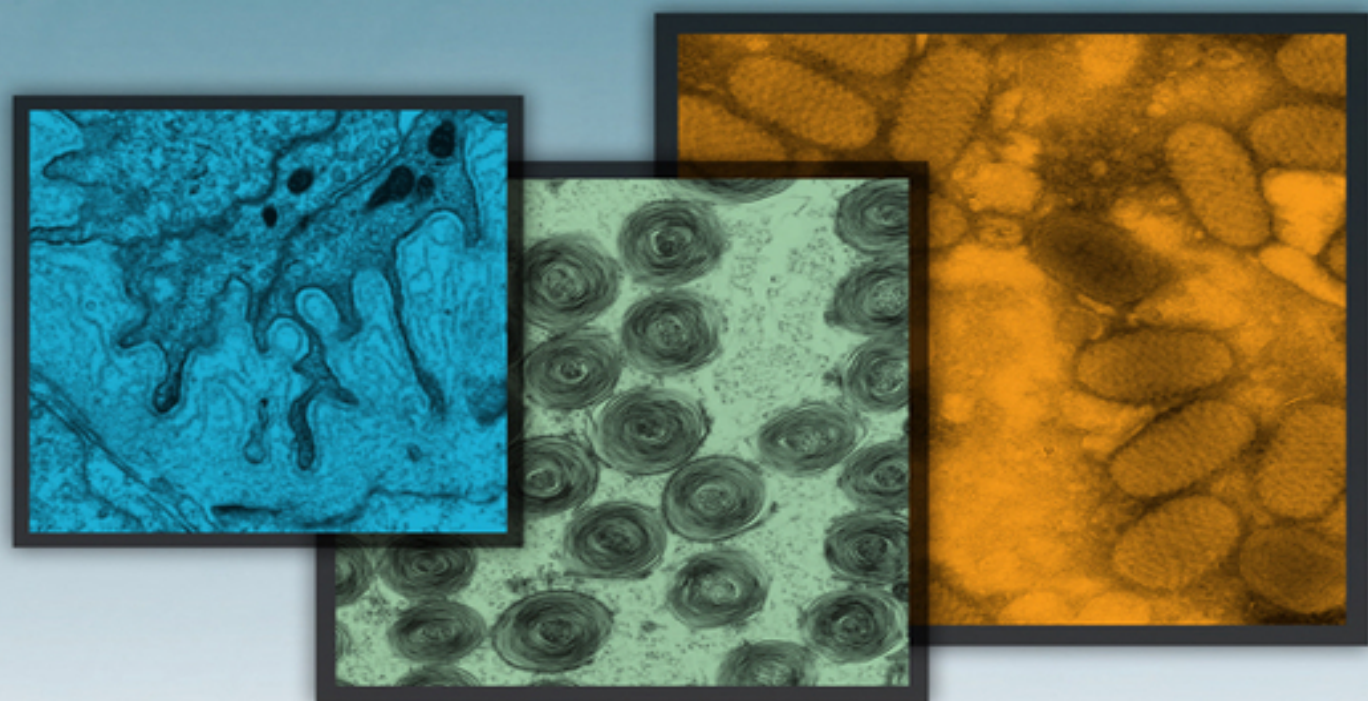


Editors

John W. Stirling, Alan Curry and Brian P. Eyden

Diagnostic Electron Microscopy



A Practical Guide to
Interpretation and Technique

 WILEY

 RMS

Table of Contents

[Series Page](#)

[Title Page](#)

[Copyright](#)

[Acknowledgements and Dedication](#)

[List of Contributors](#)

[Preface-Introduction](#)

[Diagnostic Electron Microscopy](#)

[The purpose and use of TEM](#)

[The aim and purpose of this book](#)

[Chapter 1: Renal Disease](#)

[1.1 The Role of Transmission Electron Microscopy \(TEM\) in Renal Diagnostics](#)

