CEITEC

Středoevropský technologický institut

BRNO | ČESKÁ REPUBLIKA

Electron microscopy

InnoCore project

Jiri Novacek

EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

2007–13
OP Výzkum a vývoj pro inovace

Sylabus

- **Lecture 1: Applications of electron microscopy in life-science research**
- **Lecture 2: Transmission electron microscope, cryo-electron microscopy, principles of image formation**
- **Lecture 3: Fourier transform, techniques for 3D model determination in cryo-EM**

Sylabus

- Lecture 1: Applications of electron microscopy in life-science research
- Lecture 2: Transmission electron microscope, cryo-electron microscopy, principles of image formation
- **Lecture 3: Fourier transform, techniques for 3D model determination in cryo-EM**

Content

- Fourier transform
- Contrast transfer function
- Single particle analysis
- Cryo-electron tomography

Fourier transform

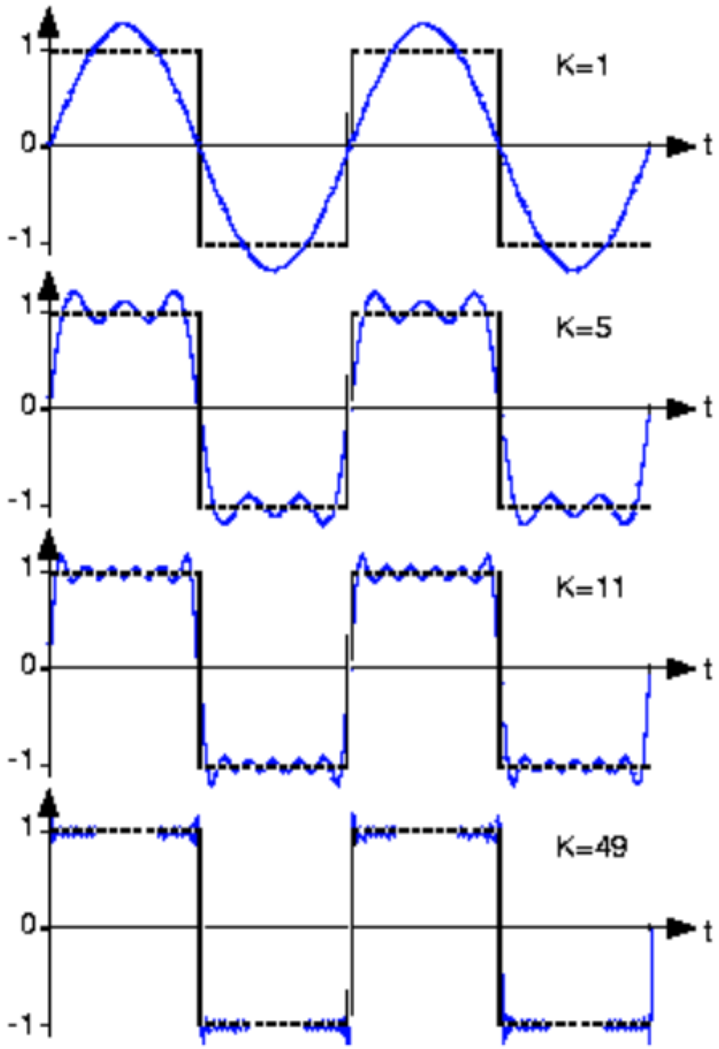
Fourier series is an expansion of a function f(x) in terms of an infinite sum of sines and cosines

$$f(x) = \frac{1}{2}a_0 + \sum_{n=1}^{\infty} a_n \cos(nx) + \sum_{n=1}^{\infty} b_n \sin(nx)$$

$$a_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\cos(nx)dx$$

$$b_n = \frac{1}{\pi}\int_{-\pi}^{\pi} f(x)\sin(nx)dx$$

The higher the spatial frequencies (i.e. higher resolution) are included, the more faithful the representation of the original signal will be.



http://cnx.org

CEITEC

Fourier transform

$$F(k) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i k x} dx$$

f: function which we are transforming (1D)

x: axis coordinate

i: √-1

k: spatial frequency

F(k): Fourier coefficient at frequency k

Euler's Formula: $e^{i\phi} = \cos\phi + i\sin\phi$

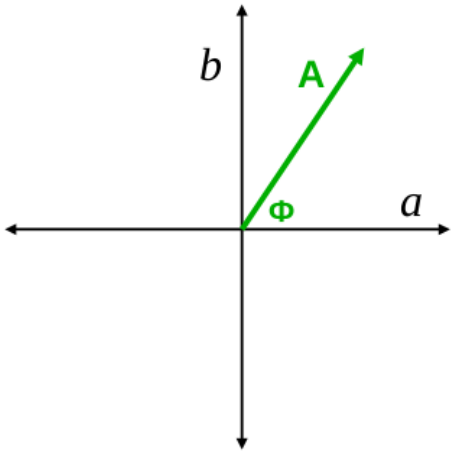
$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

a ***+i*** ***b***

Fourier transform

$$F(k) = \int_{-\infty}^{\infty} f(x)\cos(-2\pi kx)\,dx + i\int_{-\infty}^{\infty} f(x)\sin(-2\pi kx)\,dx$$

