

**Signals and Systems Tools for Advanced Nanoscale
Investigation with Atomic Force Microscopy**

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Acknowledgements

Memory of the first day of my graduate school which dates back by six and half years, seems like from yesterday. I remember the initial days when I just started working on my very first PhD research problem. I also remember the day when I found the most exciting set of results while working in my laboratory during the final years of PhD. One thing is true for sure; I was significantly different researcher during those moments. In the beginning I was uncertain, over-excited and also more anxious about every problem that I worked on. However, over the past years, I have developed considerable patience, ability to think critically, and confidence to approach problems. My PhD adviser Prof. Murti Salapaka has always been on my side and he is the one who tailored my transition from a novice to a much more matured problem solver. I deeply value the hands-on research experience that I acquired while working at the nano-dynamics systems laboratory (NDSL) and believe that these memories will keep guiding me throughout the rest of my career. I am grateful to Prof. Murti Salapaka for giving me the opportunity to expose myself to the cutting edge experimental setups at NDSL and developing very well balanced skills for theoretical as well as experimental research.

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Abstract

The atomic force microscope (AFM) is one of the major advancements in recent science that has enabled imaging of samples at the nanometer and sub-nanometer scale. Over the years, different techniques have been developed to improve the speed, resolution and accuracy of imaging using AFM. Further, the application spectrum of AFMs has extended beyond topography imaging, examples of which include material characterization, probe based data storage systems, and also single molecule force spectroscopy. In spite of the remarkable achievements by AFM technologies, many challenges exist. While majority of this thesis aims to address important challenges that exist with state of the art AFM methodologies using tools from signal processing and systems theory, it also reports some surprising new phenomena that are observed from AFM based mechanical characterization of protein molecules. The techniques developed in each chapter are extensively verified with simulation and experimental results.

A key issue that remains largely unaddressed in the AFM literature is the assessment of fidelity of the measurement data. The first contribution of this thesis is to develop a quantitative measure for the fidelity of images obtained from a fast dynamic mode AFM technique. The developed paradigm facilitates user specific priority for either detection of sample features with high decision confidence or on not missing detection of true features. The fidelity measures developed in this thesis are suitable for real-time implementation.

The second contribution of this thesis is to develop and compare the performance of different methods to characterize mechanical properties of materials utilizing the dynamic mode of AFM operation. The dynamic mode AFM is particularly suitable for investigating soft-matter. Here, an important enabler is the viewpoint of an equivalent cantilever. The parameters of the equivalent cantilever need to be estimated to derive material properties. In this thesis, we develop a new steady-state based estimation of equivalent parameters (SEEP) and compare it with the recursive estimation of equivalent parameters (REEP). We show that the SEEP is considerably simpler to implement, however, SEEP is a low bandwidth method when compared to REEP. Both methods yield material parameters that quantitatively agree in the domain of validity

of the methods. This thesis also streamlines the process of material identification and outlines the key pitfalls that need to be avoided for quantitative estimation of material parameters. Extensive design of a system identification module is reported which implements the REEP algorithm on modern field programmable gate arrays (FPGA). The step by step design procedure of the module explained in this thesis is employable to the development of a wide variety of FPGA based signal processing systems.

The third contribution of this thesis is a new system model detection technique called the innovations squared mismatch. Such detection of a model from a set of models that best describes the behavior of a system is of primary importance in many applications. Here, two discriminating signals are derived from measurements for a plant that switches between two model behaviors, where the transfer functions from inputs to the two signals are identical when one model is effective while they are negative of one another when the other model is effective. Further, we report sequence based detection approaches to extend the use of the signals for high bandwidth applications. In such applications the plant behavior can switch from one model to another at high rates and the transients from a previous behavior affect the current behavior causing inter-symbol interference (ISI). Methods developed are specialized for probe based data storage where experimental data demonstrates that they offer significant advantages over current methods.

The fourth contribution of this thesis is the first ever characterization of mechanical properties of utrophin protein molecule and its different terminal fragments using AFM based force spectroscopy experiments. Utrophin and its homologue dystrophin are proteins which are believed to play vital roles in mechanically stabilizing the muscle cells during stretch and relax cycles. These proteins are also under active research for finding possible cure for the disease muscular dystrophy. In this thesis we report markedly different mechanical characteristics for the utrophin constructs where previous thermodynamic studies measured identical thermal denaturation profiles. Our findings signify the need for force spectroscopy based characterization of molecules that are believed to play important mechanical roles in human body.

Contents

Acknowledgements	i
Abstract	iii
List of Tables	viii
List of Figures	ix
1 Introduction	1
2 Fidelity imaging techniques for AFM	6
2.1 Introduction	6
2.2 Preliminaries	9
2.3 Decision thresholds and fidelity metrics	11
2.3.1 Fidelity and threshold selection for LMP imaging	11
2.3.2 Fidelity and threshold selection for GLRT and MGLRT imaging	15
2.3.3 Determining statistics of impulsive force	18
2.4 Simulation results	20
2.5 Experimental results	26
2.6 Conclusions	31
3 Quantitative determination of material properties with dynamic mode AFM	33
3.1 Introduction	33
3.2 Preliminaries	36

3.3	Estimation of equivalent parameters	38
3.3.1	Steady-state estimation of equivalent parameters (SEEP)	38
3.3.2	Discrete-time recursive estimation of equivalent parameters (REEP)	39
3.4	Equivalence of storage and dissipative powers	40
3.5	Simulation results	44
3.6	Experimental results	46
3.6.1	Selection of system model	46
3.6.2	Estimation performance on transient data	48
3.6.3	Approach-retract experiments on mica	49
3.6.4	Stiffness and dissipation imaging of PLMA-PBMA polymer blend	54
3.7	Conclusion	56
4	Design of FPGA based material characterization module for AFM	58
4.1	Introduction	58
4.2	Design of system identification module on FPGA	60
4.2.1	Components in Main Module	62
4.2.2	Architectural backbone	66
4.2.3	Design steps	70
4.3	Results	72
4.3.1	Verification of BCEWRLS algorithm	72
4.3.2	Simulation of system identification module	73
4.3.3	Experimental implementation	75
4.4	Conclusions	78
5	Innovations squared mismatch based model detection for probe based data storage systems	80
5.1	Introduction	80
5.2	Real time plant detection with innovations squared mismatch (ISM)	84
5.2.1	Problem formulation, preliminaries and MAP	84
5.2.2	Innovations squared mismatch (ISM)	86
5.2.3	Computational complexity	91
5.3	Plant detection with known dwell interval; sequence detection	92
5.3.1	Maximum Likelihood Sequence Detection	93

5.4	Application of ISM and ISM-MLSD to probe based data storage; benefits and comparison of ISM with MAP based approach	96
5.4.1	Validation of equivalent model and detection framework	98
5.4.2	Real time detection	103
5.4.3	Symbol by symbol and sequence detection	109
5.5	Conclusions	112
6	Single molecule studies of utrophin protein constructs	114
6.1	Introduction	114
6.2	Force spectroscopy experiments on titin I27O constructs	119
6.3	Force spectroscopy studies on utrophin molecules	123
6.4	Conclusions	130
7	Conclusion and Discussion	131
	References	134
	Appendix A.	150
A.1	Derivation of steady state estimation of equivalent parameters (SEEP) .	150
A.2	Recursive update equations for BCEWRLS algorithm	153
A.3	Transfer function from $[x(t)^T, g(t)^T]^T$ to $[e_{dif}(t), e_{sum}(t)]^T$	154
A.4	Computational complexity of MAP	156

List of Tables

3.1	(Simulation data) Time constants involved in estimation of equivalent parameters (in milliseconds).	46
4.1	Number of different computational cores (latencies indicated in brackets) used in each module.	68
4.2	Number of different computational cores (latencies indicated in brackets) used in each module.	69
4.3	Timing schedule for Gain-Bias module.	70
6.1	Comparison of estimated parameters of Titin I27O molecule with specification sheet.	122

List of Figures

1.1	Atomic force microscope (AFM). A cantilever beam with a sharp tip at one end interacts with the sample. The inter-atomic forces between the tip and the sample deflects the cantilever. The cantilever deflection is monitored using a laser incident on the back of the cantilever surface that is collected by a split photodiode. A controller processes the deflection signal and actuates the piezoelectric positioner that can place the sample relative to the cantilever tip with sub-atomic resolution. In the standard contact mode operation, the sample is scanned laterally under the cantilever tip and the tip deflection is maintained at a constant set-point by the controller. The z -directional movement of the piezoelectric positioner as a function of the lateral coordinates (x - y) is interpreted as the topography of the sample. In the dynamic mode of operation, the cantilever is forced sinusoidally, typically at or near the first resonant frequency of the cantilever. During the oscillatory trajectory, the cantilever tip interacts intermittently with the sample. Such intermittent tip-sample interactions alter the amplitude and phase of the cantilever oscillations. Different dynamic mode operations exist where in a popular mode, namely, the amplitude modulation AFM (AM-AFM), the amplitude of cantilever oscillations is kept constant while actuating the z -piezo. The movement of z -piezo while scanning the sample in x - y plane reveals the topography.	3
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2.1	Fidelity imaging architecture for fast dynamic mode AFM. In dynamic mode of operation, the cantilever is excited with sinusoidal excitation g at or near its first mode resonant frequency. For fast imaging, the measured deflection y is input to an observer matched to the free-air cantilever dynamics which estimates the deflection $\hat{y}$. The tracking error $e = y - \hat{y}$ from observer is processed to generate the likelihood ratio signal l . l is compared with user adjustable threshold τ to generate the decision signal d on the presence or absence of topographic features. False alarm rate and detection accuracy of decision signal d depend on the choice of decision threshold. Further, fidelity metrics are defined that assign confidence measures on the decision signals. Final outputs of the fidelity imaging architecture are s_1 : the likelihood signal, s_2 : the decision signal and s_3 : the fidelity metric.	7
2.2	Image of DNA obtained with AFM from a $2\ \mu m \times 2\ \mu m$ area. The DNA occupies only a small fraction of the image.	8
2.3	The piecewise linear model for tip-sample interaction h which varies non-linearly with deflection of the cantilever tip p . A double spring-damper system with parameters (ω_a, c_a) for attractive spring and (ω_b, c_b) for repulsive spring models the long range attractive and short range repulsive regions of h . The cantilever model parameters, namely the spring stiffness k , damping c and mass m can be measured using frequency sweep method and thermal measurements in free air. The tip-sample model parameters (ω_b, c_b) and (ω_a, c_a) can be estimated using the harmonic balance method developed in [1].	20
2.4	(Simulation result) (a) Height image. The difference between low and high topography is 6 nm. The scale is normalized with respect to the maximum height. (b) LMP likelihood image and (c) MGLRT likelihood image. x axis represents time in the unit of cantilever oscillation period T_0 ($1/T_0=63.14$ kHz). y axis represents index of scan line. Both LMP and MGLRT images are normalized with respect to corresponding maximum values. The colorbars specify the ranges.	21

2.5	(Simulation result) The probability distribution of ν obtained from simulation. The y axis is normalized with respect to the maximum value of the distribution. The histogram is fit with a Gaussian distribution with mean ν_0 and standard deviation σ_ν	22
2.6	(Simulation result) The probability of false alarm P_{FA} and misdetection $1 - P_D$ for (a) LMP technique with decision threshold τ_{lmp} and (b) for MGLRT scheme with decision threshold τ_m . The x axes are normalized with respect to the maximum likelihood ratios received for both cases. .	23
2.7	(Simulation result) (a) LMP decision image for low threshold and (b) corresponding fidelity image. (c) LMP decision image for high threshold and (d) corresponding fidelity image. Color bars specify the ranges. . .	24
2.8	(Simulation result) (a) MGLRT decision image for low threshold and (b) corresponding fidelity image. (c) MGLRT decision image for high threshold and (d) corresponding fidelity image. The color bars specify the ranges.	25
2.9	(Experimental data) (a) Height image (nm) for silicon calibration sample taken at slow speed of 2 Hz. High speed images taken at 31.25 Hz showing (b) the estimate for strength of tip-sample interaction $\hat{\nu}$, (c) LMP likelihood and (d) MGLRT likelihood signals. $\hat{\nu}$, LMP and MGLRT images are normalized with respect to their maximum values obtained during imaging. The color bars specify corresponding ranges. x and y axes represent lengths of $2 \mu m$ and $4 \mu m$ respectively.	26
2.10	(Experimental data) Estimated distribution of the strength of tip-sample interaction ν . The y axis is normalized with respect to the maximum value of the distribution. The histogram is fit with a Gaussian distribution with mean $\nu_0 = 3.59e - 3$ and standard deviation $\sigma_\nu = 6.98e - 4$. .	27
2.11	(Experimental data) The false alarm rate P_{FA} and probability of misdetection $1 - P_D$ performance of (a) the LMP method and (b) the MGLRT method with different thresholds. The decision thresholds for both cases are normalized with respect to the maximum value of likelihood signals received from experiment.	28

2.12	(Experimental data) (a) LMP decision image and (b) fidelity image for low decision threshold. (c) LMP decision image and (d) fidelity image for relatively higher decision threshold. The color bars specify corresponding ranges.	30
2.13	(Experimental data) (a) MGLRT decision image and (b) fidelity image for low decision threshold. (c) MGLRT decision image and (d) fidelity image for relatively higher decision threshold. The color bars specify corresponding ranges.	31
3.1	Estimation of storage and dissipation properties of the sample based on identifying equivalent parameters. The system including cantilever and tip-sample interaction in the dynamic mode operation is modeled with an equivalent cantilever model which is described by parameters (ω_{eq}, Q_{eq}) with $f_{eq} = \omega_{eq}/2\pi$ as shown in the figure. The equivalent parameters (f_{eq}, Q_{eq}) are further utilized for estimating average storage power Φ_C and dissipative power Φ_D due to tip-sample interactive force $h = \phi(p, \dot{p})$	36
3.2	(Simulation data) Equivalent parameters $(f_{eq} = \omega_{eq}/2\pi, Q_{eq})$ estimated using SEEP and REEP techniques during the transient and steady-state part of the trajectory of the oscillating cantilever. When <i>sep</i> is low (high), the oscillating cantilever interacts (does not interact) with sample. In absence of sample, the cantilever is described by ideal spring-mass-damper model with resonant frequency $f_0 = \omega_0/2\pi = 63.147$ kHz and quality factor $Q_0 = 227.85$	44
3.3	(Experimental data) Frequency response of cantilever in free air. Data is fit using a plant model with $\beta_1 = 0$ denoted by Fit 1. Fit 2 is obtained using nonzero β_1 . Fit 2 better approximates the frequency response.	47
3.4	(Experimental data) (a) The input signal Z_{in} (in V) to z-piezo actuator with amplitude of cantilever oscillations <i>Amp</i> (in meter). (b) REEP estimated equivalent frequency f_{reep} and SEEP estimated equivalent frequency f_{seep} (in Hz). (b) REEP estimated equivalent quality factor Q_{reep} with SEEP estimated quality factor Q_{seep} . Time axis is in the units of T_s where $1/T_s$ is 2 MHz.	49

3.5	(Experimental data) (a) Deflection amplitude a (meter) during approach-retract cycle on freshly cleaved mica. (b) Corrected phase $\tilde{\theta}$ (degrees). (c) Equivalent frequency using REEP (f_{reep}) and SEEP method (f_{seep}) in Hz. (d) Equivalent quality factor using REEP (Q_{reep}) and by SEEP method (Q_{seep}). The reference position is chosen in free air. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.	50
3.6	(Experimental data) (a) Deflection amplitude a (meter) during approach-retract cycle on freshly cleaved mica. (b) Dissipation power using REEP (Φ_D) and by (3.21) shown as P_d (unit Watt). (c) Storage power Φ_C using REEP and using (3.23) denoted as P_{st} in Watt. The reference position is chosen in free air. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.	50
3.7	(Experimental data) (a) Oscillation amplitude a (meter) (b) Corrected phase $\tilde{\theta}$ (degrees). (c) Equivalent frequency using REEP (f_{reep}) and SEEP method (f_{seep}) in Hz. (d) Equivalent quality factor using REEP (Q_{reep}) and SEEP method (Q_{seep}). The reference position is chosen near sample to include squeeze film damping. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.	52
3.8	(Experimental data) (a) Oscillation amplitude a (meter). (b) Dissipation power using REEP (Φ_D) and P_d using (3.21) in Watt. (c) Storage power using REEP (shown as Φ_C) and using (3.23) denoted as P_{st} in Watt. The reference position is chosen near sample to include the squeeze film damping effect. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.	52
3.9	(Experimental data) (a) Height image (nm), (b) amplitude image (nm) and (b) $\tilde{\theta}$ phase image (degrees) of a $20\text{ um} \times 20\text{ um}$ PLMA-PBMA polymer sample.	54
3.10	(Experimental data) (a) Shift of equivalent frequency of oscillating cantilever around the bias frequency of 72.273 kHz estimated through REEP (f_{reep}) and (b) using SEEP method (f_{seep}) in Hz. (c) Equivalent quality factor (Q_{reep}) evaluated using REEP and (d) using SEEP method (Q_{seep}).	55
3.11	(Experimental data) (a) Storage power Φ_C evaluated through REEP and (b) P_{st} estimated using (3.23) in pW. (c) Dissipative power Φ_D estimated by REEP and (d) P_d estimated using (3.21) in pW.	56

4.1	Outline of the system identification module implemented on FPGA . . .	60
4.2	Modules and flow of signals inside the Main Module	61
4.3	Illustration of the timing of clock and count signals.	63
4.4	Amplitude demodulation scheme	65
4.5	The architectural backbone. <i>Clk</i> operates at 40.96 MHz. <i>Count</i> repeats counting from 0 to $N - 1$ with $N = 40$. MUX and DMUX represent the multiplexer and de-multiplexer blocks respectively inside a functional subunit.	67
4.6	(a) Estimated b_0 and (b) b_1 using BCEWRLS with corresponding true value from simulation. $\lambda = 0.99995$ creates very slow estimation rendering the method ineffective. $\lambda = 0.95$ yields fast but incorrect convergence. $\lambda = 0.995$ and 0.9995 create correct convergence of estimated b_0 and b_1 to their true values.	72
4.7	(a) Cantilever excitation signal (V) and (b) deflection signal (nm) acquired during an approach-retract experiment performed with the dynamic mode AFM. Comparison between the estimates of (c) b_0 and (d) b_1 generated by the system identification modules implemented in MATLAB and VHDL.	74
4.8	Comparison between (a) Equivalent frequency f_{eq} (Hz), (b) equivalent quality factor Q_{eq} , (c) conservative power Φ_C (Pico-Watt) and (d) dissipative power Φ_D (Pico-Watt) estimated by the system identification modules designed in MATLAB and VHDL.	75
4.9	(Experimental data) (a) Height (meter), (b) amplitude (meter) and (c) phase image (degree) using dynamic mode AFM obtained from a $10\mu m \times 10\mu m$ PLMA-PBMA polymer blend. The color bars specifies the ranges.	76
4.10	(Experimental data) (a) Shift of the discrete time equivalent parameter, $b_0 - b_{0,in}$, where $b_{0,in} = 0.9969$, (b) shift of parameter, $b_1 - b_{1,in}$, where $b_{1,in} = -1.8013$, (c) shift of equivalent frequency, $f_{eq} - f_0$ (in Hz), where $f_0 = 72.273$ kHz and (d) equivalent quality factor Q_{eq} obtained from a $10\mu m \times 10\mu m$ PLMA-PBMA polymer blend using the system identification module implemented on FPGA . The color bars specify the corresponding ranges.	77

4.11	(Experimental data) (a) Estimated conservative power Φ_C (in watt) and (b) dissipative power Φ_D (in watt) due to periodic tip-sample interaction estimated using the FPGA module. The color bars specify corresponding ranges.	78
5.1	The concept of equivalent cantilever model for dynamic mode AFM is explained in Section 3.2, Chapter 3. BCEWRLS method is utilized to obtain equivalent linear models when the cantilever is oscillating in free air (model 1) and when it is interacting with the sample surface at fixed oscillation amplitude (model 2). Observers generate tracking errors $e^{(1)}$ and $e^{(2)}$ that are processed using the techniques MAP and ISM for real time interpretation of topography. MLSD, ISM-MLSD further improve the detection accuracy for known dwell interval in presence of inter symbol interference.	82
5.2	(a) State transitions for $m = 1$, $m_I = 1$. At each stage i , S_i can assume four possible states. (b) Eight possible states S_i for $m = 1$, $m_I = 2$. Transition from stage S_{i-1} to S_i occurs when a new bit $a_i \in \{0, 1\}$ is input. State transitions for $a_i = 0$ ($a_i = 1$) are shown in broken (solid) lines. Increasing the assumed memories extends the complexity and memory requirements for the statistical learning step and the Viterbi detection algorithm.	95
5.3	(a) Estimation of b_0 using BCEWRLS ($\lambda = 0.995$) with ISM decision from simulation. (b) Estimation of b_1 with ISM decision from simulation. Both the estimates of b_0 and b_1 undergo lengthy transient behavior when the plant switches from one model to another. ISM decision signal is much faster and hence justifies its effectiveness for real time deciphering of the active plant model.	98

5.4	Verification of equivalent cantilever model using averaging theory technique. The oscillating cantilever is made to interact with sample for gradually ascending interaction (approach) followed by descending interaction (retract). With increasing (reducing) l_{int} , the amplitude of oscillation A_{def} reduces (increases). BCEWRLS scheme is applied to calculate equivalent cantilever parameters; equivalent resonant frequency f_{eq} and equivalent quality factor Q_{eq} . Using averaging theory f_{eq} and Q_{eq} are analytically calculated for the tip-sample interaction model. Theoretically predicted and estimated values for Q_{eq} are close match. With small interactions (low l_{int}), f_{eq} match very well with theory. For higher interactions (high l_{int}), f_{eq} deviates slightly from analytical predictions.	100
5.5	ISM system: Simulation model for verification of the switching model. .	102
5.6	(a) e_{dif} and e'_{dif} with sep from simulation. These signals have no detectable difference. (b) e_{sum} and e'_{sum} from simulation. These signals match perfectly for no interaction from sample (model 1). The phase behaviors of e_{sum} and e'_{sum} match during interaction with sample (model 2).	102
5.7	(Simulation result) (a) e_{dif} and e_{sum} oscillate in phase (out of phase) when cantilever interacts with the sample (is in free air). (b) Envelope of $(e^{(1)})^2 - (e^{(2)})^2$ changes sign as the cantilever goes from off-sample to on-sample situation and vice versa. sep high (low) implies free air oscillation (interaction).	103
5.8	(Experimental Data) (a) hgt denotes measured height of sample. The sample media changes to a raised topographic profile at $50 \mu sec$. From $0 - 50 \mu sec$ there is no interaction between the sample and the cantilever; for the rest of the duration of the experiment, cantilever interacts with the sample. e_{sum} and e_{dif} signals are in phase within a couple of cantilever oscillations of the heightened topographic profile appearing agreeing with analytical results. In the absence of the sample interaction (from $0 - 50 \mu sec$) e_{sum} and e_{dif} differ in phase by π radians. (b) Envelope of $(e^{(1)})^2 - (e^{(2)})^2$ exhibits sign inversion within a few cycles of cantilever oscillations similar to seen in simulation results.	104

5.9	(Simulation result) (a) Conventional dynamic mode AFM amplitude image (A_{def}) and ISM amplitude image ($ISM_{l_{pf}}$) with cantilever-sample separation (sep). $ISM_{l_{pf}}$ is faster and exhibits significant independence of topographical history. (b) Regular phase image (ϕ_{def}) and ISM generated phase image (ϕ_m) from simulation. ϕ_m is more suitable for high speed binary deciphering of topography.	106
5.10	(Experimental Data) (a) Conventional amplitude A_{def} and $ISM_{l_{pf}}$ from experiment. A_{def} shows an overall drift from left to right. However, $ISM_{l_{pf}}$ has more consistent levels. (b) Regular phase ϕ_{def} and ISM based phase ϕ_m signals from experiment. ϕ_m has a very consistent binary behavior and better steady state magnitudes than ϕ_{def}	107
5.11	(Simulation result) (a) MAP and (b) ISM based detection. ISM does not exhibit decision glitches as shown by MAP.	108
5.12	(Experimental Data) (a) MAP and (b) ISM based decision images d_{MAP} and d_{ISM} . d_{MAP} is severely effected by decision glitches whereas d_{ISM} has correct decision making capability.	108
5.13	(Simulation result) Bit error rate (BER) performance of symbol-by-symbol ISM and MAP with regular MLSD and ISM-MLSD as a function of interaction length l_{int} . Increasing l_{int} over the range 1 nm to 2 nm gradually improves BER performance of each scheme. Performance of ISM and MAP are close to each other. Regular MLSD lags slightly in error rate compared to other methods. However ISM-MLSD consistently outperforms all the other methods. Dwell time T is chosen as 250 micro seconds.	109
5.14	(Experimental data) Bit error rate (BER) performance of symbol-by-symbol ISM and MAP with varying threshold location. Dwell time T is chosen as 350 micro seconds.	110
5.15	(Experimental data) (a) Example mean observation vectors $\bar{\mathcal{Y}}$ for regular MLSD. (b) $\bar{\mathcal{Y}}$ for ISM-MLSD. The 3 state transitions shown here are $(S_{i-1}, S_i) \in \{(000, 001), (110, 101), (011, 110)\}$. The statistics pairs $(\bar{\mathcal{Y}}, \mathcal{C})$ in turn are used for Viterbi detection. Dwell time T is chosen as 350 micro seconds.	112

6.1	Homologous structures of dystrophin and utrophin. Both have two actin binding domains indicated by ABD1 and ABD2. In dystrophin the domains are separated by triple-helical spectrin repeats (ovals). The C-terminal (CT) domain is attached to the sarcomere. In dystrophin, unlike utrophin, ABD1 and ABD2 are separated by 10 spectrin-type repeats (STRs) [2]	115
6.2	Model for how dystrophin may function as a molecular shock absorber to dampen elastic extension during muscle stretch. I) Relaxed muscle. II) Stretch imposes forces that uncoil spring-like elements within repeats 1-10 and 18-24. III) Electrostatic interaction of basic actin binding repeats 11-17 with acidic actin filaments dampens extension of the spring-like elements (see [3]). GPC denotes the glycoprotein complex.	116
6.3	(A) A cantilever with a tip to which an end of the protein molecule with multiple domains is attached. The other end sits on a substrate. The tip deflection can be measured using a laser incident on the back of the cantilever which reflects into a quadrant photodetector. (B) The force on the molecule is estimated as kp where k is the stiffness of the cantilever flexure and p is the cantilever deflection measured by the laser-photodiode mechanism. The separation b between the substrate and the base of the cantilever support can be controlled with sub-nanometer precision using a nanopositioner. The extension of the molecule under externally applied force is denoted by sep which can be measured using $sep = b - p$. (C) In a typical puling experiment the separation between the base of the cantilever and the substrate is increased where the bonds unfold and a sawtooth pattern is observed in the force-extension data.	118
6.4	Force versus extension characteristics of titin construct I27O. The peaks in the extension data are identified as the bond unfolding events. The extension of the protein in between the bond ruptures are identified and fit with WLC models described by persistence length, P , and contour length, L_c . The x -axis denoted by sep represents the separation of the cantilever tip from the substrate (nm), which is the same as the extension of the molecule when it is taught. The y -axis represents the force acting on the molecule (pN).	120

6.5	(a) Distribution of rupture forces (N), and (b) distribution of persistence length (m) determined from force spectroscopy experiments of titin I27O molecules.	121
6.6	(a) Distribution of peak to peak distance (m), and (b) distribution of increments in contour length (m) due to bond ruptures determined from force spectroscopy experiments of titin I27O molecules.	121
6.7	(a) Maximum unfolded length (m) as a function of rupture count, and (b) relative frequency of observed rupture counts determined from force spectroscopy experiments of titin I27O molecules.	121
6.8	Full length utrophin and its different constructs investigated by force spectroscopy experiments. Molecular bond unfolding is also studied by thermal melting experiments.	123
6.9	Thermal melt curves for different utrophin constructs exhibit uniform unfolding characteristics [4].	124
6.10	Example of force spectroscopy data obtained from (a) Utr NT-R10, (b) Utr R11-CT, and (c) Utr FL protein constructs. Data suggests ‘brittle’ behavior of Utr NT-R10 versus ‘stiffening spring’ like behavior of Utr R11-CT and Utr FL constructs.	125
6.11	Estimated probability distribution of unfolding forces for (a) Utr NT-R10, (b) Utr R11-CT, and (c) Utr FL from constant speed pulling experiments.	127
6.12	Estimated distribution of unfolding forces for (a) Utr R1-R10, (b) Utr R11-R22, and (c) UtrR1-R22 from constant speed pulling experiments. Removing the terminal domains NT and CR-CT from the constructs do not alter their ‘brittle’ or ‘stiffening spring’-like characteristics.	128
6.13	Peak unfolding forces as a function of the index of rupture event for the different utrophin constructs.	129

Chapter 1

Introduction

The atomic force microscope (AFM) is widely employed to explore materials at the nanometer or sub-nanometer scale. Since the invention of the AFM [5], researchers have developed a diverse set of AFM based measurement techniques with significant impact on science and technology. For example, structure of a single pentacene molecule is revealed by AFM in [6], AFM techniques are utilized to realize data storage solutions with ultra-high densities (more than 5 Tb/in²) [7], high speed AFM imaging scheme suitable for video rate imaging of biological motors is reported in [8] and mechanical properties of interesting polymer and biological samples are investigated in [9–12]. Apart from imaging, AFMs are also the preferred instruments for force spectroscopy measurements on single protein molecules [13,14]. A detailed set of applications where AFMs are used can be found in [15,16].

The principal component of the AFM is a flexible cantilever probe which can be moved in x - y - z directions with respect to the sample surface by a piezo-electric actuator (see Figure 1.1). The cantilever has a sharp tip at one end. The atoms on the tip experience forces when in close proximity to atoms on the sample surface. The inter-atomic tip-sample forces deflect the cantilever which is measured by a laser-photodiode setup. In traditional contact mode operation, the cantilever is scanned over the sample surface in the x - y plane where the cantilever deflection is regulated at a desired set point by actuating the z -positioner. The vertical movement of the z -positioner used to regulate constant deflection of the cantilever is interpreted as the sample topography. In the contact mode of operation, considerable wear and tear is possible for both the

tip and the sample. In the dynamic mode of operation, the cantilever is sinusoidally excited at or near its first resonant frequency (see Figure 1.1). Unlike the traditional contact mode operation where the AFM probe is in continuous contact with sample, in the dynamic mode the oscillating cantilever interacts with the sample intermittently only during a very small fraction of its entire orbit. The interactive forces between tip and sample caused by gentle tapping of the sample surface change the orbit of the oscillating cantilever. These changes include alteration of the amplitude of oscillation and the phase of oscillation with respect to the drive, which are utilized for interpreting the topography and other features of the sample. Due to small duration of interaction between the tip and sample, the dynamic mode operation [15] results in minimal mechanical wear of the probe and sample material [17] compared to the contact mode of operation [18]. Thus the dynamic mode is particularly suitable for investigating soft samples including the soft polymers and biological samples.

The advantage of dynamic mode operation is clear. However, the method presently faces multiple challenges. Different chapters of this dissertation except the last chapter address such different challenges associated with the dynamic mode AFMs. The last chapter (Chapter 6) reports the first ever mechanical characterization of utrophin protein molecules utilizing AFM based force spectroscopy methods. The analytical techniques and conclusions developed in each chapter are verified extensively utilizing simulation and experimental setups. The key contributions of this thesis are summarized below:

- **Chapter 2:** The AFM is a metrology instrument with wide-spread applications in measurement science at the nano-scale. However, a key measurement metric which is lacking in most of the AFM literature is the goodness and error-bars associated with the measurement data. Such problem is particularly relevant for the dynamic mode AFM, where quantitative interpretation of measurement data is complex. Chapter 2 introduces the concept of fidelity imaging methods for dynamic mode AFM. Here, we adopt a fast dynamic mode AFM based topography measuring scheme and develop, from first principles, the fidelity measurement techniques which quantitatively measure goodness or fidelity of the measurement outcomes.

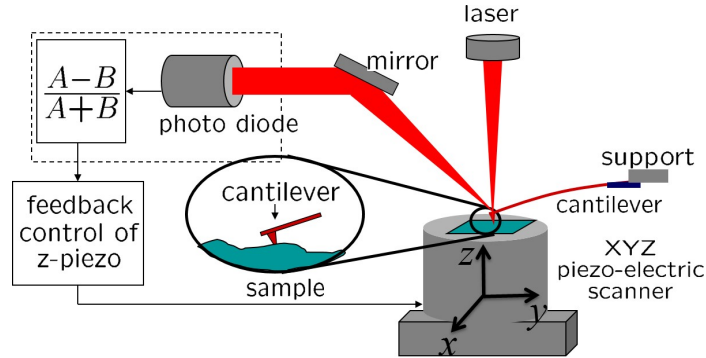


Figure 1.1: Atomic force microscope (AFM). A cantilever beam with a sharp tip at one end interacts with the sample. The inter-atomic forces between the tip and the sample deflects the cantilever. The cantilever deflection is monitored using a laser incident on the back of the cantilever surface that is collected by a split photodiode. A controller processes the deflection signal and actuates the piezoelectric positioner that can place the sample relative to the cantilever tip with sub-atomic resolution. In the standard contact mode operation, the sample is scanned laterally under the cantilever tip and the tip deflection is maintained at a constant set-point by the controller. The z -directional movement of the piezoelectric positioner as a function of the lateral coordinates (x - y) is interpreted as the topography of the sample. In the dynamic mode of operation, the cantilever is forced sinusoidally, typically at or near the first resonant frequency of the cantilever. During the oscillatory trajectory, the cantilever tip interacts intermittently with the sample. Such intermittent tip-sample interactions alter the amplitude and phase of the cantilever oscillations. Different dynamic mode operations exist where in a popular mode, namely, the amplitude modulation AFM (AM-AFM), the amplitude of cantilever oscillations is kept constant while actuating the z -piezo. The movement of z -piezo while scanning the sample in x - y plane reveals the topography.

- **Chapter 3:** Traditionally, AFMs were utilized only for measuring the topography of samples. However, with the advancement of applications of AFMs, researchers have also developed techniques for characterizing the mechanical properties of materials at nano-scale. Here, the dynamic mode of operation is suitable for characterizing soft samples. Chapter 3 develops a simple analytical method termed as the steady state estimation of equivalent parameters (SEEP) method to quantitatively estimate the

surface stiffness and dissipative properties of materials with dynamic mode AFM. The SEEP method is compared with an existing method, namely, the recursive estimation of equivalent parameters (REEP) technique which employs a system identification algorithm requiring more complex signal processing. We identify some key experimental steps that must be carried out properly for the above approaches to work. Further, we demonstrate the equivalence between the dynamic mode methods developed independently by different researchers under some key assumptions.

- **Chapter 4:** The REEP technique outlined in Chapter 3, is capable of estimating the storage and dissipative parameters even during the transient phase of cantilever oscillations. However, implementing REEP in real-time requires the design of a complex signal processing module which is explained in Chapter 4. This module is developed on modern field programmable gate arrays (FPGA), namely, the ML605 DSP development kit from Xilinx which hosts Virtex-6 generation FPGA with high speed FMC-151 ADC-DAC unit. The design steps elaborated in this chapter are employable to designing other real-time signal processing modules as well. This module can be interfaced with commercially available AFMs. It processes the raw deflection and excitation signals of the AFM cantilever in dynamic mode to yield real-time estimates of the stiffness-dissipative properties of the sample.
- **Chapter 5:** Probe based data storage systems employ flexible probes to read and write data over the topography of storage media. Such storage technology can achieve extremely high data density compared to other methods. However, a key challenge for probe based storage systems is the short device lifetime caused by mechanical wear and tear of the storage media as well as of the probe. The dynamic mode of operation can improve the device lifetime significantly. However, the dynamic mode is widely considered unsuitable due to its slow and complex data acquisition method. In Chapter 5, we investigate novel techniques of system identification, namely the innovations squared mismatch (ISM) methods for fast and accurate data readout operation using the dynamic mode. We further utilize the maximum likelihood sequence detection methods with ISM to compensate for the inter-symbol-interference that arise during fast data readout. Effectiveness of the proposed methods are corroborated via extensive simulation and experimental data.

- **Chapter 6:** Duchenne Muscular Dystrophy (DMD) is a fatal disease which presently has no cure. Research has demonstrated that abnormalities in the protein dystrophin play a key role in the disease. Dystrophin and its homologue protein utrophin are believed to play important roles in supporting the mechanical structures of muscle cells during the stretch and relax cycles. Thus it is imperative to understand the mechanical properties of dystrophin and utrophin protein molecules. However, only a few studies of the mechanical properties of some dystrophin constructs are reported in existing literature. Utrophin and its deletion constructs which are demonstrated to be effective to compensate for the dystrophin deficiency in certain cases, are under active investigation for therapeutic purposes. Presently there is no literature which details the mechanical characterization of the utrophin constructs. Chapter 6 reports the first ever mechanical characterization of full-length utrophin molecule and a number of its different deletion constructs. Such mechanical characterization is carried out utilizing AFM based force spectroscopy experiments. Our findings convey some remarkable information about the mechanical behavior of the utrophin constructs. The results also signify the need for a detailed theoretical development which can predict the mechanical behavior of these molecules as well as their roles in muscle tissues.
- **Chapter 7:** The conclusions derived from different chapters are summarized in Chapter 7.

Chapter 2

Fidelity imaging techniques for AFM

2.1 Introduction

The advantage of dynamic mode of operations over the contact mode AFM is explained in Chapter 1. However, in the dynamic mode, quantitative interpretation of the AFM data becomes challenging due to complex trajectories of AFM probe [9, 15, 19]. A natural question that arises in this context is the assessment of the *goodness* of the inferences drawn from AFM measurement data. Such emphasis on quantitative assessment of the quality of measurement data with error bounds is a standard practice in most instruments used for metrology. However, there is a lack of tools that quantify fidelity measures on the AFM measurements in real-time. Determining fidelity measures for AFM in general is a challenging problem. In this chapter, a real time method is developed to quantify fidelity of AFM measurements obtained using the dynamic mode operation and therefore is particularly suitable for studying soft matter such as biological and polymer materials [20, 21].

In related work [22], observer based architecture [23] is utilized to achieve detection of topographic features at high bandwidth. There, the tracking error generated by the observer is matched to a linear time-invariant model of the cantilever dynamics to generate a likelihood ratio based signal (see Figure 2.1). The likelihood ratio based signal is compared to a user defined threshold to decide presence or absence of topographic

features.

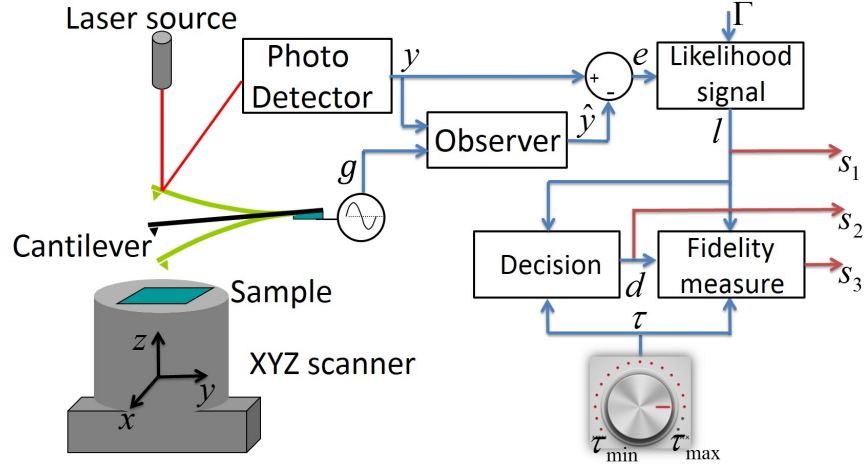


Figure 2.1: Fidelity imaging architecture for fast dynamic mode AFM. In dynamic mode of operation, the cantilever is excited with sinusoidal excitation g at or near its first mode resonant frequency. For fast imaging, the measured deflection y is input to an observer matched to the free-air cantilever dynamics which estimates the deflection $\hat{y}$. The tracking error $e = y - \hat{y}$ from observer is processed to generate the likelihood ratio signal l . l is compared with user adjustable threshold τ to generate the decision signal d on the presence or absence of topographic features. False alarm rate and detection accuracy of decision signal d depend on the choice of decision threshold. Further, fidelity metrics are defined that assign confidence measures on the decision signals. Final outputs of the fidelity imaging architecture are s_1 : the likelihood signal, s_2 : the decision signal and s_3 : the fidelity metric.

The fidelity metrics developed in this chapter assign quantitative confidence measures on the decisions. With the methods developed in this chapter it becomes possible for the decision image that shows absence or presence of a topographic feature at any pixel to be accompanied by a fidelity image. The fidelity image assigns a quantified measure of confidence on the decision reached at each pixel. The fidelity image accompanying the decision image is a function of choice of threshold selected by user. The user can prioritize detection of all true features by setting a low threshold or can emphasize that all features detected are true features by selecting a high threshold. Such facility

is particularly suitable for a two step exploration process; in the first step the user can obtain a quick overview of a large area of the sample for identifying/detecting regions that are promising for finer investigation. Here the user can set a low threshold that will help pruning out uninteresting areas of the sample with good confidence. The first exploratory step can be performed at high speeds thereby facilitating rapid pruning of areas with no features. This step can be followed by finer investigation of the areas which are left unpruned. For the second step a high threshold can be chosen to ensure that detected features are true features with high probability. We remark that often the image sought occupies a small fraction of the entire sample area (see Figure 2.2 that shows an image of a DNA obtained by an AFM with a height of 1.5 nm where the DNA strand occupies only a small fraction of the image). Thus the pruning step can identify the small region of interest which can be investigated with more attention.

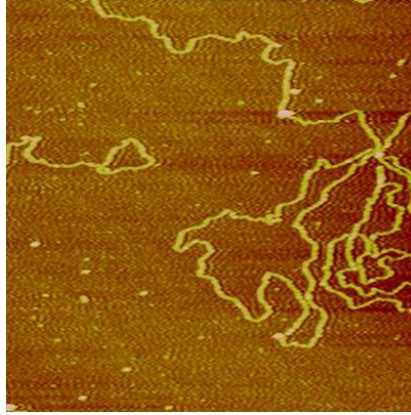


Figure 2.2: Image of DNA obtained with AFM from a $2\ \mu m \times 2\ \mu m$ area. The DNA occupies only a small fraction of the image.

Fidelity measurement for AFM was first introduced by the authors in a letter [24]. This chapter provides a detailed analysis of false alarm, misdetection rate and performance of the methods proposed in [24] which were not studied before. This chapter compares two different imaging methods, namely the locally most powerful (LMP) method [25, 26] and the generalized likelihood ratio test (GLRT) based method [25, 26] (described in detail later) with respect to threshold selection. An alternative detection scheme which is adopted in this chapter is referred to as the modified generalized likelihood ratio test (MGLRT) method. It is demonstrated that LMP based method is more

suitable than using MGLRT because the error rate performance of LMP is less sensitive to threshold selection compared to MGLRT. However, the MGLRT based method adopted in this chapter is well suited for real-time implementation in AFM setups.

This chapter is organized as follows: Section 2.2 briefly introduces the observer based architecture for fast dynamic mode AFM imaging. Section 2.3 develops and analyzes the false alarm rate, detection probability and fidelity metrics as a function of decision threshold. Section 2.4 verifies the analytical methods for fidelity imaging using realistic simulation models of the AFM setup. Section 2.5 demonstrates the efficacy of proposed methods using experimental results. Section 2.6 concludes this work.

2.2 Preliminaries

We first describe observer based dynamic mode AFM imaging methodology. The first mode approximation of the cantilever is governed by the dynamics

$$\ddot{p} + \frac{\omega_0}{Q}\dot{p} + \omega_0^2 p = f(t) = \frac{1}{m}(g + h), \quad (2.1)$$

$$h = \phi(p, \dot{p}), \quad y = p + v, \quad (2.2)$$

where p and $\dot{p}$ are the deflection and velocity of the cantilever at the tip location respectively. m is the mass of the lever. The total force per unit mass, $f(t)$, depends on the dither excitation input g and the nonlinear interactive force h between cantilever tip and sample. Typically, g is chosen to be sinusoidal with frequency close to the resonant frequency ω_0 . Q is the quality factor. ω_0 and Q can be determined from a frequency sweep method when the cantilever is free from sample. y is the measured deflection and v is the measurement noise. Equation (2.1) can be represented in state space form described by

$$\dot{x} = Ax + Bf, \quad y = Cx + v, \quad (2.3)$$

where the state is defined by $x = [p \ \dot{p}]^T$ with the matrices $A = [0 \ 1; -\omega_0^2 \ -\frac{\omega_0}{Q}]$, $B = [0 \ 1]^T$ and $C = [1 \ 0]$. In the dynamic mode of operation where the cantilever is excited by sinusoidal forcing g , the cantilever typically oscillates with a steady state amplitude ranging between 25 nm to 200 nm. The interaction with the sample occurs only during a very small fraction of cantilever orbit. Thus the force h between tip

and sample can be modeled as an impulsive force exerted by the sample on the tip when the tip is closest to the sample while oscillating. The impulsive force changes the cantilever state modeled as an instantaneous change. A discrete time model for cantilever dynamics with such impulsive interaction model can be written as [22]

$$\begin{aligned} x_{k+1} &= Fx_k + Gg_k + \delta_{\theta,k+1}\nu, \\ y_k &= Hx_k + v_k, \end{aligned} \quad (2.4)$$

where k denotes the index for discrete time. The matrices F , G and H can be obtained from continuous time model (2.3) by discretization. $\delta_{\theta,k+1}\nu$ denotes the sudden change in the state at the time of impact. ν denotes the strength of impulsive interaction and θ models the time of impact ($\delta_{i,j} = 1$ when $i = j$, else 0). A fast AFM imaging technique developed in [22] uses Kalman observer [23] for generating test signals. The Kalman observer is matched to the discrete time cantilever model described in (2.4). Here, let $\hat{x}_k$ be the estimated state from Kalman observer with observer gain L which follows the dynamics

$$\begin{aligned} \hat{x}_{k+1} &= F\hat{x}_k + Gg_k + L(y_k - H\hat{x}_k), \\ \hat{y}_k &= H\hat{x}_k, \quad e_k = (y_k - \hat{y}_k). \end{aligned} \quad (2.5)$$

From (2.4) and (2.5) it follows that, after an impact at time θ , e_k can be written as (see [22])

$$e_k = \Gamma_{k;\theta}\nu + \eta_k; \quad \Gamma_{k;\theta} := H(F - LH)^{k-\theta}, \quad (2.6)$$

where η_k is zero mean Gaussian noise with variance σ_η^2 . $\Gamma_{k;\theta}$ denotes the impulse response for the system that maps force input to the error signal e_k . Let M be the window size chosen for detection purposes. The binary hypotheses;

$$H_0 : e_k = \eta_k, \quad H_1 : e_k = \Gamma_{k,\theta}\nu + \eta_k, \quad (2.7)$$

represent absence and presence of sample with $k = 1, 2, \dots, M$. Let $\Gamma := [H, H(F - LH), \dots, H(F - LH)^{M-1}]^T$ and the observation vector $e := [e_1, e_2, \dots, e_M]^T$. Assuming the impact time $\theta = 1$, the hypotheses can be expressed in the form

$$H_0 : e = \eta, \quad H_1 : e = \Gamma\nu + \eta, \quad (2.8)$$

where η denotes the zero mean Gaussian noise vector $[\eta_1, \eta_2, \dots, \eta_M]$ with covariance matrix $V = \sigma_\eta^2 I$, I is identity matrix of size $M \times M$. The test vector e can be processed using either locally most powerful or generalized likelihood ratio techniques for which fidelity metrics are determined below.

