

Content

1	Introduction	2
1.1	Desire to magnify objects	2
1.2	Size is relative	4
2	Optics	8
2.1	Eye	8
2.1.1	Resolving power of the eye	8
2.2	Lenses and magnifying glasses	9
2.3	From the magnifying glass to the microscope	10
2.4	Optics and resolution	10
2.4.1	Resolution determines what is visible	11
2.4.2	Everything regulated: the path of light rays – from the light source to the eye	13
2.5	Lens errors	14
2.5.1	Spherical aberration	14
2.5.2	Chromatic aberration	15
2.5.3	Astigmatism	16
2.5.4	Coma	17
2.5.5	Distortions	18
2.5.6	Damage/contaminations	18
2.6	Comparison of optical methods	19
2.6.1	Bright-field microscopy (BF)	19
2.6.2	Dark-field microscopy (DF)	20
2.6.3	Phase contrast (Ph)	20
2.6.4	Differential interference contrast (DIC)	21
2.6.5	Fluorescence microscopy (FL)	22
2.7	Examples of use	23
3	Electron microscopy	24
3.1	TEM	24
3.1.1	Comparison of LM and TEM	26
3.2	Scanning electron microscope (microscopy) (SEM)	28
3.2.1	History of scanning	28

3.2.1.1	SEM – basic idea – history	28
3.2.1.2	Why is there a SEM at all?	29
3.2.2	Comparison of TEM and SEM	30
3.2.2.1	Specimens for TEM	31
3.2.2.2	Specimens for SEM	32
3.2.3	Chronology	34
4	Electrons	36
4.1	Interaction of electrons with matter	38
4.1.1	Mean free path length of electrons	39
4.2	Secondary and backscattered electrons	40
4.3	Energy distribution of SEs	42
4.4	Contrast formation	45
4.4.1	Topography contrast	46
4.4.1.1	SE signal	46
4.4.1.2	BSE signal	49
4.4.2	Material contrast	50
4.4.2.1	SE signal	50
4.4.2.2	BSE signal	51
4.4.3	Crystal orientation contrast	52
4.4.3.1	SE signal	52
4.4.3.2	BSE signal	52
4.5	Resolution	54
4.6	Charging effects	56
4.6.1	Origin of charging	56
4.6.2	Imaging the specimen chamber	60
5	Components of the EM	62
5.1	Vacuum	62
5.1.1	Mean free path of gas molecules	62
5.2	Vacuum generation	64
5.2.1	Water jet pump	64
5.2.2	Diaphragm pump	64
5.2.3	Rotary pump	65
5.2.4	Oil diffusion pump	66

5.2.5	Turbomolecular pump	67
5.2.6	Ion getter pump	69
5.3	Vacuum measurement	70
5.3.1	Pirani vacuum gauge (heat conduction vacuum gauge)	70
5.3.2	Penning vacuum gauge (cold cathode vacuum gauge)	70
5.4	Cathode as an electron source	72
5.4.1	Hairpin cathode	74
5.4.2	Tip cathode	75
5.4.3	LaB_6 cathode	75
5.4.4	Schottky emitter	76
5.4.5	Cold field emission	77
5.4.6	Comparison of cathodes	78
5.4.7	Beam generation	80
5.4.7.1	Anode and Wehnelt Cylinder	81
5.5	Electromagnetic lenses	85
5.5.1	Basic principle	87
5.5.2	Pole piece	87
5.5.3	Helical path of the electrons and rotation of the image	88
5.5.4	Hysteresis curve	89
5.5.5	Lens errors	90
5.5.5.1	Spherical aberration	90
5.5.5.2	Chromatic aberration	90
5.5.5.3	Coma	91
5.5.5.4	Astigmatism	92
5.5.5.5	Cushion distortion	93
5.5.5.6	Barrel distortion	93
5.6	Electrostatic lenses	94
5.6.1	Comparison to electromagnetic lenses	95
5.7	Scan generator	95
5.8	SE detectors	98
5.8.1	Everhart–Thornley detector	98
5.8.2	In-lens SE detector	99
5.9	BSE detectors	100
5.9.1	Autrata BSE detector	100
5.9.2	In-lens EsB detector	101
5.9.3	QBSD	101

5.10	X-rays	102
5.10.1	EDX detector	102
5.11	Sample stage	104
6	Preparation of biological samples	106
6.1	Air drying	106
6.2	Chemical fixation	108
6.2.1	Formaldehyde	108
6.2.2	Glutaraldehyde	110
6.2.3	Fixation buffers	111
6.2.4	Osmium tetroxide	112
6.2.5	Specimen preparation	114
6.2.6	Washing of samples	115
6.2.7	Dehydration of samples	116
6.2.8	Critical point drying (CPD) of samples	117
6.2.8.1	Notes on CPD	119
6.3	Drop-cryo preparation	122
6.4	Freeze fractures	124
6.5	Embedding of samples in epoxy resin	126
6.5.1	FIB pins	126
6.6	Examples of preparation	127
7.	Specimen mounting for SEM	130
7.1	Conductive silver	131
7.2	Conductive carbon cement	134
7.3	Leit-C-Plast	135
7.4	Conductive tabs (Leit-Tabs)	136
7.5	Tempfix™	138
7.6	Double-sided adhesive tape	140
7.7	Clamps	140
7.8	Adapter for CLEM	141
7.9	SEM as TEM	141
7.10	Conductive coating	142
7.10.1	Sputtering	142
7.10.1.1	Design of a sputter coater	142
7.10.1.2	Decoration effects	148
7.10.2	Carbon coating	150
7.10.2.1	Design of a carbon evaporator	150
7.11	Storage of specimens	152

