A	Bright-field microscopy, 222
Absolute quantification, 143	Buffers,
Acetone	phosphate, 4
dehydration, 114	cacodylate, 5
PLT method, 114	·
Acid phosphatase, 161–162	C
activity, 163	Cacodylate buffers, 5
Acrylic resins, 111–124	Carbon plastic
Agarose, 6, 115	butvar, 17
Alcian blue, 232	colloidon, 17
Ammonium acetate, 213, 215, 225, 232	formvar, 17
Ammonium molybdate, 15, 21, 23, 28, 29,	Carbon support films preparation, 5, 17,
33, 47	211–212
Amorphous ice, 266	Cartiladge, 84
Antibiotin, 175	Cerium-based method, 161
Antibody, 127, 142	medium, 162
gold immunolabeling, 49	Chemical fixation of sample for
penetration, 141–142	cryosectioning, 54–55
protein A-gold, 51, 190	Clathrin-coated vesicles, 191, 195
Antigenicity, 77, 99, 100, 104, 105, 107	Cold spot, 149, 151, 156
Antigens, 125, 127	Cold stage, 270
Antisera, requirements for immunolabeling,	Collagen fibres, 23
105	Combination of enzyme cytochemisry
Apoptosis, 199, 202	with immunocytochemistry, 162–163,
Arabidopsis, 90	163–164
Thuo to opolo, yo	Compartment, 129, 130, 135,
В	Contrasting and drying cryosections, 60–62
_	Counterstaining of thin sections of
BAC, for preparation of nucleic acids, 214–	epoxy resins, 9
215	Lowicryl, 116–117
Background labeling, 132	Unicryl, 116–117
Bacteria, 6, 81	Counting gold particles, 131
Bacteriophage, 234, 236	Cryoelectron microscopy, 31–48
Barley aleurone, 91	and immunolabeling, 39
Beam damage, 259	examples, 40–46
BEEM, 3, 149	Cryofixation
Biotinylated nucleotides, 167, 173	for EPXMA, 251
Blocking, 102, 104	slam-freezing, 252, 265
Bridging antibody, 67	plunge freezing, 252

Cryofixation (cont.)	Cryospecimens, 34–39
slamming against a polished metal miror,	CryoTEM-images, 40-46
252–253, 266	Cryotransfer system, 33
jet freezing, 252	Cryo-unstained vitrified, advantages, 31
high pressure freezing, 252	Cryo-workstation, 38
intensities into element concentrations,	Cytochemistry, 159–160
260	Cytochrome C, 231
of frozen-hydrated specimen, 31-48	preparation, 211
of fully hydrated specimens, 31–48	for nucleic acid spreading, 215–217
Cryogen, 265	spreading method for EM, 230–232
CryoimmunoEM (CryoImmunolabeling),	advantages, 229, 232
126	examples, 236–237, 240
Cryomicrotomy, 49–74	1 11, 11,
antibody incubation, 51, 58	D
blocking, 51, 58	Dark-field illumination, 209–210
chemical fixation, 54–55	Dark-field imaging, 212–213
double labeling, 67	examples, 216, 217, 220, 221
examples, 69, 70, 72	Dehydration
and immunolabeling, 51	for epoxy resins, 7
immunogoldlabeling, 58, 60	for Lowicryl, 117, 189
introduction, 49–50	for Unicryl, 118
knife preparation, 57	Deionized formamide, 171, 177
major equipment, 50	Denaturation, 232
specimen preparation	nucleic acids, 225
cryoprotection, 55	Dextran sulfate, 177
fixation, 54–55	Diffusion method,
freezing, 55	for nucleic acid, 217
