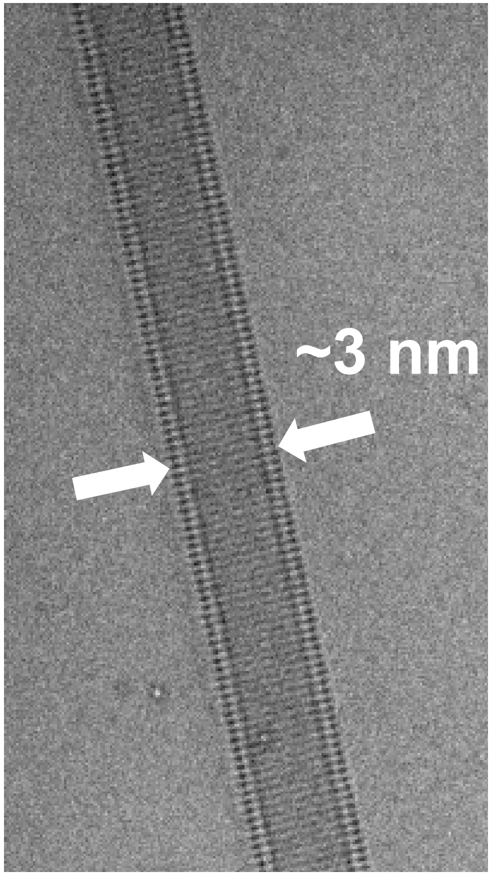
PHYS-E0525 Microscopy of Nanomaterials P (5 cr)



Prof. Janne Ruokolainen (nanotalo 102)

Email: Janne.Ruokolainen@aalto.fi

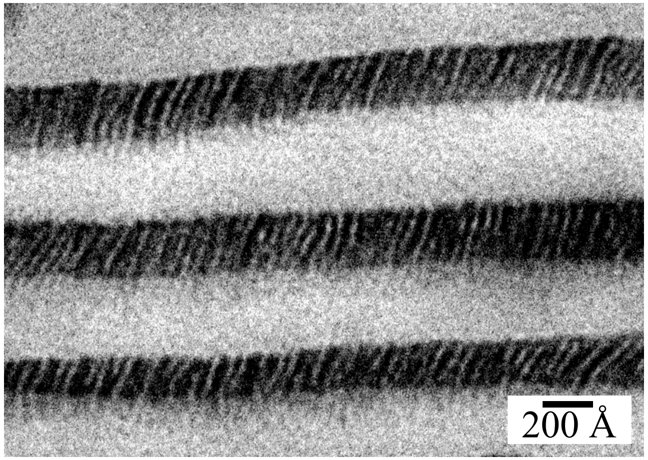
First lecture 17.1. 2023

Tuesday 12.15 – 14

Assistant: Shruti Shandilya Email: shruti.shandilya@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..



Lectures:

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

Tentative Schedule

17. 1. Introduction & Nanomicroscopy center (JR)
24. 1. TEM Basics (JR)
31. 1. Advanced TEM 1 (Hua)
7. 2. Advanced TEM 2 (Hua)
14. 2. Advanced TEM 3 (Hua)
21. 2. *no lecture (exam period at Aalto)*
28. 2. Cryo-TEM and Soft matter Sample preparation (JR)
7. 3. SEM (Ramzy)
14. 3. 3D-TEM-Tomography (Jani)
21. 3. FIB and Sample preparation (Lide)
28. 3. STM/AFM (Peter)

Summary:
Intro, Basic TEM, Cryo TEM ~ 3 lectures
Advanced TEM 2-3 lectures
(High resolution TEM and STEM, diffraction, spectroscopy EDX, EELS)
STM/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

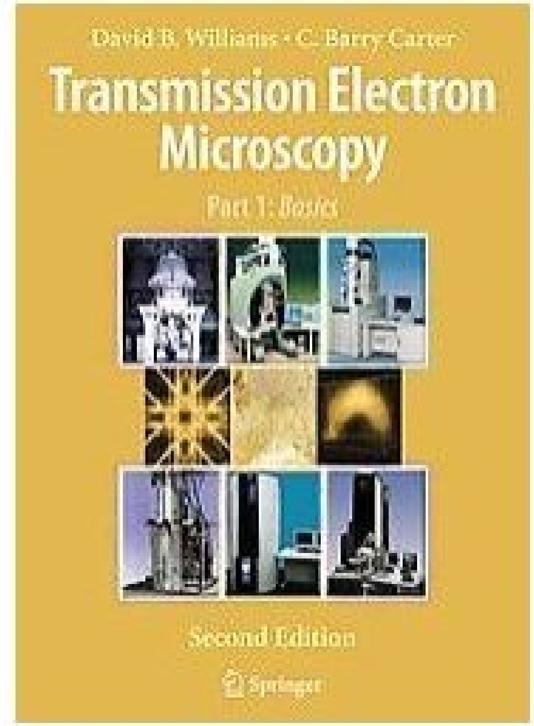
This book has *more theory,* and it also has lots of technical information about microscope (vacuum systems, electron sources, sample holders etc..) and it also has 3 other books for Advanced TEM (Part 2: Diffraction , Part 3: high resolution imaging and Part 4: Spectroscopy)

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..)

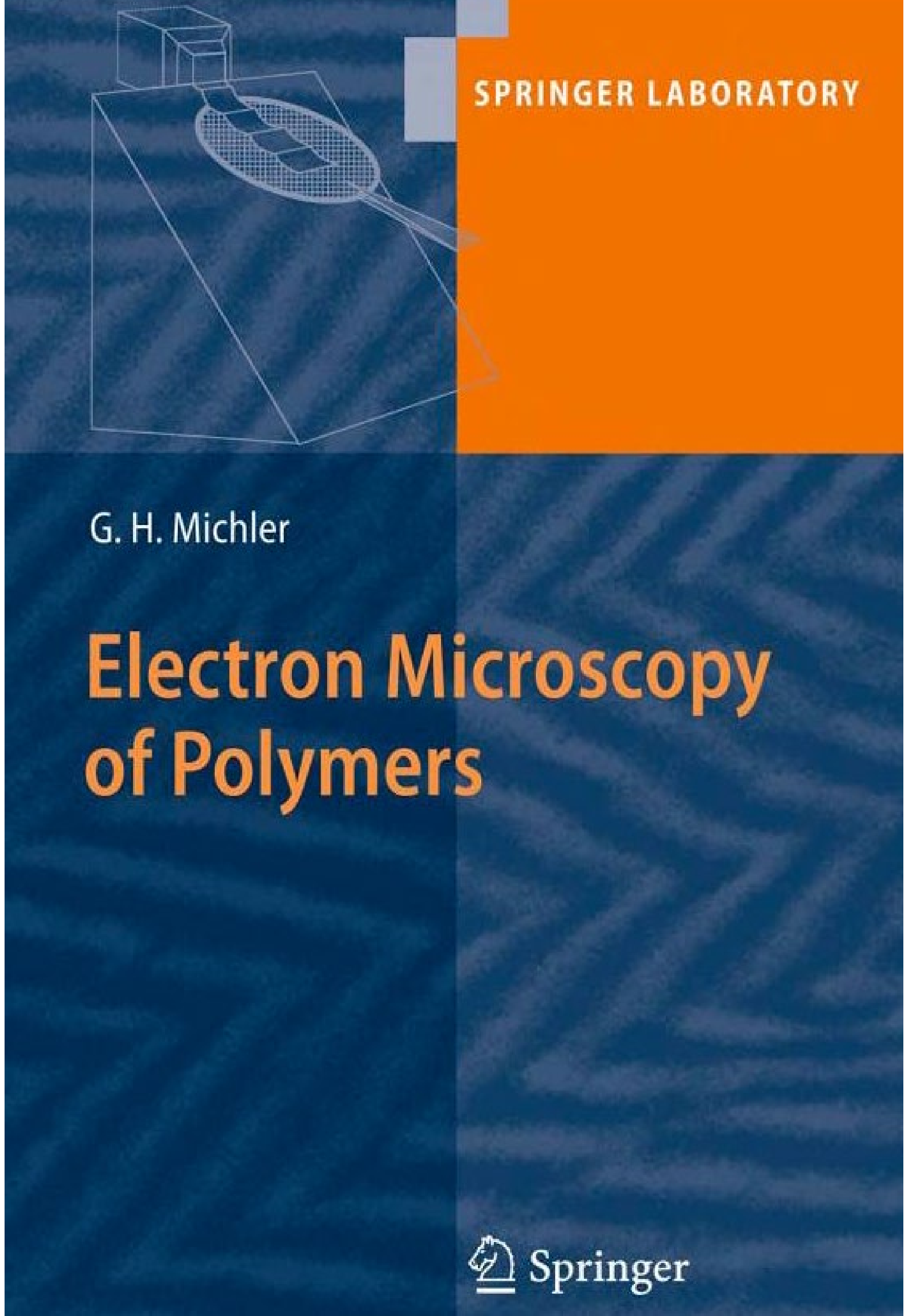
Book 3: A practical Guide to Transmission Electron Microscopy (Zhiping Luo)
This book is **most practical** especially **those who want to learn how to operate microscopes** Chapter **1-3** and (partly chapter 5?) and chapters **7-9**. (chapters 4, 5, and 6 are diffraction and perhaps more advanced level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

Book 1: Part 1: Basics

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



Book 2:



SPRINGER LABORATORY
G. H. Michler
Electron Microscopy of Polymers
Springer

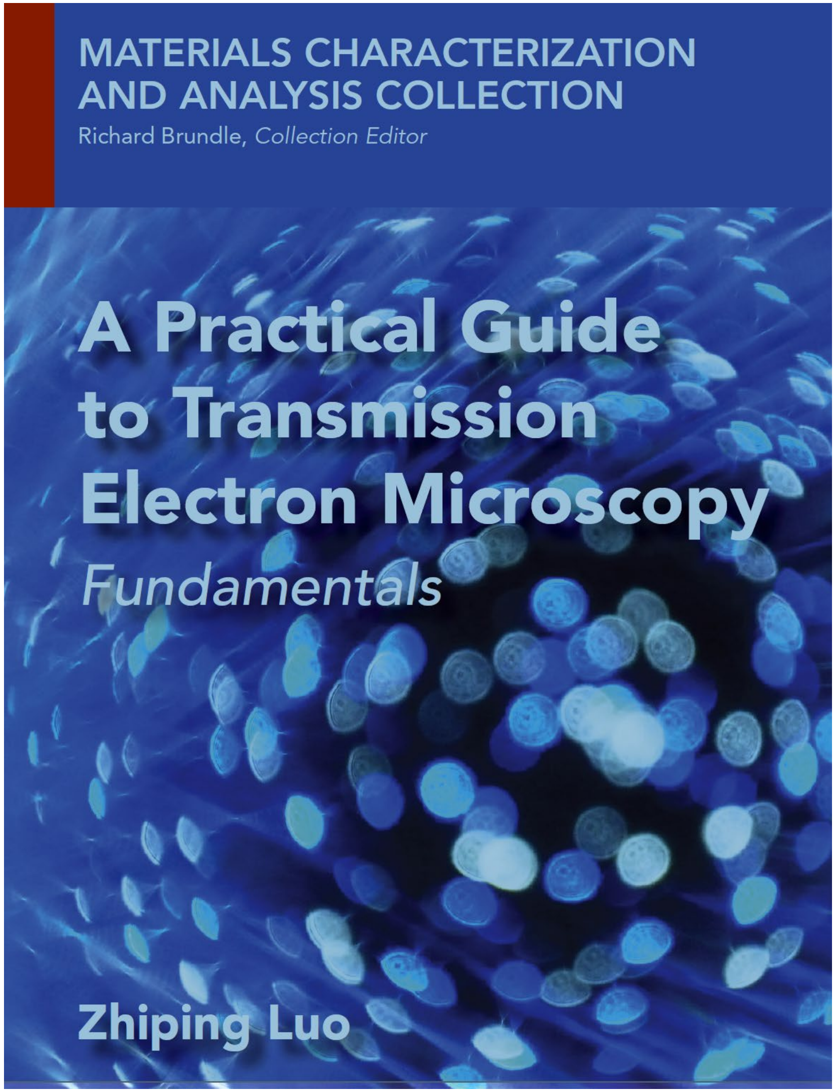
Part I Techniques of Electron Microscopy

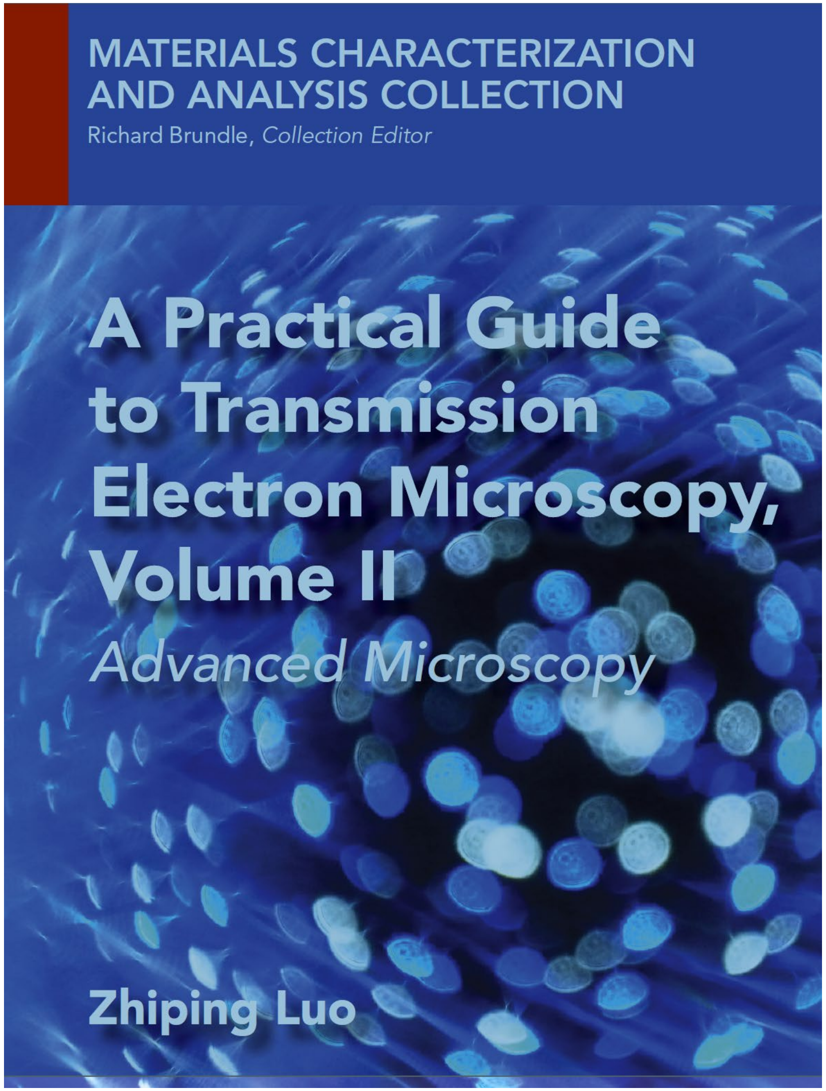
Part II in this book is mainly soft materials sample preparation – i.e. quite useful for many of you

Part III Main Groups of Polymers

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

Book 3: A Practical Guide to Transmission Electron Microscopy (2 parts)





Book 3: A Practical Guide to Transmission Electron Microscopy: ***Fundamentals*** *(chapters 1,2,3)*

And A Practical Guide to Transmission Electron Microscopy,
Volume II Advanced Microscopy (chapters 7,8 and 9)

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**)

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 6. 2. 2023

Basic exercises – Demos:

(6 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)

(3 persons per group)

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

Independent Small group exercises

(without supervision.. 3 person per group)

(2 exercises)

1) TEM imaging
2) SEM imaging
3) …

To apply for the Laboratory Course – Answer the following questions just writing a short email and send it to the nmc-contact-sci@aalto.fi deadline Friday 6. 2. 2023

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..
+ some 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 2005 - 2020
New manager 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.

