

The background of the cover is a high-magnification electron micrograph of biological tissue, likely showing cellular ultrastructure. It features various organelles such as mitochondria, endoplasmic reticulum, and large vesicles. The image is rendered in a monochromatic blue and grey tone. Several letters are overlaid on the micrograph: 'S' in the upper right, 'C' in the center, and 'V' at the bottom right.

Principles and Techniques of Electron Microscopy

Biological Applications

Fourth Edition

M. A. Hayat

CONTENTS

<i>Preface</i>	<i>page xvii</i>		
CHAPTER ONE			
HAZARDS, PRECAUTIONS, AND SAFE HANDLING OF REAGENTS	1		
BUFFERS	1	Phosphate Buffers	25
FIXATIVES	1	PIPES Buffer	25
SOLVENTS	2	Tris Buffer	25
EMBEDDING MATERIALS	2	Veronal Acetate Buffer	25
STAINS	2	Choice of Buffer	26
		Preparation of Buffers	26
		Cacodylate (0.2M)	26
		Collidine	26
		Buffer	26
		HEPES	26
		MOPS	26
		PIPES (0.3M)	27
		Phosphate	27
		Phosphate (Sörensen)	27
		Tris(hydroxymethyl)aminomethane	
		Maleate (0.2M)	27
		Veronal Acetate	27
CHAPTER TWO		ALDEHYDES	27
CHEMICAL FIXATION	4	Glutaraldehyde	28
INTRODUCTION	4	Nature of Commercial Glutaraldehyde	28
FACTORS AFFECTING THE QUALITY OF FIXATION	6	Reaction with Proteins	32
Tissue Specimen Size	7	<i>Mechanism of Protein Crosslinking</i>	33
Osmolarity and Osmolality	8	Reaction with Lipids	33
Cultured Cells	11	Reaction with Nucleic Acids	33
Vehicle Osmolarity	11	Reaction with Carbohydrates	34
Methods for Adjusting the Osmolarity	12	Osmolarity of Glutaraldehyde	34
Recommended Osmolality	12	Osmolality of Glutaraldehyde	35
Measurement of Osmolarity	13	Temperature	36
Ionic Composition of Fixative Solution	14	Concentration of Glutaraldehyde	36
Fixative pH	15	pH	37
Fixative Penetration	17	<i>Method for Using Glutaraldehyde at Higher pH Values</i>	37
Temperature of Fixation	18	<i>Changes in pH During Fixation with Glutaraldehyde</i>	37
Duration of Fixation	19	Rate of Penetration	38
Concentration of Fixative	20	Specimen Shrinkage	39
Effects of Added Substances	20	Limitations of Glutaraldehyde	39
BUFFERS	22	Storage of Glutaraldehyde	41
Buffer Types	23		
Cacodylate Buffer	24		
Collidine Buffer	24		
HEPES Buffer	24		
MOPS Buffer	25		

