

Contents

Preface *XIII*

List of Contributors *XV*

Part I **General Atomic Force Microscopy** *1*

1	AFM: Basic Concepts	3
	<i>Fernando Moreno-Herrero and Julio Gomez-Herrero</i>	
1.1	Atomic Force Microscope: Principles	3
1.2	Piezoelectric Scanners	5
1.2.1	Piezoelectric Scanners for Imaging in Liquids	8
1.3	Tips and Cantilevers	8
1.3.1	Cantilever Calibration	10
1.3.2	Tips and Cantilevers for Imaging in Liquids	11
1.3.3	Cantilever Dynamics in Liquids	13
1.4	Force Detection Methods for Imaging in Liquids	15
1.4.1	Piezoelectric Cantilevers and Tuning Forks	15
1.4.2	Laser Beam Deflection Method	17
1.4.2.1	Liquid Cells and Beam Deflection	18
1.5	AFM Operation Modes: Contact, Jumping/Pulsed, Dynamic	19
1.5.1	Contact Mode	19
1.5.2	Jumping and Pulsed Force Mode	20
1.5.3	Dynamic Modes	22
1.5.3.1	Liquid Cells and Dynamic Modes	23
1.6	The Feedback Loop	24
1.7	Image Representation	25
1.8	Artifacts and Resolution Limits	28
1.8.1	Artifacts Related to the Geometry of the Tip	28
1.8.2	Artifacts Related to the Feedback Loop	30
1.8.3	Resolution Limits	31
	Acknowledgments	32
	References	32

2	Carbon Nanotube Tips in Atomic Force Microscopy with Applications to Imaging in Liquid	35
	<i>Edward D. de Asis, Jr., Joseph Leung, and Cattien V. Nguyen</i>	
2.1	Introduction	35
2.2	Fabrication of CNT AFM Probes	37
2.2.1	Mechanical Attachment	38
2.2.2	CNT Attachment Techniques Employing Magnetic and Electric Fields	39
2.2.3	Direct Growth of CNT Tips	41
2.2.4	Emerging CNT Attachment Techniques	43
2.2.5	Postfabrication Modification of the CNT Tip	43
2.2.5.1	Shortening	43
2.2.5.2	Coating with Metal	44
2.3	Chemical Functionalization	44
2.3.1	Functionalization of the CNT Free End	45
2.3.2	Coating the CNT Sidewall	45
2.4	Mechanical Properties of CNTs in Relation to AFM Applications	46
2.4.1	CNT Atomic Structure	47
2.4.2	Mechanical Properties of CNT AFM Tips	49
2.5	Dynamics of CNT Tips in Liquid	50
2.5.1	Interaction of Microfabricated AFM Tips and Cantilevers in Liquid	50
2.5.2	CNT AFM Tips in Liquid	52
2.5.3	Interaction of CNT with Liquids	52
2.5.3.1	CNT Tips at the Air–Liquid Interface During Approach	54
2.5.3.2	CNT Tips at the Liquid–Solid Interface	56
2.5.3.3	CNT Tips at the Air–Liquid Interface during Withdrawal	58
2.6	Performance and Resolution of CNT Tips in Liquid	58
2.6.1	Performance of CNT AFM Tips When Imaging in Liquid	58
2.6.2	Biological Imaging in Liquid Medium with CNT AFM Tips	59
2.6.3	Cell Membrane Penetration and Applications of Intracellular CNT AFM Probes	60
	References	61
3	Force Spectroscopy	65
	<i>Arturo M. Baró</i>	
3.1	Introduction	65
3.2	Measurement of Force Curves	67
3.2.1	Analysis of Force Curves Taken in Air	68
3.2.2	Analysis of Force Curves in a Liquid	70
3.3	Measuring Surface Forces by the Surface Force Apparatus	70
3.4	Forces between Macroscopic Bodies	71
3.5	Theory of DLVO Forces between Two Surfaces	71
3.6	Van der Waals Forces – the Hamaker Constant	72
3.7	Electrostatic Force between Surfaces in a Liquid	72

