

# Contents

## Part I

### *Instrumentation and Methods*

#### **Scanning Probe Microscopy in Biological Research..... 3**

*Tatsuo Ushiki, Kazushige Kawabata*

Introduction.....	3
SPM for Visualization of the Surface of Biomaterials.....	4
Advantages of AFM in Biological Studies .....	4
AFM of Biomolecules .....	5
AFM of Isolated Intracellular and Extracellular Structures .....	7
AFM of Tissue Sections .....	10
AFM of Living Cells and Their Movement .....	10
Combination of AFM with Scanning Near-Field Optical Microscopy for Imaging Biomaterials .....	12
SPM for Measuring Physical Properties of Biomaterials.....	14
Evaluation Methods of Viscoelasticity .....	14
Examples for Viscoelasticity Mapping Measurements.....	17
Combination of Viscoelasticity Measurement with Other Techniques.....	20
SPM as a Manipulation Tool in Biology .....	22
Conclusion .....	24
References.....	24

#### **Scanning Probes for the Life Sciences..... 27**

*Andrea M. Ho, Horacio D. Espinosa*

Introduction.....	27
Microarray Technology .....	28
Microcontact Printing .....	29
Optical Lithography.....	30
Protein Arrays.....	32
Nanoarray Technology .....	33
The Push for Nanoscale Detection.....	33
Probe-Based Patterning .....	35
Alternative Patterning Methods .....	46
Nanoscale Deposition Mechanisms .....	48

AFM Parallelization .....	51
One-Dimensional Arrays .....	52
Two-Dimensional Arrays .....	53
Future Prospects for Nanoprobes .....	56
References .....	58
 <b>New AFM Developments to Study Elasticity and Adhesion at the Nanoscale .....</b>	 <b>63</b>
<i>Robert Szoszkiewicz, Elisa Riedo</i>	
Introduction .....	64
Contact Mechanics Theories and Their Limitations .....	65
Modulated Nanoindentation .....	67
Force-Indentation Curves .....	67
Elastic Moduli .....	70
Ultrasonic Methods at Local Scales .....	72
Brief Description of Ultrasonic Methods .....	72
Applications of Ultrasonic Techniques in Elasticity Mapping .....	75
UFM Measurements of Adhesion Hysteresis and Their Relations to Friction at the Tip-Sample Contact .....	76
References .....	78
 <b>Application of SPM and Related Techniques to the Mechanical Properties of Biotool Materials .....</b>	 <b>81</b>
<i>Thomas Schöberl, Ingomar L. Jäger, Helga C. Lichtenegger</i>	
Introduction .....	82
Typical Biotool Materials .....	84
Chemistry .....	84
Structures .....	86
Mechanical Properties .....	88
Experimental Methods and Setups .....	89
SPM and Indentation .....	89
Scratch and Wear Tests .....	91
Dynamic Modes .....	92
Fracture Toughness Tests .....	93
Samples .....	94
Choice .....	94
Storage .....	95
Preparation .....	96
Experimental Conditions .....	97
Moisture .....	97
Temperature .....	98
Probe Tips .....	98
Test Velocity .....	99

Results .....	99
Sources of Error .....	99
Interpretation .....	104
Examples from the Literature .....	105
References.....	108

## **Direct Force Measurements of Receptor–Ligand Interactions on Living Cells.....115**

*Robert H. Eibl*

Introduction.....	116
Procedure .....	122
Principle of AFM Force Spectroscopy .....	123
Cell–Cell Interactions .....	123
Cell-Substrate Measurements .....	126
Specificity and Blocking Antibodies .....	128
Activation by SDF-1 .....	131
Protocols .....	134
Cantilever Functionalization.....	134
AFM Measurement on Living Cells .....	136
Inhibition with Blocking Antibodies, Peptidomimetic Inhibitors or EDTA .....	139
Activation with $Mg^{2+}$ , $Mn^{2+}$ Ions, Activating Antibodies, Phorbol ester or Chemokines.....	140
AFM Measurement—Cell Free .....	140
Conclusion and Future Developments .....	141
References.....	143

