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Transmission electron microscopy

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7th of May, 2020

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- EDAX mapping

HR-TEM imaging II.

$\Psi(q) = \mathrm{FT}\,\Psi(r) * \mathrm{FT}\,O(r)$

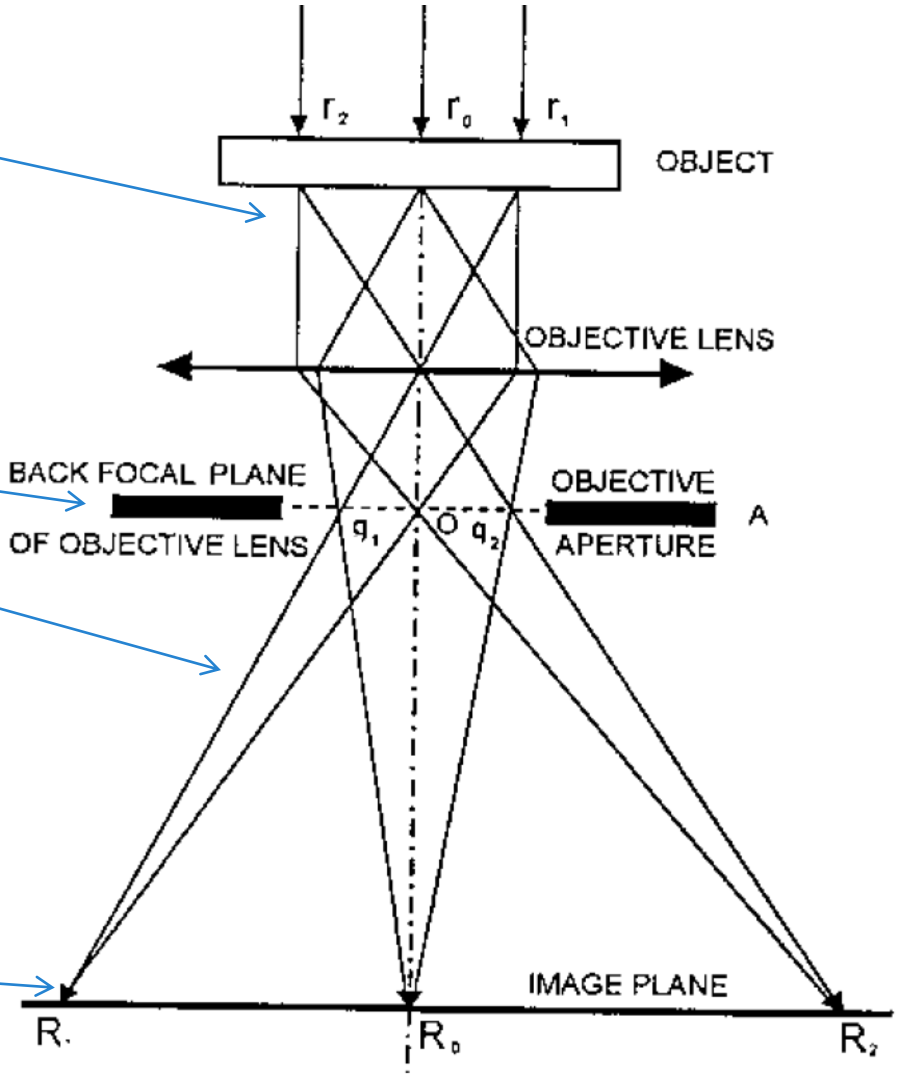
$\Psi^*(q) = A(q)$ konv. $\Psi(q)$

$\Psi_{im}(R) = \mathrm{M.FT}^{-1}\, T(q).\Psi^*(q)$

$T(q) = D(\alpha, \varepsilon, q)\exp(i\chi\,(q))$

$\chi\,(q) = \Delta\,\lambda q^2 + 0.5 C_s \lambda q^4$

$I(R) = \left|\,\Psi_{im}(R)\,\right|^2$



Contrast Transfer Function I.

- Determines the capability of microscope to transfer the spatial frequencies from sample to visualizing device:

$$I(r) = |\psi_m(r)|^2 = 1 - K(q)\cos W(q) + L(q)\sin W(q)$$

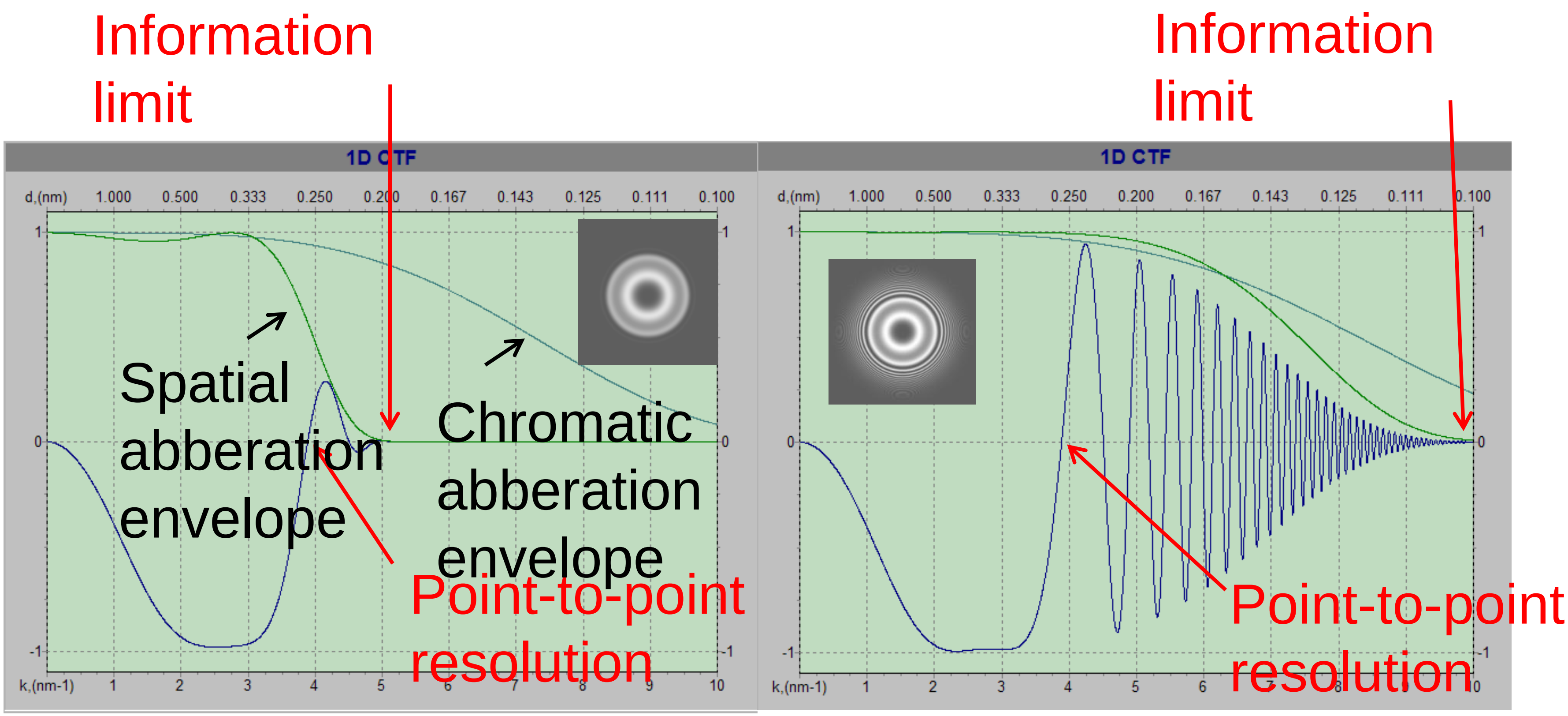
where K(q) is envelope amplitude function

L(q) is envelope frequence function

$$W(q) = \frac{\pi}{2} C_S \lambda^3 q^4 - \pi \Delta f \lambda q^2$$

- Where C_s is spherical abberation, q is spatial frequency, λ is wavelenght, Δf is defocus.

Contrast Transfer Function II.

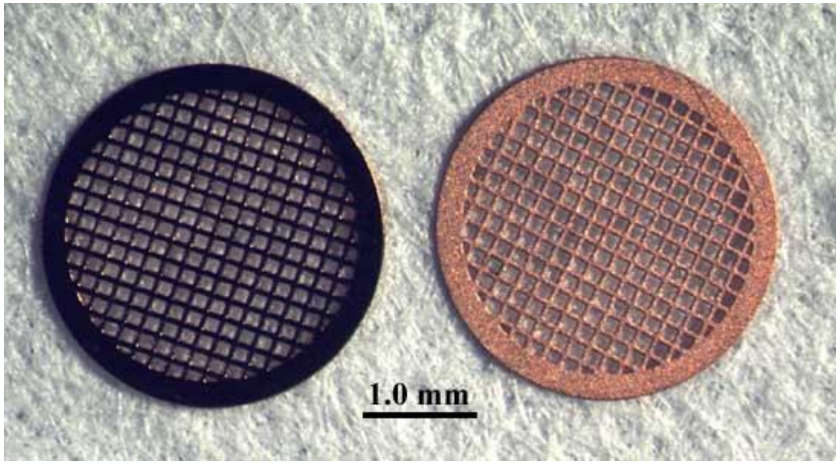


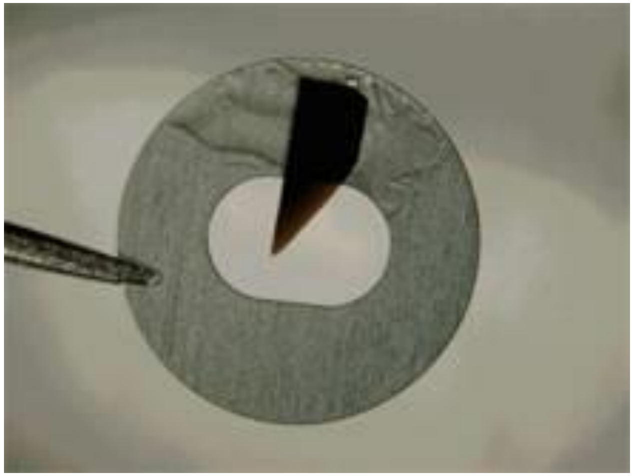
Scherzer defocus – optimal for point-to-point resolution:

$$\Delta f = -1.2\sqrt{C_s \lambda}$$

Specimens

- Thin foils, lamelas or edges of material.
- Thin foils – biologic and metalurgic application.
- Lamelas – semicondutor industry, universities, ...
- Edges – material research, universities, ...
- All of this specimens are worn on support grid.



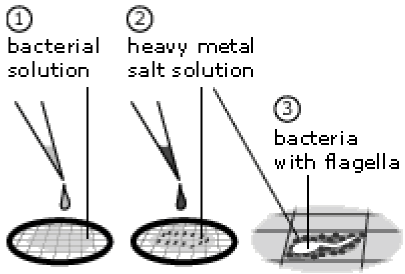


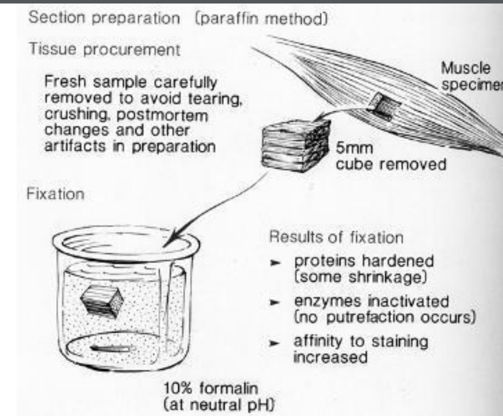
Specimen preparation

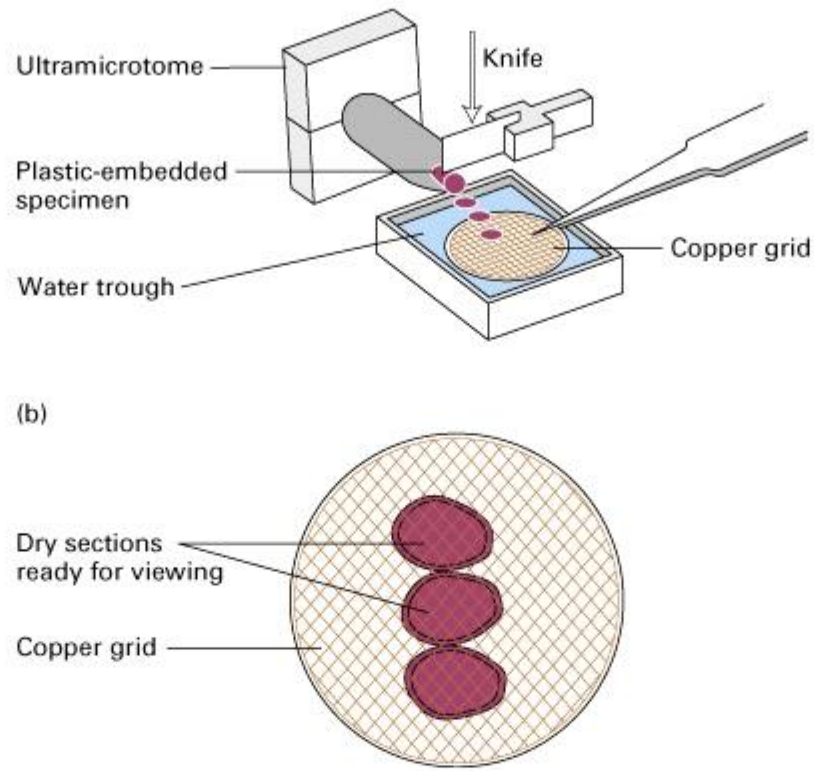
- Biological specimen – epoxy fixing or deep freezing plus microtom slicing, heavy elements marking.
- Metalurgical specimen – rolling and argon etching.
- Semiconductor specimen – acid and argon etching, SDB.
- Other – replicas, ...

- Not easy process, handy experience and knowledge required.

Bio samples preparation







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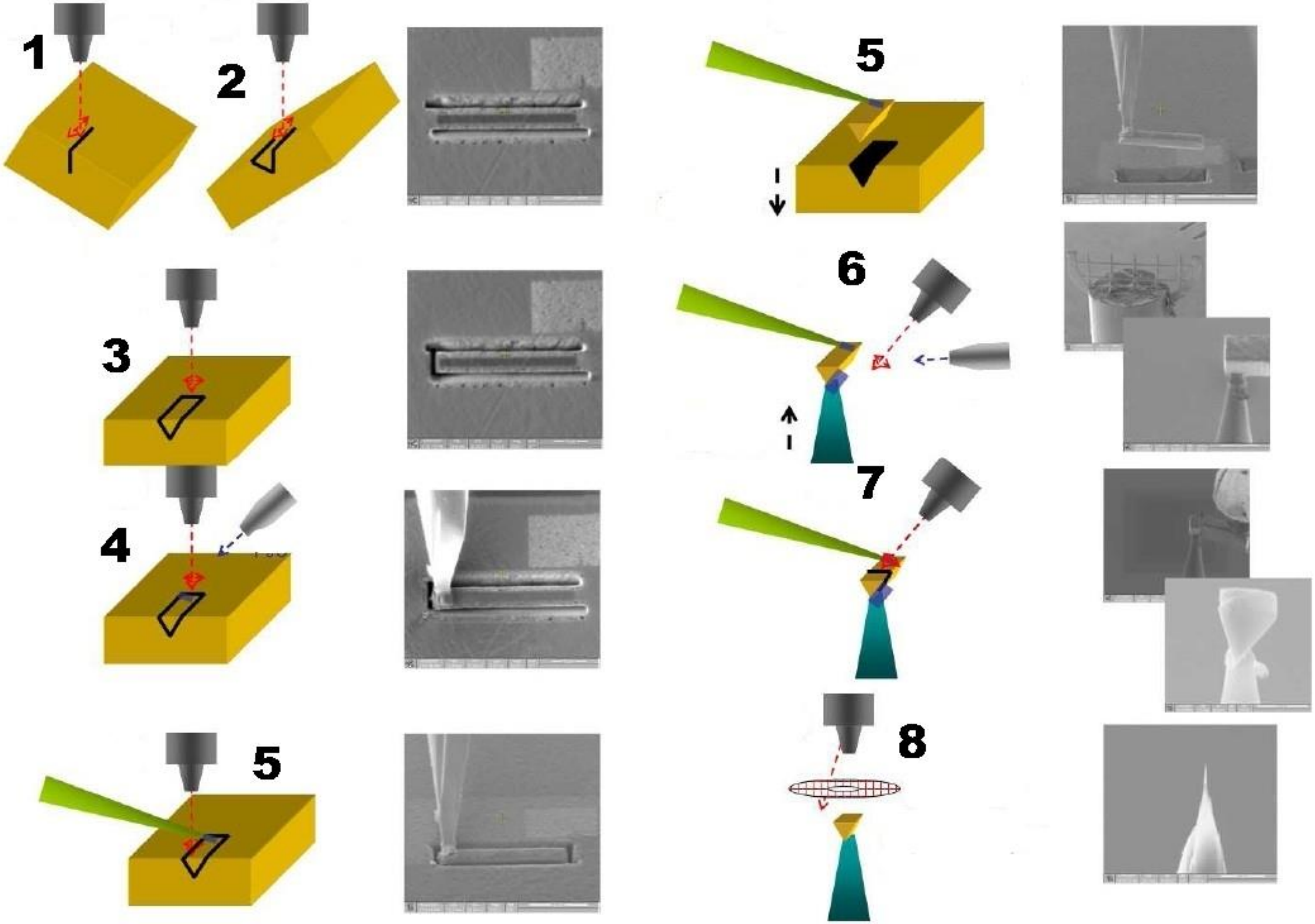
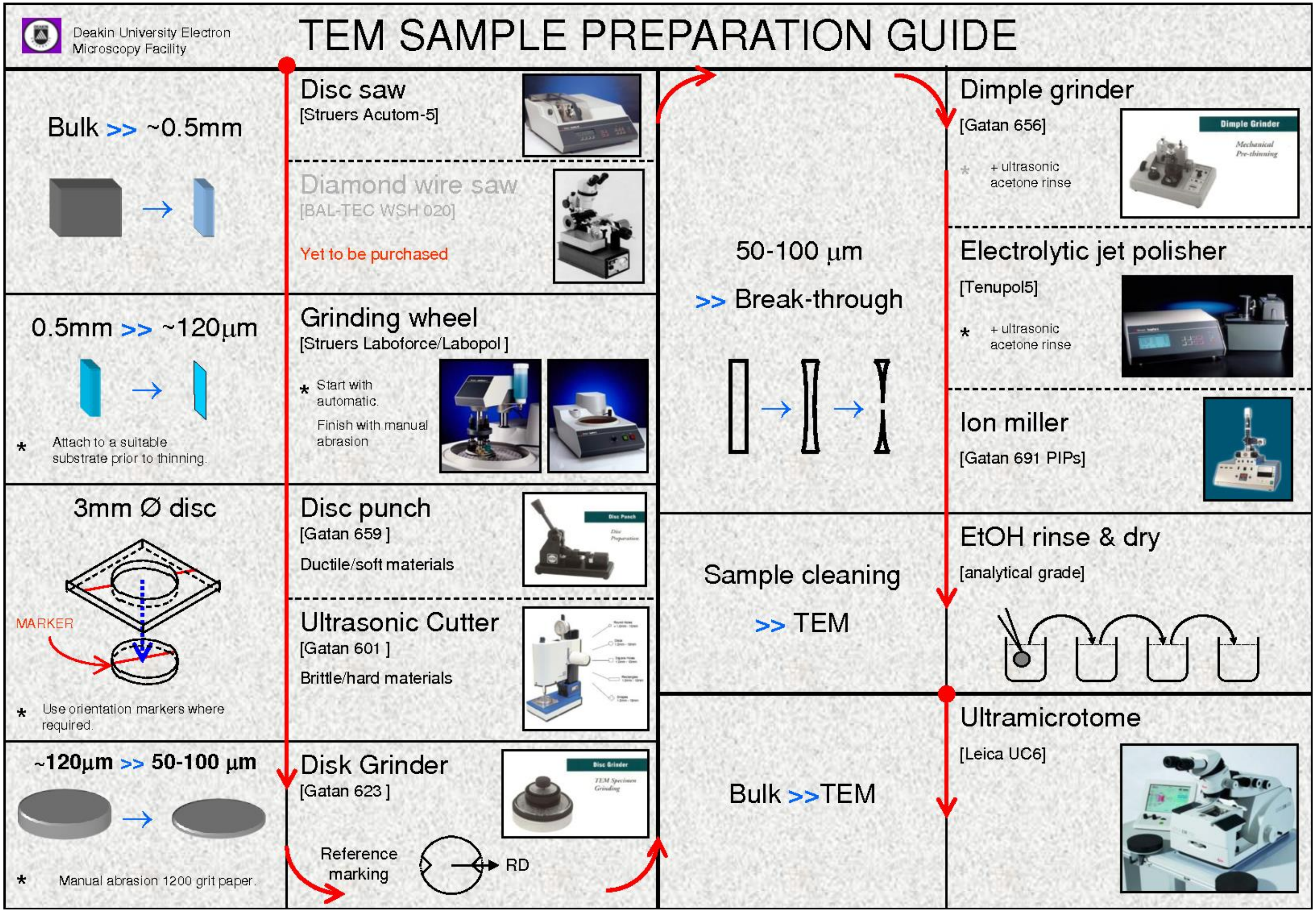
9 Lamelas preparation

