Microinjection and cell membrane strain control using vision guided robotic cell micromanipulation system

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**MICROINJECTION AND CELL MEMBRANE STRAIN
CONTROL USING VISION GUIDED ROBOTIC CELL
MICROMANIPULATION SYSTEM**

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**SCHOOL OF MECHANICAL AND AEROSPACE
ENGINEERING**

2013

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CONTROL USING VISION GUIDED ROBOTIC CELL
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Summary

This dissertation presents methodologies to measure, model and control the cell membrane strain in real-time by using a vision guided robotic cell micromanipulation system. The system includes a motion unit to control the position of the micropipette in six degrees of freedom, a vision unit to process the image information from the camera and a holding unit to immobilize the cell for strain control.

An indirect depth estimation method is proposed to place the target cell and the tip of the micropipette on the same focal plane without taking any risk of damaging the fragile tip of the micropipette, which minimizes the position error between the micropipette and the target cell.

The cell membrane deformation is estimated by the peripheral and the local deformation and a real-time machine vision algorithm is proposed to track the changes of cell membrane deformation. The proposed method speeds up the sampling rate of the cell strain control system to 10 Hz and no manual adjustment of parameters is required throughout the experiment.

A real-time cell strain control system is proposed by employing the knowledge of the strain model through the feedforward input and accounting for errors using the feedback controller. The experimental results show that the maximum error between the desired and the actual cell membrane strain is within 3%.

Chapter 1

Introduction

1.1 Background

Manipulating on biological cells involves a wide range of disciplines from engineering to biology. Optic and electric micromanipulation, magnetic micromanipulation, microelectromechanical systems (MEMS) and mechanical micromanipulation are the most common approaches for single cell manipulation. Among these approaches, mechanical micromanipulation motivates lots of engineering researchers, as it is required by a number of practical applications in the biomedical area.

1.1.1 Cell Micromanipulation

Gene delivery is the process of introducing DNA into cells to assess gene function and gene therapy. It has become a significant operation in the field of biology. The means to achieve gene transformation can be classified into three categories: virus-mediated transduction, chemical methods and physical methods. The first two methods have been well studied by biologist and promising approaches have been proposed and implemented (Watt *et al.*, 1993; Walther and Stein, 2000). The

physical gene delivery methods require specific efficient system, but the process is easier to be standardized and used for in-vitro application. Most of the physical methods use micromanipulation techniques to exert mechanical force on a local area of the cell. Hardware design, force sensing and position control are the main problems when developing the micromanipulation system.

Cell micromanipulation under microscope dates back to the last half of the nineteenth century (Desai *et al.*, 2007). Marshall A. Barber creates the pipette method to isolate bacterial cells using microinjection principle. A detail history is introduced in (Korzh and Strahle, 2002). Since its invention, biologists have conducted experiments using the conventional manual micromanipulation technique in gene injection (Kuncova and Kallio, 2004), in-vitro fertilization (Sun and Nelson, 2002), intracytoplasmic sperm injection (ICSI) (Kimura and Yanagimachi, 1995) and drug development (Nakayama *et al.*, 1998).

ICSI has been used to treat male factor infertility when conventional in-vitro fertilization (IVF) or other assisted-fertilization methods are not successful. The technique involves the direct injection of a single motile spermatozoon into the cytoplasm of a metaphase II oocyte (Joris *et al.*, 1998). Fig. 1.1 shows a typical scenario of ICSI cell injection process on a mouse's oocyte (Kimura and Yanagimachi, 1995). For manual injection, operators obtain the majority of the information from their vision and make judgement based on their intuitive feelings. It usually takes months or one year to train operators to be professional. However, the accuracy still cannot be assured due to the human fatigue or inconsistent performance. The success rates of manual cell microinjection have been reported to range from 40 – 70 % (Payne *et al.*, 1994) in the application of ICSI to merely 1 – 3 % (Bosch *et al.*, 2004) in transgenic works. In order to enable precise and efficient cell micromanipulation, the manual operation has to be replaced with automatic systems.

A few robotic cell micromanipulation systems have been built up and implemented on different types of biological cells. It plays a critical role in not

Figure 1.1. (a) Position the sperm micropipette at the edge of the oocyte. (b) Insert the micropipette deeply into the oocyte.

only engineering field, but also in the mechanobiology domain.

The typical setup of the system consists of a vision unit and two actuation units. A micropipette is mounted on each of the actuation units to simulate a chopstick manipulation (Tanikawa and Arai, 1999). Calibration of micropipette has been proposed to increase the positional accuracy (Kawaji *et al.*, 1999; Li and Xi, 2002). Recently, the interaction force sensing has also been considered to improve the reliability of the system (Kim *et al.*, 2004; Pillarisetti *et al.*, 2004). A two degrees of freedom (DOF) capacitive micro force sensor is capable of measuring the manipulating force up to $490 \mu\text{N}$ with a resolution of $0.01 \mu\text{N}$ in x direction, and up to $900 \mu\text{N}$ with a resolution of $0.24 \mu\text{N}$ in y direction (Sun *et al.*, 2002). A hybrid control system for cell micromanipulation can be developed by utilizing both force and vision feedback (Sun and Nelson, 2002). Apart from the significant improvement on the automatic system development, potential implementations have also been explored, for example, in the mechanical property characterization and elastic and viscoelastic property investigations on biological cells (Sun *et al.*, 2003; Neuman *et al.*, 2007; Tan *et al.*, 2008).

1.1.2 Mechanobiology

Cells, as the basic units of life, perform vital functions, such as extracting energy and producing materials from their environment, regulating their own internal operations, and reproducing themselves periodically. There are two types of cells — the eukaryotic cell (with nucleus) and prokaryotic cell (without nucleus). Fig. 1.2 show the basic structural elements of eukaryotic cells. They consist of three parts: the cell membrane, the nucleus and the cytoplasm. The semi-permeable boundary around the cell is the cell membrane. It selectively separates the cells contents from their environment. The nucleus is the control center of the cell, determining how the cell will function, as well as the basic structure of the cell. The cytoplasm is the gel-like fluid inside the cell. It is the medium for chemical reactions. Within the cytoplasm, organelles of distinct structures and fine filaments are distributed intricately (Boal, 2002).

Figure 1.2. Schematic diagram of a typical cell with nucleus.

Living cells possess biochemical modules and physical shapes to maintain appropriate biological functions. Meanwhile, cell membranes can sense physical stimulus from their external environment and response accordingly to reveal proper functions (Zhu *et al.*, 2000; Bausch and Kroy, 2006). It has been proposed that deformations may result in conformation changes of membrane attachment and activating cytoplasmic molecules which trigger a cascade of biochemical events (Gudi *et al.*, 1998). Significant research has been conducted to investigate the mechanotransduction mechanism. Biologists consider that the conversion process can be considered as a cyclic system of mechanosensing, mechanotransduction and mechanoresponse (Vogel and Sheetz, 2006). The mechanosensory elements affect

proteins and then drive the mechanotransduction transducing the physical changes into biochemical response. These biochemical signals can regulate cell growth, differentiation and death (Chen *et al.*, 1997; Silver and Siperko, 2003; Bustamante *et al.*, 2004; Kung, 2005).

Force and geometric shape are the primary mechanical stimulations that the cell will encounter from the exterior environment. It has been postulated that controlled functional responses can be produced by activating diversity of mechanosensory proteins through physical changes of force and shape (Chen *et al.*, 1997, 2003; Zhu *et al.*, 2000; Boal, 2002). As illustrated in Fig. 1.3, force-induced changes and deformation-induced changes are closely related to each other and will be reflected on cell functions when the changes are produced at the biochemical level (Vogel and Sheetz, 2006). Force control methodologies can be applied on biological cells to help biologists study the corresponding biochemical changes in the cell (Liu *et al.*, 2009; Zhang *et al.*, 2009). When a desired profile of force is exerted on the cell, the geometric shape of the cell is also changed. The relationship between the deformation of the cell and the force is different from one cell to another and also for the same cell in different stages. While the same trajectory of force is applied on the cell, the corresponding deformation could be different (Kim *et al.*, 2005; Lu *et al.*, 2009). Therefore, the feasibility of producing a series of predefined geometric shape by controlling the applied force cannot be assured.

Figure 1.3. Illustration of converting mechanical signal to biological signal.

1.2 Objectives

The objective of the dissertation is to develop a vision guided cell micromanipulation system capable of performing automatic cell strain control. The specific aims of the work include:

1) Propose and implement a depth estimation microscopy using autofocusing algorithms and image based visual servoing to perform micromanipulation tasks.

2) Propose and implement a real-time cell membrane strain estimation and modeling method using proposed machine vision algorithm to extract the feature parameters from the cell membrane images and model the deformation of cell membrane with respect to the displacement of the micropipette.

3) Propose and implement a cell strain control system to deform the cell membrane into desired shape.

1.3 Technical Challenges

The technical challenges to measure and control the cell membrane strain in real-time are summarized as follows:

1) Depth estimation

Manually placing the sample and the micropipette on the same focal plane is difficult as the dimension of the sample or the micropipette is in micrometers. It is also not consistent to rely on the human vision to select the optimum focused position. The depth between the micropipette and the sample cannot be always directly estimated by adjusting the position of the micropipette due to the system setup or the samples.

Either template matching technique or two cameras is used to solve this

problem by the current cell micromanipulation systems, as presented in Chapter 2. For the template matching method, a new template has to be selected when a new experiment starts. For the two camera setup, it requires accounting for the synchronization problem of the two cameras information and it is also costly to employ two cameras on the microscope. The very recent solution is to let the tip of the micropipette touch the microscope slide and even bend a few micro meters horizontally to find its maximum sharpness. This method will be introduced as the direct depth estimation method in Chapter 3 to compare with the proposed indirect depth estimation method.

2) Real-Time strain measurement

Currently, a large number of cell membrane deformation images acquired in the experiments are recorded by the camera and then post processed to extract the points on the deformed cell boundaries. Conventional adaptive thresholding algorithm and edge detection method can be combined to accomplish this task off-line. Since there will be one or two tuning parameters in the algorithm which are required to be adjusted based on different brightness or content of individual images, it is impossible to model the cell membrane deformation in real-time and the measurement process is tedious for human operators. Moreover, off-line measurements produce significant errors in the subsequent strain control process due to the poor synchronization of cell strain measurements and micropipette displacement.

One approach to solve this problem is to frequently adjust these threshold values. Another approach is to build an edge linking criteria which is analyzed from the characteristics of pixels in a small neighborhood of edge points. Magnitude and direction criteria are two principal properties used for establishing similarities of two pixels. The process is repeated at every location in the region of interest. Instead of establishing a edge linking criteria, a searching region can be defined around the deformed membrane and extract points on the deformed curve whose gray level exceed a threshold. The drawback of the above methods is that the

existence of tuning parameters in the visual module, which impedes the realization of real-time cell strain modeling and control. Some researchers also choose to use position feedback from the motor encoder instead of visual feedback to avoid the tedious image processing work and lack of synchronization.

3) Strain control

Cell membrane strain control is, to the best of our knowledge, the novel idea in the field of cell micromanipulation. As the control process is conducted after the modeling process, the elasticity of the cell membrane could have changed. Thus, the cell strain controller should be able to deal with this situation during the implementation.

1.4 Scope

Cell micromanipulation is a very broad area. The author limits the scope of the dissertation to:

1. The hardware of the vision-guided system, such as the microscope, the camera and the actuators are bought off the shelves. The author focuses on the integration of these components and development of methodology.
2. The proposed methodology of strain modeling and control is focused on the study of cell membrane deformation instead of force investigation.
3. The proposed methodology of strain modeling and control is applied on suspended circular cells only, which are assumed to be homogeneous elastic solid in the experiments. The concept can be utilized on other type of cells with the similar geometric shape.
4. The author only considers micropipette indentation of circular biological cell, aspiration experiments require different solutions. Zebrafish embryos are used

as sample cells throughout this work.

5. Novelty of this work is sought in robotic science instead of biological science. Biologists can use the proposed concept as an engineering tool to carry out their experiments, which are not easily conducted using existing approaches.

1.5 Organization

The dissertation is organized as follows:

Chapter 1 outlines the background, objectives and scope of this dissertation.

Chapter 2 introduces existing cell micromanipulation systems. The systems in the research stage are focused on the position and force control, real-time computer vision algorithm development. The concept of mechanobiology is also introduced to point out the requirement of modeling and control of the cell strain in the biological field.

Chapter 3 introduces a vision guided robotic cell micromanipulation system which is used to perform the various manipulation tasks on biological cells, e.g. microinjection, strain modeling and strain control.

Chapter 4 proposes a depth estimation method using autofocus algorithms which guides the micropipette traveling to the target position on the pixel frame. The proposed indirect depth estimation method is quantified by experiments on plant cell injection.