## **Self-Sensing Cantilever Sensor for Bioscience .....147**

*Hayato Sone, Sumio Hosaka*

Introduction.....	147
Basics of the Cantilever Mass Sensor .....	148
Finite Element Method Simulation of the Cantilever Vibration .....	151
Detection of Cantilever Deflection .....	154
Using a Position Sensor .....	154
Using a Piezoresistive Sensor .....	155
Self-Sensing Systems.....	160
Vibration Systems.....	160
Vibration-Frequency Detection Systems .....	160
Applications .....	161
Water Molecule Detection in Air.....	161
Antigen and Antibody Detection in Water .....	166
Prospective Applications .....	172
References.....	172

<b>Microfabricated Cantilever Array Sensors for (Bio-)Chemical Detection .....</b>	<b>175</b>
<i>Hans Peter Lang, Martin Hegner, Christoph Gerber</i>	
Introduction.....	175
Sensors.....	175
Cantilevers.....	176
Cantilever Operating Modes.....	178
Cantilever Arrays.....	184
Experimental Setup.....	188
Measurement Chamber.....	188
Cantilever Functionalization.....	190
Measurements .....	195
Artificial Nose for Detection of Perfume Essences .....	196
Label-Free DNA Hybridization Detection.....	198
Applications and Outlook .....	201
References.....	202
 <b>Nanomechanics and Microfluidics as a Tool for Unraveling Blood Clotting Disease .....</b>	 <b>207</b>
<i>D.M. Steppich, S. Thalhammer, A. Wixforth, M.F. Schneider</i>	
Introduction.....	207
Topography.....	208
Little Story of Blood Clotting.....	209
High-Resolution Imaging .....	212
Lab-on-a-Chip.....	219
Nanomechanical Diagnostics.....	220
Mimicking Blood Flow Conditions on a Surface Acoustic Wave-Driven Biochip.....	222
The Lab on a Chip – AFM – Hybrid.....	224
Experimental Setup.....	224
Bundle Relaxation .....	226
Stream Line Manipulation and Flow Sensoring .....	230
Summary and Outlook .....	234
References.....	235
 <b>Quantitative Nanomechanical Measurements in Biology.....</b>	 <b>239</b>
<i>Malgorzata Lekka, Andrzej J. Kulik</i>	
Stiffness of Biological Samples .....	239
Cell Structure .....	239
Determination of Young's Modulus .....	242
Brief Overview of the Application of AFM to Studies of Living Cells....	251
Summary.....	256
Friction Force Microscopy.....	258
Friction and Chemical Force Microscopy.....	259

Applications of FFM/CFM .....	263
Summary.....	270
References.....	271
 <b>Applications of Scanning Near-Field Optical Microscopy in Life Science .....</b>	<b>275</b>
<i>Pietro Giuseppe Gucciardi</i>	
Introduction.....	276
Experimental Techniques in Near-Field Optical Microscopy.....	277
Principles of Near-Field Optical Microscopy .....	277
Fluorescence Near-Field Optical Microscopy .....	279
Near-Field Optical Microscopy in Liquid.....	281
Tip-Enhanced Near-Field Optical Microscopy .....	283
Applications of Near-Field Optical Microscopy in Life Science.....	284
Infrared Imaging of Tobacco Mosaic Virus with Nanoscale Resolution.....	284
Co-Localization of Malarial and Host Skeletal Proteins in Infected Erythrocytes by Dual-Color Near-Field Fluorescence Microscopy.....	285
Co-Localization of $\alpha$ -Sarcoglycan and $\beta$ 1D-Integrin in Human Muscle Cells by Near-Field Fluorescence Microscopy.....	287
Single Molecule Near-Field Fluorescence Microscopy of Dendritic Cells.....	288
Chemical Information of Bacterial Surfaces and Detection of DNA Nucleobases by Tip-Enhanced Raman Spectroscopy .....	290
Conclusions.....	291
References.....	292
 <b>Scanning Ion Conductance Microscopy.....</b>	<b>295</b>
<i>Tilman E. Schäffer, Boris Anczykowski, Harald Fuchs</i>	
Introduction.....	295
Fundamental Principles.....	296
Basic Setup .....	296
Nanopipettes .....	299
Electrodes .....	300
Ion Currents Through Nanopipettes.....	301
Background Theory .....	301
Simple Analytical Model .....	301
Finite Element Modeling .....	303
Experimental Current-Distance Curves .....	305
Imaging with Ion Current Feedback .....	306
Advanced Techniques.....	307
Modulation Methods.....	308
Applications in Bioscience .....	310

