
Index

- Acousto-optic tunable filter (AOTF),
 quality assurance, 122, 123,
 131, 132
- AFM, *see* Atomic force microscopy
- Aldehyde fuchsin, elastic fiber staining,
 142–144
- Ammonium molybdate stain, *see*
 Electron microscopy
- Angular reconstitution, three-
 dimensional image
 reconstruction,
- AOTF, *see* Acousto-optic tunable filter
- Apolipoprotein E knockout mice, *see*
 Atherosclerotic plaque
- AQP1, *see* Aquaporin-1
- Aquaporin-1 (AQP1), zymogen granule
 association,
 evidence, 322–325
 mercury chloride sensitivity, 325,
 326
 swelling regulation, 326–328
 tritiated water entry assay, 320
- Asbestos,
 confocal laser scanning microscopy
 of lung tissue activators
 after exposure,
 double and triple labeling, 73
 markers, 68
 materials, 68, 69
 nuclei detection with fluorescent
 dyes, 73–75
 single immunofluorescence
 labeling,
 cell type markers, 71–73
 mitogen-activated protein
 kinase and protein kinase C
 antibodies, 69–71, 74, 75
 gene expression change studies in
 laser capture microdissected
 bronchiolar epithelial cells,
 DNase treatment, 235
 materials, 232, 233
 microdissection, 234, 235
 overview, 232
 reverse transcriptase-polymerase
 chain reaction, 235
 slide preparation,
 dehydration, 234
 sectioning, 233
 specimen preparation, 233
- Asthma, eosinophil analysis with laser
 scanning cytometry,
 cell quantification, 207, 209
 image acquisition, 205–207, 211
 Octospot® cytospin system, 205,
 211
 overview, 203, 204
 sputum sample preparation, 204
- Atherosclerotic plaque,
 composition analysis in
 apolipoprotein E knockout
 mice,
 confocal laser scanning
 microscopy, 145, 150, 151
 fixation and processing of tissue,
 141
 image processing and analysis,
 146, 147
 materials, 138–141, 149, 150
 overview, 138
 polarized light microscopy, 146
 sectioning, 141, 142
 staining,
 collagen with Picrosirius red,
 145, 150
 DNA, 142
 elastic fibers with aldehyde
 fuchsin, 142–144
 immunostaining, 145, 150
 lipids with Oil red O, 142, 150

- wide-field microscopy,
 - bright-field microscopy, 146, 151
 - fluorescence microscopy, 146
- types and stability, 137, 138
- Atomic force microscopy (AFM),
 - cell mechanical properties,
 - cell indentation,
 - cell culture, 342, 352, 353
 - force mapping, 343, 344, 355, 356
 - single-force curves, 342, 343, 354
 - cleaning up, 344
 - data analysis,
 - contact point identification, 345–347
 - Hertz model, 344, 356
 - pointwise elastic modulus calculation, 347, 348, 356
 - data presentation,
 - arrays of modulus curves, 350
 - elastogram, 350
 - pointwise modulus curves, 349, 350
 - functional overview, 331, 332
 - materials, 335–337, 352, 353
 - probe,
 - preparation and mounting, 337–339, 353
 - spring constant determination, 340, 341, 353, 354
 - tip geometry characterization, 341, 342, 354
- historical perspective, 296
- instrumentation, 335–337, 350–353
- porosomes,
 - immunogold localization of porosomes, 300, 305
 - immunoisolated porosomes, 311
 - pancreatic acinar cell isolation, 298
 - pits and depressions, 302, 304
 - plasma membranes,
 - findings, 307
 - preparation from pancreas, 299
 - porosome reconstitution, 299, 300
 - synaptosome and vesicle preparation, 299
 - principles, 297, 298, 332–334
 - zymogen granules, 319, 320
- AutoPix, *see* Laser capture microdissection
- Axial resolution quality assurance, *see* Confocal laser scanning microscopy
- Bead test, *see* Confocal laser scanning microscopy
- Biopsy,
 - cryofixation, *see* Microbiopsy system
 - laser scanning cytometry, 179, 180
 - reflection contrast microscopy, *see* Reflection contrast microscopy
- Calcium flux,
 - cameleons,
 - filter sets, 58, 61
 - fluorescence resonance energy transfer, 40
 - ratiometric imaging in organelles, 57–59, 61
 - subcellular targeting, 40, 57, 63
 - transfection, 47, 62, 57
 - types and calcium dissociation constants, 41–43
 - confocal laser scanning microscopy,
 - contrast and resolution, 51
 - indicators, 51
 - line-scanning mode imaging, 54, 55
 - modes for imaging, 53, 54
 - ratiometric imaging, 51, 52, 56
 - single-wavelength excitation measurements, 55, 56
 - fluorescent dye probes,