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

- 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..) >15 M€

Key Instruments:

- First lens *aberration corrected* transmission electron microscope in Finland with approximately 1 Å resolution *(JEOL 2200FS)*

(new 300 kV microscope will replace this 2024)

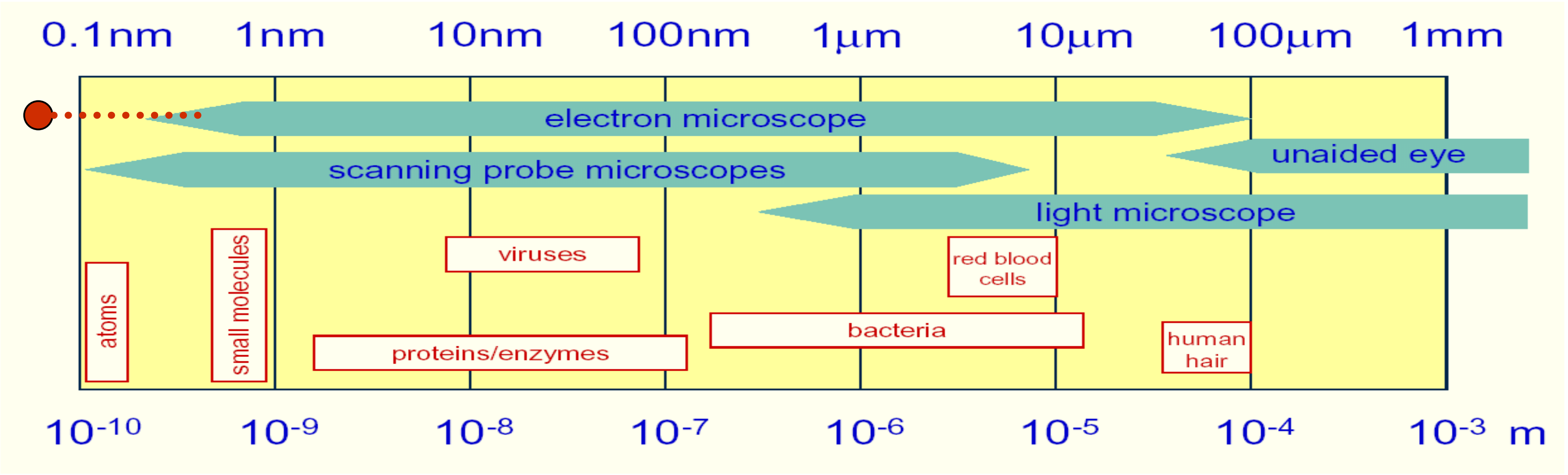
- First *Liquid helium cryo*-transmission electron microscope in Finland (operating at -255 ^{o}C or -187 ^{o}C) *(JEOL 3200FSC)*

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	>2000 k€	2009
4) 200kV FEG (S)TEM , EDX	*>1300 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 +EDX (2021)	300 k€	2011
3) Zeiss E-SEM	150 k€	2011
4) dual beam FIB-SEM (EDX, EBSD, Cryo-SEM 2021)	~1400 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,, ...	2000 k€	2009 -
	Total: >15 M€	

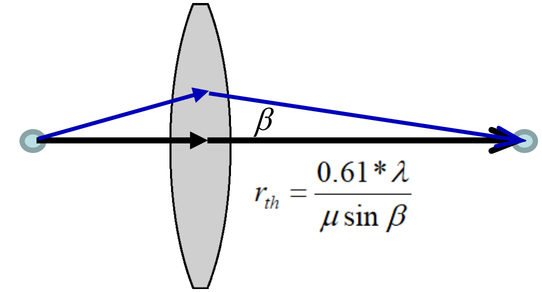
Microscopy Resolution – Different Microscopes

SEM from cm scale → up to sub nanometer resolution
TEM from micrometer scale → up to sub Ångström resolution



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

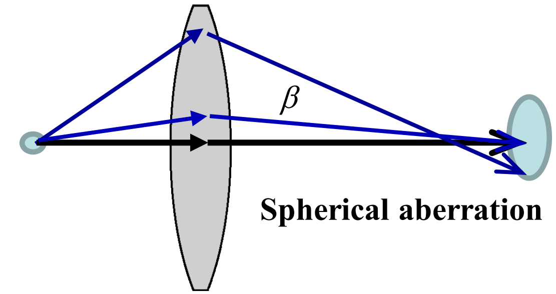
Electron microscope resolution:



In ideal case:
Theorethical resolution
(Classical Rayleight criteria)

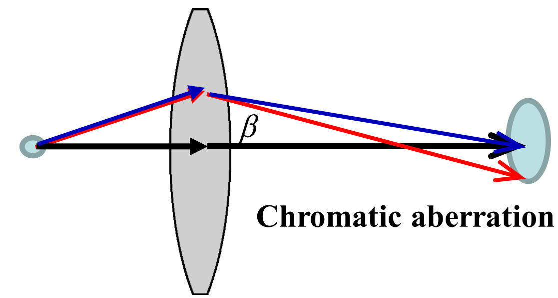
$$r_{th} = \frac{0.61 * \lambda}{\mu \sin \beta} \approx \frac{0.61\lambda}{\beta}$$

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

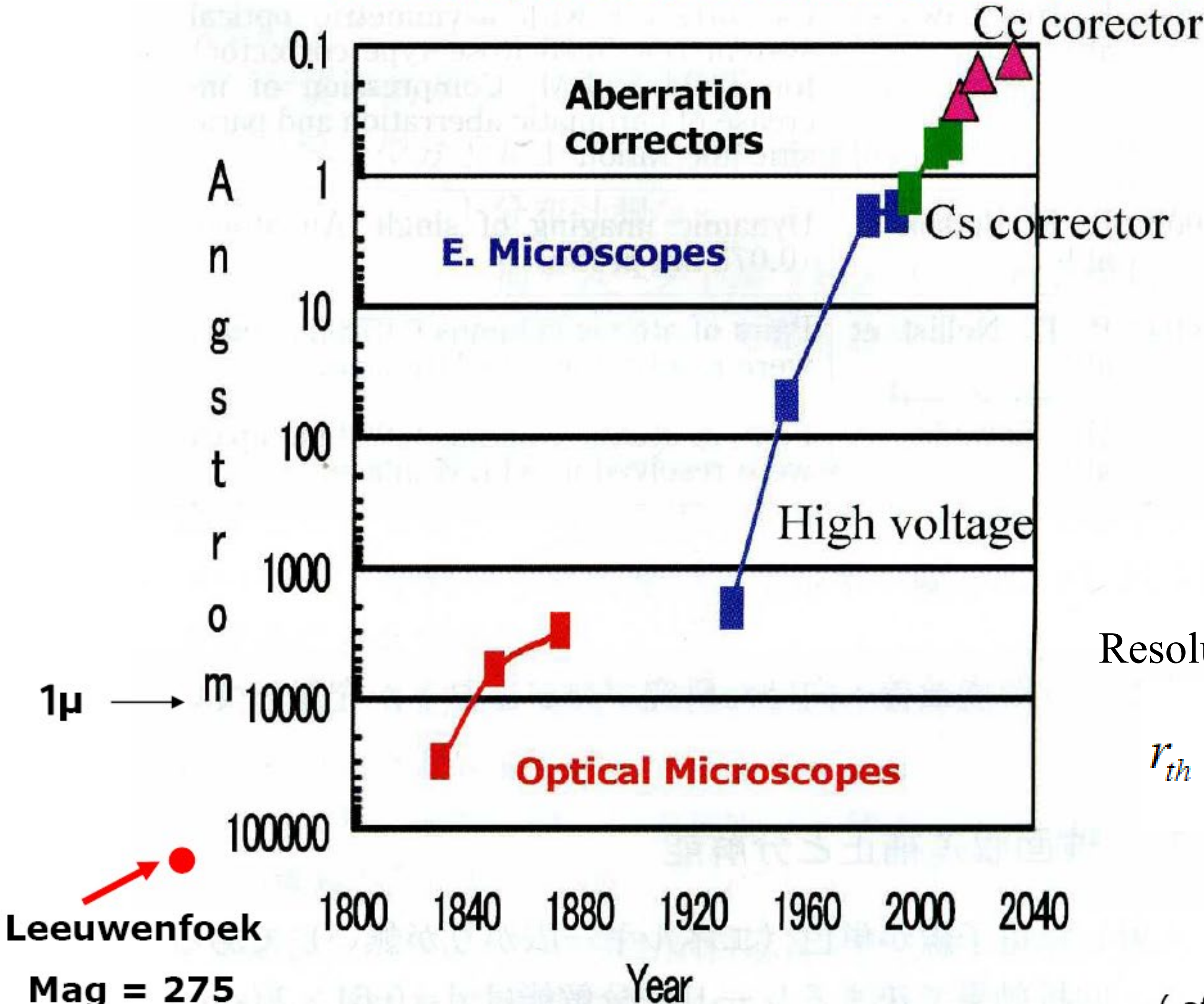
If also chromatic aberration could be corrected or minimized → resolution < 0.5 Å

Spherical aberration in **Hubble Space Telescope**

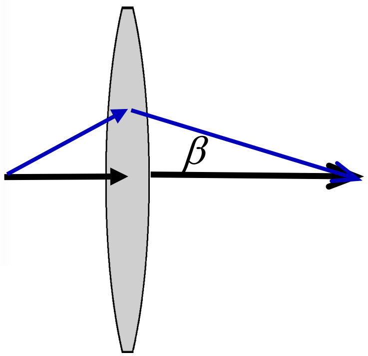
Analysis of the flawed images showed that the cause of the problem was that the primary mirror had been ground to the wrong shape. Although it was probably the most precisely figured mirror ever made, with variations from the prescribed curve of only 10 nanometers,[it was too flat at the edges by about 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)



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)

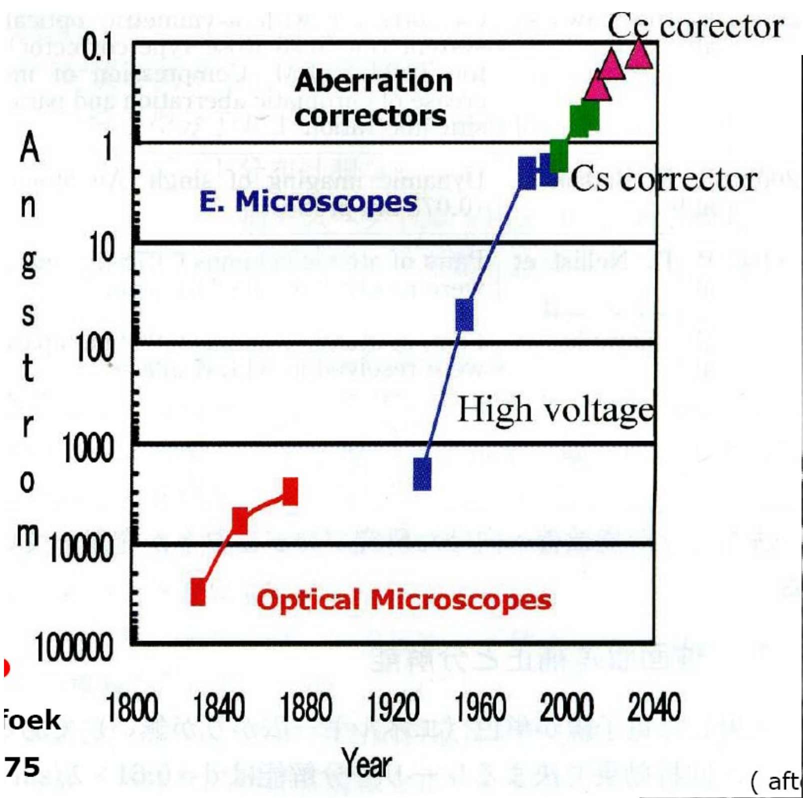


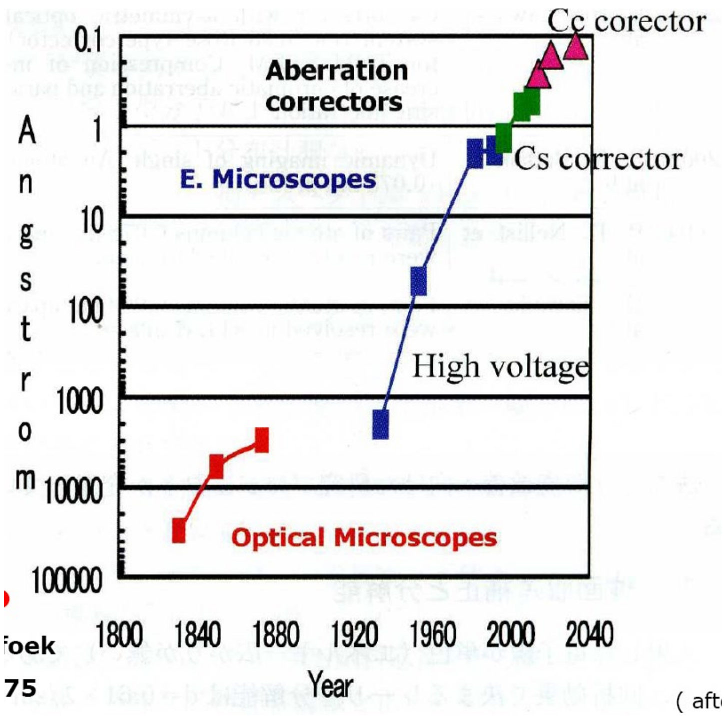
FIGURE 1.1. The electron microscope built by Ruska (in the lab coat) and Knoll, in Berlin in the early 1930s.

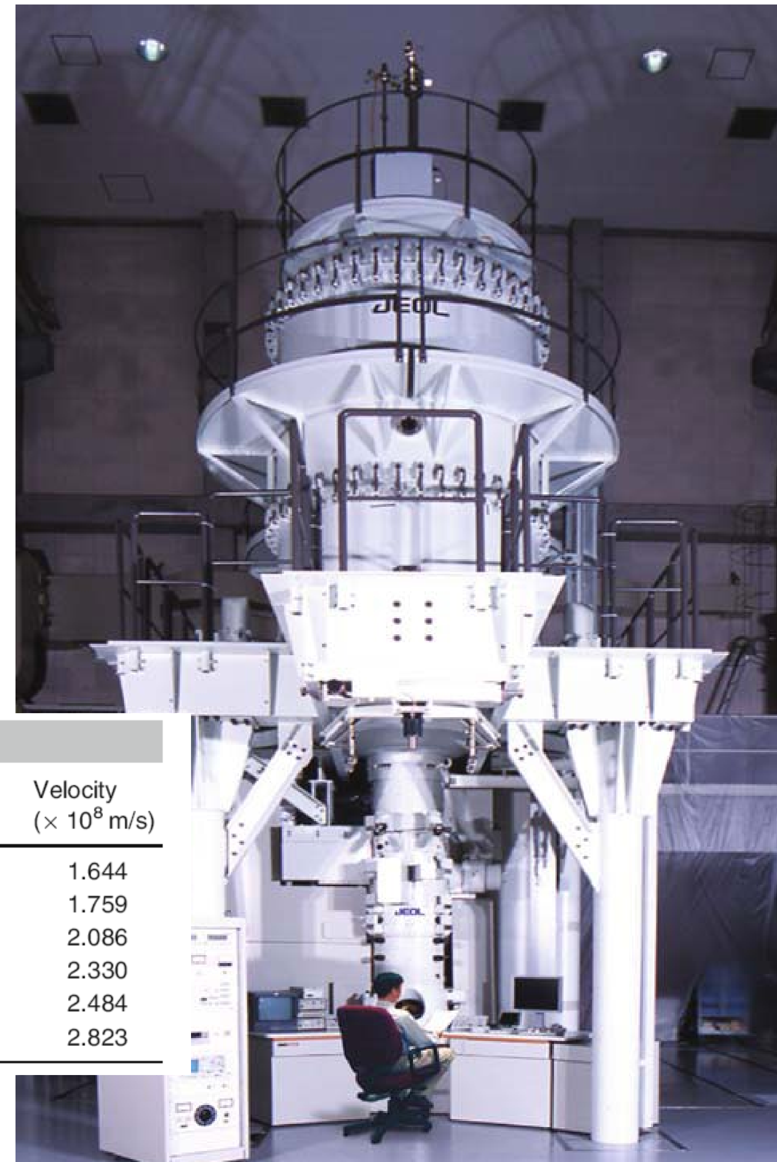
TABLE 1.2 Electron Properties as a Function of Accelerating Voltage

