Bio 5357 Lecture

(1) Basic Principles of Electron Microscopy

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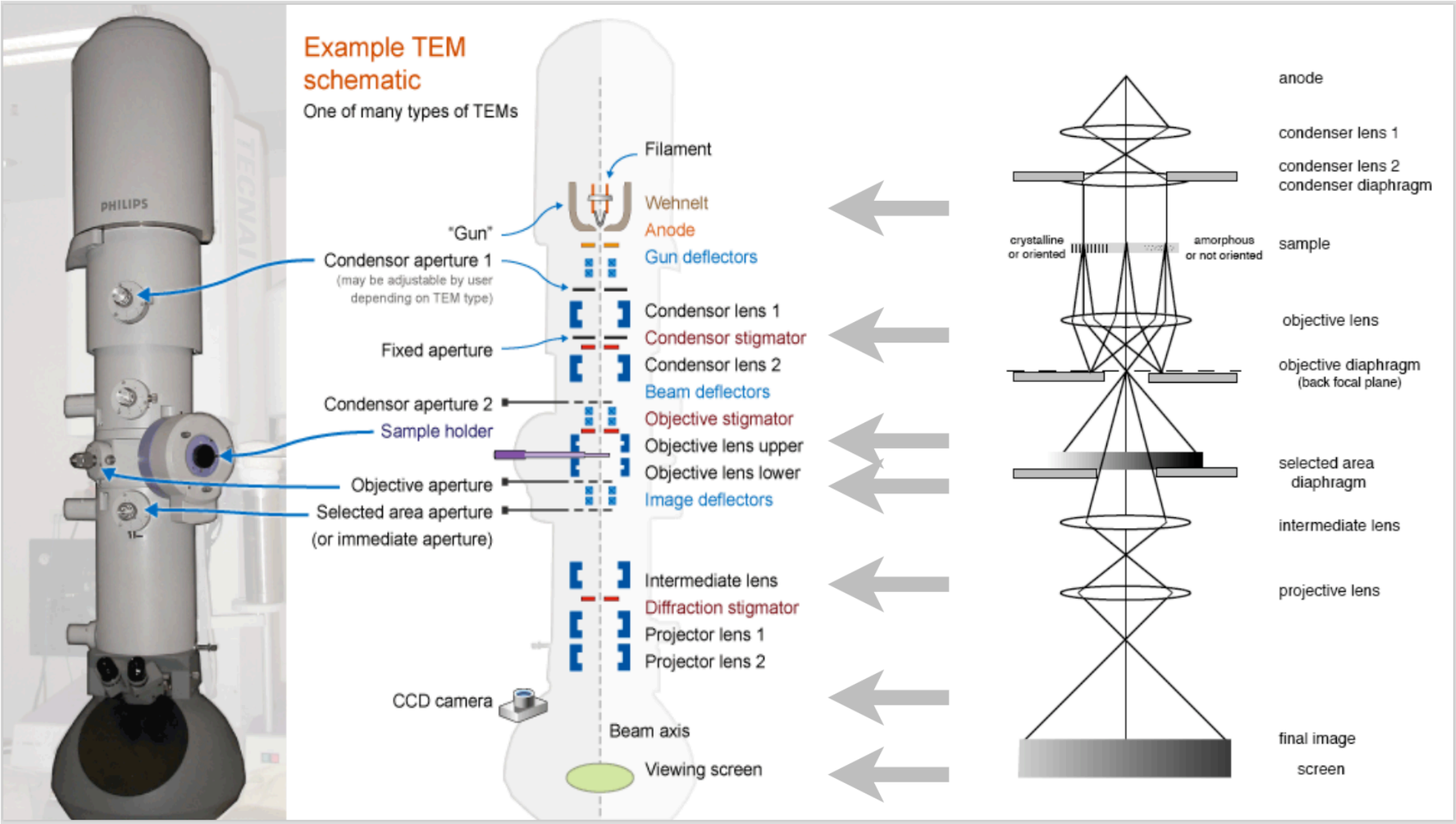
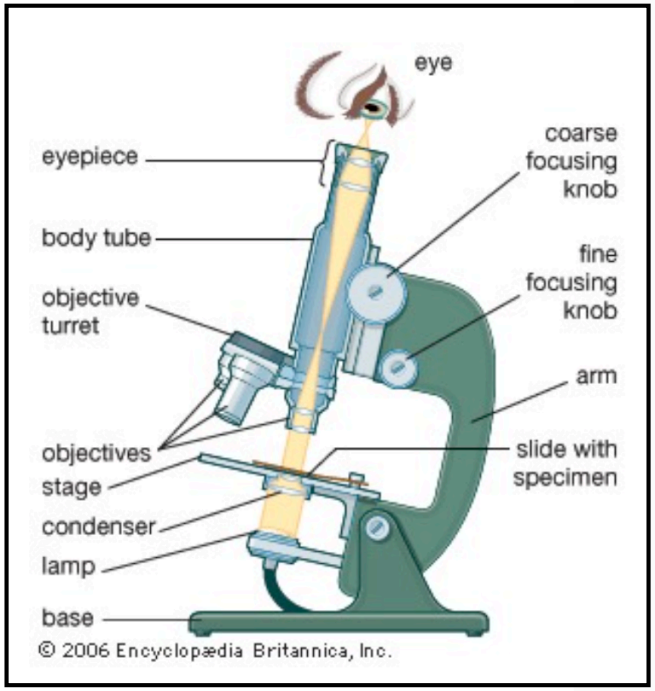
Outlines

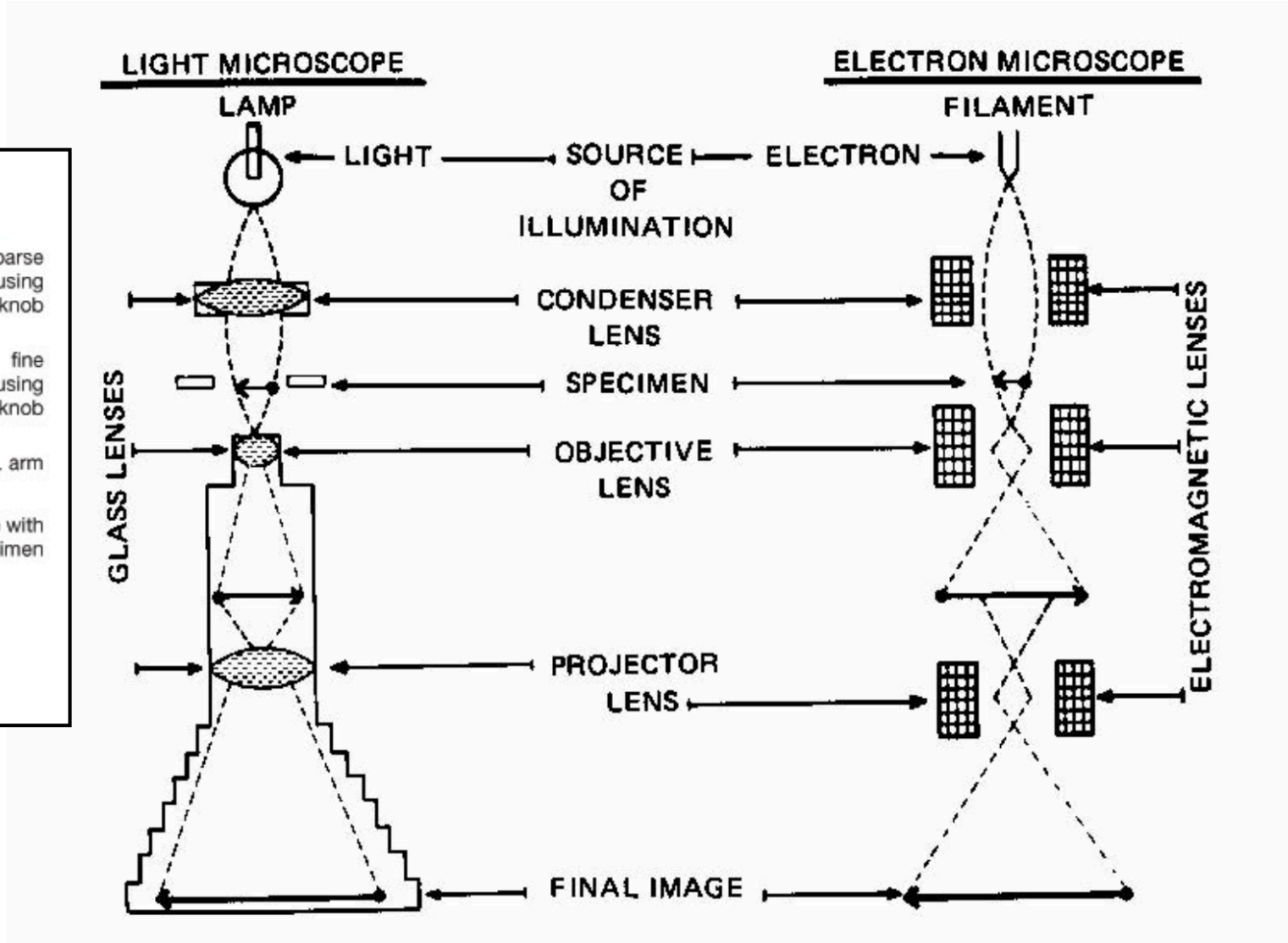
- **Transmission Electron Microscope (TEM)**
- Fourier Transform
- Contrast Transfer Function (CTF)
- 3D Reconstruction

Transmission Electron Microscope (TEM)

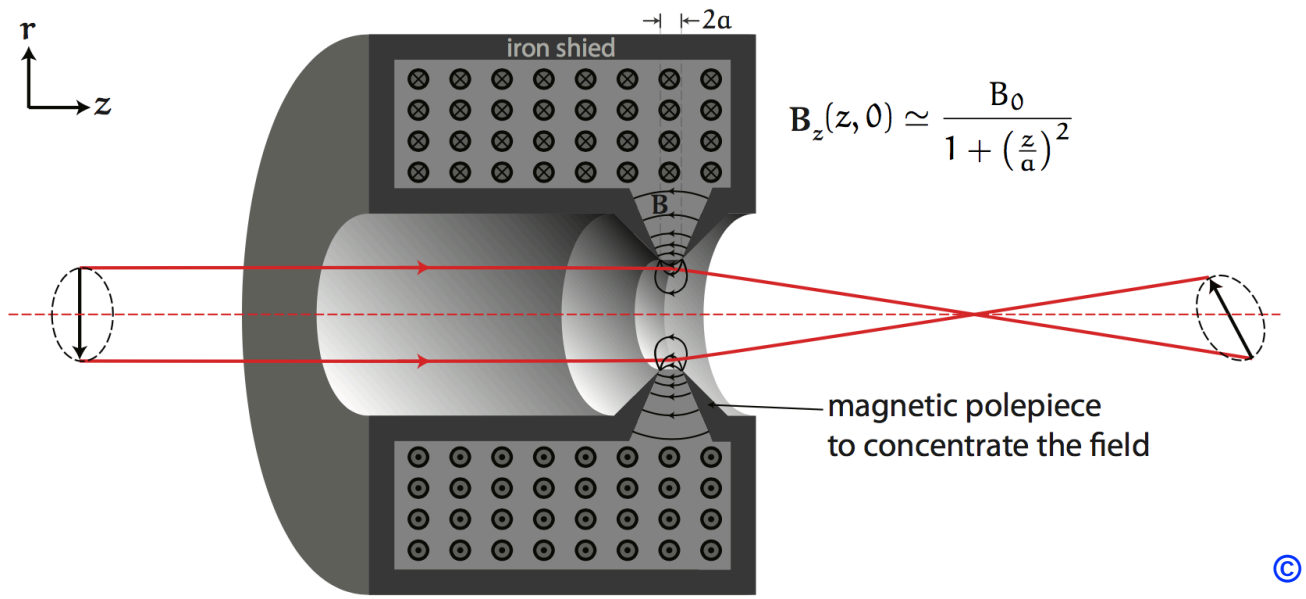


Comparison of Optics





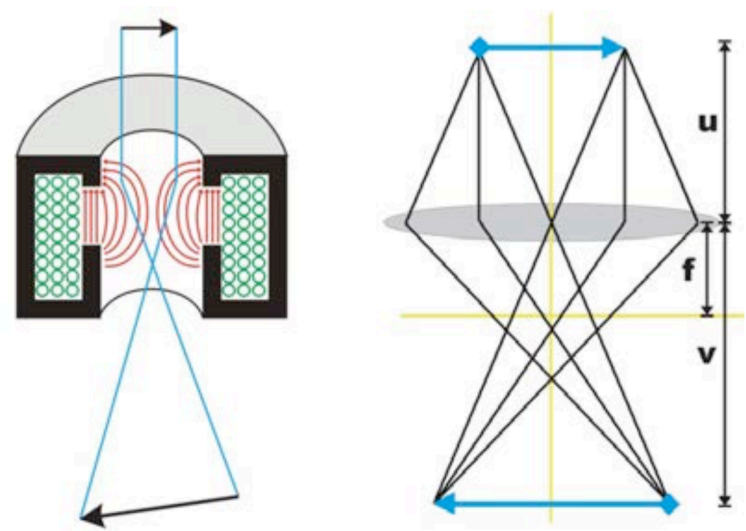
http://www.udel.edu/chem/bahnson/chem645/presentations/Bianco.pdf



