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Automatic Vision-guided Robotic Cell Micromanipulation System

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**SCHOOL OF MECHANICAL AND AEROSPACE
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Abstract

In life science research and applications such as cell mechanobiology, cell micromanipulation is skill-intensive, involving cell immobilization, micropipette positioning, cell biomembrane deformation and penetration, etc. The imprecise and inconsistent positioning in manual cell micromanipulation and biomembrane stress control demands a vision-guided robotic system to achieve high-accuracy static position control and dynamic motion tracking. This dissertation emphasizes the various techniques in realizing the aforementioned system.

Calibration of a vision system is necessary to ensure accurate coordinate systems transformation. Automatic self-calibration without the aid of calibration templates is proposed to achieve on-line calibration for real-time applications. The difference between the measured output and computed output after calibration is less than the physical resolution of the vision system.

The presence of disturbances (e.g., table vibration, electronic noise) causes the micropipette tip to deviate from the desired plane, thereby calling for a static position controller with depth deviation compensation capability. An image-based position controller with vertical direction deviation compensation capability is proposed and implemented. The position error is less than $1\text{ }\mu\text{m}$ in the image plane and less than a predefined value in the vertical direction.

Conventional hysteresis modeling of piezoelectric actuators using interferometers or capacitive sensors is performed off line. The hysteresis of the piezoelectric

actuator changes as the load acting on it or the driving frequency of the input signal alters, demanding the hysteresis of the micromanipulator be modeled on the fly whenever the driving frequency of the input voltage, or the load acting on the micromanipulator changes. The employment of interferometers or capacitive sensors to model the hysteresis of the piezoelectric actuators is not practical due to special requirements. An automatic hysteresis modeling method is proposed and implemented using vision-feedback. The method can be conducted on the fly in real time making it suitable for time critical cell micromanipulation, yet providing comparable accuracy when using interferometers.

In cell mechanobiology, controlling the indentation force on the cell biomembrane is realized indirectly via position control of the piezoelectric micromanipulator and understanding the relationship between the contact forces and the corresponding deformation. The mechanical property of the cell biomembrane changes with time, thereby requiring an on-line identification of the model of the relationship between the indentation forces and deformations so as to facilitate indentation force control according to predefined force profiles. A third-order polynomial is proposed to model the mechanical property of the cell biomembrane, making the control of mechanical forces acting on the cell biomembrane possible with average tracking error less than 6%.

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List of Symbols and Abbreviations

Symbols	Definitions
CS	Coordinate system
CCS	Camera coordinate system
ICS	Image coordinate system
WCS	World coordinate system
H_i^j	A homogeneous transformation matrix from the CS i to CS j
P^i	An point P in the coordinate system i
R_i^j	A rotational matrix from the CS i to CS j
DOF	Degree of freedom
rmse	root-mean-square error
SSD	Sum of squared difference
STD	Standard deviation
m	Ratio between displacement and voltage input ($\mu m/V$)
α	Ratio between pixels and displacement ($Pixel/\mu m$)
φ	Coordinates of the robot end-effector in WCS
ψ	A vector of image feature parameters in ICS
η	The maximum deviation allowed in the vertical direction
ρ	A regulation parameter for the PID control
ν	The resolution of the piezoelectric stage
ζ	A signal determining whether the tip is above the focal plane

Chapter 1

Introduction

1.1 Background

Cell micromanipulation includes operations such as biological cell microinjection and cell biomembrane stressing, etc. In many life science research and applications, cell micromanipulation is a skill-intensive task. The success rates of cell micromanipulation have been reported to range from a marginally acceptable 40-70% (Payne *et al.* 1994) in microinjection (e.g. in-vitro fertilization such as intracytoplasmic sperm injection (ICSI)) to a measly 1-3% (Pablo *et al.* 2004) in transgenic work for plant, animal, and human embryonic or stem cells. 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.

1.1.1 Cell Microinjection

The microinjection of living cells, which is a typical cell micromanipulation application is extensively researched recently (Sun and Nelson 2002, Xie *et al.* 2008, Wang *et al.* 2009, Sakaki *et al.* 2009). Particularly, cell dissection (e.g., sea urchin dissec-

tion), intracellular injections (e.g., ICSI) and bioelectrical signal recordings (e.g., patch-clamp) have been initiated in basic biological research, drug development, in-vitro fertilization, transgenics and other biomedical areas.

The sea urchin serves as a major research model in developmental biology, cell biology, molecular biology of gene regulation, evolutionary biology and marine biology (Coffman and Davidson 1994, Amore *et al.* 2003). The study of sea urchin involves a lot of disciplines. One important study involves dissecting of sea urchin embryonic cells at their 16-cell stage to remove and transplant the 4 micromeres, which are responsible for the growing of limbs, to the embryonic cells of other sea creatures. Successful dissection of the micromeres requires manipulation accuracy of less than 5 μm , which is impossible to achieve manually.

ICSI is another important application of cell microinjections. ICSI is a major treatment in infertility when other methods of achieving conception have failed. It was first introduced in 1992 and has since been widely used throughout the world (Palermo *et al.* 1992). The survival and fertilization rates of oocytes using ICSI varies among institutions and clinics. Reported survival rates have ranged from 80 to 90%, and fertilization rates of intact oocytes have ranged from 45 to 70% (Payne *et al.* 1994, Sherins *et al.* 1995, Tucker *et al.* 1995). The dispersion between the successful rate seems to be affected by the size and sharpness of the micropipette used for microinjection (Vanderzwalmen *et al.* 1996). There are two other factors that have significant effect on the success rates (Tan and Ng 2001). One of these is the specific medical approach used in the processing of oocytes and spermatozoa. The other is the method used for the positioning of the micropipette to the desired penetration point on the ooplasm. The first factor has been well studied by biologists and sound solutions have been proposed and used. The accuracy of the micropipette positioning and the penetration of eggs, and the repeatability of the success injection are the challenges of the second factor (Kimura and Yanagimachi 1995), it is extremely difficult to move the micropipette to the correct position manually.

Mouse embryo microinjection is another important field of cell micromanipulation (Palmiter and Brinster 1986, Jaenisch 1988, Hanahan 1989). For ethical and biological reasons, the mouse has firmly become the primary experimental mammal. Manipulating mouse embryos is the first choice in the study and understanding of the genetic properties of human beings (Yanagida *et al.* 1991). Production of transgenic mice is a typical mouse embryo microinjection application, in which any cloned gene, normal or mutant can be injected into mouse embryonic stem cells. The applications of transgenic mice include (1) the studies of tissue-specific and developmental-stage-specific gene regulation and (2) experiments of the phenotypic effects of transgene expression. A typical manual mouse embryo microinjection system is introduced by Nagy (Nagy *et al.* 2002), of which the major challenge is the efficiency of manual microinjections. Manual position control of the micropipette often results low efficiency, which is not desirable for these microinjections that must be completed in a short period of time. It is also reported that the penetration speed of the micropipette also affects the success rate (Shen *et al.* 2009), where the speed of the penetration is chosen to be 50 Hz for a single wild-type yeast cell, corresponding to 500 nm/s.

Electrophysiological techniques involve measuring bioelectrical signals in cells. Patch-clamp makes it possible to record the activities of a single membrane channel such that genetic transformation can be studied. In patch-clamp technique, an electrode is placed onto the membrane of the cell, which requires high-precision position control of the micropipette. Future versions of electrophysiological recordings include the microinjection of a compound, or different compounds, into a cell, and simultaneous measurement of the electrophysiological effects on the injected cell and its neighbours as shown in Figure 1.1 (Kallio and Kuncová 2003). The position control of the patch-clamp requires very high accuracy to avoid gaps between the patch-clamp and the cell membrane, which is a big challenge to do it manually.

Cells used for microinjection are alive, which can be suspended or adherent.

Figure 1.1. *Illustration of integrated intracellular microinjection and bioelectrical recording (Kallio and Kuncová 2003). It requires a highly skilled lab operator to move the micropipette to the desired measuring point.*

The position control of the micropipette is conducted in a 3D working space, where the microscope is often equipped with one camera only. The speed of the penetration and the biomembrane characterization of the cell also affect the overall success rate.

1.1.2 Cell Mechanobiology

Eukaryotic cells consist of an enclosing biomembrane, constructed of the cytoplasm and other internal biomembrane enclosed organelles (Alberts *et al.* 2002). It is well-accepted that external stress, such as external mechanical forces acting on the biomembrane can induce changes in the cell form and functions (Jaasma *et al.* 2007, Zhu *et al.* 2000, Wang and Thampatty 2006, Kaunas 2008), that will ultimately affect the respective tissue functionalities via mechanotransduction mechanisms (Owens 1996, Williams 1998, Duncan and Turner 1995, Mullender *et al.* 2004). One typical example is that the shape, density, and stiffness of bones change if the mechanical loading acting on them are altered.

The study of the effects of mechanical forces on cells is called mechanobiology (see Figure 1.2). The mechanical forces is usually regarded as compressive strength

(stress) acting on the cell biomembrane (see Figure 1.3), which is related to the deformation of the cell biomembrane (Sun *et al.* 2003, Kim *et al.* 2009). Thus, force (stress) control of the cell biomembrane is transformed to deformation control, which is practically the position control of the micro-tip if the force-to-deformation model of the biomembrane is identified. It is natural to stress the cell biomembrane according to some predefined dynamic force profiles, which demands a dynamic motion trajectory tracking controller.

Cell mechanobiology is relatively less explored compared to chemical biology due to the following reasons :

- The need to quantitatively analyze the changes in the tissue function when its cells are experienced mechanical forces at different developmental stages. For example, these changes could be the stiffness of red blood cell (RBC) (Cross *et al.* 2007), or the gene expression of stem cells (Engler *et al.* 2006).
- The need to quantitatively characterize the cell mechanical properties, e.g., a mathematical model $f = y(d)$ describing the deformation of the cell biomembrane and its biomembrane tension as shown in Figure 1.3, where f and d are the force and deformation, respectively.
- The need to provide controllable and reproducible dynamic microscale forces acting on the cell biomembrane using the model $f = y(d)$ according to some predefined force profiles.

It is well accepted that a cell is a very complex and dynamic system, wherein the cell's mechanical properties, for example, biomembrane elastic property, change at the different development stages (Mitchison and Swann 1954). To facilitate control of minute and intricate mechanical forces acting on cell biomembrane, micro-robotics stands as a far more superior mode of manipulation in comparison with manual operations.

There are a few common steps in cell microinjection and mechanobiology

Figure 1.2. *The first step is the mechanical property characterization of cell biomembrane. A predefined force profile is realized based on the characterization model. The comparison in terms of biological reactions towards the mechanical forces is then performed.*

as shown in Figure 1.4. The position control is defined as moving a micro-tip (e.g., micropipette, micro-force sensor, micro-holder) to the target position (e.g., the contact point on the cell biomembrane) (Sun and Nelson 2002). The biomembrane characterization is defined as the modeling of the mechanical property of the cell biomembrane when it is deformed (Lu *et al.* 2007, Huang *et al.* 2009). The dynamic position control is defined as the amplitude and frequency control of the motion trajectory of the micro-tip (Shen *et al.* 2009).

1.1.3 Proposed Solution

The difficulties in cell microinjection and mechanobiology are summarized as follows:

1. The difficulty in manually moving the micro-tip to the desired location accurately for the subsequent penetration or deformation of the cell biomembrane.

Figure 1.3. *Cell stress (strain) control: the holder (left) stabilizes the cell while the micropipette (right) deforms the cell in a predefined forces (shapes) and frequencies profiles to induce chemical changes in the cell.*

2. The difficulty in controlling the penetration speed or the dynamic motion profile of the micro-tip accurately.
3. The difficulty in providing a force controller to stress the cell biomembrane accurately.

An automatic vision-guided robotic cell micromanipulation system is proposed to achieve automatic cell microinjection and cell biomembrane force control in mechanobiology such that those major difficulties in the above applications are overcome.

1.2 Objectives

The ultimate goal of this dissertation is to provide a vision-guided micromanipulation system, which can perform cell microinjection and mechanobiology. In order to achieve this goal, the objectives of the dissertation are as follows:

Figure 1.4. *Both cell microinjection and cell mechanobiology have position control of the micro-tip. Mechanical property modeling of cell biomembrane is often employed in cell microinjection recently (Huang et al. 2009).*

1. To propose and implement a visual servoing algorithm to achieve automatic static position control of the micro-tip.
2. To propose and implement a dynamic motion trajectory tracking controller using piezoelectric actuators to achieve dynamic position control.
3. To propose and implement a force controller to mechanically stress the cell biomembrane according to predefined force profiles.

1.3 Technical Challenges

The technical challenges encountered in the proposed vision-guided robotic cell micromanipulation system are summarized as follows:

- **Static motion control with depth (z -) deviation compensation**

The static positioning error of the micro-tip in the image plane is expected to be less than 1 pixel (e.g., $1\mu m$ if the pixel size is $10\mu m \times 10\mu m$ and the optical magnification is 10.) and the deviation in the z - direction is limited to a

predefined value η (e.g., $\eta = 2\mu m$) depending on the requirements of different applications, to ensure that the micro-tip reaches the desired location.

However, only two dimensional (2D) information could be directly obtained in most of the commercial optical microscopes making the positioning of the micro-tip in the 3D working space a challenging task. The presence of disturbances (e.g., table vibration, electronic noise) makes the micro-tip deviated from the desired plane calling for a static position controller with depth deviation compensation capability.

Calibration of a vision system is necessary to ensure accurate coordinate systems transformation. The system must be re-calibrated whenever the micro-tip is dismounted and mounted, which is a tedious task. It is also noted that biological contamination is likely to be introduced using a calibration template. Thus, automatic self-calibration without calibration templates to (a) achieve on-line calibration for real-time applications and (b) minimize biological contamination caused by human intervention is desired. The difference between the measured output and computed output after calibration must be less than the resolution α (e.g., $\alpha = 1 \text{ Pixel}/\mu m$) of the vision system.

- **Dynamic motion trajectory tracking of the piezoelectric stage**

The bandwidth of the closed-loop based piezoelectric stage is usually too small to be applied in the dynamic trajectory tracking applications demanding an open-loop control of the piezoelectric stage, where hysteresis is a major limitation. Conventional hysteresis modeling of piezoelectric actuators using interferometers or capacitive sensors is often performed off line. A piezoelectric micromanipulator is obtained by assembling a few actuators using serial or parallel mechanisms (flexure).

However, the hysteresis of the micromanipulator changes as the load acting on it or the driving frequency of the input signal is altered, demanding that the hysteresis of the micromanipulator must be modeled on the fly whenever the driving frequency of the input voltage, or the load acting on the micromanipulator changes. The employment of interferometers or capacitive

sensors to model the hysteresis of the micromanipulator is not practical due to the special requirements of these instruments. For example, the micro-tip mounted on the micromanipulator must be smooth enough to provide mirror-like reflection to the incoming beam if an interferometer is employed as the position sensor; a capacitive sensor is not easily placed into the working space after the system is setup.

- **Microscale mechanical force control on cell biomembrane**

The classical force control in impedance control is puzzled by the unknown stiffness of the cell (biomembrane). The indentation force control on cell biomembrane is realized indirectly by using the position control of the micromanipulator by understanding the relationship between the contact forces and the corresponding deformations.

The mechanical property of cell biomembrane changes at different developmental stages, which requires an on-line identification of the model describing the relationship between the indentation forces to deformations, to facilitate the indentation force control according to some predefined force profiles at various developmental stages in cell mechanobiology.

1.4 Scope

Vision-guided cell micromanipulation is a very broad area. The author limits the scope of the dissertation to:

1. The proposed system is a vision-guided microscopy system, where the main components such as the microscope, the camera and the actuators are bought off the shelves. The author focuses on integration of the various systems instead of the building of each component.

2. Novelty is sought in robotics instead of biological science. The proposed concept serves as a tool for biologists to carry out various biological experiments, which are not easily conducted using non-robotic approaches.
3. The current system is designed to manipulate suspended cells only, which are assumed to be homogeneous elastic solid in the experiments. The proposed concept can be extended to other cell types with appropriate environment changes.
4. The author only considers active stressing (penetration) of cells instead of aspiration, which requires a different system setup.

Only zebrafish embryos are experimented in the dissertation. Zebrafish is considered as the model organism for vertebrates. It is the smallest vertebrate teleost with body plan similar to humans, with a neural tube, muscles, a vascular system and a beating heart, developed within 24 hours of fertilization. They also share significant genetic similarities with humans with genes and pathways well conserved.

1.5 Organization

After this chapter of introduction, the following chapters are organized as follows.

Chapter 2 reviews the state-of-art cell micromanipulation systems. Existing automatic cell micromanipulation systems are classified into teleoperated and vision-guided. Vision-guided systems equipped with a force-senor are capable of performing mechanical property characterization of cell biomembrane.

Chapter 3 describes the setup of the proposed vision-guided cell micromanipulation system. The system-wise and module-wise capabilities are introduced. The technical specifications of each component are tabulated.

