HIGH SPEED ATOMIC FORCE MICROSCOPY

DISSERTATION

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By

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ABSTRACT

Since its introduction more than twenty years ago, Atomic Force Microscopy (AFM) has extended its application areas from material science to biology or biophysics, based on its capability to image/manipulate objects in various environments with sub-nanometer spatial resolution in three dimensions. AFM has become a very useful microscopic instrument and/or manipulator of biological objects, such as proteins on membranes, in their physiological conditions.

Among the two most commonly used modes, the dynamic (tapping) mode has a great advantage over contact mode when imaging soft materials, in which the AFM cantilever is actively vibrated around its resonance frequency while the tip-sample separation is regulated such that the tip lightly taps the surface only around the lowest point of its oscillation and thus minimizes potentially destructive shear and adhesive forces on the sample. The amplitude modulation is the most commonly used control method of the tip-sample separation in the dynamic mode of AFM, in which the oscillation amplitude of the cantilever is regulated at a set-point by feeding back changes in oscillation amplitude to adjust the cantilever z-position. However, in typical implementations, due to tapping dynamics of the AFM cantilevers, the transient response of the cantilever induced by changes of the tip-sample interaction force leads to greater variations in tip-sample interaction via feedback, causing excessive tapping forces and/or
possible loss of tapping during the scanning, and thus greater sample distortions and imaging errors. In order to suppress the effect of noise in the system when measuring the oscillation amplitude, the bandwidth of the oscillation amplitude estimation was limited and so was the imaging rate. In addition, the low bandwidth of the actuators in conventional AFMs, such as the z-positioner and the raster scanner limits the scanning speed. Therefore, while dynamic mode AFM has many potential applications, the inability to achieve direct and precise control of the tip-sample interaction forces and the low bandwidth of actuators for the tip-sample separation control and the raster scanning have been key barriers which imaging rate and inhibit innovation leading to new applications.

In this research, the design, actuation and control of a new generation AFM probing system which enables high-speed and high-resolution imaging of samples are investigated. In order to achieve direct tip-sample interaction control during the scanning, a novel dynamic sensing and control method are implemented, in which the tip-sample interaction force of each tapping cycle is estimated and subsequently controlled for dynamic force microscopy. By employing collocated magnetic actuation of the AFM cantilever and dual-actuator tip-motion control scheme, the high bandwidth tip-motion control, whose bandwidth is comparable to that of the cantilever, the dynamics overdamped, and the motion range comparable to that of conventional z-scanner is achieved. For the high bandwidth raster scanning as well as high bandwidth tip-sample separation control, active multi-axis probing system is implemented, in which multi-axis magnetic actuators along with a multi-axis probe with one magnetic moment, specially designed and fabricated for the multi-axis actuation, achieves high bandwidth multi-axis tip-
motion control along the Z axis and the X axis. In order to achieve the high resolution imaging, a low noise laser measurement system is implemented and integrated to a commercial AFM (MFP3D, Asylum research). For the implementation of the direct tip-sample interaction control and high bandwidth active multi-axis probing system, high speed programmable digital controller is developed using field programmable gate array (FPGA) whose closed loop update rate is two orders of magnitude higher than commercially available ones. The results of scanning a standard grating whose pitch is 100nm and a biological grating (repeating protein structure on purple membranes) whose lateral pitch is about 6.2 nm using the high speed AFM are presented and discussed.
Dedicated to my wife Grace
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CHAPTER 1

INTRODUCTION

1.1 Background and Motivation

Since its introduction more than twenty years ago [1], Atomic Force Microscopy (AFM) have been used in a wide range of technologies benefiting the aerospace [2], biological [3,4], chemical [5,6], electronics [7], energy [8], manufacturing [9-12], and telecommunications industries [13]. It can not only image the surface variation in sub-nanometer resolution [14], but also measure the force at pico-newton scale [15]. Due to its underlying physical principle, which enables measurement of insulating materials [16] and in aqueous solutions [17], the materials being investigated include ceramics [18], polymers [19], biological membranes [20], thin [21] and thick film coatings, metals [22], and semiconductors [23]. AFM has three distinct classes of applications: 1) imaging, 2) force spectroscopy, and 3) manipulation. It has been applied to image surface topography and to study abrasion, adhesion, corrosion, friction, and lubrication [24-27].

Over the past twenty years, while several AFM operation modes have been invented [28-30] and many distinct imaging objectives [31, 32] have appeared for various purposes, the principle of AFM operation has remained very simple. The central feature of an AFM system is its local probe that has an atomically stable tip and a high-precision
manipulation device and that measures the near-field physical interactions between the probe tip and the sample lying beneath it as it moves.

The two most commonly used techniques are contact mode and dynamic (tapping) mode microscopy. In contact mode, a sharp tip is scanned over the sample surface with a feedback mechanism that regulates the tip either according to a constant force to obtain surface contour, or to a specified height above the sample to measure tip-sample interaction force [1]. Tips are typically made of $Si_3N_4$ or Si, and extended down from the end of a reflective cantilever, serving as a spring-like manipulator. A diode laser is focused onto the back surface of the cantilever. As the tip scans the sample, the laser beam is reflected off the cantilever into a photo detector, which converts the cantilever deflection to voltage that enables feedback control of the manipulator [33,34]. The resolution that can be achieved with a local probe is primarily a function of the effective tip size (feature size of the interacting point of the sharp tip), its distance from the sample, and the distance dependence of the tip-sample interaction.

In tapping mode, the cantilever is actively vibrated around its resonance frequency using either a piezo-electric actuator [28] or an electromagnetic coil [35]. The tip-sample separation is regulated such that the tip lightly taps the surface only around the lowest point of its oscillation, and thus minimizes potentially destructive shear and adhesive forces on the sample, a great advantage over contact mode when imaging soft materials. The most commonly used method is a form of amplitude modulation, in which the oscillation amplitude of the cantilever is modulated by variations of the tip-to-sample distance. By feeding back changes in oscillation amplitude to adjust the cantilever z-position, the amplitude is regulated at a set-point while scanning a surface. The resulting
changes in z-position of a piezo-based positioner yields the topography of the sample surface.

Dynamic force microscopy involves various dynamic processes. Some processes are connected with the instrument while others are related to the bandwidth of the cantilever and the tapping dynamics itself [36]. Faster changes in topography, which result from scanning the sample faster, necessitates faster response of the control system. However, in typical implementations, due to tapping dynamics and limited bandwidth of the z-positioner, the transient response of the cantilever induced by changes of the tip-sample interaction force leads to greater variations in tip-sample interaction via feedback, causing excessive tapping forces and/or possible loss of tapping during scanning, and thus greater sample distortions and imaging errors [37]. In order to suppress the effect of noise in the system when measuring the oscillation amplitude, the bandwidth of the oscillation amplitude estimation was limited and so was the imaging rate. In addition, the low bandwidth of the actuators in conventional AFMs, such as the z-positioner and the raster scanner limits the scanning speed. Therefore, while dynamic mode AFM has many potential applications, the inability to achieve direct and precise control of the tip-sample interaction forces and the low bandwidth of actuators for the tip-sample separation control and the raster scanning have been key barriers which imaging rate and inhibit innovation leading to new applications.

In order to reduce the effect of the transient dynamics of the cantilever, induced by the tip-sample interaction force during the scanning, on the scanning speed, small AFM cantilevers whose resonance frequencies are two orders of magnitude higher than usual cantilever are employed to reduce the settling time [38, 39]. But the small size of
such cantilevers, which are less than 20 μm in length, can lead to difficulties while engaging sample surface and when scanning samples having large topographic variations. For the improvement of the actuation bandwidth of the z-positioner and the raster scanner, twin piezo-actuators facing each other [40] and high stiffness structures integrated with piezo-actuators [41,42] have been successfully developed but these require fairly high-cost manufacturing process and/or complete replacement of the conventional scanners with the new one.

Therefore, the motivation of the research is to develop a next-generation AFM for high-speed/high resolution microscopic system in which the tip-sample interaction force is directly controlled and the active multi-axis probing system achieves high bandwidth tip-motion control for the tip-sample distance control and raster scanning. In order to implement the control algorithms for the direct tip-sample interaction force control and the high bandwidth multi-axis actuation, a high speed programmable digital control & display system is developed using field programmable gate array. In order to keep the high spatial resolution while achieving high temporal resolution, a low noise laser measurement system is implemented and integrated with the system.
1.2 Research Objective and Scope

The objective of this research project is to investigate the design, actuation and control of a new generation AFM probing system and to create an innovative high-speed probing system that enables high-resolution and high-speed imaging of samples. Four technological innovations along with their specific aims are identified.

**Aim #1- Dynamic force sensing and control of tip-sample interaction:** Conventional tapping mode imaging has several advantages over contact mode imaging, particularly for samples that are very soft and have large variations in topography. However, changes of the tip-sample interaction forces induced by the topography of the sample lead to transient cantilever response, significantly limiting the bandwidth of tapping dynamics and thus the maximum imaging rate. In this research, novel dynamic sensing and control methods are proposed, in which the tip-sample interaction force of each tapping cycle will be directly estimated and subsequently controlled, for dynamic force microscopy. In the proposed approach, based on the dynamic model of the cantilever, an estimator is designed and implemented to estimate the tip-sample interaction force of each tapping cycle according to its transient response. The estimated interaction force is then utilized by a model-based predictor to plan and control the next tapping through controlling the tip-to-sample distance. In order to attenuate the effects of modeling errors of the predictor, a feedback regulator is employed to directly regulate the tip-sample interaction force. Since the tip-sample interaction force of each tapping cycle is directly controlled, it does not rely on the steady-state relationship between the oscillation amplitude and the tip-to-sample distance. Therefore, tapping dynamics is irrelevant and time delay as a
result of averaging in oscillation amplitude measurement is eliminated. Consequently, precise control of the tip-sample interaction force and high imaging rate can be achieved, independent of the quality factor (Q) of the cantilever.

Aim #2- High-speed tip-position control using co-located magnetic actuation: The control objective of z-positioning and that of xy-scanning are very different. In z-positioning, the required z-motion is commanded by laser reading of the cantilever deflection (contact mode) or the oscillation amplitude (tapping mode) in real time and might contain high frequency components depending on lateral scanning rate and topographical variations. As a sample is scanned at higher speed, topographic details present themselves to the z-control loop as disturbances at higher frequencies. The bandwidth of a bulky z-scanner is about one to two orders of magnitude smaller than those of typical cantilevers. In addition, since the scanner is lightly damped structure, only a fraction of its bandwidth can be used for tracking topographic variations. Therefore, in order to track abrupt topographical variations, a dual-actuator tip-motion control scheme is proposed, in which a magnetic mode actuator is employed to achieve high bandwidth tip-motion control while the regular z-scanner provides the necessary motion range. The magnetic mode actuator aims to directly control the tip position to track the surface topography by applying magnetic forces on the cantilever using a tiny permanent-magnet particle attached to the back surface of the cantilever tip. This added actuator serves to make the entire cantilever bandwidth available for high speed imaging. In xy-scanning, the tip position is controlled to scan the sample surface on the 2D sample plane, typically by tracking two orthogonal periodic trajectories whose frequencies and magnitudes are determined by the scanning area and imaging speed. For high speed
imaging, high-bandwidth actuation scheme for the raster scanning is also required. Since the degree of the freedom for the co-located magnetic actuation can be increased by adding solenoid coils around the magnetic moment with necessary alignments, two orthogonal solenoid coils are employed so that high bandwidth tip-motion control along the two axes can be achieved by the magnetic actuation, the first of which serves for high bandwidth tip z-position control and the second of which serves for high bandwidth raster scanning.

**Aim #3- Implementation of high-speed real-time digital controller:** In order to implement the estimator which estimates the tip-sample interaction force of each tapping (Aim #1), and plan and control the next tapping through controlling tip-to-sample distance by high-speed tip-position control using co-located magnetic actuation (Aim #2), a high-speed programmable real-time digital controller is required, in which the data conversations and calculations can be completed with one to two orders of magnitude higher speed than the bandwidth of the cantilever. For high speed imaging, cantilevers with bandwidths along the Z axis of ~ hundred kHz are required. Therefore, the updated loop rate of digital controllers needs to be very high, several tens of MHz, which is very difficult to accomplish with conventional central process unit (CPU)-based digital controllers. In the typical implementation of the CPU-based digital controller, the time-sequential calculation structure and the time-delay of the data communication between the CPU and the essential peripheral devices such as analog-to-digital converter and digital-to-analog converter limit the critical time loop. Field programmable gate array (FPGA) contains multiple copies of a basic programmable logic element (LE) which can be programmed (interconnected) to implement multiple operations, enabling true parallel
processing. In addition, the logic elements can access the data ports of the peripheral devices directly without any additional time delay. These superior advantages of FPGA allow implementing an extremely high-speed real-time digital controller. A FPGA-based high-speed real-time digital controller is implemented, in which the calculation of difference equations is completed with a closed-loop rate of 20 MHz. High performance analog-to-digital converter and digital-to-analog converters are integrated into this digital controller. In addition, a high speed bi-directional data interface with a host computer is to be established for the high speed real-time data display and for further implementation of advanced algorithms.

**Aim #4- High speed/High resolution imaging:** The high speed AFM, which are embodied with the achievements of the technical innovations identified in Aim#1-3 enables high speed/ high resolution imaging of objects. In this specific aim, the developed high speed AFM is evaluated by measuring an engineered grating composed of inverted pyramids whose edge length is 50 nm and pitch is 100 nm, and a biological grating, namely, repeating structures of proteins on purple membranes whose lateral pitch is about 6.2 nm and topographic variation is several Å.
1.3 Literature Review

There had been constant effort to achieve high speed scanning in Atomic force microscopy. These efforts can be categorized into two: (1) small AFM cantilevers for high bandwidth tapping dynamics and (2) high bandwidth actuation.

1.3.1 Method to improve bandwidth of the tapping dynamics

Among the dynamics processes which the AFM probe experiences during the dynamic mode operation, the tapping dynamics, which is caused by the transient response of the probe induced by the tip-sample interaction force, limits the bandwidth of the feedback signal, namely, the tip oscillation amplitude. The bandwidth of the tapping dynamics is directly proportional to the natural frequency of the cantilever [36], and inversely proportional to its quality factor $Q$. Thus, small AFM cantilevers whose natural frequency are one or two order of magnitude higher than commercially available cantilever have been fabricated to reduce the settling time and thus improve the bandwidth of the tapping dynamics [38,39,43]. These small AFM cantilevers whose length is ~ 10 um and whose width is few um, however, require a new laser measurement system which has very small focused laser spot (~ few um in diameter) on the cantilever. They also make it difficult to engage the probe on the samples that have large topographic variation. In addition, even if the small cantilevers whose resonance frequency is ~ MHz improve the bandwidth of the tapping dynamics of the cantilever,
this method does not allow the precise tip-sample interaction control per each tapping cycle, which is very important for soft samples.

On the other hand, Active Q control method is employed to reduce the effective quality factor $Q$ of the cantilever so that its transient response vanishes quicker [44]. While this method improved scanning speed, it sacrificed the force sensitivity of the cantilever.

### 1.3.2 High bandwidth actuation

In a typical conventional AFM, the bandwidths of piezo-based z-positioner and raster scanner are several kHz and several hundred Hz respectively. This low bandwidth of the actuators of AFM is one of the important barriers which limit the scanning speed [36,37,45]. There has been a constant effort to overcome this limitation and several successful improvements of the scanning speed have been reported. Ando et al. developed high bandwidth actuators by using twin piezo-actuators facing each other. This design suppresses the lightly damped and complicated dynamics of the piezo actuator around its resonance frequency and thus, extends the useful actuation bandwidth of the piezo actuator [40]. Hansma et al. developed a high bandwidth 3 axes scanner by integrating high stiffness structures with piezo-actuators [41]. Miles et al. implemented single-axis high speed raster scanning by using a flexure-stage integrated with push-pull piezos [42]. These high bandwidth scanners require, however, a fairly high-cost manufacturing process and/or complete replacement of the conventional scanner with the new one. Humphris et al. employed economic quartz tuning forks as the high speed raster scanner by oscillating it at its resonance frequency [46]. However, the scanning
frequency gets fixed by the resonance frequency of the tuning fork thereby introducing unnecessary constraints on the scanning operation. Jeong et al. proposed a dual-mode tip-position control scheme in which collocated magnetic actuation of AFM cantilevers was employed for high bandwidth actuation along the vertical direction, whereas a conventional piezo z-positioner provides necessary actuation range [45]. In this scheme, high bandwidth collocated magnetic actuation of the cantilever was achieved by enhancing the bandwidth of the solenoid coil and replacing the lightly damped cantilever dynamics with an over-damped dynamics using model cancellation algorithms.

Recently, a new concept to modify commercially available passive AFM probes into two-axis probes was reported, in which the geometry of the cantilever was modified to have greater compliance along the desired axes and magnetic actuation was employed to apply torques about the actuation axes [47]. Even though the actuation bandwidth employed was low, it demonstrated the possibility of simultaneous multi-axis actuation of a single probe.

1.4 Dissertation Overview

In this dissertation, the design, actuation and control of a new generation AFM probing system which enables high-resolution and high-speed imaging of samples is presented. This dissertation consists of seven chapters. Chapter 1 provides the background to this research and identifies the research objective and scope. Based on the objective and the scope, a survey of prior work in related areas is conducted. Chapter 2 is devoted to the design and implementation of the direct tip-sample interaction control, which eliminates the effect of tapping dynamics and enables the precise tip-sample
interaction control per each tapping cycle. Chapter 3 presents the implementation and integration of a low noise laser measurement system. This low noise laser measurement system allows retaining the high spatial resolution of AFM while improving its temporal resolution. Chapter 4 presents design, fabrication and control of an active multi-axis probing system which enables simultaneous high bandwidth actuation of the tip along the Z axis and the X axis. The former enables high bandwidth tip-sample distance control and the latter enables high bandwidth raster scanning of the tip over the sample surface. Chapter 5 discusses the implementation of the FPGA-based high speed real-time digital controller where the direct tip-sample interaction control described in Chapter 2 and high bandwidth multi-axis actuation of a probe described in Chapter 4 are implemented. The implementation of the high speed bi-directional communication of the FPGA-based controller and a host computer is also discussed, which enables high speed data display and on-line parameter identification/modification. Chapter 6 presents high speed/ high resolution imaging using the high speed AFM developed in Chapter 2-5. In order to show the high temporal and spatial resolution of the developed high speed AFM, two grating samples, one engineered grating with 100nm pitches and one biological grating composed of a repeating structures of proteins on purple membrane patches are measured. The conclusions and remarks for future works are described in Chapter 7.
CHAPTER 2

DIRECT TIP-SAMPLE INTERACTION FORCE CONTROL FOR THE
DYNAMIC MODE ATOMIC FORCE MICROSCOPY

2.1 Introduction

Dynamic mode atomic force microscopy (AFM[1]), where a cantilever is oscillated near its resonance frequency and controlled to gently tap the sample surface, has several advantages over conventional contact mode operation. Among them, scanning with greatly reduced lateral force and imaging with less sensitivity to cantilever thermal drift are particularly significant when imaging biological samples that are really soft and that have large variations in topography. The most commonly used method in dynamic mode operation is a form of amplitude modulation, in which the oscillation amplitude of the cantilever is modulated by variations of the tip-to-sample distance. By employing changes of the oscillation amplitude as feedback signals for z-control, the oscillation amplitude is regulated and z-motion yields topographic information about the sample surface. However, in typical implementations, due to tapping dynamics and limited bandwidth of the z-scanner, the transient response of the cantilever induced by changes of
the tip-sample interaction force leads to greater variations in tip-sample interaction via feedback, causing excessive tapping forces and/or possible loss of tapping during scanning, and thus greater sample distortions and imaging errors. Therefore, while dynamic mode AFM can have many potential applications[14, 49-53], the inability to achieve precise control of the tip-sample interaction forces has been one of the key barriers that limit imaging rate and that inhibit innovation leading to more applications.

Active Q control was employed to reduce the effective quality factor Q of the cantilever so that its transient response vanishes quicker [44]. While this method improved scanning speed, it sacrificed the force sensitivity of the cantilever. Smaller cantilevers, whose resonance frequencies were two orders of magnitude higher than the usual cantilevers but having comparable stiffness, were used to reduce the settling time [38, 39]. However, the small size of the cantilever, less than 20 μm in length, could lead to difficulties while engaging sample surfaces and when scanning samples having large topographic variations. In an attempt to increase scanning bandwidth, a transient-signal-based sample detection method was developed by constructing an observer, providing an estimate of the transient state of the cantilever, to detect changes in tip-sample interaction [48].

In this Chapter, a control method for dynamic mode atomic force microscopy is presented, in which the tip-sample interaction force of each tapping cycle is directly regulated during scanning. In this method, based on a linear dynamic model of the cantilever along with its transient response, an estimator is designed and implemented to estimate the tip-sample interaction force of each tapping cycle. The estimated interaction
forces are then utilized by a model-based predictor to plan and control the next tapping by controlling the tip-to-sample distance. In order to attenuate the effects of modeling errors of the predictor, a feedback regulator is employed to directly regulate the tip-sample interaction force. Since the tip-sample interaction force of each tapping cycle is directly controlled, it does not rely on the steady-state relationship between the oscillation amplitude of the cantilever and the tip-to-sample distance. Therefore, tapping dynamics in amplitude modulation is irrelevant and time delay as a result of averaging in oscillation amplitude measurement is eliminated. Consequently, precise control of the tip-sample interaction force and high imaging rate can be achieved, independent of the quality factor Q of the cantilever.

