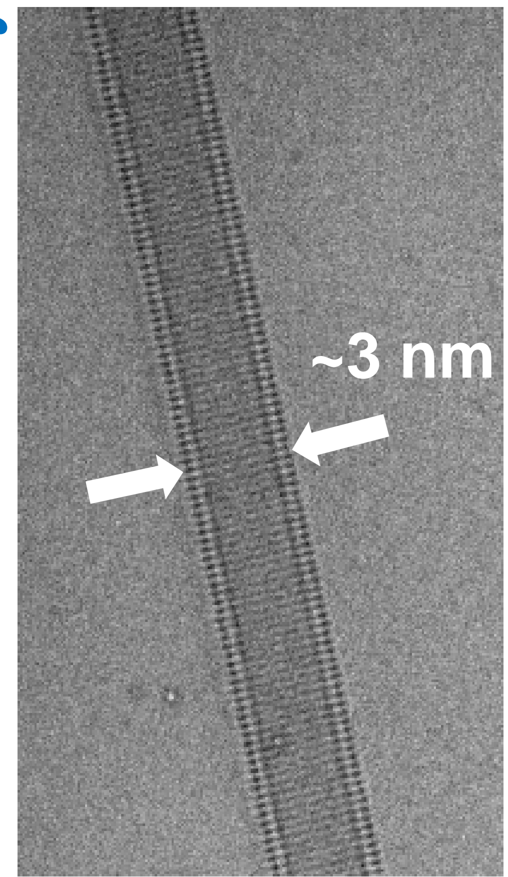
PHYS-E0525 Microscopy of Nanomaterials P (5 cr)



Prof. Janne Ruokolainen (nanotalo 107)

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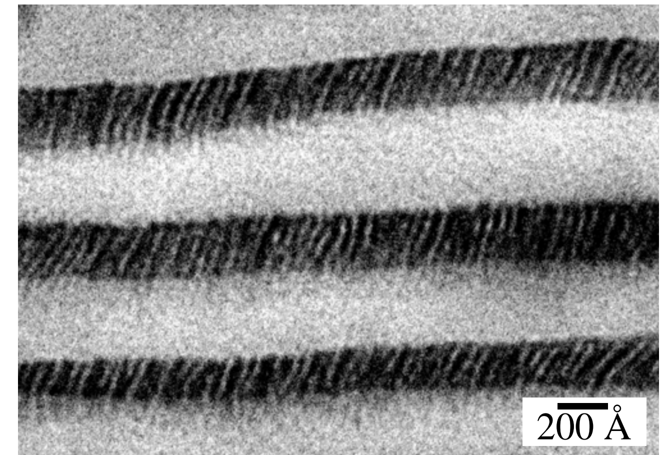
First lecture 26.1. 2021

Tuesday 12.15 – 14

Assistant: Fereshteh Sohrabi fereshteh.sohrabi@aalto.fi

Course overview:

The course gives basic knowledge of the microscopy of materials nanoscale structures - including soft and hard materials. Lectures will concentrate on transmission electron microscopy (TEM, STEM): **high resolution imaging, electron diffraction and analytical microscopy by using elemental analyses (EDX, EELS), cryo-electron microscopy and 3D electron tomography.** Additionally, scanning electron microscopy (SEM and FIB), atomic force microscopy (AFM) and methods to prepare samples are lectured..



Course Registration: WebOodi

Lectures:

Prof. Janne Ruokolainen, Dr. Hua Jiang, Dr. Jani Seitsonen, Dr. Ramzy Abdelaziz, Prof. Peter Liljeroth, Dr. Lide Yao

Tentative Schedule

26. 1.	Introduction & Nanomicroscopy center (JR)
2. 2.	SEM (Ramzy)
9. 2.	TEM Basics 1 (JR)
16. 2.	TEM Basics 2 and Cryo-TEM (JR)
23. 2.	*no lecture (exam period at Aalto)*
2. 3.	Advanced TEM 1 (Hua)
9. 3.	Advanced TEM 2 (Hua)
16. 3.	Advanced TEM 3 (Hua)
23. 3.	AFM (Peter or Ville)
30. 3.	3D-TEM-Tomography (Jani)
6. 4.	FIB and Sample preparation (Lide)

Summary:

Intro, Basic TEM, Cryo TEM ~ 3 lectures
Advanced TEM 3 lectures
(High resolution TEM and STEM, diffraction, spectroscopy EDX, EELS)
AFM
FIB/sample preparation
SEM
Tomography

Additional Literature: (optional)

Book 1: Transmission electron microscopy Basics I (David William and Barry Carter) 2nd edition

Book 2: G.H. Michler "Electron microscopy of polymers" (TEM, SEM, AFM..)

Book 3: A practical Guide to Tramsmission Electron Microscopy (Zhiping Luo)

Additional Literature if you are interested to study more in this topic..

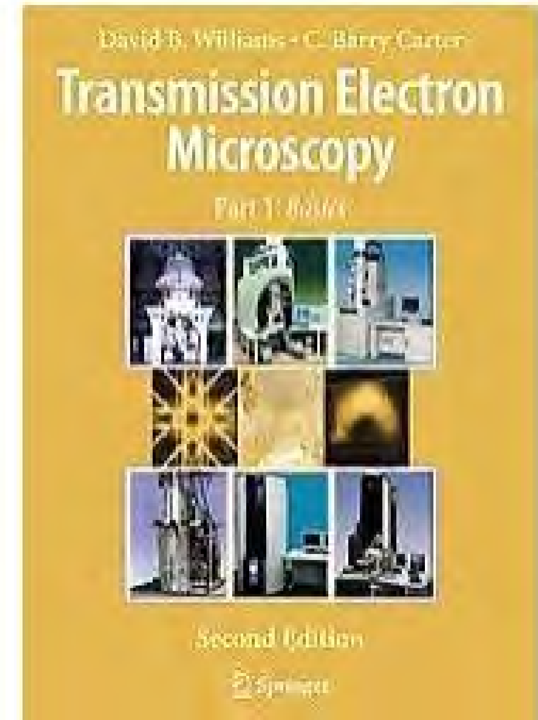
Book 1: Transmission electron microscopy Basics Part I (David William and Barry Carter) 2nd edition

This book has *more theory* and it also has lots of technical information about microscope (vacuum systems, electron sources, sample holders etc..) and it also has 3 other books for Advanced TEM (Part 2: Diffraction , Part 3: high resolution imaging and Part 4: Spectroscopy)

Book 2: G.H. Michler "Electron microscopy of polymers" (TEM, SEM, AFM..)
A bit easier book to study – more practical and has also SEM and AFM parts (First 250 pages are useful – rest are specific polymer applications..)

Book 3: A practical Guide to Transmission Electron Microscopy (Zhiping Luo)
This book is **most practical** especially **those who want to learn how to operate microscopes** Chapter 1-3 and (partly chapter 5?) and chapters 7-9. (chapters 4, 5, and 6 are diffraction and perhaps more advanced level than this course..)

Transmission Electron Microscopy
A Textbook for Materials Science
David B. Williams, C. Barry Carter



PART 1 BASICS 1

1 The Transmission Electron Microscope 3

Chapter Preview 3
1.1 What Materials Should We Study in the TEM? 3
1.2 Why Use Electrons? 4
1.2.A An Extremely Brief History 4
1.2.B Microscopy and the Concept of Resolution 5
1.2.C Interaction of Electrons with Matter 7
1.2.D Depth of Field and Depth of focus 8
1.2.E Diffraction 8
1.3 Limitations of the TEM 9
1.3.A Sampling 9
1.3.B Interpreting Transmission Images 9
1.3.C Electron Beam Damage and Safety 10
1.3.D Specimen Preparation 11
1.4 Different Kinds of TEMs 11
1.5 Some Fundamental Properties of Electrons 11
1.6 Microscopy on the Internet/World Wide Web 15
1.6.A Microscopy and Analysis-Related Web Sites 15
1.6.B Microscopy and Analysis Software 15
Chapter Summary 17

2 Scattering and Diffraction **23**

Chapter Preview 23
2.1 Why Are We Interested in Electron Scattering? 23
2.2 Terminology of Scattering and Diffraction 25
2.3 The Angle of Scattering 26
2.4 The Interaction Cross Section and Its Differential 27
2.4.A Scattering from an Isolated Atom 27
2.4.B Scattering from the Specimen 28
2.4.C Some Numbers 28
2.5 The Mean Free Path 28
2.6 How We Use Scattering in the TEM 29
2.7 Comparison to X-ray Diffraction 30
2.8 Fraunhofer and Fresnel Diffraction 30
2.9 Diffraction of Light from Slits and Holes 31
2.10 Constructive Interference 33
2.11 A Word About Angles 34
2.12 Electron-Diffraction Patterns 34
Chapter Summary 36

3 Elastic Scattering **39**

Chapter Preview 39
3.1 Particles and Waves 39
3.2 Mechanisms of Elastic Scattering 40
3.3 Elastic Scattering from Isolated Atoms 41
3.4 The Rutherford Cross Section 41
3.5 Modifications to the Rutherford Cross Section 42
3.6 Coherency of the Rutherford-Scattered Electrons 43
3.7 The Atomic-Scattering Factor 44
3.8 The Origin of $f(\theta)$ 45
3.9 The Structure Factor $F(\theta)$ 46
3.10 Simple Diffraction Concepts 47
3.10.A Interference of Electron Waves; Creation of the Direct and Diffracted Beams 47
3.10.B Diffraction Equations 48
Chapter Summary 49

4 Inelastic Scattering and Beam Damage **53**

Chapter Preview 53
4.1 Which Inelastic Processes Occur in the TEM? 53
4.2 X-ray Emission 55
4.2.A Characteristic X-rays 55
4.2.B Bremsstrahlung X-rays 60
4.3 Secondary-Electron Emission 60
4.3.A Secondary Electrons 60
4.3.B Auger Electrons 61
4.4 Electron-Hole Pairs and Cathodoluminescence (CL) 62
4.5 Plasmons and Phonons 63
4.6 Beam Damage 64
4.6.A Electron Dose 65
4.6.B Specimen Heating 65
4.6.C Beam Damage in Polymers 66
4.6.D Beam Damage in Covalent and Ionic Crystals 66
4.6.E Beam Damage in Metals 66
4.6.F Sputtering 68
Chapter Summary 68

5 Electron Sources **73**

Chapter Preview 73
5.1 The Physics of Different Electron Sources 73
5.1.A Thermionic Emission 74
5.1.B Field Emission 74
5.2 The Characteristics of the Electron Beam 75
5.2.A Brightness 75
5.2.B Temporal Coherency and Energy Spread 76
5.2.C Spatial Coherency and Source Size 77
5.2.D Stability 77
5.3 Electron Guns 77
5.3.A Thermionic Guns 77
5.3.B Field-Emission Guns (FEGs) 80
5.4 Comparison of Guns 81
5.5 Measuring Your Gun Characteristics 82
5.5.A Beam Current 82
5.5.B Convergence Angle 83
5.5.C Calculating the Beam Diameter 83
5.5.D Measuring the Beam Diameter 85
5.5.E Energy Spread 85
5.5.F Spatial Coherency 86
5.6 What kV should You Use? 86
Chapter Summary 87

6 Lenses, Apertures, and Resolution **91**

Chapter Preview 91
6.1 Why Learn About Lenses? 91
6.2 Light Optics and Electron Optics 92
6.2.A How to Draw a Ray Diagram 92
6.2.B The Principal Optical Elements 94
6.2.C The Lens Equation 94
6.2.D Magnification, Demagnification, and Focus 95
6.3 Electron Lenses 96
6.3.A Polepieces and Coils 96
6.3.B Different Kinds of Lenses 97
6.3.C Electron Ray Paths Through Magnetic Fields 99
6.3.D Image Rotation and the Eucentric Plane 100
6.3.E Deflecting the Beam 101
6.4 Apertures and Diaphragms 101
6.5 Real Lenses and their Problems 102
6.5.A Spherical Aberration 103
6.5.B Chromatic Aberration 104
6.5.C Astigmatism 106
6.6 The Resolution of the Electron Lens (and Ultimately of the TEM) 106
6.6.A Theoretical Resolution (Diffraction-Limited Resolution) 107
6.6.B The Practical Resolution Due to Spherical Aberration 108
6.6.C Specimen-Limited Resolution Due to Chromatic Aberration 109
6.6.D Confusion in the Definitions of Resolution 109
6.7 Depth of Focus and Depth of Field 110
Chapter Summary 111

7 How to 'See' Electrons **115**

Chapter Preview 115
7.1 Electron Detection and Display 115
7.2 Viewing Screens 116
7.3 Electron Detectors 117
7.3.A Semiconductor Detectors 117
7.3.B Scintillator-Photomultiplier Detectors/TV Cameras 118
7.3.C Charge-Coupled Device (CCD) Detectors 120
7.3.D Faraday Cup 121
7.4 Which Detector Do We Use for which Signal? 122
7.5 Image Recording 122
7.5.A Photographic Emulsions 122
7.5.B Other Image-Recording Methods 124
7.6 Comparison of Scanning Images and Static Images 124
Chapter Summary 125

8 Pumps and Holders **127**

Chapter Preview 127
8.1 The Vacuum 127
8.2 Roughing Pumps 128
8.3 High/Ultra High Vacuum Pumps 129
8.3.A Diffusion Pumps 129
8.3.B Turbomolecular Pumps 129
8.3.C Ion Pumps 130
8.3.D Cryogenic (Adsorption) Pumps 130
8.4 The Whole System 130
8.5 Leak Detection 131
8.6 Contamination: Hydrocarbons and Water Vapor 132
8.7 Specimen Holders and Stages 132
8.8 Side-Entry Holders 133
8.9 Top-entry Holders 134
8.10 Tilt and Rotate Holders 134
8.11 In-Situ Holders 135
8.12 Plasma Cleaners 138
Chapter Summary 138

9 The Instrument **141**

Chapter Preview 141
9.1 The Illumination System 142
9.1.A TEM Operation Using a Parallel Beam 142
9.1.B Convergent-Beam (S)TEM Mode 143
9.1.C The Condenser-Objective Lens 145
9.1.D Translating and Tilting the Beam 147
9.1.E Alignment of the C2 Aperture 147
9.1.F Condenser-Lens Defects 148
9.1.G Calibration 149
9.2 The Objective Lens and Stage 150
9.3 Forming DPs and Images: The TEM Imaging System 152
9.3.A Selected-Area Diffraction 152
9.3.B Bright-Field and Dark-Field Imaging 155
9.3.C Centered Dark-Field Operation 155
9.3.D Hollow-Cone Diffraction and Dark-Field Imaging 157
9.4 Forming DPs and Images: The STEM Imaging System . . 158
9.4.A Bright-Field STEM Images 159
9.4.B Dark-Field STEM Images 161
9.4.C Annular Dark-Field Images 161
9.4.D Magnification in STEM 161
9.5 Alignment and Stigmation 161
9.5.A Lens Rotation Centers 161
9.5.B Correction of Astigmatism in the Imaging Lenses . 162
9.6 Calibrating the Imaging System 164
9.6.A Magnification Calibration 164
9.6.B Camera-Length Calibration 165
9.6.C Rotation of the Image Relative to the DP 167
9.6.D Spatial Relationship Between Images and DPs 168
9.7 Other Calibrations 168
Chapter Summary 169

10 Specimen Preparation **173**

Chapter Preview 173
10.1 Safety 173
10.2 Self-Supporting Disk or Use a Grid? 174
10.3 Preparing a Self-Supporting Disk for Final Thinning 175
10.3.A Forming a Thin Slice from the Bulk Sample 176
10.3.B Cutting the Disk 176
10.3.C Prethinning the Disk 177
10.4 Final Thinning of the Disks 178
10.4.A Electropolishing 178
10.4.B Ion Milling 178
10.5 Cross-Section Specimens 182
10.6 Specimens on Grids/Washers 183
10.6.A Electropolishing—The Window Method for Metals and Alloys 183
10.6.B Ultramicrotomy 183
10.6.C Grinding and Crushing 184
10.6.D Replication and Extraction 184
10.6.E Cleaving and the SACT 186
10.6.F The 90° Wedge 186
10.6.G Lithography 187
10.6.H Preferential Chemical Etching 187
10.7 FIB 188
10.8 Storing Specimens 189
10.9 Some Rules 189
Chapter Summary 191

G. H. Michler

Electron Microscopy of Polymers

Part I Techniques of Electron Microscopy

1 OVERVIEW OF TECHNIQUES 7
References 14

2 TRANSMISSION ELECTRON MICROSCOPY: FUNDAMENTALS OF METHODS AND INSTRUMENTATION 15
2.1 A Brief History 15
2.2 Fundamentals of Electron Optics and Instrumentation 17
2.2.1 Some Fundamental Properties of Electrons 17
2.2.2 Electron Lenses 19
2.2.3 Electron-Optical Aberrations and Resolution 22
2.2.4 Vacuum System 26
2.3 The Instrument 27
2.3.1 Electron Gun 30
2.3.2 Illumination System 33
2.3.3 Objective Lens and Specimen Stage 35
2.3.4 Image-Forming System 36
2.3.5 Viewing Chamber and Image Acquisition 37
2.3.6 Alignment and Operation of Transmission Electron Microscopes 39
2.4 Fundamentals of Image Formation 40
2.4.1 Scattering Mechanism and Contrast Formation 41
2.4.2 Electron Diffraction and Diffraction Contrast 44
2.4.3 Fundamentals of the Imaging Process 46
References 50

3 TRANSMISSION ELECTRON MICROSCOPY: CONVENTIONAL AND SPECIAL INVESTIGATIONS OF POLYMERS 53
3.1 Conventional Investigations Utilising Mass-Thickness Contrast 53
3.2 Electron Diffraction 55
3.2.1 Selected Area Diffraction 55
3.2.2 Structure Analysis 55
3.3 High-Resolution Transmission Electron Microscopy 57
3.3.1 Introduction 57
3.3.2 Evaluation and Reduction of Radiation Damage 57
3.3.3 Application of HRTEM 58
3.4 Phase Contrast Transmission Electron Microscopy 60
3.4.1 Phase Contrast at Large Defocus Values 60
3.4.2 Phase Contrast by Means of Phase Plates 60
3.5 Electron Holography 62
3.5.1 Introduction 62
3.5.2 Image Plane Off-Axis Holography 62
3.5.3 Examples 64
3.6 Low-Voltage Transmission Electron Microscopy 64
3.6.1 Introduction 64
3.6.2 A Dedicated Low-Voltage TEM and its Application 64
3.7 High-Voltage Transmission Electron Microscopy 65
3.7.1 Introduction 65
3.7.2 Advantages and Applications of HVTEM 65
3.8 Scanning Transmission Electron Microscopy 66
3.8.1 Introduction 66
3.8.2 Similarities and Differences between STEM and TEM 67
3.8.3 Application of Bright-Field and Dark-Field Modes 68
3.9 Analytical Transmission Electron Microscopy 70
3.9.1 Introduction 70
3.9.2 EELS Investigations 72
3.9.3 Electron Spectroscopic Imaging 75
3.10 Electron Tomography 78
3.10.1 Introduction 78
3.10.2 Data Acquisition, Image Alignment and Reconstruction 79
3.10.3 Resolution of Reconstructed Data 80
3.10.4 Application of Electron Tomography 81
References 81