2.3 Decision thresholds and fidelity metrics

The locally most powerful (LMP) and generalized likelihood ratio test (GLRT) methods [25, 27] are utilized to process the tracking error e_k for detection purposes. An alternative detection method is also adopted in this chapter which is referred to as the modified generalized likelihood ratio test (MGLRT) method. These methods yield likelihood ratios that are compared with respect to user defined thresholds for deciphering presence or absence of sample. The probability of false alarm (P_{FA}) is defined to be the probability that the user decides hypothesis H_1 incorrectly where the sample is absent. The probability of detection P_D is defined to be the probability that the user correctly decides hypothesis H_1 in presence of the sample. The decision threshold τ set by the user yields a trade-off between the false alarm rate P_{FA} and probability of misdetection $1 - P_D$. Fidelity metrics, P_{FA} and P_D for LMP, GLRT and MGLRT techniques are developed below.

2.3.1 Fidelity and threshold selection for LMP imaging

The likelihood ratio test signal for LMP technique is defined as $l_{lmp} := e^T V^{-1} \Gamma$ where the matrix V and vector Γ are defined in the previous section. Both Γ and V can be determined from experimental data. Thus the hypotheses H_0 and H_1 can be represented by

$$\begin{aligned} H_0 : l_{lmp} &= \eta^T V^{-1} \Gamma, \\ H_1 : l_{lmp} &= \nu \Gamma^T V^{-1} \Gamma + \eta^T V^{-1} \Gamma. \end{aligned} \tag{2.9}$$

Let $p_{lmp}(l_{lmp}; H_0)$ denote the probability density function (pdf) of l_{lmp} under hypothesis H_0 . Notice in (2.9) that under H_1 , the pdf of l_{lmp} depends on ν . ν depends on the strength of interactive forces between cantilever tip and sample; it is assumed that ν follows a distribution $p(\nu)$. $p_{lmp}(l_{lmp}; H_1, \nu)$ is the conditional pdf of l_{lmp} under hypothesis

H_1 given a specific value ν . Since η represents a zero mean Gaussian random vector, it follows that $p_{lmp}(l_{lmp}; H_0) \sim \mathcal{N}(0, \sigma_{lmp}^2)$ and $p_{lmp}(l_{lmp}; H_1, \nu) \sim \mathcal{N}(l_{lmp}(\nu), \sigma_{lmp}^2)$ with $\sigma_{lmp} = \sqrt{\Gamma^T V^{-1} \Gamma}$ and $l_{lmp}(\nu) = \nu \Gamma^T V^{-1} \Gamma$. Let τ_{lmp} be the decision threshold. Then it follows that the probability of false alarm

$$\begin{aligned} P_{FA} &= P(\text{Decision is } H_1 | H_0) \\ &= \int_{\tau_{lmp}}^{\infty} p_{lmp}(y; H_0) dy = Q\left(\frac{\tau_{lmp}}{\sigma_{lmp}}\right), \end{aligned} \quad (2.10)$$

where $Q(x) = \frac{1}{\sqrt{2\pi}} \int_x^{\infty} e^{-y^2/2} dy$.

Similarly, probability of detection P_D can be evaluated as:

$$\begin{aligned} P_D &= P(\text{Decision is } H_1 | H_1) = \int_{\tau_{lmp}}^{\infty} p_{lmp}(y; H_1) dy \\ &= \int_{\tau_{lmp}}^{\infty} \int_{-\infty}^{\infty} p_{lmp}(y; H_1, \nu) p(\nu) d\nu dy \\ &= \int_{-\infty}^{\infty} Q\left(\frac{\tau_{lmp} - l_{lmp}(\nu)}{\sigma_{lmp}}\right) p(\nu) d\nu. \end{aligned} \quad (2.11)$$

The distribution of the impulsive forces between the tip and sample $p(\nu)$ can be estimated by gathering least square estimates of ν (denoted by $\hat{\nu}$) which is described later. The set of $\hat{\nu}$ when utilized for inferring statistical properties of ν reveals that the distribution $p(\nu)$ is mostly populated around a peak value with decaying tails lying on both sides. It is shown that such distribution $p(\nu)$ can be approximated well using a Gaussian distribution (see Section 2.4 and Section 2.5 for details). Hence, considering $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$, P_D for LMP detection algorithm can be determined numerically from (2.11).

The decision *fidelity* is defined by the conditional probability that the decision is correct given l_0 is an outcome of the random variable l_{lmp} which is denoted by:

$$P(\text{Correct decision} | l_{lmp} = l_0).$$

We consider two cases. The case 1 is given by the condition $l_0 > \tau_{lmp}$ where the decision is reached that hypothesis H_1 holds. Fidelity for case 1 is denoted by $P(H_1 | l_{lmp} = l_0, l_0 > \tau)$. Case 2 is specified by $l_0 \leq \tau_{lmp}$ where the decision is reached that hypothesis H_0 holds. Fidelity for case 2 is denoted by $P(H_0 | l_{lmp} = l_0, l_0 \leq \tau)$. The following theorem presents the methods for evaluating the fidelity measures.

Theorem 2.3.1 1. Suppose that $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$. Then

$$\begin{aligned}
 P(H_1|l_{mp} = l_0, l_0 > \tau) &= \frac{g(l_0, \nu_0, \sigma_{lmp}, \sigma_\nu)}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + g(l_0, \nu_0, \sigma_{lmp}, \sigma_\nu)}, \\
 P(H_0|l_{mp} = l_0, l_0 \leq \tau) &= \frac{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)}}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + g(l_0, \nu_0, \sigma_{lmp}, \sigma_\nu)}
 \end{aligned} \tag{2.12}$$

where $g(l_0, \nu_0, \sigma_{lmp}, \sigma_\nu)$ is defined by

$$\begin{aligned}
 g(l_0, \nu_0, \sigma_{lmp}, \sigma_\nu) &= \frac{\sigma_{lmp}}{\sqrt{\sigma_{lmp}^2 + \sigma_\nu^2 V_\Gamma^2}} e^{\left(-\frac{(V_\Gamma \nu_0 - l_0)^2}{2(\sigma_{lmp}^2 + \sigma_\nu^2 V_\Gamma^2)}\right)},
 \end{aligned} \tag{2.13}$$

with $V_\Gamma = \Gamma^T V^{-1} \Gamma$.

2. Alternatively assume that ν follows a degenerate distribution, that is $p(\nu) = \delta(\nu - \nu_0)$ where δ denotes the Dirac delta function. Then the fidelity metrics are given by

$$\begin{aligned}
 P(H_1|l_{mp} = l_0, l_0 > \tau) &= \frac{e^{\left(-\frac{(l_0 - l_{lmp}(\nu_0))^2}{2\sigma_{lmp}^2}\right)}}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + e^{\left(-\frac{(l_0 - l_{lmp}(\nu_0))^2}{2\sigma_{lmp}^2}\right)}}, \\
 P(H_0|l_{mp} = l_0, l_0 \leq \tau) &= \frac{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)}}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + e^{\left(-\frac{(l_0 - l_{lmp}(\nu_0))^2}{2\sigma_{lmp}^2}\right)}}.
 \end{aligned} \tag{2.14}$$

Proof It follows that

$$\begin{aligned}
& P(\text{Correct decision} | l_{mp} = l_0, l_0 > \tau) \\
&= P(H_1 | l_{mp} = l_0, l_0 > \tau) \\
&= \lim_{\epsilon \rightarrow 0+} P\left(H_1 | l_{mp} \in [l_0 - \frac{\epsilon}{2}, l_0 + \frac{\epsilon}{2}], l_0 > \tau\right) \\
&= \lim_{\epsilon \rightarrow 0+} \frac{\mathcal{P}_{H_1}(l_0, \epsilon) P(H_1)}{\sum_{j=0,1} \mathcal{P}_{H_j}(l_0, \epsilon) P(H_j)}, \tag{2.15}
\end{aligned}$$

where $P(H_1)$ and $P(H_0)$ are a priori probabilities for hypotheses H_1 and H_0 . The last step in the r.h.s of (2.15) follows using Bayes' rule with $\mathcal{P}_{H_j}(l_0, \epsilon)$, $j \in \{0, 1\}$ defined by $\mathcal{P}_{H_j}(l_0, \epsilon) := P(l_{mp} \in [l_0 - \frac{\epsilon}{2}, l_0 + \frac{\epsilon}{2}], l_0 > \tau | H_j)$. The individual terms in the numerator and the denominator of (2.15) can be simplified for small ϵ as:

$$\begin{aligned}
\mathcal{P}_{H_1}(l_0, \epsilon) &:= \int_{-\infty}^{\infty} \int_{l_0 - \epsilon/2}^{l_0 + \epsilon/2} p_{lmp}(l; H_1, \nu) p(\nu) dl d\nu \\
&\approx \int_{-\infty}^{\infty} \frac{\epsilon}{\sqrt{2\pi\sigma_{lmp}^2}} e^{\left(-\frac{(l_0 - l_{lmp}(\nu))^2}{2\sigma_{lmp}^2}\right)} p(\nu) d\nu. \tag{2.16}
\end{aligned}$$

Similarly,

$$\begin{aligned}
\mathcal{P}_{H_0}(l_0, \epsilon) &:= \int_{l_0 - \epsilon/2}^{l_0 + \epsilon/2} p_{lmp}(l; H_0) dl \\
&\approx \frac{\epsilon}{\sqrt{2\pi\sigma_{lmp}^2}} e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)}. \tag{2.17}
\end{aligned}$$

In the limit $\epsilon \rightarrow 0+$, the approximations in (2.16) and (2.17) can be replaced with equalities. Thus, ϵ can be canceled from the numerator and denominator in the r.h.s of (2.15). It follows that

$$\begin{aligned}
& P(H_1 | l_{mp} = l_0, l_0 > \tau) = \\
& \frac{P(H_1) \int_{-\infty}^{\infty} e^{\left(-\frac{1}{2\sigma_{lmp}^2} (l_0 - l_{lmp}(\nu))^2\right)} p(\nu) d\nu}{P(H_0) e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + P(H_1) \int_{-\infty}^{\infty} e^{\left(-\frac{(l_0 - l_{lmp}(\nu))^2}{2\sigma_{lmp}^2}\right)} p(\nu) d\nu}. \tag{2.18}
\end{aligned}$$

Evaluating (2.18) requires the a priori probabilities of the hypotheses which are $P(H_1)$

and $P(H_0)$. If the sample under study is not known a priori then it is reasonable to assume that there is equal possibility to encounter both presence and absence of sample features while imaging. Thus the hypotheses H_1 and H_0 have equal a priori probabilities. Enforcing $P(H_1) = P(H_0)$ in (2.18) it follows that,

$$P(H_1|l_{mp} = l_0, l_0 > \tau) = \frac{\int_{-\infty}^{\infty} e^{\left(-\frac{1}{2\sigma_{lmp}^2}(l_0 - l_{mp}(\nu))^2\right)} p(\nu) d\nu}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + \int_{-\infty}^{\infty} e^{\left(-\frac{(l_0 - l_{mp}(\nu))^2}{2\sigma_{lmp}^2}\right)} p(\nu) d\nu}. \quad (2.19)$$

The same steps can be carried out for Case 2 ($l_{mp} = l_0 \leq \tau$) to yield

$$P(H_0|l_{mp} = l_0, l_0 \leq \tau) = \frac{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)}}{e^{\left(-\frac{l_0^2}{2\sigma_{lmp}^2}\right)} + \int_{-\infty}^{\infty} e^{\left(-\frac{(l_0 - l_{mp}(\nu))^2}{2\sigma_{lmp}^2}\right)} p(\nu) d\nu}. \quad (2.20)$$

The r.h.s of (2.19) and (2.20) can be simplified when $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$ which is shown in (2.12). Further, assuming $p(\nu) \sim \delta(\nu - \nu_0)$, the r.h.s of (2.19) and (2.20) can be simplified to (2.14). This completes the proof. ■

2.3.2 Fidelity and threshold selection for GLRT and MGLRT imaging

The likelihood ratio test signal for GLRT technique is given by $l_{glrt} = e^T V_{\Gamma, glrt} e$ with $V_{\Gamma, glrt} = V^{-1} \Gamma \Gamma^T V^{-1}$. Since $l_{mp} = e^T V^{-1} \Gamma$ where l_{mp} is scalar, it follows that $l_{glrt} = l_{mp}^2$. It is shown before that l_{mp} follows Gaussian distribution under hypothesis H_0 where $p_{lmp}(l_{mp}; H_0) \sim \mathcal{N}(0, \sigma_{lmp}^2)$ with $\sigma_{lmp} = \sqrt{\Gamma^T V^{-1} \Gamma}$. It can be shown that under H_0 :

$$p_{glrt}(l_{glrt}; H_0) = \frac{1}{\sqrt{2\pi V_{\Gamma} l_{glrt}}} e^{-\frac{l_{glrt}}{2V_{\Gamma}}}, \quad (2.21)$$

where $V_{\Gamma} = \Gamma^T V^{-1} \Gamma$. Under H_1 , for a fixed value of the strength of tip-sample interaction ν , $p_{lmp}(l_{mp}; H_1, \nu) \sim \mathcal{N}(l_{mp}(\nu), \sigma_{lmp}^2)$ with $l_{mp}(\nu) = \nu V_{\Gamma}$. Since $l_{glrt} = l_{mp}^2$, it

follows that under H_1 :

$$p_{glrt}(l_{glrt}; H_1, \nu) = \frac{1}{\sqrt{2\pi V_\Gamma l_{glrt}}} e^{-\frac{l_{glrt} + \nu^2 V_\Gamma^2}{2V_\Gamma}} \cosh(\nu \sqrt{l_{glrt}}). \quad (2.22)$$

The false alarm rate and probability of detection for the GLRT scheme can be determined numerically using

$$P_{FA} = \int_{\tau_{glrt}}^{\infty} p_{glrt}(y; H_0) dy \text{ and } P_D = \int_{\tau_{glrt}}^{\infty} \int_{-\infty}^{\infty} p_{glrt}(y; H_1, \nu) p(\nu) d\nu dy,$$

where $p_{glrt}(l_{glrt}; H_0)$ and $p_{glrt}(l_{glrt}; H_1, \nu)$ are given in (2.21) and (2.22) respectively. ν is assumed to follow the distribution $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$.

For the GLRT technique, the matrix $V_{\Gamma, glrt}$ is aligned with the observation signal e_k during each decision window to generate the likelihood ratio $l_{glrt} = e^T V_{\Gamma, glrt} e$. For LMP imaging described before, the matrix $V^{-1}\Gamma$ must be aligned with the observation signal e_k to generate $l_{lmp} = e^T V^{-1}\Gamma$. Thus, implementation of the LMP and GLRT techniques in practice requires additional measures for synchronizing multiplication of the matrices $V^{-1}\Gamma$ and $V_{\Gamma, glrt}$ with the observation signal e_k . For AFM experimental setups, it is challenging to extract the exact timing instant where the cantilever interacts impulsively with the sample. Alternatively, define the test signal $l_m := e^T e$ which is referred to as the modified generalized likelihood ratio test (MGLRT) technique in this chapter. The MGLRT method is simpler for real time implementation in AFM setups. Here, no additional measures are needed for aligning the matrix $V_{\Gamma, glrt}$ with e_k . The test signal l_m performs well for detecting sample features [22]. Hence, in the results section we compare the LMP imaging method with the MGLRT technique instead of the original GLRT scheme. Under hypotheses H_0 and H_1 , it follows that

$$\begin{aligned} H_0 : l_m &= \eta^T \eta, \\ H_1 : l_m &= (\nu \Gamma^T + \eta^T)(\nu \Gamma + \eta). \end{aligned} \quad (2.23)$$

Under H_0 , $l_m = \eta^T \eta = \sum_{i=1}^M \eta_i^2$ where each η_i (M is the size of detection window) is independent, zero mean Gaussian random variable with variance σ_η^2 . It can be shown that l_m obeys a Chi-square distribution given by

$$p_m(l; H_0) = \frac{1}{\sigma_\eta^M 2^{M/2} \Gamma(M/2)} l^{(M/2-1)} e^{-\frac{l}{2\sigma_\eta^2}}, \quad l \geq 0, \quad (2.24)$$

where $\Upsilon(\cdot)$ denotes the standard Gamma function. Suppose that τ_m is the decision threshold. Probability of false alarm P_{FA} can be calculated analytically in the form of a finite series for even M as below

$$\begin{aligned}
P_{FA} &= \int_{\tau_m}^{\infty} p_m(l; H_0) dl \\
&= 1 - \int_0^{\tau_m} \frac{l^{\frac{M}{2}-1} e^{-\frac{l}{2\sigma_\eta^2}}}{\sigma_\eta^M 2^{M/2} \Upsilon(M/2)} dl \\
&= e^{\left(-\frac{\tau_m}{2\sigma_\eta^2}\right)} \sum_{k=0}^{M/2-1} \frac{1}{k!} \left(\frac{\tau_m}{2\sigma_\eta^2}\right)^k, \quad M \text{ is even.}
\end{aligned} \tag{2.25}$$

From (2.23), under hypothesis H_1 , $l_m = e^T e = (\nu\Gamma^T + \eta^T)(\nu\Gamma + \eta)$. Define $\eta' = \nu\Gamma + \eta = [\eta'_1, \eta'_2, \dots, \eta'_M]$. Given a specific value for ν it follows that each η'_i is a Gaussian random variable with mean $\nu\Gamma_i$ ($\Gamma_i := H(F - LH)^{i-1}$) and variance σ_η^2 . Thus $l_m = \sum_{i=1}^M (\eta'_i)^2$ follows a non central Chi-square distribution [25] with pdf given by

$$p_m(l; H_1, \nu) = \frac{1}{2\sigma_\eta^2} \left(\frac{l}{s^2}\right)^{\frac{M-2}{4}} e^{-\frac{s^2+l}{2\sigma_\eta^2}} I_{M/2-1} \left(\frac{\sqrt{l}s}{\sigma_\eta^2}\right), \tag{2.26}$$

where $s^2 = \sum_{i=1}^M \nu^2 \Gamma_i^2 = \nu^2 \Gamma^T \Gamma$. $I_\alpha(x)$ is the α^{th} order modified Bessel function of the first kind which can be represented by the infinite series $I_\alpha(x) = \sum_{k=0}^{\infty} \frac{(x/2)^{\alpha+2k}}{k! \Gamma(\alpha+k+1)}$, $x \geq 0$. Thus the probability of detection P_D is given by

$$\begin{aligned}
P_D &= \int_{\tau_m}^{\infty} \int_{-\infty}^{\infty} p_m(l; H_1, \nu) p(\nu) d\nu dl \\
&= \int_{\tau_m}^{\infty} \int_{-\infty}^{\infty} \frac{1}{\sqrt{8\pi\sigma_\eta^2\sigma_\nu}} \left(\frac{l}{\nu^2 \Gamma^T \Gamma}\right)^{(M-2)/4} \\
&\quad I_{M/2-1} \left(\frac{\sqrt{l}s}{\sigma_\eta^2}\right) e^{-\left(\frac{(\nu^2 \Gamma^T \Gamma + l)}{2\sigma_\eta^2} + \frac{(\nu - \nu_0)^2}{2\sigma_\nu^2}\right)} d\nu dl.
\end{aligned} \tag{2.27}$$

The last step in (2.27) follows from the Gaussian assumption on the distribution $p(\nu)$. The next theorem follows for GLRT and MGLRT techniques:

Theorem 2.3.2 Define the decision fidelity of the GLRT method by the probability of correct decision for a particular realization $l_{glrt} = l_0$. Similar definition holds for MGLRT method (with $l_m = l_0$). Assume equal a priori probabilities for the hypotheses H_1 and H_0 ; $P(H_1) = P(H_0)$.

1. Fidelity metrics for GLRT method can be evaluated by

$$\begin{aligned}
 P(H_1|l_{glrt} = l_0 > \tau_{glrt}) &= \\
 &= \frac{\int_{-\infty}^{\infty} p_{glrt}(l_0; H_1, \nu) p(\nu) d\nu}{\int_{-\infty}^{\infty} p_{glrt}(l_0; H_1, \nu) p(\nu) d\nu + p_{glrt}(l_0; H_0)}, \\
 P(H_0|l_{glrt} = l_0 \leq \tau_{glrt}) &= \\
 &= \frac{p_{glrt}(l_0; H_0)}{\int_{-\infty}^{\infty} p_{glrt}(l_0; H_1, \nu) p(\nu) d\nu + p_{glrt}(l_0; H_0)}.
 \end{aligned} \tag{2.28}$$

2. Further, assuming degenerate distribution of ν that is $p(\nu) = \delta(\nu - \nu_0)$ where δ denotes the Dirac delta function it follows that

$$\begin{aligned}
 P(H_1|l_{glrt} = l_0 > \tau_{glrt}) &= \\
 &= \frac{p_{glrt}(l_0; H_1, \nu_0)}{p_{glrt}(l_0; H_1, \nu_0) + p_{glrt}(l_0; H_0)}, \\
 P(H_0|l_{glrt} = l_0 \leq \tau_{glrt}) &= \\
 &= \frac{p_{glrt}(l_0; H_0)}{p_{glrt}(l_0; H_1, \nu_0) + p_{glrt}(l_0; H_0)},
 \end{aligned} \tag{2.29}$$

where the distributions $p_{glrt}(l_0; H_0)$ and $p_{glrt}(l_0; H_1, \nu_0)$ are evaluated from (2.21) and (2.22) respectively.

Replace l_{glrt} with l_m , τ_{glrt} with τ_m , $p_{glrt}(l_0; H_0)$ with $p_m(l_0; H_0)$ and $p_{glrt}(l_0; H_1, \nu_0)$ with $p_m(l_0; H_1, \nu_0)$ in (2.28) and (2.29) to obtain fidelity metrics for the MGLRT method. $p_m(l_0; H_0)$ and $p_m(l_0; H_1, \nu_0)$ are evaluated using (2.24) and (2.26) respectively.

Proof The proof is similar to the proof given for Theorem 2.3.1. ■

2.3.3 Determining statistics of impulsive force

Notice that for the Gaussian distribution assumption on $p(\nu)$, it is necessary to evaluate both ν_0 and σ_ν for determining fidelity metrics in (2.12) and (2.28). An average of recent estimates of ν can be used as estimate of ν_0 whereas standard deviation of the estimates of ν can be utilized to estimate σ_ν . A least square estimate for ν is given by

$$\hat{\nu} = (\Gamma^T \Gamma)^{-1} \Gamma^T e. \tag{2.30}$$

For the purpose of evaluating the false alarm rate P_{FA} in (2.10), (2.25) and probability of detection P_D in (2.11), (2.27) the true topographic features are assumed to be known a priori. Here, $\hat{\nu}$ is evaluated using (2.30) at the end of each decision window of size M . The set of observations $\hat{\nu}$ gathered this way is utilized for acquiring the probability distribution of ν denoted by $p(\nu)$ which is used in (2.10), (2.11), (2.25) and (2.27) for determining P_{FA} and P_D . However, in case of determining the fidelity metrics, the true topographic features are not known a priori. Thus $\hat{\nu}$ values are gathered only during the decision windows when the decision is H_1 . The latest ensemble of such estimates $\hat{\nu}$ is used to determine the probability distribution of ν with mean ν_0 and covariance σ_ν^2 which are used in (2.12) and (2.28) for fidelity measures. For the LMP technique, the Gaussian assumption allows for solutions of the decision fidelity in closed analytical form in (2.12). However, for the GLRT and MGLRT methods, Gaussian assumption leads to (2.28) in which the integration of the form $\int_{-\infty}^{\infty} p_{glrt}(l_0; H_1, \nu) p(\nu) d\nu$ must be evaluated numerically at each decision window. Thus a real time implementation is more challenging. However, note that with the degeneracy assumption to determine fidelity metrics using (2.14) and (2.29), only the estimate of ν_0 is required. Furthermore, LMP, GLRT and MGLRT fidelity metrics can be computed using closed form analytical formulas. Hence the degeneracy approximation simplifies computation of the fidelity metrics. Despite the simplification, the fidelity metrics remain effective for practical purposes that are demonstrated using simulation and experimental data in the subsequent sections.

2.4 Simulation results

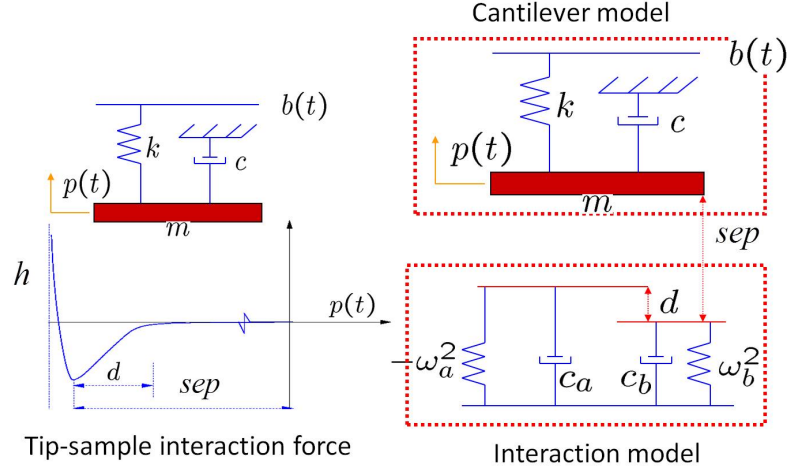


Figure 2.3: The piecewise linear model for tip-sample interaction h which varies non-linearly with deflection of the cantilever tip p . A double spring-damper system with parameters (ω_a, c_a) for attractive spring and (ω_b, c_b) for repulsive spring models the long range attractive and short range repulsive regions of h . The cantilever model parameters, namely the spring stiffness k , damping c and mass m can be measured using frequency sweep method and thermal measurements in free air. The tip-sample model parameters (ω_b, c_b) and (ω_a, c_a) can be estimated using the harmonic balance method developed in [1].

The trajectory $p(t)$ of the oscillating cantilever interacting intermittently with sample in the dynamic mode operation is simulated using a piecewise linear model for the interactive forces between cantilever tip and sample which is shown in Figure 2.3 [1]. Such a model encapsulates many experimentally observed phenomena [28].

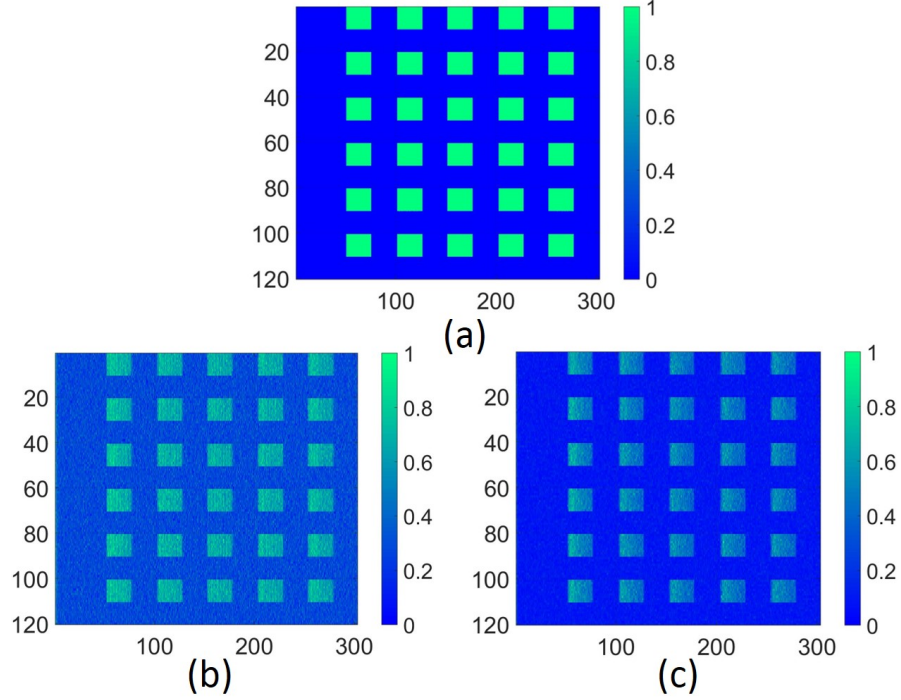


Figure 2.4: (Simulation result) (a) Height image. The difference between low and high topography is 6 nm. The scale is normalized with respect to the maximum height. (b) LMP likelihood image and (c) MGLRT likelihood image. x axis represents time in the unit of cantilever oscillation period T_0 ($1/T_0=63.14$ kHz). y axis represents index of scan line. Both LMP and MGLRT images are normalized with respect to corresponding maximum values. The colorbars specify the ranges.

The topography of the sample is shown in Figure 2.4 (a). The bright zones represent high topography whereas the dark zones correspond to depressed topography (see the associated colorbar). The difference between high and low topography levels is 6 nm. A cantilever with resonant frequency $f_0 = \omega_0/2\pi = 63.15$ kHz and quality factor $Q = 227.8$ is chosen for simulation. The excitation frequency is matched to f_0 and the oscillation amplitude (free from sample interaction) is 24 nm. The continuous time state space model in (2.3) is derived from the frequency response of the cantilever. Further, the discrete time dynamics in (2.4) is determined from the continuous time model via a zero order hold at sampling frequency of 2 MHz. A steady state Kalman observer with gain L in (2.5) is designed to match the discrete time model. The discrete time measurement

noise v_k in (2.4) is chosen to be zero mean with standard deviation σ_v such that the noise bed is retained within 100 pm ($3\sigma_v < 100$ pm) which is normally observed in our experimental data. $\sigma_v^2 = 0.5 * 10^{-3} \text{ nm}^2$ satisfies this criteria. The steady state variance σ_η^2 of the noise η_k present in the tracking error in (2.6) can be determined by solving the discrete time algebraic Riccati equation for the covariance matrix of the state estimation error of the observer in (2.5) [29]. The window size M for hypothesis testing in (2.7) is chosen to be 28 samples. The Γ vector in the hypothesis testing framework (2.8) is determined as explained in Section 2.2. LMP and MGLRT likelihood ratios l_{lmp} and l_m synthesized from the tracking error e using (2.9) and (2.23) are shown in Figure 2.4 (b) and Figure 2.4 (c) respectively.

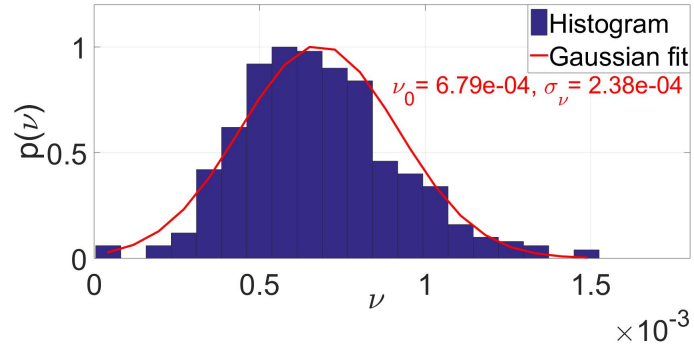


Figure 2.5: (Simulation result) The probability distribution of ν obtained from simulation. The y axis is normalized with respect to the maximum value of the distribution. The histogram is fit with a Gaussian distribution with mean ν_0 and standard deviation σ_ν .

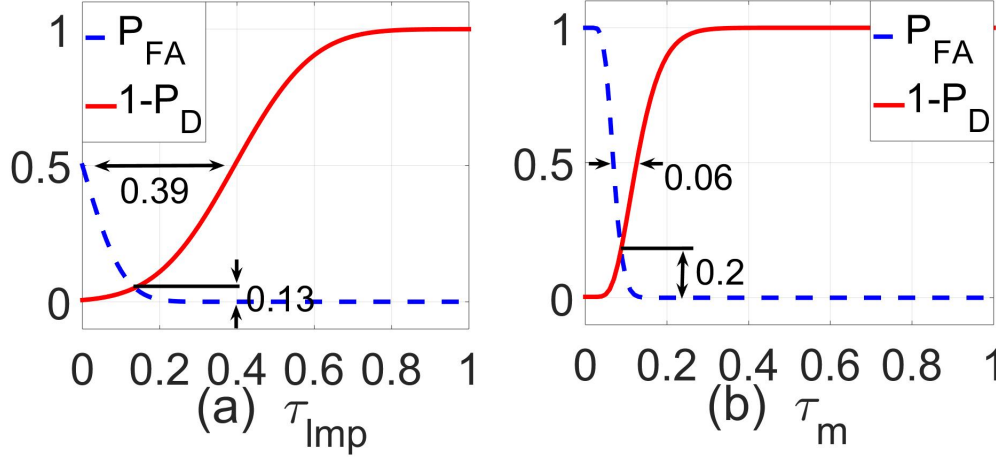


Figure 2.6: (Simulation result) The probability of false alarm P_{FA} and misdetecion $1 - P_D$ for (a) LMP technique with decision threshold τ_{imp} and (b) for MGLRT scheme with decision threshold τ_m . The x axes are normalized with respect to the maximum likelihood ratios received for both cases.

Statistics for the strength of interaction between cantilever tip and sample ν is obtained by gathering the estimates $\hat{\nu}$ using (2.30) which is shown in Figure 2.5. The histogram in Figure 2.5 is fit with a Gaussian distribution of mean $\nu_0 = 6.79e - 4$ and standard deviation $\sigma_\nu = 2.38e - 4$. The pdf $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$ is utilized for determining the false alarm rate P_{FA} using (2.10) and probability of misdetecion $1 - P_D$ using (2.11) for LMP detection technique. False alarm rate and probability of misdetecion for LMP method with different decision thresholds τ_{imp} are shown in Figure 2.6 (a). The false alarm and detection accuracy performance of MGLRT method at different decision thresholds τ_m are shown in Figure 2.6 (b). From Figure 2.6 (a) and (b), it is clear that selection of threshold controls the balance between false alarm rate and probability of misdetecion.

For LMP detection strategy, it is possible to simultaneously achieve small P_{FA} and $1 - P_D$ of the value 0.13 (see Figure 2.6 (a)). However, in Figure 2.6 (b), the best possible scenario for MGLRT with respect to P_{FA} and $1 - P_D$ is $P_{FA} = 1 - P_D = 0.2$. The transitions of the false alarm and misdetecion rates with respect to changing threshold is more drastic in MGLRT than LMP scheme. From Figure 2.6 (a), the range of threshold τ_{imp} where both P_{FA} and $1 - P_D$ are retained within 0.5 is 0.39. In contrast,

in Figure 2.6 (b), both P_{FA} and $1 - P_D$ remain below the limit 0.5 only over a narrow range of τ_m which is 0.06. Thus it is evident that LMP imaging performance is less sensitive to selection of threshold compared to MGLRT. LMP technique is also capable of better minimization of false alarm and misdetection rates. However, synthesizing the LMP likelihood signal $l_{lmp} = e^T V^{-1} \Gamma$ requires synchronization of the Γ vector precisely with the observation vector e during each decision window which is more difficult than generating the MGLRT signal $l_m = e^T e$.

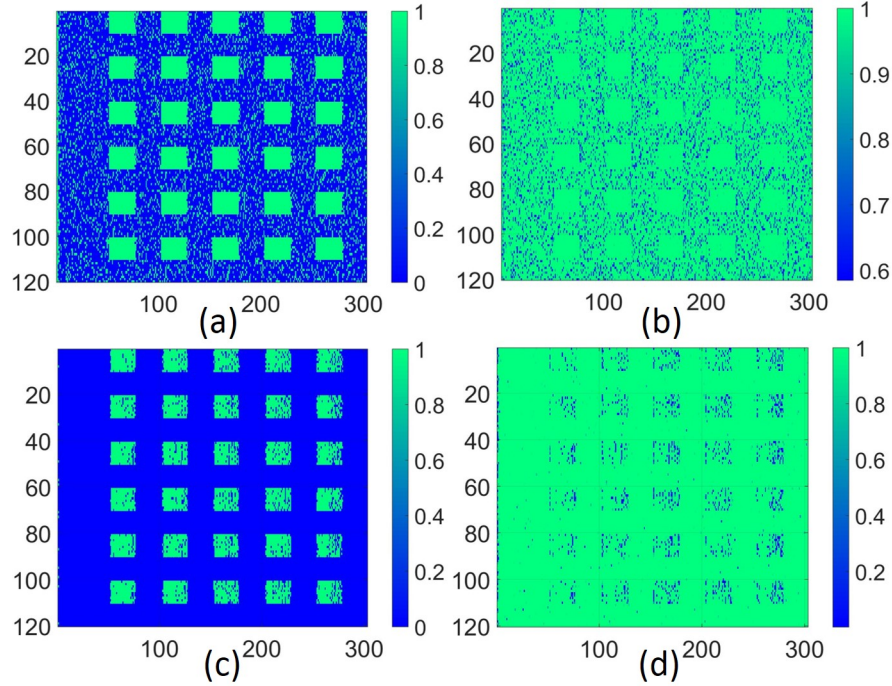


Figure 2.7: (Simulation result) (a) LMP decision image for low threshold and (b) corresponding fidelity image. (c) LMP decision image for high threshold and (d) corresponding fidelity image. Color bars specify the ranges.

The effect of selection of decision threshold on fidelity imaging is demonstrated now. Fidelity metrics for LMP and MGLRT are determined using (2.14) and (2.29) respectively. Define $\tau_{p,lmp} := (\max(l_{lmp}) + \min(l_{lmp}))/2$ and $\tau_{p,m} := (\max(l_m) + \min(l_m))/2$ which represent the mid points of the range of minimum to maximum likelihood values

obtained for LMP and MGLRT methods respectively. Figure 2.7 (a) and (b) demonstrate selection of low threshold ($\tau_{lmp} = 0.3\tau_{p,lmp}$) on LMP decision image and corresponding fidelity image. It is observed in Figure 2.7 (a) that the high topography regions are deciphered correctly but in many locations corresponding to low topography zones, bright spots appear which are *false alarms*.

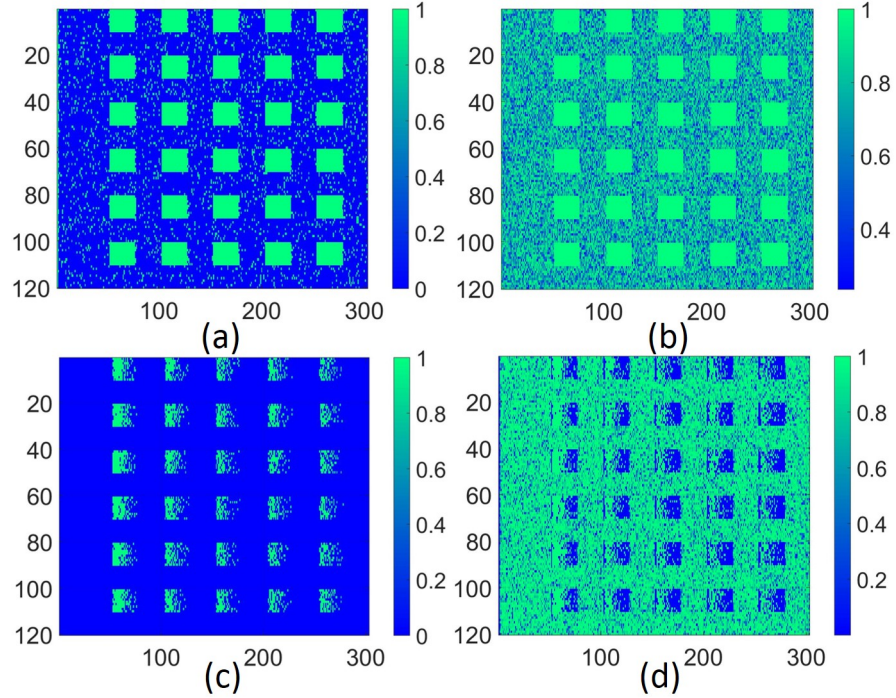


Figure 2.8: (Simulation result) (a) MGLRT decision image for low threshold and (b) corresponding fidelity image. (c) MGLRT decision image for high threshold and (d) corresponding fidelity image. The color bars specify the ranges.

The corresponding fidelity image in Figure 2.7 (b) demonstrates that there is high confidence on the decisions corresponding to high topography zones. However dark regions in fidelity image appear in Figure 2.7 (b) corresponding to the false alarm locations (compare with Figure 2.7 (a)) indicating that these decisions (which are incorrect) should be given low confidence. The effect of choosing high threshold ($\tau_{lmp} = 1.4\tau_{p,lmp}$) is demonstrated in Figure 2.7 (c) and (d). In Figure 2.7 (c), the low topography regions

are correctly detected, however, dark regions appear in the high topography regions indicating that these locations are *misdetected*. The corresponding fidelity image shown in Figure 2.7 (d) assigns low confidence on the pixels that are misdetected. Similar trends in decision images and their fidelity measures are observed in MGLRT techniques as well. Figure 2.8 (a) and (b) demonstrate MGLRT decision and fidelity images for low threshold $\tau_m = 0.4\tau_{p,m}$ whereas the effect of higher threshold $\tau_m = 1.2\tau_{p,m}$ is shown in Figure 2.8 (c) and (d).

2.5 Experimental results

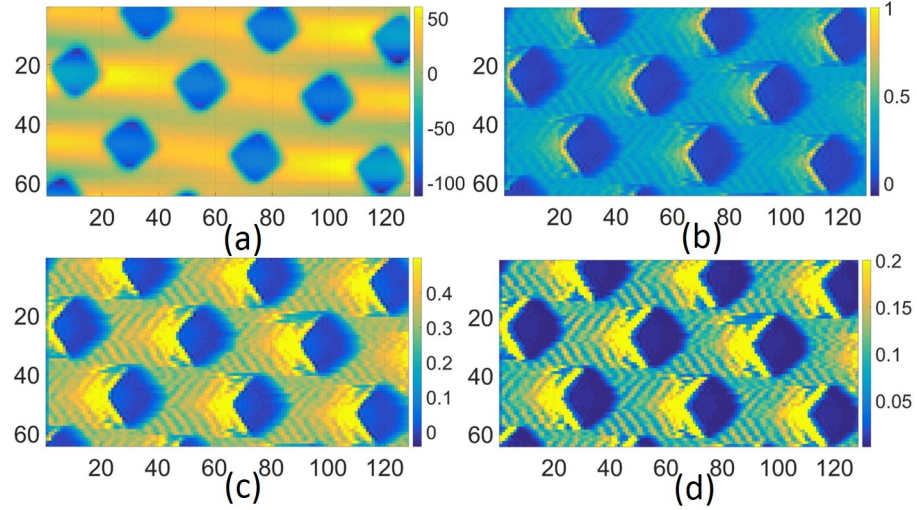


Figure 2.9: (Experimental data) (a) Height image (nm) for silicon calibration sample taken at slow speed of 2 Hz. High speed images taken at 31.25 Hz showing (b) the estimate for strength of tip-sample interaction $\hat{\nu}$, (c) LMP likelihood and (d) MGLRT likelihood signals. $\hat{\nu}$, LMP and MGLRT images are normalized with respect to their maximum values obtained during imaging. The color bars specify corresponding ranges. x and y axes represent lengths of $2 \mu m$ and $4 \mu m$ respectively.

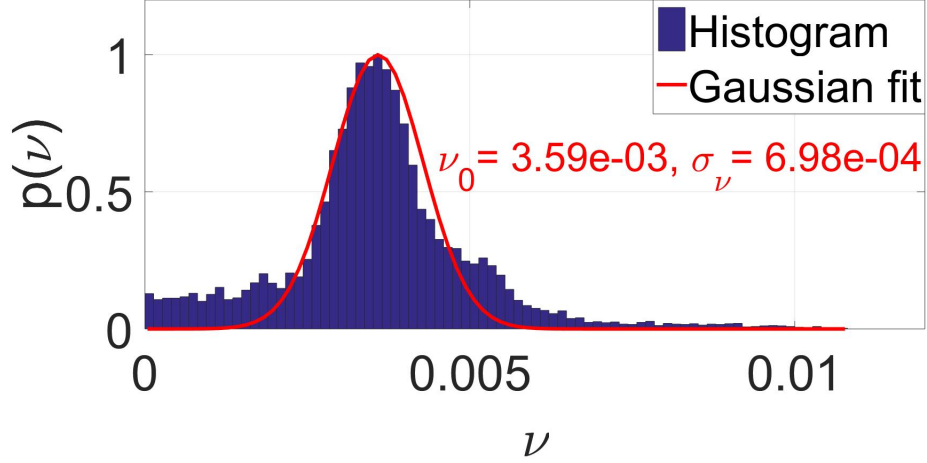


Figure 2.10: (Experimental data) Estimated distribution of the strength of tip-sample interaction ν . The y axis is normalized with respect to the maximum value of the distribution. The histogram is fit with a Gaussian distribution with mean $\nu_0 = 3.59e-3$ and standard deviation $\sigma_\nu = 6.98e-4$.

Results from experiment are presented here. AC240TS cantilever (bought from Asylum Research) with first mode resonant frequency 84.7 kHz and quality factor 254.2 is used for dynamic mode imaging with MFP 3D AFM from Asylum Research. Figure 2.9 (a) shows height image of a silicon calibration sample of size $2\mu\text{m} \times 4\mu\text{m}$ with 100 nm pits taken at slow speed of 2 Hz (2 lines are scanned on the sample per second). The height image in Figure 2.9 (a) serves as the reference for images taken at higher speed. The bright (dark) zones represent high (low) topography in Figure 2.9 (a). The high speed imaging is performed at the speed of 31.25 Hz (31.25 lines are scanned per second). The tip-sample interactive forces for the high speed image are estimated using (2.30) which are shown in Figure 2.9 (b). The estimates $\hat{\nu}$ in Figure 2.9 (b) are normalized with respect to the maximum value of $\hat{\nu}$ obtained while imaging (which is 0.0108). Corresponding likelihood images l_{mp} and l_m for LMP and MGLRT methods are demonstrated in Figure 2.9 (c) and Figure 2.9 (d) respectively. Similar to the $\hat{\nu}$ image, LMP and MGLRT images are also normalized with respect to their maximum values.

