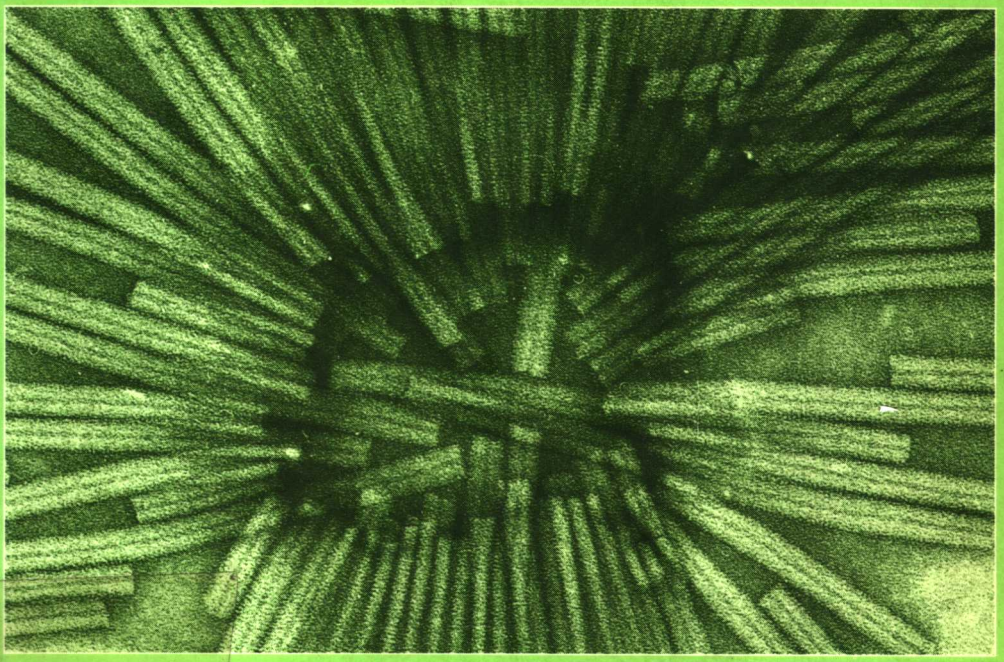
Electron Microscopy in Biology

A Practical Approach

Edited by
J. R. HARRIS



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Preface

This techniques book *Electron Microscopy in Biology* was conceived following the publication of the more specialist book within the *Practical Approach* series, entitled *Electron Microscopy in Molecular Biology*. An attempt is made here to cover some of the topics omitted or not fully covered in this previous book and to provide useful up-to-date methodological guidelines for those wishing to utilize electron microscopy as one of their research tools.

No attempt is made here to compete in terms of depth of background, experimental details, biological applications, and referencing with the other established books and series dealing with EM techniques. Indeed, several of the chapters within this book have been covered elsewhere as single or more books. Rather, each chapter presents concise accounts of the current methodology. Authors, who are specialists within their field of study, have selected what they consider to be relevant and important techniques for inclusion, most of which are presented in the 'protocol' format, supplemented by technical discussion. This being the case, there is a strong personal emphasis and flavour to almost all the contributions, which I look upon as a point of strength, within the limited framework of this short book. An attempt has been made to seek authors based within universities and research institutes throughout the world. This has been achieved to some considerable extent, although an apology is perhaps due for the predominance of UK-based contributors. The freedom given to authors with regard to chapter content has led to a small amount of technical repetition between chapters. In general this is of value, since slightly varying technical emphasis is likely to prove useful. Thus, in the main, each chapter stands as a fairly complete subject area in its own right, without the need for extensive cross-referencing to other parts of the book.

The book commences with the two fundamentally important topics of tissue fixation, covered by David Hopwood and Gordon Milne, and embedding and thin sectioning, covered by Michael Smith and Simon Croft. These two chapters represent the core strength of classical transmission electron microscopy (TEM), leading to the production of 'thin plastic/resin sections'. Many of the procedures subsequently dealt with in later chapters extend from the extensive groundwork of investigators in these areas over the past 40 years. In the third chapter, low temperature techniques are introduced as a main theme for the first time, although some mention of low temperature embedding has already appeared in the second chapter. Norbert Roos deals here with freeze-substitution and other low temperature embedding methods in an extremely competent

manner. Then comes a chapter from Alex D. Hyatt that deals very broadly with immunogold labelling techniques. It is easy to forget that immunolabelling techniques can be applied to freeze-fractured and negatively stained materials (and indeed to metal shadowed preparations) as well as to sectioned specimens. Alex Hyatt appropriately balances the subject and includes his own unique approach making use of negative staining. In the fifth chapter Gérard Morel covers in an extremely thorough manner the topic of electron microscopic autoradiography. Of equally high standard is the chapter on electron microscopical enzyme histochemistry from Cornelis Van Noorden and Caesar Hulstaert, who select for presentation the principal cytochemical techniques from what is a very large field of study. In his chapter on high resolution shadowing, Henry Slayter, places emphasis upon macromolecular studies by darkfield electron microscopy. In reality, the advanced shadowing techniques that Henry Slayter presents are likely to have a much broader usage in the future, particularly in relation to isolated organelles and membranes, and also for the study of the surface of intact cells. The field of freeze-fracture has expanded steadily and this is refected in the large number of technical procedures presented by Seizo Fujikawa. Negative staining, to some extent a neglected area in the existing texts on electron microscopical techniques, is dealt with by Bob Horne and myself. We have not attempted to cover every aspect of this subject, but have tried to give a thorough background upon which we place some of the existing negative staining techniques of tried and tested success, together with a new technique that has exciting cellular possibilities. Advancement in the field of cryoelectron microscopy of unstained frozen-hydrated specimens continues apace. This subject is handled in a brief yet professionally thorough manner by Murray Stewart in his chapter on the TEM of vitrified biological macromolecular assemblies. Emphasis upon cryotechniques continues in the chapter by Alice Warley and Brij Gupta, who present a detailed account of the procedures for performing quantitative biological X-ray microanalysis. In the final chapter of the book, Nigel James handles the difficult topic of three-dimensional electron microscopy of tissues and cells, from a mathematical yet practical point of view. Dr James indicates that the 3D reconstruction techniques available can be performed in any laboratory possessing a suitable microcomputer and prepared to purchase and utilize the necessary software.

