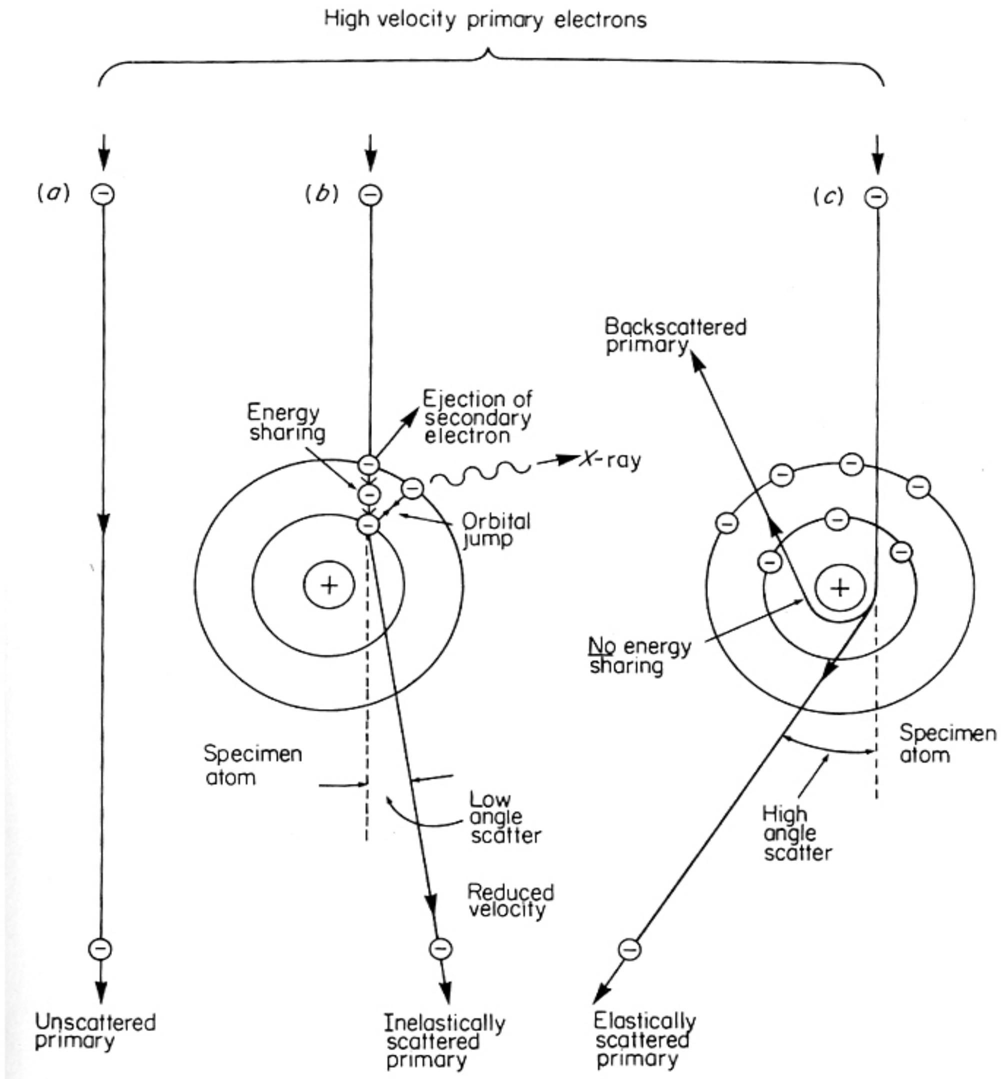
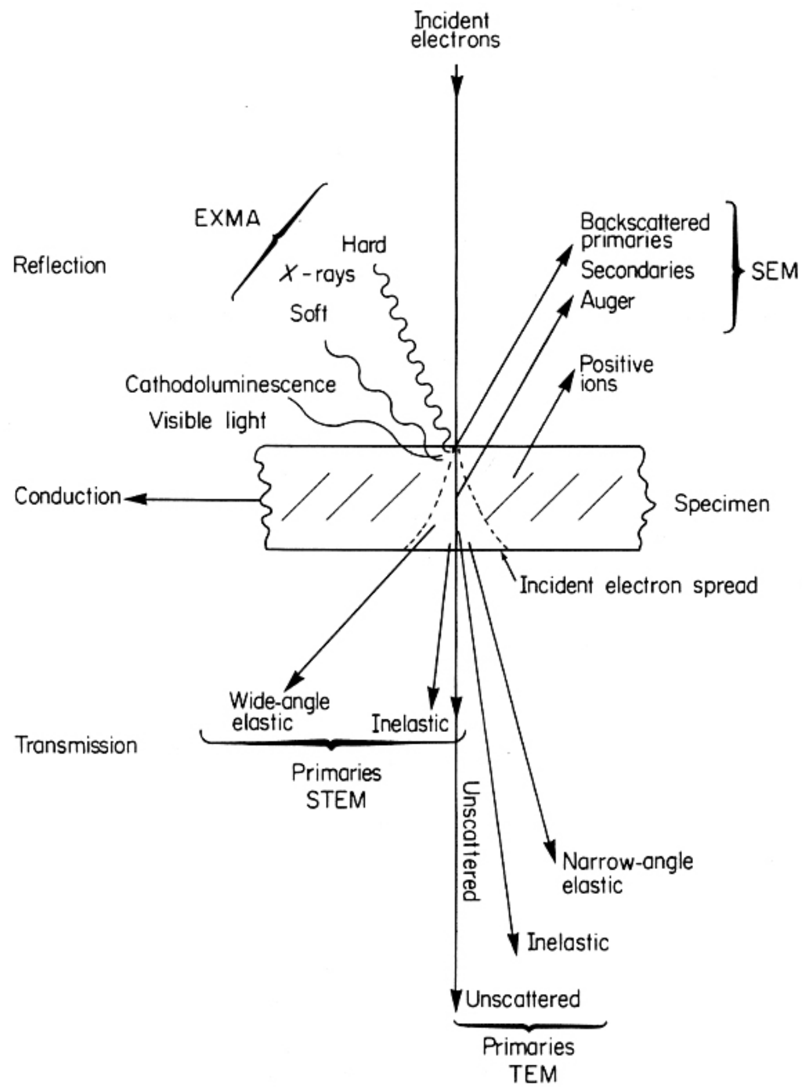
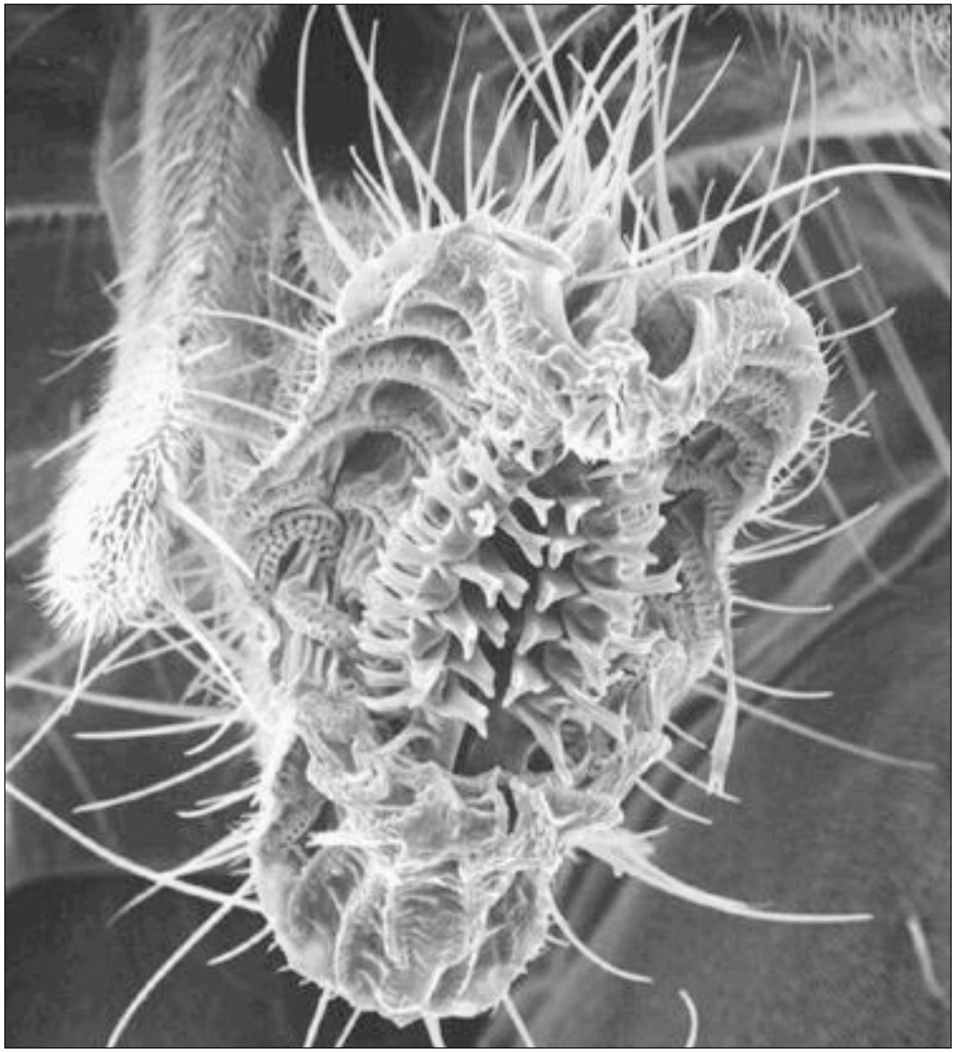
Part 3 - Image Formation