[1.2 Ultrastructural Evaluation and Interpretation](#)

[1.3 The Normal Glomerulus](#)

[1.4 Ultrastructural Diagnostic Features](#)

[1.5 The Ultrastructural Pathology of the Major Glomerular Diseases](#)

[References](#)

[Chapter 2: Transplant Renal Biopsies](#)

[2.1 Introduction](#)

[2.2 The Transplant Renal Biopsy](#)

[2.3 Indications for Electron Microscopy of Transplant Kidney](#)

[References](#)

[Chapter 3: Electron Microscopy in Skeletal Muscle Pathology](#)

[3.1 Introduction](#)

[3.2 Normal Muscle](#)

[3.3 Pathological Changes](#)

[References](#)

[Chapter 4: The Diagnostic Electron Microscopy of Nerve](#)

[4.1 Introduction](#)

[4.2 Tissue Processing](#)

[4.3 Normal Nerve Ultrastructure](#)

[4.4 Pathological Ultrastructural Features](#)

[4.5 Artefact](#)

[4.6 Conclusions](#)

[References](#)

[Chapter 5: The Diagnostic Electron Microscopy of Tumours](#)

[5.1 Introduction](#)

[5.2 Principles and Procedures for Diagnosing Tumours by Electron Microscopy](#)

[5.3 Organelles and Groups of Cell Structures Defining Cellular Differentiation](#)

References

Chapter 6: Microbial Ultrastructure

6.1 Introduction

6.2 Practical Guidance

6.3 Viruses

6.4 Current Use of EM in Virology

6.5 Viruses in Thin Sections of Cells or Tissues

6.6 Bacteria

6.7 Fungal Organisms

6.8 Microsporidia

6.9 Parasitic Protozoa

6.10 Examples of Non-enteric Protozoa

6.11 Parasitic Amoebae

6.12 Conclusions

Acknowledgements

References and Additional Reading

Chapter 7: The Contemporary Use of Electron Microscopy in the Diagnosis of Ciliary Disorders and Sperm Centriolar Abnormalities

7.1 Introduction

7.2 Ultrastructure of Motile Cilia

7.3 Genetics of PCD

7.4 Current Diagnostic Modalities

7.5 Clinical Features

7.6 Procurement and Assessment of Ciliated Specimens

[7.7 Centriolar Sperm Abnormalities](#)

[7.8 Discussion](#)

[Acknowledgements](#)

[References](#)

[Chapter 8: Electron Microscopy as a Useful Tool in the Diagnosis of Lysosomal Storage Diseases](#)

[8.1 Introduction](#)

[8.2 Morphological Findings](#)

[8.3 Conclusion](#)

[References](#)

[Chapter 9: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy \(CADASIL\)](#)

[9.1 Introduction](#)

[9.2 Diagnostic Strategies—Comparative Specificity and Sensitivity](#)

[9.3 Diagnosis by TEM](#)

[References](#)

[Chapter 10: Diagnosis of Platelet Disorders by Electron Microscopy](#)

[10.1 Introduction](#)

[10.2 TEM Preparation of Platelets](#)

[10.3 Whole-Mount EM Preparation of Platelets](#)

[10.4 EM Preparation of Bone Marrow](#)

[10.5 Pre-embed Immunogold Labelling of Von Willibrand Factor in Platelets](#)

[10.6 Ultrastructural Features of Platelets](#)

[10.7 Normal Platelets](#)

[10.8 Grey Platelet Syndrome](#)

[10.9 Arthrogryposis, Renal Dysfunction and Cholestasis Syndrome](#)

[10.10 Jacobsen Syndrome](#)

[10.11 Hermansky-Pudlak Syndrome, Chediak-Higashi Syndrome and Other Dense-Granule Deficiencies](#)

[10.12 Type 2B von Willebrand Disease and Platelet-Type von Willebrand Disease](#)

[References](#)