Chapter 4 describes the automatic self-calibration method. The vision system must be re-calibrated whenever the micro-tip is dismounted and mounted, which happens quite often for genetic material refilling in cell microinjection. Calibration of a vision system is quite time-consuming and often needs a calibration template, which could bring biological contamination during cell micromanipulation. Thus, an automatic self-calibration method without introducing calibration templates is necessary.

Chapter 5 provides the design of an image-based position controller with deviation compensation capability. Although vision-guided position control has been achieved with acceptable accuracy in existing vision-guided cell micromanipulation systems, the depth deviation due to disturbances has not been well addressed, which would cause the micromanipulation fail. An image-based position controller with depth deviation compensation capability is required for these micromanipulation ensuring that the micro-tip is always in align with the contact point of the cell on the image plane.

Chapter 6 presents the hysteresis modeling method of a piezoelectric stage using vision-feedback. The major limitation of using a piezoelectric stage in dynamic trajectory (force) tracking application is its hysteresis, which is modeled with the help of an interferometer conventionally. The rate-and-load dependent of hysteresis necessities modeling hysteresis when the load or the driving frequency changes, which is a challenging task due to the special requirements of interferometers. Thus, automatic hysteresis modeling of the piezoelectric stage using vision-feedback on-line in real time is desired.

Chapter 7 presents the mechanical force control of cell biomembrane, especially the mechanical property modeling of cell biomembrane. Automatic mechanical property modeling of cell biomembrane to (a) characterize the changes of mechanical property at different biological developmental stages and (b) facilitate mechanical stress control is necessary. The mechanical property of cell changes at different biological developmental stages, which calls for a modeling method such

that it could be implemented on-line in real time.

Chapter 8 concludes the dissertation and discusses the future work. The contributions are highlighted.

Chapter 2

Review on Cell

Micromanipulation Systems

Existing cell micromanipulation techniques can be classified into two categories: non-contact (Laser Trapping and Electro-rotation) and contact.

2.1 Non-contact Systems

Laser trapping (Ashkin 1970, Ashkin and Dziedzic 1987, Ashkin *et al.* 1987, Wright *et al.* 1990, Conia *et al.* 1997, Grover *et al.* 2001) and electro-rotation (Washizu *et al.* 1993, Washizu and Jones 1996, Arai *et al.* 1997, Nishioka *et al.* 1997) are typical non-contact cell micromanipulation techniques. Laser trapping uses laser beam to hold cells in the desired position for cell micromanipulations. However, due to high dissipation of the laser energy, cells may be destroyed or mutated during the operation. Electro-rotation uses the dielectrophoresis effect to manipulate cells. This non-contact cell manipulation technique is based on controlling the phase shift and magnitude of the electric fields to generate a torque on the cell. This method can achieve high accuracy in cell positioning but cannot hold the cell in place for penetrations and injections, because the low electric field has to be used

to ensure the viability of cells.

2.2 Contact Systems

Contact cell microinjection systems can be broadly classified into two categories: vision guided (Sun and Nelson 2002, Shim *et al.* 2004) and teleoperated (non-vision guided) (Yanagida *et al.* 1999, Tan and Ng 2001). Strictly speaking, non-vision guided cell microinjection system is not an automatic cell micromanipulation system because the positioning and penetration of the micro-tip depend on the human vision information. The parameters needed for the positioning and injection are provided by the laboratory operators' input to the computer.

2.2.1 Teleoperated Systems

The non-vision guided microinjection is still superior to manual microinjection in a sense that the positioning and penetration can be done using computer controlled actuators with better accuracy. Kimura and Yanagimachi first introduces piezo-electrically driven manipulator for the mouse embryo injection and has achieved high survival and fertilization rates (Kimura and Yanagimachi 1995). Yanagida has proved this by using the piezoelectrically driven manipulator for human ICSI (Yanagida *et al.* 1999). However, these systems face one common problem: successful injection not only depends on the controller design, but also on the computer input parameters provided by the laboratory operators.

Tan provides another method using a graphical user interface (GUI) such that the operators can select the desired motion profile (Tan and Ng 2001). This is better in a sense that the motion profile can be monitored on the computer screen. By choosing the appropriate values, the motion profiles of the manipulator are well planned. The system can only provide motion profiles specific to the type of cells, and the degree of automation is still not very high, more information is required to

be incorporated to handle operations in complex environments with multiple cells.

2.2.2 Vision-guided Systems

Vision-guided cell micromanipulation system has high degree of automation and does not require skillful laboratory operators. The parameters, for example, the positions of the target cell and the micro-tip, involved in the position controller design are determined from the visual servoing part. A vision-guided cell micromanipulation system contains an optical microscope, a camera and a computer-driven actuator. There are also some distinguished variations of the setup among the existing vision-guided cell micromanipulation systems.

The system setup depicted by Sun and Nelson (Sun and Nelson 2002) is a typical setup of a vision-guided system. The actuator used in the system is a 3-DOF microrobot with a step resolution of 40 nm in each axes. The visual information gathered is based on 2D-to-3D correspondences (Huang and Netravali 1994).

The system setup described by Shim (Shim *et al.* 2004) 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 operator can feel the status of the contact. The probability of cell damaged is greatly reduced. Moreover, two cameras are employed to reconstruct the 3D images of the cell. The penetration point can be accurately determined from the 3D images and the trajectory of the micropipette motion can be well planed. However, the system is not able to retract the micropipette tip to the desired plane if the disturbance makes the micropipette deviate from the desired plane because a two-DOF manipulator is used to accomplish the microinjection, where there is no vertical movement of the micropipette.

The system with batch cell processing capability proposed by Lu (Lu *et al.* 2007) equips with a piezoresistive micro-force sensor to characterize the mechanical

property of cell biomembrane. The normalized two-dimensional cross-correlation algorithm, which is a template-matching method, is used to identify the embryo. The penetration process is modified from purely position control (Sun and Nelson 2002) to a position control with force feedback.

Huang proposes a batch cell processing system using a rotational gel-made cell holding device. A polyvinylidene fluoride (PVDF) force sensor is mounted on the micropipette in the z-direction, along which the penetration happens. The PVDF sensor is used to monitor the penetration process, where a position and force control methodology is developed (Huang *et al.* 2009).

Wang proposes another batch cell microinjection system, where image-based PID position control is employed to control the microrobot in the microinjection process. SSD tracking algorithm is implemented to provide the vision feedback for the PID controller (Wang *et al.* 2009).

The detailed system setup and control algorithm of each system mentioned above are summarized in Table 2.1 and Table 2.2, respectively. The throughput of each system is depicted in Table 2.3.

The position controllers of the micro-tip introduced in the above systems are implemented in the image coordinate system, where the deviation caused by (1) mechanical system assembly misalignments and (2) disturbances in the operations is not considered. The deviation could make the micro-tip move away from the desired contact point resulting in a failure operation. A force sensor is included to determine whether the micro-tip has reached the desired location (Shim *et al.* 2004, Lu *et al.* 2007, Huang *et al.* 2009). However, the force sensor can only tell whether the subsequent biomembrane penetration, for example, is successful or not but cannot provide valuable information to adjust the micro-tip if the penetration fails. Thus, a static position controller with depth deviation compensation ensuring that the micro-tip reaches the desired location is required.

Calibration of a vision system is necessary to obtain precise transformation

Table 2.1. *The system setup of some micromanipulation systems. There are three typical components: vision unit (microscope & camera), cell micromanipulation unit and cell holding unit.*

System	Vision Pixel Size (μm)	Manipulation Resolution (nm)	Holding Resolution (nm)
(Sun and Nelson 2002)	one CCD cam. N.A.	3-DOF 40	3-DOF N.A.
(Shim <i>et al.</i> 2004)	two CCD cams. 8.4×9.8	2-DOF N.A.	N.A. N.A.
(Lu <i>et al.</i> 2007)	one CCD cam. 10.6×10.6	3-DOF (25)	6-DOF (37.5)
(Huang <i>et al.</i> 2009)	one CCD cam. 3×3	4-DOF N.A.	3-DOF N.A.
(Wang <i>et al.</i> 2009)	one CMOS cam. 9.2×9.2	3-DOF 40	3-DOF 40

matrices between different coordinate systems, and precise ratios between different domains, e.g., pixel to voltage, and pixel to micrometers etc. As illustrated in (Wang *et al.* 2009), the calibration of the pixel size only is repeated 100 times, which is time-consuming. The calibration must be re-conducted whenever the micro-tip is dismounted and mounted, which happens quite often in cell microinjection for the purpose of refilling the micropipette. Employing a calibration template in the conventional calibration could also bring biological contamination to the micromanipulation. Thus, automatic self-calibration without calibration templates is desired.

The mechanical forces acting on the cell biomembrane is related to the deformation of the cell biomembrane (Sun *et al.* 2003, Kim *et al.* 2009). Thus, force (stress) control of the cell biomembrane is transformed to deformation control or position control of the micro-tip. In cell mechanobiology, it is required to stress the cell biomembrane according to some predefined dynamic force profiles, which

Table 2.2. *The control algorithm of some micromanipulation systems. There are three main algorithms: feature extraction (cell & micro-tip), position control and deformation (penetration) control. CMHT: chord midpoint Hough transform, C-C: cross-correlation, C-D: convex detection, E-M: error minimization, SSD: sum of squared difference.*

System	Cell detection Tip tracking	position domain	deformation force sensing
(Sun and Nelson 2002)	Hough	E-M	precision control
	SSD	image space	no force control
(Shim <i>et al.</i> 2004)	Hough	Haptic	Haptic
	scan bar	image space	force & position
(Lu <i>et al.</i> 2007)	C-C	constant speed	hybrid control
	C-C	image space	force & position
(Huang <i>et al.</i> 2009)	CMHT	E-M	impedance control
	C-C	image space	force & position
(Wang <i>et al.</i> 2009)	C-D	PID	precision control
	SSD	image space	no force control

Table 2.3. *The average processing time for each cell in some cell micromanipulation systems. For batch cell processing, the cell holding medium is also listed.*

System	Average manipulation time	cell holding medium
(Sun and Nelson 2002)	N.A.	N.A.
(Shim <i>et al.</i> 2004)	N.A.	N.A.
(Lu <i>et al.</i> 2007)	15 s	gel-trap
(Huang <i>et al.</i> 2009)	6 s	circular-groove
(Wang <i>et al.</i> 2009)	4 s	vacuum-hole

demands a dynamic motion trajectory tracking controller of the actuator. The mechanical force control is relatively less explored compared to mechanical force characterization, to date.

Chapter 3

System Overview

The hardware setup is shown in Figure 3.1. The hardware configuration, which is a generic configuration for suspended cell micromanipulation tasks (Sun and Nelson 2002, Shim *et al.* 2004), of the proposed system is shown in Figure 3.2. The proposed system contains three units: a vision unit, a cell holding unit and a cell micromanipulation unit.

A personal computer (Pentium 4, 3G; 2G RAM) with real-time Labview installed connects the three units through a data acquisition card (DAQmx 628 NI, USA) with 4 analog output (AO) and 32 analog input (AI) channels, a piezoelectric controller (E-501, PI, Germany) and an OEM stepper motor controller (TDC001, THORLABS, USA).

3.1 Vision Unit

Manual focusing of a target in a microscopy system is time consuming and often generates subjective results, which demands an autofocus method for our application. The proposed system is able to perform autofocus with some image processing algorithms by introducing a motor-driven objective.

Figure 3.1. *The hardware setup of the proposed cell micromanipulation system. A: Vision unit; B: Holding unit; C: Micromanipulation unit.*

The vision unit consists of two cameras and a microscope (Eclipse FN1, Nikon, Japan), which equips with an optical objective attached to a stepper motor with $1\ \mu\text{m}$ resolution. The top camera (exA640m or piA640-210gc, Basler, Germany) (see Table 3.1), is utilized for visual servoing purpose and the camera (5830, OPLENIC, China) mounted on the eyepiece is utilized for video recording.

The exA640m camera is an intelligent camera with a real-time operating system (RT Linux), which is suitable for stand-alone biological phenomenon observations and the visual servoing task whose servoing rate is not a concern, e.g., system calibration and static position control. The piA640-210gc is a high frame rate camera, which is suitable for high-speed visual servoing applications, e.g., dynamic motion trajectory tracking.

Figure 3.2. The hardware configuration of the proposed system. A: Vision unit; B: Holding unit; C: Micromanipulation unit; DAC: digital-to-analog converter; ADC: analog-to-digital converter; Amp: amplifier; OEM controller: customized stepper motor controller.

Table 3.1. Parameters for the vertically mounted camera *piA640-210gc* & *exA640m*. *exA640m* is an intelligent camera with a real-time operating system that can perform on-board image processing. *piA640-210gc* is a high-speed camera with smaller pixel size, which is suitable for high-speed visual servoing applications.

Model	Resolution	Pixel Size (μm)	Max Frame Rate	Mono/Color
piA640-210gc	648 $\times$ 488	7.4 $\times$ 7.4	210	Yes/Yes
exA640-m	656 $\times$ 491	9.9 $\times$ 9.9	60	Yes/No

3.2 Cell Holding Unit

A 3-DOF computer controlled stage that can drive the micro-holder to the desired location and immobilize the cell with negative pressure generated in the micro-holder is proposed. Normally there is an angle of the tip of the micro-holder for the purpose of holding the cell tightly in the horizontal direction. In order to make the tip of the micro-holder in its favorable direction, a manual rotational stage is often mounted (see Figure 3.3).

Figure 3.3. *System design for cell holding unit. The 3-DOF coarse motorized (and manual) stage brings the micro-holder to hold the cell. The rotational stage controls the direction of the tip of the micro-holder.*

The holding unit comprises a 3-DOF motorized translation stage with a travel resolution of $1.25\ \mu\text{m}$ (7T173-50, Standa, Lithuania), a manual rotation stage (7R150-30, Standa Ltd, Lithuania) and a manual microinjector (CellTram Vario, Eppendorf, Germany).

3.3 Cell Manipulation Unit

A 3-DOF computer controlled translational micromanipulator (e.g., fine movement) that can drive the micro-tip to the desired location and perform the necessary actions such as penetrating or deforming the cell biomembrane is proposed. A 3-DOF coarse stage is required to bring the micro-tip to the field of view of the microscope. It is also necessary to put a rotational stage between the coarse and fine micromanipulator because the microinjection, for an example, is conducted in an inclined direction (see Figure 3.4).

The cell micromanipulation unit consists of a 3-DOF coarse translational stage with $10\ \mu\text{m}$ resolution (7T173-20-50, Standa Ltd, Lithuania), a coarse rotational stage with 3 arcmin resolution (7R150-30, Standa Ltd, Lithuania), a 3-DOF XYZ piezoelectric stage (P-611.3S, PI Co, Germany), and a microinjector (CellTram

Figure 3.4. *System design for cell micromanipulation unit. The 3-DOF coarse manual stage brings the micro-tip into the field of view of the microscope; the rotational stage controls the penetration (deformation) direction of cell biomembrane; the 3-DOF fine actuator controls the position of the micro-tip in the image plane and penetration or deformation of cell biomembrane.*

Figure 3.5. (a) *Cell microinjection setup*; (b) *Cell biomembrane force control setup*.

Vario, Eppendorf, Germany).

The system setup depicted in Figure 3.1 is designed to perform operations such as cell microinjection, embryo strain and stress control. For example, a micropipette with sharp tip is used for microinjection as shown in Figure 3.5(a), whilst a micro-force sensor (FT-S270, Femtotoools GmbH c/o, Switzerland) is deployed for biomembrane mechanical property characterization and cell stress control as shown

in Figure 3.5(b).

Chapter 4

Automatic Self-calibration of Vision Systems

4.1 Introduction

4.1.1 Background

Calibration of a vision system is a necessity in order to accurately infer and control the 3D location of the interested object from 2D images captured by the mounted camera(s) due to (1) misalignments of assembled mechanical stages, (2) deviations of optical specifications from claimed values, and (3) deviations in the voltage-to-displacement ratios of a voltage-based micro-positioning micromanipulator. It is common to classify the parameters that affect the position and orientation of the interested object as extrinsic parameters, and the optical and electrical scalar values as intrinsic parameters.

The identification of extrinsic and intrinsic parameters in macro-domain has been extensively studied (Tsai 1987, Weng *et al.* 1992, Soatto *et al.* 1996). However, calibration of a vision-guided microscopy system is different from that in macro-

domain in the following aspects: (1) additionally optical parameters included due to the microscope affecting the formation of images in the camera, (2) optical principles of microscopes necessitating the placement of the calibration sample (template, see Figure 4.1) parallel to the image plane, and (3) limited working space resulting the employment of only one calibration template (Zhou and Nelson 1999).

Zhou and Nelson propose a parametric model (Zhou and Nelson 1999), where the extrinsic and intrinsic system parameters are calibrated using the radial alignment constraint (Tsai 1987) and a modified iterative error minimization method, respectively. Due to the parallel alignment of the calibration template and the image plane, a sufficient number of calibration points on the template must be chosen to avoid local extremum in the iterative error minimization method.

In vision-guided cell micromanipulation systems, the vision system calibration (at least for extrinsic parameters) must be re-conducted due to the dismounting and mounting of the micro-tip, which occurs frequently in cell microinjection because of refilling the micropipette.

