Liquid Cell Electron Microscopy

The first book on the topic, with each chapter written by pioneers in the field, this essential resource details the fundamental concepts, applications, and future developments of liquid cell electron microscopy.

This book describes the techniques that have been developed to image liquids in both transmission and scanning electron microscopes, including general strategies for examining liquids, closed and open cell electron microscopy, experimental design, resolution, and electron beam effects. A wealth of practical guidance is provided, and applications are described in areas such as electrochemistry, corrosion and batteries, nanocrystal growth, biomineralization, biomaterials and biological processes, beam-induced processing, and fluid physics. The book also looks ahead to the future development of the technique, discussing technical advances that will enable higher resolution, analytical microscopy, and even holography of liquid samples.

This is essential reading for researchers and practitioners alike.

Frances M. Ross is based at the IBM Thomas J. Watson Research Center, where she has built a program around a microscope with deposition and focused ion beam capabilities and developed closed liquid cell microscopy to image electrochemical processes. Previously she worked at the National Center for Electron Microscopy, Lawrence Berkeley National Laboratory, and she has also been a Visiting Scientist at Lund University and an Adjunct Professor at Arizona State University. She received the UK Institute of Physics Boys Medal, the MRS Outstanding Young Investigator Award, and the MSA Burton Medal, holds an Honorary Doctorate from Lund University, and is a Fellow of APS, AAAS, MRS, MSA, and AVS.
Advances in Microscopy and Microanalysis

Microscopic visualization techniques range from atomic imaging to visualization of living cells at near nanometer spatial resolution, and advances in the field are fueled by developments in computation, image detection devices, labeling, and sample preparation strategies. Microscopy has proven to be one of the most attractive and progressive research tools available to the scientific community, and remains at the forefront of research in many disciplines, from nanotechnology to live cell molecular imaging.

This series reflects the diverse role of microscopy, defining it as any method of imaging objects of micrometer scale or less, and includes both introductory texts and highly technical and focused monographs for researchers and practitioners in materials and the life sciences.

Series Editors
Patricia Calarco, University of California, San Francisco
Michael Isaacson, University of California, Santa Cruz

Series Advisors
Bridget Carragher, The Scripps Research Institute
Wah Chiu, Baylor College of Medicine
Christian Collieux, Université Paris Sud
Ulrich Dahmen, Lawrence Berkeley National Laboratory
Mark Ellisman, University of California, San Diego
Peter Ingram, Duke University Medical Center
J. Richard McIntosh, University of Colorado
Giulio Pozzi, University of Bologna
John C. H. Spence, Arizona State University
Elmar Zeitler, Fritz-Haber Institute

Books in Series
Published
Heide Schatten, Scanning Electron Microscopy for the Life Sciences

Forthcoming
Nigel Browning et al., Dynamic Transmission Electron Microscopy
Michael Isaacson, Microscopic Nanocharacterization of Materials
Richard Leapman, Energy Filtered Electron Microscopy and Electron Spectroscopy
Eric Lifshin, The Scanning Electron Microscope
Chris Jacobsen and Janos Kirz, X-Ray Microscopy
Joel Kubby, Meng Cui and Sylvain Gigan, Wavefront Shaping for Biomedical Imaging
Thomas F. Kelly and Simon Ringer, Atomic-Scale Tomography
Liquid Cell Electron Microscopy

FRANCES M. ROSS
IBM Thomas J. Watson Research Center
## Contents

**List of Contributors**  
page xii  
**Preface and Acknowledgements**  
xvii

### Part I Technique

1  
**Past, Present, and Future Electron Microscopy of Liquid Specimens**  
Niels de Jonge and Frances M. Ross  
1.1 Introduction  
3  
1.2 The Rapidly Developing Liquid Cell Microscopy Technique  
4  
1.3 Liquid Cell Microscopy for Materials Science, Biology, and Beyond  
12  
1.4 Which Type of Microscopy Should I Use?  
19  
1.5 Future Prospects  
21  
1.6 Conclusions  
25  
References  
25