The estimated forces $\hat{\nu}$ between the tip and sample are arranged in a histogram plot

which is shown in Figure 2.10. From Figure 2.10, it can be observed that the significant population (80 percent) of the estimates $\hat{\nu}$ is distributed around the peak value $\nu_0 = 3.59e-3$ which can be fit with a Gaussian distribution with mean ν_0 and standard deviation $\sigma_\nu = 6.98e-4$. This observation experimentally verifies that it is a reasonable assumption that $p(\nu) \sim \mathcal{N}(\nu_0, \sigma_\nu^2)$.

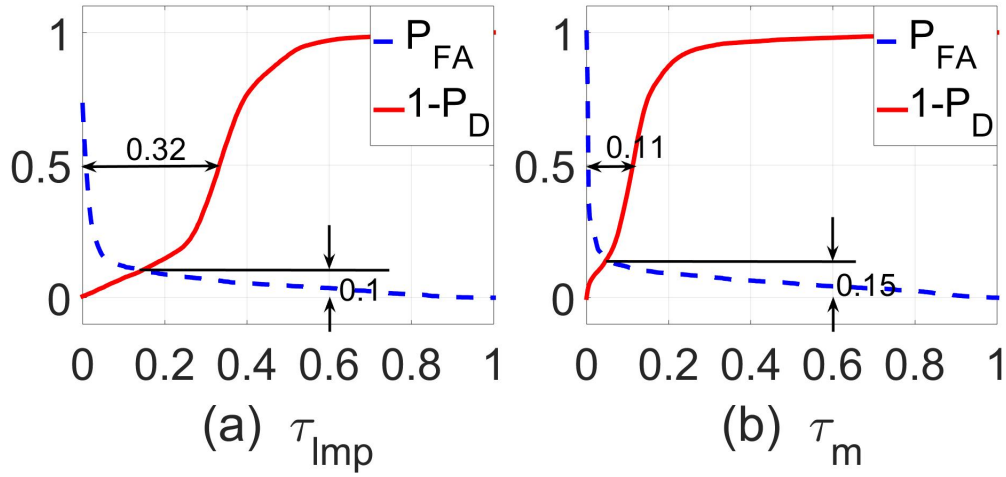


Figure 2.11: (Experimental data) The false alarm rate P_{FA} and probability of misdetection $1 - P_D$ performance of (a) the LMP method and (b) the MGLRT method with different thresholds. The decision thresholds for both cases are normalized with respect to the maximum value of likelihood signals received from experiment.

The likelihood signal images are compared with varying thresholds for decision imaging. The image of the topography of the sample taken at slow speed in Figure 2.9 (a) is accepted as the true image with respect to which false alarm and misdetection rates of the decision images are calculated. The false alarm rate P_{FA} and probability of misdetection $1 - P_D$ for LMP and MGLRT images for different choice of decision thresholds are shown in Figure 2.11 (a) and Figure 2.11 (b) respectively. The decision thresholds are normalized with respect to the maximum value of likelihood ratios received while imaging. From Figure 2.11 (a), it is evident that with LMP method it is possible to achieve $P_{FA} = 1 - P_D = 0.1$ for the choice of threshold $\tau_{imp} = 0.137$.

Whereas, in Figure 2.11 (b), the minimum error rate that is achievable with MGLRT technique is $P_{FA} = 1 - P_D = 0.15$ for the threshold $\tau_m = 0.05$. Thus, consistent to the observation from simulation results, the experimental results suggest that LMP scheme offers better decision accuracy than MGLRT technique (compare Figure 2.6 with Figure 2.11). Furthermore, from Figure 2.11 (a), the range of τ_{lmp} over which both P_{FA} and $1 - P_D$ are retained below 0.5 is 0.32. In comparison, in Figure 2.11 (b), P_{FA} and $1 - P_D$ stay below the limit 0.5 only over a range 0.11 of τ_m which is almost 67 percent less than the range offered by LMP method. Thus, similar to the simulation results, the decision accuracy of MGLRT method is found to be more sensitive to the choice of threshold than the LMP method (compare Figure 2.6 and Figure 2.11). However, as mentioned before, synthesizing the likelihood signal for the MGLRT technique developed in this chapter is more convenient than the LMP method.

The effect of choice of threshold on fidelity imaging is discussed now. Fidelity metrics for LMP and MGLRT methods are derived using (2.14) and (2.29) respectively. Figure 2.12 (a) and (b) demonstrate LMP decision and fidelity images for a low decision threshold $\tau_{lmp} = 0.03\tau_{p,lmp}$ whereas Figure 2.12 (c) and (d) demonstrate effect of relatively higher threshold $\tau_{lmp} = 0.7\tau_{p,lmp}$ ($\tau_{p,lmp} := (\max(l_{lmp}) + \min(l_{lmp}))/2$). Similar to simulation results, low thresholding creates false alarms in the LMP decisions over the depressed topographic zones in Figure 2.12 (a). The pixels that represent false alarms are assigned low decision confidence in Figure 2.12 (b). Putting a relatively higher threshold on the other hand, allows the user to detect most of the low topography zones correctly. However, some high topography zones are interpreted as low topography by the decision image (misdetections) which are demonstrated in Figure 2.12 (c). Corresponding fidelity image in Figure 2.12 (d) assigns low decision fidelity on the pixels that are misdetected. The MGLRT decision images and corresponding fidelity images are also shown in Figure 2.13 with similar effects observed for low decision threshold $\tau_m = 0.006\tau_{p,m}$ and for relatively higher decision threshold $\tau_m = 0.18\tau_{p,m}$ ($\tau_{p,m} := (\max(l_m) + \min(l_m))/2$). Fidelity images successfully assign low confidence on the misinterpreted zones that correspond to either false alarm or misdetection (compare Figure 2.13 (a) with Figure 2.13 (b) and compare Figure 2.13 (c) with Figure 2.13 (d)). Thus the fidelity imaging methodology developed in this chapter is validated by experiments.

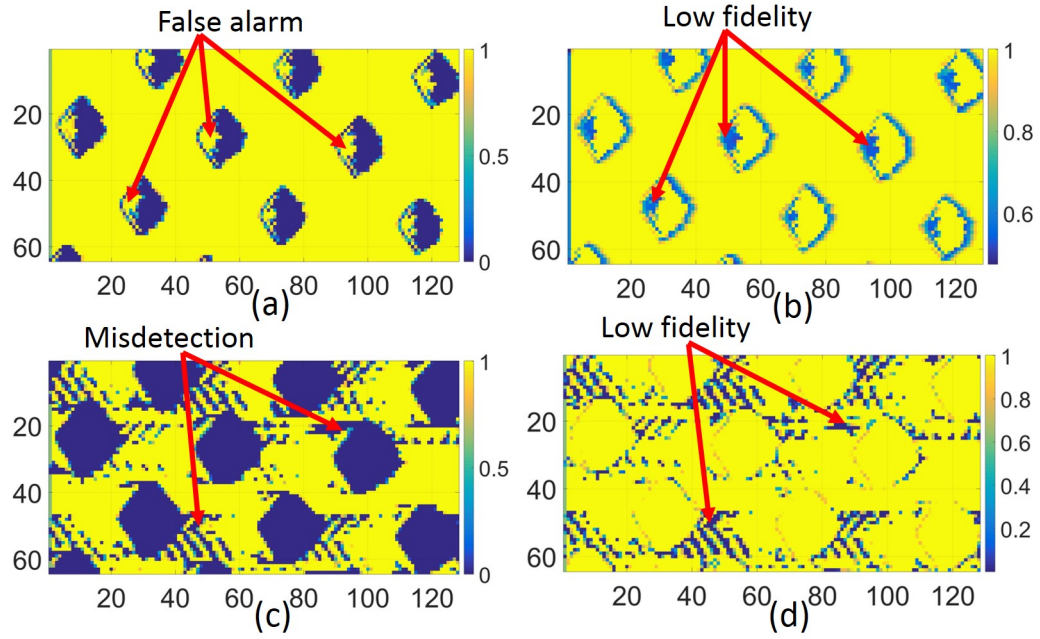


Figure 2.12: (Experimental data) (a) LMP decision image and (b) fidelity image for low decision threshold. (c) LMP decision image and (d) fidelity image for relatively higher decision threshold. The color bars specify corresponding ranges.

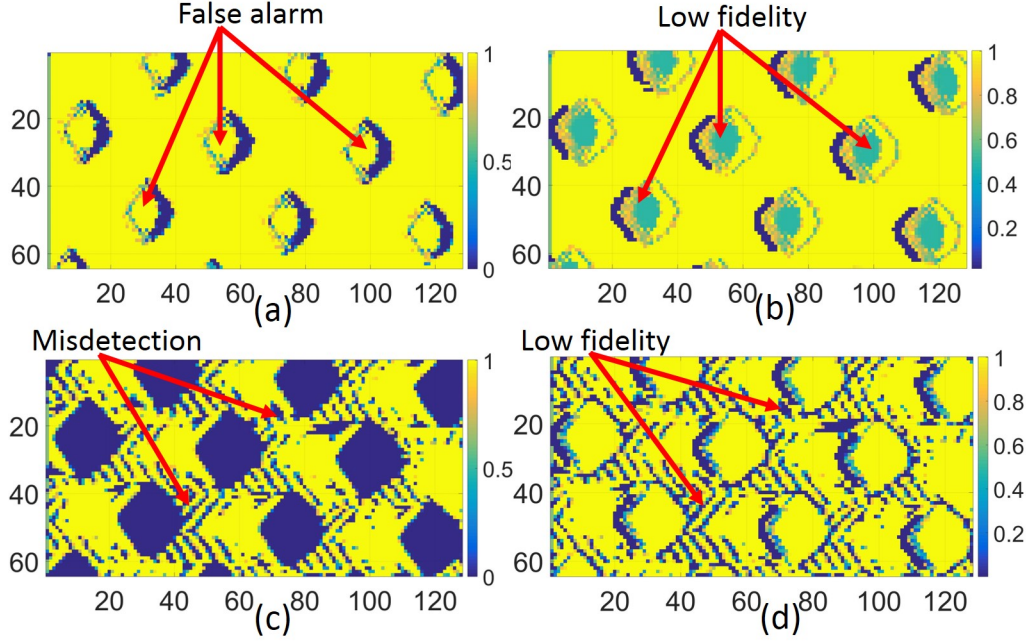


Figure 2.13: (Experimental data) (a) MGLRT decision image and (b) fidelity image for low decision threshold. (c) MGLRT decision image and (d) fidelity image for relatively higher decision threshold. The color bars specify corresponding ranges.

2.6 Conclusions

A key challenge in atomic force microscopy which is widely unaddressed is to provide fidelity metrics on the measurement data. In this chapter we developed techniques for providing fidelity measures on dynamic mode atomic force microscopy method. The method is particularly suitable for high speed deciphering of topographic features with negligible mechanical wear and tear on the sample as well as on the probe. Fidelity metrics are derived for both the locally most powerful and generalized likelihood ratio test based signal processing techniques. The trade-off between the false alarm rate and probability of detection for the choice of decision threshold is also discussed. The fidelity metrics for the decision images allow the users to observe additional channels which help to prioritize the choice of threshold; the choice is either to prioritize the high fidelity deciphering of high topographic features versus high fidelity interpretation of absence

of topographic features. Images indicating low (high) fidelity pixels corresponding to incorrect (correct) decisions on topographic features are observed from both simulation and experimental data. Thus the methods developed analytically in this chapter are verified via extensive simulation and experimental results. Development of quantitative methods for fidelity estimates of other AFM measurement techniques establishes a natural extension of this work.

Chapter 3

Quantitative determination of material properties with dynamic mode AFM

3.1 Introduction

The atomic force microscope (AFM) [5] is used for a wide variety of studies where nanometer or sub-nanometer spatial resolution is needed. The operational modes of AFM have evolved to accommodate diverse set of applications developed by a wide spectrum of researchers [30]. Recently a number of methods were devised for quantitative estimation of material properties. The principal techniques for flexure probe based material property characterization can be categorized under contact mode and dynamic mode methods. The contact mode methods include the force volume mapping method [31], band excitation method (BE) [32,33] and dual amplitude resonance tracking (DART) method [10]. Contact mode methods can be harsh on the material being imaged and the flexure probe [18]. In the dynamic mode the cantilever tip is forced, typically sinusoidally that result in the cantilever tip oscillating periodically [34]. Here, since the tip interacts with the sample intermittently, lateral frictional forces are small which results in negligible wear and tear on the sample surface and the cantilever tip [17]. Thus the dynamic mode operation is particularly suitable for investigating soft material

such as polymers and biological samples.

Different dynamic mode methods are present with a focus on material property investigation [12, 35–37]. However, interpretation of data is more complex for dynamic mode in contrast to contact mode. In a recently reported dynamic mode approach [12, 38] the dynamics of the cantilever interacting with the sample is modeled by an equivalent cantilever. Here the resonant frequency and quality factor of the equivalent cantilever depend on the material properties of the sample. The changes in the resonant frequency and quality factor are used to determine the stiffness and dissipative properties of the sample. In this chapter a simple analytical method, steady-state based estimation of equivalent parameters (SEEP), based on the assumption that the cantilever is in steady-state is developed to determine the equivalent cantilever’s resonant frequency and quality factor. SEEP relates the measured amplitude and phase of the oscillating cantilever (when being forced by a sinusoid near or at the first resonant frequency) to the parameters of equivalent cantilever. The recursive estimation of equivalent parameters (REEP) reported in earlier work [12, 38] is compared with the new method. In the recursive method presented in [38] the cantilever is forced with a sum of three sinusoidal signals with the center sinusoid close to the resonant frequency of the first mode of the cantilever. The other two frequencies are chosen symmetric around the center frequency. The recursive method requires design of additional hardware to process the cantilever deflection and excitation signals. In this chapter, it is demonstrated through experiments that the recursive method is capable of estimating equivalent parameters even when the cantilever is in a transient state. The new steady-state based method, SEEP, does not require changes to how the cantilever is forced to the conventional dynamic mode operation. Moreover, it utilizes the amplitude and phase of cantilever oscillations which are readily available in most AFM setups. However, SEEP is based on the assumption that the cantilever has reached steady state and cannot be used when the cantilever is in a transient state. Thus the choice of the SEEP versus the recursive method REEP depends on the need for simplicity in comparison to high-bandwidth needs. For both the steady-state based method and recursive method, REEP, it is shown via experimental results that how the methods are calibrated have considerable influence on the accuracy of the methods. We also demonstrate that it is important to

address the deviation of the measured cantilever response from that of an ideal spring-mass-damper (SMD) model which is often derived by approximating the behavior of the cantilever based on its first mode.

The parameters of the equivalent cantilever that models the cantilever-sample interconnected system provide important information on material properties. The dissipated power to the sample represents the power lost to the sample. The storage power that represents the conservative interaction with the sample provides important clues on the elastic behavior of the sample. Both the storage and dissipated power can be determined from equivalent parameters [12, 38, 39]. The dissipative and storage powers [36, 37] can be evaluated using conservation principles as well. Here the difference between the input power to the drive that forces the cantilever and the power dissipated due to damping yields the average dissipative power because of tip-sample interaction [36]. The storage power due to tip-sample interaction can also be estimated similarly. Both works [36] and [37] assume a sinusoidal steady state trajectory of the cantilever. We demonstrate here that the reference position where the nominal reference amplitude and phase are obtained (needed for this method) appreciably influence the inferences drawn on the material properties. We also provide a systematic method for addressing the deviation of the measured cantilever deflection from the SMD model and for determining storage and dissipated power from measured data.

This chapter is organized as the following. The equivalent cantilever model method is introduced in Section 3.2. Section 3.3 derives considerably simple semi-analytical method SEEP which is followed by description of the REEP method for estimating the equivalent parameters. In Section 3.4, existing methods that use conservation principles to estimate sample storage and dissipative powers are modified to account for the deviation of the cantilever behavior from SMD models. Simulation results are discussed in Section 3.5. Section 3.6 explains the experimental procedures and results in detail. Section 3.7 concludes with future directions of this work.

3.2 Preliminaries

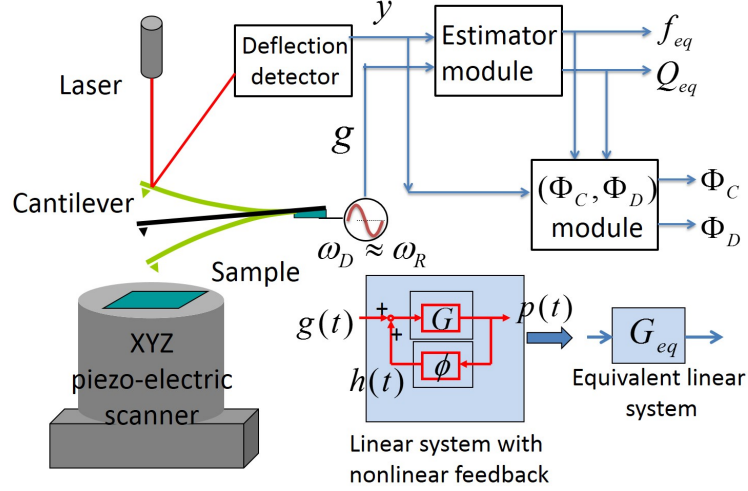


Figure 3.1: Estimation of storage and dissipation properties of the sample based on identifying equivalent parameters. The system including cantilever and tip-sample interaction in the dynamic mode operation is modeled with an equivalent cantilever model which is described by parameters (ω_{eq}, Q_{eq}) with $f_{eq} = \omega_{eq}/2\pi$ as shown in the figure. The equivalent parameters (f_{eq}, Q_{eq}) are further utilized for estimating average storage power Φ_C and dissipative power Φ_D due to tip-sample interactive force $h = \phi(p, \dot{p})$.

The cantilever dynamics when modeled as a spring-mass-damper (SMD) system is governed by:

$$\ddot{p} + \frac{\omega_0}{Q_0}\dot{p} + \omega_0^2 p = F(t) = g(t) + h(t), \quad (3.1)$$

$$h(t) = \phi(p, \dot{p}), \quad y = p + \zeta,$$

where $p, \dot{p}$ are the deflection and velocity of the cantilever tip respectively, F is the force per unit mass on the cantilever which is the sum of the dither excitation force (denoted by g) and the nonlinear tip-sample interaction force (denoted as h). y is the measured position which is the position (denoted as p) corrupted with measurement noise (denoted by ζ). ω_0 and Q_0 are the resonant frequency and the damping factor of the cantilever far from the sample. The resulting system consists of the plant G specified by (ω_0, Q_0) and the nonlinear feedback term h as shown in Figure 3.1. Closer

to the sample surface, the effective damping changes which is attributed to squeeze film damping (see Ref. [36]) where the sample interaction force h is still small. Consider a cantilever tip position (termed as the *reference position*) where the lever is at such a distance from sample that it is under the influence of squeeze film damping but the force due to the interaction with the sample is negligible. Let the resonant frequency and quality factor of the cantilever at the reference be given by ω_r and Q_r respectively (which can be different from (ω_0, Q_0)). Thus at the reference position the cantilever dynamics is described by (3.1) with ω_0 and Q_0 replaced by (ω_r, Q_r) .

Now the cantilever interacting intermittently with the sample is considered. Using tools from asymptotic methods for weakly nonlinear systems [40, 41] it can be shown that the cantilever interacting periodically with sample can be modeled by an equivalent cantilever with resonant frequency ω_{eq} and quality factor Q_{eq} . The equivalent cantilever is described by [28, 42]

$$\ddot{p} + \frac{\omega_{eq}(a)}{Q_{eq}(a)}\dot{p} + \omega_{eq}^2(a)p = g(t), \quad (3.2)$$

where ω_{eq} and Q_{eq} depend on the amplitude a of the cantilever oscillation. It can be shown that (see Ref. [12])

$$\omega_{eq}^2(a) = \omega_r^2 - \frac{2}{am}\Phi_c, \quad \frac{\omega_{eq}(a)}{2Q_{eq}(a)} = \frac{\omega_r}{2Q_r} + \frac{1}{am\omega_d}\Phi_d, \quad (3.3)$$

with

$$\Phi_c = \frac{1}{2\pi} \int_0^{2\pi} \frac{\phi(a \cos \psi, -a\omega_d \sin \psi)}{m} \cos \psi d\psi, \quad (3.4)$$

$$\Phi_d = \frac{1}{2\pi} \int_0^{2\pi} \frac{\phi(a \cos \psi, -a\omega_d \sin \psi)}{m} \sin \psi d\psi, \quad (3.5)$$

where m is the mass of the cantilever and $\omega_d = 2\pi f_d$ is the external drive frequency. The quantity $-\frac{1}{T} \int_0^T \phi(p, \dot{p})\dot{p} dt$ denotes the time-averaged power dissipation attributed to the tip-sample interaction where T is the time period of oscillation. It can be shown that $-\frac{1}{T} \int_0^T \phi(p, \dot{p})\dot{p} dt = a\omega_d\Phi_d$ (denoted by Φ_D) [12]. Similarly, the storage power due to tip-sample interaction is defined as $\frac{\omega_d}{T} \int_0^T \phi(p, \dot{p})p dt$ which can be shown to be equal to $a\omega_d\Phi_c$ (denoted by Φ_C). Thus the storage power Φ_C and dissipative power Φ_D can be evaluated using (3.3) once the equivalent parameters (ω_{eq}, Q_{eq}) and parameters at reference (ω_r, Q_r) are known. In the next section we derive techniques of ascertaining equivalent parameters ω_{eq} and Q_{eq} .

3.3 Estimation of equivalent parameters

(ω_{eq}, Q_{eq}) can be estimated using two different methods described below. The first method namely the steady-state estimation of equivalent parameters (SEEP) assumes that the scanning is slow enough such that cantilever oscillations can be approximated by its steady state behavior. The other method referred to as the recursive estimation of equivalent parameters (REEP) technique does not require the steady state assumption.

3.3.1 Steady-state estimation of equivalent parameters (SEEP)

Here we briefly outline the method of estimating parameters of the equivalent cantilever based on the assumption that the cantilever is in steady-state. Assume that the resonant frequency and quality factor of the cantilever dynamics at the reference position are known and described by the pair (ω_r, Q_r) . When the oscillating cantilever interacts with the sample, the equivalent frequency can be estimated using (see Ref. [43])

$$\omega_{eq} = \sqrt{\omega_d^2 + \frac{a_r}{a} \cos(\tilde{\theta}) \sqrt{(\omega_r^2 - \omega_d^2)^2 + \frac{\omega_r^2 \omega_d^2}{Q_r^2}}}, \quad (3.6)$$

where $\omega_d = 2\pi f_d$ is the external drive frequency, a_r is the measured cantilever oscillation amplitude at reference position, a is the amplitude of oscillation for cantilever interacting with the sample. $\tilde{\theta} := \theta + \theta_c$, where θ is the phase difference between the external drive signal and deflection of cantilever oscillations. θ_c is referred to as the bias phase which can be estimated utilizing methods outlined in Appendix A.1. Both a and θ are available in real time in standard dynamic mode AFM setups. Further, the equivalent quality factor Q_{eq} can be determined using

$$Q_{eq} = \frac{\omega_d \omega_{eq}}{(\omega_{eq}^2 - \omega_d^2) \tan(\tilde{\theta})}. \quad (3.7)$$

The derivation of (3.6) and (3.7) are detailed in Appendix A.1. It should be noted that the amplitude a and phase $\tilde{\theta}$ of cantilever oscillations are the key signals utilized in estimating ω_{eq} and Q_{eq} using the above method. Since a and θ (thus $\tilde{\theta}$) can be interpreted meaningfully only in the steady state part of the cantilever oscillations, the equivalent parameters need to be estimated in the steady state as well. ω_{eq} and Q_{eq} determined using (3.6) and (3.7) are referred to as ω_{seep} and Q_{seep} respectively in the results section.

3.3.2 Discrete-time recursive estimation of equivalent parameters (REEP)

Equivalent parameters can also be evaluated using discrete time signal processing techniques without the assumption of steady state oscillations which is described briefly below. The method in [38] utilizes a discretized model of a general second order continuous time dynamics. Such a general discretized second order dynamics is described by:

$$\begin{aligned} p(k) &= -b_1p(k-1) - b_0p(k-2) + u(k) + e(k), \\ u(k) &= a_2g(k) + a_1g(k-1) + a_0g(k-2), \\ e(k) &= \zeta(k) + b_1\zeta(k-1) + b_0\zeta(k-2), \end{aligned} \quad (3.8)$$

where $p(k)$ and $g(k)$ denote the cantilever deflection and dither forcing sampled at time $t = kT_s$ where T_s is the sampling interval. $\zeta(k)$ is zero mean white measurement noise with variance σ^2 . In the above setup, the unknown parameters can be recursively estimated by defining the unknown vector $m = [b_1 \ b_0 \ a_0 \ a_1 \ a_2]$, the observation vector $\psi(k) = [-p(k-1) \ -p(k-2) \ g(k) \ g(k-1) \ g(k-2)]^T$ and noticing that $p(k) = m\psi(k) + e(k)$. The parameter vector m can be recursively estimated using a least square approach. However, it is seen that (a_2, a_1, a_0) can be evaluated from the discrete time equivalent of the transfer function $G_r(s)$ (which is estimated by applying a frequency sweep method around first resonant frequency at the reference position). Subsequently the values of a_2 , a_1 and a_0 can be fixed to the reference values which leads to a simpler parameter identification problem. Thus (3.8) can be written as

$$\gamma(k) = \underbrace{[b_1 \ b_0]}_{\Gamma^T} \underbrace{\begin{bmatrix} -p(k-1) \\ -p(k-2) \end{bmatrix}}_{\phi(k)} + e(k), \quad (3.9)$$

where Γ represents the vector of unknown parameters and $\gamma(k) = p(k) - a_2g(k) - a_1g(k-1) - a_0g(k-2)$. A weighted least square estimate of Γ denoted as $\hat{\Gamma}_{LS}(k)$ is defined that can be computed by solving the following unconstrained optimization problem

$$\hat{\Gamma}_{LS}(k) = \arg \min_{\hat{\Gamma}(k)} \sum_{i=1}^k \lambda^{k-i} (\gamma(i) - \phi^T(i)\Gamma(k))^2, \quad (3.10)$$

where a forgetting factor $\lambda \in (0, 1)$ is introduced to emphasize on recent observations compared to the past observation data [38]. Furthermore, the speed of convergence of

solutions to (3.10) can be controlled by tuning λ . Moreover, corrections on the estimates of $\Gamma = [b_1 \ b_0]^T$ must be made since colored noise, $e(k)$, in (3.9) creates bias in the estimate [44,45]. In order to remove the bias, a bias compensated exponentially weighted recursive least square (BCEWRLS) algorithm is proposed in [38] which is adopted in this chapter. The continuous time parameters $f_{reep} = (\omega_{reep}/2\pi)$ and Q_{reep} describing the cantilever dynamics can be easily obtained from the discrete time parameters b_1, b_0 in closed form [38] using:

$$v_1 = \tanh^{-1} \left(\frac{1 - b_0}{1 + b_0} \right), \quad v_2 = \tan^{-1} \left(\frac{\sqrt{(4b_0^2 - b_1^2)}}{b_1} \right),$$

$$\omega'_{reep} = \sqrt{\left(\frac{v_1}{2}\right)^2 + v_2^2}, \quad f_{reep} = \frac{\omega'_{reep}}{2\pi T_s}, \quad Q_{reep} = \frac{\omega'_{reep}}{2v_1}. \quad (3.11)$$

Once these estimates ($\omega_{reep} = 2\pi f_{reep}, Q_{reep}$) are available, storage power Φ_C and dissipative power Φ_D are determined using $\Phi_C = a\omega_d\Phi_c$, $\Phi_D = a\omega_d\Phi_d$ where Φ_c and Φ_d are evaluated using (3.3). It is evident in the above method that there is no assumption that the data is collected from steady state. Thus transient data can be utilized as well. In principle the original five parameter identification can be recursively estimated thereby eliminating the need for determining the reference position. However, the resulting identification problem is more involved.

3.4 Equivalence of storage and dissipative powers

The power dissipation due to tip-sample interaction can also be evaluated using conservation principles [36]. Here we first provide a brief description of the approach. Let $\mathcal{F}(t)$ be the total force on the cantilever driver and $z(t)$ be its position (in most AFM setups, the driver is a dither piezo attached to the base of the cantilever and z will represent the movement of the base of the cantilever). Then $\mathcal{F}(t) = k(z(t) - p(t))$ where $p(t)$ is the position of the cantilever tip, k is the stiffness of the lever [36]. For sinusoidal excitation assume that $z = A_z \cos(\omega_d t + \theta_z)$ where A_z and θ_z represent the amplitude and phase of z at the drive frequency ω_d . Assuming sinusoidal trajectory of cantilever, $p(t) = A_p \cos(\omega_d t + \theta_p)$. The instantaneous power input to the driver is $P_{in}(t) = \mathcal{F}(t)\dot{z}(t) = k(z(t) - p(t))\dot{z}(t)$. It can be shown that average input power over

a complete cycle of duration $T = 2\pi/\omega_d$ is given by

$$\bar{P}_{in} = \frac{1}{T} \int_0^T P_{in}(t) dt = \frac{1}{2k} A_z A_p \omega_d \sin(\theta_z - \theta_p). \quad (3.12)$$

The viscous damping coefficient on the cantilever body at the reference position is c_r . The average power P_b due to background dissipation for damping during interval T can be evaluated as

$$P_b = \frac{1}{T} \int_0^T c_r \dot{p}^2(t) dt = \frac{1}{2} c_r A_p^2 \omega_d^2. \quad (3.13)$$

Using conservation of energy, $\bar{P}_{in}$ must be equal to $P_b + P_d$ where P_d is power dissipation due to tip-sample interaction. Now $c_r = (m\omega_r)/Q_r$ where (ω_r, Q_r) denote the resonant frequency and quality factor at reference position. The mass of the cantilever is $m = k/\omega_0^2$ where ω_0 is the free-air resonant frequency. From (3.12) and (3.13) it can be written as

$$\begin{aligned} P_d &= \bar{P}_{in} - P_b \\ &= \frac{k A_p^2 \omega_d}{2 Q_r} \left(\frac{A_z Q_r}{A_p} \sin(\theta_z - \theta_p) - \frac{\omega_r \omega_d}{\omega_0^2} \right). \end{aligned} \quad (3.14)$$

Note that the dynamics between the base movement z and the tip deflection p is described by (under no sample interaction)

$$\ddot{p} + \frac{\omega_r}{Q_r} \dot{p} + \omega_r^2 p = \omega_r^2 z.$$

Here it is assumed that the resonant frequency and quality factor at the reference position are available from experimental data. Thus it follows that

$$p(s) = \frac{\omega_r^2}{(s^2 + \frac{\omega_r}{Q_r} s + \omega_r^2)} z(s). \quad (3.15)$$

Since the drive frequency $\omega_d \approx \omega_r$, from (3.15) it follows that at the reference position

$$\left| \frac{p(j\omega_d)}{z(j\omega_d)} \right| = \frac{\omega_r Q_r}{\omega_d}, \text{ hence } A_z = \frac{\omega_d}{\omega_r Q_r} A_{p,r}, \quad (3.16)$$

where $A_{p,r}$ is the amplitude of p at reference position. From (3.14) and (3.16), it follows that

$$P_d = \frac{k A_p^2 \omega_d^2}{2 Q_r \omega_r} \left(\frac{A_{p,r}}{A_p} \sin(\theta_z - \theta_p) - \frac{\omega_r^2}{\omega_0^2} \right). \quad (3.17)$$

Note that in the above derivation it is hard to measure the base movement z directly; only the signal u with which the drive is excited is available. Here the frequency response from the drive u to the measurement of the tip-deflection denoted by y_m is available. We further assume that $y_m = p$. However, it is demonstrated later that the experimentally observed transfer function from u to y_m obey the following transfer function (also see Appendix A.1):

$$y_m(s) = \frac{(\alpha_1 s + \alpha_2)}{(s^2 + \frac{\omega_r}{Q_r} s + \omega_r^2)} u(s), \quad (3.18)$$

where u and y_m are the *measurable* external drive and deflection signal respectively. Hence $A_p = |Y_m(j\omega_d)| = a$ and $A_{p,r} = a_r$ where a_r is the measured deflection amplitude at reference position. Since $y_m = p$, from (3.15) and (3.18) it follows that

$$\begin{aligned} \angle(Z(j\omega_d)) - \angle(U(j\omega_d)) &= \arctan\left(\frac{\alpha_1 \omega_d}{\alpha_2}\right) = \theta_c, \\ \theta_z &= \theta_u + \theta_c, \end{aligned} \quad (3.19)$$

where θ_u is the phase angle of the phasor $U(j\omega_d)$. Thus

$$(\theta_z - \theta_p) = \underbrace{(\theta_u - \theta_p + \theta_c)}_{\theta} = \tilde{\theta}, \quad (3.20)$$

where θ is measured experimentally and θ_c is the correction term which can be determined using the methods outlined in the earlier section. $\tilde{\theta}$ is referred to as the bias corrected phase. Further using the approximation that ω_d , ω_r and ω_0 are close, from (3.17) and (3.20) it follows that [36]

$$P_d = \frac{ka^2\omega_d}{2Q_r} \left(\frac{a_r}{a} \sin(\tilde{\theta}) - 1 \right). \quad (3.21)$$

The dissipative power calculated through (3.21) utilizes the bias corrected phase $\tilde{\theta}$ and predetermined values for a_r and Q_r at the reference position.

The average storage power P_{st} due to tip-sample interaction is defined as $P_{st} = \omega_d \langle \phi \cdot p \rangle$ where $\langle \rangle$ denotes the time average and ϕ is the tip-sample interaction force. It can be shown that (see Ref. [37])

$$\begin{aligned} P_{st} &= \frac{\omega_d k A_{p,r} A_p}{2Q_r} \left(\frac{2Q_r}{A_{p,r} A_p} \langle \delta p \rangle^2 \right. \\ &\quad \left. + \frac{A_p Q_r}{A_{p,r}} \left(1 - \frac{\omega_d^2}{\omega_r^2} \right) - \cos(\theta_z - \theta_p) \right), \end{aligned} \quad (3.22)$$

where δp is the time averaged deflection (the ‘dc’ component). For usual dynamic mode operation $\delta p \ll A_{p,r}A_p$. Thus the first term on the r.h.s of (3.22) can be ignored. Further, using $(\theta_z - \theta_p) = \tilde{\theta}$ from (3.20), $A_{p,r} = a_r$, $A_p = a$, from (3.22) it follows that

$$P_{st} = \frac{\omega_d k a_r a}{2Q_r} \left(\frac{aQ_r}{a_r} \left(1 - \frac{\omega_d^2}{\omega_r^2} \right) - \cos(\tilde{\theta}) \right). \quad (3.23)$$

Thus P_{st} can be evaluated using regular dynamic mode amplitude a and corrected phase image $\tilde{\theta}$ when parameters at reference (ω_r, Q_r, a_r) are known. Suppose that ω_d , ω_r and ω_0 are close to each other. Further, assume that the cantilever oscillates in steady state. Under these assumptions, we show that the storage and dissipative powers P_{st} and P_d derived using the mechanical energy balance techniques are the same to the parameters Φ_C and Φ_D determined utilizing the SEEP method of estimating the equivalent parameters. Since $\omega_d \approx \omega_r$, the storage power in (3.23) can be simplified as

$$P_{st} = -\frac{\omega_d k a_r a}{2Q_r} \cos(\tilde{\theta}). \quad (3.24)$$

Further, the r.h.s of (3.6) can be simplified to

$$\omega_{seep} = \omega_d \sqrt{1 + \frac{a_r}{aQ_r} \cos(\tilde{\theta})}, \quad (3.25)$$

where ω_{eq} in (3.6) is replaced with ω_{seep} to indicate that the equivalent parameters are estimated using the SEEP approach. From (3.3), substituting ω_{eq} with ω_{seep} it follows that $\Phi_c = \frac{am}{2}(\omega_r^2 - \omega_{seep}^2)$. Using (3.25) and the relation $m = k/\omega_0^2 = k/\omega_d^2$ (since $\omega_d \approx \omega_0$), it can be shown that

$$\Phi_C = a\omega_d\Phi_c = -\frac{\omega_d k a_r a}{2Q_r} \cos(\tilde{\theta}), \quad (3.26)$$

which is the same as P_{st} shown in (3.24). Similarly, substituting ω_{eq} and Q_{eq} with ω_{seep} and Q_{seep} in (3.3), it follows that $\Phi_d = \frac{am\omega_d}{2}(\frac{\omega_{seep}}{Q_{seep}} - \frac{\omega_r}{Q_r})$. Further, from (3.7), $\frac{\omega_{seep}}{Q_{seep}} = \frac{(\omega_{seep}^2 - \omega_d^2)}{\omega_d} \tan(\tilde{\theta})$. Thus it follows that

$$\Phi_d = \frac{am\omega_d}{2} \left(\frac{\omega_{seep}^2 - \omega_d^2}{\omega_d} \tan(\tilde{\theta}) - \frac{\omega_r}{Q_r} \right). \quad (3.27)$$

Substituting the expression for ω_{seep} from (3.25) in (3.27), it follows that

$$\Phi_D = a\omega_d\Phi_d = \frac{ka^2\omega_d}{2Q_r} \left(\frac{a_r}{a} \sin(\tilde{\theta}) - 1 \right), \quad (3.28)$$

which is the same as the dissipative power P_d derived using the mechanical energy balance method shown in (3.21). Hence, we have demonstrated that determining storage and dissipated powers by evaluating equivalent parameters and by using conservation principles agree with each other. However, the equivalency holds under the assumption that the cantilever trajectory is in steady state and is sinusoidal. The results from the different techniques described above are presented below in a step by step approach using simulation results followed by experimental data.

3.5 Simulation results

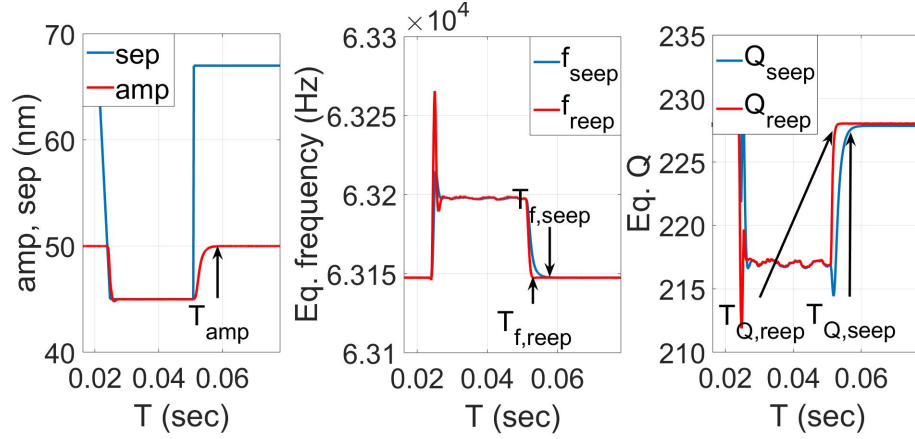


Figure 3.2: (Simulation data) Equivalent parameters ($f_{eq} = \omega_{eq}/2\pi$, Q_{eq}) estimated using SEEP and REEP techniques during the transient and steady-state part of the trajectory of the oscillating cantilever. When *sep* is low (high), the oscillating cantilever interacts (does not interact) with sample. In absence of sample, the cantilever is described by ideal spring-mass-damper model with resonant frequency $f_0 = \omega_0/2\pi = 63.147$ kHz and quality factor $Q_0 = 227.85$.

Here we first present a comparison of the SEEP method and the REEP technique of estimating the equivalent parameters using simulation. The interactive forces between the sample and tip of the cantilever in air typically follows a long range attractive and short range repulsive behavior (see Figure 2.3). This behavior is modeled with a piecewise linear model which consists of two springs and two dampers shown in Figure 2.3.

Such a model is useful to mimic interactive forces from many substrates in air [28, 42].

Let ω_B denote the bandwidth of the amplitude of the cantilever oscillations. ω_B depends on the cantilever's resonant frequency ω_0 and quality factor Q_0 by $\omega_B = \omega_0/2Q_0$. Since ω_B reduces inversely with Q_0 , high Q_0 values cause the transients in the cantilever trajectory last for longer time. $f_{eq} = \omega_{eq}/2\pi$ and Q_{eq} estimated using the SEEP method are denoted as f_{seep} and Q_{seep} respectively. Further, f_{reep} and Q_{reep} denote the corresponding parameters estimated using REEP method. The noise in the estimates f_{seep} and Q_{seep} depend on the bandwidth of the lock-in filter which is utilized to gather the amplitude a and phase signal θ from cantilever deflections. The noise variance in the REEP estimates depend on the choice of forgetting factor λ in (3.10) and bandwidth of the low pass filters that are utilized to reduce the noise contents in f_{reep} and Q_{reep} . For convenience of comparison, the bandwidth of lock-in filter for SEEP technique, the forgetting factor λ and bandwidth of low pass filters for REEP technique are chosen such that the noise levels in both SEEP and REEP estimates are similar. Although reducing the low-pass bandwidth of the lock-in filter reduces the noise in SEEP method, the bandwidth must be higher than ω_B such that the time constants of estimated amplitude (a) of cantilever oscillations are not influenced by the lock-in filter.

The transient response for three different cantilevers with the same resonant frequency $f_0 = \omega_0/2\pi = 63.147$ kHz and different quality factors $Q_0 = 227.85$, $Q'_0 = 2Q_0 = 455.69$ and $Q''_0 = 3 * Q_0 = 683.55$ are studied. These cantilevers are driven at the resonant frequency, that is $\omega_d = \omega_0$ such that the amplitude of oscillation is 50 nm. The distance between the cantilever base and the sample (shown as sep in Figure 3.2) is varied in a step wise fashion such that when sep is low (high), the oscillating cantilever interacts (does not interact) with the sample. Figure 3.2 shows the transient response from the cantilever with $Q_0 = 227.85$. When the oscillating cantilever transitions from the interaction state (sep is low) to no-interaction state (sep is high), the time to attain steady state for the parameters $f_{eq} = \omega_{eq}/2\pi$ and Q_{eq} estimated using REEP and SEEP methods are observed which are tabulated in Table 3.1. Both REEP and SEEP methods match when the cantilever has reached steady-state near-sinusoidal oscillation (see Figure 3.2). In Figure 3.2 and Table 3.1, T_{amp} denotes the time required to reach steady state by the amplitude of cantilever oscillations. $T_{f,reep}$ and $T_{Q,reep}$ denote the convergence times for equivalent frequency and quality factors estimated using

the REEP technique. Similarly, $T_{f,seep}$ and $T_{Q,seep}$ represent corresponding convergence times for the SEEP method (see Figure 3.2). For each quality factor shown in Table 3.1, the estimates using REEP method converge faster than the SEEP technique. For example, at $Q_0 = 227.85$, the SEEP method requires 5.8 ms for both the estimates f_{seep} and Q_{seep} to converge. However, the time required for both f_{reep} and Q_{reep} to converge in REEP technique is 1.8 ms which is almost 69 % smaller than the time required by the SEEP method. It should also be noted that for the SEEP method, the estimates need longer time to reach steady state with higher quality factor. However, for the REEP method, the convergence becomes slightly faster when higher quality factor cantilevers are chosen. Hence, REEP is suitable for faster estimation of equivalent parameters.

Comprehensive experimental results are presented in the next section.

Q	T_{amp} (ms)	$T_{f,reep}$ (ms)	$T_{f,seep}$ (ms)	$T_{Q,reep}$ (ms)	$T_{Q,seep}$ (ms)
227.85	4.7	1.8	3.9	1.6	5.8
455.69	7.6	1.5	8.1	1.3	10.9
683.55	11	0.84	8.6	1.1	15

Table 3.1: (Simulation data) Time constants involved in estimation of equivalent parameters (in milliseconds).

3.6 Experimental results

3.6.1 Selection of system model

In many AFM setups, the drive u to the dither piezo at the base of the cantilever and the measurement y_m of the tip-deflection are available as data which are used to infer sample properties. Under ideal conditions with the spring-mass-damper (SMD) model the transfer function from u to y_m with no sample force ($h = 0$) is given by

$$G_0(s) = \frac{\beta_2}{s^2 + \frac{\omega_0}{Q_0}s + \omega_0^2}, \quad (3.29)$$

where β_2 is a scalar, s is the Laplace variable. However, as shown here, the experimentally obtained transfer function from the drive u to measured tip-deflection y_m of the

form

$$G_0(s) = \frac{\beta_1 s + \beta_2}{s^2 + \frac{\omega_0}{Q_0} s + \omega_0^2}, \quad (3.30)$$

better explains the data. Here the numerator contains a non-zero coefficient β_1 of s . The rationale for choosing (3.30) to represent the dynamics of the cantilever is provided below.

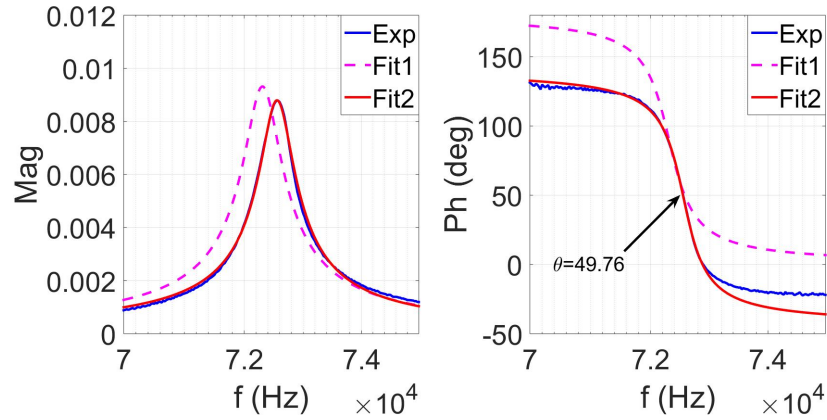


Figure 3.3: (Experimental data) Frequency response of cantilever in free air. Data is fit using a plant model with $\beta_1 = 0$ denoted by Fit 1. Fit 2 is obtained using nonzero β_1 . Fit 2 better approximates the frequency response.

Frequency response of AC240TS cantilever from Asylum Research obtained by applying frequency sweep input in free air is shown in Figure 3.3. The peak frequency $f_p = \omega_p/2\pi$ is found to be 72.55 kHz where the phase $\theta=49.76$ degrees. The frequency response is fit with plant model first described by (3.29) (called Fit 1) followed by (3.30) (called Fit 2). For Fit 2, The departure of the phase θ from 90 degrees at resonant peak can be shown equal to $\theta_c = \arctan(\beta_1\omega_p/\beta_2)$ (see Appendix A.1 for details). It is evident from Figure 3.3 that Fit 2 ($\beta_1 \neq 0$) not only better fits the frequency response data than Fit 1 ($\beta_1 = 0$), it also explains the departure of phase from 90 degrees at f_p (40.24 degrees from Figure 3.3) which is close to $\theta_c = \arctan(\beta_1\omega_p/\beta_2)=41.93$ degrees. Repeated experiments confirm the same conclusion.

