

Edited by Gerd-Joachim Krauss and Dietrich H. Nies

Ecological Biochemistry

Environmental and Interspecies Interactions

Contents

List of Contributors	<i>XVII</i>
Foreword	<i>XXI</i>
Preface	<i>XXIII</i>
Companion Website	<i>XXV</i>

Part I: Basics of Life 1

1	Basic Biochemical Roots 3
	<i>Dietrich H. Nies</i>
1.1	Chemistry and Physics of Life 3
1.2	Energy and Transport 3
1.3	Basic Biochemistry 4
2	Specialized Plant Metabolites: Diversity and Biosynthesis 15
	<i>Alain Tissier, Jörg Ziegler, and Thomas Vogt</i>
2.1	Metabolite Diversity 15
2.2	Major Classes of Plant Specialized Compounds 16
2.2.1	Terpenoids 16
2.2.2	Alkaloid Biosynthesis 20
2.2.2.1	Generation of Metabolic Diversity across Alkaloid Classes 21
2.2.2.2	Generation of Metabolic Diversity within Alkaloid Classes 23
2.2.3	Phenylpropanoid Metabolism 27
2.2.3.1	Hydroxycinnamic Acids and Lignin 27
2.2.3.2	Flavonoids, (Pro)anthocyanins, and other Chromogenic Structures 29
2.2.3.3	Simple C ₆ -C ₁ Phenolics 30
2.2.4	Glucosinolates and Cyanogenic Glucosides 31
2.3	Sites of Biosynthesis and Accumulation 33
2.3.1	Specialized Structures and Storage 33
2.3.1.1	Glandular Trichomes 33
2.3.1.2	Internal Organs 33
2.3.1.3	Vacuoles as Sites of Storage of Specialized Metabolites 34
2.3.2	Alkaloid Biosynthesis: Pathway Trafficking 34
2.4	Evolution of Specialized Pathway Genes 34
2.4.1	Gene Duplication 34
2.4.2	Chromosomal Clusters of Specialized Metabolite Pathway Genes 36
2.4.3	Convergent Evolution 36
	References 37
	Further Reading 37
3	Evolution of Secondary Metabolism in Plants 39
	<i>Michael Wink</i>
3.1	Origins of Plant Secondary Metabolism 39
3.2	Evolutionary Alternatives 41

3.3	Endophytes, Symbiotic, and Ectomycorrhizal Fungi	45
	References	47
	Part II: Ecological Signatures of Life	49
4	Systematics of Life, Its Early Evolution, and Ecological Diversity	51
	<i>Dietrich H. Nies</i>	
4.1	Cellular Life Forms and Subcellular Parasites	51
4.2	Superkingdom Archaea	51
4.2.1	General Features	51
4.2.2	Phylum Euryarchaeota (I. B)	52
4.2.3	Phylum Crenarchaeota (I. A)	53
4.2.4	Phylum Thaumarchaeota (I. E)	53
4.2.5	Other Phylae (I.C, I.D)	54
4.3	Superkingdom Bacteria	55
4.3.1	General Features	55
4.3.2	Phylum Aquificae (II. B)	56
4.3.3	Phylae Thermotogae (II. Z) and Thermodesulfobacteria (II. Y)	56
4.3.4	Chloroflexi (II. G)	56
4.3.5	Crown groups of the Bacteria	56
4.3.6	Phylum Proteobacteria (II.U)	56
4.3.7	Superphylum Bacteroidetes/Chlorobi Group (II. D)	57
4.3.8	Phylum Planctomycetes (II. T)	57
4.3.9	Gram-Positive Bacteria (II.A, II.O, II.X)	57
4.3.10	Phylum Cyanobacteria (II.I)	58
4.4	Superkingdom Eukaryota	59
4.4.1	General Features	59
4.4.2	<i>Excavata</i> : Excavates (III.O, III.P, III.Q) and Discicristates (III.R, III.S, III.T)	60
4.4.3	Chromalveolata: Kingdoms <i>Alveolata</i> (III.K), <i>Stramenopiles</i> (III.N), <i>Cryptophyta</i> (III.L), and <i>Haptophyceae</i> (III.M)	62
4.4.4	Kingdom <i>Rhizaria</i> (III.G)	64
4.4.5	<i>Archaeplastida</i> (III.H, III.I, III.J)	65
4.4.6	Kingdom <i>Amoebozoa</i> (III.A)	68
4.4.7	Kingdom <i>Opisthokonta</i> (III.E)	70
	Acknowledgment	74
	References	74
	Further Reading	74
5	Communities and Ecosystem Functioning	77
	<i>Heinz Rennenberg</i>	
5.1	Competition for, and Distribution of, Limiting Resources as a Means of Ecosystem Functioning	77
5.1.1	Light-Capturing Strategies	78
5.1.2	Competition for Nutrients	79
5.2	Joint Exploitation of Limiting Resources by Symbioses	79
5.2.1	Mycorrhiza	79
5.2.1.1	Arbuscular Mycorrhizas (AMs)	80
5.2.1.2	Ectomycorrhizas (ECMs)	83
5.2.2	Symbiotic N ₂ Fixation	86
5.2.2.1	Establishment of Symbiosis and Nodule Formation	87
5.2.2.2	Mechanism of N ₂ Fixation and Plant–Microbial Interactions in N Nutrition	88
5.3	Avoidance of Competition	89
5.4	Facilitation Mechanisms in Communities and Ecosystem Functioning	90
	References	91
	Further Reading	91