- handling, 46, 61, 62
- loading of cells, 38, 46, 62
- microinjection, 38
- organelle loading, 56, 57
- types and biophysical properties, 38, 39
- materials for quantitative imaging in cells, 44, 45
- microperfusion of cells, 47, 62, 63
- probe selection considerations, 44
- signal transduction, 37, 38
- tissue culture for imaging, 45, 46, 61
- wide-field fluorescence imaging, Fura-2 imaging, 49–51, 63
- instrumentation, 47–49
- Cameleon, *see* Calcium flux
- CF, *see* Cystic fibrosis
- CGH, *see* Comparative genomic hybridization
- CLSM, *see* Confocal laser scanning microscopy
- Coefficient of variation bead test, *see* Confocal laser scanning microscopy
- Collagen,
 - Picrosirius red staining, 145, 150
 - second-harmonic imaging, applications, 32, 33
 - detection strategies, 27, 31, 32
 - rationale, 18
 - sample preparation, 26, 27
 - signal propagation and properties, 25, 26
 - structure, 17
 - types and functions, 17, 18
- Comparative genomic hybridization (CGH),
 - microarray-based comparative genomic hybridization, 245, 246
 - principles, 240, 241
- Confocal laser scanning microscopy (CLSM),
 - advantages over wide-field imaging, 67
 - atherosclerotic plaque composition
 - analysis in apolipoprotein E knockout mice, 145, 150, 151
 - calcium flux imaging,
 - contrast and resolution, 51
 - indicators, 51
 - line-scanning mode imaging, 54, 55
 - modes for imaging, 53, 54
 - ratiometric imaging, 51, 52, 56
 - single-wavelength excitation measurements, 55, 56
 - image size, 124
 - instrumentation, 78
 - interference contrast, 125, 126
 - laser selection, 127, 128
 - lung tissue activator studies after
 - asbestos exposure,
 - double and triple labeling, 73
 - markers, 68
 - materials, 68, 69
 - nuclei detection with fluorescent dyes, 73–75
 - single immunofluorescence labeling,
 - cell type markers, 71–73
 - mitogen-activated protein kinase and protein kinase C antibodies, 69–71, 74, 75
 - optimization of images, 126, 127
 - photomultiplier tube requirements, 124
 - purchase considerations, 132, 133
 - quality assurance,
 - acousto-optic tunable filters, 122, 123, 131, 132
 - axial resolution testing,
 - bead testing, 90, 117
 - mirror testing, 87, 88, 116, 117
 - coefficient of variation bead test, cell testing, 105
 - intersystem comparisons, 124
 - photomultiplier tube testing, 101, 102, 117, 118, 124, 129, 130

