CEITEC

Central European Institute of Technology
BRNO | CZECH REPUBLIC

MUNI

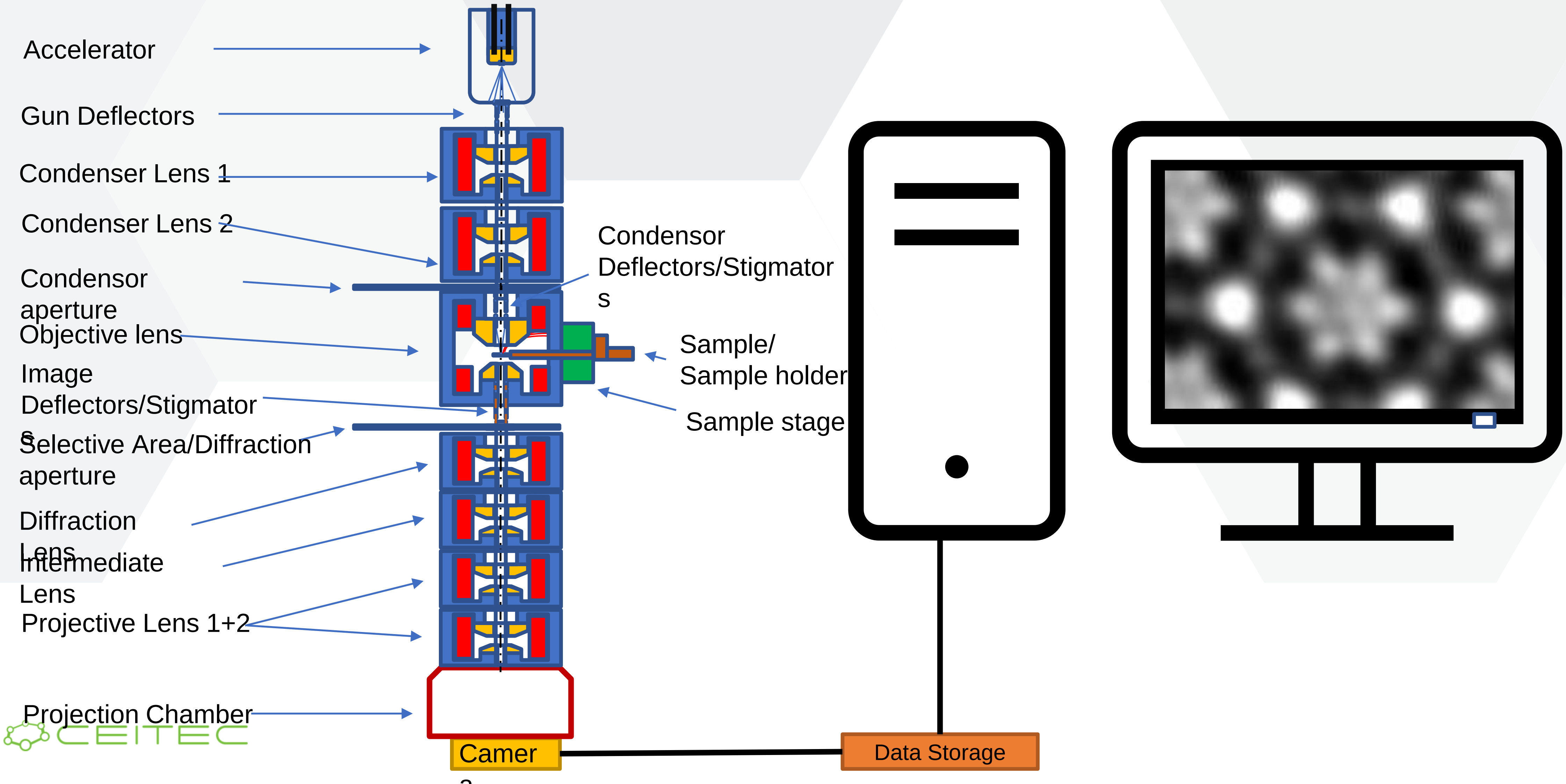
TEM imaging

Fall 2023

Ondrej L. Shanel, Ph.D.

Transmision electron microscopy - TEM

- TEM mode – Image of an illuminated sample is magnified onto a camera
- STEM Mode - Focused Beam scanning over the sample → processed signal creates an image