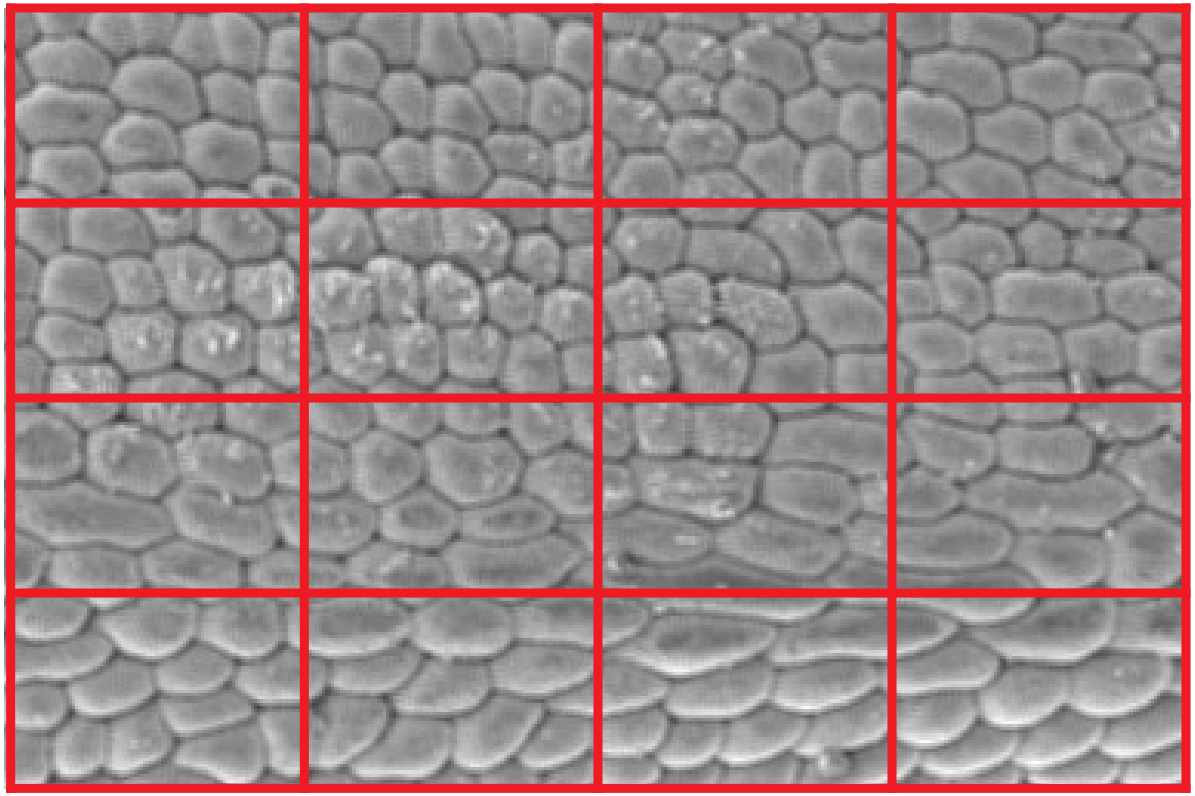
$$a \quad +i \quad b$$



Amplitude, A: $\sqrt{a^2+b^2}$

Phase, Φ: $\arctan\frac{b}{a}$

Digitization



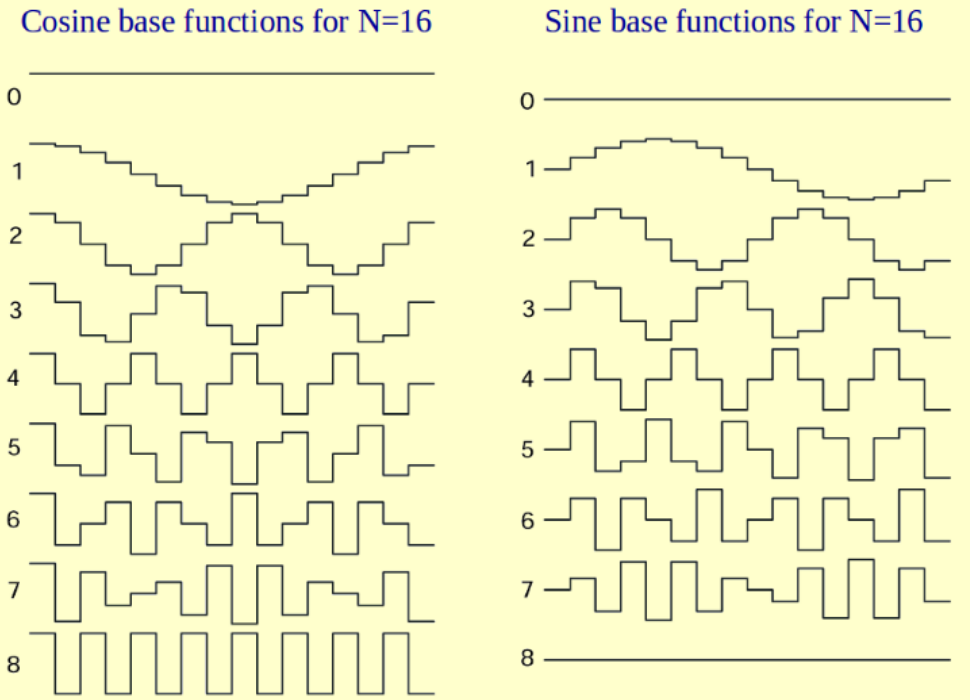
Discrete Fourier transform

- 1D discrete Fourier transform of function f(x)

$$\Phi(\omega_x) = \sum_{x=0}^{N-1} f(x)\, e^{-i(\frac{2\pi}{N}\omega_x x)}$$

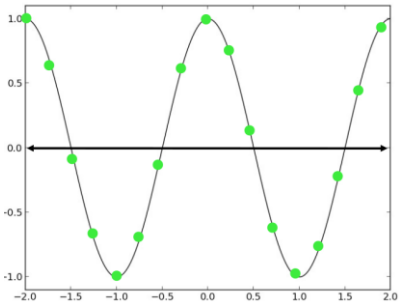
- 1D inverse discrete Fourier transform of function $\Phi(\omega_x)$

$$f(x) = \frac{1}{N} \sum_{\omega_x=0}^{N-1} \Phi(\omega_x)\, e^{i(\frac{2\pi}{N}\omega_x x)}$$

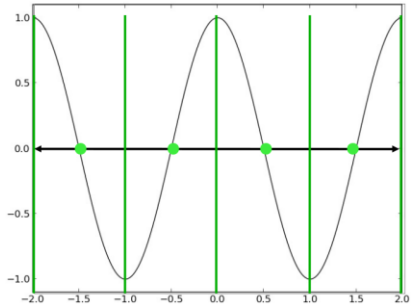


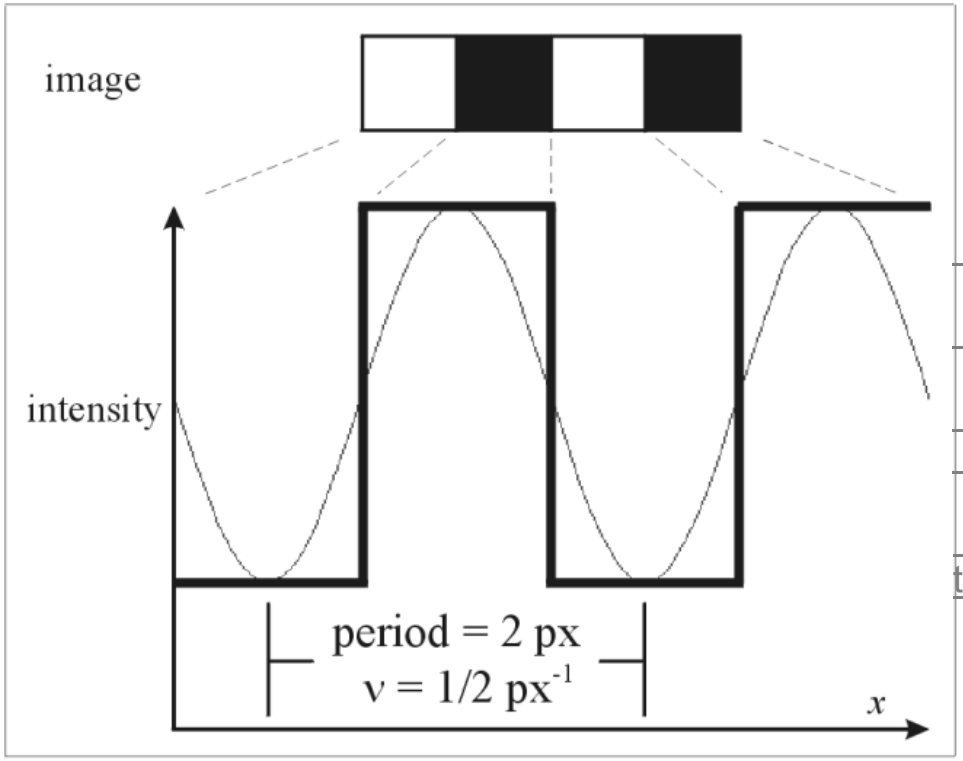
Discrete Fourier transform - sampling

Sufficient sampling



Insufficient sampling





- the period of the finest oscillation is 2 pixels

- the spatial frequency of this oscillation is $0.5px^{-1}$

- the finest detectabble oscicillation is what is known as “Nyquist frequency”