The main contributing factor for the numerator polynomial being first order and not a constant in G_0 is hypothesized as being caused by the presence of delays between

the drive and the tip-deflection which include both electronic and structural effects. Evidently, the sources of delays cannot be completely accounted for by electronic sources which should remain unchanged whether the cantilever is far away with no squeeze film damping effects or at the reference position. In this chapter we leave the study of the sources of the delays to future; here it is assumed that the transfer functions from the drive to the tip-deflection measurement when intermittently interacting with the sample and at the reference position share the same numerator polynomial.

3.6.2 Estimation performance on transient data

Results from transient response experiment are discussed now. The cantilever is driven sinusoidally near mica substrate such that it oscillates under squeeze film damping with oscillation amplitude of 122 nm. The parameters at reference position $f_r = \omega_r/2\pi$ and Q_r are found using frequency sweep method which are 73.4 kHz and 131.6 respectively. The mica substrate is actuated in the z-direction using a piezoelectric actuator. A pulse waveform (shown as Z_{in} in Figure 3.4 (a)) is applied to the piezoelectric actuator such that the mica substrate periodically nudges the oscillating cantilever out of its steady state. Both REEP and SEEP methods are employed to estimate the equivalent parameters which are shown in the Figure 3.4. In Figure 3.4 (a), it is observed that after the nudge arrives, the oscillation amplitude takes time $T_{amp} = 3.4$ ms to recover to its steady state value of 122 nm. It should be noted that the bandwidth of oscillation amplitude of the cantilever is given by $f_B = f_r/(2Q_r) = 73.4 \text{ kHz}/131.6 = 279 \text{ Hz}$. Thus the expected recovery time $t_B = 1/f_B = 3.6$ ms which is consistent to the observation $T_{amp} = 3.4$ ms. From Figure 3.4 (b) and (c), the time to reach steady state for SEEP estimates f_{seep} and Q_{seep} are observed to be $T_{f,an} = 1.4$ ms and $T_{Q,an} = 3.5$ ms respectively. Thus, interpretation of data is possible after 3.5 ms which is close to the recovery time of amplitude ($T_{amp} = 3.4$ ms). From Figure 3.4 (b) and (c), the time to attain steady state for f_{reep} and Q_{reep} which are denoted as $T_{f,reep}$ and $T_{Q,reep}$ respectively are both 1.8 ms. Indeed, REEP based estimation offers superior bandwidth than the SEEP method. When the cantilever oscillation reaches steady state, it is evident from Figure 3.4 that both REEP and SEEP methods concur with each other.

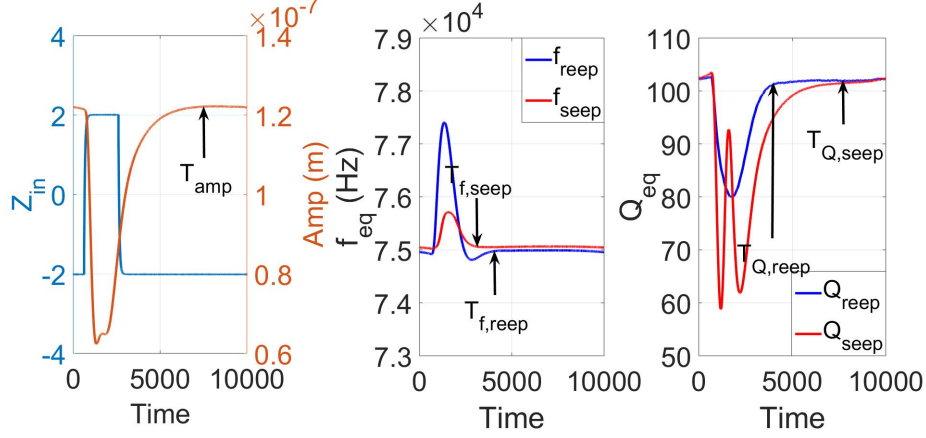


Figure 3.4: (Experimental data) (a) The input signal Z_{in} (in V) to z-piezo actuator with amplitude of cantilever oscillations Amp (in meter). (b) REEP estimated equivalent frequency f_{reep} and SEEP estimated equivalent frequency f_{seep} (in Hz). (c) REEP estimated equivalent quality factor Q_{reep} with SEEP estimated quality factor Q_{seep} . Time axis is in the units of T_s where $1/T_s$ is 2 MHz.

3.6.3 Approach-retract experiments on mica

Now we demonstrate the need for choosing proper reference position. It is observed that the transfer functions from the drive to the measured tip-deflection of the form

$$G_r(s) = \frac{\alpha_1 s + \alpha_2}{s^2 + \frac{\omega_r}{Q_r} s + \omega_r^2} \quad \text{and} \quad (3.31)$$

$$G_{eq}(s) = \frac{\alpha_1 s + \alpha_2}{s^2 + \frac{\omega_{eq}}{Q_{eq}} s + \omega_{eq}^2}, \quad (3.32)$$

provide a good fit to the data when the cantilever is at the reference position and is interacting with the sample respectively. In transfer functions $G_r(s)$ and $G_{eq}(s)$ the numerator polynomial coefficients α_1 and α_2 are the same. Note however, that the numerator polynomial coefficients β_2 and β_1 for the model $G_0(s)$ shown in (3.30) (obtained with the cantilever far away from the sample where no squeeze film damping effects are present) differ from α_2 and α_1 . Through repeated experiments it is verified that (α_1, α_2) differ consistently from (β_1, β_2) .

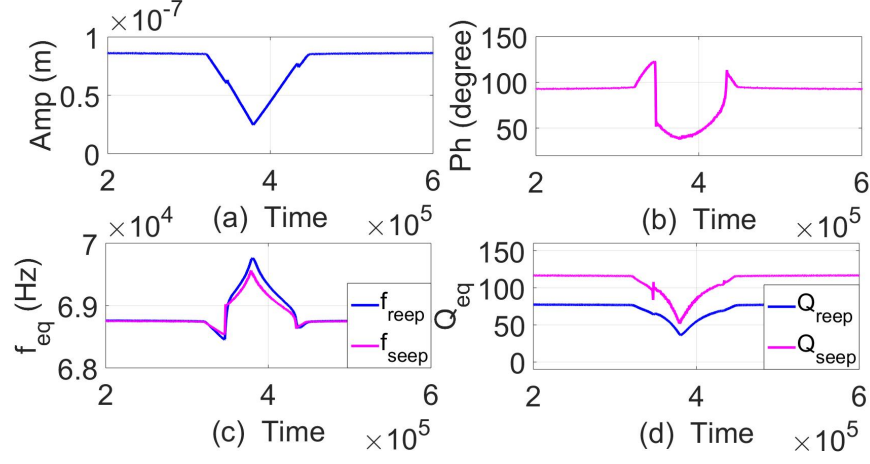


Figure 3.5: (Experimental data) (a) Deflection amplitude a (meter) during approach-retract cycle on freshly cleaved mica. (b) Corrected phase $\tilde{\theta}$ (degrees). (c) Equivalent frequency using REEP (f_{reep}) and SEEP method (f_{seep}) in Hz. (d) Equivalent quality factor using REEP (Q_{reep}) and by SEEP method (Q_{seep}). The reference position is chosen in free air. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.

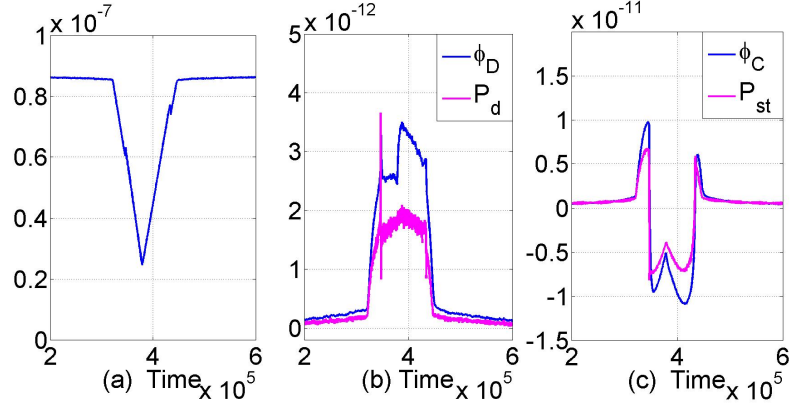


Figure 3.6: (Experimental data) (a) Deflection amplitude a (meter) during approach-retract cycle on freshly cleaved mica. (b) Dissipation power using REEP (Φ_D) and by (3.21) shown as P_d (unit Watt). (c) Storage power Φ_C using REEP and using (3.23) denoted as P_{st} in Watt. The reference position is chosen in free air. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.

Dynamic mode force curve (approach-retract) experiments are performed on freshly cleaved mica sheet using MFP-3D AFM from Asylum Research. Such approach-retract

cycles are sufficiently slow so that the cantilever oscillation can be assumed to be in steady-state. The free-air cantilever transfer function model from dither excitation signal u to the measured deflection y_m is estimated using a frequency sweep method [46]. The first mode resonant frequency and quality factor are found as $f_0 = \omega_0/2\pi = 68.765$ kHz and $Q_0 = 116.287$. The numerator coefficients of the transfer function as shown in (3.30) in free-air are estimated to be $\beta_1 = 5.6526e+03$ and $\beta_2 = -3.3618e+06$. The excitation and deflection data of the cantilever are gathered with sampling frequency of 1.024 MHz. The set of coefficients (a_2, a_1, a_0) for the discrete time plant dynamics as shown in (3.8) are used for REEP based estimation of f_{reep} and Q_{reep} . The drive frequency f_d ($= \omega_d/2\pi$) is the same as f_0 (68.765 kHz). The reference oscillation amplitude (same as free air amplitude) is set to be $a_r = 125.26$ nm. $f_{seep} = \omega_{seep}/2\pi$ and Q_{seep} are determined using (3.6) and (3.7) respectively where the parameters (ω_r, Q_r) are chosen to be the same as (ω_0, Q_0) . Figure 3.5 suggests that estimated frequencies f_{reep} and f_{seep} match well. However Q_{reep} and Q_{seep} deviate considerably from each other. In Figure 3.5 (d), the average percentage difference between Q_{reep} and Q_{seep} is 33.6 %. This discrepancy is attributed to inappropriate choice of reference position which in this case is the free-air model. The storage power Φ_C and dissipation power Φ_D are evaluated as described in Section 3.3 using REEP. Storage power P_{st} and dissipative power P_d are also evaluated utilizing conservation principles from (3.23) and (3.21) respectively which are compared in Figure 3.6. Evidently P_{st} matches with Φ_C . However Φ_D and P_d differ significantly when the oscillating cantilever engages with the sample. In Figure 3.6 (b), the average percentage error between Φ_D and P_d is estimated to be 72.3 %. Again this difference is attributed to ignoring the effect of squeeze film damping and choosing incorrect Q_r and θ_c (thus $\tilde{\theta}$).

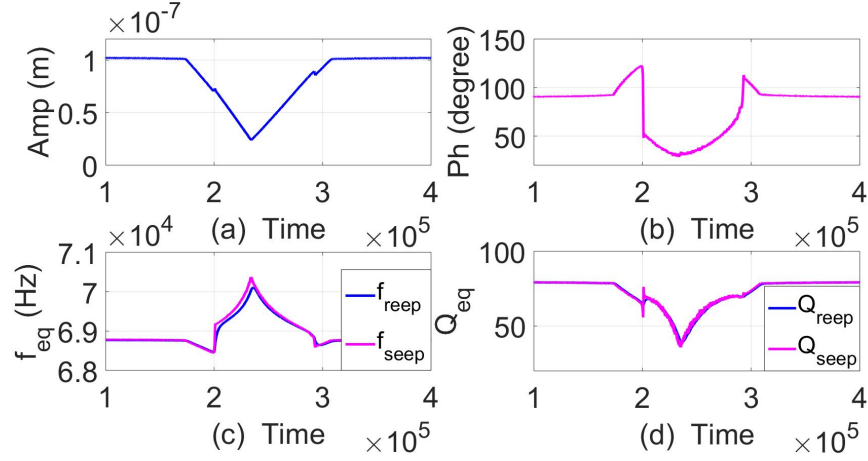


Figure 3.7: (Experimental data) (a) Oscillation amplitude a (meter) (b) Corrected phase $\tilde{\theta}$ (degrees). (c) Equivalent frequency using REEP (f_{reep}) and SEEP method (f_{seep}) in Hz. (d) Equivalent quality factor using REEP (Q_{reep}) and SEEP method (Q_{seep}). The reference position is chosen near sample to include squeeze film damping. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.

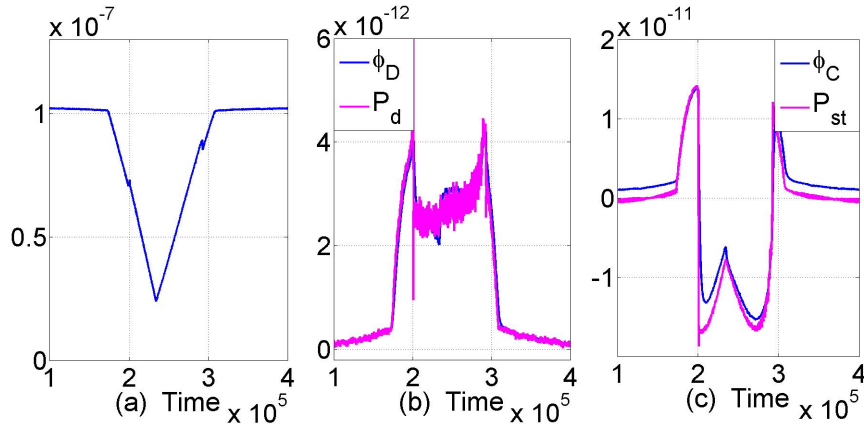


Figure 3.8: (Experimental data) (a) Oscillation amplitude a (meter). (b) Dissipation power using REEP (Φ_D) and P_d using (3.21) in Watt. (c) Storage power using REEP (shown as Φ_C) and using (3.23) denoted as P_{st} in Watt. The reference position is chosen near sample to include the squeeze film damping effect. Resolution of time axis is T_s with $1/T_s=1.024$ MHz.

Now the cantilever transfer function is estimated using frequency chirp at a reference position which is close to sample to include squeeze film damping. The reference parameters are found to be $f_r = \omega_r/2\pi = 68.857$ kHz and $Q_r = 74.61$ with the numerator coefficients $\alpha_1 = 4.455e + 03$, $\alpha_2 = 7.9461e + 08$. $f_d = \omega_d/2\pi$ is chosen as 68.784 kHz. Amplitude of oscillations at reference a_r is chosen to be 102.67 nm. (f_{seep}, Q_{seep}) are determined using (3.6) and (3.7). Discretizing the transfer function at reference position yields the parameters (a_2, a_1, a_0) which are used by REEP method. From Figure 3.7 (c) and (d), the close similarity of f_{reep} with f_{seep} and Q_{reep} with Q_{seep} can be observed. The difference between Q_{reep} and Q_{seep} from Figure 3.7 (d) is estimated to be 0.94 % which is much smaller compared to 33.6 % in Figure 3.5 (d). Further, the percentage difference between Φ_D and P_d from Figure 3.8 (b) is estimated to be just 13 % in contrast to 72.3 % observed in Figure 3.6 (b). The storage power Φ_C determined through REEP and P_{st} estimated using (3.23) also match closely in Figure 3.8 (c). Thus, selection of proper reference position plays important role in determining the storage and dissipative parameters.

3.6.4 Stiffness and dissipation imaging of PLMA-PBMA polymer blend

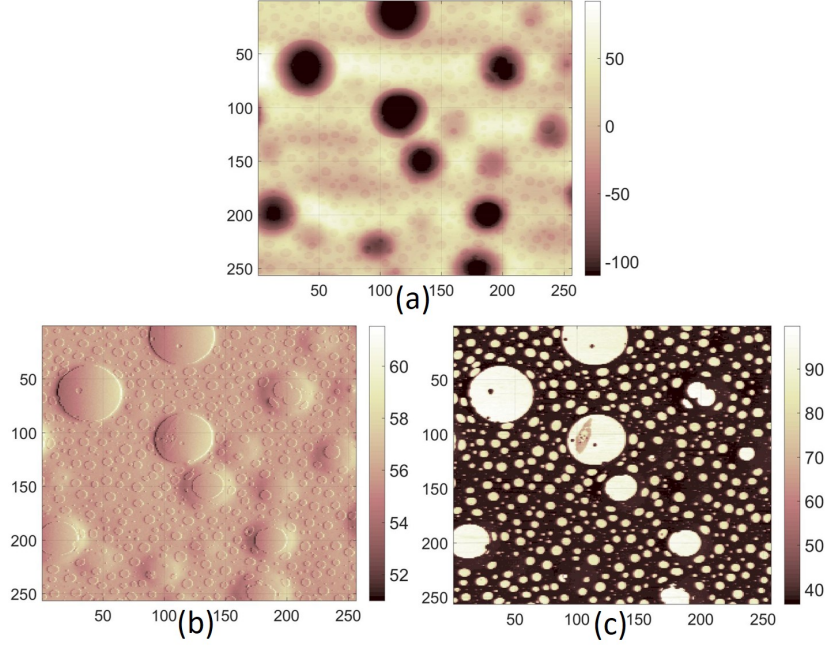


Figure 3.9: (Experimental data) (a) Height image (nm), (b) amplitude image (nm) and (c) $\tilde{\theta}$ phase image (degrees) of a 20 $\mu\text{m} \times 20 \mu\text{m}$ PLMA-PBMA polymer sample.

Real time algorithm for REEP is designed and implemented on Xilinx ML605 FPGA kit. The dither excitation and cantilever deflection data are collected through 4DSP FMC-151 ADC-DAC module that acts as an interface between the FPGA board and analog input/output ports available from the controller box of MFP-3D AFM from Asylum Research. After extensive verification of the developed FPGA design through simulations, the design is used to process dither excitation u and measured deflection y_m from MFP-3D AFM to estimate the equivalent parameters f_{reep} and Q_{reep} in real time. Dynamic mode imaging is performed on a blend of poly(lauryl methacrylate) PLMA and poly(butyl methacrylate) PBMA sample. AC240TS cantilever with free-air resonant frequency $f_0 = 72.7 \text{ kHz}$ and quality factor $Q_0 = 146.4$ is selected for imaging. The stiffness of the cantilever k is estimated as 1.75 nN/nm from thermal data in free air. The reference position is chosen near sample and the frequency tune near first mode resonance is used to estimate (α_1, α_2) and the bias phase θ_c as explained previously.

This offset correction for θ is also done in the Igor Pro software from Asylum Research during AC mode tuning near sample. The dynamic mode height, phase $\tilde{\theta}$ and amplitude images of a 20×20 micron sample is shown in Figure 3.9.

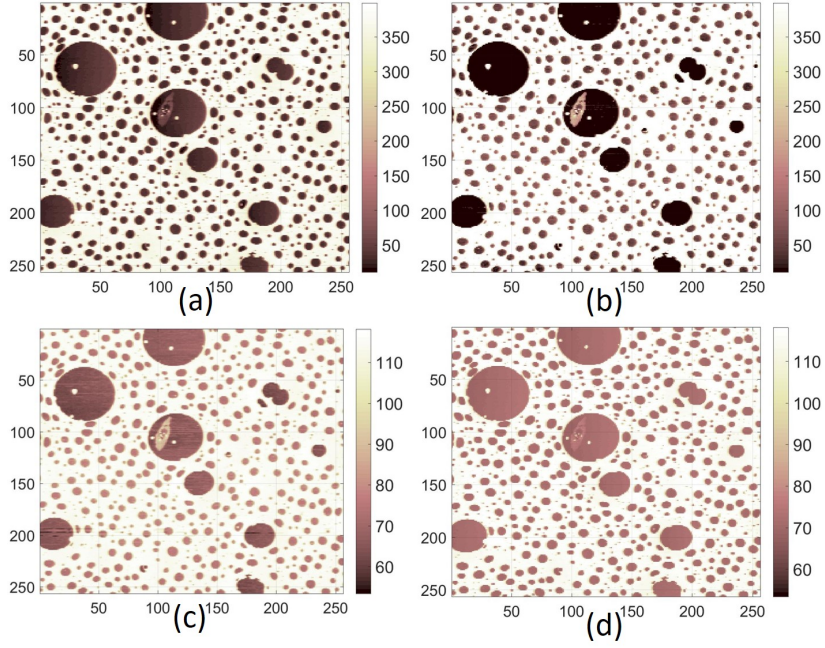


Figure 3.10: (Experimental data) (a) Shift of equivalent frequency of oscillating cantilever around the bias frequency of 72.273 kHz estimated through REEP (f_{reep}) and (b) using SEEP method (f_{seep}) in Hz. (c) Equivalent quality factor (Q_{reep}) evaluated using REEP and (d) using SEEP method (Q_{seep}).

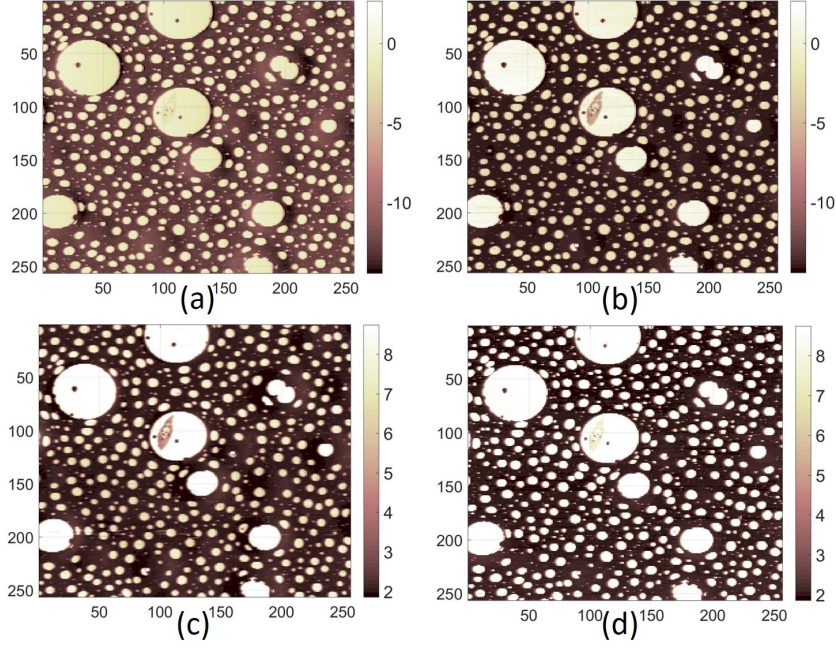


Figure 3.11: (Experimental data) (a) Storage power Φ_C evaluated through REEP and (b) P_{st} estimated using (3.23) in pW. (c) Dissipative power Φ_D estimated by REEP and (d) P_d estimated using (3.21) in pW.

Drive frequency f_d is chosen to be 72.738 kHz. Equivalent parameters estimated both using SEEP and through REEP are shown in Figure 3.10. Evidently, REEP and SEEP estimation for equivalent parameters (f_{eq}, Q_{eq}) provide a close match. Further, storage and dissipative power images are computed and shown in Figure 3.11. Close similarity between estimated storage powers Φ_C and P_{st} is evident from Figure 3.11 (a) and (b). Dissipative power Φ_D evaluated through REEP and P_d using (3.21) are shown in Figure 3.11 (c) and (d) that are also close match.

3.7 Conclusion

This chapter reports a simple steady state based method (SEEP) for estimating material properties using the dynamic mode of AFM operation. This method employs available signals used in the standard intermittent contact dynamic mode operation where the

external excitation to the cantilever need not be altered. The REEP technique is demonstrated to be capable of estimation during transient phase of cantilever oscillation. Thus REEP offers higher bandwidth of estimation than the analytical method. However, REEP requires complex hardware implementation and careful selection of excitation signals at multiple frequencies. This chapter also provides a comparison of independent approaches for estimating the storage and dissipative power components due to interaction between cantilever tip and sample. Selection of appropriate system model for interpretation of the AFM data is discussed with verification through experiments. The important role of selection of a reference position with respect to which the equivalent parameters and storage-dissipation powers should be evaluated is also emphasized with extensive results. It is demonstrated that by careful experiments, so far independent approaches for estimating storage-dissipation can yield similar results. Thus this chapter unifies the different methods available for stiffness-dissipation imaging with regular dynamic mode AFM. The step by step procedure for selection of plant model, choice of reference position and incorporation of correction terms in the phase signal can be used as recipe by AFM users for investigating sample properties with dynamic mode AFM. As an extension of this work, theoretical models should be developed for explaining the origin of the system model that better explains the cantilever dynamics.

Chapter 4

Design of FPGA based material characterization module for AFM

4.1 Introduction

System identification has played a key role in mathematical characterization of systems. Since the earlier pioneering works emphasizing mostly on identifying linear systems [47–49], techniques have broadened to characterize nonlinear and stochastic systems as well [50–54]. Further, a significant amount of research emphasizes real-time identification techniques employed for time varying systems [55–58]. This chapter details an embedded implementation of such a real-time system identification algorithm first reported in [38] (also outlined in Section 3.3.2 of Chapter 3) with application to atomic force microscopes (AFMs). This algorithm employs forgetting factor for prioritizing recent measurement data compared to the past data [57].

Since its invention [5], AFM has become a tool of choice for investigating materials in the nanometer or sub-nanometer regime. Traditionally, AFMs were primarily utilized to investigate topography of samples. In recent times, researchers have also developed AFM based methods for characterizing mechanical properties of materials at the nano-scale. Examples of such applications include but not limited to opto-electronics [59], drug delivery [60], biology [61–65] and also polymer science [66–68]. Many such applications employ either forced indentation methods [62, 64] or contact resonance mode methods [10, 61, 69, 70]. Such contact mode techniques are simple and robust, however,

they create substantial mechanical wear and tear on the sample as well as on the AFM probe [18, 71]. One promising alternative is to employ dynamic mode of AFM operation [15, 20, 72] which creates minimal mechanical wear. Recently, techniques [9, 35, 66, 73] have emerged where mechanical properties of material can be determined employing the dynamic mode of AFM operation. These techniques are particularly well suited for investigating soft-matter. Moreover, with the development of novel system theoretical perspectives of dynamic mode AFM (see [12, 28, 74, 75]), it is possible to employ real-time system identification methods for estimating the stiffness and dissipative properties of materials [12, 38] which is explained in Section 3.2 and Section 3.3.2 of Chapter 3. Such a system identification approach which we adopt in this chapter requires minimal customization of most of the commercially available AFMs.

Field Programmable Gate Arrays (FPGAs) provide attractive embedded solutions since they offer high speed of operation, supported protocols, reconfigurable capability and design reuse [76]. FPGAs are employed in a diverse spectrum of industrial applications including aircraft, automobiles, power electronics and also in areas requiring a large number of parallel computations [77–80]. Modern FPGAs can also deal with high precision data [81, 82]. Motivated by these examples, we selected the Xilinx ML605 DSP kit as the FPGA platform to host our system identification algorithm.

In this chapter, we describe the FPGA based design of a system identification module in a step by step manner. The module reported in this chapter is user friendly which can be interfaced at ease with AFMs available on the market. The design methodology developed here is not limited to the specific example provided in this chapter but applicable to a wide variety of real time signal processing algorithms which require high precision computations. We also detail the procedure for judiciously choosing the forgetting factor in the identification algorithm. As demonstrated in this chapter, high values of forgetting factor creates accurate but slow estimates (over-tuning) while the low values create fast but incorrect identification (under-tuning). Thus, appropriate tuning of the forgetting factor in the design of the system identification module plays an important role. Extensive results are presented that corroborate the effectiveness of the design.

This chapter is organized as follows: Section 4.2 details the design methodology and building blocks of the system identification module implemented on FPGA. Section 4.3

establishes the validity of the proposed design with simulation and experimental results. Section 4.4 concludes this work.

4.2 Design of system identification module on FPGA

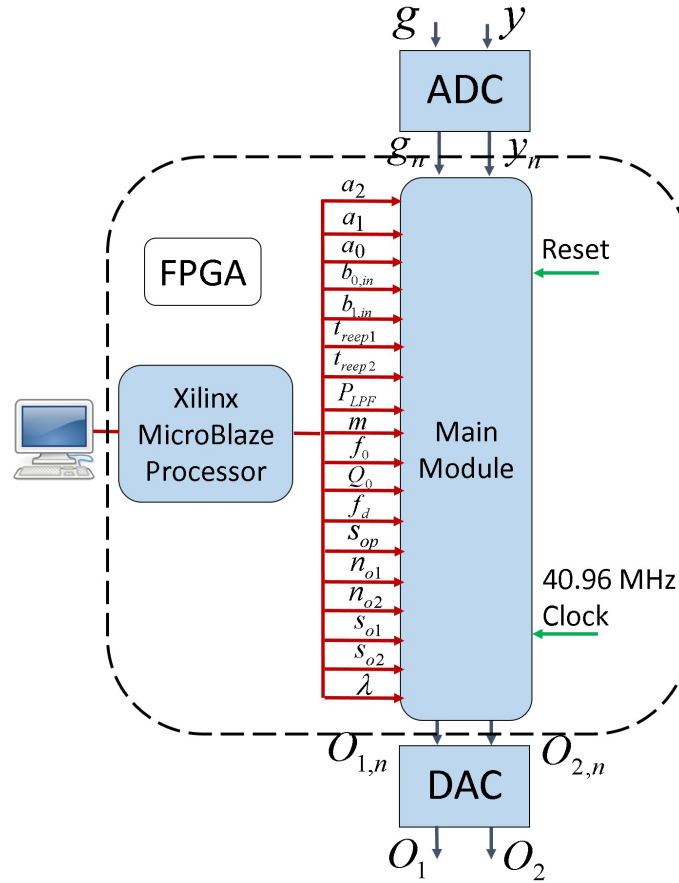


Figure 4.1: Outline of the system identification module implemented on FPGA

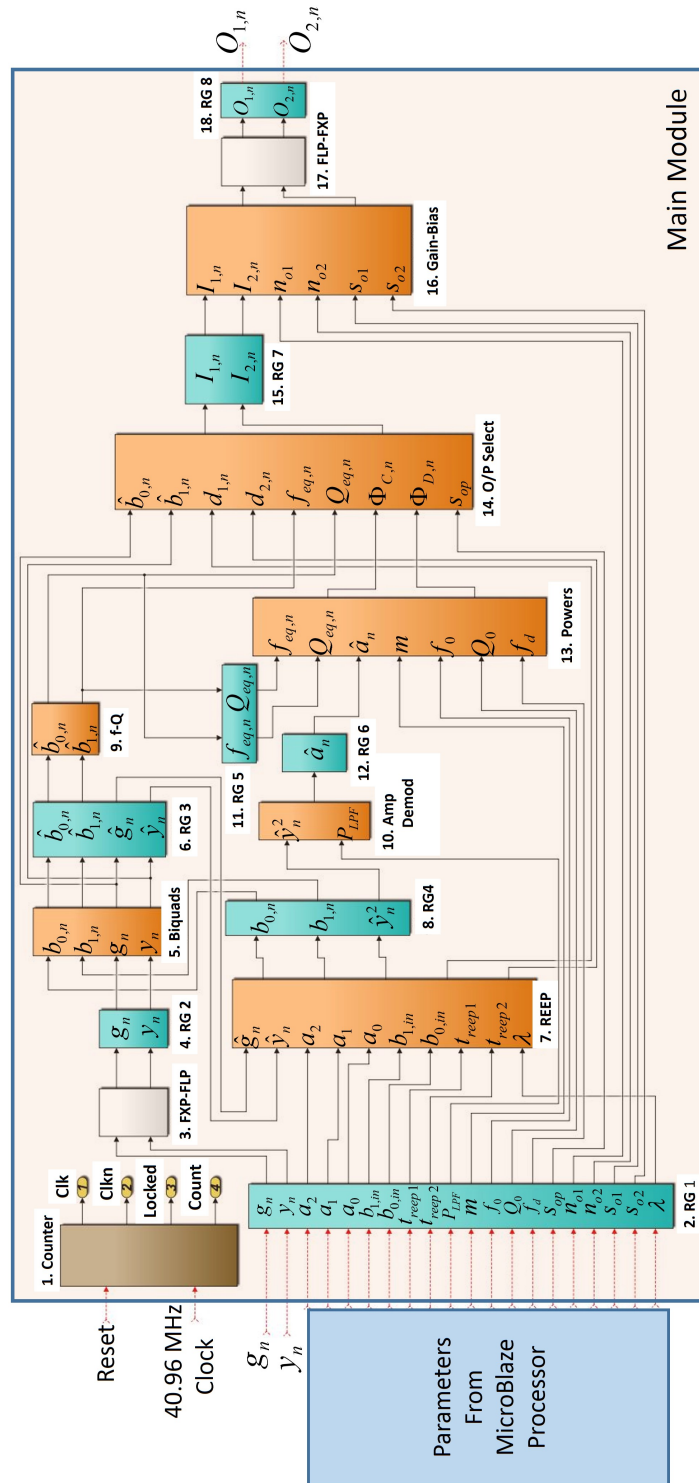


Figure 4.2: Modules and flow of signals inside the Main Module

A schematic of the system identification module is shown in Figure 4.1. This module is implemented on the target Xilinx ML605 Digital Signal Processing (DSP) development kit with FPGA Mezzanine Card (FMC) 151 ADC-DAC (analog-to-digital and digital-to-analog converter) unit. Each of the ADC and DAC contains two pairs of inputs and outputs. The block denoted as Main Module (see Figure 4.1) contains the digital design of the algorithm. Inside Main Module, computational tasks are divided in different blocks. As shown in Figure 4.1, the signals that appear as inputs to the ADC unit (top of the Figure 4.1) are g : the excitation signal of the cantilever and y : the measured deflection of the cantilever tip which are obtained from the controller box of MFP 3D AFM from Asylum Research (these signals are available as measurements in most modern AFM systems, see Figure 3.1). The analog signals g and y are applied (see Figure 3.1) to the input ports of the ADC which generates the digitally sampled signals g_n and y_n in fixed point data format. We use the subscript n to denote the discrete time signals. These signals are processed step by step by the blocks inside the Main Module. The signals with green arrows shown on the right are the Reset signal and a Clock signal of frequency 40.96 MHz. Both of these signals are generated within the FPGA and used for driving the Main Module. An embedded subsystem based on Xilinx MicroBlaze soft processor is included in our design which contains configurable memory spaces. This subsystem communicates with a personal computer through USB cable to access the 18 configurable parameters which are shown with red arrows on the left of Main Module in Figure 4.1. The user can update and store these configurable parameters in real time which are accessible to the Main Module. Such parameters include the forgetting factor, λ , in (3.10), Q_0 , f_0 , m and $f_d = \omega_d/2\pi$ in (3.3), a_2 , a_1 and a_0 in (3.8), and initial values for the unknown parameters b_0 , b_1 in (3.9) which are denoted by $b_{0,in}$ and $b_{1,in}$ respectively. The other parameters shown in Figure 4.1 will be explained in the subsequent sections. The digital outputs $O_{1,n}$ and $O_{2,n}$ from the Main Module shown in Figure 4.1 are routed to the two input ports of the DAC. The DAC outputs the analog signals O_1 and O_2 which can be accessed as the final outputs from the FPGA module.

4.2.1 Components in Main Module

The flow of signals inside the Main Module is depicted in Figure 4.2. A brief description of each of the modules inside the Main Module is provided below:

1. Counter: The Counter module accepts the Reset signal and the Clock signal (40.96 MHz) to generate a *Count* signal that repeats counting from 0 to $N - 1$ with $N = 40$ (the binary equivalent of *Count* is shown in Figure 4.3). The *Count* signal is synchronized with the buffered 40.96 MHz clock signal *Clk* generated by the Counter module. Another clock signal denoted by *Clkn* is synthesized which is shifted in phase by 180° from the *Clk* signal (see Figure 4.3). The signals *Clk*, *Clkn* and *Count* drive and maintain synchronization among all the modules inside Main Module. In order to keep the schematic simple, the routing of *Clk*, *Clkn* and *Count* to each individual module is not explicitly illustrated in Figure 4.2. However, unless mentioned otherwise, it is assumed that each of the modules is synchronous with these clocking and counting signals. The *Locked* output signal from the Counter module (see Figure 4.2 and Figure 4.3) turns from 0 to 1 when the clocking signals become ready after switching on the FPGA board. Hence, we activate the design logic of Main Module only when *Locked* is 1.

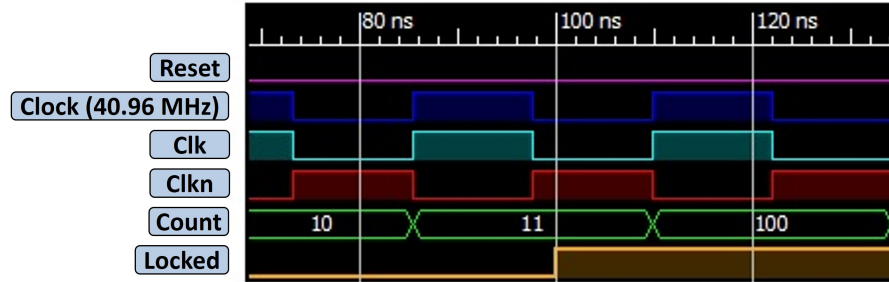


Figure 4.3: Illustration of the timing of clock and count signals.

2. RG 1 (Register module 1): The digitally sampled cantilever excitation and deflection signals i.e. g_n and y_n with the other parameters obtained from the Xilinx MicroBlaze processor are registered in this module. The registered data are accessed for subsequent processing by the rest of the modules inside the Main Module.

3. FXP-FLP (Fixed Point to Floating Point conversion module): After being registered at module RG 1, the signals g_n and y_n which are 16-bit fixed point data are routed to the FXP-FLP module where they are converted into 64-bit floating point signals. Most of the subsequent arithmetic computations on the input and intermediate signals are performed in 64-bit floating point format. These high precision computations

help to maintain computational accuracy of the system identification algorithm.

4. RG 2 (Register module 2): The signals output from FXP-FLP module are registered before being fed into the digital filters.

5. Biquads: There are two sets of biquads (digital filters) [83] which are implemented inside the Biquads module. One set includes two high pass filters (HPFs), the other set includes two low pass filters (LPFs). The HPFs are designed such that they have an equivalent cut-off frequency of 2 kHz in the analog frequency domain. These HPFs are utilized to eliminate slow changes and bias terms present in the input signals g_n and y_n to yield the outputs $\hat{g}_n$ and $\hat{y}_n$. The LPFs which have equivalent analog cut-off frequency of 2 kHz are employed to remove noise from the estimates evaluated by the REEP module as explained below. Each individual biquad is implemented using the direct form II architecture [84]. The filtered signals are routed to module RG 3.

6. RG 3 (Register module 3): The outputs of Biquads module are registered in the RG 3 module.

7. REEP (Recursive estimation of equivalent parameters module): The high pass filtered signals $\hat{g}_n$ and $\hat{y}_n$ are processed by the REEP module. This module computes the set of recursive update equations required by the BCEWRLS algorithm (see Appendix A.2). The forgetting factor λ along with other adjustable parameters are received from RG 1 module. These parameters include a_2 , a_1 , a_0 , $b_{0,in}$, and $b_{1,in}$. Further, the REEP module accepts two additional signals t_{reep1} and t_{reep2} to define two configurable test output signals denoted by $d_{1,n}$ and $d_{2,n}$. $d_{1,n}$ and $d_{2,n}$ can be configured (by selecting proper values of t_{reep1} and t_{reep2}) to access different signals internal to the REEP module. These test output signals allow the users to debug any error in the design of the REEP module. The principal outputs of the REEP module are the estimates of the discrete time parameters denoted as $b_{0,n}$, $b_{1,n}$ in Figure 4.2, and squared deflection signal $\hat{y}_n^2$. $\hat{y}_n^2$ serves as the input to the Amp Demod module outlined later. The useful frequency spectrum of the estimated parameters $b_{0,n}$ and $b_{1,n}$ lie at frequencies much lower than the excitation frequency f_d . For example, AC240TS cantilevers (supplied by Asylum Research) are excited around the first resonant frequency (f_0) which nominally lies near 70 kHz, whereas, $b_{0,n}$ and $b_{1,n}$ are contained mostly below the frequencies 2-10 kHz. Thus, as mentioned earlier, LPFs in the Biquads module are utilized to filter the high frequency noise present in these estimates.

8. RG 4 (Register module 4): $b_{0,n}$, $b_{1,n}$ and $\hat{y}_n^2$ obtained from REEP module are registered in this module before being routed to the subsequent modules.

9. f-Q: The discrete time estimated parameters $b_{0,n}$ and $b_{1,n}$, after being low pass filtered ($\hat{b}_{0,n}$ and $\hat{b}_{1,n}$ denote the corresponding LPF outputs) are input to the f-Q module. This module implements the set of equations (3.11) to determine the estimates of the continuous-time equivalent parameters, namely, $f_{eq,n}$ and $Q_{eq,n}$.

10. Amp Demod (Amplitude demodulation module): Note that in (3.3) it is necessary to know the amplitude a of the oscillating cantilever in real time to determine the storage and dissipative terms Φ_C and Φ_D . An estimate of the amplitude of cantilever oscillations denoted by $\hat{a}_n$ is evaluated in this module as explained in Figure 4.4. The parameters of the LPF shown in Figure 4.4 needed for demodulation are obtained from the parameter P_{LPF} after being registered at the RG 1 module which is shown in Figure 4.2.

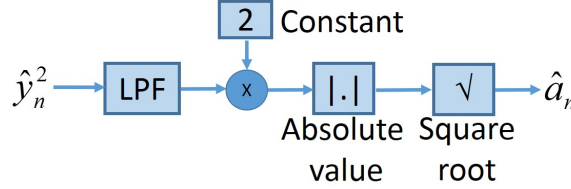


Figure 4.4: Amplitude demodulation scheme

11. RG 5 (Register module 5): The estimated equivalent parameters $f_{eq,n}$ and $Q_{eq,n}$ are registered in this module before being sent to the Powers module described below.

12. RG 6 (Register module 6): The estimate of cantilever oscillation amplitude $\hat{a}_n$ obtained from the Amp Demod module is registered here before being sent to the Powers module for further processing.

13. Powers: The Powers module determines the storage power $\Phi_{C,n}$ and the dissipative power $\Phi_{D,n}$ due to cantilever tip-sample interactions from the equivalent parameters $f_{eq,n}$, $Q_{eq,n}$ using (3.3). The parameters f_0 , Q_0 which represent the cantilever dynamics free from sample, f_d : the drive frequency and m : the mass of the cantilever are also required in (3.3). f_0 and Q_0 are determined using a frequency sweep [52] based identification of cantilever's frequency response near its first resonant frequency. m is

determined before imaging using the thermal response of the cantilever in air. f_d is set by the user (typically $f_d \approx f_0$). Thus these parameters are available before imaging and updated on the FPGA using a personal computer through the user interface depicted in Figure 4.1.

14. O/P Select (Output selection module): A 2-bit signal called s_{op} which is defined by user is input to the O/P Select module. There are 4 additional pair of inputs to the O/P Select module; $\{\hat{b}_{0,n}, \hat{b}_{1,n}\}$ from the Biquads module, $\{d_{1,n}, d_{2,n}\}$ from REEP module, $\{f_{eq,n}, Q_{eq,n}\}$ from the f-Q module and $\{\Phi_{C,n}, \Phi_{D,n}\}$ from the Powers module. The user can route any one pair out of these 4 data pairs to the next module by selecting proper values of the s_{op} signal.

15. RG 7 (Register module 7): The selected signals from O/P Select module are registered here and input to the Gain-Bias module.

16. Gain-Bias: This module is responsible for amplifying (or scaling down if necessary) the selected output data pair with appropriate gains $\{n_{o1}, n_{o2}\}$. It also allows the user to shift the output signal values with suitably chosen bias terms $\{s_{o1}, s_{o2}\}$. The gain and bias parameters are adjustable by the user.

17. FLP-FXP (Floating Point to Fixed Point conversion module): The output data from Gain-Bias module which are represented in 64-bit floating point format, enter the FLP-FXP module where they are converted into 16-bit fixed point data compatible with the DAC specifications.

18. RG 8 (Register Module 8): The fixed point output signals from FLP-FXP module get registered here. Resulting outputs exit the Main Module as the data $\{O_{1,n}, O_{2,n}\}$ which are routed to the DAC inputs.

Each of the modules inside the Main Module except the register modules and the Counter is designed with the same architectural backbone which is described below.

4.2.2 Architectural backbone

The architectural backbone is a general design framework based on which different modules are developed. Once the backbone is set, it provides a common starting point for the design of different modules. The designer develops the timing strategies of various input and output signals in the backbone and customizes some components so that a diverse set of computational targets can be accomplished. A systematic backbone also

allows for convenient timing analysis, easy customization of one module into a different module, and debugging design errors. A general outline of the architectural backbone is shown in Figure 4.5 which consists of the following components:

Input registers: The data $ip_1, ip_2, \dots, ip_i$ input to a module, where i denotes the number of inputs (see Figure 4.5), are registered at the falling edge of Clk when $Count$ is 0.

Functional subunits: The registered input data are processed by a set of functional subunits (see Figure 4.5). Each functional subunit contains one computational core [85], a multiplexer, a de-multiplexer and a set of registers which are shown in Figure 4.5. Three types of computational cores are used to design the system identification module; the arithmetic cores, special function cores and data type conversion cores.

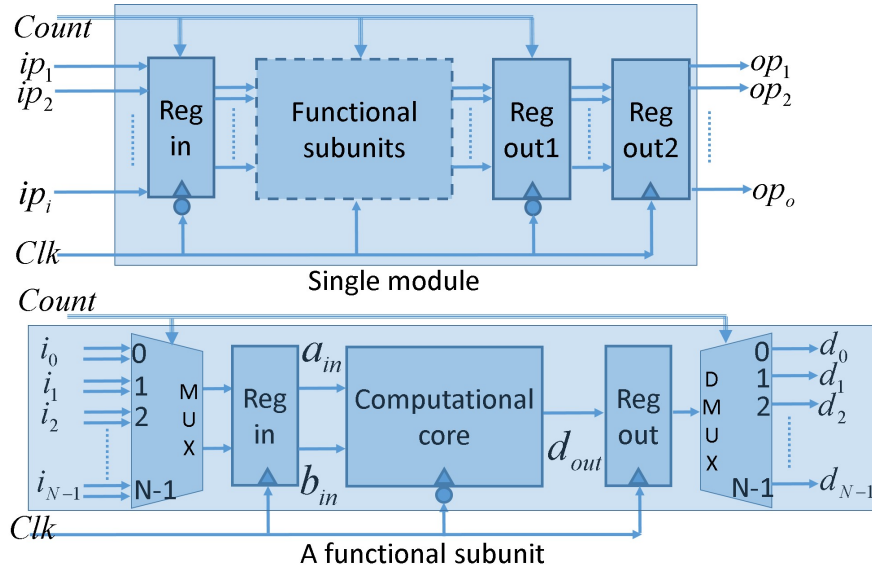


Figure 4.5: The architectural backbone. Clk operates at 40.96 MHz. $Count$ repeats counting from 0 to $N - 1$ with $N = 40$. MUX and DMUX represent the multiplexer and de-multiplexer blocks respectively inside a functional subunit.