$$B_z(z,0) \simeq \frac{B_0}{1+\left(\frac{z}{a}\right)^2}$$

© Chris Russo

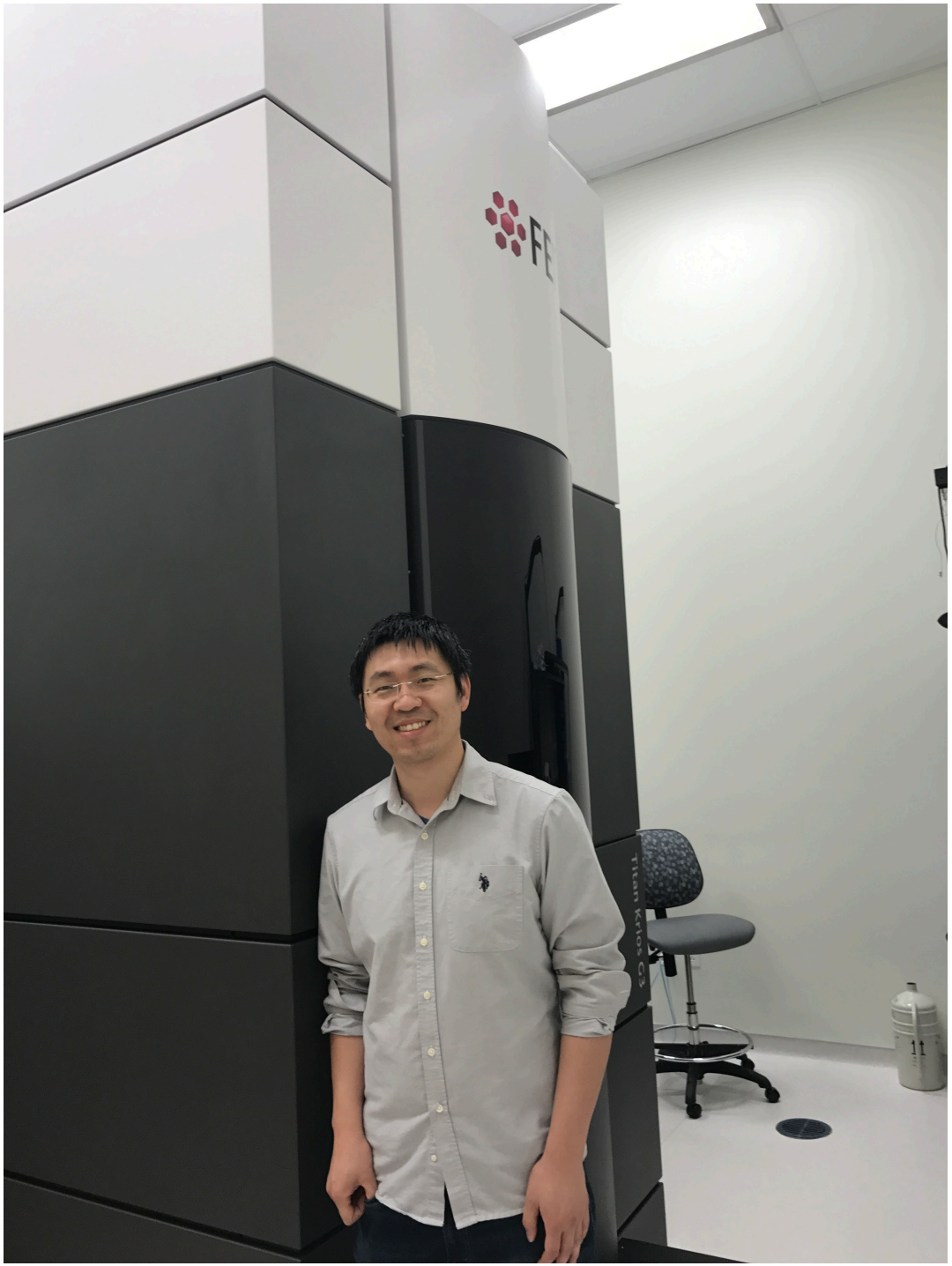
Electromagnetic lens

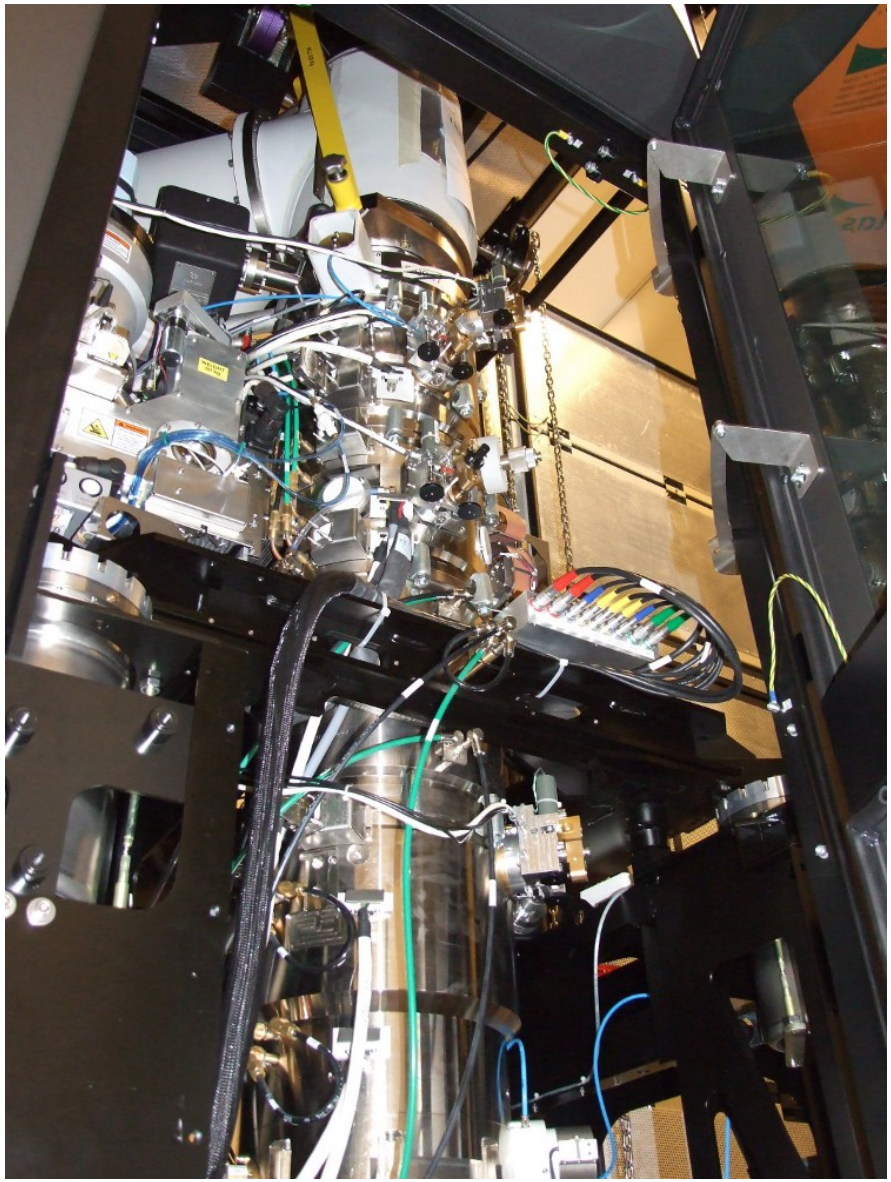


* The focal length of a electromagnetic lens can be easily adjusted by changing the lens current.

© Yifan Cheng

State-of-the-art: FEI Titan Krios





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- **Fourier Transform**
- Contrast Transfer Function (CTF)
- 3D Reconstruction

Light Scattering and Lenses are Described by Fourier Transforms

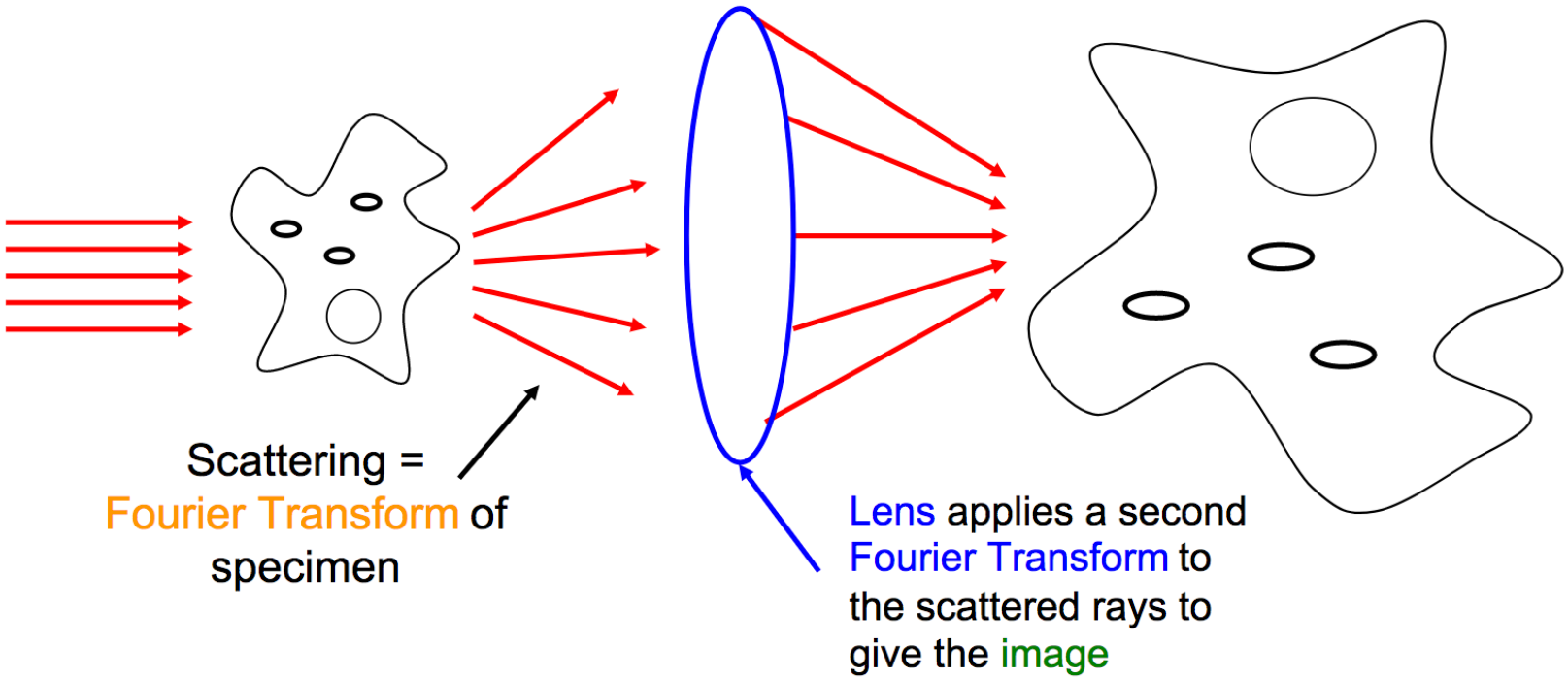
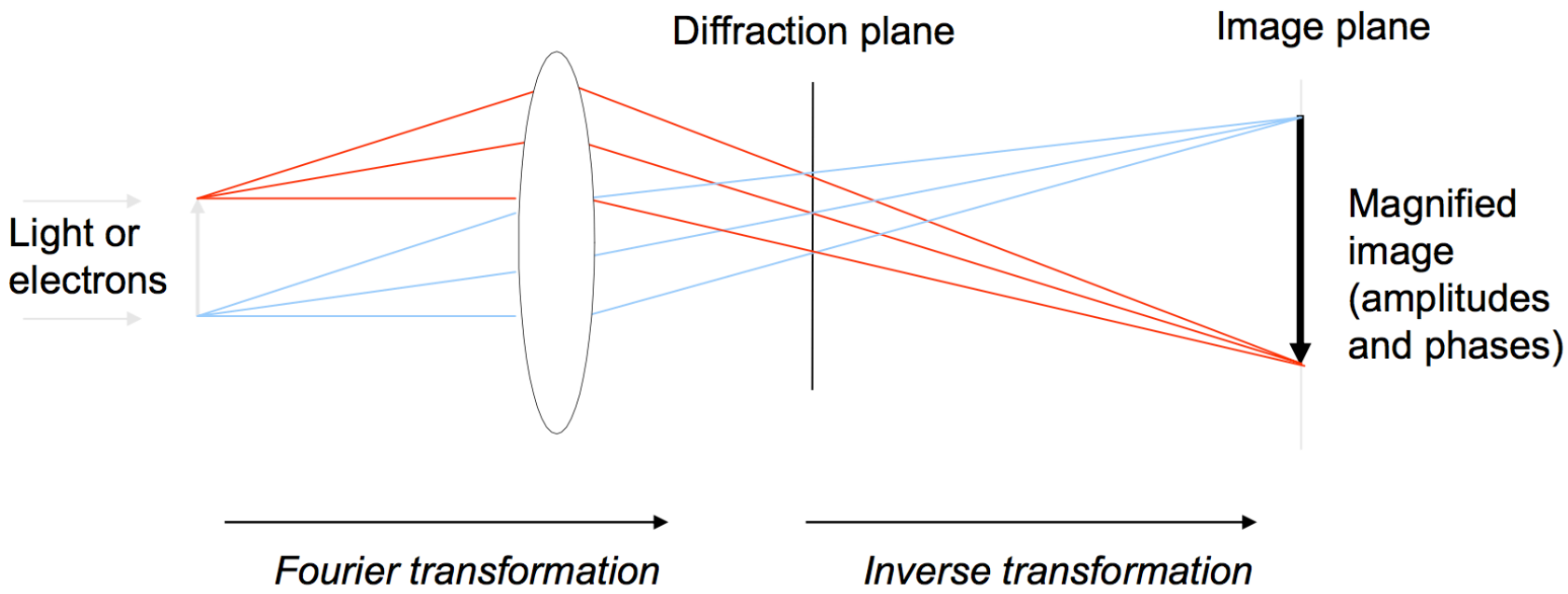
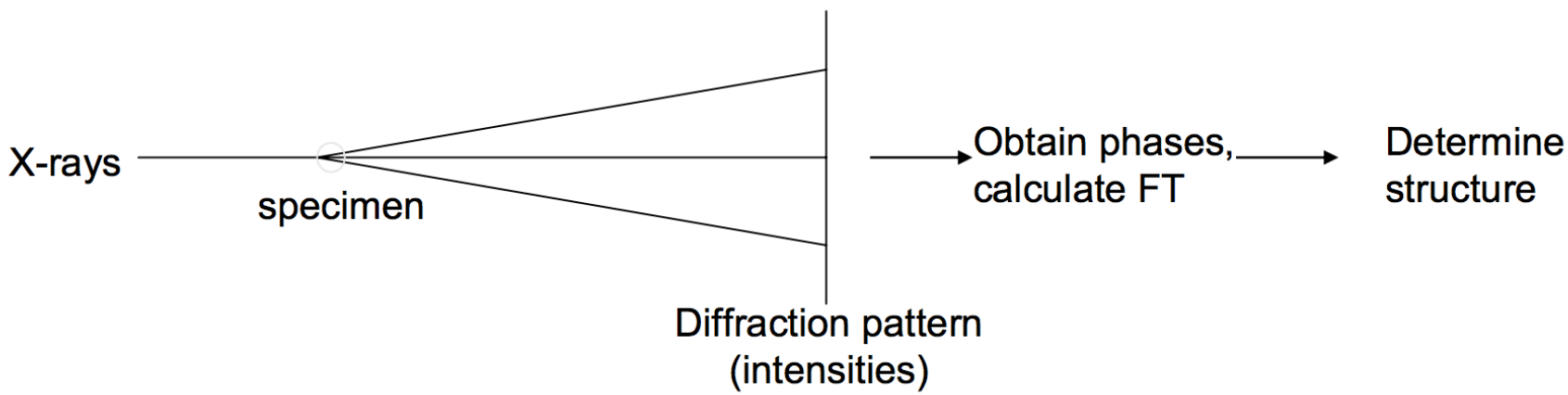
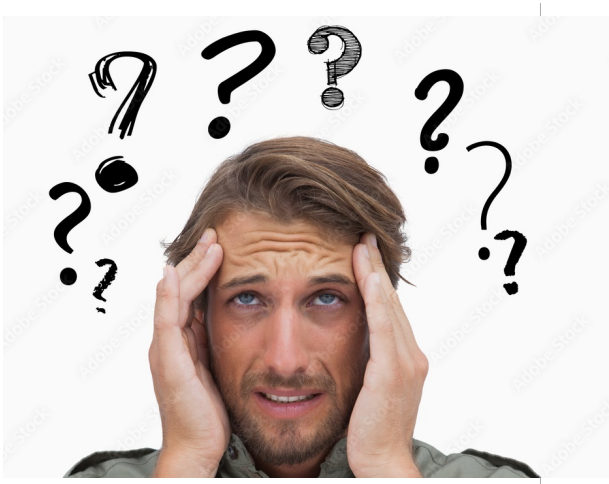