2  
**Encapsulated Liquid Cells for Transmission Electron Microscopy**  
Eric Jensen and Kristian Mølhave  
2.1 Introduction  
35  
2.2 Microfabricated Chip Designs  
37  
2.3 Other Encapsulation Methods  
45  
2.4 What Happens When the Liquid Cell Fails?  
46  
2.5 Membrane Bulging: Mitigation and Measurement  
47  
2.6 Stimuli and Correlative Measurements: Biasing, Heating, Flow, and Spectroscopy  
49  
2.7 Conclusions and Outlook  
51  
References  
52

3  
**Imaging Liquid Processes Using Open Cells in the TEM, SEM, and Beyond**  
Chongmin Wang  
3.1 Introduction  
56  
3.2 Fundamental Concepts for Open Cell Experiments in S/TEM  
57
3.3 Open Cells for Imaging Droplets, Crystal Growth, Particle Motion, and Surface Passivation 59
3.4 Open Cells for In Situ Battery Reactions 63
3.5 Extension of the Open Cell Concept to Other Imaging and Spectroscopic Techniques 72
3.6 Perspective 73
Acknowledgements 74
References 75

4 Membrane-Based Environmental Cells for SEM in Liquids 78
Andrei Kolmakov
4.1 Introduction 78
4.2 Basics of SEM through Membranes 80
4.3 Examples of Environmental Cell Designs and Liquid SEM Applications 85
4.4 Novel Two-Dimensional Materials as Electron-Transparent Membranes for Liquid SEM Cells 92
4.5 Outlook 99
Acknowledgements 101
References 101

5 Observations in Liquids Using an Inverted SEM 106
Chikara Sato and Mitsuo Suga
5.1 Introduction 106
5.2 Instrument Design and Sample Geometry of the ASEM 106
5.3 Applications of ASEM 109
5.4 Correlative Microscopy (CLEM) 116
5.5 Other SEM Techniques for Examining Liquids at Atmospheric Pressure 120
5.6 Conclusions 123
Acknowledgements 124
References 124

6 Temperature Control in Liquid Cells for TEM 127
Shen J. Dillon and Xin Chen
6.1 Introduction: Controlled Temperature Experiments 127
6.2 Electron Beam-Induced Heating 131
6.3 Temperature Measurements 132
6.4 Applications 133
6.5 Outlook 137
References 138
Contents

7 Electron Beam Effects in Liquid Cell TEM and STEM 140
Nicholas M. Schneider

7.1 Introduction 140
7.2 Electron Energy Loss in Liquids 140
7.3 Electron Beam Heating 144
7.4 Introduction to the Radiation Chemistry of Water 147
7.5 Homogeneous Irradiation 150
7.6 Finite Beam Irradiation with Diffusion 152
7.7 Practical Effects of Radiolysis 154
7.8 Radiolysis beyond Neat Water 159
7.9 Conclusions and Outlook 160
References 161

8 Resolution in Liquid Cell Experiments 164
Niels de Jonge, Nigel D. Browning, James E. Evans, See Wee Chee, and Frances M. Ross

8.1 Introduction 164
8.2 Spatial Resolution in Liquid Cell TEM 165
8.3 Spatial Resolution in Liquid Cell STEM 174
8.4 Temporal Resolution in TEM and STEM 177
8.5 Image Simulations in Liquid Cell TEM and STEM 179
8.6 Some Practicalities and Pitfalls of Liquid Cell TEM and STEM 179
8.7 Summary and Outlook 185
Acknowledgements 185
References 186

Part II Applications 189

9 Nanostructure Growth, Interactions, and Assembly in the Liquid Phase 191
Hong-Gang Liao, Kai-Yang Niu, and Haimei Zheng

9.1 Introduction 191
9.2 Formation of Nanoparticles in TEM 192
9.3 Single Particle Growth Trajectories 194
9.4 Important Factors in Nanoparticle Growth 197
9.5 Growth of Materials Architectures 200
9.6 Nanoparticle Diffusion and Assembly 201
9.7 Etching and Corrosion 205
9.8 Conclusions and Outlook 205
Acknowledgements 206
References 206
Contents