Accelerator

Gun Deflectors

Condenser Lens 1

Condenser Lens 2

Condensor aperture

Objective lens

Image Deflectors/Stigmators

Selective Area/Diffraction aperture

Diffraction Lens

Intermediate Lens

Projective Lens 1+2

Projection Chamber

Condensor Deflectors/Stigmators

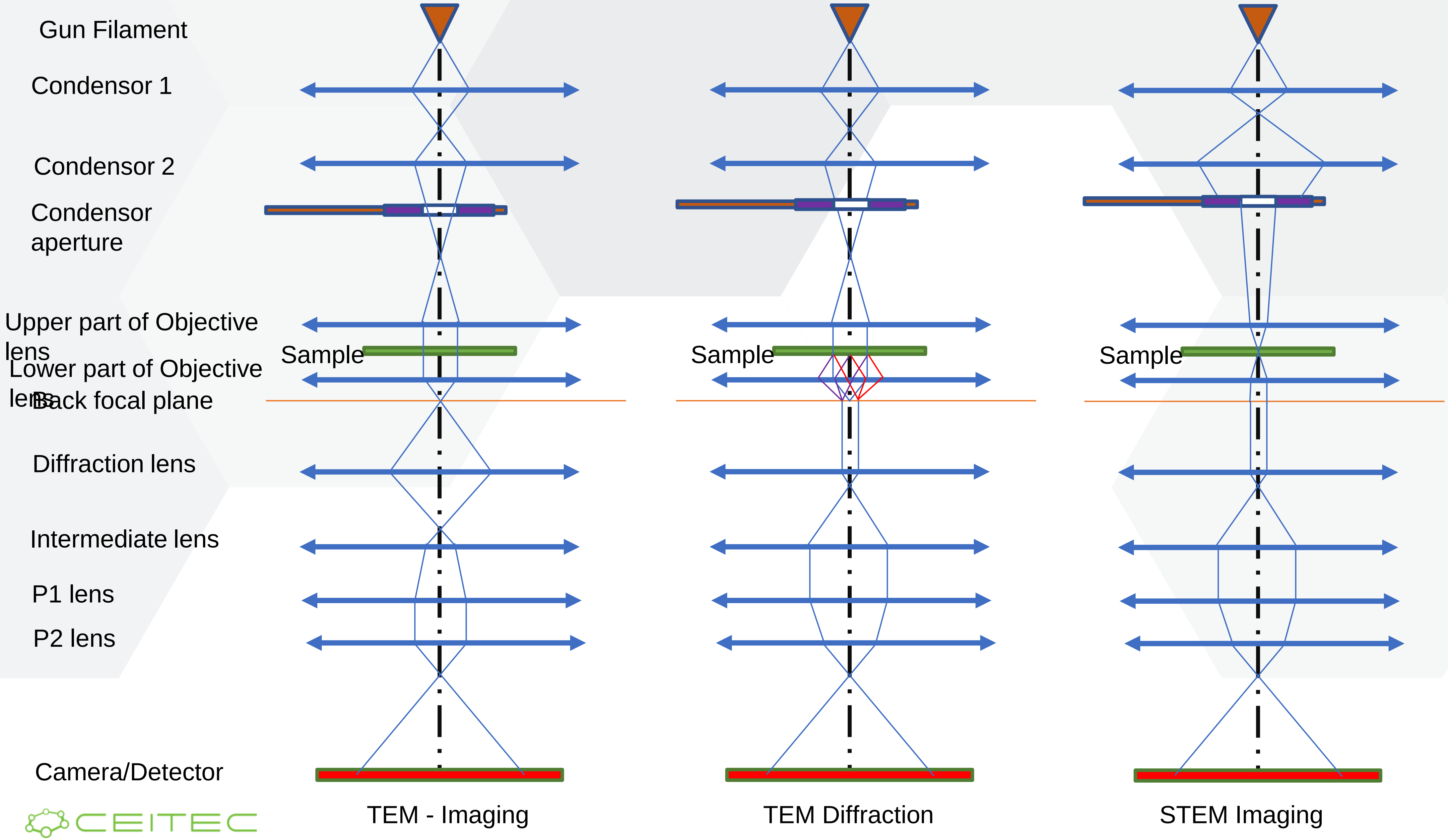
Sample/ Sample holder

Sample stage

Camera

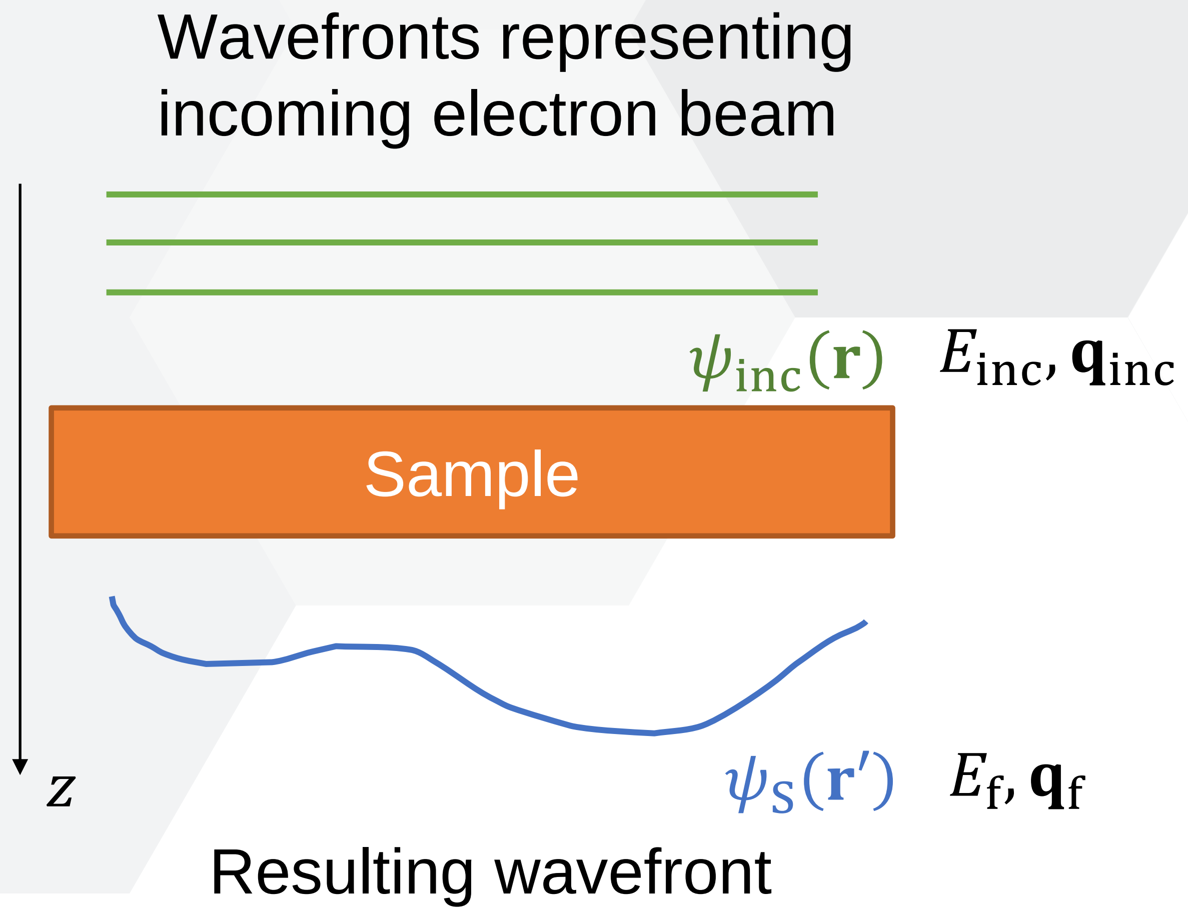
Data Storage

Transmision electron microscopy – Optical modes



CEITEC

Transmitted primary electrons



Wavefronts representing incoming electron beam

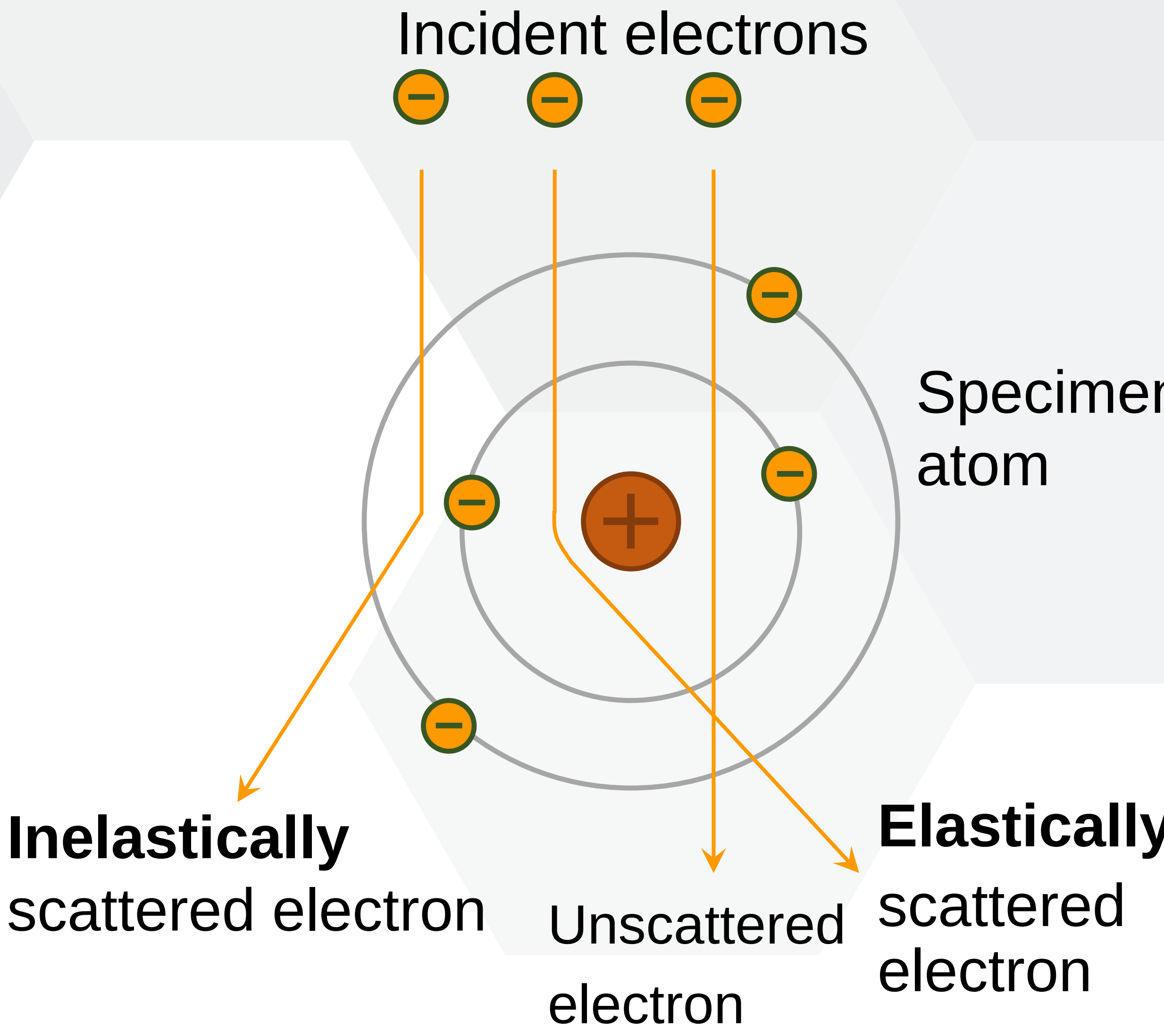
$\psi_{\text{inc}}(\mathbf{r})$ $E_{\text{inc}}, \mathbf{q}_{\text{inc}}$

Sample

z

$\psi_{\text{S}}(\mathbf{r}')$ $E_{\text{f}}, \mathbf{q}_{\text{f}}$

Resulting wavefront



Incident electrons

Specimen atom

Inelastically scattered electron

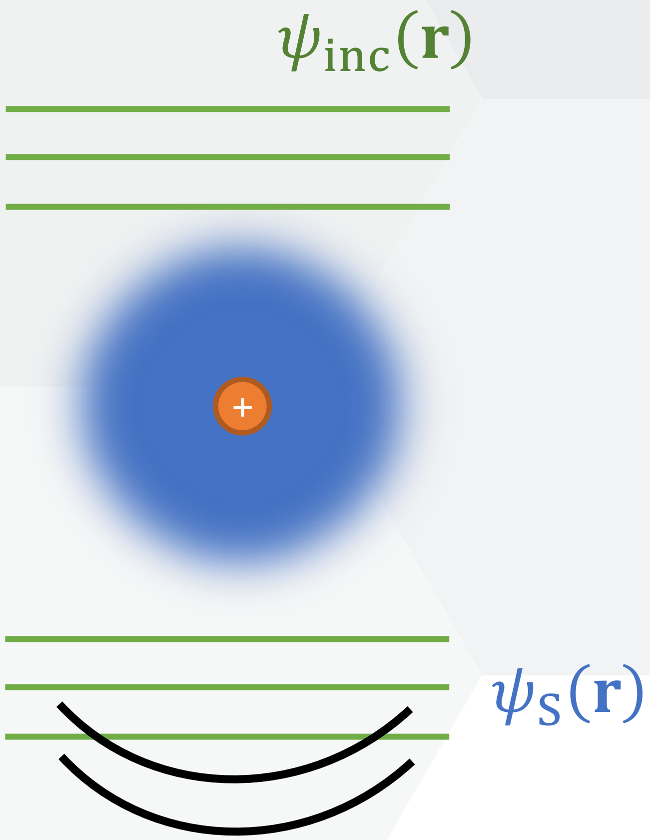
Unscattered electron

Elastically scattered electron

Elastic scattering on a signle atom

Final electron wave function after the interaction with an atom:

$$\boxed{\psi_S(\mathbf{r}) = \psi_{\mathrm{inc}}(\mathbf{r}) + f_e(q)\,\frac{\exp(\mathrm{i}\,\mathbf{q}\cdot\mathbf{r})}{r}}$$



Scattering cross section:

$$\sigma = \frac{me\lambda}{2\pi\hbar^2}$$

$$f_e(q) = \frac{2\pi\mathrm{i}}{\lambda}\int_0^\infty J_0(qr)\left\{1 - \exp\left[\mathrm{i}\sigma\int \Phi(\mathbf{r})\mathrm{d}z\right]\right\} r\,\mathrm{d}r$$

For acquiring an image, we propagate $\psi_S(\mathbf{r})$ through an electron-optical system:

$$I_{\mathrm{detector}} \propto \left|\mathrm{FT}^{-1}\{\psi_S(\mathbf{Q})\,\mathrm{TF}(\mathbf{Q})\}\right|^2$$

Elastic scattering on a signle atom

Final electron wave function after the interaction with an atom:

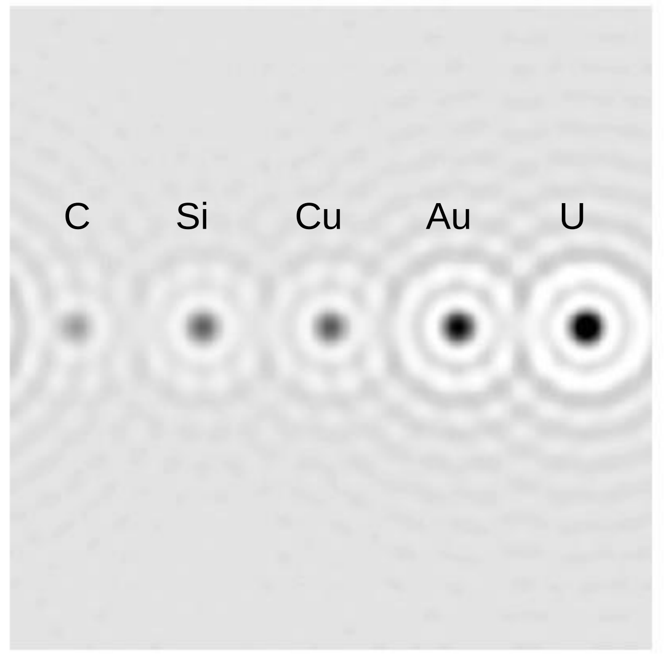
$$\boxed{\psi_S(\mathbf{r}) = \psi_{\mathrm{inc}}(\mathbf{r}) + f_e(q)\,\frac{\exp(\mathrm{i}\,\mathbf{q}\cdot\mathbf{r})}{r}}$$

Scattering cross section:

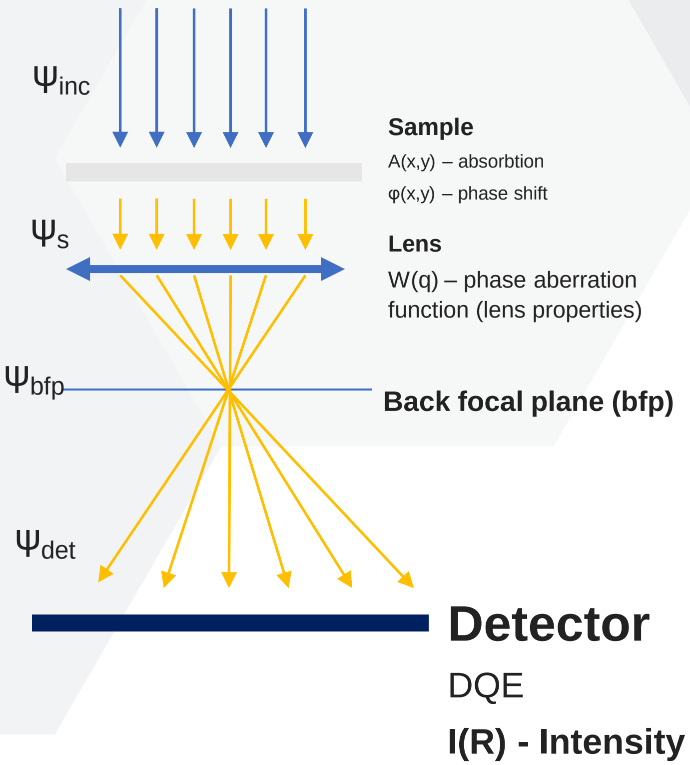
$$\sigma = \frac{me\lambda}{2\pi\hbar^2}$$

$$f_e(q) = \frac{2\pi\mathrm{i}}{\lambda}\int_0^\infty J_0(qr)\left\{1-\exp\left[\mathrm{i}\sigma\int\Phi(\mathbf{r})\mathrm{d}z\right]\right\}r\,\mathrm{d}r$$

Calculation for $\psi_{\mathrm{inc}} \propto \exp(\mathrm{i}\,2\pi z/\lambda)$
200 keV electrons
(Kirkland; Advanced computing in EM)



Transfer of Image through the optical system



Sample
A(x,y) – absorbtion
φ(x,y) – phase shift

Lens
W(q) – phase aberration function (lens properties)