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

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

(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

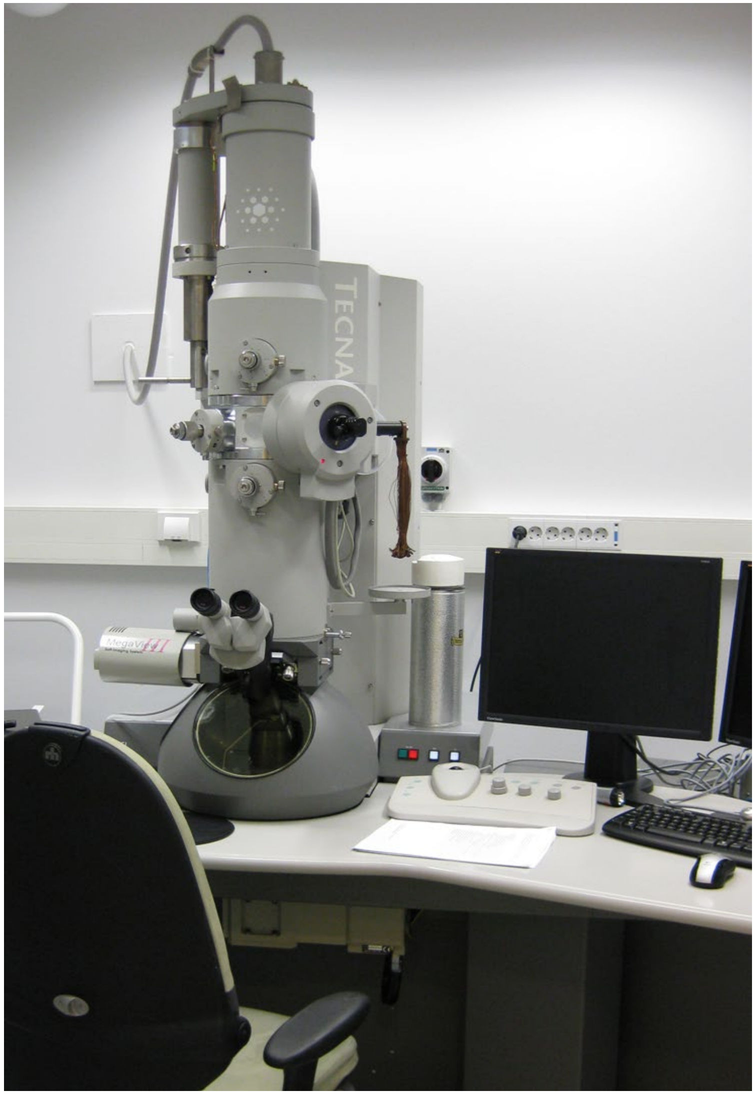
Hitachi 3.5MeV (S)TEM

Base F

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

In NMC we have 4 (5) TEM’s

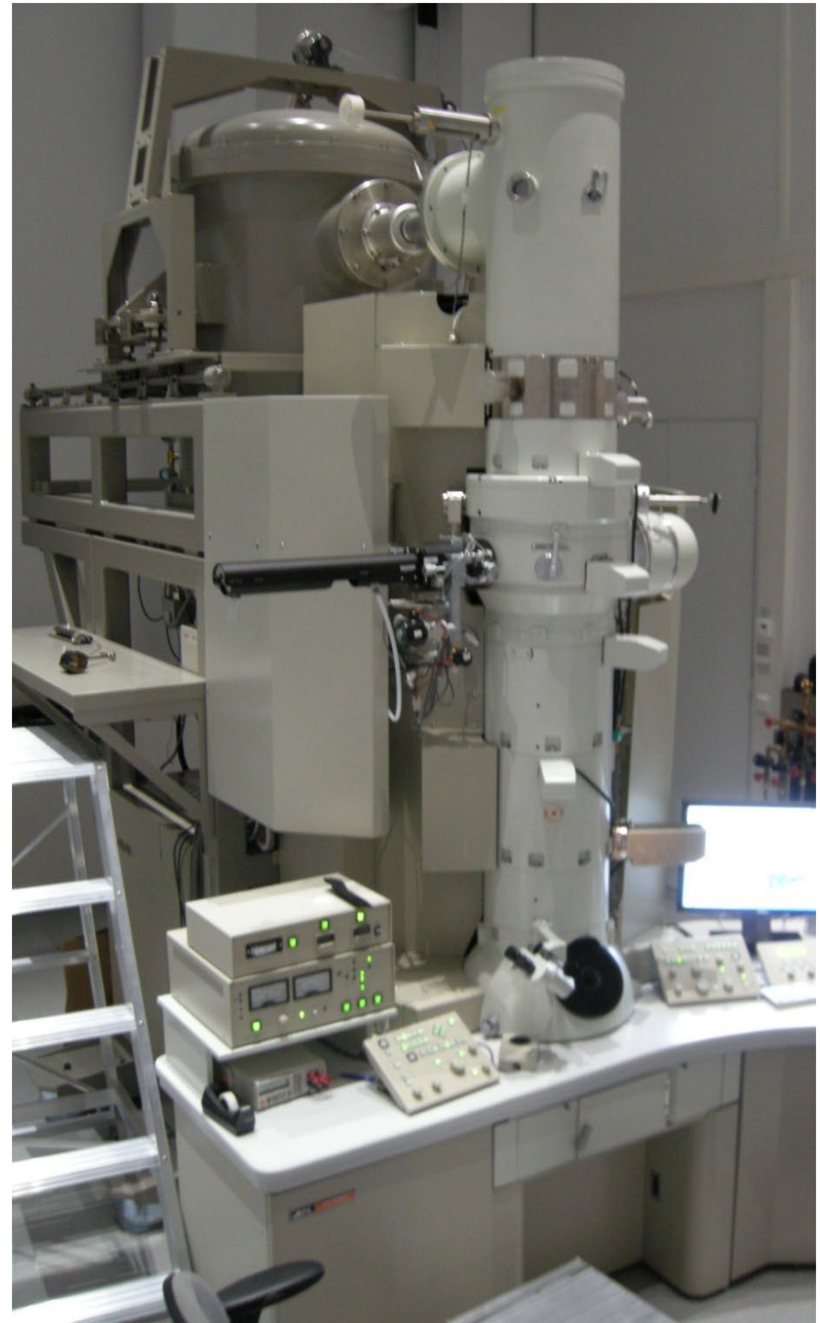
In this picture the two oldest TEM’s 120 kV TEM and 200 kV TEM are shown



FEI Tecnai T12 Installation 2003
(Will be replaced with new 200kV TEM in 2024)

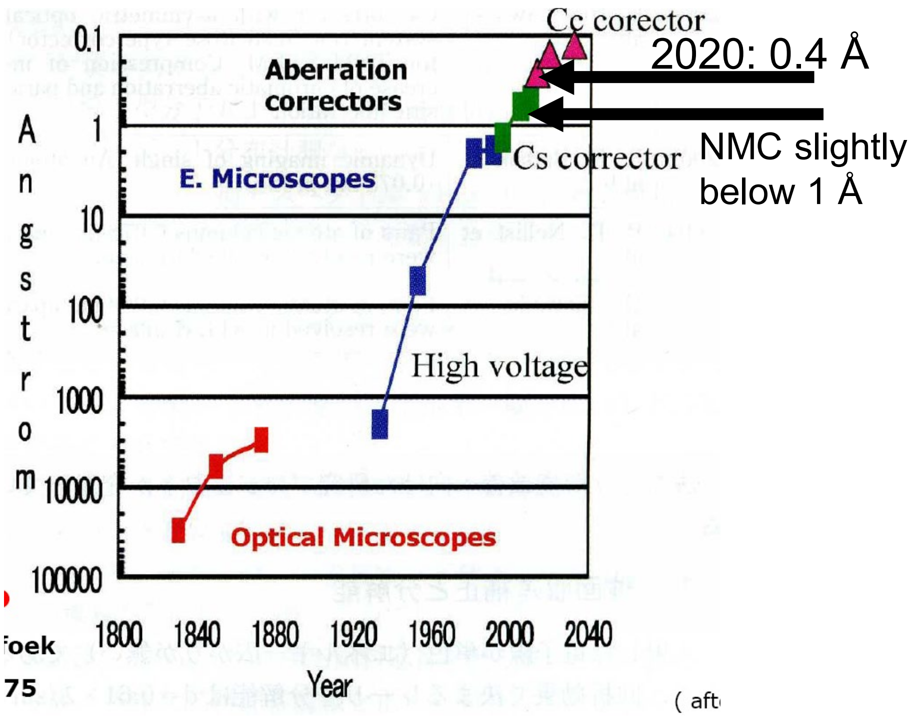
FEI Tecnai F20 Installation 1999? (Material science laboratory (Chem) and 2016 → moved to NMC)
(now permanently broken… and will be thrown away..)

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



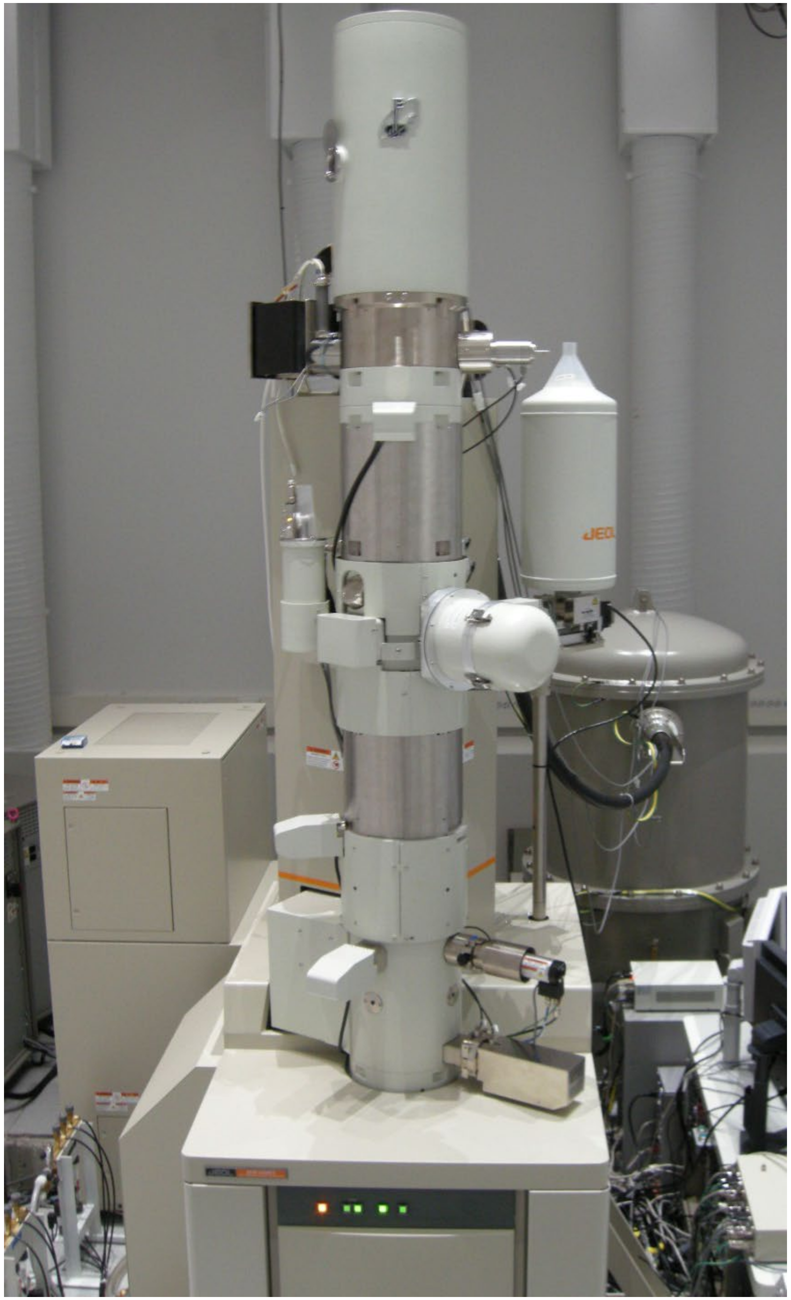
NMC highest resolution Double Cs-corrected (S)TEM

(to be replaced with new 300 kV TEM in 2024)

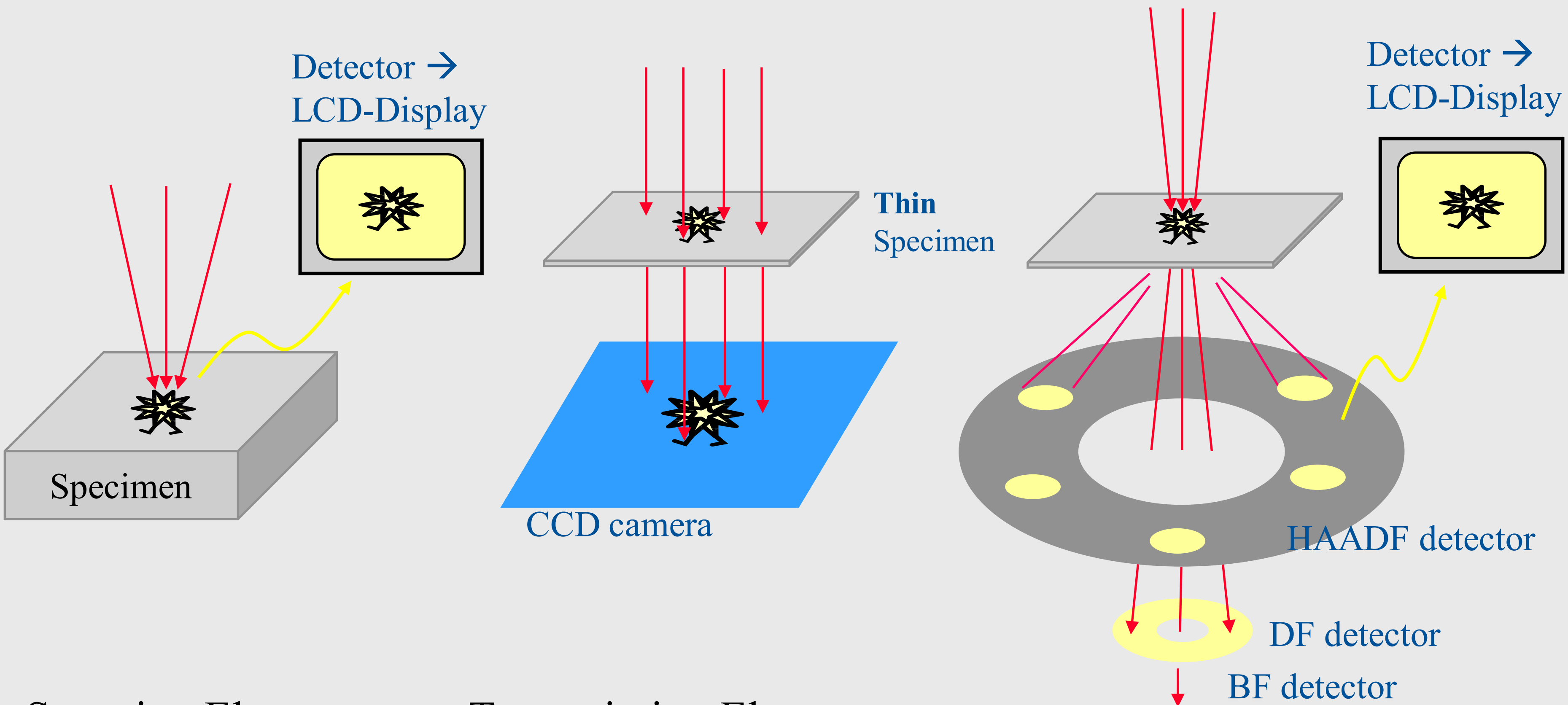


Installation 2009: TEM and STEM resolution slightly below 1 Å

Now best resolution for commercial microscopes is 0.4 Å



Brief introduction to the electron microscopy



Scanning Electron Microscopy (SEM)

Best resolution typically ~ 1 nm

Transmission Electron Microscopy (TEM)

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

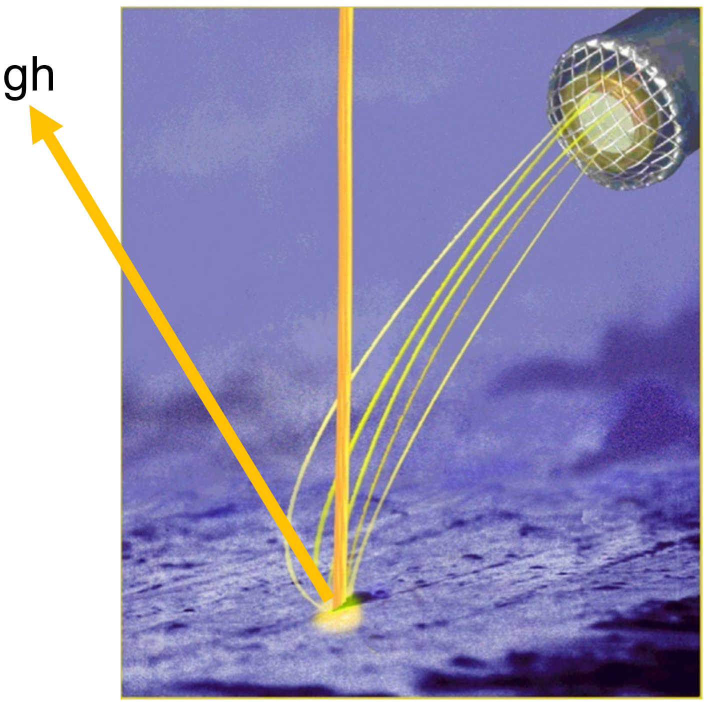
Scanning Transmission Electron Microscopy (STEM)

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

Scanning Electron Microscopy (SEM)

Primary e-beam (1-30kV)

Back scattering electron detector (high energy electrons)



Secondary electron detector (low energy electrons)

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:

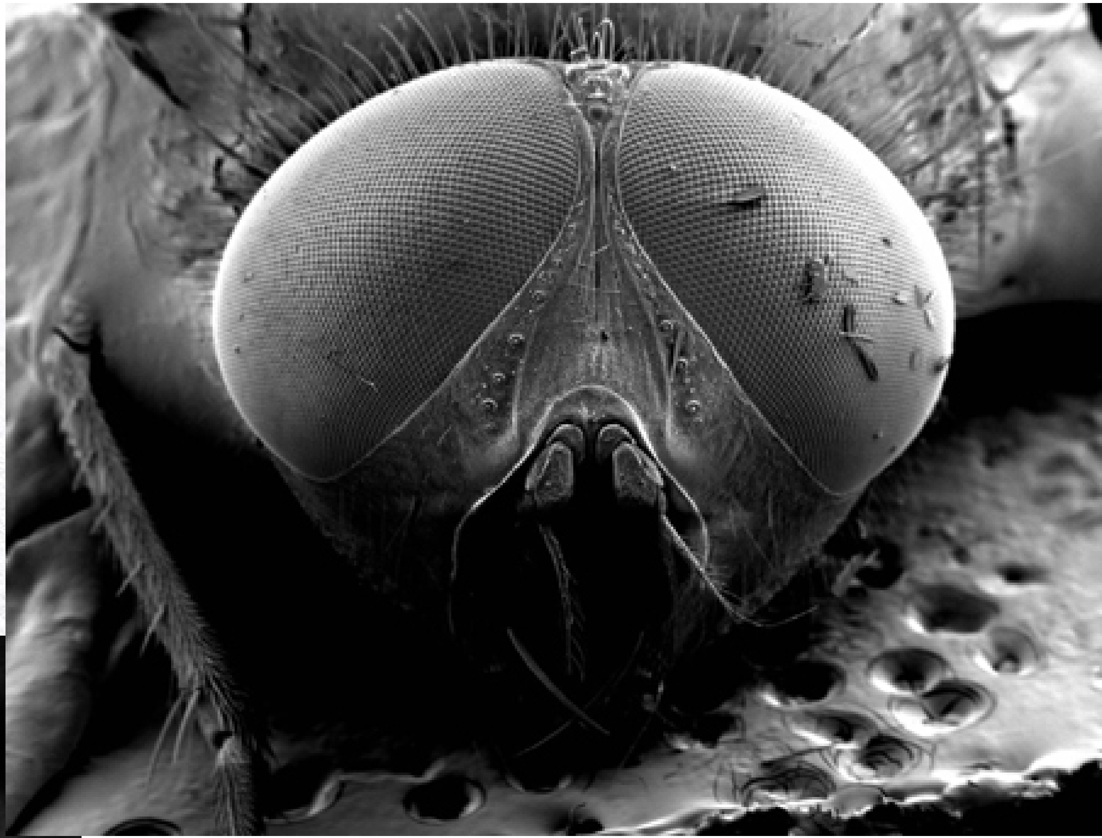
- Relatively high resolution –

  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

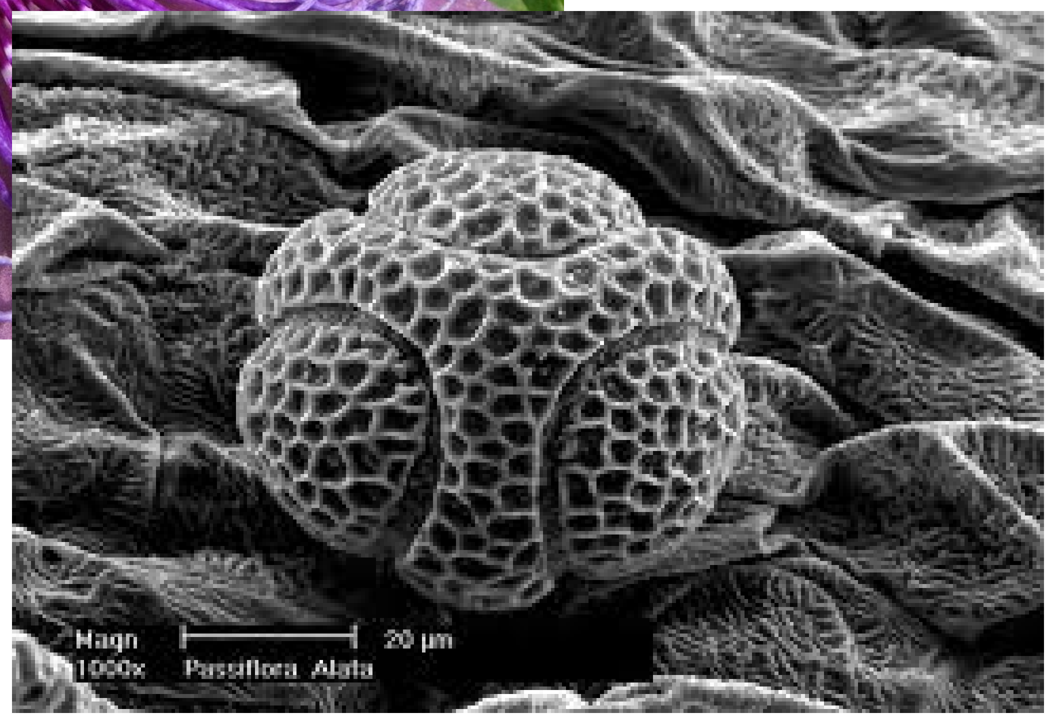
  (normally thin metal coating needed - gold/palladium, platinum, carbon, etc.)

- 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

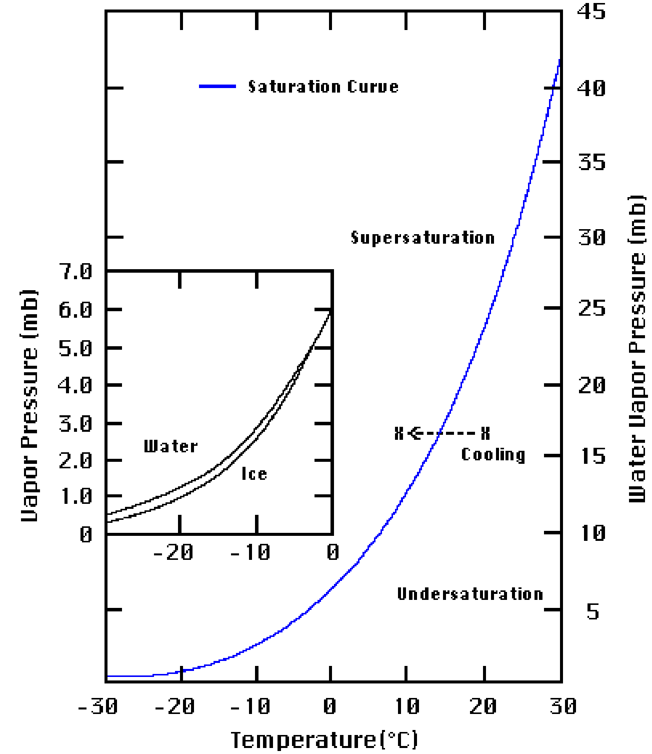
0.02 – 1.3 mbar

This mode we can reduce the charging effects for non conducting samples and even do imaging without metal/carbon coating

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)

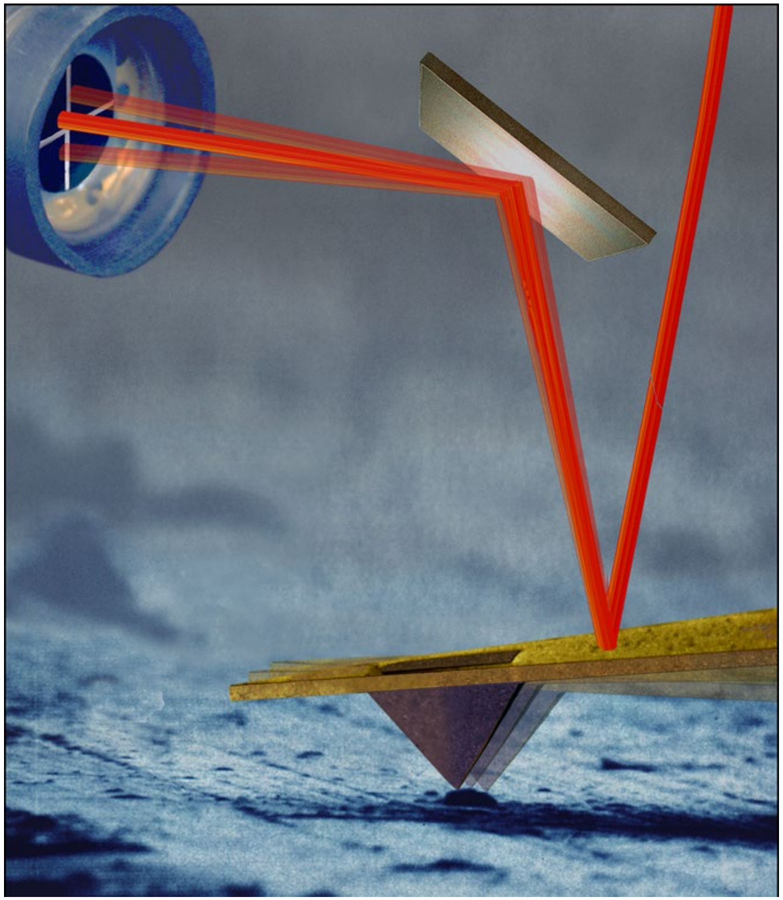


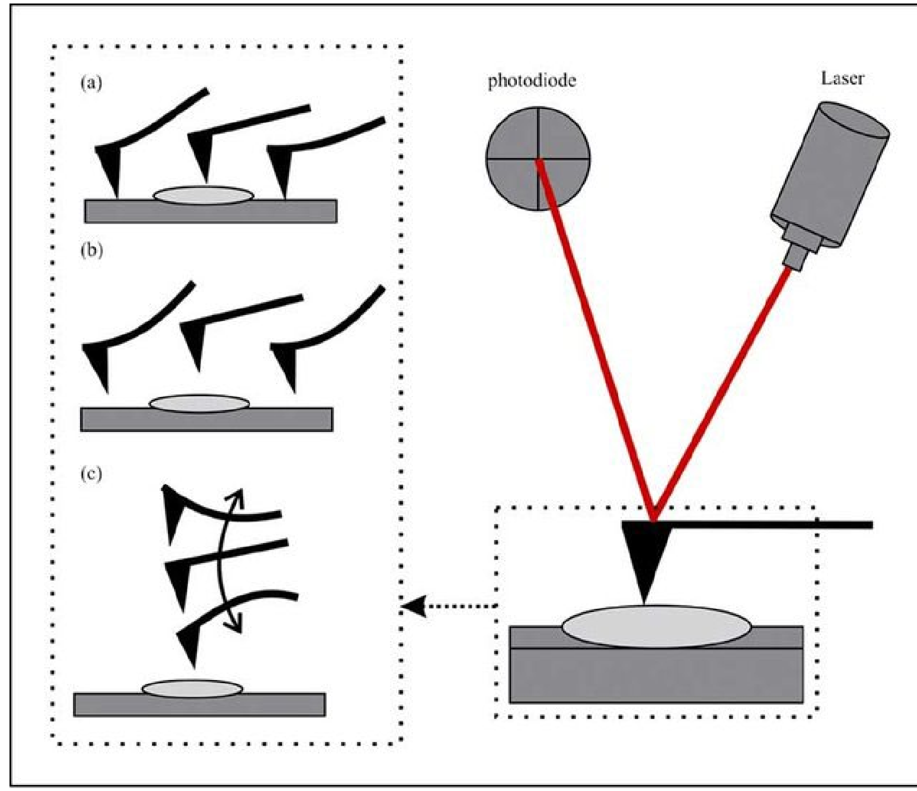
0.1 – 30 mbar

Typical working conditions for water containing samples are close to 0 degrees Celsius and 6 mbar pressures

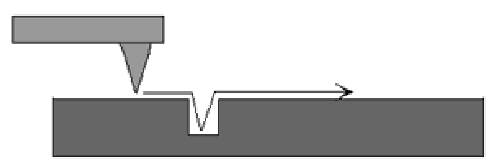
Scanning probe microscopy (SPM) historically called AFM)

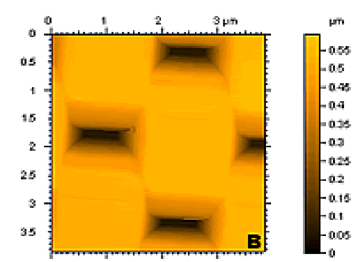
a) Contact mode
b) Non-contact mode
c) Tapping mode (tip is oscillating on surface)



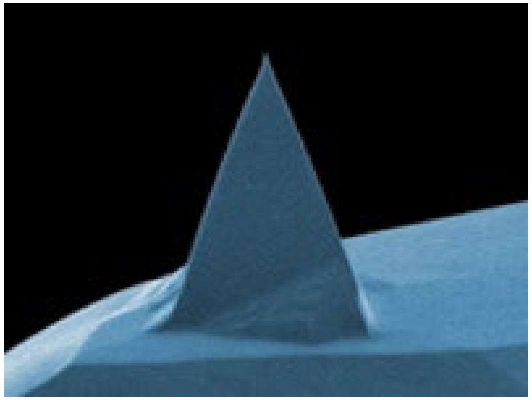


In AFM we use sharp tip to scan the sample surface to obtain information about surface topography and other properties (chemical, mechanical hardness, electrical etc.)

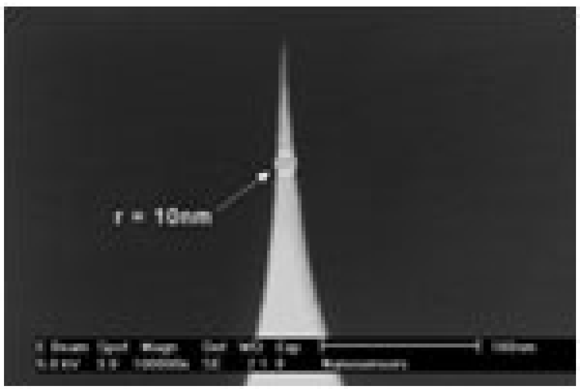




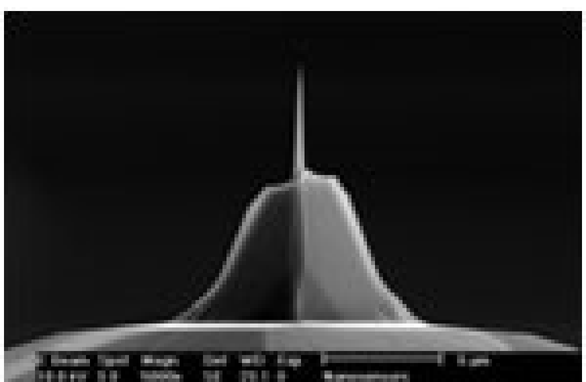
Examples AFM probes



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

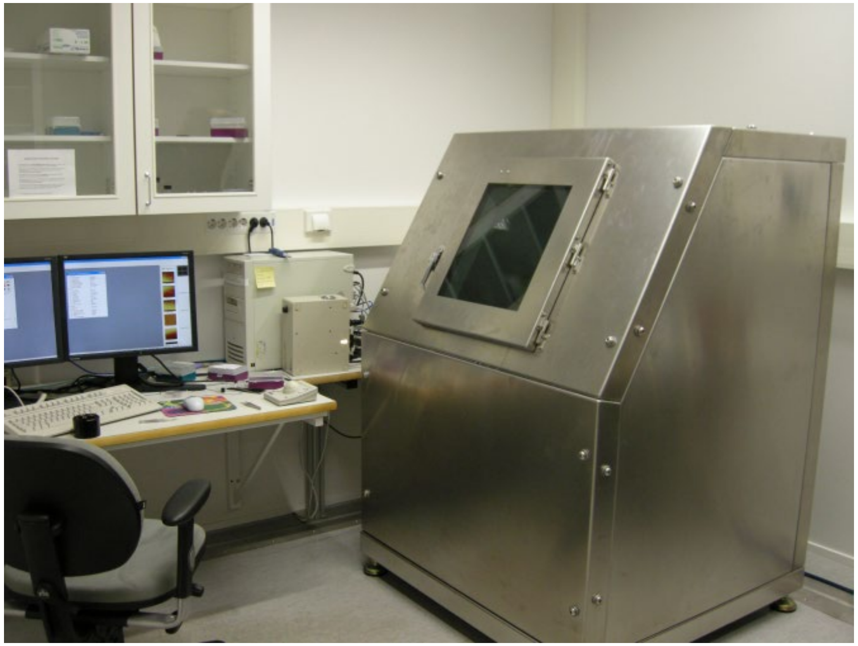


Super sharp tips: Tip radius is
typically 1nm (~100 €)



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)



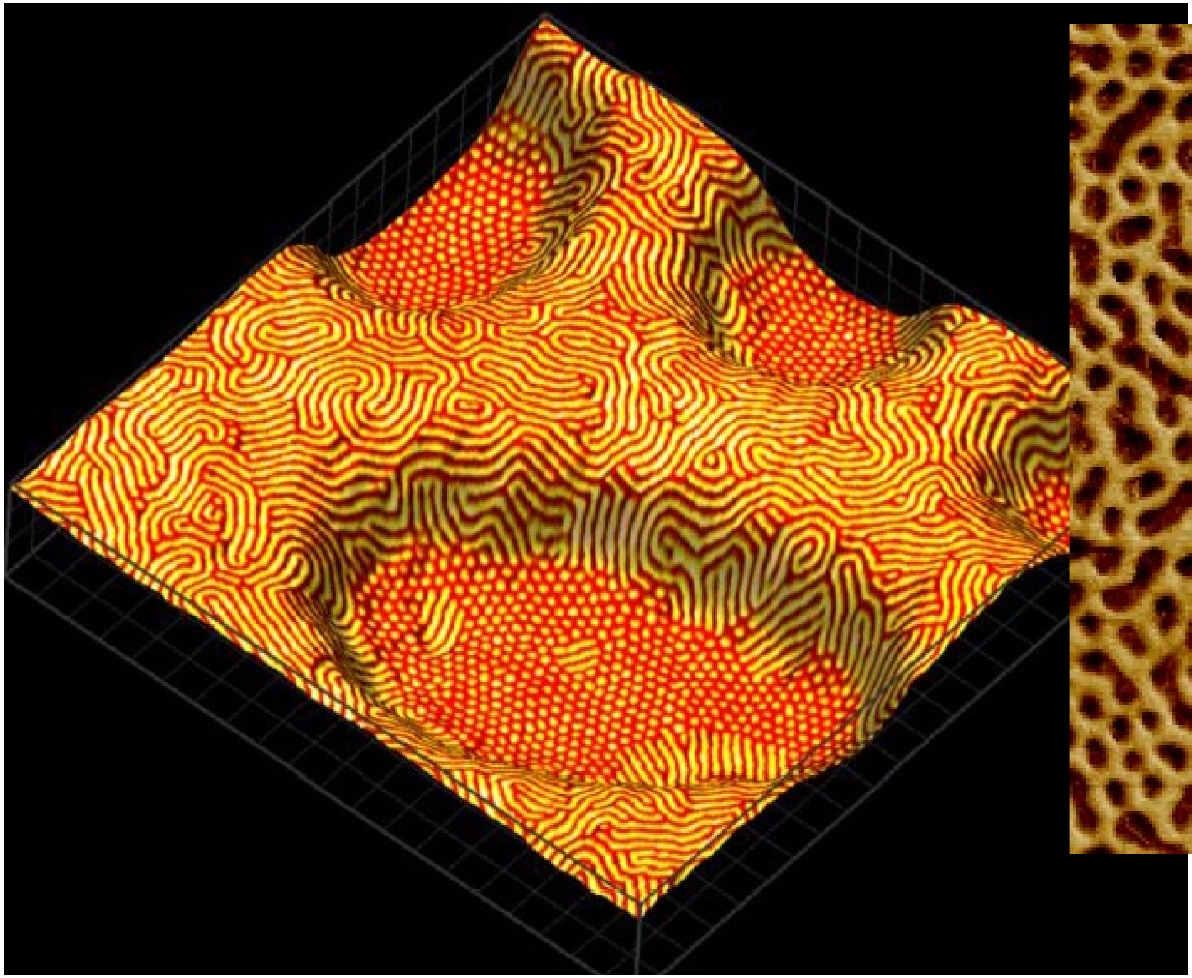
- Originally designed for semiconductor imaging.
- Capable of loading samples up to 350 mm in diameter.
- Large scanning area ~90 x 90 µm.
- Automatic measurement for up to 100 pre-selected areas.
- High pixel-density image capture 5120 x 5120 points.

