Contents

Introduction, XI

Chapter 5. Phase Contrast Microscopy, 1
  5.1. General principles, 1
  5.2. Typical phase contrast systems, 11
  5.3. Imaging properties, 15
    5.3.1. Halo, shading-off, and image fidelity, 15
    5.3.2. Resolution, 20
    5.3.3. Sensitivity, 22
    5.3.4. Influence of stray light, 25
  5.4. Nomenclature, 28
  5.5. Highly sensitive phase contrast devices, 30
    5.5.1. Optical properties of soot layers, 30
    5.5.2. Highly sensitive negative phase contrast device (KFA), 32
    5.5.3. Highly sensitive positive phase contrast device (KFS), 36
  5.6. Alternating phase contrast systems, 38
    5.6.1. Beyer's phase contrast device, 39
    5.6.2. Device with both positive and negative phase rings (KFZ), 42
  5.7. Phase contrast systems with continuously variable image contrast, 45
    5.7.1. The Polanret system, 46
    5.7.2. Nomarski's variable achromatic phase contrast system, 50
    5.7.3. Nikon interference-phase contrast device, 52
    5.7.4. Variable phase contrast device with a single polarizing phase ring, 54
  5.8. Phase contrast microscopy by using interference systems, 56
    5.8.1. Interphako, 57
    5.8.2. Variable phase contrast microscopy based on the Michelson interferometer, 61
  5.9. Stereoscopic phase contrast microscope, 66
    5.9.1. Underlying principles and mode of operation, 67
5.9.2. Lateral resolution, 70
5.9.3. Stereoscopic axial resolution, 73
5.10. Phase contrast for incident light, 74
  5.10.1. System with phase plate immediately behind the beam-splitter, 74
  5.10.2. Incident-light phase contrast microscope with internal projection system, 76
  5.10.3. System with phase plate in objectives, 77
5.11. How and where to use the phase contrast microscope, 78
  5.11.1. Adjustment of phase contrast microscopes, 79
  5.11.2. General remarks on specimens for phase contrast microscopy, 81
  5.11.3. Phase contrast microrefractometry, 85
  5.11.4. Fields of application of phase contrast microscopy, 88

Chapter 6. Amplitude Contrast, Dark-Field, Optical Staining, Modulation Contrast, and Other Related Techniques, 91

6.1. Amplitude contrast technique, 91
  6.1.1. Theoretical considerations, 91
  6.1.2. Practical implementation and properties, 96
6.2. Oblique illumination, dark-field microscopy, and related techniques, 100
  6.2.1. Oblique illumination, 101
  6.2.2. Oblique dark-field illumination, 102
  6.2.3. Dark-field microscopy with detuned interference filters, 108
  6.2.4. Central dark-field microscopy, 110
  6.2.5. Microstrioscopy, 111
  6.2.6. Ultramicroscopy, 112
6.3. Optical staining, 113
  6.3.1. Rheinberg illumination, 113
  6.3.2. Double illumination, 115
  6.3.3. Dispersion staining, 117
  6.3.4. Other optically stained images, 132
6.4. Modulation contrast microscopy, 134
  6.4.1. Theoretical principles, 134
  6.4.2. Practical implementation, 138
  6.4.3. Properties and applications, 141
6.5. Monoobjective stereoscopic microscopy, 142
Chapter 7. Differential Interference Contrast, 146

7.1. Background and principles of DIC microscopy, 146
  7.1.1. On the typical use of the Mach–Zehnder interferometer, 148
  7.1.2. Mach–Zehnder interference system used as a wavefront shear interferometer, 151
  7.1.3. DIC microscopy as a method for displaying optical gradients, 153
  7.1.4. DIC microscopy based on the double refracting interference system, 156

7.2. Nomarski DIC microscopy, 165
  7.2.1. Nomarski DIC system for transmitted light, 165
  7.2.2. Amplitude DIC microscopy, 174
  7.2.3. The image of a light point in the Nomarski DIC microscopy, 175
  7.2.4. Nomarski DIC system for reflected light, 177

7.3. Video-enhanced DIC microscopy, 181

7.4. Differential interference contrast microscopy with continuously variable wavefront shear, 183
  7.4.1. Transmitted-light VADIC system with pupilar compensation, 183
  7.4.2. Transillumination VADIC system with condenser slit diaphragm, 189
  7.4.3. VADIC system for reflected light, 192