4 SCANNING ELECTRON MICROSCOPY (SEM) 87
4.1 A Brief History of SEM 87
4.2 Fundamentals of Electron Optics and Signal Generation 88
4.2.1 Principle of SEM 88
4.2.2 The Lateral Resolution Power of SEM 89
4.2.3 Comparison of Various Cathode Types 92
4.2.4 Depth of Focus 92
4.2.5 Interaction of Primary Electrons with Sample 92
4.3 The Instrumentation of SEM 95
4.3.1 The Column 95
4.3.2 Specimen Chamber and Goniometer 97
4.3.3 Detectors 98
4.3.4 Signal Display and Magnification 99
4.4 Contrast Formation and Charging Effects 100
4.4.1 Secondary Electron Contrast 100
4.4.2 Contrast of Backscattered Electrons (Solid State Detector) . 103
4.4.3 Charging Effect 103
4.5 X-Ray Microanalysis 105
4.5.1 Physical Fundamentals of the Generation of X-Rays 105
4.5.2 X-Ray Microanalysis Techniques 107
4.5.3 Detector for EDX Analysis 108
4.5.4 Quantitative EDX Analysis 111
4.5.5 X-Ray Mapping 112
4.5.6 Wavelength Dispersive X-Ray Microanalysis (WDX) 112
4.6 Environmental Scanning Electron Microscope (ESEM™) 116
4.6.1 Low-Vacuum SEM and ESEM™ 116
4.6.2 Avoiding Charging 116
4.6.3 The Wet Mode 117
4.6.4 The Gaseous Secondary Electron Detector (GSED) 119
References 120

5 ATOMIC FORCE MICROSCOPY (AFM) 121
5.1 Introduction 121
5.2 Methodical and Instrumental Fundamentals 124
5.3 Modes of Operation 127
5.3.1 Contact Mode 127
5.3.2 Force Modulation Mode 130
5.3.3 Dynamic Operational Modes 131
5.3.4 Tapping Mode 132
5.4 Typical and Special AFM Applications 137
References 142

6 IN SITU MICROSCOPY 145
6.1 Overview 145
6.2 Micromechanical In Situ Tests 147
6.2.1 Technical Equipment 147
6.2.2 In Situ Microscopy in (E)SEM 148
6.2.3 In Situ Microscopy in (HV)TEM 150
6.3 In Situ Microscopy in AFM 154
References 158

7 IMAGE PROCESSING AND IMAGE ANALYSIS 161
7.1 Overview 161
7.2 Image Processing 162
7.3 Image Analysis 163
7.4 Fourier Transformation 165
7.5 Stereoscopic Imaging 167
References 171

Part II Preparation Techniques

8 PROBLEMS ASSOCIATED WITH THE ELECTRON MICROSCOPY OF POLYMERS 175
8.1 Overview 175
8.2 Irradiation Sensitivity of Polymers 176
8.3 Low Contrast of Polymers 177
8.4 Methods of Investigating the Morphologies of Polymers 178
8.4.1 Powders, Particles, Fibres 178
8.4.2 Bulk Polymers 178
8.5 Methods for Studying Micromechanical Processes 180
References 183

9 PREPARATION OF SURFACES 185
9.1 Overview 185
9.2 Surfaces in Their Natural Form 185
9.3 Smooth and Etched Surfaces 186
9.3.1 Chemical Etching 187
9.3.2 Physical Etching 191
9.4 Fracture Surfaces 192
9.5 Investigation of Surfaces 195
References 196

10 PREPARATION OF THIN SECTIONS: (CRYO)ULTRAMICROTOMY AND (CRYO)MICROTOMY 199
10.1 Overview 199
10.2 Instrumentation 200
10.2.1 Microtomes 200
10.2.2 Ultramicrotomes 200
10.2.3 Cryomicrotomes and Cryoultramicrotomes 200
10.2.4 Knives 200
10.2.5 Modern Trends 202
10.3 Working with a Microtome and an Ultramicrotome 203
10.4 Specimen Preparation 204
10.4.1 Embedding 204
10.4.2 Specimen Trimming 205
10.4.3 Fixation and Staining 205
10.5 Ultrathin Sectioning 206
10.5.1 Sectioning Parameters 206
10.5.2 Wet and Dry Sectioning Techniques 206
10.5.3 Room Temperature Ultramicrotomy 208
10.5.4 Cryoultramicrotomy 208
10.5.5 Collecting Sections on Grids 209
10.6 Problems, Errors and Solutions 212
10.6.1 Overview 212
10.6.2 Typical Errors and Possible Solutions 212
References 217

Part II in this book is mainly soft materials sample preparation – i.e. quite useful for many of you

11 SPECIAL PREPARATION TECHNIQUES 219
11.1 Preparation of Polymer Films from Solutions 219
11.1.1 Introduction 219
11.1.2 Solution Behaviour of Polymers 220
11.1.3 Spin-Coating 220
11.1.4 Dip-Coating 222
11.1.5 Solution Casting 223
11.1.6 Examples and Problems 224
11.2 Preparation Using the Focussed Ion Beam Technique 227
11.2.1 Introduction 227
11.2.2 Principle 228
11.2.3 Examples 229
References 230

12 PREPARATION FOR (IN SITU) DEFORMATION TESTS 231
12.1 Overview 231
12.2 Specimen Preparation 234
References 240

13 CONTRAST ENHANCEMENT 241
13.1 Overview 241
13.2 Hardening (Fixation) 241
13.2.1 Physical Hardening (Fixation) 242
13.2.2 Chemical Fixation 243
13.3 Chemical Staining Procedures 243
13.3.1 Media Used to Perform Chemical Staining of Polymers 243
13.3.2 Chemical Staining of Compact Specimens Before Sectioning 246
13.3.3 Chemical Staining of Thin Sections 248
13.4 Enhancement of Contrast Through Physical Effects 248
13.4.1 Contrast Enhancement by γ- or Electron Irradiation 248
13.4.2 Straining-Induced Contrast Enhancement 254
13.5 Problems and Artefacts 256
References 259

Part III Main Groups of Polymers

14 STRUCTURAL HIERARCHY OF POLYMERS ... 263
14.1 Overview ... 263
14.2 Macromolecular Structures ... 263
14.2.1 Constitution ... 265
14.2.2 Configuration ... 265
14.2.3 Conformation ... 265
14.2.4 Macromolecule Size ... 267
14.3 Supramolecular Structures ... 269
14.4 Morphology ... 271
14.5 Basic Relationships Between Macromolecular Parameters and (Micro)mechanical Properties ... 273
References ... 276

15 AMORPHOUS POLYMERS ... 277
15.1 Overview ... 277
15.2 Morphology ... 277
15.3 Micromechanical Behaviour ... 281
15.4 Additional Examples of Amorphous Polymers ... 287
References ... 293

16 SEMICRYSTALLINE POLYMERS ... 295
16.1 Overview ... 295
16.2 Morphology ... 296
16.2.1 Structural Hierarchy in Semicrystalline Polymers ... 296
16.2.2 Methods of Morphological Analysis ... 299
16.3 Micromechanical Behaviour ... 310
16.3.1 Brittle Behaviour ... 310
16.3.2 Ductile Behaviour ... 313
16.3.3 High-Strength Fibres and Parts ... 319
16.4 Additional Examples of Semicrystalline Polymers ... 321
References ... 327

17 POLYMER BLENDS ... 329
17.1 Overview ... 329
17.2 Morphology ... 331
17.3 Micromechanical Behaviour ... 338
17.4 Examples ... 341
17.4.1 Blends of Amorphous Polymers ... 341
17.4.2 Blends of Amorphous and Semicrystalline Polymers ... 343
17.4.3 Blends of Semicrystalline Polymers ... 343
References ... 349

Part III in this book is special applications for polymers – i.e. not the topic of this course..

18 HIGH-IMPACT RUBBER-MODIFIED POLYMERS ... 351
18.1 Overview ... 351
18.2 Morphology ... 352
18.3 Micromechanical Processes ... 357
18.4 Additional Toughening Mechanisms ... 368
References ... 370

19 BLOCK COPOLYMERS ... 373
19.1 Overview ... 373
19.2 Morphology ... 375
19.2.1 Block Copolymer Nanostructures via Self-Assembly ... 375
19.2.2 Examples of Tailored Block Copolymer Morphology ... 379
19.3 Deformation Mechanisms in Block Copolymers ... 385
19.4 Special Cases of Self-Assembly and Applications ... 389
References ... 391

20 RUBBERS AND ELASTOMERS ... 393
20.1 Overview ... 393
20.2 Morphology of Rubbers and Elastomers ... 395
20.3 Micromechanical Deformation Behaviour ... 398
References ... 402

21 POLYMER COMPOSITES ... 405
21.1 Overview ... 405
21.2 Particle-Reinforced Polymer Composites ... 406
21.3 Fibre-Reinforced Polymer Composites ... 414
References ... 417

22 POLYMER NANOCOMPOSITES ... 419
22.1 Overview ... 419
22.2 Examples of Different Classes of Nanocomposites ... 422
22.2.1 Polymer Nanocomposites Based on Zero-Dimensional Filler Particles (POSS) ... 422
22.2.2 Polymer Nanocomposites Based on One-Dimensional Filler Particles (MWCNT) ... 424
22.2.3 Polymer Nanocomposites Based on Two-Dimensional Filler Particles (MMT) ... 425
22.2.4 Polymer Nanocomposites Based on Three-Dimensional Filler Particles (SiO_2) ... 427
References ... 428

23 BIOMATERIALS ... 429
23.1 Overview ... 429
23.2 Electron Microscopy of Polymeric Biomaterials: Specific Problems and Solutions ... 431
23.3 Examples ... 432
23.3.1 Natural Biomaterials: Bone ... 432
23.3.2 UHMWPE in Orthopaedics ... 433
23.3.3 Acrylic Bone Cements ... 434
23.3.4 Bioactive Composites for Bone Replacement ... 435
23.3.5 Dental Composites ... 436
23.3.6 Sutures, Scaffolds and Meshes ... 437
23.3.7 Ureter Stents ... 439
23.3.8 Silicone-Based Tracheal Stents and Voice Prostheses ... 439
References ... 443

24 SPECIAL PROCESSING FORMS ... 445
24.1 Overview ... 445
24.2 Multilayered Films ... 446
24.2.1 Introduction ... 446
24.2.2 Morphology ... 446
24.2.3 Micromechanical Deformation Mechanisms ... 448
24.3 Hot-Compacted Self-Reinforced Polymers ... 451
24.3.1 Overview ... 451
24.3.2 Morphology ... 452
24.4 Nanofibres by Electrospinning ... 455
24.4.1 Overview ... 455
24.4.2 Morphology ... 456
24.4.3 Micromechanical Deformation Mechanism ... 458
24.5 Microformed Materials ... 460
24.5.1 Overview ... 460
24.5.2 Several Examples ... 460
References ... 462

SUBJECT INDEX ... 465

Book 3: A Practical Guide to Transmission Electron Microscopy: Fundamentals And A Practical Guide to Transmission Electron Microscopy, Volume II

Chapter 1 Introduction ... 1
1.1 Microscope Resolution ... 2
1.2 Interactions of Electrons with Specimen ... 4
1.3 Comparison of TEM with Other Microscopy Techniques ... 6
References ... 11
Chapter 2 Sample Preparation ... 13
2.1 Material Samples ... 14
2.1.1 TEM Grids ... 15
2.1.2 Ion Milling ... 20
2.1.3 Electropolishing ... 26
2.1.4 Focused Ion Beam ... 30
2.1.5 Microtomy ... 31
2.2 Biological Samples ... 36
2.2.1 Particulate Samples ... 37
2.2.2 Cells and Tissue Samples ... 39
References ... 42
Chapter 3 Instrumentation and Operation ... 45
3.1 Construction ... 45
3.1.1 Electron Gun ... 45
3.1.2 Electromagnetic Lens ... 49
3.1.3 Condenser Lenses and Condenser Apertures ... 52
3.1.4 Objective Lens and Objective Aperture ... 55
3.1.5 Intermediate Lens and Diffraction Aperture ... 57
3.1.6 Projector Lens ... 59
3.1.7 Viewing Screen and Camera ... 59
3.2 Instrument Imperfections, Alignments, Corrections, and Calibrations ... 60
3.2.1 Beam Shift and Beam Tilt ... 60
3.2.2 Spherical Aberration ... 61
3.2.3 Chromatic Aberration ... 62
3.2.4 Depth of Field and Depth of Focus ... 63
3.2.5 Specimen Height ... 64
3.2.6 Astigmatism ... 65
3.2.7 Aperture Alignment ... 68
3.2.8 Magnification Calibration ... 69
3.2.9 Camera Length Calibration ... 71
3.2.10 Magnetic Rotation Calibration ... 75
3.3 TEM Operating Procedures ... 76
3.3.1 Startup ... 76
3.3.2 Specimen Loading and Unloading ... 76
3.3.3 Alignments ... 77
3.3.4 Data Recording ... 78
3.3.5 Finishing ... 78
References ... 79
Chapter 4 Electron Diffraction I ... 81
4.1 Formation of Electron Diffraction ... 82
4.2 Reciprocal Space ... 84
4.3 Indexing of Electron Diffraction Patterns ... 88
4.3.1 Indexing of Powder Patterns ... 88
4.3.2 Indexing of Single-Crystal Diffraction Patterns ... 90
4.3.3 Indexing of Compound Patterns: Twins ... 92
4.3.4 Indexing of Compound Patterns: Multiple Phases ... 96
4.3.5 Indexing of Compound Patterns: Double Diffraction ... 98
4.4 Experimental Procedures ... 101
4.5 Simulation of Diffraction Patterns ... 103

References 105
Chapter 5 Imaging I 109
5.1 Imaging Contrast 109
5.2 Imaging with Mass-Thickness Contrast 110
5.3 Imaging with Diffraction Contrast 111
5.3.1 Formation of Diffraction Contrast 111
5.3.2 Central Dark-Field Imaging 114
5.3.3 Two-Beam Condition 115
5.3.4 Bragg-Diffracted Beam Intensity 117
5.3.5 Thickness Fringes 120
5.3.6 Bend Contours 122
5.3.7 Weak-Beam Dark-Field Imaging 122
5.3.8 Planar Defects 125
5.3.9 Dislocations 127
References 137

And A Practical Guide to Transmission Electron Microscopy, Volume II

Chapter 7 Imaging II 21
7.1 STEM Imaging 21
7.1.1 Formation of STEM Images and Optics 21
7.1.2 STEM Experimental Procedures 24
7.1.3 STEM Applications 24
7.2 High-Resolution Transmission Electron Microscopy 28
7.2.1 Principles of HRTEM 28
7.2.2 Experimental Operations 37
7.2.3 Image Interpretation and Simulation 42
7.2.4 Image Processing 45
References 48
Chapter 8 Elemental Analyses 51
8.1 X-ray Energy-Dispersive Spectroscopy 52
8.1.1 Formation of Characteristic X-Rays 52
8.1.2 EDS Detector 54
8.1.3 EDS Artifacts 57
8.1.4 Effects of Specimen Thickness, Tilt, and Space Location 59
8.1.5 Experimental Procedures 63
8.1.6 EDS Applications 64
8.2 Electron Energy-Loss Spectroscopy 73
8.2.1 Formation of EELS 73
8.2.2 EELS Qualitative and Quantitative Analyses 75
8.2.3 Energy-Filtered TEM 78
8.2.4 EFTEM Experimentation and Applications 81
References 87
Chapter 9 Specific Applications 91
9.1 Quantitative Microscopy 92
9.1.1 Quantification of Size Homogeneity 92
9.1.2 Quantification of Directional Homogeneity 96
9.1.3 Dispersion Quantification 99
9.1.4 Electron Diffraction Pattern Processing and Refinement 103
9.2 *In situ* Microscopy 107
9.2.1 *In situ* Heating 108
9.2.2 *In situ* Cooling 114
9.2.3 *In situ* Irradiation 116
9.3 Cryo-EM 117
9.4 Low-Dose Imaging 122
9.5 Electron Tomography 125
9.5.1 Experimental Procedures 125
9.5.2 Object Shapes 127
9.5.3 Nanoparticle Assemblies 133
9.5.4 Nanoparticle Superlattices 135
References 143

PHYS-E0526 Microscopy of Nanomaterials, laboratory course P (5 cr)

Assistant: Shandilay Shruti and **other teachers:** Dr. Jani Seitsonen (**TEM & Tomography**), Dr. Hua Jiang (**HR-TEM**), Dr. Ramzy Abdelaziz (**SEM**), Dr. Lide Yao (**FIB**), DR. Ville Liljeström (**AFM**)

As practical exercises nanostructured materials are studied with various microscopy methods. Course includes practical microscopy exercises by using transmission electron microscopy (TEM), scanning electron microscopy (SEM) **and Focused ion beam (FIB).**

Number of students participating to the course will be limited. (max. ~18) Based on applications… nmc-contact-sci@aalto.fi Deadline 16. 2. 2020

Basic exercises – Demos:

(4? persons per group):

1) High resolution TEM (Jeol 2200FS Cs-corrected TEM)
2) 3D tomography data collection (Jeol 3200FSC liquid helium cryo TEM or Jeol2800) + Tomography data processing (Computer room)

Small group exercises: (Select 2)

(2? persons per group)

1) basic-TEM imaging
2) SEM imaging or FIB -SEM processing/imaging
3) AFM

Independent Small group exercises

(without supervision.. 2? person per group)

(2 exercises)

1) TEM imaging
2) SEM imaging
3) …

To apply for the Laboratory Course - Send the following information by email: nmc-contact-sci@aalto.fi deadline 16. 2. 2020

1. You are a *

- [ ] graduate student
- [ ] exchange student
- [ ] Ph.D student
- [ ] post doc/other

2. Your primary affiliation is *

- ○ Aalto University
- ○ University of Helsinki
- ○ VTT
- ○ Other

3. Do you belong to a research group? If yes, who is your supervisor/instructor?

Research Group

Supervisor/Instructor

4. Write with your own words your primary motivation to participate on the laboratory course. *

5. What instrument do you plan to use in your research?

- [ ] AFM
- [ ] SEM
- [ ] TEM
- [ ] HRTEM
- [ ] Cryo-TEM
- [ ] TEM tomography
- [ ] XRAY

6. Contact Information

Firstname *

Lastname *

Email *

Student Number (if you have one)

Exam for lecture course PHYS-E0525 Microscopy of Nanomaterials…
We have multiple choice questions from TEM, SEM, FIB, AFM etc..
+ normal questions – where you write short answers

TEM Questions

1. **What is STEM?**
   - Scanning transmission electron microscopy
   - Standard transmission electron microscopy
   - Scanning and transmission electron microscopy
   - Suitable transmission electron microscopy
   - Scanning tunneling electron microscopy

2. **Bright field TEM is best described as?**
   - Microscopy where the electron beam is well aligned
   - The exclusion of the scattered electron beam while the beam is directed onto the sample
   - Any imaging that must be undertaken in a well lit room
   - The exclusion of the central beam electrons by tilting the beam, displacing the objective aperture or introducing the beam stop
   - Microscopy where the filament has not broken

3. **How can chromatic aberrations be minimized in a TEM?**
   - Use an electron gun with high energy spread and a thin specimen
   - Use an electron gun with low energy spread and a thick specimen
   - Use an electron gun with high energy spread and a thick specimen
   - Use an electron gun with medium energy spread and a thick specimen
   - Use an electron gun with low energy spread and a thin specimen

Aalto University
School of Science
and Technology

Nanomicroscopy center

Prof. Janne Ruokolainen (Director Jani Seitsonen 1. 1. 2021 →)
Aalto University, Otanano

Aalto University
School of Science
and Technology

Nanomicroscopy Center ?

