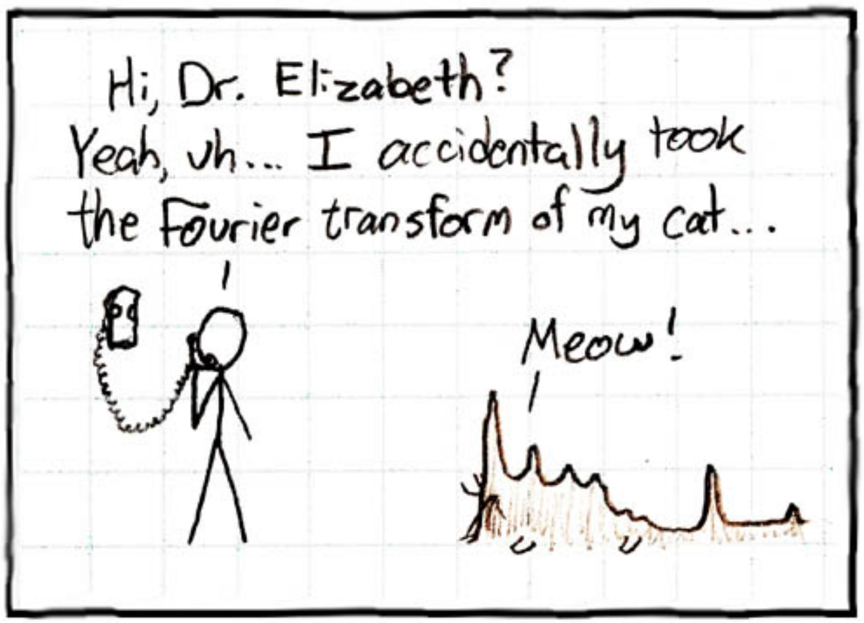
Fourier Transforms and Image Formation



One-dimensional sine waves and their sums
Concept check questions:

What four parameters define a sine wave?

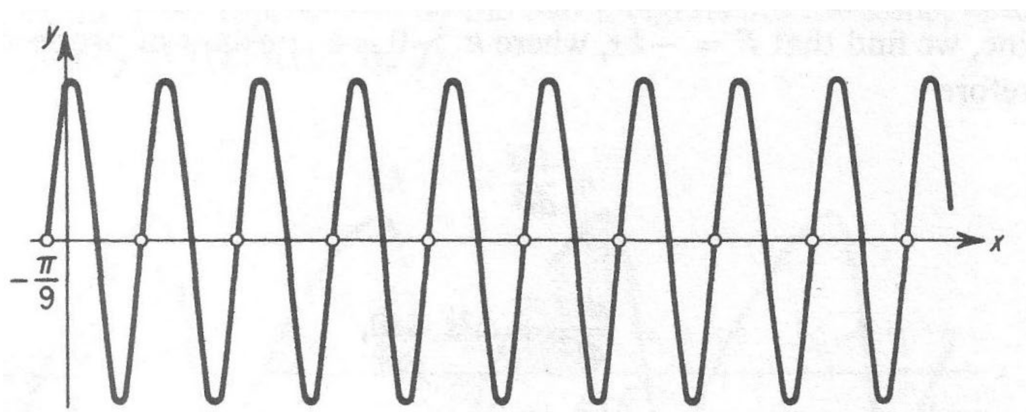
What is the difference between a temporal and a spatial frequency?

What in essence is a "Fourier transform"?

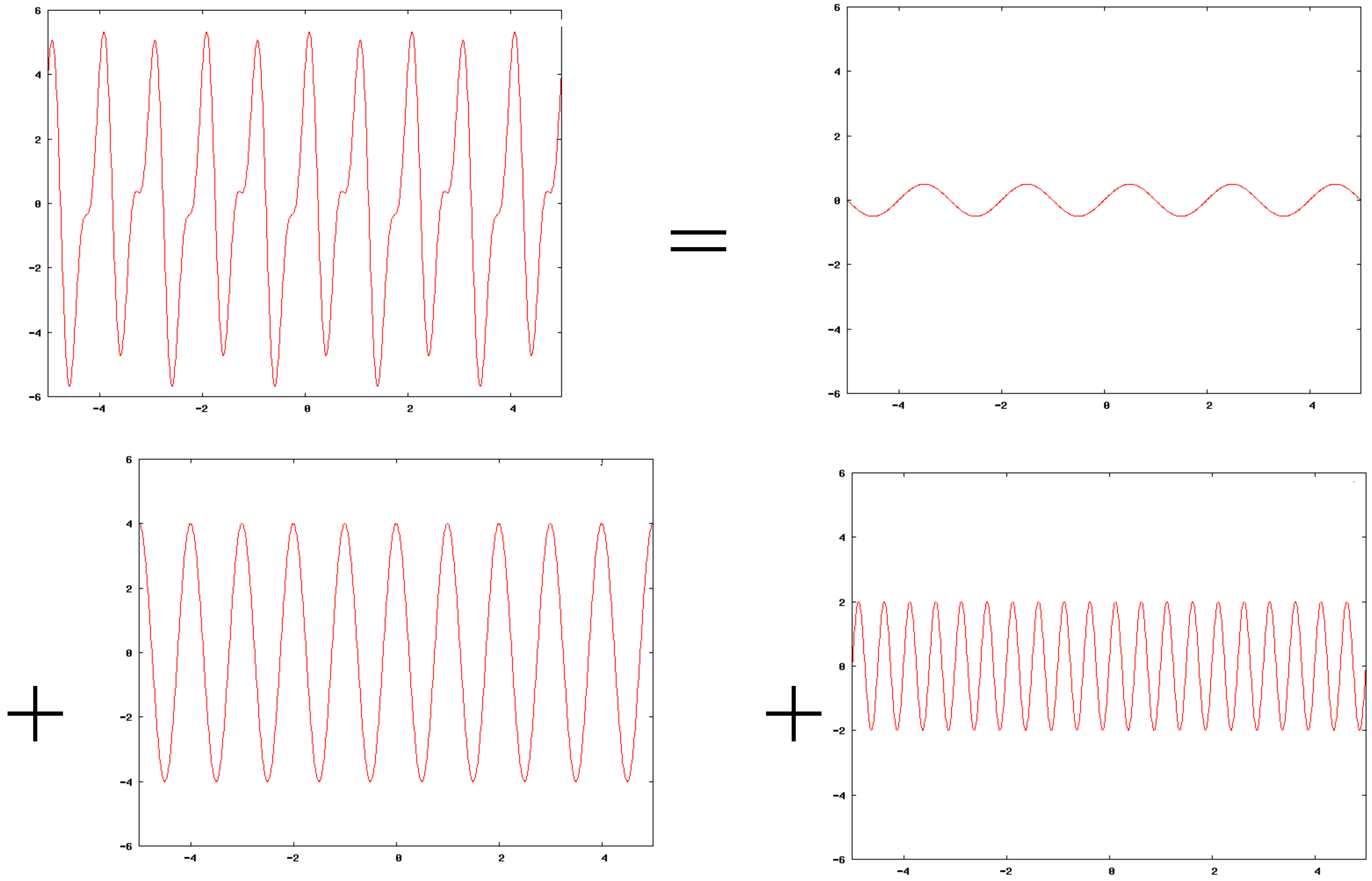
How can the amplitude of each Fourier component of a waveform be found?

Sine Wave

- $y = 2\sin(3x + \pi/3)$

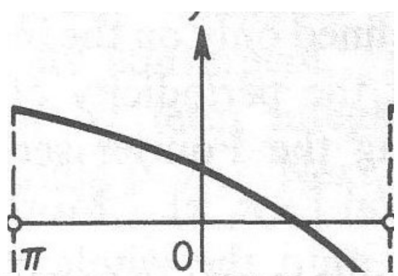


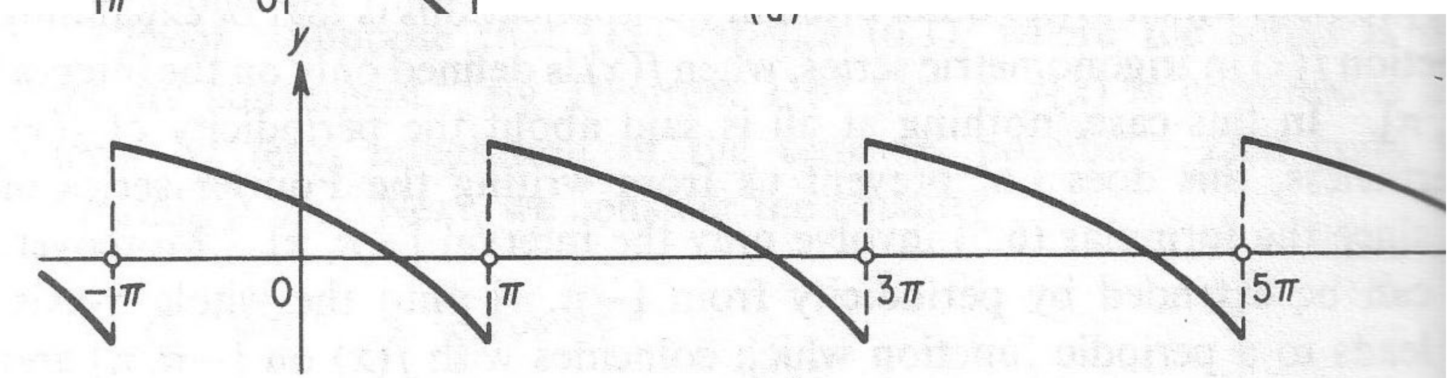
Fourier Series



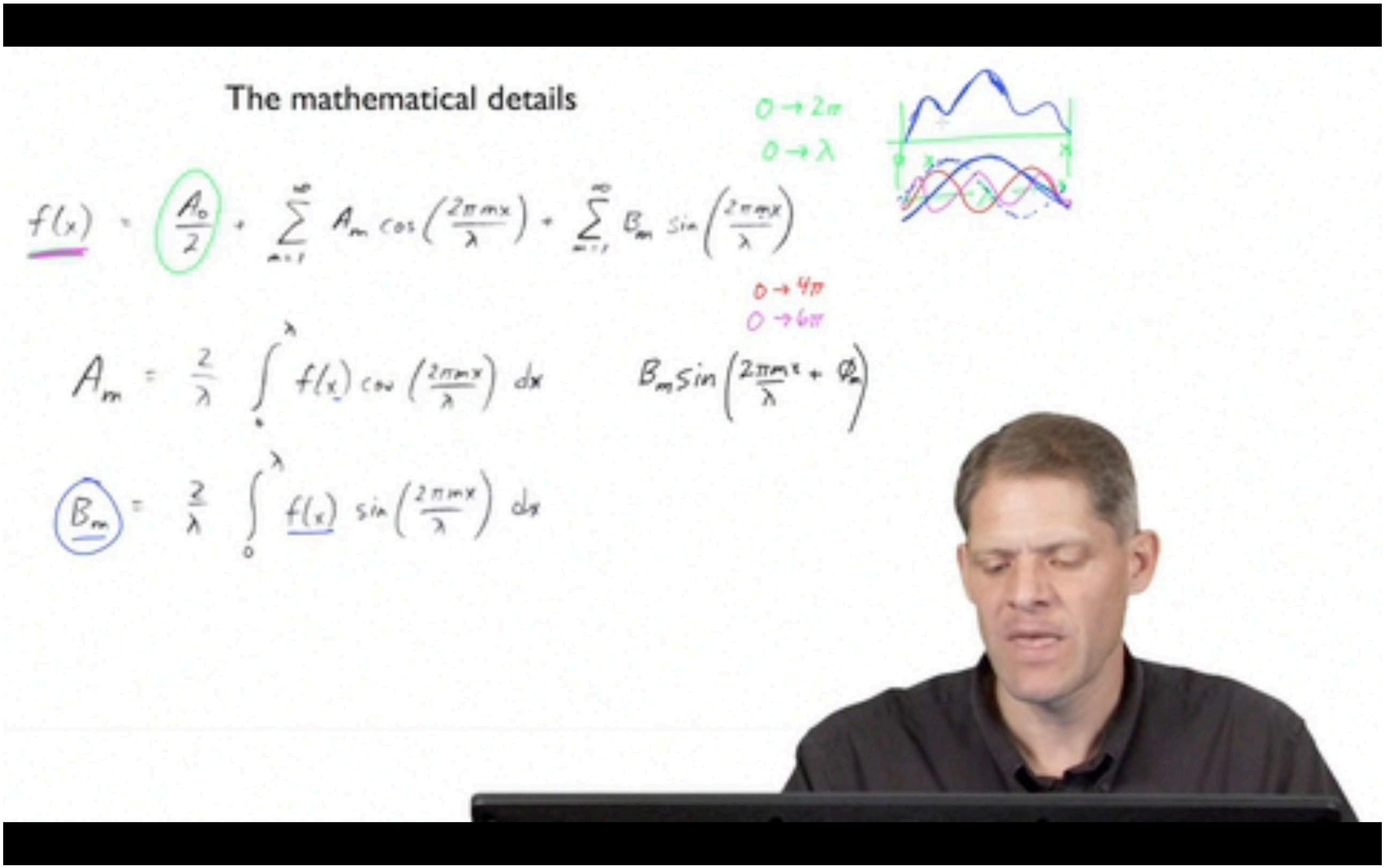
Fourier Series

- Non-periodic Functions
- For functions which are defined only on the interval [-π,π], we extend the function by periodicity onto the whole x-axis:





Details

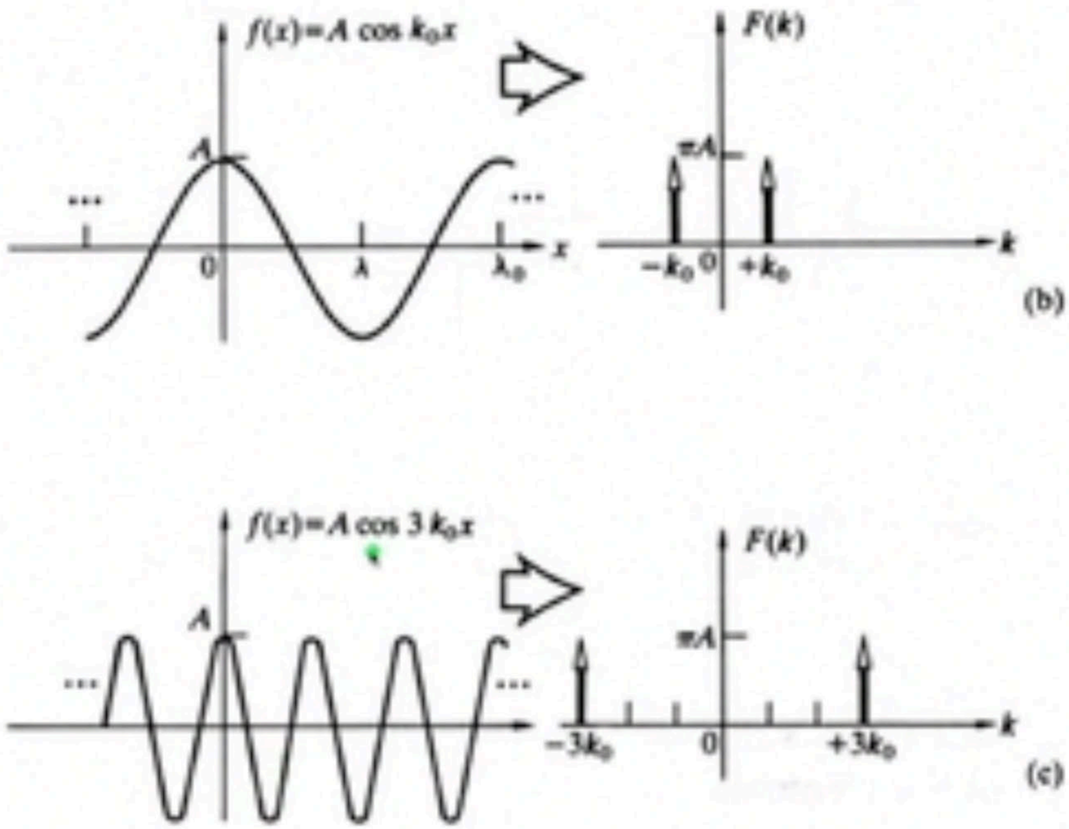


Conventions

- **Image domain**
- Real space
- $f(x,y)$
- $f(r,\theta)$
- $f(t)$

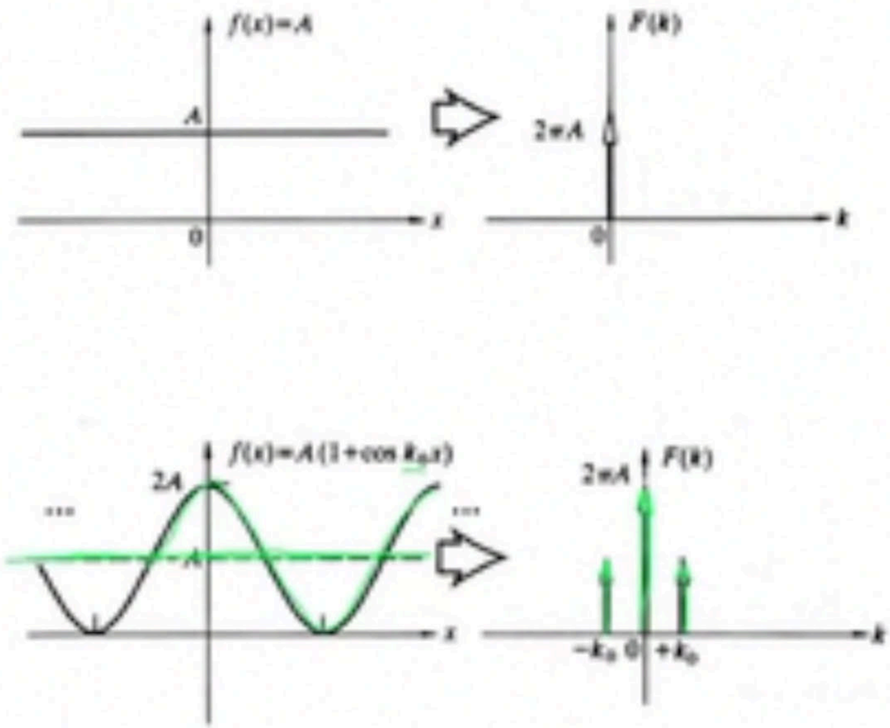
- **Fourier Domain**
- Fourier Space
- Inverse space
- Reciprocal space
- Diffraction space
- $F(X,Y)$, $F(kx,ky)$
- $F(k,\Theta)$
- $H(\omega)$

One dimensional functions and transforms (spectra)



Hecht, Fig. 11.13

One dimensional functions and transforms (spectra)