[Chapter 11: Diagnosis of Congenital Dyserythropoietic Anaemia Types I and II by Transmission Electron Microscopy](#)

[11.1 Introduction](#)

[11.2 Preparation of Bone Marrow and General Observation Protocol](#)

[11.3 CDA Type I](#)

[11.4 CDA Type II](#)

[Acknowledgements](#)

[References](#)

[Chapter 12: Ehlers-Danlos Syndrome](#)

[12.1 Introduction](#)

[12.2 Collagen Fibrils](#)

[12.3 Elastic Fibers](#)

[12.4 Nonfibrous Stroma and Granulo-Filamentous Deposits](#)

[12.5 Connective Tissue Disorders](#)

[References](#)

[Chapter 13: Electron Microscopy in Occupational and Environmental Lung Disease](#)

[13.1 Introduction](#)

[13.2 Asbestos](#)

[13.3 Hypersensitivity Pneumonitis and Sarcoidosis](#)

[13.4 Silicosis](#)

[13.5 Silicate Pneumoconiosis](#)

[13.6 Metal-Induced Diseases](#)

[13.7 Rare-Earth Pneumoconiosis](#)

[13.8 Miscellaneous Disorders](#)

[References](#)

[Chapter 14: General Tissue Preparation Methods](#)

[14.1 Introduction](#)

[14.2 Tissue Collection and Dissection](#)

[14.3 Tissue Processing](#)

[14.4 Tissue Sectioning](#)

[References](#)

Chapter 15: Ultrastructural Pathology Today—Paradigm Change and the Impact of Microwave Technology and Telemicroscopy

15.1 Diagnostic Electron Microscopy and Paradigm Shift in Pathology

15.2 Standardised and Automated Conventional Tissue Processing

15.3 Microwave-Assisted Sample Preparation

15.4 Cyberspace for Telepathology via the Internet

15.5 Conclusions and Future Prospects

Acknowledgements

References

Chapter 16: Electron Microscopy Methods in Virology

16.1 Biological Safety Precautions

16.2 Collection of Specimens

16.3 Preparation of Faeces, Vomitus or Urine Samples

16.4 Viruses in Skin Lesions

16.5 Reagents and Methods

16.6 Coated Grids

16.7 Important Elements in the Negative Staining Procedure

16.8 TEM Examination

16.9 Immunoelectron Microscopy

[16.10 Thin Sectioning of Virus-Infected Cells or Tissues](#)

[16.11 Virology Quality Assurance \(QA\) Procedures](#)
[Acknowledgements](#)

[References](#)

[Chapter 17: Digital Imaging for Diagnostic Transmission Electron Microscopy](#)

[17.1 Introduction](#)

[17.2 Camera History](#)

[17.3 The Pixel Dilemma](#)

[17.4 Camera Positioning](#)

[17.5 Resolution](#)

[17.6 Fibre Coupled or Lens Coupled?](#)

[17.7 Sensitivity, Noise and Dynamic Range](#)

[17.8 CCD Chip Type \(Full Frame or Interline\)](#)

[17.9 Binning and Frame Rate](#)

[17.10 Software](#)

[17.11 Choosing the Right Camera](#)

[References](#)

[Chapter 18: Uncertainty of Measurement](#)

[18.1 Introduction](#)

[18.2 Purpose](#)

[18.3 Factors That Influence Quantitative Measurements](#)

[18.4 How to Calculate the UM](#)

[18.5 Worked Examples](#)

[18.6 Conclusion](#)

[References](#)

[Index](#)

Current and future titles in the Royal Microscopical Society – John Wiley Series

Published

Principles and Practice of Variable Pressure/Environmental Scanning Electron Microscopy (VP-ESEM)