gelatine embedding, 55	Dimethylformamide, 164
sectioning, 57–58	Direct adsorption method for protein/DNA
section	complexes, 232–233
removal, 58	DMSO, 162, 164
retrieval, 58	DNA
Cryonegative staining, 39, 42, 43, 45	double stranded, 213, 299
Cryopliers, 252, 254, 267	plasmid, 218, 225
Cryoprotection, 55	preparation for EM, 209–227
Cryosectioned standards, 260–261	probes, 176
Cryosectioning. See also Effect of	recombination, 243
temperature on cryosectioning	repair patches, 219-222
double labeling, 51	replication, 219
manipulating samples, 58	single stranded, 213, 229, 230, 238
pick-up cryosection, 59	visualisation, 219–223
single labeling, 51, 58	DNA/protein complex, 218
specimen trimming, 57	Double immunolabeling, choice of
staining, 61	antibodies, 105
Cryosnapper, 267	Double labeling, 67
Cryospecimen holder, 33	Drosophila, 80, 85

E	Formamide, 170, 177, 213, 231
Effect of temperature on cryosectioning	Formvar, 3
quality, 64	coating specimen grids preparation, 53-
Embedding	54
in epoxy resin, 7	Freeze substitution
in gelatin, 55	description, 78
in Lowicryl, 188	making fixative for, 88–89
in LR-gold, 103	materials for, 88
problems, 10	procedure, 88
in Unicryl, 117	Freeze-drying of ultrathin crysections, 270
Embryos, 80, 85	
Endoplasmic reticulum, 191, 196	G
Enzyme cytochemistry, 159–165	Gap DNA, 241, 242
acid phosphatase, 161–162	GFP fusion protein, 200–201
activity, 163	Glow discharge of carbon-films, 214
	Glutaraldehyde 5, 6, 170, 177, 218
examples, 160, 161	monomeric vacuum-distilled, 100
and immunolabeling, 162–163	Gold labelling of specific proteins in DNA-
Epon, 3, 6, 7	protein, 233–234
Epon-Spurr mixture, 155	advantages, 230
Epoxy resins, 3, 6–7	disadvantages, 230
EPXMA (electron probe x-ray	examples, 241, 242
microanalysis), 245	Gold particles, 127
Ethanol	Gold probe
dehydration, 117	choice, 105
PLT method, 117	particle size consideration, 105, 107
Ethanol-uranyl acetate, 116	Gold-label-proteins, 233
phosphotungstic acid, 117	Golgi apparatus, 126, 127, 128, 191, 192–
Ethidium bromide, 214	193, 200
T.	Gomori method, 160
F	Gylcerol, 233
Fetal calf serum, 51	•
Filamentation, 242, 243	Н
Fixation	Hall continuum normalization method, 246
of bacteria, 6	Hemocyanin, 22, 39, 41
of cell suspensions, 6	High-pressure freezing. 77–97
chemical, for cryosectioning, 54–55	Arabidopsis, 90
of dissociated cells, 6	bacteria, 81
with formaldehyde, 55, 101	barley aleurone, 91
with glutaraldehyde, 55, 100	cartridge, 84
immersion, 5	comparison with conventional fixation,
of monolayer culture, 6	77
perfusion, 5	description, 77–78
of particulates, 6	Drosophila, 80, 85
quality, 10	embryos, 80, 85
of viruses, 6	equipment needed for, 78–79
Formaldehyde, 55, 101	examples of, 88–93

High-pressure freezing (cont.)	Labeling (cont.)
