Active Control and Adaptive Estimation of an Optically Trapped Probing System

Dissertation

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ABSTRACT

Due to its capabilities of three-dimensional (3D) non-contact manipulation and measurement with sub-picoNewton force resolution, optical trapping is a modern technique that has been particularly important for studying biological systems under physiological conditions. When a micro/nanoparticle is trapped to serve as the probe for manipulation and measurement, the major limiting factor on the performance of an optical trapping system is the probe’s Brownian motion, induced by the persistent random thermal force in the environment. In this research, the design, control, and signal processing of optical trapping systems are investigated to address the problem of Brownian motion and to expand the system’s functionality for biological researches.

An optical trapping system, composed of an FPGA-based digital controller, 3D high-speed laser measurement, and 3D rapid laser steering, is developed. The 3D steering actuators consist of a deformable mirror enabling axial actuation and a two-axis acousto-optic deflector for lateral steering. The actuation range is designed and calibrated to be over 20μm along the two lateral axes and over 10μm along the axial direction. The actuation bandwidth along lateral axes is over 50 kHz and the associated resolution is 0.016nm (1σ). The axial resolution is 0.16nm, while the bandwidth is enhanced to over 3 kHz by model cancellation method.
To enhance the manipulation resolution of the developed system, Brownian motion control is theoretically and experimentally investigated. A 1st-order ARMAX model describing the Brownian motion of an optically trapped probe is derived for controller design and analysis. The derived model is experimentally validated by proportional control results. An optimal controller based on minimum variance control theory is then designed and implemented. The theoretical analysis is validated by both experiment and simulation to illustrate the performance envelope of active control. Moreover, adaptive minimum variance control is implemented and experimentally verified to be capable of maintaining the optimal control performance in a time-varying environment.

Adaptive estimation is developed to enhance the system’s dynamic force probing capability. An adaptive observer is designed using the augmented system model that includes the dynamics of the external interaction and trapping system variation. It recursively estimates the external force and the system parameter from the noisy motion of the probe. Due to the principle of control-estimation separation, its performance of dynamic force sensing is unaffected by the manipulation and control of the system. The force probing is also corrected automatically according to the parameter estimation of the trapping dynamics. From inferring the cause of the variation, additional information of the process under investigation can be gained. Kalman filter algorithm is employed to minimize the estimation error of the designed estimator, achieving best linear unbiased
and maximum likelihood estimation when the process and measurement noises satisfy the white Gaussian condition.

The potential of the developed optically trapped probing system for biological researches is demonstrated by experiments with living cells. Intracellular trapping of an organelle is accomplished in a living CHO cell, and extracellular experiments of single-point cell pushing and cell tapping are performed to measure the mechanical property and/or the topography of living cells. The sample interference to the system’s actuation and measurement is eliminated by the modification of the sample holder and the employment of a different measurement optical path. After the non-specific binding of the probe to the cell is prevented, a topography map is obtained on a living MCF-7 cancer cell from multi-location cell tapping. The cell’s normal stiffness is also measured simultaneously, which is comparable to that of the trapping system.
Dedicated to my family
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CHAPTER 1: INTRODUCTION

1.1 Background and Motivation

Optical trapping is a modern technique that is particularly important for the study of biological systems under physiological conditions. Single-beam gradient-force optical trap [1], also known as optical tweezers, was first reported in 1986. Non-invasive trapping and manipulation of a single living cell [2] were soon demonstrated using it. A micro/nanoparticle is usually trapped in optical tweezers to serve as the probe for manipulation and measurement. Optical forces on the order of a few picoNewtons (pNs) to hundreds of pNs can be generated by a tightly focused near infrared (NIR) laser beam (e.g., at wavelength = 1064nm) and applied to that trapped individual micro-/nano- probe of dielectric material. With proper force calibration, optical tweezers can be employed as a three-dimensional (3D) force transducer to measure biological molecular interactions [3,4]. It has since been proven to be a useful tool in many research disciplines [5].

When it comes to manipulation and measurement of biological samples, optical trapping systems have distinguished themselves due to several reasons. First, they provide an unparalleled force sensing resolution of sub-pN, and have helped advance the study of molecular motor proteins at single-molecule level [3,6] significantly. Second, as 3D transducers, they are naturally attractive for research of biopolymers’ mechanical
property [7] as well as cell mechanics and mechanotransduction [8,9]. Moreover, in the absence of any mechanical link to the trapped object, more flexible 3D manipulation in a complex environment is enabled, e.g., trapping inside a living cell [10].

The major limiting factor on the performance of an optical trapping system is the probe’s Brownian motion [11], induced by the persistent random thermal force in the environment. Therefore, to address this issue and fulfill the system’s great potential in biological research, improvement of an optical trapping system’s setup is needed. Active control and dynamic force probing methods as possible ways to enhance manipulation resolution and measurement precision are also investigated to expand the functionalities of the system for future biological studies.

1.1.1 Optical trapping setup

Recently, built on optical trap and with advances in 3D position detection [12-17] as well as probe actuation and sample scanning technologies [18-23], a new scanning probe microscopy (SPM) technique is being developed. In a two-dimensional (2D) probing system, the probe’s motion is measured in real-time to sense the interaction force with the samples, and its 2D absolute position or relative position to the trapping equilibrium is regulated for various purposes, such as position clamp [29] and force clamp [20].

The positioning technologies utilized for the probing system can be classified into two categories: sample scanning and direct laser steering. Piezoelectric (PZT) sample stages equipped with capacitive sensors and computer control are a typical configuration in many setups and for different applications [18,20,24,25]. While a PZT stage can
control the relative 3D position between the probe and its samples with \(~100\mu m\) range and sub-nm resolution, its bandwidth is limited to only few tens of Hz.

On the other hand, laser beam steering, which actuates the probe directly, has demonstrated promising capabilities for control and manipulation. Three kinds of 2D transverse beam steering devices have been implemented in various optical traps: galvanometric scanning mirrors (GSM) [18,19], acousto-optic deflectors (AOD) [20,21], and electro-optic deflectors (EOD) [22]. They all alter the incident beam angle to introduce a lateral translation of the optical trap on the image plane of the objective lens, and thus change the probe’s lateral position. The bandwidth of GSM is 1–2 kHz, while that of AOD and EOD is over 50 kHz. At the expense of lower optical efficiency, a two-axis AOD is more compact and provides a greater range than an EOD.

Of late, implementations of axial actuation have been separately reported as well. In a commercial system [26], motorized lens translation was employed. Relatively low steering bandwidth was achieved, because large mass was involved and comparatively long lens stroke was required due to the design’s large axial motion reduction ratio of several thousand. Another implementation demonstrated axial actuation only [23], in which a membrane deformable mirror (DM) was employed. Although the reported range and bandwidth are fairly small, the performance of a DM actuator can be improved considerably with enhanced optical path design, modeling and control.

The 3D high-resolution motion detection in an optical trapping system has been demonstrated by a number of methods. Utilizing the off-focus image from the microscope, vision-based measurement can easily compute the 2D centroid of a micro-sized probe in the whole field of view (~100×100\(\mu m^2\) area) with sub-nm resolution [27].
The axial position detection, based on a pre-calibrated probe-specific model, also has sub-nm resolution within the calibration range of ~20μm [27]. Its measurement speed, however, is limited by the camera exposure time and image transfer rate. The measurement sampling rate therefore is up to 400Hz.

Laser-based detection systems achieve high-resolution measurement with high speed, but at the expense of much reduced measurement range of several hundreds of nanometers. They are often employed for applications such as dynamic measurement and closed-loop manipulation, where high speed is required. The most widely used laser detection method in optical trapping is back-focal-plane (BFP) interferometry [12-14], wherein a quadrant photodiode (QPD) is set downstream of a trap to sense the change in the interference pattern of the collected unscattered and forward-scattered laser light. It accomplishes 3D measurement with sub-nm resolution and over 100kHz bandwidth [14]. Back-scattered detection (BSD), as an alternative to the common BFP setup, has also been studied for its optical and mechanical conciseness [15,16]. Previously, it had relatively poor spatial resolution when compared to forward-scattered BFP detection, but was improved recently to demonstrate atomic-scale localization in all three axes [16]. Therefore, BSD may be desirable for applications in which only one side of the trap has high-quality optical access.

1.1.2 Active control

With high-speed measurement and direct steering of the probe available, the probe’s Brownian motion can be suppressed through active feedback control [19,28-30]. This technique is also employed by other type of external field tweezers, e.g. magnetic
tweezers [31] and electrokinetic traps [32,33], to improve the manipulation performance. In optical trappings, although the Brownian motion can also be reduced by increasing the laser power to reinforce the optical force field, it is not desirable due to the concern of photo damage of biological samples [34,35]. In addition, increasing laser power is not efficient when probes of sub-micrometer and nanometer scales are employed, since the stabilizing optical force declines with the volume of the probe [36] and the damping coefficient decreases with its size [5]. Feedback control, on the other hand, can enhance the trapping stability for improved trapping performance. It allows the required laser power to be reduced, so as to avoid sample damage.

Most reported systems apply 2D linear proportional (P) or proportional-integral (PI) control to reduce lateral Brownian motion, due to lack of appropriate axial actuators. Hence, the axial motion was usually not controlled, but confined passively by either uncontrolled stable trap or by specially designed sample holder. For the Brownian motion control of an optical trap, a 2D AOD actuator along with an analog control circuit was employed and one-order-of-magnitude reduction in the Brownian motion, in terms of its standard deviation, was reported [28]. When the required control force does not exceed the capability of the instrument, the theoretical power spectral density (PSD) of the motion can be used to evaluate the performance of P control [29]. It is concluded that the performance of P control is determined primarily by the delay time in the feedback loop. Nonlinear control for Brownian motion suppression was also studied by computer simulation [37]. It showed that a nonlinear controller achieving global asymptotic stability (GAS) has better performance when the applicable force generated by the setup is very small. Nonetheless, the finite response speed of the actuator and time delay of the
system were not considered in the simulation, so the improvement was overstated for real systems.

3D control of a 4.5μm magnetic bead was achieved using magnetic tweezers [31]. The performance was, however, compromised by the imaging rate of its visual measurement system. Moreover, the control of axial motion relies on the gravity force of the probe. It is therefore very difficult to be applied to particles at or below sub-micrometer scale. An optical trapping setup with 3D rapid laser steering was recently reported [30]. The steering system consists of one 2D AOD for lateral actuation and a DM for axial actuation. It is therefore capable of large range 3D steering of an optically trapped probe within its workspace. Feedback control was experimentally implemented as well, to achieve precision steering and to illustrate enhanced trapping stability through active Brownian motion suppression. This 3D control scheme restrains the entire Brownian motion volume and will greatly reduce the chance of probe escape when applied to nanoparticle trapping. However, since there is no proof that P controller can be optimal, the performance envelope of active control remains unclear. The answer to this question depends on the instrument hardware, the probe size and property, as well as the environmental condition of the manipulation. It is one subject of the research presented in this dissertation.

1.1.3 Dynamic force probing

The probe’s Brownian motion also affects the force sensing resolution of the optical trapping system. To deal with it, the measurement result is often intensively averaged or low-pass filtered for force probing [20]; the included dynamic information of
the interaction force may hence get loss. Furthermore, a 0\textsuperscript{th}-order linear spring model with constant stiffness is commonly employed to calculate the interaction force from the probe’s relative displacement to the trapping equilibrium using Hooke’s law [5], whereas the dynamics of the trapping itself is ignored. Consequently, the model is valid only for quasi-static force measurement.

Dynamic force sensing with the optically trapped probing system can facilitate the research on fast-varying biological processes. It is also the key to implementing force feedback control for regulating the interaction force, which will enable automatic scanning in unknown space, similar to what has been accomplished by atomic force microscopes (AFM) [38]. Real-time dynamic force sensing can be realized with a Luenberger observer by augmenting the interaction dynamics into the model of the probing system [39]. The observer recursively estimates the external force as a state variable of the augmented system model from the noisy motion of the probe. In addition, because of the separation principle of estimation and control [39], the performance of dynamic force sensing using such a recursive estimator is unaffected by the manipulation and control of the system. Therefore, Brownian motion control of the probe can be activated simultaneously to further enhance the manipulation resolution and accuracy of the system. In contrast, the result from the linear spring model would be disturbed when the trapping equilibrium is being steered [30] during manipulation and control, as the steering dynamics is neglected in the spring model.

Moreover, the probe’s response is non-stationary in many biological experiments, due to the time-varying trapping dynamics caused by the variation in local environment. For example, the damping coefficient of the probe is determined by the viscosity of the
surrounding medium, which is a function of local temperature [40]. This parameter is also influenced considerably by the wall effect [5] when the probe is near a fluid-solid interface. Thus, the trapping bandwidth [41] can change significantly in the experiment and influence the result of dynamic force sensing. It is, therefore, beneficial to estimate the system’s parameter variation together with the force, so as to correct the force sensing adaptively. Additional information about the experiment can be gained as well, from inferring the cause of the variation. Hence, the adaptive observer for joint state-parameter estimation is investigated in this dissertation, so that the interaction force as well as the trapping bandwidth can be measured in real time.

1.2 Research Objective and Specific Aims

The objective of this research is to develop a probing system based on optical traps for manipulation and measurement of biological samples. It requires the system to have 3D high-speed high-resolution actuation and measurement capacities on the trapped probe. In order to enhance the manipulation resolution and improve the measurement precision, real-time Brownian motion control and adaptive dynamic force probing functions also needs to be integrated in the developed system.

Four specific aims are identified. The first three are the goals of the probing system development, and the fourth is to demonstrate the potential of the developed instrument for biological applications.
(1) Development of an optical trapping system for three-axis rapid probe steering

The optical trapping setup consists of a three-axis laser steering subsystem of the trapped probe, a 3D high-resolution measurement subsystem, and a digital controller subsystem for signal processing and control, in order to implement all the desired functions of the probing system. The key design of the system is discussed in detail. Afterwards, the complete setup is calibrated to verify the system design. A series of experiments to demonstrate the resolution, working range, and bandwidth of the developed probing system is presented as well.

(2) Minimum-variance Brownian motion control

The optimal Brownian motion control problem is investigated based on accurate modeling of the optical trapping setup. The system model is verified with experiments. Then, the control performance envelope is obtained from the theoretical analysis, which is compared to experimental results for validation. The analysis is discussed for generic experiments, wherein the achievable control performance on different probes and under various experimental conditions can be predicted. Adaptive minimum-variance control demonstrates that the predicted optimal performance is maintained in a complicated environment.

(3) Adaptive dynamic force probing

The system model is employed and augmented for recursive estimator design. The dynamics of both external interactions and trapping system variation is included to expand the estimator into an adaptive observer for joint state-parameter estimation. The
observer is then optimized by Kalman filtering algorithm, in order to achieve best linear unbiased and maximum likelihood estimation when the process and measurement noises can satisfy the white Gaussian condition [42]. The performance of adaptive force probing is evaluated with experimental results.

(4) Measurement and manipulation of biological samples

The developed instrument is applied to study living cells, and the potential applications of the developed probing system is demonstrated. Trapping inside living cells illustrates the manipulation capability of the system. Non-functionalized polystyrene (PS) bead trapped by the probing system is actuated to tap living cells. The interaction force as well as the cellular response is measured through the probe’s motion. The topography map and mechanical property of the living cell is thus obtained simultaneously by tapping the probe across the cell surface.

1.3 Dissertation Overview

This dissertation presents the development of an optically trapped probing system for manipulation and measurement of biological samples. It is composed of six chapters. Chapter 1 gives the background and motivation of this research and identifies the research objectives as well as four specific aims. Chapter 2 is devoted to the design and implementation of the optical trapping system, focusing on its unique three-axis rapid laser steering capability of the optically trapped probe. System modeling and optimal Brownian motion controller design is presented in Chapter 3. Both the system model and theoretical analysis of the control performance envelope are verified with the
experimental results. The predicted optimal performance is shown to be sustainable in a complicated environment when adaptive minimum-variance control is enabled. Chapter 4 describes the adaptive estimator design for joint estimation of dynamic interaction force and trapping parameter variation. With the optimization by Kalman filtering algorithm and successful implementation in the trapping system’s digital controller, a series of experiments are conducted to evaluate the performance of the adaptive estimator. The developed probing system is then demonstrated by biological experiments, as presented in Chapter 5. Trapping capability of the system is illustrated by manipulating an organelle inside a living cell. Non-functionalized polystyrene (PS) bead trapped by the probing system are actuated to tap across living cells, and the interaction force as well as the cellular response are measured through the probe’s motion. The conclusions and remarks for future works are summarized in Chapter 6.
CHAPTER 2: OPTICAL TRAPPING SYSTEM FOR THREE-AXIS

RAPID PROBE STEERING

2.1 Introduction

This chapter begins with the overview of the optical trapping system, which is composed of a three-axis laser steering subsystem of the trapped probe, a 3D high-resolution measurement subsystem, and a digital controller subsystem for signal processing and control. The improvement of the laser measurement and the implementation of the digital control subsystem are then presented. Then the following part of the chapter focuses on the design and implementation of a three-axis steering subsystem, wherein a micro/nano particle is optically trapped and propelled to serve as a measurement probe. The actuators in the system consist of a deformable mirror (DM) enabling axial steering and a two-axis acousto-optic deflector (AOD) for lateral steering. The actuation range is designed and calibrated for all three dimensions of the probe steering. The actuation bandwidth and the associated resolution are analyzed and enhanced. The performance of the constructed optical trapping system, in terms of resolution, working range, and 3D control capability, is subsequently illustrated by three sets of experiments. First, active control of the trapped probe’s Brownian motion is
utilized to improve trapping stability. Second, large range 3D steering of a 1.87 μm probe is demonstrated by contouring a complex 3D trajectory. Third, closed-loop steering is implemented to achieve enhanced manipulation precision.

