

Contents

1	Introduction to Bioimaging	1
1.1	Color	1
1.2	Colorful Material	4
1.3	Light Source of Bioimaging	6
1.4	Subcellular Imaging	12
1.5	Cell-Selective Imaging	14
1.6	Tissue and Organ Imaging	14
1.7	Whole-Body Imaging	15
1.8	Probes in Bioimaging	15
	References	16
2	Chemical Sensors and Probes for Bioimaging	17
2.1	History of Dyes in Biological Stains	17
2.2	Blood Cell Staining	22
2.3	Bacteria Staining Using Gram Method	24
2.4	Fluorescent Sensors and Probes	25
2.5	Representative Fluorescent Compounds for Bioimaging	29
	References	33
3	Organelle-Selective Probes	35
3.1	Introduction	35
3.2	Cell Plasma Membrane	40
3.3	Endosome and Lysosome	47
3.4	Nucleus and DNA	50
3.5	Nucleolus and RNA	56
3.6	ER and Golgi Body	58
3.7	Mitochondria	62
3.8	Lipid Droplet	66
3.9	Peroxisome	67
3.10	Cytosol	68
3.11	Extracellular Vesicle	69
3.12	Non-membrane-Bound Condensate	72

3.13	Organelle Probes in Live Cells and Fixed Cells	74
3.14	Modeling for the Organelle-Selective Probes	75
	References	80
4	Live-Cell-Selective Probes	85
4.1	Protein-Oriented Live-Cell Distinction (POLD)	88
4.1.1	Embryonic Stem Cell Probe: CDy1	93
4.1.2	Neural Stem Cell Probes	99
4.1.2.1	CDr3	99
4.1.2.2	CDy5 for Neural Stem Cell Division Monitoring	103
4.1.3	Tumor-Initiating Cell Probes	105
4.1.3.1	TiY	105
4.1.3.2	TiNIR	108
4.1.4	Muscle Cell Probes	110
4.1.5	Pancreatic Cell Probes	113
4.1.5.1	Pancreatic α -Cell Probes	114
4.1.5.2	Pancreatic β -Cell Probes	114
4.1.6	Amyloid Probe: CDy11	116
4.2	Carbohydrate-Oriented Live-Cell Distinction (COLD)	118
4.2.1	Lectins	121
4.2.2	Embryonic Stem Cell Probes: CDg4 and CDb8	122
4.2.3	Gram-Positive Bacteria Probe	122
4.2.4	Biofilm Probe: CDy14 and CDr15	124
4.3	Lipid-Oriented Live-Cell Distinction (LOLD)	128
4.3.1	Filipin as a Cholesterol Probe	129
4.3.2	Lipid Droplet Probes	129
4.3.3	Neuron Probes	130
4.3.3.1	Nissl Stains as Neuron Body Probe	131
4.3.3.2	Plasma Membrane Dyes as Neuronal Network Probe	131
4.3.3.3	NeuO as a Universal Neuron Probe	132
4.3.4	B Lymphocyte Probe: CDgB	134
4.3.5	Activated CD8 ⁺ Lymphocyte Probe: Probe41	138
4.3.6	Apoptotic Cell Probe: Apo-15	139
4.4	Gating-Oriented Live-Cell Distinction (GOLD)	139
4.4.1	Cell Imaging Probes through Phagocytosis	140
4.4.2	Probes Through SLC Transporters	143
4.4.3	Probes Through Glucose Transporters	144
4.4.4	Naïve Embryonic Stem Cell Probe: CDy9	146
4.4.5	Neurotransmitter Mimetic Probes	147
4.4.6	Astrocyte Probe: SR101	149
4.4.7	Subtype-Specific Macrophage Probes: CDg16, CDr17, CDg18	150
4.4.7.1	CDg16 for Activated Macrophage	150
4.4.7.2	CDr17 for M1 Macrophage	152
4.4.7.3	CDg18 for M2 Macrophage	153
4.4.8	B-Cell-Selective Probe Through GOLD Mechanism	153

