

Contents

Preface	v
I Considerations for Routine Imaging	
1 Entering the Portal: Understanding the Digital Image Recorded Through a Microscope	3
Kristin L. Hazelwood, Scott G. Olenych, John D. Griffin, Judith A. Cathcart, and Michael W. Davidson	
1.1 Introduction	3
1.2 Historical Perspective	4
1.3 Digital Image Acquisition: Analog to Digital Conversion	4
1.4 Spatial Resolution in Digital Images	6
1.5 The Contrast Transfer Function	8
1.6 Image Brightness and Bit Depth	10
1.7 Image Histograms	11
1.8 Fundamental Properties of CCD Cameras	12
1.9 CCD Enhancing Technologies	16
1.10 CCD Performance Measures	17
1.11 Multidimensional Imaging	21
1.12 The Point-Spread Function	24
1.13 Digital Image Display and Storage	28
1.14 Imaging Modes in Optical Microscopy	29
1.15 Summary	39
1.16 Internet Resources	41
References	41
2 Quantitative Biological Image Analysis	45
Erik Meijering and Gert van Cappellen	
2.1 Introduction	45
2.2 Definitions and Perspectives	46
2.3 Image Preprocessing	48
2.3.1 Image Intensity Transformation	50
2.3.2 Local Image Filtering	50

2.3.3	Geometrical Image Transformation	53
2.3.4	Image Restoration	55
2.4	Advanced Processing for Image Analysis	57
2.4.1	Colocalization Analysis	58
2.4.2	Neuron Tracing and Quantification	58
2.4.3	Particle Detection and Tracking	60
2.4.4	Cell Segmentation and Tracking	62
2.5	Higher-Dimensional Data Visualization	63
2.5.1	Volume Rendering	64
2.5.2	Surface Rendering	64
2.6	Software Tools and Development	66
	References	68
3	The Open Microscopy Environment: A Collaborative Data Modeling and Software Development Project for Biological Image Informatics	71
	Jason R. Swedlow	
3.1	Introduction	71
3.1.1	What Is OME?	72
3.1.2	Why OME – What Is the Problem?	72
3.2	OME Specifications and File Formats	74
3.2.1	OME Data Model	74
3.2.2	OME-XML, OME-TIFF and Bio-Formats	76
3.3	OME Data Management and Analysis Software	77
3.3.1	OME Server and Web User Interface	77
3.3.2	OMERO Server, Client and Importer	84
3.3.3	Developing Usable Tools for Imaging	89
3.4	Conclusions and Future Directions	90
	References	90
4	Design and Function of a Light-Microscopy Facility	93
	Kurt I. Anderson, Jeremy Sanderson, and Jan Peychl	
4.1	Introduction	93
4.2	Users	95
4.3	Staff	96
4.3.1	Workplace Safety	96
4.3.2	User Training	97
4.3.3	Equipment Management	97
4.4	Equipment	98
4.4.1	Large Equipment	98
4.4.2	Small Equipment	99
4.4.3	Tools	100
4.4.4	Imaging Facility Layout	100
4.5	Organization	103
4.5.1	Equipment-Booking Database	103

4.5.2	Fee for Service	106
4.5.3	Cost Matrix	107
4.5.4	Advisory Committees	111
4.6	Summary	112
	References	113
 II Advanced Methods and Concepts		
5	Quantitative Colocalisation Imaging: Concepts, Measurements, and Pitfalls	117
	Martin Oheim and Dongdong Li	
5.1	Introduction	117
5.1.1	One Fluorophore, One Image?	124
5.1.2	A Practical Example of Dual-Band Detection	135
5.2	Quantifying Colocalisation	137
5.2.1	‘Colour Merging’	137
5.2.2	Pixel-Based Techniques	139
5.2.3	Object-Based Techniques	147
5.3	Conclusions	150
	References	151
 6	 Quantitative FRET Microscopy of Live Cells	 157
	Adam D. Hoppe	
6.1	Introduction	157
6.2	Introductory Physics of FRET	158
6.3	Manifestations of FRET in Fluorescence Signals	160
6.3.1	Spectral Change (Sensitized Emission)	160
6.3.2	Fluorescence Lifetime	161
6.3.3	Polarization	162
6.3.4	Accelerated Photobleaching	162
6.4	Molecular Interaction Mechanisms That Can Be Observed by FRET	163
6.4.1	Conformational Change	164
6.4.2	Molecular Association	164
6.4.3	Molecular Assembly	164
6.5	Measuring Fluorescence Signals in the Microscope	165
6.6	Methods for FRET Microscopy	167
6.6.1	Photobleaching Approaches	168
6.6.2	Sensitized Emission	170
6.6.3	Spectral Fingerprinting and Matrix Notation for FRET	173
6.6.4	Polarization	174
6.7	Fluorescence Lifetime Imaging Microscopy for FRET	175
6.8	Data Display and Interpretation	176
6.9	FRET-Based Biosensors	177