- principles, 98–101, 128, 129
- dichroic functionality, 87, 115, 116
- fiberoptic deterioration in merge modules, 121, 122
- field illumination test, 81–83, 107–110
- Focal Check bead test, 91–93
- histological power meter samples, 86, 87, 113
- importance, 78, 133
- laser adjustments, 114
- laser stability tests,
 - factors affecting stability , 18
 - heat dissipation testing, 122
 - short-term tests, 98
 - ultraviolet light, 97, 98, 121
 - visible light, 95–97, 119–121
- materials,
 - beads as test particles, 79, 80, 91–93
 - biological test slides, 80, 123, 124
 - fluorescent slides for field illumination tests, 78, 79
 - lens for laser beam shape testing, 80
 - microscope systems in study, 81
 - photomultiplier tube spectral check, 81
 - power meter reading, 79, 83
 - reflecting mirror for axial resolution test, 80
 - software, 81
 - square sampling, 80
- objectives and lens cleaning, 110, 111
- photomultiplier tube testing,
 - coefficient of variation bead test, 101, 102, 124, 129, 130
 - spectral scanning, 102, 103, 125
- power meter test, 83–86, 111–113
- sensitivity testing, 105, 106, 130, 131
- spectral registration test, 93–95
- spectral registration with 1 mm beads, 91
- square pixels and phase alignment, 90, 91, 117
- ultraviolet beam shape, 86
- ultraviolet power test, 86, 114, 115
- reflection contrast mode, 374, 391, 392, 398
- Cryoelectron microscopy, *see* Electron microscopy
- Cryofixation, *see* Microbiopsy system
- Cystic fibrosis (CF),
 - gene function and pathology, 155, 156
 - gene therapy,
 - microscopy studies,
 - overview, 153
 - resources, 162
 - vendors, 154
 - vectors,
 - adeno-associated virus, 161
 - adenovirus, 157, 160
 - cell culture studies, 156, 157
 - lentiviruses, 160, 161
 - lipofection, 157
 - pseudoviruses, 161
- prevalence, 155
- DNA microarray,
 - comparative genomic hybridization, 245, 246
- laser scanning,
 - fluorescent labels, 262, 263
 - instrumentation, 263, 264, 267
 - light source, 263
 - materials, 262
 - parameter setting, 270, 271, 273
 - photon detection, 263, 265
 - scanner comparison, 267, 270
 - signal-to-noise ratio, 267
 - simultaneous versus sequential scanning, 265, 267
- principles, 261, 262

- Doxorubicin, laser scanning cytometry
of microcapsule uptake by
breast cancer cells,
cell selection and subculture, 198,
199
doxorubicin linking to
microcapsules, 198
human serum albumin microcapsule
preparation, 198
image acquisition, 200, 201, 210,
211
instrumentation, 202
materials, 197, 198
multidrug resistance analysis, 196
overview, 196, 197
slide preparation and fluorescence
microscopy, 200, 210
uptake studies, 199, 200
- Elastogram, atomic force microscopy,
350
- Electron microscopy (EM),
alignment,
high coherence illumination
conditions,
C2 aperture settings, 417, 418
C2 lens settings, 416
gun settings, 416, 417
point mode settings at 100kV,
418
standard alignment, 414–416,
423
cryofixation, *see* Microbiopsy
system
historical perspective, 296
porosome transmission electron
microscopy,
findings, 309, 311, 320
specimen preparation, 301
zymogen granule isolation, 300,
301
radiation damage of samples, 404
reflection contrast microscopy
comparison, 363, 364, 393
three-dimensional cryoelectron
microscopy of single
particles,
applications, 403, 404
data collection,
overview, 418, 423
tilt-pairs for random conical
reconstructions, 420, 421
untilted images, 418–420
general materials and vendors, 405
holey grid preparation,
Formvar holey film
preparation, 407, 421
Formvar holey grid
preparation, 407, 408
Formvar solution preparation,
407
hole diameters, 405, 406
slide cleaning, 406
thin carbon grids, 408
image reconstruction,
angular reconstitution, 429
image processing systems, 430
random conical reconstruction,
see Random conical
reconstruction
theory, 428
tomography, 428–430
staining,
deep staining, 411, 412, 421
uranyl acetate, 411
stain preparation,
ammonium molybdate, 409
methylamine tungstate with
bacitracin, 409
uranyl acetate, 408
vitreous ice preparations, 409–
414, 421–423
zymogen granule immunoelectron
microscopy, 319
EM, *see* Electron microscopy
Eosinophil, *see* Asthma
EPON resin, *see* Reflection contrast
microscopy
ERK, *see* Extracellular signal-regulated
kinase

