Lecture 4a
Algorithms and Foundational Math, Part 1:
The contrast transfer function and Fourier transforms

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I. Phase contrast and the contrast transfer function

1. Complex numbers: review

2. Defocus contrast and the CTF (simple version)

3. Image delocalization

4. Objective lens effects on the CTF
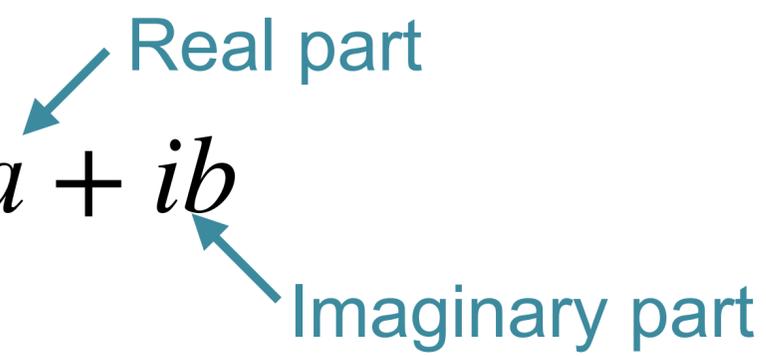
Why complex numbers?

- They make the equations simpler
- Natural for Fourier transforms
- Give us the magnitude and phase of structure factors

The unit imaginary number

$$i = \sqrt{-1}$$

A complex number

Real part

$$z = a + ib$$

Imaginary part

You can do arithmetic with complex numbers

$$z = a + ib$$
$$w = c + id$$

Add $\quad z + w = (a + c) + i(b + d)$

Multiply $\quad zw = (ab - bd) + i(ad + bc)$

Real part $\quad \mathrm{Re}(z) = a$

Imaginary part $\quad \mathrm{Im}(z) = b$

Absolute value $\quad |z| = \sqrt{a^2 + b^2}$

Conjugate $\quad z^* = a - ib$

The exponential function e^x

$$e = 2.718...$$

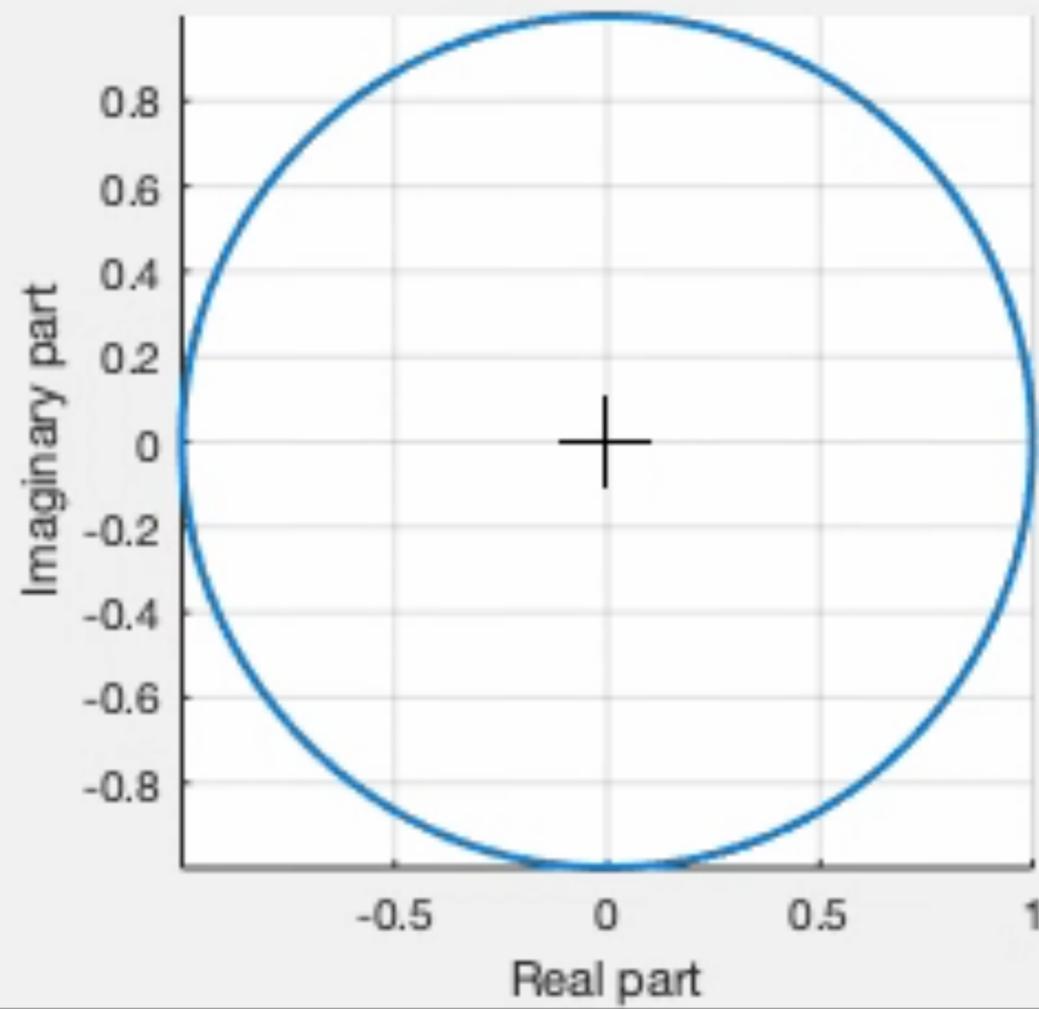$$e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{2 \times 3} + \ldots$$

A very important approximation

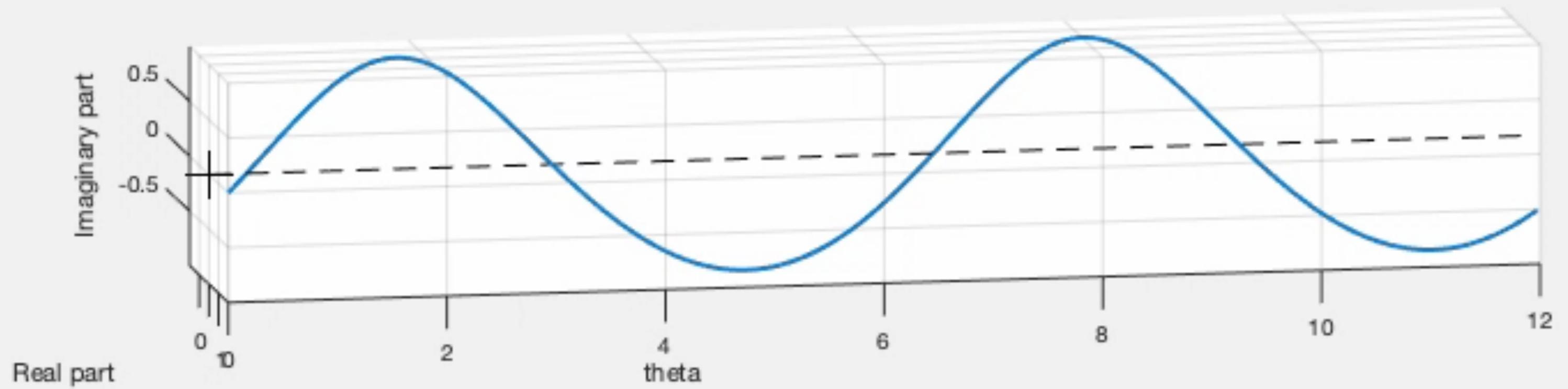$$e^x \approx 1 + x, \quad x \ll 1$$

The complex exponential

$$e^{i\theta} = \cos\theta + i\sin\theta$$
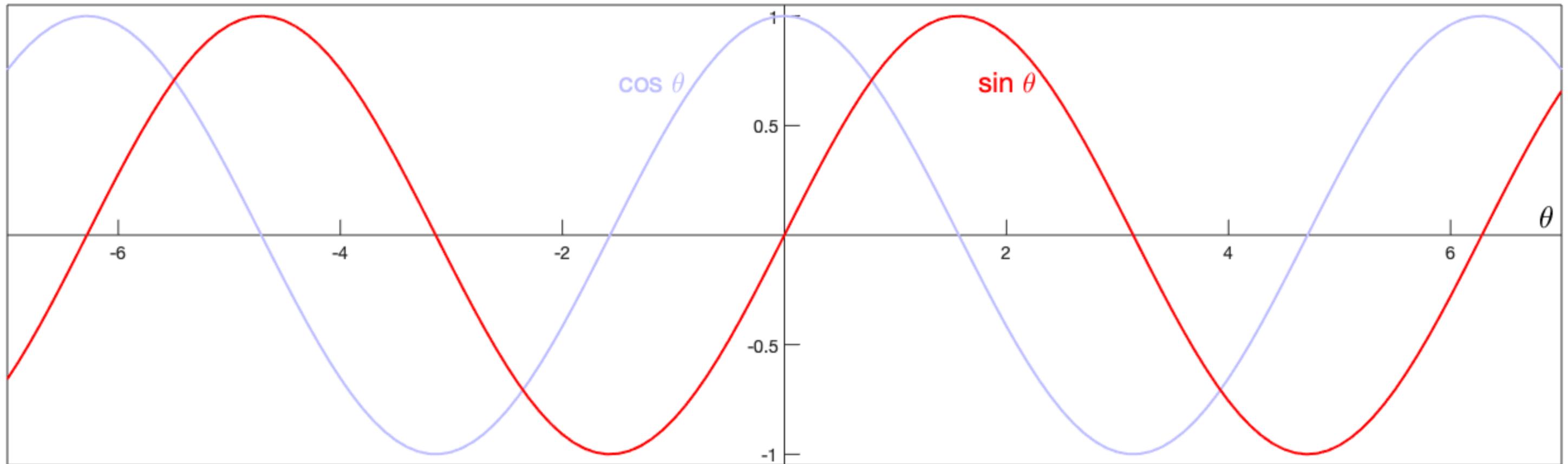
A plot of $e^{i\theta}$

A plot of $e^{i\theta}$
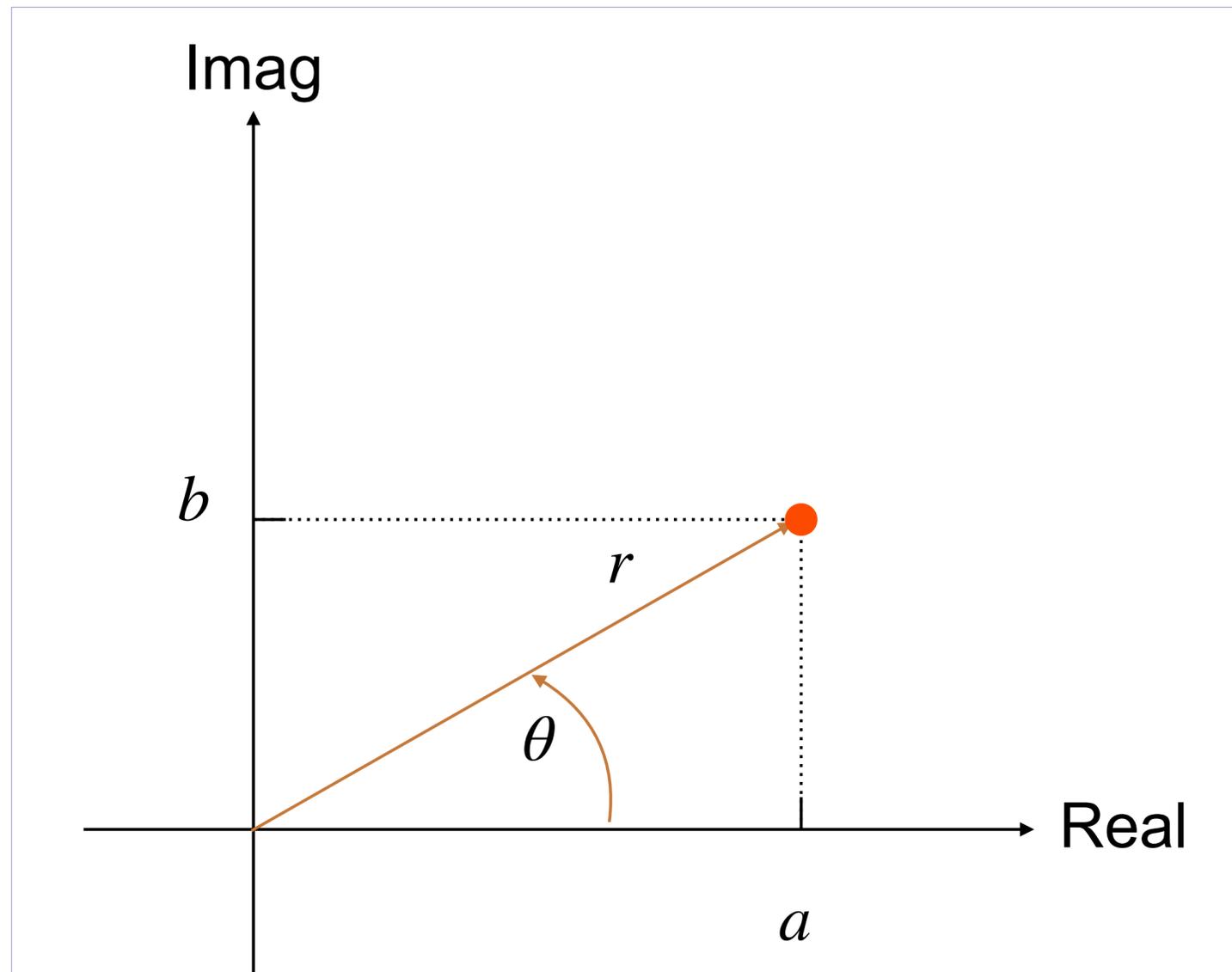
Any z can be represented as (a, b) or as (r, θ)

$$z = a + ib$$

$$z = re^{i\theta}$$

a is the real part

b is the imaginary part

r is the magnitude

θ is the phase

Recall that

$$e^x e^y = e^{x+y}$$

so, when you multiply two complex numbers, the phases add:
$$e^{i\theta_1} e^{i\theta_2} = e^{i(\theta_1 + \theta_2)}.$$

Phase contrast and the contrast transfer function

Cryo-EM specimens are imaged by phase contrast

Electrostatic potential ϕ

Elastically-scattered electron

unscattered electron
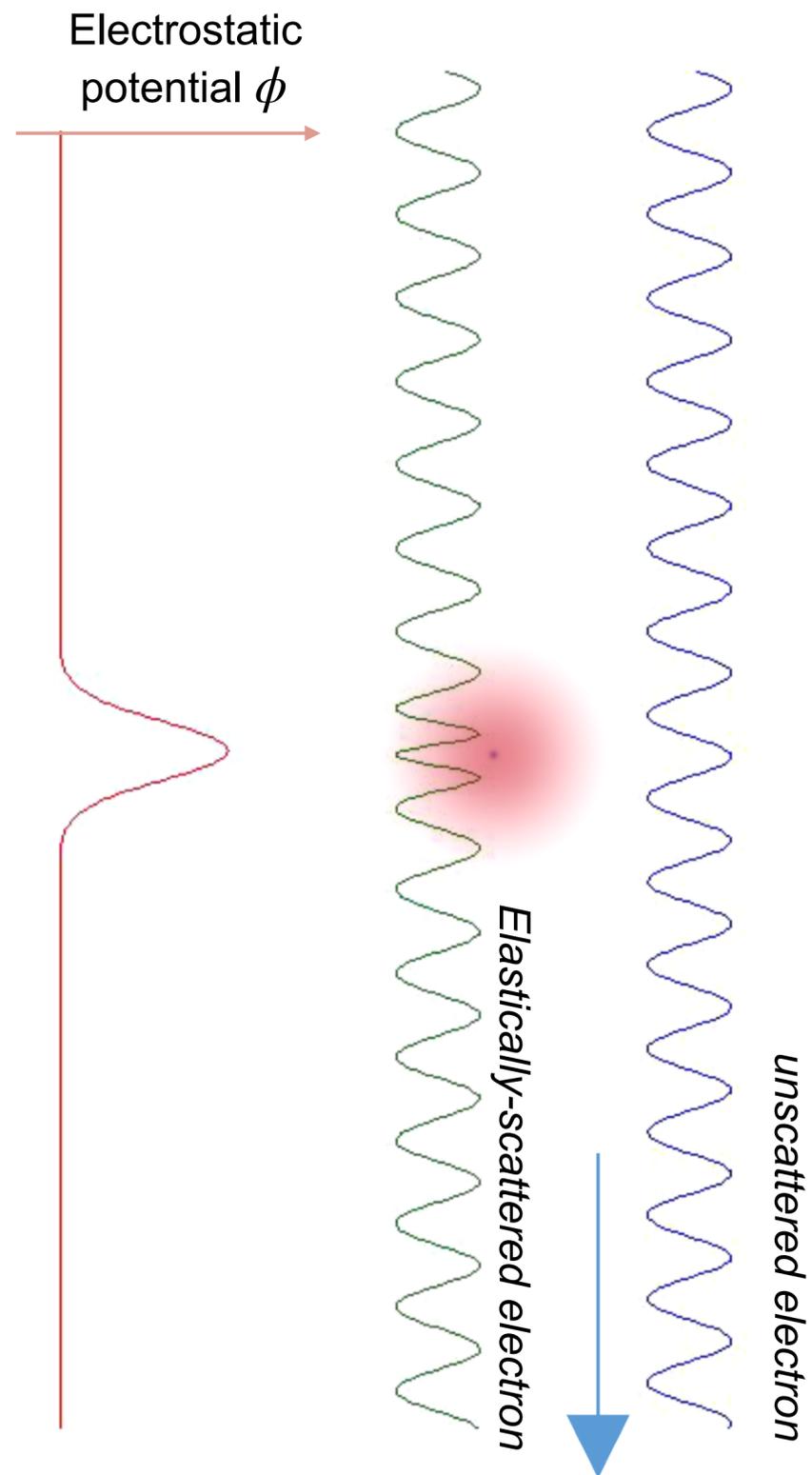
The imaging electrons are phase-shifted when passing near atomic nuclei or fixed charges.

The phase shift coefficient σ is about 0.5 milliradian per volt-angstrom of integrated potential.
The phase shift near a single atom is ~1 milliradian.
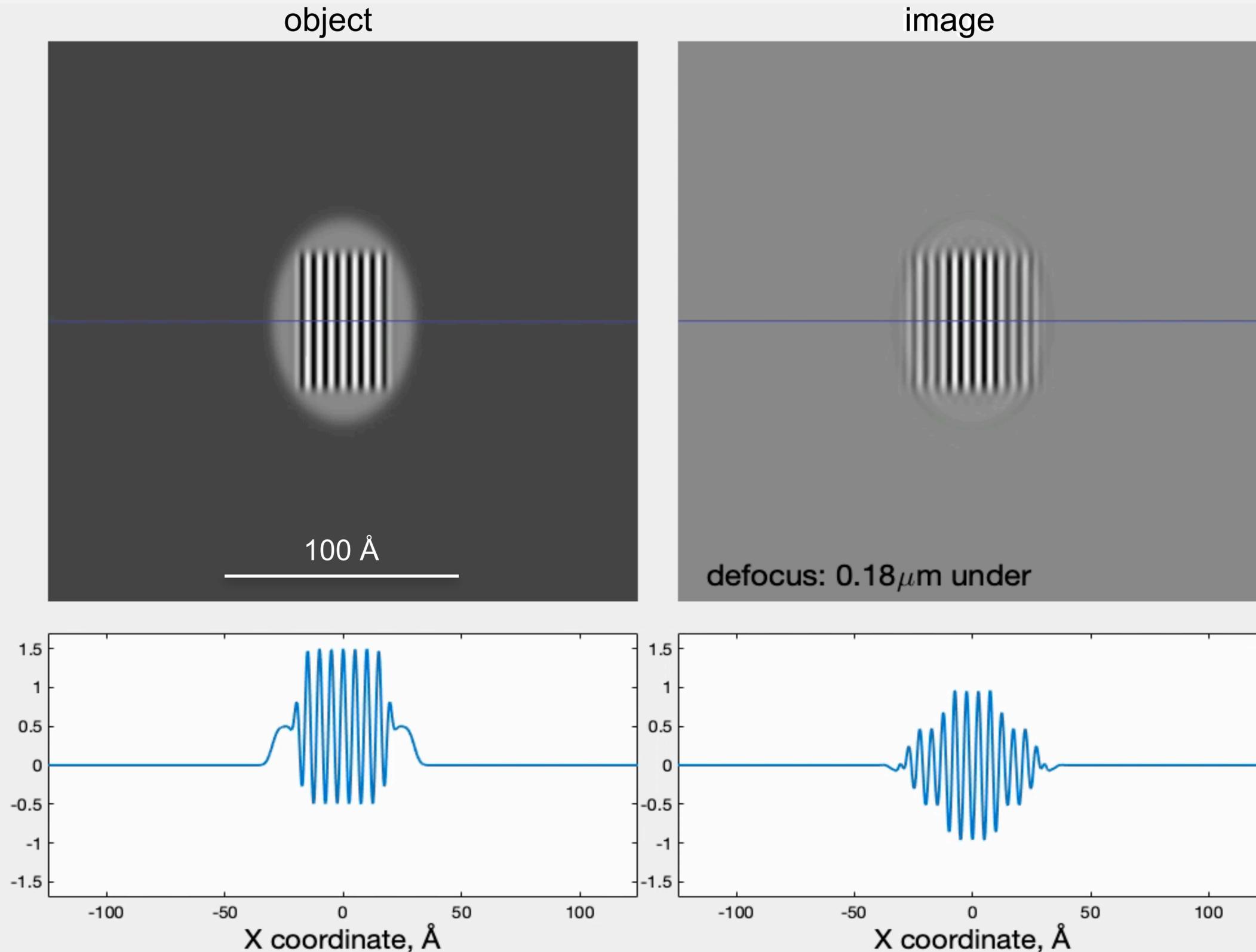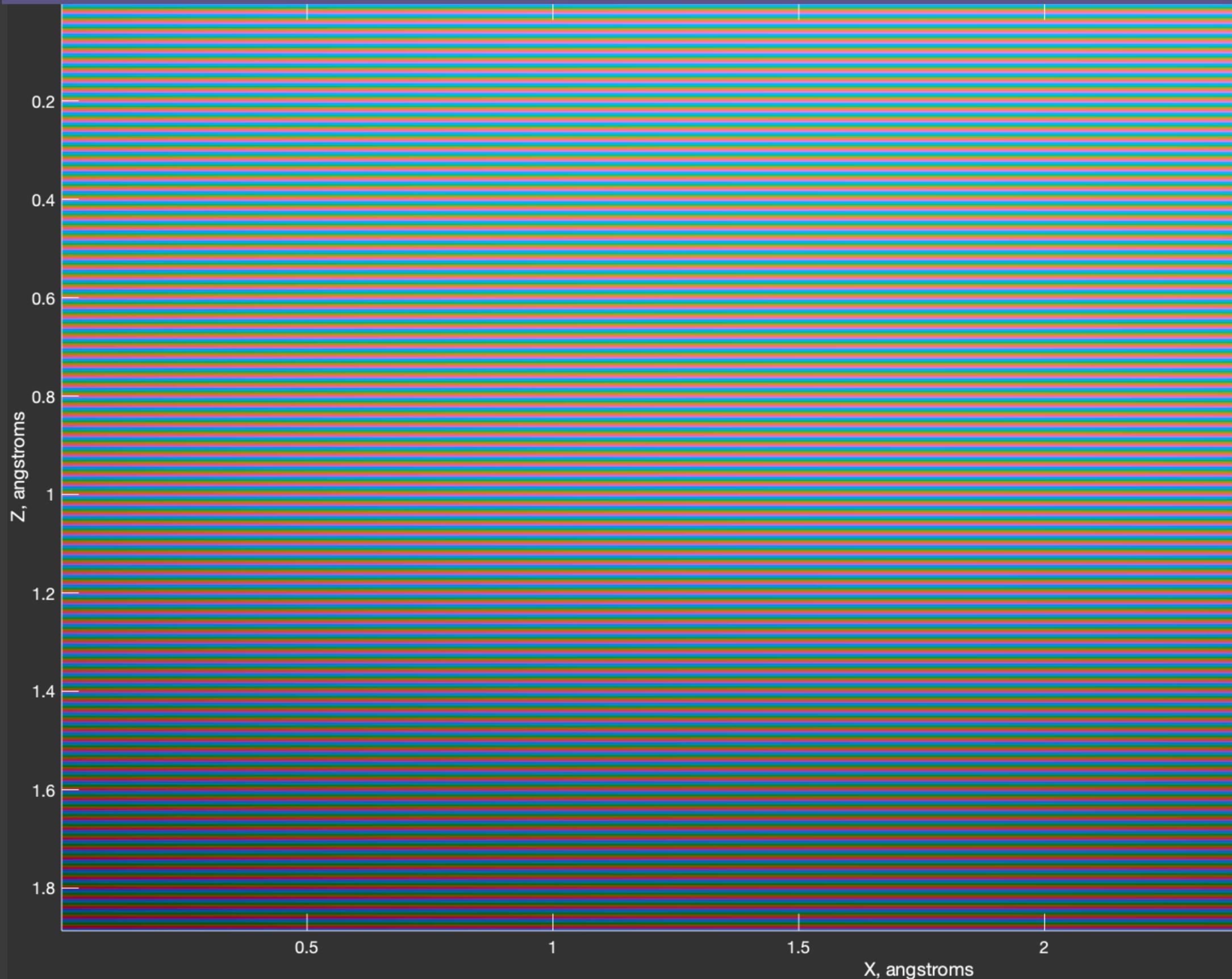
object

