

# Webinar Series in TEM: Transmission Electron Microscopy - Part 2

## Scanning, Spectroscopy, and 3D Imaging in TEM

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Material and Structural Analysis

Thermo Fisher Scientific



DIREKTORAT RISET &  
PENGEMBANGAN

**LabTEM**  
HIGH RESOLUTION

**multi**

 The world leader in serving science

HT	Size	Dwell	Coll. Angle	1 nm
80 kV	2048	10.0 μs	9 - 36 mrad	iDPC

# 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

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

# Transmission Electron Microscopy Webinar Series

## Outline: Part 03

- 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 04

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

# Transmission Electron Microscopy Webinar Series

## Outline: Part 02

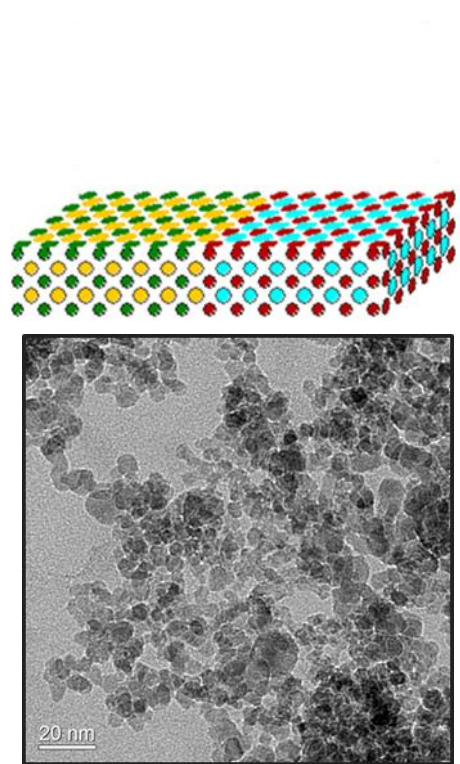
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# Scanning Transmission Electron Microscope (STEM)

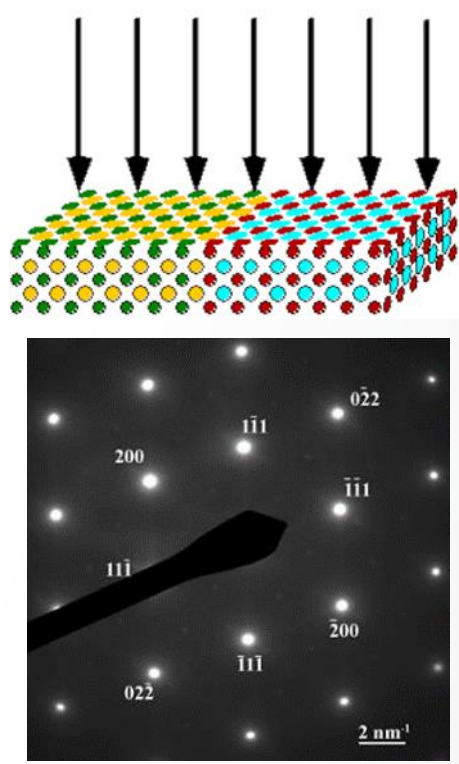
More Than a Conventional TEM



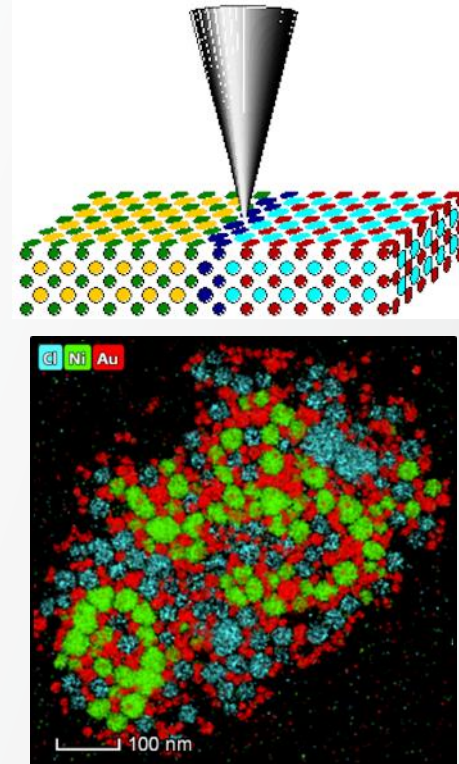
## Image



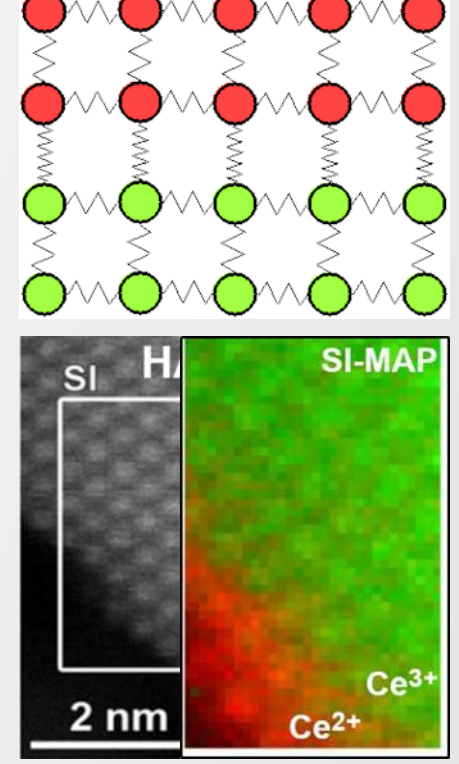
## Structure



## Chemistry



## Bonding



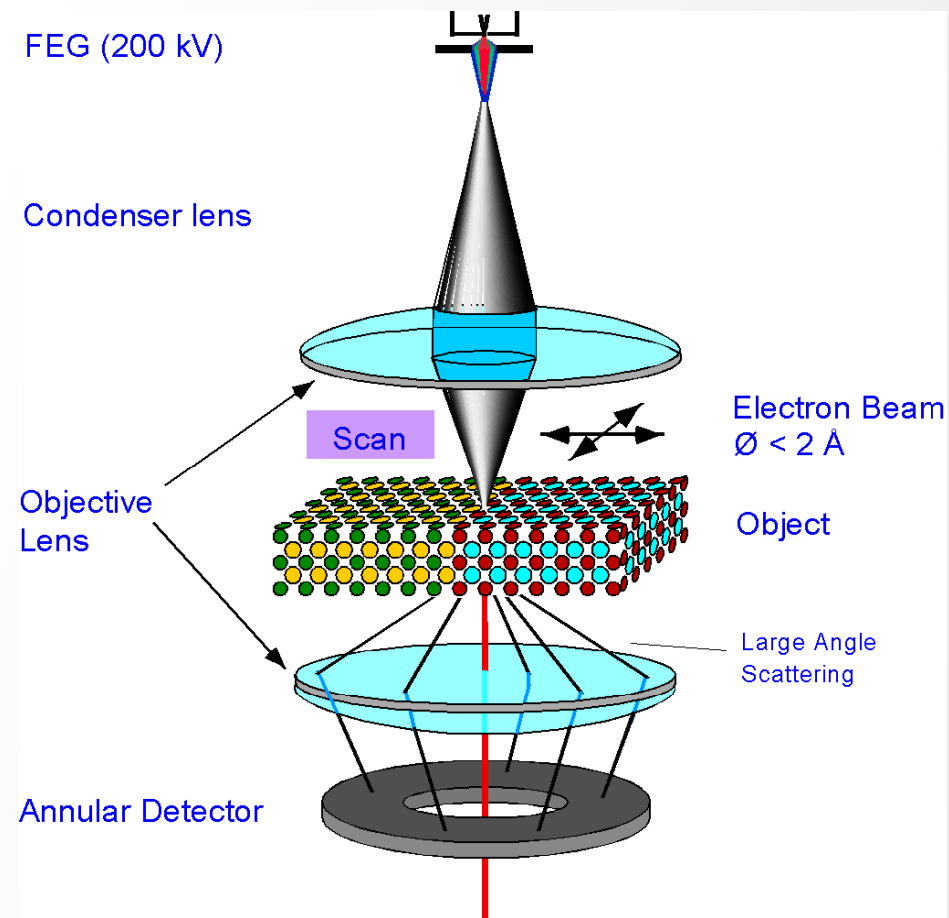
Spectra 300

An Aberration Corrected Microscope

# Scanning Transmission Electron Microscope (STEM)

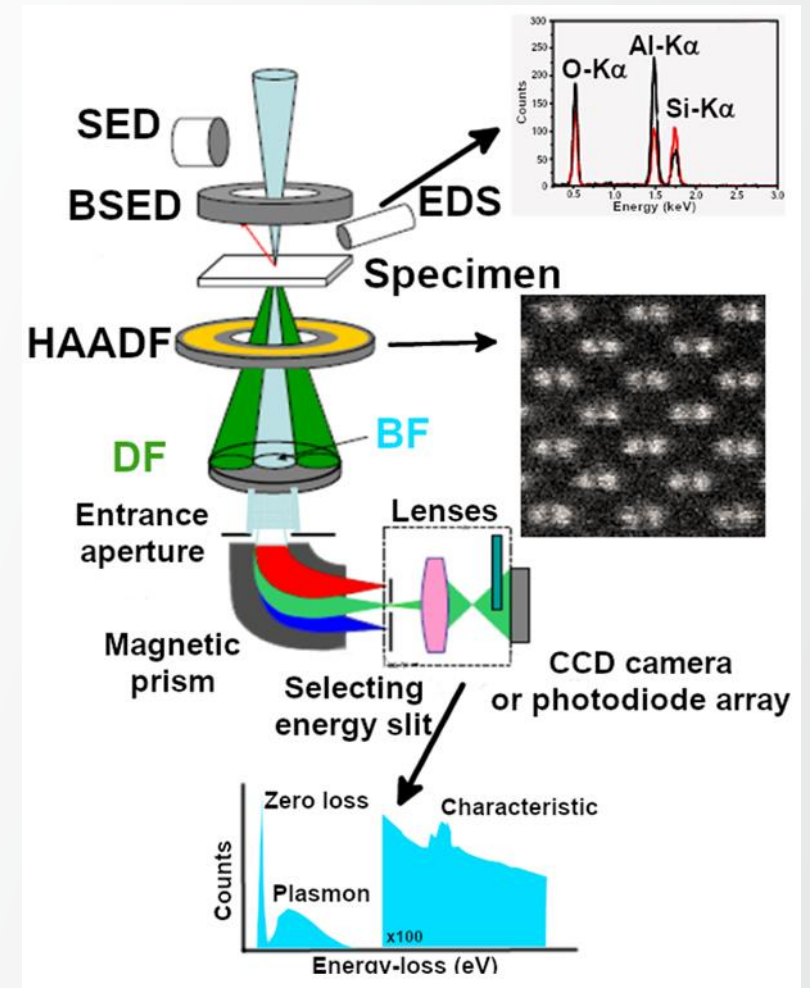
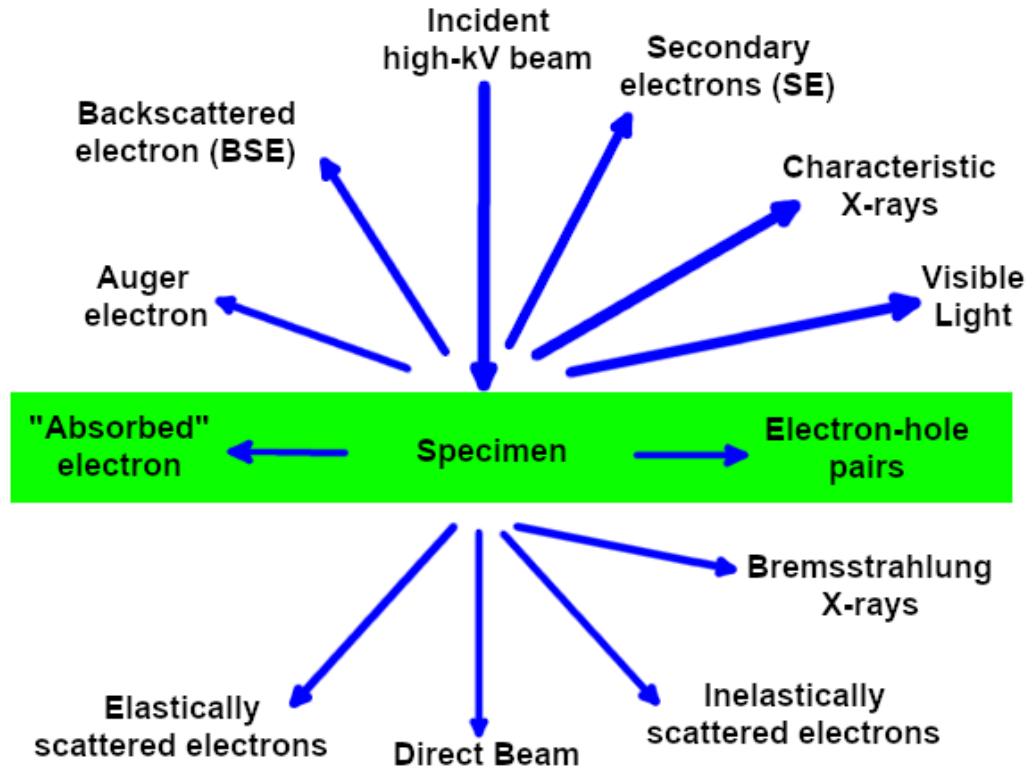
## What is STEM

- STEM is a **scanning technique** in TEM
- 'A SEM within a TEM'
- Specimen must be **small** (fitting TEM holder) and **thin** (electron transparent) because the detectors are below



# Scanning Transmission Electron Microscope (STEM)

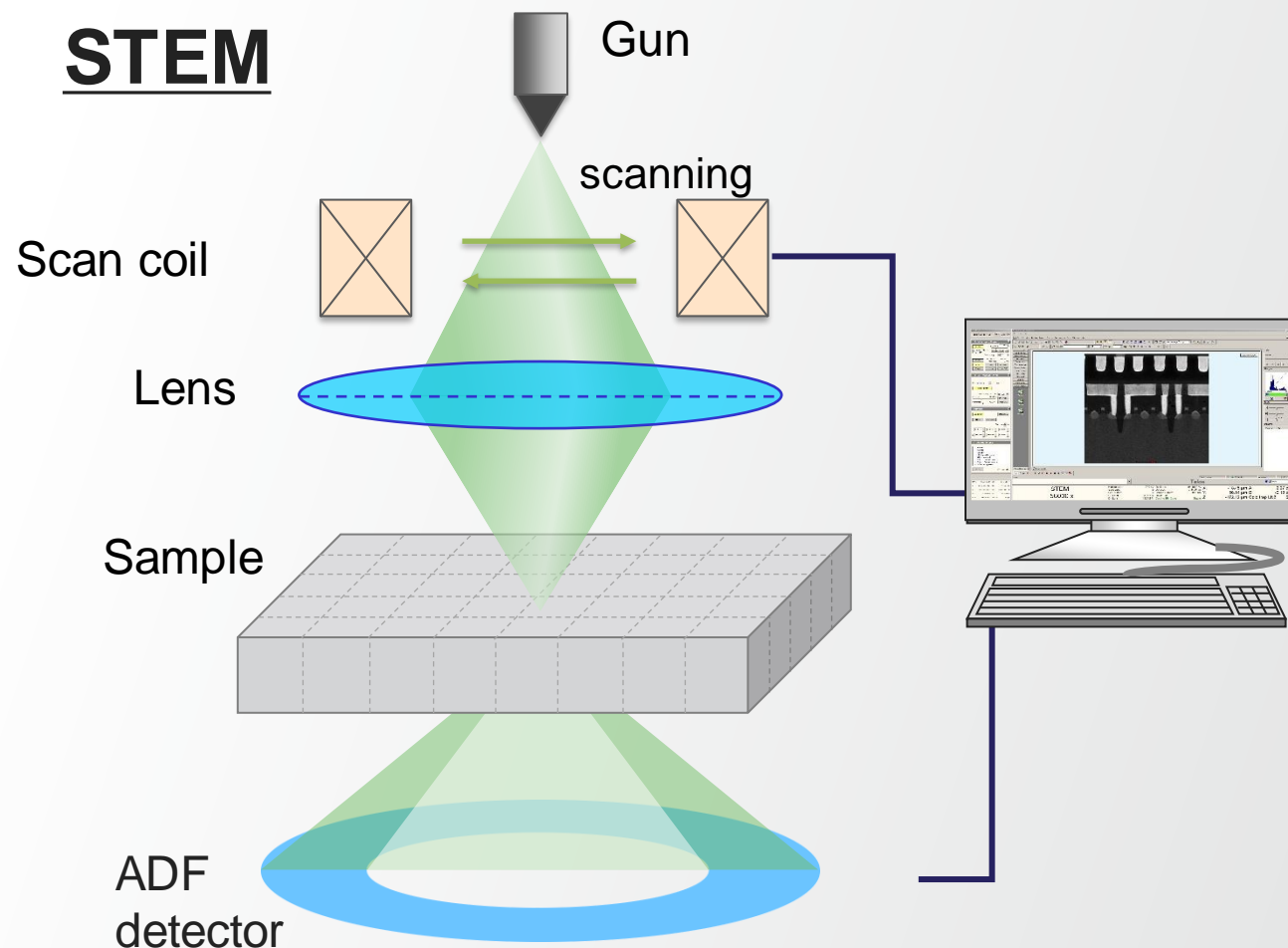
STEM and Spectroscopy: AEM (Analytical (T)EM)



# Scanning Transmission Electron Microscope (STEM)

## STEM Image Formation

- **STEM works in diffraction mode, scanning the small probe over the sample, one pixel at a time.**
- STEM detectors capture diffraction pattern information. Normally they measure the **total signal from all the electrons hitting the STEM detector.**
- Each detector covers a selected region (typically a ring) of the diffraction pattern.
- STEM mode also uses a convergent small probe so the diffraction pattern will typically consist of disks when imaging a crystalline material.





# Transmission Electron Microscopy Webinar Series

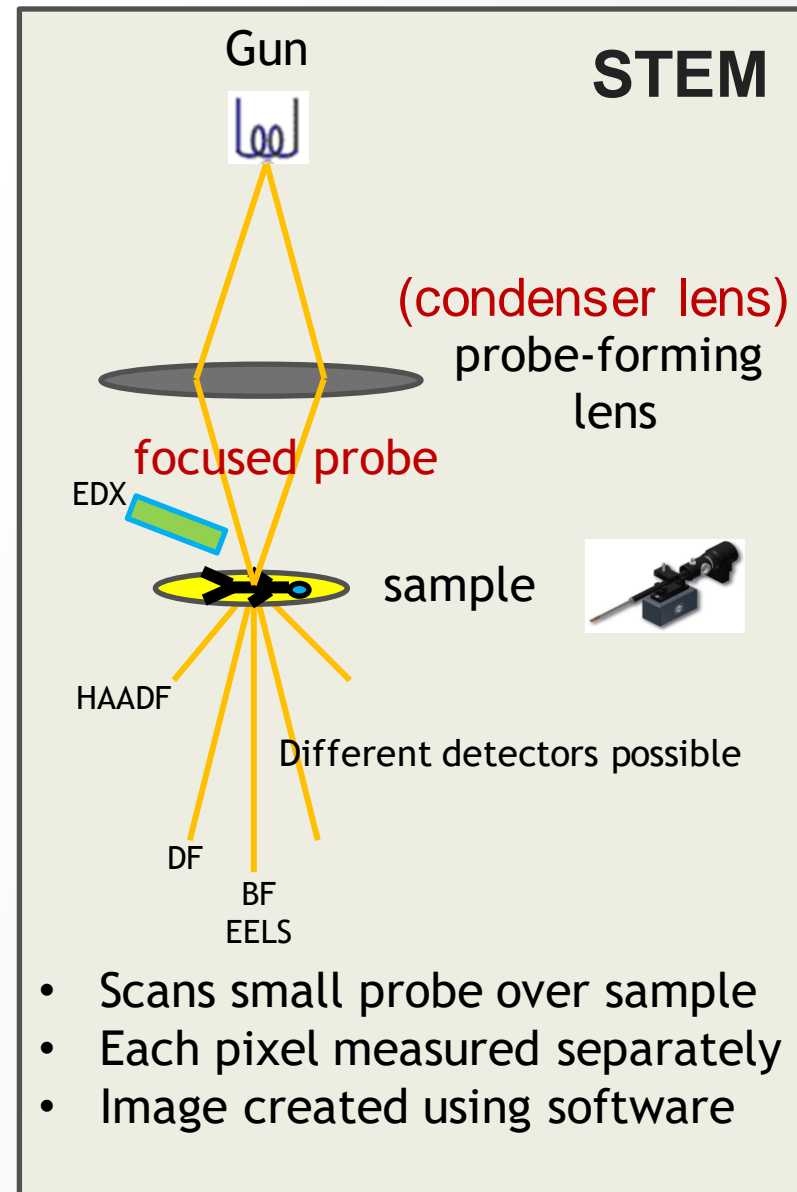
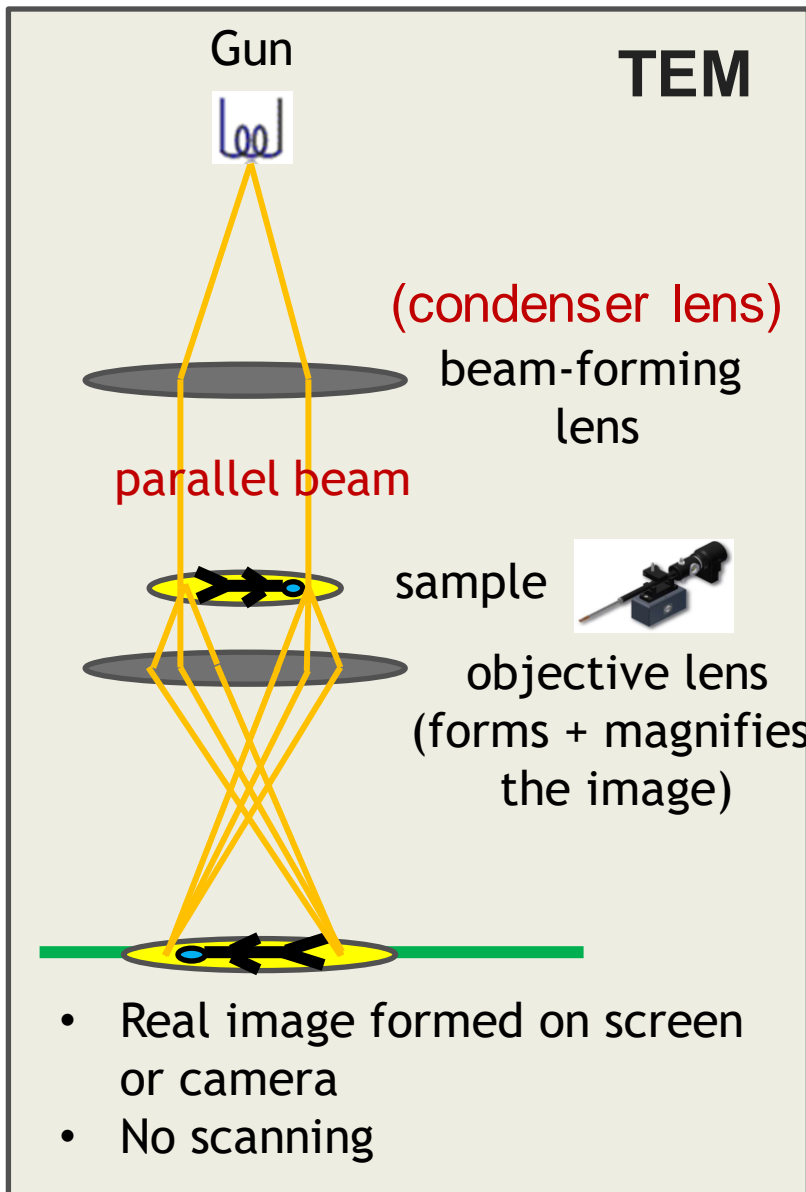
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# TEM and STEM Comparisons

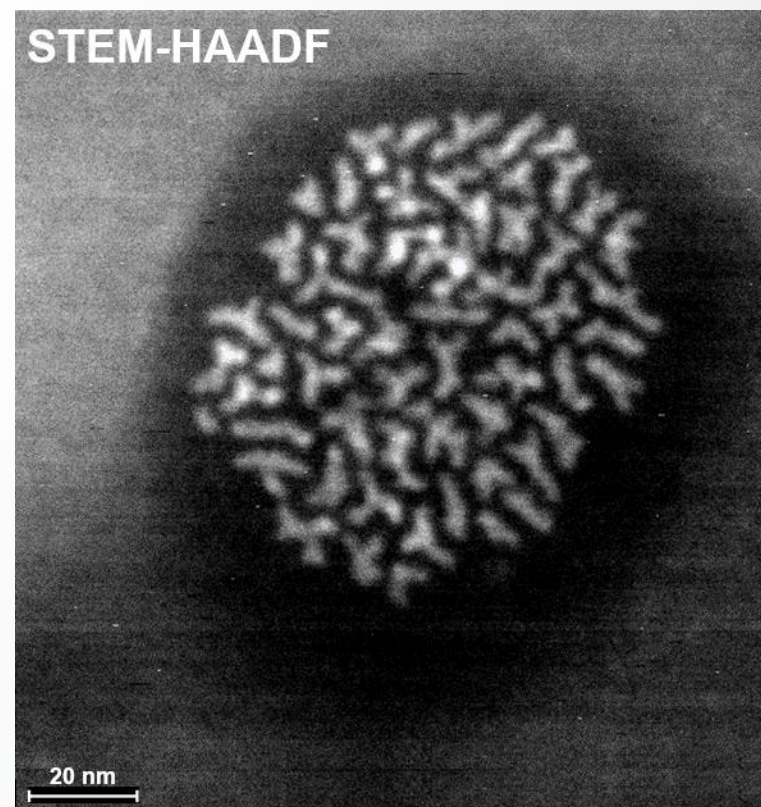
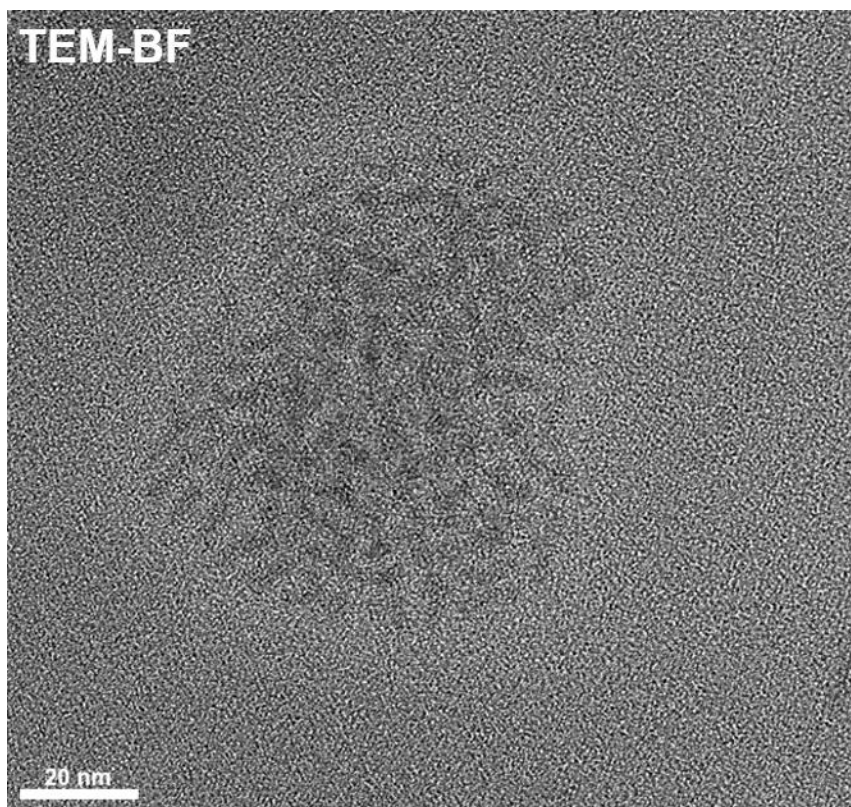
## TEM vs STEM



# TEM and STEM Comparisons

## Z-Contrast in STEM Image

- STEM-HAADF image is very sensitive to Z-contrast. Elements with higher Z will show brighter than lower Zelementst.
- CdSe nanoparticles on carbon supporting film.



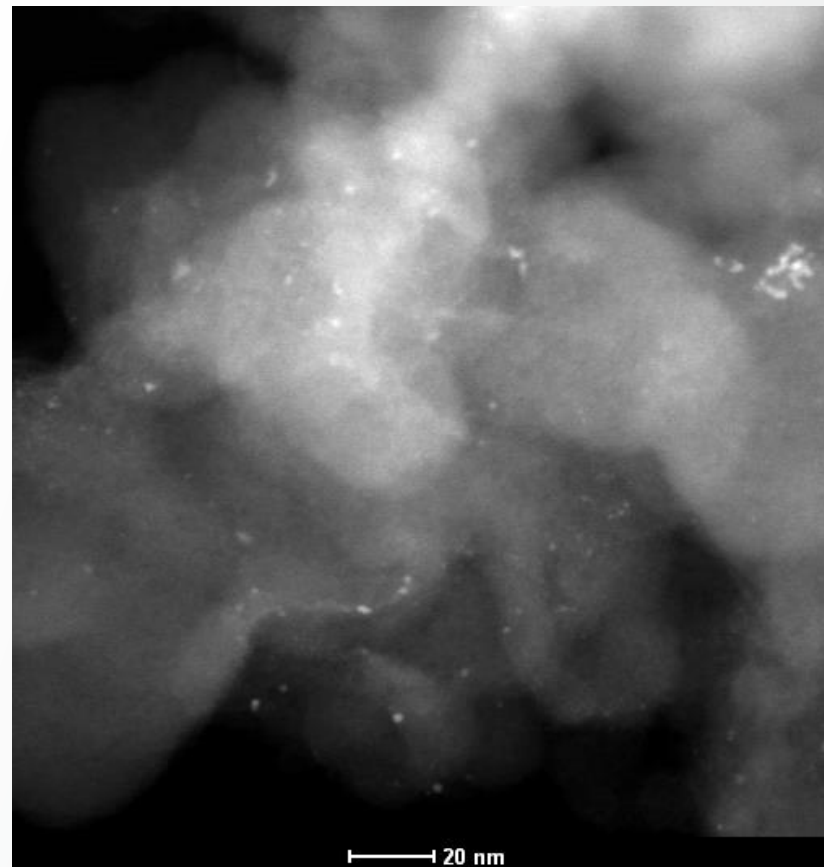
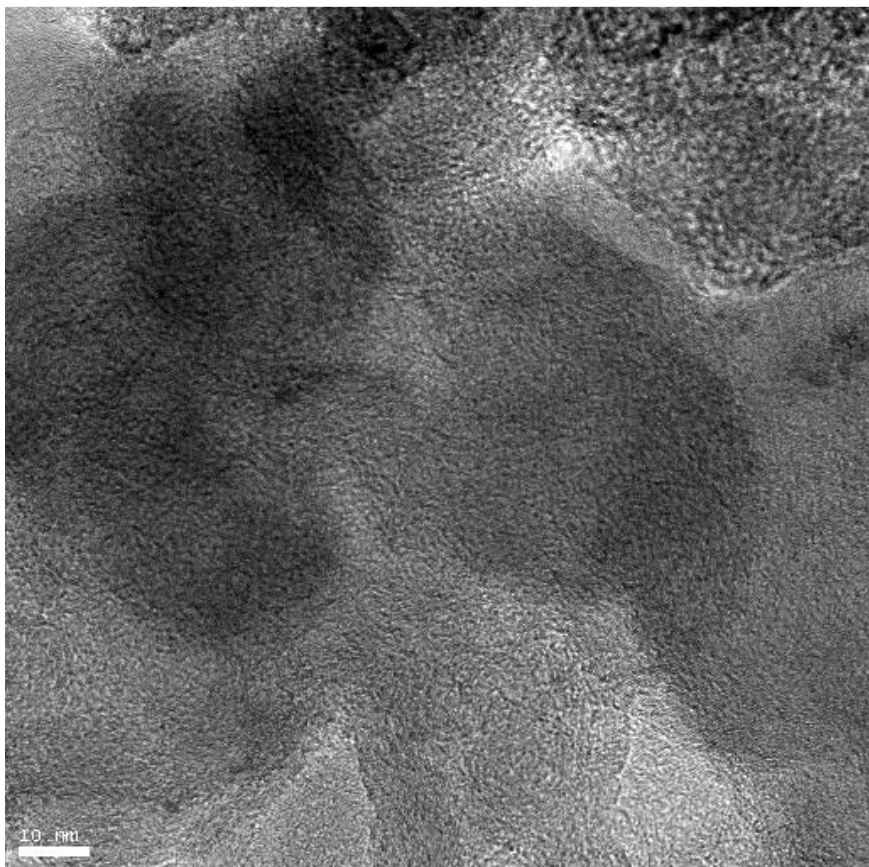


# TEM and STEM Comparisons

Combination Z-Contrast and Thick specimen

- RuPt nanoparticles on graphite

TEM



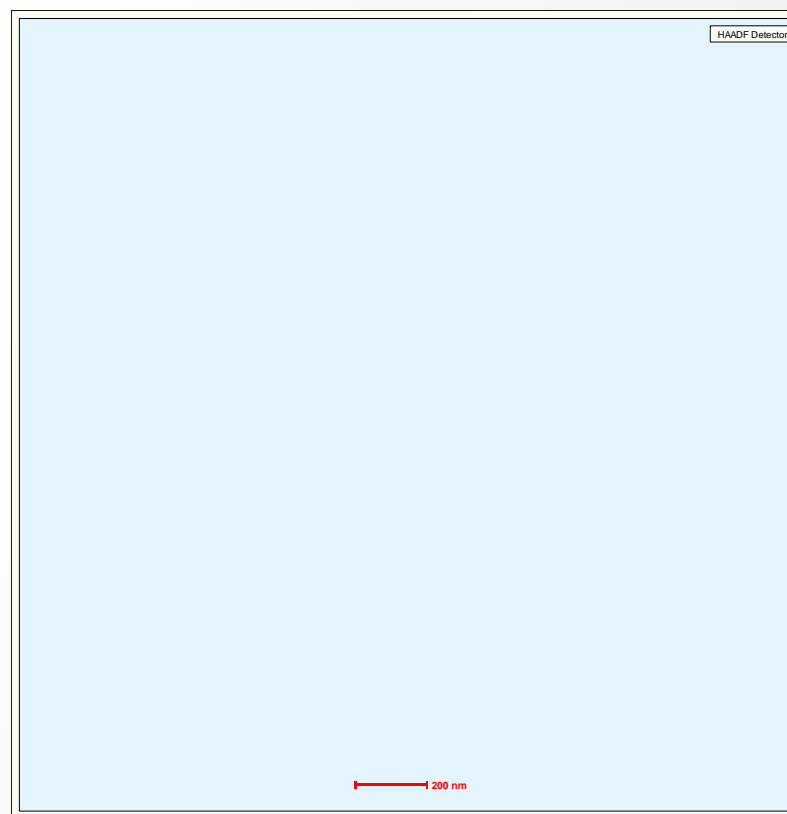
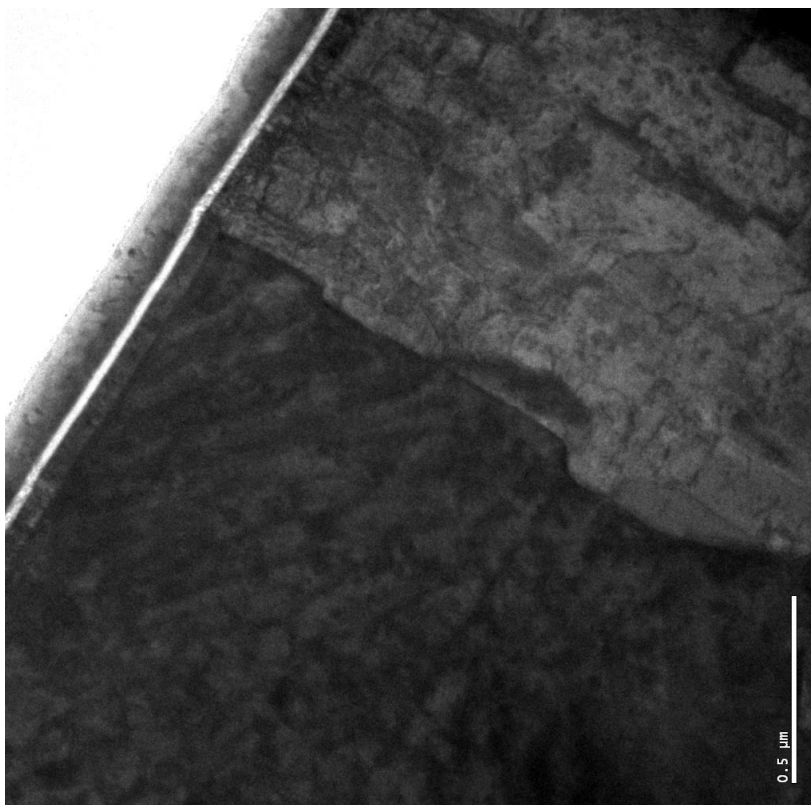
STEM

