Automatic Vision Guided Small Cell Injection: Feature Detection, Positioning, Penetration and Injection

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Abstract - Vision guided automatic cell injection system has become increasingly important in the past ten years. The existing cell injection systems mainly deal with large cells with size bigger than 40 micrometers. However, when dealing with small cells, the internal and external disturbances presented in the system may affect the success rate of injections significantly. In this paper, the system setup of vision guided cell injection system is presented. Feature tracking, micropipette positioning, cell membrane penetration and biological material injection processes for small cell with size around 20 micrometers are discussed. Sum of square difference (SSD) tracking algorithm based on 2 dimensional (2D)-to-2D feature correspondences is used to handle disturbances. A modified proportional position controller is adopted to control the micropipette. The importance of cell membrane penetration modeling is emphasized.

Index Terms – Cell micromanipulation, cell injection, penetration, position control, SSD tracking.

I. INTRODUCTION

Cell injection is a process of transforming biological material into nucleus of cells. Cell injection includes operations such as micropipette positioning, cell membrane penetration and biological material injection. In many life science research and applications, cell injection is a highly skill intensive task. The success rate of cell injection tasks have been reported to be marginally acceptable 40-70% [1]. Besides putting the blame on intrinsic genetic defects and reasons not yet known, a certain part of this inefficiency can be traced back to the manual imprecision and inconsistency of the laboratory operator. So a novel cell injection system which can improve precision and consistency is desired.

The manual procedure used in suspended cell injection is described in the flow chart as shown in Fig. 1. The technical challenges of manual procedure include:

1) **Focusing of the microscope.** For embryo pronuclei gene injection, focusing must be performed precisely on the focusing plane of the microscope. Failing to do so will cause the micropipette to skim over or damage the cell.

2) **Selection of the contact point.** The operator must decide the contact point for subsequent cell membrane penetration. If the contact point is not selected properly, the micropipette might skim over the surface of the cell.

3) **Positioning of the injection micropipette.** Due to the involuntary tremor moments from laboratory operators, it is very hard to move the micropipette to the desired contact point. This effect is exaggerated when dealing with small cells.

4) **Penetration velocity determination.** The physical properties of cell membrane vary from cell to cell. Constant injection velocity into the cell might not be suitable for certain cells. The operators need to control the injection velocity based on observation and experiences. Inappropriate injection velocity might destroy the cell.

Besides these difficulties from the manual procedure, there are other problems which are not reflected in the manual procedure. They are repeatability of successful injections and contamination introduced by direct human involvement [2].

Based on the aforementioned limitations of manual cell injection, automatic cell injection systems have been introduced by many researchers these years. Existing automatic cell injection system can be broadly classified into two categories: vision guided [2, 3] and non-vision guided [4-6]. Strictly speaking, non-vision guided cell injection system is not automatic cell injection system. However, it is still superior to manual system because the positioning and penetration can be done using computer controlled actuators.

![Fig. 1 Manual procedure for suspended cell injection](image-url)

**Fig. 1 Manual procedure for suspended cell injection**
Vision guided cell injection system has a very high degree of automation. This kind of system does not require skillful laboratory operators. All the parameters required for the controller design can be determined from the visual servoing part. Vision guided cell injection system can be subdivided into two classes: without and with force sensing.

The system setup depicted in [2] is a typical setup of vision guided injection system without force sensing element. The success rate of the penetration is very high in this system given the cell size is around 40 micrometers. The system setup described in [3] has a force sensing element together with a haptic device. The advantage of adopting a force sensor and a haptic device is that the force in the system can be measured whilst the operators can feel the status of the contact. The probability of cell damage is greatly reduced. The size of the cell used to test the system is between 700 micrometers and 900 micrometers.

Existing automatic cell injection systems have certain common limitations. They are mainly designed to deal with embryos with relatively large size. At this range, the effect of disturbances and backlash of actuators are not significant. However, as the size of the cell decreases, the effect of the disturbance and backlash of actuators will become more crucial. Thus, a vision guided cell injection system which not only can handle large sizes but also can deal with small sizes of cells is desired.