### 2.2 Optical Trapping System Overview

Fig. 2.1: An optical trapping system with three laser sources for trapping and measurement. The 830nm measurement laser with optical isolator (ISO) and two 1064nm trapping lasers are guided into the inverted microscope, combined by dichroic mirrors (DiMs), and focused by the objective lens onto the specimen plane, the position of which is controlled by a 3-axis piezo stage. They are then collected by condenser lens and projected to their respective quadrant photodiodes (QPD) for high-speed high-resolution 3D measurement.
Fig. 2.1 shows the optical trapping system, which was built in collaboration with Ms. Jingfang Wan and helped by Dr. Ming-Chieh Cheng in part of the alignment and calibration of the system. The system is laid out around an inverted microscope (Eclipse TE2000-U, Nikon, Japan) on an optical table (RS4000-48-8, Newport, Irvine, CA), and covered by a home-made acrylic enclosure to minimize the environmental disturbance. A levitated optical breadboard, as the common base of all the optical components of the system, mechanically strengthens the setup and reduces the structural drift. Three laser sources are employed for trapping and measurement. The primary trapping laser (1064 nm, CW) is a diode-pumped Nd:YAG/YVO₄ laser (IRCL-2.5W-1064-0.25%, CrystaLaser, Reno, NV) with a maximum output power of 2.5W. The red lines connected by arrows in the figure show the propagating direction of the laser beam along the designed optical path. It passes a three-axis steering system, which enables steering and active control of the optical trap, and enters the left side of the microscope. The steering system will be presented in detail in Chapter 2.4. After entering the microscope, the expanded laser beam fills the entire back aperture of a water-immersion objective lens (CFI Plan-Apo 60X/1.2 WI, Nikon, Japan) that has large numerical aperture (NA=1.2), and forms a stable trap by focusing at the specimen plane, the position of which is controlled by a three-axis piezo stage (P-517.3CL, PI, Germany) to enable 3D large range manipulation of samples over a 100×100×20μm³ volume. The secondary trapping laser (blue line) has the same wavelength but is adjusted to different polarization by a half-wave plate (HWP). The two lasers are combined by a polarized beam splitter (PBS) immediately preceding the microscope. Since the secondary trapping laser does not have an automated steering system, its optical trap is adjusted manually. Both trapping lasers
are employed to trap (functionalized or non-functionalized) polystyrene particles to serve as measurement probes. An 830nm measurement laser (LPS-830-APC-SM800-SP, Thorlabs, Newton, NJ; yellow line) is aligned with the primary trapping laser to detect the probe’s absolute position within its stationary focus. The measurement principle is based on 3D back-focal-plane (BFP) interferometry method, wherein a quadrant photodiode (QPD) detector (S4349, Hamamatsu, Japan) is used to measure the change in the interference pattern generated by the forward-scattered and unscattered laser light. It achieves sub-nm resolution and over 100 kHz bandwidth, while the measurement range is usually less than 1μm. The measurement signal is then conditioned by a circuit for enhancement and processed in the digital controller of the system for closed-loop manipulation. Based on the same measurement principle, another QPD (G6849, Hamamatsu, Japan) associated with the trapping laser is used to measure the probe’s relative position to the trapping equilibrium. It is worth mentioning that the trapping equilibrium is being steered in real-time during experiments.

In addition to laser measurement, a 3D particle tracking technique based on microscope off-focus images [27], developed by Dr. Zhipeng Zhang, was also implemented and integrated with the laser trapping system to enable large range tracking of the measurement probe and to facilitate the calibration of the three-axis steering system. This technique computes the probe’s 2D centroid with sub-nm resolution using the microscope’s off-focus image acquired through its CCD (charge-coupled-device) camera (CoolSNAP EZ, Photometrics, Tucson, AZ) for lateral position. CCD array’s pixel size along with its grid orthogonality is employed as the reference of lateral measurement. Moreover, this technique has two distinct features for axial measurement.
First, it uses all the information of the off-focus image to determine the axial position. Therefore, the effect of noise is reduced and measurement resolution is enhanced to sub-nm. Second, other than employing approximate theoretical models, a probe-specific model and matching algorithm are used to locate the axial position. The measurement accuracy is hence guaranteed over the entire measurement range at a level comparable to the calibration accuracy. The probe-specific model for the calibration of the steering system is constructed by moving a fixed probe of the same size to a set of specified axial positions with the PZT sample stage prior to the experiment. Consequently, the model and axial measurement accuracy are determined by the factory calibration of the PZT stage’s capacitive sensor, which is of high quality and NIST (National Institute of Standards and Technology) traceable.

The principal piece of electronics of the optical trapping system is a field programmable gate array (FPGA) controller (Stratix II Edition, Altera, San Jose, CA). It is connected with a six-channel 16-bit simultaneous analog-to-digital (AD) converter (ADS8365, Texas Instruments, Dallas, TX) to receive 3D reference and measurement signals. The measurement conditioning circuit (LTC1564, Linear Technology, Milpitas, CA) filters and amplifies the signal, with up to 150kHz bandwidth. Output actuation efforts are updated through peripheral 16-bit DA (digital-to-analog) channel (DAC 8814, Texas Instruments) and FPGA’s programmable digital lines to the actuator drivers for active control and direct steering. Parallel communication between the controller and a host computer is also established for data logging and analysis. The controller’s high real-time loop rate of 208.3 kHz enables fully exploiting the high-speed measurement and actuation of the system. The details of the above electronics are presented in the next
section for the enhancement of the laser measurement and the implementation of the digital controller.

2.3 Measurement and Control Subsystems

2.3.1 Optical and electronic enhancement of the laser measurement

The effort to improve the laser measurement can be divided into two parts: the first involves optical improvement of the laser beam profile and stability; the other includes the electronic conditioning of the measurement signal.

![Laser intensity fluctuation in high-frequency range.](image)

Fig. 2.2: Laser intensity fluctuation in high-frequency range. (Inset) is the zoomed-in measurement of laser fluctuation in time domain.

The original 830nm measurement laser generated from a laser diode (DL7032-001, Sanyo, Japan) contains higher order transverse electromagnetic modes (TEM) that
alters its beam profile from a smooth Gaussian function. The resultant spikes in the laser intensity distribution can directly affect the accuracy of the QPD measurement. To correct the beam profile, the laser diode output is coupled to a single-mode optical fiber, which only allows the fundamental mode to propagate. The laser after the optical filtering of the fiber is thus a TEM\(_{00}\) Gaussian beam, as the theoretical derivation of BFP method assumes [12]. In addition, the laser diode is under strict temperature control, and the optical feedback of the laser is prevented by a Faraday isolator (IO3D-830-VLP, Thorlabs, Newton, NJ) to reduce the laser’s mode-hopping noise [20].

The laser intensity fluctuation is then measured, as shown in Fig. 2.2. The dominant fluctuation is around 1.2MHz, so its adverse effect to measurement resolution can be removed by the 8\(^{th}\)-order low-pass filter in the signal conditioning circuit. The filter further enhances the measurement resolution by limiting the wideband electronic noise of the measurement circuit at the same time. The electronic noise in the QPD measurement circuit (PSS-QPD50-6SD, Pacific Silicon Sensor, CA) is also minimized with the replacement of its original operational amplifiers (op amps) by high-precision ones (OPA4132, Texas Instruments, Dallas, TX). The filtered measurement signal is finally offset and amplified in the conditioning circuit before sampled by the digital controller, so that the measurement signal’s range will match the controller’s AD conversion range to maximize the bit resolution of the AD converter. Consequently, the measurement noise level after signal conditioning is improved to the base noise level of the AD converter, as illustrated by the measured power spectrum density (PSD) [41] of the probe’s Brownian motion in Fig. 2.3.
2.3.2 Implementation of the digital control subsystem

In order to fully exploit the 3D high-speed measurement and actuation of the optical trapping systems, large rate of real-time data processing is required. FPGA-based controller can be programmed bottom-up from huge amount of basic logic elements (LE’s), all of which operate in parallel. Therefore, it can achieve high processing rate and is insensitive to the computational load. In addition, FPGA allows direct access to the peripheral devices (AD, DA, actuator drivers, etc.) through hundreds of programmable digital lines, eliminating any unnecessary time delay in the real-time loop. Conventional CPU/DSP-based digital controllers can hardly accomplish the same performance, due to their sequential processing structure and inflexible peripheral access.
Fig. 2.4: FPGA-based real-time digital controller integrated with AD and DA converters.

Fig. 2.4 shows a picture of the FPGA-based controller interconnected with the peripheral multi-channel AD and DA converters. The controller’s clock rate is 100MHz. Hence, in order to ensure the high-speed board-to-board signal’s integrity, the connections in between the FPGA board and peripheral boards (blue cables) are made through 38AWG mini-coax ribbon cable assemblies with 0.1” socket connectors (HHSC series, Samtec, New Albany, IN). The peripherals are then driven with the required clock and command signals provided by FPGA, and communicate with FPGA using the standards specified in their respective datasheet. Maximum rate of data conversion and
transfer can thus be achieved. The four 16-bit DA channels can be updated with a rate over 500kHz, whereas the 16-bit AD converter’s sampling interval is limited to 4.8μs when all six channels work simultaneously. Consequently, the FPGA controller’s loop rate is up to 208.3kHz.

The FPGA is programmed with Quartus® II design software, and the structure of the control subsystem is drawn in Fig. 2.5 to show its integration with the whole optical trapping system. The 3D measurement and reference signal come into the controller...

Fig. 2.5: Integration of FPGA-based digital controller to the optical trapping system.
through AD in the format of 16-bit signed integer. They are converted to single-precision floating numbers to be processed with the control and estimation algorithms in FPGA. The computed control efforts are then transformed to unsigned integers for update. The processed data are also transferred to a host computer (PXI-8186, National Instruments, Austin, TX) with high-speed digital I/O card (PXI-6534, National Instruments) via customized parallel communication for result logging and analysis. Moreover, the reference trajectory generation for probe scanning as well as the sample manipulation task of the PZT stage is handled in the host computer using LabVIEW software. The PZT stage’s 3D position is controlled and measured with 5kHz rate through the high-speed parallel I/O (PIO) interface [43] of its driver.

2.4 Three-axis Laser Steering Subsystem

2.4.1 Design of optical path

Fig. 2.6 illustrates the optical design of the three-axis steering system. The deformable mirror (10mm MMDM, Flexible Optical B.V., Netherlands) on the left is a membrane parabolic mirror whose focal length can be varied by electrostatic force. Due to the silicon nitride membrane’s high elasticity, small mass and thin thickness, it can produce large axial focal shift (0~1.5 diopter) with high bandwidth and is hysteresis-free, so it is selected as the axial actuator. The lateral actuator is a two-axis AOD (DTD-274HA6, IntraAction, Bellwood, IL) in between the lens pair L2:L3. It is chosen for its collective large angular range (26.9 mrad), high bandwidth (> 50 kHz), and compact size.
Fig. 2.6: Optical design of the 3D rapid laser steering system. The 1064nm trapping laser is directed by the HWP and PBS onto the DM, the axial actuator; reflected through QWP toward the two-axis AOD, the transverse actuator; focused by the microscope’s objective lens (L0) to form the optical trap; and then is collected by the condenser for measurement. HWP-PBS serves for manual adjustment of laser power. Two lens pair telescopes (L1:L2 and L3:L4) not only conjugate the two actuators with the microscope’s aperture plane set (back focal planes), but also change the laser beam size to fit each actuator’s aperture and that of the objective lens. The laser’s optical path is merged into the microscope’s imaging path without obstruction by the dichroic mirror of selected wavelength band.

In the optical path, the input trapping laser is guided to the DM along the mirror’s normal direction by a PBS. The HWP preceding the PBS manually controls the laser power being utilized with its rotation $\theta$ about the optical axis. The optical efficiency of this HWP-PBS adjustment is a function of $\cos^2(2\theta - \theta_0)$, as illustrated by the measured laser power in Fig. 2.7. By setting the rotation angle of the quarter-wave plate (QWP) between the DM and the PBS at 45° about the optical axis, the reflected laser is allowed to pass through the PBS to the succeeding optical path as the polarization of the laser beam is rotated 90° after passing the QWP twice. This orthogonal configuration is similar to the design of common interferometers [44] and eliminates any off-axis aberration that might be introduced by the DM. The following *afocal* Keplerian telescope lens pair L1:L2
adjusts the laser beam size from fulfilling the DM’s aperture to fitting that of the two-axis AOD, while the other pair $L_3:L_4$ succeeding the AOD expands the beam to slightly overfill the back aperture of the microscope’s objective lens $L_0$ to form optical trap.

![Graph](image)

**Fig. 2.7**: HWP-PBS adjustment of trapping laser power as a function of HWP’s angle about the optical axis.

One key feature of the actuation and measurement systems’ optical design is that all lenses, including the condenser (W Plan-Apo 63X/1.0 VIS-IR, Zeiss, Germany) and the lenses preceding the QPDs in Fig. 2.1, are sequentially overlaid at their respective focal points. By doing so and locating the DM, the AOD, and the two QPDs at the BFPs of the associated lenses, they are all optically conjugated with one another as well as with the aperture/back-focal plane set of the microscope, as shown in Fig. 2.6. In other words,
as illustrated in Fig. 2.8, due to this object-image conjugate relationship, the laser spot’s shape, dimension, center, as well as its level of fulfillment at the apertures of the two actuators and that of the objective lens, are invariant while the laser is being steered within the limitation of all lenses’ pupil size. So is the laser power at the focus and at the QPD if the small variation (~5%) of the AOD’s optical efficiency is neglected. The root cause of variation is therefore eliminated; and the actuation crosstalk, the trapping strength variation, as well as the trapping laser’s measurement coupling are all prevented when undertaking 3D steering. The small laser power variation caused by AOD, albeit showing no distinguishable adverse effect on the system’s calibration and manipulation in this dissertation, can be compensated by monitoring the laser power after AOD and regulating the RF (radiofrequency) power of the AOD’s driving signal accordingly.

The range of 3D laser steering and the sensitivity of actuation are calculated from the design. According to the paraxial geometric relationship shown in Fig. 2.8(a), the transverse actuation range $\delta_x$ and $\delta_y$ are determined by the AOD’s angular actuation span $\alpha$ and $\beta$ of the two axes, together with the focal length $f_i$ of the lenses $L_3$, $L_4$, and $L_0$,

$$\delta_x = \frac{f_0}{f_4} (\alpha f_3) = \frac{3.3 \text{ mm}}{250 \text{ mm}} (26.9 \text{ mrad} \times 60 \text{ mm}) = 21.3 \mu\text{m}. \quad (2.1)$$

Since the AOD’s output angle is linearly dependent on the change of its operational frequency [5], the actuation sensitivity is computed to be

$$\delta_x / \Delta f_{\text{AOD}} = 21.3 \mu\text{m} / 16 \text{ MHz} = 1.33 \mu\text{m} / \text{MHz}, \quad (2.2)$$

which will be verified by experimental calibration in Chapter 2.4.2.
The DM’s axial steering range is theoretically evaluated by analyzing the optical path with Gauss lens equation [45]. From Fig. 2.8(b), the axial shift after lens $L_1$, $\delta_f$, is derived,

$$\delta_f = \frac{f_{L_1}^2}{f_{DM}},$$

(2.3)

where the focal length of $L_1$ is $f_i=200\text{mm}$, while $1/f_{DM}$ changes from 0 to $1.5\text{m}^{-1}$ according to the DM’s specification. This axial shift induces the axial probe actuation through two pairs of lenses, $L_2:L_3$ and $L_4:L_0$, as illustrated in Fig. 2.6. The axial shift amplification of each lens pair given by geometrical optical analysis is
\[
\delta_{\text{out}} = \left( \frac{f_{\text{out}}}{f_{\text{in}}} \right)^2 \delta_{\text{in}}. \tag{2.4}
\]

Combine Eqs. (2.3) and (2.4), the total \( z \)-actuation range is

\[
\delta_z = \left( \frac{f_0 f_3}{f_4 f_2} \right)^2 / f_{DM}, \tag{2.5}
\]

in which \( f_2 = f_3 = 60\text{mm} \). The calculated range using Eq. (2.5) is 10.454\( \mu \text{m} \).

### 2.4.2 Calibration of three-axis actuation system

To calibrate lateral actuation, a 1.87\( \mu \text{m} \) polystyrene (PS) bead is trapped by the laser trapping system, and the two-axis AOD, in response to the operational frequency change, steers it along a 5×5-step raster scanning trajectory. The bead dwells at each location for 4 seconds, wherein 80 measurements of the bead’s position are acquired during this time by visual sensing under bright field illumination. Using the average of multiple measurements at each location helps reduce the adverse effect of the probe’s Brownian motion to the result of calibration. It can be clearly seen in Fig. 2.9 that measurement points aggregate at 25 distinct locations in a 5×5 format. As expected, the relationship between the actuator’s input effort and the measured motion can be fitted very well by a linear affine transformation (Fig. 2.9). From the transformation, the actuation sensitivities on \( x \)-axis and \( y \)-axis are determined to be 1.37\( \mu \text{m/MHz} \) and 1.39\( \mu \text{m/MHz} \) respectively, both of which have less than 5\% difference from the designed value of 1.33\( \mu \text{m/MHz} \). The perpendicularity of the actuation is outstanding as well: the included angle of actuation axes calibrated by the CCD grid is 90.6°, while a small
rotation between the two coordinate frames exists due to the installation of the CCD camera.

