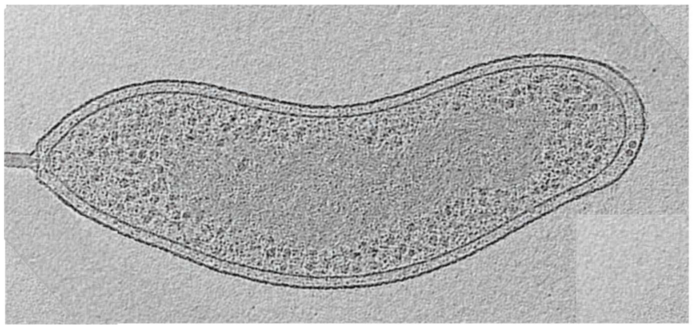
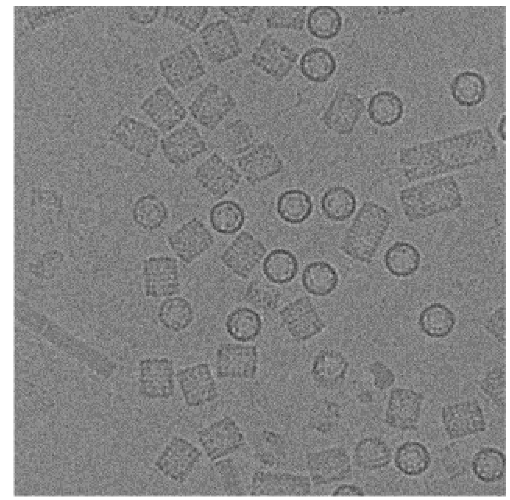
Part 7: Electron crystallography

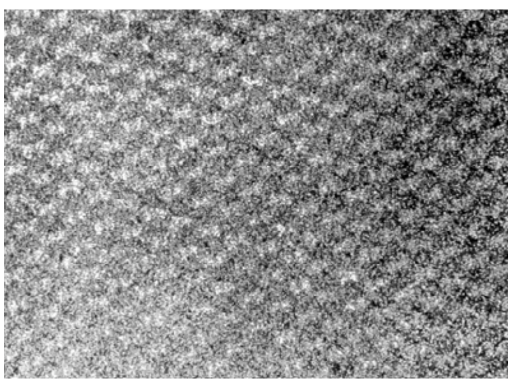
Basic approaches in cryo-EM



Tomography

Single particle analysis

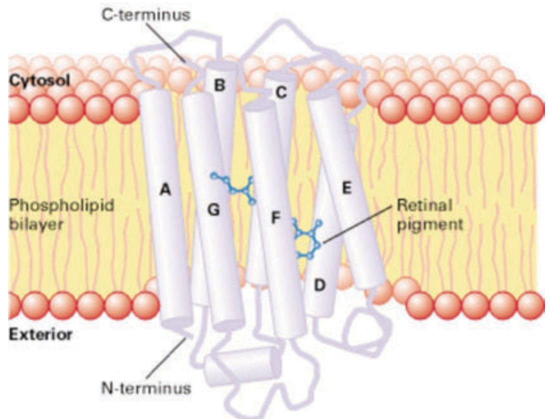




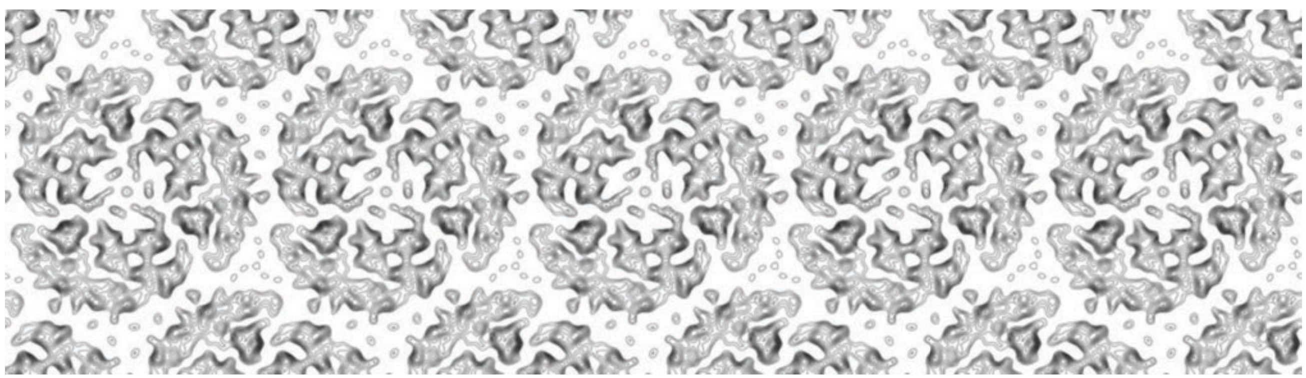
2D crystallography

Some proteins naturally assemble into 2D arrays

Example: bacteriorhodopsin

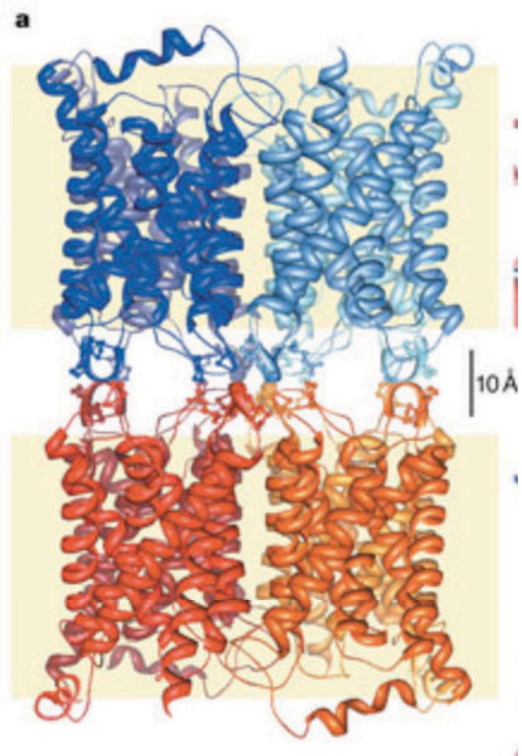


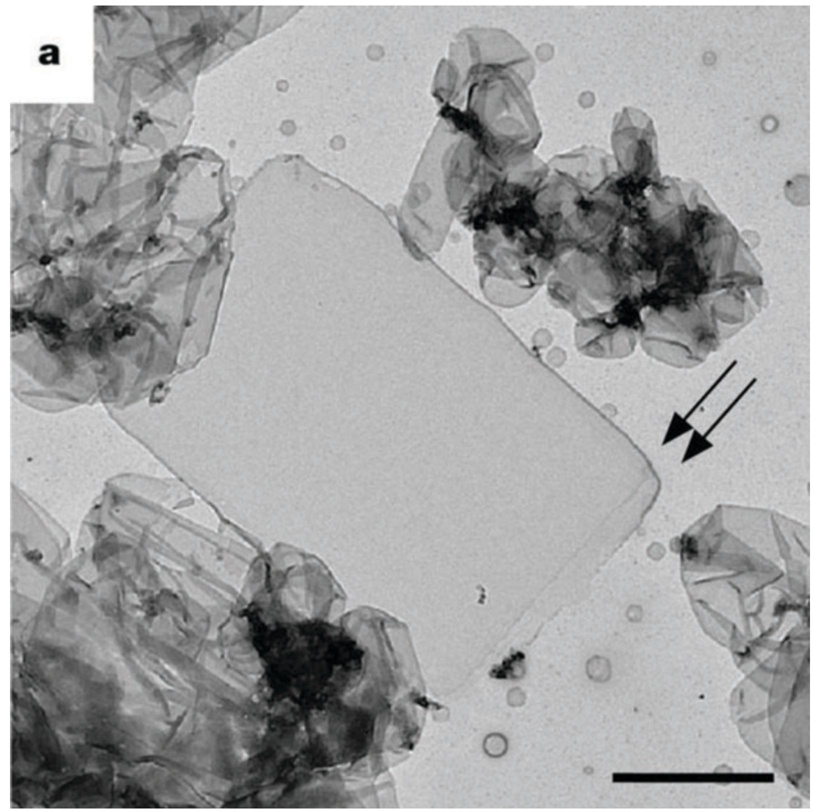
http://www.unitus.it/scienze/corsonew/lezione11.html