Three classes of scattering outcomes

Types of electron microscopes



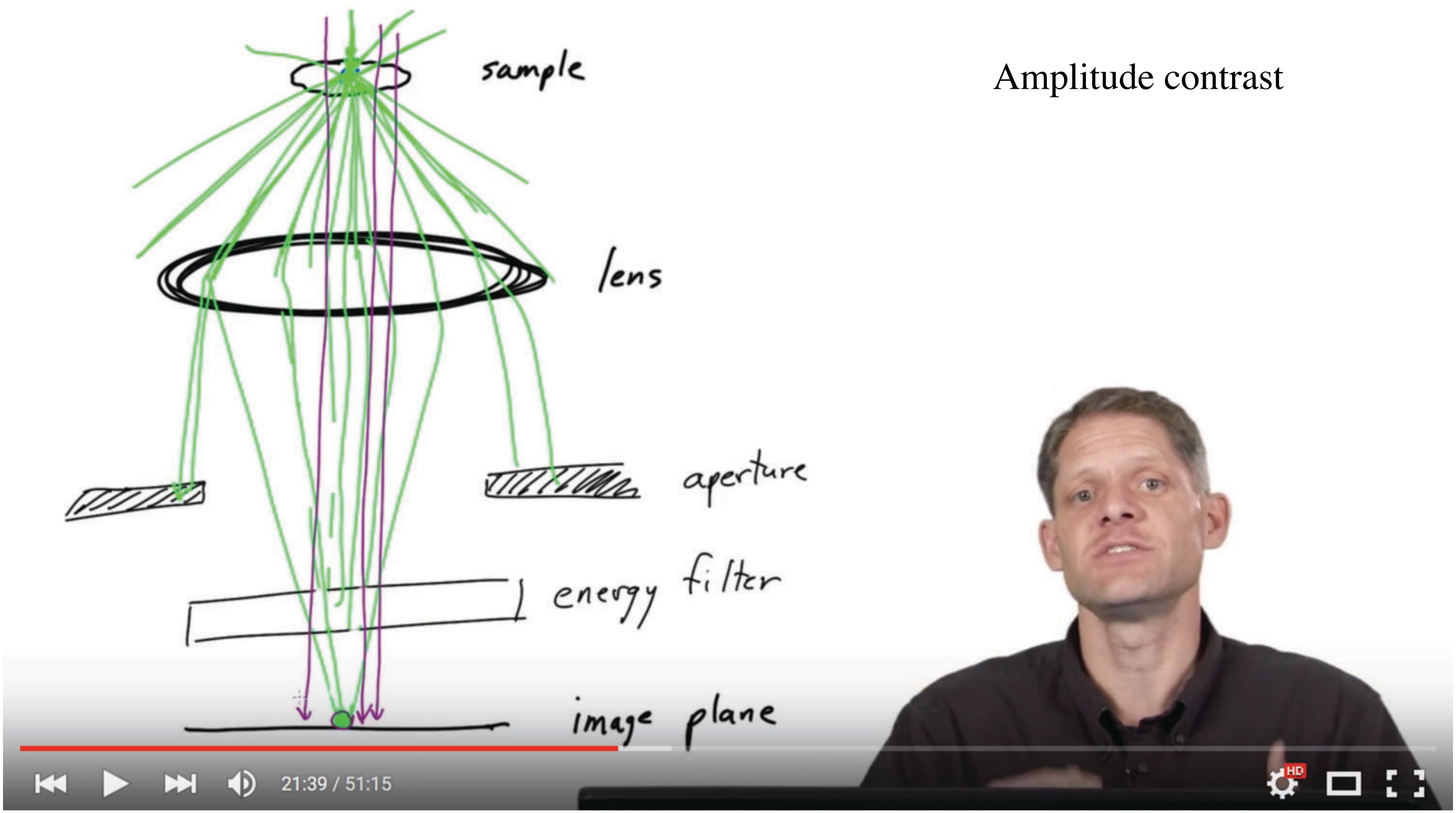


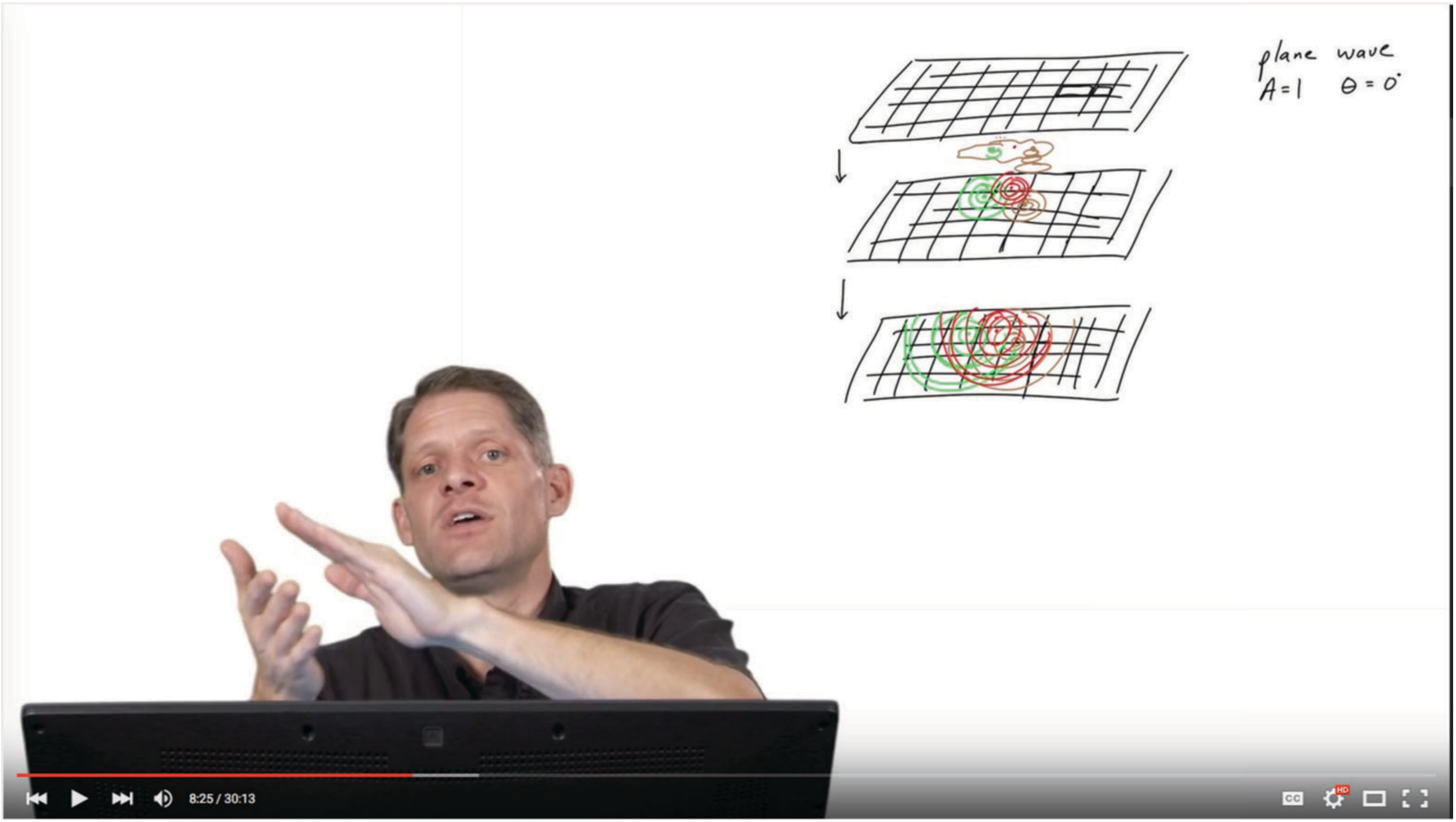
Example SEM image:
fly nose

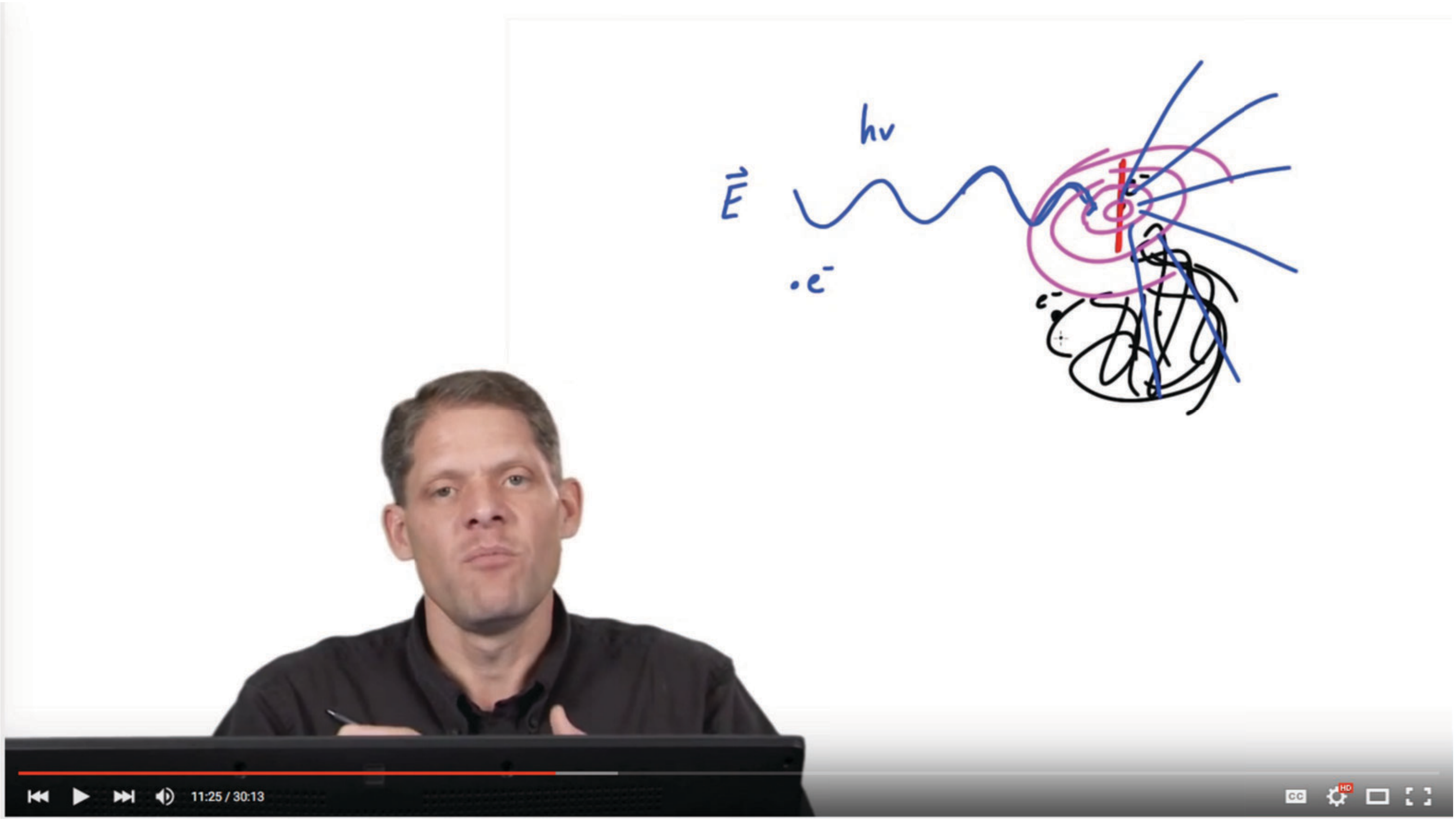
Example TEM image: muscle

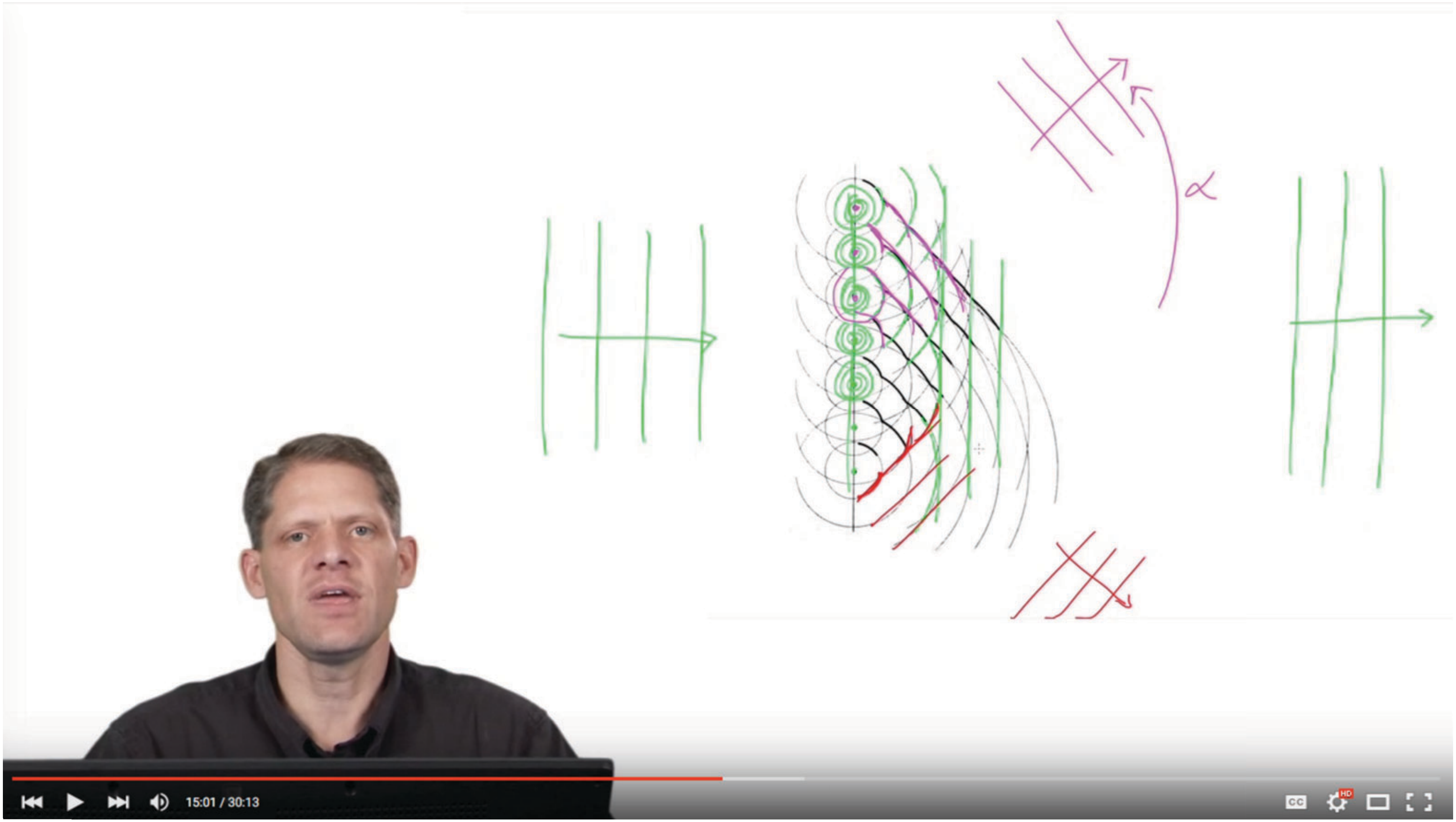
Skeletal muscle.
Cell and Tissue Ultrastructure
Mercer