2.2 Design of Direct Tip-sample Interaction Force Control

The block diagram of the direct tip-sample interaction force control is illustrated in Fig. 2.1. The augmented estimator which estimate the tip-sample interaction force and dynamic state vector of each tapping cycle is described Section 2.2.1. A model-based predictor to plan and control the next tapping by controlling the tip-to-sample distance is described in Section 2.2.2. The feedback regulator to attenuate the effects of modeling errors of the predictor is described in Section 2.2.3.
2.2.1 Tip-sample interaction force estimator

Since direct measurement is not available, in order to achieve direct control of the interaction force, the tip-sample interaction force of each tapping cycle needs to be estimated in real-time. When tapping a sample surface, the tip-sample interaction force presents itself as a disturbance to the cantilever. The dynamical state vector of the cantilever, consisting of its tip position and velocity, is augmented to include this disturbance as an additional state variable. This augmented state vector along with the dynamic model of the cantilever is employed to construct a closed-loop observer that estimates the tip-sample interaction force as well as the tip position and velocity. A feedback gain matrix is then designed for the observer to guarantee a desired rate of convergence [54]. The estimated disturbance represents the tip-sample interaction force that may include the contact repulsive force and a long range force. Based on the tip
position, the contact repulsive force of each tapping cycle can then be extracted. In addition, the sample position of the current tapping is estimated from the dynamic state vector and the estimated impulse strength. In summary, the output of the estimator for the $k^{th}$ tapping cycle includes: 1) dynamic state vector $\hat{x}_s(k,t)$ of the cantilever (tip position and velocity), 2) tip-sample interaction force $\hat{f}(k,t)$ consisting of contact repulsive force $\hat{p}(k)$ and long range force $\hat{\ell}(k,t)$, and 3) sample position $\hat{x}_s(k)$.

### 2.2.2 A Model-based Predictor

A model-based predictor is designed to plan and control the next tapping through controlling the tip-to-sample distance. Since tapping occurs near the lowest tip position of the cycle, in which the sinusoidal input force and tip velocity are small and the deflection of the cantilever stays around local maxima, all the forces except the tip-sample interaction force during the period of contact are lumped into $m \cdot a_c$, where $m$ is the lumped mass of the cantilever and $a_c$ is the expected acceleration of the tip at the lowest position when assuming no contact. By employing a spring force model (sample stiffness is $k_s$) for the tip-sample interaction along with the lumped force $m \cdot a_c$, the impulse strength, integrated over the period of contact, is approximated to be $2m(-v_m) - ma_c \pi \sqrt{m/k_s}$ for the case in which the sample stiffness is greater than that of the cantilever, where $v_m$ is the velocity with which the cantilever is incident on the sample surface. The acceleration $a_c$ can be directly predicted from the dynamic state vector $\hat{x}_s(k,t)$. It is possible to estimate the sample stiffness in real time based on the
contact impulse model along with the impulse strength $\hat{p}(k)$ from the estimator, however a calibrated value is used instead in the current implementation. Additionally, the velocity $v_{in}$ of the next tapping depends on the sample position $x_s(k+1)$, the long range force $\ell(k+1,t)$, and the z-motion $\delta z$. Therefore, for simplicity the impulse strength of the next tapping can be expressed as

$$\hat{p}(k+1) = P[\hat{x}_s(k,t), \ell(k+1,t), x_s(k+1), \delta z] .$$  \hspace{1cm} (2.1)

Using a zero-order predictor, $\hat{\ell}(k+1,t) \approx \ell(k,t)$ and $\hat{x}_s(k+1) \approx \hat{x}_s(k)$, the solution to Eq.(1) yields the desired z-motion that regulates the impulse force of the next tapping to the reference value $p_r$.

$$\delta z = P^{-1}[\hat{x}_s(k,t), \hat{\ell}(k,t), \hat{x}_s(k), p_r]$$  \hspace{1cm} (2.2)

Fig. 2.2: Model-based predictor and Z-position modification. (a) From a model-based predictor, the tip position, sample surface and the interaction force in the next tapping cycle are predicted. Gray-colored area represents the past. (b) The z-position modification regulates the tip-sample interaction force in the next tapping cycle.
This result allows us to plan the z-motion to control the tip-to-sample distance, and thus the tip-sample interaction force as illustrated in Fig. 2.2. Topographical images can then be constructed according to the estimated sample position $\hat{x}(k)$ in conjunction with the associated x-y coordinates of the scanner.

### 2.2.3 Feedback regulator

Variations in the material property of the sample surface and the degree of tip-sample engagement cause variations in the lumped stiffness of the sample. In addition, the prediction of $\tilde{x}(k+1,t)$ and $\hat{y}(k+1)$ can be erroneous, causing errors in determining the required z-motion. A feedback regulator is employed to attenuate these two effects and better regulate the tip-sample interaction force. It uses the estimated tip-sample interaction force as the feedback signal to directly control the tip-sample interaction force.

### 2.3 Scanning of a biological sample with precise tip-sample interaction control

As illustrated in Fig. 2.1, the proposed method for tip-sample interaction force control includes interaction force estimator, model-based predictor, and feedback regulator. It was implemented using a real-time control system (CP1104, dSPACE Inc.) with a closed loop rate of 20kHz, and was integrated with a commercial AFM (Picoscan 2500, Agilent). Purple membrane patches were deposited on a freshly cleaved mica surface in an absorption buffer (350 mM KCL, 10mM Tris-pH 8.0) for 10 minutes and gently rinsed with an imaging buffer (300mM KCL, 10mM Tris-pH 8.0) for three times. The topography of a purple membrane patch deposited on mica surface was measured with the direct force control method in the imaging buffer as shown in Fig. 2.3. The
stiffness of the cantilever (Type IV MAClevers) was 0.01 N/m. The scanning speed and the imaging resolution were 4 lines/sec and 256×256 pixels, respectively. The tip position and the estimated tip-sample interaction force were estimated by the estimator. The latter was then lumped into an impulse for each tapping cycle, the strength of which was employed by the feedback regulator, serving as the feedback signal (force measurement). The control of tip-sample interaction force was then achieved via z-motion control, the desired value of which was determined by the model-based predictor. In this experimental evaluation, a multi-purpose z-scanner with low coherence laser (Picoscan, Agilent) was employed to realize the required z-motion. By zooming into a very small time interval (10ms) as shown in Fig. 2.3 (c), ten tapping cycles can be clearly seen. The peak value of the estimated tip-sample interaction force is identified to be very small (around 63.5 pN). This experimental result clearly demonstrates that the direct force control method has the capability to measure the topography of the sample with very small tip-sample interaction forces (several tens pN), which is very important for biological samples.
Fig. 2.3: Experimental results of direct tip-sample interaction force control. A purple membrane patch deposited on freshly cleaved mica surface is scanned while the direct tip-sample interaction controller regulates its peak force as 63.5 pN. (a) The reconstructed topography image of a purple membrane patch. (b) Tip position, estimated tip-sample interaction force and z-motion for one line scanning. (c) Zoomed-in tip position, tip-sample interaction force and z-motion for ten tapping cycles.
2.4 Independence of cantilever’s transient dynamics

In order to illustrate the independence of the proposed method from cantilever’s transient dynamics, computer simulations were performed. Computer simulations allow us to illustrate the sole effect of transient dynamics, with no contributions from the measurement’s delay and the z-scanner’s dynamics. In the case of the amplitude modulation method (the left column in Fig. 2.4), the transient response of the oscillation amplitude led to significant variations of the tip-sample interaction force, including loss of tapping (between 0.123s and 0.137s) and introduced significant distortions in the reconstructed topography (based on the z-motion). In addition, it is evident that the reconstructed topography is sensitive to the controller gain. The right column in Fig. 2.4 shows the results of the proposed control scheme. It is seen that the impulse strength suddenly increases when the tip encounters the 2 nm step change, a result of prediction error in sample position \( \hat{\Delta}_s(k + 1) = \Delta_s(k) + 2nm \). Nevertheless, the impulse force is regulated back to the specified value rapidly. Moreover, although the transient response of the oscillation amplitude still exists, the topography reconstructed by the proposed control scheme follows the real topography right from the second tapping cycle after the step change. These results clearly show that the proposed control method is capable of directly regulating the tip-sample interaction force of each tapping cycle and that the reconstructed topography is not affected by the transient response of the oscillation amplitude.
Fig. 2.4: Computer simulations of scanning a step change (2nm) in topography with the amplitude modulation (left column) and the direct force control (right column): (a) tip position during tapping, (b) impulse strength, (c) oscillation amplitude, and (d) reconstructed topography. In these two simulations, the operation frequency of the cantilever is 1 kHz, the lumped stiffness is 0.01 N/m, and its Q factor is 20. The sample stiffness was assumed to be 0.25 N/m. The discrete time loop rate in these simulations was 50 kHz.

2.5 Conclusions and remark

A control method, in which the tip-sample interaction force of each tapping cycle is directly regulated, is proposed for dynamic mode atomic force microscopy. Since the tip-sample interaction force of each tapping cycle is directly controlled, it does not rely on the steady-state relationship between the oscillation amplitude of the cantilever and
the tip-to-sample distance. Therefore, tapping dynamics in amplitude modulation is irrelevant and time delay as a result of averaging in oscillation amplitude measurement is eliminated. The experimental result of scanning a biological sample demonstrated that the proposed control scheme allows scanning with very small tip-sample interaction force, which is important for the application to biological samples. A simulation result which illustrates the sole effect of transient dynamics during the scanning clearly show that the proposed control method is capable of directly regulating the tip-sample interaction force of each tapping cycle and that the reconstructed topography is not affected by the transient response of the oscillation amplitude.

The proposed control scheme allows the direct tip-sample interaction control of each tapping cycle and makes tapping dynamics in amplitude modulation irrelevant. In addition, the time delay caused by the estimation of the oscillation amplitude is eliminated. Therefore, this new control scheme removes two roadblocks that limit the image rate of dynamic mode AFM and enables high speed dynamic mode AFM imaging.

While precise control of tip-sample interaction force is possible, this control method necessitates rapid tip-motion control to effectively control the tip-to-sample distance, and thus the tapping force, within each tapping cycle. Therefore, the bandwidth of the z-motion control loop is fundamentally important to AFM imaging in terms of imaging rate, imaging resolution, and controlling the tip-sample interactions. In AFM imaging, as a sample is scanned at higher speed, topographic details present themselves to the z-motion control loop as disturbances at higher frequencies. Therefore, in order to track abrupt topographic variations and maintain high imaging resolution, it necessitates
higher bandwidth z-motion control. In order to meet the requirement for high bandwidth actuation, high bandwidth direct tip-position control using magnetic actuation is implemented in which the AFM cantilever not only vibrates around its resonance frequency for the dynamic mode AFM, but also its mean value changes rapidly to directly control the tip-position with the bandwidth comparable to the cantilever. The design, implementation and control of the high bandwidth probes are described in Chapter 4.

In addition to the high actuation bandwidth, the proposed control scheme in this Chapter requires large bandwidth of the measurement signal, which may result in the degradation of the resolution due to large contribution of measurement noise. In order to reduce the noise effect at the cantilever deflection measurement, a low noise deflection measurement system is implemented and integrated to the system, which reduces more than 75% of the overall noise within 1 MHz bandwidth compared to a commercial one. The details of the low noise deflection measurement are described in Chapter 3.

In order to implement direct tip-sample interaction force control for the AFM cantilever whose bandwidth is in the range of ~ hundreds kHz, a new programmable digital controller whose critical time loop is one or two order of magnitude higher than the bandwidth of the cantilever employed is required, which is difficult to achieve using a conventional CPU-based controller. To meet the update-rate requirement of the digital controller, a high speed programmable digital controller is implemented based on field programmable gate array, which shows 20 MHz. The details of the high speed digital controller are described in Chapter 5.
CHAPTER 3

LOW NOISE LASER MEASUREMENT SYSTEM

3.1 Introduction

In typical implementation of AFM, a laser measurement system is employed in order to measure the tip-position along the Z axis and/or along the other axes. The laser beam is focused on the back surface of the AFM cantilever and the position change of the reflected laser is measured by a set of photodiodes. When the cantilever is deflected about one axis (ex. X axis: the axis along the width of the cantilever), the laser incident on the cantilever changes its reflection angle and thus changes its position on the plane of a split photodiode which is located approximately normal to the laser path. The position change of the laser on the photodiode is measured in the form of difference of the two signals from the two halves of the photodiode. The split photodiodes are placed at very close distance in order to minimize the effect of common-mode noise, like power noise in the electronic circuits [55]. The thermal force from the environment excites the cantilever and causes so called thermal fluctuation of the cantilever [56]. This thermal fluctuation of the cantilever limits the positioning accuracy of the tip in the given environment. Therefore, there are two factors that affect precise measurement of tip-position, namely, measurement noise and thermal fluctuation of the tip. In order to achieve the best
measurement resolution of the tip position for a given cantilever, the laser measurement system needs to have low measurement noise so that the accuracy of the measured tip position is limited only by the thermal fluctuation of the cantilever [55, 57].

The noise density of the laser measurement system in conventional AFMs is in the range of 100–1000 fm/√Hz [57]. Fukuma, et al. analyzed the sensitivity of the laser measurement system and suggested methods to implement a low noise laser measurement system which showed the low noise density of ~ 17 fm/√Hz. The sensitivity $S_z$ of the signal measured using the laser measurement system to the tip position change is given by [57]

$$ S_z \sim \alpha R_{iV} G \frac{P_{l_f}}{d_L \ell_C}, \quad (3.1) $$

where $\alpha$ denotes the total efficiency of the laser measurement system including the efficiencies of the optical components and the conversion efficiency of the photo-diode. $R_{iV}, G, \ell_f, P, d_L$ and $\ell_C$ denote the trans-impedance of the IV converter, the total gain of the amplifier circuit, focal length of the objective lens, power of the laser beam, diameter of the collimated laser beam and length of the cantilever respectively. One of the major noise sources related to laser deflection measurement using photo-diodes is photo-diode shot noise and its voltage noise density at the output of the electric circuit is given by $n_{av} = R_{iV} G \sqrt{2e\alpha P}$ where $e$ denotes electron charge, $R_{iV}$ and $G$ denote the gains of the IV converter and the differential amplifier respectively [57]. Using the voltage noise density of the photo-diode shot noise and eq. (3.1), one can calculate the noise density $n_{sz}$ caused by the photo-diode shot noise at the deflection measurement and is given by
According to equation (3.2), the noise density of the deflection measurement of a given cantilever of length $\ell_c$ can be lowered by using a laser beam with higher power $P$, reducing the diameter of the collimated laser beam $d_L$, and increasing the focal length of the objective lens $\ell_f$. Improving the optical efficiency, which includes the conversion efficiency (light to current) of photo diodes also contributes at decreasing the deflection noise density. By making the diameter of the focused laser smaller than the cantilever width and using AFM cantilevers with back-coating of highly reflective materials such as Au and Al, the degradation of the optical efficiency can be avoided. By employing the high bandwidth electronics for IV conversion and signal amplification, and enhancing the operation bandwidth of the photo-diodes by applying reverse bias-voltage to the photo-diodes, the degradation of efficiency $\alpha$ at high frequencies can be prevented. Therefore, it is desirable to make a major modification to use a laser source with smaller diameter, increase the laser power, employ high bandwidth electronics and use reverse bias-voltage for the photo-diodes [57]. This was as illustrated in Fig. 3.1. In addition, a power-modulation is used to minimize the effect of the optical feedback. In the power-modulation method, the power to the laser is modulated at radio frequency (rf) in order to allow the laser continue to operate in its transient state. During the transient state of the operation, a single mode laser operates in its multi-modes and becomes less sensitive to the optical feedback, which causes so-called mode-hopping noise [55,58,59].
Fig. 3.1: Modification of the laser measurement system for high-sensitivity: (a) conventional laser measurement system in a commercial AFM. The beam size is larger than the reflecting surface, resulting in loss of optical efficiency. (b) Modification of the laser measurement system for higher-sensitivity. The laser source is replaced with a smaller diameter (1.1 mm) and higher power (~ few mW). In order to prevent the degradation of the sensitivity at high bandwidth, the electronic circuits consisting of high bandwidth components are employed and a reverse bias voltage is applied to the photodiodes to reduce junction capacitance. The laser power is modulated at rf-frequency (300MHz) to reduce the effect of the optical feedback and temperature change.

In this chapter, the implementation of a low noise laser measurement system along the lines described by Fukuma et.al. and its integration into a commercial AFM (MFP 3D, Asylum research) are described. The implemented laser measurement system is evaluated by measuring not only the noise density but also the overall noise level with 1 MHz bandwidth. The former is important when a band-limited signal can be used as the case of the oscillation amplitude estimation using a lock-in algorithm as described in
Appendix A. The latter is important when large measurement bandwidth is required as the case of the implementation of direct tip-sample interaction force control (Chapter 2).

This chapter is organized as follows: The implementation of the low noise laser measurement system is described in Section 3.2. The integration of the low noise laser measurement system into a commercial AFM is described in Section 3.3. The evaluation of the implemented laser measurement system in terms of the focused laser size, the noise density and overall noise level within 1MHz bandwidth is described in Section 3.4. High resolution imaging of the repeating structure of proteins on purple membranes with a stiff-AFM cantilever (40 N/m) using the new laser measurement system is described in Section 3.5. The conclusions and other remarks are added in Section 3.6.

3.2 Implementation of a low noise laser measurement system

A low noise laser measurement system for a commercial AFM (MFP3D, Asylum research) is implemented based on information reported in literatures [55, 57]. A Laser diode (VHK 635nm, Edmund Optics) with small collimated diameter of 1.1mm was employed as the laser source. A small size quadrant photo diode (S6695-01, Hamamatsu Photonics) is used in order to facilitate integration in the head scanner of MFP3D. Photo-diodes convert photonic energy into electric current. The generated currents from the photo-diode are converted into voltages by using I-V conversion circuits. Operational amplifiers with high input impedance are required for the implementation of I-V conversion circuits. Thus, operational amplifier OPA4354 (TI) with high input impedance of $10^{13}\Omega$ are employed to implement the I-V convertors for each photo-diode and the trans-impedance of the I-V convertors are designed to be 10 k$\Omega$ as shown in Fig. 3.2. The main role of the differential amplifiers connected to the I-V convertors is to
extract the position change of the laser beam on the two adjacent photo-diodes by calculating the voltage difference from two I-V converters connected to the corresponding photo-diodes. In addition, common-mode noise such as the power noise in the electric circuits and/or power fluctuation of the laser can be removed by the differential amplifier. Therefore, differential amplifiers with high common-mode rejection ratio (CMRR) are preferred. An instrumentation operational amplifier INA111 (CMRR: 110dB, TI) with gain of 10 was employed to implement differential amplification of the signal outputs from the I-V converters. Since the selected photo-diode is composed of four pieces, there are two sets of differential amplifiers for the measurement of laser position change along one direction. Thus, the outputs from the two differential amplifiers need to be added. A low noise OP amplifier (THS4032, 1.6 nV/√Hz, TI) is employed to implement the summation circuit. The output of the summation circuit is the final output voltage which measures the position of the laser beam on the photo-diodes in a differential form. The total schematic of the electronic circuits including the photo-diodes is illustrated in Fig. 3.2.

When reverse-bias voltage is applied to the photo-diodes, the dark current increases but the terminal capacitance of the photo-diodes decrease and thus improves bandwidth of the photo-diode response [60]. Therefore, for high bandwidth measurement of the laser position change, a reverse bias voltage is applied to the photo-diode as included in Fig. 3.2. In the implementation, +9V was applied as a reverse bias voltage to the photo diode through a unit-gain follower.
Fig. 3.2: Electronic circuits for I-V conversion and differential amplification of the signals. $V_{\text{def}}$ and $V_{\text{bias}}$ denote the deflection voltage output and bias voltage input respectively. All the circuits for I-V converters are identical to the one on the top and the circuits for the other three are simplified in this illustration.

3.3 Integration of the low noise laser measurement system into a commercial AFM

The integration of the implemented low noise laser measurement system to MFP3D AFM requires the addition of the optical path for the new laser source to focus it on the cantilever, and the installation of the photo-diode inside the head scanner of MPF3D AFM. In order to decide the location of the photo-diode and estimate the size of the focused laser beam on the cantilever, the size of the laser beam after passing the objective lens (10X) is measured using a beam profiler. It is worthwhile to mention here that the objective lens is originally designed for the top-view camera in MFP3D AFM. In addition, the positions of the focused laser beam on the cantilever and the reflected laser beam on the photo-diode need to be adjustable. The former is important for the optical
efficiency and the latter is important to locate the laser beam in the middle of two consecutive photo-diodes for improved rejection of the common-mode noise at the differentional amplifier. In order to meet these requirements, an optical path with adjustable angle change is added to the existing optical path in MFP3D AFM and a miniaturized 2 DOF XY stage is implemented and integrated with the photo-diode. The addition of the optical path described in Section 3.3.1. The measurement of the size of the laser beam after passing the objective lens by using a beam profiler is described in Section 3.3.2. The evaluation of the optical efficiency of the associated optical components is described in Section 3.3.3. The implementation of the miniaturized 2 DOF XY stage and its installation with the photo-diode inside of the head scanner in MFP3D AFM is described in Section 3.3.4.