Introduction

The new Nanomicroscopy Center is large microscopy clusters (even compared to other European centers). The center is now housing various microscopes able to characterize hard materials down to atomic resolution, and soft materials including biomaterials down to molecular resolution.

- Started in operation 2008 →
- Center area 1220 m^2 / 740 m^2
- Center for various different *high resolution microscopy* techniques (currently >10 different high-resolution microscopes: 5 TEM's, 3 SEM's, AFM's, 3 STM's.. + New FIB-SEM 2019
- Instrument investments (until now..) >10 M€

Key Instruments:

- First lens *aberration corrected* transmission electron microscope in Finland with approximately 1 Å resolution *(JEOL 2200FS)*
- First *Liquid helium cryo*-transmission electron microscope in Finland (operating at -255 °C or -187 °C) *(JEOL 3200FSC)*

Prof. Janne Ruokolainen +358 50 3470759
Janne. Ruokolainen@aalto.fi, http://nmc.aalto.fi

Equipment and their new values, and purchase years

Transmission electron microscopes (TEM)	**Value**	**Year**
1) 120kV TEM Tecnai 12	350 k€	2004
2) Double Cs corrected sub-Ångstrom 200kV (S)TEM, EDX, EELS	>2000 k€	2009
3) Liquid Helium 300kV cryo-TEM, EELS	>1500 k€	2009
4) 200kV FEG (S)TEM , EDX	*>1000 k€*	*2016*
5) 200kV FEG TEM (moved from Department of Materials Science and Engineering 2016)		

Scanning electron microscopes (SEM)		
1) JEOL 7500F FEG-SEM + EDX	400 k€	2008
2) Zeiss Sigma FEG-SEM	200 k€	2011
3) Zeiss E-SEM	150 k€	2011
4) dual beam FIB-SEM	~1000 k€	2019

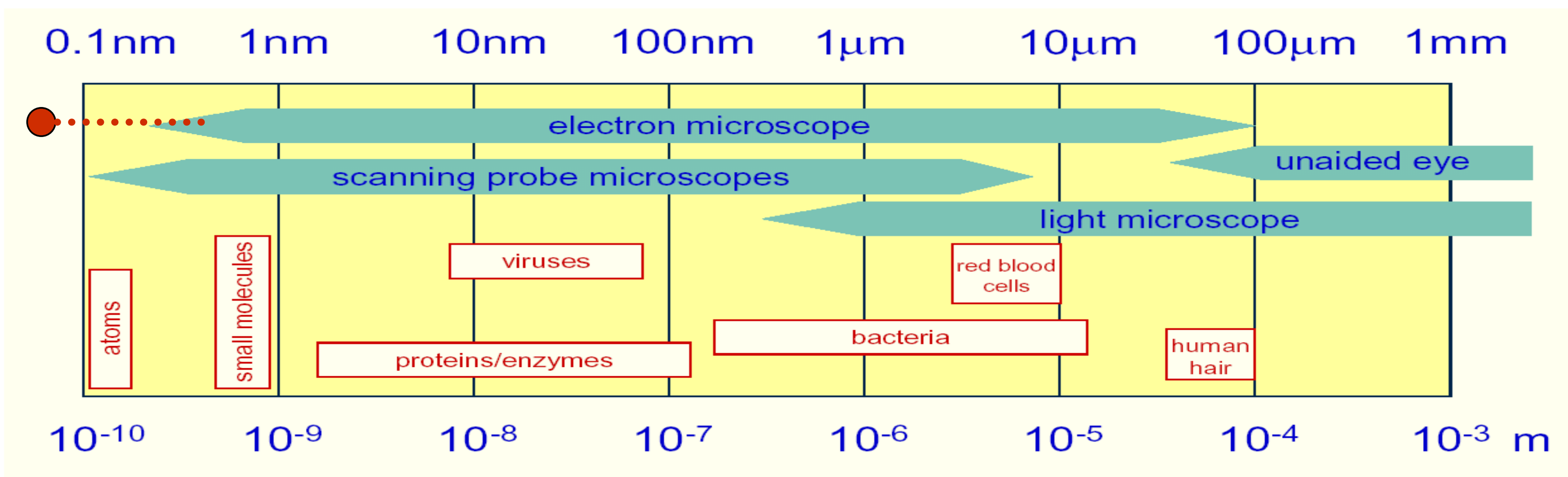
Scanning probe microscopy (AFM, STM)		
1) Veeco Dimension 5000 AFM	300 k€	2008
2) RHK UHV-750 variable temperature STM (UHV-STM)	500 k€	2005 - 2009
3) Createc LT-STM low-temperature STM	460 k€	2012
4) cryo UHV-STM	580 k€	2012

X-ray Scattering		
1) Small Angle X-ray Scattering (SAXS)	550 k€	2006 - 2008
2) Wide and Medium Angle X-ray Scattering (WAXS/MAXS)	450 k€	2008 – 2016
3) 2D-microfocus XRD	*500 k€*	*2016*
4) New-SAXS small angle X-ray setup	*500 k€*	*2020*

sample preparation equipment etc.		
Cryo vitrification (3), ultra microtoming (2), ion-milling, polishing, plasma cleaner...		
TEM holders, cross section polisher,, ...	1000 k€	2009 -

Total: >12 M€

Microscopy Resolution – Different Microscopes



Nanoparticles, carbon nanotubes	0.1 nm –
Lipids, polymer-amphiphile, liquid crystals	1 – 5 nm
Block copolymers	10 – 100 nm
Polymer blends, colloids…	50 nm – 10 μm

Electron microscope resolution:

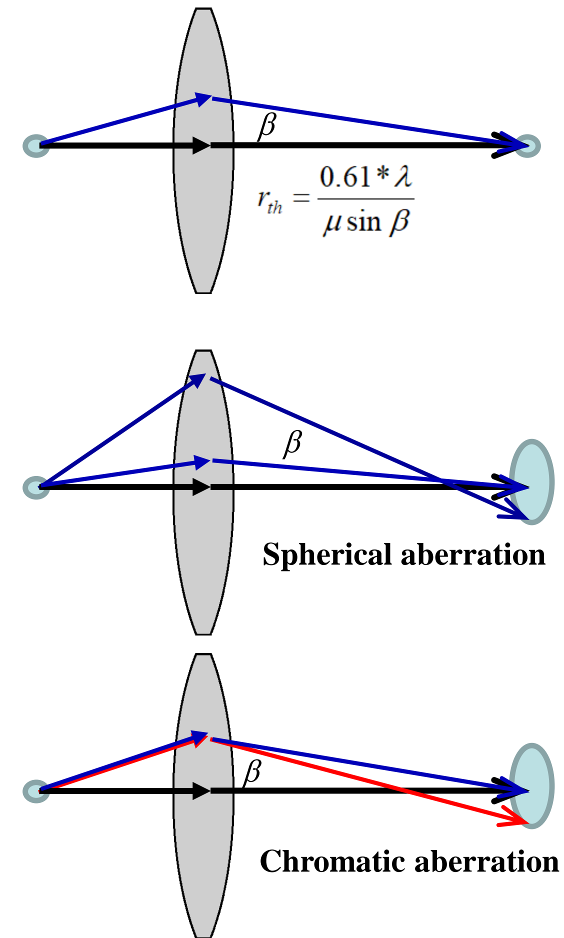
In ideal case:
Theorethical resolution
(Classical Rayleight criteria)

$$r_{th} = \frac{0.61 * \lambda}{\mu \sin \beta} \approx \frac{0.61\lambda}{\beta}$$

Light microscope wavelenght ~ 400 - 600 nm
Electron wavelenght 300 kV ~ 0.002 nm !!!

Spherical aberration $r_{sph} = C_s \beta^3$

Chromatic aberration $r_{chr} = C_c \frac{\Delta E}{E_0} \beta$



Example Jeol 3200FSC cryo-TEM:
300 kV, Cs = 4.1 mm, Cc = 3.4 mm
→point resolution ~2.6 Å

Jeol 2200FS Cs = 1.0 mm Cc = 1.4 mm, 200kV
Point resolution 2.3 Å (without Cs correctors)

Jeol 2800 200kV Cs = 0.7mm
Point resolution 2.0 Å

$$r_{total} = \sqrt{(r_{th})^2 + (r_{sph})^2 + (r_{chr})^2 + ..}$$

If spherical aberration could be corrected
Cs ~ 0 → resolution < 1 Å

If also chromatic aberration could be corrected or minimized → resolution < 0.5 Å

Spherical aberration in **Hubble Space Telescope**

Analysis of the flawed images showed that the cause of the problem was that the primary mirror had been ground to the wrong shape. Although it was probably the most precisely figured mirror ever made, with variations from the prescribed curve of only 10 nanometers,[it was too flat at the edges by about 2200 nanometers (mirror diameter 2.4 meters..). This difference was catastrophic, introducing severe spherical aberration a flaw in which light reflecting off the edge of a mirror focuses on a different point from the light reflecting off its center.

Progress of microscopes since 18 century

We stand now at a nanoworld of 0.4 Å (40pm)

Cc corector

Aberration correctors

E. Microscopes

Cs corrector

High voltage

Optical Microscopes

A n g s t r o m: 0.1, 1, 10, 100, 1000, 10000, 100000

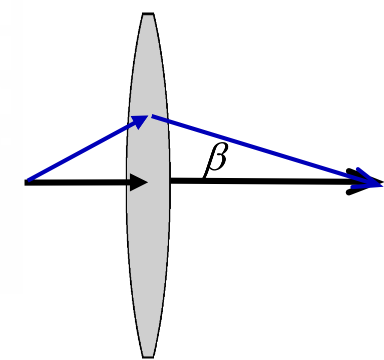
1μ →

Year: 1800, 1840, 1880, 1920, 1960, 2000, 2040

Leeuwenfoek

Mag = 275

Year 1676

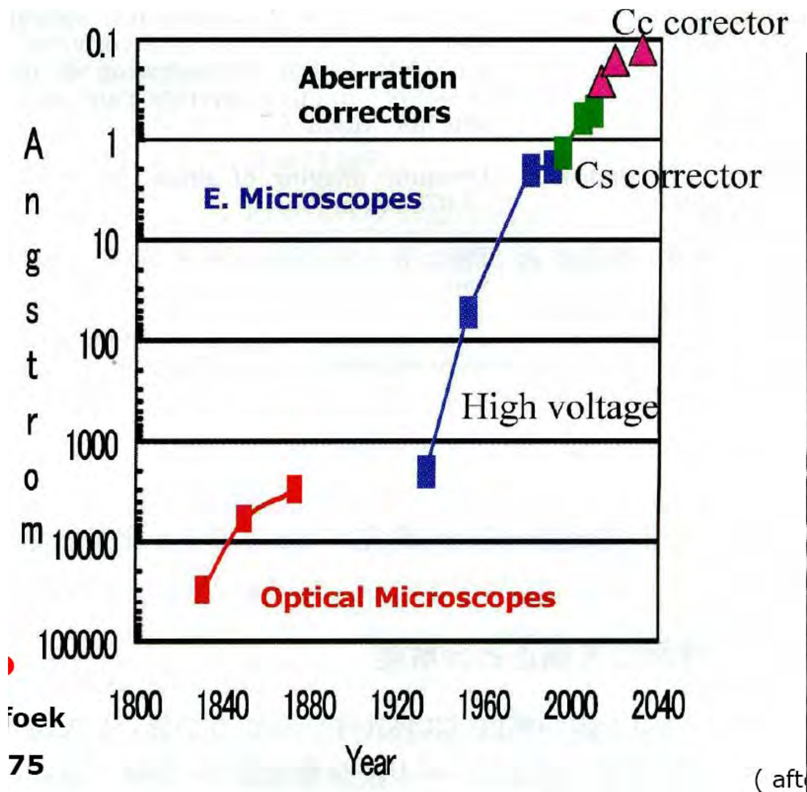


Resolution

$$r_{th} = \frac{0.61 * \lambda}{\mu \sin \beta}$$

(after Takayanagi)

Ernst Ruska and Max Knoll builtv the first electron microscope in 1931 (Nobel Prize to Ruska in 1986)



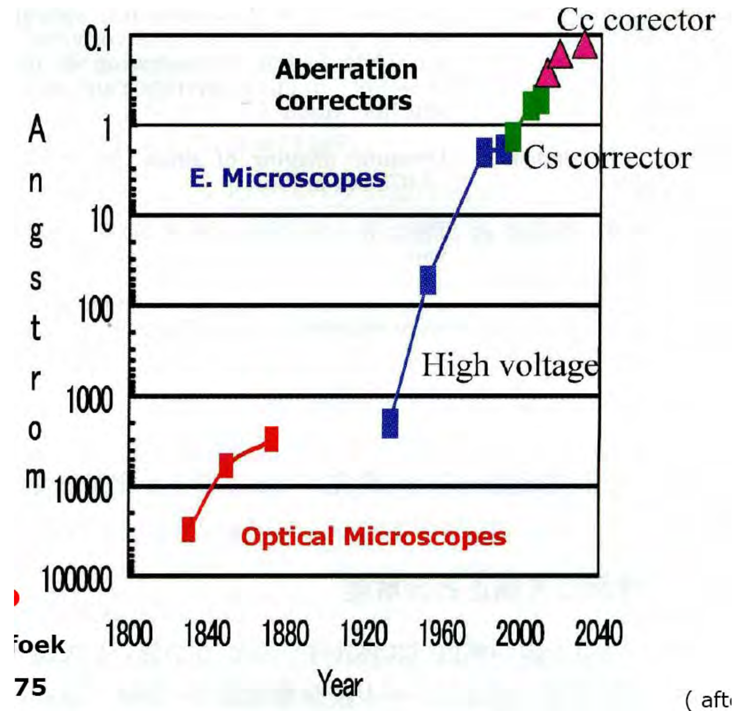
Accelerating voltage (kV)	Non-relativistic wavelength (nm)	Relativistic wavelength (nm)	Mass ($\times m_0$)	Velocity ($\times 10^8$ m/s)
100	0.00386	0.00370	1.196	1.644
120	0.00352	0.00335	1.235	1.759
200	0.00273	0.00251	1.391	2.086
300	0.00223	0.00197	1.587	2.330
400	0.00193	0.00164	1.783	2.484
1000	0.00122	0.00087	2.957	2.823

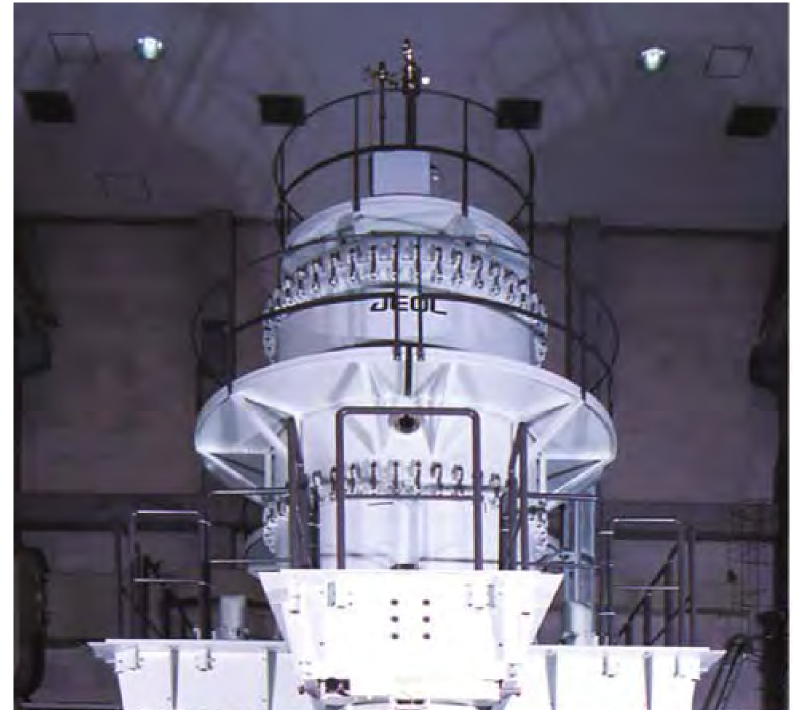
TABLE 1.2 Electron Properties as a Function of Accelerating Voltage

FIGURE 1.1. The electron microscope built by Ruska (in the lab coat) and Knoll, in Berlin in the early 1930s.

High voltage TEM (typically 400 kV -3 MV)

In early years the resolution was increased by using higher voltage TEM - since the wavelenght is smaller and therefore resolution is potentially better..