image

100 Å

defocus: 0.000μm

- At high defocus, high-resolution information in the image is strongly delocalized.

- Image processing can re-localize the signals, but at most only about half of the theoretical contrast is preserved by defocusing.

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- At high defocus, high-resolution information in the image is strongly delocalized.

- Image processing can re-localize the signals, but at most only about half of the theoretical contrast is preserved by defocusing.

1. The contrast in the image of a grating object varies with the amount of defocus.

2. The grating object produces diffracted waves with shifting phase.

3. When the diffracted waves interfere with the undiffracted waves, we have contrast.

A snapshot of an electron wave

Energy (keV)	Wavelength (Å)	Velocity (fraction of c)
120	0.033	0.59
200	0.025	0.70
300	0.020	0.78

For an electron propagating in the z direction, the time-independent wave function is

$$\Psi_0 = e^{ikz}$$

with

$$k = 2\pi/\lambda$$

The object is a grating,
$\epsilon\phi(x) = \epsilon\cos(2\pi x/d)$.

Example:

$d = 5\text{Å}$ and $\epsilon \ll 1$.

At $z = 0$,
$\Psi = e^{i\epsilon\phi(x)}$

The weak-phase approximation

- Just below the specimen, at $z = 0$, the electron wave function is $\Psi = e^{i\epsilon\phi(x)}$.

- Then, by the approximation $e^x \approx 1 + x$ we have just after the specimen

$$\boxed{\Psi \approx 1 + i\epsilon\phi(x)}$$

This is the weak phase approximation.

What are the two terms in the approximation?

- There is an undiffracted wave —essentially the same as the incident wave—of amplitude 1. We'll call this Ψ_0

- And there is a new wave combination of amplitude ϵ. In this example of a grating there are actually two diffracted waves, Ψ_+ and Ψ_-

- The full wavefunction is

$$\Psi = \Psi_0 + \Psi_+ + \Psi_-$$

Intensity at z

The grating $\phi(x)$

abs$(\Psi)^2$

Displacement z, nm

x, angstroms

$-$ Contrast $+$

Interference between the undiffracted wave and diffracted waves produces contrast.
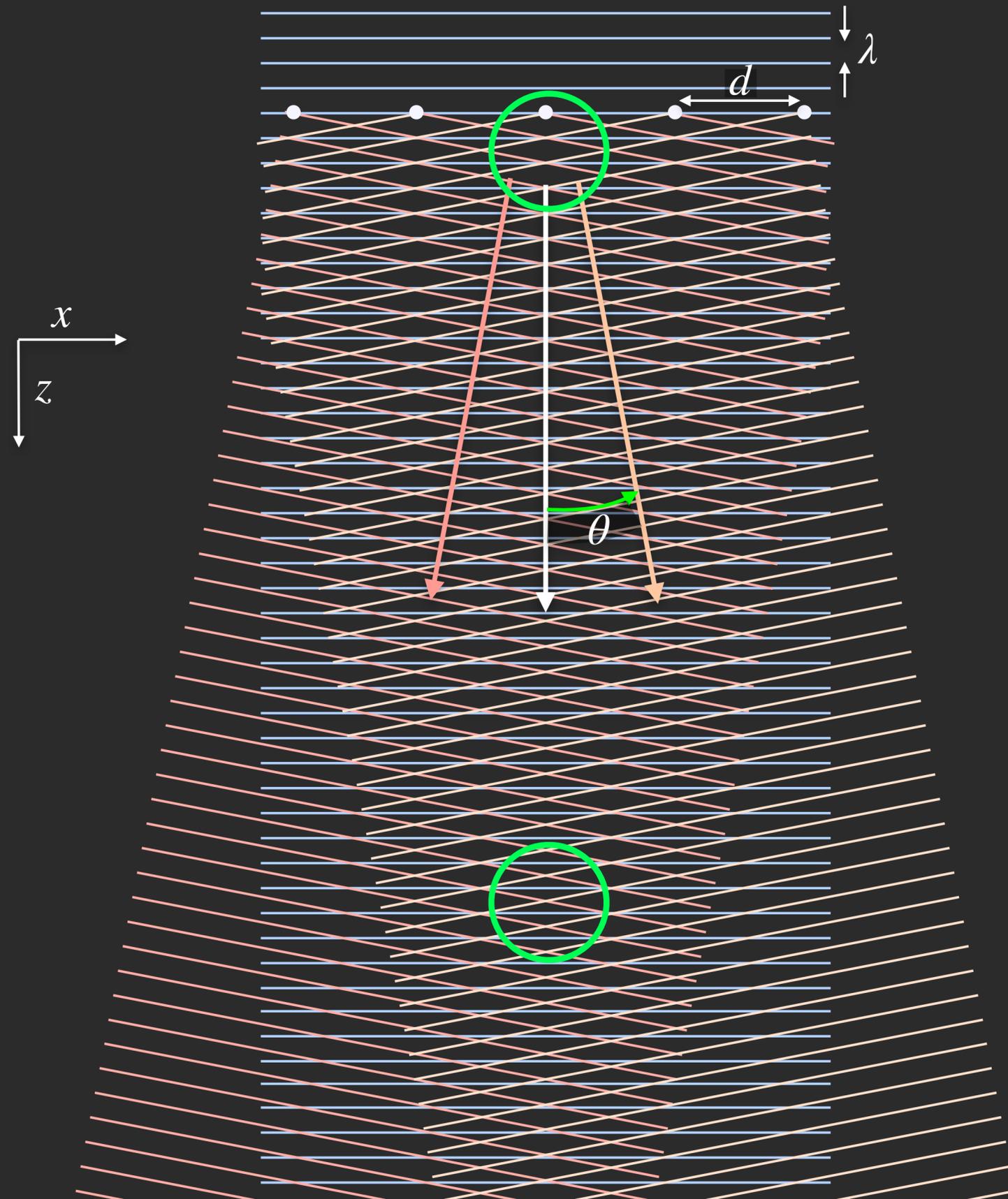
$$\sin\theta = \frac{\lambda}{d}$$
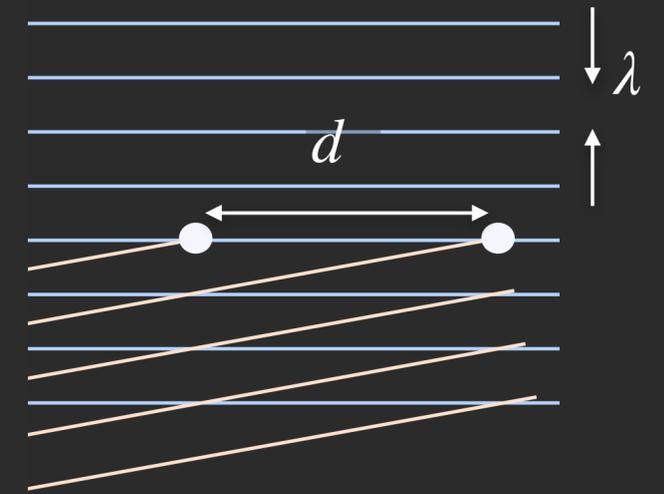
Note there's a tiny shift of wavefronts, because the diffracted waves follow slightly longer paths.

Ψ

Displacement z, nm

0

50

100

150

250

-100   -50   0   50   100

x, angstroms

$z = 0$

Ψ_+ Ψ_0

Ψ_-

θ

$\sin(\theta) = \lambda/d$

$z = 100\,\text{nm}$
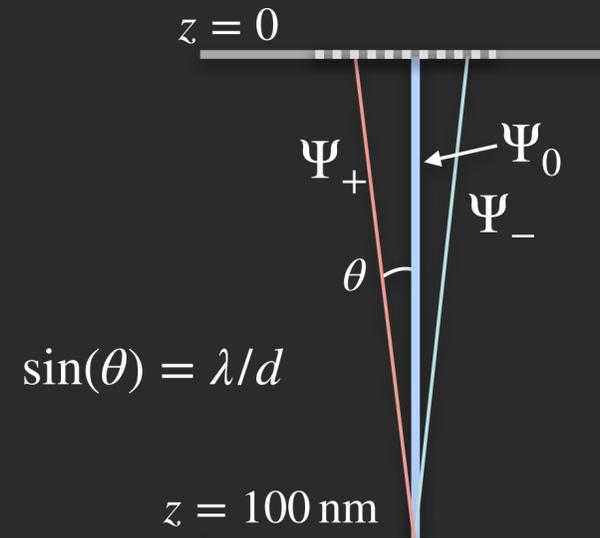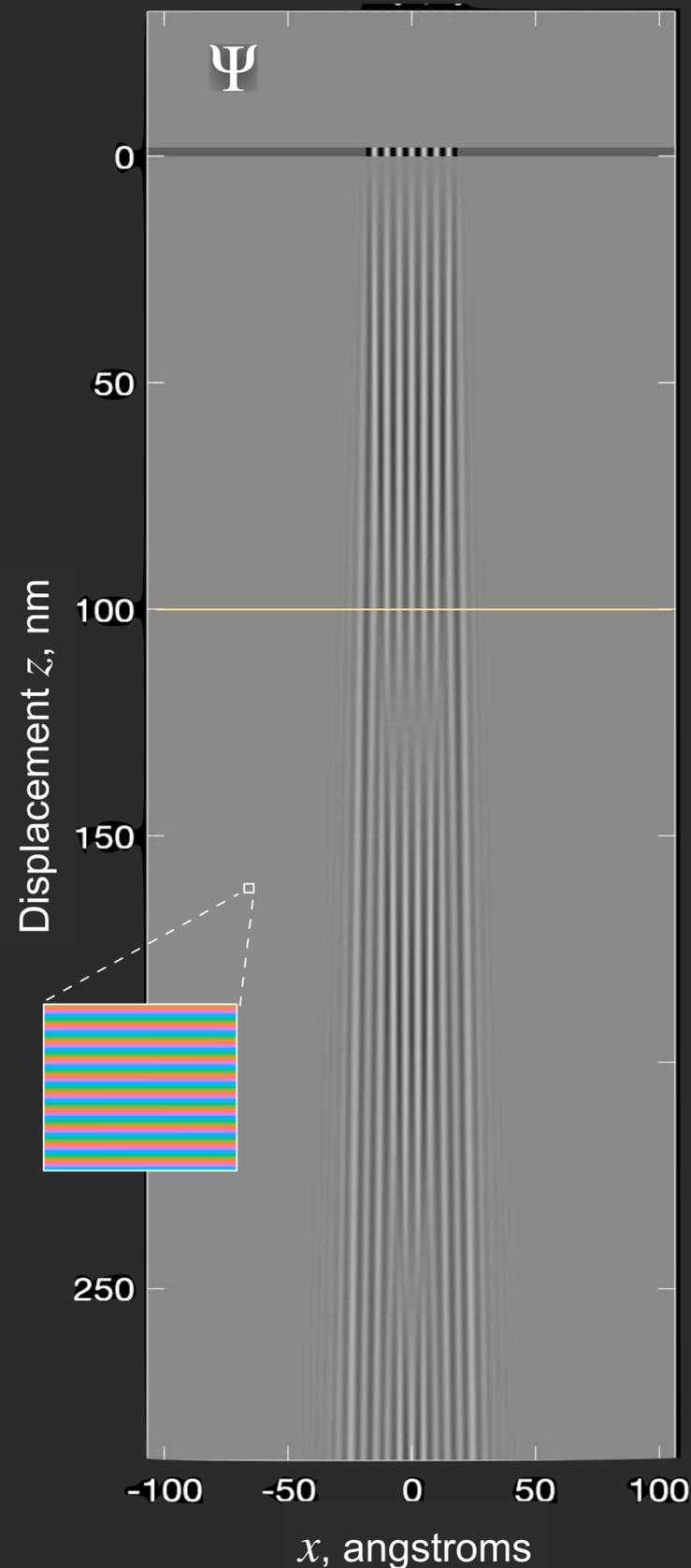
- The two diffracted waves Ψ_+ and Ψ_- travel at very small angles $+\theta$ and $-\theta$ to the undiffracted wave.

- To reach a distance z below the specimen, they take a path longer than Ψ_0 does. Let ζ = the path length difference.

$$\zeta = \frac{z}{\cos\theta} - z \approx z\lambda^2/2d^2.$$

- In our example $\lambda = .02\text{Å}$ and the grating $d = 5\text{Å}$. At the level $z = 100\,\text{nm}$, $\zeta = .008\text{Å}$, about half a wavelength.

- Define χ = the phase difference between the undiffracted and diffracted waves.

$$\chi = 2\pi\zeta/\lambda$$
$$= \pi\lambda z/d^2$$

- In this example $\chi = 0.8\pi$
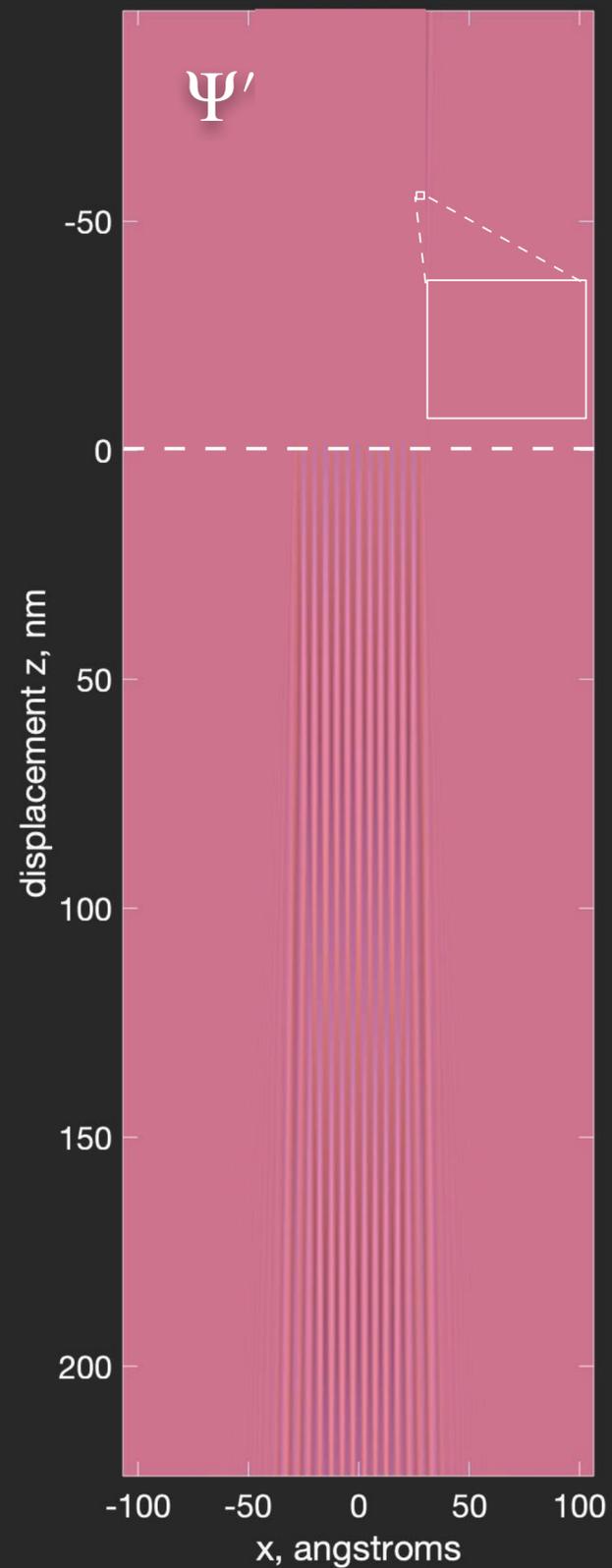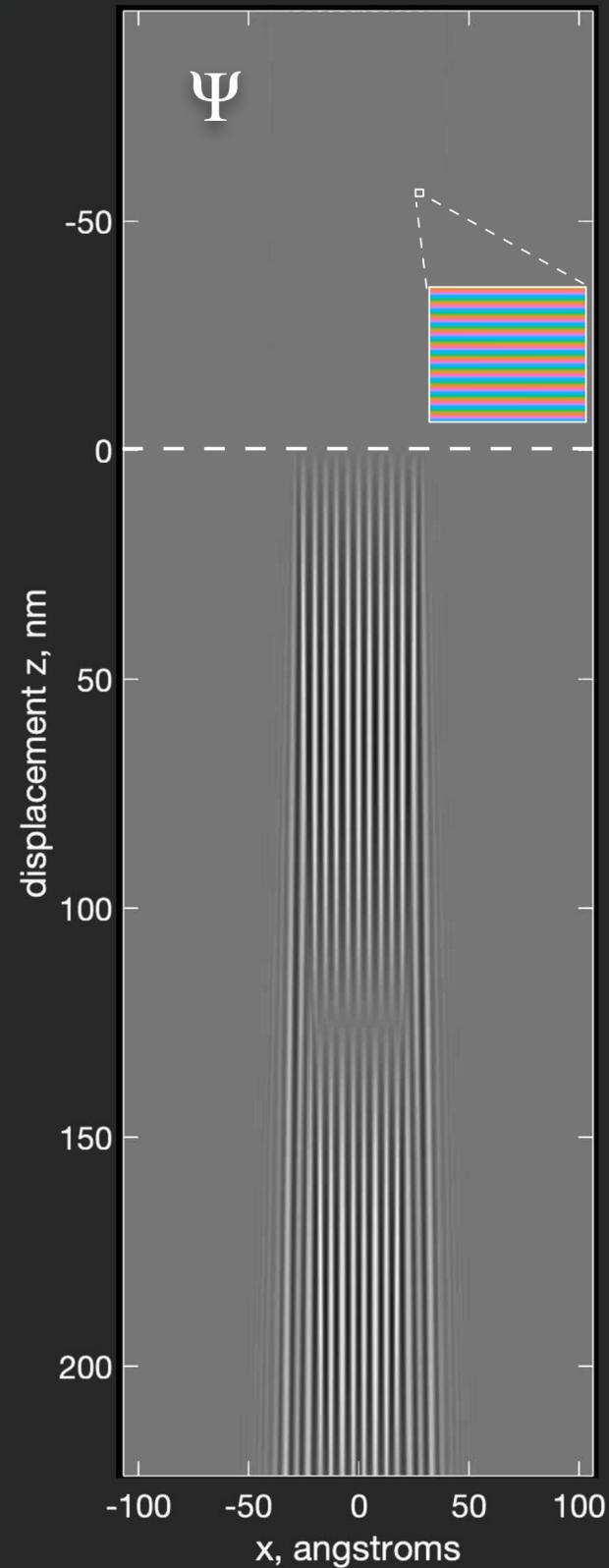
Let's unwrap the oscillations in Ψ:

