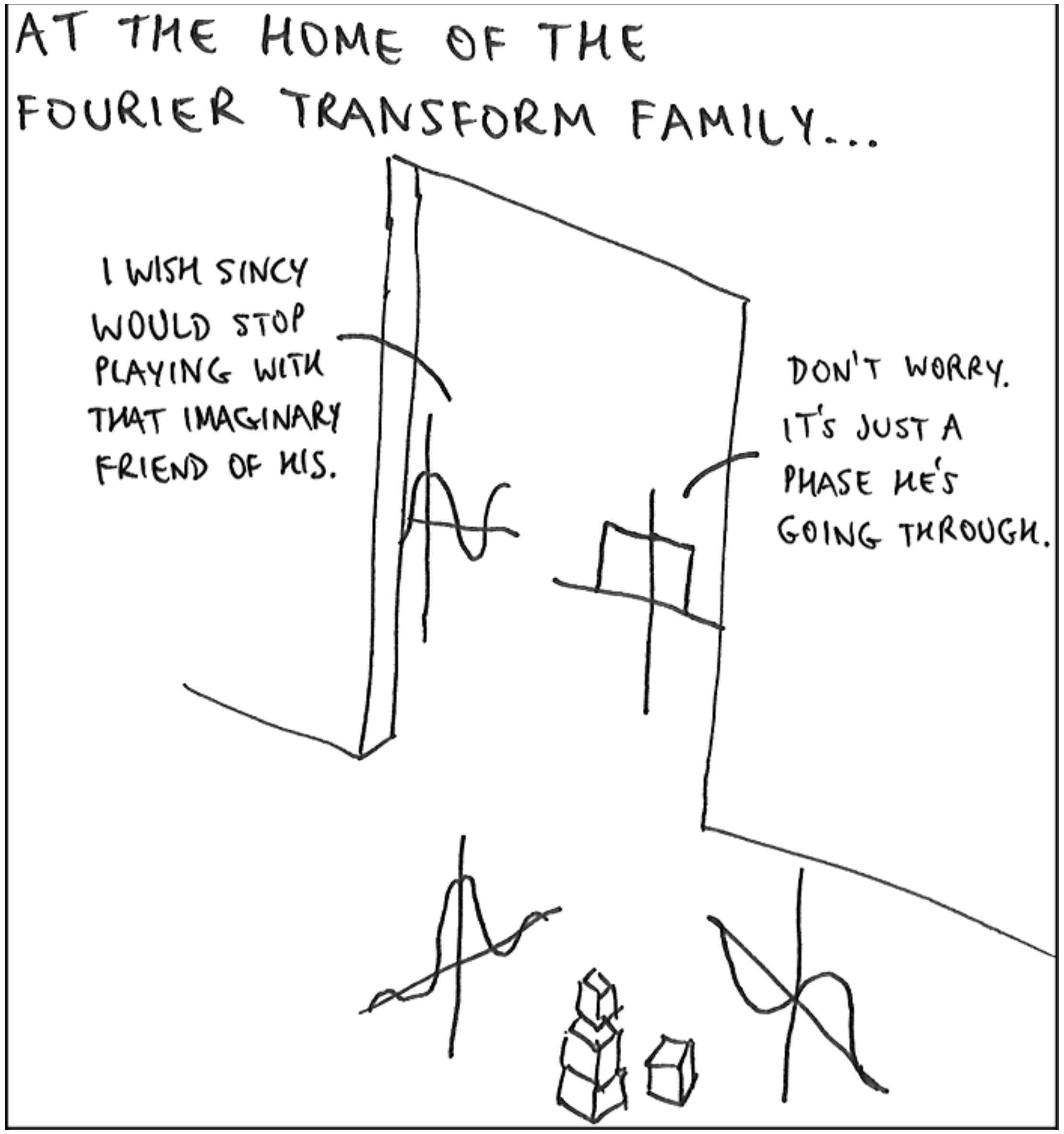
Fourier transform and Image Formation



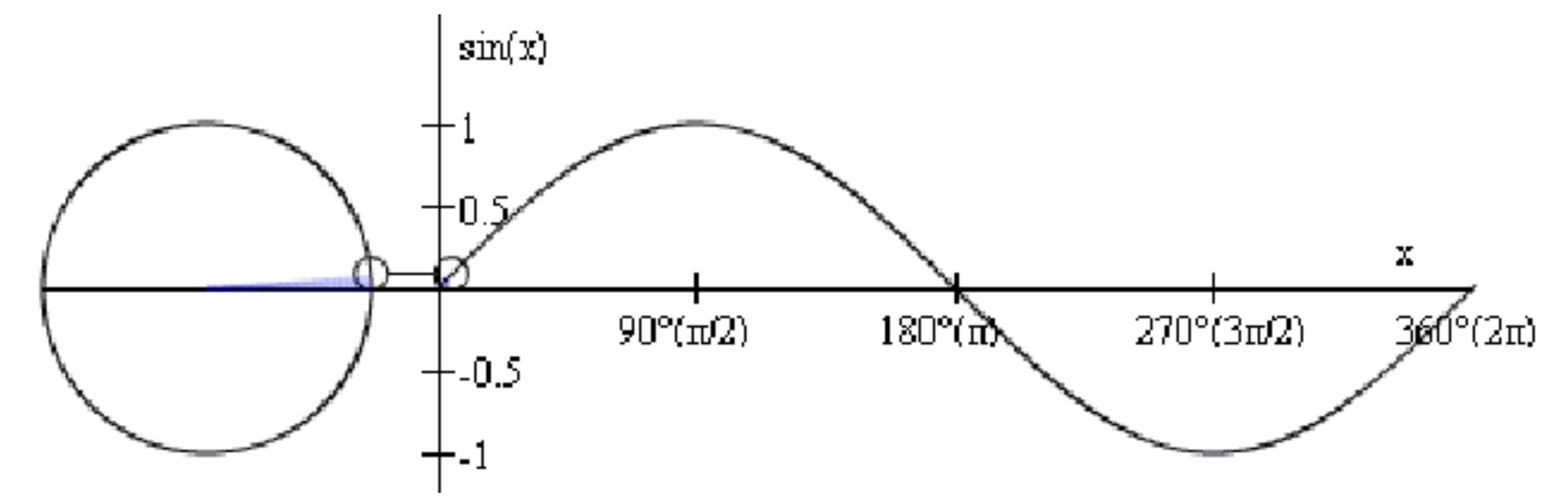
Lorenzo Finci, Ph.D.
NYSBC

Roadmap

- **Fourier transform**
  - Sine Waves, Fourier Decomposition, Nysquist, and Importance of Amplitude and Phase
  - Convolution & Cross Correlation
  - The information from FT
- **Image Formation**
  - Elastic and Inelastic scattering
  - Amplitude and Phase Contrast
  - CTF & PSF
  - Effect of Defocus on CTF
  - Estimation and correction of CTF

Sinusoid Wave

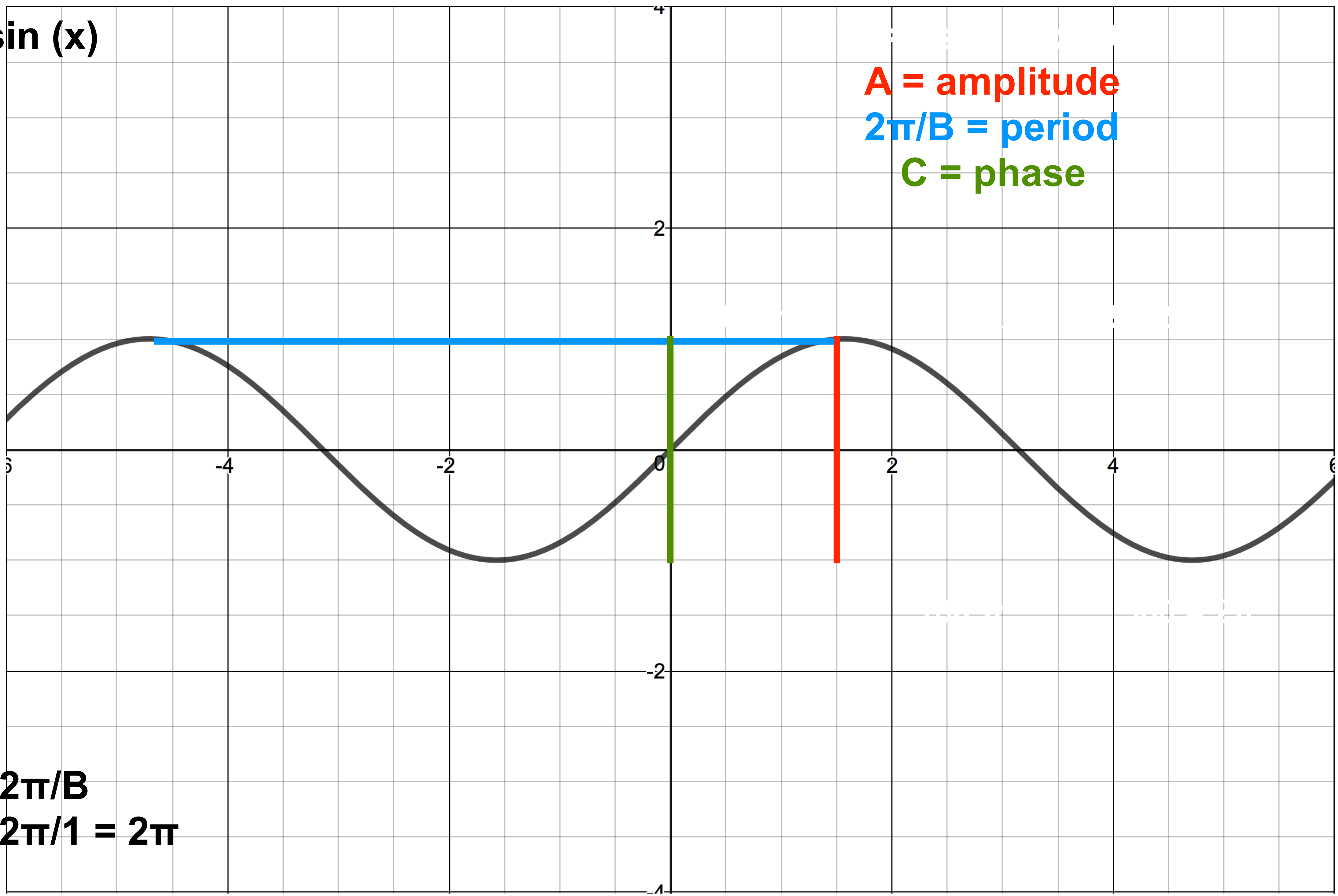
- y = A sin (Bx + C)
  - A = Amplitude
  - 2π/B = Period
  - C = Phase



www.giphy.com

Sinusoid Wave

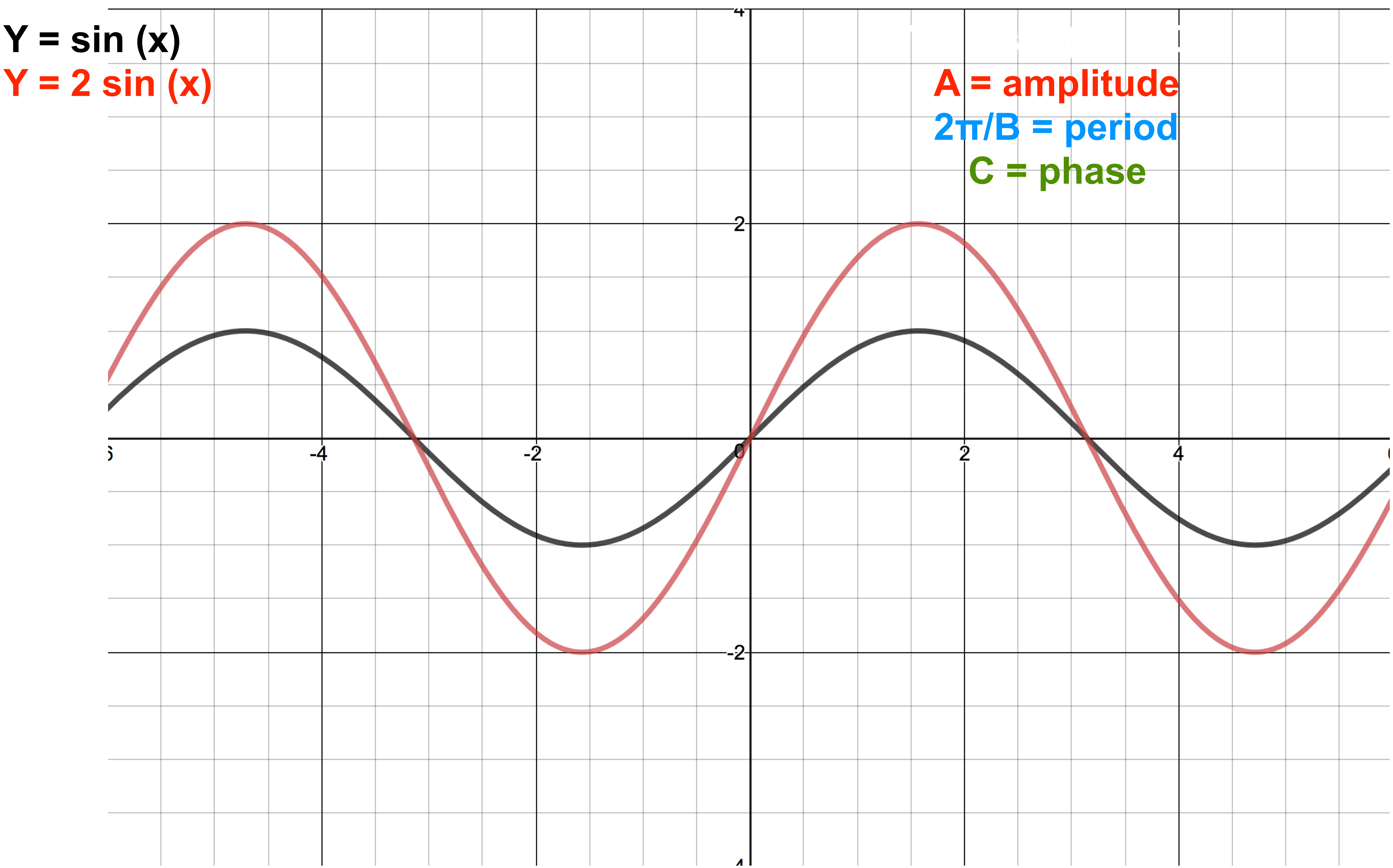
Y = sin (x)