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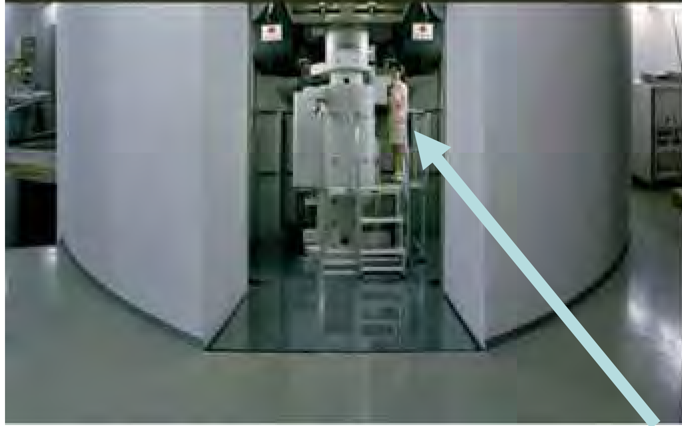
1000	0.00122	0.00087	2.957	2.823

$$r_{th} = \frac{0.61 * \lambda}{\mu \sin \beta} \approx \frac{0.61\lambda}{\beta}$$

(in TEM β is small)

Nowadays these high voltage TEM's are mostly used for material radiation damage research and some special applications where thick samples are required for imaging (high voltage electrons can penetrate thicker samples .. i.e even some micrometer thick samples .. Normal TEM's require typically <100 nm or even < 10 nm samples for high resolution work

Hitachi 3.5MeV (S)TEM

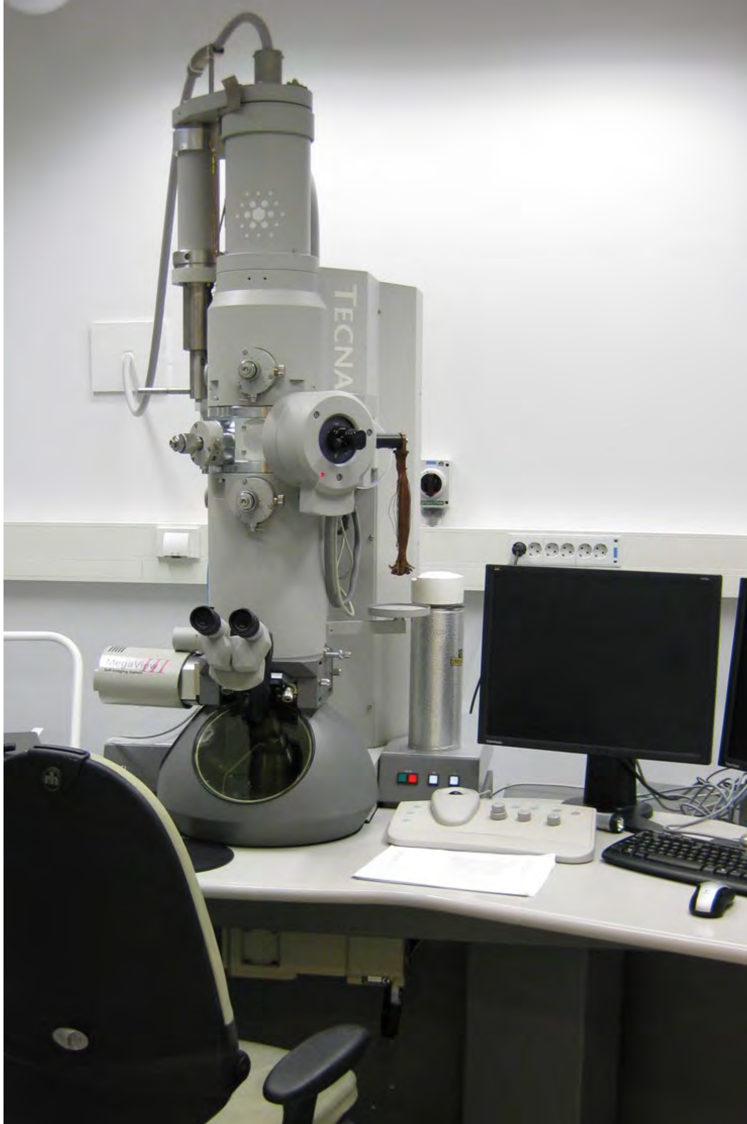


Base Fl

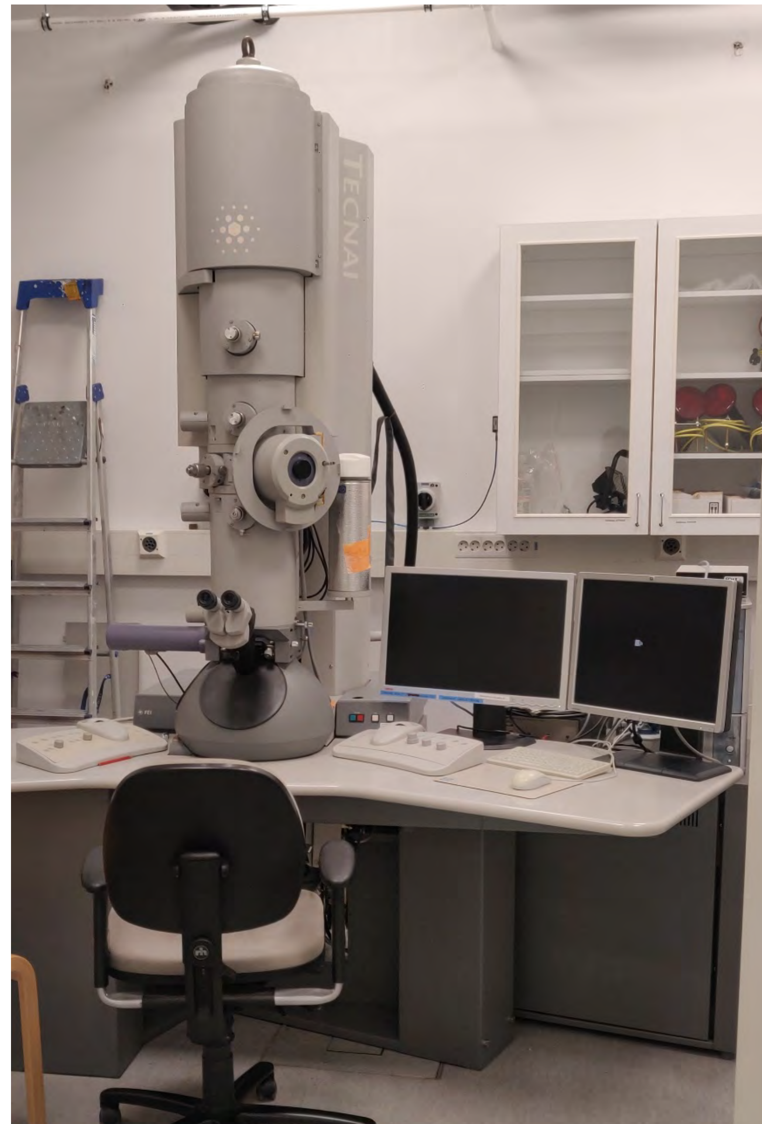
Microscopes are quite big - Notice the operator standing there..

In NMC we have 5 TEM's

In this picture the oldest TEM's 120 kV TEM and 200 kV TEM are shown



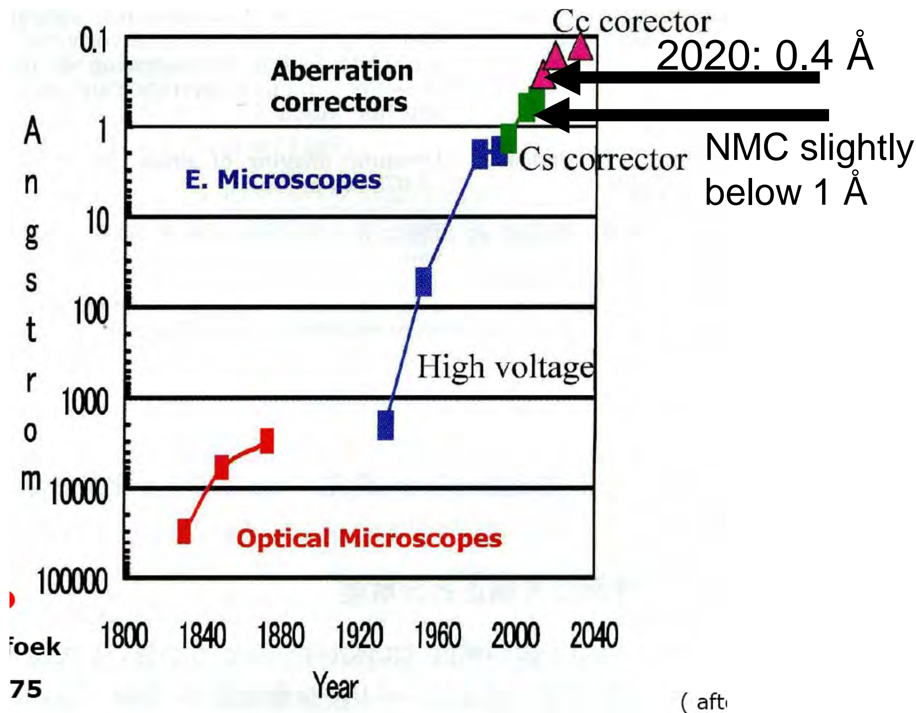
FEI Tecnai T12 Installation 2003



FEI Tecnai F20 Installation 1999? (Material science laboratory (Chem) and 2016 → moved to NMC)

NMC: new 200 kV TEM and 300 kV Cryo-TEM

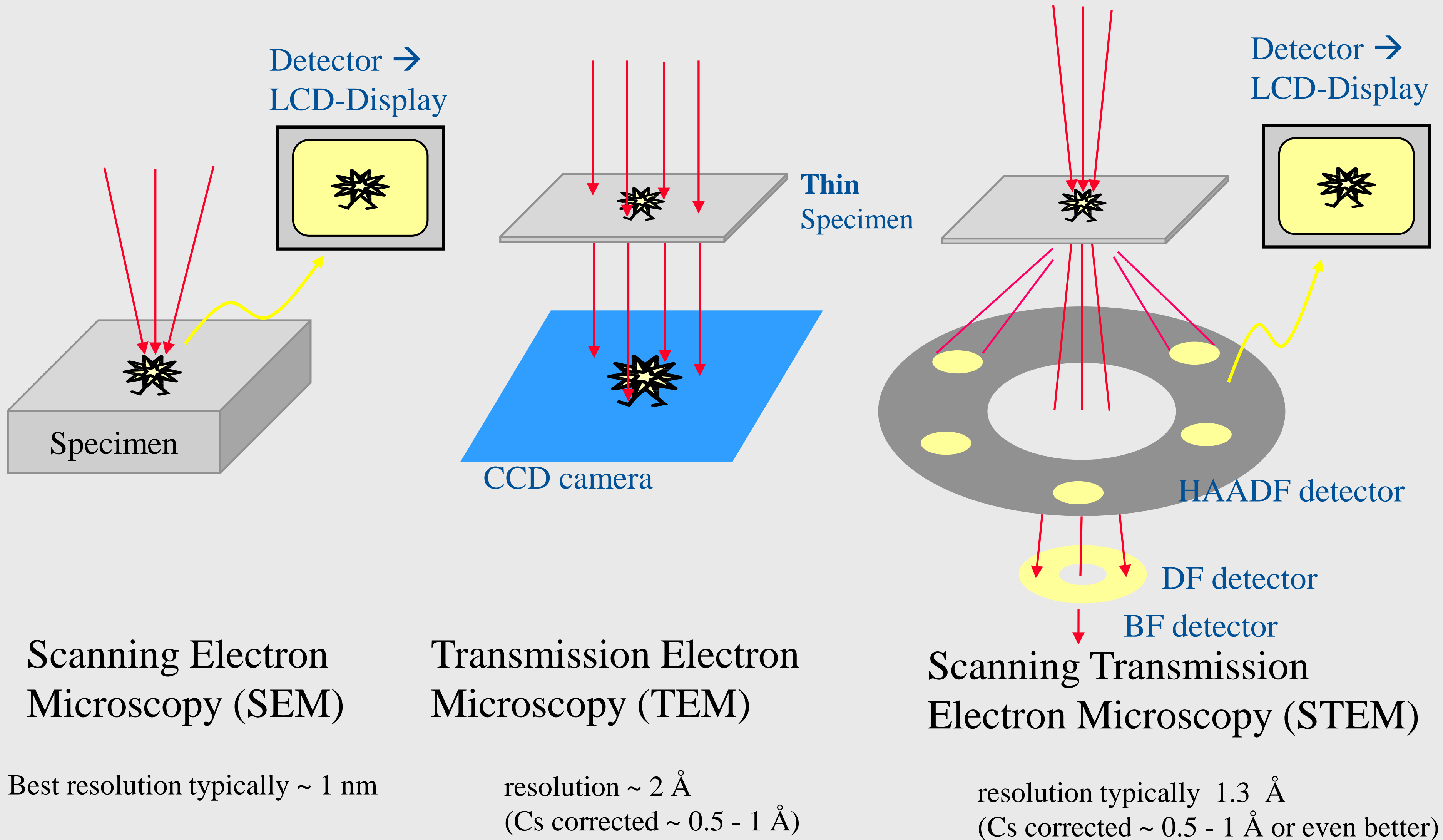
NMC highest resolution Double Cs-corrected (S)TEM



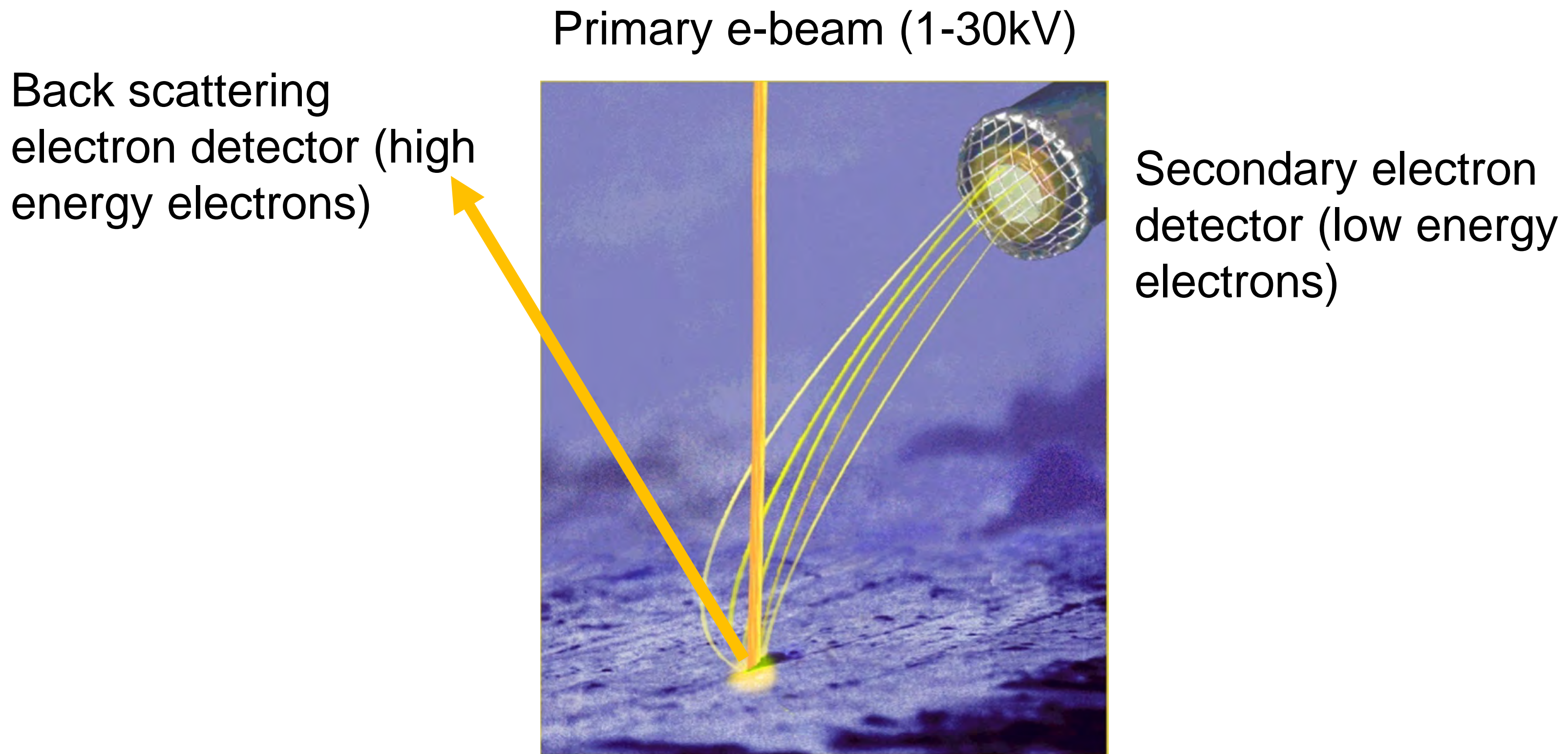
Installation 2009: TEM and STEM resolution slightly below 1 Å

Now best resolution for commercial microscopes is 0.4 Å

Brief introduction to the electron microscopy



Scanning Electron Microscopy (SEM)



The scanning electron microscope (SEM) uses a focused beam of high-energy electrons (typically 1kV to 30 kV) to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample morphology (texture), chemical composition, and crystalline structure and orientation of the materials. Electron beam is scanned across the surface point by point and each point signal is collected, and 2-dimensional image is generated.

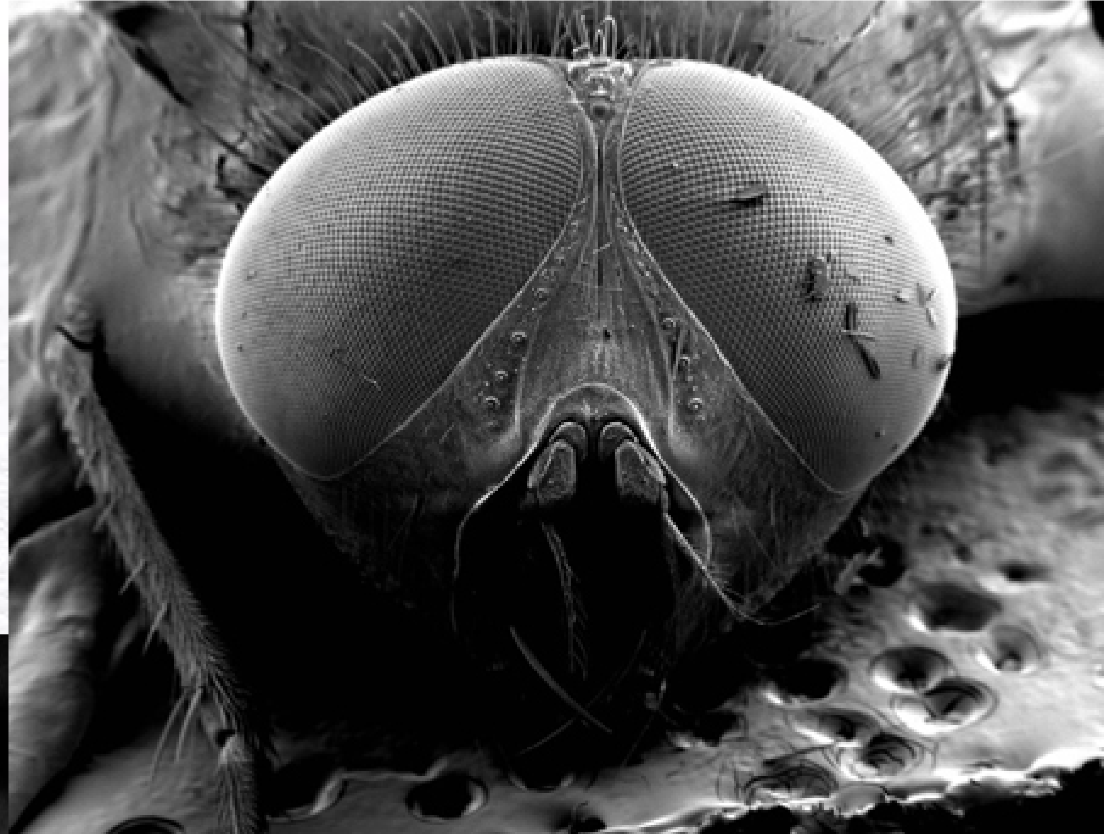
Scanning Electron Microscopy (SEM)

Surface technique:

- Relatively high resolution –
  Best case even <1nm (0.4 nm)
- Large depth of field (Large sample focus range)
- Low and high magnifications
- Large samples
- Possibility to microanalysis (EDX)
- Relatively easy sample preparation
  (normally thin metal coating needed - gold/palladium, platinum, carbon, etc.)

- Samples in vacuum (same as TEM)
- Limited to surface studies (except if STEM option is available – but then very thin samples needed… since voltage is max. 30 kV)

SEM examples: Fly head and leg



Large depth of field: sample is focus on different heights at the same time

passion flower

Nanomicroscopy center has 3 SEM's

1.)

This SEM has been the primary training and research SEM at NMC since 2008.

