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. Mhlanga
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 Kinetochore Proteins. 265
Khuloud Jaqaman, Jonas F. Dorn, and Gaudenz Danuser
9.1 Introduction. 265
9.2 Biological Problem: Deciphering the Functions of Kinetochore 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 Kinetochore 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
Rogerio 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 Athelogou, 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