Grigorieff et al., JMB 1995

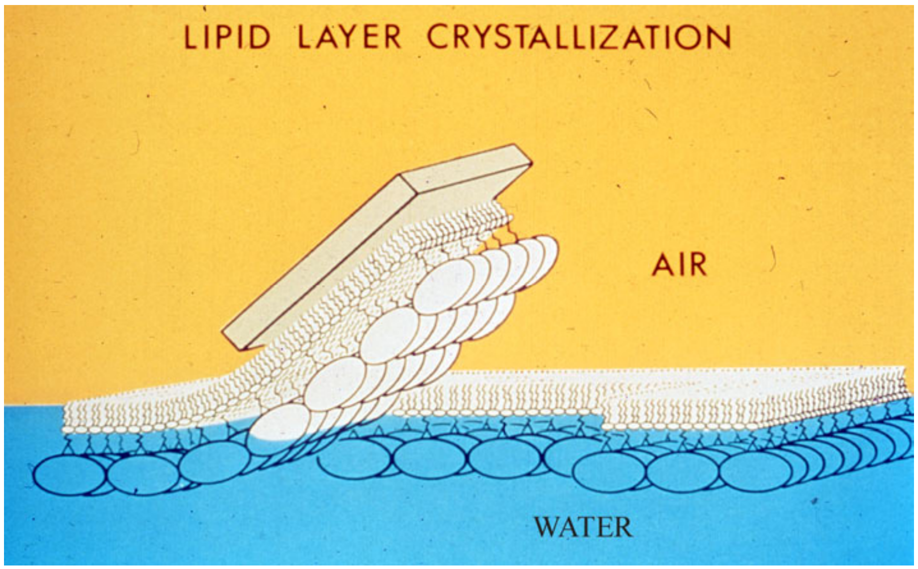
Second example: aquoporin

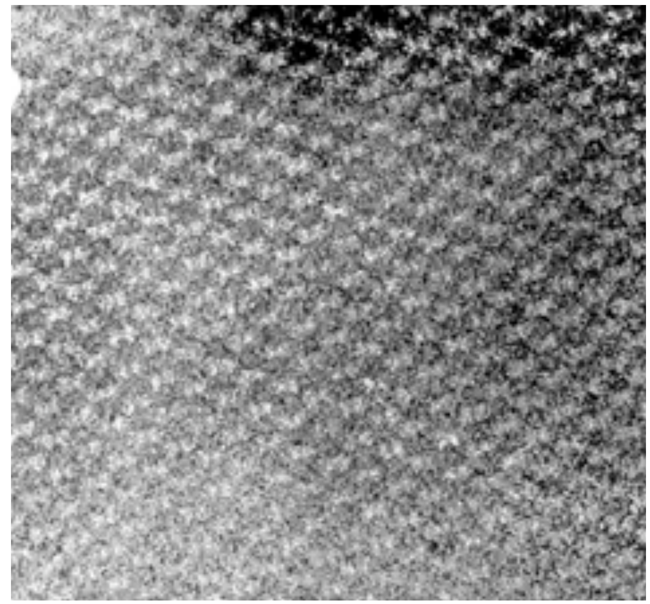




Gonen et al., Nature 2004

Others can be crystallized in-vitro

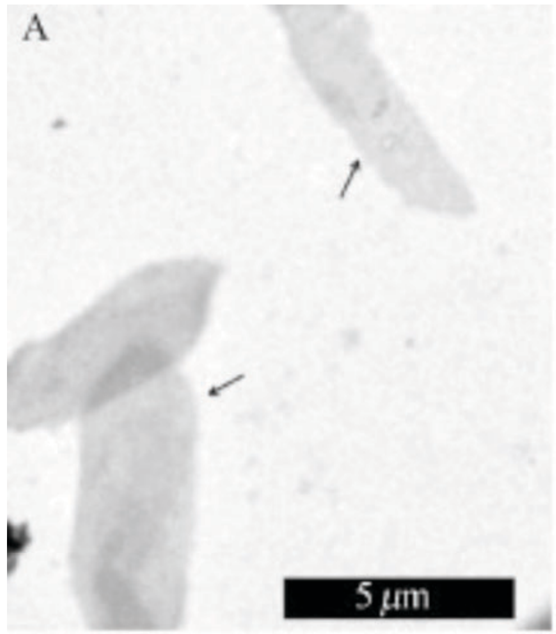




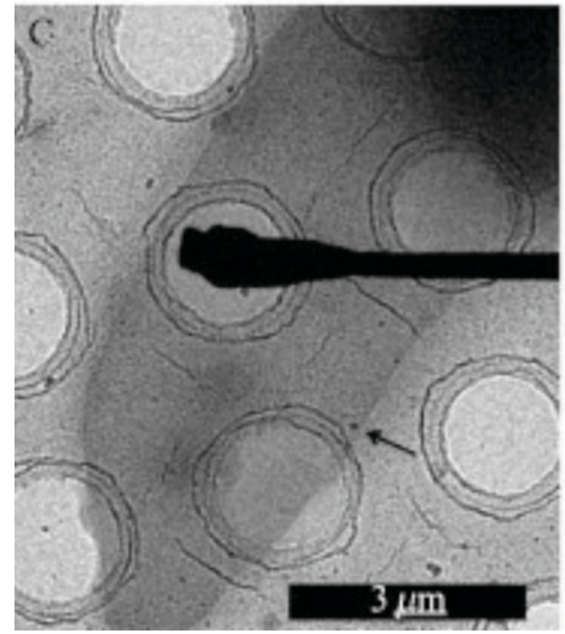
RNA polymerase

Courtesy Roger Kornberg

After formation, crystals can be embedded in a sugar like trehalose and dried, or plunge-frozen