In the space made available by the publisher not all electron microscopical techniques could be included, even within the subject areas of the chapter titles selected for inclusion in the book. Nevertheless, it is hoped that the book will prove to be useful for those performing or wishing to perform biological or biomedical experimentation using electron microscopy. All chapters are thoroughly referenced and provide an entry point to the relevant literature.

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R.H.

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Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
BPST	2-(2-benzothiazolyl)-3-(4-phthalhydrazidyl)-5-styryl-tetrazolium
BSA	bovine serum albumin
CBZ	benzyloxycarbonyl
CG	colloidal gold
Ci	Curie
Con A	concanavalin A
CRT	cathode ray tube
CTEM	conventional transmission electron microscope
CTF	contrast transfer function
2D	2-dimensional
3D	3-dimensional
DAB	diaminobenzidine
DMP	dimethoxy-methane
DMSO	dimethyl sulphoxide
d.p.m.	disintegrations per minute
EDS	energy dispersive spectrometer
EDTA	ethylene diaminetetraacetate
EDX	energy dispersive X-ray
EG	ethylene glycerol
EGTA	ethyleneglycobis (β-aminoethyl) ether *N,N,N',N'* tetra acetic acid
EM	electron microscope/microscopy
EMT	electron microscope tomography
GACH	glutaraldehyde/carbohydrazide
GMA	glyco methacrylate
eV	electronvolt
HD	half distance
HES	hydroxyethyl starch
HPL	*Helix pomatia* lectin
HR	half radius
HPL–CG	*Helix pomatia* lectin-colloidal gold
HVEM	high voltage electron microscopy
Ig–CG	immunoglobulin coated colloidal gold
IEM	immunoelectron microscopy
IGS	immunogold staining
ITP	inosine triphosphate
IVEM	intermediate voltage electron microscopy
K	kelvin
kV	kilovolts
LN_2	liquid nitrogen
L_V	length per unit volume of tissue

MNA	4-methoxy-2-naphthylamine
NADH	nicotinamide adenine dinucleotide (reduced form)
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NCIEM	negative contrast immunoelectron microscopy
nitro BT	nitro blue tetrazolium
N_V	numerical density
Pa	pascal
PA–CG	protein-A-coated colloidal gold
PBS	phosphate buffered saline
PEG	polyethylene glycol
PLT	progressive lowering temperatures
PMSF	phenyl methyl sulphonyl fluoride
PVP	polyvinyl pyrrolidone
RBC	red blood cell
RCA I	*Ricinus communis* I
RCA I–CG	*Ricinus communis* I–colloidal gold
RT	room temperature
SEM	scanning electron microscope
SNR	signal-to-noise ratio
SIEM	scanning immunoelectron microscopy
STEM	scanning transmission electron microscope
S_V	surface density
TCH	thiocarbohydrazide
TEM	transmission electron microscope
UA	uranyl acetate
UDP	uridine diphosphate
UDT	uridine triphosphate
VDU	visual display unit
V_V	volume fraction
WDS	wavelength dispersive spectrometer
WGA	wheat germ agglutinin
ZAF	atomic number (Z), absorption of X-rays (A) and secondary fluorescence (F)

Contents

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Fixation

DAVID HOPWOOD and GORDON MILNE

1. Introduction

The techniques chosen to fix tissues should enhance the sort of microscopical information which is eventually produced. Ideally, the tissue should be retained in as near life-life condition as possible, but the better the morphological preservation, the less the biological activity and vice versa. Some compromise has to be reached. Fixation should also prepare tissues to withstand the subsequent rigours of the preparative techniques (Chapter 2) and finally irradiation by the electron beam (1, 2).

Conventionally, tissues are fixed chemically and a number of chemical primary fixatives are in wide use for electron microscopy. Those most commonly used are the dialdehyde glutaraldehyde, (*para*)-formaldehyde, and osmium tetroxide. Others which may have a role include picric acid and acrolein. These fixatives are not necessarily interchangeable. Different results can be obtained in freeze-fracturing by using different fixatives (3). It is important to choose the right buffer for the fixative to be used. Some buffers will react with the fixative, thereby reducing the effective concentration of both reagents, for example, amine-containing buffers and aldehydes. Other buffers will extract material from the tissues. The most commonly used buffers for electron microscopy are sodium cacodylate and phosphate.

The temperature of fixation for electron microscopy is by convention 4°C. This will affect the rate of penetration of the fixative into the tissue, its rate of reaction with the tissues and the rate at which substances are extracted from the tissue. It is possible to use other temperatures successfully.

The rate at which fixatives diffuse into tissues is governed by

$$d = k\sqrt{t}$$

where d is the depth of penetration at positive t and k, the constant of diffusibility, a characteristic of each fixative. The values for the commonly used fixatives are given in the table below, as measured by standard techniques. The slow rate of penetration is the rationale behind small tissue blocks for electron microscopy. Even so, different layers of cells will be fixed at different rates, at times, producing a noticeable artefact.