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Webinar Series in TEM: Transmission Electron Microscopy - Part 1

A Brief Introduction to TEM

Riza Iskandar

Application Scientist

Material Science

Material and Structural Analysis - APAC

UNIVERSITAS INDONESIA | DIREKTORAT RISET & PENGEMBANGAN

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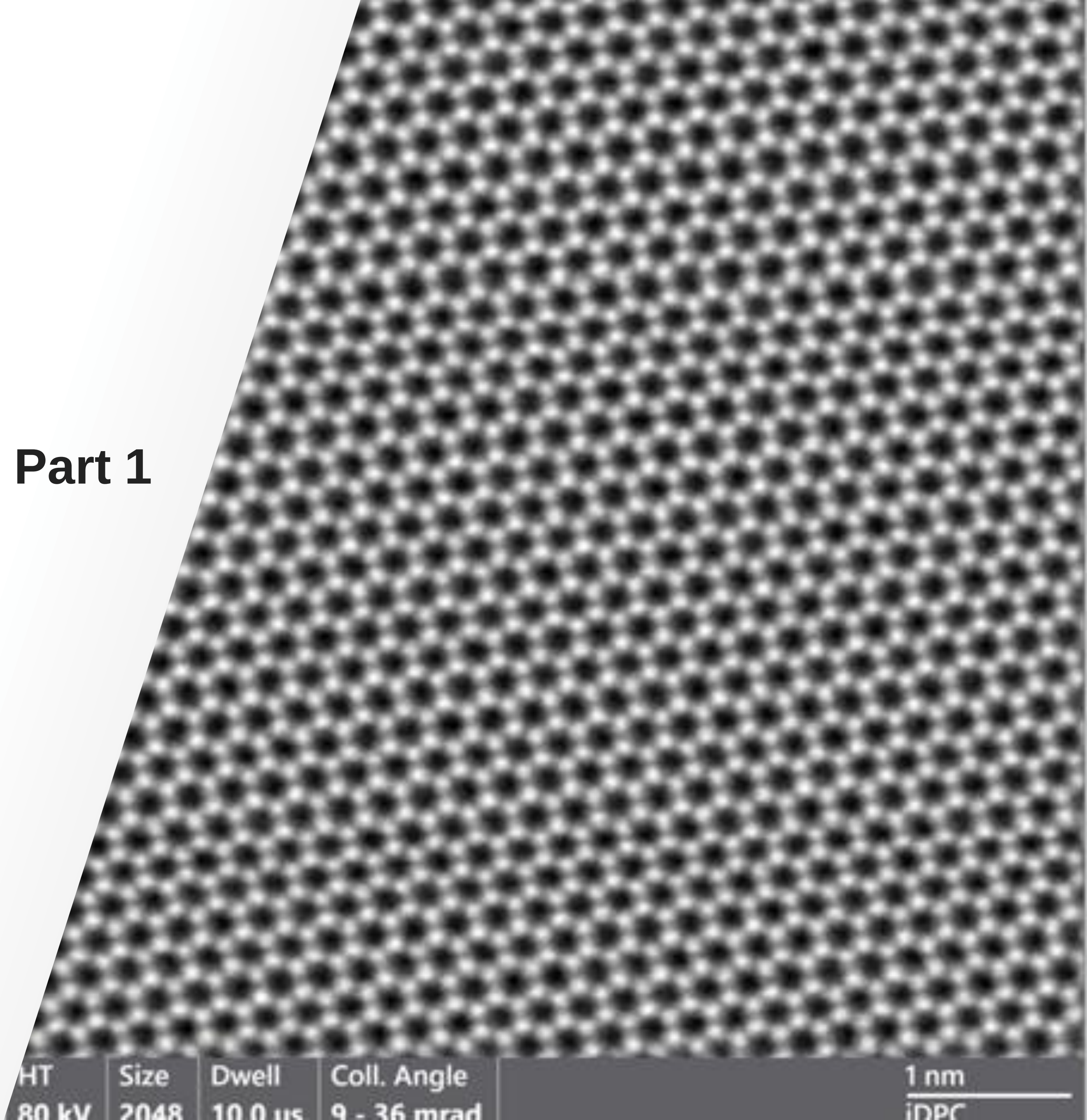

Transmission Electron Microscopy Webinar Series

Outline: Part 01

- Basic Theory of TEM
- SEM vs. TEM: What are the differences
- Conventional TEM Imaging
  - Bright-Field Imaging
  - Dark-Field Imaging
- Electron Diffraction
  - Selective Area Electron Diffraction
  - Convergence Beam Electron Diffraction
  - Nano Beam Electron Diffraction
- Advanced TEM Imaging
  - High-resolution TEM
- Special Investigation Cases
  - Magnetic Samples
  - Soft Materials
  - In-situ Investigations
  - Life-Sciences

Transmission Electron Microscopy Webinar Series

Outline: Part 02

- An Overview of TEM Sample Preparation
- Various Types of TEM Sample Preparations
  - Conventional Techniques
  - Focus Ion Beam Techniques
- Practical Aspects of TEM Sample Preparations

Transmission Electron Microscopy Webinar Series

Outline: Part 03

- Scanning Transmission Electron Microscopy (STEM)
- TEM and STEM comparisons
- High-resolution Scanning TEM (HRSTEM)
- Spectroscopy in TEM
  - Energy-Dispersive X-Rays Spectroscopy
  - Electron Energy Loss Spectroscopy
- Tomography in TEM: For 2D to 3D Imaging

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Outline: Part 04

- An Overview of TEM for Biological Materials Research
- Biological Samples Preparations
- Room Temperature Investigations
- Cryo-EM Workflow

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(A simplify) Basic Theory of TEM

Electron Microscopy History

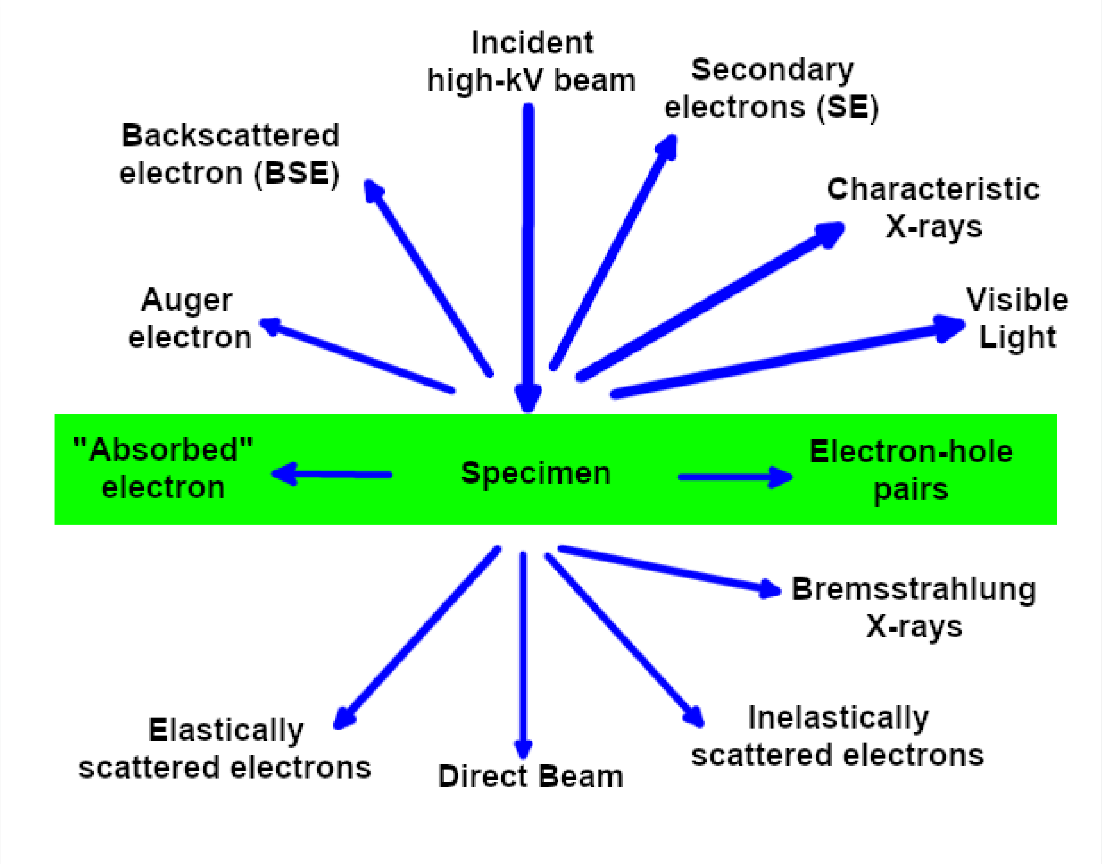
- 1897: Thompson describes the existence of negatively charged particles (electrons)
- 1925: De Broglie theorized that electrons have wave-like characteristics, addressing the wave/particle duality
- 1927: Thompson and Reid demonstrated the wave nature of electrons by diffraction experiments
- 1931: Ruska et al. build the first electron microscope (Nobel Prize in 1986)

Knoll and Ruska (in the lab coat) with the first Transmission Electron Microscope in Berlin in the early 1930s

(A simplify) Basic Theory of TEM

What is TEM?

- To see **small objects** which cannot be seen with naked eyes, light microscope or even a SEM.
- To obtain structural information of **small objects**
- To analyze the chemical compositions of **small objects**



Electron – Sample Interactions

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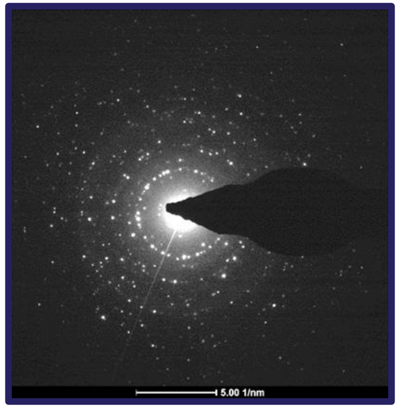
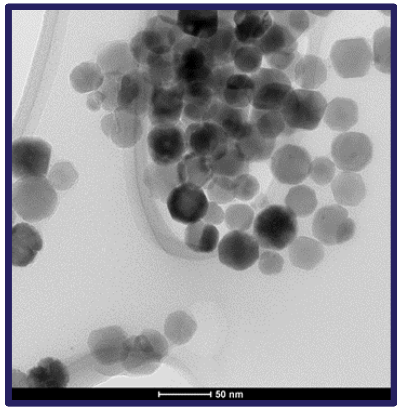
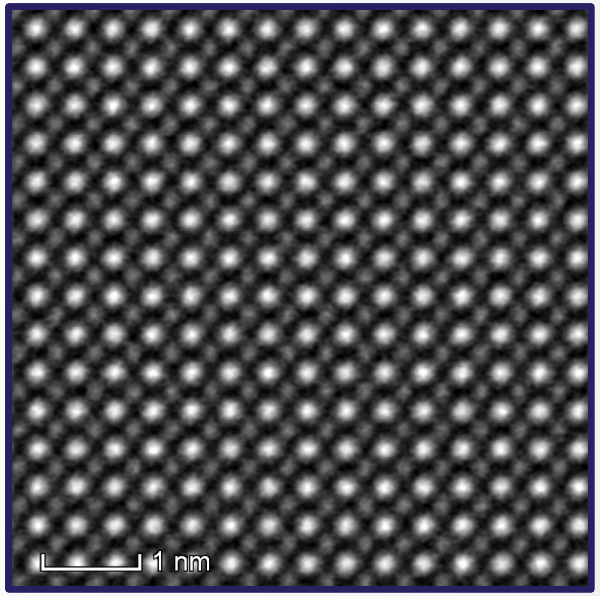
(A simplify) Basic Theory of TEM

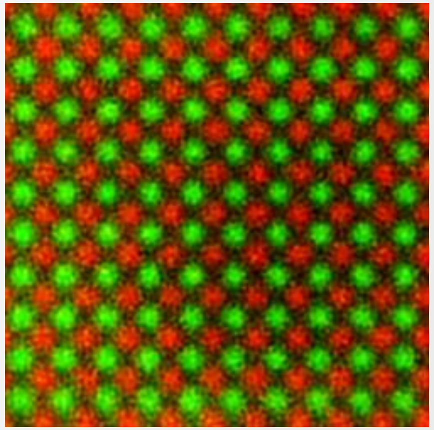
What can TEM Do?



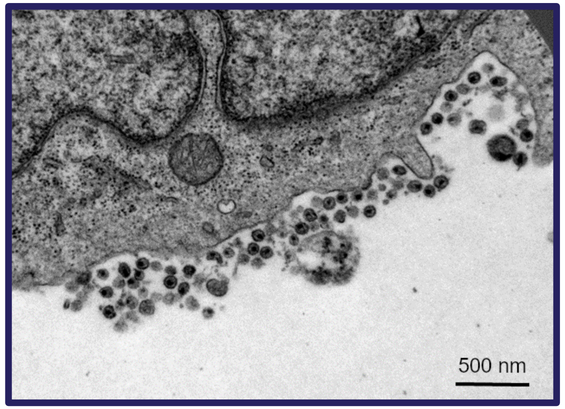


Electron Diffraction pattern (SAED) taken from nano particles (left) as shown in TEM-BF (right)

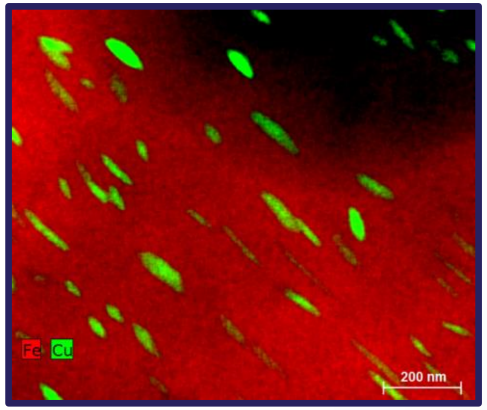




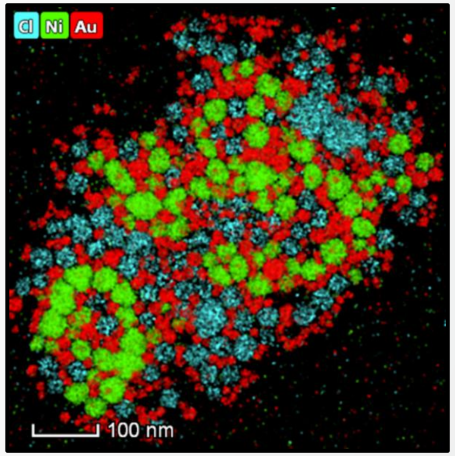
HRSTEM and HREDS $SrTiO_3$



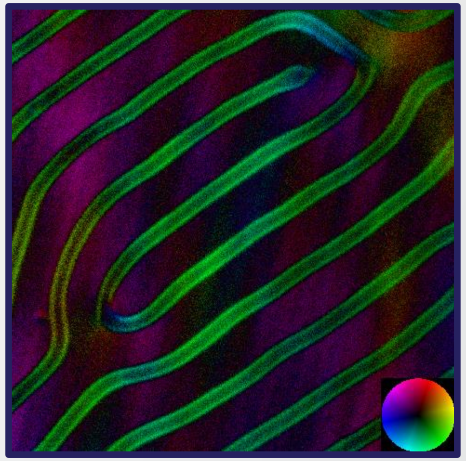
Cat bone marrow with
Feline Immunodeficiency Virus



EDX mapping of Cu and Fe elements distribution in steel



Au-Ni-Cl nanoparticles

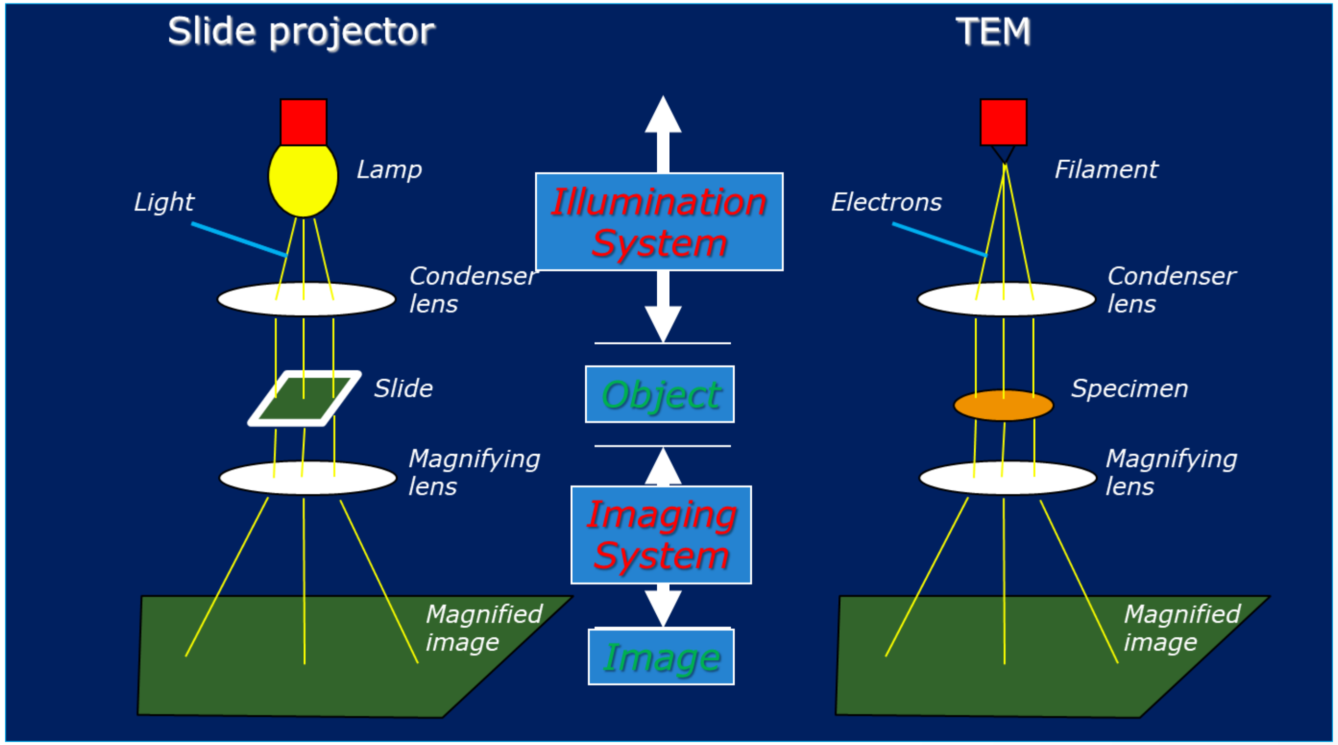


Magnetic Hexa-ferrite

(A simplify) Basic Theory of TEM

How does TEM Work?

Slide Projector vs TEM

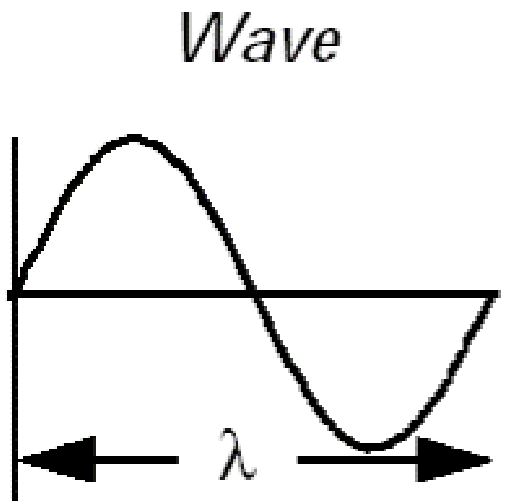


Light Microscope	Transmission Electron Microscope
Visible light	electron
Glass lenses	Electron-magnetic lenses
λ = 450-650 nm	λ = 0.0025 nm (200 kV)
spatial resolution = 100 nm ($d > \lambda$)	spatial resolution = 0.24 nm ($d \cong \lambda$)

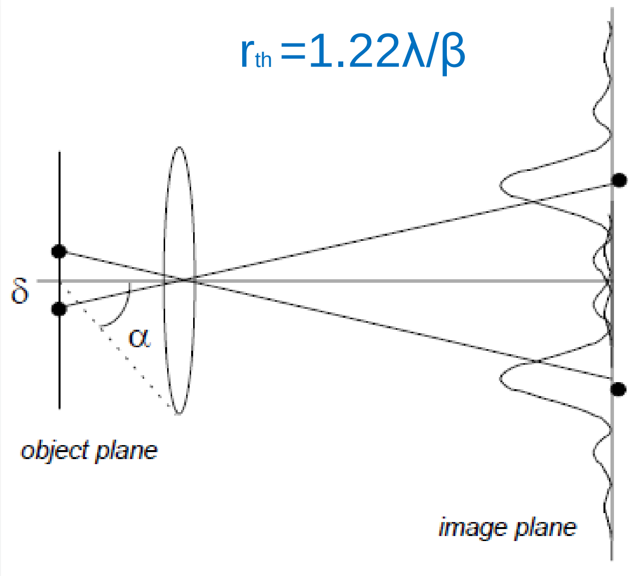
(A simplify) Basic Theory of TEM

Why Use Electrons?

- The resolution of light microscopy is limited by the wavelength of visible light (400 – 700nm)*
- Electrons, that are particles as well as a wave, have much shorter wavelength, which gives much better resolution
- De Broglie equation: $\lambda = h/mv$



U (kV)	Relativistic (λ=nm)
100	0.0037
120	0.0034
200	0.0025
300	0.0020

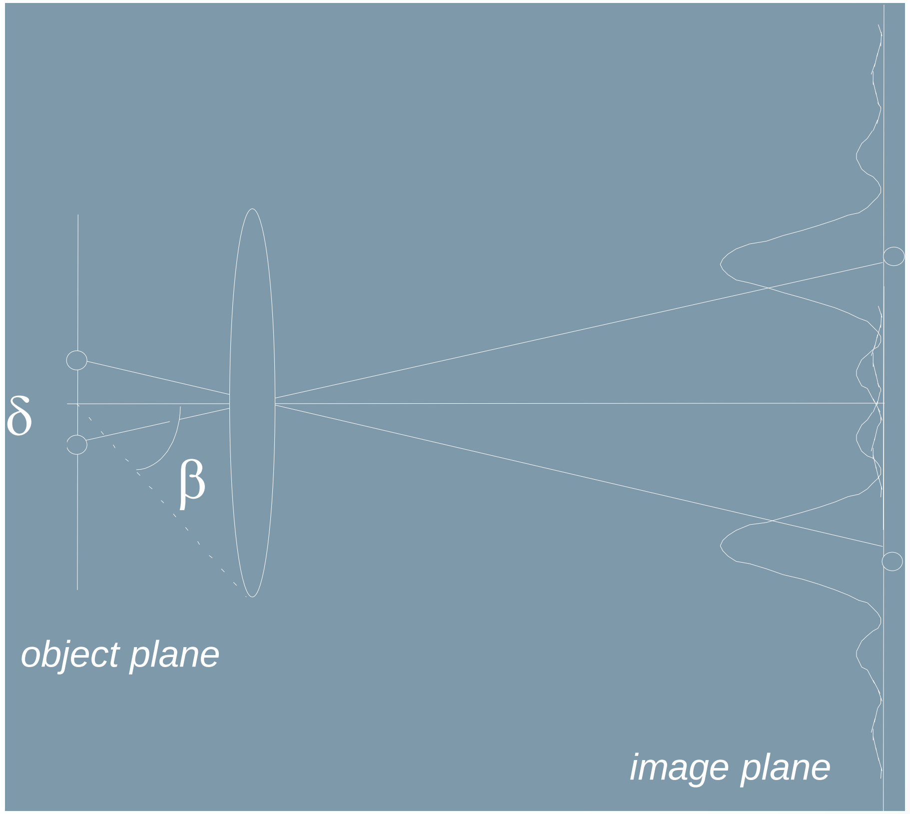


*X-ray wavelength is about 0.05-0.25 nm

(A simplify) Basic Theory of TEM

Resolution

- Resolution (or Resolving Power): the smallest distance between two points that can be resolved



Rayleigh Criterion:

$$\delta = \frac{0.61\lambda}{n \sin\beta}$$

Light microscopy:
n=1.5 (oil)
l=400 nm
b=60°
d=200 nm

(A simplify) Basic Theory of TEM

Magnification vs. Resolution

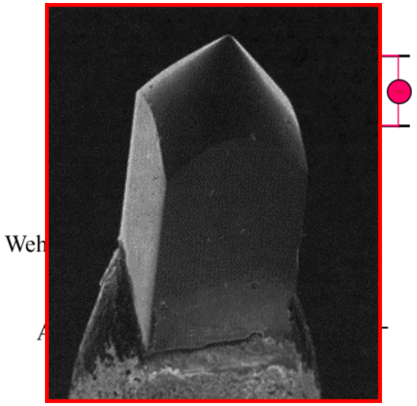
Resolution is more critical than magnification

https://vrroom.buzz/vr-news/products/japan-display-improves-vr-screens-pixel-density

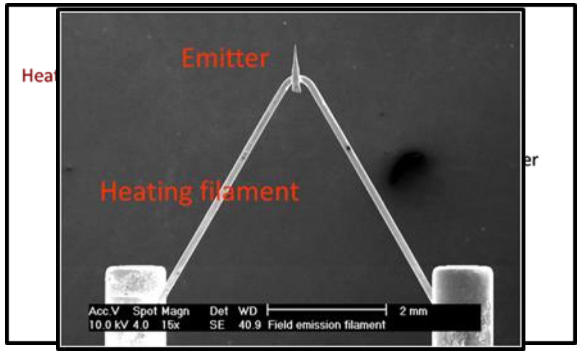
(A simplify) Basic Theory of TEM

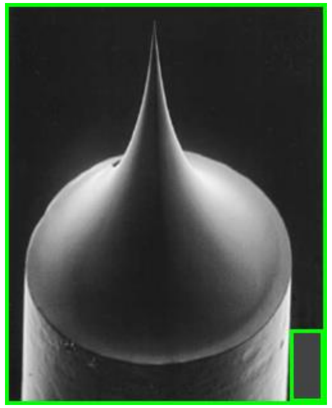
Electron Sources

Thermionic



Field Emission Gun (FEG)





	LaB_6	SFEG	XFEG	X-CFEG
Normalized brightness	1-3	250	1250	1625
Energy spread (eV)	1.1-1.5	≤0.8	≤ 0.8	≤ 0.3

Tungsten(W)/LaB_6 → SFEG → XFEG → X-CFEG

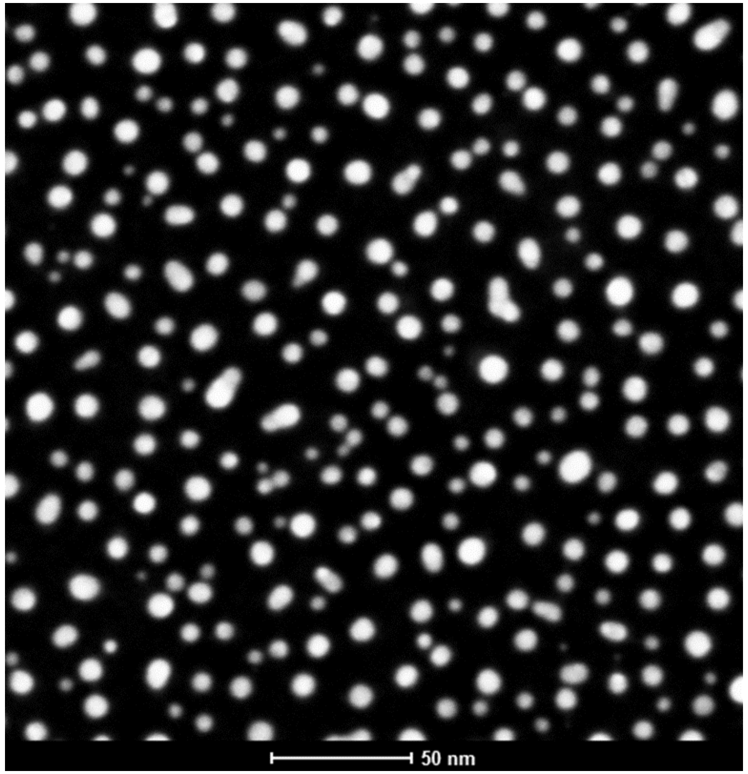
Better Performance

(A simplify) Basic Theory of TEM

Spatial Resolution in High-resolution Imaging

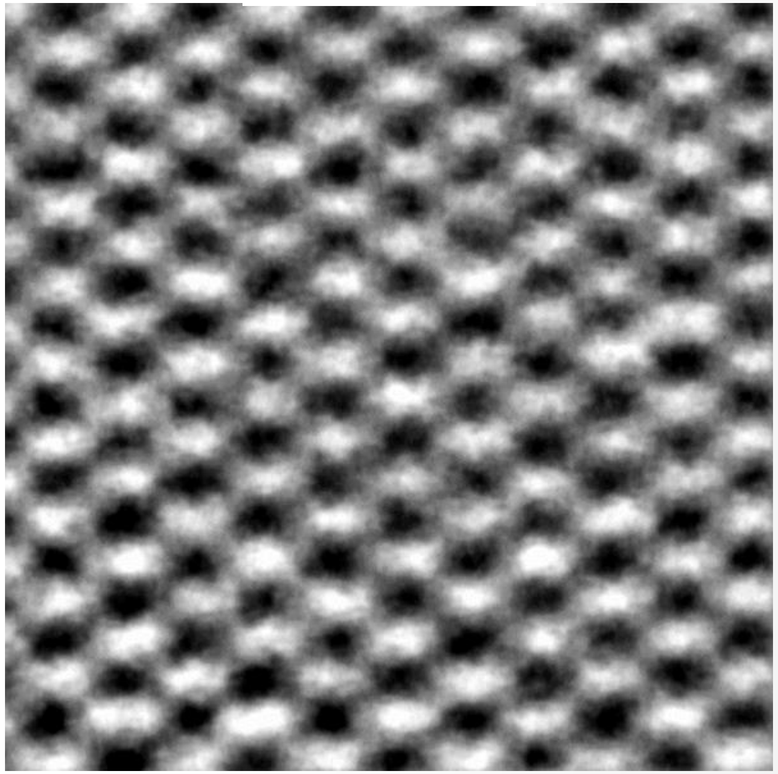
HR-STEM image taken from different TEM with different probe size