Period = 2π/B
Period = 2π/1 = 2π

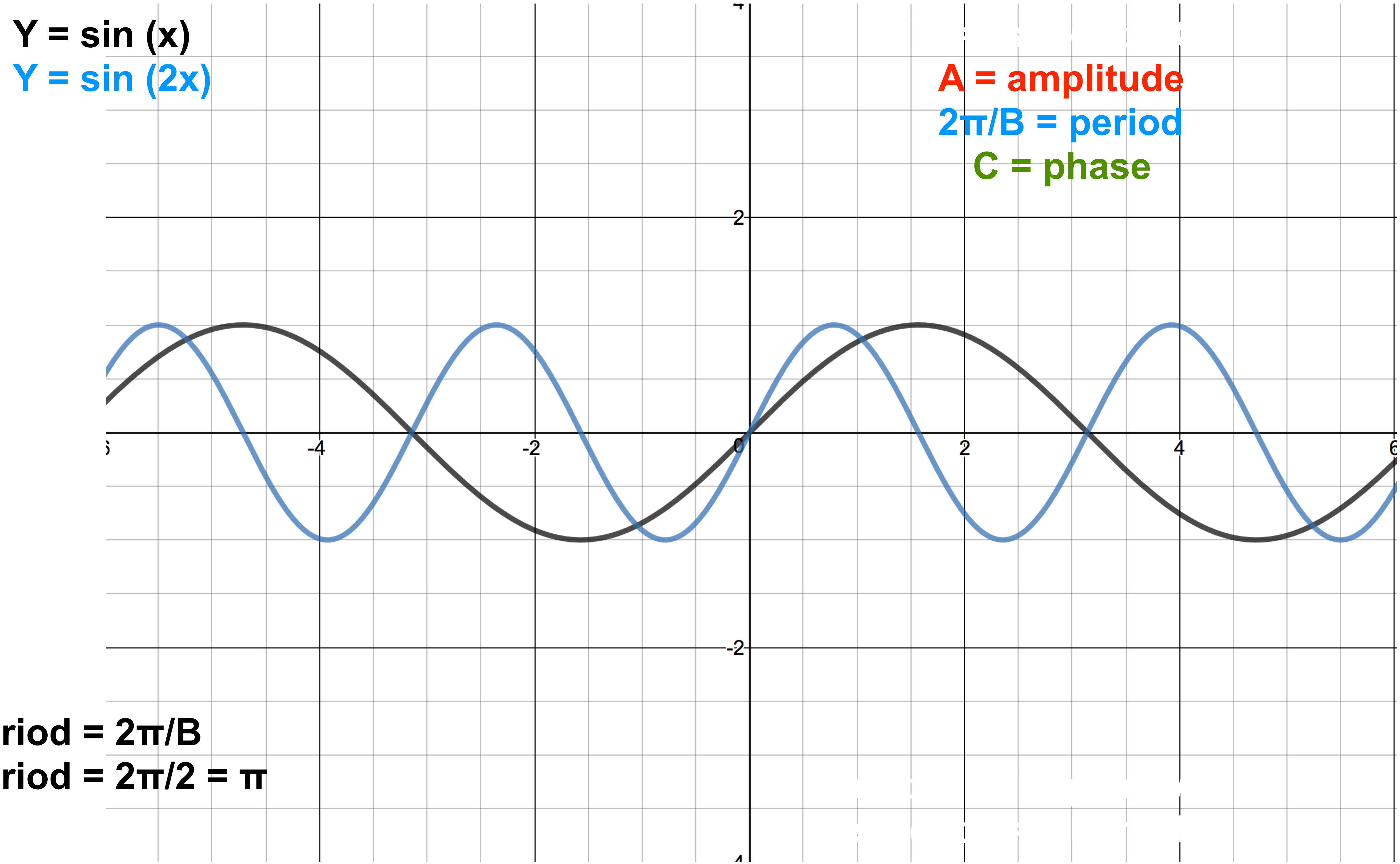
www.desmos.com/calculator

Sinusoid Wave



www.desmos.com/calculator

Sinusoid Wave



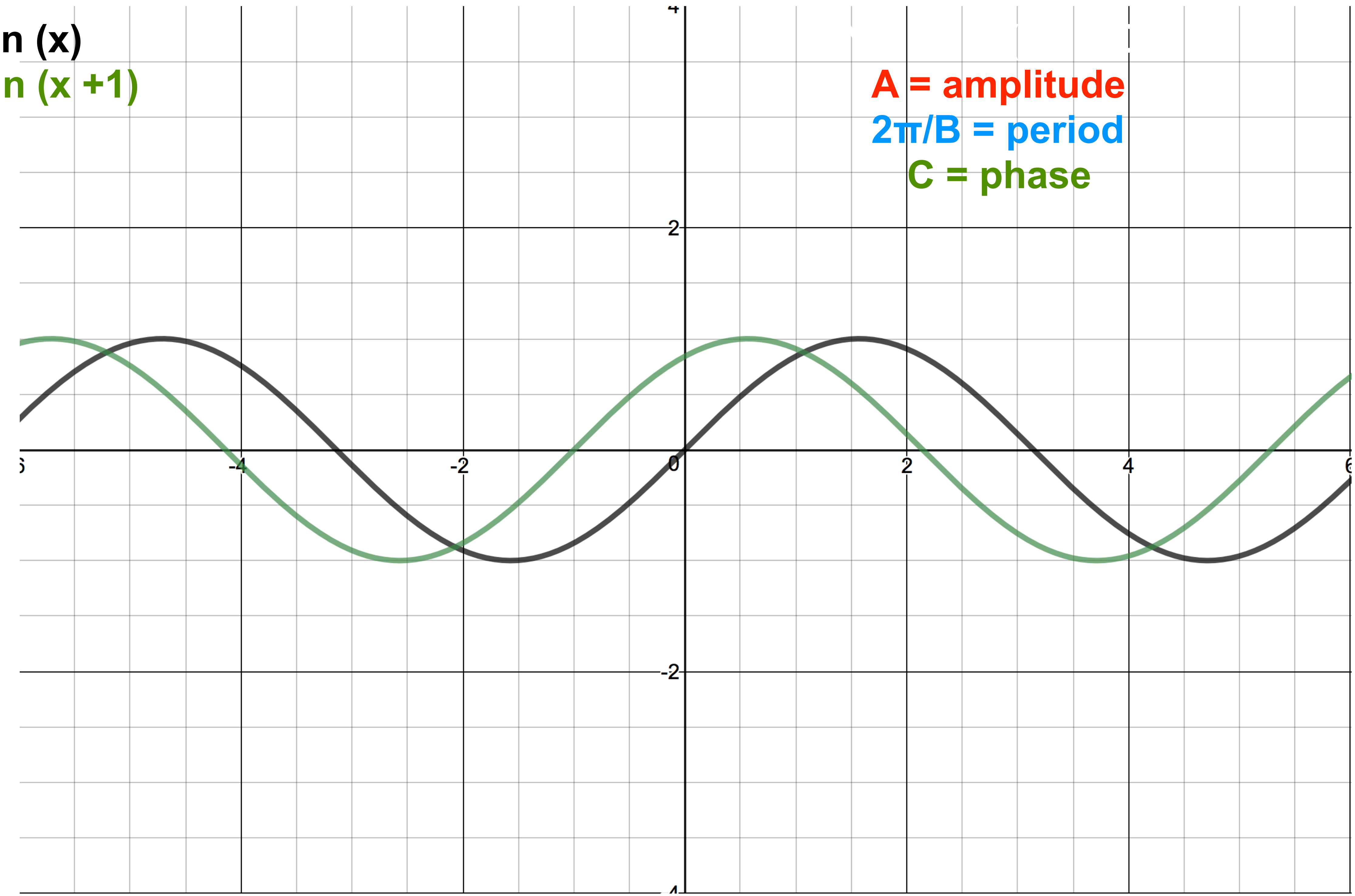
Period = 2π/B
Period = 2π/2 = π

www.desmos.com/calculator

Sinusoid Wave

Y = sin (x)
Y = sin (x +1)

A = amplitude
2π/B = period
C = phase

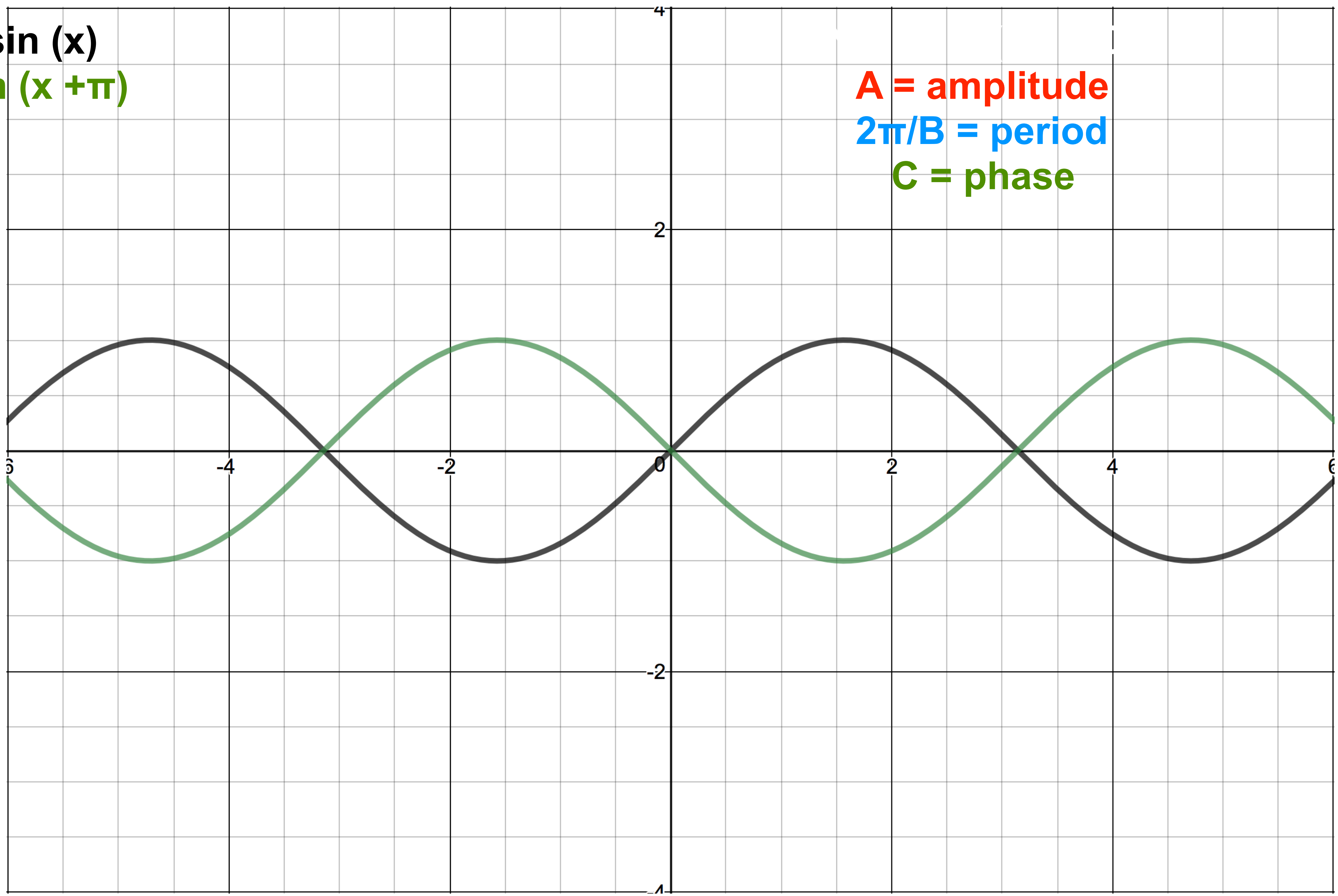


www.desmos.com/calculator

Sinusoid Wave

Y = sin (x)
Y = sin (x +π)

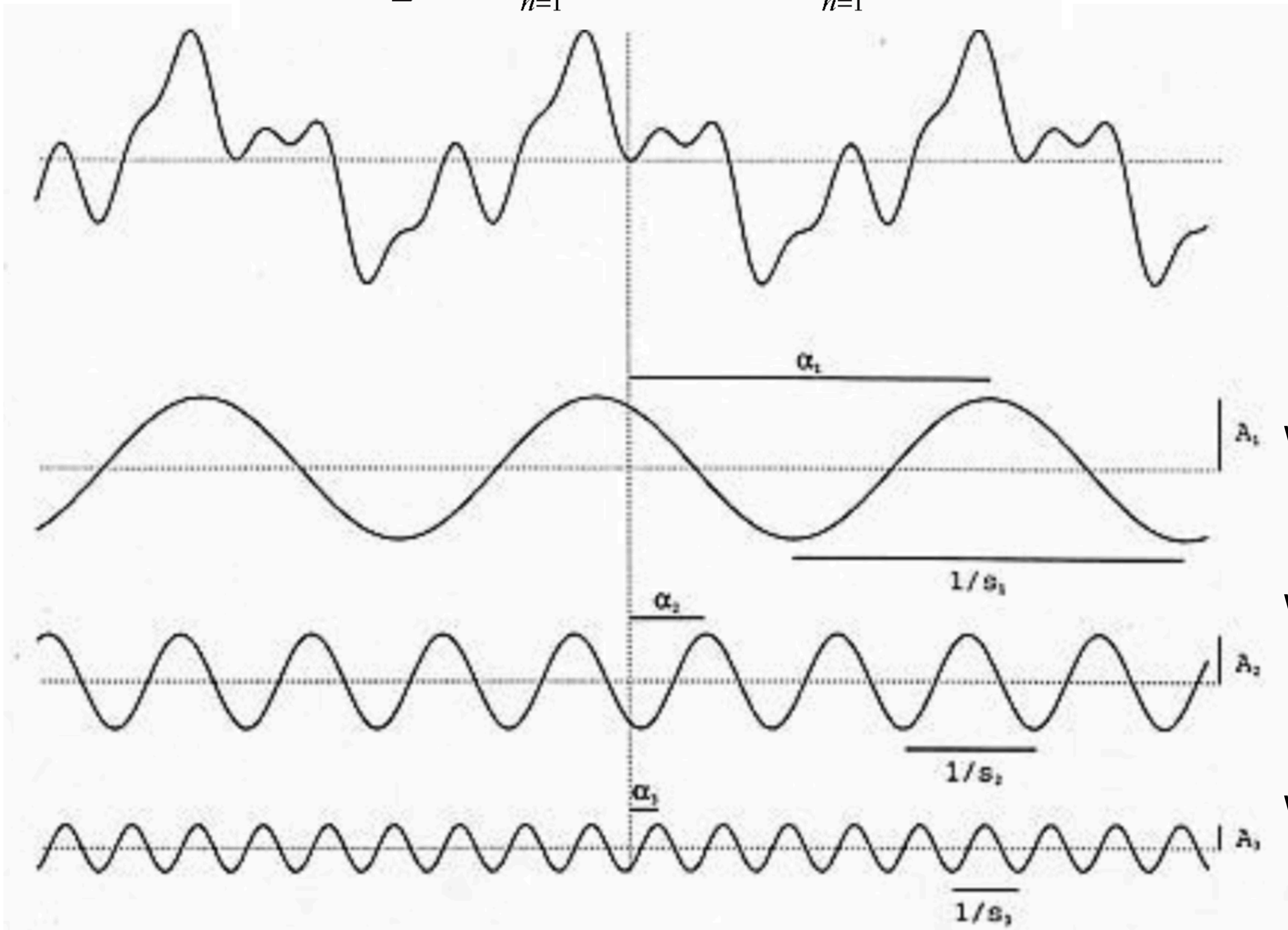
A = amplitude
2π/B = period
C = phase



www.desmos.com/calculator

Fourier Decomposition

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



Complex Wave

FT

FT^{-1}

Wave Component A

+

Wave Component B

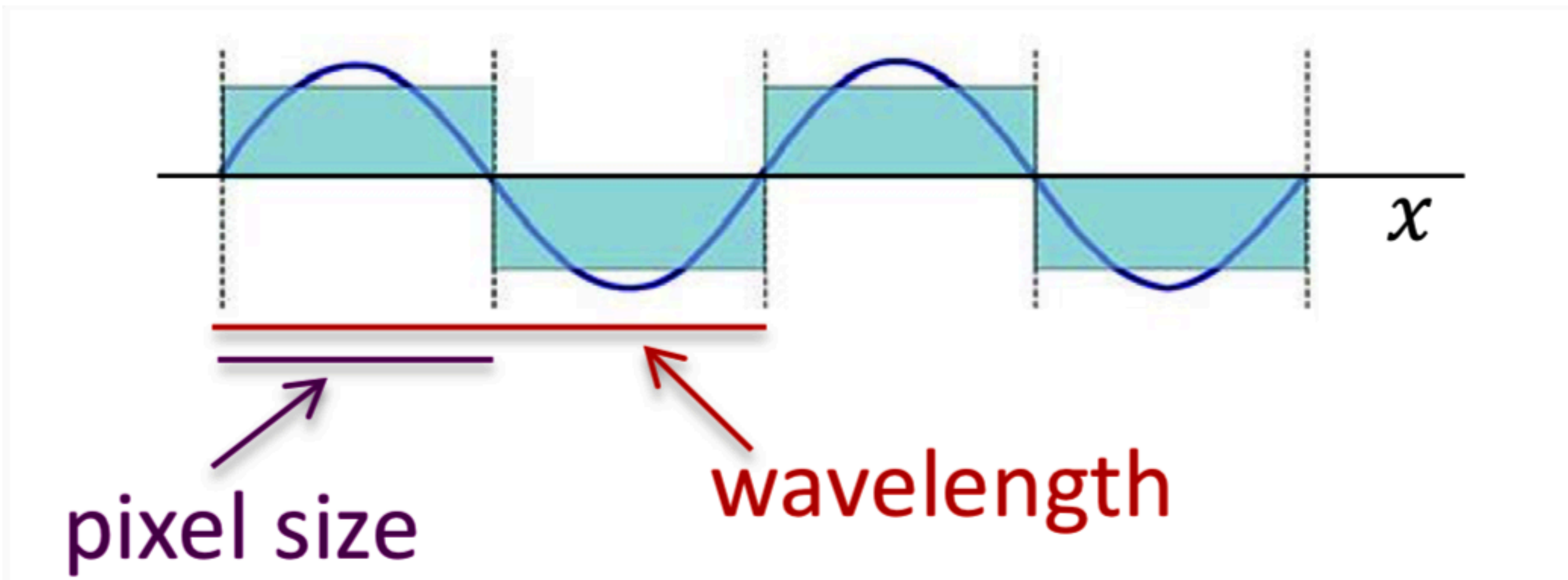
+

Wave Component C

Chiu et al Biophysics Journal 1993

I. Fourier transform

Nyquist Frequency

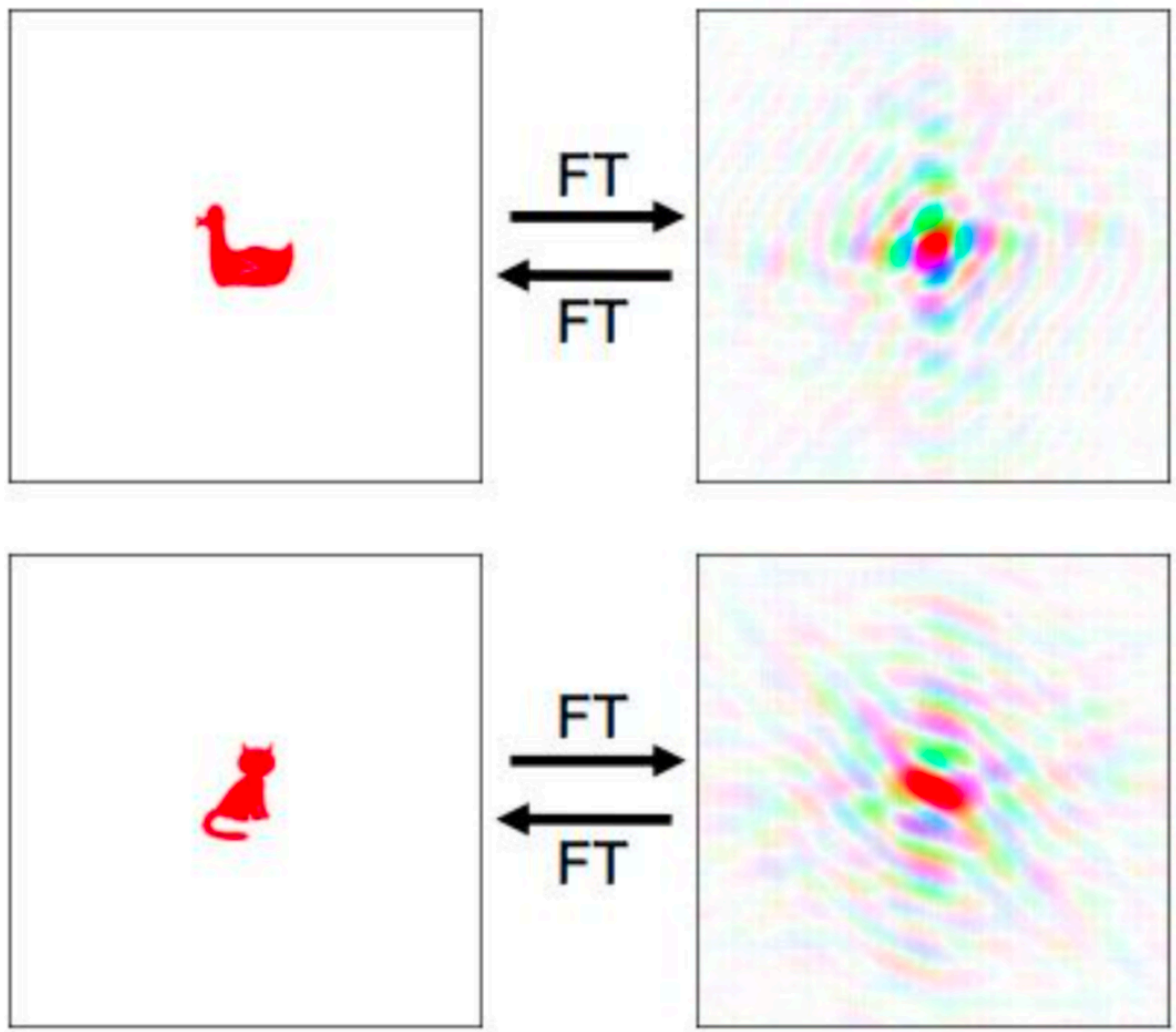


- Nyquist frequency is the highest spacial frequency that can be encoded in an image
- A waveform can only be decomposed into Fourier components with wavelengths that are at least 2x the sampling rate (pixel size)
- If the pixel size = 2Å then the smallest sine wave that can be described has a wavelength twice that value = 4Å
- 1/4 Å is Nyquist frequency