Amplitude contrast

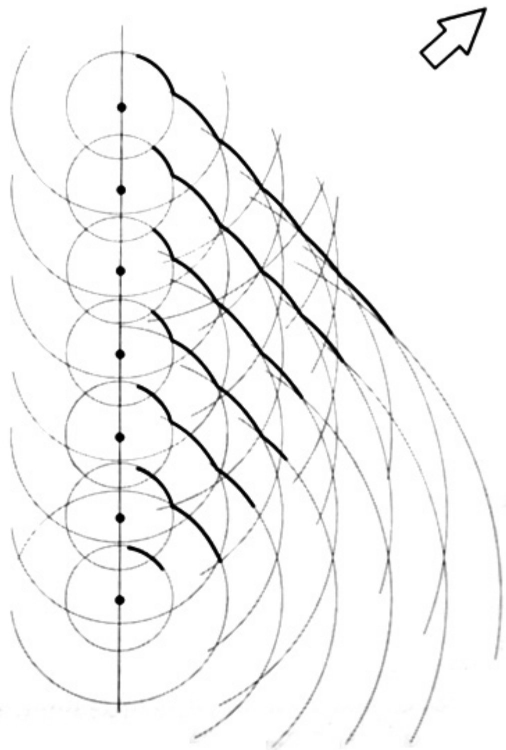


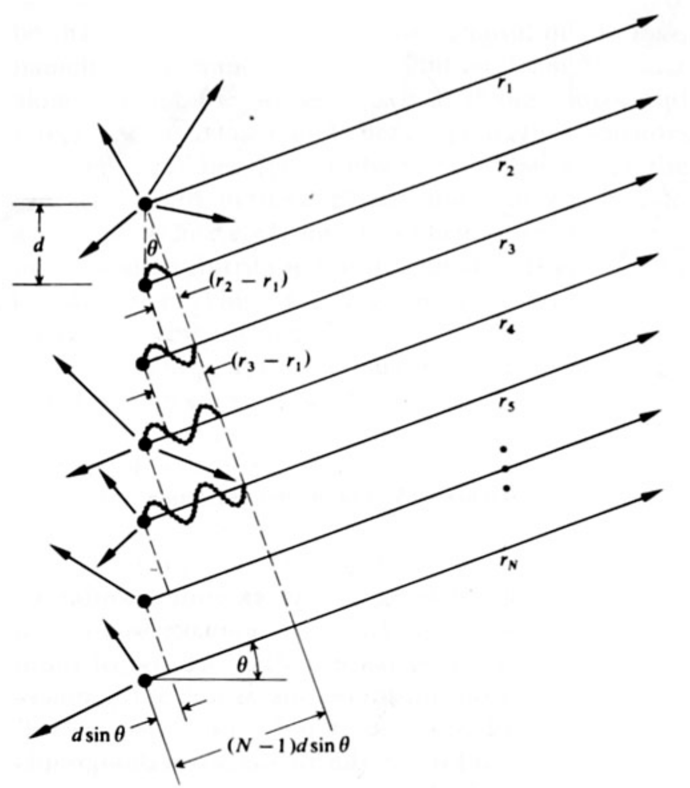

plane wave
A=1 θ=0°
8:25 / 30:13
CC
HD


hν
$\vec{E}$
$\cdot e^-$
11:25 / 30:13

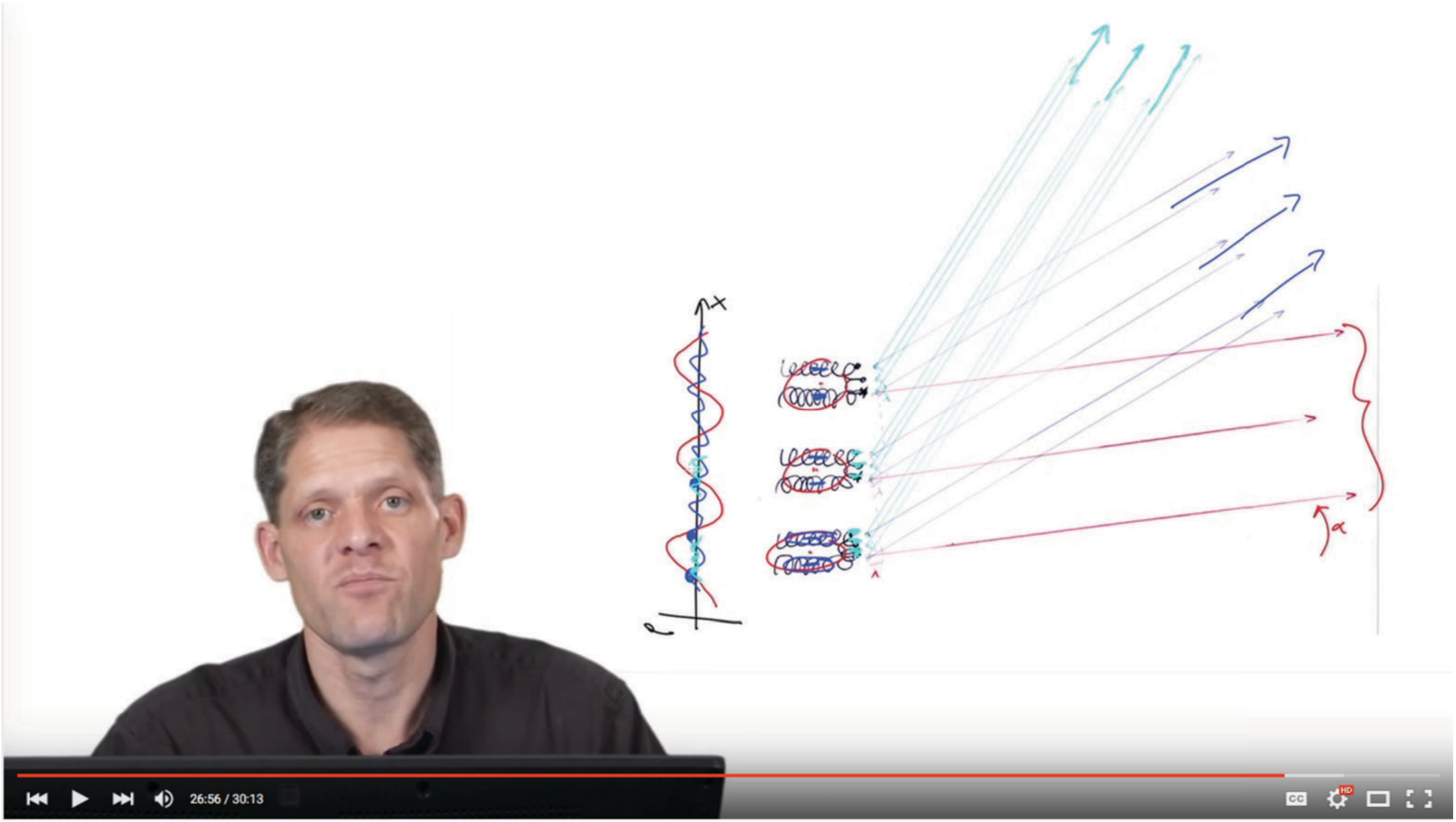

α
15:01 / 30:13
CC
HD

Diffraction can be thought about and predicted using Huygen’s construction

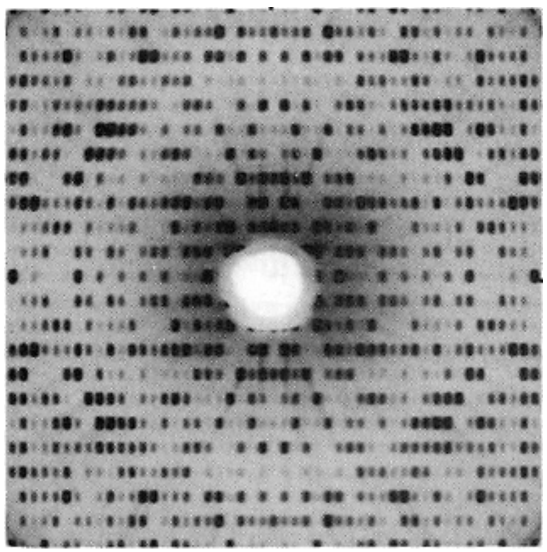




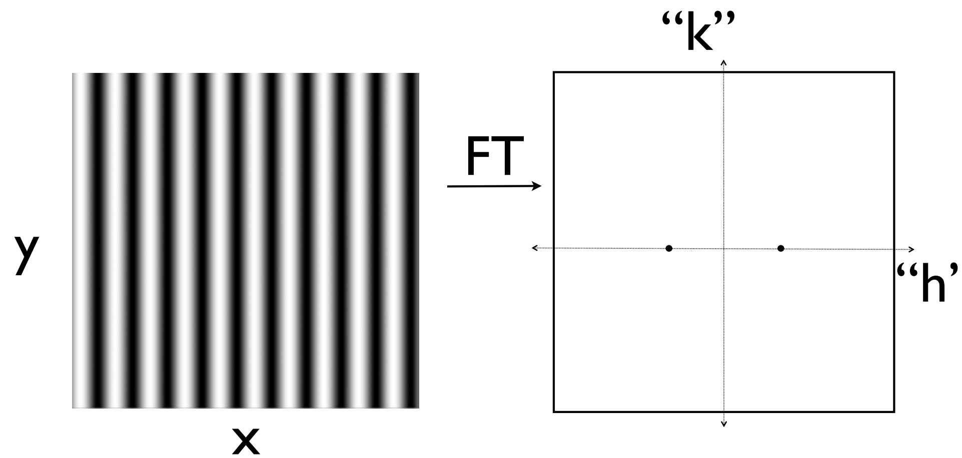
Maxima occur where $n\lambda = d \sin \theta$


x
α
26:56 / 30:13

Actual X-ray diffraction pattern



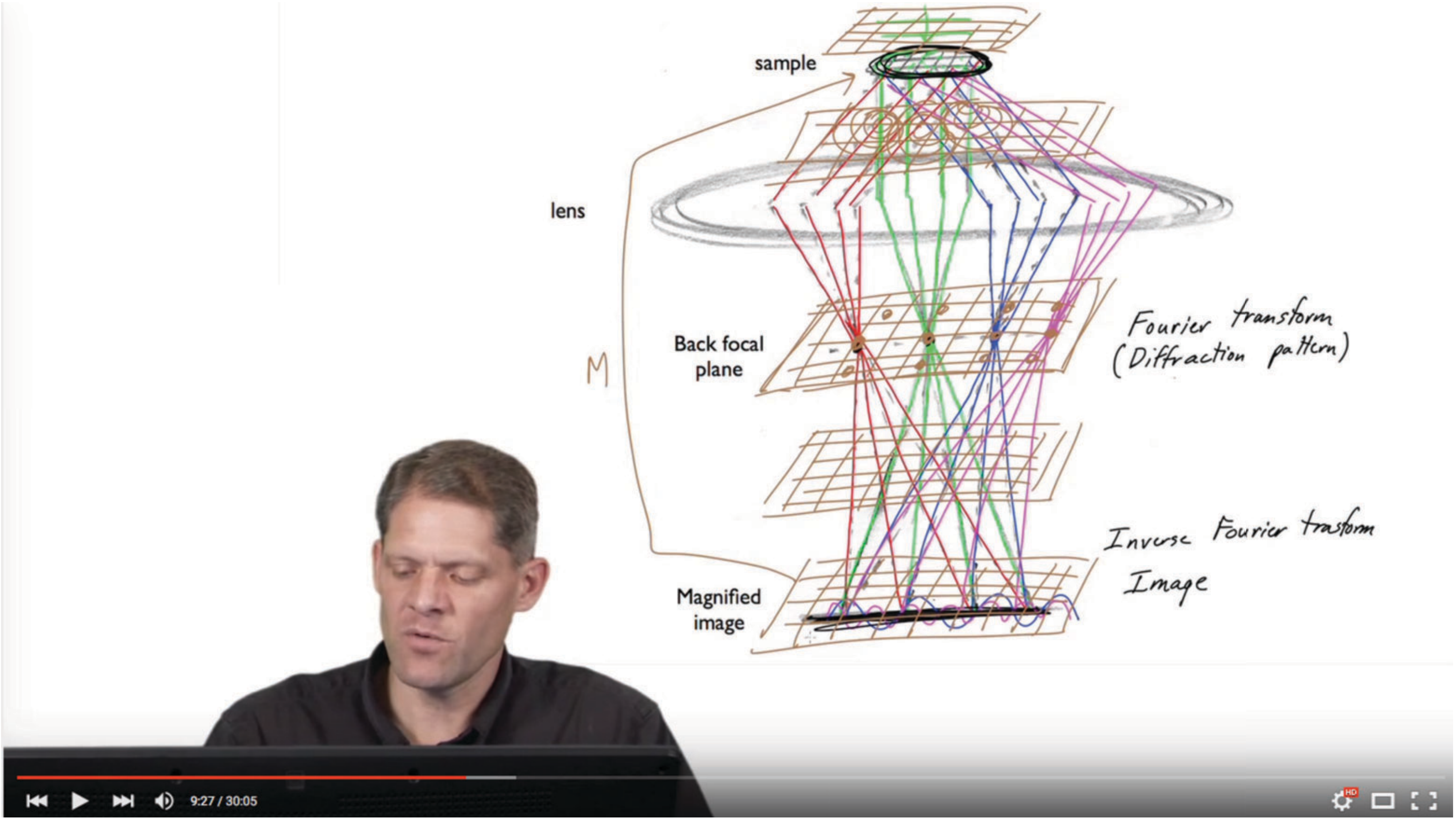
Remember this?