Glutaraldehyde-Containing Fixatives	41	Spleen	73
Formaldehyde	42	Perfusates	73
Reaction with Proteins	42	Procedure	73
Reaction with Lipids	43	Immersion Fixation	73
Reaction with Nucleic Acids	44	Dripping Method	74
Reaction with Carbohydrates	45	Injection Method	75
Preparation of Formaldehyde Solution	45	ANESTHESIA	75
ALDEHYDE MIXTURES	45	SIMULTANEOUS FIXATION FOR LIGHT AND	
OSMIUM TETROXIDE	45	ELECTRON MICROSCOPY	76
Reaction with Lipids	46	TISSUE STORAGE	76
Staining of Unsaturated Lipids	48	POSTMORTEM CHANGES	77
Reaction with Proteins	49	CRITERIA FOR SATISFACTORY SPECIMEN	
Reaction with Lipoproteins	50	PRESERVATION	79
Reaction with Nucleic Acids	50	ARTIFACTS	80
Reaction with Carbohydrates	52		
Reaction with Phenolic Compounds	53		
Method	53	CHAPTER THREE	
Reaction with Alkaloids	53	RINSING, DEHYDRATION, AND EMBEDDING	85
Loss of Lipids	54	INTRODUCTION	85
Loss of Proteins	55	RINSING	85
Changes in Specimen Volume	56	DEHYDRATION BEFORE EMBEDDING IN	
Parameters of Fixation	57	WATER-IMMISCIBLE RESINS	86
Concentration of Osmium Tetroxide	57	Acetone and Ethanol	86
Temperature of Fixation	57	Undesirable Effects of Dehydration	88
Rate of Penetration	57	Incomplete Dehydration for	
Duration of Fixation	59	Lipid Preservation	89
Removal of Bound Osmium from Sections	59	INFILTRATION OF RESIN	89
Osmium Blacks	59	POLYMERIZATION	91
Preparation and Precaution in the Handling		Polymerization by UV Irradiation	91
of Osmium Tetroxide	60	Polymerization of Epoxy Resins	
OSMIUM TETROXIDE AND		by Heat	91
GLUTARALDEHYDE MIXTURE	61	Mechanism of Crosslinking	91
PERMANGANATES	62	UNDESIRABLE EFFECTS OF INFILTRATION	
METHODS OF FIXATION	62	AND POLYMERIZATION	93
Vascular Perfusion	63	STANDARD PROCEDURE FOR FIXATION,	
Methods of Vascular Perfusion	66	RINSING, DEHYDRATION,	
General Method	66	AND EMBEDDING	94
Aorta	67	EMBEDDING MEDIA	95
Arteries	67	Water-Immiscible Embedding Media	97
Central Nervous System	68	Epoxy Resins	97
Embryo	68	Sectioning Properties	98
Heart	69	Epon 812 (LX-112, Epox 812, Polybed 812,	
Perfusates	69	Eponate 12, Taab 812, Agar 100,	
Procedure	69	Quetol 812)	98
Kidney	69	Embedding Formulation	98
Liver	70	Araldite	101
Perfusate	70	Embedding Formulations	101
Procedure	70	Vinylcyclohexene Dioxide (ERL 4206,	
Lung	71	Spurr Mixture)	102
Muscle (Skeletal Muscle of Rat Hind Limb)	72	Embedding Formulation	102
Perfusate	72	Quetol 651	103
Procedure	72	Methyl Methacrylate	103
Ovary	72	Embedding Formulation	103
Perfusate	72	Water-Miscible Embedding Media	104