An arithmetic core performs any one of the operations; multiplication, addition or subtraction, reciprocation, and square-root operation. These arithmetic operations are executed in double precision (64 bits) floating point data format [86]. Some mathematical functions including the trigonometric functions $\tan^{-1}$ and $\tanh^{-1}$ necessary in

evaluating (3.11) are implemented utilizing special function cores. Such special function cores can operate only on fixed point data. Besides, the ADC and DAC operate on fixed point data as well. Hence, data is converted from fixed point to floating point format and vice-versa when needed using the data type conversion cores.

Different modules contain different number of computational cores. Each of the computational cores requires a specific number of clock periods (called latency) to finish a computation while operating synchronously with Clk . These latencies are utilized to construct the timing schedules for assigning the inputs $\{i_0, i_1, \dots, i_{N-1}\}$ to the multiplexer and registering the outputs $\{d_0, d_1, \dots, d_{N-1}\}$ of the de-multiplexer connected to a computational core as shown in Figure 4.5. When the *Count* value is k , the MUX allows its k^{th} set of input data to be registered and fed into the computational core. During the same time, the DMUX allows its k^{th} output to update values from the output register of the computational core. TABLE 4.1 and TABLE 4.2 illustrates the number of different computational cores utilized in different modules along with corresponding latencies.

Module	Multiplication (3)	Addition (2)	Subtraction (2)	Reciprocation (5)	Square root (7)
Biquads	1	1	0	0	0
REEP	5	2	2	1	0
Amp Demod	1	1	0	0	0
f-Q	2	1	1	1	1
Powers	2	0	1	1	1
Gain- Bias	1	0	1	0	0

Table 4.1: Number of different computational cores (latencies indicated in brackets) used in each module.

Module	Tan inverse (35)	Hyperbolic tan inverse (35)	Floating to fixed point (6)	Fixed to floating point (6)
Biquads	0	0	0	0
REEP	0	0	0	0
Amp Demod	0	0	0	0
f-Q	1	1	1	1
Powers	0	0	0	0
Gain- Bias	0	0	0	0

Table 4.2: Number of different computational cores (latencies indicated in brackets) used in each module.

TABLE 4.3 illustrates an example of the timing schedule for the computational cores which are used in the Gain-Bias module. The first column shows the *Count* values at which the computations are performed. The multiplication and subtraction cores (denoted by Mult and Sub in TABLE 4.3) retain latencies of 3 and 2 respectively. Thus, after providing the inputs $I_{1,n}$ and n_{o1} to the multiplication core, the corresponding product ($I_{1,n}n_{o1}$) is available after 3 *Clk* cycles (see TABLE 4.3) which is collected in the intermediate signal mlt_4 when *Count* = 4. At the next *Clk* cycle, mlt_4 and s_{o1} are provided to the inputs of the subtraction core. Corresponding output value ($mlt_4 - s_{o1}$) is available after 2 *Clk* cycles from the output of the subtraction core which is stored in the signal sub_7 . sub_7 is eventually registered at the end of the *Count* cycle (*Count* = 39) from the Gain-Bias module as the output $O_{1,n}$. Thus $O_{1,n} = I_{1,n}n_{o1} - s_{o1}$ (n_{o1} : gain, s_{o1} : bias) which is the desired output. Other computations in the Gain-Bias module (see TABLE 4.3) as well as in other modules are performed using similar strategies.

Count	Mult Core Input 1	Mult Core Input 2	Mult Core Output	Sub Core Input 1	Sub Core Input 2	Sub Core Output
0						
1	$I_{1,n}$	n_{o1}				
2	$I_{2,n}$	n_{o2}				
3						
4			mlt_4			
5			mlt_5	mlt_4	s_{o1}	
6				mlt_5	s_{o2}	
7						sub_7 ($O_{1,n}$)
8						sub_8 ($O_{2,n}$)

Table 4.3: Timing schedule for Gain-Bias module.

Output registers: There are two output registers, one operating at the negative edge and the other operating at the positive edge of Clk through which data are output from each module. The final outputs $op_1, op_2, \dots, op_o$ of a module (o denotes the number of outputs) shown in Figure 4.5 are updated at the positive edge of Clk when *Count* is $N - 1 = 39$. These outputs, in turn, are registered by the input registers of the subsequent modules for further processing.

4.2.3 Design steps

Different modules are designed with the hardware description language VHDL [87]. Before being assembled together to construct the Main Module, each module is tested separately through simulation using the simulation tool ISim from Xilinx. The test input data is generated using MATLAB. Each design is iterated multiple times to meet the design constraints (space and timing constraints) specific to Xilinx ML605 DSP kit. Each time a design is modified, its performance is verified utilizing the simulations

tools mentioned above. The platform to read and write the configurable parameters in the FPGA is provided by the MicroBlaze embedded processor. The processor logic is developed in Xilinx Software Development Kit (SDK) using C++ programming language. The Main Module together with the MicroBlaze processor are implemented on the FPGA by completing the steps in Xilinx ISE 14.4 design suite which are mentioned briefly below:

Creating Timing Constraints: This contains a User Constraints File (UCF) that enables the user to specify timing, input-output and placement constraints through a text editor.

Synthesize: After the constraints are defined, the Xilinx Synthesis Technology (XST) synthesizes [88] the design of the system identification module to create netlist files called NGC files which retain both the logical design data as well as the constraints.

Translate: This is the first part of the implementation process where the NGC files from the previous step are merged and a Xilinx Native Generic Database (NGD) file is created.

Map: Based on the NGD file obtained in the previous step, the Map step allocates Configurable Logic Block (CLB) and Input Output Block (IOB) resources for all the primary logic elements in the design. The Map step also gets optimized depending on the target device and checks for the consistency in design rules on the final mapped netlist.

Place And Route: The Place And Route (PAR) process implements timing-driven placement and routing of the mapped netlist from the previous step.

Generate Programming File: After the PAR process is complete, the Xilinx bitstream generation program called BitGen is executed which produces a bitstream file for the Xilinx device configuration. We utilized Xilinx SDK to burn the bitstream file on FPGA. The bitstream file when burned on the FPGA, runs the system identification algorithm.

In the next section, we discuss results by first verifying the accuracy of the system identification algorithm and performance of the digital design via simulations followed by the experimental results.

4.3 Results

4.3.1 Verification of BCEWRLS algorithm

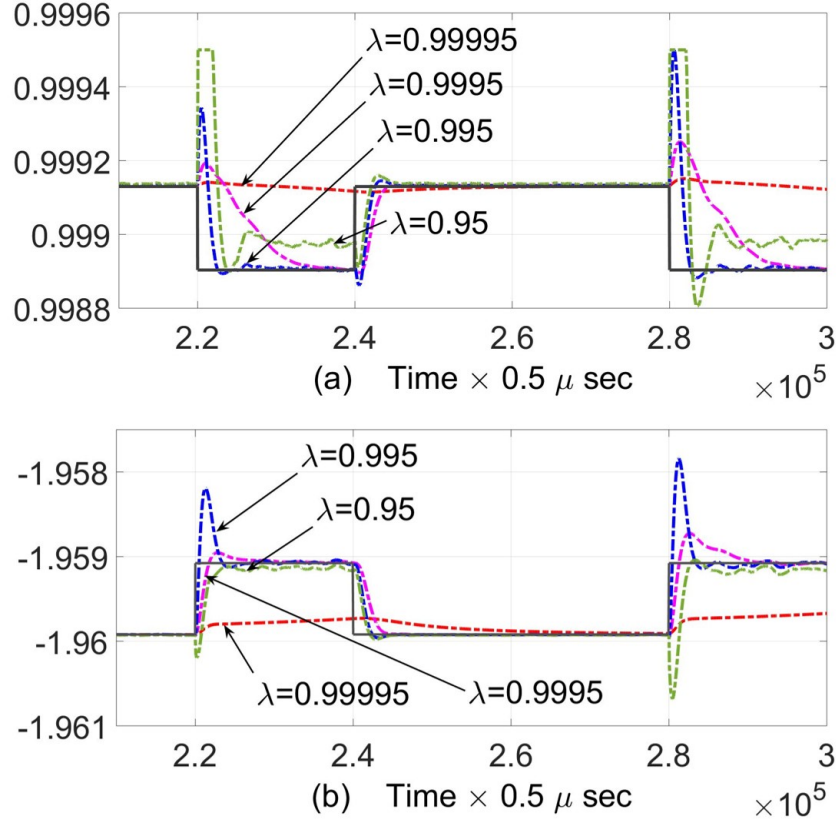


Figure 4.6: (a) Estimated b_0 and (b) b_1 using BCEWRLS with corresponding true value from simulation. $\lambda = 0.99995$ creates very slow estimation rendering the method ineffective. $\lambda = 0.95$ yields fast but incorrect convergence. $\lambda = 0.995$ and 0.9995 create correct convergence of estimated b_0 and b_1 to their true values.

The effectiveness of BCEWRLS algorithm is first verified in MATLAB with a hypothetical cantilever which switches between two known models, model 1 and model 2. These known models are useful to check the validity of the algorithm with respect to a known baseline. A cantilever with resonant frequency 63.147 kHz (f_0) and quality factor 227.849 (Q_0) is chosen as the model 1. The corresponding discrete time dynamics shown in (3.8) at sampling frequency of 2 MHz is described by the parameters $\{b_1, b_0, a_2, a_1, a_0\}$

which are given by $\{3.972\text{e-}4, -2.036\text{e-}4, -4.990\text{e-}4, -1.9599, 0.9991\}$. Another cantilever model with resonant frequency $f_0 + 0.5$ kHz and quality factor $0.8Q_0$ is chosen to represent the model 2. The parameters $\{b_1, b_0\}$ for model 2 are found to be $\{-1.9591, 0.9989\}$ while the other parameters are the same as model 1. In Figure 4.6, the hypothetical cantilever switches between model 1 and model 2 with a pseudo-random bit sequence of bit duration 10 ms. BCEWRLS algorithm is implemented in MATLAB to estimate $\{b_1, b_0\}$ for different choices of λ which are shown in Figure 4.6. Figure 4.6 suggests that choice of λ very close to 1 (0.99995) results in slow estimation process (over-tuning). When λ is lowered, the rate of convergence improves. $\lambda = 0.9995$ and 0.995 yield correct estimation within the bit duration (10 ms). However, further lowering of λ to a value of 0.95 creates incorrect estimation of b_0 and b_1 (under-tuning) which are shown in Figure 4.6 (a) and (b) respectively. Thus, appropriate choice of λ plays a crucial role in correctly estimating the parameters. In our FPGA design, we allow the user to adjust λ to yield good estimates at different imaging speeds. Typically, we found that $\lambda = 0.9995$ is a good starting point for experiments.

4.3.2 Simulation of system identification module

After verification of the BCEWRLS algorithm implemented in MATLAB as described above we verify the accuracy of the system identification module designed in VHDL for FPGA implementation (see Figure 4.2). The same module is designed in MATLAB which serves as the reference with respect to which the VHDL design is tested. The test data is acquired by performing a dynamic mode approach-retract experiment with MFP-3D AFM from Asylum Research on freshly cleaved mica sample. During such experiments, the cantilever oscillating in dynamic mode is made to approach the mica surface till the oscillation amplitude reduces due to tip-sample interactions and attains a specified value (called trigger value). Once the trigger value is reached, the cantilever is retracted from the sample till it oscillates again being free from the sample.

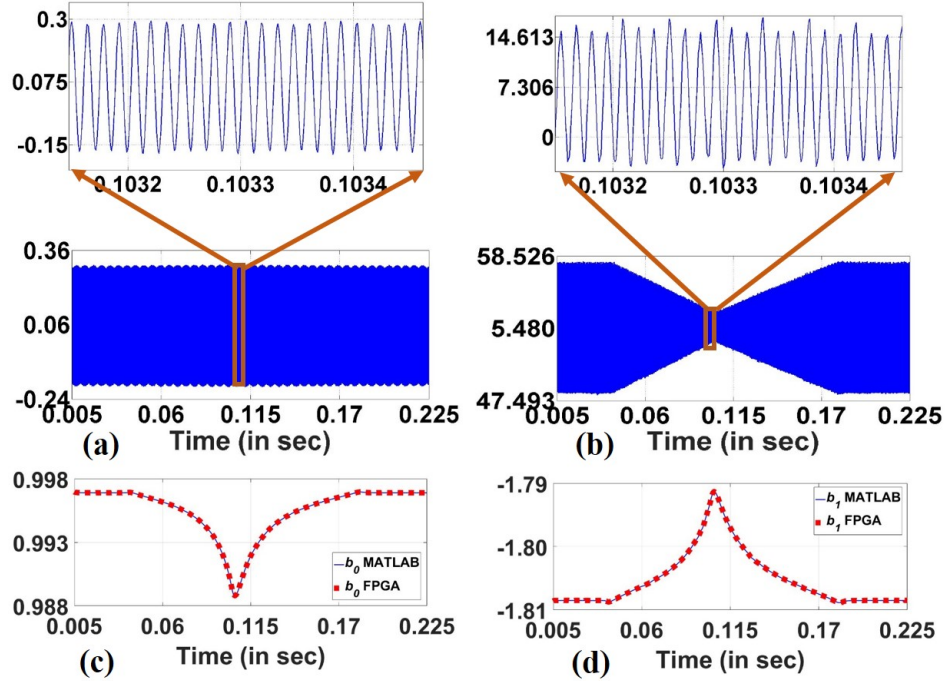


Figure 4.7: (a) Cantilever excitation signal (V) and (b) deflection signal (nm) acquired during an approach-retract experiment performed with the dynamic mode AFM. Comparison between the estimates of (c) b_0 and (d) b_1 generated by the system identification modules implemented in MATLAB and VHDL.

The cantilever excitation and the deflection signals which are shown in Figure 4.7 (a) and Figure 4.7 (b) respectively are acquired using a National Instruments BNC-2110 LabVIEW data acquisition system. The VHDL design is simulated using Xilinx ISim simulator which inputs the cantilever excitation and deflection signals acquired from the approach-retract experiment. The FPGA design outputs one of the data pairs chosen by the user: $\{b_0, b_1\}$ (the discrete time equivalent parameters), $\{f_{eq}, Q_{eq}\}$ (equivalent frequency and quality factor) or $\{\Phi_C, \Phi_D\}$ (storage and dissipative powers due to tip-sample interactions). These output data pairs are generated using ISim simulation and compared with MATLAB simulation.

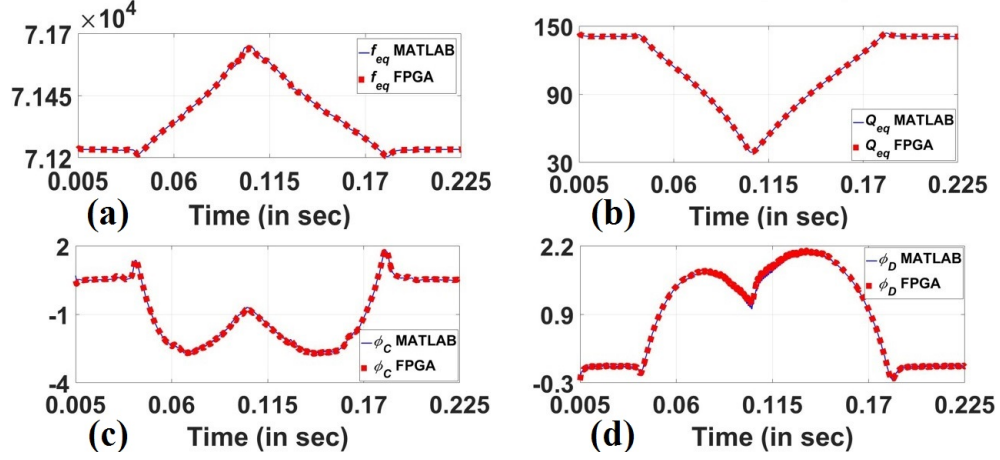


Figure 4.8: Comparison between (a) Equivalent frequency f_{eq} (Hz), (b) equivalent quality factor Q_{eq} , (c) conservative power Φ_C (Pico-Watt) and (d) dissipative power Φ_D (Pico-Watt) estimated by the system identification modules designed in MATLAB and VHDL.

Comparison of the signals $\{b_0, b_1\}$ from the MATLAB and the VHDL designs are presented in Figure 4.7 (c) and Figure 4.7 (d). Further comparisons of parameters $\{f_{eq}, Q_{eq}\}$ and $\{\Phi_C, \Phi_D\}$ are presented in Figure 4.8. Both Figure 4.7 and Figure 4.8 demonstrate that the MATLAB implementation and VHDL design yield close match. These results verify the accuracy of the VHDL design.

4.3.3 Experimental implementation

AC240TS cantilever from Asylum Research with resonant frequency of 72.7 kHz and quality factor 142.9 is utilized for dynamic mode imaging with MFP 3D AFM from Asylum Research. The sample consists of a blend of poly lauryl methacrylate (PLMA) and poly butyl methacrylate (PBMA) polymers coated over silica substrate. The conventional dynamic mode images namely the height, amplitude and phase images of a $10\mu m \times 10\mu m$ area of the sample are shown in Figure 4.9 (a), Figure 4.9 (b) and Figure 4.9 (c) respectively. These images are obtained at a scan rate of 0.25 Hz (1 horizontal line is scanned in 4 seconds). The circular valleys indicated by darker regions in the height image shown in Figure 4.9 (a) which correspond to brighter domains shown in

the phase image in Figure 4.9 (c) represent the PLMA regions which are distributed over the PBMA background. The system identification module is implemented on the FPGA with a forgetting factor $\lambda = 0.9995$. The excitation signal to the cantilever g and the measured deflection signal y are input to the FPGA. The input signals g and y are filtered with the HPFs with cut-off frequency of 2 kHz inside the system identification module (see Figure 4.2). The estimates of the parameters b_1 and b_0 in (3.9) are filtered with LPFs with cut-off frequency of 2 kHz.

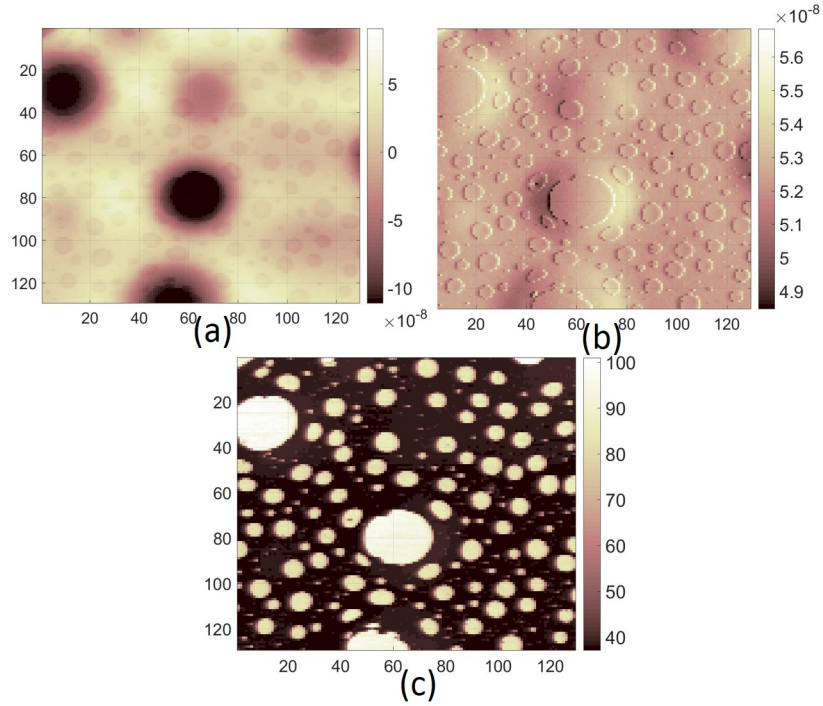


Figure 4.9: (Experimental data) (a) Height (meter), (b) amplitude (meter) and (c) phase image (degree) using dynamic mode AFM obtained from a $10\mu m \times 10\mu m$ PLMA-PBMA polymer blend. The color bars specifies the ranges.

Figure 4.10 (a) and (b) show the FPGA generated shifts of the estimates of discrete time equivalent parameters b_0 and b_1 around the sample free values which are $b_0 = 0.9969$ and $b_1 = -1.8013$ respectively. It is evident that PLMA domains represent lower b_0 values than PBMA whereas b_1 follows a reverse trend. Images for the estimates of equivalent frequency f_{eq} and quality factor Q_{eq} are shown in FIG.4.10 (c) and

FIG.4.10 (d) respectively. The conservative power Φ_C and dissipative power component Φ_D due to interactive forces between the cantilever tip and sample are found to be consistently higher on the PLMA domains compared to the PBMA film which are observed in Figure 4.11 (a) and Figure 4.11 (b). Hence, we demonstrated the effectiveness of the FPGA based system identification module. The module reported here performed successfully over multiple experimental runs conducted at different times.

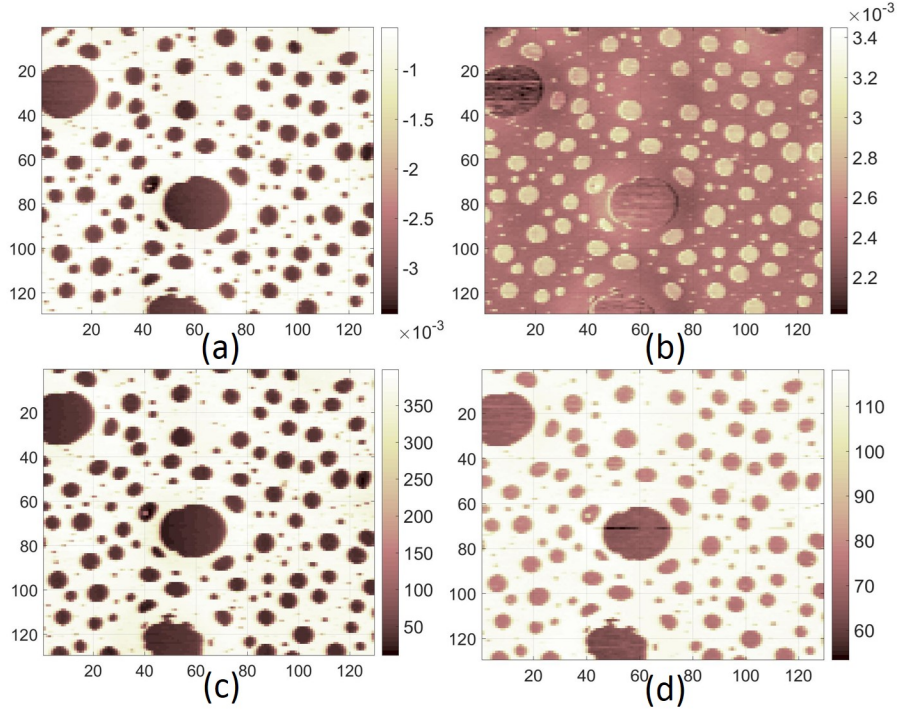


Figure 4.10: (Experimental data) (a) Shift of the discrete time equivalent parameter, $b_0 - b_{0,in}$, where $b_{0,in} = 0.9969$, (b) shift of parameter, $b_1 - b_{1,in}$, where $b_{1,in} = -1.8013$, (c) shift of equivalent frequency, $f_{eq} - f_0$ (in Hz), where $f_0 = 72.273$ kHz and (d) equivalent quality factor Q_{eq} obtained from a $10\mu m \times 10\mu m$ PLMA-PBMA polymer blend using the system identification module implemented on FPGA . The color bars specify the corresponding ranges.

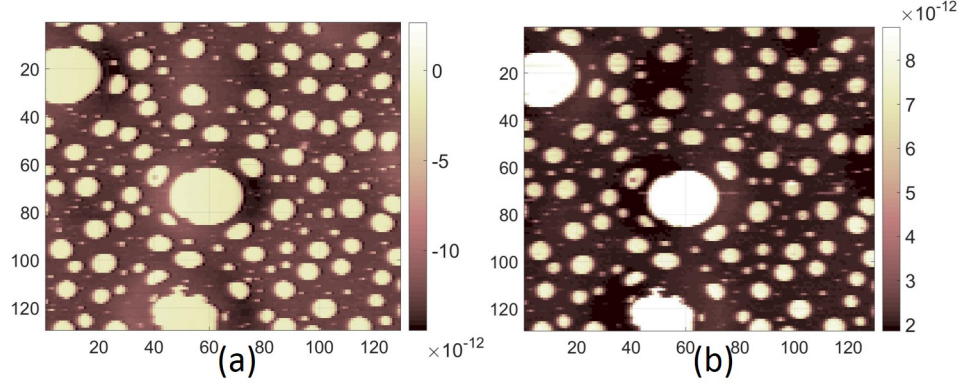


Figure 4.11: (Experimental data) (a) Estimated conservative power Φ_C (in watt) and (b) dissipative power Φ_D (in watt) due to periodic tip-sample interaction estimated using the FPGA module. The color bars specify corresponding ranges.

4.4 Conclusions

In this chapter we described the design of an FPGA based system identification module which implements BCEWRLS algorithm specialized for application with atomic force microscopy. The algorithm estimates the resonant frequency and quality factor of an equivalent cantilever which models the oscillating AFM cantilever interacting with sample in the dynamic mode of operation. The equivalent parameters are also utilized for estimating the conservative and dissipative power components due to tip-sample interaction. The systematic design procedure explained in this chapter is applicable to design of a wide variety of real time signal processing units where data is sampled from analog input signals, processed using high precision mathematical operations and final data are obtained in analog form within a few cycles of the sampling frequency. The design also enables the user to update the configurable parameters in real time through interfacing the FPGA board with personal computers. In this chapter we demonstrated the effectiveness of the design by performing a quantitative characterization of surface mechanical properties of a PLMA-PBMA polymer blend. Applicability of the FPGA module is not limited to this particular example but extendable to a wide variety of samples. Examples of such samples include different soft polymers, biological entities

like cells, DNA and proteins. The stiffness and dissipative properties of a polymer depend on its phase (glassy or rubbery) which changes around the critical temperature or humidity. Thus the system identification module can also be employed for investigating critical points of different polymers by studying the changes in stiffness and dissipative properties with varying temperature and humidity.

Chapter 5

Innovations squared mismatch based model detection for probe based data storage systems

5.1 Introduction

In many applications, the system of concern can be modeled by a set of linear models where it switches from one model to another [89]. In such systems it is often desired to identify the model that best describes the current behavior of the plant. Observer banks are widely employed to decipher plant models [90–98] for closed loop control of switched systems. In principle, the observer which generates the minimum tracking error is considered closest to the active plant model [99]. The switching index derived from tracking errors decides the closest plant. Various switching indices are proposed which include the index defined in [90,91] that integrates the square of tracking error with a tunable forgetting factor. In [92,93], a switching index is developed which uses a weighted combination of both the instantaneous and integrated value of square of tracking errors that improves the transient response of the closed-loop for uncertain plant. The switching index proposed in [93] is further modified in [94–97] which simplifies the computation of switching index by removing past observation data [100], reduces number of candidate controllers [97] and allows derivation of analytical conditions for stability of a

plant with large parametric uncertainties [96, 97]. One of the main emphases of these studies include analyzing and improving tracking performance of closed-loop switching systems while ensuring stability. This chapter, however, focuses on fast *detection* of a plant that switches between two *known* models. Such an assumption is particularly relevant for data storage application where plant characteristics when interacting with the bit 1 is different than interacting with bit 0. The maximum a posteriori probability (MAP) based methods are prevalent in bit patterned media recording and magnetic data storage [101–105] which are relatively matured technologies compared to probe based storage systems. Motivated by these examples, a recursive MAP based algorithm (see chapter 10, [29]) is developed in this chapter and applied to online plant identification for probe based storage systems. In this chapter, a new method called the innovation squared mismatch (ISM) is developed (introduced in [106] for the first time). It is demonstrated that the newly synthesized signal offers considerable advantage over MAP while being considerably simpler to implement. For high bandwidth read operation, the issue of transients caused by fast transition between bits that leads to inter symbol interference has to be overcome. In this chapter we develop dynamic programming based sequence detection schemes. Remarkably we demonstrate that bit by bit ISM based detection performs better than sequence based detection with significantly higher complexity. This performance gain is achieved by the short time scales of the transients in ISM signals. We further demonstrate that sequence detection in conjunction with ISM can lead to further significant advances in read bandwidth. An important focus of this chapter is on enabling ultra-high density storage technology. The need for such technology is evident in the connected world of data and big data age.

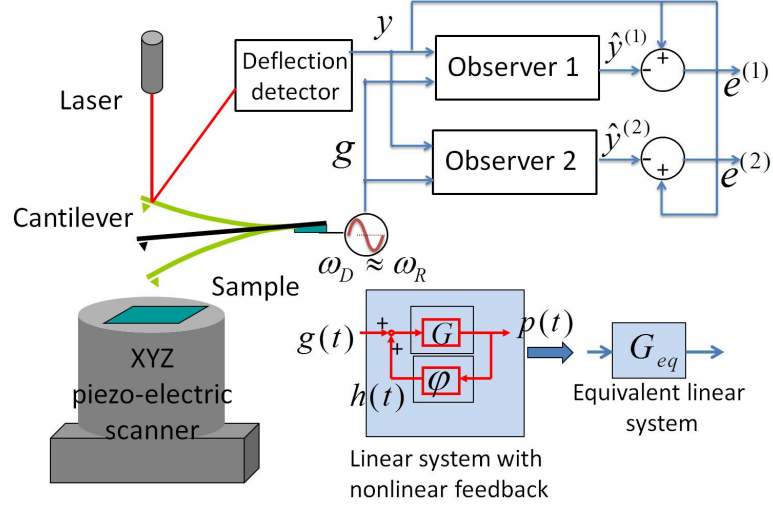


Figure 5.1: The concept of equivalent cantilever model for dynamic mode AFM is explained in Section 3.2, Chapter 3. BCEWRLS method is utilized to obtain equivalent linear models when the cantilever is oscillating in free air (model 1) and when it is interacting with the sample surface at fixed oscillation amplitude (model 2). Observers generate tracking errors $e^{(1)}$ and $e^{(2)}$ that are processed using the techniques MAP and ISM for real time interpretation of topography. MLSD, ISM-MLSD further improve the detection accuracy for known dwell interval in presence of inter symbol interference.

With data storage density for memory devices gradually increasing, the data density for the hard disk drive technology will eventually be restricted by thermal limits on magnetism (super paramagnetic limit) [107, 108]. The minimum bit size for solid state drive memories is also limited by the resolution of the lithography processes [109] where new technology for higher resolution lithography is hugely expensive. The probe based surface manipulation and reading methods offer alternative route which can pack binary data at the nanometer or sub nanometer scale. Since the invention of atomic force microscope [5], probe based investigation techniques have been employed in diverse applications [16] including probe based data storage systems [7, 108, 110]. Since the pioneering demonstrations of topographic probe based data storage [111–115] where an indentation of sample surface encodes the bit 1 and absence of indentation represents the bit 0, different physical parameters of the sample have been utilized to interpret binary data. Examples include phase-change [116, 117], magnetic properties [118, 119]

or ferro-electric characteristics [120, 121] based memories. Even though extremely high data density in the order of 3-4 Tb/ in² are reported in [115, 116, 120], a big challenge is the mechanical wear and tear of the probe as well as of the sample which severely cripples the lifetime well below the life span of present day data storage devices [111, 122]. Thus attempts have been made to develop soft samples [123] as well as to fabricate wear resistant cantilever tips [124, 125].

Most of the existing probe based data storage solutions utilize *contact mode* operation. Thus the tip of the flexure probe (see Figure 5.1) is in contact with the storage media, causing wear and tear of the media as well as of the probe. The dynamic mode operation is an alternative to the contact mode method which drastically eliminates mechanical wear and tear during the reading process. Here the flexure probe is forced sinusoidally and the probe contacts the media only intermittently. The information on the media encoded topographically modulates the flexure oscillations which is used to decipher information on the media. Due to slow read-out and complex data retrieval mechanism, the traditional dynamic mode method faces significant challenges for probe based data storage [122]. This chapter synthesizes new methods for circumventing the limited speed of bit detection with dynamic mode operation. It is demonstrated that the oscillating cantilever while interacting with raised topography assumes one model behavior (model 1) which is different than model behavior assumed by the probe when traversing over lowered topography (model 0). Thus for topographic data storage where raised and lowered topography encode binary bits, the read task becomes detection of which model is active. These models can be identified prior to the reading operation with a bias compensated recursive least square (BCEWRLS) algorithm [12, 38] (also outlined in Section 3.3.2 of Chapter 3) which remain valid for subsequent data reading. Thus it is a good assumption that the plant switches between two *known* models which need to be detected.

The conventional dynamic mode AFM (see Figure 5.1) utilizes cantilever oscillation amplitude and phase to interpret sample topography. It is demonstrated that application of MAP and ISM offers significant detection bandwidth gains over conventional dynamic mode signals. ISM offers similar detection speed, better detection accuracy and substantial computational gain over MAP. Since the ISM and MAP algorithms offer improved but *finite* detection bandwidth, the issue of inter symbol interference (ISI)

is addressed for fast bit detection using the maximum likelihood of an entire *sequence* of bits [126, 127]. In this chapter, sequence detection based on the ISM signal (the ISM-MLSD method) is developed where the time spent by the probe on single bit (the *dwell-time*) is known. ISM-MLSD consistently outperforms symbol-by-symbol ISM and MAP, however, at the expense of complex signal processing. The analytical and close to reality simulation evaluations are corroborated via exhaustive experimental results.

This chapter is organized as follows. Section 5.2 develops ISM. Modified symbol-by-symbol detection algorithms and ISM-MLSD are proposed in section 5.3. Section 5.4 provides the simulation and experimental results. Section 5.5 concludes this work.

5.2 Real time plant detection with innovations squared mismatch (ISM)

In this section, the plant is assumed to be switching between two models. The individual models can be first identified during a slower testing phase using the recursive identification method described above. Assuming that the switching plant models are already identified, a fast detection methodology called the innovations squared mismatch (ISM) is developed and compared with an existing maximum a-posteriori probability (MAP) based approach for fast real-time detection of plant models. The technique of plant parameter estimation followed by real-time plant detection is shown later to be a powerful tool with novel application to probe based storage systems.

5.2.1 Problem formulation, preliminaries and MAP

Consider a continuous time plant which is linear and time varying described by

$$\begin{aligned}\dot{x}(t) &= A(t)x(t) + B(t)g(t), \\ y(t) &= C(t)x(t) + D(t)g(t) + \zeta(t),\end{aligned}\tag{5.1}$$

where $\zeta(t)$ denotes continuous time measurement noise assumed to be zero-mean Gaussian white processes with covariances described by $E[\zeta(t)\zeta^T(t_0)] = R_n\delta(t - t_0)$. It is assumed that the quadruple $(A(t), B(t), C(t), D(t)) \in \{(A_i, B_i, C_i, D_i)\}$ with $i \in \{1, 2\}$, that is the dynamics can switch between two known linear systems. The objective of this section is to develop a simple yet fast real time detection of the plant model.

Two continuous time steady state Kalman observers with the first observer matched to the model (A_1, B_1, C_1, D_1) and the second matched to model (A_2, B_2, C_2, D_2) are realized. Let $\hat{x}^{(i)}$ denote the estimated state from the i^{th} observer and $\hat{y}^{(i)}$ denotes the corresponding estimated output. The estimated states and outputs are governed by

$$\begin{aligned}\dot{\hat{x}}^{(i)}(t) &= A_i \hat{x}^{(i)}(t) + B_i g(t) + L_i(y(t) - \hat{y}^{(i)}(t)), \\ \hat{y}^{(i)}(t) &= C_i \hat{x}^{(i)}(t) + D_i g(t),\end{aligned}\tag{5.2}$$

where $i = 1, 2$ represent the first and the second observer respectively. Let $\tilde{x}^{(i)}$ denote the error between the original and the estimated states, i.e. $\tilde{x}^{(i)} = x - \hat{x}^{(i)}$ and $e^{(i)}$ denote the error between the plant output and the estimated output, i.e. $e^{(i)} = y - \hat{y}^{(i)}$. It follows from (5.1) and (5.2) that the error dynamics is described by

$$\begin{aligned}\dot{\tilde{x}}^{(i)}(t) &= ((A(t) - A_i) - L_i(C(t) - C_i))x(t) \\ &\quad + (A_i - L_i C_i)\tilde{x}^{(i)}(t) \\ &\quad + (B(t) - B_i + L_i D_i - L_i D(t))g(t) \\ &\quad - L_i \zeta(t).\end{aligned}\tag{5.3}$$

The output error (also referred in this chapter as innovation) is given by

$$\begin{aligned}e^{(i)}(t) &= (C(t) - C_i)x(t) + C_i \tilde{x}^{(i)}(t) \\ &\quad + (D(t) - D_i)g(t) + \zeta(t).\end{aligned}\tag{5.4}$$

A prevalent approach for detecting which plant model is active is based on a maximum a posteriori probability (MAP) based approach [29]. Here, detection method based on MAP is summarized. Consider a discrete time plant which stays in model i given by

$$\begin{aligned}x[m+1] &= A_{d,i}x[m] + B_{d,i}g[m], \\ y[m] &= C_{d,i}x[m] + D_{d,i}g[m] + \zeta[m],\end{aligned}\tag{5.5}$$

with $\zeta \sim N(0, R_{d,i})$. A steady state discrete-time Kalman observer matched to model i with gain $L_{d,i}$ is governed by

$$\begin{aligned}\hat{x}^{(i)}[m+1|m] &= A_{d,i}\hat{x}^{(i)}[m|m-1] + B_{d,i}g[m] + L_{d,i} \\ &\quad \left(y[m] - C_{d,i}\hat{x}^{(i)}[m|m-1] - D_{d,i}g[m]\right), \\ \hat{y}^{(i)}[m] &= C_{d,i}\hat{x}^{(i)}[m|m-1] + D_{d,i}g[m].\end{aligned}\tag{5.6}$$

Let θ_i denote model i and let $\mathcal{Y}_m$ be the sequence of observations $\mathcal{Y}_m = \{y[0], y[1], \dots, y[m]\}$. The a posteriori probability that the plant parameter is θ_i given the observation vector $\mathcal{Y}_m$ is denoted by $p(\theta_i|\mathcal{Y}_m)$. The decision θ_{MAP} is chosen from the candidates θ_i so that $p(\theta_i|\mathcal{Y}_m)$ is maximized. It can be shown that (see [29])

$$p(\theta_i|\mathcal{Y}_m) = \frac{p(y_m|\mathcal{Y}_{m-1}, \theta_i)p(\theta_i|\mathcal{Y}_{m-1})}{\sum_{j=1}^2 p(y_m|\mathcal{Y}_{m-1}, \theta_j)p(\theta_j|\mathcal{Y}_{m-1})}. \quad (5.7)$$

From [29], it follows that $p(y_m|\mathcal{Y}_{m-1}, \theta_i)$ obeys a Gaussian distribution with mean $\hat{y}_i[m] \in \mathbb{R}^q$ and covariance $\Omega_{m/i}$ where

$$p(y_m|\mathcal{Y}_{m-1}, \theta_i) = \frac{1}{(2\pi)^{q/2} |\Omega_{m/i}|^{1/2}} \exp \left\{ -\frac{1}{2} \tilde{y}_{m,i}^T \Omega_{m/i}^{-1} \tilde{y}_{m,i} \right\}. \quad (5.8)$$

As a result, $p(\theta_i|\mathcal{Y}_m)$ can be updated from $p(\theta_i|\mathcal{Y}_{m-1})$ using

$$p(\theta_i|\mathcal{Y}_m) = c_m |\Omega_{m/i}|^{-\frac{1}{2}} \exp \left\{ -\frac{1}{2} \tilde{y}_{m,i}^T \Omega_{m/i}^{-1} \tilde{y}_{m,i} \right\} p(\theta_i|\mathcal{Y}_{m-1}), \quad (5.9)$$

where $\tilde{y}_{m,i} = y - \hat{y}_{m,i}$. c_m is a normalization constant set to satisfy $\sum_{i=1}^2 p(\theta_i|\mathcal{Y}_m) = 1$. The output covariance $\Omega_{m/i}$ in (5.9) and covariance of state estimation error $\Sigma_{m+1/m,i} = E \left[\tilde{x}_{m+1,i} \tilde{x}_{m+1,i}^T \right]$ with $\tilde{x}_{m+1,i} = (x[m+1] - \hat{x}^{(i)}[m+1|m])$ can be updated recursively using

$$\begin{aligned} \Sigma_{m+1/m,i} &= (A_{d,i} - L_{d,i}C_{d,i})\Sigma_{m/m-1,i}(A_{d,i} - L_{d,i}C_{d,i})^T \\ &\quad + B_{d,i}Q_{d,i}B_{d,i}^T + L_{d,i}R_{d,i}L_{d,i}^T, \end{aligned} \quad (5.10)$$

$$\Omega_{m/i} = C_{d,i}\Sigma_{m/m-1,i}C_{d,i}^T + R_{d,i}. \quad (5.11)$$

Equations (5.9), (5.10) and (5.11) provide the way to recursively estimate the probability of the plant being either in model 1 or 2.