Chapter 5 presents the measurement method of cell deformation and develops a cell strain modeling and control system to produce a predefined strain profile on biological cells. The measurement method is capable to characterize the deformed cell membrane and the computer vision algorithm is able to measure the deformation in real-time. Experiments are carried out on zebrafish embryos

using the proposed methodology.

Chapter 6 concludes the dissertation and provides the possible improvements of the current system and suggests future directions that this project can move towards to.

Chapter 2

Literature Review

2.1 Existing Cell Micromanipulation Systems and Methodologies

2.1.1 Overview of Cell Micromanipulation

Cell micromanipulation comprises four main types of experimental techniques. First, localized probing of a small portion of the cell, such as atomic force microscopy (AFM) method. In AFM, a sharp tip at the free end of a flexible cantilever generates a local deformation on the cell surface, as shown in Fig. 2.1 (a). The second type is loading a mechanical force on an entire cell, such as micropipette aspiration. As shown in Fig. 2.1 (b), a cell is deformed by applying suction through a micropipette placed on the surface of the cell. Fig. 2.1 (c) shows that the cell also can be deformed using optical tweezers and dielectric beads. The force generated between the bead and the laser beam pull the bead towards the focal point of the trap. The fourth type is deforming a population of cells by simultaneous mechanical stressing, such as shear flow method shown in Fig. 2.1 (d). It is usually conducted with a device which can apply laminar and turbulent flows, or a parallel-plate

flow chamber in which cells are subjected to laminar flow (Bao and Suresh, 2003). Currently, the majority of automatic cell micromanipulation systems are developed

Figure 2.1. Schematic representation of different types of cell micromanipulation techniques (Bao and Suresh, 2003). (a) AFM. (b) Aspiration. (c) Optical trap. (d) Shear flow.

to perform microinjection and indentation deformation tasks, which belong to the first type of manipulation.

2.1.2 Existing Usage of Automatic Cell Micromanipulation Systems

Sun proposes an automatic microrobotic system to conduct automatic pronuclei DNA injection of mouse embryos (Sun and Nelson, 2002). The system consists of an injection unit, a vision unit and a software unit. The injection part is installed on a 3-DOF microrobot to control the motion of the injection pipette. The holding module is mounted on a manual 3-DOF translation stages. The vision unit includes an inverted microscope with a 400 $\times$ objective, a CCD camera, a PCI frame grabber and a host computer to track the image features. The setup of the system is shown in Fig. 2.2.

Figure 2.2. Setup of Sun's system (Sun and Nelson, 2002).

Hough transform is used for this automatic cell microinjection system to locate the nuclei. Hough transform is a machine vision algorithm to detect line features such as straight line, circle, ellipse or arc (Gonzalez and Woods, 2002). Classical Hough transform requires the desired features be specified in some parametric form like a straight line or a circle, etc. Both the membrane of the embryos and the nuclei are considered as a circle. The computational complexity of the 3-DOF Hough transform is avoided by using an approximation of $10\text{ }\mu\text{m}$ for the diameter of the nuclei. Sum-of-squared-differences (SSD) algorithm is used to track the movement of the injection micropipette for visual servoing (Papanikolopoulos, 1995). SSD

algorithm is easy to implement, but it is sensitive to the brightness changes, image rotations and scale changes in the image sequence. The author uses a template matching technique to prevent the tip of the injection micropipette from sliding over the top of the embryo, which reduces the depth between the tip of the injection pipette and the target cell.

The work has been further improved to perform high-throughput automatic cell microinjection by using a vacuum-based cell holding device to immobilize a large number of cells (Wang *et al.*, 2009; Liu and Sun, 2009). Mechanical properties of mouse embryos and zebrafish embryos are also investigated by using the above system (Sun *et al.*, 2003; Kim *et al.*, 2006). The indentation of the cell membrane is estimated by combining the three geometric parameters, i.e. the radius of the dimple, the depth into the cell and the radius of the semicircular curved surface. These features can be used to describe the shape of the deformed cell membranes, but it is hard to control all the variables if a desired deformation is required.

Shim proposes a vision-guided biological cell manipulation system which is composed of two parts, a vision processing part and a motion control part (Shim *et al.*, 2004). To increase the survival rate of the cells, a force sensing unit with a resolution of 0.01×10^{-6} kgf is included in the motion control module. It is the attractive contribution of this new microinjection system. Two CCD cameras are applied in the vision part to reconstruct the 3D shape of the cell. One camera is mounted on the top of the optical microscope, the other is mounted in the horizontal direction with respect to the biological cells. With these two cameras, the depth between the pipette tip and the target cell is not required to be estimated. From the top and the front view of the image, both the tip and the cell positions are obtained directly. The injection is performed from the center of cell as stated by the author. Only thinning algorithm is applied during the image processing procedure. The injection angle and the linear motion of the injection pipette towards the biological cell are controlled by two manipulators in the motion control part. The setup of the system is shown in Fig. 2.3.

Figure 2.3. Setup of Shim's system (Shim *et al.*, 2004).

The system is capable to detect the penetrating force and prevent damage of the cell from excessive force. However, the hardware of this cell microinjection system is expensive and complicated. It requires one more camera comparing with the previous system. The camera quantity could be reduced by implementing advanced image processing method, such as depth estimation method based on the autofocusing algorithms the report proposes.

Lu proposes a micromanipulation system with force-feedback which can perform automatic microinjection for a batch of cells (Lu *et al.*, 2006, 2007). To achieve batch microinjection, cells are held in multiple rows by parallel V-grooves. The grooves are made in a petri dish in an upright optical microscope. The petri dish is mounted on a holder at a certain angle to reduce the mobility of the cell. The petri dish holder and the modified piezoresistive force sensor are mounted on a 6-DOF high precision stage. Fig. 2.4 illustrates the setup of this micromanipulation system for batch cell microinjection.

The normalized cross correlation algorithm is used to identify the target cell and the tip of the injection micropipette. This method is easy to implement but it is sensitive to image rotations and scale changes. By analyzing the force trajectory with respect to the time on a batch of cells, the penetration point is decided by detecting the sharp drop in the penetration force after the initial rise.

The main contribution of this work is the setup of an automatic microinjection system for a batch of cells is introduced. But the micro force sensor used in this work cannot be applied in practical work, as the cavity is blocked by the glue that bonds the sensor to the micropipette.

In (Huang *et al.*, 2006, 2007, 2009b), an automatic microinjection system and a force control algorithm based on the deformation of the cell are proposed. The system consists of an executive, sensory and control module. Similar to the previous systems, a injection manipulator, a holding unit, a vision system and a motion controller are included in this microinjection system, as shown in Fig. 2.5.

One of the differences in this work is that the target cell is fixed on a working plate whose height is precisely calibrated instead of using a holder. A visual-based impedance control algorithm is proposed to regulate the injection force in the out-of-plane scenario. The vision system is relatively more important than the previous system for more information is acquired from the image. The displacement of the tip is used to represent the cell deformation and estimate the relationship

Figure 2.4. Setup of Lu's system (Lu *et al.*, 2007).

between the changes of cell shape and applied force, as shown in Fig. 2.6. Instead of using the displacement, the deformed area should be more precisely measured.

A hybrid position and force control has also been proposed in Huang's work for batch microinjection of cells (Huang *et al.*, 2009a). A cell holding device made of agarose gel is developed to carry a number of cells at one time. Instead of using

(a)

(b)

Figure 2.5. Setup of Huang's system (Huang *et al.*, 2006).

Figure 2.6. Sketch of the deformed cell after being injected (Huang *et al.*, 2007).

the visual feedback throughout the entire injection process, the position of the injection tip on the 2D plane is acquired from the motor encoders to speed up the sampling frequency. Based on the assumption that the same batch of cells have approximately the same membrane dynamic characteristics, the depth position of the tip is inferred from the empirical relationship between the injection force and injection trajectory.

Recently, Xie has proposed a force control approach to regulate the penetrating force during the cell injection process based on a force-sensing structure using PVDF(polyvinylidene fluoride) film (Xie *et al.*, 2010, 2011). In her work, a polynomial model is also used to model the cell membrane.

From the above reviews on the existing automatic cell micromanipulation systems, it is noticed that the main contributions in this field are on position and force control for microinjection tasks. The accuracy and efficiency for cell microinjections have been significantly improved in recent years by the above great works. Few works have also been done to characterize the mechanical properties of biological cells by modeling the relationship between the mechanical force and cell deformation (Sun *et al.*, 2003; Tan *et al.*, 2008). Measurement of cell membrane deformation is not only important for building the mechanical model of biological cells, but also one of the critical factors that determine the biochemical changes of cells.

2.2 Cell Membranes

In general, Cell membranes cover the outer layer of an organ or organelle and protect the cellular components from extra cellular matrix (ECM). Substrate for embedded membrane proteins, receptors and enzymes are provided by cell membranes to enable active transport of matters from and to the cell (Alberts *et al.*, 2002). A biological cell membrane is composed of two layers of fluid lipids

with hydrophobic and hydrophilic proteins embedded in them (Lodish *et al.*, 2004), as shown in Fig. 2.7.

Figure 2.7. The basic structure of a cell membrane. Cell function is associated with protein-protein interactions (Lodish *et al.*, 2004).

Cell membranes that are defined as embryonic coats or cover the early fertilized embryos are also called chorion for teleosts or zona pellucida for mammals. Functions of these types of membranes are protecting the inner yolks from toxins, bacteria and virus, assistance of ovulation and preventing of polyspermy (Herrler and Beier, 2000). They are composed of fibrous matrices with many important proteins housing inside (Rawson *et al.*, 2001).

The membrane proteins are the prerequisite components in formatting of the coat and processing cell signal throughout the embryonic development. They also provide a special protection by holding the blastomeres together when the embryo reaches the blastula stage. Fish embryos, such as zebrafish embryos, will undergo a softening process through a certain protease enzymatic reaction and prepare for the hatching in the later stage. This reaction will be influenced if a mechanical force applies on the chorion, which will further affect the hatching condition for the fish (Bonsignorio *et al.*, 1996; Denker, 2000).

2.3 Mechanobiology

All cells and organisms from most primitive to the most complex are sensitive to the mechanical force (Ingber, 2006; Orr *et al.*, 2006). This common character requires cells to rely on physical stimulations either from their external environment, or from internally generated contractile force, so that essential biological response can be driven to support cell development and homeostasis (Chen *et al.*, 2004; Wozniak and Chen, 2009). The process of conversion the mechanical stimulation into biological signals can be considered as a cycle of mechanosensing, mechanotransduction and mechanoreponse.

Based on the extensive studies, force and geometry changes at nano-to-micrometer level are the main physical stimulations that cells can sense. By integrating more complex physical parameters, such as matrix rigidity or micrometer-level geometric changes, transduction process of cellular mechanotransduction system can be deeply investigated (Vogel and Sheetz, 2006).

It has also been postulated that cytoskeletal filaments in the cell membrane can propagate geometric changes over long distances, which induce interactions of other distant mechanosensory and signalling components (Ingber, 2005; Wang and Suo, 2005). Considering the existence of various mechanosensory proteins, controlled functional responses might be produced through activation of many signalling pathways during the transduction process from mechanosensing to mechanoreponse. Initial cellular response can occur in seconds to minutes and subsequent cellular response can induce a second cycle of reactions. Therefore, cells behavior can be significantly changed after cells are deformed and maintained in culture for days (Vogel and Sheetz, 2006).

Recent studies determine that sensing of geometric changes at cellular level is a crucial component of changing the cell function. Same matrix of protein will induce a different cell function when it is organized from flat surface to filaments (Katz *et al.*, 2000; Cukierman *et al.*, 2001, 2002). It has also been found that

cells can differentiate between a concave and a convex membrane curvature. The natural ECM of the cell membrane is a complex 3D meshwork which is composed of wide distributive fibres and gaps. The 3D matrix fibres can sense the geometric changes and provide complex biochemical and physical cues to the cell. As the curvature of the membrane changes, the cell will sense the 3D fibres differently from the same matrix molecules (Vogel and Sheetz, 2006).

Studies of endocytosis show that BAR-domain (Bin, amphiphysin, Rvs domain) proteins can recognize concave membranes curvature and increase small G protein activity (Zimmerberg and Mclaughlin, 2004; Habermann, 2004). In Fig. 2.8 (a), releasing of Rac might lead to further enhancement of traction force. For convex membrane curvature, it can affect the local lipid composition, spatial clustering and segregation of transmembrane proteins, which induce the opening of mechanogated ion channels, such as K^+ channels (Patel *et al.*, 2001), as shown in Fig. 2.8 (b). Vogel proposes that concave and convex membrane could be sensed by two different mechanisms. Concave curvature which is produced by the external posts can be sensed by BAR domain proteins, while the convex curvature produced by the indentation deformation could be sensed by opening of channels (Vogel and Sheetz, 2006).