We'll define $\Psi' = \Psi/\Psi_0$

Complex number color scheme
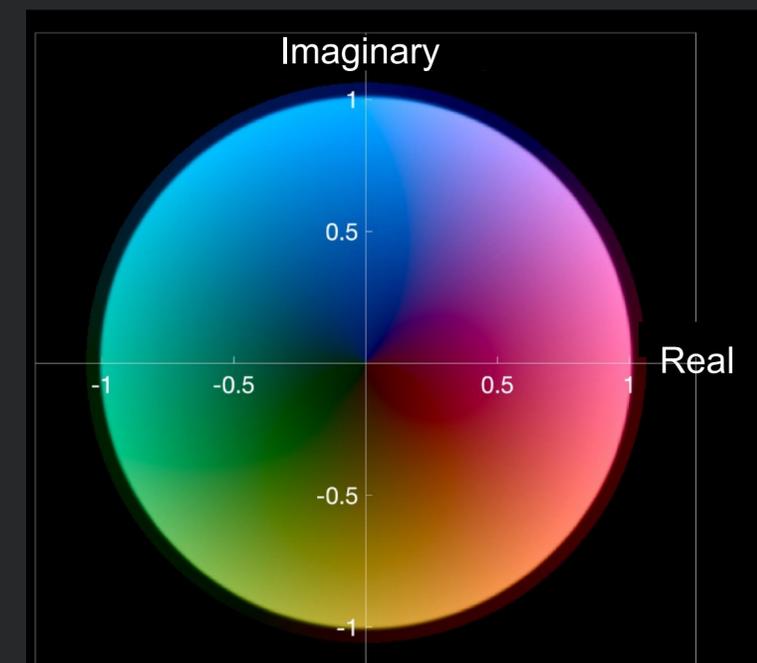
Ψ

$\Psi' - 1$

displacement z, nm

displacement z, nm

x, angstroms

x, angstroms
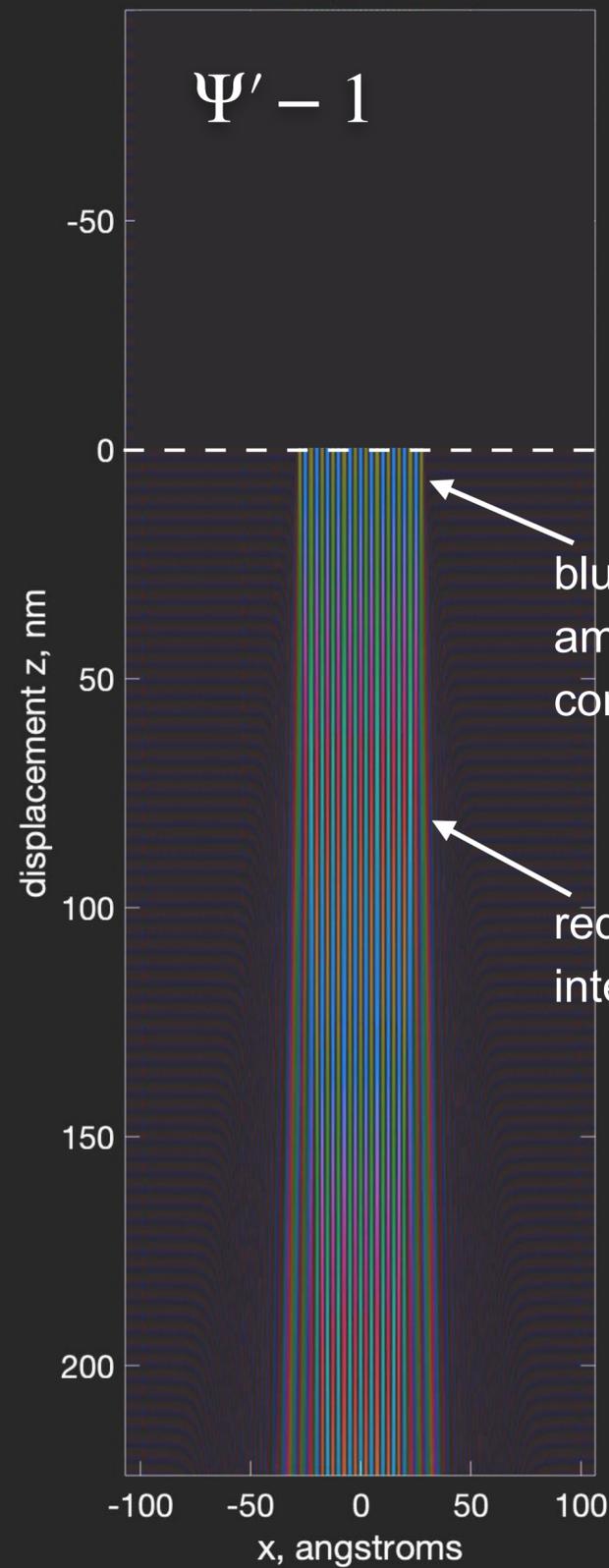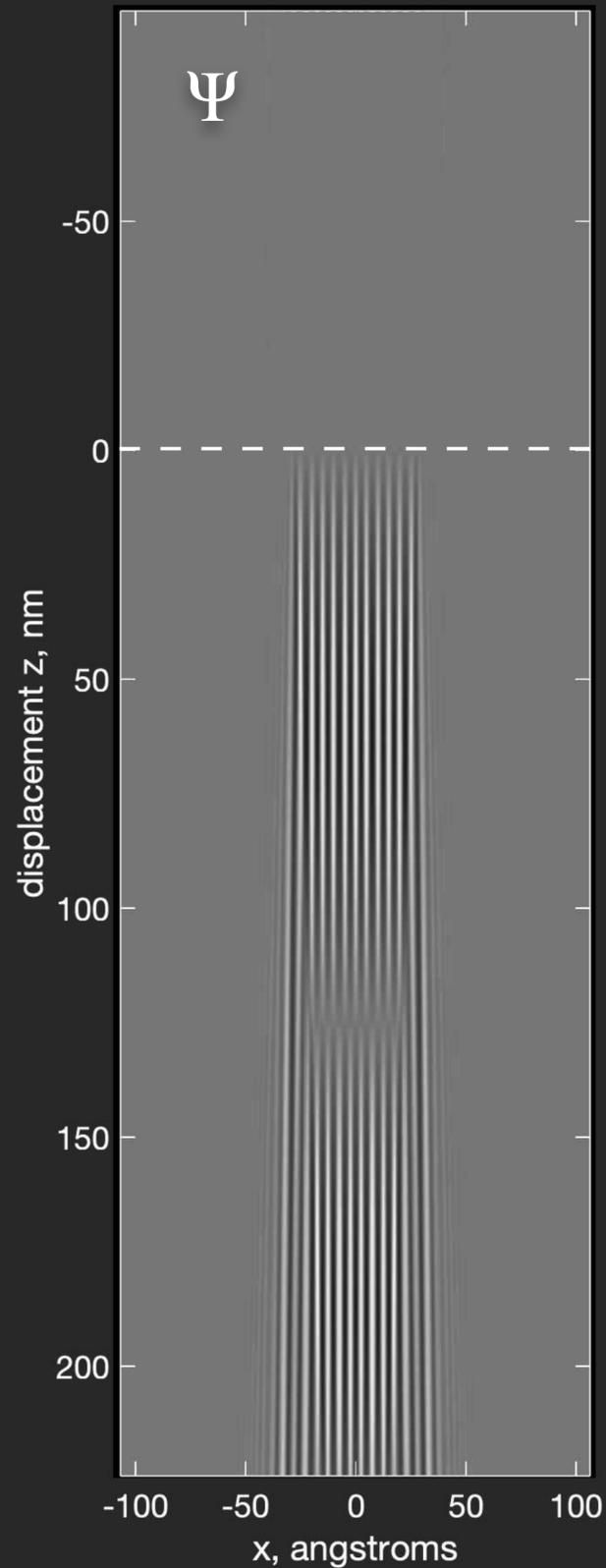
Let's unwrap the oscillations in Ψ:

We'll define $\Psi' = \Psi/\Psi_0$

Next, let's remove the undiffracted wave, so we have just the diffracted waves,

$$(\Psi' - 1) = \Psi'_+ + \Psi'_-$$

blue / yellow: imaginary amplitude, no interference, no contrast

red / green: real amplitude; interference

Complex number color scheme

Imaginary

Real
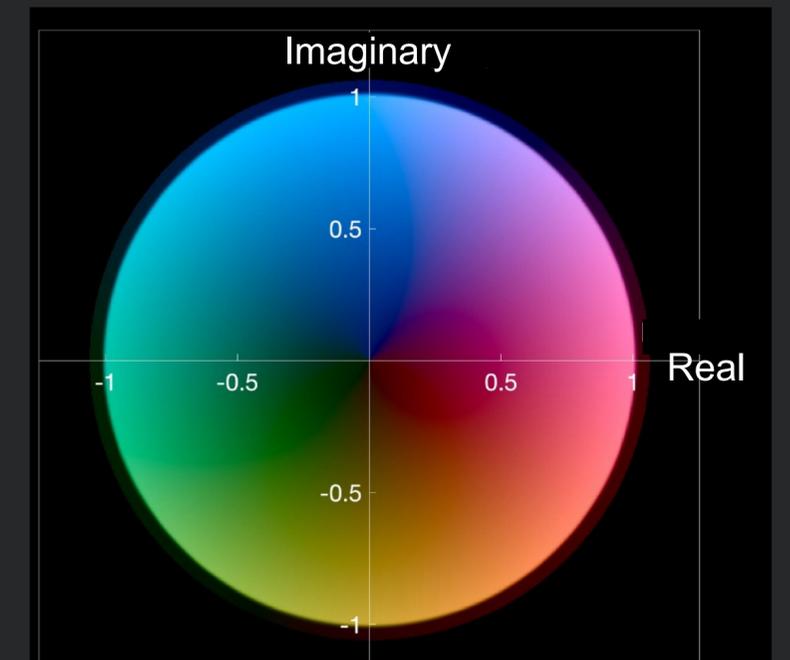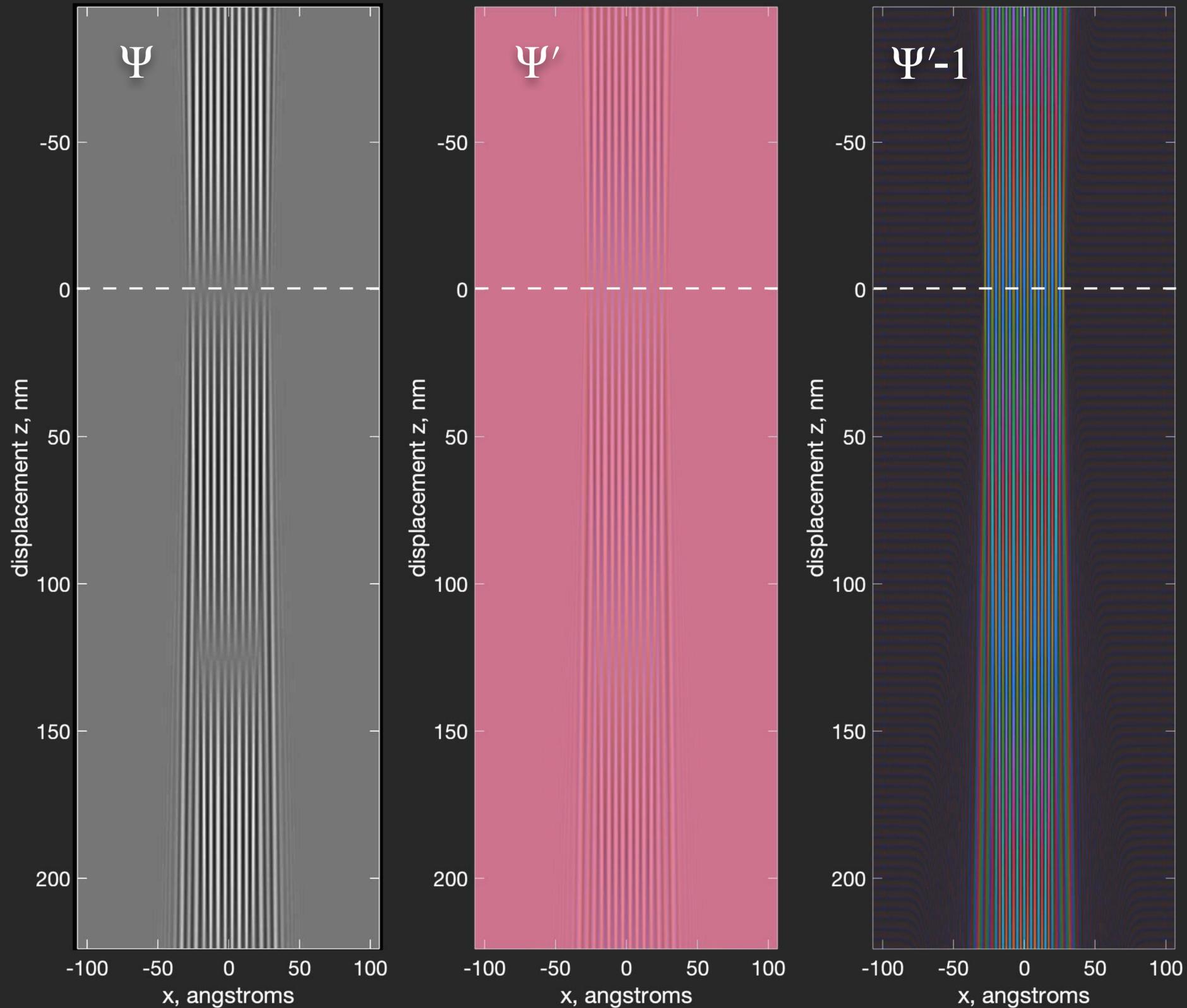
What wavefunction above the specimen would give rise to what we see below it?

We can back-propagate Ψ: this is what the objective lens "sees"

Intensity at z

The grating $\phi(x)$

abs(Ψ)^2

Displacement z, nm

x, angstroms

− Contrast +

dark (neg) | bright (pos)
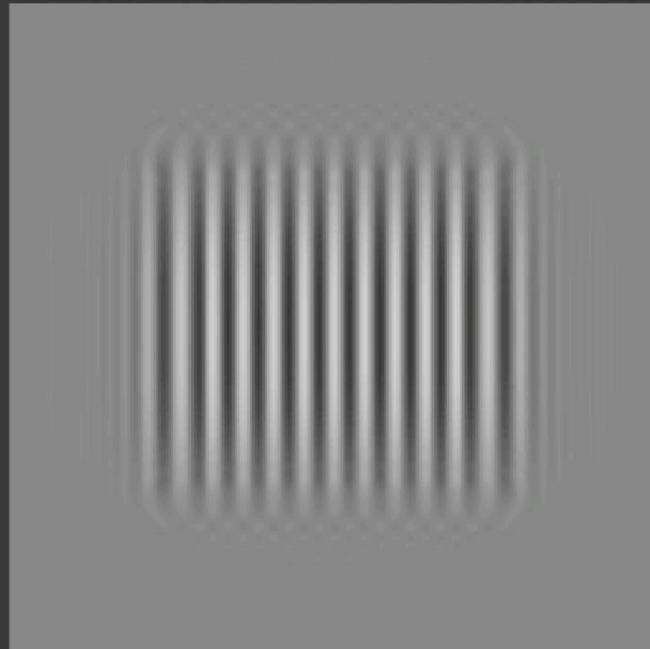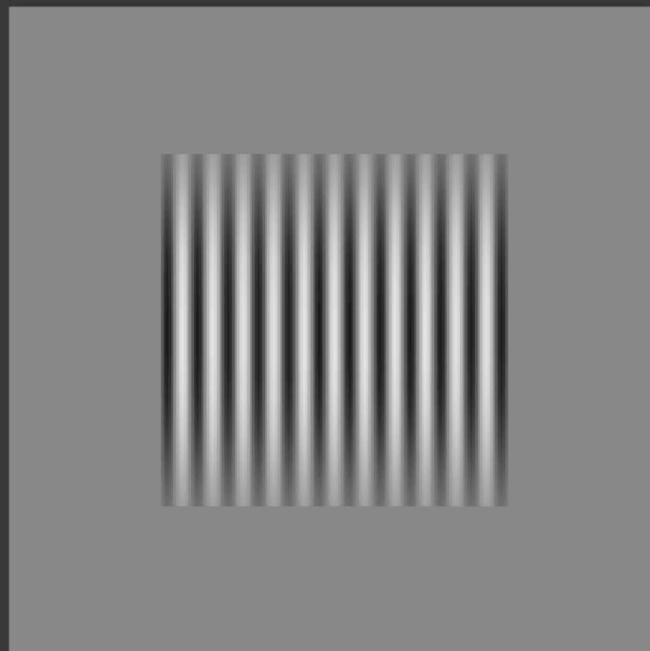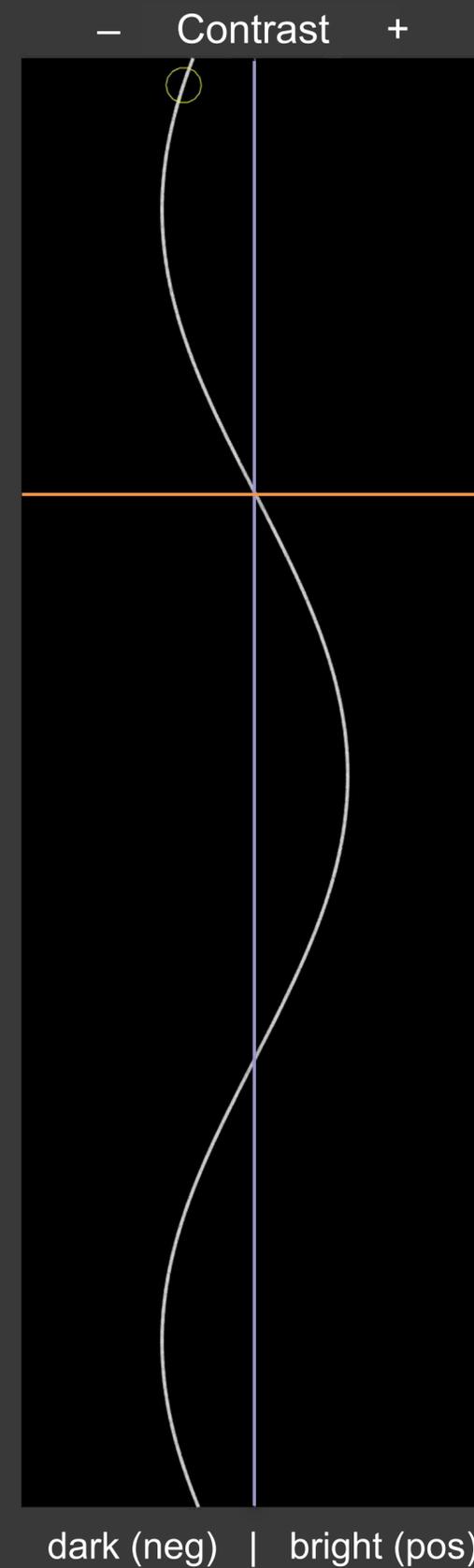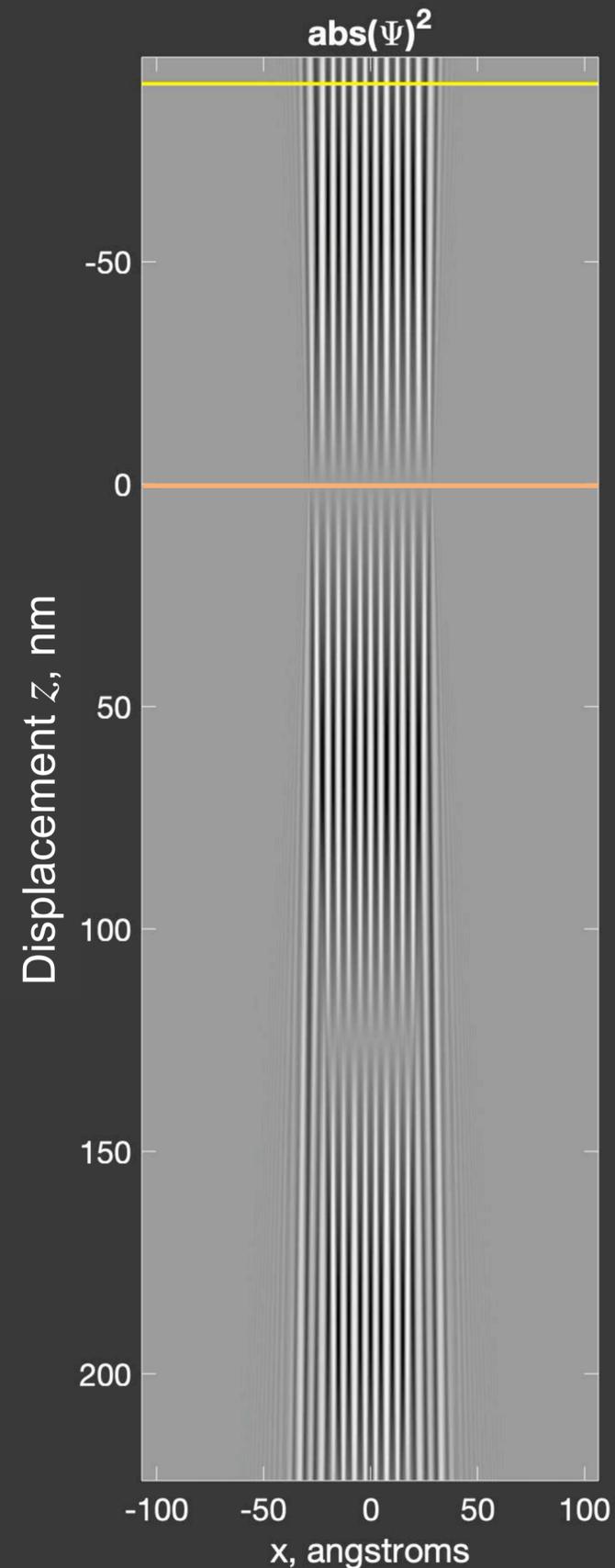
Terminology

• "Underfocus" is focusing the objective lens above the specimen.

• By convention, defocus values δ are positive for underfocus:

$$\delta = -z$$

• Spatial frequency is $s = 1/d$

• The phase shift χ is proportional to δ.

