

Index

a

absorption 14, 16–18, 61, 67, 85–89,
106, 136, 140, 141, 149, 153, 157,
166, 188, 208, 212–214, 218, 223,
226, 230, 236, 255, 274, 276, 348,
389, 394, 397, 408, 410–412, 416,
419, 420, 448, 449

Abbe's diffraction limit 368

absorption dipole moment 416

acceptor density 426–428

acceptor emission filter 431

acceptor fluorophores 413

acousto-optical modulator (AOM) 345

Airy disk 38, 41–44, 48

Airy disk radius 458

Airy pattern 173, 174

angle of incidence 376

angle of refraction 377

animalcules 165

astigmatic imaging approach 281–284

astigmatism 21

ATTO647N 325, 337

autocollimation telescope 468

autocorrelation function (ACF) 188,
189, 191, 192, 199

autofluorescence 381

Avalanche photodiode (APD) 114

Avogadro's number 412

axial resolution 45, 47, 173–179

b

back focal plane (BFP) 249, 472–473

ballistic light 377

bandpass filter 324, 326

Bayesian statistics 404

beam aberrations 332

beamlets 466

beam propagation 466

beam splitter 311

O² benzylcytosine (BC) 145

Bessel beam 262

Bessel function 38

bioluminescence 95

binning 106

biplane imaging approach 284

birefringence 13

bleaching 120

blue-shifted dye 334

Bragg condition 33

Braun tubes 181

bright field 31–32, 44, 45, 61, 70, 71,
73, 74

brightness 28, 29, 57–62, 67, 74, 76, 80

c

camera acquisition software 456

carbocyanines 275, 281

cell membrane glycans 283

centrosomes 287

charge-coupled device (CCD) 26, 104,
107, 112, 273, 386–387

Chinese hamster ovary (CHO) 381

chromatic reflectors 18–19

chromophore 274

click chemistry 281–283

cluster size 397

coherent beams 295

colliding-pulse mode-locked (CPM)
218

- complementary
 - metal–oxide–semiconductor (CMOS) 111, 112, 388
 - confocal effect 346
 - confocality 302
 - confocal laser scanning microscope (CLSM) 342
 - confocal scanning microscopy 165, 247, 261, 429
 - conjugation process, synthetic
 - fluorophores 142
 - continuous fluorescence
 - microphotolysis (CFM) 343
 - continuous fluorescence photobleaching 343
 - continuous illumination 358
 - continuous wave (CW) 167, 172, 329
 - conventional dual-color imaging scheme 334
 - conventional fluorescence microscopy
 - techniques 264
 - convolution 41
 - convolution theorem 294, 298
 - copper-catalyzed azide–alkyne cycloaddition 282
 - Coulombic coupling 411, 414
 - Coulomb's law 414, 418
 - coupling FRET 425
 - critical angle 378
 - critical illumination 28–30
 - crystallography techniques 422
 - cut-off frequency 295, 302
 - cyan fluorescent protein (CFP) 152, 423
 - cylindrical lens 246, 248
- d**
- DAOSTORM 287
 - Davson–Danielli–Robertson model 341
 - defocused imaging 395
 - Deinococcus radiodurans 150
 - depth of field (DOF) 49, 81, 392
 - depth of focus 49, 256, 313, 392
 - detection, PSFs 175
 - development, confocal microscopy 169
 - dichroic mirror 96, 97, 102, 375
 - dichromatic beam splitter 468
 - dielectric mirrors 16–17
 - differential interference contrast (DIC)
 - 13, 23
 - interpretation 81
 - optical setup 77
 - differential interference microscopy 77–80
 - different lens configurations 259–260
 - diffraction 1, 7, 323, 324, 326, 327
 - coefficients 341, 342, 346
 - limit 269, 270
 - pattern 33, 34, 36
 - diffusion-enhanced energy transfer 424
 - diffusion-reaction equation 351, 355
 - diffusion time 188
 - digital camera, fluorescence microscopy 113
 - digitally scanned light sheet microscopy (DSLM) 246, 248
 - 4,4'-diisothiocyanatostilbene-2,2'-disulfonic (DIDS) 352
 - dipole–dipole distance 414
 - dipole moments 414, 416
 - Dirac delta distribution 298
 - discovery, fluorescence of 91–92
 - discriminator 172
 - dispersion 9
 - donor fluorophores 425
 - donor emission spectrum 412
 - donor excitation filter 431
 - donor quantal spectrum 412
 - donor quantum yield 412, 417, 431, 438, 440
 - donor quenching (DQ) 428–429, 432
 - double helix PSF 284
 - double-sided illumination 253, 254, 258
 - doughnut-shaped depletion beam 335
 - dSTORM 275, 282
 - dual-color imaging 334
 - dual-view system 375
 - dynamic imaging 373