Hecht, Fig. 11.38

Fourier Transforms

The two equations

$$F(X)=\int_{-\infty}^{\infty} f(x)e^{-2\pi iXx}\,dx$$

$$f(x)=\int_{-\infty}^{\infty} F(X)e^{2\pi ixX}\,dX$$

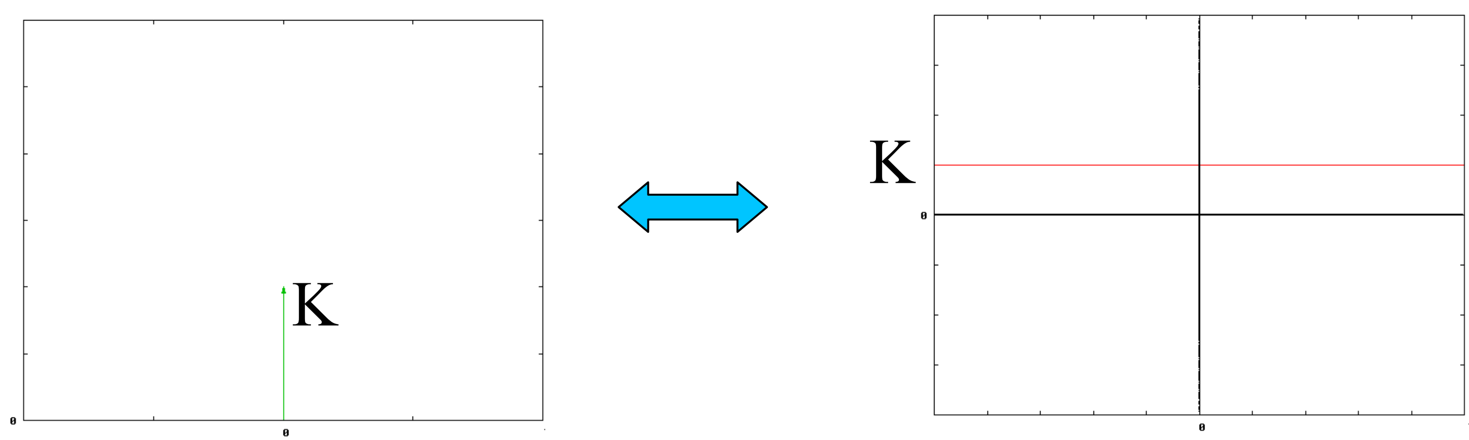
are mates, and let you convert from real space to frequency space and back

Fourier transform of a delta function

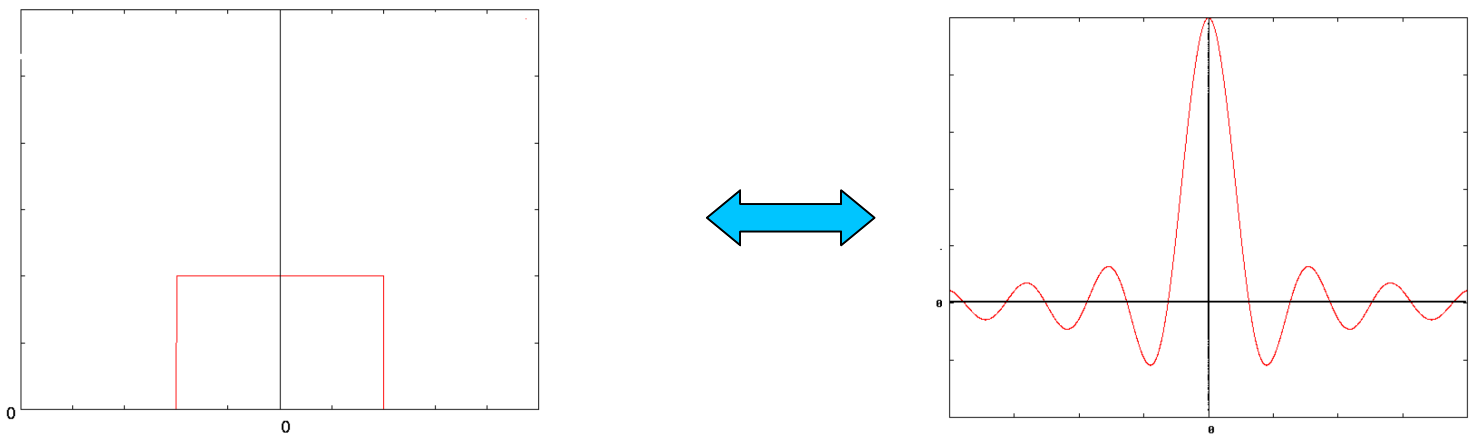
$$h(t) = K\delta(t)$$

$$H(f) = \int_{-\infty}^{\infty} K\delta(t) e^{-2\pi i f t}\, dt$$

$$H(f) = K$$



Top-hat function

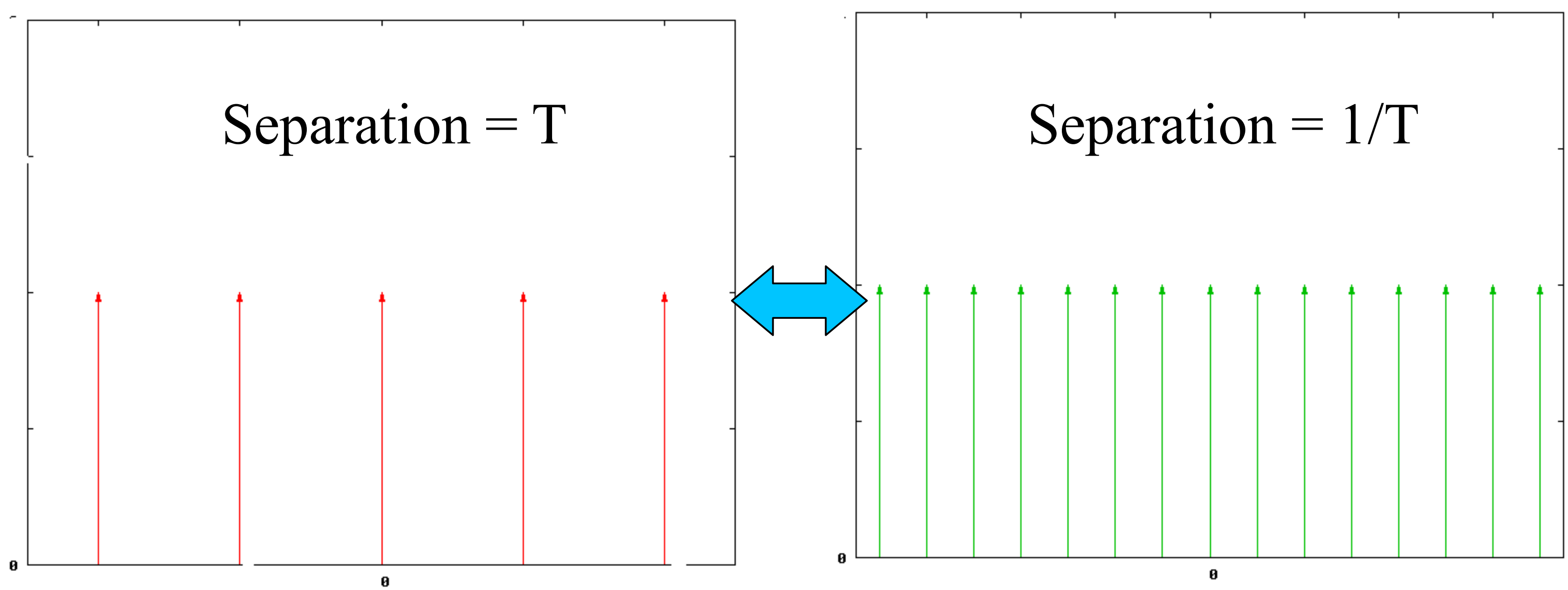


$$H(X) = \frac{\sin(X)}{X}$$

sinc function

Comb function

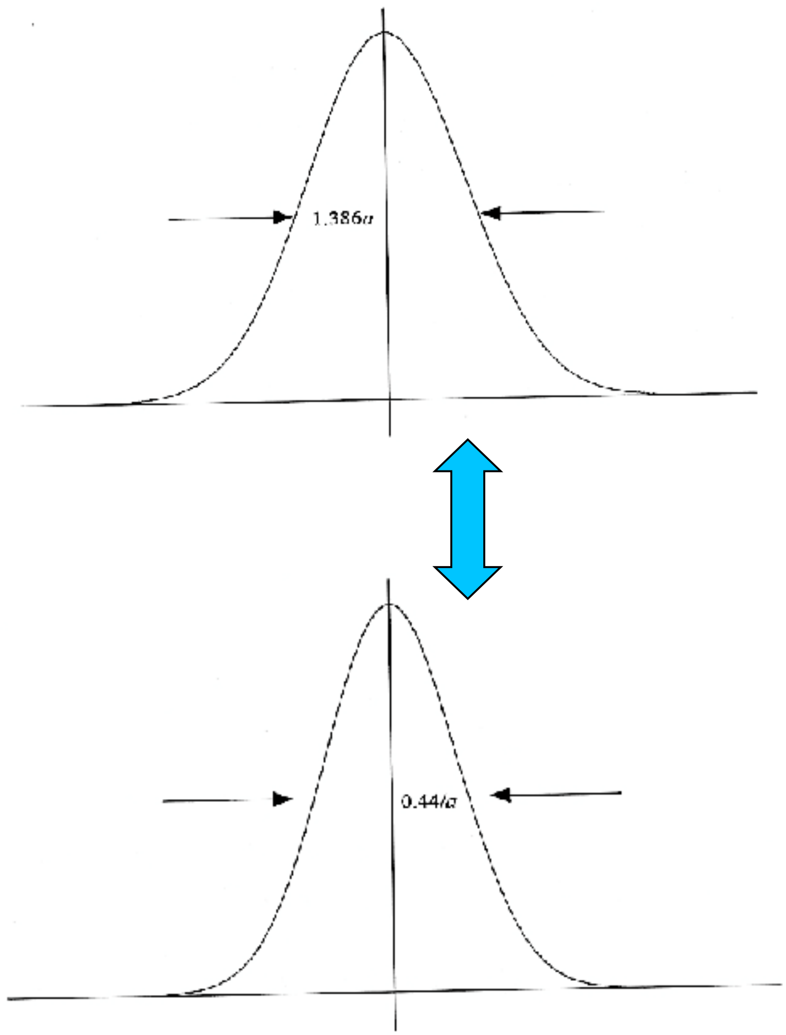
- Infinite points on comb



$$h_\infty(t) = \sum_{n=-\infty}^{\infty} \delta(t - nT)$$

$$H_\infty(f) = \frac{1}{T} \sum_{n=-\infty}^{\infty} \delta\left(f - \frac{n}{T}\right)$$

Gaussian Function

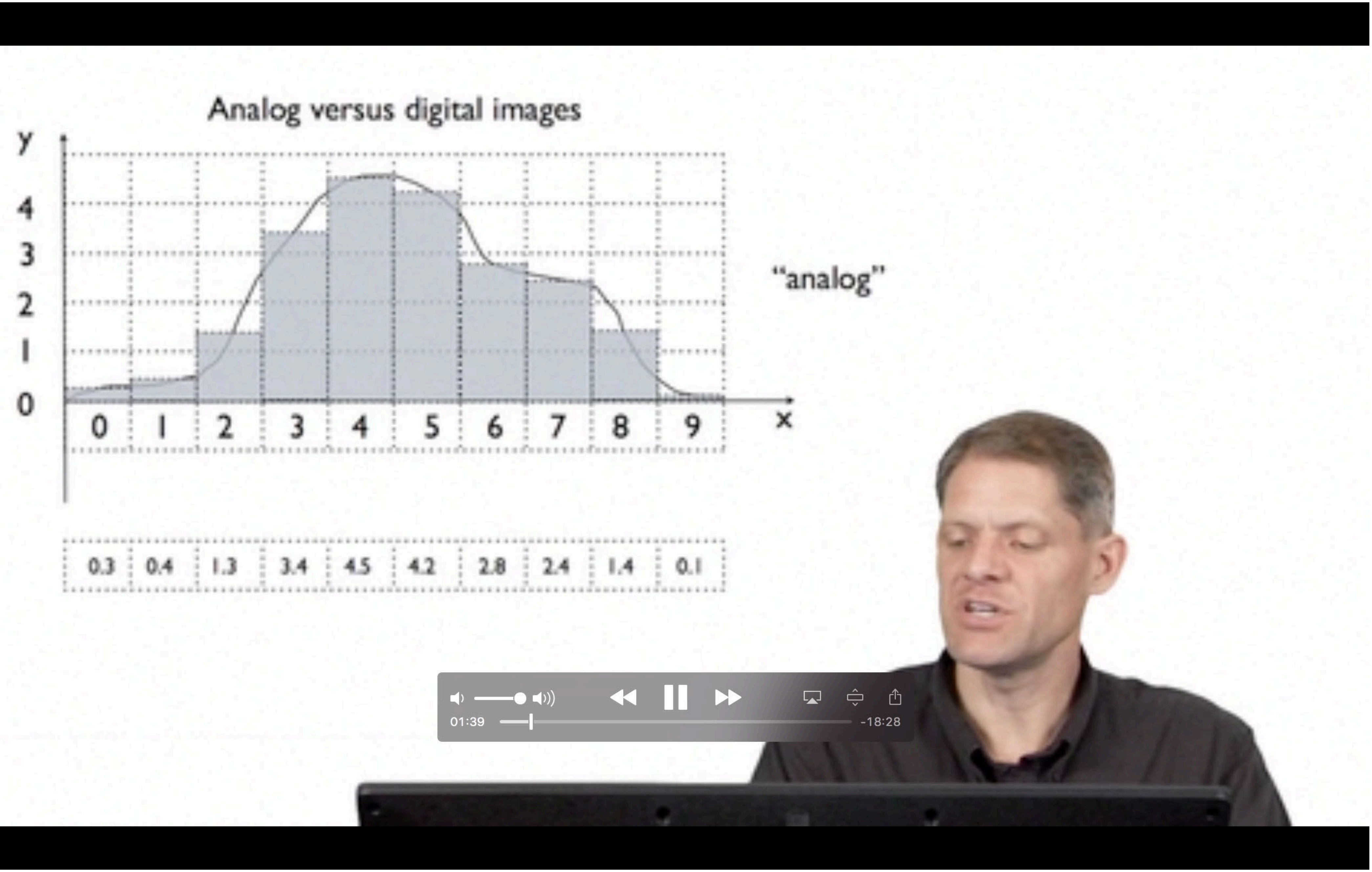


width=a

width=1/a

One-dimensional reciprocal space
Concept check questions:

- What is the difference between an "analog" and a "digital" image?
- What is the "fundamental" frequency? A "harmonic"? "Nyquist" frequency?
- What is "reciprocal" space? What are the axes?
- What does a plot of the Fourier transform of a function in reciprocal space tell you?


Analog versus digital images
y
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9
x
"analog"
0.3 0.4 1.3 3.4 4.5 4.2 2.8 2.4 1.4 0.1
01:39
-18:28

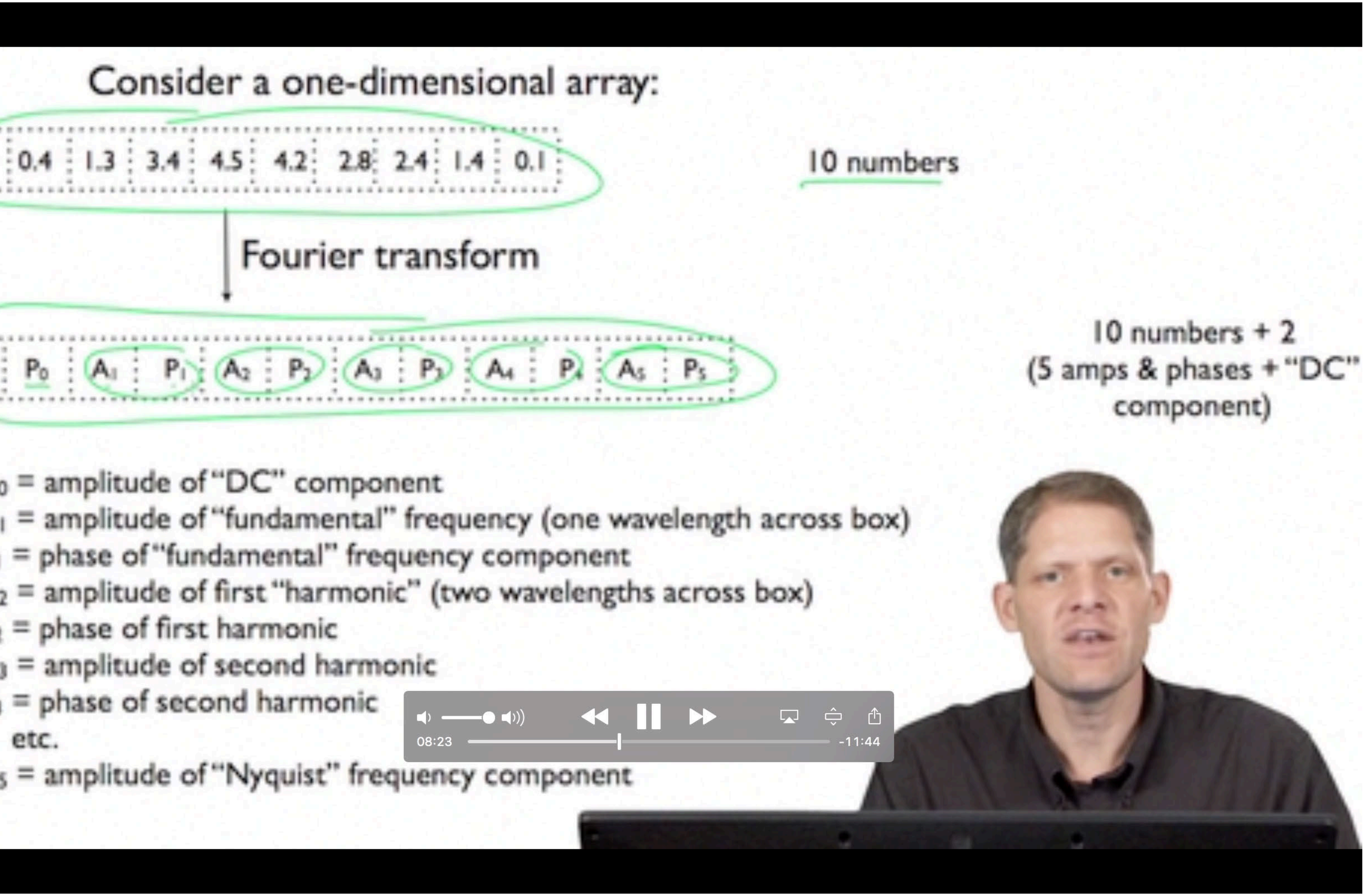
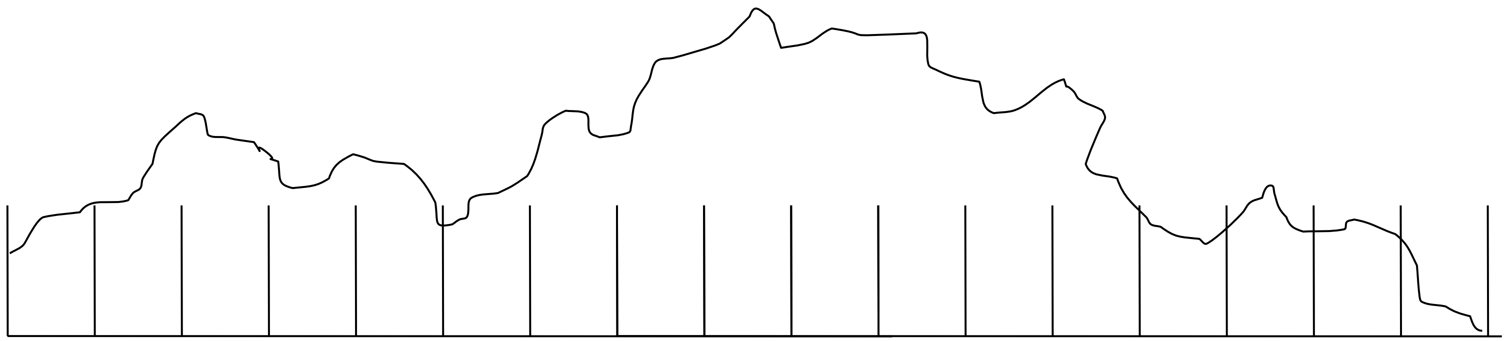
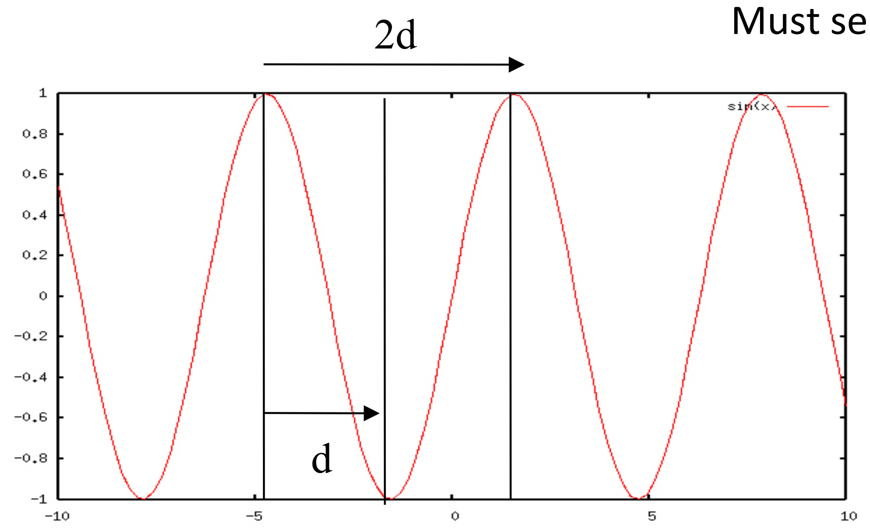