Adapted from Paula da Fonseca

Significance of Phase & Amplitude

Duck



Duck FT

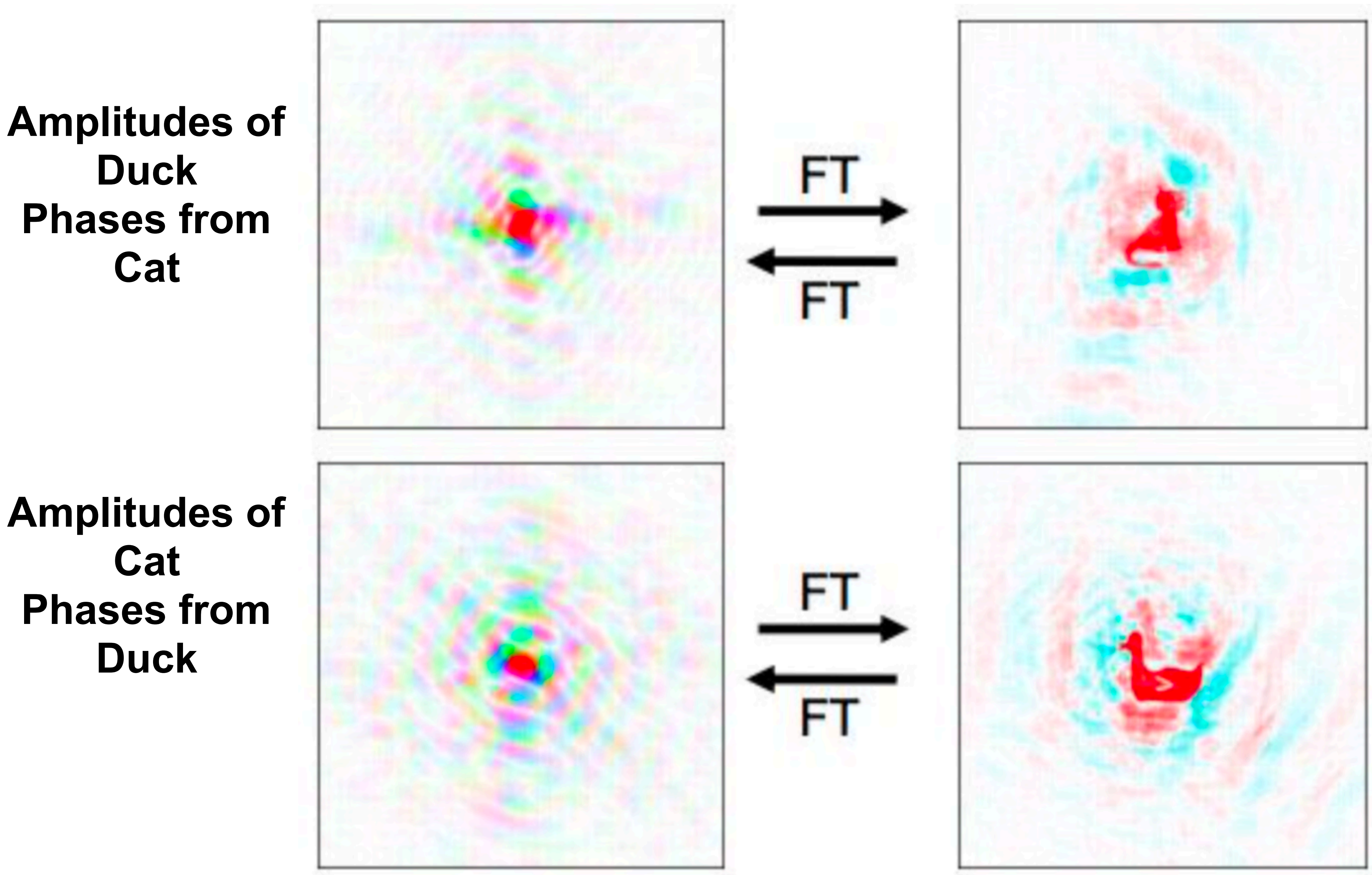
Cat

Cat FT

I. Fourier transform

From Kevin Cowtan's "Book of Fourier"

Significance of Phase & Amplitude



I. Fourier transform

From Kevin Cowtan's "Book of Fourier"

Convolution & Cross-Correlation

Convolution Theorem

$$G(u) = F(u)\,H(u)$$

Convolution

$$g(x) = f(x) \odot h(x) = \int_{-\infty}^{\infty} f(s)\,h(x-s)\,\mathrm{d}s$$

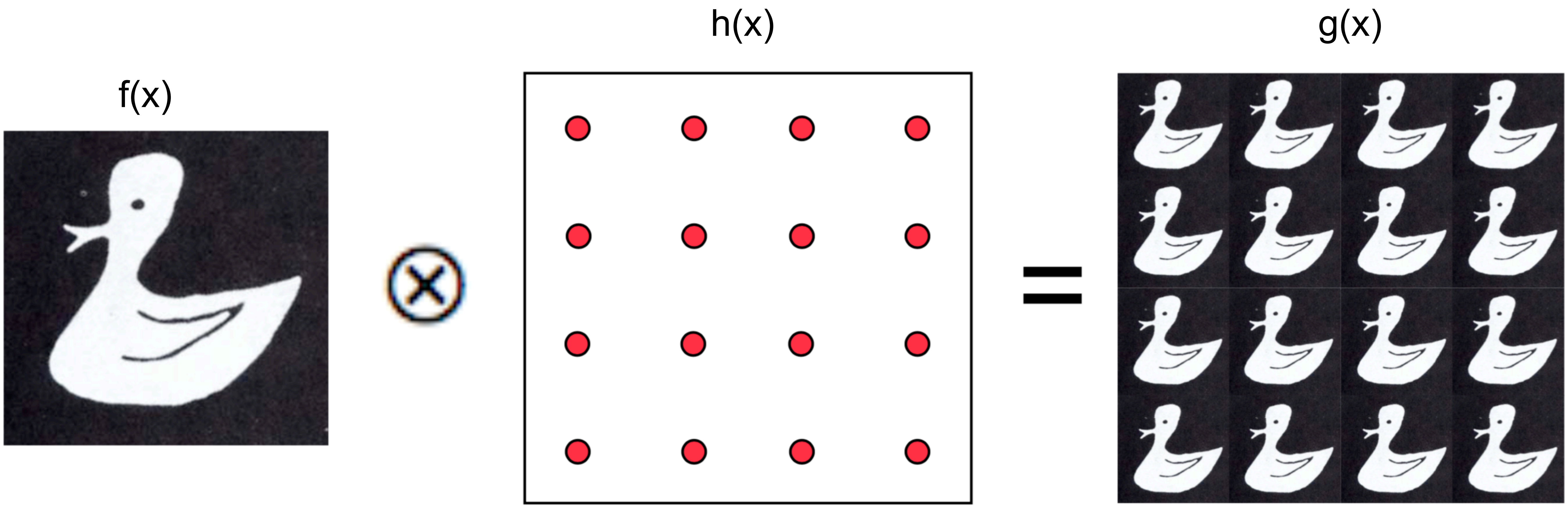
Correlation

$$c(x) = f(x) \otimes h(x) = \int_{-\infty}^{\infty} f(s)\,h^*(s-x)\,\mathrm{d}s$$

$$\begin{aligned} G(u) &= \mathcal{F}\{g(x)\} \\ F(u) &= \mathcal{F}\{f(x)\} \\ H(u) &= \mathcal{F}\{h(x)\} \end{aligned}$$

- The **convolution** operation may be considered the area of overlap between the function f(x) and the spatially reversed version of the function h(x)
- In the **correlation** operation two functions are shifted and the area of overlap formed by integration, but this time without the spatial reversal involved in convolution.
- The **convolution theorem** relates the convolution between the real space domain to a multiplication in the Fourier domain

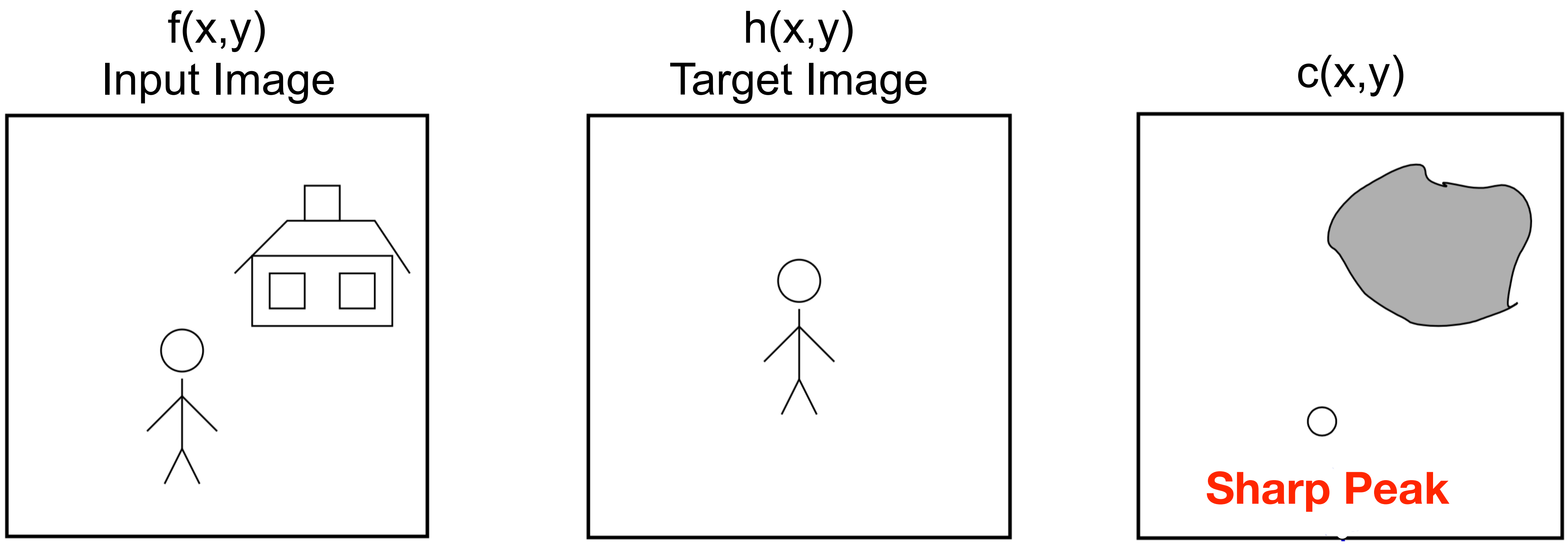
Convolution



- Combine two functions f(x) and h(x) such that a copy of one function is placed at each point in space, weighted by the value of the second function at the same point.

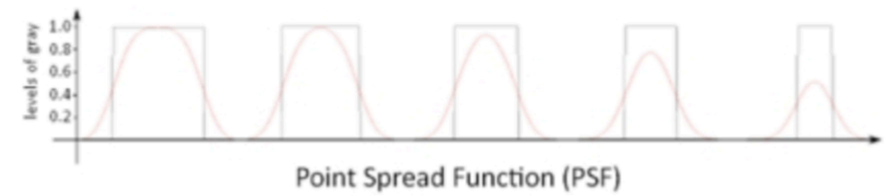
Adapted from Grant Jensen Lecture

Cross-Correlation

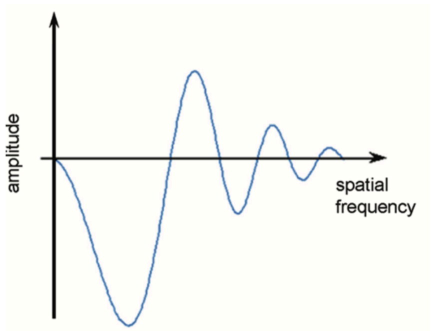


- Combine two functions f(x,y) and h(x,y) such that a copy of one function is placed at each point in space, weighted by the value of the second function at the same point
- Take an input image f(x, y) and a target image h(x, y), then the correlation is formed by taking a shifted version of h(x, y), the target, and placing over the input image f(x, y).
- When the man is located over man there is a good match so that multiplying and summing will give a sharp peak. This height of the peak will give the degree at match between the target and the input image and the location of the peak will give the location of the target.

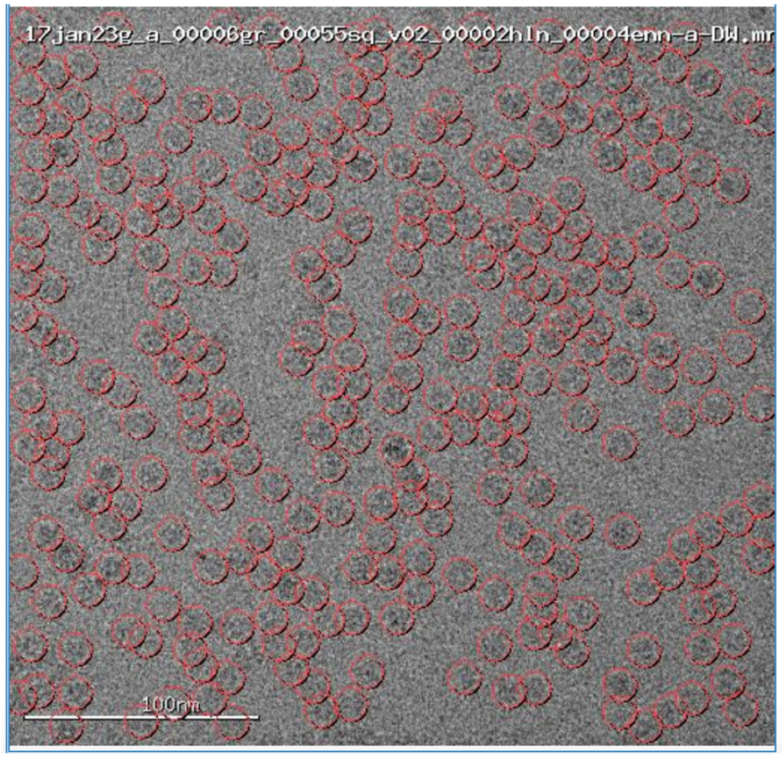
Convolution & Cross-correlation



- **Applications of Convolution**
  - Describes PSF
  - Describes effects of CTF

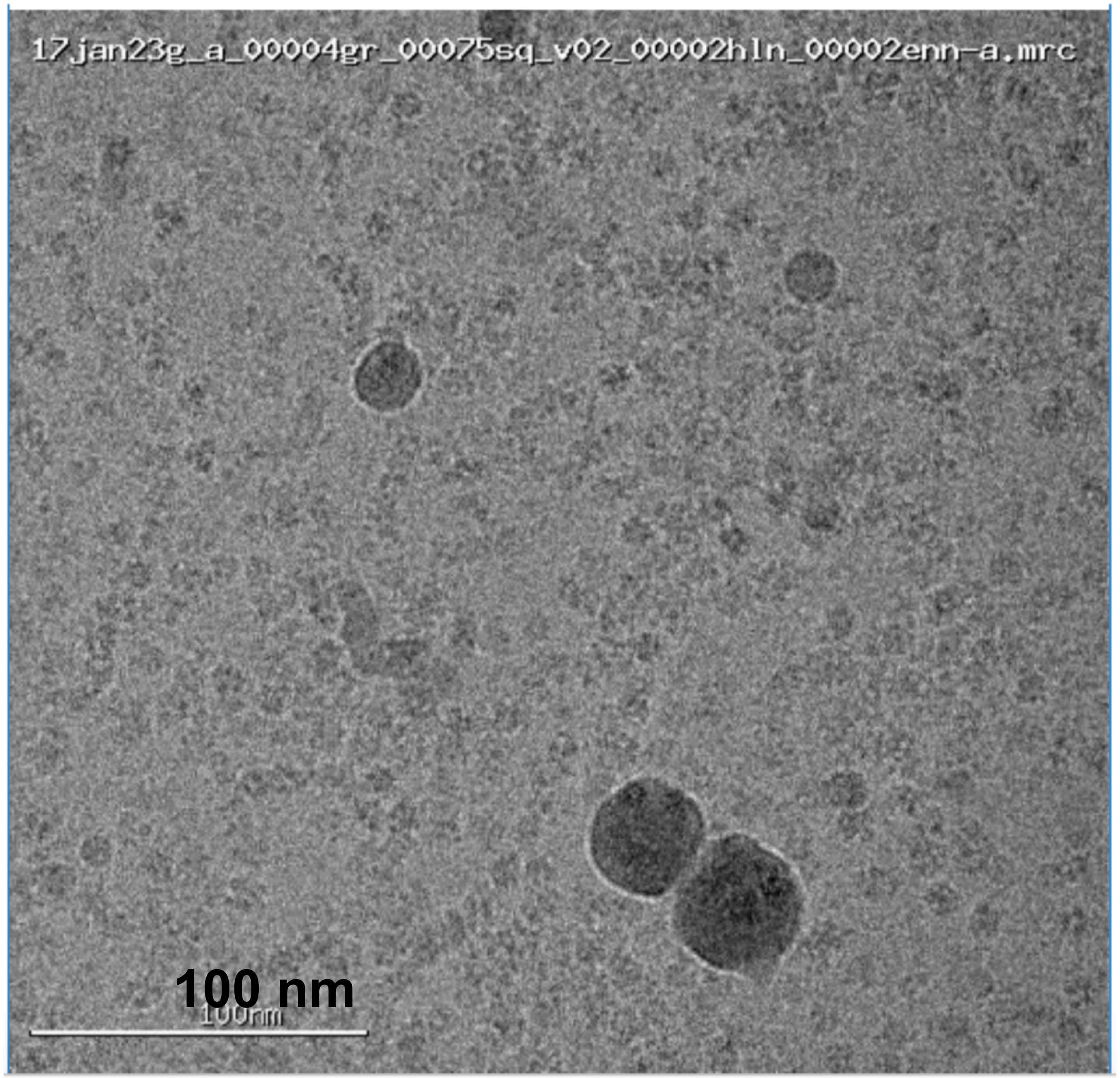


- **Applications of Cross-correlation**
  - Particle picking
  - Alignment of images