4.4.9	Bacteria Probes Through Transporters	154
4.4.10	Probes Through ABC Transporters	155
4.4.11	Background-Free Tame Dye	157
4.5	Metabolism-Oriented Live-Cell Distinction (MOLD)	160
4.5.1	Substrate for Proteases in Extracellular Matrix	160
4.5.1.1	MMP12 Substrate for Activated Macrophage Probe	162
4.5.1.2	Cathepsin S Substrate for Tumor-Associated Macrophage Probe	162
4.5.1.3	Elastase Substrate for Neutrophil Probe	163
4.5.1.4	Granzyme Substrate for Natural Killer and Cytotoxic T Cell Probe	163
4.5.2	Microglia Probe: CDr10 and CDr20	165
4.5.2.1	CDr10a and b for Microglia Imaging among Brain Cells	165
4.5.2.2	Microglia Probe CDr20 through Ugt1a7c	169
4.5.3	Neutrophil Probe: NeutropG	170
	References	172
5	Ex Vivo Tissue Imaging Probes	179
5.1	Immunohistochemistry	181
5.2	Tissue Imaging with Nucleic Acid Probes	186
5.3	Tissue Imaging with Small-Molecule Probes	186
5.3.1	Pancreatic Islet Imaging	188
5.3.2	Neuronal Tissue Imaging	191
5.4	Organoid as Model of Tissue and Organ	194
5.4.1	Blood Vessel 3D Model	195
5.4.2	Tumor Organoid for Drug Screening	196
	References	196
6	In Vivo Whole-Body Imaging Probes	199
6.1	ElaNIR for Elastin Imaging in Mouse	200
6.2	Probes for Exposed Neuron in Zebrafish Embryo	201
6.3	NeuO for Whole-Body Neuron Imaging in Zebrafish	202
6.4	LipidGreen for Fatty Tissue Imaging in Zebrafish	203
6.5	Blood Vessel Imaging in Zebrafish	204
6.6	Probes for Bone Imaging	205
6.7	Probes for Pancreatic Islet Imaging	206
6.8	Probes for Eye Imaging	208
6.8.1	Optical Coherence Tomography for Retina	209
6.8.2	Fundus Photography for Blood Vessel Imaging in Retina	210
6.8.3	Neuron Imaging on Retina	210
6.8.4	Bacterial Infection on Cornea	211
6.9	Macrophage Imaging in Ischemia and Inflammation	213
	References	214
7	Imaging for Biological Environment and Function	217
7.1	pH	218
7.2	Metal Ions	221

7.2.1	Na ⁺ and K ⁺	222
7.2.2	Ca ²⁺	225
7.2.3	Mg ²⁺	227
7.2.4	Metal Ion Selectivity of Fluorescent Sensors	228
7.2.5	Iron Ion	230
7.2.6	Zn ²⁺	230
7.2.7	Copper Ion	231
7.3	Metabolites	232
7.3.1	ATP	232
7.3.2	NADH	233
7.3.3	Histamine	234
7.4	Viscosity	234
7.5	Temperature	238
7.5.1	ER Thermometer	238
7.5.2	Mitochondrial Thermometer	240
7.5.3	Organelle-Specific Fluorescent Thermometers	241
7.6	Reactive Oxygen Species and Reactive Nitrogen Species	241
7.6.1	Superoxide	243
7.6.2	H ₂ O ₂	245
7.6.3	ONOO ⁻	247
7.6.4	HOCl and Hypochlorite	249
7.6.5	Hydroxyl Radical	251
	References	252
8	Imaging for Disease	259
8.1	Introduction	259
8.2	Cancer Imaging	260
8.2.1	Imaging by Cancer-Specific Biomarker Binding	261
8.2.2	Imaging by Cancer-Specific Metabolism	263
8.2.3	Imaging by Cancer-Specific Transporter	267
8.2.4	Imaging by the Changed Environment of Cancer	268
8.2.5	Circulating Tumor Cell (CTC)	269
8.2.6	Cancer Cell Line for Imaging	269
8.2.7	Animal Models of Tumor Imaging	271
8.2.8	Ex Vivo 3D Tumor Culture Model	275
8.2.9	Clinical Imaging of Tumor for Diagnosis and Prognosis	277
8.2.10	Intraoperative Imaging of Tumor	278
8.3	Neurodegenerative Disease Imaging	278
8.3.1	AD Imaging Through A β Amyloid Aggregates	278
8.3.2	AD Imaging Through Tau	281
8.3.3	Animal Model for AD	283
8.4	Inflammation Imaging	284
8.4.1	Inflammation Imaging by Environmental Changes	284
8.4.2	Inflammation Imaging Through Immune Cells	285
8.4.3	Inflammation Animal Model	286

8.5	Diabetes Imaging	286
8.6	Liver Disease Imaging	288
8.7	Aging	289
8.8	Theranostics	289
	References	290
9	Non-optical Imaging Probes	297
9.1	Ultrasound Imaging Probes	297
9.2	X-Ray Contrast Agents	298
9.3	MRI Contrast Agents	301
9.4	SPECT Probes	303
9.5	PET Probes	306
9.5.1	PET Probes for Tumor	307
9.5.2	PET Probes for Brain Function	309
9.6	Multimodality	310
	References	311
10	Fluorescence Imaging Techniques and Analysis Methods	313
10.1	Multicolor Imaging	313
10.2	Ratiometric Measurement	315
10.3	Fluorescence Lifetime Imaging Microscopy	316
10.4	Confocal Fluorescence Microscopy	316
10.5	Two-Photon Excitation Fluorescence Imaging and Harmonic Generation	317
10.6	Selective Plane Illumination Microscopy	318
10.7	Total Internal Reflection Fluorescence Microscopy	319
10.8	Super-Resolution Imaging	320
10.9	Single-Molecule Imaging	322
10.10	Photoactivation of Caged Molecule	323
10.11	Fluorescence Recovery After Photobleaching	324
10.12	Flow Cytometry Technique	325
	References	327
11	Perspectives for Future Probe Development	329
11.1	Design of Selective Probes	329
11.2	Discovery of Selective Probes by Screening	331
11.3	Future Probe Development	336
	References	337
	Appendix	339
	Index	341