

Contents

Preface *xi*

1	Super-Resolution Microscopy (SRM): Brief Introduction	1
	<i>Zhigang Yang, Soham Samanta, and Yingchao Liu</i>	
1.1	Optical Microscopy	1
1.1.1	History and Background	1
1.2	Specialized Optical Microscopes	3
1.2.1	Inverted Microscopes	4
1.2.2	Confocal Microscopes	4
1.3	Optical Diffraction Limit	5
1.4	Super-Resolution Microscopy: Overcoming the Diffraction Limit	6
1.5	Near-Field Scanning Optical Microscopy	7
1.6	Far-Field Super-Resolution Microscopy	8
1.7	Fluorescent Probes for Super-Resolution Microscopy	9
1.8	Image Analysis Algorithms	10
1.9	Applications	11
1.10	Outline of the Content of Succeeding Chapters	11
	Acknowledgment	11
	References	12
2	Point Spread Function Engineering SRM	15
	<i>Wei Yan, Luwei Wang, Yinru Zhu, Jialin Wang, and Ruijie Xiang</i>	
2.1	Stimulated Emission Depletion Microscopy (STED)	15
2.1.1	Principles of STED	15
2.1.2	Three-Dimensional STED	16
2.1.3	Multi-Color and Multi-Photon STED	18
2.1.4	Strategies to Reduce STED Power	20
2.1.4.1	Time-Gated STED Technology	21
2.1.4.2	Offline Gated STED Technology	22
2.1.4.3	Phasor-Plot Analysis of STED-FLIM	23
2.1.4.4	STED Super-Resolution Imaging with Quantum Dots	24
2.1.4.5	Temporal and Spatial Modulation STED	26
2.1.4.6	STED Super-Resolution Imaging Based on Adaptive Optics	27

2.1.5	Live Cell Imaging	29
2.2	Ground State Depletion (GSD) Microscopy	32
2.2.1	Principles of GSD	32
2.2.2	Advantages and Disadvantages of GSD	33
2.2.3	Applications of GSD	34
2.3	Reversible Saturable Optical Fluorescence Transition Microscopy	34
2.3.1	Improvement in the RESOLFT System	36
2.3.1.1	Parallelized RESOLFT Microscopy	36
2.3.1.2	Two-Photon RESOLFT	37
2.3.1.3	Dual-Channel RESOLFT Imaging	37
2.3.1.4	Three-Dimensional Imaging	37
2.3.2	Fluorescent Probe for RESOLFT Microscopy	38
2.3.2.1	Early-Stage: Fluorescent Protein	38
2.3.2.2	Improvement Based on Fluorescence Dynamics	39
2.3.2.3	Improvement in Other Properties	39
2.3.2.4	Organic Fluorophores	41
2.3.3	Advances in RESOLFT Application	42
2.3.3.1	Application in Life Science	42
2.3.3.2	Application in Writing and Manufacturing at the Nanoscale	43
2.4	Conclusion	44
	Acknowledgment	44
	References	45
3	Single-Molecule Localization Microscopy (SMLM)	51
	<i>Danying Lin, Yingying Jing, Pengfa Chen, Zekai Wu, Zhenquan Gong, Jiao Zhang, Arup Tarai, and Xuehua Wang</i>	
3.1	Main Idea of SMLM	51
3.2	Stochastic Optical Reconstruction Microscopy (STORM)	53
3.2.1	Implementation of STORM	53
3.2.1.1	Typical Optical Setup	53
3.2.1.2	Two Key Steps	54
3.2.1.3	Derivative Forms	56
3.2.2	Key Consideration in STORM	57
3.2.3	Multi-Color STORM	59
3.2.4	Three-Dimensional STORM	61
3.2.4.1	PSF Engineering	63
3.2.4.2	Multi-Focal Plane Imaging	67
3.2.4.3	Other Methods	68
3.2.5	Live Cell STORM Imaging	69
3.3	Photo-Activated Localization Microscopy (PALM)	72
3.3.1	Basic Principle of PALM and Differences with STORM	72
3.3.2	Single-Particle Tracking PALM (sptPALM)	73
3.4	Point Accumulation for Imaging in Nanoscale Topography (PAINT)	75
3.4.1	Basic Principle, Advantages, and Disadvantages of PAINT	75