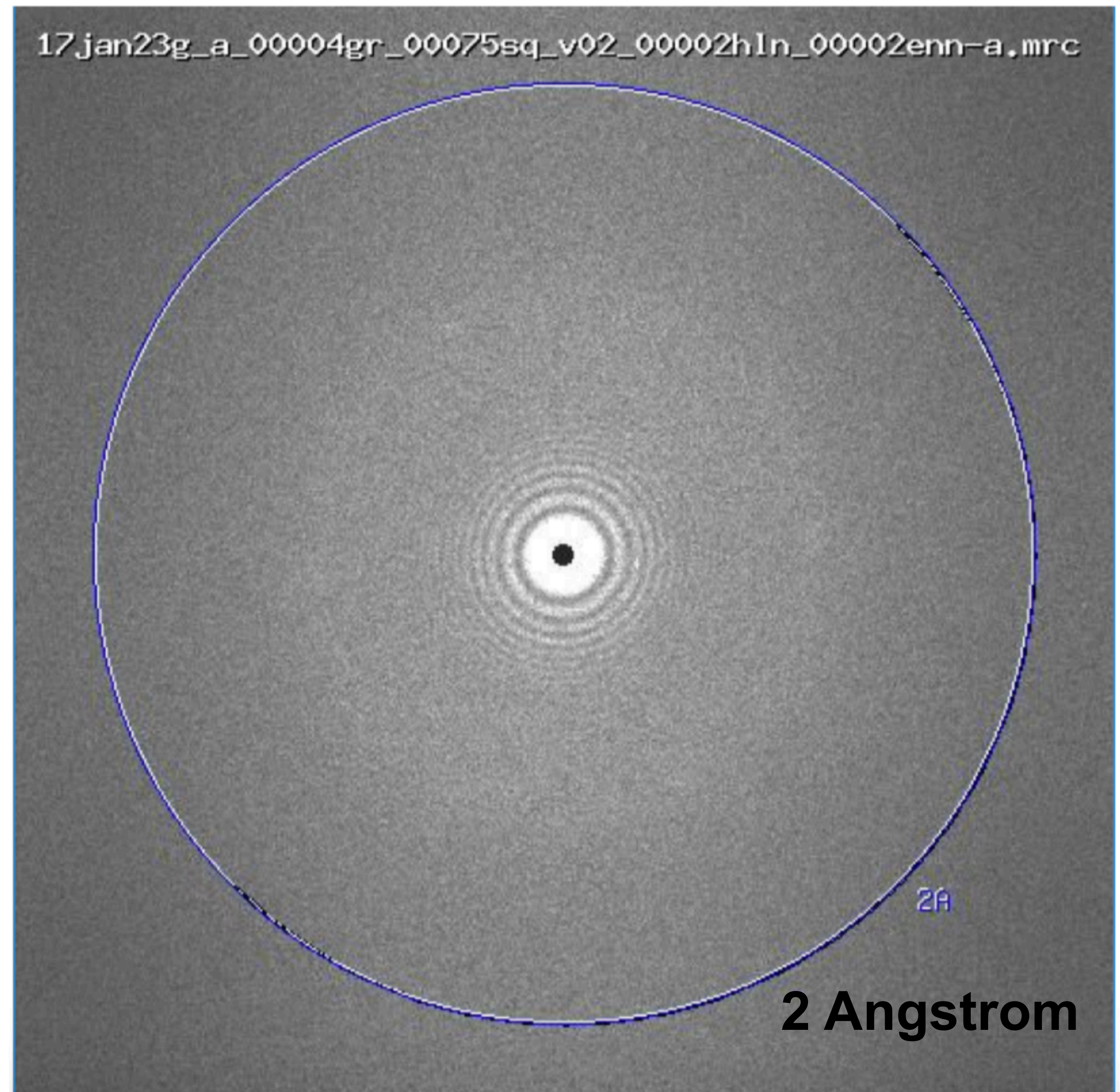


Information from Fourier Transform

Micrograph



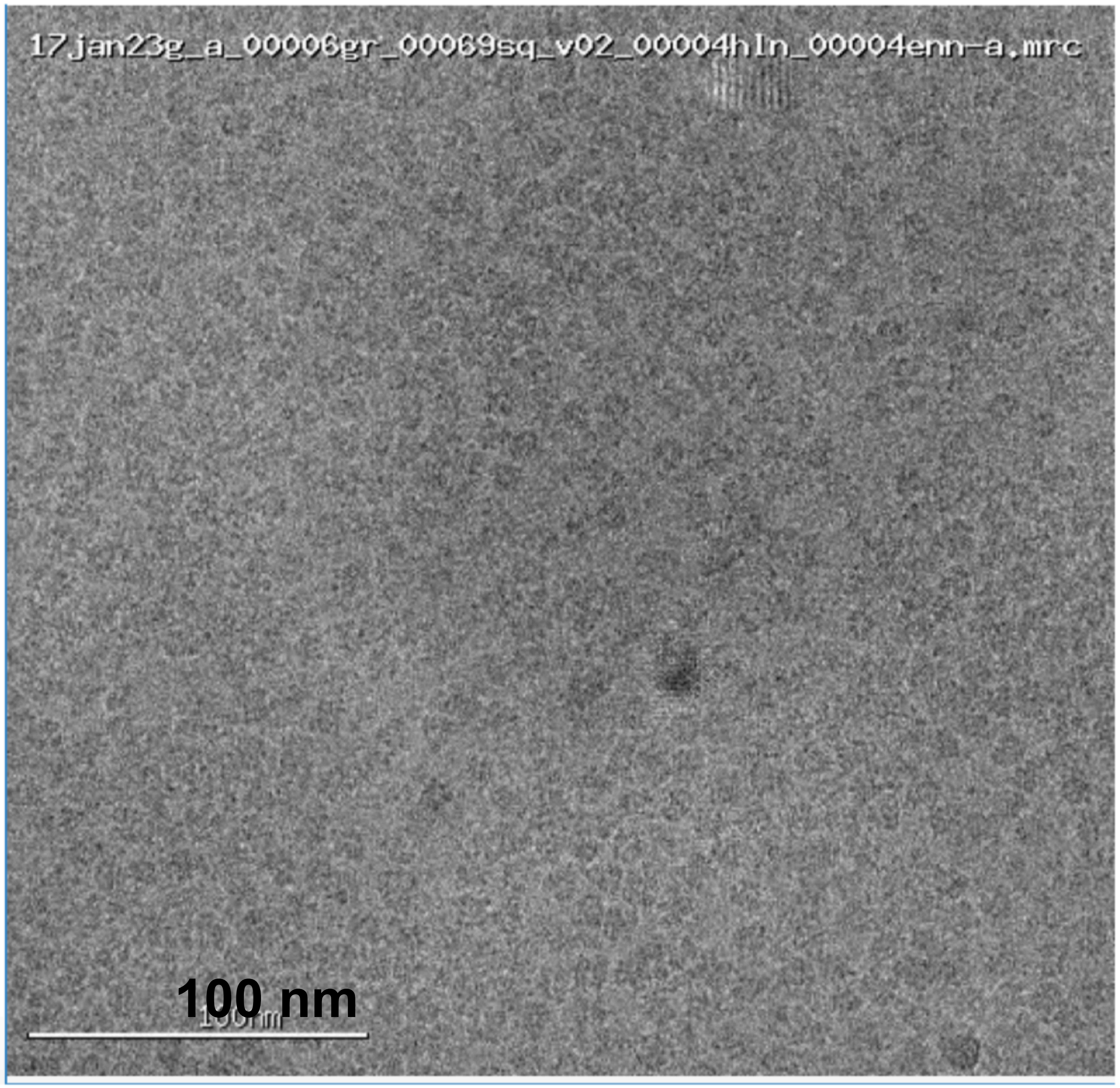
Power Spectrum of Micrograph



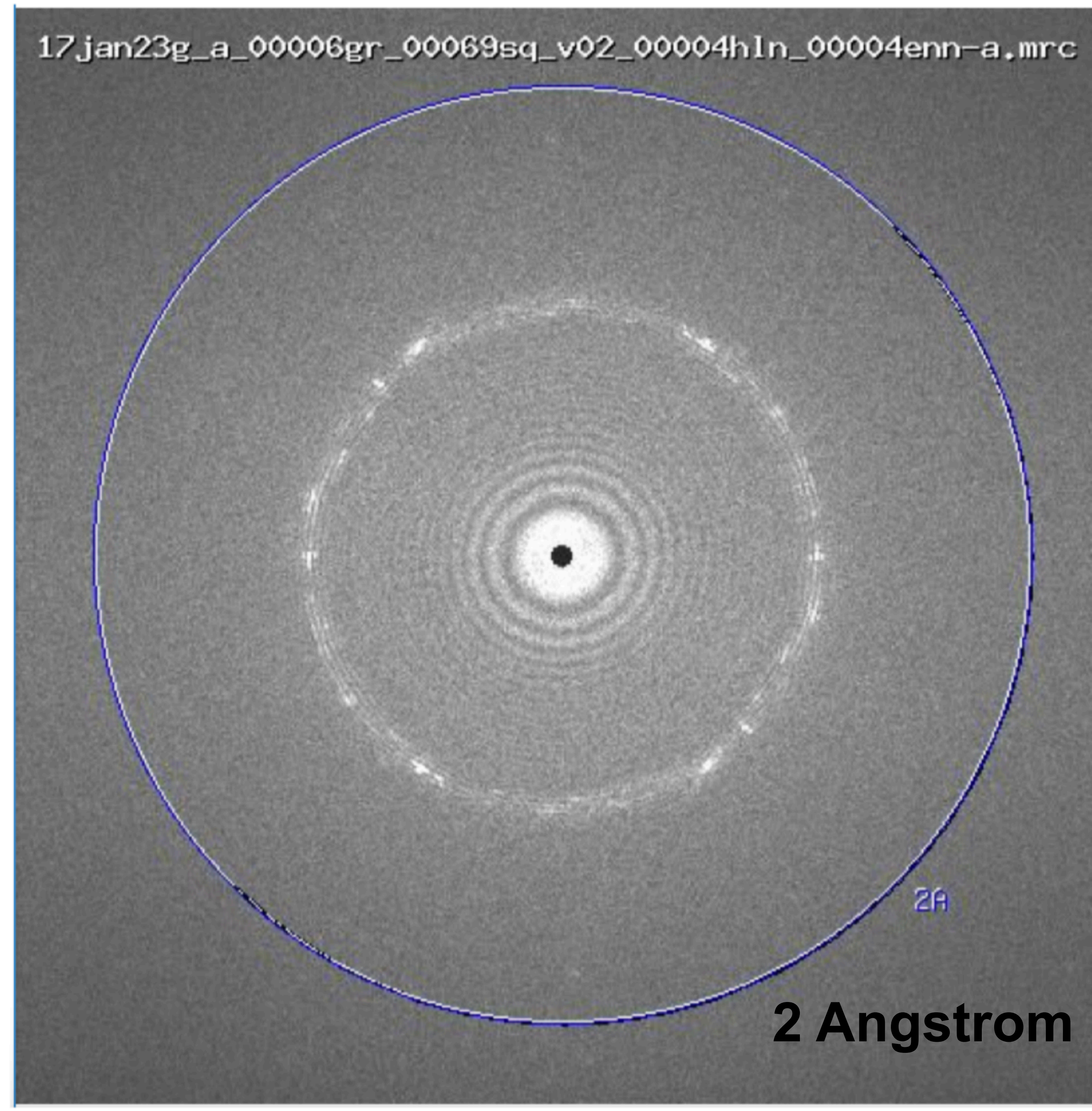
I. Fourier transform

Information from Fourier Transform

Micrograph



Power Spectrum
Of Micrograph



I. Fourier transform

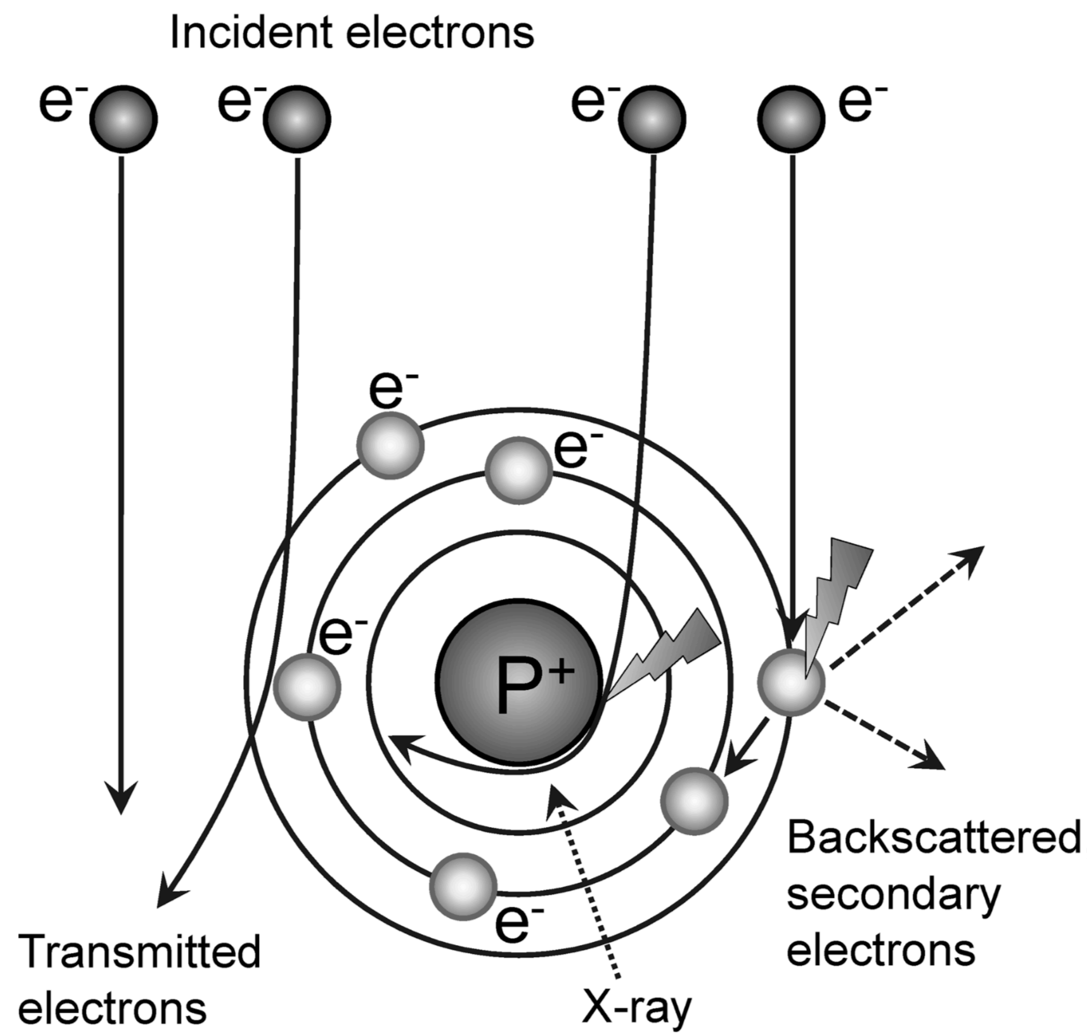
Roadmap

- **Fourier transform**
    - FT, Sine Waves and Importance of Amplitude and Phase
    - Convolution & Cross Correlation
    - The information from FT
- **Image Formation**
    - Elastic and Inelastic scattering
    - Amplitude and Phase Contrast
    - CTF & PSF
    - Effect of Defocus on CTF
    - Estimation and correction of CTF

Electron Wave Particle Duality

- Electrons are negatively charged particles
    - Interact with other particles
    - Elastic vs Inelastic scattering
- An electron wave is almost a plane wave
    - Diffracted by atoms
    - Determined by atomic scattering factor Z

Electron Scattering



Electrons have 3 types of interactions:

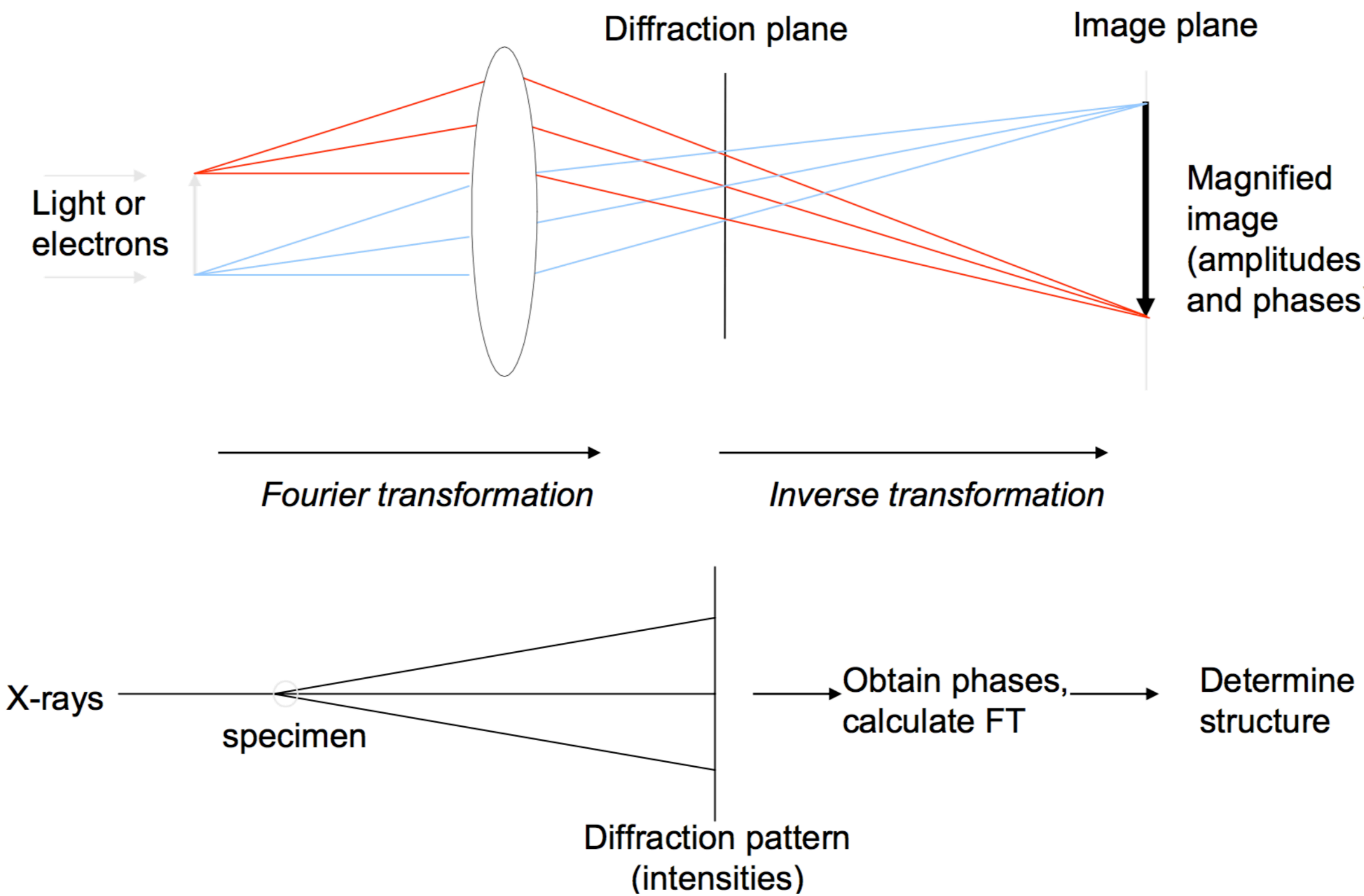
1. Non interacting
2. Elastic Scattering - Deflection by electron cloud - Maintain Energy - Image
3. Inelastic Scattering - Collision of beam electrons with nuclei - Radiation
4. Inelastic Scattering - Collision of beam electrons and atomic electrons - Radiation

Orlova & Saibil et al 2011, Chem Reviews

Electrons vs X-rays

- X-rays are scattered by the electrons in the specimen
    - The sample electrons oscillate with the period of the incoming X-ray wave
    - Electrons are scattered by the electromagnetic field (caused by nuclei and electrons)
- Electrons are scattered much stronger than X-rays

Electrons vs. X-rays

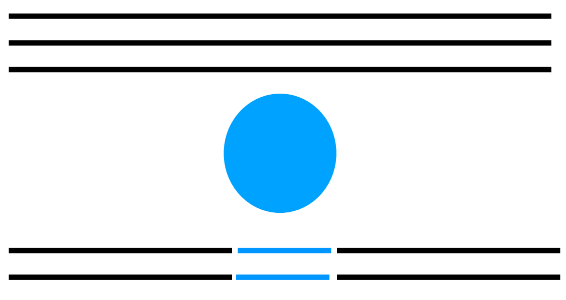


Amplitude and Phase Contrast

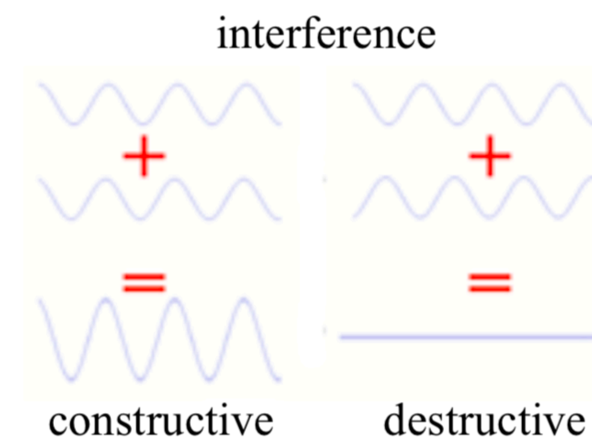
- **Amplitude Contrast**
    - Some parts can be electron transparent while rest is not electron transparent
    - Contrast is produced because of differences in the mass or thickness of the objects.
- **Phase Contrast**
    - Objects are electron transparent
    - Contrast is produced because of the differences in the phases of the scattered electrons compared to un-scattered electrons.

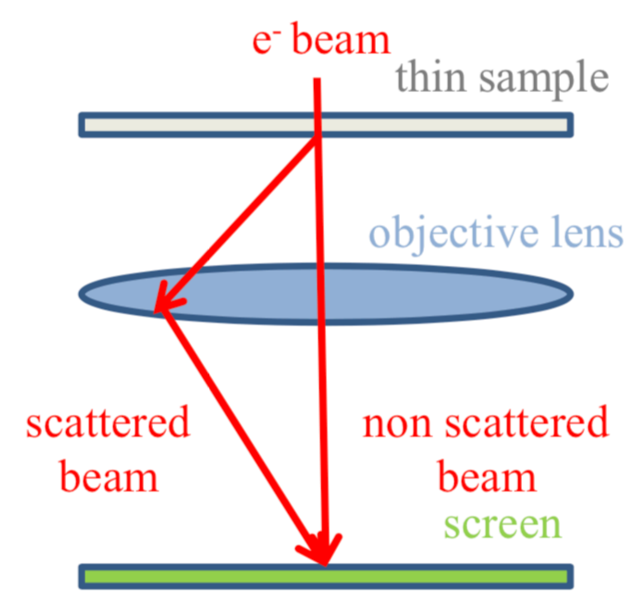
Amplitude and Phase Contrast

Electron as particle
Amplitude Contrast



Electron as wave
Phase Contrast



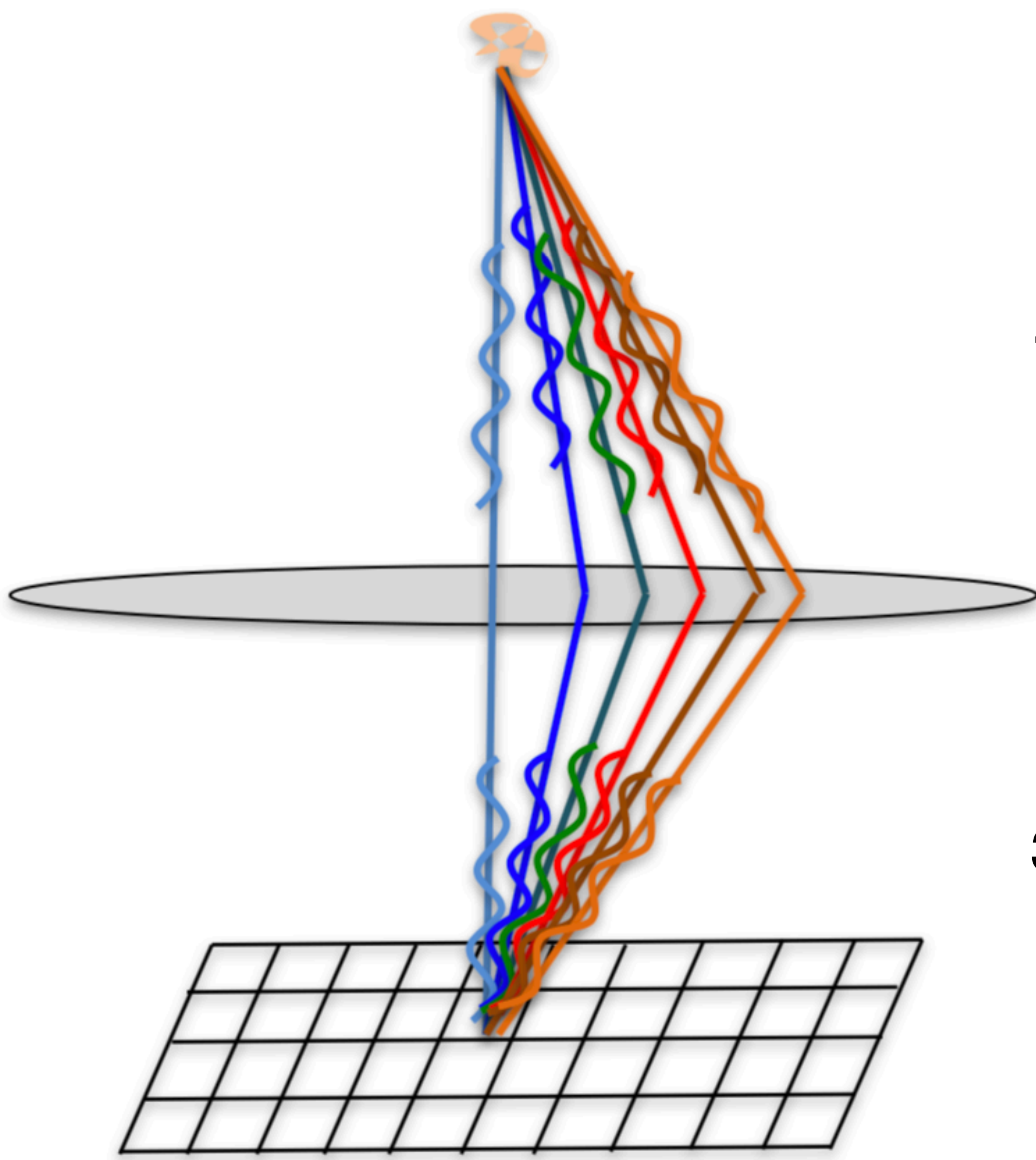


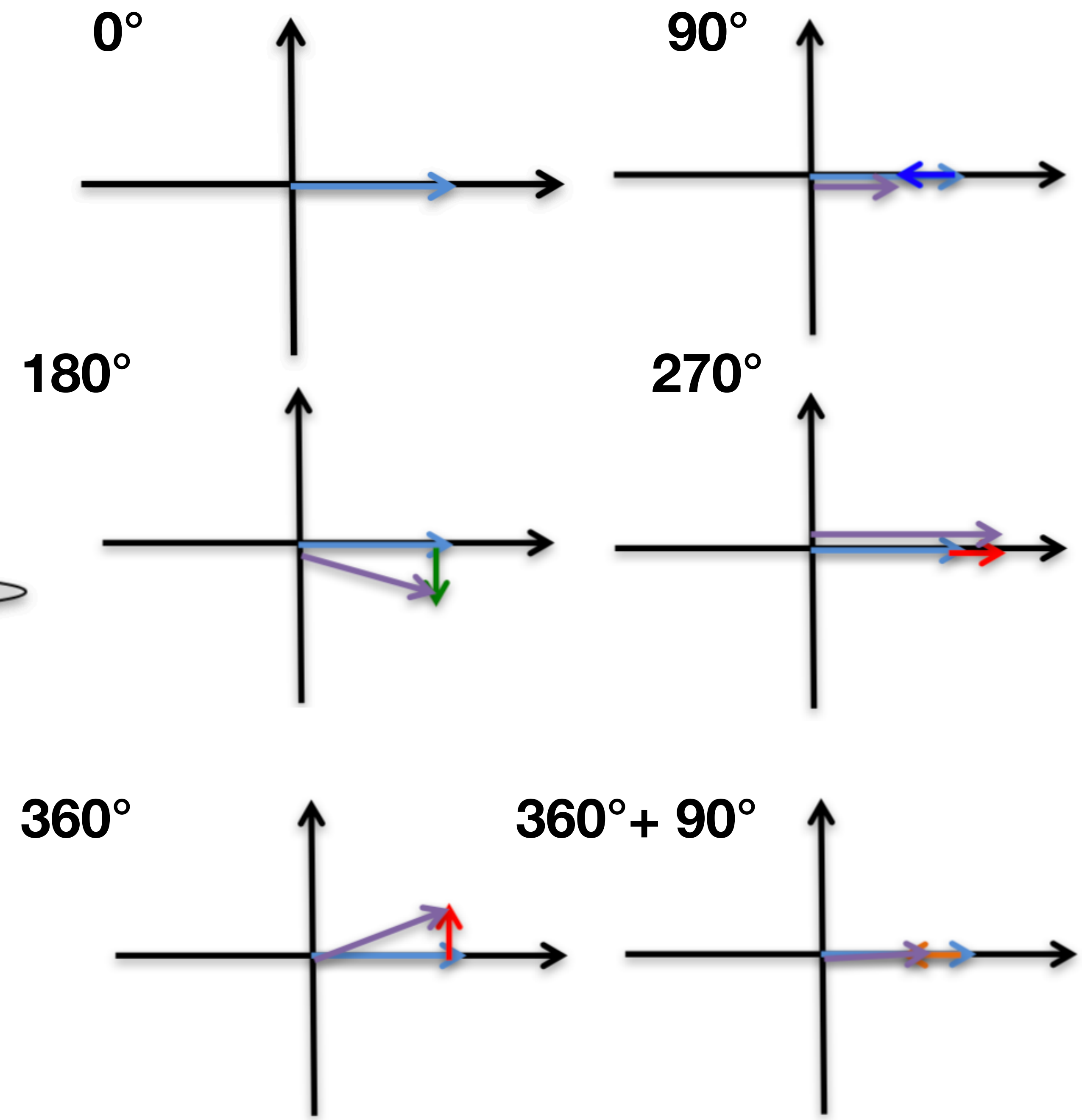
Amplitude Contrast
- Transmitted electrons produce an image
- Thicker regions produce more scattering
- 7% in CryoEM, 25% in NS EM

Phase Contrast
- Scattered and un-scattered electrons do not have the same phase
- Their interference produces difference in intensity
- Thin biological samples