Glycol Methacrylate	105	CHAPTER FOUR	139
<i>Limitations of Glycol Methacrylate</i>	106	SECTIONING	139
Serial Sections	107	INTRODUCTION	139
Embedding Formulations	107	GLASS KNIVES	140
Prepolymerization Procedure	108	Selection of Plate Glass	141
Dehydration and Polymerization	108	Preparation of Glass Knives	141
LOWICRYLS	109	Examination of Glass Knives	143
Properties of Lowicryls K4M, K11M, HM20, and HM23	109	Defects on Glass Knives	143
Progressive Lowering of Temperature Dehydration and Embedding in Lowicryls	111	Tungsten Coating of Glass Knives	144
Limitations of Lowicryls	111	DIAMOND KNIVES	144
Comparison of Lowicryl K4M with Epon	112	Examination of Diamond Knives	147
LR GOLD	114	Care of Diamond Knives	148
<i>Procedure</i>	114	Cleaning the Cutting Edge	149
LR WHITE	114	SAPPHIRE KNIVES	151
POLYETHYLENE GLYCOL (CARBOWAX)	115	MECHANISM OF THIN SECTIONING	152
MIXED RESIN EMBEDDING	116	SECTION THICKNESS	152
Vinylcyclohexene Dioxide- <i>n</i> -Hexenyl Succinic Anhydride	117	Relation Between Section Thickness and Interference Color	155
PROPERTIES OF THE FINAL RESIN BLOCK	118	Intrasection Variation in Thickness	156
Hardeners	118	Measurement of Section Thickness	157
Modifiers	118	SECTIONING ANGLES	159
Catalysts	119	Clearance Angle	159
VISCOSITY AND AGITATION	119	Knife Angle	159
SPECIMEN ORIENTATION	121	SPECIMEN BLOCK	160
Flat Embedding	123	Trimming and Preparation of Block Face	160
Labeling	124	Hand Trimming	161
RAPID EMBEDDING	124	Mechanical Trimming	163
GRADUAL, PROGRESSIVE DEHYDRATION AND EMBEDDING	127	Mesa Technique	164
LOW DENATURATION EMBEDDING	129	Mounting the Specimen Block	165
REVERSIBLE EMBEDDING	130	PREPARATION OF TROUGHS	166
Methods	131	MOUNTING THE KNIFE	167
REMBEDDING	133	TROUGH FLUIDS	170
Reembedding of Tissue Poorly Embedded in Resin	133	CUTTING SPEED	171
Reembedding of Thick Resin Sections	134	SECTION FLOTATION	171
Method 1	134	SECTION VIEWING	174
Method 2	134	SELECTION AND HANDLING OF GRIDS	175
Method 3	134	SECTION COLLECTION	178
Reembedding of Paraffin-Embedded Tissue in Resin	134	Mechanical Devices to Collect Sections	181
Two-Step Method	134	Static Electricity	181
One-Step Method	134	SECTIONING PROCEDURE	182
Reembedding of Paraffin-Embedded Tissue Sections in Resin	135	IMPROVEMENTS IN SECTIONING	184
Method 1	135	SURFACE CHARACTERISTICS OF THIN SECTIONS	184
Method 2	136	Section Relief	186
Method 3	136	SECTION DEFORMITIES	186
Pop-Off Method for Reembedding	137	Normal Surface Damage	186
Reembedding of Tissue Culture Cells	137	Section Compression	191
		Factors Affecting the Compression	191
		Removal of Section Compression	192
		Section Wrinkling	193
		Section Shrinkage	194
		Chatter	195
		SECTION CONTAMINATION AND DAMAGE	197
		SERIAL SECTIONING	199

Transfer of Serial Sections	200	Carbon Films for Cryoelectron Microscopy	228
Section Thickness for Serial Sectioning	203	Procedure	229
Three-Dimensional Reconstruction	204	GRAPHITE FILMS	229
ELECTRON MICROSCOPE TOMOGRAPHY	205	Preparation of Graphite Oxide	230
SEMITHIN SECTIONING	206	QUARTZ FILMS	230
Ralph Knife	207	PERFORATED FILMS	231
Sectioning	208	Perforated Formvar Films	233
Section Transfer	208	Method I	233
CORRELATIVE MICROSCOPY	209	Method II	233
		Method III	233
		Method IV	224
CHAPTER FIVE		Support Film with Large Holes (Micronet)	234
SUPPORT FILMS	211	Perforated Carbon Film	234
INTRODUCTION	211	Perforated Collodion-Carbon-Graphite	
MATERIALS FOR SUPPORT FILMS	212	Oxide Film	235
PLASTIC FILMS	212	Holey Support Films for Cryoelectron Microscopy	235
Estimation of Plastic Film Thickness	213	Procedure	236
Preparation of Plastic Films	213	WETTABILITY OF SUPPORT FILMS	237
Formvar Film Cast on Glass	214	Construction (Figs. 5.12 and 5.13)	238
Formvar Film Cast on Water	216	Operation	239
Formvar Film Cast on Mica	217	ADSORPTION PROPERTIES OF	
Collodion Film Cast on Glass	217	SUPPORT FILMS	241
Collodion Film Cast on Water	217		
Butvar B-98 Support Film	218		
Preparation of Butvar Film	218	CHAPTER SIX	
Polystyrene Films	218	POSITIVE STAINING	242
Transfer of Plastic Films onto Grids	219	INTRODUCTION	242
Plastic Coating of Single-Hole or Slotted Grids	219	IMAGE CONTRAST	247
Repairing of Plastic Films	220	FACTORS AFFECTING CONTRAST	248
VERMICULITE FILMS	220	DURATION OF STAINING	249
CARBON FILMS	220	SIZE OF STAIN AGGREGATES	250
Surface Topography of Carbon Films	221	STAIN PENETRATION	250
Preparation of Carbon Films	222	STAIN SPECIFICITY	251
Carbon Films Prepared by Evaporation		STAINS	252
of the Carbon Filament	223	Alcian Blue	253
Substrates for Carbon Evaporation	223	Purification of Alcian Blue	253
Carbon Film Deposited Directly on		Chemical Composition	253
Sections	223	Mechanism of Staining	254
Preparation of Adhesive-Coated Grids		Reaction with Nucleic Acids	254
for Carbon Coating	224	Critical Electrolyte Concentration	255
Carbon Film Cast on Glycerin	224	Role of pH	256
Carbon Film Prepared on Glass	224	Rate of Staining	256
Procedure	225	Fixation and Staining Procedures	257
Carbon Film Prepared on Mica	225	Bismuth	257
Procedure 1	225	Mechanism of Staining	259
Procedure 2	225	Fixation and Staining Procedures	260
Carbon Film Deposited on a Plastic Substrate	226	General Staining	260
Procedure	226	Staining of Mucosubstances, Glycoproteins,	
Method	226	and Polysaccharides	260
Carbonized Plastic Film	227	Staining of Polysaccharides without	
Procedure	227	Periodic Acid	260
Carbon Film Supported by Perforated Plastic		Selective Staining of Nucleoproteins	
Substrate	228	(Locke and Huie, 1977)	260
Procedure	228	Staining of Synapses	260