# TEM and STEM Comparisons

Thick specimen

- On the same FIB-made thick sample, the TEM image looks not focused due to chromatic aberration, but the STEM image still looks sharp

TEM



STEM

# Transmission Electron Microscopy Webinar Series

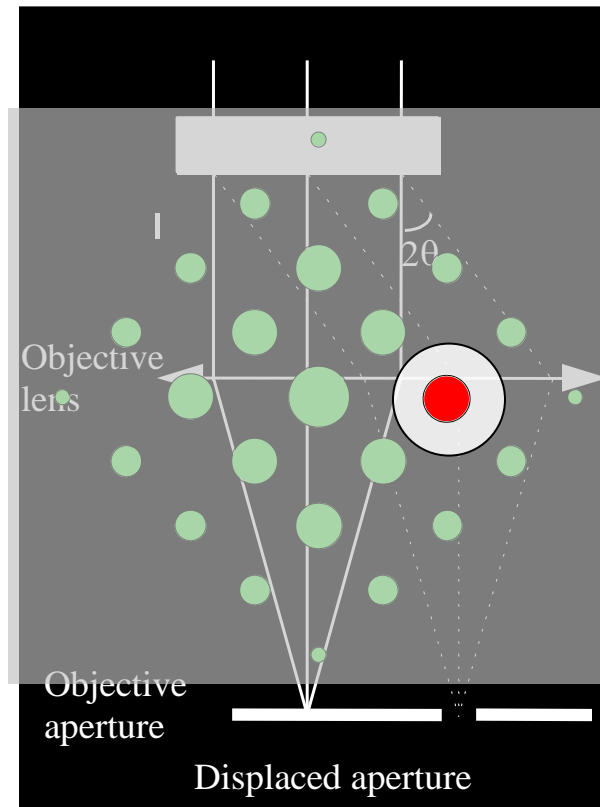
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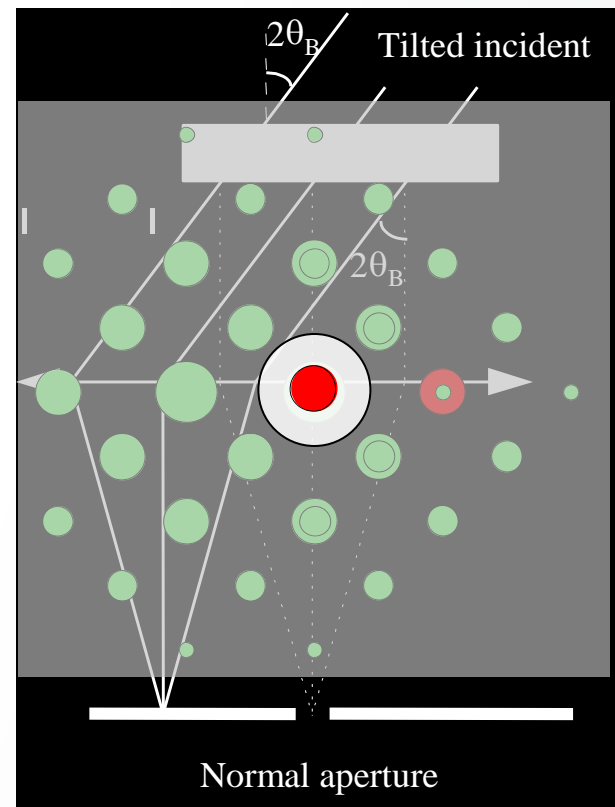
# Conventional TEM Imaging: Bright and Dark Field Imaging

## Dark Field Imaging

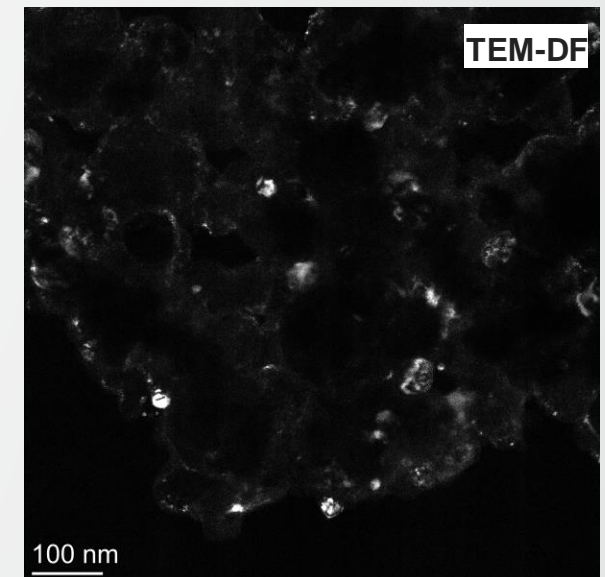
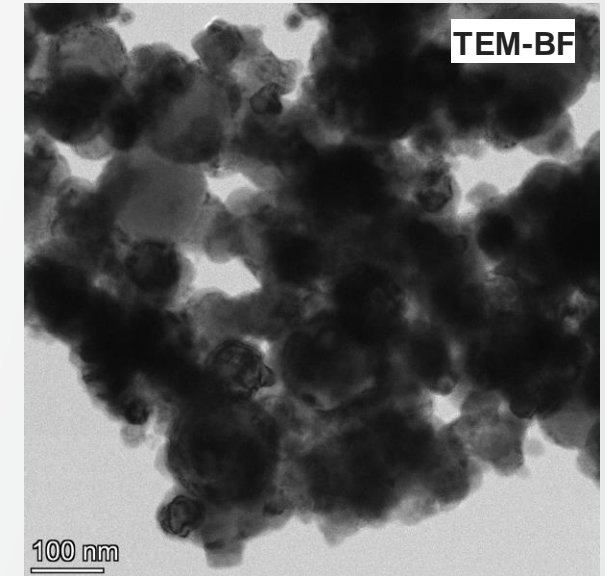
- Standard mode for imaging without the transmitted beam
- Loss of resolution due to higher  $C_s$  at off-axis positions
- Two types of DF-Images: Off-axis and On-axis DF



Off-axis DF



On-axis DF

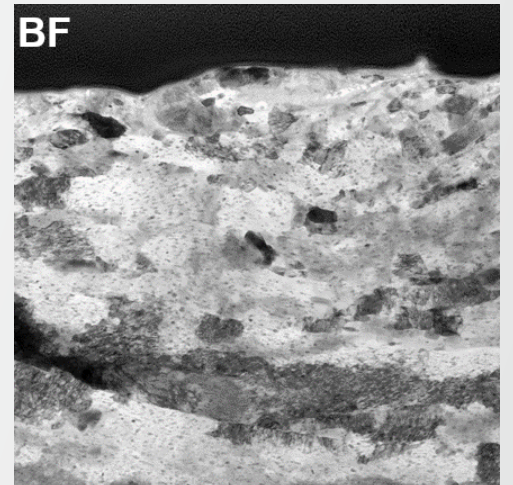
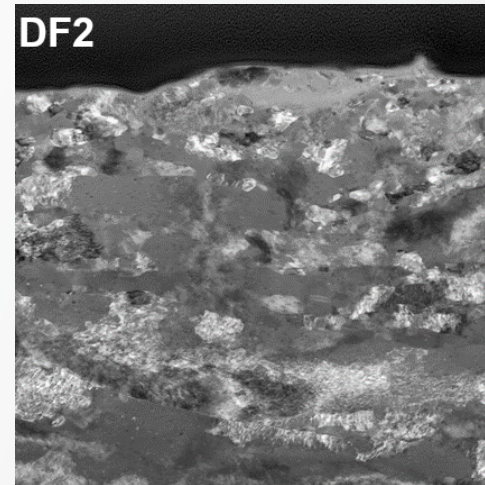
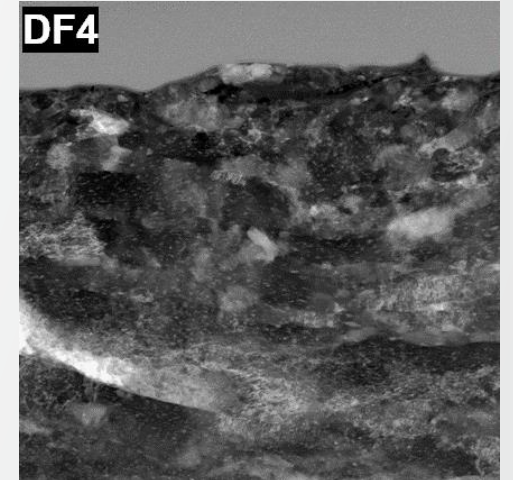
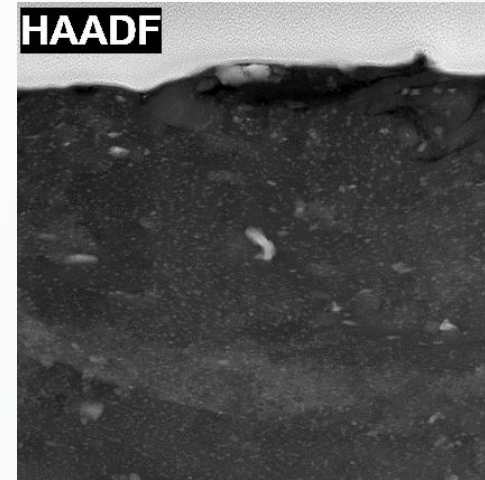
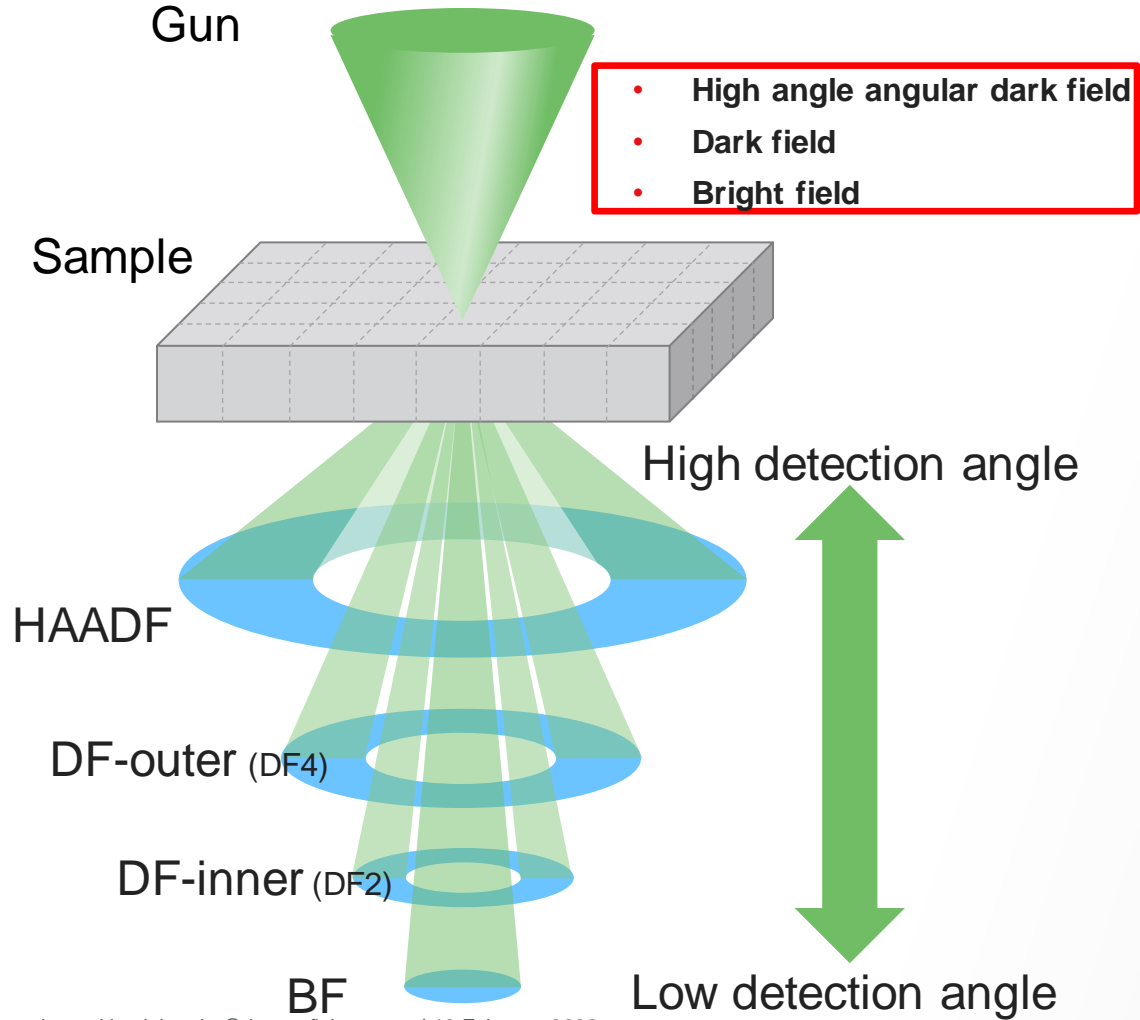




# STEM Imaging

## Image Detectors in STEM

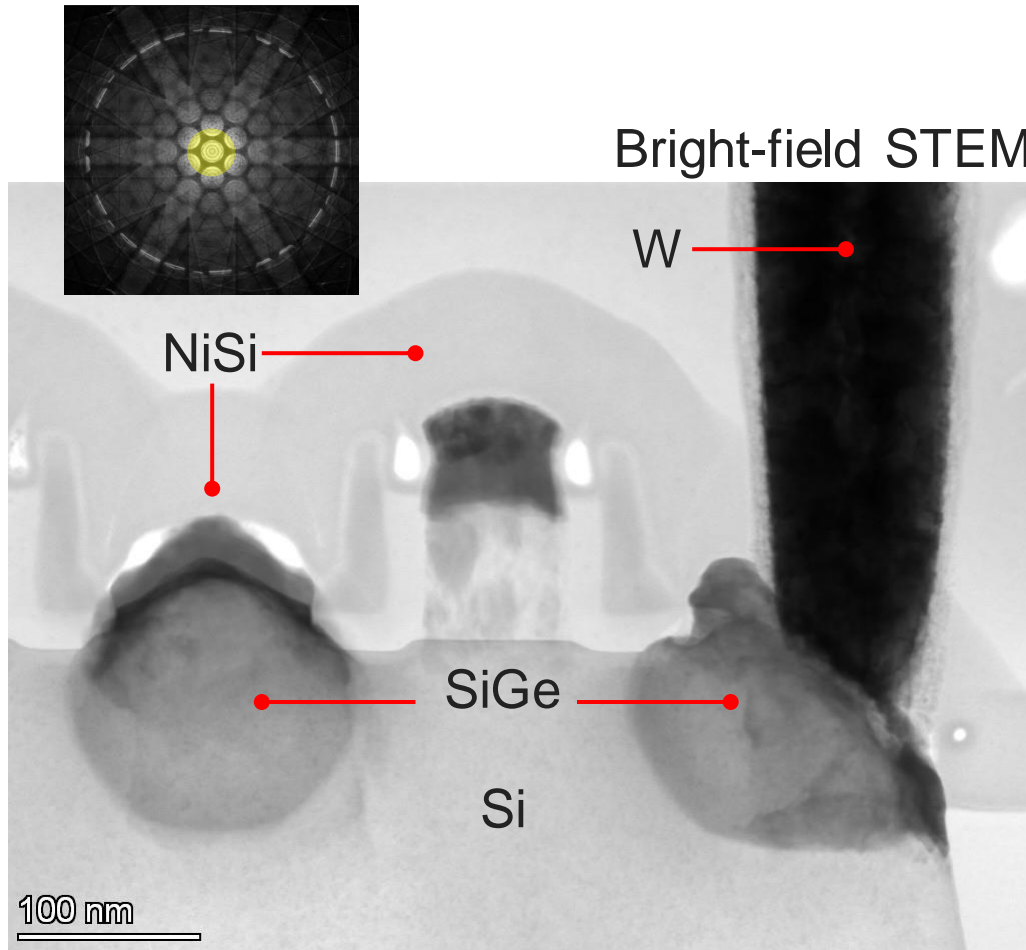
- A range of different contrast and information is available with different detector geometry
- STEM Detectors- Shot-peened Aluminum alloy 7075





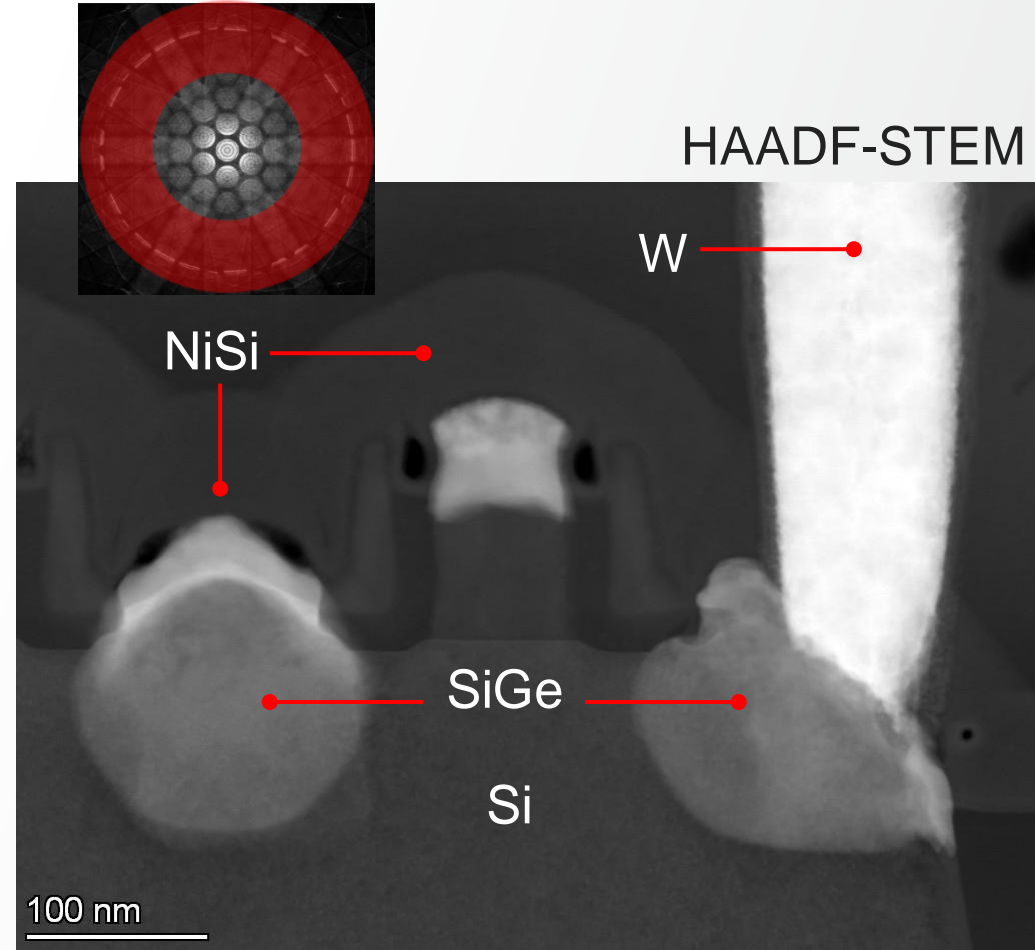
# STEM Imaging

Bright-Field STEM vs (High Angle Angular) Dark-Field Image



**Bright-field STEM**

Heavy regions are dark, thin and vacuum regions are bright



**Dark-field STEM**

Heavy regions are bright, thin regions are dark, vacuum is black

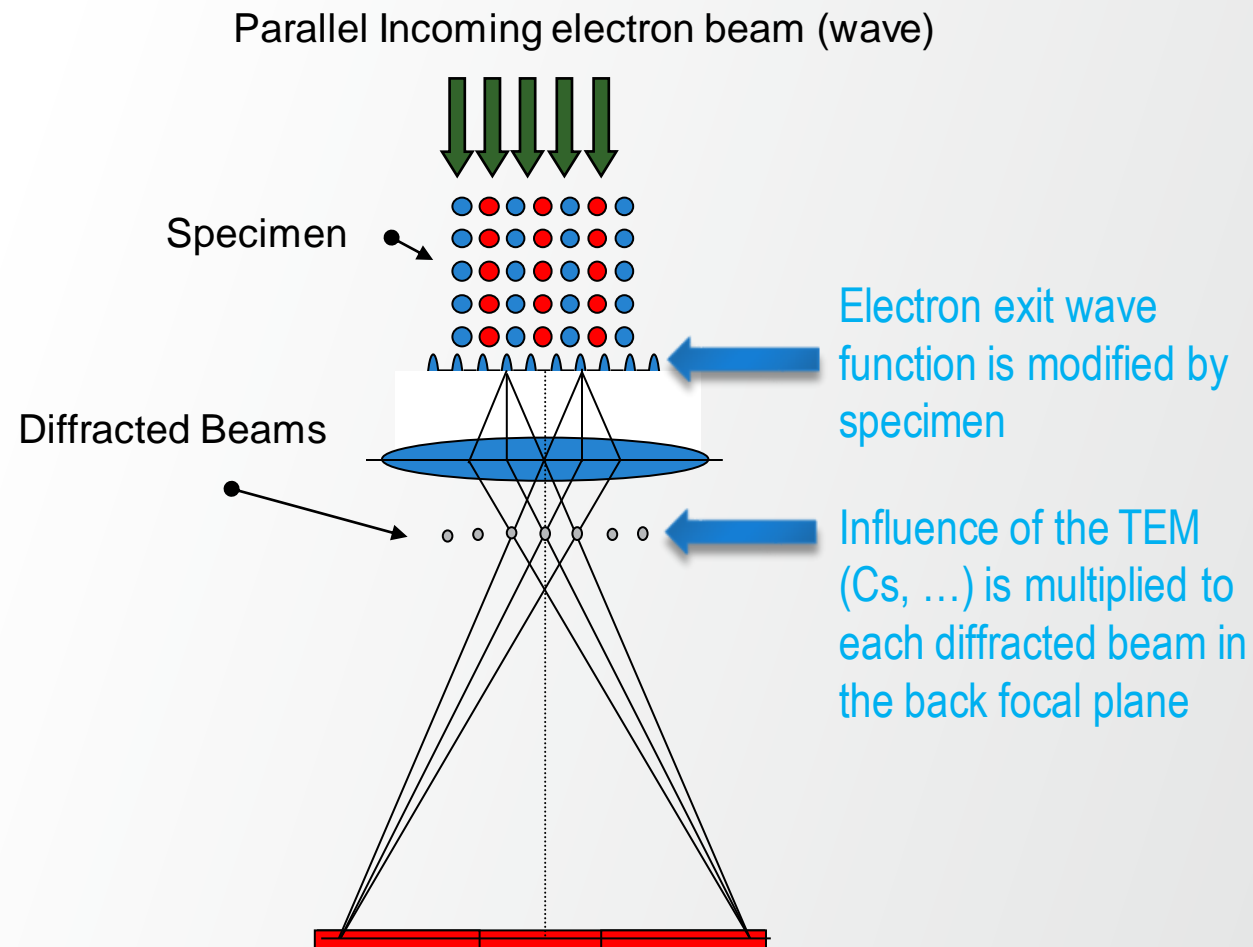
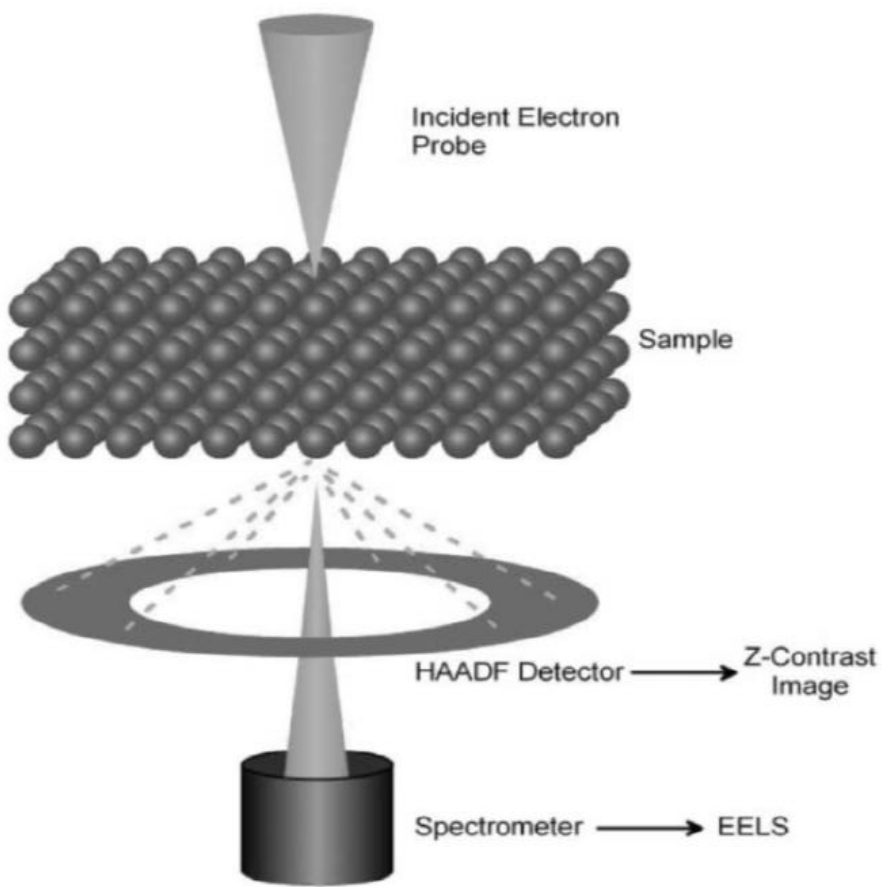
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# High-resolution Scanning TEM (HRSTEM) Imaging

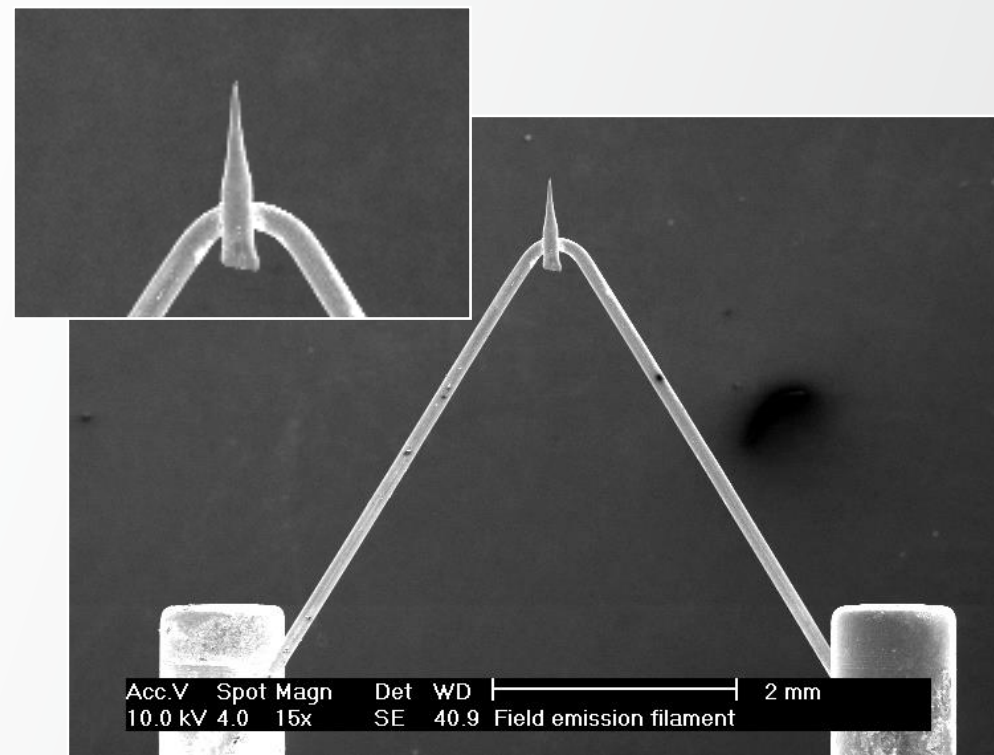
Small Probe for a Better Spatial Resolution



# High-resolution Scanning TEM (HRSTEM) Imaging

## Electron Source – Field Emission Gun

- STEM (HR) needs a bright source of electron:
  - The source must be demagnified to produce a very small focused electron probe; intensity or beam current is lost in the process.
- The only source which can be used is a field emission source:
  - A very sharp tungsten point which only operates in an ultrahigh vacuum environment ( $10^{-6}$  -  $10^{-8}$  Pa).
  - Very long lifetimes: a couple or more years continuously.

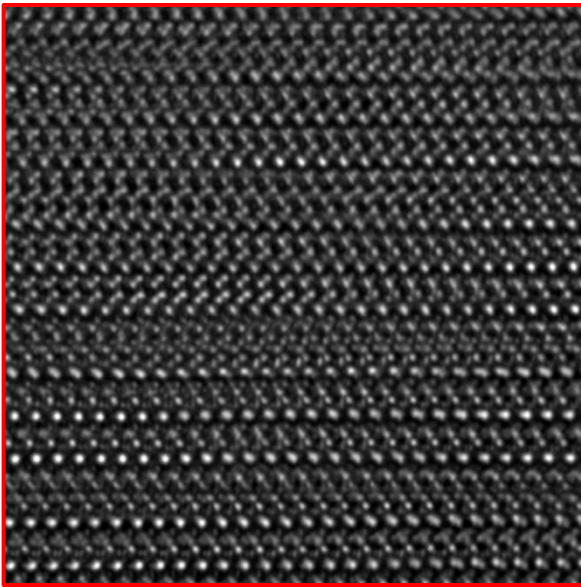




# High-resolution Scanning TEM (HRSTEM) Imaging

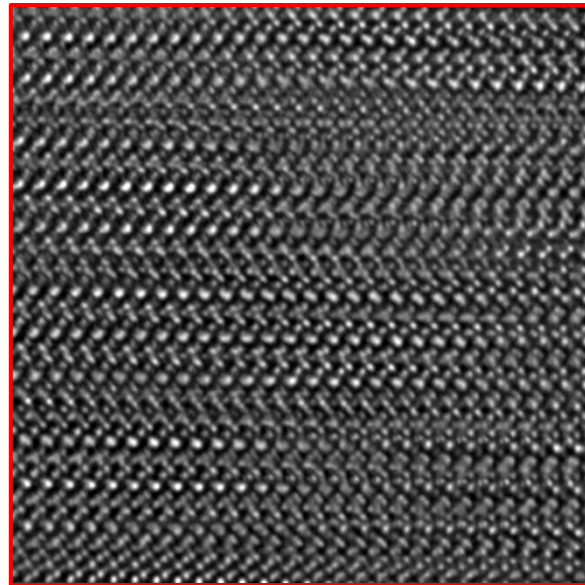
Less Defocus Dependent

Defocus 0 nm

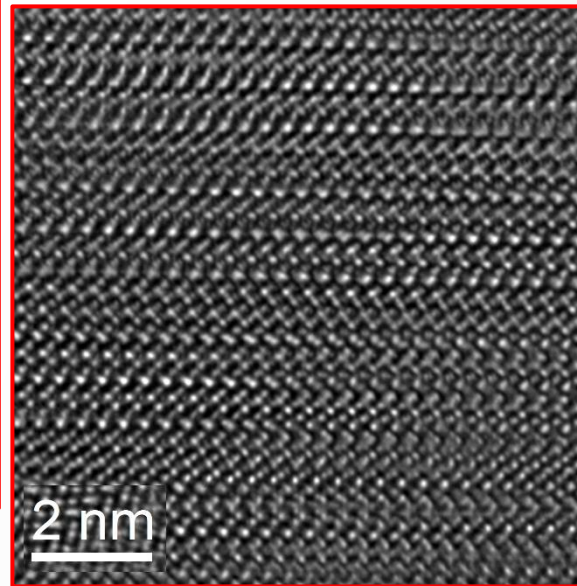


HR-TEM

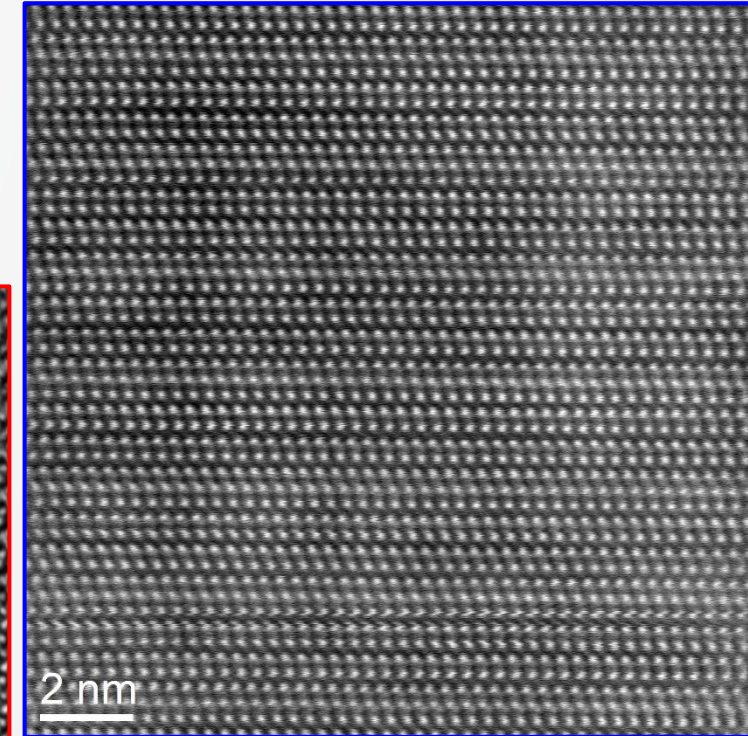
Defocus 1 nm



Defocus 2 nm

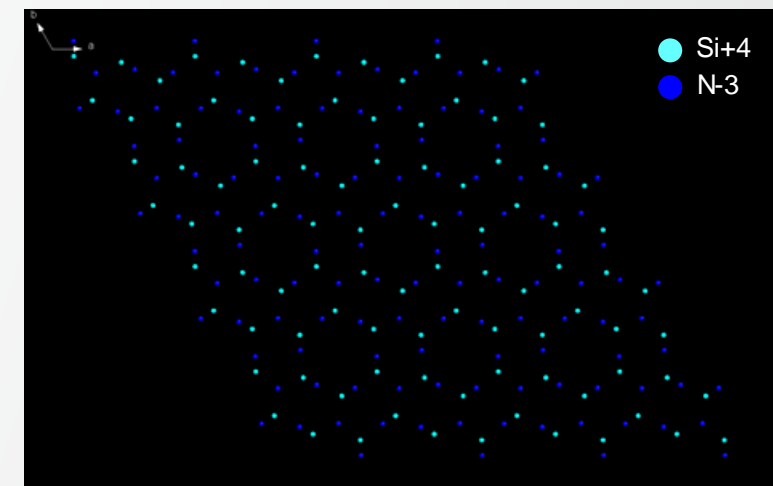
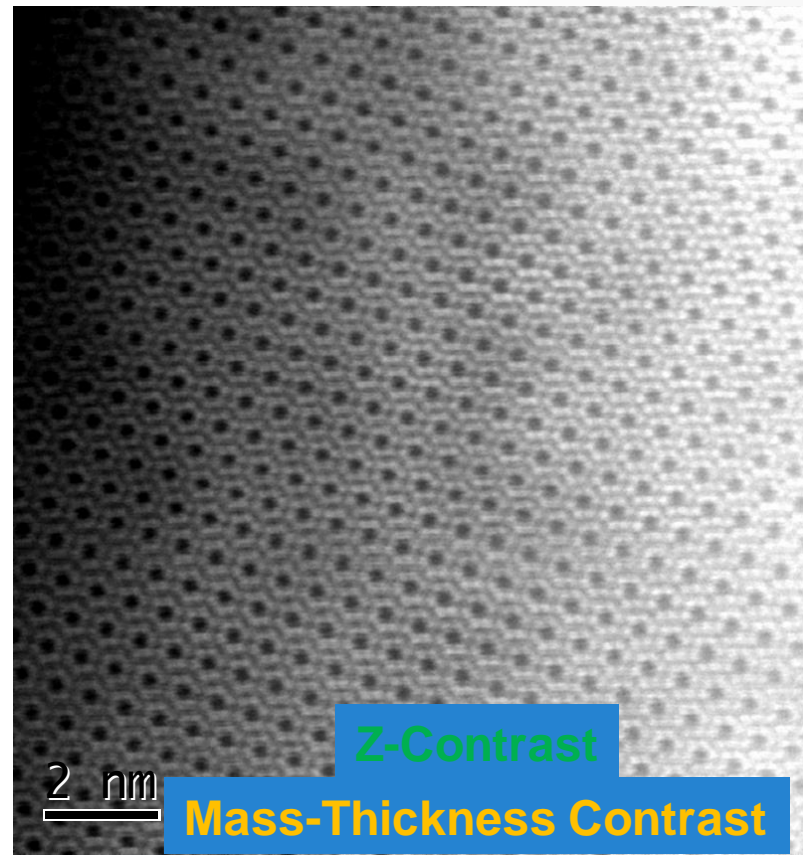
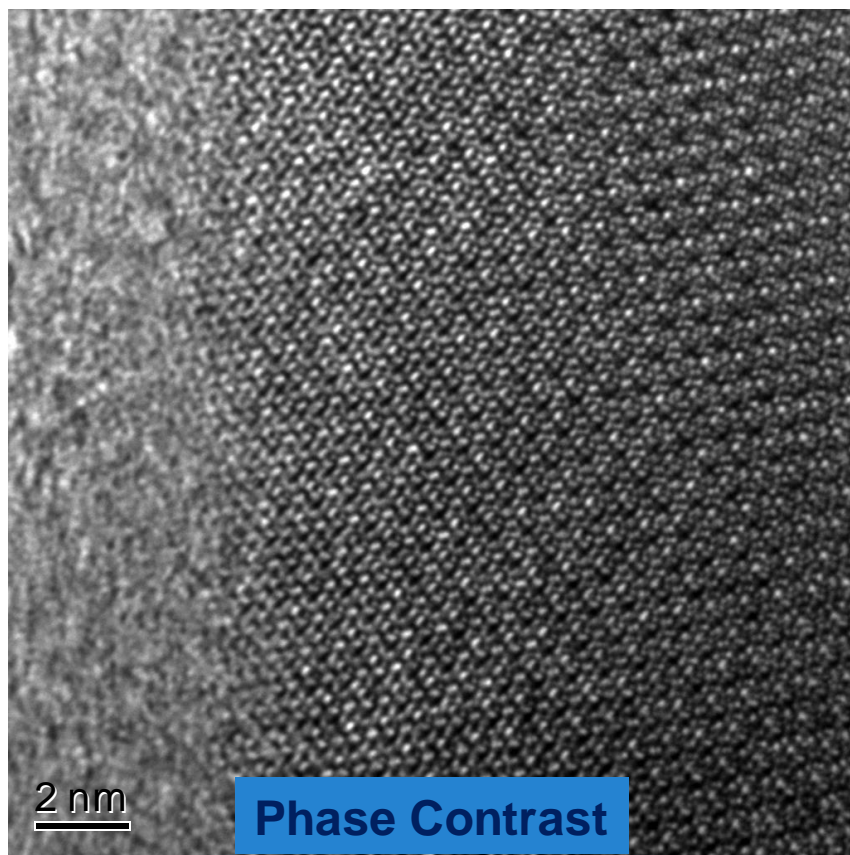