Fig. 2.9: Measured AOD raster scanning and calibration result on lateral AOD actuation: at each of the 5×5 locations, ~80 measurements are computed from microscope images (black dots). The linear transformation between the actuation effort (not shown) and the measured position is then obtained, which resolves the two actuation sensitivities as well as the included angle of the two actuation axes. The superimposed grid lines in the figure are calculated from the actuation effort using the obtained transformation, to be compared with the raw position data for validation.
Fig. 2.10: (a) Measured axial motion of a 1.87μm probe: the DM’s virtual actuation effort is a triangular wave with 30% p-p amplitude and 5s period; (b) Measured motion of multiple cycles versus virtual actuation effort: a linear fitting is utilized to project the full actuation range, and to illustrate its high quality as well.
The axial manipulation range is calibrated by measuring the trapped probe’s motion subject to a triangular wave of the DM’s input actuation effort. During calibration, the DM is directly driven by a high voltage (HV) up to 235V, generated from its FPGA-controlled HV amplifier. The mirror deformation and the resulting shift \(1/f_{DM}\) are, however, proportional to the voltage square [46]. Thus, a variable of 0~1 is used in the FPGA controller as the steering reference, which is regarded as the virtual effort for actuator linearization, because the driving voltage is scaled up from its square root in FPGA to achieve a linearized input-output relationship in the axial steering. A 50% constant offset is also added to the virtual effort to enable bi-directional operation since the mirror can only deform concavely. The triangular-wave motion of a 1.87\(\mu\)m probe with 30% peak-to-peak (p-p) amplitude is measured with multiple cycles using the visual sensing system [Fig. 2.10(a)], and drawn against the DM’s virtual actuation effort in Fig. 2.10(b). The full axial actuation range projected by linear fitting of this measurement is 10.478\(\mu\)m, in close accordance with the design, and the fitting residue is about \(\pm1\%\) of the motion amplitude. The repeatability of the measurement is affected by the probe’s Brownian motion, but it is low-pass filtered by the image integration during camera exposure, and is less than 30nm, or 1% of the motion range in the experiment. The crosstalk of axial actuation on transverse plane recorded by the visual sensing system is also within \(\pm1\%\). It demonstrates very good performance of the steering system. In addition, the residual actuation crosstalk can be compensated in two ways: a) enable feedback control on probe position during the steering; b) in open-loop steering, the crosstalk can be calibrated in advance, and feedforward compensation in the steering reference will cancel it.
2.4.3 Bandwidth and resolution of lateral actuation

The AOD’s bandwidth is decided by how fast the stable acoustic wave can be established in its acousto-optic crystal after changing the driving frequency. Due to the wave propagation speed (0.625mm/μs) in the crystal and the crystal size, the operational delay of the AOD is 15μs, including the actuation effort updating time, as measured from the synchronized response of a trapped probe. When further considering the AD’s 4.8μs conversion time in the feedback loop, lateral bandwidth for closed-loop steering is higher than 50 kHz, already comparable to the loop rate of the system’s digital controller.

Fig. 2.11: Laser pointing stability in low-frequency range measured by a spectrum analyzer.

On the other hand, the analog driver of the AOD is modified to enhance the actuator’s resolution. The original frequency sources inside the AOD driver are analog voltage-controlled oscillators (VCOs), but are found to introduce frequency instability. The laser beam, which is measured a spectrum analyzer (Model 5820A, Wavetek/Rockland) and shows no intensity fluctuation preceding the AOD, hence shows
pointing drift at power line frequency of 60Hz and its odd harmonics, as well as an increase of noise level around the entire low frequency range below 300Hz, as shown in Fig. 2.11. This results in reduced lateral steering resolution.

![5nm AOD Stepping Measured by QPD](image)

**Fig. 2.12:** Lateral steering by the AOD results in 5nm stepping of a 1.87μm probe. Measurement is obtained using the 830nm measurement laser. A moving average with a 0.4-second long window frame is applied to suppress the probe’s Brownian motion and to demonstrate the actuation resolution. The data are plotted after downsampling.

The VCOs are, therefore, replaced by direct digital synthesizers (DDS, LMDD_0040_01, General Electronic Devices, San Marcos, CA), whose frequency stability is determined by their high precision clock, and is as good as ±1ppm (part per million). The frequency resolution of the employed DDS is 10Hz, corresponding to high actuation resolution of 14pm (picometer) while fluctuation caused by the clock precision
is calculated to be less than 98pm p-p (±3σ). After the replacement, the measured probe motion demonstrates no sign of fluctuation at 60Hz or any other frequency. The resolution of open-loop lateral steering of the probe is demonstrated in Fig. 2.12. With the suppression of the probe’s Brownian motion by moving average, 5nm stepping is clearly seen, although the averaged motion is still subject to low-frequency disturbance on the probe.

2.4.4 Resolution and bandwidth of axial actuation

![20nm DM Stepping Measured by QPD](image_url)

Fig. 2.13: Axial steering by the DM results in 20nm stepping of a 1μm probe. The data are plotted after moving average and downsampling to illustrate the actuation resolution.
Fig. 2.14: Dynamic response enhancement of DM actuator: (a) Bode plots of the original DM response (measurement) with 8th-order model fitting, and the enhanced dynamic response (simulation); and (b) step responses of the DM before as well as after dynamic compensation.
The FPGA’s 16-bit DA channel drives the DM is. However, due to the voltage-square relationship, the displacement resolution varies with different actuation effort and its offset. The worst case occurs when the actuation effort is the highest, wherein the resolution is one bit lower: $10.478\mu m/2^{15}=0.32nm$; when the effort is offset at 50%, as the normal operation does, the resolution is 16-bit equivalence, or 0.16nm. This axial resolution is illustrated in Fig. 2.13, which shows the result of open-loop axial steering of the probe. Although the motion is affected more by thermal noise and other disturbance due to smaller axial trapping stiffness [18], the 20nm stepping of the probe is evident.

The trapping stability and steering performance can be improved through feedback control, whereas the actuator’s bandwidth is a major limiting factor of the improvement [29]. The DM’s dynamic response is therefore measured and enhanced.

A stationary $1.87\mu m$ probe fixed on a cover slip is aligned in the trapping laser focus by the PZT sample stage. The laser focus is then axially steered by the DM to scan over the probe. With frequency-sweep excitation, the amplitude and phase of the relative z-motion between the fixed probe and the laser focus are acquired from the trapping laser’s QPD and recorded by a lock-in amplifier (Model 7280, PerkinElmer, Waltham, MA). Hence the Bode plot of the DM can be drawn in Fig. 2.14(a). This frequency response is fitted by an 8th-order linear model [Fig. 2.14(a)]. The actuator’s bandwidth is shown to be limited by the first mode of the model. Its undamped natural frequency is 1.6 kHz, and the large damping ratio of 0.65 is due to the effect of the surrounding air. A non-minimum phase zero [47] at 3.5kHz causes undershoot in the DM’s step response, as verified in Fig. 2.14(b), where the $43.2\mu s$ pure time delay is also illustrated. The major part of this delay comes from the delay of the measurement filter, which can be reduced
by increasing the filter bandwidth up to 150kHz, whereas the FPGA controller’s calculation and AD/DA operation cause the remaining delay of 9.6μs.

The DM’s response is swift when compared to the axial Brownian motion of the probe, whose bandwidth is a few hundred Hz at most, but the response can be further enhanced by dynamic compensation. First, the original HV amplifier of the DM is replaced by a faster one (APEX PA90, Cirrus Logic, Austin, TX) to ensure quick electronic reaction. Then employing the model cancellation method [48], a 6\textsuperscript{th}-order compensator is designed based on the identified dynamic model of the DM and implemented in FPGA. It actively adjusts the HV amplifier’s voltage and cancels the dynamics of slow poles and zeroes, replacing them with desired ones. The red line in Fig. 2.14(a) shows the enhanced dynamic response. The effectiveness of this 6\textsuperscript{th}-order compensator is experimentally confirmed by measuring the step response of the DM after compensation [Fig. 2.14(b)]. Although the enhancement is restricted by the actuator’s non-minimum phase zero and time delay, the ±1% settling time reduces from 0.64ms to 0.33ms, achieving 94% improvement at the cost of a little worse undershoot. It extends the bandwidth of the axial steering to over 3 kHz and will help improve the closed-loop performance of the steering system.

2.5 Experimental Evaluation of the Optical Trapping System

The performance and potential applications of the constructed optical trapping system are illustrated by three sets of experiments for its resolution, working range, and 3D control capability.
2.5.1 3D active control of Brownian motion

Feedback control can greatly enhance the trapping stability of the probing system. It reduces the probe’s Brownian motion, increases optical trap’s effective stiffness, and raises signal-to-noise-ratio (SNR) for force probing and manipulation. Preliminary experimental results of 3D Brownian motion control are shown in Fig. 2.15, where the data are offset for comparison. A probe (1.87μm) was initially trapped with high laser power (~80mW at focus); its Brownian motion was measured using the measurement laser and shown in the figure (green color). The laser power was decreased to about 10mW at focus and the measured Brownian motion (blue color) showed significant increase, roughly tripling in terms of the motion’s standard deviation σ in all three dimensions. Active control with a simple proportional control law was then enabled. The control objective was to steer the probe to a specified equilibrium point, and better trapping stability was illustrated by much reduced Brownian motion (red color), as the σ after control is improved to the level of the open-loop trapping using eight times of laser power.
Fig. 2.15: Brownian motion of the probe with and without active control. The data are offset for comparison, wherein the respective standard deviations of each case’s Brownian motion are: 1) 6.4nm, 5.8nm, and 18nm along x-, y-, and z-axis in high-power (~80mW at focus) open-loop trapping; 2) 19nm, 15nm, and 58nm in low-power (~10mW at focus) open-loop trapping; and 3) 6.4nm, 4.6nm, and 20nm in low-power proportionally controlled trapping.
2.5.2 Large range 3D open-loop steering

Fig. 2.16: Large range 3D open-loop steering: a 1.87μm probe is steered in a 6×6×4μm³ volume to form the outline of a vase shape. (Inset) is a compound image of three probe positions, illustrating the image change due to 3D steering.

Using our 3D particle tracking program, Fig. 2.16 shows the detected motion of a 1.87μm probe, which is steered in a 6×6×4μm³ volume to contour a complex 3D trajectory. In the experiment, the trapping laser was commanded to trap the probe and move it along a predetermined complex 3D trajectory, which forms the outline of a vase. As is seen in the figure, the probe’s motion precisely follows the complex 3D trajectory and the steering performance of the system is uniform in the whole space. The inset
compound picture in the figure shows three probe positions along the path, illustrating the image change caused by 3D steering. Other open-loop steering of smaller probes with larger range have also been successfully achieved.

2.5.3 3D closed-loop steering

The performance of 3D steering is further improved by employing high-speed feedback control, implemented with the FPGA controller and absolute position detection of the 830nm laser. Fig. 2.17 compares the outcome of 3D open- and closed-loop steering of a 1.87 μm probe, wherein the probe moves along each axis back and forth with 200nm p-p amplitude. The laser power used in open-loop steering is eight times of that used in closed-loop steering; the Brownian motions in the two cases are, however, similar. The moving-averaged trajectories (black lines) also show that closed-loop control enables achieving better precision along and less crosstalk among the three axes, as low-frequency disturbances are rejected by feedback control [47].
Fig. 2.17: 3D (a) open- and (b) closed-loop steering of a 1.87 μm probe: the probe moves along each axis back and forth with 200 nm p-p amplitude. The laser power used in open-loop steering is eight times of that used in closed-loop steering; the Brownian motions in the two cases are, however, similar. The moving-averaged trajectories (black lines) also show that closed-loop control enables achieving better precision along and less crosstalk among the three axes.
2.6 Conclusions and Remarks

The design and implementation of an optical trapping system with direct laser steering is presented in this chapter. In order to achieve high-resolution and high-speed motion control, the system’s laser measurement is improved and an FPGA-based digital controller with 208.3kHz loop rate is implemented to fully exploit the high-speed measurement and actuation of the system. In the steering subsystem, a two-axis acousto-optic deflector is selected for lateral steering and a deformable mirror for axial actuation. Through optical path design, the actuation range of 21.3μm along x-, y-, and 10.5μm along z-axis is accomplished. The optical path design, if perfectly aligned, eliminates actuation crosstalk among the three axes and coupling between actuation and relative position detection of the trapping laser as well. The 3D actuation system is calibrated and the design in terms of motion range and actuation crosstalk is verified. The lateral resolution of the steering system is determined to be 16pm (1σ) and its bandwidth is over 50 kHz; the axial resolution is 0.16nm with enhanced bandwidth of over 3 kHz. The performance of the constructed optical trapping system is illustrated by three sets of experiments. First, 3D active control of the trapped probe’s Brownian motion is utilized to enhance trapping stability. Second, large range 3D steering is illustrated by maneuvering a 1.87μm probe along a complex 3D trajectory in a 6×6×4μm³ volume. Third, closed-loop steering is implemented to achieve improved precision for manipulation.

The experimental setup demonstrates the capability of rapid 3D probe steering, measurement, and control with optical trapping. This capability will not only facilitate automated 3D calibration of the trapping system [21], but also enable researches
involving 3D probing and scanning of biological samples, such as live cells. Using feedback control to enhance the stability of the optical trap will lower the required laser power, and thus reduce the temperature rise of the probe as well as that of the sample being probed. Laser-induced heating [49,50] will therefore be considerably relieved, and optical damage in biological applications [34,35] will be minimized. The system can also be employed to improve nanoparticle trapping [51] with the trapping stability in all three dimensions being enhanced, wherein unlike using other 2D controlled traps, the chance of the probe’s axial as well as lateral escape will be reduced all together.
CHAPTER 3: MINIMUM-VARIANCE BROWNIAN MOTION

CONTROL

3.1 Introduction

As demonstrated in Chapter 2.5.1, the high-speed measurement and direct laser steering capabilities of the constructed optical trapping system can be utilized to suppress the probe’s Brownian motion through active feedback control. However, the performance envelope of active control remained unclear with only a simple proportional control law implemented.

This chapter thus presents theoretical and experimental investigation on the Brownian motion control of an optically trapped probe. The Langevin equation is employed to describe the motion of the probe, when experiencing random thermal force and optical trapping force. Since active feedback control is applied to suppress the probe’s Brownian motion, actuator dynamics and measurement delay are included in the equation. The equation of motion is simplified to a first-order linear differential equation and transformed to a discrete model for the purpose of controller design and data analysis. The established 1st-order ARMAX (autoregressive moving average with exogenous input) model [52] captures the discrete nature of the sampled motion and digital control. It is
experimentally verified by comparing the model prediction to the measured response of a 1.87μm trapped probe under proportional control. It is then employed to design the optimal controller that minimizes the variance of the probe’s Brownian motion. Minimum variance control (MVC) theory [53] is investigated and theoretical analysis is derived to evaluate the control performance of a specific optical trap. Both experiment and simulation are used to validate the design and its theoretical analysis, and to illustrate the performance envelope of the active control as well. The theoretical analysis can therefore be applied to identify influential factors and their impact on the performance of Brownian motion control. Moreover, adaptive minimum variance control is implemented to maintain the optimal performance in the case that the system is operated in a complex time-varying environment.

3.2 Modeling and System Identification

In this section, the Langevin equation is employed to describe the motion of the optically trapped probe, when experiencing random thermal force and optical trapping force. Since active feedback control is applied to suppress the probe’s Brownian motion, actuator dynamics and measurement delay are included in the equation. The equation of motion is simplified to a first-order linear differential equation and transformed to a discrete model for the purpose of controller design and data analysis. The derived model is then experimentally verified.
3.2.1 Equation of motion for an optically trapped probe

The optical force exerted on an optically trapped probe has two components, namely scattering force and gradient force [36]. In a stable optical trap, an equilibrium point is reached under the balance of these two forces, whereas the random thermal force $F_L$, also known as the Langevin force, tends to destabilize it and causes Brownian motion around the equilibrium. The probe’s motion is therefore governed by the Langevin equation [11], in which the 3D motion can be decomposed into three components, along $xyz$ directions. For simplicity, only the equation of motion along the $x$ direction is presented,

$$m\ddot{x}(t) = - \gamma \dot{x}(t) + F_L(t) + F_{OT}(x-u) + F_E(t).$$

(3.1)

As the probe’s mass $m$ is very small, the inertia force $m\ddot{x}$ is negligible when compared to the viscous drag $\gamma \dot{x}$. According to the Stoke’s law [5], the drag coefficient $\gamma (= 6\pi\eta a)$ is proportional to the probe’s radius $a$ and medium viscosity $\eta$. The white Gaussian thermal force $F_L$ is characterized by its flat double-sided power spectral density (PSD) in the frequency domain, $S_f(f) = 2\gamma k_B T$ [54], where $k_B = 1.38 \times 10^{-23} \text{J} \cdot \text{K}^{-1}$ is the Boltzmann constant and $T$ the absolute temperature. When the focal center $u$ of the laser beam is fixed, the optical trapping force $F_{OT}$ acts as a passive restoring force that stabilizes the probe’s motion and confines it to be around the focus. Through feedback control, the focal center $u$ can be rapidly altered according to the probe’s position such that the optical force exerting on the probe attempts to actively balance the thermal force.
and leads to the reduction of the probe’s Brownian motion. This control concept is illustrated by the block diagram shown in Fig. 3.1.

