## CONTENTS

**Preface** xv

**Acknowledgments** xvii

### 1. Introduction 1

1.1 Biophotonics—A New Frontier 1
1.2 An Invitation to Multidisciplinary Education, Training, and Research 2
1.3 Opportunities for Both Basic Research and Biotechnology Development 4
1.4 Scope of this Book 5

### 2. Fundamentals of Light and Matter 11

2.1 Nature of Light 12
  2.1.1 Dual Character of Light 12
  2.1.2 Propagation of Light as Waves 14
  2.1.3 Coherence of Light 17
  2.1.4 Light as Photon Particles 19
  2.1.5 Optical Activity and Birefringence 20
  2.1.6 Different Light Sources 21
2.2 Quantized States of Matter 21
  2.2.1 Introductory Concepts 21
  2.2.2 Quantized States of Atoms 24
  2.2.3 Quantized States of Molecules: Partitioning of Molecular Energies 27
  2.2.4 Electronic States of a Molecule 29
  2.2.5 Bonding in Organic Molecules 35
  2.2.6 Conjugated Organic Molecules 37
  2.2.7 Vibrational States of a Molecule 39
2.3 Intermolecular Effects 41
2.4 Three-Dimensional Structures and Stereoisomers 43
Highlights of the Chapter 46
References 48
3. Basics of Biology  
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Introductory Concepts</td>
<td>51</td>
</tr>
<tr>
<td>3.2 Cellular Structure</td>
<td>52</td>
</tr>
<tr>
<td>3.3 Various Types of Cells</td>
<td>58</td>
</tr>
<tr>
<td>3.4 Chemical Building Blocks</td>
<td>60</td>
</tr>
<tr>
<td>3.5 Interactions Determining Three-Dimensional Structures of Biopolymers</td>
<td>68</td>
</tr>
<tr>
<td>3.6 Other Important Cellular Components</td>
<td>72</td>
</tr>
<tr>
<td>3.7 Cellular Processes</td>
<td>73</td>
</tr>
<tr>
<td>3.8 Protein Classification and Function</td>
<td>82</td>
</tr>
<tr>
<td>3.9 Organization of Cells into Tissues</td>
<td>85</td>
</tr>
<tr>
<td>3.10 Types of Tissues and Their Functions</td>
<td>87</td>
</tr>
<tr>
<td>3.11 Tumors and Cancers</td>
<td>88</td>
</tr>
</tbody>
</table>

Highlights of the Chapter 89
References 91

4. Fundamentals of Light-Matter Interactions  
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Interactions Between Light and a Molecule</td>
<td>93</td>
</tr>
<tr>
<td>4.1.1. Nature of Interactions</td>
<td>93</td>
</tr>
<tr>
<td>4.1.2. Einstein's Model of Absorption and Emission</td>
<td>95</td>
</tr>
<tr>
<td>4.2 Interaction of Light with a Bulk Matter</td>
<td>97</td>
</tr>
<tr>
<td>4.3 Fate of Excited State</td>
<td>99</td>
</tr>
<tr>
<td>4.4 Various Types of Spectroscopy</td>
<td>102</td>
</tr>
<tr>
<td>4.5 Electronic Absorption Spectroscopy</td>
<td>105</td>
</tr>
<tr>
<td>4.6 Electronic Luminescence Spectroscopy</td>
<td>109</td>
</tr>
<tr>
<td>4.7 Vibrational Spectroscopy</td>
<td>113</td>
</tr>
<tr>
<td>4.8 Spectroscopy Utilizing Optical Activity of Chiral Media</td>
<td>117</td>
</tr>
<tr>
<td>4.9 Fluorescence Correlation Spectroscopy (FCS)</td>
<td>122</td>
</tr>
</tbody>
</table>