3.3.1 Addition of an optical path for the new laser beam

In order to integrate the implemented new laser measurement system without disturbing original laser measurement system, a new optical path was added utilizing the optical path for the top-view camera system in MFP3D as shown in Fig. 3.3. Fig. 3.3 (a) shows the optical path in MFP3D of the top view camera. The illumination light is introduced using a beams splitter and is focused on the plane of the cantilever using an objective lens (10x). The reflected light traverses the same path in the reverse direction and reaches the top view camera. Since these optical components for the top-view camera system are designed to focus the illumination light on the cantilever and capture its reflected light using a camera, they can also be used to focus an additional laser beam on the cantilever if the laser beam is introduced along the same path as the illumination light as shown in Fig. 3.3 (b). The wave length of the laser beam employed is 635nm. In order
to introduce this laser beam along the optical path of the top-view camera, a 45 deg red-reflector (FM02, Thorlabs) is used as shown in Fig. 3.3 (b). This red-reflector also blocks a part of the spectrum of light from the illumination source but does not disturb the function of the top-view camera. For the adjustment of the position of the focused laser beam on the cantilever, a home-made 2 DOF angular adjustment which changes the angle of red reflector is integrated together with the red reflector as shown in Fig. 3.4.

Fig. 3.3: The Optical path in MPF 3D for the top view camera and its modification for a new laser measurement system: (a) original optical path for the top view camera, (b) the modification of the optical path in order to introduce a laser source. The red-reflector directs the laser source to the optical path such that the laser beam is focused on the cantilever back-side.
Fig. 3.4: The installation of the red-reflector on a home-made 2 DOF angular adjustment: (a) an additional optical path for the new laser, (b) the installed red reflector at a commercial AFM (MPF3D, Asylum research). The position of the focused laser is adjusted by 2DOF angular adjustment where the red reflector is attached.

3.3.2 The measurement of the laser beam size versus the distance

In order to estimate the size of the focused laser on the cantilever and design the distance of the photo-diode from the cantilever at which the laser beam size is smaller than the photo-diode, the size of the laser beam was measured by using a beam profiler (BP109-VIS, Thorlabs) at various positions. The configuration of the beam size measurement and estimation of the size of the laser reflected on the cantilever are illustrated in Fig. 3.5. The measured size of the laser beam versus distance is shown in Fig. 3.6. According to the measured beam profile, the diameter of the focused laser was estimated to be 23.4um which is small enough to locate on the back-side of the commercial AFM cantilevers. In addition, the required distance of the photo-diode from the cantilever which assures that the size of the laser spot on the photo-diode plane is smaller of that of photo-diodes (2 mm x 2mm) was estimated to be within 13 mm.
Fig. 3.5: The evaluation of the laser size change by the focus lens: (a) the size of the laser beam after the focus lens was measured by using a beam profiler at various distances from the location of the cantilever. (b) From the slope between the laser size and the distance, the required distance of the photo-diode $\ell_{PD}$ from the cantilever location which makes the beam size is smaller than that of photo-diode is estimated to be within 13 mm.

Fig. 3.6: The variation of the size of the laser beam versus the relative distance along the path of the focused laser beam. The original beam size is 1.1 mm and the size of the focused laser is estimated as 23.4 um using the slope between the relative distance and ratio of the beam size.
3.3.3 Evaluation of the optical efficiency of the associated components

By comparing the optical power measured by the beam profiler after each component, the optical efficiencies of the associated optical components could be evaluated as shown in Fig. 3.7. Since it is very difficult to measure the power of the laser reflected on the back surface of a cantilever because the large size of the beam profiler, the optical efficiency of the back surface of the cantilever could not be evaluated. Instead, the only cantilevers whose back surface are coated with gold (Au) or Aluminum (Al) are employed in the experiments in order to minimize the degradation of the optical efficiency from the cantilever, which is directly related to the deflection noise density as described in Section 3.1. The overall optical efficiency of the associated components was measured to be higher than 86% as illustrated in Fig. 3.7.

Fig. 3.7: The evaluation of the optical efficiency of the optical components for the new laser measurement system.
3.3.4 Miniaturized 2DOF XY stage for the adjustment of the photo-diode position

In order to adjust the location of the reflected laser on the photo-diode, 2 DOF miniaturized XY stage was designed and fabricated as illustrated in Fig. 3.8. This miniaturized XY stage is small enough to insert into the inside of the head scanner of MFP 3D AFM and its position along the two axes can be adjusted by corresponding screws. The travel ranges of the miniaturized XY stage were designed to be 0.8 mm along the two axes. In order to hold the position of the XY stage and minimize mechanical drift, small permanent magnets and metal sheet were attached to the slider and the stator respectively in order firmly hold the two sliding surfaces of the assembled XY stage. Figure 3.8 (b) shows the installation of the photo-diode assembled on the miniature XY stage into the inside the head scanner of MFP3D AFM.

![Fig. 3.8: The installation of the miniaturized XY stage for adjustment of the laser on the Photo-diode: (a) the design of the 2 DOF XY manual stage. The positions along the two axes are adjusted by two screws along with the taps machined on the moving parts. (b) The installation of the photo-diode assembled with the 2DOF XY stage into the head scanner of MPF3D AFM.](image-url)
3.3.5 Laser beam size reduction with two focus lenses

According to the equation (3.2), the deflection noise density can be decreased by reducing the diameter $d_L$ of the collimated laser beam. In order to modify the diameter of the laser beam, two focus lenses with different focal length were employed as illustrated in Fig. 3.9. One pair of focus lenses (CAY046: focal length 46mm and CAX100: focal length 100mm, Thorlabs) are integrated to reduce the diameter of the collimated laser beam by half. On the other hand, this method can also be used for reducing the size of the focused laser by increasing the diameter of the collimated laser beam. This enables their use for AFM cantilevers with smaller width. The effect of the beam reducer on the noise density is evaluated and described in Section 3.4.2.

3.4 Evaluation of the implemented laser measurement system

The new laser measurement system integrated in MFP3D AFM was evaluated by measuring the thermal fluctuation of a commercial AFM cantilever (NCHAuD, NanoSensors) whose nominal stiffness is 40 N/m. The evaluation of the size of the focused laser beam is described in Section 3.4.1. The evaluation of the noise level with the new laser measurement system is described in Section 3.4.2.
3.4.1 Evaluation of the size of the focused laser

Fig. 3.10. Bottom view camera image of the cantilever and focused laser. The focused laser on the cantilever plane was captured by a bottom view camera through an objective lens (LUCPlanFL N, 20X, Olympus). The image pixels where the focused laser beam stays were saturated and its diameter gives information of the diameter of the focused laser beam. The nominal width of the AFM cantilever (NCHAuD) is 40um.

The new laser measurement system integrated in MFP3D AFM is evaluated measuring the thermal fluctuation of a commercial AFM cantilever (NCHAuD, NanoSensors) whose nominal stiffness is 40 N/m. Firstly, the size of the focused laser beam on the cantilever plane was evaluated with a transmitted light image of the cantilever and a focused laser beside the cantilever, measured by a bottom view camera (C4742-95, Hamamatsu) through an objective lens (LUCPlanFL N, 20X, Olympus) as shown in Fig. 3.10. In Fig. 3.10, a bright solid circle beside a transmitted light image of the cantilever represents the saturated image pixels by the focused laser beam and the diameter of the focused laser beam is estimated as 28.29 um from the diameter of the area with saturated image pixels, which is close to the value estimated by a beam profiler (Section 3.3.2).
3.4.2 Evaluation of the noise level with the new laser measurement system

The measured cantilever deflection without any external excitation (except the thermal force) includes the thermal fluctuation of the cantilever caused by the thermal energy, and the measurement noise. At cantilever deflection measurement, both of them are considered as noise which limits the resolution.

There are two purposes of using the new laser measurement system: (1) the reduction of the overall noise level within large bandwidth (e.g., 1MHz). (2) the reduction of the noise level around the resonance frequency of the cantilever. The former is essential to achieve high resolution imaging in operation mode of the AFM which requires large bandwidth such as the direct tip-sample interaction control described in Chapter 2. The latter is useful for the operation which allows band-limited measurement around the resonance frequency of the cantilever like the amplitude modulation mode. The standard deviation of the measured deflection signal of the cantilever without external excitation within 1MHz bandwidth was measured in order to evaluate the overall noise level of the measurement system. For the evaluation of the noise level near the resonance frequency of the cantilever, the noise density was evaluated. The noise density represents the power spectral density of the measured cantilever deflection without any external excitation.

Figure 3.11 shows the direct comparison of the standard deviation of the measured signals using the new laser measurement system. The new laser measurement system shows 74.33% reduction of the standard deviation of the cantilever deflection measurement compared to that by the laser measurement system in MFP3D AFM. Since it can be assumed that the contribution of the thermal fluctuation of the cantilever to the
noise levels measured by the two different measurement systems is consistent, this reduction of the noise level in the new laser measurement system is mostly due to the suppression of the noise in the measurement system itself.

The measurement noise includes noise from the AD converter and the other electronic circuits. In order to evaluate the noise from the AD converter and analog circuits which convert the voltage inputs to differential current sources as a part of AD converter, the input of the AD converter was connected to the signal ground while the signal was measured from the AD converter. For the evaluation of the noise of the other circuits including the photo-diode, IV converter, differential amplifier and summation circuit, all the light sources around the photo-diode were blocked while the output of the laser measurement system was measured with the AD converter. In this cases, the noise in the voltage output of the measurement system includes the dark current generated from photo-diodes, and the noise from the other electronic circuits.

The standard deviations of the noise in the signals from the AD converter and from the circuits in the dark room condition are also shown in Fig. 3.11. The contributions of the AD converter and the electronic circuits in the dark room condition to the overall noise level with the new laser measurement system were 9.7% and 36.5% respectively as compared with the values in parentheses. Therefore, the standard deviation of the deflection measurement of the cantilever without the external excitation using the new laser measurement system is dominated by the thermal fluctuation of the cantilever.
Fig. 3. 11: Comparison of the accumulated overall noise level (standard deviation) within 1 MHz bandwidth. The new laser measurement system shows 74.33% reduction of the standard deviation of the cantilever deflection measurement compared to that by the laser measurement system in MFP3D AFM. The contributions of the AD converter and the electronic circuits in the dark room condition to the overall noise level with the new laser measurement system were 9.7% and 36.5% respectively as compared with the values in parentheses.

In order to evaluate the effect of the reverse bias voltage to the photo-diodes, the standard deviation of the measured deflection within 1 MHz bandwidth and the noise density near the resonance frequency of the cantilever were evaluated as shown in Fig. 3.12 and Fig. 3.13 respectively. When the reverse bias voltage is applied to the photo-diodes, the junction capacitance decreases [60] and prevents the saturation of the photo-diodes [57] especially at the high bandwidth. Therefore, the reduction of the noise level
(standard deviation) over the large bandwidth and noise density at high frequency is expected. Four different reverse bias voltages namely 0V, 3V, 9V and 12V were applied to the photo-diode and the standard deviations of the measured free deflection of the cantilever (NCHAuD, Nanosensors) in the air were compared. While the standard deviation of the measured free deflection of the cantilever from MFP3D was 256.16 pm, the standard deviations of the measured deflection signal in the new laser measurement with reverse bias voltages 0V, 3V, 9V and 12V were 99.72 pm, 63.41 pm, 59.48 pm and 59.92 pm respectively. The decrease of the standard deviation with the reverse bias voltage higher than 3V was not significant. When the reverse bias voltage was 9V, the standard deviation was the smallest (59.48 pm) and the reduction percentage from one by MFP3D was 76.78%.

The noise density near the resonance frequency of the cantilever (NCHAuD, Nanosensors) was also compared as shown in Fig. 3.13. The noise density around the resonance frequency of the cantilever is important for the estimation of the oscillation amplitude using a lock-in amplifier. When the new laser measurement system was used with zero reverse bias voltage, the noise density of the measured deflection signal of the cantilever was $61 \text{fm}/\sqrt{\text{Hz}}$. When the bias-voltages higher than 3V were applied to the photo-diodes, the noise density was reduced to be $38 \text{fm}/\sqrt{\text{Hz}}$. On the other hand, the noise density of the laser system in MFP 3D AFM was measured to be around $200 \text{fm}/\sqrt{\text{Hz}}$. The difference between the noise densities with the laser measurement system in MFP 3D and the new laser measurement system shows the improvement of the deflection measurement with the new laser measurement system.
Fig. 3.12: The overall noise level evaluated using the standard deviation of the measured signal within 1 MHz bandwidth with various reverse bias voltages. The standard deviations of the measured deflection signal within 1 MHz bandwidth were 99.72 pm, 63.41 pm, 59.48 pm and 59.92 pm when the reverse bias voltages of 0V, 3V, 9V and 12V were induced to the photo-diodes respectively in the new laser measurement system. The measured data points are indicated with circles. The standard deviation of the measured deflection signal of the cantilever (NCHAuD) with the laser measurement system in MFP 3D AFM was measured to be 256.16 pm.
Fig. 3.13: The comparison of noise densities of the measured deflection signal of the cantilever (NCHAuD, NanoSensors) in the air near its resonance frequency.

In order to evaluate the effect of reduction of the diameter of the input laser beam, one pair of focus lenses (CAY046: focal length 46mm and CAX100: focal length 100mm, Thorlabs) were employed to implement a beam reducer with the gain of 0.46. The implemented beam reducer was inserted between the collimated laser beam source and the red-reflector, and the noise density of the free deflection of a cantilever (NCHAuD) measured in air with the new laser measurement system (Reverse bias voltage 9V) was evaluated around the resonance frequency of the cantilever. With the beam reducer gain of 0.43, the lower noise density ($24 \text{ fm} / \sqrt{\text{Hz}}$) could be achieved. The resulting frequency spectrum is shown in Fig. 3.14.
Fig. 3.14: The noise density after the reduction of the diameter of the laser beam using beam reducer composed of two focus lenses. The noise density around the resonance frequency of the cantilever (NCHAuD) was measured to be $24 \text{ fm} / \sqrt{\text{Hz}}$.

3.5 High resolution imaging with the new laser measurement system in dynamic mode AFM

For high speed AFM imaging, the high bandwidth AFM cantilevers are required in order to have high image point rate (pixel rate) which limited by the tapping frequency of the cantilever [45]. Among the commercially available cantilevers, cantilevers whose bandwidth is high ($> 100$ kHz) usually also have high stiffness ($> 40$N/m). When cantilevers whose stiffness is high are employed, the measurement noise level becomes more important because the reduced thermal fluctuation of the cantilever due to its high stiffness can easily cause the measurement noise to dominate in the deflection measurement and thus, degrade the measurement resolution. Subsequently, the degradation of the deflection measurement resolution will result in the degradation of the force sensitivity of the cantilever. However, if the measurement noise level is
significantly smaller than the thermal fluctuation of the cantilever employed, it does not cause degradation of the deflection measurement resolution and thus, the force sensitivity of the cantilever can be retained [56].

In order to evaluate the effect of the implemented low noise laser measurement system, a soft biological sample was scanned using a commercial AFM cantilever with high stiffness. A commercial AFM cantilever (NCHAuD) whose nominal stiffness is 40N/m is modified to have a magnetic moment on it. Here, the magnetic moment is a mean to excite the cantilever for dynamic mode AFM. Firstly, the effect of noise on the estimation of the oscillation amplitude using the lock-in amplifier is evaluated when the new laser measurement system is used. Secondly, the protein structures on the purple membranes are measured using the stiff-cantilever along with the new laser measurement system. The evaluation of the noise effect on the amplitude oscillation estimation is described in Section 3.5.1. The successful imaging of the purple membrane with the new laser measurement system is described in Section 3.5.2.

3.5.1 Noise level of the oscillation amplitude estimation with the new laser measurement system

In the oscillation amplitude estimation scheme used in a lock-in amplifier, a low pass filter (cutoff frequency: \( \omega_c \)) is designed to extract from modulated signals the oscillation amplitude and the phase, and has a band-pass filtering effect of the noise. The center frequency of the band pass filter is the operation frequency of the cantilever and the bandwidth is double of the cutoff frequency (\( 2\omega_c \)) of the low pass filter. This is described in greater detail in Appendix A. In order to evaluate the effect of the noise on the estimation of the oscillation amplitude of the cantilever using the lock-in amplifier,
the noise density of the free deflection of the cantilever in liquid was measured with the new laser measurement system and was band-pass filtered in order to calculate its standard deviation. The center frequency and the bandwidth of the band pass filter were the resonance frequency of the cantilever and 2 kHz since the low pass filter in the lock-in amplifier has the cut-off frequency of 1 kHz. Figure 3.15 shows the noise density of the deflection measurement of the cantilever in water and the standard deviation of the band-pass filtered signal. As shown in Fig 3.15 (b), the standard deviation of the oscillation amplitude estimation was estimated to be very small as 7.6125 pm with the new laser measurement system.

Fig. 3.15: Noise density of a cantilever with an attached magnetic particle in liquid and the accumulated standard deviation of the band pass filtered signal up to 1 MHz bandwidth. (a) Noise density of the cantilever (NCHAuD) in liquid around its resonance frequency. (b) The effect of noise at the estimation of the oscillation amplitude using a lock-in amplifier.
3.5.2 High resolution imaging of the purple membrane in dynamic mode AFM

Purple membrane patches deposited on freshly cleaved mica surface was measured using the amplitude modulation mode in a commercial AFM (MFP3D, Asylum research) along with the new low noise laser measurement system. Purple membrane patches were deposited on a fleshly cleaved mica surface in an absorption buffer (350 mM KCL/Tris-pH 8.0) for 10 minutes and were rinsed gently three times with an image buffer (150 mM KCL/Tris-pH 8.0). The AFM cantilever (NCHAuD, 40 N/m) with the magnetic moment attachment was excited around its resonance frequency using a solenoid coil located under the sample plate. The free oscillation amplitude of the cantilever was very small (0.22 nm ~ 0.26 nm) and the scanning frequency was 3 lines/sec. Figure 3.16 shows the measured images of the surface of purple membrane patches. The repeating structures of the protein whose lateral pitch is about 6.2 nm and vertical variation is several Angstrom are clearly visible in the images. This repeating structure on the purple membranes is composed of the proteins, which are very soft and small. Therefore, it requires both high spatial resolution and high force sensitivity for imaging. The success at imaging these protein structures by using an AFM cantilever with high stiffness proves that the low noise laser deflection measurement system allows the usage of high bandwidth cantilevers without degradation of the force sensitivity, which is very important for biological applications.
Fig. 3.16: High resolution imaging of purple membrane patches with a stiff commercial cantilever (40 N/m) using the new laser measurement system in dynamic mode AFM. A commercial AFM cantilever (NCHAuD, 40 N/m) was modified to carry a magnetic particle which was used for excitation and the images were acquired by the scanning in the amplitude modulation mode of commercial AFM (MPF3D, Asylum research) along with the new laser measurement system. (a) The image when the free oscillation amplitude was 0.22 nm (b) The image when the free oscillation amplitude was 0.26 nm. The reduction of the oscillation amplitude during the scanning of both patches was 20–30 pm.
3.6 Conclusions

In order to measure the deflection of the cantilever with an improved noise level, a new laser measurement system was implemented and integrated into a commercial AFM (MFP3D, Asylum research). The diameter of the new focused laser on the cantilever was measured to be 28.29 um. This is smaller than the width of the typical commercial AFM cantilevers, minimizing the loss of optical power. The overall noise level was evaluated of using the standard deviation of the measured free deflection of a commercial AFM cantilever (NCHAuD, nominal stiffness: 40 N/m). The new laser measurement system reduces the standard deviation of the deflection signal by more than 75% relative to the original laser measurement system in MFP3D (256.16 pm). The standard deviation of the measured free deflection of the cantilever (NCHAuD) within 1 MHz bandwidth was 59.48 pm when 9V reverse bias voltage was applied to the photodiode. The new laser measurement system showed very low noise density of 24 fm/\sqrt{Hz} around the resonance frequency of the cantilever employed. The effect of the noise on the estimation of the oscillation amplitude of a commercial cantilever (NCHAuD) measured using a lock-in amplifier was estimated to be very small (7.625 pm) when the new laser measurement system was used. Using the new high sensitivity laser measurement system, the repeating structures of the proteins on the purple membrane patches were successfully measured using a commercial cantilever with high stiffness (40N/m) in the amplitude modulation mode of the MFP3D. This showed that the
important of low noise measurement system at achieving high bandwidth – high resolution imaging of AFM.