• The contrast transfer function is given by

$$\mathrm{CTF} = \sin(\chi)$$
$$= \sin(-\pi\lambda\delta\, s^2)$$

Defocus 0.25 μm

CTF = sin(χ)

$$\text{CTF} = \sin(\chi)$$
$$= \sin(-\pi\lambda\delta s^2)$$

Ψ

Ψ'
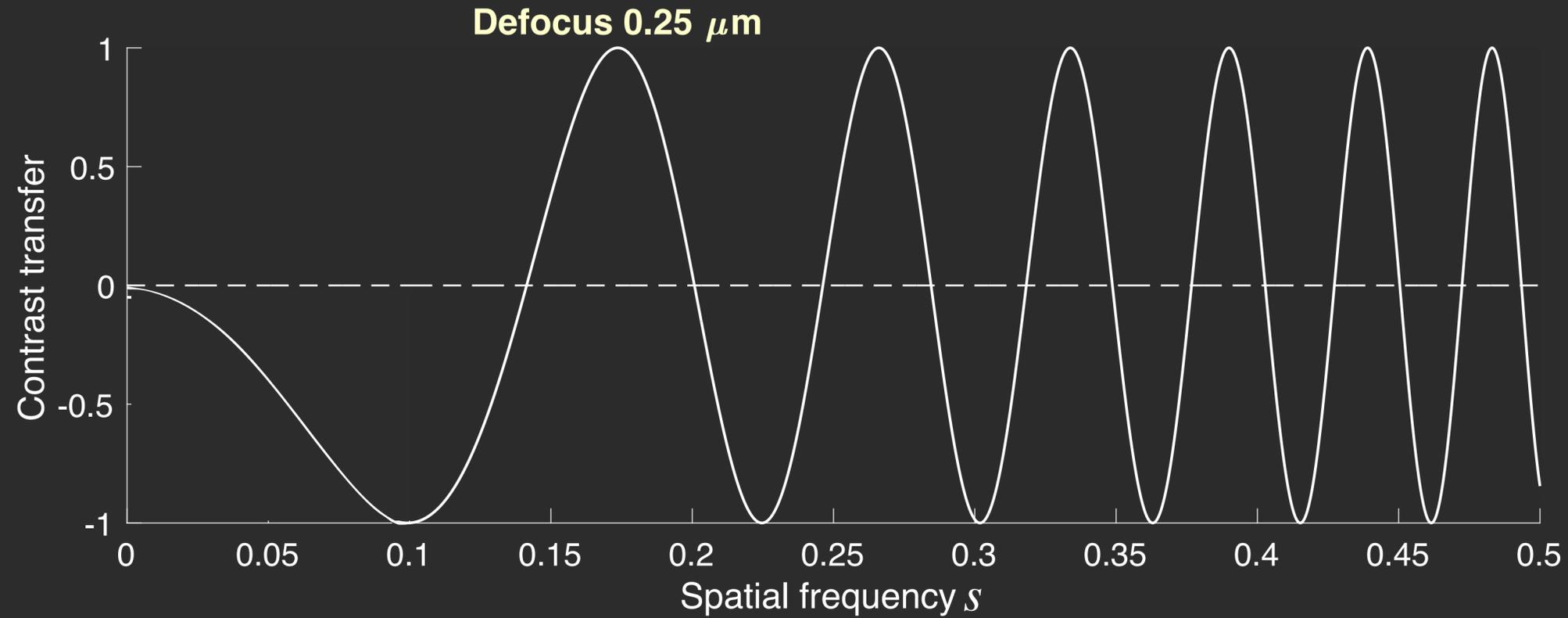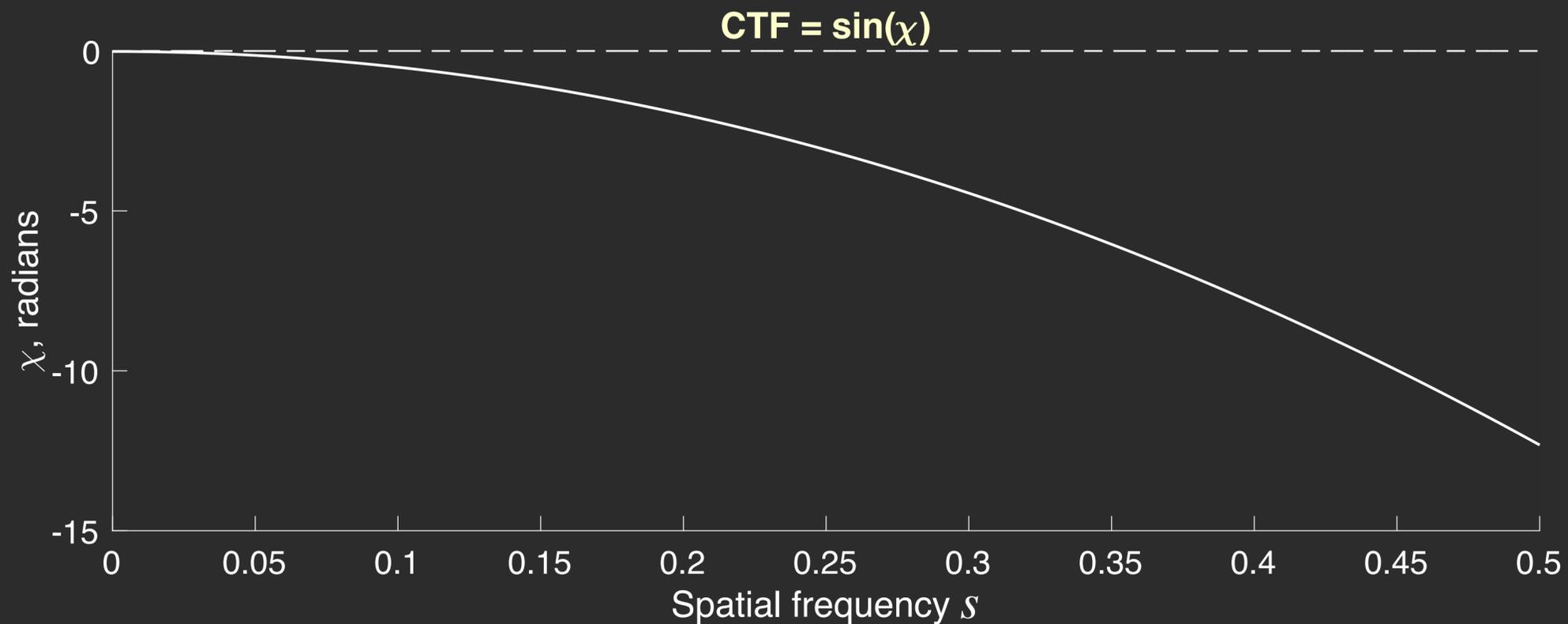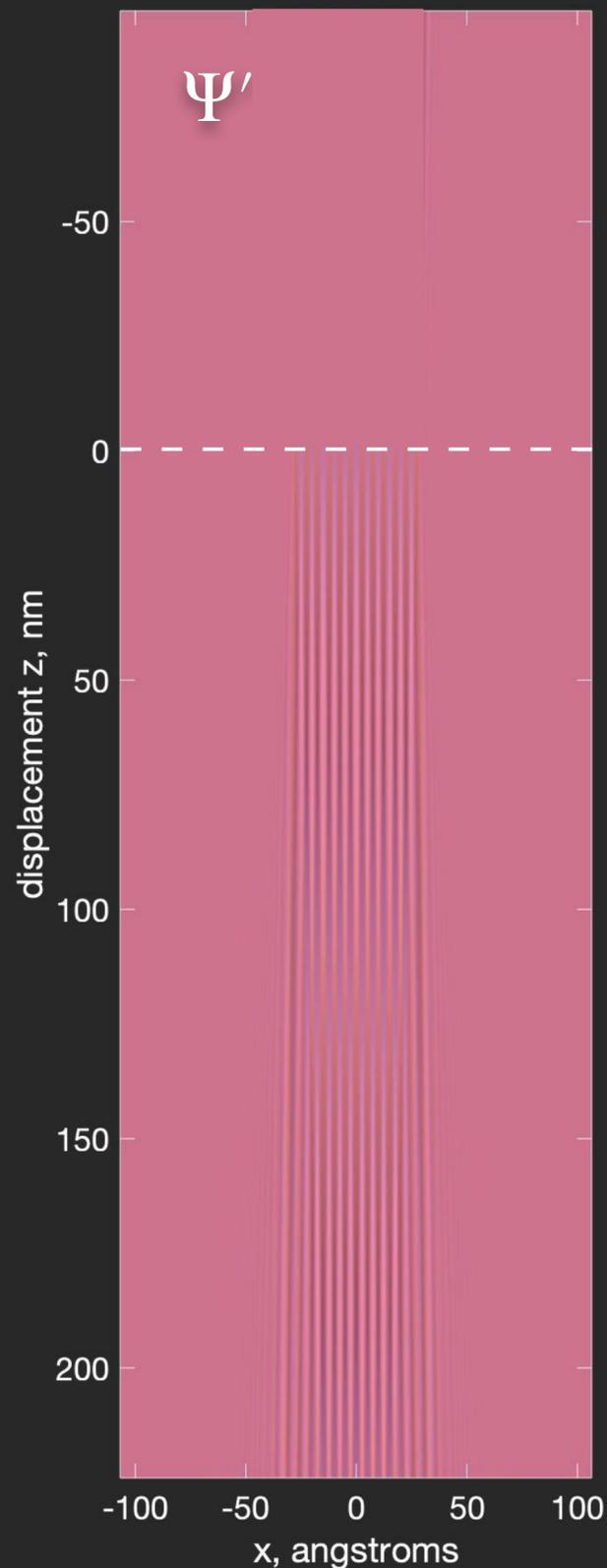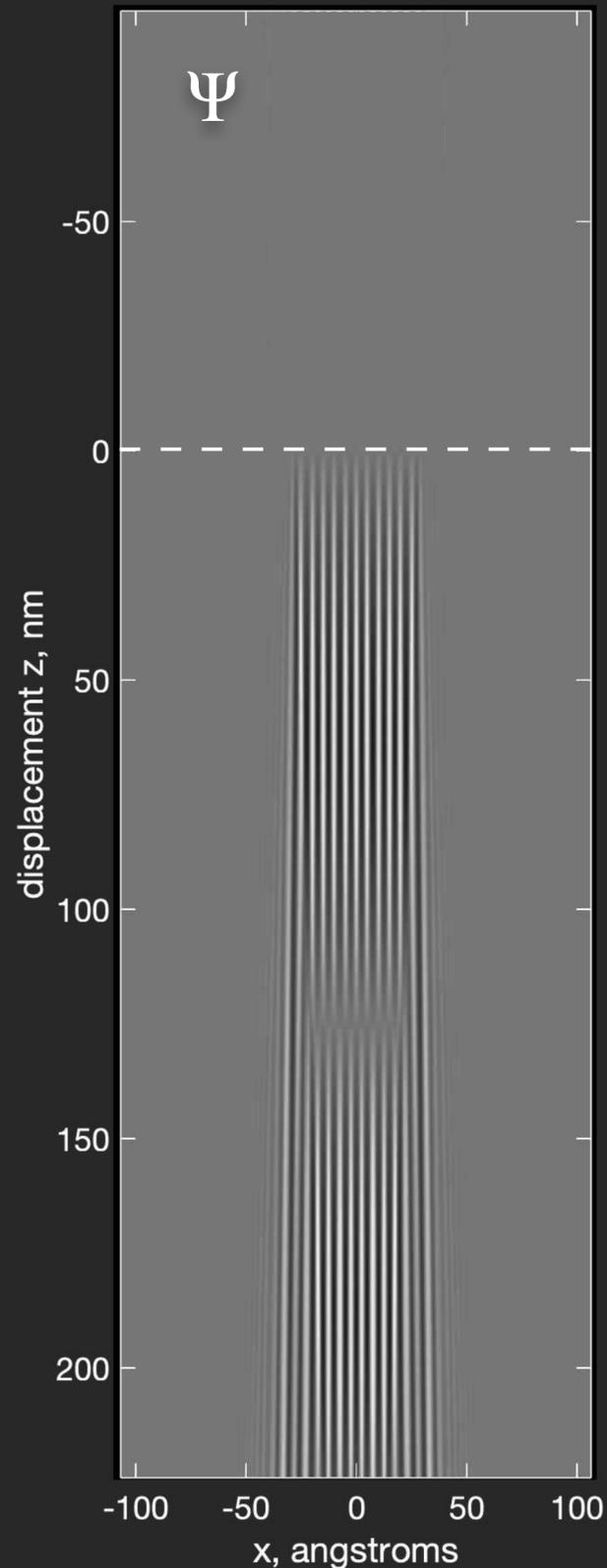
We define

$$\Psi' = \Psi/\Psi_0$$

$$\Psi' = 1 + ie^{-ik\zeta} \cdot \epsilon \cos(2\pi x/d)$$

and can be written as

$$\Psi' = 1 + ie^{-i\chi} \epsilon \phi(x).$$

Grating object:

$$\epsilon\phi(x) = \epsilon \cos(2\pi x/d)$$

Electron propagation:

$$k = 2\pi/\lambda$$

Diffracted wave path difference:

$$\zeta \approx -\delta\lambda^2/2d^2$$

Wave aberration function:

$$\chi = k\zeta \approx -\pi\lambda\delta/d^2$$

$$\Psi' = 1 + ie^{-i\chi}\,\epsilon\phi(x).$$

The measured intensity is

$$|\Psi|^2 = |\Psi'|^2 = (\text{real part})^2 + (\text{imag part})^2$$

and

$$|\Psi'|^2 = \left[\,1 + \sin(\chi)\,\epsilon\phi(x)\,\right]^2 + \left[\,\cos(-\chi)\,\epsilon\phi(x)\,\right]^2$$

$$= \left[\,\boxed{1 + 2\sin(\chi)\,\epsilon\phi(x)} + \mathcal{O}\epsilon^2\,\right] + \left[\,\mathcal{O}\epsilon^2\,\right].$$

Grating object:

$$\epsilon\phi(x) = \epsilon\cos(2\pi x/d)$$

Wave aberration function:

$$\chi \approx -\pi\lambda\delta/d^2$$

$$\approx -\pi\lambda\delta s^2$$

In practice

• We ignore the constant background intensity.

• Everyone ignores the factor of 2 also.

• So we say the transfer from phase shift to intensity change
 —the contrast transfer function—is

$$\boxed{\text{CTF} = \frac{\Delta\text{Intensity}}{\Delta\text{Electron phase}} = \sin(\chi)}$$
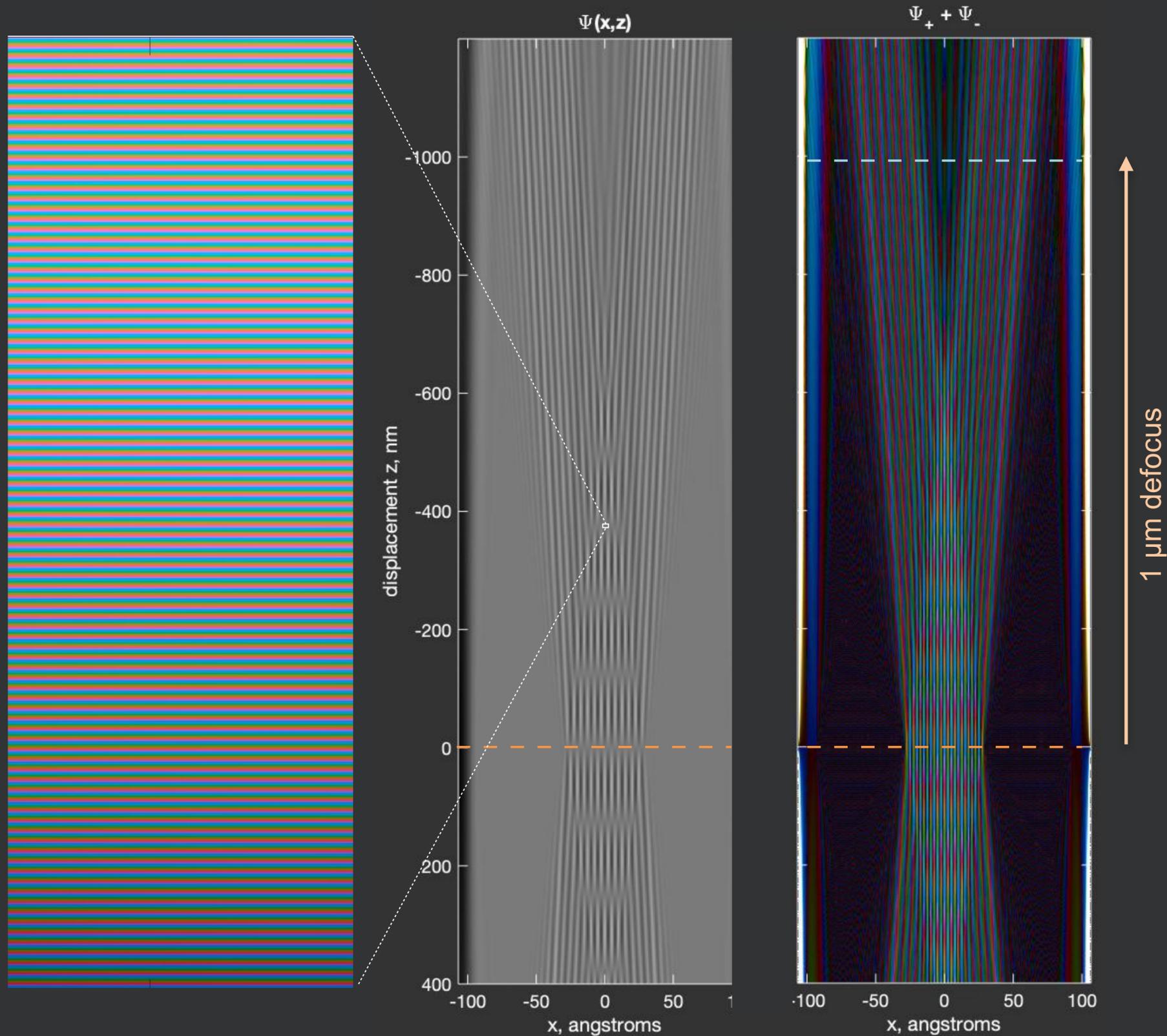
1 μm—a moderate defocus for cryo-EM imaging–is 500,000 wavelengths!

This has ramifications regarding

- beam coherence
- specimen charging
- delocalization

The dispersion radius is given by

$$r = \delta \tan \theta$$

$$= \delta \lambda / d \quad \text{(small angle approx*)}$$

For example at 3µm defocus and 3Å resolution

$$\delta = 3 \times 10^4 \text{Å}$$

$$\lambda = .02 \text{Å}$$

$$d = 3 \text{Å}$$

then

$$r = 200 \text{Å}$$

In this case one would want 200Å of space in the box around each particle image.

_____

*Note: beyond about 3Å resolution, spherical aberration should be taken into account too.
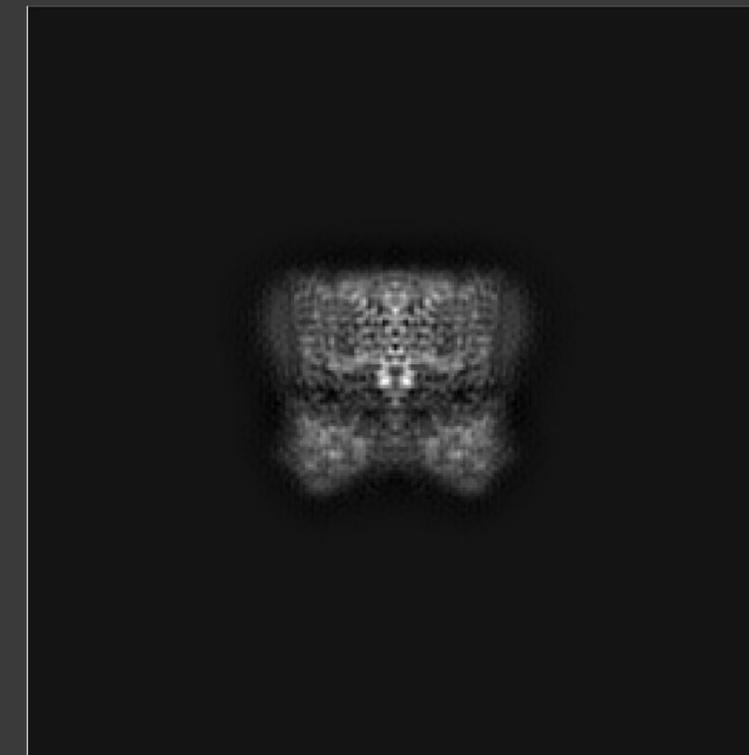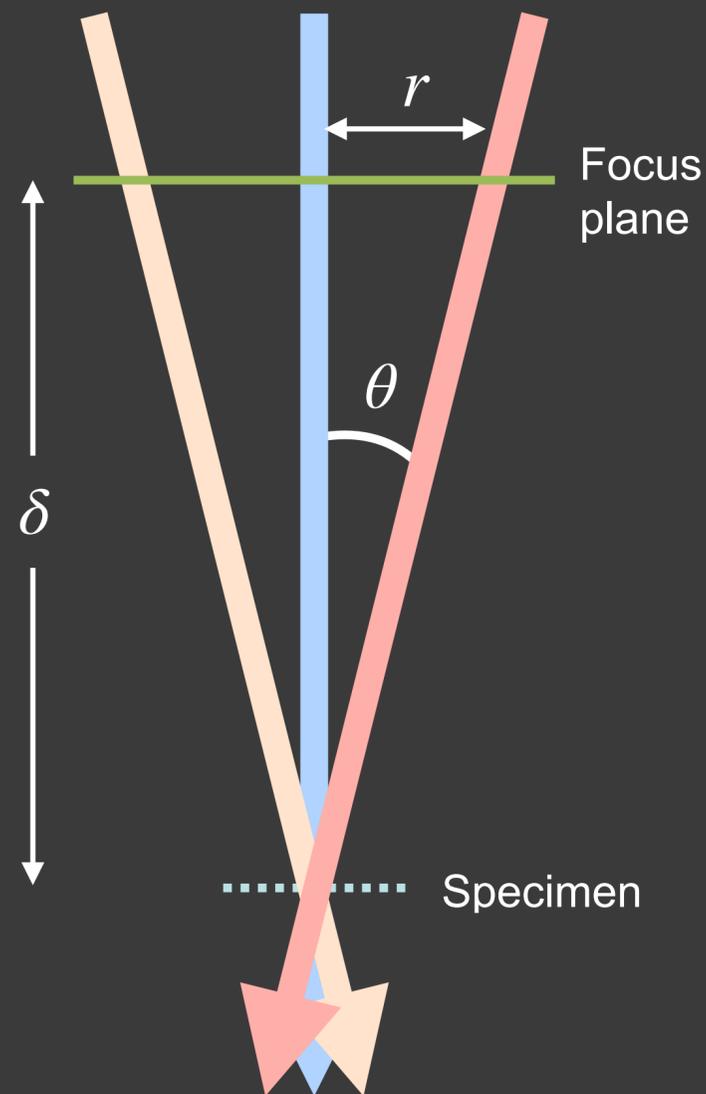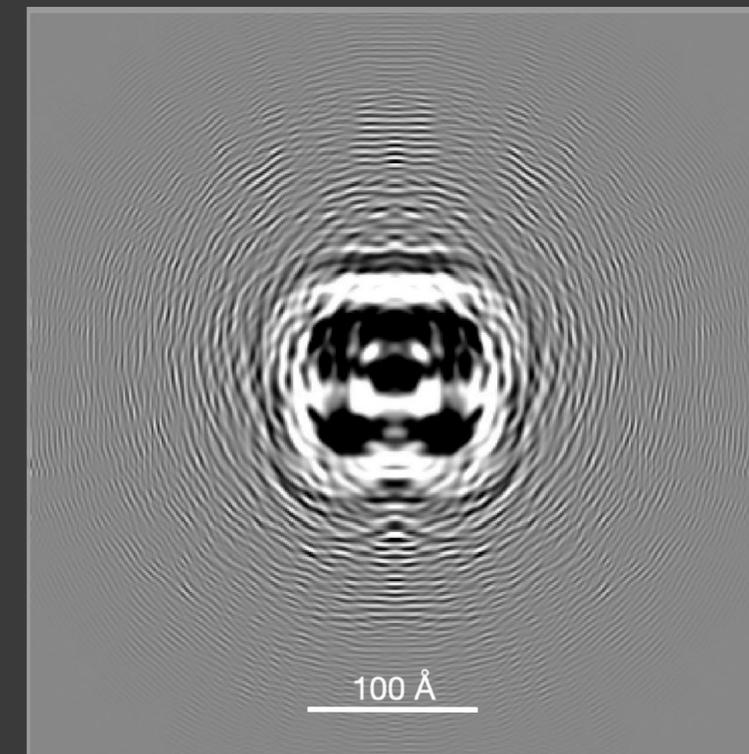
r