Combination with Other Scanning Techniques .....	311
Combination with Atomic Force Microscopy .....	312
Application in Material Science .....	312
Combination with Shear Force Microscopy .....	315
Application in Bioscience.....	318
Outlook .....	319
References.....	320

## **Scanning Probe Lithography for Chemical, Biological and Engineering Applications .....325**

*Joseph M. Kinsella, Alben Ivanisevic*

Introduction.....	326
Modeling of the DPN Process.....	328
Patterning of Biological and Biologically Active Molecules.....	331
DNA Patterning .....	332
Protein Patterning .....	334
Peptide Patterning.....	337
Patterning of Templates for Biological Bottom-Up Assembly .....	339
Chemical Patterning.....	341
Thiols .....	341
$\omega$ -Substituted Thiols .....	342
Silanes and Silazanes.....	343
Deposition of Solid Organic Inks .....	344
Polymers .....	345
Polyelectrolytes .....	347
Dendrimers .....	347
Deposition of Supramolecular Materials .....	348
Deposition of Metals.....	349
Deposition of Solid-State Materials.....	350
Deposition of Magnetic Materials .....	351
Engineering Applications of DPN .....	352
Future Challenges and Applications .....	354
Conclusions.....	355
References.....	355

## **Scanning Probe Microscopy: From Living Cells to the Subatomic Range .....359**

*Ilse C. Gebeshuber, Manfred Drack, Friedrich Aumayr,  
Hannspeter Winter, Friedrich Franek*

Introduction .....	359
Cells <i>In Vivo</i> as Exemplified by Diatoms .....	360
Introduction to Diatoms .....	360
SPM of Diatoms .....	362
Interaction of Large Organic Molecules .....	365

Nanodefects on Atomically Flat Surfaces.....	369
Ion Bombardment of Highly Oriented Pyrolytic Graphite (HOPG) .....	370
Bombardment of Single Crystal Insulators with Multicharged Ions.....	374
Subatomic Features.....	377
Atom Orbitals .....	377
Single Electron Spin Detection with AFM and STM .....	379
Conclusions and Outlook.....	382
References.....	383

## Part II

### *AFM of Biomolecules*

#### **Atomic Force Microscopy of DNA Structure and Interactions .....389**

*Neil H. Thomson*

Introduction: The Single-Molecule, Bottom-Up Approach .....	389
DNA Structure and Function .....	391
The Atomic Force Microscope .....	393
Binding of DNA to Support Surfaces .....	399
Properties of Support Surfaces for Biological AFM.....	399
DNA Binding to Surfaces.....	400
DNA Transport to Surfaces .....	404
AFM of DNA Systems .....	405
Static Imaging versus Dynamic Studies .....	405
The Race for Reproducible Imaging of Static DNA.....	406
Applications of Tapping-Mode AFM to DNA Systems .....	408
Outlook .....	419
References.....	421

#### **Nanostructuration and Nanoimaging of Biomolecules for Biosensors.....427**

*Claude Martelet, Nicole Jaffrezic-Renault, Yanxia Hou,  
Abdelhamid Errachid, François Bessueille*

