Transmission Electron Microscopy of Polymer Materials

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The lecture was created for courses on Polymer Morphology.
Great majority of information in this lecture holds for non-polymeric materials as well.

Focus of the lecture:
(1) the basic methods of TEM, important for polymers (BF, DF, SAED, EDX)
(2) how to interpret/get information from the micrographs of polymer systems
* other methods of TEM not so important for polymer systems just briefly listed
* basic theory of electron microscopy has been explained in the 1st lecture of the course

Background of the slides:
blue = theory; green = examples; yellow = calculations; grey = supplements

Micrographs in this lecture:
(Almost) all micrographs in this lecture come from our laboratory + majority of samples from IMC $\Rightarrow$ we can discuss/collaborate on whatever will be shown in the presentation.

Part 1

Brief Introduction to TEM

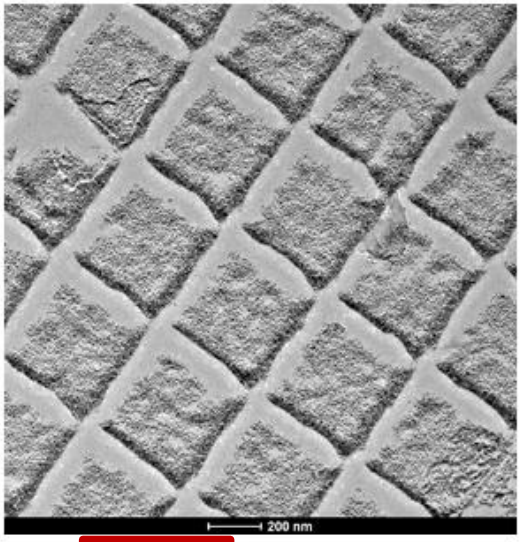
Contents

- ❖ Four basic modes of TEM
- ❖ Signal in TEM from in macro-, microscopic and atomistic view

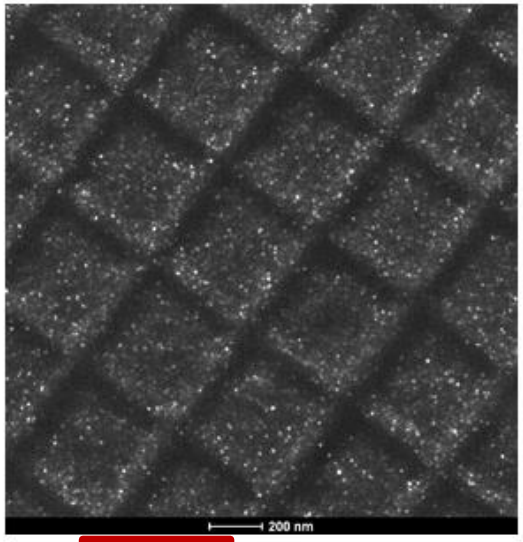
Notes:

- Supplement #1 gives overview of other TEM methods and possibilities
- Supplement #2 gives basic information about SEM hardware components

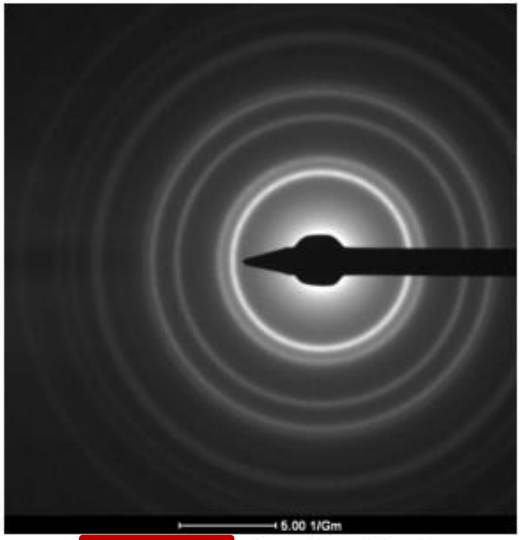
TEM microscopy :: four main modes



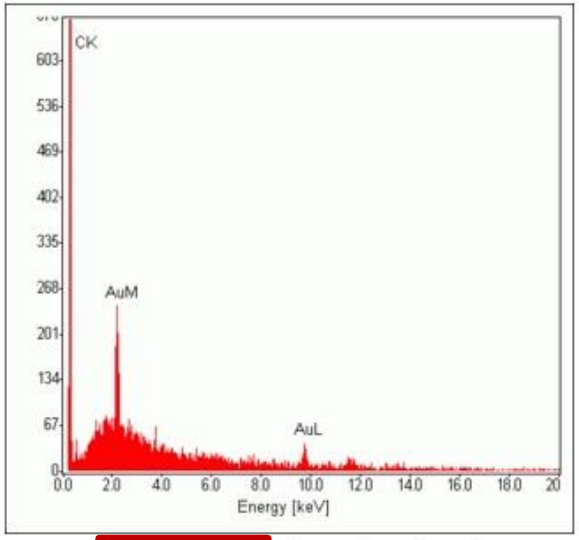
TEM/BF Au standard
dark Au particles on light C film



TEM/DF Au standard
white Au particles on dark C film



TEM/ED Au standard
electron diffraction of Au on C



TEM/EDS Au_standard
elemental analysis of Au on C

TEM/BF

- transmitted electrons, direct image (bright field)
- >90% of applications in polymer science

TEM/DF

- diffracted electrons, direct image (dark field)
- special applications connected with crystal structure

TEM/ED

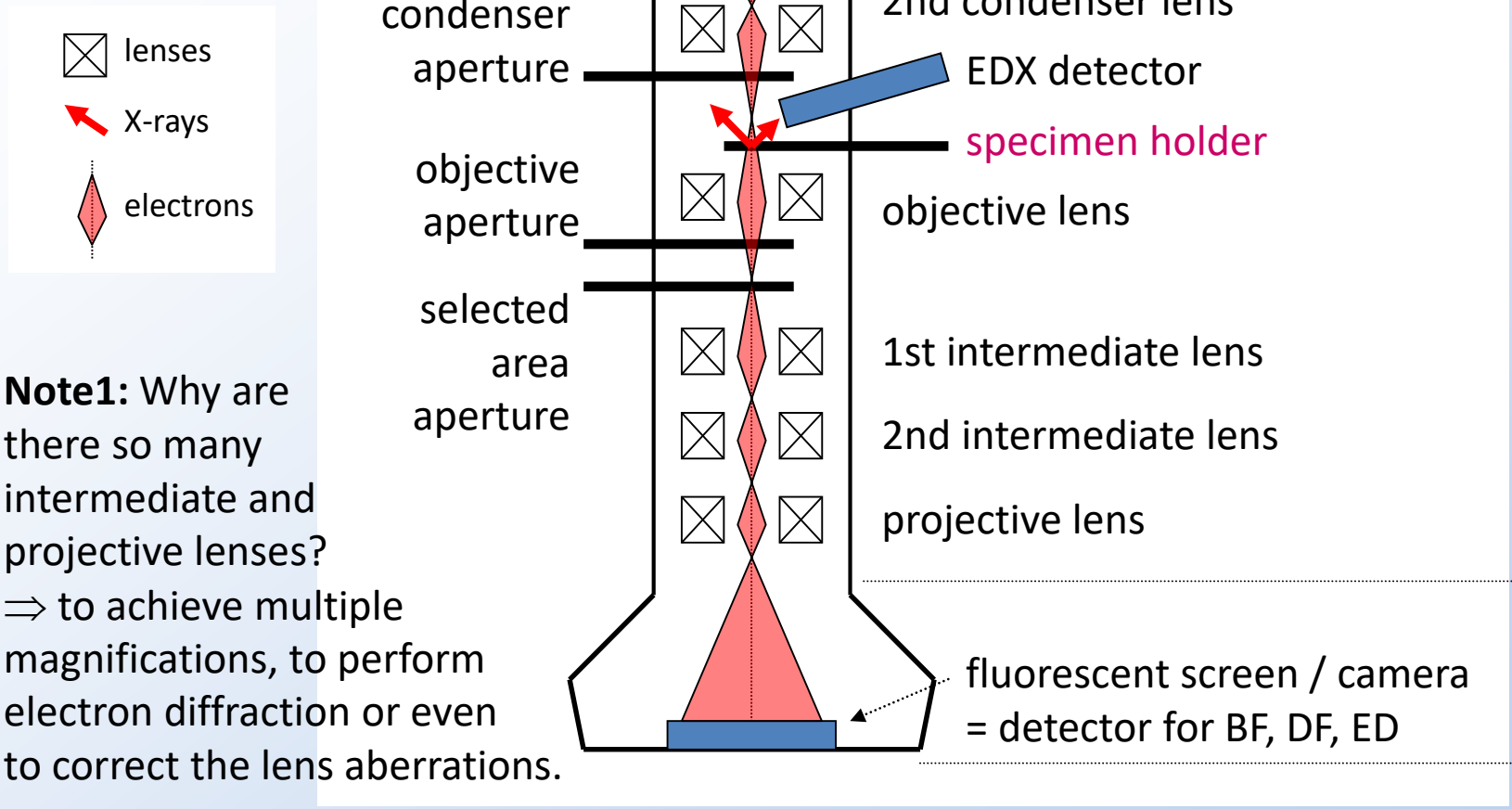
- electron diffraction = diffracted electrons
- nanocrystals (inorganics, metals, polymer fillers...)

TEM/EDX

- characteristic X-rays
- microanalysis (like in SEM, but in nanometer scale)

TEM :: macroscopic view :: scheme of the microscope

Schematic view of a standard TEM microscope

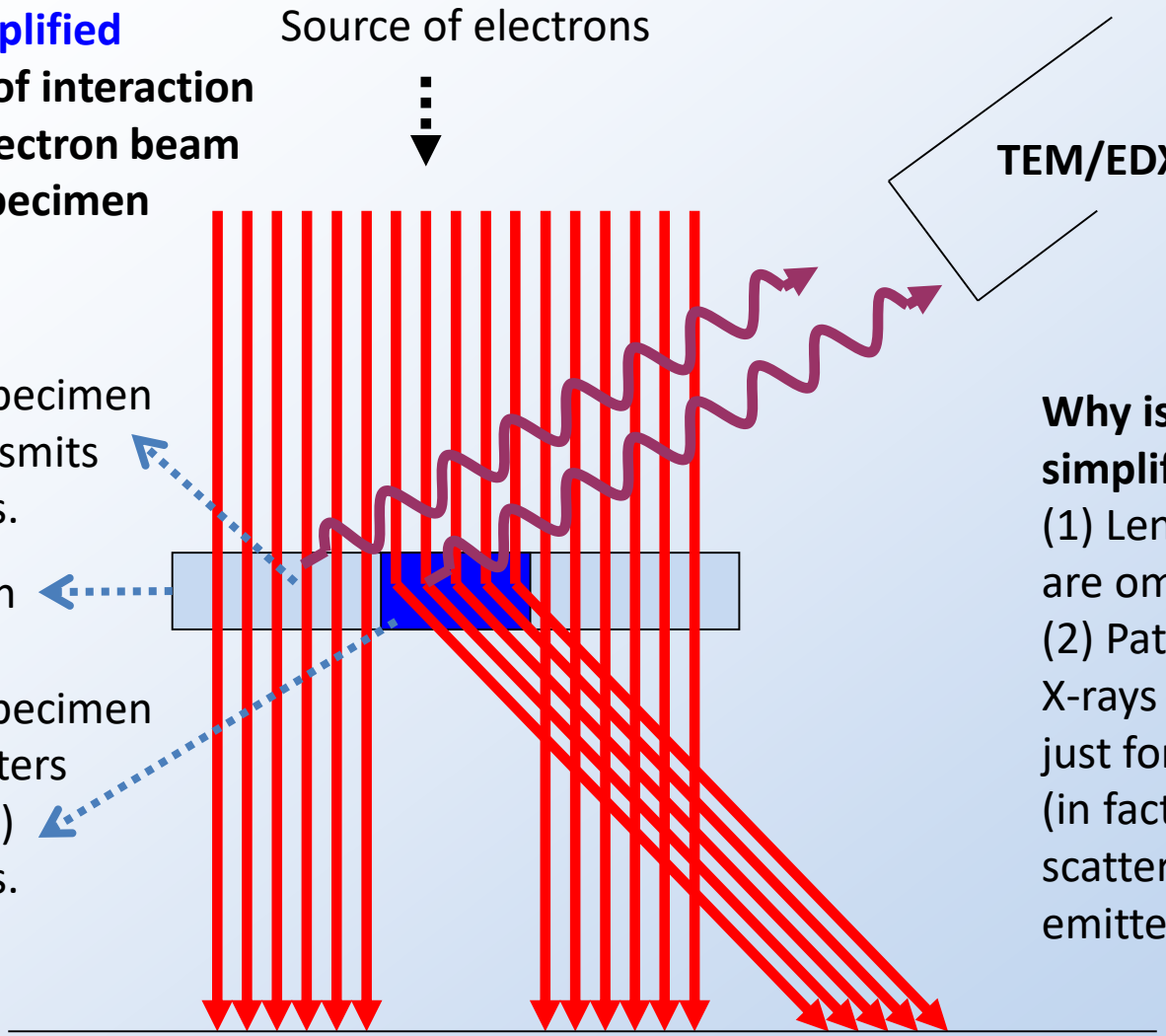


Note1: Why are there so many intermediate and projective lenses?
⇒ to achieve multiple magnifications, to perform electron diffraction or even to correct the lens aberrations.

Note2: TEM is more similar to LM than to SEM.
The image is formed in one step like in LM, not point-by-point like in SEM.

TEM :: microscopic view :: beam–specimen interactions

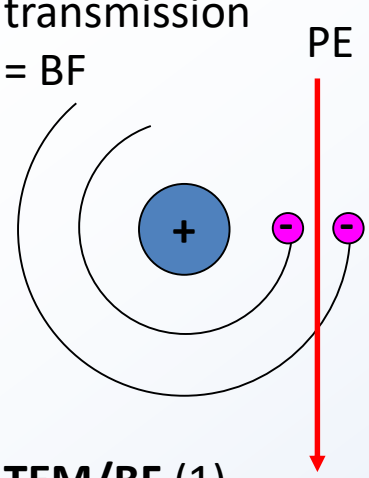
Very simplified scheme of interaction of the electron beam with a specimen in TEM.



Why is this scheme so simplified:
(1) Lenses and apertures are omitted.
(2) Path of electrons and X-rays shown schematically just for one direction.
(in fact the electrons are scattered and the X-rays are emitted to all directions).

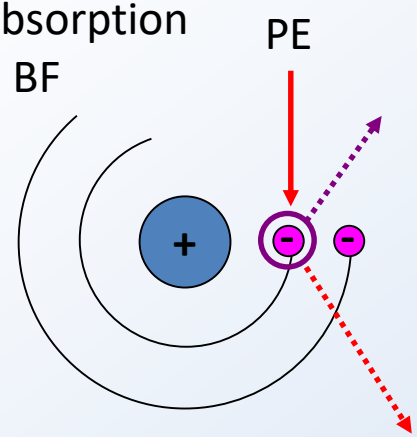
TEM :: atomistic view :: electron–atom interactions

transmission
= BF



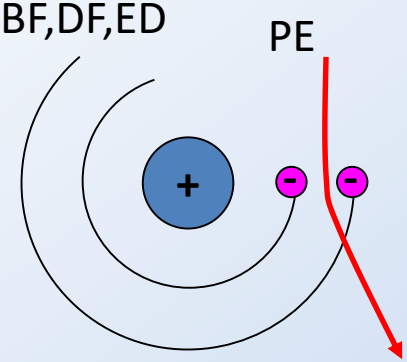
TEM/BF (1)

absorption
= BF



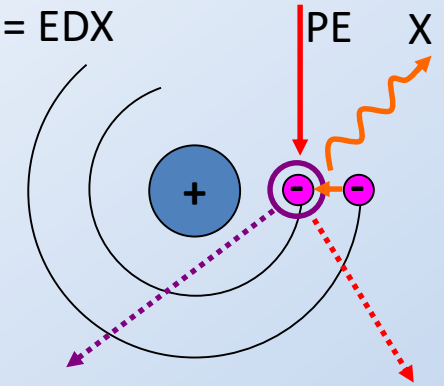
TEM/BF (1)

elastic scattering
= BF,DF,ED



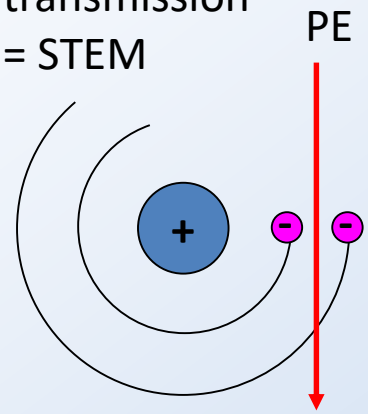
TEM/BF+DF+ED (2)

characteristic X-rays
= EDX



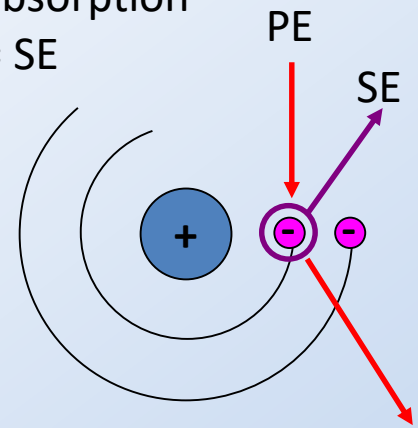
TEM/EDX (3)

transmission
= STEM



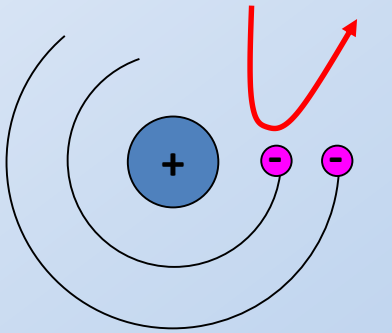
SEM/STEM (4)

absorption
= SE



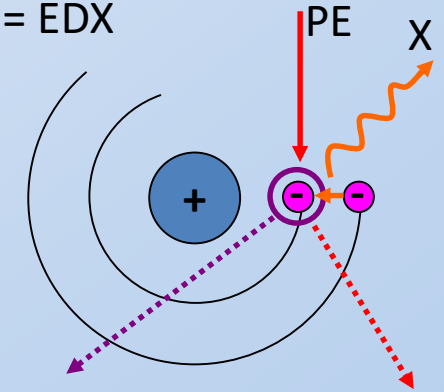
SEM/SE (1)

back scattering
= BSE



SEM/BSE (2)

characteristic X-rays
= EDX



SEM/EDX (3)

Comparison of signals in TEM (upper row) and SEM (lower row).

Supplement #1 :: TEM :: summary of methods

These are the main methods used for polymer systems
⇒ main part of this lecture

1) Conventional TEM (CTEM; covered in this lecture):
   standard methods : BF, DF, ED ~ SAED
   useful supplement: EDX ~ EDS ~ microanalysis

2) Analytical TEM (ATEM; mostly for inorganics, but also organics/polymers):
   ATEM = CTEM + STEM mode + analytical methods such as EDX, EELS
   => ATEM = maps of elemental composition in nanometer scale

3) High-resolution TEM (HRTEM; almost exclusively for inorganic materials):
   HRTEM = atomic resolution due to combination of direct + diffraction imaging
   => HRTEM = micrographs showing individual atoms (mostly in stable crystals)

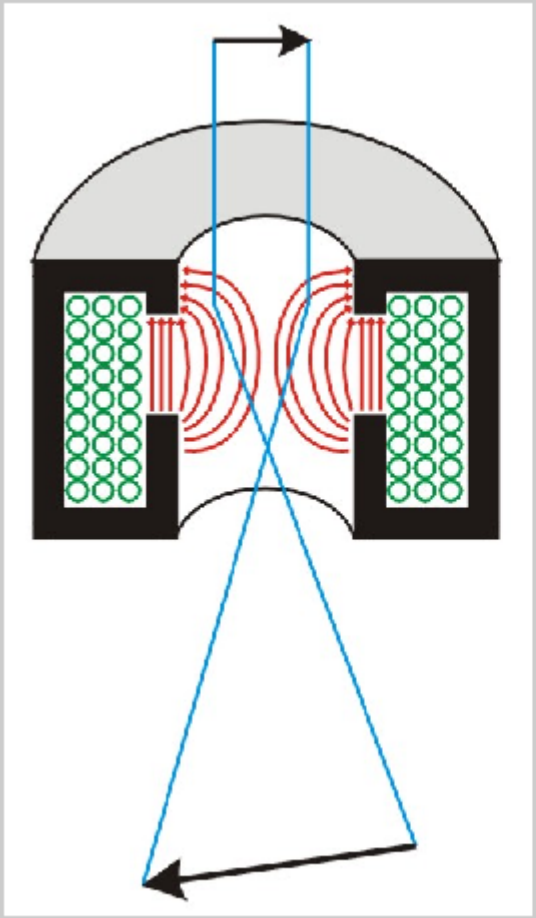
4) Cryogenic-TEM (Cryo-TEM, mostly in biology, also polymer solutions):
   Cryo-TEM = TEM + device for freezing/imaging samples at LN2 temperature
   => Cryo-TEM = imaging of bio-objects or polymer nanoparticles in solution

5) 3D-TEM (mostly in biology, also other fields):
   3D-TEM = [CTEM or ATEM] + [stage for high tilts] + [SW for 3D-reconstruction]
   => 3D-TEM = 3D-models of microscopic objects such as viruses, organelles...

* TEM sample holders: single/double-tilt, low-background, cryo-holder...
* TEM other methods: E-TEM (environmental TEM: sample in 'natural' conditions), EFTEM (better contrast + analysis), HVTEM (high voltage & resolution, obsolete), Cs/Cc-aberration corrected TEM (corrections => even higher atomic resolution)
* TEM combinations: e.g. HRTEM + EFTEM + aberration corrections
* TEM other methods: (HAADF, EELS, CBED, PED, SE...) => see Wikipedia, textbooks

Supplement #2a :: TEM components

Electromagnetic lenses (analogous to SEM)



A magnetic lens consists of a coil of copper wires inside the iron pole pieces. A current through the coils creates a magnetic field (symbolized by red lines) in the bore of the pole pieces. The rotationally symmetric magnetic field is inhomogeneous in such a way that it is weak in the center of the gap and becomes stronger close to the bore. Electrons close to the center are less strongly deflected than those passing the lens far from the axis. The overall effect is that a beam of parallel electrons is focused into a spot (so-called cross-over).

In a magnetic field, an electron experiences the Lorentz force F:

$$\mathbf{F} = -e\,(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$
$$|\mathbf{F}| = evB\sin(\mathbf{v},\mathbf{B}) \quad -eE$$

E: strength of electric field
B: strength of magnetic field
e/v: charge/velocity of electrons

The focusing effect of a magnetic lens therefore increases with the magnetic field B, which can be controlled via the current flowing through the coils. As it is described by the vector product, the resulting force **F** is perpendicular **v** and **B**. This leads to a helical trajectory of the electrons and to the magnetic rotation (image is rotated in respect of the object).

Particle with charge Q is moving with velocity **v** in the electromagnetic field; electric and magnetic forces are acting on the particle:

$$\mathbf{F} = \mathbf{F}_E + \mathbf{F}_M$$

Electric force $\mathbf{F}_E$ is given by the intensity of electric field **E**:

$$\mathbf{F}_E = Q \cdot \mathbf{E}$$

Magnetic force $\mathbf{F}_M$ is given by magnetic induction **B**:

$$\mathbf{F}_M = Q \cdot (\mathbf{v} \times \mathbf{B})$$

Force acting on electron:

$$\mathbf{F} = -e \cdot (\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

Connection with real life: electromagnetic lens deflects electrons due to inhomogeneous magnetic field; strength of the lens can be adjusted by changing current (logically: $B \propto N \times I$).

** Moreover, electron trajectories can be calculated – this is employed in construction of microscopes.*

Supplement #2b :: TEM components

Electron guns, Apertures and Detectors

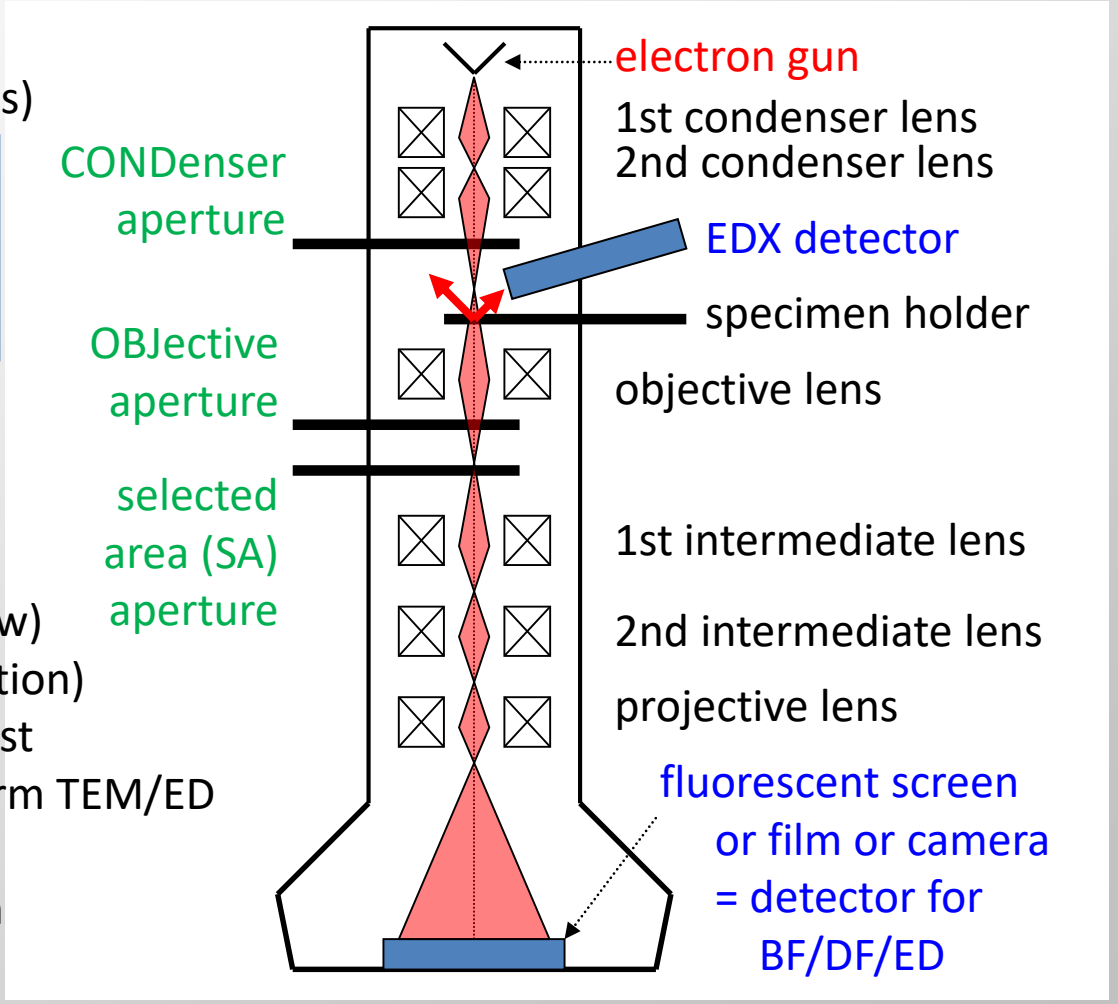
Electron guns (analogous to SEM)
(anything that easily emits electrons)

- W-filament
- LaB_6 crystal
- hot FEG
- cold FEG

increase in:
quality, lifetime
required vacuum
price

Apertures (different from SEM)

- more important than in SEM
- **COND** is usually changed when we go to STEM mode (below) (narrower beam → higher resolution)
- **OBJ** is inserted to increase contrast in TEM/BF and removed to perform TEM/ED
- **SA** is used in TEM/SAED to select the area of interest for diffraction



Detectors (different from SEM)

- common TEM has just one detector for BF/DF/ED – camera at the bottom (+ possibly EDX)
- analytical TEM (ATEM) usually has additional detectors (EDX – obligatory) + EELS, HAADF...

Part 2

More theory
and resolution of TEM

Contents

- Basic calculations (analogous to SEM):
  (Velocities, wavelengths and penetration depths of electrons
- TEM specific: electron diffraction
  (Why it cannot be observed in SEM?
- Contrast, magnification and resolution in TEM
  (Finishing of the story from the introductory lecture...

TEM :: Velocity, wavelength and penetration of electrons

Calculations like in SEM – just the relativistic corrections are more important.

❖ **Why are the relativistic corrections more important in TEM?**
typical SEM: accelerating voltage up to 30 kV – velocity ~ 0.3c – corrections not critical
typical TEM: accelerating voltage 200–300 kV – velocity close to c – corrections needed

❖ **Velocity of electrons:**
(2nd equation with rel. corrections

$$v(U) = \sqrt{\frac{2eU}{m_e}}$$

$$v(U) = c \cdot \sqrt{1 - \frac{1}{\left(1 + \frac{eU}{m_e c^2}\right)^2}}$$

❖ **Wavelength of electrons:**
(2nd equation with rel. corrections

$$\lambda(U) = \frac{h}{\sqrt{2m_e eU}}$$

$$\lambda(U) = \frac{h}{\sqrt{2m_e eU}} \cdot \frac{1}{\sqrt{1 + \frac{eU}{2m_e c^2}}}$$

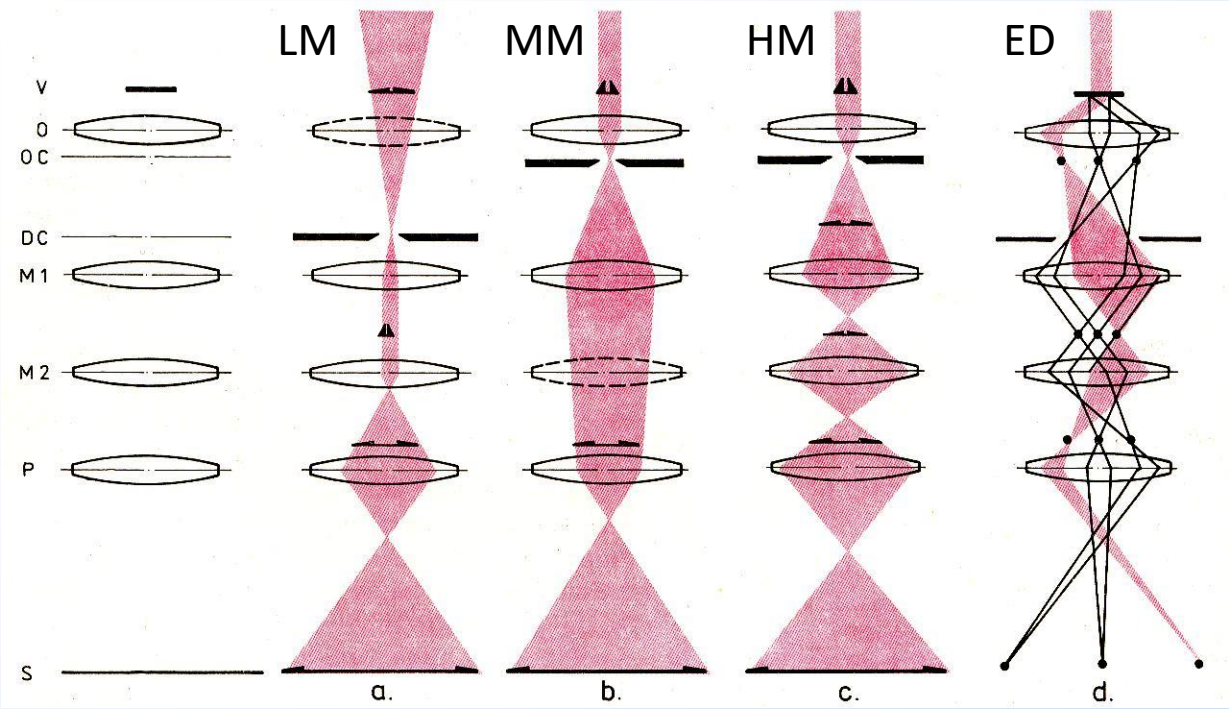
❖ **Penetration depth of electrons:**
- no corrections needed in this case
- ultrathin sections for TEM ~50nm
  $\Rightarrow$ less than R (almost) all cases

$$R(M, \rho, Z, U) = 27.6 \frac{MU^{5/3}}{\rho Z^{8/9}}$$

❖ **Technical notes:**
- For sample calculations and justifications of the non-relativistic formulas see the previous lecture on SEM.
- Relativistic formulas taken from: [Reimer, Kohl: Transmission electron microscopy, 2008].

TEM :: magnification

Why does a TEM microscope contain so many lenses?



Magnification modes of TEM:
- LM = Low Magnification
- MM = Medium...
- HM = High...

What is the difference?
...in modern TEM
there is no difference from the point of view of user;
the switching among the modes is automatic.
...but alignments for are usually separated.

Why is there so many lenses in TEM?

...among other things because of the magnification modes (and also because of ED...).

Is it possible to add one more lens and get higher magnification? ...Yes, of course.

Therefore, can we increase the resolution to infinity? ...No, of course not.

Why not? ...after exceeding the resolution limit, we get no further details in the image.

TEM :: contrast

Resolution in TEM is usually sufficient for polymer materials → contrast is the key.

Contrast × sample preparation:

Sample preparation is the key factor.

In biology and **polymer science** (where the contrast is usually weak)

we use **staining** (treating of specimen with staining agents = substances with high Z)

- **OsO_4** – specific staining of polymers with C=C bonds
- **RuO_4** – not-so-specific, less predictable, less reproducible, but (almost) universal method
- numerous other stains with heavy elements (uranyl acetate, phosphotungstic acids)...

Contrast × objective aperture:

Discussed in detail in previous slides.

The smaller the aperture, the higher the contrast.

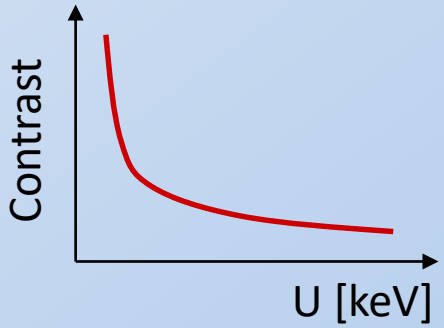
BUT too small aperture → low signal + low resolution → see next.

Contrast × accelerating voltage:

In general: lower U ⇒ higher contrast.

BUT: too low U ⇒ low resolution & high sample damage.

Note: in SEM the lower U usually means lower sample damage.



Contrast × other factors:

1) You can buy a TEM objective lens optimized for higher contrast at the cost of resolution .

2) You can switch from TEM/BF to TEM/DF: higher contrast at the cost of signal – see next.

TEM :: resolution

Summary: resolution in various modes and types of TEM.

- **Theoretical TEM resolution = diffraction condition:** $d = \lambda/2 \approx 0.003$ nm (C-C bond ~ 0.15nm). BUT this resolution cannot be achieved due to lens aberrations (more details = next slides).

  If your own eye lens was as good as our best electromagnetic lens, then you'd be legally blind!

  The best electromagnetic lens is equivalent to the bottom of a Coke bottle if used as a magnifying glass.

- **CTEM microscopes = modern TEM's** in TEM/BF $\rightarrow$ $d \approx 0.3$ nm @ 100kV.
  - Moreover, approximate rule says that the best resolution ≈ 1/10 of sample thickness.
  - Typical thickness of polymer samples (UMT ≈ 50nm) $\Rightarrow$ resolution ≈1/10*50nm = 5nm.
  - Conclusion: CTEM resolution is Ok for micro/nanostructures but not for individual atoms.

- **HRTEM microscopes = High-Resolution TEM microscopes:**
  - HRTEM's use higher accelerating voltages ($d = \lambda/2$; $\lambda = h/\sqrt{2meU}$), some imaging tricks and/or (expansive) correctors of lens aberrations (Cs, Cc) $\Rightarrow$ $d \approx 0.1$ nm @ 200-300kV.
  - Conclusion: in HRTEM, you can see individual atoms (in thin, beam-resistant samples).

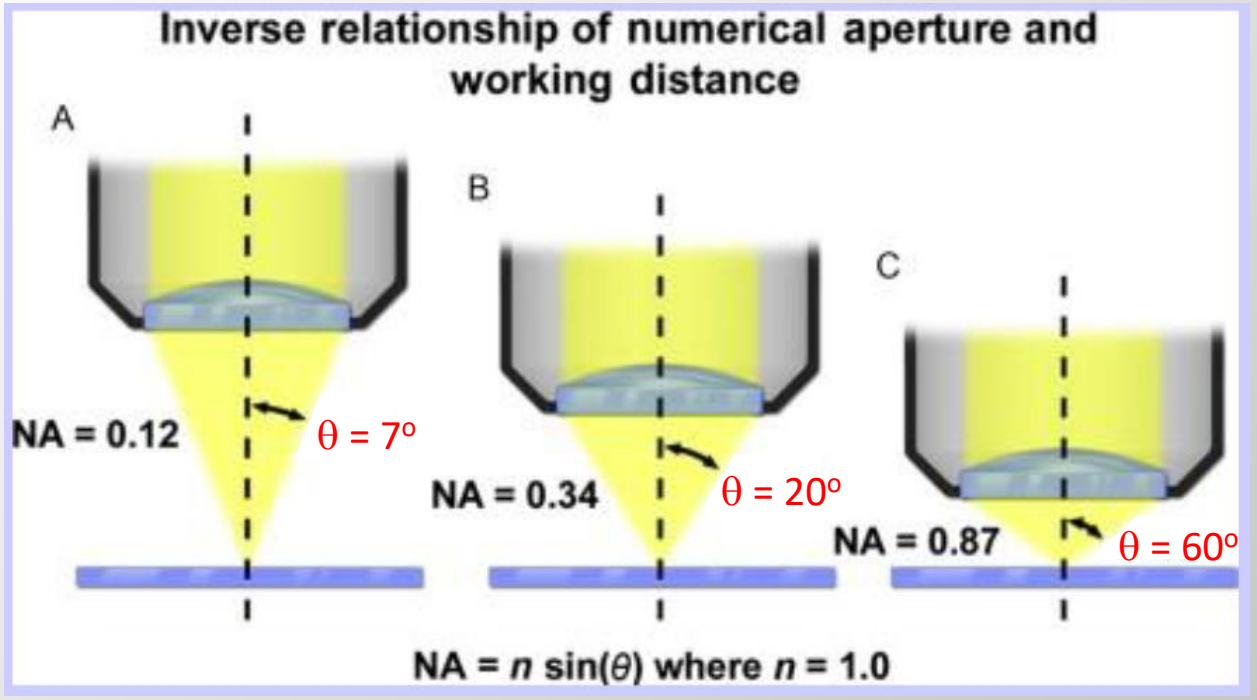
- **ATEM microscopes = Analytical TEM microscopes:**
  - Modern ATEMs (= microscopes with STEM + EDX...) are usually also HRTEM microscopes.
  - Conclusion: in top ATEMs, you can achieve atomic resolution also in STEM, EDX, EELS...
  - This is always connected with STEM mode in TEM:
    scanning of very thin specimen (note that max.resolution ≈ 1/10 of specimen thickness)
    in a top TEM microscope (you need all possible corrections and optimizations)
    and you get the images of atoms (but your material must withstand this!)

Suppl. #3 :: Why TEM resolution is worse than λ/2?

Part 1: Numerical aperture & marginal rays.

- **Numerical aperture (NA)**
  is a dimensionless number that characterizes the range of angles over which the system can accept or emit light (the exact definition of NA may vary for different fields of optics).

- **Numerical aperture in microscopy**
  LM: NA = n ∗ sinθ = refractive index of the medium ∗ sin(semi-angle of the objective lens)
  TEM: NA = sinα → n for vacuum is 1; aperture angle θ is frequently denoted as α in TEM



* NA in microscopy is always defined with respect to given point, typically to the center of the focused sample = to the central point at working distance.

* Refractive index is a part of the definition, as denser medium with higher n refracts light and effectively increases the acceptance angle (Snell's law).