5.2.2 Innovations squared mismatch (ISM)

In this part, a new method termed the innovations squared mismatch (ISM) method is developed. Consider the continuous time varying plant described in (5.1). A new set of

state space variables will be defined such that the observer outputs in terms of the new variables follow a sign changing behavior depending on the present plant model. This sign changing phenomenon in turn will be utilized for plant model detection. The state space equations describing the original time varying plant together with two observers matched to models 1 and 2 contain the dynamical equations of $x(t)$, $\tilde{x}^{(1)}(t)$ and $\tilde{x}^{(2)}(t)$ given by

$$\begin{aligned}
\begin{bmatrix} \dot{x}(t) \\ \dot{\tilde{x}}^{(1)}(t) \\ \dot{\tilde{x}}^{(2)}(t) \end{bmatrix} &= \begin{bmatrix} A(t) \\ ((A(t) - A_1) - L_1(C(t) - C_1)) \\ ((A(t) - A_2) - L_2(C(t) - C_2)) \end{bmatrix} x(t) \\
&+ \begin{bmatrix} 0 & 0 \\ (A_1 - L_1 C_1) & 0 \\ 0 & (A_2 - L_2 C_2) \end{bmatrix} \begin{bmatrix} \tilde{x}^{(1)}(t) \\ \tilde{x}^{(2)}(t) \end{bmatrix} \\
&+ \begin{bmatrix} B(t) \\ (B(t) - B_1 + L_1(D_1 - D(t))) \\ (B(t) - B_2 + L_2(D_2 - D(t))) \end{bmatrix} g(t) \\
&+ \begin{bmatrix} 0 \\ -L_1 \\ -L_2 \end{bmatrix} \zeta(t). \tag{5.12}
\end{aligned}$$

From (5.4), the output errors can be represented by

$$\begin{aligned}
\begin{bmatrix} e^{(1)}(t) \\ e^{(2)}(t) \end{bmatrix} &= \begin{bmatrix} (C(t) - C_1) \\ (C(t) - C_2) \end{bmatrix} x(t) \\
&+ \begin{bmatrix} C_1 & 0 \\ 0 & C_2 \end{bmatrix} \begin{bmatrix} \tilde{x}^{(1)}(t) \\ \tilde{x}^{(2)}(t) \end{bmatrix} \\
&+ \begin{bmatrix} D(t) - D_1 \\ D(t) - D_2 \end{bmatrix} g(t) + \begin{bmatrix} 1 \\ 1 \end{bmatrix} \zeta(t). \tag{5.13}
\end{aligned}$$

A new set of variables z_1 and z_2 are derived from the state space vectors $\tilde{x}^{(1)}(t)$ and $\tilde{x}^{(2)}(t)$ via $z_1(t) = (\tilde{x}^{(1)}(t) - \tilde{x}^{(2)}(t))$ and $z_2(t) = (\tilde{x}^{(1)}(t) + \tilde{x}^{(2)}(t))$. The dynamics of the

new variables is given by

$$\begin{aligned}
\begin{bmatrix} \dot{z}_1(t) \\ \dot{z}_2(t) \end{bmatrix} &= \begin{bmatrix} (L_2 - L_1)C(t) - 2M_2 \\ 2A(t) - C(t)(L_1 + L_2) - 2M_1 \end{bmatrix} x(t) \\
&+ \begin{bmatrix} M_1 & M_2 \\ M_2 & M_1 \end{bmatrix} \begin{bmatrix} z_1(t) \\ z_2(t) \end{bmatrix} \\
&+ \begin{bmatrix} B_2 - B_1 + L_1D_1 - L_2D_2 \\ 2B(t) - (B_1 + B_2) + L_1D_1 + L_2D_2 \\ +(L_2 - L_1)D(t) \\ -(L_1 + L_2)D(t) \end{bmatrix} g(t) \\
&+ \begin{bmatrix} (L_2 - L_1) \\ -(L_1 + L_2) \end{bmatrix} \zeta(t), \tag{5.14}
\end{aligned}$$

where matrices M_1 and M_2 are defined by $M_1 = \frac{1}{2}[(A_1 + A_2) - (L_1C_1 + L_2C_2)]$ and $M_2 = \frac{1}{2}[(A_1 - A_2) - (L_1C_1 - L_2C_2)]$. The output vectors e_{dif} and e_{sum} are defined by $e_{dif}(t) = e^{(1)}(t) - e^{(2)}(t)$ and $e_{sum}(t) = e^{(1)}(t) + e^{(2)}(t)$ where it follows from (5.13) that

$$\begin{aligned}
\begin{bmatrix} e_{dif}(t) \\ e_{sum}(t) \end{bmatrix} &= \begin{bmatrix} C_2 - C_1 \\ 2C(t) - C_1 - C_2 \end{bmatrix} x(t) \\
&+ \begin{bmatrix} \frac{(C_1+C_2)}{2} & \frac{(C_1-C_2)}{2} \\ \frac{(C_1-C_2)}{2} & \frac{(C_1+C_2)}{2} \end{bmatrix} \begin{bmatrix} z_1(t) \\ z_2(t) \end{bmatrix} \\
&+ \begin{bmatrix} D_2 - D_1 \\ D(t) - D_1 - D_2 \end{bmatrix} g(t) + \begin{bmatrix} 0 \\ 2 \end{bmatrix} \zeta(t). \tag{5.15}
\end{aligned}$$

Let $N_1 := \frac{1}{2}(C_1 + C_2)$, $N_2 := \frac{1}{2}(C_1 - C_2)$, $A_{m1} := ((A_2 - A_1) + L_2(C_1 - C_2))$, $A_{m2} := ((A_2 - A_1) + L_1(C_1 - C_2))$, $B_{m1} := (B_2 - B_1 + L_2(D_1 - D_2))$, $B_{m2} := (B_2 - B_1 + L_1(D_1 - D_2))$, $C_{m1} = C_{m2} := (C_2 - C_1)$ and $D_{m1} = D_{m2} := (D_2 - D_1)$. Hence, the dynamics of the system in the new coordinates for model i is given by

$$\begin{aligned}
\begin{bmatrix} \dot{z}_1(t) \\ \dot{z}_2(t) \end{bmatrix} &= \begin{bmatrix} A_{mi} \\ (-1)^i A_{mi} \end{bmatrix} x(t) + \begin{bmatrix} M_1 & M_2 \\ M_2 & M_1 \end{bmatrix} \begin{bmatrix} z_1 \\ z_2 \end{bmatrix} \\
&+ \begin{bmatrix} B_{mi} \\ (-1)^i B_{mi} \end{bmatrix} g(t) + \begin{bmatrix} (L_2 - L_1) \\ -(L_1 + L_2) \end{bmatrix} \zeta(t). \tag{5.16}
\end{aligned}$$

Thus, e_{dif} and e_{sum} in model i are

$$\begin{bmatrix} e_{dif}(t) \\ e_{sum}(t) \end{bmatrix} = \begin{bmatrix} C_{mi} \\ (-1)^i C_{mi} \end{bmatrix} x(t) + \begin{bmatrix} N_1 & N_2 \\ N_2 & N_1 \end{bmatrix} \begin{bmatrix} z_1 \\ z_2 \end{bmatrix} + \begin{bmatrix} D_{mi} \\ (-1)^i D_{mi} \end{bmatrix} g(t) + \begin{bmatrix} 0 \\ 2 \end{bmatrix} \zeta(t). \quad (5.17)$$

Suppose that $\mathcal{G}_1(s)$ denotes the transfer function from $[x(s)^T, g(s)^T]^T$ to $[e_{dif}^T(s), e_{sum}^T(s)]^T$ in model 1. It is derived in Appendix A.3 that $\mathcal{G}_1(s)$ can be written as

$$\mathcal{G}_1(s) = \begin{bmatrix} \mathcal{G}_{m1}^{(1)}(s) & \mathcal{G}_{m1}^{(2)}(s) \\ -\mathcal{G}_{m1}^{(1)}(s) & -\mathcal{G}_{m1}^{(2)}(s) \end{bmatrix}, \quad (5.18)$$

where $\mathcal{G}_{m1}^{(1)}(s)$ and $\mathcal{G}_{m1}^{(2)}(s)$ are defined by

$$\begin{aligned} \mathcal{G}_{m1}^{(1)}(s) &= (N_1 \mathcal{S}_1 + N_2 \mathcal{S}_2 - (N_1 \mathcal{S}_2 + N_2 \mathcal{S}_1)) A_{m1} + C_{m1}, \\ \mathcal{G}_{m1}^{(2)}(s) &= (N_1 \mathcal{S}_1 + N_2 \mathcal{S}_2 - (N_1 \mathcal{S}_2 + N_2 \mathcal{S}_1)) B_{m1} + D_{m1}. \end{aligned} \quad (5.19)$$

Further, when the plant is on model 2, the transfer function from $[x(s)^T, g(s)^T]^T$ to $[e_{dif}^T(s), e_{sum}^T(s)]^T$ is denoted by $\mathcal{G}_2(s)$ as shown below

$$\mathcal{G}_2(s) = \begin{bmatrix} \mathcal{G}_{m2}^{(1)}(s) & \mathcal{G}_{m2}^{(2)}(s) \\ \mathcal{G}_{m2}^{(1)}(s) & \mathcal{G}_{m2}^{(2)}(s) \end{bmatrix}. \quad (5.20)$$

$\mathcal{G}_{m2}^{(1)}(s)$ and $\mathcal{G}_{m2}^{(2)}(s)$ are defined as

$$\begin{aligned} \mathcal{G}_{m2}^{(1)}(s) &= (N_1 \mathcal{S}_1 + N_2 \mathcal{S}_2 + N_1 \mathcal{S}_2 + N_2 \mathcal{S}_1) A_{m2} + C_{m2}, \\ \mathcal{G}_{m2}^{(2)}(s) &= (N_1 \mathcal{S}_1 + N_2 \mathcal{S}_2 + N_1 \mathcal{S}_2 + N_2 \mathcal{S}_1) B_{m2} + D_{m2}. \end{aligned} \quad (5.21)$$

It is derived in Appendix A.3 that the matrices $\mathcal{S}_1$ and $\mathcal{S}_2$ are defined by

$$\begin{aligned} \mathcal{S}_1 &= ((sI - M_1)M_2^{-1}(sI - M_1) - M_2)^{-1}(sI - M_1)M_2^{-1}, \\ \mathcal{S}_2 &= ((sI - M_1)M_2^{-1}(sI - M_1) - M_2)^{-1}. \end{aligned} \quad (5.22)$$

It can also be shown that the transfer function matrix from the measurement noise $\zeta(s)$ to $[e_{dif}^T(s), e_{sum}^T(s)]^T$ is given by $[\mathcal{G}_{(dif,\zeta)}^T, \mathcal{G}_{(sum,\zeta)}^T]^T$ where $\mathcal{G}_{(dif,\zeta)} = (N_1 \mathcal{S}_1 + N_2 \mathcal{S}_2)(L_2 -$

$L_1) + (N_1\mathcal{S}_2 + N_2\mathcal{S}_1)(L_1 + L_2)$ and $\mathcal{G}_{(sum,\zeta)} = (N_1\mathcal{S}_2 + N_2\mathcal{S}_1)(L_2 - L_1) - (N_1\mathcal{S}_1 + N_2\mathcal{S}_2)(L_1 + L_2) + 2$. Summarizing, in model i , it follows that:

$$\begin{aligned} e_{dif}(s) &= e_{mi}(s) + \zeta_{dif}(s), \text{ and} \\ e_{sum}(s) &= (-1)^i e_{mi}(s) + \zeta_{sum}(s), \end{aligned} \quad (5.23)$$

where $e_{mi} = \mathcal{G}_{mi}^{(1)}x + \mathcal{G}_{mi}^{(2)}g$ represents the *signal* component. $\zeta_{dif} = \mathcal{G}_{(dif,\zeta)}\zeta$ and $\zeta_{sum} = \mathcal{G}_{(sum,\zeta)}\zeta$ denote the noise components in e_{dif} and e_{sum} respectively. Notice that e_{mi} is multiplied with $(-1)^i$ in (5.23), which suggests that when the system is in model 2, the transfer functions from $[x^T(s), g^T(s)]^T$ to $e_{dif}(s)$ and $e_{sum}(s)$ have the same value and the same sign, opposite to the case when the system is in model 1. Thus, it is expected that when the signal components in $e_{dif}(t)$ and $e_{sum}(t)$ reach steady-state after plant switching, the relative sign between them can be employed to infer the active plant model. A simple discrete time implementation for checking relative sign between e_{dif} and e_{sum} can be achieved by sampling the signals over a time window of size M and then correlating the sampled signals. Let the test signal corresponding to sample index m be denoted by $T[m]$ described by

$$\begin{aligned} T[m] &= e_{dif}[m]e_{sum}[m] + e_{dif}[m-1]e_{sum}[m-1] + \\ &\quad \cdots + e_{dif}[m-M+1]e_{sum}[m-M+1]. \end{aligned} \quad (5.24)$$

If $T[m] > 0$ decision is made that plant is in model 2, otherwise it is in model 1. It should be noted that the window size M strikes a balance between the noise level and detection speed of the test signal $T[m]$. Typically, increasing M tends to average out (thus reduce) the noise present in $T[m]$. However, increasing M also reduces the detection speed.

Here the case when $g(t)$ being sinusoidal is further analyzed. As a consequence of (5.23), when the system is in model 1, signal components in $e_{dif}(t)$ and $e_{sum}(t)$ will be exactly out of phase in steady state. Conversely, when the plant follows dynamics described by model 2, signal components in e_{dif} and e_{sum} will be in phase in steady state. An analog phase demodulator can be used to extract the phase of the sinusoids e_{dif} and e_{sum} and the resulting phase signals will differ by the angle π when the system is in model 1 and angle 0 when the system is in model 2. Hence, the difference of the phase signals, denoted by ϕ_m , can be used for real time detection of system model.

Another simple way to extract the model information is to use the envelope of the signal $(e^{(1)}(t))^2 - (e^{(2)}(t))^2$. Assume that in model 1, in steady state, $e_{dif}(t) = \mathcal{A}_{m1}(t) \sin(\omega t + \phi_1(t)) + \zeta_{dif}(t)$, where $\mathcal{A}_{m1}(t)$ and $\phi_1(t)$ denote the amplitude and phase of the signal component in $e_{dif}(t)$ respectively. Then, (5.23) suggests that $e_{sum} = -\mathcal{A}_{m1}(t) \sin(\omega t + \phi_1(t)) + \zeta_{sum}(t)$. Hence, in steady state, the following holds true.

$$\begin{aligned} (e^{(1)}(t))^2 - (e^{(2)}(t))^2 &= e_{dif}(t)e_{sum}(t) \\ &= -\mathcal{A}_{m1}^2(t) \sin^2(\omega t + \phi_1(t)) + \zeta_{ISM,1}(t) \\ &= -\frac{\mathcal{A}_{m1}^2(t)}{2}(1 - \cos(2\omega t + 2\phi_1(t))) + \zeta_{ISM,1}(t). \end{aligned} \quad (5.25)$$

In model 2, if $e_{dif}(t) = \mathcal{A}_{m2}(t) \sin(\omega t + \phi_2(t)) + \zeta_{dif}(t)$, then $e_{sum}(t) = \mathcal{A}_{m2}(t) \sin(\omega t + \phi_2(t)) + \zeta_{sum}(t)$. Then,

$$\begin{aligned} (e^{(1)}(t))^2 - (e^{(2)}(t))^2 &= e_{dif}(t)e_{sum}(t) \\ &= \mathcal{A}_{m2}^2(t) \sin^2(\omega t + \phi_2(t)) + \zeta_{ISM,2}(t) \\ &= \frac{\mathcal{A}_{m2}^2(t)}{2}(1 - \cos(2\omega t + 2\phi_2(t))) + \zeta_{ISM,2}(t). \end{aligned} \quad (5.26)$$

Here, $\zeta_{ISM,i}(t)$, $i \in \{1, 2\}$ denotes the noise components. A low pass filter (LPF) with appropriate bandwidth can be used to remove the high frequency term $\cos(2\omega t + 2\phi_{i \in \{1,2\}}(t))$ from $(e^{(1)}(t))^2 - (e^{(2)}(t))^2$ as shown in (5.25) and (5.26). As a consequence, in steady state, an ideal LPF results in

$$\left((e^{(1)}(t))^2 - (e^{(2)}(t))^2 \right)_{lpf} = \begin{cases} -\frac{\mathcal{A}_{m1}^2(t)}{2} + \zeta'_{ISM,1}(t), & \text{in model 1} \\ +\frac{\mathcal{A}_{m2}^2(t)}{2} + \zeta'_{ISM,2}(t), & \text{in model 2} \end{cases} \quad (5.27)$$

Here, $\zeta'_{ISM,i}(t)$ denotes the low pass filtered noise components. It can be seen that $((e^{(1)}(t))^2 - (e^{(2)}(t))^2)_{lpf}$ should have negative sign in model 1 and positive sign in model 2 and this sign inversion can be used in real time to interpret the model of the system. In the context of probe based imaging, the phase difference signal between e_{dif} and e_{sum} is called the ISM based phase image: ϕ_m . Similarly, the test signal $((e^{(1)}(t))^2 - (e^{(2)}(t))^2)_{lpf}$ is referred to as the ISM based amplitude image: ISM_{lpf} .

5.2.3 Computational complexity

A comparison of the computational effort required by each of the two methods MAP and ISM is discussed here. Assume the following dimensions of the matrices in (5.6), (5.10)

and (5.11); $A_{d,i} \in \mathbb{R}^{\mathbf{m}_A, \mathbf{m}_A}$, $B_{d,i} \in \mathbb{R}^{\mathbf{m}_A, \mathbf{n}_B}$, $C_{d,i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{m}_A}$, $D_{d,i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{n}_B}$, $L_{d,i} \in \mathbb{R}^{\mathbf{m}_A, \mathbf{m}_C}$, $\Sigma_{m+1/m,i} \in \mathbb{R}^{\mathbf{m}_A, \mathbf{m}_A}$, $Q_{d,i} \in \mathbb{R}^{\mathbf{n}_B, \mathbf{n}_B}$, $R_{d,i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{m}_C}$, $\Omega_{m/i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{m}_C}$. Define a floating point multiplication, division, addition or subtraction operation as a flop. It is derived in Section A.4 that total number of flop counts required per update for (5.9), (5.10) and (5.11) is $2(N_\Sigma + N_\Omega + N_p)$ where

$$N_\Sigma = \begin{cases} \left(\frac{3}{2}\mathbf{m}_A^4 + \mathbf{m}_A^2 \left(\frac{3}{2}(\mathbf{m}_C^2 + \mathbf{n}_B^2) + 2\mathbf{m}_C + 1 \right) \right. \\ \left. + \mathbf{m}_A \left(\frac{3}{2}(\mathbf{m}_C + \mathbf{n}_B) - \frac{1}{2} \right) \right), & \text{for first update} \\ \frac{3}{2}(\mathbf{m}_A^4 + \mathbf{m}_A^2), & \text{for subsequent updates} \end{cases} \quad (5.28)$$

with $N_\Omega = \frac{3}{2}(\mathbf{m}_C^2 \mathbf{m}_A^2 + \mathbf{m}_C \mathbf{m}_A)$ and $N_p = \mathbf{m}_C^3 + \frac{5}{2}\mathbf{m}_C^2 + \frac{7}{2}\mathbf{m}_C + 4$. The flop counts required for discrete time implementation of ISM is determined now. The test signal $T[m]$ defined in (5.24) required for ISM can be recursively updated using

$$\begin{aligned} T[m+1] &= T[m] + e_{dif}[m+1]^T e_{sum}[m+1] \\ &\quad - e_{dif}[m-M+1]^T e_{sum}[m-M+1]. \end{aligned} \quad (5.29)$$

Here, $e_{dif}[m], e_{sum}[m] \in \mathbb{R}^{\mathbf{m}_C}$ can be determined from e_1 and e_2 using $\mathbf{m}_C$ subtractions and $\mathbf{m}_C$ additions, thus $2\mathbf{m}_C$ flops. Multiplication $e_{dif}[m]^T e_{sum}[m]$ needs $\mathbf{m}_C$ multiplications and $(\mathbf{m}_C - 1)$ additions thus $(2\mathbf{m}_C - 1)$ flops. Hence, update of $T[m+1]$ from $T[m]$ needs $2(2\mathbf{m}_C - 1) + 2\mathbf{m}_C + 2 = 6\mathbf{m}_C$ flops. The immense computational benefits achieved by ISM over MAP is evident. The computational complexity for MAP depends on the dimension of the output vector as $\mathbf{m}_C$ by $O(\mathbf{m}_C^3)$. Besides, the dependence on the size of the state space matrix, namely $\mathbf{m}_A$ is of $O(\mathbf{m}_A^4)$. Whereas, computational complexity of ISM is independent of $\mathbf{m}_A$ and the dependence on $\mathbf{m}_C$ is only $O(\mathbf{m}_C)$.

5.3 Plant detection with known dwell interval; sequence detection

In many applications including the probe based data storage system shown later in this chapter each symbol is encoded in the media for an equivalent duration of T seconds on the read operation. Thus the probe encounters one kind of interaction during entire

T seconds and the interaction behavior can change only after every T seconds. Here during the dwell-time the probe interacts with the media for deciphering the same bit of information and the bit detection performance can be improved by averaging the measured interaction to reduce the affects of noise.

The test signals for each of the schemes ISM and MAP have a finite response time; $T[m]$ in (5.24) and $p(\theta_i|\mathcal{Y}_m)$, $i \in 1, 2$ from (5.9) take time to change to new steady state values when the plant changes from model 1 to model 2 and vice versa. The effect of contributions of past interactions on the present interaction causes inter symbol interference (ISI). ISI is more severe when the plant switches fast, where the dwell interval T is small. A significant gain over symbol-by-symbol schemes can be achieved by deciphering the *sequence* of plant models from the observation data. A widely accepted sequence detection technique in the communication systems and data storage community is the Maximum Likelihood Sequence Detection (see [126]) method which is discussed next. As demonstrated here, Maximum Likelihood Sequence Detection (MLSD) can be applied in the context of binary plant model detection as well.

5.3.1 Maximum Likelihood Sequence Detection

In this chapter MLSD is applied to process sequence of samples from the test signal $s_m(t)$ generated by two ways; firstly $s_m(t)$ is generated by suitably low pass filtered $(e^{(1)})^2$ which is termed as regular MLSD and secondly $s_m(t)$ is generated using low pass filtered $(e^{(1)})^2 - (e^{(2)})^2$ signal which is termed as ISM-MLSD. Both these methods decipher the maximum likelihood sequence of active plant models. It is further demonstrated in the simulation and experimental results section that ISM-MLSD provide significant performance benefit over symbol-by-symbol ISM, MAP based detections and also regular MLSD. Let q be the number of samples of the test signal gathered in single dwell interval T . Let $\bar{a} = [a_0, a_1, \dots, a_{N-1}]^T$ denote the bit sequence such that $a_k = 0$ when model 1 is active ($a_k = 1$ for model 2) in the time interval $t \in (kT, (k+1)T]$ with $k = 0, 1, \dots, N-1$. The sampled sequence of $s_m(t)$ for MLSD detector is denoted by the vector $\bar{z} = [z_0, z_1, \dots, z_{(Nq-1)}]^T$. Let $f(\bar{z}|\bar{a})$ be the conditional probability density function (pdf) of the output vector $\bar{z}$ given a source bit sequence $\bar{a}$. The maximum

likelihood bit sequence is given by

$$\hat{a} = \arg \max_{\bar{a} \in \{0,1\}^N} \prod_{i=0}^{N-1} f(\bar{z}_i | \bar{a}, \bar{z}_0^{i-1}), \quad (5.30)$$

where $\bar{z}_j^i$ is defined $\bar{z}_j^i = [z_{jq}, z_{jq+1}, \dots, z_{iq-1}]^T$. Define $\bar{z}_i = [z_{iq}, z_{iq+1}, \dots, z_{(i+1)q-1}]^T$ as the sampled vector corresponding to i^{th} dwell interval. The i^{th} state S_i is defined as

$$S_i = [a_{i-m-m_I+1}, a_{i-m-m_I+2}, \dots, a_i]^T, \quad (5.31)$$

where m is the system memory and m_I is the inter symbol interference memory (see [27]). Hence, the problem of identifying the maximum likelihood sequence of plant models can be recast as the problem of identifying the maximum likelihood sequence of states $\hat{S}_i$. The detection strategy can be modified by increasing the total memory $m + m_I$ to improve the accuracy. However, the number of possible states S_i at each stage i is given by $2^{(m+m_I)}$ (see Figure 5.2) which increases and extends the computational complexity of the algorithm. For practical purposes increasing the memory beyond a certain value does not improve the detection performance much. The conditional pdf $f(\bar{z}_i | \bar{a}, \bar{z}_0^{i-1})$ can be simplified by removing the history of the source bits $\bar{a}$ and observed outputs $\bar{z}$ beyond the assumed memories. In many data retrieving applications it is assumed that $f(\bar{z}_{i-m_I}^i | S_i, S_{i-1})$ is Gaussian i.e. $f(\bar{z}_{i-m_I}^i | S_i, S_{i-1}) \sim N(\bar{\mathcal{Y}}(S_i, S_{i-1}), \mathcal{C}(S_i, S_{i-1}))$ with the mean vector $\bar{\mathcal{Y}}(S_i, S_{i-1})$ and the covariance matrix $\mathcal{C}(S_i, S_{i-1})$. Even though the distribution may not be exactly Gaussian, this assumption helps to render the MLSD problem mathematically tractable. For each state transition as shown in Figure 5.2, $\bar{\mathcal{Y}}$ and $\mathcal{C}$ can be estimated by a statistical learning step before employing it for the read operation.

Theorem 5.3.1 *Let $\hat{S} = \arg \max_{\bar{S}} f(\bar{z} | \bar{S})$. Then*

$$\begin{aligned} \hat{S} = \arg \min_{\bar{S}} \sum_{i=0}^{N-1} \log \left(\frac{\mathcal{C}(S_i, S_{i-1})}{c(S_i, S_{i-1})} \right) \\ + (\bar{z}_{i-m_I}^i - \bar{\mathcal{Y}}(S_i, S_{i-1}))^T \mathcal{C}(S_i, S_{i-1})^{-1} \\ (\bar{z}_{i-m_I}^i - \bar{\mathcal{Y}}(S_i, S_{i-1})) \\ - (\bar{z}_{i-m_I}^{i-1} - \bar{\mathcal{Y}}(S_i, S_{i-1}))^T c(S_i, S_{i-1})^{-1} \\ (\bar{z}_{i-m_I}^{i-1} - \bar{\mathcal{Y}}(S_i, S_{i-1})), \end{aligned} \quad (5.32)$$

where $c(S_i, S_{i-1})$ is upper $m_I q \times m_I q$ principal minor of $\mathcal{C}(S_i, S_{i-1})$ and $\bar{y}(S_i, S_{i-1})$ is the first $m_I q$ elements of the mean vector $\mathcal{Y}(S_i, S_{i-1})$.

Proof:

$$\begin{aligned}
 \hat{\bar{S}} &= \arg \max_{\bar{S}} f(\bar{z}|\bar{S}) \\
 &= \arg \max_{\bar{S}} \prod_{i=0}^{N-1} f(\bar{z}_i|S_i, S_{i-1}, \bar{z}_{i-m_I}^{i-1}) \\
 &= \arg \max_{\bar{S}} \prod_{i=0}^{N-1} \frac{f(\bar{z}_{i-m_I}^i|S_i, S_{i-1})}{f(\bar{z}_{i-m_I}^{i-1}|S_i, S_{i-1})}
 \end{aligned} \tag{5.33}$$

Since $f(\bar{z}_{i-m_I}^i|S_i, S_{i-1}) \sim N(\bar{\mathcal{Y}}(S_i, S_{i-1}), \mathcal{C}(S_i, S_{i-1}))$, (5.32) follows immediately from (5.33). ■

With the help of this cost function (5.32), the maximum likelihood sequence of plant models can be found employing the Viterbi algorithm (see [128]).

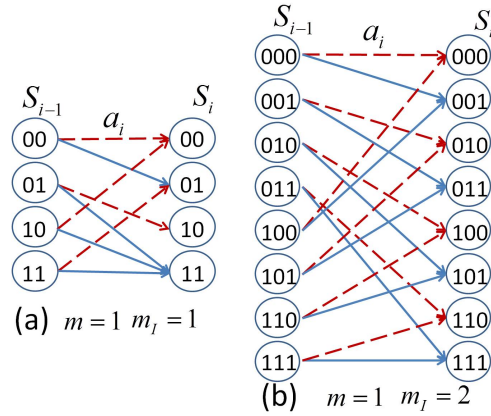


Figure 5.2: (a) State transitions for $m = 1$, $m_I = 1$. At each stage i , S_i can assume four possible states. (b) Eight possible states S_i for $m = 1$, $m_I = 2$. Transition from stage S_{i-1} to S_i occurs when a new bit $a_i \in \{0, 1\}$ is input. State transitions for $a_i = 0$ ($a_i = 1$) are shown in broken (solid) lines. Increasing the assumed memories extends the complexity and memory requirements for the statistical learning step and the Viterbi detection algorithm.

In the next sections, the technique of real time plant detections with ISM and MAP

are applied for high speed topography detection with atomic force microscopy (AFM). Further for AFM based data storage applications, performance of ISM-MLSD with regular MLSD and symbol-by-symbol ISM and MAP are compared. The advantages of ISM-MLSD over regular MLSD and symbol by symbol detection methods are further reinforced by simulations and experimental data.

5.4 Application of ISM and ISM-MLSD to probe based data storage; benefits and comparison of ISM with MAP based approach

The operation of dynamic mode AFM and concept of the equivalent cantilever perspective are explained in Sections 3.1 and 3.2, Chapter 3. As outlined in Section 5.1, for probe based data storage applications [111], [27], a high topography is used to encode the bit 1 and a depressed topography for the bit 0. Binary topography detection technique, where the detection of bits is the main task, in the context of the dynamic mode AFM is developed here.

A continuous time state-space model for the free air cantilever dynamics as specified in (3.1) can be easily constructed. The state space model consists of the matrices (A_1, B_1, C_1) that can be considered as model 1 for switched detection framework. The BCEWRLS method is used to estimate the discrete-time parameters of the equivalent cantilever dynamics (3.8) when the cantilever interacts with the sample for a specified set point amplitude. Once these equivalent parameters are known they can be easily converted to continuous-time domain to construct another set of state space matrices (A_2, B_2, C_2) (see [106]) that represent the model 2 in the model detection strategy. Here it is assumed that the system dynamics instantaneously changes to the equivalent cantilever (nominal cantilever) model when it starts (stops) interacting with the sample. It also holds true that the equivalent cantilever model differs from the free air model only in the A matrix. So that $B_1 = B_2$ ($:= B$) and $C_1 = C_2$ ($:= C$). Hence from (5.16),

it follows that

$$\begin{aligned} \begin{bmatrix} \dot{z}_1(t) \\ \dot{z}_2(t) \end{bmatrix} &= \underbrace{\begin{bmatrix} M_1 & M_2 \\ M_2 & M_1 \end{bmatrix}}_{A_{ISM}} \begin{bmatrix} z_1(t) \\ z_2(t) \end{bmatrix} \\ &+ \underbrace{\begin{bmatrix} (A_2 - A_1) & 0 & (L_2 - L_1) \\ 0 & (A_2 - A_1) & -(L_1 + L_2) \end{bmatrix}}_{B_{ISM}} \begin{bmatrix} x(t) \\ b_m x(t) \\ \zeta(t) \end{bmatrix}, \end{aligned} \quad (5.34)$$

where the variable $b_m = -1$ when the cantilever oscillates in air and $b_m = +1$ when the cantilever follows the equivalent dynamics. The output equations for e_{dif} and e_{sum} are governed by

$$\begin{bmatrix} e_{dif}(t) \\ e_{sum}(t) \end{bmatrix} = \underbrace{\begin{bmatrix} C & 0 \\ 0 & C \end{bmatrix}}_{C_{ISM}} \begin{bmatrix} z_1 \\ z_2 \end{bmatrix} + \underbrace{\begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 2 \end{bmatrix}}_{D_{ISM}} \begin{bmatrix} x(t) \\ b_m x(t) \\ \zeta(t) \end{bmatrix}. \quad (5.35)$$

The system described by (5.34) and (5.35) is used for verifying the switching model for the AFM system. Discrete time equivalent systems of (A_i, B, C) , $i \in \{1, 2\}$ are used for MAP based detection as shown in (5.9).

It should be noted that although the BCEWRLS scheme is extremely effective for measurements of equivalent parameters, the speed of parameter convergence is slower than what can be achieved by a detection scheme like ISM or MAP. Thus typically, we estimate the nominal and equivalent plant dynamics from a slower learning phase using BCEWRLS algorithm, and subsequently utilize the detection strategies.

5.4.1 Validation of equivalent model and detection framework

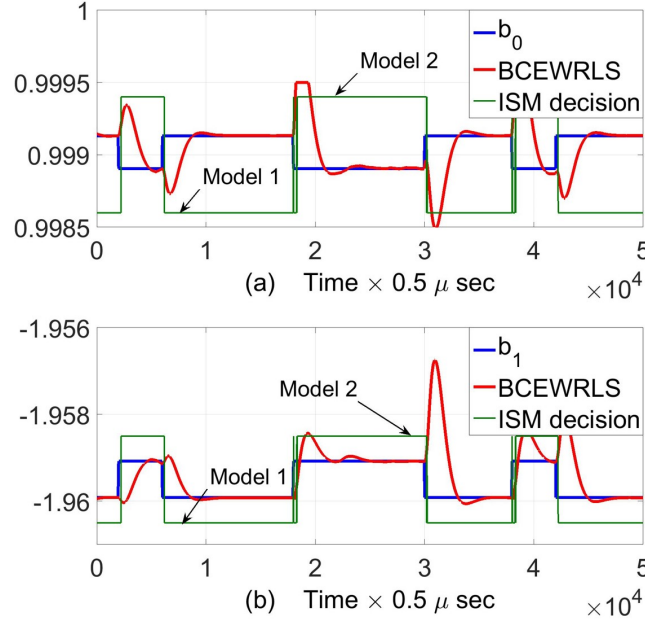


Figure 5.3: (a) Estimation of b_0 using BCEWRLS ($\lambda = 0.995$) with ISM decision from simulation. (b) Estimation of b_1 with ISM decision from simulation. Both the estimates of b_0 and b_1 undergo lengthy transient behavior when the plant switches from one model to another. ISM decision signal is much faster and hence justifies its effectiveness for real time deciphering of the active plant model.

Here we justify the utilization of a detection framework over an estimation framework alone for the purpose of fast identification of plant models in real time. The transfer function of an AFM cantilever with resonant frequency of 63.147 kHz (f_0) and quality factor 227.849 (Q_0) is chosen as the model 1 for switching plant. A discrete time plant can be easily obtained from the continuous time model corresponding to sampling frequency chosen as 2 MHz. The discrete time model 1 is described by parameters $\{b_1, b_0, a_2, a_1, a_0\}$ found to be $\{3.972 \times 10^{-4}, -2.036 \times 10^{-4}, -4.990 \times 10^{-4}, -1.9599, 0.9991\}$. Another cantilever transfer function model with resonant frequency of $f_0 + 0.5$ kHz and quality factor $0.8 \times Q_0$ is chosen to represent the model 2. The parameters $\{a_2, a_1, a_0\}$ are the same in both models while $\{b_1, b_0\}$ for model 2 are found to be $\{-1.9591, 0.9989\}$. In Figure 5.3,

the plant is made to switch randomly between model 1 and 2 at a bit duration of 2 ms. The BCEWRLS algorithm (outlined in Section 3.3.2 of Chapter 3) shown in Figure 5.3 works with $\lambda = 0.995$. ISM based detection algorithm as described in Section 5.2.2 is also employed. The decision variable $T[m]$ for discrete time implementation of ISM (5.24) is calculated and decision is made to be model 2 when $T[m] > 0$, otherwise model 1. Figure 5.3 shows the significant speed improvement of implementing ISM algorithm over BCEWRLS scheme for plant detection purposes. Near the time 0.9 micro sec, plant switches from model 1 to model 2. At that time both the estimates of b_0 and b_1 undergo transient states in Figure 5.3 (a) and (b). However, during the time scales shown in Figure 5.3 (a) and (b), ISM based detection algorithm is observed to catch up with plant changes at much faster rates than the estimation scheme. As example, in Figure 5.3 (b), near time 0.9 μ sec, b_1 changes from low to high value. The ISM decision changes within 0.0175 μ sec to the correct decision value. However the BCEWRLS algorithm takes almost 0.350 μ sec to reach steady value. Hence a speed improvement order of $0.35/0.0175=20$ can be achieved using ISM based detection over BCEWRLS method alone. Even though the exact convergence time may vary from one example to another, ISM based detection is consistently observed to be faster than BCEWRLS based estimation.

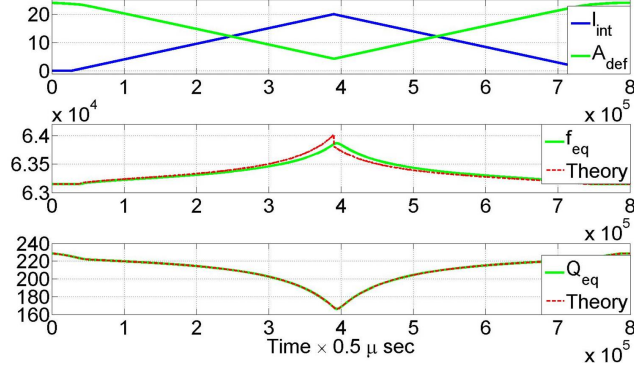


Figure 5.4: Verification of equivalent cantilever model using averaging theory technique. The oscillating cantilever is made to interact with sample for gradually ascending interaction (approach) followed by descending interaction (retract). With increasing (reducing) l_{int} , the amplitude of oscillation A_{def} reduces (increases). BCEWRLS scheme is applied to calculate equivalent cantilever parameters; equivalent resonant frequency f_{eq} and equivalent quality factor Q_{eq} . Using averaging theory f_{eq} and Q_{eq} are analytically calculated for the tip-sample interaction model. Theoretically predicted and estimated values for Q_{eq} are close match. With small interactions (low l_{int}), f_{eq} match very well with theory. For higher interactions (high l_{int}), f_{eq} deviates slightly from analytical predictions.

A ‘close to reality’ tip-sample interconnected system is accepted for verification of the effectiveness of BCEWRLS in estimating the equivalent cantilever parameters. A piecewise linear model developed in [1] is utilized to model the tip-sample interaction (also see Figure 2.3 in Chapter 2) which works reasonably well for capturing interactions with hard substrates like mica. The interaction model parameters are chosen so that the cantilever tip-sample interaction is predominantly repulsive (see [45]). A cantilever with first resonant frequency of 63.15 kHz and quality factor 227.85 is chosen for simulation. The cantilever is oscillated with free air oscillation amplitude of 24 nm. The oscillating cantilever is gradually moved towards and away from the sample surface to increase the interaction length l_{int} followed by reducing it. In this chapter, l_{int} is defined as the difference between the steady state amplitude of oscillations of the cantilever in free air and that of the cantilever interacting with an infinitely hard sample. As shown in Figure 5.4 when l_{int} increases, the amplitude of oscillation A_{def} reduces and vice

versa. Equivalent cantilever parameters (f_{eq} and Q_{eq}) corresponding to each cantilever oscillation amplitude are obtained from analytical expressions derived using averaging theory techniques (see [45]) and compared with estimated values using BCEWRLS with $\lambda = 0.995$. It is evident from Figure 5.4 that the equivalent cantilever model is indeed a very useful tool to model oscillating cantilever interacting with a sample. Further, BCEWRLS estimated values are close match with theoretical predictions.

The validity of the switching model discussed before is explained now. The separation between the cantilever base and the sample (called *sep*) is raised and lowered with a pseudo random bit pattern of bit duration $200 \mu s$. A high value of *sep* in Figure 5.6 indicates model 1 (free air oscillations) and a low value indicates model 2 (interaction state). A frequency sweep method is applied to obtain the frequency response of the cantilever around the first resonance frequency and from the frequency response the state space matrices (A_1, B, C) are obtained. In order to get the model 2, the cantilever tip is made to interact with the sample with an interaction length (l_{int}) of 2 nm. The equivalent cantilever model corresponding to l_{int} of 2 nm is obtained using the BCEWRLS scheme with forgetting factor $\lambda = 0.995$. From the estimated equivalent parameters, A_2 matrix for the continuous time equivalent system is obtained. From the state space models described by model 1 (A_1, B, C) and model 2 (A_2, B, C), the system ($A_{ISM}, B_{ISM}, C_{ISM}, D_{ISM}$) as shown in (5.34) and (5.35) can be constructed. Now, the objective is to compare the outputs e'_{dif} and e'_{sum} from the ISM system (see Figure 5.5) to the outputs e_{dif} and e_{sum} obtained from the ‘close to reality’ cantilever-sample interconnected system together with the two observers.

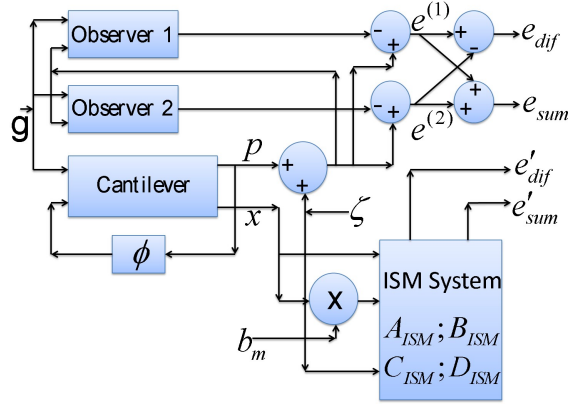


Figure 5.5: ISM system: Simulation model for verification of the switching model.

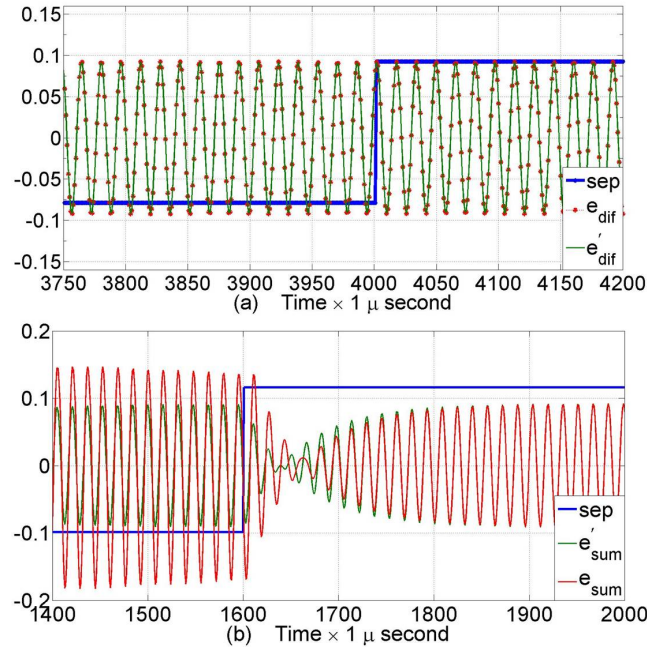


Figure 5.6: (a) e_{dif} and e'_{dif} with sep from simulation. These signals have no detectable difference. (b) e_{sum} and e'_{sum} from simulation. These signals match perfectly for no interaction from sample (model 1). The phase behaviors of e_{sum} and e'_{sum} match during interaction with sample (model 2).

From Figure 5.6 (a) it can be observed that whether the model is 1 (sep high) or

2 (*sep* low), e_{dif} and e'_{dif} are always perfect match with each other. From Figure 5.6 (b), it is clear that e_{sum} and e'_{sum} match correctly in model 1. In model 2, the phase behaviors of e_{sum} and e'_{sum} are very close match. However, the amplitude behaviors are not the same. A better model than a simple switching model is required for predicting the behavior of amplitude of e_{sum} . Nevertheless, the switching model is accepted since it allows to have a nice balance between simplicity and a reasonable ground for analytical understanding.

5.4.2 Real time detection

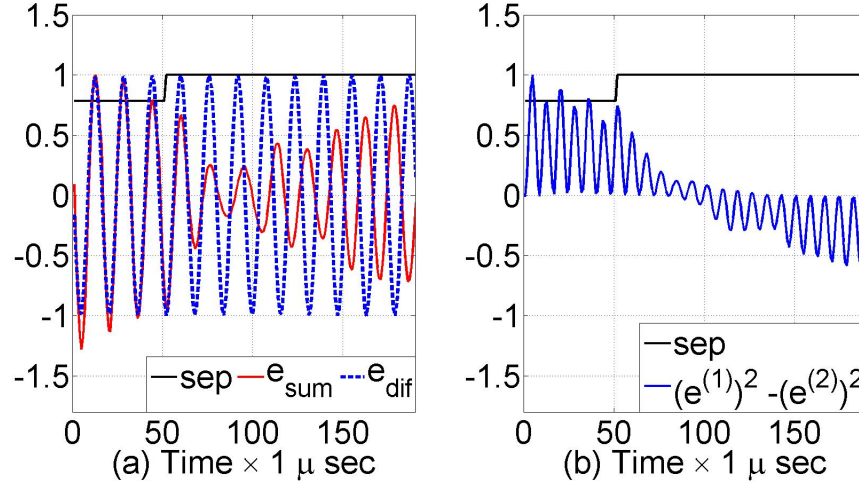


Figure 5.7: (Simulation result) (a) e_{dif} and e_{sum} oscillate in phase (out of phase) when cantilever interacts with the sample (is in free air). (b) Envelope of $(e^{(1)})^2 - (e^{(2)})^2$ changes sign as the cantilever goes from off-sample to on-sample situation and vice versa. *sep* high (low) implies free air oscillation (interaction).

The effectiveness of the model detection methods developed in the chapter are assessed below in the context of AFM based material investigation. In simulation studies the separation between the cantilever base and sample (*sep*) is raised and lowered with a pseudo random bit pattern of bit duration $200 \mu\text{s}$. High *sep* in Figure 5.7 indicates model 1 (free air oscillations) and low *sep* indicates model 2 (interaction). ISM detectors are designed by matching the free air cantilever model (model 1; (A_1, B, C)) and BCEWRLS

estimated equivalent model (model 2; (A_2, B, C)) obtained using $l_{int} = 2$ nm. It is evident from Figure 5.7 (a), when sep is low (interaction), both e_{sum} and e_{dif} oscillate in phase. However, high sep (free air oscillations) cause e_{dif} and e_{sum} to oscillate 180 degrees out of phase confirming result (5.23). In Figure 5.7 (b), the envelope of $(e^{(1)})^2 - (e^{(2)})^2$ (referred to as ISM_{lpf}) follows the analytically predicted behavior in Section 5.2; ISM_{lpf} is negative when the media presents depressed topography (the equivalent dynamics follows model 1) and is positive when the media presents a raised topography with an interaction length of 2 nm (the equivalent dynamics follows model 2).

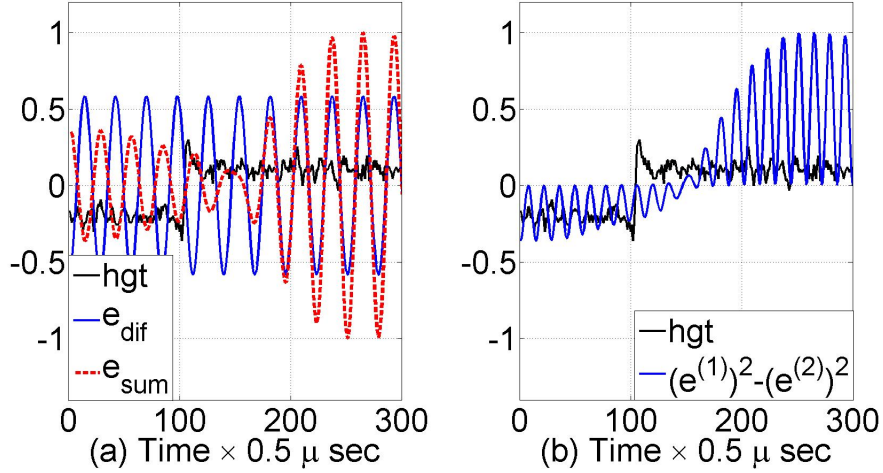


Figure 5.8: (Experimental Data) (a) hgt denotes measured height of sample. The sample media changes to a raised topographic profile at $50 \mu sec$. From $0 - 50 \mu sec$ there is no interaction between the sample and the cantilever; for the rest of the duration of the experiment, cantilever interacts with the sample. e_{sum} and e_{dif} signals are in phase within a couple of cantilever oscillations of the heightened topographic profile appearing agreeing with analytical results. In the absence of the sample interaction (from $0 - 50 \mu sec$) e_{sum} and e_{dif} differ in phase by π radians. (b) Envelope of $(e^{(1)})^2 - (e^{(2)})^2$ exhibits sign inversion within a few cycles of cantilever oscillations similar to seen in simulation results.

In the following the detection method is experimentally assessed. A field-programmable

gate array (FPGA) based circuit actuates the piezoelectric nano-positioning system according to a pseudo random bit pattern of bit duration $350 \mu s$. In Figure 5.8 (a) and (b), the signal hgt denotes the height of the sample. High hgt implies interaction and low hgt implies free air cantilever oscillations where there is no interaction with the sample. The innovation signal $e^{(1)}$ is obtained from observer matched to the free air cantilever model ($f_0 = 71.73$ kHz, $Q_0 = 130.21$). The second observer is designed to match the equivalent cantilever model where f_{eq} is determined (using BCEWRLS method) to be 100 Hz less than f_0 . Q_{eq} is determined to be same as Q_0 . Behaviors of e_{sum} , e_{dif} and envelope of $(e^{(1)})^2 - (e^{(2)})^2$ resemble remarkably closely with theoretical predictions and simulation results (compare Figure 5.7 and Figure 5.8). The high bandwidth detection capability is also evident from experimental data where the sign inversion occurs within a few cycles of the cantilever oscillations (with time period $\approx 1/f_0$). Moreover the experimental results also support the robustness and applicability of the methods where the estimated equivalent models are utilized in the ISM method and are used in real experimental conditions thereby confirming that deviations of the estimated models from the physical dynamics do not pose limitations on the ISM based method.

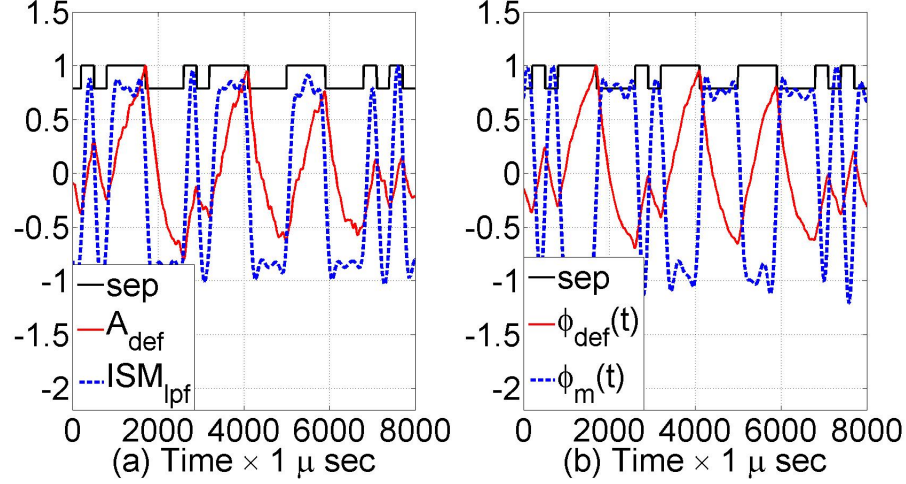


Figure 5.9: (Simulation result) (a) Conventional dynamic mode AFM amplitude image (A_{def}) and ISM amplitude image (ISM_{lpf}) with cantilever-sample separation (sep). ISM_{lpf} is faster and exhibits significant independence of topographical history. (b) Regular phase image (ϕ_{def}) and ISM generated phase image (ϕ_m) from simulation. ϕ_m is more suitable for high speed binary deciphering of topography.

A comparison of ISM with conventional AFM signals (the amplitude and phase of cantilever oscillations, explained in Section 3.2 and Section 3.3 in Chapter 3) is presented below. A_{def} and ϕ_{def} in Figure 5.9 (a) and (b) denote the demodulated amplitude and phase of cantilever oscillations. ISM generated amplitude (ISM_{lpf}) and the phase difference between e_{dif} and e_{sum} (referred to as ϕ_m) signals are also shown in Figure 5.9 (a) and (b). All the data are scaled and shifted appropriately to fit in the same figure. In the time scale shown in Figure 5.9 (a) and (b), A_{def} and ϕ_{def} are always in their transient region. However, in contrast to ISM based signals which reach their steady state. Further, traditional images are dependent on history of the past topography encountered since a history of consistent high topography lowers the amplitude A_{def} . However, ϕ_m and ISM_{lpf} follow a consistent binary behavior independent of history.