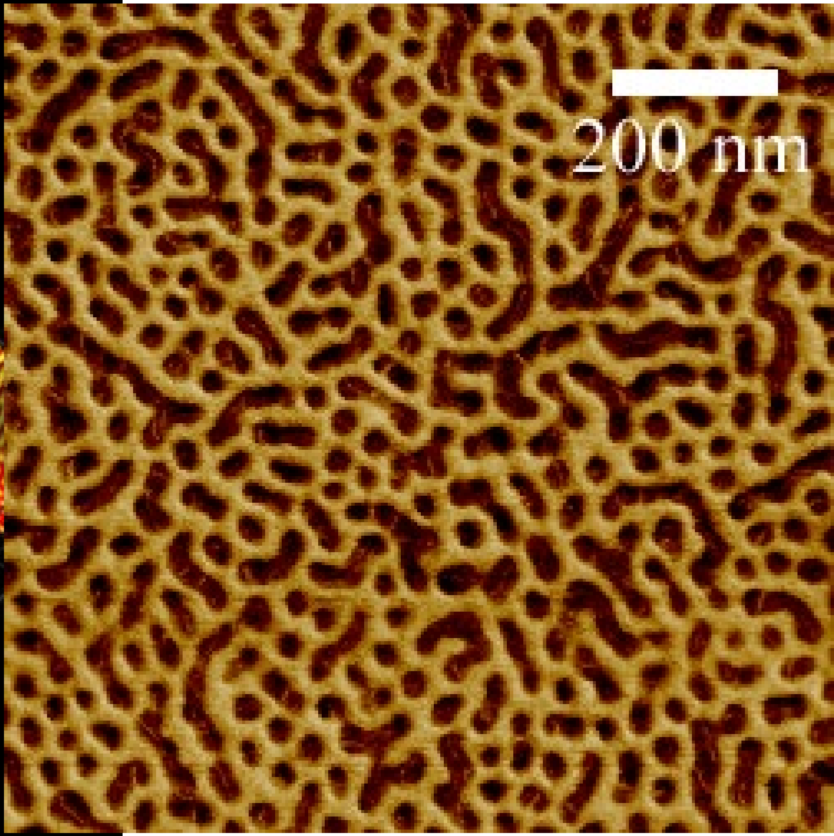
High resolution, easy to use, large samples..

Unfortunately currently broken ..

But Micronova and Chemistry department has other good AFM's

AFM image examples - Polymer films:

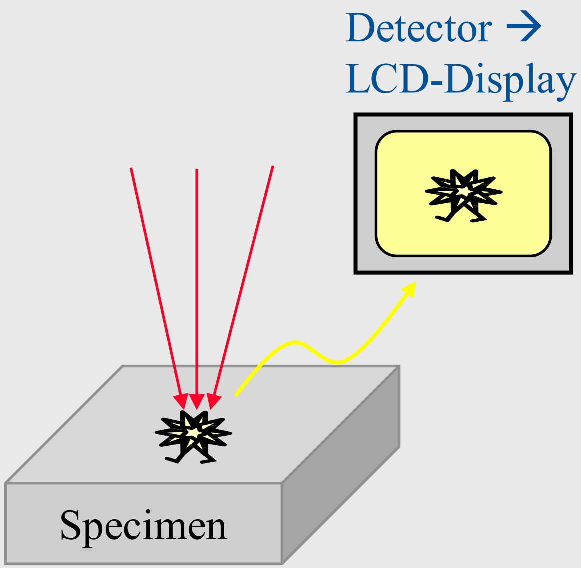




This image dark domains are polyethylene (soft) and gray domains Polystyrene which is glassy polymer.

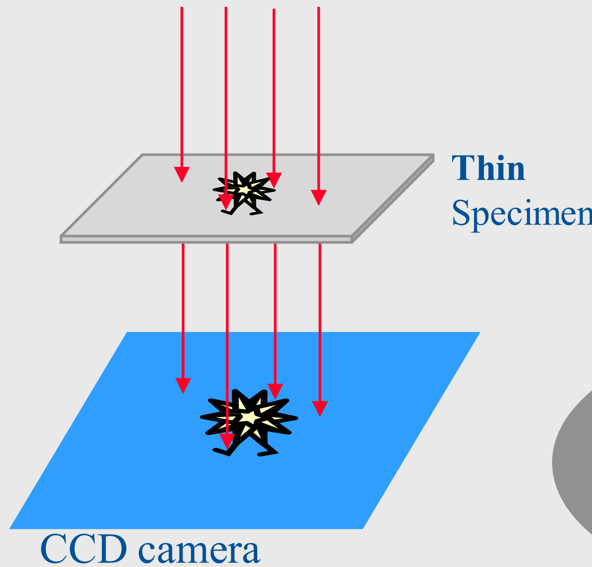
Topography (height) and composition imaging (soft and hard domains) – in tapping mode imaging one can get at the same time the surface topographical information and contrast due to mechanical properties (example hard of soft domains)

Brief introduction to the electron microscopy



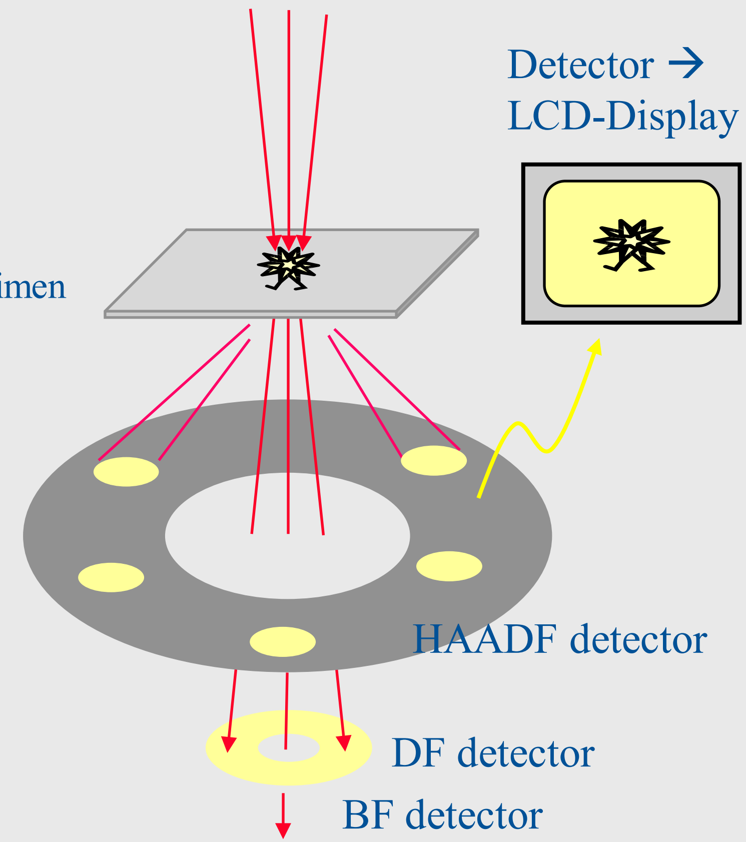
Scanning Electron Microscopy (SEM)

Best Practical resolution ~ 1 nm



Transmission Electron Microscopy (TEM)

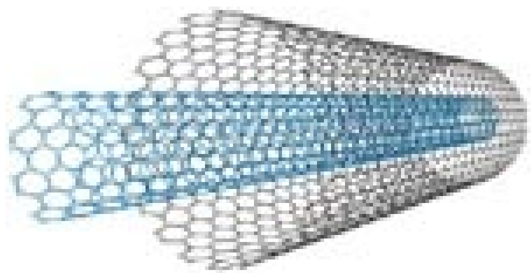
resolution ~ 2 Å
(Cs corrected ~ 0.5 - 1 Å)

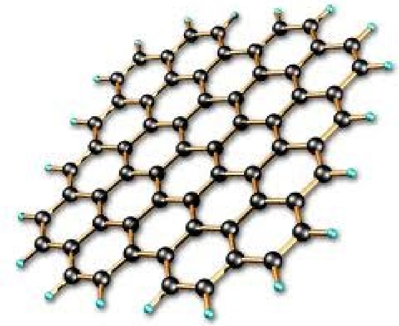


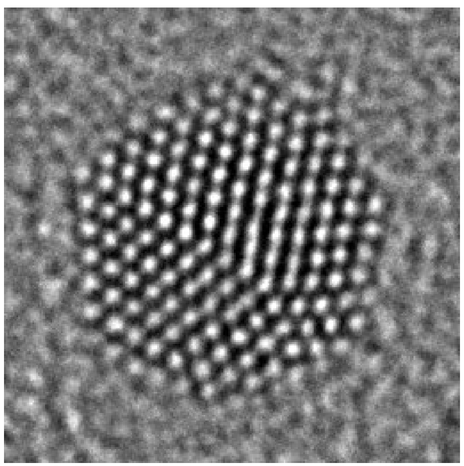
Scanning Transmission Electron Microscopy (STEM)

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

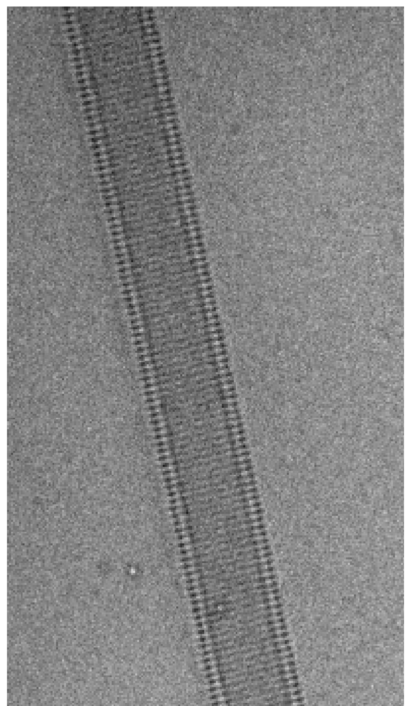
Nowadays it is possible to obtain atomic resolution images even from low atomic number materials such as Carbon nanotubes and single graphene sheets..

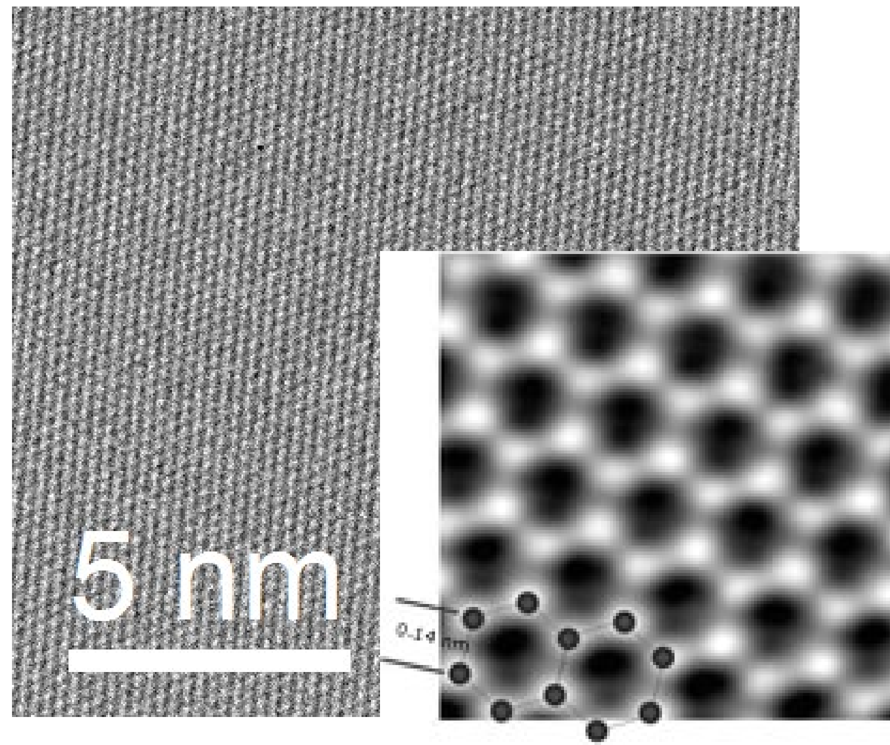






High resolution TEM image from Gold nanoparticles

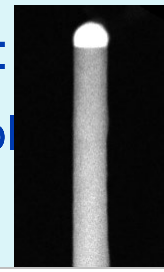


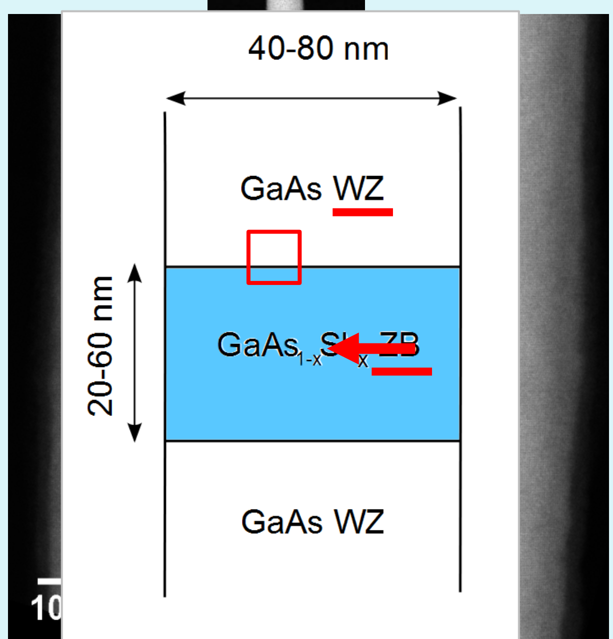


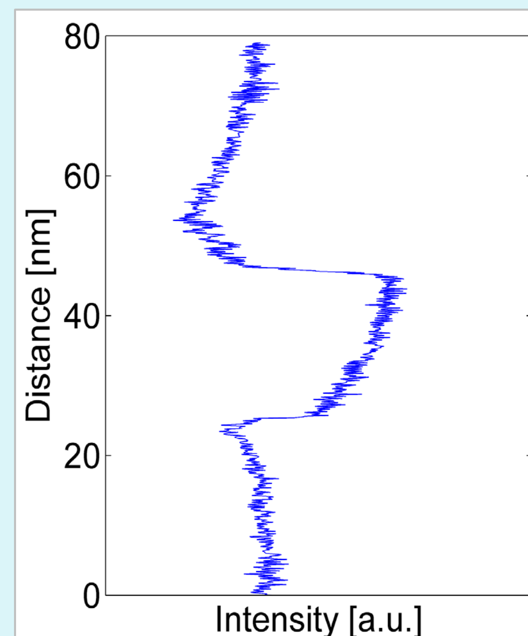
Another examples: Heterostructured GaAs nanowires

- 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:

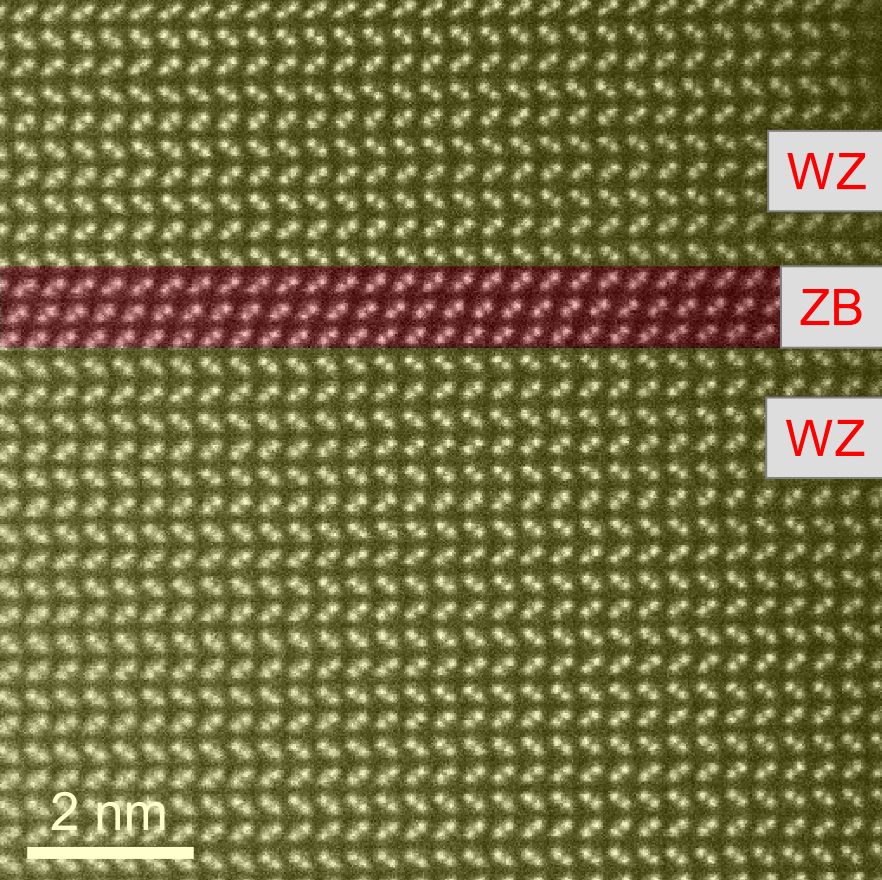
  $GaAs_{1-x}Sb_x$ insert : Zinc bl **ZB**) structure (闪锌矿结构, 立方相)