Back focal plane (bfp)

Detector

DQE

I(R) - Intensity

Incoming Wave $\psi_{inc}(r)$

$$\psi_S(\mathbf{r}) = \psi_{\mathrm{inc}}(\mathbf{r}) + f_e(q)\frac{\exp(\mathrm{i}\,\mathbf{q}\cdot\mathbf{r})}{r}$$

Weak Phase Approximation

Sample Amplitude Influence $A(r)$

Sample Phase Influence $\varphi(r) = f_e(q)$

Exit Wave $\psi_s(r) = A(r)\psi_{inc}(r)e^{i\varphi(r)}$

when $A(r) \ll 1$ *and* $\varphi(r) \ll 1, \varepsilon(r) = lnA(r)$
and assumption $\psi_{inc}(r) = 1\ (parallel\ illumination)$

Exit Wave $\psi_s(r) = \psi_{inc}(r)[1 + \varepsilon(r) + i\varphi(r)]$

$$\psi_{bfp}\,(q) = FT\{\psi_s(r)\}$$

$$\psi_{bfp}\,(q) = \delta(q) + E(q) + i\Phi(q)$$

Aberrations addition $W(q) = \frac{\pi}{2}(C_{3,0}q^4\lambda^3 + C_{1,0}q^2\ \lambda\ )$

$C_{3,0}$ - spherical aberration
$C_{1,0}$ - defocus

$$\psi_{bfp,ab}\,(q) = \delta(q) + E(q)e^{-iW(q)} + i\Phi(q)e^{-iW(q)}$$

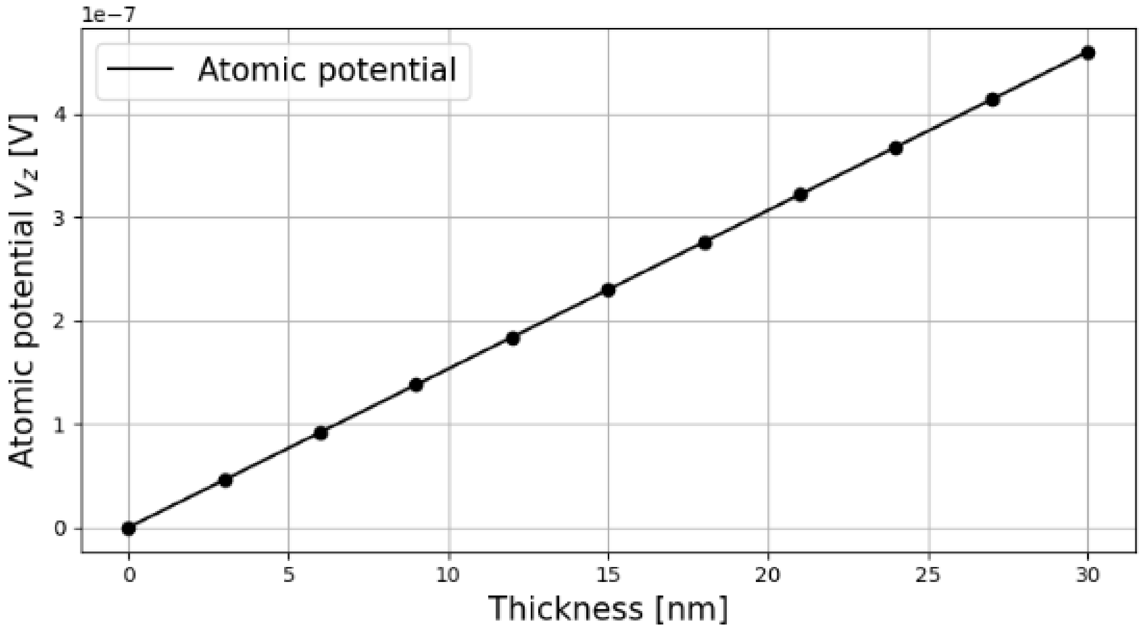
Optical Intensity at Image Plane

$$I(R) = |\psi_m(Rde_t)|^2 = FT\psi_{bfp,ab}\overline{FT\psi_{bfp,ab}}$$

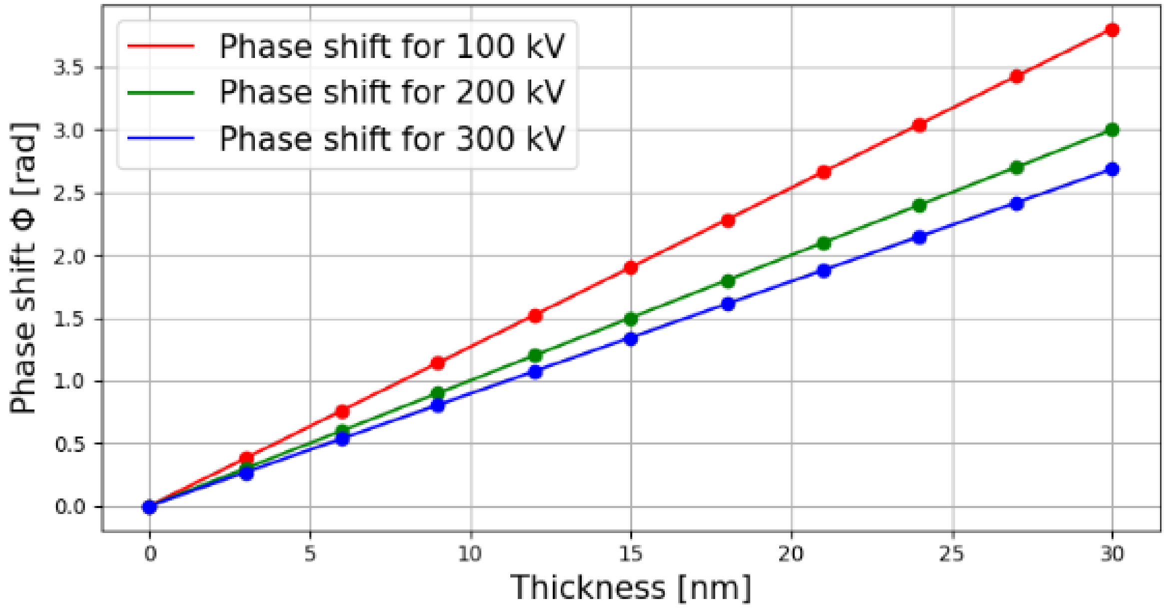
Optical Intensity at Image Plane with Dumping Envelope (Systém imperefections)

$$I(R) = |\psi_m(Rde_t)|^2 = E_t * E_sE_dE_u\{1 - 2\varphi(Q)\sin\big(W(Q)\big) + 2\varepsilon(Q)\cos(W(Q)\}$$

Phase shift – Carbon sample



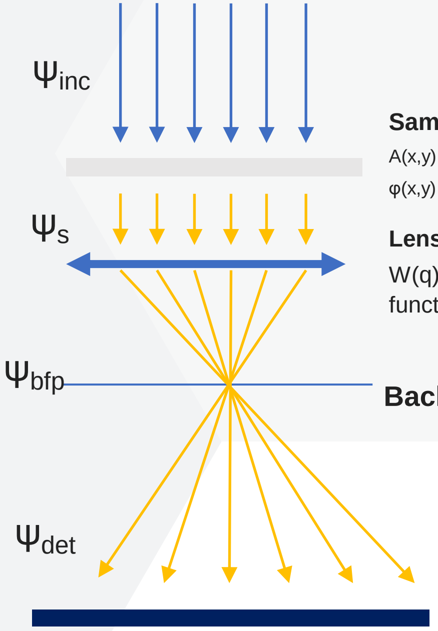
(a) Atomic potential of amorphous carbon.



(b) Phase shift of amorphous carbon for different accelerating voltages.

Michal Brzica bachelor thesis – derived from RICOLLEAU, C., et al. Random vs realistic amorphous carbon models for high resolution microscopy and electron diffraction. Journal of Applied Physics, 2013, 114.21: 213504. ISSN 0021-8979. Available from DOI: 10.1063/1.4831669.

Contrast Transfer Function



Sample

A(x,y) – absorbtion

φ(x,y) – phase shift

Lens

W(q) – phase aberration function (lens properties)

Back focal plane (bfp)

Detector

DQE

I(R) - Intensity

$$I(R) = |\psi_m(Rde_t)|^2 = FT\psi_{bfp,ab}\overline{FT\psi_{bfp,ab}}$$

$$I(R) = |\psi_m(Rde_t)|^2 = \{1 - 2\varphi(Q)\sin\big(W(Q)\big) + 2\varepsilon(Q)\cos(W(Q)\}$$

Contrast Transfer Function (CTF)

- Describing optical property of TEM

$$\mathrm{CTF}(\vec{q}\,')=E_\mathrm{t}(q')E_\mathrm{s}(\vec{q}\,')E_\mathrm{d}(\vec{q}\,')E_\mathrm{u}(\vec{q}\,')\cdot\mathrm{Intenzita}(\vec{q}\,')\in\langle -1;1\rangle$$

where

$E_\mathrm{t}(q')$ - *temporal coherency* $E_\mathrm{t}(\vec{q}\,') = e^{-(\pi\lambda q^2 H/4)^2/ln2}, H(\Delta E, \Delta U, \Delta I)$

$E_\mathrm{s}(\vec{q}\,')$ - *spatial coherency* $E_\mathrm{s}(\vec{q}\,') = e^{-\pi^2(C_{3,0}\lambda^2 q'^3 - C_{1,0}q')^2\alpha_i^2/ln2}$

$E_\mathrm{d}(\vec{q}\,')$ - drift impact

$E_\mathrm{u}(\vec{q}\,')$ - *vibration dumping*

Observed Intensity on PC

CTF is not seen directly on our PC!

$$\mathrm{Intensity}_{\mathrm{ob}}(\vec{r'}) = \mathrm{I}_{\mathrm{rn}} + \mathrm{I}_{\mathrm{dc}} + \mathrm{CF} \cdot \mathrm{IFT}\left[\mathrm{FT}\left[P_{\mathrm{oiss}}\left(\Phi_{\mathrm{e}} \cdot \mathrm{IFT}^{-1}\left[\mathrm{CTF}_{\mathrm{optical}}(\vec{q'})\sqrt{\mathrm{DQE}(\vec{q'})}\right]\right)\right] \cdot \mathrm{NTF}(\vec{q'})\right]$$

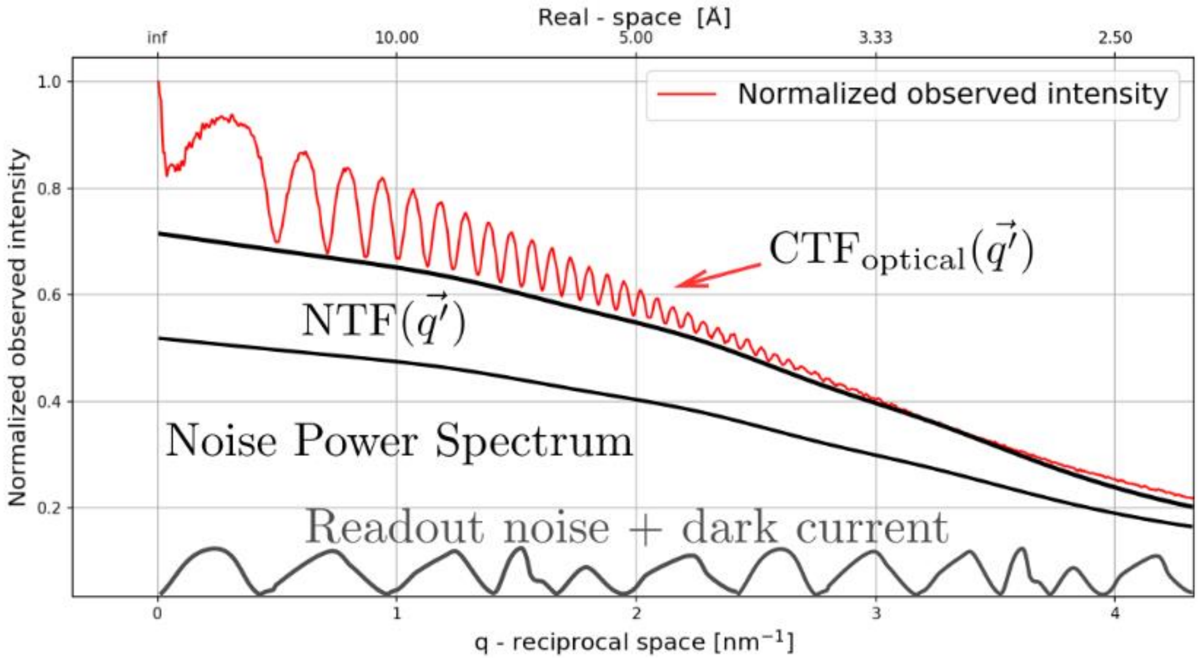


Figure 4.2: Scheme of the normalized observed intensity.