Mutations and modifications of cells produced by mechanical stimulations could also lead to a variety of diseases (Jaalouk and Lammerding, 2009). Table 2.1 lists some of the diseases that are resulted by the certain types of deformed cells.

Table 2.1. *Disease associated with affected cells*

<i>Disease</i>	<i>Cells affected</i>
Deafness	Hair cells in the inner ear
Arteriosclerosis	Endothelial and smooth muscle cells
Polycystic kidney disease	Epithelial cells
Cancer	Multiple cell types and tissues

Disruption of the complex force transmission between ECM, the cytoskeleton

Figure 2.8. Mechanisms of form sensing (Vogel and Sheetz, 2006). (a) Concave curvature of membrane could cause BAR- domain proteins to release small GTPases Rac. (b) Convex membrane could activate the opening of an ion channel.

Figure 2.9. Transmission of external stimulations between the ECM and the nucleus (Jaalouk and Lammerding, 2009).

and the nucleus is the major reason of many mechanobiology diseases. External stimulations are transmitted through specific proteins in the ECM, such as collagen, laminin and fibronectin. ECM and the cytoskeleton are physically linked by adhesion fibres in the membrane. The configuration and binding affinity of these complexes can transmit the extracellular force to the intracellular force, which is further delivered to the cytoskeletal network. The cytoskeleton interacts with the nucleus through nesprins and other proteins on the nuclear membrane. Nuclear proteins which bind to nuclear pore complexes can lead to nuclear cytoskeletal coupling and finally form stable nuclear structures and bind to DNA. Thus, the physical changes of membrane is transmitted from ECM to nuclear interior, as shown in Fig. 2.9. During this process, any mutation in these components, or cellular environment could disturb the mechanotransduction and result in cell function changes.

Based on the effects brought by the mechanical stimulations, it shows that the cell membrane deformation produced by the physical force is important to cell function changes. Quantification of geometric changes of cell membranes could estimate the biochemical reactions.

2.4 Summary

From the above reviews of the existing cell micromanipulation systems and the concept of mechanobiology, we propose to develop a robotic cell micromanipulation system which is capable to provide a predefined deformation for cell membrane. Although the automatic cell micromanipulation systems have been significantly improved in recent years, none of the systems is able to measure and control the cell membrane deformation in real-time. By building a control system for cell membrane strain, i.e. changes of cell membrane deformation, biologists could produce a controlled functional response by giving a desired change to the cell.

Chapter 3

Vision Guided Robotic Cell Micromanipulation System

The proposed methodology is implemented on a vision guided robotic cell micromanipulation system. The basic structure of the system includes a motion unit, a vision unit and a holding unit. As there are two implementations on different specimens, i.e. orchid petals and zebrafish embryos, different hardware components are employed.

3.1 Plant Cell Injection System

The automatic plant cell injection system with autofocusing characteristics of plant cell images is composed of vision unit and motion unit. The configuration of the system is described in Fig. 3.1.

The vision unit consists of one upright microscope (Eclipse FN1, Nikon, Japan) fitted with a motorized objective (CFI Plan Fluor 10x, Nikon, Japan) and one CCD camera (scA640-74fm/fc, Basler, Germany) mounted on the rear port of the microscope. The motion of the objective is controlled by a stepper motor

Figure 3.1. The schematic representation of the system configuration implemented for automatic plant cell injection. The motorized XYZ table is composed by three motorized translational stages. The camera is mounted on the rear port of the microscope, which can also refer to Fig. 3.2.

(ECO-MOT, Lang, Germany) attached to the fine focus adjusting knob of the microscope with a resolution of $0.5 \mu\text{m}$. The camera has a resolution of 659×494 , pixel size of $9.9 \times 9.9 \mu\text{m}$ and a maximum frame rate of 74 frames per second.

The motion unit includes an injection micropipette (Sterile Femtotips, Eppendorf, Germany), with $1 \mu\text{m}$ diameter, mounted on the micromanipulator, a NanoCube XYZ piezoelectric stage (P-611.3S, PI, Germany), a mini rotational stage with 3 arcmin resolution (7R174-AA, Standa, Lithuania) mounted a 3-DOF motorized translational stages (8MT173-20-50, Standa, Lithuania) with a traveling range of $50 \times 50 \times 50 \text{ mm}$ and $1.25 \mu\text{m}$ resolution. The micromanipulator is installed on the piezoelectric stage with a workspace of $100 \times 100 \times 100 \mu\text{m}$ and resolution of 1 nm. The rotational and the piezoelectric stage are connected through an adapter to

provide an injection angle of 30 degrees to the target plant cell. The platform keeps stationary throughout the experiments. The position of the injection micropipette is controlled by the motorized translational stages and the rotational stage.

A host computer with LabVIEW installed connects the vision and motion unit through a Gigabit ethernet interface and a piezoelectric controller (E-501, PI, Germany). Autofocusing algorithm is programmed in LabVIEW to find the best focused image.

3.2 Cell Strain Modeling and Control System

The hardware configuration of the automatic cell strain modeling and control system is shown in Fig. 3.2. It consists of three parts: the motion unit, the vision unit and the holding unit. The same vision components which are used in the plant cell experiments are adopted excluding the replacement of a lower magnification objective (CFI Plan 4x, Nikon, Japan).

In the motion unit, a motorized rotational stage (PRM1-Z7E, Thorlabs, USA) with 1 arcsec resolution is attached to the 3-DOF motorized translational stage (MM-4M-F-50, National Aperture, USA) with the traveling range of $50 \times 50 \times 50$ mm and 1 μm resolution. It controls the angle of the micropipette with respect to the platform whose position is fixed. Instead of using piezoelectric actuator, the micromanipulator is directly mounted on the rotational stage and a micropipette (Sterile VacuTips, Eppendorf, Germany) with 100 μm diameter and 35 degree angle is used for the experiments. As shown in Fig. 3.3, the rotational stage and the vertical axis of the translational stage are joint with an angle of $\theta = 35^\circ$ to let the tip of the micropipette be horizontal with respect to the platform. The position of the micropipette tip which is controlled by the motorized translational stages and the rotational stage has six degrees of freedom.

The holding unit employs a 3-DOF motorized translational stage (7T173-50,

Figure 3.2. Experimental setup of the cell membrane strain modeling and control system. The system is composed of motion, vision and holding units.

Standa, Lithuania) with the range of $50 \times 50 \times 50$ mm and a resolution of $5 \mu\text{m}$. A manual rotational stage (7R150-30, Standa, Lithuania) is mounted on the vertical axis of the stage. A vacuum pump (CellTram Vario, Eppendorf, Germany) is connected to the holding device to provide negative pressure for immobilizing cells. The tip of the holding pipette is made up of a pulled glass capillary with an angle to let the pipette tip be horizontal to the platform, as shown in Fig. 3.4.

Real-Time LabVIEW operation system, embedded in NI PXI-1042 chassis, integrates the motion and vision modules through a gigabit ethernet interface (PXI-8231, National Instruments, USA), a servo motion controller (PXI-7354, National Instruments, USA) and a multifunction amplifier (MC-4SA, National Aperture, USA), as shown in Fig. 3.5. LabVIEW applications are developed on the host PC and loaded to the Real-Time system to execute. Graphical user

Figure 3.3. The rotational stage and the vertical axis of the XYZ table are joint with an angle of $\theta = 35^\circ$. The motorized XYZ table is composed by three motorized translational stages.

Figure 3.4. The tip of the micropipette and holding pipette are horizontal to the platform.

interface, status of the stages and output data are managed on the host PC.

Figure 3.5. Hardware architecture of the strain modeling and control system. The motion and vision units are integrated in Real-Time LabVIEW platform through an image acquisition card, a servo motion controller and a multi function amplifier.

Chapter 4

Visual Servoing in Microscopy

4.1 Depth Estimation

4.1.1 Depth and Sharpness Function

As shown in Fig. 4.1, the tip of the micromanipulator is initially positioned above the cell to avoid damaging the tip. When the cell manipulation process starts, both the tip and cell must be in focus to proceed with the 2D visual servoing scheme in the next step. However, it is difficult to manually move the tip down to eliminate the depth between the tip and the cell, as the depth value is normally in hundreds of micro meters. The tip may be damaged if it is moving too much towards the microscope slide. Hence, the depth between the tip and the cell, d , has to be estimated to place the tip and the cell in the same focal plane using a robotic system.

The cell micromanipulation systems reviewed in Chapter 2 utilize template matching technique or two cameras to solve the above problem (Sun and Nelson, 2002; Shim *et al.*, 2004). For the template matching method, a new template is required to select when a new experiment starts. For the two cameras setup, it

Figure 4.1. Illustration of the depth between the tip and the target cell. The micropipette must be positioned above the cell to avoid damaging the tip before the deforming process starts. The depth should be estimated to proceed with the subsequent tasks. The platform is fixed throughout the experiments.

requires to account for the synchronization problem of the two cameras information and it is also costly to employ two cameras on the microscope.

It is noticed that the degree of focus of the tip image is related to the value of d . As the objective of the microscope is positioned to focus the cell image, the tip image will become more defocused as d increase. Thus, the sharpness of the image is quantified to estimate the value of depth.

The sharpness function is used to compute the degree of focus of an image or a region of an image. The maximum value of the sharpness function corresponds to the best focused image. There are many different sharpness functions in the literature (Sun *et al.*, 2004). The performance of sharpness functions is different for different image contents. No single sharpness function is suitable to all types of images (Chern *et al.*, 2001; Huang and Jing, 2006). Variance function is utilized to compute the degree of focus for images contain a single micropipette. Comparing with Tenengrad, Fast Fourier Transform and Sum Modulus Difference autofocusing

functions, Variance function has a better performance in the accuracy and efficiency for micropipette images (Yu *et al.*, 2010). And the Tenengrad method is employed to compute the images with texture content for its less complex computation.

For variance function, let $I(x, y)$ be the gray level intensity of the pixel (x, y) in an image. The expression of the gray level variance for the $M \times N$ image $I(x, y)$ is

$$Variance = \frac{1}{M \times N} \sum_x \sum_y (I(x, y) - \bar{I})^2 \quad (4.1)$$

where

$$\bar{I} = \frac{1}{M \times N} \sum_x \sum_y I(x, y) \quad (4.2)$$

It calculates the differences between the gray level of every pixels and the average gray level of the image.

In the Tenengrad method, the Sobel edge detection masks I_x and I_y are used as the operators to find the horizontal and vertical gradients (Gonzalez and Woods, 2002). I_x and I_y are defined by

$$\mathbf{I}_x = \begin{bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{bmatrix}, \mathbf{I}_y = \begin{bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix} \quad (4.3)$$

The sharpness for every $M \times N$ image I_k is defined as

$$L_k = \sum_{x=1}^{M-1} \sum_{y=1}^{N-1} S_k(x, y), \text{ for } S > T, \quad (4.4)$$

where

$$S_k(x, y) = \{\nabla S_x^2 + \nabla S_y^2\}_k, \quad (4.5)$$

T is the threshold value which is set to zero as suggested by Krotkov (Krotkov, 1989). ∇S_x and ∇S_y are computed by

$$\begin{aligned} \nabla S_x &= I(x-1, y+1) + 2I(x, y+1) + I(x+1, y+1) - \\ &\quad I(x-1, y-1) - 2I(x, y-1) - I(x+1, y-1) \\ \nabla S_y &= I(x-1, y-1) + 2I(x-1, y) + I(x-1, y+1) - \\ &\quad I(x+1, y-1) - 2I(x+1, y) - I(x+1, y+1) \end{aligned} \quad (4.6)$$

The best focused image I_k happens if L_k is maximized.

4.1.2 Direct Estimation of Depth

In some case, the microscope slide is placed on a motorized platform, as shown in Fig. 4.2 (a), the depth can be directly estimated by computing the image sharpness while moving up the platform (Nayar and Nakagawa, 1994). The value of the depth is considered as the distance between the starting position of the platform and the position where the maximum sharpness value locates, as shown in Fig. 4.2 (a) and (b).

Figure 4.2. Direct estimation of depth by adjusting the position of the motorized platform or the micropipette. From (a) and (b), the displacement of the platform to get the micropipette focused is the depth between the tip and the target cell. From (a) and (c), the tip of the micropipette has to contact with the microscope slide and even move a few micro meters horizontally to find its focused position (Wang *et al.*, 2007).

Procedure of direct estimation of depth is illustrated in Fig. 4.3. The similar steps can be used on flexible or stiff end-effectors if they are capable to sustain the contactness with the microscope slide surface (Wang *et al.*, 2007), as the tip has to touch the microscope slide and even bend a few micro meters horizontally to find

its maximum sharpness value, as shown in Fig. 4.2 (c). Successfully estimate the depth by moving the tip cannot be assured if a fragile glass pipette or a delicate force sensor is used.