- Extracellular signal-regulated kinase (ERK), confocal laser scanning microscopy studies of asbestos injury in lung, 68–70
- FIA, *see* Fluorescence image analysis
- Fiber-FISH, *see* Fluorescence *in situ* hybridization
- FISH, *see* Fluorescence *in situ* hybridization
- Flow cytometry,
 laser scanning cytometry advantages, 165, 166, 193, 202
 limitations, 165, 166
- Fluo-3, *see* Calcium flux
- Fluorescence image analysis (FIA), laser scanning cytometry, 169
- Fluorescence *in situ* hybridization (FISH),
 denaturation of target DNA, 250
 fiber-FISH, 245
 formalin-fixed, paraffin-embedded tumor samples, 251–253
 hybridization conditions, 250, 253
 interphase FISH,
 clinical applications, 241, 242
 paraffin-embedded tissues, 242, 243, 245
 specimen preparation, 249, 253
 laser capture microdissected nuclei analysis, 252–254
 laser scanning cytometry, 169, 174, 181
 microarray-based comparative genomic hybridization, 245, 246
 multicolor FISH of metaphase preparations,
 comparative genomic hybridization, 240, 241
 overview of techniques, 239, 240
 specimen preparation,
 fibroblast culture, 248, 253
 lymphocyte culture, 248
 spectral karyotyping, 240
 telomere analysis, 241
 near-field scanning optical microscopy of metaphase chromosomes, 283, 285, 286, 290
 principles, 237, 238
 probes,
 centromeric probes, 238
 gene-specific probes, 239
 labeling,
 direct versus indirect labeling, 249
 materials, 246, 247
 nick translation, 249, 253
 telomere probes, 239
 whole-chromosome paints, 239
 visualization,
 counterstaining, 251, 253
 posthybridization washing for
 direct labeling, 251
 primary antibody reaction for
 indirectly labeled slides, 250
- Formvar holey grid, *see* Electron microscopy
- Fourier shell correlation, *see* Random conical reconstruction
- Fura-2, *see* Calcium flux
- Fusion pore, *see* Porosome
- Gene expression profiling, *see* DNA microarray; Laser capture microdissection
- Gene therapy, *see* Cystic fibrosis
- G protein, zymogen granule association, 320, 321
- H&E staining, *see* Hematoxylin and eosin staining
- Hematoxylin and eosin (H&E) staining,
 formalin-fixed paraffin-embedded tissue sections, 217, 218
 frozen tissue sections, 216, 217, 225
- Holey grid, *see* Electron microscopy

- iGeneration, laser scanning cytometers, 183, 184
- Image reconstruction, *see* Electron microscopy; Random conical reconstruction
- Immunoelectron microscopy, *see* Electron microscopy
- Interphase FISH, *see* Fluorescence *in situ* hybridization
- JNK, *see* Jun N-terminal kinase
- Jun N-terminal kinase (JNK), confocal laser scanning microscopy studies of asbestos injury in lung, 69, 70
- Ki-67, confocal laser scanning microscopy studies of asbestos injury in lung, 68, 73
- Laser capture microdissection (LCM), asbestos-induced gene expression change studies in bronchiolar epithelial cells, DNase treatment, 235 materials, 232, 233 microdissection, 234, 235 overview, 232 reverse transcriptase-polymerase chain reaction, 235 slide preparation, dehydration, 234 sectioning, 233 specimen preparation, 233 fluorescence *in situ* hybridization analysis of nuclei, 252–254 principles, 213–215, 224, 225, 231, 232 protein analysis of microdissected frozen tissue sections, automated laser capture microdissection with AutoPix system, 223, 224, 227 frozen tissue sectioning, 216, 225 hematoxylin and eosin staining, formalin-fixed paraffin-embedded sections, 217, 218 frozen sections, 216, 217, 225, 226 manual laser capture microdissection, PixCell system operation, 218–223, 226, 227 sample storage, 223 saving images, 223 slide preparation, 219 materials, 215, 216, 225
- Laser quality assurance, *see* Confocal laser scanning microscopy
- Laser scanning cytometry (LSC), advantages over flow cytometry, 166, 193, 202 cell-cell interaction studies, 181, 182 clinical pathology applications, biopsy analysis, 179, 180 doxorubicin microcapsule uptake by breast cancer cells, cell selection and subculture, 198, 199 doxorubicin linking to microcapsules, 198 human serum albumin microcapsule preparation, 198 image acquisition, 200, 201, 210, 211 instrumentation, 202 materials, 197, 198 multidrug resistance analysis, 196 overview, 196, 197 slide preparation and fluorescence microscopy, 200, 210 uptake studies, 199, 200 eosinophil analysis in asthma, cell quantification, 207, 209