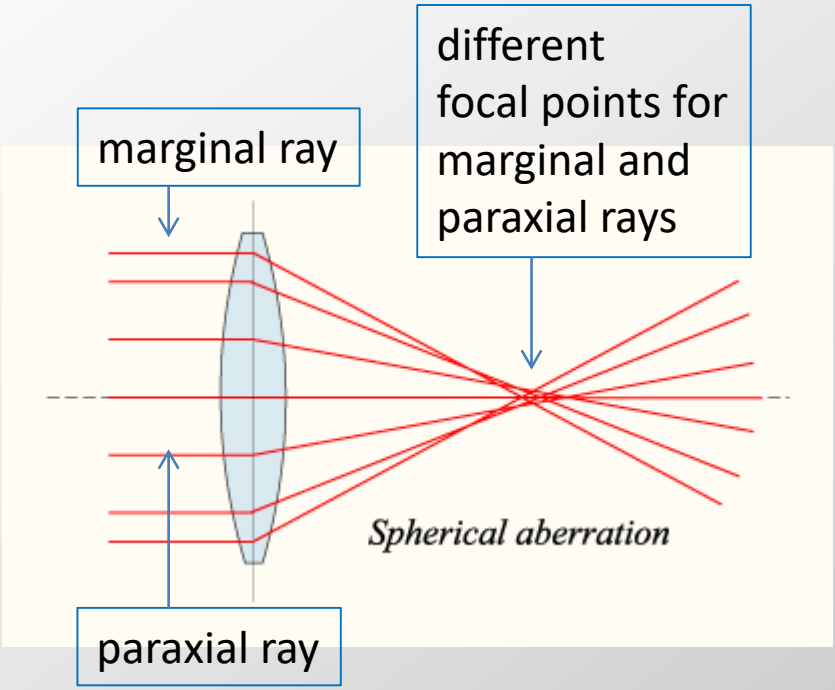
- **Higher NA ~ θ ~ α ⇒ less paraxial rays = more marginal rays = more lens aberrations.**
  (lens aberrations = non-ideal imaging → see next slide)

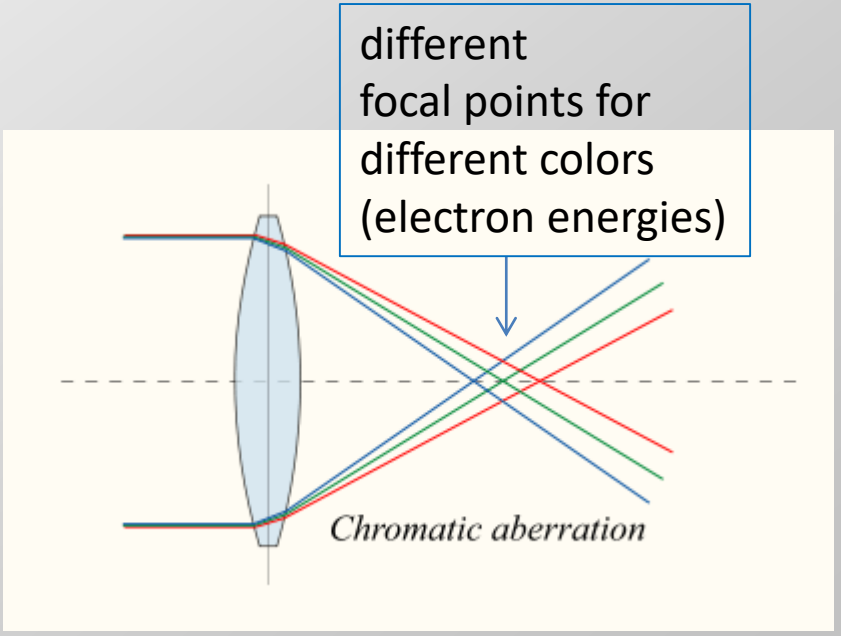
Suppl. #3 :: Why TEM resolution is worse than λ/2?

Part 2: Marginal rays and lens aberrations.

- **Glass lenses for LM are almost perfect.**
  Moreover, they can be combined together to compensate for the aberrations.
  Result: aplanatic/achromatic objective → LM resolution is limited by $d \approx \lambda/2 \approx 200nm$.

- **Electromagnetic lenses for TEM are very poor.**
  Moreover, their improvement is complicated (in the past: impossible, now: very costly).
  Result: the resolution in TEM is much worse than $d \approx \lambda/2 \approx 0.03/2 \approx 0.015Å$





- **Spherical aberration is the more critical in TEM**
  (logical: colors ≈ energies of electrons are more-or-less similar)

Suppl. #3 :: Why TEM resolution is worse than λ/2?

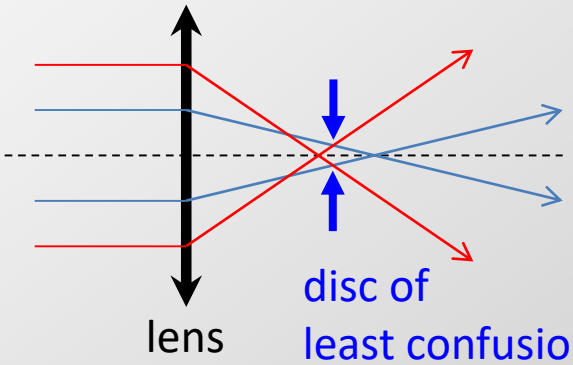
Part 3: End of the story from the introductory lecture.

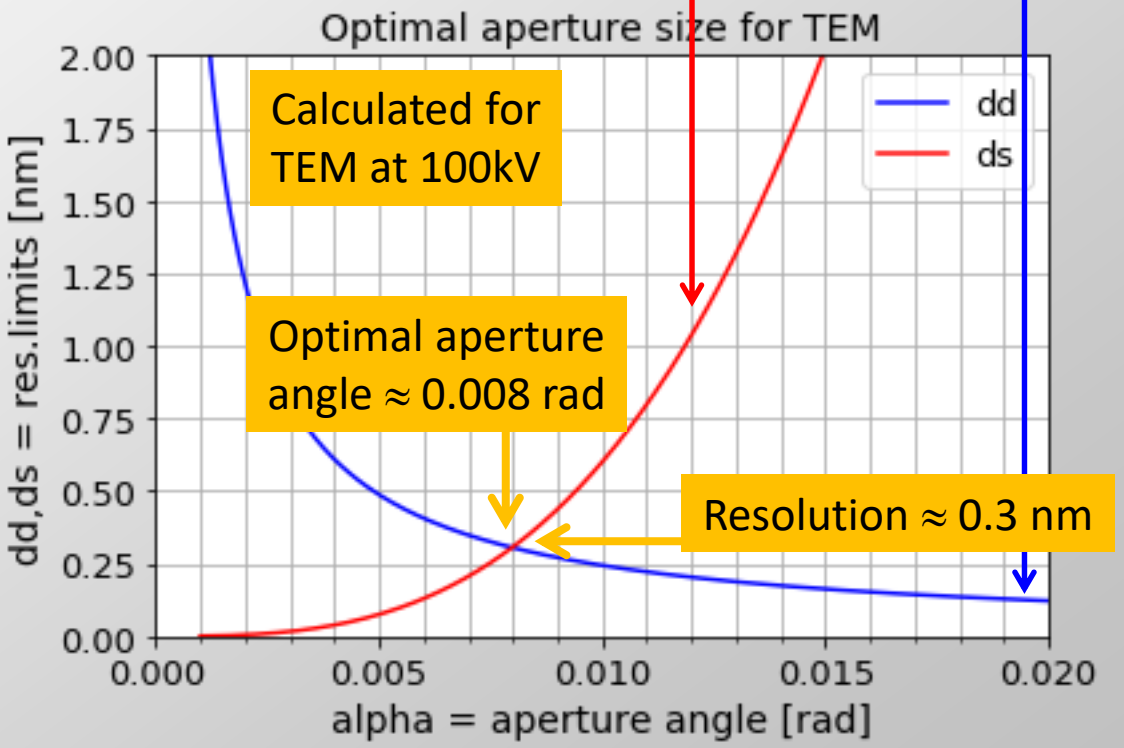
Diffraction-limited resolution in TEM (Rayleigh): $\delta_d = \lambda/2\sin(\alpha) = 0.50\lambda/\sin(\alpha) \approx 0.61\lambda/\alpha$

- derivation of the basic formula for δ_d = diffraction limit $d=\lambda/2$ → introductory lecture
- plus: finite aperture: sin(α); Abbe(0.50) → Rayleigh(0.61); small angles: sin(α) ≈ α

Spherical aberration and the radius of the disc of the least confusion: $\delta_s \approx C_s\alpha^3$

- spherical aberration of modern HRTEM microscopes ≈ 0.6mm = 6×10^5nm
- meaning of δ_s and C_s graphically (the higher C_s, the bigger disc of least confusion and δ_s):





Logical ↑ higher aperture ⇒ more marginal rays

Calculation of real resolution ↑ in Jupyter/Python.

* Theory: Brandon & Kaplan: Microstructural Characterization of Materials (2008), pp. 188-189.

Part 3

TEM/BF – interpretation of micrographs

Contents

- Theory:
  Explanation of contrast in TEM/BF using ray optics
  → nice application of theory from the introductory lecture!
- Examples:
  Application of TEM/BF for polymer systems
  Visualization of nanoparticles/nanofillers and ultrathin sections

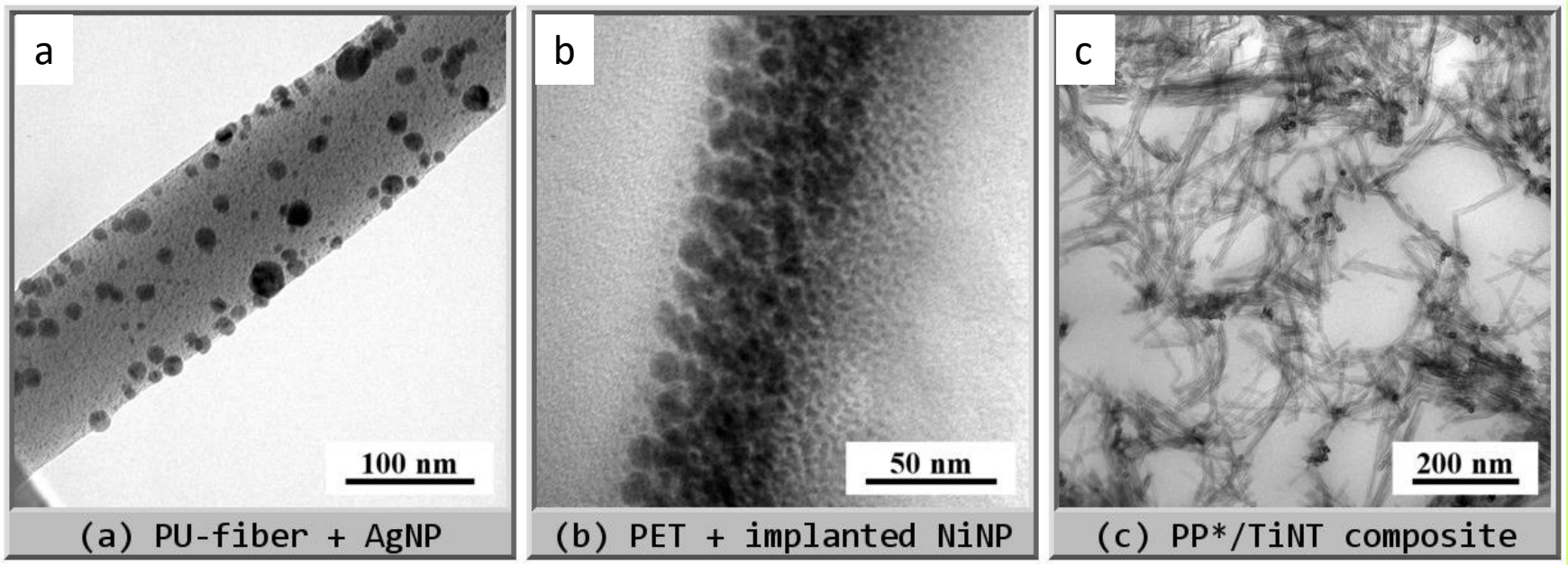
TEM/BF :: typical applications (for polymer systems)

Two main types of contrast in TEM/BF:

(1) mass-thickness contrast = thicker/higher-Z areas appear darker

(2) diffraction contrast = diffracting/scattering areas appear darker

Important advantage of TEM/BF in comparison with STEM/BF: higher resolution



TEM/BF micrographs of various polymer systems:

(a) morphology nanoparticles – polyurethane nanofibers decorated with Ag nanoparticles

preparation: 2uL of the nanofiber suspension onto Cu-grid with C-film, left to evaporate

(b) subsurface morphology of bulk polymers – PET surface implanted with Ni ions

preparation: cut small piece of sample, embed in epoxy resin, cut cross-section of the surface with UMT

(c) internal morphology of bulk polymers – dispersion of TiNT in e-beam modified PP:

preparation: cut square pyramid with upper surface 0.1x0.1mm, cut with Cryo-UMT, observe in TEM

TEM/BF :: two main types of contrast (for polymer systems)

Two main types of contrast in TEM/BF:

(1) mass-thickness contrast: higher mass (= higher Z) or thicker areas ⇒ darker

(2) diffraction contrast: areas that diffract (or scatter) more electrons ⇒ darker

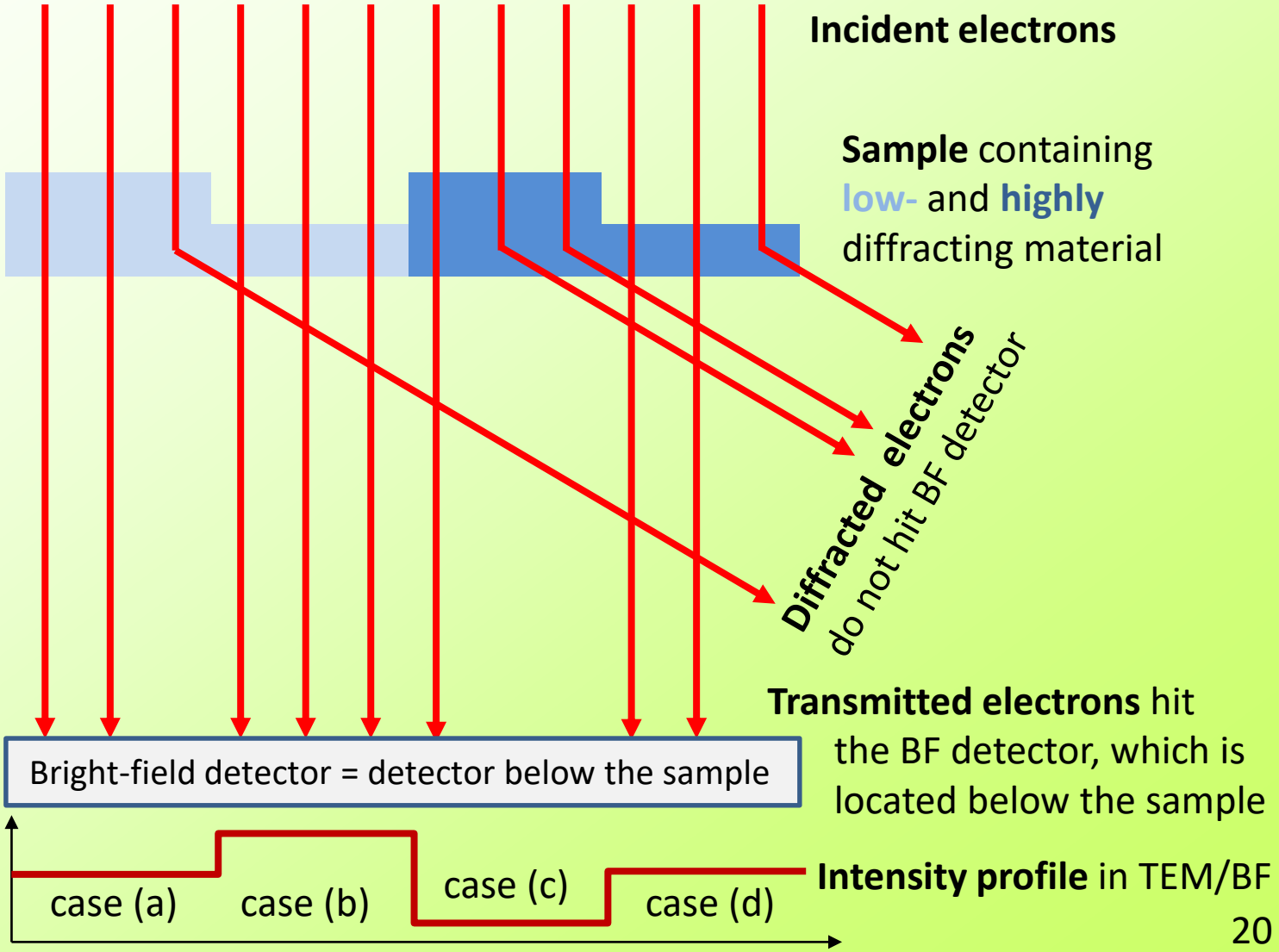
(a) low-diffracting and thick sample: **medium intensity**

(b) low-diffracting and thin sample: **high intensity**

(c) highly-diffracting and thick sample: **low intensity**

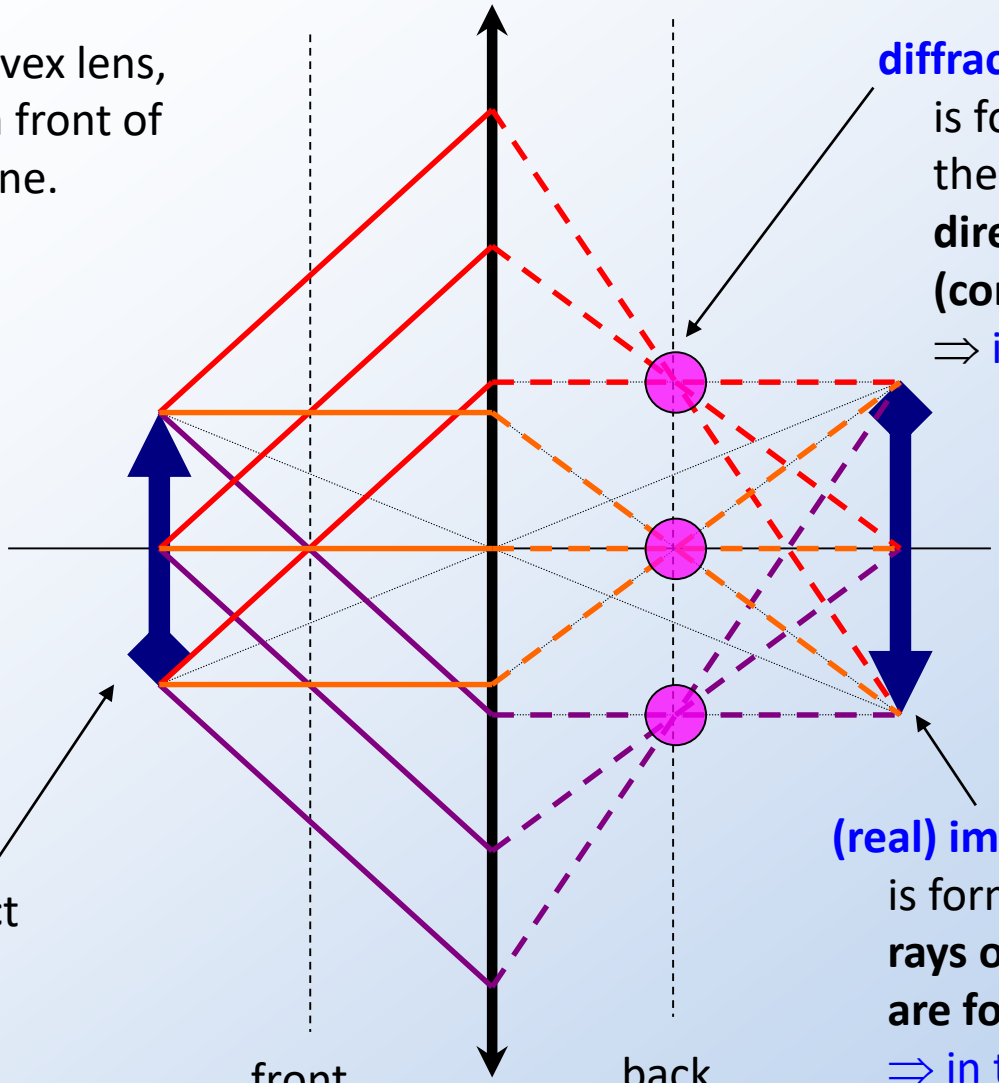
(d) highly-diffracting and thin sample: **medium intensity**

Note: for inorganic materials, textbooks usually differentiate more types of diffraction contrast!



TEM/BF :: Theory :: Lenses, images, and diffraction

Thin convex lens, object in front of focal plane.



diffraction pattern
is formed at the location where the **rays passing in the same direction are focused (come together)**
⇒ in back focal plane

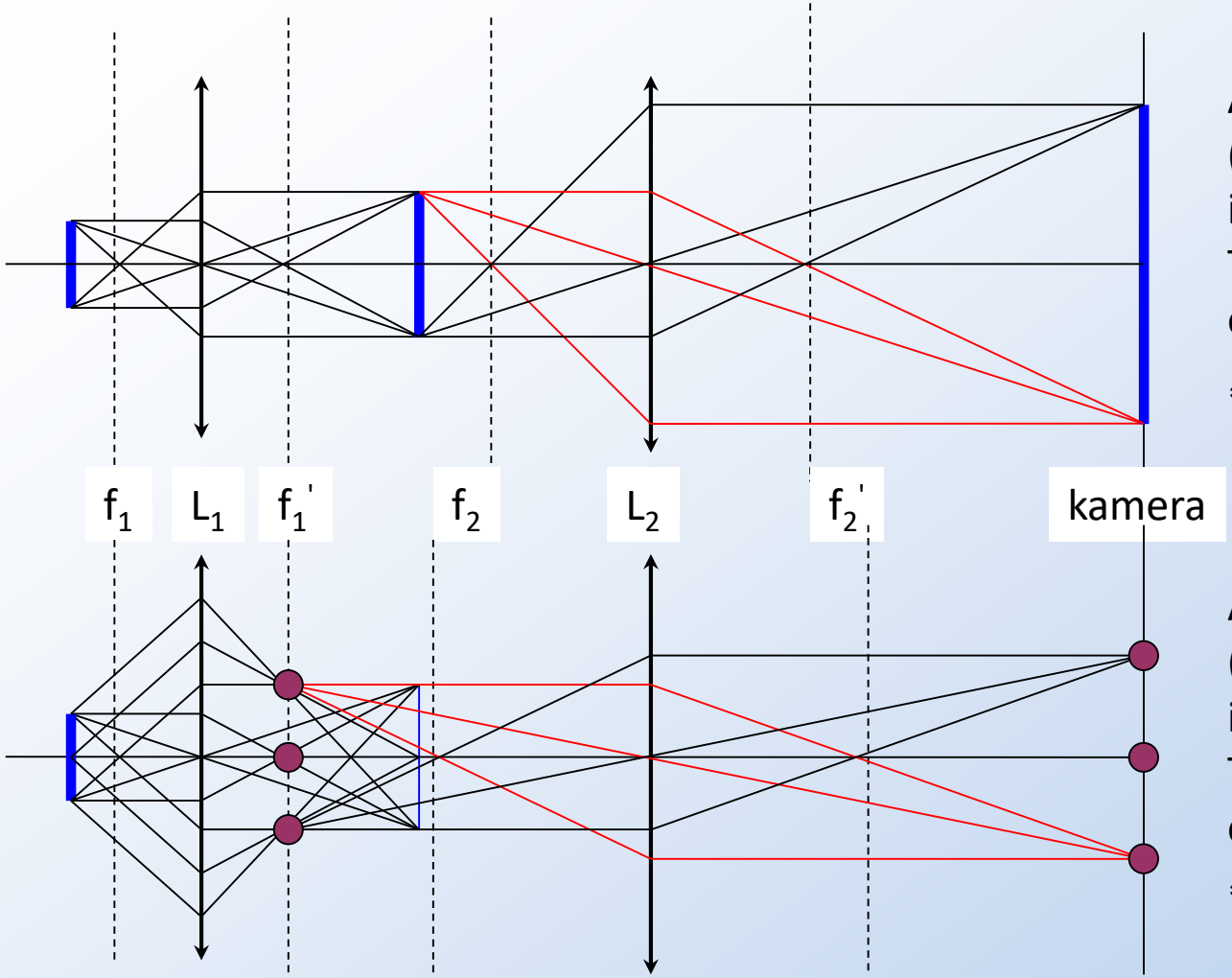
object

front focal plane

back focal plane

(real) image
is formed at the location where the **rays originating in the same place are focused (come together)**
⇒ in the image plane

TEM/BF :: Ray optics :: Imaging and diffraction mode



A two-lens microscope (from previous slide) in imaging mode. The 2nd lens (L_2) is focused on (real) image.

* lens L_2 is stronger than in diffraction mode (Power = $1/f_2$)

A two-lens microscope (the same as above) in diffraction mode. The 2nd lens (L_2) is focused on diffraction pattern.

* lens L_2 is weaker than in imaging mode (Power = $1/f_2$)

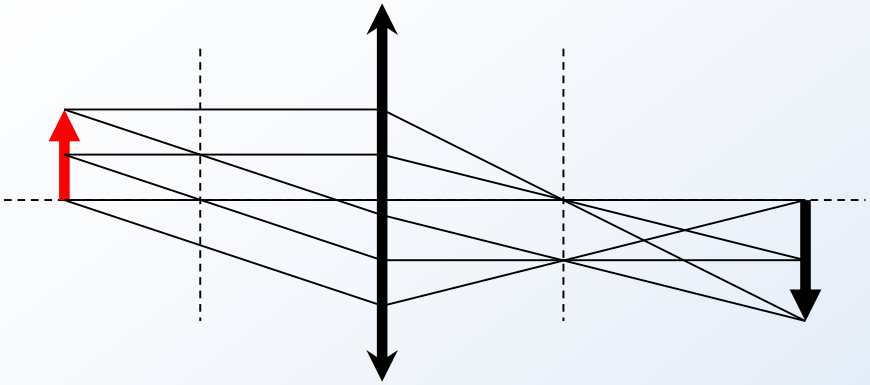
sample and its images

diffraction pattern

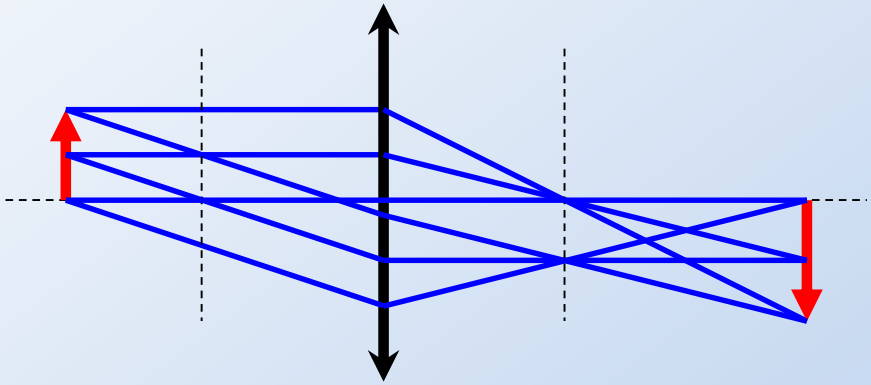
Note: power of the lens ~ 1/[focal length] in EM can be altered by changing the strength of the current.

TEM/BF :: Theory :: Mass-thickness and diffraction contrast

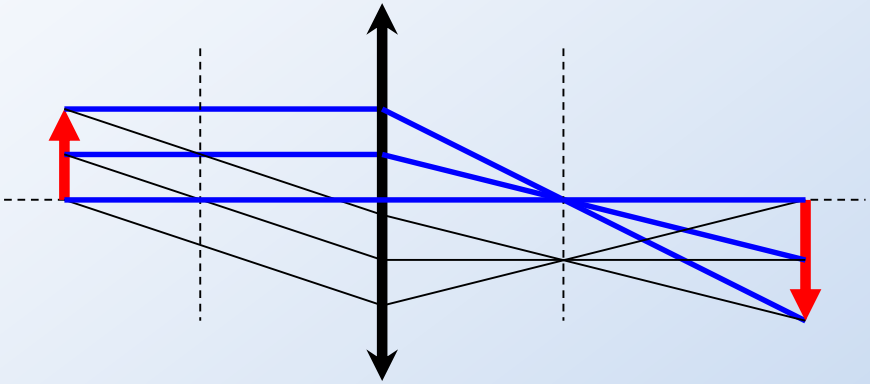
TEM without objective aperture – just mass-thickness contrast.



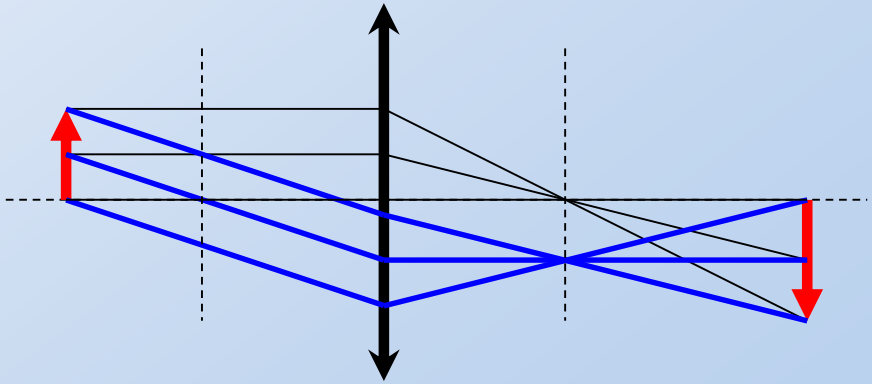
Object that neither transmits nor diffracts electrons ⇒ black.



Object that both transmits and diffracts electrons ⇒ white.



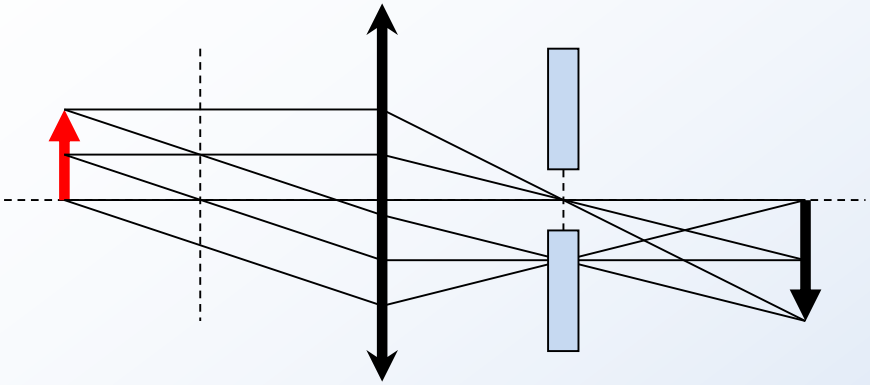
Object that transmits but does not diffract electrons ⇒ white.



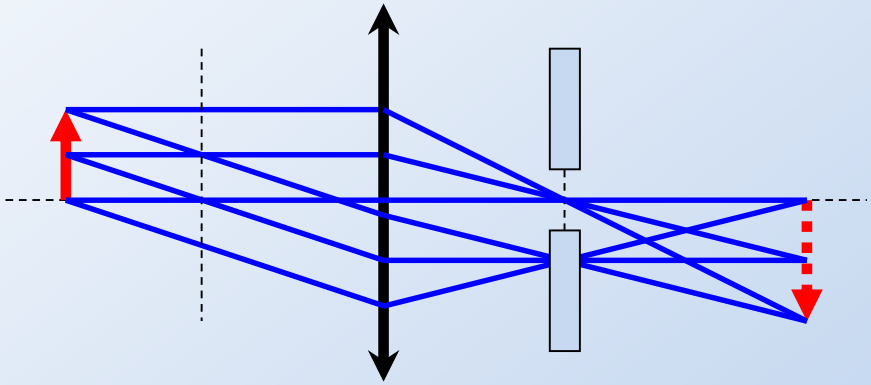
Object that does not transmit but diffracts electrons ⇒ white.

TEM/BF :: Theory :: Mass-thickness and diffraction contrast

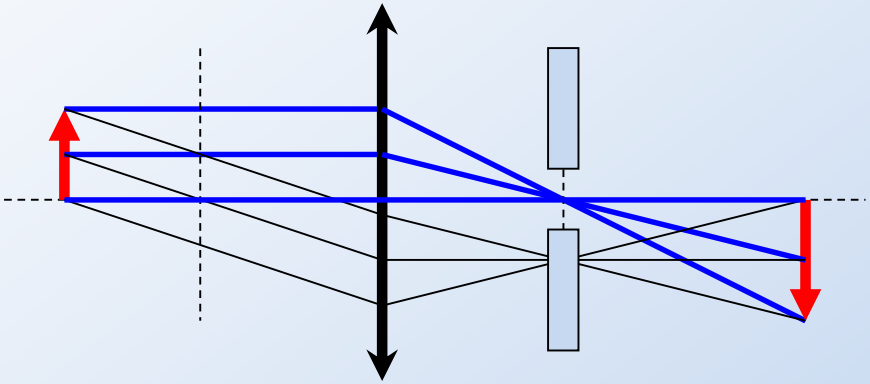
TEM with the objective aperture in the center – bright field imaging.



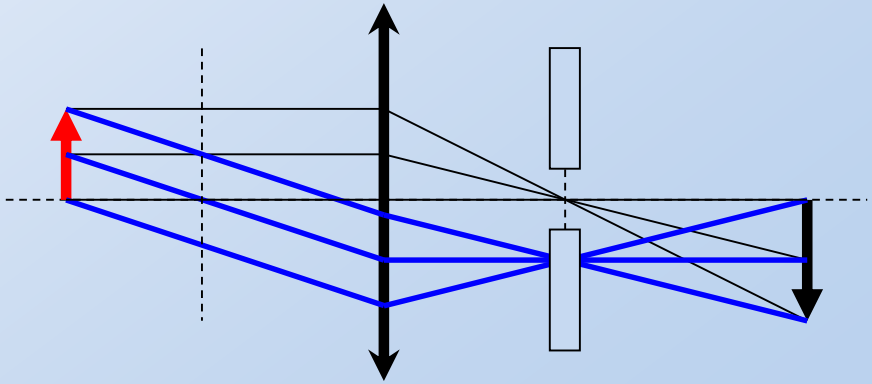
Object that neither transmits nor diffracts electrons ⇒ black.



Object that both transmits and diffracts electrons ⇒ gray. → contrast in TEM/BF



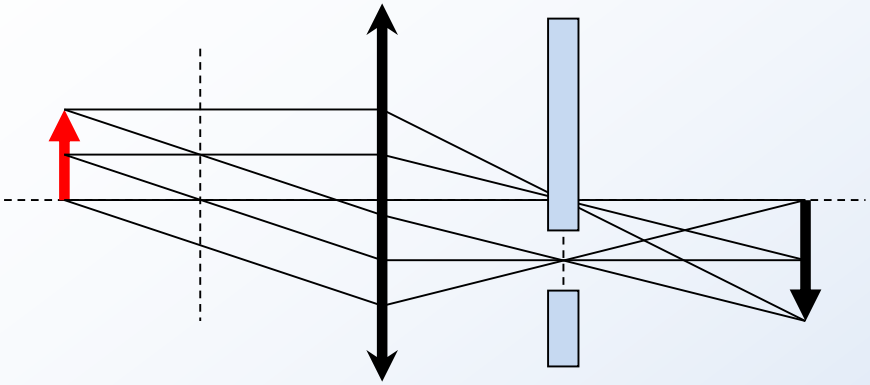
Object that transmits but does not diffract electrons ⇒ white. → contrast in TEM/BF



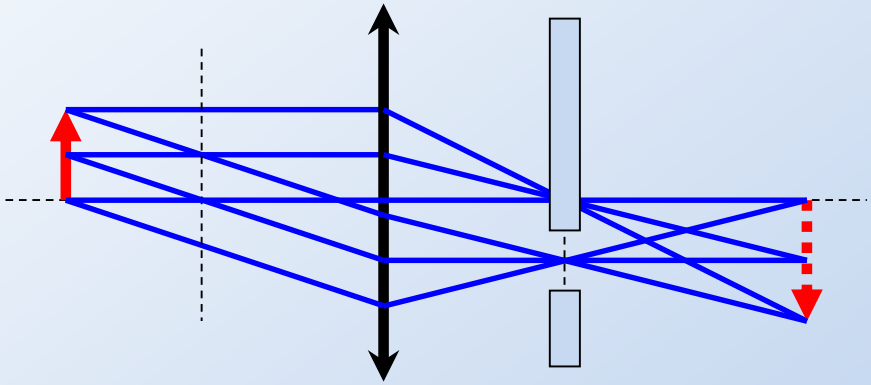
Object that does not transmit but diffracts electrons ⇒ black. → contrast in TEM/BF

TEM/BF :: Theory :: Mass-thickness and diffraction contrast

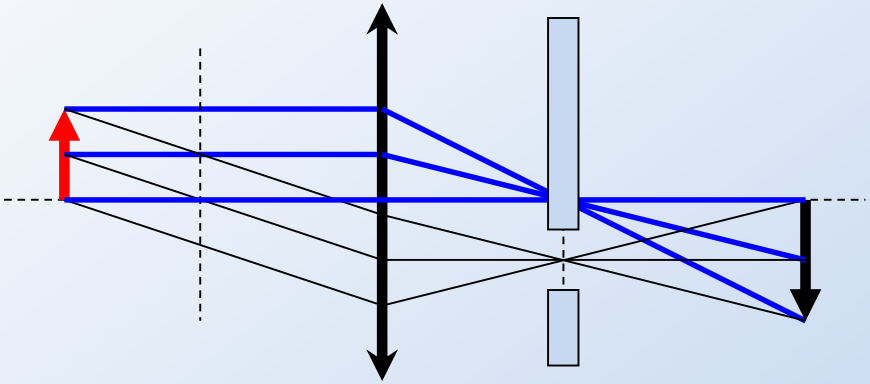
TEM with the objective aperture outside center – dark field imaging.



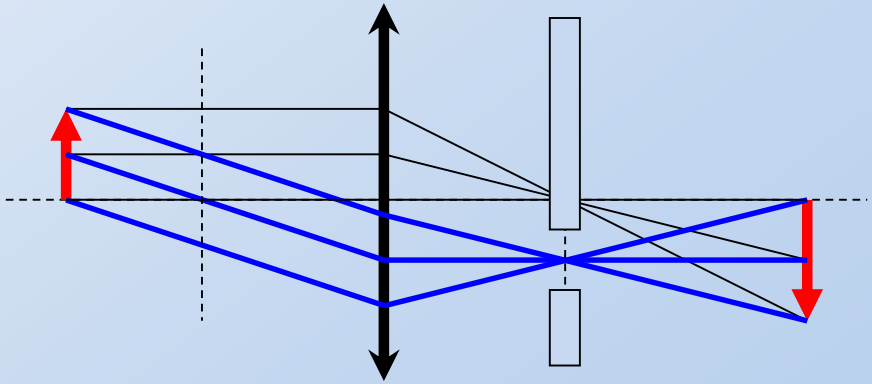
Object that neither transmits nor diffracts electrons ⇒ black.



