

<b>1</b>	<b>An Introduction to Electron Microscopy (EM)</b> . . . . .	<b>1</b>
1.1	Imaging Methods in Electron Microscopy . . . . .	1
1.1.1	Conventional Transmission Electron Microscopy (TEM) . . . . .	1
1.1.1.1	Bright Field Electron Microscopy . . . . .	1
1.1.1.2	Low Dose Transmission Electron Microscopy . . . . .	2
1.1.1.3	Dark Field Electron Microscopy . . . . .	3
1.1.2	Conventional Scanning Electron Microscopy (SEM) . . . . .	5
1.1.2.1	Imaging with Secondary and Back-Scattered Electrons . . . . .	5
1.1.2.2	Scanning Electron Microscopy at Low Accelerating Voltages . . . . .	8
1.2	Preparation Procedures in TEM . . . . .	10
1.2.1	Overview . . . . .	10
1.2.2	Structural Preservation During Fixation, Dehydration and Embedding of Biological Objects . . . . .	12
1.3	Imaging Problems . . . . .	13
1.3.1	On the Interpretation of TEM Images . . . . .	13
1.3.2	On the Interpretation of SEM Images . . . . .	15
1.4	Support Films . . . . .	16
1.4.1	Grids for TEM and Their Pretreatment . . . . .	16
1.4.2	Formvar Films . . . . .	16
1.4.3	Collodion Films . . . . .	17
1.4.4	Hydrophilisation of Films . . . . .	18
1.4.5	Support Films with Holes . . . . .	18
1.4.6	Carbon Films . . . . .	19
<b>2</b>	<b>Methods for TEM</b> . . . . .	<b>23</b>
2.1	Fixation, Dehydration and Embedding . . . . .	23
2.1.1	Chemical Fixations . . . . .	23
2.1.1.1	General Comments . . . . .	23
2.1.1.2	Fixatives: Properties and Preparation . . . . .	24
2.1.1.3	Composition of Fixation Solutions . . . . .	27
2.1.1.4	The Fixation of Animal Cells . . . . .	31
2.1.1.5	The Fixation of Plants and Microorganisms . . . . .	38

2.1.1.6	The Fixation of Isolated Organelles . . . . .	41
2.1.1.7	Fixing for Immunocytochemistry . . . . .	42
2.1.2	Dehydration . . . . .	43
2.1.3	Embedding . . . . .	43
2.1.3.1	Embedding Media: General Usage and Precautions . . . . .	44
2.1.3.2	Conventional Embedding . . . . .	44
2.1.3.3	Water-Soluble Embedding Media . . . . .	46
2.1.3.4	Embedding for Immunocytochemistry . . . . .	47
2.1.3.5	Embedding Moulds and Specimen Orientation . . . . .	51
2.1.3.6	Embedding of Monolayer Cell Cultures . . . . .	52
2.2	Ultramicrotomy . . . . .	53
2.2.1	Trimming of Blocks . . . . .	53
2.2.1.1	General . . . . .	53
2.2.1.2	Controlled Trimming: Production and Staining of Semi-Thin Sections . . . . .	53
2.2.2	Preparing Glass Knives . . . . .	55
2.2.2.1	Preparation of the Glass Strips . . . . .	55
2.2.2.2	Breaking Glass Squares . . . . .	57
2.2.2.3	Making Knives . . . . .	57
2.2.2.4	Judging the Quality of a Glass Knife . . . . .	58
2.2.2.5	Attaching Troughs . . . . .	58
2.2.2.6	Storing Glass Knives . . . . .	59
2.2.3	Diamond Knives and Their Care . . . . .	60
2.2.4	Conventional Sectioning . . . . .	61
2.2.4.1	Trough Liquids . . . . .	61
2.2.4.2	Using an Ultramicrotome . . . . .	61
2.2.4.3	Section Thickness . . . . .	62
2.2.4.4	Picking Up Sections . . . . .	62
2.2.4.5	Sectioning Problems . . . . .	64
2.2.5	Cryo-ultramicrotomy . . . . .	66
2.2.5.1	Freezing the Sample . . . . .	66
2.2.5.2	Sectioning the Frozen Sample . . . . .	66
2.2.5.3	Picking up Frozen Sections . . . . .	67
2.2.6	Staining Sections . . . . .	67
2.2.6.1	Staining Solutions . . . . .	67
2.2.6.2	Procedure for Double Staining Sections . . . . .	70
2.2.6.3	Staining Sections of Material Embedded for Immunocytochemical Purposes . . . . .	71
2.2.6.4	Staining Cryosections . . . . .	72
2.2.6.5	Block Staining . . . . .	74
2.3	Macromolecular EM . . . . .	75
2.3.1	Isolated Proteins and Protein Aggregates . . . . .	75
2.3.1.1	Preparation of Specimens . . . . .	75
2.3.1.2	Negative Staining Techniques . . . . .	76