10  Quantifying Electrochemical Processes Using Liquid Cell TEM 210
    Frances M. Ross
    10.1 Introduction 210
    10.2 Design of Liquid Cells for Quantitative Electrochemical Experiments 211
    10.3 Electrochemical Nucleation and Growth in Plan View 219
    10.4 Growth Front Propagation via Lateral Measurements 224
    10.5 Experimental Challenges 228
    10.6 Outlook 232
    References 233

11  Application of Electrochemical Liquid Cells for Electrical Energy Storage and Conversion Studies 237
    Raymond R. Unocic and Karren L. More
    11.1 Introduction 237
    11.2 Electrical Energy Storage and Conversion Systems: Challenges and Opportunities 237
    11.3 Closed Cell Electrochemical-S/TEM for Energy Storage and Conversion Studies 238
    11.4 Electroanalytical Measurement Techniques 242
    11.5 Application of Electrochemical-S/TEM for Battery Research 244
    11.6 Application of ec-S/TEM for Fuel Cell Research 252
    11.7 Summary 254
    Acknowledgements 254
    References 255

12  Applications of Liquid Cell TEM in Corrosion Science 258
    See Wee Chee and M. Grace Burke
    12.1 Introduction 258
    12.2 Studying Corrosion in Aqueous Environments 259
    12.3 Studies of Corrosion using Liquid Cell TEM 261
    12.4 Considerations Pertaining to Studying Corrosion with Liquid Cell TEM 266
    12.5 Microfluidic Cell Design for Electrochemical Corrosion Experiments 270
    12.6 Outlook 271
    Acknowledgements 272
    References and Notes 272

13  Nanoscale Water Imaged by In Situ TEM 276
    Utkur Mirsaidov and Paul Matsudaira
    13.1 Introduction 276
    13.2 Interfacial Fluids 277
    13.3 Nanodroplet Condensation 280
    13.4 Fluids in Nanochannels 282
## Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>Voids and Nanobubbles in Liquid Films</td>
<td>285</td>
</tr>
<tr>
<td>13.6</td>
<td>Outlook</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>287</td>
</tr>
<tr>
<td>14</td>
<td>Nanoscale Deposition and Etching of Materials Using Focused Electron Beams and Liquid Reactants</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>Eugenii U. Donev, Matthew Bresin, and J. Todd Hastings</td>
<td></td>
</tr>
<tr>
<td>14.1</td>
<td>Overview of Gas-Phase Focused Electron Beam-Induced Processing (FEBIP)</td>
<td>291</td>
</tr>
<tr>
<td>14.2</td>
<td>Methods for LP-FEBIP</td>
<td>293</td>
</tr>
<tr>
<td>14.3</td>
<td>Survey of LP-FEBID of Transition Metals</td>
<td>299</td>
</tr>
<tr>
<td>14.4</td>
<td>Multi-element LP-FEBID</td>
<td>303</td>
</tr>
<tr>
<td>14.5</td>
<td>Liquid-Phase Focused Electron Beam-Induced Etching (LP-FEBIE)</td>
<td>306</td>
</tr>
<tr>
<td>14.6</td>
<td>Mechanisms for LP-FEBIP</td>
<td>309</td>
</tr>
<tr>
<td>14.7</td>
<td>Outlook</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>310</td>
</tr>
<tr>
<td>15</td>
<td>Liquid Cell TEM for Studying Environmental and Biological Mineral Systems</td>
<td>316</td>
</tr>
<tr>
<td></td>
<td>Michael H. Nielsen and James J. De Yoreo</td>
<td></td>
</tr>
<tr>
<td>15.1</td>
<td>Introduction</td>
<td>316</td>
</tr>
<tr>
<td>15.2</td>
<td>Mechanisms of Mineral Formation</td>
<td>317</td>
</tr>
<tr>
<td>15.3</td>
<td>Liquid Holder Design</td>
<td>319</td>
</tr>
<tr>
<td>15.4</td>
<td>Calcium Carbonate Formation Pathways</td>
<td>321</td>
</tr>
<tr>
<td>15.5</td>
<td>Nucleation within an Organic Matrix</td>
<td>324</td>
</tr>
<tr>
<td>15.6</td>
<td>Particle-Based Crystallization</td>
<td>326</td>
</tr>
<tr>
<td>15.7</td>
<td>Conclusions and Future Applications</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>330</td>
</tr>
<tr>
<td>16</td>
<td>Liquid STEM for Studying Biological Function in Whole Cells</td>
<td>334</td>
</tr>
<tr>
<td></td>
<td>Diana B. Peckys and Niels de Jong</td>
<td></td>
</tr>
<tr>
<td>16.1</td>
<td>Introduction</td>
<td>334</td>
</tr>
<tr>
<td>16.2</td>
<td>Liquid STEM Technology</td>
<td>334</td>
</tr>
<tr>
<td>16.3</td>
<td>Studying Membrane Proteins in Whole Cells in Liquid</td>
<td>339</td>
</tr>
<tr>
<td>16.4</td>
<td>Live Cell Liquid STEM</td>
<td>344</td>
</tr>
<tr>
<td>16.5</td>
<td>Gold Nanoparticle Uptake Studied in Whole Cells</td>
<td>347</td>
</tr>
<tr>
<td>16.6</td>
<td>Comparison with Cryo-TEM</td>
<td>348</td>
</tr>
<tr>
<td>16.7</td>
<td>Conclusions and Outlook</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>351</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>351</td>
</tr>
</tbody>
</table>
## Contents

