be found. Some use the Hough circle transformation [14-17] and ellipse fitting [18] to locate the polar body. Such methods usually approximate the oocyte to a circle and use the Hough circle to cover the cell. And the remaining part of oocyte is regarded as polar body, but when the polar body area is small, this method is often unable to detect. SVM [19] and semantic segmentation [20-21] are used to locate the polar body. The accuracy is high, but because the characteristics of the polar body itself are relatively close to the main part of oocyte,
in order to distinguish the polar body from the oocyte, a large number of features need to be used to distinguish, which leads to an increase in calculation and have no real-time performance. Zennan Wang proposed an edge-based polar body detection method [22], this method requires high accuracy of edge extraction, but the traditional algorithm used in the edge extraction process of this method is not reliable.

This paper proposes a real-time algorithm that can accurately locate the polar body. This method uses a lightweight U-Net network to semantically segment the oocyte image and extract its edges, then it performs polar body detection based on the cell edge and optimizes the edge-based polar body detection method. This method was verified by 524 pictures of mice oocyte.

2. Method
The polar body detection algorithm in this paper is mainly divided into two steps: semantic segmentation of oocyte image; edge-based polar body search algorithm. The complete oocyte’s edge is obtained through image semantic segmentation, and then the edge-based polar body search algorithm is used to detect polar body.

2.1 Image segmentation with U-Net

U-Net neural network is used to semantic segmentation, which is currently widely used in medical image [21] [23-25]. The structure of U-Net is shown in Fig 1 [26]. The main structure of U-Net is U-shaped and there are layer jump connections. This structure can combine the low-resolution information and high-resolution information of the image. Because the edge of oocyte is blurred, more high-resolution information is required for accurate segmentation; however, the structure of oocyte is relatively unchanged, and the images’ semantics are simple and clear, low-resolution information is useful for target Recognition. All in all, U-Net is very suitable for oocyte image segmentation.
The segmentation in paper is only a simple binary-segmentation (Fig 2), so only a few features are needed to achieve a good segmentation effect. We lighten the original U-Net network weight (Fig 3). Reduce the calculation time while ensuring the segmentation effect.

2.2 Polar body search algorithm

As shown in Fig 4, based on the output of U-Net [Fig 4(a)], we extract edge of the whole connected domain, and it is easy to find that there are two inflection points [see Fig 4 (b)]. If the inflection point does not exist, the polar body does not exist; on the contrary, if the inflection point exists and the positions of the two inflection points are located, the position of the polar body can be accurately located.
2.2.1 Locate inflection points
In order to locate inflection points, the following methods are adopted:

As shown in Fig 4(c): Take a pixel point on the edge as point B; pass N (N will be determined below) pixels along the edge, take the second pixel point as point C; pass N points along the edge in the opposite direction, take the third point as point A. It is easy to find that when the three points A, B, and C are on the part of edge with no polar body, the direction of \( A \rightarrow B \rightarrow C \) is clockwise; when the three points are on the part of edge with polar body, seeing points D, E, and F, the direction of \( D \rightarrow E \rightarrow F \) is anticlockwise.

\[ \text{Fig 5. Angle between vector } \overrightarrow{AB} \text{ and vector } \overrightarrow{BC} \]

Suppose point A position is \((x_A, y_A)\), point B position is \((x_B, y_B)\), and point C position is \((x_C, y_C)\). Vector \( \overrightarrow{AB} \) is \((x_B - x_A, y_B - y_A)\), vector \( \overrightarrow{BC} \) is \((x_C - x_B, y_C - y_B)\). The deflection angle of vector \( \overrightarrow{BC} \) relative to \( \overrightarrow{AB} \) is \( \theta \) (shown in Fig 5). If the direction of \( A \rightarrow B \rightarrow C \) is clockwise, \( \theta > 0 \), otherwise \( \theta < 0 \).

According to linear algebra theory we know:

\[
\begin{vmatrix}
  x_A & y_A & 1 \\
  x_B & y_B & 1 \\
  x_C & y_C & 1 \\
\end{vmatrix} = |\overrightarrow{AB}| |\overrightarrow{BC}| \sin(\theta)
\]

Then we define a new variable \( P \):

\[
P = \sin(\theta) = \frac{\begin{vmatrix}
  x_A & y_A & 1 \\
  x_B & y_B & 1 \\
  x_C & y_C & 1 \\
\end{vmatrix}}{|\overrightarrow{AB}| |\overrightarrow{BC}|} = \frac{x_A y_2 + x_2 y_1 + x_2 y_1 - x_1 y_2 - x_1 y_1 - x_1 y_2}{\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \sqrt{(x_2 - x_3)^2 + (y_2 - y_3)^2}}
\]

If \( P < 0 \), the direction of \( A \rightarrow B \rightarrow C \) is clockwise;
If \( P > 0 \), the direction of \( A \rightarrow B \rightarrow C \) is unclockwise;
If \( P = 0 \), point A, B and C are collinear.

Traverse each pixel on the edge and calculate the corresponding \( P \). As shown in Fig 6, taking \( N = 10 \) as an example, the two valley points of the \( P \) curve graph correspond to the two ends of the polar body. If there is no polar body, all pixels’ corresponding \( P \) are greater than zero, as shown in Fig 7.
2.2.2 Determine value of $N$

The value of $N$ has a great influence on polar body search algorithm. As shown in Fig 8, when $N = 1$, it is obvious that only three possible values ($\sin \left(\frac{\pi}{4}\right), \sin \left(-\frac{\pi}{4}\right), 0$) $P$ would be; however, if $N = 50$, all pixels’ corresponding $P$ are greater than zero. It is important to determine the value of $N$ before we begin polar body search algorithm.