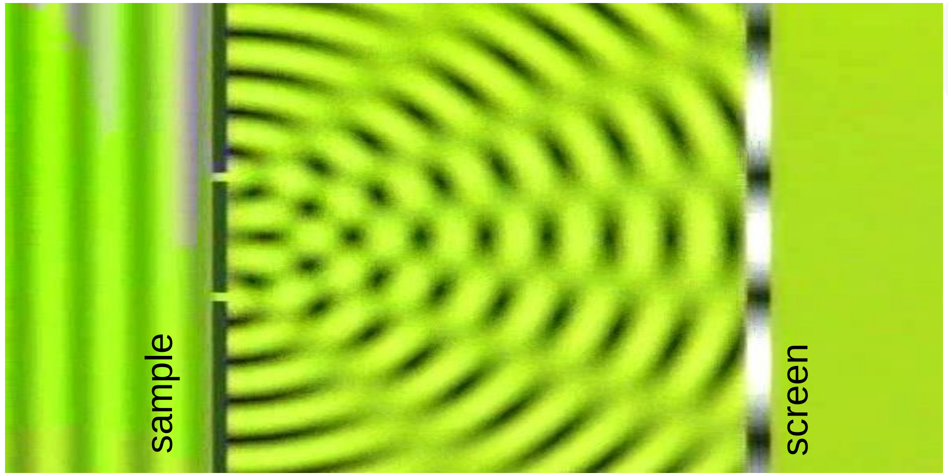
I_{rn} – Read-out noise
I_{dc} – dark current
CF – Conversion ration e/signal
Φ_{e} – Primary electron number
CTF – Contrast Transfer Function
DQE – Detector Quantum Efficiency
NTF – Noise Transfer Function

Michal Brzica bachelor thesis – derived VULOVIĆ, Miloš, et al. Image formation modeling in cryo-electron microscopy. Journal of structural biology, 2013, 183.1: 19-32. ISSN 1047-8477. Available from DOI: 10.1016/j.jsb.2013.05.008.

TEM – phase contrast I.

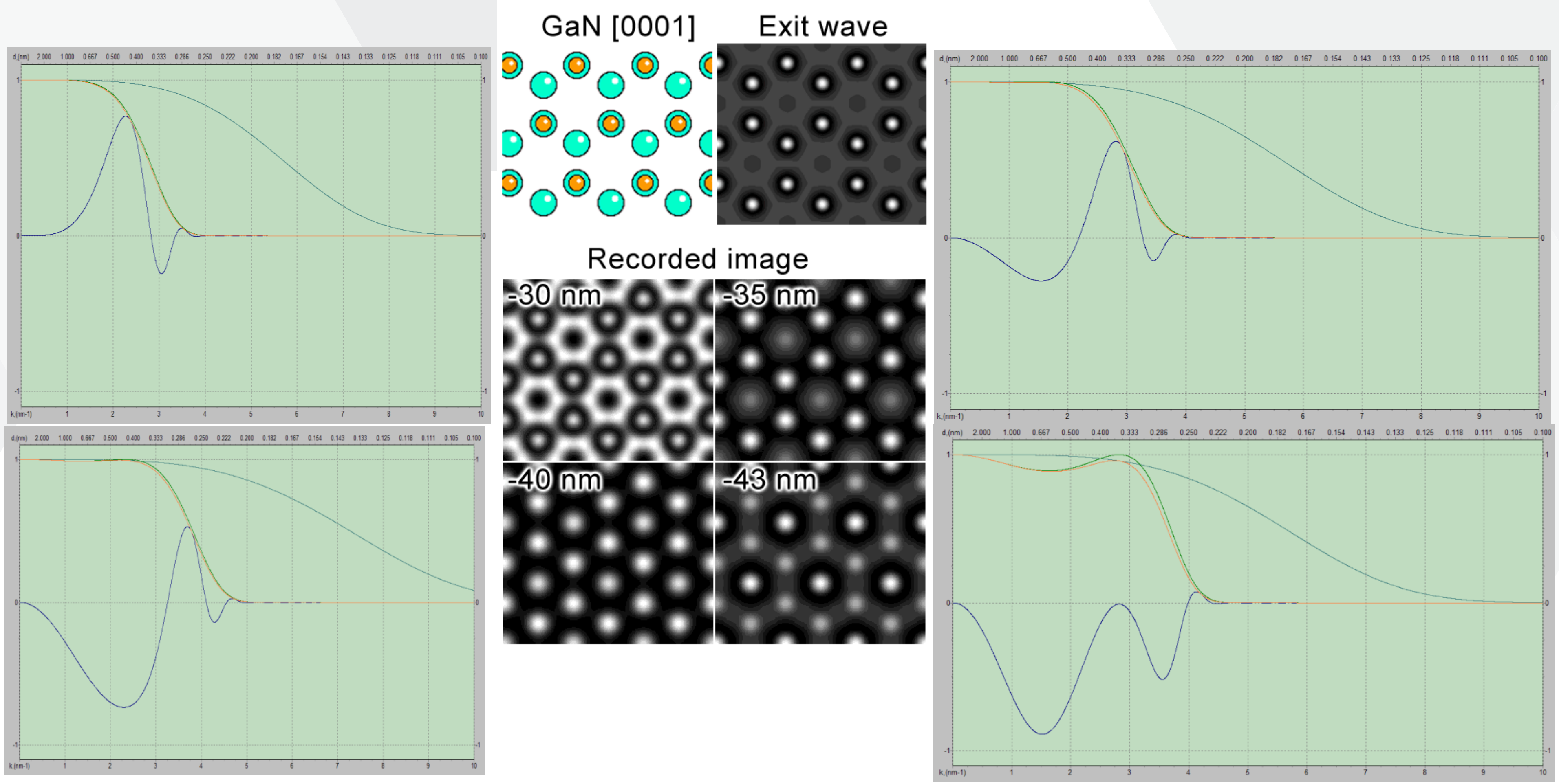
Based on electron interference – sample is pattern.
Using phase part of CFT.
Main role above magnification 300kx.
Non-trully atomic resolution – vacancy atoms are not clear visible – only decreasing of intensity is detected.
This contrast is used in HR-TEM imaging.

$$I(R) = |\psi_m(Rde_t)|^2 = Et * \{1 - 2\boxed{\varphi(Q)\sin\left(W(Q)\right) + 2\varepsilon(Q)\cos(W}(Q)\}$$



TEM – phase contrast II.

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



TEM imaging – Influence of Defocus

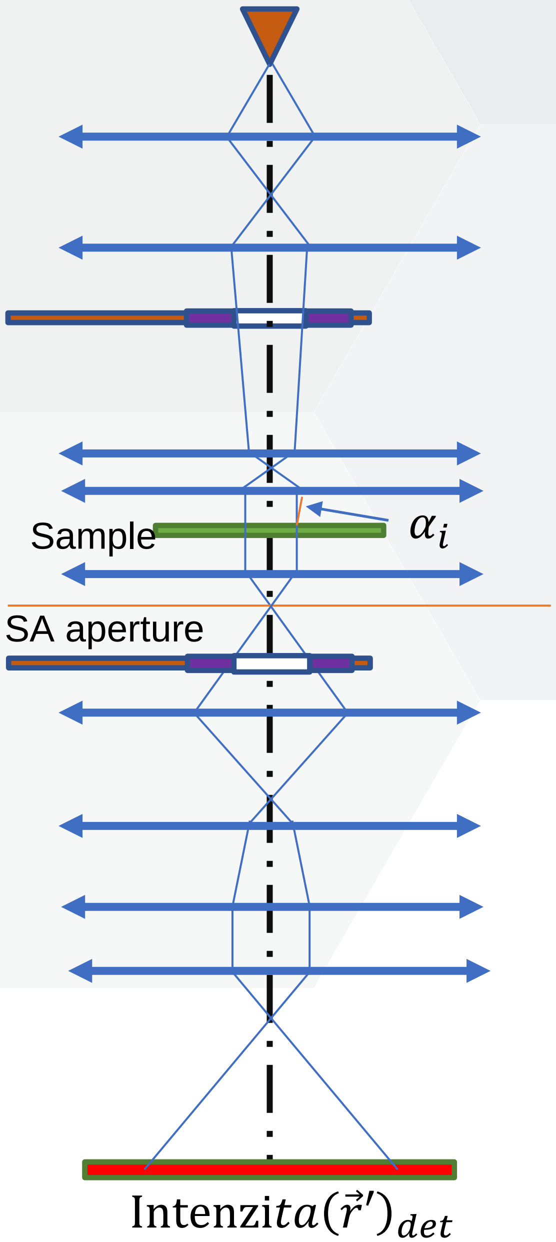
- Modulating CTF

$$W(\vec{q}) = \frac{\pi}{\lambda} C_{1,0}\lambda^2\vec{q}^2 + \frac{1}{2} C_{3,0}\lambda^4\vec{q}^4$$

$$\psi_{\mathrm{bfp,ab}}(\vec{q}) = \delta(\vec{q}) + \mathrm{E}(\vec{q})\mathrm{e}^{-\mathrm{i}W(\vec{q})} + \mathrm{i}\Phi(\vec{q})\mathrm{e}^{-\mathrm{i}W(\vec{q})}$$

$$\mathrm{Intenzita}(\vec{q}\,')_{\mathrm{det}} = \left(\mathrm{IFT}\{\psi_{\mathrm{bfp,ab}}(\vec{q}/M)\}\right)^2$$

$$\mathrm{Intenzit}a(\vec{q}\,')_{det} = E_{\mathrm{t}}(q')E_{\mathrm{s}}(\vec{q}\,')E_{\mathrm{d}}(\vec{q}\,')E_{\mathrm{u}}(\vec{q}\,') \cdot \mathrm{Intenzita}(\vec{q}\,')$$