Probe size <<<



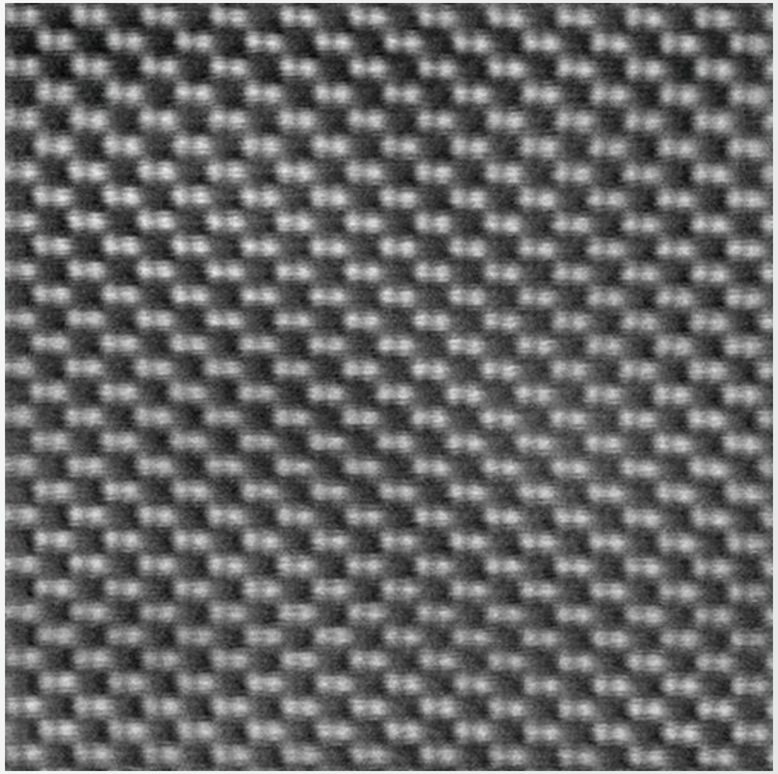
Maximum Magnification

Au nano particle @ 120 kV
STEM resolution ≤ 1 nm



Highest Magnification

The Si <110> dumbbells are seen as one atom @200kV

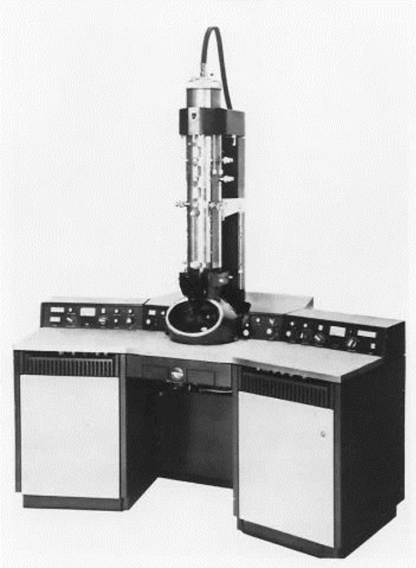


Better spatial resolution

The dumbbells are clearly resolved to be separated

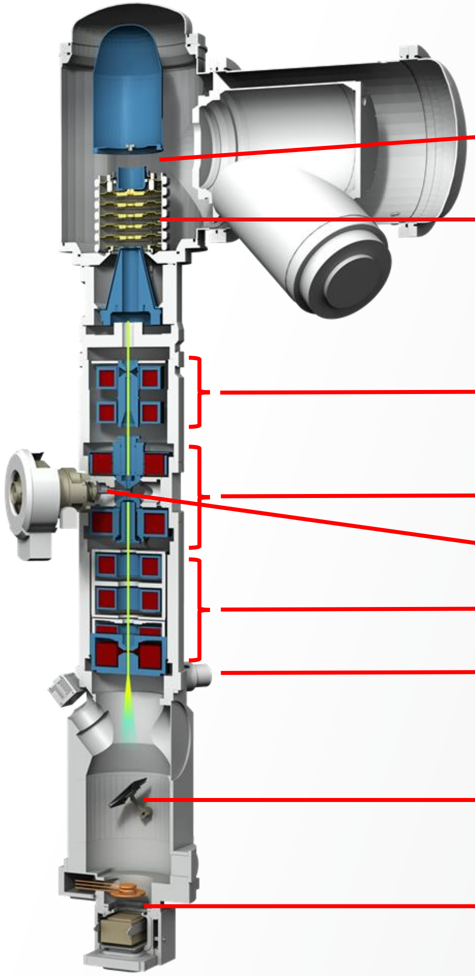
(A simplify) Basic Theory of TEM

Parts of the TEM



Philips Eindhoven 1966

Talos F200X



EELS

Electron Gun – LaB6, FEG, XFEG (CFEG, X-CFEG, monochromator)	Produces electrons
Accelerator (200 kV))	Accelerates the electrons to high energy
Condenser lens Condenser aperture	Control illumination on the sample
Objective lens Sample / Holder / EDS detectors	Sample and main imaging lens + EDS (objective lens)
Diffraction and Projection lens (magnification)	Controls magnification and image/diffraction mode
STEM detectors: HAADF, 4DSTEM Detectors for imaging formation	
Flu-Screen	Flu-screen image viewed using a camera + monitor
TEM camera STEM detectors (BF/DF)	TEM and STEM detectors
All in high vacuum	Whole column under high vacuum

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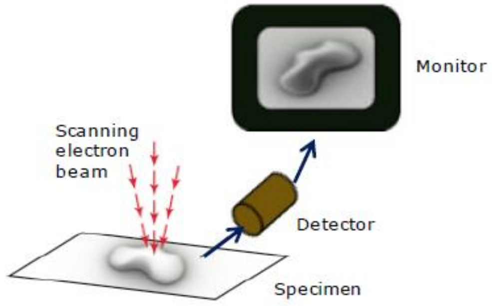
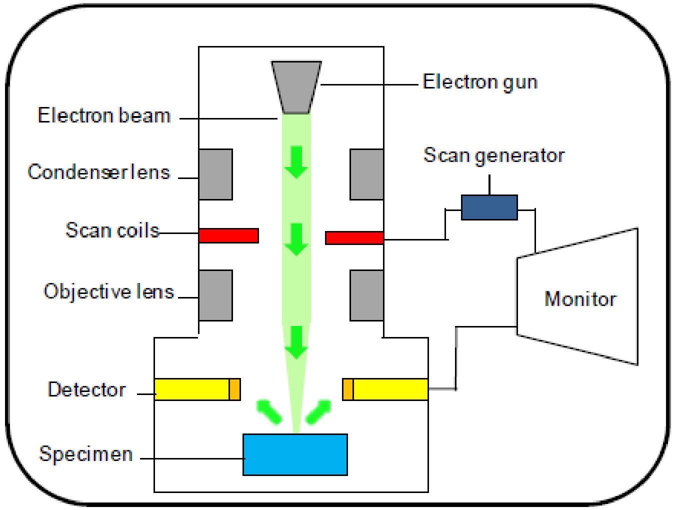
SEM vs TEM

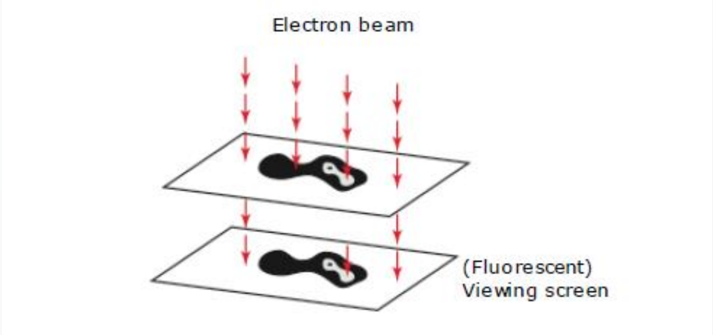
What are the differences?

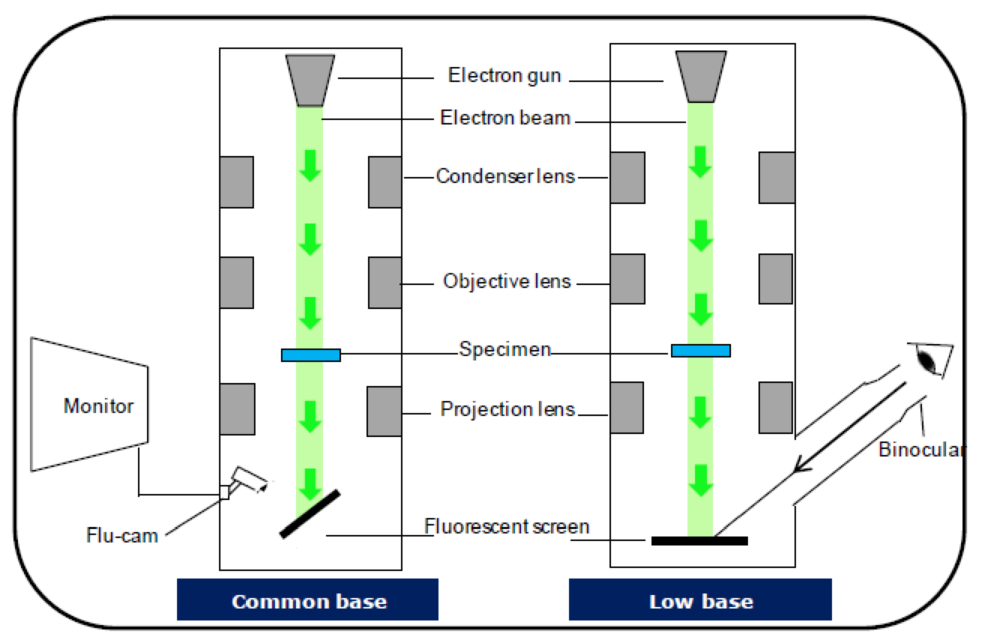
SEM: Scanning Electron Microscope





TEM: Transmission Electron Microscope



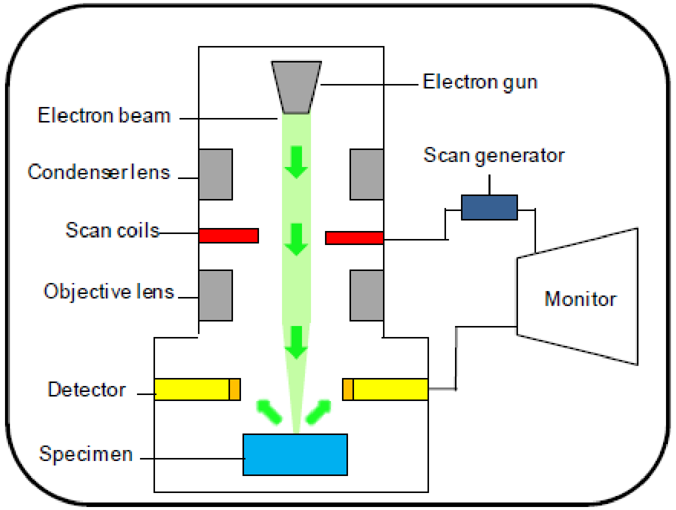


SEM vs TEM

What are the differences?

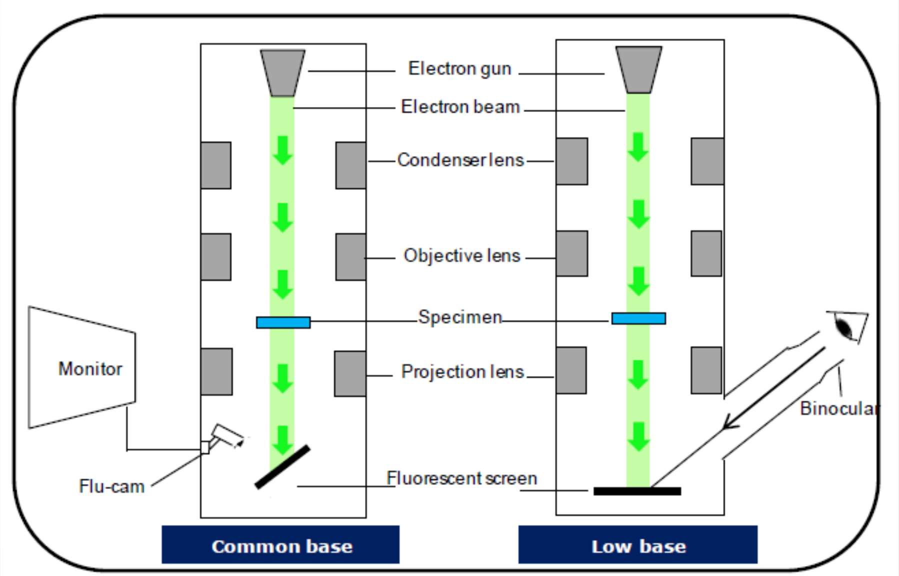
SEM: Scanning Electron Microscope

- Smaller/shorter
- Acceleration voltage: 5kV – 30 kV
- Resolution ≥ 0.7 nm
- Focused scanning beam
- Larger specimen chamber
- Larger samples



Transmission Electron Microscope

- Larger/taller
- Acceleration voltage: 60-300kV or *30kV – 1MV**
- Resolution ≤ 0.1 nm
- Broad static beam and *focused scanning beam (STEM)**
- Smaller specimen chamber
- Thin samples of ≈ 100 nm



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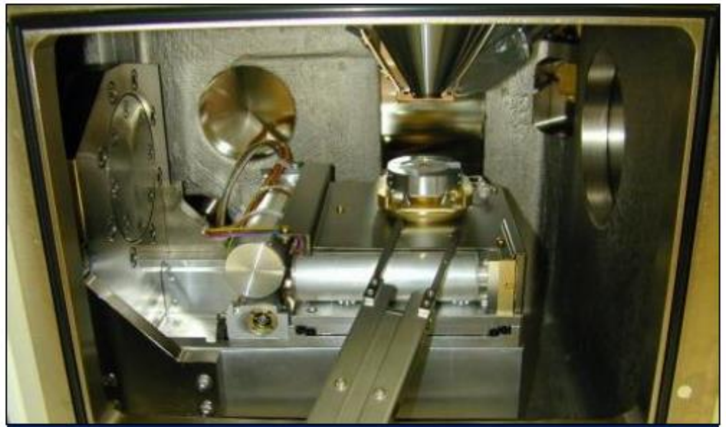
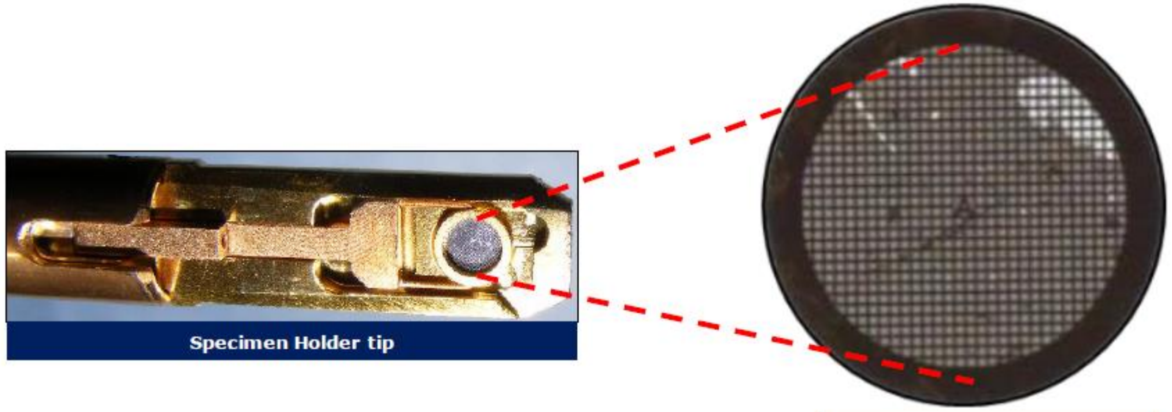
SEM vs TEM

Samples


SEM stage

SEM Specimen


Specimen Holder tip

TEM Specimen

- Diameter: 2-3 mm
- Thickness: 100 – 150 nm

SEM vs TEM

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What is their differences?

- SEM
  - Controlling the electrons to scan the sample surface: Scanning Electron Microscope (SEM)
  - Bulk sample, chamber constrain the sample's size and dimension
  - Sample preparation: "Relatively" simple and straight forward
  - Operating Voltage: 5 – 30 keV
  - Most detectors are located above the sample
  - Resolution achieved ≥ 0.7 nm
  - Capable to produce 2D imaging

- TEM
  - Transmitted electrons through the sample: Transmission Electron Microscope (TEM)
  - The sample must be very thin: Electron transparent sample (≈50 nm)
  - Sample preparation: More complicated than SEM
  - Operating Voltage: 30 – 300 kV - *(1000 kV)**
  - Most detectors are located below the sample
  - Resolution achieved ≤ 0.1 nm
  - Capable to produce 2D and 3D imaging

*) The 1000 kV, known as HVTEM, was very popular to achieve a high resolution. But nowadays with the help or aberration-corrected microscope the high-resolution image can be acquired with much lower operating voltage.

SEM vs TEM

What is their differences?

- SEM
  - Typical imaging techniques available:
    - Secondary Electron (SE) Imaging: Surface Morphology
    - Back Scattering Electron(BSE) Imaging: Sample Composition
    - Scanning Transmission (ST) Imaging*: Sample Composition
    - Electron Back Scattering Diffraction (EBSD): Crystallography
    - Energy Dispersive Spectroscopy (EDS), Wavelength Dispersive Spectroscopy (WDS): Element Mapping

- TEM
  - Typical imaging techniques available:
  - Bright-Field (BF) and Dark-Field (DF) Imaging:
  - Morphology, Grain orientation
  - Electron Diffraction: Crystallography Structure Information
  - High Angle Angular Dark Field Image (HAADF): Z-Contrast/Chemical Contrast Image

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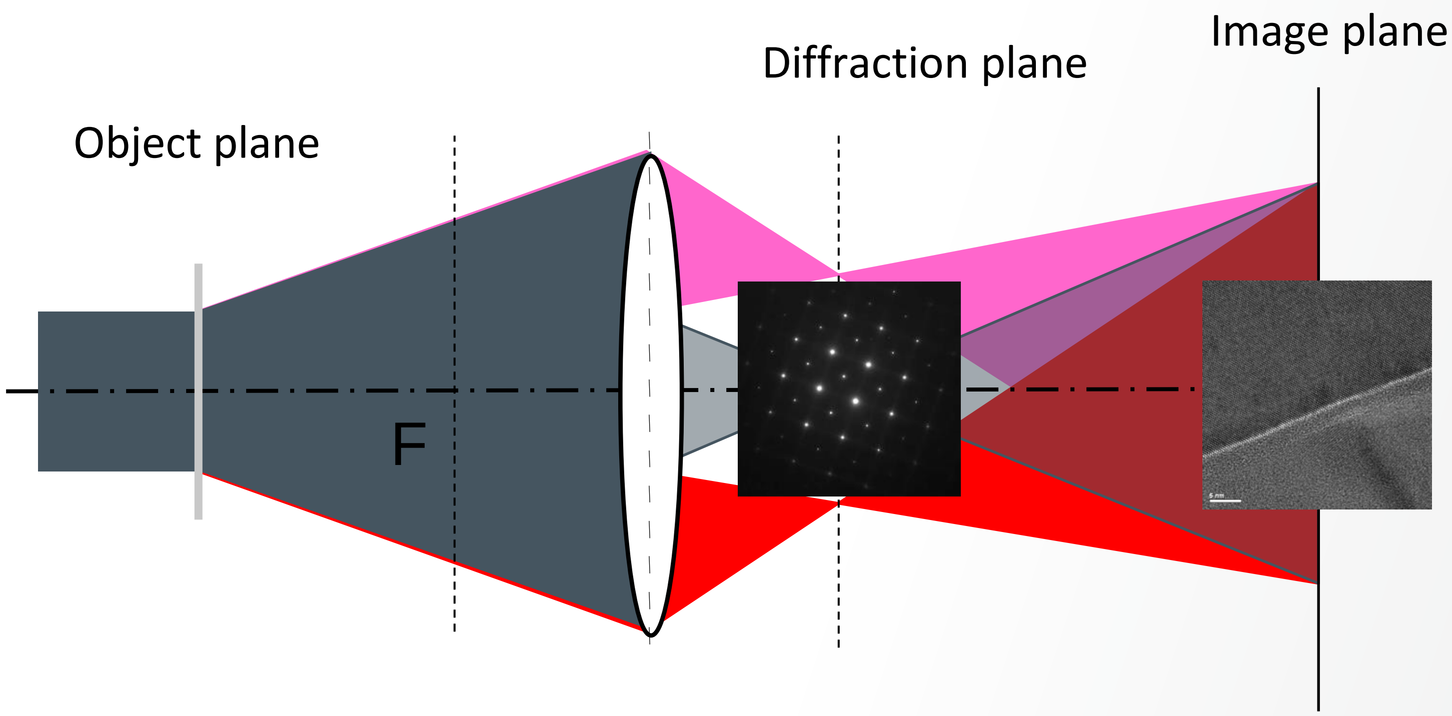
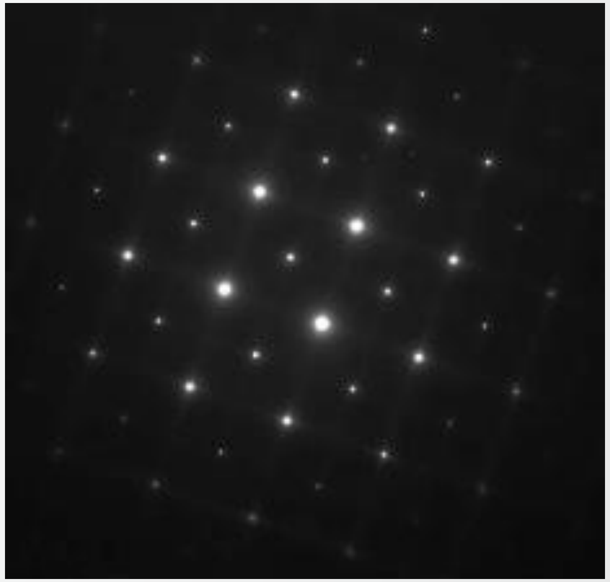
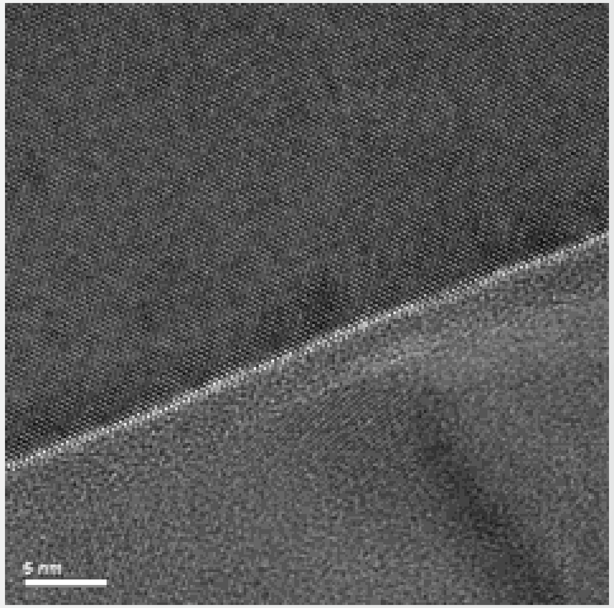
Conventional TEM Imaging

Optics: Image and Diffraction Formation



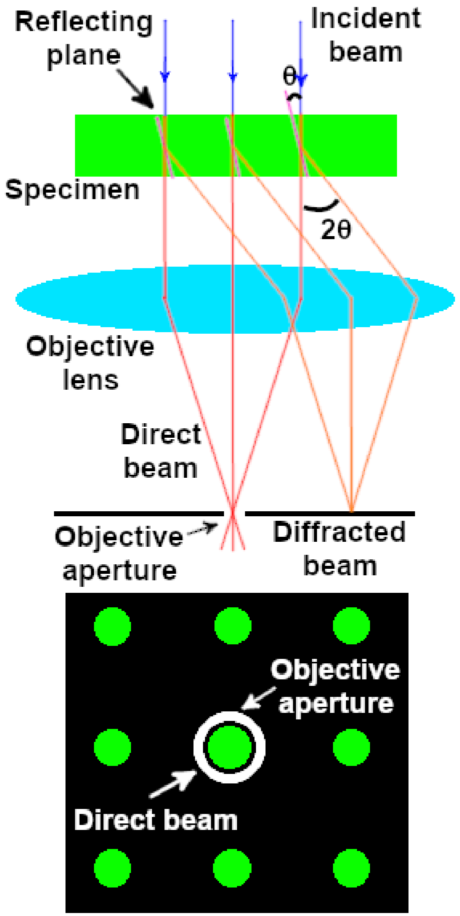


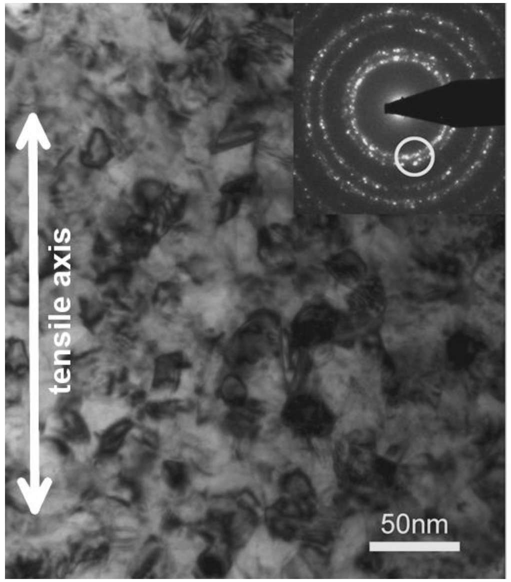


Conventional TEM Imaging

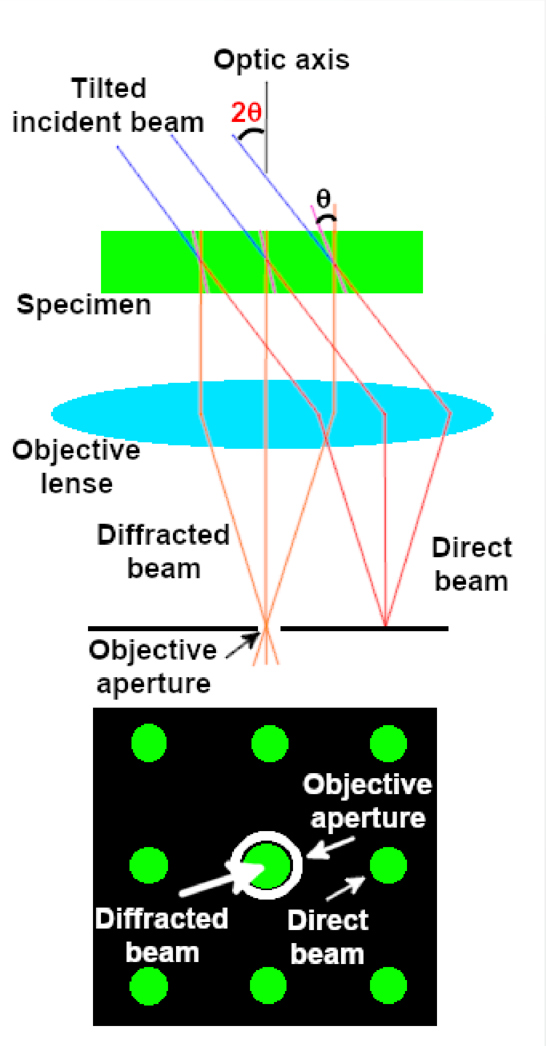
Bright and Dark Field Imaging

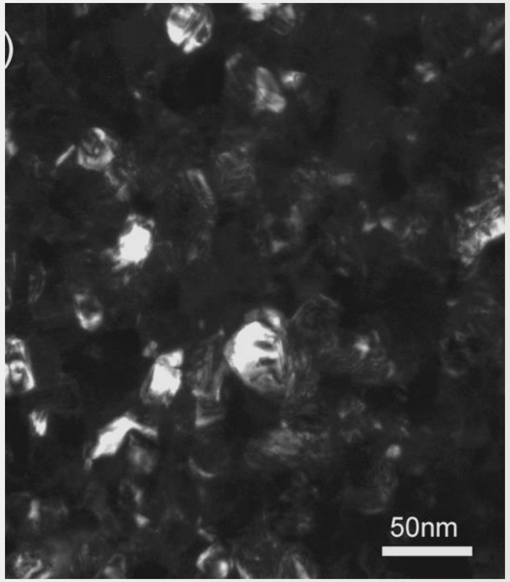
Bright and Dark Field (BF and DF) images of plan-view FIB sample prepared from a 310 nm thick Pd film deformed at 4%.