3.8	Spatially Resolved Force Spectroscopy	76
3.9	Force Spectroscopy Imaging of Single DNA Molecules	78
3.10	Solvation Forces	79
3.11	Hydrophobic Forces	81
3.12	Steric Forces	81
3.13	Conclusive Remarks	83
	Acknowledgments	83
	References	83
4	Dynamic-Mode AFM in Liquid	87
	<i>Takeshi Fukuma and Michael J. Higgins</i>	
4.1	Introduction	87
4.2	Operation Principles	88
4.2.1	Amplitude and Phase Modulation AFM (AM- and PM-AFM)	88
4.2.2	Frequency-Modulation AFM (FM-AFM)	89
4.3	Instrumentation	90
4.3.1	Cantilever Excitation	90
4.3.2	Cantilever Deflection Measurement	91
4.3.3	Operating Conditions	93
4.3.4	AM-AFM	93
4.3.4.1	FM-AFM	95
4.3.4.2	PM-AFM	96
4.4	Quantitative Force Measurements	97
4.4.1	Calibration of Spring Constant	98
4.4.2	Conservative and dissipative forces	101
4.4.3	Solvation Force Measurements	103
4.4.3.1	Inorganic Solids in Nonpolar Liquids	104
4.4.3.2	Measurements in Pure Water	106
4.4.3.3	Solvation Forces in Biological Systems	106
4.4.4	Single-Molecule Force Spectroscopy	108
4.4.4.1	Unfolding and “Stretching” of Biomolecules	108
4.4.4.2	Ligand–Receptor Interactions	110
4.5	High-Resolution Imaging	110
4.5.1	Solid Crystals	112
4.5.2	Biomolecular Assemblies	113
4.5.3	Water Distribution	114
4.6	Summary and Future Prospects	116
	References	117
5	Fundamentals of AFM Cantilever Dynamics in Liquid Environments	121
	<i>Daniel Kiracofe, John Melcher, and Arvind Raman</i>	
5.1	Introduction	121
5.2	Review of Fundamentals of Cantilever Oscillation	122
5.3	Hydrodynamics of Cantilevers in Liquids	123

5.4	Methods of Dynamic Excitation	126
5.4.1	Review of Cantilever Excitation Methods	128
5.4.2	Theory	130
5.4.2.1	Direct Forcing	130
5.4.2.2	Ideal Piezo/Acoustic	132
5.4.2.3	Thermal	132
5.4.2.4	Comparison of Excitation Methods	133
5.4.3	Practical Considerations for Acoustic Method	135
5.4.4	Photothermal Method	137
5.4.5	Frequency Modulation Considerations in Liquids	140
5.5	Dynamics of Cantilevers Interacting with Samples in Liquids	140
5.5.1	Experimental Observations of Oscillating Probes Interacting with Samples in Liquids	141
5.5.2	Modeling and Numerical Simulations of Oscillating Probes Interacting with Samples in Liquids	142
5.5.3	Compositional Mapping in Liquids	145
5.5.4	Implications for Force Spectroscopy in Liquids	148
5.6	Outlook	150
	References	150
6	Single-Molecule Force Spectroscopy	157
	<i>Albert Galera-Prat, Rodolfo Hermans, Rubén Hervás, Ángel Gómez-Sicilia, and Mariano Carrión-Vázquez</i>	
6.1	Introduction	157
6.1.1	Why Single-Molecule Force Spectroscopy?	157
6.1.2	SMFS in Biology	158
6.1.3	SMFS Techniques and Ranges	158
6.2	AFM-SMFS Principles	159
6.2.1	Length-Clamp Mode	160
6.2.2	Force-Clamp Mode	163
6.3	Dynamics of Adhesion Bonds	165
6.3.1	Bond Dissociation Dynamics in Length Clamp	165
6.3.2	General Considerations	167
6.3.3	Bond Dissociation Dynamics in Force Clamp	168
6.3.3.1	The Need for Robust Statistics	169
6.4	Specific versus Other Interactions	169
6.4.1	Intramolecular Single-Molecule Markers	170
6.4.1.1	The Wormlike Chain: an Elasticity Model	170
6.4.1.2	Proteins	171
6.4.1.3	DNA and Polysaccharides	174
6.4.2	Intermolecular Single-Molecule Markers	174
6.5	Steered Molecular Dynamics Simulations	176
6.6	Biological Findings Using AFM–SMFS	177
6.6.1	Titin as an Adjustable Molecular Spring in the Muscle Sarcomere	177