![Block Diagram](image)

**Fig. 3.1: Brownian motion control of an optically trapped probe.**

The external force \( F_E \) in Eq. (3.1) usually represents the probe-sample interaction in the experiment. According to the measured motion of the probe, it is possible to estimate the probe-sample interaction force in real time to enable other force probing and regulation objectives, which will be discussed later in the next chapter, whereas the interaction is precluded in the analysis and experiment of this chapter, as the focus is on the Brownian motion control of the optically trapped probe.
Fig. 3.2 shows the measurement of the optical force exerted on a 1.87 μm-diameter polystyrene (PS) probe along the $x$-axis. It was obtained by performing a step response test similar to the experiment reported in [28]. The laser power was tuned to 80mW at focus. A sequence of step changes ($\pm 600$ nm) were applied to the optical trap and the resulting step responses of the probe were recorded. The averaged step response was used to construct the curve $(\gamma \hat{x})$ vs. $(\bar{x} - u)$, in which the effect of the thermal force (zero-mean) was cancelled out and $\gamma \hat{x}$ converged to the optical force $F_{\text{opt}}$, as derived from Eq. (3.1). It can be clearly seen that the force field is very linear within $\pm 300$ nm range, and
the peak force, whose amplitude is proportional to the power of the trapping laser, occurs at around ±500nm. The radius of the monotonic trapping force region is roughly half the probe’s radius. This result agrees well with the escape distance measurement concluded for micron-sized beads [28]. Hence, as long as the required control force does not exceed this range, the nonlinear optical force can be modeled as a linear restoring force, acting like a linear spring force characterized by a constant trapping stiffness $k_{OT}$. Consequently, Eq. (3.1) is linearized,

$$\gamma \ddot{x} + k_{OT} x = k_{OT} u + F'_L(t).$$

(3.2)

When trapping a nanoparticle whose radius is smaller than the wavelength of the trapping laser, instead of being related to the particle’s size, the radius of the monotonic trapping force region is about half of the focused beam waist [36], which is about ±220nm in our trapping system. The force field can, nevertheless, be determined using the same method presented above. The same criterion that requires the control force to be smaller than the escape force can then be applied to verify whether the linearized equation is suitable for controller design and result analysis.

3.2.2 Actuator dynamics and measurement delay

The block diagram in Fig. 3.1 and Eq. (3.2) can be employed to investigate the active control of the probe’s Brownian motion, whereas the control law needs be designed, and the actuator dynamics of the laser steering system and the measurement delay need be included in the analysis. As presented in Chapter 2, the three-axis laser steering system consists of a 2D AOD for lateral actuation and a DM for axial actuation. The signal conditioning circuit cascaded to the QPD device of the high-speed
measurement system includes an amplifier with tunable gain, employed to adjust the measurement sensitivity according to the probe’s size, and an 8th-order low-pass filter whose bandwidth is up to 150 kHz. The sampling rate of the FPGA-based digital controller is up to 208.3 kHz, limited by its AD conversion time, which is much higher than the bandwidth of the probe’s Brownian motion. The bandwidth of the low-pass filter is set to be over the digital controller’s Nyquist frequency. It improves the measurement resolution mainly by limiting the laser’s high frequency fluctuation (over 500 kHz) as well as electronic noise in the measurement signal.

Since the low-pass filter’s passing band is greater than the controller’s Nyquist frequency and in this band the measurement filter has a nearly linear phase [55], its dynamics is simplified as a pure time delay $\tau_m$, which is inversely proportional to its bandwidth:

$$x_m(t) = x(t - \tau_m), \quad (3.3)$$

where $x_m$ is the measured motion shown in Fig. 3.1. In the case that the filter’s bandwidth is 150kHz, the delay time $\tau_m$ is 6.7μs. Moreover, the digital controller’s pipeline delay $\tau_c$ is deterministic. It comprises 4.8μs for AD conversion and 4.8μs for performing calculation and updating control effort. Hence, for a general discrete control law $u_c(x_r - x_m)$ designed with the sampling time interval $\tau_s = t_k - t_{k-1}$ for zero-order hold (ZOH) [39], and the reference position $x_r = 0$, the control effort is constant within the time interval,

$$u_c(t) = u_c[x_m(t_k - \tau_c)], \quad t \in [t_{k-1}, t_k)_{k=1, 2, \ldots}.$$  \quad (3.4)
The actuator dynamics was analyzed in Chapter 2.4 as well. The DM for axial actuation was modeled as an 8\textsuperscript{th}-order system via experimental calibration and model fitting, and the AOD for lateral actuation as a pure time delay $\tau_a$ ($\approx 12 \mu s$ when excludes the actuation effort updating time) due to the time required for the acoustic wave propagation in the actuator. The laser steering block in Fig. 3.1 for the $x$-axis is thus represented by

$$u(t) = u_c(t - \tau_a).$$

Substituting Eqs. (3.3)–(3.5) into Eq. (3.2) and defining the total loop delay time as $\tau_T = \tau_m + \tau_c + \tau_a$, the equation that describes the measured probe motion $x_m$ with the control law $u_c(x_m)$ can be derived,

$$\gamma \ddot{x}_m(t) + k_{OT} x_m(t) = k_{OT} u_c \left[ x_m(t_{k-1} - \tau_T) \right] + F_L(t), \quad t \in [t_{k-1}, \ t_k], \ k=1, 2, \ldots, \quad (3.6)$$

in which $F_L(t) = F_L(t - \tau_m)$ is the modified thermal force. It is evident that the design and analysis for the control of the lateral motion are characterized by the total delay time. For the control of the axial motion, the actuator dynamics is more complex. Nevertheless, the same approach can be applied as long as the actuator dynamics is included.

3.2.3 Discrete time system model and data analysis

The solution to Eq. (3.6) is the probe’s motion subject to digital control, whereas the measured motion is the sampled data at a series of discrete instants. Therefore, a discrete time system model can be derived by transforming Eq. (3.6) to an equivalent difference equation that establishes the relationship between the sampled motion and the discrete control effort. It enables the design of optimal controllers based on digital control.
theory and the development of discrete data analysis methods. When the discrete control effort \( u_c \) is kept constant in between each sampling, Eq. (3.6) is a linear equation and the principle of superposition is applicable. The sampled motion at each time \( t_k \) is therefore decomposed into two components, i.e. \( x_m[k] = x_{md}[k] + v[k] \), where \( x_{md}(t) \), driven by the optical trapping force, is the deterministic motion from preceding sampled position \( x_m[k-1] \) toward the focal center \( u \) during the sampling time interval \( [t_{k-1}, t_k) \), and \( v \) is the additive displacement of the probe due to the random thermal force within the time interval. The two components are the solutions to the following two equations at time \( t_k \), given their respective initial conditions at time \( t_{k-1} \), namely \( x_{md}(t_k) = x_m[k-1] \) and \( v(t_{k-1}) = 0 \),

\[
\begin{align*}
\gamma \dot{x}_{md}(t) + k_{\text{opt}} x_{md}(t) &= k_{\text{opt}} u_c \left[ x_m(t_{k-1} - \tau) \right], \quad t \in [t_{k-1}, t_k) \\
\gamma \dot{v}(t) + k_{\text{opt}} v(t) &= F_L(t) U(t-t_{k-1}), \quad t \in [t_{k-1}, t_k) 
\end{align*}
\]

(3.7)

The use of unit step function \( U(t-t_{k-1}) \) and zero initial condition in the second equation of Eq. (3.7) indicates that the additive displacement \( v \) is solely caused by the thermal noise between two consecutive samplings, whereas the thermal force’s effect before time \( t_{k-1} \) has been reflected in the initial probe position \( x_m[k-1] \) at \( t_{k-1} \). In the case that the sampling time interval \( \tau_s \) is chosen such that \( \Delta = \tau_s / \tau_t \) is an integer, the resulting difference equation is

\[
x_m[k] = \phi x_m[k-1] + (1-\phi) u_c \left[ x_m[k-1-\Delta] \right] + v[k]
\]

(3.8)

where the state transition coefficient,
\[ \varphi = \exp\left(-\frac{k_{OT}}{\gamma} \tau_s\right) = \exp(-2\pi f_c \tau_s), \]  

(3.9)

is associated with the open-loop trapping bandwidth [41]

\[ f_c = \frac{k_{OT}}{2\pi \gamma}. \]  

(3.10)

The difference equation represents a 1st-order ARMAX model that relates the sampled probe motion to the discrete control effort of the trapping system and the persistent thermal noise. The noise term \( v \) is characterized by its variance

\[ \sigma_v^2 = \frac{k_B T}{k_{OT}} (1 - \varphi^2) \leq \frac{k_B T}{\gamma} \tau_s, \]  

(3.11)

which is derived from Eq. (3.7) with the autocorrelation of the thermal force [41],

\[ R_f(\tau) = 2\gamma k_B T \cdot \delta(\tau). \]  

(3.12)

Furthermore, since \( v[k] \) for each \( k \) is the cumulative effect of independent white noise within its own time interval, it can be proven that its discrete autocorrelation is

\[ R_v[i, j] = \sigma_v^2 \cdot \delta[i, j] = \begin{cases} \frac{k_B T}{k_{OT}} (1 - \varphi^2), & i = j, \\ 0, & i \neq j \end{cases}, \]  

(3.13)

and by taking the z-transform [56] its discrete PSD is

\[ S_v(z) = \frac{k_B T}{k_{OT}} (1 - \varphi^2), \]  

(3.14)

where \( z \) is the complex frequency-associated variable in z-transform.

With the ARMAX system model, any causal digital controller can be directly designed using digital control theory, no matter it is linear or not, and the theoretical PSD of the actively controlled motion can be derived to benchmark the controller’s
performance. For a linear controller whose transfer function is \( C(z) \), i.e. \( U_c(z) = -C(z)X_m(z) \), the control system’s discrete transfer function is

\[
H(z) = \frac{X_m(z)}{V(z)} = \frac{1}{1-\varphi z^{-1} + (1-\varphi)C(z)z^{-(\Delta+1)}}. \tag{3.15}
\]

The discrete PSD of the controlled motion is thus derived to be \( S_\omega(z) = S_e(z)\cdot H(z)H(z^{-1}) \) \[57\], according to a corollary of the Wiener-Khinchin theorem. As a consequence of the Poisson summation formula, the Brownian motion’s double-sided PSD in frequency domain is \[57\]

\[
S_{\omega\omega}(f) = \tau_x \cdot S_e[z \mid z = \text{exp}(j2\pi f \tau_s)]_f \quad f \in \left(-\frac{1}{2\tau_s}, \frac{1}{2\tau_s}\right] \]

\[
= \frac{k_g T(1-\varphi^2) \tau_x}{k_{oT} \left| 1-\varphi e^{-j2\pi f \tau_s} + (1-\varphi)e^{j2\pi f \tau_s}e^{-j(\Delta+1)2\pi f \tau_s} \right|^2}. \tag{3.16}
\]

### 3.2.4 Proportional control experiment and model validation

Proportional control experiments using the controller \( C_p(z) = K_p \) were conducted and Eq. (3.16) was employed to validate the derived discrete time model. A stable open-loop trap of a 1.87μm probe with ~32mW laser power at focus was established initially. System parameters, \( k_{oT} \) and \( \varphi \), in Eq. (3.16) were obtained through the calibration of the open-loop trapping based on PSD fitting method \[41\]. The total loop delay \( \tau_T \) is about 28.8μs as analyzed earlier, so \( \Delta = 2 \) when the sampling time interval \( \tau_s \) is set to 14.4μs.
Fig. 3.3: Comparison of experimental PSDs and theoretical predictions of proportionally controlled Brownian motion of a 1.87\(\mu\)m probe, wherein a number of control gains are employed. The experimental PSDs are calculated using FFT algorithm and averaged in frequency domain from 100 sets of data to enhance the result. They are overlaid with their corresponding theoretical predictions (black solid lines) based on Eq. (3.16) and open-loop system calibration.

A number of control gains were implemented. The experimental PSDs were acquired from the measured Brownian motion of the probe with the help of FFT (fast Fourier transform) algorithm and averaged in frequency domain to enhance the result. They are overlaid in Fig. 3.3 with the theoretical predictions based on Eq. (3.16), in which the environmental temperature \(T\) is 296.15K (23°C). The close resemblance of the experimental PSDs and the analytical predictions in Fig. 3.3 validates the derived discrete-time model. It can thus be used to predict the performance of a given controller design. It is worth noting that a similar comparison based on a continuous-time model
was reported in [29]. The total system delay as a lumped parameter in that model was obtained by fitting the experimental PSDs. The delay, therefore, implicitly includes the delay of each component in Fig. 3.1 and the equivalent half-step sampling delay introduced by the digital controller’s ZOH [39] as well. The ARMAX model derived in this paper captures the discrete nature of the system’s digital controller and the sampled probe motion. It facilitates the development and analysis of various control and estimation objectives. Specifically, its application to the design and implementation of minimum-variance Brownian motion control is discussed in the next section.

3.3 Minimum Variance Control Analysis and Experiments

In this section, after the investigation on minimum variance control theory, the optimal controller that minimizes the probe’s Brownian motion is designed and implemented according to the derived discrete ARMAX model. The theoretical analysis is then compared to the experimental result as well as the simulation to illustrate the performance envelope of active control. Adaptive minimum variance control is also implemented to sustain optimal performance when operating the actively controlled optical trap in a complex environment.

3.3.1 Minimum variance control theory

Minimum variance control (MVC) analysis for an ARMAX system that is subject to loop delay and stochastic disturbance was developed by Astrom [53]. A general $i^{th}$-order ARMAX model that has $k$-step loop delay is

$$A(z^{-1})y[n+k] = B(z^{-1})u[n] + D(z^{-1})e[n+k],$$

(3.17)
where \( A(z^{-1}) \), \( B(z^{-1}) \), and \( D(z^{-1}) \) are polynomials of time-shifting variable \( z^{-1} \) with no more than \( i \)th-orders and \( e[n] \) is a discrete white noise. An admissible feedback control law to minimize the variance of the system output must be causal [56]. In order to make the causal controller design easy, Eq. (3.17) needs to be rewritten with respect to the available information of the output \( y \) and control effort \( u \) no later than sequence \( n \). Hence the following identity is defined [53]:

\[
1(z^{-1}) = A(z^{-1})F(z^{-1}) + z^{-i}G(z^{-1}), \tag{3.18}
\]

with \( F(z^{-1}) \) a \((k-1)\)th-order polynomial and \( G(z^{-1}) \) \((i-1)\)th-order. The total \((i+k)\) coefficients of the two polynomials can be solved from the identity, whose order is \((i + k - 1)\). Employing the identity, Eq. (3.17) can be transformed as

\[
y[n+k] = F(z^{-1})e[n+k] + \frac{G(z^{-1})}{D(z^{-1})}y[n] + \frac{B(z^{-1})F(z^{-1})}{D(z^{-1})}u[n]. \tag{3.19}
\]

The noise term \( F(z^{-1})e[n+k] \) is a linear function of white noise vector \( \{e[n+k], \ldots, e[n+1]\}^T \), and is independent of the output and control effort at or before sequence \( n \). Therefore, the zero-mean output’s variance is

\[
\begin{align*}
E\left\{y[n+k]\right\}^2 & = E\left\{F(z^{-1})e[n+k]\right\}^2 + E\left\{\frac{G(z^{-1})}{D(z^{-1})}y[n] + \frac{B(z^{-1})F(z^{-1})}{D(z^{-1})}u[n]\right\}^2 \\
& \geq \sigma_e^2 \sum_{j=0}^{k-1} f_j^2 \tag{3.20}
\end{align*}
\]

where \( \sigma_e^2 \) is the variance of \( e[n] \), and \( f_j \) is the \( j \)th coefficient of \( F(z^{-1}) \). Then the minimum variance controller is designed to make the equality hold in above inequality by forcing the controller-dependent part to zero:
It is seen from Eq. (3.8) that for our optical trapping system, $A(z^{-1}) = 1 - \varphi z^{-1}$, $B(z^{-1}) = 1 - \varphi$, and $D(z^{-1}) = 1$. Then using Eq. (3.18), $F(z^{-1}) = 1 + \varphi z^{-1} + \cdots + \varphi^\lambda z^{-\lambda}$ and $G(z^{-1}) = \varphi^{\lambda+1}$ are derived. Therefore, the minimum variance $\sigma^2_x$ as well as the optimal controller $C_M(z)$ can be determined from Eqs. (3.20) and (3.21) for Brownian motion control with our trapping system.

3.3.2 Theoretical minimum-variance Brownian motion control analysis

As shown above, the optimal Brownian motion controller can be designed according to the MVC theory. The system output’s variance is directly set by the control objective as the cost function to be minimized, and the optimal controller is designed such that the variance of the output’s controller-dependent part is forced to zero. Therefore, the performance envelope of active control of a specific optical trap can be evaluated. In the analysis, the effect of measurement noise is ignored as the measurement system achieves sub-nanometer resolution [14]; i.e., measurement noise is very small when compared to the Brownian motion’s amplitude, which is typically between several nanometers and hundreds of nanometers.