embedded in trehalose between two layers of continuous carbon film

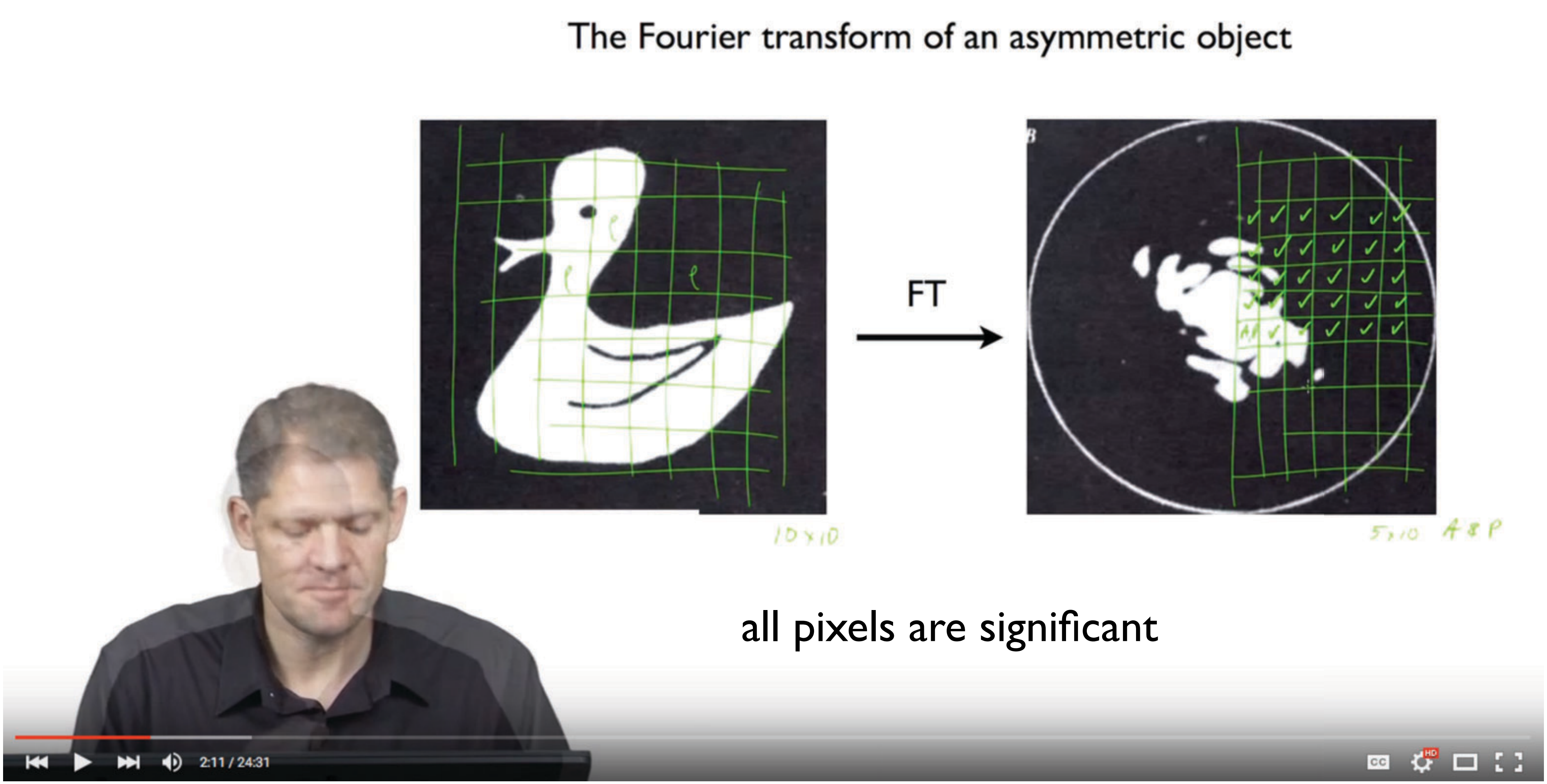


adsorbed onto holey carbon film and plunge-frozen

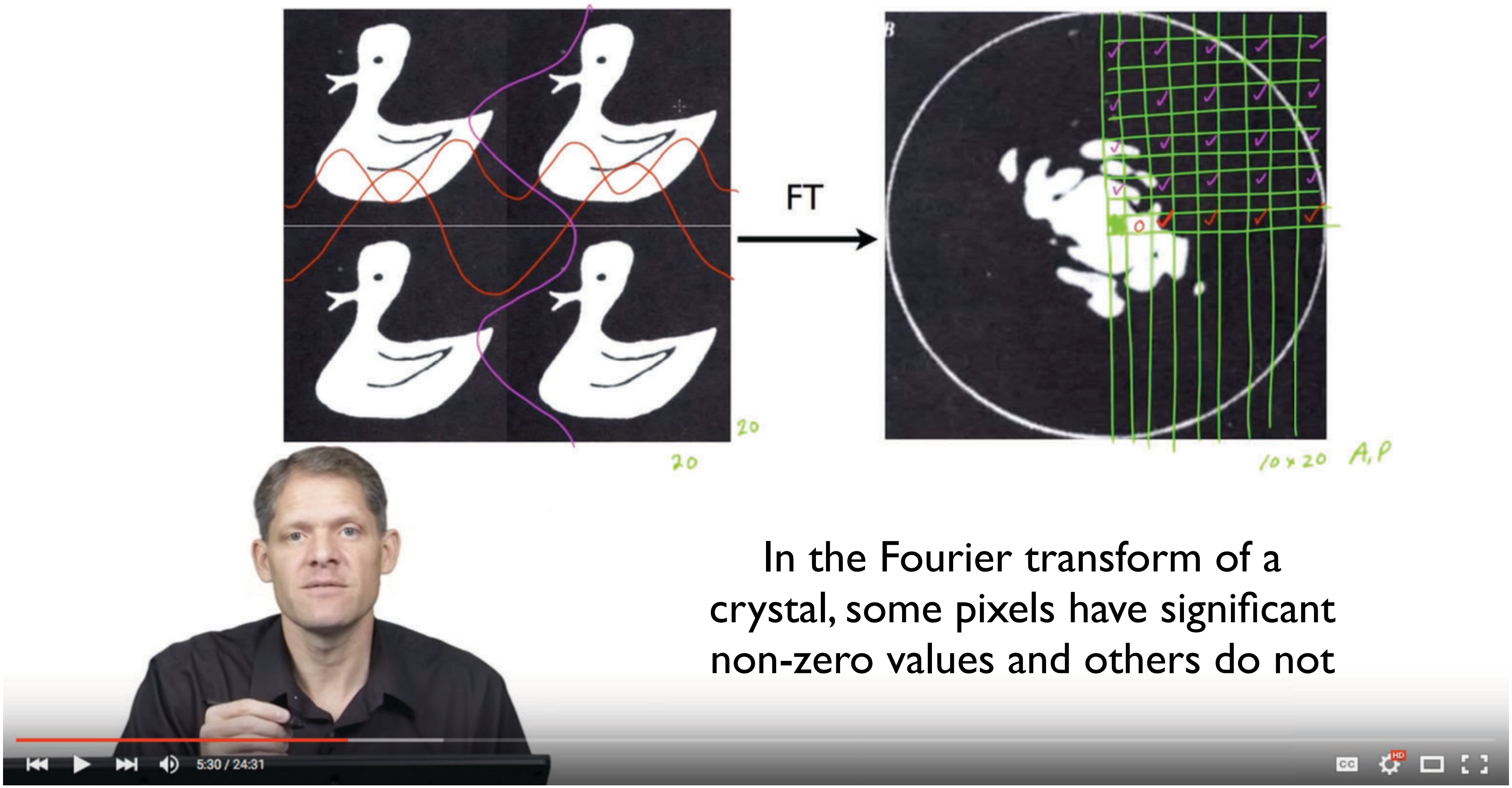
Abeyrathne et al., MIE 2010

2-D crystallography - Intro and sample prep
Concept check questions:

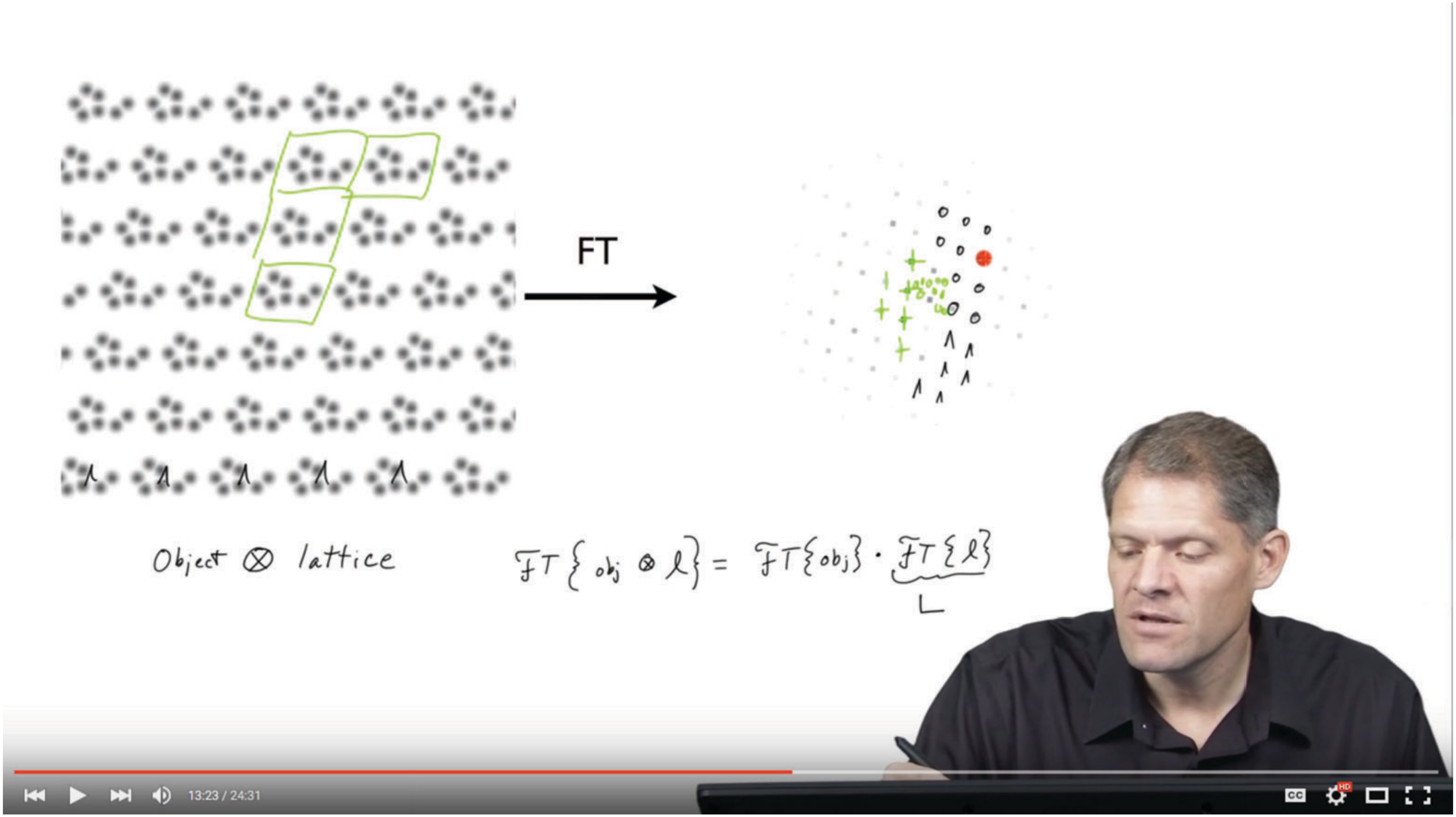
- What is a “2-D crystal”?
- When is 2-D crystallography the cryo-EM approach of choice?
- Describe a method for inducing a protein of interest to form a 2-D crystal.
- In addition to plunge-freezing, what other way have 2-D crystals been stabilized for EM imaging?