The distinguished characteristic of vision-guided cell micromanipulation systems demands a self-calibration method such that the vision system calibration can be conducted on the fly without a calibration template, preferably with less computational time by reducing the number of calibration points detection.

The self-calibration technique proposed by Chesi and Hashimoto assumes that the camera is mobile (Chesi and Hashimoto 2002). However, in microscopy systems, cameras are normally stationary along the optical path of the microscope. Extensive studies about system self-calibration are carried out by Hemayed (Hemayed 2003), these methods cannot be applied because at least one of the conditions is not satisfied in cell micromanipulation systems.

Figure 4.1. *The calibration template could contain different markers, e.g., square and circle.*

4.1.2 Objective of Calibration

The following definitions are emphasized in order to clarify the calibration objective:

Nominal values of parameters:

The extrinsic parametric matrix obtained by the D-H (Denavit and Hartenberg 1955) transformation and intrinsic values provided by hardware manufacturers' specifications.

Calibrated values of parameters:

The extrinsic parametric matrix and intrinsic values obtained through the calibration method.

Known input:

A set of values used to drive the micromanipulator (e.g., the piezoelectric stage).

Nominal output:

The displacement of the micro-tip computed using all the nominal values of parameters and the known input.

Measured output:

The real displacement of the micro-tip measured through the image processing algorithm.

Calibrated output:

The displacement of the micro-tip computed using all the calibrated values of parameters and the known input.

The objective of a calibration method is to minimize the difference between the measured output and the calibrated output, e.g., the calibrated values of the parameters are correct.

In this chapter, a fast self-calibration method for cell micromanipulation systems is proposed. Each parameter in the system is composed of an nominal value and an unknown deviation called uncertainty. All the system parameters' uncertainties are consolidated into a matrix. The difference between the measured and nominal output is assumed to be caused by the matrix (i.e., system parameters' uncertainties). This matrix is solved by collecting at least three pairs of known input and the corresponding output differences.

4.2 Consolidated-matrix Parametric Model

The relationship between the input of the piezoelectric stage (Voltage) and the displacement (Pixel) of the feature in the image plane can be described using the following equation.

$$\begin{bmatrix} r & c \end{bmatrix}^T = m\alpha M_c^i H_p^c V \quad (4.1)$$

where $\begin{bmatrix} r & c \end{bmatrix}^T \in \mathbb{R}^{2 \times 1}$ is the coordinates of the micro-tip with respect to the image coordinate system (ICS); $H_p^c \in \mathbb{R}^{4 \times 4}$ and $M_c^i \in \mathbb{R}^{2 \times 4}$, respectively, are homogeneous transformation matrices from the piezoelectric stage coordinate system, also regarded as the world coordinate system (WCS), to the camera coordinate

Figure 4.2. *Kinematic representations of the micromanipulator. (a) The micro-tip in the world coordinate system; (b) WCS w.r.t. CCS with uncertainty; (c) world w.r.t. camera under nominal scenario; (d) image coordinate system.*

system (CCS) and CCS to ICS; $V = [v_x \ v_y \ v_z \ 1]^T \in \mathbb{R}^{4 \times 1}$ represents the homogeneous control input to the piezoelectric stage; m ($\mu m/V$) and α ($Pixel/\mu m$) are two scalars representing the ratios between displacement in CCS and voltage input, pixels in ICS and displacement in CCS, respectively.

If the image plane is chosen to be on the x - y plane of CCS and put WCS and CCS at the same origin, from Figure 4.2(b) to (d), Equation 4.1 can be rewritten as Equation 4.2.

$$[r \ c]^T = m\alpha \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} R_p^c V \quad (4.2)$$

where $R_p^c \in \mathbb{R}^{3 \times 3}$ is the rotational matrix from WCS to CCS. If m , α and R_p^c can be identified, the exact location of the micro-tip is obtained through Equation 4.2.

The parameters m , α and R_p^c are each composed of two values called the nominal value and uncertainty. The nominal values are obtained from hardware specifications and readings of the respective translational and rotational stages. For example, the transformation matrix is derived from the reading of the rotational stage using D-H convention.

Uncertainties for parameters m , α and R_p^c come from various sources. For example, there may be a DC offset in the piezoelectric micromanipulator controller,

and m will have uncertainty. The uncertainty for α comes from the optical aspects of the microscope and camera, for example, the pixel size of the camera and the focal length of the objective. The uncertainty for R_p^c is introduced, for example, from mechanical assembly. Since m and α are scalars, all the uncertainties can be consolidated together with R_p^c and renamed $R_d \in \mathbb{R}^{3 \times 3}$. Figure 4.2(a) is transformed to (b) and rewrite Equation 4.2 as follows:

$$[r \ c]^T = m_n \alpha_n \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} (R_n + R_d)V \quad (4.3)$$

where m_n , α_n and R_n are nominal values of m , α and R_p^c , respectively. The deviation caused by R_d is written as:

$$[\Delta r \ \Delta c \ \Delta d]^T = m_n \alpha_n R_d V \quad (4.4)$$

$$R_d = \begin{bmatrix} r_{11} & r_{12} & r_{13} \\ r_{21} & r_{22} & r_{23} \\ r_{31} & r_{32} & r_{33} \end{bmatrix} \quad (4.5)$$

where $[\Delta r \ \Delta c \ \Delta d]^T$ is the deviation of the micromanipulator tip at x_c , y_c and z_c direction, respectively. Since R_d contains nine unknowns, at least three independent sets of correspondences between $[\Delta r \ \Delta c \ \Delta d]^T$ and V must be obtained.

The unit for Δd is μm , while Δc and Δr are in pixels. Equation 4.4 is modified so that all the terms have the same unit.

$$[\Delta r/\alpha_n \ \Delta c/\alpha_n \ \Delta d]^T = m_n R_d V \quad (4.6)$$

4.3 Template-free Calibration

4.3.1 Estimation of Δd

In a single camera vision system using an optical microscope, extracting the depth information is a challenging task (Wilczkowiak *et al.* 2001), especially when the

camera is stationary . The depth information is estimated based on the analysis of expectation maximization (Vitria and Llacer 1996), where the image is represented as a function of the distance between the objective and the focusing plane. The 3D information of the object under an optical microscope is also obtained through the analysis of so called section images (Verbeek and Boon 2002), where each section represents one level of depth. The relationship between the focus and depth is explored by Ohba (Ohba *et al.* 2003), which serves as basis for our study.

The method proposed here is modified from the microscope autofocusing research (Vollath 1987, Santos *et al.* 1997, Lin *et al.* 2002, Hülsen *et al.* 2004, Sun *et al.* 2004). Autofocusing can identify the best position of the objective to obtain the best focused image. Thus, a sequence of images (I) are captured and the characteristic values, for example, two dimensional (2D) standard deviation (STD)(S), with respect to the objective positions (D) are extracted as shown in Figure 4.3. A suitable mathematical model (f) is set up to fit the data. The inverse model (f^{-1}) can provide the objective position based on the characteristic values from the subsequent captured images with the same system setup.

$$\begin{aligned}
 S_i &= \frac{1}{H \cdot W} \sum_H \sum_W (I_i(r, c) - \mu_i)^2 \quad i = 1 \dots N \\
 \Delta d_j &= f^{-1}(D, S, S_j) - f^{-1}(D, S, \max(S)) \\
 S &= \{S_i | i = 1 \dots N\} \\
 D &= \{D_i | i = 1 \dots N\}
 \end{aligned} \tag{4.7}$$

where H and W are the height and width of the region of interest (ROI) in the image, respectively; D_i is the objective position corresponding to image i (I_i); μ_i is the mean gray value of I_i ; Δd_j is the difference between the objective position of I_j and the best focused image, respectively.

Figure 4.3. The difference in the normalized STD (NSTD) value when the objective moves downwards and upwards. The traveling range of the objective in both downwards and upwards is 1000 μm with a step resolution of 1 μm .

4.3.2 Estimation of Δr and Δc

In a single camera vision system, The location of the micro-tip, named $[r_m \ c_m]^T$, can be traced using shape-based (Rosenhahn *et al.* 2006), component-based (Hussain and Eakins 2004) or gray-value-based feature matching algorithms. Sum of squared difference (SSD) is a typical gray-value-based feature tracking algorithm suitable for images with consistent background (Nelson *et al.* 1996). It should be noted that the SSD tracking algorithm can only provide 2D information corresponding to x and y values in CCS. The nominal tip location can be obtained by applying the nominal values of m , α and R_p^c in Equation 4.2. The difference between the measured and nominal location is as follows:

$$[\Delta r \ \Delta c]^T = [r_m \ c_m]^T - m_n \alpha_n \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} R_n V \quad (4.8)$$

Three sets of input ($[v_{x1} \ v_{y1} \ v_{z1}]^T$, $[v_{x2} \ v_{y2} \ v_{z2}]^T$ and $[v_{x3} \ v_{y3} \ v_{z3}]^T$) are used to excited on the piezoelectric stage. Three sets of corresponding deviations ($[\Delta r_1 \ \Delta c_1 \ \Delta d_1]^T$, $[\Delta r_2 \ \Delta c_2 \ \Delta d_2]^T$ and $[\Delta r_3 \ \Delta c_3 \ \Delta d_3]^T$) are col-

lected. $\hat{R}_d$, which is a reshape of R_d , is derived according to the following formula.

$$\hat{R}_d = m_n^{-1} \hat{V}^{-1} \hat{D} \quad (4.9)$$

where

$$\hat{V} = \begin{bmatrix} v_{x1} & v_{y1} & v_{z1} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & v_{x1} & v_{y1} & v_{z1} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & v_{x1} & v_{y1} & v_{z1} \\ v_{x2} & v_{y2} & v_{z2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & v_{x2} & v_{y2} & v_{z2} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & v_{x2} & v_{y2} & v_{z2} \\ v_{x3} & v_{y3} & v_{z3} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & v_{x3} & v_{y3} & v_{z3} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & v_{x3} & v_{y3} & v_{z3} \end{bmatrix} \quad (4.10)$$

$$\hat{R}_d = [r_{11} \ r_{12} \ r_{13} \ r_{21} \ r_{22} \ r_{23} \ r_{31} \ r_{32} \ r_{33}]^T \quad (4.11)$$

$$\hat{D} = [\Delta r_1/\alpha_n \ \Delta c_1/\alpha_n \ \Delta d_1 \ \Delta r_2/\alpha_n \ \Delta c_2/\alpha_n \ \Delta d_2 \ \Delta r_3/\alpha_n \ \Delta c_3/\alpha_n \ \Delta d_3]^T \quad (4.12)$$

Singularity of $\hat{V}^{-1}$ prevents $\hat{R}_d$ from being identified. More than three pairs of independent input and Pseudoinverse (Campbell and Meyer 1991) based on Moore-Penrose matrix inverse are used to provide better approximations.

It should be noted that the above calibration is repeated whenever the micro-tip is dismounted and mounted, because the position and orientation of the micro-tip change.

Each STD value in Figure 4.3 corresponds two positions of the objective except at the extreme points. The actual position is obtained by moving the lens a step downwards, the changes in the STD value determines whether the target is above or below the focal plane, which is discussed in more detail in Chapter 5.

Besides comparing the difference between the calibrated and measured output with the physical resolution in each direction, another parameter e is defined to evaluate the performance of the proposed method as follows:

Figure 4.4. *Automatic template-free self-calibration of vision-guided microscopy systems, (TP: tip point of the sensor; ZFE: zebra fish embryo). The template-free calibration method is feasible if the difference between the measured output and the calibrated output is reduced to the desired value.*

$$e = \frac{\|\text{Calibrated output} - \text{Measured output}\|}{\|\text{Nominal output}\|} \quad (4.13)$$

4.4 Experiments on the Proposed Method

4.4.1 Experimental Setup

The system setup is similar as shown in Figure 3.1. In order to demonstrate that the template-free calibration method can be conducted on the fly during cell micromanipulation, the micro-force sensor (FT-S270, Femtotools GmbH c/o, Switzerland) acts as the micro-tip in an experiment where a zebra fish embryo and a microholder are present as shown in Figure 4.4. The target is to verify the feasibility of the proposed calibration method, e.g., whether the difference between the measured output and the calibrated output is reduced to the desired value, which is a combination effect of various hardware specifications.

Table 4.1. *The achievable resolutions in x – (y –) and z – direction in the camera coordinate system are the ratio between the pixel size and the magnification of the objective, and the step resolution of the motor driving the vertical objective and the micromanipulator, respectively. It should be noted that the resolution of the micromanipulator in the nanometer range according to the specification. Therefore, if the camera is kept stationary while moving the micromanipulator, the achievable resolution is limited by the optical property of the objective, which has to be identified experimentally.*

Axes	Physical achievable resolution (μm)
x	0.99
y	0.99
z	1.0

The actual resolution of a CCD or CMOS camera is determined by the number of photodiodes and their size relative to the image projected onto the surface of the imaging array by the microscope optical system (e.g., the magnification and numerical aperture of the objective). In this experiment, the resolution of the optical microscope using a 10X objective with numerical aperture (NA) of 0.3 is $0.92 \mu m$. The pixel size of the camera (exA640m, Basler, Germany) is $9.9 \mu m \times 9.9 \mu m$. The step resolution of the motor driving the vertical objective is $1 \mu m$. With these specifications, the desired values of the micro-tip in the image plane and the vertical direction are expected to be smaller than the physical resolutions tabulated in Table 4.1.

The 3-DOF piezoelectric stage (P-611.3S, PI Co, Germany) driving the micro-tip has 2 nm resolution according to the specifications, which is much smaller than the resolution of the imaging system in the x –, y – and z – direction. The nominal angle between the piezoelectric stage and the camera coordinate system is 30° (with 3 arcmin resolution) as shown in Figure 4.5, e.g., R_n in Equation 4.3 is as follows:

$$R_n = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(30^\circ) & \sin(30^\circ) \\ 0 & \sin(30^\circ) & -\cos(30^\circ) \end{bmatrix} \quad (4.14)$$

Figure 4.5. *The camera coordinate system is obtained by rotating the world coordinate system by 30° along its x direction.*

m_n ($\mu\text{m}/V$) is the voltage-to-displacement ratio, which is 10 for the given piezoelectric stage controller (E-501, PI Co, Germany). α_n is 1.01, which is the reciprocal of the physical achievable resolution in the image plane.

4.4.2 Experiment Procedure

The experiment is conducted in six steps as shown in Figure 4.6, which include cell immobilization, STD model identification, piezoelectric stage excitation, deviations extraction, parameters computation and validation.

A suspended zebra fish embryo is placed into a petri dish on the platform and focused manually. The microholder is brought to the field of view to immobilize the embryo. After the cell is immobilized, the micro-force sensor is brought to the field of view of the microscope for STD model identification. The objective (lens) is first driven to an upper position where the micro-force sensor and the cell are defocused. It is then driven downwards till the scene is focused again as shown in

Figure 4.6. *The experiment for the proposed self-calibration is conducted in six steps.*

Figure 4.7 and 4.8. A fifth-order polynomial (see Figure 4.9) is employed to fit the STD values with respect to the positions of the objective.

The measured position of the micro-force sensor is obtained using the SSD tracking algorithm and inverse STD model. The differences between the measured and the nominal output are used to compute R_d using Equation 4.9. In order to validate R_d , another set of input is used to excite the piezoelectric stage, the differences between the calibrated output and the measured output imply the correctness of the proposed calibration method.

4.4.3 Experiment Result

The STD values and objective positions are obtained by collecting 51 images at different positions of the objective as shown in Figure 4.8. The relationship between the STD values and the objective positions is derived by fitting a fifth-order

Figure 4.7. The optical objective is driven downwards to get a series of defocused-focused-defocused images.

Figure 4.8. Images captured at different positions of the objective, from left to right: 1st, 11th, 21st, 31st, 41st and 51st. The number below the image is the normalized STD value.

polynomial. The polynomial coefficients are as follows:

$$p = [-0.1128 \times 10^{-10} \quad 0.1267 \times 10^{-7} \quad -0.4349 \times 10^{-5} \quad 0.3049 \times 10^{-3} \quad 0.0461 \quad 111.8] \quad (4.15)$$

A set of input with 10 data samples is used to excite the piezoelectric stage. The data collected is shown in Table 4.2. The matrix R_d is computed as follows:

$$R_d = \begin{bmatrix} 0.33 & 0.16 & 0.14 \\ 0.19 & 0.09 & 0.08 \\ -0.25 & -0.02 & 0.25 \end{bmatrix} \quad (4.16)$$

Another four input data samples are utilized to evaluate R_d . The differences

Figure 4.9. A fifth-order polynomial is employed to fit the NSTD values.

between the measured and calibrated output based on Equation 4.3, are shown in Table 4.3.

4.5 Discussion

The actual achievable resolution of a vision-guided microscopy system is determined by a number of factors such as the optical resolution of the microscope, the pixel size of the camera as well as the resolutions of the driving sources. For example, if the minimum traveling distance, which is distinguishable from the vision system is $1 \mu\text{m}$ in the 3D working space, a calibration method is feasible as long as the difference between the measured and calibrated output is less than $1 \mu\text{m}$.