JEOL JSM-7500FA Information Card	
Manufacturer	JEOL
Model	JSM-7500F (later upgraded to JSM-7500FA)
Emitter	Cold FEG
Installation	2008
Detectors	In-column SE ETSE BSE (2 segment) EDX (JEOL)
Resolution	0.6 nm @ 30 kV 1.4 nm @ 1 kV
Acceleration voltage	0.1 - 30 kV
Probe current	-
Operating modes	High vacuum (10^{-5} Pa)
Stage	Motorized 5 axis (compucentric) X & Y 50 mm Z 25 mm T -5 - 70° R 360°
Specimen size	max. 100 mm diameter max. 10 mm height

2.)

This SEM is going to be the primary training SEM for Nanomicroscopy Center SEM users.

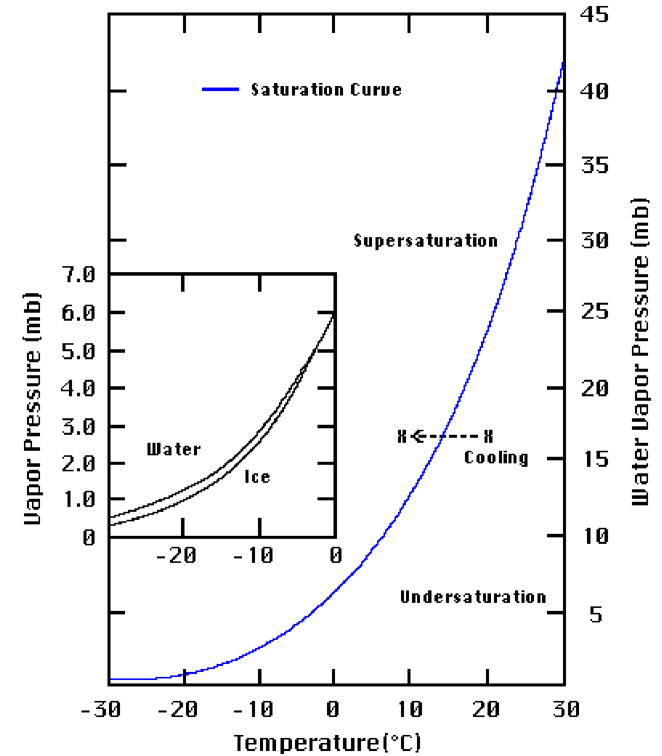
Zeiss Sigma VP Information Card	
Manufacturer	Zeiss
Model	Σigma VP
Emitter	Schottky FEG
Installation	2011
Detectors	In-column SE ETSE BSE (5 segment) VPSE STEM
Resolution	1.3 nm @ 20 kV 2.8 nm @ 1 kV 2.5 nm @ 30 kV (VPSE)
Acceleration voltage	0.1 - 30 kV
Probe current	4 pA - 20 nA
Operating modes	High vacuum (10^{-5} Pa) Variable pressure (2 - 133 Pa, N_2)
Stage	Motorized 5 axis (compucentric) X & Y 125 mm Z 50 mm T -10 - 90° R 360°
Specimen size	max. 250 mm diameter max. 145 mm height

3.)

Zeiss EVO HD15 Information Card

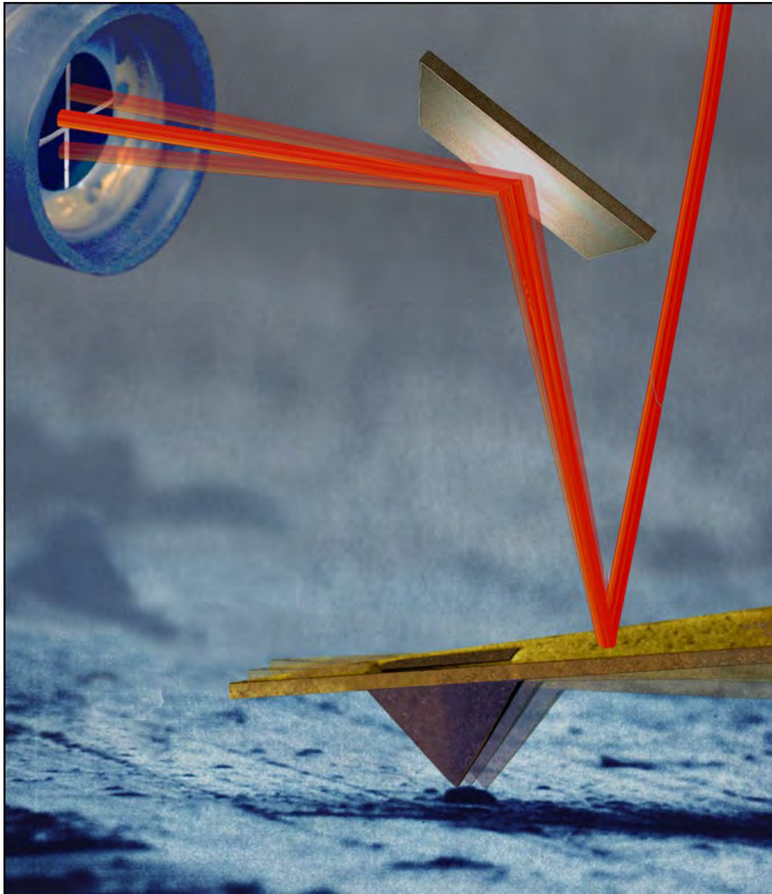
Manufacturer	Zeiss
Model	EVO HD15
Emitter	LaB_6 with “HD” technology
Installation	2011
Detectors	ETSE BSE (5 segment) VPSE EPSE STEM
Resolution	1.9 nm @ 30 kV 3 nm @ 30 kV (1 nA) 5 nm @ 3 kV 8 nm @ 1 kV 3 nm @ 30 kV (VPSE)
Acceleration voltage	0.2 - 30 kV
Probe current	0.5 pA - 1 uA
Operating modes	High vacuum (10^{-5} Pa) Variable pressure (10 - 133 Pa, N_2, air, H_2O) Extended pressure (10 - 3000 Pa, N_2, air, H_2O)
Stage	Motorized 5 axis (compucentric) X & Y 125 mm Z 50 mm T -10 - 90° R 360°
Specimen size	max. 250 mm diameter max. 145 mm height

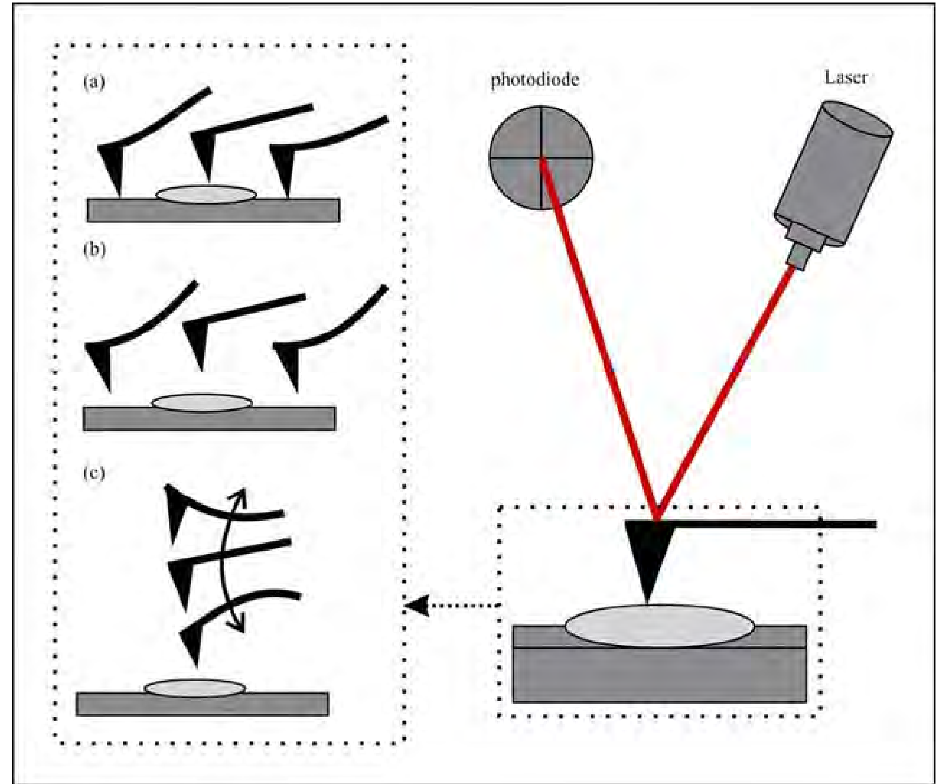
The vapor pressure of ice and water between -30° and 30° (mb = millibar). (Berner and Berner 1987)



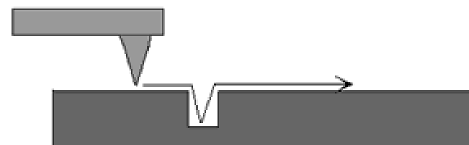
Scanning probe microscopy (SPM) historically called AFM)

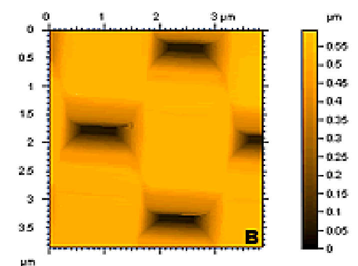
a) Contact mode
b) Non-contact mode
c) Tapping mode (tip is oscillating on surface)





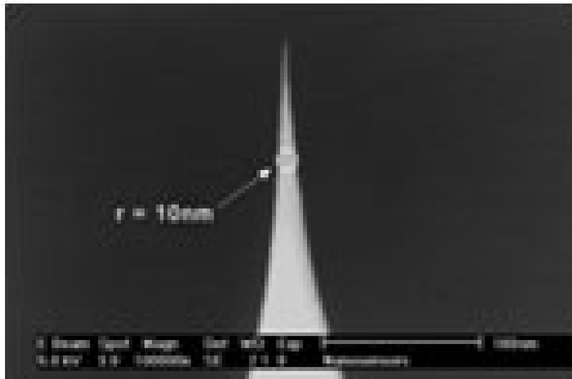
In AFM we use sharp tip to scan the sample surface to obtain information about surface topography and other properties (chemical, mechanical hardness, electrical etc.)



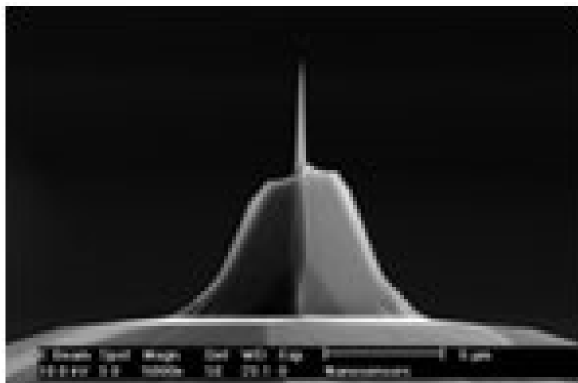


Examples AFM probes

Standard Tip radius typically 8-10 nm
(cost 10 -20 euros per each)



Super sharp tips: Tip radius is
typically 1nm (~100 €)



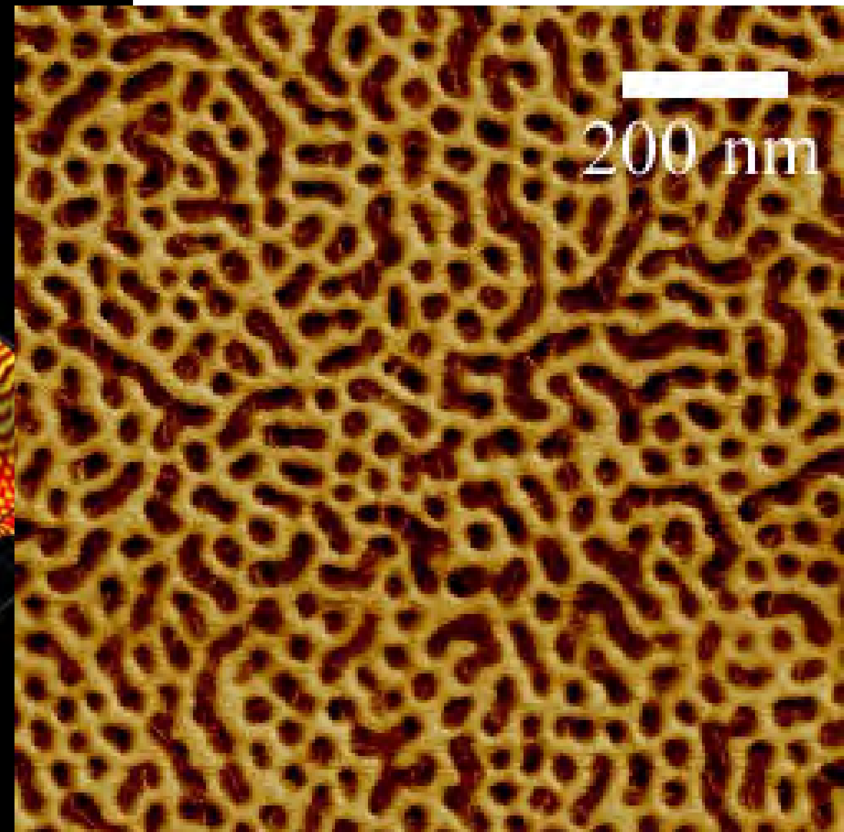
High aspect ratio tips:
The length of the high aspect portion of
the tip is larger than 1.5 μm

NMC instruments:
AFM: Veeco Dimension 5000 SPM (installed 1/2008)

- Originally designed for semiconductor imaging.
- Capable of loading samples up to 350 mm in diameter.
- Large scanning area ~90 x 90 μm.
- Automatic measurement for up to 100 pre-selected areas.
- High pixel-density image capture 5120 x 5120 points.

High resolution, easy to use, large samples..

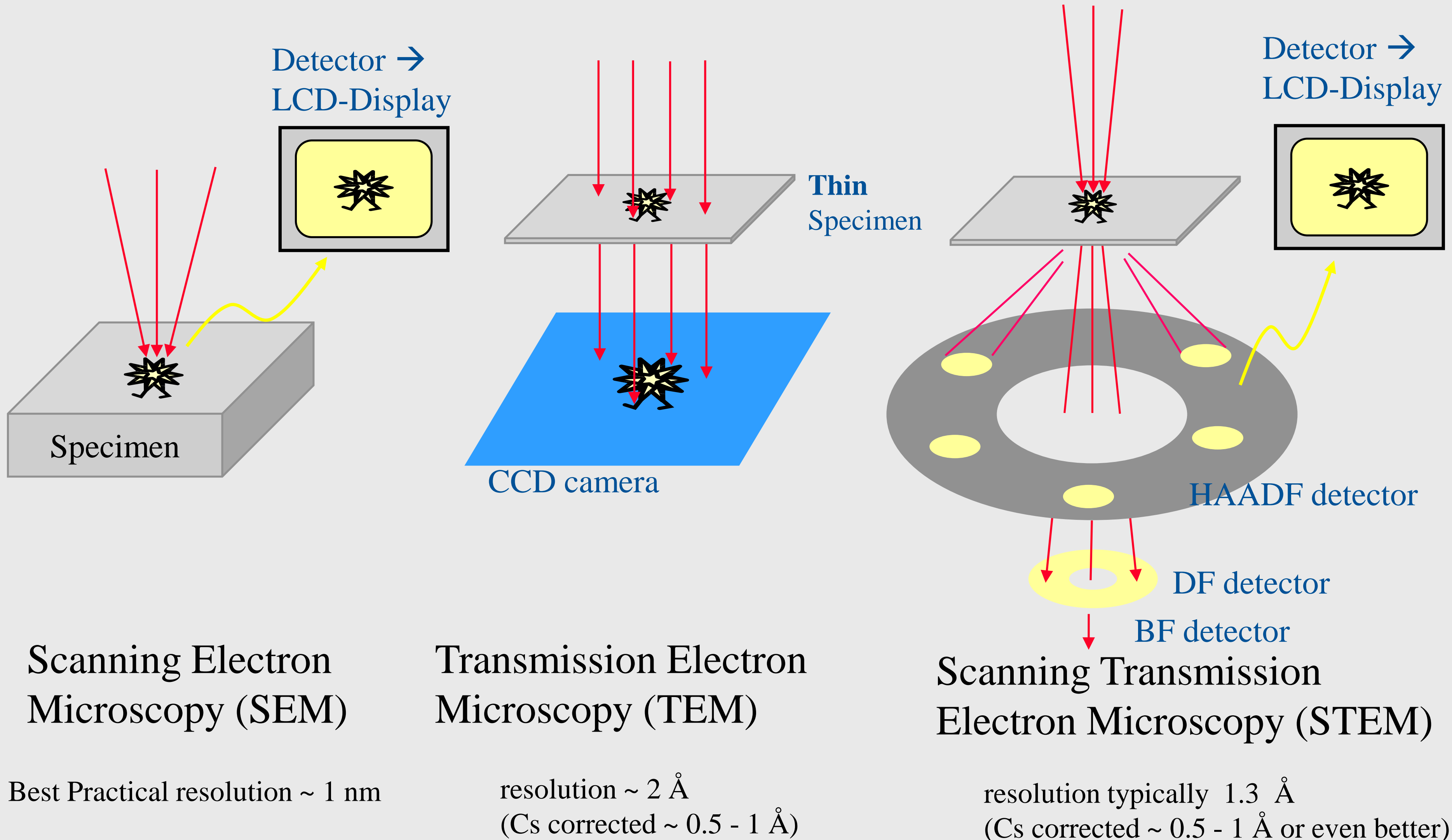
AFM image examples - Polymer films:



This image dark domains are polyethylene (soft) and gray domains Polystyrene which is glassy polymer.