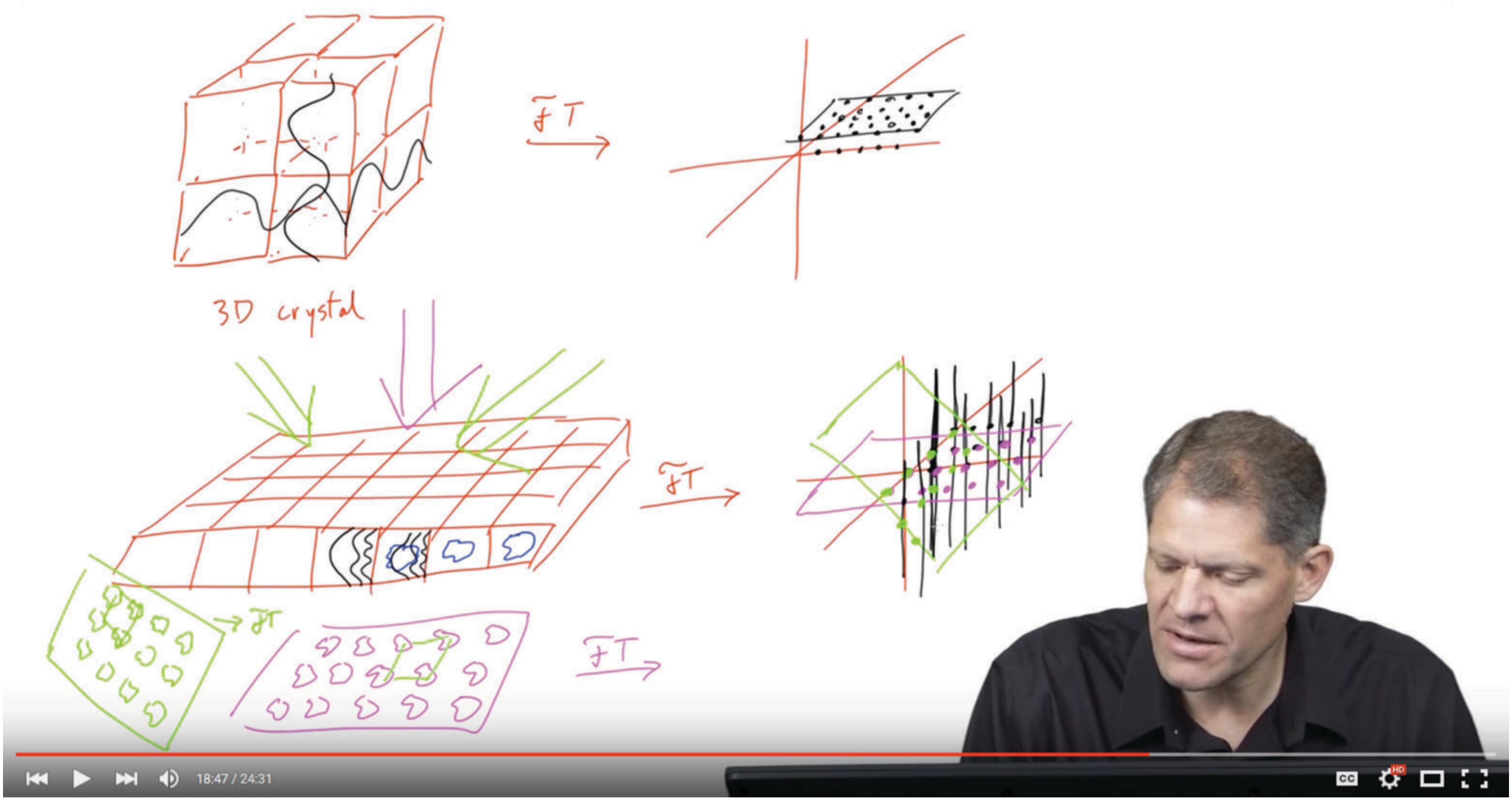


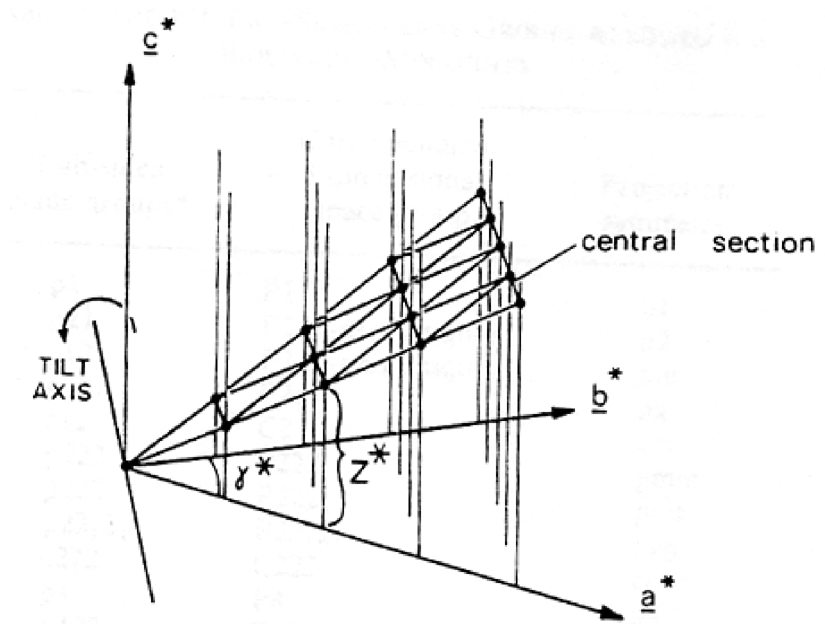
The Fourier transform of an asymmetric object
FT
10x10
5x10
all pixels are significant
2:11 / 24:31



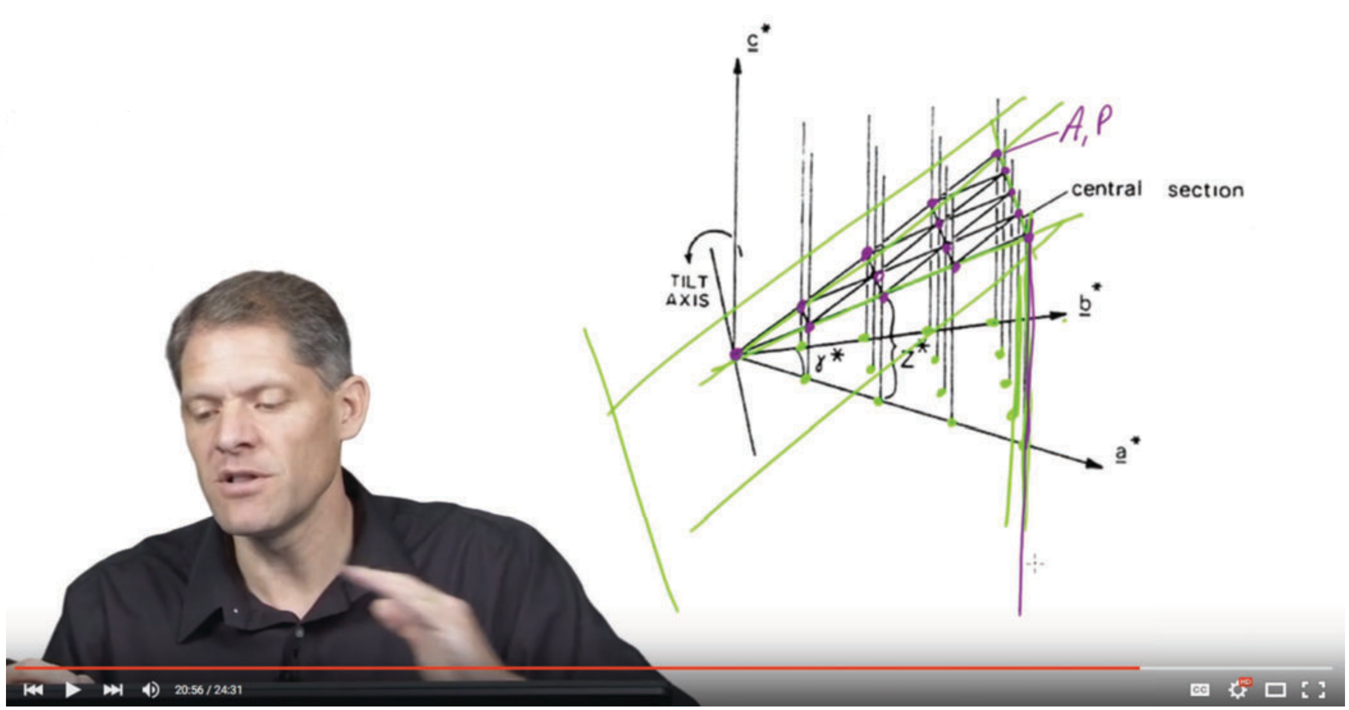
In the Fourier transform of a crystal, some pixels have significant non-zero values and others do not

