VISION CONTROL OF A HYBRID ROBOTIC SYSTEM FOR CELL INJECTION

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ABSTRACT. In this contribution, an approach for two-dimensional vision control of the cell injection process realized by a hybrid-robotic system is presented. The hybrid system is combined of one large range robot and one low range manipulator with high-precision. A glass pipette mounted on the high-precision robot realizes cells penetration and injection of different substances. Suitable optical system has been designed to provide high-resolution imaging of the injection pipette over the working area defined by the cell holder dimensions. Numerical algorithms for pipette point detection, auto focusing and tracking during the working process are developed. The sub-pixel accuracy of these algorithms and high precision linear measuring system integrated in the large range robot allow precise calibration of the image space. In this way, the visual feedback controls the whole robot system and the pipette position with respect to the target cell. Once the cell’s position is detected and defined in the image space, the injection process could be completely automated.

KEY WORDS: 2-D vision control; hybrid-robotic system; biological cells injection; sub-pixel accuracy.

1. Introduction

Nowadays biological cell injection is widely used to perform many tasks in favor of gene engineering, in-vitro fertilization, molecular biology and drug manufacturing. Manual performance of these procedures is time consuming, low efficient, and with moderate accuracy due to various subjective factors. To increase

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the success rate a few micro-robotic systems for automated microinjection and controlled penetration have been proposed recently [1, 2, 3].

One of the most important challenges ahead the modern bioengineering is to inject a great number of cells in a short time. For this purpose, the cells should be set in a mesh holder where immobilized. The system should detect each cell, position the pipette next to it, and penetrate the cell’s membrane. This procedure is repeated for each cell in the holder. Therefore, a complete automation of the injection process is strongly recommended.

Multi cell injection approach seriously provokes the robotic system because it should be precise enough while covering the entire cells holder. In such cases, a hybrid robotic system is sufficient as it combines large range robot and a low range manipulator with high-precision.

To automate the whole injection process a remote vision control is the most adequate way. It could be performed in real time and provides reliable information about the pipette point position in the image space with respect to the target cells.

Here we present an approach for precise two-dimensional vision control of the pipette motion realized by a hybrid-robotic system. It could be successfully applied to inject single or many cells fixed in a mesh holder.

2. Hybrid robotic system

To inject cells we use a hybrid robotic system with large working range having high precision and integrated positioning sensors. The large range robot provides working space of the glass-pipette with dimensions up to \(100 \times 100 \times 50\) mm\(^3\) and realizes rough positioning to the cell membrane with accuracy of \(1\) µm. The integrated linear measuring system is used to provide the robot control system with 0.1 µm resolution feedback. The large range robot also allows injection pipette to be easily replaced away from the cells and accurately placed back afterward.

Fine positioning, orientation and cell-penetration are realized by micro-manipulator, actuated by 3 piezo-stack actuators equipped with positioning sensors with resolution of 0.6 nm. Detailed description of the hybrid robotic system is made in the following paper [4].

3. Vision control system

Vision control consists of optical, computer and robot communication sub-systems. The optical sub-system provides high-resolution imaging of the pipette point over the whole working area. Numerical algorithms detect, focus, and track the pipette point in the image space. Simultaneously, the computer sub-system reads the robot positioning sensors and establishes a one-to-one correspondence between the robot and image space.

3.1. Optical set-up
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The optical system should fulfill many contradictive requirements arising from the specificity of this task. First, the optical magnification and image resolution must be high enough to resolve the pipette point itself. Second, the field of view has to cover the entire mesh holder with cells. Third, the distance between the objective and cells should be large enough since the injection pipette has to access the cells easily. Distances in such systems are crucial and finding the right balance between all parameters is essential. Formula (3.1) gives approximate relation between the main optical quantities:

\[
\frac{1}{f} = \frac{1}{L} + \frac{1}{l}, \quad \frac{D}{d} = \frac{L}{l} = \frac{L - f}{f},
\]

\[
m = \frac{d}{D}, \quad d = Np, \quad CR = 2p, \quad SR = \frac{CR}{m},
\]

where \(l\) is the distance from the objective plane to the object, \(L\) – objective length, \(D\) – field of view, \(d\) – camera sensor width, \(f\) – focal distance of the lens, \(N\) – number of pixels across the sensor width, \(p\) – pixel size, \(m\) – optical magnification, CR – camera resolution, and SR is the system resolution.