Object that both transmits and diffracts electrons ⇒ gray. → contrast in TEM/DF



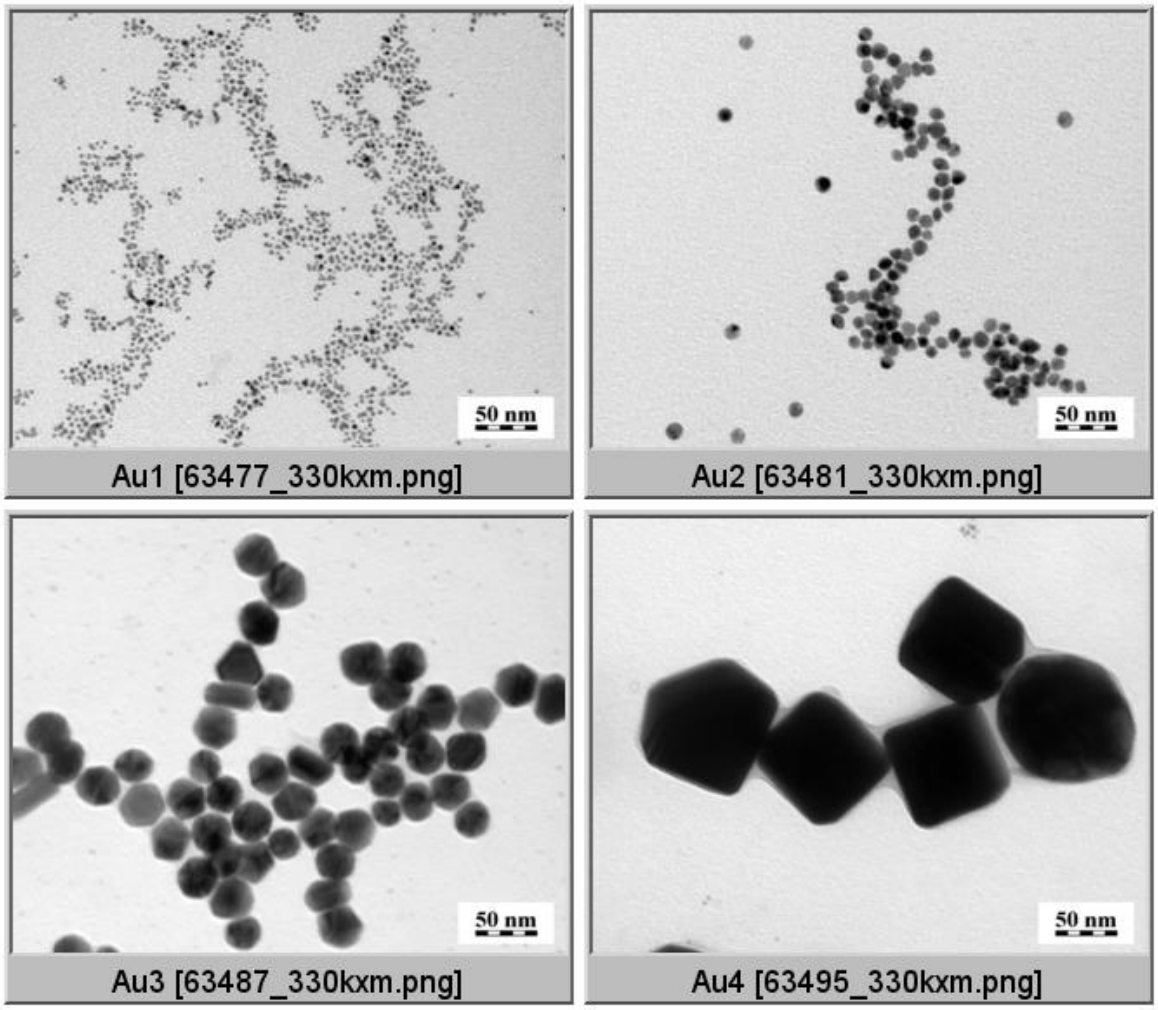
Object that transmits but does not diffract electrons ⇒ black. → contrast in TEM/DF



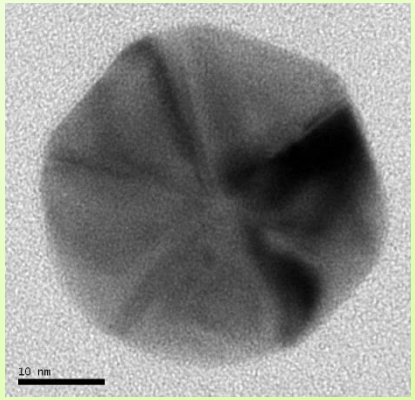
Object that does not transmit but diffracts electrons ⇒ white. → contrast in TEM/DF

TEM/BF :: Examples

Visualization of nanoparticles: **mass-thickness contrast** (+ diffraction contrast).



TEM/BF micrographs: Au-NP with tunable size on C-film.
Mass-thickness contrast dominates (proof: OBJ aperture).



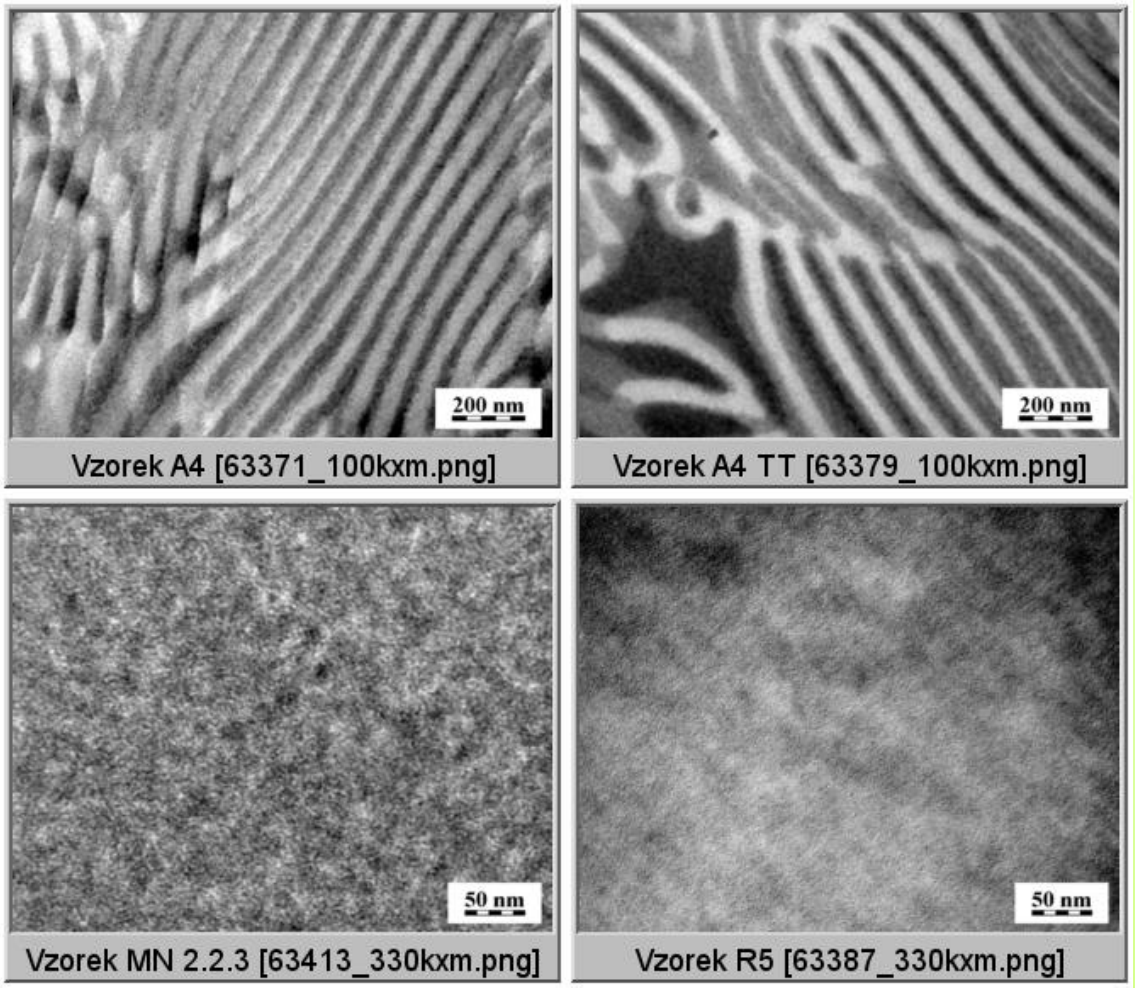
TEM/BF, higher magnification.

Sample preparation:

1) Preparation of Au-NC.
   (controlled reduction of Au^{3+}
2) Preparation of C-films.
   (vacuum evaporation device
3) Transfer of C-films on Cu grids.
   (alternative: buy the grids
4) Drop 2µl of Au colloid
   onto carbon-coated Cu-grid
   suck off the excess of solution.
5) Insert the grid into TEM
   and observe in BF ☺

TEM/BF :: Examples

Microscopy of ultrathin sections: **diffraction contrast** (+ mass-thickness contrast).



TEM/BF of several PS-based block copolymers stained with RuO_4, showing microphase-separated blocks.

Sample preparation:

1) Preparation of ultrathin sections and their capture onto microscopic Cu-grids (ultramicrotome @ RT)
2) Staining of samples on the grids by RuO_4 vapors. (as usual, one phase is stained (faster than the other by RuO_4
3) Insertion of the grid into TEM and observation in BF.

How can we verify if our sample exhibits also absorption contrast?

- remove OBJ aperture
- look if you can still differentiate the structure:
  $\rightarrow$ yes $\Rightarrow$ mass-thickness contrast
  $\rightarrow$ no $\Rightarrow$ only diffraction contrast
- this case:
  after removing OBJ aperture the structure still partially visible

Part 4

TEM/DF – interpretation of micrographs

Contents

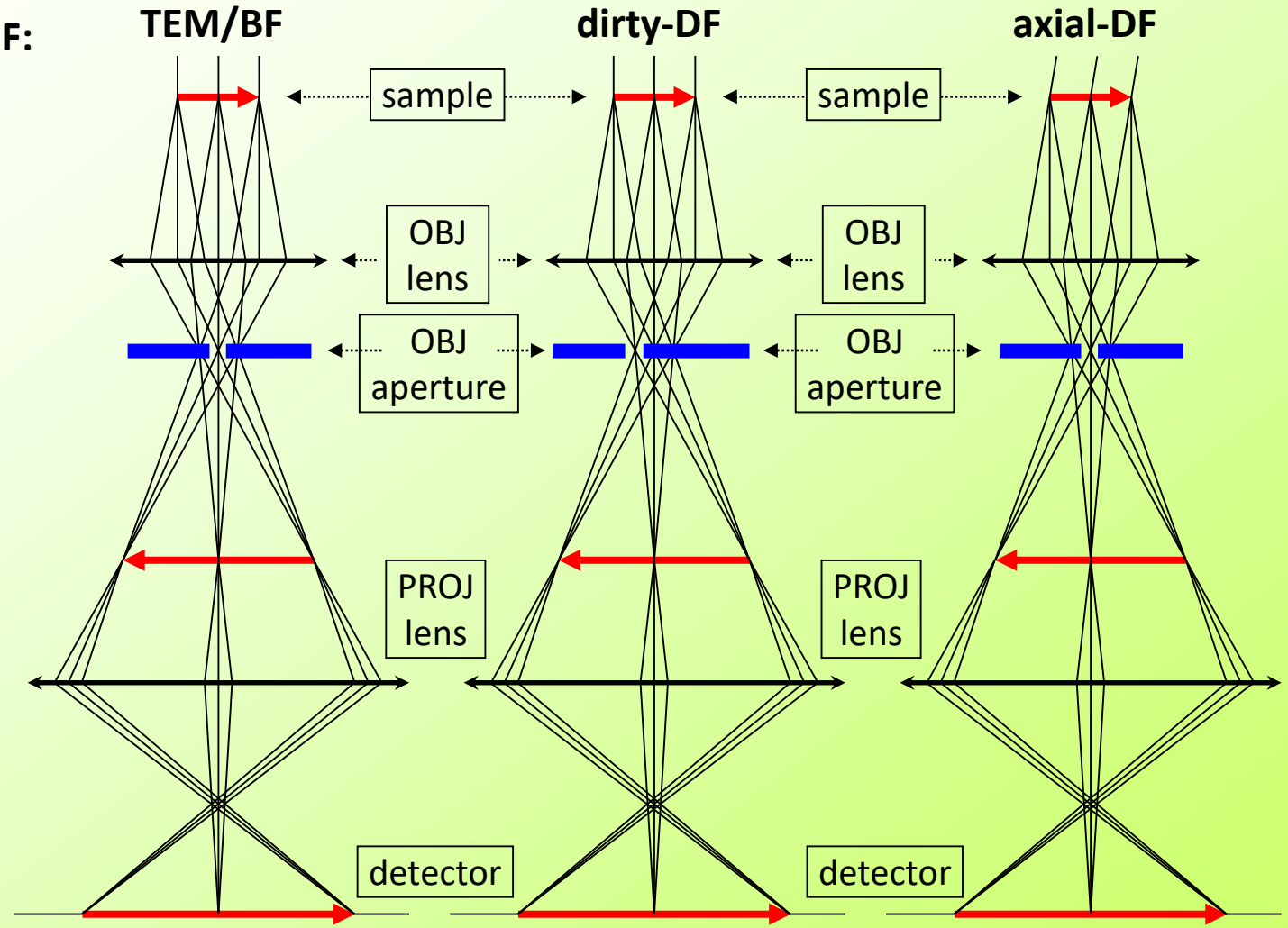
- Theory:
  explanation of contrast in TEM/DF using ray optics
  → simple ray-tracing diagrams of TEM/BF vs. TEM/DF
  another instructive application of ray optics in TEM
- Examples:
  1) application of TEM/DF in order to increase contrast
  2) application of TEM/DF in order to real structure of crystals

TEM/DF :: Theory :: Ray-tracing diagram of the microscope

Principle of DF. types of DF. Jak získat TEM/DF mikrofotografii?

Physical principle of DF:
We detect real image formed only by diffracted beams.

Types of DF:
We need to transmit just diffracted beams. This can be achieved in two main ways: dirty-DF (is easier to achieve and understand) and axial-DF (yields better micrographs). The modes of DF are best shown in ray-tracing diagrams.



How to get TEM/DF micrograph: 1) get TEM/BF, 2) for axial-DF, incline the PE beam, 3) switch to ED, 4) select a diffracted beam by OBJ-aperture, 5) switch back to BF ⇒ because we have selected diffracted beams (instead of transmitted beams), we get TEM/DF (instead of BF).

TEM/DF :: Examples

Example 1. Application of TEM/DF in order to increase contrast.

Practical example ↓ taken from [Transmission Electron Microscopy and Diffractometry, str.76].

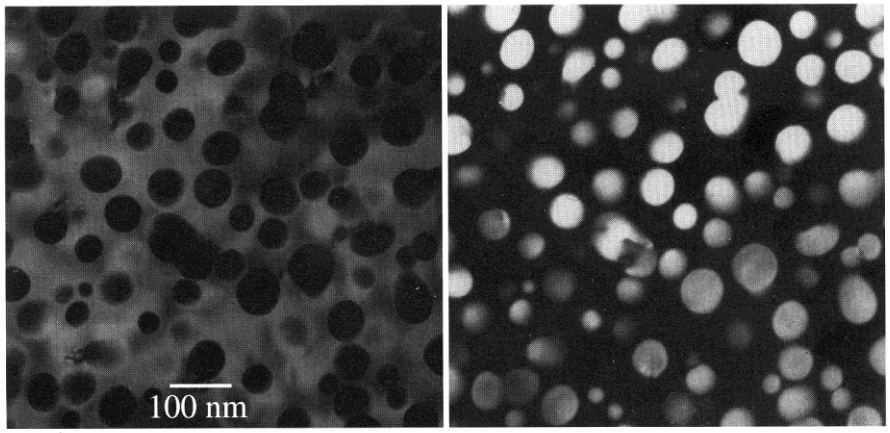
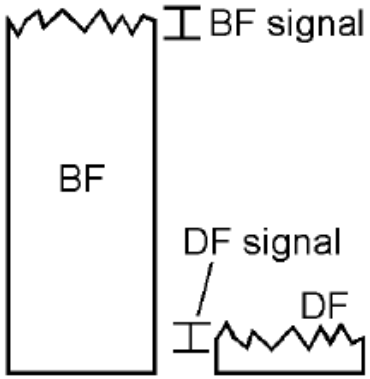


Fig. 2.14. Spherical δ' precipitates in an Al-Li alloy at 80,000 X magnification. Left: BF image. Right: DF image from (100) diffraction spot, unique to δ' precipitates.

Theoretical explanation ↓ taken from [TEM Tecnai manual, Chapter: Modes, page: 21].



The origin of the high contrast is shown schematically in the figure to the left. Bright-field as well as dark-field images display changes in intensity across the image. In both cases the total range of intensities is roughly similar. In bright-field images, however, the changes in intensity come on top of a high and unvarying signal – the undiffracted electrons. If one attempts to expose for a longer time to improve the signal, the negative becomes overexposed. In the case of the dark-field the background signal is much lower, leading to a much higher contrast in the image.

Note: With negatives the inherent lower contrast of the bright-field image is inescapable. With slow-scan CCD images it is however possible to subtract the uniform background from the image and stretch the contrast.

TEM/DF :: Examples

Example 2: Application of TEM/DF for visualization of real structure of crystals.

TEM/DF is frequently used for study of specific structures/dislocations of real crystals.

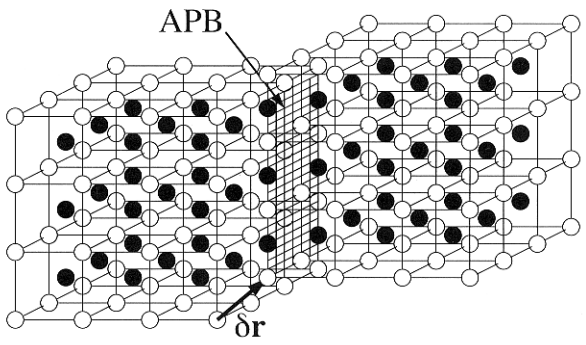


Fig. 7.59. Illustration of an APB with $\delta r = a/2\langle 111\rangle$ in the B2 structure.

The basic principle is not difficult:

- specific dislocations give specific diffractions
- if we select a specific diffraction and visualize only the beams from this diffraction, we see the dislocation

Typical procedure: we visualize the dislocation in TEM/BF, switch to TEM/SAED in order to get the diffractogram from selected area, localize the specific diffraction(s), select them by means of OBJ aperture and switch to TEM/DF mode.

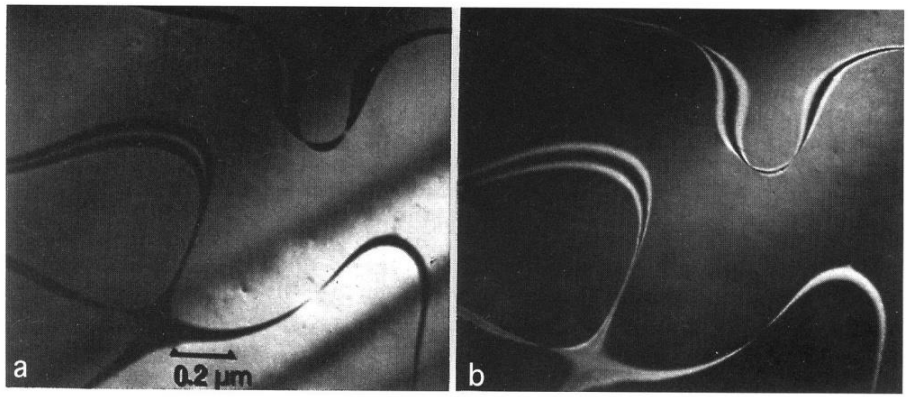


Fig. 7.60. (**a**) Bright-field and (**b**) axial dark-field images of APBs in ordered Fe_3Al using a (100) superlattice diffraction. After [7.2].

By comparing TEM/BF and TEM/DF we can determine the dislocation type. In this slide, we show APB disclocation (upper image), which is manifested on TEM/BF and TEM/DF micrographs (lower image) with inverted contrast (the inverted contrast is typical of APB).

Part 5

TEM/SAED – interpretation of diffractograms

Diffraction :: Level 1 = distances
1) crystals and crystallographic planes
2) crystallographic planes and Bragg's Law
3) Bragg's Law and diffraction experiments
4) Bragg's Law and TEM/SAED => camera equation
5) TEM/SAED in the microscope => ray-tracing

Types of diffractograms, examples
* types: monocrystal, polycrystalline, and oriented samples
* examples: identification of structures, limitations...

Diffraction :: Level 2 = distances + positions
(optional – just in Appendix – better understanding of TEM/SAED
(examples: calibration and indexing of diffraction patterns

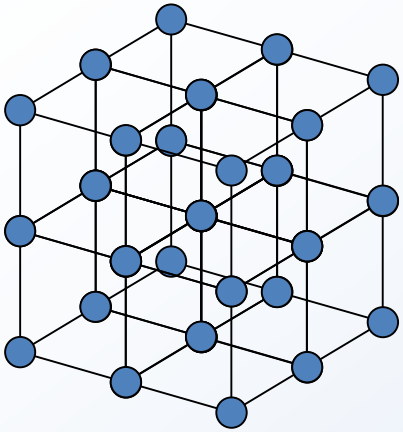
Diffraction :: Level 3 = distances + positions + intensities
(optional – just in Appendix – brief intro to diffraction theory
(example: *ab initio* calculation of powder diffraction with Jupyter

Diffraction :: Level 4...
(just mentioned here for the sake of completeness
(structure analysis, dynamic theory, magnetic structures...

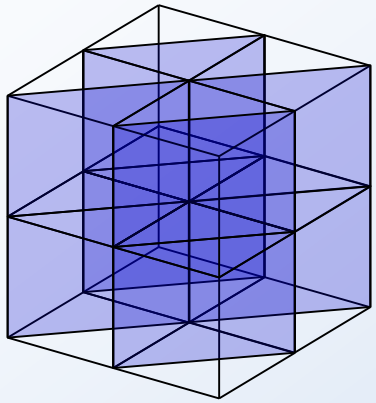
Note: distances, positions and intensities = distances, positions and intensities of the diffractions spots/rings on the diffraction pattern with respect to the origin = to the position of primary beam.

TEM/ED :: Theory :: Level 1

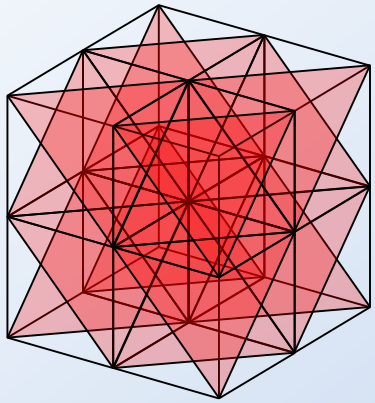
Crystals and crystallographic planes (revision of general chemistry).



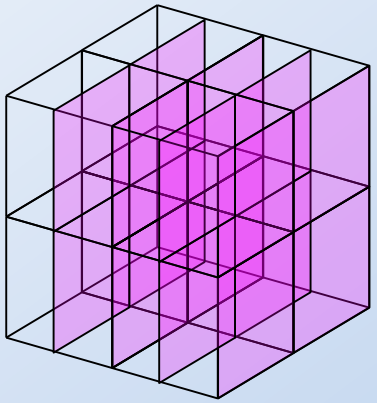
Crystal:
3D-periodic structure.



Lattice
planes (110).



Lattice
planes (111).



Lattice
planes (200).

Materials can crystallize in one of **seven crystal systems**: cubic, hexagonal, trigonal, tetragonal, orthorhombic, monoclinic, and triclinic.

In crystal lattices we define **lattice planes**, defined by Miller and/or **diffraction indexes** (hkl).

Interplanar spacing d_{hkl} (i.e. the distance between [hkl] planes) can be calculated

d_{hkl} = function(a,b,c,α,β,γ,h,k,l)

(justification → consult XRD textbooks

(calculation in Jupyter: → Ex.1

$$\frac{1}{d_{hkl}^2} = \left[\frac{h^2 \sin^2\alpha}{a^2} + \frac{k^2 \sin^2\beta}{b^2} + \frac{l^2 \sin^2\gamma}{c^2} + \frac{2hk}{ab}(\cos\alpha \cos\beta - \cos\gamma) + \frac{2kl}{bc}(\cos\beta \cos\gamma - \cos\alpha) + \frac{2lh}{ca}(\cos\gamma \cos\alpha - \cos\beta)\right] / (1 + 2\cos\alpha \cos\beta \cos\gamma - \cos^2\alpha - \cos^2\beta - \cos^2\gamma)$$

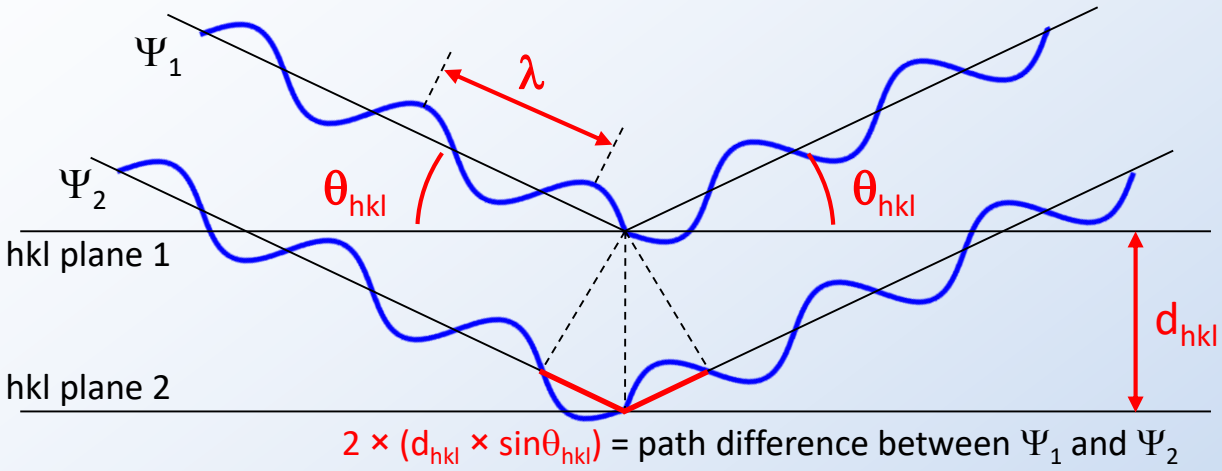
TEM/ED :: Theory :: Level 1

Lattice planes and Bragg's Law (revision from the introductory lecture).

Bragg's Law in words:

Maximal interference = diffraction of lattice plane (hkl) is observed just at specific angles ($2d\sin\theta = n\lambda$).

Bragg's Law graphically:



BL and Physics:

1) Ray optics
   (wave reflection
2) Wave optics
   (interference

BL and Mathematics:

$2d_{hkl}\sin\theta_{hkl} = n\times\lambda$

path difference between waves Ψ_1 a Ψ_2 — integer multiplication of λ

Bragg's Law and lattice planes:

A crystal contains plains: d_{hkl} = function(a,b,c,α,β,γ,h,k,l) – see previous page.

BL says: diffraction will be observed only for θ_{hkl} such that d_{hkl} will obey $2d_{hkl}\sin\theta_{hkl} = n\times\lambda$

Limitations of Bragg's Law:

[1] Distances of diffractions from the center (of diffractogram): direct information $\Rightarrow \theta_{hkl}$.

[2] Positions of diffractions: indirect information $\Rightarrow$ just intuitively, based on law of reflection.

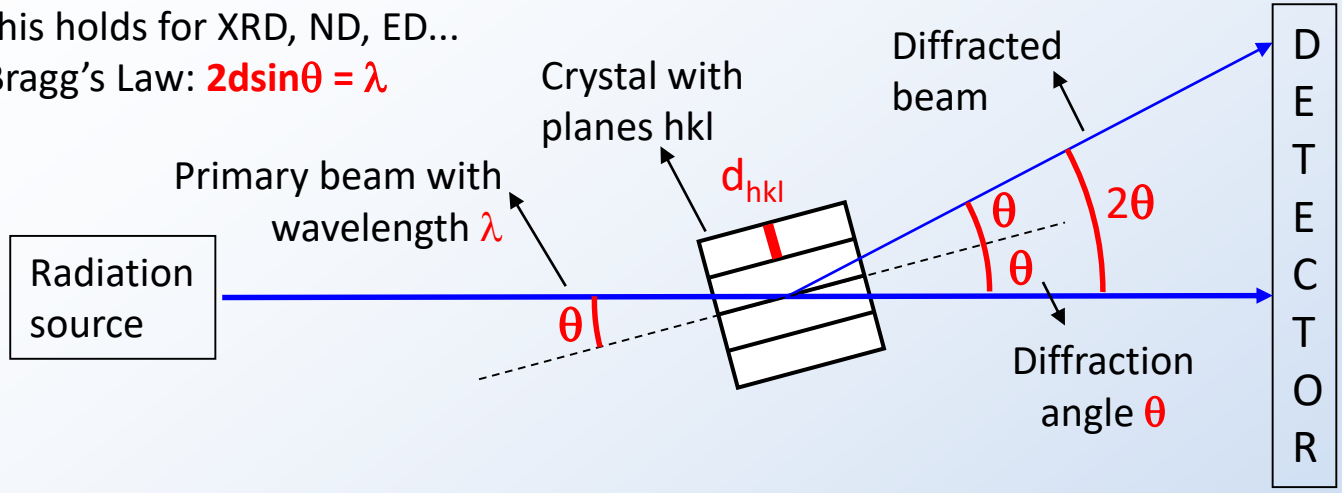
[3] Intensity: no information $\Rightarrow$ just some sophisticated considerations about atoms in planes..

TEM/ED :: Theory :: Level 1

Bragg's law and diffraction experiments.

General diffraction experiment (with a crystal):

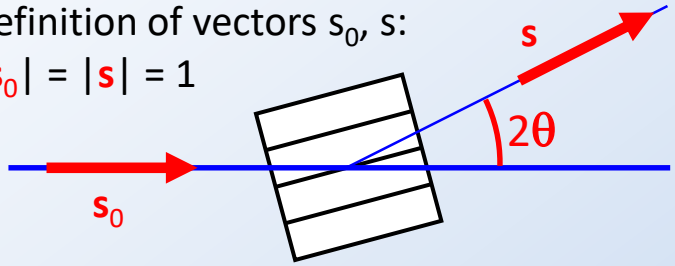
(this holds for XRD, ND, ED...
(Bragg's Law: **$2d\sin\theta = \lambda$**



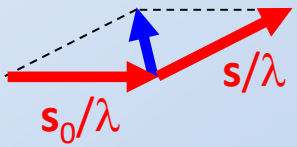
Important conclusions:
[1] One lattice plane yields one diffr. spot.
[2] Bragg's law gives:
a) distance of the spot from the primary beam
b) estimate of position from the exp. geometry
NO info about intensity!

Bragg's law & relations θ × S × q

Definition of vectors s_0, s:
$|\mathbf{s_0}| = |\mathbf{s}| = 1$



Definition of diffraction vectors:
$\mathbf{S} = (\mathbf{s}-\mathbf{s_0})/\lambda$
$\mathbf{q}=2\pi\mathbf{S}$



The magnitudes of diffraction vectors **S** and **q** increase with increasing diffraction angle θ.

→ see Ex.2

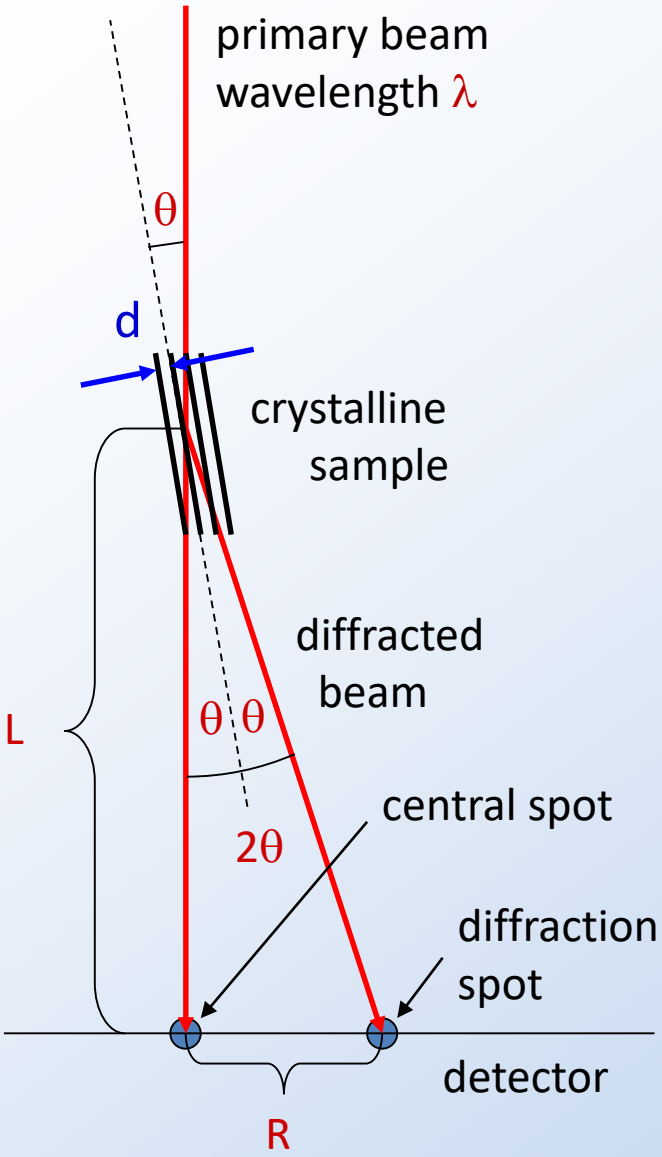
Alternative forms of Bragg's law:

$2d\sin\theta = n\lambda$ ← basic form, sometimes with diffraction indices : $2d_{hkl}\sin\theta_{hkl} = n\lambda$
$2d\sin\theta = \lambda$ ← without n, because it holds, for example: $2d_{100}\sin\theta_{100}=2\lambda \equiv 2d_{200}\sin\theta_{200}=1\lambda$
$dq = 2\pi n$ ← with q, because $q = 4\pi\sin\theta/\lambda$
$dS = n$ ← with S, because $S = 2\sin\theta/\lambda$

→ see Ex.2

TEM/ED :: Theory :: Level 1

Bragg's law and diffraction experiments in TEM.



Input data:

d = interplanar spacing → this is what we want

R = distance of diffraction from the center → measure

θ = diffraction angle → to be calculated

L = this is *camera constant* → has to be calibrated

λ = wavelength of electrons → can be calculated
but it is calibrated together with L

Important prerequisites:

Electrons diffract at very low angles and,
as a result: $\sin\theta \approx \tan\theta \approx 1/2*\tan(2\theta)$ → Ex.3

Calculation of d:

(1) Bragg's law: $2d*\sin\theta = \lambda$

(2) Geometry of the experiment: $\tan(2\theta) = R/L$.

(3) Prerequisite – low angles: $\tan(2\theta) = 2\sin\theta = R/L$

(4) Combination of (1+3): $Rd = \lambda L$ (camera equation)

(5) The constant λL is determined from calibration,
i.e. from the diffraction with a known sample.

Recalculations d × θ × S × q:

Important in practice: see previous page.

TEM/ED :: Theory :: Level 1

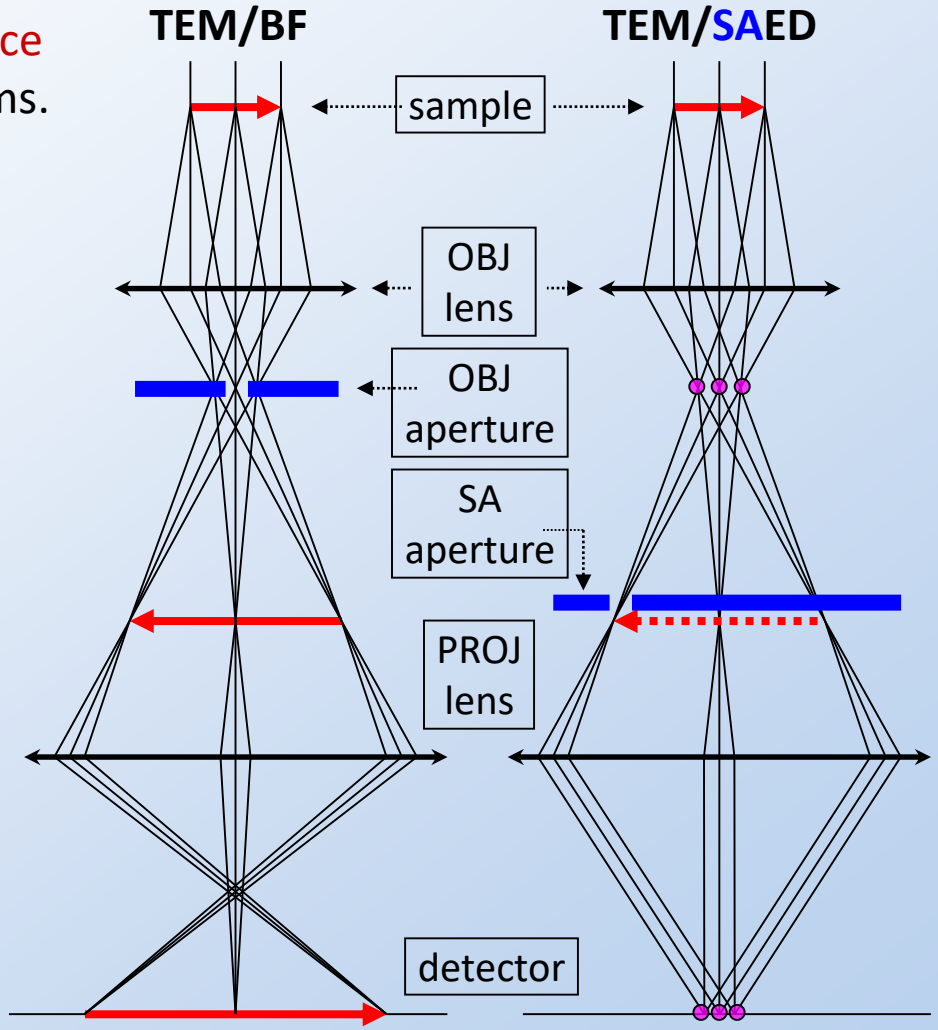
Ray-tracing diagram for TEM/SAED experiment.

Physical principle of ED: scattering + interference

1) Electrons are elastically scattered on atoms.
2) After scattering, the waves interfere and give rise to diffraction patterns.

Types of electron diffraction:

1) The basic type of diffraction in TEM is
   SAED = selected-area ED
   → the area for diffraction is selected by SA aperture
2) Modern TEM microscopes have also
   ED = aperture-less ED
   → the area for diffraction is selected directly by e-beam
3) And many other types...
   CBED = Convergent Beam ED
   NBD = NanoBeam ED
   PED = Precession ED ...

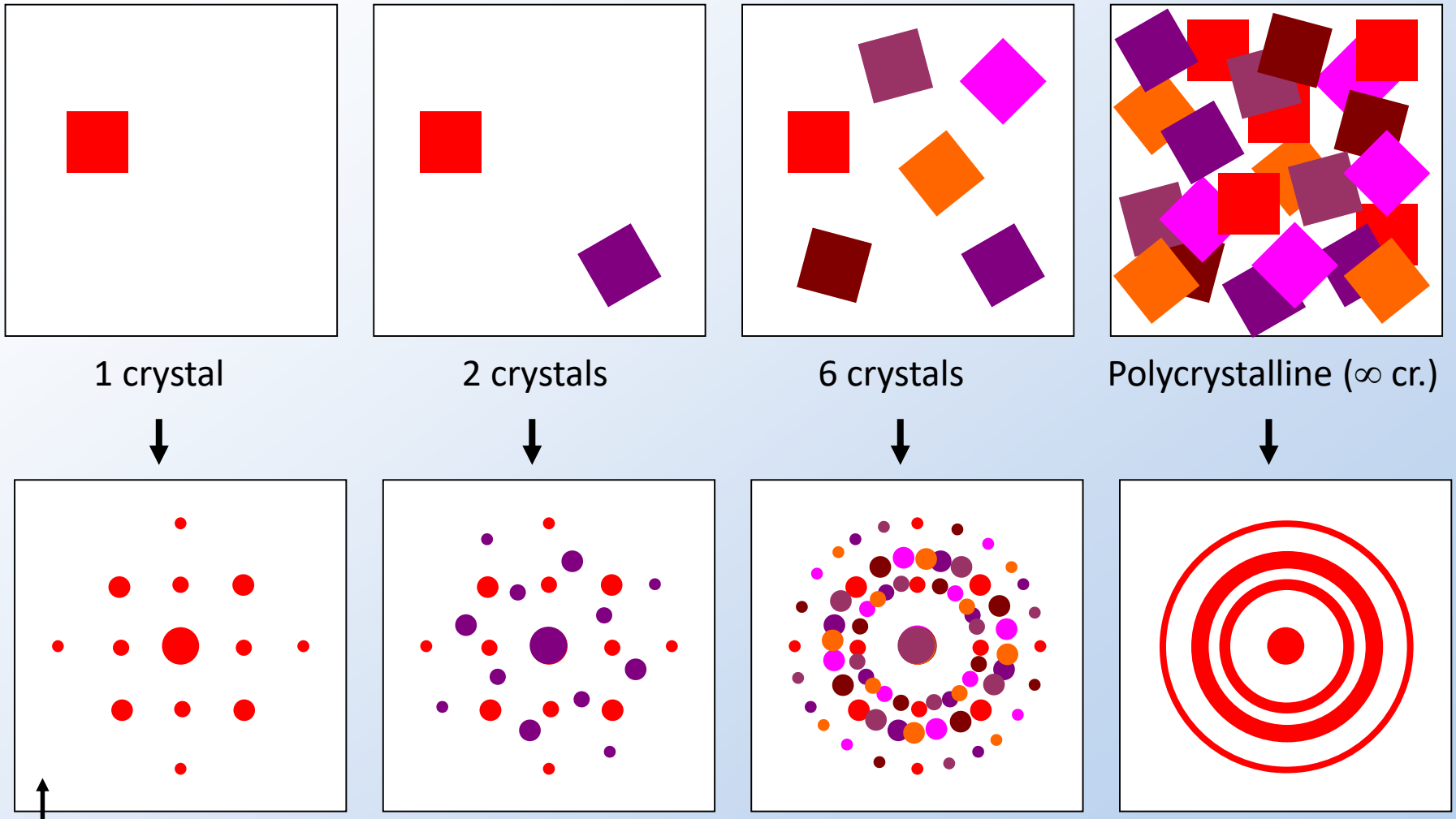


How to get TEM/(SA)ED pattern: (1) Get BF image, (2) remove OBJ aperture (3) select area for diffraction (in SAED: using SA-aperture; in ED: using e-beam directly), (4) switch to diffraction and (5) carefully magnify/focus/record the diffraction pattern.