Topography (height) and composition imaging (soft and hard domains) – in tapping mode imaging one can get at the same time the surface topographical information and contrast due to mechanical properties (example hard of soft domains)

Brief introduction to the electron microscopy

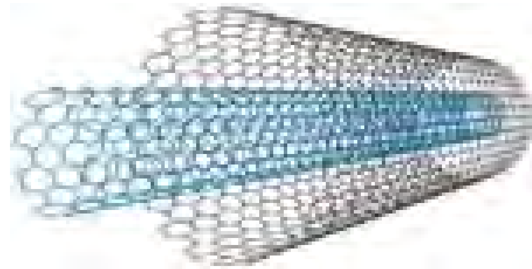


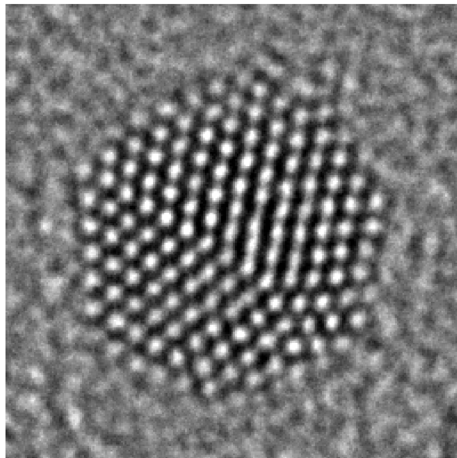
Best Practical resolution ~ 1 nm

resolution ~ 2 Å
(Cs corrected ~ 0.5 - 1 Å)

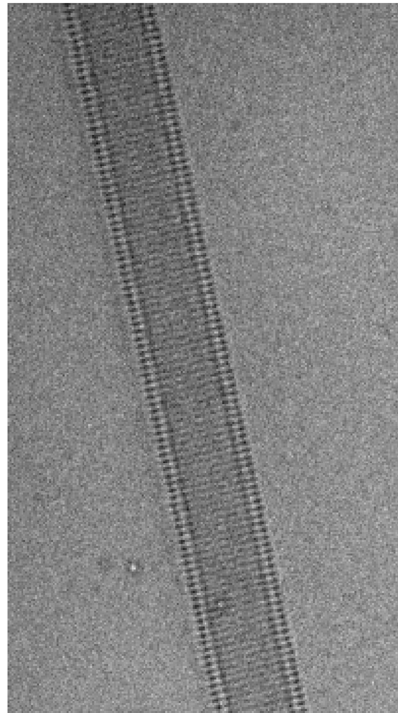
resolution typically 1.3 Å
(Cs corrected ~ 0.5 - 1 Å or even better)

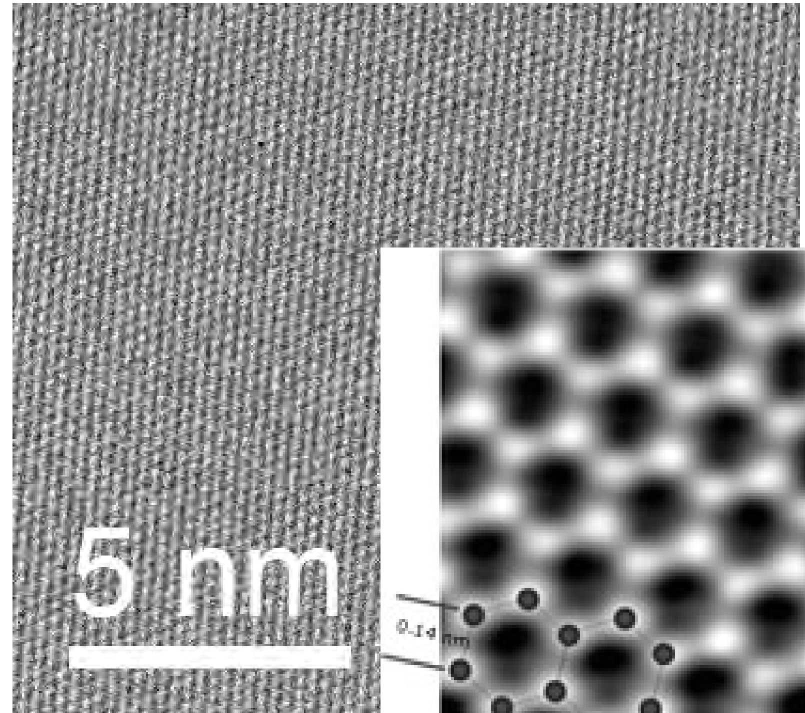
Nowadays it is possible to obtain atomic resolution images even from low atomic number materials such as Carbon nanotubes and single graphene sheets..





High resolution TEM image from Gold nanoparticles

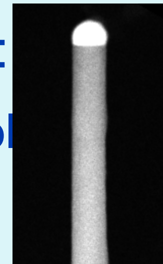


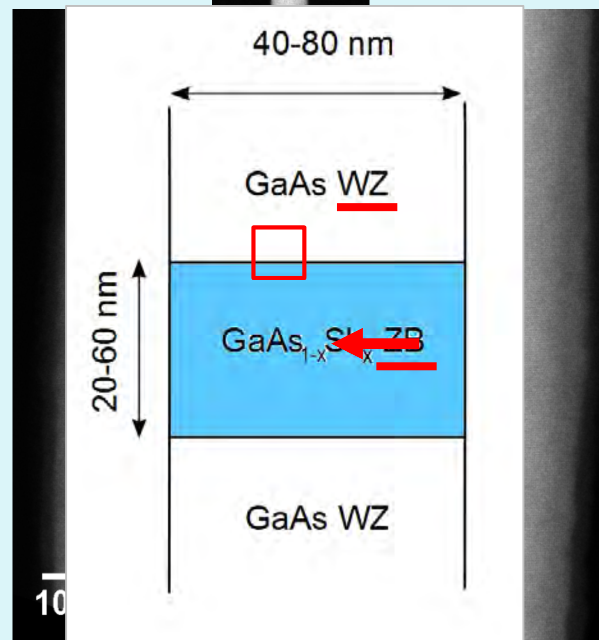


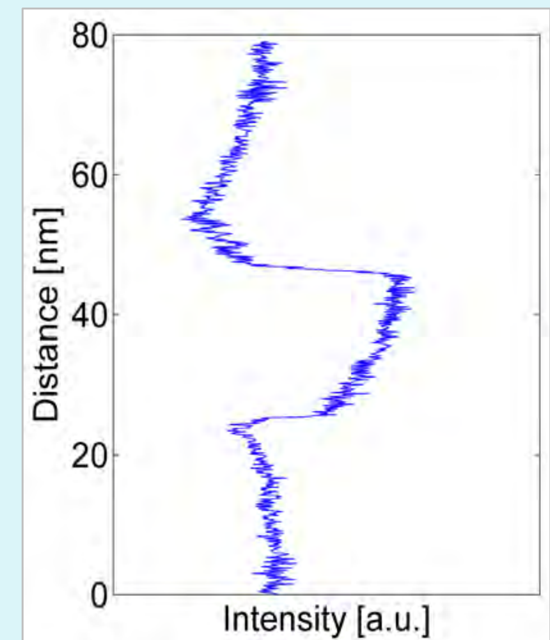
Another examples: Heterostructured GaAs nanowires

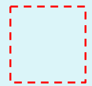
- GaAs nanowires (NWs)
- Molecular beam epitaxial (MBE) growth
- Pure GaAs NWs: Wurtzite (**WZ**) structure (纤锌矿结构，六方相)
- Partially alloyed with Sb:

  $GaAs_{1-x}Sb_x$ insert : Zinc bl **ZB**) structure (闪锌矿结构, 立方相)

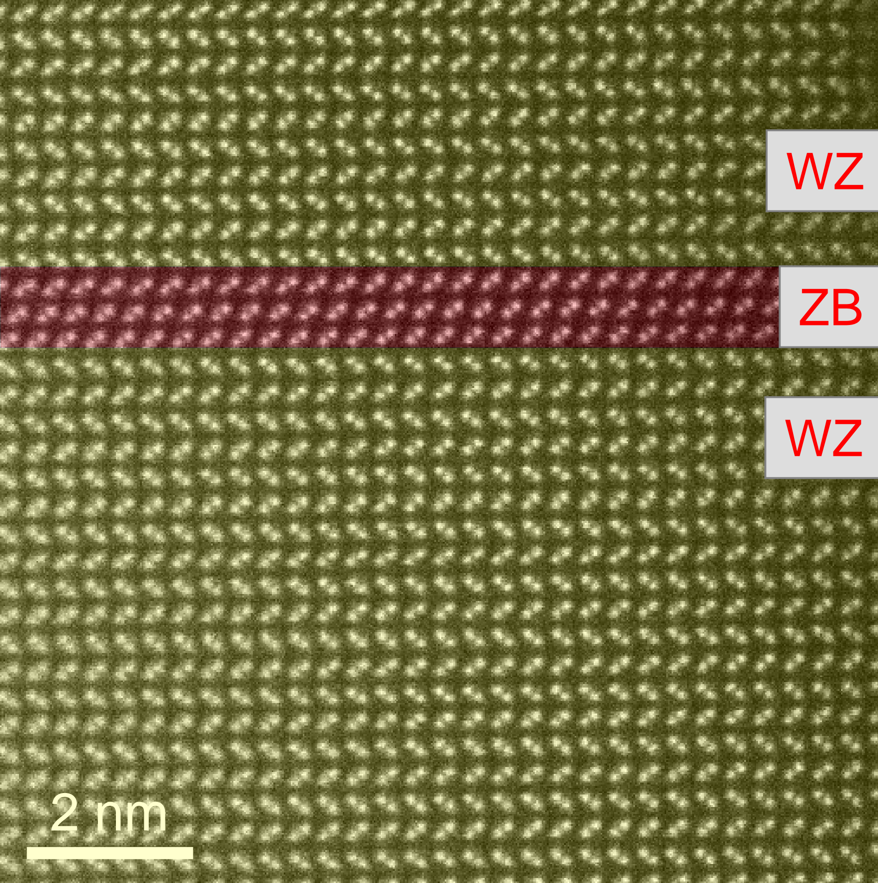


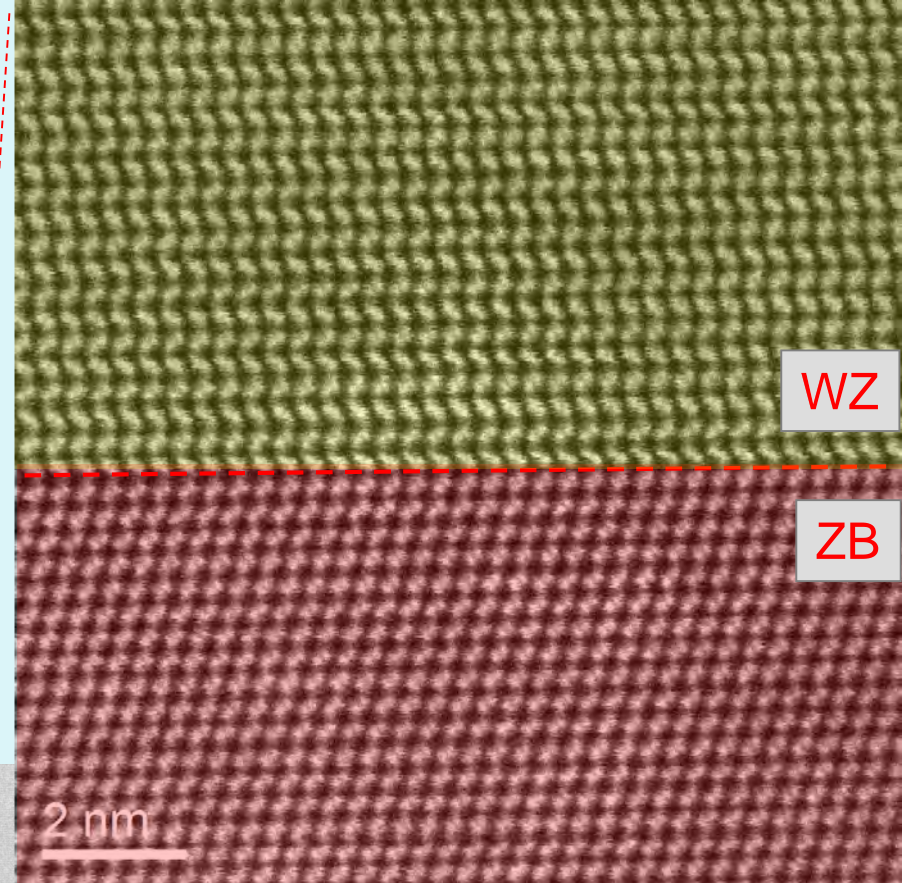


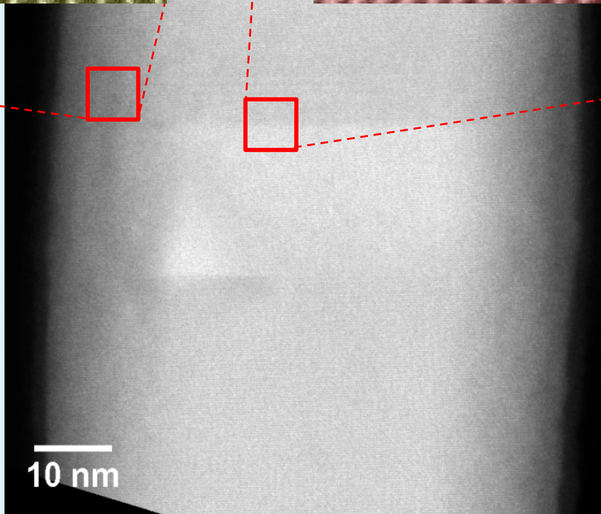




Sample: Hanne Kauko, NTUT





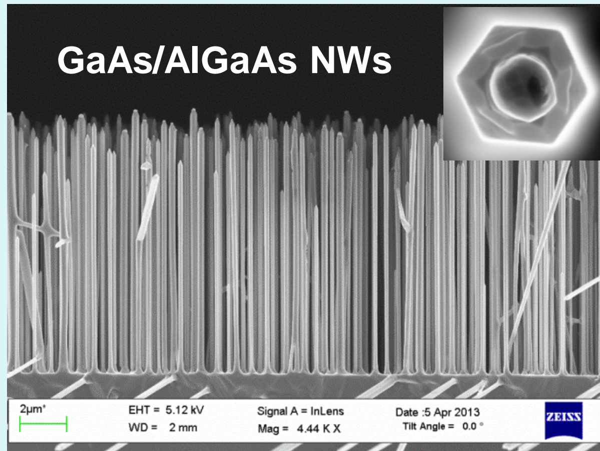


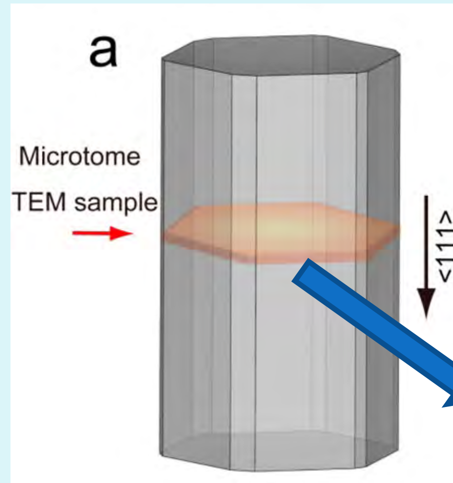
- WZ and ZB coexist;
- Ga and As columns well separated, 1.55 Å;
- Ga and As columns has different intensities.

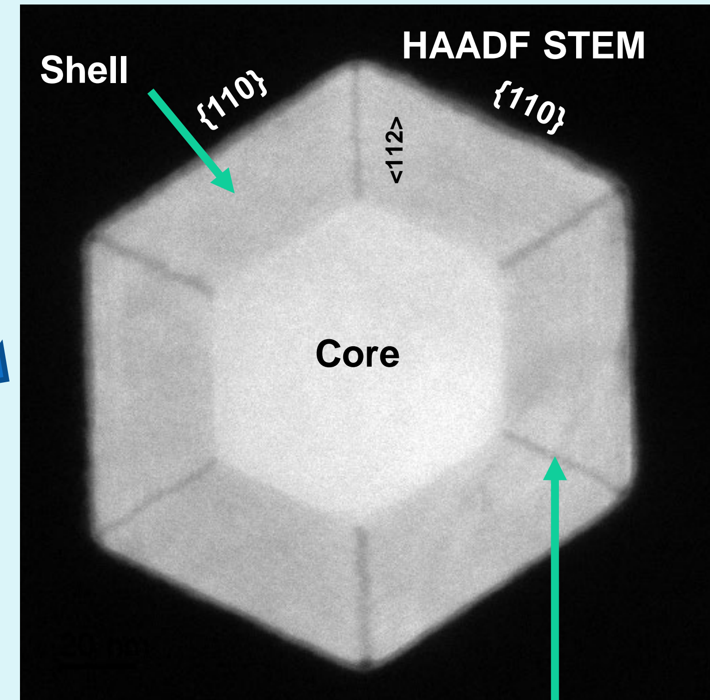
Sample: Hanne Kauko, NTUT

- Wurtzite structure [11-20] orientation
- Zinc Blende structure [110] orientation

TEM characterization of III-V nanowires (HAADF STEM)

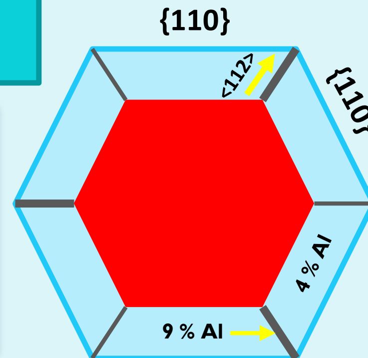






Core-shell GaAs/AlGaAs nanowires grown using MOCVD
60 nm core, 30 nm shell

Al segregation in AlGaAs shell along 6 dark lines
3-fold rotational symmetry (3 thin and 3 thick lines

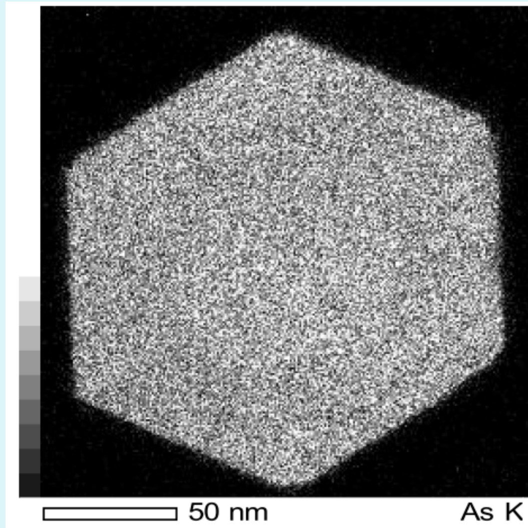


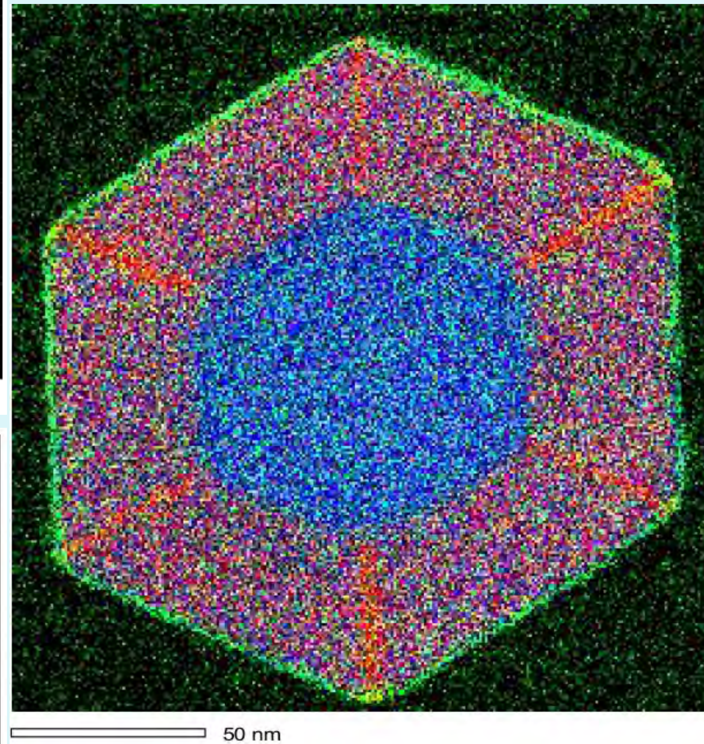
Dark lines (Al segregation)
Cross section view