Image formation and diffraction





The Fourier Transform



To get the weights (amount of each frequency): $\mathcal{F}$

$$F(s) = \int_{-\infty}^{\infty} f(t) e^{-i2\pi st} dt$$

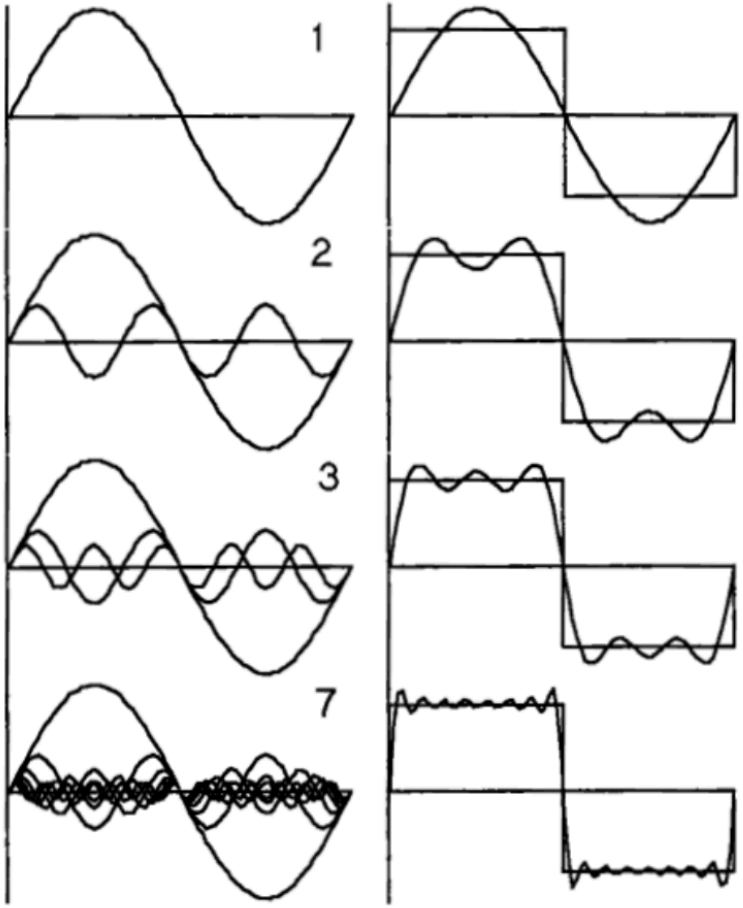
$F(s)$ is the Fourier Transform of $f(t)$: $\mathcal{F}(f(t)) = F(s)$

To convert weights back into a signal (invert the transform):

$$f(t) = \int_{-\infty}^{\infty} F(s) e^{i2\pi st} ds$$

$f(t)$ is the Inverse Fourier Transform of $F(s)$: $\mathcal{F}^{-1}(F(s)) = f(t)$

Fourier synthesis - adding up waves to build a shape



constituent waves summed waves

Fourier analysis - determining the waves components of a shape

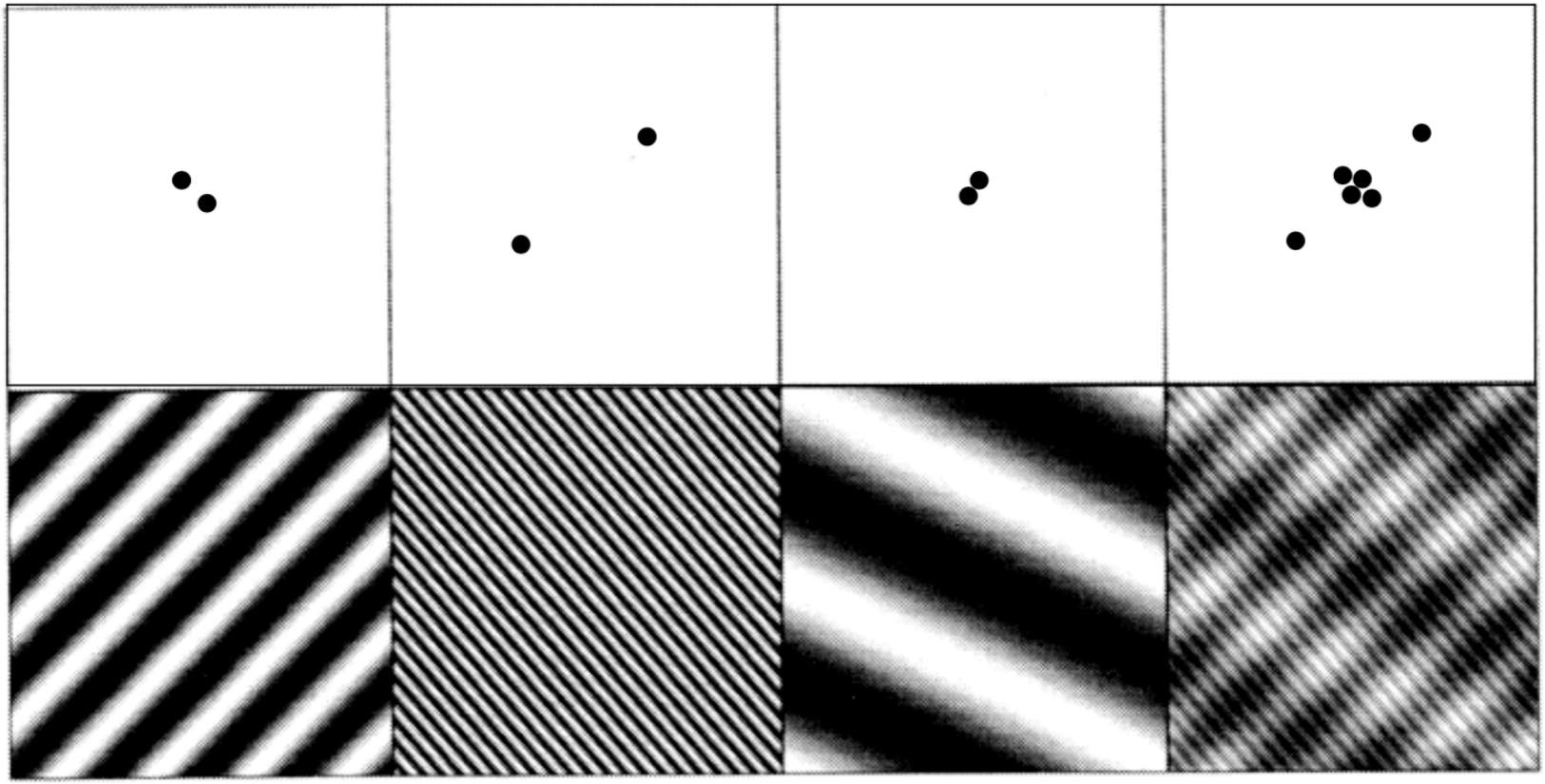
Understand Fourier Transform Intuitively

	Real space	Reciprocal space
Coarse features	Large distances ⟷	Low frequencies 
Fine details	Small distances ⟷	High frequencies 
	object	diffraction pattern