TEM/ED :: Types of diffraction patterns

Monocrystal vs. polycrystalline samples.

Simple intuitive model:

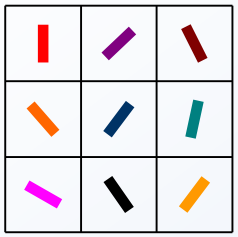


Why the monocrystal diffractogram looks like this? ⇒ see next.

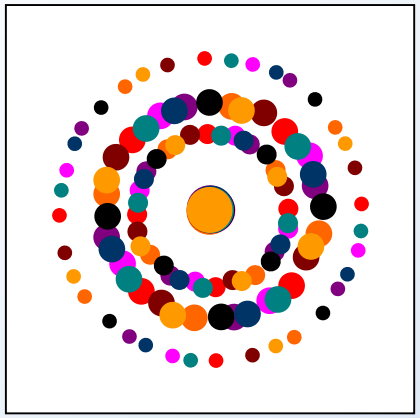
TEM/ED :: Types of diffraction patterns

Unoriented and oriented polycrystalline samples.

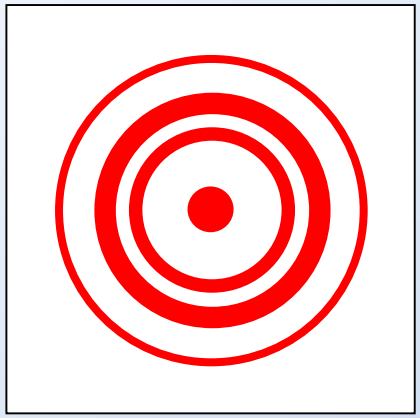
Simple intuitive model:



Polycrystalline sample (unoriented)

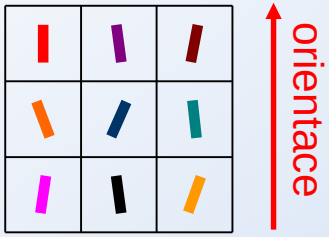


Diffraction pattern

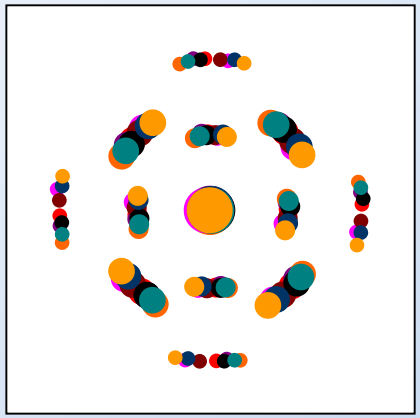


∞ crystals

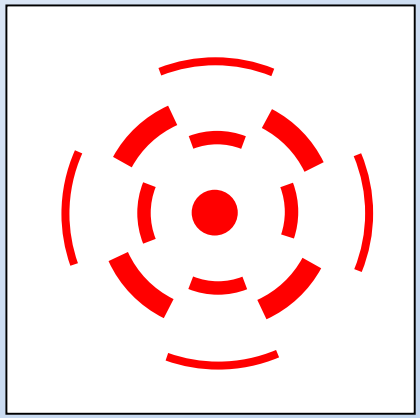
Well known fact: unoriented polycrystalline sample yields diffractogram with diffraction rings.



Polycrystalline sample. (oriented)



Diffraction pattern

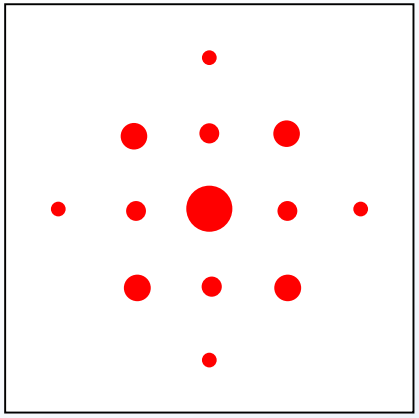


∞ crystals

Less known fact: with increasing orientation of crystallites in the sample, the diffraction rings change to half-moons.

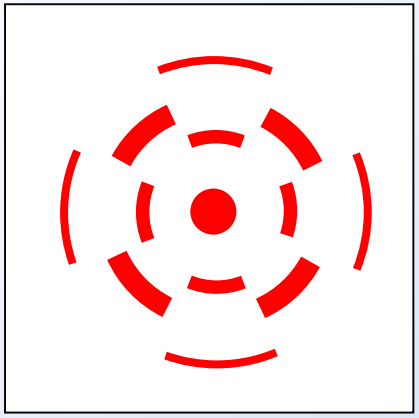
TEM/ED :: Types of diffraction patterns

Summary: three basic types of diffractograms we can observe in TEM.



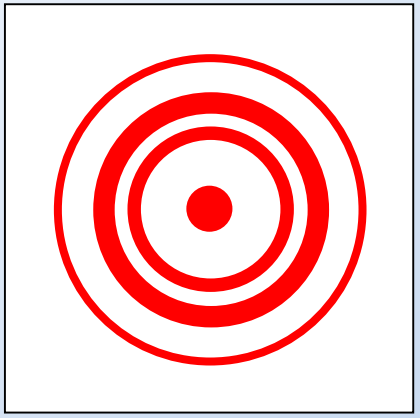
Monocrystal.
= single crystal.

- Diffraction pattern is composed of diffraction spots.
- Each lattice plane gives rise to one spot.
- The distance of the spot from the center is given by Bragg's Law or its TEM equivalent: Camera equation.



Oriented polycrystalline.
= sample consists of many small crystals with some preferred orientation.

- Diffraction pattern is composed of half-moons.
- The position and intensity of half-moons is linked with the orientation.



Polycrystalline sample.
= sample consists of many small crystals with random orientation.

- Diffraction pattern is composed of rings.
- Each ring is formed by many diffractions from the small crystals.

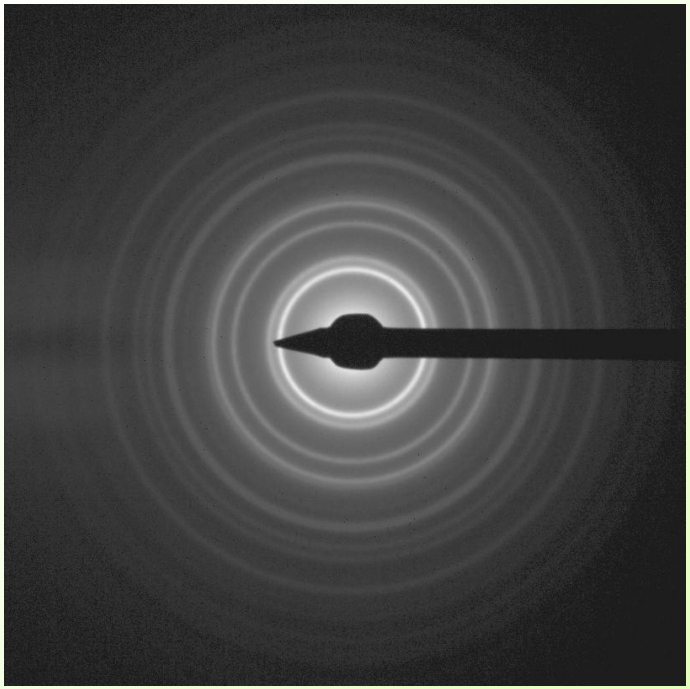
Precise explanation = Theory/Level 2. In this course, we skip level 2, but see texbooks → Laue diffraction condition, Ewald sphere..

TEM/ED :: Examples

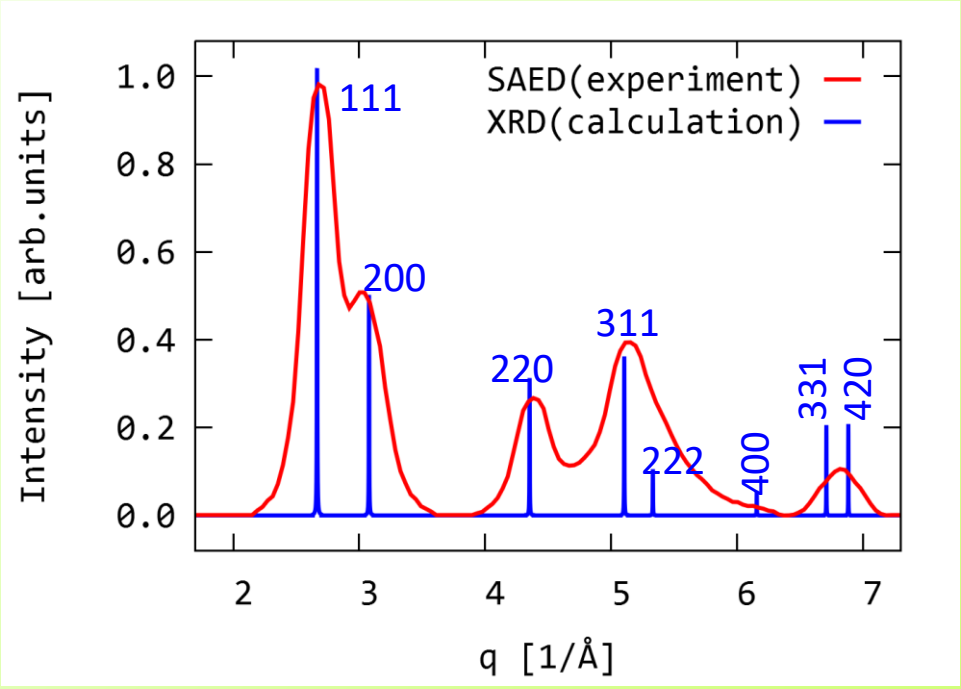
[Au standard]

Example 1: Identification of known samples + principle of TEM calibration.

Sample: Au nanoparticles.
TEM/SAED diffraction pattern.



ED experiment versus XRD calculation.
Conclusion: good agreement ⇒ it is Au, fcc.



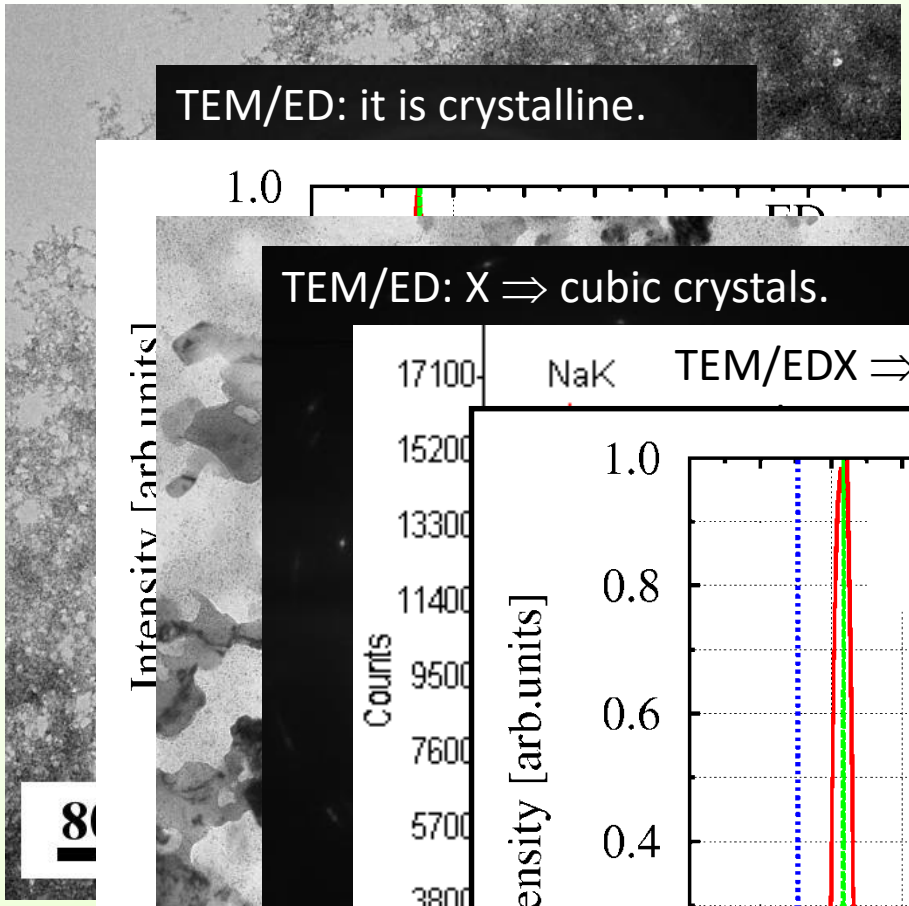
This is also a principle of the TEM/SAED calibration using Camera equation $Rd = \lambda L$:
⇒ R is measured (directly on 2D-diffractogram), d is calculated (easy – as already shown).
⇒ Then we can determine λL = CC = camera constant and L = CL = CC/λ = camera length.

We need at least two pieces of software to do such things:
(1) [Red program] converts 2D-diffraction pattern to 1D-diffraction pattern (red curve)
(2) [Blue program] for calculation of theoretical 1D powder diffraction pattern (blue curve)

TEM/ED :: Examples

[TiO_2 + X]

Example 2: Identification of unknown/unexpected substances (whose structures are known).



TEM/BF, final TiO_2 synthesis at Charles University.
Problem: What is the crystal modification?

Comparison SAED × PXRD: anatase + **X**

TEM/BF: TiO_2 before purification.

TEM/EDX (before purification)

Final comparison
TEM/SAED × PXRD
⇒ anatase + **NaCl**

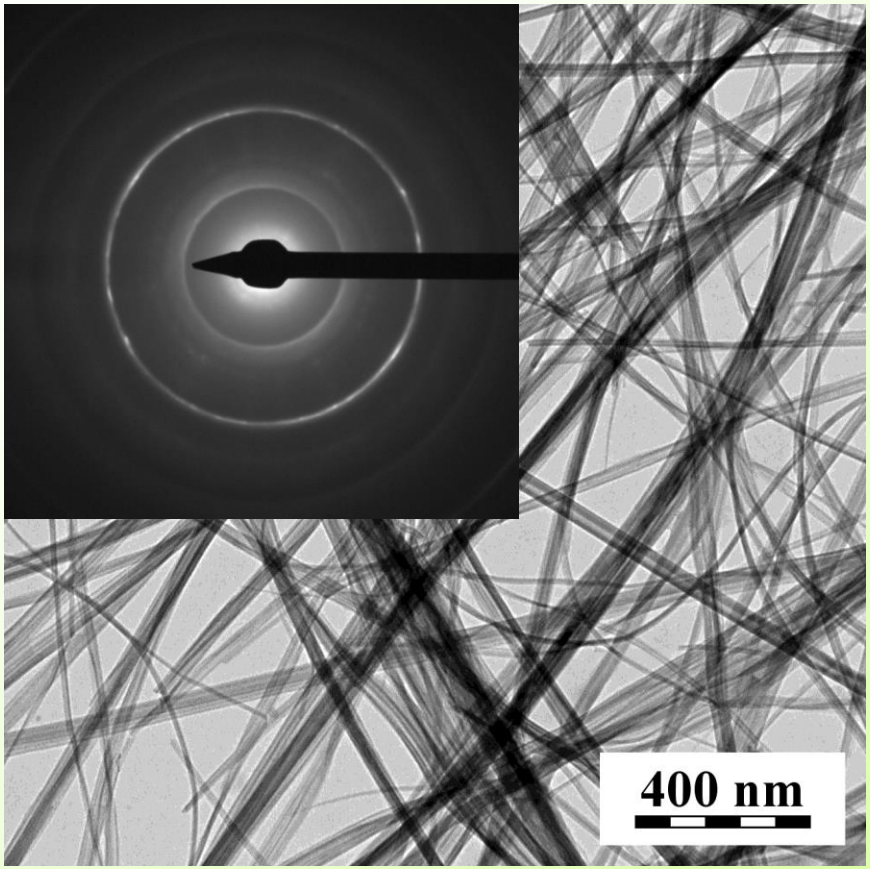
Conclusions

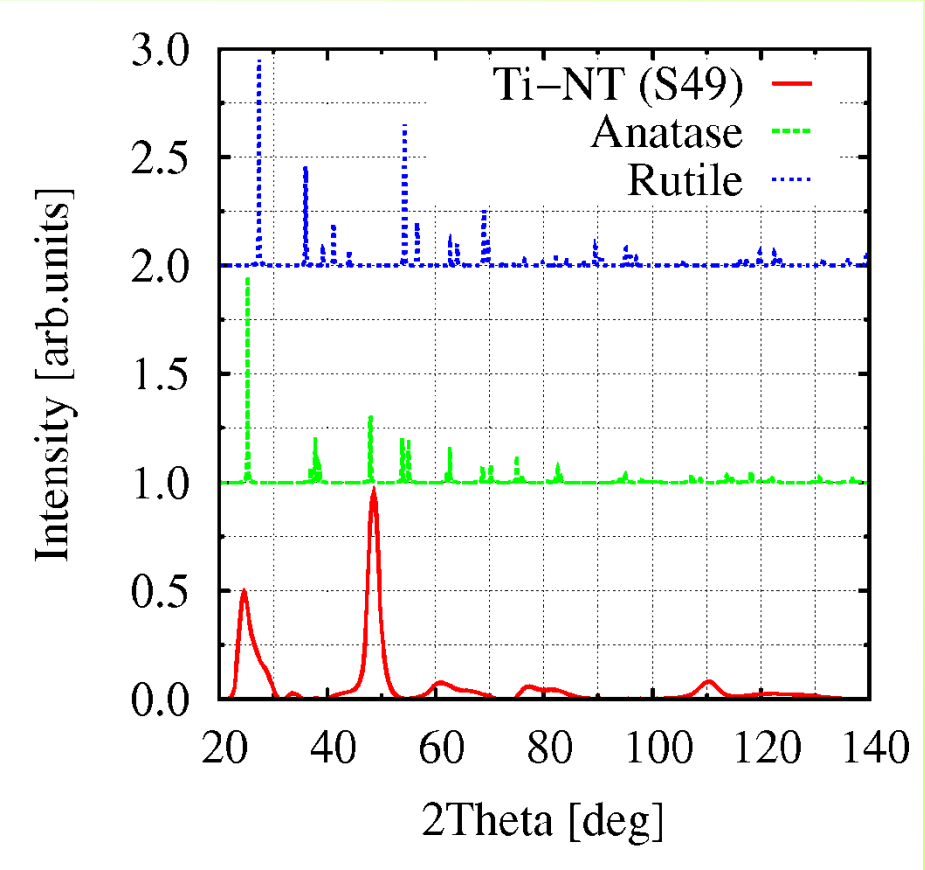
1) The prepared TiO_2 nanopowder is anatase.
2) Po syntéze zbyla v produktu nečistota = NaCl.

Note: this is the SAED pattern of intentionally non-purified sample.

TEM/ED :: Examples

Example 3: Identification of new/unknown compounds (their structures not in databases).





TEM/BF micrograph of TiNT.

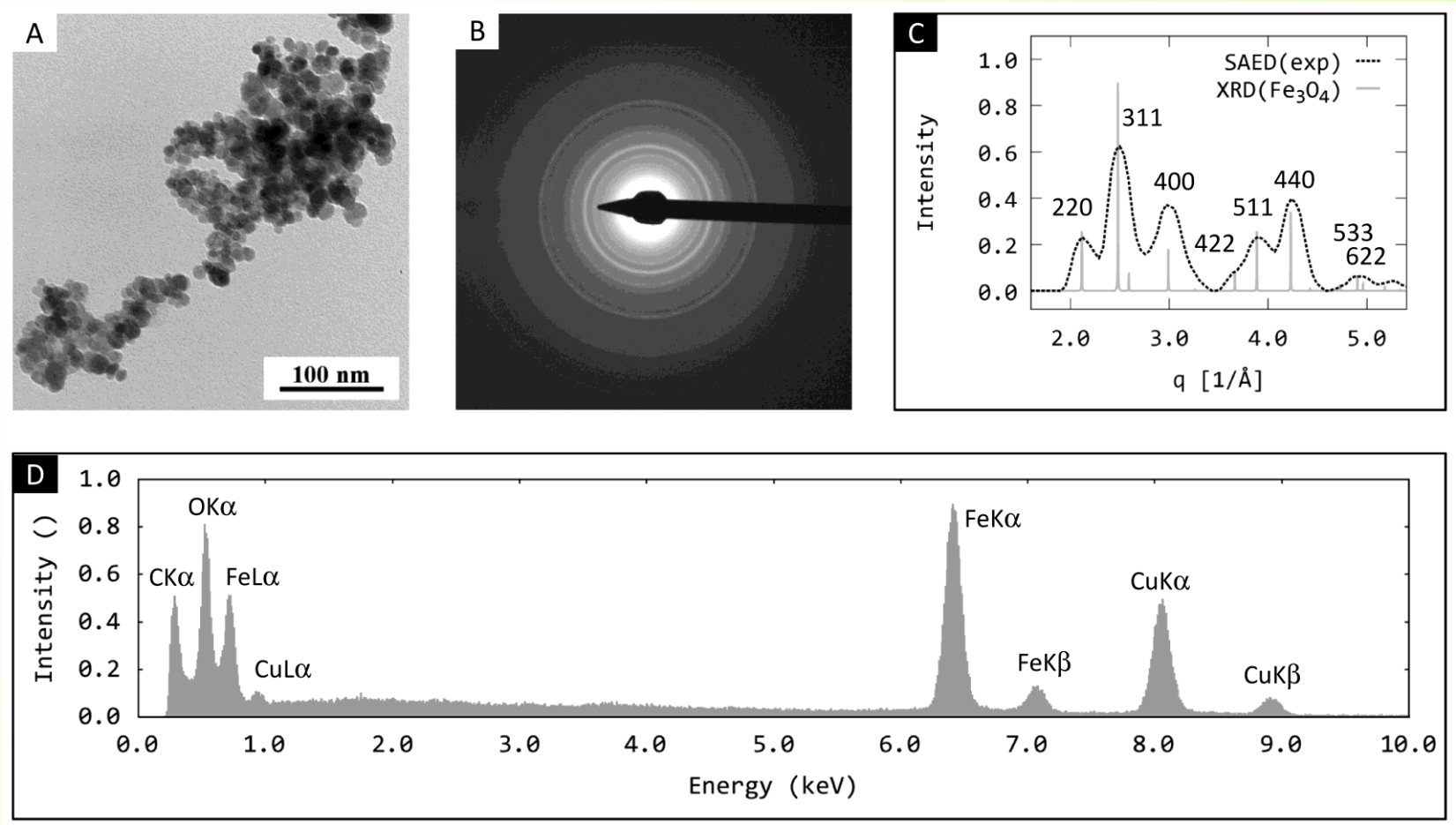
⇒ new crystalline modification based on TiO_2 (original synthesis from our laboratory @ IMC).

TEM/ED × PXRD(anatas,rutil).

⇒ we cannot say what it IS, but we can claim what it IS NOT, i.e. we can claim it is a new structure.

TEM/ED :: Examples

Example 4: Complete (simple) TEM/BF-EDX-SAED analysis – Fe_3O_4 nanoparticles.

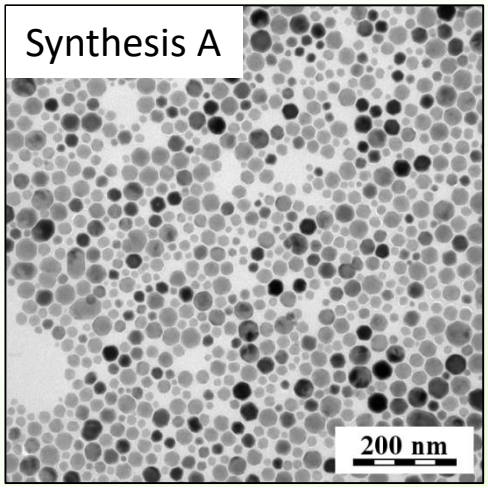


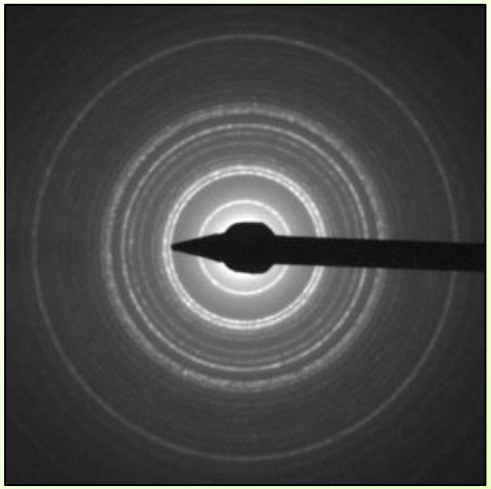
(a) TEM/BF shows the nanoparticle morphology (b) TEM/SAED confirms nanocrystalline character, (c) comparison of calculated PXRD and experimental SAED proves that the nanoparticles are magnetic form of iron oxide, and (d) TEM/EDX verifies the composition.

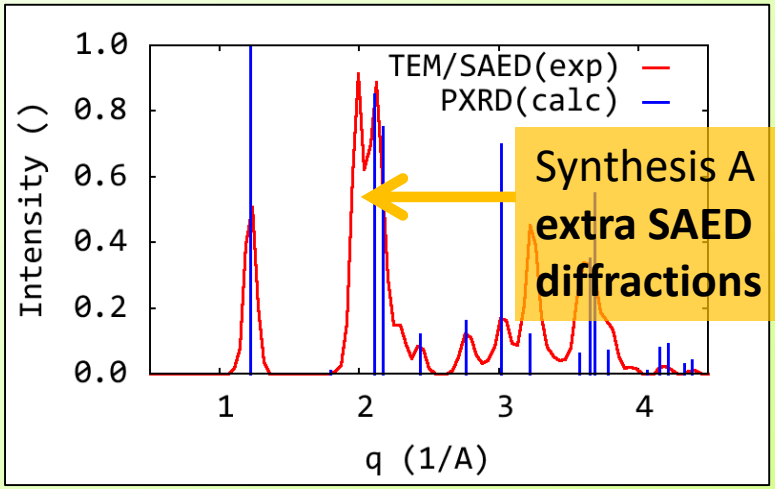
J Nanopart Res (2016) 18:301. DOI 10.1007/s11051-016-3615-7

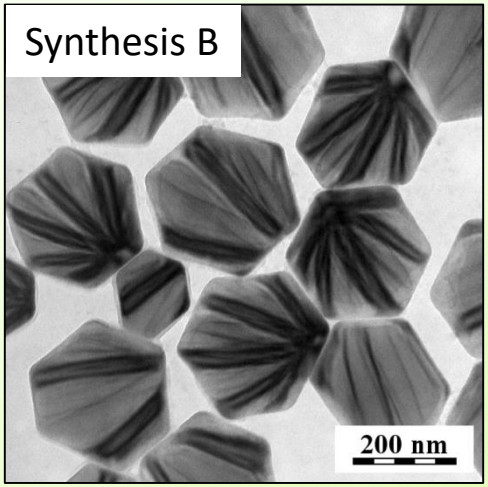
TEM/ED :: Examples

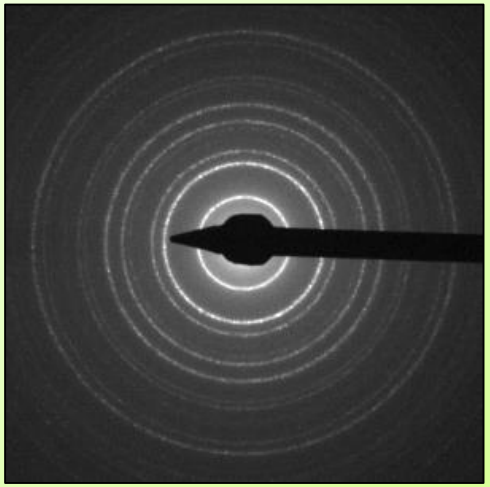
Example 5: Complete (more difficult) TEM/BF-EDX-SAED analysis – $NaYF_4$.

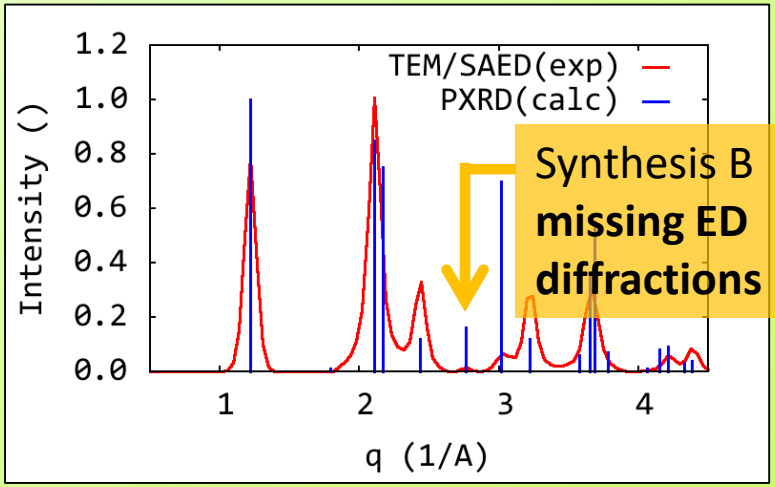












TEM/BF: different for very similar syntheses.

TEM/SAED: different, but TEM/EDX: identical!

Comparison SAED(exp) x PXRD(calc): some extra/missing diffractions!?

Nanoscale 7 (2015) 18096. DOI: 10.1039/c5nr05572e

Detailed explanation →

Supplement :: TEM/SAED :: Homework

HW #1:

Let us have Au crystal, cubic structure, unit cell parameter a = 4,08 Å.
The type of crystalline structure = fcc = face-centered cubic, allowed diffractions have diffraction indexes (hkl) = (111), (200), (220), (311), (222), (400), (331), (420), (422), ...
Calculate diffraction angles θ and the magnitudes of diffraction vectors S=|**S**| a q=|**q**| for:

a) XRD, radiation CuKα (λ = 1.54 Å)
b) TEM/ED, electrons @ accelerating voltage 120 kV

Motivation:

1) Write a small, but real program in Python (the result should be a table like this).
2) Verify, if diffraction angles and vectors depend on wavelength.

Hint for HW:

All formulas (calculation of d(hkl), λ...) have been given in previous slides/lectures.
Table in Python can be made using print+formatting, NumPy or Pandas $\rightarrow$ check www.

HW #2:

- In [Ex.3] we have a "Graphical proof" that for small angles: $2\sin(\theta) = \tan(2\theta) = 2\theta$.
- Make an analogous "Symbolic proof" using Taylor series (the result should look like this).
- **Motivation:**

1) Using Taylor series to prove approximate relations is quite common trick in Physics.
2) Make sure that you understand the Taylor series trick (you may add a graphical proof).
3) Practice Python/SymPy – using SymPy, you can solve similar problems in the future...

Part 7

TEM/EDX – interpretation of spectra

Contents

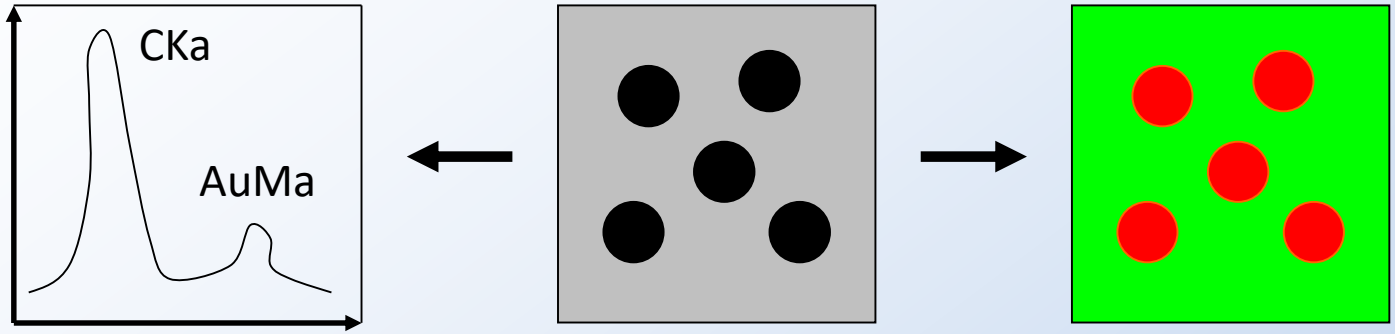
- Theory: just brief overview
  (because the principle of TEM/EDX ≈ SEM/EDX
- Examples: just one case study
  (illustrating improved resolution in TEM/EDX
  (even in a TEM microscope without STEM mode

TEM/EDX :: Theory

Principle of TEM/EDX, types of EDX, and higher resolution in comparison with SEM.

Principle of TEM/EDX: → exactly the same as in SEM/EDX.

Types of TEM/EDX: → two basic types of TEM/EDX, like in SEM



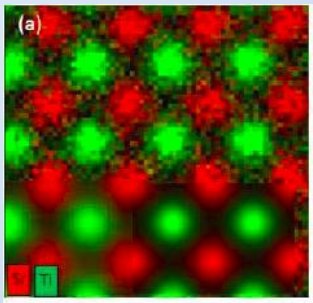
TEM/EDX spectrum | TEM/BF: Au on C | TEM/EDX map

How to get the spectrum and the map?

- Spectrum: (1) BF, (2) select area with beam (3) remove OBJ, (4) insert EDX det. (5) measure
- EDX-map: collect in STEM mode, like in the case of SEM/EDX mapping ⇒ you need ATEM

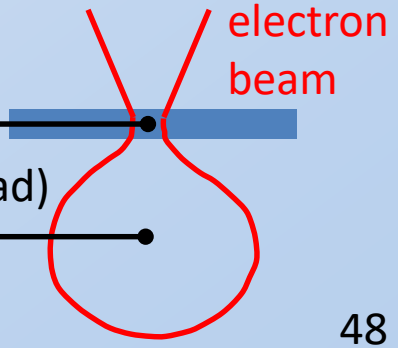
Higher resolution of TEM/EDX in comparison with SEM/EDX.

ATEM/EDX map of $SrTiO_3$.
with atomic resolution.
Result from EMC2012.
Microscope: FEI Titan G2.



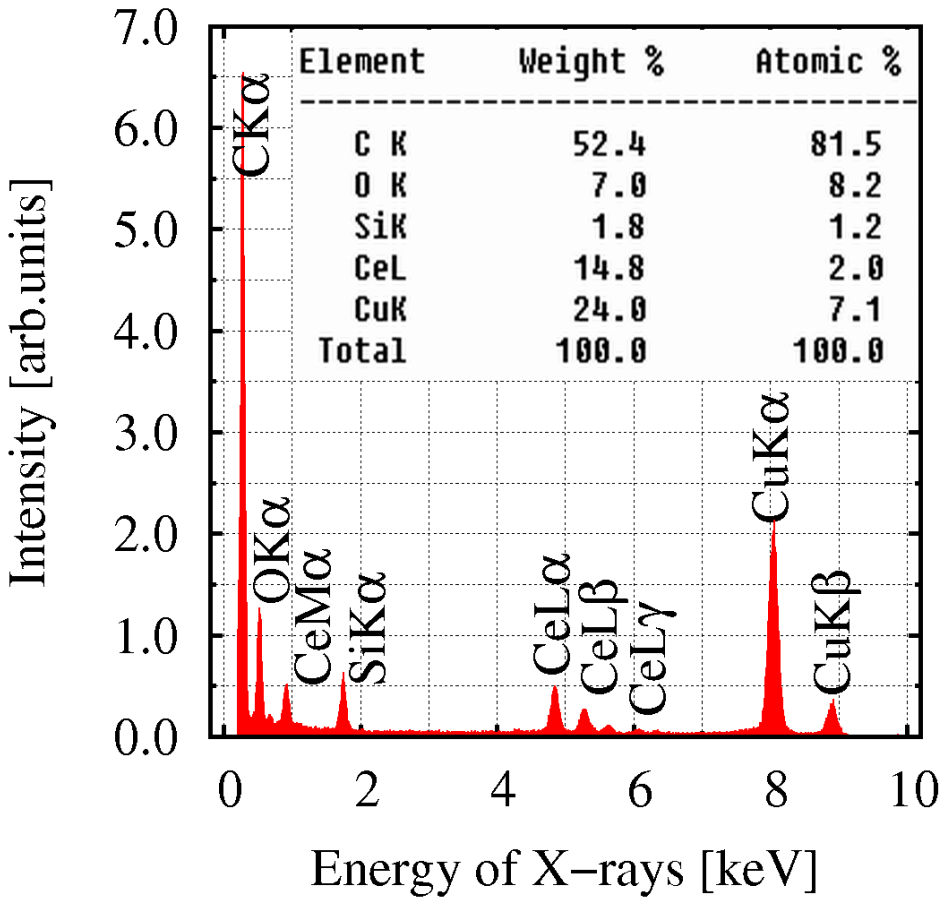
How is this possible?

- TEM/EDX
  very thin sample (no spread)
- SEM/EDX
  infinitely thick sample



TEM/EDX :: Examples

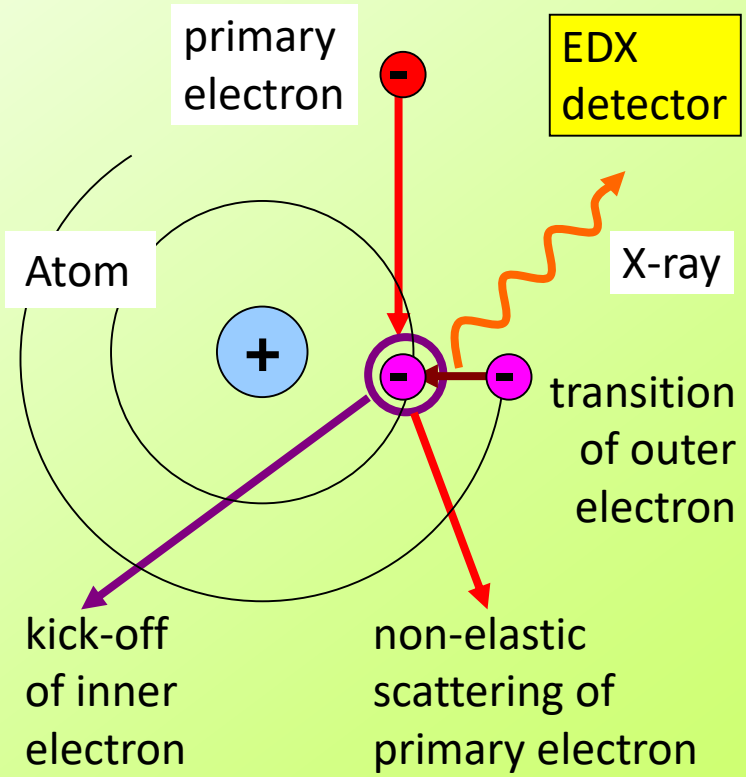
Example 1: Revision of EDX and illustration that the spectra look like from SEM.



TEM/EDX of CeO_2 nanocubes for immunolabeling.