Figure 4.3. Flow chart of direct estimation of the depth. The depth can be directly estimated by computing the image sharpness while moving up the platform. The depth is the distance between the starting position of the platform and the position where the maximum sharpness value locates.

4.1.3 Indirect Estimation of Depth

The depth cannot be directly estimated using the above method, when the system platform is fixed or the end-effector is too fragile to contact with the microscope slide.

In the experiments, we find that there is a certain relationship between the change of the image sharpness by adjusting the objective and the micropipette, respectively. Therefore, the indirect depth estimation method is proposed based on this phenomenon.

Figure 4.4. Displacements of the micropipette and the displacements of the objective bring different changes to the image sharpness. From the same degree of focus, the micropipette travels D_i to get the tip image in focus while the objective needs to travel D_j .

Fig. 4.4 plots the change of sharpness value while adjusting the vertical position of micropipette and adjusting the vertical position of the objective, respectively. The two profiles start from the same degree of focus and the sharpness value is computed using Variance function. While the objective travels D_j to focus the tip, the micropipette needs to travel D_i to get the tip in focus.

Fig. 4.5 shows the image formation process to explain the above phenomenon. The light rays that pass through the object point O and are refracted by the lens are converged at point O_s on the sensor plane, which is consistent with the camera position. A focused image is formed if the sensor plane coincides with the image plane, as shown in Fig. 4.5. For a perfect thin lens with object distance of o , focal

length of f and image distance of s , we have

$$\frac{1}{o} + \frac{1}{s} = \frac{1}{f} \quad (4.7)$$

As the 3D cell is focused on a 2D plane, the circular section which has the biggest area and horizontal with the microscope slide is considered as the object here.

Moving point O closer to the lens, from O to O' for instance, will cause a defocused image on the sensor plane, which also happens when the target cell is focused whereas the tip is not. Consider O is on the plane of the target cell and O' is on the plane of the defocused tip, the distance from O to O' is the depth we need to estimate,

$$d = o - o' \quad (4.8)$$

Figure 4.5. Illustration of image formation geometry. Consider O is on the plane of the target cell and O' is on the plane of the defocused tip, the distant from O to O' is the depth we need to estimate. The relationship between the value of d and Δo is used to estimate the depth.

Since the position of the camera does not change, a focused image of O'_s can be obtained on the same sensor plane by varying the lens distance from the sensor

plane. Then, the Gaussian lens law can be written as,

$$\frac{1}{o' + \Delta o} + \frac{1}{s - \Delta o} = \frac{1}{f} \quad (4.9)$$

where Δo is the displacement of the lens to get the point O' focused on the sensor plane. From equation (4.7) and (4.9), we have,

$$\begin{aligned} s - \Delta o &< s \\ o' + \Delta o &> o \\ \Delta o &> d \end{aligned} \quad (4.10)$$

Therefore, the objective should travel a relatively larger distance than the depth between the target cell and the tip to get the tip focused, which coincides with the profile shown in Fig. 4.4.

However, a focused image may not be obtained by varying the position of the objective when the object distance is smaller than the focal length of the objective. Hence, the depth between the tip and the target cell cannot be too large. In the experiment, the tip is positioned above the target cell by $1 - 2 \text{ mm}$ to assure the possibility of obtaining a focused tip image.

For the profiles shown in Fig. 4.4, the image sharpness and the displacement has a linear relationship before the maximum value is reached. Consider the micropipette is moving while the objective keeps stationary, before the sharpness value of the tip image reaches its maximum, the relationship between the image sharpness and the displacement of the micropipette is represented by

$$L_i = \kappa_1 D_i \quad (4.11)$$

where D_i represents the displacement of the micropipette, L_i represents the corresponding sharpness value.

Instead of moving the micropipette, the objective is moved while the micropipette keeps still. Until the focused image is located, the relationship between the sharpness value and the displacement of the objective is

$$L_j = \kappa_2 D_j \quad (4.12)$$

Figure 4.6. Positions of the tip and the objective in the process of indirect depth estimation. The objective travels d_α to focus the tip image from which we obtain the depth d_β . The micropipette does not move before d_β is estimated.

where D_j represents the displacement of the objective, L_j represents the corresponding sharpness value.

The above operations are conducted in the room temperature and an environmental chamber is used to prevent cells from contamination. Experiments are carried out successively to eliminate the effect by other factors. By assuming the experimental conditions keep consistent, the difference of the displacement between the micropipette and the objective is due to their effects on image sharpness.

Let the target cell be focused and the tip of the micropipette is defocused with the sharpness value of L_0 , as the setup shown in Fig. 4.6 (a). Consider d_α is the displacement traveled by the objective to focus the tip, the micropipette needs to travel for a displacement of

$$d_\beta = \kappa_2 d_\alpha / \kappa_1 \quad (4.13)$$

to focus the tip. In other words, the depth between the micropipette and the cell is

Figure 4.7. Procedures of indirect estimation of depth. The objective is controlled by a stepper motor attached to the fine focus adjusting knob of the microscope, as shown in Fig. 3.1.

d_β . Therefore, we estimate the depth by moving the objective without the risk of damaging either the micropipette or the cell. Positions of the tip and the objective during the estimation are illustrated in Fig. 4.6.

Procedure to indirectly estimate the depth is summarized in Fig. 4.7. The relationship between the image sharpness and the displacement of the objective and the relationship between the image sharpness and the micropipette are studied to compute the value of κ_1 and κ_2 . The target cell is manually focused before adjusting the position of the objective and computing the corresponding sharpness value of the tip image. After identifying the displacement of the objective for focusing the

tip image, the depth can be estimated by using the equation in (4.13).

4.1.4 Validation

The following steps are carried out to evaluate the indirect depth estimation method:

(a) Place the tip of the micropipette in the defocused position. For a upright microscope, the objective has to move away from the tip to get the tip in focus.

(b) Study the relationship between the image sharpness and the objective position.

(c) Return the objective to the original position.

(d) Study the relationship between the image sharpness and the tip position.

Table 4.1 shows the evaluation results of the indirect depth estimation method. The error between the actual depth and calculated depth is within 10%.

4.2 2-DOF Position Control

4.2.1 Visual Servoing

Visual servoing is used in a variety of applications such as seam tracking for arc welding, navigation for vehicles and manipulation of objects. In a robotic system, it proves to be very useful for position error compensation and trajectory tracking by using this type of exteroceptive noncontact sensing. The main advantage is that the visual feedback is adaptable to environmental uncertainties. A comprehensive literature review has been introduced in the form of a tutorial in (Hutchinson *et al.*, 1996). Two critical issues involved in visual servoing are feature estimation and

Table 4.1. *Evaluation of the indirect depth estimation method.*

<i>Sets</i>	κ_1	κ_2	<i>Actual Depth</i> (μm)	<i>Calculated Depth</i> (μm)	<i>Difference</i> (%)
1	59.93	49.90	159	169.03	6.31
2	59.94	50.40	159	169.01	6.30
3	59.62	50.20	160	170.08	6.30
4	58.98	49.75	162	172.08	6.22
5	58.67	49.56	163	173.16	6.23
6	59.01	49.13	162	172.34	6.38
7	58.71	49.08	163	173.05	6.17
8	58.02	48.89	165	175.29	6.24
9	47.86	40.92	133	146.21	9.93
10	48.30	40.90	132	144.82	9.71

control generation.

The feature includes the visual information extracted from pertinent images such as optical flow, position and orientation of an object or features of points or lines (Espiau *et al.*, 1992). Issues related to feature estimation are: camera configuration and adopted images processing techniques. In cell micromanipulation, positions of the micropipette and the target cell, deformation of the cell membrane are the features we need to measure. The method to automatically extract these information will be presented in next chapter.

The control scheme is closely related to the visual-motor model estimation. Kinematic model of the manipulator, perspective projection of the robot in the image and camera parameters are used to calibrate the visual-motor model. Based on the representation mode of the feedback and calibration between the camera and manipulator frame, the visual servoing systems are categorized into position based, image based and 2-1/2-D systems (Hutchinson *et al.*, 1996; Malis *et al.*, 1999). They all require that the adopted control law should be simple enough

to be compatible with the bandwidth of the control system, but, robust to the uncertainties in the sensor and environment.

Position based visual servoing is also referred to as 3-D visual servoing, as the error between the current and the desired pose of the target is defined in 3D Cartesian space. The image measurements are used to compute the pose of the target with respect to the camera. It relies on the knowledge of the geometric model of the object and an accurate calibration of the camera to obtain unbiased results. The process of calibration could be tedious and the task cannot be carried out with errors.

2-1/2-D visual servoing approach is proposed for an eye-in-hand camera configuration. The method is a compromise of the position based and image based approaches. The input is expressed in part of the 3-D Cartesian space and in part of the 2-D image space. In this work, since the cell micromanipulation system uses a monocular stand-alone camera, this approach is not adopted.

In an image based control system, the input is computed in 2-D image space. The error is simply defined as the difference between the current and the desired feature parameters. Hence, the cell micromanipulation is conducted using this approach.

4.2.2 Image-based Control

A block diagram of the image based visual servoing approach is presented in Fig. 4.8.

The image based control system may use either a fixed camera or an eye-in-hand camera. In either case, motion of the manipulator causes feature changes in the image observed via the camera. The objective of the image based visual servoing system is to minimize the error function $\mathbf{E}$,

$$\mathbf{E}(\mathbf{f}) = \mathbf{F}^c - \mathbf{F}^* \quad (4.14)$$

Figure 4.8. The block diagram of the image based visual servoing.

where $\mathbf{F}[F_1, F_2, \dots, F_k]$ represents a vector of image features, k is the number of image features. $\mathbf{F}^c$ and $\mathbf{F}^*$ are current and desired image features, respectively. Since the error function is defined in the image space while the control input of the manipulator is defined in the task coordinate system, the image Jacobian is used to relate the image feature parameters to the motion of the manipulator.

Consider $\mathbf{q}$ represents the coordinates of the end-effector in some parameterization of the task space Γ , m is the dimension of the task space Γ , $\dot{\mathbf{q}}$ represents the corresponding end-effector velocity and $\dot{\mathbf{F}}$ represents the rate of changes of the image feature parameters. The image Jacobian, J , is the differential relationship between the task frame and the image frame,

$$J(\mathbf{q}) = \left[\frac{\partial \mathbf{F}}{\partial \mathbf{q}} \right] = \begin{bmatrix} \frac{\partial F_1(\mathbf{q})}{\partial q_1} & \cdots & \frac{\partial F_1(\mathbf{q})}{\partial q_m} \\ \vdots & \ddots & \vdots \\ \frac{\partial F_k(\mathbf{q})}{\partial q_1} & \cdots & \frac{\partial F_k(\mathbf{q})}{\partial q_m} \end{bmatrix} \quad (4.15)$$

Then the relationship between the velocity of the manipulator with respect to the global frame and rates of change of the image parameters is given by,

$$\dot{\mathbf{F}} = J\dot{\mathbf{q}} \quad (4.16)$$

Let $\mathbf{f} = [u, v]^T$ be the position of the micropipette in the pixel frame and $\mathbf{M} = [X, Y, Z]^T$ represent the corresponding coordinates vector in the manipulator frame $\Gamma \in \mathbb{R}^3$. By using a classical perspective projection model, we have,

$$\mathbf{f} = P\mathbf{M} = P_p^c N P_c^m \mathbf{M} \quad (4.17)$$

where:

- P_p^c represents the transformation from camera frame to pixel frame:

$$P_p^c = \begin{bmatrix} f_l/S & 0 & 0 \\ 0 & f_l/S & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (4.18)$$

f_l represents the focal length, $S(\mu m/pixel)$ is the effective size of the pixel in the horizontal or vertical direction.

- N is the normalized perspective projection matrix:

$$N = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \quad (4.19)$$

- P_c^m represents the transformation from manipulator frame to camera frame:

$$P_c^m = \begin{bmatrix} R_c^m & T_c^m \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad (4.20)$$

The image Jacobian matrix can be derived in the form of (Aloimonos and Tsakiris, 1991; Hutchinson *et al.*, 1996),

$$J = \begin{bmatrix} \frac{\lambda}{z} & 0 & -\frac{u}{z} & -\frac{uv}{\lambda} & \frac{\lambda^2+u^2}{\lambda} & -v \\ 0 & \frac{\lambda}{z} & -\frac{v}{z} & \frac{-\lambda^2+v^2}{\lambda} & \frac{uv}{\lambda} & u \end{bmatrix} \quad (4.21)$$

where $\mathbf{M}^c = [x, y, z]^T$ is the coordinates of $\mathbf{M}$ in the camera frame and $\lambda = f_l/S$. The value of z can be calculated using the forward kinematics described in (4.17).