Focus plane

θ

δ

Specimen

Object

3 µm defocus

100 Å

Phase contrast and the contrast transfer function

1. Complex numbers: review

2. Defocus contrast and the CTF (simple version)

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4. Objective lens effects on the CTF

In focus

Underfocus

Camera'

Specimen

Specimen

Camera

Camera

The camera "looks" above
the specimen

Specimen'
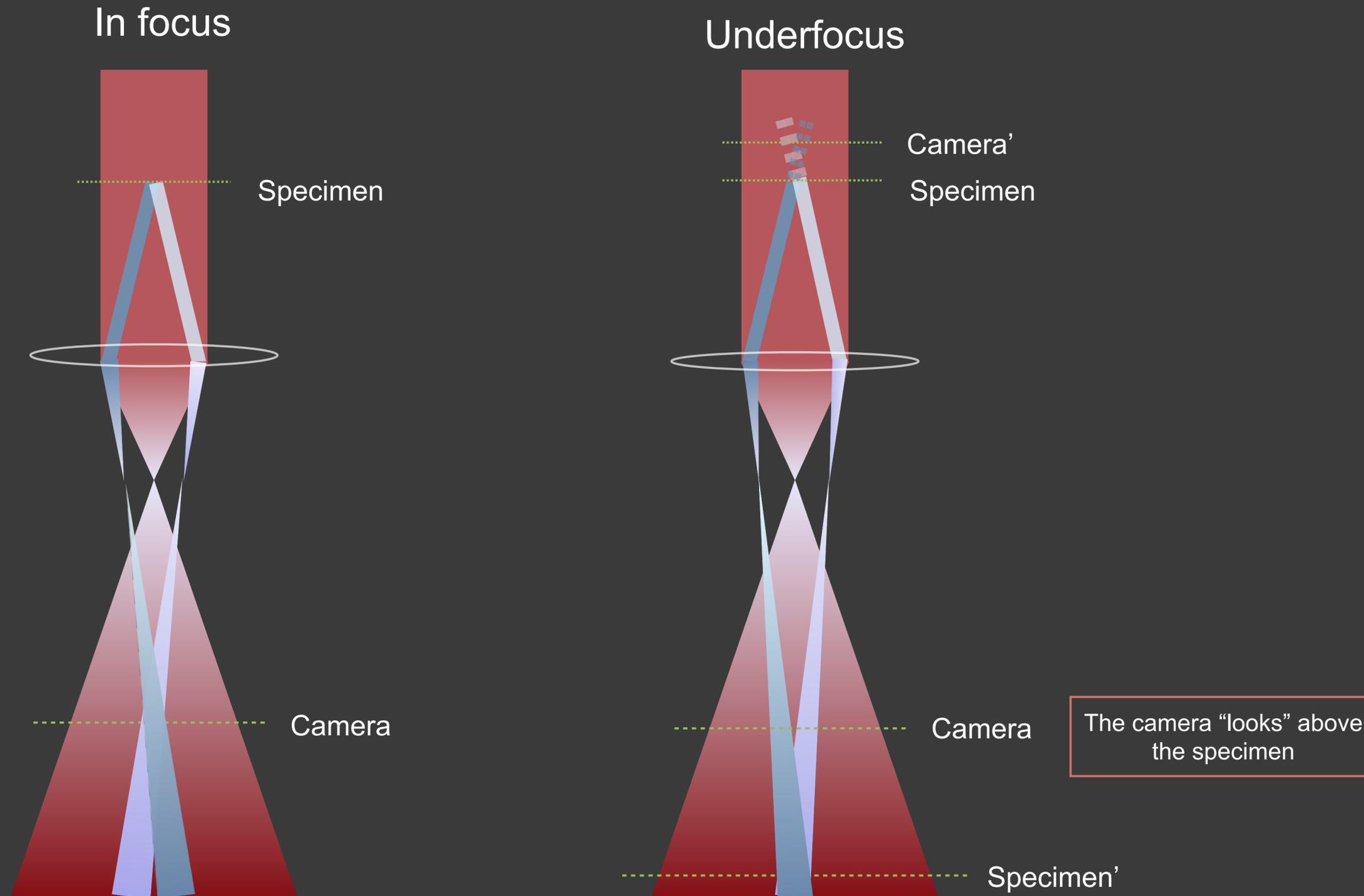
Spherical aberration changes the defocus by

$$\delta' = -C_s\lambda^2 s^2/2.$$

The contrast transfer function now includes δ',

$$\mathrm{CTF} = \sin(-\pi\lambda\,(\delta + \delta')\,s^2)$$

or, expanded,

$$\mathrm{CTF} = \sin(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\,\lambda^3 s^4)$$

The coefficient C_s is typically ~2mm. Spherical aberration typically becomes important for $s \gtrsim 0.25\mathring{A}^{-1}$, or about $4\mathring{A}$ resolution.

Underfocus

Specimen

Specimen'
(at high s)

Camera

Specimen'
(Gaussian focus)
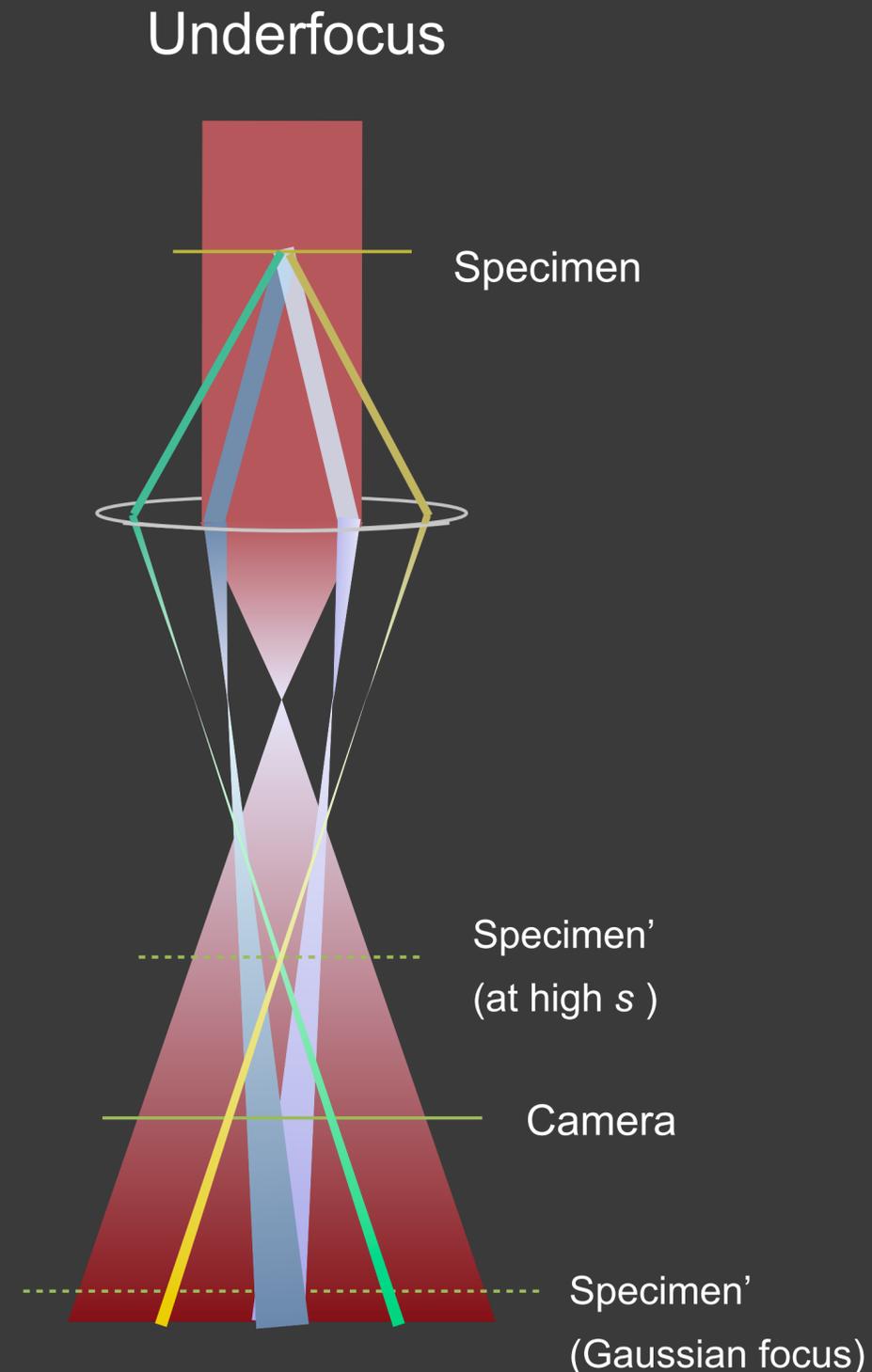
Electrons that pass very close to an atomic nucleus are scattered at high angles, and are caught by the objective aperture.

- The loss of these electrons results in a small amount of negative amplitude contrast.

- For proteins α is typically around 0.05.

- The amplitude contrast term allows the CTF to have a small negative value even at zero spatial frequency.

Combining all these terms, the contrast transfer function is given by

$$\mathrm{CTF} = \sin(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\lambda^3 s^4 - \alpha)$$

defocus   *sphere abb.*   *amplitude*

Specimen

Objective aperture

Camera

$$\text{CTF} = \sin(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\lambda^3 s^4 - \alpha)$$

defocus   sphere abb.   amplitude

Defocus only
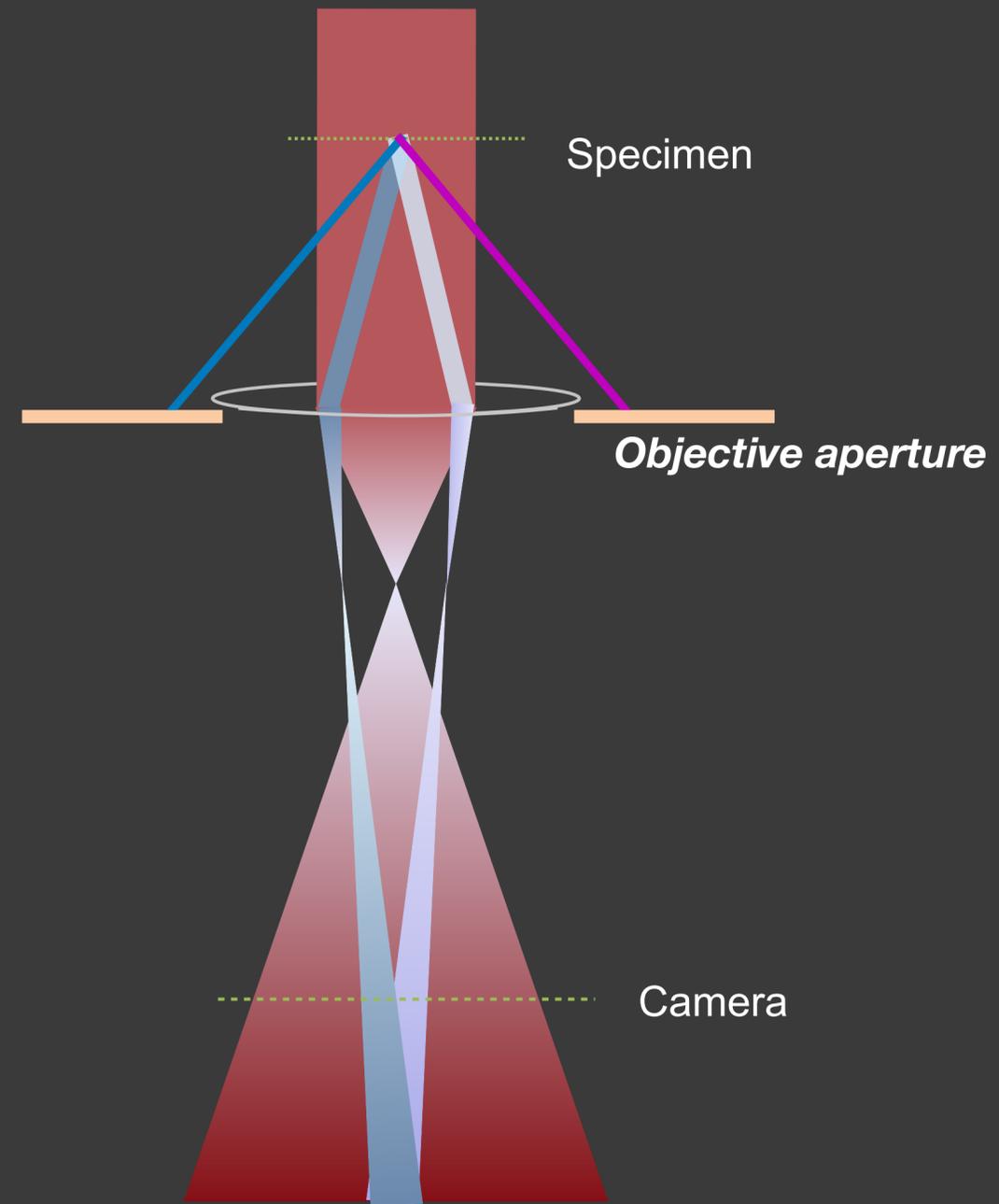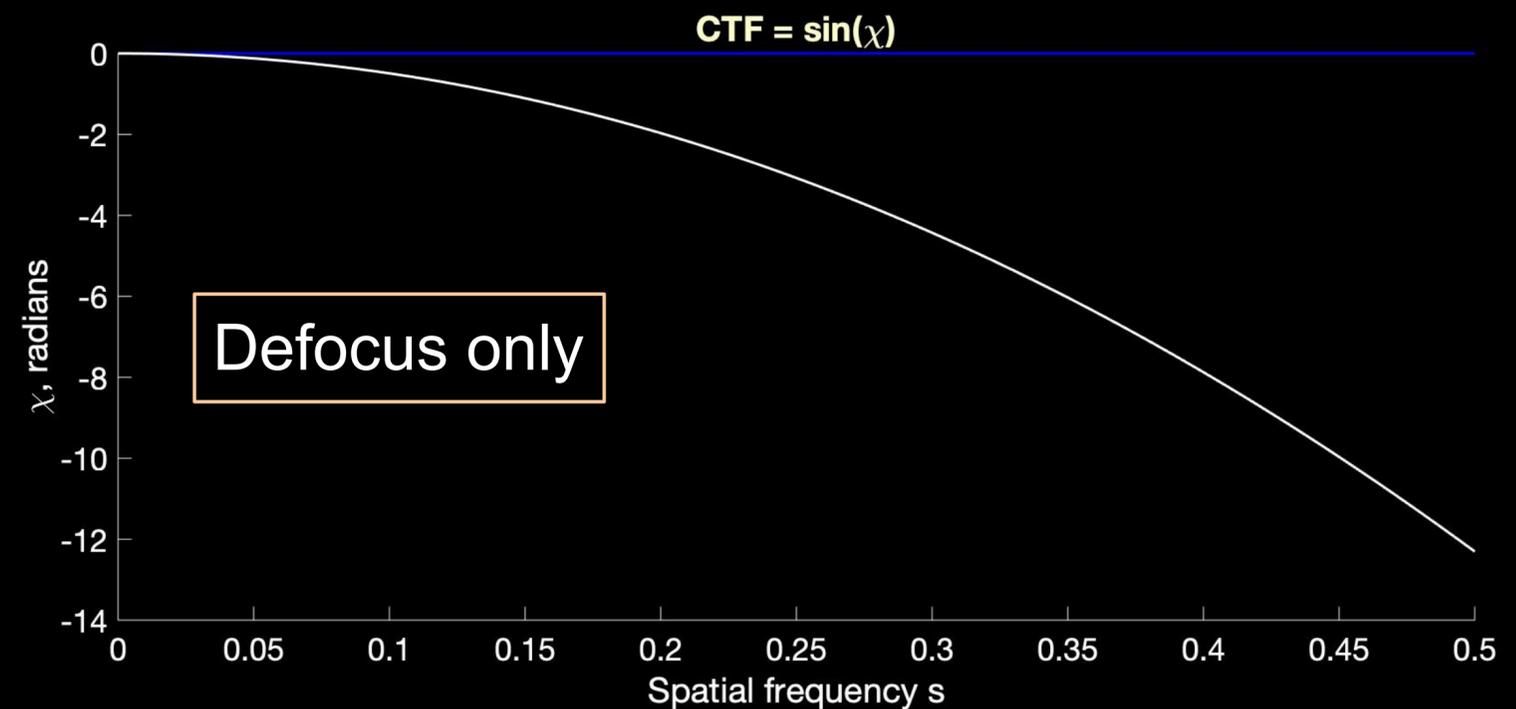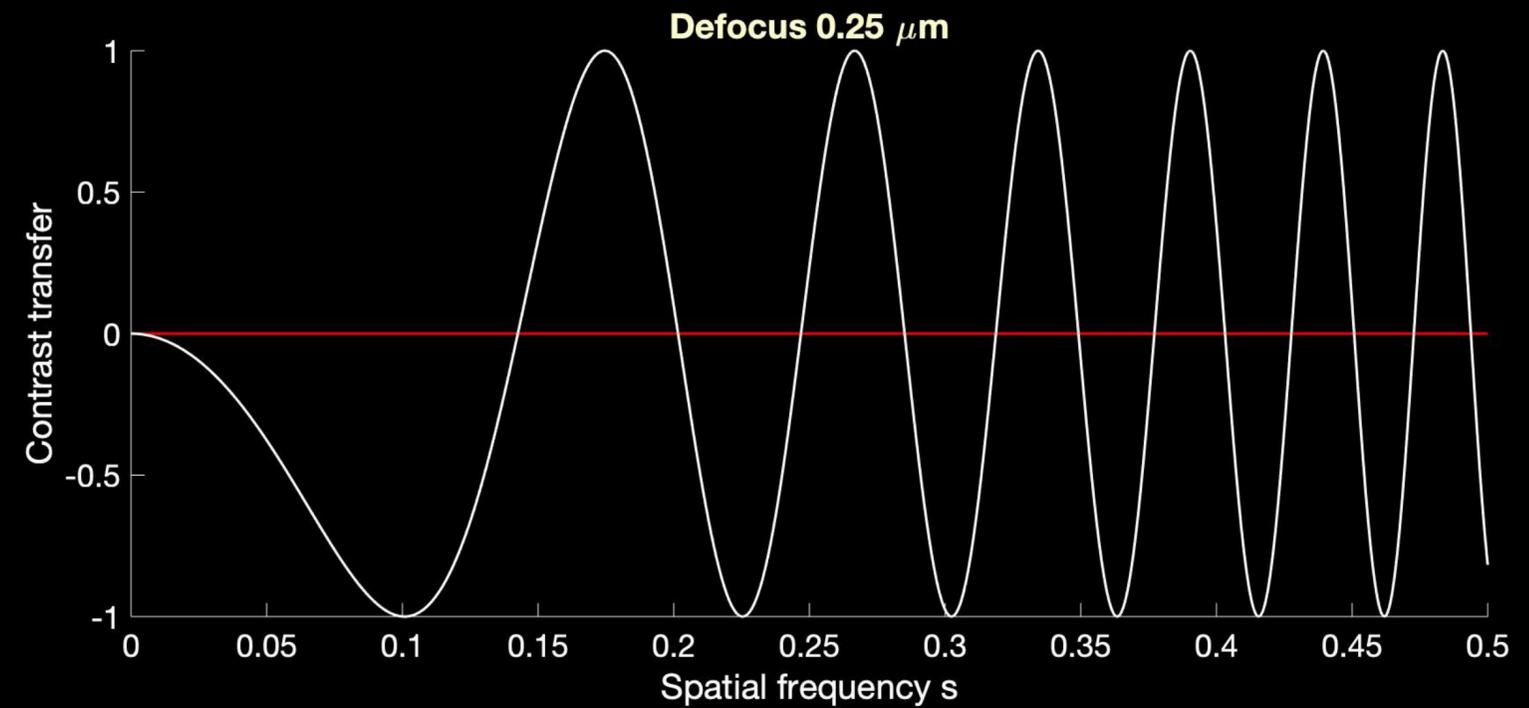
Here you can see why everyone uses underfocus: the amplitude contrast and defocus contrast are additive in this case.