Bright field image





Dark field image

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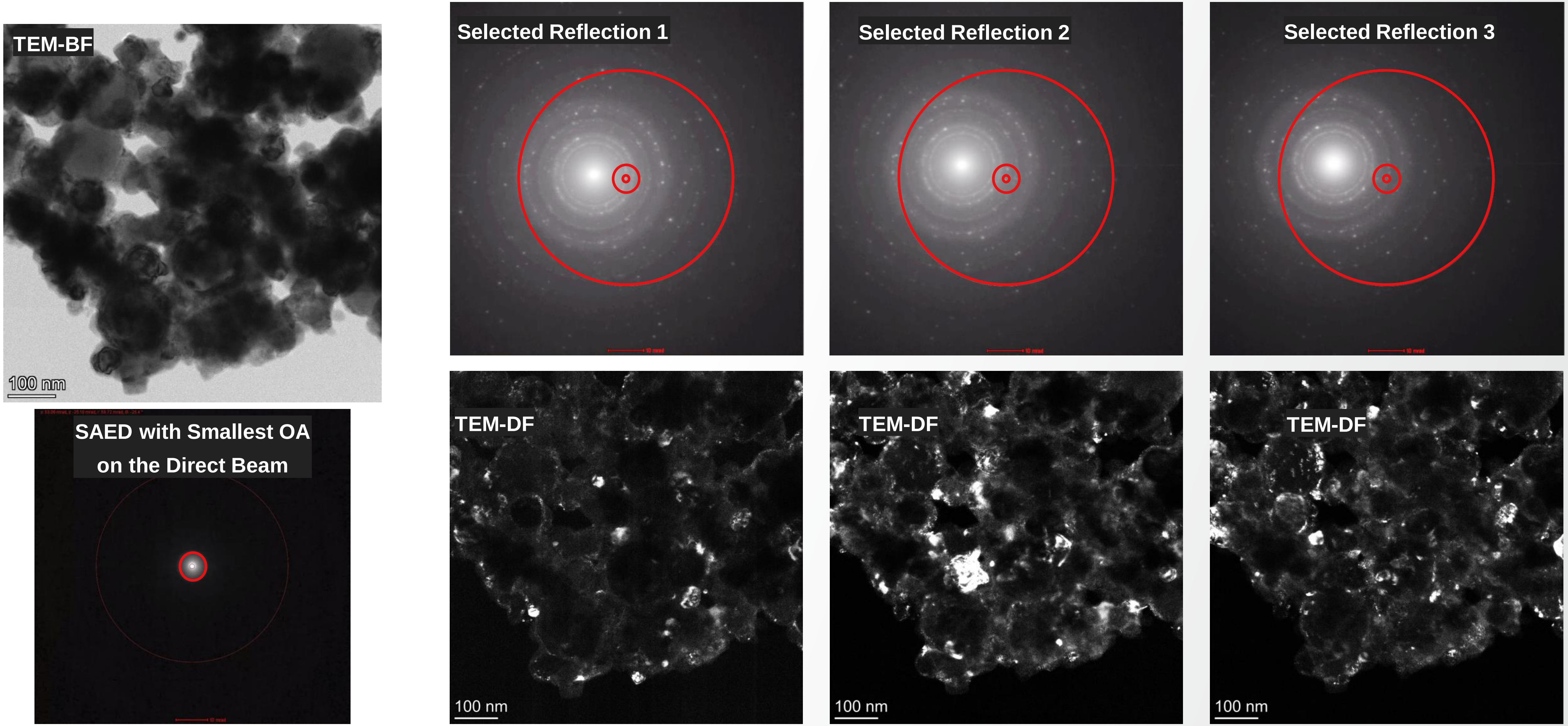
Conventional TEM Imaging

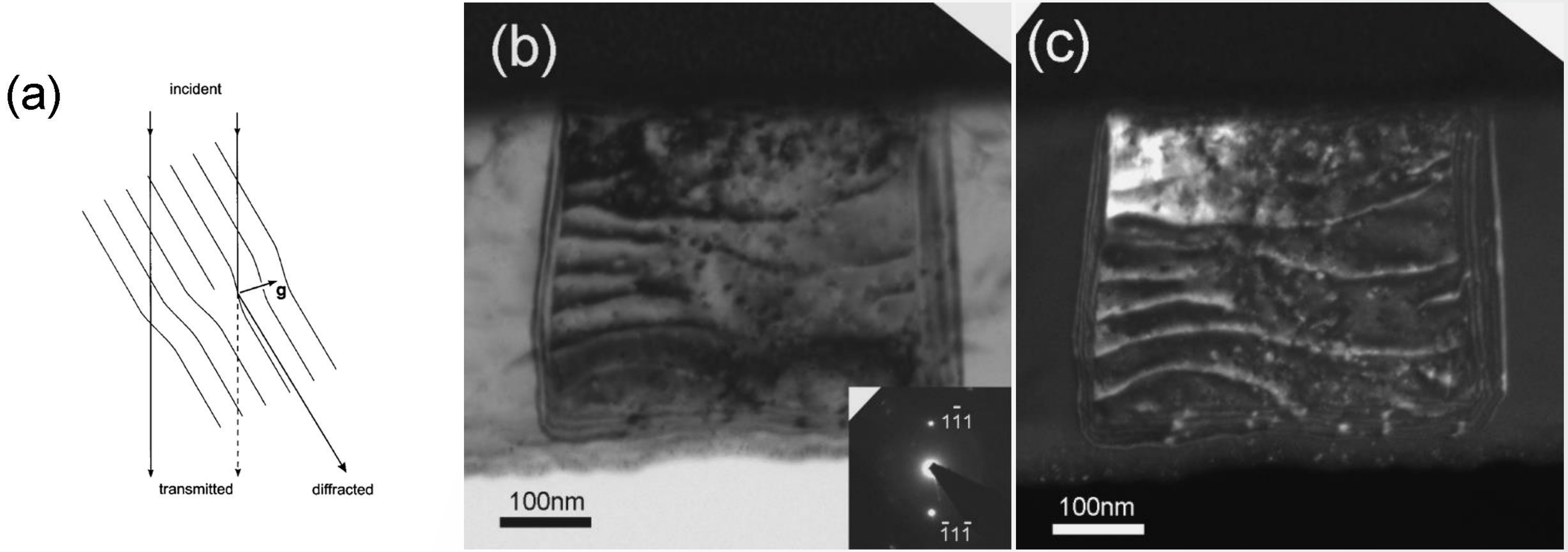
Bright and Dark Field Imaging



Conventional TEM Imaging

Benefit of Dark Field Image

- Grain Orientation
- Defect Analysis
- Phase formation



(a) Planes near an edge dislocation bend into the orientation for diffraction (b) BF image and (c) DF image of dislocations under a two-beam condition in an Al thin film. The inset in (b) shows the SAED pattern indicating the orientation condition for BF imaging.

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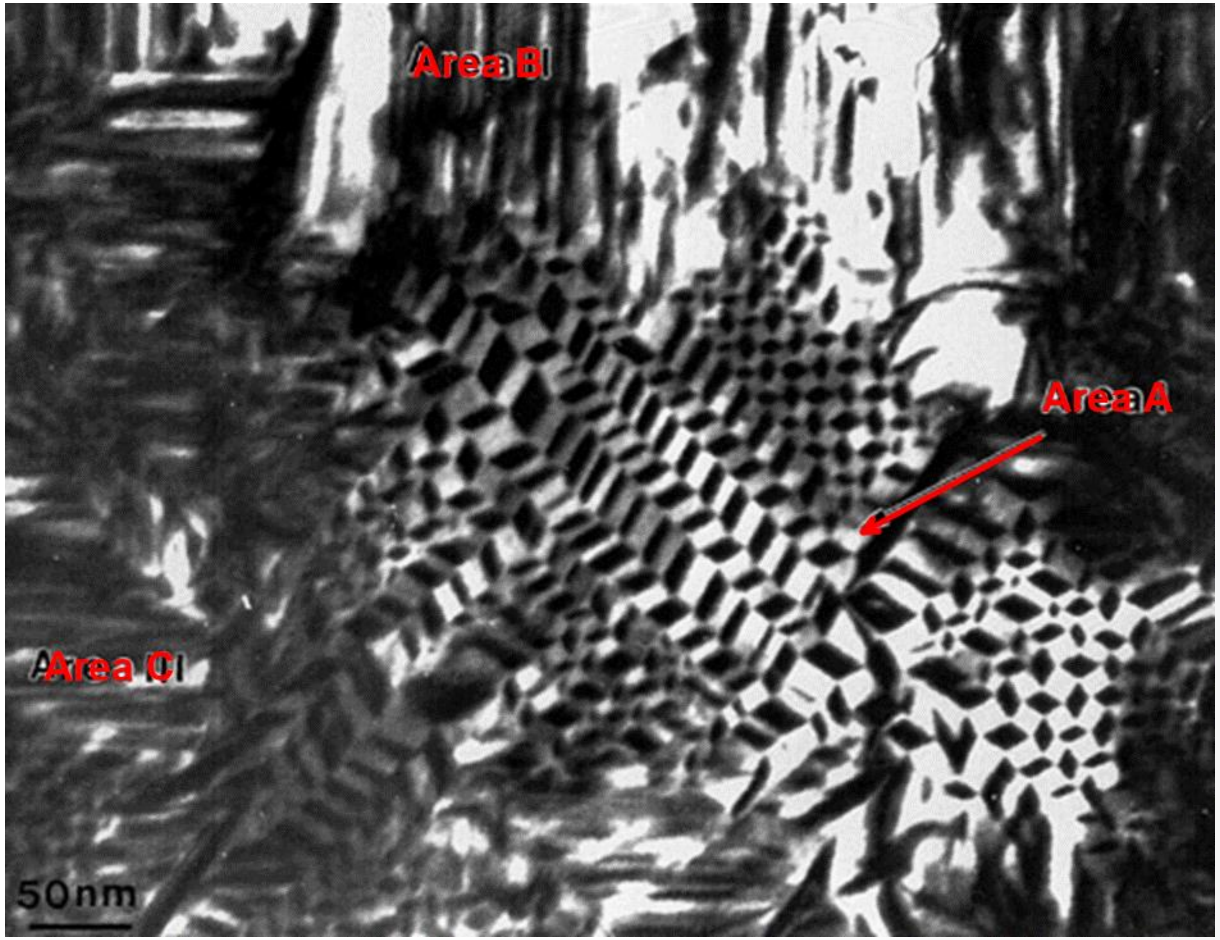
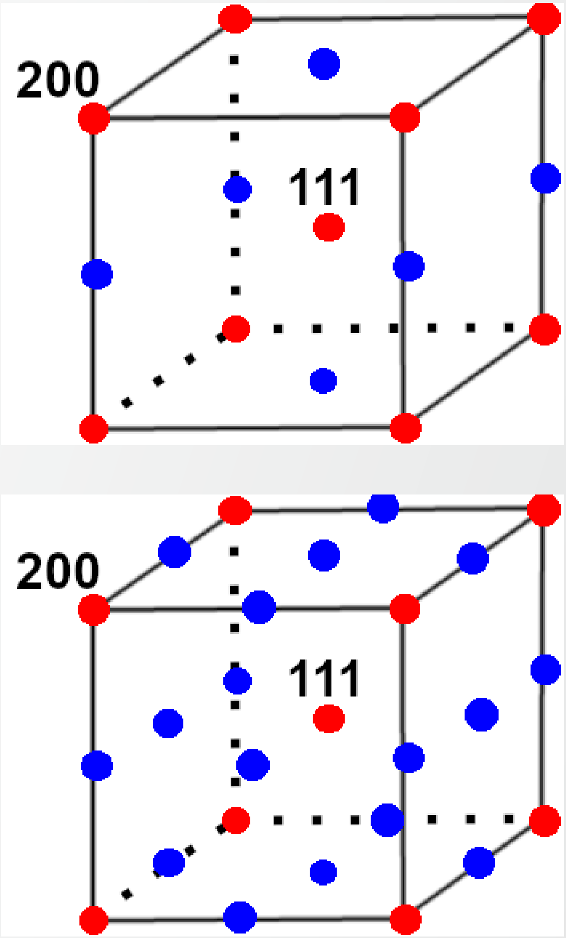
Conventional TEM Imaging

Benefit of Dark Field Image

Regions of one variant or one phase are made visible by selecting a single Bragg reflection for imaging

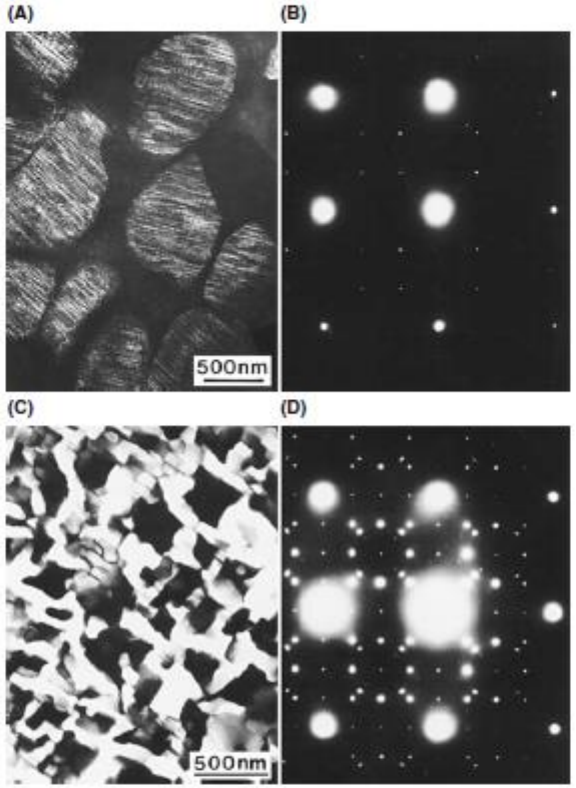




Conventional TEM Imaging

Benefit of Dark Field Image

(A) DF image of ordered V6C5 and (B) accompanying DP. (C) DF image of V8C7 and (D) DP. In both carbides, the ordering is due to vacancies on the C sublattice



Dislocation walls in Al which have been heavily deformed by directional rolling. (200-keV electrons but super specimen.)

D.B. Williams and C. B. Carter, Transmission Electron Microscopy, Springer

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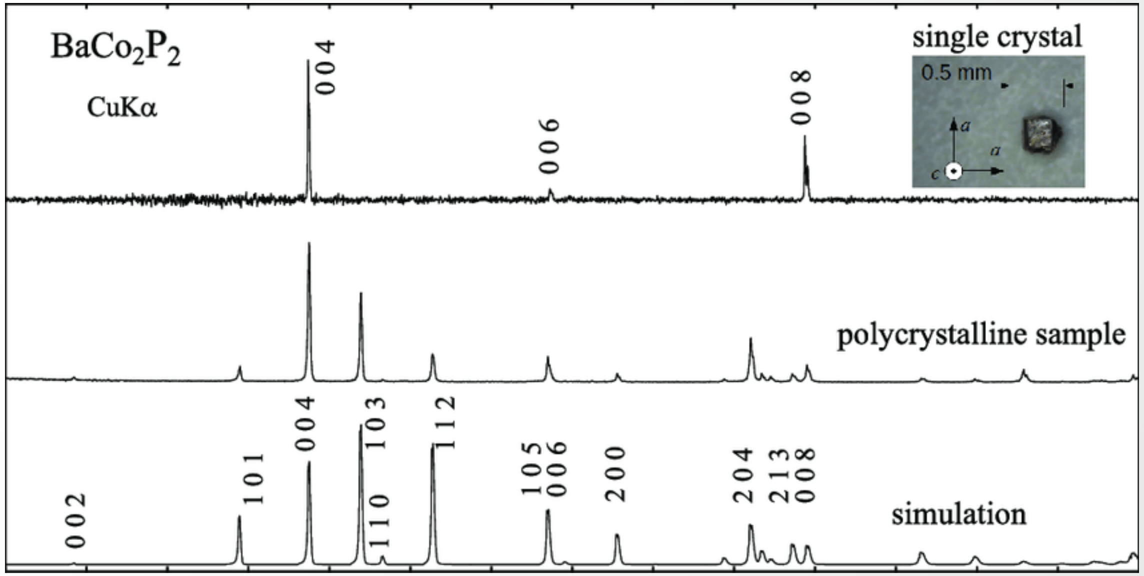
Electron Diffraction

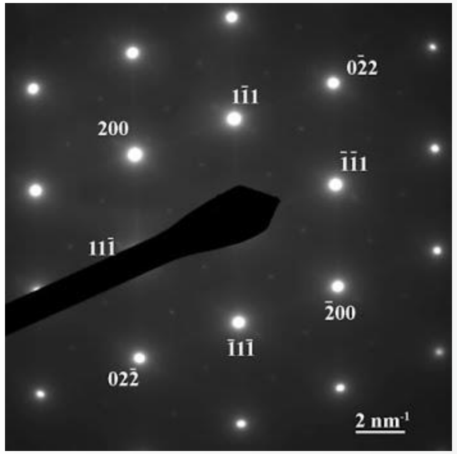
X-rays vs Electron Diffraction

X-rays vs Electron Diffraction		
Parameter	**X-rays**	**Electron**
Analysis	Intensity and position	Position
Type of wavelength for single crystal	Multi-wavelength	Single-wavelength
Acquisition time	Minutes to hours	Less than second

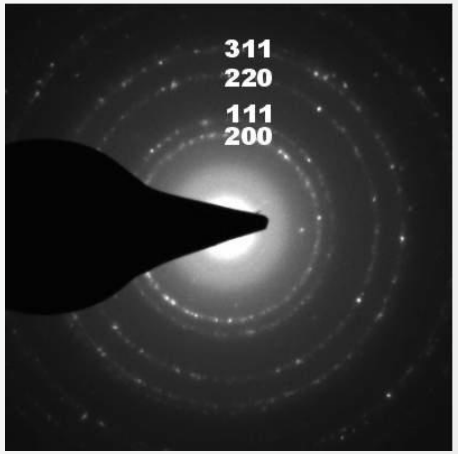


Important facts about electron for diffraction:

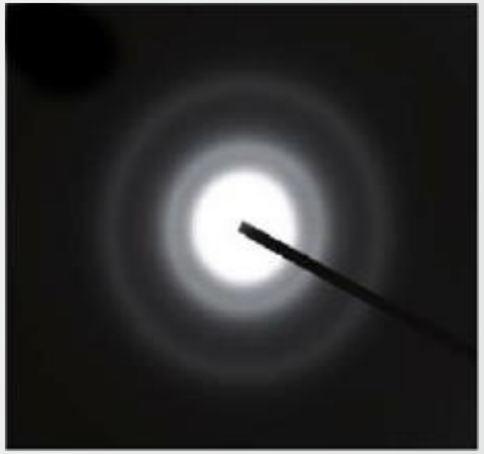
- Electrons have shorter λ than X-rays
- Electron scattered more strongly
- Electron beams are easily directed



Single crystalline SAED pattern



Poly-crystalline SAED pattern



Amorphous SAED pattern

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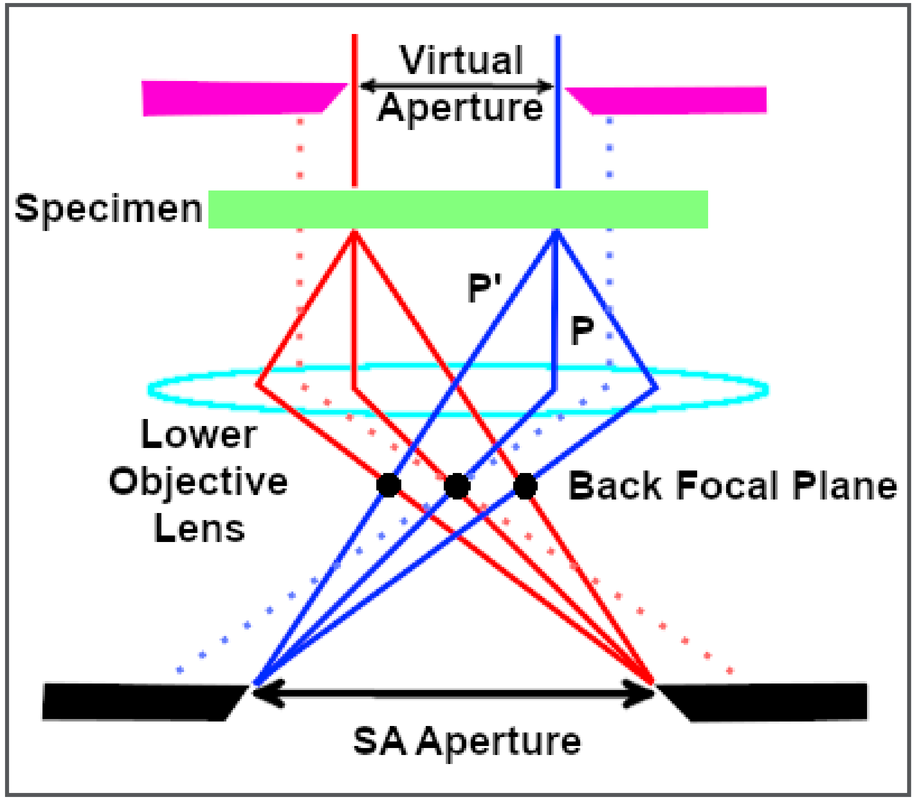
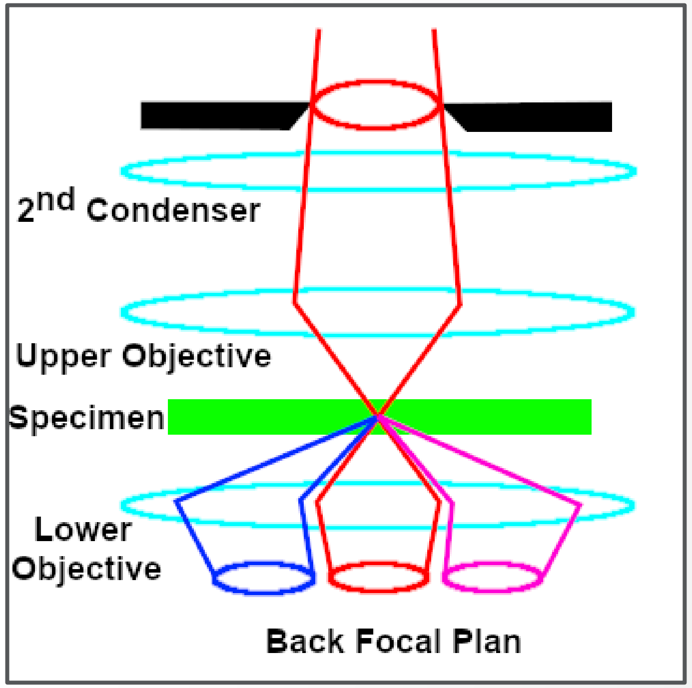
Electron Diffraction

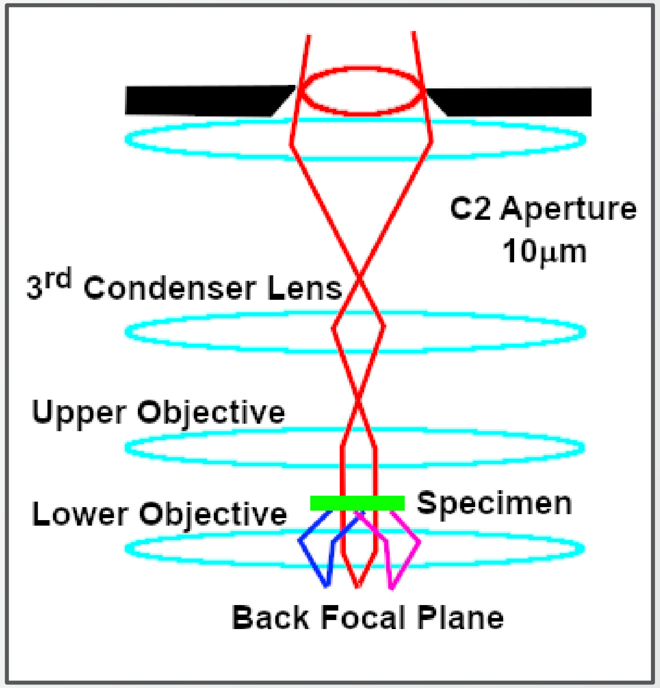
Type of Electron Diffraction in TEM



Selected Area Electron Diffraction (SAED)



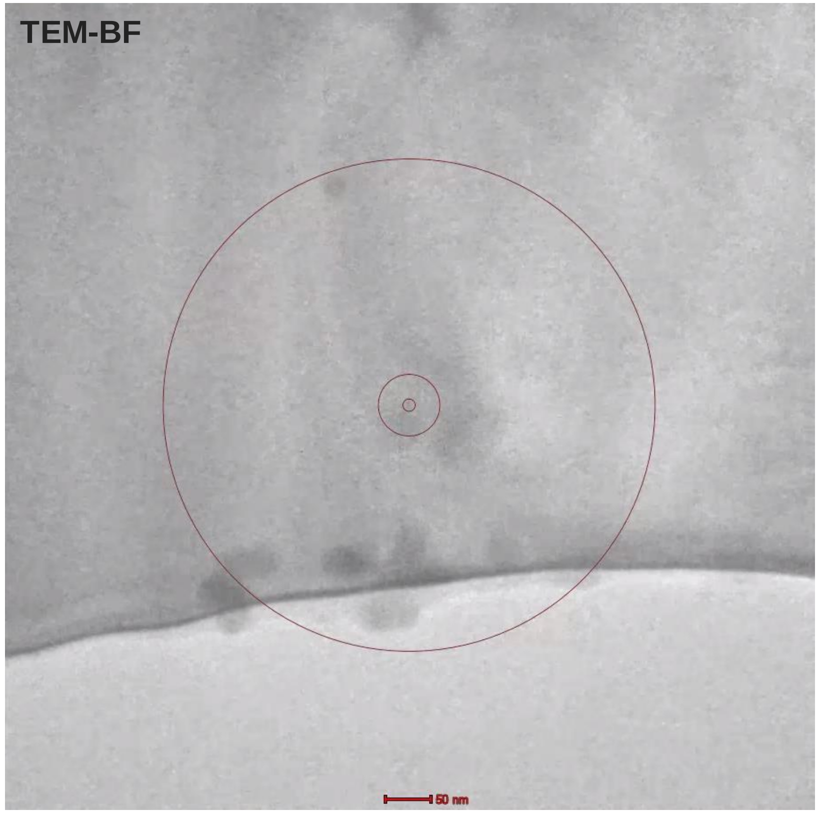
Convergence Beam
Electron Diffraction (CBED)