Consider a one-dimensional array:
0.4 1.3 3.4 4.5 4.2 2.8 2.4 1.4 0.1
10 numbers
Fourier transform
P0 A1 P1 A2 P2 A3 P3 A4 P4 A5 P5
10 numbers + 2
(5 amps & phases + "DC" component)
= amplitude of "DC" component
= amplitude of "fundamental" frequency (one wavelength across box)
= phase of "fundamental" frequency component
= amplitude of first "harmonic" (two wavelengths across box)
= phase of first harmonic
= amplitude of second harmonic
= phase of second harmonic
etc.
= amplitude of "Nyquist" frequency component
08:23
-11:44

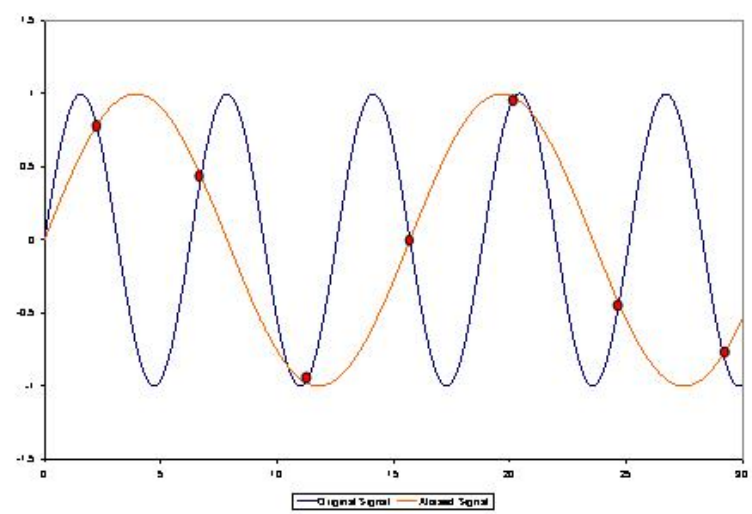
Image sampling
(for digital FT)

Shannon-Nyquist sampling limit:
Finest spatial period must be sampled >2x
Otherwise → aliasing



Must see peaks and valleys of a feature

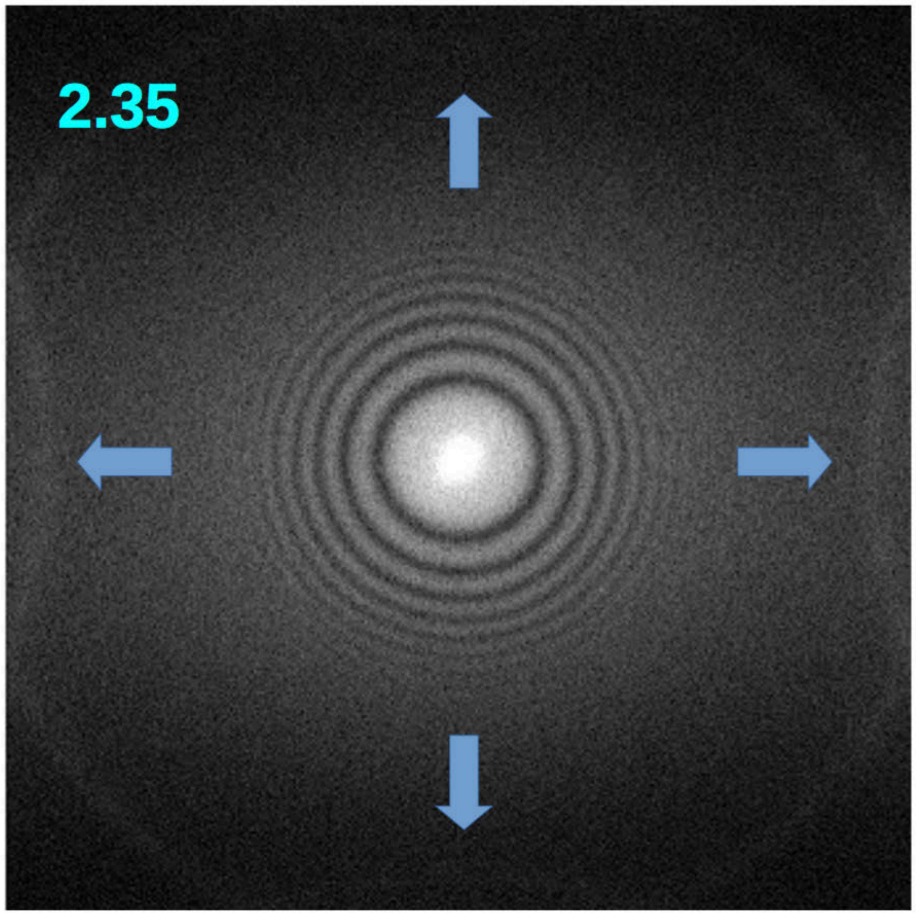




A minimally sampled image

Undersampled

30K: Gold Aliases Back into Image



Pixel Size: 1.2 A

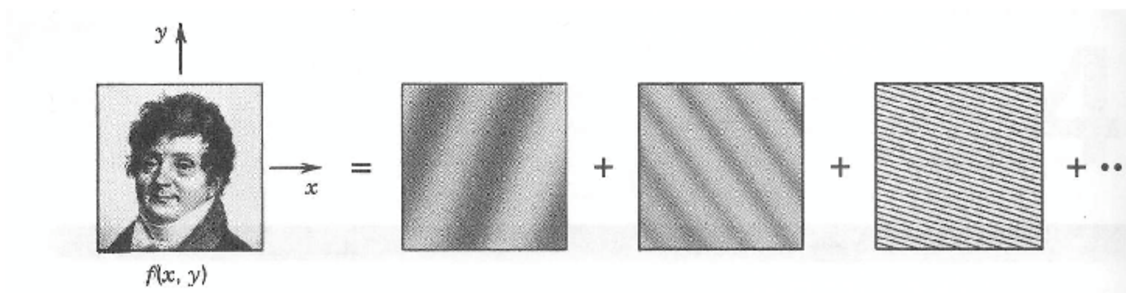
Two-dimensional waves and images
Concept check questions:

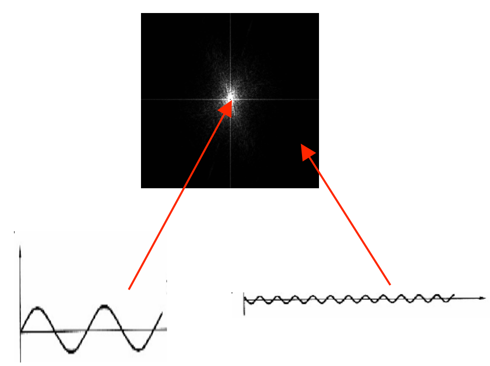
- What does a two-dimensional sine wave look like?
- What do the "Miller" indices "h" and "k" indicate?

Images: Extending transforms into 2d

- Consider images as a 2 dimensional function of x and y, where the value of f(x,y) is represented as brightness

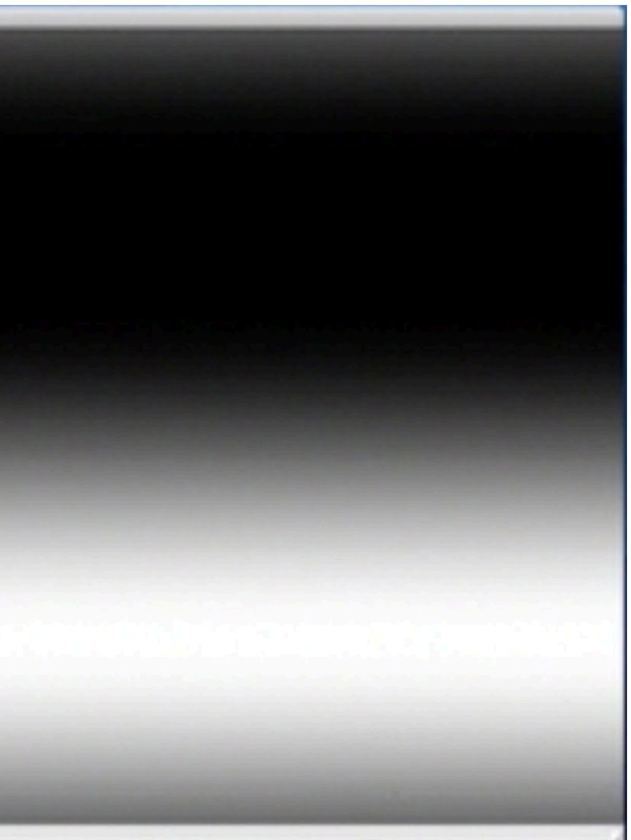
2D Fourier transform



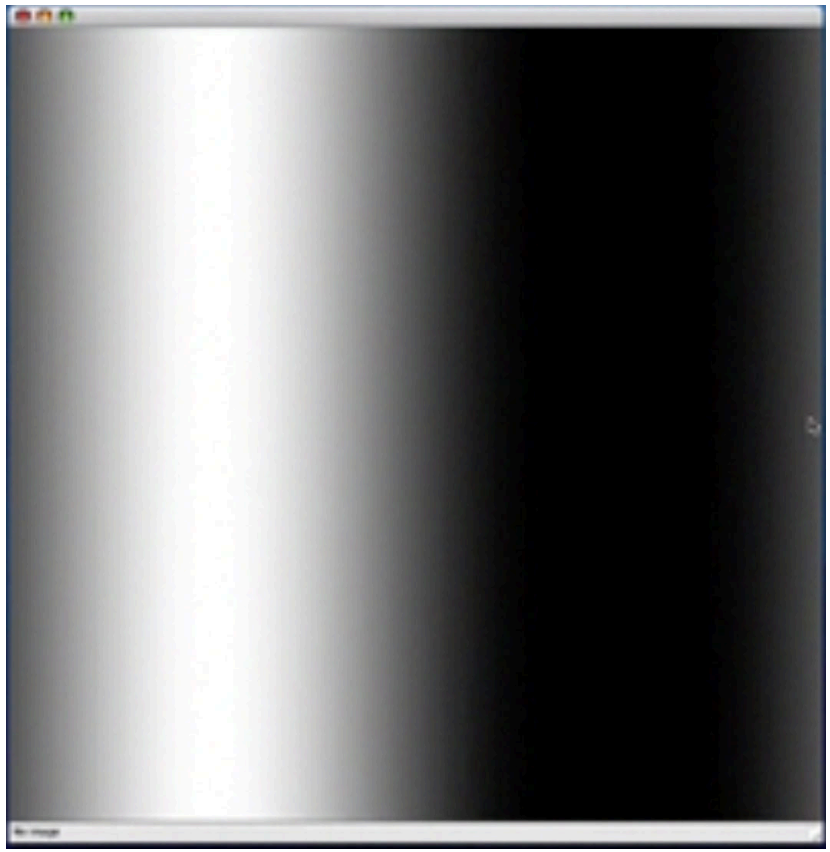


$$F(K_x, K_y) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} f(x,y) \exp(i(K_x x + K_y y)) dxdy$$

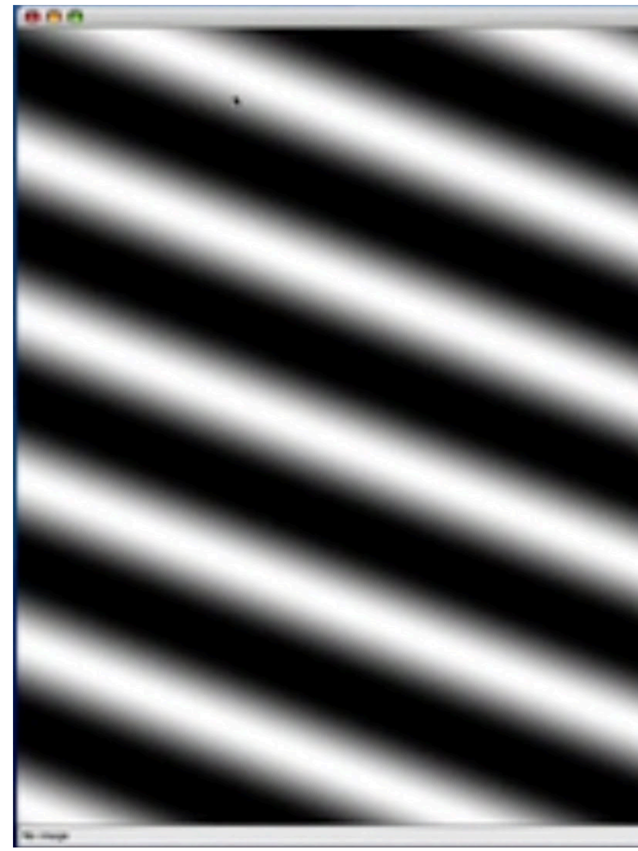
(0,1)



(1,0)

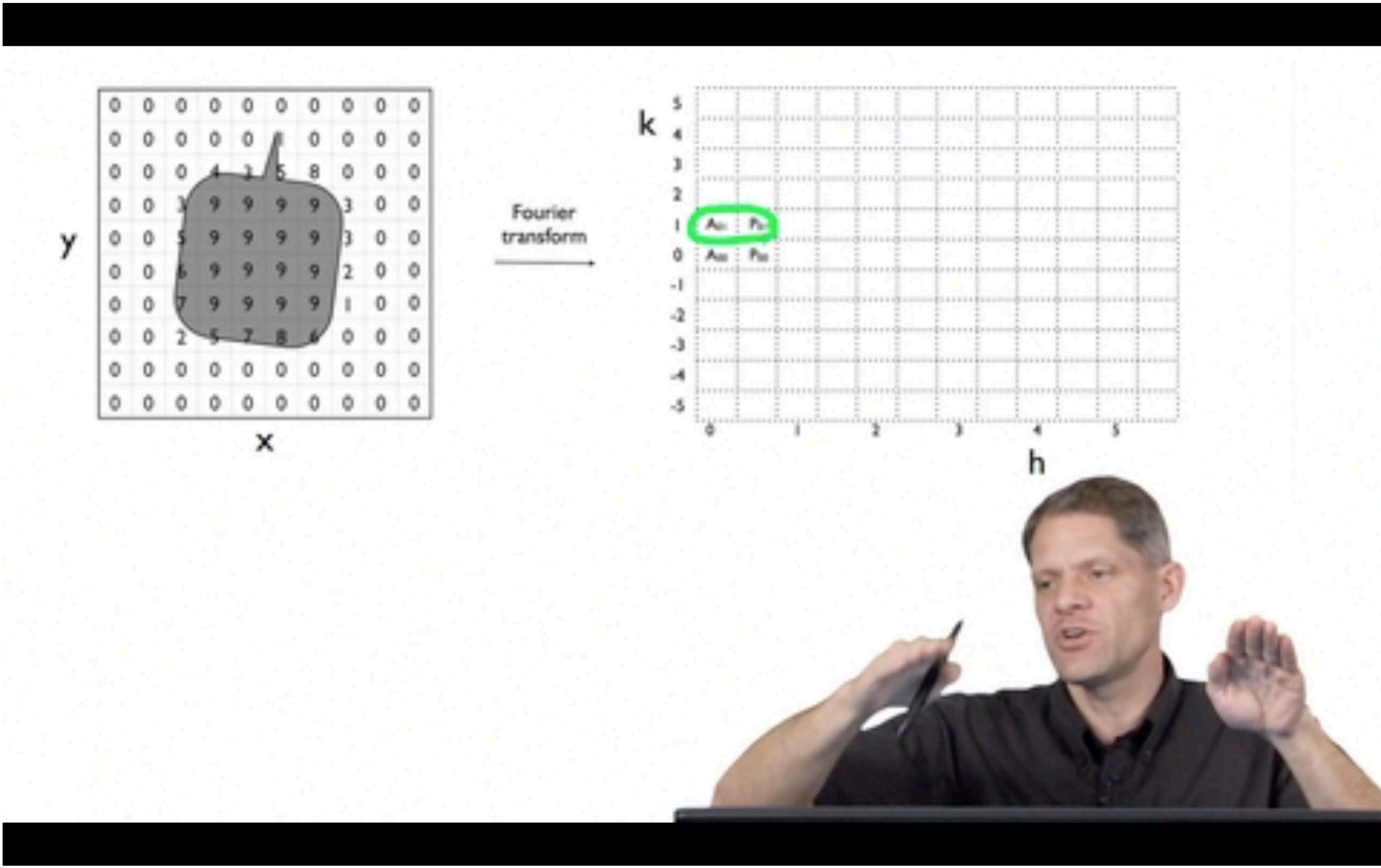


(2,5)