Adapted from Irina Gutsche

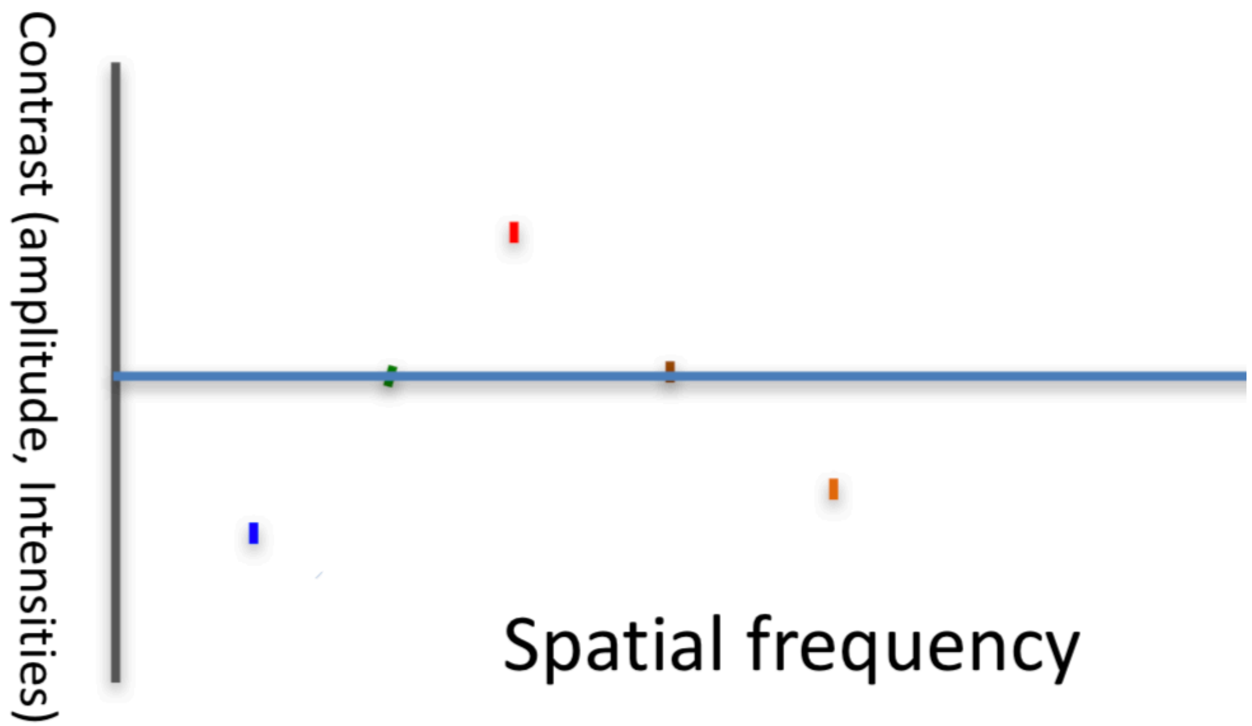
Wave Propagation & Phase Contrast

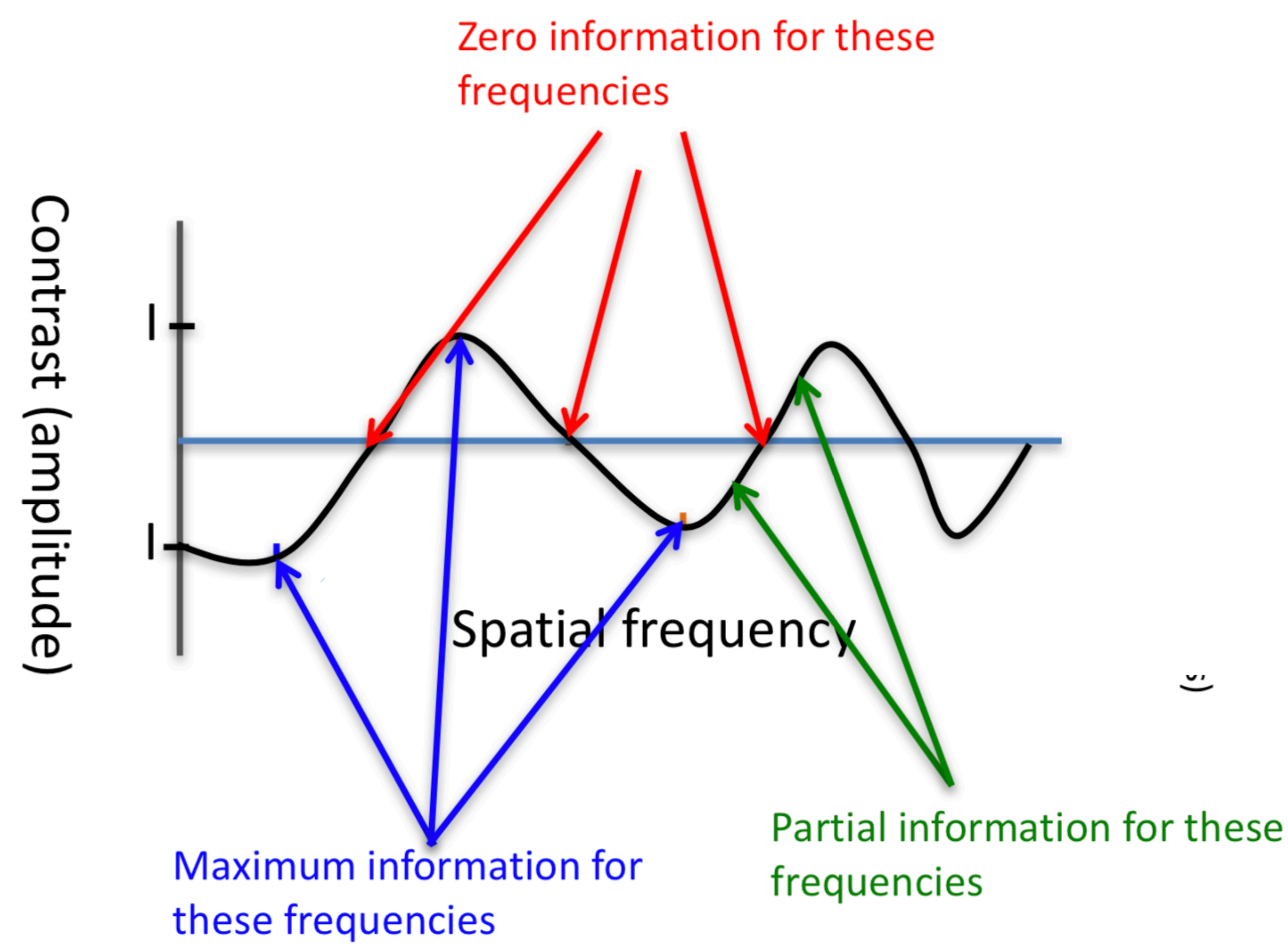




Adapted from Shashi Bhushan

Wave Propagation & Phase Contrast

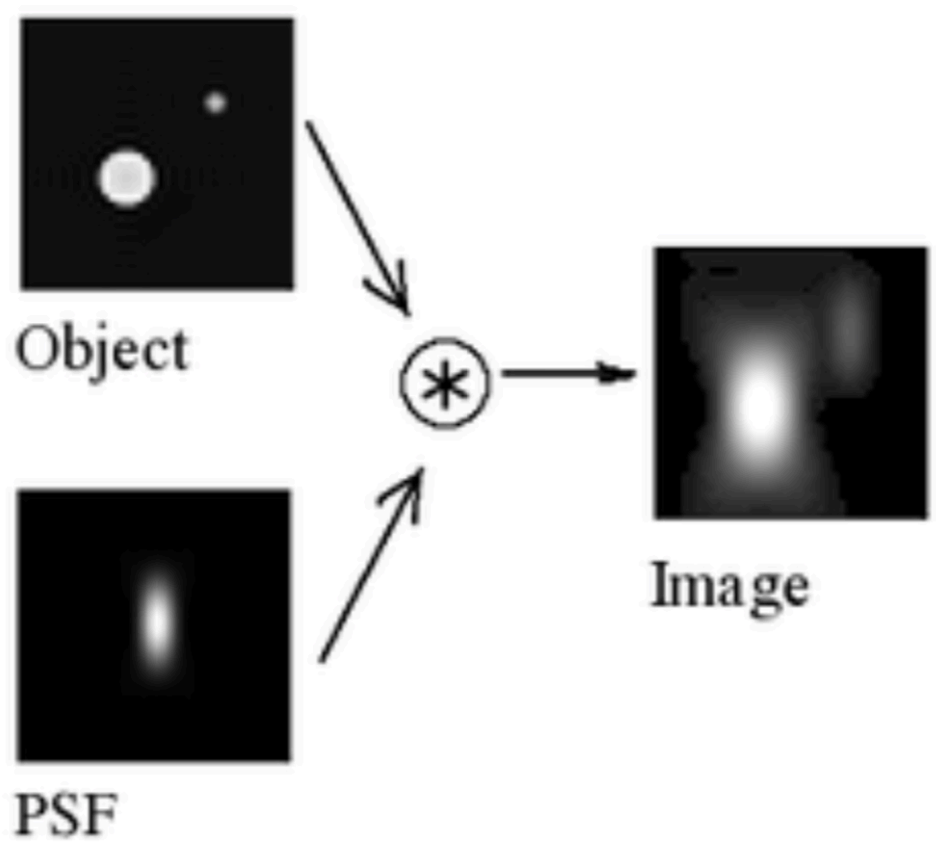




1. CTF is an oscillatory function
2. CTF describes how contrast (information) is transferred to image in terms of spatial frequency (Fourier components)
3. Each frequency components represents information: low frequency - lower resolution, high frequency -higher resolution)

Adapted from Shashi Bhushan

Point Spread Function



- The Point Spread Function (PSF) represents microscope aberrations
- In cryo-EM, images are blurred by convolution with a point spread function arising from the detector as well as imperfections in the imaging system
- Convolution of the Object (FT) with the PSF (FT) generates image Imperfections transferred to image

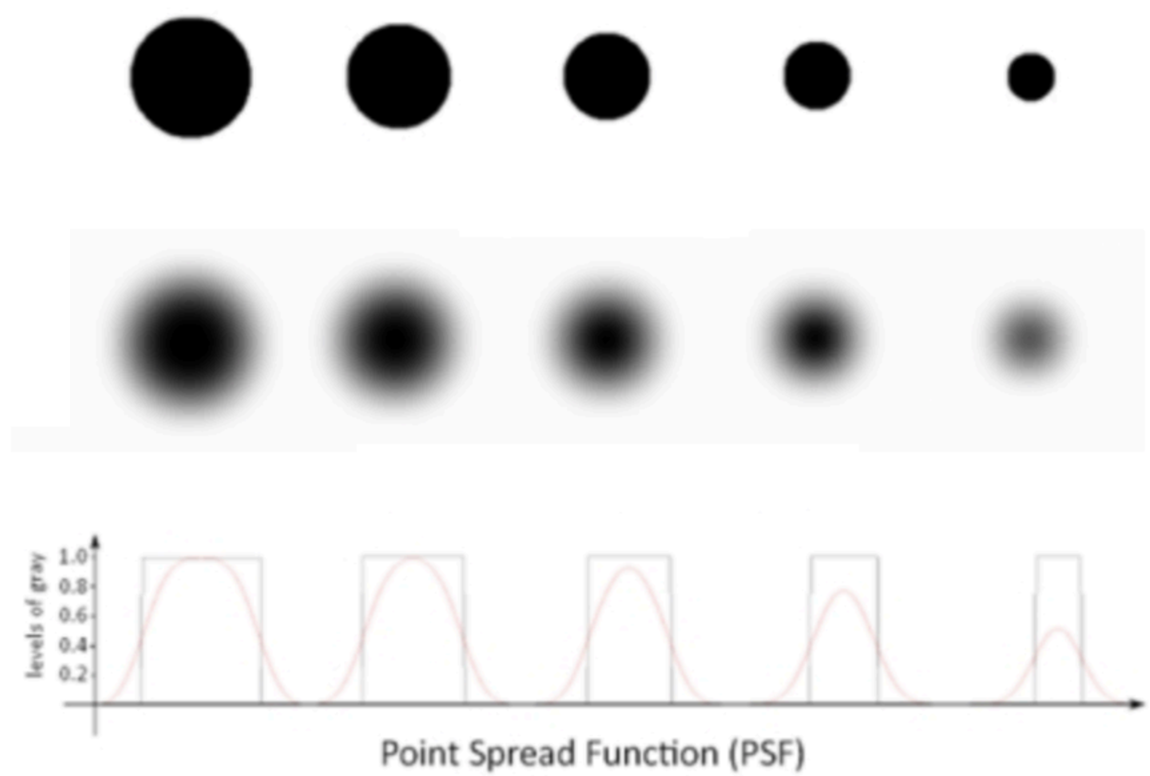
Orlova & Saibil et al 2011, Chem Reviews

Point Spread Function

Object

Image

PSF



CTF Correction:
Object = FT-1 [FT(Image)]/CTF

Object **PSF** **Image**

- Point spread function is the Fourier transform of CTF
- Image is equal to the Object convolved with the PSF
- The object is equal to the inverse Fourier transform of the Fourier transform of the Image/CTF

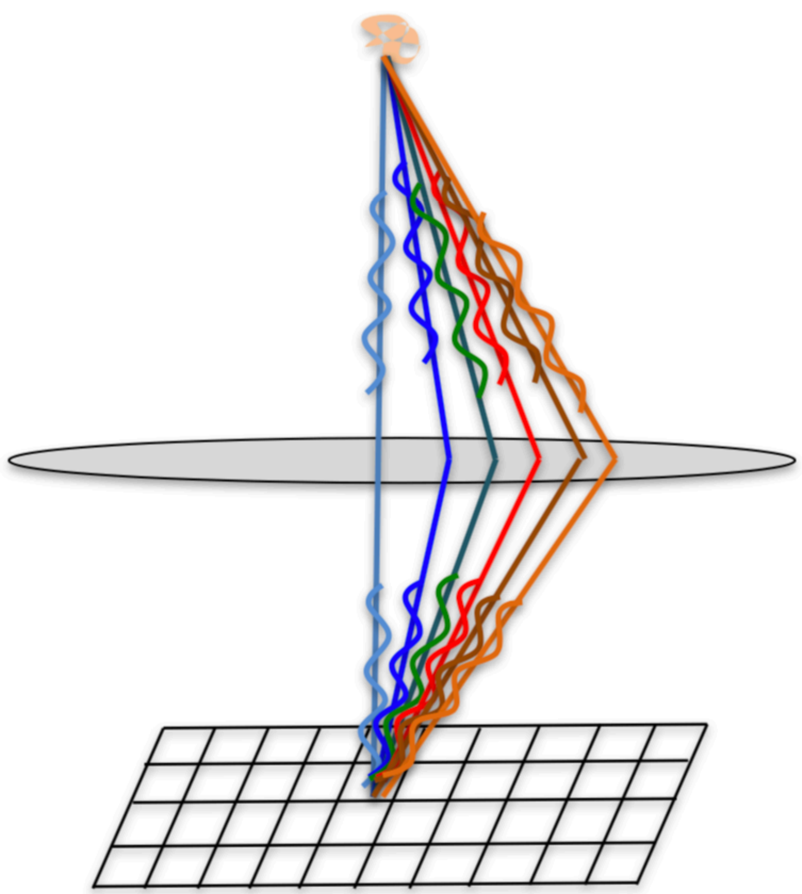
Adapted from Shashi Bhushan

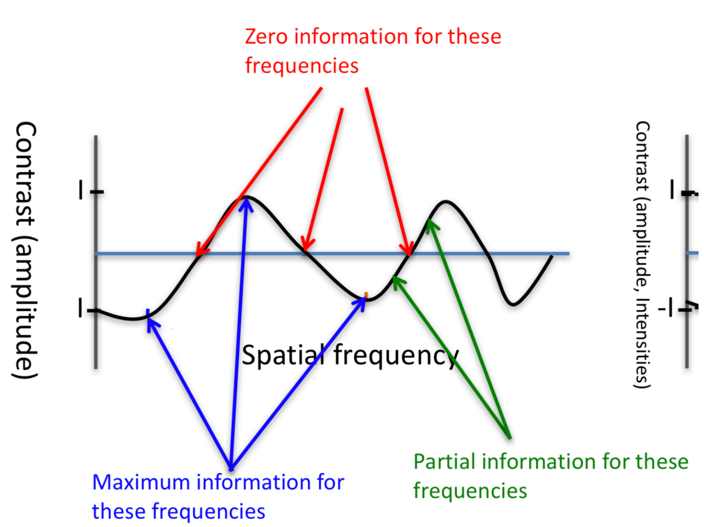
Contrast Transfer Function (CTF)

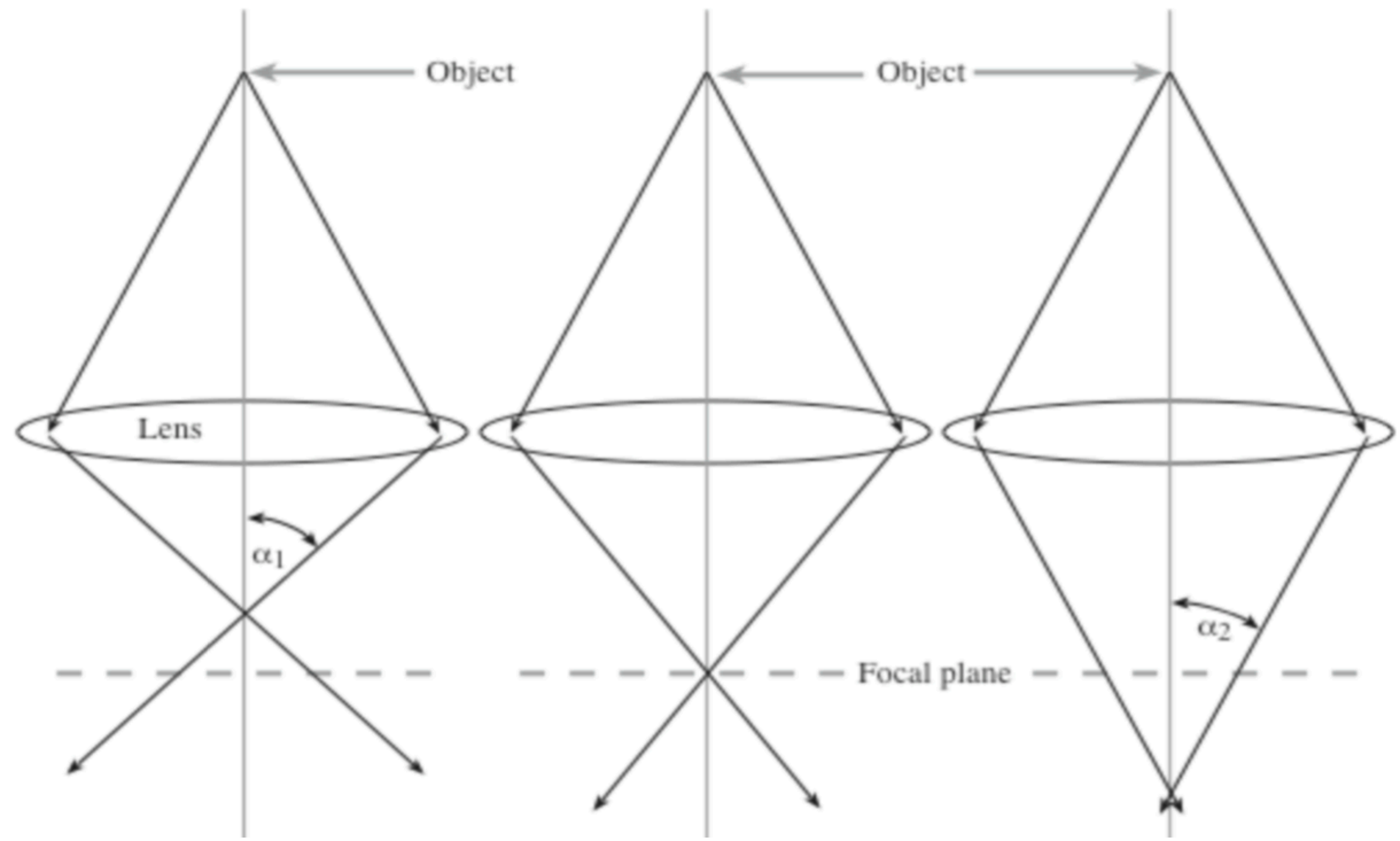
$$CTF(\nu) = \sin\left[\frac{2\pi}{\lambda}\left(\frac{Cs \cdot \lambda^4 \cdot \nu^4}{4} - \frac{\Delta f \cdot \lambda^2 \cdot \nu^2}{2}\right)\right]$$

- The contrast transfer function (CTF) mathematically describes how aberrations in a transmission electron microscope modify the image of a sample
- CTF depends on several parameters:
    - Spherical aberration coefficient (known variable)
    - Defocus (must be determined)
    - Apertures
- **Parameters:**
    - C_s = the quality of objective lens defined by spherical aberration coefficient)
    - λ = electron wavelength defined by accelerating voltage
    - Δf = the defocus value
    - v = spatial frequency
-

Effect of Focus on CTF







Overfocus Focus Underfocus

- To apply defocus, reduce the strength of the lens by reducing the current

Adapted from Shashi Bhushan & Rui Zhang

Effect of Focus on CTF

- By changing the defocus, path lengths of all the waves will be changed at the image plane. This will introduce additional phase shifts.