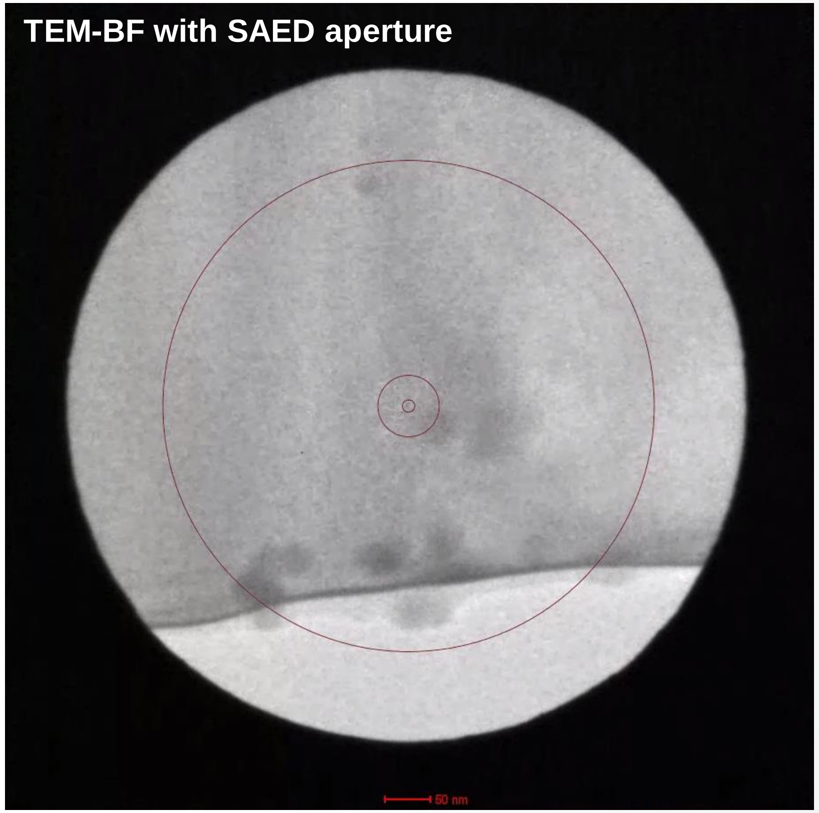


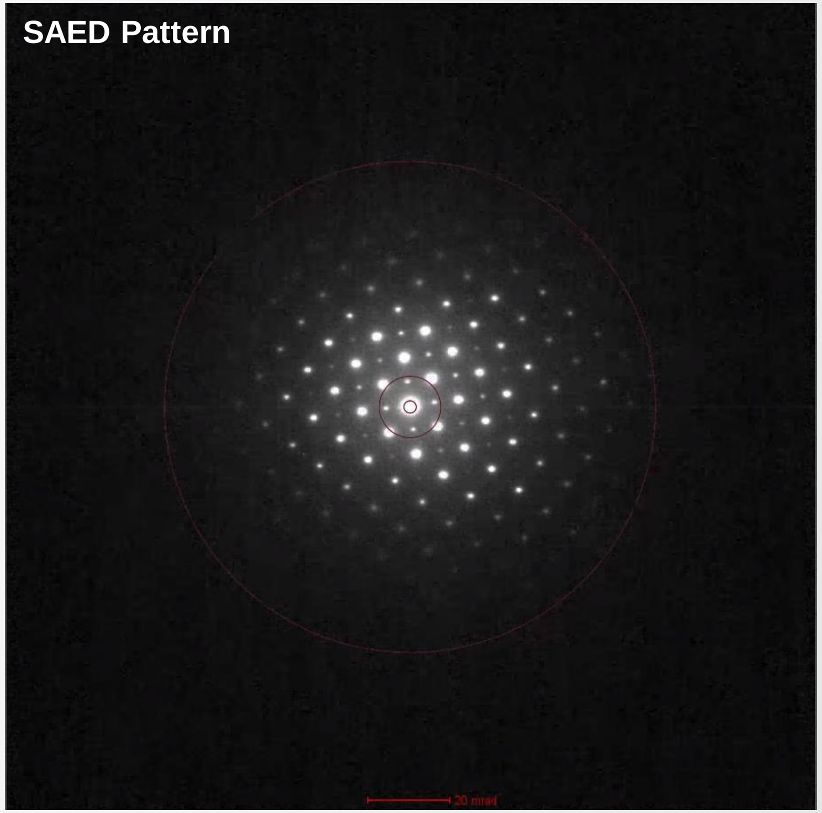
Nano Beam
Electron Diffraction (NBED)

Electron Diffraction

Selected Area Electron Diffraction (SAED)



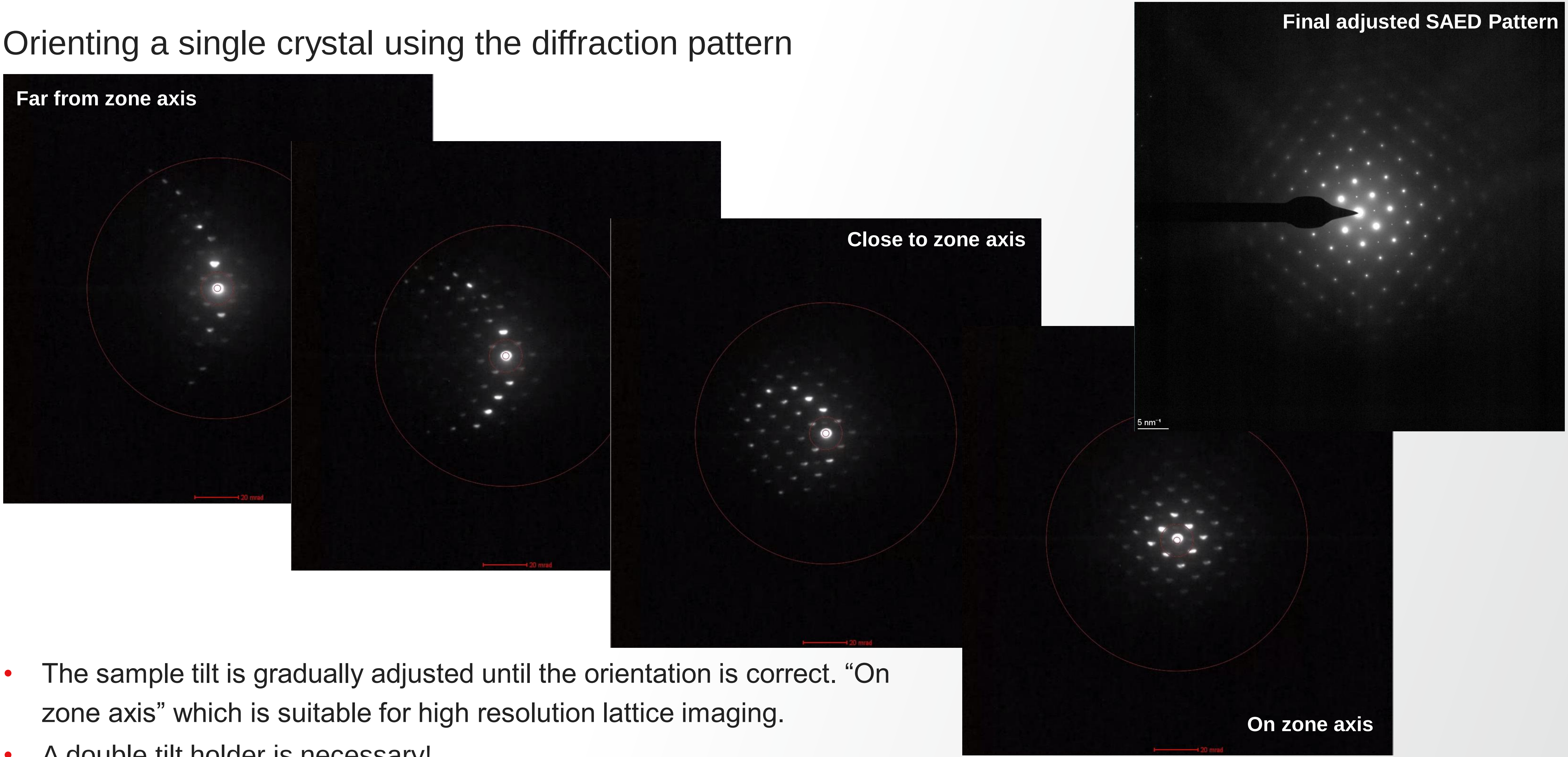




As the name, the aperture is always needed to select an area and produce the Electron Diffraction Pattern

Electron Diffraction

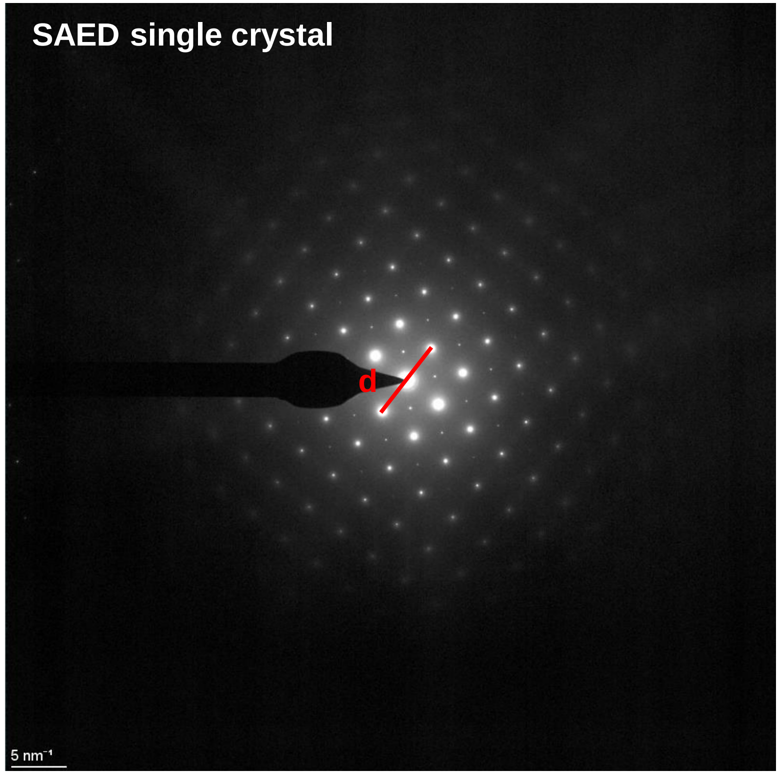
Orienting a single crystal using the diffraction pattern

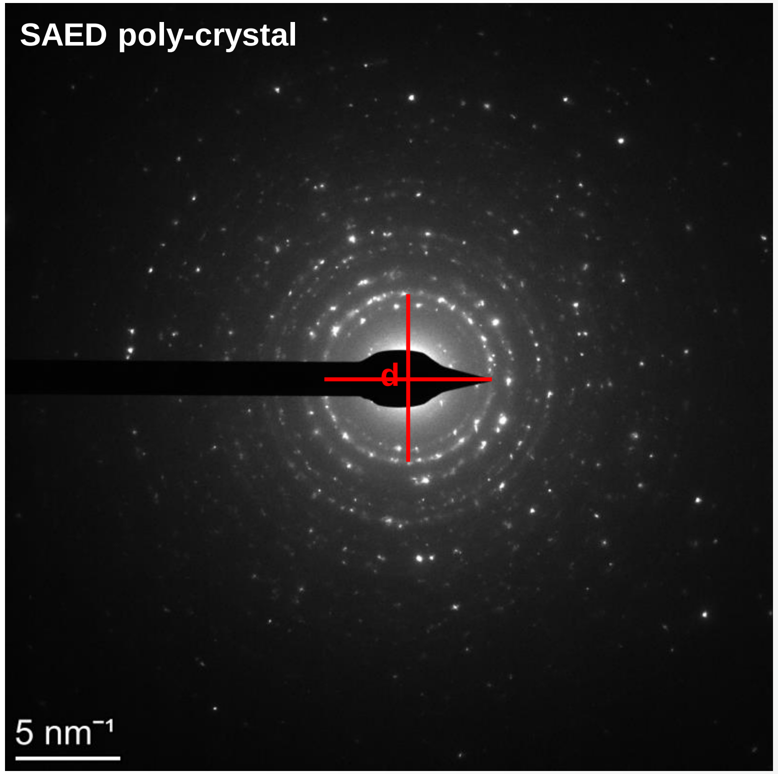


- The sample tilt is gradually adjusted until the orientation is correct. “On zone axis” which is suitable for high resolution lattice imaging.
- A double tilt holder is necessary!

Electron Diffraction

(An Overview) SAED Pattern Analysis



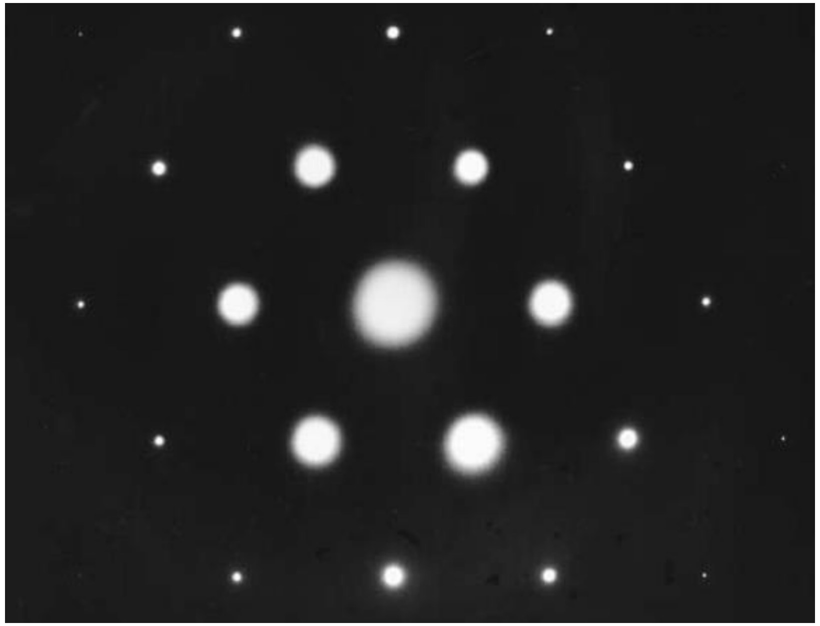


- Make sure that the microscope is calibrated
- Measure the distance from reflections or rings to the main spot (d/2)
- Inversed the measured value
- Compare the data (d-spacing) with database to find the phase presence

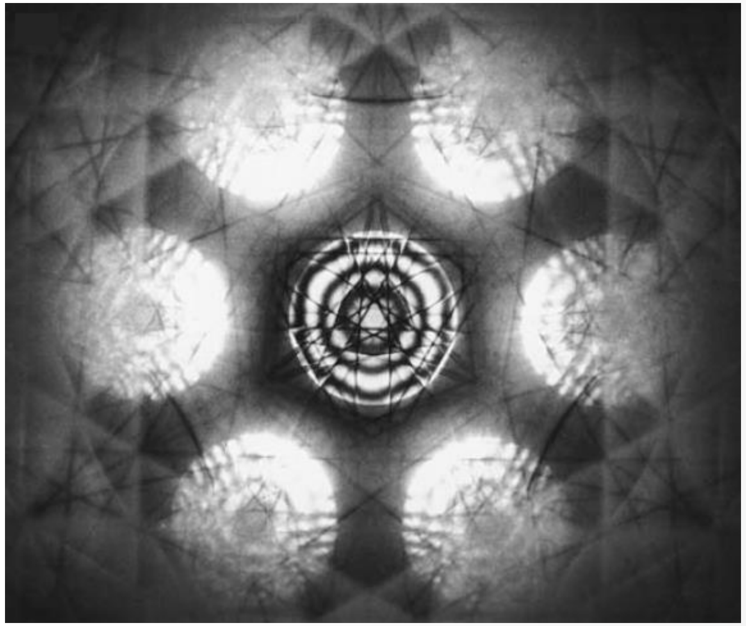
Electron Diffraction

Convergence beam electron diffraction (CBED)

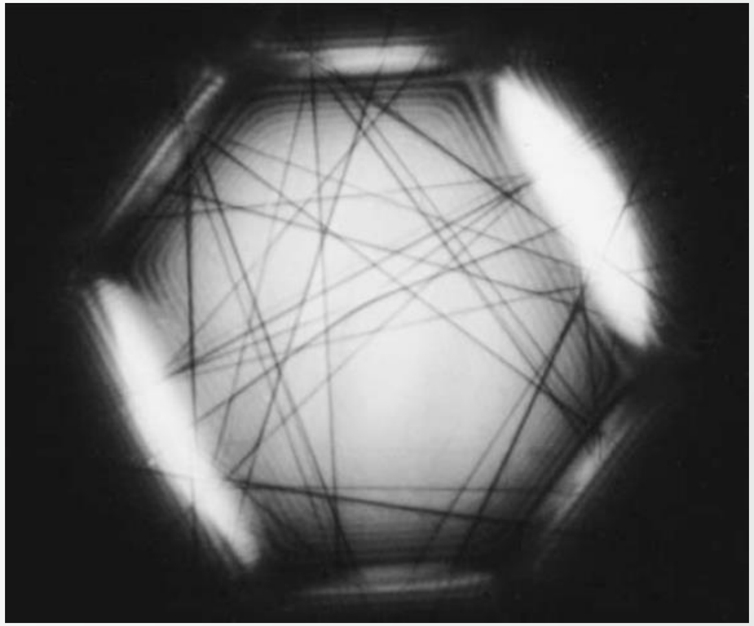
SAED pattern of Si <111>



CBED pattern of Si <111>



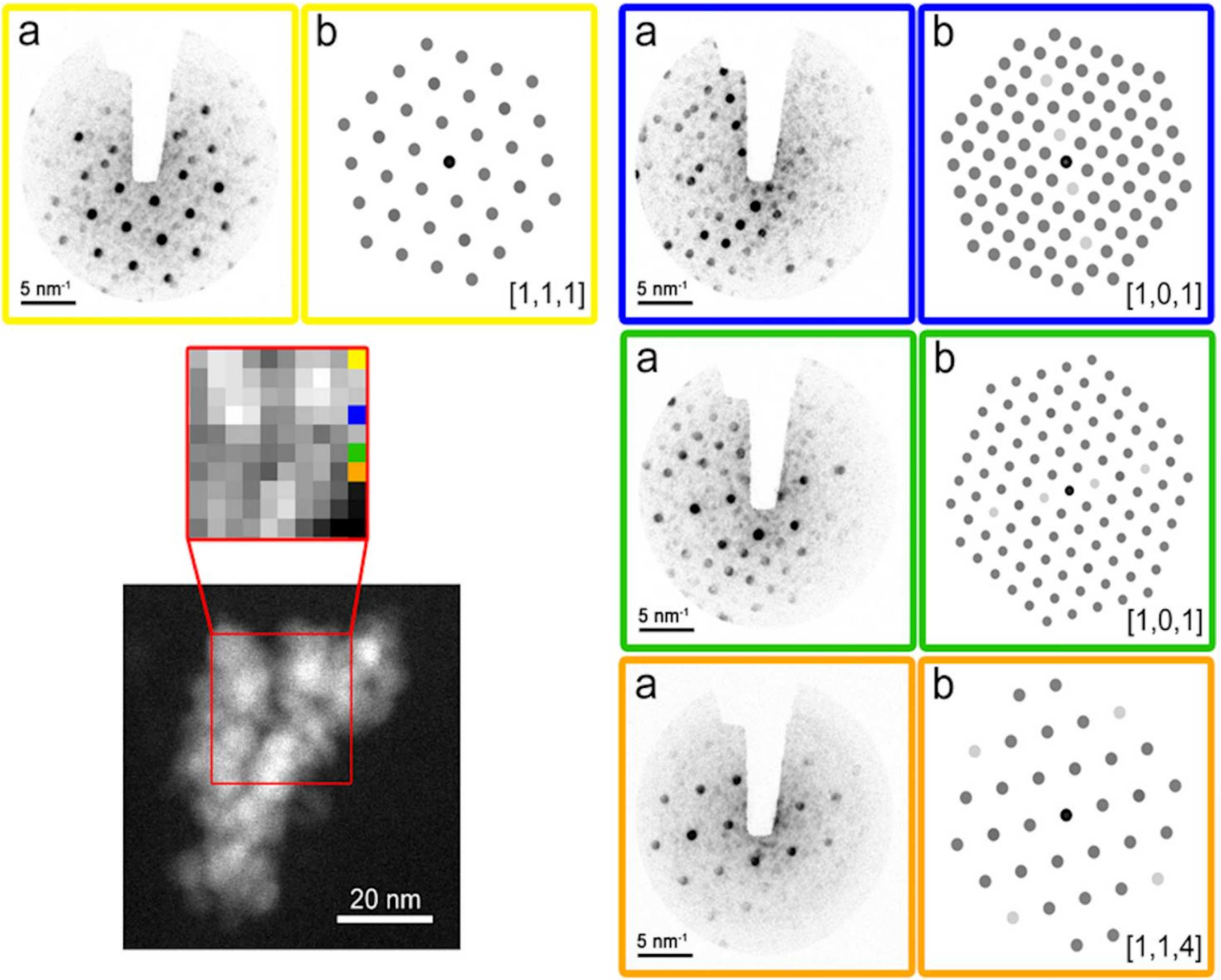
Al HOLZ line pattern



- Small area ≤ 100 nm
- CBED gives quantitative data on
    - Specimen thickness
    - Crystallographic data such as unit cells, Bravais lattice, crystal system and 3D full symmetry
    - Precise lattice-strain measurements
    - Valence-electron distribution, structure factor, and chemical bonding
    - Characterization online and planar defects

Electron Diffraction

Nano Beam Electron Diffraction

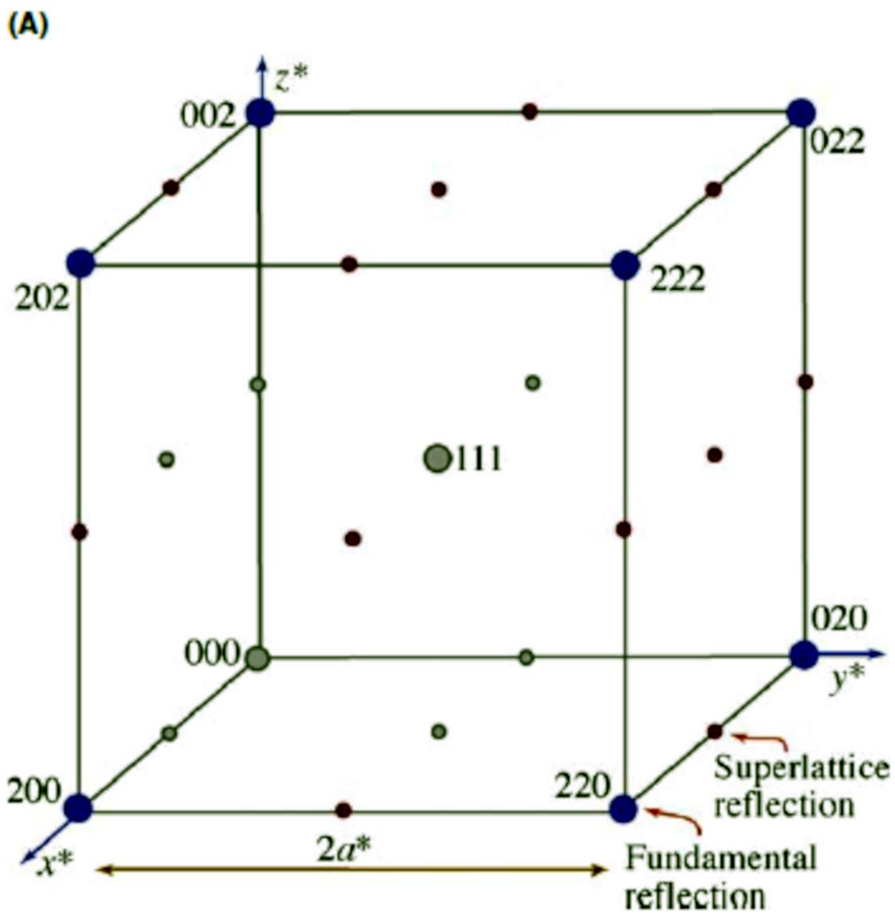


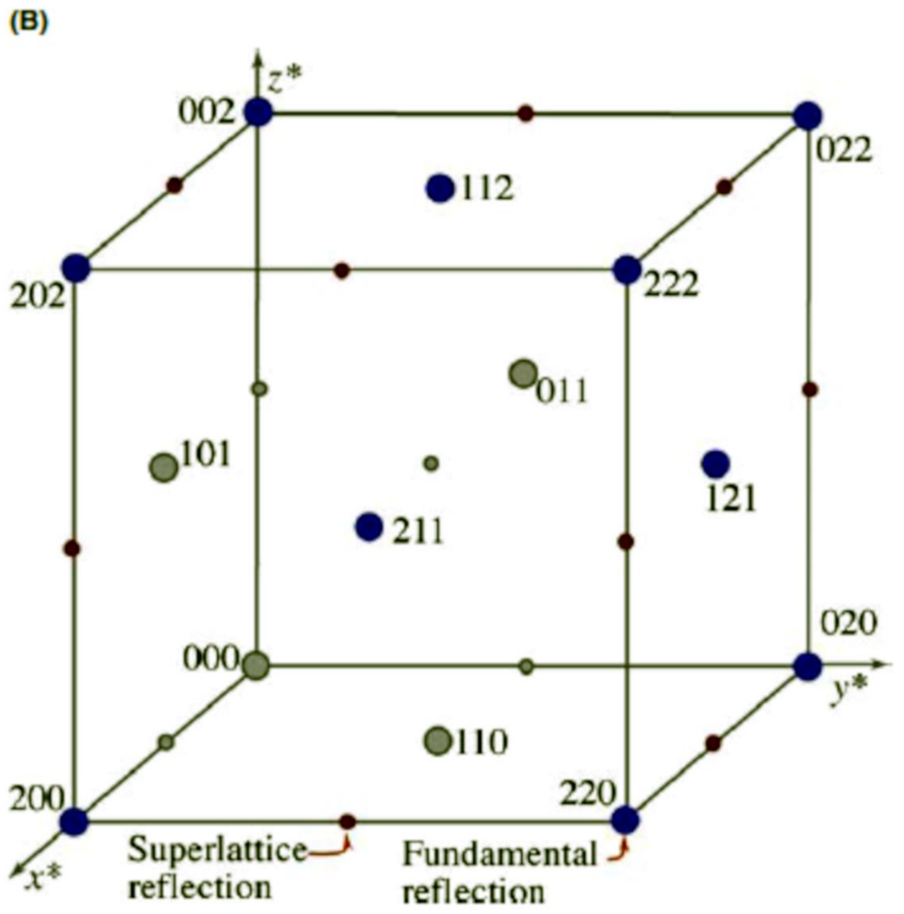
- Nanocrystals of Fe_3O_4 (magnetite), which have been incorporated in melt spinning polyvinylidene fluoride (PVDF) fibers.
- Experimental NBED in STEM mode (a) with corresponding simulated pattern (b).
- The frame colors refer to the position of electron beam during acquisition of the diffraction patterns.
- Domains or particles can be analyzed at nm-range by collection electron diffraction pattern.

Data countersy of N.Wirch Central Facility for Electron Microscopy, RWTH Aachen University

Electron Diffraction

Information from Electron Diffraction (SAED) – Superlattice Reflections

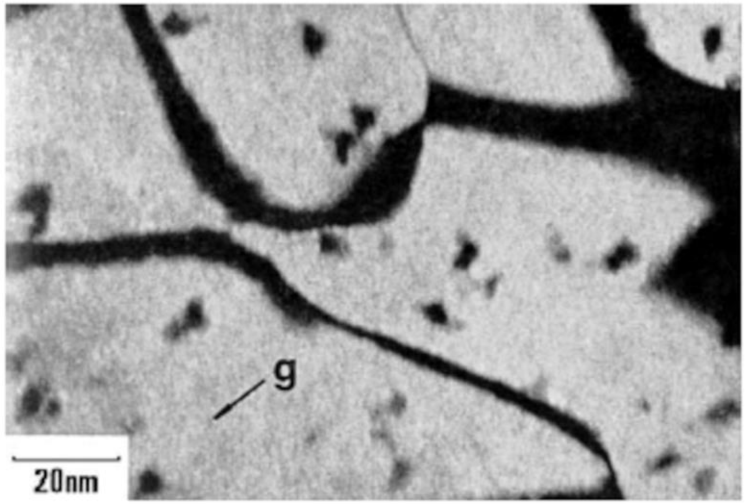




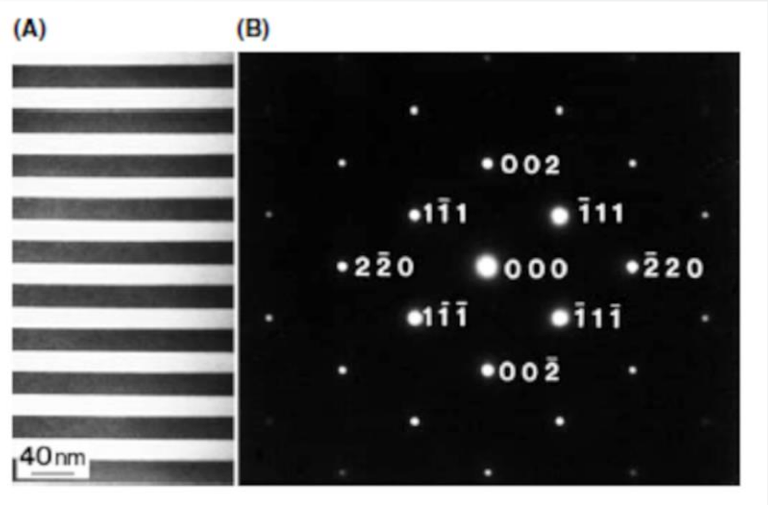
The reciprocal lattice for (A) the Ni_3Al and (B) the NiAl structures. In (A), Ni_3Al is fcc, so the fcc-forbidden reflections (h, k, l mixed even and odd) are allowed and become chemically sensitive (superlattice) reflections. In (B), NiAl is bcc, so the bcc-forbidden reflections (if h + k + l odd) are now allowed superlattice reflections.