The proportional image-based controller can be written as,

$$\mathbf{u} = \mathbf{K}J^{-1}\mathbf{e}(\mathbf{f}) \quad (4.22)$$

where $\mathbf{u}$ is the control input to the actuator, $\mathbf{K}$ is the constant gain matrix and $\mathbf{e}(\mathbf{f})$ is the position error of the end-effector. There are three cases to be considered to compute the inverse Jacobian matrix:

When $k = m$ and J is nonsingular, J^{-1} exists.

When $k > m$, there are more feature parameters than the degrees of freedom of the task space. The pseudoinverse of J is computed by

$$J^+ = (J^T J)^{-1} J^T \quad (4.23)$$

When $k < m$, the pseudoinverse of J is computed by

$$J^+ = J^T (J J^T)^{-1} \quad (4.24)$$

To proceed the cell micromanipulation task, we consider the three dimension manipulator frame as the global frame. The image Jacobian matrix can be written as,

$$J = \begin{bmatrix} \frac{\lambda}{z} & 0 & -\frac{u}{z} \\ 0 & \frac{\lambda}{z} & -\frac{v}{z} \end{bmatrix} = \frac{1}{z} \begin{bmatrix} \lambda & 0 & -u \\ 0 & \lambda & -v \end{bmatrix} = \frac{1}{z} J_m \quad (4.25)$$

Therefore, the controller is implemented in the form of

$$\mathbf{u} = \mathbf{K} J_m^+ \mathbf{e}(\mathbf{f}) = \mathbf{K} J_m^T (J_m J_m^T)^{-1} \mathbf{e}(\mathbf{f}) \quad (4.26)$$

We have $k = 2$, i.e. $k < m$, as image feature is the coordinate in the image frame. So (4.24) is used to compute J_m^+ .

The advantages of using image-based control for the cell micromanipulation tasks are:

a) Image based visual servoing systems is robust with respect to the camera calibration errors (Espiau, 1993). Approximated values of intrinsic parameters provided by the camera and microscope supplier can be directly used.

b) By simplifying the image Jacobian matrix for cell micromanipulation task, the image based controller is not influenced by the calibration error of the extrinsic parameters, as there are no parameters of focal length, f_l , and pixel size, $S(\mu m/pixel)$, in equation (4.26).

The convergence of the image based visual servoing system is ensured in a region around the desired position and it is influenced by the value of proportional

gain. The gain value can be first determined by trial and error in the simulation before the experiments. The simulated gain value which can make the maximum error between the desired position and the actual position within 1 pixel is chosen.

4.3 Experiments

The proposed position control method using autofocusing algorithms and image based visual servoing is implemented on plant cell injections. Petal cells of white orchid are used as the injection targets. As the injection pipette is very easily damaged, indirect estimation is used to find the depth value. Experimental procedure and results are presented in the following sections.

4.3.1 Experimental Procedure

Experimental procedure is summarized in Fig. 4.9. Seven steps are carried out to position the target plant cell and injection pipette in the pre-injection position.

First of all, the orchid petal is stuck to the microscope slide by hydrogels, without any treatment, and positioned on the platform. The fine focus knob is then manually adjusted until the tip of the micropipette comes into focus. As shown in Fig. 4.10, the plant cells are too blur to be seen because of the depth difference between the micropipette and the petal, which may range from hundreds to thousands of micrometers.

After the above manual operations, the following procedure is carried out automatically. The coefficients of κ_{n1} and κ_{n2} are computed to represent the relationship between the image sharpness and the displacement of injection pipette and the objective, respectively. The tip position on the pixel frame is detected. The objective is moved with known steps by controlling the stepping motor and saving corresponding images with different degrees of focus on the host computer for

Figure 4.9. Procedure to perform plant cells injection based on indirect depth estimation. Image sharpness also refers to the degree of focus of an image.

sharpness computation. The objective displacement, d_α , where the cell image can be focused is associated to the maximum magnitude value of the image sharpness. Hence, the value of depth d is estimated accordingly by

$$d = \kappa_{n2}d_\alpha/\kappa_{n1} \quad (4.27)$$

Since the degree of focus of the tip image is changing from focused to defocused and followed by traveling a distance of d to re-focus the tip image, the coefficients of κ_{n1} and κ_{n2} represent the decreasing image sharpness against the displacement of injection pipette and objective, respectively.

After the depth estimation and identification of the target cell, the objective

Figure 4.10. The tip of the injection pipette is detected with the background of defocused plant cells.

Figure 4.11. Illustration of the pre-injection position. The final shot is executed by the piezoelectric stages by moving $2\Delta d$ along its x-axis with an angle of 30 degrees (Han *et al.*, 2008).

travels back to focus the tip in order to move the injection pipette above the target cell using the image-based visual servoing. The injection pipette travels down by $d - \Delta d$ to position at the pre-injection point. The final shot is executed by the piezoelectric stages by moving $2\Delta d$ along its x-axis with an angle of 30 degrees (Han *et al.*, 2008), as shown in Fig. 4.11. In the experiments, an experience value of $20 \mu\text{m}$ is used for Δd .

The reasons to complete the injection using the piezoelectric stage are:

(a) Unlike a single cell, it is difficult to inject one plant cell from arrays of cells horizontally by changing the tip of the injection pipette or cell holding device.

(b) For the dimension constrain of the working space, the traveling range of the piezoelectric stage is $100\text{ }\mu\text{m}$ which is smaller than the depth between the target cell and the tip.

During the experiments, changing sequence of the objective positions and the associated images content are illustrated in Fig. 4.12. Defocused projection of the injection pipette tip are represented by dash lines.

4.3.2 Results

Fig. 4.13 shows the distribution of the image sharpness values against the objective positions. The objective is moved in an interval of $10\text{ }\mu\text{m}$ and the image sharpness is quantified using Tenengrad method. In Fig. 4.13, the maximum value of the sharpness corresponds to the frame 33 which has the clearest plant cell image based on the observation of the images on the host computer.

A defocused plant cell and focused micropipette are captured when the autofocusing process starts to estimate the value of depth. After the value of depth is obtained, the micropipette is moved to the pre-injection position and both target cell and micropipette can be captured in the image, as shown in Fig. 4.14.

Table 4.2 shows the result of depth estimated from the displacement of objective.

Figure 4.12. Changing sequence of the objective positions and the associated images content in the field of view. In (a), the tip of the injection pipette is focused. In (b), the displacement of the objective is computed to focus the plant cell. In (c), the tip is re-focused to place it at the target cell position using image-based visual servoing. In (d), the tip is positioned at the pre-injection position by traveling down $d - \Delta d$.

4.3.3 Discussion

The experimental results demonstrate that the value of depth ranges from $300 \mu\text{m}$ to $400 \mu\text{m}$ subjected to the initial position of the objective which is positioned by

Figure 4.13. Magnitude of sharpness for plant cell images at different objective position. Tenengrad method is used to find the best focused image and the corresponding objective displacement, as its less complex computation for texture content images.

Figure 4.14. Image content when the autofocusing process finishes. Micropipette is located at the pre-injection position.

manual adjustment. A clear tip image with a defocused plant cell as the background is confirmed as the initial position which is achieved by manual adjustment.

Table 4.2. *Depth estimation in plant cell injection based on autofocusing algorithm. The objective is controlled by a stepper motor attached to the fine knob of the microscope with a resolution of $0.5 \mu\text{m}$*

<i>Displacement of objective</i> (μm)	κ_{n1}	κ_{n2}	<i>Depth</i> (μm)	Difference (μm) (%)	
330	-14.82	-14.12	314.41	15.59	4.96
330	-14.53	-13.87	315.01	14.99	4.76
330	-14.77	-13.99	312.57	17.43	5.58
330	-13.91	-13.10	310.78	19.22	6.18
320	-13.96	-13.12	300.75	19.25	6.40

Table 4.2 shows that the difference between the estimated depth and the displacement of the objective is around $20 \mu\text{m}$ which is within 10 % of estimated depth. The relatively small difference is due to the times of magnification of the objective. As the magnification time increases, the difference between κ_{n1} and κ_{n2} is reduced. For example, for 4 times magnification, we have $\kappa_{n2}/\kappa_{n1} = 0.61$ while we have $\kappa_{n2}/\kappa_{n1} = 0.95$ for 20 times magnification under the same experimental condition. In this work, 20 times is used for the plant cell injection and 4 times is used for the single cell strain control.

As the depth of field of the objective is $10 \mu\text{m}$, the objective is also moved in the same interval, i.e. $10 \mu\text{m}$, to find the best focused image. Experimental results show that $10 \mu\text{m}$ accuracy is sufficient for the plant cell injection. Visual inspection of the cell piercing is considered as the successful injection. However, if the specimen is changed to a cluster of small cells with only a few micrometers in diameter, the step of the objective travels should regulate accordingly.

The above depth estimation procedure can be achieved via offline calibration to ensure the automatic injection or strain control in the next stage. But, it is necessary to adjust the starting position and estimate the depth for the injector when the sample cell is changed. Because the tip should be moved up to give enough

space for demounting and mounting the microscope slide to avoid damaging the tip.

4.4 Contributions

A depth estimation method is realized by the vision guided robotic cell micromanipulation system. The proposed indirect estimation method is capable of positioning the tip and the target cell in the same focal plane by adjusting the position of the objective without taking any risk of damaging the fragile tip.

Chapter 5

Real-time Control of Cell Membrane Strain

5.1 Definition of Cell Membrane Deformation

Cell membrane deformations can be approximated by different means. For a circular cell, Sun uses three parameters to describe the indented cell membrane shape, the radius of the semicircular curved surface, the radius and depth of the indentation created by the micromanipulator (Sun *et al.*, 2003). To sufficiently describe the deformability of the cell membrane, these three values must be extracted from the image. Tan suggests to use cylindrical coordinates to describe the contact region and noncontact region of deformed cells (Tan *et al.*, 2008). Segments are considered distinctly in each region. Ordinary differential equations are derived from the equilibrium equation in the contact and noncontact regions. A complex numerical computation procedure is required to solve the equations.

Since the objective of measuring the cell membrane deformation in this work is to control the cell strain, i.e. changes of the deformation, less variable should be introduced to reduce the complexity of the strain. We propose another estimation

method to quantify the indentation deformation and simplify the strain model.

Fig. 5.1 shows the comparison between the original cell membrane and the deformed cell membrane. As the micropipette presses on the cell towards the center of the cell along the horizontal axis, the membrane is strained and stressed along the vertical and horizontal axes, respectively. Based on this phenomenon, we estimate the cell deformation from both macro and micro aspects. The peripheral deformation which is plotted in the gray solid line in Fig. 5.1 represents the change of cell shape in the macro manner. The local deformation plotted in the dark solid curve describes the deformed membrane in the micro manner. The intersections of the two curves are determined by the traveling distance of the micropipette. The connection between the intersection and the destination of the micropipette is parallel with the x-axis.

Figure 5.1. Comparison between the original and deformed single circular cell membranes. The peripheral and local deformations are described by the gray solid line and dark solid line, respectively.

Consider a denotes the length of the vertical axis of the cell membrane and it will be stretched to a' after being deformed. The y -axis of the membrane will be

compressed from b to b' . The peripheral strain is defined by

$$\varepsilon = \left| \frac{\Delta a}{a} \right| + \left| \frac{\Delta b}{b} \right| \quad (5.1)$$

where

$$\varepsilon_1 = \frac{|a' - a|}{a} \times 100\% \quad (5.2)$$

represents the cell strain in vertical direction.

$$\varepsilon_2 = \frac{|b' - b|}{b} \times 100\% \quad (5.3)$$

represents the cell strain in horizontal direction. ε represents the changes of cell profile in both vertical and horizontal directions.

To calculate the length of the vertical and horizontal axes, an ellipse is employed to fit the peripheral deformation using the least square method. As shown in Fig. 5.1, the major axis of the ellipse represents the length of the vertical axis of the cell membrane. The minor axis represents the length of the horizontal axis. Comparing to other geometries, the reasons of using ellipse are listed as follows:

- (i) The initial status of the circular cell membrane can be exactly estimated by ellipse.
- (ii) When the cell is pressed using a micropipette, the shape of the ellipse is a more accurate approximation of the actual profile of the peripheral deformation.
- (iii) The mathematics is less complex so that it reduces the computational time of the image processing algorithm.

Then, the local deformation is estimated by zooming in the contact area between the cell and the micropipette, i.e. the dark solid curves shown in Fig. 5.1.