INLO 2013 revision 3.mp4

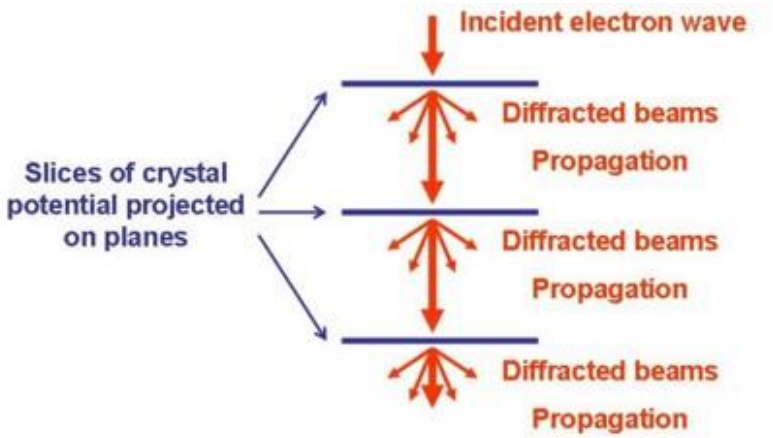


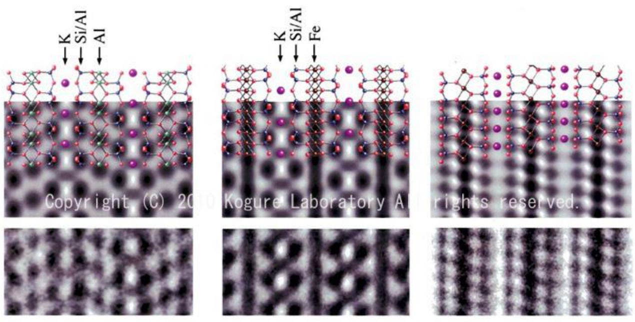
Material science – standard method



HR-TEM imaging I.

- Image is created as interaction of electron beam with specimen and microscope optic including its abberation and phase shifts.
- To calculate exact image wave function is used.
- Interaction of electron with specimen is multiscattered act.





dioctahedral mica (muscovite) [100]

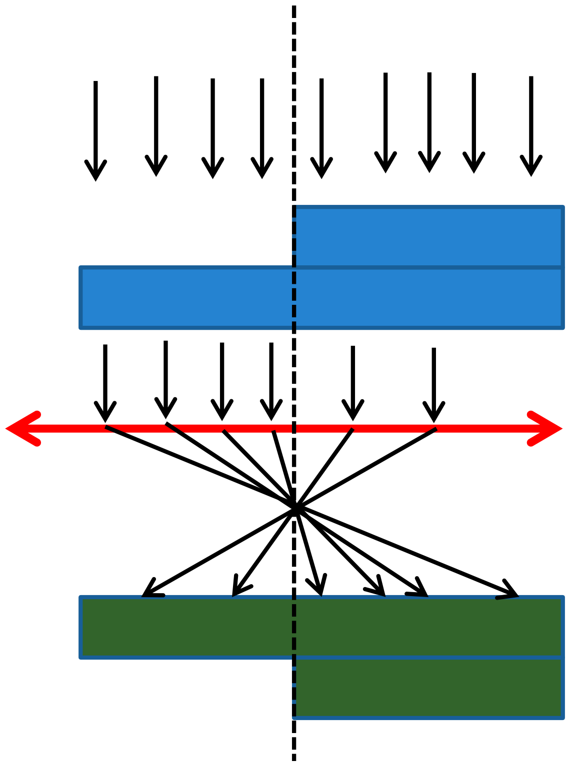
trioctahedral mica (annite) [100]

trioctahedral mica (annite) [010]

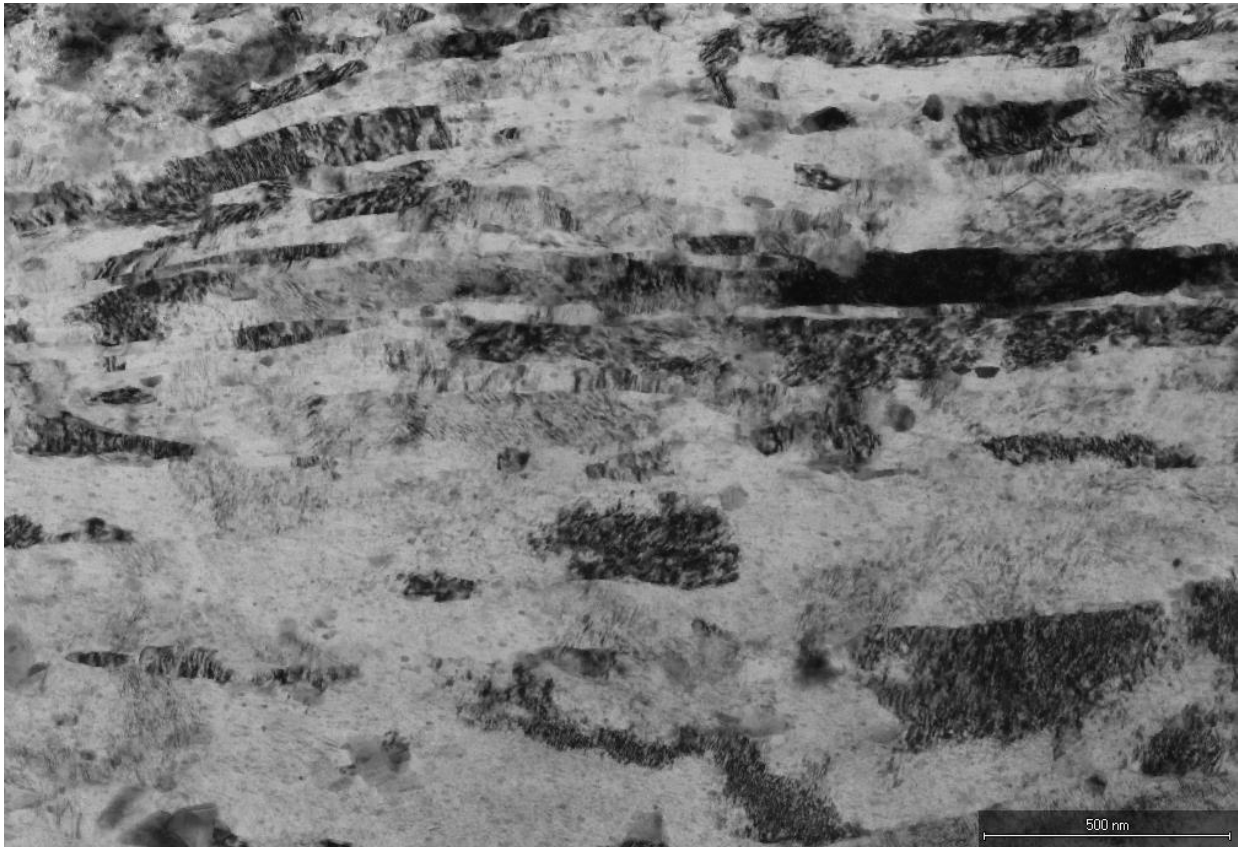
TEM – mass contrast.

- Based on material absorbtion and reflection of electrons.
- Cannot distinguish between material differences and thickness.
- Main role to mag. 100kx.
- Using amplitude envelope of CTF.
- Most usage in biology.