Electron Diffraction

Information from Electron Diffraction (SAED) – Superlattice Reflections



DF image from a chemically sensitive 110 reflection showing bright ordered domains in Cu_3Au. The dark areas in the bright domains are regions of local disorder induced by ion beam damage



(A) DF image from 002 chemically sensitive reflection in GaAs/$Al_xGa_{1-x}As$. The $Al_xGa_{1-x}As$ is the lighter region because Al has replaced Ga in the GaAs (darker region). (B) DP showing the less intense 002 and other superlattice reflections.

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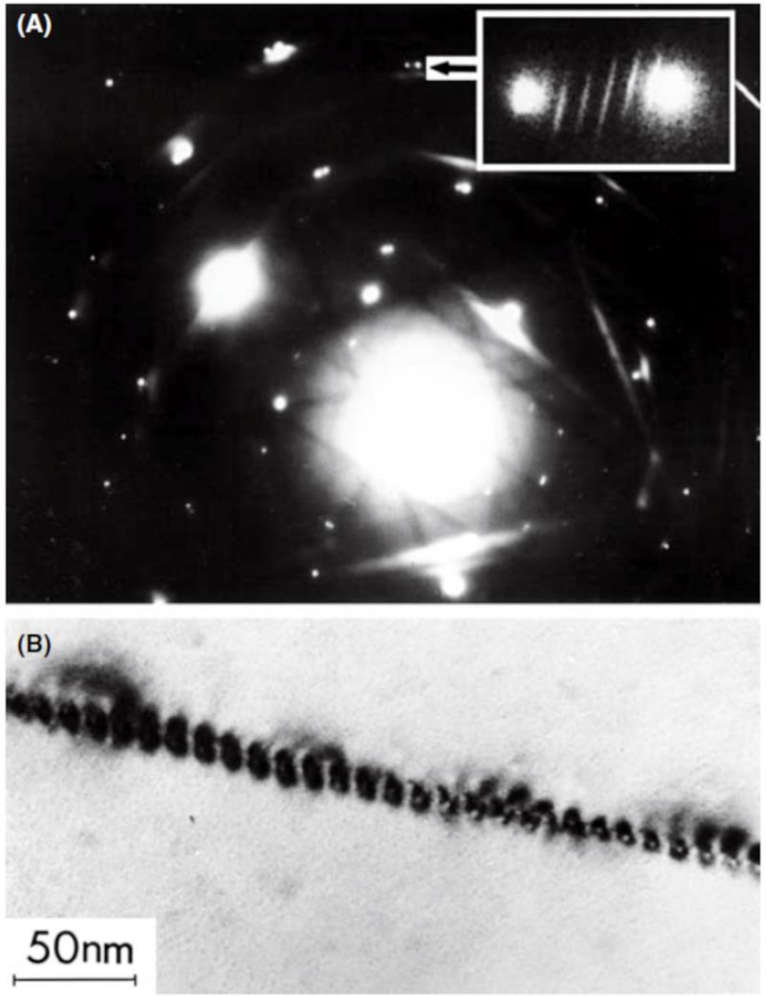
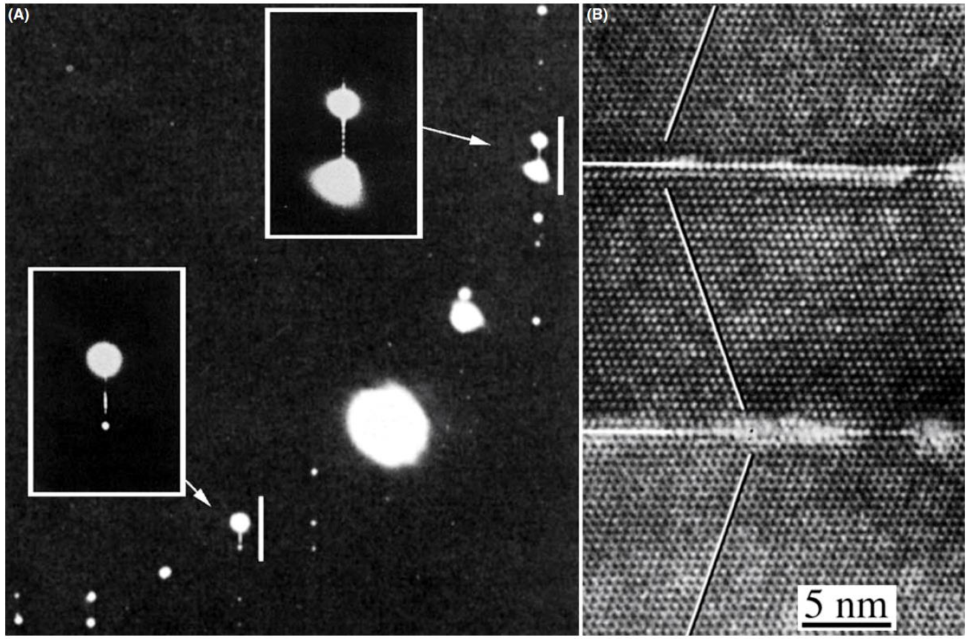
Electron Diffraction

Information from Electron Diffraction (SAED) – Extra Spots



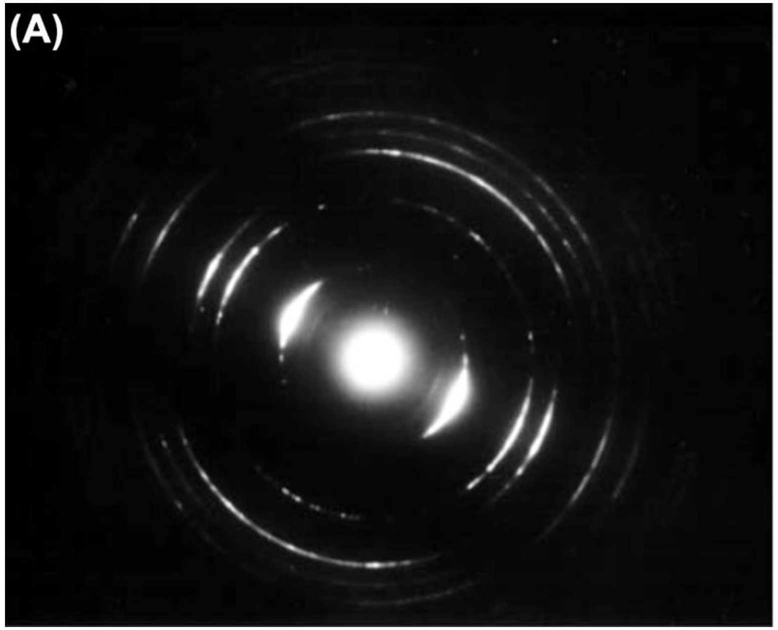
(A) The set of streaks from an array of dislocations in Al_2O_3 lying parallel to the electron beam. The distance between the streaks is inversely related to the spacing of the dislocations shown in image (B)

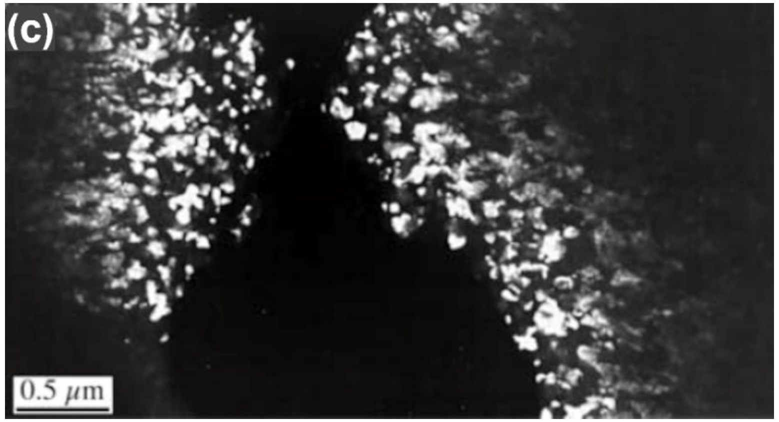


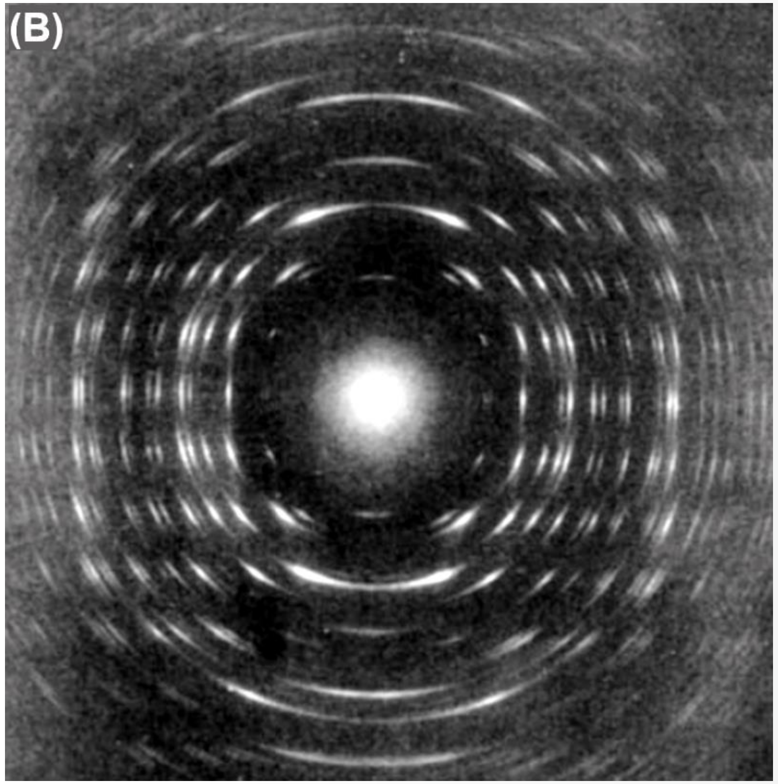
Extra spots can be formed in the DP (A) when only two defects are scattering in phase. The separation of the extra spots is related to the inverse separation of the two twin boundaries seen in image (B).

Electron Diffraction

Information from Electron Diffraction (SAED) – Texture ED Patterns







(A) A texture ring pattern where the rings are more intense over a certain angular range. (B) The corresponding interception of the Edwald sphere (plane) with the reciprocal lattice. (C) A DF image of the texture grains, taken from a brighter position of one of the hkl rings, shows an equiaxed structure. In (D), the specimen is textured about a direction at an angle to the beam, so the Edwald sphere creates elongated spots or arcs in the DP.

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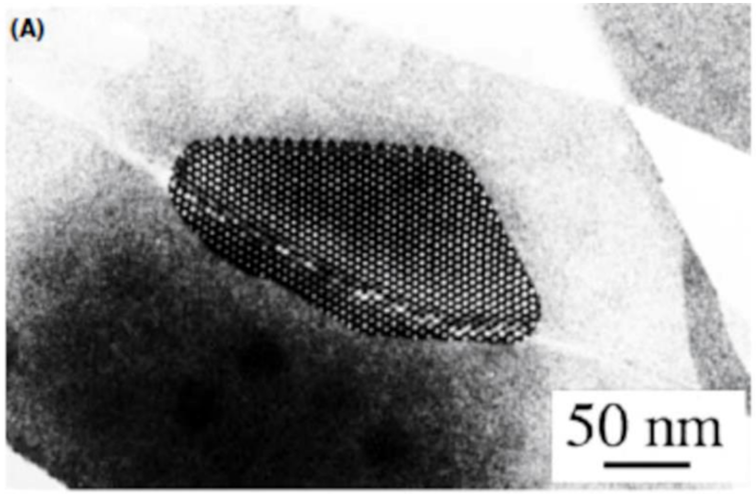
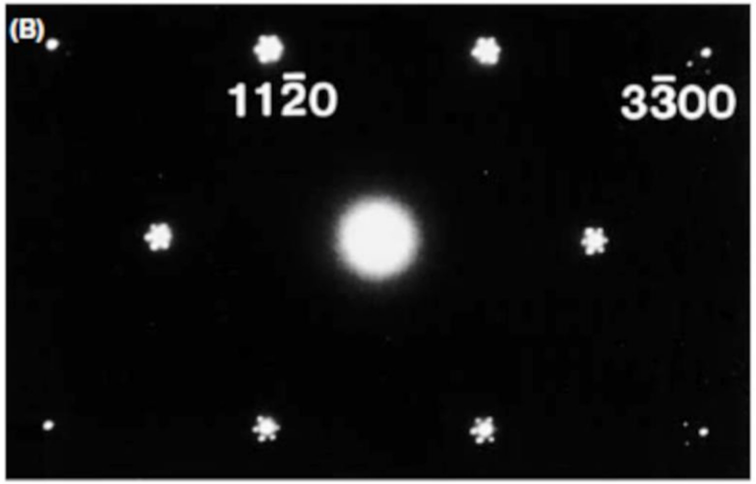
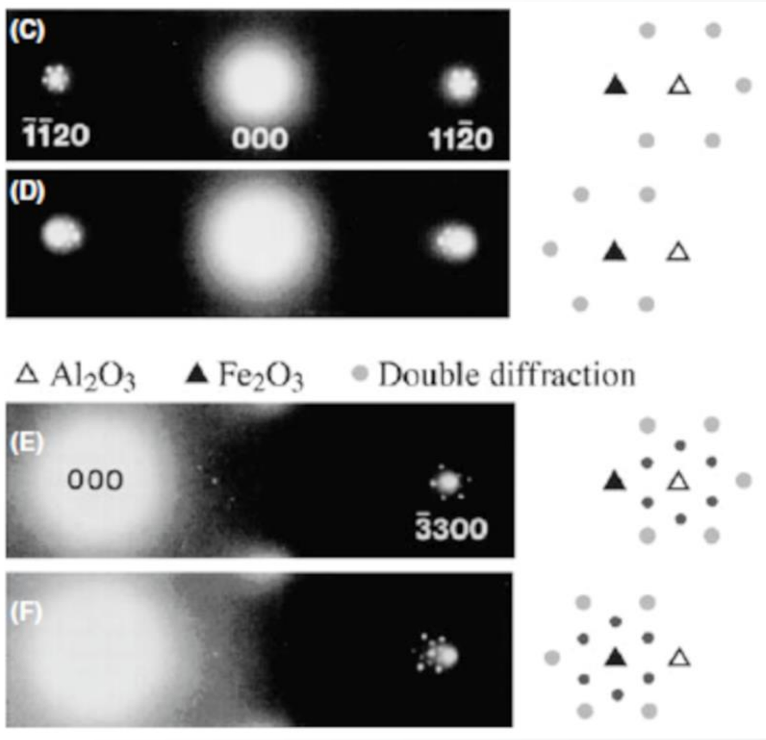
Electron Diffraction

Information from Electron Diffraction (SAED) – Double-Diffraction







(A) BF on-axis image of a particle of α-Fe_2O_3 on α-Al_2O_3.

(B) [0001] SADP from α-Fe_2O_3 showing double-diffraction spots around the [11-20] and [3-300] reflections.

(C) Enlargements of regions near the [11-20] reflections when hematite island is on the top surface.

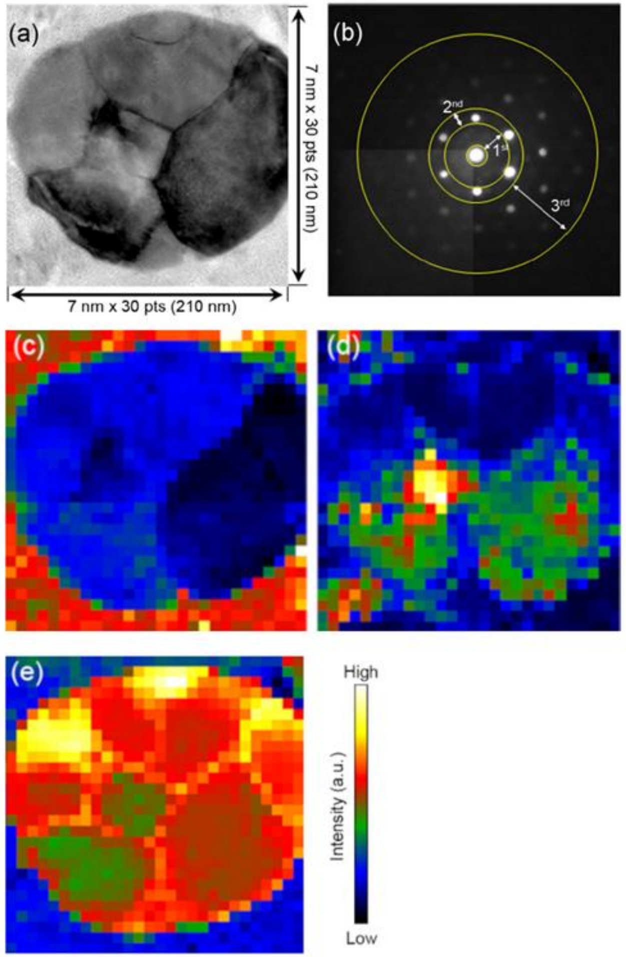
(D) Enlargements of the region near the [11-20] reflections when the hematite island is on the bottom.

(E) Enlargements of regions near the [3-300] reflections when the hematite island is on the top surface.

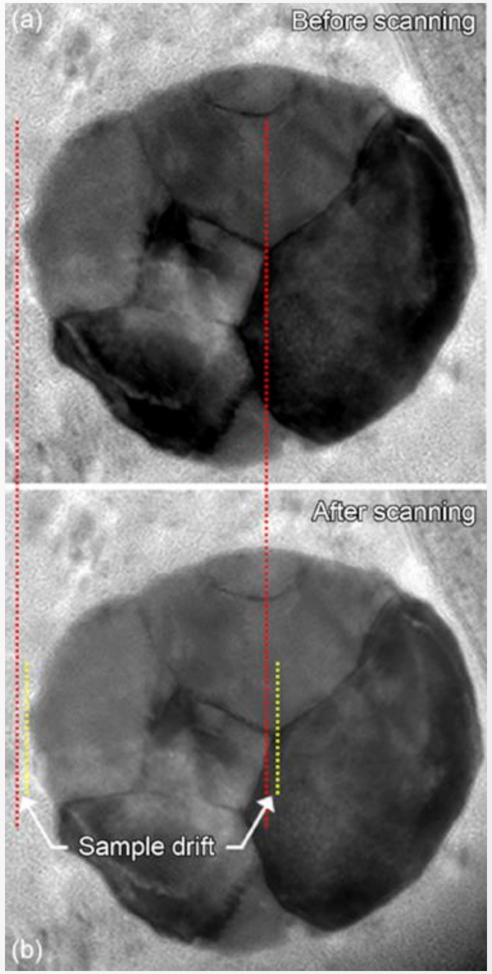
(F) Enlargements of regions near the [3-300] reflections when the hematite island is on the bottom.

Electron Diffraction

Scanning Nano Electron Diffraction (SNED) of Nanostructured Au Disk



(a) A BF image of nanostructured Au disk and (b) selected diffraction pattern acquired from SEND. The diffraction intensity is integrated for the areas of 1,2, and 3 represented in (b). The corresponding maps are shown in (c), (d) and (e), respectively.



Sample drift during SEND.
(a) is the initial position, and (b) is the final position after SEND

Note: SNED requires the Scanning capability in TEM

K.-H. Kim et al., Micron 71 (2015) 39-45

Transmission Electron Microscopy Webinar Series

Thermo Fisher SCIENTIFIC

Outline: Part 01

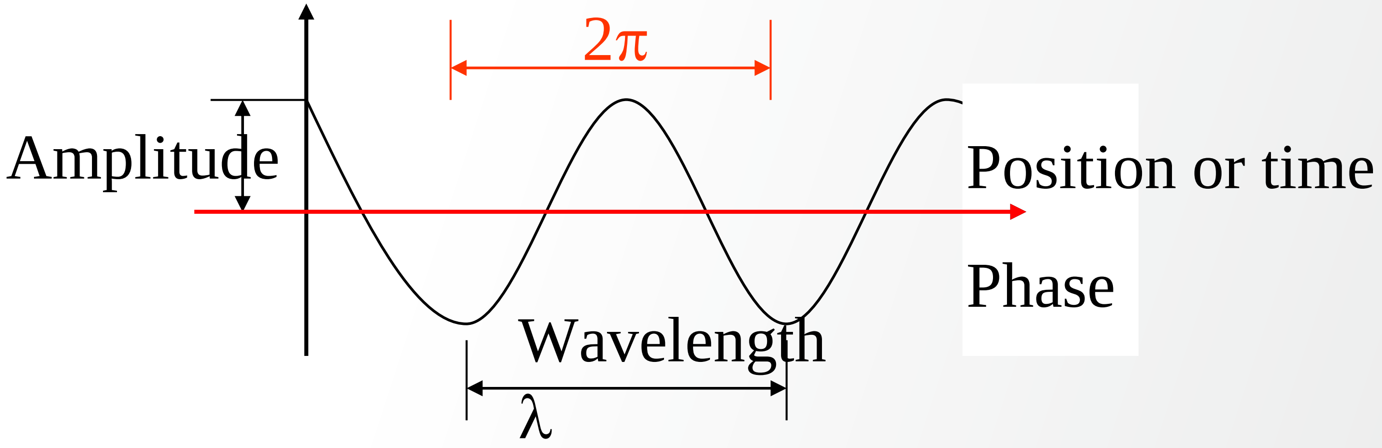
- Basic Theory of TEM
- SEM vs. TEM: What are the differences
- Conventional TEM Imaging
  - Bright-Field Imaging
  - Dark-Field Imaging
- Electron Diffraction
  - Selective Area Electron Diffraction
  - Convergence Beam Electron Diffraction
  - Nano Beam Electron Diffraction
- Advanced TEM Imaging
  - High-resolution TEM
- Special Investigation Cases
  - Magnetic Samples
  - Soft Materials
  - In-situ Investigations
  - Life-Sciences

Advanced TEM Imaging: High-resolution TEM

Thermo Fisher SCIENTIFIC

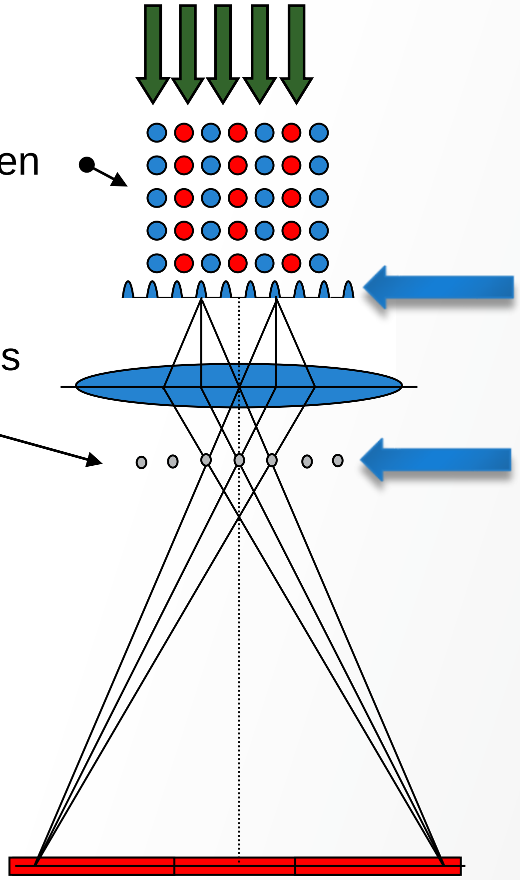
High-resolution TEM

- Electron beam is a wave with *amplitude (A) and phase (φ)*
- The periodicity is the wave-length (e.g. 0.0025nm at 200kV) or, in terms of phase, 2π



Advanced TEM Imaging: High-resolution TEM

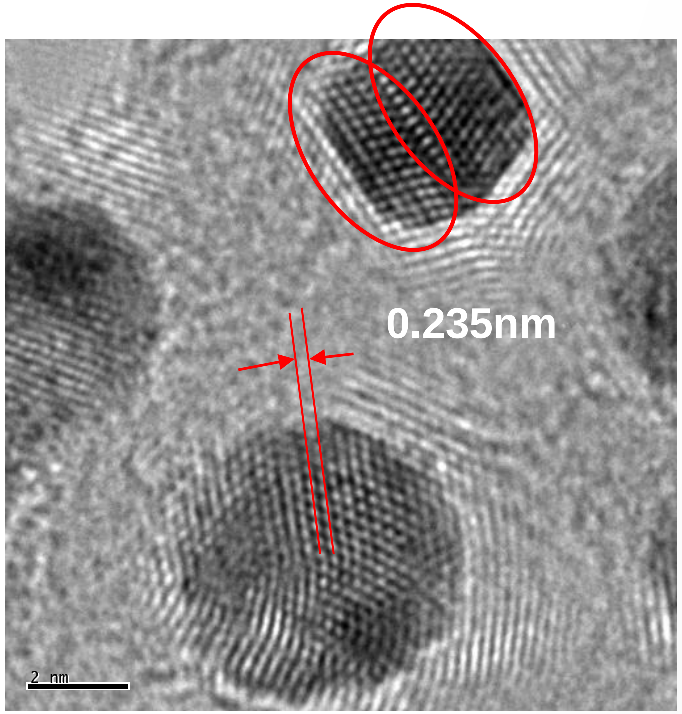
High-resolution TEM: Imaging Formation

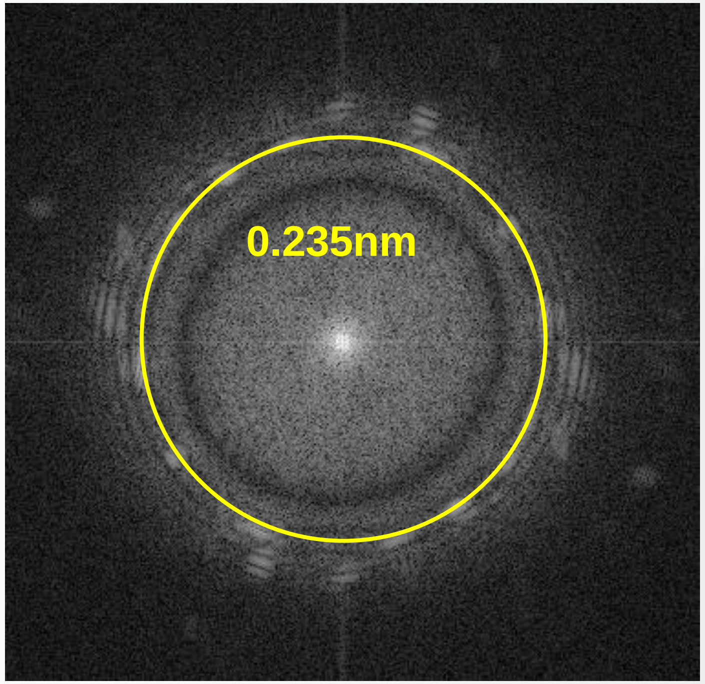


Advanced TEM Imaging: High-resolution TEM

High-resolution TEM: What do we See on a HRTEM image?