The reduction of the noise level using the new laser measurement system is important not only for the operation mode which allows limiting the bandwidth of the measured signal like the amplitude modulation, but also for the operation mode which requires large bandwidth of the measured signal. The low noise deflection measurement system is one of the key components which allow both the high speed and high resolution imaging of the AFM.
CHAPTER 4

DESIGN, FABRICATION AND CONTROL OF ACTIVE MULTI-AXIS PROBES FOR HIGH SPEED ATOMIC FORCE MICROSCOPY

4.1 Introduction

Since its invention more than 20 years ago, the application areas of atomic force microscopy (AFM) have extended from material science to biology or biophysics, based on its capability to image/manipulate objects in various environments with sub-nanometer spatial resolution in three dimensions [50-52]. AFM has become a very useful microscopic instrument and/or manipulator of biological objects, such as proteins on membranes, in their physiological conditions. The native shape of fine protein structures on lipid bi-layer membranes has been successfully observed without any preprocessing steps which add artificiality to the samples [14, 53]. But its slow scanning speed, which is in the range of ~ several minutes per frame, has prevented expansion of its applications to imaging of dynamic processes. The low bandwidth of the actuators of AFM, such as the z-positioner and the raster scanner, is one of the important barriers which limit the scanning speed [36, 45]. In a typical conventional AFM, the bandwidths of piezo-based z-positioner and raster scanner are several kHz and several hundred Hz respectively.

In addition, for the implementation of direct tip-sample interaction force control
algorithm described in Chapter 2, high bandwidth z-positioner whose bandwidth is comparable to that of cantilever is required.

There has been a constant effort to overcome the bandwidth limitation of the actuators in AFM and several successful improvements of the scanning speed have been reported. Ando et al. developed high bandwidth actuators by using twin piezo-actuators facing each other. This design suppresses the lightly damped and complicated dynamics of the piezo actuator around its resonance frequency and thus, extends the useful actuation bandwidth of the piezo actuator [40]. Hansma et al. developed a high bandwidth 3 axes scanner by integrating high stiffness structures with piezo-actuators [41]. Miles et al. implemented single-axis high speed raster scanning by using a flexure-stage integrated with push-pull piezos [42]. These high bandwidth scanners require, however, a fairly high-cost manufacturing process and/or complete replacement of the conventional scanner with the new one. Humphris et al. employed economic quartz tuning forks as the high speed raster scanner by oscillating it at its resonance frequency [46]. However, the scanning frequency gets fixed by the resonance frequency of the tuning fork thereby introducing unnecessary constraints on the scanning operation. Recently, a new concept to modify commercially available passive AFM probes into two-axis probes was reported, in which the geometry of the cantilever was modified to have greater compliance along the desired axes and magnetic actuation was employed to apply torques about the actuation axes [47]. Even though the actuation bandwidth employed was low, it demonstrated the possibility of simultaneous multi-axis actuation of a single probe.
In this chapter, the design, fabrication and control of a high bandwidth active multi-axis probing system for high speed AFM are presented. The active multi-axis probing system employs a multi-axis compliant manipulator [47] that is optimally designed in terms of its geometry for high bandwidth actuation. It is actuated by two magnetic actuators to control the tip position along the vertical direction and the lateral direction simultaneously for realizing high speed tip-to-sample distance control and high speed raster scanning respectively.

This magnetic actuator overcomes the bandwidth limitations of the conventional z-scanner and does not introduce undesirable under-damped dynamics [36]. Moreover, since the magnetic force is applied directly at the location where the motion being controlled, it is much easier and reliable to use a model cancellation method to compensate the dynamics of the cantilever and to enlarge the bandwidth of the tip-motion control loop.

For the high bandwidth tip-motion control along the vertical direction, which enables the entire cantilever bandwidth available for tip-motion control, a model cancellation method is employed for enhancing bandwidth of the actuation-coil and replacing the under-damped cantilever dynamics with an over-damped one. In addition, a dual-mode actuation scheme is employed for high bandwidth tip-motion control with the large travel range, in which collocated magnetic actuation of AFM cantilevers serves for high bandwidth actuation, whereas a conventional piezo z-positioner provides necessary actuation range [45].

For the high bandwidth magnetic actuation of the tip along the lateral direction, the bandwidth of the magnetic actuator is also enhanced using a model cancellation
method. In this way, simultaneous two axes high bandwidth tip-motion control is achieved.

Since the solenoid coils for the multi-axis high bandwidth magnetic actuation are integrated in a small space, the heat generated from the magnetic coils is not negligible. In order to remove the heat generated from the solenoid coils, a water circulation cooling system is designed and integrated with the multi-axis solenoid coils.

A fast programmable electronics board (Field Programmable Gate Array: FPGA) was employed to implement the model cancellation algorithms and dual-mode actuation scheme. Since the implemented control system is programmable, all the control algorithms in FPGA can be easily modified according to the parameters of the selected cantilever and those of the designed magnetic actuators. The details of the FPGA-based digital controller are described in Chapter 5.

This chapter is organized as follows: The design of the active multi-axis probe based on static and dynamic analyses is described in Section 4.2. The fabrication of the designed active multi-axis probe and its evaluation are described in Section 4.3. High bandwidth multi-axis magnetic actuator is described in Section 4.4. The evaluation of the fabricated multi-axis probe using the multi-axis magnetic actuator is described in Section 4.5. The details of the high bandwidth magnetic actuation of the tip along the Z axis are described in Section 4.6. The conclusion and remarks are added in Section 4.7.

4.2 Design of the multi-axis probe for the high speed AFM

The configuration of an active multi-axis probing system for high speed AFM is shown in Fig. 4.1. The active multi-axis probe consists of a “Head” which includes the tip of the probe, a magnetic moment attached to it and the reflective surface for the laser
measurement, a “Neck” which is comprised mainly of three thin beams: one aligned along the Y axis and two aligned along X axis, and “Body” which is attached to the base. The neck is designed to have multiple thin beams aligned along two different axes in order to separately design the angular stiffness of the probe about each axis. This is discussed in Section 4.2.1. The magnetic moment of the particle attached to the head is aligned along the Y axis. Two sets of solenoid coils, aligned along the X axis and Z axis, are used for simultaneous control of the tip along the X axis and the Z axis respectively, by means of multi-axis torsional magnetic actuation of the magnetic moment. Whereas X-actuation enables scanning a sample, Z-actuation enables control of tip-sample interaction.

Fig. 4.1: The configuration of an active multi-axis probing system for high speed AFM.
High speed AFM imaging demands high speed control of tip position along the Z- and the X axes. In a typical dynamic mode operation, the tapping frequency of the probe is chosen to be near its resonance frequency. In the limiting case that achieves maximum imaging rate, every tapping point can be used for the image pixel point. Therefore, the resonance frequency of the probe along the Z axis limits the maximum imaging rate. One can calculate the required minimum resonance frequency of the probe along the Z axis from the desired imaging (pixel) rate. If the pixel resolution is \( n_r \times n_s \) pixels where \( n_r \) and \( n_s \) denote the pixel number along the raster scanning direction and the slow scanning direction respectively, and the desired frame rate is \( f \), then the imaging pixel rate, which sets the required minimum resonance frequency of the probe, is calculated as \( n_r \times n_s \times f \). The minimum bandwidth of the probe along the X axis can be calculated from the raster scanning frequency, which is related to the pixel number along the slow scanning direction and the desired frame rate and is equal to \( n_s \times f \). As an example, if the desired frame rate is 10 frames/sec and the pixel resolution is 100×100 pixels, the required minimum bandwidths of actuation along the Z axis and X axis are calculated to be ~100 kHz and ~1 kHz respectively.

On the other hand, the scanning size along the raster scanning direction is inversely proportional to the stiffness of the probe along the X axis. Therefore, as long as the bandwidth requirement is met, it is good to select a low stiffness for the probe along the X axis in order to realize large scanning range. In addition, in order to minimize tip motion along other axes due to structural coupling or/and actuation coupling, it is desirable to design the stiffness of the probe along the other axes to be high. Thus, with
the configuration of the actuation shown in Fig. 4.1, the stiffness of the probe about the Y axis needs to be designed to be high so as to minimize torsional motion of the probe.

4.2.1 Static Analysis of a Multi-axis Probe

Since the neck, which is composed of three thin beams, has the lowest stiffness among the three parts, viz. head, neck and body along all axes, only the effect of the neck is considered for the static analysis of the multi-axis probe. Thus, a simplified model of the multi-axis probe is employed as shown in Fig. 4.2, in which the body and the head are modeled as rectangular rigid bodies and the neck is modeled as a structure composed of three thin compliant beams. The width, length and the thickness of the head are defined as $w_H$, $\ell_H$ and $t_H$, respectively. A tip whose height is $\ell_T$ is rigidly attached at the free end of the head. The neck has three thin beams. The one which is aligned along the Y axis has width $w_n$, length $\ell_n$ and thickness $t_n$ and the two which are aligned along the X axis have width $w_w$, length $\ell_w$ and thickness $t_w$. For the simplicity, it is assumed that the two beams of the neck along the X axis start from the location $X=0$ and that the contribution of the structures connecting these beams to the head to the overall compliance is negligible.
The angular deformation of the probe about each axis $[\theta_x \ \theta_y \ \theta_z]^T$ caused by the input torque $[\tau_x \ \tau_y \ \tau_z]^T$ is decided by the angular stiffness along each axis:

$$\begin{bmatrix} \theta_x \\ \theta_y \\ \theta_z \end{bmatrix} = \begin{bmatrix} 1/k_{xx} & 0 & 0 \\ 0 & 1/k_{yy} & 0 \\ 0 & 0 & 1/k_{zz} \end{bmatrix} \begin{bmatrix} \tau_x \\ \tau_y \\ \tau_z \end{bmatrix}, \quad (4.1)$$

where $k_{xx}$, $k_{yy}$, and $k_{zz}$ are angular stiffness about the X, Y, and Z axis respectively. The lumped angular stiffness of the neck in the simplified model about each axis can be calculated by superposition of the angular stiffness of the three thin beams. Their expressions are given below:

$$k_{xx} = \frac{E}{12} \frac{w_n t_n^3}{\ell_n} + \frac{2G}{3} \frac{t_n w_w^3}{\ell_w}, (w_w << t_w) \quad (4.2)$$

$$k_{yy} = \frac{G}{3} \frac{t_n w_n^3}{\ell_n} + \frac{E}{6} \frac{w_n t_n^3}{\ell_w} \quad (4.3)$$

$$k_{zz} = \frac{E}{12} \frac{t_n w_n^3}{\ell_n} + \frac{E}{6} \frac{t_w w_n^3}{\ell_w}. \quad (4.4)$$
It is worthy of noting that the contribution of the two beams along the X axis to the angular stiffness $k_{xx}$ and $k_{zz}$ is small if the width $w_\omega$ is designed to be very small when compared to its thickness $t_\omega$ and length $\ell_\omega$. By doing this, it is possible to make each angular stiffness have one dominating parameter, which are $t_n, t_w$ and $w_n$ for $k_{xx}, k_{yy}$ and $k_{zz}$ respectively. Thus, independent choice of the dominant parameters allows each angular stiffness term to be designed independently.

If the angular deformation of the neck about each axis is small, from simple kinematic relationship, the tip position changes along the Z axis $z_t$ and along the X axis $x_t$ due to orientation changes $[\theta_x \theta_y \theta_z]^T$ are equal to $(\ell_n + \ell_H) \cdot \theta_x$ and $-\ell_T \cdot \theta_y - (\ell_n + \ell_H) \cdot \theta_z$ respectively. Combining this with (4.1), the tip position change along the X and Z axis caused by the input torques is given by:

$$z_t = (\ell_n + \ell_H) / k_{xx} \cdot \tau_x \quad (4.5)$$

$$x_t = -\ell_T / k_{yy} \cdot \tau_y - (\ell_n + \ell_H) / k_{zz} \cdot \tau_z \cdot (4.6)$$

Since the head is modeled as a rigid body, the kinematic relationship between the equivalent force at the end of tip $\vec{F}_z$ along the Z and the torque $\tau_x$ is given by

$$\vec{F}_z = \tau_x / (\ell_n + \ell_H).$$

Similarly, the equivalent force $\vec{F}_{x-\gamma}$ along the X axis caused by $\tau_y$ and the force $\vec{F}_{x-z}$ along the X axis caused by $\tau_z$ are given by $\vec{F}_{x-\gamma} = -\tau_y / \ell_T$ and $\vec{F}_{x-z} = -\tau_z / (\ell_n + \ell_H)$ respectively.

From these equivalent forces acting at the end of the tip and from (4.5) and (4.6), one can calculate the equivalent linear stiffness as:
\[ k_z = k_{zz} \left( \ell_n + \ell_H \right)^2 \]  \hspace{1cm} (4.7) \\
\[ k_{x<z} = k_{xy} \ell_x^2 \] \hspace{1cm} (4.8) \\
\[ k_{x<z} = k_{xz} \left( \ell_n + \ell_H \right)^2 \] \hspace{1cm} (4.9)

where \( k_z \) denotes the equivalent linear stiffness of the probe along the Z axis, \( k_{x<z} \) and \( k_{x<z} \) denote the equivalent linear stiffness of the probe along the X axis caused by the angular stiffness about the Y axis and Z axis respectively.

In order to estimate the static deflection of the probe along the actuation axis for a given magnetic field, the magnetic moment is modeled as a spherical magnetic particle whose magnetization is \( M \) and radius is \( R_m \). Then, the magnetic moment is calculated as \( 4/3 \cdot \pi R_m^3 M \) \cite{45}. If the magnetic moment is aligned along the Y axis and rigidly attached to the head of the probe, in the presence of magnetic field \( B_x \) along the X axis and \( B_z \) along the Z axis, it experiences torques \( \tau_z \) about the Z axis and \( \tau_x \) about the X axis given by: \( \tau_z = -4/3 \cdot \pi R_m^3 MB_z \) and \( \tau_x = 4/3 \cdot \pi R_m^3 MB_x \) \cite{45}. In addition, the torque about the Y axis does not exist \( (\tau_y = 0) \). Therefore, from (4.5) and (4.6), the static deflections of the probe along the Z axis and the X axis caused by the applied magnetic field \( B_z \) and \( B_x \) respectively are given by

\[ z_i = \frac{4\pi R_m^3 M \cdot (\ell_n + \ell_H)}{3k_{xx}} B_z \] \hspace{1cm} (4.10) \\
\[ x_i = \frac{-4\pi R_m^3 M \cdot (\ell_n + \ell_H)}{3k_{zz}} B_x \] \hspace{1cm} (4.11)

Equation (4.11) enables direct estimation of the scanning range of the multi-axis
probe and also provides guidelines for the design of the magnetic actuator which generates the X-magnetic field, in order to achieve the desired scanning range.

### 4.2.2 Dynamic Analysis of the Multi-axis Probe

In order to design and evaluate the bandwidth of the multi-axis probe, a modal analysis of the multi-axis probe using the finite element method (FEM, Ansys 7.0) was performed.

A commercial AFM probe (NCHAuD, Nanosensors), whose nominal thickness and length are 4 μm and 130 μm respectively, was chosen to design an active multi-axis probe whose actuation bandwidth meets the requirement to achieve imaging speed of 10 frames/sec with the pixel resolution of 100×100 pixels. The geometries of the head and the neck of a multi-axis probe are designed through the static analysis such that \( k_{xx} \) and \( k_{yy} \) have large values in order to achieve high actuation bandwidth along the Z axis and minimize structure coupling respectively. In addition, \( k_{zz} \) is designed to be small to obtain large scanning range. The detailed dimensions of the head and neck and the estimated static parameters are summarized in Table I. In order to estimate the scanning range of the designed probe from (4.11), a magnetic particle whose radius is 14 μm is attached on the head with a longitudinal offset of 18um from the end of the neck and a vertical offset of 10 μm above the surface of the head. It is possible to locate the particle with a vertical offset smaller than its radius by making a hole on the head, which is described in Section 4.3. Based on the residual induction of the magnetic particle to be used, the magnetization \( M \) is assumed to be \( 1/\mu_0 \), where \( \mu_0 \) denotes the permeability of free
space. The kinematic compliance $C_{xx}$ along the X axis, which is calculated from (4.11) as $|x_r/B_x|$, is estimated to be $18.924 \times 10^4$ [nm/T]. This gives the scanning range along the X axis of 378.4 nm when a magnetic field of $20 \times 10^{-4}$ T is applied.

Fig. 4.3 shows first three modes of the designed multi-axis probe obtained from modal analysis using FEM. In this analysis, the geometry of the neck is same as that given in Table I. The geometry of the head is carefully designed to reflect the actual geometry of a commercial AFM probe, but with reduced thickness and width, whereas retaining the length ($\ell_H$) given in Table I. As the result of modal analysis, the resonance frequencies of the first three modes were identified to be at 44.5 kHz, 107.87 kHz and 359.49 kHz respectively. The first mode and the second mode shapes of the multi-axis probe represent the raster scanning of the tip and the high bandwidth tip-to-sample distance control respectively. It is seen that the identified frequencies meet the design requirement.

<table>
<thead>
<tr>
<th>Angular stiffness</th>
<th>Equivalent stiffness</th>
<th>Kinematic compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[nNm/rad]$</td>
<td>$[N/m]$</td>
<td>$\times 10^4$ [nm/T]</td>
</tr>
<tr>
<td>$k_{xx}$</td>
<td>$\tilde{k}_z$</td>
<td>$C_{zz}$</td>
</tr>
<tr>
<td>83.44</td>
<td>19.44</td>
<td>0.503</td>
</tr>
<tr>
<td>$k_{yy}$</td>
<td>$\tilde{k}_{xe-y}$</td>
<td>298.58</td>
</tr>
<tr>
<td>58.52</td>
<td>0.50</td>
<td>$C_{xx}$</td>
</tr>
<tr>
<td>$k_{zz}$</td>
<td></td>
<td>18.924</td>
</tr>
<tr>
<td>2.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Head and tip: $\ell_H = 60 \mu m$, $\ell_T = 14 \mu m$
*Magnetic particle: $R_m = 14 \mu m$
*Neck: $w_n = 0.5 \mu m$, $t_n = 4 \mu m$, $\ell_n = 5.5 \mu m$, $w_w = 0.5 \mu m$, $t_w = 4 \mu m$, $\ell_w = 16 \mu m$
*Material Property: $E = 169 \times 10^9 N/m^2$

Where $C_{xx} = |x_r/B_x|$ and $C_{zz} = |z_r/B_z|$.
Fig. 4.3: Modal analysis of a multi-axis probe using a finite element method. The first three modes were evaluated: (a) the first mode shape, (b) the second mode shape and (c) the third mode shape.

4.3. Fabrication and implementation of the active multi-axis probes

Fabrication of the active multi-axis probe consists of machining of the geometry of a conventional AFM probe by using a focused ion beam (FIB) milling, the attachment of a magnetic particle on the head, and magnetization of the magnetic particle along the designed direction.

Figure 4.4 shows the procedure of the fabrication of the designed multi-axis probe. At the first stage of FIB milling (Helios Nanolab 600, FEI COMPANY) of the cantilever (Fig. 4.4 (b)), the head is machined to reduce its thickness from 4 um to 1.5um
and a hole of diameter 20um is created on it. This hole is the area where a magnetic particle (MQP-S-11-9, Magnequench) will be placed and glued to the head. The hole allows accurate placement of the particle at the designed location and also improves the quality of attachment of the particle to the head by increasing the contact area between the magnetic particle and the epoxy. A water- and chemical-resistant epoxy (801055 BA, Emerson & Cuming) is injected around the hole through a micro-syringe pump or dropped by a micro-pipette which was dipped into the epoxy beforehand (Fig. 4.4 (c)). Subsequently (Fig. 4.4 (d)), a micro-pipette whose end is gently coated with oil is used to pick up a particle from a freshly cleaved mica surface and the particle location is precisely controlled manually by using 3-dimentional micro stages under the optical microscope, as it approaches the hole. As soon as the particle contacts the epoxy, it is detached from the micro-pipette because the adhesive force of the epoxy is much stronger than that of the oil. After the epoxy is completely cured, the magnetic particle is magnetized within an electromagnet (3470, GMW Associates) along the Y-Z plane with a tilt angle of 10~15 degree about X axis (Fig. 4.4 (e)). This tilt angle is added to compensate for the misalignment of the magnetic moment caused by the engagement angle of the probe on the sample surface. At the stage of the second FIB milling (Fig. 4.4 (f)), the neck of the probe is carefully machined using low powered ion beam. The neck is machined from the bottom side of the probe and at an angle -10 ~ -15 degree about the X axis in order to compensate for the probe engagement angle on the sample surface and thus align the beam structure along the actuation coordinates. During FIB milling, exposure of the tip to the FIB is carefully avoided in order to retain the sharpness of the tip.
Fig. 4.4: Fabrication of the multi-axis probe by using focused ion beam milling and subsequent particle attachment: (a) A bare cantilever, (b) FIB milling for reduction of the head thickness, and thus its mass, and creation of a hole for later particle attachment, (c) deposition of epoxy around the hole, (d) particle attachment, (e) magnetization of the particle and (f) FIB milling of the neck.
Fig. 4.5: A fabricated multi-axis probe for high-speed AFM: (a) bottom view (SEM image), (b) side view of the tilted probe (SEM image). The dotted line through the particle represents the estimated direction of the magnetic moment from the distorted image around the particle, (c) top view (SEM image), and (d) neck viewed along the machining axis (FIB image). The scale bars in (a), (b) and (c) represent 30 $\mu$m whereas the scale bar in (d) represents 5 $\mu$m.
Fig. 4.5 shows the SEM (S-4300, HITACHI) and FIB images of a fabricated active multi-axis probe. From the bottom view (Fig. 4.5 (a)) acquired using the SEM and especially with the FIB image (Fig. 4.5 (d)) acquired by the reflected focused ion beam along the FIB milling axis, the direct inspection of the machined area is possible during and/or after the FIB milling. In addition, from the SEM bottom view (Fig. 4.5 (a)), the tip condition and the quality of the particle attachment can be evaluated. When the particle is well placed on the epoxy, the epoxy forms a ring shape around the boundary of the hole. The side view SEM image (Fig. 4.5 (b)) shows image distortion around the magnetic particle due to the magnetic field generated by the particle. This distorted image contains information about the direction of the magnetic field and thus, is useful to evaluate the direction of the magnetic moment of the particle. The top view image (Fig. 4.5 (c)) allows the evaluation of the particle location on the head and the quality of the back surface of the cantilever where the laser will be reflected for the measurement of the deflection of the probe.