$$I(r) = |\psi_m(r)|^2 = \boxed{1 - K(q)\cos W(q)} + L(q)\sin W(q)$$

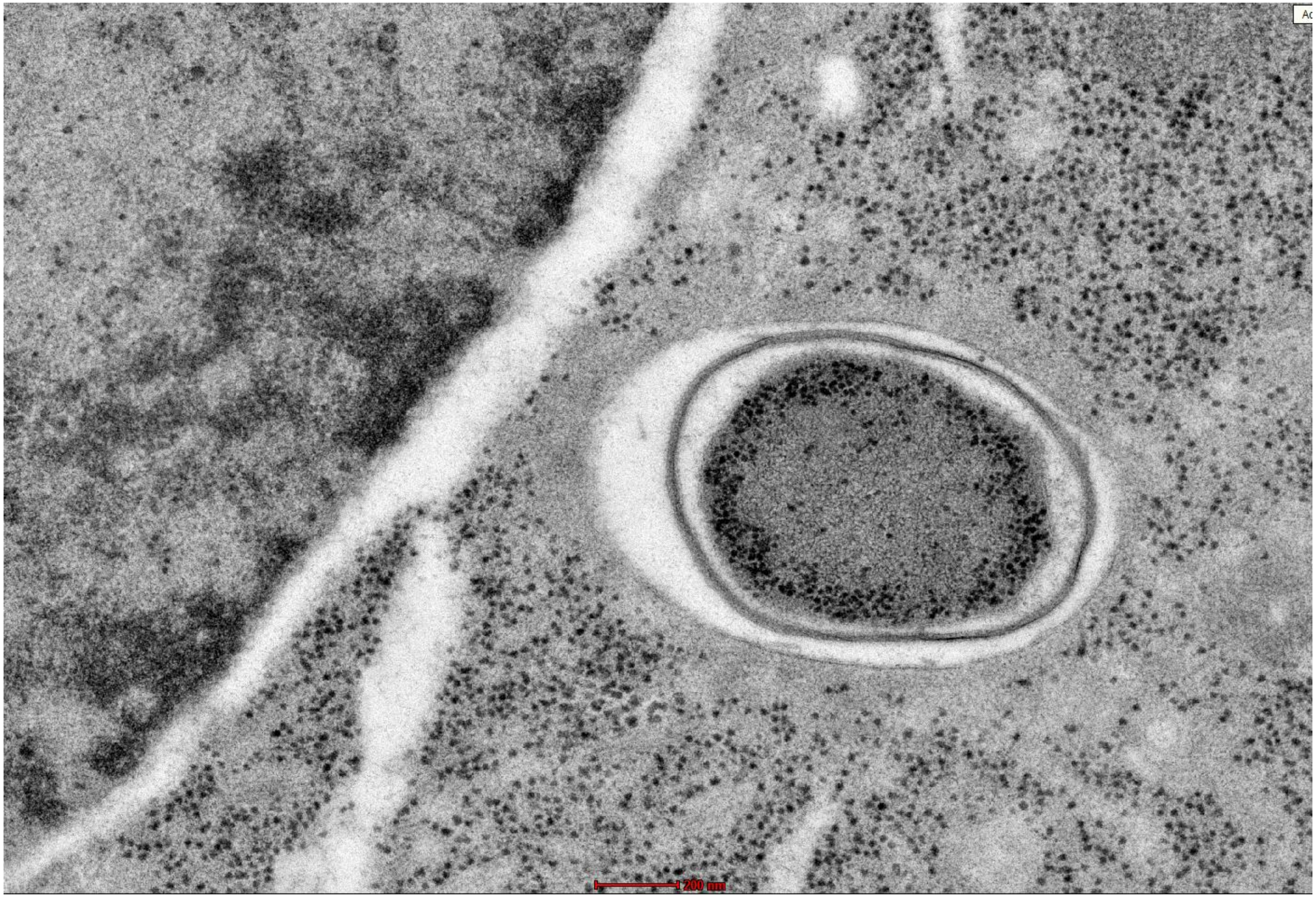


TEM bright Field – Mass contrast



Aluminium 7075

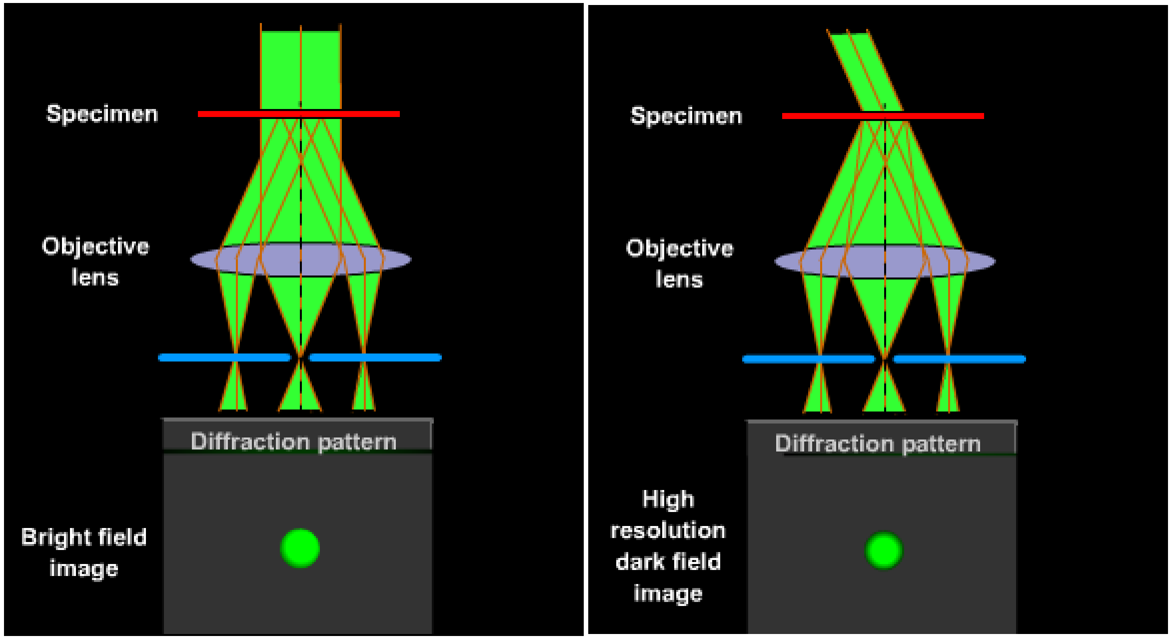
TEM bright Field - BioSpecimen



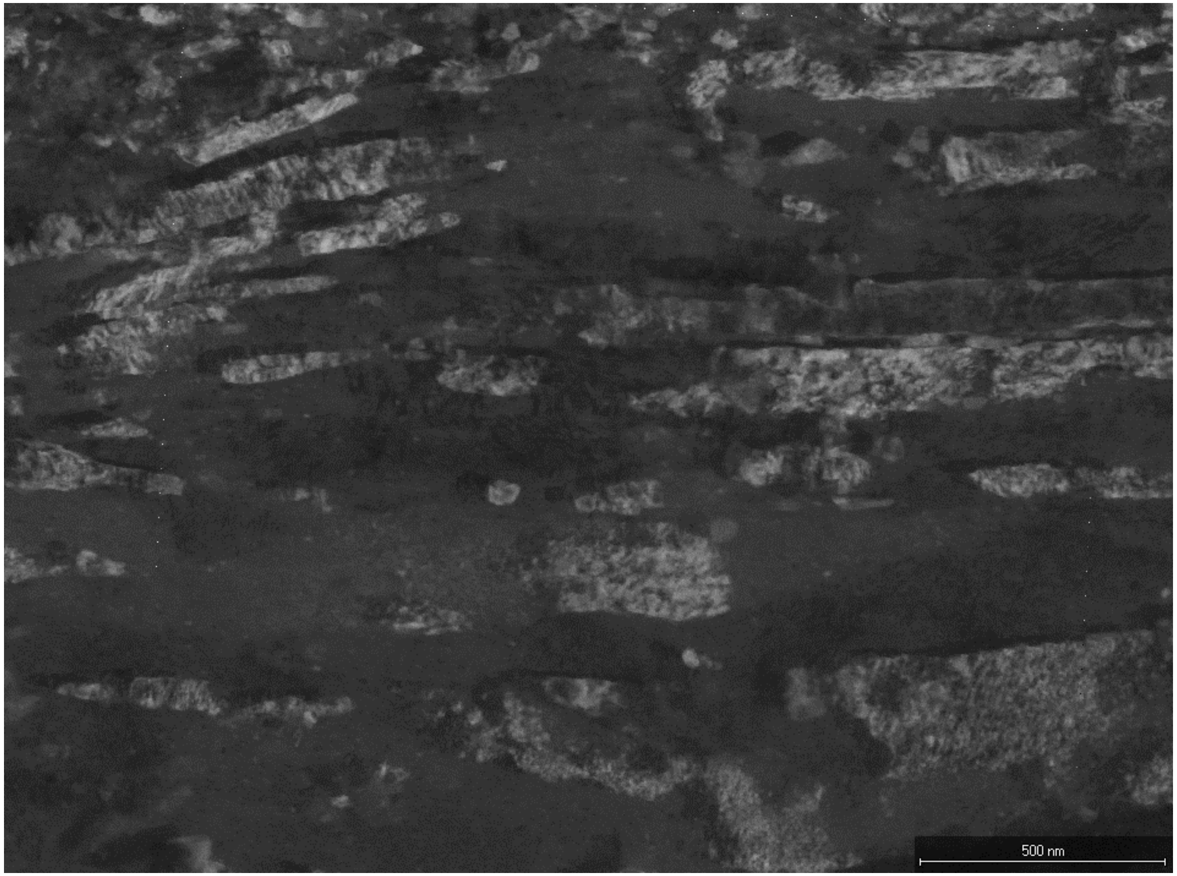
Bakterie syfilisu – *(2017) O.L.Sháněl*

TEM – dark field.

- Exploiting only deflected electrons to enhance the image contrast for different techniques (phase, mass contrast).
- Using tilted illumination and objective apperture.
- Use in defect imaging, HR-TEM.

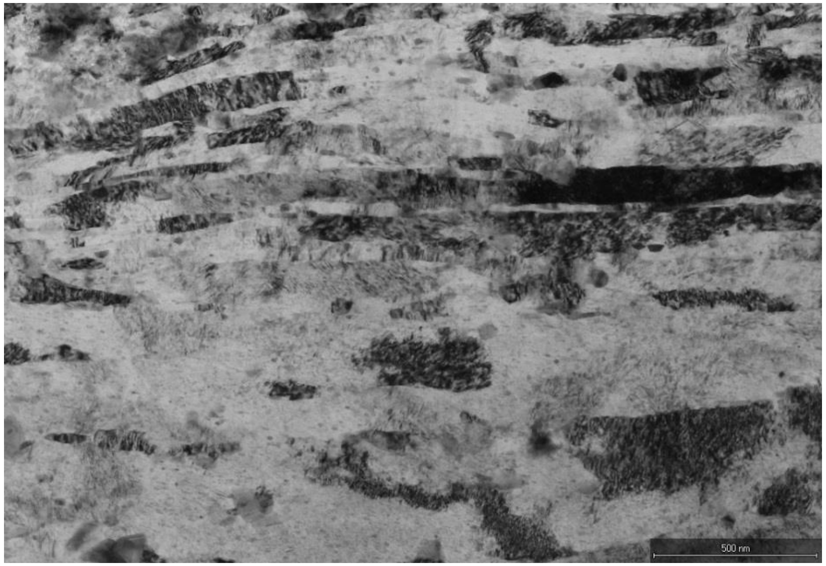


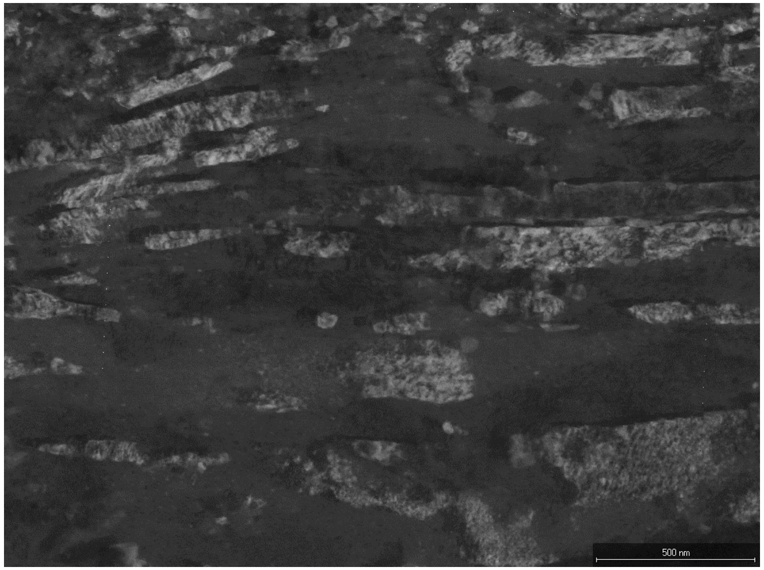
TEM Dark Field – Mass contrast



Aluminium 7075

TEM Bright vs Dark Field

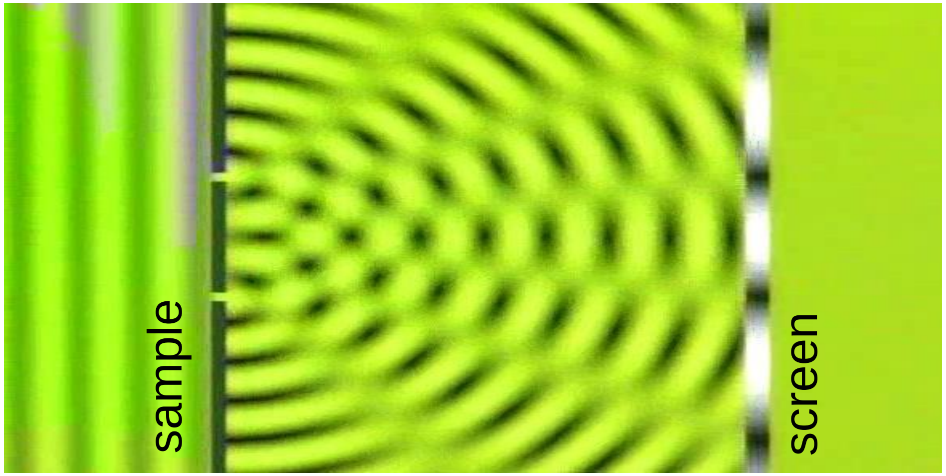




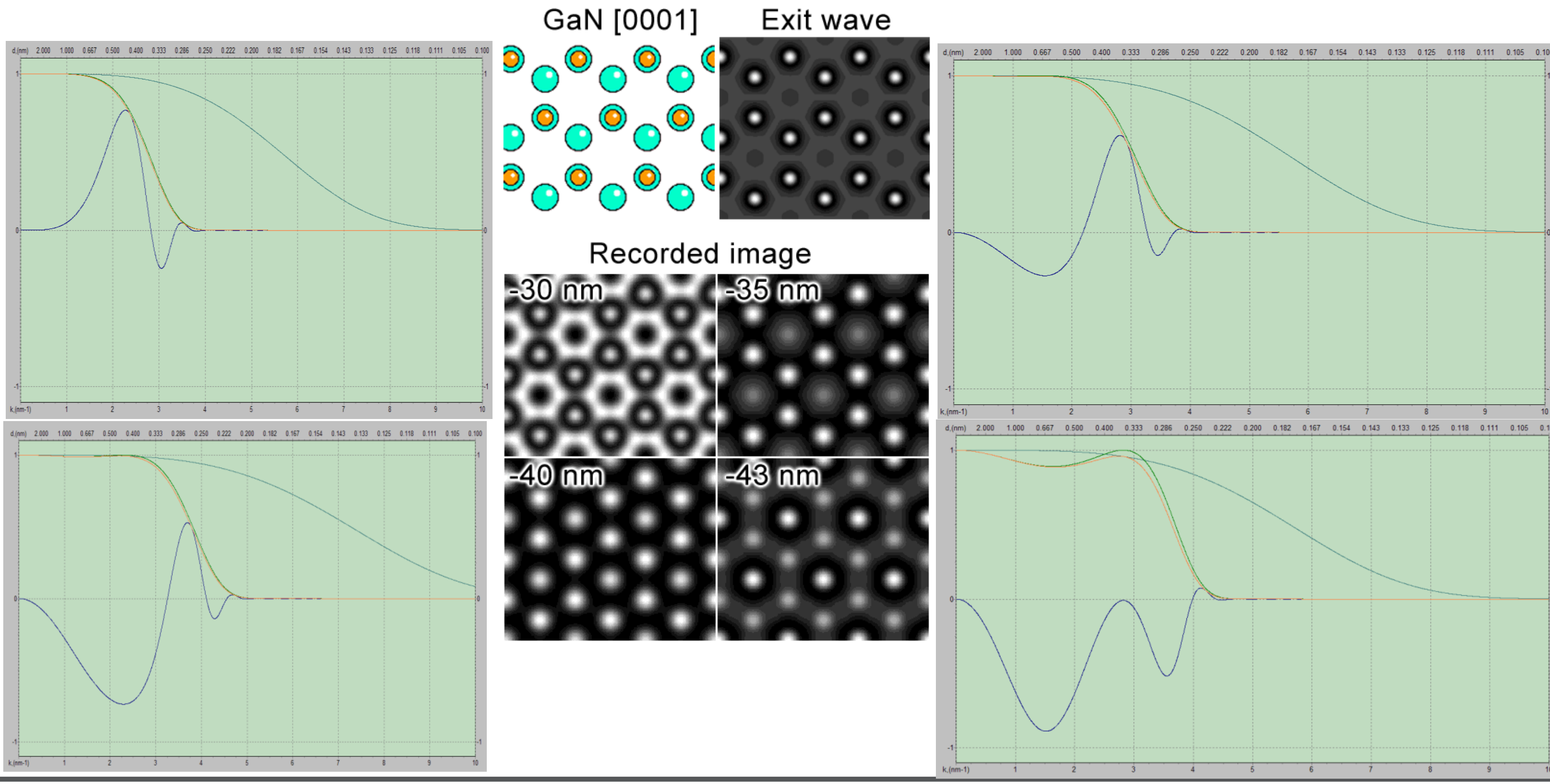
Bright Field - Aluminium 7075 - Dark Field

TEM – phase contrast I.

- Based on electron interference – sample is pattern.
- Using phase part of CFT. $I(r) = |\psi_m(r)|^2 = 1 - K(q)\cos W(q) + \boxed{L(q)\sin W(q)}$
- Main role above magnification 300kx.
- Non-trully atomic resolution – vacancy atoms are not clear visible – only decreasing of intensity is detected.
- This contrast is used in HR-TEM imaging.

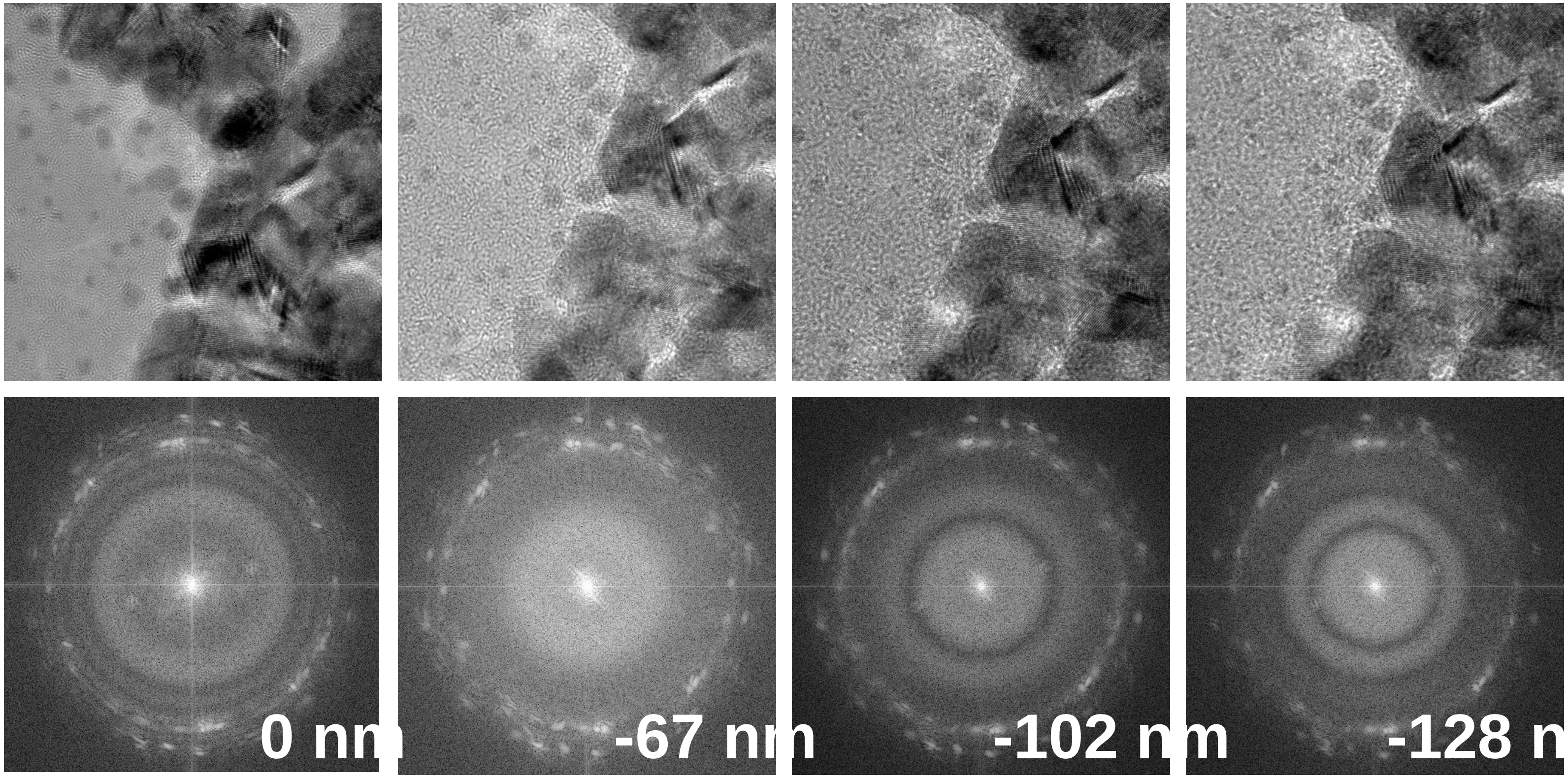


- Interpretation of image is not easy.
- Importance to know what it should be seen – theoretical calculation.

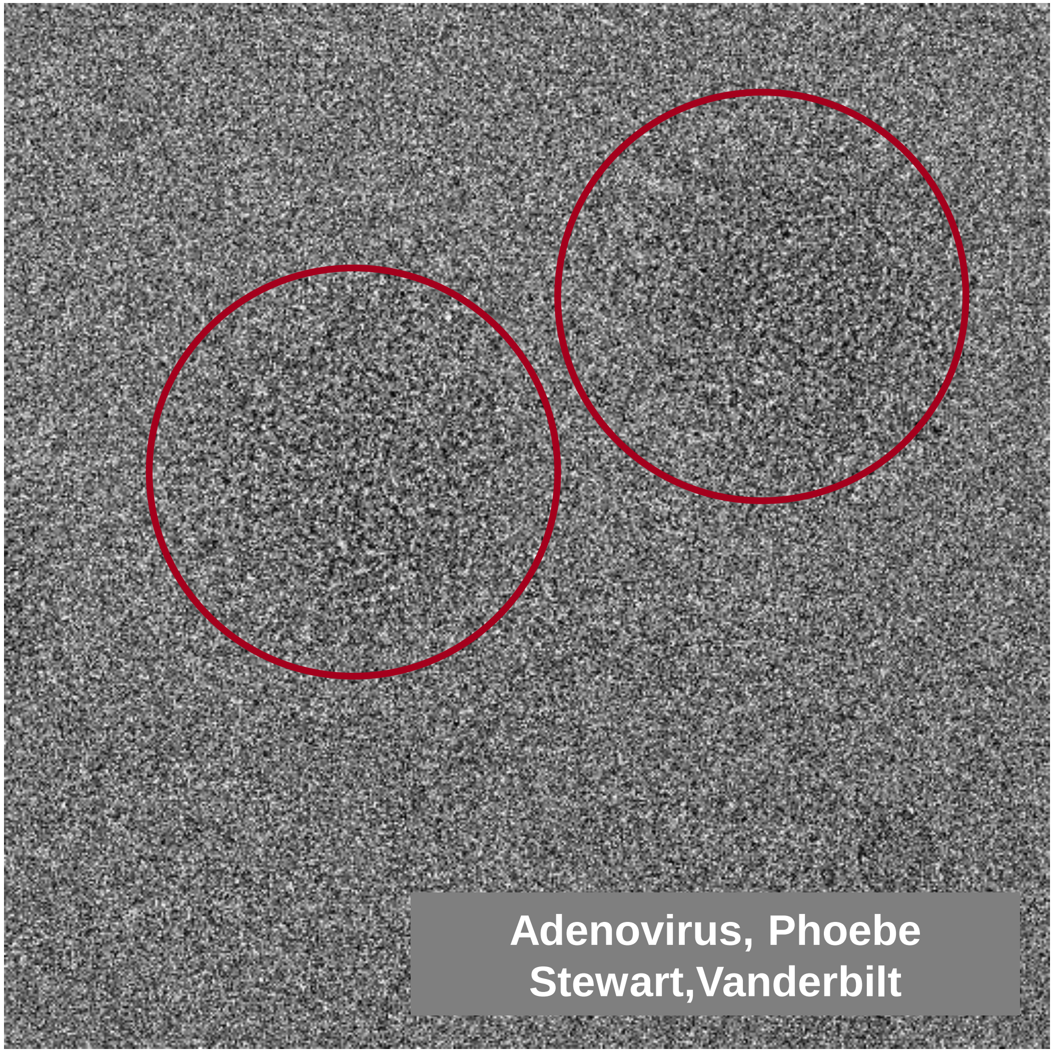


TEM – phase contrast III.