Debbie Stokes

Aberration-Corrected Analytical Electron Microscopy

Edited by Rik Brydson

Diagnostic Electron Microscopy—A Practical Guide to Interpretation and Technique

Edited by John W. Stirling, Alan Curry & Brian Eyden

Forthcoming

Low Voltage Electron Microscopy: Principles and Applications

Edited by David C. Bell & Natasha Erdman

Atlas of Images and Spectra for Electron Microscopists

Edited by Ursel Bangert

Understanding Practical Light Microscopy

Jeremy Sanderson

Focused Ion Beam Instrumentation: Techniques and Applications

Dudley Finch & Alexander Buxbaum

*Electron Beam-Specimen Interactions and Applications in
Microscopy*

Budhika Mendis

Diagnostic Electron Microscopy – A Practical Guide to Interpretation and Technique

Edited by

John W. Stirling

The Centre for Ultrastructural Pathology, Adelaide, Australia

Alan Curry

Manchester Royal Infirmary, Manchester, UK

and

Brian Eyden

Christie NHS Foundation Trust, Manchester, UK

*Published in association with the Royal
Microscopical Society*

Series Editor: Susan Brooks

 **WILEY**

A John Wiley & Sons Ltd Publication

This edition first published 2013

© 2013 John Wiley & Sons Ltd.

Registered office

John Wiley & Sons Ltd, The Atrium, Southern Gate,
Chichester, West Sussex, PO19 8SQ, United Kingdom

For details of our global editorial offices, for customer
services and for information about how to apply for
permission to reuse the copyright material in this book
please see our website at www.wiley.com.

The right of the author to be identified as the author of this
work has been asserted in accordance with the Copyright,
Designs and Patents Act 1988.

All rights reserved. No part of this publication may be
reproduced, stored in a retrieval system, or transmitted, in
any form or by any means, electronic, mechanical,
photocopying, recording or otherwise, except as permitted
by the UK Copyright, Designs and Patents Act 1988, without
the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic
formats. Some content that appears in print may not be
available in electronic books.

Designations used by companies to distinguish their
products are often claimed as trademarks. All brand names
and product names used in this book are trade names,
service marks, trademarks or registered trademarks of their
respective owners. The publisher is not associated with any
product or vendor mentioned in this book. This publication is
designed to provide accurate and authoritative information
in regard to the subject matter covered. It is sold on the
understanding that the publisher is not engaged in
rendering professional services. If professional advice or
other expert assistance is required, the services of a
competent professional should be sought.

The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of fitness for a particular purpose. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for every situation. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read. No warranty may be created or extended by any promotional statements for this work. Neither the publisher nor the author shall be liable for any damages arising herefrom.

Library of Congress Cataloging-in-Publication Data

Diagnostic electron microscopy : a practical guide to interpretation and technique / edited by John W. Stirling, Alan Curry, and Brian Eyden.

p. ; cm.

Includes bibliographical references and index.

ISBN 978-1-119-97399-7 (cloth)

I. Stirling, John W. II. Curry, Alan. III. Eyden, Brian.

[DNLM: 1. Diagnostic Imaging-methods. 2. Microscopy,
Electron, Transmission. WN 180]

616.07'54—dc23

2012027835

A catalogue record for this book is available from the British
Library.

ISBN: 978-1-119-97399-7

Acknowledgements and Dedication

All three editors wish to thank the many individuals who have helped to make this volume possible. Firstly, they would like to express their appreciation to all the authors for their hard work and generosity in sharing their professional experience, as well as all the 'behind-the-scenes' staff and colleagues without whom this book could not have been produced.

John Stirling thanks the staff of the Centre for Ultrastructural Pathology, SA Pathology, Adelaide, for their support and photographic contributions—especially Alvis Jaunzems and Jeffrey Swift—and Dr Sophia Otto of the Department of Surgical Pathology, SA Pathology, for her advice and for proofreading.

Alan Curry acknowledges the contributions to his work of the pathologists, particularly Dr Helen Denley and Dr Lorna McWilliam, and technical staff of the Manchester Royal Infirmary, as well as two inspirational organisations—the Public Health Laboratory Service Electron Microscopy network and the Manchester Electron Microscope Society.