The organization of the paper is as follows: section II describes the system setup of typical cell injection systems. Section III presents the feature tracking algorithms. Section IV discusses the position controller. Section V emphasizes the importance of cell membrane penetration. Section VI presents the experimental results and the last section concludes the paper.

II. System Setup

A vision guided cell injection system often consists of a holding unit, an injection unit, an imaging unit shown in Fig. 2, and a software unit planted in a personal computer.

The piezoelectric stack actuators shown in Fig. 3 is a well known commercially available device for managing extremely small displacements with a resolution in the order of a nanometer, has a bandwidth in the order of a kilohertz and can provide mechanical power in the order of several watts. Since piezoelectric stack actuators are monolithic and have no sliding or rolling parts, they exhibit no significant mechanical backlash [7]. These advantages make piezoelectric actuators the first choice in computer controlled biological operations.

The injecting unit consists of motorized or manual coarse translational and rotational stages, a piezo-driven actuator, and an air or oil injector. These coarse translational and rotational stages can bring the micropipette to the field of view of the camera. The piezo-driven actuator is a programmable actuator which can perform positioning and penetration tasks. A manual piston pump in the injection unit enables easy and accurate transfer of biological materials.

Fig. 2 Typical setup for a vision guided cell micromanipulation system

Fig. 3 Piezoelectric actuator used in microinjection

The holding unit often has similar setup as the injection unit except that the micropipette injector is replaced by the micropipette holder.

The imaging unit contains an inverted microscope, a CCD camera which can provide large frame rate.

The software unit is planted in the computer. This unit incorporates every process in the visual servoing loop as shown in Fig. 4.

III. Modified 2D-to-2D SSD Feature Tracking

The visual servoing in an automatic vision guided cell injection system often exhibits some important characteristics which make the designing process of the position controller different from normal visual servoing systems. They are:

1) The fine movements in vision guided cell micromanipulations are often translational movements. One of the reasons is that the travelling range of the rotational piezo-driven micropipette is too small for micro-manipulations. The second reason is that, 3 dimensional (3D) translational setup can fulfill the requirements of most micromanipulations.

2) The values of the extrinsic and intrinsic calibration parameters must be accurate enough to avoid deviations during the micropipette positioning phase. A small error in the calibration parameters can easily cause the micromanipulation to fail.
3) The system is noise sensitive. Internal noise such as electrical noise and external disturbance often easily make the pipette deviate from the desired position up to a few micrometers. This deviation can cause the positioning and penetration to fail. The feature tracking algorithm must be able to monitor the deviation so that the controller can compensate the noise at each time instance.

Visual servoing system can be classified into three categories [8] depending on feature to feature correspondences. They are 3D-to-3D correspondences, 3D-to-2D correspondences and 2D-to-2D correspondences. 3D-to-3D correspondences requires the reconstruction of 3D objects using 2D images. 3D-to-2D correspondences can be adopted only if system is properly calibrated and the calibration results are repeatable. 2D-to-2D correspondences is quite suitable for the position control for cell injection systems because the depth of field of the lens is normally around a few micrometers. The positioning can be assumed on the focusing plane. The experimental result shows that this assumption is valid up to 90% cases with 5% confidence interval (CI).

In vision guided systems, features are used to design the position controller. There are several feature tracking algorithms including moment invariants [9-13], decision theory [14, 15], SSD [16, 17], neural networks [18] etc.

The measurement of the motion of the features on the image plane must be finished continuously and quickly. SSD is a fast feature tracking algorithm. In this paper, SSD is not only used to track the feature, but also the SSD value is used by the controller to adjust the micropipette. This is a new application of SSD algorithm.

Mathematically, a template window of N x N pixels window is selected around the feature in the initial chosen focusing image called template image. The feature is normally a point, an angle or a line. For every successive image called searching image captured, a searching window is placed at each location in the searching image to find the matched pattern to the template window. The searching window is selected only when it produces the smallest SSD value. Mathematically,

$$SSD(Ar, Δc) = \sum_{i,j\in N}(SI(r_i + Δr + i, c_i + Δc + j) − TP(r_i + i, c_i + j))^2$$

where $SI$ is the current searching image, $TP$ is the template window, $(r_i, c_i)$ is the origin of the template window in the template image, $(Δr, Δc)$ is the movement of the searching window with respect to the origin of $SI$. $(r_i + Δr, c_i + Δc)$ gives the absolute position of searching window with respect to the origin of $SI$.