4.4 High bandwidth multi-axis magnetic actuation

In this section, the design and implementation of high bandwidth multi-axis magnetic actuator for the multi-axis probe is described.

Instead of using piezoelectric actuator to position the AFM tip from the base, direct control of the z-position of the tip can be achieved by means of magnetic actuation, e.g. by attaching a magnetic moment to the end of the cantilever, the tip-position can be controlled by means of an externally applied magnetic field. In the presence of a uniform magnetic field $B$ (gradient = zero), a magnetic moment $m$ experiences magnetic torque
\( \tau \) which is given by \( \tau = m \times B \). As an example, if the magnetic moment is aligned along the Y axis in the cantilever coordinate system as shown in Fig. 4.6 (a) and the magnetic field is aligned along the Z axis, the magnetic moment experiences magnetic torque about the X axis, which causes the tip of the cantilever moves along the Z axis. On the other hand, if the magnetic field is aligned along the X axis while the magnetic moment retains its alignment along the Y axis as shown in Fig. 4.6 (b), the magnetic torque is generated about the Z axis and this causes the tip moves along the X axis. If the deflections of the probe are small, the change of the magnetic moment alignment is negligible and thus independent two-axis magnetic actuation by using two sets of magnetic coils are possible. Fig. 4.6 (c) shows the configuration of a two-axis magnetic actuation of the tip along the Z axis and the X axis by using two sets of magnetic coils. High bandwidth tip-motion controls along the Z axis and the X will be used for the high bandwidth tip-sample distance control and the high speed raster scanning respectively.

![Fig. 4.6: Multi-axis magnetic actuation for high bandwidth tip-sample distance control and the high speed raster scanning.](image)
Fig. 4.7: Configuration of the multi-axis magnetic actuator: Two sets of the solenoid coils are integrated with a water circulation cooling system. In this design, the optical path through the coil along the Z axis is allowed for the use of an inverted microscope (Olympus IX81) integrated with the commercial AFM (MPF3D, Asylum research).

Since the magnetic coils need to be integrated in a small space near the sample plate and the multi-axis probe, the heat generated from the magnetic coils are not negligible. If the temperature difference between the magnetic coils and the sample plate is large, it causes thermal gradient around the multi-axis probe and thus significant thermal deflection of the multi-axis probe. For samples sensitive to the temperature of the environment such as live biological samples, this generated heat can degrade their quality. Therefore, in the design of the multi-axis high bandwidth magnetic actuation, not only high bandwidth actuation of each axis but also efficient cooling of the solenoid coil is considered. A water-circulation thermal cooling method was employed for removal of the heat from the solenoid coils. In addition, for simultaneous usage of the inverted microscope (IX81, Olympus) integrated with the AFM (MFP3D, Asylum Research), an optical path is included in the magnetic actuator. Fig. 4.7 shows the schematic of the designed multi-axis magnetic actuator considering these requirements.
Fig. 4.8: Implementation of the high bandwidth multi-axis magnetic actuator with water-circulation cooling system: (a) Implemented multi-axis magnetic actuator installed a commercial AFM (MPF3D, Asylum research). The rectangular dots and the circular dot represent the locations of the X coils and the Z coil inside of the package respectively. The shape of a glass plate is modified to place it between the two X solenoid coils. (b) The configuration of the water-circulation cooling system.
Fig. 4.8 (a) shows the implemented multi-axis magnetic actuator installed on a commercial AFM (MPF3D, Asylum research). The cooling system circulates water around the solenoid coils using a peristaltic pump (913, HITYFLEX) and exchanges heat with the surroundings through a copper tube as shown in Fig. 4.8 (b). In order to minimize the effect of vibration, two home-made pressure dampers were also included in the circuit.

Fig. 4.9: The enlarged bandwidths of the magnetic actuators for Z axis and X axis: (a) the enlarged bandwidth of the solenoid coil dynamics along the Z axis, (b) the enlarged bandwidth of the solenoid coil dynamics along the X axis. The identified coil dynamics are replaced with ones with higher bandwidth (800kHz and 70kHz for the Z axis coil and for the X axis coil respectively).
The bandwidths of the solenoid coil are enhanced by using the model cancellation method described in Section 4.6.3 such that the replaced bandwidths of the solenoid coil dynamics for the Z axis and the X axis are 800 kHz and 70kHz respectively as shown in Fig. 4.9. The model cancellation algorithms for the multi-axis high bandwidth magnetic actuator are implemented in a high speed digital controller based on field programmable gate array (FPGA, stratiX-II, 100 MHz, Altera), whose closed loop update rate is 20Mhz. The details of this FPGA-based digital controller with 20Mhz update rate are described in Chapter 5. A high bandwidth power amplifier (PA119, 900 V/us, 4A, Apex) was employed to amplify the signal output from the digital controller and drive the solenoid coils in the multi-axis magnetic actuator.

4.5 Evaluation of the fabricated multi-axis probe

In this section, the fabricated active multi-axis probing system is evaluated experimentally. The deflections of the probe along the actuation axes are measured using a home-made low noise laser measurement system described in Chapter 3 and the measured signals are calibrated using quasi-static methods. The bandwidths of the multi-axis probe along the actuation axis are evaluated from the frequency responses of the probe. The resonance frequency of the probe along the non-actuation axis is identified using the noise density of the thermal fluctuation of the probe.

4.5.1 Static calibration of the fabricated probe

In order to measure the deflections of the multi-axis probe, a home-made low noise laser measurement system is employed which measures the angular deflections of the probe about the X axis and Y axis. In a conventional AFM, the former signal allows
the measurement of the translation of the probe-tip along the Z axis, and the latter so called “lateral signal” measures the twist of the probe about the Y axis. Strictly speaking, in the presence of small misalignment about the Y-axis, the lateral signal also measures the lateral translation of the probe. However, in a conventional probe, this translation is negligible, owing to its high stiffness along this axis. In the case of a multi-axis probe, on the other hand, where the lateral stiffness is designed to be very small when compared to the torsional stiffness about the Y axis, the deflection of the probe along the X axis can also contribute significantly to the lateral signal of the laser measurement.

Calibration of the laser measurement of the probe deflection along the Z axis is achieved by gently pushing the cantilever from the base using a calibrated z-positioner when the tip is touching a hard surface like mica and measuring the deflection of the probe along the Z. Using this method, the sensitivity of the laser measurement for the probe deflection along the Z axis is calibrated to be 57.50 mV/nm. For calibration of the lateral deflection of the probe along the X axis, an image processing method which estimates the translational motion of a registered image with sub-pixel resolution is employed. Transmitted light images of the fabricated probe were measured with a digital camera (C4742-95, Hamamatsu) through the objective lens of an inverted microscope (Olympus 81X) whose magnification is 10X. When the magnetic field along the X axis was applied as a sinusoidal wave form of frequency 0.5 Hz, the image of the probe was captured by the digital camera at the speed of ~ 10 frames/sec. The translational motion of the registered image (A small box in Fig. 4.10 (a)) around the tip position is estimated by comparing the sequence of captured images with the original image.
Fig. 4.10: Calibration of the lateral deflection using image processing method: (a) A transmitted-light image of the fabricated probe obtained from an inverted microscope with 10x magnification. The dotted rectangle represents the image registration for image processing. (b) A transmitted-light image of the probe when the magnetic field along the X axis is oscillated at the lateral resonance frequency of the probe. The blurred image of the head is due to the large lateral deflection of the probe. (c) The lateral deflection estimated from image processing.

Fig. 4.10 (c) shows the result of the lateral deflection motion estimated with the image processing method. By comparing this estimated lateral deflection from image processing method with the simultaneously measured lateral signal of the laser measurement, the sensitivity of the laser measurement for the probe deflection along the X axis is calibrated to be 0.79 mV/nm. The low value of this sensitivity is due to the alignment of the X-translational motion of the probe nearly normal to the laser path. Thus, open loop control of the lateral tip position is used for the raster scanning.
4.5.2 Evaluation of the dynamics of the multi-axis probe

In order to evaluate the resonant frequencies of the fabricated multi-axis probe, the frequency response of the probe along each axis was obtained by using a lock-in amplifier. For the evaluation of the bandwidth of the probe along the Z axis, Z-deflection of the laser measurement was recorded while the frequency of the actuation along the Z axis was swept between 20 kHz and 140 kHz. To estimate the bandwidth of the probe for lateral deflections along the X axis, the lateral signal of the laser measurement was analyzed while the magnetic field along the X axis was swept in the frequency range from 1 kHz to 90 kHz. Fig. 4.11 (a) shows the frequency responses of the probe along two different actuation axes. The resonance frequencies of the probe along the X axis and the Z axis are identified as 46.40 kHz and 101.5 kHz respectively, which are very close to the values estimated (44.5 kHz and 107.87 kHz) using modal analysis. Fig. 4.10 (b) shows the image of the probe when the lateral deflection of the probe was excited at its resonance frequency (46.4 kHz). The blurring of the image, caused by the large resonant response of the probe, serves to confirm that this mode corresponds to lateral oscillation.

The expected third mode of the probe i.e. the angular motion of the probe about the Y axis cannot be excited using the magnetic actuation scheme employed here. Therefore, in order to evaluate the resonance frequency of the probe about the Y axis, the thermal fluctuation of the probe in the air about this axis is measured and its noise density is analyzed. The noise density shows a sharp peak at a frequency of 278.36 kHz (Fig. 4.11 (b)) which corresponds with the estimated resonance frequency (359.49 kHz) of the third mode obtained from the modal analysis.
Fig. 4.11: Dynamic responses of the fabricated multi-axis probe (experimental result): (a) the frequency responses of the probe along the actuation axes (i.e., the X axis and Z axis). (b) Noise density of the thermal fluctuation of the probe about the Y axis in air. The dimension of the Y axis is $m/\sqrt{Hz}$. The downward triangles denote the location of the resonance frequencies of the first three modes estimated by the modal analysis.
4.5.3 High bandwidth probe-motion control

Fig. 4.12: High bandwidth actuation of the multi-axis probe: (a) the configuration of the implemented model cancellation algorithms for high bandwidth actuation. The inverse probe dynamics represents the model cancellation algorithm which replaces the lightly damped probe dynamics with an over damped dynamics, whereas inverse coil dynamics is the one to enhance the bandwidth of the coil dynamics. The signal route with a sinusoidal wave bypasses the inverse probe dynamics, whereas the one with a step-wise wave goes through both the inverse probe dynamics and inverse coil dynamics. (b) Rapid step response of the probe along the Z axis. The probe was vibrated around its resonance frequency along the Z axis and the mean position of the probe was moved up by 20 nm within one cycle.

In order to make the entire Z axis bandwidth of the probe available for tip-motion (along the Z axis) control, a model cancellation algorithm, which replaces the lightly
damped probe dynamics with an over-damped one, is implemented in the FPGA as illustrated in Fig. 4.12 (a). The model cancellation algorithm enables the rapid position change of the probe without introducing unwanted transient dynamics. The details of the implementation of the model cancellation algorithm are described in Section 4.6.4. Fig. 4.12 (b) shows a rapid step response of the probe motion along the Z axis. In this experiment, the probe was vibrated around its resonance frequency using the magnetic actuator through a signal route that did not include the inverse probe dynamics. The same magnetic actuator was also used to change the mean position of the probe by 20 nm within one cycle, without introducing additional oscillatory motion. For this rapid mean position change of the probe, the signal route that included the inverse probe dynamics was used. This experimental result illustrates that the magnetic actuator, along with the model cancellation method, leads to a tip-motion control system, the bandwidth of which is comparable to that of the probe. If a dual-actuation control scheme [45] is employed, large travel range can be achieved by using a regular piezo scanner whereas magnetic actuation enables high bandwidth tip-motion control. The details of the dual-mode actuation scheme are described in Section 4.6.5.

4.5.4 Conclusion and remarks

In Sections 4.2~4.5, the design and fabrication of an active multi-axis probing system for high speed AFM is presented. The static deflection and the dynamic bandwidth of the fabricated probe were evaluated experimentally by using an image processing method with sub-pixel resolution and a laser measurement system. The fabricated probe has the required bandwidth to achieve image frame rate of 10 frames/sec.
with pixel resolution of 100×100 pixels. The design and fabrication process described in this chapter can be applied to fabricate active multi-axis probes of even higher bandwidth like 30 frames/sec by employing AFM cantilevers with larger thickness (ex. ~ 7um) and further reducing the mass at the head. The fabrication and evaluation of multi-axis probes for higher bandwidth (> 200 kHz) are added in Appendix B.

4.6 High bandwidth magnetic actuation of the tip along the Z axis

In this chapter, the details of the implementation of the high bandwidth magnetic actuation of the tip along the Z axis whose bandwidth is comparable to that of cantilever are described.

The magnetic actuator is designed for the maxima cantilever deflection and its bandwidth is enhanced by using a model cancellation method (Section 4.6.1). In order to make the whole bandwidth of the cantilever useful for tip-position control, a model cancellation method which replaces lightly damped cantilever dynamics with an over-damped one is implemented (Section 4.6.4). A dual-actuator motion control scheme is implemented, in which a magnetic mode actuator is introduced to work together with the regular z-scanner to achieve rapid tip positioning with a large working range (Section 4.6.5).

The high bandwidth magnetic actuation of the tip was applied to image purple membrane (Halobacterium) patches which have very fine repeating structures of proteins in aqueous solution to illustrate its ability to track high spatial-frequency surface topography when increasing imaging speed (Section 4.6.6).
4.6.1 Co-located Magnetic Actuation

As shown in Fig. 4.13, in the presence of a magnetic field $\mathbf{B} = B\hat{z}$, a magnetic moment $\mathbf{m} = m\hat{y}$, aligned along the length of the cantilever, experiences a torque given by

$$\tau = \mathbf{m} \times \mathbf{B} = mB\hat{x}. \quad (4.12)$$

If the moment is rigidly attached to a cantilever to avoid physical rotation of the moment due to the torque, it transmits the torque to the cantilever [36]. The equivalent $Z$-force, producing the same amount of tip-displacement along the $Z$-axis of a cantilever of length $\ell$ as the torque, is given by [63]

$$\mathbf{F}_{eq} = -\frac{3mB}{2\ell}\hat{z}. \quad (4.13)$$
A rectangular cantilever of width \( w \), length \( \ell \), thickness \( t \) and modulus of elasticity \( E \) has a \( Z \)-stiffness, \( k \), equal to \( Ewt^3 / 4\ell^3 \). The cantilever tip deflection due to the force, given by

\[
\Delta z = -\frac{6mB\ell^2}{Ewt^3},
\]

allows us to control the tip \( Z \)-position by means of an externally applied field \( B \). In this scheme, a magnetic particle is attached to the tip of the cantilever. A solenoid coil positioned below the cantilever applies magnetic field to the magnetic particle and deflects the cantilever in proportion to the field. This approach has three advantages. Firstly, the bandwidth of actuation is limited by the bandwidth of the coil. Thus, with careful design, the coil bandwidth can be made comparable to the fastest component of the \( z \)-motion control loop, namely the AFM cantilever. Secondly, the second-order dynamics of the usual piezoelectric \( z \)-scanner is replaced by the first-order dynamics of the coil, improving the gain margin and hence stability of the feedback system. Thirdly, compared to controlling the tip position from the base (the other end of the cantilever), the proposed approach achieves co-located control. Therefore, it is much easier and reliable to use a model cancellation method to compensate the dynamics of the cantilever and to enlarge the bandwidth of the tip-position control, the details of which are described in Section 4.6.4.

There are two objectives when designing the magnetic actuator, namely maximizing the deflection of the cantilever and extending the bandwidth of the actuator dynamics. The maximum deflection is proportional to the maximum electromagnetic force and directly related to the achievable range of tip position control with the magnetic
actuator. According to (4.14), the electromagnetic force is proportional the magnetic moment \( m \) which is mostly determined by the size of the particle, and magnetic field \( B \) which is generated by the coil. A magnetic particle whose magnetization is \( M \) has the magnetic moment expressed as

\[
m = \frac{4}{3} \pi r_m^3 M ,
\]

(4.15)

where \( r_m \) is the radius of the particle. The magnetization of the particle can be estimated from the residual induction of the magnetic particle employed and is assumed to be \( 1/\mu_0 \) for the magnetic particle selected (MQP-S-11-9, Magnequench), where \( \mu_0 \) is the magnetic permeability in free space.

4.6.2 Design of the magnetic actuator for Maxima Cantilever Deflection

Stronger magnetic field can be achieved using core material inside the magnetic coil, whose magnetic permeability is much larger than that of air. However, using magnetic core material limits bandwidth due to the effects of eddy current and/or hysteresis [14]. Therefore, no magnetic core material is employed in the design. The magnetic field, generated by a coil, at the location of the magnetic moment above the coil is given by

\[
B = G \frac{\mu_0 K I}{d} ,
\]

(4.16)

where \( d \) is the diameter of and \( K \) is the layer number of the wire, and \( G \) is a gain determined by the geometric design parameters, including the size and shape of the coil and the relative distance between the magnetic moment and the coil. The electric current,
$I$, passing through the coil is determined by the induced voltage and electrical impedance of the designed coil.

There are several constraints to achieving strong magnetic field in designing the coil. They include geometrical constraints for its installation on an AFM system and operational constraints of the electrical components. The maximum current passing through the coil needs to be limited, considering the operational current range of the power amplifier. Even though the heat generated from the solenoid coils can be removed by a water-circulation cooling system which is described in Section 4.4, it is still useful to minimize the current passing through the coil within the operational range of the power amplifier, in order to have large nominal operation range of the designed magnetic actuation and minimize the creation of temperature gradient around the sample due to the residual heat in the coil. By considering these constraints, one can design a coil, which maximizes the strength of the magnetic field, and thus the deflection of the cantilever using (4.14), (4.15) and (4.16). In the design of the magnetic coil as an example, the maximum current passing through and the maximum voltage induced in the coil were set as 0.5A and 30V respectively. Figure 4.14 shows the maximum deflection ($\Delta z$) of the cantilever as the design parameters (length of coil, diameter of coil, number of wire layer, total resistance) are varied within constraints. The maximum deflection is shown to be 141 nm when the length of coil, diameter of coil, number of wire layer and total resistance are 10.8 mm, 4.9 mm, 5 and 9.5 $\Omega$ respectively. The designed and implemented coil generates a magnetic field of 23.03 gauss at the location of the magnetic moment and achieves dynamic bandwidth around 5 kHz.
The bandwidth of this coil dynamics (input: voltage, output: current) can be enlarged by using model cancellation method described in Section 4.6.3. In this implementation, the solenoid coil for the tip position control along the Z axis was driven by a power amplifier circuit (OPA 549, Texas Instruments).

4.6.3 Bandwidth Enlargement of the magnetic actuator

The magnetic actuator along the Z axis serves two purposes: 1) providing sinusoidal excitation to the cantilever at near its resonance frequency for dynamic mode AFM, and 2) achieving rapid tip-motion control with a bandwidth comparable to that of the cantilever. For these two purposes, the bandwidth of the coil dynamics needs to be at least comparable to that of the cantilever. The resonance frequency of typical cantilevers and that of fabricated multi-axis probes for high speed AFM, however, are much higher.
than the dynamic bandwidth of the designed coil and lie between several tens kHz and several hundreds kHz [64, 65].

The coil dynamics (input: voltage, output: current) is expressed as the first order system, the bandwidth of which is determined by the time constant, \( \tau_c = L / R \), where \( L \) and \( R \) denote the inductance and resistance of the coil, respectively. In order to utilize the entire bandwidth of the cantilever to achieve rapid tip-motion control, the bandwidth of the magnetic actuation needs to be enlarged, improving from the designed coil bandwidth to at least the resonance frequency of the cantilever. A model based compensation scheme is adopted to realize this objective.