8	CLEM	156
8.1	Slides with coordinates	159
8.2	Critical point drying	160
8.3	Flat embedding	161
8.4	Tissue sections	164
8.5	Cryo samples	164
8.6	Semithin and thick sections	168
8.6.1	Mounting of larger slide pieces	170
8.7	Stamp	170
8.8	Workflow for CLEM	173
9.	Microscopy	176
9.1	Choice of the acceleration voltage	176
9.2	Selecting the working distance	182
9.3	Choice of aperture	186
9.4	Choice of the tilt angle	188
9.5	Scanning	190
9.5.1	Selection of the scan area	190
9.5.2	Selecting the scan mode	192
9.5.3	Pixel averaging	193
9.5.4	Line averaging	194
9.5.5	Frame averaging (continuous averaging)	195
9.5.6	Frame integration	196
9.6	Characteristics of the detectors	198
9.6.1	Chamber SE detector	198
9.6.2	In-lens SE detector	200
9.6.2.1	In-lens SE imaging in the FIB mode	202
9.6.3	Autrata BSE detector	204
9.6.4	QBSD	206
9.6.4.1	Distribution of BSE according to energy, take-off angle, and angle of impact	208
9.6.5	In-lens EsB detector	208
9.6.6	Imaging in the FIB mode	216
9.7	Image optimization	220
9.7.1	Brightness and contrast	220
9.7.2	Direction of illumination	224
9.7.3	Scan rotation or specimen rotation?	226
9.7.4	Image esthetics	227
9.7.5	Scales	228

10	Resolution	230
10.1	Beam size – spot size	231
10.2	Pixel size – image pixel size	231
10.3	Resolution parameters	234
10.4	Signal-to-noise ratio	236
10.5	Limiting the resolution	238
10.5.1	Empty magnification	240
10.6	Impairment of image quality	241
10.6.1	Astigmatism	241
10.6.2	Distortions	243
10.6.3	Moiré effect	246
11	Analytics	248
11.1	Material contrast	248
11.1.1	SE signal	248
11.1.2	BSE signal	250
11.1.3	Metal impregnations	252
11.1.3.1	Osmium tetroxide	252
11.1.3.2	rOTO	253
11.1.3.3	Platinum blue	254
11.1.3.4	Diaminobenzidine (DAB)	256
11.1.4	Immunogold labeling	258
11.2	Simultaneous acquisition of several signals	261
11.2.1	Image acquisition	261
11.2.2	Signal mixing	262
12	EDX	264
12.1	X-ray analysis	264
12.2	X-ray spectrum	266
12.3	EDX operation	268
12.3.1	Operating parameters for EDX	269
12.3.2	Acceleration voltage	270
12.3.3	Area analysis/point analysis	271
12.3.4	Distribution analysis: line scan	271
12.3.5	Distribution analysis: mapping	274
12.3.6	EDX mapping in cryo-SEM	277
12.3.7	EDX mapping on ultrathin sections	278

13	FIB/SEM	280
13.1	Design of the ion gun	280
13.2	Gallium	280
13.2.1	Gallium emitter	281
13.2.2	Mode of action	284
13.2.3	Detection of the ions	284
13.2.4	Crystal orientation contrast with the FIB	284
13.3	FIB operation	286
13.3.1	Conductive mounting	286
13.3.2	Coating with carbon	286
13.4	Ablation with the ion beam	288
13.4.1	Selection of the ion current	288
13.4.2	Checking the ion current	288
13.4.3	Checking the beam profiles	289
13.4.4	Beam profile and ablation profile	290
13.5	Milling a trench with a ramp	292
13.5.1	Geometry of the Redeposition	295
13.6	Milling on a vertical wall	296
13.7	Tilt compensation	298
13.8	Dynamic focus	298
13.9	Track WD	299
13.10	Curtaining	300
13.11	Rippling	302
13.12	Gas injection system (GIS)	304
13.12.1	Electron beam deposition with platinum	305
13.12.2	Ion beam deposition with platinum	306
14	Serial block face SEM (SBF)	310
14.1	Preparation	311
14.2	Microscopy	314
14.3	Comparison of SBF and FIB/SEM	316
15	Array tomography	318
15.1	Basic obstacles	319
15.2	Trimming and sectioning	320
15.3	SFB–SEM vs. ATUM	321
15.4	Multi-beam documentation	324

16	Cryo-SEM	326
16.1	Cryo fixation	326
16.2	Cryo-fracture	328
16.3	Conductive coating	333
16.4	Cryomicroscopy	333
16.5	Cryo-3D	339
17	VP-SEM	342
17.1	VP Setup	342
17.2	VP operation	345
18	Stereo SEM	352
18.1	Stereoscopic vision	352
18.2	Moving the specimen with stage shift	354
18.3	Moving the image with beam shift	356
18.4	Specimen tilting	357
18.4.1	Selection of the tilt angle	357
18.5	Specimen rotation	361
18.6	Mounting stereo images	365
18.7	Selection of the image detail	366
18.8	Anaglyph images	367
18.8.1	Red–green	368
18.8.2	Red–cyan (red–blue)	368
18.8.3	Mounting anaglyph images	368
Glossary		374
References		386
Index		388