

Contents

Abbreviations	xi
---------------	----

PART 1: BASIC PRINCIPLES AND METHODS

1. What Sort of Microscopy Should I Use?	1
-------------------------------------------------	----------

2. Microscope Optics	7
Properties of lenses	7
Resolution, magnification and numerical aperture	9
References	12

3. Components of a Microscope	13
Objectives	13
Condenser	17
Eyepieces	19
Focus controls	20
Bertrand lens, phase telescope and magnification changer	20
Beam splitter	20
Field aperture	20
Light source	21
Photo tube	21
Camera	22

4. Types of Imaging with the Light Microscope	23
Bright field (Köhler) illumination	23
Phase contrast microscopy	25
Fluorescence microscopy	27
Dark field microscopy	30
Polarized light microscopy	32
Reflected light (epi-polarization microscopy and reflection contrast microscopy)	36
Nomarski microscopy	37

Other techniques	40
References	40
<hr/>	
5. Three-dimensional (3D) Microscopy	43
<hr/>	
Optical sectioning	43
Confocal microscopy	44
Types of confocal microscope	46
Optical sectioning with a conventional microscope	47
Three-dimensional reconstruction	48
References	49
PART 2: TECHNIQUES AND APPLICATIONS	
<hr/>	
6. How to Get the Best Image	51
<hr/>	
Preparation of unfixed specimens	51
Preparation of fixed specimens	52
Fixation	52
Tissue handling	53
Dehydration and embedding	53
Sectioning	54
Sticking tissue to slides	55
Staining and labeling	55
Mounting media	56
Cover slips	57
Immersion media	58
Use of microscope controls	58
Use of objectives	58
Use of the condenser	59
Use of eyepieces	59
Use of the specimen stage	61
Use of the focus controls	62
Bulb centering	63
Use of the Bertrand lens or phase telescope	63
Maintenance	64
References	65
<hr/>	
7. Setting Up the Microscope	67
<hr/>	
Bright field (Köhler) illumination	67
Equipment required	67
Setup procedure	67
Low power observation	73
Oiling the condenser	73
Effect of the condenser aperture	73

Colored filters	76
Troubleshooting	76
Phase contrast microscopy	80
Equipment required	80
Setup procedure	82
Advantages and disadvantages of phase contrast	84
Fluorescence microscopy	88
Equipment required	88
Choice of objective	88
Anti-fade mountants	89
Practical hints	89
Dark field illumination	91
Equipment required	91
Setup procedure	91
Practical hints	92
Nomarski (DIC) microscopy	92
Equipment required	92
Setup procedure	92
Interpreting the image	93
Polarized light microscopy	94
Equipment required	94
Setup procedure	94
Reflected light illumination	94
Equipment required	94
Setup procedure	95
Confocal and three-dimensional microscopy	96
Equipment required	96
Practical hints	97
Optical sectioning	97
References	98

8. Case Studies **99**

Mitotic chromosomes: labeling the ends	99
Floral apex: detection of mRNA	99
Human lung epithelial cells:	
cytoskeletal networks	102
Pond alga: a model	
light microscope specimen	105
References	106

9. Measuring Down the Microscope **107**

Counting	107
Lengths and areas	107
References	111

10. Photomicrography	113
Cameras and exposure systems	113
Taking good photomicrographs	114
Choice of film	115
Magnification	118
Printing	118
Stereo pairs for three-dimensional images	119
References	120
11. Video Microscopy	121
Types of video camera	121
Fitting a video camera to a microscope	122
Image capture, display, processing and analysis	123
Magnification, pixel size and resolution	123
Recording dynamic processes	124
References	125
Appendices	127
Appendix A. Further reading	127
Appendix B. Glossary	128
Appendix C. Fluorescence filter sets	134
Appendix D. Suppliers	136
Index	139