Introduction and Definition of Biosensors.....	427
Definition.....	427
Biosensor Components .....	427
Immobilization of the Bioreceptor.....	428
Langmuir–Blodgett and Self-Assembled Monolayers as Immobilization Techniques.....	429
Langmuir–Blodgett Technique .....	429
Self-Assembled Monolayers.....	438
Characterization of SAMs and LB Films.....	450
Prospects and Conclusion .....	455
References.....	457

## Part III

*AFM of Biological Membranes, Cells and Tissue***Imaging Chemical Groups and Molecular Recognition Sites****on Live Cells Using AFM .....463**

*David Alsteens, Vincent Dupres, Etienne Dague, Claire Verbelen,  
Guillaume André, Grégory Francius, Yves F. Dufrêne*

Introduction.....	463
Chemical Force Microscopy .....	464
Methods .....	464
Probing Hydrophobic Forces.....	465
Chemical Force Microscopy of Live Cells .....	468
Molecular Recognition Imaging .....	472
Spatially Resolved Force Spectroscopy.....	473
Immunogold Imaging .....	475
Conclusions.....	477
References.....	477

**Single-Molecule Studies on Cells and Membranes Using the Atomic****Force Microscope.....479**

*Ferry Kienberger, Lilia A. Chtcheglova, Andreas Ebner,  
Theeraporn Puntheeranurak, Hermann J. Gruber, Peter Hinterdorfer*

Introduction.....	480
Principles of Atomic Force Microscopy .....	481
Imaging of Membrane-Protein Complexes .....	482
Membranes of Photosynthetic Bacteria and Bacterial S-Layers.....	482
Nuclear Pore Complexes .....	484
Cell Membranes with Attached Viral Particles.....	484
Single-Molecule Recognition on Cells and Membranes.....	488
Principles of Recognition Force Measurements .....	488
Force-Spectroscopy Measurements on Living Cells.....	491
Unfolding and Refolding of Single-Membrane Proteins .....	495
Simultaneous Topography and Recognition Imaging on Cells (TREC) .....	497
Concluding Remarks.....	500
References.....	501

**Atomic Force Microscopy: Interaction Forces Measured in****Phospholipid Monolayers, Bilayers, and Cell Membranes .....505**

*Zoya Leonenko, David Cramb, Matthias Amrein, Eric Finot*

Introduction.....	505
Phase Transitions of Lipid Bilayers in Water .....	507
Morphology Change During Lamellar Phase Transition .....	508
Change in Forces During Phase Transition .....	510
Force Measurements on Pulmonary Surfactant Monolayers in Air.....	517
Adhesion Measurements: Monolayer Stiffness and Function.....	519



Repulsive Forces: The Interaction of Charged Airborne Particles with Surfactant.....	520
Interaction Forces Measured on Lung Epithelial Cells in Buffer .....	522
Cell Culture/Force Measurement Setup.....	523
Mechanical Properties .....	525
Conclusions.....	528
References.....	529
 <b>Atomic Force Microscopy Studies of the Mechanical Properties of Living Cells .....</b>	<b>533</b>
<i>Félix Rico, Ewa P. Wojcikiewicz, Vincent T. Moy</i>	
Introduction.....	533
Principle of Operation.....	534
AFM Imaging .....	536
Force Measurements.....	536
Cell Viscoelasticity .....	537
AFM Tip Geometries.....	538
Elasticity: Young's Modulus .....	538
Viscoelasticity: Complex Shear Modulus.....	540
Cell Adhesion .....	542
Concluding Remarks and Future Directions.....	548
References.....	549
 <b>Application of Atomic Force Microscopy to the Study of Expressed Molecules in or on a Single Living Cell.....</b>	<b>555</b>
<i>Hyonchol Kim, Hironori Uehara, Rehana Afrin, Hiroshi Sekiguchi, Hideo Arakawa, Toshiya Osada, Atsushi Ikai</i>	
Introduction.....	556
Methods of Manipulation To Study Molecules in or on a Living Cell Using an AFM .....	557
AFM Tip Preparation To Manipulate Receptors on a Cell Surface .....	557
Analysis of Molecular Interactions Where Multiple Bonds Formed .....	559
Measurement of Single-Molecule Interaction Strength on Soft Materials .....	561
Observation of the Distribution of Specific Receptors on a Living Cell Surface .....	562
Distribution of Fibronectin Receptors on a Living Fibroblast Cell .....	562
Distribution of Vitronectin Receptors on a Living Osteoblast Cell .....	565
Quantification of the Number of Prostaglandin Receptors on a Chinese Hamster Ovary Cell Surface.....	567
Further Application of the AFM to the Study of Single-Cell Biology.....	570
Manipulation of Expressed mRNAs in a Living Cell Using an AFM .....	570
Manipulation of Membrane Receptors on a Living Cell Surface Using an AFM .....	576
References.....	579