HR-STEM  $-20 < \text{Defocus} < 20$  nm



# High-resolution Scanning TEM (HRSTEM) Imaging

HRTEM vs HRSTEM Imaging



$\text{Si}_3\text{N}_4$  structure model

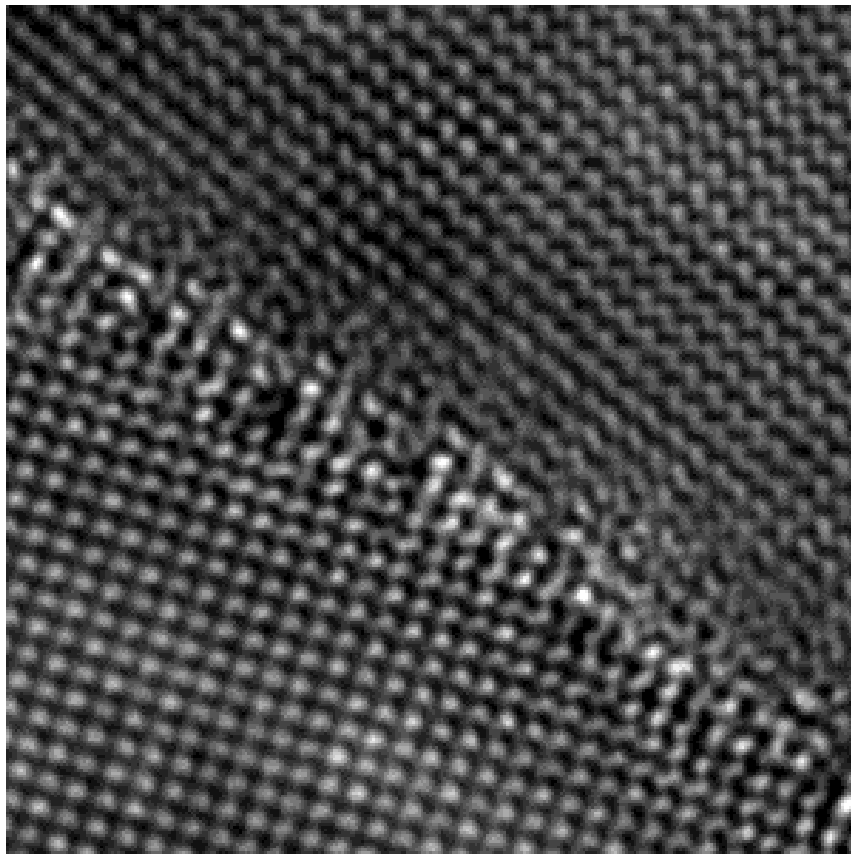
HRTEM and HR-STEM images showing the atomic structure of  $\text{Si}_3\text{Ni}_4$



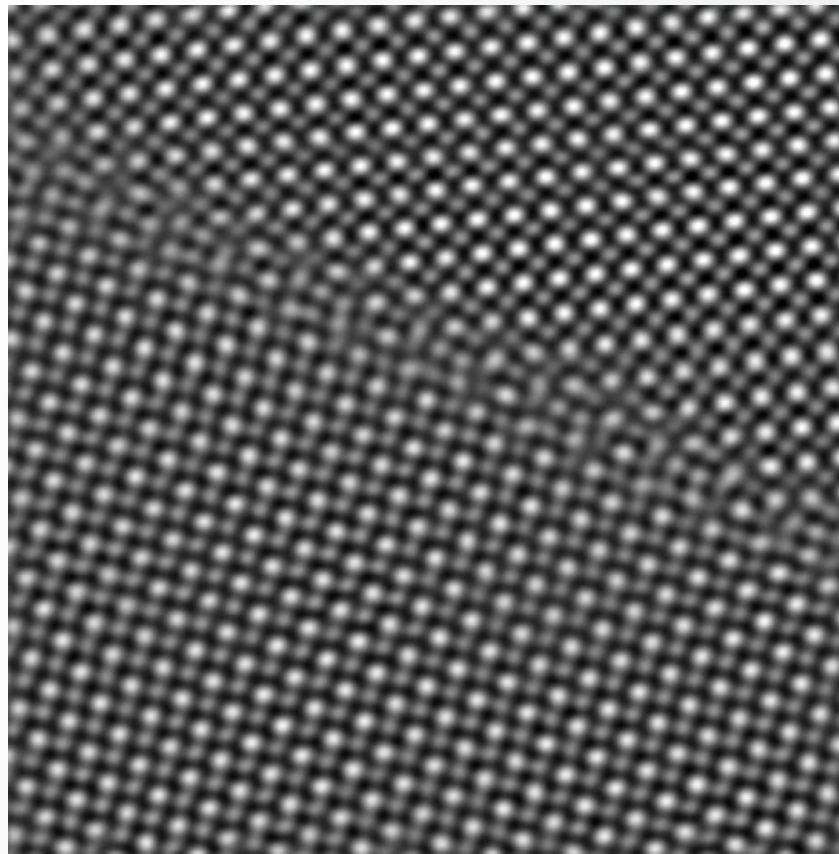
# High-resolution Scanning TEM (HRSTEM) Imaging

HRTEM and HRSTEM images from the same  $\text{SrTiO}_3$  bi-crystal boundary

HRTEM



HRSTEM

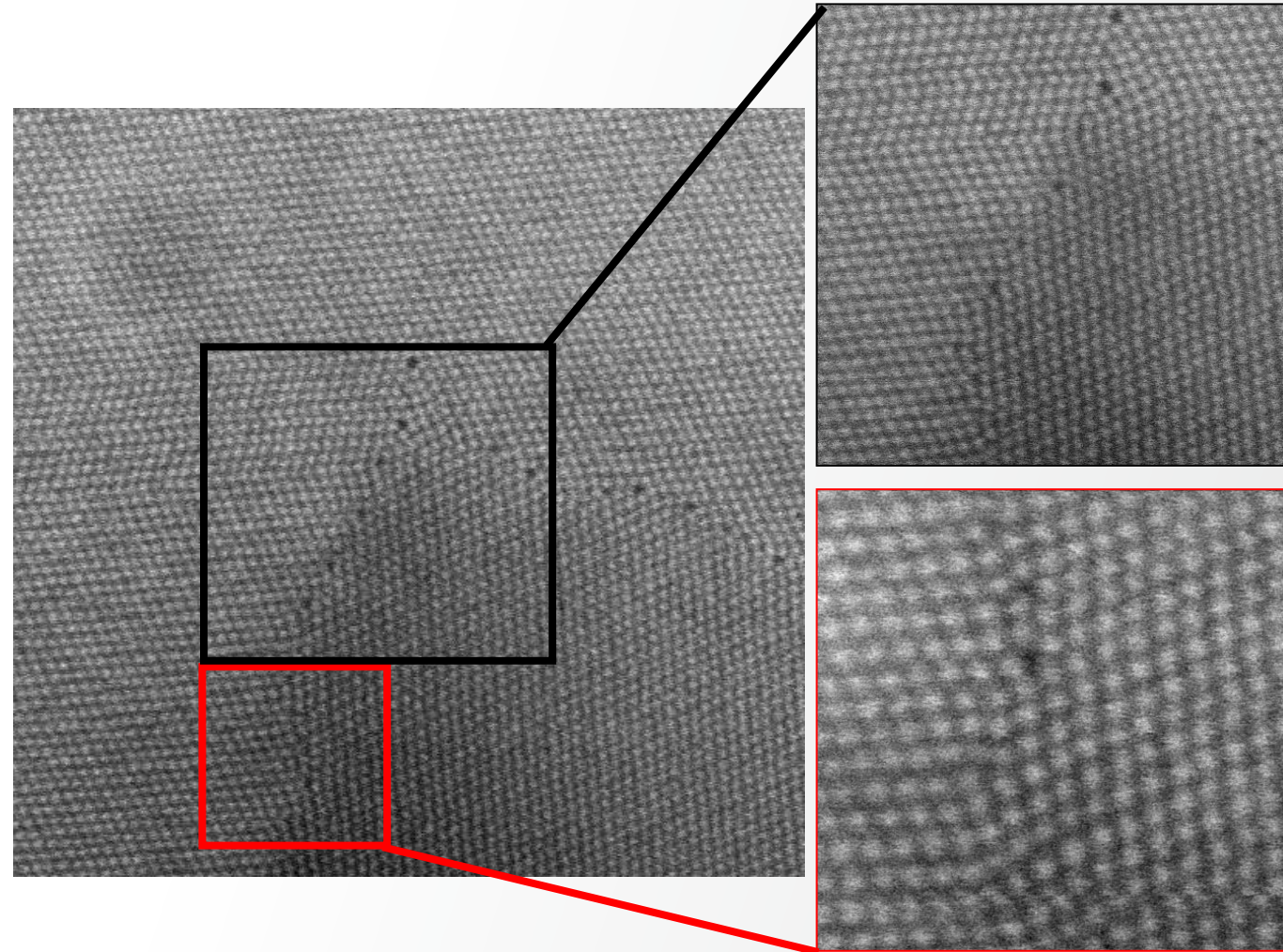




# High-resolution Scanning TEM (HRSTEM) Imaging

## Point Defects investigation

High Resolution ADF  
STEM image of a  
triple-junction in Au  
poly-crystal.  
Numerous voids  
occur at defects at  
the interface.



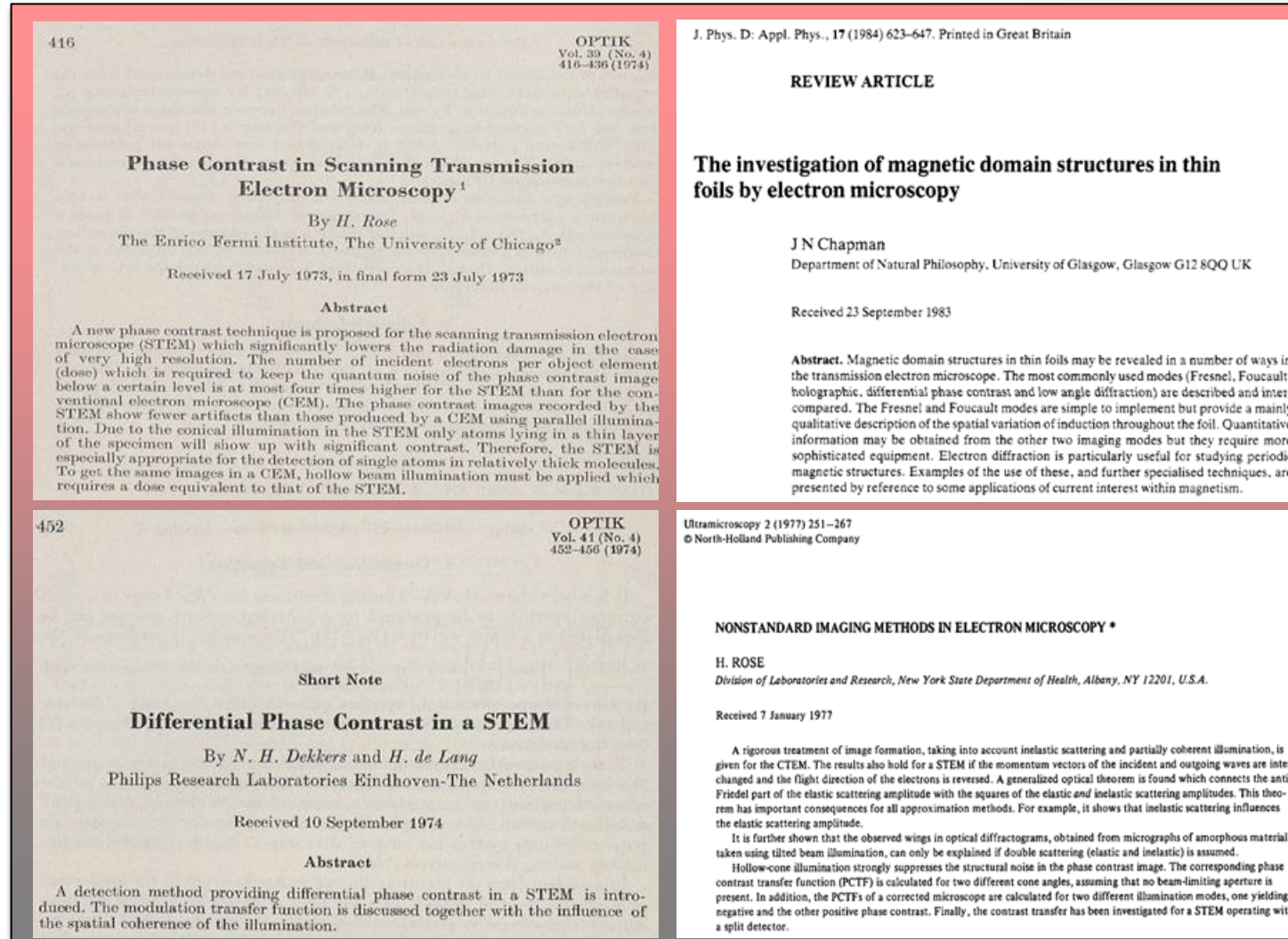
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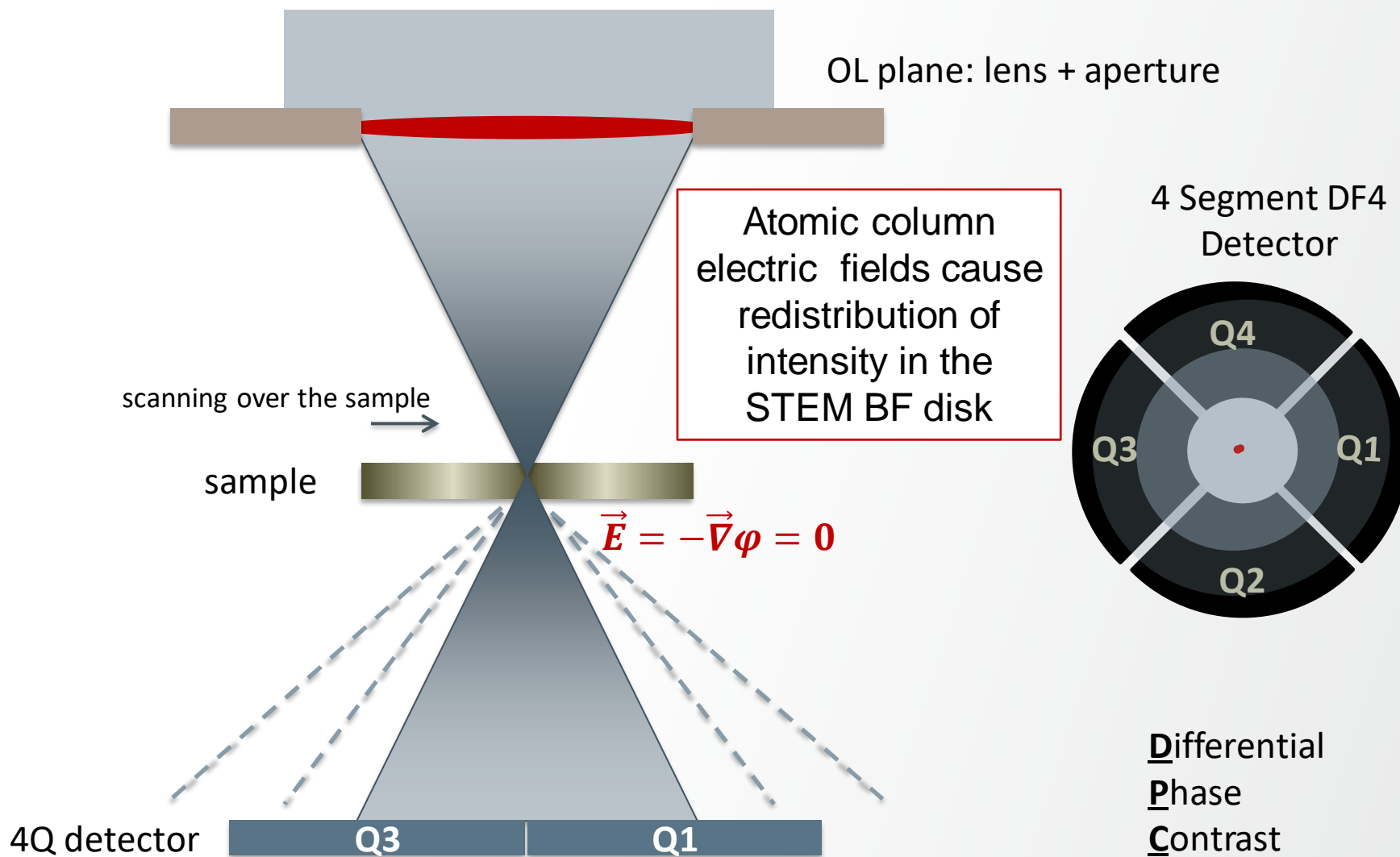
# Differential Phase Contrast Imaging (DPC)

## An Old Non-Standard Imaging Methods in Electron Microscopy



# Differential Phase Contrast Imaging (DPC)

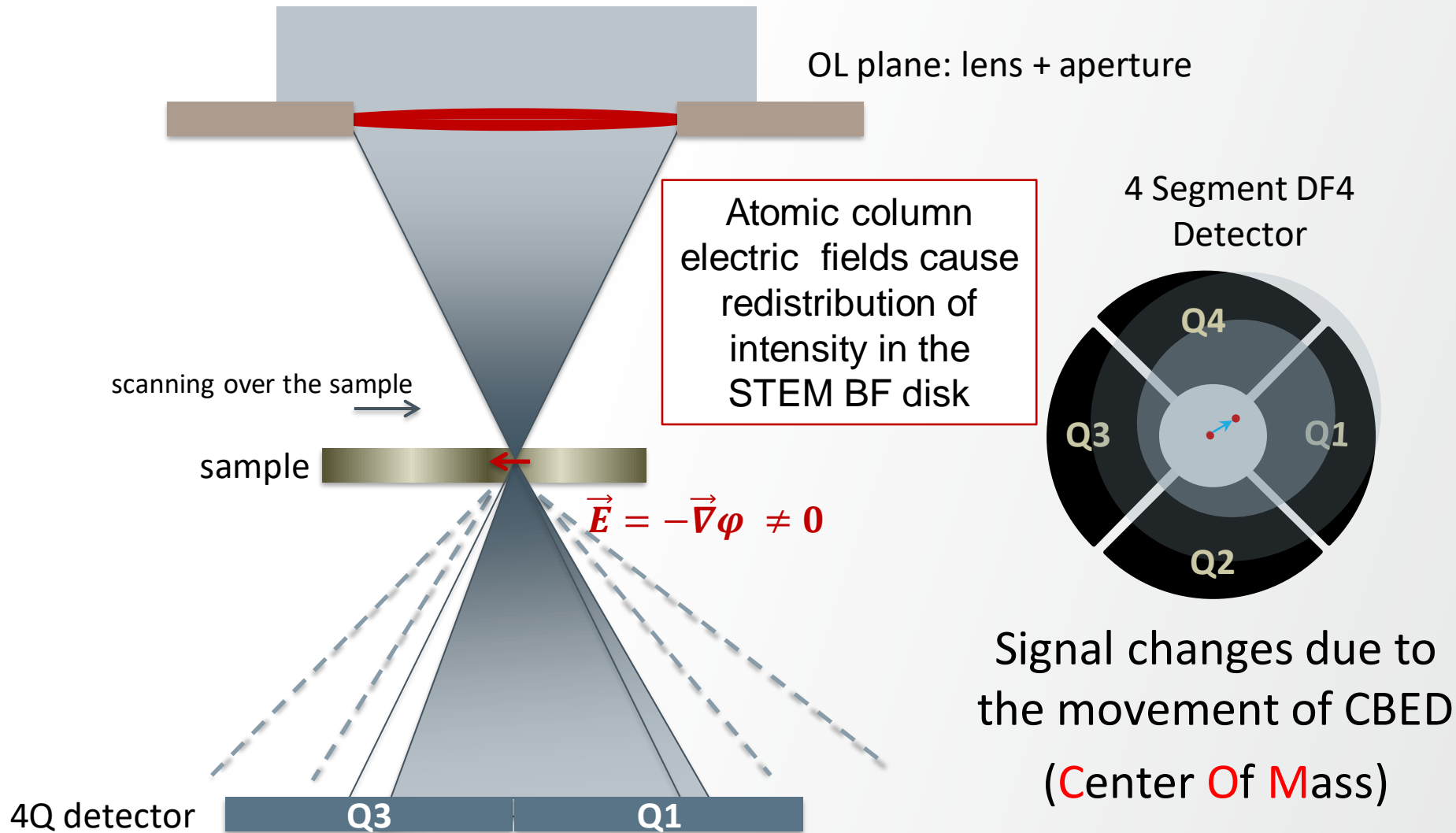
## DPC Detector Configuration





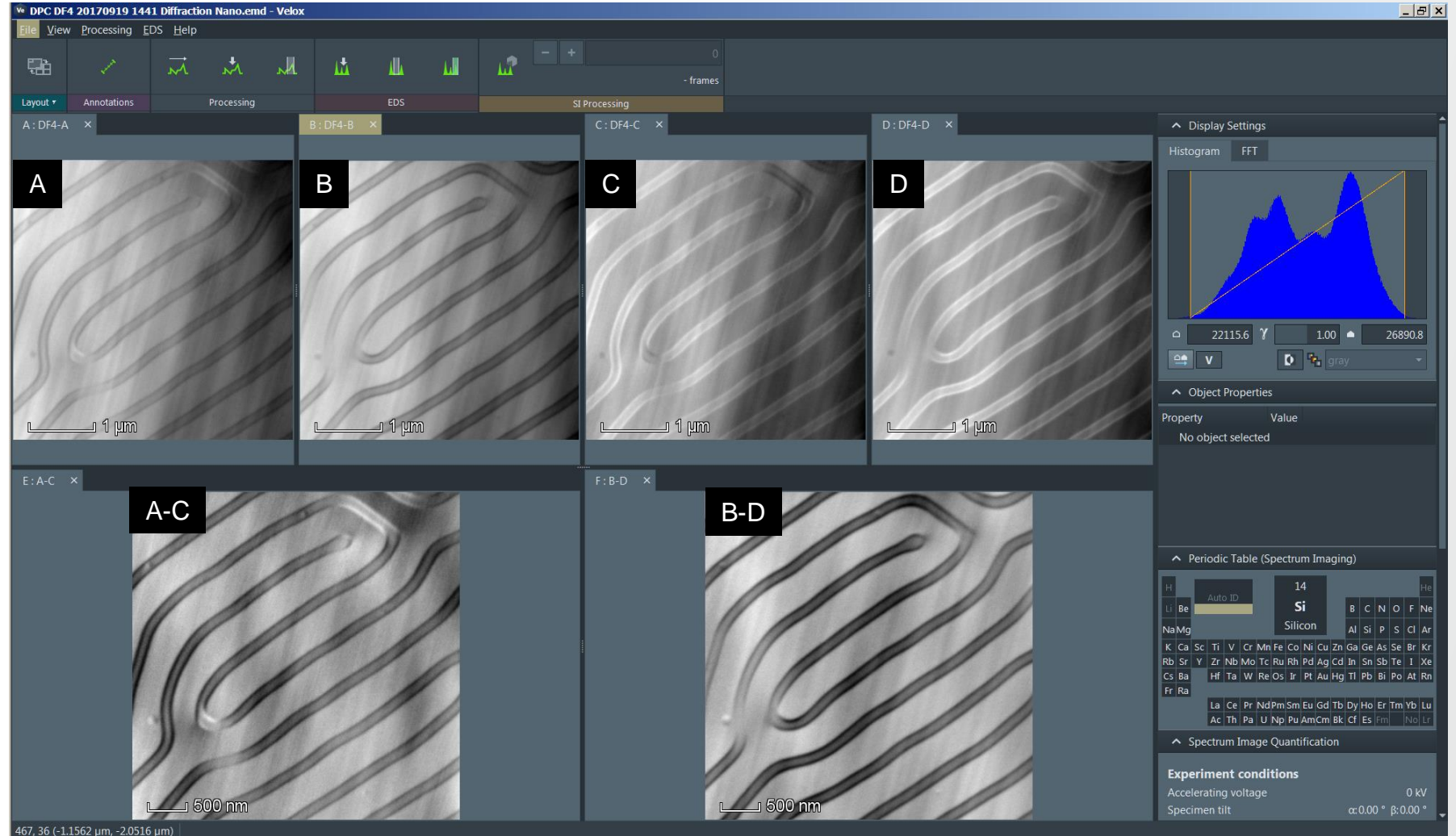
# Differential Phase Contrast Imaging (DPC)

## DPC Detector Configuration



# Differential Phase Contrast Imaging (DPC)

## DPC Imaging of Ferrite

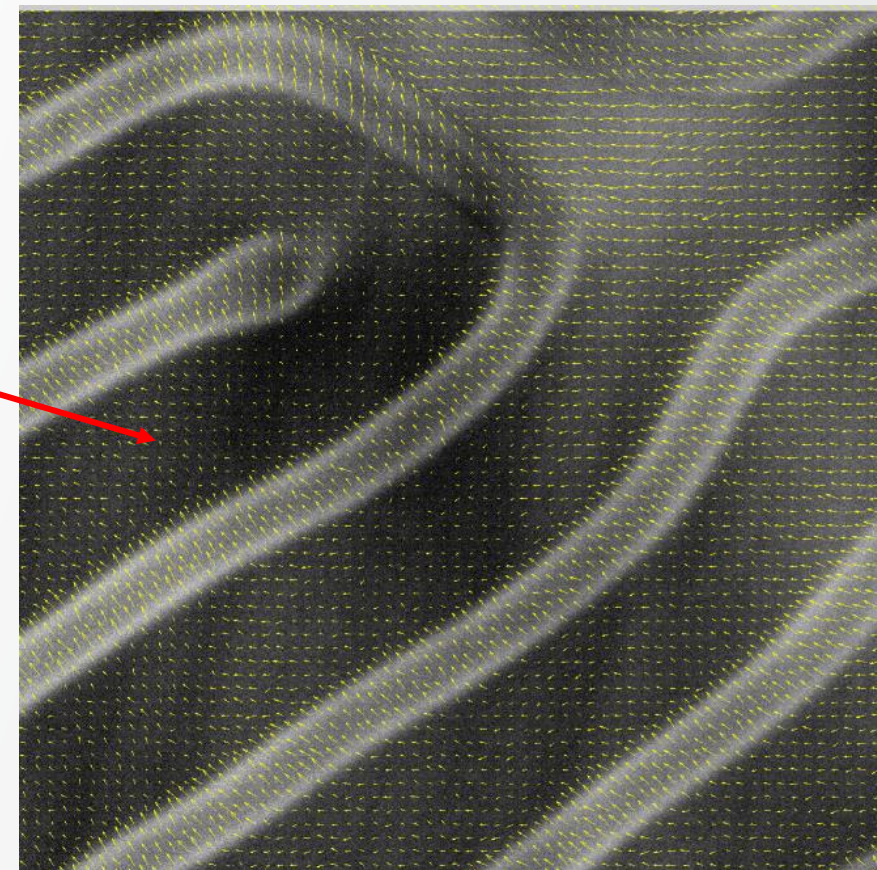
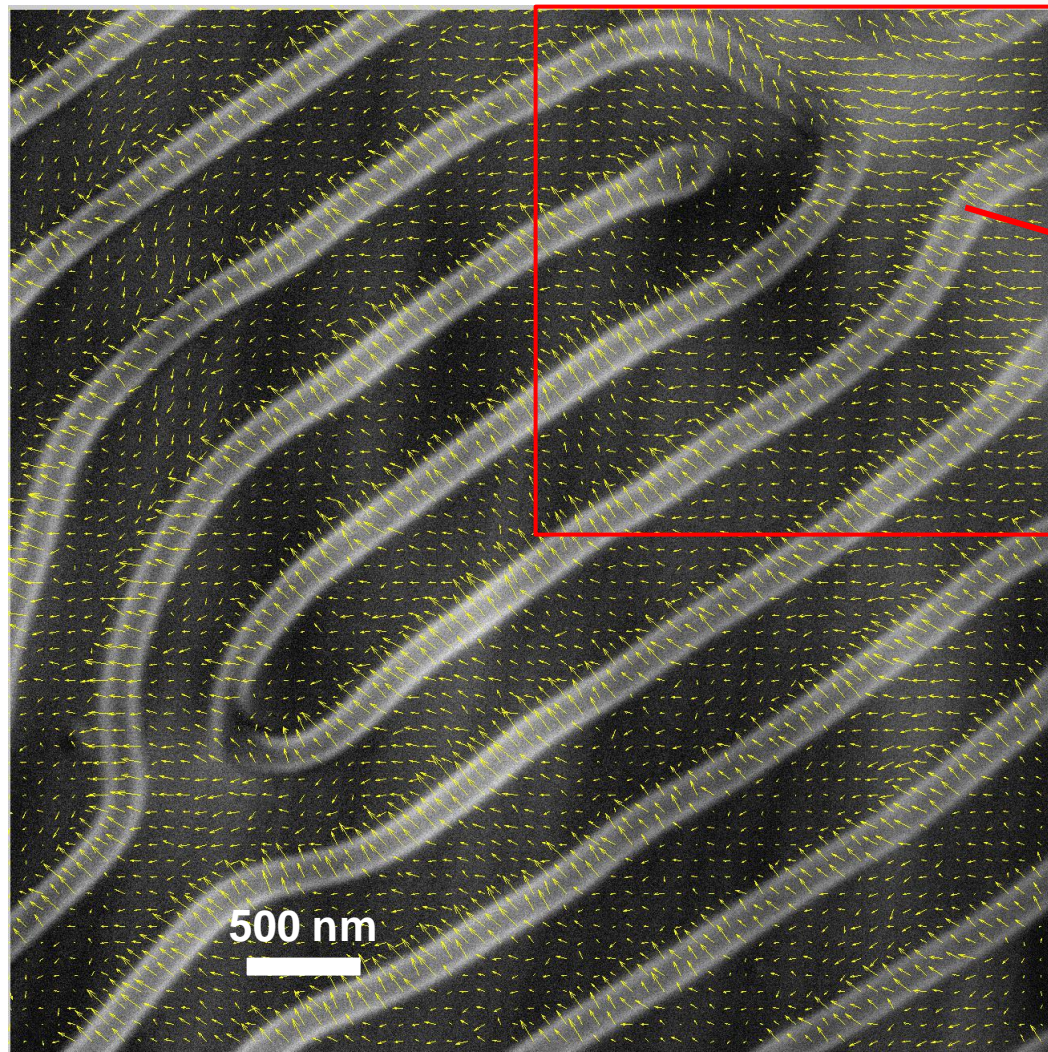