plant tissue, 84	density, 132
procedure, 80–85	efficiency, 127
specimen carriers, 81–82, 83, 93	nonspecific, 107
starfish oocyte Golgi apparatus, 92	particle size, 105, 107
yeast, 81, 89, 93, 193, 194, 197, 198, 199	quantitative evaluation, 125-143
HIV-1, 170	LAMMA, 246
HIVDNA, 170	Lead citrate, 9, 116
Hybridization agents, 171	make up, 102
double labeling, 175	Lead-based method, 159
method	medium, 162
direct, 174, 175	Linear density, 138
indirect, 175	Low electron dose, 14, 38
probe, 174	Low-temperature embedding, 111–124
•	examples, 120–121
I	Lowicryl, 188
Ice-damaged, 247, 264, 266	K4M, 113–114, 115–116, 168, 169, 170,
IgG-gold, 105	172, 175
Image recording, 38	HM20. 113–114, 115–115, 188–190
Immunocytochemistry using cryosections,	LR gold resin,
49–76	embedding, 103, 108
common problems	examples, 106
during immunolabeling, 62	embedding, 103, 108
with morphology, 71–73	for immunogold labeling, 99–110
Immunogold labeling	polymerisation, 103, 108
controls, 204	
cryosections, 51	section post-staining, 104
of DNA-repair patches, 218–222	LR white resin, 155, 156, 162, 164
of resin-embedded, 99–110, 118–119	Lysosomes, 160
Immunolabeling,	M
blocking incubation, 102	Magnesium acetate, 218
of cryothin section, 58, 59	Mass in an electron beam, 270
fixation consideration, 103	Methacrylate resin, 168
negative results, 107–108	Methanol
nonspecific labeling, 107	
Immunonegative staining, 24–27	dehydration, 189
examples, 27, 29	PLT method, 189
In situ molecular hybridization, 167–181	Methyl cellulose, 51, 59
examples, 168–170	and uranyl acetate, 51, 59
Infiltration, 7	Mica, 231, 232
problems, 10	Microwave processing techniques, 145–158
Intercellular compartments, 135	aldehyde fixation, 148, 151
Intersection method, 133	cavity, 149
	dehydration, 149
L	embedding, 149, 153
Labeling	Epon-Spurr mixture, 155
antibody choice, 105	LR white resin, 155, 156
background, 132	UNICRYL, 155, 156

Microwave processing techniques (cont.)	Organelles, labelling density, 134
examples, 146 four-hour protocol, 147–154	Osmium tetroxide (OSO ₄), 3, 6, 87, 90, 91, 92
and immunocytochemistry, 147	92
irradiation, 149	P
osmium fixation, 148, 152, 156	Paraformaldehyde, 5, 6, 101, 115, 188
polymerization, 149, 154, 156	Particulates, 13–30
processor, 147–148	Peroxisomes, 197
temperature, 151	Phosphatases, 159, 160
Mitochondria, 169, 175	Phosphate buffers, 4
Mitotic spindle, 199	Pick-up cryosection, 59
Mu phage, 235, 236	Pioloform, 3, 5
	PIXE, 246
	Platinum, 232, 233
N	PLT technique, 111, 172
Negative staining, 13–30	Plunge-freezing, 33, 34, 35
carbon film procedure, 20–21	Point Hi method, 132, 133
combinations with	Polyclonal antisera, 105
carbohydrate, 15	Polymerisation, 8
PEG, 15	for Epon resins, 8
droplet procedure, 20–21	for Lowicryl, 117
examples, 22–24, 28	problems. 10
methods,	for UNICRYL, 118, 119 Polymerization chamber, 177
carbon-films, 21–22	Post-embedding methods, 77–124
immunonegative staining, 24–27	Preparation and staining of sections,
single droplet, 20–21	1–11
mica spreading procedures, 21–24	Preparation
sample material, 19	of cryospecimens of
solutions, 15	stained thin-film, 39–40
Nick-translation buffer, 171	unstained thin-film, 34–39
Nucleic acids	of DNA and RNA molecules for EM,
spreading methods	209–227
diffusion method, 217	of the fission yeast, 183-207
using BAC, 214–215 using cytochrome C, 215, 217	of the Lowicryl resins, 115–116
watch glass, 218	Presentation of results
denaturation, 232, 235	by cumulative, 139
hybridization, 167–181	by standard deviation, 139
replicating DNA, 219–221	Pretreatment of carbon-coated grids
protein binding complexes, 229–243	by ethidium bromide, 214
Nucleolus, 168	by glow discharge, 214
Tracteoras, 100	Propane, 265
	Protein A-gold, 51, 52, 60, 67, 105
0	Proteins. See specific proteins.
Operating the analytical transmission	Protein kinase C, 191, 194–195
electron microscope, 257–260	Protein-DNA binding, visualisation of,
Steetfolf Inferescope, 237 200	223–224

Protein-DNA complexes, 229–243	RNA (cont.)