e

- electrically tunable lenses (ETLs) 248, 256
- electromagnetic radiation 391, 408
- electronic delay 332
- electronic filters 103
- electron-multiplying charge-coupled device (EM-CCD) 109, 387
- emission dipole 416, 417
- emission spectrum 90, 99, 411
- epifluorescence 98
- epi-illumination 31–32
- equilibrium properties, fluorescence emission 88–89
- excitation, PSFs 175
- excitation filter 101
- excitation light distribution 311–313
- excited state 325, 327
- exciting light intensity 99, 122–123
- exciting photon 89

f

- Fermi's golden rule 414, 416
- field of view (FOV) 247, 248
- finite optics setup 25
- FLAsH 148
- fluid mosaic model 341
- fluorescein 410
- fluorescence confinement 326
- fluorescence correlation spectroscopy (FCS) 172, 354
- fluorescence cross-correlation spectroscopy (FCCS) 190
- fluorescence depletion 326
- fluorescence emission 272, 300
 - depolarization of 428
- fluorescence excitation 293
- fluorescence filter 246
- fluorescence intensity, measurements of 119–121
- fluorescence labeling 368
- fluorescence lifetime changes 437–441
- fluorescence markers 157
- fluorescence microscopy 86, 90–95, 299
- fluorescence photobleaching recovery (FPR) 341

- fluorescence photobleaching techniques
 - CLSMs-assisted photobleaching methods
 - implementation 358–359
 - opportunities 359
 - concepts and procedures
 - complexity from bottom up 346–347
 - principle and several modes 340–345
 - set up 345–346
 - continuous fluorescence
 - microphotolysis (CFM) background and data evaluation 354–357
 - combination of 357
 - variants 357–358
 - fluorescence recovery after photobleaching (FRAP)
 - binding 350–351
 - diffusion measurements 347–350
 - membrane transport 351–354
- fluorescence photon 88
- fluorescence quantum yield 136, 147
- fluorescence recovery after photobleaching (FRAP) 160, 341
- fluorescent labels
 - characteristics 134
 - properties 133, 135, 142
 - sensors in cell 160
- fluorescent protein (FPs) 152, 156, 324, 329, 337
- fluorophore 270, 407
 - classes of 337
 - emission spectrum 326, 336
 - excitation 326
 - kinetics 328
 - photoactivatable/photoconvertible 272
 - photobleaching 270, 355
 - photon number 280
 - photoswitchable 273
 - property of 342
 - reaction-induced photoswitching 273