- the edge of the Fourier transform corresponds to Nyquist frequency

CEITEC

Nyquist frequency

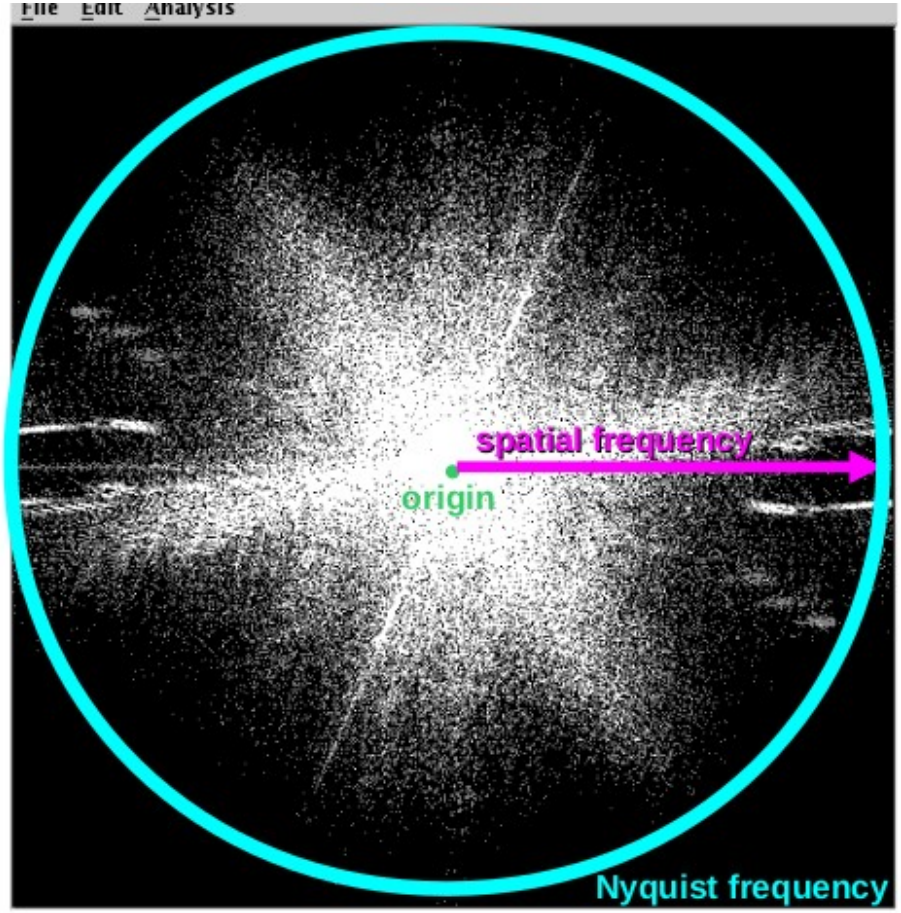


Image formation

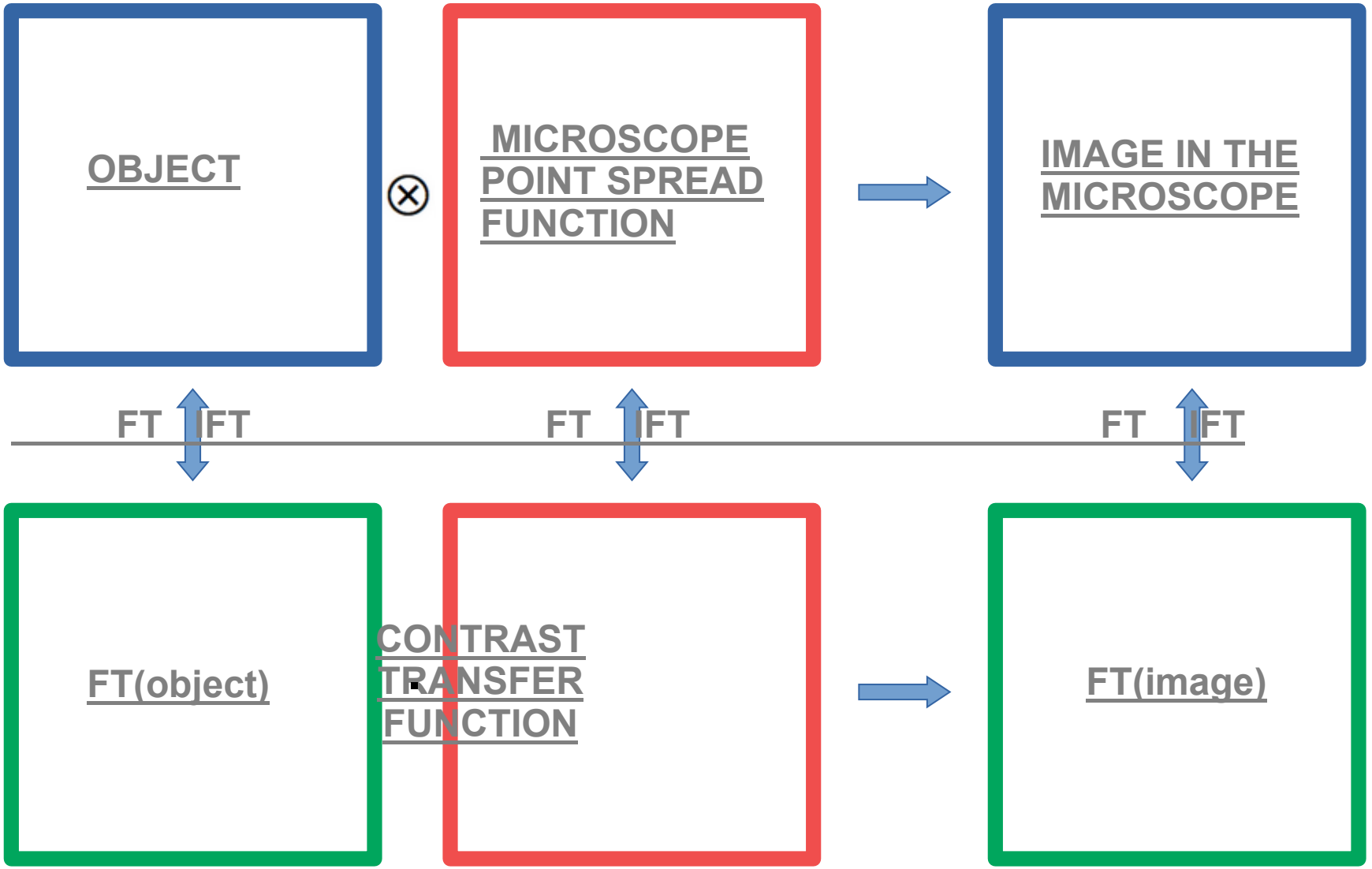
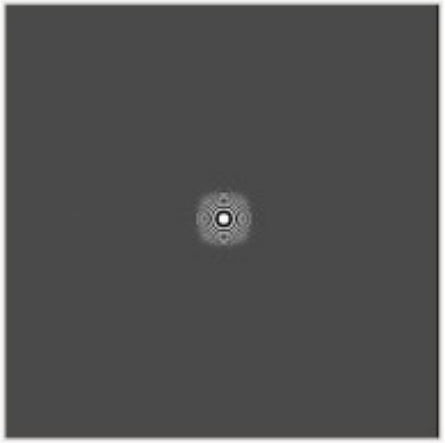
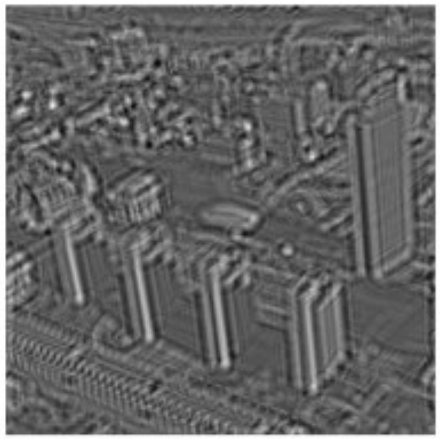