Also, Cs has the effect of reversing some of the oscillations in the CTF.

Combining all these terms, the contrast transfer function is given by

$$\text{CTF} = \sin(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\lambda^3 s^4 - \alpha)$$

defocus   sphere abb.   amplitude

Defocus 0.25 μm

α

Contrast transfer

Spatial frequency s

CTF = sin(χ)

With α and C_s

C_s effect

χ, radians
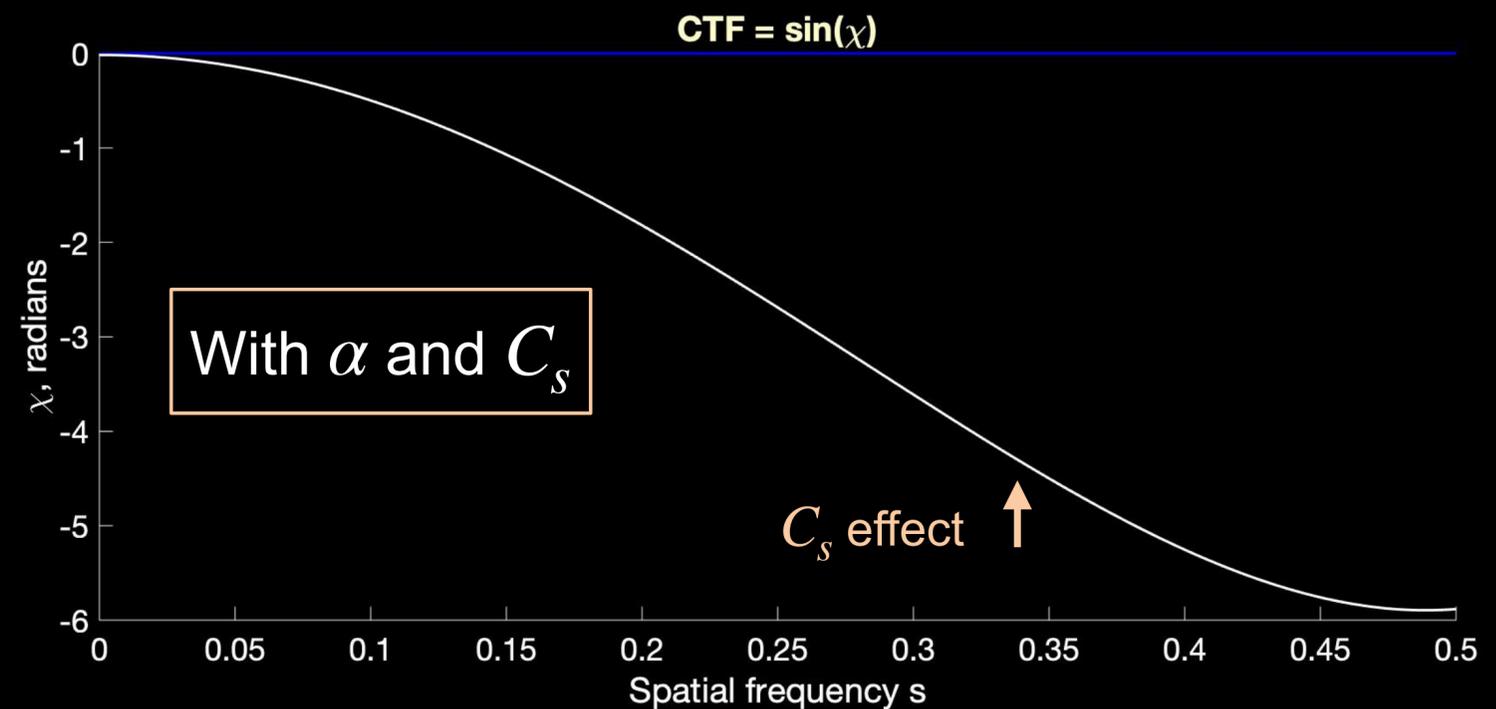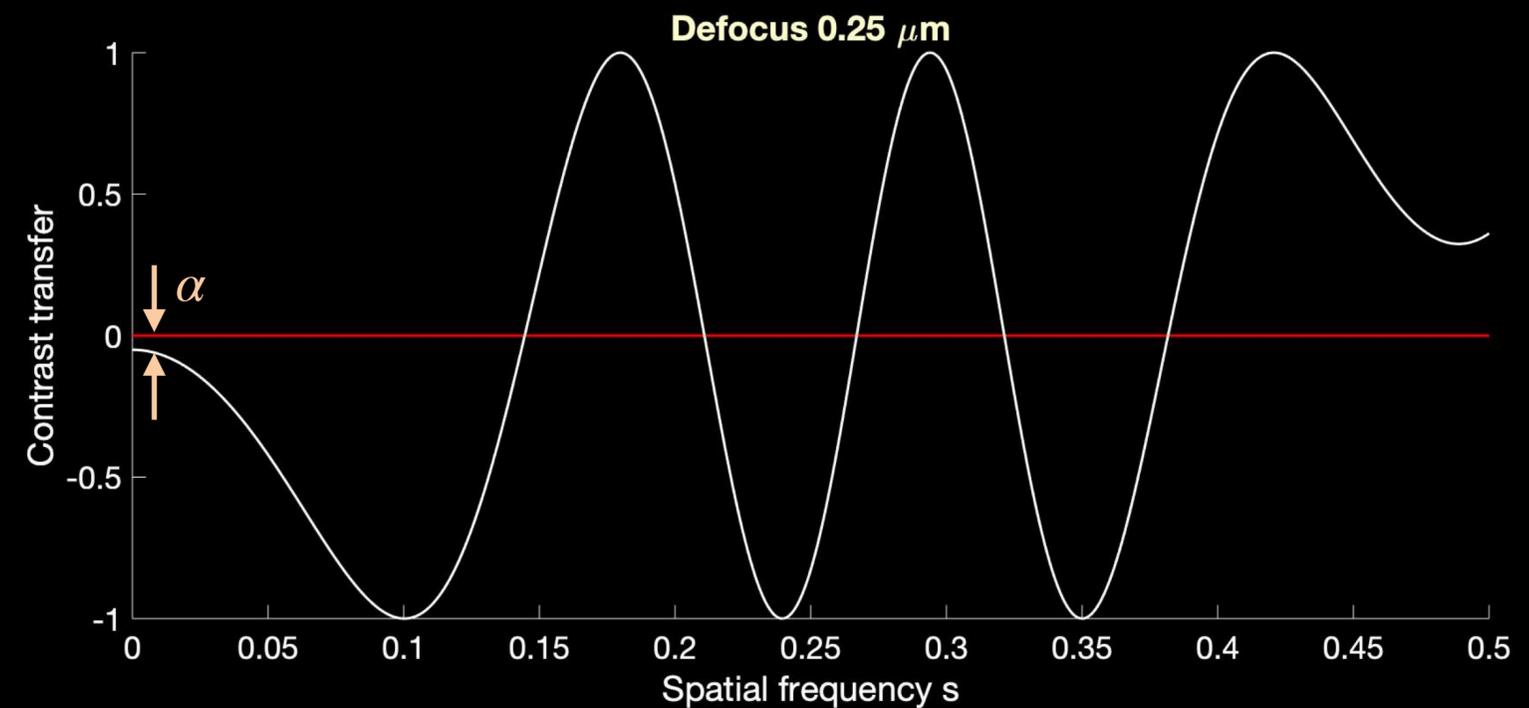
Spatial frequency s

If we're not using image processing to remove CTF effects, Scherzer defocus is a good solution: just enough defocus to give signal over a broad range of spatial frequencies.

It's popular in materials science but not much for cryoEM: the contrast transfer at low frequencies is very poor.
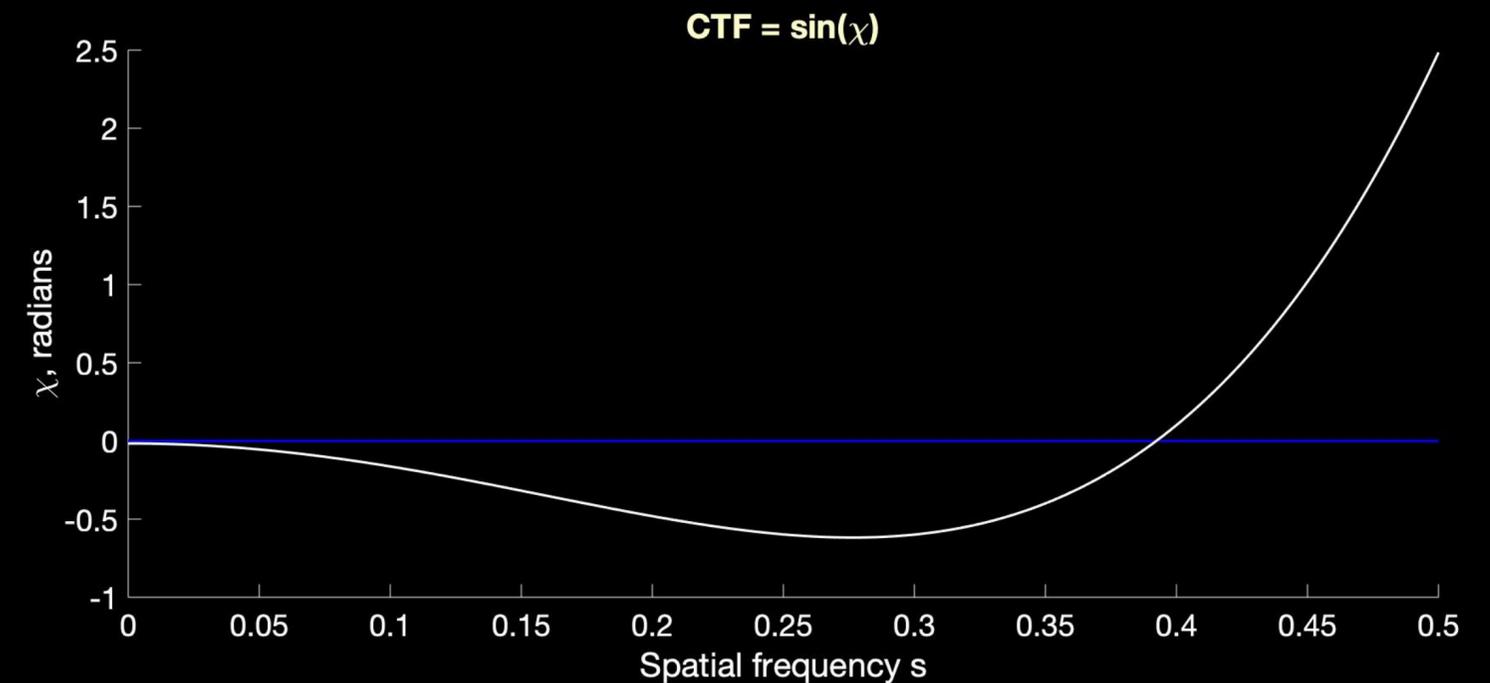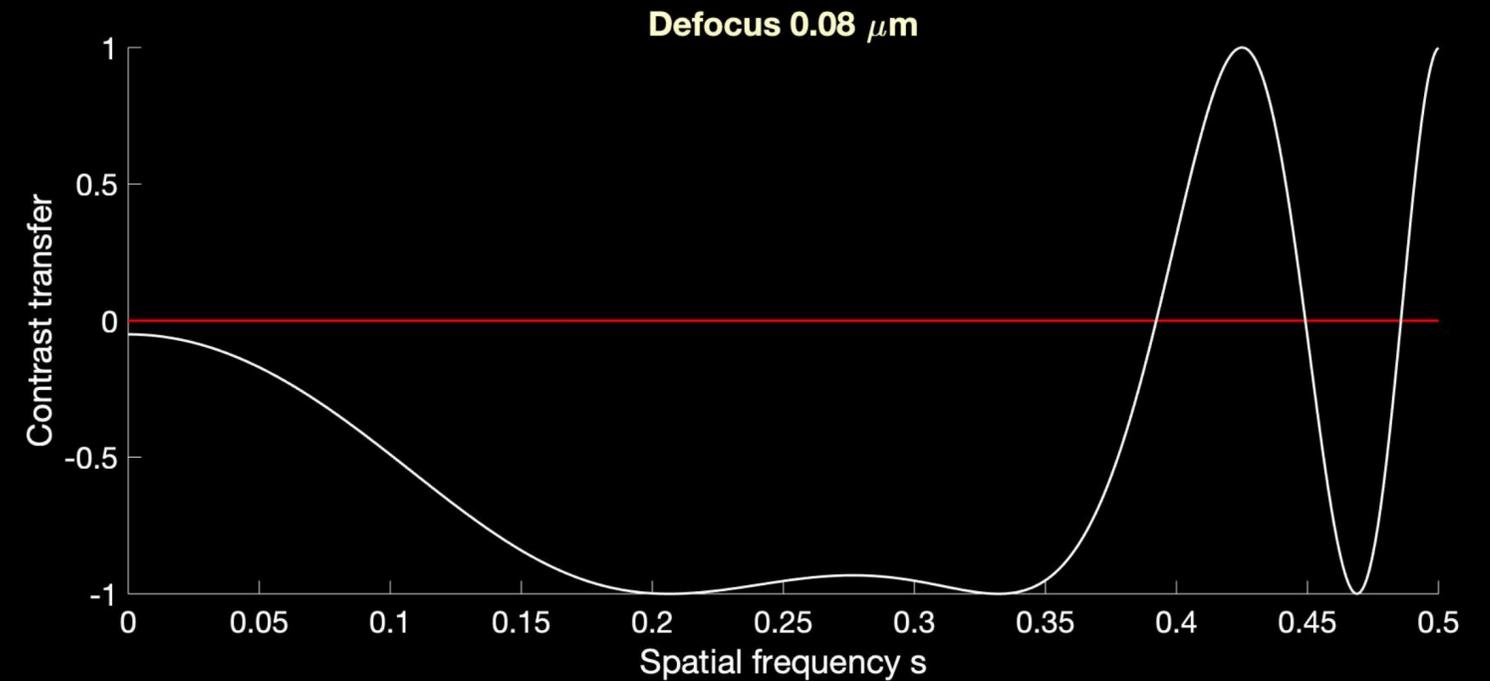
A phase plate modifies the interference of electron waves at the camera

In focus

Phase plate

Specimen

Diffraction plane

Phase Plate

Camera
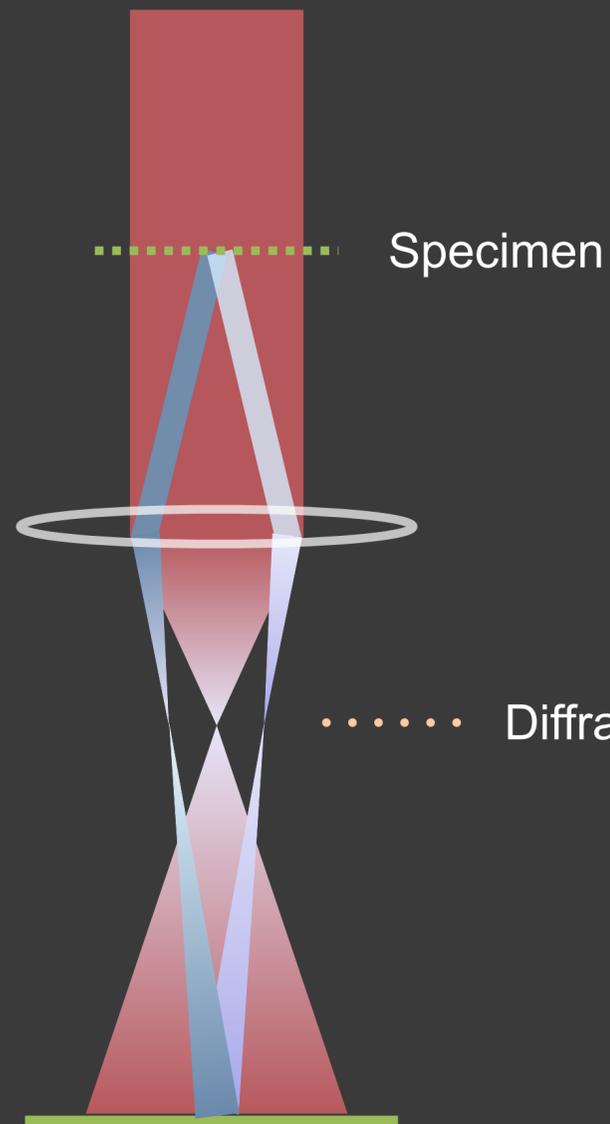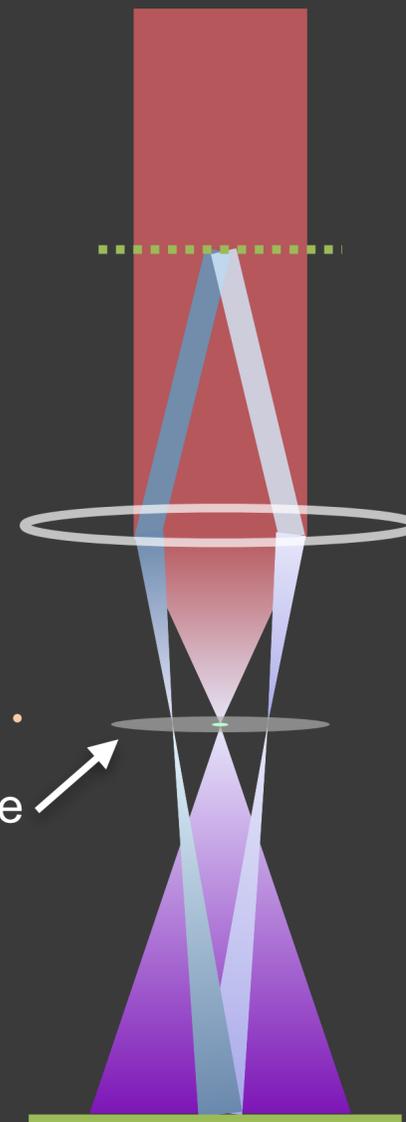
The phase plate shifts the phase of the undiffracted beam Ψ_0 by some angle ϕ.

Then $\mathrm{CTF} = \sin(\chi - \phi)$.

If $\phi = 90^{\mathrm{o}}$ then
$$\mathrm{CTF} = -\cos(\chi)$$

Cryo-EM single particle analysis with the Volta phase plate

Radostin Danev*, Wolfgang Baumeister

Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany

eLife 2016

- The better low-frequency contrast makes particles much more visible.