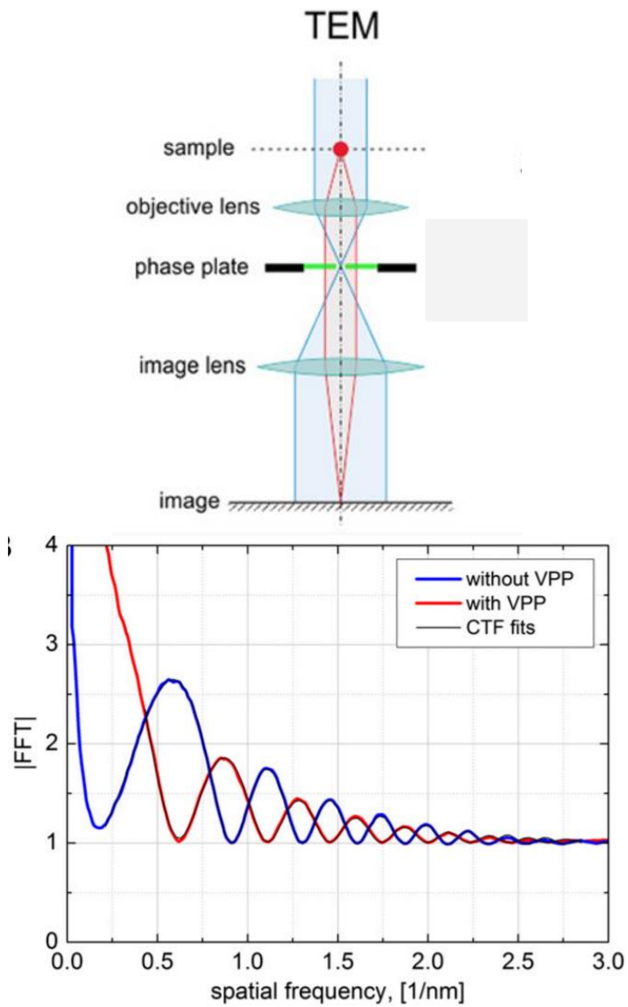
• Gold particles on thin carbon film imaged by 200kV SuperTWIN FEG under different defocus.



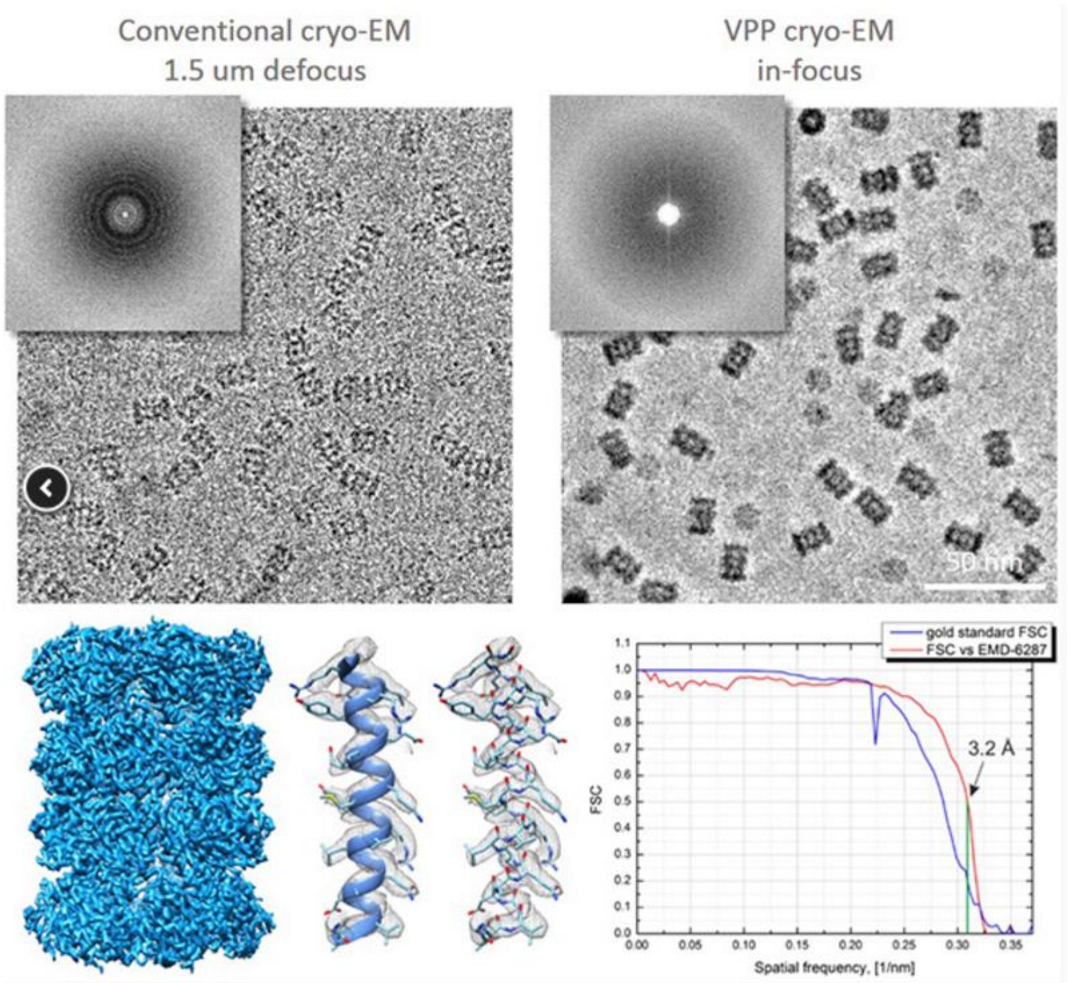
Low Contrast specimen – Phase contrast



Phase Plate



Danev, R. et al. (2014) *PNAS,* 111, 15635



Danev, R. and Baumeister, W. (2016) *eLife,* 5, e13046

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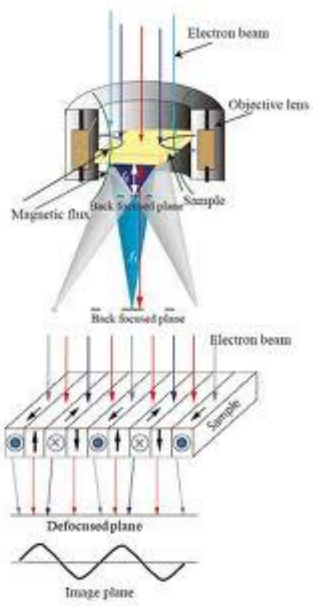
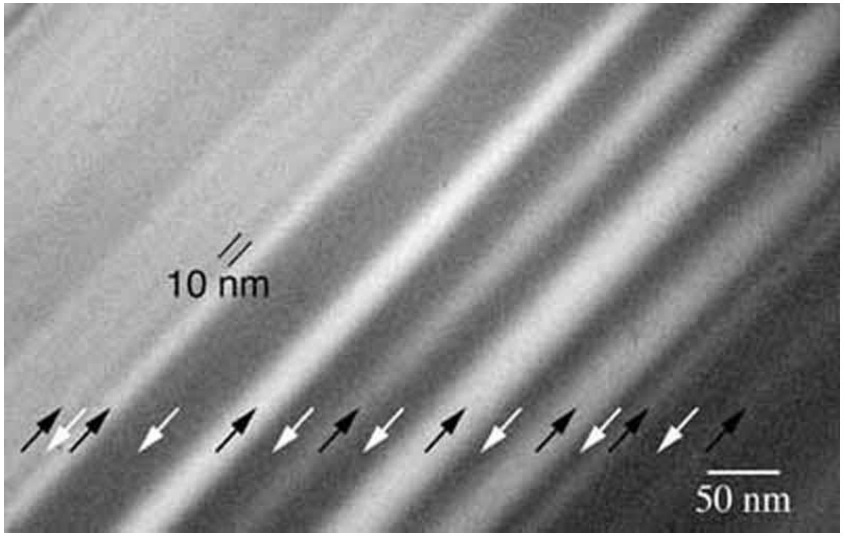
Lorentz microscopy

- To image magnetic or electrostatic specimen.
- Specimen inserted out of magnetic lens field.
- Resolution down to 0.34nm.





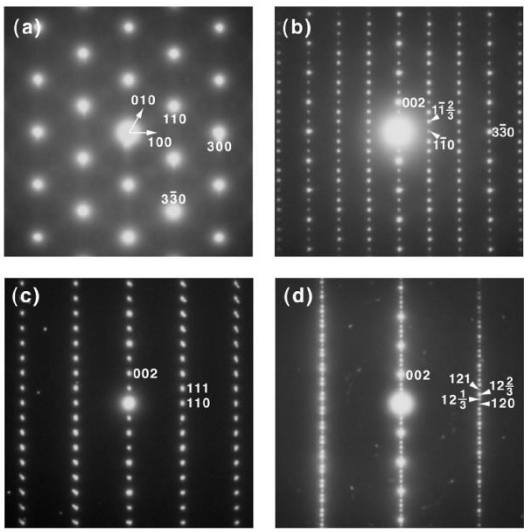
Enviromental TEM

- Specimen is inserted in special capsule where gases can be introduced.
- Maximal pressure varies from 100Pa to 600Pa.
- Resolution 0.344 nm.
- Usage for lifetime experiment observation.
- http://www.youtube.com/watch?v=sHtKG-Z-AVI

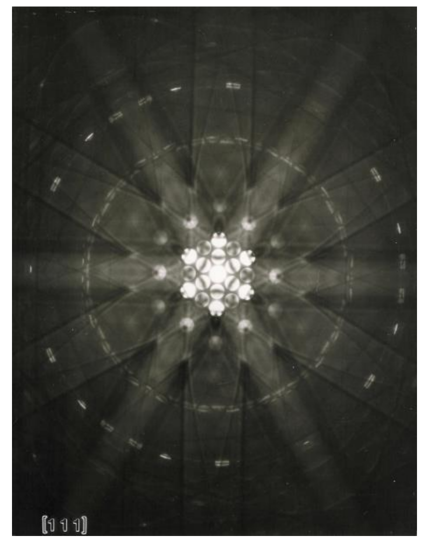
Diffraction

- Focal plane is imaged instead of image itself.
- Two types - SAED – Illumination with plane wave.

  - CBED – Illumination with focused beam.



SAED – YMnO3 (Figure 1: Sets of electron diffraction patterns of $YMn_{0.75}Ti_{0.25}O_3$



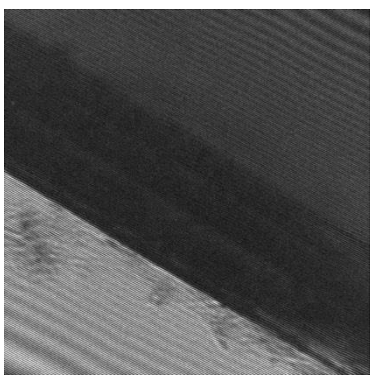
CBED - $Fe_{0.7}Pb_{1.3}Sb_20_7$ (pyrochlore-type) along [111].

TEM – potential contrast.

- Using holography
- Added phase shi

$I_{hol} = 1 + A^2(\vec{r}) + 2\mu A(\vec{r})\cos($

$\varphi(\vec{r}) = C_E V_{proj}(r) - 2\pi\Phi_{mag}(r$



Original Image (called 'the hologram')

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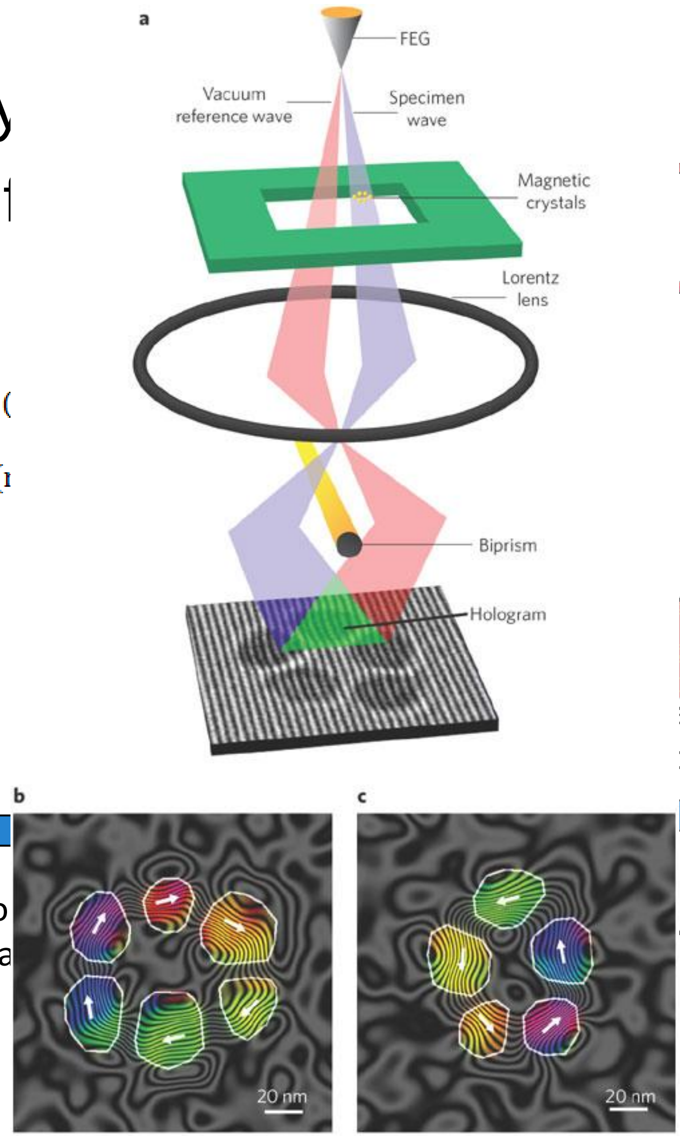

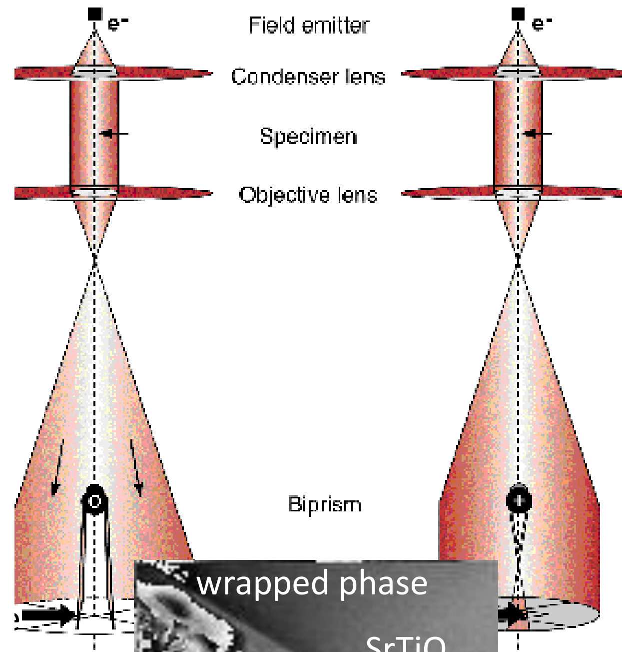

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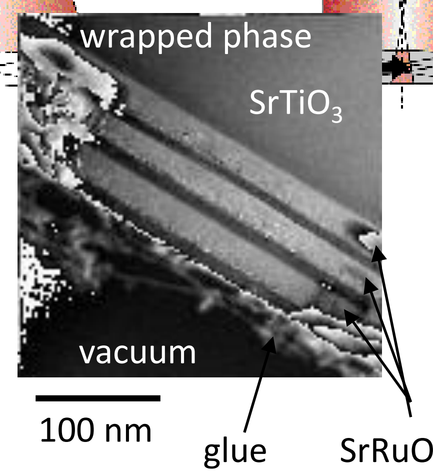
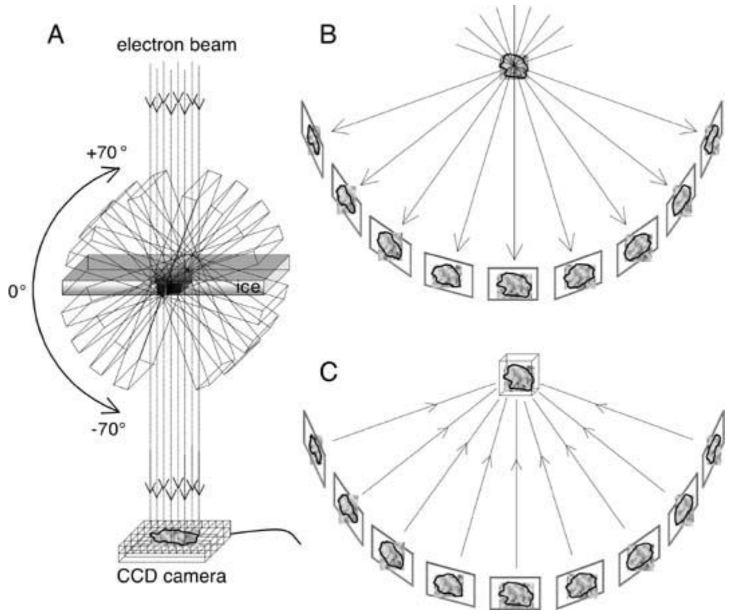
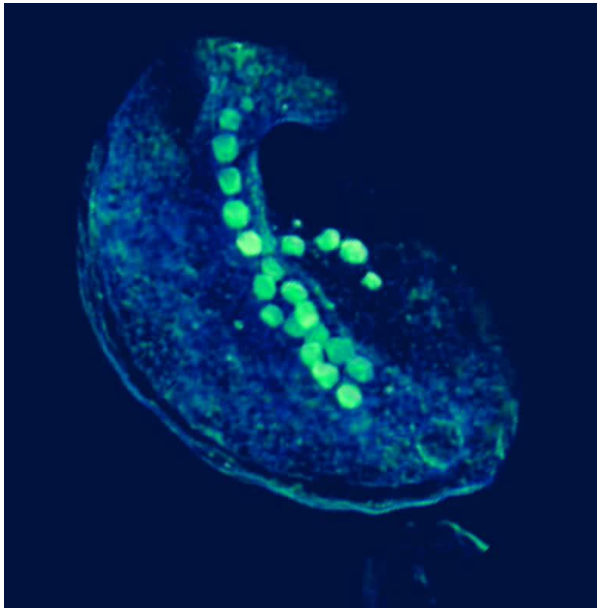


Tomography

- Specimen is imaged under different angles and then is constructed with mathematical module to 3D image.
- Resolution down to 0.5 nm (limited by specimen stage shift and tilt accuracy).