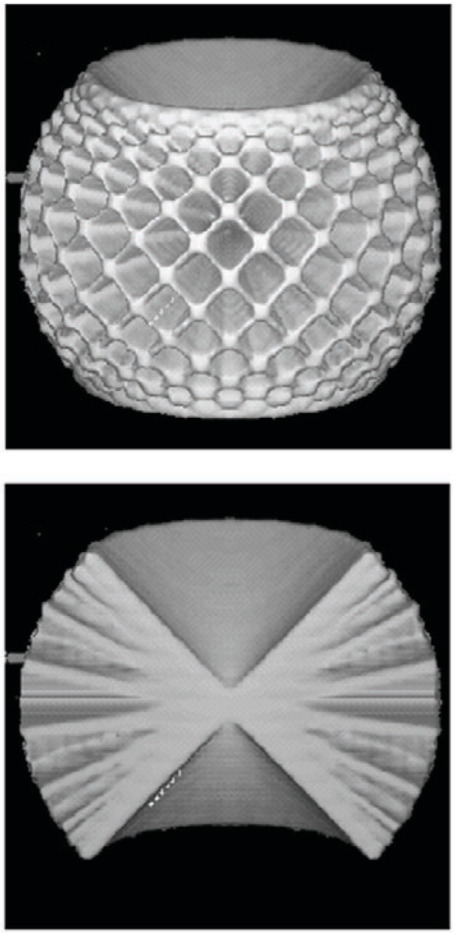

FT
Object ⊗ lattice
FT{obj ⊗ l} = FT{obj} · FT{l}
L
13:23 / 24:31


FT
3D crystal
FT
FT
FT
18:47 / 24:31

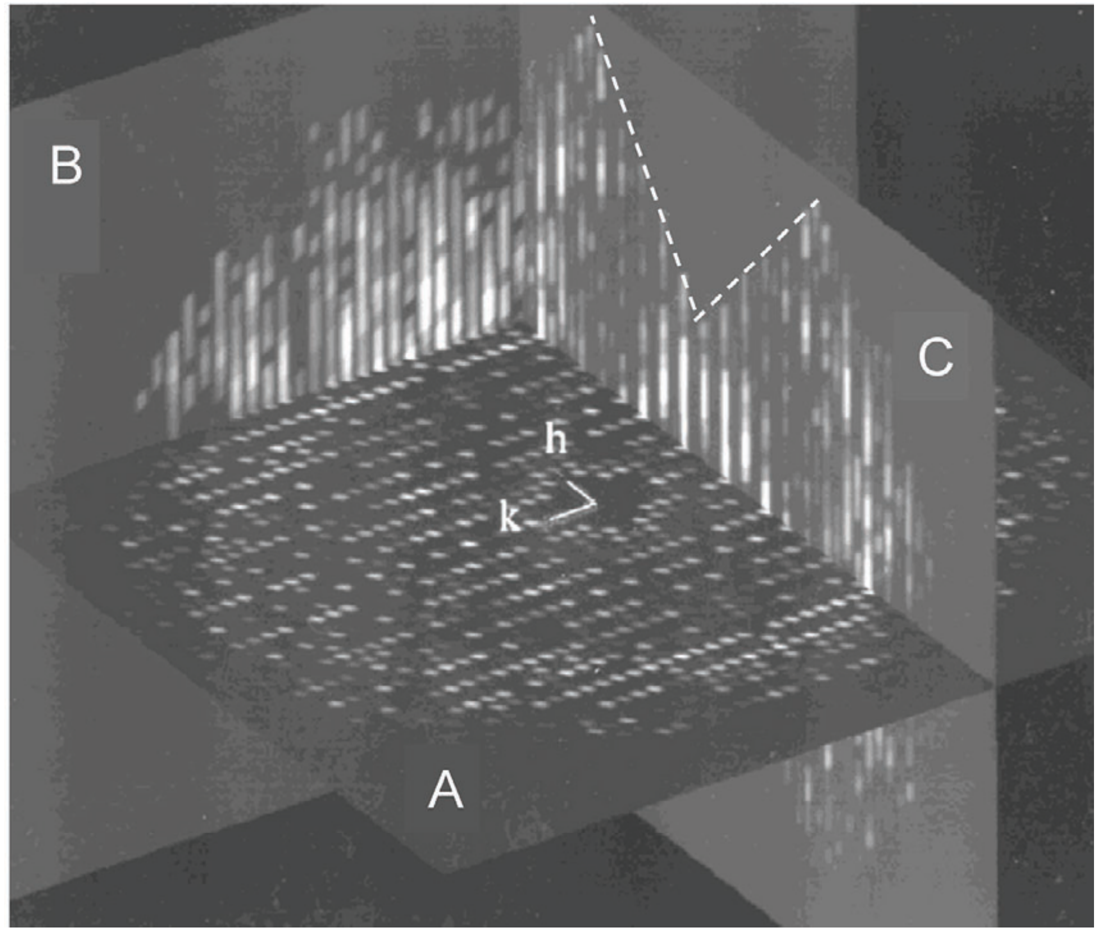


How the Fourier transforms of images of 2-D crystals sample the 3-D Fourier transform of the object