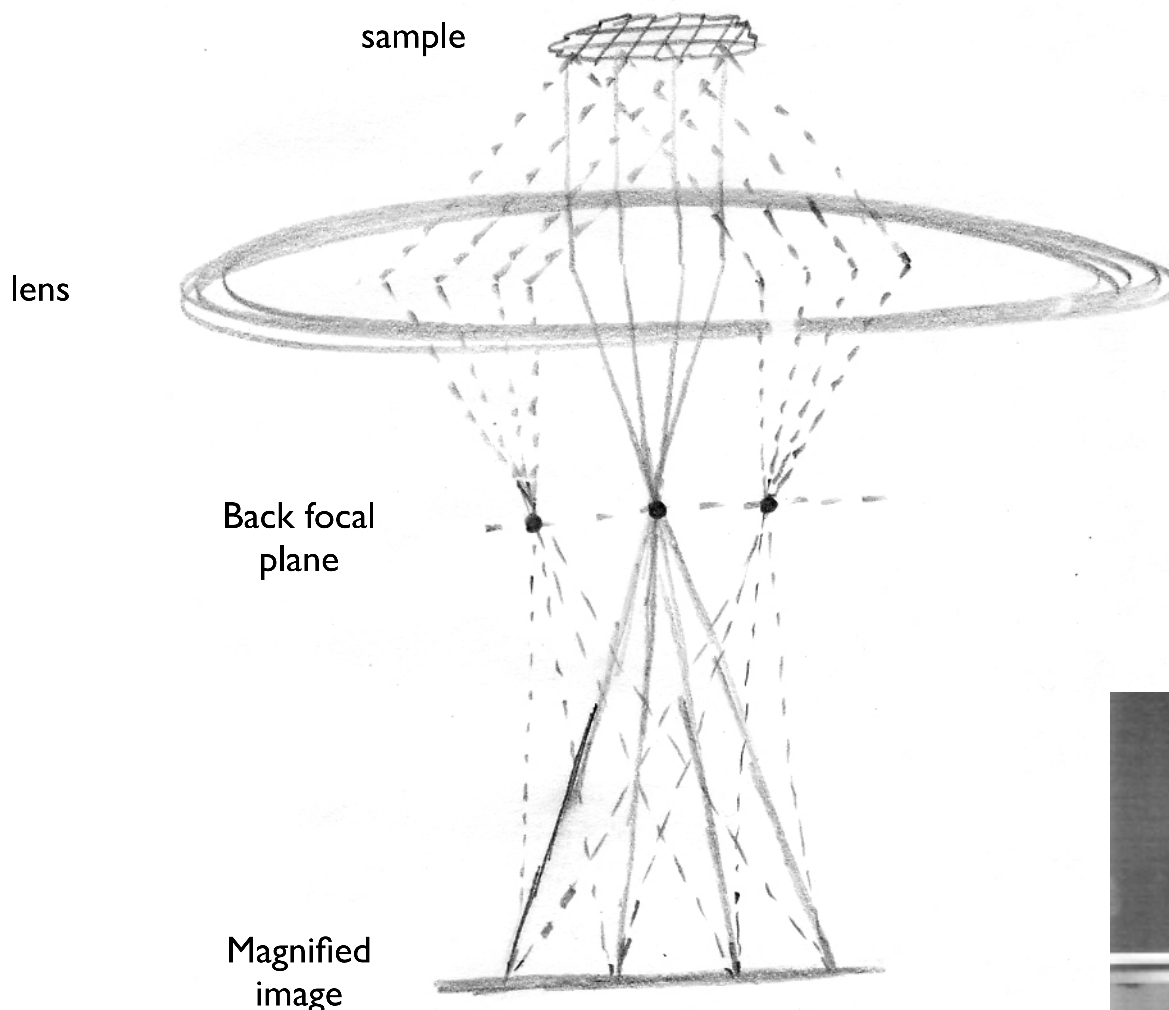


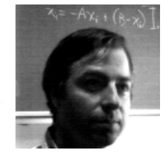
- Each spot represents a Fourier component (a 3-D sine wave)
- Identified by (h, k, l) indices
- Each has an amplitude and a phase
- Both must be known to recalculate the “real space” object

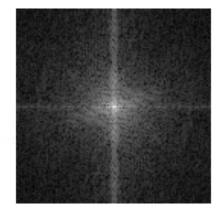
Amplitude and phase contrast
Concept check questions:

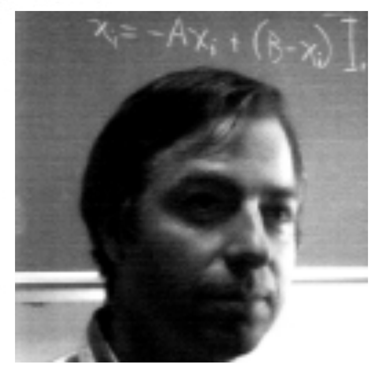
- In what ways are inelastic and elastic scattering different? What causes them?
- What signals emerge from scattering events in the electron microscope that can be measured, and how do they lead to the three main types of electron microscopy?
- How does amplitude contrast arise?
- Why does phase contrast require us to think of imaging electrons as waves?
- What is a "plane wave"? What about a plane wave changes as it travels through a vacuum?
- Explain how/why atoms scatter X-rays.
- Why are there discrete peaks in the scattering from crystals?
- What information is delivered by each peak?

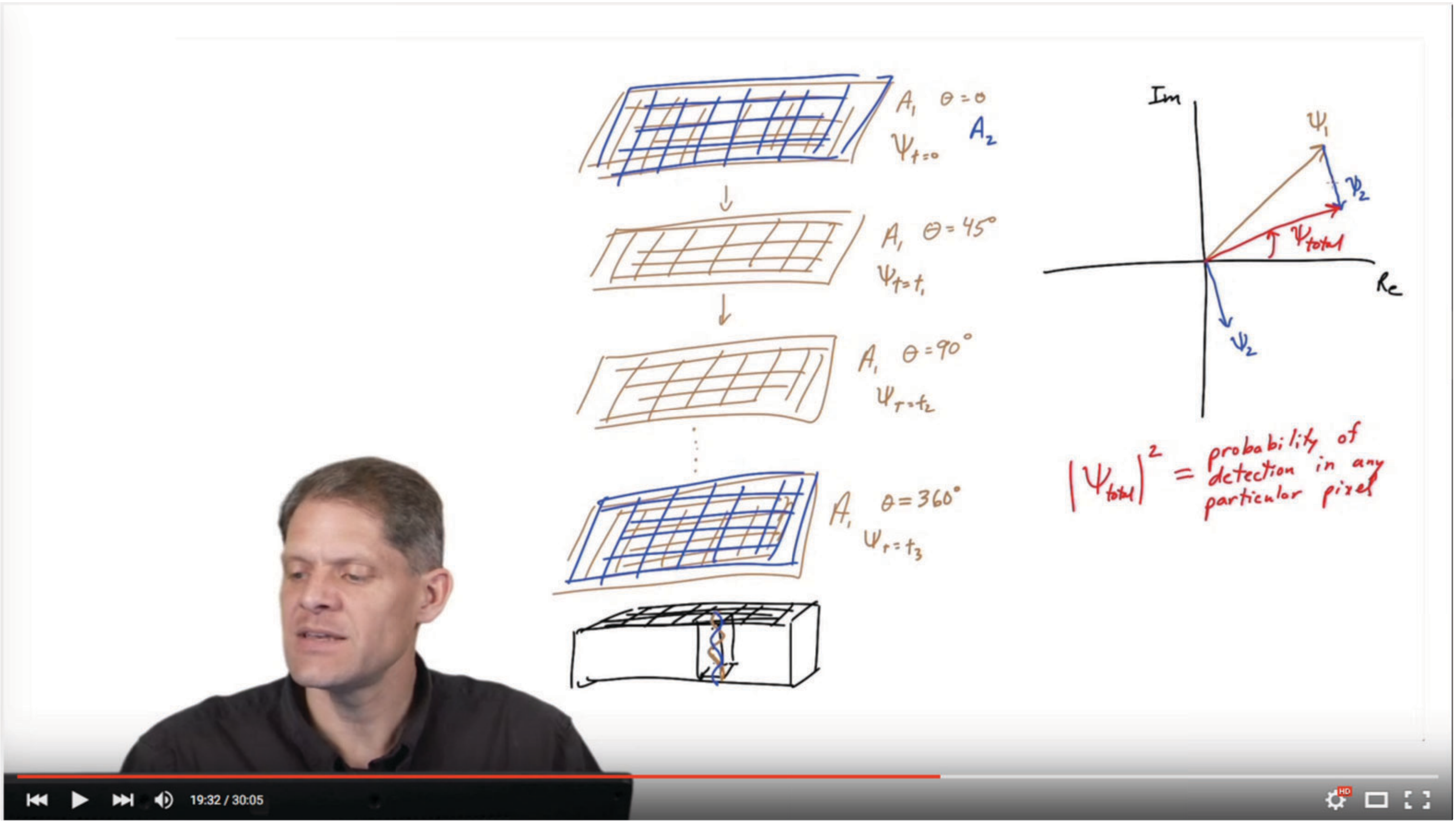

sample
lens
M
Back focal plane
Fourier transform (Diffraction pattern)
Inverse Fourier trasform
Image
Magnified image
9:27 / 30:05