Fig. 1. Experimental set-up. Left – scheme of the optical set-up, right – picture of the experimental arrangement

A sketch of the optical set-up is shown in Fig. 1 - left, and a picture of the whole system – in Fig. 1 - right. This set-up provides resolution of 4 µm – same as the pipette point diameter, field of view of 1.4 × 1.0 mm², and working distance of 35
mm. The camera sensor is CMOS with $2592 \times 1944$ pixels and the pixel dimensions are $2.2 \times 2.2$ µm$^2$. The frame rate at full resolution is 3 frames per second (fps) and at VGA – 30 fps. It is time to mention that the frame rate defines how many times per second we can inspect the system. This parameter together with the computational power and algorithm effectiveness define the vision control speed. If we have efficient numerical algorithms and fast computer sub-system, the vision control is limited only by the camera frame rate.

Another significant parameter is the camera noise, it affects directly the tracking and focusing precision. Therefore knowing exactly the camera noise level is an advantage [5] which allows increasing the control accuracy.

### 3.2. Pipette point detection

First goal ahead the vision control is to detect the pipette point. For this purpose, we remove the mesh holder and everything behind the pipette. In this way, only the reflected light from the pipette is captured and it appears bright on dark background. Let us assume that pipette point is in the image space, almost focused and its orientation is known.

There are many different methods to find the pipette point, some of them utilize Laplace’s differential operator, or calculate image difference taken before and after small robot’s movement. To detect the pipette point we leave the robot at rest and take a picture. Than a snake scanning for intensity increment in the captured image is performed (Fig. 2 - left). First intensity increment larger than the noise level presumes that the pipette point is in the near surrounding. A small sub-image surrounding is chosen and a passive focusing in this sub-image, presented in the next section, is performed. Once the pipette point is focused, the snake scanning procedure is repeated again to mark finally its position.

### 3.3. Pipette point focusing

Most focusing algorithms measure the sharpness relying on gradients [6], spectrum analysis [7], image correlation [8], and image variance [9].

We use the image variance method since its computational efficiency and robustness against noise has been previously noted [10]. The variance sharpness measure is defined as:

\[
V = \frac{1}{MN} \sum_{x=1}^{N} \sum_{y=1}^{M} \left( I(x, y) - \bar{t} \right)^2 ,
\]

where $I(x, y)$ is an sub-image, $\bar{t}$ is the mean value of this sub-image, and $M, N$ are the image dimensions in pixels. Actually only the pipette point should be focused that is why the variance sharpness is calculated over a small sub-image surrounding the pipette point depicted with white rectangle in Fig. 2 - right.
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Correct focusing is a crucial task in view of the fact that it can 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 experimentally verified that the smallest change of the pipette height that can be detected with the proposed focusing method is less than 7 μm. Therefore, we can control the pipette height with this accuracy.

3.4. Pipette point tracking

Once the pipette point is detected and focused a small rectangular sub-image surrounding is chosen. It is shown with white rectangle in Fig. 3 – left. This sub-image is used as a sample for the numerical algorithm which searches the entire image finding the best match. In this way, the pipette point can be tracked in the image space.