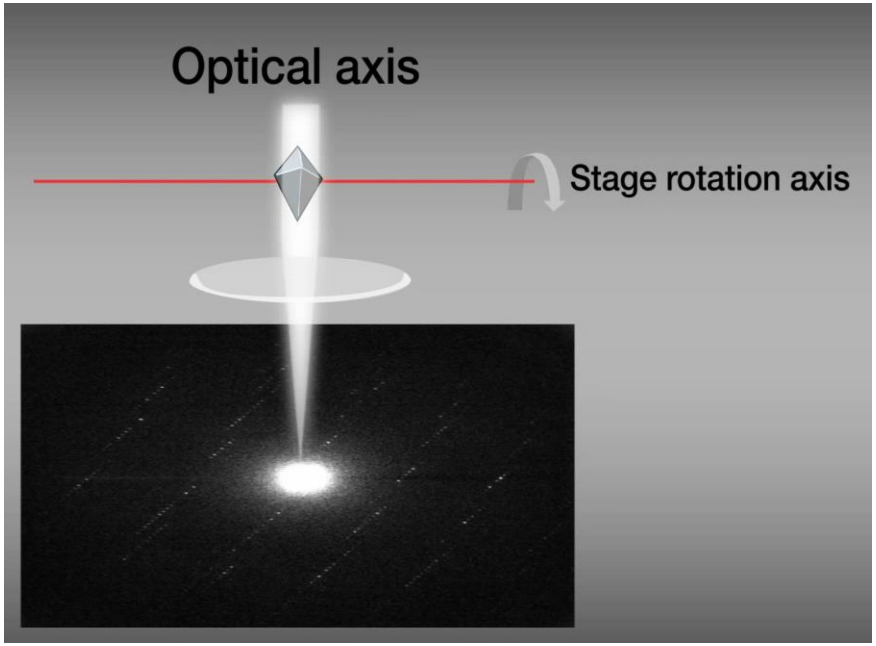




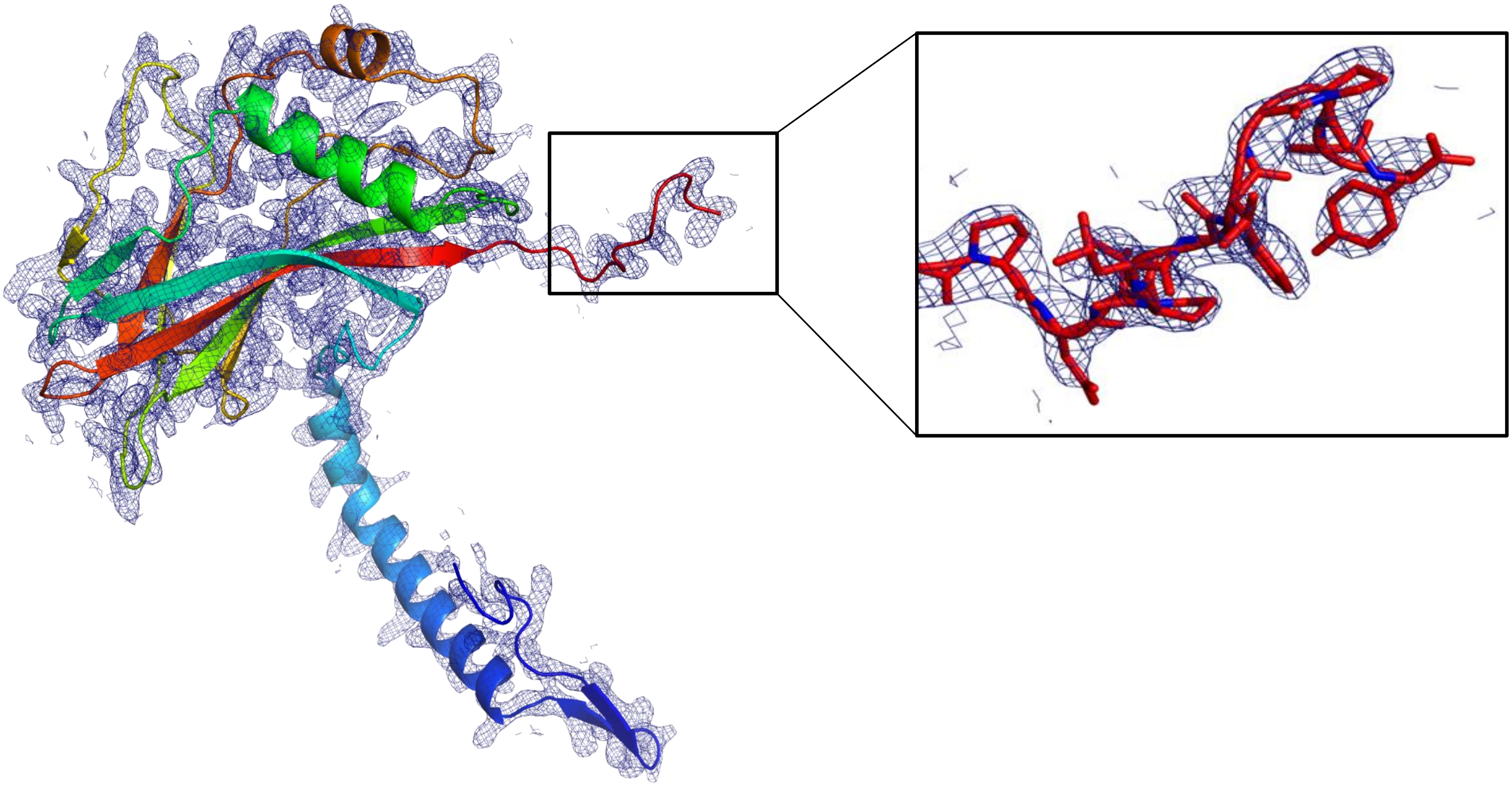
MicroED

Vitrification of small crystals → TEM low-dose screening → TEM diffraction screening → Diffraction tilt series data collection → Reconstruction

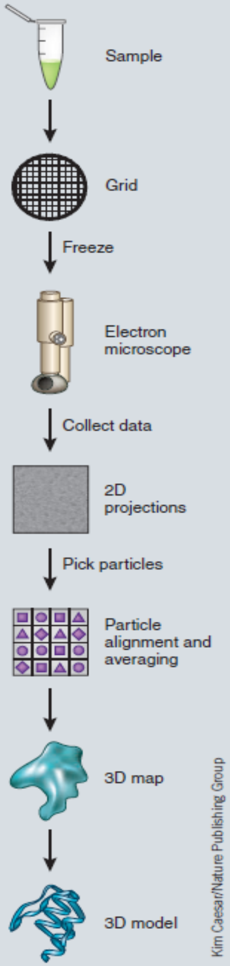
- Small crystals
- Needs few crystals
- Low dose imaging (1.5 - 3 el/Å^2)
- Crystals imaged under cryo-conditions
- Provides high resolution



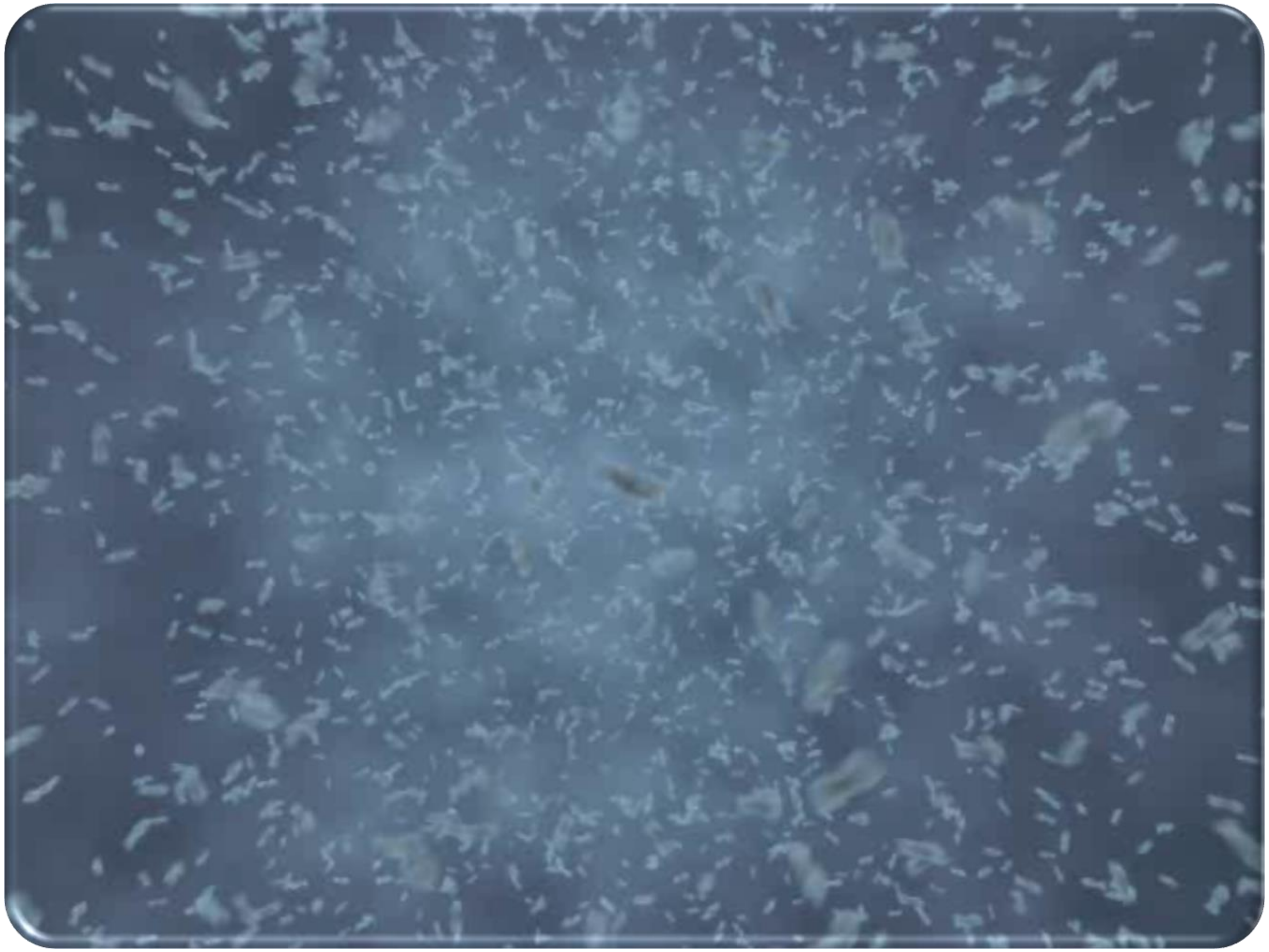
MicroED



Single Particle CryoEM Workflow



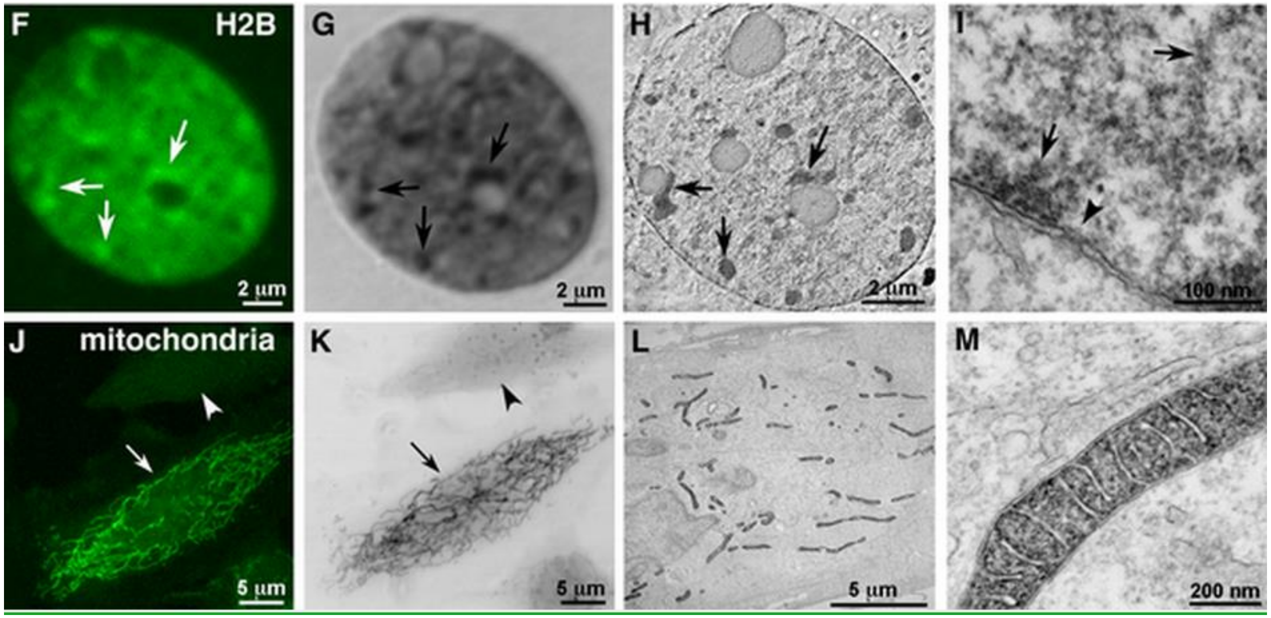
The overall single-particle cryo-EM workflow, from protein sample to 3D model.



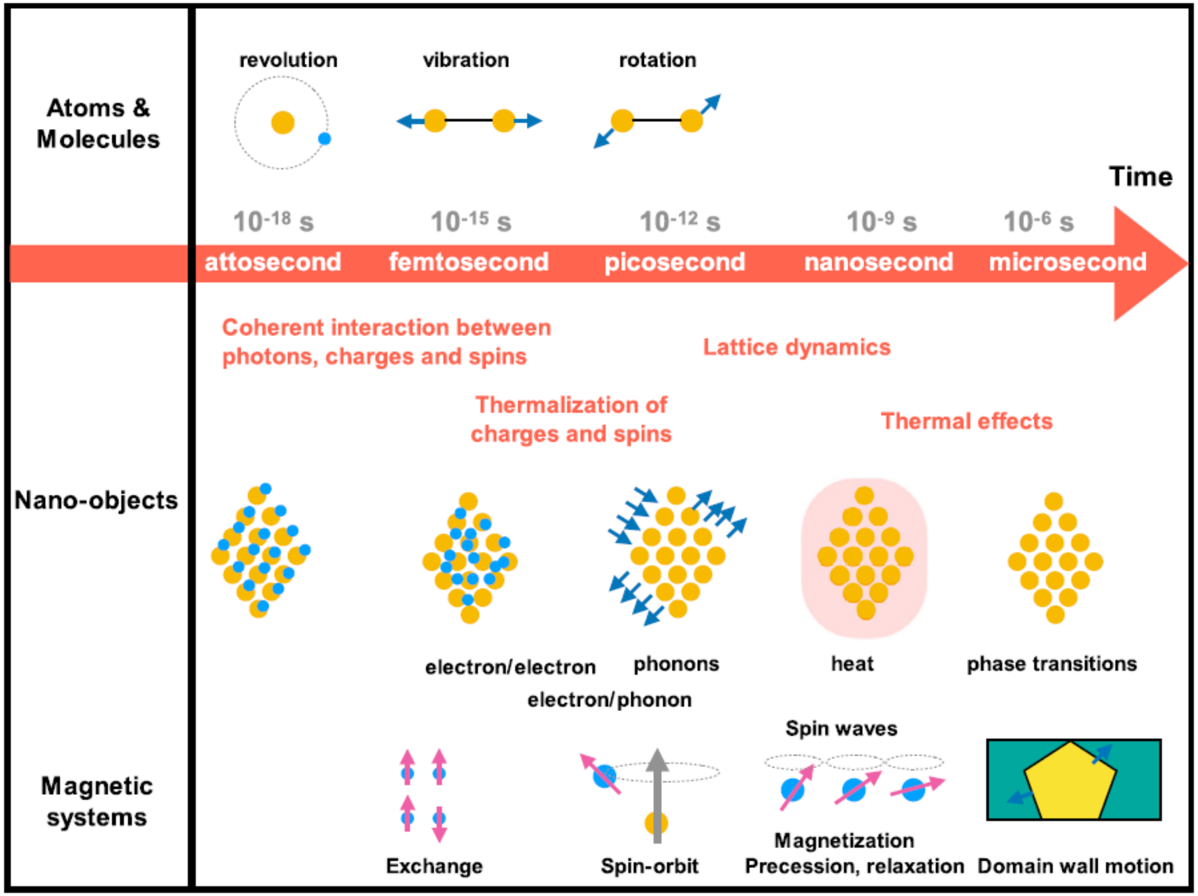
Animace se svolením Max Planck Institutu Biochemie, Martinsried, Germany

Correlative EM-light microscopy

- Imaging with light microscopy first (fluorescence or reflection mode) – up to 1μm details.
- EM imaging for more details up to 1nm.