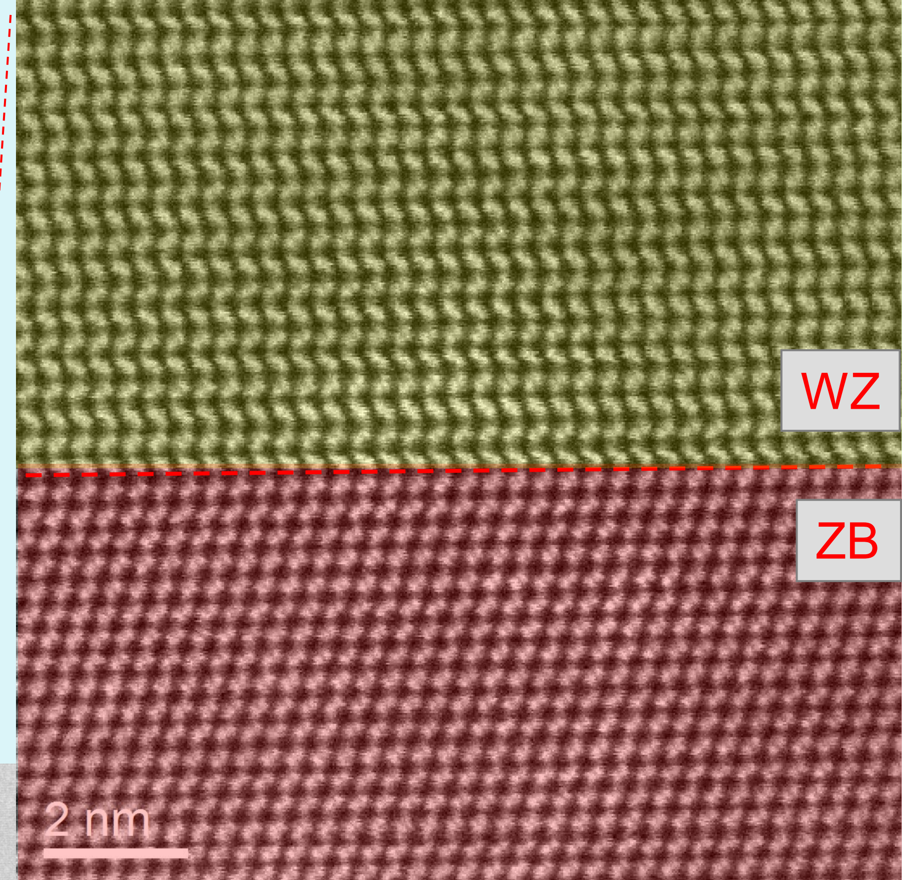


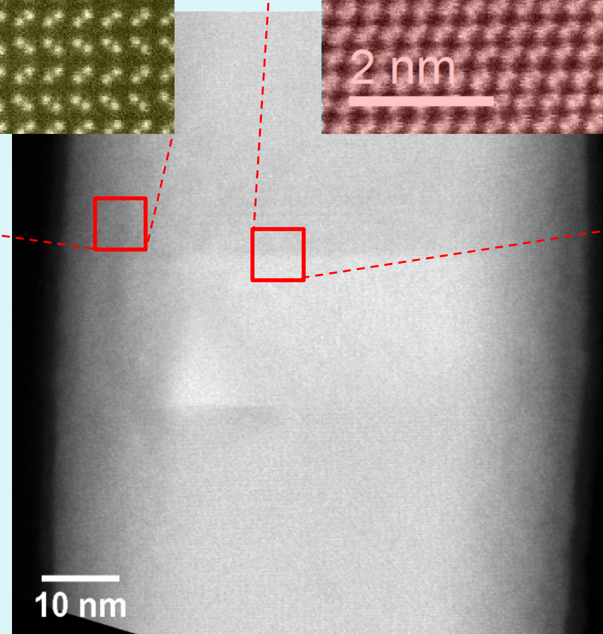




Sample: Hanne Kauko, NTUT





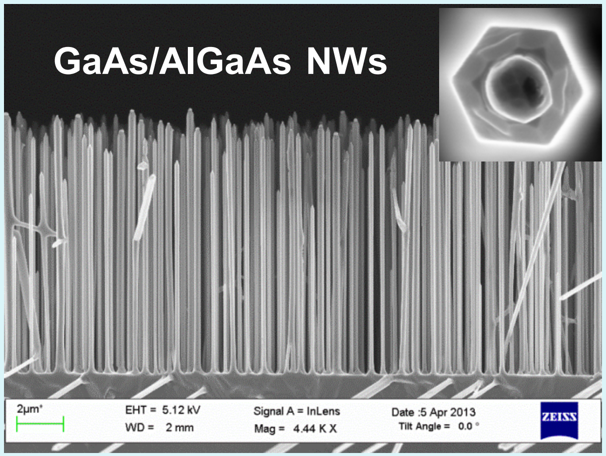


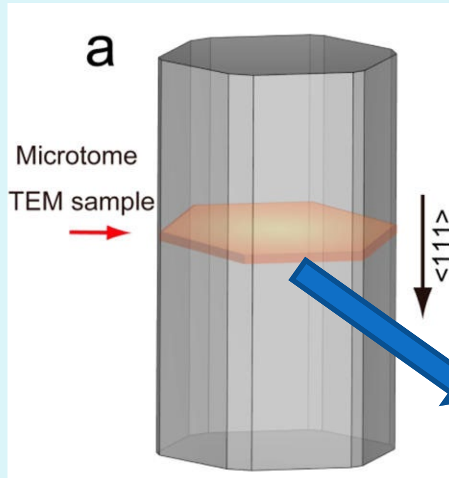
- WZ and ZB coexist;
- Ga and As columns well separated, 1.55 Å;
- Ga and As columns has different intensities.

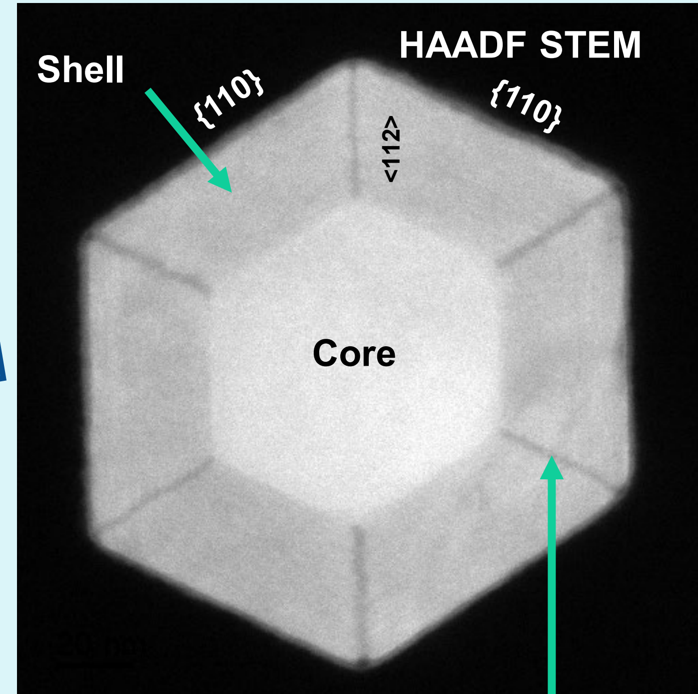
Sample: Hanne Kauko, NTUT

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

TEM characterization of III-V nanowires (HAADF STEM)

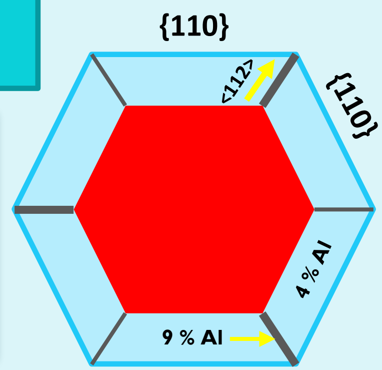






Core-shell GaAs/AlGaAs nanowires grown using MOCVD
60 nm core, 30 nm shell

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

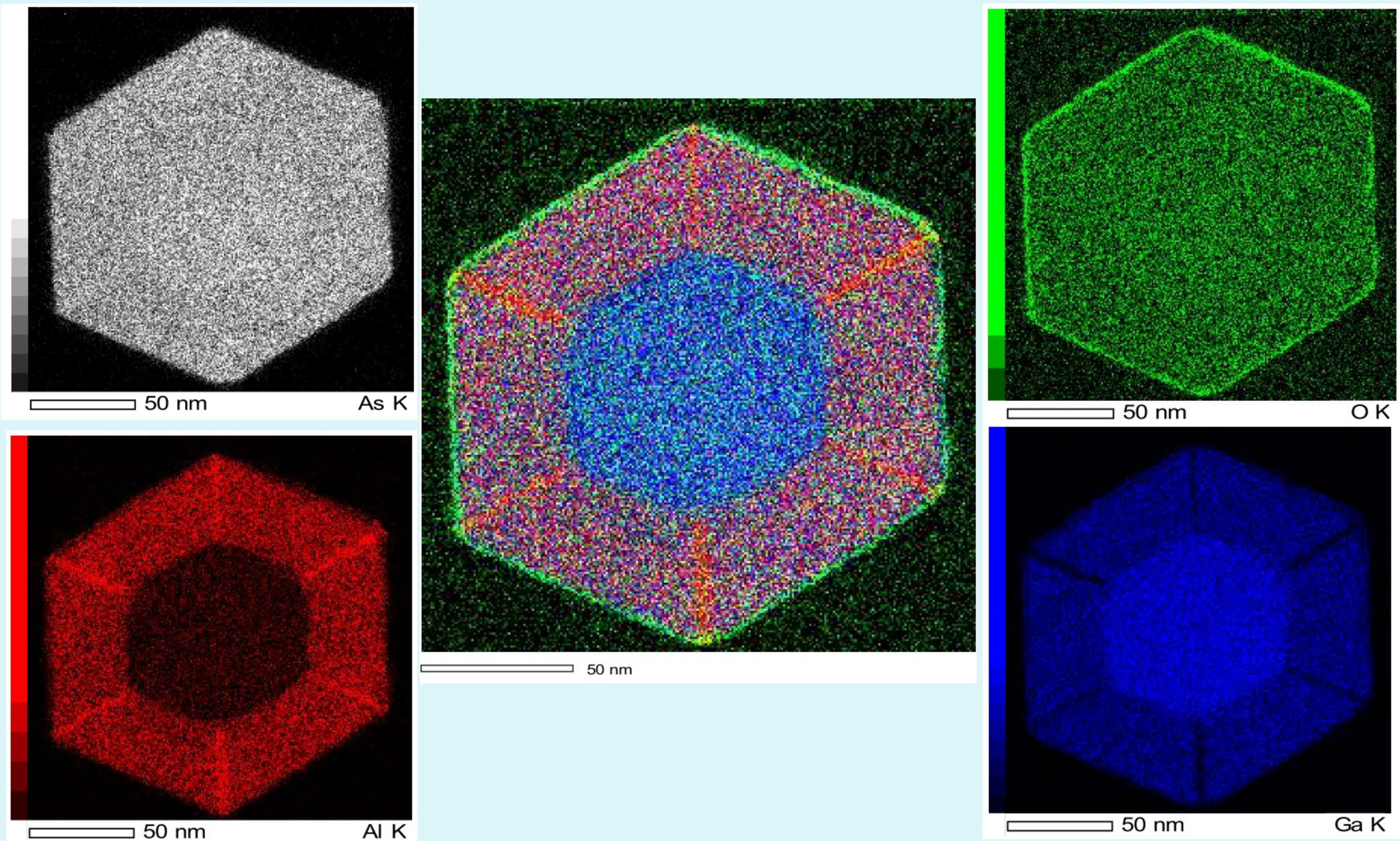


Dark lines (Al segregation)
Cross section view

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

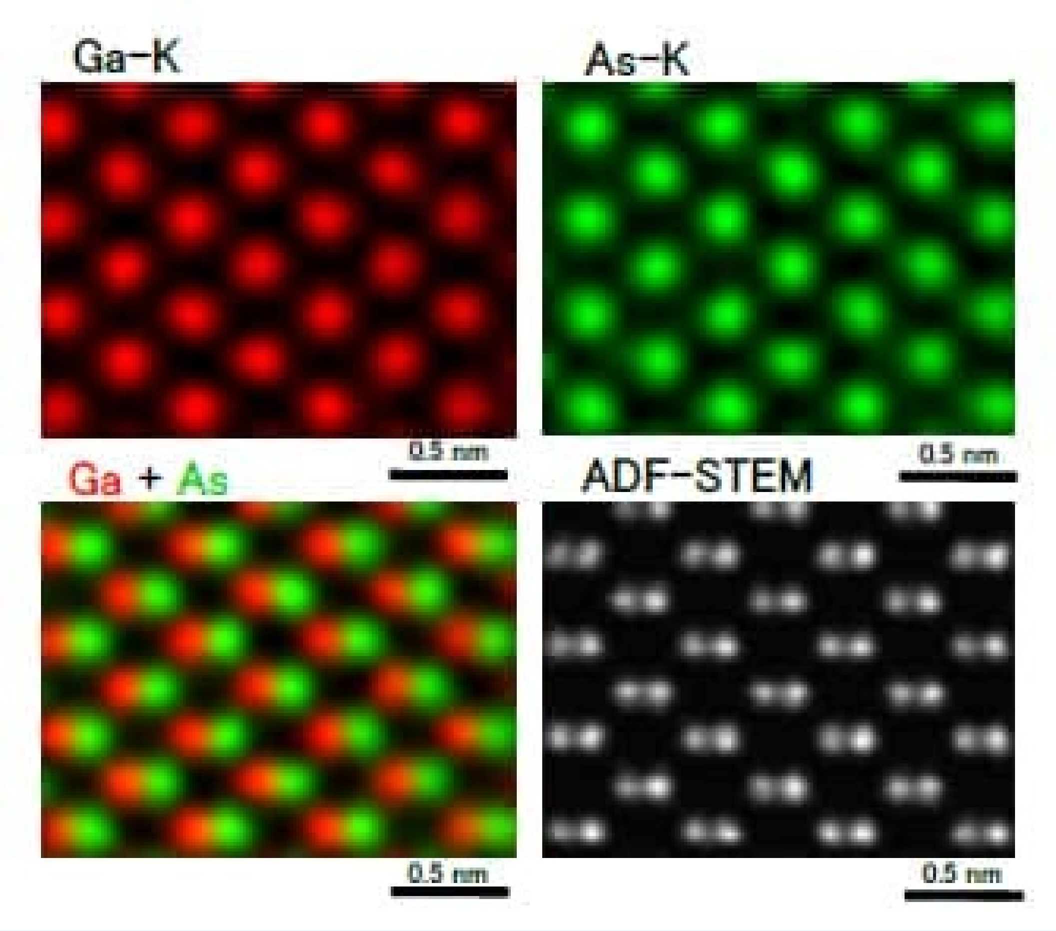
Elemental Mapping of cross-sectional GaAs nanowires

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..



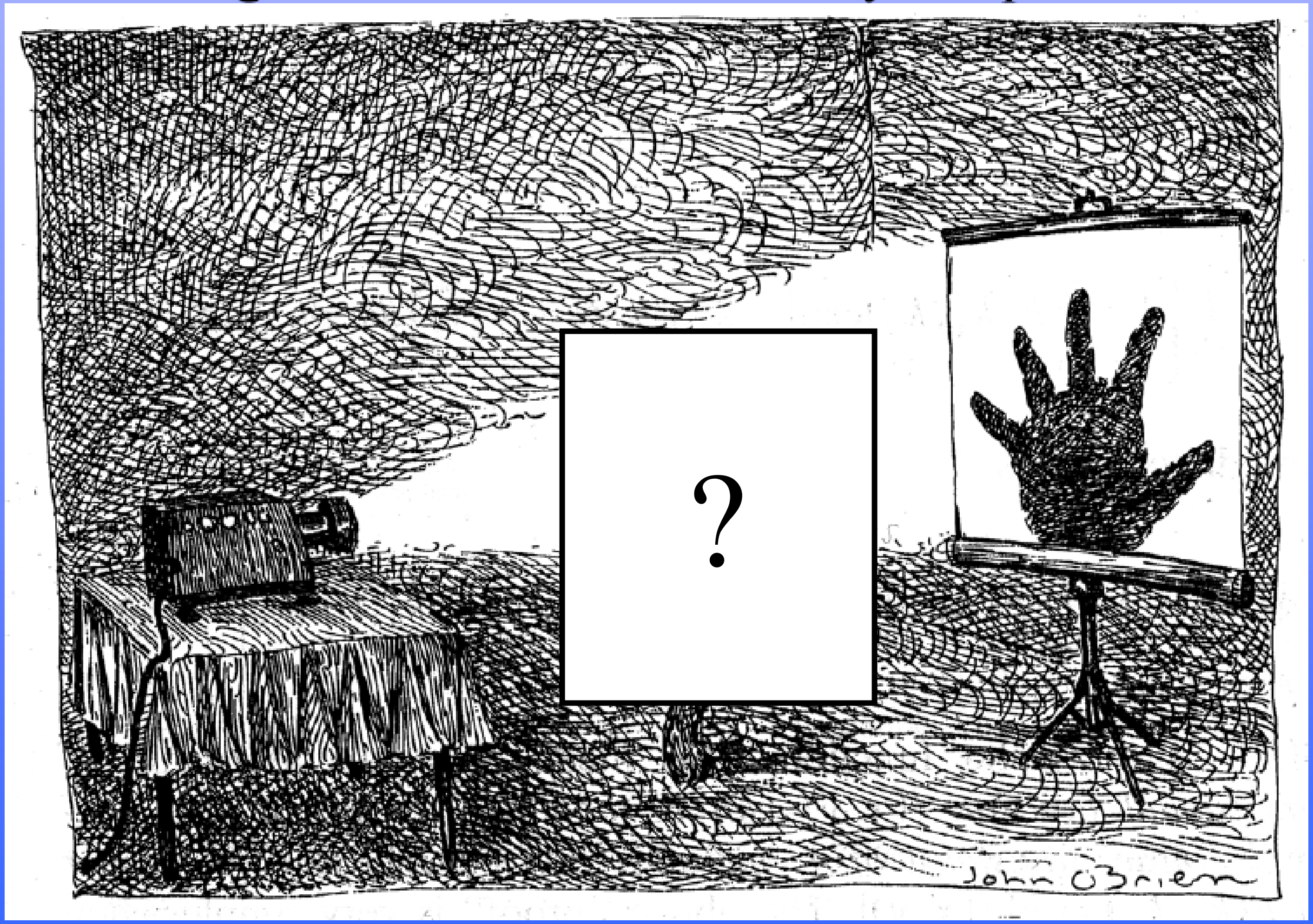
Atomic-level-resolution EDX elemental map of a gallium arsenide monocrystal <011>