Instead of explicitly finding the exact values for the extrinsic and intrinsic parameters, which is conducted in most of the existing calibration methods, a calibration method is also acceptable if a matrix, which accurately represents the transformations from the world coordinate system to the camera coordinate system is identifiable because the ultimate aim of calibration in vision-guided system is to determine the relationship between the two coordinate systems. Moreover, mathematically speaking, the transformation matrix is obtained by incorporating the extrinsic and intrinsic parameters into the D-H convention (Tsai 1987).

Table 4.2. *Calibration result: Input (V) is the control input applied to the piezo-electric stage, $[\Delta r \ \Delta c \ \Delta d]^T$ is the difference between the nominal and measured values.*

Input(V)			Difference(μm)		
v_x	v_y	v_z	Δr	Δd	Δc
3	2	1.15	2.5	-0.17	-0.59
4	3	1.73	2.5	-0.18	0.36
5	4	2.31	2.5	-1.17	1.31
5	5	2.89	2.5	-0.10	-0.24
6	5	2.89	2.5	0.08	-0.24
7	6	3.46	2.5	-0.60	0.72
8	7	4.04	2.5	-1.36	1.67
2	8	4.62	2.5	-1.38	2.62
5	8	4.62	2.5	-1.92	2.62
7	8	4.62	2.5	-1.78	2.62

The experimental results demonstrate that the proposed self-calibration method can make the difference between the measured and the calibrated output smaller than the physical achievable resolutions as shown in Table 4.1 and 4.3, which implies the feasibility of this method for the calibration of such microscopy systems.

The proposed method is a self-calibration method resulting that the time spent (e.g., average lead time is about 2 minutes.) on the calibration is greatly reduced because the preparation of a suitable calibration template for the current system configuration is eliminated. The calibration procedure is easily re-conducted on the fly when the micro-tip is dismounted and mounted while the target cells can remain on the platform, which happens quite often in cell microinjection for the purpose of refilling the micropipette. Thus, the proposed self-calibration method is suitable for vision-guided cell micromanipulation systems.

Table 4.3. *Evaluation result: Input (V) is the control input applied to the piezo-electric stage, $[\Delta r \ \Delta c \ \Delta d]^T$ is the difference between the calibrated and measured values. $\max|\Delta|$ is the maximum difference among the three directions.*

Input(V)			Difference(μm)			$e(\%)$	$\max \Delta (\mu m)$
v_x	v_y	v_z	Δr	Δd	Δc		
6	2	1.15	-0.1	-0.13	0.02	0.27	0.13
5	7	4.04	-0.21	-0.08	-0.04	0.39	0.21
8	7	4.04	-0.3	0.98	-0.46	1.28	0.98
6	8	4.62	-0.42	0.38	0.44	1.04	0.44

The proposed method is only applicable when the origins of the world and camera coordinate systems are at the same place, e.g., only rotational relationship exists between the two coordinate systems. A linear transformation is required if the two origins are separated.

4.6 Conclusion

A self-calibration method is proposed in this thesis. There are several advantages over the conventional calibration methods: (1) calibration templates are not required; (2) only one matrix is required to be calibrated; (3) it is suitable for systems that require frequent calibration; (4) it is simple to implement in practical applications. The algorithms adopted in the proposed method are also useful for feature tracking, microscope focusing, position control of the micro-tip in vision-guided cell micromanipulation systems. The experimental results have also demonstrated the effectiveness of this method.

Chapter 5

Image-based Position Control with Deviation Compensation

5.1 Introduction

Positioning the micropipette or micro-force sensor to the desired position, such as the contact point in Figure 5.1 is one of the key steps to ensure successful penetration or deformation of the cell biomembrane. Due to the lack of 3D information of the micro-tip during the cell micromanipulation, the changes of the depth of the micro-tip are usually ignored in the position control in the image coordinate system.

In order to verify the above assumption, a comprehensive test is conducted where

- A position controller based on the minimization of an objective function whose inputs are the current and desired position of the micropipette in the image space is implemented (Sun and Nelson 2002).
- A position controller based on a second-order closed-loop dynamics where the

Figure 5.1. (a) The cell, holder and micropipette are positioned into the same focal plane; (b) The micromanipulator deviates from the plane as the cell while traveling resulting in the wrong indentation point.

Table 5.1. Δx (Pixel), Δy (Pixel) and Δz (μm) are deviations of the micro-tip from the desired position using two controllers. It should be noted that the deviation values, which are not published in the corresponding authors' articles, are obtained using the proposed system in Chapter 3. The calibration effect introduced in Chapter 4 is also investigated.

Method	Without calibration			With calibration		
	Δx	Δy	Δz (μm)	Δx	Δy	Δz (μm)
(Sun and Nelson 2002)	1	< 1	5.78	< 1	< 1	2.92
(Huang <i>et al.</i> 2009)	< 1	1	-4.23	< 1	< 1	-2.12

motion trajectory is assumed to be in the image space of the camera frame is implemented (Huang *et al.* 2009).

- The micro-tip is required to travel by 30 pixels in x - and y -direction in the image space using a 10X optical objective.
- The deviation in z -direction is expected to be zero.

Base on the z -deviation estimation method introduced in Chapter 4, the deviation of two controllers are summarized in Table 5.1 and Figure 5.2.

A few conclusions are drawn based on the test result:

Figure 5.2. Two existing position controllers are implemented and their deviations in z -direction are recorded, which are expected to be below certain values to ensure successful indentation. *: (Sun and Nelson 2002); **: (Huang et al. 2009).

1. The existing tested controllers prove their correctness in the task space, e.g., the image coordinate systems. The x - and y -deviations are less than 1 pixels if the calibration of the vision system is performed.
2. The deviations of the micro-tip in the z -direction do exist in the position control, which may result in the wrong contact point on the cell biomembrane, for example.
3. The calibration method introduced in Chapter 4 reduces the position error in x -, y - and z -direction up to 50%.
4. However, the calibration only provides accurate fixed relations between different coordinate systems, for example, the disturbance present still causes the micro-tip deviated from the desired position.

In this chapter, an image-based controller with depth deviation compensation capability is introduced. Before that, a review on visual servoing is given.

5.2 Review on Visual Servoing

Visual servoing is realized in many areas primarily due to advanced camera technology, and the increasing computational power of personal computers that allows analysis of scenes at a sufficient rate to "servo" a robotic manipulator. Visual servoing is the combination of different disciplines ranging from high-speed image processing, kinematics, dynamics, control theory to real-time computing (Malis *et al.* 1999). Hutchinson gives an excellent tutorial on visual servoing, which describes a complete picture of visual servoing control (Hutchinson *et al.* 1996). In this section, a brief overview of some basic knowledge related to cell micromanipulation is presented. Four visual servoing architectures are discussed.

5.2.1 Camera Projection Model

The geometric configuration of image sensors in the visual servoing system is subject to applications. To date, there are three geometric configurations: perspective projection, scaled orthographic projection and affine projection as shown in Figure 5.3.

Perspective projection has a view point (or called center of projection) at a finite distance (λ) from the image plane (or called projection plane). Orthographic projection is independent of the distance between the object and the image plane. This can be realized by putting the view point at infinite distance from the image plane. Affine projection is a linear projection where the geometric aspect is not taken into account. What the projection performs is to collect a few pair of corresponding coordinates of the same points with respect to the camera and image coordinates systems.

Figure 5.3. *The coordinate frame for the camera (optical objective) system (Hutchinson et al. 1996).*

5.2.2 Visual Servoing Architectures

There are four classes of visual servoing architectures based on two criteria: (1) the control structure (joint controller or visual servoing) and (2) domain of the error signal (position-based or image-based).

One type of control structures utilizes joint controllers to control the joint movements as shown in Figure 5.4. There is a closed loop inside the joint controller. The vision part provides necessary parameters to the joint controller. The joint controller controls the end-effector until the desired tasks have been successfully fulfilled. During this process, no image is captured until the joint controller has finished these required movements. Another image will be captured followed by image processing. Parameters such as the current position of the robot end-effector will be passed to the joint controller again and this process repeats until the final goal is reached. The advantages of this control structure are: (1) the existing robotic controllers can be easily adopted; (2) high sampling rate for image capturing is not required.

Visual servoing eliminates the robot joint controller entirely in the second type of configuration, and computes the joint inputs directly to stabilize the system.

Figure 5.4. *Image-based visual servoing system using a joint controller.* I_d is the desired position in the image coordinate system, I_f is the current position and I_e is the error, H_f^j is a transformation matrix from the feature space to the joint space.

High image sampling rate is required to provide the necessary visual feedback. This type of control is suitable for vision guided cell micromanipulation systems, since visual information is used as the feedback, any unforeseen events present during the microinjection can be immediately detected and necessary corrections are performed.

Position-based systems or image-based systems can be defined in a simple manner. In a position-based system, image features are extracted and utilized by the geometric model for transforming the features to the corresponding target pose with respect to the camera model. The controller in this kind of system is designed in the object working space. It is preferable that the target object is static and only the camera is moving in this configuration (Feddema and Lee 1990, Lee *et al.* 1998, Sim *et al.* 2002, Yilmaz *et al.* 2004).

In an image-based system, parameters are extracted from the image directly. These parameters are fed into the visual controllers to control the movement of the micropipette. Clearly, more identification work is required in an image-based system and often, adaptive control is needed (Sanderson and Weiss 1983, Astolfi

Figure 5.5. *Functional units of visual servoing.*

et al. 2001, Shen and Pan 2003, Zachi *et al.* 2004).

Feature-to-feature correspondence is another way to classify visual servoing system. Huang classifies the visual servoing system into three categories according to their applications (Huang and Netravali 1994). There are 3D-to-3D feature correspondences, 2D-to-3D and 2D-to-2D correspondences. 3D-to-3D feature correspondences is suitable for those applications with stereo or other range finding devices (Vogl *et al.* 2004). 2D-to-3D feature correspondences is widely used in many macro-domain and some micro-domain applications (Tsai 1987). 2D-to-2D feature correspondences is applicable for applications in which the pose of the object or target is hard to estimate.

5.2.3 Feature Detection

In visual servoing and digital image processing, the concept of feature detection refers to computing abstractions of image information and making local decisions at every image point (pixel) whether there is an image feature of a predetermined type at the point (pixel). The resulting features will be subsets of the image domain, often in the form of isolated points, continuous curve or connected regions. Feature detection is the first step in visual servoing controller design, but the last step in digital image processing as shown in Figure 5.5.

Morphological processing is the process of extracting image components that are useful in the representation and description of the interested shape such as pointers, corners, boundaries, etc. The inputs of morphological processing are im-

ages and the outputs are attributes from those images (Bala and Wechsler 1992, Loncaric and Dhawan 1994, Ong 1995). The step immediately following morphological processing is image segmentation. It is not surprising that sometimes morphological processing and image segmentation are mixed together.

Segmentation subdivides an image into its constituent regions or interested objects. The level to which the subdivision is preceded depends on the requirements of the application. The segmentation should stop as long as the interested object is identified and isolated. Point detection (Teh and Chin 1988, Shufelt 1999), line detection (Li and Jing 2004, Zhao *et al.* 2006) and edge detection are the preliminary steps in segmentation. Edge linking should be carried out immediately after the points or lines have been detected.

Usually, a biological cell is spherical in shape (Shim *et al.* 2004). Thus a segmentation method which can detect a circle is desired. Hough transform is an excellent method of identifying edges such as circle. Hough transform was first introduced to detect straight lines in 1962 and then it is found that Hough transform is applicable to any function of the form $g(v,c)=0$, where v is a vector of coordinates and c is a vector of coefficients.

Li utilizes a hierarchical approach to obtain fast Hough transform, in which the computations is greatly reduced (Li *et al.* 1986). Xu then introduces a randomized approach to improve the performance of Hough transform in some complex images (Xu *et al.* 1990). This is further improved by using fuzzy randomized Hough transform (Han *et al.* 1994, Chatzis and Pitas 1996, Chatzis and Pitas 1997). The common problem encountered in the above methods is the expensive computation required.

Many approaches including decision theory (Highleyman 1962, Moon and Budge 1994), optimum statistical classifier (Kwan and Cai 1994, Miller *et al.* 1995, Ustun 1999, Jain *et al.* 2000), moment invariants (Hu 1962, Dudani *et al.* 1977, Keyes and Winstanley 2001, Mercimek and Mumcu 2005), sum of squared

difference (SSD), optimization and neural network have been investigated to perform image tracking. SSD tracking algorithm (Papanikolopoulos 1995, Nelson *et al.* 1996) is an effective method for feature tracking in a structured environment where image patterns do not change considerably between successive frames of images.

5.3 Imaged-based Position Control and Deviation Compensation

The major difficulty of image-based visual servoing control comes from the fact that the robot end-effector might deviate from the desired plane which the target is on during the positioning phase and cause the end-effector to skim over or damage the target. This effect is exaggerated in micro-domain applications where the noise tolerance level is limited. For example, if the penetration point must be precisely chosen at the particular location with $1\ \mu m \times 1\ \mu m$ area on the biomembrane, any deviation greater than $1\ \mu m$ of the micropipette in the vertical direction causes the penetration fail. Thus, the feature processing algorithm is expected to monitor the changes of the depth of the micropipette and (or) the target to keep the interested objects on the desired plane.

In image-based visual servoing tasks, the error is defined in terms of the image features. The task is to drive the micropipette to a desired position such that the error is minimized. For example, as shown in Figure 5.7, the distance, d , from the tip of the micropipette to the contact point of the target is defined on the 2D image in the desired plane, $\varepsilon = \sqrt{\varepsilon_x^2 + \varepsilon_y^2}$. The visual servoing controller should be designed in a way such that ε is minimized; at the same time, the micropipette is still in the plane where cell is focused, e.g., the deviation in the z -direction caused by disturbances is compensated.

A hybrid image-based position controller is proposed, whose block diagram

Figure 5.6. Control diagram for the image-based position control with deviation compensation. The upper loop performs the position control while the lower loop performs the deviation compensation.

is shown in Figure 5.6. The hybrid controller contains two loops. The upper loop is in charge of the 2D position control and the other loop is for z -deviation compensation:

1. In the first loop, an image-based position controller using Jacobian matrix, on which an constraint is explicitly imposed, is implemented to control the movement of the micro-tip in the image coordinate system.
2. In the second loop, a STD-based deviation controller is implemented to compensate the deviation of the micro-tip, such that the deviation is kept in a predefined range.

5.3.1 Image-based Position Control

The image Jacobian matrix relates the velocity of the robot end-effector in the task space and image feature space. Let $\varphi \in \mathbb{R}^j$ represent coordinates of the robot end-effector in some parameterizations of the task space $T \in \mathbb{R}^j$ and $\dot{\varphi} \in \mathbb{R}^j$ represent the corresponding robot end-effector velocity. Let $\psi \in \mathbb{R}^k$ represent a

Figure 5.7. *Image-based motion control. The objective is to bring the micropipette to the contact point in the image plane, yet the depth deviation is constrained.*

vector of image feature parameters and $\dot{\psi} \in \mathbb{R}^k$ the rates of change, where j is the dimension of the task space and k is the length of the image feature parameters vector.

$$\dot{\psi} = J(\varphi)\dot{\varphi} \quad (5.1)$$

where $J_v \in \mathbb{R}^{k \times j}$, and

$$J(\varphi) = \left[\frac{\partial \psi}{\partial \varphi} \right] = \begin{bmatrix} \frac{\partial \psi_1(\varphi)}{\partial \varphi_1} & \cdots & \frac{\partial \psi_1(\varphi)}{\partial \varphi_j} \\ \vdots & \ddots & \vdots \\ \frac{\partial \psi_k(\varphi)}{\partial \varphi_1} & \cdots & \frac{\partial \psi_k(\varphi)}{\partial \varphi_j} \end{bmatrix} \quad (5.2)$$

For example, in a case where only a rotational transformation is considered in Figure 5.8, the image features is the tip of the robot end-effector (micropipette) in the image frame. The image Jacobian describing the relationship between the velocity of image features $[x_c \ y_c \ z_c]^T$ and the velocity of the tip with respect to the global coordinate system $[x_w \ y_w \ z_w]^T$ is as follows:

$$\begin{bmatrix} x_c \\ y_c \\ z_c \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \\ 0 & -\sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix} \quad (5.3)$$

Figure 5.8. *The relationship between the world coordinate system and camera coordinate system. (a) Due to mechanical assembly misalignments, there may exist a few rotations between the two coordinate systems; (b) It is assumed that the camera coordinate system is obtained by rotating the world coordinate system by its x -direction by an angle θ .*

Differentiating both sides:

$$\begin{bmatrix} \dot{x}_c \\ \dot{y}_c \\ \dot{z}_c \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \\ 0 & -\sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} \dot{x}_w \\ \dot{y}_w \\ \dot{z}_w \end{bmatrix} \quad (5.4)$$

And

$$[r \ c]^T = m * [x_c \ y_c]^T \quad (5.5)$$

So the image Jacobian is

$$J(\varphi) = m * \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \end{bmatrix} \quad (5.6)$$

which relates the velocity of the tip in image plane with the velocity in the robot end-effector domain or global domain, in this case, m ($Pixel/\mu m$) is the ratio of pixel to distance (micrometers).