### 17 Visualizing Macromolecules in Liquid at the Nanoscale

Andrew C. Demmert, Madeline J. Dukes, Elliot Pohlmann, Kaya Patel, A. Cameron Varano, Zhi Sheng, Sarah M. McDonald, Michael Spillman, Utkur Mirsaidov, Paul Matsudaira, and Deborah F. Kelly

17.1 Introduction: The Critical Need for Imaging Dynamic Events in Life Sciences 356

17.2 Recent Technical Advances: How Liquid Cell TEM Can Address This Critical Need 357

17.3 The Affinity Capture Technique to Tether Unlabeled Biological Complexes onto Si$_x$N$_y$ 357

17.4 Correlative Nanoscale Imaging: What Information Can We Learn from Combining Liquid Cell TEM and Cryo-EM? 361

17.5 New Directions: Use of Direct Electron CMOS Detectors to Acquire “Molecular Movies” of Fundamental Processes 368

Acknowledgements 369
References 369

### 18 Application of Liquid Cell Microscopy to Study Function of Muscle Proteins

Haruo Sugi, Shigeru Chaen, Tsuyoshi Akimoto, Masaru Tanokura, Takuya Miyakawa, and Hiroki Minoda

18.1 Introduction: Our Motivation for Liquid Cell Microscopy of Muscle Contraction 371

18.2 Experimental Methods for Recording Myosin Head Movement 375

18.3 ATP-Induced Movement of Individual Myosin Heads 381

18.4 Conclusions and Outlook 388

Acknowledgements 389
References 389

### Part III Prospects

#### 19 High Resolution Imaging in the Graphene Liquid Cell

Jungwon Park, Vivekananda P. Adiga, Alex Zettl, and A. Paul Alivisatos

19.1 Introduction to Graphene Liquid Cells: Advantages, Opportunities, and Fabrication Methods 393

19.2 Studying Growth Mechanisms in Atomic Detail by GLC-TEM 396

19.3 Applications of GLC-TEM in Biological Studies 400

19.4 Future Directions 404

References 406

#### 20 Analytical Electron Microscopy during *In Situ* Liquid Cell Studies

Megan E. Holtz, David A. Muller, and Nestor J. Zaluzec

20.1 Introduction 408

20.2 Electron Energy Loss Spectroscopy 413
20.3 X-ray Energy Dispersive Spectroscopy 420
20.4 Summary 431
Acknowledgements 432
References 432