Highlights of the Chapter 124
References 127

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Principles of Lasers</td>
<td>130</td>
</tr>
<tr>
<td>5.1.1 Lasers: A New Light Source</td>
<td>130</td>
</tr>
<tr>
<td>5.1.2 Principles of Laser Action</td>
<td>131</td>
</tr>
<tr>
<td>5.1.3 Classification of Lasers</td>
<td>135</td>
</tr>
<tr>
<td>5.1.4 Some Important Lasers for Biophotonics</td>
<td>139</td>
</tr>
<tr>
<td>5.2 Current Laser Technologies</td>
<td>139</td>
</tr>
<tr>
<td>5.3 Quantitative Description of Light: Radiometry</td>
<td>142</td>
</tr>
<tr>
<td>5.4 Nonlinear Optical Processes with Intense Laser Beam</td>
<td>143</td>
</tr>
<tr>
<td>5.4.1 Mechanism of Nonlinear Optical Processes</td>
<td>143</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.4.2 Frequency Conversion by a Second-Order Nonlinear Optical Process</td>
<td>145</td>
</tr>
<tr>
<td>5.4.3 Symmetry Requirement for a Second-Order Process</td>
<td>146</td>
</tr>
<tr>
<td>5.4.4 Frequency Conversion by a Third-Order Nonlinear Optical Process</td>
<td>148</td>
</tr>
<tr>
<td>5.4.5 Multiphoton Absorption</td>
<td>149</td>
</tr>
<tr>
<td>5.5 Time-Resolved Studies</td>
<td>152</td>
</tr>
<tr>
<td>5.6 Laser Safety</td>
<td>154</td>
</tr>
<tr>
<td>Highlights of the Chapter</td>
<td>156</td>
</tr>
<tr>
<td>References</td>
<td>157</td>
</tr>
<tr>
<td>6. Photobiology</td>
<td>159</td>
</tr>
<tr>
<td>6.1 Photobiology—At the Core of Biophotonics</td>
<td>160</td>
</tr>
<tr>
<td>6.2 Interaction of Light with Cells</td>
<td>160</td>
</tr>
<tr>
<td>6.2.1 Light Absorption in Cells</td>
<td>161</td>
</tr>
<tr>
<td>6.2.2 Light-Induced Cellular Processes</td>
<td>163</td>
</tr>
<tr>
<td>6.2.3 Photochemistry Induced by Exogenous Photosensitizers</td>
<td>167</td>
</tr>
<tr>
<td>6.3 Interaction of Light with Tissues</td>
<td>168</td>
</tr>
<tr>
<td>6.4 Photoprocesses in Biopolymers</td>
<td>175</td>
</tr>
<tr>
<td>6.4.1 The Human Eye and Vision</td>
<td>176</td>
</tr>
<tr>
<td>6.4.2 Photosynthesis</td>
<td>181</td>
</tr>
<tr>
<td>6.5 In Vivo Photoexcitation</td>
<td>186</td>
</tr>
<tr>
<td>6.5.1 Free-Space Propagation</td>
<td>186</td>
</tr>
<tr>
<td>6.5.2 Optical Fiber Delivery System</td>
<td>187</td>
</tr>
<tr>
<td>6.5.3 Articulated Arm Delivery</td>
<td>189</td>
</tr>
<tr>
<td>6.5.4 Hollow Tube Waveguides</td>
<td>190</td>
</tr>
<tr>
<td>6.6 In Vivo Spectroscopy</td>
<td>190</td>
</tr>
<tr>
<td>6.7 Optical Biopsy</td>
<td>191</td>
</tr>
<tr>
<td>6.8 Single-Molecule Detection</td>
<td>195</td>
</tr>
<tr>
<td>Highlights of the Chapter</td>
<td>197</td>
</tr>
<tr>
<td>References</td>
<td>199</td>
</tr>
<tr>
<td>7. Bioimaging: Principles and Techniques</td>
<td>203</td>
</tr>
<tr>
<td>7.1 Bioimaging: An Important Biomedical Tool</td>
<td>205</td>
</tr>
<tr>
<td>7.2 An Overview of Optical Imaging</td>
<td>206</td>
</tr>
<tr>
<td>7.3 Transmission Microscopy</td>
<td>209</td>
</tr>
<tr>
<td>7.3.1 Simple Microscope</td>
<td>209</td>
</tr>
<tr>
<td>7.3.2 Compound Microscope</td>
<td>210</td>
</tr>
<tr>
<td>7.3.3 Kohler Illumination</td>
<td>212</td>
</tr>
<tr>
<td>7.3.4 Numerical Aperture and Resolution</td>
<td>214</td>
</tr>
</tbody>
</table>
7.3.5 Optical Aberrations and Different Types of Objectives 215
7.3.6 Phase Contrast Microscopy 216
7.3.7 Dark-Field Microscopy 216
7.3.8 Differential Interference Contrast Microscopy (DIC) 217
7.4 Fluorescence Microscopy 219
7.5 Scanning Microscopy 220
7.6 Inverted and Upright Microscopes 221
7.7 Confocal Microscopy 221
7.8 Multiphoton Microscopy 223
7.9 Optical Coherence Tomography 225
7.10 Total Internal Reflection Fluorescence Microscopy 228
7.11 Near-Field Optical Microscopy 232
7.12 Spectral and Time-Resolved Imaging 234
7.12.1 Spectral Imaging 235
7.12.2 Bandpass Filters 235
7.12.3 Excitation Wavelength Selection 236
7.12.4 Acousto-Optic Tunable Filters 236
7.12.5 Localized Spectroscopy 237
7.13 Fluorescence Resonance Energy Transfer (FRET) Imaging 237
7.14 Fluorescence Lifetime Imaging Microscopy (FLIM) 238
7.15 Nonlinear Optical Imaging 240
7.15.1 Second-Harmonic Microscopy 241
7.15.2 Third-Harmonic Microscopy 243
7.15.3 Coherent Anti-Stokes Raman Scattering (CARS) Microscopy 243
7.16 Future Directions of Optical Bioimaging 245
7.16.1 Multifunctional Imaging 245
7.16.2 4Pi Imaging 245
7.16.3 Combination Microscopes 246
7.16.4 Miniaturized Microscopes 246
7.17 Some Commercial Sources of Imaging Instruments 246
Highlights of the Chapter 246
References 249