<b>Towards a Nanoscale View of Microbial Surfaces Using the Atomic Force Microscope.....</b>	<b>583</b>
<i>Claire Verbelen, Guillaume Andre, Xavier Haulot, Yann Gilbert, David Alsteens, Etienne Dague, Yves F. Dufrêne</i>	
Introduction.....	583
Imaging.....	584
Sample Preparation.....	584
Visualizing Membrane Proteins at Subnanometer Resolution.....	584
Live-Cell Imaging.....	585
Force Spectroscopy.....	588
Customized Tips.....	588
Probing Nanoscale Elasticity and Surface Properties.....	589
Stretching Cell Surface Polysaccharides and Proteins.....	591
Nanoscale Mapping and Functional Analysis of Molecular Recognition Sites.....	592
Conclusions.....	595
References.....	596
 <b>Cellular Physiology of Epithelium and Endothelium .....</b>	 <b>599</b>
<i>Christoph Riethmüller, Hans Oberleithner</i>	
Introduction.....	599
Epithelium.....	600
Transport Through a Septum.....	600
In the Kidney .....	602
Endothelium.....	608
Paracellular Gaps.....	609
Cellular Drinking.....	611
Wound Healing.....	614
Transmigration of Leukocytes.....	615
Technical Remarks .....	616
Summary.....	617
References.....	617
 <b>Nanotribological Characterization of Human Hair and Skin Using Atomic Force Microscopy (AFM).....</b>	 <b>621</b>
<i>Bharat Bhushan, Carmen LaTorre</i>	
Introduction.....	621
Human Hair, Skin, and Hair Care Products .....	625
Human Hair and Skin .....	625
Hair Care: Cleaning and Conditioning Treatments, and Damaging Processes.....	632
Experimental Techniques.....	637
Experimental Procedure.....	639
Hair and Skin Samples.....	643

Results and Discussion .....	645
Surface Roughness, Friction, and Adhesion for Various Ethnicities of Hair.....	645
Surface Roughness, Friction, and Adhesion for Virgin and Chemically Damaged Caucasian Hair (with and without Commercial Conditioner Treatment) .....	656
Surface Roughness, Friction, and Adhesion for Hair Treated with Various Combinations of Conditioner Ingredients .....	664
Investigation of Directionality Dependence and Scale Effects on Friction and Adhesion of Hair .....	671
Surface Roughness and Friction of Skin.....	684
Closure.....	684
References.....	688
Appendix.....	689

### **Evaluating Tribological Properties of Materials for**

<b>Total Joint Replacements Using Scanning Probe Microscopy.....</b>	<b>691</b>
<i>Sriram Sundararajan, Kanaga Karuppiyah Kanaga Subramanian</i>	

Introduction.....	691
Total Joint Replacements.....	691
Social and Economic Significance.....	692
Problems Associated with Total Joint Replacements .....	692
Tribology .....	694
Materials .....	694
Lubrication in Joints—the Synovial Fluid.....	695
Conventional Tribological Testing of Material Pairs for Total Joint Replacements .....	696
Wear Tests .....	696
Friction Tests .....	696
Scanning Probe Microscopy as a Tool to Study Tribology of Total Joint Replacements .....	696
Nanotribology of Ultrahigh Molecular Weight Polyethylene.....	697
Fretting Wear of Cobalt–Chromium Alloy .....	705
Summary and Future Outlook.....	709
References.....	710

