

Contents

Contributors	xiii
Preface	xix
CHAPTER 1 Concepts in Quantitative Fluorescence Microscopy	1
Jennifer C. Waters, Torsten Wittmann	
1.1 Accurate and Precise Quantitation	2
1.2 Signal, Background, and Noise	3
1.3 Optical Resolution: The Point Spread Function	7
1.4 Choice of Imaging Modality	7
1.5 Sampling: Spatial and Temporal	8
1.6 Postacquisition Corrections	12
1.7 Making Compromises	15
1.8 Communicating Your Results	16
Acknowledgment	16
References	16
CHAPTER 2 Practical Considerations of Objective Lenses for Application in Cell Biology	19
Stephen T. Ross, John R. Allen, Michael W. Davidson	
Introduction	20
2.1 Optical Aberrations	20
2.2 Types of Objective Lenses	22
2.3 Objective Lens Nomenclature	25
2.4 Optical Transmission and Image Intensity	25
2.5 Coverslips, Immersion Media, and Induced Aberration	27
2.6 Considerations for Specialized Techniques	31
2.7 Care and Cleaning of Optics	32
Conclusions	34
References	34
CHAPTER 3 Assessing Camera Performance for Quantitative Microscopy	35
Talley J. Lambert, Jennifer C. Waters	
3.1 Introduction to Digital Cameras for Quantitative Fluorescence Microscopy	36
3.2 Camera Parameters	37
3.3 Testing Camera Performance: The Photon Transfer Curve	44
References	52

CHAPTER 4	A Practical Guide to Microscope Care and Maintenance	55
	Lara J. Petrak, Jennifer C. Waters	
	Introduction	56
4.1	Cleaning	58
4.2	Maintenance and Testing	66
4.3	Considerations for New System Installation	74
	Acknowledgments	75
	References	75
CHAPTER 5	Fluorescence Live Cell Imaging	77
	Andreas Ettinger, Torsten Wittmann	
5.1	Fluorescence Microscopy Basics	78
5.2	The Live Cell Imaging Microscope	79
5.3	Microscope Environmental Control	83
5.4	Fluorescent Proteins	87
5.5	Other Fluorescent Probes	92
	Conclusion	93
	Acknowledgments	93
	References	93
CHAPTER 6	Fluorescent Proteins for Quantitative Microscopy: Important Properties and Practical Evaluation	95
	Nathan Christopher Shaner	
6.1	Optical and Physical Properties Important for Quantitative Imaging	96
6.2	Physical Basis for Fluorescent Protein Properties	99
6.3	The Complexities of Photostability	101
6.4	Evaluation of Fluorescent Protein Performance <i>in Vivo</i>	106
	Conclusion	108
	References	109
CHAPTER 7	Quantitative Confocal Microscopy: Beyond a Pretty Picture	113
	James Jonkman, Claire M. Brown, Richard W. Cole	
7.1	The Classic Confocal: Blocking Out the Blur	114
7.2	You Call that Quantitative?	118
7.3	Interaction and Dynamics	123
7.4	Controls: Who Needs Them?	125
7.5	Protocols	127
	Conclusions	133
	References	133

CHAPTER 8	Assessing and Benchmarking Multiphoton Microscopes for Biologists.....	135
	Kaitlin Corbin, Henry Pinkard, Sebastian Peck, Peter Beemiller, Matthew F. Krummel	
	Introduction: Practical Quantitative 2P Benchmarking	136
8.1	Part I: Benchmarking Inputs.....	136
8.2	Part II: Benchmarking Outputs.....	144
8.3	Troubleshooting/Optimizing.....	150
8.4	A Recipe for Purchasing Decisions.....	150
	Conclusion.....	151
	Acknowledgments	151
	References.....	151
CHAPTER 9	Spinning-disk Confocal Microscopy: Present Technology and Future Trends.....	153
	John Oreopoulos, Richard Berman, Mark Browne	
9.1	Principle of Operation	153
9.2	Strengths and Weaknesses.....	155
9.3	Improvements in Light Sources	157
9.4	Improvements in Illumination	157
9.5	Improvements in Optical Sectioning and FOV.....	162
9.6	New Detectors.....	166
9.7	A Look into the Future	167
	References.....	171
CHAPTER 10	Quantitative Deconvolution Microscopy	177
	Paul C. Goodwin	
	Introduction.....	178
10.1	The Point-spread Function.....	180
10.2	Deconvolution Microscopy.....	182
10.3	Results	187
	Conclusion.....	191
	References.....	191
CHAPTER 11	Light Sheet Microscopy	193
	Michael Weber, Michaela Mickoleit, Jan Huiskens	
	Introduction.....	194
11.1	Principle of Light Sheet Microscopy	195
11.2	Implementations of Light Sheet Microscopy	198
11.3	Mounting a Specimen for Light Sheet Microscopy.....	203
11.4	Acquiring Data.....	205
11.5	Handling of Light Sheet Microscopy Data	210
	References.....	212