6.10	FRET Microscopy for Analyzing Interaction Networks in Live Cells	178
6.11	Conclusion	180
	References	180
7	Fluorescence Photobleaching and Fluorescence Correlation Spectroscopy: Two Complementary Technologies To Study Molecular Dynamics in Living Cells	183
	Malte Wachsmuth and Klaus Weisshart	
7.1	Introduction	183
7.1.1	FRAP and Other Photobleaching Methods	184
7.1.2	FCS and Other Fluctuation Analysis Methods	186
7.1.3	Comparing and Combining Techniques	187
7.2	Fundamentals	189
7.2.1	Fluorescent Labelling	189
7.2.2	Microscope Setup	191
7.2.3	Diffusion and Binding in Living Cells	193
7.2.4	Fluorescence, Blinking, and Photobleaching	194
7.2.5	Two-Photon Excitation	195
7.3	How To Perform a FRAP Experiment	196
7.3.1	The Principle of Imaging-Based FRAP	196
7.3.2	Choosing and Optimising the Experimental Parameters	197
7.3.3	Quantitative Evaluation	200
7.3.4	Controls and Potential Artefacts	203
7.4	How To Perform an FCS Experiment	205
7.4.1	The Principle of FCS	205
7.4.2	Instrument Alignment and Calibration	208
7.4.3	Setting Up an Experiment	212
7.4.4	Types of Applications	213
7.4.5	Potential Artefacts	215
7.5	How To Perform a CP Experiment	217
7.5.1	The Principle of CP	217
7.5.2	Choosing and Optimising the Experimental Parameters	218
7.5.3	Quantitative Evaluation	219
7.5.4	Controls and Potential Artefacts	220
7.6	Quantitative Treatment	221
7.6.1	Fluorescence Recovery After Photobleaching	221
7.6.2	Fluorescence Correlation Spectroscopy	223
7.6.3	Continuous Fluorescence Photobleaching	226
7.7	Conclusion	227
	References	227
8	Single Fluorescent Molecule Tracking in Live Cells	235
	Ghislain G. Cabal, Jost Enninga, and Musa M. Mhlana	
8.1	Introduction	235

8.2	Tracking of Single Chromosomal Loci.	236
8.2.1	General Remarks.	236
8.2.2	In Vivo Single Loci Tagging via Operator/Repressor Recognition.	237
8.2.3	The Design of Strains Containing TetO Repeats and Expressing TetR–GFP	238
8.2.4	In Vivo Microscopy for Visualization of Single Tagged Chromosomal Loci	244
8.2.5	Limits and Extension of Operator/Repressor Single Loci Tagging System	246
8.3	Single-Molecule Tracking of mRNA	247
8.3.1	Overview.	247
8.3.2	The MS2–GFP System	247
8.3.3	The Molecular Beacon System.	248
8.3.4	Setting Up the Molecular Beacon System for the Detection of mRNA	250
8.3.5	Ensuring the Observed Fluorescent Particles in Vivo Consist of Single Molecules of mRNA.	251
8.4	Single-Particle Tracking for Membrane Proteins	253
8.4.1	Overview.	253
8.4.2	Quantum Dots As Fluorescent Labels for Biological Samples	254
8.4.3	Functionalizing Quantum Dots To Label Specific Proteins	255
8.4.4	Tracking the Glycin Receptor 1 at the Synaptic Cleft Using Quantum Dots	257
8.5	Tracking Analysis and Image Processing of Data from Particle Tracking in Living Cells	258
8.6	Conclusion	258
8.7	Protocols for Laboratory Use	259
8.7.1	Protocol: Single-Molecule Tracking of Chromosomal Loci in Yeast	259
8.7.2	Protocol: Single-Molecule Tracking of mRNA – Experiment Using Molecular Beacons	259
	References	261
9	From Live-Cell Microscopy to Molecular Mechanisms: Deciphering the Functions of Kinetochores Proteins.	265
	Khuloud Jaqaman, Jonas F. Dorn, and Gaudenz Danuser	
9.1	Introduction.	265
9.2	Biological Problem: Deciphering the Functions of Kinetochores Proteins.	268
9.3	Experimental Design.	269
9.4	Extraction of Dynamics from Images.	273
9.4.1	Mixture-Model Fitting	274

9.4.2	Tag Tracking	275
9.4.3	Multitemplate Matching	275
9.5	Characterization of Dynamics	276
9.5.1	Confined Brownian Motion Model.	277
9.5.2	Simple Microtubule Dynamic Instability Model	278
9.5.3	Autoregressive Moving Average Model	279
9.5.4	Descriptor Sensitivity and Completeness	280
9.6	Quantitative Genetics of the Yeast Kinetochores	282
9.7	Conclusion	284
	References	284