Sample Courtesy: H. Nakajima and S. Mori, Osaka Prefecture University

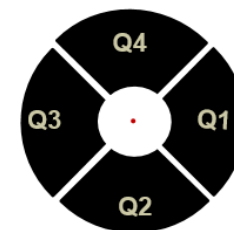


# Differential Phase Contrast Imaging (DPC)

DPC of Ferrite Magnetic Field



4 Segment DF4 Detector

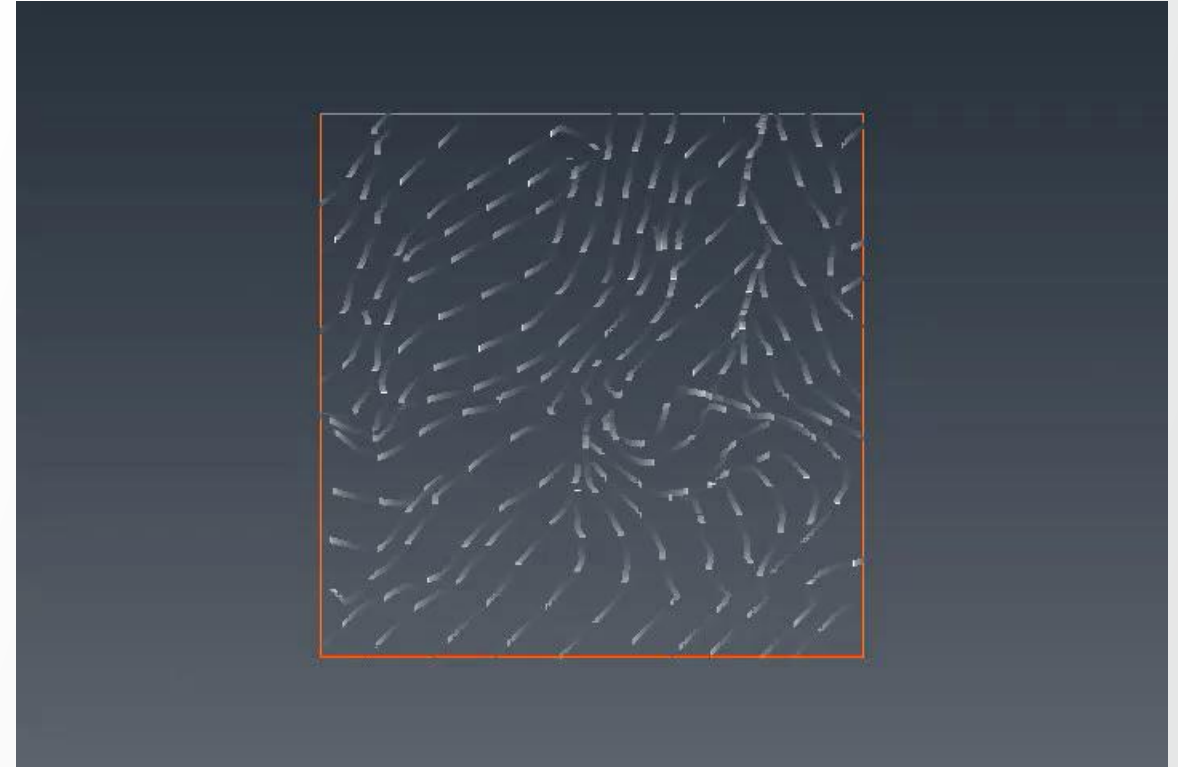
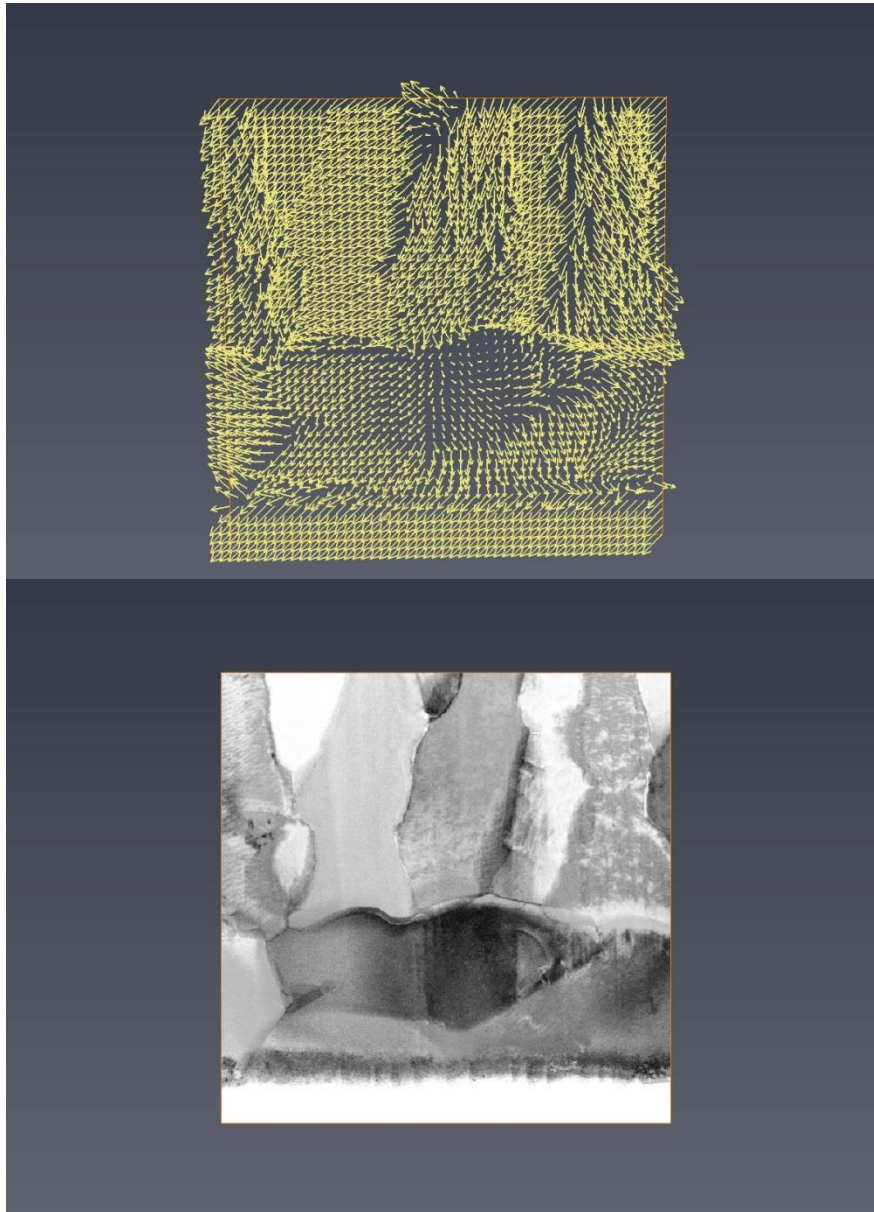


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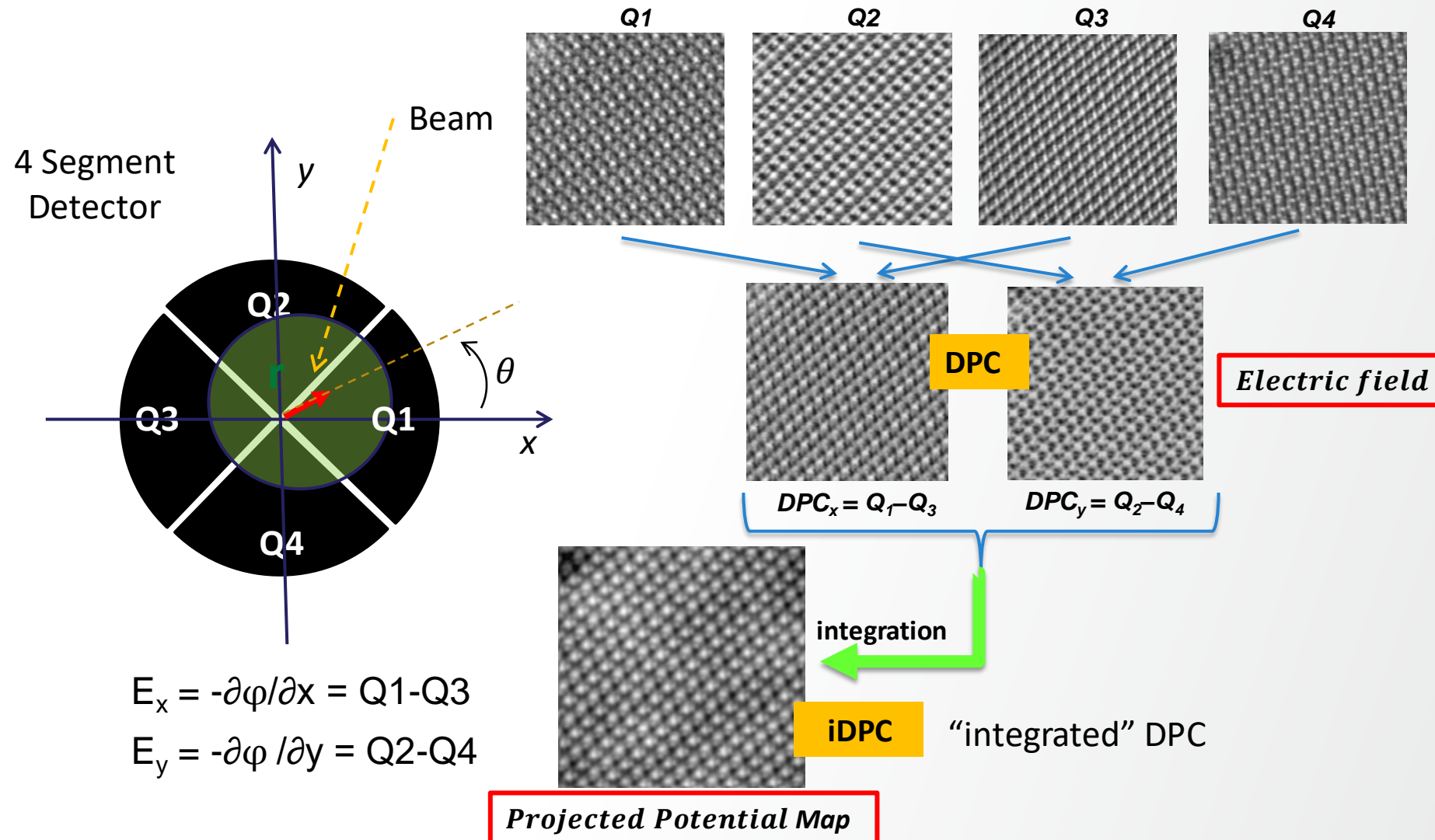
# Differential Phase Contrast Imaging (DPC)

Magnetic Field of Fe<sub>2</sub>O<sub>3</sub> Thin Film



# Integrated Differential Phase Contrast Imaging (iDPC)

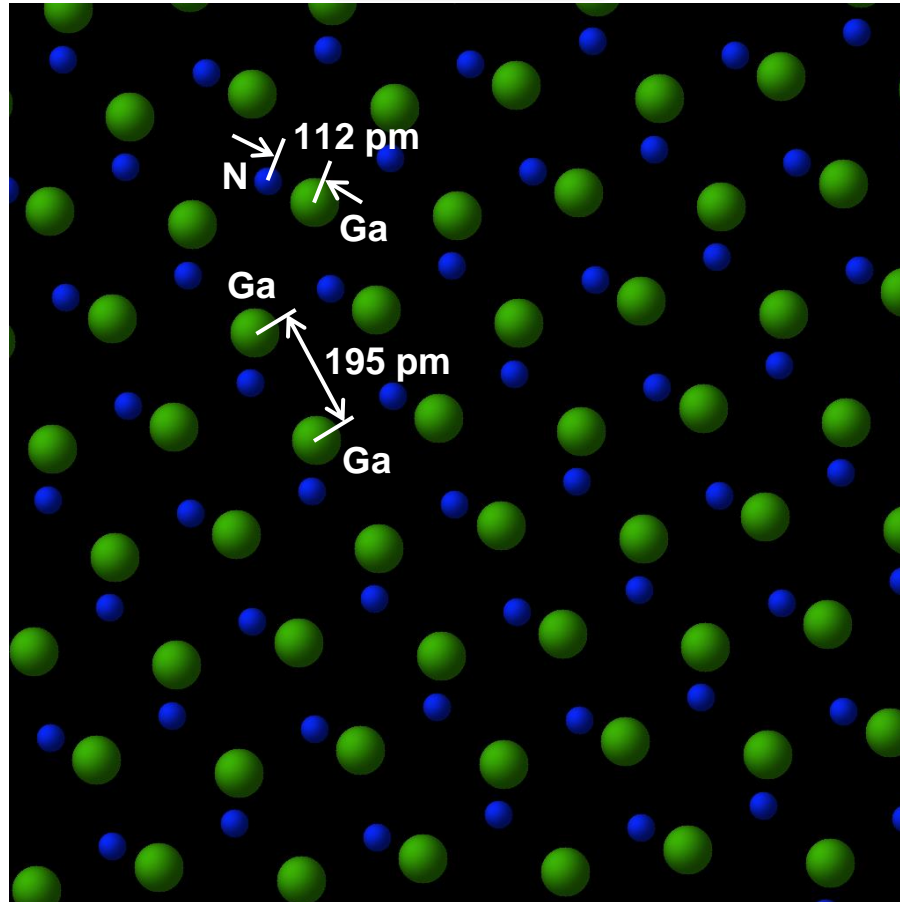
GaN 1120 Experimental DPC and iDPC images – Light Element Imaging with iDPC STEM



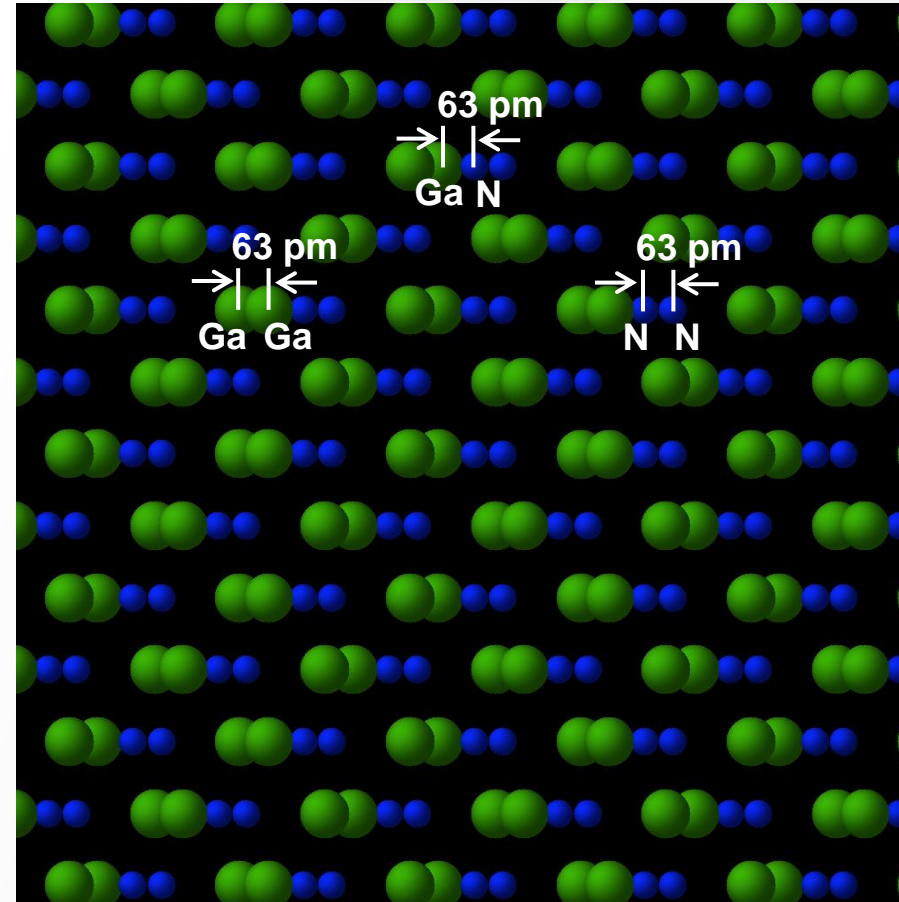
# Integrated Differential Phase Contrast Imaging (iDPC)

## GaN iDPC – Imaging

GaN [110]



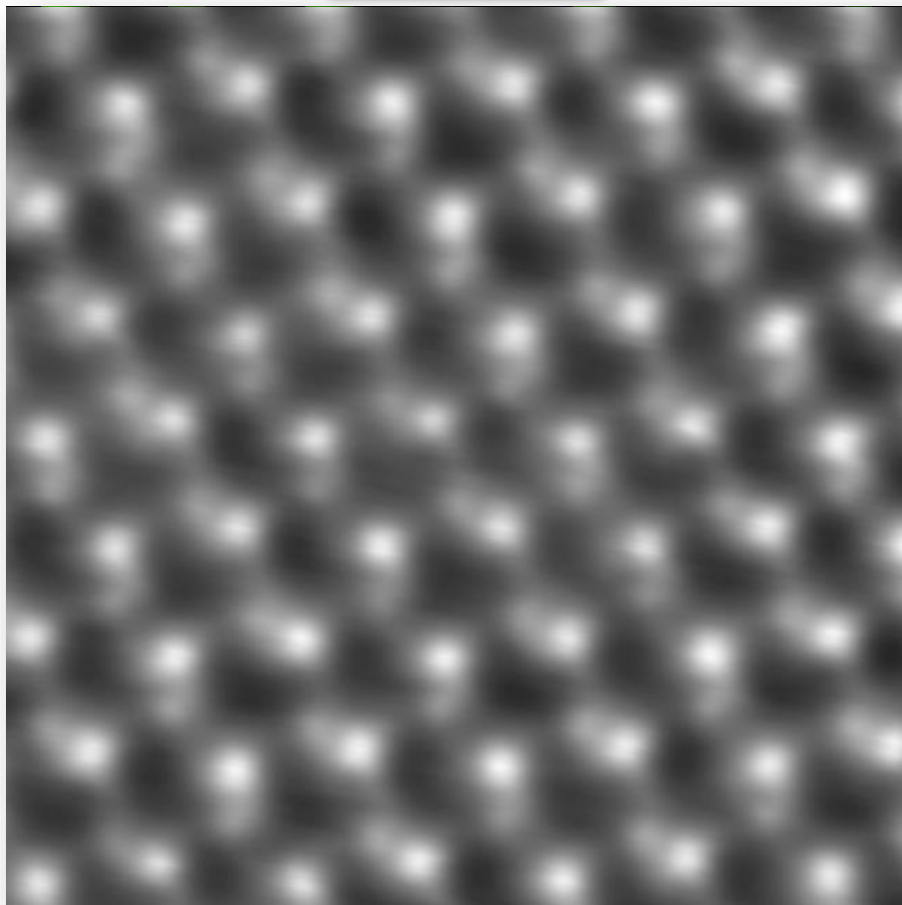
GaN [211]



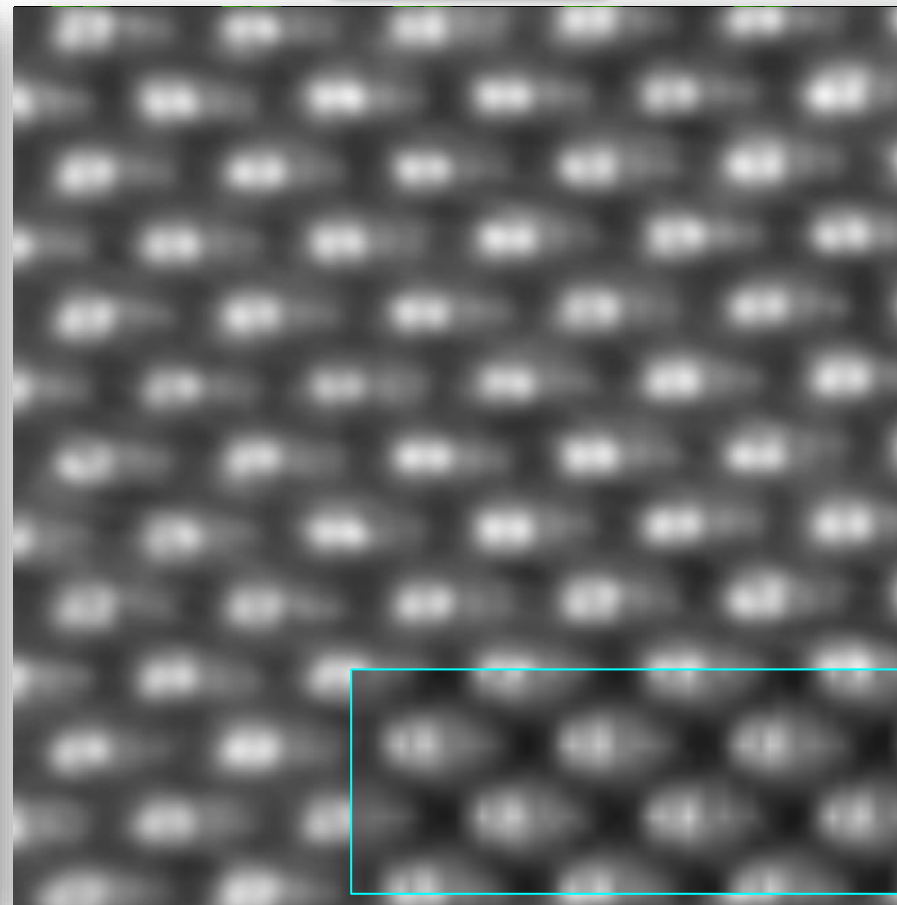
# Integrated Differential Phase Contrast Imaging (iDPC)

GaN iDPC – Imaging

GaN [110]



GaN [211]

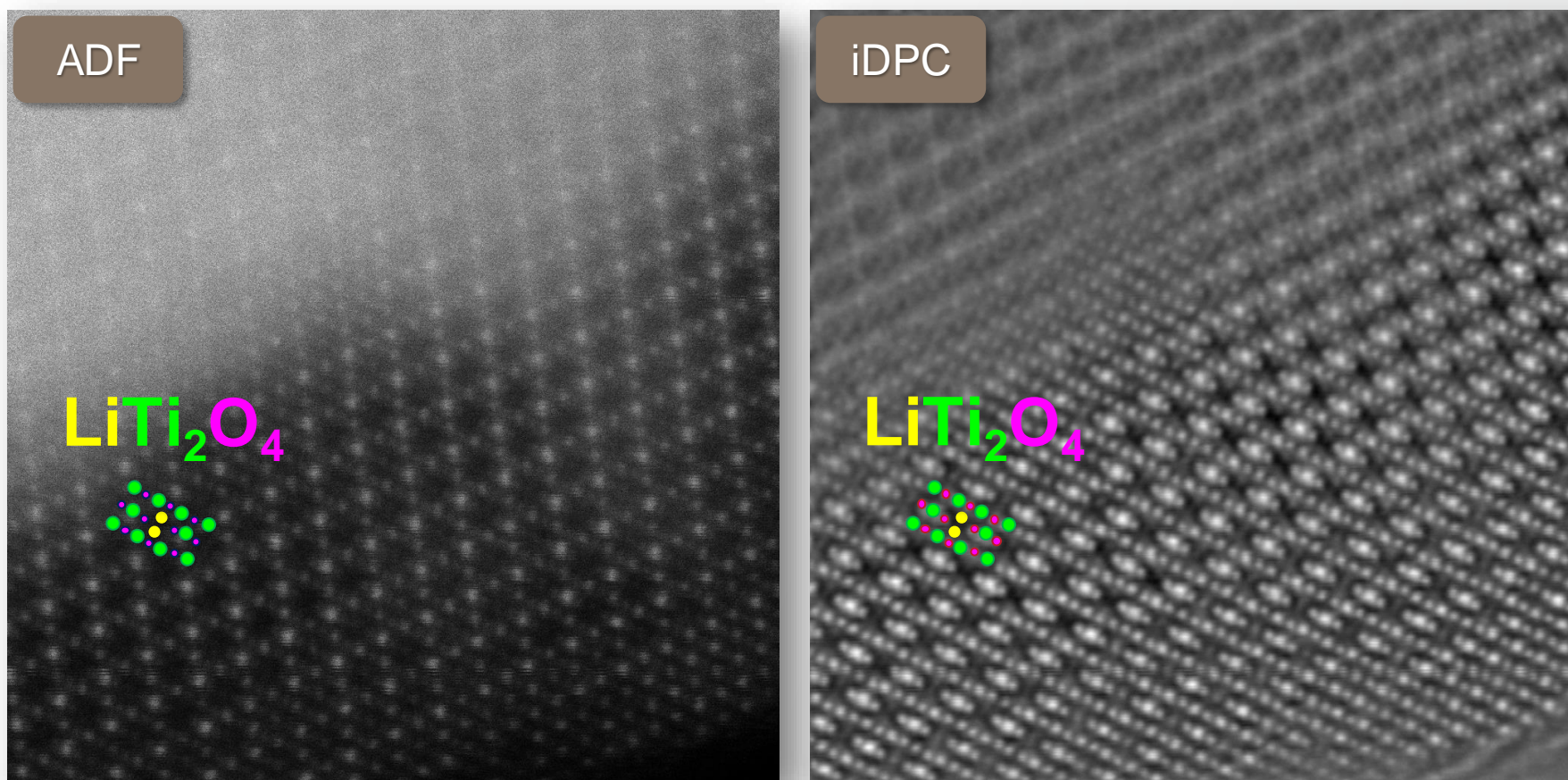


*Images and Simulation: Emrah Yücelen*



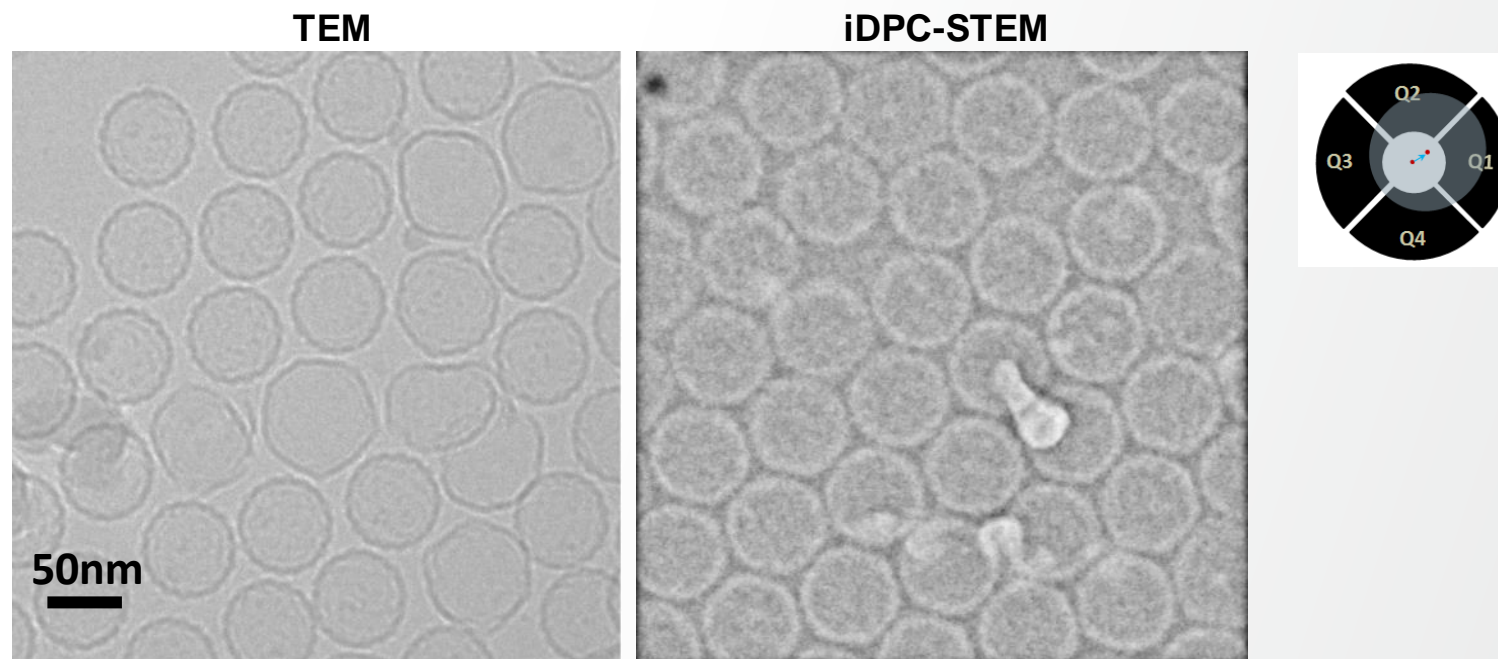
# Integrated Differential Phase Contrast Imaging (iDPC)

iDPC for Light Element Imaging



# Integrated Differential Phase Contrast Imaging (iDPC)

Low-Dose Cryo Imaging with iDPC – Virus Imaging on TEM and iDPC-STEM



Both images with dose  $\sim 100 \text{ e}/\text{\AA}^2$

Courtesy: K. Sader, B. Buijsse

Phase Contrast STEM for thin samples: Integrated differential Phase Contrast

Ivan Lazić, Eric Bosh, and Sorin Lazar, *Ultramicroscopy* 160 (216) 265-280

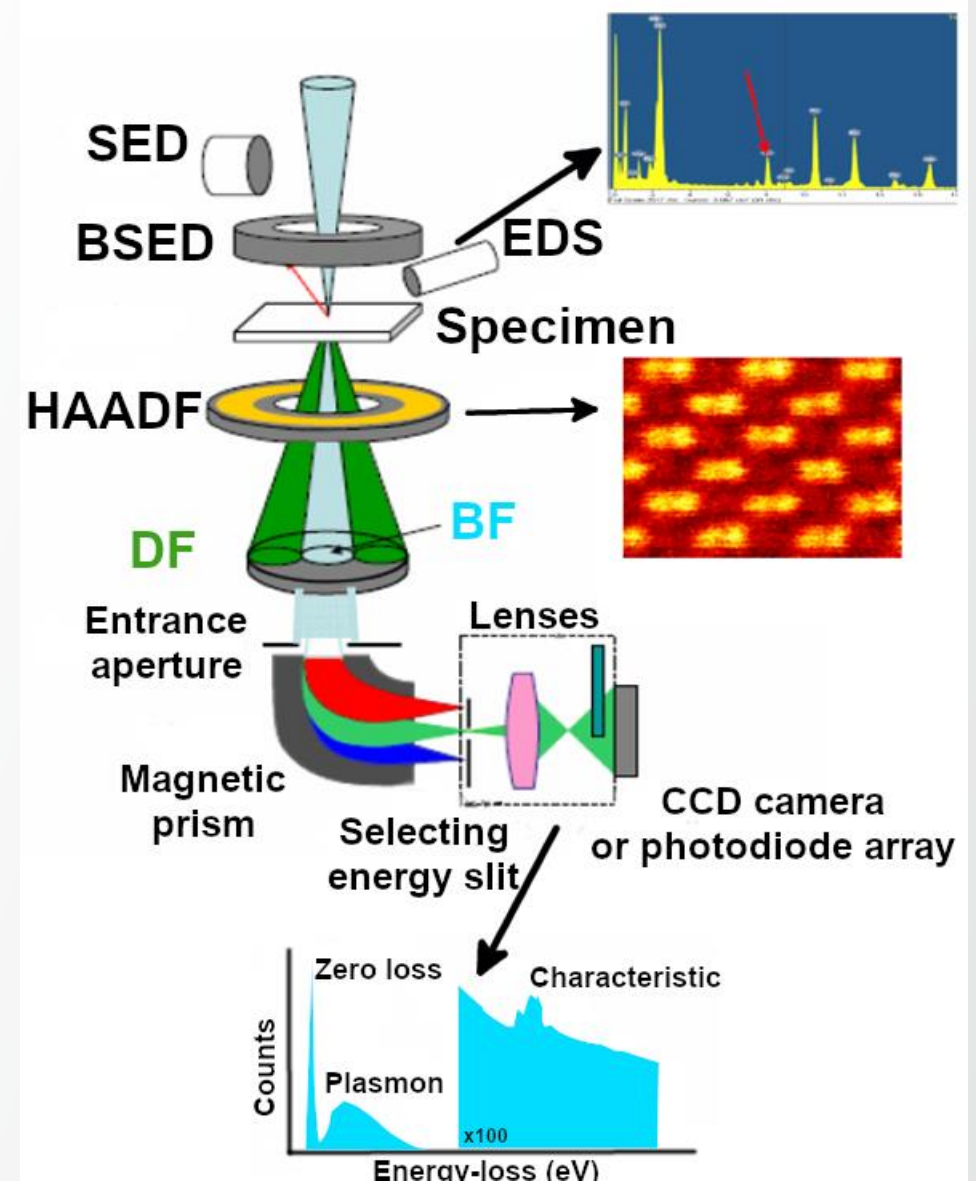
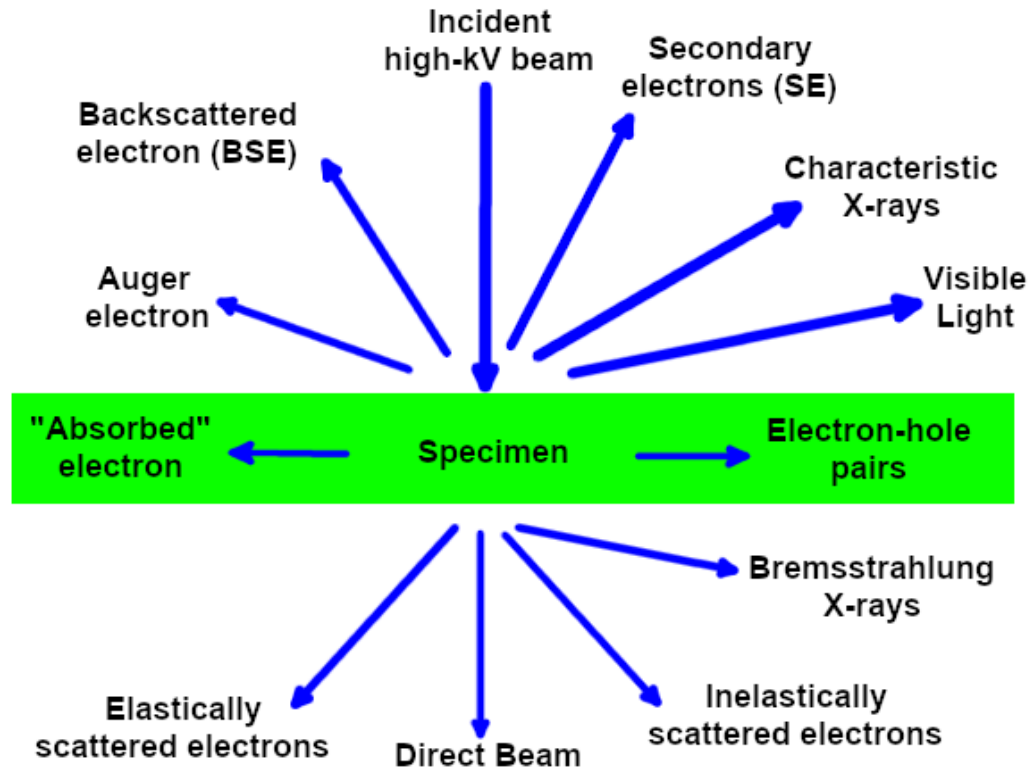
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# Spectroscopy in TEM