Image formation

$f(x)$

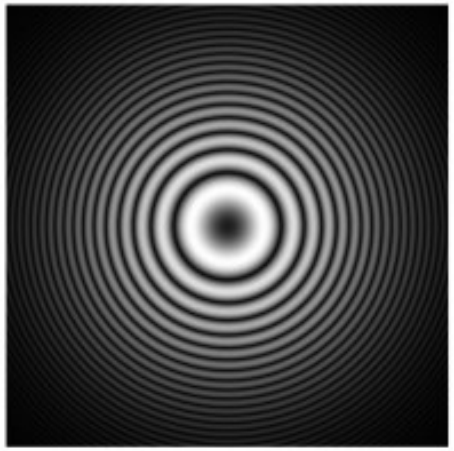


$g(x)$

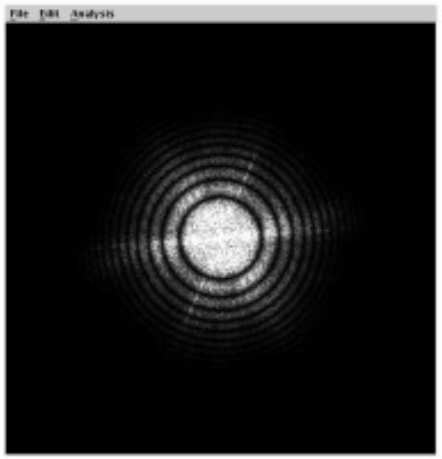


$f(x) \bullet g(x)$

$F(X)$



$G(X)$



$F(X)\ G(X)$

Contrast transfer function

$$\mathrm{CTF}\ (\vec{s}) = -\sqrt{1 - A^2} \cdot \sin(\gamma(\vec{s})) - A \cdot \cos(\gamma(\vec{s}))$$

$$\gamma(\vec{s}) = \gamma(s, \theta) = -\frac{\pi}{2} C_s \lambda^3 s^4 + \pi \lambda z(\theta) s^2$$