- fluorophore (*contd.*)
 - rhodamine 275
 - ROP 354
 - for STED microscopy 337
 - stimulated emission depletion 324
 - flux equation 356
 - focal lengths 251
 - focal plane 250, 260, 471–472
 - Förster distance 413, 420
 - Förster equation 414–418
 - Förster radius 399, 400
 - Förster resonance energy transfer (FRET) 155, 191, 369
 - Fourier frequency 36
 - Fourier theory 41
 - Fourier-transformed illumination pattern 299
 - 4Pi microscopy 357
 - Fraunhofer diffraction pattern 38
 - frequency-domain FLIM 443–444
 - frequency spectra 415
 - fringe distance 315
 - fringe period 313
 - fringe projection, *see* two-beam interference
 - full quantitation 433–434
 - full width at half-maximum (FWHM) 175, 372
 - functional magnetic resonance imaging (fMRI) 237
- g**
- Gaussian beam 250–252
 - Gaussian filters 462
 - Gaussian function 272, 394
 - Gaussian intensity profile 348
 - Gaussian photon distribution 372
 - Gaussian-shaped signal distribution 390
 - geometric-optical term 176
 - global fitting approaches 446
 - gratings 34, 37, 38, 57, 330
 - constant 8, 33, 35
 - distance 299, 312
 - structure 34
 - gray values 189, 317, 455
 - green fluorescent protein (GFP) 150, 281, 368, 410
 - grid projection 295
- h**
- hetero-FRET 413
 - high-pressure mercury vapor
 - arc-discharge lamps (HBO) 99
 - burners 100
 - high-resolution information 300–301
 - homo-FRET 414
 - Huygens–Fresnel integral equation 35
 - Huygens wavelet 37
- i**
- illumination intensity (I) 381
 - illumination microscopy 296
 - illumination pattern 298–301, 303–307, 313
 - illumination photons 324
 - illumination time 381, 382
 - image brightness 57
 - image contrast 90–93
 - image deconvolution 184, 462
 - image integration time 109
 - image processing operations
 - focal plane 37
 - pixel groups 461–462
 - single pixels 460–461
 - imaging artifacts 278
 - imaging contrast, measures improving 285
 - immersion media 62–65
 - immunofluorescence 281
 - immunoglobulin G antibodies 147, 282
 - induced acceptor emission 429–432
 - infinity space 24
 - instrument response function (IRF) 172
 - integrator 172
 - intensified charge-coupled device (ICCD) 104, 107, 109
 - intensity-based measurements, FRET 432–433
 - intensity distribution 173
 - intensity histograms 288, 459–460

- intensity zero 332
 - interference effects
 - light rays 11
 - pattern 304
 - interference microscopy 77
 - interferometer 303, 304, 309
 - plane 306
 - setup 308
 - interferometric imaging 284–285
 - intermediate image 25
 - intrinsically photoswitchable probes 274
 - inverted microscopes 32, 473
 - in vivo/in vitro* measurements, FRET 401
 - IrisFP 154
 - isoSTED configuration 336
- j**
- Jablonski diagram 92, 325
- k**
- Köhler illumination 28–30, 379, 383, 384
 - kinetic measurements, FRET 401
 - kinetics, fluorescence emission 88–89
- l**
- Lambert radiator 58
 - Lampyris noctiluca 93
 - laser scanning microscopes (LSMs) 180, 362
 - label size vs. structural resolution 280–282
 - lateral resolution 41, 175
 - coherent light sources 43
 - incoherent light sources 41
 - optical units 47
 - lens alignment 468
 - lens classes 61–62
 - lens design 53–57
 - light-emitting diodes (LEDs) 100, 101
 - light microscopy 372
 - angular aperture 27
 - apertures 50
 - components 23
 - contrast 67
 - dark-field 68
 - DIC 77
 - interference contrast 74
 - optical contrast methods 68
 - phase contrast, *see* phase contrast
 - phase objects 67
 - entrance pupil 50
 - exit pupil 50, 51
 - field-of-view 28
 - high resolution objectives 65
 - illumination system 28
 - beam path 30
 - image path 24
 - magnifications 26
 - CCD 26
 - focal length 27
 - magnifying glass 27
 - multiview imaging 257–259
 - numerical aperture 27
 - telecentricity 51
 - wave optics and resolution 32
 - light propagation 377
 - light sheet fluorescence microscopy (LSFM) 246
 - light sheet illumination 254, 259, 263
 - light sheet microscopy
 - construction and working 248–249
 - photobleaching and toxicity 247
 - polarization 253
 - principle of 246–247
 - 3D imaging 255–357
 - water-dipping illumination lens 250
 - light sources, STED 332–333
 - light waves 2
 - circular polarized 4
 - on interference 2
 - plane wave 7
 - transverse wave 3
 - description 3
 - linear 299
 - polarization 378
 - lipofuscin 316, 318
 - live-cell dynamics 160
 - live-cell labeling 282
 - LivePalm 286
 - localization errors 280
 - localization precision (LP) 280, 374