Signal Produced during Electron – Sample Interactions

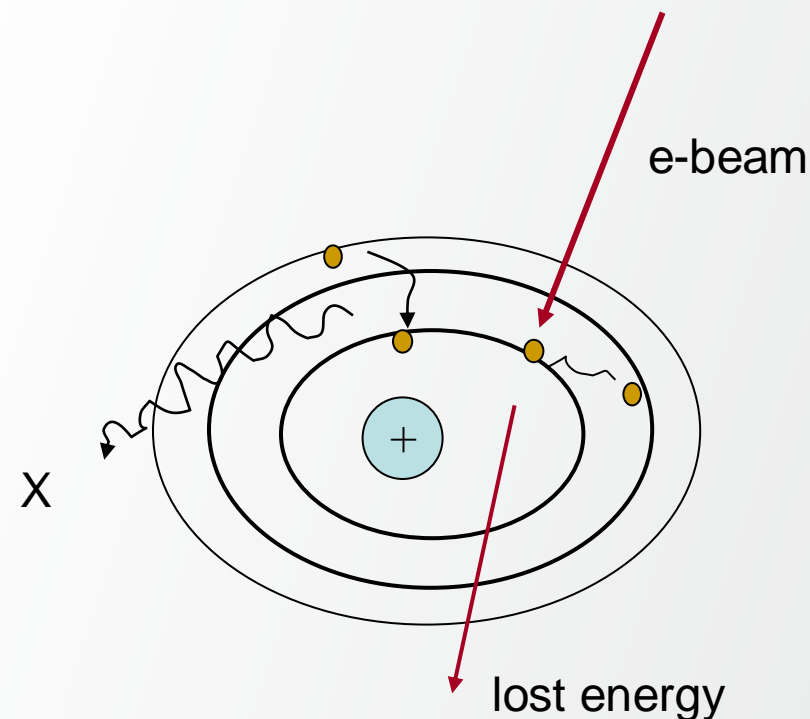




# Spectroscopy in TEM

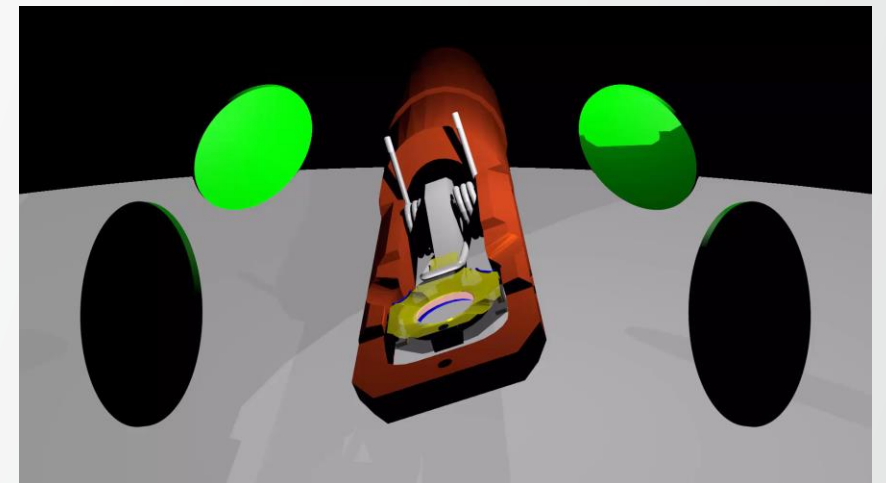
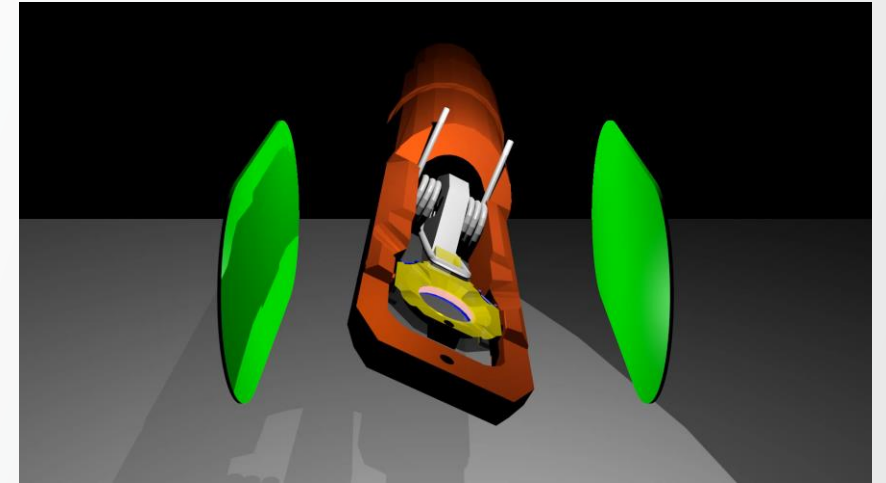
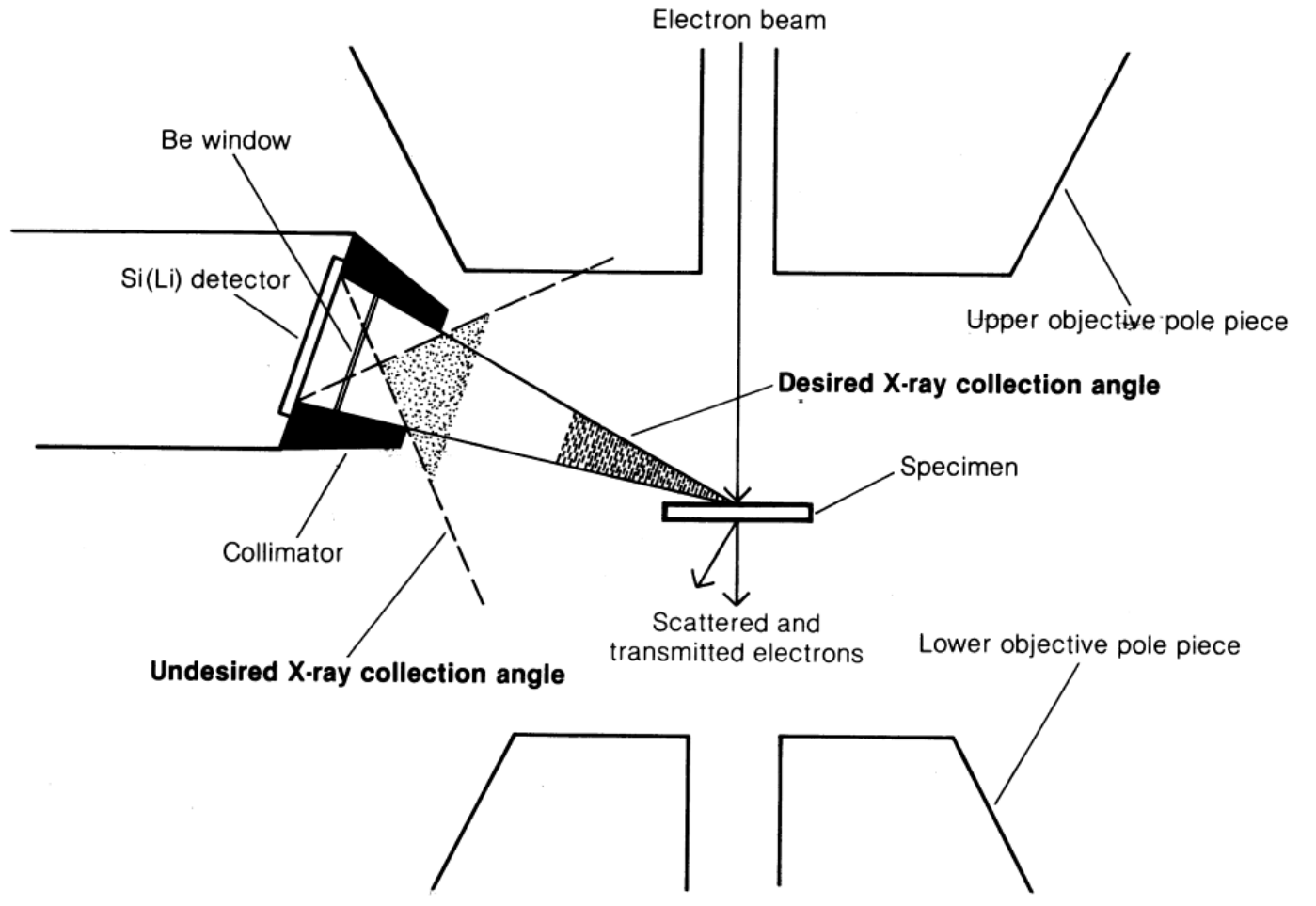
## Spectroscopy: Inelastic Effects

- Energy Dispersive X-ray Analysis (EDS)
  - Detecting X-rays from excited atoms
    - Chemical Information
    - Heavy Elements
    - 120 eV resolution, 0-2 keV range
- Electron Energy Loss Spectroscopy (EELS)
  - Measuring Energy Loss of Primary Electrons
    - Chemical Information
    - Electronic Information
    - Light Elements
    - 1 eV resolution, 0 – 2 keV range



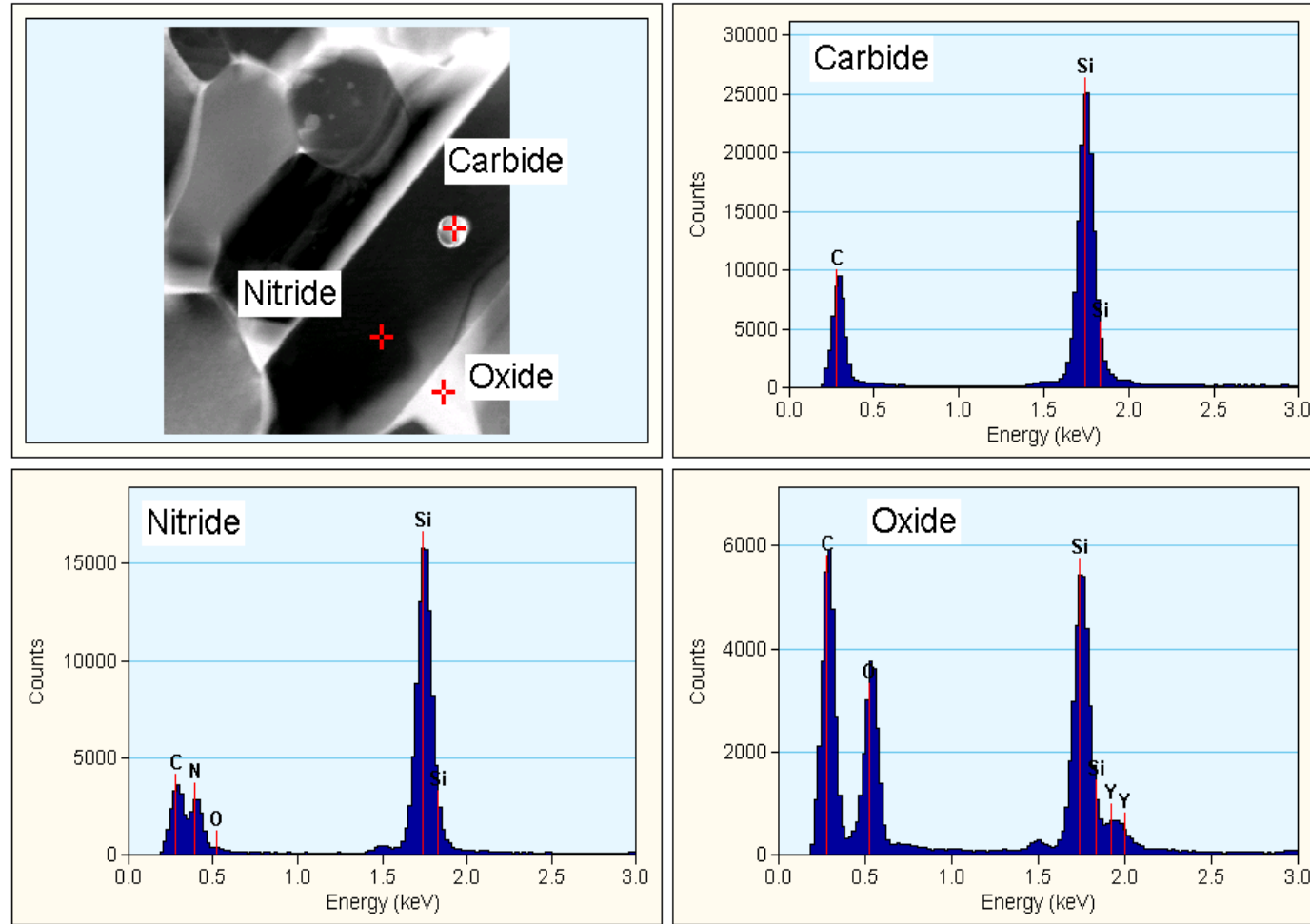
# Spectroscopy in TEM - EDS

## Energy Dispersive X-Rays Spectroscopy in TEM: EDS Setup



# Spectroscopy in TEM - EDS

## STEM and EDS Analysis: Spot Analysis

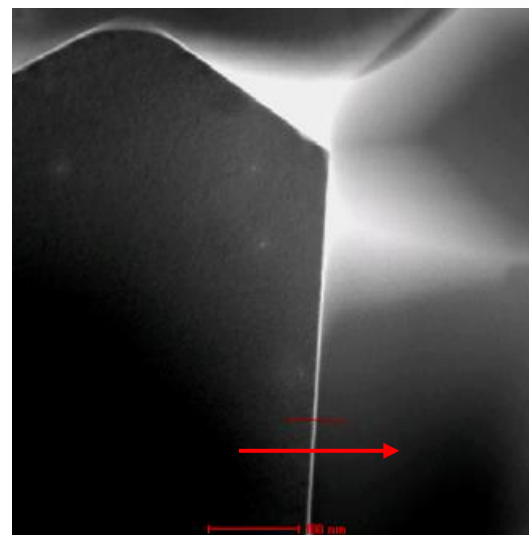
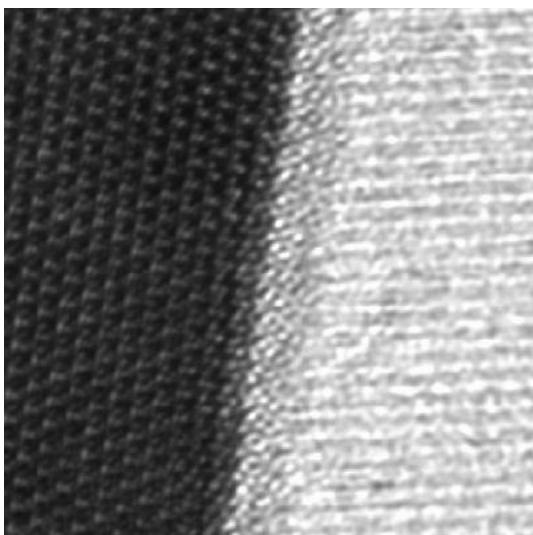


# Spectroscopy in TEM - EDS

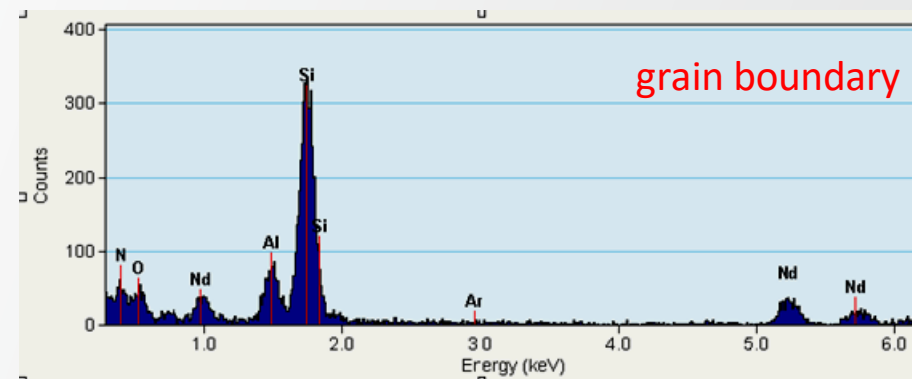
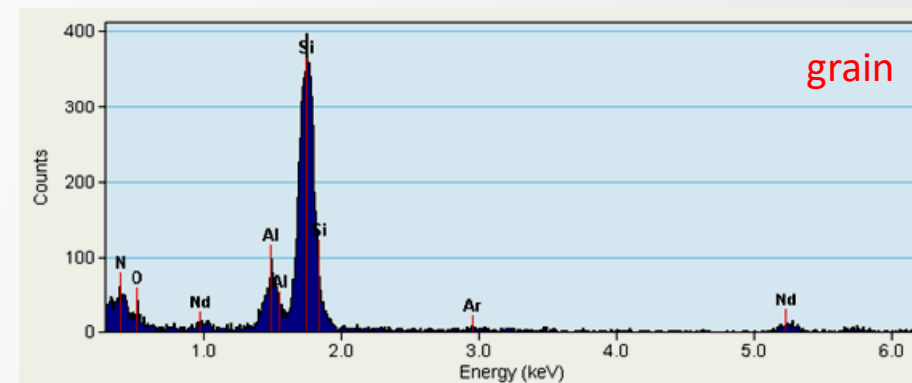
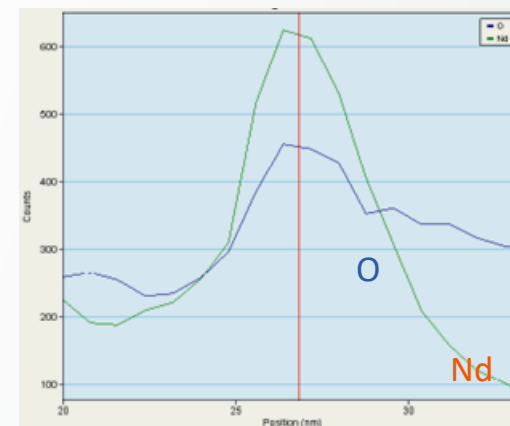
STEM and EDS Analysis: Spectrum Profiling (Line Scan)

Spectrum profile across a grain boundary in a SiAlON ceramic.

The Nd bearing amorphous grain boundary is about 2 nm wide.



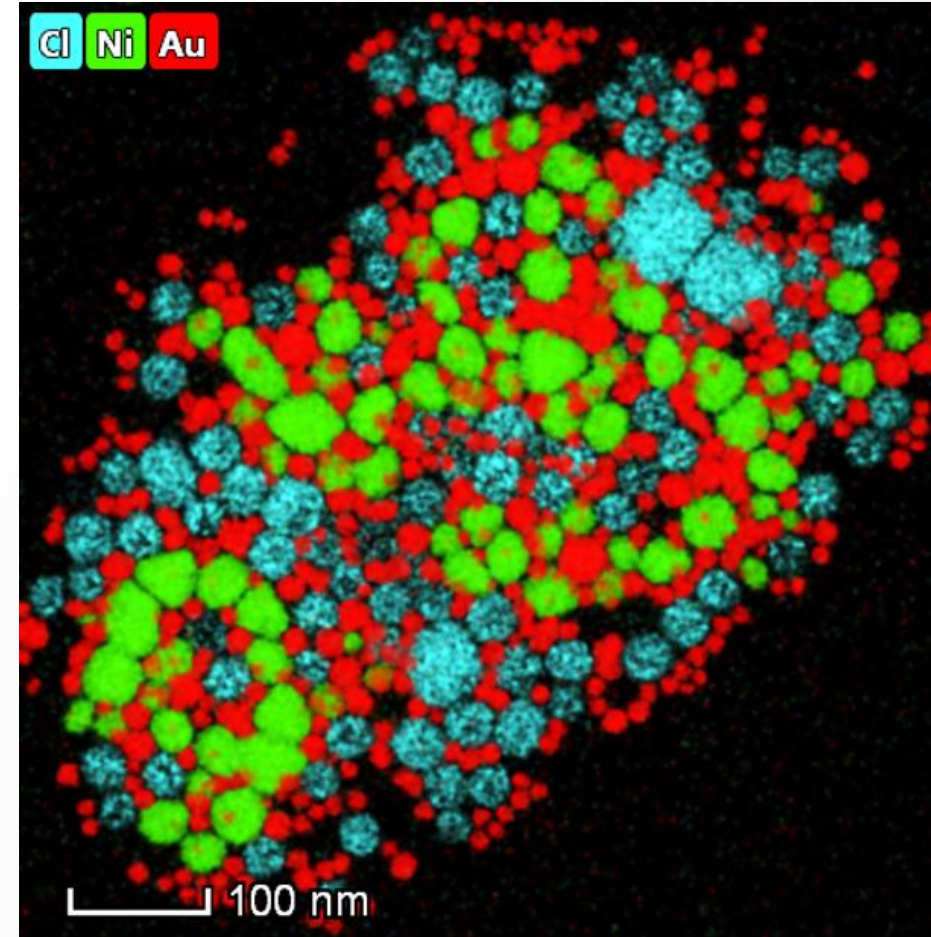
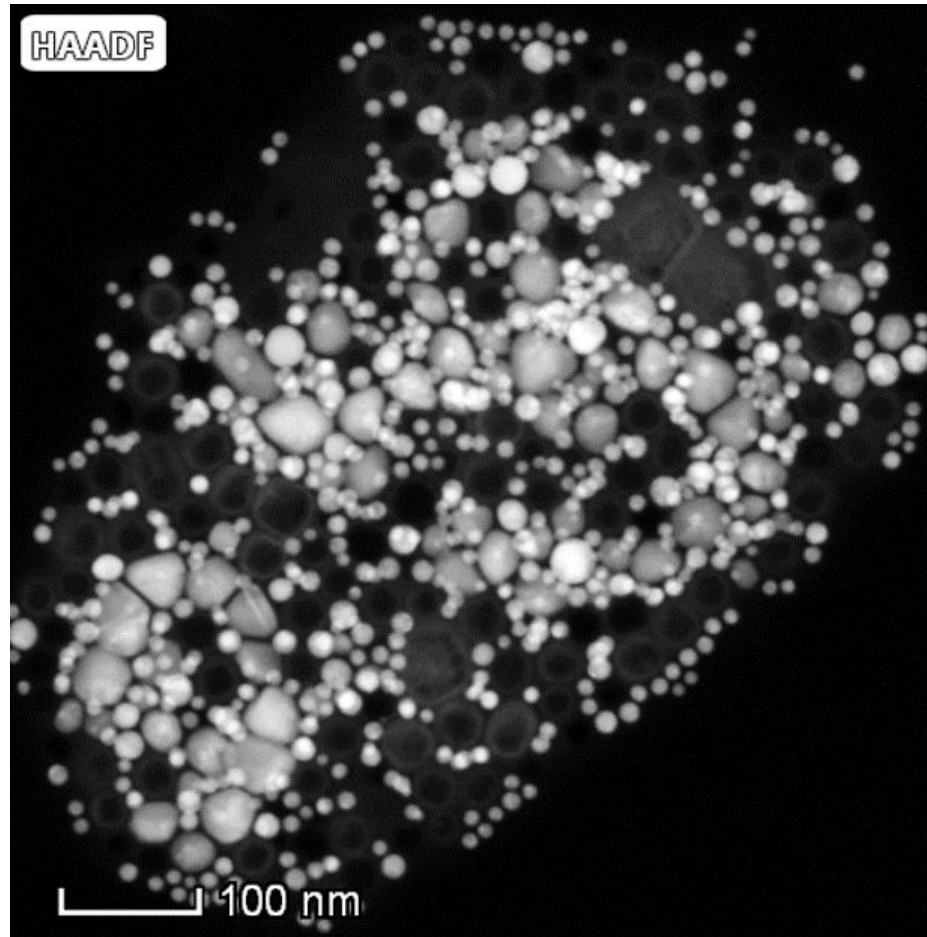
Tecnai F20 S-TWIN





# Spectroscopy in TEM - EDS

STEM and EDS Analysis: Spectrum Imaging (EDS Mapping)

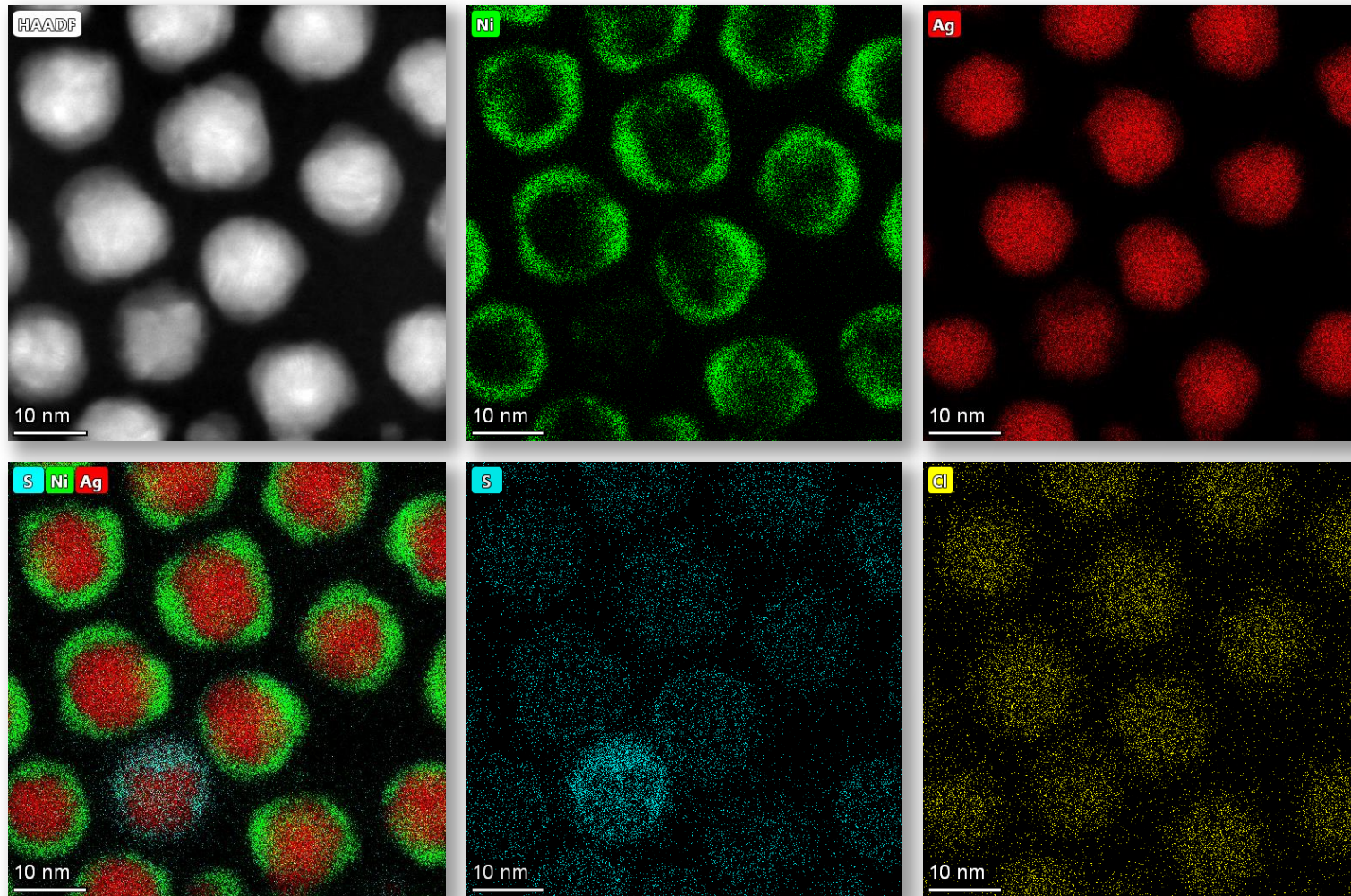


Sample courtesy: J. Bursik, Institute of Physics of Materials, Brno

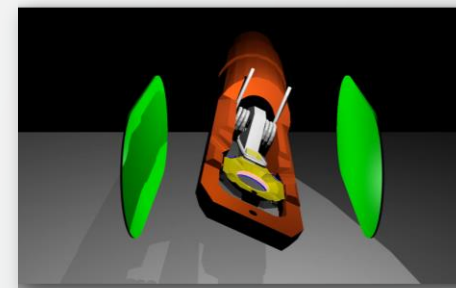


# Spectroscopy in TEM - EDS

## AgNi Core-Shell Nanoparticles: Dual-X EDS



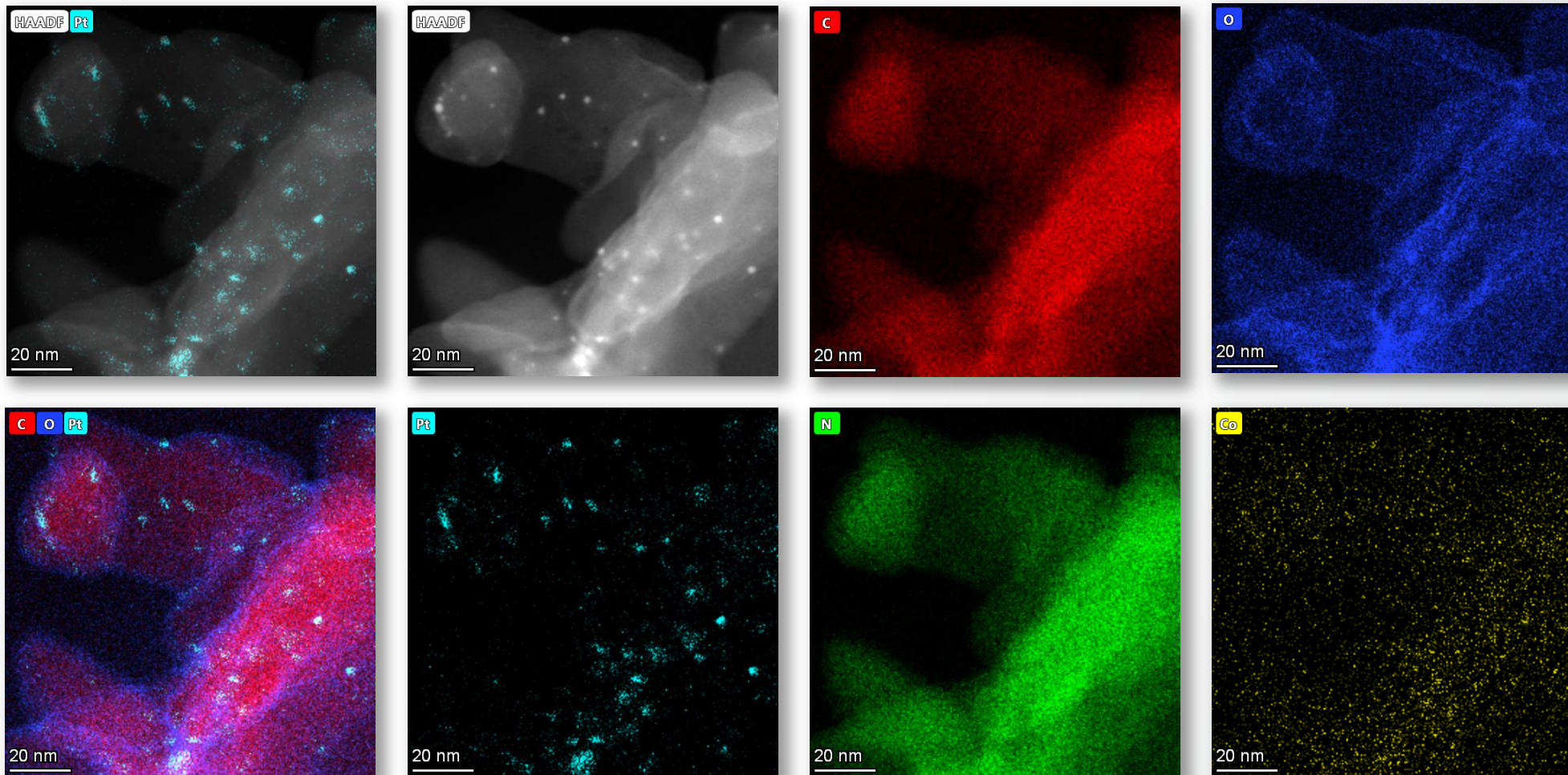
AgNi NPs may be effectively used as catalysts for reducing nitro compounds and degrading organic dyes. System  $\text{Ag}_{0.6}\text{Ni}_{0.4}$  showed the highest catalytic activity for the reduction and degradation reaction of nitro compounds and organic dyes. AgNi NPs were also studied as catalysts for hydrogen generation, and the hydrogen generation rate of AgNi NPs was found to be much higher than Ag and Ni NPs of similar size. Sulfur is unwanted.



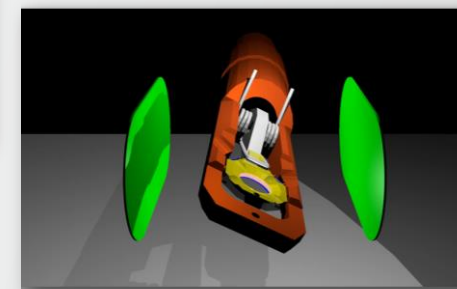


# Spectroscopy in TEM - EDS

$C_3N_4$ (Co)-Pt Beam Sensitive and Thick Dirty Matrix: Dual-X EDS



Photocatalytic hydrogen evolution. The small nanoparticles (Pt) acted as active sites for the improved photocatalytic reaction. Pt on the surface. Co atomically dispersed or located with a diameter less than 1 nm.