III Cutting Edge Applications & Utilities

10	Towards Imaging the Dynamics of Protein Signalling	289
	Lars Kaestner and Peter Lipp	
10.1	Spatiotemporal Aspects of Protein Signalling Dynamics.	289
10.2	How To Be Fast While Maintaining the Resolution.	290
10.3	How To Make Proteins Visible	299
10.4	Concepts To Image Protein Dynamics	303
10.5	Concepts To Image Protein–Protein Interactions	305
10.6	Concepts To Image Biochemistry with Fluorescent Proteins.	309
	References	311
11	New Technologies for Imaging and Analysis of Individual Microbial Cells	313
	Byron F. Brehm-Stecher	
11.1	Introduction.	313
11.2	Live-Cell Imaging.	314
11.3	Imaging Infection	315
11.4	Imaging Single Molecules (Within Single Cells).	318
11.5	Measuring Discrete Cell Properties and Processes.	319
11.6	“Wetware”.	321
11.7	Hardware and Applications	323
11.7.1	Nonphotonic Microscopies.	323
11.7.2	Image Analysis	324
11.7.3	Spectroscopic Methods.	325
11.8	Fluorescence Correlation Spectroscopy	326
11.9	A Picture is Worth a Thousand Dots – New Developments in Flow Cytometry	330
11.10	Strength in Numbers – Highly Parallel Analysis Using Cellular Arrays	334
11.11	Nontactile Manipulation of Individual Cells and “Wall-less Test Tubes”	335
11.12	Conclusions.	337
	References	338

12 Imaging Parasites in Vivo	345
Rogério Amino, Blandine Franke-Fayard, Chris Janse, Andrew Waters, Robert Ménard, and Freddy Frischknecht	
12.1 Introduction	345
12.2 The Life Cycle of Malaria Parasites	346
12.3 A Very Brief History of Light Microscopy and Malaria Parasites	348
12.4 In Vivo Imaging of Luminescent Parasites	349
12.5 In Vivo Imaging of Fluorescent Parasites	350
12.6 Imaging Malaria Parasites in the Mosquito	351
12.7 Imaging Malaria Parasites in the Mammalian Host	354
12.8 Towards Molecular Imaging in Vivo	358
12.9 A Look at Other Parasites	359
12.10 Conclusion	360
References	360
13 Computer-Assisted Systems for Dynamic 3D Reconstruction and Motion Analysis of Living Cells	365
David R. Soll, Edward Voss, Deborah Wessels, and Spencer Kuhl	
13.1 Introduction	365
13.2 Approaches to 3D Reconstruction and Motion Analysis	366
13.3 Obtaining Optical Sections for 3D Reconstruction	368
13.4 Outlining	368
13.5 Reconstructing 3D Faceted Images and Internal Architecture	373
13.6 Quantitative Analyses of Behavior	373
13.7 3D-DIASemb	375
13.8 Resolving Filopodia	377
13.9 The Combined Use of LSCM and 3D-DIAS	380
13.10 Reasons for 3D Dynamic Image Reconstruction Analysis	381
References	382
14 High-Throughput/High-Content Automated Image Acquisition and Analysis	385
Gabriele Gradl, Chris Hinnah, Achim Kirsch, Jürgen Müller, Dana Nojima, and Julian Wölcke	
14.1 The Driving Forces for High-Throughput/High-Content Automated Imaging	385
14.2 Confocal Imaging in High Throughput – The Principles Available	386
14.3 Resolution and Sensitivity	389
14.4 Measurements	392
14.5 Where Is the Signal and How To Focus?	393
14.6 Plates and Lenses	394
14.7 Image Analysis	395
14.8 Throughput: How To Acquire and Analyze Data Rapidly	399

14.9	Screening Examples	401
	References	404
15	Cognition Network Technology – A Novel Multimodal Image Analysis Technique for Automatic Identification and Quantification of Biological Image Contents	407
	Maria Athelougou, Günter Schmidt, Arno Schäpe, Martin Baatz, and Gerd Binnig	
15.1	Introduction	407
15.2	Cognition Network Technology and Cognition Network Language	409
15.2.1	Cognition Networks	409
15.2.2	Input Data and Image Object Hierarchy	410
15.2.3	Features and Variables	411
15.2.4	Classes and Classification	413
15.2.5	Processes	414
15.2.6	Domains	414
15.2.7	Using CNT-CNL for Image Analysis	415
15.2.8	Application Notes	417
15.3	Discussion	421
	References	421
16	High-Content Phenotypic Cell-Based Assays	423
	Eugenio Fava, Eberhard Krausz, Rico Barsacchi, Ivan Baines, and Marino Zerial	
16.1	A New Tool for Biological Research and Drug Discovery	423
16.2	What Is High-Content Screening and How Can Biologists Use It?	424
16.3	Assay Design: First Think, Then Act	425
16.4	Assay Optimization	426
16.5	Cell Culture	426
16.6	Cell Vessels	428
16.7	Cellular Imaging	428
16.8	Autofluorescence	430
16.9	Image Analysis	431
16.10	Transfection Optimization for RNAi-Based Assays	431
16.11	Escapers and Silencing Efficiency	432
16.12	Toxicity	435
16.13	Off-Target or Unspecific Reactions	436
16.14	Assay Quality	437
16.15	Assay Validation	438
16.16	Conclusion and Outlook	440
	References	440
	Index	443

Imaging Cellular and Molecular Biological Functions

Shorte, S.L.; Frischknecht, F. (Eds.)

2007, XXII, 450 p. 138 illus., 82 illus. in color., Hardcover

ISBN: 978-3-540-71330-2