6	Food Chains and Nutrient Cycles 93
	<i>Felix Bärlocher and Heinz Rennenberg</i>
6.1	Basic Concepts 93
6.1.1	Food Chains and Food Webs 93
6.1.2	Grazing vs. Detritus Food Chains and the Microbial Loop 94
6.1.3	Role of Parasites 95
6.1.4	Metabolic Theory and Ecological Stoichiometry 95
6.1.5	Terrestrial Versus Aquatic Habitats 95
6.1.6	General Principles of Nutrient Cycles 96
6.2	Aquatic Systems 97
6.2.1	Important Physicochemical Properties of Water 98
6.2.2	Marine Systems 99
6.2.2.1	Density Gradients and Pycnoclines 99
6.2.2.2	The Euphotic Zone 99
6.2.2.3	The Pelagial Zone 99
6.2.2.4	Hydrothermal Vents and Methane Seeps 100
6.2.2.5	The Benthic Habitat 100
6.2.2.6	Mangroves 100
6.2.2.7	Salt Marshes 101
6.2.2.8	Coral Reefs 102
6.2.3	Freshwater Systems 102
6.2.3.1	Temperature Gradients and Circulation in Lakes 102
6.2.3.2	Oxygenic and Anoxygenic Photosynthesis 103
6.2.3.3	The Freshwater Pelagial Zone 103
6.2.3.4	The Freshwater Benthic Zone and Freshwater Marshes 103
6.2.3.5	Running Waters 104
6.2.4	Nutrient Cycles 106
6.2.4.1	The Nitrogen Cycle 106
6.2.4.2	The Sulfur Cycle 107
6.2.4.3	The Phosphorus Cycle 107
6.2.4.4	The Carbon Cycle 109
6.3	Terrestrial Systems 109
6.3.1	Trophic Cascades 109
6.3.2	Community Dynamics and Its Regulation 111
6.3.3	Nutrient Cycles 112
6.3.3.1	The Nitrogen Cycle 113
6.3.3.2	The Sulfur Cycle 116
6.3.3.3	The Phosphorus Cycle 119
6.3.3.4	The Carbon Cycle 120
	References 120
	Further Reading 120
	Part III: Biochemical Response to Physicochemical Stress (Abiotic Stress) 123
7	Information Processing and Survival Strategies 125
	<i>Ingo Heilmann</i>
7.1	The Stress Concept – Plants and Their Environment 125
7.2	Plant Signal Transduction and the Induction of Stress Responses 126
7.3	Phytohormones 130
7.3.1	Functionality of Phytohormones 130
7.3.2	Signal Transduction through Nuclear Derepression of Gene Expression 131
7.3.2.1	Auxins 131
7.3.2.2	Jasmonic Acid 133
7.3.2.3	Gibberellins 135
7.3.3	Signal Transduction through Other, Nonmembrane-Associated Receptors 136
7.3.3.1	Abscisic Acid 136