- image acquisition, 205–207, 211
- Octospot® cytospin system, 205, 211
- overview, 203, 204
- sputum sample preparation, 204
- histologic section analysis, 180, 181
- overview, 195, 196
- coupling with flow cytometry and confocal microscope, 183
- fluorescence image analysis, 169
- fluorescence *in situ* hybridization, 169, 174, 181
- instrumentation, 166, 167, 182, 194, 195, 183, 184
- ligand-receptor association studies, 181
- maximal pixel of fluorescence
 - intensity applications,
 - apoptosis assays, 170, 173, 174
 - cell cycle analysis, 169, 170
 - protein translocation, 170, 171
 - white blood cell identification, 170
- microgravity conditions and
 - liquidless staining, 183
- micronucleus assay, 172, 173
- nuclear versus cytoplasmic
 - localization of fluorescence, 171, 172
- principles, 193, 194
- recorded parameters, 167–169
- relocation feature applications,
 - enzyme kinetics, 179
 - sequential analysis of same cells
 - with different probes, 178, 179
 - visual cell examination, 177, 178
- research applications,
 - live-cell studies, 184
 - tissue microarray analysis, 184–186
- WinCyte software applications,
 - clonogenicity assay, 175, 176
 - cytogenetic studies, 174
 - immunophenotyping of cells, 176, 177
 - protein translocation between nucleoli and nucleoplasm, 174, 175
- LCM, *see* Laser capture microdissection
- Leica DMR microscope, reflection contrast microscopy adaptation, 389
- Leica EM PACT, *see* Microbiopsy system
- Lowicryl K4M resin, *see* Reflection contrast microscopy
- LSC, *see* Laser scanning cytometry
- Lung injury, *see* Confocal laser scanning microscopy
- Maximal pixel of fluorescence
 - intensity, *see* Laser scanning cytometry
- Messenger RNA detection, *see* Molecular beacons
- Methylamine tungstate stain, *see* Electron microscopy
- mfold*, molecular beacon secondary structure prediction,
 - accessible target region selection criteria, 4, 5, 11
 - file output, 4, 11
 - parameters, 4, 9, 11
 - server, 3, 4
- Microarrays, *see* DNA microarray; Tissue microarray
- Microbiopsy system,
 - advantages, 472
 - anesthesia of rats, 467, 473
 - biopsy excision, 469
 - cryofixation advantages, 463
 - cryofixation in Leica EM PACT, 471
 - electron microscopy of samples, 472