The assumption made for SSD is that searching patterns (micropipette tip) do not change considerably between two successive frames. This assumption is valid for most cases because we have a predictable environment and constant illumination model. However, if the system disturbance is beyond the tolerance limit, which means the micropipette is deviated from the designed plane too much, the operation has to stop. In some cases, it is not affordable to stop and restart the experiment. Thus, there should be an algorithm which can retract the pipette to the initial focusing plane to continue the micromanipulation. The algorithm used to retract the micropipette defines a parameter $ζ$ relating to minimum SSD value called SSD$_m$

$$ζ = \frac{SSD_m(Ar, Δc)}{(TP(r_i + i, c_i + j))^2} \in [0,1]$$

$ζ$ defines the degree of similarity between two patterns in two frames. $ζ = 0$ means the patterns in two frames are the same except that the positions may be different. Depending on the size of the cell, the tolerance level for $ζ$ is different. For example, based on the experimental results, for plant cells, such as orchid petal cells, $ζ < 0.1$ is good enough to do the positioning and penetration. For animal cells such as Chinese Hamster Ovary (CHO) cells, $ζ < 0.02$ is required to ensure the successful penetration. If we define the previous step movement of the micropipette in the camera coordinate system be $(Δx, Δy, Δz, Δ$c$)$, the algorithm works in such way:

1) Define the upper limit for $ζ$ to be $ψ$, and then calculate $ζ$ for current searching image.
2) If $ζ ≤ ψ$, the micropipette is still in the desired plane statistically. Then the controller prepares the next step movement $(Δx, Δy, Δz, Δ$c$)$.
3) If $ζ > ψ$, the micropipette is deviated from the desired plane. Before preparing the next step movement, the
controller must be able to retract the micropipette to the desired plane. In order to do this, the controller first moves the micropipette in $z_c$ direction by $\Delta z_c/10$ amount; recalculate the $\zeta$ value, if the $\zeta$ decreases, then we carry on the movement until $\zeta$ stops decreasing. If $\zeta \leq \varphi$ now, we have successfully moved the micropipette back to the desired plane, we can carry on the next movement to the target position. However, when the micropipette moves in $z_c$ direction, the $\zeta$ increases or $\zeta > \varphi$ after a few consecutive movements in $z_c$ direction, the controller needs to move the micropipette in $y_c$ direction in the same manner as in the $z_c$ direction and same as in $x_c$ direction if $\zeta > \varphi$ until $\zeta \leq \varphi$.

4) If $\zeta > \varphi$, the controller can also move the micropipette in $z_c$ direction by $-\Delta z_c/10$ amount, and the process repeats similar as step (3) for other directions.

This iterative adjustment of the micropipette has some advantages. First, the experiment doesn’t have to be stopped even if the noise is beyond the tolerance level. This is crucial because vision guided cell injection system is very sensitive to noise, especially using optical lens with large magnifications for small cells. Second, the calibration of the system is greatly simplified. In this system, we didn’t carry out any calibration; we follow the readings from the translational and rotational stages to obtain the extrinsic parameters, we used those relevant parameters provided by manufacturers as the intrinsic parameters. Third, although this is an iterative algorithm, it works very fast. Because between two successive image frames, $\zeta$ doesn’t change too much, a few trials can make $\zeta \leq \varphi$. The tracking algorithm can find the feature in 95% of the experiments with 2% confidence level.

IV. MODIFIED PROPORTIONAL POSITION CONTROLLER

For a 2D-to-2D feature correspondences, normally at least two feature points are needed in order to uniquely locate the micropipette. We know that there are an infinite number of positions in the world coordinate system to which the object could be driven so that the feature is at the desired image coordinates. However, if we use the feature tracking algorithm described above, we understand that although the features of these infinite points locate at the same image coordinates, yet they have different light intensities. The above SSD algorithm together with the initial chosen template image can successfully distinguish these points. Thus, only one feature point is used as a feature point in the controller design. This simplifies the controller design and reduces the computational time by half.