The image Jacobian matrix relates the velocity of the robot end-effector in the image frame to the global frame. However, the inverse Jacobian matrix is required in the controller design. There are three cases where the inverse Jacobian matrix can be determined.

$k = m$ the matrix is nonsingular, the inverse exists

$$\dot{\varphi} = J^{-1}(\varphi)\dot{\psi} \quad (5.7)$$

$k > m$

$$J^+ = (J^T J)^{-1} J^T \quad (5.8)$$

$$\dot{\varphi} = J^+(\varphi)\dot{\psi} \quad (5.9)$$

$k < m$

$$J^+ = J^T (J J^T)^{-1} \quad (5.10)$$

In Equation 5.10, although $k < m$, we have another physical constraint. The movement of the image features of the robot end-effector in the vertical direction is expected to be zero at each sampling instant. That is

$$z_c = -y_w \sin\theta + z_w \cos\theta = 0 \quad (5.11)$$

Solving this equation

$$z_w = y_w \tan\theta \quad (5.12)$$

We get

$$\begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \tan\theta \end{bmatrix} \begin{bmatrix} x_c \\ y_c \end{bmatrix} \quad (5.13)$$

Thus, the inverse of the image Jacobian matrix can be obtained directly

$$J^{-1} = \frac{1}{m} \begin{bmatrix} 1 & 0 \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \tan\theta \end{bmatrix} \quad (5.14)$$

After obtaining the inverse of the image Jacobian matrix, the next step is to design the image-based controller. For example, if only a proportional controller

is required and the designed image features is ψ_d , the proportional controller can be written in such way

$$V = KJ^{-1}(\psi_d - \psi) \quad (5.15)$$

where K is the proportional gain. Denote $\psi_d = [r_t \ c_t]^T$, $\psi = [r \ c]^T$, and $V = [v_x \ v_y \ v_z]^T$, Equation 5.15 are written in the discrete control input domain of the piezoelectric stage.

$$\begin{bmatrix} v_x((j+1)\Delta T) \\ v_y((j+1)\Delta T) \\ v_z((j+1)\Delta T) \end{bmatrix} = \begin{bmatrix} v_x(j\Delta T) \\ v_y(j\Delta T) \\ v_z(j\Delta T) \end{bmatrix} + \frac{K}{m\alpha} \begin{bmatrix} 1 & 0 \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \tan\theta \end{bmatrix} \begin{bmatrix} r_t - r \\ c_t - c \end{bmatrix} \quad (5.16)$$

where α ($\mu/Volt$) is displacement-to-voltage ratio of the piezoelectric stage, and ΔT is the sampling period.

Stability Analysis

The stability analysis of the static positioning control in discrete visual servoing system in has its own characteristic: the next visual feedback is only performed after the actuator reaches its steady state. A few assumptions are made in order to analyze the stability:

1. The closed-loop version of the piezoelectric stage, with high linearity and repeatability in absolute position control is employed. The performance of the closed-loop at steady state is shown in Figure 5.9, whose response time is around 40ms.
2. The system sampling period ΔT is greater than the response time of the closed-loop in the piezoelectric stage, which implies that the image-based controller is time independent.

Figure 5.9. *The output of the closed-loop piezoelectric stage is linearly dependent on the input at steady state.*

The image-based control system is linear based on the first assumption and it is always stable according to the stability criteria of a bounded-input-bounded-output (BIBO) system.

Performance Analysis

The proportional gain K in Equation 5.16 determines the time needed to complete the position control, given a fixed sampling period ΔT . It is worth to note that the camera must be able to "see" the micro-tip in order to provide valuable visual feedback, thus the operation will fail if the micro-tip deviated from the field of view of camera due to some unknown disturbances (e.g., the change in the DC offset in the piezoelectric micromanipulator controller, electrical noise and mechanical disturbance in the environment.) present in the sharp movement of the tip, which may happen when the tip just starts to move.

This distinguished observation calls for a smoother movement of the micro-tip. Instead of using a fixed K value in the proportional controller throughout the

Figure 5.10. The parameter ρ is able to make the movement of the micro-tip smooth to avoid the sharp movement in a PID control.

whole positioning process, another parameter is introduced:

$$\begin{bmatrix} v_x((j+1)\Delta T) \\ v_y((j+1)\Delta T) \\ v_z((j+1)\Delta T) \end{bmatrix} = \begin{bmatrix} v_x(j\Delta T) \\ v_y(j\Delta T) \\ v_z(j\Delta T) \end{bmatrix} + \frac{K\rho}{m\alpha} \begin{bmatrix} 1 & 0 \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \\ 0 & (\cos\theta + \tan\theta \sin\theta)^{-1} \tan\theta \end{bmatrix} \begin{bmatrix} r_t - r \\ c_t - c \end{bmatrix} \quad (5.17)$$

where ρ is an increasing function as shown in Figure 5.10, whose input is the ratio of the distance between the current position to the desired position, and the initial position $[r_0 \ c_0]^T$ to the desired position.

$$\rho = e^{\sqrt{(r-r_0)^2 + (c-c_0)^2} / \sqrt{(r_d-r_0)^2 + (c_d-c_0)^2}} \quad (5.18)$$

5.3.2 z -deviation Compensation

The compensation action starts only when the deviation ΔZ in the vertical direction is greater than the predefined threshold η . For example, the threshold η can be set same as the step resolution of the compensation movement of ν of the

Figure 5.11. The STD values of the micro-tip are STD_1 and STD_2 at position P_1 and P_2 , respectively.

micromanipulator. The control input for the vertical deviation compensation is defined as:

$$v_z = \begin{cases} 0, & |\Delta Z| < \eta \\ \zeta \nu, & |\Delta Z| \geq \eta \end{cases} \quad (5.19)$$

The ζ value is determined by current STD value STD_1 and the next STD value STD_2 by moving the optical objective downwards as shown in Figure 5.11.

$$\zeta = \begin{cases} -1, & SSD_1 > SSD_2 \\ 1, & SSD_1 < SSD_2 \end{cases} \quad (5.20)$$

The micro-tip is above the desired plane if $SSD_1 > SSD_2$ when the optical objective moves downwards, demanding the micro-tip moving downwards by $\nu \mu m$, for example. Instead, the micro-tip moves upwards if $SSD_1 < SSD_2$.

The values of ν and η in Equation 5.19 have some physical meanings. The threshold η determines the tolerance level of the depth deviation of the micro-tip. Smaller value of η often requires heavy compensations in a noisy system, resulting in longer time in the positioning process. The compensation movement of η in the z -direction regulates the traveling distance of the actuator in z -direction, which is often set to be a multiple times of the resolution of the actuator.

Table 5.2. Δx (Pixel), Δy (Pixel) and Δz (μm) are deviations of the micro-tip from the desired position using the proposed controller.

Method	Δx (Pixel)	Δy (Pixel)	Δz (μm)	Time (s)
Without compensation	< 1	< 1	2.07	≈ 3
With compensation	< 1	< 1	1.11	≈ 5

5.4 Experiments

Two experiments are conducted on the proposed visual servoing method. The first one evaluates the proposed position controller with the vertical deviation compensation using a piezoelectric stage (P-611.3S, PI Co, Germany). The other one shows the repeatability of the controller, which is critical for cell microinjection and cell stress control. The system setup is shown in Figure 3.1.

5.4.1 Experiment 1: z -deviation Compensation

In this experiment, the micro-force sensor (see Figure 3.5(b)) acts as the micro-tip, which is required to travel by 30 pixels in x - and y -direction in the image plane under 10x magnification with a CCD camera (exA640m, Basler, Germany). The data is tabulated in Table 5.2 similar in the way as in Table 5.1. The tolerance of depth change is set to $\eta = 2 \mu m$.

The proportional gain K in Equation 5.17 is chosen to be 0.1, the parameter value in deviation compensation control input is chosen to be 100. e.g, the minimum adjustment in the vertical direction is $0.2 \mu m$. The parameters m and α are the same as those in Chapter 4.

Figure 5.12. *The force-deformation profile for one zebra fish embryo at Zygote stage.*

5.4.2 Experiment 2: Deformation Force Profiles Comparison

In order to test the repeatability of the proposed controller, The piezoelectric stage is excited by a known input to deform the cell biomembrane after the micro-force sensor is brought to the contact point. The positioning of the micro-force sensor and the deformation of cell biomembrane is repeated 10 times consecutively for each zebra fish embryo at its Zygote stage. The differences in their force profiles (see Figure 5.12) demonstrate whether the sensor has reached to the desired contact point (Lu *et al.* 2007, Huang *et al.* 2009), because the force profiles change sharply at different contact points.

The 10 average values of each force profile for each cell are computed (see Figure 5.13). The mean values and standard deviations of the 10 average values are shown in Table 5.3.

Figure 5.13. *The average value for each force profile is very close to each other, which implies that the indentation point for each deformation is also very close to each other.*

Table 5.3. *The mean and standard deviation of the 10 average values for 5 zebra fish embryos.*

Experiment set	mean (μN)	Standard deviation
1	106.35	1.85
2	107.82	1.76
3	108.37	1.75
4	107.85	1.82
5	106.79	1.79

5.5 Discussion

5.5.1 Deviations comparison

The Δx and Δy values in Table 5.2 indicate that the proposed image-based positional controller is suitable for static position control in vision-guided cell micro-manipulation. The PID-type controller makes itself easily implement in real-time applications.

Comparing Δz values in Table 5.2 and Table 5.1, the vertical deviation has

been reduced up to 50%, where $\Delta z = 1.11\mu m < \eta = 2\mu m$ demonstrates the feasibility of the image-based deviation compensation controller.

It is worth to point out η cannot be chosen arbitrarily small due to the hardware constraint, each optical objective has its depth of field where the images in the camera won't change significantly to obtain the STD difference.

Due to the deviation compensation process involved, which is an iterative adjustment method for the micro-tip, the time spent on the position control increases. One possible way of shortening the time is to perform the deviation compensation only when the micro-tip approaches the target, which is only applicable when the target cell is stationary, e.g., the value $[r_t \ c_t]^T$ is constant, otherwise the vertical deviation might be accumulated as shown in Figure 5.2 resulting in even longer compensation time.

5.5.2 Deformation Force Profiles Comparison

The standard deviations in Table 5.3 shows the high repeatability of the proposed controller. Compare with the cell penetration controller in (Lu *et al.* 2007, Huang *et al.* 2009), where another closed-loop force controller is needed to determine whether the contact point is the suitable penetration point, the proposed controller can guarantee that the real contact point is the desired position. No additional checking procedure is required, which makes it cost-effective since a micro-force sensor is not required to equipped with the micropipette in cell microinjection.

Incorporating a force control in cell microinjection helps the users to determine whether the microinjection is successful or not (Lu *et al.* 2007, Huang *et al.* 2009) through the penetration force profiles. However, the force profiles only is not enough to tell whether the micropipette is above or below the penetration point in a failure experiment unless the vision feedback is analyzed, which is the role that the deviation compensation controller plays. Thus, the proposed position controller

with deviation compensation is suitable for those cell micromanipulations where the micro-tip must reach the indentation point accurately.

5.6 Conclusion

A static image-based position controller with depth deviation compensation capability is proposed in this chapter. The experiments results demonstrate its feasibility and advantages in cell micromanipulation, where the changes in depth is not tolerated.

The closed-loop system of the piezoelectric stage is employed in the static position with sampling period greater than 40 *ms*. However, in cell mechanobiology, for example, deforming the cell following a 2 Hz sinusoidal force profiles sampled at 40 Hz (25 *ms* sampling period), the closed-loop piezoelectric stage will fail. An open-loop system of piezoelectric stage is required. The major limitation of the open-loop system is its hysteresis problem, which demands a method to model the hysteresis.

Chapter 6

Dynamic Motion Tracking of Piezoelectric Micromanipulator

6.1 Introduction

In cell mechanobiology, controlling the indentation force on the cell biomembrane is realized indirectly via position control of the piezoelectric micromanipulator and understanding the relationship between the contact forces and the corresponding deformation (Sun *et al.* 2003, Kim *et al.* 2009). Thus, to stress the biomembrane according to certain force profiles in cell mechanobiology requires the dynamic motion tracking control of the micromanipulator.

Piezoelectric actuator (see Figure 6.1) is preferred over conventional stepper motors in micro- and nano- positioning systems due to its inherent ultrafine resolution (unlimited resolution theoretically) of piezoelectric ceramic, fast response, high force generation, no magnetic fields generated and low power consumption (PI 2009). A micromanipulator driven by piezoelectric actuators is often employed in cell micromanipulation systems (Tan and Ng 2001).

Motion tracking of a micromanipulator driven by piezoelectric actuators de-

mands the modeling of the actuators and the tracking control strategy selection. A number of modeling methods (Smith and Hom 2000, Goldfarb and Celanovic 1997, Adriaens *et al.* 2000, Janocha and Kuhnen 2000) and control strategies (Yi and Veillette 2005, Shieh *et al.* 2004, Ang *et al.* 2007, Xu and Abidi 2008, Liaw and Shirinzadeh 2008a, Liaw and Shirinzadeh 2008c) for piezoelectric actuators have been investigated.

The dynamics of piezoelectric actuators is governed by a set of linear piezoelectricity equations when the driving voltage has relatively lower frequency and amplitude (Zhong and Yao 2008). However, the nonlinear multi-path hysteresis of the piezoelectric materials causes severe positioning errors as the frequency of the driving voltage increases (Tan *et al.* 2009).

Various closed-loop controllers such as PID controllers are explored to compensate the hysteretic effect. However, due to the phase lag in such controllers as well as the noise introduced in the servo-electronics of the position sensor (PI 2009), the open-loop controller stands as a superior approach in terms of real-time active noise or vibration compensation (Ang *et al.* 2007), although a closed-loop feedback controller may have a better tracking accuracy.

The hysteresis of the piezoelectric actuator is modeled by means of position sensors with high-accuracy and bandwidth such as interferometers (non-contact position sensor) (see Figure 6.2(a)) and capacitive sensors (contact position sensor) (see Figure 6.2(b)). The conventional way of hysteresis modeling of the piezoelectric actuator is demonstrated in Figure 6.3: a known input is applied to the piezoelectric actuator, the displacements measured by either an interferometer or a capacitive sensor are utilized for the model identification (Ang *et al.* 2007, Yu *et al.* 2002). The whole process is often conducted off line.

However, in order to apply these methods for the piezoelectric micromanipulator in cell micromanipulation systems, it is required that the micro-tip (see Figure 6.4) mounted on the micromanipulator must be smooth enough to provide

Figure 6.1. (a) A structure diagram of a stacked piezoelectric actuator ; (b) A stacked piezoelectric actuator (Physik Instruments (PI) GmbH & Co. KG).

mirror-like reflection to the incoming beam if an interferometer is employed as the position sensor or certain space is needed to place the capacitive sensor if it is deployed as the position sensor. The rigid requirements of these positions sensors are hard to be satisfied if the displacement measurement is to be made on the end-effector (e.g., the micro-tip) mounted on the micromanipulator.

The other reasons preventing the conventional way being employed are: (1) Hysteresis of a piezoelectric actuator is momentum-dependent, which means that the hysteretic behavior changes as the load acting on the piezoelectric actuators or the driving frequency of the input voltage changes as shown in Figures 6.5-6.7; e.g., the hysteresis increases with the rate of the control input or the load; (2) The coupling mechanism (flexure) as shown in Figure 6.8 has its own non-linearity, which contributes to the overall hysteresis of the micromanipulator.

It is necessary to model the micromanipulator instead of using the model of the piezoelectric actuator. The hysteresis modeling of the micromanipulator has to be re-conducted whenever the load or the driving frequency of the input voltage changes unless a rate-and-load dependent hysteresis model is available.

Figure 6.2. (a) *Hysteresis modeling using interferometers requires that the micro-tip must be able reflect the incoming beam to the receiver; (b) Hysteresis modeling using capacitive sensors needs certain space to mount the sensors. PEA: piezoelectric actuator.*

Figure 6.3. *The conventional way of hysteresis modeling with the help of an interferometer or an capacitive sensor. A known input is applied to the piezoelectric actuator, the displacements measured by either an interferometer or a capacitive sensor are utilized for the model identification.*

Most commercial systems provide closed-loop displacement control using internal mounted sensors, for example, strain gauge to deduce the displacement. This strategy is excellent for static position control. However, for dynamic trajectory-tracking applications, there are two major limitations: (1) the phase lag; (2) the longer response time as shown in Figure 6.9, which demands a feedforward controller.