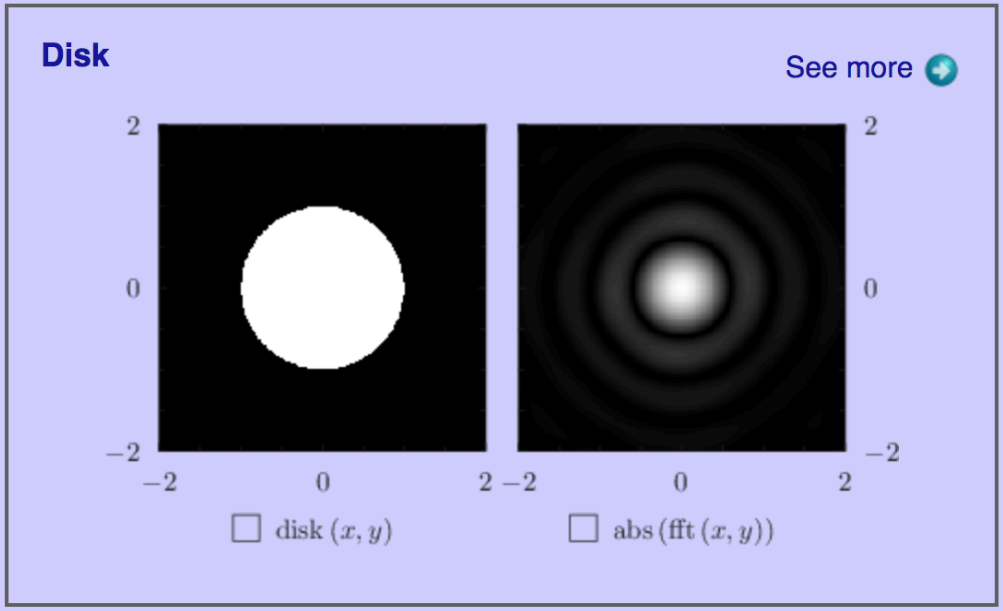
Properties of FTs

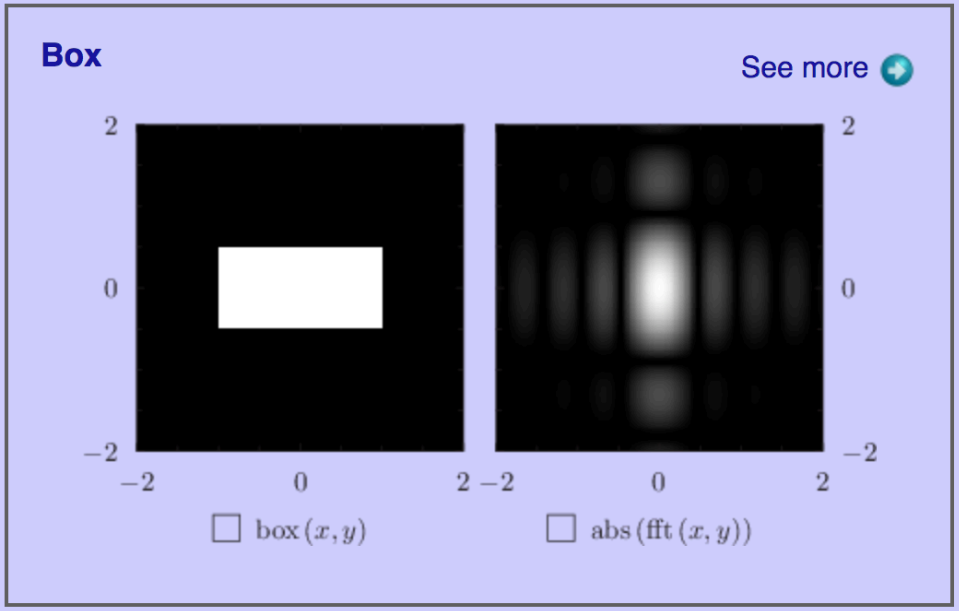
Rotation

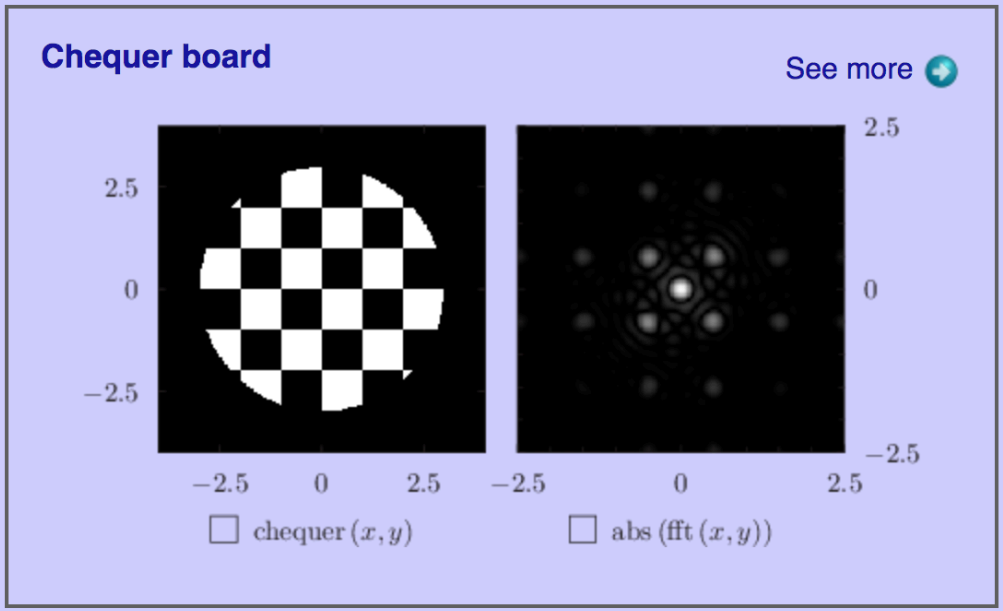
Addition



2D Fourier Transform of different shapes







http://pyxplot.org.uk/examples/08fourier/index.html

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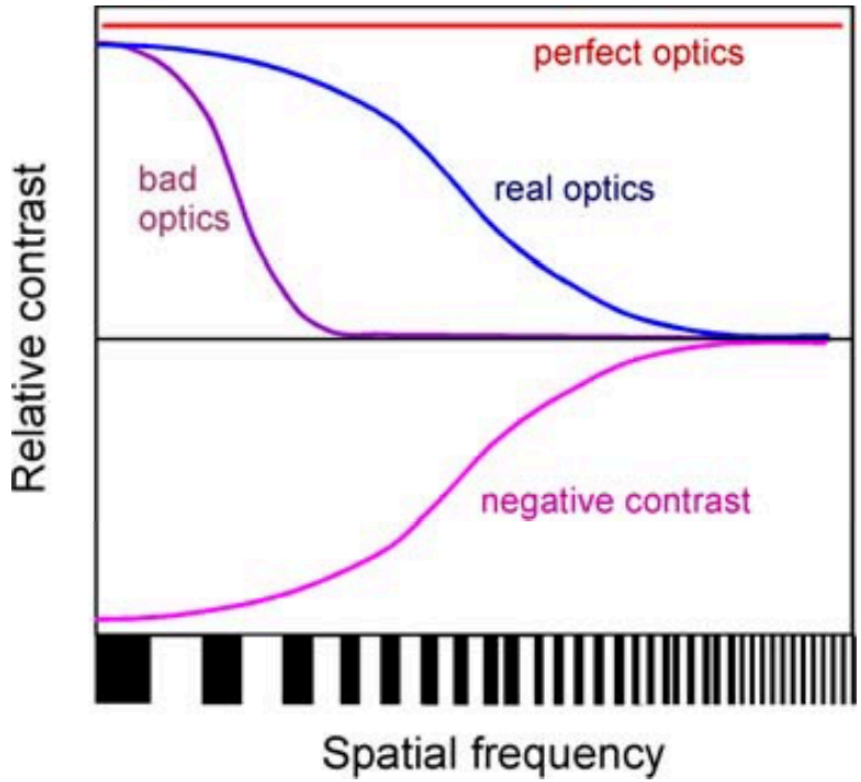
- Transmission Electron Microscope (TEM)
- Fourier Transform
- **Contrast Transfer Function (CTF)**
- 3D Reconstruction

Contrast transfer



perfect optics | normal optics | bad optics | negative contrast

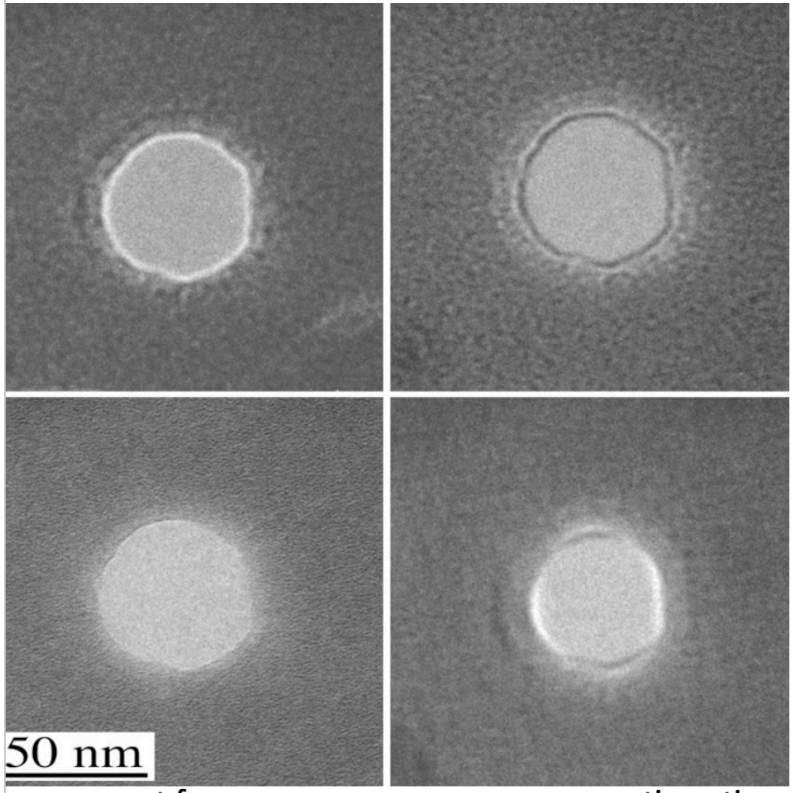
Phase CTF formula from the weak phase approximation

$$\text{Phase CTF} = -2 \sin\left[\pi\left(\Delta z \lambda q^2 - C_s \lambda^3 q^4/2\right)\right]$$

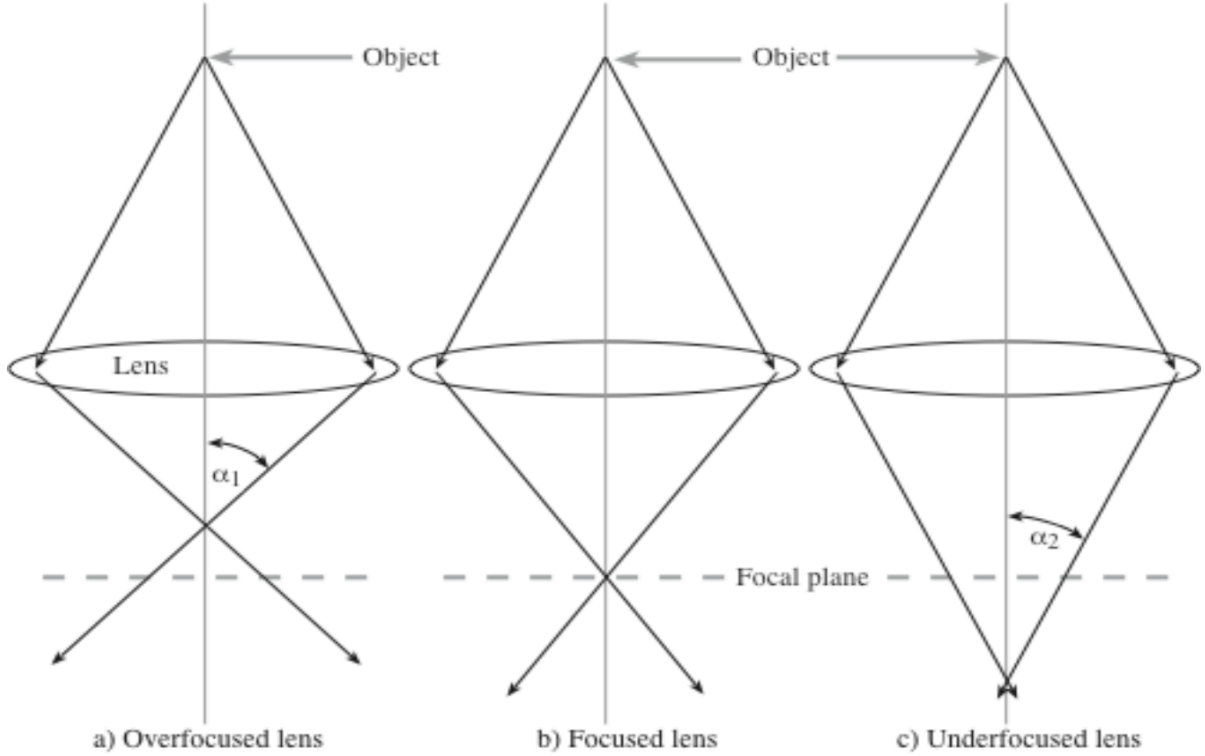
C_s – spherical aberration coefficient
ΔZ – defocus
q – spatial frequency
λ – electron wavelength