- localization-based super-resolution microscopy
 - experimental setup for 275–277
 - fluorescence labeling 280–285
 - imaging contrast, measures for improving 285
 - intrinsically photoswitchable probes 274
 - optical resolution and imaging artifacts 278–280
 - organic fluorophores, photoswitching of 275
 - photoactivatable and photoconvertible probes 274
 - single-molecule localization microscopy (SMLM)
 - quantification of 288–289
 - reference structures for 287–288
 - software 285–287
 - lock-in techniques 384
 - Lorentzian model function 327
 - low-pass filters 462
- m**
- magnification, optical system 48–49
 - maleimides 143
 - mean-square displacement (MSD) 400
 - metal halide lamps 99
 - Michaelis–Menten equation 353
 - microchannel plate 107
 - Micrographia 165
 - microtubulin 277, 287, 288
 - mirror alignment 467–468
 - modulation contrast 314, 315
 - Moiré effect 300, 301
 - molecular brightness 136, 196
 - molecular complexes, stoichiometry of 371
 - Monte Carlo simulations 425
 - multicolor imaging 373
 - multidirectional SPIM (mSPIM) 255
 - multiparameter fluorescence detection (MFD) 193–195
 - multiple acceptors 424–425
 - multiple donor–acceptor pairs 424
- n**
- nanocrystals 140
 - nanometers 377
 - near-field scanning optical microscopy (NSOM) 383
 - near-infrared (NIR) region 86
 - negative sign, image formation 35
 - N*-hydroxysuccinimide (NHS) esters 142, 143, 146–149
 - Nipkow disk 181, 183
 - nitriлотriacetic acid 144
 - NMR 422
 - noise detector, EM-CCD 389
 - noninvasive measurements 402
 - nonlinear microscopies 203, 239
 - nonlinear optics 206
 - non-natural amino acids 146
 - nonscanning applications 186
 - normalization factor 178
 - number and brightness analysis (N&B analysis) 195
 - numerical aperture (NA) 165, 247, 372
 - Nyquist–Shannon sampling theorem 278
 - Nyquist theorem 384, 458
- o**
- objective lens 326
 - objective-type TIR 378
 - object size estimation 313
 - occupancy errors 434
 - oblique angles 466
 - optical aberrations 20
 - optical components, light microscopes 26
 - optical delay 332
 - optical elements 13–19
 - optical filters 17–18
 - optical lenses 13–15
 - optical metallic mirrors 15–16
 - optical parametric oscillator (OPO) 217
 - optical path length difference 37
 - optical projection tomography (OPT) 262
 - optical resolution 126, 278
 - optical sub-diffraction techniques 422