By applying the minimum variance (MV) controller design, Eq. (3.21), to the discrete ARMAX model, Eq. (3.8), the transfer function of the optimal controller for Brownian motion control with the optical trapping system is

$$C_M(z) = -\frac{U(z)}{Y(z)} = \frac{G(z^{-1})}{B(z^{-1})F(z^{-1})}.$$  (3.21)
\[
C_m(z) = \frac{\varphi^{\Delta+1}}{1 - \varphi} \frac{1}{1 + \varphi z^{-1} + \cdots + \varphi^\Delta z^{-\Delta}}. \tag{3.22}
\]

Substituting Eq. (3.22) to Eq. (3.15), the control system’s discrete transfer function from noise \( v \) to measured motion \( x_m \) is
\[
H_m(z) = \frac{X_m(z)}{V(z)} = 1 + \varphi z^{-1} + \cdots + \varphi^\Delta z^{-\Delta}. \tag{3.23}
\]

It is evident that the Brownian motion’s mean is zero as \( v \) is zero-mean noise. The Brownian motion’s variance is therefore equal to the motion’s mean square, which can be derived using Eqs. (3.13) and (3.23),
\[
\sigma_x^2 = E\left\{ \left[ v[k] + \varphi v[k-1] + \cdots + \varphi^\Delta v[k-\Delta] \right]^2 \right\} \\
= \frac{k_B T}{k_{OT}} \left[ 1 - \varphi^{2(\Delta+1)} \right] \leq 2 \frac{k_B T}{\gamma} \left( \tau_r + \tau_s \right). \tag{3.24}
\]

This value equals to the variance of the trapped probe’s incremental displacement, caused by the cumulative effect of the random thermal force lasting for a time period of \((\tau_r + \tau_s)\). This period is actually the time consumed by the control loop from sensing the motion to making the feedback control effort effective; therefore, the effect of the thermal noise during this latency is irrepressible. As a result, the performance of optimal Brownian motion control is dictated mainly by the system’s loop delay, and to a less extent by the sampling time (ZOH time) in the case that \( \tau_s \) is much smaller than \( \tau_r \).

Moreover, the Brownian motion’s PSD after minimum variance control is derived from Eqs. (3.16) and (3.22),
\[
S_m(f) = \frac{k_B T (1 - \varphi^2) \tau_s}{k_{OT}} \left| 1 + \varphi e^{-j2\pi\tau_r} + \cdots + \varphi^\Delta e^{-j2\pi\tau_s} \right|^2. \tag{3.25}
\]
Theoretical prediction using Eq. (3.25) will be compared to the experimental PSD later to validate the MVC analysis.

One constraint of the above analysis arises from the fact that the optical trapping force is finite, as mentioned in Chapter 3.2.1. Hence, it is necessary to establish an applicability criterion to determine whether the required control force exceeds the capability of the optical trap. The control force $F_{OT} = -k_{OT}(x - u)$ can be written with respect to the discrete white noise $\nu[k]$ using Eqs. (3.22) and (3.23). Its variance is then derived,

$$\sigma_{F_{OT}}^2 = E \left( k_{OT}^2 \left( \nu[k] + \varphi \nu[k-1] + \cdots + (\varphi^{\Delta+1}) \nu[k-\Delta] \right)^2 \right)$$

$$= k_b T k_{OT} \left[ 1 - \varphi^{2(\Delta+1)} + 2\varphi^{2\Delta+1}(1+\varphi) + \frac{\varphi^{2(\Delta+1)}(1+\varphi)}{1-\varphi} \right] \leq \frac{2k_bT\gamma}{\tau_s} . \quad (3.26)$$

Consequently, an applicability criterion is proposed, wherein the analysis of Eqs. (3.22)~(3.25) should hold if the $\pm 3\sigma_{F_{OT}}$ force range is within the two extremes of the trapping force field. Otherwise, it may be necessary to employ nonlinear control laws to improve the trapping stability, as suggested by the simulation in [37]. In case of controlling a 1.87\,\mu m probe with ~32mW laser power at focus, the two trapping force extremes scaled from Fig. 3.2 is $\pm 20$pN. According to Eq. (3.26), the required $3\sigma$ force range is determined to be $\pm 8.4$pN. The MV controller of Eq. (3.22), therefore, meets the applicability criterion. It is implemented and the experimental result is subsequently compared to the MVC analysis for the purpose of verification.
3.3.3 Validation of the theoretical analysis

Fig. 3.4: Experimental standard deviations of a 1.87\(\mu m\) probe’s Brownian motion after control. Each data is statistically observed from measured motion of 5s long, and drawn against the respective controllers’ DC gain. The gain is \(K_p\) for P control, and derived from Eq. (3.22) for MVC. As is seen, the DC gain of best P controller is the same as that of the minimum variance controller.

The experimental standard deviations of a 1.87\(\mu m\) probe’s motion, after P control and MVC with \(\sim32\text{mW}\) laser power at focus, are drawn against their respective controllers’ DC gain in Fig. 3.4 to demonstrate the performance envelope of the system. For P controller, the gain is \(K_p\), and that of MV controller is shown by Eq. (3.22) to be dictated by \(\varphi\). The best P control achieves a 4.4-nm \(\sigma_x\) of the controlled motion with the gain experimentally determined to be 7, as illustrated in the figure. This gain is the same
as that of the MV controller, designed by Eq. (3.22), wherein the \( \sigma \), after control is 3.8~3.9nm. The PSD of the experimental MVC result is then compared to its theoretical prediction and that of the best P control result to validate the analysis in Chapter 3.3.2.

Fig. 3.5: Comparison of analytical and experimental PSDs of the controlled Brownian motion of a 1.87\( \mu \)m probe between the best P controller and the MV controller.

As indicated by Eq. (3.23), the controlled Brownian motion using MV controller is theoretically the weighted moving average of discrete white noise \( \nu[k] \). The weighting factors are characterized by \( \phi \), which is very close to 1 due to the high sampling rate of the control system. Therefore, according to Eq. (3.25), the theoretical PSD of the controlled motion has a shape similar to the square of a sinc function [56]. It is illustrated
by the black solid curve in Fig. 3.5 for the case of controlling a 1.87μm probe with ~32mW laser power at focus. The experimental PSD (blue curve) is drawn in Fig. 3.5 as well for comparison. Except the small discrepancy around the roll-off of the curve and that close to the Nyquist frequency, the two agree well with each other. This comparison again demonstrates the accuracy of the theoretical analysis. The best result of P control, with the gain $K_p = 7$, is also included in Fig. 3.5 for comparison. The two PSDs coincide in low-frequency range due to the same DC gain of the controllers, as is seen from Fig. 3.4. However, the P controller results in a small peak at higher frequency, the value of which is decided by the loop delay of the system [29]. The MV controller suppresses the motion’s high frequency response with phase-lead compensation and larger AC gain, as illustrated by the controller’s Bode plot calculated using Eq. (3.22) (Fig. 3.6). In terms of the standard deviation $\sigma_s$ of the controlled motion, the theoretical $\sigma_s$ of the best P control, obtained by integrating the analytical PSD [57], is 4.8nm; and that of MVC, predicted by Eq. (3.24), is 4.3nm. Both theoretical values are a little larger than their respective experimental results.
Fig. 3.6: The Bode plot of MV controller for controlling a $1.87\mu m$ probe’s Brownian motion. The controller’s phase-lead compensation and larger AC gain are clearly demonstrated.

The slight error in the theoretical analysis is further explained by computer simulation, through which the applicability criterion is validated as well. There are two simplifications on the trapping system model in the theoretical analysis. First, the 3D nonlinear optical force field is decomposed into three independent linear spring forces in the analysis. Second, the dynamics of the measurement filter is simplified as a pure time delay. The effects of these simplifications can be examined using time-domain simulation.
A Matlab Simulink® simulator of the MVC was built based on Eqs. (3.1), (3.3) ~ (3.5), and (3.22), as shown in Fig. 3.7. In addition to including the actuator dynamics and measurement delay, the trapping force nonlinearity as well as the coupling of the lateral and axial force field is depicted by a gradient force model [36], wherein the trapping force is calculated from the simulated probe motion and control effort. The simulated trapping force is added to the thermal force, generated according to the theoretical autocorrelation of the thermal noise of Eq. (3.12) by a Band-Limited White Noise block in Simulink®. The correlation time of the thermal force cannot be infinitesimal in the simulation, but is selected to be 10ns (nanosecond), three orders shorter than the system’s sampling time \( \tau_s \). Therefore, the accuracy of the simulation is guaranteed [58]. The total force is subsequently scaled by the damping coefficient \( \gamma \) of the probe and integrated to obtain its controlled Brownian motion.

The PSD of the simulated motion (red dotted line in Fig. 3.5) precisely overlaps the theoretical prediction from Eq. (3.25), and the motion’s \( \sigma_x \) equals to the theoretical
value of 4.3nm, as predicted in Eq. (3.24). It is hence concluded that as long as the applicability criterion holds, trapping force nonlinearity does not affect the control law design and the result of Brownian motion control. Subsequently, the measurement delay in the simulation was replaced by an 8th-order Butterworth filter model with 150 kHz bandwidth. Although it is not the exact model of the system’s measurement filter, which is unavailable, the PSD obtained from the probe’s motion in the new simulation (green dotted line in Fig. 3.5) is seen to better agree with the experimental PSD. It is evident that the slight discrepancy between the theoretical analysis and the experimental result is primarily due to the simplification of the filter model. In addition, the probe’s $\sigma_x$ in the new simulation is 3.9nm, which is very close to the experimental result of the MVC.

3.3.4 Adaptive minimum variance control experiment

Besides the design of the MV controller, Eq. (3.24) indicates that the variance of the controlled motion of a specific optical trap also depends on the state transition coefficient $\phi$ of the system. As shown in Eq. (3.9), the parameter $\phi$ is a variable of the trapping force stiffness $k_{ot}$ and the probe’s damping coefficient $\gamma$, which changes according to the temperature and varies significantly due to wall effect [5]. The system is hence likely time-varying in many applications. In order to maintain the optimal control performance, adaptive MVC is employed.

The basic concept of adaptive MVC is to estimate the parameter $\phi$, and update the controller, Eq. (3.22), accordingly. Theoretical studies of this type of self-tuning control structures exist in literature [59,60], where both estimation convergence and control stability have been proven. Both estimation and adaptive control algorithms were
implemented in our FPGA controller. The implemented algorithms are based on the weighted recursive least squares (WRLS) algorithm [61], in which appropriate weighting factor is selected to estimate the time-varying parameter. It can be shown that the estimation turns out to be the best linear unbiased estimation (BLUE) [42]. Moreover, in order to estimate the probe-sample interaction simultaneously, the estimator was expanded into an adaptive observer to enable joint state-parameter estimation [62], in which the probe-sample interaction is modeled as an external disturbance. When employing a proper disturbance model and optimized by the Kalman filter algorithm, the joint estimation is BLUE as well as maximum likelihood (ML) [42], as to be discussed in Chapter 4. In this way, the estimated probe-sample interaction can be utilized to enable dynamic force probing and force feedback control, whereas this chapter focuses on the adaptive MVC that maintains the optimal performance of Brownian motion control even when the probe’s damping coefficient varies significantly.
Fig. 3.8: (a) Measured motion of a 1.87μm probe and (b) standard deviation of the motion with and without adaptive control. Each data point in (b) is statistically determined from observing 0.3-second long data in (a) of the corresponding time.
Fig. 3.8 shows the measured motion of a 1.87μm probe and its standard deviation in an adaptive minimum-variance Brownian motion control experiment. In the experiment, the laser power was ~32mW at focus and the probe was initially stabilized by the trapping laser with MVC. The sample stage was moved upward toward the probe, stayed at a constant height for 1.5 seconds, and then retreated back to the initial position. At the highest position of the stage, the probe was about 600nm above the cover slip placed on the sample stage. According to Faxen’s law [5], the damping coefficient $\gamma$ increased as the stage moved upward and decreased as it retreated, and so did $\phi$. Fig. 3.9 shows the stage’s trajectory and the real-time estimate of $\phi$. As can be seen in Fig. 3.8 and Fig. 3.9, the Brownian motion’s standard deviation $\sigma_x$ [green curve in Fig. 3.8(b)] decreased with the increase of estimated $\phi$ (green curve in Fig. 3.9), when the stage was closer to the probe. This is due to the fact that the adaptive MVC is able to tune the control gain according to the estimated $\phi$ and that according to Eq. (3.24), the closer is $\phi$ to 1 the smaller the Brownian motion. The experiment was repeated with the feedback control turned off. Concluded from the equipartition theorem [5], $\frac{1}{2}k_{0r}\langle x^2 \rangle = \frac{1}{2}k_BT$, and the PSD analysis [57] the Brownian motion’s variance is expected to be independent of $\gamma$’s variation without active control, as is verified in Fig. 3.8. The results shown in Fig. 3.8 and Fig. 3.9 validate the theoretical analysis and demonstrate the effectiveness of the adaptive MVC.
3.4 Conclusions and Remarks

Theoretical and experimental investigation on the Brownian motion control of an optically trapped probe is presented in this chapter. For the purpose of digital controller design and discrete data analysis, a 1st-order ARMAX model is derived to describe the Brownian motion of an optically trapped probe with active control. It establishes the relationship between the probe’s motion and the controller, characterized by the effects of the feedback loop’s time delay. The derived model is experimentally validated using the proportional control result of a 1.87µm trapped probe. It is then employed to design the optimal controller that minimizes the variance of the probe’s Brownian motion. In order
to evaluate the control performance, analysis is derived based on minimum variance control theory. The theoretical analysis is then compared to the experimental result and the simulation to illustrate the performance envelope of active control. The minimum standard deviation of a 1.87μm probe’s Brownian motion, achieved with our current setup, is shown to be 3.8~3.9nm. Moreover, adaptive minimum variance control is implemented and experimentally verified. It is capable of maintaining the optimal control performance in the case that the actively controlled optical trap is operated in a complex time-varying environment.

Based on the analysis derived in this chapter, the performance of the Brownian motion control of a specific optical trap can be predicted and optimized. When the applicability criterion is met, according to Eq. (3.24), the variance of the probe’s motion is primarily dictated by the probe’s damping coefficient $\gamma$, the system loop delay $\tau_T$, and sampling time interval $\tau_s$. While the trapping stiffness $k_{ot}$ also has slight effect, the trapping laser power can be reduced to the level decided by the applicability criterion, and thus the damage of biological samples induced by laser heating can be minimized. Since $\gamma$ is proportional to the bead size, so is the trapping force to the bead volume for nanoparticles, it is therefore more difficult to achieve stable trapping of particles at nanometer scale.

Reducing loop delay and sampling time of the system will improve the trapping stability. For example, if $\tau_T$ reduces from 14.4μs to 4.8μs, the minimum standard deviation of a proportionally controlled 1.87μm probe’s motion improves from 4.4nm to 4.1nm experimentally, as shown in Fig. 3.10. It, however, requires faster devices for
actuation, measurement, and control. Moreover, according to Eq. (3.26), raising the
system’s sampling rate increases the required optical force for control, since the force for
MVC is roughly \( \propto \tau^{-1/2} \). In this case, the regulating force needs to be restrained in the
controller from exceeding the trapping capability. It can be proven by Lyapunov 2nd
theorem [39] that the nanoparticle trapping will be globally asymptotically stable (GAS)
using the modified control law with restricted trapping force, even when the applicable
optical force is very small. The new 3D control scheme can then be applied to stabilize
the nanoparticles that were not possible to trap previously in room temperature [51].

Fig. 3.10: Experimental standard deviations of a 1.87\( \mu \)m probe’s \( x \)-axis and \( y \)-axis
Brownian motion after P control. The sampling time \( \tau_s=4.8\)\( \mu \)s, and the best results of both
axes, in terms of the motion’s standard deviation \( \sigma \), are 4.1nm. Due to the slight
asymmetry of the open-loop trapping, the initial \( \sigma \)’s without control for the two axes are
different, so are the optimal gains of the controllers.
CHAPTER 4: ADAPTIVE ESTIMATION FOR DYNAMIC FORCE PROBING

4.1 Introduction

Due to its capabilities of three-dimensional non-contact manipulation and measurement with sub-pN force resolution, optically trapped probing system is an important research tool for biological studies. The persistent thermal noise in the system is the primary source that affects its measurement resolution. In addition, quasi-static linear spring model, which is commonly employed with the probing system for force measurement, limits its dynamic sensing applications. Real-time adaptive estimation is therefore implemented to optimize force probing with the system. It will facilitate the research on time-varying biological processes.

A recursive adaptive observer is designed based on the augmented system model. It solves two major issues to improve the dynamic force measurement. First, the dynamics of the external force as well as the trapping system variation are included for accurate force estimation by means of a Luenberger observer. The observer will recursively estimate the external force as an augmented state variable of the system from the noisy motion of the probe, and due to the principle of control-estimation separation,
its performance of dynamic force sensing is unaffected by the manipulation and control of the system. Second, parameter estimation in the developed adaptive observer detects the variation of trapping dynamics to correct the dynamic force sensing automatically. From inferring the cause of the variation, additional information of the process under investigation can be gained as well. Kalman filter algorithm [42], as an efficient recursive algorithm evolved from least squares solution for estimating the state variables of the system with noisy measurements, is employed to minimize the estimation error. Consequently, it results in the best linear unbiased and maximum likelihood estimation if the process and measurement noises satisfy the white Gaussian condition.