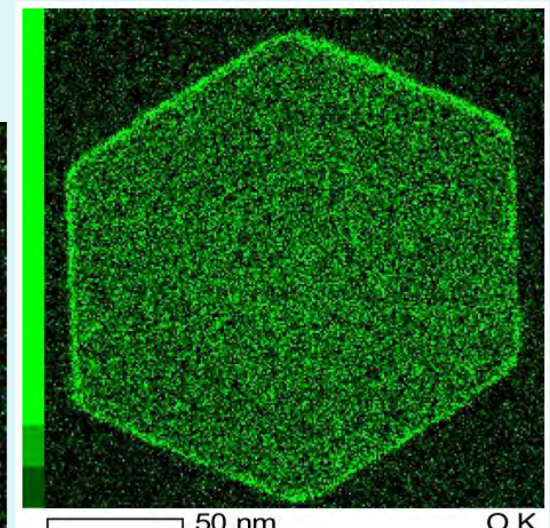
Dhaka et al., Nano Letters, 2013, 13 (8), pp 3581–3588

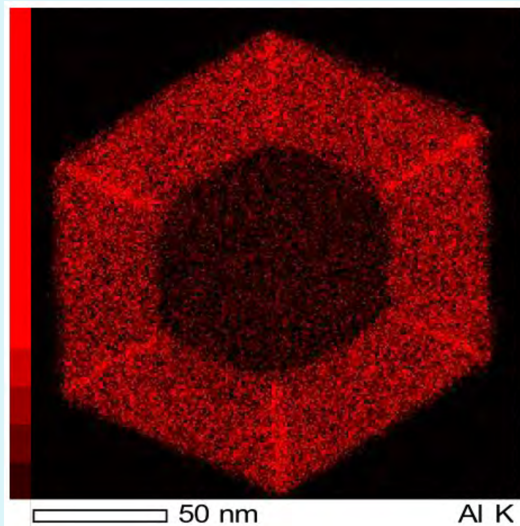
Elemental Mapping of cross-sectional GaAs nanowires

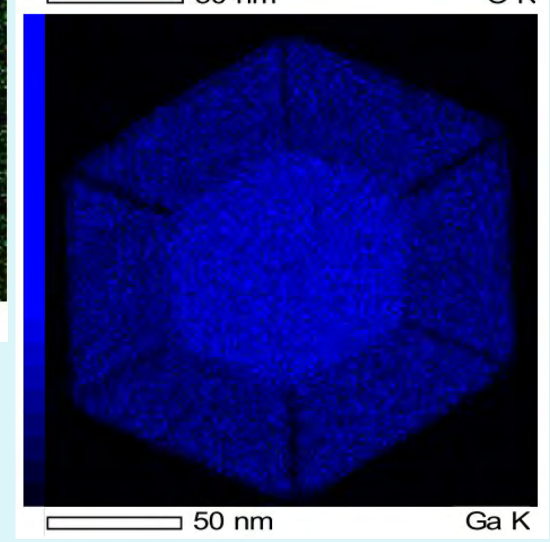
by analysing the X-ray signals generated in the sample – atomic composition can be analysed (EDX-spectroscopy)





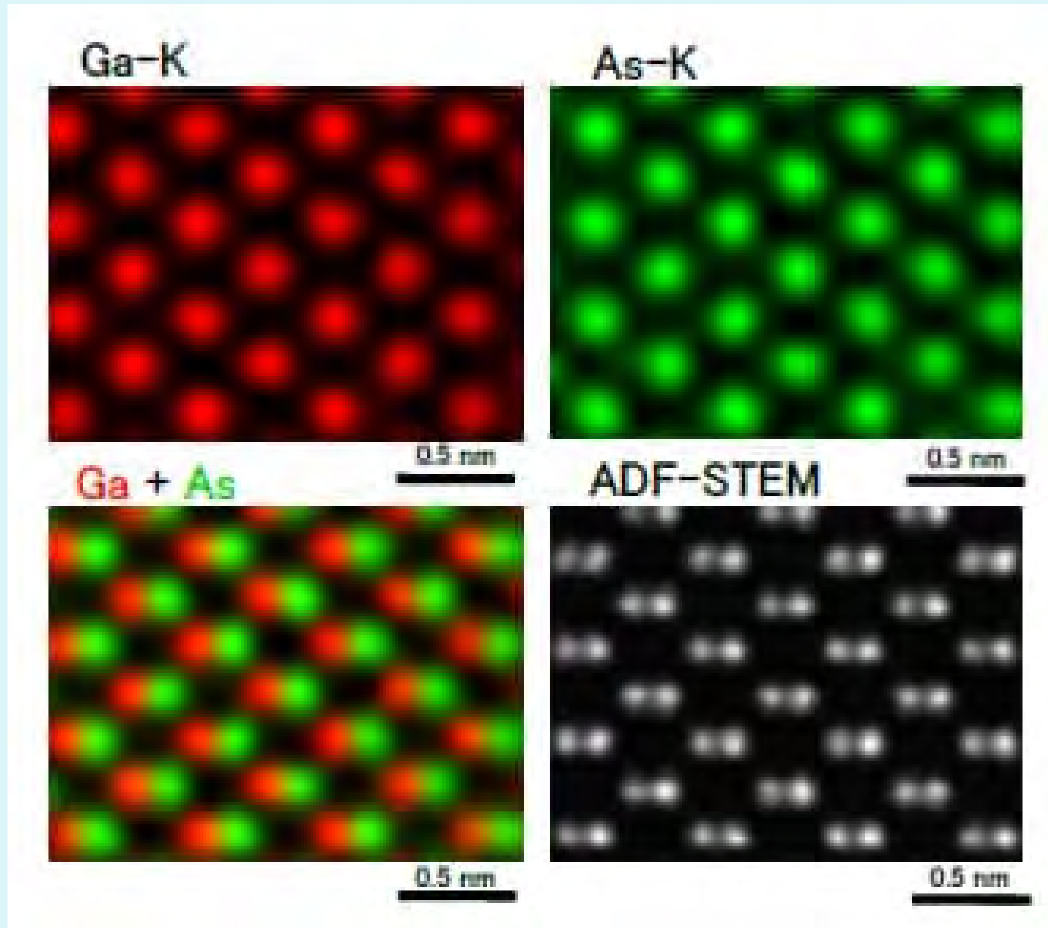






Sample: Veer Dhaka, Aalto U.

Atomic resolution elemental analysis is also possible with modern high resolution microscopes..



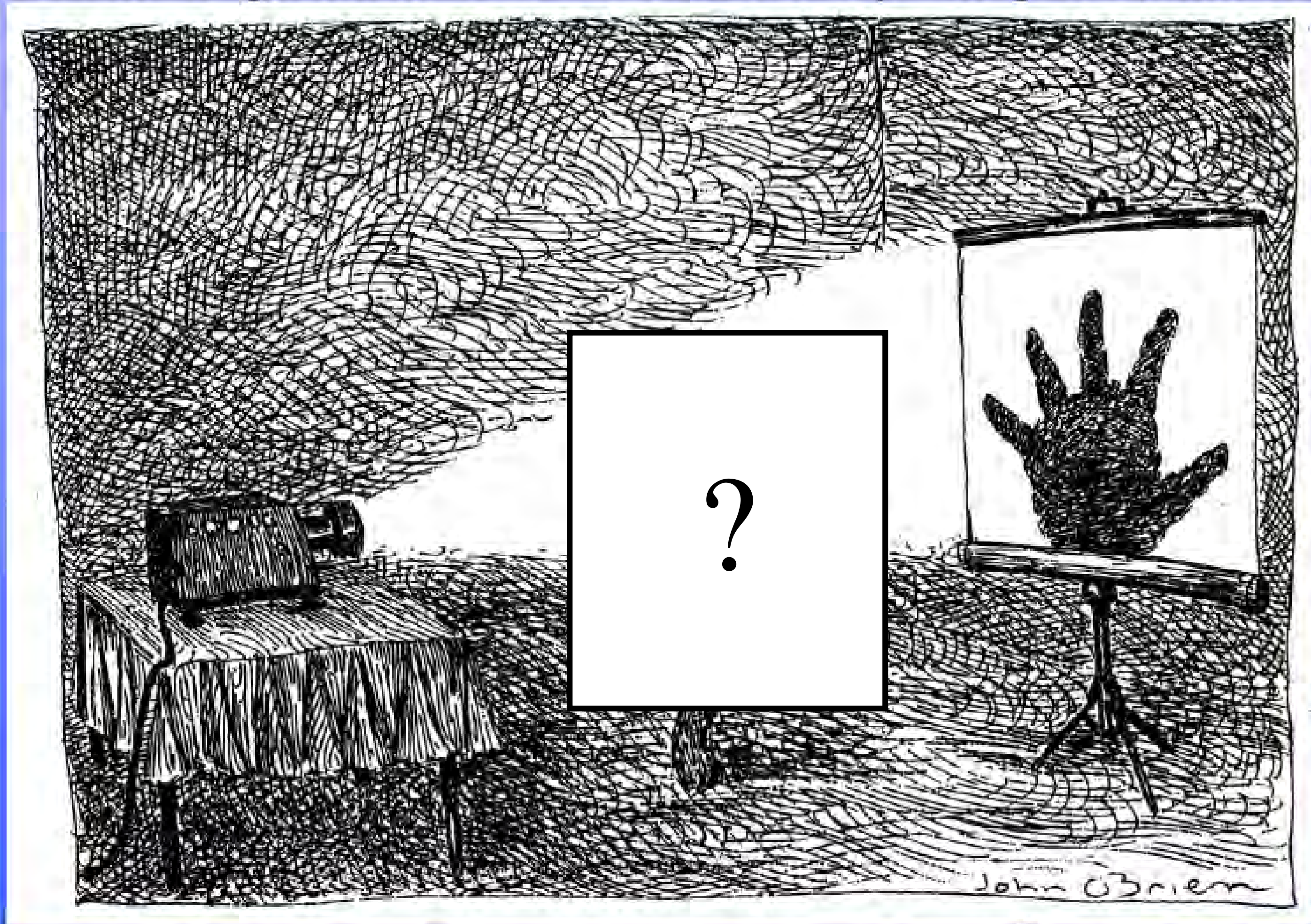
Atomic-level-resolution EDX elemental map of a gallium arsenide monocrystal <011>

3D tomography – Why important?

(Jani Seitsonen will give lecture on this topic later in the spring)

TEM image is projection from 3D → 2D

- Micrograph represents a projection image of the specimen. So features at different depths in the structure are all superimposed. Hence cannot generate a 3D structure by simple visual inspection.



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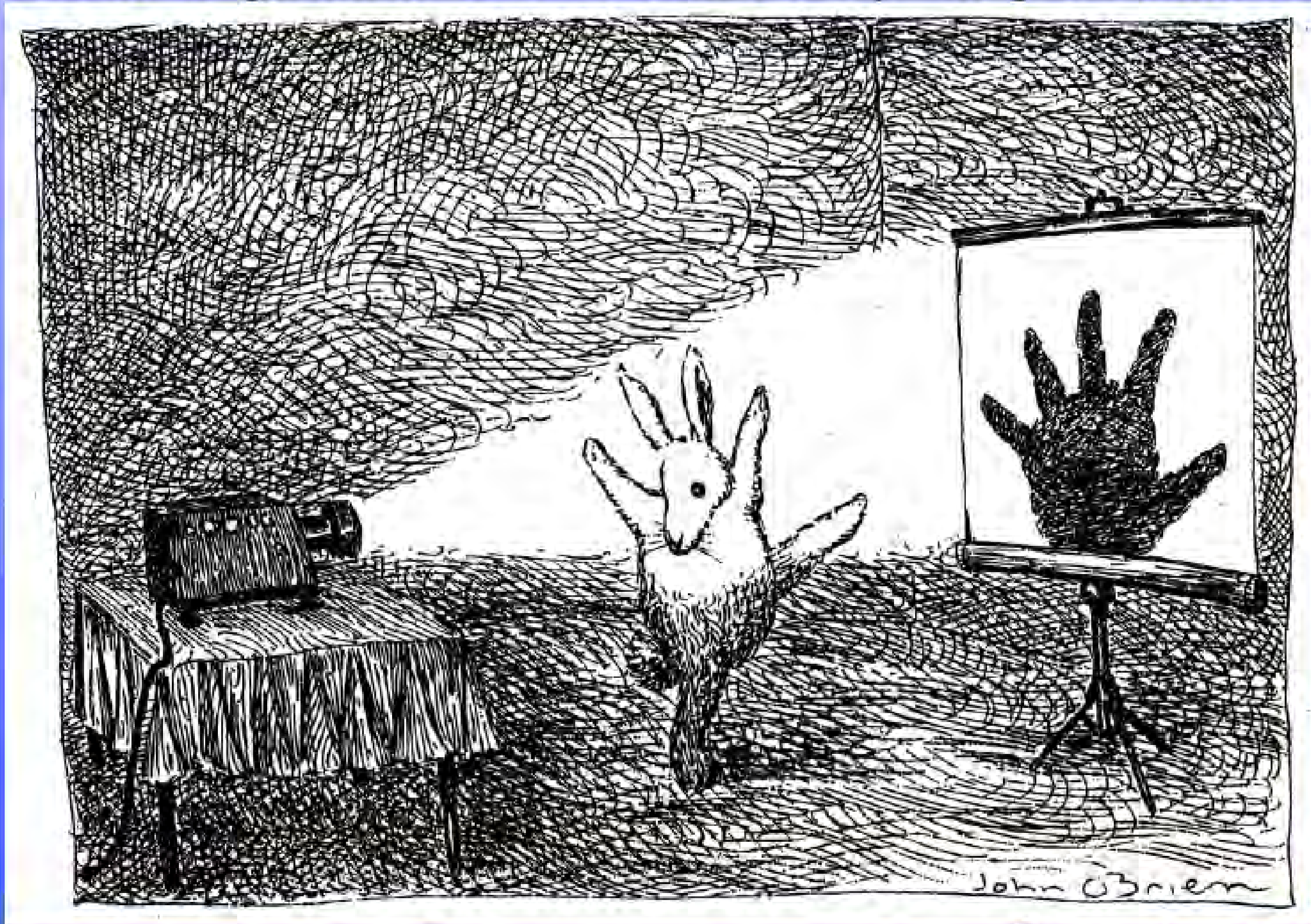
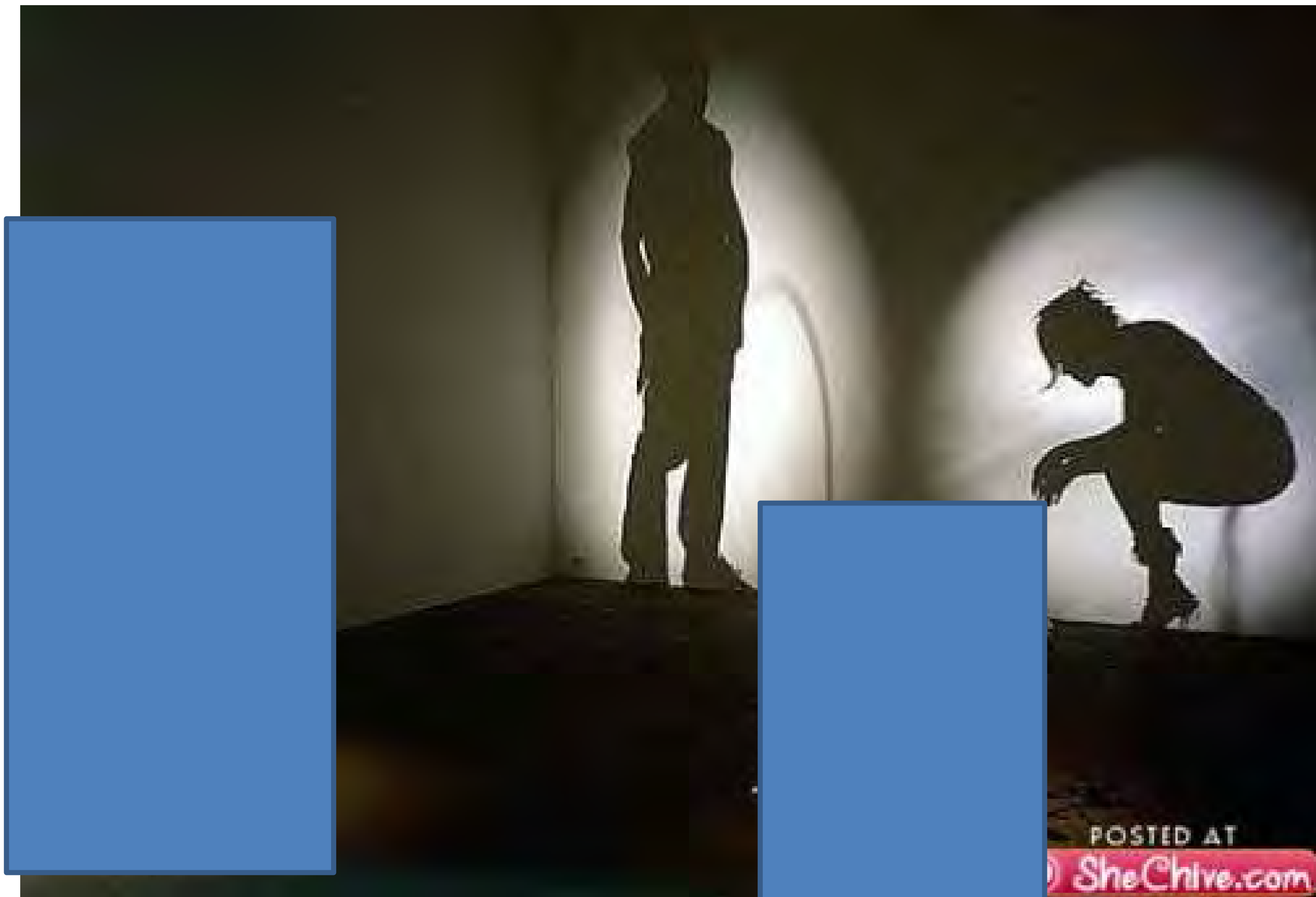


Another example of projection from 3D → 2D



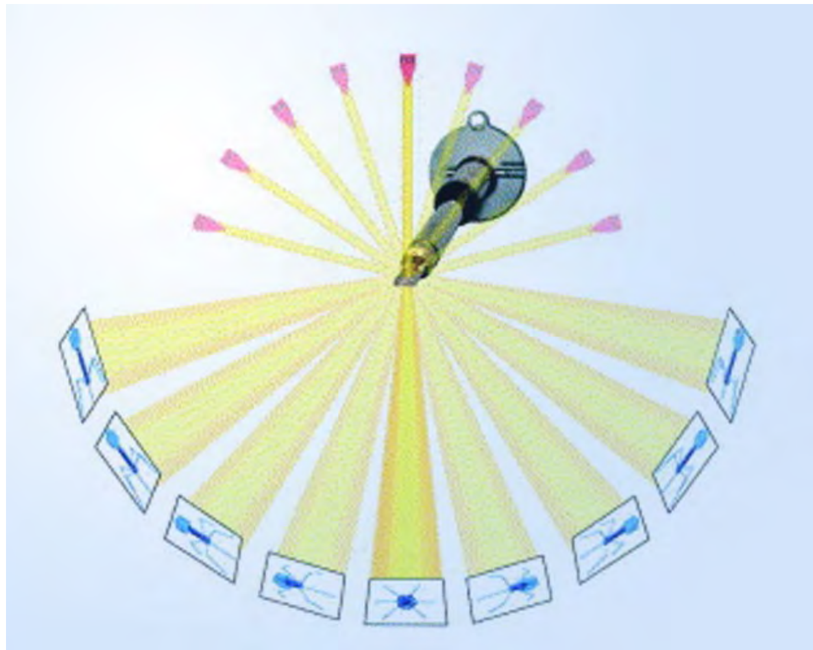
Another example of projection from 3D → 2D