The parabola is used to model the segment at the indentation area. The concavity with respect to the displacement of the micropipette tip is used to estimate the local deformation. The parabola may be written as

$$v = pu^2 + qu + r \quad (5.4)$$

where p represents the concavity of the parabola, (u, v) is the pixel coordinates of the local deformed curves, $(-q/2p, (4pr - q^2)/4p)$ is the coordinate of the vertex. The local deformation is approximated using parabola at different indentation depth in Fig. 5.2. As the displacement of the micropipette increases, the concavity of the parabola increases. While the coefficients of q and r are related to the position of the vertex, the concavity of the parabola is only determined by p . Therefore, the local strain is represented by

$$\gamma = \frac{|p' - p|}{p} \times 100\% \quad (5.5)$$

where p' is the concavity after deformation.

Figure 5.2. Magnification of the local deformation at different indentation depth. The deformed cell membrane shown in this figure is fitted by parabola p_4 .

Since the micropipette is controlled to move towards the center of the cell along the horizontal direction, the local deformation curves may be considered to be symmetrical. Therefore, only one of the local deformed curves is analyzed here. By modeling both the left and the right local deformed curves, as shown in Fig. 5.3, Table 5.1 shows that the difference between the left and the right curves throughout the experiments is within 10%.

The peripheral and local strain are then combined, $[\varepsilon, \gamma]^T$, to denote the cell membrane strain. The next section will introduce how to detect the peripheral and local boundary by real-time image processing. Advantages of this cell strain estimation method are listed as follows:

Figure 5.3. Modeling both the left and the right local deformed curves of a single circular cell.

- (i) The shape of the deformed cell membrane is comprehensively estimated by measuring the deformed membrane in both macro and micro manner.
- (ii) Parameters used to represent the cell strain are easy to compute from the images.
- (iii) Provided that the position of the micropipette and the original dimension of the cell membrane are known, the shape of the deformed cell membrane can be recovered from the value of ε and γ .

5.2 Detection of Cell Membrane Deformation

5.2.1 Challenge of Real-Time Boundary Extraction

The feasibility of the cell strain control is determined by the accuracy of the micropipette displacement, the accuracy of the cell strain parameters calculation, i.e. changes of cell shape parameters and the synchronization of the displacements

Table 5.1. *The symmetry test of the left and the right curves throughout the experiments. The difference can maintain within 10%. Therefore, symmetric deformation is assumed.*

<i>Indentation depth (μm)</i>	<i>p_{left}</i>	<i>p_{right}</i>	<i>Difference</i>
0	0.0048	0.0052	8.3%
30	0.0058	0.0063	8.6%
60	0.0068	0.0074	8.8%
90	0.0074	0.0081	9.5%
120	0.0080	0.0085	6.3%
150	0.0083	0.0086	3.6%
180	0.0084	0.0087	3.6%
210	0.0085	0.0088	3.5%
240	0.0086	0.0089	3.5%

and the resulting strain changes of the cell membrane. Currently, cell deformation images are recorded and processed off-line in which cell boundary is manually measured by adjusting certain thresholding parameters or simply taking the displacement of the micropipette as the deformation. Cell boundary measurements are tedious for human operators, making the cell deformation modeling a time consuming task. Moreover, off-line measurements produce significant errors in the subsequent strain control process due to the poor synchronization of cell strain measurements and micropipette displacement.

Conventionally, an edge detection method is used to find the object boundary. The optimal Canny edge detector suppresses the non-maxima gradients of an image that convolved with first derivative of Gaussian. Weak edges are eliminated by hysteresis thresholding with two constant threshold values. The high threshold, T_h , decides the starting point of the strong edges and the edge tracking continues until the gradients fall below the low threshold, T_l . This method can extract the cell boundary directly for the majority of images. However, it fails to work in some situations and produces lots of breaks on the edge, as shown in Fig. 5.4 (c), due to

the changes of the brightness and the degree of focus when the micropipette moves into the cell.

One approach to solve this problem is to frequently adjust these two constant threshold values. Another approach is to build an edge linking criteria which is analyzed from the characteristics of pixels in a small neighborhood of edge points. Magnitude and direction criteria are two principal properties used for establishing similarities of two pixels. The process is repeated at every location in the interested region. Instead of establishing an edge linking criteria, Lu defines a searching region around the deformed membrane and extracts points on the deformed curve whose gray level exceed a threshold (Lu *et al.*, 2009). These two strategies are summarized in Fig. 5.5. The drawback of the above methods is that the existence of tuning parameters in the visual module impedes the implementation of the real-time cell strain modeling and control.

Image thresholding algorithms can also be used to directly extract the cell contour rather than starting from edge detection followed by a series of operations which require adjusting parameters. Otsu's method is one of the most popular conventional methods for image global thresholding by maximizing the between-class variance without tuning any parameters. But Otsu's method does not always produce desired results, as shown in Fig. 5.4 (d). Although the thresholding value can be manually adjusted to extract the local deformed curves for a specific image, the contact portion of the micropipette and the membrane is totally blind, as shown in Fig. 5.6 (c).

A local thresholding method is superior to the global methods for images with uneven brightness. However, the majority of the local thresholding methods have manually tuned parameters that need to be adjusted for different images by trial and error method (Niblack, 1986; Sezgin, 2004).

In this chapter, we propose a real-time machine vision algorithm to track the coordinates of the boundary points without off-line tuning parameters and a

(a) Original image. Pixel size is $3.571 \times 3.571 \mu\text{m}$ with $4\times$ magnifications and the C-mouse connector, which is mounted between the camera and the microscope.

(b) Cropped image.

(c) Canny detection.

(d) Otsu thresholding.

(e) Recommended method.

Figure 5.4. Problems by using conventional method to extract the cell boundary.

Figure 5.5. Procedures of extracting the points on the cell boundary off-line.

(a) Original image.

(b) Otsu thresholding.

(c) Otsu thresholding after adjusting.

(d) Recommended method.

Figure 5.6. Problems by using conventional thresholding method.

Kalman filter algorithm is developed to distinct the deformed local curves in the contact area between the micropipette and the cell membrane.

5.2.2 Extraction of Deformed Cell Membrane Boundary

Fig. 5.7 shows the flow chart of the proposed cell boundary extraction process. First of all, the cell images are processed using adaptive thresholding algorithm. The proposed adaptive image thresholding method is able to apply on all the images captured in the deforming process to extract the deformed cell boundary in real-time. The coordinates of the boundary points are then located by boundary tracing.

Figure 5.7. Flow chart of proposed real-time cell boundary extraction method.

An adaptive local image thresholding algorithm based on energy minimization is applied on the cell images. The weighting parameters for the data and the regularization terms in the cost function are solved using variational minimax optimization which does not require manual adjustments (Saha and Ray, 2009). The numerical implementation is modified to find the solution in less iterations which improves the efficiency of the algorithms.

Let $I(u, v)$ and $T(u, v)$ represent the two dimensional image and threshold surface functions. The intersection of $I(u, v)$ and $T(u, v)$ is considered to be the object boundary, as shown in Fig. 5.8.

Figure 5.8. Segment an image using adaptive threshold surface. The gray level that above the threshold surface are considered as the object.

The cost function is in the form of,

$$E(T, \alpha) = \sqrt{1 - \alpha^2} E_1(T) + \alpha E_2(T) \quad (5.6)$$

where

$$E_1(T) = \frac{1}{2} \int \int h(u, v) (I(u, v) - T(u, v))^2 du dv \quad (5.7)$$

$$E_2(T) = \frac{1}{2} \int \int |\nabla T(u, v)|^2 du dv \quad (5.8)$$

E_1 is the data term, which includes an edge indicator $h(u, v) = \frac{|\nabla I(u, v)|}{\max(|\nabla I(u, v)|)}$. Minimization of $E_1(T)$ requires the threshold surface to intersect the original image at high gradient places. $E_2(T)$ is the regularization term. Minimization of $E_2(T)$ helps to smooth the threshold surface. $\alpha \in [0, 1]$ is the weighting parameter that balances the contribution of $E_1(T)$ and $E_2(T)$. If more weight is put on the data term, the threshold surface may suffer from discontinuity and spurious segmentation. If more weight is put on the regularization term, the image may result in under segmentation.

It is noticed that the cost function E is a strictly concave function with respect to α and a strictly convex function with respect to T . Thus, the optimum threshold surface function is computed using the minimax principle by

$$T^* = \min_T \max_{\alpha} E(T; \alpha) \quad (5.9)$$

The maximum value α is calculated by $\frac{\partial E}{\partial \alpha} = 0$. The energy value E is minimized using gradient descent technique to find the optimum T . The solution is written as follows,

$$\alpha^* = \frac{E_2(T)}{\sqrt{(E_1(T))^2 + (E_2(T))^2}} \quad (5.10)$$

$$\frac{\partial T}{\partial t} = \sqrt{1 - (\alpha^*)^2} (h(u, v)(I(u, v) - T(u, v))) + \alpha^* (\nabla^2 T(u, v)) \quad (5.11)$$

Procedure to optimize the cost function is shown in Fig. 5.9. The original image is assigned as the threshold surface for initialization. E_1 , E_2 and α^* are calculated via equations (5.7), (5.8) and (5.10), respectively. The threshold surface function is updated using equation (5.11) until the cost function is minimized to the predefined tolerance or reaches the maximum iterations.

Figure 5.9. Procedure to optimize the cost function E .

Explicit numerical method is used to update T via equation (5.11):

$$T_{r,c}^{i+1} = T_{r,c}^i + \tau \sqrt{1 - (\alpha^*)^2} (h_{r,c}(I_{r,c} - T_{r,c}^i)) + \tau \alpha^* (T_{r+1,c}^i + T_{r-1,c}^i + T_{r,c+1}^i + T_{r,c-1}^i - 4T_{r,c}^i) \quad (5.12)$$

where τ is the step length for successive updates, i is the number of iterations, r and c represent the row coordinate and column coordinate of the image. The steepest descent technique is implemented to find the optimum value of τ at each iteration (Saha and Ray, 2009). The search direction at iteration k is defined as

$$\Delta T_{r,c}^k = \sqrt{1 - (\alpha^k)^2} (h_{r,c}(I_{r,c} - T_{r,c}^k)) + \alpha^k (T_{r+1,c}^k + T_{r-1,c}^k + T_{r,c+1}^k + T_{r,c-1}^k - 4T_{r,c}^k) \quad (5.13)$$

The step length τ^k is determined by minimizing $E(T^k + \tau \Delta T^k)$ with respect to τ^k using line search strategy (Fletcher, 1987). Substitute $T = T + \tau \Delta T$ into equation (5.6), we have

$$E(T + \tau \Delta T) = \frac{\sqrt{1 - (\alpha^k)^2} h(u, v)}{2} \sum (I - T - \tau^k \Delta T)^2 + \frac{\alpha^k}{2} \sum \|\nabla(T + \tau \Delta T)\|^2 \quad (5.14)$$

By rearranging the above equation, we obtain

$$E(T + \tau \Delta T) = M_2 (\tau^k)^2 + M_1 \tau^k + M_0 \quad (5.15)$$

where

$$M_2 = \frac{\sum [\sqrt{1 - (\alpha^k)^2} h(u, v) (\Delta T)^2 + \alpha^k \|\nabla \Delta T\|^2]}{2}$$

$$M_1 = \sum [\alpha^k (\nabla T \cdot \nabla (\Delta T)) - \sqrt{1 - (\alpha^k)^2} h(u, v) (I - T) \Delta T] \quad (5.16)$$

$$M_0 = \frac{\sum [\alpha^k \|\nabla T\|^2 + \sqrt{1 - (\alpha^k)^2} h(u, v) (I - T)^2]}{2}$$

Minimizing E by $\frac{dE}{d\alpha^k} = 0$, we can get the search step

$$\tau^k = -\frac{M_1}{2M_2} \quad (5.17)$$

Fig. 5.10 shows the changes of step length by implementing above optimization procedures on a cell-micropipette image. Although the algorithm requires no adjustment of parameter during the operation, the number of iterations will effect the efficiency of the machine vision algorithm to find the cell boundary. Hence, the step length to update the threshold surface is modified to speed up the minimization process of the cost function E .

Figure 5.10. Search steps for 50 iterations.

It is noticed that large changes of step length τ actually result in more adjustment of the energy value to reach its minimum. Therefore, the value of τ is modified according to its previous changes. When

$$\tau^k - \tau^{k-1} \geq \tau_T \quad (5.18)$$

the update of threshold surface is slow down by

$$\tau_m^k = \tau^k / p_\tau \quad (5.19)$$

Meanwhile, it also has $\tau_m^k \geq 0.25$ at every iteration according to the convergence analysis of the explicit method (Evans *et al.*, 1999). The value of E updating through τ^k and τ_m^k are compared in Fig. 5.11, where $\tau_T = 0.5$ and $p_\tau = 2$. The efficiency of the optimization process has improved. The energy value E , which takes 16 iterations to reach by τ^k , requires 11 iterations by using updating step of τ_m^k . It takes less than 70% time to extract the boundary from the cell image. The constrain of $\tau_m^k \geq 0.25$, $1 \leq p_\tau \leq 3$ is used in the experiments as the improvements is not obvious for a larger value of p_τ .