3.4.2	Modifications of PAINT	76
3.4.2.1	uPAINT	76
3.4.2.2	DNA-PAINT and Exchange-PAINT	76
3.5	Single-Molecule Localization Algorithms	78
3.5.1	Algebraic Algorithms	78
3.5.2	Single-Emitter Fitting Algorithms	79
3.5.3	Multi-Emitter Fitting Algorithms	80
3.5.4	CS Algorithms	82
3.5.5	Other Methods	83
3.6	MINFLUX	84
3.7	Conclusion	84
	Acknowledgment	85
	References	85
4	Fluorescence Fluctuation-Based Super-Resolution Imaging	93
	<i>Xuehua Wang and Bin Yu</i>	
4.1	Stochastic Optical Fluctuation Imaging (SOFI)	94
4.1.1	XC-SOFI	95
4.1.2	bSOFI	96
4.1.3	fSOFI	96
4.1.4	Speckle SOFI	97
4.2	Other Techniques	99
4.2.1	VISion	99
4.2.2	Bayesian Analysis of Blinking and Bleaching (3B)	99
4.2.3	Super-resolution Radial Fluctuations (SRRF)	100
4.2.4	Entropy-Based Super-Resolution Imaging (ESI)	101
4.2.5	Multiple Signal Classification Algorithm for Super-resolution Fluorescence Microscopy (MUSICAL)	102
4.3	Applications of Fluorescence Fluctuation-Based SRM Methods	102
4.4	Conclusion	103
	Acknowledgment	104
	References	104
5	Structured Illumination Microscopy	107
	<i>Bin Yu, Siwei Li, Faiz Wali, and Rong Xu</i>	
5.1	Introduction	107
5.2	Wide-field SIM	107
5.2.1	Basics of SIM	108
5.2.2	SR-SIM	110
5.2.2.1	Conventional Grating-Based SIM	111
5.2.2.2	Blind SIM	113
5.2.2.3	Grazing Incidence SIM (GI-SIM)	116
5.2.2.4	Hessian-SIM	117
5.2.3	Summary	118

5.3	Point-Scanning SIM	118
5.3.1	Principle of PS-SIM	119
5.3.2	PS-SIM Based on the Digital Method	121
5.3.3	PS-SIM Based on the Optical Method	123
5.3.4	Special PS-SIM	126
5.3.5	Summary	127
5.4	Conclusions and Future Prospects	128
	Acknowledgement	129
	References	129
6	Deep Learning-Based SR Microscopy	135
	<i>Jia Li and Jianhui Liao</i>	
6.1	Introduction	135
6.2	Fundamentals of Deep Networks	135
6.2.1	Neural Networks	136
6.2.2	Activation Function and Layers	137
6.2.2.1	Sigmoid	138
6.2.2.2	Softmax	139
6.2.2.3	Rectified Linear Unit (ReLU)	139
6.2.2.4	Leaky ReLU	140
6.2.3	Training and Data	141
6.2.3.1	Gradient Descent	141
6.2.3.2	Backpropagation	142
6.2.3.3	Data	143
6.2.4	Loss Functions	144
6.3	Deep Learning for SR Image Reconstruction	144
6.3.1	2D Reconstruction Methods	145
6.3.1.1	Convolutional Neural Networks (CNNs)	145
6.3.1.2	Convolutional Layer	146
6.3.1.3	Pooling Layer	147
6.3.1.4	Properties	147
6.3.1.5	SR Image Reconstruction with CNN	148
6.3.1.6	Generative Adversarial Networks (GANs)	149
6.3.1.7	Game Theory	150
6.3.1.8	Architecture	150
6.3.1.9	Training	150
6.3.1.10	SR Image Reconstruction with GAN	151
6.3.2	3D Reconstruction Methods	153
6.4	Challenges of Deep Learning-Based Methods	153
6.4.1	Data Limitations	154
6.4.2	Training Obstacles	154
6.4.3	Result Reliability	155
6.5	Conclusion	156
	References	158

7	Fluorescent Materials for Super-Resolution Imaging	163
	<i>Zhigang Yang and Soham Samanta</i>	
7.1	Fluorescent Probes for Super-Resolution Imaging	163
7.2	Fluorescent Proteins	164
7.2.1	FPS for STED and RESOLFT Nanoscopy	164
7.2.2	FPS for SMLM-Based SRM	169
7.2.3	FPS for SIM and Other New SRM Techniques	176
7.3	Small-Molecule Fluorescent Probes	176
7.3.1	Organic Fluorescent Probes for STED	176
7.3.1.1	Rhodamine-Based Fluorescent Probes for STED Imaging	177
7.3.1.2	Diverse Fluorescent Probes for STED Imaging	179
7.3.1.3	Phosphole-Based Fluorescent Probes for STED Imaging	183
7.3.2	Organic Fluorescent Probes for SMLM	185
7.3.2.1	Xanthene/Rhodamine Dyes	185
7.3.2.2	Cyanine Dyes	191
7.3.2.3	BODIPY and Oxazine/Spiropyran Dyes	194
7.3.2.4	Other Dyes (2-dithienylethenes and Cicyanodihydrofurans)	198
7.3.3	Organic Fluorescent Probes for SIM	199
7.4	Fluorescent Metal Complexes for SRM	202
7.4.1	Fluorescent Metal Complexes for STED	202
7.4.2	Fluorescent Metal Complexes for SMLM	203
7.4.3	Fluorescent Metal Complexes for SIM	204
7.5	Fluorescent Nanomaterials (Nanoparticles/Quantum Dots/Carbon Nanotubes/Carbon Dots (CDs)/Polymers Dots) for SRM	204
7.5.1	Fluorescent Nanomaterials for STED	205
7.5.2	Organic Nanoparticles	205
7.5.3	Inorganic Nanoparticles	211
7.5.4	Fluorescent Nanomaterials for SMLM	213
7.5.5	Fluorescent Nanomaterials for SIM	216
	Acknowledgment	218
	References	219
8	Conclusion and Future Perspectives	229
	<i>Zhigang Yang, Soham Samanta, and Junle Qu</i>	
	Index	235