Lens terminology

underfocus overfocus

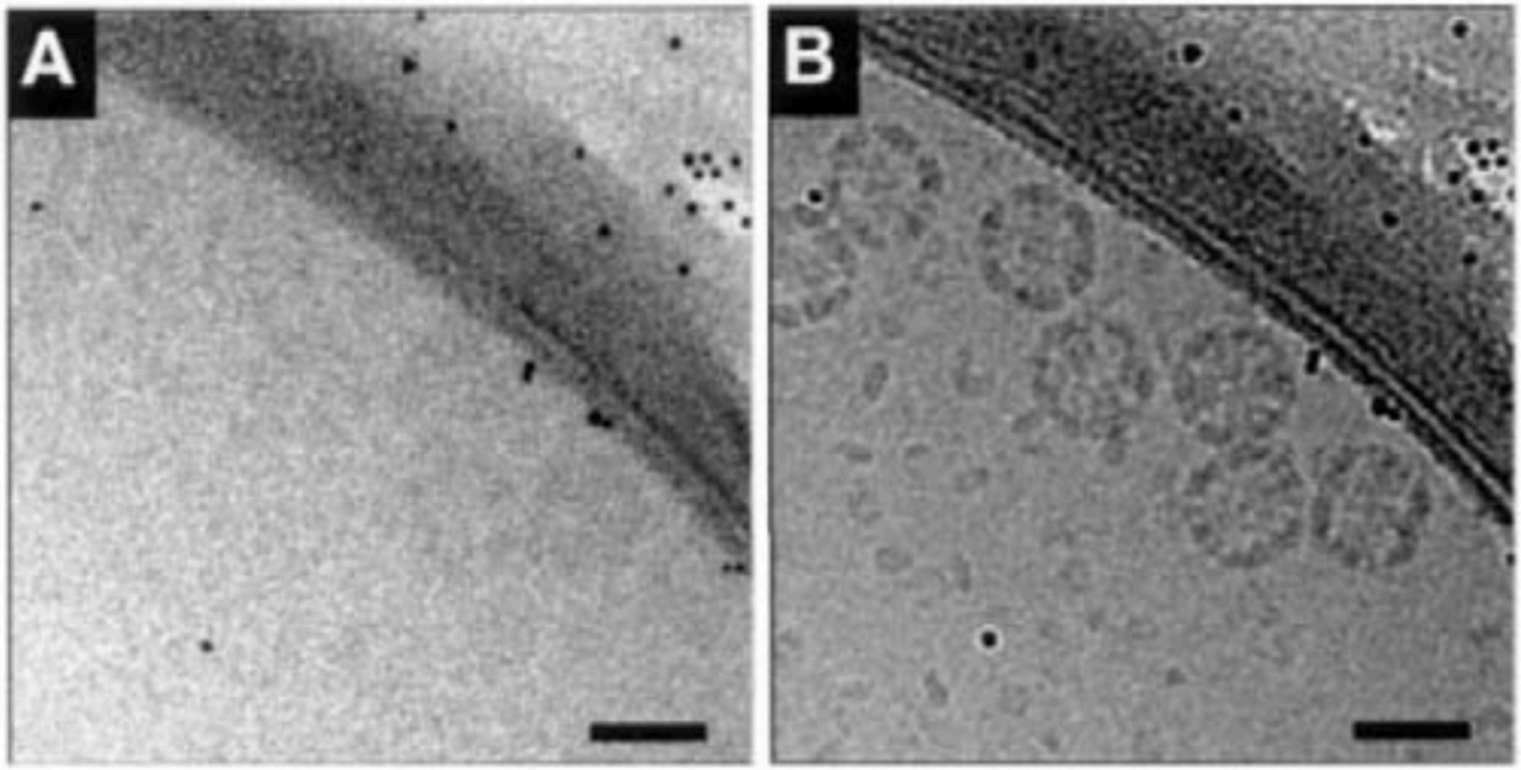


exact focus astigmatism



Too strong Just right Too weak

Why do we need to bother with defocus?



2 μm 7 μm

Tricorn protease, Walz, J et al (1997) Mol Cell 1, 59-65

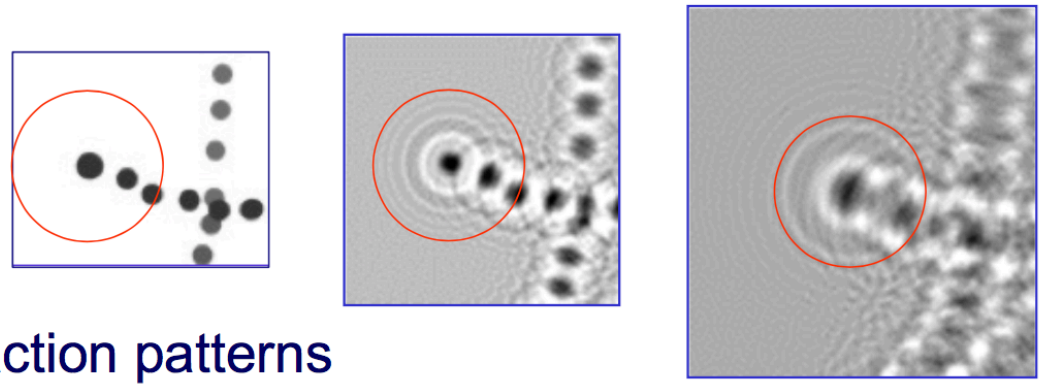
The CTF is the FT of the Point Spread Function

In focus

Defocus 1

Defocus 2

Point spread functions

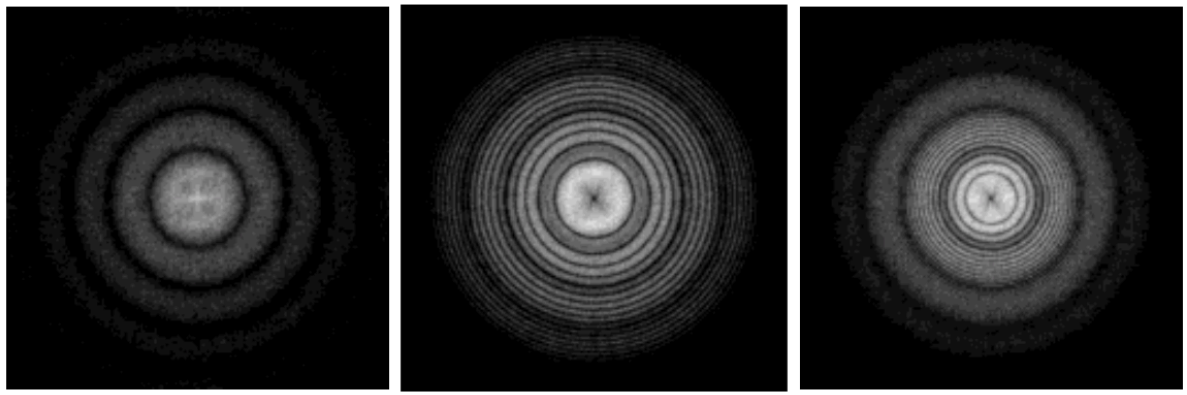


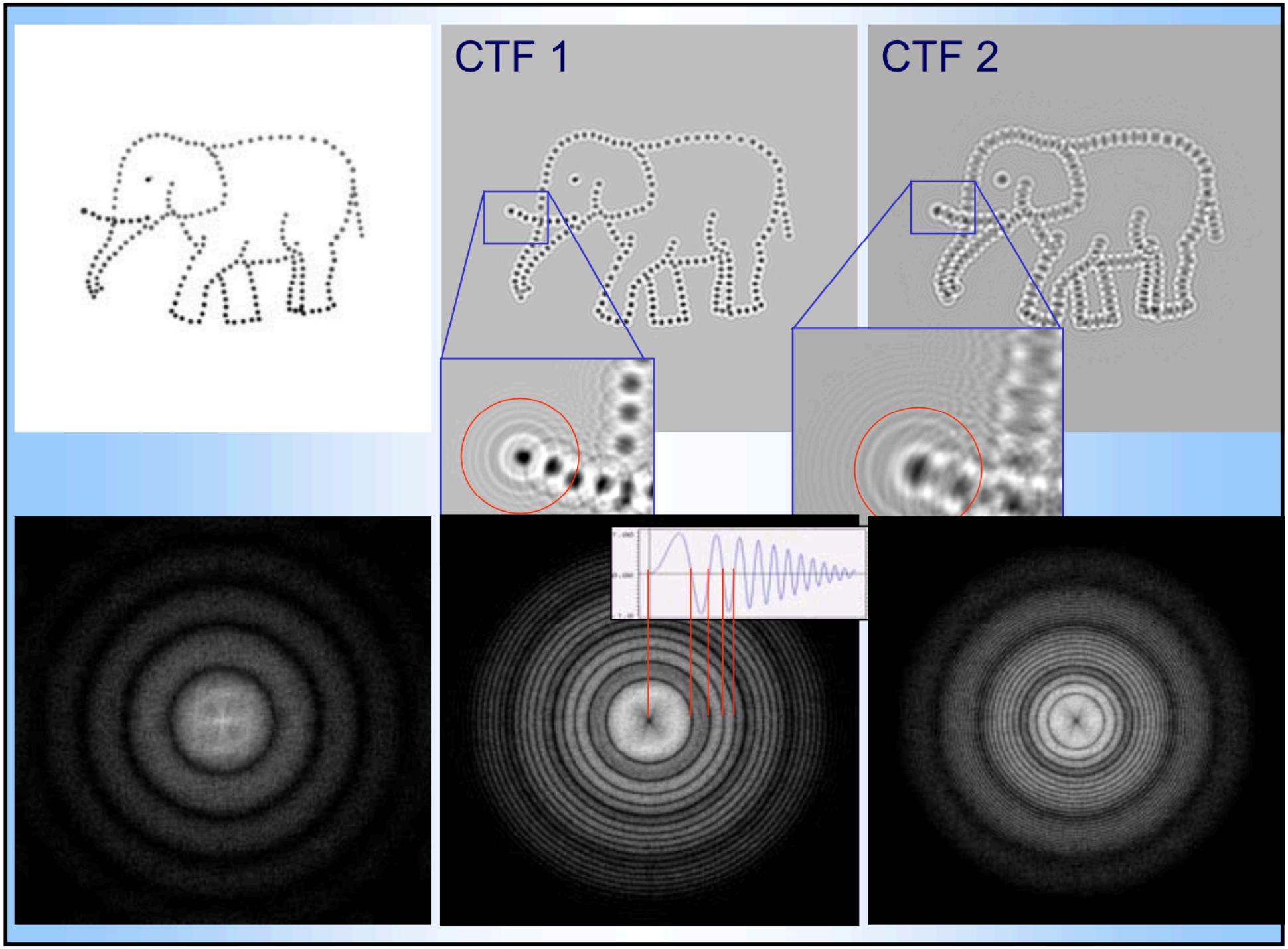
PSF

FT

CTF

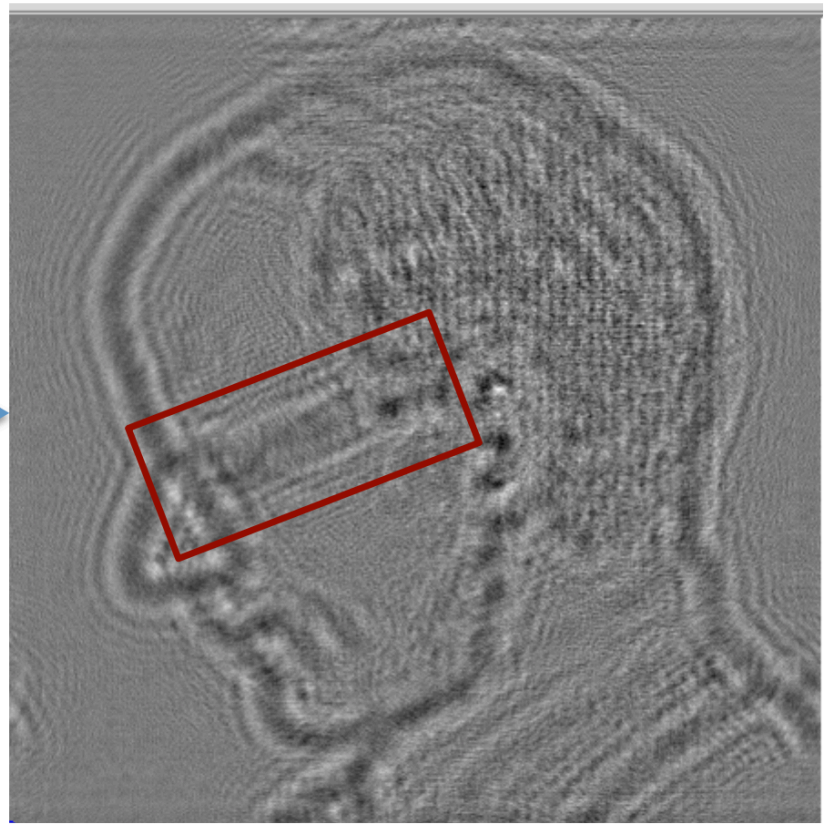
Diffraction patterns





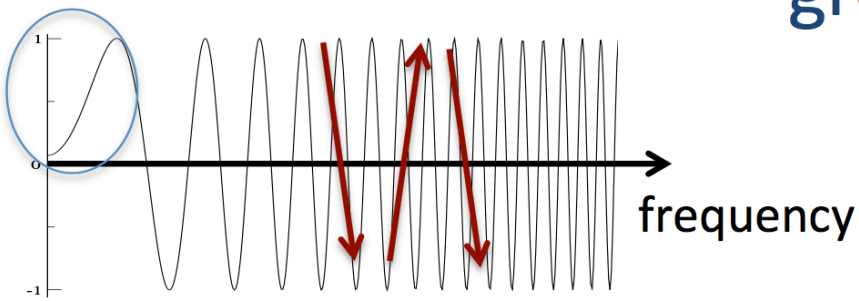
CTF effects

Delocalisation!