- Inset = final table from standardless ZAF analysis
- **Quantitative analysis – limited precision!**

Reminder: SEM/EDX and TEM/EDX have common principle ↓

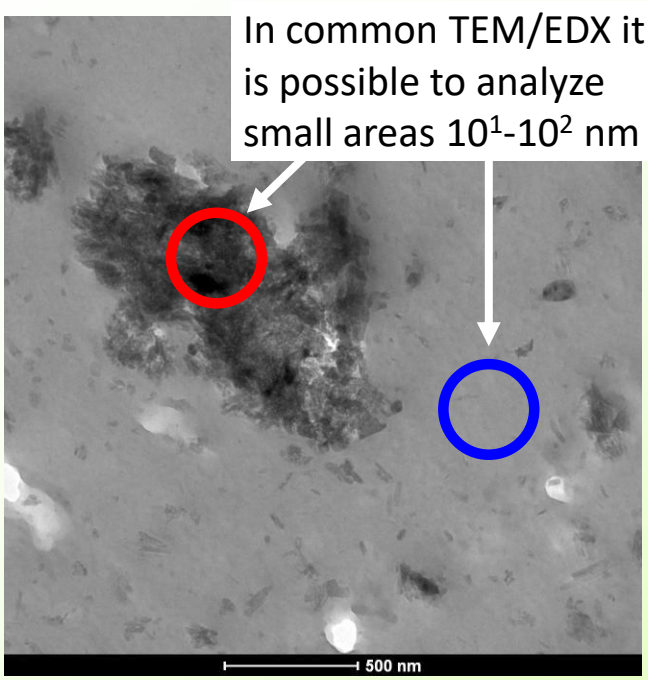


Qualitative evaluation of EDX:

Ce,O	CeO_2 nanocrystals
C,Cu	supporting C-film on Cu-grid
Si,O	silicon oil, dust

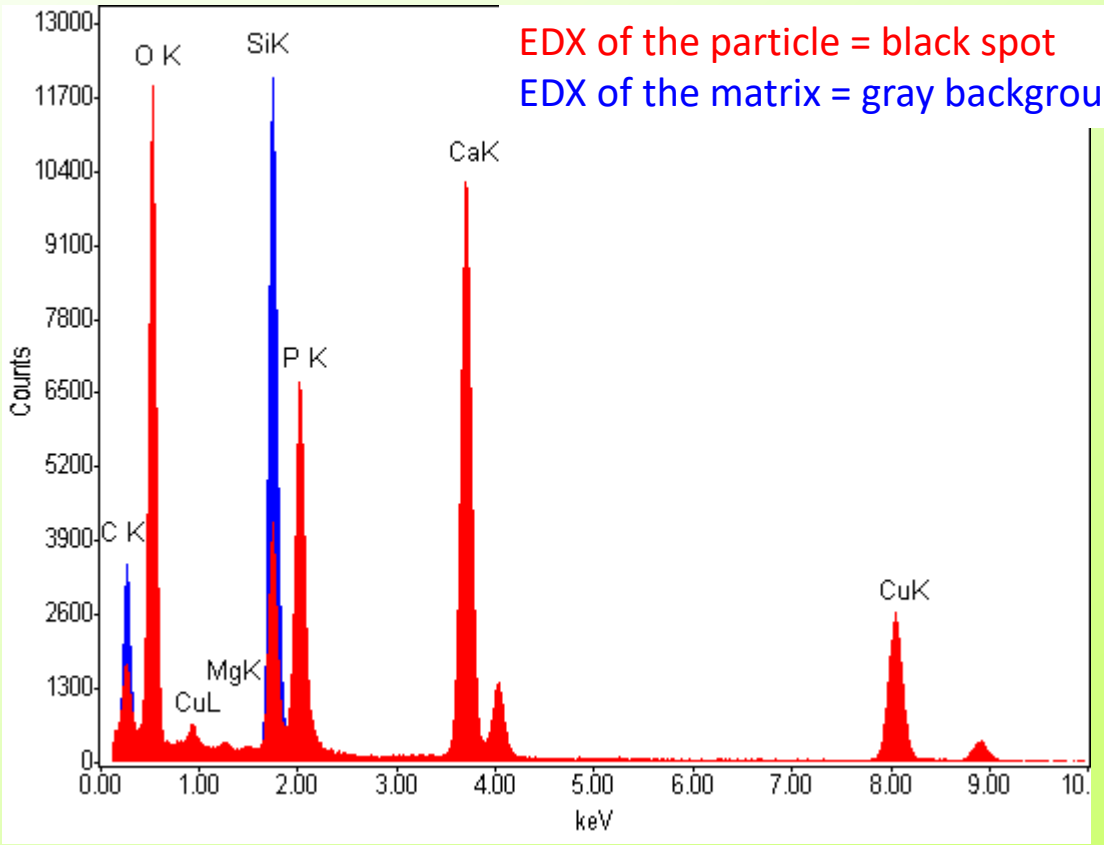
TEM/EDX :: Examples

Example 2: High resolution of TEM/EDX even without STEM – HAP nanocomposite.



TEM/BF micrograph showing siloxane filled with nanocrystals of hydroxyapatite (HAP).
[job ID 876-4]

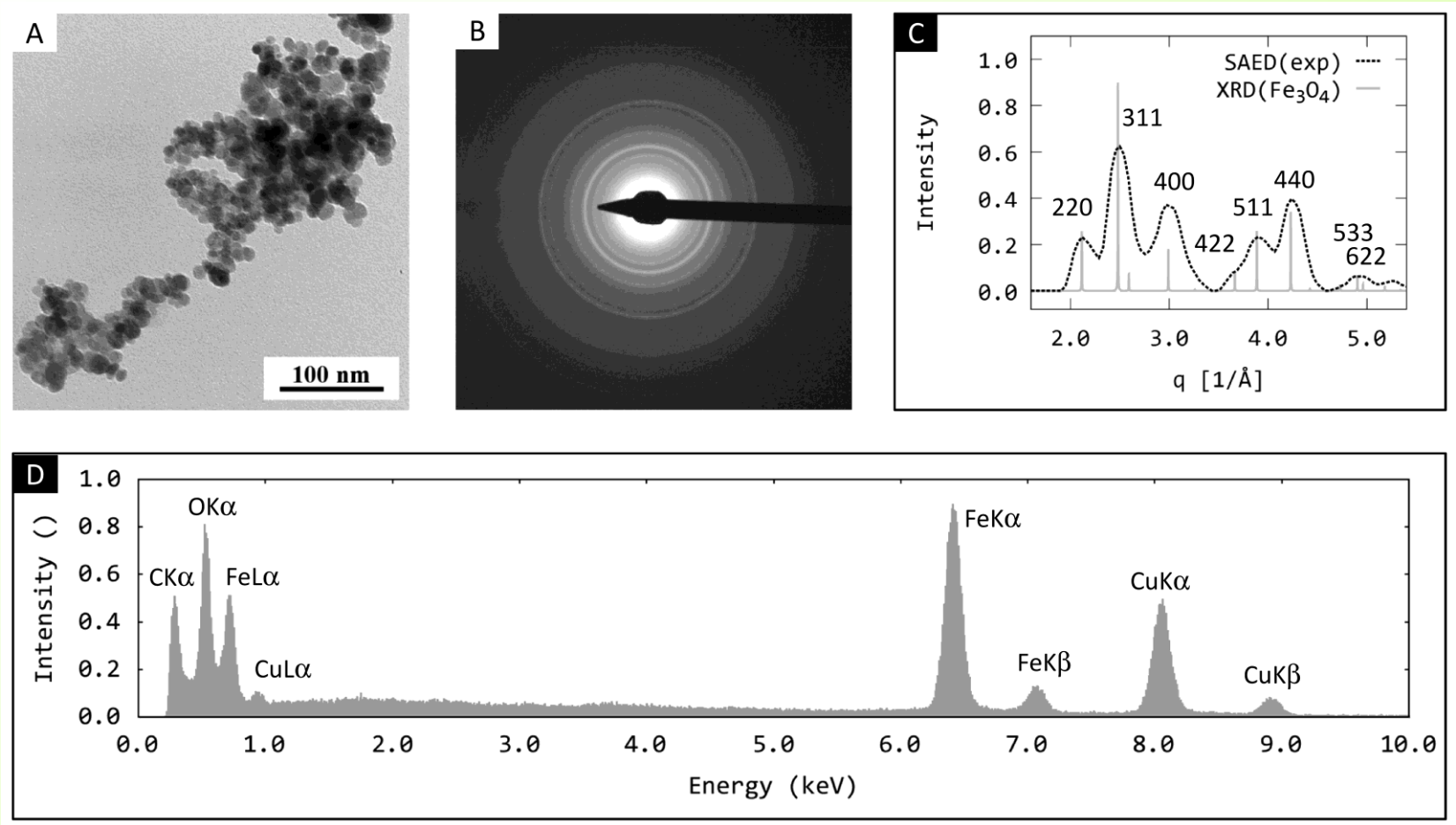
Problem: the black spots are agglomerates of HAP, or some impurities from the sample preparation?



Answer - TEM/EDX: black spots are HAP.
Matrix (light gray) contains just C, Si, O.
Particles (black spots) contain also Ca, P.
(chemical formula of hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$
(H has no EDX signal, Cu is the grid, Mg is an admixture.

TEM/EDX :: Examples

Example 3: Complete TEM/BF-EDX-SAED analysis – Fe_3O_4 nanoparticles.



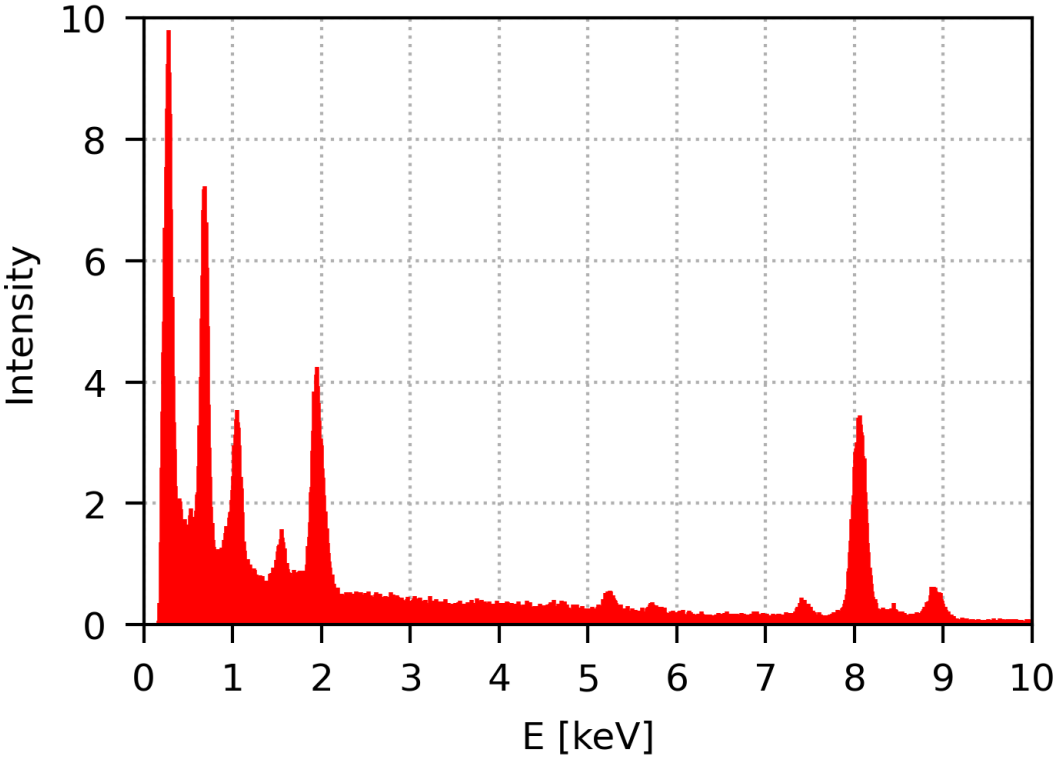
(a) TEM/BF shows the nanoparticle morphology (b) TEM/SAED confirms nanocrystalline character, (c) comparison of calculated PXRD and experimental SAED proves that the nanoparticles are magnetic form of iron oxide, and (d) TEM/EDX verifies the composition.

J Nanopart Res (2016) 18:301 DOI 10.1007/s11051-016-3615-7

Supplement :: TEM/EDX :: Homework

HW #3:

Download the spectrum below from www-pages of the course and index = mark all peaks.



Preliminary information is a key for interpretation of EDX spectra, in our case we knew the following:

- The analyzed nanoparticles should be: $NaYF_4:Yb^{3+}/Er^{3+}@NaYF_4:Nd^{3+}$
- The nanoparticles were deposited on a C-coated Cu-grid for TEM.
- The concentration of Er^{3+} is very low, maybe below detection limit, but Yb^{3+} and Nd^{3+} should be visible.
- The nanoparticles are enveloped by biopolymers and used for light-upconverting applications (biolabeling).

Motivation: (1) Practice EDX analysis and (2) working with a Jupyter templates.
Hints: Employ the EDX-table from EM1:SEM + Jupyter template from www of the course.
Note: The rules explained in the previous lecture EM1:SEM hold for TEM/EDX as well.

Part 8

TEM - supplements

Contents

- ❖ Special sample holders
- ❖ Analytical TEM microscopes
- ❖ Sample preparation for TEM (in Polymer science)

Supplement :: Special sample holders

Single-tilt holder = standard holder, position for one sample, tilt only along one axis
It is used for BF and DF; possible for ED of micro/nanopowders and basic EDX analyses.

Multiple holder = the same as above, but positions for more samples (usually 2-5).

Double-tilt holder = tilt along two axes ⇒ primarily for ED of monocrystals.

Low-background holder = holder made of Be – no background in EDX ⇒ for microanalysis.

Note: there are various combinations, frequent is low-background double-tilt holder.

Cryo-holders.

Cryo-holder + other HW and SW in TEM + equipment for flash-freezing ⇒ CryoTEM.
Cryo-TEM is used for sensitive biological specimen to minimize sample damage (especially in combination with 3D-microscopy, which requires many tilts = high exposure of the sample) or for studies of nanoparticles in (flash-frozen) solutions.

Straining/deformation holders.

Straining/deformation holders are used to elongate/deform electron transparent specimens in order to study deformation mechanisms *in situ*. This is used mostly for metals (other specimens are difficult or even impossible to fix reasonably if they are ultrathin).

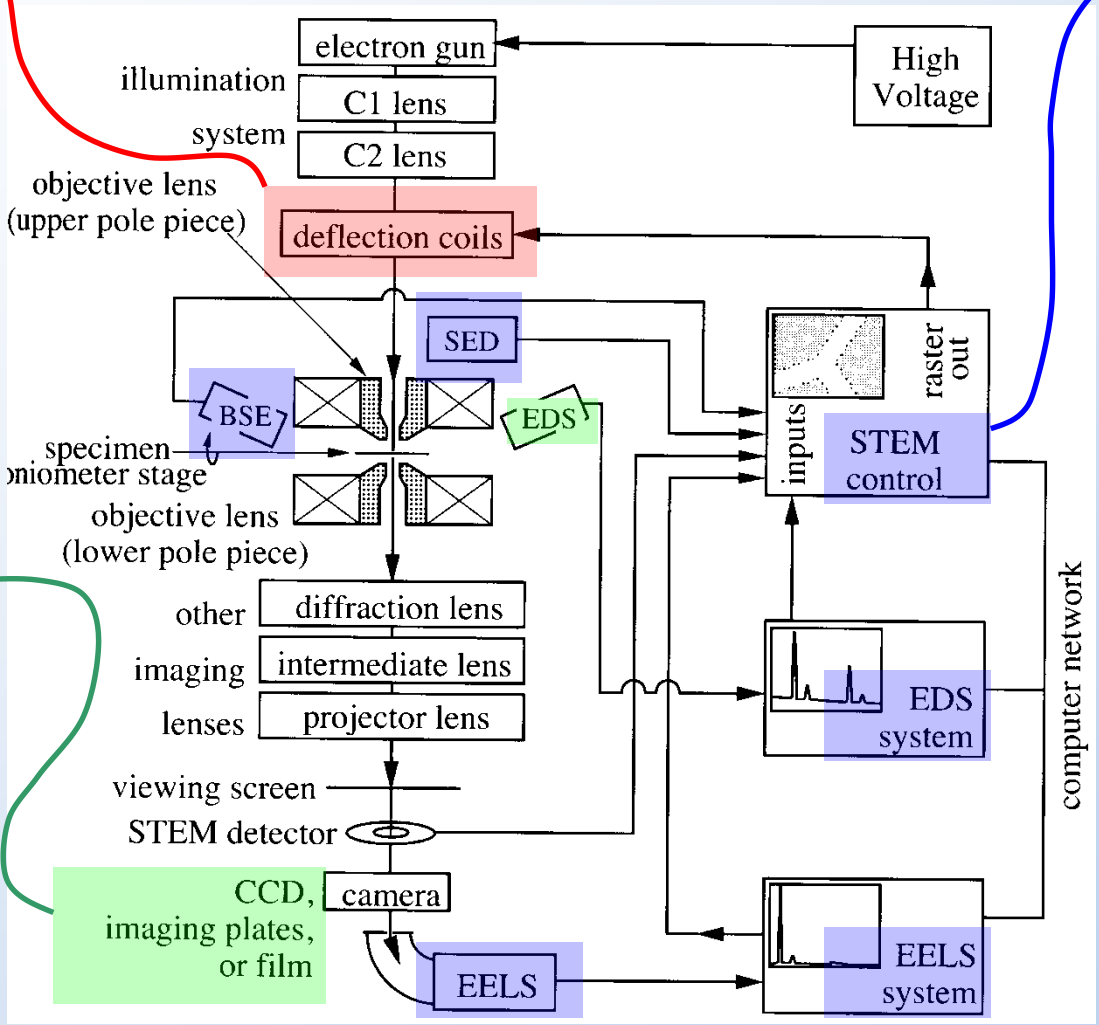
Supplement :: Scheme of analytical TEM = ATEM

What are the advantages of STEM in TEM microscopes?

ATEM microscope has scanning coils in comparison with standard TEM.

Standard TEM microscope has three modes, occasionally supplemented with micro-analysis (EDX).

Scheme of ATEM = TEM with STEM mode.



ATEM microscope can control the scanning of the beam on the sample, which adds new possibilities:

a) STEM itself (useful in DF

b) EDX mapping (high resolution

c) detection of other signals that does not make much sense in standard TEM: EELS, SE, BSE...

Supplement :: Sample preparation for TEM.

Key problem: how to make samples transparent for electrons.

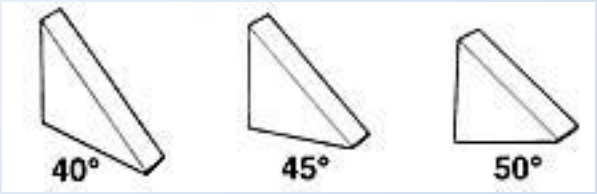
See also sample preparation in SEM lecture!

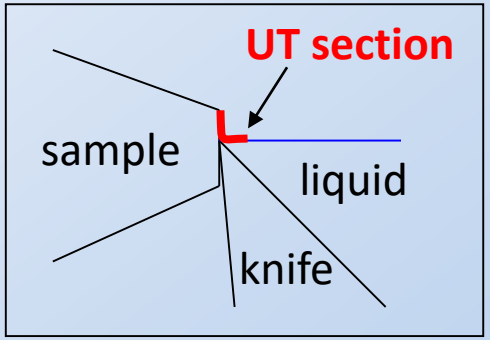
(1) Nanopowders: transparent “by themselves" – deposit on Cu-grid with C-film and observe.

(2) Micropowders: a) if possible, pulverize into nanopowder and proceed like in item (1).
b) if not, embed in an epoxy resin and proceed like in items (3,4) below.

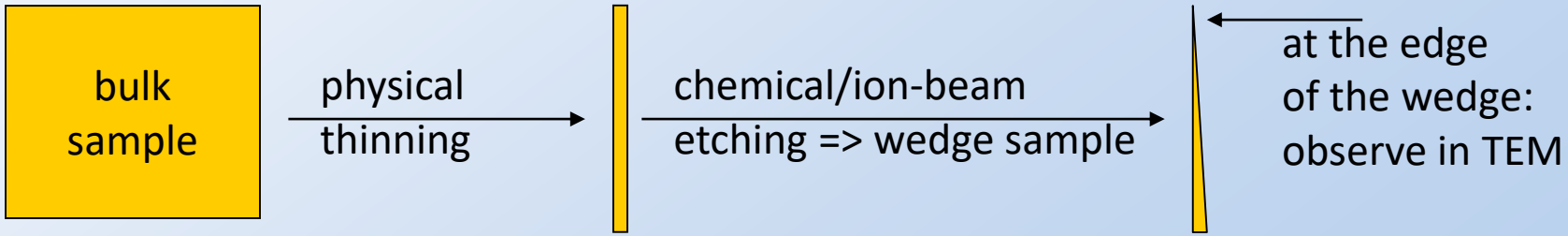
(3) 3D-samples, soft: (almost all biology and polymer science) ⇒ ultramicrotomy

ultramicrotome, knives
and principle of UT sectioning





(4) 3D-samples, hard: (chemistry, physics, geology...) ⇒ cutting, thinning, etching.

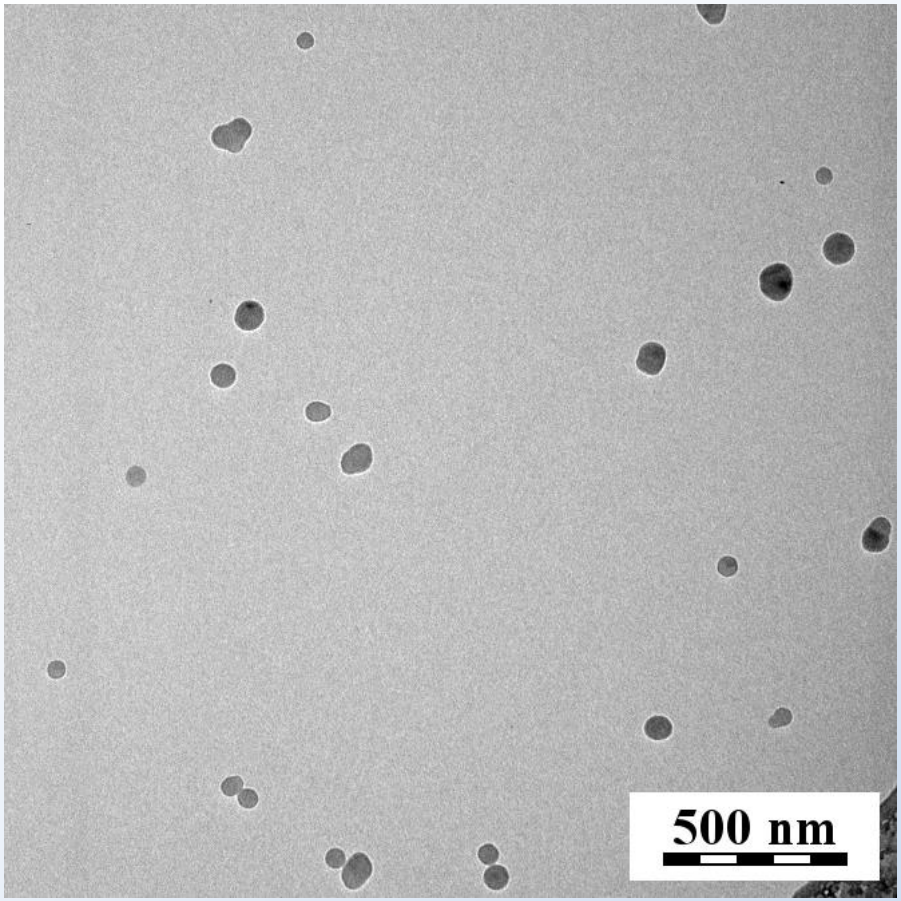


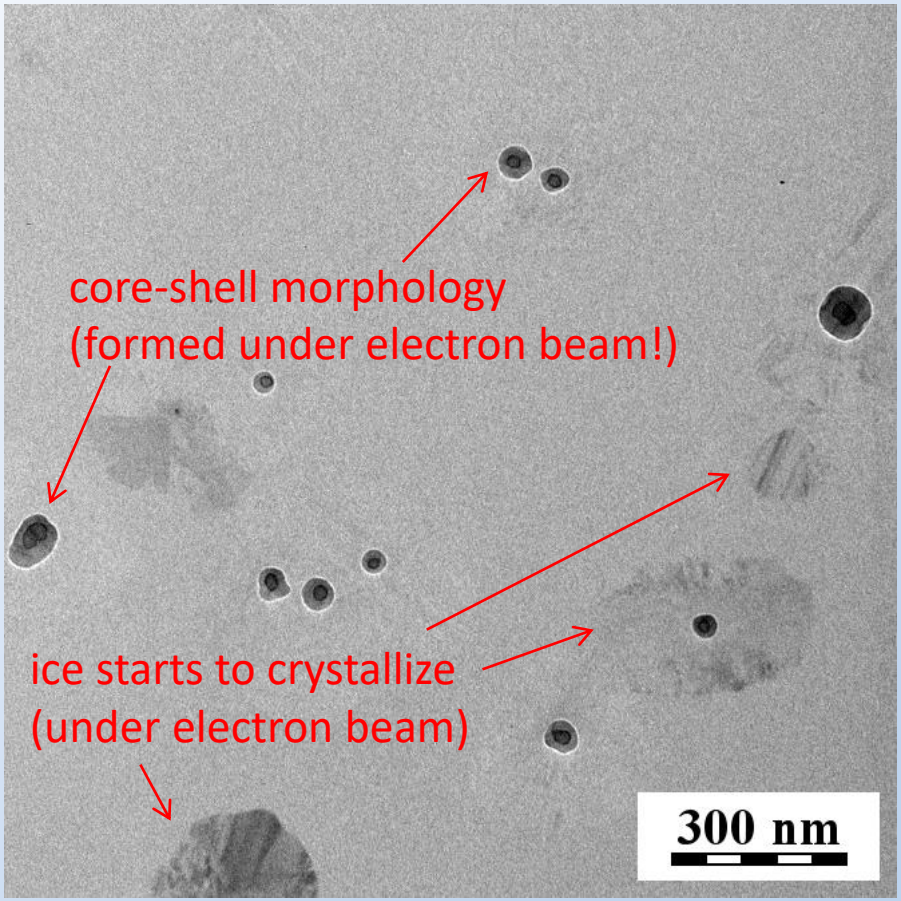
(5) Old, interesting, but rarely used technique: replication (see older textbooks).

(6) New, recent, modern technique: FIB microscopy (see newer textbooks and www).

Supplement :: Cryo-TEM :: Chitosan-g-ssDNA in water

TEM of (nano)particles in solution is more-and-more requested – so at least one example.





Cryo-TEM (short exposure, good results):

- clear background = thin amorphous ice
- particles visible (no core-shell structure)
- size of particles agrees with prev. results

Cryo-TEM (longer exposure ⇒ artifacts!):

- gray striped zones/areas – crystalline ice
- core-shell structure of particles – artifact
- both artifacts observed at longer exposure

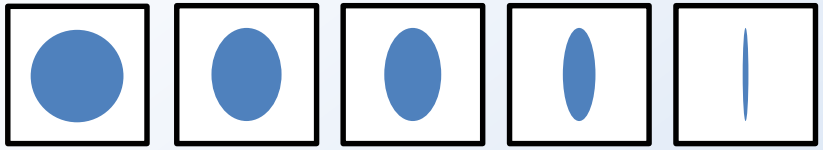
Supplement :: 3D-TEM :: principle

3D-TEM is more-and-more popular, namely in biology – so at least the principle.

The 1st way how to get 3D-information about an object: series of tilts.

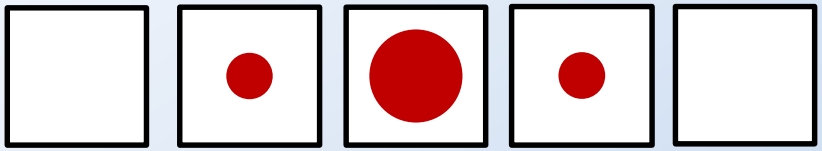
- five tilts of a sphere

- five tilts of a thin disk

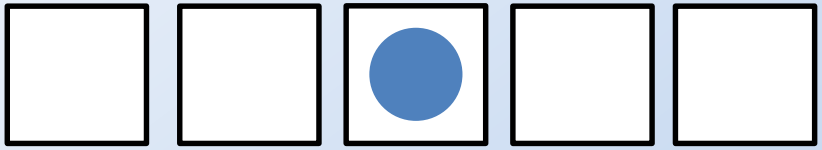


The 2nd way how to get 3D-information about an object: series of sections.

- five sections of a sphere



- five sections of a disk



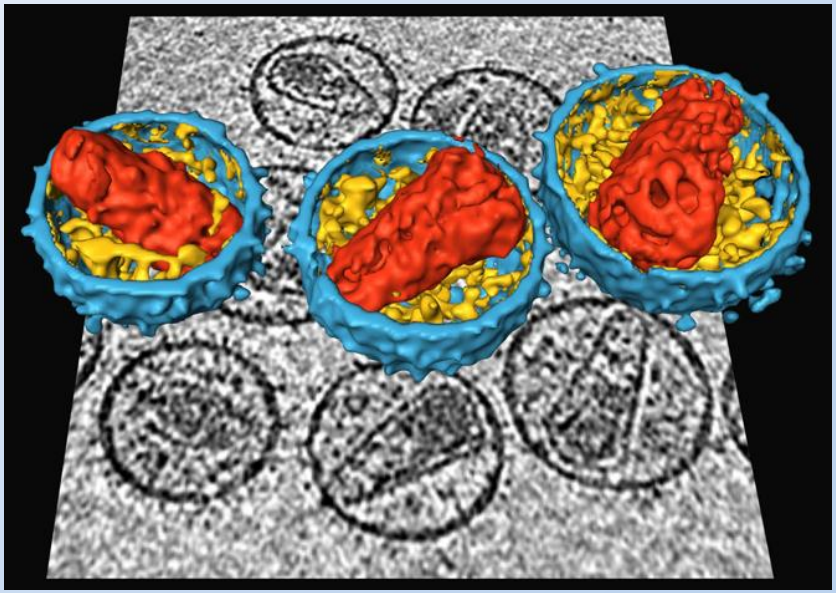
The 3rd way how to get 3D-information about an object: the same object in many copies and random orientations (bio-objects).

Note #1: SW for 3D-reconstructions – complex!

Note #2: Many tilts → e-beam damage → CryoTEM.

Example illustrating that 3D-TEM works.

↓ http://www.embl.de/research/units/scb/briggs/



3D reconstruction of HIV-1 virions using cryo-electron microscopy.

Conclusions & summary

- **We have explained the four basic methods of TEM microscopy:**
  - TEM/BF = mass-thickness & diffraction contrast
  - TEM/DF = image formed by diffracted electrons
  - TEM/ED = diffraction pattern (formed by diffracted electrons)
  - TEM/EDX = microanalysis, analogy to SEM/EDX, but higher resolution

- **The lecture was focused on understanding and interpretation of TEM micrographs and diffraction patterns.**

- **Other pieces of information:**
  - We have learnt more about magnification, contrast and resolution of TEM
  - We have also shown some calculations connected with TEM/SAED
    → basic calculations: d(hkl), Bragg's Law & camera equation
    → advanced calculations: KDT, calculation of diffraction pattern of Au nanocrystals
  - We have discussed some other aspects of TEM, such as: key importance of sample preparation for TEM studies and briefly also advanced TEM microscopes such as ATEM, HRTEM...

[Example 1] Calculation of d(hkl) in Jupyter

Note: illustration that complex formulas are easier/more readable in Jupyter than in Excel…

In [1]:
```
# Calculate interplanar spacing dhkl for arbitrary crystal
# (dhkl = function of nine variables, manual calculation is tedious and error-prone
```

In [2]:
```
from math import pi,sin,cos,sqrt
```

In [3]:
```
# Input parameters - unit cell and diffraction idexes
(a,b,c)             = (6.7, 20.8, 6.5)
(alpha,beta,gamma) = (90.0, 99.6, 90.0)
(h,k,l)             = (1, 1, 0)
```

In [4]:
```
# Convert unit cell angles to radians
(alpha,beta,gamma) = (alpha*pi/180, beta*pi/180, gamma*pi/180)
```

In [5]:
```
# Define function that calculates dhkl
def dhkl(a,b,c,alpha,beta,gamma,h,k,l):
    dhkl_rec_2 = (
        (h*sin(alpha)/a)**2 + (k*sin(beta)/b)**2 + (l*sin(gamma)/c)**2
        + (2*h*k)/(a*b) * (cos(alpha)*cos(beta)  - cos(gamma))
        + (2*k*l)/(b*c) * (cos(beta) *cos(gamma) - cos(alpha))
        + (2*l*h)/(c*a) * (cos(gamma)*cos(alpha) - cos(beta) )
    ) / (
        1 - 2*cos(alpha)*cos(beta)*cos(gamma) - cos(alpha)**2 - cos(beta)**2 - cos(gamma)**2
    )
    dhkl = 1 / sqrt(dhkl_rec_2)
    return(dhkl)
```

In [6]:
```
# Calculate dhkl
dhkl(a,b,c,alpha,beta,gamma,h,k,l)
```

Out[6]:
```
6.296242378027061
```

[Example 2] Diffraction experiments vs. $\theta \times S \times q$.

$2d*\sin\theta = n\lambda$ ...basic form of Bragg's Law = BL

$2d*\sin\theta = \lambda$ ...BL with omitted n

...this is quite common, as it holds (for example): $d_{200} = d_{100}/2$

...therefore : $2d_{200}*\sin\theta_{200} = \lambda \Leftrightarrow d_{100}*\sin\theta_{100} = 2*\lambda$

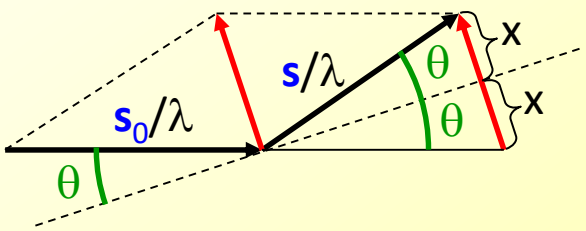
Relation [θ-S-q] i.e. among:

- diffraction angle θ
- diffraction vector **S**
- diffraction vector **q**

Definition #1: $|\mathbf{s}_0|=|\mathbf{s}|=1$

($\mathbf{s}_0$,**s** = just directions of beams

Definition #2: $\mathbf{S} = (\mathbf{s}-\mathbf{s}_0)/\lambda$



Definition #3: $\mathbf{q} = 2\pi\mathbf{S}$

Calculation #1: $|\mathbf{S}| = 2x$

$\sin\theta = x / (|\mathbf{s}|/\lambda) = x / (1/\lambda)$

$\sin\theta = x*\lambda \Rightarrow x = \sin\theta/\lambda$

$|\mathbf{S}| = 2x = 2 * \sin\theta / \lambda$

Calculation #2: $\mathbf{q} = 2\pi\mathbf{S}$

$|\mathbf{q}| = 4\pi * \sin\theta / \lambda$

$d*q = 2\pi$ ...BL: we exchanged diffraction angle θ with (magnitude of) diffraction vector q

$d*S = 1$ ...BL: we exchanged diffraction angle θ with (magnitude of) diffraction vector S

Diffraction angle θ may be at most 90° (2θ = 180°) – logical: see the image above.

In range 0-90° it holds: increase in θ ⇒ increase in sinθ ⇒ increase in S ⇒ increase in q.

⇒ i.e. there is a proportionality among diffraction angle and diffraction vectors.

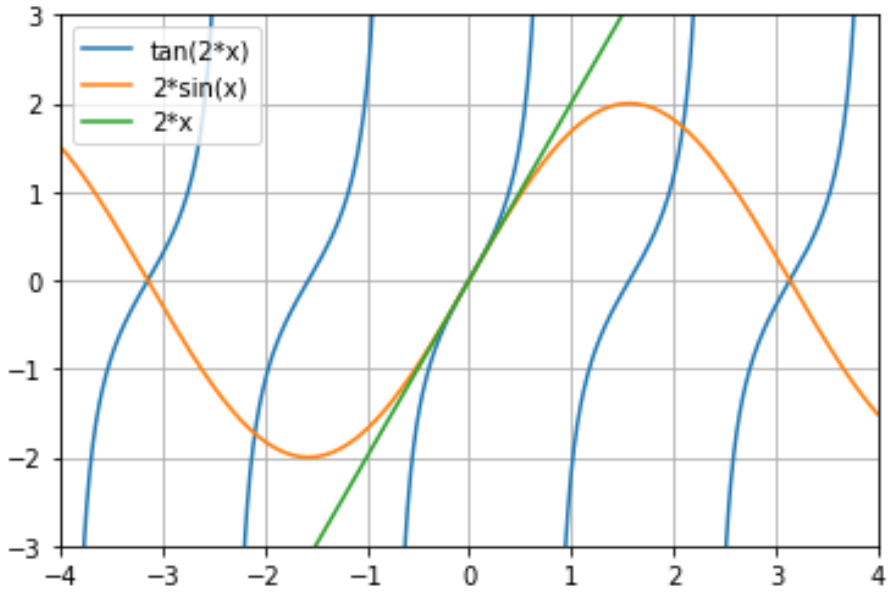
[Example 3] Graphical proof that for small angles: 2sin(θ) = tan(2θ)

```
def f1(x): return( tan(2*x) )
def f2(x): return( 2*sin(x) )
def f3(x): return( 2*x      )
```

```
# Trick: standard plotting of tangent would join points at -oo and +oo
def f1_modif(x): return( np.where(np.abs(f1(x)>10),np.nan,f1(x)) )
```

```
plt.plot(X,f1_modif(X), label='tan(2*x)')
plt.plot(X,f2(X),       label='2*sin(x)')
plt.plot(X,f3(X),       label='2*x')
plt.xlim(-4,4)
plt.ylim(-3,3)
plt.legend()
plt.grid()
plt.show()
```

← **Part of the script in Jupyter/Python:** graphs of functions f(θ) = 2sin(θ), tan(2θ) a 2θ showing that it holds **2sin(θ) ≈ tan(2θ) ≈ 2θ** for small θ



Link to a several scripts, which show:

0) Calculation of d(hkl).
1) Fact that diffraction angles in ED are low.
2) Fact that for low diffraction angles the camera equation holds, using...
   ...numerical verification of the relation 2sin(θ) ≈ tan(2θ) ≈ 2θ
   ...symbolic verification of the relation using Taylor expansions
   ...graphical verification of the relation which is illustrated in this slide

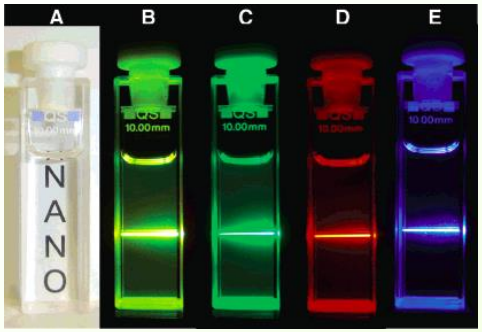
Appendix

Complete TEM-BF-EDX-SAED analysis

Illustration of a more complex problem
This is extended example from the first part of the presentation

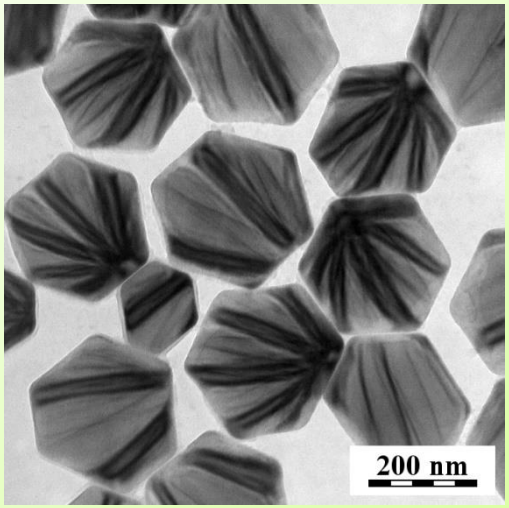
Case study :: Light upconverting $NaYF_4$ nanoparticles

Introduction & description of the problem from the point of view of microscopy.



Colloidal solutions of light upconverting nanocrystals: NIR light excites their luminescence. *J Am Chem Soc* **128** (2006) 7444-7445.

- **Luminescence:** emission of light by a substance.
- **Luminescent nanoparticles:** nanoparticles that luminise.
- **Usage of common luminescent nanoparticles** (such as QD): solar cells, data storage, photocatalysis, bioimaging...
- **Disadvantage of common nanoparticles in bioimaging**: luminescence induced by UV light, which...
  ...damages biological tissues + has low penetration depth
  ...causes high background autofluorescence
- **Advantage of light upconverting nanoparticles in bioimaging**: luminescence induced by NIR light, which...
  ...produces visible light by energy transfer upconversion (ETU)
  ...easily penetrates and does not damage biological tissues



$NaYF_4:Yb^{3+}/Er^{3+}$ nanocrystals, prepared at our Institute.
Purpose: cover with a polymer, fill into microspheres...
(origin of samples: Dept. of polymer particles, IMC, Czech Rep.)

Tasks for the microscopic part of the work:

(1) Visualize the nanoparticles.