In order to obtain a feedforward controller for dynamic motion tracking of the piezoelectric micromanipulator, the hysteresis of the micromanipulator is ideally

Figure 6.4. *Three types of micromanipulator. (a) Microgripper (Femtotools GmbH); (b) Micro-force sensor (Femtotools GmbH); (c) Microinjector (Eppendorf).*

Figure 6.5. *The responses of a piezoelectric stage at two different driving frequencies. The hysteresis loop becomes larger at higher driving frequency due to the structural vibration.*

modeled on the fly whenever the driving frequency of the input voltage, or the load acting on manipulator changes. Hence, the objectives of this chapter are to provide an automatic hysteresis modeling and compensation method such that:

- Hysteresis modeling can be conducted on the fly in real time to cope with rate-and-load changes;
- The micro-tip mounted on the micromanipualtor can be any shape ideally;
- The displacement measurement of the micromanipulator can be obtained from the camera instead of interferometers or capacitive sensors.

Figure 6.6. *The responses of a piezoelectric stage at different loads. (a) There is no external load acting on the micromanipulator besides the sensor and the connector; (b) An external load (100 gram) is acting on the stage.*

Automatic hysteresis modeling and compensation of a piezoelectric micromanipulator using Prandtl-Ishlinskii (PI) model in vision-guided cell micromanipulation system is proposed and implemented in this thesis. A camera acts as the displacement sensor to replace interferometers or capacitive sensors.

6.2 Hysteresis Modeling and Compensation

It is believed that there exist a small percent of wrongly oriented polarized unit cells even after the piezoelectric ceramics undergo poling process in a strong electric field (see Figure 6.10). This group of cells causes hysteresis at various electric fields in many applications. Many strategies have been developed to model and compensate hysteresis, which can be classified as follows:

1. Microscopic theories
2. Electromechanical model
3. Phenomenological models

Figure 6.7. *The responses of a piezoelectric stage at two different loading conditions. The hysteric loop becomes larger at higher load due to the motion momentum.*

6.2.1 Microscopic Theory

Understanding the process of making piezoelectric ceramics facilitates the modeling and compensation of hysteresis. Before polarization, the cubic cells as shown in Figure 6.10 have symmetric structure, which become asymmetric at temperatures below the Curie temperature. Individual asymmetric crystal cell unit exhibits piezo effect. No macroscopic piezo effect exists in the ceramics due to the random distribution of orientations of the individual asymmetric crystal cells unless a poling process is performed where a strong electric field is applied to permanently align the cells. The poled ceramics exhibits macroscopic polarization or called piezo effect when an electric potential is applied.

However, it is recognized that some crystal cells are not properly aligned during the poling process. This group of cells exhibits "switching effects" under electric potential, which causes the presence of hysteresis in piezoelectric materials. The study of the behaviors of the crystal cells is called microscopic theory (Landauer *et al.* 1956).

Figure 6.8. (a) A structure diagram of a piezoelectric stage with serial kinematics flexure ; (b) A XYZ piezoelectric stage with serial kinematics flexure (Physik Instruments (PI) GmbH & Co. KG).

Smith and Hom points out that a ceramic crystal is a lattice of cells, where each cell has electric polarization. The domain wall is formed by the collections of neighboring cells which have the same polarization. The electric-mechanical behavior of the domain wall with external electric field as well as the interactions between cells in the domain are modeled. Combining with macroscopic constitutive relations and phase transitions, only five parameters are required to be estimated for a given material (Smith and Hom 2000).

Smith also proposes a rate-dependent hysteresis model for low frequencies applications, where hysteresis is shown to be rate dependent even at low frequencies. The proposed hysteresis model in this reference is derived primarily from the probability analysis of how the crystal cell changes its orientation due to the applied electric field (Smith *et al.* 2000).

Physical hysteresis models of piezoelectric material are obtained in microscopic theory, which characterize the material property at a low level. These models are suitable for real-time applications and able to accommodate the changing conditions in real operations. However, firstly, in the modern fabrication of piezoelectric actuators and stages where the ceramics are interacting with other mechanism,

Figure 6.9. The response time of a piezoelectric stage under open-loop and closed-loop control.

it is a challenging task to measure the polarization. Secondly, the models identified using microscopic theory are mainly for low-frequency applications but not for dynamic tracking applications, where high-frequency components might exist.

6.2.2 Electromechanical Model

Most mechanical systems can be explained as a mass-spring-damper system. The electromechanical model of a piezoelectric actuator as shown in Figure 6.11 based on Generalized Maxwell Slip theory is extensively investigated (Goldfarb and Celanovic 1997, Adriaens *et al.* 2000). This model consists of six equations:

$$V_{in} = V_h + V_z \quad (6.1)$$

$$V_h = H(q) \quad (6.2)$$

$$q = CV_z + q_z \quad (6.3)$$

$$q_z = T_{em}x \quad (6.4)$$

$$f_z = T_{em}V_z \quad (6.5)$$

$$m_z\ddot{x} + b_z\dot{x} + k_zx = f_z - f_{ext} \quad (6.6)$$

where V_{in} , V_h and V_z represent the total applied input voltage, voltage excited on the capacitor, and voltage caused by the hysteresis, respectively. The parameters

Figure 6.10. (a) A crystal unit cell of PZT ceramic in symmetric state has no piezo effect; (b) A polarized unit cell of PZT has piezo effect.

q and q_z are the total charge in the ceramic, and charge related to the capacitor C , which is parallel to the electromechanical transformer with ratio T_{em} , respectively. The parameter H is the hysteretic operator. The parameters f_z and f_{ext} are the transduced force from the ceramic, and external force excited on the ceramic, respectively. The parameters m_z , k_z and b_z are mass, spring, stiffness, respectively. The parameter x is the displacement of the ceramic in the desired direction.

A sliding mode controller with a dynamic compensator is proposed to perform dynamic motion tracking of the piezoelectric actuation system (Liaw *et al.* 2007), where the hysteresis is treated purely as an external disturbance, regardless its rate-depended behavior. An adaptive tracking controller using only displacement measurements is formulated (Shieh *et al.* 2004), where a friction model is employed to produce the hysteresis function.

Figure 6.11. *Electromechanical model of a piezoelectric actuator; The left side is an electric model; The right side is a mechanical model and the middle rectangle describes the relationships between the two models.*

6.2.3 Phenomenological Model

Microscopic theory and electromechanical model try to understand the behavior of piezoelectric ceramics from the material formation, and a mass-spring-damper system characteristics, respectively. The hysteretic loops in many piezoelectric actuators demonstrate that there is a special pattern inside the loop. Phenomenological modeling of hysteresis by means of so-called relay hysterons, which extended to hysteresis operators in 1970s, started as early as in 1938 by P. Preisach. In the discrete space, the Preisach model is a summation of many parallel hysterons as shown in Figure 6.12. The mathematical representation of the Preisach model in the continuous space is:

$$y(t) = \int \int_{\alpha < \beta} \mu(\alpha, \beta) R_{\alpha\beta}[\mu(t)] d\alpha d\beta \quad (6.7)$$

where α and β are the ON-OFF switching values in Figure 6.12(a), $R_{\alpha\beta}$ is the transfer function in the hysterons with an output either 0 or 1, $\mu(\alpha, \beta)$ is the weight and y is the output. The discrete Preisach model described in Equation 6.8 is obtained by summing the outputs from the $\alpha - \beta$ domain where $\alpha < \beta$ as shown in Figure 6.13(a). The accuracy of the Preisach model is dependent on the number

Figure 6.12. (a) The ON-OFF hysteron is an elementary component of the Preisach model ; (b) The Preisach model is a summation of many parallel hysterons.

of hysterons as shown in Figure 6.13(b) as well as the distribution of α and β .

$$y = \sum_{i=1}^N \mu(\alpha_i, \beta_i) R_{\alpha_i\beta_i}[x] \quad (6.8)$$

where x and y are the input and output, respectively.

Hu and Mrad discover that the classical Preisach model for piezoelectric actuators is only accurate when the frequency and the load are limited to a narrow band (Hu and Mrad 2003). Yu presents a modified Preisach model to describe the hysteresis and a numerical implementation method (Yu *et al.* 2002). Bernard introduces the magnetic flux density and its rate as inputs in the dynamic Preisach-based hysteresis model, where the inverse model is easily identified due to the odd symmetry of the Preisach model (Bernard *et al.* 2002). Song integrates a PD/lead-lag feedback controller using a classic inverse Preisach model in the feedback controller to achieve displacement tracking (Song *et al.* 2005). Ge and Jouaneh employs a PID controller in the closed-loop system (Ge and Jouaneh 1996).

Ang explains the challenging of classical Preisach model and implements the Prandtl-Ishlinshii (PI) model, which is a subclass of the Preisach model (Ang *et al.* 2007). The PI operator is computational efficient and the inverse model can

Figure 6.13. (a) The $\alpha - \beta$ plane of the Preisach model with 45 hysterons connected in parallel; (b) The accuracy of the Preisach model is dependent on the number of hysterons as well as the distribution of α and β .

be computed analytically, which is quite suitable for on-line real-time micromanipulation. Kuhunen carries out extensive studies on the employment of PI model for hysteresis modeling and compensation (Krejci and Kuhnen 2001, Janocha and Kuhnen 2000, Kuhnen and Janocha 2001, Kuhnen 2003, ?). The detail about the employment of PI model is given in the following section.

A non-contact displacement sensor, for example, an impedance transducer or interferometer, with specific spacial configuration with the piezoelectric actuator is usually required in the existing hysteresis modeling methods. The front moving surface of the piezoelectric actuator must be flat enough to work with the displacement sensor. Hysteresis in a piezoelectric micromanipulator is frequency and load dependent, unless a rate-and-load dependent hysteresis model is proposed, the modeling process must be re-conducted when the working frequency or load changes.

The hysteresis in piezoelectric actuators exists between the input voltage and the output displacement, but not in charge and displacement (Yi and Veillette 2005). However, the limitation of using charge control is the special requirement

of charge measuring and excitation equipments, which makes it less attractive for commercial systems.

The author proposes an automatic hysteresis modeling and compensation method using vision-feedback. This method can be implemented on the fly in real time. It eliminates the critical requirements of using an interferometer or a capacitive position sensor.

6.3 Automatic Hysteresis Modeling using Prandtl-Ishlinskii (PI) Operators

6.3.1 Hysteresis Modeling by Modified PI operator

An elementary PI operator is defined as:

$$\begin{aligned} y(t) &= H_r[x, y_0](t) \\ &= \max\{x(t) - r, \min\{x(t) + r, y(t - T)\}\} \end{aligned} \quad (6.9)$$

where x and y are the control input and output, respectively, r is the control input threshold value and T is the sampling period. Equation 6.9 is a recursive equation with its initial value $y(0)$ defined as:

$$y(0) = \max\{x(0) - r, \min\{x(0) + r, y_0\}\} \quad (6.10)$$

where $y_0 \in \Re$ is initialized to 0 if the system starts in its deenergized state. The control input threshold value r in Equation 6.9 determines the hysteretic curve width as shown in Figure 6.14(a). To control the slopes of rising (and decreasing) lines in the hysteretic loop as shown in Figure 6.14(b), a weight w_h is multiplied with the elementary PI operator to obtain the generalized PI operator:

$$y(t) = w_h H_r[x, y_0](t) \quad (6.11)$$

PI hysteresis model is obtained by summing many generalized PI operators with

Figure 6.14. *The behavior of the PI hysteresis operator depends on r and w values. (a) The hysteretic loop becomes wider with larger r value; (b) The slope of the rising (and decreasing) line becomes deeper with larger w value.*

different r and w_h values:

$$y(t) = \vec{w}_h^T \vec{H}_r[x, \vec{y}_0](t) \quad (6.12)$$

where $\vec{w}_h^T = [w_{h0} \dots w_{hn}]$ and $\vec{H}_r[x, \vec{y}_0](t) = [H_{r0}[x, y_{00}](t) \dots H_{rn}[x, y_{0n}](t)]^T$, the threshold vector $\vec{r} = [r_0 \dots r_n]^T$ with $0 = r_0 < \dots < r_n$ and $\vec{y}_0 = [y_{00} \dots y_{0n}]^T$. The parameter n is the number of the generalized PI operators. If the actuator starts in its deenergized state (e.g., $\vec{y}_0 = \vec{0}_{n \times 1}$), the output would be 0 when $t = 0$ as shown in Figure 6.15.

A few observations can be made from Equation 6.12 and Figure 6.15:

1. The number of PI operators n determines the smoothness of the hysteretic curve, which behaves similarly as the classic Preisach model but the number of PI operators needed is much smaller than that of hysterons.
2. The hysteresis curve is characterized by the initial loading curve. Assume $y(0) = 0$, $\vec{r} = [r_0 \ r_1 \ \dots \ r_i]^T$ and $\vec{w} = [w_{h0} \ w_{h1} \ \dots \ w_{hi}]^T$, the following deriva-

Figure 6.15. The initial loading curve and hysteretic loop of the PI hysteresis model with $n=6$. The output won't return to zero when hysteresis is present. The hysteresis model is characterized by the initial loading curve.

tion holds

$$y(x) = y_0(x) + y_1(x) + \dots + y_i(x) \quad (6.13)$$

$$= w_{h0} * (x - r_0), \quad \text{if } r_j < r_{j+1} \text{ and } r_0 \leq x < r_1; \quad j = 0 \dots i - 1$$

Expands the above equation to obtain the general equation for the initial loading curve as well as its slope.

$$y(x) = \sum_{j=0}^i w_{hj}(x - r_j), \quad r_i \leq x \leq r_{i+1} \quad (6.14)$$

$$W_{hi} = \frac{d}{dx}y(x) = \sum_{j=0}^i w_{hj} \quad (6.15)$$

3. The hysteretic curve does not return to the starting point in the initial loading curve, but the shape repeats in the subsequent loops.
4. The hysteretic loop is symmetric after the initial loading curve. Since the width of a hysteresis operator is $2r$ as shown in Figure 6.14, the maximum value of the backlash operator r in Equation 6.12 is half of the maximum control input.

5. The hysteresis in a real system might not be symmetric, so it is necessary to incorporate a transformation of Equation 6.12 to suit the non-symmetric curve.

There are two ways of choosing the value for each threshold of r in Equation 6.14, namely linear and importance-sampling. For example, when $n = 10$ and $x_{max} = 5$, where x_{max} is the maximum control input. The threshold r is determined by:

Linear sampling

The input threshold vector r is uniquely determined by sampling the range $0 \sim x_{max}$ with equal intervals, e.g. sample $0 \sim x_{max}$ equally with 10 numbers, $r = [0 \ 0.2778 \ 0.5556 \ 0.8333 \ 1.1111 \ 1.3889 \ 1.6667 \ 1.9444 \ 2.2222 \ 2.5000]^T$.

Importance-sampling

When n is relatively small, the importance-sampling is preferred over linear sampling because more samples are dedicated to describe the turning point area, e.g., $r = [0 \ 0.1 \ 0.2 \ 0.3 \ 0.5 \ 0.8 \ 1.2 \ 1.8 \ 2.2 \ 2.5]^T$, where the slope changes more frequently comparing to the changes of the slope of the curve further away from the turning points as shown in Figure 6.15. The difference of the accuracy of the curve fitting diminishes as n increases, but the computational time to identify the weights increases.

The hysteretic curve described using the linear weighted superposition of general PI operators is always symmetrical due to the inherited symmetry property of the elementary PI operator as shown in Figure 6.14. However, the hysteretic loops of most real actuators are not symmetric. In order to solve the symmetrical problem in the PI hysteresis model, a general saturation operator which is a linearly weighted superposition of elementary dead-zone operators as shown in Figure 6.16

Figure 6.16. (a) One-sided dead-zone operator is characterized by the threshold d and the weight w_s . (b) A saturation operator with 3 deadzone operators, e.g., $p = 2$. The slope of the piecewise linear curve at interval i is the sum of the weights up to i , which is similar as the PI hysteresis model.

is introduced. They are defined as:

$$S_d[y](t) = \begin{cases} \max\{y(t) - d, 0\}, & d > 0 \\ y(t), & d = 0 \end{cases} \quad (6.16)$$

$$z(t) = \vec{w}_s^T \vec{S}_d[y](t) \quad (6.17)$$

where y and z are the output from the PI model and the actuator, respectively. The parameter p is the number of deadzone operators. The vectors $\vec{w}_s^T = [w_{s0} \dots w_{sp}]$ and $\vec{S}_d[y](t) = [S_{d0}[y](t) \dots S_{dp}[y](t)]^T$ are the weight vector and the corresponding one-sided dead-zone operators with $\vec{d} = [d_0 \dots d_p]^T$, respectively. As shown in Figure 6.17, a general saturation operator can alter the shape of the hysteretic curve to better model a real hysteretic loop. Since the control input to the saturation operator is the displacement of the micromanipulator and always in the positive domain, it is required that $0 = d_0 < d_1 < \dots < d_p$.

The modified PI model is defined as

$$z(t) = \Gamma[x][t] = \vec{w}_s^T \vec{S}_d[\vec{w}_h^T \vec{H}_r[x, y_0]](t) \quad (6.18)$$

Figure 6.17. The result of a modified PI hysteresis model. The original PI hysteretic loop is symmetric, which is not true in real applications. The saturation operator makes the loop non-symmetric.