Experiments of adaptive dynamic force probing on known interaction forces as well as adaptive estimation of a non-stationary process are conducted to demonstrate the estimator’s performance. The resolution and accuracy of the estimation is illustrated by comparing the force probing results to the given inputs. Control-estimation separation is validated, wherein the estimator works consistently when the various controls for manipulation enhancement and force regulation are enabled. The convergence of the adaptive estimation is verified as well, by the comparison between estimated variation of trapping dynamics and its theoretical prediction.

4.2 Adaptive Estimation Algorithm

In this section, the discrete system model developed in Chapter 3.2 is first augmented with the dynamics of the external force and trapping system variation. A reduced-order adaptive observer is then designed for the joint state-parameter estimation of the external force and the trapping system model. To optimize the adaptive dynamic
force probing result, Kalman filtering algorithm is employed, and the complete recursive formulation is given for the implementation in the FPGA controller of the trapping system.

4.2.1 Discrete system model

In Chapter 3.2, a discrete model that describes the sampled probe motion was established, whereas the external interaction force $F_E$ was absent in the scenario of pure Brownian motion control. In order to measure and regulate the interaction force involved in dynamic biological processes, the model is modified to include the force and a recursive estimator is designed accordingly for the purpose. The block diagram illustrating the combined estimation and control concept of the developed probing system is drawn in Fig. 4.1.

![Fig. 4.1: The combined estimation and control structure of the optically trapped probing system.](image-url)
When the force \( F_E \) is present, the linearized equation of motion of the trapped probe, Eq. (3.2), becomes
\[
\gamma \ddot{x} + k_{OT} x = k_{OT} u + F_E(t) + F_L(t).
\] (4.1)

According to the analysis in Chapter 3.2.2, the actuator dynamics and measurement delay needs to be included. For the actuation and measurement along lateral axes, they can be lumped into a single parameter of total loop delay \( \tau_L \), which is 28.8μs for current trapping setup. Then the new equation that describes the measured probe motion \( x_m \) with the control law \( u_c(x_m) \) and the external force to be estimated \( F_E \) can be derived,
\[
\gamma \dot{x}_m(t) + k_{OT} x_m(t) = k_{OT} u_c \left[ x_m(t_{k-1} - \tau_L) \right] + F_{E'}(t) + F_{L'}(t), \quad t \in [t_{k-1}, t_k), \quad k = 1, 2, \ldots,
\] (4.2)
in which \( F_{E'}(t) = F_E(t - \tau_m) \) and \( F_{L'}(t) = F_L(t - \tau_m) \) are the modified forces due to the measurement delay \( \tau_m \), and the system’s sampling time interval is \( \tau_s = t_k - t_{k-1} \). The axial DM actuator’s dynamics is more complicated, which was calibrated to be 8th-order in Chapter 2.4.4. Nevertheless, as long as the actuator dynamics is included in the model, the same approach for estimator design as presented in this chapter can be applied.

Similar to the procedure employed in Chapter 3.2, the new discrete system model is established from Eq. (4.2), which is linear between each sampling. Based on superposition principle, the sampled motion at each time \( t_k \) is decomposed into three components instead of two, i.e.
\[
x_m[k] = x_m[k] + d[k] + v[k],
\]
where the additional disturbance term \( d \) is the probe’s displacement due to the external force in each sampling time interval. Therefore, \( d[k] \) is the solution to the following equation at time \( t_k \), given the initial condition \( d(t_{k-1}) = 0 \), whereas the other two are still the solutions to Eq. (3.7):
\[
\gamma \dot{d}(t) + k_{OT} d(t) = F_E(t)U(t-t_{k-1}), \quad t \in [t_{k-1}, t_k).
\]

(4.3)

The use of unit step function \( U(t-t_{k-1}) \) and zero initial condition in Eq. (4.3) again reflects the fact that the initial probe position \( x_m[k-1] \) at \( t_{k-1} \) has been the integrated consequence of all the forces before time \( t_{k-1} \). Hence when the sampling time interval \( \tau_s \) is chosen such that \( \Delta = \tau_f / \tau_s \) is an integer, the new difference equation that incorporates the effect of external force is

\[
x_m[k] = \varphi x_m[k-1] + (1-\varphi)u_c \{x_m[k-1-\Delta]\} + d[k] + \nu[k],
\]

(4.4)

where \( \varphi \) is the same state transition coefficient defined in Eq. (3.9) and \( \nu \) has the same autocorrelation as Eq. (3.13). Solving from Eq. (4.3),

\[
d[k] = \int_{t_{k-1}}^{t_h} h(t)F_E(t-\tau)dt,
\]

(4.5)

in which

\[
h(t) = \frac{1}{\gamma} \exp[-\frac{k_{OT}}{\gamma}(t-t_{k-1})]
\]

(4.6)

is the impulse response function of Eq. (4.3). If \( F_E \) varies slowly compare to the system’s high sampling rate, it can be measured by its mean value during each sampling time interval, which, as shown by the convolution of Eq. (4.5), can be scaled from \( d[k] \):

\[
F_E(t-t_{m_{\|t_{k-1}<t\|}}) \approx \overline{F_E}[k] = \frac{k_{OT}}{1-\varphi} d[k].
\]

(4.7)

4.2.2 System augmentation and adaptive observer design

As seen from Eq. (4.7), in order to compute the dynamic interaction force \( F_E \), both the disturbance \( d \) and the parameter \( \varphi \) in the system model, Eq. (4.4), need to be
estimated in real time. The trapping stiffness $k_{OT}$ in the equation, which can be calibrated beforehand, is invariant if the laser power is unchanged. Adaptive estimation of $d$ and $\phi$ is therefore investigated in this section.

A well-known approach of disturbance estimation is to augment the system model with the dynamics of the disturbance, so that it becomes a virtual state variable to be estimated by the designed observer [39]. The same methodology can be applied to parameter estimation, with the inclusion of a suitable update law for parameter variation in the system model [63]. Hence, as long as the convergence of the estimator can be proven, adaptive estimation is feasible. The detail of using above method to design the adaptive observer in our probing system is presented below.

The disturbance dynamics can be described with an arbitrary linear model. Although a priori knowledge of the actual dynamics is desirable for accurate estimation, it is not mandatory [39]. The only constraint to assure the existence of convergent estimators, $d$ to be observable, is evident in Eq. (4.4). Thus, a $n^{th}$-order model that fits a dynamic process with a $(n-1)^{th}$-order polynomial function of time is employed [64]:

$$d[k] = \sum_{m=1}^{n} (-1)^{n-1} C^n_m d[k-m] + w_1[k], \quad (4.8)$$

where $C^n_m$ is the binomial coefficient, and $w_1$ is process noise with variance $\sigma_1^2$. $w_1$ includes all unmodeled dynamics as well as any random disturbance source. For a slow-varying external force, the 1st-order model of Eq. (4.8), i.e.,

$$d[k] = d[k-1] + w_1[k], \quad (4.9)$$

is appropriate. Similarly, since parameter variation caused by the environmental change is often even slower, it is represented by a 1st-order model of the same type,
\[
\varphi[k] = \varphi[k-1] + w_2[k].
\] (4.10)

Then the system model, Eq. (4.4), can be expanded and a recursive estimator, also known as a Luenberger observer [39], can be designed. It is worth noting that in Eq. (4.4), the state \( x \) is measured with high resolution; subsequently, it is convenient to construct a reduced-order estimator [39] and estimate \( d \) and \( \varphi \) only. In this case, the augmented system is modeled as

\[
\begin{pmatrix}
    d[k] \\
    \varphi[k]
\end{pmatrix} = \Phi_d
\begin{pmatrix}
    d[k-1] \\
    \varphi[k-1]
\end{pmatrix} +
\begin{pmatrix}
    w_1[k] \\
    w_2[k]
\end{pmatrix}.
\] (4.11)

The first equation of Eq. (4.11), \( d[k] = \Phi_d d[k-1] + w[k] \), is the state-space model of the variables to be estimated. \( \Phi_d \) is a \( 2 \times 2 \) identity matrix \( I_2 \) for slow-varying variables if Eqs. (4.9) and (4.10) are employed. When more accurate dynamics of the variables are available, the model can be revised accordingly. The second part of Eq. (4.11), \( y[k] = H_y[k] d[k] + \nu[k] \), is transformed from Eq. (4.4) and written as a measurement equation to reduce the estimator’s order. Therefore, a Luenberger observer for adaptive estimation with the probing system is formulated to correct the model-based prediction with measurement innovations:

\[
\hat{d}[k] = \Phi_d \hat{d}[k-1] + L[k] \left( y[k] - H_y[k] \Phi_d \hat{d}[k-1] \right).
\] (4.12)

\( L \) in Eq. (4.12) is a \( 2 \times 1 \) feedback gain vector of the estimator, which, depending on the design, may or may not be constant.
When employing Eq. (4.12), the convergence of the estimation on \(d\) is easily guaranteed by choosing proper gains, whereas the convergence of \(\hat{\phi}\) demands additional persistent excitation condition in \(H_d\) [62]. This condition, which is typically required for system identification [63], is satisfied in our system due to the fact that the probe’s motion \(x\) is subject to persistent thermal force excitation. Hence, unbiased adaptive estimation of the dynamic interaction force can be obtained from Eqs. (4.7) and (4.12).

To optimize the adaptive estimation result from Eq. (4.12), Kalman filter algorithm is employed for computing the gain of the estimator. The algorithm will produce the best linear unbiased as well as maximum likelihood estimation (MLE), if \(w\) is white Gaussian and uncorrelated to the white Gaussian measurement noise \(v\) [42]. Consequently, the estimation error will be minimized in the sense of its mean square, and the estimator is asymptotically efficient [42] among all possible ones, whether linear or nonlinear.

Adapted from standard Kalman filter algorithm [39], the gain update equation for Eq. (4.12) is

\[
L[k] = P[k]H_d^T[k]R_{\alpha}^{-1},
\]

(4.13)

where

\[
R_{\alpha} = \sigma_v^2 = R_v[0,0] = \frac{k_s}{k_{or}}(1 - \hat{\phi}^2)
\]

(4.14)

and

\[
P[k] = E(\varepsilon[k]\varepsilon^T[k]) = M[k] - \frac{M[k]H_d^T[k]H_d[k]M[k]}{H_d[k]M[k]H_d^T[k] + R_{\alpha}}.
\]

(4.15)
\( \mathbf{P} \) is the 2×2 error covariance matrix of the estimation after measurement update. It is an indicator of the estimation accuracy as well. In Eq. (4.15),

\[
\mathbf{e}[k] = \mathbf{d}[k] - \hat{\mathbf{d}}[k]
\]

(4.16)
is the 2×1 estimation error vector, and \( \mathbf{M} \) is the propagated value of \( \mathbf{P} \) from previous step,

\[
\mathbf{M}[k] = \Phi_d \mathbf{P}[k-1] \Phi_d^T + \mathbf{R}_{w_0},
\]

(4.17)

with the process noise covariance matrix \( \mathbf{R}_{w_0} \) defined as

\[
\mathbf{R}_{w_0} = \begin{pmatrix}
\sigma_{w_1}^2 & 0 \\
0 & \sigma_{w_2}^2
\end{pmatrix}.
\]

(4.18)

Since \( \mathbf{H}_d \) is related to the persistently excited motion \( x \) by its definition in Eq. (4.11), the gain \( \mathbf{L} \) computed by Eq. (4.13) is time-varying, and steady-state Kalman filter does not exist for the adaptive estimation. The determination of \( \mathbf{L} \) by Kalman filter algorithm leads to whitening the innovations process \( \left( y[k] - \mathbf{H}_d[k] \Phi_d \hat{\mathbf{d}}[k-1] \right) \) [42] and results in optimal tracking performance on the variation of \( \mathbf{d} \) [65].

4.3 Experimental Results

The developed adaptive estimation algorithm of Eqs. (4.12), (4.13), (4.15), and (4.17) is implemented in the probing system’s FPGA controller, and a series of experiments were conducted to assess its performance. The experiments of dynamic force probing of known external interactions are presented in Chapter 4.3.1 and adaptive estimation of a non-stationary process is shown in Chapter 4.3.2, demonstrating the real-time measurement capability of the probing system.
4.3.1 Force sensing experiments

The dynamic response of the adaptive force sensing is illustrated by using the probing system to measure given external forces. The external force on the probe is generated from controlled viscous drag [66,67], which is often employed for force calibration of optical traps. According to Stoke’s law [66],

\[ F_{\text{drag}} = 6\pi \eta a v_m, \]  

(4.19)

where \( a \) is the trapped probe’s radius, \( \eta \) is the viscosity of surrounding medium, and \( v_m \) is the flow speed of the medium. The medium flow is induced by the motion of the setup’s piezo stage, with the probe held still by trapping. The external force can thus be computed from the speed of the stage measured by its own capacitance sensor, and compared to the adaptive force sensing result of the system as the benchmark.

In the first experiment, a 1.87\( \mu \)m-diameter probe was trapped in open loop by the system with \( \sim32 \)mW laser at focus. The piezo stage was commanded to move along \( y \)-axis with a velocity of 250\( \mu \)m/s for 0.4 second, stop for 0.8 second, and then retreat to the initial position with the same speed [Fig. 4.2(b) inset]. This motion exerted \( \sim4\)pN drag force on the probe, as the calculated force (black dashed line) in Fig. 4.2(b) shows. The probe’s motion and the adaptively estimated force, converted using Eq. (4.7) from the estimation result of Eq. (4.12), are drawn in solid blue lines in Fig. 4.2(a) and (b) respectively. Since the trap is not regulated, the external force displaces the probe until new force balance is established [Fig. 4.2(a)]. However, due to the Brownian motion, direct force measurement with the linear spring model using the probe’s position would be very noisy. The adaptive force sensing in Fig. 4.2(b) shows much improved result, having more than one-order-of-magnitude better resolution. In addition, the estimation,
with its settling time (within 5%) <30ms, tracks the force variation in real time. The system is sensitive to any external force, so if any small unknown disturbance happens, it is sensed, causing the estimated force to deviate from the calculated one in some occasions in Fig. 4.2(b).

The same external force was estimated when the control was enabled to validate that the adaptive estimation works independently of the control in the probing system. There are two control schemes activated in the experiment. The first is a proportional Brownian motion control [30] to enhance manipulation resolution by suppressing the probe’s Brownian motion. As a result, the thermal fluctuation of the probe, shown with solid red line in Fig. 4.2(a), is reduced over 50% from the previous open-loop case (blue line), in terms of its standard deviation (STD). The second control is force regulation for disturbance rejection [39]. This is one type of force feedback control, in which the estimated state of the external force is utilized to generate additional trapping force for dynamic balance of the total force on the probe. Consequently, the probe’s collective displacement, caused by the external drag force, disappears. Instead, barely noticeable impulses occur [red line in Fig. 4.2(a)] at the time when the external force is applied or unloaded. Despite the very different probe motion, the red line in Fig. 4.2(b) illustrates that the adaptive estimation is unaffected by control and shows consistent performance. Moreover, the external force and trapping dynamics in this experiment are exactly modeled by Eqs. (4.9) and (4.10), so \( \mathbf{w} \) contains only white Gaussian noise, and the result of Fig. 4.2(b) is optimal.
Fig. 4.2: Adaptive force sensing on step force changes along $y$-axis. (a) The probe’s $y$-axis positions in open-loop and regulated cases; (b) force estimation results of the two cases compared to the computed external force, and (inset) shows the stage’s $y$-axis motion that generates the external drag force.
Fig. 4.3: Adaptive force sensing on step force changes along z-axis. (a) The probe’s z-axis positions in open-loop and regulated cases; (b) force estimation results of the two cases compared to the computed external force, and (inset) shows the stage’s z-axis motion that generates the external drag force.
Adaptive force probing along $z$-axis demonstrates comparable performance to the probing along $x$- and $y$-axis, after the inclusion of axial DM’s dynamics. To take the $z$-actuator dynamics into account, $u_c[k - \Delta - 1]$ in Eq. (4.11) is replaced by $\hat{u}[k - \Delta_z - 1]$, which is calculated from $u_c$ using the 8th-order model of the DM. Because the dynamic model of DM is open-loop stable, $\hat{u}$ converges to the actually applied control effort along $z$-axis with any initial condition; therefore, the estimation is unaffected by the actuator dynamics. Its performance is demonstrated by a similar set of experiments as shown in Fig. 4.2. The stage’s speed along $z$-axis was set to 100$\mu$m/s during 0.2-second ramp up and ramp down, with a 0.4-second-long stop in between. The drag force exerted on the probe is $\sim1.7$pN, as shown in Fig. 4.3(b) by the theoretical calculation as well as the estimation results under both open-loop trapping and closed-loop regulation. Due to the smaller axial trapping stiffness, the probe was displaced more by the force in open-loop, but the displacement was rejected when the force regulation was enabled. However, as a result of smaller variance $\sigma^2_{w_i}$ of the process noise employed in the estimator, the settling time of the estimation becomes longer. The control performance of force regulation is therefore affected due to this and the dynamics of axial DM actuator, which is slower than that of the lateral AOD. Consequently, the force feedback control takes longer time to reject the impact from the external force, as the red line in Fig. 4.3 shows. In addition, the probe’s Brownian motion in $z$ after control is not as small as its motion in lateral plane. This is mainly due to the slower actuator dynamics, as analyzed in Chapter 3.
Fig. 4.4: Adaptive force sensing of sinusoidal force change along $x$-axis. (a) The probe’s $x$-axis positions in open-loop and regulated cases; (b) force estimation results of the two cases compared to the computed external force, and (inset) shows the motion of the stage along $x$-axis for drag force generation.
When applying the adaptive observer to study an unknown process, due to the lack of *a priori* knowledge on the dynamics of the process, the estimation using Kalman filter algorithm may only be suboptimal. The estimator’s performance in this situation is evaluated as well. A 5Hz cosine drag with ~5pN peak force was generated by oscillating the stage with 10μm amplitude along $x$-axis. The accurate model of simple harmonic vibration for $d$ should be

$$d[k] = 2\cos(\omega_d \tau_s) d[k-1] - d[k-2] + w_1[k],$$ (4.20)

where $\omega_d$ is the angular frequency of the force and equals to $10\pi$/s in this experiment. If Eq. (4.12) continues to be employed with $\Phi_d = \Phi$, $w_1$ is no longer white Gaussian because of the modeling error. The resultant force sensing is hence not optimal. Nevertheless, the adaptive observer still works properly and delivers satisfactory estimation results from both controlled and uncontrolled probe motion, as illustrated in Fig. 4.4(b). The unregulated probe shows large force-induced vibration in Fig. 4.4(a) (blue line), whereas it was nearly rejected by control, as illustrated by the red line with slight residual vibration. This imperfect regulation is the consequence of suboptimal estimation, employed to generate balancing force in disturbance rejection. To improve the dynamic force probing on an arbitrary oscillatory process, the 1st-order Eq. (4.9) can be replaced by the 2nd-order model of Eq. (4.8) the adaptive observer design, as a better approximation of the disturbance dynamics.