7.3.3.2	Salicylic Acid	137
7.3.4	Signal Transduction through Transmembrane-Receptors	138
7.3.4.1	Cytokinins	138
7.3.4.2	Ethylene	139
7.3.4.3	Brassinosteroids	141
7.4	Other Signaling Molecules	141
7.4.1	Phytochromes and Cryptochromes	141
7.4.2	Strigolactones and Nodulation Factors	141
7.4.3	Reactive Oxygen Species and Nitric Oxide	142
7.4.4	Peptide Signals	143
7.4.5	Volatile Signals	143
7.4.6	Polyamines	145
7.4.7	Phosphoinositides and Inositolpolyphosphates	145
7.4.8	Guanine Nucleotide-Binding Proteins	146
7.4.9	Ion Channels	148
7.5	Signal Transduction by Protein Phosphorylation	148
7.6	The Calcium Signaling Network	149
7.7	Stress-Induced Modulation of Gene Expression by microRNAs	150
	References	152
	Further Reading	152
8	Oxygen	155
	<i>Karl-Josef Dietz</i>	
8.1	Chemical Nature of Oxygen and Reactive Oxygen Species	155
8.2	Oxygen Metabolism	156
8.2.1	Photosynthesis and Oxygen Metabolism	156
8.2.2	Mitochondrion and Respiration	158
8.2.3	Oxygen Metabolism in Peroxisomes	159
8.2.4	Membrane-Associated Oxygen Metabolism	159
8.3	Oxygen Sensing	160
8.4	Antioxidant Defense	161
8.5	Reactive Oxygen Species in Abiotic Stresses	162
8.6	Reactive Oxygen Species in Biotic Interactions	164
8.7	Cell Signaling Function of Reactive Oxygen Species	165
	References	168
	Further Reading	168
9	Light	171
	<i>Thomas Kretsch</i>	
9.1	Principles of Light Detection and Photoreceptor Function	171
9.1.1	Modular Domain Structure of Photoreceptors	171
9.1.2	Identification and Classification of Photoreceptor Molecules	171
9.1.3	COP1-Containing E3 Ubiquitin Ligase Complexes: Central Components of Light Signaling in Plants	173
9.2	Sensing of UV-B Light	175
9.2.1	Effects of UV-B Light	175
9.2.2	UV-B Light Reception and Signal Transduction	175
9.3	The LOV Domain: A Variable Molecular Building Block of Many Blue and UV-A Light Sensors	176
9.3.1	LOV Chromophore-Binding Domains	176
9.3.2	Phototropins and Neochromes	176
9.3.3	Zeitlupe-Like Proteins	176
9.3.4	White Collar Light Sensors	178
9.4	Cryptochromes	179
9.4.1	Physiological Functions	179
9.4.2	Cryptochrome Structure and Light Signaling	179

9.5	Phytochromes	180
9.5.1	The Light Sensor Module of Phytochromes	180
9.5.2	Physiological Functions of Phytochromes	181
9.5.3	Light Signal Transduction of Phytochromes	182
9.5.4	The Unique Function of Phytochrome A	183
9.6	Other Photoreceptor Systems	185
9.7	Flavonoid Biosynthesis in Plants – a Model for a Light-Regulated Adaptation Process	185
9.7.1	Flavonoids as Light Protectors	185
9.7.2	Light Regulation of the Flavonoid Biosynthesis Pathway	186
	References	187
	Further Reading	188
10	Water	191
	<i>Wiebke Zschiesche and Klaus Humbeck</i>	
10.1	Water: the Essence of Life	191
10.2	Water Balance in Plants	192
10.2.1	Water Flow	192
10.2.2	Loss of Water by Stomatal Transpiration	193
10.3	Drought Stress	194
10.3.1	Drought Stress, a Worldwide Challenge	194
10.3.2	Cellular Disturbances	194
10.3.3	Adaptations to Survive Drought Stress	194
10.3.4	Molecular Mechanism of Drought Stress Tolerance	197
10.3.4.1	Downstream Signal Transduction	197
10.3.4.2	Role of Abscisic acid	198
10.3.4.3	Transcriptional Control Mechanism	198
10.3.4.4	Drought Stress Genes	199
10.4	Cold Stress and Freezing	200
10.4.1	Lowering of Temperature	200
10.4.2	Requirements of Ice Crystal Formation	200
10.4.3	Strategies to Survive Low Temperatures	200
10.5	Salinity	201
10.5.1	Salinity, an Increasing Problem in Agriculture	201
10.5.2	Salt Stress Alters Plant Functions	201
10.5.3	Salinity Tolerance	202
10.5.4	Signal Sensing and Transcriptional and Posttranscriptional Control	202
10.5.5	Target Processes	203
10.6	Flooding Stress	205
10.6.1	Causes of Flooding	205
10.6.2	Effects on Cellular Energy Status	205
10.6.3	Acclimation to Hypoxia	205
	References	206
	Further Reading	206
11	Mineral Deficiencies	209
11.1	Mineral Requirement and Insufficiencies	209
	<i>Edgar Peiter</i>	
11.1.1	The Essentiality of Mineral Nutrients for Plants	209
11.1.2	The Availability of Mineral Nutrients in the Soil	211
11.1.3	Mineral Nutrient Movement Toward and Uptake by the Plant Root	212
11.1.4	Plant Responses to a Variable Mineral Nutrient Availability	214
11.1.4.1	Nitrogen	214
11.1.4.2	Phosphorus	215
11.1.4.3	Iron	218
11.1.4.4	Zinc	221