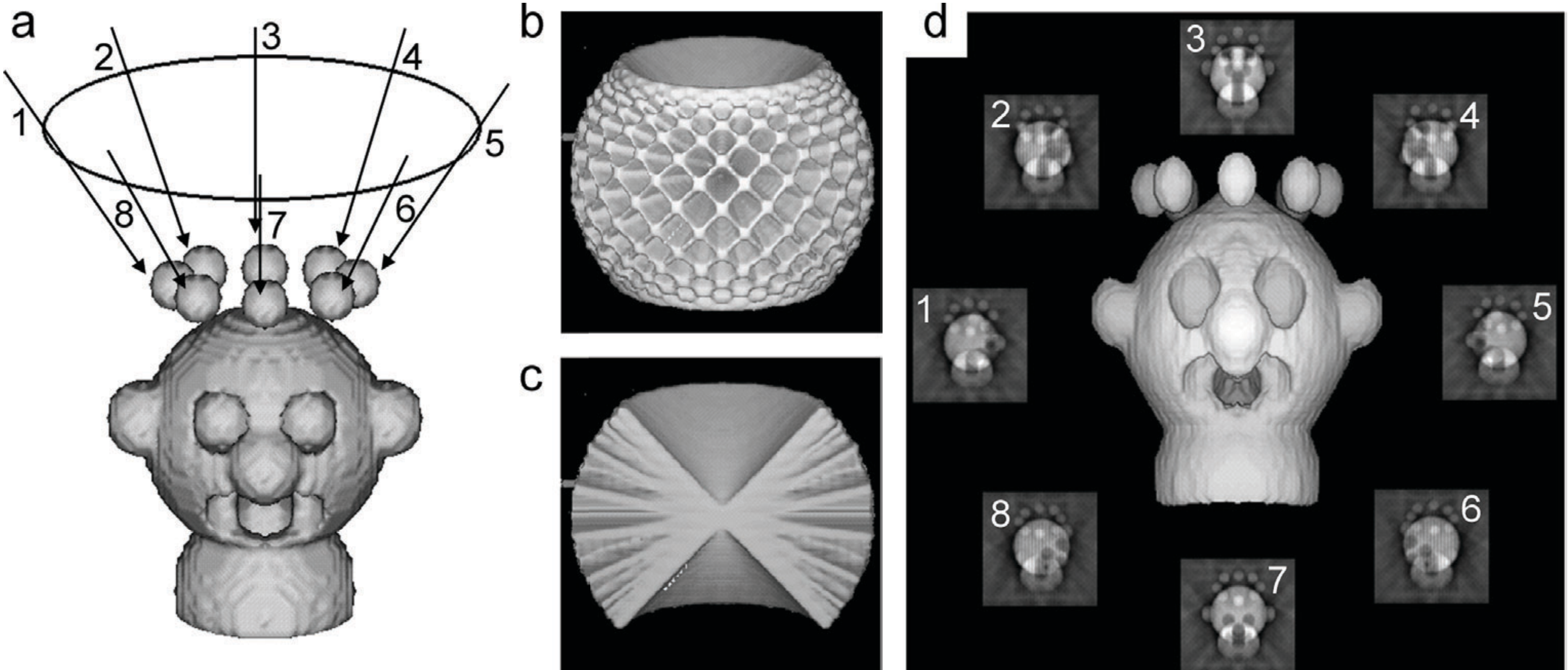




Boisset et al.,
Ultramicroscopy 1998



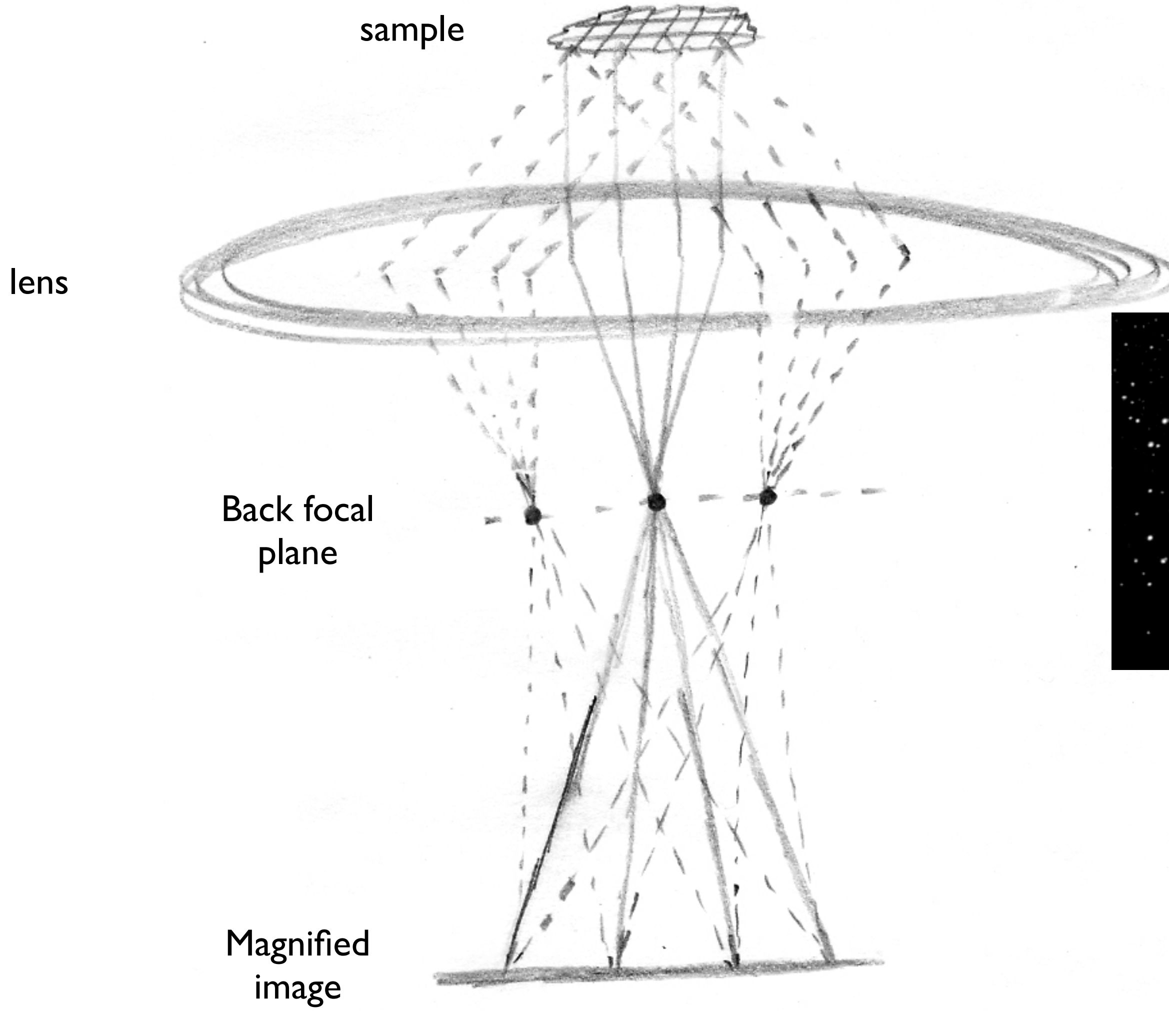
Orlova and Saibil,
Chemical Reviews 2011

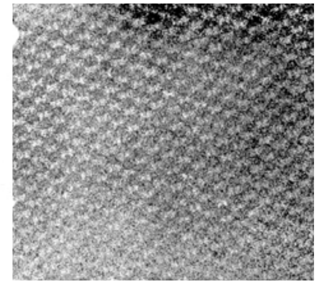


Boisset et al., Ultramicroscopy 1998

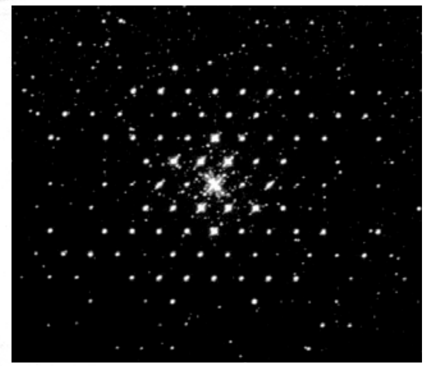
Fourier transform of a 2-D crystal
Concept check questions:

- Why does the Fourier transform of a crystalline object have discrete spots separated by pixels with near-zero amplitudes?
- What is the convolution theorem, and what does it have to do with crystallography?
- What does the Fourier transform of a 2-D crystal look like?
- What is the "missing cone," why is it "missing," and what effect does it have on 2-D crystallographic reconstructions?



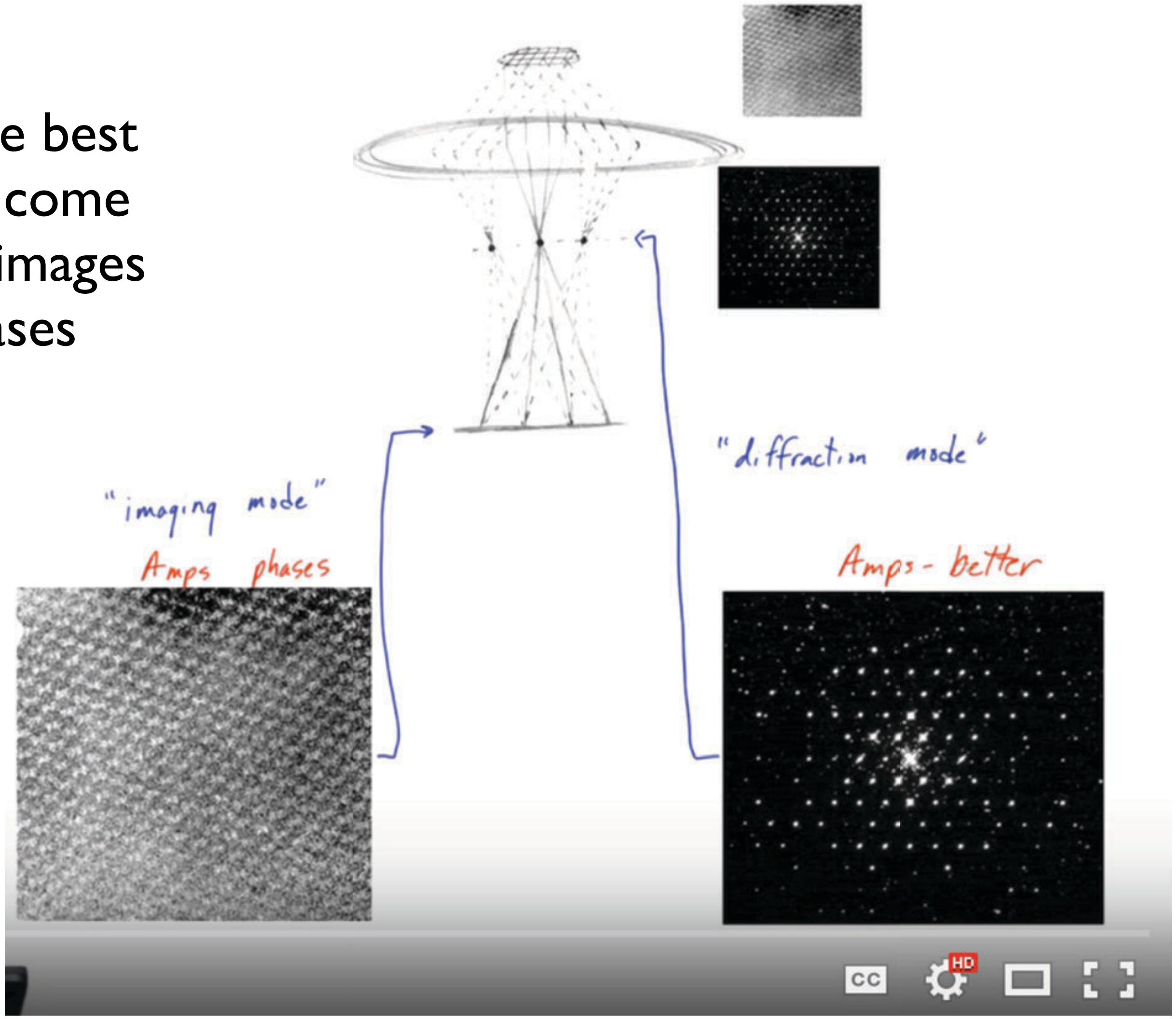


2D crystal

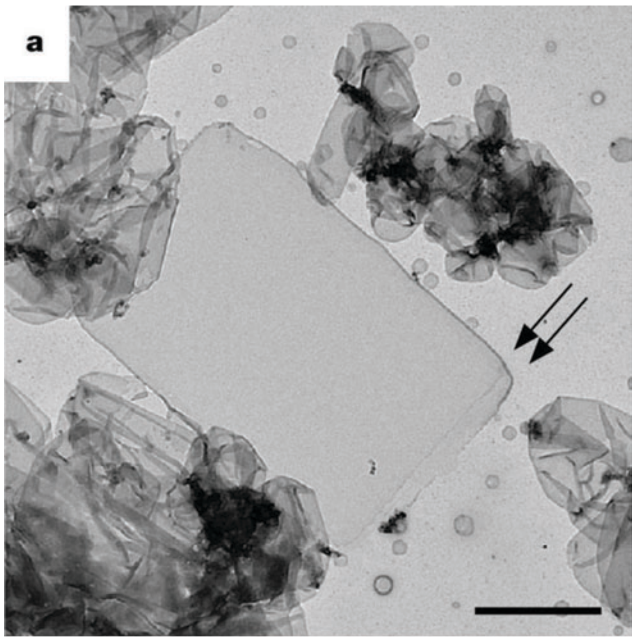


diffraction pattern on back focal plane

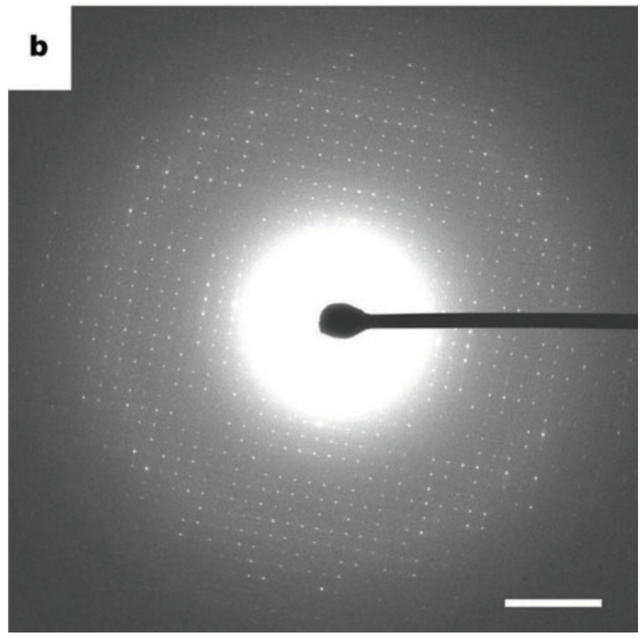
In electron crystallography, the best measurements of amplitudes come from diffraction patterns, but images are recorded to obtain phases