- The defocus value must be precise within 60 nm in order to get 4 Å resolution.
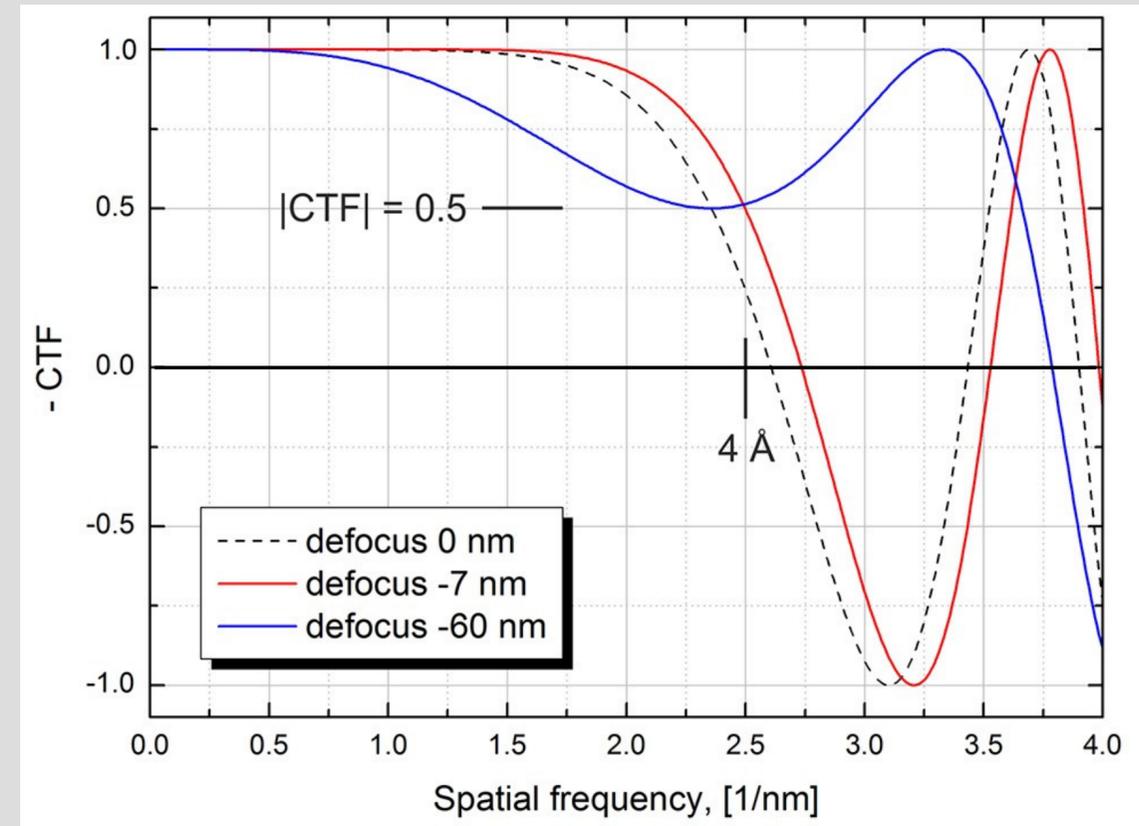
In-focus phase plate
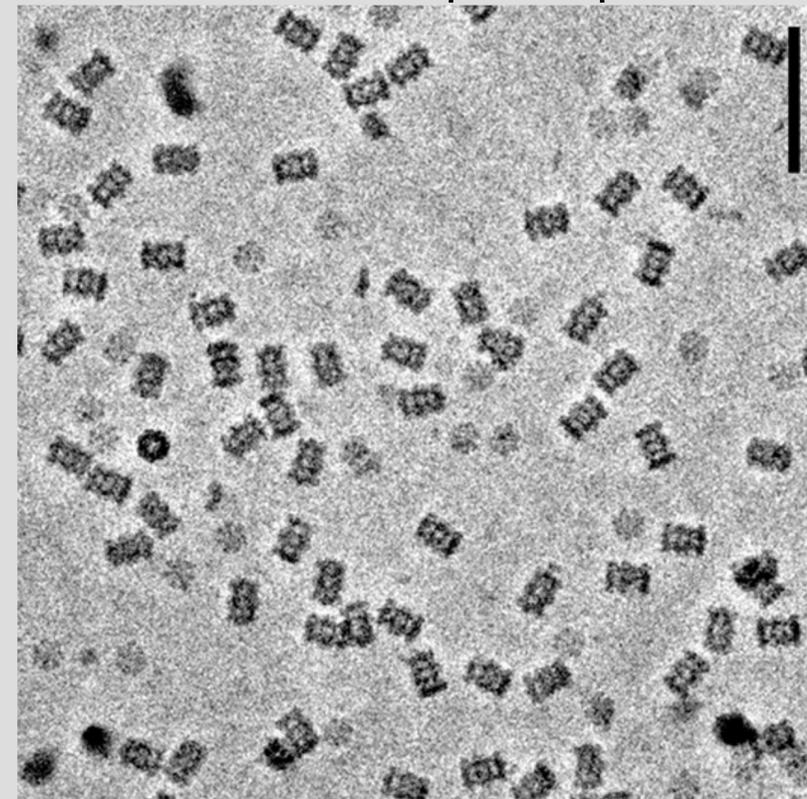
Defocus contrast

2D CTF
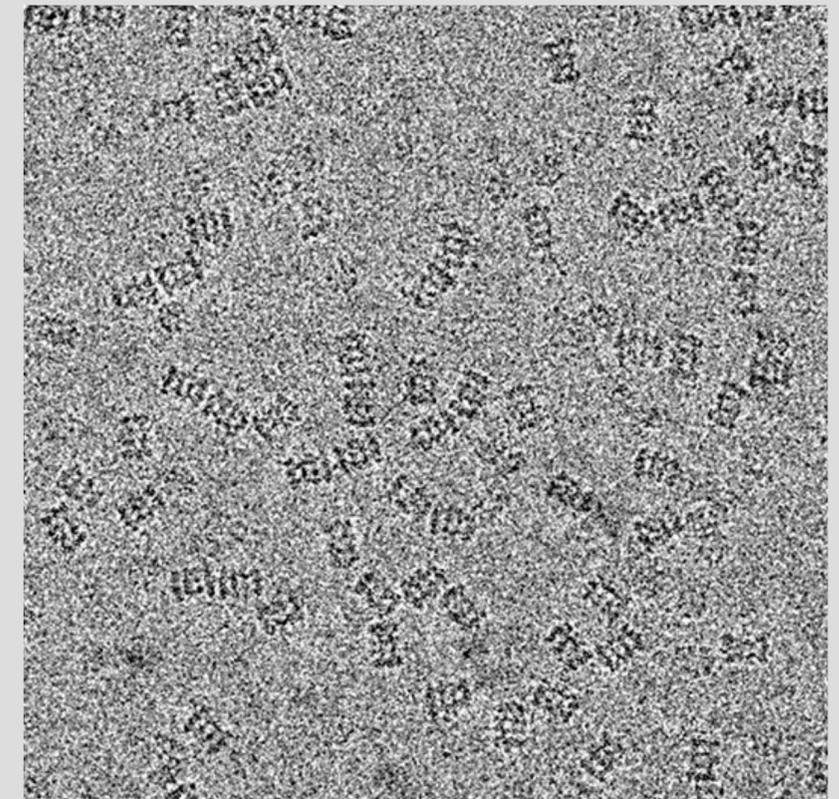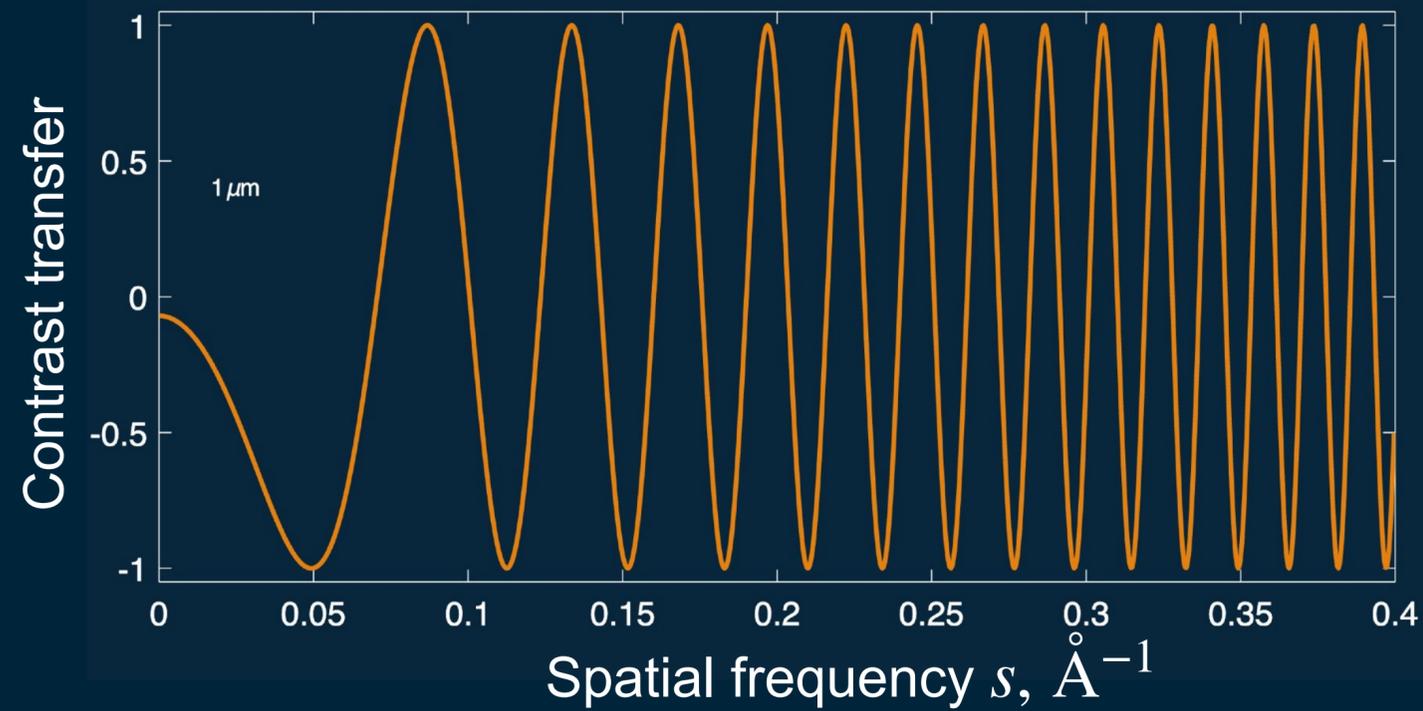
$$\text{CTF} = \sin(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\lambda^3 s^4 - \alpha)$$

defocus   sphere abb.   amplitude

$$\text{CTF} = \sin(-\pi\lambda\delta(s_x^2 + s_y^2) + \frac{\pi}{2}C_s\lambda^3(s_x^2 + s_y^2)^2 - \alpha)$$
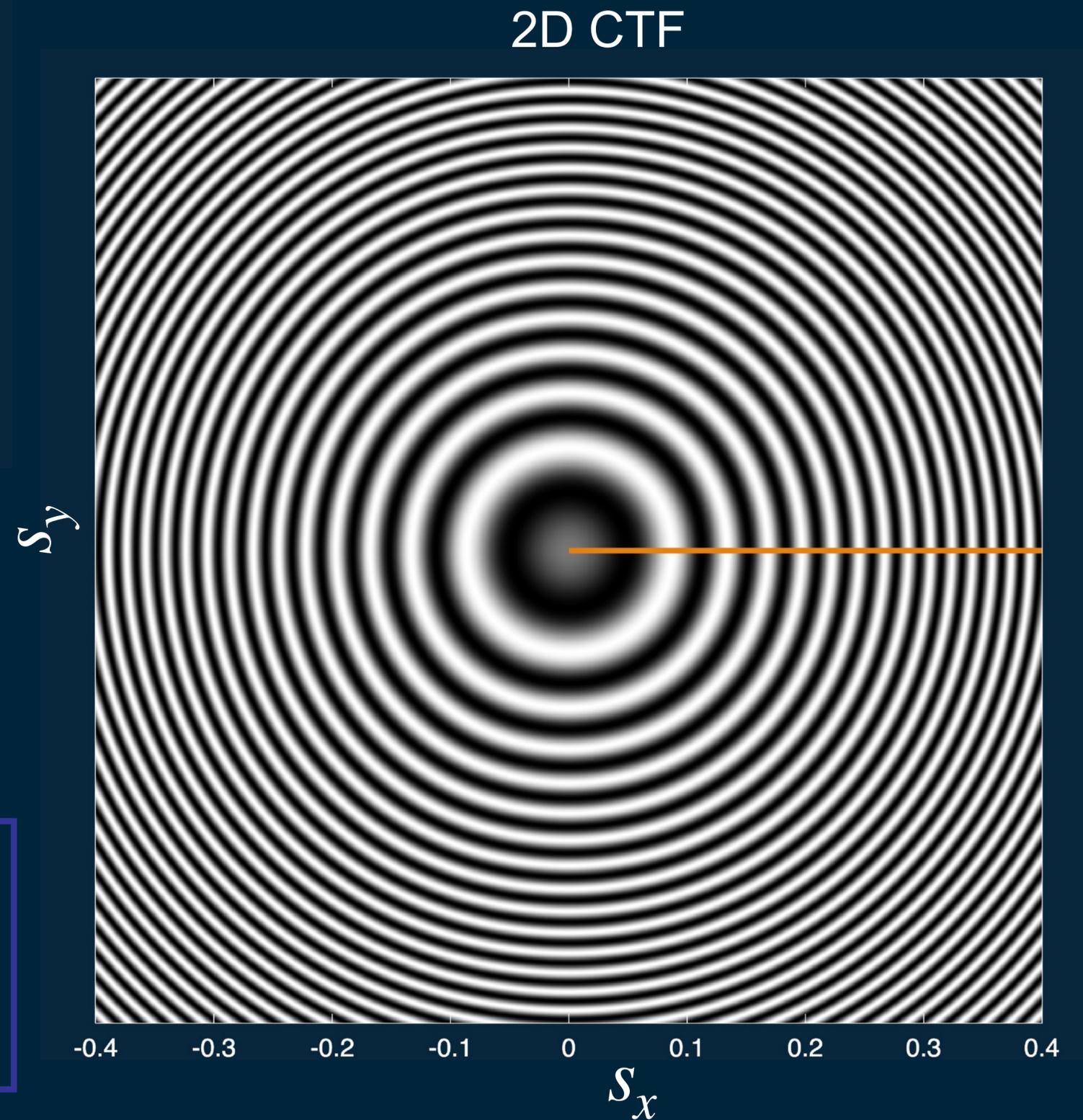
Astigmatism yields a varying defocus depending on the angle

The defocus and other CTF parameters can be estimated by curve fitting.
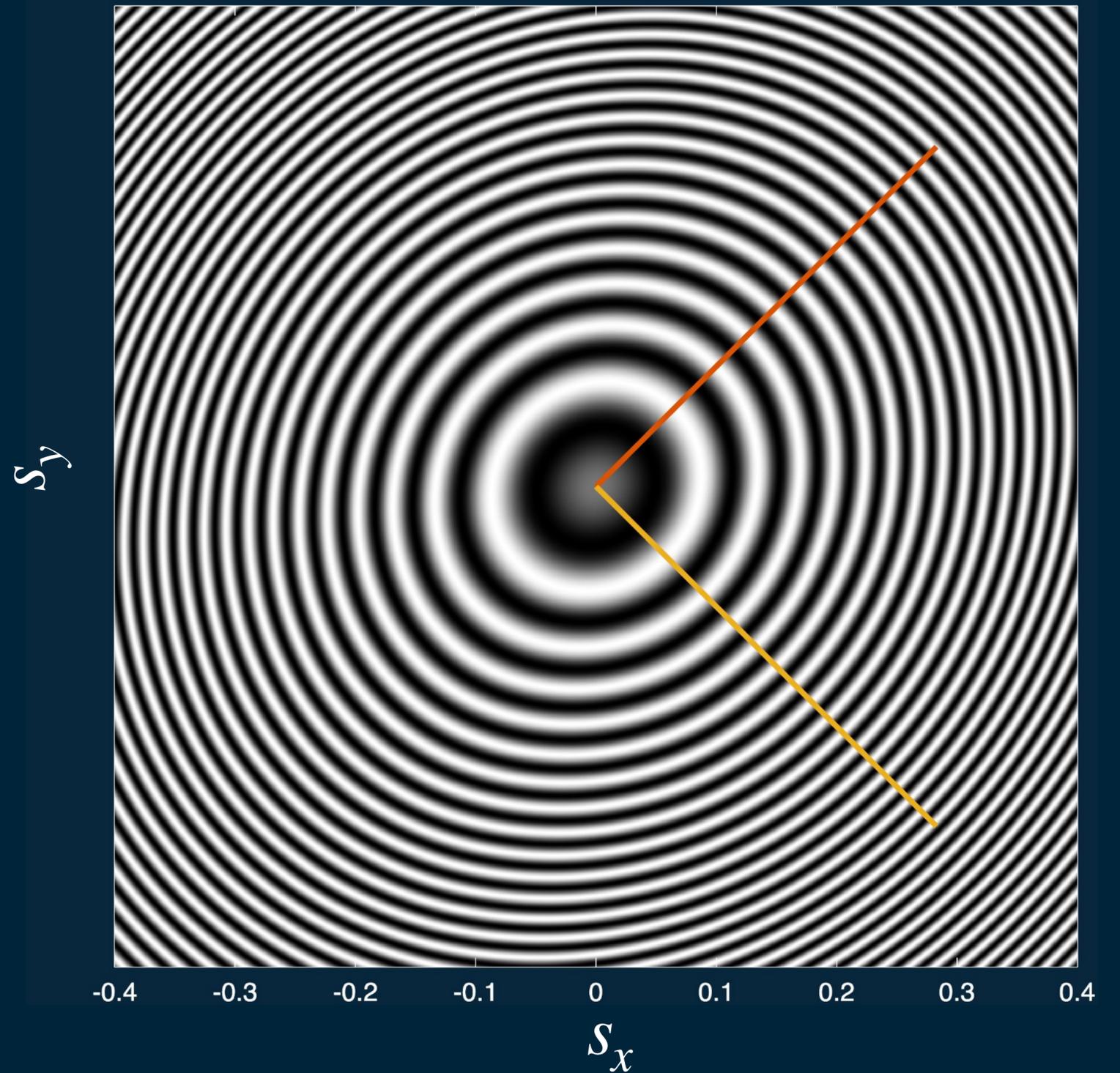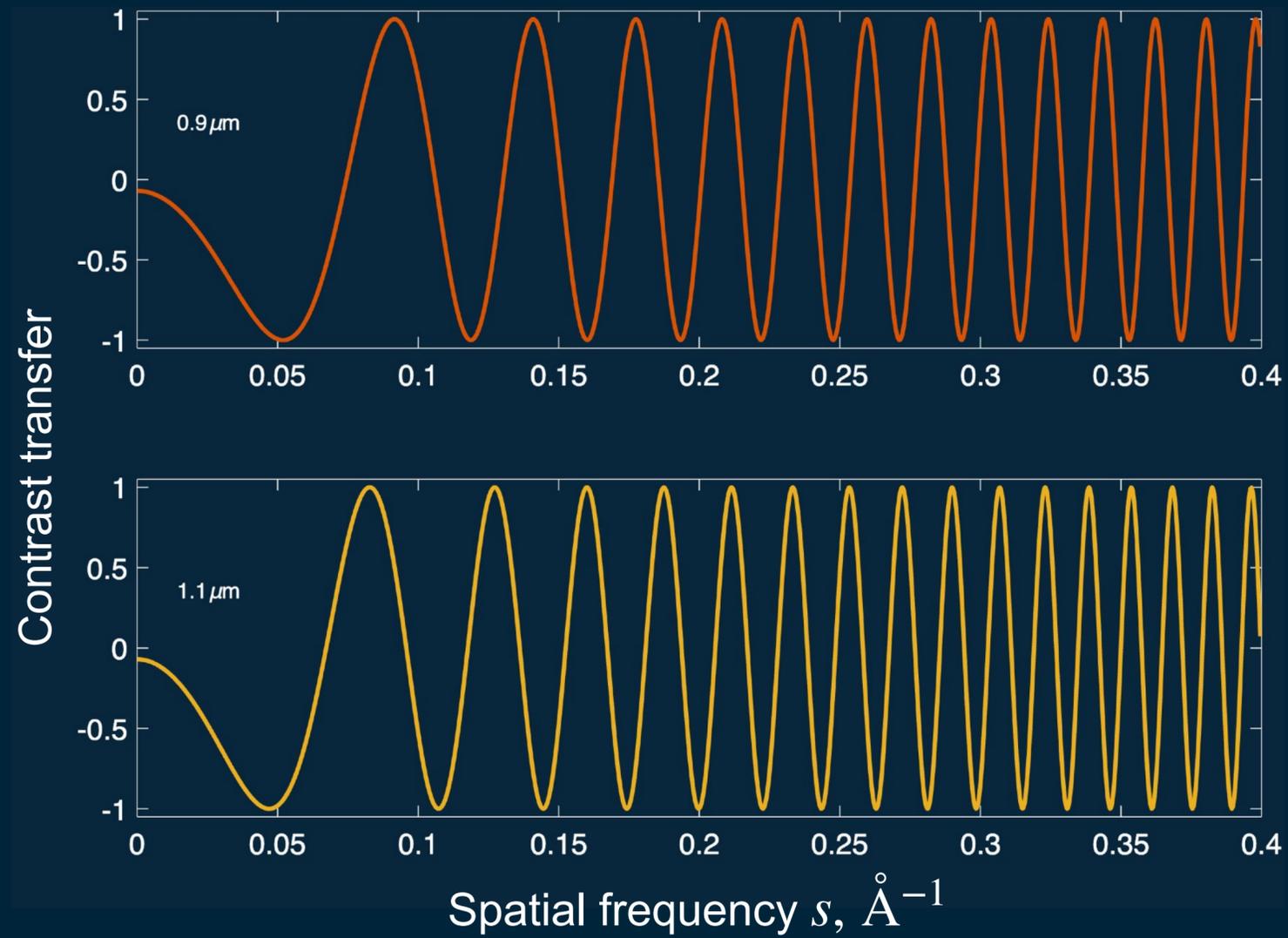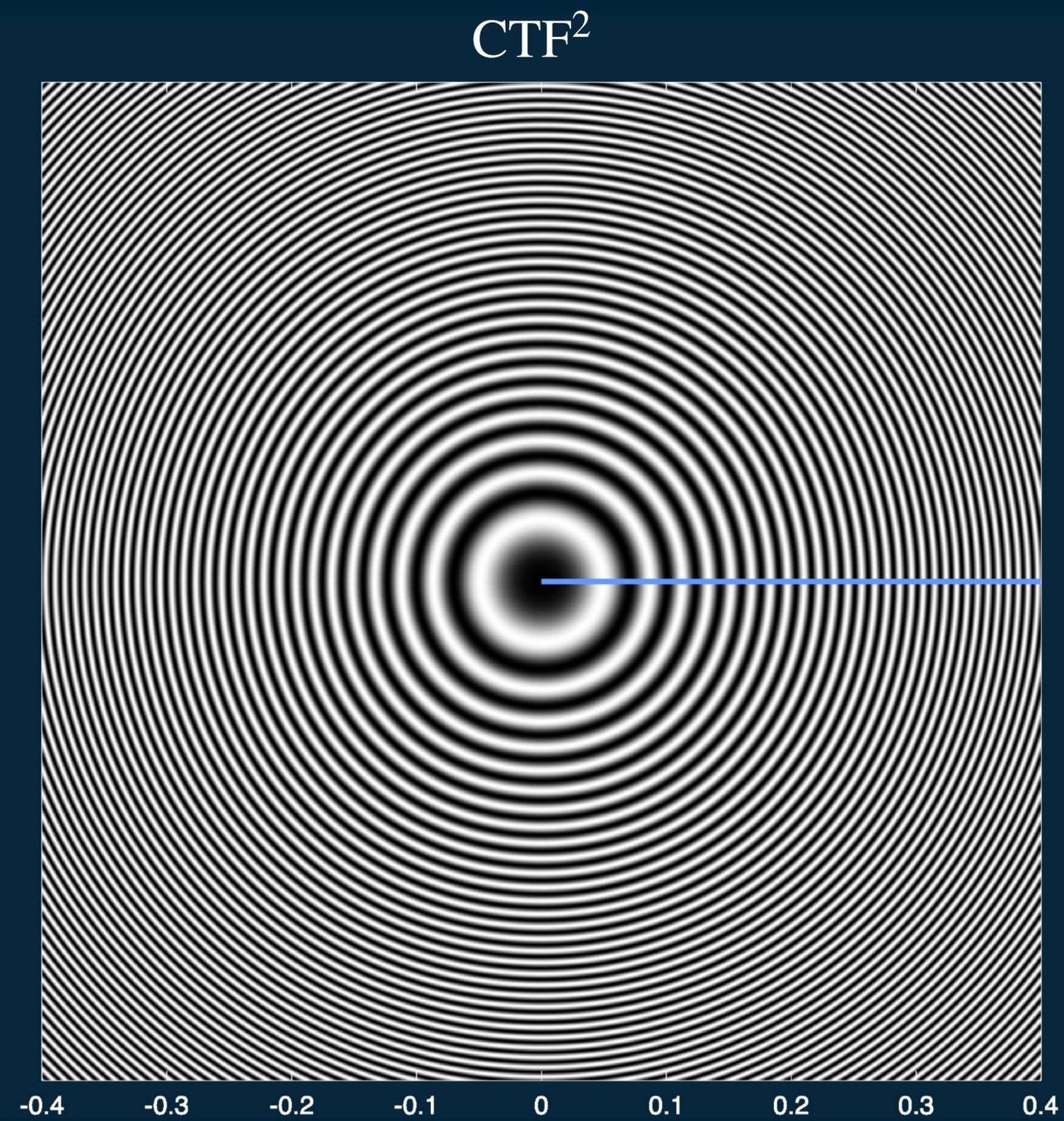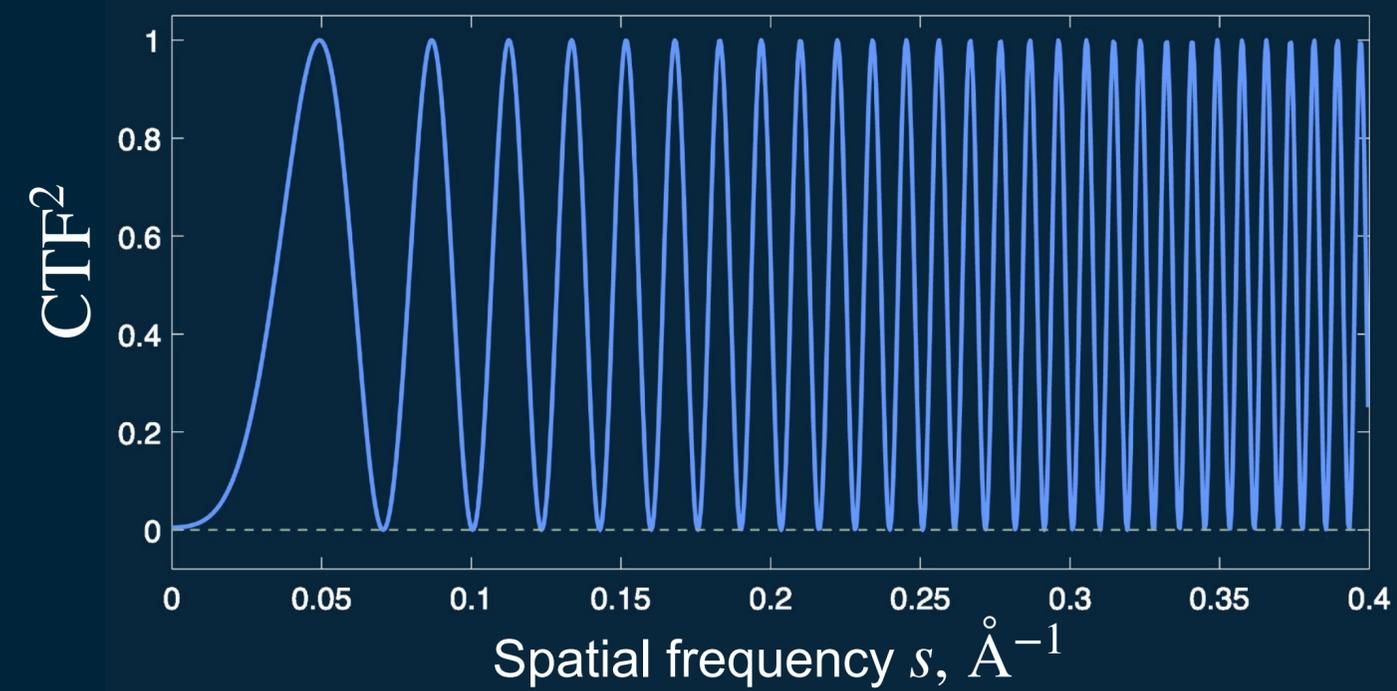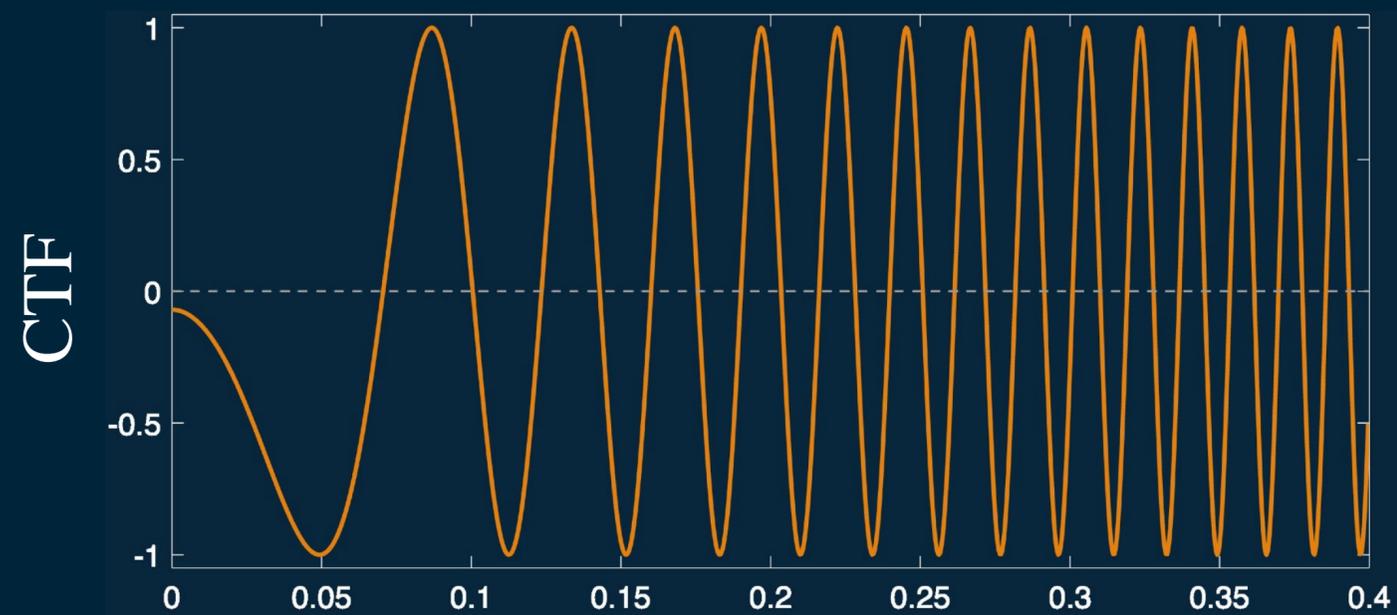
The power spectrum shows the magnitude of an image's FT