The need of ultrafast



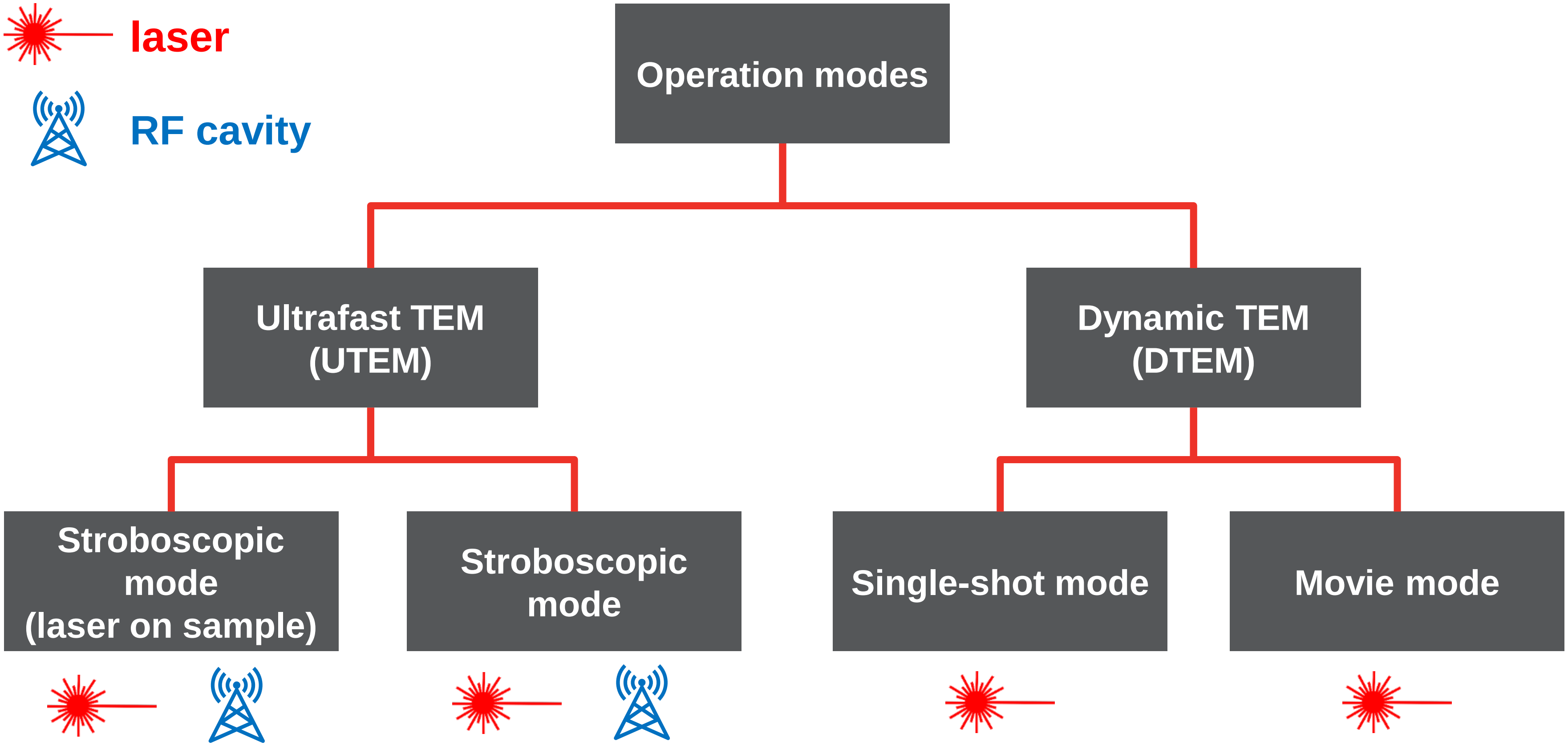
Time resolution

TEM: miliseconds (camera frame rate limit)

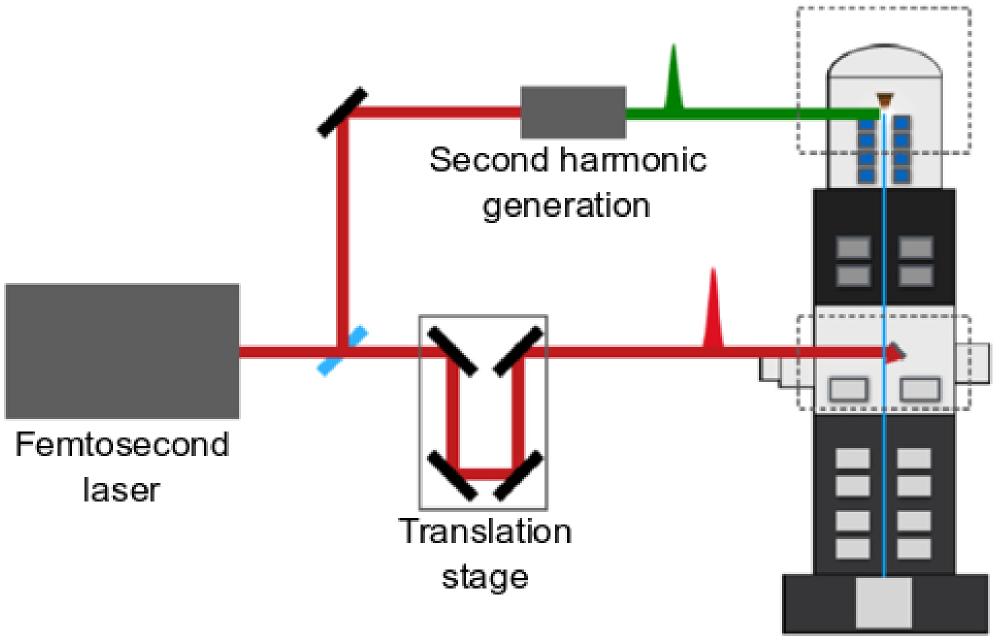
UEM: femtoseconds (pulse length limit)

Ziegler A., Graafsma H., Zhang X. F., Frenken J. W. M.: *In-situ Materials Characterization: Across Spatial and Temporal Scales*, (2014)

Operation modes



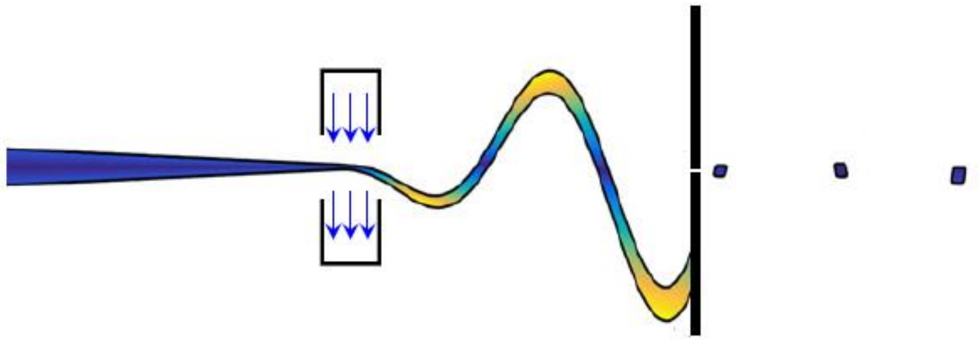
Schematic overview of UEM setup with laser induced/assisted emission



Arbouret A.,Caruso G. M., Houdellier F.: *Advances in Imaging and Electron Physics*, **207**, (2018)

Realization of femtosecond electron pulses

- RF-cavity beam deflection: pulses are created by deflection of the continuous beam over a slit   aperture using RF-cavity

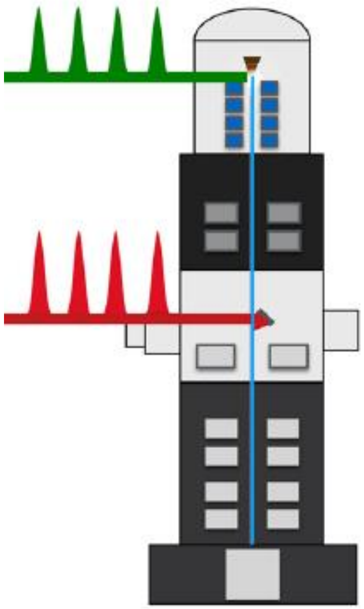


Verhoeven W. et al: *In-situ High quality ultrafast transmission electron microscopy using resonant microwave cavities*, (2018)

Operation modes

Stroboscopic mode

- also called *Ultrafast TEM* (UTEM)
- millions of electron pulses are detected to create a single image
- time delay between laser hitting the sample and electron pulse arrival is kept unchanged during one image capture
- pulse repetition rate – order of **MHz**
- pulse duration – order of **100 fs** (laser pulse duration limit)
- no significant change in spatial resolution



Arbouret A.,Caruso G. M., Houdellier F.: *Advances in Imaging and Electron Physics*, **207**, (2003)

UTEM - applications

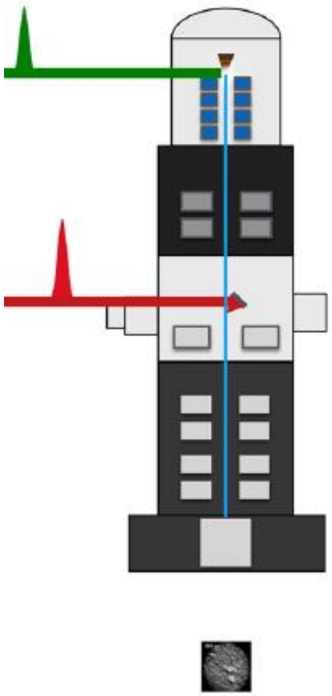
ONLY REVERSIBLE PROCESSES

- nanostructures melting and recrystallization
- dynamics of laser beam induced phase transitions and atomic structural expansion
- debeye-waller factor measurement

Barwick B. et al:*4D Imaging of Transient Structures and Morphologies in Ultrafast Electron Microscopy*, **322**, (2008)

Single-shot mode

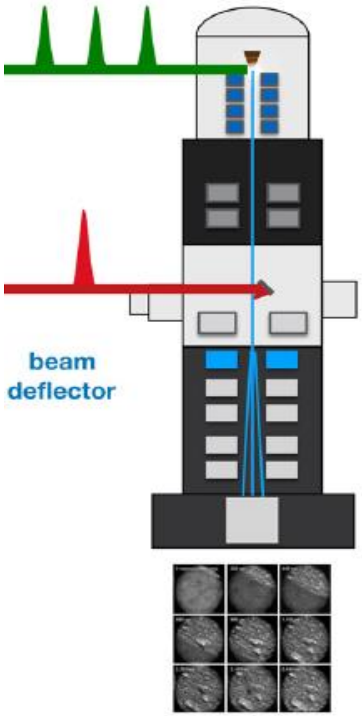
- also called *Dynamic TEM* (DTEM)
- irreversible processes
- millions (typically 10^8) electrons in a single pulse
- spacecharge effect (Boersch effect) results in limited spatial resolution (typically **10-200 nm**) and temporal resolution (typically **10-50 ns**) which is no longer determined by laser pulse length



Arbouret A.,Caruso G. M., Houdellier F.: *Advances in Imaging and Electron Physics*, **207**, (2003)

Movie mode

- also called *Movie DTEM*
- electron pulse train is created and individual pulses are then deflected to different sections of camera
- sample is still illuminated by a **single pulse**
- same limitations as for the case of the single-shot mode



Arbouret A.,Caruso G. M., Houdellier F.: *Advances in Imaging and Electron Physics*, **207**, (2018)

DTEM and movie DTEM - applications

IRREVERSIBLE PROCESSES

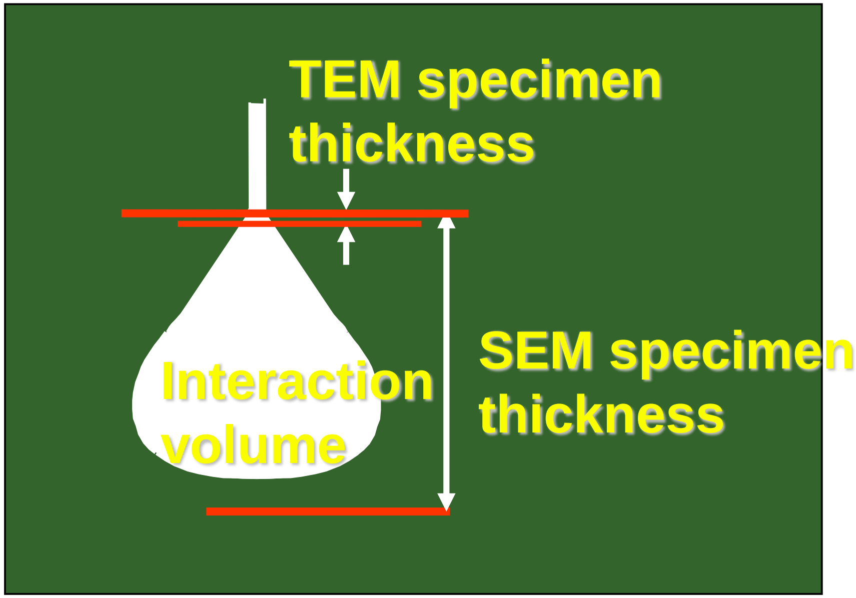
- reactive multilayer foils – reaction wavefront propagation
- various irreversible chemical reactions
- study of biological reactions: utilizing also *in-situ* liquid microscopy or cryo-electron microscopy

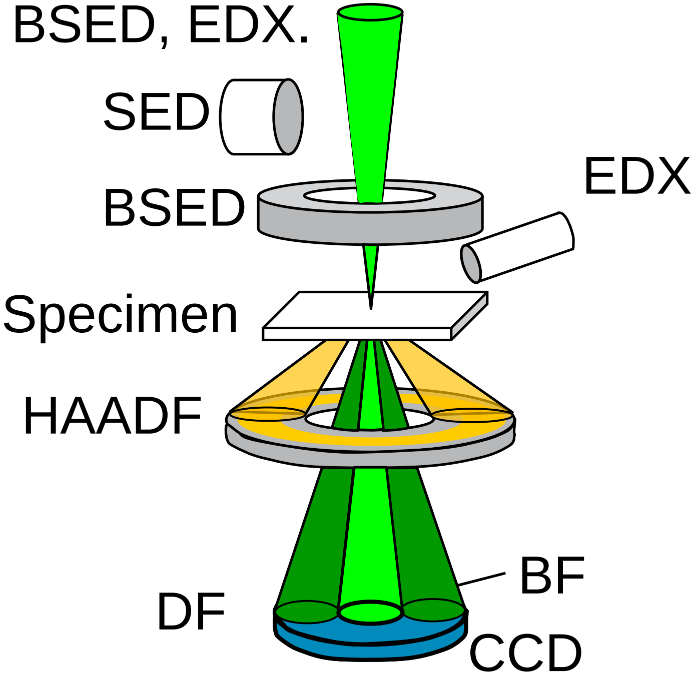
Kim J. S., et al: *Imaging of Transient Structures Using Nanosecond in Situ TEM*, **321**, (2008)

Evans J. E.,Browning N. D.: *Enabling direct nanoscale observations of biological reactions with dynamic TEM*, **62**, (2013)

STEM - principle

- Scaning with small probe through sample – collection of signal gone through the sample on the detector below imaging system.
- STEM and SEM is not the same technique. STEM is of course much better!
- Almost true atomic resolution – easier interpretation regarding to HR-TEM.
- Many detection techniques – BF, DF, SED, BSED, EDX.