- optical transfer function (OTF) 40–41, 294, 298
 - organic dyes 138
 - organic fluorophores, photoswitching of 275
 - orthogonal-plane fluorescence optical sectioning (OPFOS) 245
 - oscillation probability 418
 - oscillator strength function 416
 - oversampling 50
- p**
- PA-green fluorescent protein (PA-GFP) 234
 - Palm3d 286
 - paraffin 316
 - particle 1, 2, 6
 - parallel data acquisition 455
 - patterned techniques, application of 315
 - Perrin formula 448
 - Perrin's equation 414, 415
 - Perrin's estimation 411
 - phase contrast 69
 - conjugate planes 70
 - focal plane 73
 - phasor diagrams 72
 - phase ring 73
 - properties 74
 - Zernike's experiments 70
 - phase fluorimetry 443
 - phase modulation technique 443
 - phasor plot 447
 - phosphorescence 88
 - photoactivatable fluorescent proteins (PA-FPs) 277
 - photoactivatable (PA) 151, 274, 369
 - photobleaching 124, 138, 329, 337
 - CLSMs 358
 - curve 397
 - effects 299
 - localization microscopy 374
 - quantum efficiency 342
 - technique 435
 - photoconvertible probes 274
 - photodetectors 104, 346
 - photomultiplier tube (PMT) 113–114, 169, 342, 432
 - photon antibunching 397
 - photon noise/shot noise 117
 - photoswitchable fluorophores 273
 - phototoxicity 125, 137
 - phycobiliproteins 149
 - physical grating 306
 - picture elements, camera 105
 - pinhole 169
 - pivoting light sheet 254
 - pixel
 - oversampling 384
 - size 384
 - undersampling 384
 - pixelation noise 390
 - planar distribution 425
 - Planck constant 414, 448
 - plane of incidence 11
 - plano-convex lenses 466
 - pointillism 271
 - point spread function (PSF) 38, 40–42, 48–50, 65, 67, 294, 297, 314, 323, 357
 - Poissonian signal fluctuations 390
 - polarization microscopy 395
 - polarization vector 305
 - precise measurements 399–400
 - primary image plane 25
 - principles, confocal microscopy of 166
 - prism-type TIR 378
 - protein–lipid ratio 341
 - pulsed interleaved excitation (PIE) 191
 - pulsed irradiation 358
 - pulsed lasers 328
 - diodes 332
 - laser systems 332
 - pulse synchronization 332
- q**
- Quantenspektrum 416
 - quantification methods 425
 - quantitative measurements 401
 - quantum efficiency 89
 - quantum yields 399, 412, 413, 415, 417
 - QuickPALM 284

r

radial resolution 173–179
 radiative lifetime 440
 radical anion 275
 read noise 118
 rapid diffusion limit 424
 rapid lifetime determination 445
 rapidSTORM 286
 raster image correlation spectroscopy (RICS) 198
 rate of transfer 413, 414
 ratiometric measurements (pH) 121
 Rayleigh distance 294
 Rayleigh length 251
 reaction-induced photoswitching 273
 reactive oxygen species (ROS) 137
 ReAsH 148
 redox chemistry 275
 red-shifted dye 334
 reducing and oxidizing system (ROXS) 337
 reflected light image 97
 reflection 10–11
 refraction 9–10
 refractive index 9
 region of photolysis (ROP) 342, 343, 346
 relaxation 325
 resolution limit, microscope 35
 reversible photoswitching 274
 reversible saturable optical fluorescence transitions (RESOLFT) 161
Rhodococcus rhodochrous 145
 rigorous approach, optical resolution 33

s

saturated structured illumination microscopy (SSIM) 161
 saturation effects 299, 372
 scanning confocal imaging 179, 195
 scanning tunneling microscopy (STM) 269
 scientific complementary metal-oxide-semiconductor (sCMOS) 247, 277
 secondary image plane 25

sensitive detection 94
 selective plane illumination microscopy (SPIM) 206, 246
 sensitized emission 429
 sensors
 CCD 107
 FRET 431
 sequential data acquisition 457–458
 Shannon/Nyquist sampling frequency 333
 shear plate 465
 signal fluctuation 389
 signal-to-noise ratio
 detector noise 389
 signal fluctuation 389
 silicon–rhodamine (SiR) dyes 145, 337
 sine condition 59–60
 single-molecule emitter 397
 single-molecule fluorescence resonance energy transfer 400
 single-molecule localization microscopy (SMLM) 324
 principle of 271, 272
 quantification of 288
 reference structures for 287
 software 285
 single-molecule methods, mobility/anisotropy 371
 single-molecule microscopy
 collection efficiency 377
 dual-view system 375
 dynamic imaging 373
 magnification/resolution 376
 multicolor imaging 373
 photoactivation/photoswitching 373
 photobleaching localization microscopy 374
 single-molecule/-particle tracking 374
 super-resolution imaging 372
 single-molecule signals
 brightness 394
 color 395
 defocused imaging 395
 intensity pattern 393
 orientation 394