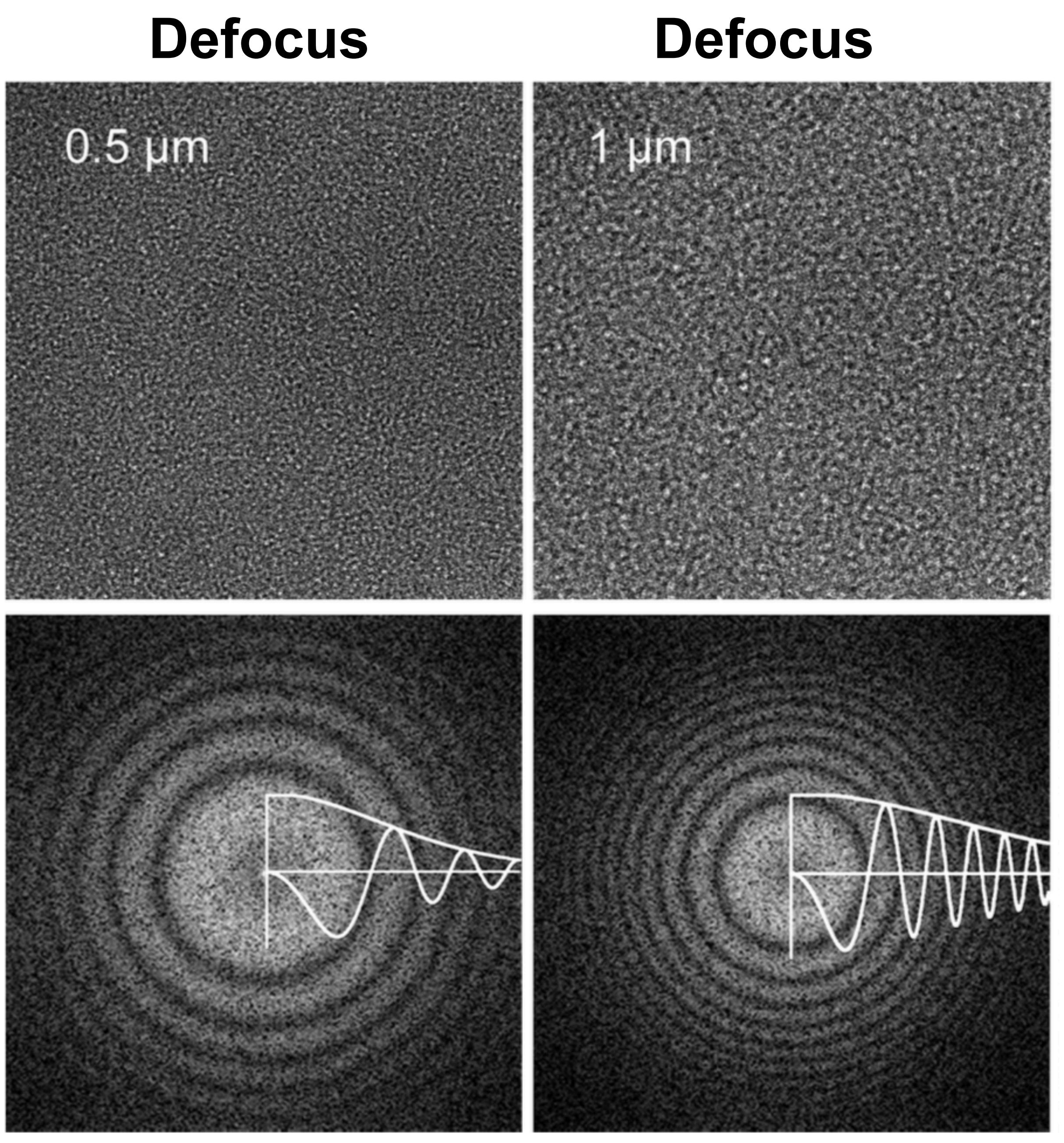
- The wave functions at the image plane will be different now.

- Certain waves which were not detected earlier will now be detected and vise versa.

- By applying a range of defocus, information lost at zeros can be retrieved.

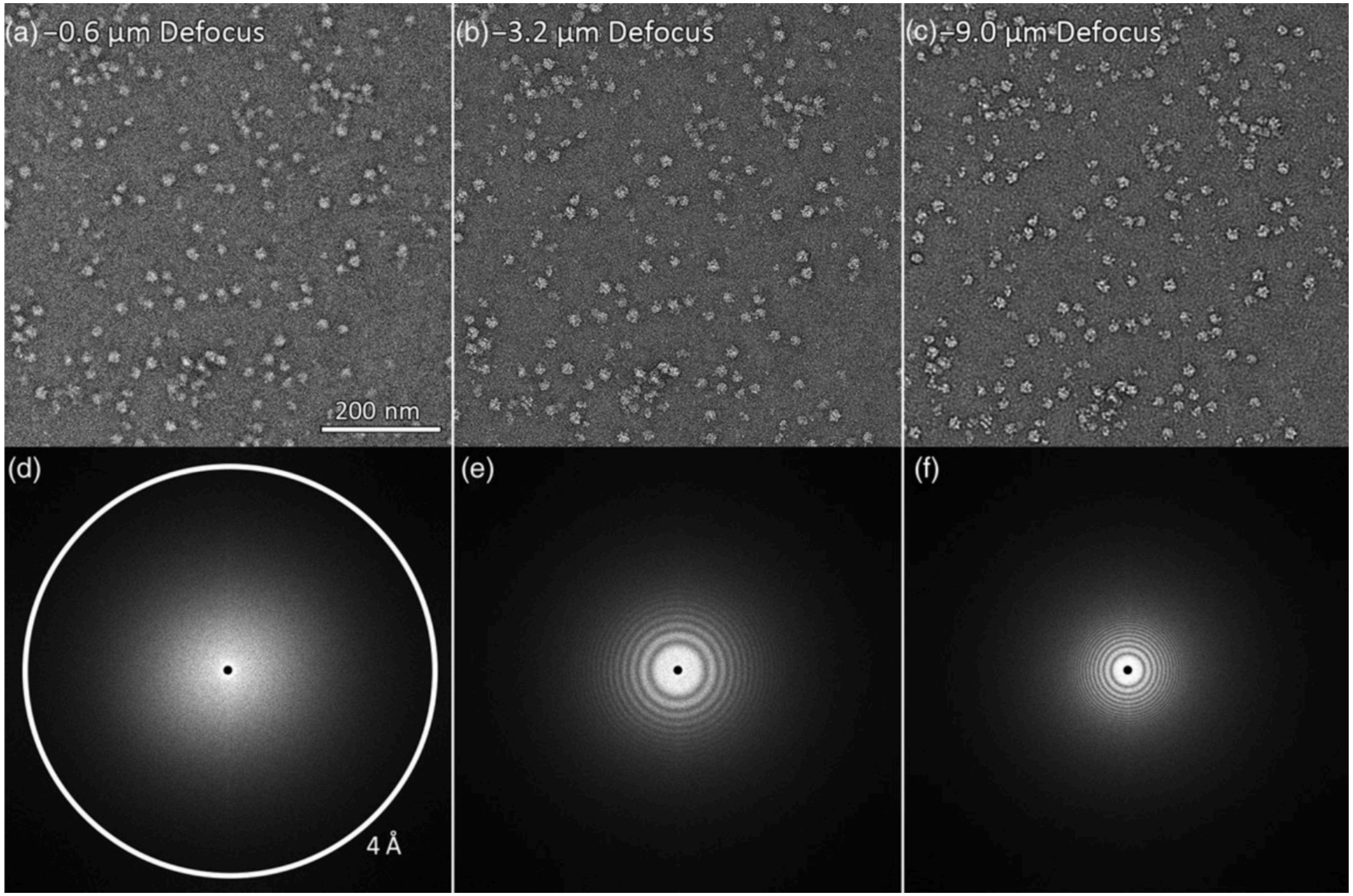
Effect of Focus on CTF

- Images of carbon film and their power spectra (FT), showing Thon rings and CTF curves
- Thon rings of the second image are located closer to the origin and oscillate more rapidly
- The rings alternate between positive and negative contrast, as seen in the plotted curves



Orlova & Saibil et al 2011, Chem Reviews

Effect of Focus on CTF



- Negative stain electron micrographs at different defocus and corresponding FT

- Micrograph 1 is set closest to focus followed by sequentially higher defocus

YZ Tan et al 2015, Journal of EM

Effect of Focus on CTF

Phil. Trans. Roy. Soc. Lond. B. **261**, 105–118 (1971) [105]
Printed in Great Britain

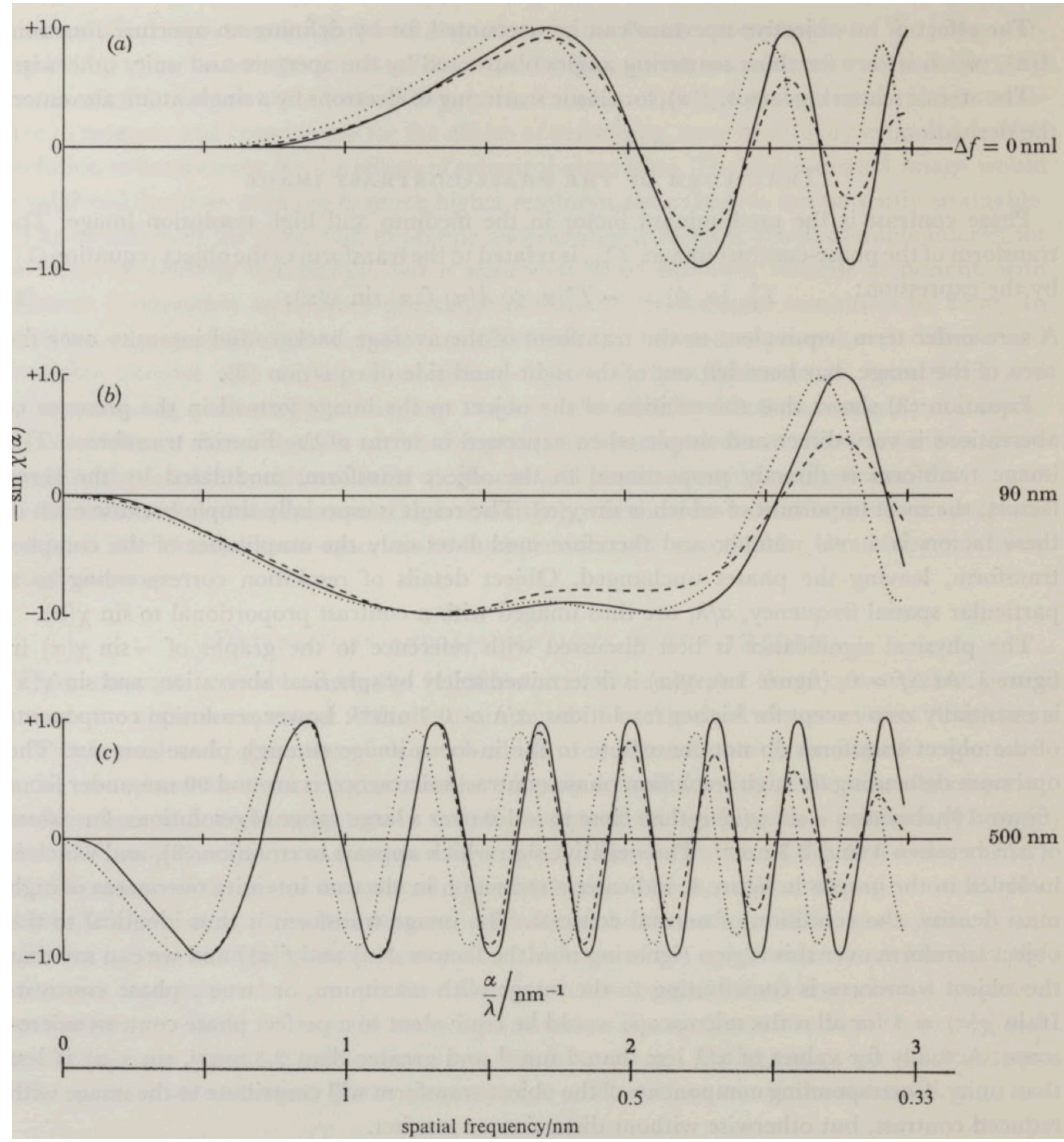
Measurement and compensation of defocusing and aberrations by Fourier processing of electron micrographs

BY H. P. ERICKSON AND A. KLUG, F.R.S.
Medical Research Council Laboratory of Molecular Biology, Cambridge

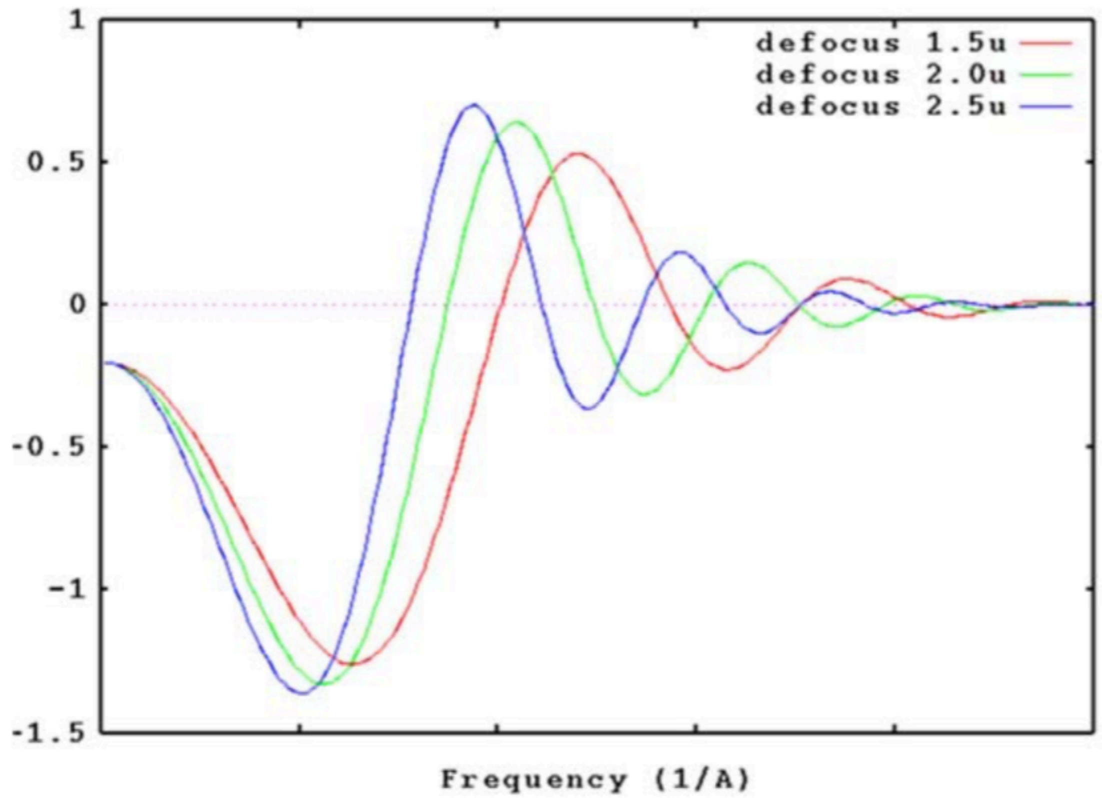
- Underfocusing will be advantageous as there is a contrast increase over the entire transform and a relative enhancement in the high resolution components with respect to the lower
- This is why we use underfocus!

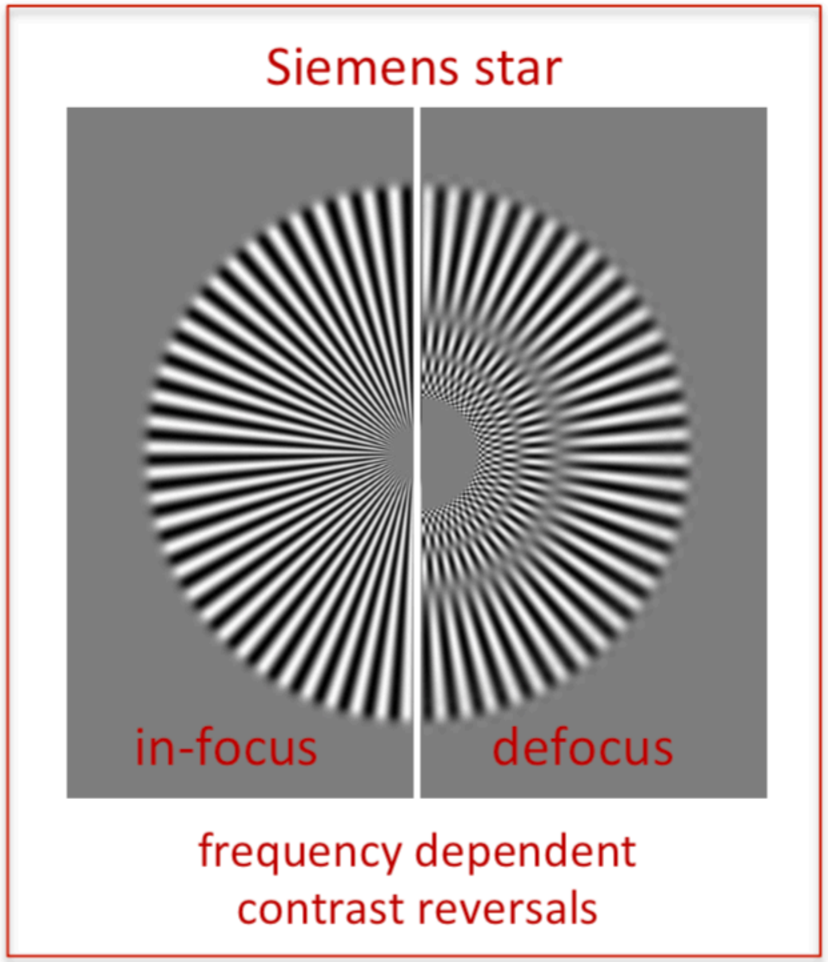
Effect of Focus on CTF

- **A. In focus image**
    - Lower resolution components do not contribute
- **B. 90 nm underfocus**
    - High resolution components begin to contribute
    - No high resolution artifacts
- **C. 500 nm under focus**
    - CTF becomes sharper
    - When one wants to obtain a high contrast image of details in this medium resolution range, and is not concerned about artifacts at higher resolution.