2.3.1.3	High Resolution Metal Shadowing . . . . .	81
2.3.1.4	Preparation and Imaging of Two-Dimensional Protein Crystals . . . . .	82
2.3.1.5	Making a "Tilt Series" . . . . .	84
2.3.2	Isolated Nucleic Acids . . . . .	84
2.3.2.1	Problems and Aims . . . . .	84
2.3.2.2	Specimen Preparation . . . . .	85
2.3.2.3	Spreading and Diffusion Techniques Which Employ Cytochrome c . . . . .	87
2.3.2.4	"BAC" Technique . . . . .	90
2.3.2.5	Partial Denaturing, Heteroduplex and R-Loop Techniques . . . . .	91
2.3.3	Nucleic Acid-Protein Complexes . . . . .	95
2.3.3.1	Specimen Preparation . . . . .	95
2.3.3.2	Production and Staining of NA-Protein Complexes . . . . .	95
2.4	Immunoelectron Microscopy (IEM) . . . . .	96
2.4.1	Principle Requirements . . . . .	96
2.4.1.1	Antigens . . . . .	96
2.4.1.2	Antibodies . . . . .	97
2.4.2	Labelling of Antigens in Cells and Cell Fractions . . . . .	99
2.4.2.1	Ferritin-Labelled Antibodies . . . . .	99
2.4.2.2	Immunolabelling with Protein A-Gold . . . . .	100
2.4.3	Localization of Protein Subunits with Specific IgG Antibodies . . . . .	103
2.4.3.1	Preparation and Visualization of the Protein-Antibody Complex . . . . .	103
2.5	Autoradiography . . . . .	104
2.5.1	General Background . . . . .	104
2.5.1.1	Physical Basis . . . . .	104
2.5.1.2	Chemical Basis . . . . .	106
2.5.2	Choice and Dosis of Radioactive Compounds . . . . .	108
2.5.2.1	Choosing a Radioactive Precursor . . . . .	108
2.5.2.2	Dosage . . . . .	109
2.5.3	Working with Isotopes-Radiation Protection . . . . .	110
2.5.4	Preparation of Radio-Labelled Cells/Tissues for Electron Microscopy . . . . .	111
2.5.5	Photographic Emulsions and Autoradiography . . . . .	112
2.5.5.1	Apparatus Required . . . . .	112
2.5.5.2	Choice of Emulsion; Consequences for Resolution . . . . .	112
2.5.5.3	Preparation of Sections . . . . .	114
2.5.5.4	LM Autoradiography . . . . .	114
2.5.5.5	Emulsion, Coating Techniques . . . . .	116
2.5.6	Exposing, Developing and Fixing . . . . .	121
2.5.6.1	Exposing . . . . .	121
2.5.6.2	Developing and Fixing . . . . .	122
2.5.6.3	Future Developments in Autoradiography . . . . .	123
2.6	Freeze (Fracturing) Etching . . . . .	124
2.6.1	Introduction . . . . .	124

2.6.2	Freezing . . . . .	125
2.6.2.1	Theoretical Background . . . . .	125
2.6.2.2	Cytoprotectants . . . . .	126
2.6.2.3	Supports . . . . .	127
2.6.2.4	Cryogenes and Freezing Methods . . . . .	128
2.6.2.5	Storage of Frozen Specimens . . . . .	130
2.6.3	Fracturing . . . . .	130
2.6.3.1	Transfer of the Object into the Vacuum Recipient . . . . .	130
2.6.3.2	The Fracturing Process . . . . .	132
2.6.3.3	Fracture Planes in Biological Material . . . . .	133
2.6.4	Etching . . . . .	135
2.6.4.1	The Purpose of Etching . . . . .	135
2.6.4.2	Theory and Practice . . . . .	135
2.6.5	Shadowing and Replica Formation . . . . .	137
2.6.5.1	Resistance-Heating Evaporation . . . . .	138
2.6.5.2	Electron Beam Evaporation . . . . .	139
2.6.5.3	Measurement of Replica Thickness . . . . .	139
2.6.6	Cleaning the Replica . . . . .	140
2.6.7	Artifacts in Freeze Etching . . . . .	140
2.6.8	Using a Freeze-Etch Machine: a Practical Description . . . . .	141
<b>3</b>	<b>Methods for SEM . . . . .</b>	<b>145</b>
3.1	Conventional Methods of Preparation . . . . .	146
3.1.1	Introduction . . . . .	146
3.1.2	Specimen Size; Handling Specimens and Exposing Surfaces . . . . .	148
3.1.2.1	Cleaning Surfaces . . . . .	148
3.1.3	Stabilization . . . . .	151
3.1.3.1	Chemical Fixations . . . . .	151
3.1.3.2	Cryofixation . . . . .	154
3.1.4	Dehydration . . . . .	154
3.1.5	Drying . . . . .	155
3.1.5.1	Critical Point Drying . . . . .	155
3.1.5.2	Freeze Drying . . . . .	159
3.1.6	Mounting Specimens . . . . .	160
3.1.7	Increasing Conductivity . . . . .	162
3.1.7.1	Sputtering . . . . .	163
3.1.7.2	Evaporating . . . . .	164
3.2	Storage of Specimens . . . . .	164
3.3	Demonstration of Surfaces via Replicas and Casts . . . . .	164
3.4	Visualization of Internal Surfaces Through Sectioning and Dry-Fracturing (Dry-Cleaving) . . . . .	166
3.5	Element Analysis . . . . .	167

<b>4</b>	<b>Evaluation of Micrographs</b> . . . . .	173
4.1	Morphometry . . . . .	173
4.1.1	Problems and Solutions . . . . .	173
4.1.2	Measurement: Some General Points . . . . .	173
4.1.3	Stereology: General Principles . . . . .	174
4.1.4	Collection and Evaluation of Data; Statistical Treatments . . . . .	174
4.2	Averaging and Image Reconstruction . . . . .	176
4.2.1	General . . . . .	176
4.2.2	Markham Rotation . . . . .	176
4.2.3	Principles of Light Optical Diffraction . . . . .	177
4.2.4	Principles of Computer-Assisted Image Reconstruction . . . . .	179
	<b>Appendix: Buffers in Electron Microscopy</b> . . . . .	181
	<b>Subject Index</b> . . . . .	183