- point spread function 391
 - polarization microscopy 394
 - single-molecule tracking 260
 - single-photon avalanche diode (SPAD) 401
 - single-sided illumination 254, 258
 - solvent relaxation 88
 - small objects, misrepresentation 127–128
 - Snell's law 377
 - spatial frequency 36, 294
 - spatial resolution 270
 - fluorescence microscope 280
 - spatially modulated illumination
 - excitation light distribution 311
 - object size estimation 313
 - overview 309
 - setup 311
 - specificity, fluorescence labeling 93–94
 - spectral emission bands 431
 - spectrofluorometry 428
 - spinning disk confocal microscope (SDCM) 181, 184
 - stage scanning 179–180
 - standard deviation 372
 - stimulated emission depletion (STED) 155, 324, 357
 - applications
 - fluorophore, choice of 336
 - labeling strategies 337
 - experimental setup
 - axial resolution, improving 335
 - multicolor imaging 334
 - scanning and speed 333
 - fundamental concepts 324
 - key parameters in 328
 - stochastic optical reconstruction
 - microscopy (STORM) 161, 275, 422
 - stochastic switching processes 324
 - Stokes' shift 409–411, 420, 448
 - structured illumination microscopy (SIM)
 - high-resolution information 300
 - illumination pattern 303
 - image generation in 297
 - interference pattern 304
 - optical sectioning 301
 - super-resolution microscopy
 - resolution limit 294
 - spatially modulated illumination (SMI)
 - excitation light distribution 311
 - object size estimation 313
 - overview 309
 - setup 311
 - superman cape function 419
 - synchronization, STED 332
 - synthetic grating 306
- t**
- tetracysteine 144
 - thiols 275
 - transfection 159
 - three-beam interference 295, 302
 - three-dimensional SMLM 283
 - three-dimensions, fluorescence
 - microscopy 121–122
 - time-correlated single-photon counting 444–445
 - time-domain FLIM 442, 444
 - time-of-flight camera 444
 - total internal reflection (TIR) 378
 - total internal reflection fluorescence (TIRF) microscopy 354
 - collimation 384
 - illumination time (t_{ill}) 382
 - intensity 380
 - polarization 382
 - uniformity 379
 - wavelength 383
 - two-beam illumination 302
 - two-beam interference 295, 297, 298, 301
 - two-dimensional (2D) grating 301
 - two-photon excitation microscope 203, 329
 - absorption 212
 - advantages 220, 238
 - caged compounds 233
 - continuous wave 208
 - depth of imaging 230
 - description 205

two-photon excitation microscope

(contd.)

- detection strategies 219
- focal volume 210
- history and theory 207
- in vivo* image 229
- lasers 216
 - CPM 218
 - OPO 217
 - pulse widths 218
- limitations 222
 - diphenylhexatriene 224
 - heating, laser powers 223
 - hyperspectral image 229
 - photobleaching 227
 - spatial resolution 223
- PA-GFP 234
- photochemistry 231
- photobleaching 211
- quantum dots 239
- scattering 213
- UV excitation 231
- UV fluorophores 231
- Tyndall effect 85

U

- undersampling 50

V

- Venus-YFP (yellow fluorescent protein) 423
- von Bieren condition 33, 35, 60

W

- water-dipping lenses 259
- wave 1–11, 13–18, 20, 23, 32–50, 57, 67, 70–80
- wavelength effects 330
- wavelength fluorophores 419
- widefield fluorescence microscope 271
- widened parallel laser beam 465–467
 - single lens 468–470
- Wollaston prism 375

X

- X-ray crystallography 372

Z

- zero-order diffraction 307