COLLOIDAL GOLD	260	Staining Specificity for Synaptic Vesicles	282
Advantages of Colloidal Gold	261	Staining Solutions	283
Stabilization of Colloidal Gold	262	<i>Zinc Iodide Solution</i>	283
Size of Colloidal Gold Particles	263	<i>Final Solution</i>	283
Determination of Gold particle size	264	Sodium Iodide-Osmium Tetroxide	285
<i>Procedure</i>	264	IRON	285
Preparation of Colloidal Gold	264	Mechanism of Staining	285
Determination of Optimal pH for Preparing		pH	287
Gold Sol	265	Rate of Penetration	288
Influence of Embedding Media on the Colloidal		Mode of Staining	288
Gold Method	265	Staining Solutions	288
Influence of Other Factors on the Colloidal		Colloidal Ammonium Ferric Glycerate	289
Gold Method	266	Positive Ferric Oxide Solution	289
Protein A	266	Negative Ferric Oxide Solution	289
Preparation of Protein A-Gold Complex	267	Negative Colloidal Ferric Hydroxide	290
pH	267	Iron Diamine	291
Determination of Optimal pH for		High Iron Diamine	291
Preparing Protein A-Gold Complex	267	Low Iron Diamine	291
Determination of Optimal Stabilizing		LANTHANUM	292
Amount of Protein A	268	Mechanism of Staining	292
<i>Procedure for Preparing Protein A-Gold</i>		Fixation and Staining Procedures	293
Complex	268	LEAD	294
Labeling	269	Mechanism of Staining	294
Nonspecific Labeling	269	Reaction with Membranes	296
Considerations in the Use of Protein A-Gold		Reaction with Glycogen	296
Complex	269	Reaction with Other Cell Components	297
Possible Limitations of the Protein A-Gold		Lead Acetate	297
Complex Method	270	Lead Aspartate	298
Multiple Immunogold Staining Methods	271	Lead Citrate	299
<i>Procedure 1</i>	272	Lead Hydroxide	300
<i>Procedure 2</i>	273	Lead Tartrate	301
<i>Procedure 3</i>	274	<i>En Bloc</i> Staining with Lead	301
Immunogold-Silver Method	274	Double-Lead-Staining Method	302
Procedures	276	Glycogen Staining	302
<i>Thin Resin Sections</i>	276	Tricomplex Fixation and Staining	302
<i>Thick Resin Sections</i>	276	OSMIUM TETROXIDE	303
Immunoglobulin-Colloidal Gold		OSMIUM TETROXIDE-IMIDAZOLE	
Method	277	COMPLEXES	304
Immunolabeling of Thin Cryosections	277	Osmium Tetroxide-Imidazole	305
Lectin-Colloidal Gold Complex	277	Osmium Tetroxide-3-amino-1,2,4-triazole	305
Lectin-Horseradish Peroxidase-Colloidal		Potassium Osmate-3-amino-1,2,4-triazole	305
Gold Method	278	OSMIUM TETROXIDE-POTASSIUM	
Lectin-Horseradish Peroxidase-Colloidal		FERRICYANIDE OR FERROCYANIDE	305
Gold-Ruthenium Red Method	279	Fixation and Staining Procedures	307
Enzyme-Colloidal Gold Method	279	OXALATE-GLUTARALDEHYDE	308
Preparation of Enzyme-Gold Complex	279	PHOSPHOTUNGSTIC ACID	309
Labeling	280	Mechanism of Staining	309
Light and Electron Microscopic		Fixation and Staining Procedures	313
Immunocytochemistry on the Same		POTASSIUM PERMANGANATE	315
Section	280	POTASSIUM PYROANTIMONATE	316
DIAMINO BENZIDINE-OSMIUM TETROXIDE	281	Mechanism of Staining	316
IODIDE-OSMIUM TETROXIDE MIXTURES	281	Effects of Fixation	317
Zinc Iodide-Osmium Tetroxide	281	Specificity of Reaction	317
Mechanism of Staining	281	Reproducibility of Results	318