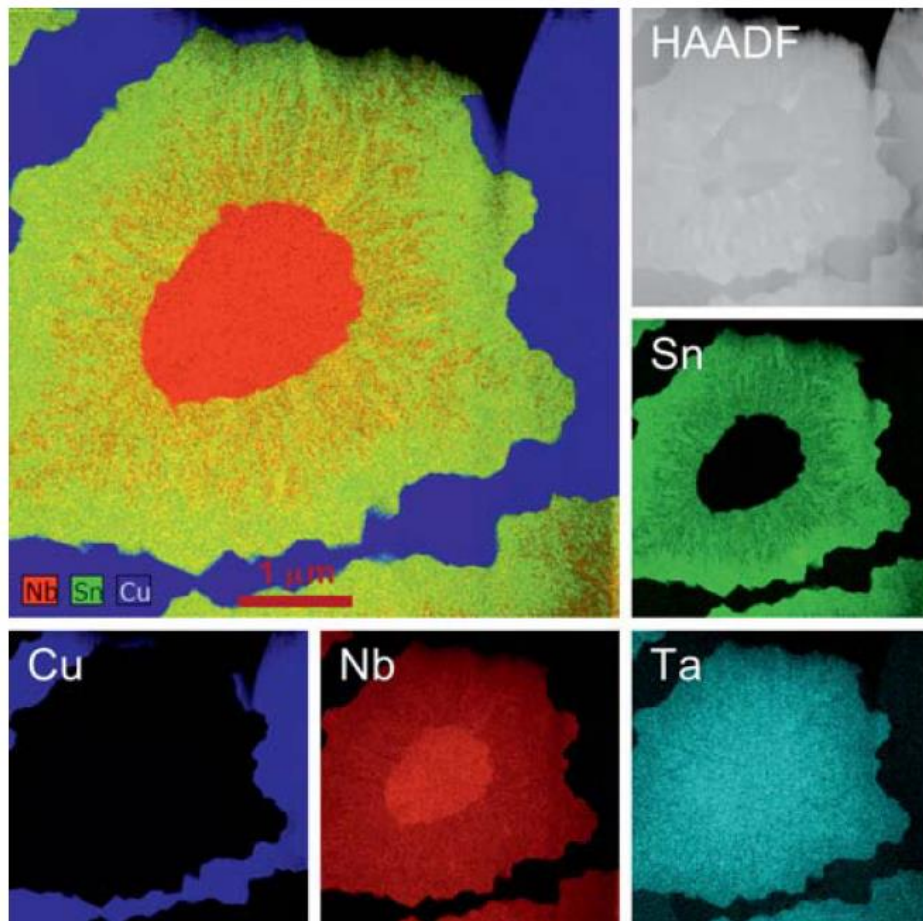




# Spectroscopy in TEM - EDS

## Super-X Fast EDS Mapping

**Nb<sub>3</sub>Sn superconducting cable**, 11 min acquisition time, 400\*400 pixels, 2.5nA probe current

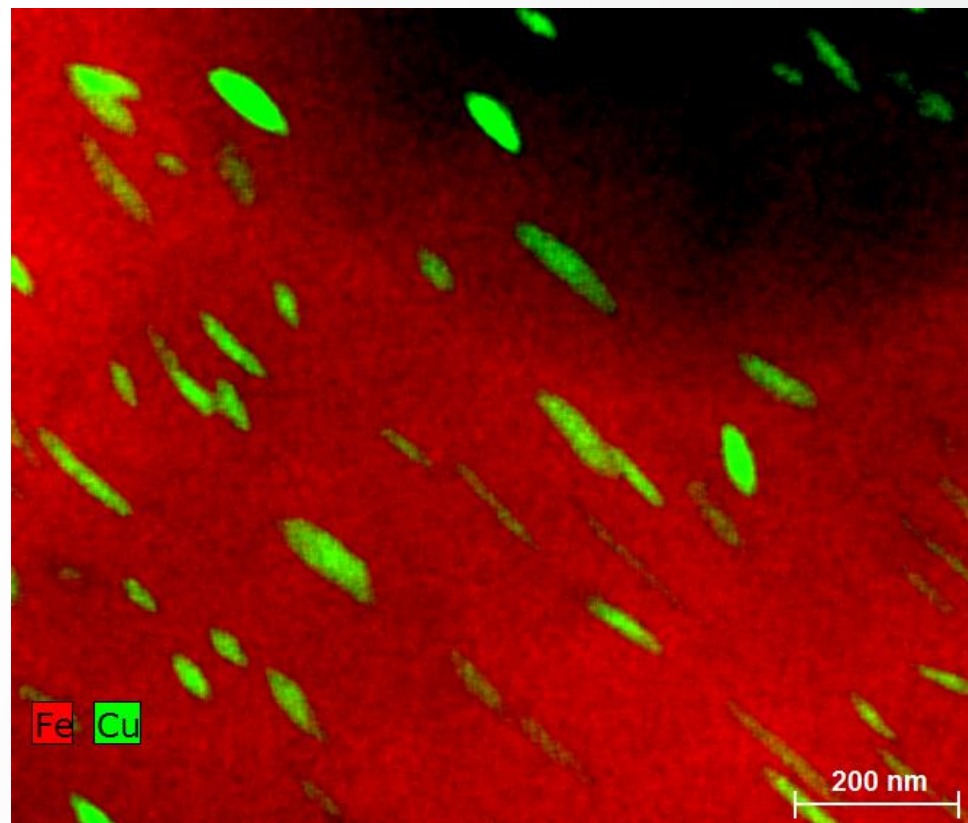


Sample courtesy: Marco Cantoni (EPFL Lausanne, Switzerland)

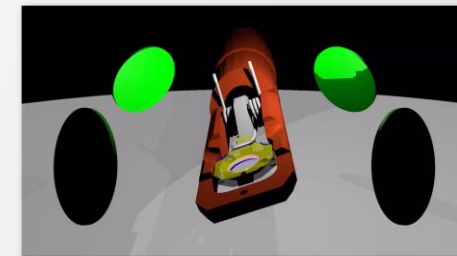


KYUSHU UNIVERSITY

**Rolled steel with copper precipitates.**  
Acquired in 5min with >200kcps X-ray count rate



Sample courtesy: Dr. Satoshi Hata and Dr. Toshihiro Tsuchiyama, Kyushu University and Dr. Mitsuhiro Murayama, Virginia Tech





# Spectroscopy in TEM - EDS

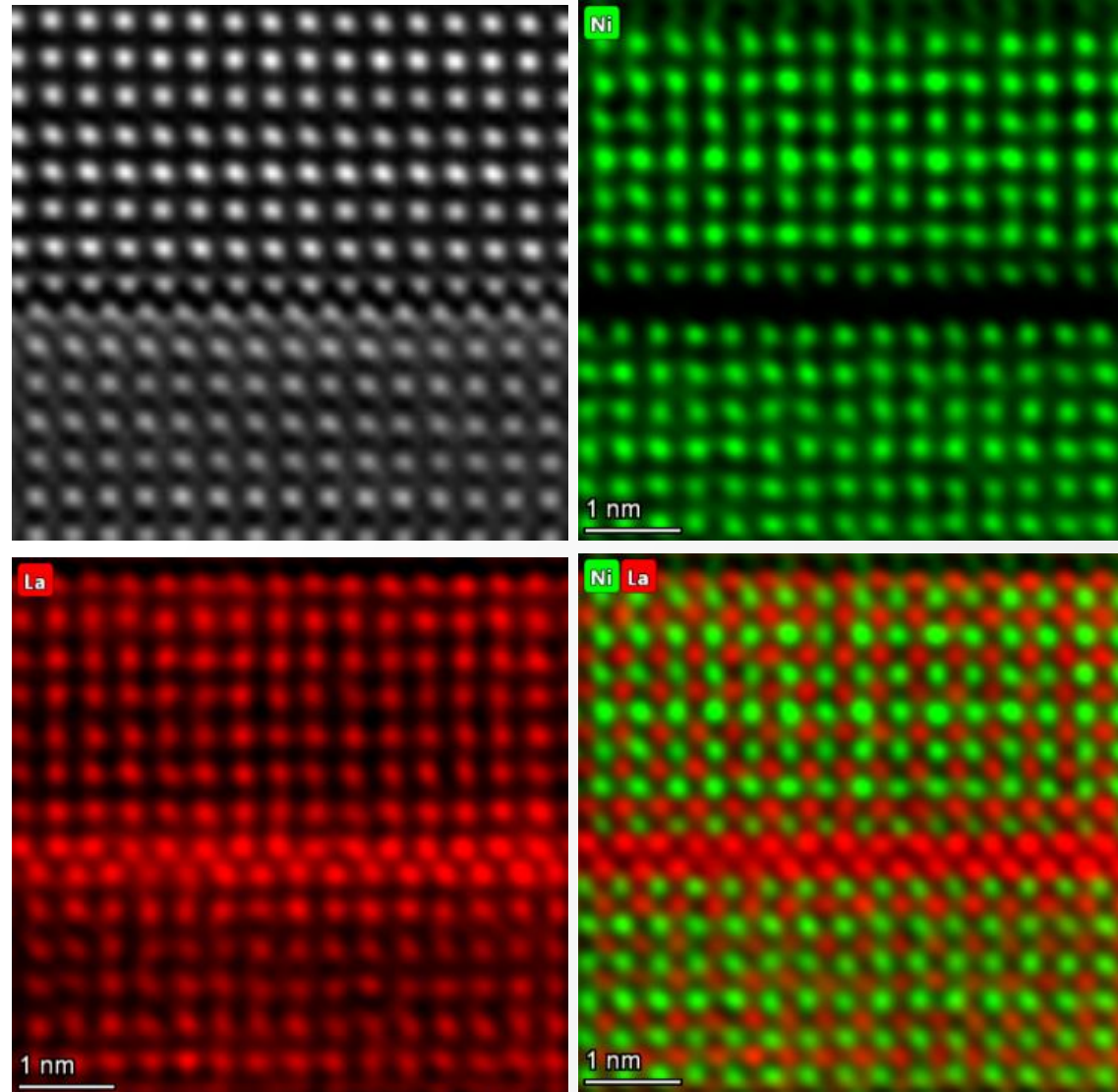
## EDS Mapping at Atomic Resolution



- Rh too low to get spatial resolution
- O is light elements, need more time to get the spatial resolution



Talos F200X

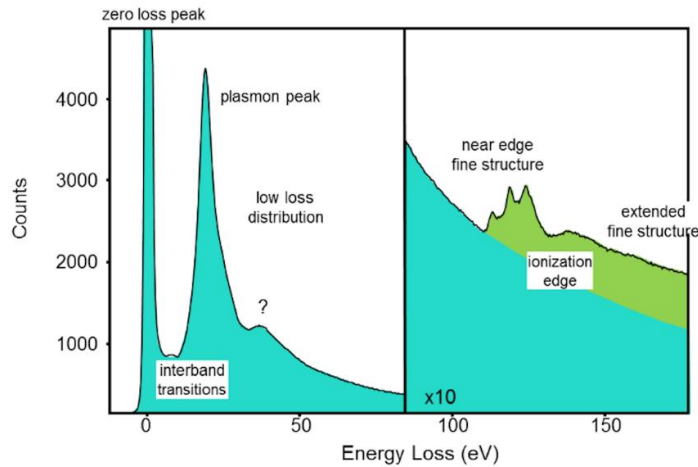
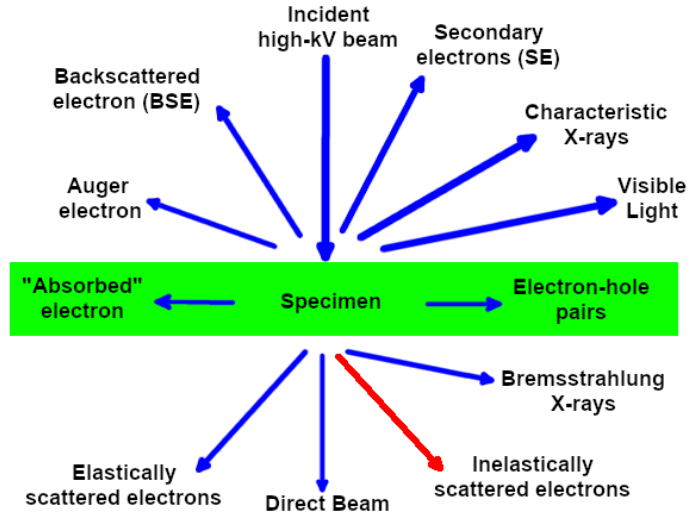


Sample courtesy Nikolla Lab, Wayne State University



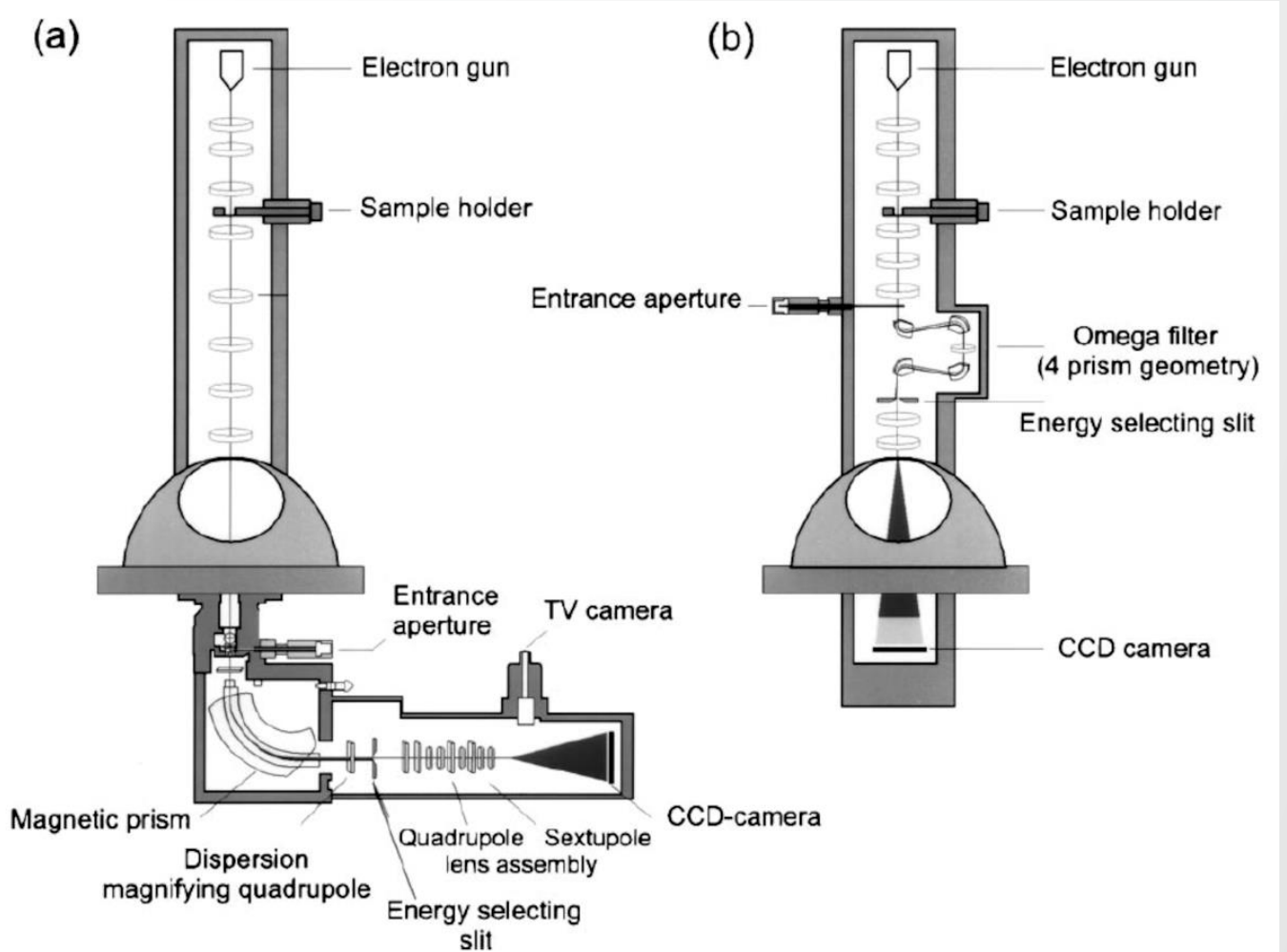
# Spectroscopy in TEM - EELS

## Electron Energy Loss Spectroscopy (EELS) in TEM: EELS Setup



Post-Column Filter

In-Column Omega Filter

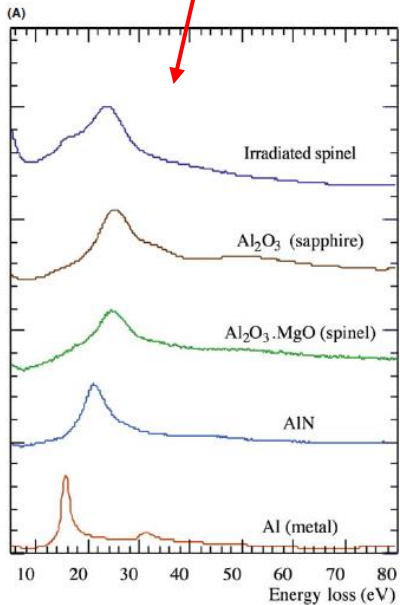
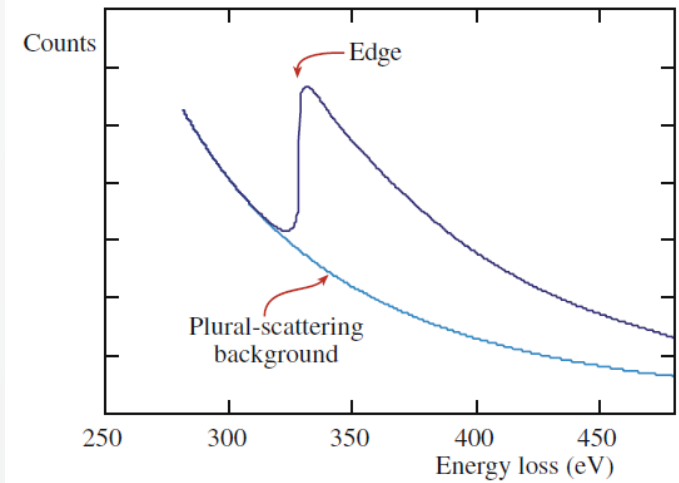
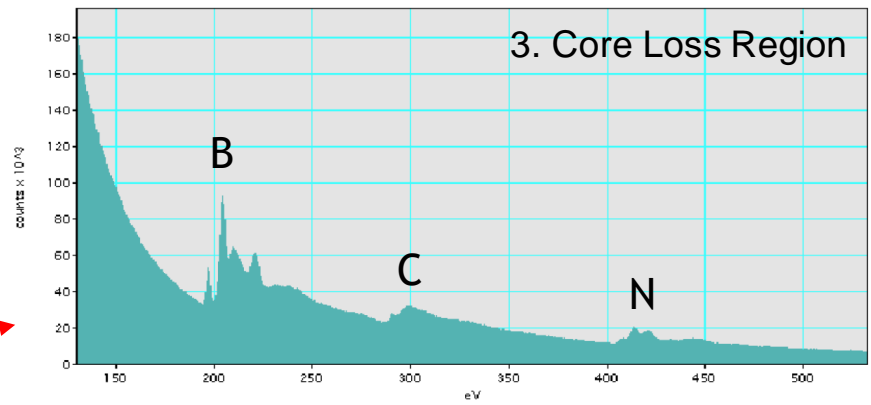
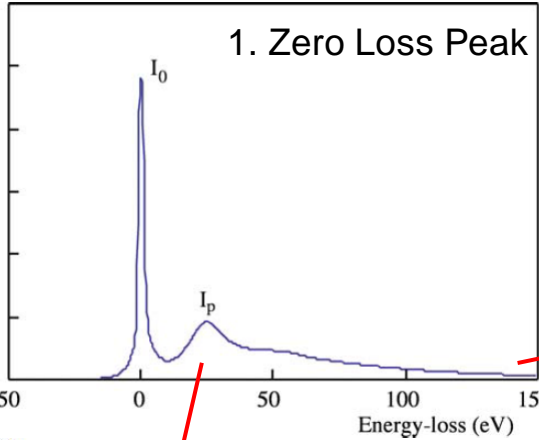


<https://eels.info/about/overview>

F. Hofer, *Mikrochim, Acta* 132, 273-288 (2000)

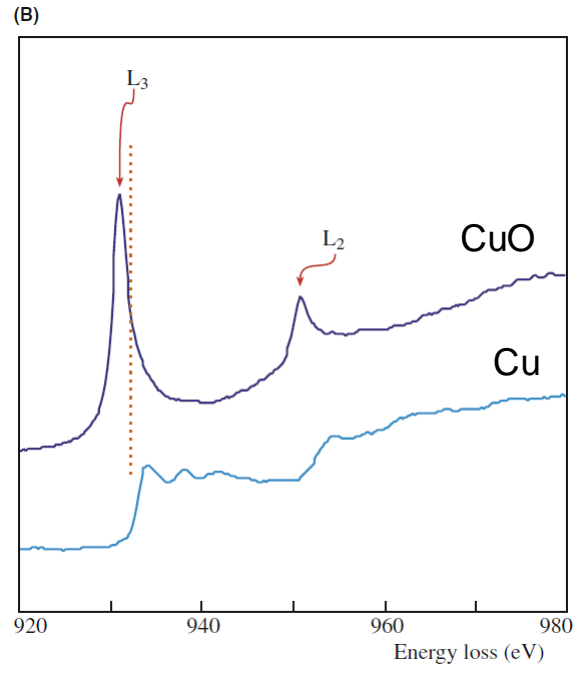
# Spectroscopy in TEM - EELS

## EELS Spectrums Regions



2. Low Loss Region  
1eV~75eV

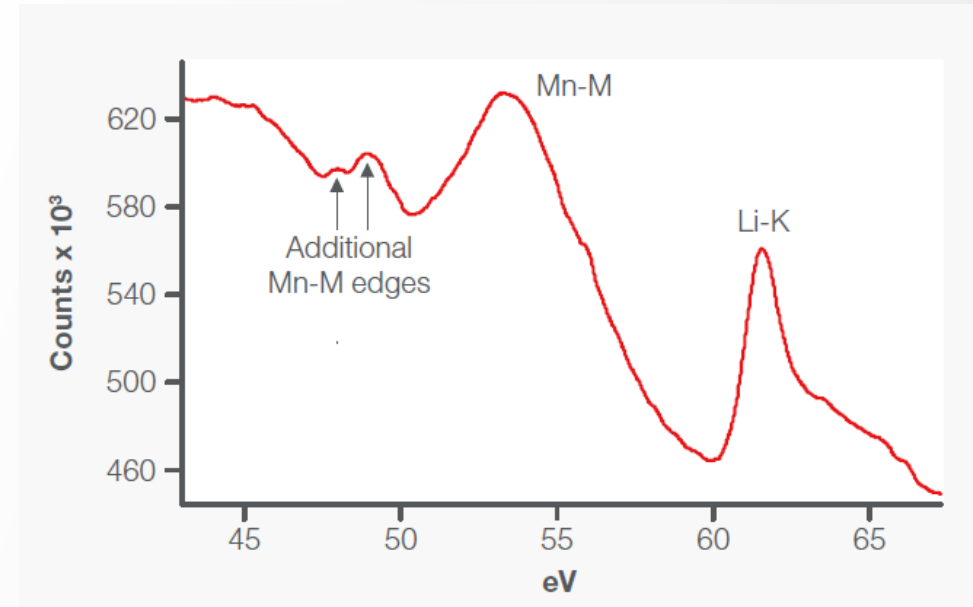
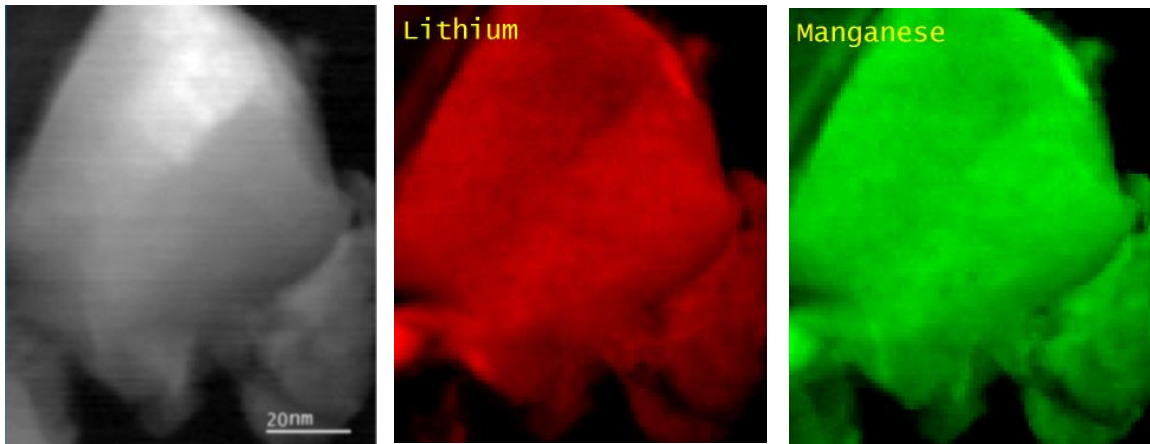
Change in the Cu L<sub>2,3</sub> edge structure as Cu metal is oxidized



An “edge” in the spectrum corresponds to energy absorption from an electron in a certain element.

# Spectroscopy in TEM - EELS

## STEM and EELS Analysis: Spectrum Imaging (EELS Mapping)



The Mn-M edge fine structure and Li-K edge in the Li battery are distinguished.



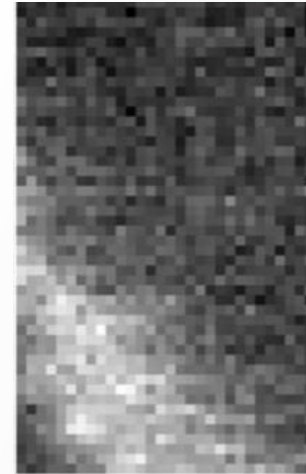
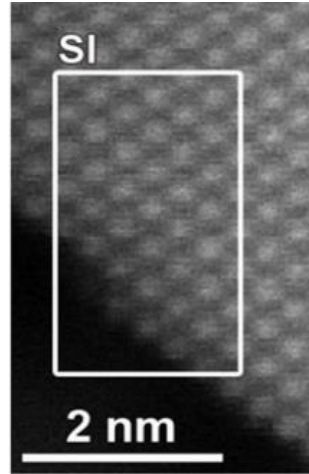
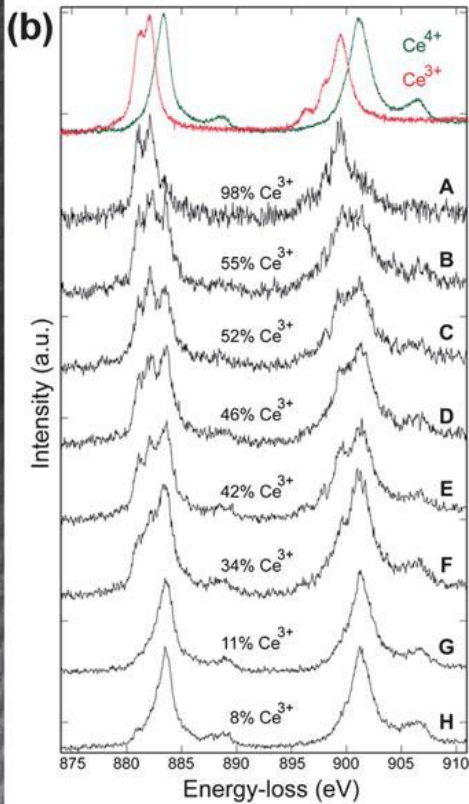
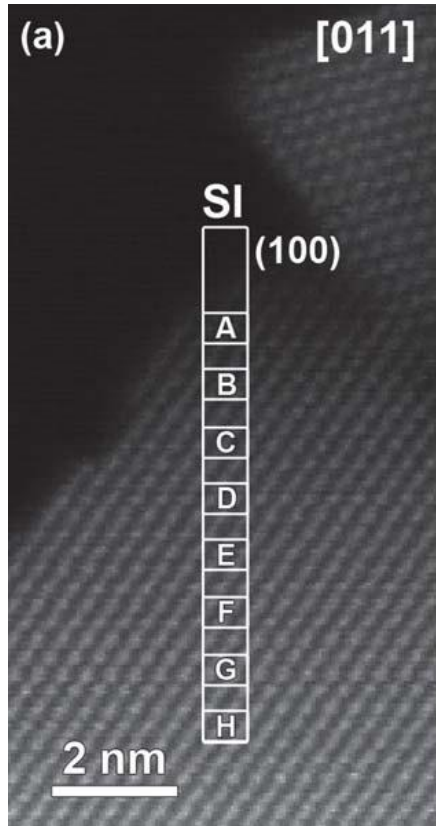
Talos F200X

Sample courtesy: Dr. Chongmin Wang, at Pacific Northwest National Laboratory (PNNL).

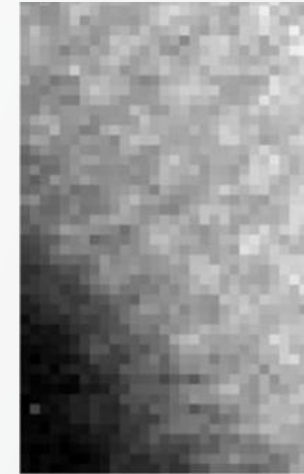


# Spectroscopy in TEM - EELS

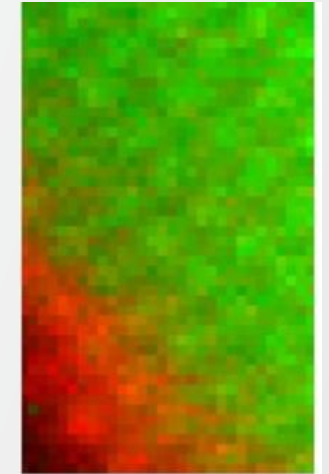
## STEM and EELS Analysis: Oxidation State Mapping



Ce<sup>3+</sup>



Ce<sup>4+</sup>



- 0.2 eV
- 60 pA
- 0.1 nm resolution

Spectra Ultra  
X-FEG + Monochromator

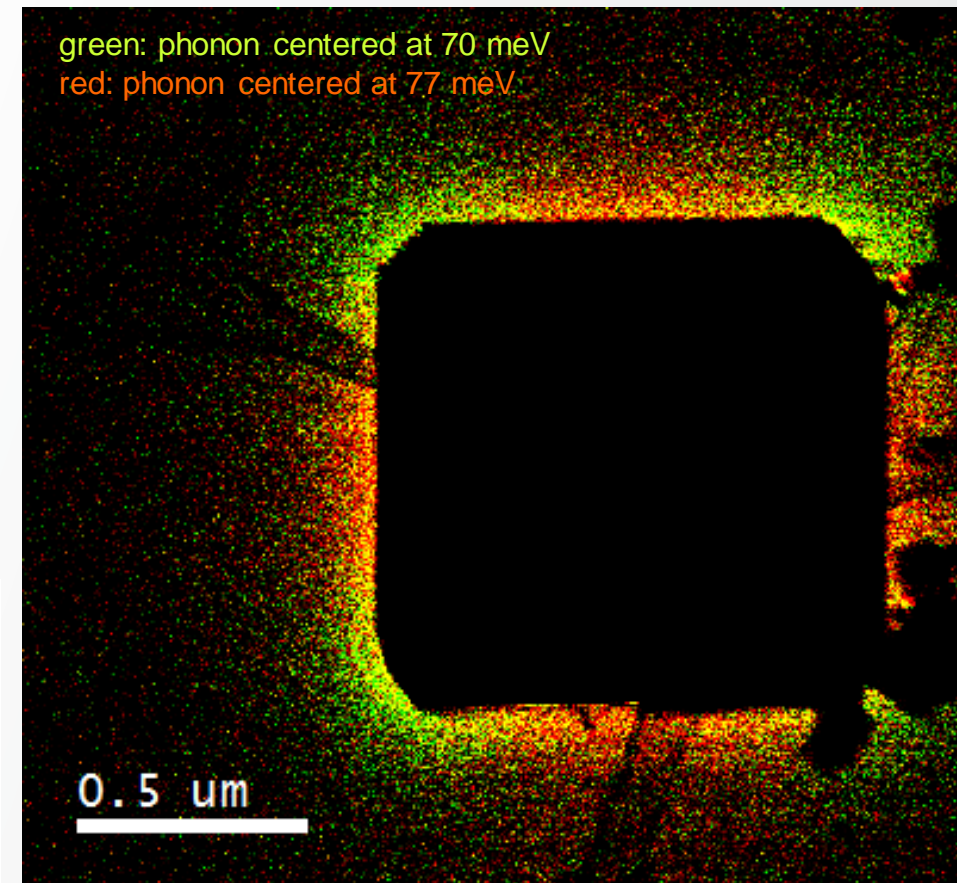
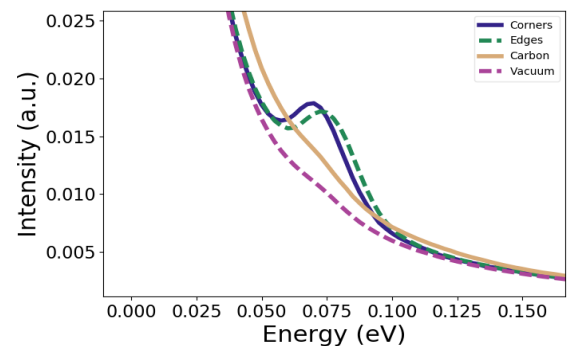
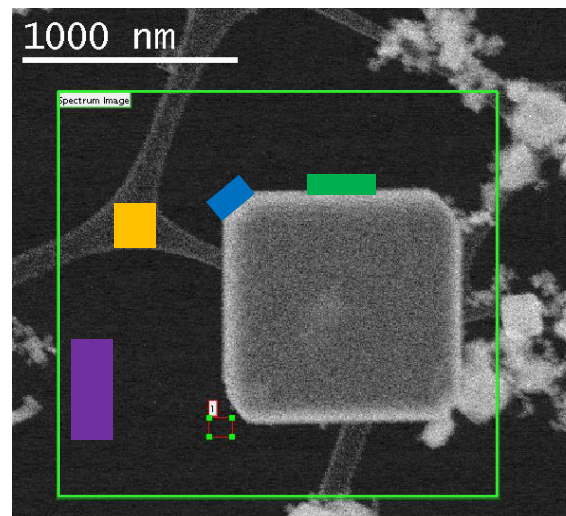
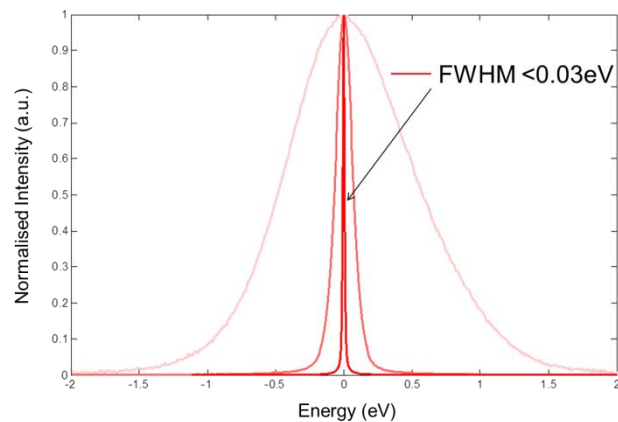


# Spectroscopy in TEM - EELS

## STEM and EELS Analysis: Surface Phonons

### Surface phonons:

- Energy spread  $<0.025$  eV
- De-scan coils standard on Spectra 300 column
- Continuum 1066
- 8-minute acquisition



MgO Surface Phonons imaged at 60kV

Spectra Ultra - X-FEG + Monochromator

Sample: Prof. G. Botton  
Canadian Centre of Electron Microscopy, McMaster University

# Transmission Electron Microscopy Webinar Series

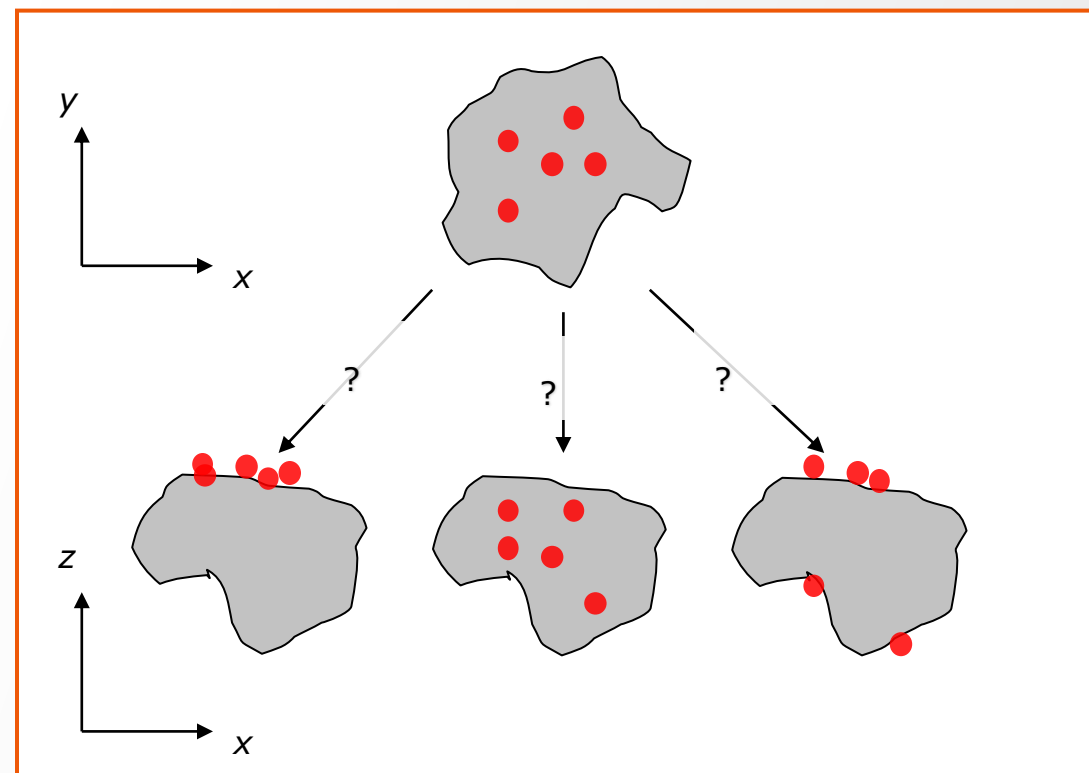
## Outline: Part 02

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

# Tomography in TEM: For 2D to 3D Imaging

## Third Dimension on TEM Specimens

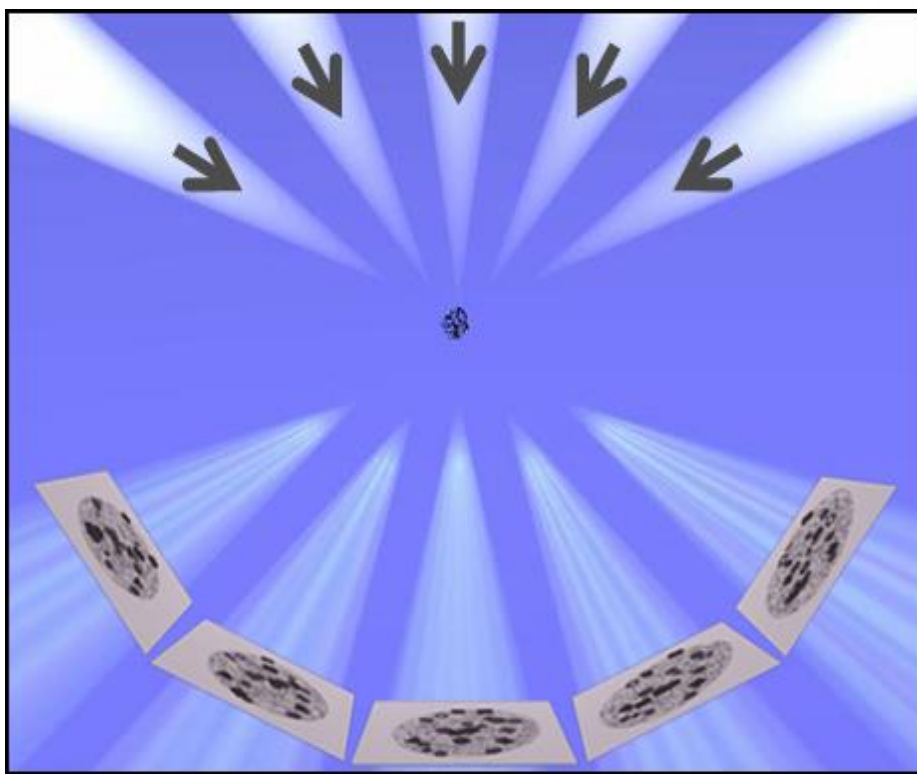
- 2D projections:
  - Miss out on important information
  - May give erroneous information