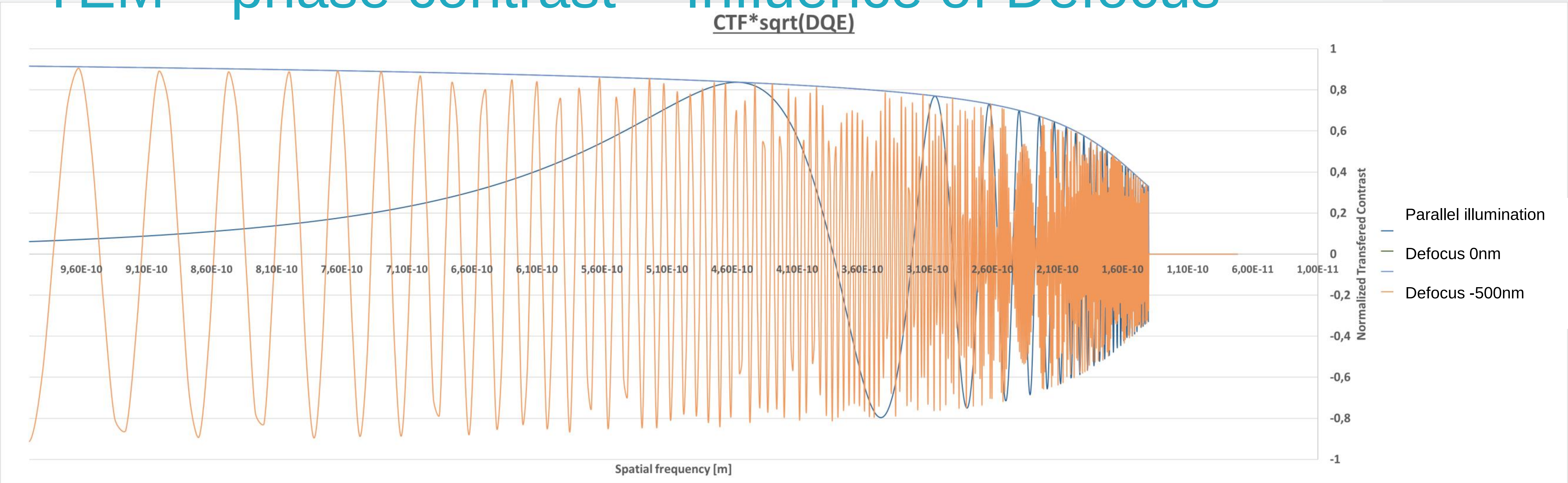
Spatial dumping envelope

$$E_{\mathrm{s}}(\vec{q}\,') = e^{(C_{3,0}\lambda^2 q'^3 - C_{1,0} q')^2 \alpha_i^2 / ln2}$$

α_i is convergent angle of illumination
$C_{3,0}$ - spherical aberration
$C_{1,0}$ - defocus

- Practical hint:
  - Higher defocus promote contrast in low frequecies (lost at higher)
  - Work in Parallel illumination

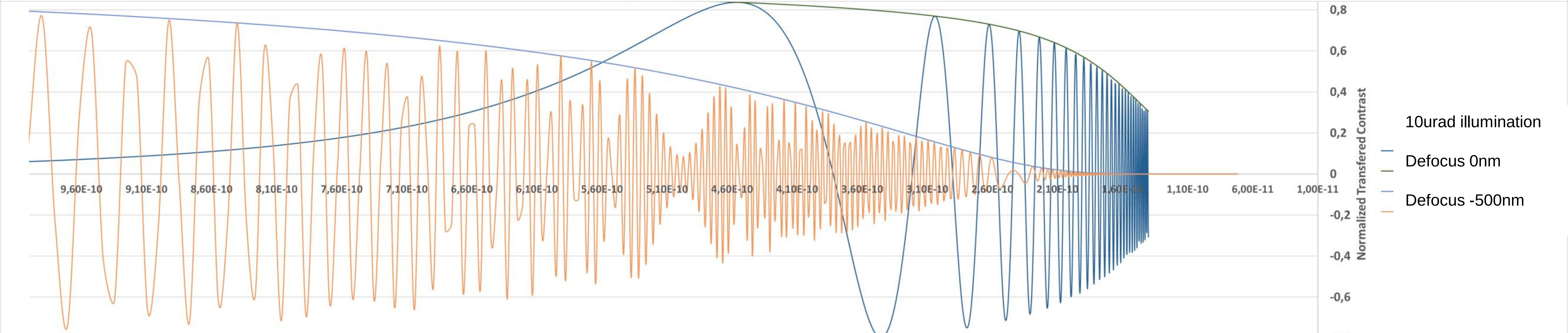
TEM – phase contrast - Influence of Defocus



Parallel illumination

Defocus 0nm

Defocus -500nm

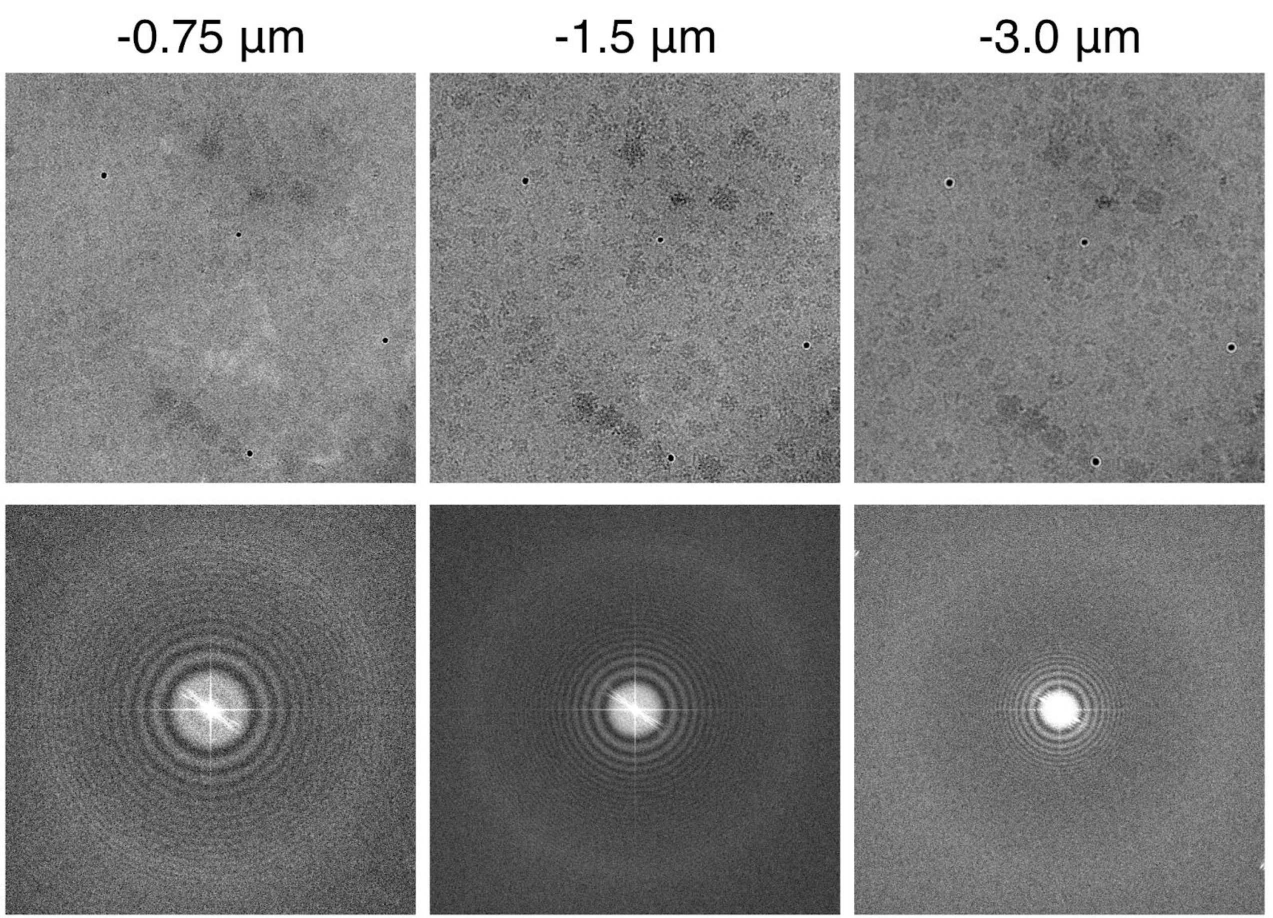


10urad illumination

Defocus 0nm

Defocus -500nm

TEM – phase contrast III.



SPA particle with different defocus

https://cryoem101.org/chapter-5/

TEM imaging – Influence of Cond Aperture

- Definition of illuminating area + convergent angle

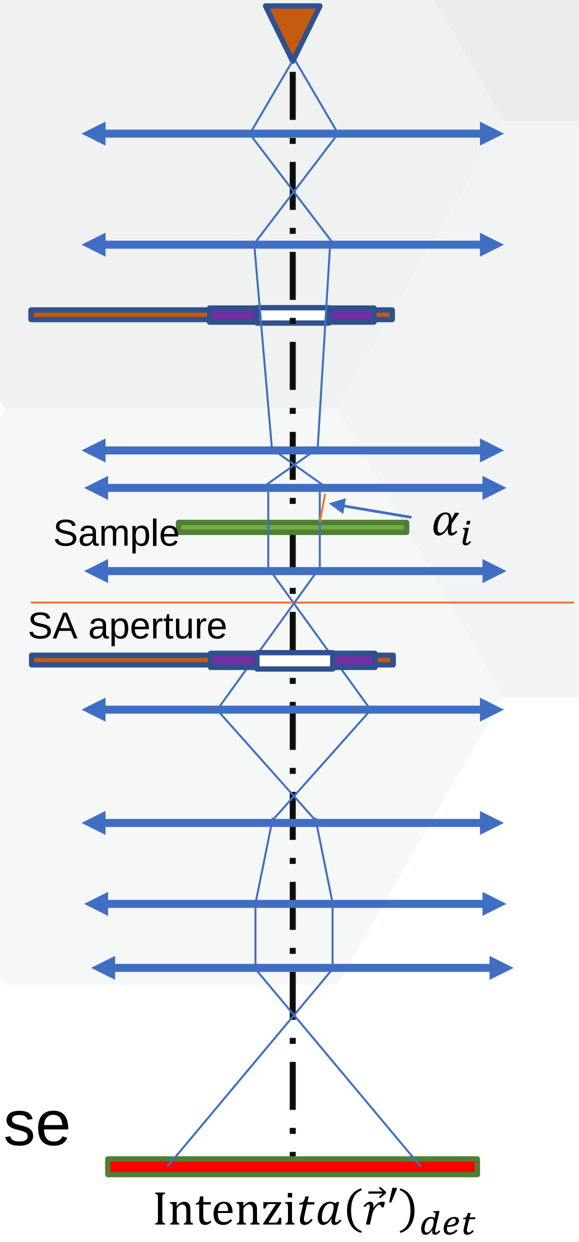
$$\mathrm{Intenzita}(\vec{q}')_{det} = E_{\mathrm{t}}(q')E_{\mathrm{s}}(\vec{q}')E_{\mathrm{d}}(\vec{q}')E_{\mathrm{u}}(\vec{q}') \cdot \mathrm{Intenzita}(\vec{q}')$$