Based on the identified coil dynamics, a model cancellation method enables dynamic compensation, in which a manipulated voltage is generated to cancel the original coil dynamics and replace it with a desired first order dynamics, and thus enlarge the bandwidth of the magnetic actuator. The approach and implementation of model cancellation are illustrated in Fig. 4.15 (a). The transfer function of the compensator is \( C_{coil}(s) = \left\{ (L / R)s + 1 \right\} / (\tau_a s + 1) \), where \( \tau_a \) is determined according to the desired bandwidth. This compensator dynamically adjusts the voltage output of the power amplifier to cancel the original dynamics of the coil and replace it with the one characterized by \( G_a(s) = C_{coil}(s) \cdot G_{coil}(s) \approx 1 / (\tau_a s + 1) \). The enlarged bandwidth was experimentally verified. A comparison between the frequency response of the compensated system and that of the original coil is shown Fig. 4.15 (b), illustrating more than 10 times enlargement in this example.
The maximum bandwidth enlargement using the model cancellation method is limited by the operational voltage range of the power amplifier which drives required current in the coil at a given frequency. Therefore, at the design of the magnetic coil, this operational voltage of the amplifier is considered as one of constraints at the optimization of design parameters for the maximization of the cantilever deflection (Section 4.6.2). The bandwidth enlargement of the coil dynamics for the cantilever whose bandwidth is about hundreds kHz was described in Section 4.4. This model cancellation algorithm is implemented with a FPGA (Field Programmable Gate Array: Cyclone-II, Altera) board as shown in Fig. 4.16. A fast DAC (Digital to Analog Converter; DAC5672, Texas Instruments) is added to the FPGA and 2MHz DAC update rate, including the floating point calculation for the model cancellation algorithms, is achieved. A recent version using fixed point calculation (20MHz) is described in Chapter 5.
Fig. 4.16: FPGA board (Cyclone-II, Altera) and DA converter board (DAC5672, 14bit, Texas Instruments). Both are operated by a synchronized 100MHz clock source. In this version of the FPGA-based digital controller, the floating point calculation method was employed in order to implement model cancellation algorithms and its maximum closed-loop update was 2 MHz.

4.6.4 **High bandwidth tip-motion control using a model cancellation method**

When a rare-earth permanent magnetic particle (MQP-S-11-9, Magnequench) with the diameter of around 35 μm was attached on the backside of a cantilever (AC240, Olympus) whose nominal stiffness is 2 N/m, the damped resonance frequency of the cantilever with the magnetic particle was measured as 34.83 kHz and its quality factor was identified as 267.7 in ambient condition. This high quality factor of the cantilever is advantageous for dynamic mode AFM, however it has a detrimental effect on tip-motion control.
The dynamic model of the cantilever was identified and used to implement the model cancellation algorithm. In this case, the model cancellation algorithm is designed to replace the lightly damped cantilever dynamics $G_{\text{can}}(s)$ by an over-damped system ($\zeta > 1$) $G_c(s) = C_{\text{can}}(s) \cdot G_{\text{can}}(s) \approx 1/(1/\omega_c^2 \cdot s^2 + 2\zeta / \omega_c \cdot s + 1)$.

The compensator $C_{\text{can}}(s)$ allows the cantilever to position the tip very rapidly without introducing unwanted transient dynamics. This model cancellation algorithm is also implemented in the FPGA board. In the implementation, the input to the magnetic actuator has two signal routes, one for tip-motion control and the other for dynamic mode excitation. Employing dynamic compensation for both the coil and the cantilever renders the compensated magnetic actuator for tip-motion control high bandwidth and over-damped dynamics,

$$G_M(s) = C_{\text{can}}(s) \cdot C_{\text{coil}}(s) \cdot G_{\text{coil}}(s) \cdot G_{\text{can}}(s) \approx \frac{1}{(\tau_d s + 1)(1/\omega_c^2 \cdot s^2 + 2\zeta / \omega_c \cdot s + 1)}.$$ \hspace{1cm} (4.17)

On the other hand, the signal route for the dynamic mode excitation of the AFM cantilever employs dynamic compensation for the coil only, does not pass $C_{\text{can}}(s)$.

Therefore, it maintains the characteristics of cantilever dynamics,

$$G_D(s) = C_{\text{can}}(s) \cdot G_{\text{coil}}(s) \cdot G_{\text{can}}(s) \approx \frac{1}{(\tau_d s + 1)} G_{\text{can}}(s).$$ \hspace{1cm} (4.18)

Experiments were conducted to verify the effectiveness of model cancellation methods and the performance of tip-motion control using the proposed magnetic actuator. Three frequency response curves are shown in Fig. 4.17 (a). Among them, two are computed curves based on $G_D(j\omega)$ and $G_M(j\omega)$ to compare the distinct dynamic characteristics between dynamic mode excitation and tip-motion control and to illustrate
the effectiveness of model cancellation methods. The third one is an experimental result that verifies the desired dynamics of the implemented tip-motion control. It shows that the tip-motion control possesses an improved frequency response, which has no resonance peak, i.e. over-damped, with a bandwidth comparable to that of the cantilever. Fig. 4.17 (b) shows a rapid step response of the tip motion. In the experiment, while the cantilever was vibrated around its resonance frequency using the magnetic actuator, the same actuator was also used to move up the mean position of the tip by 20nm within one cycle. This experimental result illustrates that the magnetic actuator along with model cancellation methods elevates the bandwidth of the tip-motion control by more than two orders of magnitude when compared to that of a regular z-scanner. Since the implemented control system is programmable, all the control algorithms in FPGA can be easily modified according to the parameters of the selected cantilever.

Fig. 4.17: Tip-motion control and dynamic mode excitation: (a) comparison of frequency response, and (b) rapid step response.
4.6.5 Dual-mode Actuation scheme along the Z axis

When using the magnetic actuator alone, the achievable travel range in z-motion control is limited due to the operational range of the power amplifier. When using cantilevers with higher resonance frequency so as to achieve higher imaging rate, the cantilever stiffness is higher and the designed coil is necessarily smaller in order to possess a larger dynamic bandwidth. The achievable travel range in tip-motion control using magnetic actuation becomes much smaller due to lower magnetic force coupled with higher cantilever stiffness.

Therefore, in order to track abrupt and large topographic variations, a dual-actuator tip-motion control scheme is adopted. Since the piezoelectric z-scanner employed in the dual-actuator scheme has a large travel range (≈15μm) but a small bandwidth, it is used to track slow changes in topography that typically occur at larger length scales. This z-scanner not only provides the necessary motion range, but also significantly reduces the mean current in the magnetic coil, allowing the electric current varies around its zero mean and thus further reducing the heat generation. On the other hand, the fast changing topography is tracked by the magnetic actuator that serves to make the entire cantilever bandwidth available for tip-motion control. It can rapidly control the tip-position of the cantilever to maintain the specified tip-sample interaction.

Fig. 4.18 shows a block diagram of the dual-actuator tip-motion control scheme. The desired tip position $Z_d(s)$ has two signal routes that are input into the control loop. First, it passes through a low-pass filter $L(s)$ and then commands the z-scanner $G_z(s)$ to
track slow changes in the desired tip position. Second, it is compared with the z-motion achieved by the z-scanner, the difference between the two is fed directly to the magnetic actuator $G_M(s)$ such that the response speed of the overall control system is not limited by the slow z-scanner. It is worthy of noting that an identified z-scanner model $\hat{G}_Z(s)$ is used to obtain the z-motion achieved by the z-scanner. The dual-actuator control scheme renders the tip of the cantilever to follow the desired tip position over a large frequency range specified by $G_M(s)$,

$$Z_l(s) = G_M(s)Z_d(s) + \delta G_Z(s)L(s)Z_d(s),$$  \hspace{1cm} (4.19)

where $\delta G_Z(s) = G_Z(s) - \hat{G}_Z(s)$ denotes the modeling error of the z-scanner, whose effect is further suppressed by the low pass filter and can be negligible. Working together with the z-scanner, the magnetic actuator picks up high spatial-frequency surface topography while the regular z-scanner provides the necessary motion range. Since the bandwidth of magnetic actuation $G_M(s)$ is designed to be at least comparable to that of the cantilever, it makes the entire cantilever bandwidth available for tip-motion control.

![Block diagram](image)

Fig. 4.18: A block diagram illustrating the dual-actuator tip-motion control scheme.
Two experiments were conducted to illustrate the capabilities of proposed dual-actuator tip-motion control in terms of response speed and travel range. In the first experiment, similar to the step response reported in Fig. 4.17, the tip was commanded to move up by 30 nm. The major difference is that this was done using the two-actuator control scheme rather than using the magnetic actuator alone. Figure 4.19 shows three results: a) tip deflection caused by the magnetic actuator, b) z-motion achieved by the z-scanner, and c) the tip-position which is the combination of the two. It is seen that while the tip motion is a rapid step response, very similar to the one in Fig. 4.17, the tip deflection caused by the magnetic actuator decreases gradually to zero when the z-motion achieved by the z-scanner increases to 30 nm. This result is significant as the coil current decreases to zero when using the proposed two-actuator control scheme. The decreasing rate is dictated by the bandwidth of the low-pass filter $L(s)$. The lower is the bandwidth, the slower is the decreasing rate. However, if the bandwidth is not low enough, compared to that of the z-scanner, resonant vibration of the z-scanner can occur. As shown in Fig. 4.19 (b), a minor resonance oscillation can be seen in the motion of the z-scanner. Nevertheless, this minor resonance is fully compensated by the magnetic actuator. In the second experiment, the tip was commanded to ramp up from its initial position by 1000 nm in 10 milliseconds and stay at the final position thereafter. Three results are again shown in Fig. 4.20 and are used to illustrate the response speed and travel range of the two-actuator tip-motion control scheme. It was shown that the controlled tip position (Fig. 4.20 (c)) was able to follow the commanded input precisely while the z-scanner contributed to the large ramp and the magnetic actuator helped the tip with rapid movement.
Fig. 4.19: Step response of the dual-actuator control: a) tip deflection caused by the magnetic actuator, b) z-scanner position, and c) tip position as the summation of the two.

Fig. 4.20: Ramp up from the initial position by 1000 nm in 10 milliseconds and stay at the final position thereafter: a) tip deflection caused by the magnetic actuator, b) z-scanner position, and c) tip position as the summation of the two.
4.6.6 High Spatial Frequency AFM Imaging

In order to illustrate its ability to track high spatial-frequency surface topography when increasing imaging speed, the dual-actuator tip-motion control scheme was applied to image purple membrane (Halobacterium) patches. Purple membrane patches compose of repeating structures of identical membrane proteins due to its naturally crystallized bacteriorhodopsins, the lateral dimension of each of whose trigonal structures is about 6.2 nm [66, 67]. Since the sharpness of the tip and the material property of the cantilever are also important to imaging the trigonal structures, a silicon-nitride cantilever with sharp tip (TR800PSA, 0.57N/m, Olympus) was modified to have a magnetic moment on its backside for magnetic actuation and was installed on a regular z-scanner (MFP3, Asylum Research). The damped resonance frequency and the quality factor of the modified cantilever in imaging buffer were measured as 23.28 kHz and 3.4, respectively. These parameters were used in model cancellation algorithms implemented in FPGA. The imaging pixel rate is given by \( f_p = 2nf_s \), where \( n \) and \( f_s \) denote the pixel number along the fast (raster) scanning direction and its scanning frequency respectively. It should not exceed the tapping frequency of the cantilever on the sample. Therefore, the scanning frequency was set as 20 lines/sec, considering the tapping frequency of the cantilever (~20kHz) and the pixel number (512).

Purple membrane patches were deposited on a freshly cleaved mica surface for 10 minutes in an absorption buffer (300mM KCL, 10mM Tris-HCL, pH8.0) and rinsed three times gently with the imaging buffer (150 mM KCL, 10mM Tris-HCL, pH8.0) before the scanning. A purple membrane patch (90 nm × 90 nm) was imaged with the regular z-scanner using AM (Amplitude Modulation) mode while a larger membrane patch (120
nm × 120 nm) was measured with the dual-actuator using the direct tip-sample interaction force control mode [37] which is described in Chapter 2. In this experiment, the conventional XY stage in the MFP 3D AFM was used for both the raster scanning and the slow scanning in this experiment. The results are shown in Fig. 4.21. At the imaging rate of 20 lines/sec, the image obtained with the regular z-scanner did not show any details of the topography except the slope, Fig. 4.21 (a), while the image with the dual-actuator showed the repeating bacteriorhodopsin structures clearly, Fig. 4.21 (b).

Fig. 4. 21: Images of purple membrane patches using z regular z-scanner and the dual-actuator scheme: (a) image with a regular z-scanner, and (b) image with the dual-actuator scheme. A sample tilt angle of ~3 degrees was removed after the measurement. A feed-forward controller [Appendix C] was employed to control the x-y scanner along the fast scanning direction.
4.7 Conclusions and remarks

For high speed AFM, high bandwidth tip-motion control along the Z axis and the X axis is required for the tip-sample distance control and the fast raster scanning respectively. In order to achieve rapid tip positioning with a large working distance along the Z axis, a dual-actuation tip-motion control scheme was employed. In this scheme, a magnetic mode actuator makes the entire cantilever bandwidth available for tip-motion control by using model cancellation methods for enhancing the bandwidth of the magnetic actuator and replacing the lightly damped cantilever dynamics with an over-damped one. During AFM imaging, it tracks high spatial-frequency surface topography while the regular z-scanner provides the necessary motion range and tracks low-frequency topography.

For fast raster scanning, magnetic actuator along X axis is also added in order to build a multi-axis magnetic actuator which controls tip-motion of a multi-axis probe both along the Z axis and along the X axis simultaneously. The bandwidths of both magnetic coils for the Z axis and the X axis are enhanced using the model cancellation method. In addition, to remove the heat generated by solenoid coils that are confined to a small space, water-circulation cooling system was designed and integrated into the multi-axis magnetic actuator.
The control algorithms for the high bandwidth tip-motion control and large travel range are implemented in a FPGA-based high speed digital controller. Since the implemented control system is programmable, all the control algorithms can be easily modified according to the parameters of the selected cantilever and/or those of the designed magnetic actuator. In other words, the developed control system can be readily applied to realize various imaging objectives using multi-axis probes which have distinct geometric and dynamic parameters.
CHAPTER 5

HIGH SPEED REAL-TIME DIGITAL CONTROL & DISPLAY SYSTEM

5.1 Introduction

In order to implement the estimator which estimates the tip-sample interaction force of each tapping cycle, and plan and control the next tapping through controlling tip-to-sample distance by high-speed tip-position control using co-located magnetic actuation, a high speed programmable digital controller is required, in which the data conversions and the calculations can be completed with the speed of one or two orders of magnitude higher than the bandwidth of the cantilever. For high speed imaging, cantilevers with high bandwidth along the Z axis (~ hundred kHz) are required. Therefore, the update loop rate of the digital controller needs to be very high, on the order of several tens MHz. This is very difficult to accomplish with conventional central process unit (CPU)-based digital controller.

In addition, as the scanning speed increases, the image pixel rate also increases. Therefore, in addition to a high speed programmable digital controller, a high speed image display system is also required for high speed atomic force microscopy.
In this chapter, the implementation of a new high speed real-time digital controller based on field programmable gate array is described, whose closed loop update rate is 20Mhz. In addition, the implementation of the high speed bi-directional communication between the FPGA-based digital controller and a host computer is described, which achieves a data transfer rate of 3.476 Mbit/s. By using this high speed communication channel, high speed data display is also implemented.

This chapter is organized as follows: The implementation of the FPGA-based high-speed digital controller is described in Section 5.2. The implementation of the high speed bi-directional communication between the FPGA-based digital controller and the host computer is described in Section 5.3. The integration of the FPGA-based digital controller to a commercial AFM is described in Section 5.4. The conclusions and remarks are presented in Section 5.5.

5.2 Implementation of FPGA-based high-speed Digital Controller

FPGA contains multiple copies of a basic programmable logic element (LE) which can be programmed (interconnected) by end-users to implement basic algebraic computations such as summation, subtraction, multiplication and division [61, 62]. Since the controllers expressed as transfer functions in discrete-time domain can be converted into difference equations involving multiplications and summations, it is possible to implement the control algorithms in the form of difference equations in FPGA. Furthermore, since the implementation of the control algorithms in FPGA are completely independent, the true parallel computation of multiple codes can be achieved, in which the closed loop rate is insensitive to the computation load. In addition, FPGA allows
direct access to the peripheral device such as analog-to-digital converter and digital-to-analog converter without any data buffers which add the delays in communication. Therefore, FPGA-based digital controller can achieve very high closed loop rate along with the direct access to the peripheral devices.

The implementation of the FPGA-based high speed digital controller is composed of the two parts: (1) the implementation of the computational algorithms in the form of recursive difference equations and (2) the integration of the FPGA board with high bandwidth analog-to-digital converter and digital-to-analog converter. The first part is described in Section 5.2.1 and the second part is described in Section 5.2.2.

5.2.1 Implementation of recursive computational algorithms

A FPGA board (Stratix-II, Altera) is employed to implement a FPGA-based high-speed real-time digital controller. The computational algorithms were designed and implemented in a graphic programming environment (Quartus-II, Altera) for a hardware description language (VHDL) [62]. In order to synchronize the computational algorithms and implement closed loop form of computations for the recursive difference equations, periodic triggers were generated by using a fixed point up-counter and fixed point comparators. The fixed point up-counter is designed to be reset by a comparator when the counter value reaches a given value so that the counter value increases monotonically at fixed time intervals. This periodic counter value can serve as a time-reference for subsequent triggers. By using comparators along with a counter output (value), triggers whose period is same as that of the counter, and whose triggering times are determined by the reference values given to each comparator are generated. These triggers serve as time reference for time sensitive components in the implementation of the recursive
computation such as data latch and data update. Figure 5.1 shows one example of generating periodic triggers using an up-counter and comparators. The reset period of the counter decides the closed loop rate of the computations implemented in the FPGA.

Fig. 5.1: The generation of the periodic triggers using an up-counter and fixed point comparators: (a) An up-counter is reset by a comparator whenever its value (COUNT_FT) reaches a given value (RST_COUNTER) with a synchronized form (sclr). (b) Comparators to generate triggers at a given reference value. (c) Periodic triggers generated from the comparators along with the counter output (COUNT_FT). The blocks displayed in (a) and (b) resemble graphic representation of the programming available in Quartus-II environment.
There are two methods to implement the algebraic calculation in a FPGA: (1) calculation using floating point variables and (2) calculation using fixed point variables. The calculation with floating point variables has the advantage of holding the same bit numbers for the precision of the variables during calculation. But if variables from ADC or to DAC are included in the calculation, as is usually the case in digital control, it is necessary to include variable conversion between the fixed point variables and floating point variables because ADC and DAC only handle fixed point values. In addition, the required clock numbers to complete one process of multiplication and summation is larger than that with fixed point calculations. The default clock numbers for multiplication and summation in floating point calculation are five and six respectively while in the fixed point calculation, both require a default time of one clock cycle. Therefore, for the higher closed loop rate recursive calculations, implementation of the calculations with fixed points has an advantage even though it requires careful handling of the scales of each variable in order to keep essential bit numbers for the precision during/after the calculation and to avoid the data overflow. A method to minimize the loss of precision using fixed point variables with adjustable scales during the fixed point calculation is described in Appendix D.

Both calculation methods, i.e., with floating point variable and fixed point variables, are implemented and the required numbers of clock cycles (clock period) to complete the calculation for the one cycle of recursive difference equations for each case were designed to be 50 and 5 respectively. Within this clock period, not only the computation of the algebraic calculation but also the essential data update (data backup for the delayed variables such as x(k-1), x(k-2).. ) is processed. When the frequency of
the clock source for the FPGA is 100 MHz, the update rate of the calculation of recursive
difference equations with the floating point numbers and the fixed point numbers are 2
MHz and 20 MHz respectively. This is at least two orders of magnitude faster than
commercially available digital controllers. The block diagram for the calculation with the
fixed point number of the recursive difference equation is shown in Fig. 5.2. For the
timing of data latch or data update, the periodic triggers shown in Fig. 5.1 are used. The
coefficients in the recursive difference equations represented as $N^T$ and $D^T$ in Fig. 5.2
can be modified on-line by a host computer using a bi-directional communication channel
implemented with USB 1.1 protocol. The details of the bi-directional communication
between FPGA and a host computer are described in Section 5.3.

Fig. 5.2: Fixed point calculation of a recursive difference equation: $clk$ denotes the clock
timing. Using Hardware Description Language, the computation of a recursive difference
equation can be completed in 5 clock cycles. The block diagram shows the calculation of
a recursive difference equation given by $y(k) = N^T X(k) - D^T Y(k - 1)$.
5.2.2 Integration of FPGA with high bandwidth ADC and DAC

For the implementation of the calculation of recursive difference equations described in the previous section (5.2.1), a FPGA development board (Stratix-II, EP2S180F, Altera) is employed. The clock source of the board is 100 MHz and has a large number of 9-bit DSP block elements (768). The 9-bit DSP block elements are especially important for the implementation of the multiplication function. To convert analog data from the measurement system into fixed point values in discrete time domain for the calculation in the FPGA, and the fixed point values from the FPGA into analog signals, ADC and DAC need to be integrated in the FPGA board. In order to implement a high speed real-time digital controller, ADC and DAC whose sampling rates are higher than the desired closed loop rate and whose group delays at data transfer are small are required. Thus, a fast analog-to-digital converter (ADS 5424, Texas Instruments, 14bit, 105 MSPS) and a fast digital-to-analog converter (DAC 5687, Texas Instruments, 16bit, 500 MSPS) are employed and integrated with the FPGA board as shown in Fig. 5.3. The clock source (100 MHz) in the FPGA board is shared with the ADC and DAC so that the data conversions are synchronized with the calculation in the FPGA. To drive the solenoid coils for multi-axis magnetic actuation, high bandwidth power amplifiers (PA119, 900 V/us, 4A, Apex) are connected to the output of the DAC.
Fig. 5.3: The integration of the FPGA board with high bandwidth ADC and DAC. The ADC and DAC are synchronized with the clock source in the FPGA board (100 MHz). In order to convert the current outputs from the DAC into voltage output, a differential type OP amplifier (INA 111, TI) is added to the outputs of DAC.