A – amplitude contrast

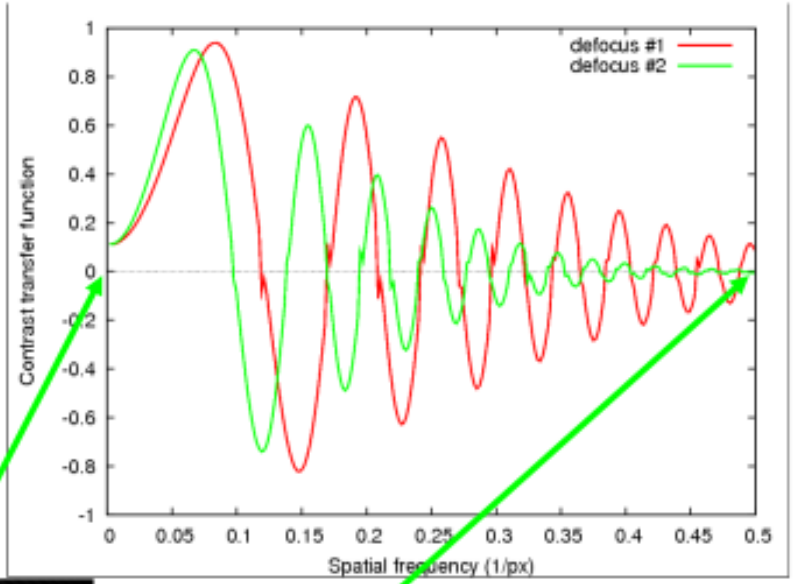
s – spatial frequency

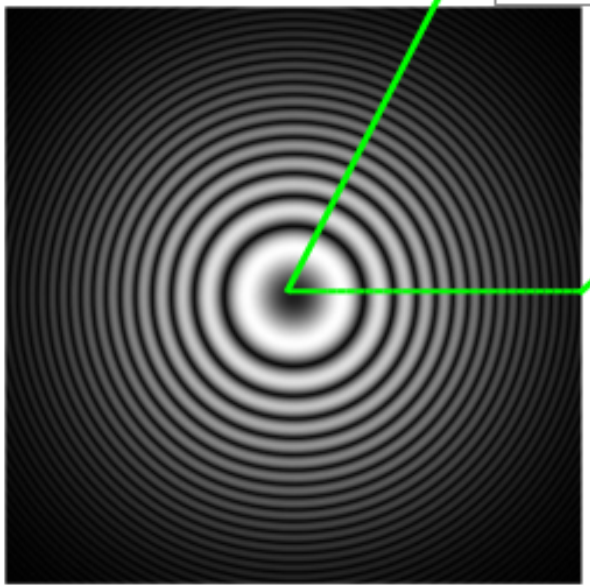
Cs – spherical abberation

λ – electron wavelength

z – defocus

1D profile

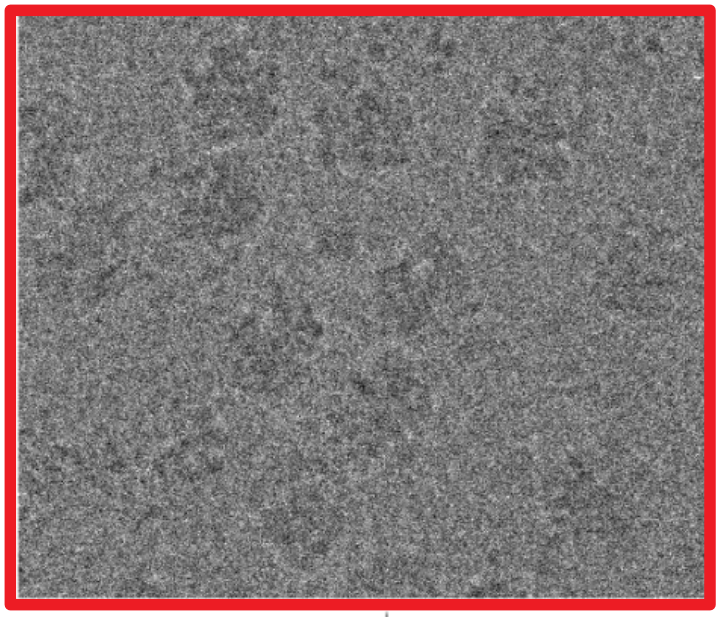


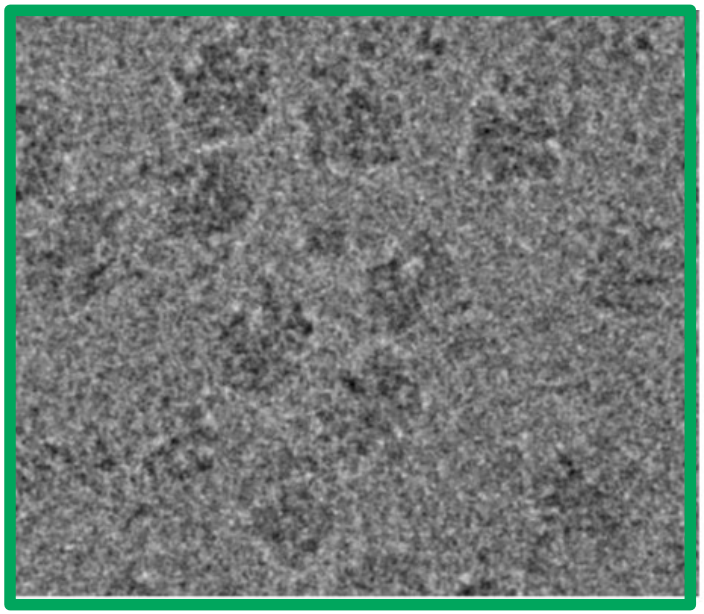


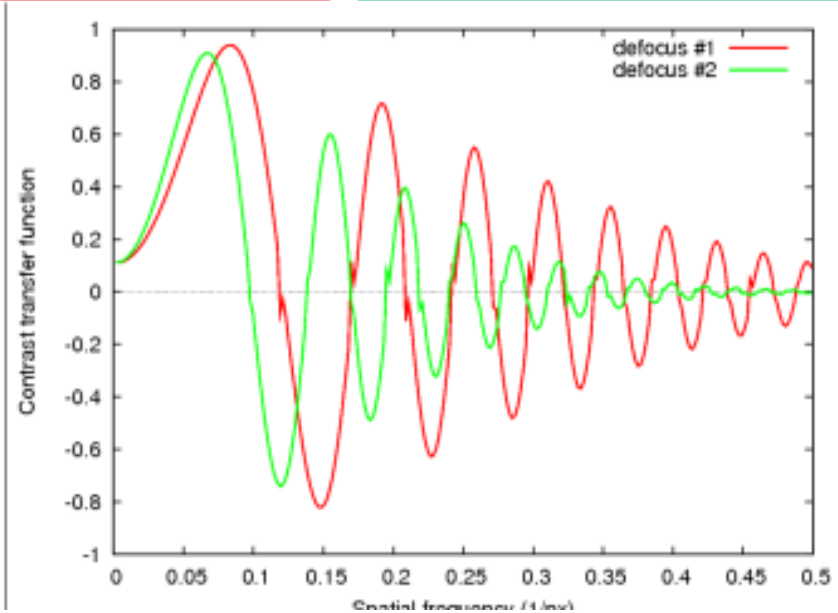
2D power spectrum
$G(X)$

Image formation

Low defocus **High defocus**







CEITEC

Parameters required for 3D reconstruction

Two translational:

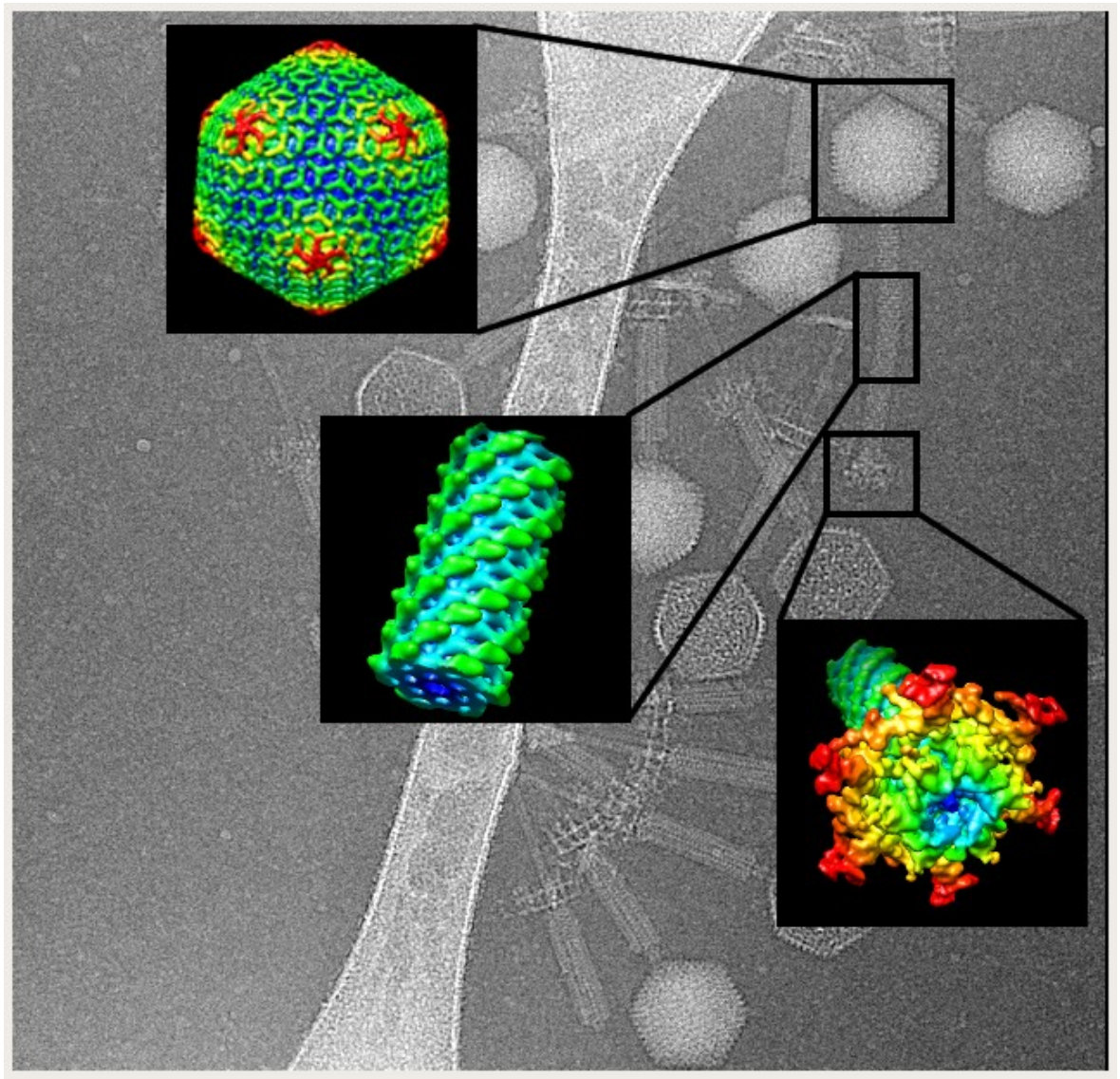
- Δx
- Δy

Three orientational (Euler angles):

