CONTENTS

Contributors *xi*
Preface *xiii*

1. Direct Electron Detectors **1**

G. McMullan, A.R. Faruqi, and R. Henderson

1. Introduction 1
2. Past 2
3. Present 4
4. Future 13

References 15

2. Specimen Behavior in the Electron Beam **19**

R.M. Glaeser

1. Introduction 20
2. High-Energy Electrons Are a Form of Ionizing Radiation as Well as Being a Form of Short-Wavelength Radiation That Can Be Focused 21
3. Biological Molecules Become Structurally Damaged When Irradiated 25
4. Vitreous Ice Also Becomes Structurally Damaged by Ionizing Radiation 30
5. Bubbling of Hydrated Biological Specimens Becomes Apparent at High Electron Exposure 34
6. Cryo-Specimens Exhibit Collective Beam-Induced Movement When Irradiated 37
7. More Than One Mechanism May Contribute to Beam-Induced Motion 40
8. Irradiation Can Produce Electrostatic Charging of the Specimen 42
9. Summary and Future Directions 46

Acknowledgments 47

References 47

3. Specimen Preparation for High-Resolution Cryo-EM **51**

L.A. Passmore and C.J. Russo

1. Introduction 52
2. A Systematic Approach to Specimen Preparation 53
3. Support Choice, Handling, and Storage 62
4. Contamination and Cleaning 65
5. Continuous Films of Amorphous Carbon and Graphene 67
6. Surface Treatments 73

7. Vitrification 76
8. Data Collection 80
Acknowledgments 84
References 84

4. Strategies for Automated CryoEM Data Collection Using Direct Detectors 87

A. Cheng, Y.Z. Tan, V.P. Dandey, C.S. Potter, and B. Carragher

1. Introduction 88
2. Practical Considerations in Instrumentation Configuration 90
3. Automated Data Acquisition Using DD Cameras 93
4. Challenges Arising from DDs and High-Resolution Biological CryoEM 95
5. Future Outlook for DD Integration with Automated Data Collection 100
Acknowledgments 101
References 101

5. Processing of Cryo-EM Movie Data 103

Z.A. Ripstein and J.L. Rubinstein

1. Introduction 103
2. Motioncorr 107
3. *alignframes_lmbfgs* and *alignparts_lmbfgs* 109
4. Unblur 112
5. Optical Flow 114
6. Particle Polishing in *Relion* 116
7. Summary 121
8. Future Prospects 122
Acknowledgments 123
References 123

6. Processing of Structurally Heterogeneous Cryo-EM Data in RELION 125

S.H.W. Scheres

1. Introduction 126
2. New Algorithmic Concepts 128
3. A Typical High-Resolution Structure Determination Procedure 134
4. Dealing with Structural Heterogeneity 142
5. Outlook 154
Acknowledgments 154
References 154

7. Single-Particle Refinement and Variability Analysis in EMAN2.1 159

S.J. Ludtke

1. Introduction 160
2. Single-Particle Reconstruction 164
3. Assessing a Refinement and Identifying Variability 167
4. Conformational and Compositional Variability 172
5. Interactive Tools 184
6. Conclusion 186

Acknowledgments 187
References 187

8. Frealign: An Exploratory Tool for Single-Particle Cryo-EM 191

N. Grigorieff

1. Introduction and Philosophy 192
2. Frealign Elements at a Glance 193
3. Algorithms 199
4. Typical Application Scenarios 201
5. Tuning Options 220
6. Related Software 223

References 224

9. Testing the Validity of Single-Particle Maps at Low and High Resolution 227

P.B. Rosenthal

1. Introduction 228
2. Validation at Low Resolution: Tilt-Pair Analysis and Orientation Determination 230
3. Validation of Map Resolution 239
4. Resolution Assessment of a Map with an Atomic Coordinate Model 245
5. Contrast Restoration 246
6. Local Resolution Assessment 247
7. Summary of Validation Tests and Future Prospects 249

Acknowledgments 250
References 250

10. Tools for Model Building and Optimization into Near-Atomic Resolution Electron Cryo-Microscopy Density Maps 255

F. DiMaio and W. Chiu

1. Introduction 255
2. De Novo Model Building 258

3. Model Optimization 262
4. Model Validation 269
5. Discussion 273
Acknowledgments 274
References 274

11. Refinement of Atomic Structures Against cryo-EM Maps 277
G.N. Murshudov

1. Introduction 278
2. Target Function 281
3. Variance of Observational Noise 286
4. Map Calculation 288
5. Crossvalidation 290
6. Effect of Oversharpening 292
7. $2F_o-F_c$ and Other Crystallographic Maps 294
8. Form Factors 295
9. Conclusions 299
Acknowledgments 302
References 302

12. Cryo-EM Structure Determination Using Segmented Helical Image Reconstruction 307
S.A. Fromm and C. Sachse

1. Introduction 308
2. Architecture of Helical Assemblies 310
3. Segmented Helical Image Analysis 311
4. Iterative Helical Structure Refinement 315
5. High-Resolution Helical Image Reconstructions 317
6. The Use of Direct Electron Detectors for Helical Structures 319
7. Validation of Helical Structures 322
8. Conclusion 323
Acknowledgments 324
References 325

13. Cryo-Electron Tomography and Subtomogram Averaging 329
W. Wan and J.A.G. Briggs

1. Introduction 330
2. Tomographic Data Collection 333
3. Tilt-Series Processing and Tomogram Reconstruction 337

4. Subtomogram Averaging 345
5. Classification in Subtomogram Averaging 351
6. Maximum-Likelihood Approaches for Subtomogram Alignment and Classification 355
7. Combinations of Subtomogram Averaging and 2D Approaches 357
8. Software for Subtomogram Averaging 360
9. Conclusions 361
Acknowledgments 362
References 362

14. High-Resolution Macromolecular Structure Determination by MicroED, a cryo-EM Method 369
J.A. Rodriguez and T. Gonen

1. Introduction 370
2. Background 371
3. Sample Preparation 378
4. Instrumentation and Data Collection 380
5. Processing of MicroED Data 384
6. Summary and Outlook 388
Acknowledgments 388
References 389

15. Databases and Archiving for CryoEM 393
A. Patwardhan and C.L. Lawson

1. Introduction 394
2. Resources 399
3. Deposition and Annotation 402
4. Recent Community-Wide Initiatives 403
5. Challenges and Opportunities 408
Acknowledgments 409
References 410

Author Index *413*
Subject Index *431*