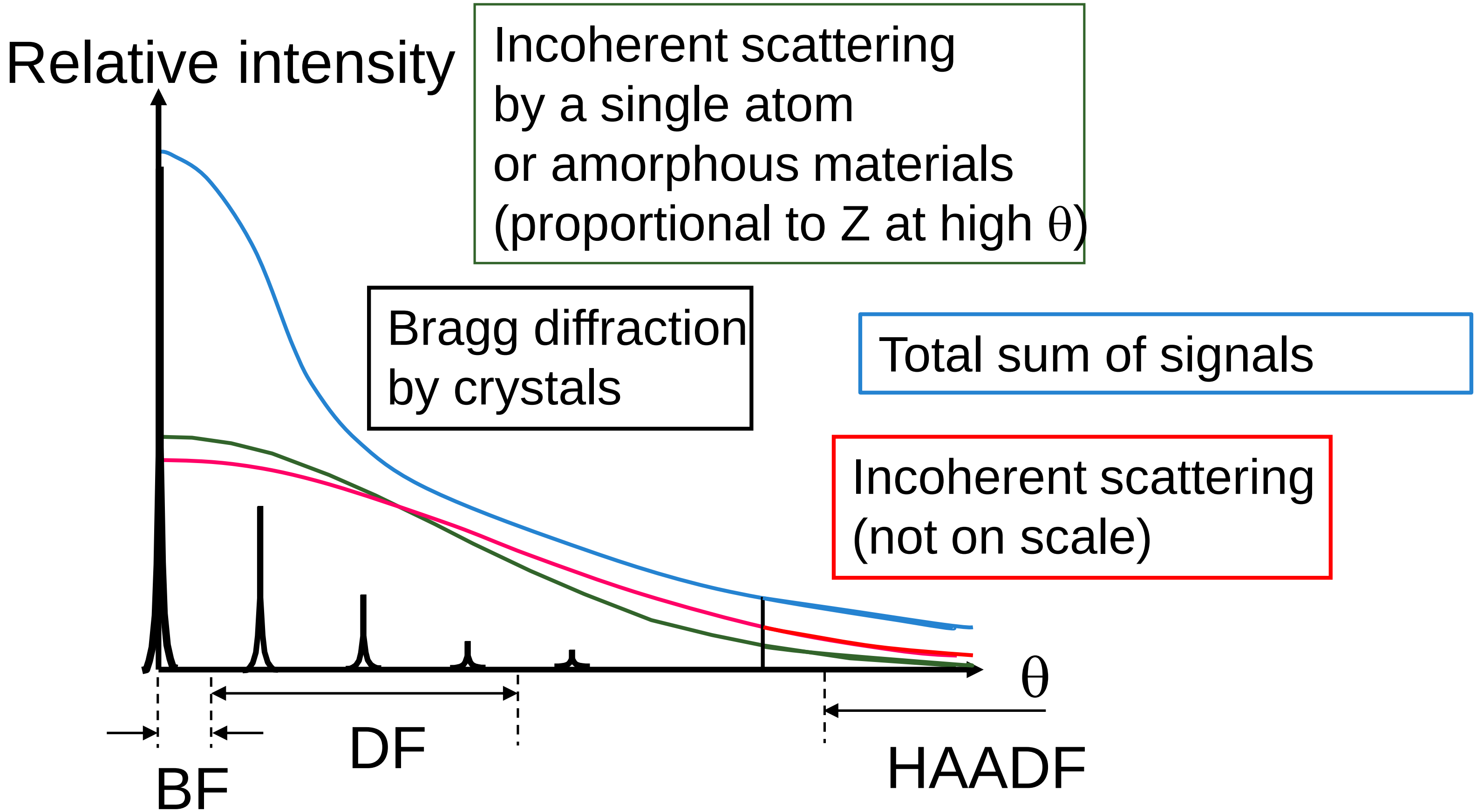


STEM – image creation.

- STEM image is influenced by three main factors:
  1.) Sample-electron interaction.
  2.) Detector type (BF, DF, HAADF).
  3.) Diffraction camera lenght.
- Image contrast is defined by:
  1.) Sample thickness – BF (transmitted primary).
  2.) Elements composition – DF (diffraction on cryst. structure).
  3.) Elements weight – HAADF (Z-contrast).

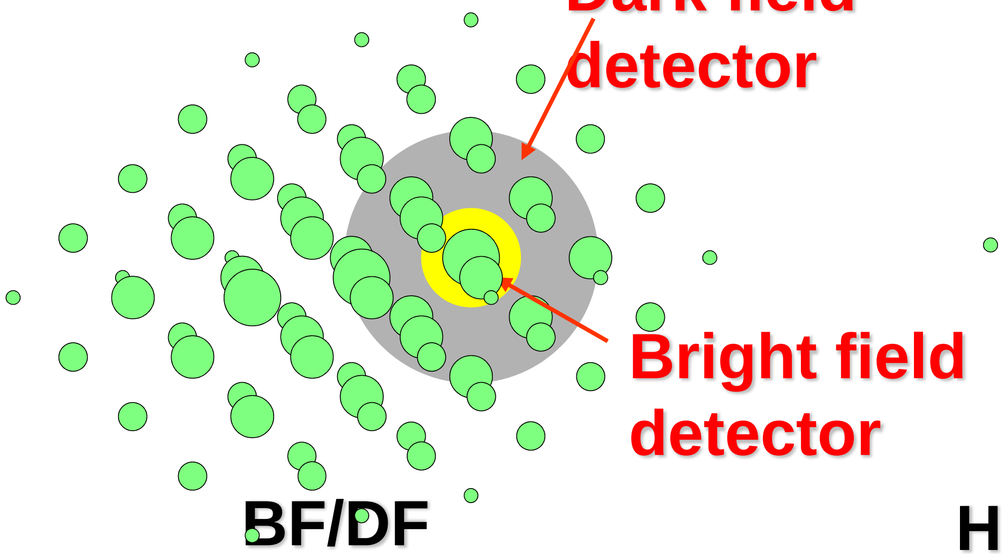
STEM – electron sample interaction.

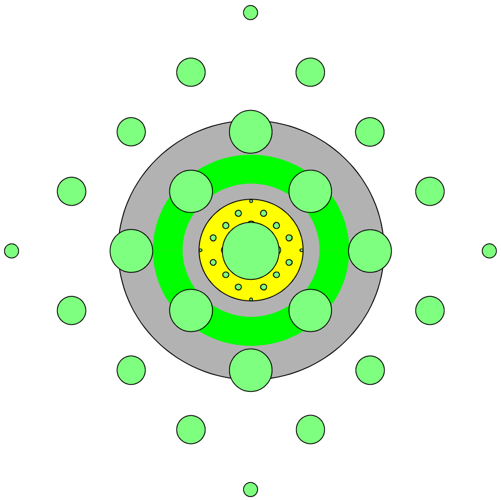
- Electron sample interaction change the energy of primary beam differently based on atom weight and crystal structure.



STEM detectors

- Three main detectors collect different spectrum of deflected beam:
- BF – transmitted primary beam.
- DF – deflected beam on crystall structures.
- HAADF – high angle delflected beam – Z-contrast.





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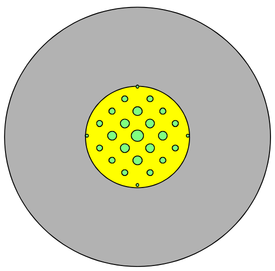
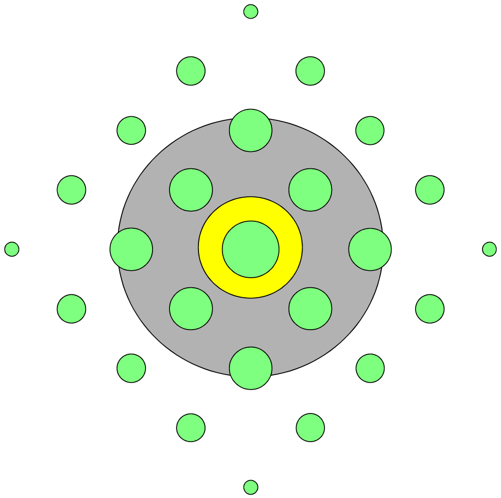
STEM – camera lenght

- The diffraction camera lenght significantly influences the detected signal.
- With camera lenght changing is possible to obtain a different contrast using the same detector.



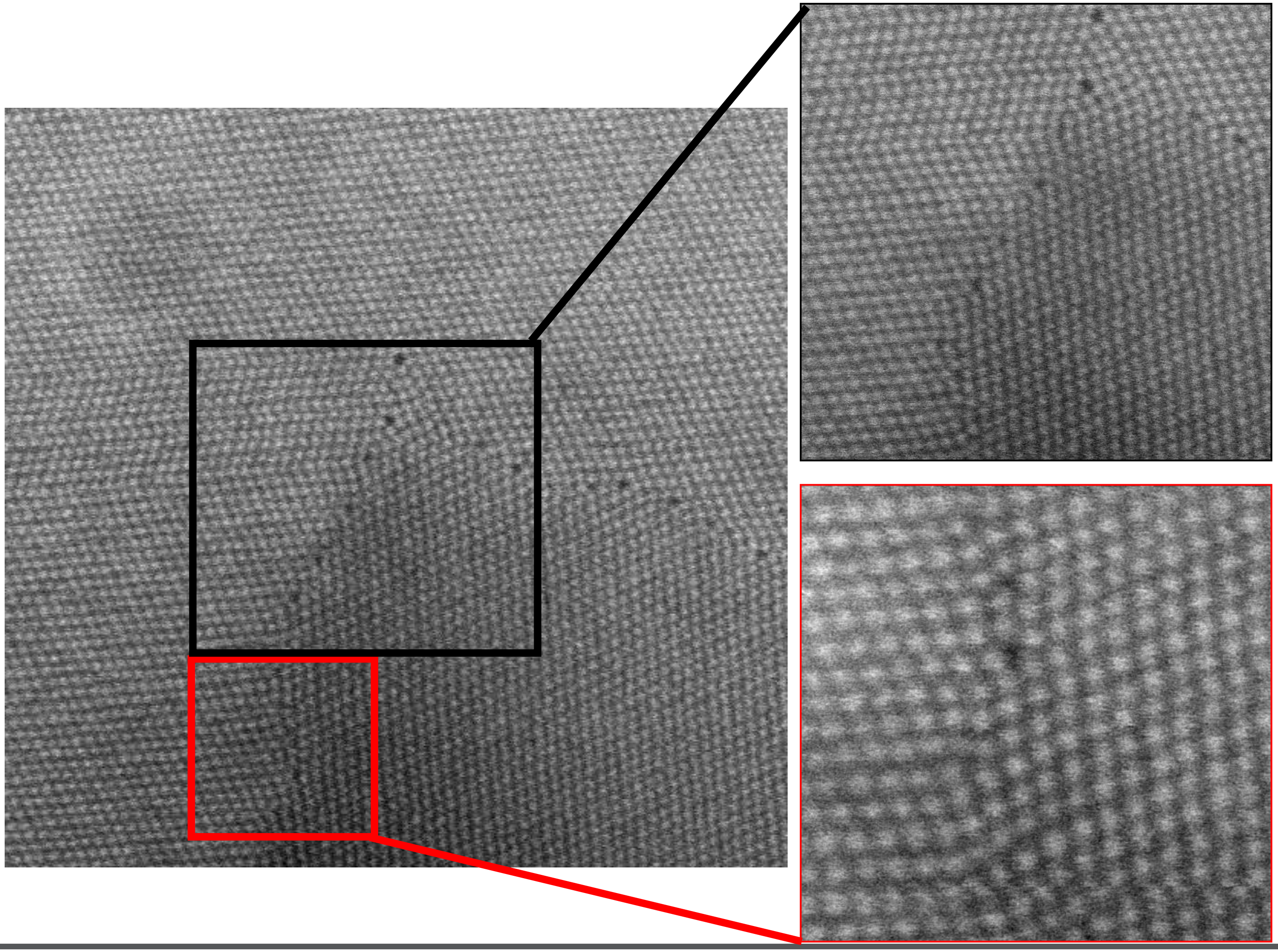
camera lenght 100 mm



camera lenght 300 mm

HR-STEM II.

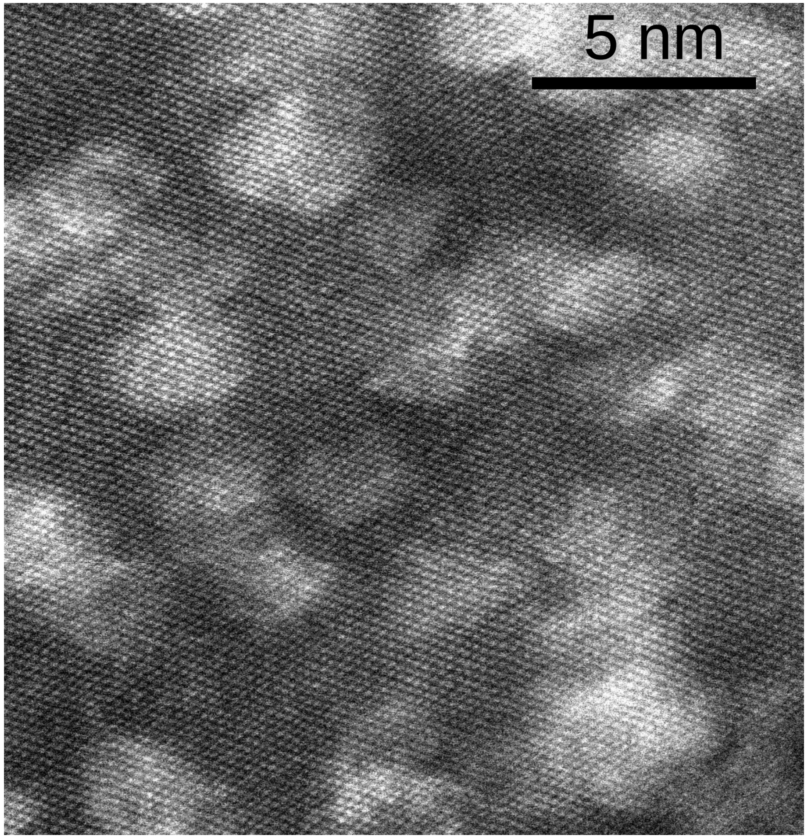
High Resolution ADF STEM image of a triple-junction in Au poly-crystal. Numerous voids occur at defects at the interface.



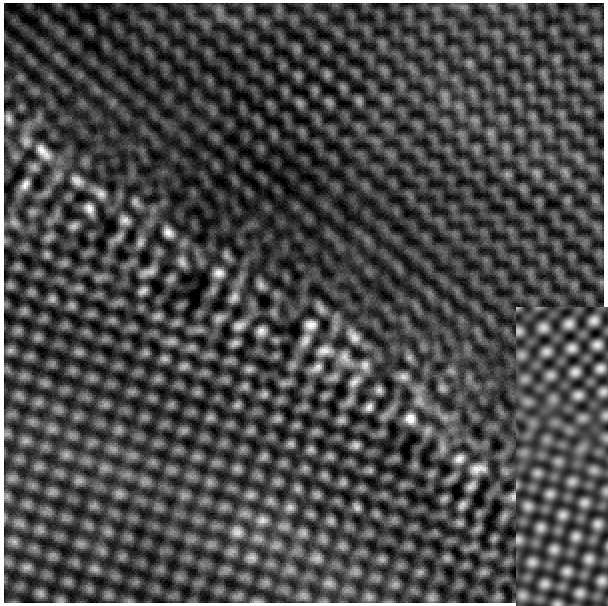
Sample courtesy of Dr. T. Radetic, U.Dahmen & C. Kisielowski, NCEM Berkeley, USA

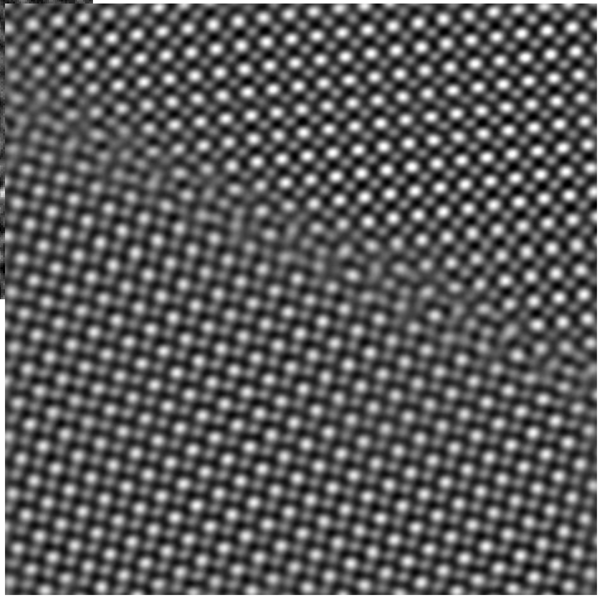
HR-STEM II.

- Ag concentration in individual atomic columns of Al



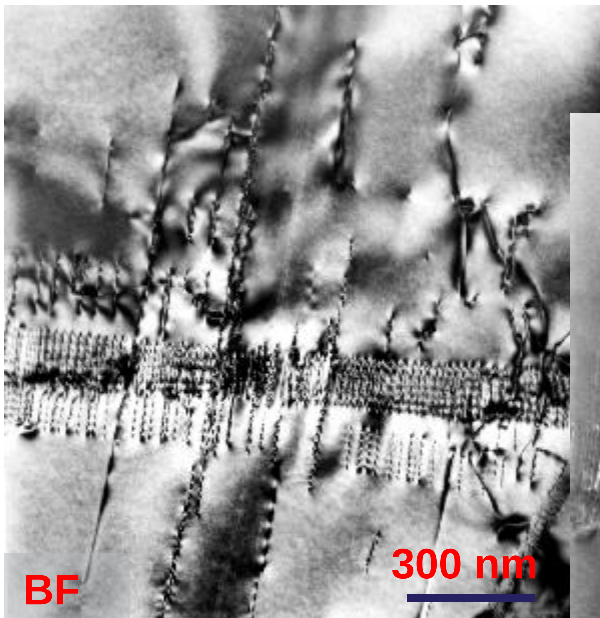
HR-TEM vs. HR-STEM I.