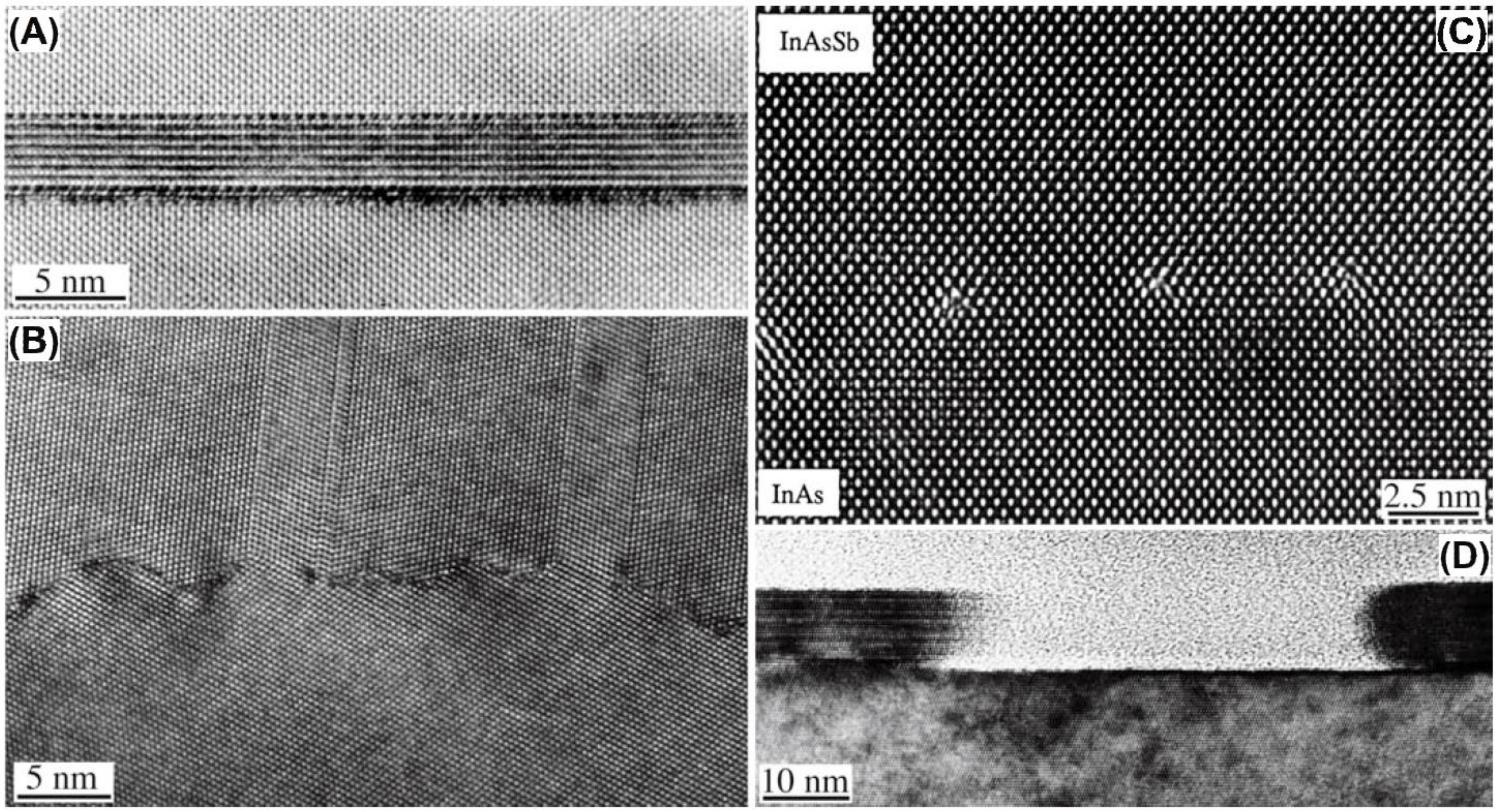
Usually, you can not say where the atom is, but you can tell the distance on atomic scale and crystal defects





Advanced TEM Imaging: High-resolution TEM

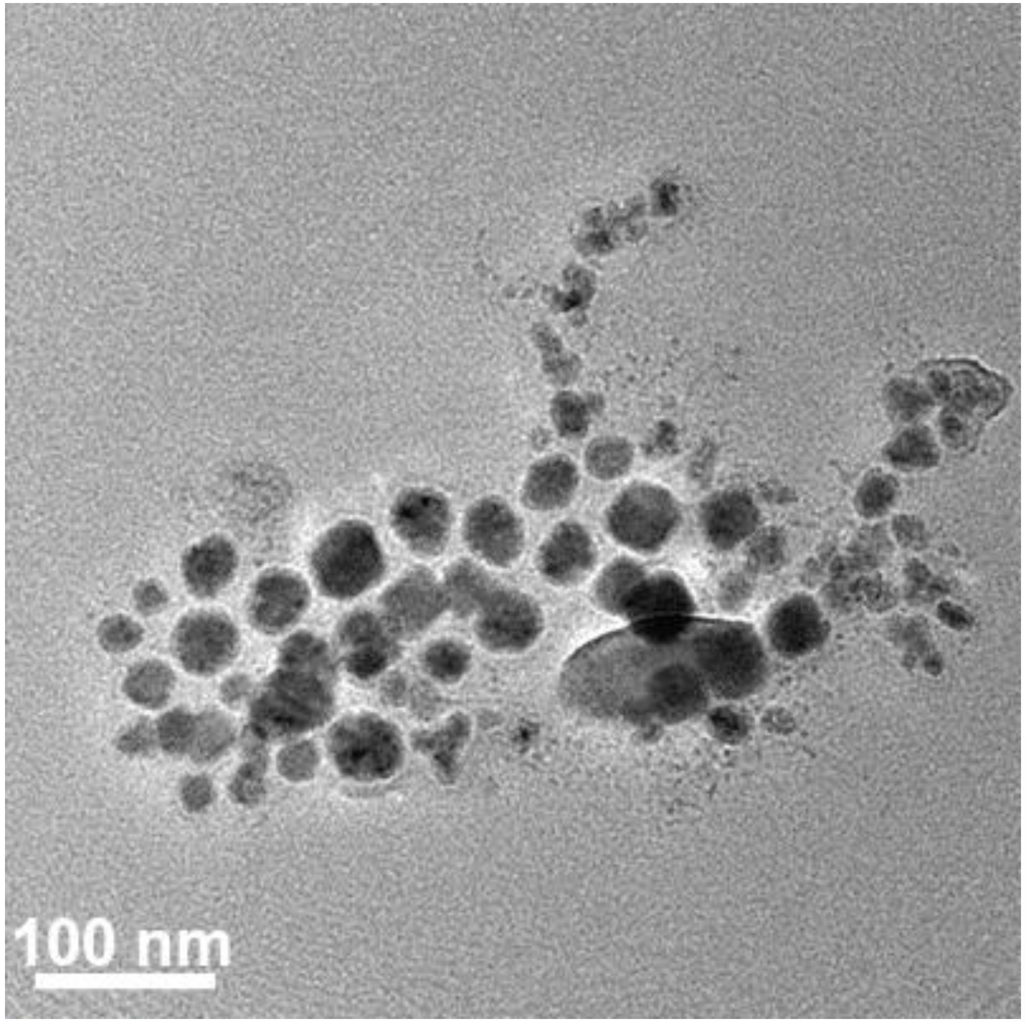
Dislocation on Lattice Images

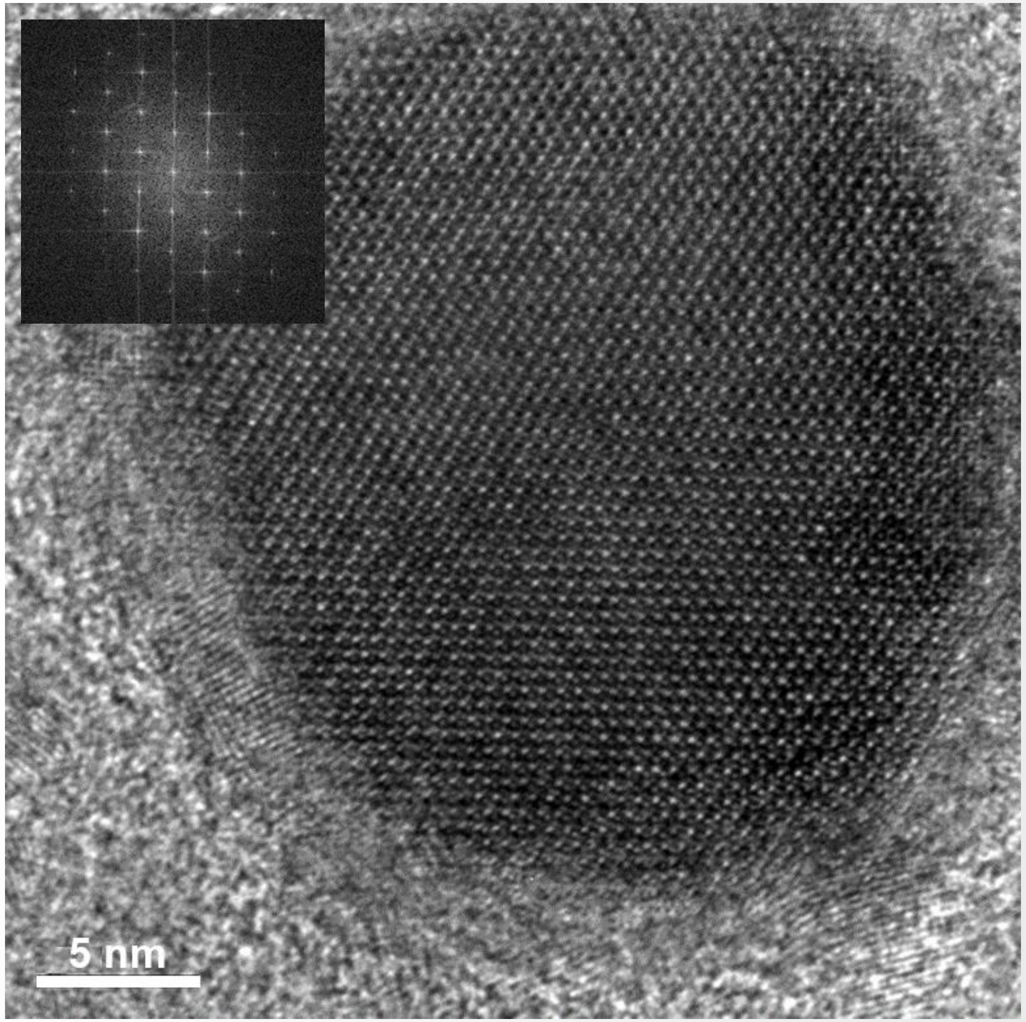


Illustrations of lattice images that contain easily interpreted information. (A) The spinel/olivine interface; (B) dislocation at a heterojunction between InAsSb and InAs; (C) a grain boundary in Ge faceting on an atomic scale; (D) a profile view of a faceted surface.

Advanced TEM Imaging: High-resolution TEM

Core-Shell Structure of Nanoparticles

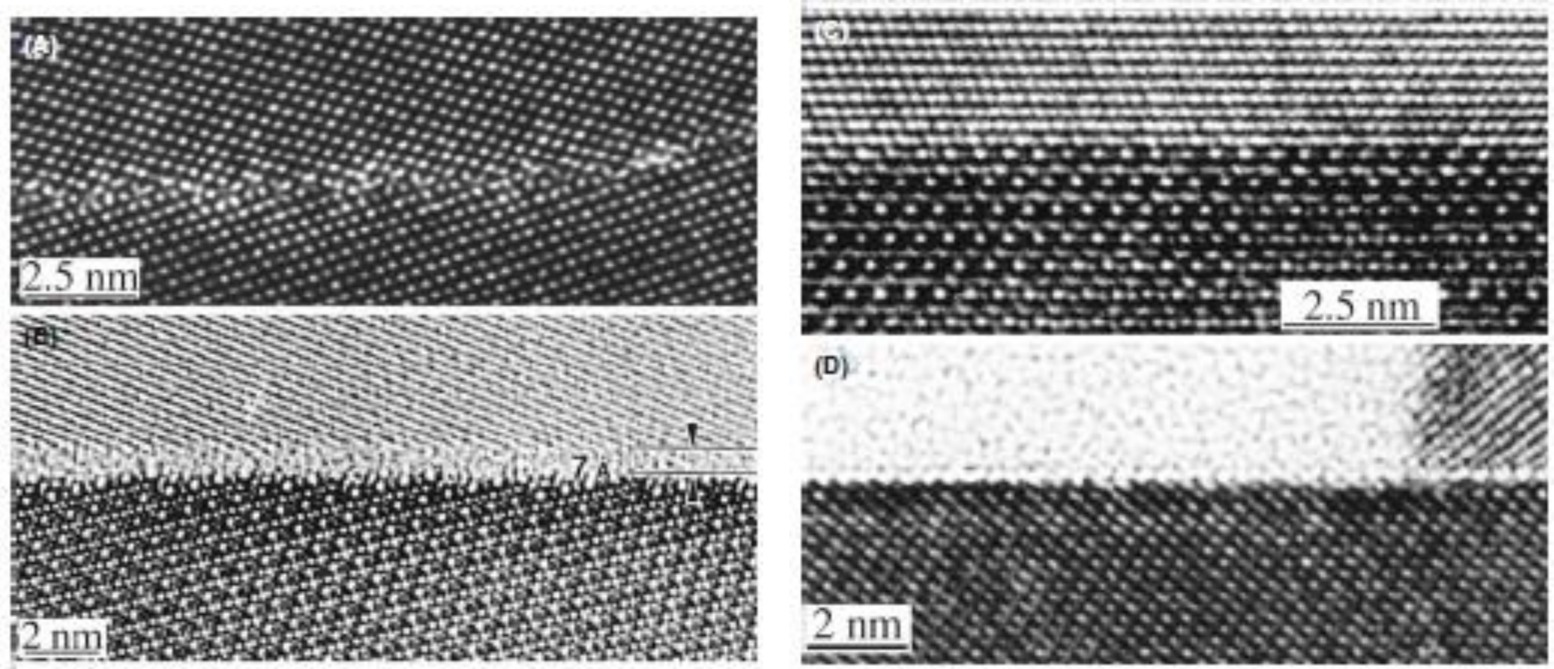




Co/CoO nanoparticles

Advanced TEM Imaging: High-resolution TEM

Planar Interface Investigations



Examples of HRTEM images of planar interfaces.

(A) Grain boundary in Ge;

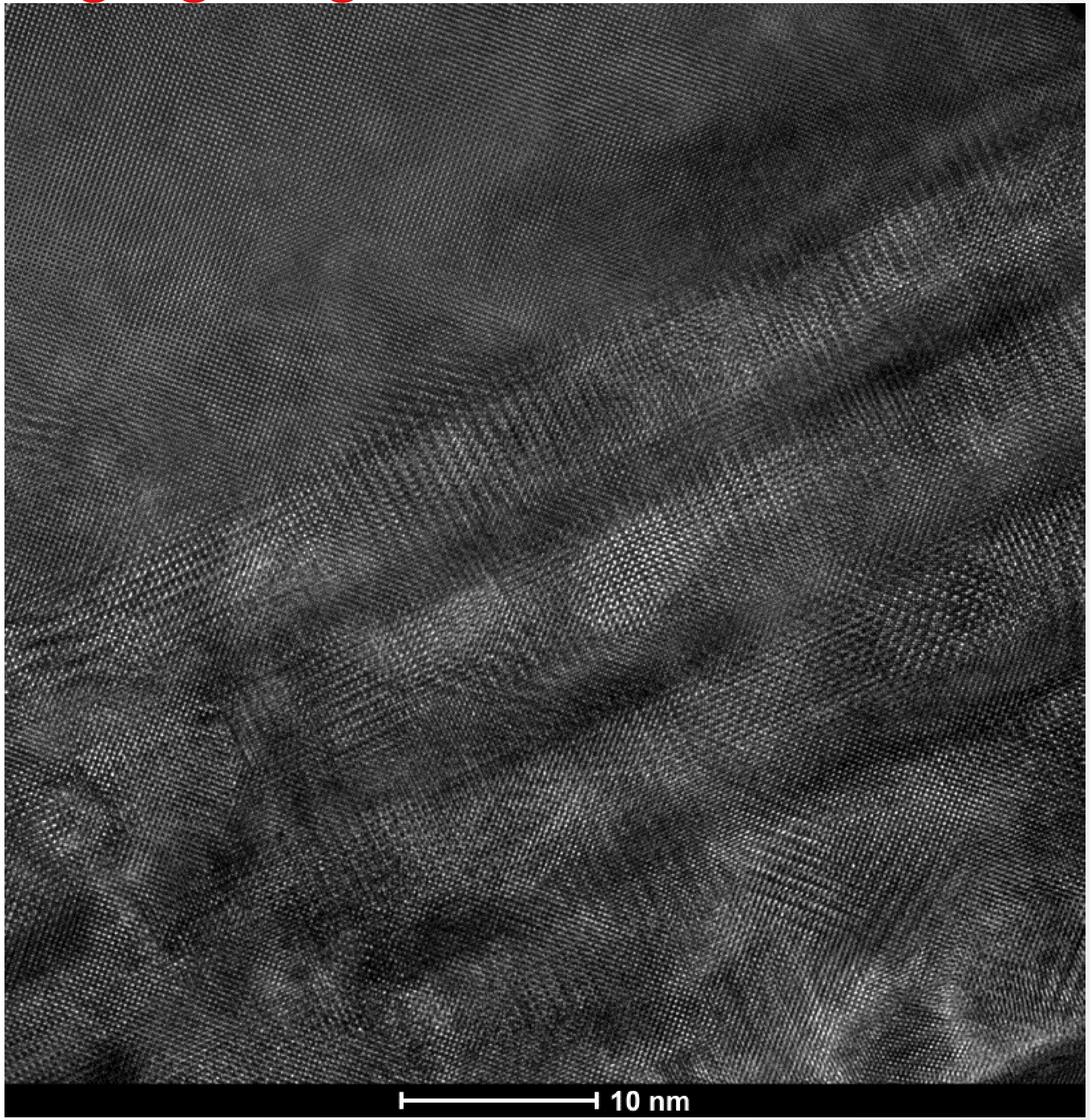
(B) Grain boundary in Si_3N_4 with a layer of glass along the interface

(C) Phase boundary separating NiO and $NiAl_2O_4$

(D) Profile images of the (0001) surface of Fe_2O_3.

Advanced TEM Imaging: High-resolution TEM

Multi Layer $SrTiO_3$



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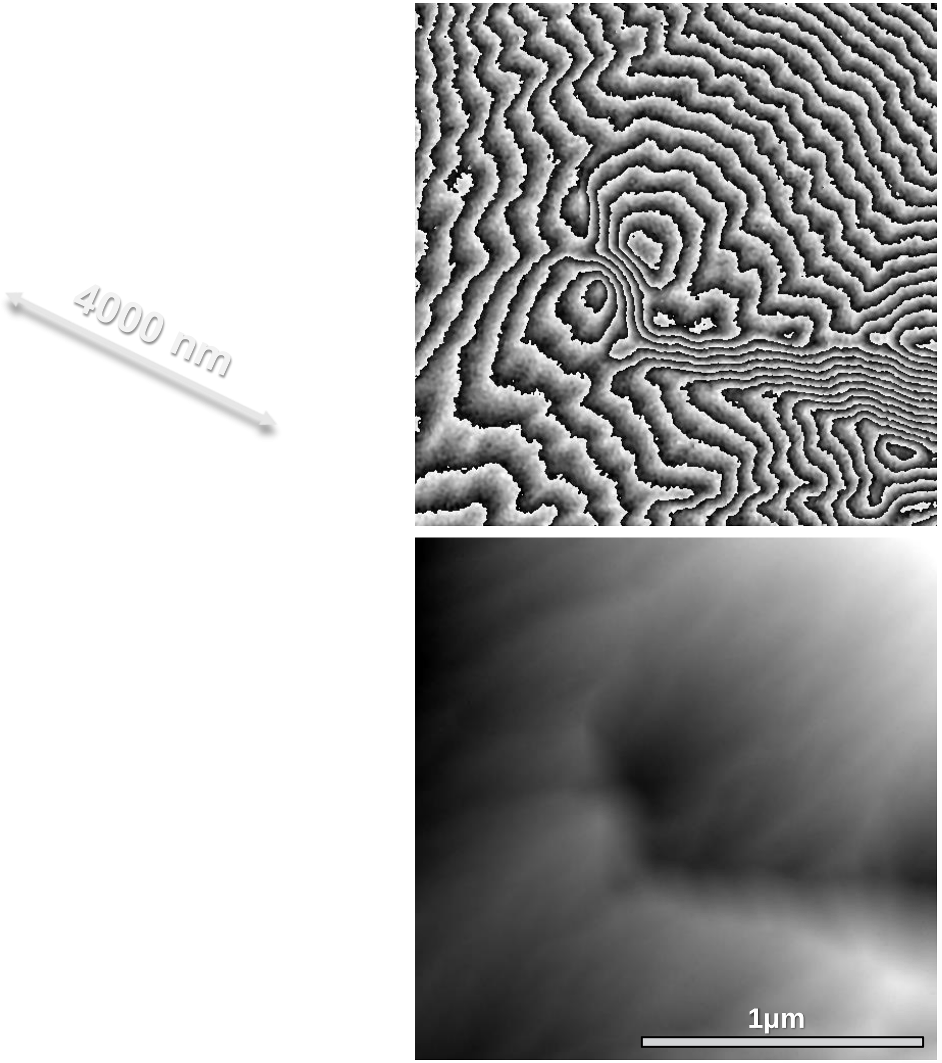
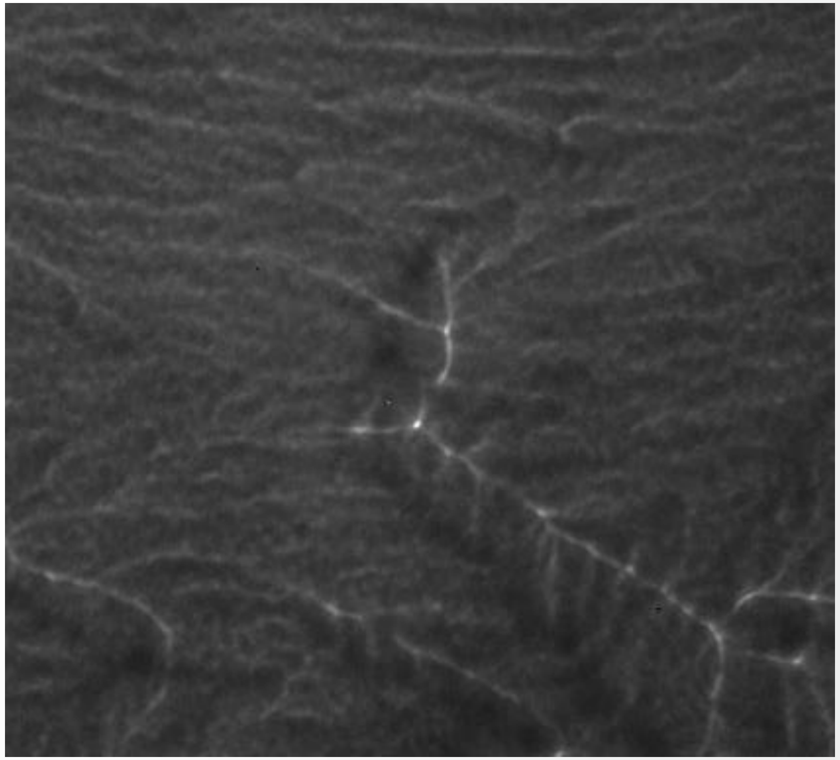
- Special Investigation Cases
  - Magnetic Samples
  - Soft Materials
  - In-situ Investigations
  - Life-Sciences