CHAPTER 12 DNA Curtains: Novel Tools for Imaging Protein–Nucleic Acid Interactions at the Single-Molecule Level	217
Bridget E. Collins, Ling F. Ye, Daniel Duzdevich, Eric C. Greene	
Introduction	218
12.1 Overview of TIRFM	219
12.2 Flow Cell Assembly	220
12.3 Importance of the Lipid Bilayer	221
12.4 Barriers to Lipid Diffusion	222
12.5 Different Types of DNA Curtains	223
12.6 Using DNA Curtains to Visualize Protein–DNA Interactions	226
12.7 Future Perspectives	232
Acknowledgments	232
References	232
CHAPTER 13 Nanoscale Cellular Imaging with Scanning Angle Interference Microscopy	235
Christopher DuFort, Matthew Paszek	
Introduction	236
13.1 Experimental Methods and Instrumentation	241
13.2 Image Analysis and Reconstruction	250
Conclusion	250
Acknowledgments	251
References	251
CHAPTER 14 Localization Microscopy in Yeast	253
Markus Mund, Charlotte Kaplan, Jonas Ries	
Introduction	254
14.1 Preparing the Yeast Strain	256
14.2 Considerations for the Choice of a Labeling Strategy	257
14.3 Preparing the Sample	260
14.4 Image Acquisition	264
14.5 Results	265
Summary	267
Acknowledgments	269
References	269
CHAPTER 15 Imaging Cellular Ultrastructure by PALM, iPALM, and Correlative iPALM-EM	273
Gleb Shtengel, Yilin Wang, Zhen Zhang, Wah Ing Goh, Harald F. Hess, Pakorn Kanchanawong	
Introduction	274

15.1 Principles.....	275
15.2 Methods.....	277
15.3 Future Directions	290
Acknowledgments	291
References.....	292
CHAPTER 16 Seeing More with Structured Illumination Microscopy.....	295
Reto Fiolka	
Introduction.....	296
16.1 Theory of Structured Illumination.....	297
16.2 3D SIM.....	302
16.3 SIM Imaging Examples	307
16.4 Practical Considerations and Potential Pitfalls	310
16.5 Discussion	311
References.....	312
CHAPTER 17 Structured Illumination Superresolution Imaging of the Cytoskeleton.....	315
Ulrike Engel	
Introduction.....	316
17.1 Instrumentation for SIM Imaging.....	316
17.2 Sample Preparation.....	322
17.3 Minimizing Spherical Aberration.....	324
17.4 Multichannel SIM.....	327
17.5 Live Imaging with SIM	330
Acknowledgments	331
References.....	331
CHAPTER 18 Analysis of Focal Adhesion Turnover: A Quantitative Live-Cell Imaging Example	335
Samantha J. Stehbens, Torsten Wittmann	
Introduction to Focal Adhesion Dynamics	335
18.1 FA Turnover Analysis	337
Acknowledgments	346
References.....	346
CHAPTER 19 Determining Absolute Protein Numbers by Quantitative Fluorescence Microscopy.....	347
Jolien Suzanne Verdaasdonk, Josh Lawrimore, Kerry Bloom	
Introduction.....	348
19.1 Methods for Counting Molecules.....	348
19.2 Protocol for Counting Molecules by Ratiometric Comparison of Fluorescence Intensity	356