8. Bioimaging: Applications 255
8.1 Fluorophores as Bioimaging Probes 256
8.1.1 Endogenous Fluorophores 256
8.1.2 Exogenous Fluorophores 257
8.1.3 Organometallic Complex Fluorophores 264
8.1.4 Near-IR and IR Fluorophore 265
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1.5</td>
<td>Two-Photon Fluorophores</td>
<td>265</td>
</tr>
<tr>
<td>8.1.6</td>
<td>Inorganic Nanoparticles</td>
<td>269</td>
</tr>
<tr>
<td>8.2</td>
<td>Green Fluorescent Protein</td>
<td>269</td>
</tr>
<tr>
<td>8.3</td>
<td>Imaging of Organelles</td>
<td>271</td>
</tr>
<tr>
<td>8.4</td>
<td>Imaging of Microbes</td>
<td>273</td>
</tr>
<tr>
<td>8.4.1</td>
<td>Confocal Microscopy</td>
<td>273</td>
</tr>
<tr>
<td>8.4.2</td>
<td>Near-Field Imaging</td>
<td>274</td>
</tr>
<tr>
<td>8.5</td>
<td>Cellular Imaging</td>
<td>276</td>
</tr>
<tr>
<td>8.5.1</td>
<td>Probing Cellular Ionic Environment</td>
<td>276</td>
</tr>
<tr>
<td>8.5.2</td>
<td>Intracellular pH Measurements</td>
<td>277</td>
</tr>
<tr>
<td>8.5.3</td>
<td>Optical Tracking of Drug-Cell Interactions</td>
<td>279</td>
</tr>
<tr>
<td>8.5.4</td>
<td>Imaging of Nucleic Acids</td>
<td>281</td>
</tr>
<tr>
<td>8.5.5</td>
<td>Cellular Interactions Probed by FRET/FLIM Imaging</td>
<td>287</td>
</tr>
<tr>
<td>8.6</td>
<td>Tissue Imaging</td>
<td>289</td>
</tr>
<tr>
<td>8.7</td>
<td>In Vivo Imaging</td>
<td>294</td>
</tr>
<tr>
<td>8.8</td>
<td>Future Directions</td>
<td>301</td>
</tr>
<tr>
<td>8.9</td>
<td>Commercially Available Optical Imaging Accessories</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td>Highlights of the Chapter</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>306</td>
</tr>
<tr>
<td>9.1</td>
<td>Biosensors: An Introduction</td>
<td>312</td>
</tr>
<tr>
<td>9.2</td>
<td>Principles of Optical Biosensing</td>
<td>314</td>
</tr>
<tr>
<td>9.2.1</td>
<td>Biorecognition</td>
<td>314</td>
</tr>
<tr>
<td>9.2.2</td>
<td>Optical Transduction</td>
<td>316</td>
</tr>
<tr>
<td>9.2.3</td>
<td>Fluorescence Sensing</td>
<td>317</td>
</tr>
<tr>
<td>9.2.4</td>
<td>Fluorescence Energy Transfer Sensors</td>
<td>317</td>
</tr>
<tr>
<td>9.2.5</td>
<td>Molecular Beacons</td>
<td>320</td>
</tr>
<tr>
<td>9.2.6</td>
<td>Optical Geometries of Biosensing</td>
<td>321</td>
</tr>
<tr>
<td>9.3</td>
<td>Support for and Immobilization of Biorecognition Elements</td>
<td>323</td>
</tr>
<tr>
<td>9.3.1</td>
<td>Immobilization</td>
<td>323</td>
</tr>
<tr>
<td>9.4</td>
<td>Fiber-Optic Biosensors</td>
<td>327</td>
</tr>
<tr>
<td>9.5</td>
<td>Planar Waveguide Biosensors</td>
<td>331</td>
</tr>
<tr>
<td>9.6</td>
<td>Evanescent Wave Biosensors</td>
<td>334</td>
</tr>
<tr>
<td>9.7</td>
<td>Interferometric Biosensors</td>
<td>338</td>
</tr>
<tr>
<td>9.8</td>
<td>Surface Plasmon Resonance Biosensors</td>
<td>339</td>
</tr>
<tr>
<td>9.9</td>
<td>Some Recent Novel Sensing Methods</td>
<td>343</td>
</tr>
<tr>
<td>9.10</td>
<td>Future Directions</td>
<td>347</td>
</tr>
<tr>
<td>9.11</td>
<td>Commercially Available Biosensors</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>Highlights of the Chapter</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>352</td>
</tr>
</tbody>
</table>
## 10. Microarray Technology for Genomics and Proteomics