11.1.4.5	Copper	222
11.2	Carnivorous Plants and Fungi	224
	<i>Gerd-Joachim Krauss and Gudrun Krauss</i>	
11.2.1	Habitats, Diversity and Evolution	224
11.2.2	Trapping Devices	226
11.2.3	Prey Digestion and Nutrient Utilization	229
11.2.4	Coevolutionary Strategies of the Carnivorous Life Style: Pitcher Plants	232
	References	233
	Further Reading	234
12	Excess of Metals	237
	<i>Dietrich H. Nies, Eva Freisinger, and Gerd-Joachim Krauss</i>	
12.1	Properties of Transition Metals	237
12.2	Metal Transport through Cell Membranes	238
12.3	Biochemistry of the Minor Biometals: Essential, Desired, but Also Toxic	240
12.3.1	Oxyanions: Molybdate and Its Neighbors	240
12.3.2	Manganese: the Electron Buffer	240
12.3.3	Transition Metal Cations in Octahedral Complexes	241
12.3.4	Iron: Transmitter of Single Electrons	241
12.3.5	Cobalt and Nickel: between Iron and Zinc	242
12.3.6	Copper: the Oxygen-Handle	243
12.3.7	Zinc (and Exceptionally Cadmium): Non Redox-Active Transition Metals	244
12.4	Biochemistry of Chemical Elements Without Known Biological Functions	244
12.4.1	Cadmium and Lead	244
12.4.2	The Noble Metals Silver and Gold	245
12.4.3	Mercury	245
12.4.4	Arsenate, Arsenite and Antimonite	246
12.5	Metal-Binding Peptides and Proteins Involved in Transition Metal Homeostasis	246
12.5.1	Function of Intracellular Metal-Binding Polypeptides	246
12.5.2	Copper Chaperones	246
12.5.3	COG0523 Metal Chaperones	246
12.5.4	Glutathione and Related Compounds	247
12.5.5	Phytochelatins	248
12.5.6	Metallothioneins and Their Ubiquitous Features	248
12.6	Interaction of Plants and Fungi with Metals	251
12.6.1	Avoidance and Tolerance Mechanisms in Plants	251
12.6.2	Metal Hyperaccumulators	252
12.6.3	Interaction of Plants with Individual Metals	252
12.6.4	Avoidance and Tolerance Mechanisms in Fungi	254
	References	256
	Further Reading	256
13	Xenobiotics from Human Impacts	259
	<i>Magali Solé and Dietmar Schlosser</i>	
13.1	Xenobiotics: from Emission to Cellular Uptake	259
13.1.1	Emission, Dispersal, Fate Processes, and Bioavailability of Xenobiotics	259
13.1.2	Cellular Uptake of Xenobiotics	260
13.2	Adverse Effects of Xenobiotics: from Cells to Ecosystems	265
13.2.1	Effects at the Level of Individuals and Below	265
13.2.2	Effects at Higher Levels of Biological Organization: Populations, Communities, and Ecosystems	267
13.3	Organismal Responses: Biochemical Elimination of Xenobiotics	268
13.3.1	General Aspects of Biodegradation and Biotransformation Reactions	268
13.3.2	Initial Biochemical Attack on Xenobiotics – Phase I Reactions	273
13.3.3	Conjugate Formation from Functionalized Xenobiotics – Phase II Reactions	273

- 13.3.4 Further Modification, Excretion, and Deposition of Xenobiotics and Their Metabolites – Phase III Reactions 274
- References 274
- Further Reading 274