Fig. 5.4: FPGA-based high speed digital controller integrated with high bandwidth ADC and DAC. The development board of Stratix-II (Altera) is used for the implementation of computation and the data interface with peripheral devices.
Figure 5.4 shows one version of the implemented FPGA-based high speed digital controller integrated with high bandwidth ADC (ADS 5424, TI) and DAC (DAC 5687, TI). The rate of the data sampling in the ADC and the data conversion in the DAC are each 100 MHz.

5.3 High speed bi-directional communication with a host computer

One of the disadvantages of using FPGA is its long compilation time, which ranges between few minutes to a few hours. In addition, the implementation of complicated mathematical functions such as trigonometric functions or exponential functions in FPGA is not easy.

In order to avoid time-inefficient compilations of the codes that enable modification of the coefficients used in the control algorithms implemented in FPGA, a USB-based high speed bi-directional communication between the FPGA board and a host computer is established. This high speed bi-directional communication with a host computer can also enable implementation of dual-form of calculation for complicated algorithms that are less time sensitive, so that the host computer computes complicated algorithms while the FPGA performs time-sensitive functions. Furthermore, this high speed communication channel between the FPGA and a host computer can be used for high speed display of the data generated by FPGA, in a host computer.

The implementation of high speed bi-directional communication between the FPGA and a host computer is described in Section 5.3.1. The on-line parameter modification using the high speed communication channel is described in Section 5.3.2. High speed display of data obtained from high speed scanning is described in Section
5.3.3. The implementation of the on-line parameter identification in the form of dual-computation is described in Section 5.3.4.

5.3.1 Implementation of a high speed bi-directional communication between FPGA and a host computer

USB1.1 IP core (USB 3 point core, SLS) is employed to run on an embedded processor (NIOS-II, 48MHz) implemented in FPGA in order to achieve communication with a host computer using USB communication protocol. The embedded processor, NIOS-II is implemented in the FPGA by using SOPC (System-On-a-Programmable-Chip) builder and it runs independently in the same FPGA board where the control algorithms of the digital controller are implemented. This embedded processor (NIOS-II) runs in the FPGA in order to take care of the data traffic through the USB core. Within the FPGA, a method to establish unsynchronized data communication between the digital controller and embedded processor NIOS-II is required, because these two parts operate based on different clock frequencies, even though they run in the same FPGA board. The implemented digital controller runs with an update frequency of 2MHz (floating point calculation) or 20MHz (fixed point calculation) while the embedded processor runs with the clock frequency of 48 MHz. To achieve unsynchronized communication between the two, dual-port RAMs are employed. The dual-port RAMs allow simultaneous data reading and writing through two ports and thus, enable unsynchronized data communication between two parts with different clock frequencies. The overall configuration of the implemented data communication using USB 1.1 core is illustrated in Fig. 5.5.
In the host computer, the counter part of the communication using USB protocol is programmed with Windows APIs. Considering the available software environment of commercial AFM (MFP3D, Asylum Research) into which the FPGA-based high speed digital controller is integrated, the software (Igor-pro, Wavemetrics) used in MFP3D for the graphic user interface is selected as the end-user software for the bi-directional communication with FPGA. The implementation of the bi-directional communication with the FPGA is, however, not limited to Igor-pro only. Any software which calls the functions in dynamic link library (DLL) can replace the current implementation of the end-user panels using Igor-pro. Igor-pro software enables accessing external functions in DLL through XOP codes. Therefore, the XOP codes which enable the data communication between the Igor-pro and external dynamic links are also developed. The implemented data transfer rate between the FPGA to a host computer via USB 1.1
IP core was measured as 3.476 MBit/s. This data bit speed allows high speed data display of 35 frames/sec with an image pixel resolution of 100×100 pixels.

5.3.2 On-line parameter modification

When modification of parameters in the FPGA-based high speed digital controller is required, the modified parameters are sent to FPGA through USB protocol and the embedded processor NIOS-II reads the data through USB 1.1 IP core and writes the data on the dual port RAMs with the corresponding addresses. In the digital controller, all the data written in the dual port RAM are read periodically using an automatic address generator and data are assigned to the parameters which have matching addresses. In this way, on-line modification of the parameters for the control algorithms implemented in FPGA is achieved using the data communication between a host computer and FPGA. The configuration of the on-line parameter modification is illustrated in Fig. 5.6.

Fig. 5.6: On-line parameter modification of FPGA-based high speed digital controller.
5.3.3 High-speed data display

In order to display data generated in the FPGA-based digital controller, high speed data flow from the FPGA to a host computer is required. The direction of the data flow for the high speed data display is the reverse of the one needed for on-line parameter modification. The image data, such as the reconstructed topography, generated from the high-speed digital controller are written in a dual port RAM dedicated for the image display along with the addresses generated from a periodic address generator. Before the data are written in the dual port RAM, the data can be modified through a simple image processing algorithm in order to remove sloping trends from the image. When the embedded processor NIOS-II receives the request from the host computer to send out the data, NIOS-II reads the data from the dual port RAM and sends them to the host computer through USB protocol. When the host computer receives the data, it is written in a Bitmap, which is a dedicated memory space for high speed image display. Finally, the image data on the Bitmap memory are displayed in a sub-window of Igor pro. The whole data flow in high speed image display is illustrated in Fig. 5.7.

When one pixel of the image map is expressed with a variable with 8 bits and the pixel resolution is 128×96 pixels, the required data transfer rate, for an image display rate of 30 frames/sec, is about 3 Mbit/s. The established bi-directional communication between FPGA to a host computer showed a maximum data bit transfer rate of 3.476 MBit/s. Therefore, the established communication channel can support the high speed image display with the display rate higher than 30 frames/sec.
5.3.4 **On-line parameter identification**

By using USB 1.1 IP core, high speed bi-directional communication between the FPGA-based high speed digital controller and a host computer is established. This bi-directional communication channel can be used not only for the on-line parameter modification in the FPGA-based digital controller or high speed data display, but also for the implementation of the dual-computation scheme of advanced functions. The FPGA-based digital controller is an independent high speed real-time digital control system which runs the control algorithms and handles the signal in/out through the ADC and DAC separately from any host system. However, programming complicated algorithms in FPGA, which require more than just arithmetic functions is still very difficult due to its limited functions (IP cores) and long compilation time.
Therefore, for the implementation of on-line model parameter identification and modification, a dual-computation scheme is employed in which the FPGA-based digital controller logs and sends data to the host computer, and the host computer identifies the model parameters using the data received from the FPGA-based digital controller, generates the system parameters for the controller and send them back to the FPGA-based digital controller. Subsequently, the FPGA-based digital controller processes the control algorithms with the updated parameters. The configuration of the on-line parameter identification and modification is illustrated in Fig. 5.8.

Fig. 5.8: The configuration of the data flow for the on-line parameter identification and modification. Simultaneous multi-channel data log is added to log the data in the FPGA and send them to the host computer. In the host computer, model parameters are identified using the data from the FPGA, and the updated parameters for the high speed digital controller are sent back to the FPGA.
5.4 Integration of the FPGA-based digital controller to a conventional AFM

The FPGA-based high-speed digital controller is integrated with a commercial AFM (MPF3D, Asylum research). The FPGA-based high speed digital controller controls the high bandwidth tip-motion along the Z axis and the X axis for tip-sample distance control and raster scanning respectively. It also controls a conventional piezo Z-positioner in MPF3D for implementation of the dual-mode actuation scheme. The MPF3D system controls the conventional piezo XY scanner for slow scanning. The high speed image generated from the high speed digital controller is sent to a host computer and displayed in it. The key part of the integration of the two systems is the synchronization of actuation of the slow scanning operation controlled by MPF3D with the raster scanning operation controlled by the high speed digital controller. For this synchronization, a digital signal from the MPF3D is connected to the high speed digital controller, which generates a trigger signal whenever the new frame of image begins in the slow scanning actuation. Based on this information, the high speed digital controller generates a new synchronized trajectory at each image frame for the high bandwidth raster canning and controls the tip-motion along the X axis accordingly. As the result of this implementation, the FPGA-based high speed digital controller serves as an add-on high performance digital controller to a commercial AFM for high speed AFM.
Fig. 5.9: Integration of the FPGA-based digital controller with a commercial AFM. Using a digital line, the actuation synchronization for the high speed scanning is achieved.

5.5 Conclusions

In order to implement the control algorithms for direct tip-sample force control and high bandwidth multi-axis actuation of a probe, a new high speed real-time digital controller is implemented using FPGA board, with integrated high bandwidth ADC and DAC, which achieves closed loop update rate of 20 MHz.

For on-line parameter modification, high speed data display and on-line parameter identification/modification, bi-directional communication between the high speed digital controller and a host computer is implemented by employing USB 1.1 IP core. The maximum data transfer rate achieved was 3.476 Mbit/s which is fast enough to
support high speed image display of ~35 frames/sec with image resolution of 100×100 pixels.

The implemented high speed digital controller can be integrated into a commercial AFM with proper synchronization of actuation. As the result of this implementation, the FPGA-based high speed digital controller along with the high bandwidth active multi-axis probing system serves as an add-on high bandwidth control & actuation system to a commercial AFM for high speed atomic force microscopy.
CHAPTER 6

HIGH SPEED, HIGH RESOLUTION AFM IMAGING

6.1 Introduction

The capability of AFM to image/manipulate objects in various environments with sub-nanometer spatial resolution in three dimensions has made it a unique and useful microscopic instrument and/or manipulator in many areas ranging from material science to biology or biophysics. But its slow scanning speed, which is in the range of several minutes per frame, has prevented expansion of its further applications. The research topics described in Chapter 2 through Chapter 5 are intended to develop a high speed AFM, which overcomes this temporal limitation of the conventional AFMs, while retaining its high resolution capability.

In this chapter, the integration of the components of the proposed high speed AFM with a commercial AFM (MFP3D, Asylum research) is first described. The integrated high speed AFM is composed of namely two parts, namely, the high bandwidth components designed and developed in this research, and slow bandwidth components from a commercial AFM.

In order to evaluate the potential of the proposed high speed AFM, two experiments are performed. Firstly, a standard engineering grating composed of periodic
inverted pyramids whose edge length and pitch are 50nm and 100nm respectively is measured using the integrated high speed AFM at high speed. This enables evaluation of its temporal resolution. Secondly, a biological membrane on which proteins are arranged in a repeating structure with lateral pitch of about 6.2 nm and topographic variation of several Å is measured with high temporal-spatial resolution using the proposed high speed AFM. The image is compared with the one measured by slow scanning in a conventional AFM (Chapter 3).

This chapter is organized as follows: The integration of each component of high speed AFM with the MFP3D AFM is described in Section 6.2. High speed imaging of an engineering grating is described in Section 6.3. High speed – high resolution imaging of the repeating protein structure on purple membranes is described in Section 6.4. The conclusion and remarks are added in Section 6.5.

6.2 Integrated high speed AFM

Fig. 6.1 shows the configuration of the integrated high speed AFM proposed in this research work. The implementation of the high speed AFM is achieved by integrating the high bandwidth components developed as part of the research work described in this dissertation with the low bandwidth components of a commercial AFM (MFP3D, Asylum research). The details of the role of each component are as follows: for multi-axis tip-motion control with high bandwidth and large travel range, an active multi-axis AFM probe, two high bandwidth magnetic actuators (Z coil and X coil) and two low bandwidth conventional piezo actuators (Z positioned and XY scanner) are used. The main role of piezo XY scanner is to control the tip motion along the slow scanning trajectory, although it also contributes to the large travel range when selecting the
scanning area for high-speed scanning. In order to achieve high bandwidth multi-axis tip-motion control using high bandwidth magnetic actuators, an FPGA-based high speed digital controller is included. This high speed digital controller also enables the high speed data display on a host computer. A low noise laser measurement system is included in order to reduce the measurement noise without sacrificing bandwidth and facilitate achievement of high speed–high resolution AFM imaging.

Fig. 6.1: The configuration of the integrated high speed AFM. (a) The high speed digital controller and high speed display system is integrated with MFP3D controller. (b) Two high bandwidth magnetic actuations (Z coil, X coil) and two low bandwidth conventional piezo actuations (Z-positioner and XY scanner) are combined together to achieve the required bandwidth of actuation for high speed scanning and travel range. The dashed boxes represent components from MFP3D AFM, while the solid boxes are the newly developed ones for high speed AFM.
6.3 High speed imaging of an engineering grating

A standard grating (2D100, Nanosensors) which consists of inverted square pyramids whose edge length is approximately 50nm and pitch is 100 nm is employed as an engineering sample in order to evaluate the temporal resolution of the proposed high speed AFM. A commercial AFM cantilever (ATEC-NC, 45 N/m) was fabricated into a multi-axis probe with an attached magnetic moment. It was vibrated near its resonance frequency (70khz) in liquid with an amplitude of 10.7nm. At first, the grating surface was scanned using the conventional scanning mode in the amplitude modulation, with scanning frequency of 3 lines/sec. For this scanning operation, the piezo XY scanner was controlled by MFP3D AFM for along both the raster scanning and slow scanning directions, while the tip motion along the Z axis was controlled by the dual-actuation scheme in a high speed digital controller. The image acquired with the conventional scanning mode was subsequently used as an image map to select an area for high speed imaging. Once the area for the high speed imaging is selected, the active multi-axis probe is automatically moved to the selected area by the piezo XY scanner and begins to scan the selected area in the high-speed scanning mode. The piezo Y scanner controls the sample stage motion according to the required slow scanning trajectory while the multi-axis probe controls the tip-motion along the X axis for the raster scanning and along the Z axis for tip-sample distance control in the dual-actuation scheme. The image generated from the high speed digital controller is sent to the host computer and displayed in a sub-window of Igor-pro software. In this experiment, the standard grating was scanned with two different raster scanning frequencies: 175 lines/sec and 872 lines/sec.
Figure 6.2 (a) and (b) show high speed AFM images using the proposed high speed AFM with a raster scanning frequency of 175 lines/sec and 872 lines/sec respectively. With a pixel resolution of 128×96 pixels, it took 0.547 second and 0.110 second per frame for the respective scanning frequencies. There are two images in Fig. 6.2 (a) and (b), which look similar to one another. However, the images at right hand side were obtained one frame after those on the left hand side were obtained. In both of the cases, the grating is clearly visible, showing the improvement of the temporal resolution at AFM imaging by using the proposed high speed AFM.

Fig. 6.2: AFM images of a standard grating (2D100) composed of inverted pyramids with 50nm edge length and 100nm pitch in water. (a) High speed AFM imaging. The raster frequency was 175 lines/sec and it took 0.547 second per frame. (b) High speed AFM imaging with the raster frequency of 872 lines/sec. It took 0.110 second per frame. The white spot at the left-top edge in images (b) are debris on the grating. For both, the active probe was vibrating at the frequency of 70 kHz with the oscillation amplitude of 10.7 nm.
6.4 High speed - high resolution imaging of purple membrane patches

The repeating structures of proteins on the purple membrane (*Halobacterium*) patches are measured with the proposed high speed AFM. Purple membrane patches compose of repeating structures of identical membrane proteins, namely, naturally crystallized bacteriorhodopsins, the lateral dimension of each of whose trigonal structures is about 6.2nm [15,16] and its vertical variation is several Å. Due to its naturally repeating structures with small pitch along the lateral directions and small variation along the vertical direction, it can be considered as a biological grating composed of proteins, which requires very high spatial resolution and force sensitivity for the measurement.

Purple membranes were deposited on a freshly cleaved mica surface in absorption buffer (350 mM KCL/Tris-ph 8.0) for 10 min. and were rinsed gently three times with imaging buffer (150 mM KCL/Tris-ph 8.0). A commercial AFM cantilever (NCHAuD) was fabricated into an active multi-axis probe and its damped resonance frequency measured in water was 69 kHz. The multi-axis probe was vibrated at 68 kHz with the oscillation amplitude of 1.04 nm. The images were measured in high speed AFM with the amplitude modulation mode in the imaging buffer. For the amplitude modulation mode in the high speed AFM, a digital lock-in amplifier was implemented in the high speed digital controller. Fig. 6.3 (a) shows the repeating structures on a purple membrane patch with the raster frequency of 48 lines/sec obtained using the developed high speed AFM. The imaging time per frame was 1.97 second with the pixel resolution of 128×96 pixels. In these images, the repeating structure of the proteins is clearly visible. Fig. 6.3 (b) is the high resolution image of a purple membrane structure shown in Section 3.5 (Chapter 3), added for the comparison of the structure of purple membrane. This
image was acquired at 3 lines/sec raster frequency and the time to acquire one frame of
the image, with a pixel resolution of $512 \times 512$ pixels, was 2.84 minutes. The images
measured with the proposed high speed AFM shows the repeating structure clearly. This
experimental result shows the potential of the proposed system to go to higher scanning
speed while retaining its spatial resolution.

Fig. 6.3: AFM images of the repeating structure of the protein on the purple membrane
patch in imaging buffer (150 mM KCL/Tris-ph 8.0). (a) High resolution image of the
purple membrane using the high speed AFM. The raster scanning frequency was 48
lines/sec (scanned with the active multi-axis probe) and it took 1.97 seconds per frame of
$128 \times 96$ pixel-resolution. The oscillation amplitude was 1.04nm. The Y-size of the image
is 50 nm. (b) The high resolution image of the purple membrane with a conventional
AFM using the low noise laser measurement system that was presented in Section 3.5
(Chapter 3). The raster scanning frequency was 3 lines/sec and image resolution was
$512 \times 512$ pixels. In both (a) and (b), the pixel number per spatial period of repeating
proteins along the raster scanning direction is about 25.
6.5 Conclusions

In this chapter, the potential of the proposed high speed AFM was evaluated using two experiments. Firstly, by using an engineering grating sample, the temporal resolution of the proposed high speed AFM was evaluated, which achieved one image at 0.110 second with the high raster scanning frequency 872 lines/sec. The measurement of a biological grating (repeated protein structure on purple membrane) with the proposed high speed AFM proved its potential to achieve high temporal – spatial resolution for biological samples.
CHAPTER 7

CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The main focus of this dissertation was design and development of a high speed AFM, which retains its high spatial resolution capability. Detailed objectives defined to achieve high speed atomic force microscopy were as follows: 1) to design a new control method for the AFM which allows precise and direct tip-sample interaction force control of each tapping cycle and does not rely on steady-state parameters. 2) to design and develop a high bandwidth multi-axis probing system, which overcomes the limitations of the conventional piezo-based actuation system. 3) to design and implement a new high speed digital control & display system which allows the implementation of control algorithms for the new control method and high bandwidth multi-axis tip-motion control. 4) to evaluate the proposed high speed AFM in terms of scanning speed and image resolution. In order to minimize the effect of the measurement noise for larger measurement and actuation bandwidths, the implementation & integration of a low noise laser measurement system for deflection measurement was also undertaken. This dissertation described and discussed the achievement of each these objectives.
By using direct tip-sample interaction control, a biological sample was scanned with very small interaction force, with a peak value of about 63.5 pN (Chapter 2). The new laser measurement system enabled reduction of the standard deviation of the measured free deflection of a commercial AFM cantilever (NCHAuD, 40 N/m) by more than 75% and achievement of a low noise density of 24 fm/√Hz around the cantilever’s resonance frequency (Chapter 3). Using the new laser measurement system, the repeating protein structure on purple membrane patches was successfully measured at high resolution with an AFM cantilever with high stiffness (40 N/m) thereby providing evidence for the possibility of achieving high speed - high resolution AFM imaging with high bandwidth AFM cantilevers. By fabricating a commercial AFM cantilever and attaching a magnetic moment to the head of the fabricated cantilever, an active high bandwidth multi-axis probing system was successfully implemented, along with the high bandwidth multi-axis magnetic actuators. As an example, an active high bandwidth multi-axis probe was fabricated and evaluated with bandwidth along the Z axis and X axis higher than 100 kHz and 40 kHz respectively (Chapter 4). It was also demonstrated that high bandwidth actuation of the multi-axis probe is possible, whose bandwidth is comparable to that of the cantilever, by using the control algorithms based on a model cancellation method. A new FPGA (Stratix-II, Altera)-based programmable real-time digital controller was developed, whose closed loop rate is 20 MHz and to which high bandwidth peripheral devices such as analog-to-digital converter (ADS5424, 105 MSPS, TI) and digital-to-analog converter (DAC5687, 500 MSPS, TI) were integrated (Chapter 5). By implementing high speed bi-directional communication with a host computer, on-line parameter modification, high speed image display and on-line model parameter
identification & modification were implemented. The developed high speed AFM was integrated to a commercial AFM (MFP3D, Asylum research) and a standard engineering grating composed of inverted pyramids with edge lengths of about 50 nm was scanned with the integrated high speed AFM. The time to acquire one frame of the image (128 × 96 pixels) was 0.110 second. The repeating structure of the proteins on a purple membrane patch was scanned and the time to acquire one frame of image (128 × 96 pixels) was 1.97 seconds, showing the potential to achieve high speed imaging without losing high spatial resolution.