6.6.2	Monitoring the Folding Process by Force-Clamp Spectroscopy	180
6.6.3	Intermolecular Binding Forces and Energies in Pairs of Biomolecules	180
6.6.4	New Insights in Catalysis Revealed at the Single-Molecule Level	181
6.7	Concluding Remarks	182
	Acknowledgments	182
	Disclaimer	182
	References	182
7	High-Speed AFM for Observing Dynamic Processes in Liquid	189
	<i>Toshio Ando, Takayuki Uchihashi, Noriyuki Kodera, Mikihiro Shibata, Daisuke Yamamoto, and Hayato Yamashita</i>	
7.1	Introduction	189
7.2	Theoretical Derivation of Imaging Rate and Feedback Bandwidth	190
7.2.1	Imaging Time and Feedback Bandwidth	190
7.2.2	Time Delays	191
7.3	Techniques Realizing High-Speed Bio-AFM	192
7.3.1	Small Cantilevers	192
7.3.2	Fast Amplitude Detector	194
7.3.3	High-Speed Scanner	194
7.3.4	Active Damping Techniques	196
7.3.5	Suppression of Parachuting	198
7.3.6	Fast Phase Detector	199
7.4	Substrate Surfaces	200
7.4.1	Supported Planar Lipid Bilayers	200
7.4.1.1	Choice of Alkyl Chains	201
7.4.1.2	Choice of Head Groups	201
7.4.2	Streptavidin 2D Crystal Surface	201
7.5	Imaging of Dynamic Molecular Processes	203
7.5.1	Bacteriorhodopsin Crystal Edge	203
7.5.2	Photoactivation of Bacteriorhodopsin	204
7.6	Future Prospects of High-Speed AFM	206
7.6.1	Imaging Rate and Low Invasiveness	206
7.6.2	High-Speed AFM Combined with Fluorescence Microscope	206
7.7	Conclusion	207
	References	207
8	Integration of AFM with Optical Microscopy Techniques	211
	<i>Zhe Sun, Andreea Trache, Kenith Meissner, and Gerald A. Meininger</i>	
8.1	Introduction	211
8.1.1	Combining AFM with Fluorescence Microscopy	214
8.1.1.1	Epifluorescence Microscopy	214
8.1.2	Examples of Applications	215
8.1.2.1	Ca ²⁺ Fluorescence Microscopy	215
8.1.2.2	AFM – Epifluorescence Microscopy	217

- 8.2 Combining AFM with IRM and TIRF microscopy 217
 - 8.2.1 Interference Reflection Microscopy 217
 - 8.2.1.1 Optical Setup 218
 - 8.2.2 Total Internal Reflection Fluorescence Microscopy 218
 - 8.2.2.1 Optical Setup 218
 - 8.2.2.2 Applications of Combined AFM–TIRF and AFM–IRM Microscopy 220
 - 8.3 Combining AFM and FRET 221
 - 8.3.1 FRET 221
 - 8.3.2 FRET and Near-Field Scanning Optical Microscopy (NSOM) 222
 - 8.4 FRET-AFM 222
 - 8.5 Sample Preparation and Experiment Setup 223
 - 8.5.1 Cell Culture, Transfection, and Fura-Loading 223
 - 8.5.2 Cantilever Preparation 224
 - 8.5.3 Typical Experimental Procedure 225

Part II Biological Applications 231

- 9 **AFM Imaging in Liquid of DNA and Protein–DNA Complexes** 233
Yuri L. Lyubchenko
 - 9.1 Overview: the Study of DNA at Nanoscale Resolution 233
 - 9.2 Sample Preparation for AFM Imaging of DNA and Protein–DNA Complexes 234
 - 9.3 AFM of DNA in Aqueous Solutions 236
 - 9.3.1 Elevated Resolution in Aqueous Solutions 236
 - 9.3.2 Segmental Mobility of DNA 237
 - 9.4 AFM Imaging of Alternative DNA Conformations 239
 - 9.4.1 Cruciforms in DNA 239
 - 9.4.2 Intramolecular Triple Helices 244
 - 9.4.3 Four-Way DNA Junctions and DNA Recombination 245
 - 9.5 Dynamics of Protein–DNA Interactions 247
 - 9.5.1 Site-Specific Protein–DNA Complexes 247
 - 9.5.2 Chromatin Dynamics Time-Lapse AFM 251
 - 9.6 DNA Condensation 253
 - 9.7 Conclusions 254
 - Acknowledgments 254
 - References 255
- 10 **Stability of Lipid Bilayers as Model Membranes: Atomic Force Microscopy and Spectroscopy Approach** 259
Lorena Redondo-Morata, Marina Inés Giannotti, and Fausto Sanz
 - 10.1 Biological Membranes 259
 - 10.1.1 Cell Membrane 259
 - 10.1.2 Supported Lipid Bilayers 259