In order to design the position controller, first, we set up a relationship between the electrical control input and the movement of the micropipette in the global coordinate system.

$$\begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix} = k \begin{bmatrix} u_x \\ u_y \\ u_z \end{bmatrix}$$  \hspace{1cm} (3)

where $(x_w, y_w, z_w)$ is the feature point in world coordinate system and $(u_x, u_y, u_z)$ is the controller input in X, Y and Z direction also in world coordinate system. $k$ $(k=10$ for the employed piezoelectric actuator) is the ratio between the distance in terms of micrometers and the computer analog output (Volt).

The unit of the word coordinate is micrometer; the unit of the control input is volt. The value of $k$ is provided by the manufacturer. Second, we transform the point from world coordinate system to camera coordinate system. In our system setup, the relationship of the two coordinate systems is defined in Fig. 6(a). The rotational matrix (4) has to be calibrated out before designing the controller. However, using the iterative SSD tracking algorithm described above, and if the system is carefully set up, we can use the relationship from Fig. 6(b) to derive the rotational matrix (5). The angle $\theta$ can be obtained directly from the reading.

![Fig. 6. The relationship between world coordinate system and camera coordinate system. (a) original relationship (b) simplified relationship.](image)

$$(x_c, y_c, z_c)$$ is the feature point in camera coordinate system.

$$\begin{bmatrix} x_c \\ y_c \\ z_c \end{bmatrix} = \begin{bmatrix} r_1 & r_2 & r_3 \\ r_4 & r_5 & r_6 \\ r_7 & r_8 & r_9 \end{bmatrix} \begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix}$$  \hspace{1cm} (4)

$$\begin{bmatrix} x_c \\ y_c \\ z_c \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos \theta & \sin \theta \\ 0 & -\sin \theta & \cos \theta \end{bmatrix} \begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix}$$  \hspace{1cm} (5)

Since we use 2D-to-2D correspondences, the focal length and other intrinsic parameters are not used in the controller design. We need to know the rough relationship between the camera coordinate system and the image frame system in order to control the step movement.

$$\begin{bmatrix} r \\ c \end{bmatrix} = M \begin{bmatrix} x_c \\ y_c \end{bmatrix}$$  \hspace{1cm} (6)

where M depends on the magnification of optical lens and camera specifications. $(r, c)$ is the feature point in image frame coordinate system and $(x_c, y_c)$ in camera coordinate system. The target position can be chosen manually by the user or automatically using some image processing algorithms [2]. Denote the target position and the initial position of the micropipette be $[r_t \ c_j]^T$ and $[r_0 \ c_0]^T$, respectively.
In order to improve the success rate of the proposed SSD feature tracking, conventional proportional controller must be modified due to its nature. SSD feature tracking can perform better if the pattern is undergoing a smooth movement. Thus, instead of using a constant feedback gain, we incorporate time varying increasing coefficients. Define

\[
D_x = \sqrt{(t_x - r_x)^2} \quad (7)
\]
\[
D_y = \sqrt{(t_y - r_y)^2} \quad (8)
\]
\[
d_x = \sqrt{(c_x - c_x)^2} \quad (9)
\]
\[
d_y = \sqrt{(c_y - c_y)^2} \quad (10)
\]

Now we define two functions:

\[
\psi_x = e^{(\alpha \psi_x / D_x)} \quad (11)
\]
\[
\psi_y = e^{(\beta \psi_y / D_y)} \quad (12)
\]

where \(\alpha\) and \(\beta\) are parameters determining the gradient of \(\psi_x\) and \(\psi_y\), respectively. The profile of \(\psi_x\) or \(\psi_y\) is shown in Fig. 7. The purpose of this function is to ensure the smooth movement of the micropipette, such that the micropipette will not be lost during the visual interpretation.