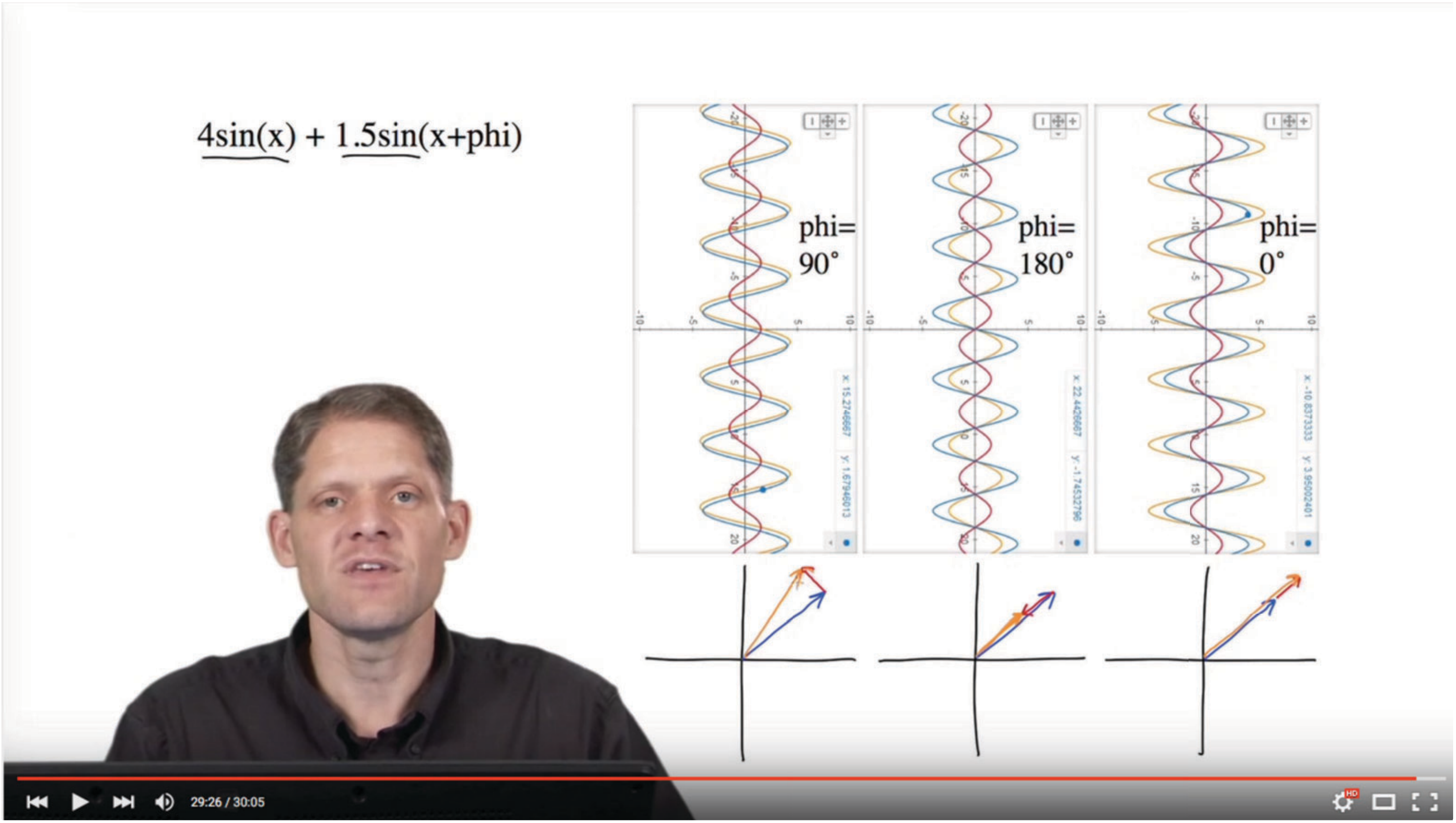

sample
lens
Back focal
plane
Magnified
image







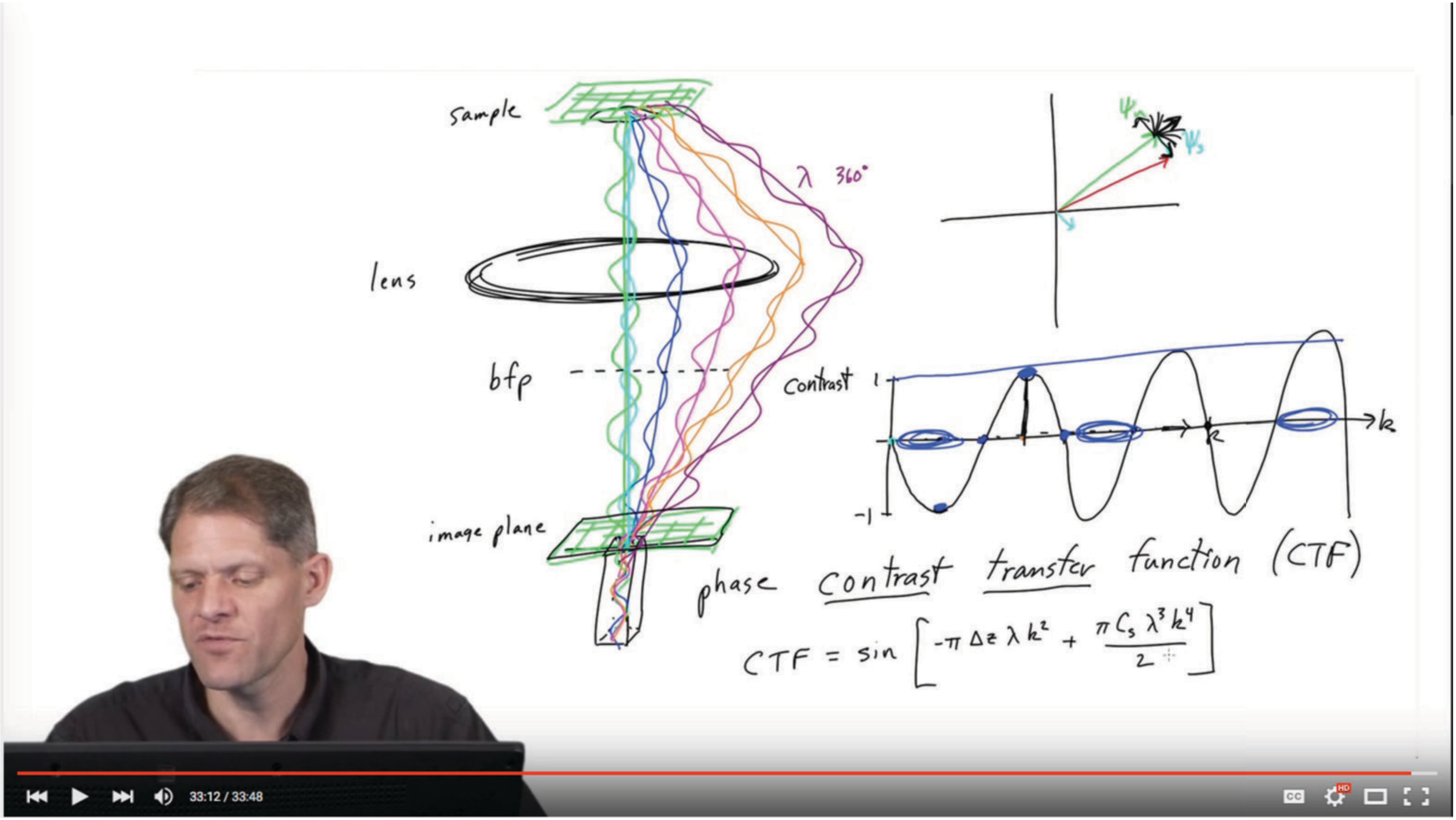

A1 θ=0
Ψt=0
A2
A1 θ=45°
Ψt=t1
A1 θ=90°
Ψt=t2
A1 θ=360°
Ψt=t3
Im
Re
Ψ1
Ψ2
Ψtotal
|Ψtotal|² = probability of detection in any particular pixel
19:32 / 30:05



4sin(x) + 1.5sin(x+phi)
phi=
90°
phi=
180°
phi=
0°
29:26 / 30:05

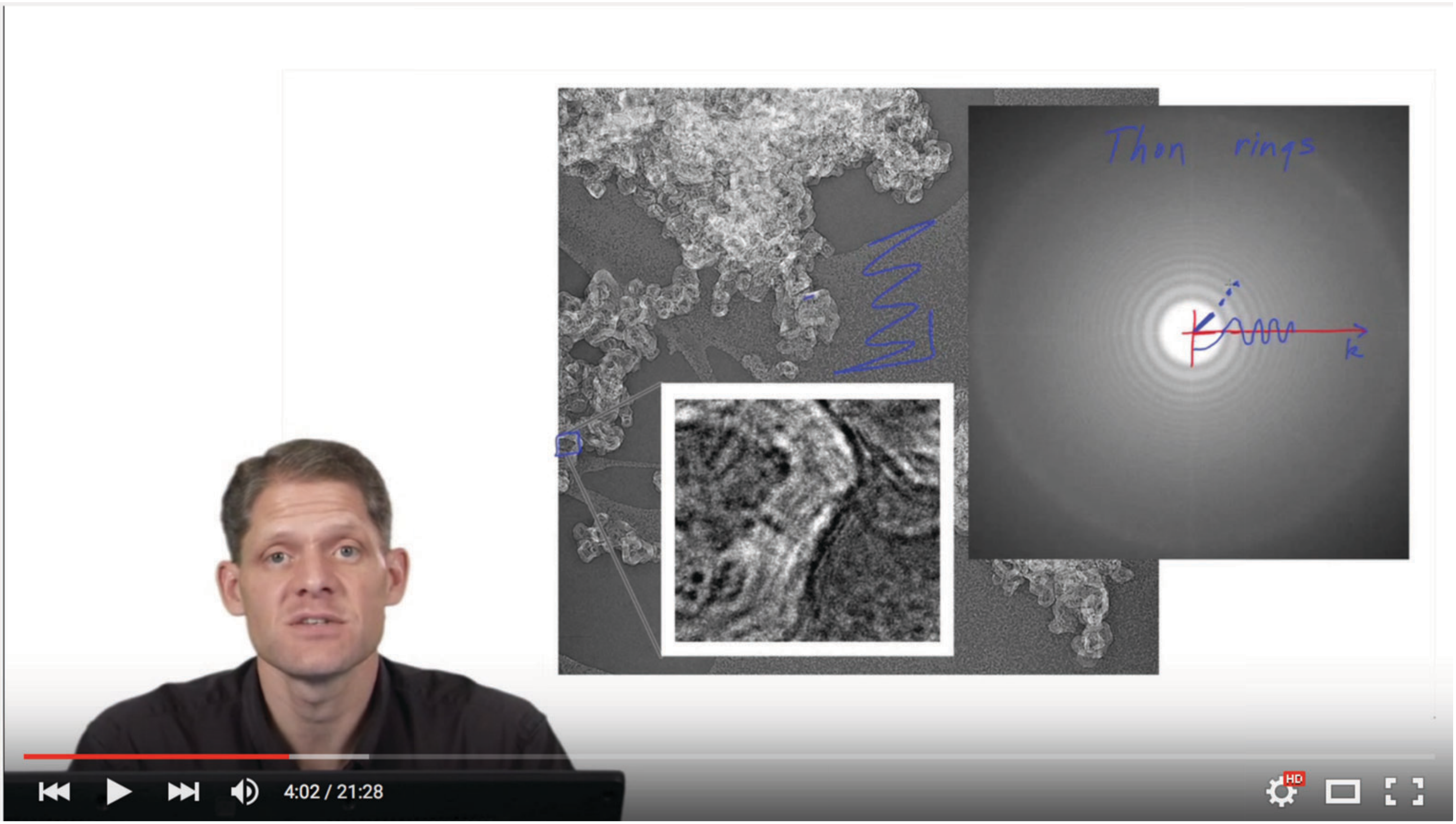
Wave propagation and phase shifts
Concept check questions:

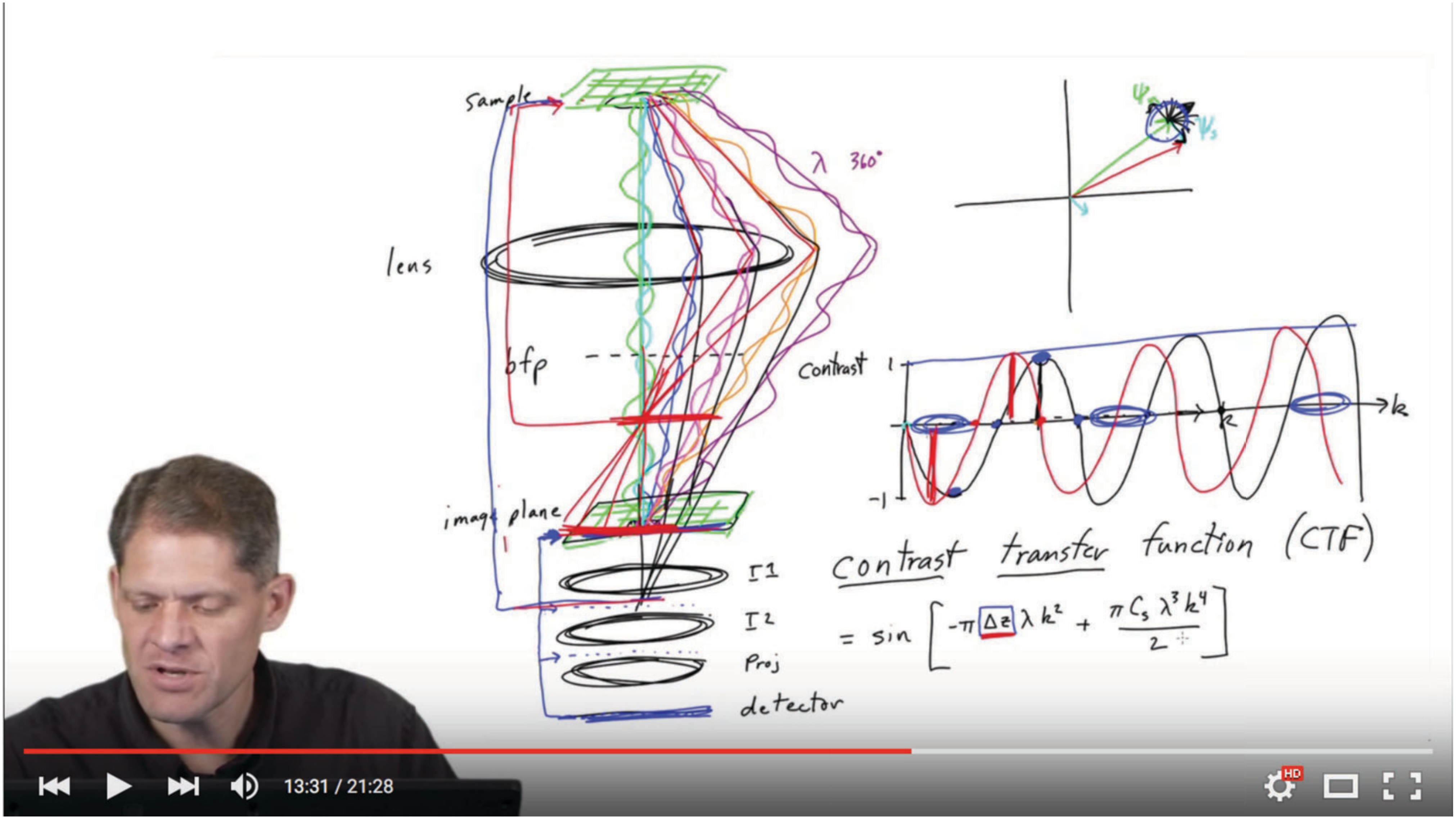
- How is the scattering from an object converted into an image in a microscope?
- What is the relationship between the density of the sample and the wavefunction present on the back focal plane of the objective lens? The image plane? Can you draw a picture showing why?
- How are plane waves represented in an "Argand" diagram? What are the axes?
- Why were Argand diagrams introduced (how do they help us understand wave propagation and interference)?
- How does the phase difference between two waves of identical frequency effect their interference?
- What property of an electron wave gives the probability of its detection at each position?