### 4.3.2 Adaptive estimation of a time-varying process

One advantage of adaptive force sensing is that it can detect the parameter variation of the probing system and automatically correct the force sensing result in the
real-time. This capability is especially helpful for biological research of living cell samples. Morphological change and energy transfer occur all the time in living cells; trapping dynamics is therefore often non-stationary in these applications due to environmental variation. In this case, adaptive probing can be employed to improve the estimation performance, and additional information of the process under investigation can be gained from inferring the cause of the trapping variation. The effectiveness of adaptive estimation is experimentally verified in this section by varying the boundary condition of the trapping during force probing and comparing the estimation result to the corresponding theoretical analysis.

When a probe approaches a hard surface, its damping coefficient \( \gamma \) is dictated by the probe’s interaction with the boundary layer of water. According to Eq. (3.10), the open-loop trapping bandwidth \( f_c \) will hence vary significantly with the change of the boundary position. \( \gamma \)'s value during the boundary change can be determined by Faxen’s law [5]:

\[
\gamma = \frac{6 \pi \eta a}{1 - \frac{9a}{16h} + \frac{a}{8h} - \frac{45a^4}{256h^4} - \frac{a^5}{16h^5}},
\]

(4.21)

where \( h \) is the distance from the center of the probe to the surface. During an adaptive probing experiment, \( h \) can be controlled by the piezo sample stage. Hence, the theoretical variation of \( \gamma \) is obtained from Eq. (4.21). The estimated trapping bandwidth \( f_c \) can also be employed to infer the change of \( \gamma \), the result of which can be compared to the theoretical prediction for the validation purpose.
Fig. 4.5: Adaptive force sensing of a time-varying process. (a) Real-time estimated open-loop trapping bandwidth along $x$- and $y$-axis; (b) force estimation results of the two axes, and (inset) shows the $z$-axis stage motion, which alters the boundary condition of the trapping. The probe is subject to Brownian motion control and force regulation during the estimation.
Fig. 4.5 shows the adaptive estimation result of such a time-varying process. A 1.87μm probe was trapped with ~32mW laser power at focus when Brownian motion control as well as force regulation were activated simultaneously for manipulation enhancement. The boundary of the surrounding medium, as formed by a cover slip fixed on the piezo sample stage, was moved 5μm axially by the stage toward the trapped probe in 3 seconds, stayed about 200nm away from the probe’s lowest point for 1.5 seconds, and then retreated back to its initial position. The trap’s open-loop bandwidth \( f_c \) is expected to decline and then recover in this experiment. It was computed from the estimated state transition coefficient \( \hat{\phi} \) using the relationship

\[
\hat{f}_c = -\frac{\ln \hat{\phi}}{(2\pi\tau_s)},
\]

derived from Eq. (3.9), and drawn in Fig. 4.5(a). As illustrated by the figure, \( f_c \)’s of both x- and y-axis change up to almost 50%, and synchronize with the stage motion shown in the inset of Fig. 4.5(b). Since no external force was intentionally applied in lateral plane, the force sensing result on both axes in Fig. 4.5(b) shows only sub-pN uncontrolled disturbance and estimation noise.

The adaptive estimation result of this non-stationary process is further validated in two ways. First, the controls were disabled and the recorded probe position was transformed to its double-sided PSD using FFT algorithm for calibration. The open-loop trapping bandwidth \( f_c \) at the initial position of the experiment can thus be obtained using PSD fitting method [41]. The PSD of x-axis is illustrated in Fig. 4.6, where the fitted \( f_c \) is 435.7Hz. The x-axis adaptive estimation result at the beginning and end of the controlled probing experiment, shown in Fig. 4.5(a), gives the same value. It proves the
convergence of the adaptive estimator, and demonstrates that this convergence is independent of the manipulation and control of the probing system.

Fig. 4.6: Power spectrum analysis of $x$-axis position of an open-loop trapped 1.87$\mu$m probe at the initial position of the probing experiment.
Fig. 4.7: Comparison of estimated damping coefficient $\gamma$ and its theoretical prediction during an adaptive probing of a non-stationary process. The theoretical prediction is delayed 140ms so that the two estimates of $x$- and $y$-axis can match it exactly along the time axis.

Then the change in the estimated $\hat{c}_f$ was employed to infer the variation of the probe’s damping coefficient $\gamma$ for comparison with its theoretical prediction. Since the laser power is unchanged during the experiment, the trapping stiffness is constant and was calibrated beforehand. The estimated $\hat{c}_f$ can hence be converted to $\gamma$ using Eq. (3.10), as drawn in Fig. 4.7. The obtained $\gamma$ is compared to its theoretical prediction from Eq. (4.21), in which $h$ is related to the stage’s $z$-axis motion. As shown in the figure, the estimated $\gamma$’s from both $x$- and $y$-axis data are very close to the theoretical value (black dashed line), even though $\hat{c}_f$’s of the two axes in Fig. 4.5(a) are different. This difference
in trapping bandwidth is due to the slight asymmetry of the trapping laser focus. The two estimates also match the theoretical $\gamma$ along the time axis exactly, after it is delayed 140ms in Fig. 4.7. The convergence time of the parameter estimation accounts for this delay, which decides the tracking performance of the adaptive estimator on trapping variation. In addition, the two estimates show larger fluctuation when the probe is closer to the medium boundary. This fluctuation of the estimation results from the probe-surface distance variation caused by the probe’s Brownian motion, whose amplitude after control is $\sim \pm 40$nm peak-to-peak along $z$-axis, as shown in Fig. 4.3. According to Eq. (4.21), small change in $h$ will alter $\gamma$ significantly when the probe is in the vicinity of the surface boundary.

### 4.4 Conclusions and Remarks

Optically trapped probing system, as a versatile 3D nanomanipulator and force transducer with sub-pN resolution, has been intensely studied and applied for biological research on a variety of topics, ranging from single molecules to cellular mechanics. However, thermal noise imposes limitation on the resolution and accuracy of its measurement as well as of the closed-loop manipulation that employs the measurement. In addition, the system’s dynamic probing applications are limited by the widely used quasi-static linear spring model for force measurement. A novel real-time signal processing algorithm is implemented in this chapter to deal with the problems, and the performance of the technique is experimentally demonstrated.

The discrete system model is augmented with the dynamics of the external interaction and trapping system variation for the adaptive observer design. The adaptive
estimation solves two major issues to improve the dynamic force measurement. First, after model augmentation, the external force is recursively estimated in the designed Luenberger observer as a virtual state variable of the system from the noisy motion of the probe. Due to the principle of control-estimation separation, its performance of dynamic force sensing is unaffected by the manipulation and control of the system. Second, parameter estimation in the developed adaptive observer detects the variation of the trapping dynamics and corrects the force sensing result automatically. From inferring the cause of the variation, additional information of the process under investigation can be gained as well. Furthermore, as evolved from recursive least squares solution for estimation, Kalman filter algorithm was employed to minimize the estimation error. Consequently, it results in the best linear unbiased and maximum likelihood estimation if the process and measurement noises satisfy the white Gaussian condition.

Experiments of adaptive dynamic force probing on known interaction forces as well as adaptive estimation of a non-stationary process are conducted to demonstrate the estimator’s performance. The resolution and accuracy of the estimation are high, as illustrated by the comparison between the force probing results and the generated external drag force. Control-estimation separation is validated, wherein the estimator works consistently when the various controls for manipulation enhancement and force regulation are enabled. The convergence of the adaptive estimation was verified as well, by the close agreement between the estimated trapping dynamics variation of a non-stationary process and its theoretical prediction.

With the successful implementation of adaptive force sensing in the system, force feedback controller for automatic 3D scanning can be designed. It will transform the
optically trapped probing system into a new scanning probe microscope (SPM), and may greatly enhance its applicability for biological research on living cells. The adaptive estimation is also readily developed into adaptive control, which has been shown in Chapter 3.3.4 to maintain optimal control performance in the case that the trapping is time-varying, e.g., when the system is operated in a complex environment.
CHAPTER 5: BIOLOGICAL APPLICATIONS

5.1 Introduction

Optical trapping systems have been employed in many biological research areas, from single-molecule study of motor proteins [3,6] to cell mechanics and mechanotransduction [8,9]. In cytological research, the knowledge of micromechanical properties of living cells is essential for understanding their functions. Mechanical forces are found to not only change the cell’s morphology, but also induce a variety of cellular and biomolecular responses, the mechanisms of which are still unclear in many cases [8]. For example, tumor metastasis is a consequence of invasive and motile tumor cells that spread, adapt, and proliferate in selected foreign environments. During the process, the tumor cells are continuously exposed to various physical forces, and can dynamically react and adapt to the forces with active change of their morphology as well as mechanical properties. Accordingly, both intracellular and extracellular responses of a tumor cell to mechanical loading may signify its metastatic potential. The possible applications of the developed optically trapped probing system to investigate mechanical responses of living cells for future research on tumor metastasis are demonstrated by the preliminary experiments in this chapter.
Intracellular trapping of an organelle was accomplished in a living cell, illustrating the potential of probing intracellular mechanical properties [9] and in vivo molecular responses [68]. Extracellular experiments were conducted in several ways, for measuring the mechanical reactions as well as mapping the topography of living cells, using non-functionalized polystyrene (PS) microbeads as the probes for manipulation and measurement. In the first single-point cell pushing experiment, the probe was held by optical trapping, and the cell sample was brought into contact with the probe by the piezo stage. The interaction force was measured from the probe displacement, while the cell deformation was obtained from the difference between the probe motion and that of the stage. In the second set of experiments, the probe was actuated axially by the optical trapping system to tap on living cells. The cell response was directly measured from the probe’s position, and the force involved was estimated from the collective information of the manipulation effort and probe motion. The experimental setup was further improved to eliminate sample interference by inverting the sample chamber and measuring the probe’s motion with its epi-fluorescence signal [69] using visual sensing method, which was developed by Dr. Zhipeng Zhang. Consequently, a topography map can be obtained simultaneously with the mechanical property measurement on a living cell. In addition to the acquired preliminary manipulation and measurement results that may lead to insights of cellular functions, the capabilities of current optically trapped probing system are clearly demonstrated through these experiments, and possible improvement of the system for future applications can be identified.

The living cell samples were cultured and prepared by Dr. Yanhui Shi and Mr. Lu Zhang, wherein all the related experiments were co-supported by Professor Sissy Jhiang.
in Department of Physiology and Cell Biology of The Ohio State University. Ms. Jingfang Wan took part in the experiment in Chapter 5.3.1, and Dr. Zhipeng Zhang participated in the cell tapping experiments of Chapter 5.3.2.

5.2 Intracellular Trapping Experiment

Live Chinese hamster ovary (CHO) cells were treated with 0.1% Janus Green B for 10 minutes to stain mitochondria for better visibility, and the sample was observed under the bright field illumination. Most visible organelles were 300nm to 1μm in size and confined locally, with a few that can move around in several microns range. In order to demonstrate the trapping capability inside the live cell, optical trapping was applied onto a moving organelle. The organelle's Browninan motion was immediately constrained around an equilibrium point. The relative motion between the trapped organelle and the rest of the cell was introduced for manipulation by moving the cell with the microscope's piezo stage while clamping the trapped organelle around the laser focus.

Fig. 5.1: Establish a stable trapping inside a cell and alter the relative position between the trapped organelle and the rest of the cell.
with the optical trapping force (Fig. 5.1). The motion control has sub-nm resolution, but in order to illustrate visible motion through the optical microscope, 500 nm step size in both $x$- and $y$- directions was provided. After the experiment, the cell morphology showed no noticeable damage.

High-speed active control and dynamic force probing of the optical trapping system can be implemented for this type of intracellular trapping experiments. The objective is to achieve more sophisticated direct manipulation of active organelles inside live cells. The organelle can then be employed to probe the intracellular mechanical properties, such as the frequency-dependent viscoelasticity [9]. Alternatively, the active response of the organelle due to chemical or mechanical stimulus, e.g. its intracellular transport [68], can be measured.

5.3 Extracellular Manipulation and Measurement Experiments

5.3.1 Single-point cell pushing

In the single-point cell pushing experiment, a 1.87 μm non-functionalized probe (Fluoresbrite® YG microspheres, Polysciences Inc., Warrington, PA) was held still by the trapping system with 80mW laser power at focus. The axial trapping stiffness was calibrated to be 18.5pN/μm. The probe was brought into contact with living human embryonic kidney 293 (HEK293) cells at different locations by moving the cells with the piezo sample stage. In each axial approach/withdrawal, the probe position was measured by the 830nm measurement laser. Since the trapping equilibrium is stationary and the sample manipulation is slow in pushing experiments, the probe-cell interaction force is estimated by the trapping force calculated with a linear-spring force model from the
measured absolute z-position of the probe $z_m$, $F_e = k_{OT} z_m$. When the probe and the cell are in contact, the cell deformation can be derived from the difference between the probe motion and that of the stage, $z_m - z_c$, which is defined as the probe-sample distance. This distance is zero when the two get into contact without cell deformation, whereas its negative value is the opposite of the cell deformation. Hence, the interaction force versus probe-sample distance is drawn, and the cell’s normal stiffness can be fitted from the second quadrant portion of this force-distance curve. A typical measurement of the force-distance curve is shown in Fig. 5.2. The curve was measured at an overlapping area between three cells, wherein the normal stiffness of the sample was estimated to be 45.3pN/μm. This value, which is much smaller from the stiffness measured (in Chapter 5.3.2) on a hard surface, is in the same order as the trapping stiffness, so the result is one of high fidelity. In addition, the repeatability of the probe’s approach/withdrawal is quite good, as shown in Fig. 5.2. Another force-distance curve was measured at the top of a living HEK293 cell (Fig. 5.3). The measurement was again very repeatable, but the fitted sample stiffness from the curve was 8.19pN/μm, even smaller than previous case. The maximum interaction force in these measurements is merely around 10pN. Therefore, the measurement perturbation to the living cells is minimized for the faithful acquisition of the cells’ undisturbed response under physiological conditions.
Fig. 5.2: Single-point cell pushing experiment. (a) Microscope image of the overlapped living HEK293 cells under investigation; and (b) force-distance curve measured from the probe motion as well as that of the stage for estimating the sample’s normal stiffness.
5.3.2 Cell tapping

Eliminating sample interference

A living cell as an active system composed of composite materials can react and adapt to mechanical stimuli, and its mechanical property is frequency-dependent as well. To introduce dynamic excitation and measure the dynamic response of living cells, the probe can be actuated axially by the trapping system to tap on the cell. The cell’s response is directly measured by the probe’s motion when the two are in contact, and as discussed in Chapter 4, the force involved can be estimated from the collective information of the manipulation effort and probe motion. Both the cell response and force
estimation would now be based on the same single measurement of the probe position, which can be calibrated each time before the experiment with the laser steering system. The measurement accuracy on the cell’s mechanical property can thus be improved. However, before dynamic measurement is performed, the sample’s interference to the actuation and measurement optical path needs to be solved to enhance the accuracy of the manipulation and measurement.

The sample interference is due to the fact that the cell samples are usually mounted on the cover slip below the foci of lasers, so both trapping and measurement lasers pass the sample before steering the probe or measuring its motion. Since the optical property of a cell is different from the surrounding medium, and is highly inhomogeneous and time-varying, this interference disturbs the trapping dynamics and produces spurious signals in the measurement. The cell sample, therefore, must be separate from the optical path of the probing system to completely solve the problem. The cells can be inverted and mounted on a top cover slip instead of the bottom one. A spacer can then be put in between the two cover slips to form a sealed chamber that holds the culture medium. The medium provides the physiological environment for the living cells to function as desired. As long as the interior height of the chamber is less than the working distance of the microscope’s objective lens, the trapped probe can reach the cells to perform designed manipulation tasks without sample interference to the trapping. To remove the measurement interference, current measurement optical path should be re-designed, so that backscattered laser light can be employed for 3D measurement [16]. Consequently, the sample is able to be utterly isolated from the system’s optical path, and high quality extracellular manipulation and measurement with living cell samples can be
accomplished. The inverted sample chamber and the separation of the sample from the system’s optical path are illustrated in Fig. 5.4.