Limitation of the Method	319	Uranyl Acetate	342
Fixation and Staining Procedures	320	Uranyl Nitrate	343
RUTHENIUM RED	320	Mechanism of Staining	343
Penetration	321	Reaction with Nucleic Acids	344
Mechanism of Staining	321	Reaction with Proteins	345
Fixation and Staining Procedures	323	Reaction with Lipids and Membranes	345
SILVER	324	Factors Affecting Uranyl Staining	346
Mechanism of Staining	324	pH	346
Role of Fixation	326	Buffer Types	347
Fixation and Staining Procedures	327	Fixation and Embedding Methods	347
Ammoniacal Silver	327	Overall Effect on Tissues	347
Silver Methenamine	327	Staining Solutions	348
Silver Nitrate	330	Uranaffin Reaction	350
Impregnation Techniques	330	Procedure	350
Silver Proteinate	331	STAINING PROCEDURES	350
Periodic Acid-Silver Method	332	Double Staining with Uranyl Acetate and	
Periodic Acid-Chromic Acid-Silver Method	332	Lead Citrate	350
Staining of Nucleolar Organizer Region	332	Effect of Washing on Staining	351
Silver Staining <i>In Situ</i>	333	Multiple-Grid Staining	352
Silver Staining on Sections	333	MULTIPLE STAINING	353
SILVER LACTATE-OSMIUM TETROXIDE	334	SECTION CONTAMINATION AND	
SODIUM TUNGSTATE	334	ITS REMOVAL	353
TANNIC ACID	334	Precautions to Minimize Section Contamination	354
Reaction with Proteins	335	Removal of Section Contamination	356
Reaction with Carbohydrates	336	SELECTIVE HEAVY METAL STAINING	
Reaction with Lipids	336	FOR HIGH-RESOLUTION	
Fixation and Mordanting Effects		ELECTRON MICROSCOPY	357
of Tannic Acid	336	STAINING FOR HIGH-VOLTAGE	
Penetration	337	ELECTRON MICROSCOPY	357
Negative Staining with Tannic Acid	337	Staining Procedures	358
Fixation and Staining Procedures	337	STAINING OF THIN CRYOSECTIONS	359
General Procedures	337	STAINING OF SEMITHIN SECTIONS	360
Visualization of Mucosubstances	337	Solutions	363
Visualization of Collagen and Elastin	337	Procedure	363
Visualization of Cholinergic Synaptic Junctions	338	Selected Staining Methods for Semithin	
Visualization of Exocytosis	339	Sections	363
Tannic Acid-Glutaraldehyde-OsO ₄		Azure B for Plant Tissues	364
Method	339	Procedure	364
Tannic Acid in Ringer's Solution		Results	364
Method	339	Basic Fuchsin and Methylene Blue	364
TARI Method by Vascular Perfusion	339	Staining Solution	364
Modified TARI Method	340	Procedure	364
Tannic Acid Medium	340	Results	364
Procedure	340	Methylene Blue-Azure II-Basic Fuchsin	364
THIOSEMICARBAZIDE AND		Results	364
THIOCARBOHYDRAZIDE	340	Hematoxylin-Malachite Green-Basic Fuchsin	364
Periodic Acid-Thiosemicarbazide or		Staining Solutions	364
Thiocarbohydrazide-Silver Proteinate	340	Procedure	365
Periodic Acid-Thiosemicarbazide or		Results	365
Thiocarbohydrazide-Osmium Tetroxide	341	Hematoxylin and Phloxine B	365
Modified Method	341	Procedure	365
Sodium Periodate-Thiosemicarbazide-Osmium		Results	365
Tetroxide	341	Methyl Green and Methyl Violet	366
URANYL PREPARATIONS	342	Staining Solution	366