- Spatial dumping envelope

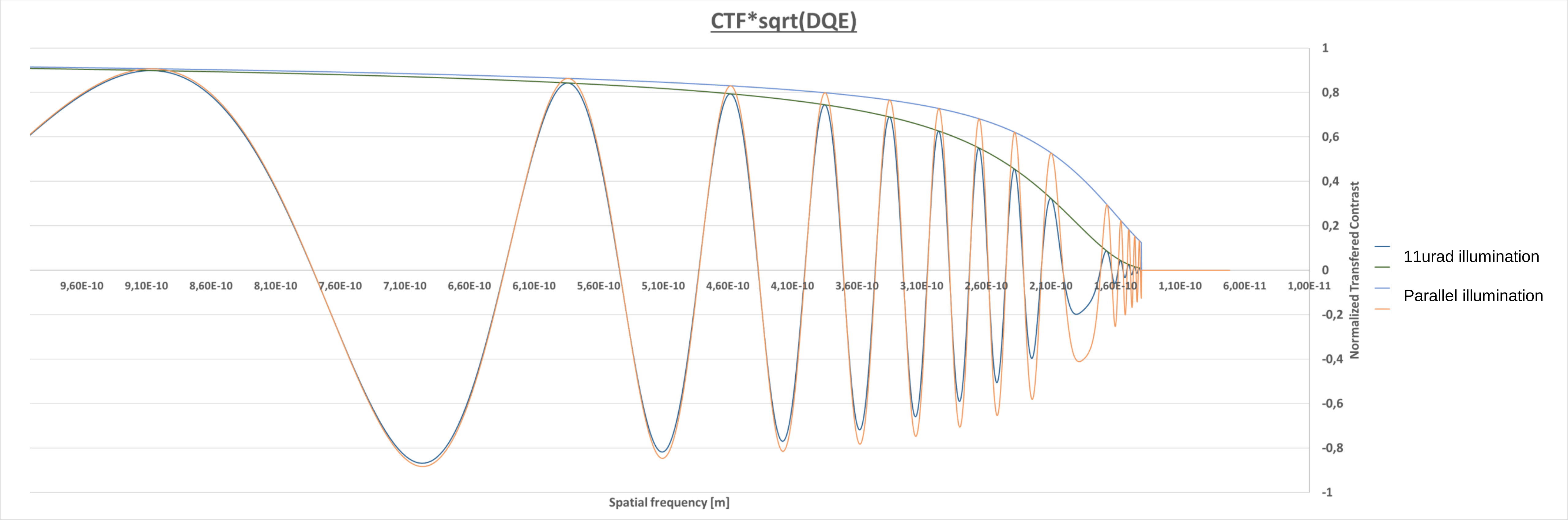
$$E_{\mathrm{s}}(\vec{q}') = e^{(C_{3,0}\lambda^2 q'^3 - C_{1,0} q')^2 \alpha_i^2 / ln2}$$

α_i is convergent angle of illumination
$C_{3,0}$ - spherical aberration
$C_{1,0}$ - defocus

- Practical hint:
  - Work as close to parallel illumination – feel free to use Gun lens or spot size to manage your dose



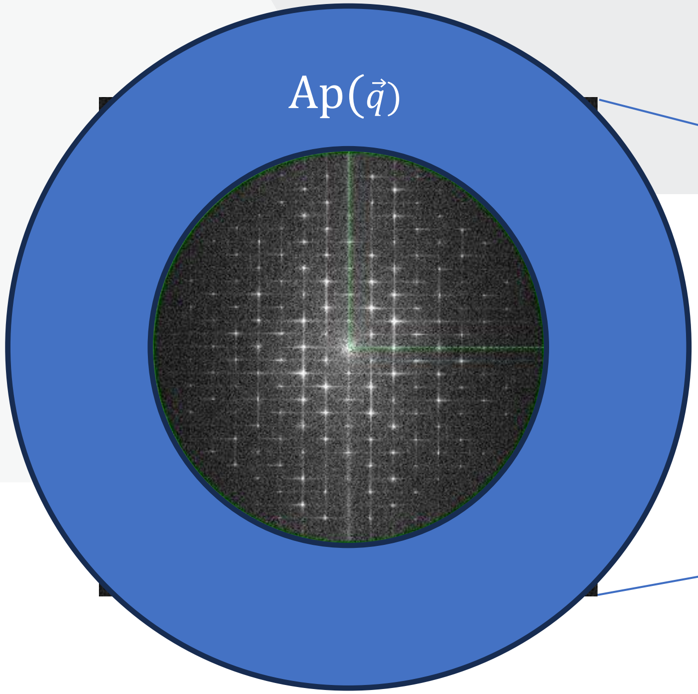
TEM imaging – Influence of Cond Aperture

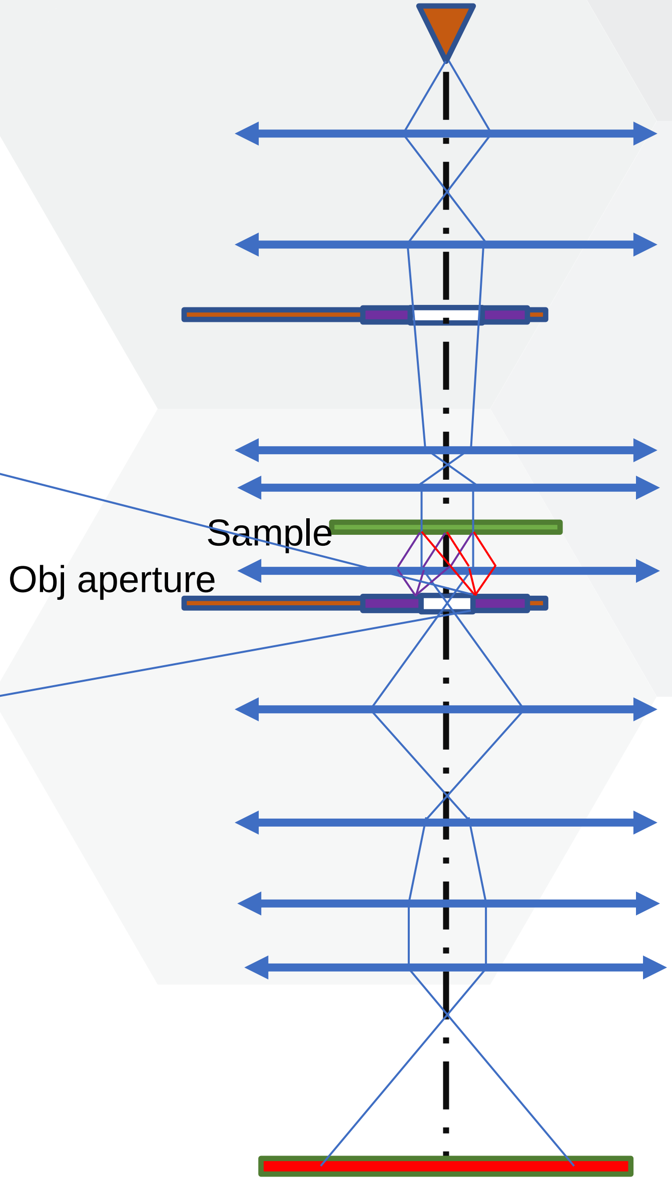


TEM imaging – Influence of Obj Aperture

- Limiting max transfer angle/reciprocal frequency

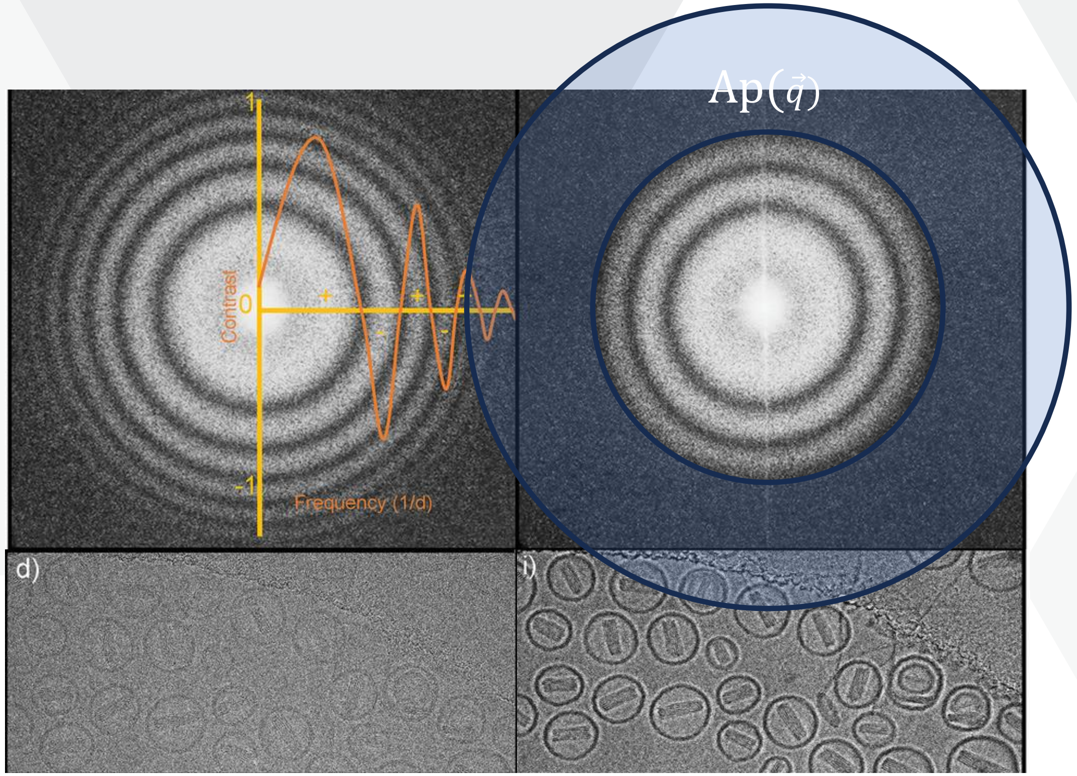
$$\psi_{\mathrm{bfp}}(\vec{q}) = \mathrm{FT}\{\psi_{\mathrm{out}}(\vec{r})\} = \mathrm{Ap}(\vec{q})\mathrm{FT}\left\{\psi_0\left(\vec{r},\vec{k}\right)(1 + \epsilon(\vec{r}) + \mathrm{i}\varphi(\vec{r}))\right\}$$





- Practical hint
  - Always image stigmate after objective ap insertion
  - Using objective aperture to verify non-linear imaging