Fig. 5.6 (d) shows the processed image by the adaptive thresholding method. Before the micropipette presses into the cell yolk, the coordinates of the deformed

Figure 5.11. Optimization process in different updating steps.

cell curve can be extracted by tracing the pixels with the gray level of 1 from a specific starting point and direction. For example, the tracing starts from the rightmost point on the last column with the gray level of 1 towards west direction to locate the coordinates of the right deformed local curves. The micropipette position is obtained by tracing the tip boundary from the rightmost point on the last row with the gray level of 1 towards north direction. The intersection of the above two curves are the position of the micropipette, as shown in Fig. 5.12.

One problem to find the coordinates of the deformed cell membrane is to separate the micropipette tip from the deformed curves, when the tip presses into the cell yolk. Tracing the pixels with gray level of 1 cannot distinguish the deformed local curves and tip position from the yolk. Thus, the local deformed curve cannot be entirely extracted, as shown in Fig. 5.13 (a).

A Kalman filter tracking algorithm is developed to detect the tip position from its surrounding noisy pixels and the deformed local curves is extracted further according to the tip position. In other words, the Kalman filter is not used to do tracking, but to detect the tip position which is blocked by the cell yolk. A state

Figure 5.12. The intersection of the local curve and the tip boundary is the tip position when the micropipette does not press into the cell yolk.

vector

$$X_t = \begin{bmatrix} u_t & v_t \\ u_{t-1} & v_{t-1} \\ u_{t-2} & v_{t-2} \end{bmatrix} \quad (5.20)$$

is used to represent the estimated position of the micropipette tip in the last three images as the tip still outside the cell yolk. Assuming the micropipette is moving with constant acceleration, a transition matrix is used to model the dynamics of the tip position.

$$A = \begin{bmatrix} \lambda_a & -\lambda_a & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} \quad (5.21)$$

where $\lambda_a(\mu\text{m}/\text{pixel})$ is defined as the pixel size under the current magnification.

The predicted state X'_t given by the Kalman step is

$$X'_t = AX_{t-1} \quad (5.22)$$

Updating of X_t is conducted according to

$$X_t = X'_t + K_t(z_t - HX'_t) \quad (5.23)$$

(a)

(b)

Figure 5.13. Extract the deformed curve in the contact area of the micropipette and the cell membrane.

where $K_t = (1, 0, 0)^T$ is the Kalman gain, $\mathbf{z}_t = (u_z \ v_z)$ is the tip position measured from the actuator and $\mathbf{H} = (1 \ 0 \ 0)$ (Maybeck, 1979). Finally, the current real tip position is determined by

$$\mathbf{z}'_t = \mathbf{H} \mathbf{X}_t \quad (5.24)$$

Fig. 5.14 shows the tip position detected by the Kalman filter tracking algorithm. By combining coordinates of the tip position and the deformed local curves outside the cell yolk, the parabola curve is fitted using least square method. As shown in Fig. 5.13 (b), the deformed local curves can be entirely extracted and measured when the tip is pressed into the cell yolk.

(a)

(b)

Figure 5.14. Tip position detected by the Kalman filter.

The following three steps summarize the automatic cell boundary detection scheme:

(i) Transform gray scale image to the binary image based on the adaptive thresholding method with the modified updating step for threshold surface. Let the gray value of the foreground and background be 1 and 0, respectively.

(ii) Determine the starting and the end points of the peripheral boundary (PB) and the local boundary (LB). In Fig. 5.15, PB is composed of two curves, $\widehat{AB}$ and $\widehat{CD}$. The starting points of the PB, i.e. A and C , are around the contact point of the holding pipette and the cell. Since the position of the holding pipette is known and fixed as (u_h, v_h) , the starting points A and C are defined as the first

and last point on the column of $v_h + 5$ with a gray value of 1, respectively. The end points, B and D , are the same as the starting point of the LB.

The column coordinates of B and D are the same as the termination of the micropipette. Therefore, the row coordinates for B and D are the first and last point on this column with a gray value of 1. The end point of the LB is acquired from the tip of the micropipette.

(iii) Trace the boundary curves from every starting point towards their end points. Pixels with value of 1 are recorded.

Boundaries of the cell membrane are extracted in Fig. 5.15 by implementing the proposed strategy. Real-time LabVIEW platform is adopted for the experiments. The processing speed is 2 frames/second for the peripheral deformation and 10 frames/second for the local deformation.

Figure 5.15. Results of using the proposed real-time cell boundary detection scheme. The inverse image is shown for the presentation purpose.

5.3 Micromanipulation on Zebrafish Embryos

Zebrafish embryos are used as the sample to qualify the feasibility of the proposed cell membrane strain modeling and control system. The zebrafish are bred and embryos are prepared in standard condition (27°C , $\text{PH}=7.0$) and procedures according to (Westerfield, 2000).

Figure 5.16. Embryonic developmental stages of the zebrafish, adopted from bioimages of department of biological sciences of Vanderbilt University. (a) Zygote stage. (b) Partial cleavage. (c) Cleavage stage. (d) Blastula stage. (e) Oblong stage. (f) Gastrula stage.

Zebrafish is considered as the model organism for vertebrates. Their body structure is similar to humans with a neural tube, muscles, a vascular system and a beating heart. They also have significant genetic similarities with humans in genes and pathways (Stainier, 2000; Lodish *et al.*, 2004). Recently, zebrafish is also employed as a model for investigation of cancer, neurobiology, infection and genetic diseases of the muscle (Deiters and Yoder, 2006). In addition to these, zebrafish embryos have the advantage of rapid developmental cycles and it is easy to observe their growth in different developmental stages.

The dimension of zebrafish embryos range from 700 to 1300 μm in diameter which is an appropriate size to test the feasibility of micromanipulation technologies. The embryogenesis is classified into broad stages of zygote, cleavage, blastula, gastrula, segmentation, pharyngula and the hatching periods. Fig. 5.16 shows the embryonic developmental stages of the zebrafish. The developmental condition can be check in a few hours after the experiments.

Fig. 5.17 shows the biological cycle of a zebrafish embryo (Kimmel *et al.*, 1995). It takes 3 – 4 months for a embryo developing to an adult fish.

Figure 5.17. Biological cycle of a zebrafish, from embryo to adult fish (Kimmel *et al.*, 1995).

5.4 Experiments on Real-Time Cell Strain Measurement

5.4.1 Experimental Procedure

Embryos are divided into two hemispheres: the animal pole and the vegetal pole. Since the animal pole forms the primary germinal epitheliums during animal embryogenesis and eventually generates the organs of the fish, the deformation is produced at the vegetal pole to enhance the survival rate of the embryo, as shown in Fig. 5.18.

Figure 5.18. Experiments on different embryonic developmental stages of the zebrafish. Cell division takes place above the yolk. (a) Experiment on zebrafish embryo at cleavage stage. (b) Experiment on zebrafish embryo at blastula stage.

The following steps are conducted to collect the experimental embryos:

- (a) Select one female zebrafish with broad abdomen and one male fish.
- (b) Separate them with a partition and keep them in the dark environment for 12 – 14 hours.
- (c) 12 – 14 hours later, remove the partition and switch on the light.
- (d) Zebrafish will chase each other and lay embryos in around 40 minutes.

(e) Separate the zebrafish from the embryos and transfer the embryos to a clean petri dish.

Experimental procedure is illustrated in Fig. 5.19. The embryo is immersed into culture medium and fixed by the holding pipette. Manually focus the embryo and move the tip of the micropipette close to the embryo. The depth between the tip and the embryo is determined by the proposed indirect depth estimation method. The proposed automatic boundary detection method is used to detect the tip of the micropipette. Then, the micropipette is moved to the starting position using the image-based controller and starts the deformation process. Images are processed to measure the cell strain and compute the parameters in the strain model in real-time.

Figure 5.19. Procedures to measure the cell strain.

5.4.2 Results

Table 5.2 shows the results of depth estimation. The coefficients κ_{p1} and κ_{p2} describe the relationship of image sharpness with respect to the displacement of

the micropipette and the objective of the microscope, respectively.

Table 5.2. *Depth estimation for controlling of the cell membrane strain. The depth between the target cell and the tip is estimated by the displacement of the objective using the indirect estimation method.*

<i>Displacement of objective</i> (μm)	κ_{p1}	κ_{p2}	<i>Depth</i> (μm)	Difference	
				(μm)	(%)
1310	2.30	1.50	854.35	455.65	53.33
1510	2.16	1.42	992.69	517.31	52.11
1330	2.55	1.61	839.73	490.27	58.38
1430	2.33	1.51	926.74	503.26	54.30
1320	2.51	1.55	815.14	504.86	61.94

Fig. 5.20 shows the tip position detected based on the proposed boundary extraction method and the starting point of stressing, which is the right vertex of the horizontal axis of the fitted ellipse.

The automatic cell boundary detection scheme is implemented to test the accuracy of the proposed peripheral and local deformation model. Fig. 5.21 shows the fitting ellipse based on the extracted points on the peripheral boundary and the parabola fitted from the extracted points on the local boundary.

Table 5.3 summarizes the modeling results on 10 embryos.

5.4.3 Discussion

Table 5.2 shows that the difference between the estimated depth and the displacement of the objective is from 50% to 60% which further proves that the displacement of the objective cannot be considered as the distance that the micropipette travels to get the focused tip image. Table 5.3 shows that the average R-square value of using ellipse to model the peripheral deformation is 0.96. The average R-square value of using parabola to model the local deformation is 0.99.

Figure 5.20. Illustration of the tip position and the starting point of stressing. The tip position is detected based on the boundary extraction method, while the starting point is the right vertex of the horizontal axis of the fitted ellipse.

The maximum error of ellipse estimation for peripheral deformation is within 2.5% and parabola estimation for local deformation is within 5%. The experimental results show that the shape of the deformed cell can be recovered by combining the estimated peripheral boundary and local boundary.

As the image sharpness is affected by the changes of image brightness, the coefficients κ_{p1} and κ_{p2} are required to be recalibrated in the depth estimation step when the cell is changed.

One of the factors that determining the feasibility of the proposed cell boundary extraction algorithm is the image brightness. Noisy pixels will be produced if the background is too dark, which affects the accuracy of the extracted boundary. This phenomenon happens when the cell is placed on the microscope slide without or with less solution. Therefore, both the cell and the tip must immerse into enough solution throughout the experiments to provide a bright

Figure 5.21. Shape of the deformed embryo can be recovered by combining the estimated ellipse and parabola. The cell membrane is deformed by the cell strain control system.

background for the object, i.e. the cell and the tip. The proposed real-time cell strain measurement method is capable of detecting the indentation deformation of a circular cell with the diameter of hundreds micrometers. When a smaller cell whose diameter is within $100\text{ }\mu\text{m}$ is used, the same methodology can be used to extract the boundary and compute the cell membrane strain with recalibration of pixel size for transition matrix in equation (5.21) and changes of the size of the holding pipette and deforming micropipette.

5.5 Modeling of Cell Membrane Strain

In Section 5.1, $\phi = [\varepsilon, \gamma]^T$ is used to represent the cell membrane strain. To proceed the control action on the cell strain in the next stage, the magnitude,

Table 5.3. *Experimental results of modeling changes of cell shape using peripheral and local deformation on 10 embryos. By assuming the estimated column positions of the deformed curves are identical to the actual column positions, the root mean square error is computed by $RMSE = \sqrt{\frac{\sum (u_{e,i} - u_{a,i})^2}{n_u}}$, the max error is computed by $\max\{|\frac{u_{e,i} - u_{a,i}}{u_{a,i}}|\}$, where u_e and u_a represent the estimated and actual row positions of the deformed curves, respectively. n_u is the number of pixels on the deformed curves.*

Cells	Peripheral deformation		Local deformation	
	RMSE(pixels)	Max Error(%)	RMSE(pixels)	Max Error(%)
1	4	1.7	0.5	3.5
2	3.5	1.6	0.5	2.5
3	3	1.9	0.5	3.2
4	3	1.6	0.5	2.1
5	3.5	1.5	0.5	4.5
6	3	1.5	0.5	2.2
7	3.5	1.7	0.5	2.2
8	3.5	1.8	0.5	3.5
9	4	1.8	0.5	4.0
10	4	2.3	1	5.0

$\phi = (\varepsilon^2 + \gamma^2)^{1/2}$, is used to quantify the changes of cell strain and model with respect to the displacement of the end-effector by the second order polynomial, in the form of,

$$\phi = m_2\omega^2 + m_1\omega + m_0 \quad (5.25)$$

where ω is the depth that the end-effector traverses into the cell membrane, m_i with $i = 0, 1, \&2$, can be computed from a set of images with associated cell strain and corresponding depth.