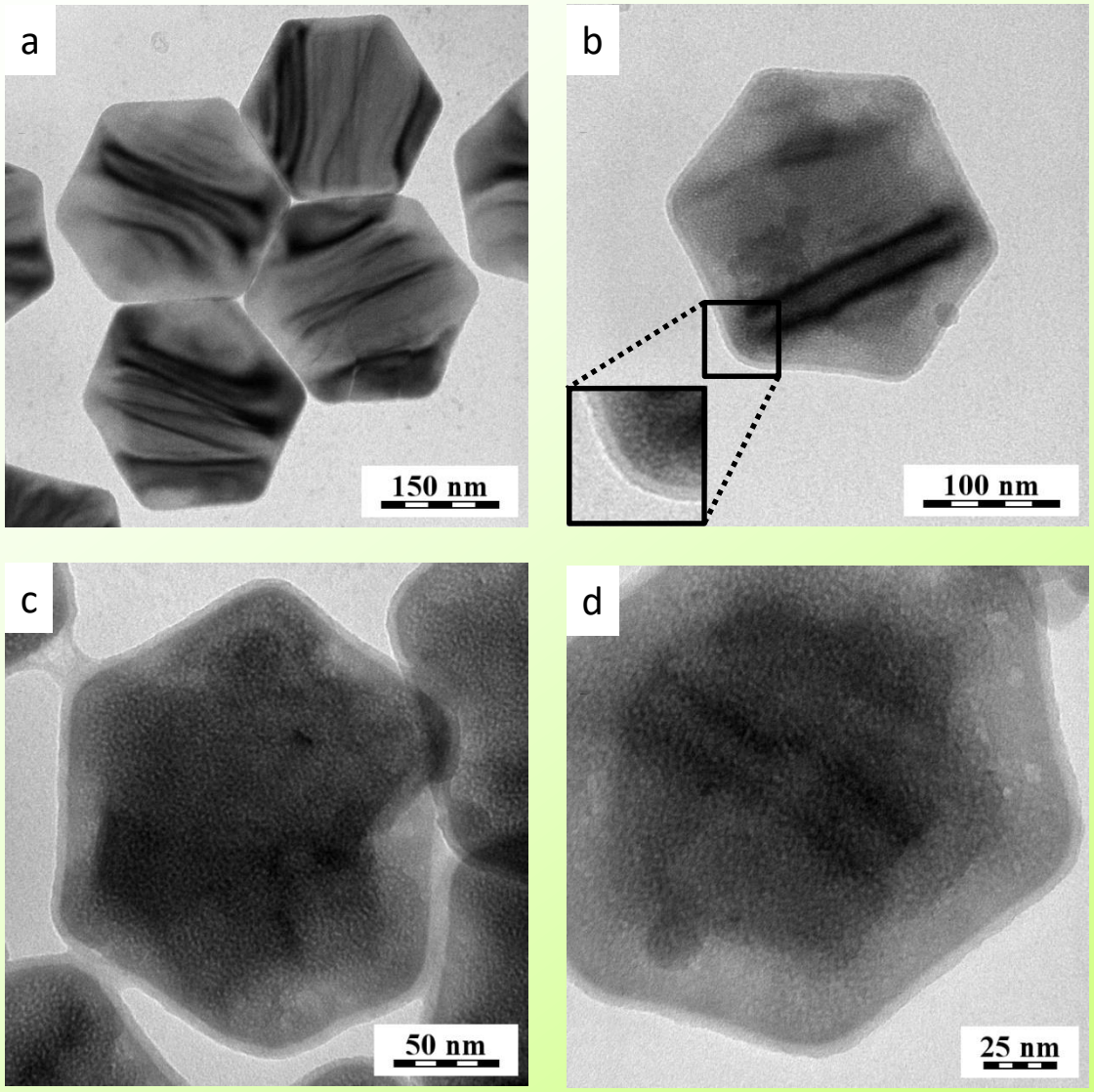
(2) Visualize the polymer shell around the nanoparticles.

(3) Are the nanoparticles hexagonal or cubic form of $NaYF_4$?

Light upconverting $NaYF_4$ nanoparticles

Problem #1: Simultaneous visualization of the nanoparticles and their shell.

Solution: TEM/BF at **multiple** magnifications → example of microscopic scaling problem.



Prepared nanoparticles (summary):

- Inorganic matrix (IUPAC name) sodium yttrium fluoride (1:1:4) **$NaYF_4$**
- Doping with Yb^{3+} and Eu^{3+} in order to get upconversion: **$NaYF_4$:Yb^{3+}/Er^{3+}** (Y:Yb:Eu=57:39:4)
- Covering with silica and its amino derivative in order to improve biocompatibility: **$NaYF_4$:Yb^{3+}/Er^{3+}&SiO_2** **$NaYF_4$:Yb^{3+}/Er^{3+}&SiO_2-NH_2**

Visualization of nanoparticles:

- easy: 2µL on C-coated Cu-grid
- TEM/BF @ 120 kV
- BUT: difficult to see nano-particles + shell simultaneously

⇒ here: multiple magnifications

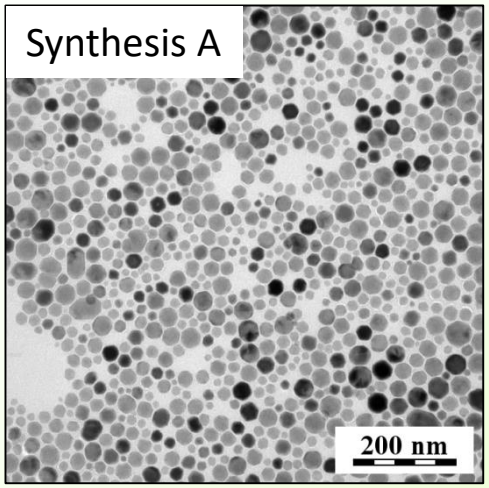
⇒ general solution: see next

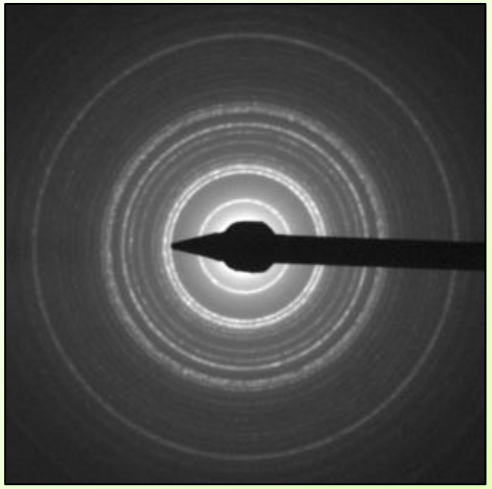
Light upconverting $NaYF_4$ nanoparticles

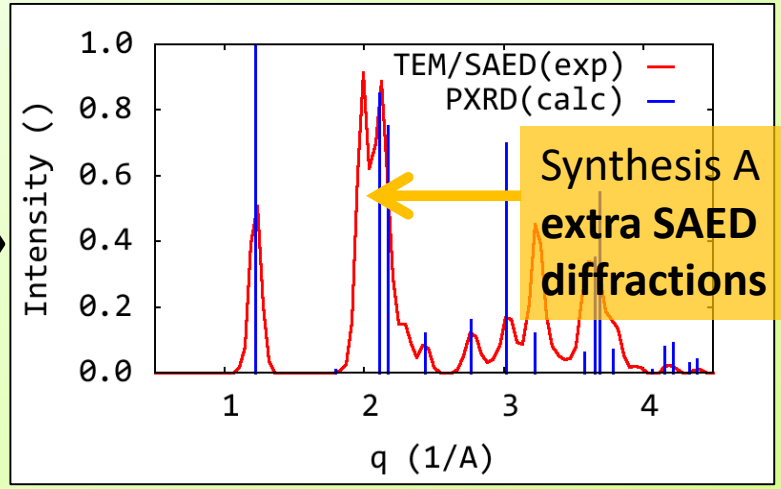
Problem #2: Verification, if the nanoparticles exhibit the expected $NaYF_4$ structure.

Note: Similar syntheses of $NaYF_4$ give quite different results, situation gets complicated...

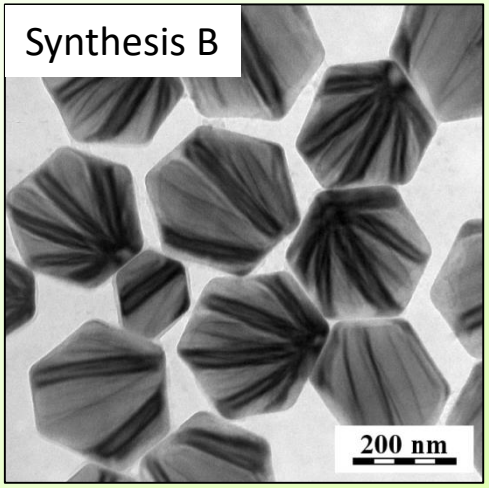
Synthesis A

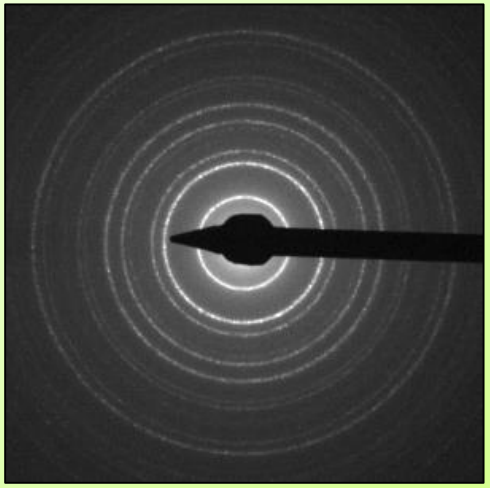


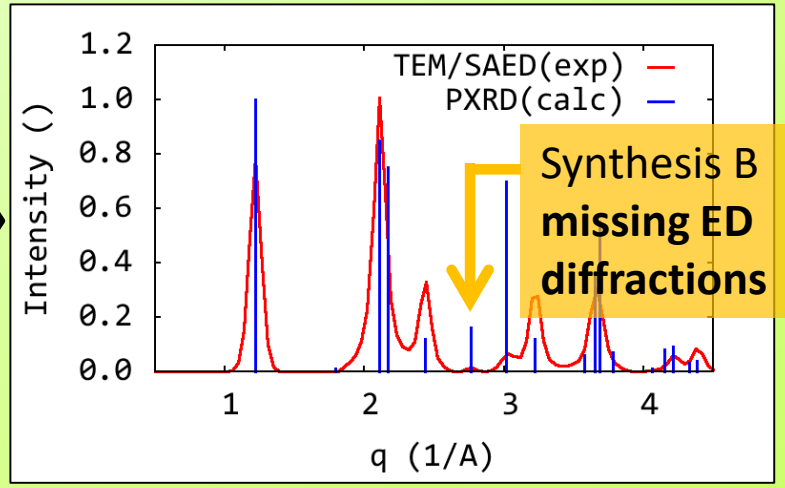




Synthesis B







TEM/BF: different for very similar syntheses.

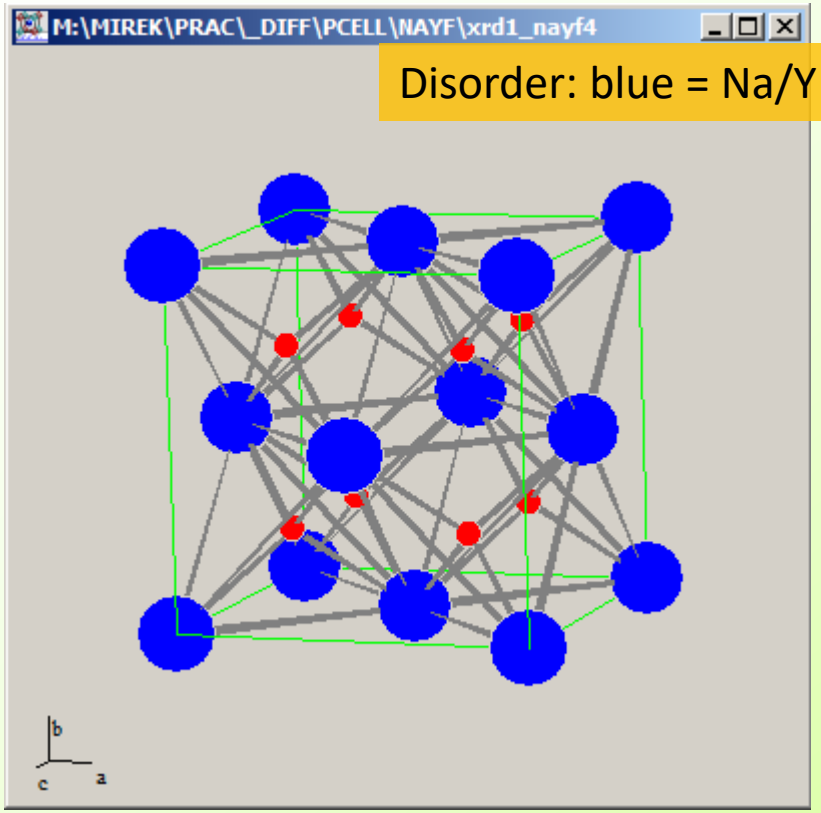
TEM/SAED: different, but TEM/EDX: identical!

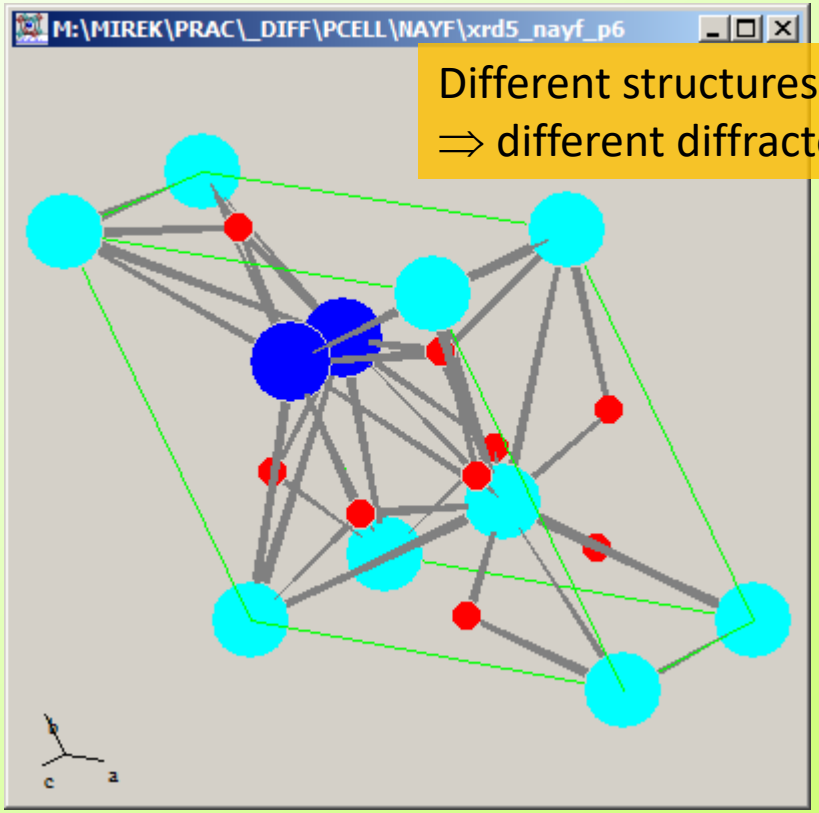
Comparison SAED(exp) x PXRD(calc): some extra/missing diffractions!?

Light upconverting $NaYF_4$ nanoparticles

Problem #2: Verification, if the nanoparticles exhibit the expected $NaYF_4$ structure.

Explanation – important additional information: there are two crystal structures of $NaYF_4$...





α-$NaYF_4$ - unexpected
cubic, space group $Fm\bar{3}m$
unit cell parameters: a = 5.47 Å
less efficient in light upconverting

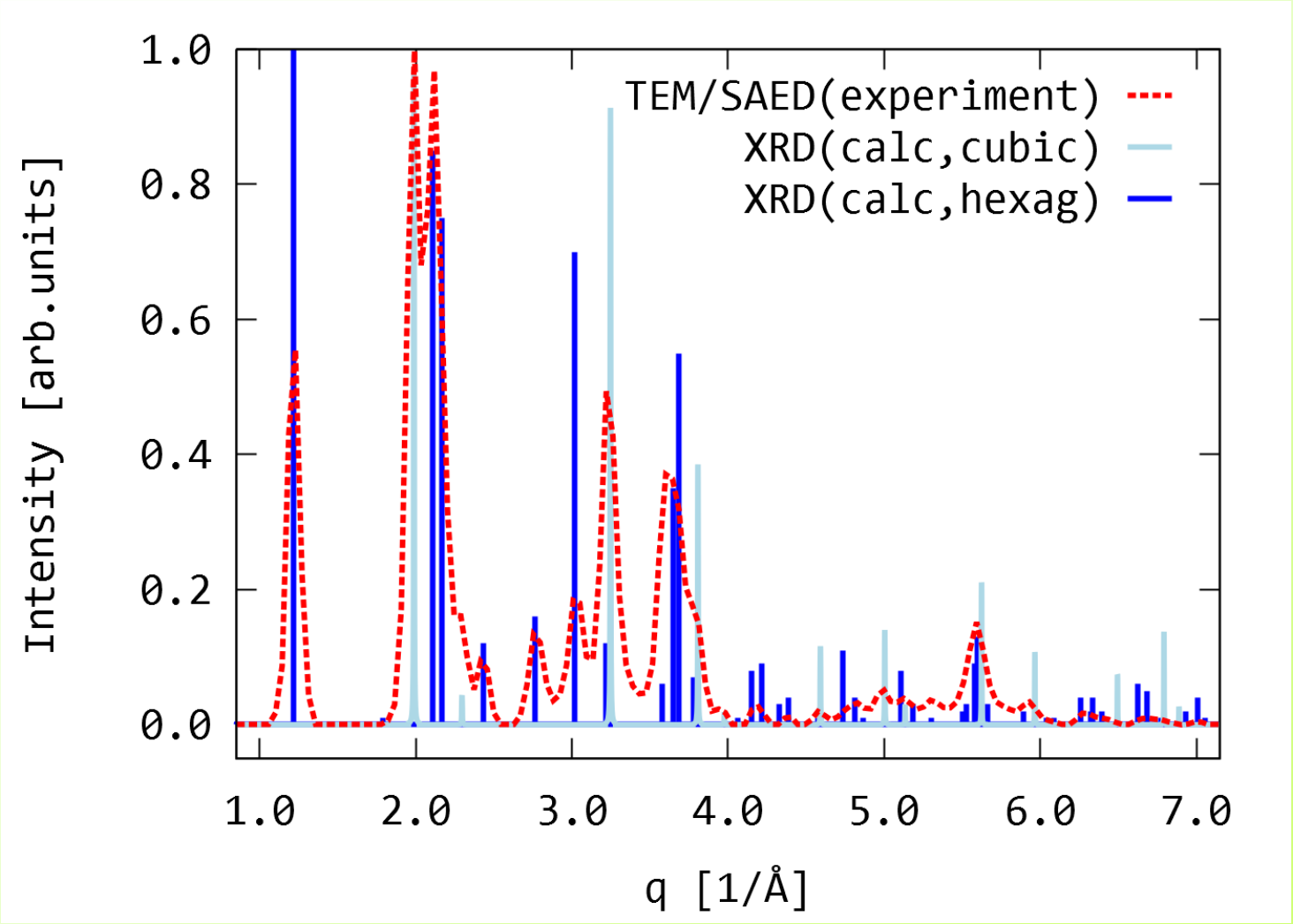
β-$NaYF_4$ – expected acc.to synthesis conditions
hexagonal, space group $P\bar{6}$ ($P\bar{6}2m$, $P6_3/m$)
unit cell parameters: a = 5.96 Å, c = 3.51 Å
more efficient in light upconverting

* Outputs from **PowderCell** – a freeware program, used here for PXRD calculations.

Light upconverting $NaYF_4$ nanoparticles

Problem #2: Verification, if the nanoparticles exhibit the expected $NaYF_4$ structure.

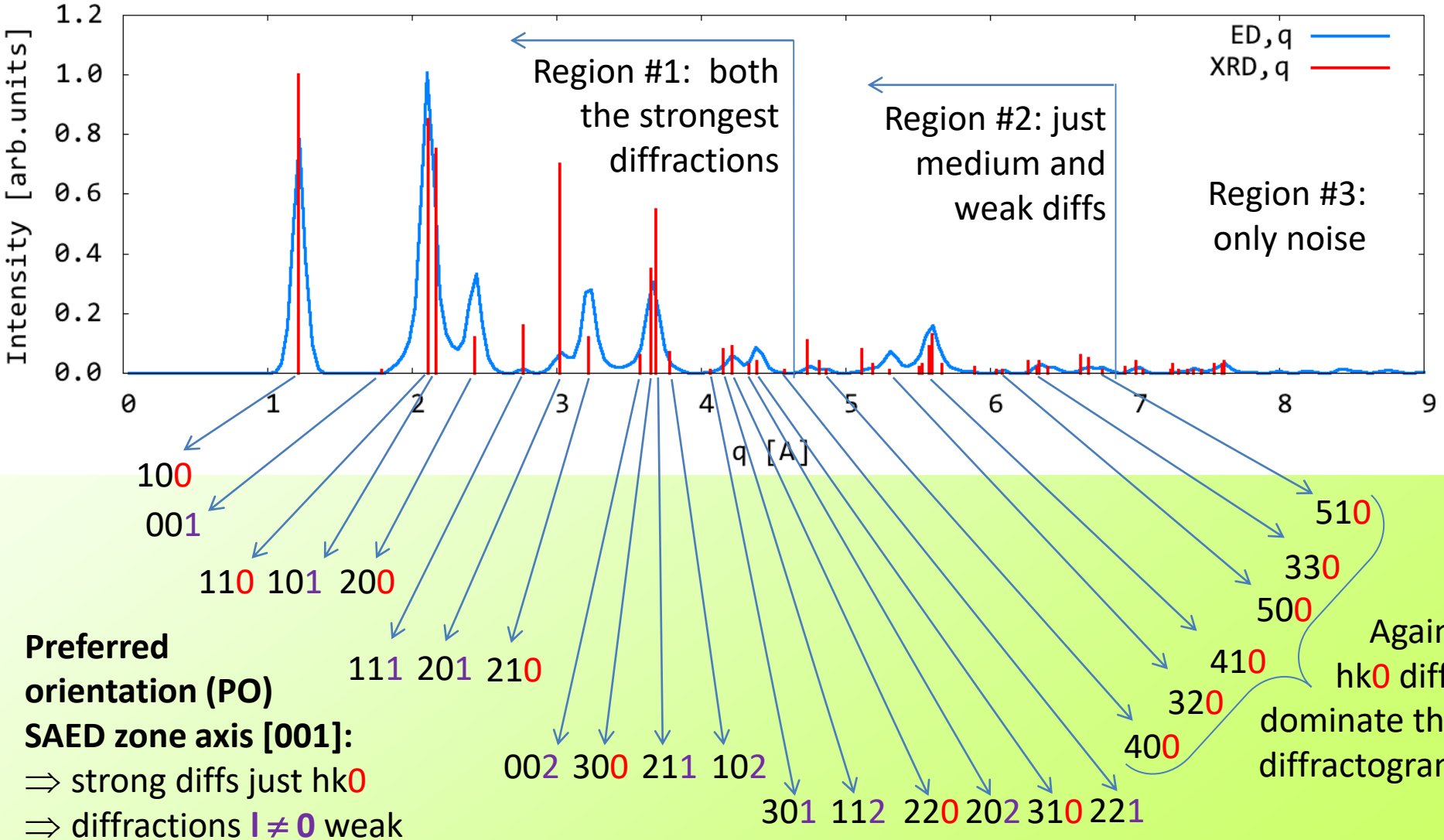
Explanation for TEM/SAED of synthesis A $\Rightarrow$ mixture of cubic and hexagonal phase.



Light upconverting $NaYF_4$ nanoparticles

Problem #2: Verification, if the nanoparticles exhibit the expected $NaYF_4$ structure.

Explanation for TEM/SAED of Synthesis B ⇒ pure hexagonal phase + preferred orientation.



Preferred orientation (PO)
SAED zone axis [001]:
⇒ strong diffs just hk0
⇒ diffractions **l ≠ 0** weak

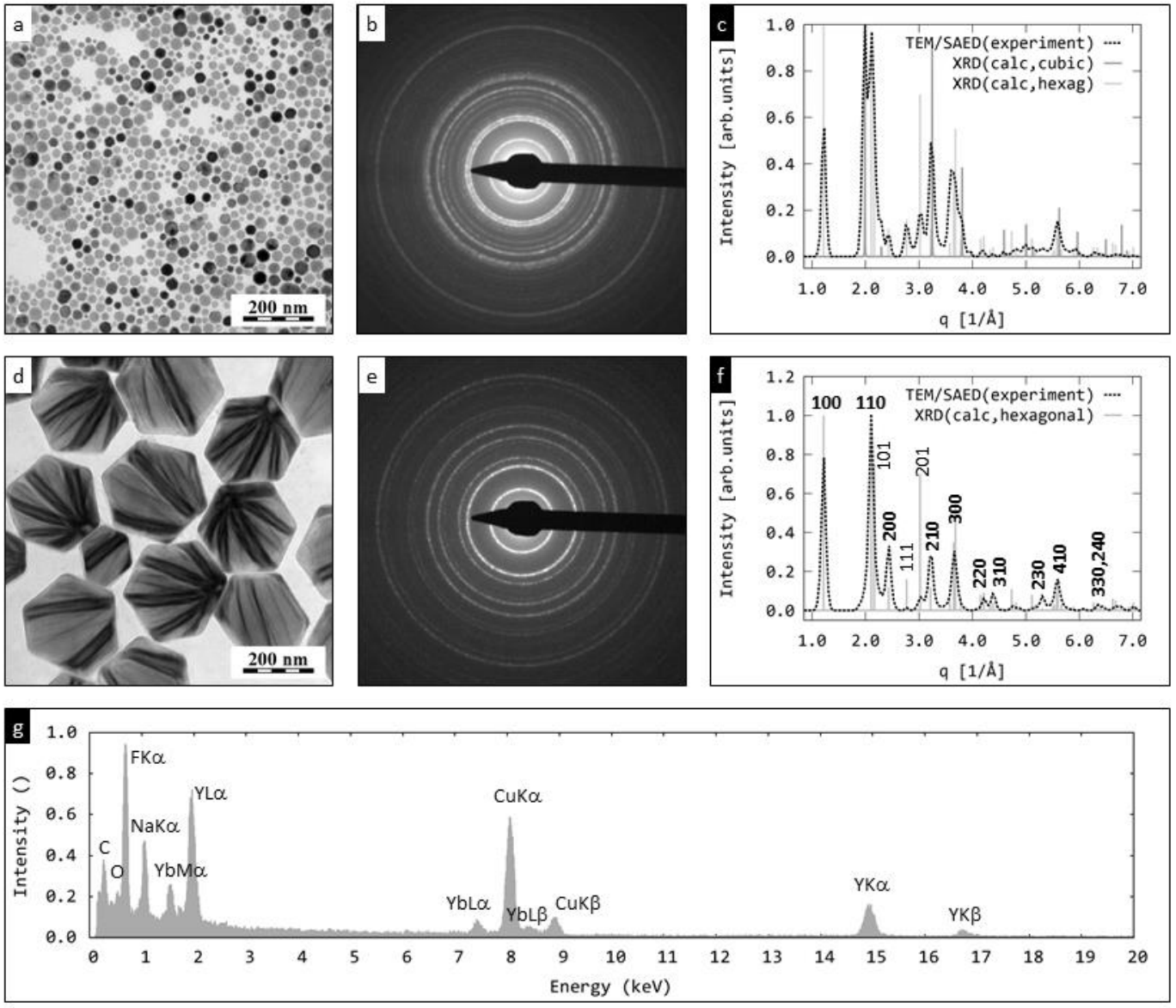
Again: hk0 diffs dominate the diffractogram

Conclusion: the missing diffractions are due to extremely strong preferred orientation.

Light upconverting $NaYF_4$ nanoparticles

Problem #2: Verification, if the nanoparticles exhibit the expected $NaYF_4$ structure.

Final image from the publication → everything is crystal-clear in the end...



Synthesis A

- molar ratio: Ln^{3+}/Na^{+} = 1/2.5
- mixture of cubic and hexagonal crystal modifications

Synthesis B

- molar ratio: Ln^{3+}/Na^{+} = 1.5/2.5
- pure hexagonal modification with strong PO/texture

TEM/EDX spectrum from both syntheses was (logically) almost exactly the same

Appendix

Diffraction level 1 = distances of diffractions from the center

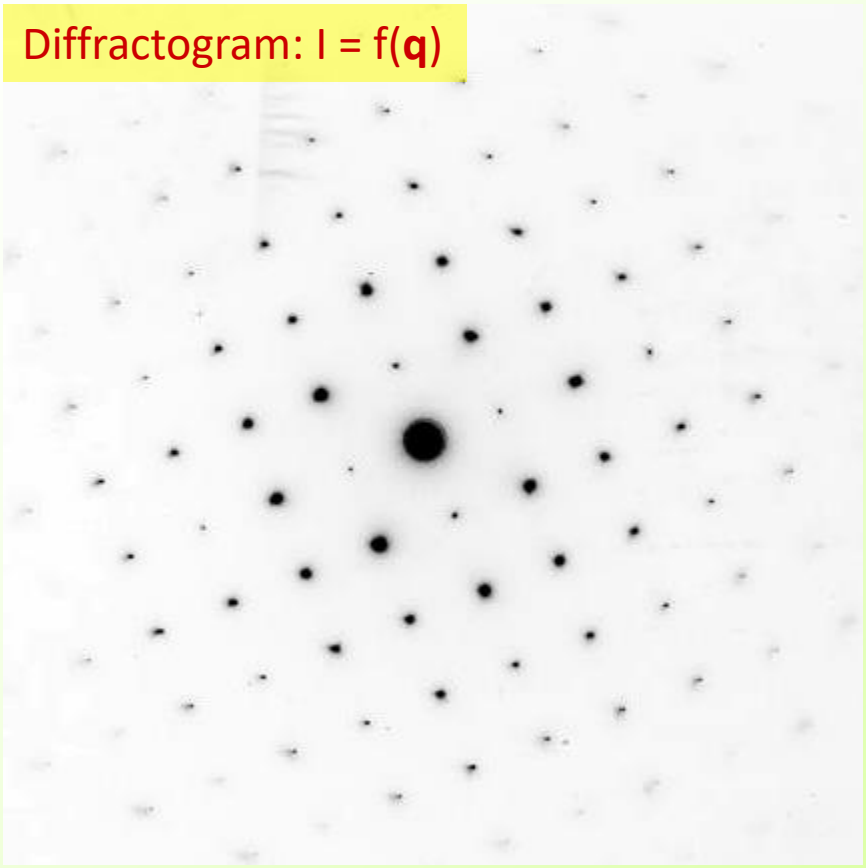
- This is just a summary – levels of diffraction theory:
  Level 1 (main lecture) = **distances** of diffractions (from the central spot)
  Level 2 (see below) = **positions** of diffractions (optional, not at exams)
  Level 3 (see below) = **intensities** of diffractions (optional, not at exams)
- Example what it all means → next slide.
- More details about diffraction theory – numerous textbooks and www.

Technical note:
A = common font = scalar (usually real number, but it may be complex as well)
A = bold font = vector (magnitude of the vector is marked with common font)
A = extra bold = complex number → emphasizing that the number is complex

Diffraction theory :: Level 1 vs. higher levels

Example: SAED pattern of SnO_2 monocrystal (cassiterite, tetragonal mineral).

Diffractogram: I = f(**q**)



Note: We have learnt quite a lot about diffraction, but we still cannot answer the blue questions on the right….
→ but see Appendixes.

What can we say about the diffrattogram?

1) Overall appearance:
   Diffraction spots ⇒ monocrystal.
   Reminder: each spot = one lattice plane.
   This is explained by Bragg's Law.
2) Overall symmetry:
   The crystal was probably cubic or tetragonal
   (symmetry of crystal vs. diffractogram?
3) Distances of diffractions:
   These are explained by Bragg's Law.
   (why can we see ALL spots AT ONCE?
4) Positions of the diffractions:
   These are explained by Laue diffraction condition (→ appendix: Diffraction, level 2)
5) Intensity of the diffractions:
   These can be calculated (to the 1st approximation) by kinematic diffraction theory (→ appendix: Diffraction, level 3).
6) How to calculate the structure from its diffractogram? (Level 4, beyond our course)

Appendix

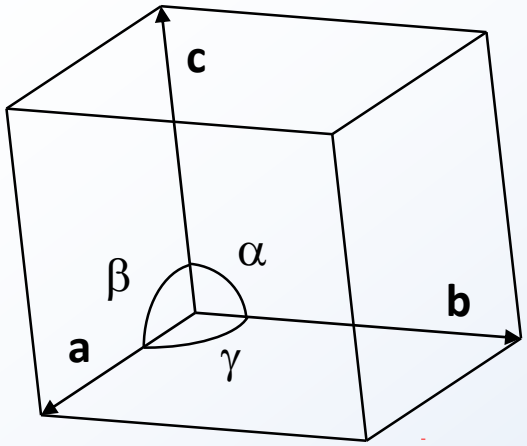
Diffraction level 2 = positions of diffractions

- ❖ This part is optional (not at exams)
- ❖ Introduction/revision for those, who are interested in TEM/SAED.
- ❖ More details about diffraction theory – numerous textbooks and www.

Technical note:
A = common font = scalar (usually real number, but it may be complex as well)
A = bold font = vector (magnitude of the vector is marked with common font)
A = extra bold = complex number → emphasizing that the number is complex

Diffraction theory :: Level 2

Step 1: Direct lattice and reciprocal lattice (DL and RL); RL vectors $\mathbf{G}^*_{hkl}$.



Direct lattice (DL):
triclinic unit cell
of direct lattice in 3D.

Reciprocal lattice (RL):
RL cell is defined by vectors **a***, **b***, **c*** which are defined as follows:

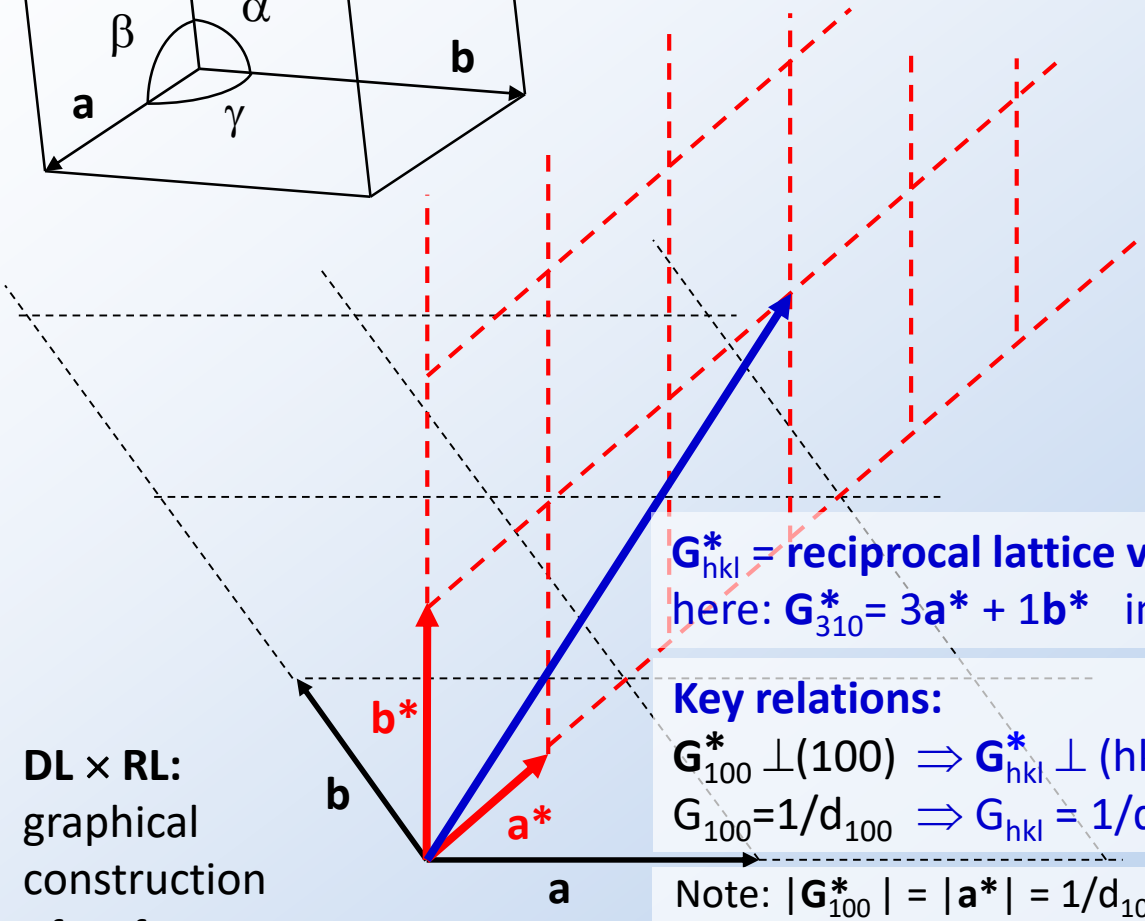
a) length of RL vectors:

$|\mathbf{a}^*| = 1/d_{100}$
$|\mathbf{b}^*| = 1/d_{010}$
$|\mathbf{c}^*| = 1/d_{001}$

In general: $|\mathbf{a}^*| \neq 1/a$ as $1/d_{100} = 1/a$ only in "rectangular" DL's.

b) direction of RL vectors:
each RL vector is perpendicular to the other two DL vectors;
example: ($\mathbf{a}^* \perp \mathbf{b}$) and ($\mathbf{a}^* \perp \mathbf{c}$)

c) orientation of RL vectors
right-hand rule (→ Wikipedia)



DL × RL:
graphical
construction
of RL from given DL.

$\mathbf{G}^*_{hkl}$ = **reciprocal lattice vector**
here: $\mathbf{G}^*_{310} = 3\mathbf{a}^* + 1\mathbf{b}^*$ in general: $\mathbf{G}^*_{hkl} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$

Key relations:
$\mathbf{G}^*_{100} \perp (100) \Rightarrow \mathbf{G}^*_{hkl} \perp (hkl)$
$G_{100} = 1/d_{100} \Rightarrow G_{hkl} = 1/d_{hkl}$

Note: $|\mathbf{G}^*_{100}| = |\mathbf{a}^*| = 1/d_{100}$
More details → textbooks

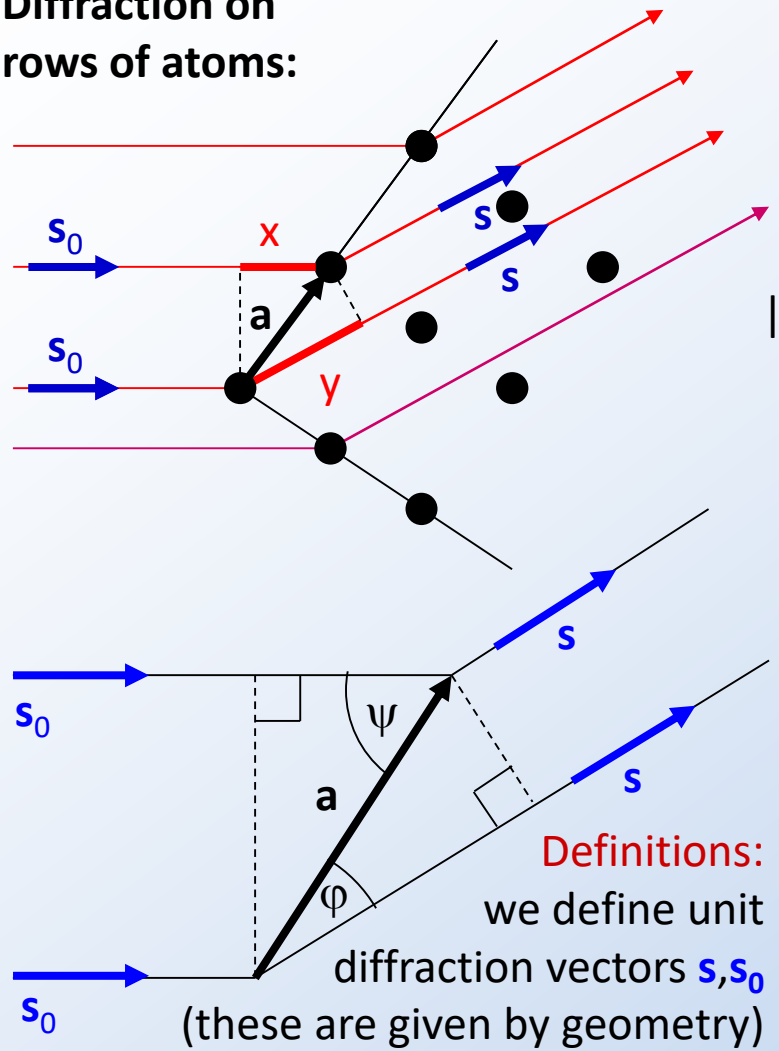
Further important relations:

$$\begin{pmatrix} \mathbf{aa^*} & \mathbf{ab^*} & \mathbf{ac^*} \\ \mathbf{ba^*} & \mathbf{bb^*} & \mathbf{bc^*} \\ \mathbf{ca^*} & \mathbf{cb^*} & \mathbf{cc^*} \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

Diffraction theory :: Level 2

Step 2: Direct lattice, reciprocal lattice and Laue diffraction condition (LDC).