6.3.2 Inverse Feedforward Controller

The inverse feedforward controller as shown in Figure 6.18 and 6.19, is derived as:

$$z(t) = \Gamma[\Gamma^{-1}[\hat{z}]](t) = I[\hat{z}](t) = \hat{z}(t) \quad (6.19)$$

where $\hat{z}(t)$ and $z(t)$ are the desired and actual system output, respectively, Γ^{-1} is the inverse modified model, I is the linearized model.

The inverse of the modified PI model is the reflection of the hysteretic loops about the 45° line as shown in Figure 6.20. The parameters can be written as:

$$\Gamma^{-1}[\hat{z}](t) = \vec{w}_h'^T \vec{H}_{r'} [\vec{w}_s'^T \vec{S}_{d'} [\hat{z}], \vec{y}_0'](t) \quad (6.20)$$

Figure 6.18. *The linearization of the modified PI model. Given a desired periodic actuator output $\hat{z}(t)$, the inverse modified PI model Γ^{-1} computes the real control input to the micromanipulator, which produces the output $z(t)$ from the hysteresis model Γ . The vectors $\vec{y}_0'$ and $\vec{y}_0$ are the initial values of the inverse and the PI model, respectively.*

where the parameters in the inverse model can be computed as follows:

$$\begin{aligned}
 w'_{h0} &= \frac{1}{w_{h0}} \\
 w'_{hi} &= \frac{-w_{hi}}{\sum_{j=0}^i w_{hj} \sum_{j=0}^{i-1} w_{hj}} \\
 r'_i &= \sum_{j=0}^i w_{hj} (r_i - r_j) \\
 y'_{0i} &= \sum_{j=0}^i w_{hj} y_{0i} + \sum_{j=i+1}^n w_{hj} y_{0i}, \quad i = 1 \dots n
 \end{aligned} \tag{6.21}$$

$$\begin{aligned}
 w'_{s0} &= \frac{1}{w_{s0}} \\
 w'_{si} &= \frac{-w_{si}}{\sum_{j=0}^i w_{sj} \sum_{j=0}^{i-1} w_{sj}} \\
 d'_i &= \sum_{j=0}^i w_{sj} (d_i - d_j), \quad i = 1 \dots p
 \end{aligned} \tag{6.22}$$

6.3.3 Vision-based Parameters Identification

Identifying the modified PI model involves three tasks as shown in Figure 6.21. In the first step, a suitable discrete control input which is the desired output of the system, is fed into the piezoelectric stage. The images associated with each

Figure 6.19. The feedforward controller of the piezoelectric micromanipulator. The desired output $\hat{z}(t)$ is specified by the user. The actual input $x(t)$ is computed using inverse of the modified PI operator. The real output $z(t)$ is expected to resemble $\hat{z}(t)$.

discrete input are captured and processed to obtain the real output in the second step. The control input and the computed output are employed to identify the necessary parameters using a least squares minimization method.

A novel method based on Hough Transform that extracts the line of the micro-tip is implemented, where a micro-force sensor (see Figure 6.4(b)) is mounted on the micromanipulator. The position of the sensor can be determined in the following ways: (1) the vertical line located on the extracted edges as shown in Figure 6.22(b); (2) the intersection of two edges (lines) as shown in Figure 6.22(d).

Locating the front line as shown in Figure 6.22(b) gives the absolute position of the sensor, e.g., the x coordinate of the middle point on the line can represent the position of the sensor if the camera and the sensor is carefully aligned and the vision system is calibrated. This method is computationally less expensive and suitable for the cases where the front line can be uniquely determined. This method will fail if the front line is not unique, for example, some particles contaminate the

Figure 6.20. The inverse of the modified PI operator Γ^{-1} , which is the reflection of the hysteresis model Γ about the 45° line.

Figure 6.21. The main procedures for PI model identification.

micro-force sensor.

An alternative method is to compute the intersection of two non-parallel lines as shown in Figure 6.22(c) & (d), which gives a relative position of the sensor. Although this method is computationally more expensive due to the intersection point extraction, it is less likely affected by particles appearing on the edges, for example, because the noisy sections on the two lines can be removed without affecting the line detection.

The above position tracking methods are only applicable when the piezoelectric stage moves in the x - and y - direction as shown in Figure 6.23, because the vertical position z is assumed to be constant. The identification of the hysteresis

Figure 6.22. The position of the micro-force sensor is obtained using Hough transform. (a) the original image and the desired edges; (b) the detected edges; (c) the important edges; (d) the intersection of the two important edges.

model in z - direction will be explained in more details in the discussion section.

By sending a known control input at the desired working frequency of the micromanipulation, the corresponding positions of the micro-force sensor are extracted. With suitable choices of the order of PI operator (n) and the saturation operator (p) in Equation 6.18, and the corresponding initial values of the threshold vector $\vec{r}$ and $\vec{d}$, the weight vector $\vec{w}_h$ and $\vec{w}_s$ are identified using a least-squares fit of the following error function, which is the difference of the real and desired

Figure 6.23. *The xy movement of the micro-tip. It is assumed that there is no vertical movement.*

output.

$$E[x, z](\vec{w}_h, \vec{w}_s, t) = \vec{w}_s^T \vec{S}_d [\vec{w}_h^T \vec{H}_r[x, \vec{y}_0]](t) - z(t) \quad (6.23)$$

The inverse modified PI model is found using Equation 6.21 and 6.22. The linearized model of the piezoelectric stage in x (or y) direction is characterized by these parameters $\vec{r}$, $\vec{d}$, $\vec{w}_h$, $\vec{w}_s$, $\vec{y}_0$, $\vec{r}'$, $\vec{d}'$, $\vec{w}_h'$, $\vec{w}_s'$ and $\vec{y}_0'$.

6.4 Experiments

6.4.1 Experimental Setup

A two-loop programming hierarchy is implemented for the hysteresis model identification, where one loop works in real time for the input signal (voltage) excitation and output signal (image) capturing, the other loop is for the image processing and model identification (least-squares fitting). The two loops are both implemented in the real-time PC (RT Labview) as shown in Figure 6.24. For the motion tracking experiment, one more loop is added for the verification purpose as shown in Figure 6.25

Figure 6.24. *Real-time implementation of hysteresis modeling and compensation (AO: analog output). Input signal excitation and output image capturing are performed in the real-time loop. Feature extraction and model identification are implemented in the non real-time loop.*

The micromanipulator is a 3-DOF XYZ piezoelectric stage (P-611.3S, PI Co, Germany), on which a micro-force sensor (FT-S270, Femtotoools GmbH c/o, Switzerland) is mounted. The frame rate of the camera is 210 fps. In the experiment, the sampling rate is set at 40 Hz.

The input signal used for the hysteresis model identification is a 1 Hz sinusoidal signal with a $p - p$ value of 4 Volt as shown in Figure 6.26. The output from the image processing algorithm is converted to voltage in order to perform the least-squares optimization. Dynamic motion tracking is performed using a 1 Hz sinusoidal signal immediately after the model identification.

Figure 6.25. After the model is identified, the Model Identification block is replaced by a Model Verification block, where an input signal with the same frequency as that in the identification step is excited. The real output is collected.

6.4.2 Experiment Result

Model Identification

The hysteretic curves and the fitted model using linear sampling and importance-sampling for $n = 10$ are shown in Figure 6.27. The corresponding linearized models are shown in Figure 6.28.

The same procedure for the model identification is repeated with $n = 20$. The linearized models for linear and importance-sampling are shown in Figure 6.29.

Figure 6.26. *The input signal for the hysteresis modeling. The sampling frequency is 40 Hz with p-p value 4 Volt in the periodic motion. There is a 10x amplifier in the piezoelectric micromanipulator controller so that the real input to the piezoelectric micromanipulator is 40 Volt (p-p).*

Dynamic Motion Trajectory Tracking

The dynamic trajectory tracking using the inverse feedforward controller is conducted for $n = 10$ and $n = 20$. The tracking performance using linear and importance-sampling for $n = 10$ is shown in Figure 6.30 and 6.31. The performance for $n = 20$ is shown in Figure 6.32 and 6.33. The tracking rmse reduction is shown in Table 6.1.

6.5 Discussion

6.5.1 The Accuracy of the Dynamic Motion Tracking

The rmse and its reduction using the importance-sampling when $n = 10$ or $n = 20$ indicate that the inverse feedforward dynamic motion tracking controller based on PI hysteresis model using vision feedback is suitable for microscopy applications,

Figure 6.27. *Hysteresis model identification when $n = 10$. (a) Linear sampling; (b) Importance-sampling. Both the linear and importance-sampling methods fit the hysteretic curve with fitting error less than 2%.*

as long as the micro-tip can be tracked in the images. The requirement of employing position sensors such as interferometers or capacitive sensors in the system is eliminated resulting that the identification can be done on the fly in real time for the loaded micromanipulator.

The rmse (%) is a slightly bigger than that using interferometers (Ang *et al.* 2007), which is due to the resolution limitation of the vision system. The rmse is around $0.80 \mu m$ when the displacement-to-pixel ratio is $0.79 \mu m$, thus it is expected that rmse can be further reduced if the displacement-to-pixel ratio becomes smaller, which can be achieved by (1) smaller pixel size of the camera and (2) large magnification the optical objective.

Hysteresis modeling is conducted on the fly, making the feedforward control suitable for a range of frequencies as well as different loads. The maximum sampling frequency of the camera is around 200 Hz, ensuring that the motion tracking frequency can be around 100 Hz ideally according to Nyquist criteria. Practically, it can achieve 20 Hz due to the overhead of data communications. It should be noted that the maximum sampling frequency can only be achieved when the image

Figure 6.28. The linearized models for (a) linear and (b) importance-sampling methods when $n = 10$. Although both and linear and importance-sampling methods provide acceptable curve fitting results, the linearized models are quite different.

Table 6.1. Experimental results of the inverse feedforward motion trajectory tracking control using linear and importance-sampling with different number of PI hysteresis operators.

	$n = 10$			$n = 20$		
	Without	Linear	Importance	Without	Linear	Importance
rmse (μm)	6.12	2.86	1.27	6.33	1.17	0.80
$\frac{\text{rmse}}{\text{p-p amplitude}}$ (%)	15.30	7.15	3.18	15.83	2.92	2.0
max error (μm)	12.00	3.80	2.24	12.08	2.23	1.09
$\frac{\text{max error}}{\text{p-p amplitude}}$ (%)	30.0	9.50	5.60	30.20	5.58	2.73

processing is carried out off line because feature extracting is a time-consuming task.

Figure 6.29. *The linearized models for linear and importance-sampling methods when $n = 20$. The difference in terms of linearization of the linear and importance-sampling diminishes as n increases.*

6.5.2 The Effect of PI Operators

The number of PI operators and the way of sampling the input threshold r are crucial in the modeling and compensation of hysteresis. Linear sampling of input threshold r has poorer performance when only limited number of PI operators are chosen as shown in Figure 6.30, although the fitting result is comparable with the importance-sampling as shown in Figure 6.27. As shown in Equation 6.14, the number of PI operators determines the slope of the hysteretic curve expecting that more input threshold samples are desired in the turning point neighbors of the curve for better performance.

However, as the number of PI operators increases, the difference between the linear and importance-sampling diminishes because there is enough number of samples to describe the turning point neighbors as shown in Figure 6.29 and 6.33. There is a tradeoff between choosing importance-sampling and larger number of hysteresis operators: additional effort incurred to do importance-sampling in contrast to expensive computational works required for larger number of hysteresis operators.

Figure 6.30. *The performance of the inverse feedforward tracking controller using linear and importance-sampling when $n = 10$. Although both linear and importance-sampling methods provide acceptable curve fitting results, the tracking performance of the importance-sampling is better than that of the linear sampling.*

6.5.3 z -direction Modeling

The proposed image-based tracking algorithm can only provide the x - and y - positions of the micromanipulator. The hysteresis modeling of the piezoelectric stage in z -direction could be performed based on STD model introduced in Chapter 4. However, that method would only work when the stage travels in a small range when it still can be detected by the camera.

A horizontal mounting lens and camera can be equipped so that the z -direction movement with respect to the vertical lens becomes x - (or y -) movement of the horizontal camera. The image tracking algorithm proposed in this chapter is applied. Additional cost and space are needed for the lens and camera.

Figure 6.31. The rmse of linear and importance-sampling when $n = 10$. The rmse of the importance-sampling is nearly half of that of the linear sampling. The mean values for linear and importance-sampling are $2.78 \mu m$ and $1.25 \mu m$, the standard deviations are 0.11 and 0.07 , respectively.

6.5.4 Singularity and Rate-dependent

The modified PI hysteresis modeling method necessitates that the slope of the hysteretic curve (e.g., the sum of the weights at any input) must be positive, which leads to singularity (Tan *et al.* 2009) when the slope of the real hysteresis curve is non-positive. Although the fitting result of the modified hysteresis model may present very attractive results, the singularity problem brings on poor performance. Even a checking procedure which decides the sign of the sum of the weights is implemented to prevent singularity, the accuracy of hysteresis compensation is not guaranteed.

The model proposed is rate-independent, while hysteresis is rate-dependent. Ang proposes and implements a rate-dependent model to model those non-stationary signals, which is suitable for rate-dependent systems (Ang *et al.* 2007). Tan proposes and implements a singularity-free PI hysteresis model by transforming the non-positive slopes to a positive domain (Tan *et al.* 2009). The incorporation of these algorithms will be performed in the future work.

Figure 6.32. The performance of the inverse feedforward tracking controller using linear and importance-sampling when $n = 20$. The tracking capability of the linear sampling improves.

Figure 6.33. The rmse of linear and importance-sampling when $n = 20$. The difference in the rmse of the importance and linear sampling diminishes. The mean values for linear and importance-sampling are $1.17 \mu\text{m}$ and $0.81 \mu\text{m}$, the standard deviations are 0.05 and 0.04 , respectively.

6.6 Conclusion

The rate-and-load dependent property of hysteresis in the piezoelectric micromanipulator desiderates an easy-to-implement hysteresis modeling and compensation method. The conventional way of employing an interferometer in the system is difficult in implementation because of the special requirements. Thus, a vision-based modified PI hysteresis modeling and compensation method is proposed and implemented in this chapter.

In cell mechanobiology, stressing the cell biomembrane is directly linked to the deformation of the biomembrane (Sun *et al.* 2003). Thus, the proposed hysteresis modeling and compensation makes the position control of the piezoelectric stage possible at the desired frequency. Nevertheless, stress the cell biomembrane also requires that the mechanical property model of the membrane is provided, which is investigated in the next chapter.

Chapter 7

Mechanical Force Control on Cell Biomembrane

7.1 Introduction

Eukaryotic cells consist of an enclosing biomembrane (Alberts *et al.* 2002). It is well-accepted that mechanical forces acting on the biomembrane can induce changes in the cell form and functions (Jaasma *et al.* 2007, Kaunas 2008, Wang and Thampatty 2006), that will ultimately affect the respective tissue functionalities (Duncan and Turner 1995, Williams 1998, Mullender *et al.* 2004, Owens 1996).

Control of contact forces through impedance control with kinematic constraints on rigid targets are extensively investigated (Hogan 1985, Colgate 1987, S.P. and Liaw 1996, Takubo *et al.* 2002, Liaw and Shirinzadeh 2008*b*). However, the classical force control in impedance control is challenged by the unknown stiffness of the cell (biomembrane). To make the matter worse, the mechanical property of the cell biomembrane changes at its different developmental stages (Mitchison and Swann 1954). In cell mechanobiology, controlling the indentation force on the cell biomembrane is realized indirectly via position control of the piezoelectric

micromanipulator and understanding the relationship between the contact forces and the corresponding deformation.

The advancement in vision-guided microrobotics or more specifically in micromanipulator, micro-force sensors and imaging technology contributes to the possibility of analyzing individual cells rather than averaging of properties over a population (Tan and Ng 2001, Shim *et al.* 2004). Sun utilizes vision-guided microrobotics to mechanically characterize and quantify the dramatic hardening in the zona pellucida of mouse oocyte immediately after fertilization (Sun *et al.* 2003). A biomembrane point-load model is proposed to describe the measured mechanical forces and cell biomembrane deformations. This model is also applied in zebra fish embryo chorion mechanical property characterization (Kim *et al.* 2004, Kim *et al.* 2005). The work done by Sun and Kim primarily focuses on the measurement and characterization of biomembrane mechanical property, where the control of mechanical forces acting on the biomembrane is not undertaken.

This chapter extends the above works to include automatic control of mechanical forces acting on cell biomembranes, which consists of the following main components: automatic vision-guided position control of the holder and the micro-force sensor (Chapter 4 & 5), dynamic motion tracking of the piezoelectric micromanipulator (Chapter 6), automatic characterization of cell membrane mechanical properties, and lastly automatic control of mechanical forces acting on cell biomembranes. Experiments are performed on various developmental stages of zebra fish embryos. The proposed prototype would benefit biologists in complex analysis of the biological reactions of cells as a result of stimuli from external mechanical forces.