<i>Procedure</i>	366	<i>Sputum</i>	392
<i>Results</i>	366	<i>Tears</i>	393
Toluidine Blue and Acid Fuchsin	366	<i>Tissue Scrapings</i>	393
<i>Procedure</i>	366	<i>Urine</i>	393
<i>Results</i>	366	GENERAL METHODS FOR PLANT VIRUSES	393
CHAPTER SEVEN		<i>Rapid Procedures</i>	393
NEGATIVE STAINING	367	<i>Viruses in Crude Extract</i>	393
INTRODUCTION	367	IMMUNOELECTRON MICROSCOPY	394
MECHANISM OF NEGATIVE STAINING	368	<i>Classical Immunoelectron Microscopy</i>	394
HIGH-RESOLUTION ELECTRON		<i>Immunosorbent Electron Microscopy</i>	395
MICROSCOPY	370	<i>Antigen-Coated-Grid Method</i>	395
SPECIMEN PREFIXATION	370	<i>Procedure</i>	395
<i>Negative Staining after Fixation Technique</i>	371	<i>Protein A-Coated-Grid Method</i>	395
NEGATIVE STAINS	372	<i>Serum-In-Agar-Diffusion Method</i>	396
<i>Uranyl Acetate</i>	372	<i>Procedure</i>	396
<i>Uranyl Citrate</i>	374	<i>Protein A-Coated-Bacteria Technique</i>	396
<i>Uranyl Formate</i>	374	<i>Procedure</i>	397
<i>Potassium (or Sodium) Phosphotungstate</i>	374	<i>Virus-Immune Complex Electron Microscopy</i>	397
GENERAL METHODS	377	<i>Procedure</i>	397
<i>Basic Considerations</i>	377	<i>Immunogold Staining Method</i>	398
<i>One-Step (Simultaneous) Method</i>	377	<i>On-Grid Method</i>	398
<i>Negative Stain-Carbon Method</i>	378	<i>Procedure</i>	399
<i>Two-Step (Sequential) Method</i>	379	<i>Suspension Method</i>	399
<i>Single- or Double-Carbon-Layer Method</i>	381	<i>Grid-Cell-Culture Technique</i>	399
<i>One-Sided Negative Staining Method</i>	382	<i>Procedure</i>	399
<i>Paper-Filtration Method</i>	382	CHAPTER EIGHT	
<i>Pseudoreplica Method</i>	383	LOW TEMPERATURE METHODS	400
<i>Agar-Filtration Method</i>	383	INTRODUCTION	400
<i>Freeze-Dry Negative Staining</i>	386	CRYOFIXATION	400
<i>Covering Method for Thin Cryosections</i>	386	<i>Advantages of Cryofixation</i>	402
<i>Cryonegative Staining</i>	386	CRYOPROTECTANTS	402
<i>Cryoelectron Microscopy</i>	388	<i>Penetrating Cryoprotectants</i>	403
<i>Procedure</i>	388	<i>Nonpenetrating Cryoprotectants</i>	404
GENERAL METHODS FOR HUMAN		VITRIFICATION	404
VIRUSES	389	LIQUID CRYOGENS	405
<i>Allantoic Fluid</i>	390	RATE OF COOLING	406
<i>Biopsy or Autopsy Tissues</i>	390	METHODS OF FREEZING	406
<i>Blister Fluids</i>	390	<i>Conventional Freezing Procedure</i>	406
<i>Brain Tissue</i>	390	<i>Plunge-Freezing Method for Conventional and</i>	
<i>Breast Milk</i>	390	<i>Ultrarapid Freezing</i>	407
<i>Cell Cultures</i>	390	<i>Procedures</i>	407
<i>Cerebrospinal Fluid</i>	391	<i>Cold Metal Block Freezing Method</i>	408
<i>Eye Biopsy</i>	391	<i>Procedure</i>	409
<i>Feces</i>	391	<i>Cryogen-Jet-Freezing Method</i>	410
<i>Hard Tissues</i>	391	<i>Procedure</i>	411
<i>Liver or Kidney Biopsy</i>	391	<i>Spray-Freezing Method</i>	411
<i>Nodules</i>	391	<i>Procedure</i>	412
<i>Respiratory Secretions</i>	392	<i>High-Pressure-Freezing Method</i>	412
<i>Serum</i>	392	<i>Specimen Holders</i>	414
<i>Skin Lesions</i>	392	<i>Limitations of High-Pressure Freezing</i>	414
<i>Skin Tumors</i>	392	<i>Popsicle-Freezing Method</i>	415
<i>Skin Warts</i>	392	<i>Punch-Freezing Methods</i>	415