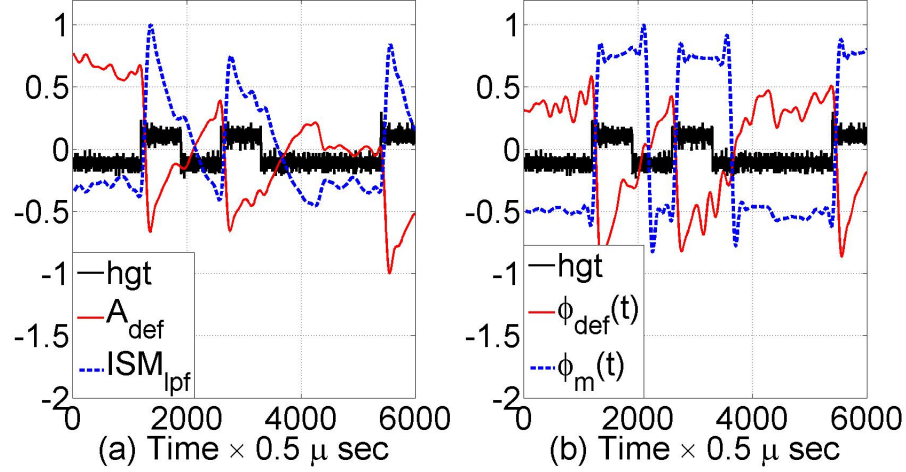


Figure 5.10: (Experimental Data) (a) Conventional amplitude A_{def} and ISM_{lpf} from experiment. A_{def} shows an overall drift from left to right. However, ISM_{lpf} has more consistent levels. (b) Regular phase ϕ_{def} and ISM based phase ϕ_m signals from experiment. ϕ_m has a very consistent binary behavior and better steady state magnitudes than ϕ_{def} .

Similar images are generated from experiments and compared (see Figure 5.10). An interesting observation is that ϕ_m exhibits better steady behavior than ISM_{lpf} in experiment. In Figure 5.10 (a), value of A_{def} shows a downward drift from left to right. However, the magnitude levels of ISM_{lpf} are seen to be consistent implying that a threshold based detection is difficult from A_{def} but it is possible from ISM_{lpf} . ϕ_m is found to detect the topography changes at significantly higher bandwidth. When hgt changes, ϕ_m switches value almost seven times faster than ϕ_{def} (see Figure 5.10 (b)). Clearly ISM based detection outperforms conventional AFM images for binary detection of topography.

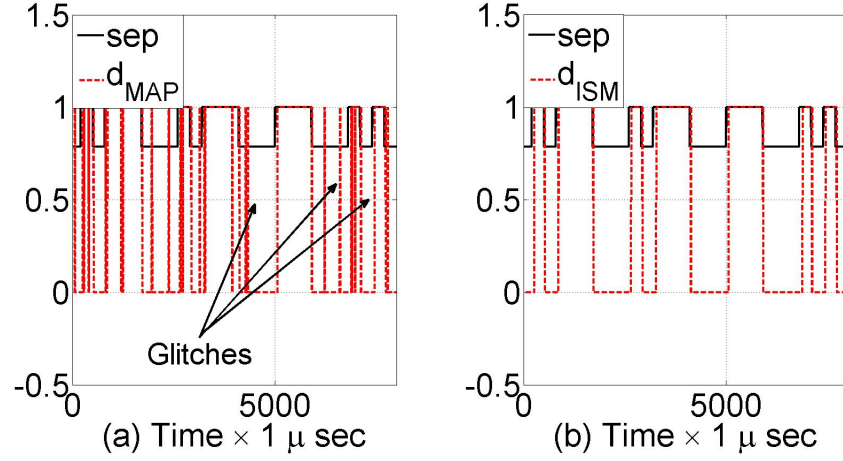


Figure 5.11: (Simulation result) (a) MAP and (b) ISM based detection. ISM does not exhibit decision glitches as shown by MAP.

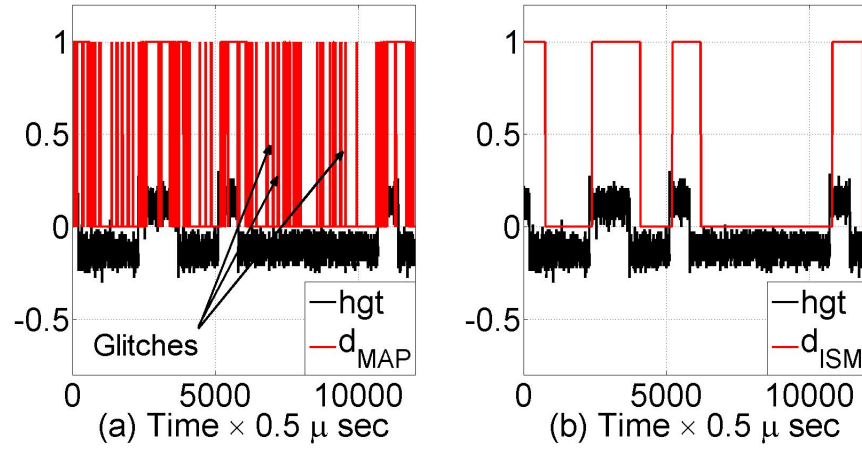


Figure 5.12: (Experimental Data) (a) MAP and (b) ISM based decision images d_{MAP} and d_{ISM} . d_{MAP} is severely effected by decision glitches whereas d_{ISM} has correct decision making capability.

A comparison of recursive MAP (5.9) and discrete time implementation of ISM (5.24) is described here. For time varying systems, the MAP algorithm requires periodic refreshing of $p(\theta_1|\mathcal{Y}_m)$ and $p(\theta_2|\mathcal{Y}_m)$ (see [29]). A time window of 40 samples for periodic refreshing is found suitable for MAP algorithm. Such periodic refreshment is

not necessary for ISM. In Figure 5.11 (a), the decision variable for MAP shown as d_{MAP} is set to 1 when $p(\theta_1|\mathcal{Y}_m) > p(\theta_2|\mathcal{Y}_m)$ otherwise 0. ISM signal $T[m]$ is gathered using a window size $M = 15$. d_{ISM} in Figure 5.11 (b) is set to 1 when $T[m] < 0$ otherwise 0. d_{MAP} as shown in Figure 5.11 (a) produces glitches in the decision even when sep is steady. However, d_{ISM} shows better binary behavior without any errors in Figure 5.11 (b). Figure 5.12 (a) and (b) show a comparison of experimentally obtained decision images by recursive MAP and ISM. It is evident that the decision image from MAP results in considerable fraction of incorrect decisions (see Figure 5.12 (a)). However, from Figure 5.12 (b) it is observed that ISM yields high fidelity results.

5.4.3 Symbol by symbol and sequence detection

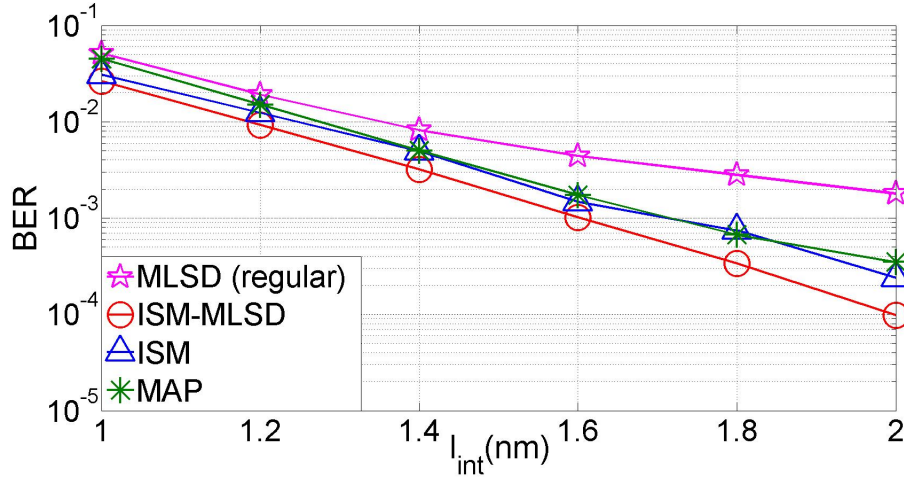


Figure 5.13: (Simulation result) Bit error rate (BER) performance of symbol-by-symbol ISM and MAP with regular MLSD and ISM-MLSD as a function of interaction length l_{int} . Increasing l_{int} over the range 1 nm to 2 nm gradually improves BER performance of each scheme. Performance of ISM and MAP are close to each other. Regular MLSD lags slightly in error rate compared to other methods. However ISM-MLSD consistently outperforms all the other methods. Dwell time T is chosen as 250 micro seconds.

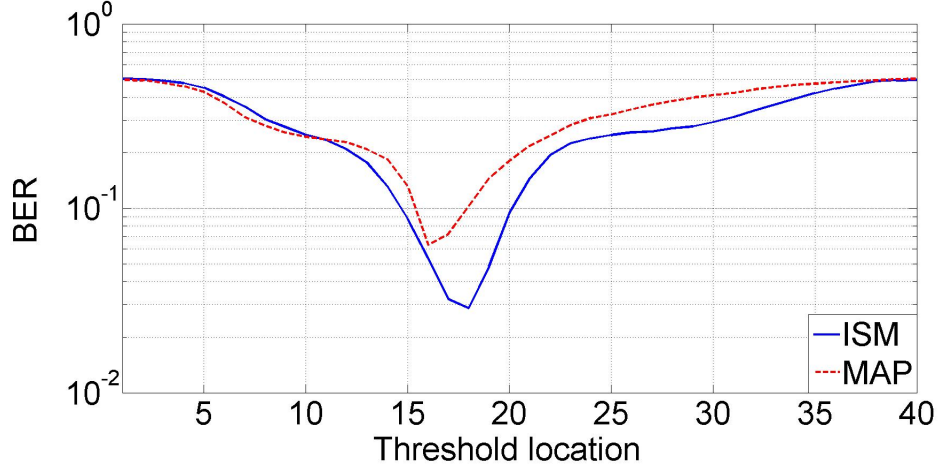


Figure 5.14: (Experimental data) Bit error rate (BER) performance of symbol-by-symbol ISM and MAP with varying threshold location. Dwell time T is chosen as 350 micro seconds.

A comparison of detection of plant models with finite dwell time as discussed in Section 5.3 is presented here. For simulations, sep is varied with a dwell time T (or equivalently bit duration) of 250 micro sec. Test signals for ISM in (5.24) and MAP in (5.9) are averaged over this time interval T before making decisions for symbol-by-symbol detection of plant models. In the previous work on MLSD for probe based data storage (see [27]), the test signal $s_m(t)$ is chosen by applying a matched filter on the innovation signal $e^{(1)}$. Apart from the assumption of known dwell time T , in Ref. [27] it is also assumed that the exact time instant when the oscillating cantilever tip impulsively interacts with the sample (q times during dwell interval T) is known and outputs of the matched filter are collected at their maximum value during each successive tip-sample interaction cycle. In Ref. [106] it is shown that ISM performs as good as MLSD applied on test signal generated through processing $e^{(1)}$ as recommended in Ref. [27]. However, from the perspective of implementation, having accurate knowledge of q instants where the cantilever interacts with the sample during each dwell time T is a difficult task. Hence in this chapter regular MLSD and ISM-MLSD are applied on test signals $s_m(t)$ chosen as appropriately low pass filtered $(e^{(1)})^2$ and $(e^{(1)})^2 - (e^{(2)})^2$ signals respectively. This method is simpler to implement and the q samples during each interval T can be

collected just by uniform sampling of the observation data without knowing the time instants of cantilever tip-sample interaction. The decision error rates (also referred to as bit error rate; BER) of each method are observed where the bits are generated pseudo randomly. Average BERs are obtained for fixed on-sample interaction length l_{int} which is further varied from 1 nm to 2 nm. For all these cases, ISM signals are generated using the fixed equivalent model of $l_{int} = 2$ nm. The resulting BER vs l_{int} plots are compared in Figure 5.13. Clearly, tapping the surface harder by increasing l_{int} improves the detection schemes. In Figure 5.13 ISM-MLSD performs better than other methods. Similar simulations are performed by varying the dwell interval T and for all cases ISM-MLSD is observed to consistently outperform other methods.

Results from experiment are presented now. The sample height hgt is actuated with FPGA based actuator with a pseudo random bit pattern of bit duration $T = 350$ micro sec. Symbol-by-symbol test signals for ISM and MAP are obtained. The ranges of obtained values for ISM and MAP are divided into 40 grid points and they are used as decision thresholds for bit detection. Experimentally obtained BERs for ISM and MAP for various threshold locations are shown in Figure 5.14. It is clear that the optimum threshold for detection (where BER is minimized) is slightly off from the mid-point of range of test values. The minimum BER for ISM and MAP for optimum choice of thresholds are obtained as 2.88 and 6.32 percent respectively. Hence more than 2-times improvement of accuracy is obtained by ISM over MAP.

Statistics for sequence detectors is gathered for known bit sequence with assumptions of system memory $m = 1$ and inter symbol interference memory $m_I = 2$. As shown in Figure 5.2, there are 8 possible states S_i with 16 possible state transitions (S_{i-1}, S_i) . For each possible state transition, average observation vector $\bar{\mathcal{Y}}$ and corresponding covariance matrix $\mathcal{C}$ are determined. Example of such experimentally gathered mean observation vectors for regular MLSD and ISM-MLSD corresponding to three possible state transitions $(S_{i-1}, S_i) \in \{(000, 001), (110, 101), (011, 110)\}$ are shown in Figure 5.15. The set of statistics pairs $(\bar{\mathcal{Y}}, \mathcal{C})$ for 16 possible state transitions are utilized for Viterbi sequence detections using regular MLSD and ISM-MLSD. Interestingly, regular MLSD on the experimental data yields BER of 0.14 percent whereas ISM-MLSD yields only 0.07 percent BER. These experimental data suggest that MLSD and ISM-MLSD are

capable of improvement of accuracy almost 20 times and 41 times than the best possible situation for symbol-by-symbol detection. The improvement of sequence detection over symbol by symbol method is achieved by correcting for the moderate ISI which is observed in the experimentally obtained test signals $(e^{(1)})^2$ and also $(e^{(1)})^2 - (e^{(2)})^2$. However, implementation of MLSD and ISM-MLSD are much more intricate compared to simple symbol-by-symbol methods and require a statistical learning step with known sequence of bits before detection of unknown bit sequence.

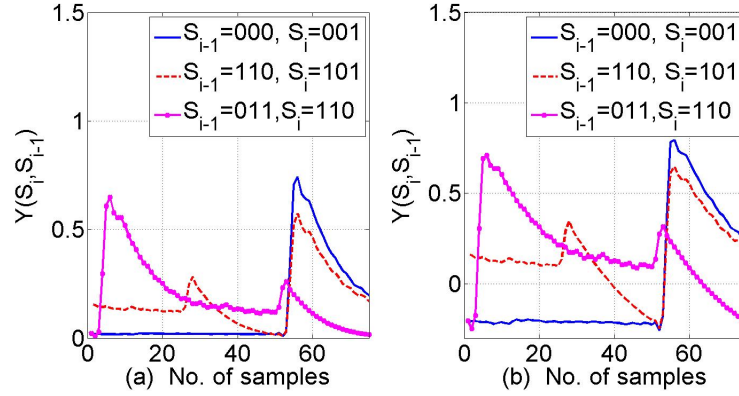


Figure 5.15: (Experimental data) (a) Example mean observation vectors $\bar{\mathcal{Y}}$ for regular MLSD. (b) $\bar{\mathcal{Y}}$ for ISM-MLSD. The 3 state transitions shown here are $(S_{i-1}, S_i) \in \{(000, 001), (110, 101), (011, 110)\}$. The statistics pairs $(\bar{\mathcal{Y}}, \mathcal{C})$ in turn are used for Viterbi detection. Dwell time T is chosen as 350 micro seconds.

5.5 Conclusions

In this chapter a real time switching-model detection strategy ISM is developed for a system that switches between two models. The concept of utilizing the known plant models and sign-inverting test signals for ISM is developed. A MAP based detection algorithm from existing literature is briefly outlined and compared with ISM. Detection schemes are modified for the situation when a dwell time with which the plant model switches is known a priori. Sequence detection method namely MLSD on innovation squared mismatch signal is proposed to further improve detection performance in the presence of inter symbol interference. In the application part, the dynamic mode AFM

based fast topography detection system is cast as a binary switched system. Effectiveness and accuracy of the equivalent cantilever and switched plant model are discussed with theoretical predictions. The results indicate the superior speed of operation and the steady-state value advantages of plant model detection approach over the conventional AFM imaging. The simulation and experimental results also suggest that ISM is simpler to implement yet performs better than recursive MAP for real time detection. Furthermore the robustness of ISM against the plant uncertainties, i.e., the situation when the observers are not accurately matched to the plant models and noise is present is borne out by thorough experimental data. Performance of sequence based detections are compared with ISM and MAP based symbol-by-symbol detections when the bit duration is known. It is verified that the proposed combination of ISM-MLSD offers significant gain in detection accuracy compared to other methods through simulation and experiments.

Chapter 6

Single molecule studies of utrophin protein constructs

6.1 Introduction

Duchenne muscular dystrophy (DMD) is the most prevalent and severe form of human muscular dystrophy. DMD is caused by a defective gene for dystrophin (see Figure 6.1). It usually affects boys where they have a 50% chance of having the disease if the mother is a carrier whereas a similar percentage of daughters of carriers will be carriers of the disease. The disease affects 1 in 5000 boys who lose the ability to walk by twelve years and have a life expectancy of less than 25 years. While clinical descriptions of DMD date back to the 1850s, over 100 years passed before evidence suggested that the muscle cell plasma membrane, or sarcolemma, is compromised in DMD muscle. There is currently no cure for DMD. Investigations into the molecular basis for DMD were greatly facilitated by identification of the defective gene and its major protein product, dystrophin [129]. Pioneering biochemical studies [130], [131], [132] revealed the tight association of dystrophin with a multi-subunit complex, the so-named dystrophin and glycoprotein complex (GPC).

Since its description, the dystrophin-GPC has emerged as an important structural unit of muscle and also as a critical nexus for understanding a diverse array of muscular dystrophies arising from defects in nearly all genes encoding DGC subunits [133].

Increased expression of the fetal dystrophin homolog utrophin can compensate for dystrophin deficiency in mouse models of DMD [134], so efforts to upregulate utrophin expression to substitute for dystrophin deficiency in DMD are underway in many laboratories [135]. Dystrophin and utrophin are frequently hypothesized to function as shock absorbers or molecular springs that protect the plasma membrane from rupture during muscle contraction or stretch [136] (see Figure 6.2). Even though dystrophin and utrophin play important mechanical roles, the mechanical properties of either protein remain to be characterized.

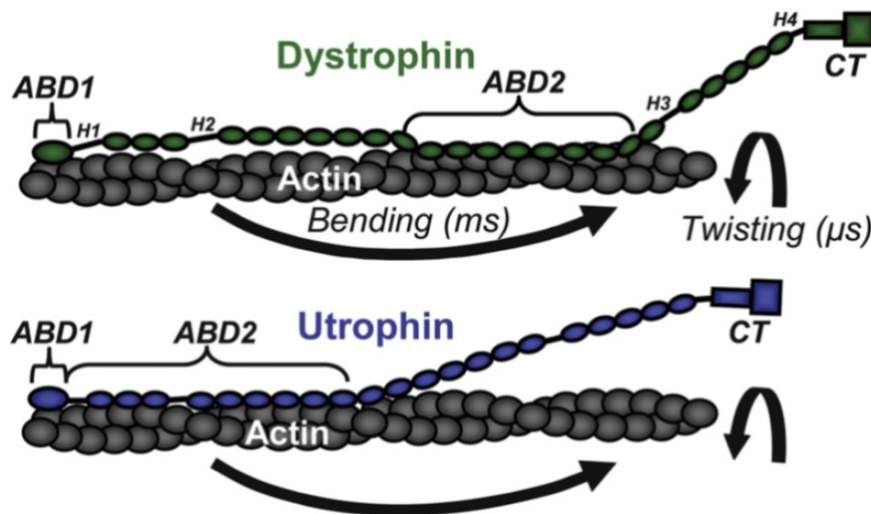


Figure 6.1: Homologous structures of dystrophin and utrophin. Both have two actin binding domains indicated by ABD1 and ABD2. In dystrophin the domains are separated by triple-helical spectrin repeats (ovals). The C-terminal (CT) domain is attached to the sarcomere. In dystrophin, unlike utrophin, ABD1 and ABD2 are separated by 10 spectrin-type repeats (STRs) [2]

Methods exist to recombinantly express and purify full-length dystrophin and utrophin in quantities that enabled rigorous biochemical characterization [137–139]. While *in vivo* data demonstrate that utrophin can functionally compensate for dystrophin-deficiency at least in dystrophic mice [134], studies have demonstrated clear differences between dystrophin and utrophin with respect to their molecular mechanisms [137], [138], [139].

In brief, dystrophin is a 427-kDa protein whereas utrophin is simpler 400-kDa homolog. In Figure 6.1 homologous structures of dystrophin and utrophin are shown. Both have two actin binding domains; ABD1 and ABD2. Here the first actin binding domain is located at the N-terminus (NT). The C-terminal (CT) domain is attached to the β -dystroglycan in the sarcolemma (sarcolemma is the cell membrane of striated muscle cells). In dystrophin the domains are separated by triple-helical spectrin repeats (ovals). In both dystrophin and utrophin, ABD2 is made up by spectrin like repeats. However, in dystrophin, unlike utrophin, ABD1 and ABD2 are separated by 10 spectrin-type repeats (STRs).

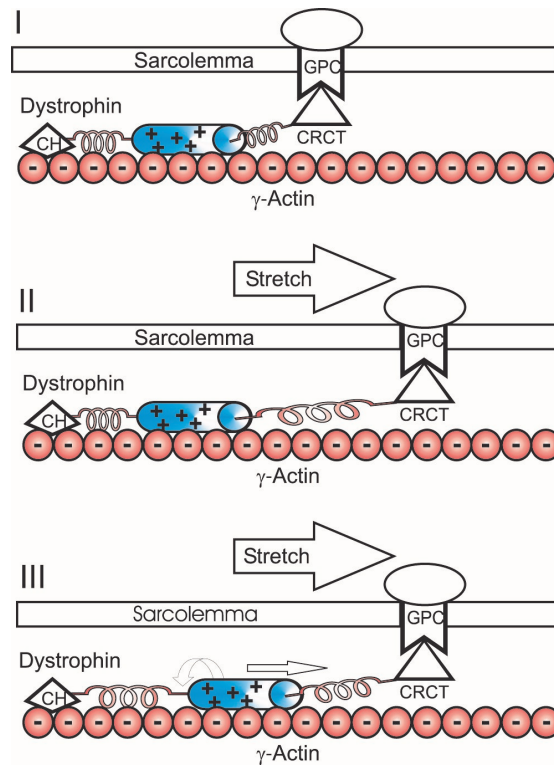


Figure 6.2: Model for how dystrophin may function as a molecular shock absorber to dampen elastic extension during muscle stretch. I) Relaxed muscle. II) Stretch imposes forces that uncoil spring-like elements within repeats 1-10 and 18-24. III) Electrostatic interaction of basic actin binding repeats 11-17 with acidic actin filaments dampens extension of the spring-like elements (see [3]). GPC denotes the glycoprotein complex.

Both dystrophin and utrophin are too large for most gene therapy vectors and thus considerable effort is ongoing on smaller constructs, which are suitable as candidates for gene therapy. Here it is possible that constructs derived from the simpler protein utrophin can be more effective than those derived from dystrophin. For example, in [2], it is established that mini-dystrophin without one of the actin binding domains is less effective in regulating actin dynamics whereas a micro-utrophin derived from utrophin with more extensive internal deletions is significantly more effective in regulation of actin dynamics. These and similar results further motivate the need to determine mechanical properties of smaller constructs of both utrophin and dystrophin and compare their behavior with full-length dystrophin and utrophin. Only a few studies have reported on the mechanical properties of dystrophin [140, 141] and no studies have reported on the mechanical properties of utrophin.

The atomic force microscope [5] is utilized for single molecule force spectroscopy. Such technique has been extensively used to study mechanical proteins such as titin [142, 143] and spectrin [144–146]. In this chapter we report the first ever mechanical characterization of full length utrophin and its functionally relevant terminal fragments utilizing MFP-3D atomic force microscope (AFM) from Oxford Instruments Asylum Research. Typical experimental setup for protein studies is explained in Figure 6.3. The AFM setup contains a flexible cantilever, a laser-photodiode based sensor which measures the position of the cantilever tip, and a piezo electric nano-positioner which can move a substrate in three spatial directions with respect to the cantilever base [5]. A droplet of the protein solution is employed on the substrate and kept for some time so that some proteins get adsorbed on the substrate surface. The cantilever is approached towards the substrate surface, pressed against the surface for some time (known as dwell time) and then retracted with a specified retract velocity. The approach-retract cycle is repeated. During such a cycle, if one end of a protein molecule gets attached to the cantilever tip with the other end adsorbed on the substrate, it is stretched during the retract phase of the cantilever. The pulling force on the molecule is balanced by the force on the cantilever which can be measured by observing the deflection of the cantilever tip. Forced extension causes the bonds in the molecule to unfold (also referred to as bond rupture events) which can be detected by a characteristic saw-tooth pattern observed in the cantilever deflection [144]. Data from typically 200-500 successful force spectroscopy

experiments are collected for each protein construct which reveal the statistical nature of the force vs extension properties of these molecules.

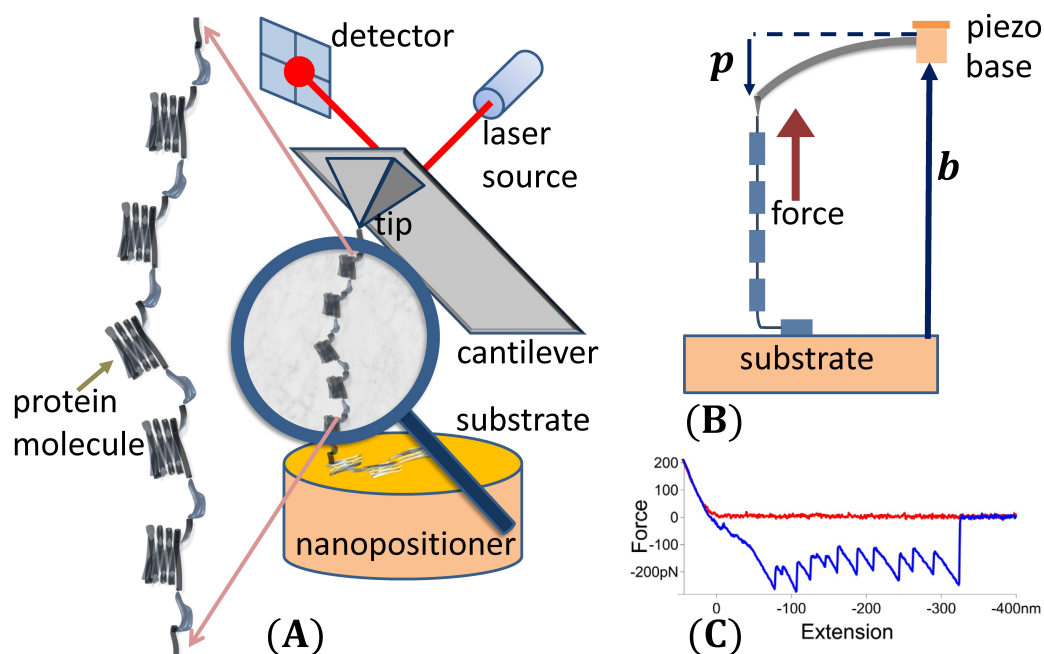


Figure 6.3: (A) A cantilever with a tip to which an end of the protein molecule with multiple domains is attached. The other end sits on a substrate. The tip deflection can be measured using a laser incident on the back of the cantilever which reflects into a quadrant photodetector. (B) The force on the molecule is estimated as kp where k is the stiffness of the cantilever flexure and p is the cantilever deflection measured by the laser-photodiode mechanism. The separation b between the substrate and the base of the cantilever support can be controlled with sub-nanometer precision using a nanopositioner. The extension of the molecule under externally applied force is denoted by sep which can be measured using $sep = b - p$. (C) In a typical pulling experiment the separation between the base of the cantilever and the substrate is increased where the bonds unfold and a sawtooth pattern is observed in the force-extension data.

This chapter is organized as follows: Section 6.2 validates our experimental setup by studying the well studied protein titin [142] and matching with the existing literature. Section 6.3 explains our surprising findings of the mechanical behavior of utrophin protein segments. Section 6.4 concludes with future directions of this work.

6.2 Force spectroscopy experiments on titin I27O constructs

Titin is critical for proper functionality of muscles. It provides support to the skeletal and cardiac muscle sarcomere and restores the sarcomere to its original dimension after relaxation of the muscle where it acts like a molecular spring (see [147, 148]). Single molecule force spectroscopy studies of titin have resulted in deep insights in how it achieves its functionality. In [149] the mechanical unfolding properties of individual domains was determined and used to reconstruct the force-extension curve of the complete titin molecule. Nuanced insights were achieved such as: muscle elasticity at low forces of less than 4 pN is due to the compliant N2B and PEV regions and that under high or forces that persist for long times, a mechanical buffer was provided by the Ig domains in a tandem array. Given the wealth of data available on titin, for the single molecule force spectroscopy methods, we use titin I27O constructs [150] to validate our experimental setup.

The I27O molecule contains eight repeats of I27 domains of human titin. The stock protein is diluted to a concentration of 100 $\mu\text{g/ml}$ in phosphate buffer saline (PBS, pH level 7.3). 100 μl of the diluted protein solution is applied on gold substrate. An Olympus biolever (long) of stiffness 6.67 pN/nm is employed for constant speed pulling experiments where the cantilever base is moved away from the gold substrate at speed of 1 $\mu\text{m/sec}$. Figure 6.4 shows example data of a successful pulling experiment. The force versus extension characteristics of a wide variety of protein molecules including titin is fit with worm like chain model [142] which is described by:

$$F_{pr} = \frac{K_B T}{P} \left[\frac{1}{4} \left(1 - \frac{d}{L_c} \right)^{-2} + \frac{d}{L_c} - \frac{1}{4} \right], \quad (6.1)$$

where L_c is the contour length, P is the persistence length, and F_{pr} is the force (the tension) in the molecule. d is the extension of the molecule, k_B is the Boltzmann constant and T denotes the absolute temperature. The parameters that best fit the worm like chain like chain (WLC) model provide key insights into the mechanical properties of the protein molecule.

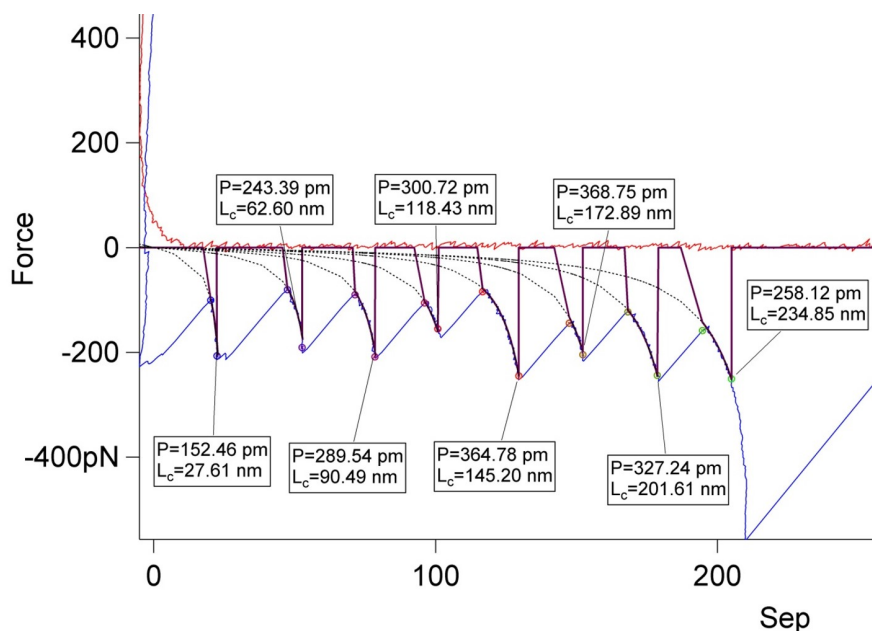


Figure 6.4: Force versus extension characteristics of titin construct I27O. The peaks in the extension data are identified as the bond unfolding events. The extension of the protein in between the bond ruptures are identified and fit with WLC models described by persistence length, P , and contour length, L_c . The x -axis denoted by sep represents the separation of the cantilever tip from the substrate (nm), which is the same as the extension of the molecule when it is taught. The y -axis represents the force acting on the molecule (pN).

The bonds unfolding during forced extension are identified by the peaks in the extension curve (see Figure 6.4). The extension of the molecule in between such consecutive bond unfolding is fit with the WLC model as shown in Figure 6.4 using Igor Pro software from Oxford Instruments Asylum Research. Collective data from the successful pulling experiments reveal the statistical behavior of the unfolding forces, persistence lengths, unfolding lengths, and also the contour lengths which are demonstrated in Figure 6.5, Figure 6.6, and Figure 6.7.

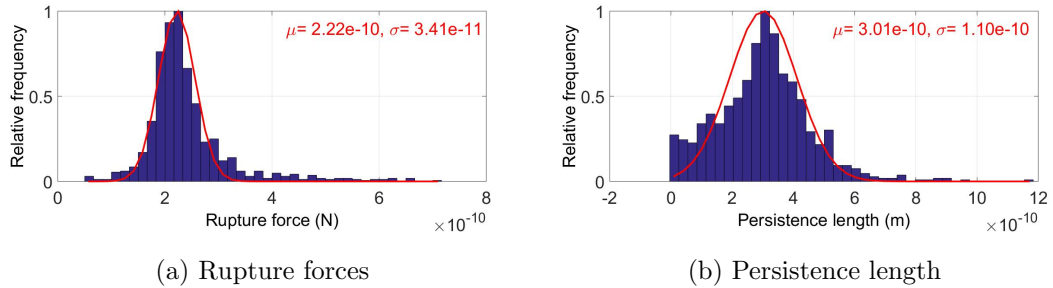


Figure 6.5: (a) Distribution of rupture forces (N), and (b) distribution of persistence length (m) determined from force spectroscopy experiments of titin I27O molecules.

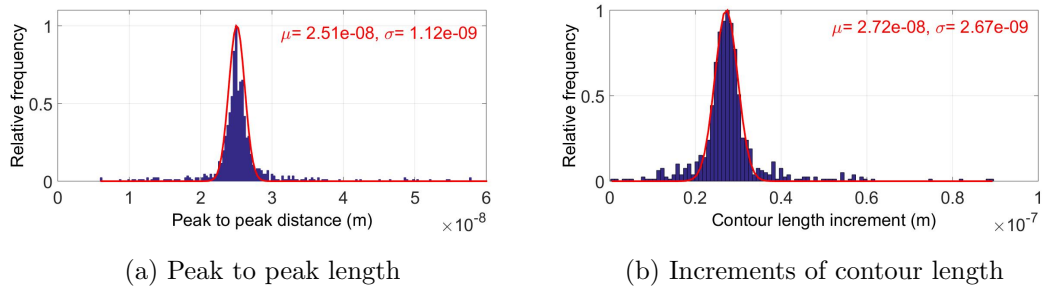


Figure 6.6: (a) Distribution of peak to peak distance (m), and (b) distribution of increments in contour length (m) due to bond ruptures determined from force spectroscopy experiments of titin I27O molecules.

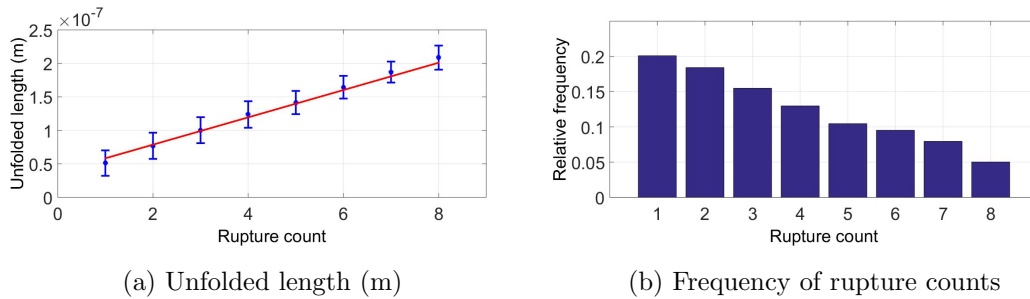


Figure 6.7: (a) Maximum unfolded length (m) as a function of rupture count, and (b) relative frequency of observed rupture counts determined from force spectroscopy experiments of titin I27O molecules.

The experimentally obtained histograms shown in Figure 6.5 and Figure 6.6 are fit with Gaussian distributions. The peaks and standard deviations obtained from the Gaussian fits are also demonstrated in the corresponding figures. A comparison of the statistics of the estimated distributions with the specifications detailed in existing literature [143,150] for a pulling speed of $1\mu\text{m/s}$ is presented in Table 6.1. The close proximity between our results with the data reported in existing literature is evident from Table 6.1. We also shared our data with the vendor of I27O protein [150] and affirmed the accuracy of our estimated parameters. This validates our AFM experimental setup and we move on to investigating the utrophin molecules.

Parameter	Estimated	Specification sheet
Persistence length	$0.30 \pm 0.05 \text{ nm}$	$0.30 \pm 0.07 \text{ nm}$
Rupture force	$222 \pm 17 \text{ pN}$	$228 \pm 29 \text{ pN}$
Peak to peak length	$25.1 \pm 0.56 \text{ nm}$	$24.1 \pm 0.34 \text{ nm}$
Increment in contour length	$27.2 \pm 1.3 \text{ nm}$	$24.1 \pm 0.34 \text{ nm}$

Table 6.1: Comparison of estimated parameters of Titin I27O molecule with specification sheet.

It should be noted in Figure 6.7a that the maximum unfolded length increases linearly as more bonds unfold. This observation is consistent with the fact that the I27O molecule is synthesized by concatenating identical I27 domains. These domains when unfold, contribute to uniform increment in the extendable length of the molecule. Again, due to the identical nature of the constituent bonds, the increments in the contour length for bond ruptures are tightly distributed around the peak value of 27.2 nm as illustrated in Figure 6.6b. Figure 6.7b represents the relative frequency of observed bond rupture counts from successful pulling experiments. It is evident from Figure 6.7b, that the number of successful pulling experiments where higher number of bonds rupture, are much lower compared to the number of experiments where at least one or two rupture events are observed.

6.3 Force spectroscopy studies on utrophin molecules

Here we present the results obtained from utrophin protein constructs. The domains in the full length utrophin protein molecule and its different deletion constructs that we investigate in this chapter are shown in Figure 6.8. As explained earlier, the full length utrophin (also referred to as Utr FL) contains two halves; the fragment that binds with actin filaments contains the N-terminus and spectrin repeats R1 to R10 (denoted by Utr NT-R10), the other fragment containing the repeats R11 to R22 and the CR-CT terminus (denoted by Utr R11-CT) connects to the dystrophin-glycoprotein complex. We study each of the constructs Utr FL, Utr NT-R10 and Utr R11-CT. It is hypothesized that the terminal domains NT and CR-CT may influence the mechanical behavior of utrophin constructs through long range intra-molecular communication. In order to verify this hypothesis, we also investigate the full length utrophin, the actin binding fragment and the dystroglycan binding fragment by removing the terminal domains which are referred to as Utr R1-22, Utr R1-10 and Utr R11-22 respectively.

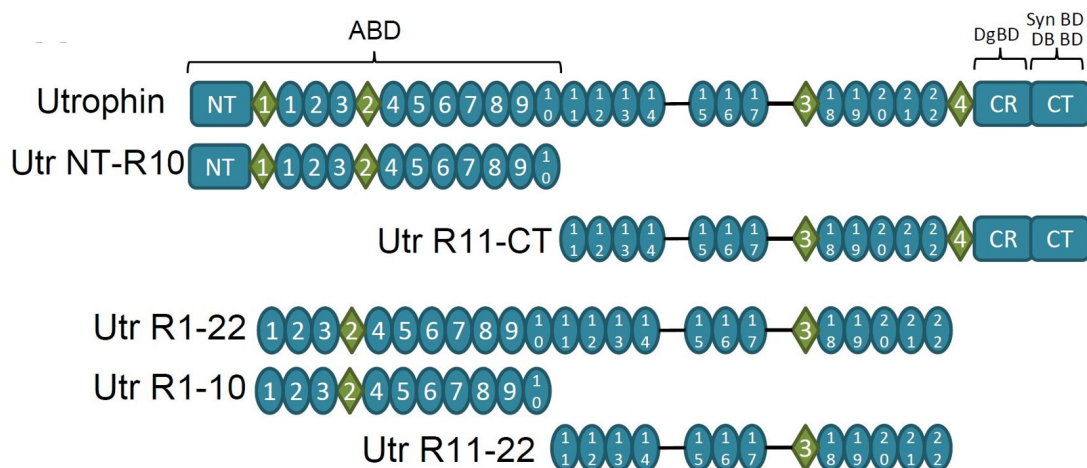


Figure 6.8: Full length utrophin and its different constructs investigated by force spectroscopy experiments. Molecular bond unfolding is also studied by thermal melting experiments.

Thermal melting experiments conducted using circular dichroism techniques reveal that the full-length and truncated utrophin constructs exhibit uniform thermal stability

from N- to C-terminus [4, 151] which is shown in Figure 6.9. From Figure 6.9, it is evident that the constituent molecular bonds unfold uniformly upon thermal excitation all across the utrophin molecule. Thus it intuitively follows that the bond unfolding events from different utrophin constructs would maintain similar trends upon externally applied force. However, our force spectroscopy studies reveal that utrophin constructs behave mechanically very differently than what we expected.

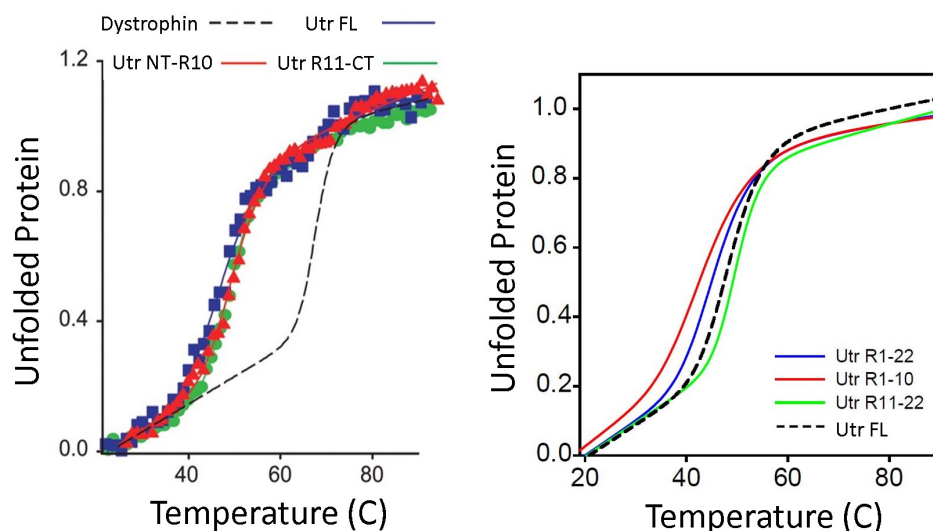
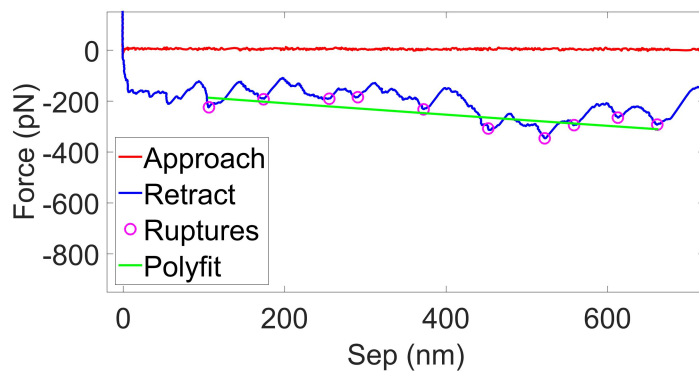
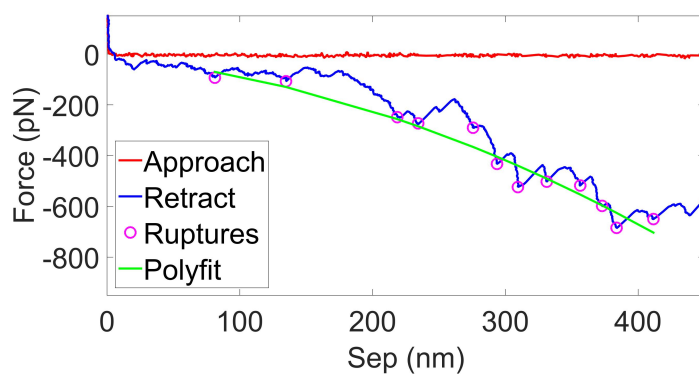


Figure 6.9: Thermal melt curves for different utrophin constructs exhibit uniform unfolding characteristics [4].

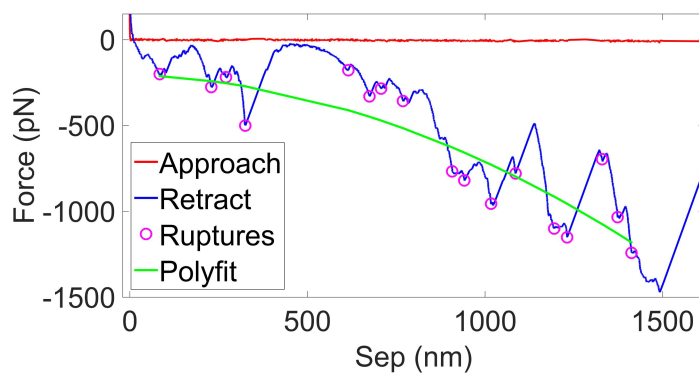
The utrophin constructs shown in Figure 6.8 are employed on freshly cleaved mica samples in phosphate buffer saline (PBS, pH 7.5) solutions. Constant speed protein pulling experiments are conducted using Olympus biolevers (long) at a pulling speed of $1 \mu\text{m/s}$. Example of force versus extension curves of the constructs Utr NT-R10, Utr R11-CT and full length utrophin (Utr FL) are shown in Figure 6.11a, Figure 6.11b and Figure 6.11c respectively. A key aspect of the mechanical behavior of Utr R11-CT evident from Figure 6.11b is that, as the bonds unfold, Utr R11-CT offers more and more restoring forces so that, the molecule behaves as a ‘stiffening spring’. In contrast, observation of Figure 6.11a, suggests that the different bond unfolding events in Utr NT-R10 occur over similar force ranges. Thus the force versus extension characteristics of Utr NT-R10 exhibits a flat trend which we refer to as the ‘brittle’ behavior.