Effect of Focus on CTF





- A data-set should comprise images with an adequate range of defocus levels in order to recover information in the zones of zero crossings of the CTF
- Siemens star is a graphical object with a continuous decrease in spacings
- The pixel resolution of the image corresponds to a recorded micrograph

Orlova & Saibil et al 2011, Chem Reviews

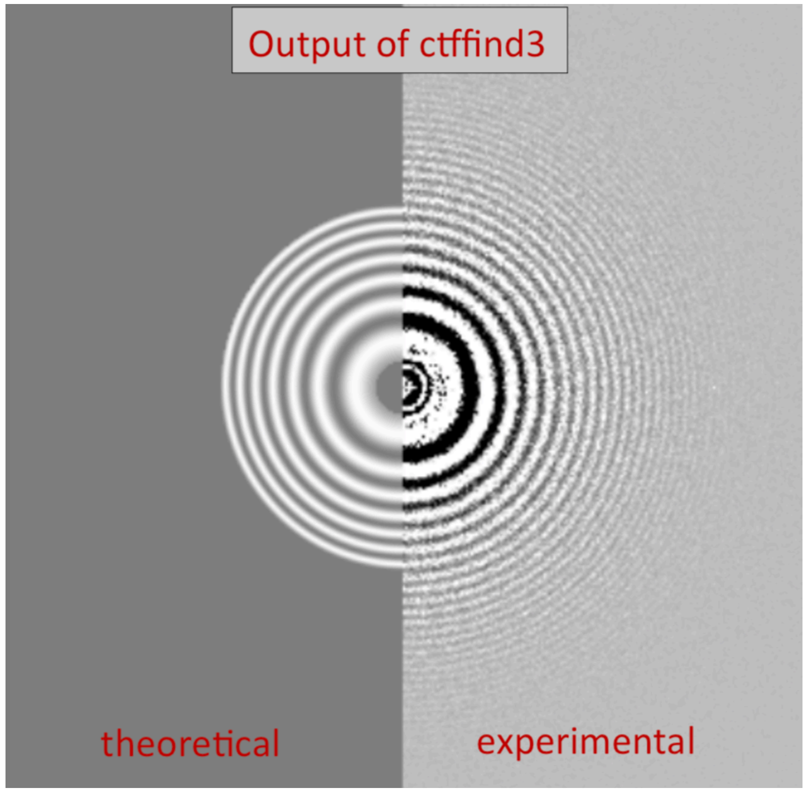
Adapted from Paula da Fonseca

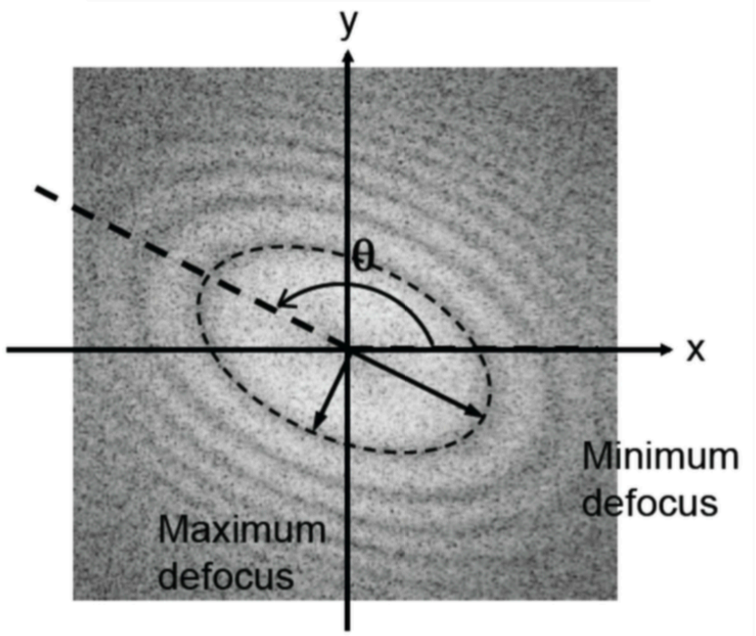
CTF Estimation

- The Defocus is estimated by comparing theoretical CTF curves, at different defocus, with the experimental image power spectra

- The experimental power spectrum must have clearly identifiable Thon rings for accurate defocus determination

- Astigmatism correction requires the determination of the minimum and maximum defocus directions and of the astigmatism angle

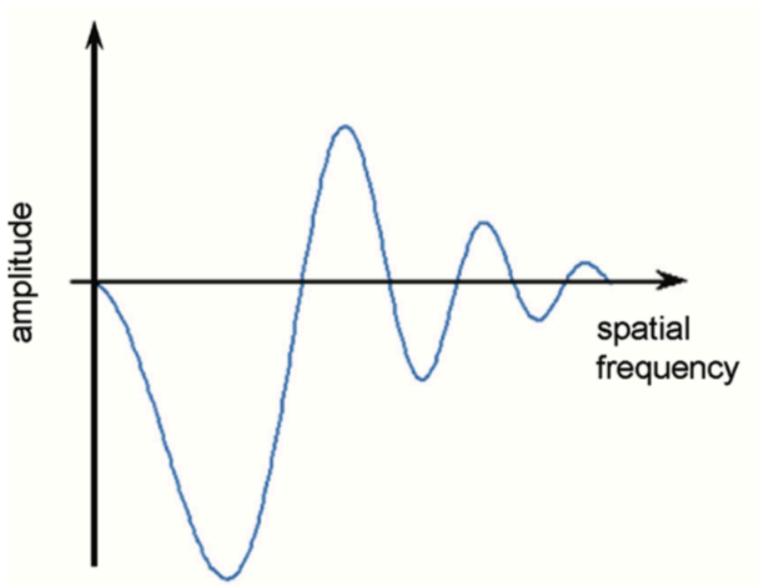




cmfind3: hLp://grigoriefflab.janelia.org/cm

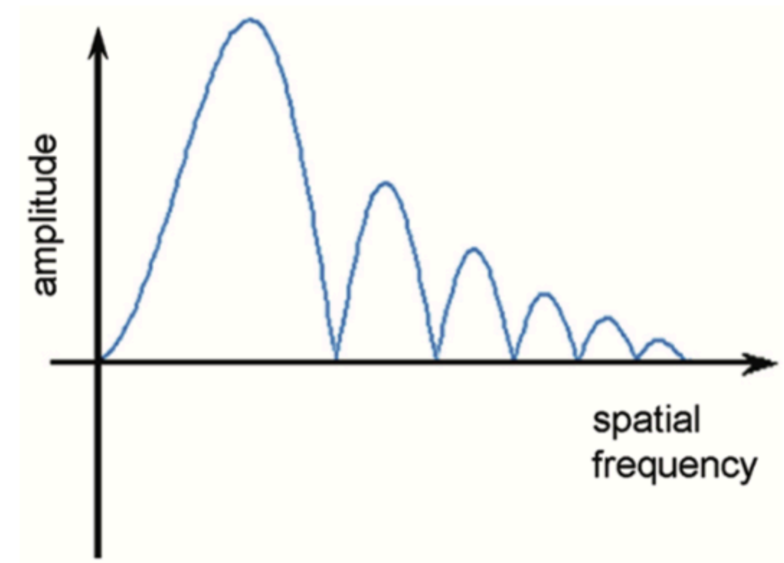
CTF Correction

CTF of uncorrected data



Phase Flipping

CTF of phase corrected data



- After accurate measurement of the defocus levels in the image, the contrast reversals associated with the CTF can be corrected by phase flipping (adding 180^{o} to the phases)
- This will not affect the amplitudes (these are better corrected later in the processing)

Orlova & Saibil et al 2011, Chem Reviews

Thanks for your attention!

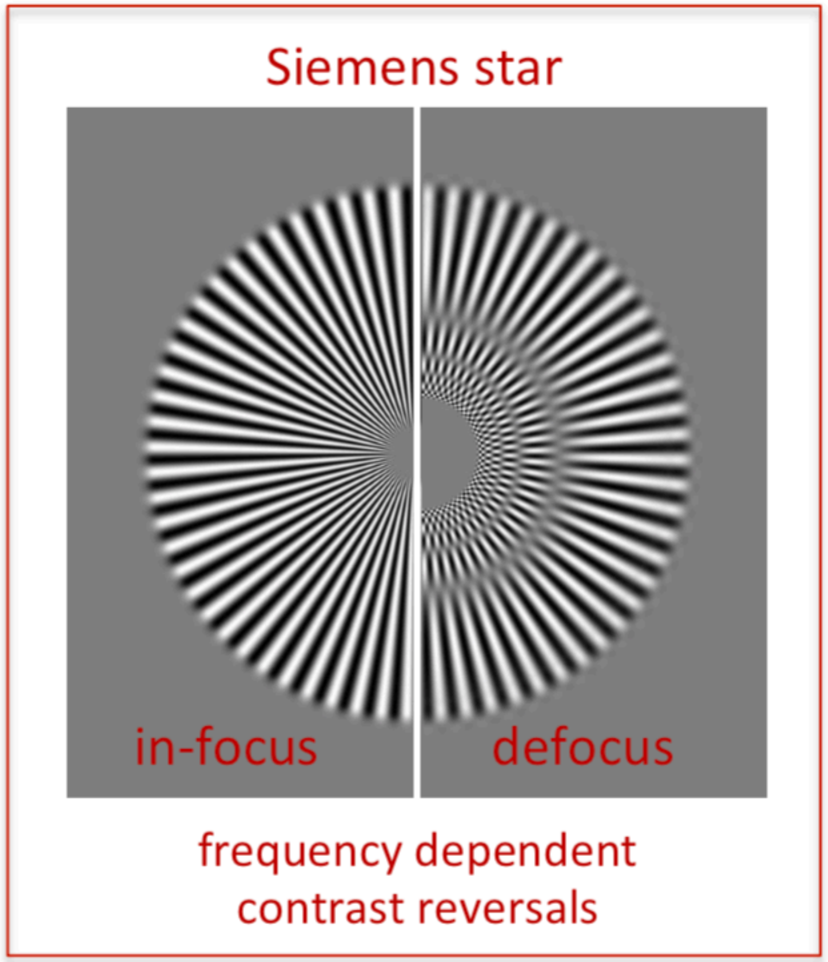
Fourier transform

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

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

- A Fourier series is an expansion of a periodic function f(t), into an infinite series of sine and cosines

CTF Correction

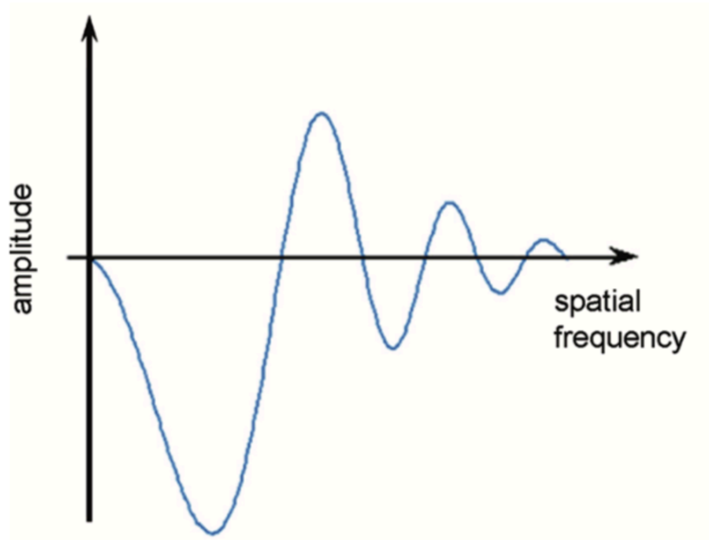


- Siemens star is a graphical object with a continuous decrease in spacings
- The pixel resolution of the image corresponds to a recorded micrograph

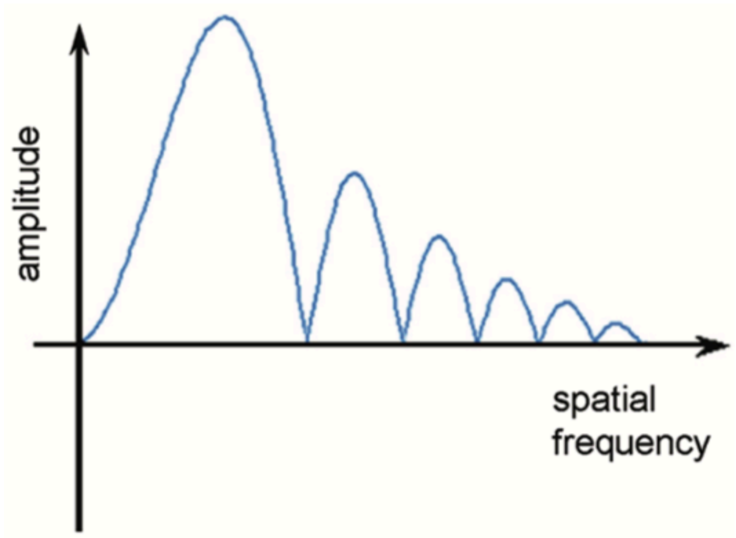
- Higher defocus, particles are more easily seen
- Higher defocus, need larger box size to prevent aliasing
- Defocus values can vary in x and y direction
- Defocus values can also vary in z direction
- Underfocus is used because it gives slightly more contrast
- Range of defocus used during collection to fill in zeroes that occur for any one defocus value

CTF Correction

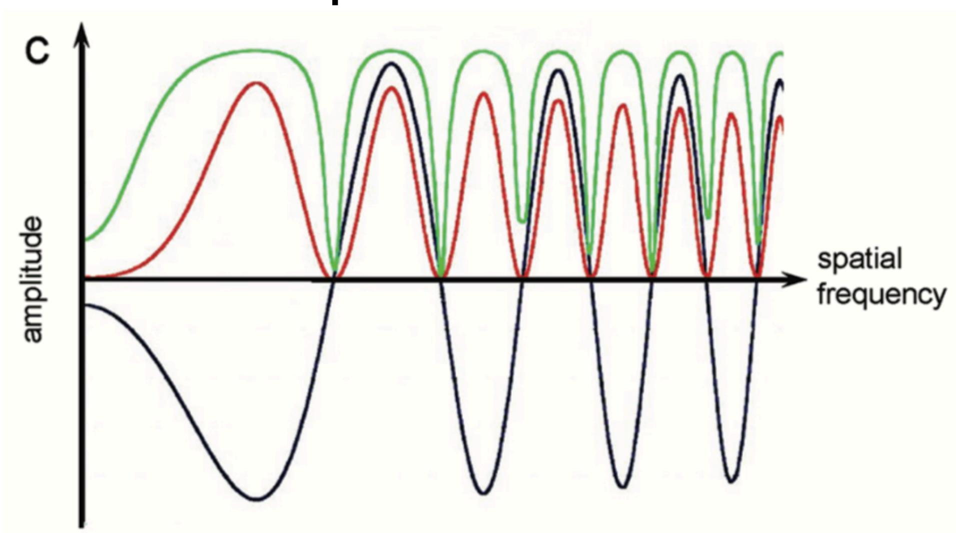
CTF of uncorrected data



CTF of phase corrected data



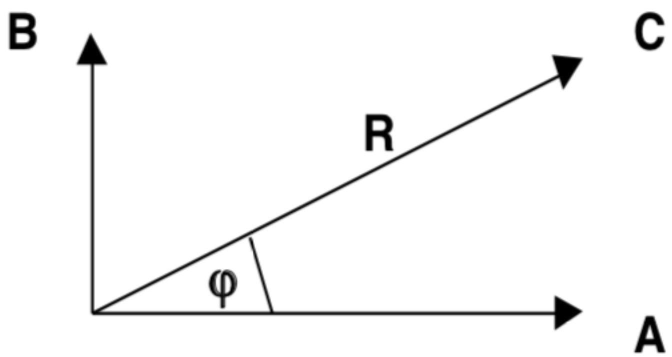
CTF of amplitude corrected data



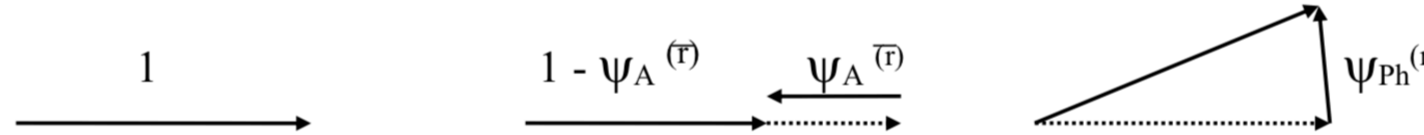
Orlova& Saibil et al 2011, Chem Reviews

- Phase of scattered electron is shifted:
    - By the object - carries structural information
    - By the inner potential of the specimens - 90 degrees for all scattered waves
    - By the spherical aberration of the objective lens - depending on scattering angle = resolution

- Nyquist
- Aliasing
- Projection Theorem



$$e^{i.\varphi} = \mathrm{Cos}\,(\varphi) + i\,.\,\mathrm{Sin}\,(\varphi)$$



$$O(\mathbf{r}) = \psi_A\,(\mathbf{r})\,.\,e^{\{-i\,.\,\psi_{Ph}\,(\mathbf{r})\}}$$

$$I(\mathbf{r}) = O(\mathbf{r})^2 = \psi^2{}_A\,(\mathbf{r})$$