The contributions of this research work are summarized below:

(1) Design and development of direct tip-sample interaction force control which directly controls the tip-sample interaction at each tapping cycle and does not rely on steady state parameters.

(2) Design and implementation of a high bandwidth multi-axis probing system for high speed atomic force microscopy. In this implementation, the bandwidth of tip-motion control along the Z axis is comparable to that of the cantilever. The designed bandwidth of the tip-motion along the X axis is several tens kHz.

(3) Design and implementation of a tip-motion control scheme high bandwidth positioning and large travel range. Namely, a dual-mode actuation scheme in which high bandwidth magnetic actuation allows rapid tip-position change and a conventional piezo actuator provides large travel range.

(4) Design and implementation of a high speed programmable real-time digital control & display system. This real-time digital controller provides very high temporal resolution.
for the implementation of control algorithms for direct tip-sample interaction control and high bandwidth actuation of the probes.

(5) With the integrated high speed AFM along with a multi-axis probe (NCHAuD), high temporal resolution was achieved, with which one image \((128 \times 96\) pixels) was displayed at 0.110 second with the high raster scanning frequency 872 lines/sec. For high speed, high resolution imaging, the repeating structure of the proteins on a purple membrane patch was scanned and one image \((128 \times 96\) pixels) with clear repeating structures was achieved at 1.97 seconds, showing the potential to achieve high speed imaging without losing high spatial resolution.

### 7.2 Recommended future work

The following are the list of recommended future research topics:

(1) The development of a method to attach single-walled carbon nano tube to the fabricated multi-axis probes:

In order to achieve AFM imaging of biological samples with high spatial resolution in three dimensions, not only high force sensitivity along the tip-sample interaction direction but also very sharp geometry of the tip (i.e., high lateral resolution along the scanning directions) is required. The former primarily determines the resolution along the Z direction (height in topography) whereas the latter determines the resolutions along the other two directions (X and Y). The requirement for the high force sensitivity can be accomplished by employing a low noise deflection measurement system [56]. To obtain a sharp geometry for the tip, oxide-sharpening method is commonly used in fabrication of the commercial AFM cantilevers. But if during the scanning, the geometry
of the tip changes due to attachment of small objects to the tip or wear, the spatial resolution in the image will be degraded accordingly. Therefore, for imaging with high spatial resolution and ever higher speed, a method to retain the sharp geometry of the tip is required. Single walled carbon nano tube has many merits due to its narrow geometry (~ 4nm in diameter) with high aspect ratio, high strength and stiffness [68, 69]. If a reliable method can be developed to attach single-walled carbon nano tube to the fabricated multi-axis probes, it will contribute to minimization of lateral-resolution issues due to tip geometry degradation and thus, will improve the spatial resolution at imaging fine biological samples with high speed scanning.

(2) Simultaneous functional mapping, such as the material property map and specific recognition map:

The augmented state estimator designed to estimate the tip-sample interaction force of each tapping cycle is not only useful for the direct interaction force control but also useful for other applications which require the reconstruction of the tip-sample force versus the tip-sample distance. Since the estimator is designed to estimate the tip-sample interaction force along with other state variables (position and velocity of the tip), it can directly reconstruct the force-distance curves at every tapping cycle.

For example, the material property of the sample can be estimated from the variation of the reconstructed force versus the distance [72, 73]. In addition, this reconstructed force-distance curve can be used to recognize the specific bindings between anti-gen and anti-body [74]. This additional information analyzed from estimated force will allow the generation of functional maps (material property map or/and recognition
map) simultaneously with the topography of the scanned area in high speed. The high speed-high resolution imaging and simultaneous functional maps such as material property map or recognition map will facilitate its application to studies on biological samples.


[64] [https://www.veecoprobes.com/](https://www.veecoprobes.com/)

[65] [http://www.nanoandmore.com/USA/afm_probes.php](http://www.nanoandmore.com/USA/afm_probes.php)


APPENDIX A

EFFECT OF THE NOISE ON THE ESTIMATION OF THE OSCILLATION AMPLITUDE USING A LOCK-IN AMPLITUDE ALGORITHM
Fig. A.1: The block diagram for the implementation of a lock-in amplifier to estimate the oscillation amplitude and the phase of signal in \( y(t) \) with the frequency \( \omega / 2\pi \). The cutoff frequency \( \omega_c / 2\pi \) of the Low Pass Filter is designed to be much smaller the carrier frequency \( \omega / 2\pi \).

There are several methods to extract the amplitude and phase of the measured signal using a lock-in amplifier. Figure A.1 shows one of the methods to implement the lock-in amplifier algorithm to extract the oscillation amplitude and/or the phase of the signal \( y(t) \) at a frequency \( \omega / 2\pi \). The estimated oscillation amplitude of the signal from the lock-in amplifier is used as a feedback variable in the amplitude modulation mode operation.

If the input signal is assumed to be composed of the steady state response of the system to a sinusoidal signal \( \sin(\omega t) \) and the noise in the position measurement, the input signal \( y(t) \) is given by

\[
y(t) = a \sin(\omega t + \phi) + \sum_{i=0}^{\infty} n_i \sin(\omega_i t + \phi_i) + C
\]  
(A.1)
where \( a \) and \( \phi \) denote the oscillation amplitude and the phase of the steady state response of the system. \( \sum_{i=0}^{\infty} n_i \sin(\omega_i t + \phi_i) \) and \( C \) denote the harmonic components and the DC component of the noise in the position measurement respectively. The noise in the position measurement includes the thermal fluctuation of the cantilever caused by the thermal force from the environment and the noise in the measurement system.

Multiplying the signal input \( y(t) \) with \( \sin(\omega t) \), the following result is obtained:

\[
y(t) \sin \omega t = a \sin(\omega t + \phi) \sin \omega t + \sum_{i=0}^{\infty} n_i \sin(\omega_i t + \phi_i) \sin \omega t + C \sin \omega t
\]

(A.2)

By the expansion of the trigonometric functions, the equation (A.2) can be expressed as

\[
y(t) \sin \omega t = \frac{-a \cos \phi}{2} + \frac{a \cos \phi}{2} \cos 2\omega t + \frac{a \sin \phi}{2} \sin 2\omega t
\]

\[
+ \sum_{i=0}^{\infty} n_i \frac{\cos \phi}{2} \cos(\omega_i + \omega) t + \sum_{i=0}^{\infty} n_i \frac{\sin \phi}{2} \sin(\omega_i + \omega) t + \sum_{i=0}^{\infty} \frac{n_i}{2} \cos((\omega_i - \omega) t - \phi_i) \].
\]

(A.3)

When \( y(t) \sin \omega t \) in equation (A.3) passes through a low pass filter whose cut-off frequency \( \omega_c \) is much smaller than \( \omega \), the DC component and other components whose frequency is smaller than \( \omega_c \) emerge un-attenuated while the rest are greatly attenuated. Thus, the low pass filtered version of \( y(t) \sin \omega t \) is calculated as

\[
LPF_{\omega_c < \omega}[y(t) \sin \omega t] = \frac{-a \cos \phi}{2} - LPF_{\omega_c < \omega} \left[ \frac{\sum_{i=0}^{\infty} n_i}{2} \cos((\omega_i - \omega) t - \phi_i) \right].
\]

(A.4)
where the first part on the right hand side includes the oscillation amplitude and the phase of the steady state response of the system and the second part includes the effect of the noise in position measurement.

Since the second part of equation (A.4), contains only the components whose frequency $|\omega - \omega_i|$ is smaller than the cutoff frequency $\omega_c$, the low pass filter has a band pass filtering effect on the amplitude measurement, whose center frequency and the bandwidth are $\omega$ and $2\omega_c$ respectively. Therefore, the equation (A.4) can be expressed with a band pass filter as

$$
LPF\left[a(t)\sin \omega t\right] = \frac{a \cos \phi}{2} - BPF_{\omega,2\omega_c}\left[\sum_{i=0}^{\infty} \frac{n_i}{2} \cos \{\omega_i t - \phi_i\}\right].
$$  \hspace{1cm} (A.5)

Noting that $BPF_{\omega,2\omega_c}\left[\sum_{i=0}^{\infty} \frac{n_i}{2} \cos \{\omega_i t - \phi_i\}\right] = BPF_{\omega,2\omega_c}\left[\sum_{i=0}^{\infty} \frac{n_i}{2} \sin \{\omega_i t + \pi/2 - \phi_i\}\right]$ and the phase offset of the noise has no effect on its contribution to the measurement system, equation (A.5) can be modified as

$$
LPF\left[a(t)\sin \omega t\right] = \frac{a \cos \phi}{2} - BPF_{\omega,2\omega_c}\left[\sum_{i=0}^{\infty} \frac{n_i}{2} \sin \{\omega_i t + \phi_i\}\right].
$$  \hspace{1cm} (A.6)

Likewise, the low pass filtered $y(t)\cos \omega t$ can be calculated and is expressed as

$$
LPF\left[a(t)\cos \omega t\right] = \frac{a \sin \phi}{2} + BPF_{\omega,2\omega_c}\left[\sum_{i=0}^{\infty} \frac{n_i}{2} \sin \{\omega_i t + \phi_i\}\right].
$$  \hspace{1cm} (A.7)

From equations (A.6) and (A.7), it is clear that the low pass filtering of the modulated signal $y(t)\sin \omega t$ and $y(t)\cos \omega t$ gives a constant component which includes the oscillation amplitude and the phase, and the band-pass filtered noise. The effect of the low pass filter whose cutoff frequency is $\omega_c$ on the noise component in the position measurement...
measurement is the same as that of a band pass filter whose center frequency and bandwidth are $\omega$ and $2\omega$, respectively. Therefore, one can calculate the contribution of the noise in estimation of the oscillation amplitude using the lock-in amplifier from the band pass filtered (center frequency: $\omega$, bandwidth: $2\omega$) noise density of the free deflection of the cantilever.
APPENDIX B

ACTIVE MULTI-AXIS PROBE FOR THE HIGH BANDWIDTH (>200kHz)
In order to achieve higher bandwidth actuation, an active multi-axis whose resonance frequency is higher is required. Since the resonance frequency of the multi-axis probe can be improved greatly by using thicker cantilevers, a commercial AFM cantilever (TAP525A, Veeco) whose nominal thickness is 7um is employed in this design. In addition, by placing the magnetic particle as close as possible to the neck and reducing the mass of the head, the resonance frequency of the multi-axis probe can be improved. Figure B.1 shows the designed multi-axis probe for higher bandwidth.

According to the design shown in Fig. B.1, the AFM cantilever with 7 um thickness is fabricated as shown in Fig. B.2. In order to evaluate the bandwidth of the fabricated multi-axis probe, the resonance frequency of the fabricated probe was analyzed in the air and water. The resonance frequencies of the fabricated multi-axis probe in the air and water were identified as 290 kHz and 240 kHz respectively.

Fig. B.1: The modified design of the geometry of a multi-axis probe for high bandwidth (> 200 kHz).
Fig. B.2: The fabricated multi-axis probe whose resonance frequencies in air and water are 290 kHz and 240 respectively. The unnecessary parts of the head and the magnetic particle were removed by FIB milling.
APPENDIX C

FEED-FORWARD CONTROL OF THE CLOSED LOOP PIEZO XY SCANNER
FOR THE IMPROVED TRACKING PERFORMANCE
To control the motion along the slow scanning direction at high frame rates, a feed-forward controller is added to the closed loop piezo XY scanner. The controller is designed based on the pole/zero cancellation method achieves zero-phase lag in the system response. By using the actual response of the closed loop XY scanner for a given scanning frequency along with the time-domain data of the reference trajectory at that frequency, the model of the closed loop piezo XY scanner was identified, and subsequently used to build the feed-forward controller.

The slow scanning frequency is directly determined by the required frame rate, which, in a conventional AFM, is very slow (typically ~ few mHz). But for the high speed AFM, the frame rate can be ~ ten Hz and thus the required slow scanning frequency become much higher than the case of conventional AFMs. A closed loop control scheme is used for the piezo XY scanner in a commercial AFM (MFP3D, Asylum Research) in order to reduce the nonlinear effect of the piezo actuators such as the drift and hysteresis. The useful achievable actuation bandwidth which does not introduce significant phase delay using the feedback controller gain is a few Hz. Higher bandwidth can be achieved for the closed loop piezo XY scanner by using higher feedback controller gains. However, this sacrifices the position accuracy because the higher feedback gains also increases the contribution of noise in the feedback signal to the closed loop system. Therefore, a feed-forward controller based on pole/zero cancellation of the identified closed loop system model is employed in order to improve the tracking performance of the closed loop XY scanner. In the presence of the zeros in the model which cannot be cancelled using pole/zero cancellation such as a non-minimum zeros or lightly damped zeros, the phase cancellation method based on Zero-
Phase-Error-Tracking-Controller is used [70, 71] in order to ensure zero phase shift between the desired output and the actual output. The closed loop system response of the XY scanner is modeled as a 3rd order discrete pole/zero model given by

\[
X_a(k) = \frac{n_0 + n_1 z^{-1} + n_2 z^{-2} + n_3 z^{-3}}{1 + d_1 z^{-1} + d_2 z^{-2} + d_3 z^{-3}} X_r(k),
\] (C.1)

where \(X_a(k)\) and \(X_r(k)\) denote the actual closed loop system response and the reference trajectory at time step \(k\) respectively. \(d_1, d_2\) and \(d_3\) are the coefficients of the denominator while \(n_0, n_1, n_2\) and \(n_3\) are the coefficients of the numerator of the discrete transfer function. There are many methods to identify the coefficients in the numerator and denominator of the model including evaluation of the closed loop frequency response of the XY scanner. The method adopted in this implementation used the time-domain technique. For a given frame rate and scanning size, the scanning trajectory of the closed loop XY scanner is generated in advance considering the required scanning frequency and the scanning size and sent to the controller for feedback control. Since the generated reference trajectory is available and the actual response of the closed loop XY scanner for the reference trajectory is measurable in time domain, the time domain data is used to identify model parameters by using a least-square algorithm. The discrete time domain transfer function in (C.1) can be converted into a difference equation form shown below.

\[
X_a(k) + d_1 X_a(k-1) + d_2 X_a(k-2) + d_3 X_a(k-3) = n_0 X_r(k) + n_1 X_r(k-1) + n_2 X_r(k-2) + n_3 X_r(k-3).\] (C.2)

From the knowledge of the reference trajectory and the measured actual system response at the time step \(k\), one can find the coefficients of equation (C.2) which minimize the root-mean square of the error, namely,
\[
\begin{bmatrix}
    n_0 \\
n_1 \\
n_2 \\
n_3 \\
d_1 \\
d_2 \\
d_3
\end{bmatrix} = \left(M^T M \right)^{-1} M^T \begin{bmatrix}
    X_a(4) \\
X_a(5) \\
. \\
. \\
. \\
. \\
.
\end{bmatrix},
\]

(C.3)

where \(M = \begin{bmatrix}
    X_r(4) & X_r(3) & X_r(2) & X_r(1) & -X_a(3) & -X_a(2) & -X_a(1) \\
X_r(5) & X_r(4) & X_r(3) & X_r(2) & -X_a(4) & -X_a(3) & -X_a(2) \\
. & . & . & . & . & . & . \\
. & . & . & . & . & . & . \\
. & . & . & . & . & . & . \\
. & . & . & . & . & . & . \\
\end{bmatrix}\).

Figure C.1 shows one example of model identification using the time-domain data and the reconstruction of the frequency response of the closed loop XY scanner. At a scanning frequency of 31.25Hz, the actual trajectory of the closed loop XY scanner shows large tracking error (Fig. C.1(a)). By using the reference trajectory and the corresponding closed loop system response shown in Fig. C.1 (a), the coefficients of the model of the closed loop XY scanner were identified using eq. (C.3). In order to evaluate the identified model, the frequency response of the closed loop XY scanner was reconstructed using the identified model and compared with the frequency response of the closed loop XY scanner obtained by directly sweeping the excitation frequency.

A feed-forward controller is designed based on the pole/zero cancellation using the identified closed loop XY scanner. Uncancellable zeros in the model were not included the pole/zero cancellation scheme. Instead, the same normalized zeros with additional preview steps of the order of the unacceptable zeros were multiplied to the feed-forward controller [70, 71]. As the result, a zero-phase tracking error of the XY
scanner can be expected. Figure C.2 (a) shows the overall configuration of the feed-forward and feed-back controller. The former is added for realizing Zero-phase error tracking control; the latter was already a part of the XY scanner of a commercial AFM (MFP3D, Asylum research). Figure C.2 (b) shows the modified reference trajectory after the feed-forward control.

Fig. C.1: Closed loop system model identification using time-domain data: (a) the reference trajectory and the actual closed loop response of the XY scanner. The solid line represents the reference trajectory with the scanning frequency of 31.25Hz and the line with circles represents the actual closed loop response of the scanner. (b) The reconstructed frequency response of the closed loop system response of the scanner. The dots denote the reconstructed frequency response using the model identified from the time-domain data. The inverted solid triangle shows the scanning frequency which is the primary frequency of the triangular wave with smoothed corners.
Fig. C.2: The configuration of the feed-forward control and the trajectory modification by the feed-forward controller: (a) the configuration of the feed-forward/back control for the piezo XY scanner. (b) The trajectory modification from the feed-forward controller.

Figure C.3 shows the comparison of the tracking performances with the closed loop only (left) and with the feed-forward and feed-back controller (right). As the scanning frequency increases, the poor tracking performance of the closed loop XY scanner becomes more obvious but the modified control scheme with both the feed-forward and feedback controllers (right) show consistently good tracking performance.
Fig. C.3: The improved tracking performance of the closed loop XY scanner using a feed-forward controller based on pole/zero cancellation method and zero-phase tracking control: (a) Tracking performance comparison with the scanning frequency of 10 lines/sec. (b) Tracking performance comparison with the scanning frequency of 40 lines/sec. Tracking performance with a feedback controller using default gains of MFP3D XY scanner is displayed at left column while the improved tracking performance with a feedforward controller along with the feedback controller is displayed at the right column.
APPENDIX D

ADJUSTABLE SCALE FIXED POINT CALCULATION
The calculation of multiplication and summation with fixed point variables can be completed in one clock cycle in FPGA while the calculation with floating point variables takes at least 5 clock cycles. Therefore, the implementation of recursive difference equations with fixed point variables has the advantage at reducing the total clock number required for the computation and thus increasing the update loop rate. However, since the scale of the fixed points is fixed, it is easy to lose the precision of the variables during or after the calculation. In order to reduce the loss of the precision, it is necessary to modify scales of fixed point variables during and/or after the calculation.

For the efficient modification of the scale of the fixed point variables, variables with fixed point format (1 bit for sign + precision bits) are expressed in FPGA along with corresponding scale values which can be easily adjusted from a host computer through the communication channel between the computer and FPGA. From the computer simulation of the calculation with fixed point variables, the scale of each variable which minimizes the precision loss during/after the calculation can be determined in advance. In addition, the recursive variables in a recursive difference equation can be designed to have larger bit numbers than other variables for the further preservation of the precision during the calculation.

As an example, a simple recursive difference equation is given by

\[ y(k + 1) = a \cdot x_1(k) + b \cdot x_2(k) + c \cdot y(k) \]  \hspace{1cm} (D.1)

where \( a, b \) and \( c \) are fixed point constants and \( x_1(k), x_2(k) \) are fixed point variables. In this equation, \( y(k) \) is a recursive variable.

If a scale up parameter \( S \) is multiplied to both sides of (D.1), it can be expressed with a scaled up recursive variable \( Y(k) = S \cdot y(k) \) as
\[
Y(k+1) = (S \cdot a) \cdot x_1(k) + (S \cdot b) \cdot x_2(k) + c \cdot Y(k) .
\] (D.2)

In this case, \( Y(k) \) contains the scaled up value of \( y(k) \) and \( S \) is its scale. The total precision bit number of \( Y(k) \) is the summation of the bit number of \( y(k) \) and that for \( S \). The former contains the value larger than or equal to 1 while the latter contains value smaller than 1 when \( Y(k) \) is scaled down to \( y(k) \). From the computer simulation of (D.2), in which the results from fixed point calculation with the possible variations of \( x_1(k) \) and \( x_2(k) \) are compared with the ones from floating point calculation, one can find a scale value \( S \) within the given bit number, which minimizes the loss of precision during the calculation. After the calculation, \( y(k) \) can be expressed with scaled fixed point \( y_s(k) \) and the scale \( S_y \) in order to keep the best precision of \( Y(k) \) within the bit number of \( y(k) \), which are calculated as

\[
y_s(k) = Y(k) \cdot S_y / S = y(k) \cdot S_y .
\] (D.3)

All the fixed point variables during/after the calculation are expressed with fixed point values and their adjustable scales. Whenever coefficients \( a \), \( b \) and \( c \) need to be modified, the scale of each fixed point variable is calculated again in the computer simulation in advance for the minimization of the precision loss and is updated through the communication along with the coefficients. In this way, it is possible to minimize the loss of precision of fixed point variables during/after fixed point calculation.