(a) Utr NT-R10



(b) Utr R11-CT

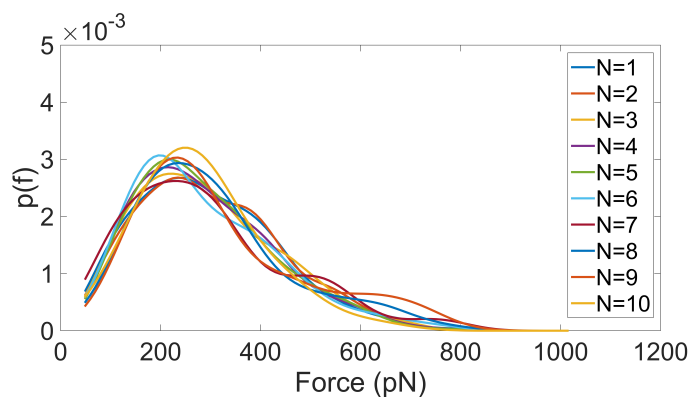


(c) Utr FL

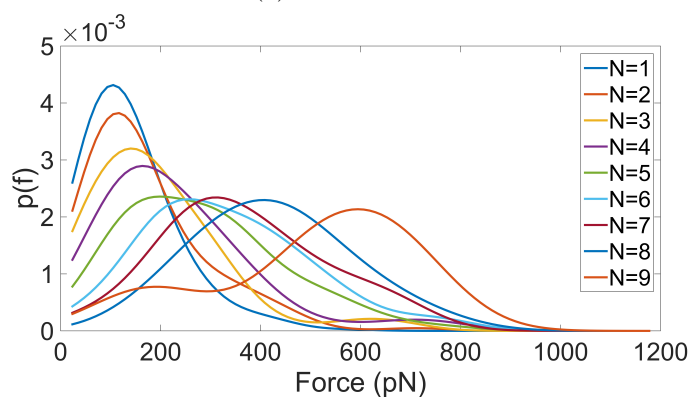
Figure 6.10: Example of force spectroscopy data obtained from (a) Utr NT-R10, (b) Utr R11-CT, and (c) Utr FL protein constructs. Data suggests 'brittle' behavior of Utr NT-R10 versus 'stiffening spring' like behavior of Utr R11-CT and Utr FL constructs.

The full length molecule Utr FL behaves mechanically as a stiffening spring (see Figure 6.11c) which is similar to the trend observed in Utr R11-CT (see Figure 6.11b), however, much larger unfolding forces are observed in Utr FL when more number of bonds unfold. Thus the overall resistive force that Utr FL can withstand is much higher than either Utr NT-R10 or Utr R11-CT can.

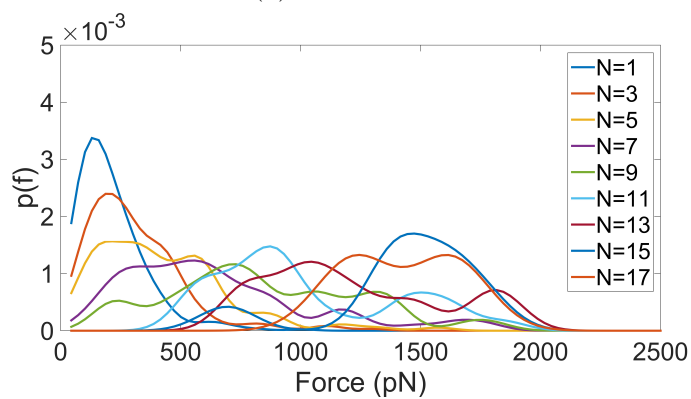
In order to substantiate these observations statistically, we collected data from all the successful pulling experiments and estimated the probability distribution of the unfolding forces for each of the fragments as a function of the index of unfolding events. Here, we define the index of unfolding event, denoted by N , as the N^{th} bond unfolding that occurs in pulling experiments. Figure 6.11a, Figure 6.11b and Figure 6.11c demonstrate the estimated probability distributions of the unfolding forces determined, separately for each N , for the constructs Utr NT-R10, Utr R11-CT and Utr FL respectively. The estimated force distributions further validate our initial observation of the ‘brittle’ versus ‘stiffening spring’ behavior of the utrophin fragments. The unfolding force distributions at different values of N for Utr NT-R10 in Figure 6.11a overlap on each other. Thus the peaks in the distribution of unfolding forces observed in Figure 6.11a are similar. Besides, from Figure 6.11a, it is observed that the widths of the distributions are uniform as well. Thus, irrespective of the index of bond unfolding, the uncertainties in the unfolding forces are similar. In contrast, the force distributions observed in Figure 6.11b shift towards higher forces for higher values of N . The peaks forces in the distributions increase consistently as N increases (see Figure 6.11b). Besides, for Utr R11-CT, the width of the distribution also increases with N , indicating that for higher unfolding events, there is more uncertainty in the unfolding forces that are observed. The distribution of unfolding forces for full length molecule Utr FL is demonstrated in Figure 6.11c. Evidently, the shift of distributions towards higher forces with increasing N suggests that the stiffening spring-like behavior, similar to Utr R11-CT, is followed by Utr FL. However, the distributions corresponding to the higher values of N range over much higher forces that can not be observed in either Utr NT-R10 or Utr R11-CT. Our findings are particularly surprising given the fact that Utr NT-R10, Utr R11-CT and Utr FL behave almost identically under thermal melting experiments illustrated in Figure 6.9.



(a) Utr NT-R10

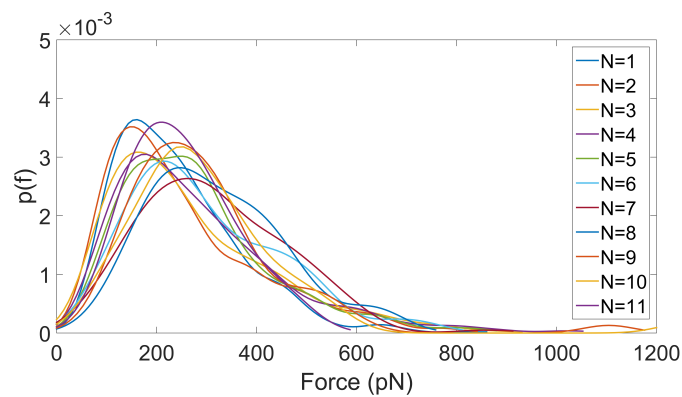


(b) Utr R11-CT

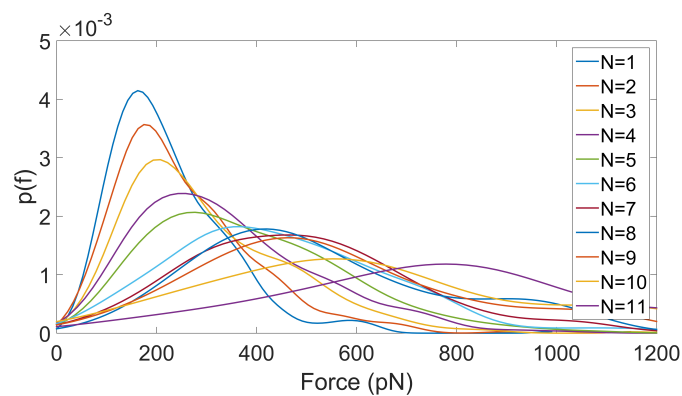


(c) Utr FL

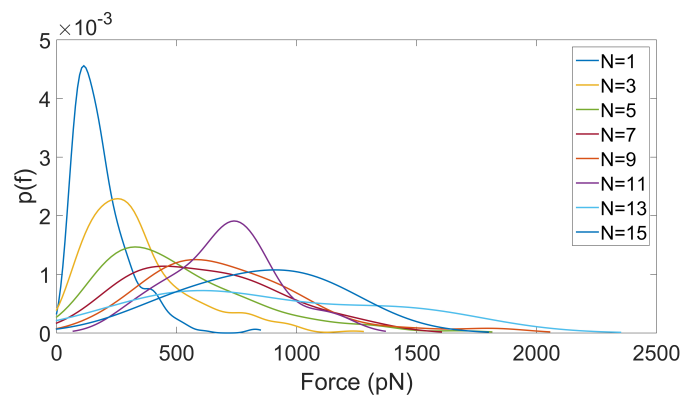
Figure 6.11: Estimated probability distribution of unfolding forces for (a) Utr NT-R10, (b) Utr R11-CT, and (c) Utr FL from constant speed pulling experiments.



(a) Utr R1-R10



(b) Utr R11-R22



(c) Utr R1-R22

Figure 6.12: Estimated distribution of unfolding forces for (a) Utr R1-R10, (b) Utr R11-R22, and (c) Utr R1-R22 from constant speed pulling experiments. Removing the terminal domains NT and CR-CT from the constructs do not alter their ‘brittle’ or ‘stiffening spring’-like characteristics.

Since, both Utr NT-R10 and Utr R11-CT contain structurally similar spectrin type repeats except the terminal domains NT and CR-CT, we hypothesized that the terminal domains might dictate the dissimilar mechanical behaviors of these constructs. Thus, we also investigated unfolding forces for the constructs Utr R1-10, Utr R11-22 and Utr R1-22 which do not contain the terminal domains NT and CR-CT. From Figure 6.12a, Figure 6.12b and Figure 6.12c, it is evident that removing the terminal domains do not influence the characteristic brittle or stiffening spring-like behaviors of the protein constructs. Hence we remove the possibility that the termini NT or CR-CT influence the mechanical unfolding behavior through any long range intra-protein communication. For convenience in comparison, the peak unfolding forces in the estimated distributions for all the fragments are shown in Figure 6.13 as a function of the index of rupture event, N . In comparison to previously reported unfolding forces for homologous proteins comprised of spectrin like repeats [140,141,144–146,152], our studies indicate much higher unfolding forces. Thus our findings necessitate deeper investigation of possible reasons and analytical models that can explain such radically different mechanical unfolding characteristics of protein constructs that otherwise exhibit uniform unfolding under thermal excitation.

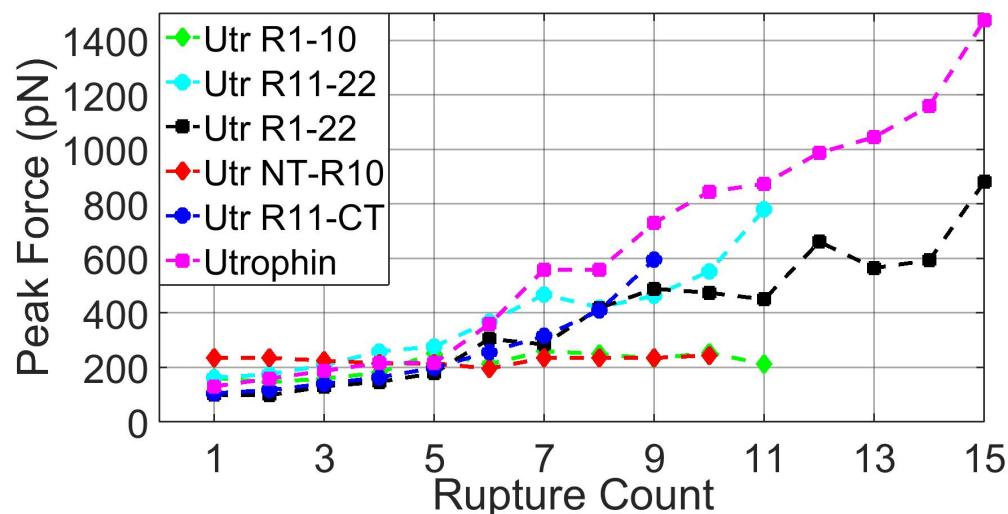


Figure 6.13: Peak unfolding forces as a function of the index of rupture event for the different utrophin constructs.

6.4 Conclusions

In this chapter we report the first ever mechanical characterization of full length utrophin and its relevant terminal fragments with experiments conducted using AFM. The experimental setup is validated by experiments conducted on the well studied I27O titin constructs. From our investigation of utrophin constructs, we observe much higher unfolding forces compared to what are reported for similar proteins with spectrin like repeats. Further, our studies reveal some surprising facts about the mechanical behavior of these protein constructs, particularly, the heterogeneity that these constructs exhibit under externally applied force while their unfolding behaviors under thermal excitation follow a uniform trend. The possibility of influence of terminal domains over the mechanical behavior of these constructs is also investigated. Results suggest that the terminals do not dictate the characteristic brittle or stiffening spring-like behaviors exhibited by the actin-binding and the dystroglycan-binding halves of utrophin constructs. A deeper understanding and theoretical modeling are necessary to predict the observed phenomena. Further investigation of the mechanical behavior of full length dystrophin and its different deletion constructs is needed to acquire better comprehension of the molecular unfolding events. Moreover, our results emphasize the significance of force spectroscopy studies of protein molecules which play important mechanical roles in the human body.

Chapter 7

Conclusion and Discussion

A diverse set of problems related to atomic force microscopy is addressed in this thesis mostly utilizing tools from signal processing and systems theory. The topics include determining confidence measures on the measurement data of atomic force microscope, quantitatively estimating mechanical properties of materials using dynamic mode AFMs, development of novel algorithms for probe based data storage, and also studying protein molecules.

Chapter 2 develops measures for fidelity applicable to images obtained using a fast dynamic mode atomic force microscopy method. A comparison of different techniques namely the LMP, GLRT and MGLRT methods for fast detection of topography is presented. LMP is observed to be better performing because the false alarm rate and probability of detection is less sensitive to selection of threshold. However, LMP and also GLRT require synchronization of the decision window with observation data which is difficult to achieve in experimental setups. Thus the developed algorithm, MGLRT, is better suited for practical applications because it is less sensitive to synchronization. Closed form analytical results are derived to measure the fidelity of decisions arising from LMP, GLRT and MGLRT methods. The fidelity measuring algorithm is well suited for real time applications.

Chapter 3 deals with the means for quantitative estimation of material properties such as stiffness and dissipation with nanometer resolution. Such material characterization is an important prerequisite to fulfill the promise of nano-scale material design. A

simple steady state based method referred to as SEEP is reported which estimates material stiffness-dissipative properties using the dynamic mode of AFM operation. This method employs available signals, namely the amplitude and phase used in the standard dynamic mode operation where the external excitation to the cantilever need not be altered. Another technique, referred to as the REEP method is demonstrated to be capable of estimation during transient phase of cantilever oscillation. Thus REEP offers higher bandwidth of estimation than SEEP method. However, REEP requires complex hardware implementation and careful selection of excitation signals at multiple frequencies. Chapter 3 also provides a comparison of independent approaches for estimating the storage and dissipative power components due to interaction between cantilever tip and sample. Selection of appropriate system model and reference position for interpretation of the AFM data is discussed with verification through experiments. The step by step procedure for selection of system model, choice of reference position and incorporation of correction terms in the phase signal can be used as recipe by AFM users for investigating sample properties with dynamic mode AFM.

In Chapter 4 we report on embedded implementation of the real-time system identification algorithm (referred to as the BCEWRLS algorithm) employed for the REEP technique described in Chapter 3. We choose Xilinx Virtex-6 generation FPGAs as the host platforms to develop the system identification module. The design methodology explained in Chapter 4 is conveniently applicable to development of a diverse set of signal processing systems. The reported module is user friendly which can be interfaced with AFMs to estimate surface mechanical properties of materials.

In Chapter 5, a switching-model detection strategy named ISM is developed for a system that switches between two models. The concept of utilizing the sign-inverting test signals for ISM is developed. A MAP based detection algorithm from existing literature is briefly outlined and compared with ISM. Further, employment of maximum likelihood sequence detection (MLSD) techniques is proposed which improves the detection performance in presence of fast plant switching by compensating for the inter-symbol-interference. In the application part, the dynamic mode AFM based fast topography detection system is cast as a binary switched system. The switched models are firstly identified with BCEWRLS algorithm (outlined in Chapter 3) followed by usage of detection algorithms MAP and ISM. The results indicate the superior speed of

operation and the steady-state value advantages of plant model detection approach over the conventional AFM imaging. The simulation and experimental results also suggest that ISM is simpler to implement yet performs better than recursive MAP. Furthermore, the robustness of ISM against the plant uncertainties, i.e., the situation when the observers are not accurately matched to the plant models and noise is present is borne out by thorough experimental data. A combination of ISM with MLSD, referred to as the ISM-MLSD technique is demonstrated to achieve the best bit detection performance compared to previously reported methods for probe based data storage applications.

Chapter 6 deals with studying protein molecules using AFM based force spectroscopy methods. Patients with the disease Duchenne muscular dystrophy (DMD) lack the protein dystrophin. The fetal homologue of dystrophin, utrophin, is both structurally and functionally similar to dystrophin and is under active investigation as a therapeutic replacement for dystrophin in patients with DMD. In Chapter 6 we report the first ever mechanical characterization of full-length utrophin and functionally relevant terminal fragments using atomic force microscopy (AFM). We observe markedly different mechanical characteristics for constructs where previous thermodynamic studies measured identical thermal denaturation profiles. Our observations suggest that the terminal fragment of utrophin which binds actin filaments exhibit a ‘brittle’ behavior under externally applied force. However, the other fragment which connects to the dystroglycan complex behave mechanically as a ‘stiffening spring’. We also verify our AFM experimental setup by investigating the well studied protein construct titin I27O and matching data with existing literature.

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Appendix A

A.1 Derivation of steady state estimation of equivalent parameters (SEEP)

Remark 1 *This step guides selection of a reference position. Perform one approach-retract (force curve) experiment on the sample in the contact mode. From the force curve experiment, choose a set-point deflection of the cantilever such that when the cantilever is engaged in contact mode, it resides at the beginning of the repulsive region of tip-sample interaction. Engage the cantilever in contact mode with the selected set-point deflection. Now retract the cantilever base by a distance which is just larger than the amplitude of oscillations that will be chosen at the reference position. Typical amplitude of oscillations can be chosen ranging from 40 nm to 150 nm. For example, choose 100 nm to be the amplitude of cantilever oscillations at reference. Then the cantilever base can be retracted by 120 nm from the engaged position in the contact mode. Choose this retracted position as the reference position.*

Suppose that the frequency response of the cantilever $G_r(j\omega)$ at the reference position is known. Note that if $|U(j\omega_d)|$ denotes the drive amplitude and $|Y_r(j\omega_d)|$ denotes the *steady state* amplitude of the cantilever oscillating sinusoidally at frequency ω_d then $|G_r(j\omega_d)| = \frac{|Y_r(j\omega_d)|}{|U(j\omega_d)|}$. The phase of $G_r(j\omega_d)$ can be determined by evaluating the phase difference between the cantilever deflection and the drive signal given by $\angle Y_r(j\omega_d) - \angle U(j\omega_d)$. Thus the following can be evaluated experimentally at the reference

position:

$$\left| \frac{Y_r(j\omega_d)}{U(j\omega_d)} \right| = |G_r(j\omega_d)| = \frac{\sqrt{\alpha_2^2 + \alpha_1^2 \omega_d^2}}{\sqrt{(\omega_r^2 - \omega_d^2)^2 + \frac{\omega_d^2 \omega_r^2}{Q_r^2}}}, \quad (\text{A.1})$$

$$\begin{aligned} (\angle Y_r(j\omega_d) - \angle U(j\omega_d)) &= \angle G_r(j\omega_d) = \arctan\left(\frac{\alpha_1 \omega_d}{\alpha_2}\right) \\ &\quad - \arctan\left(\frac{\omega_d \omega_r}{Q_r(\omega_r^2 - \omega_d^2)}\right). \end{aligned} \quad (\text{A.2})$$

Here for each new experiment it is assumed that the gain and phase of $G_r(j\omega_d)$ at reference are determined.

The equivalent model for oscillating cantilever interacting with the sample is governed by the dynamics $G_{eq}(s) = (\alpha_1 s + \alpha_2)/(s^2 + \frac{\omega_{eq}}{Q_{eq}}s + \omega_{eq}^2)$, where we have assumed that the numerator of G_{eq} and G_r remain the same throughout the rest of the imaging process. It follows that

$$\left| \frac{Y_m(j\omega_d)}{U(j\omega_d)} \right| = |G_{eq}(j\omega_d)| = \frac{\sqrt{\alpha_2^2 + \alpha_1^2 \omega_d^2}}{\sqrt{(\omega_{eq}^2 - \omega_d^2)^2 + \frac{\omega_d^2 \omega_{eq}^2}{Q_{eq}^2}}}, \quad (\text{A.3})$$

$$\begin{aligned} \angle G_{eq}(j\omega_d) &= (\angle Y_m(j\omega_d) - \angle U(j\omega_d)) \\ &= \arctan\left(\frac{\alpha_1 \omega_d}{\alpha_2}\right) - \arctan\left(\frac{\omega_d \omega_{eq}}{Q_{eq}(\omega_{eq}^2 - \omega_d^2)}\right), \end{aligned} \quad (\text{A.4})$$

where $|Y_m(j\omega_d)|$ and $\angle Y_m(j\omega_d)$ denote the amplitude and phase of measured deflection of the cantilever.

Thus taking the ratio $\frac{|G_{eq}(j\omega_d)|}{|G_r(j\omega_d)|}$, it follows from (A.1) and (A.3) that

$$\left| \frac{Y_r(j\omega_d)}{Y_m(j\omega_d)} \right| = \sqrt{\frac{(\omega_{eq}^2 - \omega_d^2)^2 + \omega_d^2 \omega_{eq}^2 / Q_{eq}^2}{(\omega_r^2 - \omega_d^2)^2 + \omega_d^2 \omega_r^2 / Q_r^2}}. \quad (\text{A.5})$$

In the dynamic mode of operation the phase difference between the input excitation and output deflection oscillations θ is measured experimentally given by $\theta = (\angle U(j\omega_d) - \angle Y_m(j\omega_d))$. Let $\theta_c := \arctan(\alpha_1 \omega_d / \alpha_2)$ with $\tilde{\theta} = (\theta + \theta_c)$. From (A.4) it follows that

$$\tan(\tilde{\theta}) = \frac{\omega_{eq} \omega_d}{Q_{eq}(\omega_{eq}^2 - \omega_d^2)}. \quad (\text{A.6})$$

Using the identity $\sec(\tilde{\theta}) = \sqrt{1 + \tan^2(\tilde{\theta})}$ it follows that

$$\sec(\tilde{\theta}) = \frac{\sqrt{(\omega_{eq}^2 - \omega_d^2)^2 + \omega_{eq}^2 \omega_d^2 / Q_{eq}^2}}{(\omega_{eq}^2 - \omega_d^2)}. \quad (\text{A.7})$$

Hence, from (A.5) and (A.7) we have

$$\begin{aligned} \left| \frac{Y_r(j\omega_d)}{Y_m(j\omega_d)} \right| &= \frac{\sec(\tilde{\theta})(\omega_{eq}^2 - \omega_d^2)}{\sqrt{(\omega_r^2 - \omega_d^2)^2 + \omega_r^2 \omega_d^2 / Q_r^2}}, \\ \omega_{eq} &= \sqrt{\omega_d^2 + \frac{a_r}{a} \cos(\tilde{\theta}) \sqrt{(\omega_r^2 - \omega_d^2)^2 + \frac{\omega_r^2 \omega_d^2}{Q_r^2}}}, \end{aligned} \quad (\text{A.8})$$

where $a_r = |Y_r(j\omega_d)|$ and $a = |Y_m(j\omega_d)|$. Further, from (A.6) it follows that

$$Q_{eq} = \frac{\omega_d \omega_{eq}}{(\omega_{eq}^2 - \omega_d^2) \tan \tilde{\theta}}. \quad (\text{A.9})$$

Thus ω_{eq} and Q_{eq} can be estimated experimentally using (A.8) and (A.9) which are referred to as ω_{seep} and Q_{seep} respectively. The steps for determining equivalent parameters using the approach above is summarized in Algorithm 1.

An alternative method for evaluating θ_c when (α_1, α_2) are not exactly known is discussed now. It can be seen that the peak frequency ω_p where the magnitude $|G_r(j\omega)|$ attains the maximum satisfies the equation

$$\frac{2\omega_p^2 + \omega_r^2 \left(\frac{1}{Q_r^2} - 2 \right)}{(\omega_r^2 - \omega_p^2)^2 + \frac{\omega_p^2 \omega_r^2}{Q_r^2}} = \frac{\alpha_1^2}{\omega_p^2 \alpha_1^2 + \alpha_2^2}. \quad (\text{A.10})$$

It is experimentally observed that α_1 has a significantly smaller magnitude than α_2 and the right hand side (r.h.s) of (A.10) can be approximated to be zero. For sufficiently large values of Q_r which is the case for most of high Q cantilevers, $(\frac{1}{Q_r^2} - 2) \approx -2$. Thus from (A.10), with r.h.s set to zero, the peak frequency $\omega_p \approx \omega_r$. This peak frequency can be found by applying a frequency sweep method. Also, by replacing $\omega_{eq} = \omega_r$ and $Q_{eq} = Q_r$ in (A.9) for the reference position we have $\tan \tilde{\theta} = \frac{\omega_d \omega_r}{(\omega_r^2 - \omega_d^2)/Q_r}$. Thus for drive frequency $\omega_d = \omega_r$ at the reference position, $\tilde{\theta} = \arctan(\infty) = 90$ degrees and $\theta = 90 - \theta_c$. Hence at resonance the departure of the measured value of θ from 90 degrees can be interpreted as θ_c . Since α_1 and α_2 are assumed to be fixed during experiment, θ_c remains constant as well. Subsequently when θ_c is known, it can be added to experimentally measured θ to calculate $\tilde{\theta}$ during imaging. Thus, all the terms in the r.h.s of (A.8) are known and ω_{eq} can be analytically estimated. Further, Q_{eq} is evaluated using (A.9). With these assumptions the simplified algorithm is described as Algorithm 2.

A.2 Recursive update equations for BCEWRLS algorithm

Let $\hat{\Gamma}_{LS}$ and $\hat{\Gamma}_c$ denote the least square and bias compensated estimates of the unknown parameter vector Γ shown in (3.9). As outlined in Section 3.3.2, $\hat{\Gamma}_{LS}$ contains bias due to colored noise $e(k)$ in (3.9). Thus, additional steps are required to remove the bias and generate $\hat{\Gamma}_c$. $\lambda \in (0, 1)$ denotes the forgetting factor in the estimation problem (3.10). The set of recursive update equations for bias compensated and exponentially weighted recursive least squares (BCEWRLS) technique [45] which evaluates $\hat{\Gamma}_c$ can be summarized as:

$$\begin{aligned} \hat{\Gamma}_{LS}(k) = & \hat{\Gamma}_{LS}(k-1) \\ & + P(k)\phi(k) \left[y(k) - \phi^T(k)\hat{\Gamma}_{LS}(k-1) \right], \end{aligned} \quad (\text{A.11})$$

$$P(k) = \frac{1}{\lambda} \left(P(k-1) - \frac{P(k-1)\phi(k)\phi^T(k)P(k-1)}{\lambda + \phi^T(k)P(k-1)\phi(k)} \right), \quad (\text{A.12})$$

$$J(k) = \lambda \left(J(k-1) + \frac{\left[y(k) - \phi^T(k)\hat{\Gamma}_{LS}(k-1) \right]^2}{\lambda + \phi^T(k)P(k-1)\phi(k)} \right), \quad (\text{A.13})$$

$$\hat{\sigma}^2(k) = \frac{\left(\frac{1-\lambda}{1-\lambda^k} \right) J(k)}{1 + \hat{\Gamma}_C(k)\Lambda\hat{\Gamma}_{LS}(k)}, \quad (\text{A.14})$$

$$\hat{\Gamma}_C(k) = \hat{\Gamma}_{LS}(k) + \hat{\sigma}^2(k) \left(\frac{1-\lambda^k}{1-\lambda} \right) P(k)\Lambda\hat{\Gamma}_C(k-1), \quad (\text{A.15})$$

where k denotes the index of recursive update. The initial conditions are set by $\hat{\Gamma}_{LS}(0) = \mathbf{0}$, $P(0) = P_0I$, $J(0) = 0$, and $\hat{\Gamma}_C(0) = \mathbf{0}$. P_0 is chosen to be a big number (example, $P_0 = 1e6$). $\Lambda = \begin{bmatrix} I & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{bmatrix}$, where I is an identity matrix of size $n \times n$, and n defines the number of output delays in the SISO dynamics shown in (3.8) (For our case, $n=2$). $\hat{\sigma}^2(k)$ represents an estimate of the variance of the measurement noise, ζ , in (3.8). Convergence speed and accuracy of $\hat{\Gamma}_c$ depend on appropriate tuning of λ which is discussed in Section 4.3.1 of Chapter 4.

A.3 Transfer function from $[x(t)^T, g(t)^T]^T$ to

$$[e_{dif}(t), e_{sum}(t)]^T$$

The system specified by (5.16) and (5.17) is driven by the signal input $[x(t)^T, g(t)^T]^T$ whereas $\zeta(t)$ govern the noise in the dynamics. Using the linearity property, the contribution due to signal and noise can be separately calculated. The signal component due to the input $[x(t)^T, g(t)^T]^T$ is evaluated first. Putting $i = 1$ for model 1 and ignoring $\eta(t)$, $\zeta(t)$ it follows that

$$\begin{aligned} \begin{bmatrix} \dot{z}_1(t) \\ \dot{z}_2(t) \end{bmatrix} &= \underbrace{\begin{bmatrix} M_1 & M_2 \\ M_2 & M_1 \end{bmatrix}}_{A_{big1}} \begin{bmatrix} z_1(t) \\ z_2(t) \end{bmatrix} \\ &+ \underbrace{\begin{bmatrix} A_{m1} & B_{m1} \\ -A_{m1} & -B_{m1} \end{bmatrix}}_{B_{big1}} \begin{bmatrix} x(t) \\ g(t) \end{bmatrix}, \end{aligned} \quad (A.16)$$

$$\begin{aligned} \begin{bmatrix} e_{dif}(t) \\ e_{sum}(t) \end{bmatrix} &= \underbrace{\begin{bmatrix} N_1 & N_2 \\ N_2 & N_1 \end{bmatrix}}_{C_{big1}} \begin{bmatrix} z_1(t) \\ z_2(t) \end{bmatrix} \\ &+ \underbrace{\begin{bmatrix} C_{m1} & D_{m1} \\ -C_{m1} & -D_{m1} \end{bmatrix}}_{D_{big1}} \begin{bmatrix} x(t) \\ g(t) \end{bmatrix}. \end{aligned} \quad (A.17)$$

The transfer function from $[x(t)^T, g(t)^T]^T$ to $[e_{dif}, e_{sum}]^T$ is given by

$$\mathcal{G}_1(s) = C_{big1}(sI - A_{big1})^{-1}B_{big1} + D_{big1}. \quad (A.18)$$

It follows that with invertible matrices U and W the following identity is satisfied

$$\begin{bmatrix} U & W \\ W & U \end{bmatrix}^{-1} = \begin{bmatrix} (UW^{-1}U - W)^{-1}UW^{-1} \\ (W - UW^{-1}U)^{-1} \\ (W - UW^{-1}U)^{-1} \\ (UW^{-1}U - W)^{-1}UW^{-1} \end{bmatrix}. \quad (A.19)$$

Hence, the following equations can be written with the assumption of nonsingular $(sI - M_1)$ for all $s \in \mathbb{C}$ and nonsingular M_2

$$(sI - A_{big1}) = \begin{bmatrix} (sI - M_1) & -M_2 \\ -M_2 & (sI - M_1) \end{bmatrix},$$

$$\text{Implies, } (sI - A_{big1})^{-1} = \begin{bmatrix} \mathcal{S}_1 & \mathcal{S}_2 \\ \mathcal{S}_2 & \mathcal{S}_1 \end{bmatrix}, \quad (\text{A.20})$$

with $\mathcal{S}_1$ and $\mathcal{S}_2$ defined as

$$\mathcal{S}_1 = ((sI - M_1)M_2^{-1}(sI - M_1) - M_2)^{-1}(sI - M_1)M_2^{-1},$$

$$\mathcal{S}_2 = ((sI - M_1)M_2^{-1}(sI - M_1) - M_2)^{-1}. \quad (\text{A.21})$$

From (A.18) it follows that

$$\begin{aligned} \mathcal{G}_1(s) &= \begin{bmatrix} N_1 & N_2 \\ N_2 & N_1 \end{bmatrix} \begin{bmatrix} \mathcal{S}_1 & \mathcal{S}_2 \\ \mathcal{S}_2 & \mathcal{S}_1 \end{bmatrix} \begin{bmatrix} A_{m1} & B_{m1} \\ -A_{m1} & -B_{m1} \end{bmatrix} \\ &+ \begin{bmatrix} C_{m1} & D_{m1} \\ -C_{m1} & -D_{m1} \end{bmatrix} \\ &= \begin{bmatrix} \mathcal{G}_{m1}^{(1)}(s) & \mathcal{G}_{m1}^{(2)}(s) \\ -\mathcal{G}_{m1}^{(1)}(s) & -\mathcal{G}_{m1}^{(2)}(s) \end{bmatrix}, \end{aligned} \quad (\text{A.22})$$

where $\mathcal{G}_{m1}^{(1)}(s)$ and $\mathcal{G}_{m1}^{(2)}(s)$ are defined by

$$\mathcal{G}_{m1}^{(1)}(s) = (N_1\mathcal{S}_1 + N_2\mathcal{S}_2 - (N_1\mathcal{S}_2 + N_2\mathcal{S}_1))A_{m1} + C_{m1},$$

$$\mathcal{G}_{m1}^{(2)}(s) = (N_1\mathcal{S}_1 + N_2\mathcal{S}_2 - (N_1\mathcal{S}_2 + N_2\mathcal{S}_1))B_{m1} + D_{m1}. \quad (\text{A.23})$$

It can be observed that when the system is in model 1 the transfer functions corresponding to e_{dif} and e_{sum} have same value but opposite signs. Similarly, for model 2 the transfer function from $[x(t)^T, g(t)^T]^T$ to $[e_{dif}, e_{sum}]^T$ can be derived as:

$$\mathcal{G}_2(s) = \begin{bmatrix} \mathcal{G}_{m2}^{(1)}(s) & \mathcal{G}_{m2}^{(2)}(s) \\ \mathcal{G}_{m2}^{(1)}(s) & \mathcal{G}_{m2}^{(2)}(s) \end{bmatrix}. \quad (\text{A.24})$$

$\mathcal{G}_{m2}^{(1)}(s)$ and $\mathcal{G}_{m2}^{(2)}(s)$ are shown below

$$\mathcal{G}_{m2}^{(1)}(s) = (N_1\mathcal{S}_1 + N_2\mathcal{S}_2 + N_1\mathcal{S}_2 + N_2\mathcal{S}_1)A_{m2} + C_{m2},$$

$$\mathcal{G}_{m2}^{(2)}(s) = (N_1\mathcal{S}_1 + N_2\mathcal{S}_2 + N_1\mathcal{S}_2 + N_2\mathcal{S}_1)B_{m2} + D_{m2}. \quad (\text{A.25})$$

A.4 Computational complexity of MAP

The estimate of the computational effort required by each step of the recursive update for the MAP algorithm is derived here. Each update involves matrix multiplications and additions. Let us consider the state covariance update equation (5.10). The last term on the right hand side of equation (5.10) is $(L_{d,i}R_{d,i}L_{d,i}^T)$. Let us write $L_{d,i}^T = [v_1, v_2, \dots, v_{\mathbf{m}_A}]$ where v_j represents the j th column of $L_{d,i}^T$ matrix. Then, the (j, k) th component of the matrix $(L_{d,i}R_{d,i}L_{d,i}^T)$ is of the form $(v_j^T R_{d,i} v_k)$. When v_j and v_k are not equal, $(v_j^T R_{d,i} v_k) = \sum_{m=1}^{\mathbf{m}_C} \sum_{n=1}^{\mathbf{m}_C} v_j(m) R_{d,i}(m, n) v_k(n)$ where $v_j(m)$ is the m th element of the vector v_j and $R_{d,i}(m, n)$ is the (m, n) th component of the matrix $R_{d,i}$. Clearly, for calculating $(v_j^T R_{d,i} v_k)$ ($v_j \neq v_k$) $2\mathbf{m}_C^2$ multiplications and $(\mathbf{m}_C^2 - 1)$ additions are required. As a result, $2\mathbf{m}_C^2 + (\mathbf{m}_C^2 - 1) = 3\mathbf{m}_C^2 - 1$ flops are required. For symmetric matrix $R_{d,i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{m}_C}$ and any vector $v_j \in \mathbb{R}^{\mathbf{m}_C}$, the product of the form $(v_j^T R_{d,i} v_j)$ requires $(\frac{3}{2}\mathbf{m}_C^2 + \frac{3}{2}\mathbf{m}_C - 1)$ flop counts (see [153]). $(L_{d,i}R_{d,i}L_{d,i}^T)$ being a symmetric matrix, only $\mathbf{m}_A$ diagonal elements of the form $(v_j^T R_{d,i} v_j)$, $j = 1, 2, \dots, \mathbf{m}_A$ need to be computed each requiring $(\frac{3}{2}\mathbf{m}_C^2 + \frac{3}{2}\mathbf{m}_C - 1)$ flops. The $\mathbf{m}_A(\mathbf{m}_A - 1)/2$ off-diagonal terms need $(3\mathbf{m}_C^2 - 1)$ flops. As a result, computation of $(L_{d,i}R_{d,i}L_{d,i}^T)$ needs $(\mathbf{m}_A(\frac{3}{2}\mathbf{m}_C^2 + \frac{3}{2}\mathbf{m}_C - 1) + (\mathbf{m}_A(\mathbf{m}_A - 1)/2)(3\mathbf{m}_C^2 - 1)) = (\frac{3}{2}\mathbf{m}_A^2\mathbf{m}_C^2 + \frac{3}{2}\mathbf{m}_A\mathbf{m}_C - \frac{1}{2}\mathbf{m}_A - \frac{1}{2}\mathbf{m}_A^2) = N_{\Sigma_1}$ (say) flops. Similarly, it can be derived that the matrix product $(B_{d,i}Q_{d,i}B_{d,i}^T)$ requires $(\frac{3}{2}\mathbf{m}_A^2\mathbf{n}_B^2 + \frac{3}{2}\mathbf{m}_A\mathbf{n}_B - \frac{1}{2}\mathbf{m}_A - \frac{1}{2}\mathbf{m}_A^2) = N_{\Sigma_2}$ flops. Let us compute flops necessary to calculate $((A_{d,i} - L_{d,i}C_{d,i})\Sigma_{m/m-1,i}(A_{d,i} - L_{d,i}C_{d,i})^T)$. It is easy to verify that flop count needed for computation of $(A_{d,i} - L_{d,i}C_{d,i})$ is $\mathbf{m}_A^2 + \mathbf{m}_A^2(2\mathbf{m}_C - 1) = 2\mathbf{m}_C\mathbf{m}_A^2$. Once the matrices $(A_{d,i} - L_{d,i}C_{d,i})$ and $\Sigma_{m/m-1,i}$ are available, flop counts for the product matrix $((A_{d,i} - L_{d,i}C_{d,i})\Sigma_{m/m-1,i}(A_{d,i} - L_{d,i}C_{d,i})^T)$ can be calculated the same way it is done for $(L_{d,i}R_{d,i}L_{d,i}^T)$ and the count is $(\frac{3}{2}\mathbf{m}_A^4 + \mathbf{m}_A^2 - \frac{1}{2}\mathbf{m}_A) = N_{\Sigma_3}$. So, computation of $((A_{d,i} - L_{d,i}C_{d,i})\Sigma_{m/m-1,i}(A_{d,i} - L_{d,i}C_{d,i})^T)$ needs $N_{\Sigma_3} + (2\mathbf{m}_C\mathbf{m}_A^2)$ flops. Finally, denote the total flops necessary for evaluating the expression on the right hand side of (5.10) by N_{Σ} . Flop counts needed for adding the three symmetric component matrices $((A_{d,i} - L_{d,i}C_{d,i})\Sigma_{m/m-1,i}(A_{d,i} - L_{d,i}C_{d,i})^T)$, $(B_{d,i}Q_{d,i}B_{d,i}^T)$ and $(L_{d,i}R_{d,i}L_{d,i}^T)$ when they are available is $\mathbf{m}_A(\mathbf{m}_A + 1)$. The matrices $(A_{d,i} - L_{d,i}C_{d,i})$, $(B_{d,i}Q_{d,i}B_{d,i}^T)$ and $(L_{d,i}R_{d,i}L_{d,i}^T)$ are time invariant and need to be computed only once for the first instance of the recursive update. Clearly, for the first recursive update $N_{\Sigma} = N_{\Sigma_1} +$

$N_{\Sigma_2} + N_{\Sigma_3} + (2\mathbf{m}_C \mathbf{m}_A^2) + \mathbf{m}_A(\mathbf{m}_A + 1)$. From the second recursive stage onwards, the matrices $(B_{d,i}Q_{d,i}B_{d,i}^T + L_{d,i}R_{d,i}L_{d,i}^T)$ and $(A_{d,i} - L_{d,i}C_{d,i})$ are already available implying $N_{\Sigma} = N_{\Sigma_3} + \mathbf{m}_A(\mathbf{m}_A + 1)/2$. As a result

$$N_{\Sigma} = \begin{cases} \left(\frac{3}{2}\mathbf{m}_A^4 + \mathbf{m}_A^2 \left(\frac{3}{2}(\mathbf{m}_C^2 + \mathbf{n}_B^2) + 2\mathbf{m}_C + 1 \right) \right. \\ \left. + \mathbf{m}_A \left(\frac{3}{2}(\mathbf{m}_C + \mathbf{n}_B) - \frac{1}{2} \right) \right), \text{ for first update} \\ \frac{3}{2}(\mathbf{m}_A^4 + \mathbf{m}_A^2), \text{ for subsequent updates} \end{cases} \quad (\text{A.26})$$

Let N_{Ω} denote the flop counts necessary per update for (5.11) and it can be easily shown to be

$$N_{\Omega} = \frac{3}{2}(\mathbf{m}_C^2 \mathbf{m}_A^2 + \mathbf{m}_C \mathbf{m}_A). \quad (\text{A.27})$$

Finally, let us compute flop counts necessary for the recursive probability calculation (5.9) and denote it by N_p . Since, the matrix $\Omega_{m/i}$ is a covariance matrix it is positive semidefinite. Further, assume that $\Omega_{m/i}$ is invertible and hence it is positive definite. A widely accepted and convenient way to calculate the determinant and inverse of positive definite matrices is to use the Cholesky decomposition. Let the Cholesky decomposition of the positive definite $\Omega_{m/i} \in \mathbb{R}^{\mathbf{m}_C, \mathbf{m}_C}$ matrix be given by $\Omega_{m/i} = L_{\Omega} L_{\Omega}^T$ where L_{Ω} is lower triangular and a standard algorithm for the Cholesky factorization requires $(\frac{1}{3}\mathbf{m}_C^3 + \frac{1}{2}\mathbf{m}_C^2 + \frac{1}{6}\mathbf{m}_C)$ flops (see [153]). Then, $|\Omega_{m/i}|^{-\frac{1}{2}} = |L_{\Omega}|^{-1}$. Since, L_{Ω} is lower triangular, $|L_{\Omega}|$ can be computed by multiplying its diagonal elements needing $(\mathbf{m}_C - 1)$ multiplications. So, when the matrix L_{Ω} is available then $|\Omega_{m/i}|^{-\frac{1}{2}}$ needs $(\mathbf{m}_C - 1 + 1) = \mathbf{m}_C$ flops. Now, $\Omega_{m/i}^{-1} = L_{\Omega}^{-T} L_{\Omega}^{-1}$. Inversion of lower triangular L_{Ω} needs $(\frac{1}{3}\mathbf{m}_C^3 + \frac{2}{3}\mathbf{m}_C)$ flops and computing the product $L_{\Omega}^{-T} L_{\Omega}^{-1}$ needs $(\frac{1}{3}\mathbf{m}_C^3 + \frac{1}{2}\mathbf{m}_C^2 + \frac{1}{6}\mathbf{m}_C)$ flops [153]. So, $|\Omega_{m/i}|^{-\frac{1}{2}}$ and $\Omega_{m/i}^{-1}$ can be computed using $(\frac{1}{3}\mathbf{m}_C^3 + \frac{1}{2}\mathbf{m}_C^2 + \frac{1}{6}\mathbf{m}_C) + (\mathbf{m}_C) + (\frac{1}{3}\mathbf{m}_C^3 + \frac{2}{3}\mathbf{m}_C) + (\frac{1}{3}\mathbf{m}_C^3 + \frac{1}{2}\mathbf{m}_C^2 + \frac{1}{6}\mathbf{m}_C) = (\mathbf{m}_C^3 + \mathbf{m}_C^2 + 2\mathbf{m}_C)$ flops. Evaluation of $(-\frac{1}{2}\tilde{\mathbf{y}}_{m,i}^T \Omega_{m/i}^{-1} \tilde{\mathbf{y}}_{m,i})$ needs $1 + (\frac{3}{2}\mathbf{m}_C^2 + \frac{3}{2}\mathbf{m}_C - 1) = \frac{3}{2}(\mathbf{m}_C^2 + \mathbf{m}_C)$ flops when $\Omega_{m/i}^{-1}$ and $\tilde{\mathbf{y}}_{m,i}$ are available. As a result, total number of flops required for computation of $p(\theta_i | \mathcal{Y}_m)$ is given by

$$\begin{aligned} N_p &= (\mathbf{m}_C^3 + \mathbf{m}_C^2 + 2\mathbf{m}_C) + \frac{3}{2}(\mathbf{m}_C^2 + \mathbf{m}_C) + 3 + N_{exp} \\ &= \mathbf{m}_C^3 + \frac{5}{2}\mathbf{m}_C^2 + \frac{7}{2}\mathbf{m}_C + 3 + N_{exp}. \end{aligned} \quad (\text{A.28})$$

Here, N_{exp} is the flop counts required to compute $\exp(\cdot)$ operation. N_{exp} may vary depending on the algorithm used to implement $\exp(\cdot)$. However, for simplicity assume that $N_{exp} = 1$. So, from (A.28) it follows that

$$N_p = \mathfrak{m}_C^3 + \frac{5}{2}\mathfrak{m}_C^2 + \frac{7}{2}\mathfrak{m}_C + 4. \quad (\text{A.29})$$

Since, the plant involved is assumed to be binary model switching, it is necessary to evaluate a posteriori probabilities for two models ($i \in 1, 2$). Which implies that total number of flop counts per recursive update necessary for binary plant detection using MAP is $2(N_\Sigma + N_\Omega + N_p)$.