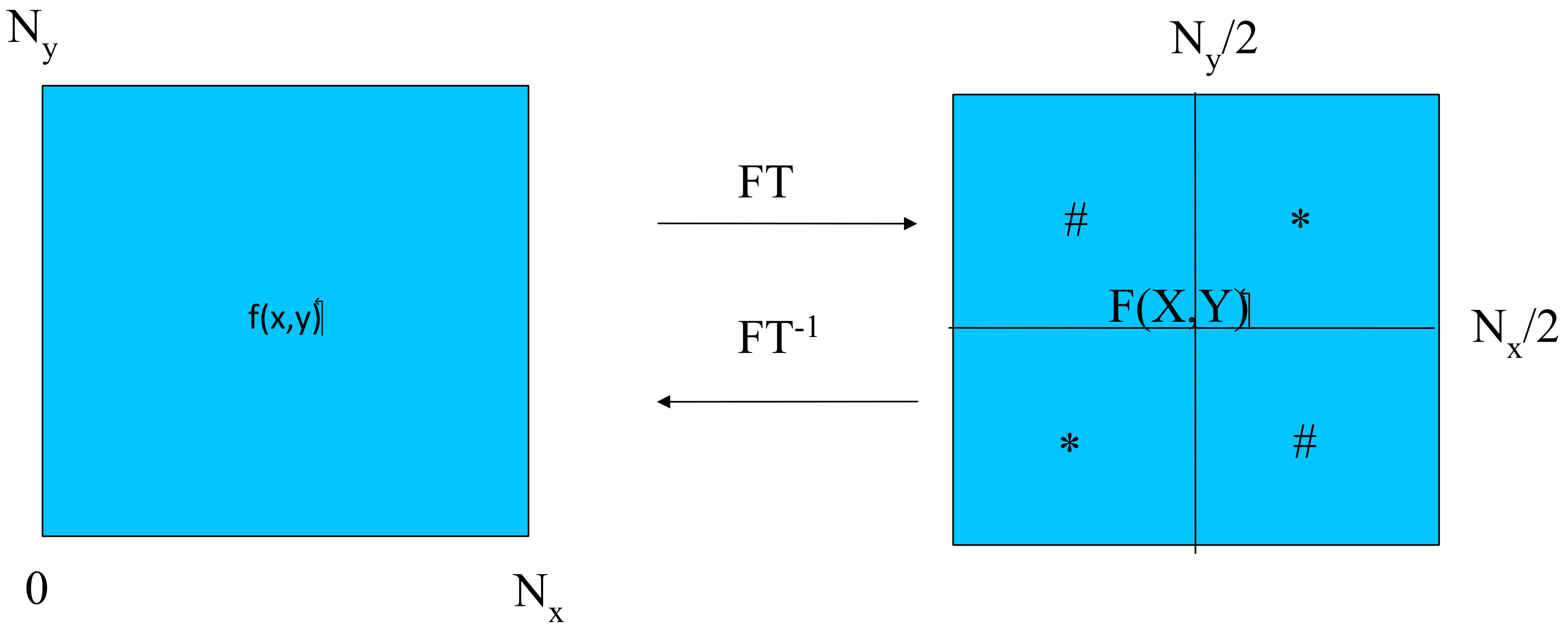


Two-dimensional transforms and filters
Concept check questions:

- In the Fourier transform of a real image, how much of reciprocal space (positive and negative values of "h" and "k") is unique?
- If an image "I" is the sum of several component images, what is the relationship of its Fourier transform to the Fourier transforms of the component images?
- What part of a Fourier transform is not displayed in a power spectrum?
- What does the "resolution" of a particular pixel in reciprocal space refer to?
- What is a "low pass" filter? "High pass"? "Band pass"?


y
x
Fourier
transform
k
h

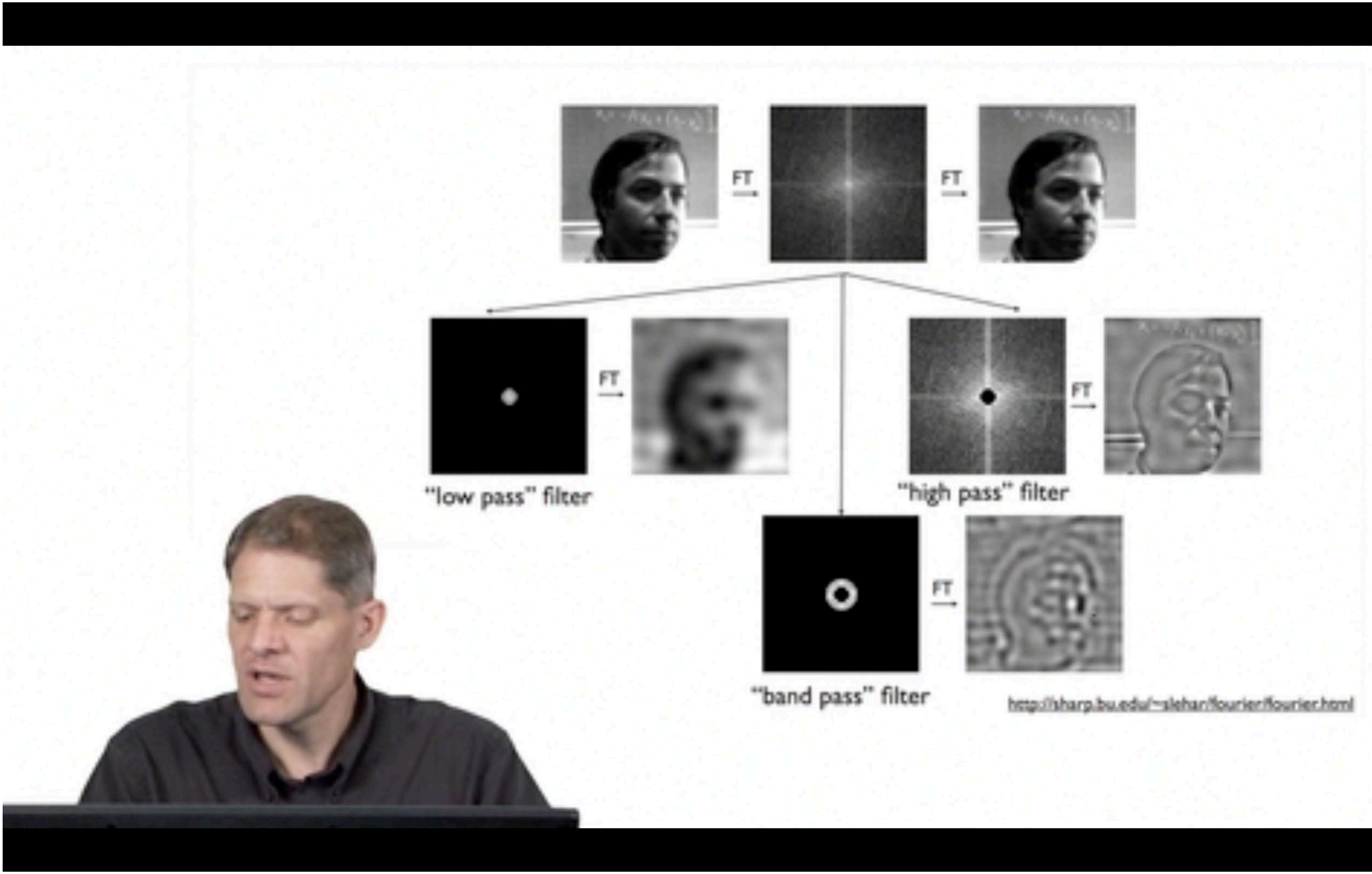
Fourier transform is an invertible operator



*, # - Friedel mates

Fourier Transforms of Images

- Computationally, images are a discrete matrix of points, and so the computer actually calculates the discrete Fourier transform (DFT)
- We are back to sums instead of integrals
- The Fast Fourier transform (FFT) is efficient: on order n log(n) rather than n^2 (Cooley and Tukey, 1965; Gauss 1805)
- Originally for n of power of 2, it can be calculated for all n, though power of 2 is simpler (small prime factors better)
- Generally only the amplitude is displayed


FT
FT
FT
FT
FT
"low pass" filter
"high pass" filter
"band pass" filter
http://sharp.bu.edu/~slehar/fourier/fourier.html

Importance of Phase and Amplitude

1 

Calculate FFT Keep amplitude

2 

Calculate FFT Keep phase

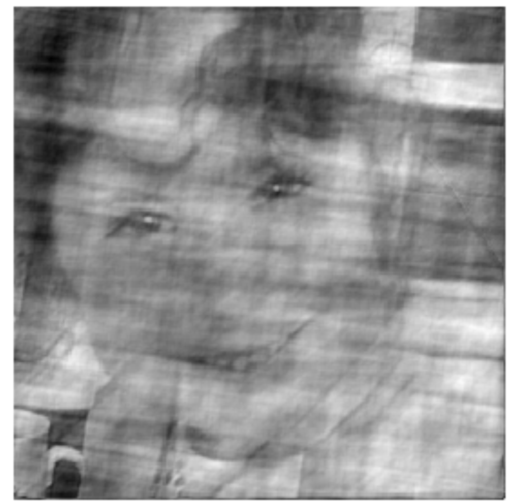
Importance of Phase and Amplitude

1

2

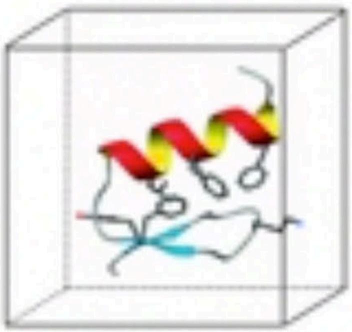
Amplitude of object 1
Phase of object 2

Inverse FT

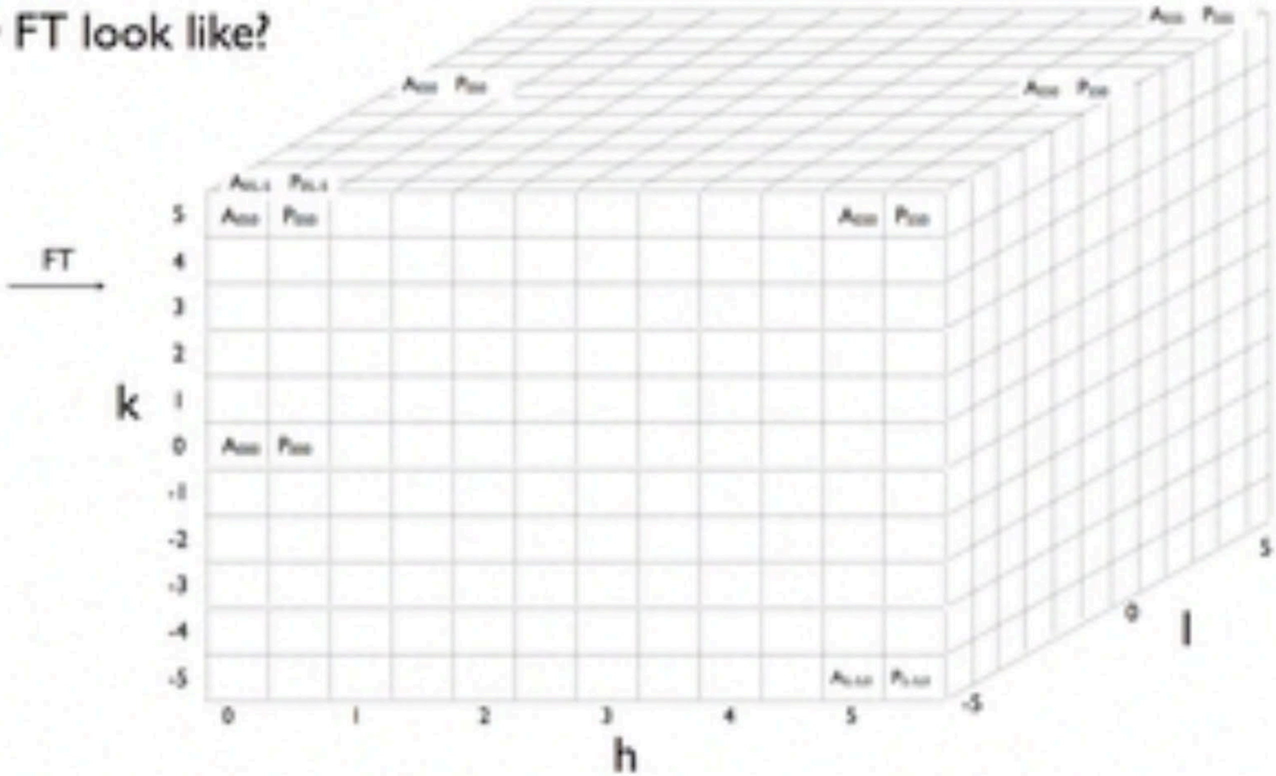


Reconstituted image is dominated by phase

What does a 3-D FT look like?

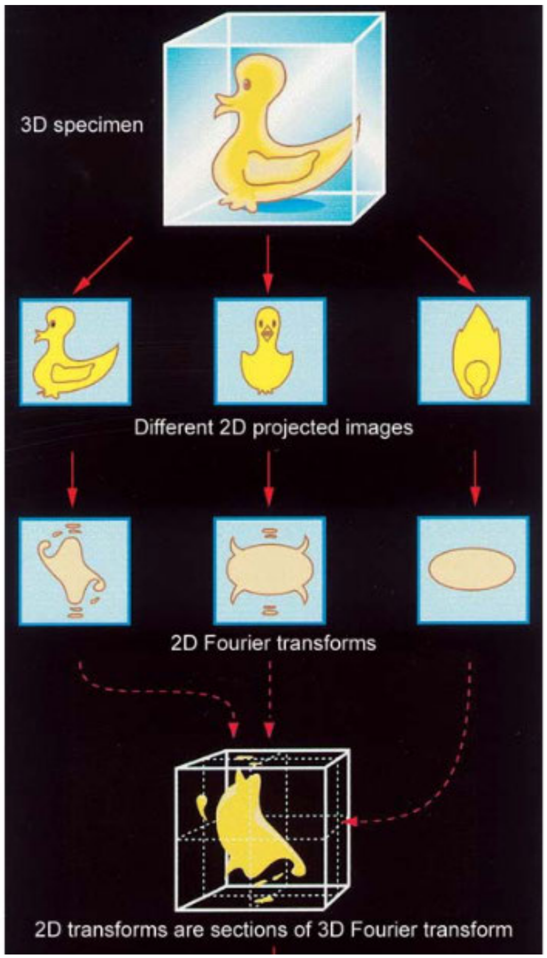


N^3 numbers



$\sim N^3$ numbers

Projection Theorem (Central Slice Theorem)

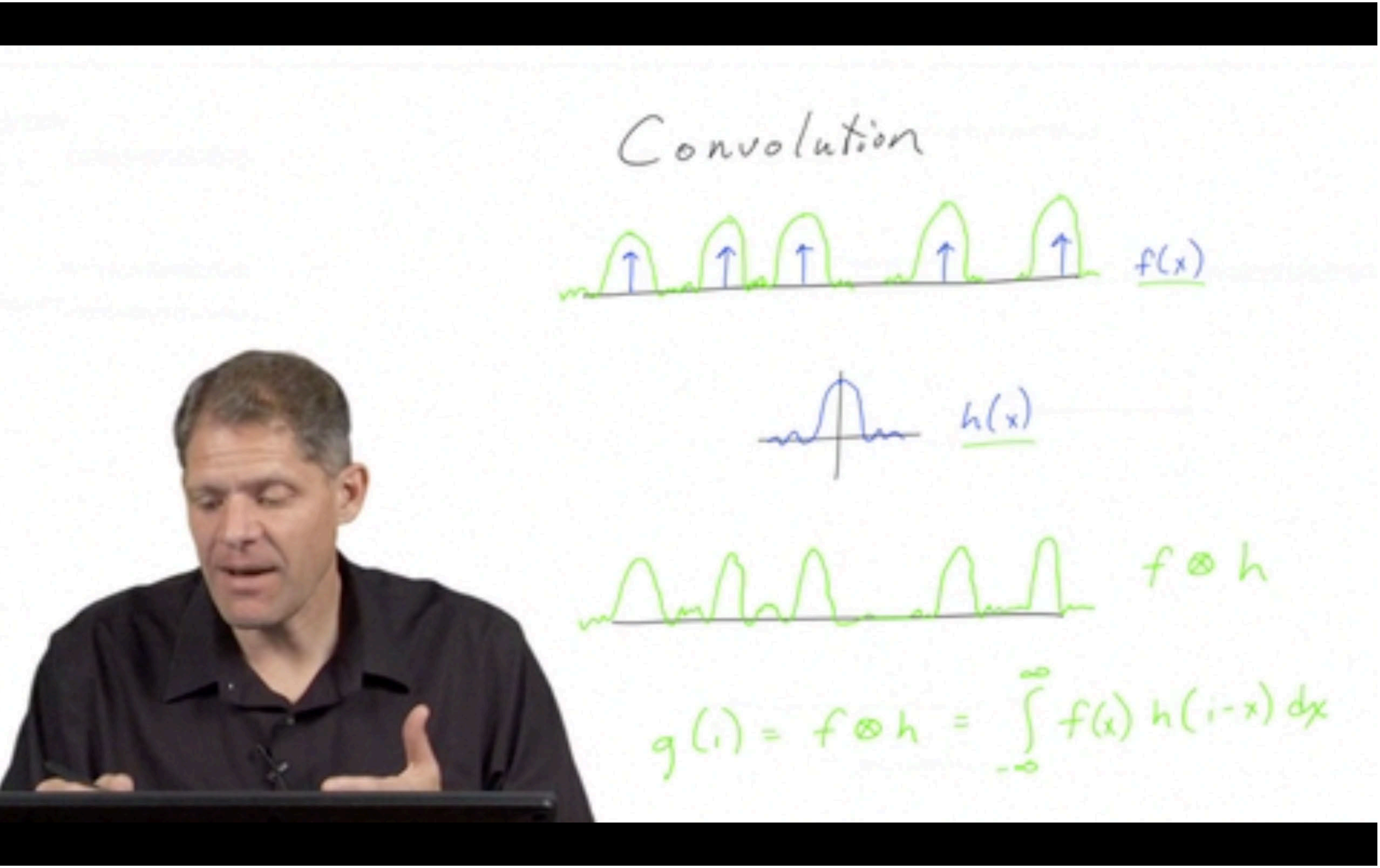


From Baker and Henderson (2012), International Tables of Crystallography Vol. F, Ch. 19.6, pp. 593-614.