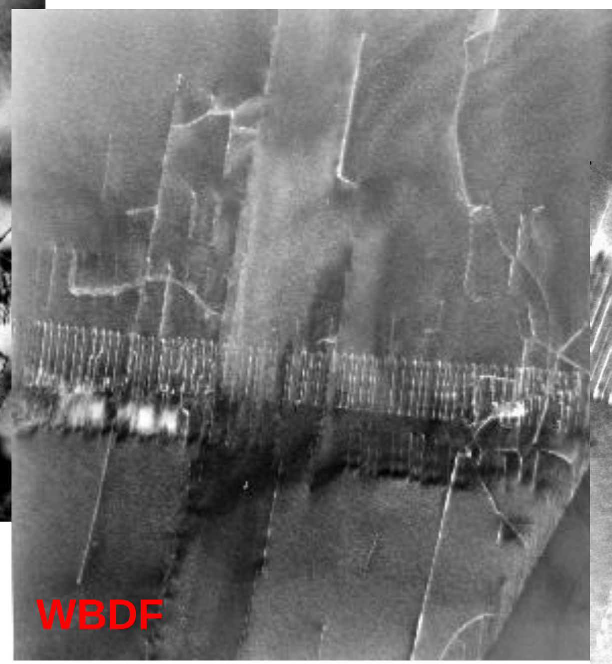


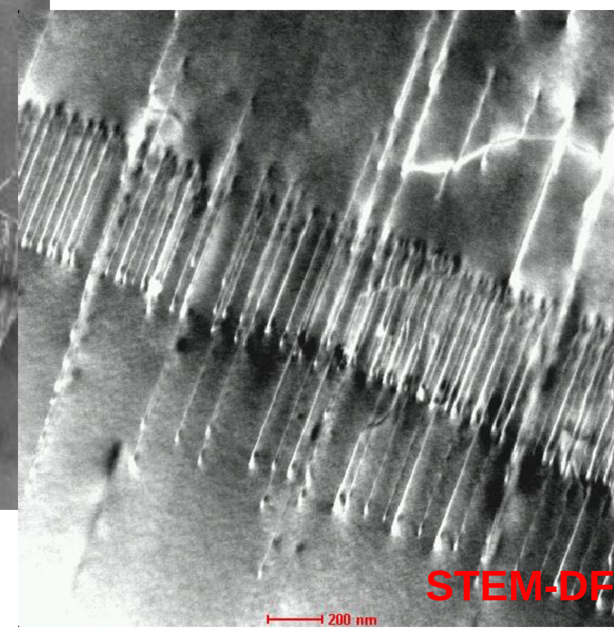


- HREM and HR-STEM images from the same $SrTiO_3$ bi-crystal boundary

HR-TEM vs. HR-STEM II.



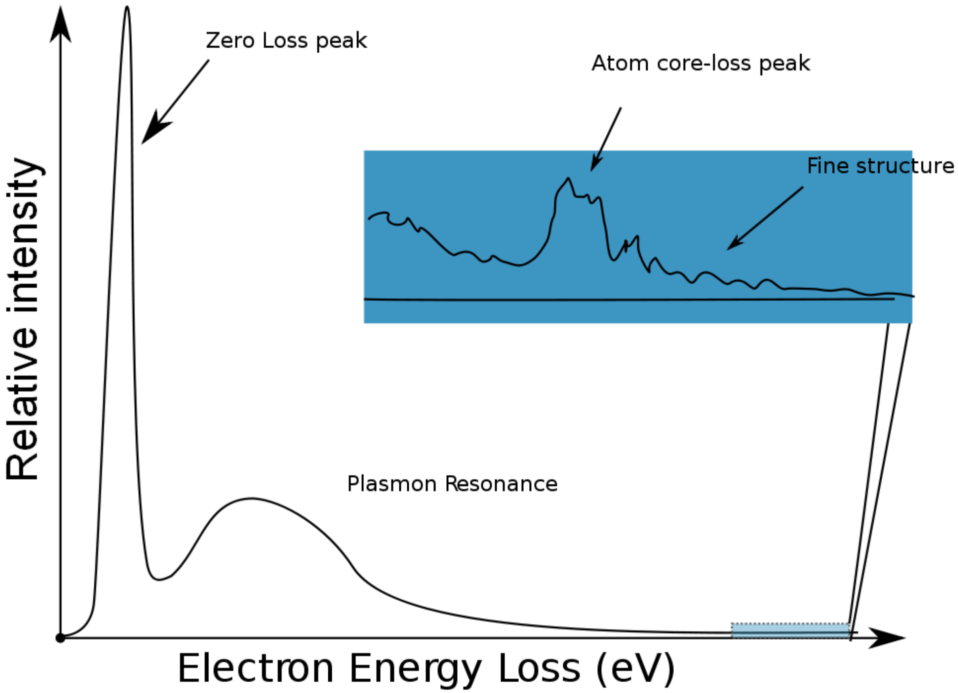




Visualization of dislocation in silicon using TEM and STEM techniques

Electron Energy Loss Spectroscopy I

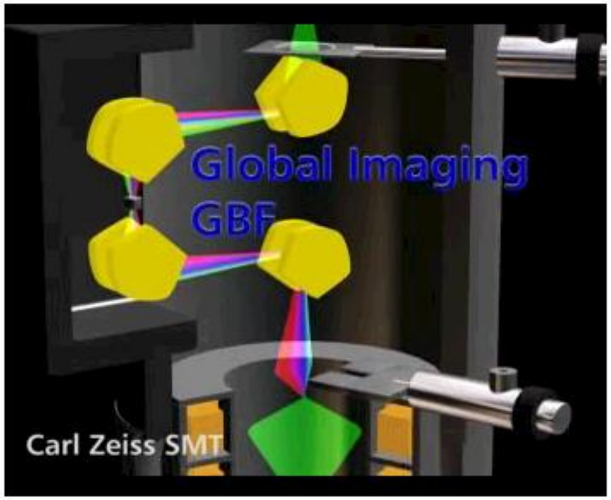
- Electron energy filtering for spectroscopy or imaging.
- Resolution to 0.004 eV.
- Possible to distuingish element type, plasmon energy, type of atomic band.

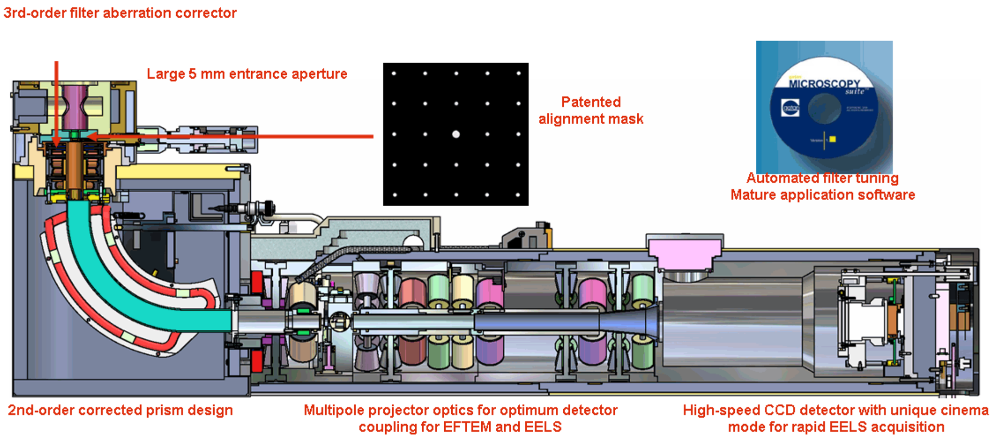


Electron Energy Loss Spectroscopy II

- Using magnetic Biprism.
- Two HW solution – in-column (Zeiss, Jeol).
  - under column (Gatan – FEI, Jeol).

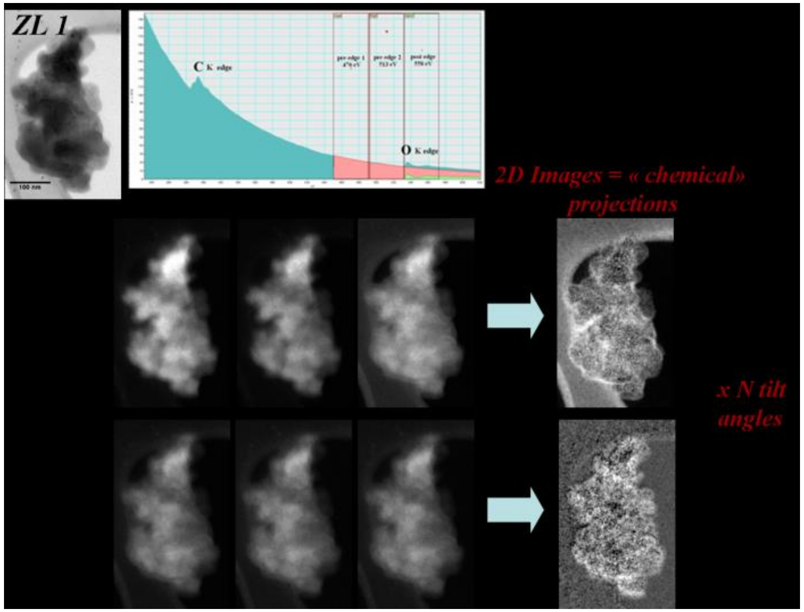
LIBRA® 200 C_S
- Corrected View of the Sub-Ångstrom World -

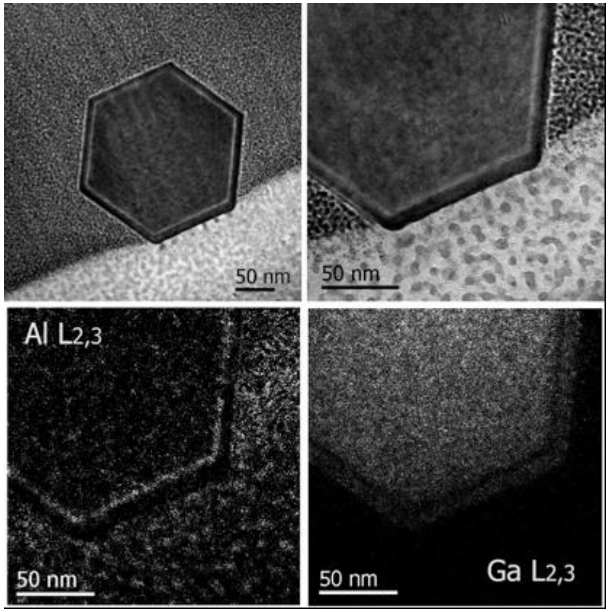




Energy Filtering TEM

- Used same filtering technique as EELS.





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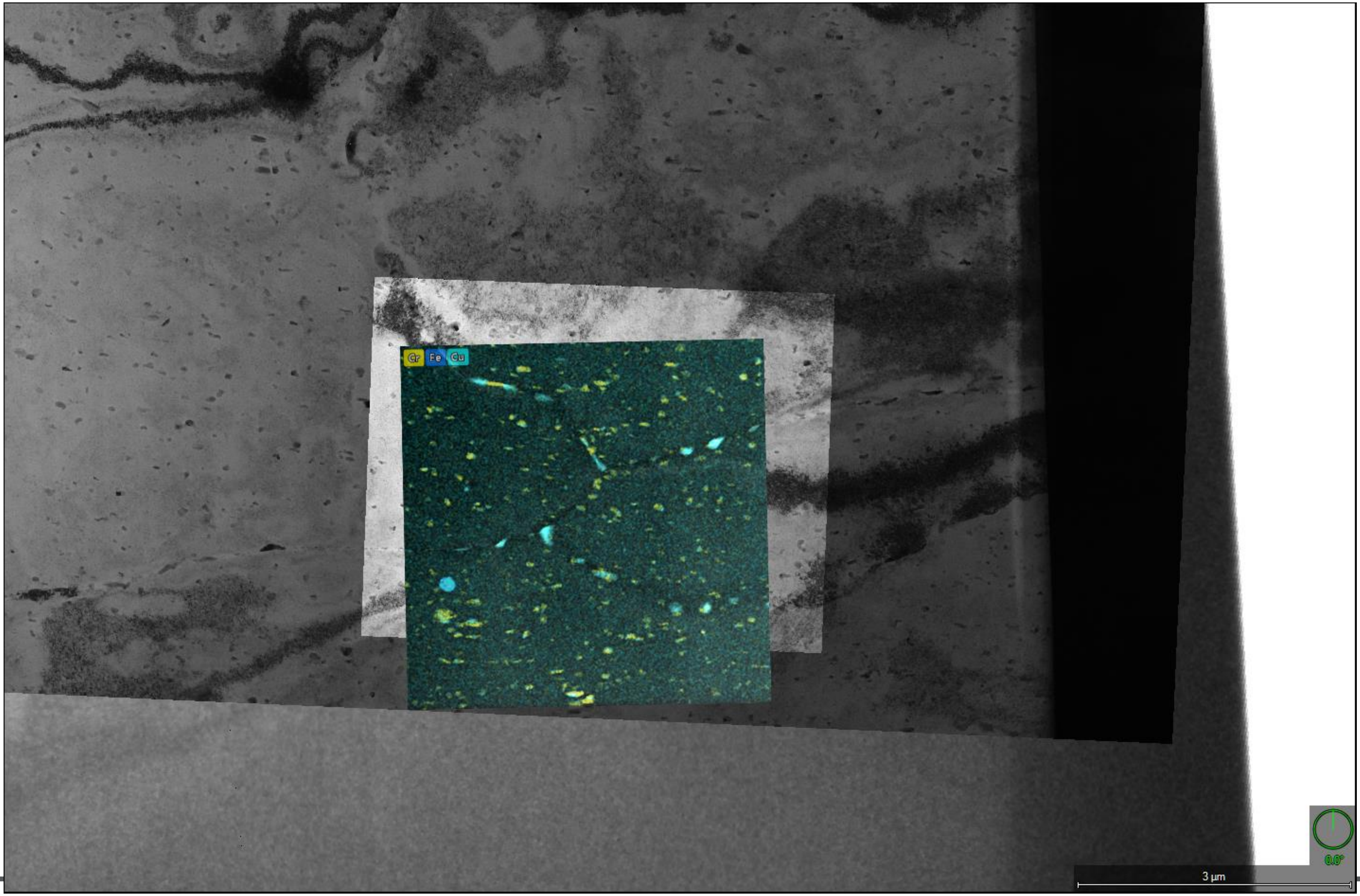
EDAX mapping



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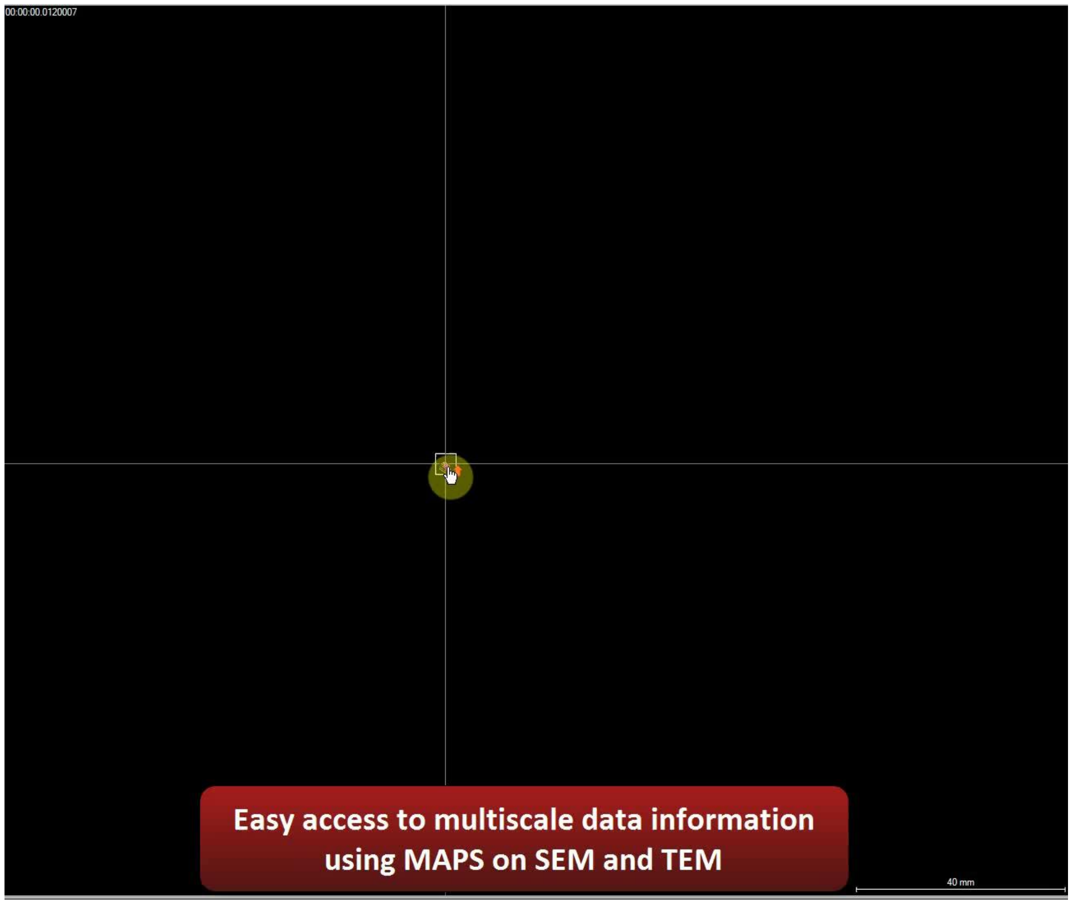
Big data


00:00:00.0120007
Easy access to multiscale data information
using MAPS on SEM and TEM
40 mm

Conclusion

- TEM and STEM offer extremly powerful scientific tool.
- There are many possibilities how to create image under different contrast conditions – enable to distinguish many types of physical properities of sample:

  1.) Atomic structure.

  2.) Electric and magnetic potential distribution.

  3.) Bound type and its energy.

  4.) Thickness.

  5.) Elements distribution over sample.