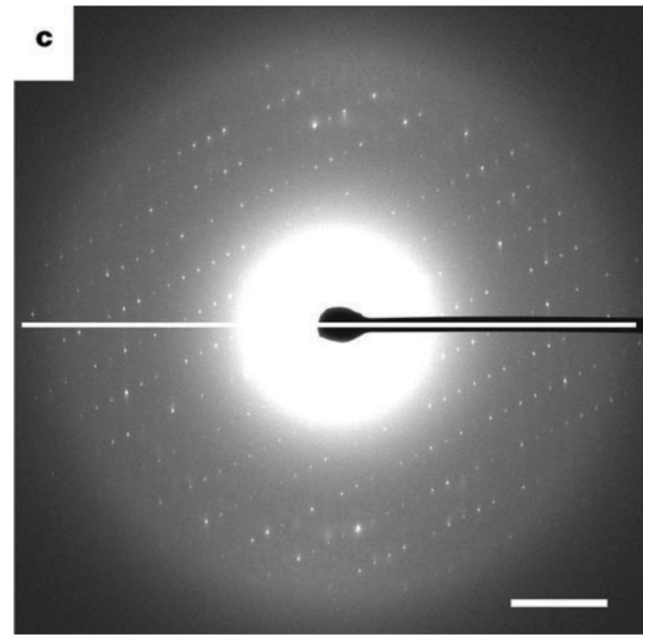
Example images and diffraction patterns from aquaporin crystals



Aquaporin crystal



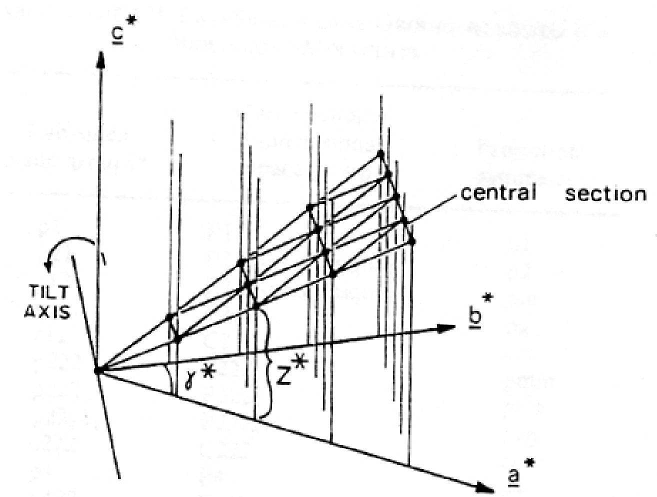
Electron diffraction pattern of an untilted crystal

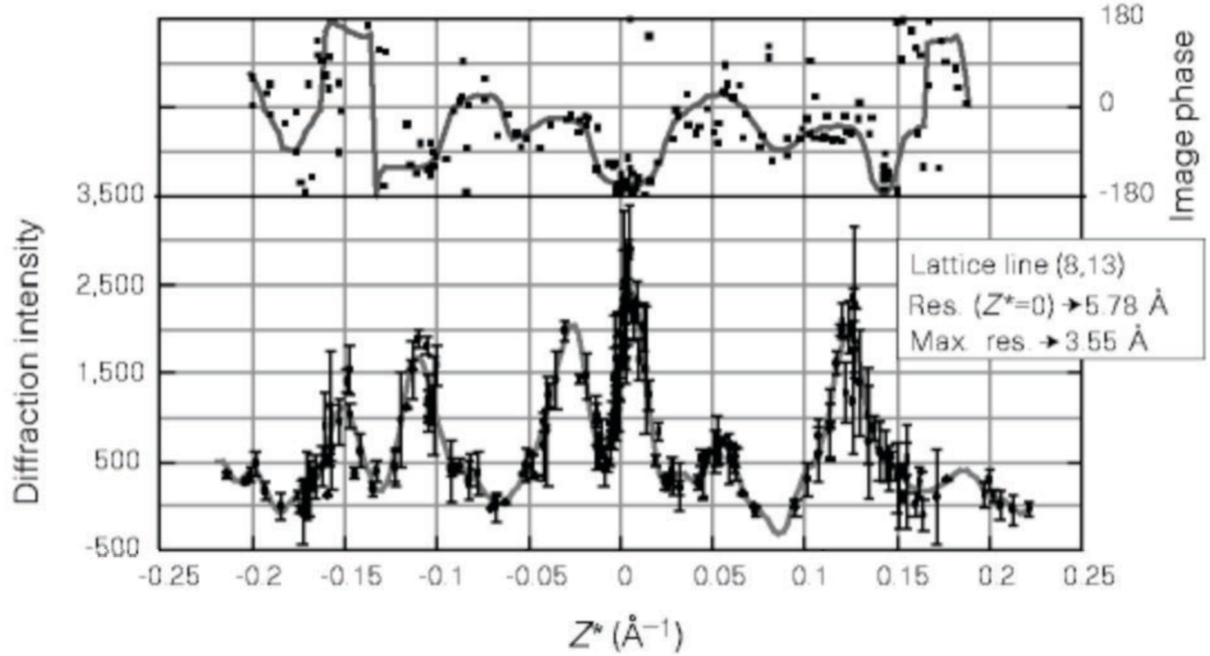


Electron diffraction pattern of a crystal tilted to 70°

Gonen et al., Nature 2004

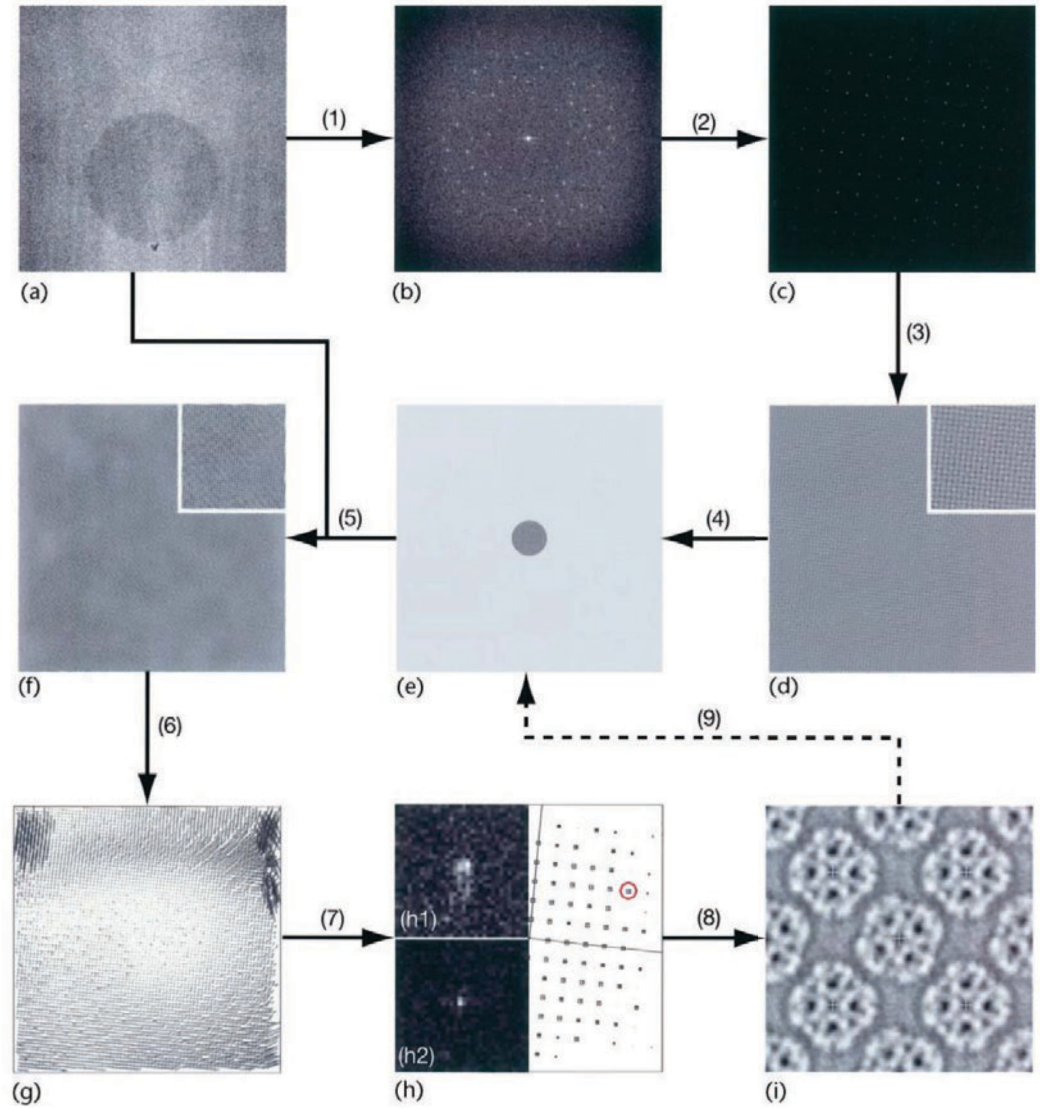
Example lattice line data (amplitude and phase) and curve fitting