10.2	Mechanical Characterization of Lipid Membranes	263
10.2.1	Breakthrough Force as a Molecular Fingerprint	263
10.2.2	AFM Tip-Lipid Bilayer Interaction	265
10.2.3	Effect of Chemical Composition on the Mechanical Stability of Lipid Bilayers	267
10.2.4	Effect of Ionic Strength on the Mechanical Stability of Lipid Bilayers	268
10.2.5	Effect of Different Cations on the Mechanical Stability of Lipid Bilayers	271
10.2.6	Effect of Temperature on the Mechanical Stability of Lipid Bilayers	273
10.2.7	The Case of Phase-Segregated Lipid Bilayers	274
10.3	Future Perspectives	279
	References	279
11	Single-Molecule Atomic Force Microscopy of Cellular Sensors	285
	<i>Jürgen J. Heinisch and Yves F. Dufrêne</i>	
11.1	Introduction	285
11.1.1	Mechanosensors in Living Cells	285
11.1.2	Yeast Cell Wall Integrity Sensors: a Valuable Model for Mechanosensing	286
11.2	Methods	288
11.2.1	Atomic Force Microscopy of Live Cells	288
11.2.2	AFM Detection of Single Sensors	290
11.2.3	Bringing Yeast Sensors to the Surface	291
11.3	Probing Single Yeast Sensors in Live Cells	292
11.3.1	Measuring Sensor Spring Properties	292
11.3.2	Imaging Sensor Clustering	295
11.3.3	Using Sensors as Molecular Rulers	298
11.4	Conclusions	302
	Acknowledgments	303
	References	303
12	AFM-Based Single-Cell Force Spectroscopy	307
	<i>Clemens M. Franz and Anna Taubenberger</i>	
12.1	Introduction	307
12.2	Cantilever Choice	310
12.3	Cantilever Functionalization	310
12.4	Cantilever Calibration	311
12.5	Cell Attachment to the AFM Cantilever	311
12.6	Recording a Force–Distance Curve	313
12.7	Processing F–D Curves	315
12.8	Quantifying Overall Cell Adhesion by SCFS	317
12.9	SFCS with Single-Molecule Resolution	320
12.10	Dynamic Force Spectroscopy	321

12.11	Measuring Cell–Cell Adhesion	325
12.12	Conclusions and Outlook	326
	References	327
13	Nanosurgical Manipulation of Living Cells with the AFM	331
	<i>Atsushi Ikai, Rehana Afrin, Takahiro Watanabe-Nakayama, and Shin-ichi Machida</i>	
13.1	Introduction: Mechanical Manipulation of Living Cells	331
13.2	Basic Mechanical Properties of Proteins and Cells	331
13.3	Hole Formation on the Cell Membrane	332
13.4	Extraction of mRNA from Living Cells	334
13.5	DNA Delivery and Gene Expression	335
13.6	Mechanical Manipulation of Intracellular Stress Fibers	338
13.6.1	AFM Used as a Lateral Force Microscope	338
13.6.2	Force Curves and Fluorescence Images under Lateral Force Application	340
13.6.2.1	Case 1	340
13.6.2.2	Case 2	340
13.7	Cellular Adaptation to Local Stresses	343
13.8	Application of Carbon Nanotube Needles	344
13.9	Use of Fabricated AFM Probes with a Hooking Function	346
13.9.1	Result for a Semi-Intact Cell	348
13.9.2	Result for a Living Cell	348
13.10	Membrane Protein Extraction	348
13.11	Future Prospects	350
	Acknowledgments	350
	References	350
	Index	355