Specimen Preparation by Sandwich Freezing	415	Callose	451
Sandwich Freezing of Monolayer Cell Cultures	416	Immunogold Labeling of Callose	451
Freezing Tissues <i>In Situ</i>	416	Cytochemical Localization of Callose in the Seed Coat	452
CRYOPRESERVATION IN THE PRESENCE OF MICROWAVE IRRADIATION	417	Cellulose	452
STORAGE OF FROZEN SPECIMENS	418	Enzyme-Linked Colloidal Gold Localization of Cellulose	452
FREEZE DRYING	419	Preparation of Enzyme-Gold Complex	452
Procedure	419	Cutin	453
FREEZE SUBSTITUTION	421	Procedures	454
Method for Epoxy Resins	423	Hemicelluloses	454
Method for Lowicryls	423	Procedure	455
FREEZE FRACTURING	424	Immunogold Labeling of Xylans	455
Procedure	425	Immunogold-Silver Staining of Glucuronoxylan for Light Microscopy	456
Cleaning of Replicas	426	Lignin	456
FREEZE ETCHING	427	Procedures	457
Procedure	427	Immunocytochemical Studies of Three Lignins	458
EMBEDDING AT LOW TEMPERATURES	428	Immunogold Labeling of Lignin in Woody Tissues	458
Freeze Drying and Embedding	428	Pectins	458
Freeze Substitution and Embedding	428	Immunogold Labeling of Pectin in Tissues	460
LIMITATIONS OF LOW-TEMPERATURE METHODS	429	Procedure 1: JIM7 or JIM5 Antibody	460
HAZARDS	431	Procedure 2: RG-1 Antibody	460
CRYOULTRAMICROTOMY	431	Immunogold Labeling of Rhamnogalacturonan II	461
Quality of Thin Cryosections	432	Enzyme-Colloidal Gold Labeling of Homogalacturonic Acid	462
Section Transfer	433	Immunogold Labeling of Pectin in Cultured Cells	463
Preparation of Thin Cryosections	433	Proteins	463
Procedure	434	Expansins	464
Section-Cutting Artifacts	435	Antibodies	465
Limitations of Cryoultramicrotomy	436	Immunogold Labeling of Hydroxyproline-Rich Glycoprotein	465
CRYOTRANSMISSION ELECTRON MICROSCOPY	436	Immunogold Labeling of Extensin	465
		Immunogold Labeling of β -(1 \rightarrow 4) and β -(1 \rightarrow 6)-D-Galactan	465
CHAPTER NINE		Suberin	466
PLANT TISSUES	439	Conventional Method	467
INTRODUCTION	439	Iodine Potassium Iodide Method	467
CELL WALL	440	Hydrogen Peroxide Method	467
Mechanism of Cell Wall Expansion	440	Potassium Permanganate Method	467
VACUOLAR SYSTEM	443	SPECIFIC METHODS	467
PROBLEMS IN PROCESSING PLANT TISSUES	444	Dry Plant Specimens	467
Bubble Problem	446	Epidermal Hair of Seeds	468
Permeabilization of Cell Walls	446	Leaves	468
Vacuum Infiltration	448	Plant Tissues (Hard)	468
GENERAL METHOD OF FIXATION AND EMBEDDING	448	Plant Tissues (Soft)	468
GENERAL METHOD OF FIXATION AND EMBEDDING OF WOODY TISSUES	449	Plasmodesmata	468
MICROWAVE-ASSISTED FIXATION	449	Pollen Grains (Fossil Material)	469
METHODS FOR STUDYING CELL WALL COMPONENTS	449	Pollen Walls	469
Antibodies	450	Potato Tuber	470
Antibodies to Localize Cell Wall Components	450	Roots	470