Fig. 2. Illustration of the numerical routines to trace the pipette point

Fig. 3. Picture of the pipette point. Left – the white rectangle presents the sub-image; Right – the beam of a blue DPSS laser is driven through the pipette canal

Numerical tracking routine is based on the cross-correlation [11, 12] between the captured image and the sub-image sample.
Normalized cross correlation between an image and sub-image sample can be described as:

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

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

Sharpest peak location of the normalized cross-correlation presents the pipette point position. For sub-pixel detection, a biparabolic interpolation of the cross-correlation peak is proposed [13]. Sub-pixel accuracy depends on many parameters, two of them are the sub-image size and the information contained inside. Larger informative sub-image size leads to better sub-pixel accuracy. We can simply increase the sub-image size but it will not increase the accuracy. The reason is that information inside will remain the same since the black background is not informative. The achieved accuracy using this method is 10% of the pixel size. To increase the informative sub-image size we introduce an optical fiber connection between the pipette and a laser light source (see Fig. 1 - left).

In this way the point of the pipette produce a speckle pattern which moves together with the pipette (see Fig. 3 - right.). Therefore, the whole image carries information about the pipette and the correlation can be presented as [13]:

\[
C(x,y) = F\left\{\frac{F[f(x,y)]^* F[t(x,y)]]}{|F[f(x,y)]| F[h_2(x,y)]^{0.5}}\right\}^{-\alpha}
\]

where \( F \) denotes the Fourier transform and \( \alpha \) is an appropriate constant \((0 < \alpha < 1)\). Sub-pixel accuracy of this approach can reach 3% of the pixel size. Another advantage is the faster computation due to the used FFT (Fast Fourier Transform) algorithm. In order to estimate the system noise we leave it at rest and start calculating pipette point position. In this way the error due to the system vibrations and tracking algorithm error are assessed.

As soon as we have a reliable approach to track the pipette point, we can establish a one-to-one correspondence between the image and the robot space. For this purpose the robotic system moves in small steps covering the entire field of view. For each step, the robot’s positioning sensors are read and the corresponding position of the pipette point in the image space is calculated. This process is usually called image space calibration.
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3.5. Cells detection

In this section we shall not discuss in details the cell detection algorithms, we shall only highlight a few possibilities.

Biological cells have many different shapes and colors. One large group of cells has a spherical shape and appears circular in the image. Therefore, a Hough circle detection [14] as one of the most common 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 [15]. Another fast and simple approach is to illuminate the cells at a certain angle and to look for bright spots in the image.

4. Application

The proposed vision control system has been experimentally tested on glass particles and Xenopus Oocyte cells. We used small spherical particles with diameters ranging between 50 $\mu$m and 80 $\mu$m. During the presented in Fig. 4 test three small particles have been put on focus in the field of view of the optical system. The borders of these spherical parts then are at the seam height with the pipette point. Their positions in the image space have been detected and the pipette point positioned subsequently next each particle. To simulate an injection the pipette point is translated quickly at the particle's center. Since the glass particles can not be penetrated, only a small displacement of the particles is observed after the contact. Six pictures illustrate the precise positioning of the pipette point with respect to each particle and its displacement after a contact simulating an injection.

Fig. 4. Vision controlled positioning and injection simulation with tree spherical glass particles with diameters of 50 $\mu$m to 80 $\mu$m
Next, the developed vision control has been applied in close to the designed robotic system genuine purpose – automated injection of living cells. Three pictures in Fig. 5 illustrate the vision controlled cell injection. Left one shows the pipette point captured in the working field of view before reaching the cell’s surface. On the middle one the pipette point is positioned next to the cell’s membrane. Right picture finely presents the penetrated cell.

**Fig. 5.** Tree steps of vision controlled penetration in a Xenopus Oocyte cell

### 5. Conclusion

In this paper, we have presented shortly a 2-D vision control system for robotic cell injection and some experimental verifications of its potential. The system includes an appropriate optical set-up for high-resolution imaging, computer, and numerical algorithms for pipette point detection, auto focusing, and tracking with sub-pixel accuracy. We have introduced an optical fiber connection between the pipette and a coherent light source. In this way we have increased significantly the sub-pixel tracking accuracy. Two examples of a number of performed tests (with glass particles and Xenopus Oocyte cell) are illustrated.

This 2-D vision control system logically could be extended to become a 3-D system. An appropriate incorporation of a second micro-objective, camera, and sufficient numerical algorithms will provide more accurate 3-D control.

**REFERENCES**


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