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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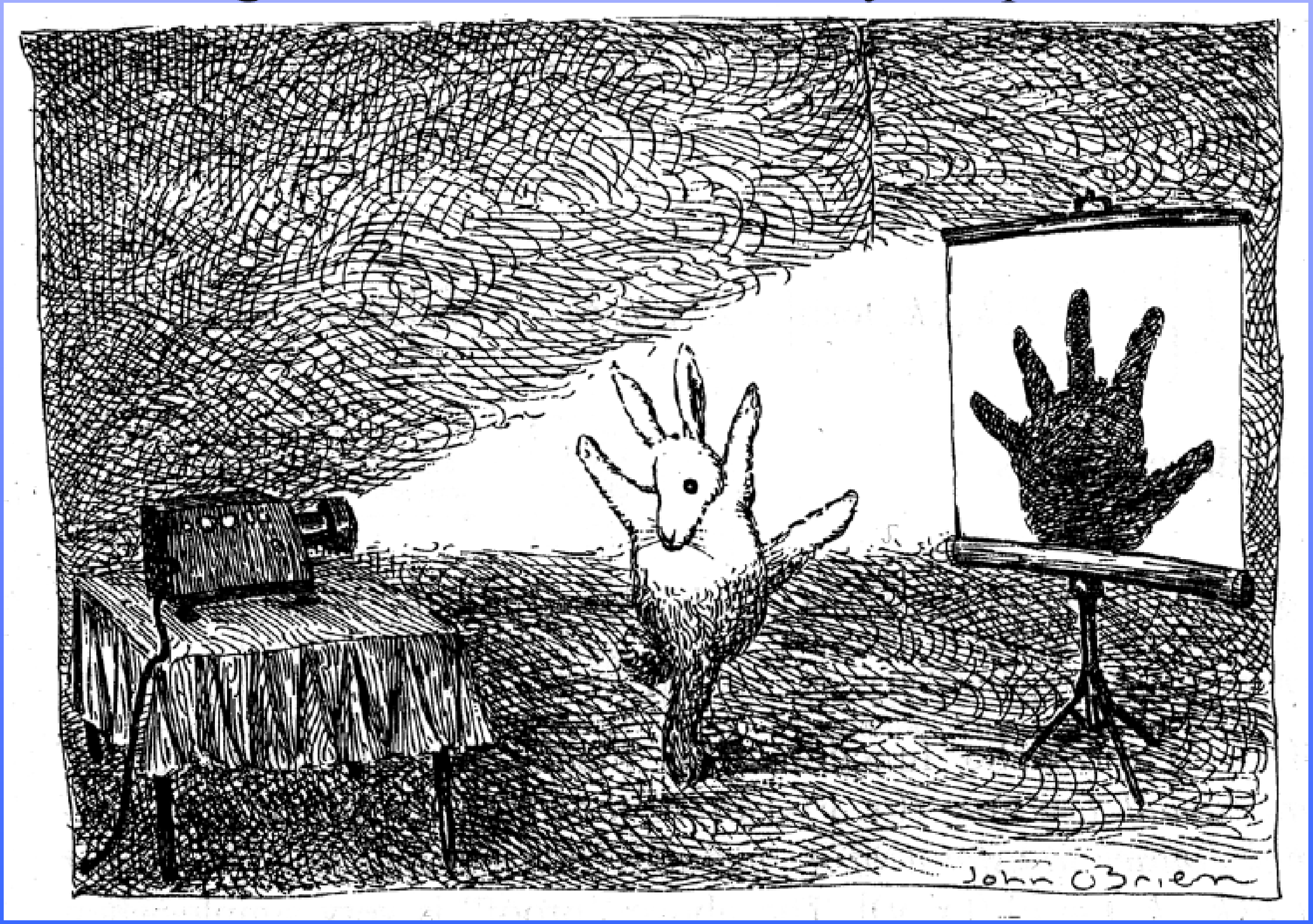


Another example of projection from 3D → 2D

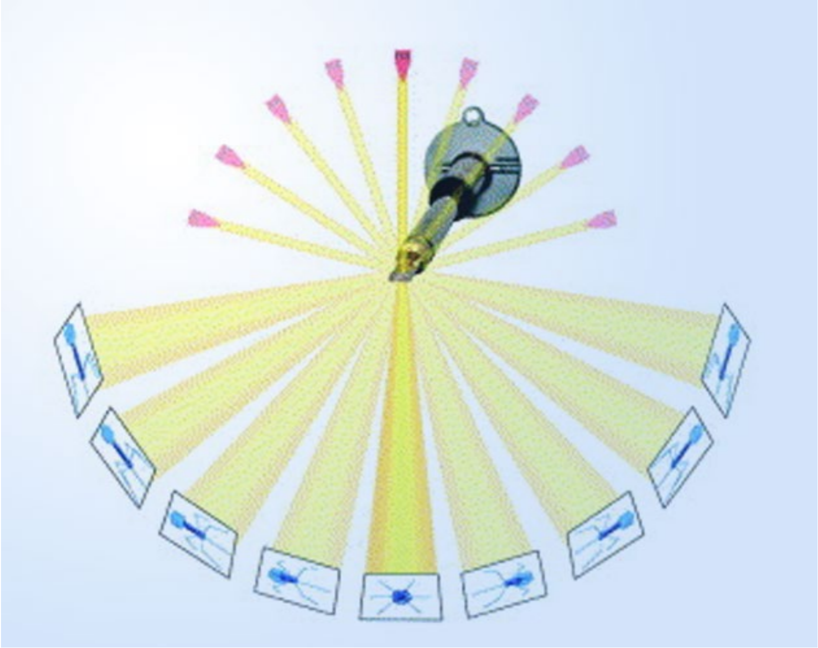
Another example of projection from 3D → 2D


POSTED AT
SheChive.com

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

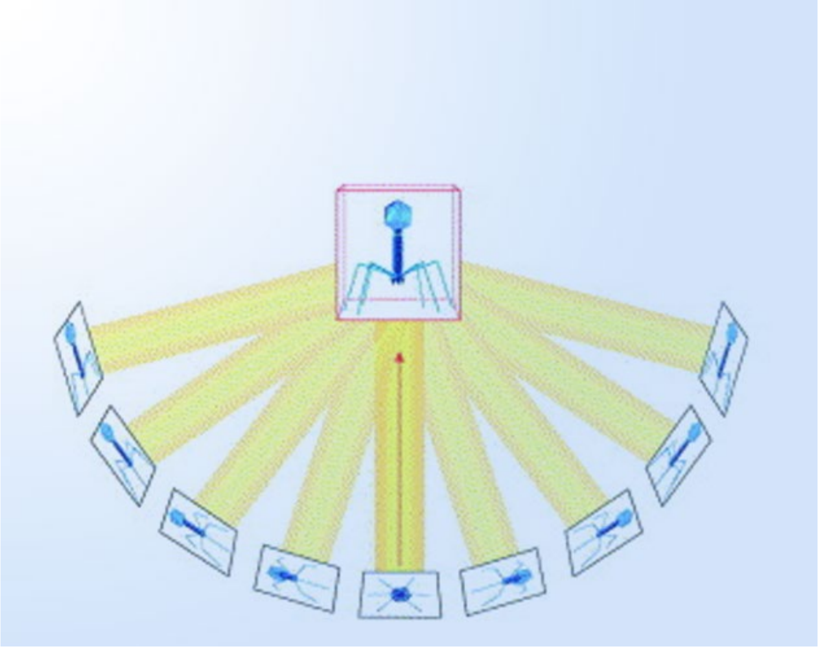
Principle of electron tomography:

DATA COLLECTION



3D object => 2D-projections

RECONSTRUCTION



2D-projections => 3D-reconstruction

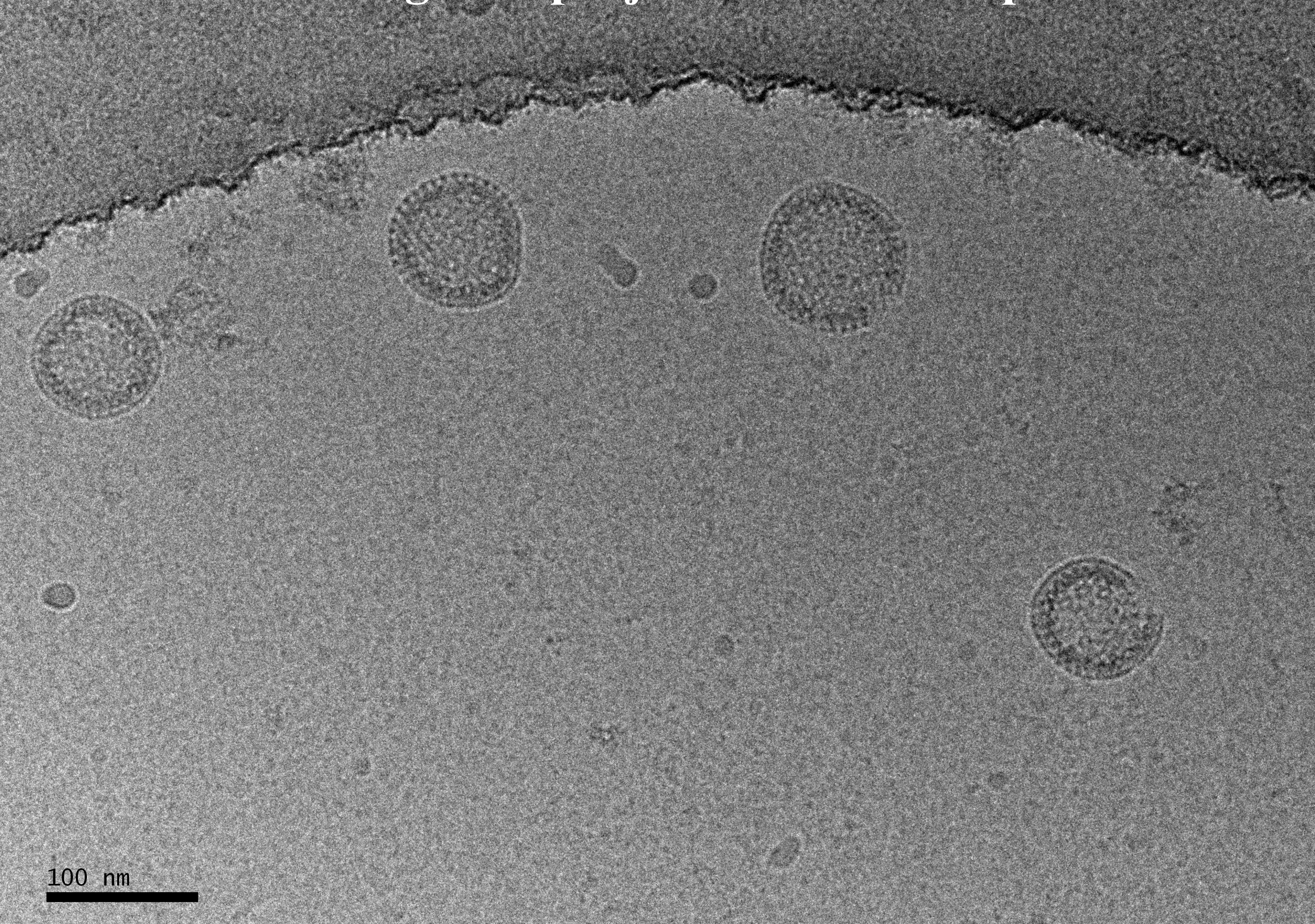
Cryo-Tomography

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

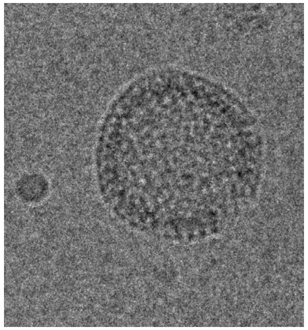
Sample: *in vitro* assembled **V**irus-**L**ike **P**articles of **M**ason-**P**fizer **M**onkey **V**irus

(Pasi Laurinmäki, Institute of Biotechnology
University of Helsinki)