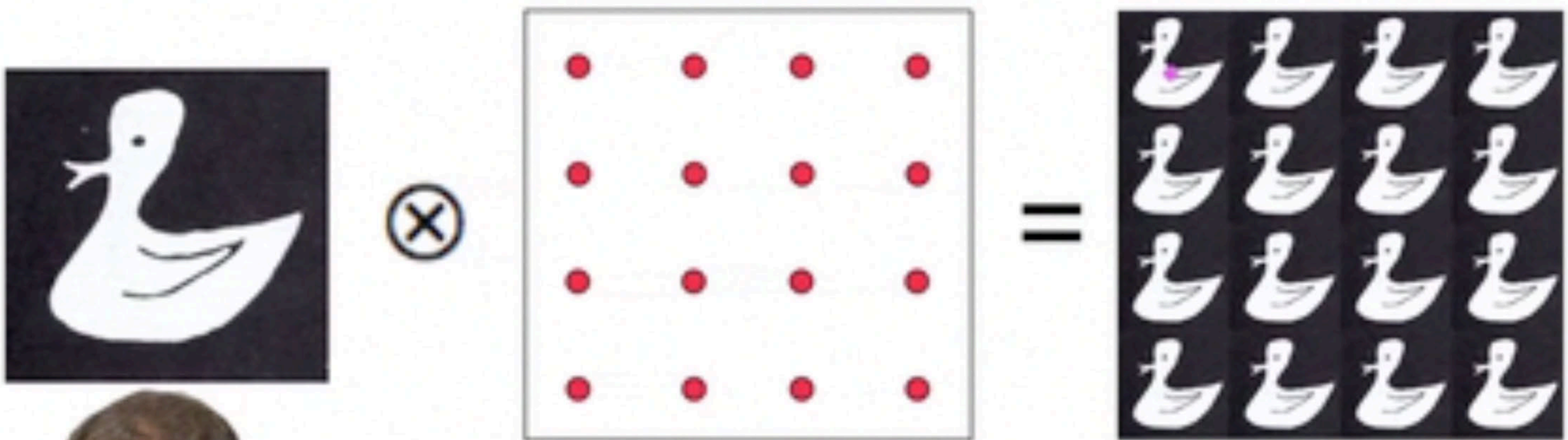
Fourier Inversion

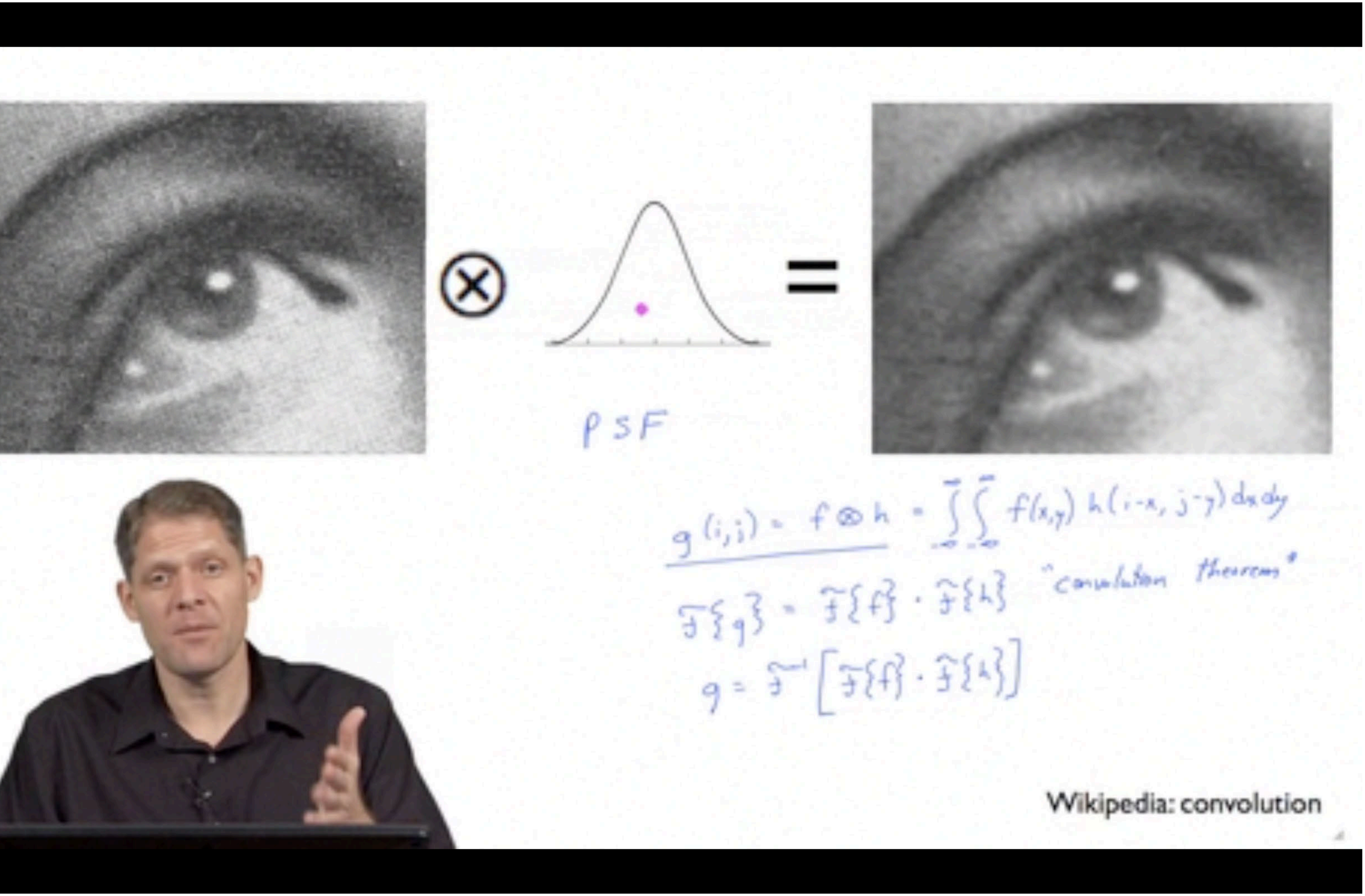
Convolution and cross-correlation
Concept check questions:

- What is a "convolution"?
- What is the "convolution theorem"?
- What is a "point spread function"?
- What does convolution have to do with the structure of crystals?
- What is "cross-correlation"?
- How might cross-correlations be used in cryo-EM?


Convolution
f(x)
h(x)
f ⊗ h
g(i) = f ⊗ h = ∫_{-∞}^{∞} f(x) h(i-x) dx

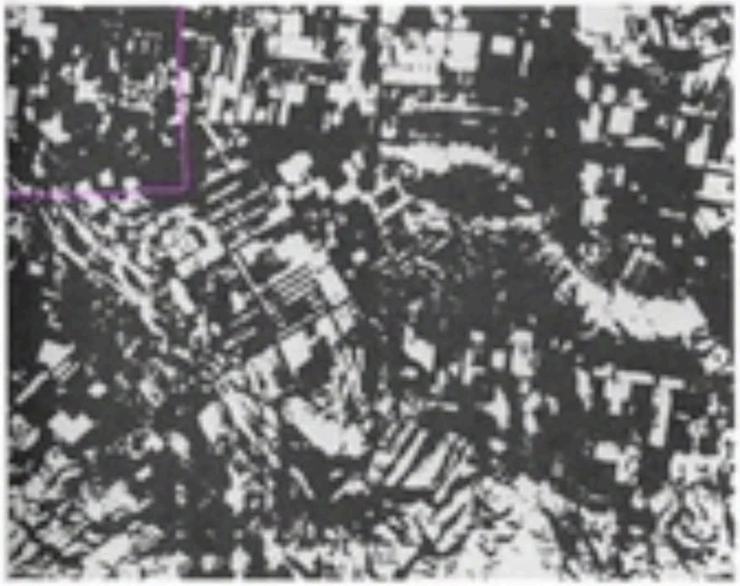
Convolution





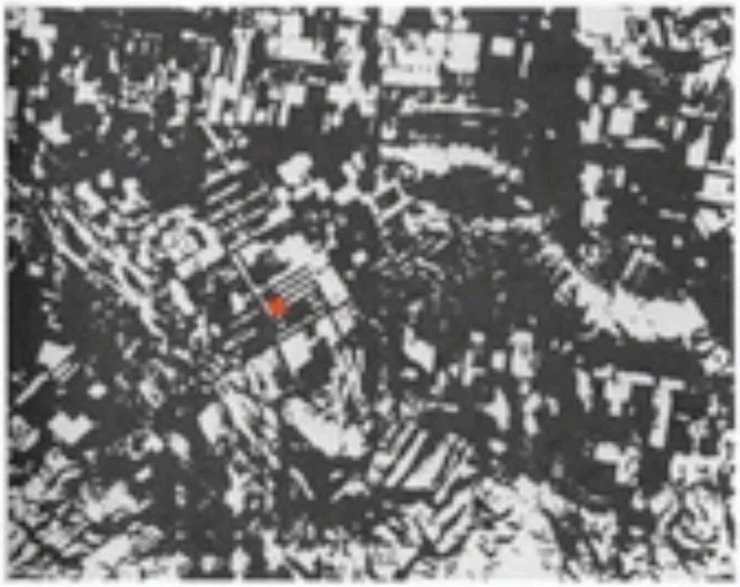
PSF
$g(i,j) = f \otimes h = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x,y)\, h(i-x, j-y)\, dx\, dy$
$\mathcal{F}\{g\} = \mathcal{F}\{f\} \cdot \mathcal{F}\{h\}$ "convolution theorem"
$g = \mathcal{F}^{-1}\left[\mathcal{F}\{f\} \cdot \mathcal{F}\{h\}\right]$
Wikipedia: convolution

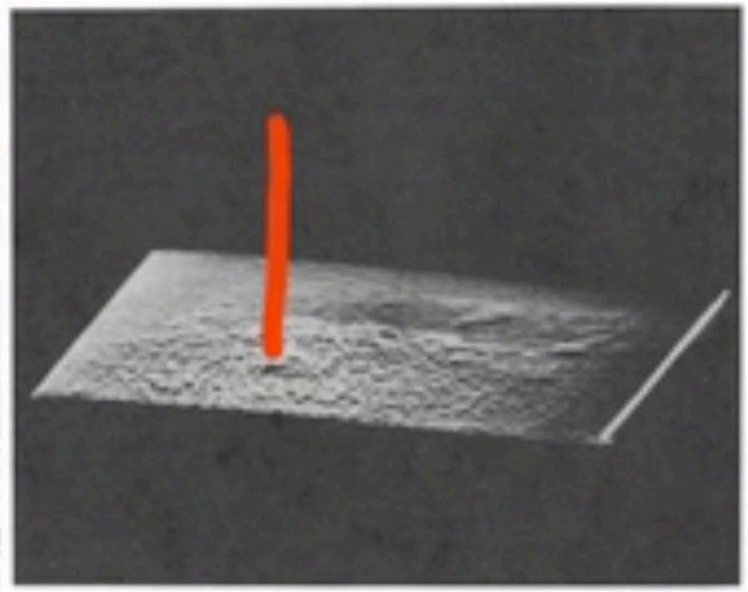
Cross-correlation



Hecht, Optics

Cross-correlation





Hecht, Optics

Correlation and Convolution

$h(x)=\int_{-\infty}^{\infty} f(x')g(x-x')dx$ Convolution

$p(x)=\int_{-\infty}^{\infty} f(x')g(x'-x)dx$ Correlation

Convolution Theorem

$h(x)=FT^{-1}\{F(X)G(X)\}$

$p(x)=FT^{-1}\{F(X)G^*(X)\}$

$G^*(X)$: complex conjugate of G

Complex conjugate:
if $x=a+ib$, $x^* = a-ib$

mage Formation

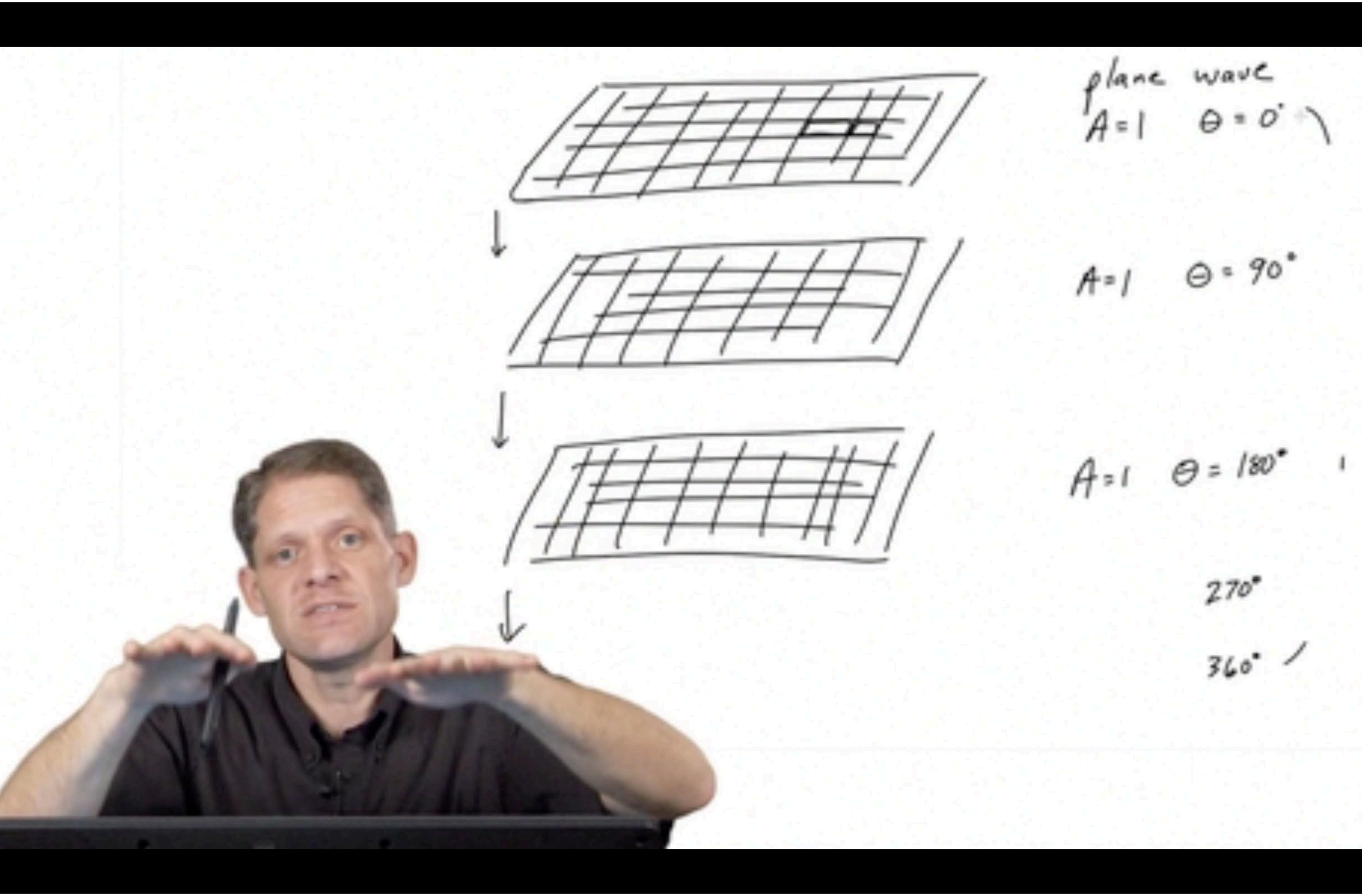
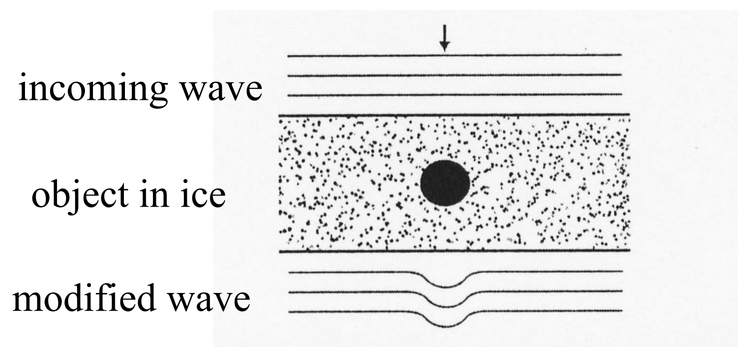

plane wave
A=1 θ=0°
A=1 θ=90°
A=1 θ=180°
270°
360°

Image formation

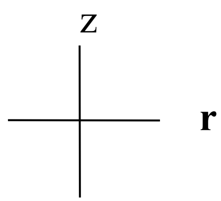
- Object is modeled as a weak-phase object
- Consider only phase contrast at first
- Will add amplitude contrast later

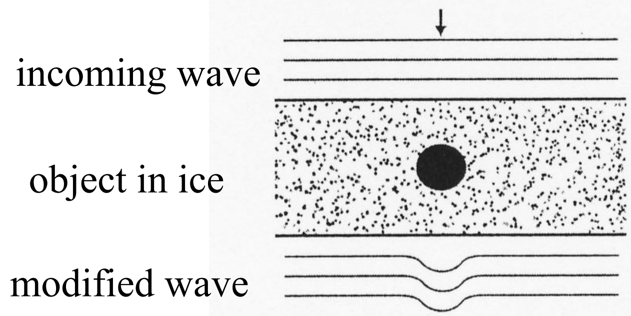


Observe: projected density of object

Frank, 2006

Image Formation





$\psi_0 = \exp(ikz)$

$$\Phi(\mathbf{r}) = \int C(\mathbf{r}, z)\, \mathrm{d}z$$

$$\Psi(\mathbf{r}) = \Psi_0 \exp[i\Phi(\mathbf{r})]$$

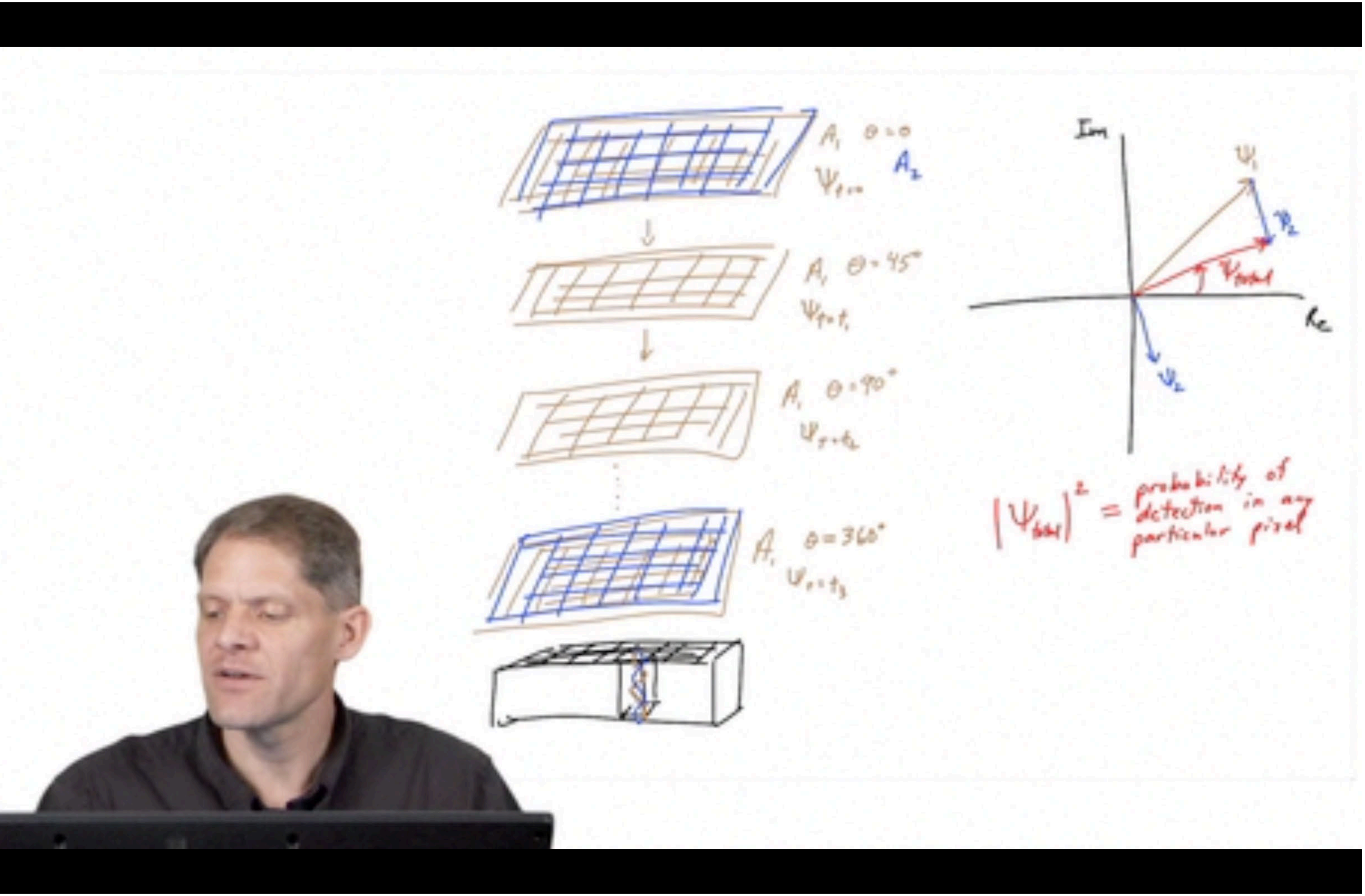
Taylor Expansion of modified wave equation: $$\Psi(\mathbf{r}) = \Psi_0 \left[1 + i\Phi(\mathbf{r}) - \frac{1}{2}\Phi(\mathbf{r})^2 + \cdots \right]$$