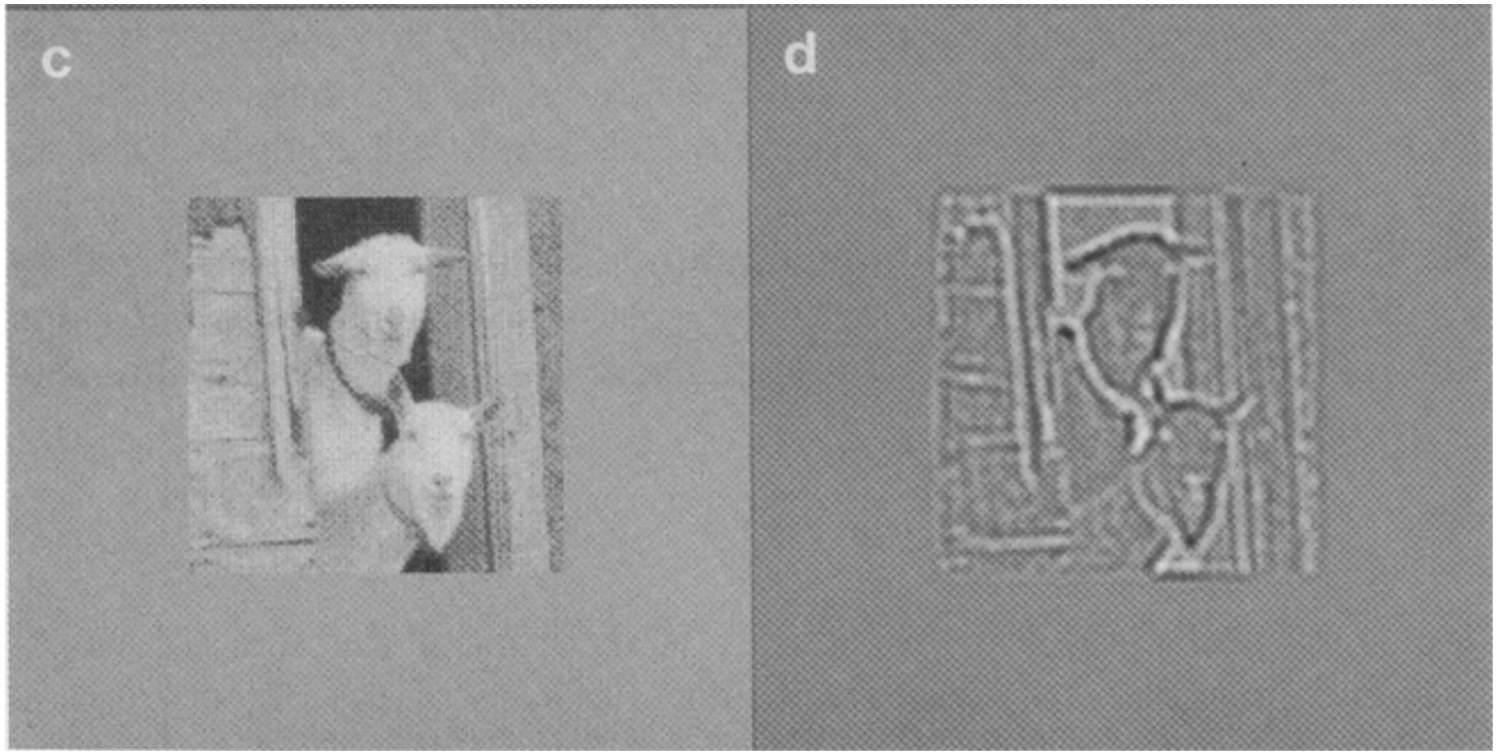


Black-white

grey



What is this CTF thing anyway and why do I care?



Distortions of CTF to the image are:
1) Contrast reverve of large area; 2) diminished contrast in large area; 3) edge enhancement and 4) appearance of fringes along the borders.

From Joachim Frank

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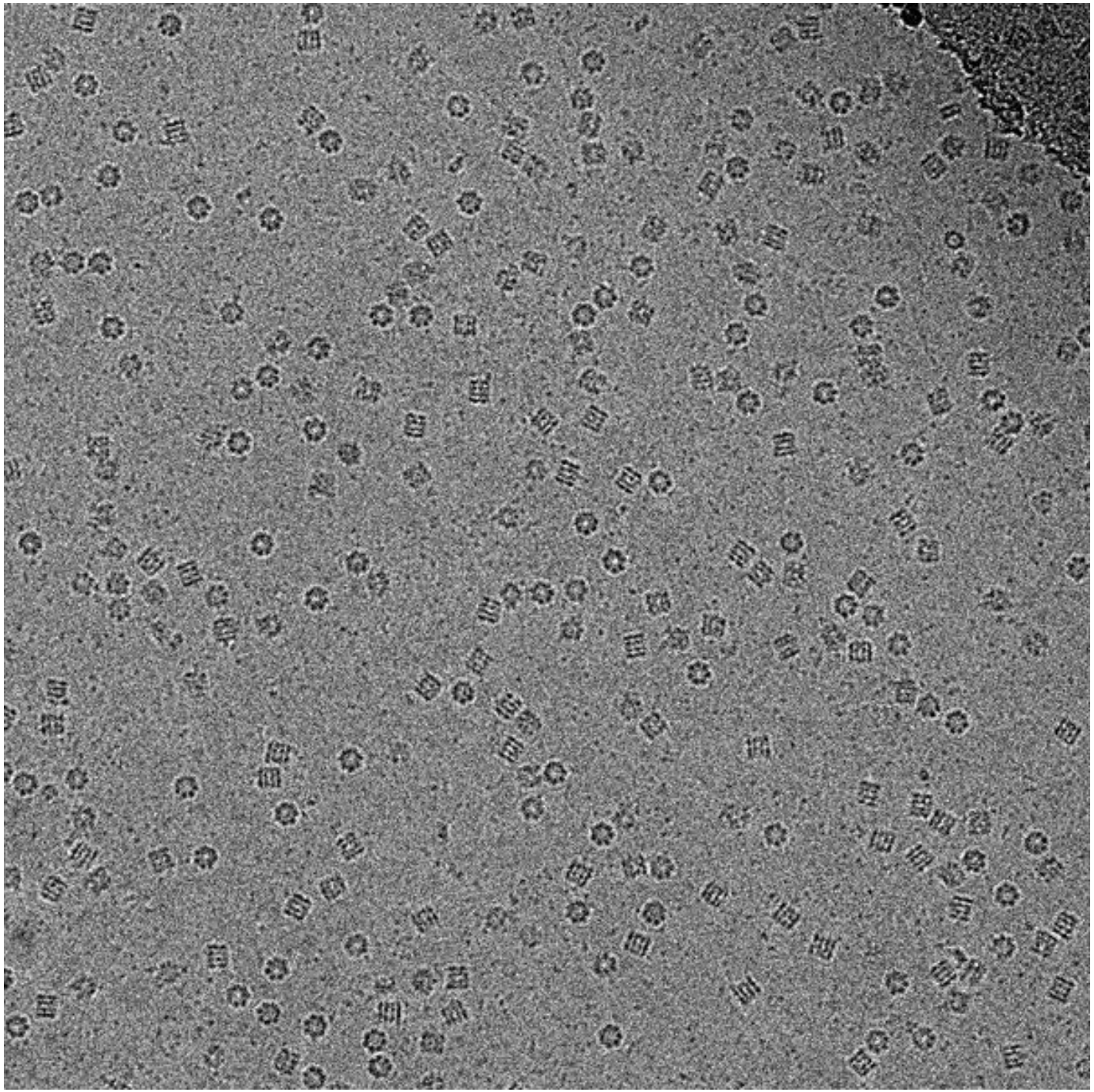
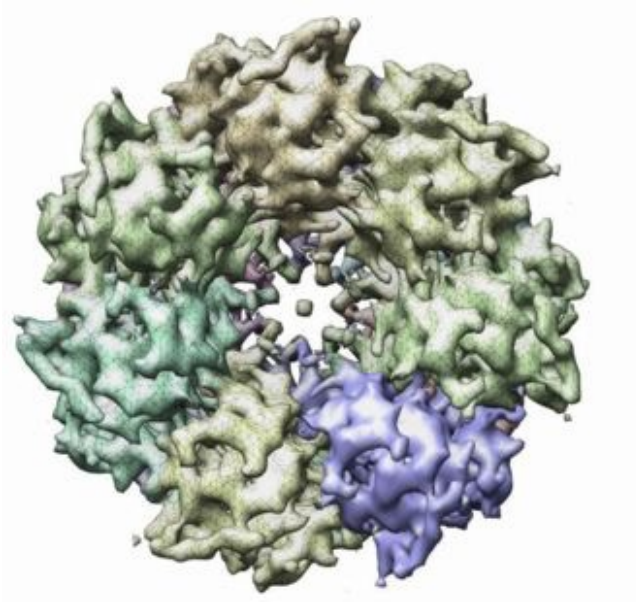
- Fourier Transform
- Contrast Transfer Function (CTF)
- **3D Reconstruction**