The advantages of the proposed model are:

- (a) The model can be derived for individual cell membrane in a real-time

experiment regardless changes of cell membrane mechanical properties.

(b) The inverse model can be acquired from straightforward numerical computations which will determine the cell strain controller in the subsequent phase.

5.6 Control of Cell Membrane Strain

Based on the polynomial model obtained in (5.25), the strain controller is computed based on the inverse model $f^{-1}(\omega, \phi)$,

$$u_\phi = f^{-1}(\omega, \phi)\phi_d \quad (5.26)$$

where u_ϕ is the actual position of the end-effector, ϕ_d is the desired strain. However, the control task cannot be successfully achieved if it only relies on open-loop strain controller. The cell strain model shows discrepancy even for the same cell as the time changes. Accuracy of the strain model can affect the output strain significantly.

To manage the unforeseen errors during the control process, an addition of feedback proportional controller, represented by K_s , is integrated in the system. The block diagram of the cell strain control system is shown in Fig. 5.22. The system employs the knowledge of the strain model through the feedforward input and accounts for errors using the feedback. The objective is to minimize the strain error e_s within the predefined tolerance.

A three degrees of freedom motorized translational stage is the actuator of the system, which will drive the motion of the end-effector. Its travel distance is controlled by the strain controller and the current strain error. On the feedback loop, the cell strain is measured using the real-time machine vision algorithm proposed in Chapter 5 to obtain the current strain error and adjusts the motion of the end-effector. The end-effector will be accurately positioned on the pixel

Figure 5.22. Block diagram of the cell strain control system. An addition of feedback proportional controller, K_s , is integrated in the system to manage the uncertainties of the strain model. The image-based controller in the inner loop accurately position the end-effector on the pixel frame.

frame by the image-based controller presented in Chapter 4. The control strategy is written as,

$$\omega_{real} = (f^{-1}(\omega, \phi) + K_s)(\phi_d - \phi_a) \quad (5.27)$$

where ω_{real} , the real position of the end-effector, is the control variable, ϕ_d and ϕ_a are the desired and actual measured cell strain, respectively.

The sequence to realize real-time cell strain control is summarized as follows,

The cell membrane is stressed by moving the end-effector with a sinusoidal profile. A series of images are captured and processed in real-time to measure the strain of the cell membrane. The model of the cell strain with respect to the displacement of the end-effector is obtained and used to compute the strain controller. Given a desired profile of cell strain, the cell membrane is deformed to the desired value according to the proposed control scheme.

Figure 5.23. The sequence of the real-time cell strain control, from identification to control.

5.7 Experiments on Real-Time Cell Strain Modeling and Control

5.7.1 Experimental Results

In the real-time cell strain control process, a micropipette with a tip outer diameter of $100\ \mu\text{m}$ is used to indent the cell. The objective is to deform the local strain of the embryo in a pre-defined sinusoidal profile.

Fig. 5.24 shows the modeling of cell strain with respect to the micropipette displacement. The maximum displacement is $300\ \mu\text{m}$ to avoid penetration of cell membrane. The numerical inverse of the model is then directly computed to acquired the control input.

The cell strain controller is calculated based on the model parameters and the proposed strain control scheme is implemented to generate desired strain to the zebrafish embryo. The sampling rate of the system is 10 Hz due to the strain

calculation in the visual module.

Fig. 5.25 plots strain control results on one embryo. It shows that the controlled strain can trace the desired strain quite well. Fig. 5.26 shows the deformation of zebrafish embryos membranes during the control process. Errors of cell strain control on ten zebrafish embryos are shown in Table 5.4. The maximum normalized RMSE and maximum error on 10 set of experiments are summarized in Fig. 5.27.

Table 5.4. *Experimental results of controlling the cell strain on 10 embryos. s_d and s_a represent the desired and actual strain, respectively. n_{s_d} represents the number of desired strains.*

<i>Cells</i>	<i>RMSE</i> $(\sqrt{\frac{\sum (s_{d,i} - s_{a,i})^2}{n_{s_d}}})$	<i>Normalized RMSE (%)</i> $(\frac{RMSE}{s_{a,max} - s_{a,min}})$	<i>Max Error (%)</i> $(\max\{ \frac{s_{d,i} - s_{a,i}}{s_{d,i}} \})$
1	0.2332	0.49	2.30
2	0.1996	0.42	2.09
3	0.2248	0.47	2.40
4	0.1290	0.27	1.12
5	0.0795	0.17	0.75
6	0.0799	0.16	0.48
7	0.1223	0.25	0.73
8	0.1792	0.37	1.97
9	0.1138	0.23	0.78
10	0.1766	0.36	1.52

5.7.2 Discussion

The experimental results show that the maximum error between the modeled and actual strain is 6.3%. The difference between the controlled and desired cell strain does not exceed 3%. In the experiments, we find that the parameters of the strain

model will change from one embryo from another and also for the same embryo in its different developmental stages, as shown in Fig. 5.28. This is due to the different mechanical properties or health conditions of the embryos. Therefore, the strain model must be identified before proceeding with the control process when the embryo is changed.

The proposed real-time strain measurement and control method can also be implemented on other types of circular cells with diameter from 350 μm to 1700 μm subjects to the pixel size of the camera and objectives magnification. By employing the objective with 20 times magnification, the camera is able to measure submicrometer changes.

Only one cycle of indentation deformation, i.e. one back and forth movement, is conducted to obtain the strain model to reduce the influence of the uncertainties brought by the elastic properties changes of cell membrane. The control procedure is carried out once the strain model is obtained. Therefore, the strain model needs to be recalibrated if the cell is used for a few set of experiments. The sampling rate of the control system is 10 Hz due to the real-time strain measurement in the feedback loop. The efficiency of the machine vision algorithm still needs to be improved to reach 24 Hz sampling rate.

5.8 Contributions

Real-time cell strain measurement is realized using the vision guided robotic cell micromanipulation system. The proposed methodology requires no manual adjustment of parameters, reduces the image processing time and is capable of detecting the blocked tip to improve the feasibility and efficiency of modeling the cell membrane deformation in real-time applications. A cell strain control scheme is proposed to manage the unforeseen error after the modeling process. The system is capable to model and control the deformation of the cell membrane in real-time.

(a) Comparison between the measured strain and the modeled strain.

(b) Modeling error, standard deviation $\sigma_m = 1.45$.

(c) Standard deviation of modeling errors for 10 zebrafish embryos.

Figure 5.24. Embryo strain is modeled with respect to the displacement of the tip using second order polynomial.

(a) Comparison between the desired strain and the actual controlled strain.

(b) Controlling error, standard deviation $\sigma_c = 0.6329$.

(c) Standard deviation of controlling errors for 10 zebrafish embryos.

Figure 5.25. Deforming the zebrafish embryo into desired strain. The strain model is identified using the proposed real-time strain measurement algorithm and the strain control scheme is utilized to stress the embryo into predefined strain.

(a) Deformed membrane with the strain of 24.11%.

(b) Deformed membrane with the strain of 50.74%.

Figure 5.26. Deformed membrane with the strain of 24.11% and 50.74%, respectively.

Figure 5.27. The maximum normalized RMSE and maximum error for 10 sets of experiments. Strain control on 10 embryos is conducted for every set of experiments. The mean value of maximum normalized RMSE and maximum error are 0.441 and 2.154, respectively. The standard deviation of maximum normalized RMSE and maximum error are 0.039 and 0.1326, respectively.

Figure 5.28. The cell membrane strain increases as the cell develops, i.e. the cell membrane becomes softer as it develops to the later stage.

Chapter 6

Conclusion and Future work

6.1 Conclusion

The dissertation has presented depth estimation, cell strain measurement and real-time cell strain control by using a vision guided robotic cell micromanipulation system.

Estimation the depth between the target cell and the micropipette tip is an important preliminary work before conducting any manipulation on biological cells. Chapter 4 has proposed a depth estimation microscopy based on autofocusing algorithms and image based visual servoing to guide the tip of the micropipette.

Off-line processing of cell deformation images is a tedious work for human operators and makes the real-time cell deformation control impossible. Chapter 5 has proposed a real-time machine vision algorithm to extract the feature parameters from the cell membrane images and model the change of cell membrane in real-time.

By using the real-time cell strain measurement method, Chapter 5 has also proposed a cell strain control scheme to model and control the cell shape into

desired strain.

A series of experiments have conducted from Chapter 4 to Chapter 5 to implement the proposed methodologies on both plant and animal cells using an automatic cell micromanipulation system.

6.2 Contributions

The major contributions of the dissertation are listed as follows:

1) Depth estimation in cell micromanipulation

A depth estimation microscopy is realized on the vision guided robotic cell micromanipulation system. The indirect estimation method is capable of positioning the tip and the target cell in the same focal plane by adjusting the position of the objective without taking any risk of damaging the fragile tip.

2) Real-time cell membrane strain measurement

Real-time cell strain measurement is realized using the vision guided robotic cell micromanipulation system. The proposed methodology requires no manual adjustment of parameters, reduces the image processing time and is capable of detecting the blocked tip to improve the feasibility and efficiency of modeling the cell membrane deformation in real-time applications.

3) Real-time cell strain modeling and control

A cell strain control scheme is proposed to manage the unforeseen error after the modeling process. The system is capable to model and control the deformation of the cell membrane in real-time.

6.3 Future Work

Based on this dissertation, potential future works are proposed to improve the current work:

1) Non-Contact Force Sensing

The relationship of the applied force and the cell strain could be characterized to model the viscoelastic behavior of cell by using a micro force sensor and the proposed real-time cell strain measurement. With the static and dynamic behavior of the biological cells, a non-contact force sensing technique may be developed. The vision-based force sensing method has great potential to improve the robustness and feasibility of micromanipulation tasks. This would overcome the engineering difficulty of integrating a micro force sensor with the end-effector, which limits the current robotic micromanipulation techniques (Sun and Nelson, 2002; Lu *et al.*, 2007; Shen *et al.*, 2007).

A few trials have been done on a dozen of zebrafish embryos and the experimental data shows that the relationship between the cell membranes reaction force and the cell strain can be modeled by using a power function in the form of,

$$f_r = \sigma_2 s^{\rho(c)} + \sigma_1 \quad (6.1)$$

where f_r is the applied force, σ_2 is the time related coefficient and $s = |\varepsilon| + |\gamma|$ and $\rho(c)$ is the function of cycles that the cell has been stressed, which is in the form of,

$$\rho = \xi_3 c^3 + \xi_2 c^2 + \xi_1 c + \xi_0 \quad (6.2)$$

Fig. 6.1(a) shows the measured force and compares with the proposed force model in equation (6.1) by using a different embryo. The power coefficients in five continuous cycles are estimated using a cubic function, as shown in Fig. 6.1(b).

As the response of cell membrane changes from different cells or cells at different developmental stages, the same applied force may induce different strain.

(a)

(b)

Figure 6.1. Force modeling on a zebrafish embryo. (a) Measured force and the modeled force are compared under repeat compressive loading. One sinusoidal displacement of sensor tip is considered as one cycle. (b) Changes of coefficient ρ in 5 continuous cycles can be fitted using a cubic function.

The average error between the predicted force and the measured force is 10.07%, with up to 20% maximum error. It is necessary to study the viscoelastic behavior of the cell membrane for at least four cycles to compute the coefficients of the cubic

function and σ_2 should be modeled with respect to the time. It could be a good extension of our current work.

2) Suggestions for Hardware Improvements

The cell strain model could be improved by increasing the frequencies of the end-effector displacement. For an embryonic sample in $350 - 1700 \mu\text{m}$, 3-DOF piezoelectric stages with traveling range of hundreds micrometers could comprehensively characterize the static and dynamic changes of cell deformation. This would make the dynamic control of cell strain possible and meanwhile it requires improvements of real-time machine vision algorithms for cell strain measurements.

3) Plant Cell Characterization

Implementation of automatic micromanipulation system on plant cells has become interests in recent years. However, characterization of plant cell is much more difficult than a single cell, as plant cells are distributed in arrays. It introduces special difficulties in machine vision algorithms development and experimental setup. We suggest to investigate the deformation and force sensing on edge cells first.

4) Developmental Biology

In the experiments, we also find that some of the embryos that have been deformed grow to larger size of zebrafish comparing with those embryos that never have been deformed, as shown in Fig. 6.2. The range of the end-effector traverses into the embryo and the number of times that embryos have been stressed and strained are all related to the phenomenon that the zebrafish reveals. The biological significance of the deformation effect still requires a systematic and rigorous investigation in the future.

(a)

(b)

Figure 6.2. Comparing the zebrafish after breeding for 90 days. (a) Zebrafish that never being deformed at the embryonic stage. (b) Zebrafish that being deformed at blastula stage.

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