POSTED AT
SheChive.com

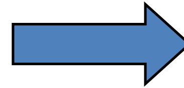
When 2D-projections are not enough: TOMOGRAPHY (or single particle reconstruction)

Principle of electron tomography:

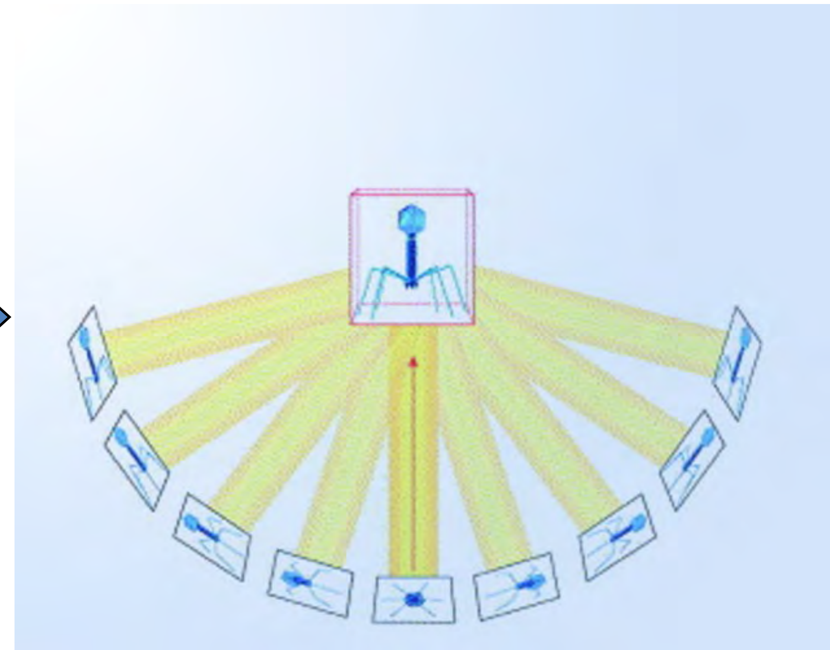
DATA COLLECTION



3D object => 2D-projections



RECONSTRUCTION



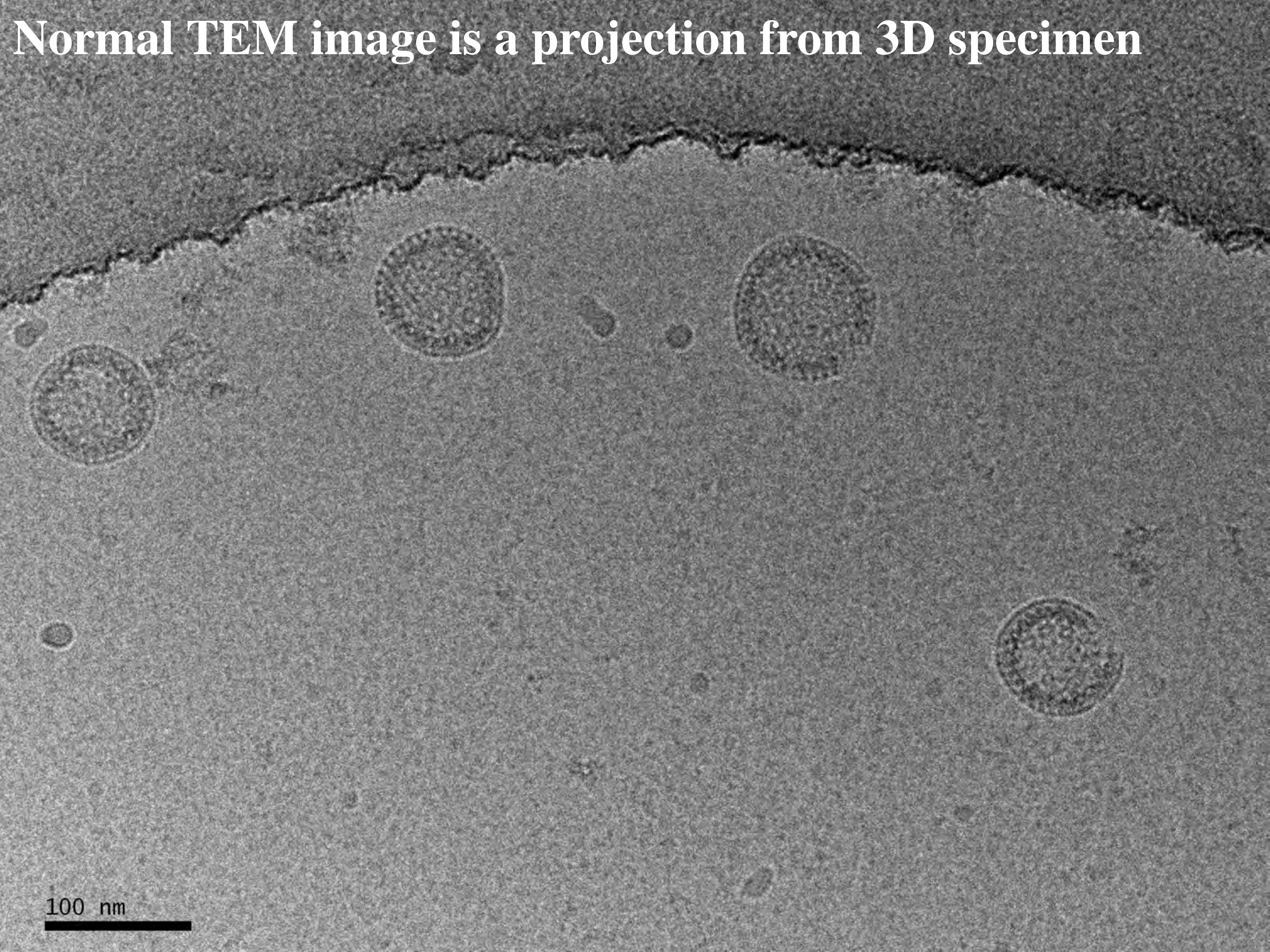
2D-projections => 3D-reconstruction

Cryo-Tomography

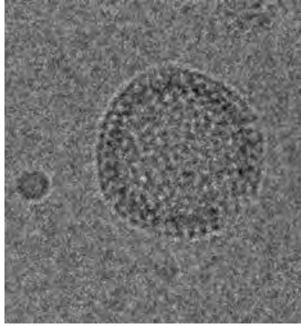
Case study: Cryo-EM structure of M-PMV VLPs

Sample: *in vitro* assembled **V**irus-**L**ike **P**articles of **M**ason-**P**fizer **M**onkey **V**irus

(Pasi Laurinmäki, Institute of Biotechnology
University of Helsinki)


Normal TEM image is a projection from 3D specimen
100 nm

3D cryo-EM: aligned tomographic tilt series

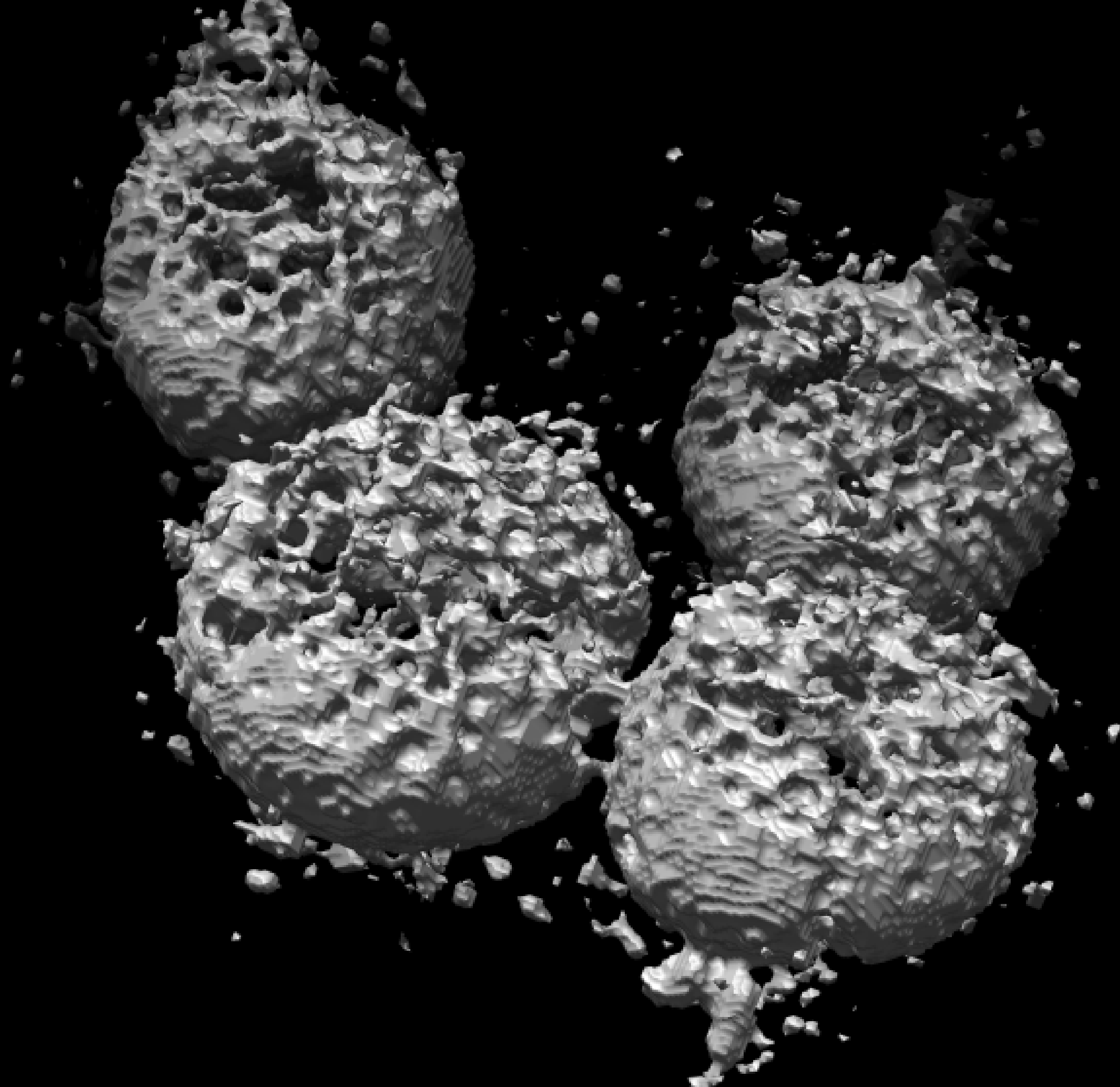


Low-dose series of *in vitro* assembled proCANC M-PMV particles

Diameter of the large round hole is about 2um

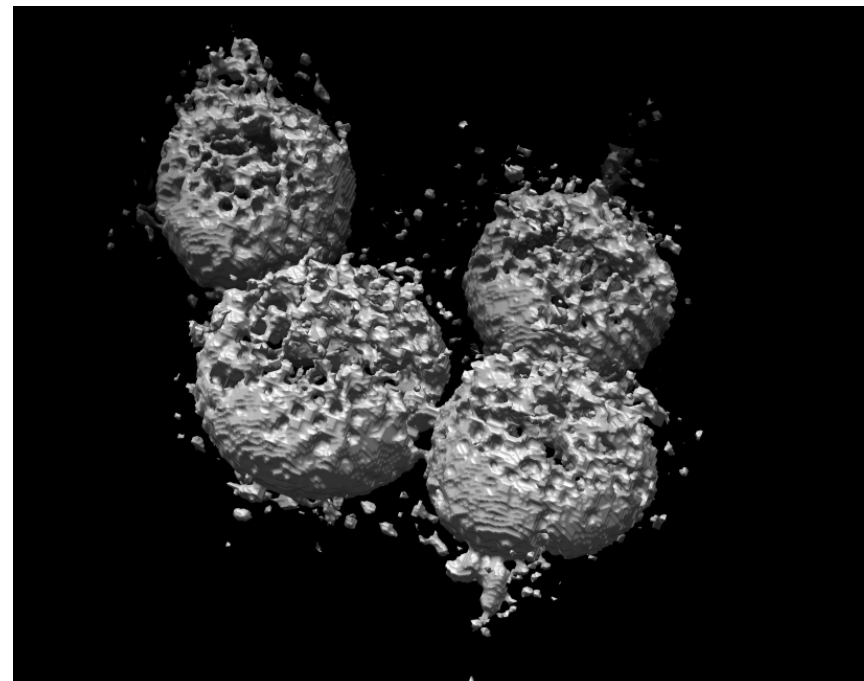
Dense dots are 10nm gold particles used as markers to align the images

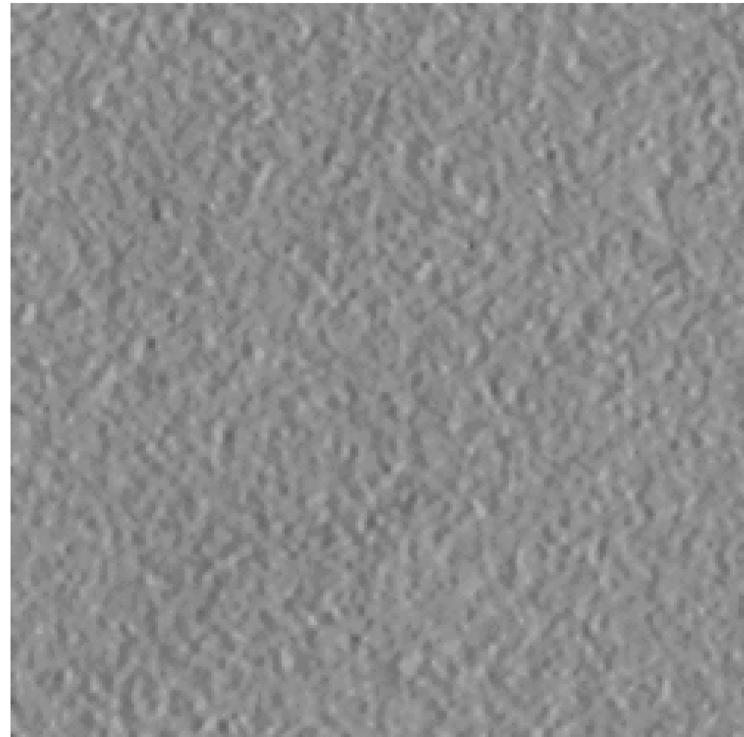
Then we just take images from -70 to +70 degree tilt and do 3D recontruction



Surface representation of the reconstructed density

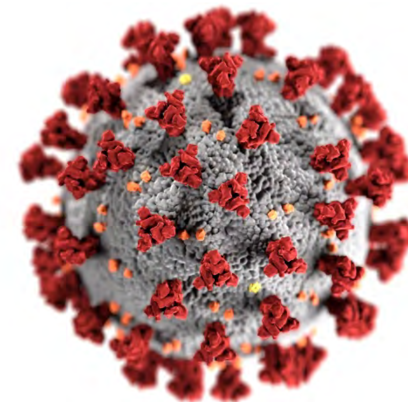
Serial sections through the reconstructed density





3D structure visualization by using computers and 3D glasses..

Using similar 3D imaging methods also the structure of the **SARS-CoV-2** virus was resolved (single particle reconstruction)



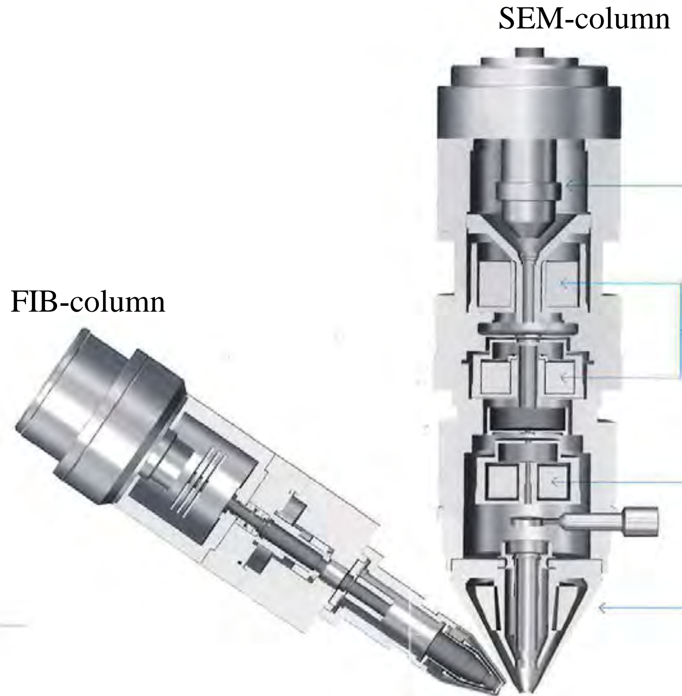
Sample preparation for TEM: THIN SPECIMENS

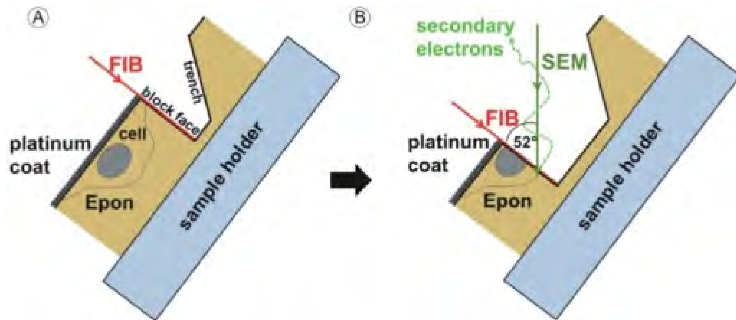
A major limitation of the TEM is we need thin specimens.
Methods to prepare thin specimens exist for almost all materials, and we talk about them specific lecture. But as a general rule, the thinning processes that we use do affect the specimens, changing both their structure and chemistry. So you need to be aware of the drawbacks of specimen preparation and learn to recognize the artifacts introduced by standard preparation methods.

Ultramicrotoming, ion milling, Focused ion beam, cryo sample preparation etc..

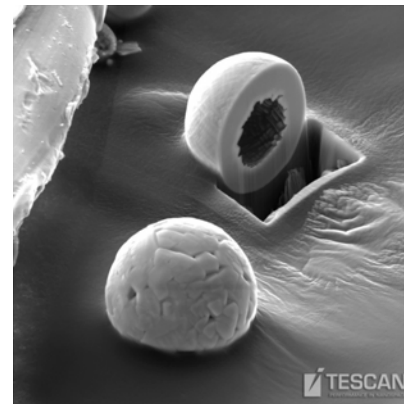
Planar Surface Preparation for SEM Cross Section Viewing

New Dual beam focused ion beam system in Nanomicrosopycenter (installation finnished by June 2019): Main applications TEM sample preparation, cross-section imaging and 3D imaging





3D imaging



Here is the example of cross-section imagin: FIB column is used to cut the cross-section and SEM column for imaging

Example: FIB cross-section imaging from soldering tin particles

TEM sample preparation using Focused ion beam..

FIB is very usefull for hard materials thin cross-section sample preparation – after final polishing << 100 nm meter thick sample can be done