The inverse of the image Jacobian matrix of the system is

\[
J_p^{-1} = \begin{bmatrix}
1 & 0 & 0 \\
0 & (\cos \theta + \tan \theta \sin \theta)^{-1} & 0 \\
0 & (\cos \theta + \tan \theta \sin \theta)^{-1} \tan \theta & 0
\end{bmatrix} \quad (13)
\]

Thus, a modified proportional position controller is proposed as follows:

\[
\begin{bmatrix}
u_x \\
u_y \\
u_z
\end{bmatrix} = \frac{1}{Mk} \begin{bmatrix}
t_x \psi_x & 0 & 0 \\
0 & t_y \psi_y & 0 \\
0 & 0 & t_z \psi_z
\end{bmatrix} J_p^{-1} \begin{bmatrix}
t_x - r_x \\
0 \\
0
\end{bmatrix} \quad (14)
\]

where \(t_x\), \(t_y\), and \(t_z\) are proportional controller gain whose values can be determined by users depending on the type of application and \(t_x \psi_x = t_y \psi_y\) in order to ensure 0 movement in \(z\) direction. \([u_x \quad u_y \quad u_z]^T\) is the control input applied to the the piezoelectric actuator.

V. CELL MEMBRANE PENETRATION MODELING

Cell membrane penetration modeling is a very new research topic. Cell membrane penetration modeling becomes important when automatic cell injection systems are used. The ideal cell membrane penetration process is described in Fig. 8.

Cell penetration modeling can provide biologist a better understanding of the cells’ physical characteristics. A cell penetration modeling could substantially improve the success rate of transgenic micromanipulations. A cell penetration modeling holds the potential of revolutionizing micromanipulation standards and protocols for cells and microorganisms in life science research and applications. It also allows microbiologists to attempt experiments and new procedures that are otherwise hindered by the inherent limitation of human in manual micromanipulation at the microscopic level.

Force sensors are often adopted when modeling the membrane strength [3]. The force sensor can be placed between the actuator and the micropipette in order to measure the contact force between the tip of micropipette and the cell membrane. A penetration controller which determines the optimal penetration speed and acceleration based on the contact force is designed. The major difficulty of using a force sensor is because of the elasticity of the cell membrane. Due to this elasticity, the penetration controller may not provide an accurate penetration force and this could destroy the cell.

![Fig. 7 Profile of the increasing function](image)

![Fig. 8 Ideal cell membrane penetration process](image)
Another method which we are proposing now is based on pure image processing techniques together with some experimental results. From the images captured, the deformation of the cell membrane is calculated. Comparing the deformation with some experimental data, a new penetration will be carried out. This method involves large amount of experimentations. We aim to setup a database for some typical cells considered in future work.

VI. EXPERIMENTAL RESULTS

Two set of experiments were carried out to test the effectiveness of the position controller using 2D-to-2D feature correspondences. The first set made use of orchid petal cells which are not suspended cells. Fluorescent materials were injected into the petal cells. The second set of experiments made use of CHO cells, a kind of cancer cells. CHO cells are much smaller than the orchid petal cells. In order to make sure the micropipette is located at the correct position for penetration, the positional controller must be very robust to noise. Experimental result also shows that the position controller incorporating with SSD feature tracking algorithm can successfully fulfill the tasks. $\zeta$ values were calculated statistically based on 100 trials for each set of experiment as shown in Table.I in order to ensure successful penetration.

VII. CONCLUSION

The general steps including feature detection, micropipette positioning, and cell membrane penetration of vision guided cell injection are introduced. Biological material injection is the final step in vision guided injection system. The typical system setup of vision guided cell injection systems is presented. 2D-to-2D feature correspondence SSD tracking algorithm is discussed together with the disturbance handling techniques. The advantage of using SSD feature tracking algorithm is also discussed. A simple but easy to implement robust proportional position controller is designed. The importance of cell membrane penetration modelling is emphasized. Possible modelling methods are discussed.

Future research work possibly includes setting up a general cell membrane penetration model to further study the cell membrane characteristics. It is also a desired goal to perform other cell micromanipulations such as cell dissections using the designed system.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>VALUES FOR ORCHID PETAL CELLS AND CHO CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trials</td>
<td>Orchid petal cells</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
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</table>

REFERENCES