Brian Eyden wishes to thank all of the Pathology Department staff at the Christie NHS Foundation Trust (Manchester), without whose technical and light microscopic input the interpretation of tumour ultrastructure would be compromised, if not, in some instances, impossible.

Secondly, the editors wish to recognise the support and encouragement of their families in this endeavour. John Stirling thanks his partner, Jill, and expresses a special appreciation of his teachers and mentors, particularly Alec Macfarlane who helped him achieve his dream of a career in biology and Andrew Dorey who introduced him to electron microscopy and the wonders of cell ultrastructure. Alan

Curry thanks his wife, Collette (particularly for her exceptional computer skills), and Brian Eyden thanks his wife, Freda, for understanding the needs of a writing scientist.

Finally, the editors dedicate this book to diagnostic electron microscopists—wherever they may be—who continue to make uncertain diagnoses more precise as a result of their labours, which, in turn, help clinicians to treat their patients better, the ultimate purpose of our work.

List of Contributors

Joseph Alroy, Department of Pathology, Tufts University Cummings's School of Veterinary Medicine, Grafton, Massachusetts, United States and Department of Pathology and Laboratory Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, Massachusetts, United States

John Brealey, Centre for Ultrastructural Pathology, Surgical Pathology—SA Pathology (RAH), Adelaide, Australia

Hilary Christensen, Program in Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada

Alan Curry, Health Protection Agency, Clinical Services Building, Manchester Royal Infirmary, Manchester, United Kingdom

Elizabeth Curtis, Muscle Biopsy Service/Electron Microscope Unit, Department of Cellular Pathology, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom

Gary Paul Edwards, Chelford Barn, Stowmarket, Suffolk, United Kingdom

Brian Eyden, Department of Histopathology, Christie NHS Foundation Trust, Manchester, United Kingdom

Pierre Fillion, Electron Microscopy Section, Division of Anatomical Pathology, PathWest Laboratory Medicine, QE II Medical Centre, Nedlands, Australia

A. Hadjisavvas, Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Trinh Hermanns-Lê, Department of Dermatopathology, University Hospital of Liège, Liège, Belgium

Walter H.A. Kahr, Division of Haematology/Oncology, Program in Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada and Departments of Paediatrics and Biochemistry, University of Toronto, Toronto, Ontario, Canada

Rosalind King, Institute of Neurology, University College London, London, United Kingdom

K. Kyriacou, Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

M. Nearchou, Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Rolf Pfannl, Department of Pathology and Laboratory Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, Massachusetts, United States

Gérald E. Piérard, Department of Dermatopathology,
University Hospital of Liège, Liège, Belgium

Claudine Piérard-Franchimont, Department of
Dermatopathology, University Hospital of Liège, Liège,
Belgium

Marie-Annick Reginster, Department of
Dermatopathology, University Hospital of Liège, Liège,
Belgium

Victor L. Roggli, Department of Pathology, Duke
University Medical Center, Durham, North Carolina,
United States

Yong-xin Ru, Institute of Haematology & Blood Diseases
Hospital, Chinese Academy of Medical Sciences and
Peking Union Medical College, Tianjin, China

Josef A. Schroeder, Zentrales EM-Labor, Institut für
Pathologie, Klinikum der Universität Regensburg,
Regensburg, Germany

Caroline Sewry, Wolfson Centre for Inherited
Neuromuscular Diseases, RJA Orthopaedic Hospital,
Oswestry, United Kingdom and Dubowitz Neuromuscular
Centre, Institute of Child Health and Great Ormond
Street Hospital, London, United Kingdom

John W. Stirling, Centre for Ultrastructural Pathology,
IMVS—SA Pathology, Adelaide, Australia

Angelo A. Ucci, Department of Pathology and
Laboratory Medicine, Tufts Medical Center and Tufts