- embedding of samples, 472
- freeze substitution of samples, 471, 472
- materials, 465, 467
- transfer,
 - station preparation, 467–469, 473–475, 477
 - tissue, 469, 477
- Micronucleus assay, laser scanning cytometry, 172, 173
- Molecular beacons,
 - composition and nuclease resistance, 3
 - hybridization and fluorescence, 1
 - length and GC composition, 6, 12
 - melting profile determination, 7
 - oskar messenger RNA detection in *Drosophila* oocytes, 9, 12, 13
 - purity analysis with signal-to-background ratios, 6, 7
 - target RNA site selection,
 - OligoWalk analysis, 5, 6
 - overview, 2, 3
 - secondary structure prediction using *mfold*,
 - accessible target region selection criteria, 4, 5, 11
 - file output, 4, 11
 - parameters, 4, 9, 11
 - server, 3, 4
 - testing in vitro, 7, 9
- Near-field scanning optical microscopy (NSOM),
 - applications and advantages, 279, 287, 288
 - cell surface imaging, 281–283, 289, 290
 - diffraction limit, 275, 276
 - far-field microscopy combination, 278, 289
 - image acquisition, 281, 289
 - instrumentation, 279, 280, 289
 - meiotic chromosomes, 286, 287, 290, 291
 - metaphase chromosomes after fluorescence *in situ* hybridization, 283, 285, 286, 290
 - principles, 276–278, 288, 289
 - solutions, 280, 281
 - specimen requirements, 278, 279, 289
- NSOM, *see* Near-field scanning optical microscopy
- Octospot® cytospin system, eosinophil analysis in asthma, 205, 211
- Oil red O, lipid staining, 142, 150
- OligoWalk, molecular beacon analysis, 5, 6
- oskar, messenger RNA detection in *Drosophila* oocytes with molecular beacons, 9, 12, 13
- PCR, *see* Polymerase chain reaction
- Pericam, *see* Calcium flux
- Photomultiplier tube (PMT),
 - DNA microarray laser scanning and photon detection, 263, 265
 - quality assurance, *see* Confocal laser scanning microscopy,
- Picrosirius red, collagen staining, 145, 150
- PixCell, *see* Laser capture microdissection
- PKC, *see* Protein kinase C
- PMT, *see* Photomultiplier tube
- Pointwise modulus curve, atomic force microscopy, 349, 350
- Polymerase chain reaction (PCR),
 - reverse transcriptase-polymerase chain reaction of laser capture microdissected lung cells, 235
- Porosome,

- atomic force microscopy,
 - immunogold localization of
 - porosomes, 300, 305
 - immunoisolated porosomes, 311
 - pancreatic acinar cell isolation, 298
 - pits and depressions, 302, 304
 - plasma membranes,
 - findings, 307
 - preparation from pancreas, 299
 - porosome reconstitution, 299, 300
 - synaptosome and vesicle
 - preparation, 299
- discovery, 297
- function, 311–314
- immunoprecipitation and Western
 - blot analysis, 301, 302, 307–309
- pathology, 295, 296
- proteins, 307–311
- size, 297, 312
- transient fusion, 313, 314
- transmission electron microscopy,
 - findings, 309, 311
 - specimen preparation, 301
 - zymogen granule isolation, 300, 301
- Projection onto convex sets, *see*
 - Random conical reconstruction
- Protein kinase C (PKC), confocal laser scanning microscopy studies
 - of asbestos injury in lung, 68, 69, 71
- Radon inversion, *see* Random conical reconstruction
- Random conical reconstruction,
 - angle assignment on tilt images, 443, 457
 - centration of tilt images, 442, 443
 - class separation, 443, 444
 - digitization of images, 433, 455
 - file types and data management, 431–433
 - image alignment,
 - centration, 435, 436, 457
 - rotational and translational alignment,
 - reference-based alignment, 436, 437
 - reference-free alignment, 437
 - simultaneous rotational/translational alignment, 437, 438
 - image classification,
 - correspondence analysis, 439–442
 - self-organizing maps, 438, 439
 - image processing systems, 430, 431
 - image recording and negative selection, 431, 455
 - merging of volumes with different orientations,
 - alignment of volumes, 449
 - filtering, 448, 449
 - merging, 449, 450
 - overview, 448, 449
 - multireference alignment, 442
 - particle extraction,
 - selection of particle, 433, 434, 455, 456
 - windowing of untilted images,
 - contrast normalization, and tilt axis rotation, 434, 435, 456
 - principles, 429, 430
 - projection alignment refinement,
 - correction and iteration of refinement process, 446, 457
 - Radon/Fourier space, 445, 446
 - reprojection methods, 445
 - signal-to-noise ratio correction, 446, 447, 457
- projection onto convex sets,
 - mask application in real space, 444, 445

