

Contents

Contributors	xi
Preface	xvii

CHAPTER 1 Mitotic live-cell imaging at different timescales1

**Anna-Maria Olziersky, Chris A. Smith, Nigel Burroughs,
Andrew D. McAinsh, Patrick Meraldi**

1. Introduction	2
2. Automated High-Resolution Kinetochore Tracking	3
3. Monitoring Chromosome Segregation and Spindle Organization Over Several Hours	12
4. Monitoring Mitotic Cell Fate Over Several Days.....	19
5. Conclusion.....	22
Acknowledgments	22
References.....	23

CHAPTER 2 Correlating light microscopy with serial block face scanning electron microscopy to study mitotic spindle architecture.....29

Nicholas I. Clarke, Stephen J. Royle

1. Introduction	30
2. Correlating Light With SBF-SEM in Mitotic Cells.....	31
3. Visualization of Mitotic Spindle Architecture in 3D.....	36
4. Discussion	40
Acknowledgments	41
References.....	41

CHAPTER 3 Quantification of three-dimensional spindle architecture.....45

**Norbert Lindow, Stefanie Redemann, Florian Brünig,
Gunnar Fabig, Thomas Müller-Reichert, Steffen Prohaska**

1. Introduction	46
2. Rationale	47
3. Methods.....	47
4. Instrumentation	61
5. Discussion and Outlook.....	61
Acknowledgments	63
References.....	63

CHAPTER 4 Quantitative live and super-resolution microscopy of mitotic chromosomes	65
<i>Nike Walther, Jan Ellenberg</i>	
1. Introduction	66
2. FCS-Calibrated Confocal Imaging of mEGFP-Tagged Proteins on Mitotic Chromosomes in Living Cells	67
3. STED Microscopy of Fixed and Immunostained mEGFP-Tagged Proteins on Mitotic Chromosomes.....	76
4. Materials and Instrumentation.....	82
5. Conclusion and Outlook	87
Acknowledgments	88
References.....	89
CHAPTER 5 Live imaging of cell division in 3D stem-cell organoid cultures	91
<i>Ana C.F. Bolhaqueiro, Richard H. van Jaarsveld, Bas Ponsioen, René M. Overmeer, Hugo J. Snippert, Geert J.P.L. Kops</i>	
1. Introduction	92
2. Conclusion.....	104
Acknowledgments	104
References.....	104
CHAPTER 6 Choosing the right microscope to image mitosis in zebrafish embryos: A practical guide.....	107
<i>Iskra Yanakieva, Marija Matejčić, Caren Norden</i>	
1. Introduction	108
2. Overview of Three Microscope Systems Well Suited for Imaging Mitosis During Zebrafish Development	110
3. Determining the Mitotic Index in Zebrafish Tissues Using an LSCM.....	112
4. Imaging Intracellular Components During Mitosis in Zebrafish Tissues With High Temporal Resolution Using SDCM.....	116
5. Tracking Cells Over Several Cell Cycles for Lineage Tracing Using a LSFМ	121
6. Conclusion.....	125
Acknowledgments	125
References.....	125
CHAPTER 7 Advanced microscopy methods for bioimaging of mitotic microtubules in plants	129
<i>Petra Vyplelová, Miroslav Ovečka, George Komis, Jozef Šamaj</i>	
1. Introduction	130

2. Preparation of Molecular Markers for the Visualization of Mitotic Microtubule Arrays	139
3. Use of <i>Arabidopsis</i> Mutants to Dissect Mitotic Progression in Plants.....	139
4. Materials.....	140
5. Conclusions	151
Acknowledgments	152
References.....	152
CHAPTER 8 Microsurgery and microinjection techniques in mitosis research.....	159
Charles A. Day, Jessica Hornick, Alyssa Langfald, Christopher Mader, Edward H. Hinchcliffe	
1. Introduction	160
2. Cell Culture	160
3. Imaging/Manipulation Chambers	161
4. Manipulation/Injection Microscope Setup	162
5. Manipulator and Injector	163
6. Needles.....	163
7. Imaging Microscope	165
8. Microinjection Protocol	166
9. Micromanipulation Protocol	168
Acknowledgments	170
References.....	170
CHAPTER 9 Analyzing the micromechanics of the cell division apparatus	173
Yuta Shimamoto, Tarun M. Kapoor	
1. Introduction	174
2. Optical Trap-Based “Mini-Spindle” Assay	175
3. Microneedle-Based “Whole-Spindle” Assay	181
Acknowledgments	188
References.....	188
CHAPTER 10 Optogenetic reversible knocksideways, laser ablation, and photoactivation on the mitotic spindle in human cells	191
Ana Milas, Mihaela Jagrić, Jelena Martinčić, Iva M. Tolić	
1. Introduction.....	192
2. Optogenetic Reversible Knocksideways	195
3. Construction of Plasmids for Optogenetic Experiments.....	196
4. Sample Preparation	197
5. Imaging	198