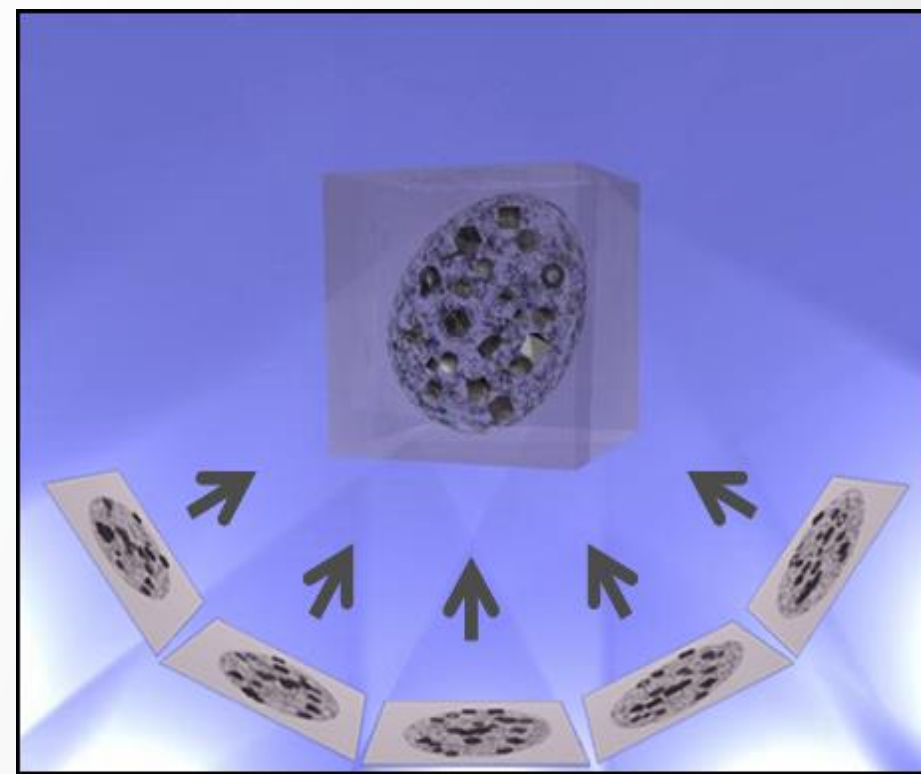


# Tomography in TEM: For 2D to 3D Imaging

From 2D to 3D Information



3D object  $\Rightarrow$  set of 2D projections

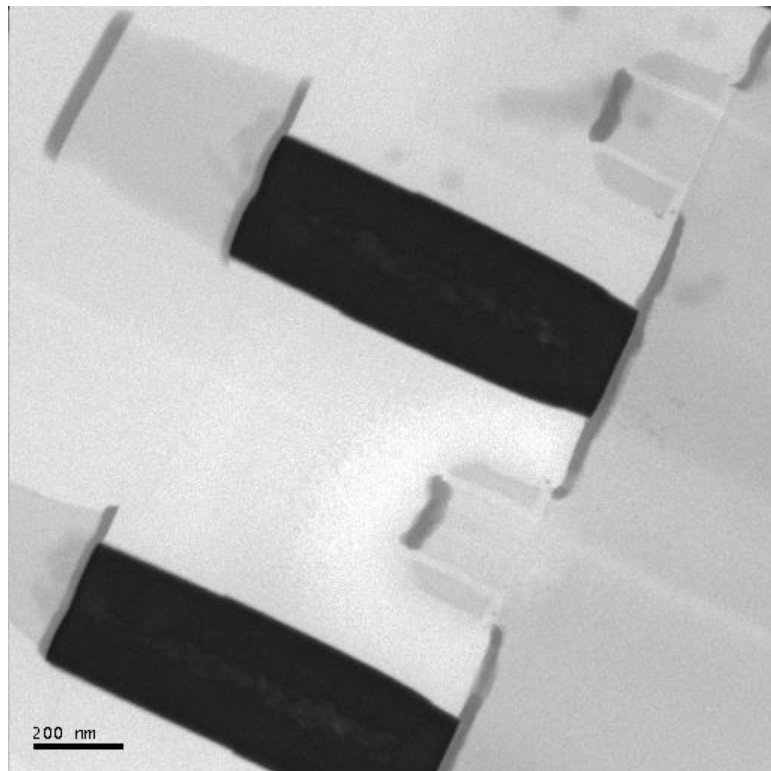


2D projections  $\Rightarrow$  3D reconstruction

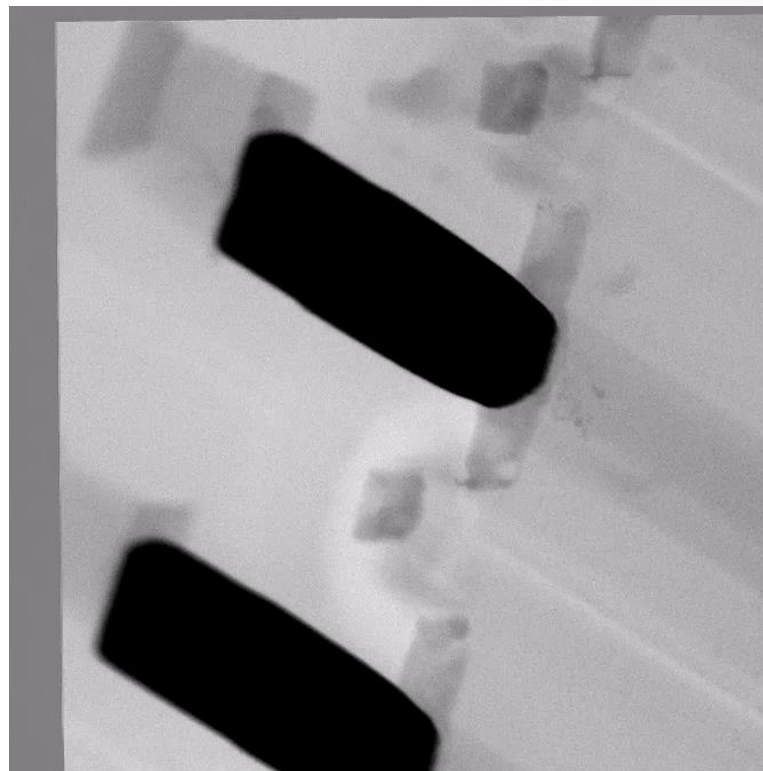
Common acquisition:  $\pm 70$  deg,  $1^\circ$  increments, 141 images

# Tomography in TEM: For 2D to 3D Imaging

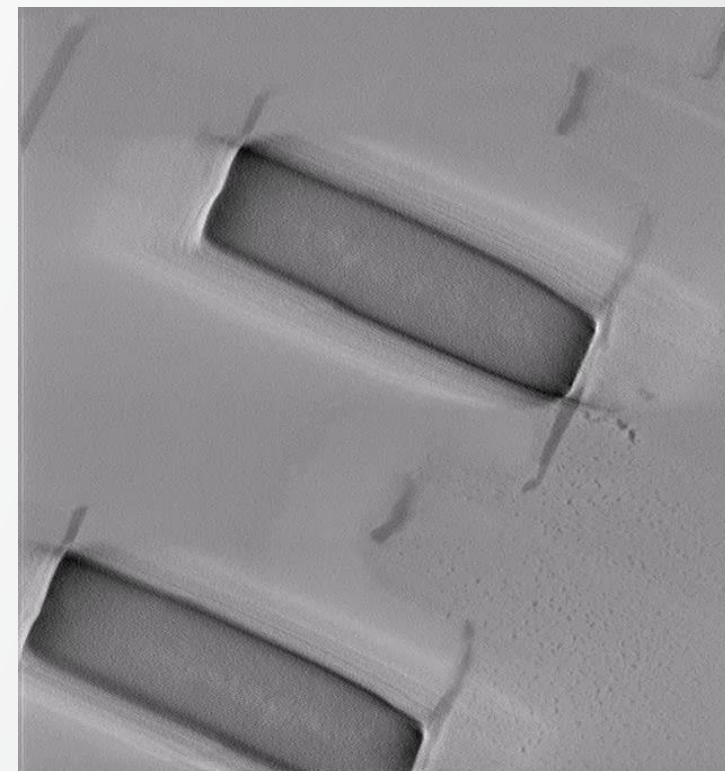
2D vs 3D Imaging: High Crystalline Semiconductor Device



STEM-BF



Tilt-Series

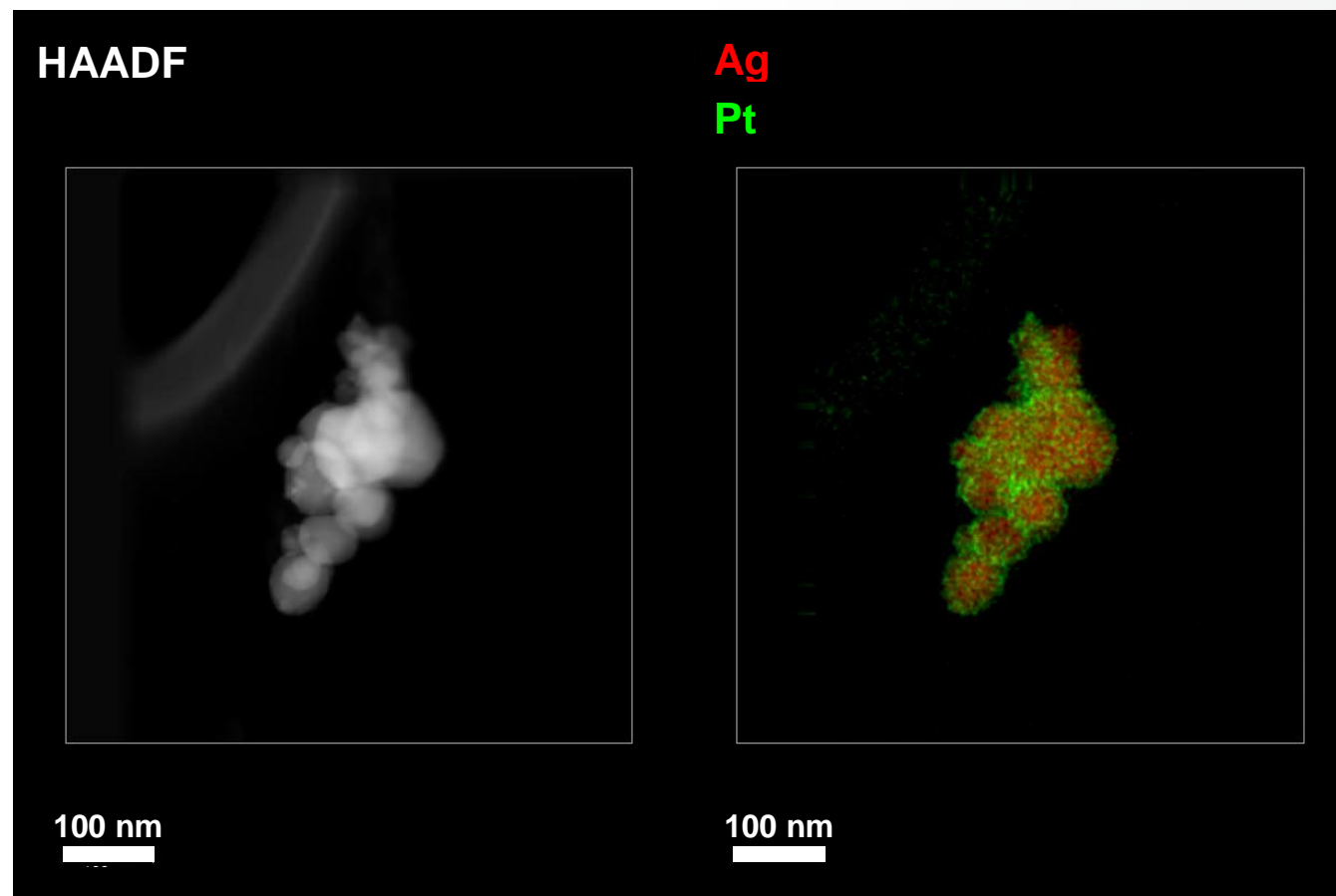


Reconstruction

Tecnai F20

# Tomography in TEM: For 2D to 3D Imaging

High resolution; Ag-Pt core-shell catalyst particles

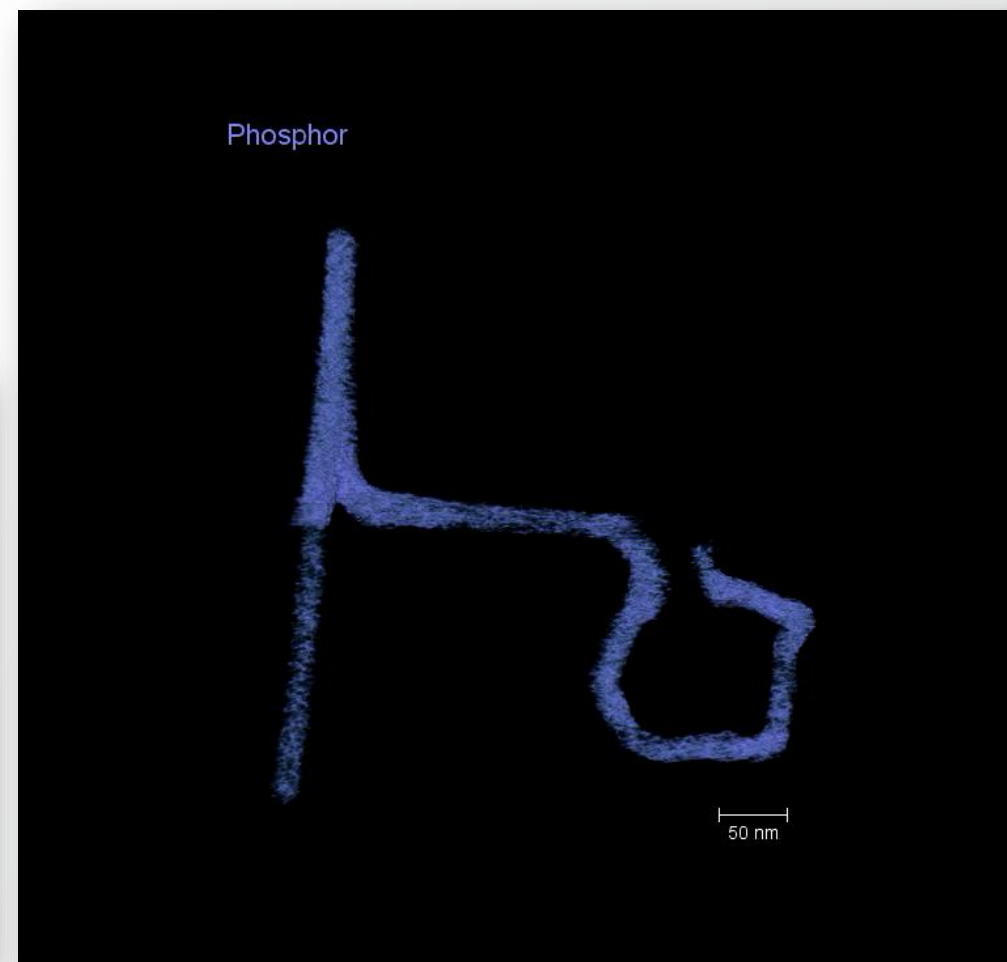
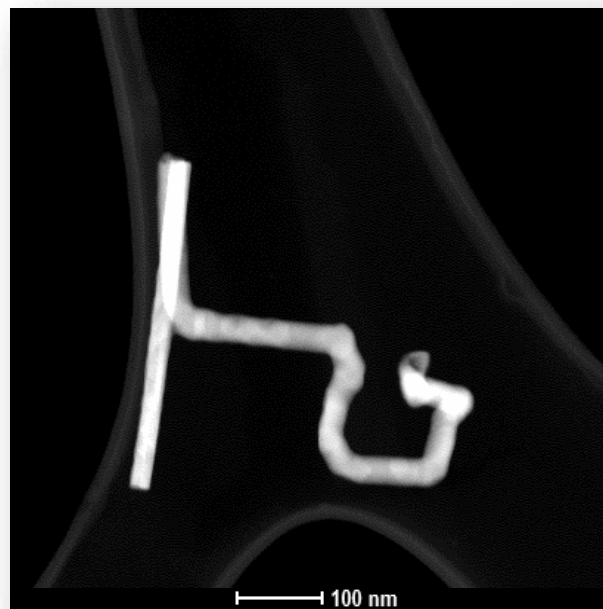


*Aligned Tilt Series*

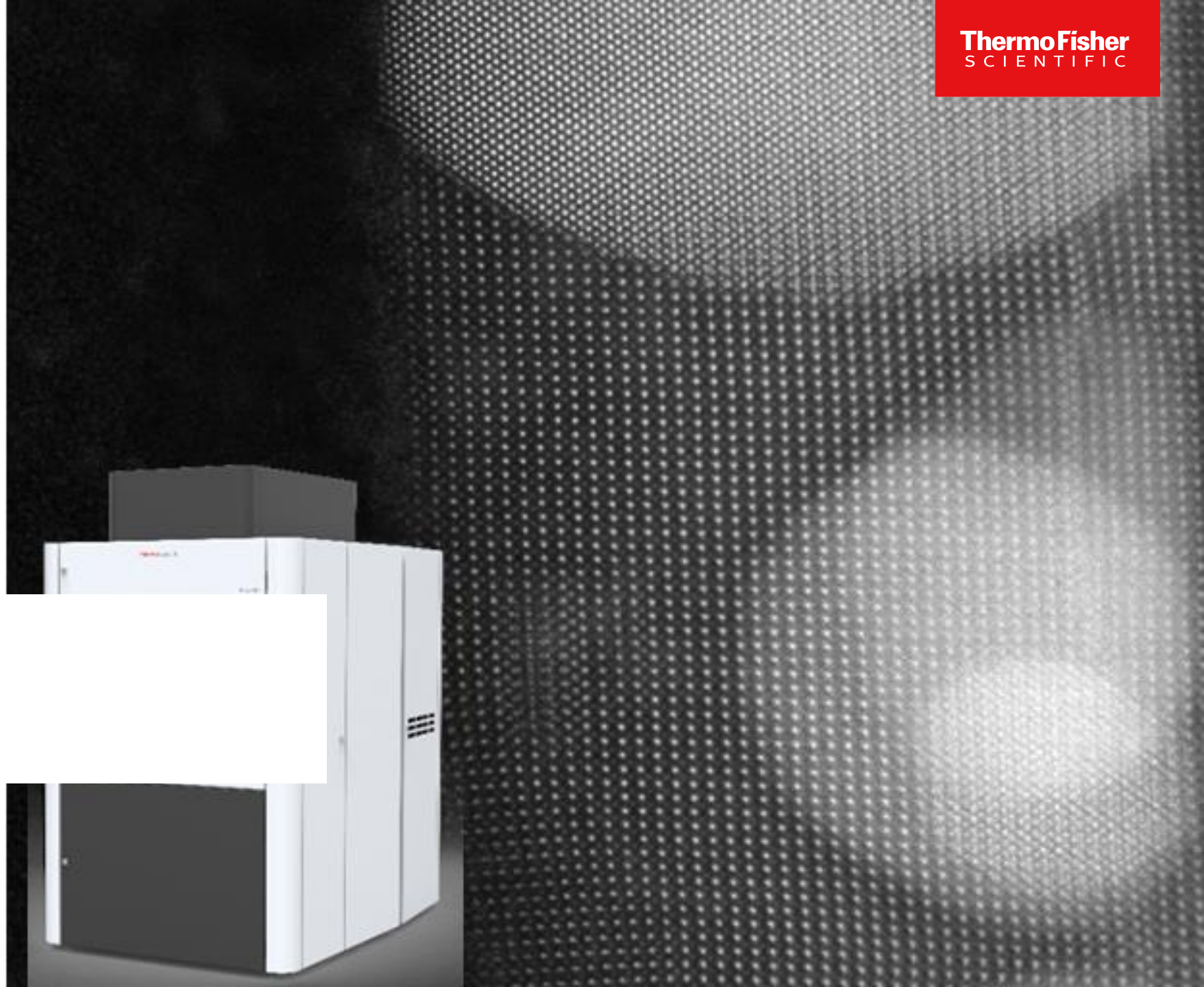
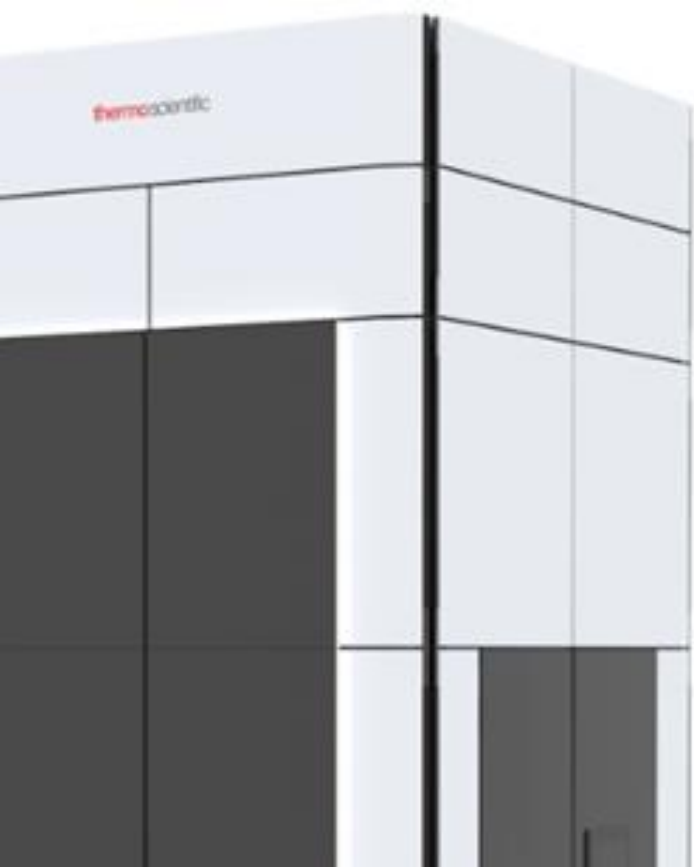
Sample courtesy Prof. Yi Ding and Prof. Jun Luo, Center for Electron Microscopy, Tianjin University of Technology

# Tomography in TEM: For 2D to 3D Imaging

Electrode material for Na-ion and Li-ion batteries







## Examples



# Video on TEM Operational – Talos F200X

Talos F200X



# Video on TEM Operational – Talos F200X

## Holder Insertion



# Video on TEM Operational – Talos F200X

## STEM Imaging

The screenshot displays the ThermoFisher TEM software interface, split into two main sections. The left section is the 'TEM User Interface' with various control panels:

- Vacuum (Supervisor):** Status: All Vacuum (Opened). Parameters: Acceleration: 1 kV, Column: 2, Detector Unit: 20, Buffer tank: 52, Backing line: 59, Nitrogen level: 59.
- FEG Control (Expert):** Gun lens: 3, Fine: . Extractor: 3950. Extraction (Standard mode): 2950 V. FEG Emission: 339 uA. Status: Operate.
- High Tension:** High Tension: 200 kV. Free high tension: .
- FEG Registers:** Table with columns for ID, Location, and Date.

The right section is the 'Acquisition' window, showing a STEM image of a sample. The image is a grayscale micrograph with a 1 µm scale bar. The right sidebar contains 'Display Settings' and 'Detector Settings'.

**Display Settings:** Histogram, FFT, Show spatial frequency (126 µm).

**Detector Settings:** Detector: HAADF, Gain: 26332, Offset: 42.890.

**DPC Settings:** DPC Only.

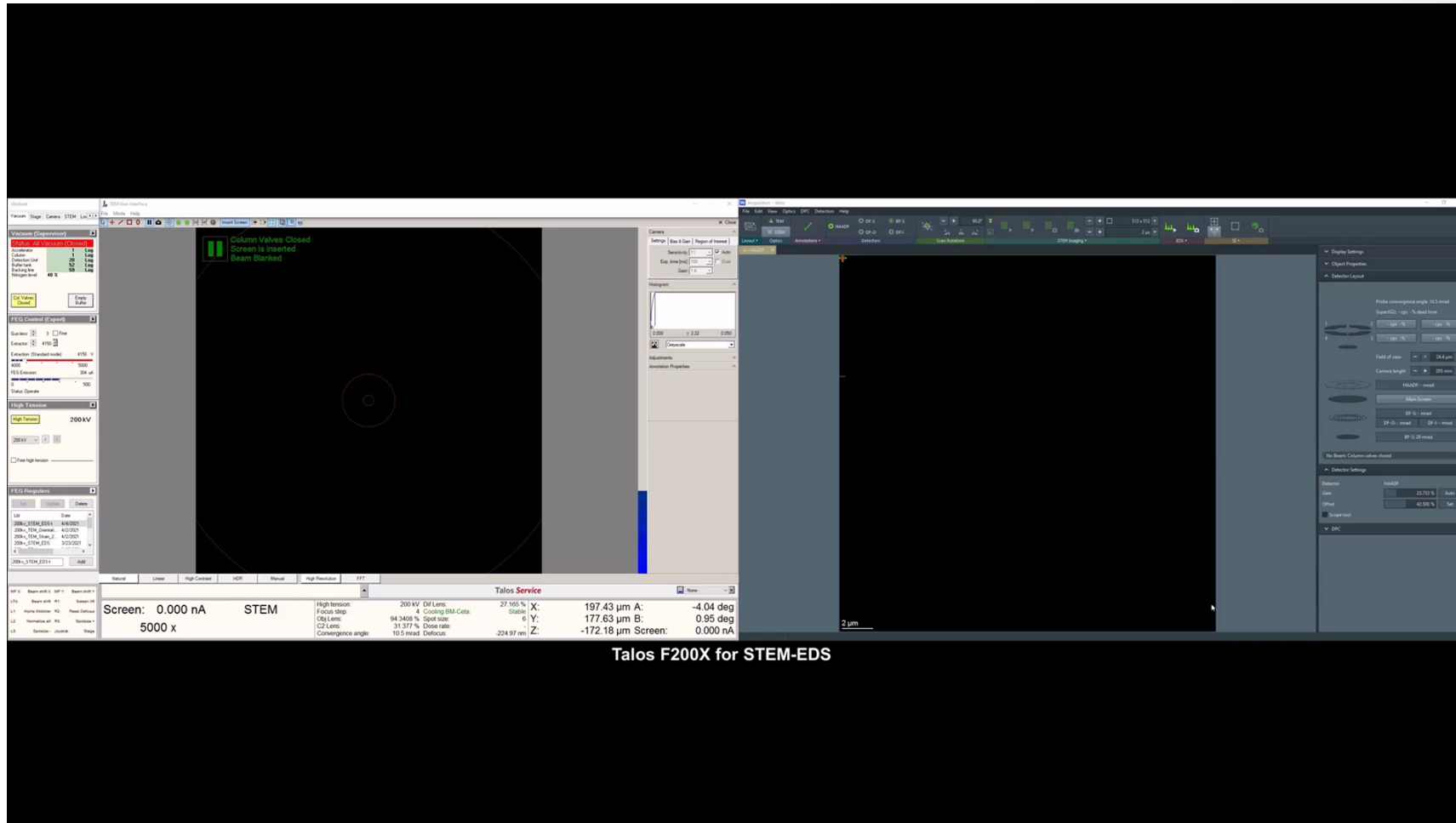
**System Information (Bottom Panel):**

HT:	200 kV	STEM	Spot size:	9	Convergence angle:	10.5 mrad	X:	39.49 µm	A:	1.41 deg
	9900 x	Screen: 0.000 nA	C2 Lens:	28.273 %	Focus step:	1	Y:	-124.51 µm	B:	-4.25 deg
			Obj Lens:	94.6380 %	Dose rate:		Z:	-209.30 µm	Defocus:	52.64 nm
			Dif Lens:	27.074 %	Screen current:	0.000 nA				

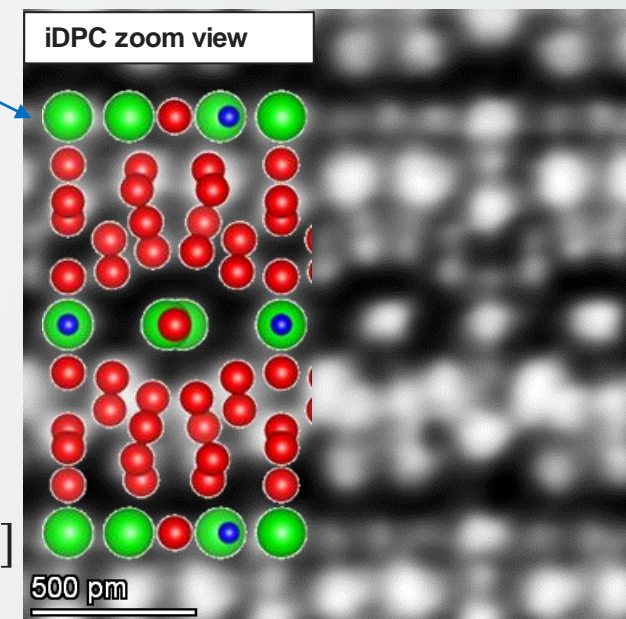
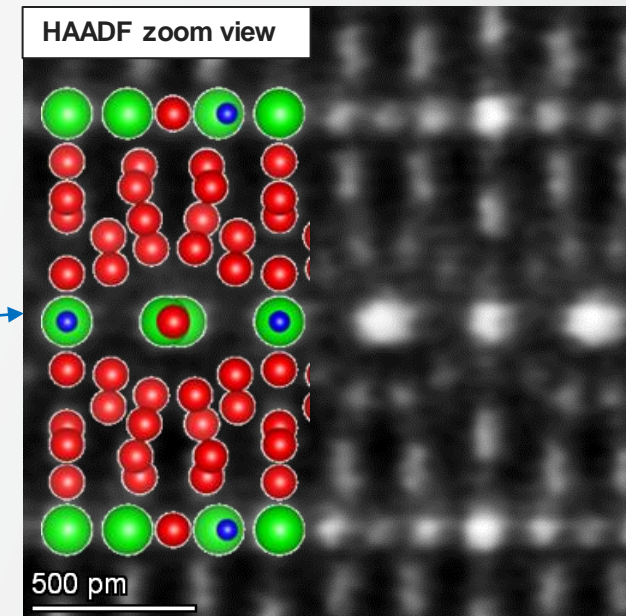
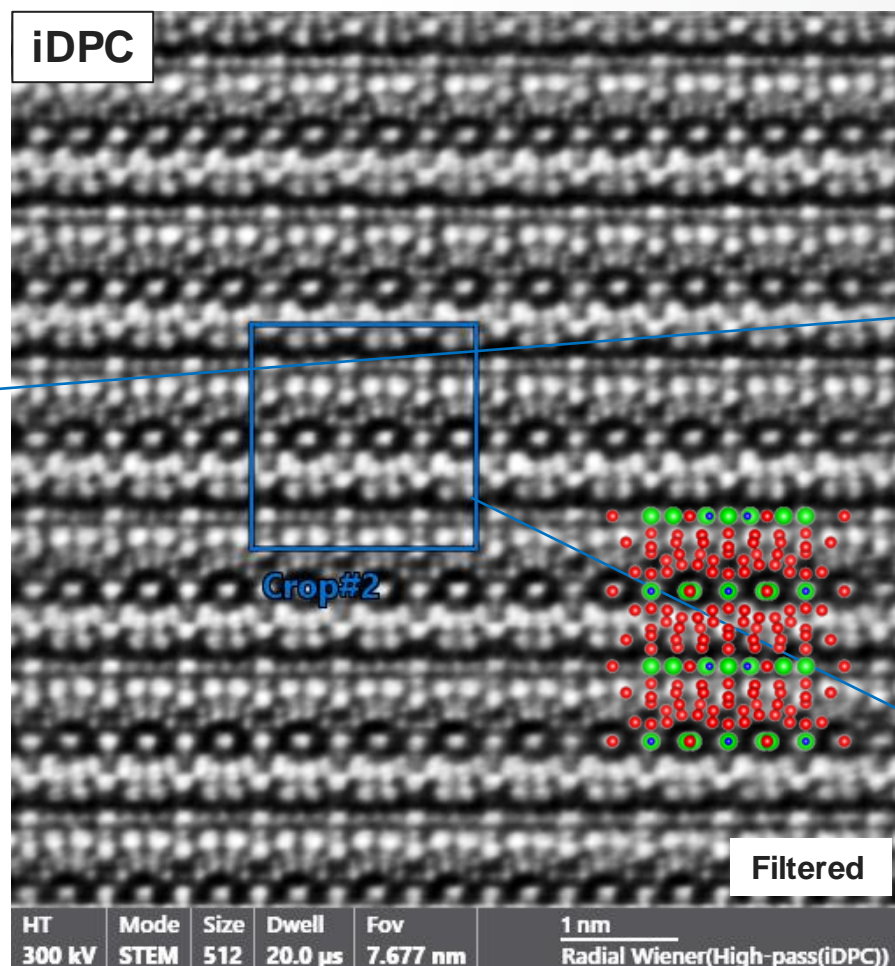
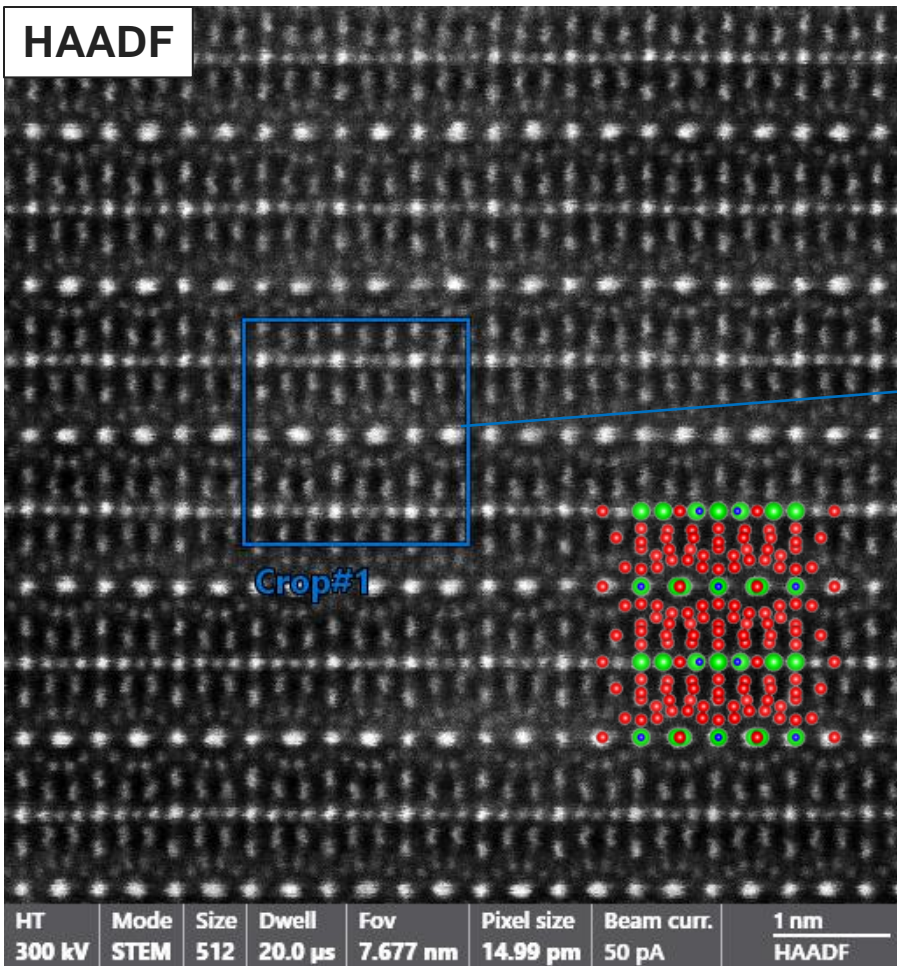