Because the number of edge pixels of different oocyte images is inconsistent, the $N$ determined by different oocyte images has no reference significance for other images. To ensure the consistency, here we introduce a new normalization coefficient $\alpha$:

$$\alpha = \frac{N}{M}$$  \hspace{1cm} (3)

Where $M$ is the number of edge pixels.
Observing Fig 6 (middle), we can see that if the polar body exists, the P-value curve will fluctuate greatly, in order to quantify the degree of fluctuation. Here defines a coefficient $\beta$:

$$\beta = \frac{\max(P) - \min(P)}{2\sigma}$$ (4)

$\beta$ represents the ratio of the maximum amplitude to the average amplitude of the P-value curve. $\sigma$ is the standard deviation of P-value curve, $\max(P)$ is the maximum value of P-value curve, and $\min(P)$ is the minimum value of P-value curve. $2\sigma$ is the average amplitude of P-value curve, and $\max(P) - \min(P)$ is the maximum amplitude of P-value curve. The larger $\beta$ is, the greater the fluctuation is. Fig 9 is a graph about the relationship between $\alpha$ and $\beta$ of some oocytes with polar-bodies. We can find that when the value of $\alpha$ is between 0.015 and 0.035, $\beta$ is in the maximum range. Considering that the larger the $N$, the less the interference of some non-polar body on the edge on the P-value curve, we take $\alpha = 0.035$, therefore, for all oocyte images: $N = \lfloor 0.035M \rfloor$.

![Fig 9. curve graph of $\alpha - \beta$](image)

### 2.2.3 Valley point search based on Fibonacci searching

According to the determined edge point interval $N$, the P value corresponding to the edge pixel point is obtained, as shown in Fig.10(a). By determining the two valley points of P-value curve, polar body can be located. Here, we use Fibonacci searching method to search for the two valley points.

Fibonacci searching method is a search technique for unimodal function in a limited interval, and it is also the search algorithm with the highest compression ratio in one-dimensional search. The Fibonacci sequence is denoted as $\{F_k\}$, where the Fibonacci number: $F_0 = 1, F_1 = 1$, when $k \geq 2$, $F_k = F_{k-1} + F_{k-2}$.

Suppose there is a certain interval, denoted as $L_1$, the first valuation points are:

$$x_1 = \frac{F_{k-2}}{F_k} \times L_1, \quad x_2 = \left(1 - \frac{F_{k-2}}{F_k}\right) \times L_1$$ (5)

Where $\frac{1}{F_k}$ should be equal to or less than the expected accuracy of the search.

If $f(x_1) \leq f(x_2)$, delete interval $[x_2, 1]$, otherwise delete $[0, x_1]$. The left new interval is denoted as $L_2$. The second valuation points are:

$$x_1 = \frac{F_{k-2}}{F_{k-1}} \times L_2, \quad x_2 = \left(1 - \frac{F_{k-2}}{F_{k-1}}\right) \times L_2$$ (6)

The same to first step: If $f(x_1) \leq f(x_2)$, delete interval $[x_2, 1]$, otherwise delete $[0, x_1]$. The left new interval is denoted as $L_3$.

Repeat the above operation until the denominator used for iteration becomes $F_2$.

Since the Fibonacci searching method is a single-peak search method, before using it to locate the valley points, P-value curve (suppose P-value curve is connected end to end) needs to be processed as follows:

a. For values greater than or equal to 0 on P-value curve, set all to 0, as shown in Fig 10(b);

b. Find the midpoints of the uninterrupted continuous 0-value interval on the P-value curve, and divide the P-value curve into multiple intervals with these midpoints as interval points (the interval number is denoted as $Num_{interval}$), as shown in Fig 10(b);
c. Smooth $P$-value curve, as shown in Fig 10(c);

d. Use the Fibonacci search method to find the minimum points in the respective intervals. The initial $F_k$ is determined by the length of the intervals, as shown in Fig 10(d);

e. If $\text{Num}_{\text{interval}}$ is equal to 2, the two finally determined pixels are the target pixels; if $\text{Num}_{\text{interval}} \geq 3$, among the finally determined $\text{Num}_{\text{interval}}$ pixels, the two pixels with the smallest corresponding $P$ value are seemed as the target pixels.

![Fig 10. Valley point search based on Fibonacci searching](image)

3. Result

In this paper, the input of U-Net network are grayscale images and batch size is 4. The accuracy of the verification set basically does not increase after more than 70 epochs (shown in Fig 11), and the final accuracy is 0.984. After getting the edge of output image, the polar body search algorithm is verified, and the final success rate is 99.43%. Because part of the image’s polar body area is too small (shown in Fig 12), the overall $P$ value curve is greater than 0, and the polar body search algorithm fails. The entire polar body detection algorithm (including image segmentation and polar body search) takes 0.048s (CPU: i7-10710U, GPU: NO) in average, and the CCD sampling rate is 15FPS, so the entire polar body detection algorithm meets the real-time requirements.
4. Conclusion
The method proposed in the paper is used to determine whether there is a polar body in the plane and locate it. U-Net network performance well in semantic segmentation even with changes of image contrast and exposure. In addition, the lightweight U-Net network only needs a CPU to meet real-time requirements. After the polar body is detected, it should be rotated to the specified position. In further work, we are going to study the real-time in-plane polar body tracking algorithm. It can provide a technical support for the realization of sperm and oocyte automated injection.

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