6. Optogenetic Reversible Knocksideways of TACC3 in Metaphase	199
7. Optogenetic Reversible Knocksideways of KIF18A in Metaphase	203
8. Combination of Different Optical Approaches for Studying Mitotic Spindle Dynamics	204
9. Conclusion.....	209
Acknowledgments	210
References.....	210
CHAPTER 11 Live imaging of <i>C. elegans</i> oocytes and early embryos	217
Kimberley Laband, Benjamin Lacroix, Frances Edwards, Julie C. Canman, Julien Dumont	
1. Introduction	218
2. In Utero Imaging of Oocyte Meiotic Divisions	221
3. Ex Utero Imaging of Oocyte Meiotic Divisions and Early Embryo Mitosis.....	227
4. Conclusions	233
Acknowledgments	234
References.....	234
CHAPTER 12 Combining microscopy and biochemistry to study meiotic spindle assembly in <i>Drosophila</i> oocytes	237
Pierre Romé, Hiroyuki Ohkura	
1. Introduction	238
2. Methods.....	239
References.....	247
CHAPTER 13 Methods to study meiosis in insect spermatocytes ..	249
James R. LaFountain Jr., Christopher S. Cohan	
1. Introduction	250
2. Methods for the Study of Meiosis in Populations of Spermatocytes.....	252
3. Methods for Preparing Spermatocytes for Live-Cell Imaging ..	254
4. Method for Ionophoretic Microinjection of Charged Molecules Into the Cytoplasm of Living Cells.....	259
5. Method for Pressure Microinjection of Cell-Permeable Probes Into Interstitial Space Surrounding Spermatocytes In Vitro.....	264
References.....	267

CHAPTER 14 Live-cell microscopy of meiosis in spermatocytes ..269**Hiroki Shibuya, Yoshinori Watanabe**

1. Introduction	270
2. Materials.....	270
3. Methods.....	272
4. Notes	274
Acknowledgments	276
References.....	276

CHAPTER 15 Live imaging of cell division in preimplantation**mouse embryos using inverted light-sheet****microscopy****Judith Reichmann, Manuel Eguren, Yu Lin, Isabell Schneider,
Jan Ellenberg**

1. Introduction	280
2. Preimaging Preparations	283
3. Live-Cell Visualization.....	286
4. Materials.....	291
5. Conclusion.....	291
Acknowledgments	292
Conflict of Interest.....	292
References.....	292

CHAPTER 16 Correlated light and electron microscopy of cell**division in large marine oocytes, eggs,****and embryos.....****293****Mariia Burdyniuk, Natalia Wesolowska, Michal Fleszar,
Matthia A. Karreman, Pedro Machado, Joana Borrego-Pinto,
Bernhard Ruthensteiner, Yannick Schwab, Péter Lénárt**

1. Introduction	294
2. Protocol 1	296
3. Protocol 2	302
4. List of Equipment	310
5. List of Reagents	311
Acknowledgments	311
References.....	312

CHAPTER 17 A microscopy-based approach for studying meiosis**in live and fixed human oocytes****315****Agata P. Zielinska, Melina Schuh**

1. Introduction	316
2. Oocyte Collection and Culture	317

3. Studying Meiosis in Live Human Oocytes by Confocal Microscopy	321
4. Super-Resolution Microscopy of Immunolabeled Samples.....	327
Acknowledgments	333
References.....	333
CHAPTER 18 Superresolution imaging of the synaptonemal complex	335
Katharina Schücker, Markus Sauer, Ricardo Benavente	
1. Introduction	336
2. Materials.....	338
3. Methods.....	340
4. Conclusions	345
Acknowledgments	345
References.....	345
CHAPTER 19 Cryo-electron tomography of SYCP3 fibers under native conditions.....	347
Daniel Bölschweiler, Laura Radu, Luca Pellegrini	
1. Introduction	348
2. Methods.....	350
3. Discussion	369
References.....	370