7.5. A DIC microscope for testing cross-sections of optical fibres, 196

Chapter 8. Reflection Contrast Microscopy, 198

8.1. Optical principles of RC microscopy, 198
  8.1.1. Light reflection and interference in RC image formation, 198
  8.1.2. Discrimination between pure reflection and interference, 202
  8.1.3. Evaluation of the RC image by measurement of reflectivity, 203

8.2. Instrumentation of RC microscopy, 204
  8.2.1. Leitz–Ploem RC microscopy, 204
  8.2.2. Other systems for RC microscopy, 207

8.3. Applications of RC microscopy, 208

Chapter 9. Fluorescence Microscopy, 211

9.1. General principles and types of fluorescence microscopy, 212

9.2. Immunofluorescence microscopy, 216

9.3. Instrumentation of fluorescence microscopy, 222
  9.3.1. Light sources for fluorescence excitation, 222
9.3.2. Exciter and barrier filters, 228
9.3.3. Dichroic mirrors, 238
9.3.4. Objectives and condensers for fluorescence microscopy, 240
9.3.5. Ploemopak epi-illuminator, 242
9.3.6. Other devices and facilities, 246
9.4. Fluorescence combined with other microscopical techniques, 248
9.5. Laser fluorescence microscopy, 252
9.6. Total internal reflection fluorescence microscopy, 256
9.7. Surgical fluorescence microscopy, 257
9.8. Fields of application of fluorescence microscopy, 262
9.8.1. Application in biology and medicine, 262
9.8.2. Application in materials sciences, 263

Chapter 10. Ultraviolet and Infrared Microscopy, 264
10.1. Observation of UV and IR images, 264
10.2. UV microscopy and its applications, 268
10.3. IR microscopy and its applications, 272
10.4. Thermal microscopy, 278

Chapter 11. Holographic Microscopy, 282
11.1. A bird's eye view of the history of holographic microscopy, 283
11.2. Principles of holographic microscopy, 285
11.2.1. Recording of typical holograms of transparent objects, 286
11.2.2. Holographic image reconstruction, 289
11.2.3. Holography of light-reflecting objects, 292
11.2.4. Classification of holograms, 295
11.3. Holographic microscopy without objective lenses, 296
11.3.1. In-line microholographic system, 296
11.3.2. Off-axis microholographic system, 300
11.3.3. Other lensless holographic systems for microscopy, 301
11.4. Holographic microscopy combined with objective lenses, 302
11.4.1. Microholographic systems with premagnification and direct wavefront reconstruction, 302
11.4.2. Holographic microscopy with premagnification and reversed wavefront reconstruction, 308
11.4.3. Other lens-assisted holographic systems for microscopy, 311
11.5. Problems of coherent noise elimination from holographic microscopy, 312
CONTENTS

11.5.1. Coherent noise and speckle patterns, 312
11.5.2. Techniques for coherent noise reduction, 314
11.5.3. Holographic microscopy with unidirectional suppression of coherent noise, 317
11.6. Holographic interference microscopy, 325
   11.6.1. Advantages of holographic interference microscopy, 326
   11.6.2. Real-time holographic interference methods, 329
   11.6.3. Double-exposure holographic interference microscopy, 332
   11.6.4. It is possible to accept holographic interference microscopy without coherent noise reduction? 335
11.7. Holographic phase contrast microscopy, 336
11.8. Applications of holographic microscopy, 337
   11.8.1. Examples of biological applications, 338
   11.8.2. Applications in materials sciences, 339
   11.8.3. Holographic imaging and analysis of three-dimensional distribution of particles, 340

Chapter 12. Laser Projection, Scanning, and Other New Microscope Systems, 353
12.1. Laser projection microscope with brightness amplifier, 353
12.2. Scanning optical microscopy, 355
   12.2.1. Confocal scanning microscopy, 356
   12.2.2. Laser-scan microscope developed by C. Zeiss Oberkochen, 363
   12.2.3. Tandem scanning reflected-light microscope, 365
   12.2.4. Other scanning light microscopes, 368
12.3. Nonlinear microscopy with second harmonic generation, 371
12.4. Raman microscopy, 373
12.5. Optoacoustic and photothermal microscopy, 375

Epilogue to Volume 2, 380
References, 384
Index of Names, 421
Subject Index, 424
Plates, 447