# Video on TEM Operational – Talos F200X

## STEM EDS



# Complex Structure Investigation



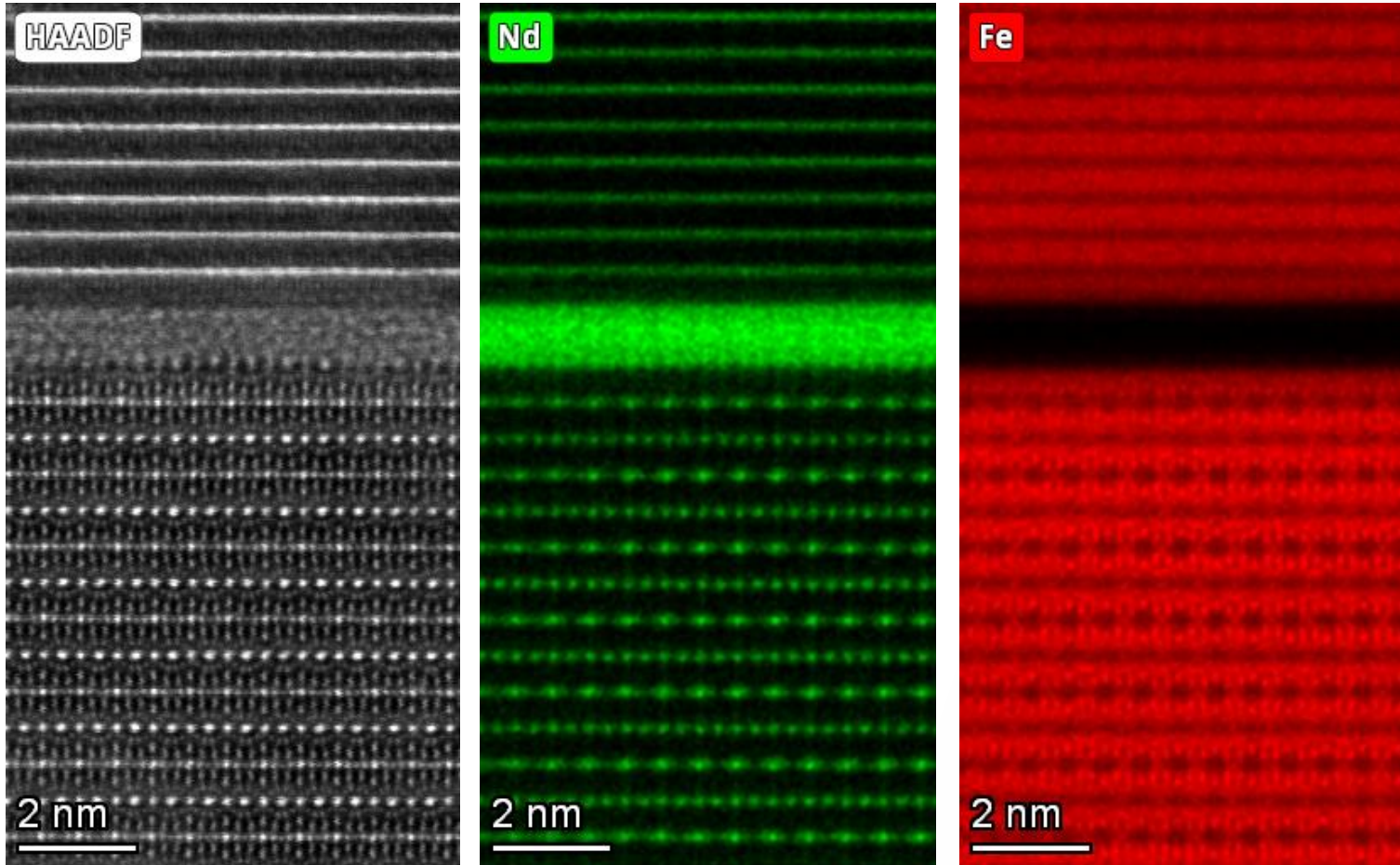
HAADF/iDPC STEM





# Complex Structure Investigation

$\text{Nd}_2\text{Fe}_{14}\text{B}$

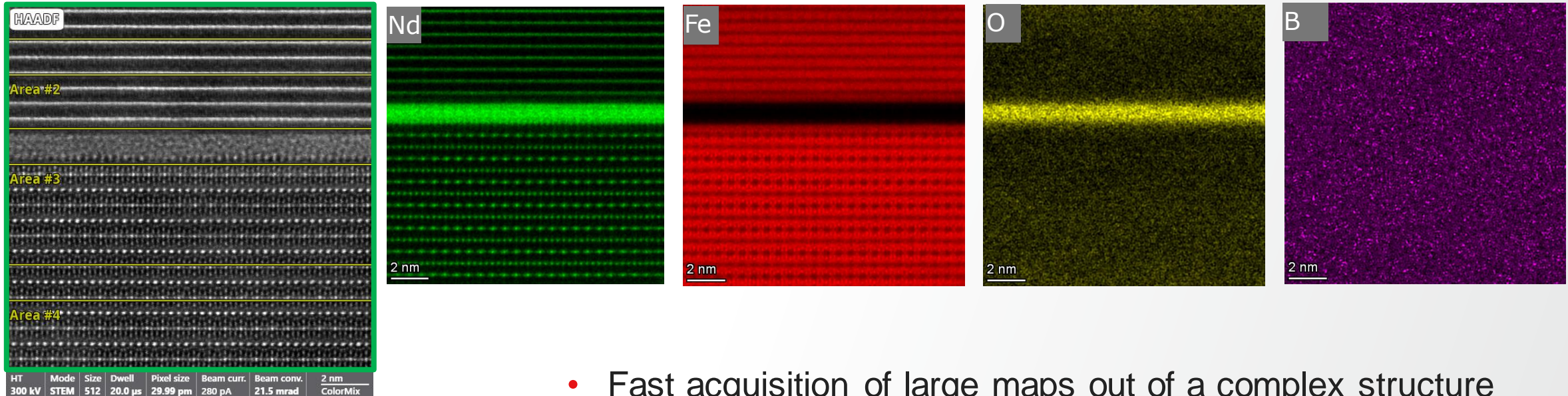


System	Spectra Ultra X-CFEG, 300 kV
Beam current	280 pA
Acquisition time	9' 18"
Filter	Gaussian & radial Wiener Filter

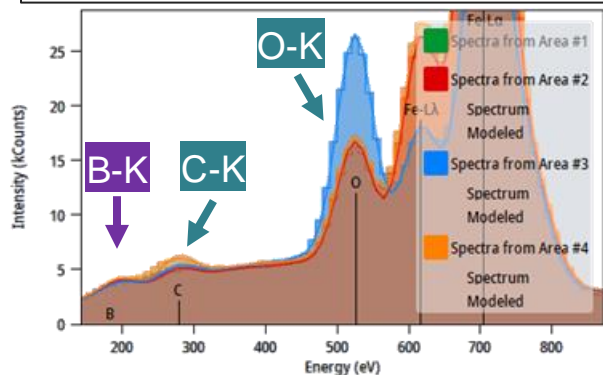
- Fast acquisition of large maps out of a complex structure

# Complex Structure Investigation

$\text{Nd}_2\text{Fe}_{14}\text{B}$



Extracted EDX spectra from regions 2, 3 and 4



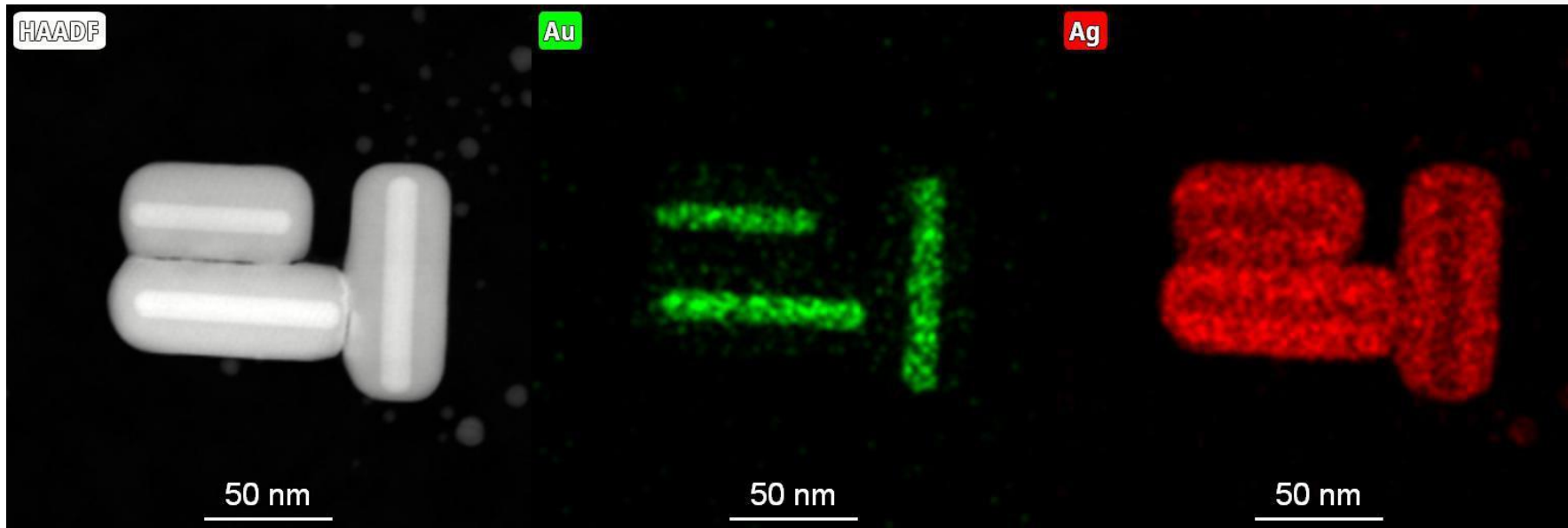
No significant variation of boron content is observed across the material.

- Fast acquisition of large maps out of a complex structure and lightweight elements sensitivity.



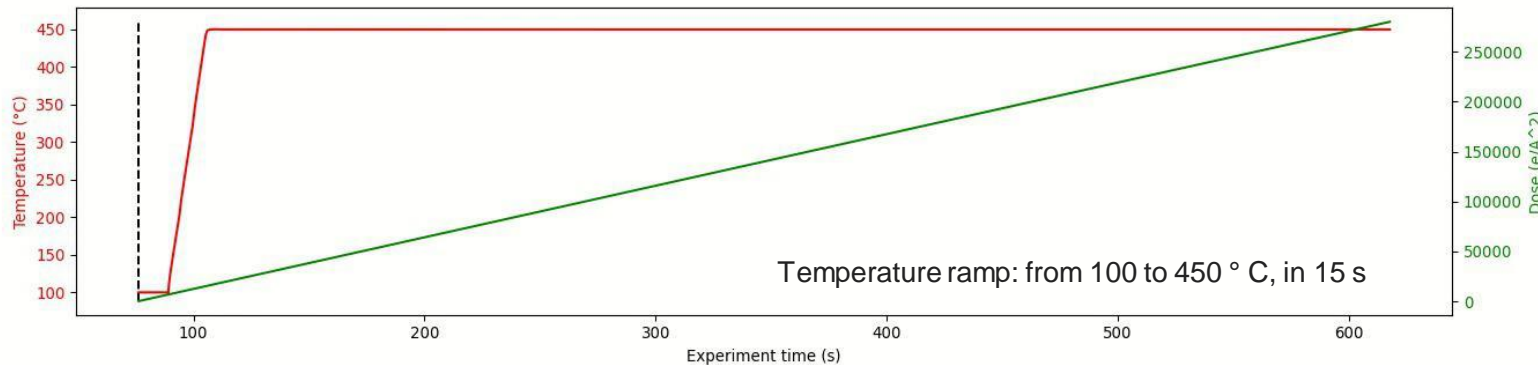
# Dynamic in situ EDS

## Au-Ag Nanorods in situ Heating Experiments



System	Spectra Ultra X-CFEG, 300 kV
In situ holder	DENSolutions Wildfire
Acquisition time	Total: 490 s, <b>EDS frames: 7.8 s each</b>
Maps size	256x256 pixels, pixel 818 pm
Dose per EDS frame	<b>4500 e<sup>-</sup> / A<sup>2</sup></b>
Filter	Gaussian & radial Wiener

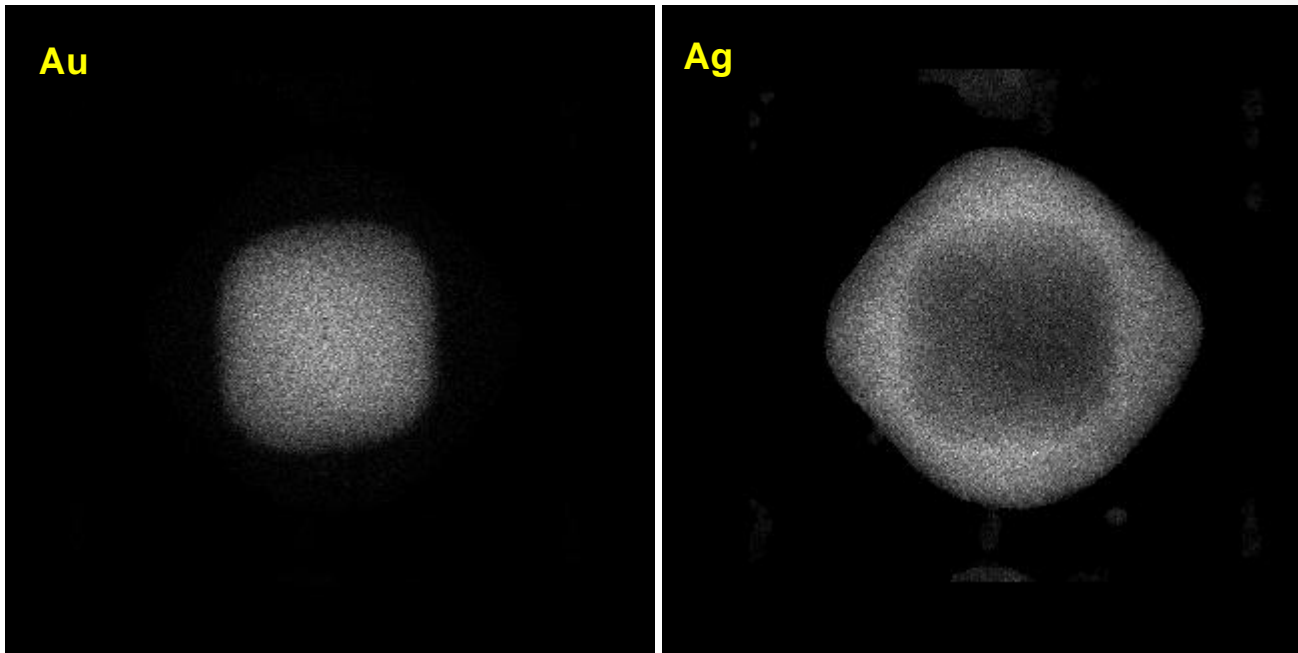
- The Ultra X EDS detector enables TRUE live chemical in situ imaging, fast and with the lowest electron dose.



# EDS Tomography

## AgAu-Octahedral Core-Shell

	HAADF-STEM	EDS maps
HT / Beam Current	200 kV, 150 pA	200 kV, 150 pA
Tilt Range	-72°~72° (3° step)	-70°~70° (10° step)
Size	1024*1024	256*256
Frame time	4 s	5 min
Pixel size	0.193 nm	0.386 nm



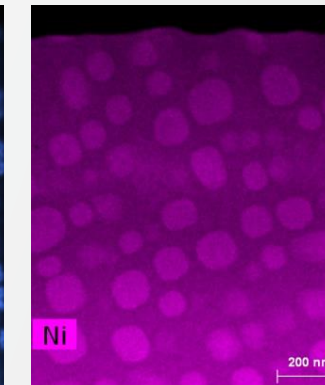
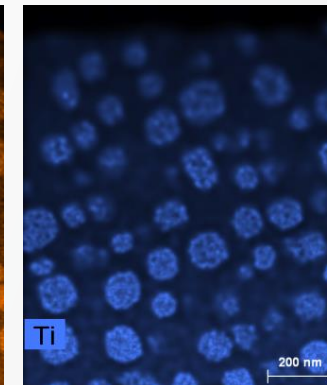
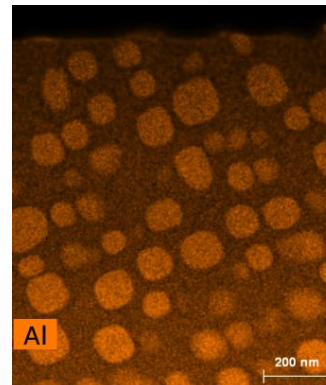
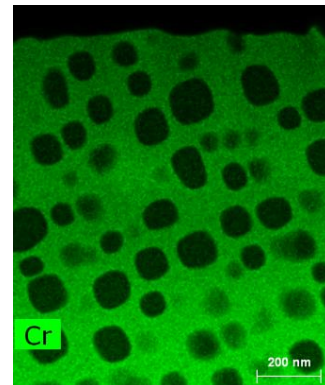
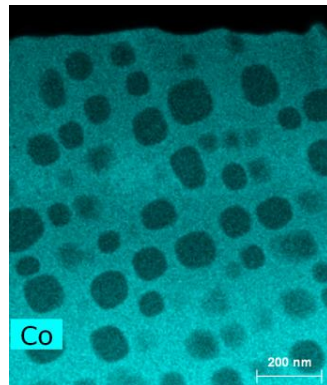
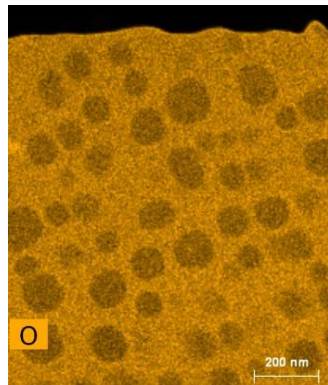
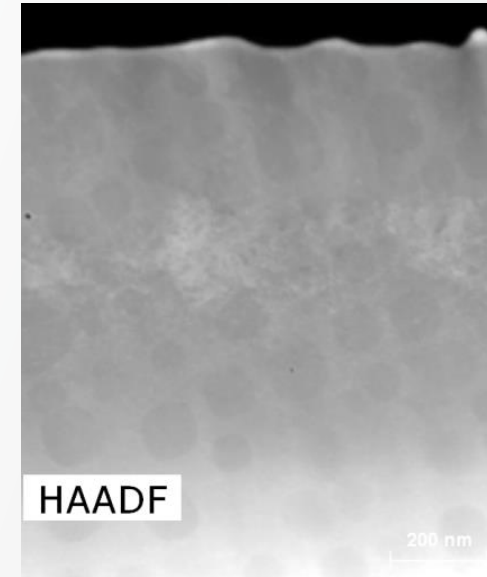
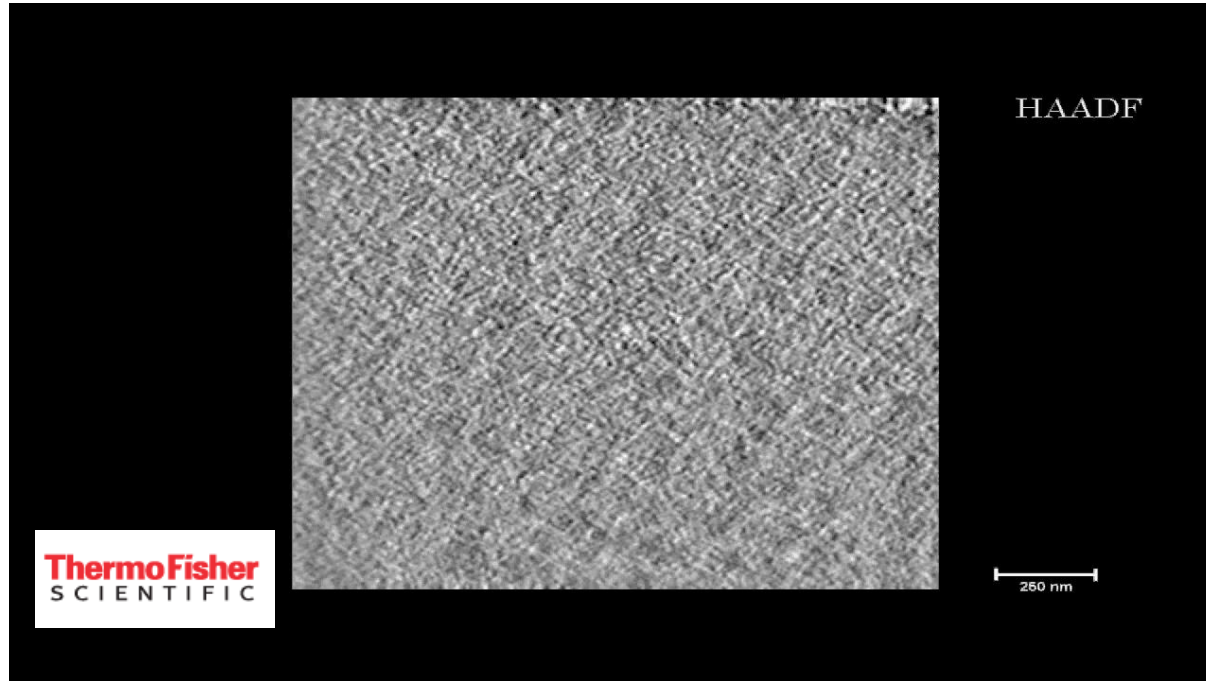
Quantified reconstructed EDS volume

Samples: Prof. Luis Liz-Marzán

Data analysis: Prof. S. Bals

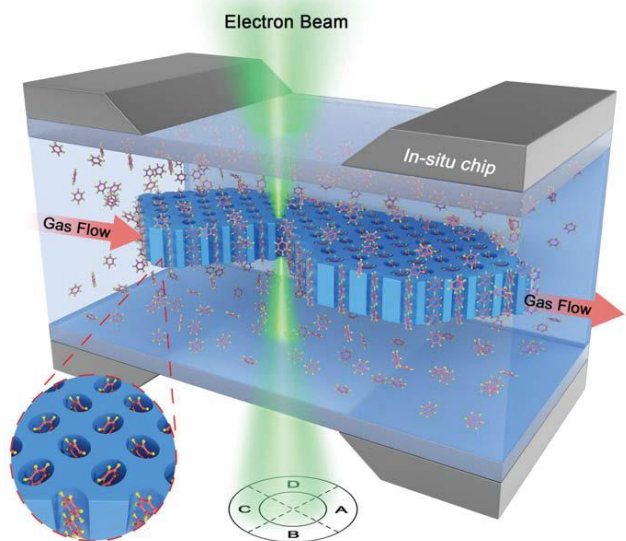
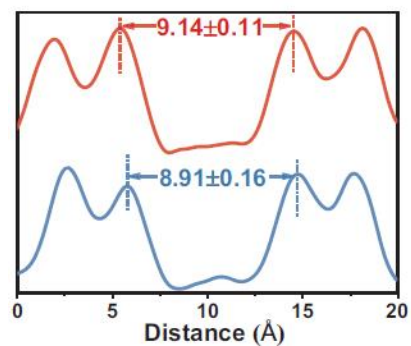
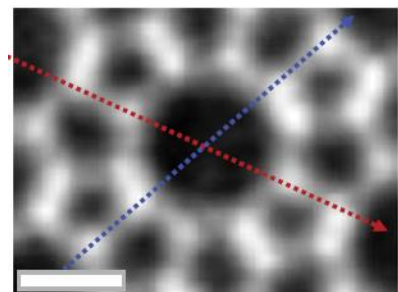
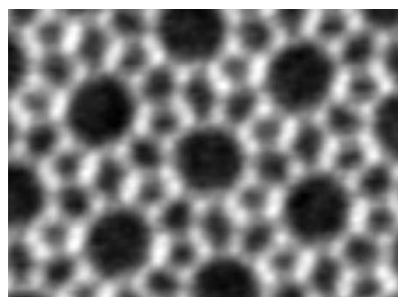
# EDS Tomography

## 3D EDS Compositional Map for the Super Alloy Sample

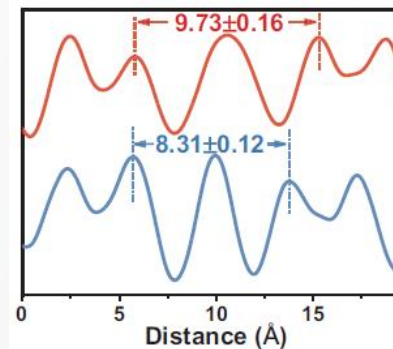
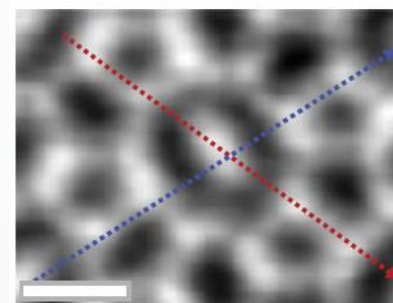
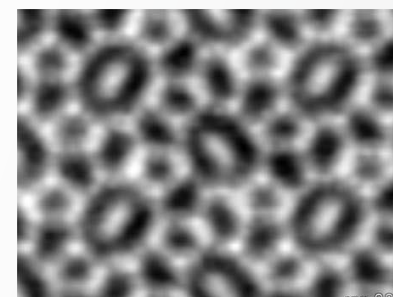


# In Situ iDPC STEM

## High Contrast Low Dose Imaging



The adsorption-induced deformation of the pores, occurring when benzene is flown over the ZSM-5 zeolite, can easily be tracked by live i-DPC.

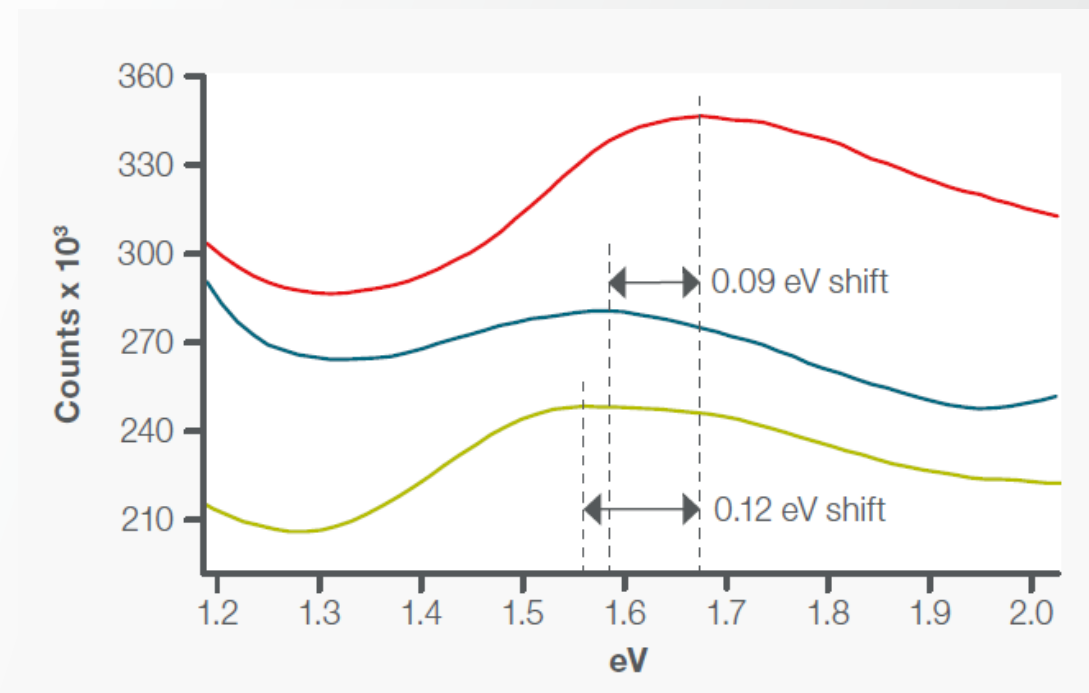


Accelerating Voltage	300 kV
Beam Current	0.1 pA
Total Dose	<b>636 e<sup>-</sup> / Å<sup>2</sup></b>



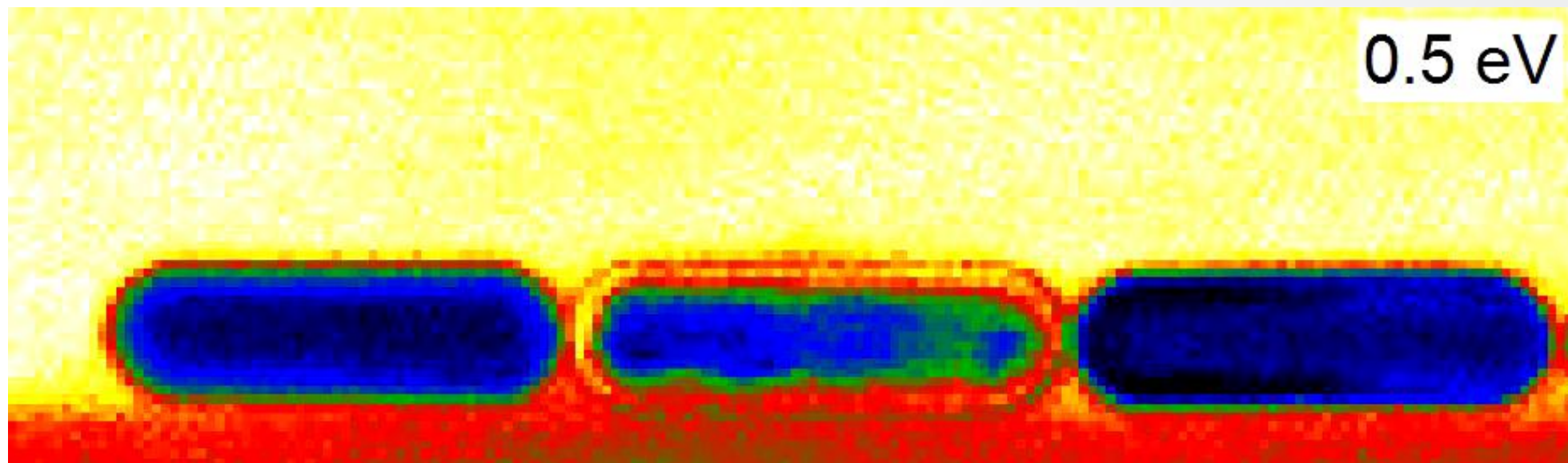
# High-Resolution EELS

## Gold Nanorods: Surface Plasmon Resonances



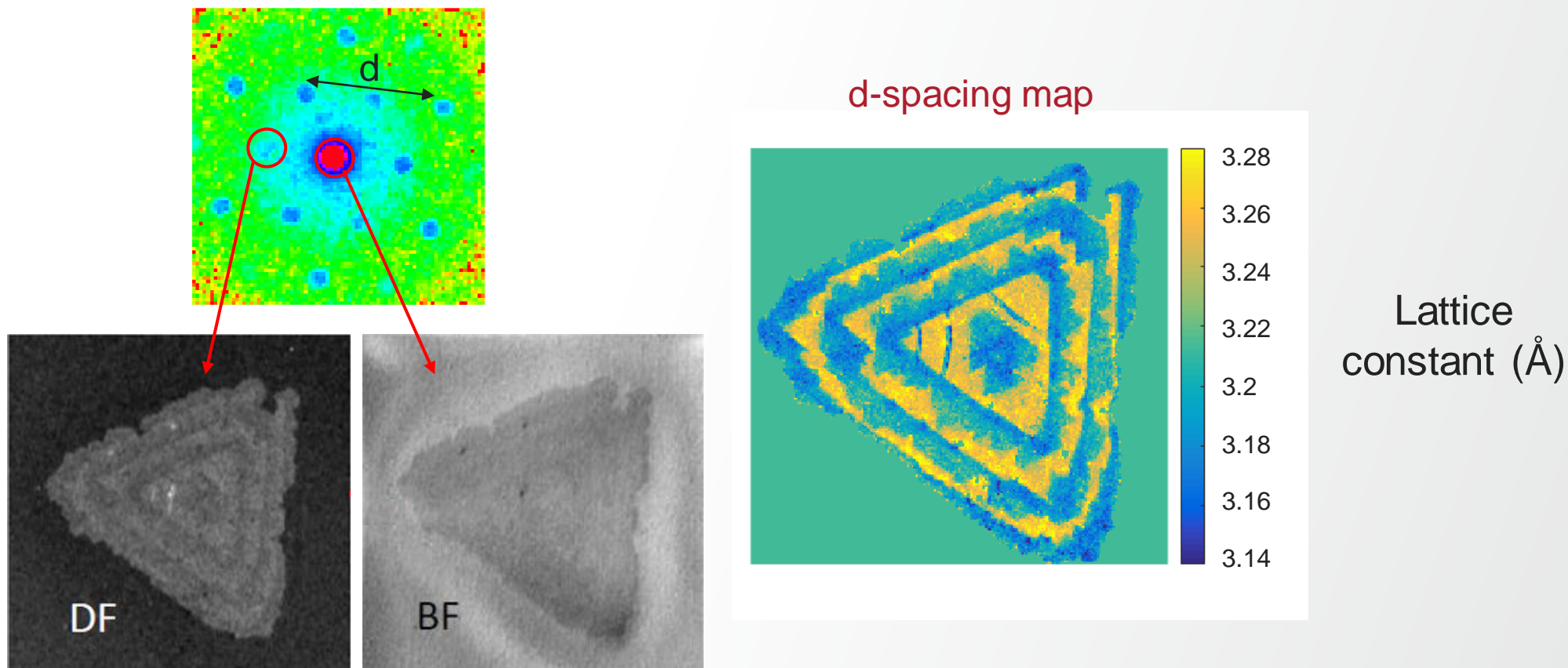
# High-Resolution EELS

## Gold Nanorods: Surface Plasmon Resonances



# Electron Microscope Pixel Array Detector (EMPAD)

EMPAD Diffraction Mapping: Monolayer  $WS_2/WSe_2$  Heterostructure on 10 nm Silicon Nitride



# Thank you