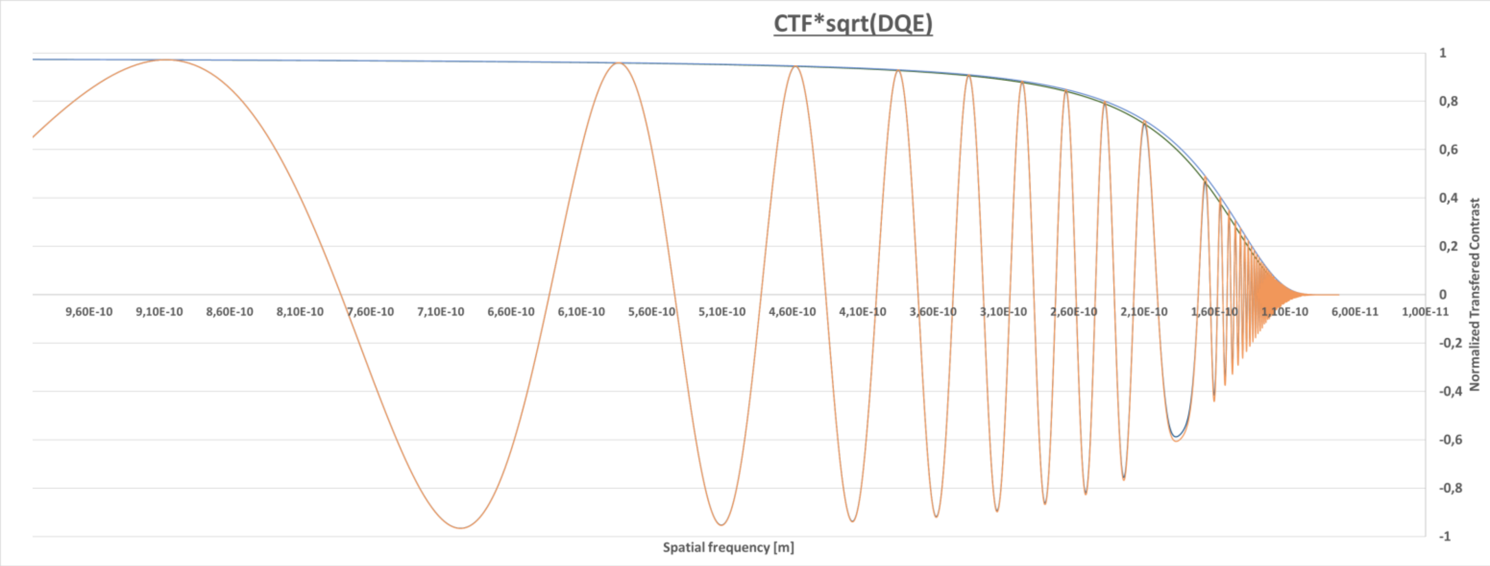
TEM imaging – Influence of Obj Aperture



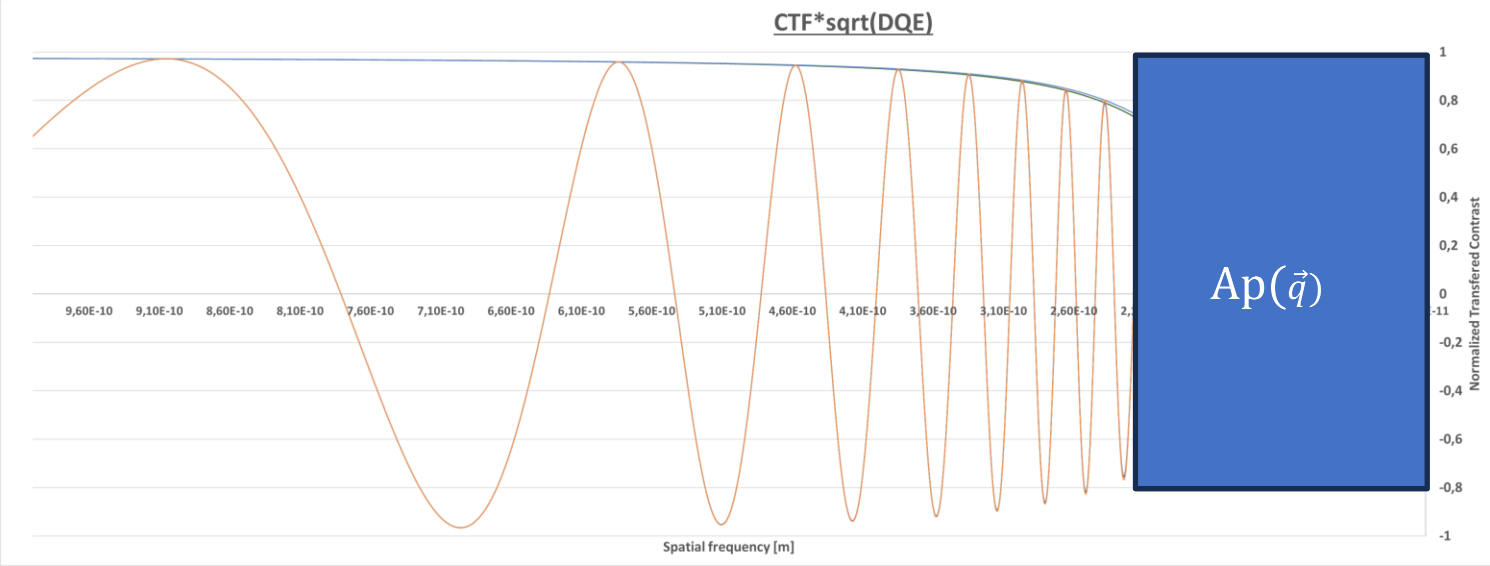
No objective aperture

Objective aperture inserted

TEM imaging – Influence of Obj Aperture



Not limited CTF

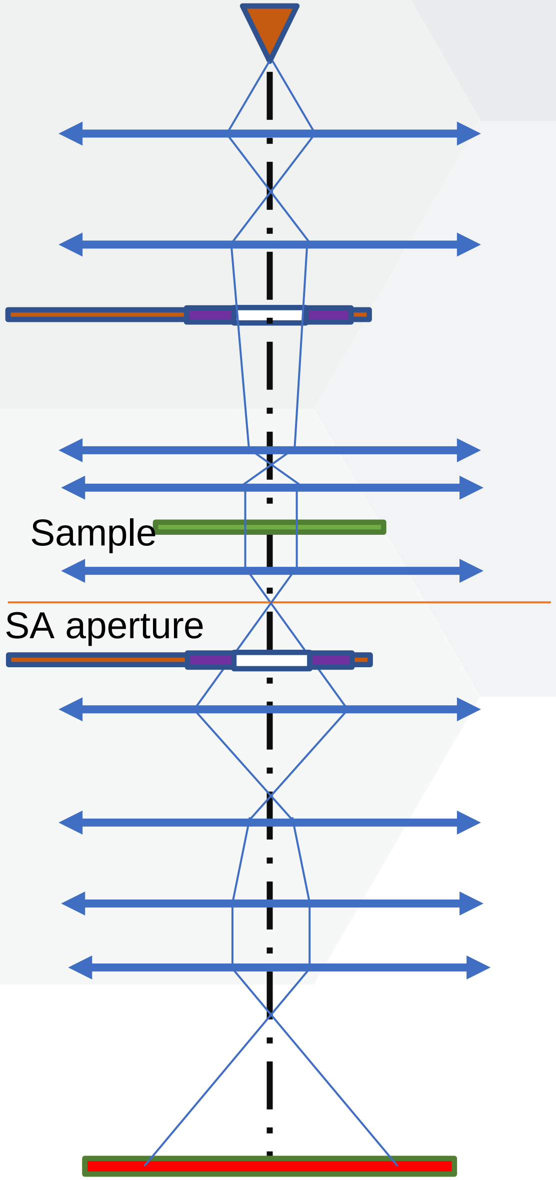


CTF limited by Obj. aperture

TEM imaging – Influence of SA Aperture

- Limiting FOV($\vec{r}$)
- No advantage for TEM imaging

$$\psi_{\mathrm{out}}(\vec{r}) = 1 + \underline{\epsilon}(\vec{r}) + \mathrm{i}\underline{\varphi}(\vec{r})$$



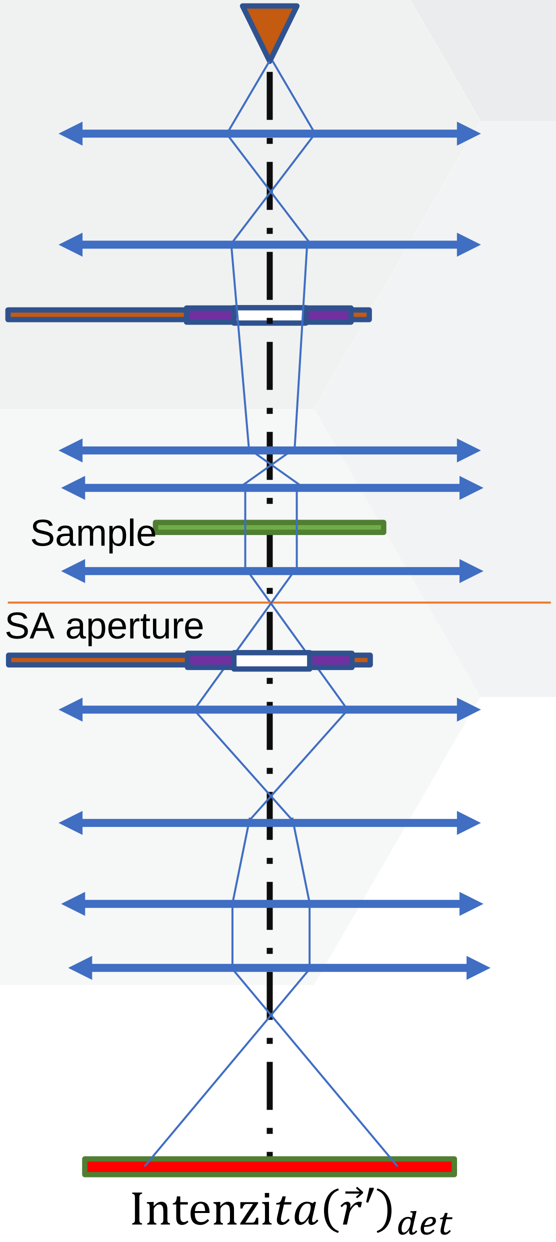
TEM imaging – Influence of Detector

- Critical for TEM imaging

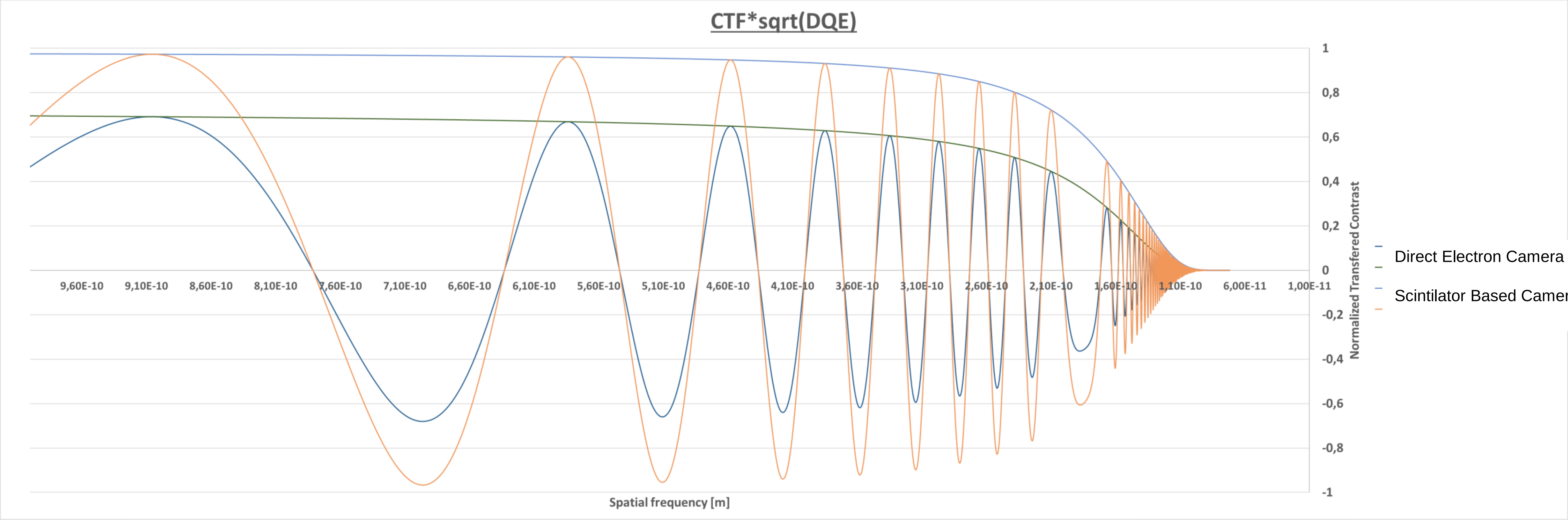
$$\mathrm{Intensity}_{\mathrm{ob}}(\vec{r}\,') = \mathrm{I}_{\mathrm{rn}} + \mathrm{I}_{\mathrm{dc}} + \mathrm{CF} \cdot \mathrm{IFT}\left[\mathrm{FT}\left[P_{\mathrm{oiss}}\left(\Phi_{\mathrm{e}} \cdot \mathrm{IFT}^{-1}\left[\mathrm{CTF}_{\mathrm{optical}}(\vec{q}\,')\sqrt{\mathrm{DQE}(\vec{q}\,')}\right]\right)\right] \cdot \mathrm{NTF}(\vec{q}\,')\right]$$