Special Investigation Cases

Magnetic Materials



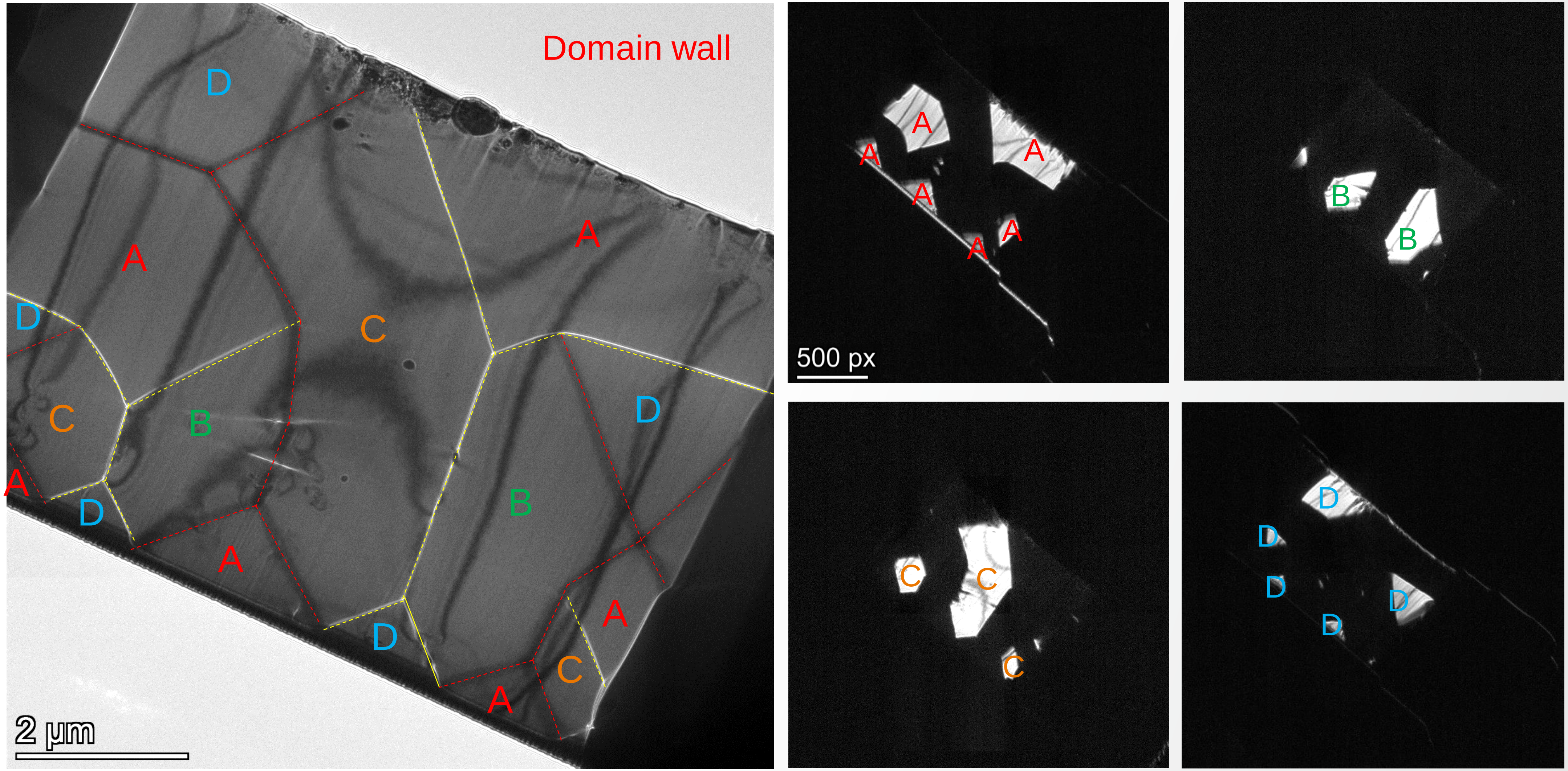


Direct imaging of complex magnetic materials

Sample courtesy: Dr. Andras Kovacs, Forschungszentrum Jülich

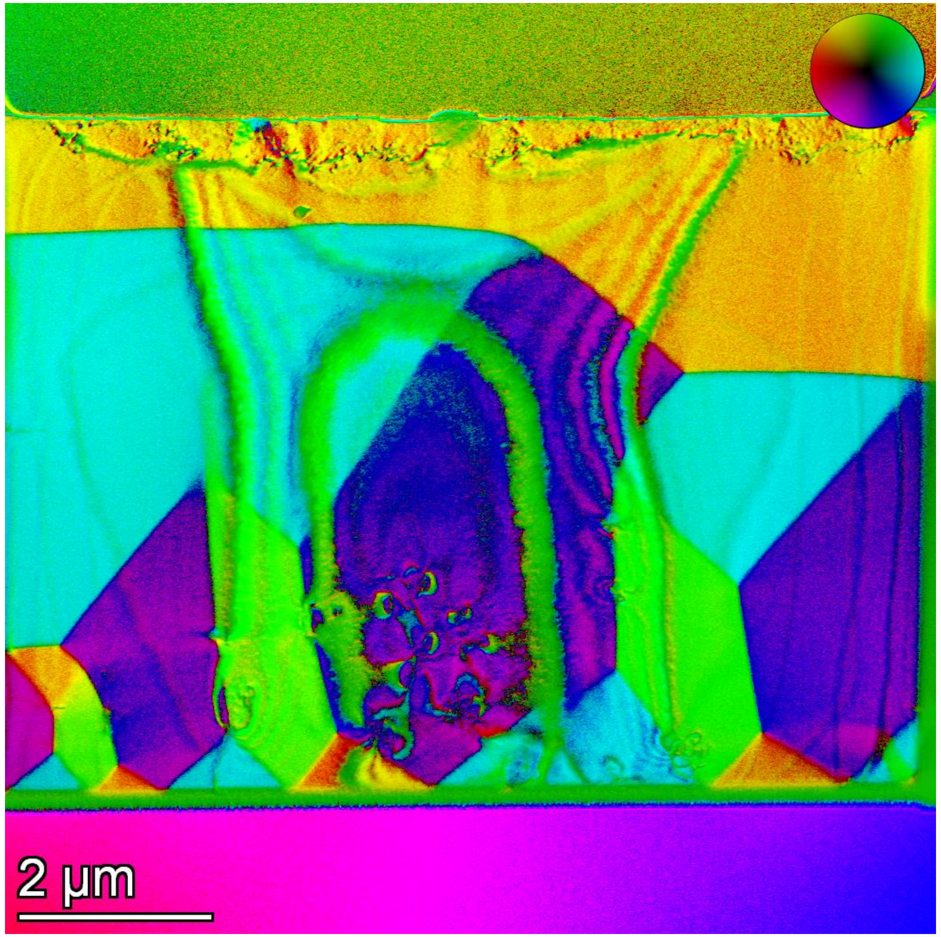
Special Investigation Cases

Magnetic Materials – Lorentz Foucault Imaging

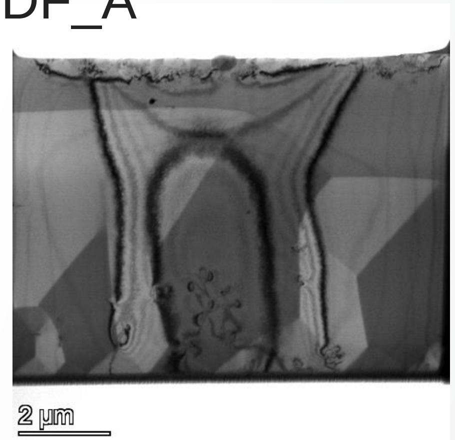


Special Investigation Cases

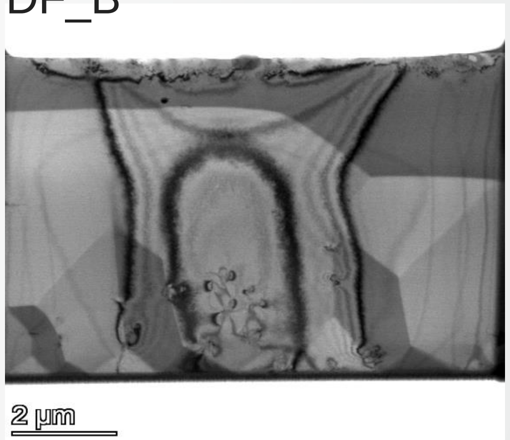
Magnetic Materials – LM DPC



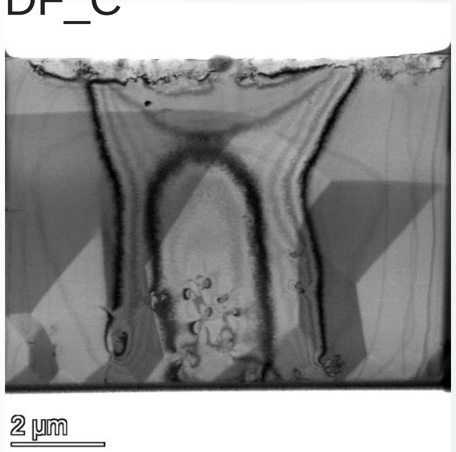
DF_A



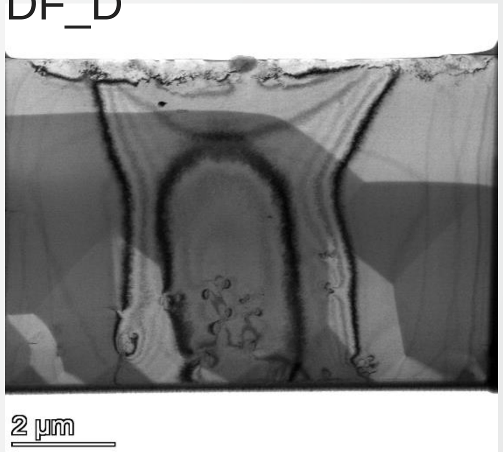
DF_B



DF_C

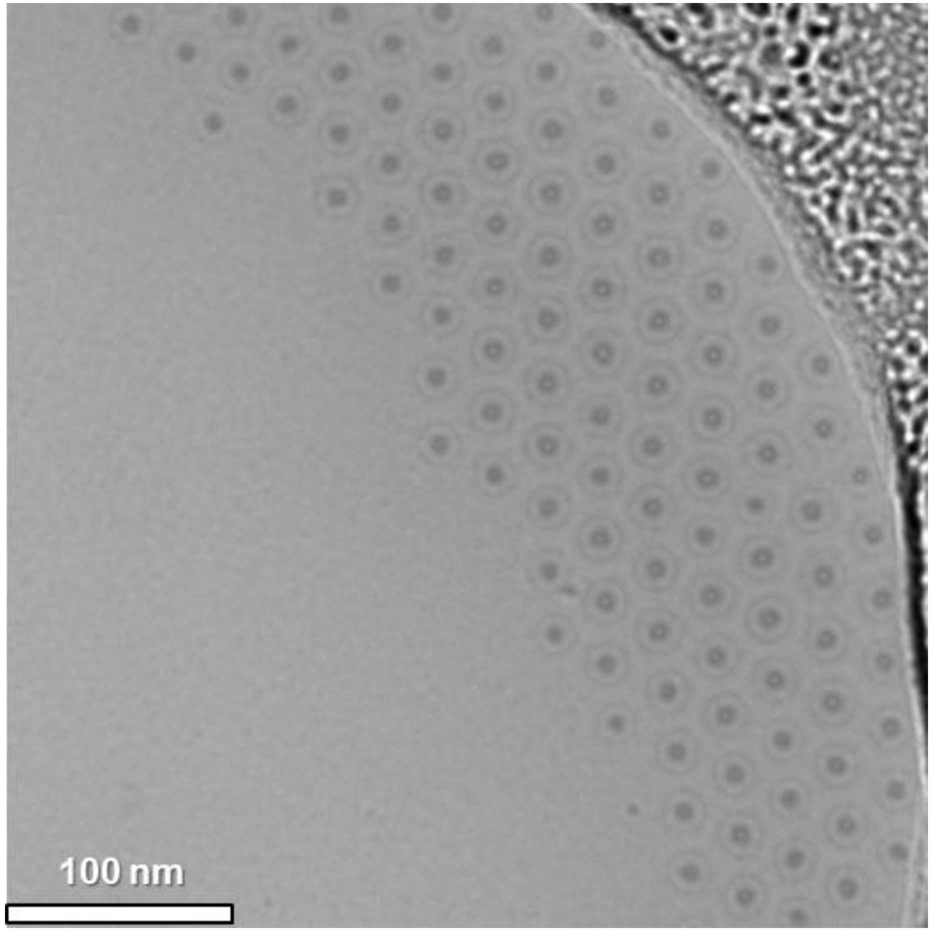


DF_D

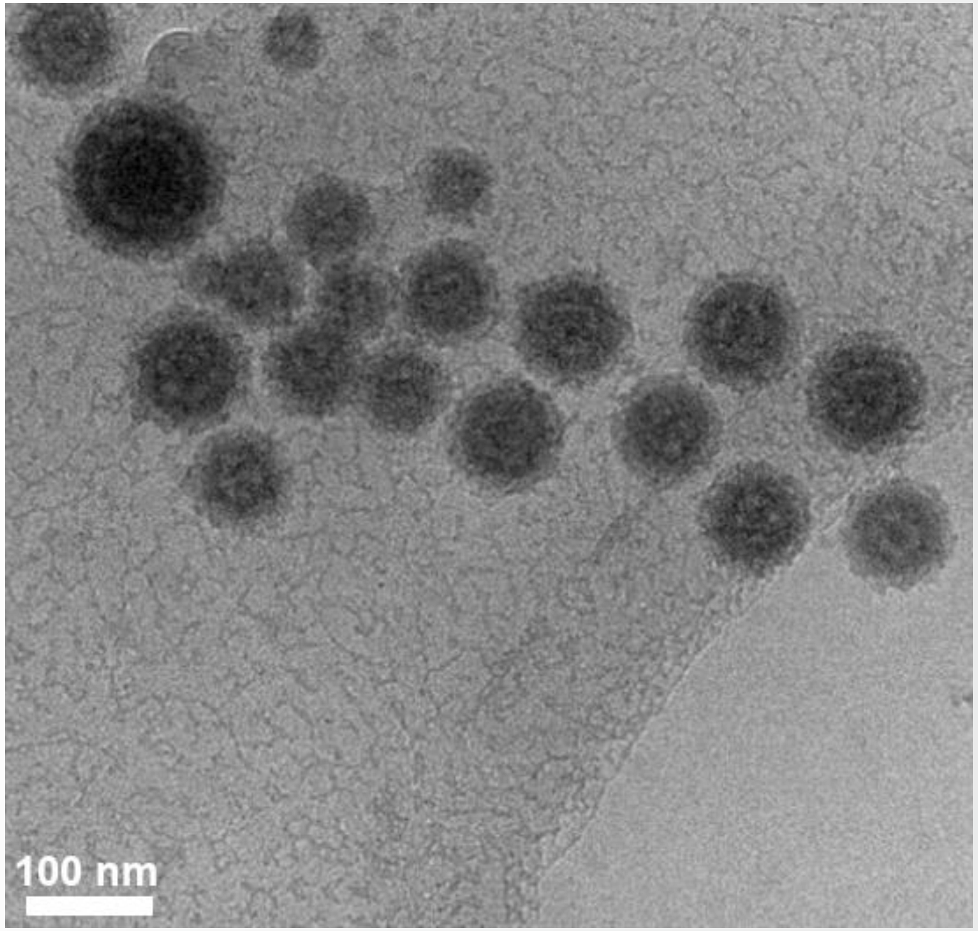


Special Investigation Cases

Soft Materials - Polymer



Block copolymer micelle in water, sphere-sphere packing assembled from blends of PAA-PI-PS and PAA-PS (150/30 nm small spheres)

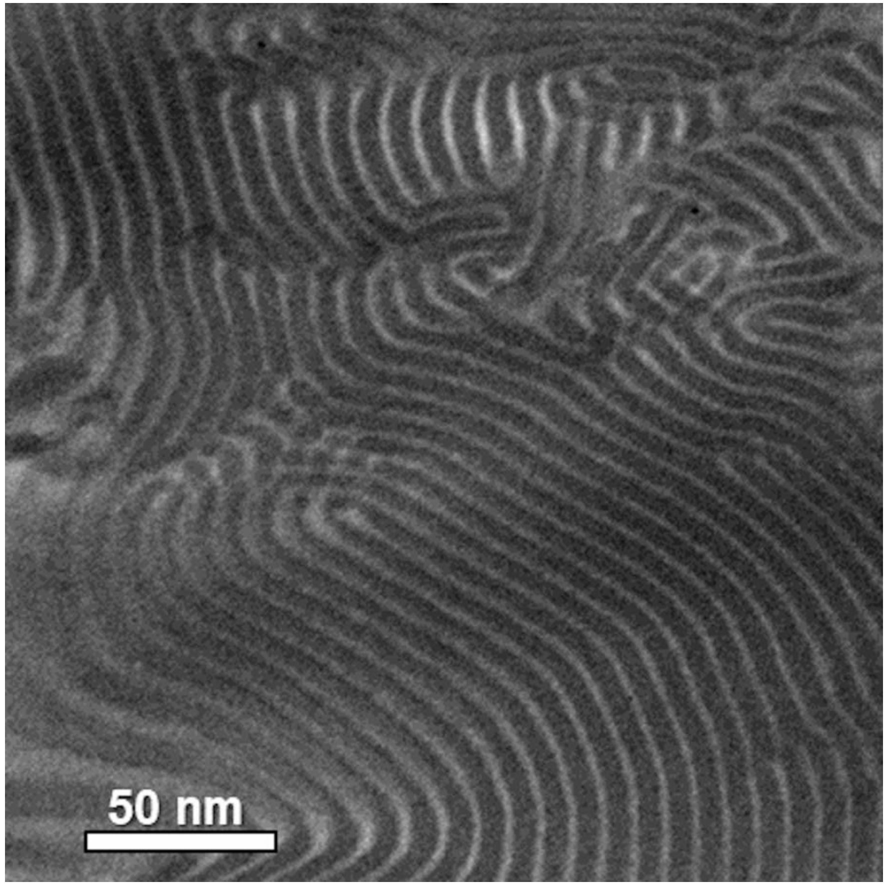


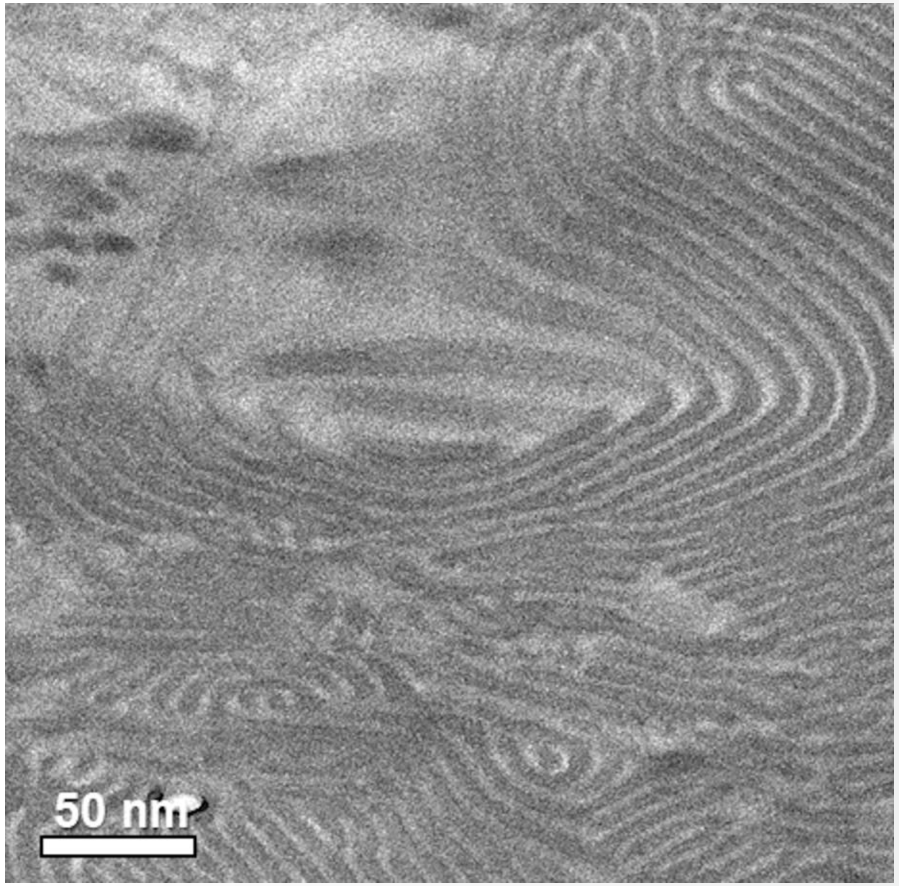
Block copolymer giant spherical aggregations in water, with internal phase separation assembled from PPA-PI-PS

Sample courtesy: Prof. Thomas Epps, III, University of Delaware

Special Investigation Cases

Soft Materials - Polymer



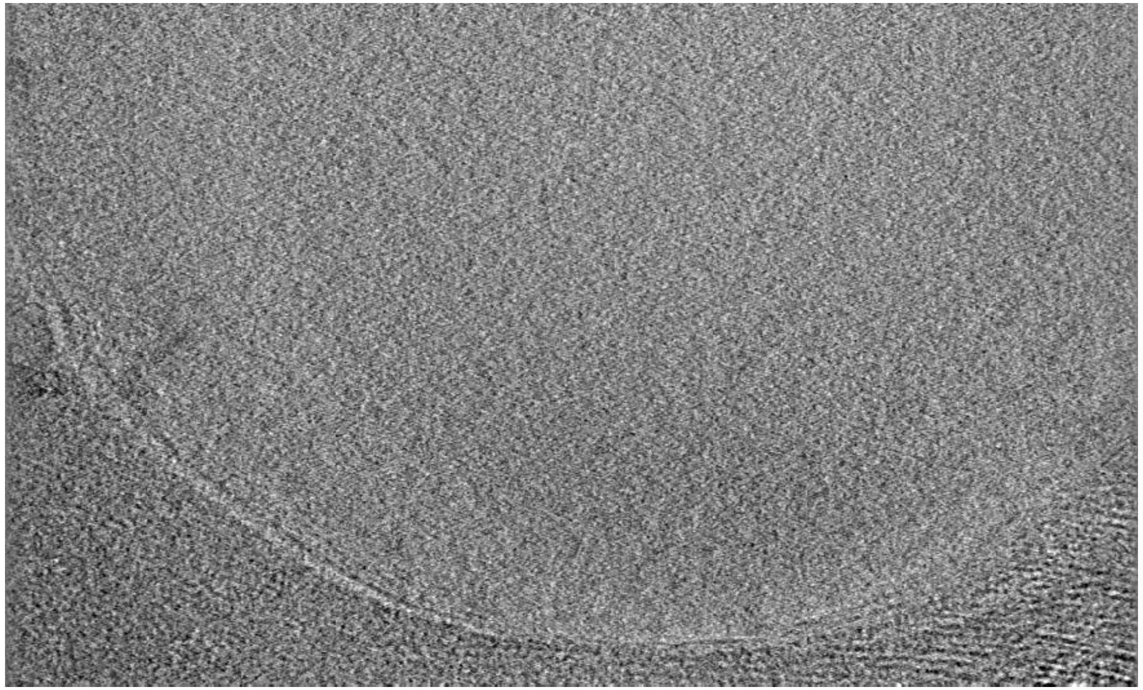


PS-PMMA block copolymer; Lamellar structure with PS units exhibiting light and PMMA units exhibiting dark contrast

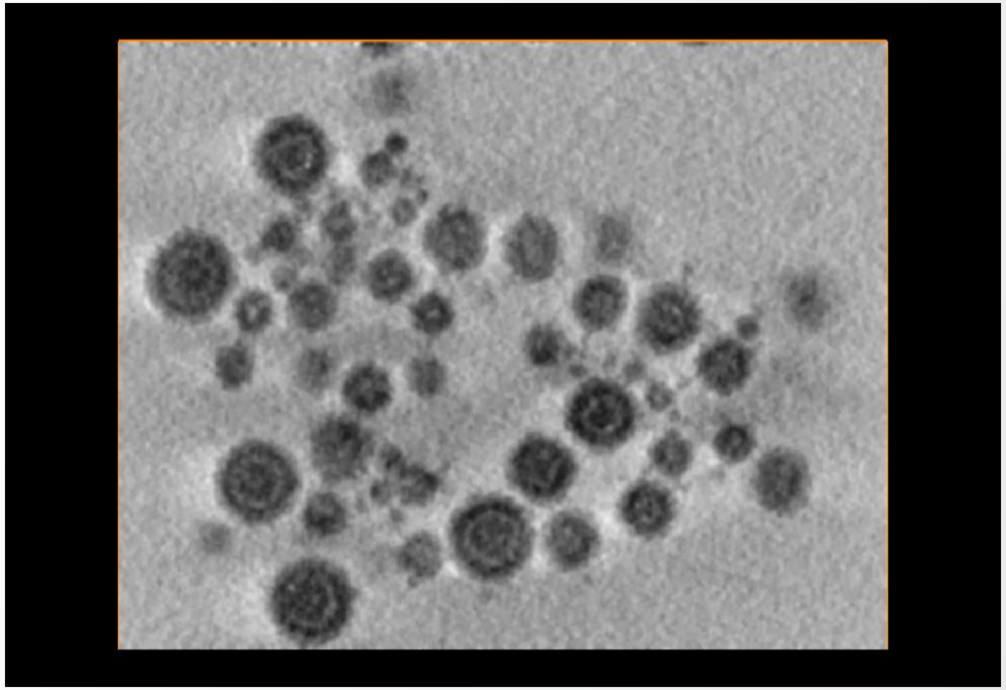
Sample courtesy: A.Prof. Kevin Jack and A.Prof. Idriss Blakey, University of Queensland

Special Investigation Cases

Soft Materials – Polymer with Cryo Tomography



Block copolymer micelle in water, sphere-sphere packing assembled from blends of PAA-PI-PS and PAA-PS (150 & 30 nm spheres).

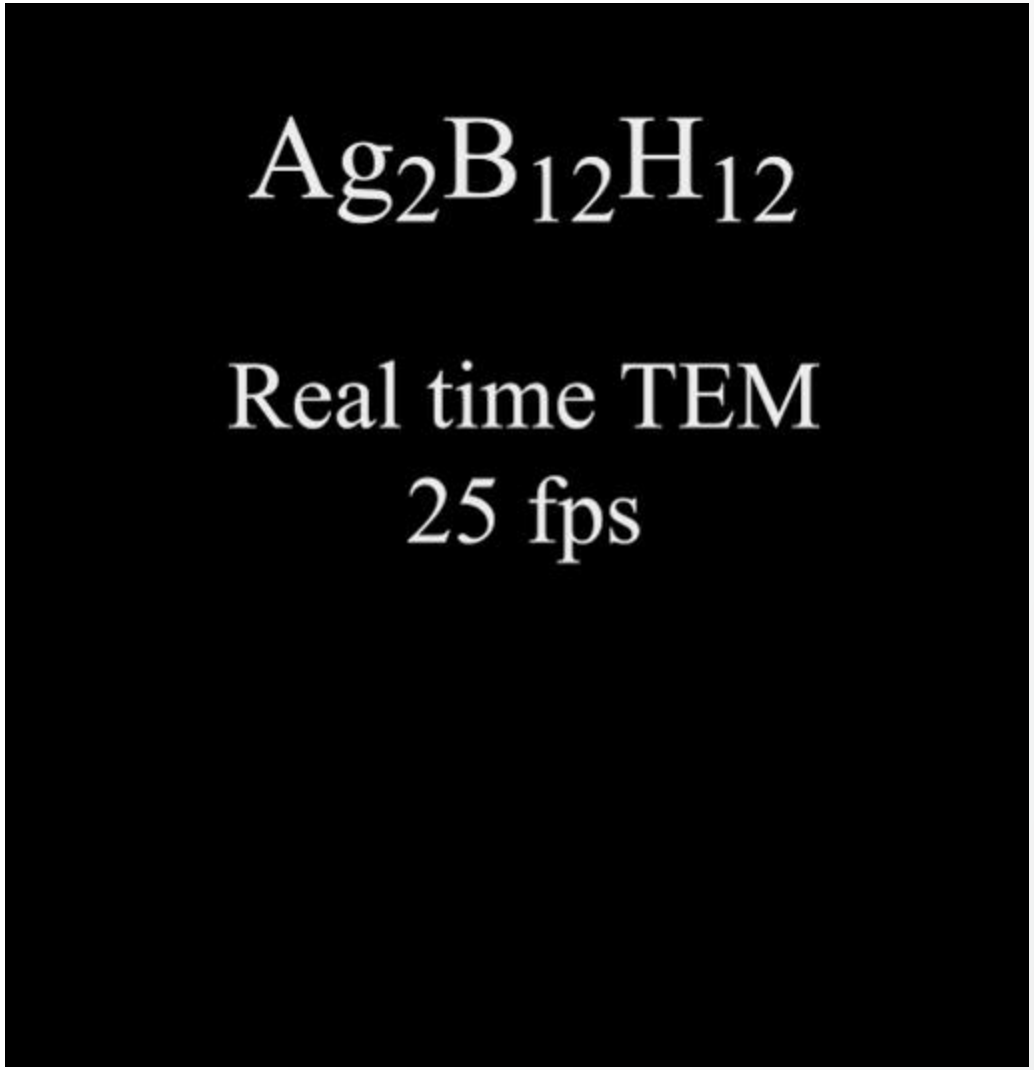


Block copolymer giant spherical aggregations in water, with internal phase separation assembled from PAA-PI-PS (100 - 200nm spheres)

Sample courtesy: Prof. Thomas Epps, III, University of Delaware

Special Investigation Cases

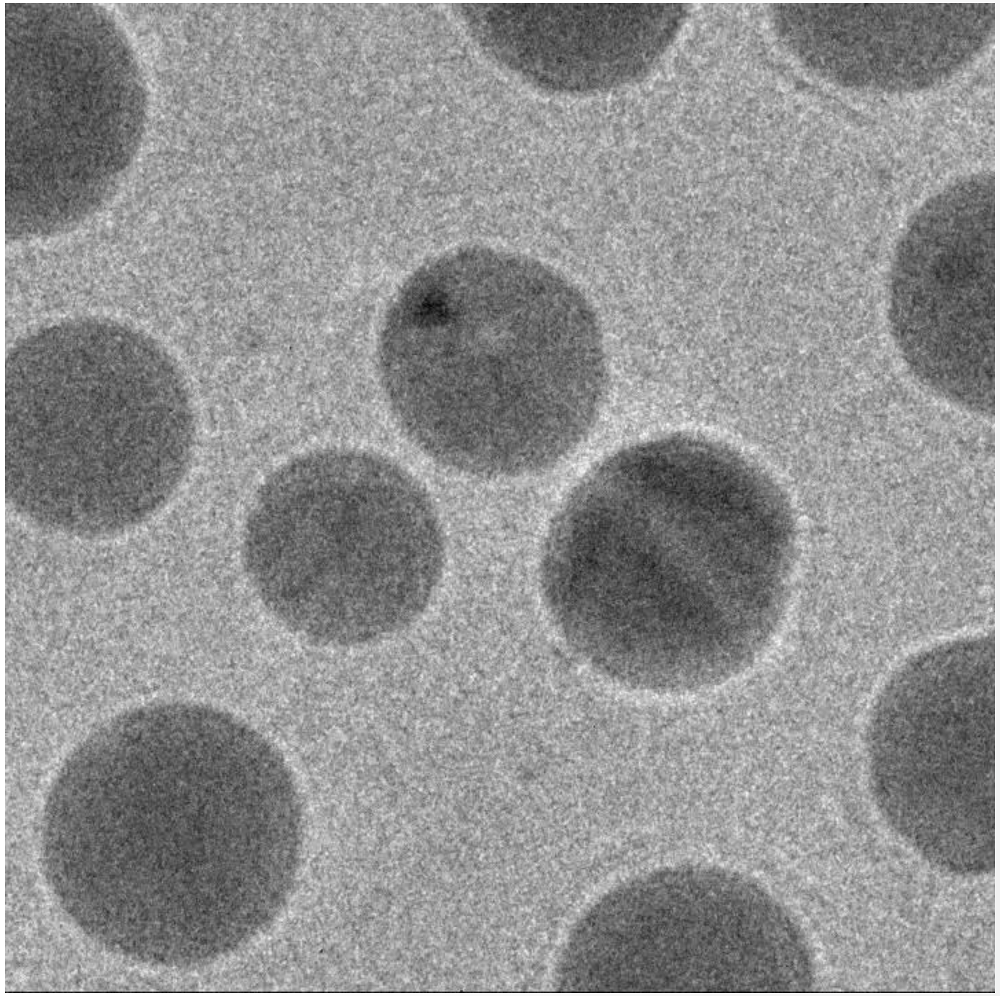
In-situ Experiments



*M. Paskevicius, B.R.S. Hansen, M. Jørgensen, B. Richter & T.R. JensenNature Communications **8**, Article number: 15136 (2017)*

Special Investigation Cases

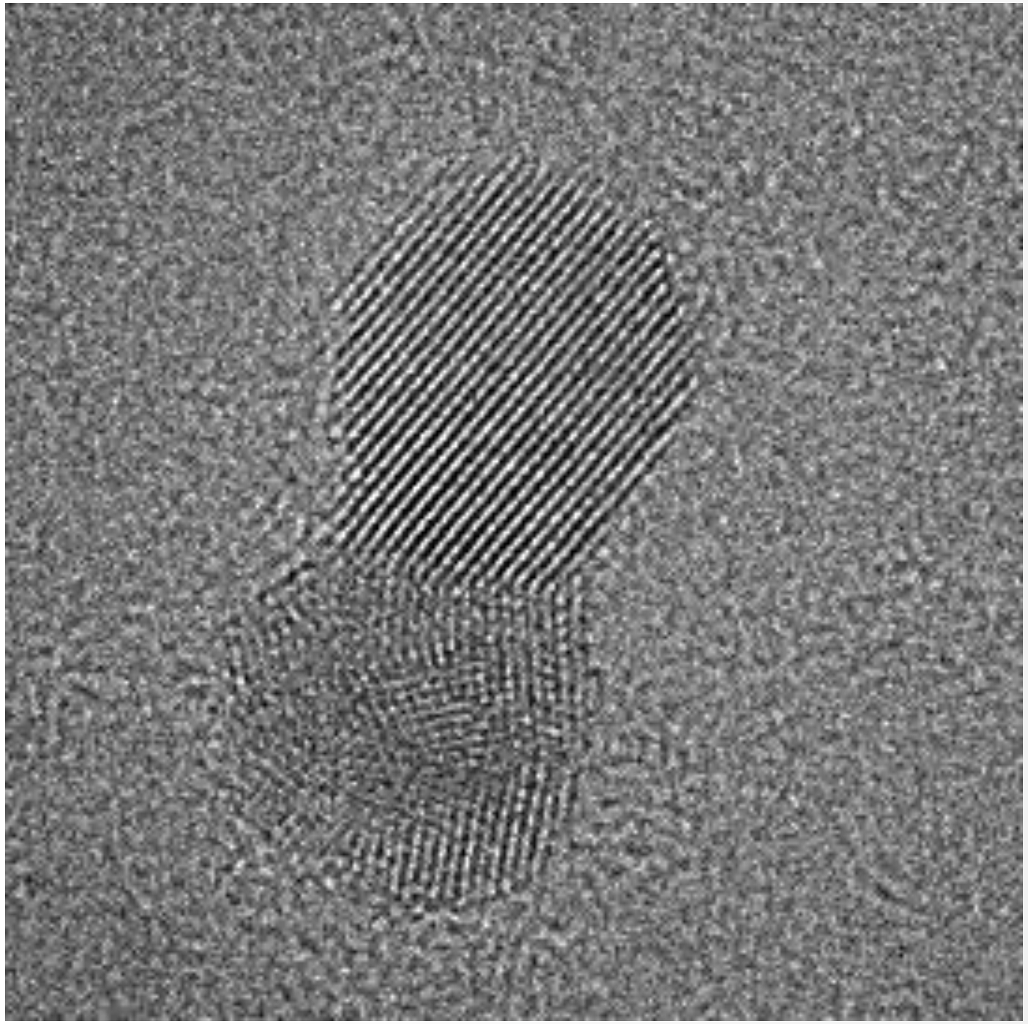
In-situ Experiments



Movie of Au nanoparticles coalescence @ 900°C with 30 fps (4k x 4k) - video running at half speed