University School of Medicine, Boston, Massachusetts,
United States

P. Yiallourous, Cyprus International Institute, Cyprus
University of Technology, Limassol, Cyprus

Preface-Introduction

John W. Stirling, Alan Curry and Brian Eyden

Diagnostic Electron Microscopy

Science progresses as a result of a variety of factors. Critical to progress, however, is the invention and availability of appropriate tools and techniques that can completely transform our ability to investigate and understand the world around us—without such tools our ability to investigate even basic phenomena would be severely restricted. One such ‘transformational’ technology is the electron microscope. Although transmission electron microscopy (TEM) is now taken for granted, its application to the biological and medical sciences in the late 1950s and early 1960s ranks as one of the single most important factors that has impacted on our knowledge in biology and medicine. The resolving power of the transmission electron microscope (~ 0.2 nm as compared with the light microscope with a resolution of ~ 200 nm) made two important things possible for the first time, these being the visualisation of: (1) cell organelles and cytoplasmic structures at the macromolecular level (both useful indicators of cell differentiation) and; (2) viruses and microorganisms in general. Thus, TEM gave us new fundamental insights into cell structure and function, histogenesis and differentiation, and, following from this, our understanding of disease and disease processes.

TEM was quickly taken up as a diagnostic tool. In the clinical setting, electron microscopy has been used to

improve diagnostic precision and confidence in many fields, including renal disease, neuromuscular disease, microbiology (particularly virology), tumour pathology, skin diseases, industrial diseases, haematology, metabolic storage diseases and conditions involving abnormalities of cilia and sperm. A number of encyclopaedic atlases of normal and pathological tissues quickly followed the introduction of electron microscopy and the medical literature contains many articles describing diagnostic applications of TEM in a wide range of conditions and specialist areas. Diagnostic TEM reached a zenith during the 1980s; however, since then, the introduction of new methodologies (particularly molecular techniques and affinity labelling systems) has reduced the need for TEM, particularly in tumour diagnosis. Despite this, TEM continues to play a significant and important role in pathology, and techniques continue to develop and improve. For example, the introduction of microwave processing and digital cameras has transformed tissue processing and screening so that 'same-day' reporting is easily achieved.

The purpose and use of TEM

The purpose of TEM is to diagnose disease based on the ultrastructural features of the tissue. These features include:

- 1.** The presence (or sometimes the absence) of specific or characteristic cellular structures or organelles that indicate cell differentiation
- 2.** The general ultrastructural architecture, including the identity, location and morphology of specific structural features that may be associated with pathology, or indicate disease.

In general, the use of TEM will be predetermined either as a stand-alone protocol (e.g., CADASIL) or as part of a broad integrated diagnostic strategy (e.g., renal biopsies). However, TEM can also be applied on an *ad hoc* basis whenever there is a chance it will give an improved diagnosis (and therefore better patient care). The general criteria indicating the use of TEM may be summarised simply as follows:

- 1.** When it provides useful (complementary) structural, functional or compositional information in respect to diagnosis, differential diagnoses or disease staging
- 2.** When only atypical features or minor abnormalities are visible by light microscopy despite clear clinical evidence of disease (e.g. some renal diseases)
- 3.** When affinity labelling results are equivocal (e.g. renal disease and tumours)
- 4.** When there is no realistic alternative diagnostic technique or a 'simple' test is not available or feasible (e.g. genetic diseases with multiple mutations such as CADASIL and primary ciliary dyskinesia)
- 5.** The investigation and diagnosis of new diseases and microorganisms
- 6.** When it is time and/or cost effective in respect to alternative techniques.

The aim and purpose of this book

The prime aim and purpose of this book is to summarise the current interpretational applications of TEM in diagnostic pathology. In this respect, we have not attempted to reproduce previous encyclopaedic texts but to provide what we regard as a working guide to the main, or most useful,

applications of the technique given the limited space available in a text of this size. In addition, we have also included practical topics of concern to laboratory scientists, including brief guides to traditional tissue and microbiological preparation techniques, microwave processing, digital imaging and measurement uncertainty.