21 Spherical and Chromatic Aberration Correction for Atomic-Resolution Liquid Cell Electron Microscopy 434
Rafal E. Dunin-Borkowski and Lothar Houben
21.1 Introduction 434
21.2 Spherical Aberration Correction in the TEM 435
21.3 Spherical Aberration Correction in STEM 444
21.4 Chromatic Aberration Correction in the TEM 446
21.5 Conclusions 452
Acknowledgements 453
References 453

22 The Potential for Imaging Dynamic Processes in Liquids with High Temporal Resolution 456
Nigel D. Browning and James E. Evans
22.1 Introduction 456
22.2 Why Do We Need Better Temporal Resolution? 457
22.3 Hardware/Software Developments for Fast Temporal Resolution 460
22.4 Materials and Biological Examples 466
22.5 Conclusions 471
Acknowledgements 471
References 472

23 Future Prospects for Biomolecular, Biomimetic, and Biomaterials Research Enabled by New Liquid Cell Electron Microscopy Techniques 476
Taylor Woehl and Tanya Prozorov
23.1 Introduction 476
23.2 Visualizing Protein Structure in Liquid Water at High Resolution 476
23.3 Elucidating Fundamental Biomineralization Mechanisms via In Vivo Imaging 479
23.4 Visualizing Electromagnetic Fields and Nanoparticle Interactions in Biomolecular Systems 483
23.5 Biomimetics 488
23.6 Mesocrystal Formation 491
23.7 Conclusions 495
Acknowledgements 495
References 495

Index 501
Contributors

Vivekananda P. Adiga
University of California, Berkeley

Tsuyoshi Akimoto
Teikyo University

A. Paul Alivisatos
Lawrence Berkeley National Laboratory

Matthew Bresin
University of Illinois at Urbana-Champaign

Nigel D. Browning
Pacific Northwest National Laboratory

M. Grace Burke
The University of Manchester

Shigeru Chaen
Nihon University

See Wee Chee
National University of Singapore

Xin Chen
East China University of Science and Technology

Andrew C. Demmert
Virginia Tech Carilion Research Institute

Niels de Jonge
INM – Leibniz Institute for New Materials
List of Contributors

James J. De Yoreo
Pacific Northwest National Laboratory

Shen J. Dillon
University of Illinois at Urbana-Champaign

Eugenii U. Donev
Sewanee: The University of the South

Madeline J. Dukes
Protochips, Inc.

Rafal E. Dunin-Borkowski
Forschungszentrum Jülich

James E. Evans
Pacific Northwest National Laboratory

J. Todd Hastings
University of Kentucky

Megan E. Holtz
Cornell University

Lothar Houben
Forschungszentrum Jülich

Eric Jensen
Technical University of Denmark

Deborah F. Kelly
Virginia Tech Carilion Research Institute

Andrei Kolmakov
National Institute of Standards and Technology

Hong-Gang Liao
Lawrence Berkeley National Laboratory

Paul Matsudaira
National University of Singapore

Sarah M. McDonald
Virginia Tech Carilion Research Institute
List of Contributors

Hiroki Minoda
Tokyo University of Agriculture and Technology

Utkur Mirsaidov
National University of Singapore

Takuya Miyakawa
University of Tokyo

Kristian Mølhave
Technical University of Denmark

Karren L. More
Oak Ridge National Laboratory

David A. Muller
Cornell University

Michael H. Nielsen
Lawrence Livermore National Laboratory

Kai-Yang Niu
Lawrence Berkeley National Laboratory

Jungwon Park
Harvard University

Kaya Patel
Virginia Tech Carilion Research Institute

Diana B. Peckys
INM – Leibniz Institute for New Materials

Elliot Pohlmann
Virginia Tech Carilion Research Institute

Tanya Prozorov
Ames DOE Laboratory

Frances M. Ross
IBM T. J. Watson Research Center

Chikara Sato
National Institute of Advanced Industrial Science and Technology (AIST)
List of Contributors

Nicholas M. Schneider
University of Pennsylvania

Zhi Sheng
Virginia Tech Carilion Research Institute

Michael Spillman
Direct Electron, LP

Mitsuo Suga
JEOL Ltd.