Special Investigation Cases

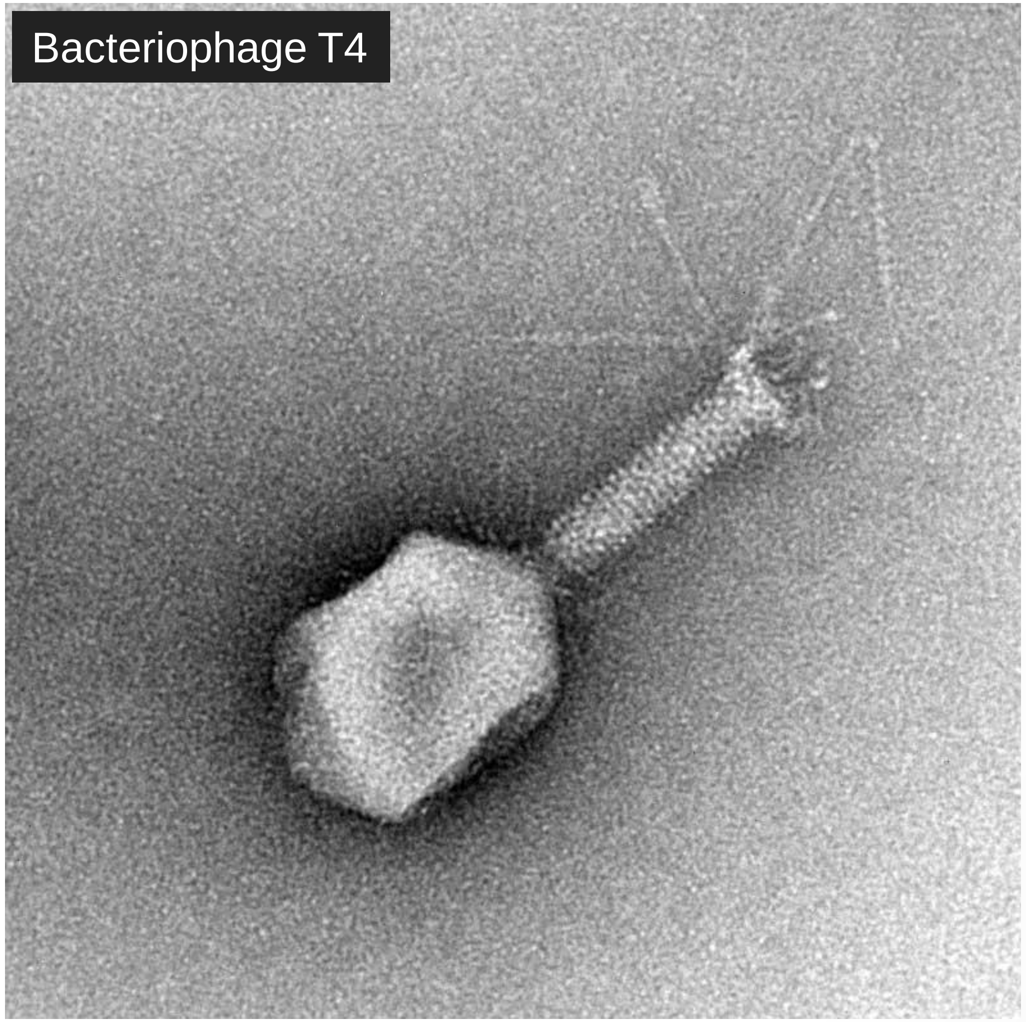
In-situ Experiments

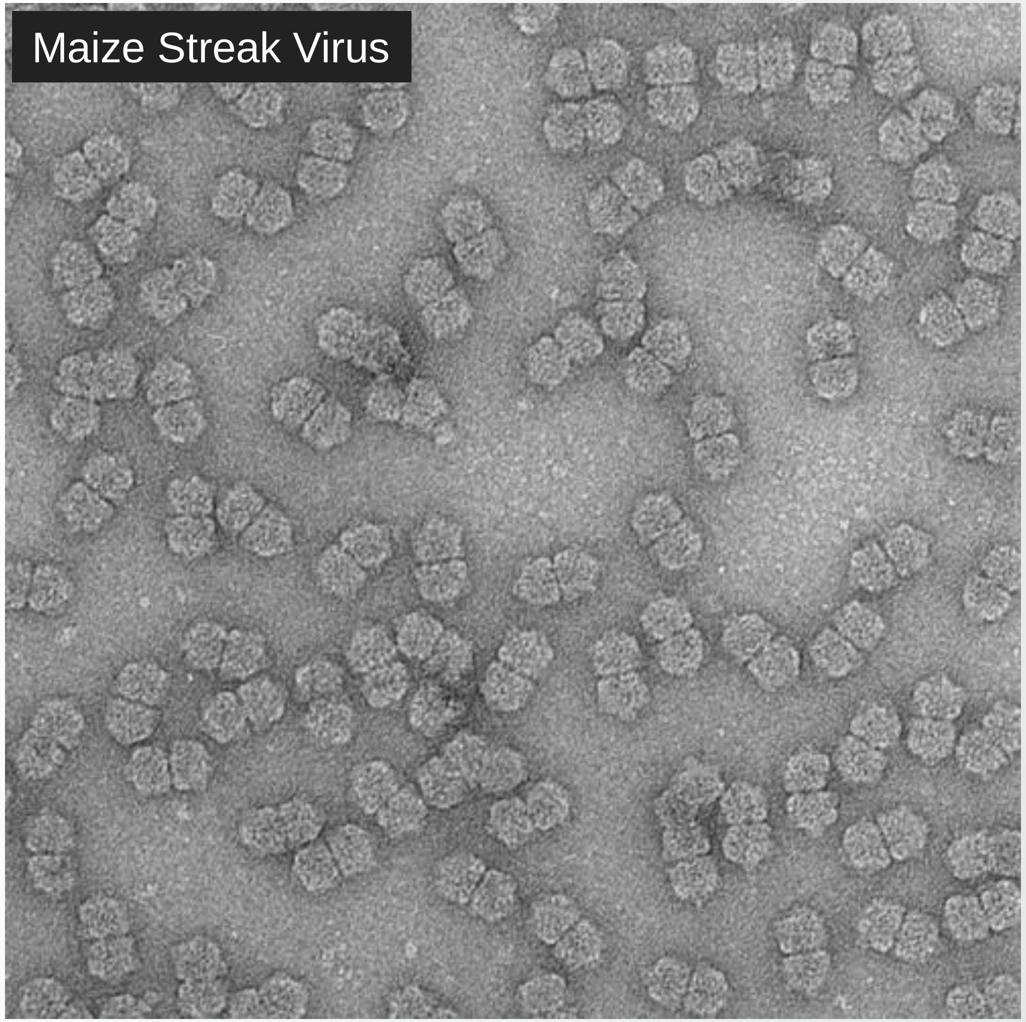


Movie of Au nanoparticles sintering in the beam with 25 fps

Special Investigation Cases

Application for Life Science – Negative Stain (Room Temperature)





Special Investigation Cases

Application for Life Science – Positively Stained Renal Biopsy Section (Room Temperature)

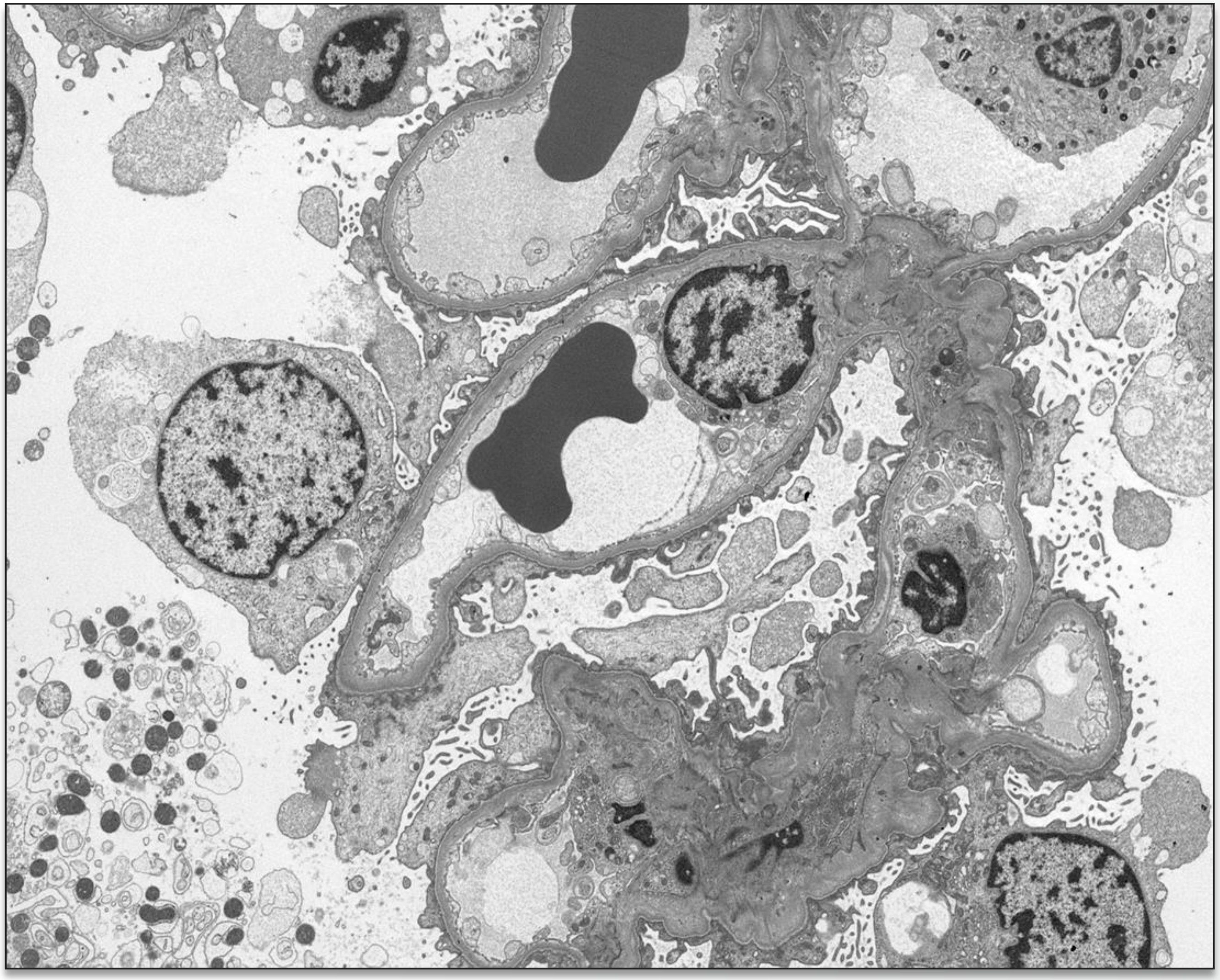
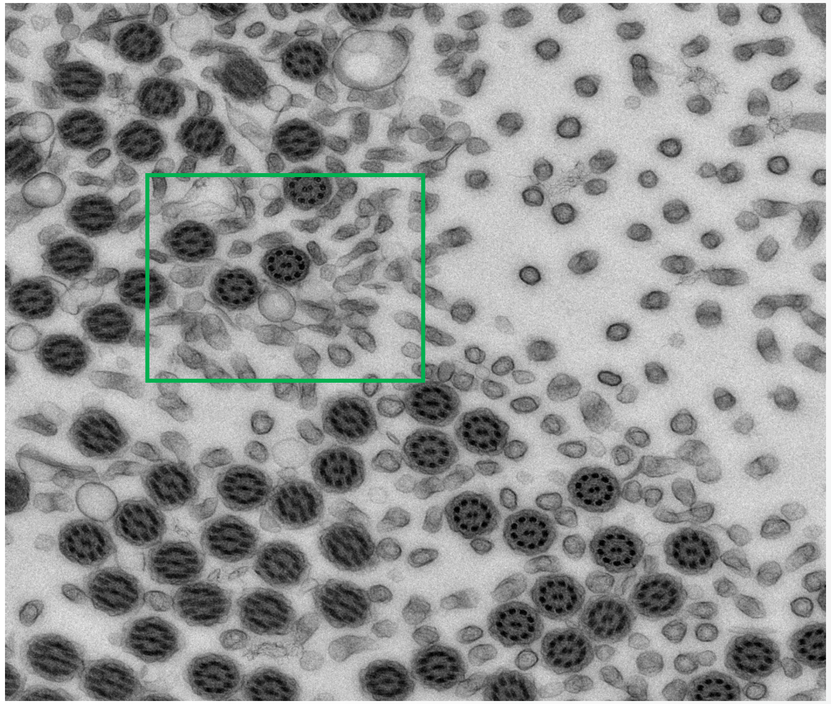


Image courtesy of Dr. Ito, Yorkhill Hospital, Glasgow, UK

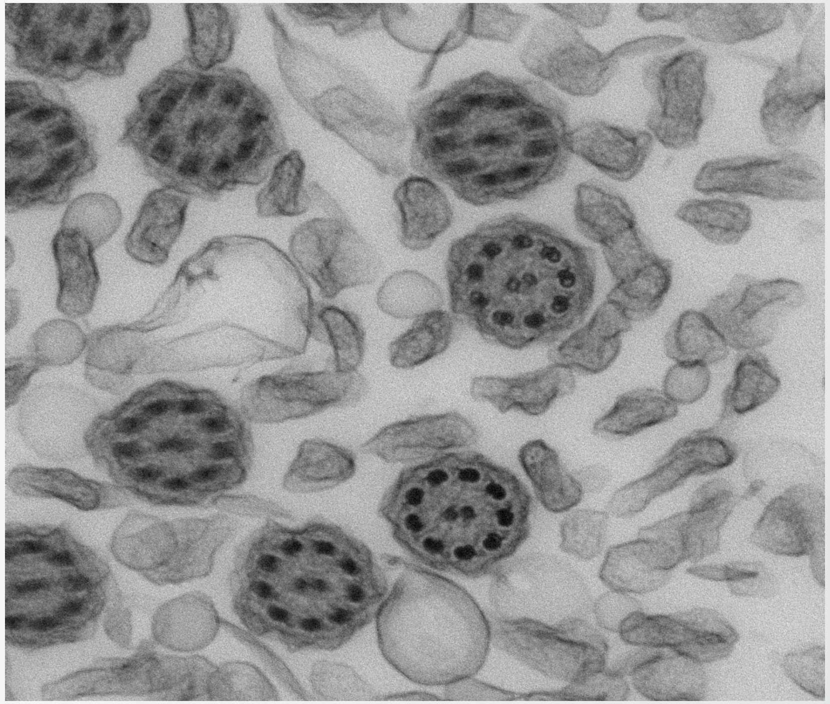
Special Investigation Cases

Application for Life Science – Lung Epithelium (Room Temperature)

TEM at 120kV			
Image size:	4kx4k	Exposure time:	1 s
Detector:	Ceta 16M	Magnification:	1600x
Pixel size:	6.3 nm		



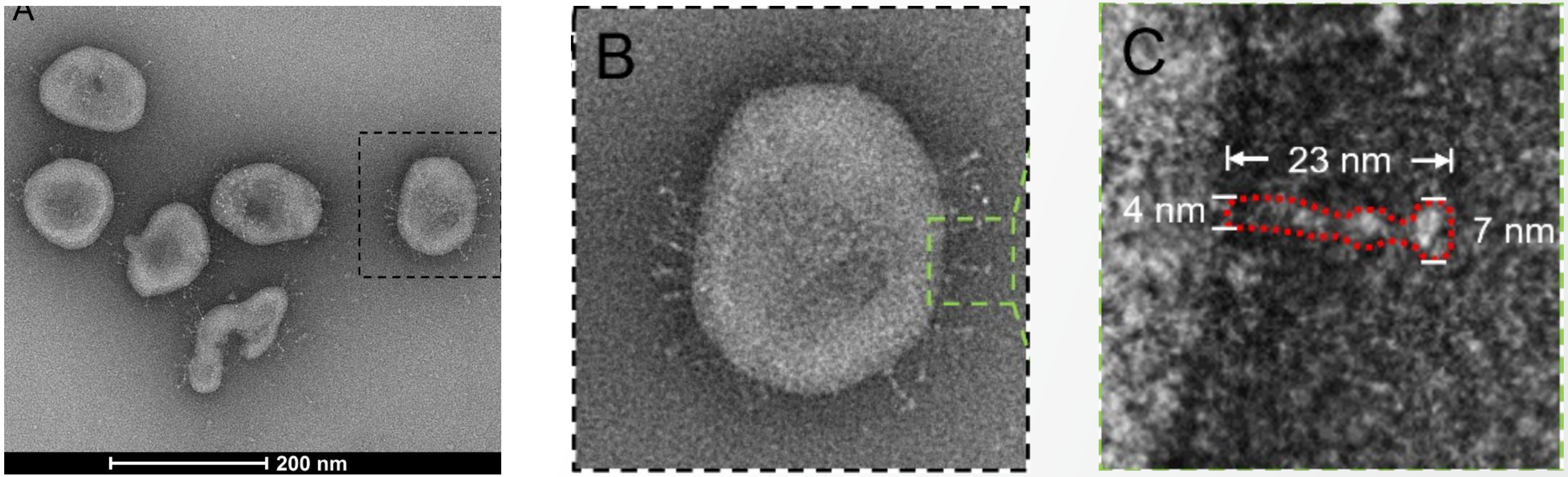
TEM at 120kV			
Image size:	4kx4k	Exposure time:	1 s
Detector:	Ceta 16M	Magnification:	3400x
Pixel size:	3.0 nm		

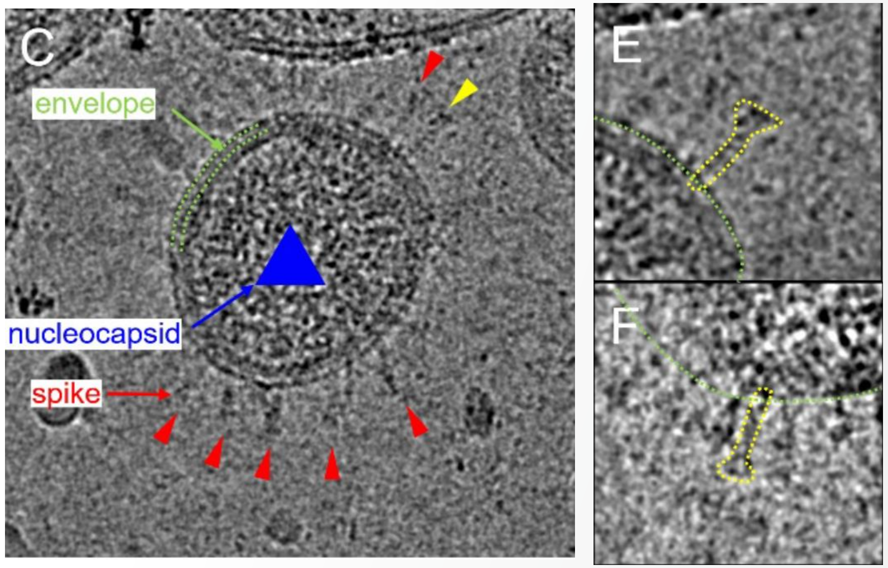


TEM at 120kV			
Image size:	4kx4k	Exposure time	1 s
Detector:	Ceta 16M	Magnification	28000x
Pixel size:	0.36nm		

Special Investigation Cases

Application for Life Science – Negative stain EM results of SARS-CoV-2.





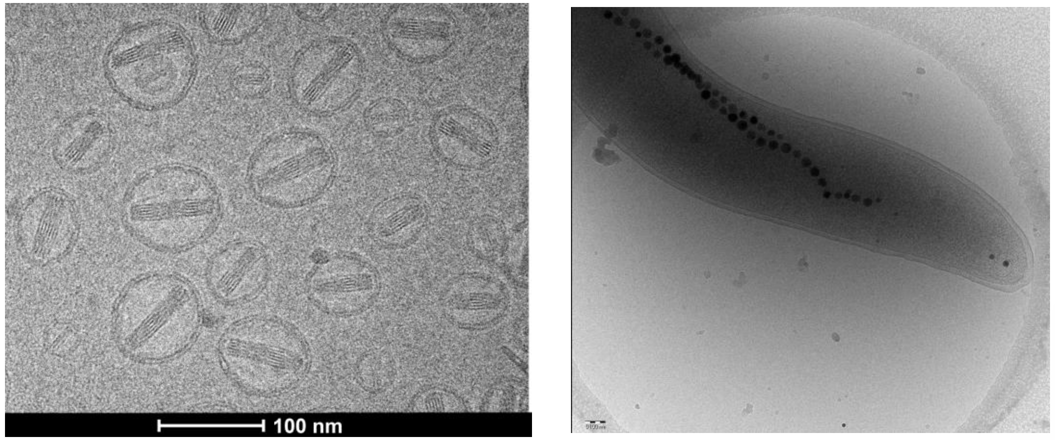
Negative stain EM results of SARS-CoV-2.

(A). Image of negative stained SARS-CoV-2. Nail-like spikes can be seen.
(B). Enlarged view of virion boxed in (A).
(C) Zoom-in view of a spike boxed in (B).
A red dot line depicts the shape. Length, the diameter of the stem, and the spike's head are 23nm, 4nm, and 7nm, respectively.

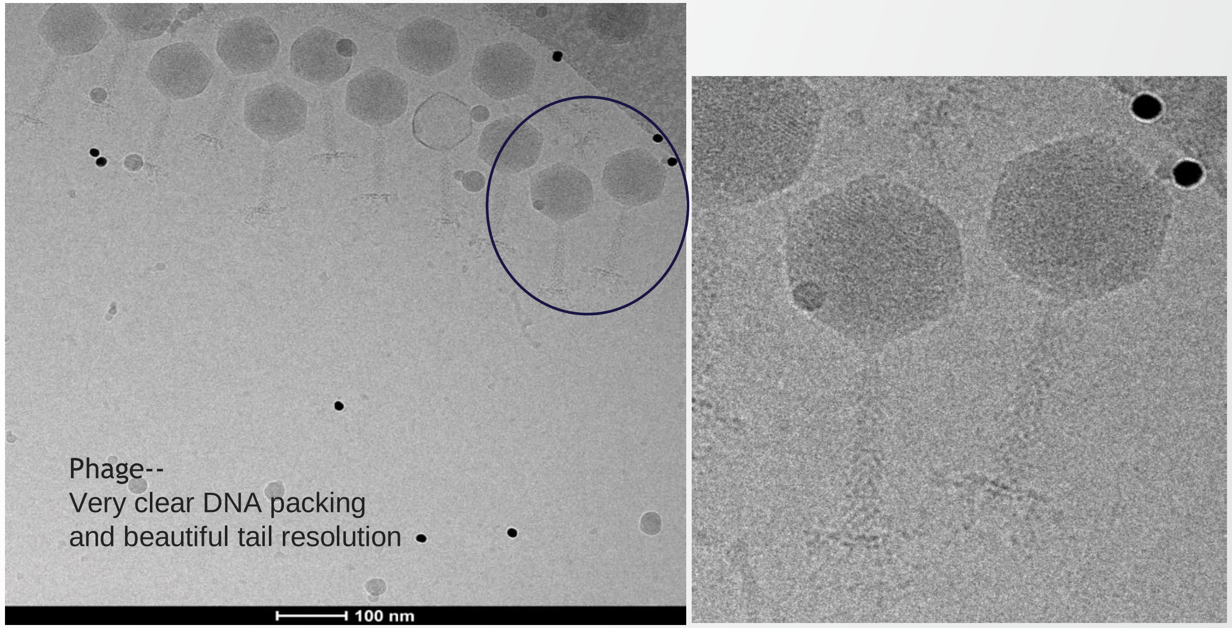
BioRxiv preprint doi: https://doi.org/10.1101/2020.03.02.972927

Special Investigation Cases

Application for Life Science – Cryogenic Condition



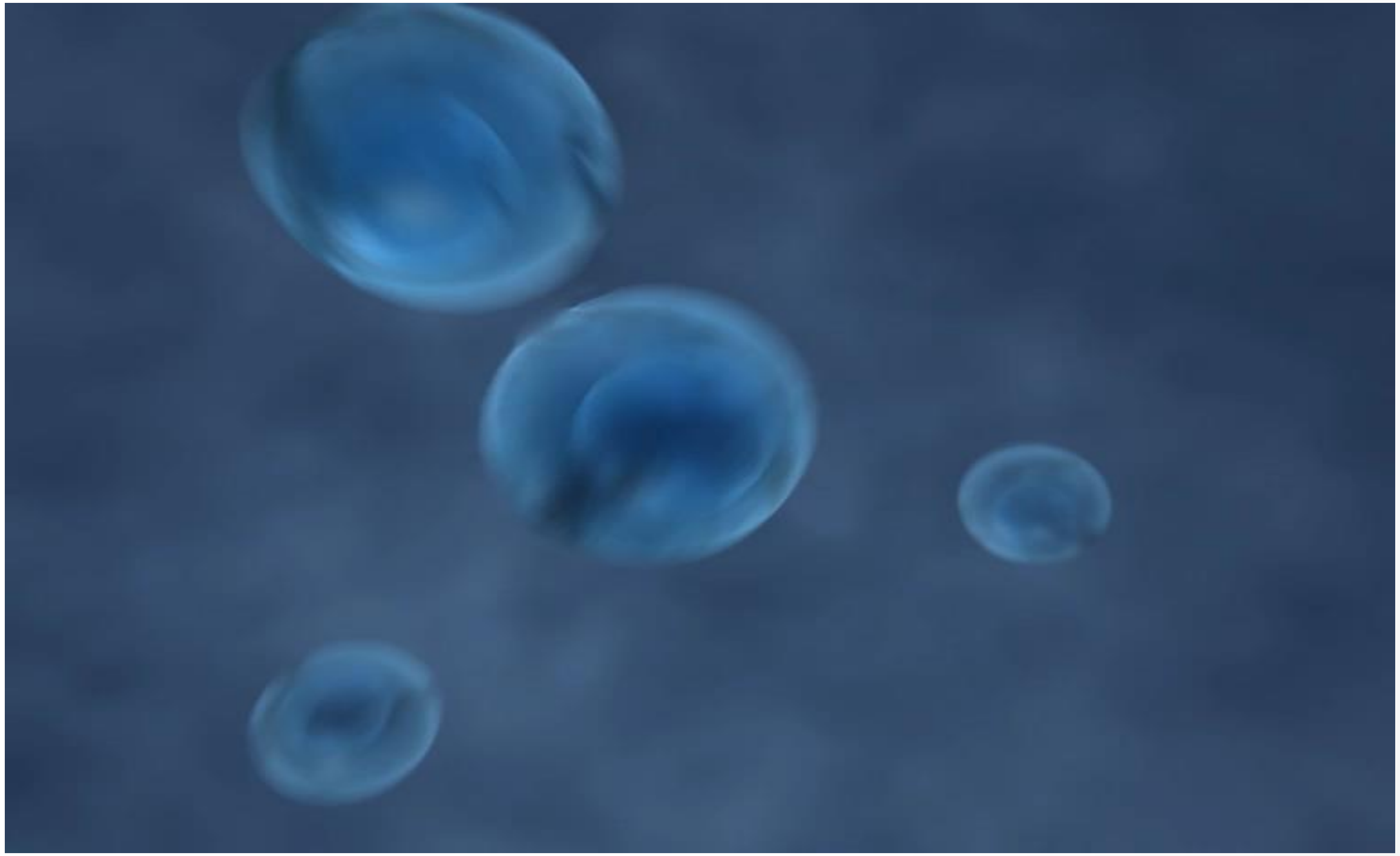
Drug delivery Liposome



Bacteriophage T4

Special Investigation Cases

Application for Life Science – Cryo Tomography



- Does not rely on labelling or fixation.
- The cells are not fixed, not stained or permeabilized in any way.
- Instead, the sample is preserved in the native state by a very fast freezing technique which is called vitrification
- Provide the context of Cellular environment
- Higher Resolution

Thank you