Figure 7.1. *The overall sequence of the automatic control of mechanical forces acting on cell biomembranes.*

7.2 Overall Sequence

The overall sequence consists of nine main modules apart from cell preparation as shown in Figure 7.1. The first six modules are dedicated to vision-guided static position control of the micro-force sensor and holder as mentioned in the previous chapters. The seventh module presents the hysteresis modeling of the piezoelectric stage, which is needed for the dynamic motion tracking control. The eighth module performs the modeling of the measured mechanical forces and deformations of cell biomembranes. The automatic control of mechanical forces acting on cell biomembranes is conducted in the last module.

The automatic vision-guided position control of the micro-force sensor and the holder consists of vision system calibration, autofocusing of the cell, holder and the micro-force sensor, and position control of the micro-force sensor and holder. The template-free vision system calibration method proposed in Chapter 4, which would minimize external biological contamination, is implemented for general time-critical vision-guided microscopy systems. An optimal autofocusing strategy for multiple objects under the microscope is also introduced. The image-based controller discussed in Chapter 5 is implemented to position the micro-force sensor and the holder. The motion tracking controller discussed in Chapter 6 is also implemented.

After the cell is held in place by the holder and the micro-force sensor positioned to the desired location, an automatic cell membrane mechanical property characterization procedure is performed to obtain the mathematical model represented by a third order polynomial. This model is inversed immediately to perform automatic control of mechanical forces acting on the cell biomembrane.

Autofocusing is widely adopted in microscopy systems (Hülßen *et al.* 2004, Sun *et al.* 2004, Santos *et al.* 1997, Vollath 1987, Lin *et al.* 2002, Boddeke *et al.* 1997). Autofocusing of a single object can be conducted by either moving the objective or the target in the vertical direction, provided that the objective (or the target) is mounted on a motorized stage. The best focal plane of the target is obtained from the images' standard deviations (STD) and the objective-to-target distances profile as shown in Figure 4.3.

However, positioning multiple objects into the same focal plane is a challenging task due to: (1) interference in STD from the previously focused images might affect the subsequent objects autofocusing; (2) there are multiple objects excited by independent driving sources with different resolutions, needed to be controlled. In the proposed prototype, it is required that the cell, the micro-force sensor and the holder are positioned into the same focal plane. If the cell is focused improperly, the sensor would indent the cell at an unfavorable position resulting in inconsistency of the experimental results.

An efficient way of positioning the cell, holder and micro-force sensor into the same focal plane is as follows: The cell is first autofocused into the focal plane by moving the optical objective. The holder is manually moved to the field of view of the camera and then positioned to the same focal plane as the cell by controlling the motorized translation stages. The holder is positioned to the cell and negative pressure in the holder is applied to hold the cell. Finally, the micro-force sensor is manually moved to the field of view of the camera and then positioned to the same focal plane by controlling the piezoelectric micromanipulator. Images are decomposed to minimize the image processing time and the interference from the

Figure 7.2. *Autofocusing of multiple objects using image decomposition. (a) The image is decomposed into A1 and A2 to perform holder autofocusing; (b) the image is decomposed into B1 and B2 to perform the micro-force sensor autofocusing.*

previously focused images as shown in Figure 7.2.

7.3 Force and Deformation Modeling

Mechanical property characterization is an important study in the comprehension of the cell functions, commencing as early as in the 1950s (Mitchison and Swann 1954). A few existing bicapsular elastic models are reviewed by Sun (Sun *et al.* 2003). In their work, a biomembrane point-load model is proposed and this model can describe the relationship between mechanical forces and cellular deformations:

$$F = \frac{2\pi Ehw^3}{a^2(1-\nu)} \left[\frac{3 - 4\varsigma^2 + \varsigma^4 + 2\ln\varsigma^2}{(1 - \varsigma^2)(1 - \varsigma^2 + \ln\varsigma^2)^3} \right] \quad (7.1)$$

where $\varsigma = \frac{c}{a}$, F is the measured force from the micro-force sensor, a and w are extracted from the captured images, E , ν and h are the membrane elastic modulus, Poisson ratio and membrane thickness respectively and c is the tip radius of the sensor.

In the zebrafish embryo, the outer embryonic coat called the chorion, a thick acellular envelop of the teleost embryos, composed mainly of proteins and glycoproteins undergoes a process called "softening" which is known to be due to certain protease enzymatic reactions. This softening prepares the embryo for the hatching

in the later developmental stages. The biomembrane point-load model can fairly describe the membrane characteristic of the zebrafish embryo.

Another model describing the injection force and the deformation of the cells in microinjection is proposed by Tan (Tan *et al.* 2008). The cell deformation and the required injection force are expressed in two equations making the model suitable for describing the force-deformation characteristics. However, in the case of real-time systems, there are too many parameters to be estimated before applying the model.

In Equation 7.1, only a , w and F can be measured during the experiments, with the rest of the parameters unknown. The elasticity E can be numerically obtained given some estimations of ν and h . However, because the elasticity and Poisson ratio change at various development stages of the cell, it makes the identification of this model a challenging task.

When the holder size and the sensor radius are fixed (e.g., a and c are constants), ς is a constant. If we assume that deformation w is the distance traversed by the micro-force sensor, the previous mentioned model (Sun *et al.* 2003) can be reduced to a third-order polynomial function by rearranging the parameters as follows:

$$F = \left[\frac{2\pi E h}{a^2(1-\nu)} \frac{3 - 4\varsigma^2 + \varsigma^4 + 2\ln\varsigma^2}{(1-\varsigma^2)(1-\varsigma^2 + \ln\varsigma^2)^3} \right] w^3 \\ \approx p_3 w^3 + p_2 w^2 + p_1 w + p_0 \quad (7.2)$$

where p_3 , p_2 , p_1 and p_0 can be numerically determined by applying a series of input values to the piezoelectric micromanipulator, e.g., a few pairs of deformations of the cell biomembrane and the corresponding measured forces can produce the values for the four coefficients using the polynomial curve fitting method.

The modified model has the following advantages: (1) it is deduced directly from the biomembrane point-load model with the exception of the parameters regrouping, which takes its values from the experimental data, ensuring the accu-

racy of the model; (3) the inverse model can be determined numerically such that the controller design in the subsequent force control phase is less complex, which facilitates the desired real-time force control.

7.4 Automatic Control of Mechanical Forces

The force and deformation modeling provides the relationship between the deformation of the cell biomembrane and the measured applied mechanical forces. The natural subsequent step would be controlling the mechanical forces acting on the cell biomembranes based on desired force profiles. Based on the polynomial model $f(w, F)$ obtained, an open-loop controller is designed based on the inverse model $f^{-1}(w, F)$, which is an inverse function of $f(w, F)$:

$$w_{real} = f^{-1}(w, F) * F_{desired} \quad (7.3)$$

where $F_{desired}$ is the desired force profile and w_{real} is the real position of the micro-force sensor. The sum of squared differences between the measured forces $F_{measure}$ and the desired forces $F_{desired}$ is used to evaluate the polynomial model.

A linearized model proposed in Chapter 6 is used to control the piezoelectric micromanipulator because it is capable of compensating the hysteresis at the desired frequency. The mechanical force control acting on the cell biomembrane is transformed to a dynamic motion trajectory control of the piezoelectric stage.

7.5 Experiments

The objectives of the experiment are as follows: (1) to demonstrate the correctness of the modified modeling method of the relationship between the measured mechanical forces and cell biomembranes deformations; (2) to explore the capability of the automatic control of the mechanical forces acting on cell biomembranes.

7.5.1 Materials and Methods

The experiments are conducted on the early developmental stages of zebra fish embryos from the Zygote period to Oblong phase of the Blastula period (Kimmel *et al.* 1995). Three embryos are experimented. Five data samples are collected from each phases, and only two phases of each embryo are considered. Thus, ten sets of data are collected from each embryo.

The procedures described in Figure 7.1 are followed. In order to minimize the exposure of the embryo to the light source of the microscope, the total time spent on each phase is restricted to within five minutes.

7.5.2 Experiment Result

The coefficients in Equation 7.2 for the modified point-load model are shown in Table 7.1. The error in the last column represents fitting error of the polynomial. The last row in the table represents the average model in each phase as shown in Figure 7.3. The average models from all phases are shown in Figure 7.4.

A sinusoidal force profile is fed in as the desired force profile in each phase. The frequency of the profile is only 1 Hz with 40 Hz sampling frequency and a peak-to-peak value of $200 \mu N$. The result is shown in Figure 7.5. The errors of each control between the measured and desired forces are shown in Table 7.2.

7.6 Discussion

The results shown in Table 7.1 demonstrate that the modified model can fit the biomembrane force-to-deformation profile with an average error of less than 5%. This model is modified from the physical biomembrane point-load model and obtained using pure numerical estimations. Hence, the three parameters in the point-

Table 7.1. *Parameters for the modified biomembrane point-load model for embryo I at Zygote Stage. p_3 , p_2 , p_1 and p_0 represent the coefficients in Equation 7.2. The Error is the relative difference between the fitted model and the experimental data.*

	p_3	p_2	p_1	p_0	Error (%)
	0.0004	-0.0165	4.1014	-0.8086	1.25
	0.0004	-0.0211	4.2231	-1.7941	0.66
	-0.0001	0.0274	3.2591	-0.0060	0.56
	-0.0001	0.0221	3.1989	1.8558	1.26
	0.0001	0.0104	3.5074	0.1169	1.22
Average	0.0001	0.0044	3.6579	-0.1270	0.72

Table 7.2. *Results for the control of mechanical forces acting on the cell biomembranes. The absolute and relative error is the absolute and relative difference between the desired and measured mechanical forces.*

Cell Type	Absolute Error (μN)	Error (%)
Zygote Stage	39.2058	3.58
Cleavage Stage	46.3722	4.23
Blastula I Stage	53.7242	4.90
Blastula II Stage	50.5463	4.61
Blastula V Stage	55.4264	5.06
Oblong Stage	33.6974	3.08

load model can be eliminated. Furthermore, the model can be estimated in real time, thus enabling its application to real-time characterization of the mechanical properties change of the biomembrane. For example, the polynomials in Figure 7.4 can clearly define the mechanical properties change of the zebra fish embryos biomembrane.

The third order polynomial also facilitates the design and implementation of the open loop biomembrane force controller. The results in Figure 7.5 and Table 7.2 demonstrate that the average normalized error is less than 5.1%, also proving

Figure 7.3. *An average force-deformation curve of a zebra-fish embryo at Zygote stage. The maximum and minimum values from the five data sets are also plotted.*

that a consistent force control on the biomembrane can be achieved.

An interesting observation is that although the models in (Tan *et al.* 2008, Sun *et al.* 2003) claim an upperhand in terms of accuracy in the number of parameters involved, it can be established that a third order polynomial currently derived can be fit into the same force profiles as above. Hence, it can be concluded that a third-order polynomial fit does not alter the accuracy of the model.

Further advanced improvisations of the prototype can be undertaken to enhance the control of the mechanical forces. One of the possible improvements is to employ multi-axes force sensors in the system such that when the cell is under indentation on one side, the mechanical property in the orthogonal direction can be quantified and modeled.

7.7 Conclusion

In this chapter, the automatic control of mechanical forces acting on cell biomembranes is proposed using a vision-guided microrobotic system. A template-free

Figure 7.4. *Force-deformation curves from six different phases of developmental stages of three different zebra fish embryos.*

vision system calibration, multiple objects autofocusing, vision-guided position control of the holder and the sensor, on-line cell mechanical property modeling, dynamic motion control of piezoelectric micromanipulator and cell biomembrane force control are implemented. Experimental results based on zebra fish embryos show the feasibility of the proposed prototype for dynamic force control acting on cell biomembranes in cell mechanobiology.

Figure 7.5. *Results of mechanical forces acting on cell biomembranes from three different zebra fish embryos. a1 & a2: cell 1 at two stages; b1 & b2: cell 2 at two stages; c1 & c2: cell 3 at two stages; x: time; y: measured forces and desired forces.*

Chapter 8

Conclusion

This dissertation has presented the design and control of a vision-guided cell micromanipulation system in order to reduce the difficulties in various cell micromanipulation tasks such as microinjection and cell biomembrane stress control in cell mechanobiology.

The cell micromanipulation system proposed is composed of three major units as described in Chapter 3. The vision unit makes the autofocusing of the targets possible. The holding and cell micromanipulation units make the immobilization and the micromanipulation of the cell, respectively, in computer-controlled manners.

Vision system calibration is a tedious and challenging task, where a calibration template is often required. The calibration must be re-conducted if the micro-tip is dismounted and mounted. In Chapter 4, an automatic calibration method, where no calibration template is required, is proposed and implemented. With this method, the vision system calibration can be carried out on the fly in real time.

Static position control of the micromanipulator in a visual serving system is a must. An image-based position controller with z -direction deviation compensation

capability is proposed and implemented in Chapter 5. The static positioning error of the micro-tip in the image plane is expected to be less than 1 pixel and the deviation in the z -direction is limited to a predefined value depending on the requirements of the application.

Hysteresis is a common limitation when applying piezoelectric stages in microscale dynamic trajectory tracking applications. An interferometer is usually equipped to perform the hysteresis modeling, which is not realistic in cell micromanipulation system due to the critical requirements of the interferometer. In Chapter 6, an automatic hysteresis modeling method is proposed and implemented using vision-feedback. The method can be conducted on the fly in real time, which is suitable for time critical cell micromanipulation.

A cell is a very complex and dynamic system, wherein the cell's mechanical properties changes at the different developmental stages. It is necessary to identify the cell mechanical property model at different stages to facilitate cell biomembrane stress control. A third-order polynomial is proposed to model the mechanical property of cell membrane in Chapter 7, making the control of mechanical forces acting on cell biomembrane possible with high accuracy.

The major contributions about the dissertation are summarized as follows:

Automatic Self-calibration of Vision-guided Microscopy Systems

Only one matrix is required to be calibrated and no calibration template is required. It is simple to be implemented in practical applications and suitable for systems that require frequent calibration.

Image-based Position Control with Deviation Compensation

A static image-based position controller with depth deviation compensation capability is realized, The experiment results demonstrate its feasibility and advantages in cell microinjection and cell biomembrane stress control.

Dynamic Motion Tracking of a Piezoelectric Micromanipulator

Hysteresis is one of the limitations when applying piezoelectric stages in microscale dynamic trajectory tracking applications. An automatic vision-based hysteresis modeling method, which is performed on the fly in real time is realized without using interferometers.

Automatic Mechanical Force Control on Cell Biomembrane

Automatic cell mechanical property modeling and cell biomembrane force control are realized. It would benefit biologists in complex analysis of the biological reactions of cells as a result of stimuli from external mechanical forces.

Future Work

The proposed system is capable of performing many cell micromanipulation tasks. However, there are a few improvements that could be made to improve the accuracy and the throughput.

Firstly, from the system point of view, a motorized 2D platform is desired such that cell micromanipulation could be performed continuously for batch-cell processing. As seen in the experiments in Chapter 7, the next interested cell must be manually transported to the field of view of the camera for the purpose of cell holding and micromanipulation. If there are multiple cells required to be experimented in the petri dish, this process is time consuming. Incorporating a motorized 2D stage in the system could solve this problem. Alternatively, the batch-cell holding devices (Lu *et al.* 2007, Huang *et al.* 2009) are worth to be investigated.

Secondly, the modified PI model is not singularity-free (Tan *et al.* 2009) resulting in poor trajectory tracking performance when the slope of the hysteresis curve is non-positive as shown in Figure 8.1 due to the nature of the PI model. The singularity could make the dynamic motion trajectory tracking fail. Thus, a singularity-free hysteresis model is desired to achieve better curve tracking.

Figure 8.1. *Singularity occurs when the slope of the hysteresis curve is non-positive.*

Thirdly, the proposed hysteresis modeling method is implemented for a particular frequency, which implies that the working frequency of proposed dynamic motion (force) trajectory tracking controller is also limited to the the frequency. Thus, a rate-dependent (Ang *et al.* 2007) dynamic motion (force) trajectory tracking controller for non-stationary signals is desired.

Appendix

Refereed Journal:

- Y. L. Zhang, M. L. H. amd C. Y. Shee amd T. F. CHIA, and W. T. Ang, Self-calibration method for vision-guided cell micromanipulation systems, Journal of Microscopy, vol. 233, no. 2, pp. 340C345, 2009.
- Y. L. Zhang, M. L. Han, J. Vidyalakshmi, C. Y. Shee, and W. T. Ang Automatic control of mechanical forces acting on cell biomembranes using a vision-guided microrobotic system in Computer Microscopy, Journal of Microscopy, vol. 236, no. 1, pp. 70-78, 2009.
- Y. L. Zhang, M. L. Han, M. Y. Yu, C. Y. Shee, and W. T. Ang Automatic hysteresis modeling of piezoelectric stage using vision-feedback, IEEE Transaction on Mechatronics (submitted).

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- Y. Zhang, M. Han, C. Y. Shee, and W. T. Ang, Calibration of piezoelectric actuator-based vision guided cell microinjection system, in IEEE/ASME Intl. Conf. Advanced Intelligent Mechatronics, Xian, China, Jul. 2008, pp. 808C812.
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