Part IV: Organismal Interactions (Biotic Stress) 277

- 14 The Biofilm Mode of Life 279
 - Hans-Curt Flemming*
 - 14.1 What are Biofilms? 279
 - 14.2 Environmental Roles of Biofilms 280
 - 14.3 Life Cycle of Biofilms 281
 - 14.4 Investigation of Biofilms 283
 - 14.5 The Matrix: Extracellular Polymeric Substances 284
 - 14.5.1 The Extracellular Polymeric Substances (EPS) 284
 - 14.5.2 Predation and Antagonism 285
 - 14.5.3 VBNC Forms and Persisters 285
 - 14.5.4 Motility within Biofilms 285
 - 14.5.5 Matrix Stability 286
 - 14.5.6 Gradients and Heterogeneity in the Biofilm Matrix 287
 - 14.6 Communication in Biofilms 287
 - 14.7 Enhanced Resistance of Biofilm Organisms 288
 - 14.8 Emergent Properties of the Biofilm Mode of Life 290
 - References 291
 - Further Reading 291
- 15 Rhizosphere Interactions 293
 - Silvia D. Schrey, Anton Hartmann, and Rüdiger Hampp*
 - 15.1 Bacterial Communities in the Rhizosphere 294
 - 15.1.1 Plant Growth Promoting Rhizobacteria 294
 - 15.1.2 Plant Disease Suppression by Rhizobacteria – Indirect Plant Growth Promotion 297
 - 15.1.3 Nitrogen-Fixing Plant–Bacterium Symbiosis 299
 - 15.1.4 Actinobacteria: Prolific Producers of Natural Compounds 300
 - 15.1.5 Plant Pathogenic Soil Bacteria 300
 - 15.1.6 Plant-Associated Bacteria as (Opportunistic) Human Pathogens 301
 - 15.2 Fungi of the Rhizosphere 303
 - 15.2.1 Mycorrhiza: Chemical Dialogue between Plants and Mycorrhizal Fungi 303
 - 15.2.2 Chemical Cross Talk between Plant Roots and Pathogenic Fungi: Signaling Involved in Recognition 304
 - 15.2.3 Fungus–Bacterium Interactions 305
 - 15.2.3.1 Mycorrhiza Helper Bacteria 306
 - 15.2.3.2 Bacterial Mycophagy 306
 - 15.3 Plant–Plant Interactions 306
 - 15.3.1 Plant–Plant Interaction via Fungal Networks 307
 - 15.3.2 Parasitic Plants 307
 - 15.3.3 Allelopathy 308
 - References 310
 - Further Reading 310
- 16 Plant-Animal Dialogues 313
 - Susanne Preiß, Jörg Degenhardt, and Jonathan Gershenzon*
 - 16.1 The Flower Pollinator System 313
 - 16.1.1 General Aspects 313
 - 16.1.2 Flower Color 313
 - 16.1.3 Nectar 317
 - 16.1.4 Floral Scent 317

16.1.5	Flower Pollinator Interactions Are Guided by Complex Patterns of Biochemical Cues	318
16.2	Ant–Plant–Fungus Mutualism, a Three-Way Interaction	319
16.3	Phenolics in the Interaction between Plant and Animals	320
16.3.1	Salicin – a Defense Compound in <i>Salix</i> Species	320
16.3.2	Flavonoid Signals Modulate Herbivore Behavior	321
16.4	Alkaloids in the Interaction between Plants and Animals	321
16.4.1	The Monarch Butterfly	321
16.4.2	Optimal Defense in the Wild Ragwort <i>Senecia jacobea</i>	322
16.4.3	Endophytes and Plant Parasitic Nematodes Mediate Plant–Herbivore Interactions	323
16.4.4	Glucosinolates Are Pungent Antiherbivore Defenses of Mustards and Related Plant Species	324
16.5	Terpenes in Plant Defense	325
16.5.1	Monoterpene-Based Defenses of Thyme	325
16.5.2	Mammals, Wood Ants, and Scots Pine Trees	326
16.5.3	Ecological Role of Herbivore-Induced Plant Volatiles	326
16.5.4	Tritrophic Interactions with Herbivores and Herbivore Enemies	326
16.5.5	Interference of Plant Volatile Terpenes with Insect Pheromones	328
16.5.6	Terpene-Mediated Interactions between Plants Affect Herbivores	329
	References	330
	Further Reading	330