examples, 234–243,	double-stranded, 235, 237
methods for studying,	spreading methods, 213
cytochrome-C-spreading method,	Reo virus, 235, 237
230–232	RNAse H, 169, 174, 178
procedure, 231	Rotary shadowing
direct absorption method, 232–233	of nucleic acid, 210, 218
gold labelling of specific proteins in	of protein-nucleic acid complexes, 232,
DNA-protein, 233–234	233, 234, 236, 237, 240, 241, 242
Proximity Process, 233 231	233, 231, 230, 237, 210, 211, 212
of DNA and protein within	S
bacteriophages, 234	-
of RNA and proteins within Reo virus,	Sectioning problems, 10
235	Shadowing. See Rotary shadowing.
233	SIMS, 246
Q	Single-stranded DNA, 213
Ouantification	Slam-freezing, 252, 265
absolute, 143	Sodium metaperiodate, 11
background, 132	Sodium/potassium phosphotungstate, 15
calculation, 136	Sodium/potassium silicotungstate, 15
examples, 133–136	Sodoim ethoxide, 10
limitation and alternatives, 140	Specific gold labeling, 134–135
methods	Specimens. See also Preparation.
	for cryoelectron microscopy, 31-48
intersection method, 133 point hit, 132, 133, 134	transfer to precooled cryochamber, 55-56
-	Spreading nucleic acid
and multiple labeling, 141	by BAC, 214–215
problems, 141	by cytochrome C, 215–216
steric hindrance, 141	by diffusion method, 217
sampling, 128	solution for DNA and RNA, 213
specimen, 131	watch glass technique, 218
Quantification aspect of immunogold	Spurr's resin, 6, 162
labeling, 125–144	SSB protein, 241
Quantitative EPXMA, 246	Staining
R	with phosphotungstic acid, 15
RecA protein, 238–242	with uranyl acetate, 15, 21, 22, 25, 28
*	problems, 10
DNA filaments, 238–239, 242	Standard deviation, 139
RecF protein, 241, 243	Standard error of the mean, 140
RecO protein, 238–242	Streptavidin, 169, 171, 174, 175
RecR protein, 238–243	Sulfonated probe, 176
Relative distribution, 136–137	Support films, 3, 17, 53–54
Reo virus, 235, 237	Support IIIIIs, 3, 17, 33–34
RNA	TD.
bateriophage, 132	T
denaturation	Temperature
using formamide, 213	advantages of Lowicryl resins, 111
using urea-formamide, 213	cryosubstitution, 88

Thin-film of frozen specimen, 31–48 of vitrified specimens, 31–48 Thin-section hybridization procedures, 174 3-D electron microscopy, 159 Time-resolved studies, 253–257 Toluidene blue, 4 Trehalose, 15, 28 Trehalie, 100	Virus (cont.) Tobacco mosaic, 39, 40 Tomato bushy, 45 wasp, 237–240 Wasp virus, 237–240 Watch glass technique, 218 X-Y Xenopus, 219, 220, 221
Tubulin, 199 2–D crystal formation, 15, 19, 22, 23	X-ray
U Ultramicrotomy, 8 problems, 11 Ultrathin cryosection	continuum, 246 count rate, 259 cryomicrotomy, 269 distribution map, 271 elemental mapping, 272
freezedrying, 270 goldlabeling, 49–76 preparation, 54–58	Yeast apoptosis, 199, 202 cell wall, 191–192, 194, 202
UNICRYL resin, 114, 155, 156 embedding, 117	endoplasmic reticulum, 191, 196 examples, 190–203
Uranyl acetate, 9, 116, 232	fixation, 188
UV light, 115, 189	Golgi, 191, 192–193, 200
V-W	growing cells, 188 immunogold labelling procedure, 190 media, 186–187
Vesicles, 191, 194–195 Viral	mitotic spindle, 199 morphology, 189
DNA, 238, 239, 240 RNA, 170, 237	peroxisomes, 197
Virus, 6 cryonegative staining, 39–45 Reo, 237 Rota, 43, 44	preparation by cryosectioning, 189 by high-pressure freezing, 89, 189 by PLT method, 188–189 spore formation, 197–198