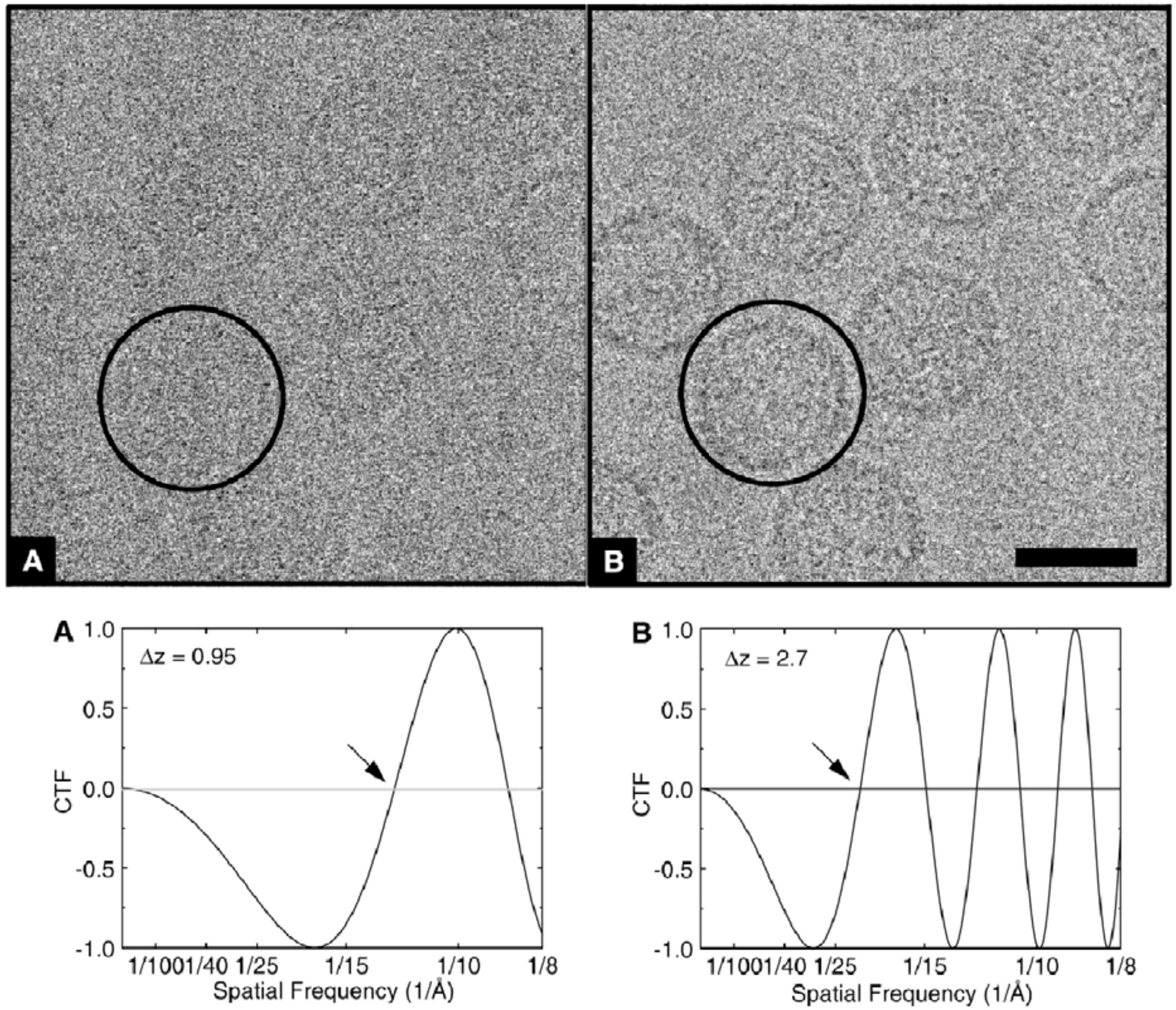

sample
λ 360°
lens
bfp
contrast
1
-1
k
image plane
phase contrast transfer function (CTF)
$CTF = \sin\left[-\pi \Delta z \lambda k^2 + \frac{\pi C_s \lambda^3 k^4}{2}\right]$
33:12 / 33:48

The contrast transfer function
Concept check questions:

- What two factors make the phase of a scattered component of a wave different from that of an unscattered component?
- The contrast transfer function is typically plotted as a sinusoidally-varying function of what variable (what is the horizontal axis)? What quantity is plotted on the vertical axis?
- What is the CTF's domain and range?
- What does a "contrast transfer" of 1.0 mean? -1? 0?
- Why does the CTF oscillate sinusoidally?
- What four variables appear in the argument of the sine function?


Thon rings
k
4:02 / 21:28
HD

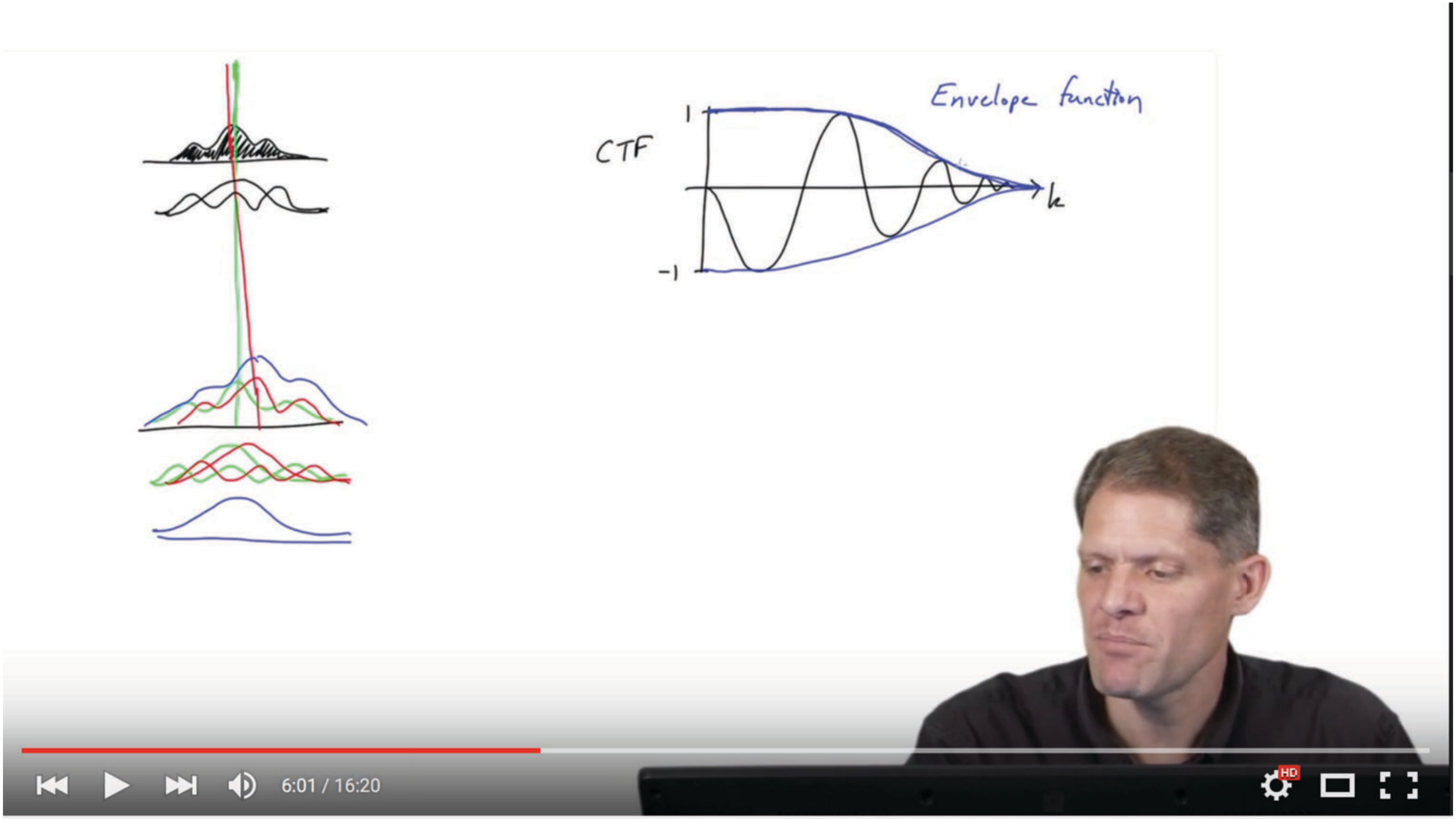

sample
lens
bfp
image plane
I1
I2
Proj
detector
λ 360°
Contrast
1
-1
k
Contrast transfer function (CTF)
$= \sin\left[-\pi \boxed{\Delta z} \lambda k^2 + \frac{\pi C_s \lambda^3 k^4}{2}\right]$
13:31 / 21:28



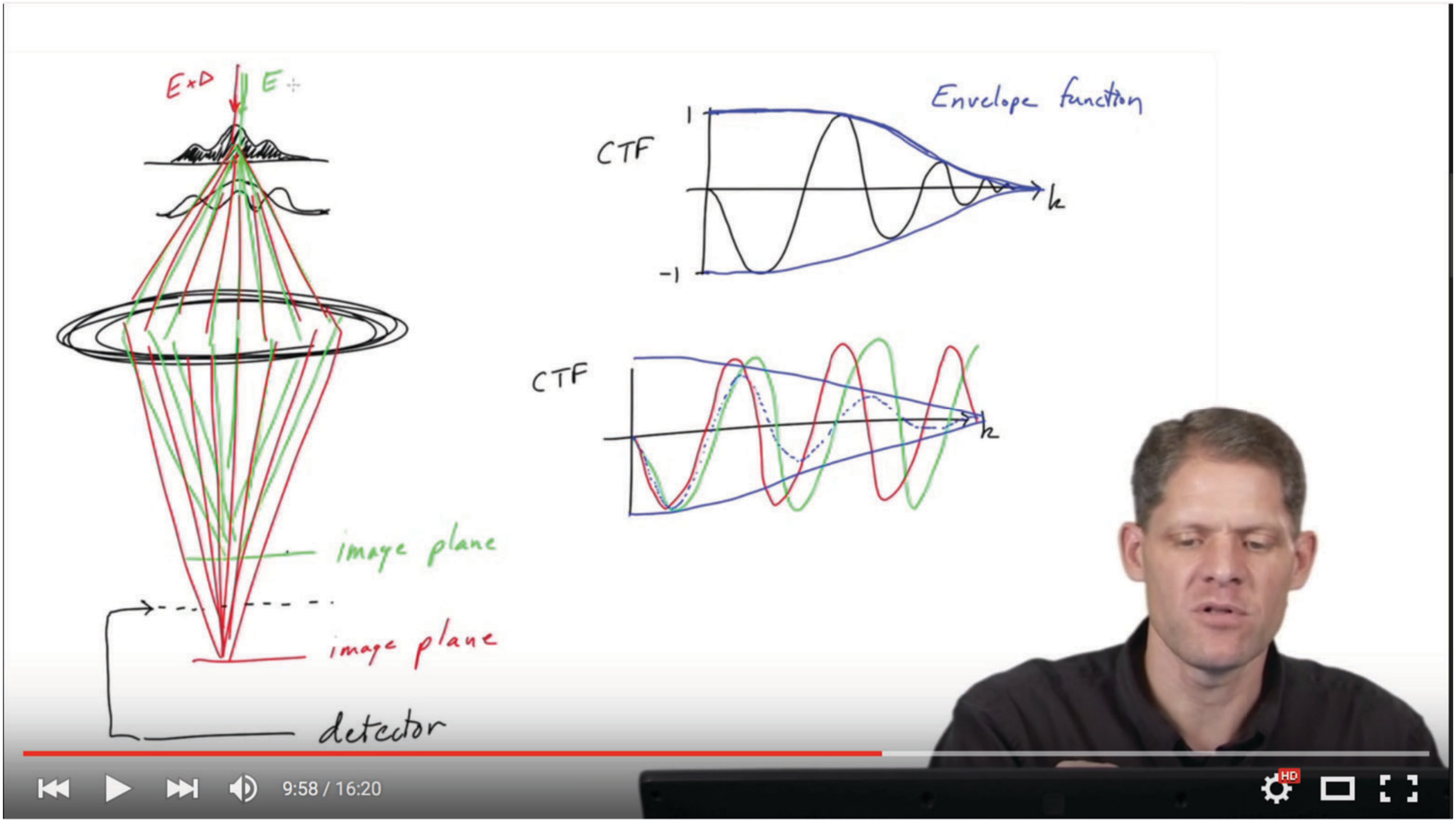
Thuman-Commike and Chiu, *Micron* **31**:687

Defocus and its effects
Concept check questions:

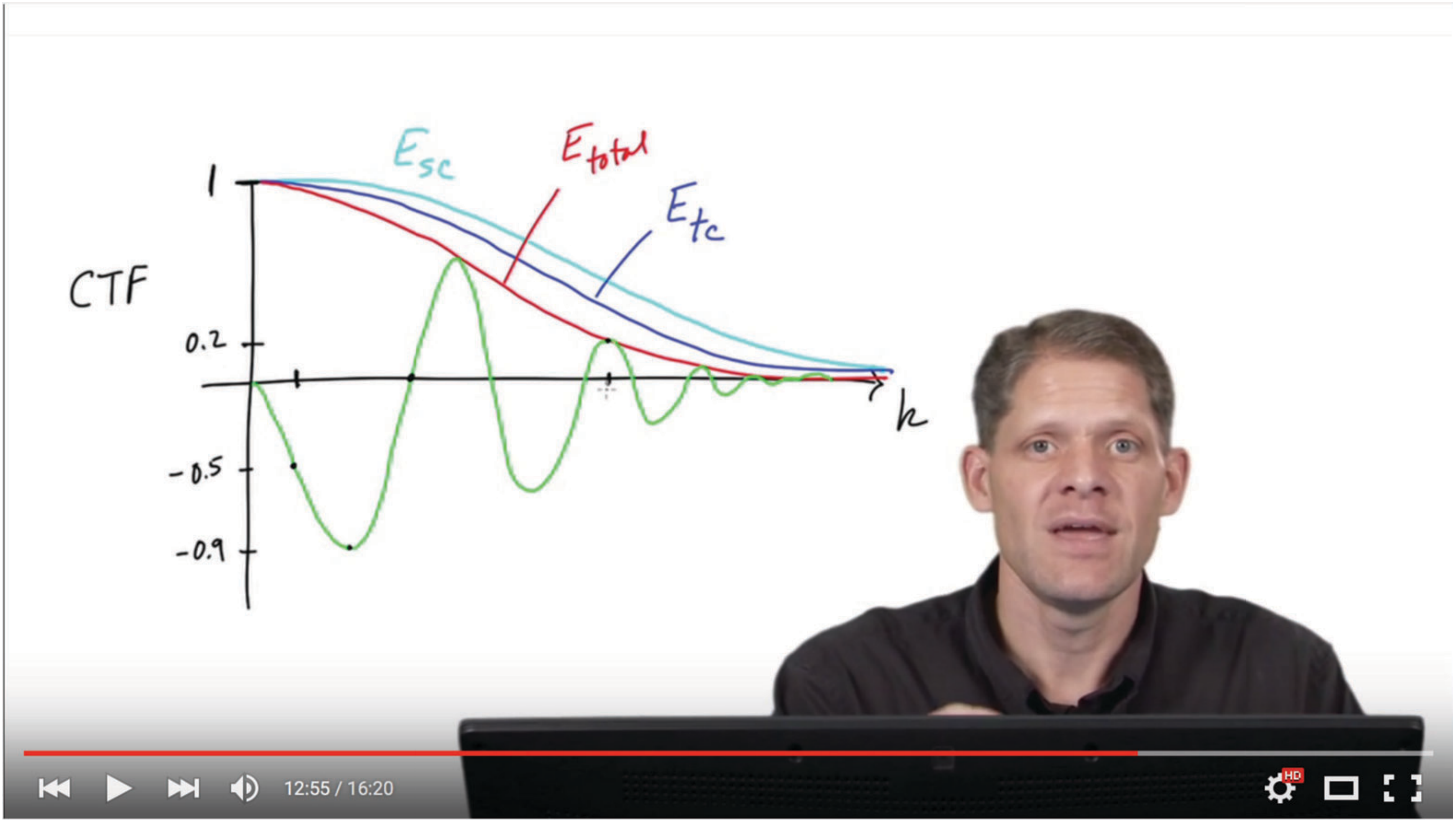
- What is a "Thon" ring?
- How can the defocus of a TEM image be determined?
- Why is defocus part of the argument of the CTF sine function?
- Does increasing the current in the objective lens make the image more or less defocussed?
- What is "over-focus"?
- How do heavily defocussed images look different than "closer-to-focus" images?
- What are the advantages of taking pictures far from focus? close to focus?



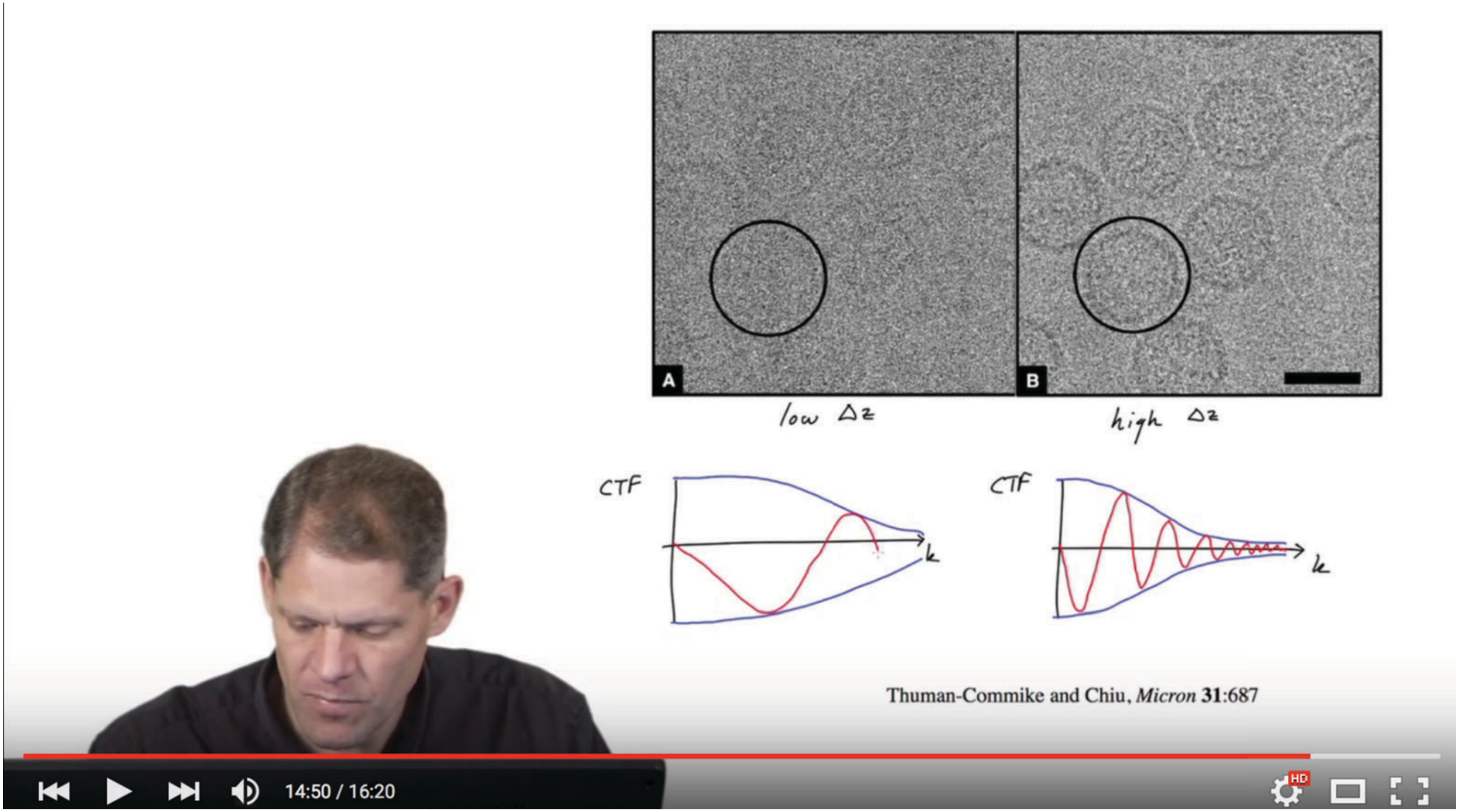
Envelope function
CTF
1
-1
k
6:01 / 16:20
HD



E×D
E
Envelope function
CTF
1
-1
k
CTF
k
image plane
image plane
detector
9:58 / 16:20
HD

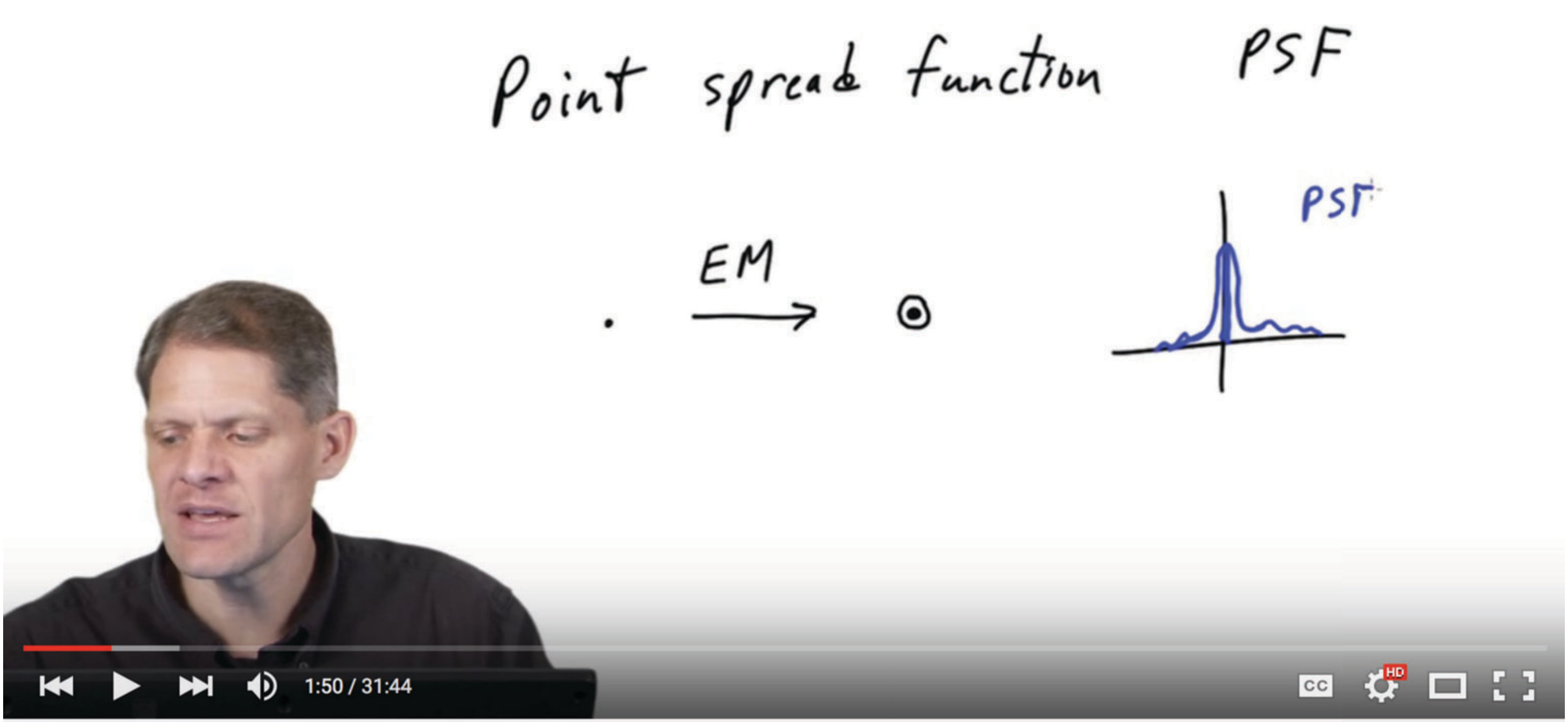


CTF
1
0.2
-0.5
-0.9
k
E_{sc}
E_{total}
E_{tc}
12:55 / 16:20
HD


A
B
low Δz
high Δz
CTF
k
CTF
k
Thuman-Commike and Chiu, *Micron* **31**:687
14:50 / 16:20
HD

Envelopes
Concept check questions:

- What effect does partial spatial coherence have on the CTF? Why?
- What effect does partial temporal coherence have on the CTF? Why?
- What is their combined effect?
- How do these effects depend on defocus?



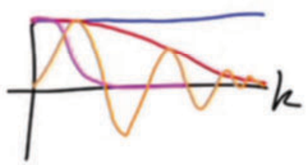
Point spread function
PSF
EM
PSF
1:50 / 31:44
CC
HD

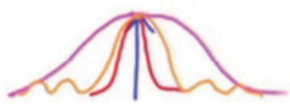
$$PSF = \mathcal{F}\{CTF\}$$

$\uparrow$ $\qquad\qquad$?