I_{rn} - camera read-out noise
I_{dc} - dark current
CF - Conversion factor – how much primar electrons are count as 1 signal
Φ_e - number of primary electron on a pixel
$DQE = \frac{(MTF)^2}{PowerSpectrum}$ = „how well camera can trasfer details“
NTF – Noise Transfer Function (related to non-elastic scattering of the sample

- Practical hint:
  - Make your Dark current calibration each 2 weeks or monthly
  - Check your camera cooling stability
  - Do not overexpose your camera



TEM imaging – Influence of Detector



Diffraction imaging – Influence of SA Aperture

- SA aperture selecting Sample region for Diffraction
- SA selection vs Nano Beam Diffraction
  - No fringes in SA image
  - Illuminating whole sample

$$\psi_0(\vec{r}) = 1$$

$$\psi_{\mathrm{out}}(\vec{r}) = 1 + \epsilon(\vec{r}) + \mathrm{i}\varphi(\vec{r})$$

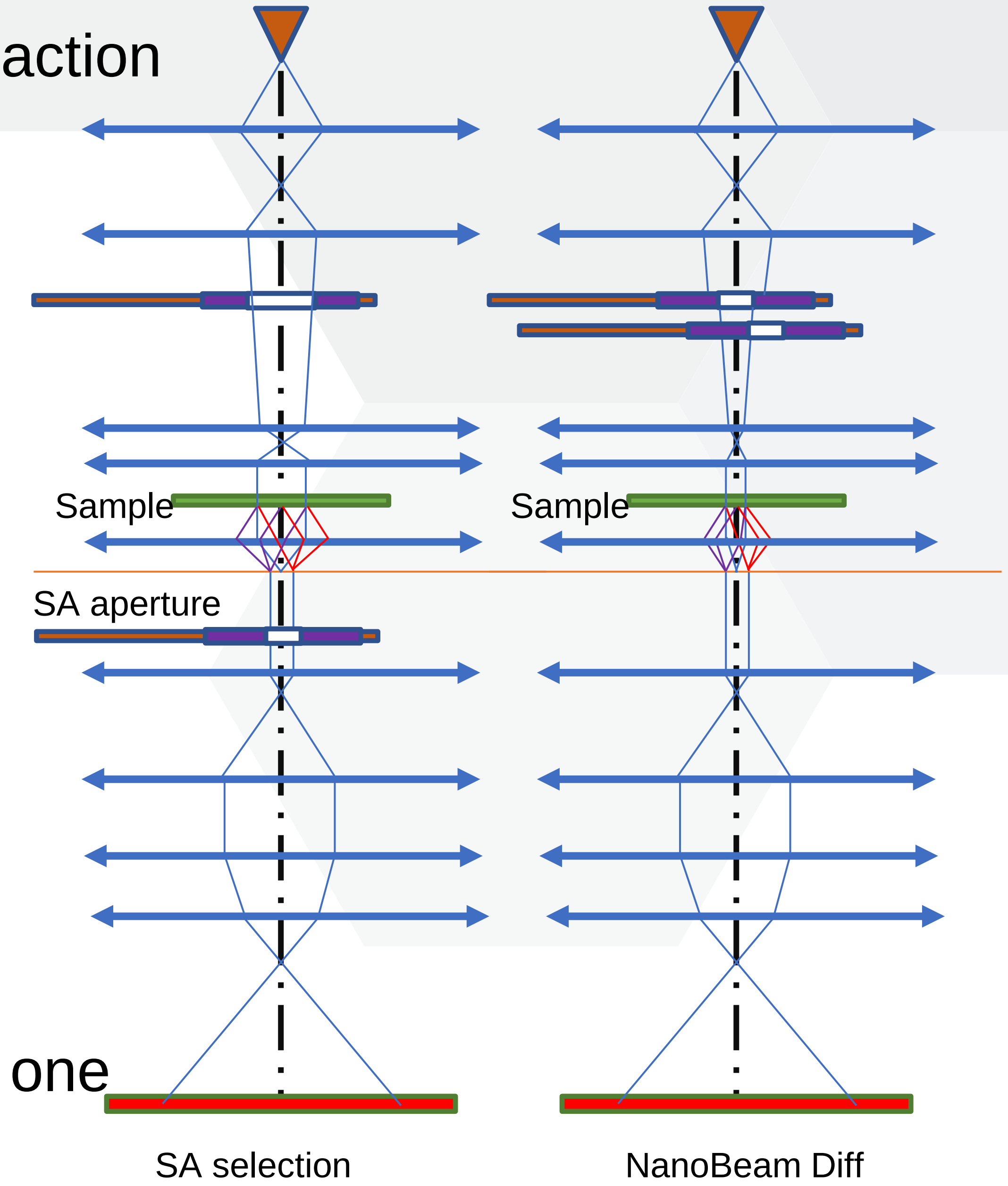
  - Fringes in SA image
  - Illuminating only selected area

$$\psi_0(\vec{r}) = \mathrm{A}/\sqrt{r^2+z^2}\,e^{\pm i\vec{k}\,\overrightarrow{\sqrt{r^2+z^2}}}$$

$$\psi_{\mathrm{out}}(\vec{r}) = 1 + \epsilon(\vec{r}) + \mathrm{i}\varphi(\vec{r})$$

- Practical hint fo SA
  - use C2 overfocus in diffraction to localize your particle accuratelly

- Allways stigmate first cond stigmator then diffr. one



Diffraction imaging – Influence of Detector

- Not so Critical for Diffraction imaging
- DO NOT OVERSATURATE THE CENTRAL SPOT!!!

→ RISK OF CAMERA DAMAGE!!!

$$\mathrm{Intensity}_{\mathrm{ob}}(\vec{r}') = \mathrm{I}_{\mathrm{rn}} + \mathrm{I}_{\mathrm{dc}} + \mathrm{CF} \cdot \mathrm{IFT}\left[\mathrm{FT}\left[P_{\mathrm{oiss}}\left(\Phi_{\mathrm{e}} \cdot \mathrm{IFT}^{-1}\left[\mathrm{CTF}_{\mathrm{optical}}(\vec{q}')\sqrt{\mathrm{DQE}(\vec{q}')}\right]\right)\right] \cdot \mathrm{NTF}(\vec{q}')\right]$$

I_{rn} - camera read-out noise

I_{dc} - dark current

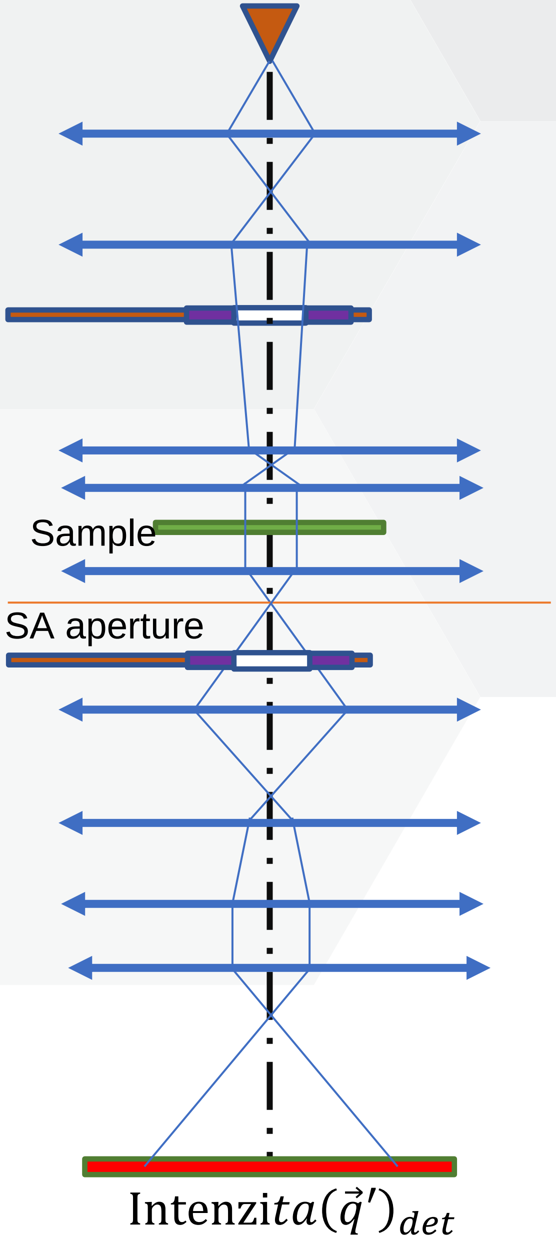
CF - Conversion factor – how much primar electrons are count as 1 signal

Φ_e - number of primary electron on a pixel

$DQE = \frac{(MTF)^2}{PowerSpectrum}$ = „how well camera can trasfer details“

NTF – Noise Transfer Function (related to non-elastic scattering of the sample

- Practical hint:
  - Shield your Zero order diffraction peak to avoid damaging camera
  - CL as magnification



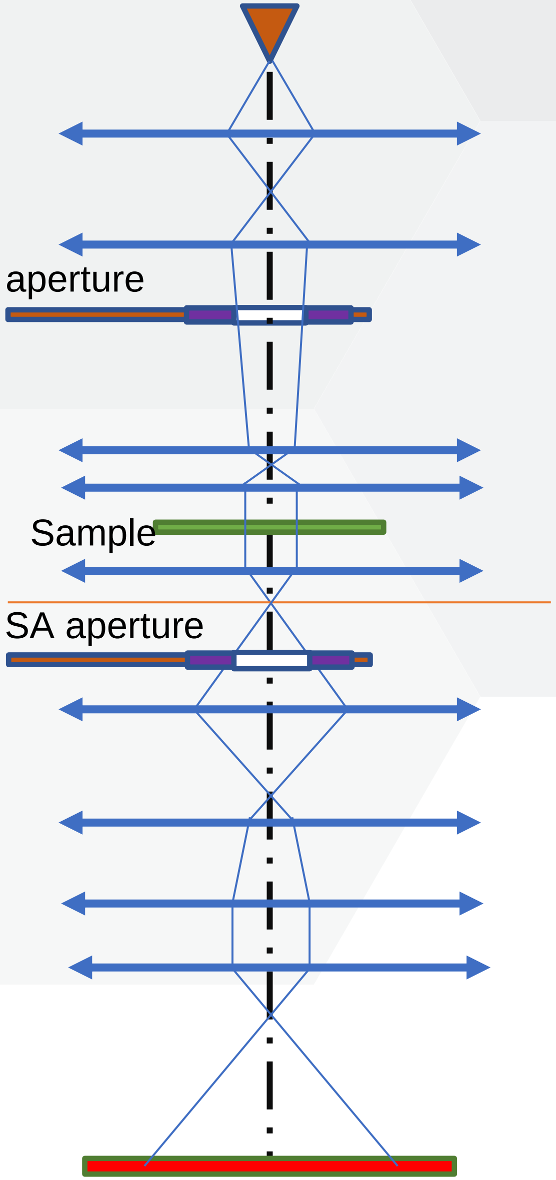
Diffraction imaging – Influence of Cond Aperture

- Non-critical for SAED – defining mainly illuminated FOV
- Critical for CBED
  - Definition of Ronchigram size

$$D_{ronchi}(\vec{q}) = D_{ap}(r)/f$$

$D_{ronchi}\ (\vec{q})$ - size of ronchigram in reciprocal space
$D_{ap}\ (\vec{r})$ - size of C2 aperture
f - focal lenght of condensor

- Practical hint
  - Allways condensor stigmate on Ronchigram



Conclusion

TEM imaging providing atomic resolution

Knowledge of your system setup is crucial for image interpretation (further processing)