Haruo Sugi
Teikyo University

Masaru Tanokura
University of Tokyo

Raymond R. Unocic
Oak Ridge National Laboratory

A. Cameron Varano
Virginia Tech Carilion Research Institute

Chongmin Wang
Pacific Northwest National Laboratory

Taylor Woehl
Ames DOE Laboratory

Nestor J. Zaluzec
Argonne National Laboratory

Alex Zettl
University of California, Berkeley

Haimei Zheng
Lawrence Berkeley National Laboratory
Preface and Acknowledgements

Electron microscopy amazes and educates us with its images of atomic level structure, measurements of nanoscale chemistry, and movies showing otherwise invisible phenomena. However, electron microscopy is generally used only on solid samples, since liquids, particularly those with low vapor pressure such as water, are challenging to image within the microscope vacuum. The difficulty of dealing with water and other liquids was recognized early on in the development of electron microscopy, but practical solutions to the engineering challenges appeared only recently with the advent of microfabrication and micromanipulation techniques. We can now build liquid cells that allow us to apply the powerful capabilities of the electron microscope to imaging and analysis of liquid specimens. This has opened up wonderful opportunities ranging from nanoscale materials and processes in liquids to biological structures imaged at high resolution without freezing or drying. Physical and chemical changes taking place within batteries during operation, the attachment of atoms during the self-assembly of nanocrystals, and protein structures within whole cells in liquid water are examples in which a microscopic view is providing unique insights.

This book describes the practical aspects of liquid cell electron microscopy. We start with a historical perspective, then consider several different strategies for experimental design as well as resolution and electron beam effects. We next describe research results in fields as diverse as energy storage, biomineralization, fluid physics, and the structure of different types of biomaterials in their native, hydrated state. We finally look forward to future experiments with improved spatial and temporal resolution or analytical capabilities that probe increasingly complex materials and processes.

The motivation for bringing these discussions together is the exciting current state of liquid cell electron microscopy. Since the equipment is not too expensive and generally works in existing electron microscopes, many programs involving electron microscopy of liquids have developed around the world. These projects have provided unique, high quality scientific information that frequently appears in high impact journals. Within just the last few years, electron microscopy experiments involving liquids have become a regular component of materials science and microscopy conferences, generating lively and occasionally confrontational discussions. Liquid cell electron microscopy in all its different variations is well positioned to improve its reach still further. The ease of use of the equipment is improving continuously, our understanding is becoming more quantitative, and new capabilities are appearing that allow further exploration of frontiers in materials, environmental science, life sciences, and beyond.
Combining the expertise of many materials scientists, physicists, biologists, and microscopists in this edited volume enables us to address two aims. One is to help electron microscopists by providing guidance on how to obtain meaningful data from experiments on liquid samples. This is addressed through the chapters on experimental design, artifacts, and quantification. The other aim is to provide information that will help scientists who are not microscopists to decide for themselves the value of adopting or collaborating on electron microscopy techniques for imaging and analysis of liquids. This is addressed through the descriptions of scientific questions that have already been answered, as well as the discussions of the current status and future capabilities for obtaining information on liquid samples. By drawing on the experience of many of the leaders in this field we hope this book will fulfill these two objectives. While we cover a broad range of topics, our intention is that not too much specialized knowledge is required in any one of them.

I am grateful for all the help I have received during this project. I would especially like to thank my family for their patience and enthusiastic support and my colleagues at IBM and Cambridge University Press for their understanding and help as this book came together. It has been a great pleasure and a powerful learning experience to collaborate with colleagues around the world. Although there are omissions—a field moving this quickly cannot be captured in a book—I hope that we can nevertheless give a snapshot of the evolution, current position, and future of liquid cell electron microscopy, and preview the remarkable opportunities that I believe will be realized in the future.

Frances M. Ross
Yorktown Heights, New York