10.1 Microarrays, Tools for Rapid Multiplex Analysis
10.2 DNA Microarray Technology
   10.2.1 Spotted Arrays
   10.2.2 Oligonucleotide Arrays
   10.2.3 Other Microarray Technologies
10.3 Protein Microarray Technology
10.4 Cell Microarray Technology
10.5 Tissue Microarray Technology
10.6 Some Examples of Application of Microarrays
10.7 Future Directions
10.8 Companies Producing Microarrays
Highlights of the Chapter
References

## 11. Flow Cytometry

11.1 A Clinical, Biodetection, and Research Tool
11.2 Basics of Flow Cytometry
   11.2.1 Basic Steps
   11.2.2 The Components of a Flow Cytometer
   11.2.3 Optical Response
11.3 Fluorochromes for Flow Cytometry
11.4 Data Manipulation and Presentation
11.5 Selected Examples of Applications
   11.5.1 Immunophenotyping
   11.5.2 DNA Analysis
11.6 Future Directions
11.7 Commercial Flow Cytometry
Highlights of the Chapter
References

## 12. Light-Activated Therapy: Photodynamic Therapy

12.1 Photodynamic Therapy: Basic Principles
12.2 Photosensitizers for Photodynamic Therapy
   12.2.1 Porphyrin Derivatives
   12.2.2 Chlorins and Bacteriochlorins
   12.2.3 Benzoporphyrin Derivatives
   12.2.4 5-Aminolaevulinic Acid (ALA)
   12.2.5 Texaphyrins
   12.2.6 Phthalocyanines and Naphthalocyanines
   12.2.7 Cationic Photosensitizers
   12.2.8 Dendritic Photosensitizers

---

**Page Numbers:**
- 10. Microarray Technology for Genomics and Proteomics: 357
- 11. Flow Cytometry: 390
- 12. Light-Activated Therapy: Photodynamic Therapy: 433
12.3 Applications of Photodynamic Therapy 447
12.4 Mechanism of Photodynamic Action 450
12.5 Light Irradiation for Photodynamic Therapy 453
  12.5.1 Light Source 453
  12.5.2 Laser Dosimetry 454
  12.5.3 Light Delivery 455
12.6 Two-Photon Photodynamic Therapy 455
12.7 Current Research and Future Directions 457
Highlights of the Chapter 460
References 461

13. Tissue Engineering with Light 464
  13.1 Tissue Engineering and Light Activation 465
  13.2 Laser Tissue Contouring and Restructuring 467
  13.3 Laser Tissue Welding 472
  13.4 Laser Tissue Regeneration 475
  13.5 Femtolaser Surgery 476
  13.6 Future Directions 478
Highlights of the Chapter 479
References 480

14. Laser Tweezers and Laser Scissors 482
  14.1 New Biological Tools for Micromanipulation by Light 483
  14.2 Principle of Laser Tweezer Action 487
  14.3 Design of a Laser Tweezer 490
  14.4 Optical Trapping Using Non-Gaussian Beams 495
  14.5 Dynamic Holographic Optical Tweezers 496
  14.6 Laser Scissors 499
    14.6.1 Laser Pressure Catapulting (LPC) 500
    14.6.2 Laser Capture Microdissection (LCM) 502
  14.7 Selected Examples of Applications 502
    14.7.1 Manipulation of Single DNA Molecules 502
    14.7.2 Molecular Motors 506
    14.7.3 Protein–Protein Interactions 507
    14.7.4 Laser Microbeams for Genomics and Proteomics 509
    14.7.5 Laser Manipulation in Plant Biology 510
    14.7.6 Laser Micromanipulation for Reproduction Medicine 511
  14.8 Future Directions 512
    14.8.1 Technology of Laser Manipulation 513
    14.8.2 Single Molecule Biofunctions 513
  14.9 Commercially Available Laser Microtools 514