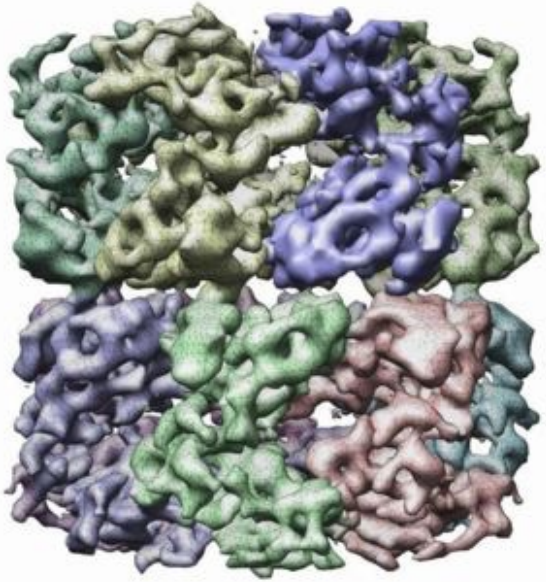
Cryo-EM image and structure of GroEL

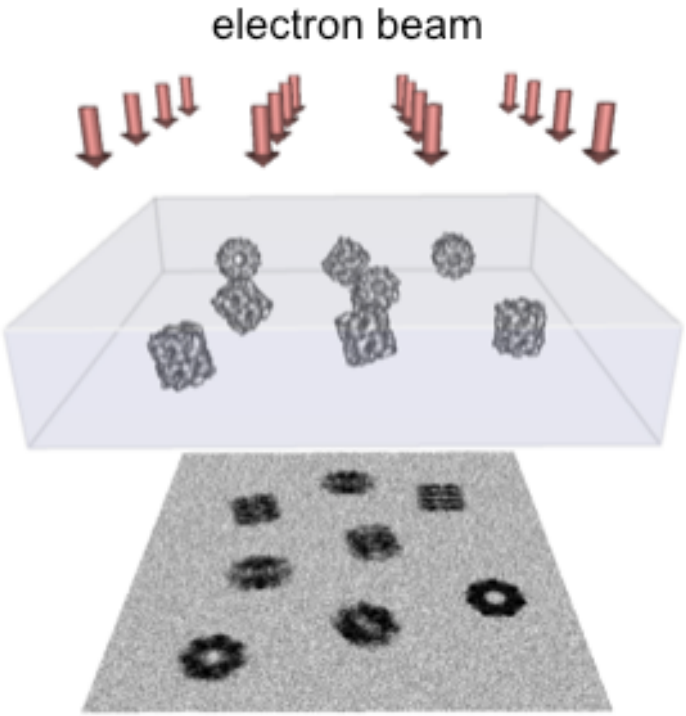


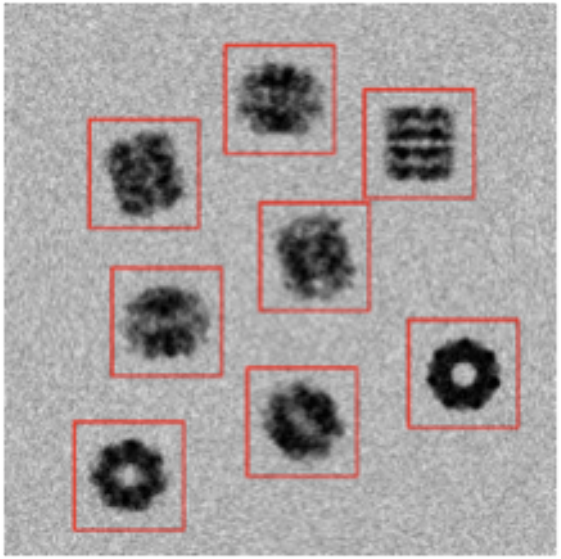
top view

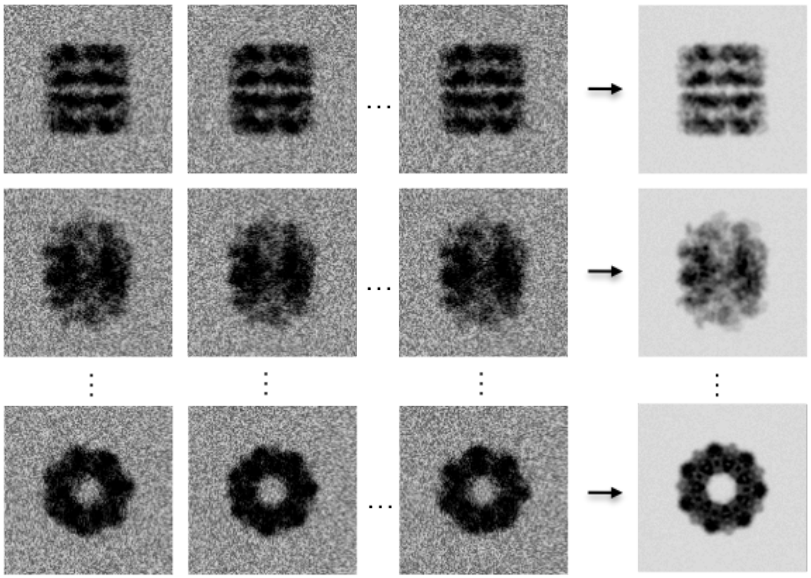


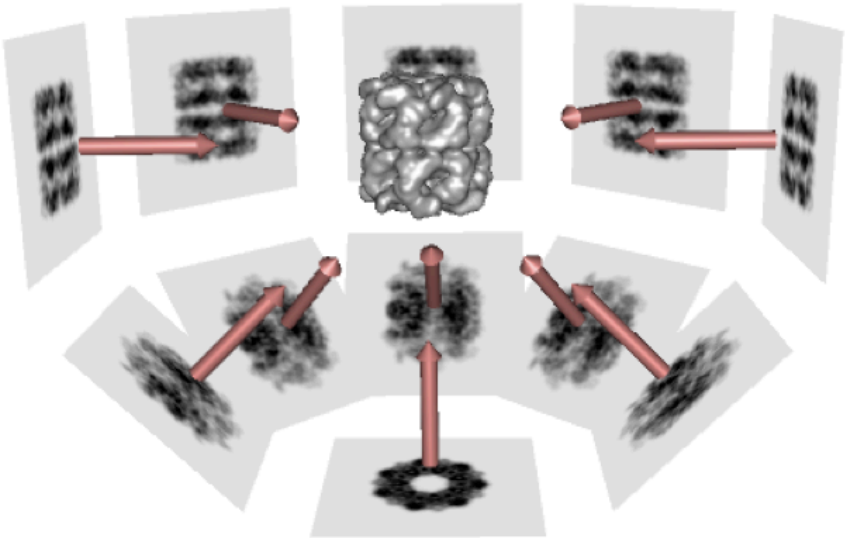
side view





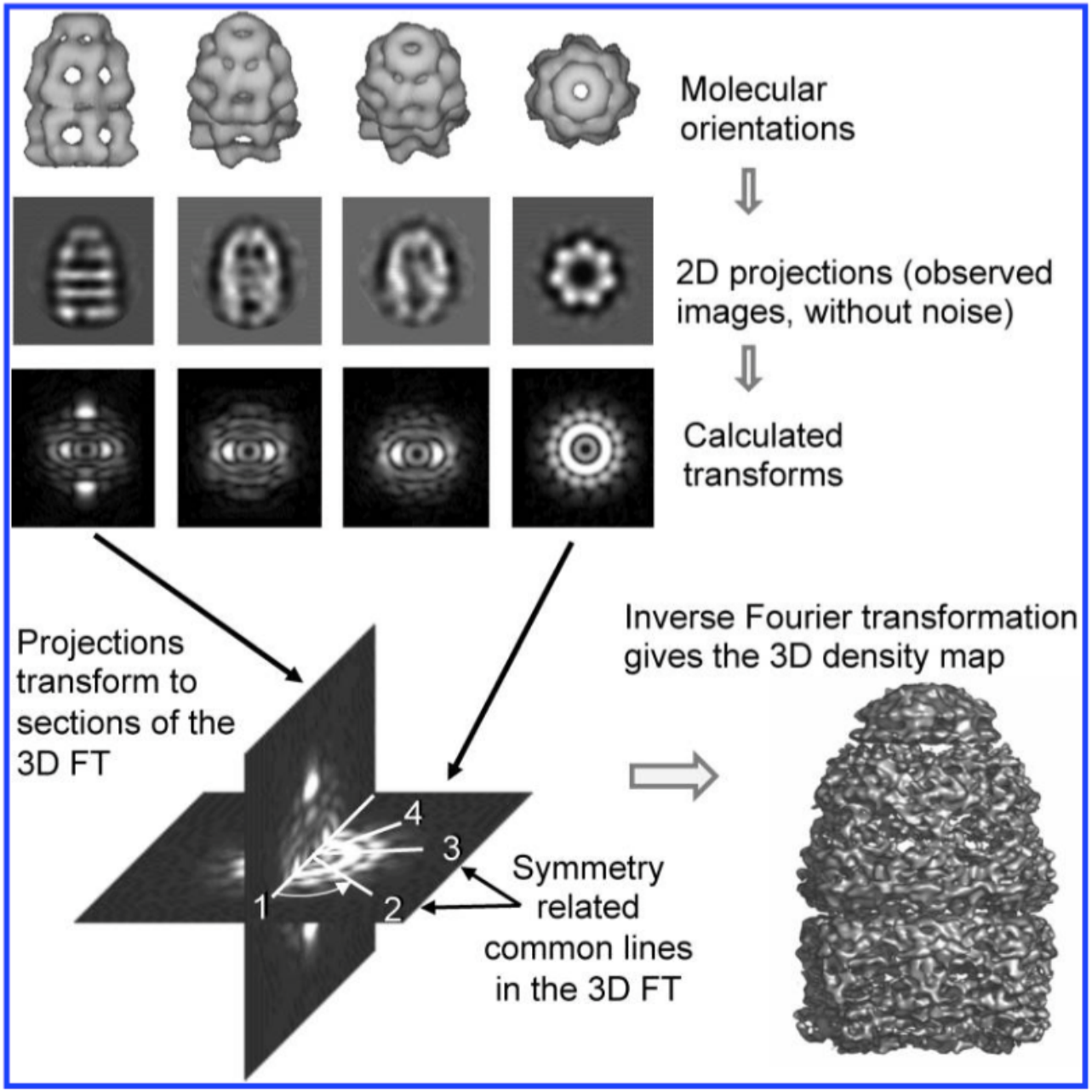






Projection-Slice Theorem

- The F.T. of a 2D projection of a 3D object is a central slice through the 3D F.T. of that object
- Vector normal to slice = projection direction



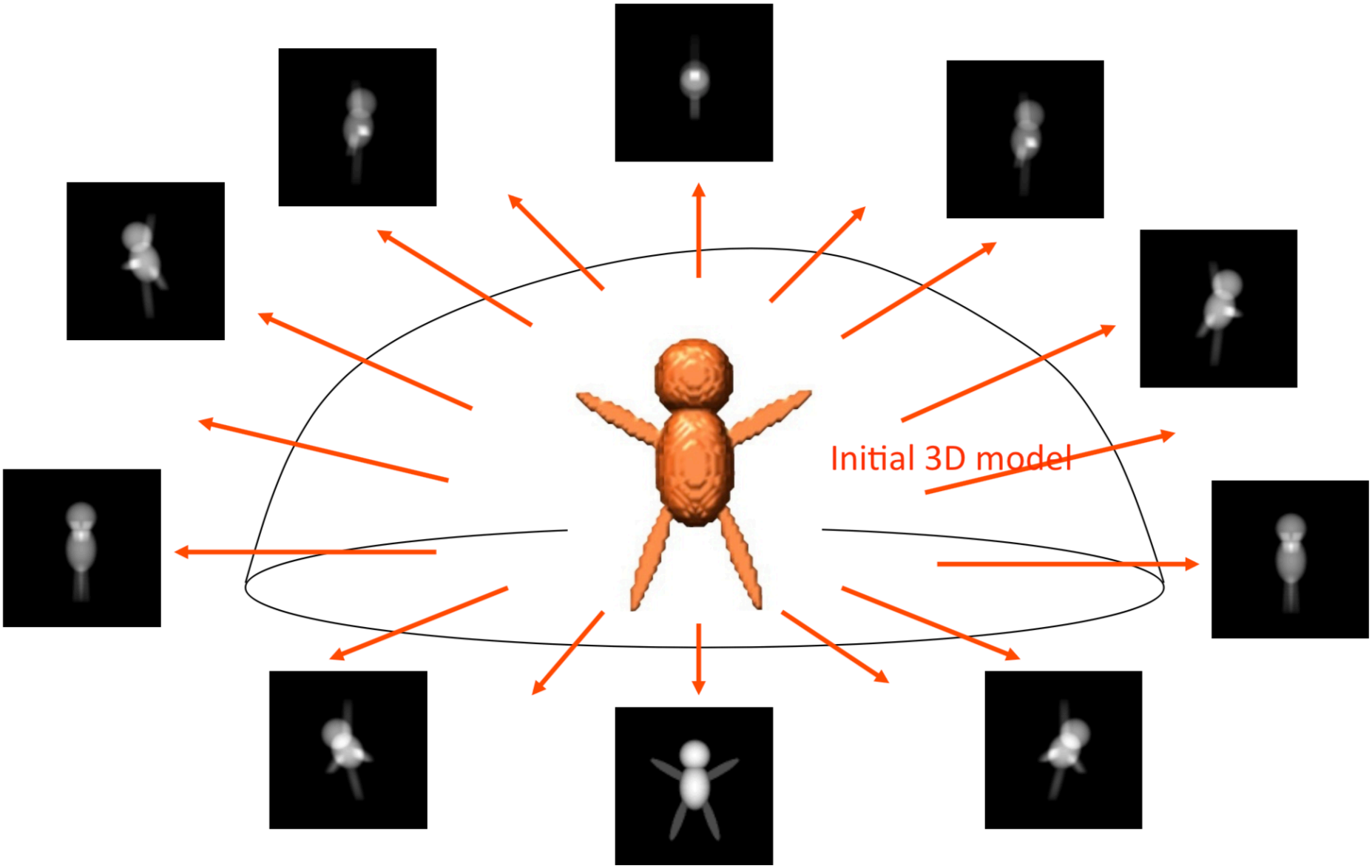
Orlova, E. V. & Saibil, H. R. (2011) Chem. Rev.

Single particle analysis

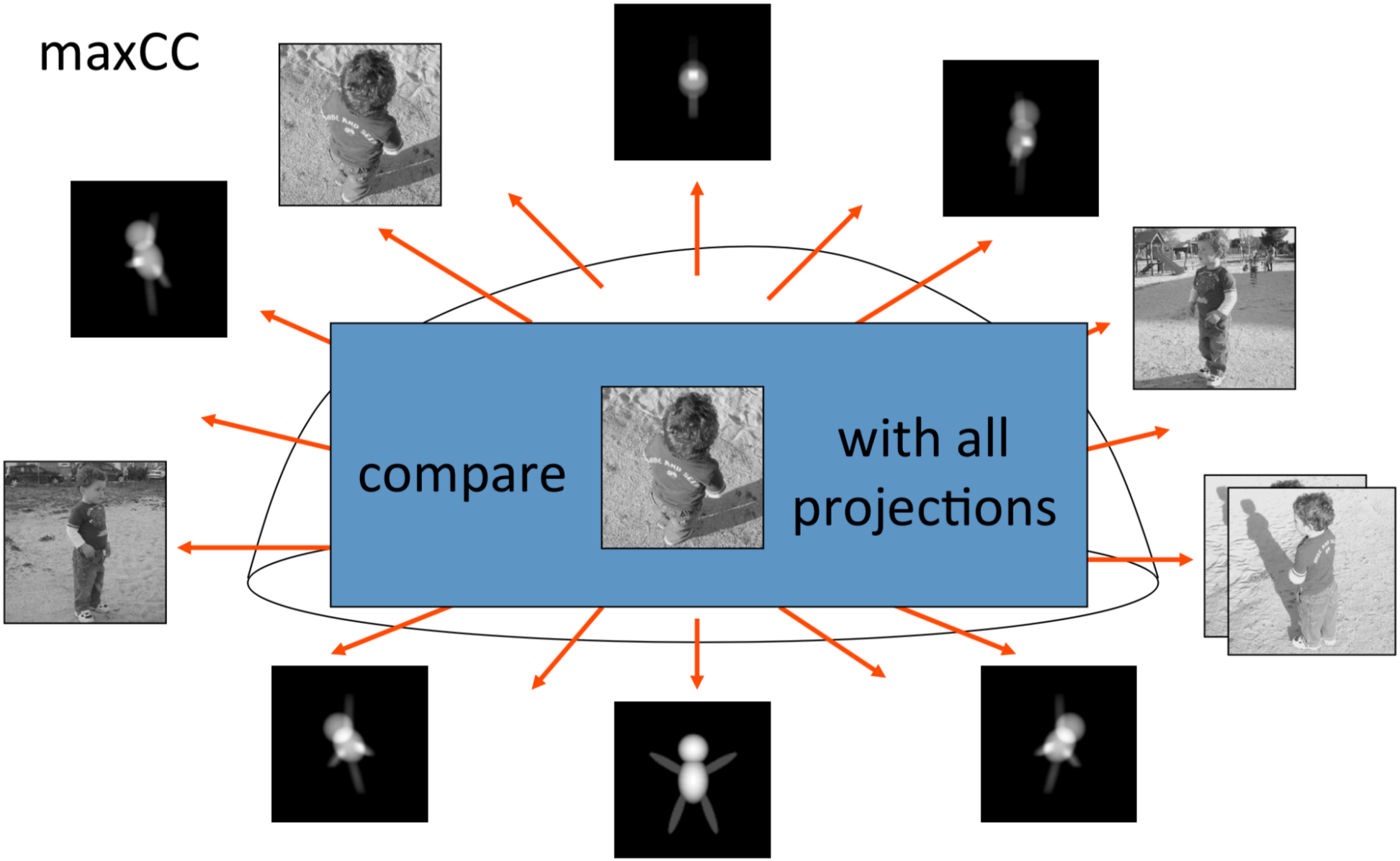
- Embedded in ice: many unknown orientations