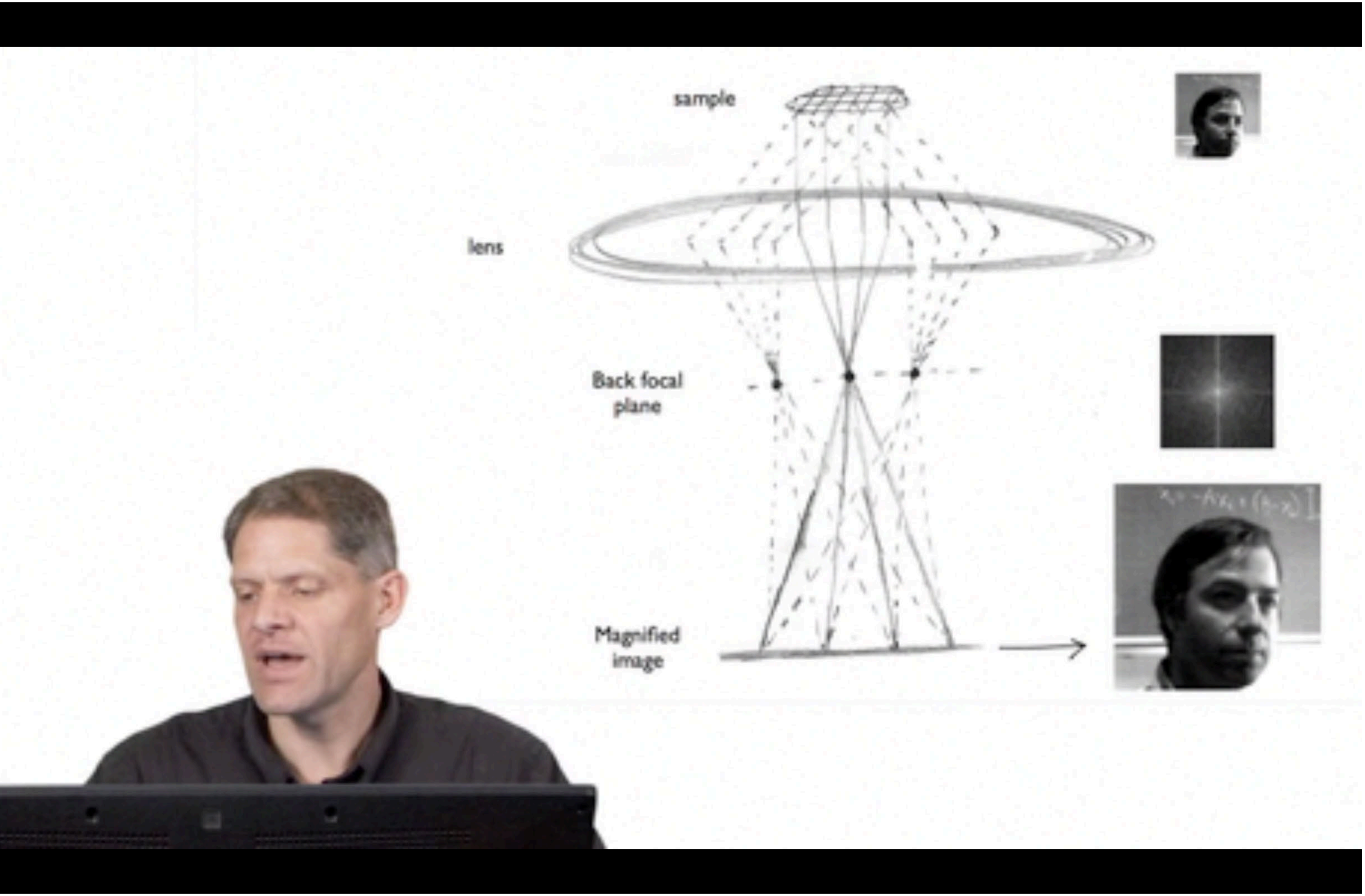
Weak phase approximation: $\phi(\mathbf{r}) \ll 1$ $$\Psi(\mathbf{r}) = \Psi_0 \left[1 + i\Phi(\mathbf{r}) \right]$$

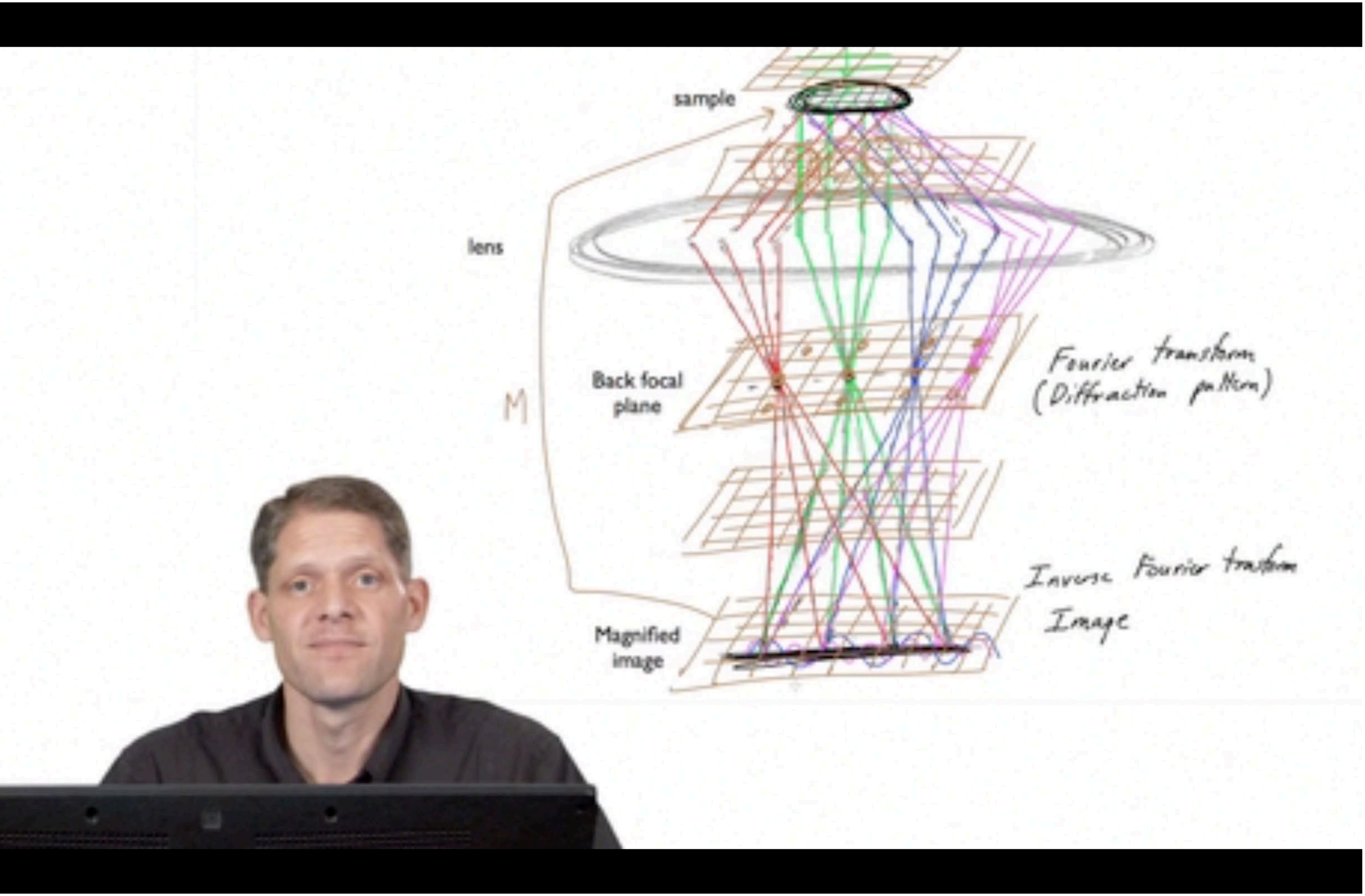
unmodified wave

scattered wave
(90° phase shift)

k, 2006


A1 θ=0
A2
Ψt=0
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


sample
lens
Back focal
plane
Magnified
image


sample
lens
Back focal plane
Magnified image
M
Fourier transform
(Diffraction pattern)
Inverse Fourier transform
Image

Contrast Transfer Function (CTF)

- Observed image:
  - $f(x) = o(\mathbf{x}) * psf(\mathbf{x}) * env(\mathbf{x}) + n(\mathbf{x})$
    - $f(\mathbf{x})$: observed projection of image
    - $o(\mathbf{x})$: true projection of image
    - $psf(\mathbf{x})$: point-spread function
    - $env(\mathbf{x})$: envelope function of microscope
    - $n(\mathbf{x})$: noise
  - FT:
    - $F(k) = O(\mathbf{k}) \times CTF(\mathbf{k}) \times ENV(\mathbf{k}) + N(\mathbf{k})$

Image Formation

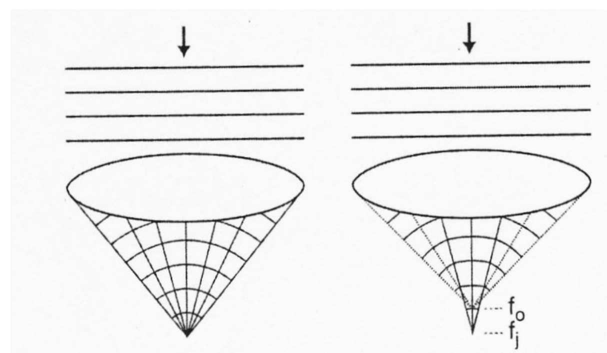
Lens aberrations and defocusing shift the phase of the scattered wave, as described by γ(k)

$$\gamma(\mathbf{k}) = 2\pi\chi(\mathbf{k})$$

$\chi(\mathbf{k})$ is called the wave aberration function
Define χ in polar coordinates as follows:

$$k = |\mathbf{k}|, \quad \phi = a\tan(k_x, k_y)$$

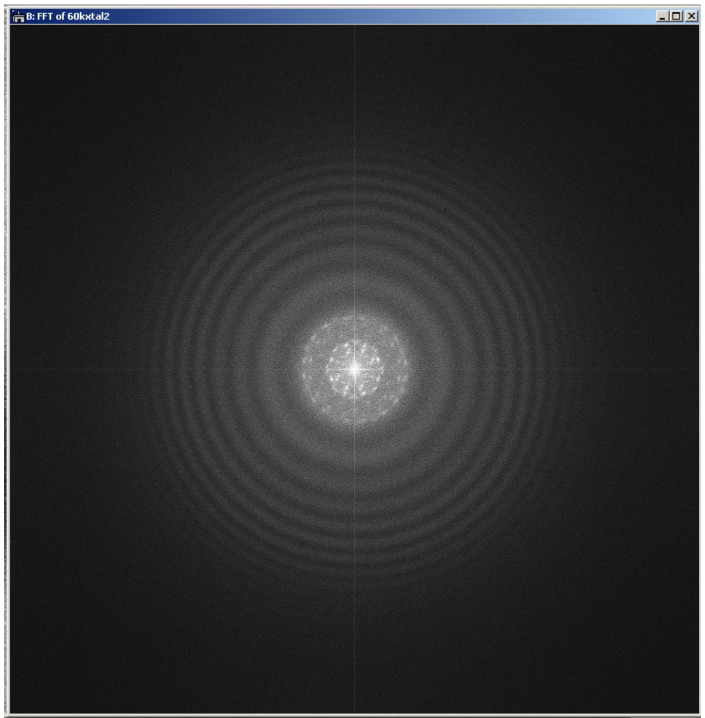
$$\chi(k,\phi) = -\frac{1}{2}\lambda\left[\Delta z + \frac{z_a}{2}\sin 2(\phi - \phi_0)\right]k^2 + \frac{1}{4}\lambda^3 C_s k^4$$

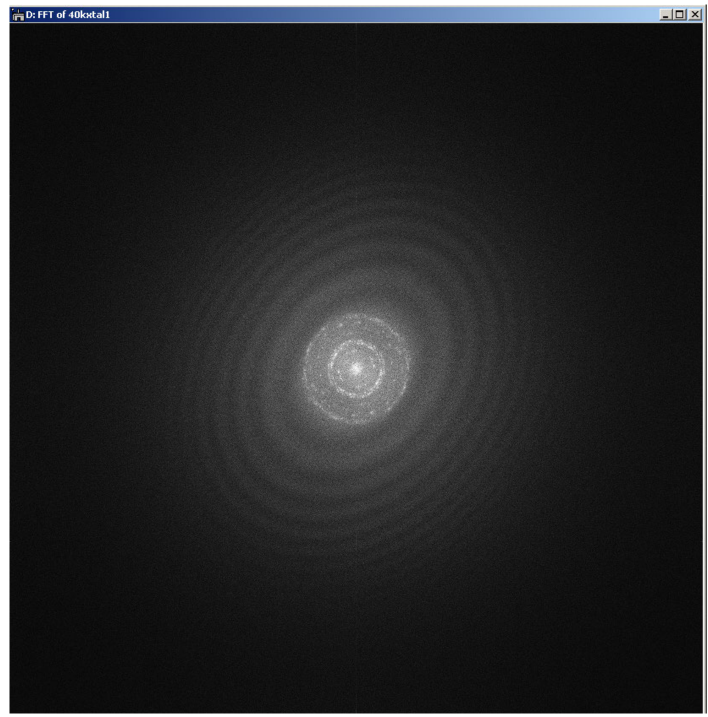


defocus   astigmatism   spherical aberration

ank, 2006

CTF Visualization: FFT of Image





good image

Astigmatism

Contrast Transfer Function

Compute FT of image: $$F(\mathbf{k}) = O(\mathbf{k})A(\mathbf{k}) \sin \gamma(\mathbf{k})$$

where

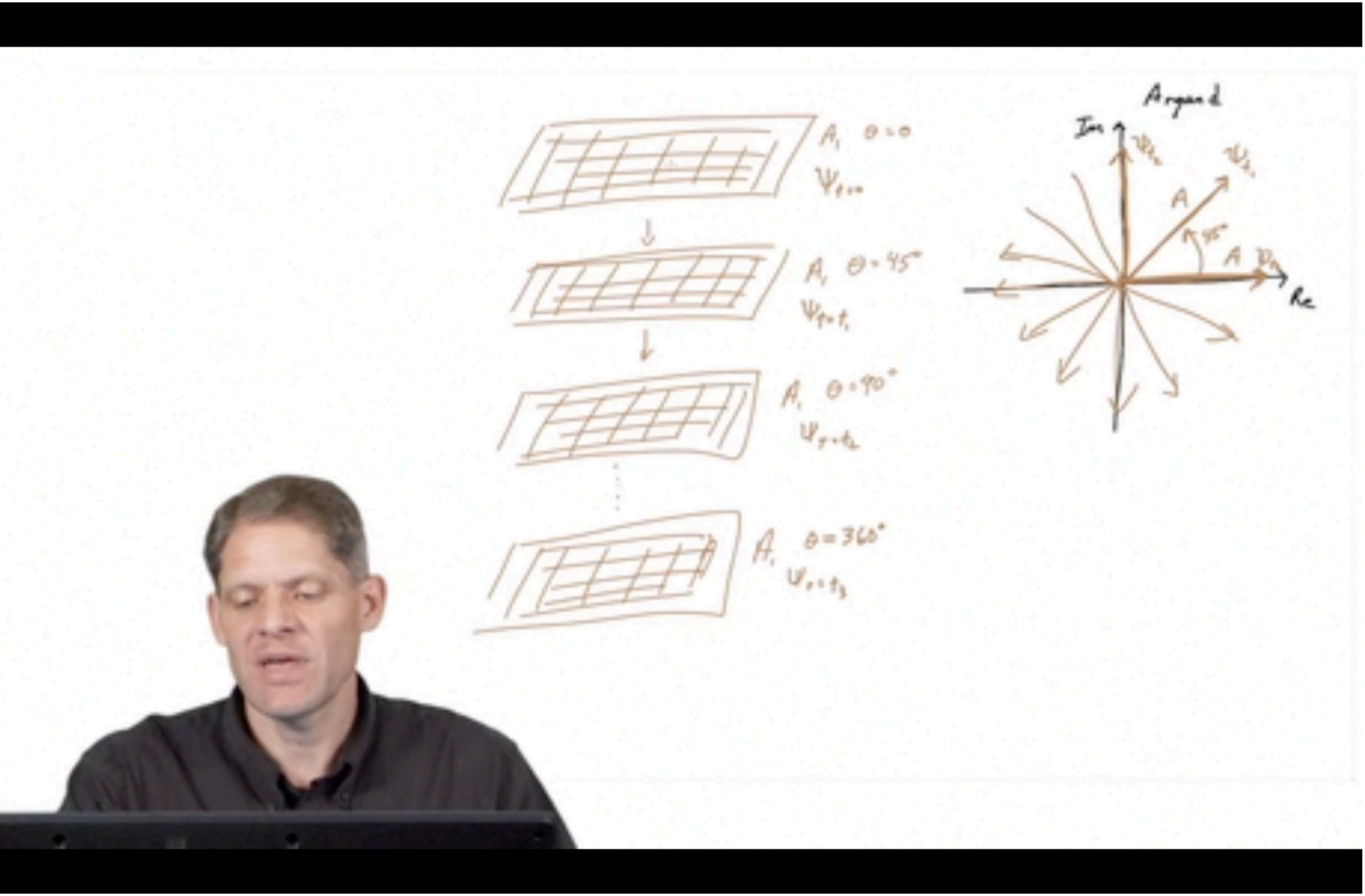
$O(k)=FT\{\psi(\mathbf{r})\}$ $A(\mathbf{k})$ = aperture function

$$\gamma(k,\Delta z) = (\pi \Delta z\, k^{2} + \pi/2\, k^{4})$$

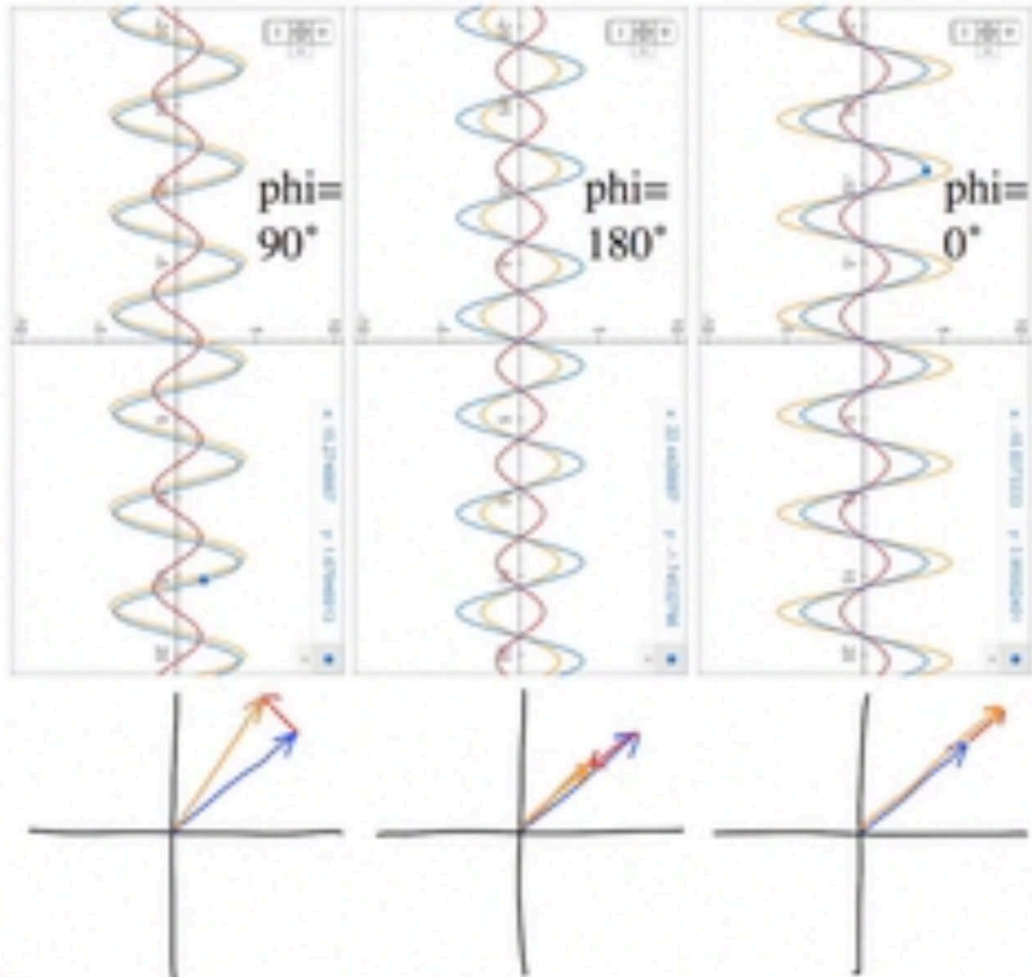
(ignoring astigmatism and using generalized defocus and frequency)