Diffraction on rows of atoms:



Definitions:
we define unit diffraction vectors **s**,**s**$_0$ (these are given by geometry) and diffraction vector **S** = (**s**-**s**$_0$)/λ (this is defined by the above formula)

...in order to get maximum interference on one row of atoms, the following has to hold:

$$\begin{aligned} \text{path\_difference} &= \text{integer\_multiple\_of\_}\lambda \\ y - x &= h*\lambda \quad \dots h = \text{integer} \\ |a|\cos\varphi - |a|\cos\psi &= h*\lambda \\ |a||s|\cos\varphi - |a||s_0|\cos\psi &= h*\lambda \\ \mathbf{a}(\mathbf{s}-\mathbf{s}_0) &= h*\lambda \end{aligned}$$

$$\boxed{\begin{aligned} \mathbf{a}*\mathbf{S} &= h \\ \mathbf{b}*\mathbf{S} &= k \\ \mathbf{c}*\mathbf{S} &= l \end{aligned}}$$

...one row of atoms
+ the second row
+ the third row

If all three conditions hold we get maximal interference on the whole crystal.
⇒ diffraction occurs only under these conditions

Now we express **S** by means of arbitrary vector defined in RL: **S** = p**a***+q**b***+r**c***...
...we insert S into eqs. in rectangle above and get:
(p=h ∧ q=k ∧ r=l) ⇒ diffraction occurs only at ...

Laue diffraction condition: $\mathbf{S} = \mathbf{G}^*_{hkl}$

Diffraction theory :: Level 2

Step 3: Laue diffraction condition (LDC) and Ewald construction (EC).

Laue diffraction condition: $\mathbf{S} = \mathbf{G}^*_{hkl}$

If you want to know more details:
wikipedia.org → Ewald sphere

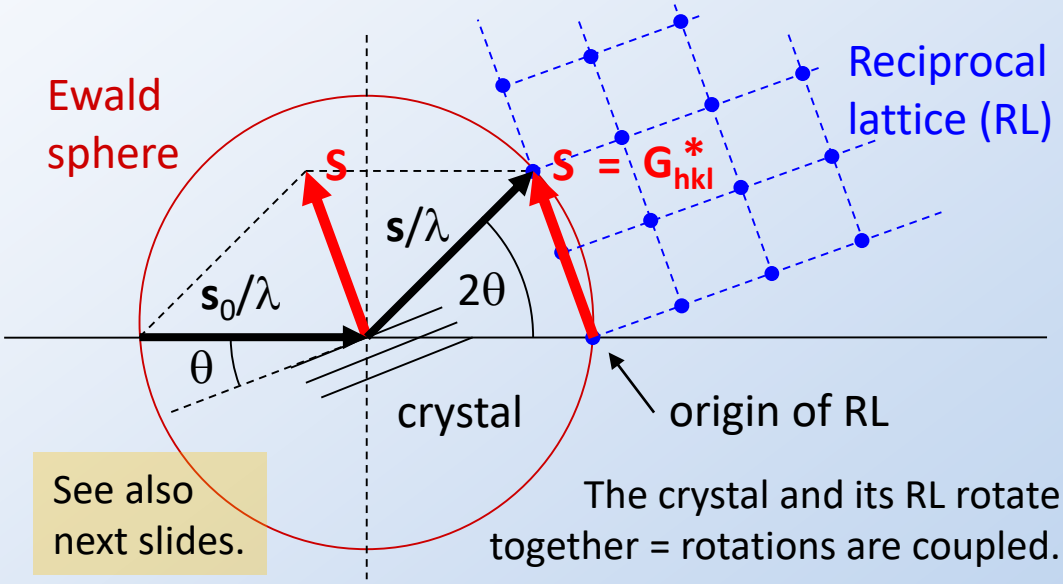
What LDC means in real life?

⇒ Diffraction occurs ONLY on condition that our diffraction vector **S** is equal to some reciprocal lattice vector $\mathbf{G}^*_{hkl}$.

Ewald construction is a graphical representation of LDC.

⇒ EC is just a clever image, which shows when and where the diffraction occurs.

⇒ Good message: EC can be used and understood even if you do not know all math behind. BUT you must know how to draw it and remember the key conclusion:



See also next slides.

The crystal and its RL rotate together = rotations are coupled.

The image shows EC for a crystal, which is just diffracting.

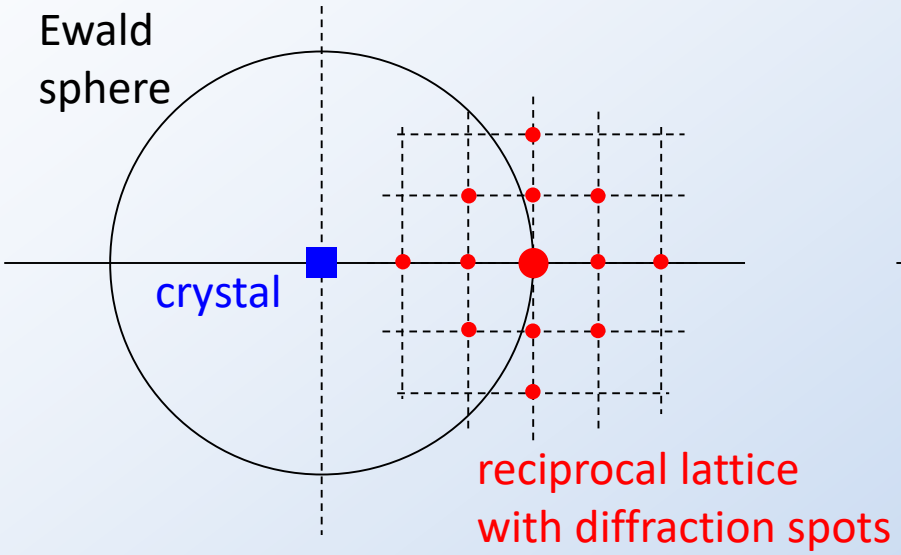
Key conclusion:
Diffraction occurs only if some RL point lies on the Ewald sphere. (i.e. if $\mathbf{S} = \mathbf{G}^*_{hkl}$ ⇔ LDC)

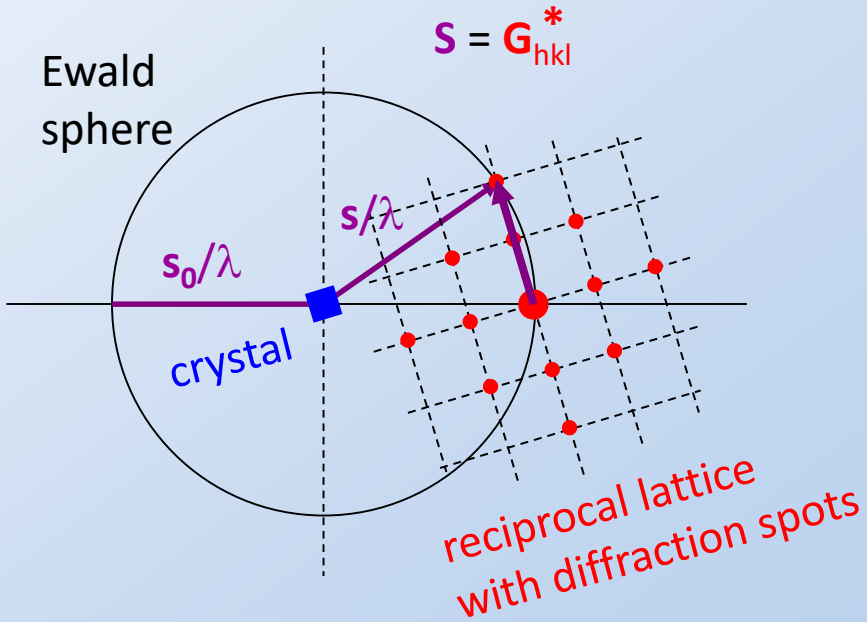
Diffraction theory :: Level 2

Step 4: Ewald construction → XRD pattern of a monocrystal (= single crystal XRD).

XRD of cubic monocrystal.
One axis of the monocrystal parallel with $\mathbf{s_0}$.
⇒ no RL point lies on Ewald sphere under this condition
⇒ LDC is not fulfilled – no RL vector is identical to **S**
⇒ no diffraction occurs

In order to get diffraction, the crystal must be rotated. At suitable rotation LDC can be fulfilled as shown below:





Conclusions:

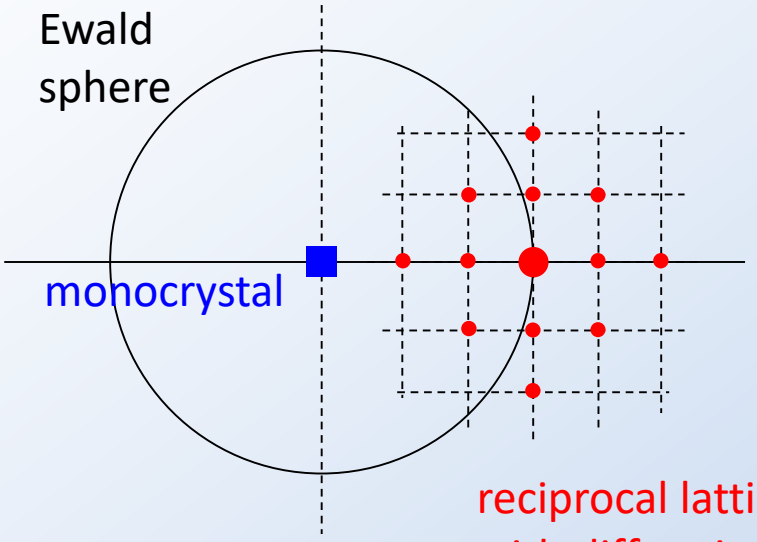
1) XRD with single crystal: it is necessary to rotate the crystal ⇒ four-circle diffractometers.
2) Typical XRD diffractogram = file, which gradually grows and contain lines: h, k, l, I(hkl)...

Diffraction theory :: Level 2

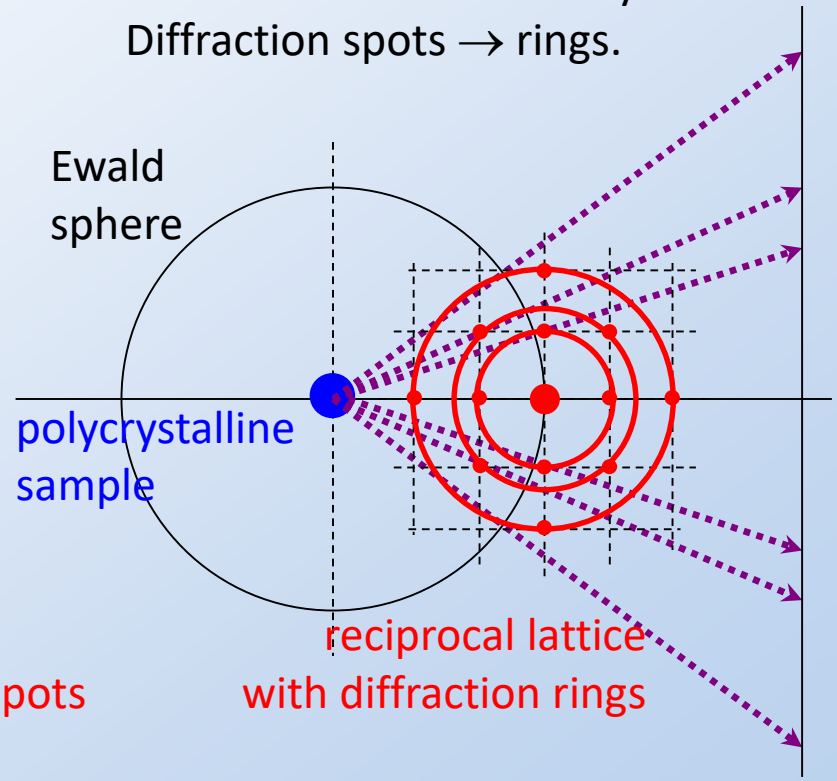
Step 5: Ewald construction → XRD of polycrystalline sample (= powder XRD = PXRD).

PXRD, cubic polycrystalline sample = powder sample.

(1) Reminder:
XRD of a monocrystal
(no diffraction; see previous slide)



(2) Different situation for powder sample:
Random orientation of crystallites.
Diffraction spots → rings.



Conclusions:

1) XRD with powder sample: LDC fulfilled automatically ⇒ fixed 2D-detectors are sufficient.

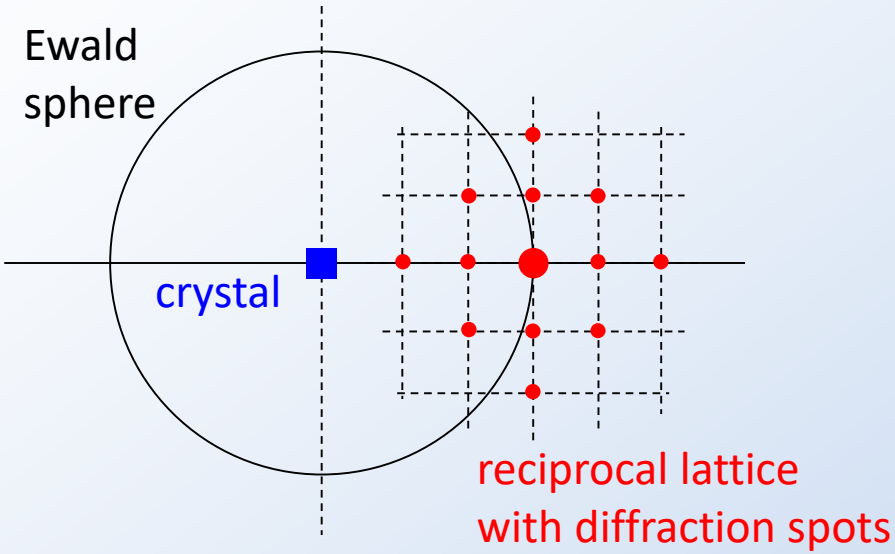
2) Typical PXRD diffractogram (with 2D-detector) = rings (intersections of ES and RL spheres).

Diffraction theory :: Level 2

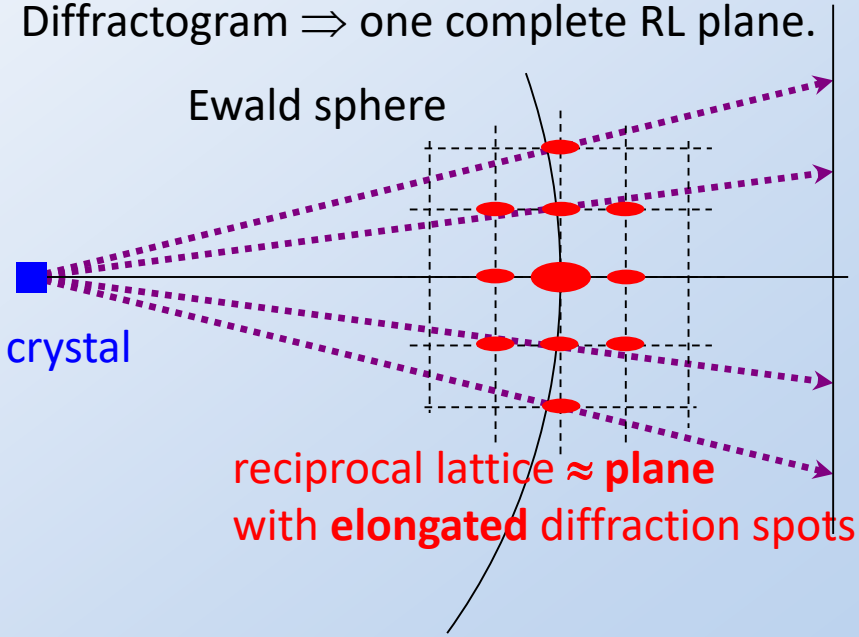
Step 6: Ewald construction → ED pattern of a monocrystal (= single crystal ED).

ED, cubic monocrystal, oriented with one axis along the incident beam = with vector $\mathbf{s}_0$.

(1) Reminder:
XRD of a monocrystal
(no diffraction, see previous slides)



(2) Different situation for a monocrystal in ED:
Radius of ES = $1/\lambda \Rightarrow$ ES ≈ almost plane!
Moreover: elongated spots $\Rightarrow \varepsilon_{hkl} \approx 1/L_{hkl}$
Diffractogram ⇒ one complete RL plane.



Two main differences between XRD and ED:

1) Ewald sphere radius = $1/\lambda$; for X-rays: $\lambda \approx 1$Å, for elns: $\lambda \approx 0.03$Å ⇒ XRD sphere → ED plane.
2) Diffraction spots are elongated for thin crystals $\Rightarrow \varepsilon_{hkl} \approx 1/L_{hkl} \Rightarrow$ XRD spots → ED ellipsoids.

Conclusions:

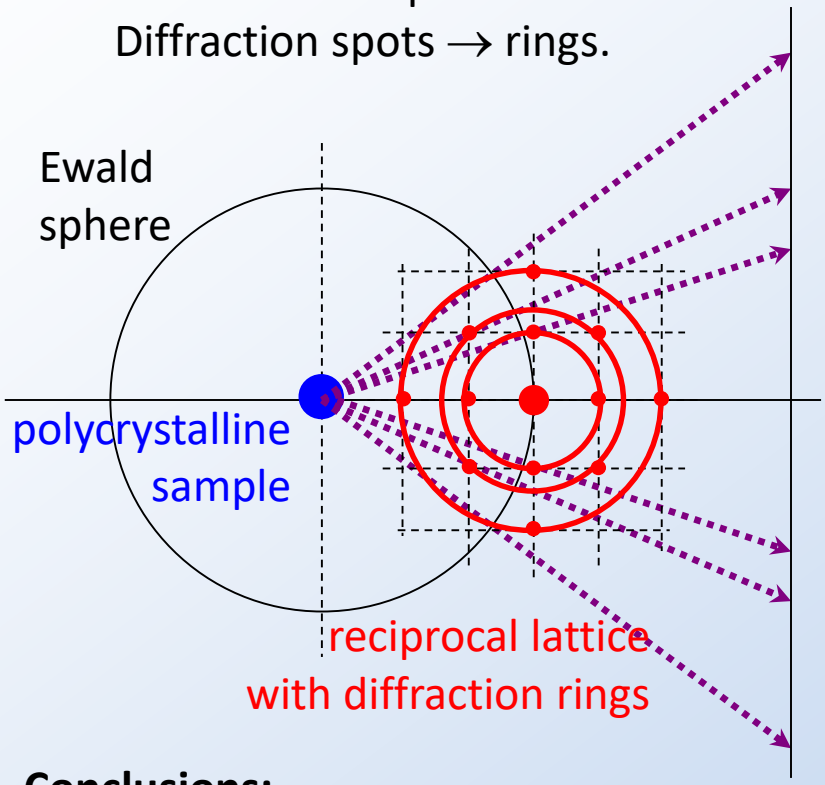
1) For ED of a monocrystal, LDC is easily fulfilled due to short λ a elongated diffraction spots.
2) Typical ED diffractogram of a monocrystal = spots = image of one reciprocal lattice plane.

Diffraction theory :: Level 2

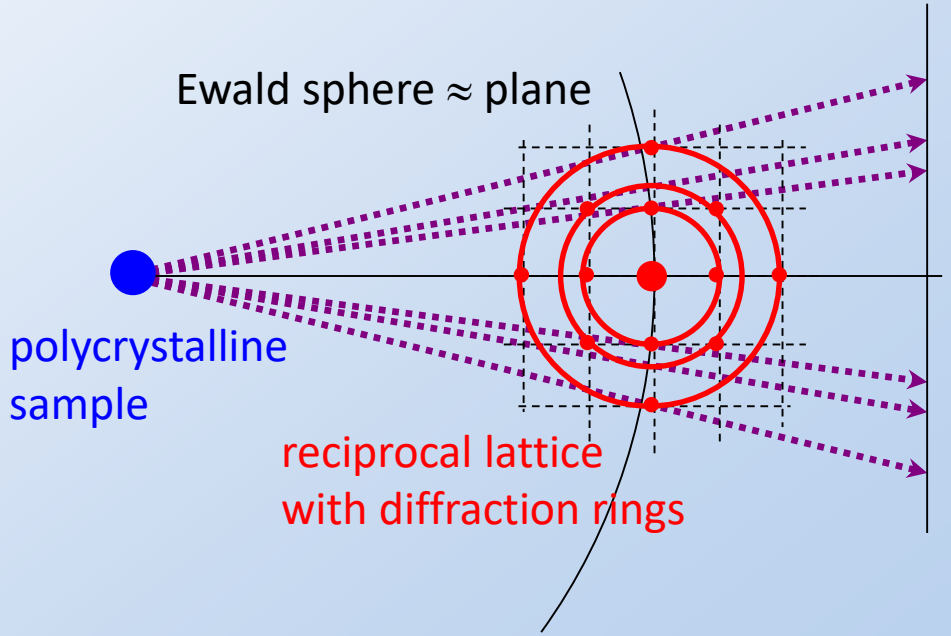
Step 7: Ewald construction → ED pattern of a polycrystalline sample (= powder ED).

ED, cubic polycrystalline sample = powder sample.

(1) Reminder:
PXRD = XRD of powder
Diffraction spots → rings.



(2) ED of powder = like PXRD, only...
...ES has higher radius (lower diff. angles)
...diffraction spots elongated (not shown ↓)
elongated + broader spots = broader peaks



Conclusions:

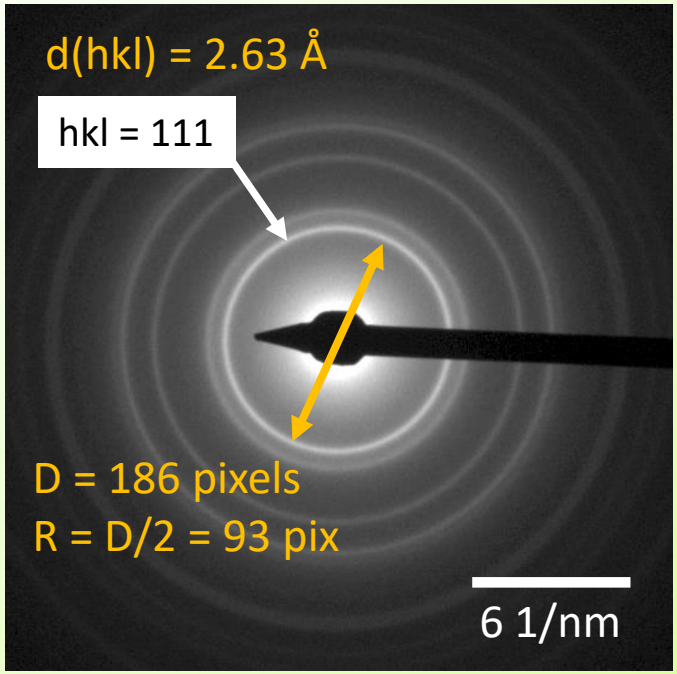
1) ED of powder/polycrystalline sample is completely analogous to PXRD.

2) Typical ED powder diffractogram: diffraction rings on 2D-camera in the TEM microscope.

Example 1 :: Calibration of TEM/SAED patterns

What do we know from previous slides?

- LDC = Laue Diffraction Condition: Diffraction occurs only if $\mathbf{S} = \mathbf{G}^*_{hkl}$
- Single crystal electron diffraction patterns show spots at positions = $\mathbf{G}^*_{hkl}$
- Powder electron diffraction patterns show rings (~multi-spots) at distances = $|\mathbf{G}^*_{hkl}|$
- Magnitude of $|\mathbf{G}^*_{hkl}| = 1/d_{hkl} \Rightarrow$ scalebars in diffractograms are in units [1/nm] or [1/Å].
- Typical calibration procedure:
  (1) Find a spot/ring with known *hkl.* (2) Calculate d_{hkl} in [Å]. (3) Measure $|\mathbf{G}^*_{hkl}|$ in [pixels].
  (4) Calibrate the image by means of relation: $|\mathbf{G}^*_{hkl}|$[pixels] = $1/d_{hkl}$ [1/Å] (typically in ImageJ).



Calibration of SAED diffractogram of Au (fcc, a=4.08Å)

(1) The first diffraction corresponds to plane (111).
(this can be found in the literature or calculated

(2) The corresponding d(hkl) = d(111) = 2.36[Å]
(literature or calculation → calculation in iPython

(3) The corresponding G*(hkl) = G*(111) = 186/2 = 93[pix]
(this we measure directly in ImageJ

(4) The calibration is then calculated in usual way:
G*(111) = 93[pixel] ~ 1/d(111) = 1/2.36 = 0.42[1/Å]
93[pix] = 0.42[1/Å] = 4.2[1/nm] ⇒ 6[1/nm] = 133[pix]
and the scalebar is inserted with ImageJ → IJM macro
the only not-so-common thing: reciprocal units

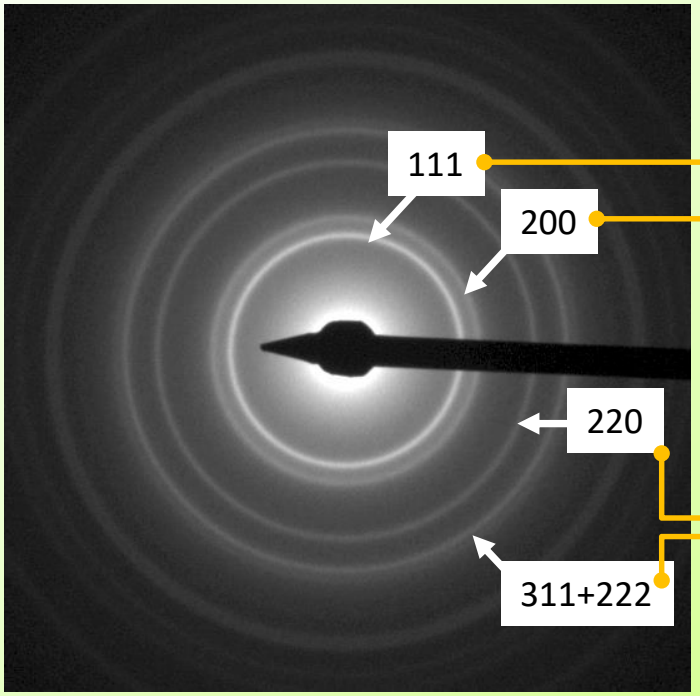
! Note: Common units in 2D-diffractograms: [1/nm] (diff.vector S ~ G^*_{hkl}), but in 1D: [1/Å] (q = 2πS).

Example 2 :: Indexing of powder TEM/SAED diffraction patterns

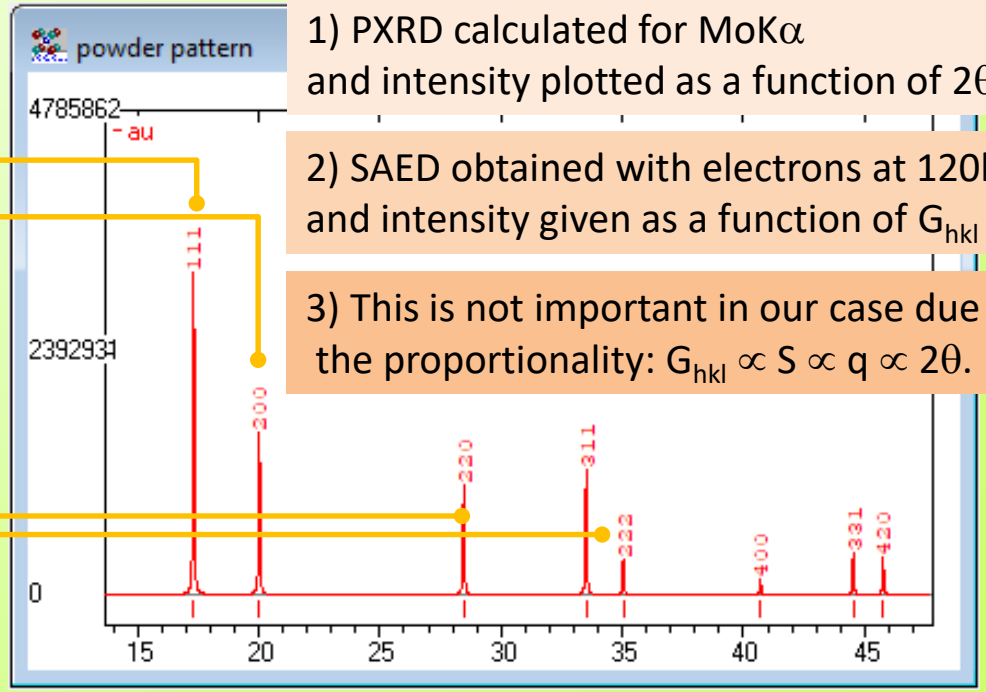
What do we know from previous slides?

- All that was summarized in the previous Example 1 concerning RL vectors.
- Namely: Powder SAED patterns show diffraction rings at distances $|\mathbf{G}^*_{hkl}| = 1/d_{hkl}$.
- Moreover: $|\mathbf{G}^*_{hkl}|$ is proportional to S and other diffraction vectors/angles $|\mathbf{G}^*_{hkl}| \propto S \propto q \propto 2\theta$.
- Typical indexing procedure:
  (1) Just compare experimental 2D-SAED with (2) calculated 1D-PXRD (x-axis in q or S or 2θ).

Experiment: 2D-TEM/SAED of Au



Theory: Calculation of 1D-PXRD in PowderCell



1) PXRD calculated for MoKα and intensity plotted as a function of 2θ.

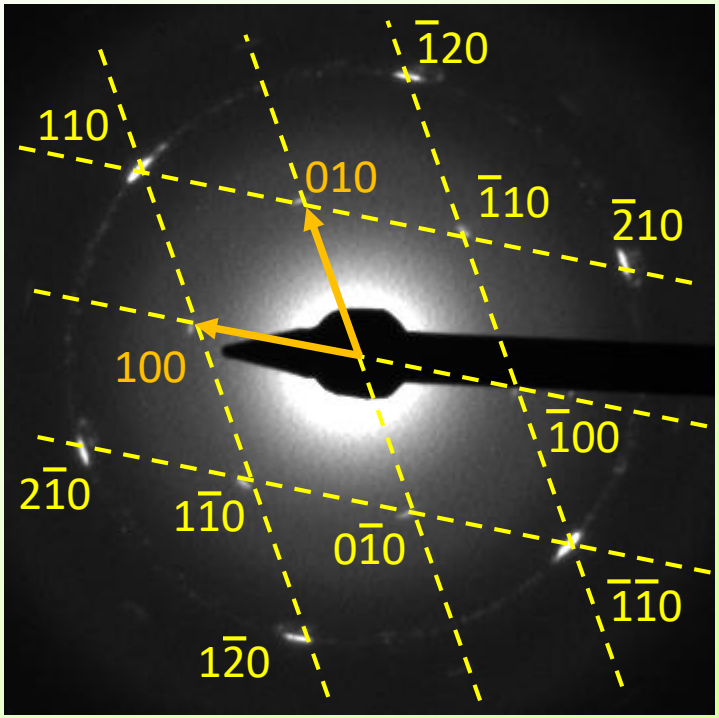
2) SAED obtained with electrons at 120kV and intensity given as a function of $G_{hkl} \propto S$

3) This is not important in our case due to the proportionality: $G_{hkl} \propto S \propto q \propto 2\theta$.

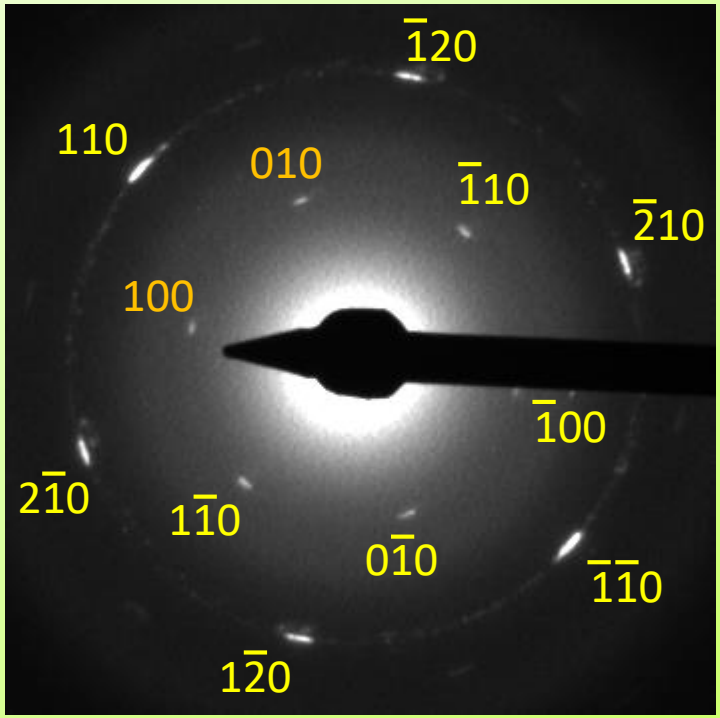
Example 3 :: Indexing of single-crystal TEM/SAED diffraction patterns

What do we know from previous slides?

- All that was summarized in the previous Example 1 concerning RL vectors.
- Single crystal ED patterns (with defined orientation) show images of defined RL planes.
- Single crystal ED patterns (with random orientation) show random sections through 3D RL.
- Typical indexing procedure (for oriented ED patterns):
  (1) Find two independent RL vectors and (2) Index the spots using vector addition.



Indexing of MXene monocrystal.
Plane with zone axis 001.



Final result (without RL vectors and lines).
More about MXenes → wiki, literature...

Appendix

Diffraction level 3 = intensities of diffractions

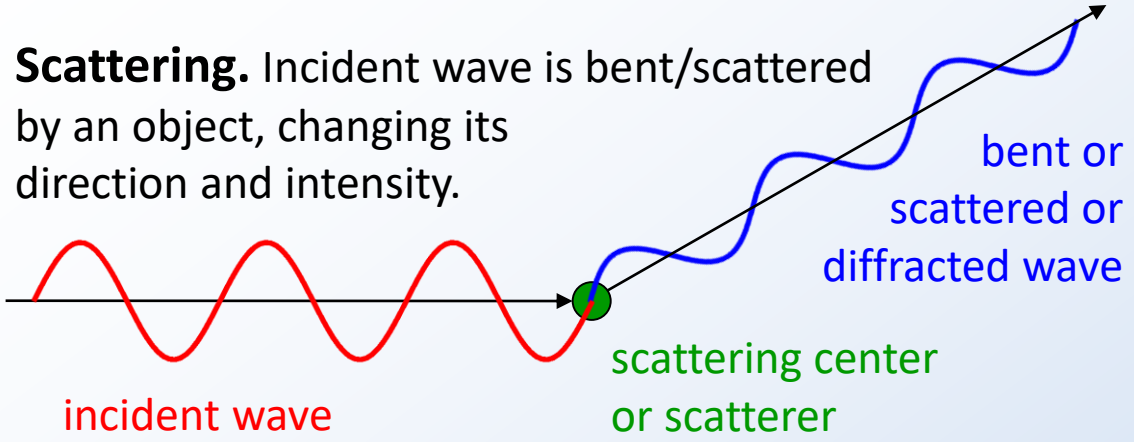
- ❖ This part is optional (not at exams)
- ❖ Introduction/revision for those, who are interested in TEM/SAED.
- ❖ More details about diffraction theory – numerous textbooks and www.

Technical note:
A = common font = scalar (usually real number, but it may be complex as well)
A = bold font = vector (magnitude of the vector is marked with common font)
A = extra bold = complex number $\rightarrow$ emphasizing that the number is complex

Diffraction :: Level 3 :: Basic formula: $\mathbf{A}(\mathbf{q}) = \int \rho(\mathbf{r}) \exp[i\mathbf{q}\mathbf{r}]\, d\mathbf{r}$

Step 1: Scattering by one volume element dV = d**r**.

Scattering. Incident wave is bent/scattered by an object, changing its direction and intensity.



Wave-particle duality.
Particles may have wave aspect and vice versa.
Examples:
light × photons
electron beam × electrons
De Broglie waves:
$\lambda = h/mv$

Incident wave	Incident particle	λ [Å]	Scatterer	Methods
X-ray beam	X-ray photon	0.5-2	electron	XRD, WAXS, SAXS..
neutron beam	neutron	0.5-6	atom nucleus	ND, WANS, SANS..
electron beam	electron	0.001-2	el. potential	ED, SAED, CBED, NBD..
light beam	photon	4000-8000	different n	LS, SALS, WALS, QELS..

Final result = scattering by one volume element d**r**

$I(\mathbf{q}) \approx |A(\mathbf{q})|^2$ ...intensity of the scattered wave depends on its direction $\approx \theta \approx \mathbf{S} \approx \mathbf{q}$

$A(\mathbf{q})$ = const * number_of_scatterers_in_unit_volume * volume_element

$A(\mathbf{q}) = b * n(\mathbf{r}) * d\mathbf{r}$ ...b = diffraction length = different for X-rays, neutrons, electrons...

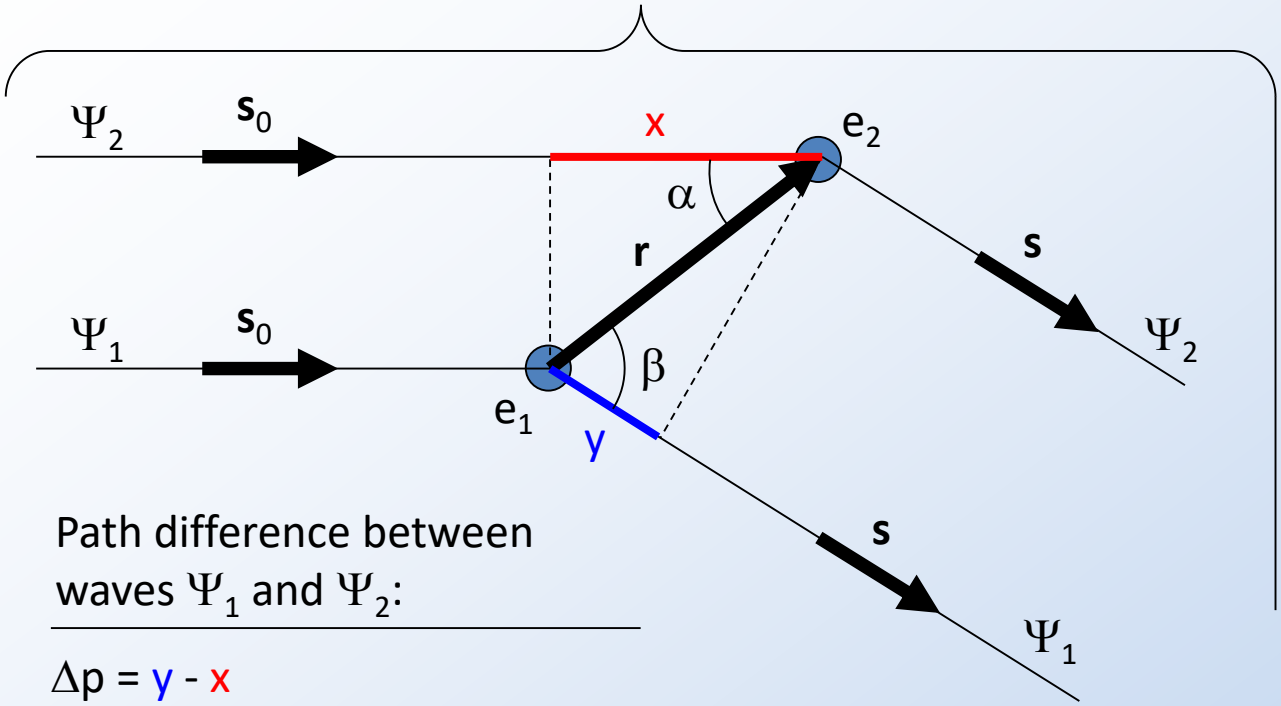
$A(\mathbf{q}) = \rho(\mathbf{r}) * d\mathbf{r}$ ...$\rho(\mathbf{r}) = b * n(\mathbf{r})$ = electron density in XRD, nuclear density in ND...

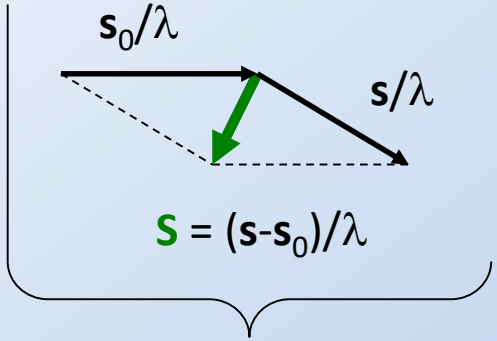
The above formulas ↑ are presented here without justification, but they are quite logical. More details are given in good (select carefully!) textbooks about diffraction.

Diffraction :: Level 3 :: Basic formula: $\mathbf{A(q)} = \int \rho(\mathbf{r}) \exp[i\mathbf{qr}]\, d\mathbf{r}$

Step 2: Scattering by two centers - path difference.