Fig. 5.4: (a) Inverted cell chambers, wherein the sealed medium can be clearly seen. The chamber’s height is 120μm, less than the 220μm working distance of the objective lens. (b) Separation of the sample from the system’s optical path.

Quasi-static tapping

The backscattered laser measurement is to be implemented. In this section, a visual sensing measurement based on the epi-fluorescence signal of the probe is employed to demonstrate the above separation concept for improvement. Utilizing an optical path similar to that of the backscattered laser measurement, the probe’s fluorescent light is excited by the illumination from the objective lens and the emitted light goes back without passing through the cell, when inverted sample chamber is used. By cutting off bright field illumination of the sample during measurement, the fluorescent position sensing is rendered free from the sample interference. Hence it is employed to measure the cell response subject to normal probe tapping as described below.
A 1.87μm non-functionalized probe was trapped with 80mW laser power at focus, and actuated to move axially in a triangular waveform of 2μm p-p amplitude and 5-second period. Under fluorescence illumination, the probe’s off-focus image was recorded by the microscope’s CCD camera. A method extending the 3D particle tracking technique employed for the steering calibration under bright field illumination (Chapter 2.4.2) was used to compute the probe’s 3D position from its off-focus image [69]. The algorithm is robust to the photobleaching of the probe’s fluorescent signal and to the variation in the intensity of the excitation light. Its measurement resolution is enhanced as well, by the method’s best linear unbiased estimation of the probe’s axial position. The sampling rate of the measurement, however, is limited by the camera’s data transfer rate to 25Hz. Therefore, only quasi-static tapping was measured with the method. Before tapping on a living cell, the probe was first engaged to a cover slip surface for test. Fig. 5.5 demonstrates the resulting probe motion and the derived interaction force versus probe displacement curve, both of which are acquired via single fluorescence visual measurement, as described at the beginning of Chapter 5.3.2. It is apparent that the hard surface stops the probe with the fast-growing interaction force when the two come into contact. The fitted sample stiffness, which is obtained from the part of the force-displacement curve with notable interaction force, is 662pN/μm and over 35 times larger than the trapping stiffness. It is high as expected, but is too large to be estimated accurately.
Fig. 5.5: (a) Probe’s motion before and after tapping on a cover slip surface; (b) the derived force-displacement curve from the probe’s motion during tapping.
Fig. 5.6: (a) Probe’s motion from the MCF-7 cell’s engagement to its withdrawal; (b) the derived force-displacement curve when the probe is tapping on the cell, and (inset) is the bright field image of the cell overlapped with the fluorescent image of the probe, indicating the location of the tapping. The red arrows in two figures indicate the corresponding data of the first cycle of tapping after cell engagement.
The probe was subsequently commanded to tap on a MCF-7 cell, which is a non-invasive breast cancer cell. The probe’s motion starting from the cell engagement to its withdrawal is shown in Fig. 5.6(a). The first reduction in tapping amplitude happened during the cell engagement, while the cell was kept stationary for the rest of the tapping until it was withdrawn. The cell’s deformation was clearly demonstrated by the probe’s motion when the two got into contact. However, due to non-specific binding force, the probe adhered to the cell after three and half cycles of tapping, and the trapping force was not large enough to dislocate it, as illustrated in the figure. The cell’s stiffness can be estimated from the probe’s tapping before the binding event, and the corresponding force-displacement curve of the tapping is drawn in Fig. 5.6(b). Except the first cycle of tapping after the cell engagement, the following two and half cycles are highly repeatable. The estimated sample stiffness from the later cycles is 79.7pN/μm, whereas the cell seems softer during the first cycle. This may imply that the cell adapted to the external force, even though the applied force was only a few pNs.

**Cell mapping**

The non-specific binding issue encountered above needs to be solved to enable the mapping of the cell’s mechanical property at different locations. The binding is mainly due to the hydrophobic property of the probe, and sometimes can be caused by charge-based attractions [70]. The probe preparation is therefore improved to alleviate the problem. 1.95μm carboxylate-modified fluorescent microspheres (FluoSpheres®, Invitrogen Corp., Chicago, IL) are selected as measurement probes. Their surfaces are modified with carboxylic acid group (-COOH) to make them relatively hydrophilic and
negatively charged in medium solution. The high density of surface charge also helps reduce the attraction to cells, which are often negatively charged. As a further prevention of non-specific binding, additives such as bovine serum albumin (BSA) or dextrans are introduced to block the hydrophobic or charged binding sites on the probe’s surface. Hence, the probe and the cultured cell are prepared with the following procedure before the experiment.

1. Dilute the original 2% (weight/volume) distilled water solution of 1.95μm microspheres 10000 times with 0.1M phosphate buffered saline (PBS, pH=7.4). (2) Add BSA to make 3% w/v final concentration of it. (3) Incubate the solution at 37ºC with 5% CO2 for 30 minutes. (4) Incubate the cells, already cultured 24 hours in complete culture medium with serum, in serum-free culture medium for 30 minutes. (5) Wash the cells with serum-free culture medium 3 times. (6) Apply the Secure Seal™ spacer to the cover slip that grows the cells and add 6μl diluted microsphere solution in the middle. (7) Seal the spacer with a 25mm×25mm No. 1 cover slip (0.15mm thick) to form the cell chamber. (8) Invert the chamber and mount it onto the microscope for experiment.

Subsequently, after the probe’s tapping period is reduced to 0.4 second and the probe-cell contact time is less than 0.2 second in each tapping cycle, irreversible non-specific binding events seldom occur. The tapping experiment can then be extended to map the cell’s topography and mechanical property at a series of locations. The xy positioning of the scan in this case is achieved by the system’s laser steering of the probe. The steering is synchronized with the z-tapping so that the probe moves laterally only when it is away from the sample. Any friction force that might be induced in the scan is thus eliminated.
Fig. 5.7: Tapping living MCF-7 cells at different locations for cell topography mapping. (a) Measured $z$-position of the probe; (b) Microscope image of the scanned sample area; (c) Measured cell topography, where black circles are raw data and the smooth surface is interpolated from the averaged raw data.
Fig. 5.7 shows the mapping experimental result of living MCF-7 cells at 5×5 locations separated by 600-nm intervals. The laser power applied to the 1.95μm probe was 80mW at focus, generating a z-trapping stiffness of 18.9pN/μm, and the triangular tapping amplitude was 1μm p-p. An overlapping area of several cells was measured, which roughly covers the lower left quadrant of the square in Fig. 5.7(b). At each location, the probe tapped 5 times on the cell. Hence, depending on the cell’s local height, the peak position of the probe reduced from the original extreme before the cell engagement and varied every 5 cycles, as shown in Fig. 5.7(a). The reduction of each tapping cycle is drawn against the corresponding lateral position of the probe in Fig. 5.7(c) (black circles) as a representation of the sample’s topography, and a smooth surface was interpolated using the 25 averaged local height measurement and the lateral probe positions. The cell height varies ~500nm in the 2.4×2.4μm² scanning area, wherein the measurement repeatability is quite good. The standard deviation of the measured height at each location is 4~20nm and that of lateral positioning is mostly 3~5nm, both of which are dictated by the probe’s Brownian motion.

The mechanical property map of the scan, however, can’t be obtained completely. Due to the cell’s topography and lack of force feedback control for adaptation of the large topography change in the scanning, the tapping depth changes significantly. In some locations, the depth is too small to obtain good stiffness measurement; while if it was too large, the probe would be pushed out of the trap, causing scanning failure. Nevertheless, the cell stiffness can still be estimated simultaneously on a number of occasions during the mapping experiment. One instance of the estimated force-displacement curves is drawn in Fig. 5.8 from the data measured at the 24th location of the experiment shown in
Fig. 5.7. The fitted sample stiffness, 79.4pN/μm, is comparable to previous tapping measurement on MCF-7 cells, whereas the measured data points are sparse due to the increased tapping frequency.

![Force-displacement curve](image)

**Sample stiffness is 7.94e-005N/m**

Fig. 5.8: Force-displacement curve acquired at the 24th location of the mapping experiment shown in Fig. 5.7.

### 5.4 Conclusions and Remarks

This chapter presented some preliminary experimental results to show the potential of the developed optically trapped probing system for biological research. Its applications to investigate cell mechanics and mechanotransduction in tumor metastasis are of special interest. Therefore, both intracellular and extracellular experiments were
performed to illustrate the system’s current capability, as well as to demonstrate needed capacity for future research.

Intracellular trapping of an organelle was accomplished in a living CHO cell to demonstrate the potential of probing intracellular mechanical properties and in vivo molecular responses. Extracellular experiments of single-point cell pushing and cell tapping were conducted to measure the mechanical property and/or the topography of living cells, using non-functionalized PS microbeads as the probes for manipulation and measurement. In the single-point cell pushing experiment, the interaction force was sensed from the probe displacement, and the cell deformation was measured by the probe-sample distance, which is related to the difference between the probe motion and that of the stage. The resultant force-distance curve was employed to estimate living HEK293 cells normal stiffness. This stiffness was shown to be comparable to the trapping stiffness of the system. The sample interference to the system’s actuation and measurement optical path was eliminated by inverting the cell chamber and measuring the probe’s motion with its epi-fluorescence signal. After the non-specific binding of the probe to the cell was prevented, a topography map was obtained for a living MCF-7 cancer cell from tapping at a series of locations of the cell. The cell’s mechanical property was simultaneously measured at some places of the map.

The experiments demonstrate that the system can measure a living cell’s response with minimized perturbation under physiological conditions, wherein the measured living cells’ normal stiffness is in the comparable range to that of the trapping system. Sample interference can be solved for sophisticated biological experiments, but its dynamic manipulation and measurement capability is currently limited by the employed
fluorescent position detection technique. In the future, backscattered 3D laser measurement [16] can be integrated with the system for dynamic measurement of the cell response with high speed and high accuracy. In addition, to achieve robust topography scanning with simultaneous mechanical property measurement of living cells, force feedback control is necessary for the adaptation of topography change in the scanning, the feasibility of which has been illustrated by AFM-based technologies, when operate on a similar principle [38].
CHAPTER 6: CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

The main focus of this dissertation is the design and development of an optically trapped probing system with active control and adaptive estimation. The motivation for this research is to enhance manipulation and measurement capabilities of the-state-of-the-art optical trapping systems for biological applications. The Brownian motion of the system’s trapped probe, induced by random thermal force in the environment, is identified as the major limiting factor of the system’s performance. Therefore, the design, control, and signal processing of the optical trapping system are investigated to address the problem and to expand the system’s functionality for biological researches.

Four specific aims were defined and accomplished in this dissertation to implement and demonstrate the improved optically trapped probing system. (1) Develop an optical trapping system with 3D rapid laser steering and high-speed position detection. (2) Implement minimum variance control to suppress the probe’s Brownian motion. (3) Design adaptive estimation algorithm for adaptive dynamic force probing with the system. (4) Demonstrate the system’s manipulation and measurement capability for potential biological applications.
In the developed optical trapping system, 3D laser measurement is enhanced and an FPGA-based digital controller with 208.3kHz loop rate is implemented to fully exploit the high-speed measurement and actuation of the system. The 3D actuators consist of a deformable mirror enabling axial actuation and a two-axis acousto-optic deflector for lateral steering. Through optical path design, an actuation range of 21.3μm along x-, y-, and 10.5μm along z-axis is accomplished. The optical path design, if perfectly aligned, eliminates actuation crosstalk among the three axes as well as the coupling between actuation and relative position detection of the trapping laser. The 3D actuation system is calibrated, and the design in terms of motion range and actuation crosstalk is verified. The lateral resolution of the steering system is determined to be 16pm (1σ) and its bandwidth is over 50 kHz; the axial resolution is 0.16nm with enhanced bandwidth of over 3 kHz. The performance of the constructed optical trapping system is illustrated by three sets of experiments. First, 3D active control of the trapped probe’s Brownian motion is utilized to enhance trapping stability. Second, large range 3D steering is demonstrated by maneuvering a 1.87μm probe along a complex 3D trajectory in a 6×6×4μm³ volume. Third, closed-loop steering is implemented to achieve improved precision for manipulation.

To enhance the manipulation resolution of the developed system, Brownian motion control of an optically trapped probe is theoretically and experimentally investigated. A 1st-order ARMAX model describing the Brownian motion of an optically trapped probe subject to active control is derived for digital controller design and discrete data analysis. It establishes the relationship between the probe’s motion and the control effort and characterizes the effect of the feedback loop’s time delay. The derived model is
experimentally validated by the proportional control results of a 1.87μm trapped probe. It is then employed to design the optimal controller that minimizes the variance of the probe’s Brownian motion. In order to evaluate the control performance, analysis based on minimum variance control theory is performed. The theoretical analysis is then compared with the experimental result and the simulation to illustrate the performance envelope of active control. The minimum standard deviation of a 1.87μm probe’s Brownian motion, achieved with the current setup, is shown to be 3.8–3.9nm. Moreover, adaptive minimum variance control is implemented and experimentally verified. It is capable of maintaining the optimal control performance in the case that the system is operated in a time-varying environment. The control analysis developed in this dissertation can also be applied to predict and optimize the Brownian motion control performance of generic trapping systems.

The resolution and accuracy of the trapping system’s dynamic force probing is limited by the probe’s Brownian motion as well, and the widely used linear spring model is valid only for quasi-static force measurement. A novel real-time signal processing algorithm is implemented to deal with the problems, and the performance of the technique is experimentally demonstrated. The discrete system model is augmented with the dynamics of the external interaction and that of the trapping system variation. An adaptive observer is then designed to recursively estimate the force and the system parameter from the noisy motion of the probe. Due to the principle of control-estimation separation, its performance of dynamic force sensing is unaffected by the manipulation and control of the system. The force probing is also corrected automatically according to the parameter estimation of the trapping dynamics. From inferring the cause of the
variation, additional information of the process under investigation can be gained. Kalman filter algorithm is employed to minimize the estimation error of the designed adaptive observer. If the process and measurement noises satisfy the white Gaussian condition, it results in the best linear unbiased and maximum likelihood estimation of the dynamic force. As demonstrated by experiment, the resolution and accuracy of the estimation are high and the convergence of the adaptive estimation is verified.

To demonstrate the potential applications of the developed optically trapped probing system for biological research, experiments with living cell samples were performed. Intracellular trapping of an organelle was accomplished in a living CHO cell, and extracellular experiments of single-point cell pushing and cell tapping were conducted to measure the mechanical property and/or the topography of living cells. The sample interference to the system’s actuation and measurement was eliminated via the modification of the sample holder and the employment of a different measurement optical path. After the non-specific binding of the probe to the cell is prevented, a topography map is obtained on a living MCF-7 cancer cell from multi-location cell tapping. The cell’s normal stiffness is also shown to be in a range comparable to that of the trapping system.

### 6.2 Recommended Future Work

The following is a list of recommended research topics that can be addressed in the future.
(1) Development of optically trapped scanning probe system

As implied by the biological experiments and discussed in Chapter 5, backscattered 3D laser measurement can be integrated with the system in the future as an alternative dynamic measurement method with high speed and high accuracy. Its optical and mechanical conciseness makes it amenable to a variety of applications [71]. Specifically, in extracellular experiments on living cell samples, backscattered detection can help eliminate sample interference to the system’s measurement while simultaneously enable high-speed feedback control. Depending on control objectives, the manipulation resolution can be enhanced by active Brownian motion suppression and the dynamic interaction force estimated by the system can be regulated to enable automatic scanning. During scanning, force feedback control is necessary in order to adapt to the topography change of the sample, as illustrated by many other SPM techniques as well. Robust topography scanning with simultaneous mechanical property mapping of living cells can then be achieved with a dual control loop. The inner loop is the motion control to enhance the manipulation resolution and tapping bandwidth. The outer loop is a 3D force-feedback scanning control. It has two objectives: (a) according to the estimated tapping force, adjust the reference motion of the probe in the normal direction of the sample’s local tangent plane, so as to accommodate the probe to the sample topography; (b) generate scanning trajectory along the surface tangent that is synchronized with the tapping, which eliminates any friction force on the sample. The scanning’s spatial resolution can be further enhanced by extracting statistical information of the probe’s Brownian motion with, e.g., thermal noise imaging method [18,72]. Hence, a new optically trapped scanning probe system can be developed for biological research.
(2) Extracellular/intracellular scanning

Employing the optically trapped scanning probe system, 3D mapping of cellular mechanical property can be acquired. The high-speed manipulation and measurement of the system will allow the dynamic mechanical property of the sample to be probed. Consequently, localized changes of the cell’s mechanical phenotype can be recognized and quantified by mapping the membrane viscoelastic property over the cell surface. Cellular adaptation to mechanical loading in the form of shape variation and stiffness adjustment can also be monitored by measuring cell stiffness and topography in real time over various locations.

In addition, 3D visualization of structural geometry and its change in the interior of living cells can be performed. The intracellular probes can be either naturally existent organelles [9,68], or intentionally introduced micr-/nano-beads through various means, such as micro-injection [73] and cell phagocytosis [74]. 3D dynamic force sensing along with force feedback control will help maneuver the probe inside the cell, and 3D dynamic interaction force induced by its surrounding medium or structures will be sensed. Being able to probe the interior of living cells, to examine the viscoelastic property of specified cytoskeleton bundles, and to visualize the intracellular structural geometry as well as its change will be fundamental to better understanding of cell functions.
BIBLIOGRAPHY