- Radon transform, 445
- resolution,
 - improvement, 451–455
 - measurement with Fourier shell correlation, 447, 448, 457
- three-dimensional projection
 - alignment, 450, 457, 458
- transfer function,
 - correction in tilt images, 454
 - correction in untilted images, 453, 454
 - fitting to tilt images, 454
 - fitting to untilted images,
 - astigmatism value fitting, 452, 453
 - averaged periodogram
 - calculation, 451, 452
 - defocus value fitting, 452
- two-step Radon inversion, 444
- weighted back-projection, 444
- RCM, *see* Reflection contrast microscopy
- Reflection contrast microscopy (RCM),
 - advantages and comparison with other microscopy techniques, 363, 364, 393
- applications, 365
- confocal laser scanning microscope
 - in reflection mode, 374, 391, 392, 398
- fluorescence microscope adaptation,
 - equipment, 373, 374, 388, 389, 397
- Leica DMR microscope, 389
- Zeiss Axioskop microscope, 389, 398
- image recording,
 - conventional photography, 392, 398
 - digital recording, 392, 393
 - materials, 374, 375
- interpretation, 393
- living cell imaging, 388
- microscope operation, 389–391, 398
- observation chambers, 373
- principles, 366–368
- specimen preparation,
 - dehydration and embedding,
 - EPON, 380, 381, 395
 - Lowicryl K4M, 381, 395
 - Unicryl, 381, 395
- immunostaining, 383–385, 396
- materials, 369–372
- overview, 365, 366, 368, 369, 375, 376, 394
- sectioning,
 - collection and storage, 381–383, 395
 - cryosectioning, 383, 395
- single-cell experiments,
 - gold tracer endocytosis, 387, 396, 397
 - immunogold staining, 385, 387, 396, 397
 - peroxidase-conjugated antibody internalization, 387, 388
- slide cleaning and coating, 376
- small cube formation,
 - monolayer cultures, 378, 395
 - single-cell suspensions, 377, 378, 394
- small biopsies, 377, 394
- tissue, 376, 394
- transwell cultures, 378, 379, 394
- tissue processing for pre-embedding, 379, 394, 395
- RNA localization, *see* Molecular beacons
- Second-harmonic imaging,
 - collagen,
 - applications, 32, 33
 - detection strategies, 27, 31, 332
 - rationale for second-harmonic imaging, 18
 - sample preparation, 26, 27

- signal propagation and properties, 25, 26
- generation of signal, 15–17, 33
- instrumentation,
 - coupling system, 20
 - laser, 18–21, 33
 - microscope, 21, 23, 25
- Secretory vesicles, *see* Porosome; Zymogen granule,
- Spectral karyotyping, principles, 240
- Spectral registration quality assurance, *see* Confocal laser scanning microscopy
- Three-dimensional cryoelectron microscopy, *see* Electron microscopy; Random conical reconstruction
- Tissue microarray, laser scanning cytometry analysis, 184–186
- Tomography, three-dimensional image reconstruction, 428–430
- Transmission electron microscopy, *see* Electron microscopy
- Unicryl resin, *see* Reflection contrast microscopy
- Uranyl acetate stain, *see* Electron microscopy,
- Western blot,
 - porosomes, 301, 302, 307–309
 - zymogen granules, 318, 319
- WinCyte software, *see* Laser scanning cytometry
- Zeiss Axioskop microscope, reflection contrast microscopy adaptation, 389, 398
- Zymogen granule,
 - aquaporin-1 association, evidence, 322–325
 - swelling regulation, 326–328
 - tritiated water entry assay, 320
 - atomic force microscopy, 319, 320
 - cell fractionation, 318
 - G protein association, 320, 321
 - GTP-induced swelling, mercury chloride sensitivity, 325, 326
 - immunolectron microscopy, 319
 - plasma membrane fusion assays, 318
 - secretory vesicle swelling and exocytosis, 317, 318
 - size distribution, 320
 - transmission electron microscopy, findings, 309, 311, 320
 - isolation, 300, 301
 - specimen preparation, 301
 - Western blot, 318–321