2 waves (Ψ_1,Ψ_2), which are scattered by 2 centers (e_1,e_2):





Definition of scattering vector **S**.
(λ = wavelength
($\mathbf{s}$,$\mathbf{s_0}$ = unit vectors
($\mathbf{S} = (\mathbf{s}-\mathbf{s_0})/\lambda$

Path difference between waves Ψ_1 and Ψ_2:

$\Delta p = y - x$

$\Delta p = |\mathbf{r}|*\cos(\beta) - |\mathbf{r}|*\cos(\alpha)$

$\Delta p = |\mathbf{r}|*|\mathbf{s}|*\cos(\beta) - |\mathbf{r}|*|\mathbf{s_0}|*\cos(\alpha)$ ... trick1: $\mathbf{s}$, $\mathbf{s_0}$ = scattering vectors of unit length!

$\Delta p = \mathbf{r}*\mathbf{s} - \mathbf{r}*\mathbf{s_0}$ .... trick2: vector multiplication: $|\mathbf{a}||\mathbf{b}|\cos(\gamma) = \mathbf{a}*\mathbf{b}$

$\Delta p = \mathbf{r}(\mathbf{s} - \mathbf{s_0})$

Final result:

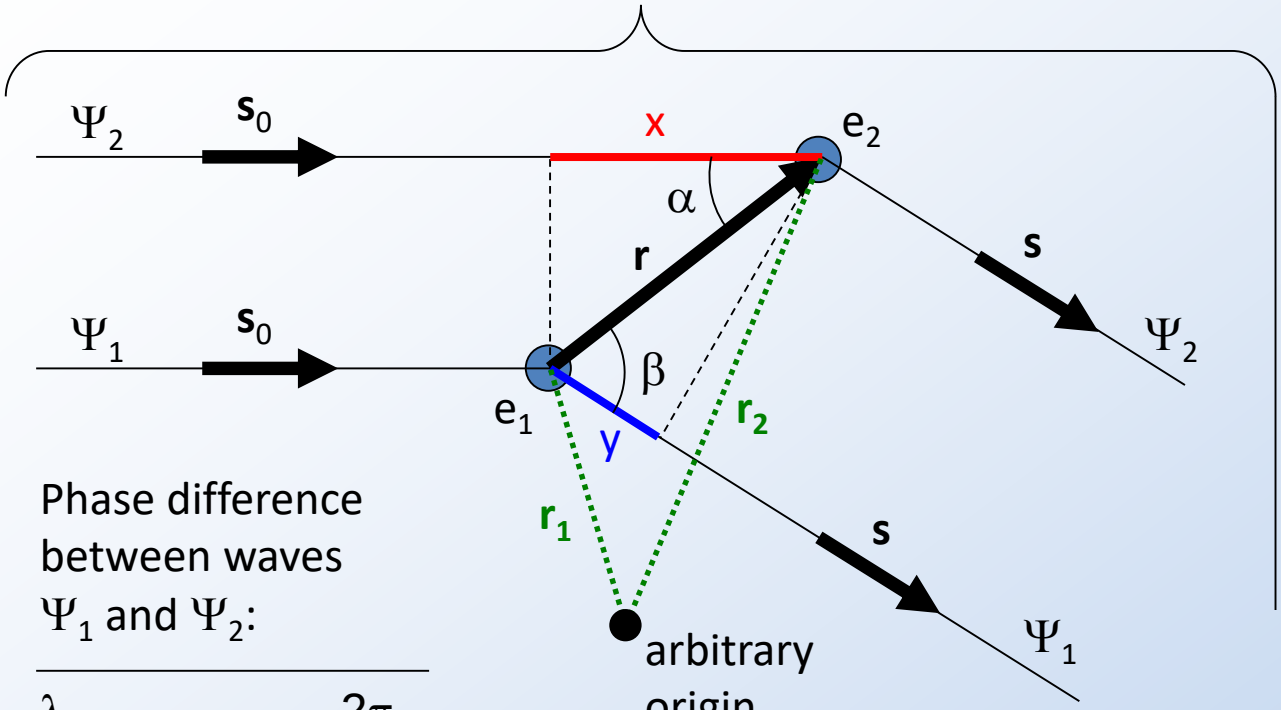
Note: The justification holds for any scatterers and/or geometry.

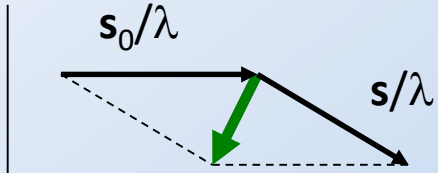
The difference between paths lenghts of 2 waves (Ψ_1,Ψ_2) and **arbitrary** 2 scatterers (e_1,e_2) is $\Delta p = \mathbf{r}(\mathbf{s} - \mathbf{s_0})$ = the additional distance that Ψ_1 has to travel in comparison with Ψ_2.

Diffraction :: Level 3 :: Basic formula: $\mathbf{A(q)} = \int \rho(\mathbf{r}) \exp[i\mathbf{qr}]\, d\mathbf{r}$

Step 3: Scattering by two centers - phase difference.

2 waves (Ψ_1,Ψ_2), which are scattered by 2 centers (e_1,e_2):





Definition of scattering vector **S**.
(λ = wavelength
(**s**,**s**$_0$ = unit vectors
(**S** = (**s**-**s**$_0$)/λ
(**q** = 2π**S**

Phase difference between waves Ψ_1 and Ψ_2:

λ 2π
Δp ..... $\Delta\Phi$

$\Delta\Phi = 2\pi/\lambda * \Delta p = 2\pi/\lambda * \mathbf{r}(\mathbf{s}-\mathbf{s}_0) = 2\pi\mathbf{r} * (\mathbf{s}-\mathbf{s}_0)/\lambda = 2\pi\mathbf{r} * \mathbf{S} = \mathbf{q}*\mathbf{r}$

$\Delta\Phi = \mathbf{qr}$ ..phase of the second wave Ψ_2 with respect to the first wave Ψ_1

Δp from previous page

Final result (including generalization):

$\Phi_1 = \mathbf{qr}_1$ ..phase of the fist wave Ψ_1 with respect to [arbitrary origin]
$\Phi_2 = \mathbf{qr}_2$ ..phase of the fist wave Ψ_2 with respect to [arbitrary origin]

The justification of the phase shifts with respect to [arbitrary origin] is the same.

Diffraction :: Level 3 :: Basic formula: $\mathbf{A}(\mathbf{q}) = \int \rho(\mathbf{r}) \exp[i\mathbf{qr}]\, d\mathbf{r}$

Step 4: Generalization for scattering by N scattering centers.

Summation of two exponential waves (the same amplitudes):

$$\Psi_3 = \Psi_1 + \Psi_2 = [A\exp(i\phi_1) + A\exp(i\phi_2)] \times \exp(iX)$$

Summation of two exponential waves (different amplitudes):

$$\Psi_3 = \Psi_1 + \Psi_2 = [A_1\exp(i\phi_1) + A_2\exp(i\phi_2)] \times \exp(iX)$$

Derivation from EM0, Appendix B.

Re-writing for two scattered waves ($\Phi_i = \mathbf{qr}_i$):

$$\Psi_3 = \Psi_1 + \Psi_2 = [A_1\exp(i\mathbf{qr}_1) + A_2\exp(i\mathbf{qr}_2)] \times \exp(iX)$$

Combine previous eq. and previous slide.

Complex amplitude of two scattered waves:

$$\mathbf{A}_3 = \mathbf{A}_1 + \mathbf{A}_2 = [A_1\exp(i\mathbf{qr}_1) + A_2\exp(i\mathbf{qr}_2)]$$

Final formula for amplitude of 2 scattered waves.

Generalization for N waves with different amplitudes:

$$\Psi = \sum_{j=1}^{N} \Psi_j = \left[\sum_{j=1}^{N} A_j \exp(i\mathbf{qr}_j)\right] \times \exp(iX)$$

$$\mathbf{A} = \sum_{j=1}^{N} \mathbf{A}_j = \sum_{j=1}^{N} A_j \exp(i\mathbf{qr}_j)$$

Generalization: amplitude of N scattered waves.

Diffraction :: Level 3 :: Basic formula: $\mathbf{A}(\mathbf{q}) = \int \rho(\mathbf{r}) \exp[i\mathbf{q}\mathbf{r}]\, d\mathbf{r}$

Step 5: Generalization for scattering by any object (*within RDG approximation!*)

Amplitude of the scatterred wave from a single volume element $d\mathbf{r}$ ($\rho(\mathbf{r})$ = density of scatterers):

$$A = \rho(\mathbf{r}) \cdot d\mathbf{r} \qquad (1)$$

Step 1

Amplitude of scattering from two volume elements (A_i are the amplitudes given by Eq. 1, the final amplitude is a complex number because of the phase shifts):

$$\mathbf{A}(\mathbf{q}) = \mathbf{A}_1(\mathbf{q}) + \mathbf{A}_2(\mathbf{q}) = A_1 \exp(i\mathbf{q}\mathbf{r}_1) + A_2 \exp(i\mathbf{q}\mathbf{r}_2) \qquad (2)$$

Step 4

Direct generalization of Eq. (2) for the scattering by N volume elements:

$$\mathbf{A}(\mathbf{q}) = \sum_{j=1}^{N} \mathbf{A}_j(\mathbf{q}) = \sum_{j=1}^{N} A_j \exp(i\mathbf{q}\mathbf{r}_j) \qquad (3)$$

Gene-ralized.

Direct generalization of Eq. (3) for the scattering of a continuous object with volume V (note: summation $\rightarrow$ integration; $A_i \rightarrow \rho(\mathbf{r})d\mathbf{r}$ according to Eq. (1)):

$$\mathbf{A}(\mathbf{q}) = \int_V \rho(\mathbf{r}) \exp(i\mathbf{q}\mathbf{r})\, d\mathbf{r} \qquad (4)$$

Final formula

Q.E.D. → Final intensity of diffraction is calculated according to: $I(\mathbf{q}) \propto |\mathbf{A}(\mathbf{q})|^2$

Example 1 :: Basic formula: $\mathbf{A(q)=\int\rho(r)\exp[iqr]dr}$ → in SAXS

SAXS = Small-Angle X-ray Scattering (objects: 1000-10A; diffraction angles: <2deg for CuKα)

Dilute solution ⇒ spheres scatter independently ⇒ particulate scattering.

[1] Scattering of sphere with radius R can be derived from key formula:

$$A(\mathbf{q}) = \int_V \rho(\mathbf{r}) \exp[i\mathbf{qr}]\, d\mathbf{r}$$

[2] The formula is re-written in polar coordinates:

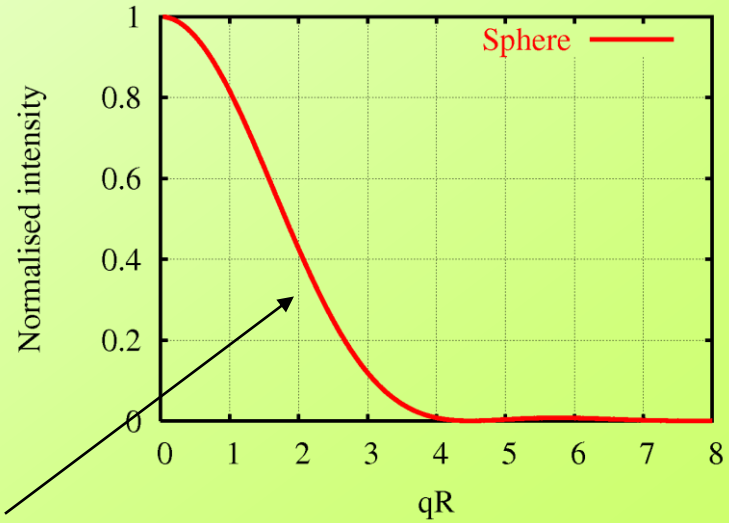
$$A(\mathbf{q}) = \int_{r=0}^{+\infty} \int_{\Theta=0}^{\pi} \int_{\Phi=0}^{2\pi} \rho(r,\Theta,\Phi) \exp[iqr\cos\Theta]\, r^2 \sin\Theta\, dr\, d\Theta\, d\Phi$$

[3] The rest is only mathematics; results:

$$A(q) = \rho_0 V \frac{3(\sin(qR) - qR\cos(qR))}{(qR)^3}$$

$$I(q) = |A(q)|^2 = \rho_0^2 V^2 \frac{9(\sin(qR) - qR\cos(qR))^2}{(qR)^6}$$

Complete derivation.



Conclusion: If the scattering curve has this shape, scattering objects are homogeneous spheres.

Example 2 :: Basic formula: $\mathbf{A}(\mathbf{q})=\int\rho(\mathbf{r})\exp[i\mathbf{qr}]d\mathbf{r}$ → in WAXS

WAXS = Wide-Angle X-ray Scattering (objects: crystals; diffraction angles 5-90deg for CuKα)

General diffraction theory

! RDG approximation

⇒ no multiple scattering

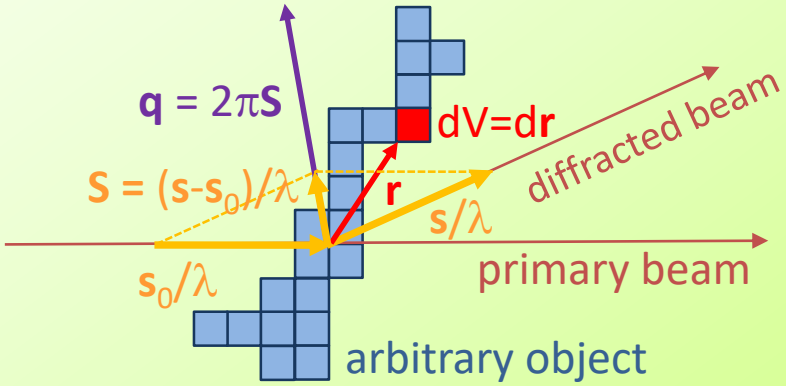
⇒ scatterers = arbitrary objects

$$I(\mathbf{q}) \propto |\mathbf{A}(\mathbf{q})|^2$$

$$\mathbf{A}(\mathbf{q}) = \int_V \rho(\mathbf{r}) \exp(i\mathbf{qr})\, \mathrm{d}\mathbf{r}$$

We sum waves of all volume elements d**V** of an object, considering phase shifts.

(ρ(**r**)d**r** = contribution of 1 volume element)



Note – volume element: dV = dx·dy·dz = d**r**

Diffraction by crystals

Kinematic diffraction theory = KDT

⇒ no multiple scattering

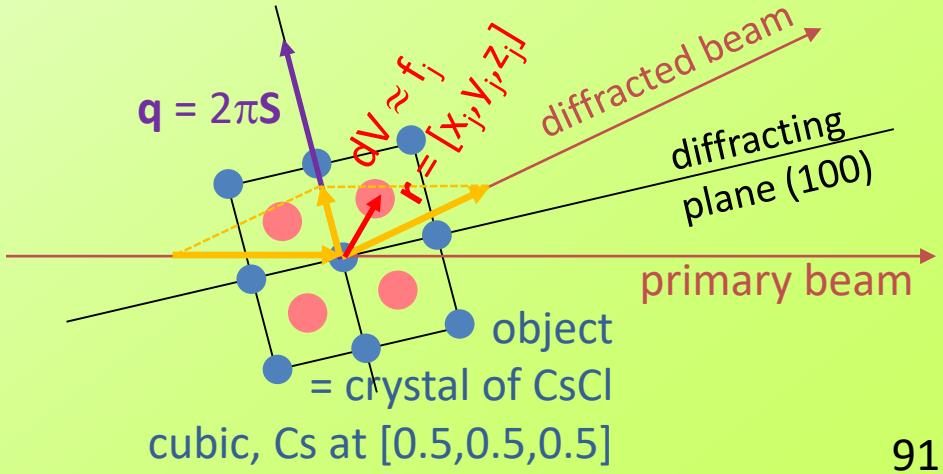
⇒ scatterers = atoms

$$I(hkl) \propto |F(hkl)|^2$$

$$F(hkl) = \sum_{j=1}^{N} f_j \exp[2\pi i(hx_j + ky_j + lz_j)]$$

We sum waves from all atoms in the unit cell, considering their phase shifts ~ positions.

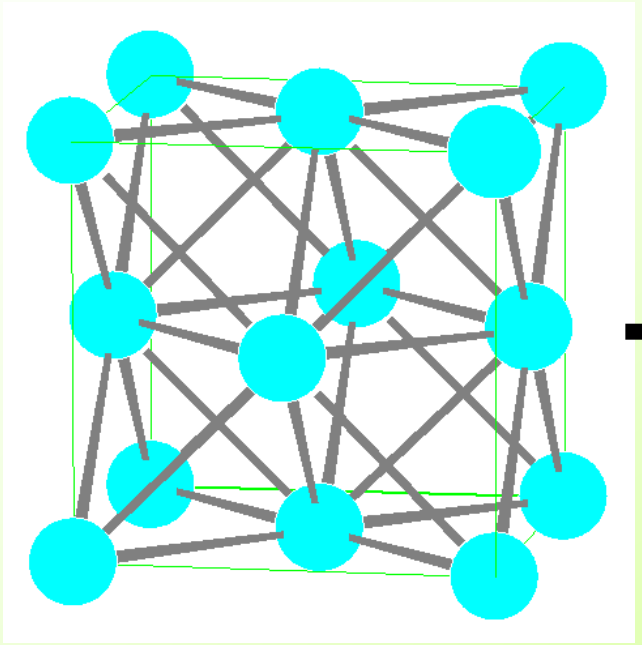
(f_j = atom scattering factor = contribution of an atom)



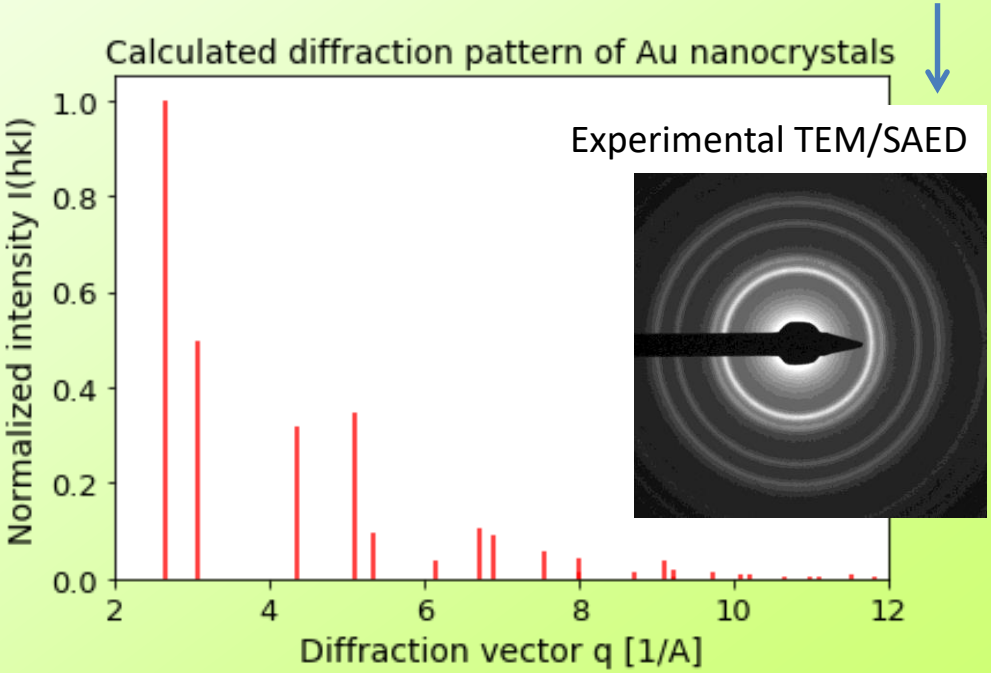
Example 3 :: Calculation of diffraction pattern

Calculation of TEM/SAED powder pattern for Au nanocrystals in Python/Jupyter.

(1) Input for our calculation:
Known crystal structure of Au
cubic structure, fcc, a = 4.08 Å



(3) Output of our calculation:
Calculated PXRD diffractogram of polycrystalline Au
(and its comparison with experimental TEM/SAED)



(2) Program and formulas for our calculation:

- Program: Jupyter/Python (without any specific modules)
- Basic formulas: only those shown in the introductory lecture of this course
- Supplementary data: atomic scattering factors + corrections for LP, vibrations, multiplicity

Link to calculation in Jupyter

Note1 :: Our *ab initio* PXRD calculation in Python

We should know that the calculation based on our own code has serious limitations...

- **We have learnt how to calculate powder diffraction patterns *ab initio*** (success!), and our calculation was quite precise, **BUT...**
- Limitation #1: We used a lot of simplifications/approximations:
  - kinematic diffraction theory, atom scattering factors from XRD, T(isotropic)...
  - BUT the result was good (small nanocrystals: dynamic effects weak, approximations Ok)
- Limitation #2: The atom positions were hardcoded, symmetry not taken into account...
  - our code is rather inflexible, more demo than real program
  - for more complex structures this would be very impractical
- Limitation #3: We have re-invented the wheel, because there are professional, better, more effective and user-friendly programs for this task, such as:
  - PowderCell, Vesta, and others → GoogleSearch: simulate powder diffraction pattern
  - Recently also Python solutions to do this → see next slide
- Limitation #4: We should not forget that this **was not the structure analysis**:
  - our calculation: known structure → diffraction pattern (this is straightforward)
  - real life: experimental diffraction pattern → solve the structure (this is more difficult)
- **CONCLUSIONS:**
  Comparison of experimental and calculated diffraction patterns = **structure identification.**
  Determination of unknown structure from its diffraction pattern = **structure analysis**.
  We did structure identification – for structure analysis see textbooks of crystallography.

Note2 :: Better, universal PXRD calculation in Python

Calculation based on standard Python modules = universal + just 10 lines of code!

Spyder (Python 3.10)

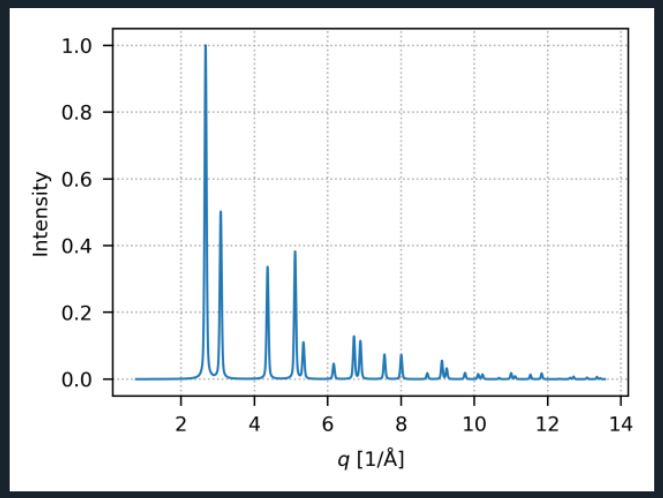
File Edit Search Source Run Debug Consoles Projects Tools View Help

M:\MIREK.PRE\0_EM\2_TEM\DATA\SPYDER.PXRD

M:\MIREK.PRE\0_EM\2_TEM\DATA\SPYDER.PXRD\01_pxrd_calc.py

01_pxrd_calc.py

```
''' Calculate theoretical PXRD diffractogram from CIF file '''

# CALCULATION is performed by means of EDIFF/PXRD module
#  1. Get CIF file for Au and save it to current directory
#  2. Adjust sections [0,1,2], run this script and see outputs
#  3. Help: place cursor or any function and type Ctrl+I
# CIF files can be obtained from various databases or publications:
#  http://www.crystallography.net/cod/
#  https://en.wikipedia.org/wiki/Crystallographic_database

import ediff.pxrd

# [0] Crystal structure
# is defined by means of CIF = Crystallographic Information File.
CIF_FILE = r'./au_9008463.cif'

# [1] Crystal, experimental and plot parameters
# are defined as objects XTAL, EPAR, PPAR and CALC, respectively.
XTAL = ediff.pxrd.Crystal(structure=CIF_FILE, temp_factors=0.8)
EPAR = ediff.pxrd.Experiment(wavelength=0.71, two_theta_range=(5,100))
PPAR = ediff.pxrd.PlotParameters(x_axis='q')

# [2] PXRDcalculation object
# calculates PXRD during initialization to be used in the next steps.
CALC = ediff.pxrd.PXRDcalculation(XTAL, EPAR, PPAR, peak_profile_sigma=0.2)

# [3] Show/save CALCulation results,
# the default outputs can be further processed with Python or other SW.
CALC.print_diffractions()
CALC.save_diffractions('pxrd_au.py.diff')
CALC.plot_diffractogram('pxrd_au.py.png')
CALC.save_diffractogram('pxrd_au.py.txt')
```



Help Variable Explorer Plots Files

Console 1/A

18	77.762	6	4	0	0.566	1.768	11.110	0.847
19	81.295	6	4	2	0.545	1.835	11.529	1.352
20	83.922	7	3	1	0.531	1.883	11.834	1.733
21	88.274	8	0	0	0.510	1.962	12.325	0.113
22	90.879	7	3	3	0.498	2.007	12.611	0.397
23	91.748	6	4	4	0.495	2.022	12.705	0.760
24	95.228	6	6	0	0.481	2.081	13.073	0.485
25	97.850	7	5	1	0.471	2.124	13.342	0.674
26	98.727	6	6	2	0.468	2.138	13.431	0.279

In [8]:

IPython Console History

custom () Completions: custom LSP: Python Line 1, Col 1 ASCII CRLF RW Mem 86%

Spyder, script using EDIFF (https://pypi.org/project/ediff), which is build on pymatgen.

Appendix

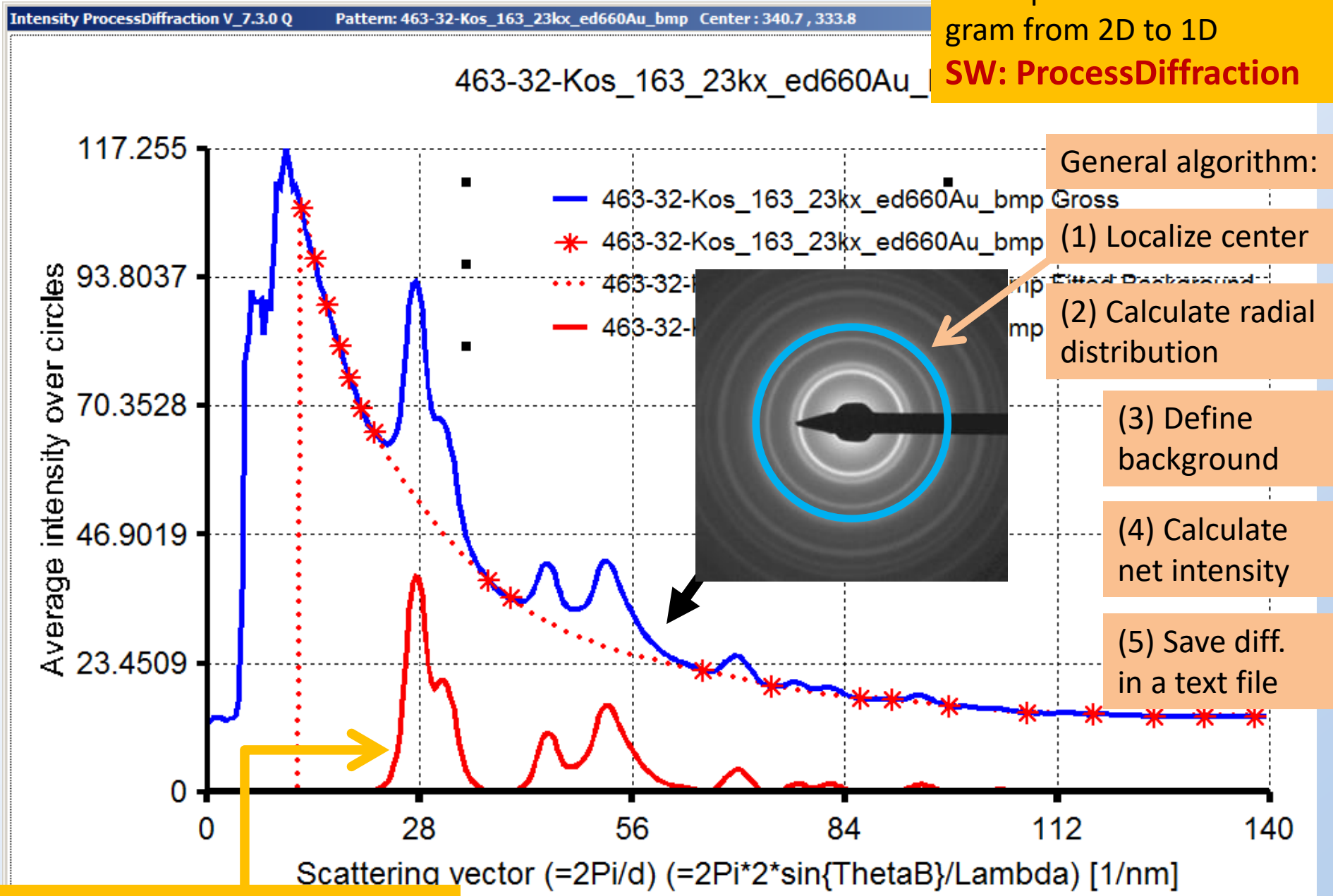
Powder TEM/SAED diffractograms – standard processing

- This part is optional (not at exams)
- Previous appendix = theory + sample calculation in Python/Jupyter
- This appendix = real life = how it can be done with standard software

TEM/SAED :: Standard processing (step 1)

In the first step, we convert the experimental diffracto-gram from 2D to 1D

SW: ProcessDiffraction



General algorithm:

(1) Localize center

(2) Calculate radial distribution

(3) Define background

(4) Calculate net intensity

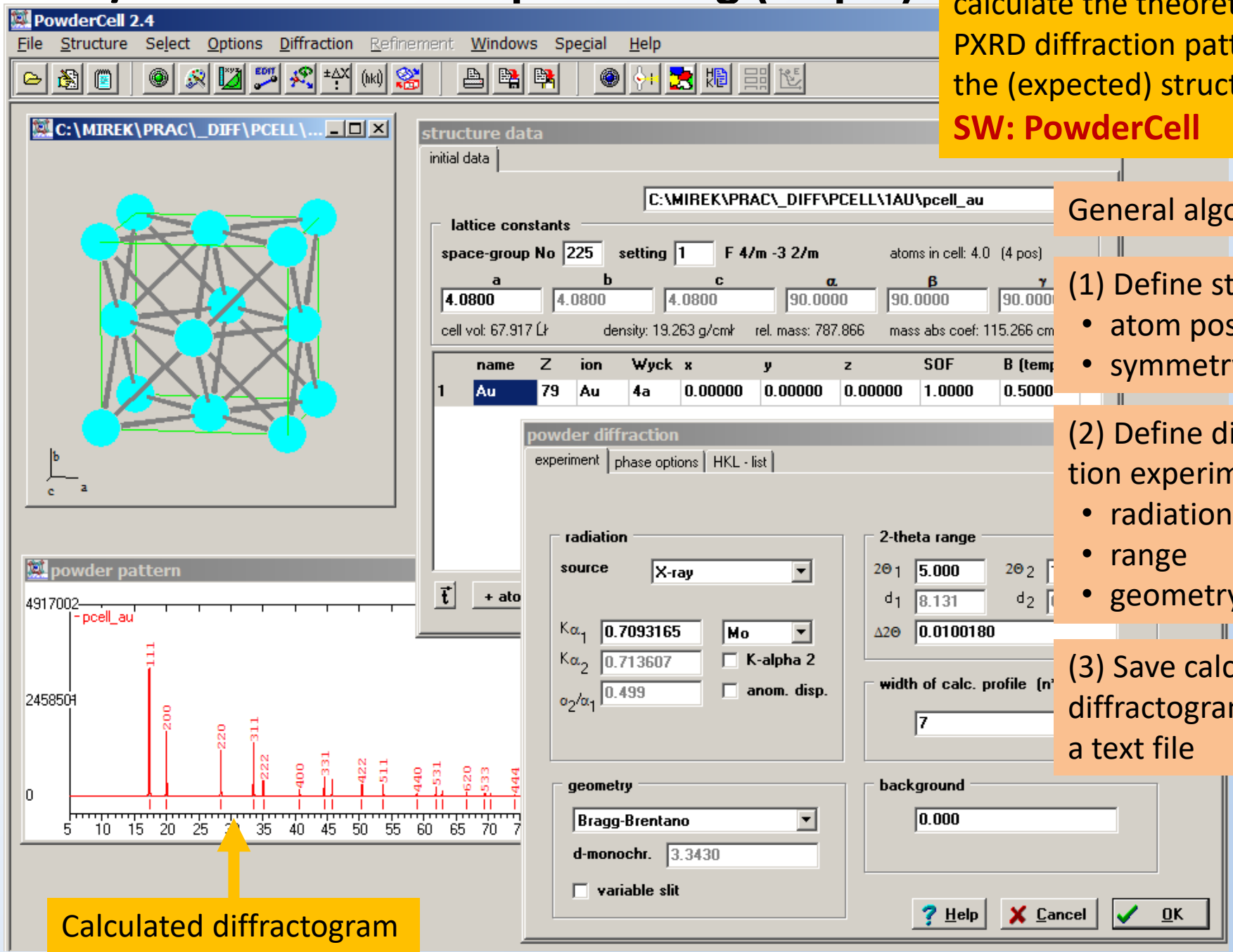
(5) Save diff. in a text file

Final 1D diffraction pattern

TEM/SAED :: Standard processing (step 2)

In the second step, we calculate the theoretical PXRD diffraction pattern of the (expected) structure

SW: PowderCell



Calculated diffractogram

General algorithm:

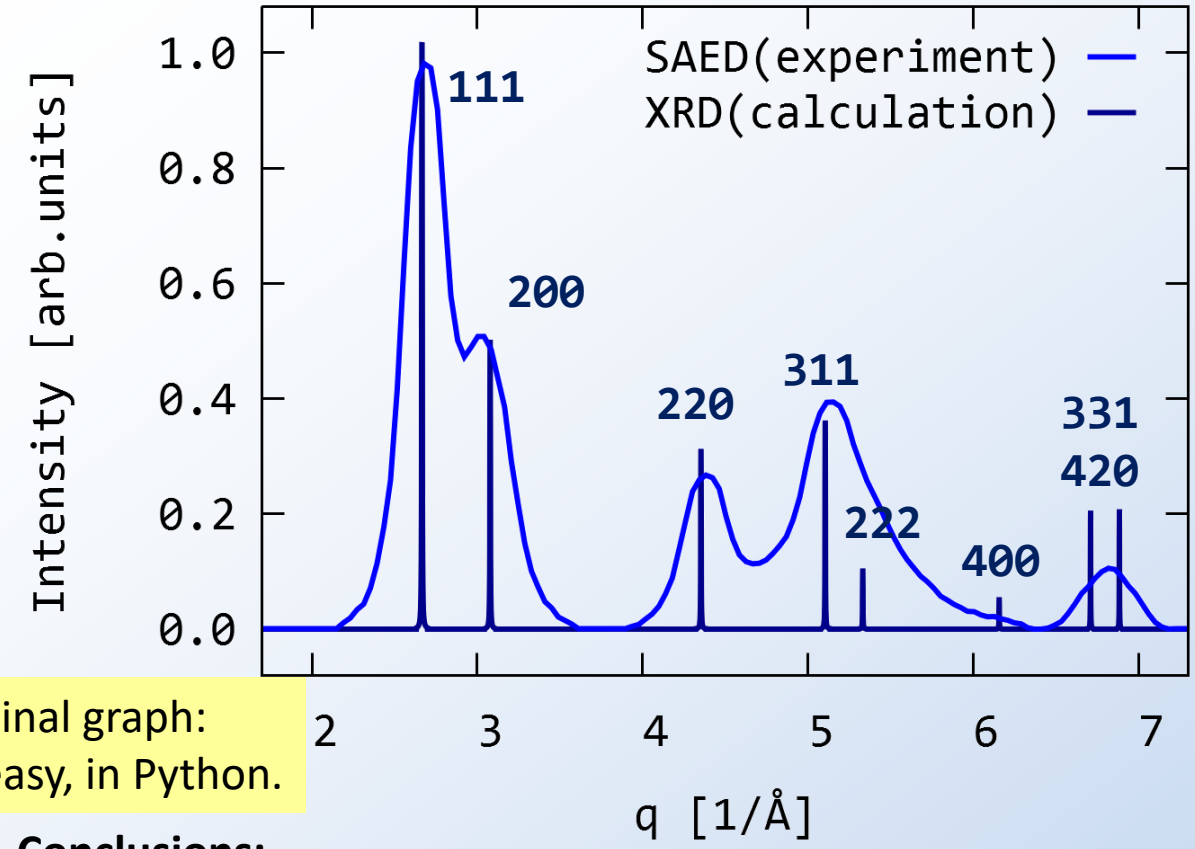
(1) Define structure
- atom positions
- symmetry

(2) Define diffraction experiment:
- radiation
- range
- geometry

(3) Save calculated diffractogram in a text file

TEM/SAED :: Standard processing (step 3)

In the third step, we compare the experimental and calculated diffraction pattern; the structure is identified if they match.



Final graph: easy, in Python.

Conclusions:

1) We should re-emphasize that this is not a structure analysis.
2) In fact we do identification of the structures by fingerprint method – each substance has more-or-less unique unit cell.
3) On the other hand, we should not that this type of simple analyses is quite frequent in TEM – see lecture about IMA.
4) Finally, this could be a basis for more complex TEM/ED work.

Notes:

- We can index the diffractions, because PowderCell does the indexation automatically
- Even if not, we could do it ourselves, as we can calculate distances (Ex.1) and recalculate diffraction vectors (Ex.2).
- This diffractogram re-confirms that the intensities from XRD and SAED are quite comparable, if the investigated crystals are small.

Appendix

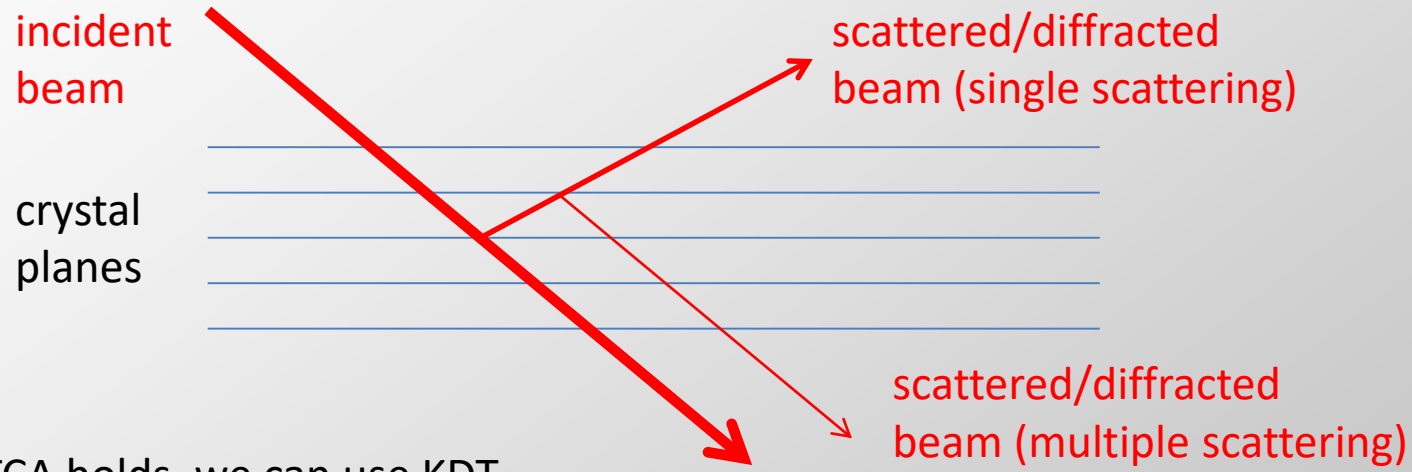
Kinematic vs. dynamic diffraction theory

❖ This part is optional (not at exams)

Supplement :: Kinematic vs. dynamic theory of diffraction

- The formulas derived here were based on Raylegh-Debye-Gans approximation (RDG)
  (RDG comes from general theory of diffraction
  (Assumption: negligible multiple scattering in the diffracting object

- In crystallography, the equivalent to RDG is thin crystal approximation = TCA
  (Assumption: negligible multiple scattering in the crystal



- If TCA holds, we can use KDT.
  If multiple scattering occurs, we must use Dynamic Diffraction Theory (DDT)

- XRD: multiple scattering mostly negligible, KDT is a good approximation.
  ED: multiple scattering usually not negligible $\Rightarrow$ DDT gives more precise results.

- BUT here we use KDT for electrons – why?
  $\Rightarrow$ kinematic theory it is easier for understanding/calculations than dynamic theory
  $\Rightarrow$ for nanocrystals (used as fillers for polymers) KDT holds quite well – proof: Ex. 6/6