



Victor J. Morris
Andrew R. Kirby
A. Patrick Gunning

Atomic Force Microscopy for Biologists

Second Edition

Imperial College Press

CONTENTS

Acknowledgements	xiii
CHAPTER 1 AN INTRODUCTION	1
CHAPTER 2 APPARATUS	5
2.1. The atomic force microscope	5
2.2. Piezoelectric scanners	7
2.3. Probes and cantilevers	10
2.3.1. Cantilever geometry	10
2.3.2. Tip shape	12
2.3.3. Tip functionality	14
2.4. Sample holders	14
2.4.1. Liquid cells	15
2.5. Detection methods	16
2.5.1. Optical detectors: laser beam deflection	16
2.5.2. Optical detectors: interferometry	18
2.5.3. Electrical detectors: electron tunnelling	19
2.5.4. Electrical detectors: capacitance	20
2.5.5. Electrical detectors: piezoelectric cantilevers	21
2.6. Control systems	21
2.6.1. AFM electronics	21
2.6.2. Operation of the electronics	24
2.6.3. Feedback control loops	25
2.6.4. Design limitations	27
2.6.5. Enhancing the performance of large scanners	28
2.7. Vibration isolation: thermal and mechanical	28
2.8. Calibration	30
2.8.1. Piezoelectric scanner non-linearity	30
2.8.2. Tip related factors: convolution	31
2.8.3. Calibration standards	32
2.8.4. Tips for scanning a calibration specimen	33
2.9. Integrated AFMs	34
2.9.1. Combined AFM-light microscope (AFM-LM)	34
2.9.2. 'Submarine' AFM — the combined AFM-Langmuir Trough	35
2.9.3. Combined AFM-surface plasmon resonance (AFM-SPR)	36
2.9.4. Cryo-AFM	36
CHAPTER 3 BASIC PRINCIPLES	41
3.1. Forces	41
3.1.1. The Van der Waals force and force-distance curves	41
3.1.2. The electrostatic force	44

3.1.3.	Capillary and adhesive forces	44
3.1.4.	Double layer forces	46
3.2.	Imaging modes	47
3.2.1.	Contact dc mode	47
3.2.2.	Ac modes: Tapping and non-contact	47
3.2.3.	Deflection mode	54
3.3.	Image types	55
3.3.1.	Topography	55
3.3.2.	Frictional force	56
3.3.3.	Phase	56
3.4.	Substrates	58
3.4.1.	Mica	58
3.4.2.	Glass	58
3.4.3.	Graphite	58
3.5.	Common problems	59
3.5.1.	Thermal drift	59
3.5.2.	Multiple tip effects	59
3.5.3.	The 'pool' artifact	61
3.5.4.	Optical interference on highly reflective samples	61
3.5.5.	Sample roughness	62
3.5.6.	Sample mobility	63
3.5.7.	Imaging under liquid	64
3.6.	Getting started	65
3.6.1.	DNA	65
3.6.2.	Troublesome large samples	68
3.7.	Image optimisation	70
3.7.1.	Grey levels and colour tables	70
3.7.2.	Brightness and contrast	71
3.7.3.	High and low pass filtering	71
3.7.4.	Normalisation and plane fitting	71
3.7.5.	Despike	71
3.7.6.	Fourier filtering	72
3.7.7.	Correlation averaging	73
3.7.8.	Stereographs and anaglyphs	73
3.7.9.	Do your homework!	74
CHAPTER 4 MACROMOLECULES		76
4.1.	Imaging methods	76
4.1.1.	Tip adhesion, molecular damage and displacement	76
4.1.2.	Depositing macromolecules onto substrates	77
4.1.3.	Metal coated samples	78
4.1.4.	Imaging in air	79
4.1.5.	Imaging under non-aqueous liquids	80

4.1.6.	Binding molecules to the substrate	81
4.1.7.	Imaging under water or buffers	85
4.2.	Nucleic acids: DNA	86
4.2.1.	Imaging DNA	87
4.2.2.	DNA conformation, size and shape	88
4.2.3.	DNA-protein interactions	94
4.2.4.	Location and mapping of specific sites	99
4.2.5.	Chromosomes	102
4.3.	Nucleic acids: RNA	105
4.4.	Polysaccharides	106
4.4.1.	Imaging polysaccharides	107
4.4.2.	Size, shape, structure and conformation	108
4.4.3.	Aggregates, networks and gels	117
4.4.4.	Cellulose, plant cell walls and starch	122
4.4.5.	Proteoglycans and mucins	128
4.5.	Proteins	130
4.5.1.	Globular proteins	131
4.5.2.	Antibodies	136
4.5.3.	Fibrous proteins	139
CHAPTER 5 INTERFACIAL SYSTEMS		181
5.1.	Introduction to interfaces	181
5.1.1.	Surface activity	181
5.1.2.	AFM of interfacial systems	184
5.1.3.	The Langmuir trough	185
5.1.4.	Langmuir-Blodgett film transfer	186
5.2.	Sample preparation	188
5.2.1.	Cleaning protocols: glassware and trough	188
5.2.2.	Substrates	189
5.2.3.	Performing the dip	191
5.3.	Phospholipids	192
5.3.1.	Early AFM studies of phospholipid films	193
5.3.2.	Modification of phospholipid bilayers with the AFM	194
5.3.3.	Studying intrinsic bilayer properties by AFM	196
5.3.4.	Ripple phases in phospholipid bilayers	199
5.3.5.	Mixed phospholipid films	202
5.3.6.	Effect of supporting layers	205
5.3.7.	Dynamic processes of phospholipid layers	208
5.4.	Liposomes and intact vesicles	211
5.5.	Lipid-protein mixed films	213
5.5.1.	High resolution studies of phospholipid bilayers	217
5.6.	Miscellaneous lipid films/surfactant films	219