- Combine all 2D projections into a 3D reconstruction

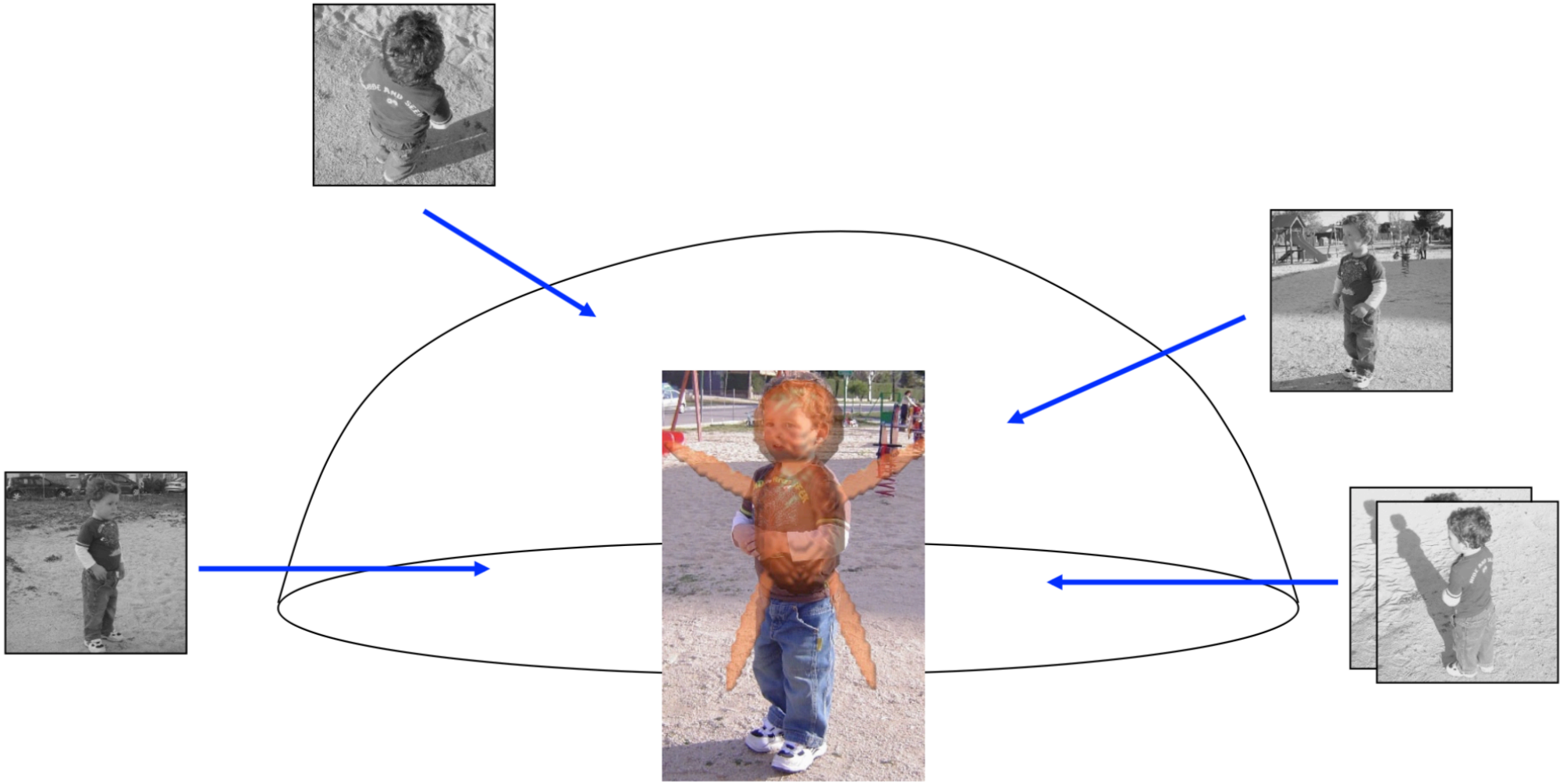
Projection matching



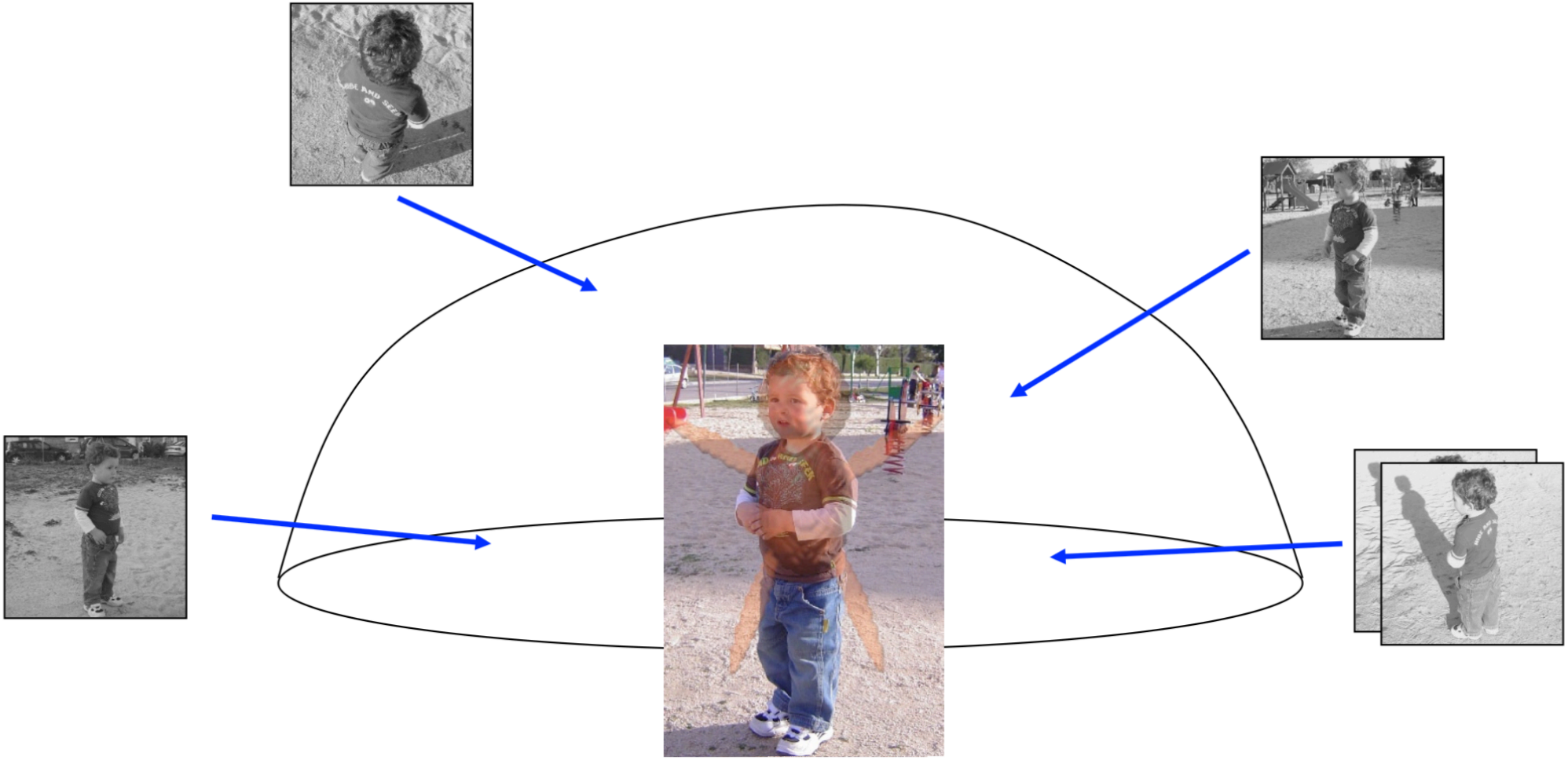
Projection matching



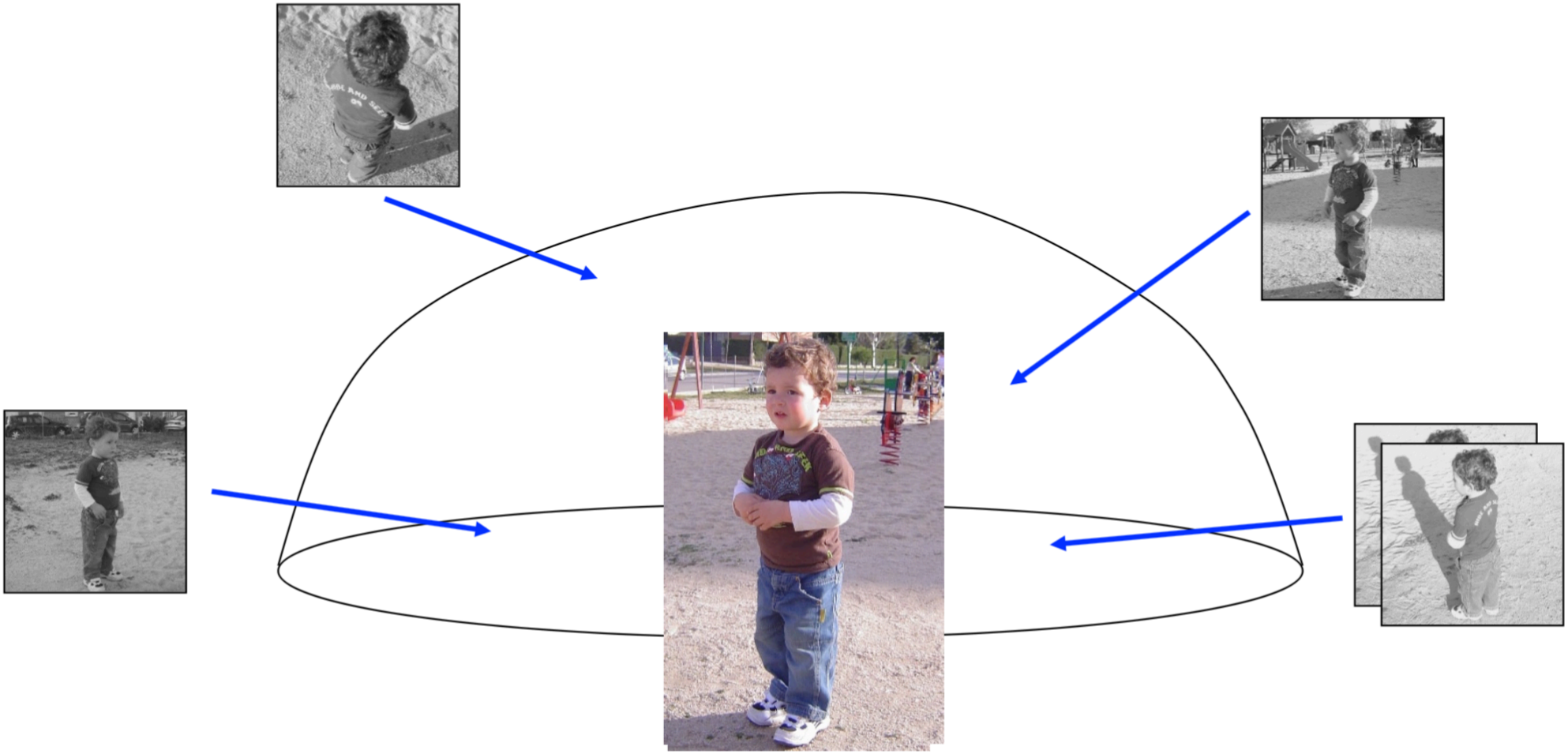
3D reconstruction



Iterative refinement



Iterative refinement



Thank You!