$\sin \gamma(k)$ -- **Contrast Transfer Function**

FT of image is multiplied by CTF
Image is convoluted with CTF
FT of CTF is a point-spread function


Argand
Im
Re
A
45°
A, θ=0
A, θ=45°
A, θ=90°
A, θ=360°

$4\sin(x) + 1.5\sin(x+phi)$



Amplitude Contrast

- Due to loss of electrons due to
  - scattering outside of aperture
  - removal by inelastic scattering
- ratio of amplitude to phase contrast depends on atomic weight
- Assuming homogeneous specimen, get modified CTF:

$$H'(\mathbf{k}) = \sin\gamma(\mathbf{k}) - Q_0 \cos\gamma(\mathbf{k})$$

Q_o: % amplitude contrast (~7% for cryo)

Coherence and Envelope Functions

CTF is dampened because of partial coherence of beam

a) finite source size of beam

$$E_i(k) = \exp\left[-\pi^2 q_0^2 (C_s \lambda^3 k^3 - \Delta z \lambda k)^2\right]$$

defocus dependent

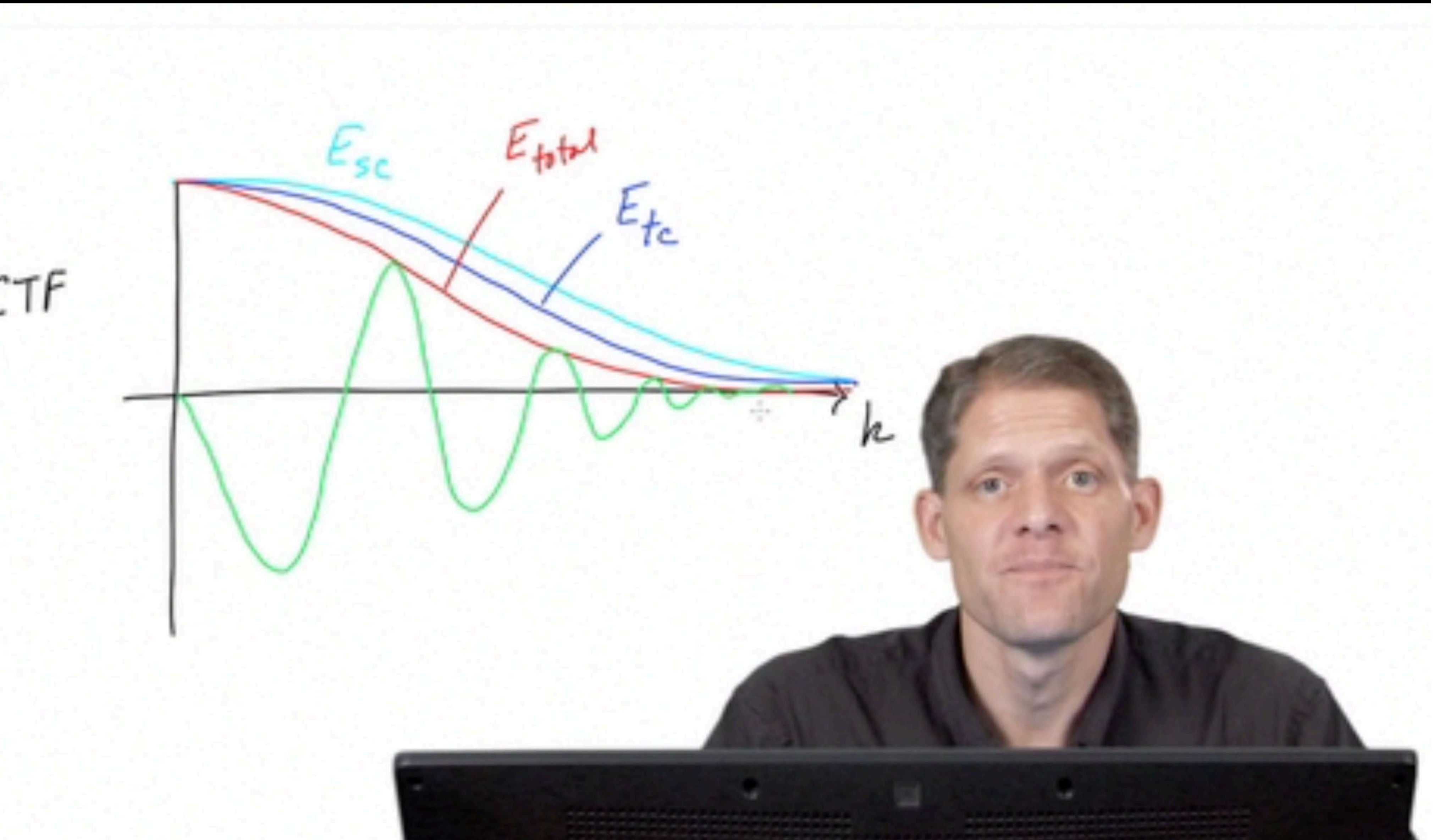
b) energy spread of beam

$$E_e(k) = \exp\left[-(\pi \delta z \lambda k^2/2)^2\right]$$

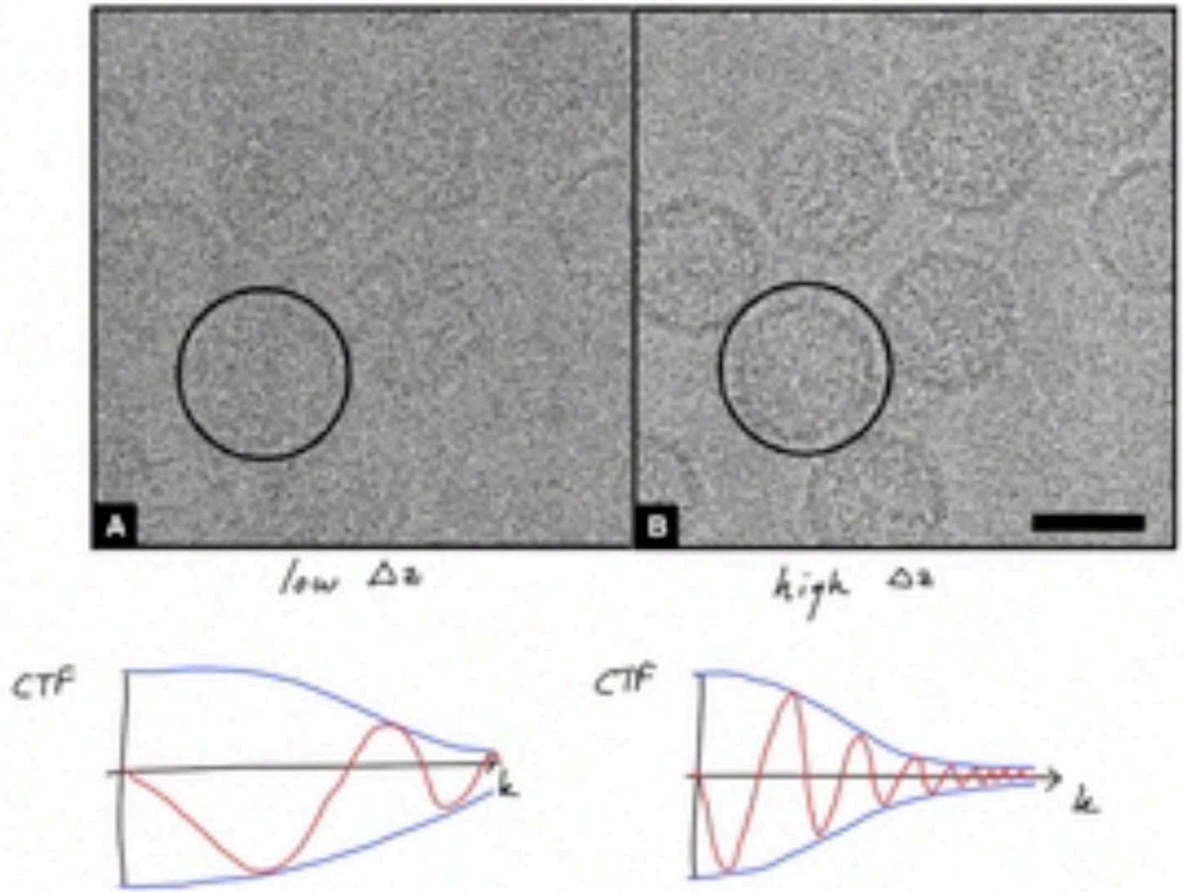
defocus independent

c) other effects: energy fluctuations, coolant fluctuations, mechanical movement, ... approximate as Gaussian B factor $\exp(-Bk^2)$

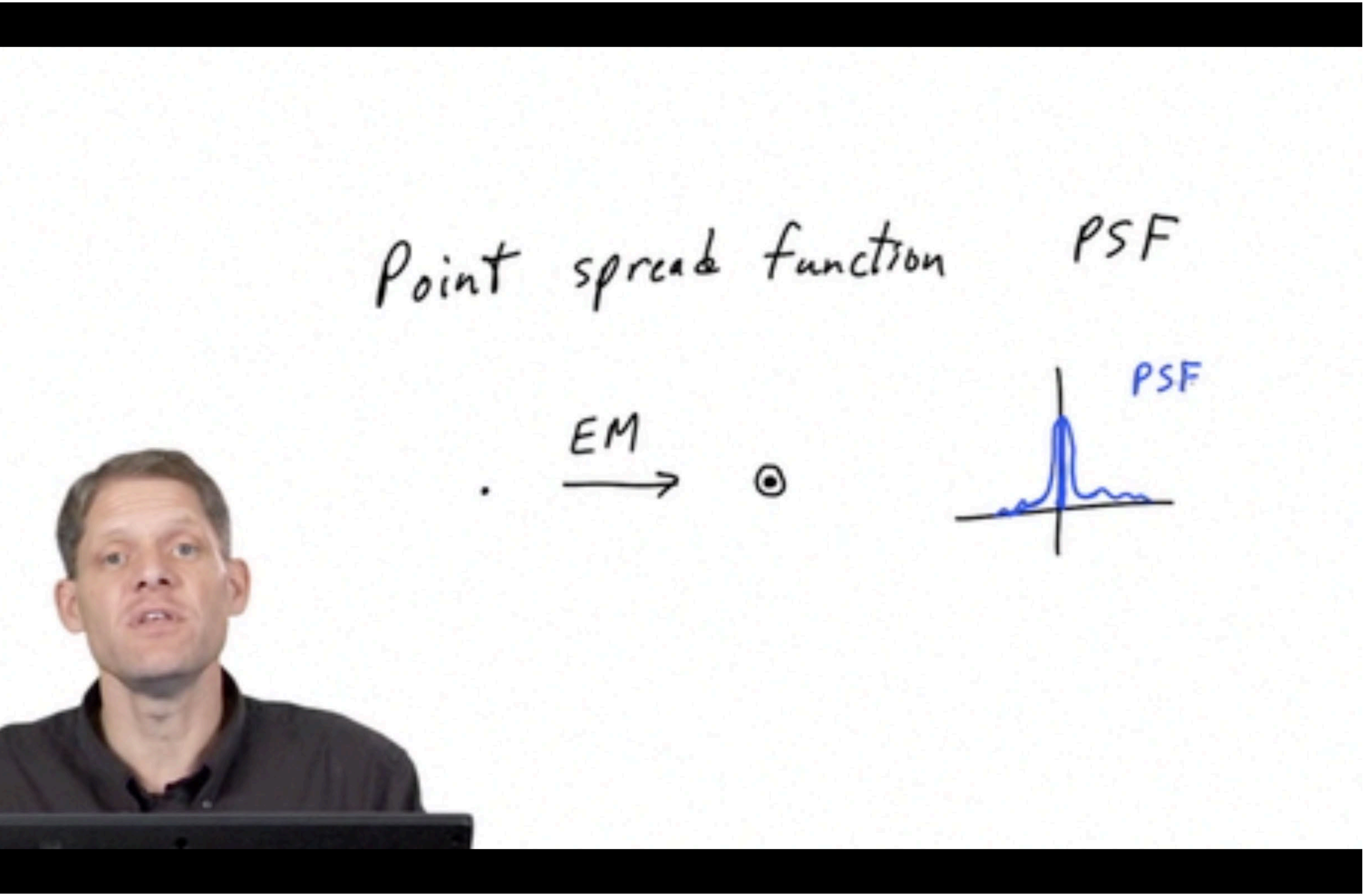
$$ENV(k) = E_i(k) E_e(k) E_B(k)$$

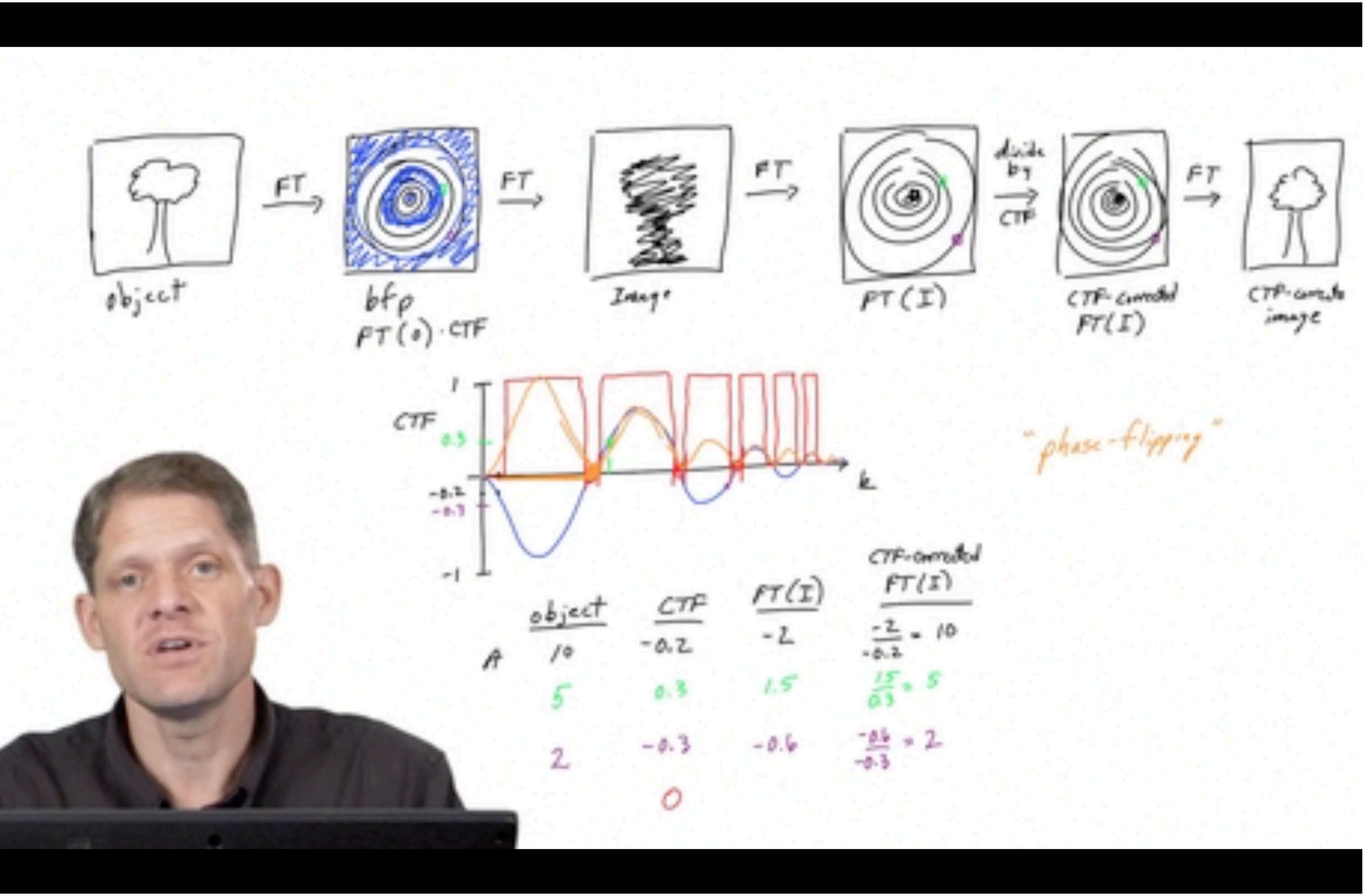


CTF
E_{sc}
E_{total}
E_{tc}
k



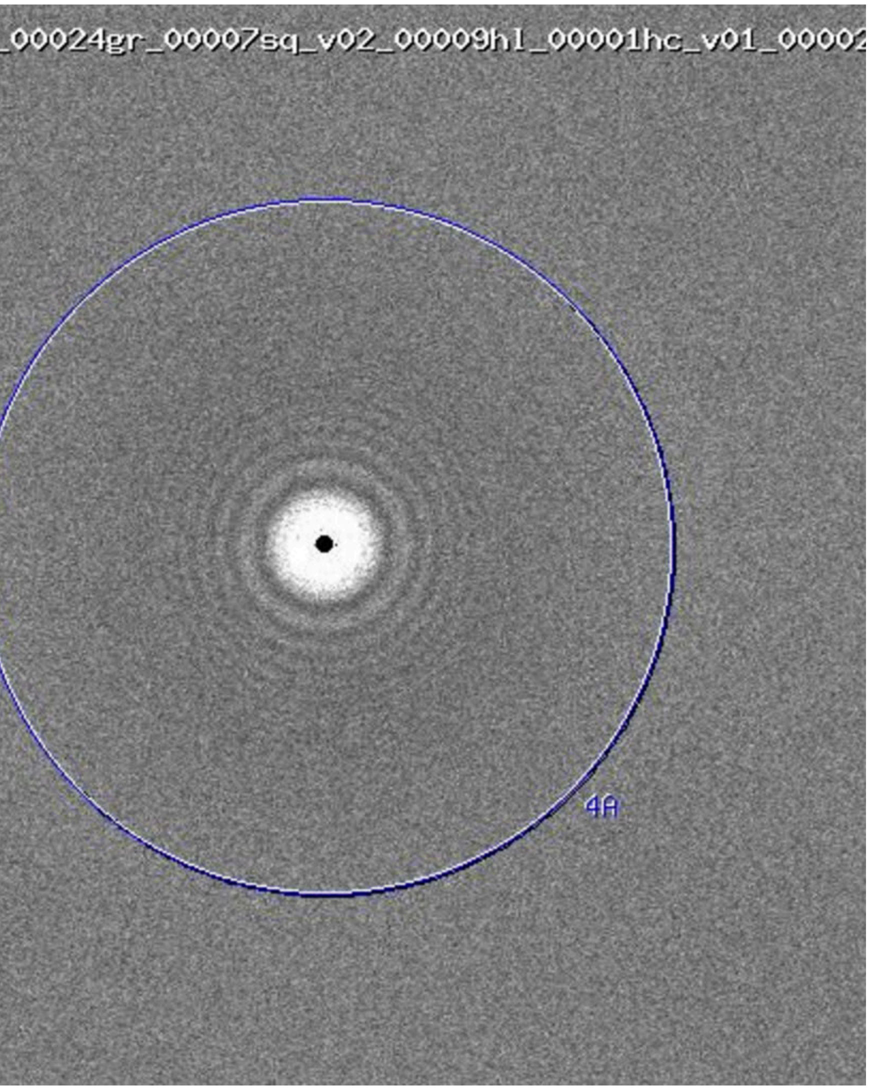
Thuman-Commike and Chiu, *Micron* **31**:687