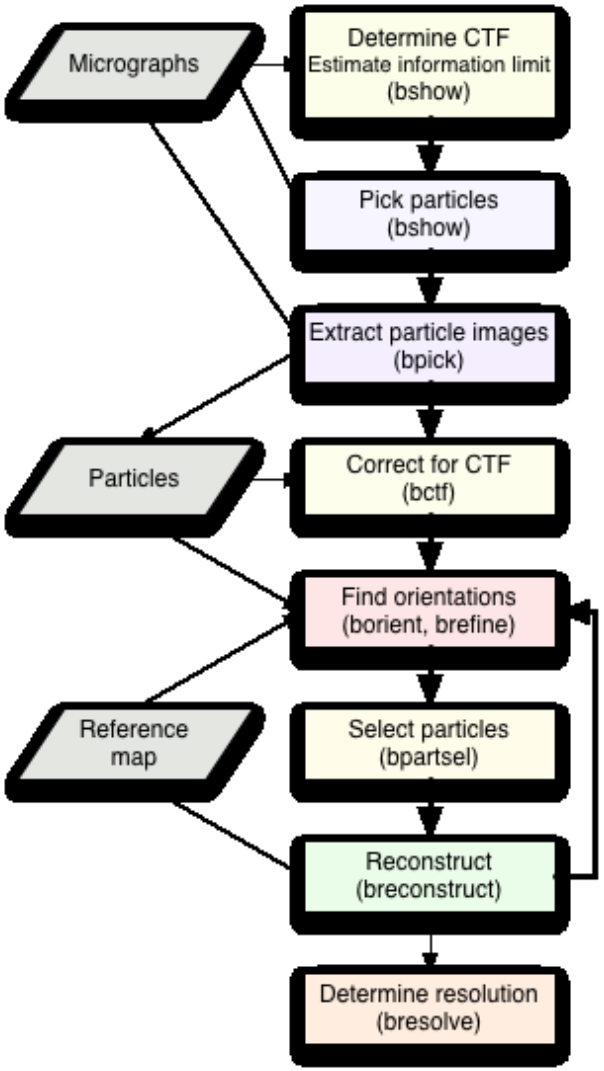
- phi (about z axis)
- theta (about y)
- psi (about new z)

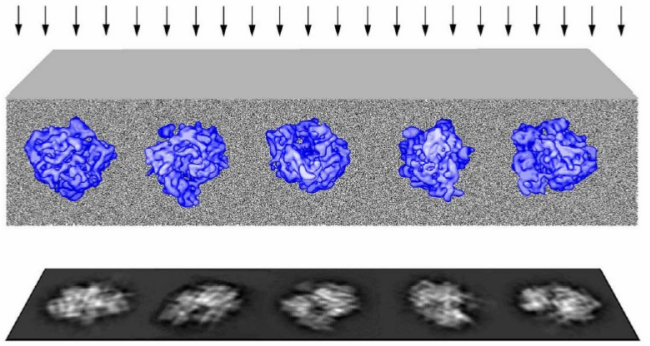
These are determined in 2D.

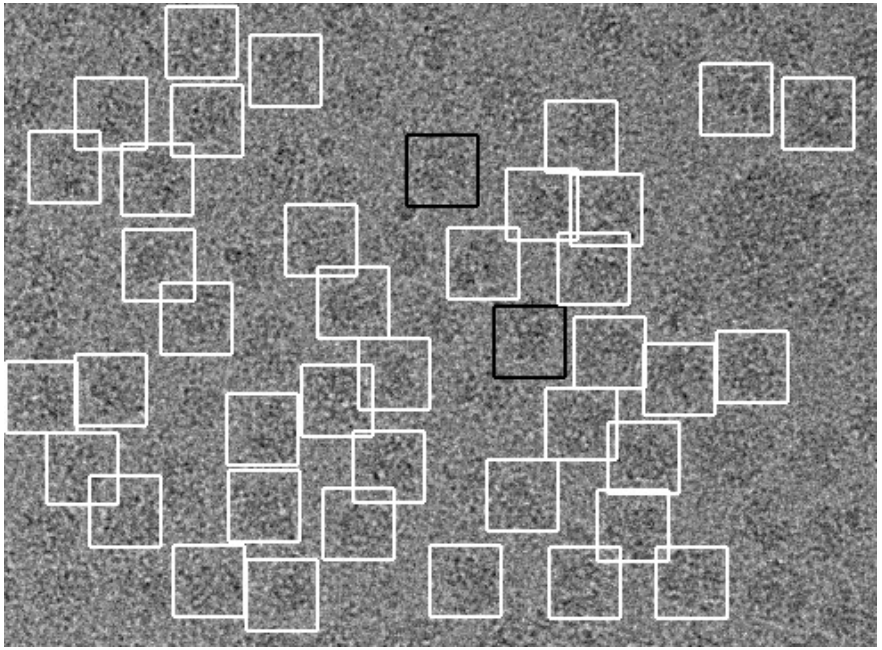
These are determined in 3D.