Conclusions	361
References	361
CHAPTER 20 High-Resolution Traction Force Microscopy	367
Sergey V. Plotnikov, Benedikt Sabass, Ulrich S. Schwarz, Clare M. Waterman	
Introduction	368
20.1 Materials	374
20.2 Methods	381
References	392
CHAPTER 21 Experimenters' Guide to Colocalization Studies: Finding a Way Through Indicators and Quantifiers, in Practice	395
Fabrice P. Cordelières, Susanne Bolte	
Introduction	396
21.1 An Overview of Colocalization Approaches	397
Conclusion	406
References	407
CHAPTER 22 User-Friendly Tools for Quantifying the Dynamics of Cellular Morphology and Intracellular Protein Clusters	409
Denis Tsygankov, Pei-Hsuan Chu, Hsin Chen, Timothy C. Elston, Klaus M. Hahn	
Introduction	410
22.1 Automated Classification of Cell Motion Types	411
22.2 GUI for Morphodynamics Classification and Ready Representation of Changes in Cell Behavior Over Time	415
22.3 Results of Morphodynamics Classification	417
22.4 Geometry-based Segmentation of Cells in Clusters	418
22.5 GUI for Cell Segmentation and Quantification of Protein Clusters	421
22.6 Results for Quantifying Protein Clusters	424
22.7 Discussion	424
Acknowledgments	426
References	426
CHAPTER 23 Ratiometric Imaging of pH Probes	429
Bree K. Grillo-Hill, Bradley A. Webb, Diane L. Barber	
Introduction	430
23.1 Currently Used Ratiometric pH Probes	430

23.2 Applications	435
23.3 Protocols.....	438
Acknowledgments	445
References.....	445
CHAPTER 24 Toward Quantitative Fluorescence Microscopy with DNA Origami Nanorulers	449
Susanne Beater, Mario Raab, Philip Tinnefeld	
Introduction.....	450
24.1 The Principle of DNA Origami.....	452
24.2 Functionalizing DNA Origami Structures.....	452
24.3 DNA Origami as Fluorescence Microscopy Nanorulers	455
24.4 Brightness References Based on DNA Origami	457
24.5 Applications of DNA Origami Nanorulers for Visualizing Resolution.....	458
24.6 How to Choose an Appropriate Nanoruler for a Given Application.....	461
References.....	463
CHAPTER 25 Imaging and Physically Probing Kinetochores in Live Dividing Cells.....	467
Jonathan Kuhn, Sophie Dumont	
Introduction.....	468
25.1 Spindle Compression to Image and Perturb Kinetochores	469
25.2 Imaging Kinetochores Dynamics at Subpixel Resolution Via Two-Color Reporter Probes.....	477
Conclusion and Outlook	484
Acknowledgments	485
References.....	485
CHAPTER 26 Adaptive Fluorescence Microscopy by Online Feedback Image Analysis	489
Christian Tischer, Volker Hilsenstein, Kirsten Hanson, Rainer Pepperkok	
Introduction.....	490
26.1 Requirements for Adaptive Feedback Microscopy	492
26.2 Selected Applications	493
Acknowledgments	501
References.....	501

CHAPTER 27 Open-Source Solutions for SPIMage Processing.....	505
Christopher Schmied, Evangelia Stamatakis,	
Pavel Tomancak	
Introduction.....	506
27.1 Prerequisites	509
27.2 Overview of the SPIM Image-Processing Pipeline.....	512
27.3 Bead-Based Registration.....	513
27.4 Multiview Fusion.....	518
27.5 Processing on a High-Performance Cluster	524
27.6 Future Applications.....	525
References.....	527
CHAPTER 28 Second-Harmonic Generation Imaging of Cancer	531
Adib Keikhosravi, Jeremy S. Bredfeldt, Md. Abdul Kader	
Sagar, Kevin W. Eliceiri	
Introduction.....	532
28.1 SHG Physical and Chemical Background.....	532
28.2 SHG Instrumentation	533
28.3 Collagen Structure as a Biomarker	533
28.4 SHG in Cancer Research	535
28.5 Quantitative Analysis of SHG Images	541
Conclusion.....	541
References.....	542
Index.....	547