Part V: The Methodological Platform 331

17	Sensing of Pollutant Effects and Bioremediation	333
	<i>Gerd-Joachim Krauss and Dietmar Schlosser</i>	
17.1	Pollutant Effect and Approaches to Characterize Exposure	333
17.2	Ecological Restoration and Bioremediation	335
17.2.1	Biological Ecosystem Components Mitigating Environmental Pollution	335
17.2.2	Present and Future Directions	338
	References	339
	Further Reading	340
18	The -Omics Tool Box	343
	<i>Dirk Schaumlöffel</i>	
18.1	Genomics	343
18.1.1	First-Generation Sequencing	343
18.1.2	Next-Generation Sequencing (NGS)	344
18.1.2.1	Pyrosequencing: 454/Roche FLX™ Pyrosequencer	344
18.1.2.2	Reversible Dye Terminator Technology: Illumina Genome Analyzer™	344
18.1.2.3	Sequencing by Ligation: Applied Biosystems SOLiD™ Sequencer	345
18.1.3	Understanding the Genome: Genes and Their Functions	345
18.2	Transcriptomics	345
18.2.1	DNA Microarrays	346
18.2.2	Transcriptome Sequencing by NGS Platforms	346
18.3	Proteomics	346
18.3.1	Proteomic Strategies	347
18.3.1.1	Sample Preparation for Proteomics	347
18.3.1.2	Protein Separation	347
18.3.1.3	Differential Proteome Analysis	348
18.3.1.4	Identification of Proteins	348
18.3.1.5	Analytical Protein Microarrays	348
18.3.1.6	Bottom-Up Proteomics	348
18.3.1.7	Shotgun Proteomics	349
18.3.1.8	Top-Down Proteomics	350
18.3.1.9	<i>De novo</i> Sequencing of Proteins	350
18.3.2	Protein Mass Spectrometry	350

18.3.2.1	Ion Sources for Biomolecules	350
18.3.2.2	Mass Analyzers	351
18.3.2.3	Tandem Mass Spectrometry	352
18.3.2.4	Element Mass Spectrometry: ICP MS	353
18.3.3	Quantitative Proteomics	354
18.3.3.1	Label-Free Relative Quantification	354
18.3.3.2	Chemical and Metabolic Labeling with Stable Isotopes and Metals for Relative Quantification	354
18.3.3.3	Absolute Quantification Strategies	354
18.3.4	Determination of the 3D Protein Structure and Functional Evaluation	355
18.3.4.1	X-Ray Crystallography and Protein NMR	355
18.3.4.2	Functional Protein Microarrays	355
18.3.5	Meta-Omics Approaches	355
18.4	Metabolomics	356
18.4.1	Analytical Strategies	356
18.4.2	Metabolomics in Single Cells	358
18.4.3	Integrating -Omics Techniques	360
18.5	Metallomics	360
18.5.1	Analytical Strategies	361
18.5.1.1	Element Mass Spectrometry (ICP MS)	361
18.5.1.2	Coupling Techniques	361
18.5.1.3	Elemental Imaging Techniques	361
18.5.1.4	Bioinformatic Approaches	362
18.5.2	Functional Connections between DNA, Proteins, Metabolites, and Metals	362
18.5.3	Metallothiolomics	362
	References	365
	Further Reading	365
19	Microscope Techniques and Single Cell Analysis	367
	<i>Bettina Hause and Gerd Hause</i>	
19.1	Visualization Principles	367
19.1.1	Light Microscopy	367
19.1.1.1	Bright Field Microscopy	367
19.1.1.2	Dark Field Microscopy	370
19.1.1.3	Fluorescence Microscopy	370
19.1.2	Advanced Fluorescence Techniques/Generation of Optical Sections	370
19.1.2.1	Confocal Laser Scanning Microscopy (CLSM)	370
19.1.2.2	Multiphoton Microscopy	371
19.1.2.3	Spinning Disk Microscopy	371
19.1.2.4	Stimulated Emission Depletion (STED), 4Pi Microscopy, and 4Pi-STED-Microscopy	371
19.1.2.5	Photo-Activated Localization Microscopy (PAL-M)/Stochastic Optical Reconstruction Microscopy (STORM)	371
19.1.2.6	Structured Illumination Microscopy (SIM)	372
19.1.3	Electron Microscopy	372
19.1.3.1	Transmission Electron Microscopy (TEM)	372
19.1.3.2	Scanning Electron Microscopy (SEM)	373
19.1.4	Scanning Probe Microscopy	373
19.2	Preparation of Biological Materials	373
19.2.1	Chemical Fixation	373
19.2.2	Physical Fixation/Cryofixation	374
19.3	Detection Methods – from Macromolecules to Ions	375
19.3.1	Histological Staining	375
19.3.2	Autoradiography	375
19.3.3	Immunocytochemistry (IC)	376

19.3.4	<i>In situ</i> Hybridization (ISH)	377
19.3.5	Reporter Molecules in Transgenic Approaches	378
19.4	Single Cell Technologies	380
	References	382
	Further Reading	382
	Glossary	383
	Index	397