Point spread function PSF
EM
PSF

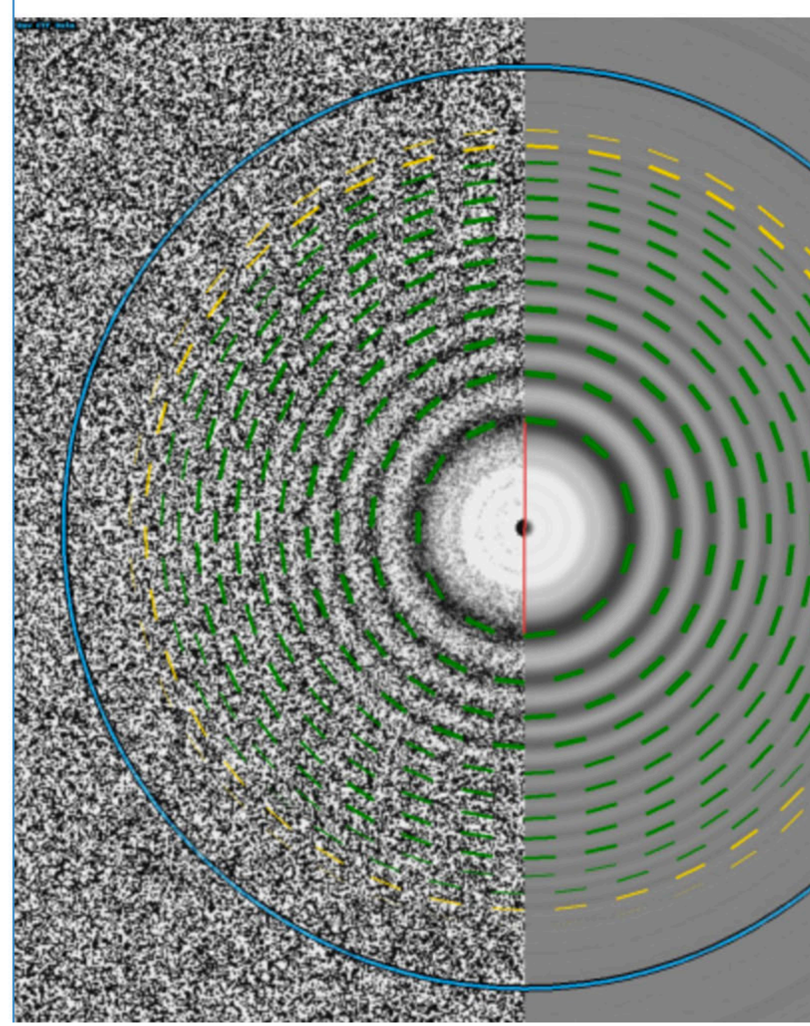


FT
object
bfp
FT(o)·CTF
Image
FT(I)
divide by CTF
CTF-corrected FT(I)
CTF-corrected image
CTF
1
0.3
-0.2
-0.3
-1
k
"phase-flipping"
object
CTF
FT(I)
CTF-corrected FT(I)
A
10
-0.2
-2
-2/-0.2 = 10
5
0.3
1.5
1.5/0.3 = 5
2
-0.3
-0.6
-0.6/-0.3 = 2
0

CTF Determination Example: CTFFIND3/4



runname: ctffind4run1 **nomDef:** -1.03 µm **def1:** 1.75 µm **def2:** 1.77 µm **θ**
amp con: 7.00E-2 **cs:** 2
res (0.8): 6.18 **res (0.5):** 5.32 **conf (30/10):** 0.99 **conf (5 peak):** 0.99 **conf**



MTF

Modulation Transfer Function of the detector

Measures response of the detector in the frequency domain

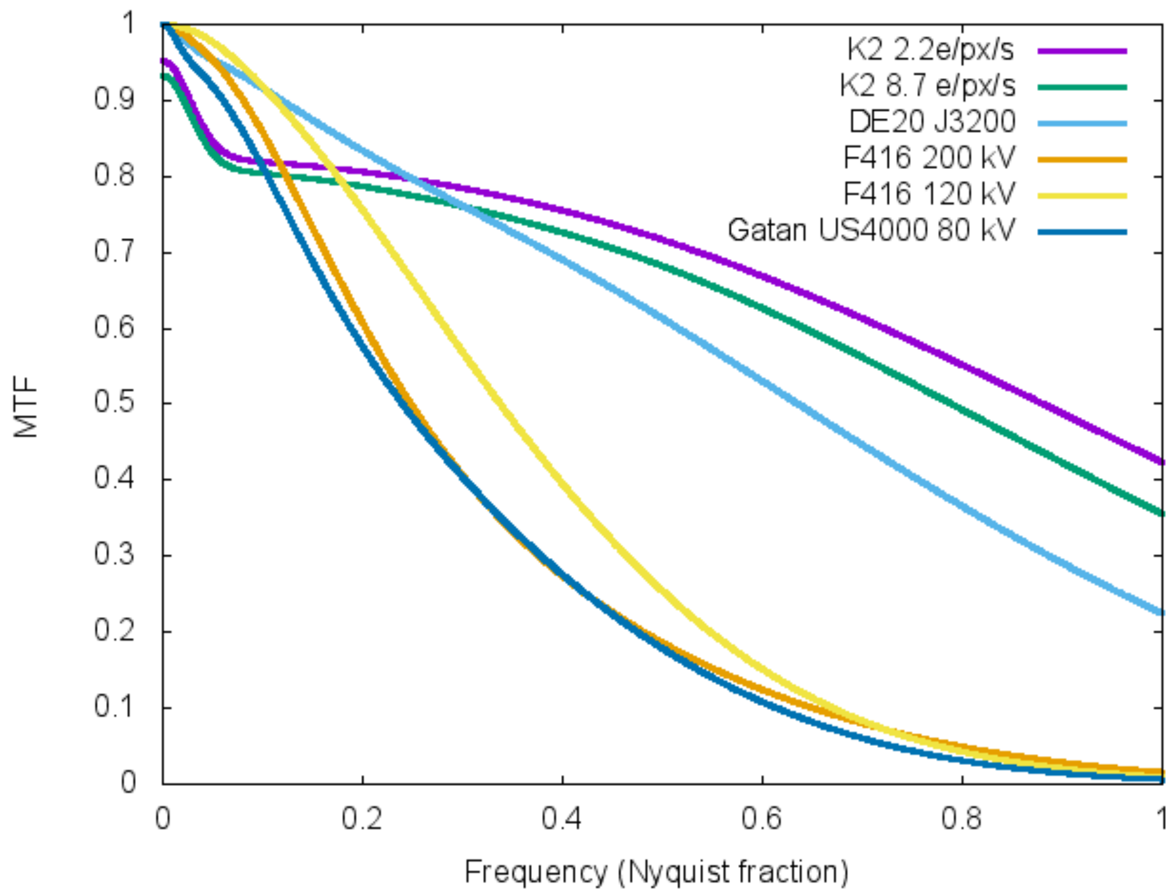
- A measure of how much contrast is transferred to the image at each resolution $\mathrm{MTF}(\omega) = \sqrt{\frac{P(\omega)_{\mathrm{out}}}{P(\omega)_{\mathrm{in}}}}$

Point Spread Function: The “blurring kernel” of the detector

- $\mathrm{Image}_{\mathrm{Out}} = \mathrm{PSF} \otimes \mathrm{Image}_{\mathrm{In}}$
- $\mathrm{MTF}(\omega) = FT(\mathrm{PSF}(r))$
- $\mathrm{FT}(\mathrm{Image}_{\mathrm{out}}) = \mathrm{MTF} \cdot \mathrm{FT}(\mathrm{Image}_{\mathrm{in}})$

MTF ⟷ PSF

MTF: All Cameras at NYSBC

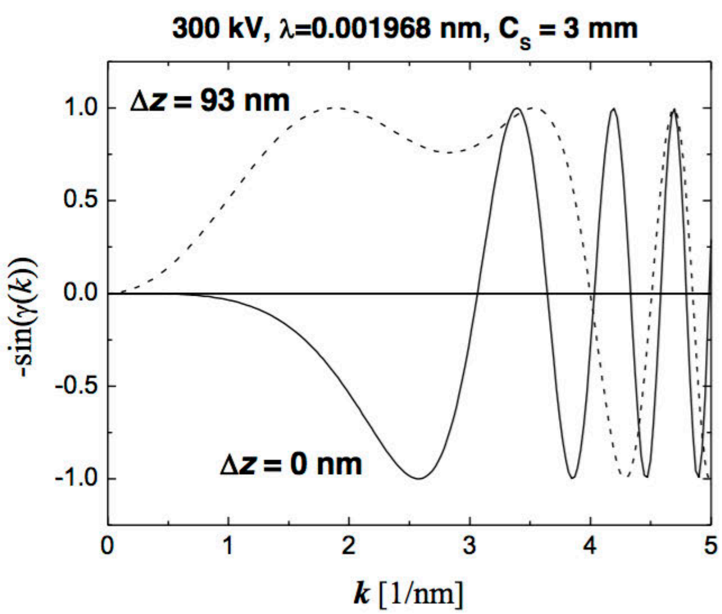


Courtesy of Radostin Danev

Contrast transfer function

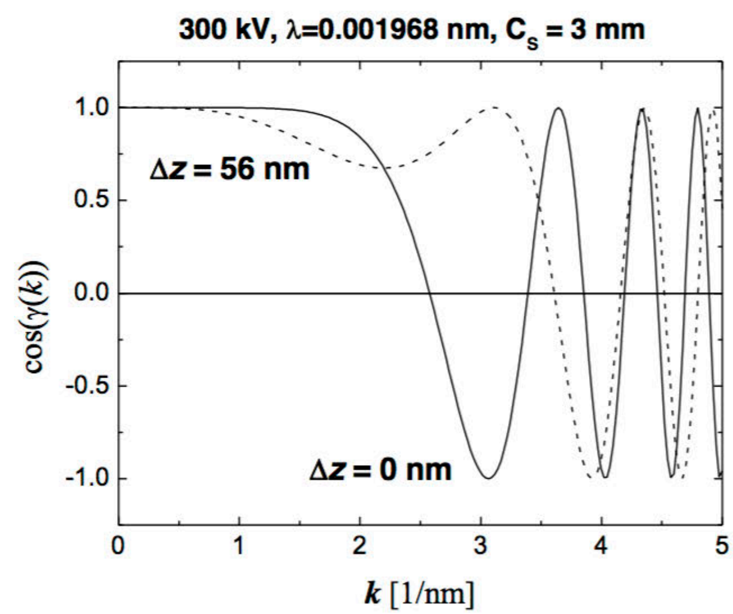
conventional TEM

$$\sin(\gamma(\mathbf{k}))$$



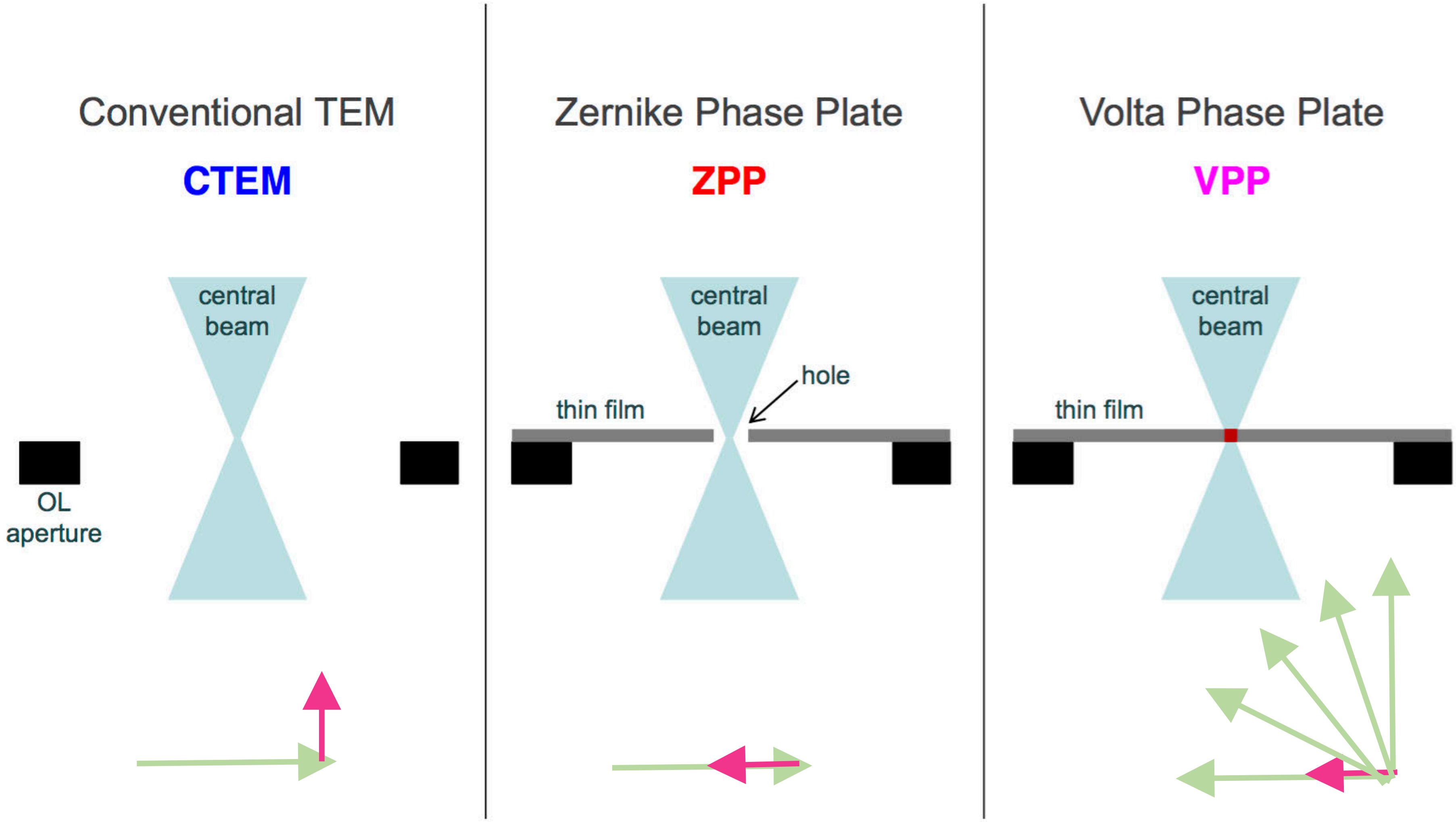
phase plate TEM

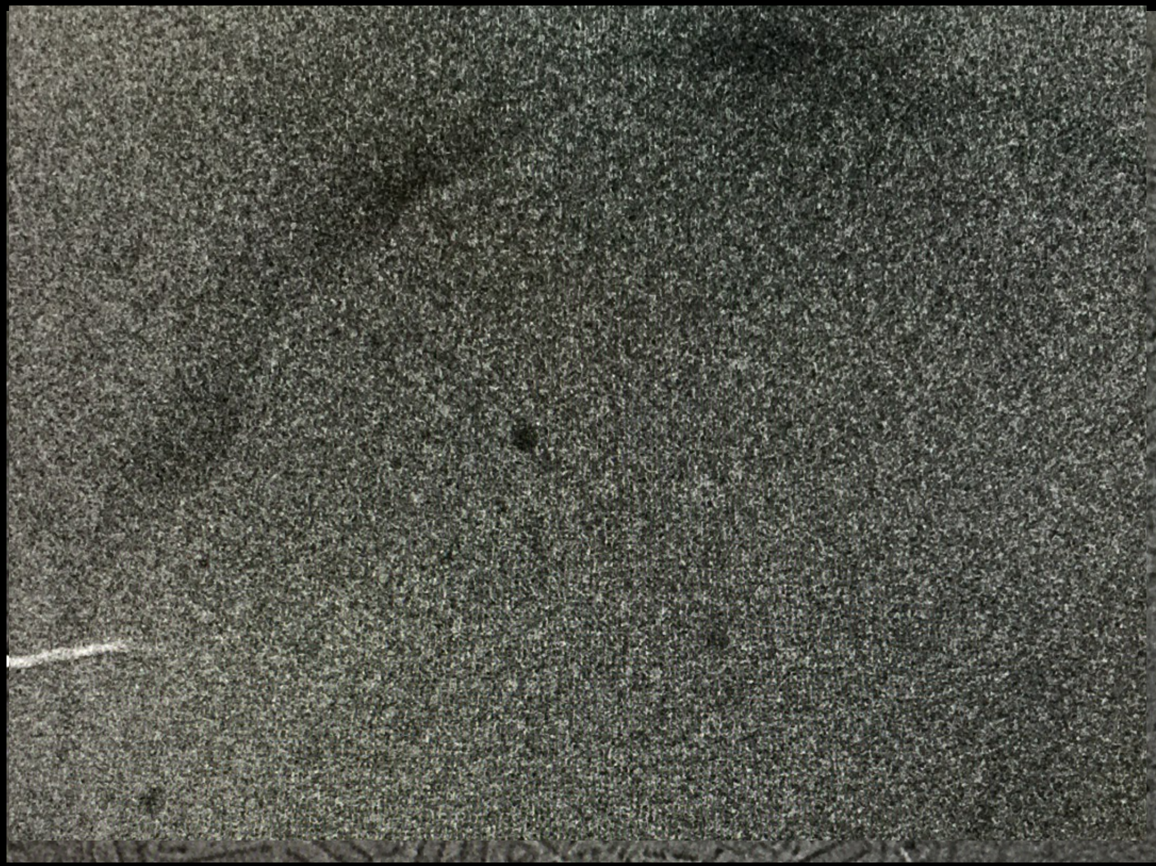
$$\cos(\gamma(\mathbf{k}))$$



Courtesy of Radostin Danev

TEM imaging modes





IMAGING OBJECT AS IS

Practical Phase Plate for cryo-EM

Further Reading

Frank, J. (2006). Three Dimensional Electron Microscopy of Macromolecular Assemblies (Chapter 2)

Galeser, R.M., Downing, K., DeRosier, D., Chiu, W., and Frank, J. (2007). Electron Crystallography of Biological Macromolecules

Steward, E.G. (2011) Fourier Optics: An Introduction (2nd Ed)