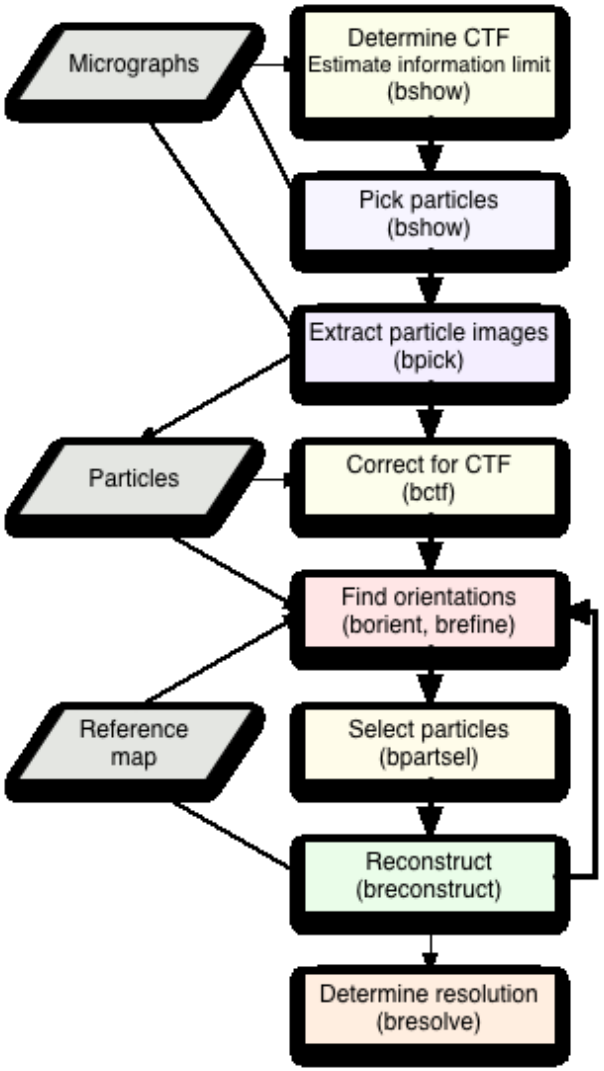
Single particle analysis

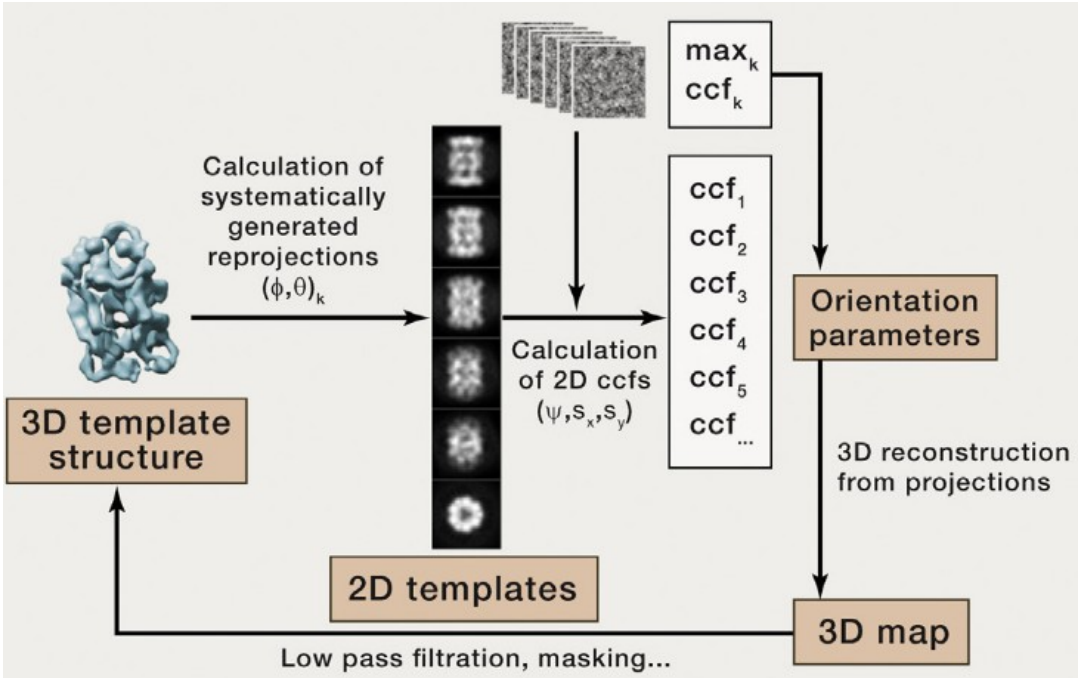


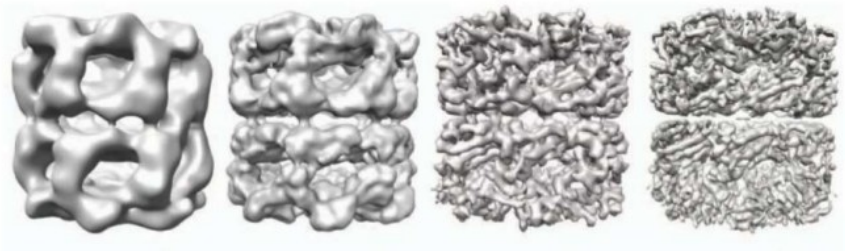




Single particle analysis

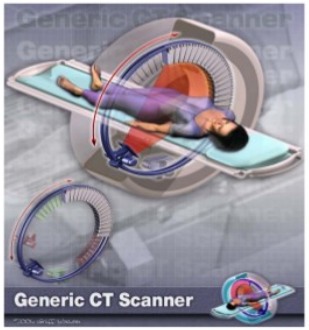




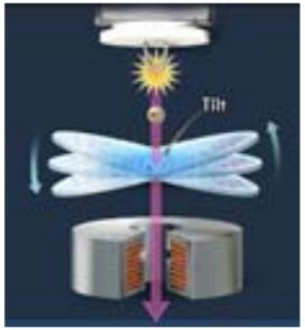


Electron tomography

Computer Tomography



Electron Tomography



Normalize micrographs (bnorm)

↓

Seed fiducial markers (bshow, btrack)

↓

Track fiducial markers (btrack)

↓

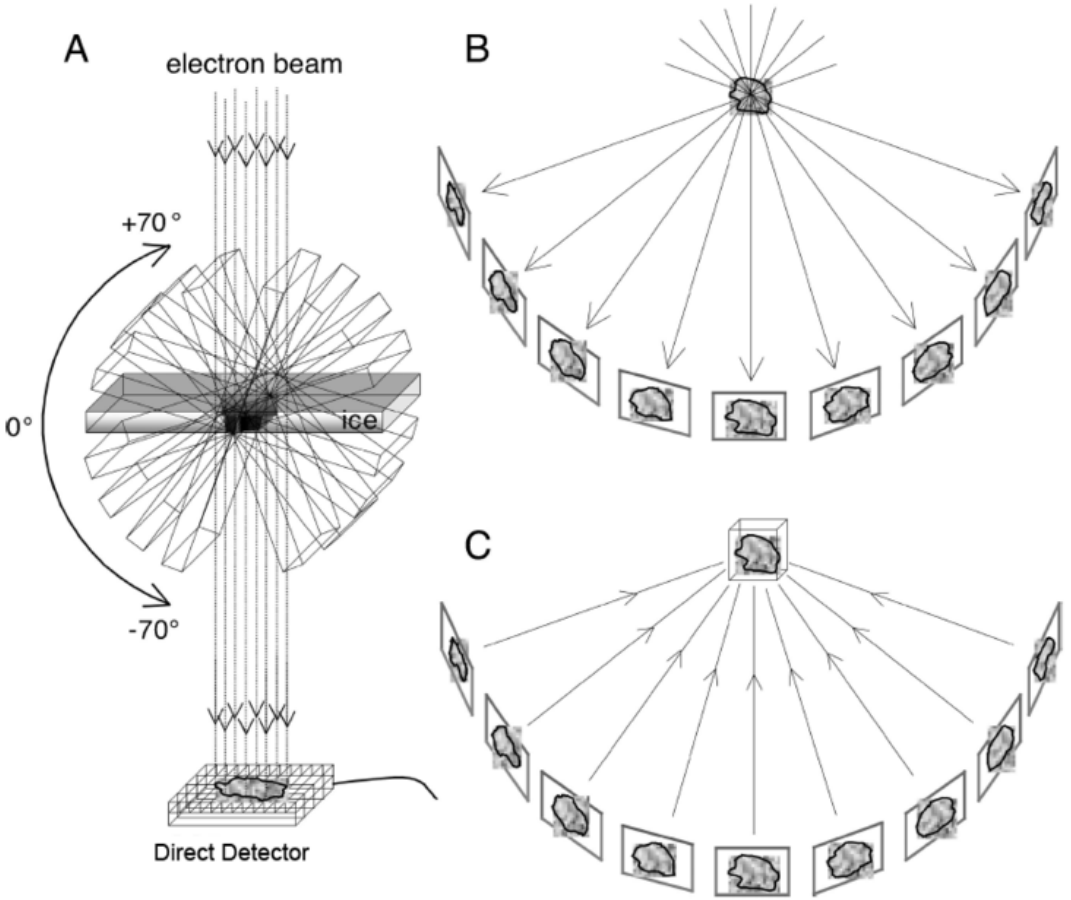
Refine alignment (bshow, btrack)

↓

Reconstruct tomogram (bmgft, btomrec, bzfft, bpatch)

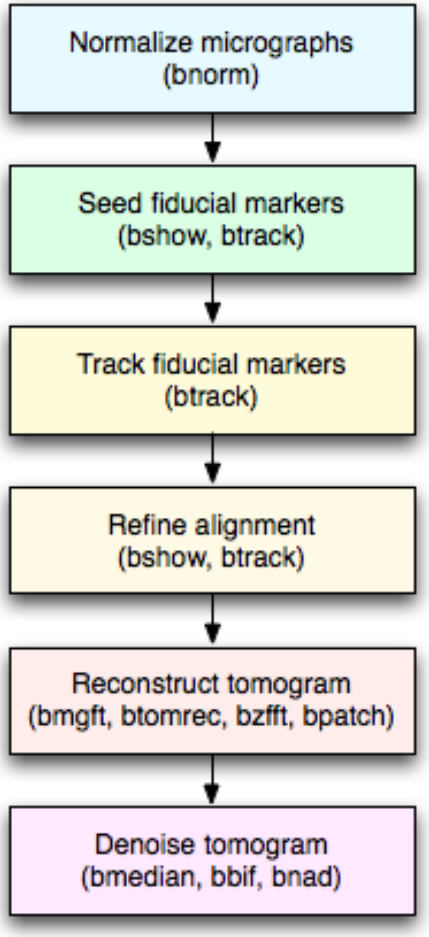
↓

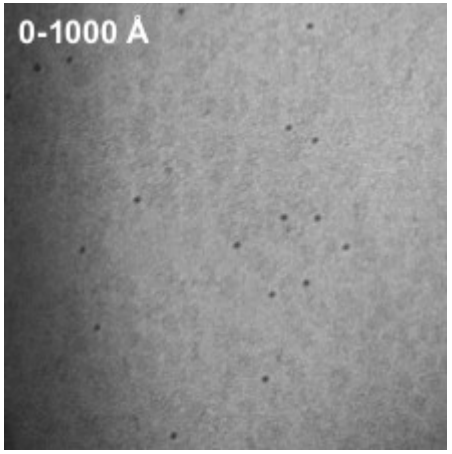
Denoise tomogram (bmedian, bbif, bnad)