<b>Atomic Force Microscopy in Nanomedicine.....</b>	<b>713</b>
---	------------

*Dessy Nikova, Tobias Lange, Hans Oberleithner, Hermann Schillers, Andreas Ebner, Peter Hinterdorfer*

AFM in Biological Sciences .....	713
Plasma Membrane Preparation for AFM Imaging.....	716
Introduction .....	716
Plasma Membrane Preparation .....	717
Atomic Force Microscopy .....	719

Molecular Volume Measurements of Membrane Proteins .....	719
AFM Imaging .....	719
AFM Imaging of CFTR in Oocyte Membranes .....	722
Introduction .....	723
Does the CFTR Form Functional Assemblies? .....	723
Two CFTRs are Better Than One .....	725
Single Antibody–CFTR Recognition Imaging .....	728
Introduction .....	728
Tethering of Antibodies to AFM Tips .....	729
AFM Imaging and Recognition .....	729
A Single Antibody Sees a Single CFTR .....	729
Single Cell Elasticity: Probing for Diseases .....	731
Introduction .....	731
Force–Mapping AFM .....	732
Can One Protein Change Cell Elasticity? .....	733
Summary .....	736
References .....	736

#### Part IV

#### *Functional Bio(-inspired) Surfaces*

<b>Lotus Effect: Roughness-Induced Superhydrophobicity .....</b>	<b>741</b>
<i>Michael Nosonovsky, Bharat Bhushan</i>	
Introduction .....	741
Contact Angle Analysis .....	744
Homogeneous Solid–Liquid Interface .....	745
Composite Solid–Liquid–Air Interface .....	748
Stability of the Composite Interface .....	751
Calculation of the Contact Angle for Selected Rough Surfaces and Surface Optimization .....	759
Two-Dimensional Periodic Profiles .....	760
Three-Dimensional Surfaces .....	763
Surface Optimization for Maximum Contact Angle .....	769
Meniscus Force .....	771
Sphere in Contact with a Smooth Surface .....	771
Multiple-Asperity Contact .....	773
Experimental Data .....	774
Closure .....	777
References .....	778

<b>Gecko Feet: Natural Attachment Systems for Smart Adhesion—Mechanism, Modeling, and Development of Bio-Inspired Materials .....</b>	<b>781</b>
<i>Bharat Bhushan, Robert A. Sayer</i>	
Introduction.....	781
Tokay Gecko.....	782
Construction of Tokay Gecko.....	782
Other Attachment Systems .....	785
Adaptation to Surface Roughness.....	787
Peeling .....	788
Self-Cleaning.....	790
Attachment Mechanisms.....	792
Van der Waals Forces.....	792
Capillary Forces.....	793
Experimental Adhesion Test Techniques and Data .....	794
Adhesion Under Ambient Conditions.....	795
Effects of Temperature .....	797
Effects of Humidity .....	798
Effects of Hydrophobicity .....	798
Adhesion Modeling.....	799
Spring Model.....	801
Single Spring Contact Analysis .....	801
The Multilevel Hierarchical Spring Analysis .....	803
Adhesion Results for the Gecko Attachment System Contacting a Rough Surface.....	806
Capillarity Effects.....	810
Adhesion Results that Account for Capillarity Effects.....	811
Modeling of Biomimetic Fibrillar Structures.....	814
Fiber Model .....	814
Single Fiber Contact Analysis .....	814
Constraints .....	815
Numerical Simulation.....	819
Results and Discussion .....	821
Fabrication of Biomimetic Gecko Skin .....	828
Single-Level Hierarchical Structures.....	829
Multilevel Hierarchical Structures.....	833
Closure.....	835
Appendix.....	836
References.....	839