Seeds	470	APPLICATION OF MICROWAVE HEATING	
Seeds (Dry)	470	TO EPITOPE RETRIEVAL	483
Seeds (Water-Impermeable Coat)	470	Duration of Microwave Heating	483
Wood	471	EPITOPE RETRIEVAL METHODS	484
		Nonheating Methods	484
CHAPTER TEN		Detergents	484
APPLICATIONS OF MICROWAVE HEATING		Procedures	484
TO MICROSCOPY	472	Proteolytic Enzyme Digestion	485
INTRODUCTION	472	Procedures	486
MECHANISM OF MICROWAVE HEATING	472	Ultrasound Treatment	486
FIXATION	473	Procedure	486
Effect of Microwave Heating on Formaldehyde		Heating Methods	487
Fixation	475	Wet Autoclave Treatment	487
Effect of Heating on Fixation with		Procedure	487
Glutaraldehyde	475	General Procedure for Epitope Retrieval	
Osmium Tetroxide-Microwave Heating	477	by Microwave Heating	487
ROLE OF MICROWAVE HEATING IN		Epitope Retrieval with Enzyme Digestion and	
ENZYME CYTOCHEMISTRY	477	Microwave Heating	490
DEHYDRATION AND EMBEDDING		Epitope Retrieval with Microwave Heating	
FOR LIGHT MICROSCOPY	477	and Ultrasound	490
MICROTOMY OF PARAFFIN SECTIONS	479	EPITOPE RETRIEVAL ON RESIN SECTIONS	
RESIN EMBEDDING IN MICROWAVE		BY MICROWAVE HEATING	491
OVENS	479	Procedure for Electron Microscopy	492
USE OF HEAT FOR STAINING	479	Rapid, Cold Fixation with Microwave Heating	
IMMUNOHISTOCHEMISTRY AND		for Electron Microscopy	493
IMMUNOCYTOCHEMISTRY	480	Fixative (pH 7.4)	493
PROBLEM OF EPITOPE RETRIEVAL		Microwave Oven	493
STANDARDIZATION	481	Procedure	493
ROLE OF MICROWAVE HEATING			
IN EPITOPE RETRIEVAL	482	References	495
CARE AND USE OF A MICROWAVE OVEN	482	Index	533