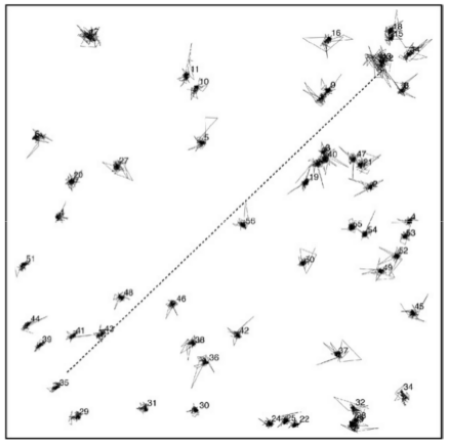


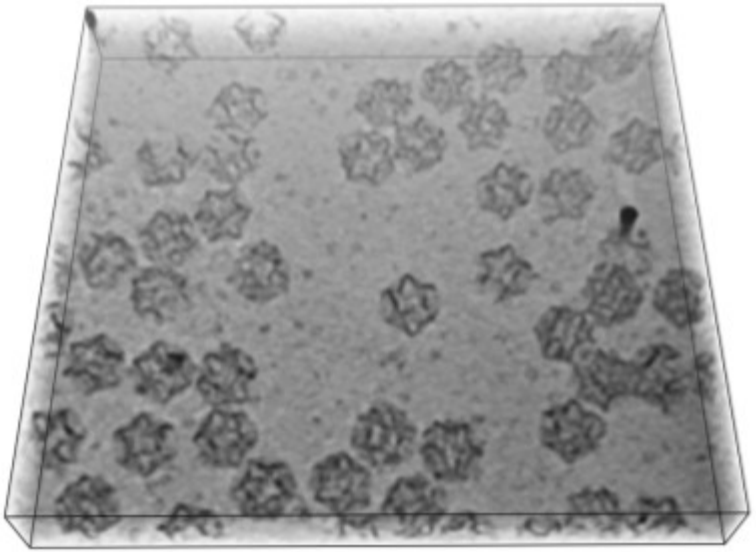
CEITEC

Electron tomography

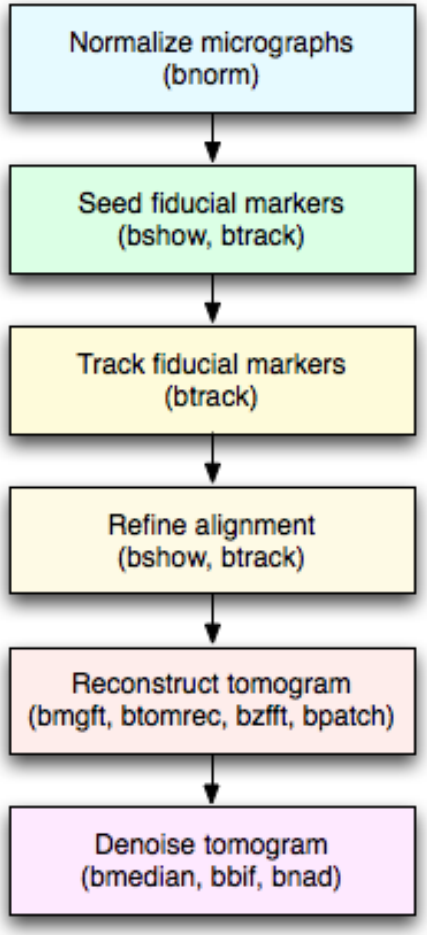


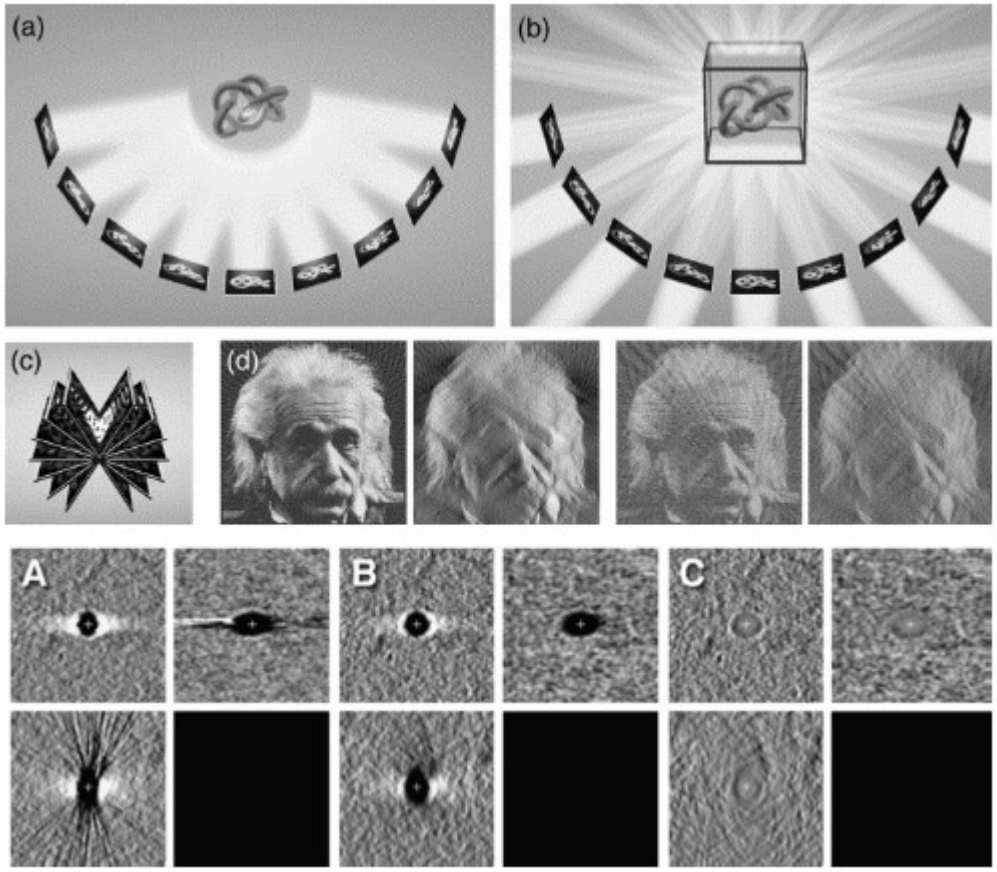






Electron tomography





CEITEC

Thank you for attention

jiri.novacek@ceitec.muni.cz

CIISB
Czech Infrastructure for Integrative Structural Biology

instruct ERIC

iNEXT DISCOVERY