Normal TEM image is a projection from 3D specimen



3D cryo-EM: aligned tomographic tilt series

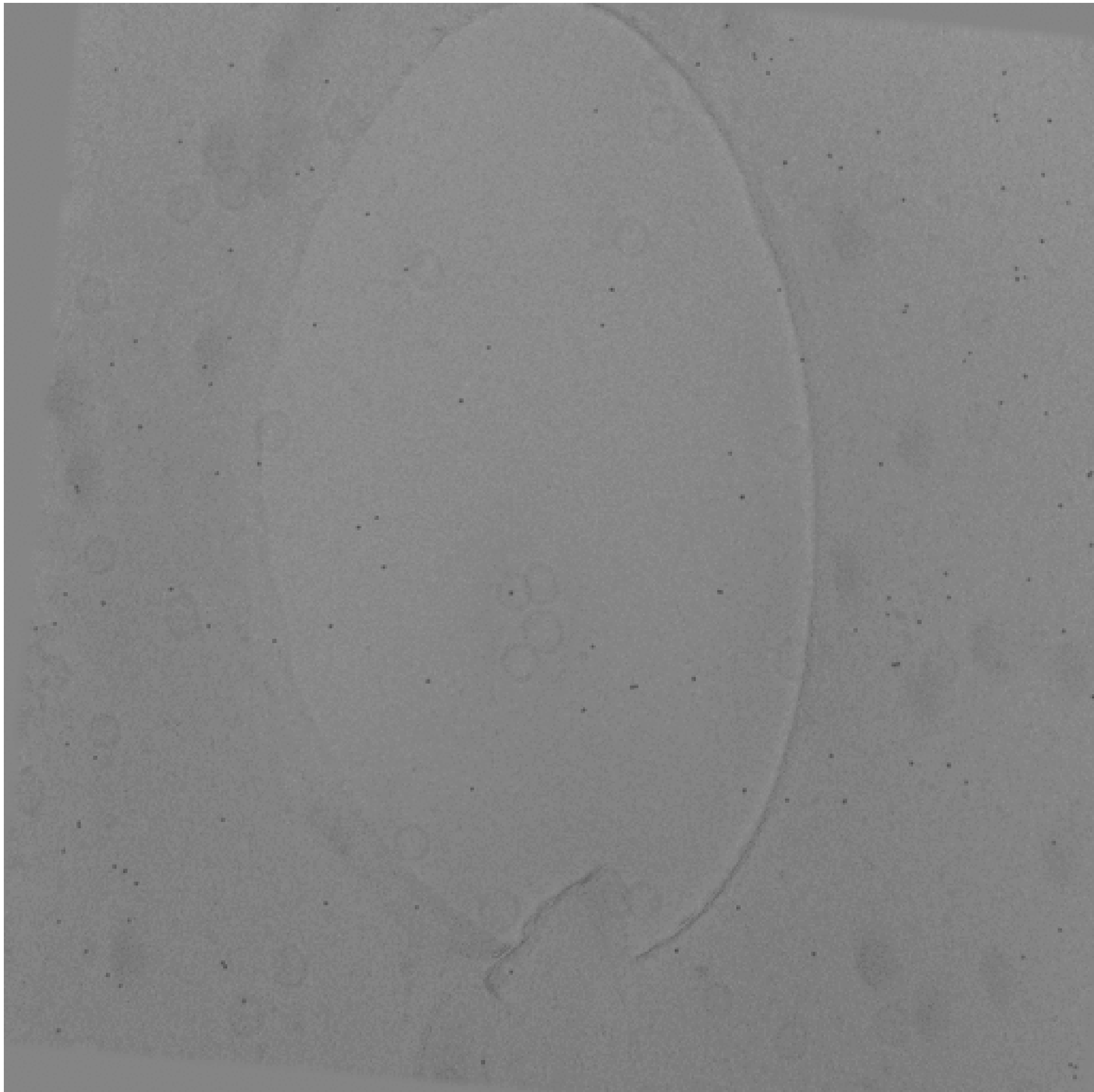


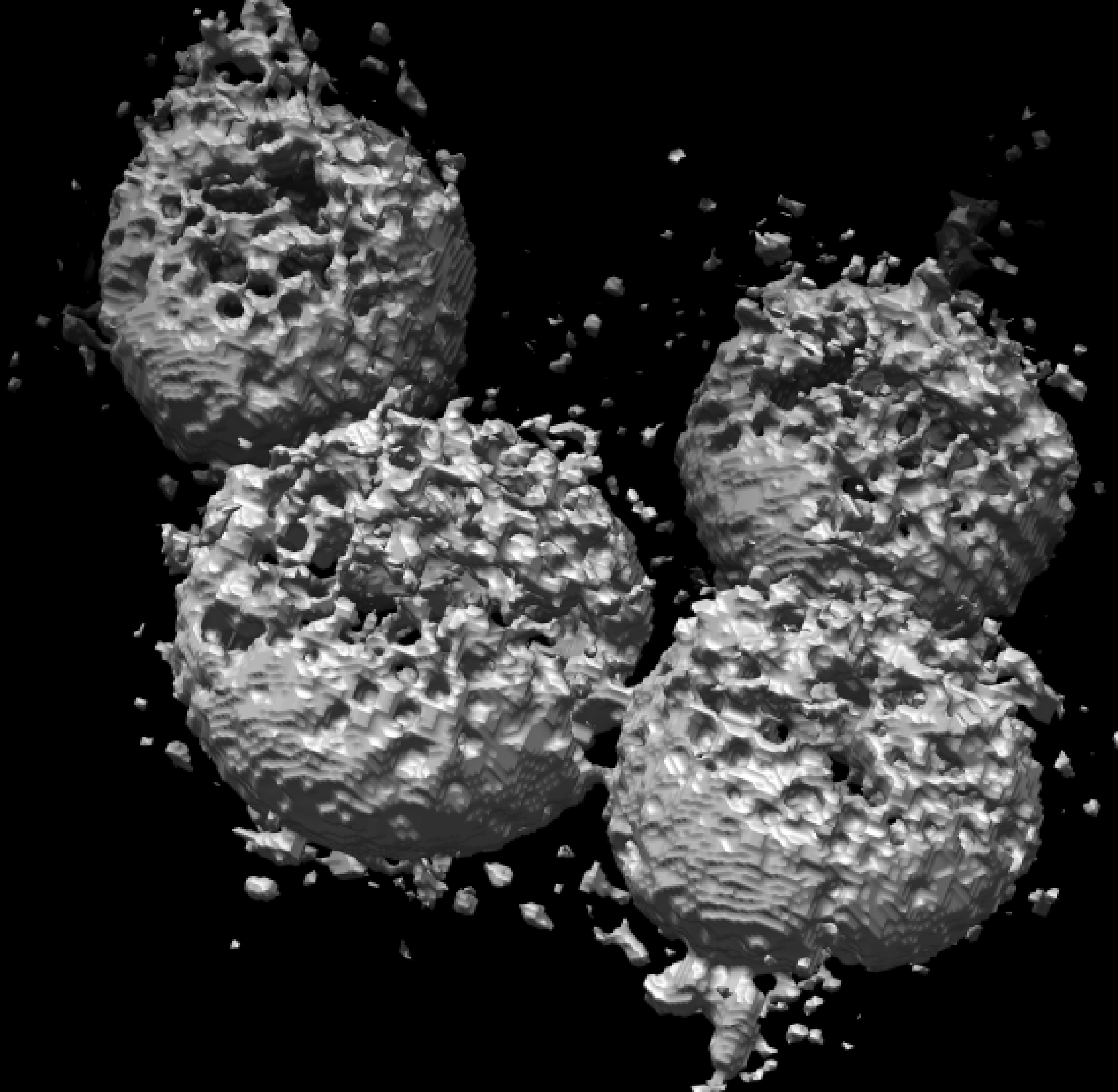
Low-dose series of *in vitro* assembled proCANC M-PMV particles

Diameter of the large round hole is about 2um

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

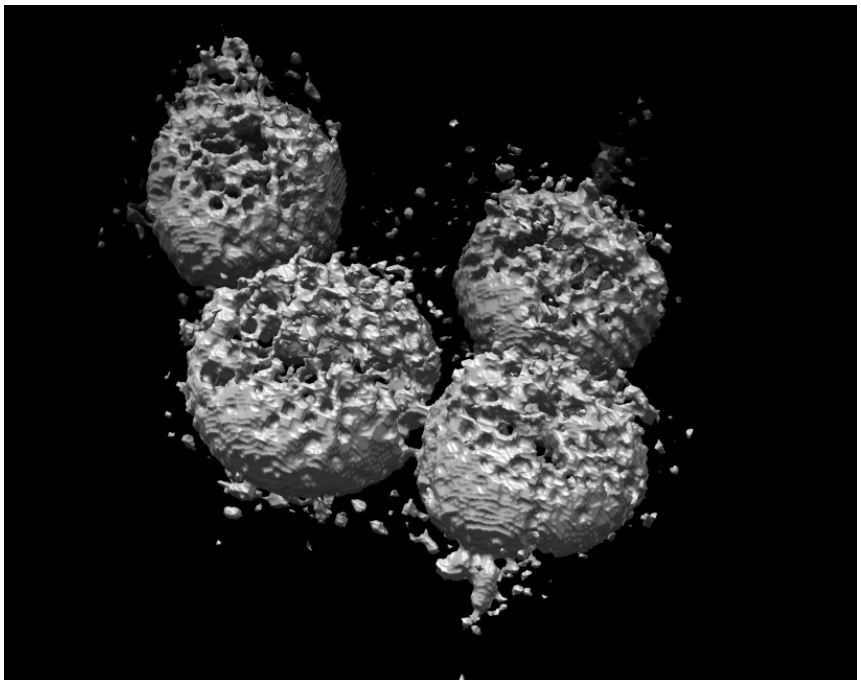
Then we just take images from -70 to + 70 degree tilt and do 3D recontruction

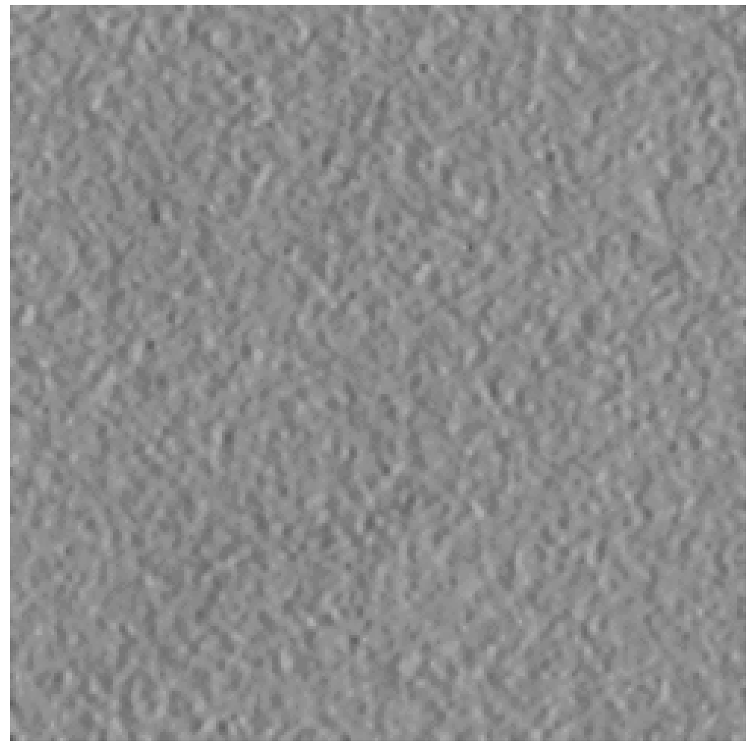




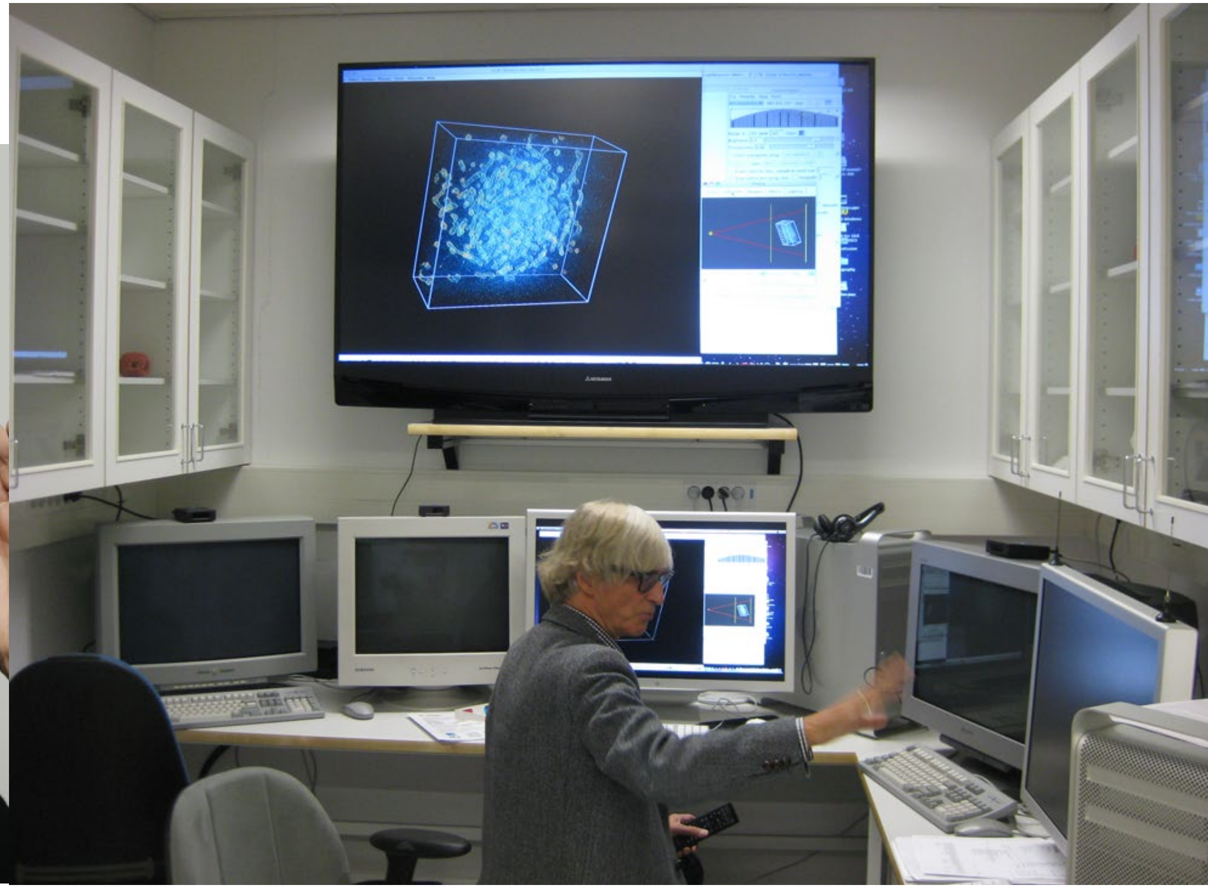
Surface representation of the reconstructed density

Serial sections through the reconstructed density

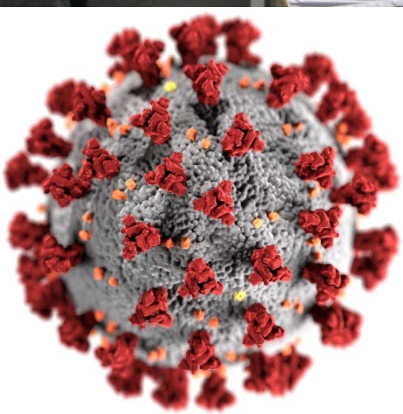




3D structure visualization by using computers and 3D glasses..



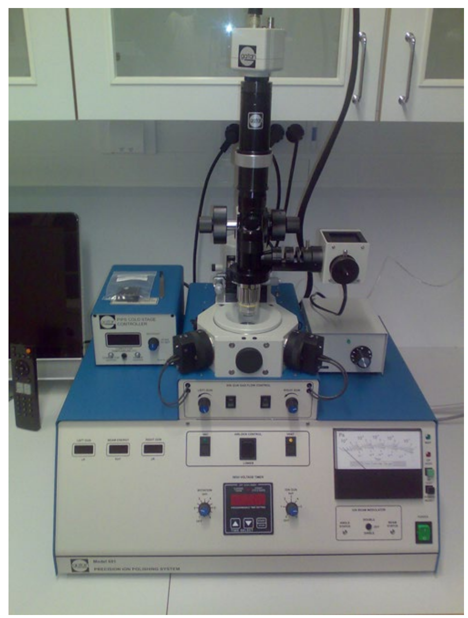
Using similar 3D imaging methods also the structure of the **SARS-CoV-2** virus was resolved (single particle reconstruction)

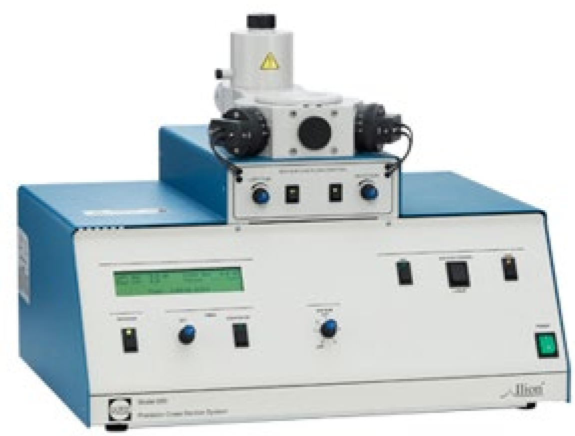


Sample preparation for TEM: THIN SPECIMENS

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

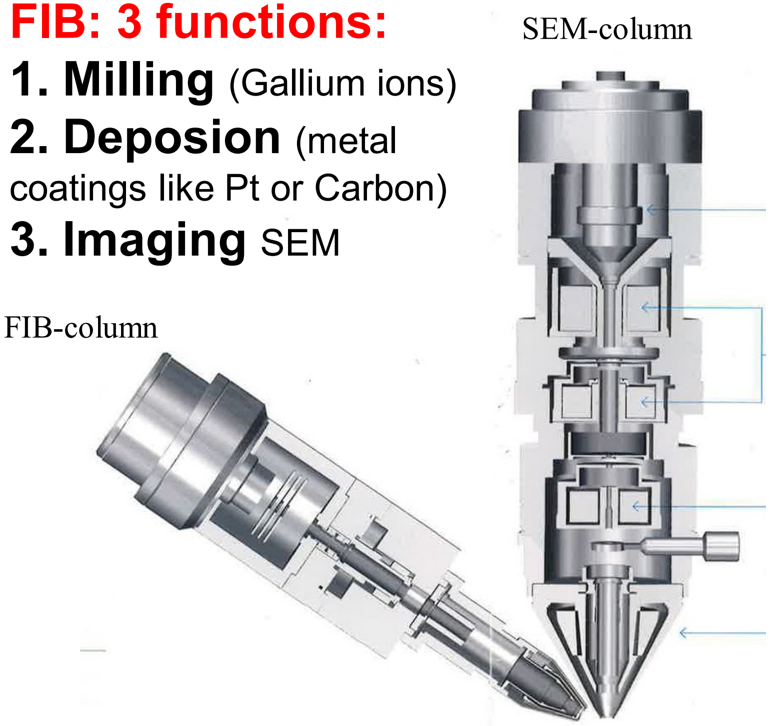
New Dual beam focused ion beam system in Nanomicrosopycenter (installation finnished by June 2019): Main applications TEM sample preparation, cross-section imaging and 3D imaging … (also nano fabrication and IC device failure analyses)

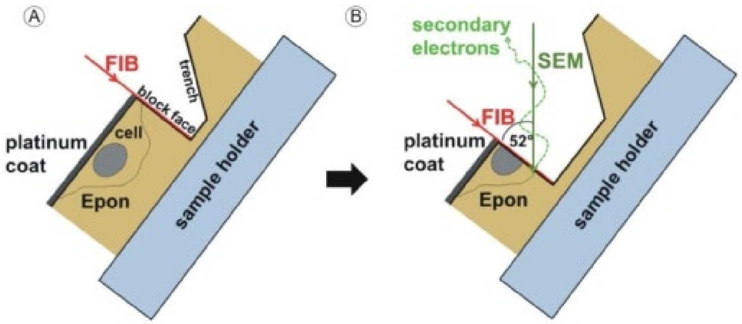
FIB: 3 functions:

1. Milling (Gallium ions)

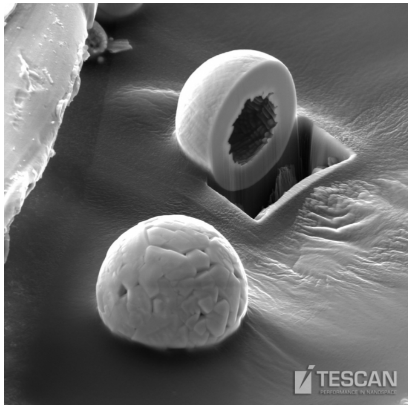
2. Deposion (metal coatings like Pt or Carbon)

3. Imaging SEM



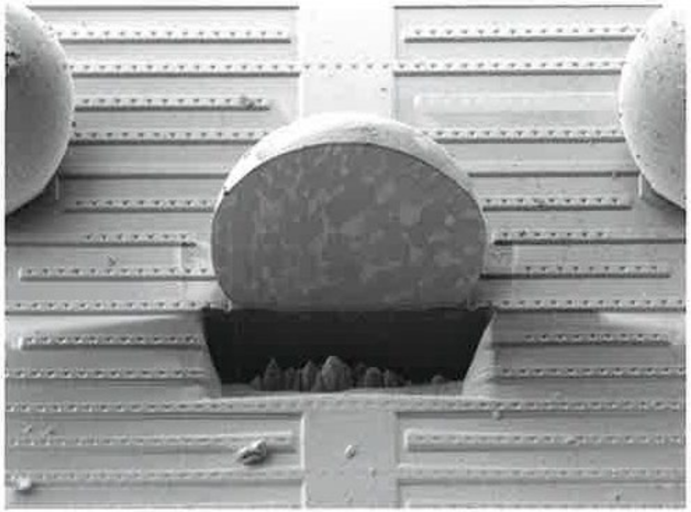


3D imaging



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