5.7.	Interfacial protein films	219
5.7.1.	Specific precautions	220
5.7.2.	AFM studies of interfacial protein films	222
CHAPTER 6 ORDERED MACROMOLECULES		231
6.1.	Three-dimensional crystals	231
6.1.1.	Crystalline cellulose	231
6.1.2.	Protein crystals	232
6.1.3.	Nucleic acid crystals	235
6.1.4.	Viruses and virus crystals	236
6.2.	Two dimensional protein crystals: an introduction	240
6.2.1.	What does AFM have to offer?	241
6.2.2.	Sample preparation: membrane proteins	243
6.2.3.	Sample preparation: soluble proteins	244
6.3.	AFM studies of 2D membrane protein crystals	246
6.3.1.	Purple membrane (bacteriorhodopsin)	246
6.3.2.	Gap junctions	249
6.3.3.	Photosynthetic protein membranes	252
6.3.4.	ATPase in kidney membranes	252
6.3.5.	OmpF porin	253
6.3.6.	Bacterial S layers	254
6.3.7.	Bacteriophage $\phi 29$ head-tail connector	257
6.3.8.	AFM imaging of membrane dynamics	259
6.3.9.	Force spectroscopy of membrane proteins	261
6.3.10.	Gas vesicle protein	261
6.4.	AFM studies of 2D crystals of soluble proteins	262
6.4.1.	Imaging conditions	264
6.4.2.	Electrostatic considerations	266
CHAPTER 7 CELLS, TISSUE AND BIOMINERALS		276
7.1.	Imaging methods	276
7.1.1.	Sample preparation	277
7.1.2.	Force mapping and mechanical measurements	278
7.2.	Microbial cells: bacteria, spores and yeasts	290
7.2.1.	Bacteria	290
7.2.2.	Yeasts	300
7.3.	Blood cells	302
7.3.1.	Erythrocytes	302
7.3.2.	Leukocytes and lymphocytes	304
7.3.3.	Platelets	304
7.4.	Neurons and Glial cells	306
7.5.	Epithelial cells	307

7.6. Non-confluent renal cells	309
7.7. Endothelial cells	311
7.8. Cardiocytes	313
7.9. Other mammalian cells	314
7.10. Plant cells	317
7.11. Tissue	321
7.11.1. Embedded sections	321
7.11.2. Embedment-free sections	322
7.11.3. Hydrated sections	323
7.11.4. Freeze-fracture replicas	324
7.11.5. Immunolabelling	324
7.12. Biominerals	325
7.12.1. Bone, tendon and cartilage	325
7.12.2. Teeth	327
7.12.3. Shells	328
CHAPTER 8 OTHER PROBE MICROSCOPES	342
8.1. Overview	342
8.2. Scanning tunnelling microscope (STM)	342
8.3. Scanning near-field optical microscope (SNOM)	345
8.4. Scanning ion conductance microscope (SICM)	347
8.5. Scanning thermal microscope (SThM)	349
8.6. Optical tweezers and the photonic force microscope (PFM)	351
CHAPTER 9 FORCE SPECTROSCOPY	356
9.1. Force measurement with the AFM	356
9.2. First steps in force spectroscopy: from raw data to force-distance curves	357
9.2.1. Quantifying cantilever displacement	357
9.2.2. Determining cantilever spring constants	359
9.2.3. Anatomy of a force-distance curve	362
9.3. Pulling methods	364
9.3.1. Intrinsic elastic properties of molecules	364
9.3.2. Molecular recognition force spectroscopy	369
9.3.3. Chemical force microscopy (CFM)	373
9.4. Pushing methods	374
9.4.1. Colloidal probe microscopy (CPM)	374
9.4.2. How to make a colloid probe cantilever assembly	377
9.4.3. Deformation and indentation methods	380
9.5. Analysis of force-distance curves	381
9.5.1. Worm-like chain and freely jointed chain models	382
9.5.2. Molecular interactions	384
9.5.3. Deformation analysis	387
9.5.4. Adhesive force at pull-off	388

xii *Contents*

- 9.5.5. Elastic indentation depth, δ , and contact radius, a , during deformation 388
- 9.5.6. Contact radius at zero load 389
- 9.5.7. Colloidal forces 389

SPM Books 397

Index 399