Nogales et al., Nature 1998

Crystal "unbending"



Braun and Engel,
Encyclopedia of Life Sciences 2005

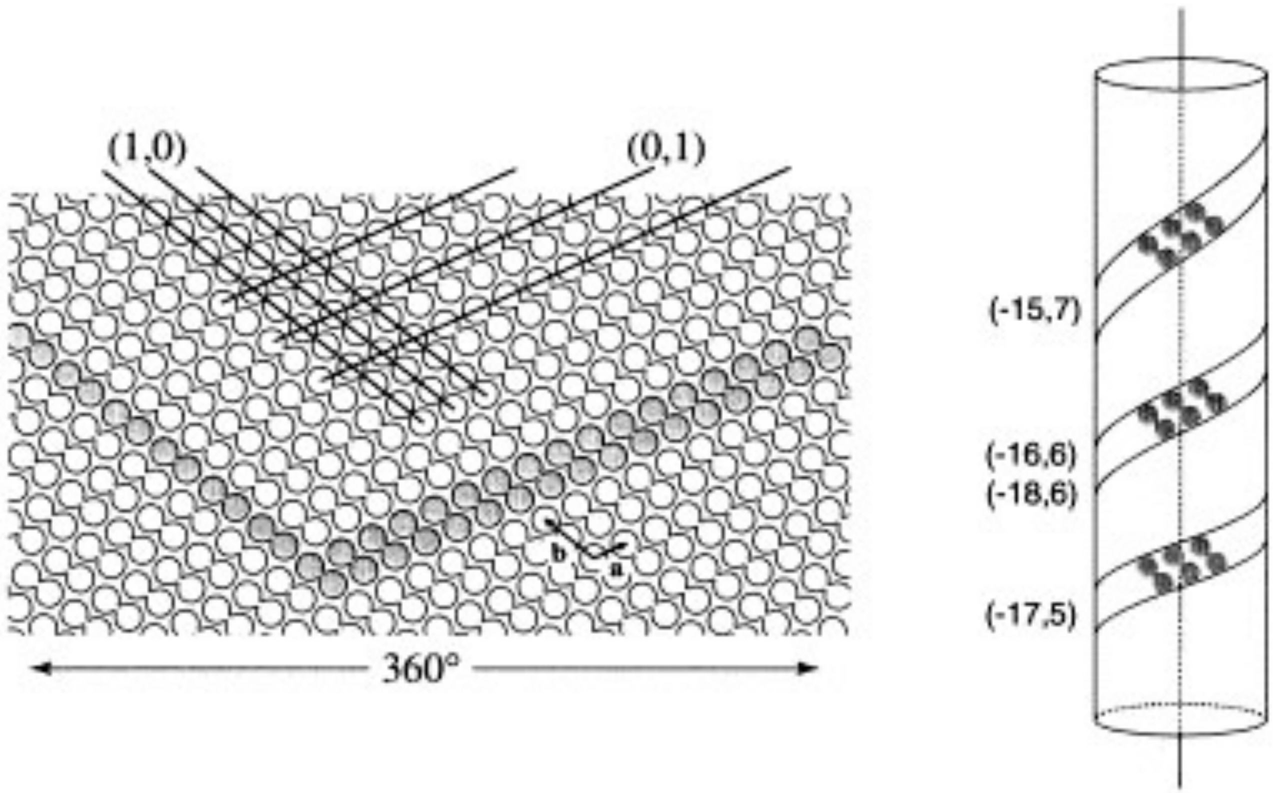
Challenges in 2D crystallography

- Hard to get well-ordered crystals
- Hard to get flat crystals
- Charging, beam-induced movement can blur images
- “Missing cone”

2-D crystallography - Data collection and reconstruction
Concept check questions:

- What is the difference between "imaging" and "diffraction" modes on an EM?
- Why are both images and diffraction patterns of 2-D crystals recorded in a 2-D crystallography project?
- Why are images of both untilted and tilted samples recorded?
- How is all the data from all these images and diffraction patterns merged to produce the reconstruction?
- What is crystal "unbending"? How and why is it done?
- Describe four common challenges in 2-D crystallography projects.

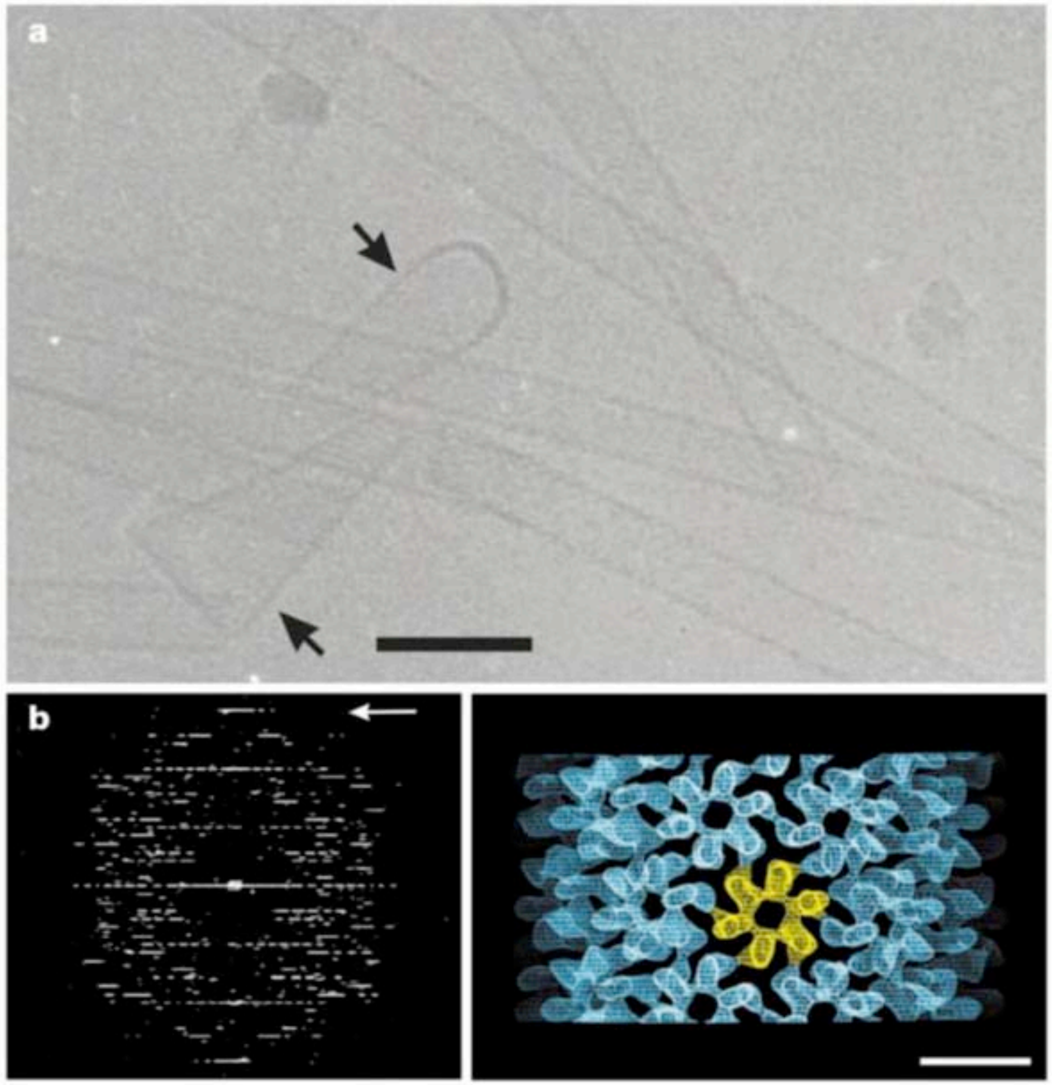
Helical tubes are "rolled up" versions of 2-D crystals,
can be rolled up into different families of tubes with different pitches



Miyazawa et al., JMB (1999)

Cryo-EM projection image of a helical tube of purified HIV CA protein

Su Li et al. Nature 2000



Power spectrum shows “layer lines”

3D reconstruction

Helical tubes
Concept check questions:

- How are helical tubes related to 2-D crystals?
- Why are helical tubes particularly good samples for cryo-EM reconstruction?
- What does the diffraction pattern of a helical tube look like?