Chapter 1

Renal Disease

John W. Stirling¹ and Alan Curry²

¹*Centre for Ultrastructural Pathology, IMVS—SA Pathology
Adelaide Australia*

²*Health Protection Agency, Clinical Sciences Building
Manchester Royal Infirmary, Manchester United Kingdom*

1.1 The Role of Transmission Electron Microscopy (TEM) in Renal Diagnostics

The ultrastructural examination of renal biopsies has made a significant contribution to our understanding of renal disease and is fundamental to accurate diagnosis. For overall tissue evaluation, light microscopy (LM), immunolabelling and transmission electron microscopy (TEM) are generally combined as an integrated protocol. LM is used to make an assessment of overall tissue morphology and to identify the major pathological processes present. Immunolabelling (preferably using immunofluorescence or by the immunoperoxidase technique) is used to determine the composition and location of glomerular immune

deposits. Local practices vary, but an antibody panel can contain antibodies directed against IgG, IgA, IgM, complement (C3, C1q and sometimes C4), κ and λ light chains and albumin. TEM can play a major role when LM and immunolabelling findings are normal, only mildly atypical or equivocal and difficult to interpret, particularly in respect to conditions where there may be similar LM or immunolabelling findings. Thus, the technique is particularly useful in the setting of familial disease where the structural abnormalities in the glomerular basement membrane (GBM) cannot be resolved by LM (e.g. Alport's syndrome). TEM can also provide critical information not revealed by the other methodologies to identify underlying primary disease and unexpected concomitant disease. Similarly with immunolabelling, the full classification and staging of deposits require ultrastructural analysis. Some transplant biopsies can also benefit from ultrastructural evaluation (see Chapter 2); however, TEM rarely contributes to the diagnosis of tubular, vascular or interstitial disease. Overall, ultrastructural screening is essential; it can change the diagnosis in ~25% of cases and provides 'useful' information in ~66% of cases (Pearson *et al.*, 1994; Elhefnawy, 2011).

1.2 Ultrastructural Evaluation and Interpretation

Examination of glomeruli (and other areas, if necessary) should be thorough and systematic with all components being evaluated for possibly significant features or changes. During screening, a range of representative images should be taken. These should include low-power images to show

overall glomerular morphology, plus a representative selection of higher power images to show the specific and critical diagnostic features. In some instances, it may also be important to show that certain features are, in fact, absent (e.g. deposits) or normal (e.g. foot processes). The principal elements that should be examined are (i) the location, size and morphology of immune-related deposits and other inclusions; (ii) the thickness, overall morphology and texture of the GBM; (iii) the size and morphology of the mesangial matrix and (iv) the number and morphology of the cellular components of the glomerulus (Stirling *et al.*, 2000). Sclerotic glomeruli should be avoided, and only well-preserved functional (or significantly functional) glomeruli should be examined. It is also important to ensure that the glomeruli screened are representative of the LM findings: this means that, ideally, the choice of glomeruli to be screened (from semithin sections) should be done in collaboration with the reporting pathologist. Finally, it should be stressed that screening should be unbiased, although some knowledge of the pathology and immunolabelling results may be useful if the features expected are minor or uncommon. The vascular pole should be avoided during ultrastructural evaluation as it may contain misleading nonpathologic deposits, and likewise Bowman's capsule which has no real diagnostic value, although the presence of crescents can be confirmed.

Following evaluation, representative images and findings should be communicated to the reporting pathologist, the latter verbally or in a concise written report. If the initial evaluation does not correspond with the LM evaluation (e.g. the electron microscopy (EM) samples only a tiny fraction of the available tissue), then the specimen should be re-examined or additional glomeruli observed to increase diagnostic confidence.