Cell Imaging Techniques

Methods and Protocols

Edited by

Douglas J. Taatjes and Brooke T. Mossman*Department of Pathology, University of Vermont, Burlington, VT*

Cell imaging methodologies have now become essential research tools for a variety of disciplines that traditionally had not relied on them. In *Cell Imaging Techniques: Methods and Protocols*, distinguished international researchers describe in detail their state-of-the-art methods for the microscopic imaging of cells and molecules. The authors cover a wide spectrum of complementary techniques, including such methods as fluorescence microscopy, electron microscopy, atomic force microscopy, and laser scanning cytometry. Additional protocols on confocal scanning laser microscopy, quantitative computer-assisted image analysis, laser-capture microdissection, microarray image scanning, near-field scanning optical microscopy, and reflection contrast microscopy round out this eclectic collection of cutting-edge imaging techniques now available. The authors also discuss preparative methods for particles and cells by transmission electron microscopy. The protocols follow the successful *Methods in Molecular Biology*™ series format, each offering step-by-step laboratory instructions, an introduction outlining the principles behind the technique, lists of the necessary equipment and reagents, and tips on troubleshooting and avoiding known pitfalls.

Timely and highly practical, *Cell Imaging Techniques: Methods and Protocols* provides researchers and clinicians with a richly useful guide to selecting and performing the best imaging method from a bewildering variety of microscopy-based techniques.

FEATURES

- Basic and clinical microscopic techniques found in today's core cell imaging facilities
- Light microscopic techniques to observe mRNA, calcium, and collagen molecules
- Preparative methods for transmission electron microscopy of particles and cells
- Tutorials to provide background for the more complex imaging techniques
- Step-by-step instructions to ensure successful results
- Tricks of the trade and notes on troubleshooting and avoiding known pitfalls

CONTENTS

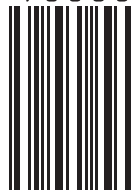
Molecular Beacons: *Fluorescent Probes for Detection of Endogenous mRNAs in Living Cells*. Second-Harmonic Imaging of Collagen. Visualizing Calcium Signaling in Cells by Digitized Wide-Field and Confocal Fluorescent Microscopy. Multifluorescence Labeling Techniques and Confocal Laser Scanning Microscopy on Lung Tissue. Evaluation of Confocal Microscopy System Performance. Quantitative Analysis of Atherosclerotic Lesion Composition in Mice. Applications of Microscopy to Genetic Therapy of Cystic Fibrosis and Other Human Diseases. Laser Scanning Cytometry: *Principles and Applications*. Near-Clinical Applications of Laser Scanning Cytometry. Laser Capture Microdissection. Analysis of Asbestos-Induced Gene Expression Changes in Bronchiolar Epithelial Cells Using Laser Capture Microdissection and Quantitative Reverse Transcriptase-Polymerase Chain Reaction. New Approaches to Fluorescence *In Situ* Hybridization. Microarray Image Scanning. Near-Field Scanning Optical

Microscopy in Cell Biology and Cytogenetics. Porosome: *The Fusion Pore Revealed by Multiple Imaging Modalities*. Secretory Vesicle Swelling by Atomic Force Microscopy. Imaging and Probing Cell Mechanical Properties With the Atomic Force Microscope. Reflection Contrast Microscopy: *The Bridge Between Light and Electron Microscopy*. Three-Dimensional Analysis of Single Particles by Electron Microscopy: *Sample Preparation and Data Acquisition*. Three-Dimensional Reconstruction of Single Particles in Electron Microscopy: *Image Processing*. A New Microbiopsy System Enables Rapid Preparation of Tissue for High-Pressure Freezing. Index.

ISBN 1-58829-157-X



9 0000



Methods in Molecular Biology™ • 319

CELL IMAGING TECHNIQUES: METHODS AND PROTOCOLS

ISBN: 1-58829-157-X E-ISBN: 1-59259-993-1

ISSN: 1064-3745 humanpress.com