object $\longrightarrow$ image

CTF $\qquad\qquad$ PSF





convolution

$$I = O \otimes PSF$$

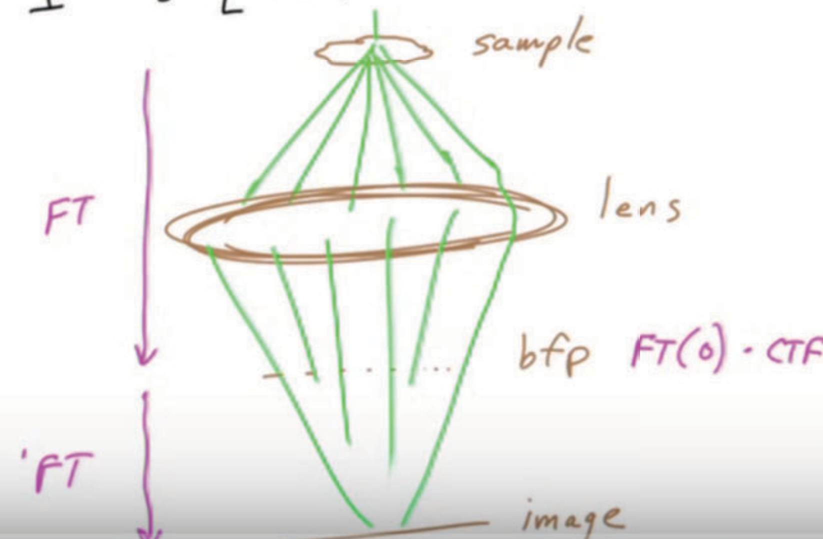
$$I = O \otimes PSF$$

$$\mathcal{F}\{I\} = \mathcal{F}\{O \otimes PSF\}$$

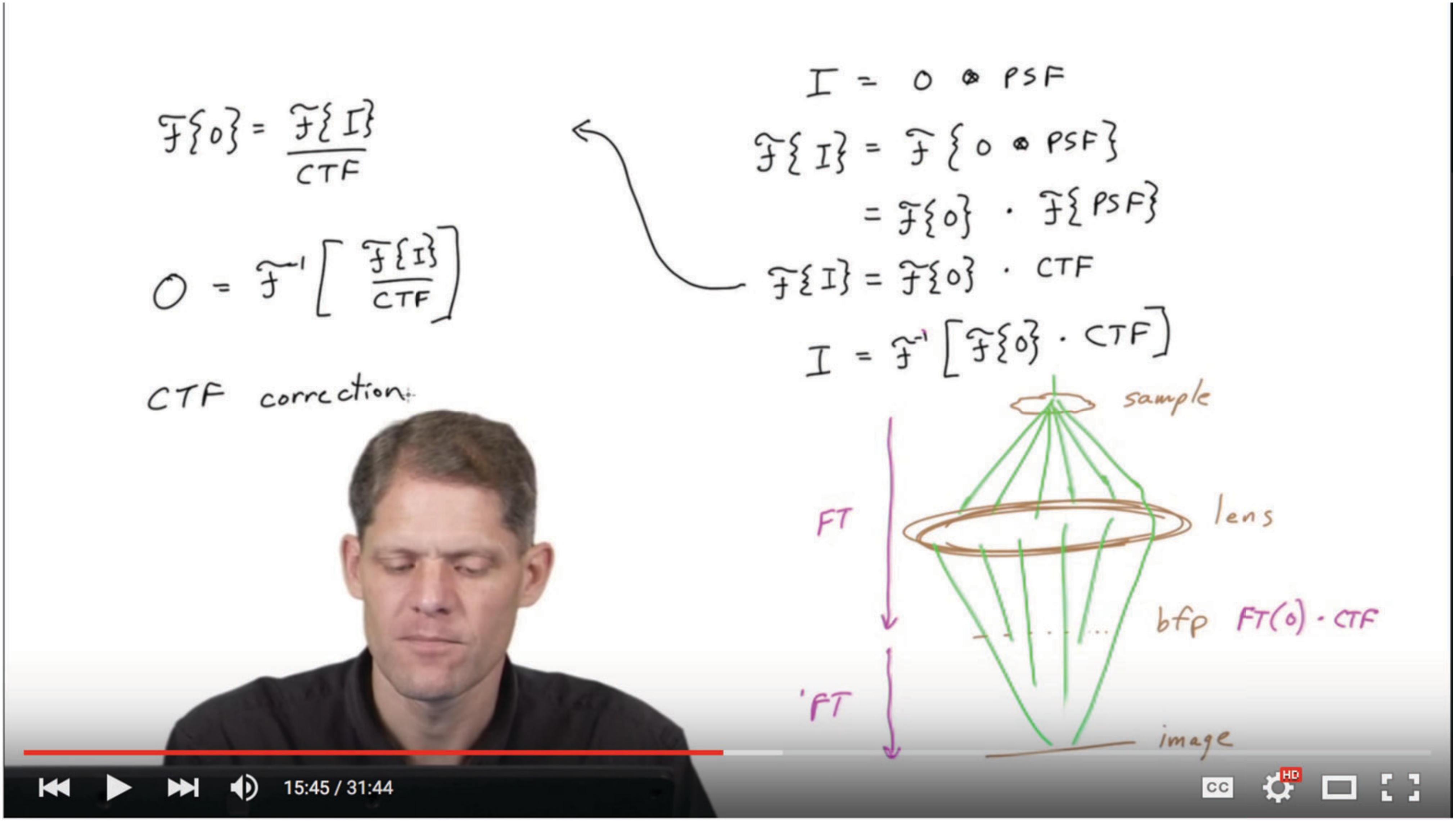
$$= \mathcal{F}\{O\} \cdot \mathcal{F}\{PSF\}$$

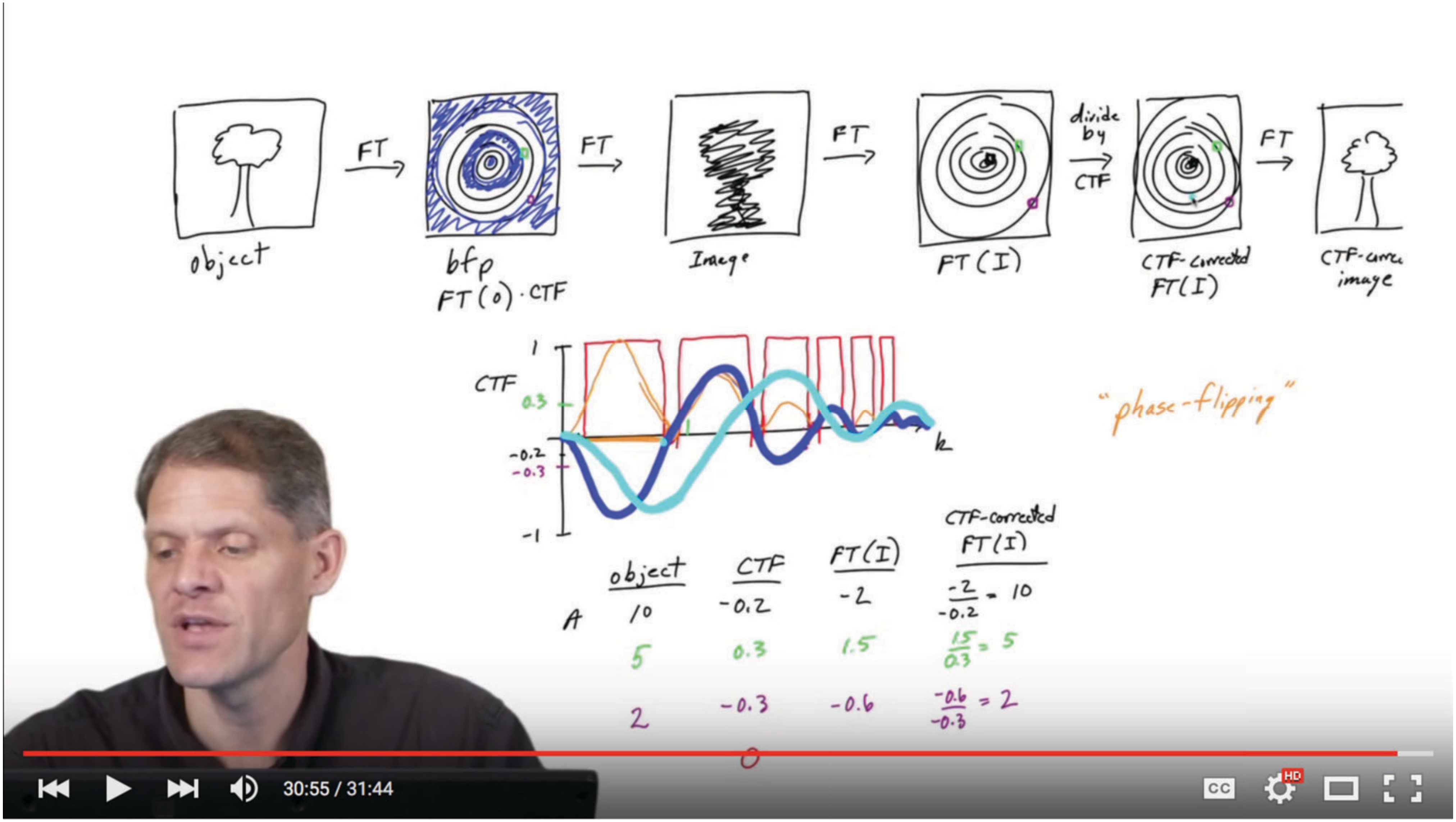
$$\mathcal{F}\{I\} = \mathcal{F}\{O\} \cdot CTF$$

$$I = \mathcal{F}^{-1}\left[\mathcal{F}\{O\} \cdot CTF\right]$$



13:55 / 31:44


I = O ⊗ PSF
F{I} = F{O ⊗ PSF}
= F{O} · F{PSF}
F{I} = F{O} · CTF
I = F⁻¹[F{O} · CTF]
F{O} = F{I} / CTF
O = F⁻¹[F{I} / CTF]
CTF correction
sample
lens
FT
bfp FT(O) · CTF
'FT
image
15:45 / 31:44
CC
HD


object
FT
bfp
FT(o)·CTF
FT
Image
FT
FT(I)
divide by CTF
CTF-corrected FT(I)
FT
image
CTF
1
0.3
-0.2
-0.3
-1
k
"phase-flipping"
object
CTF
FT(I)
CTF-corrected FT(I)
A
10
-0.2
-2
-2/-0.2 = 10
5
0.3
1.5
1.5/0.3 = 5
2
-0.3
-0.6
-0.6/-0.3 = 2
0
30:55 / 31:44
CC
HD

CTF correction

Record image

↓

Calculate Fourier transform

↓

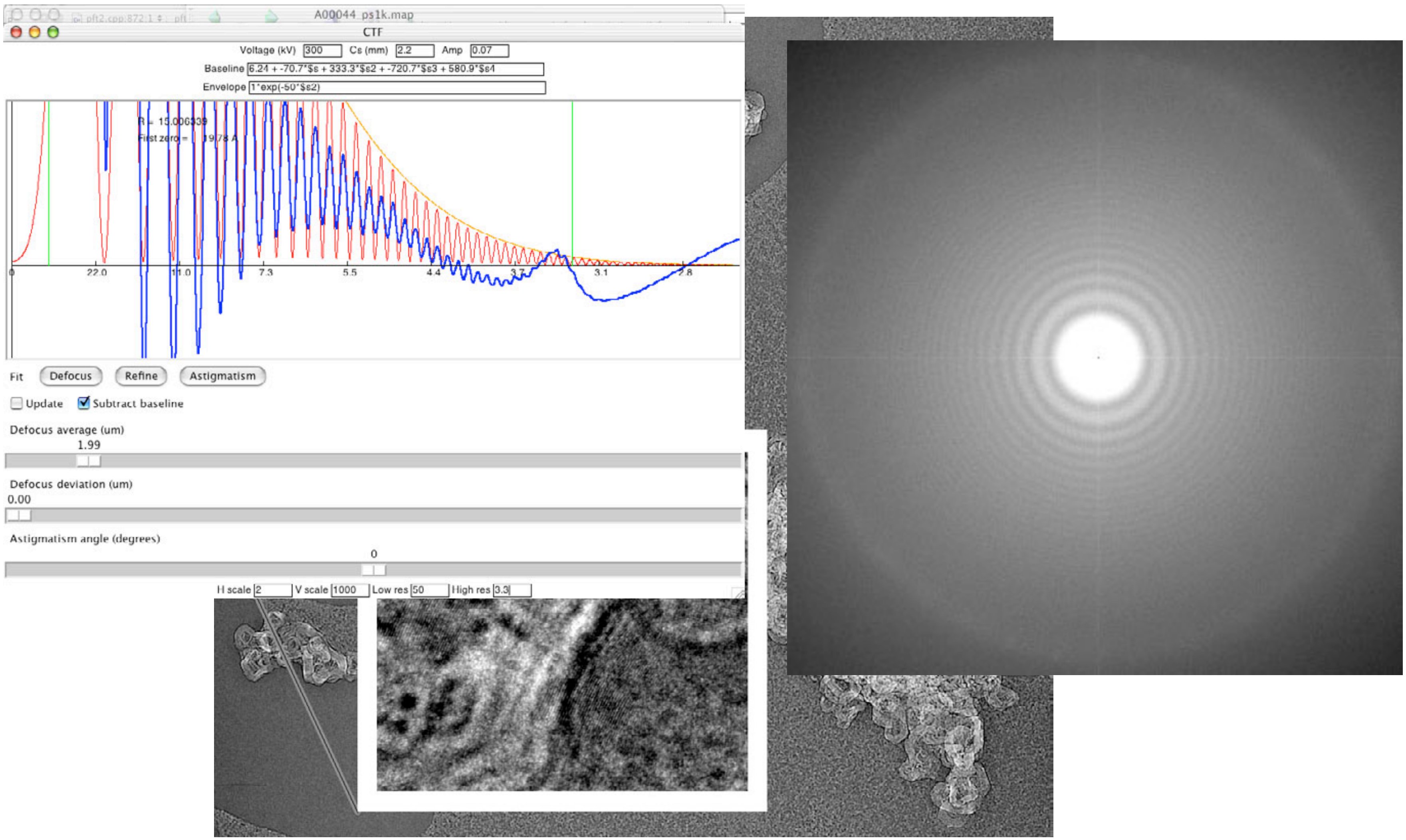
Fit power spectrum to theoretical CTF

↓

Divide amplitudes by CTF (taking care not to divide by zero)

↓

Inverse Fourier transform result



A00044_ps1k.map
CTF
Voltage (kV) 300 Cs (mm) 2.2 Amp 0.07
Baseline 6.24 + -70.7*$s + 333.3*$s2 + -720.7*$s3 + 580.9*$s4
Envelope 1*exp(-50*$s2)
0 22.0 11.0 7.3 5.5 4.4 3.7 3.1 2.8
Fit Defocus Refine Astigmatism
Update Subtract baseline
Defocus average (um)
1.99
Defocus deviation (um)
0.00
Astigmatism angle (degrees)
0
H scale 2 V scale 1000 Low res 50 High res 3.3

CTF correction
Concept check questions:

- What is a “point spread function”?
- How is the point spread function related to the CTF?
- What is the relationship between the wavefunction that exists on the back focal plane of the microscope and the Fourier transform of the recorded image?
- How (conceptually) can EM images be “CTF-corrected”?
- How can the CTF of a TEM image be determined?
- What special issue arises at CTF-zeros? How can it be handled?
- What would it mean if someone said they “CTF-corrected by phase-flipping only”?
- How can the information loss at CTF-zeros be overcome?