Power spectrum

Grating at the spatial frequency $s = (s_x, s_y)$
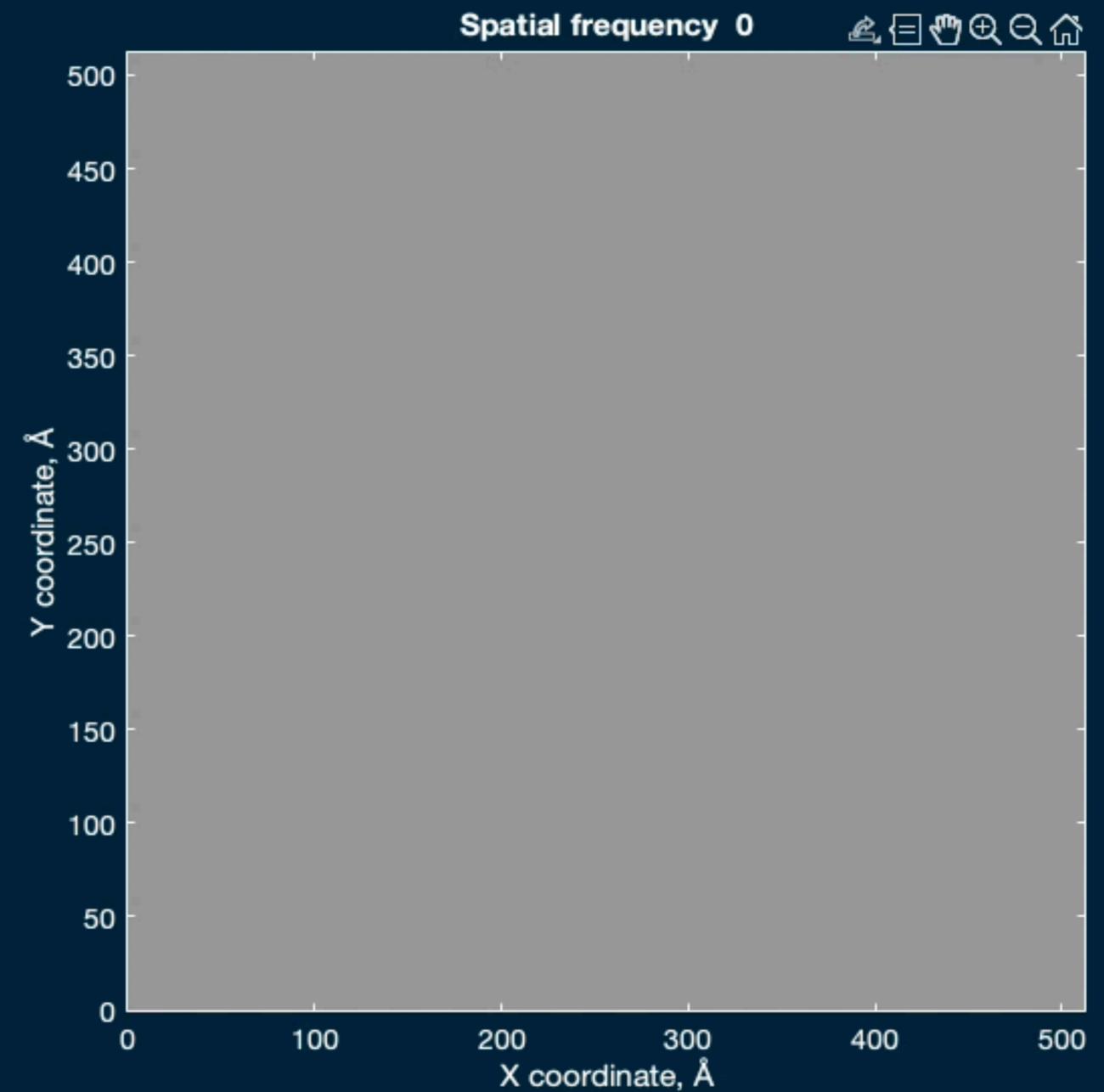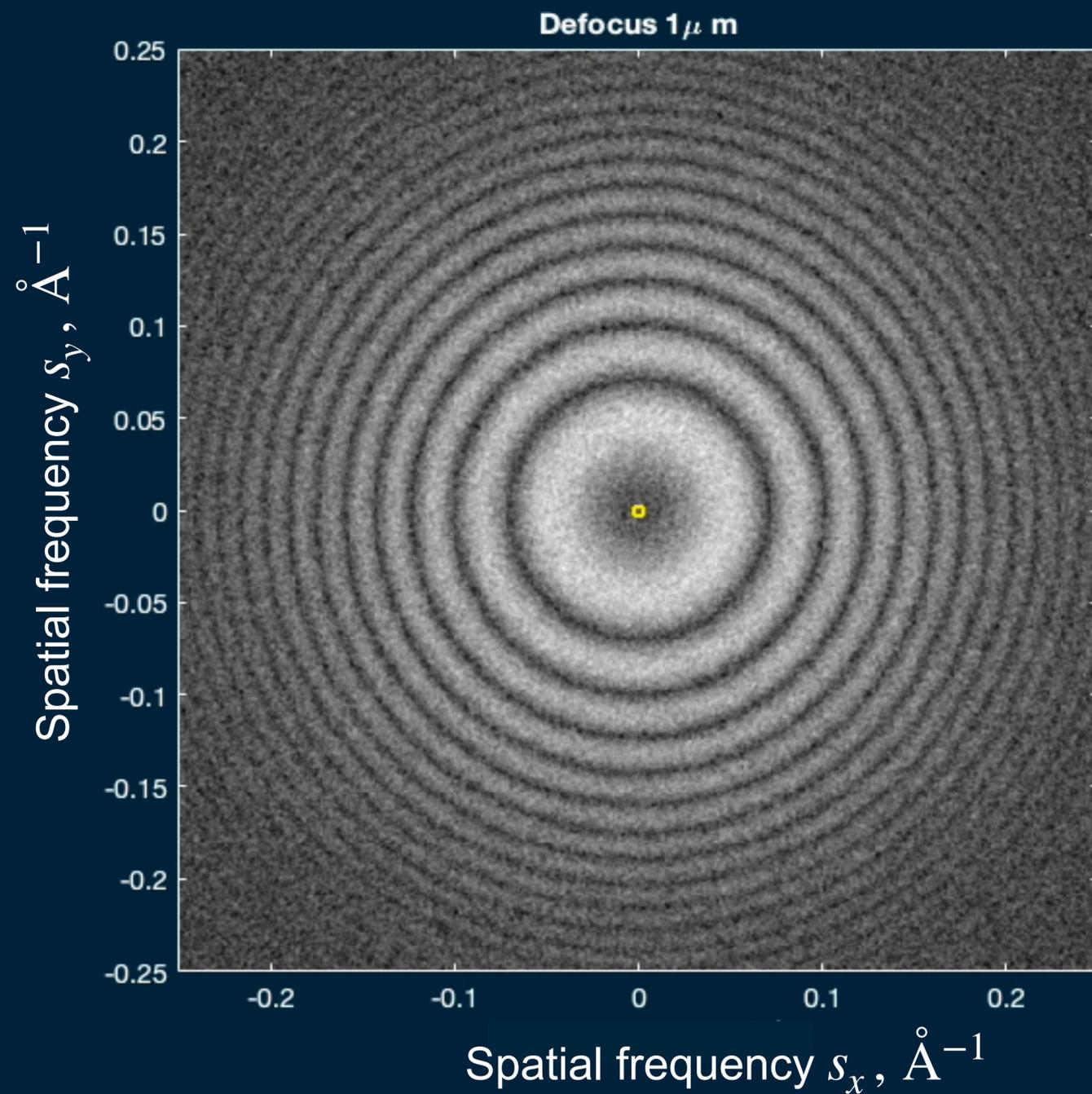
Defocus contrast: a summary

1. Electrons have really short wavelengths, and they travel through the column one by one.

2. The grating object produces diffracted waves with shifting phase

3. When the phase of the diffracted waves is right, we have contrast.

4. A lens reproduces the wavefronts at the image plane.

5. Spherical aberration and amplitude contrast introduce new terms in the CTF.

6. A phase plate alters the wavefronts after they've passed through the lens.

II. Fourier transforms and their properties

1. Fourier transform in 1D

2. Fourier transform in 2D

3. The Fourier slice theorem and reconstruction

Reconstruction of a Gaussian function from cosine waves
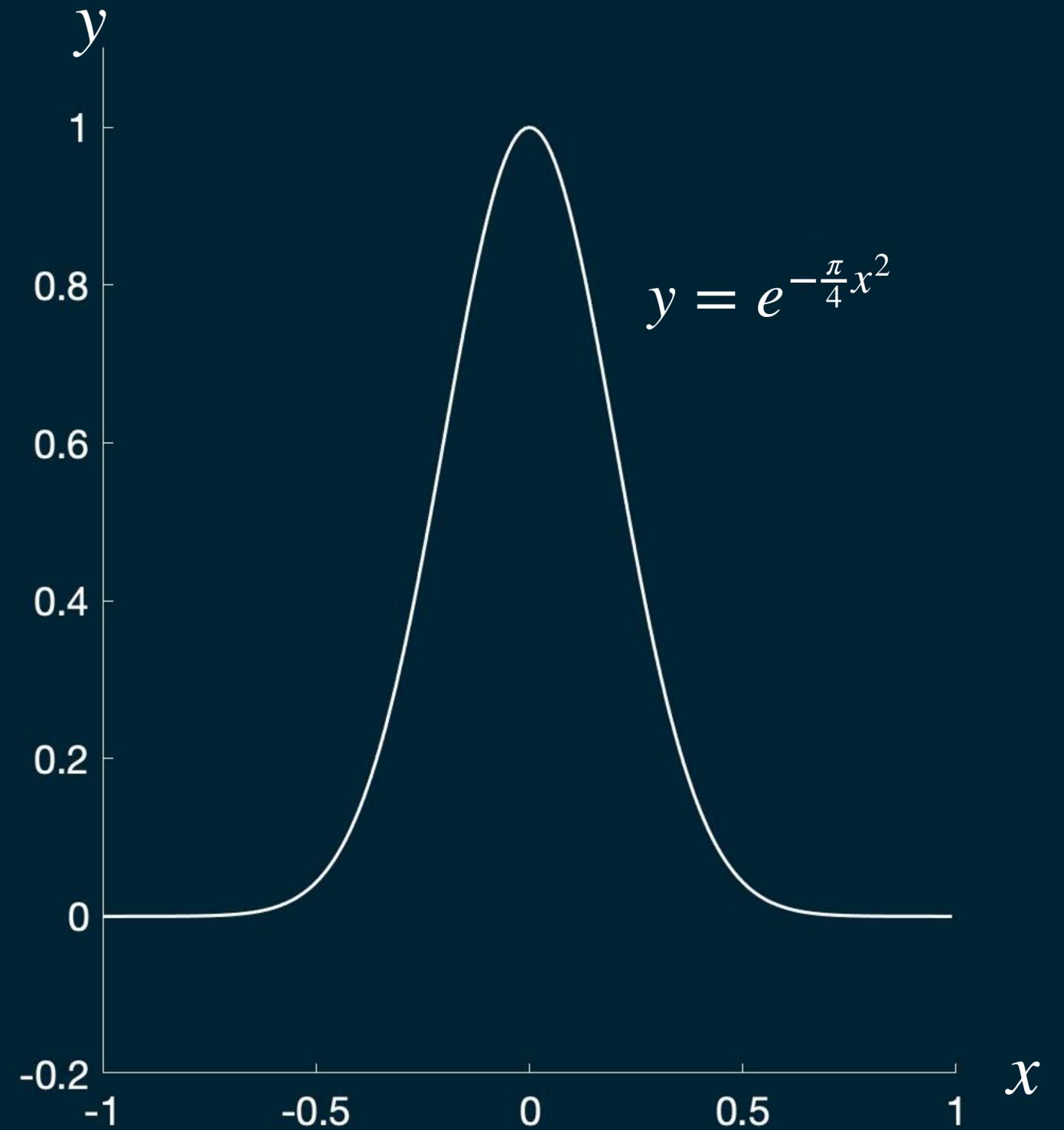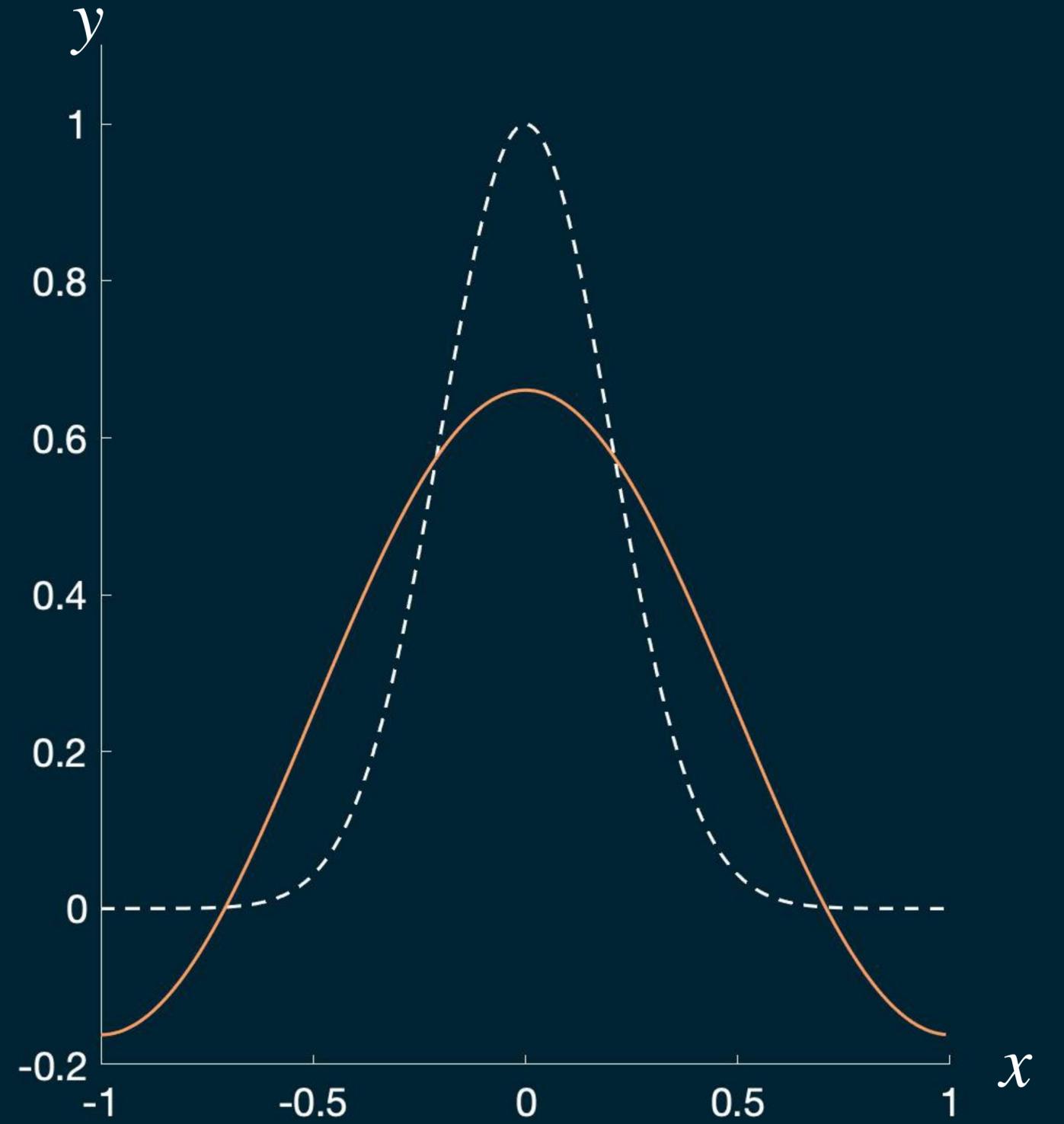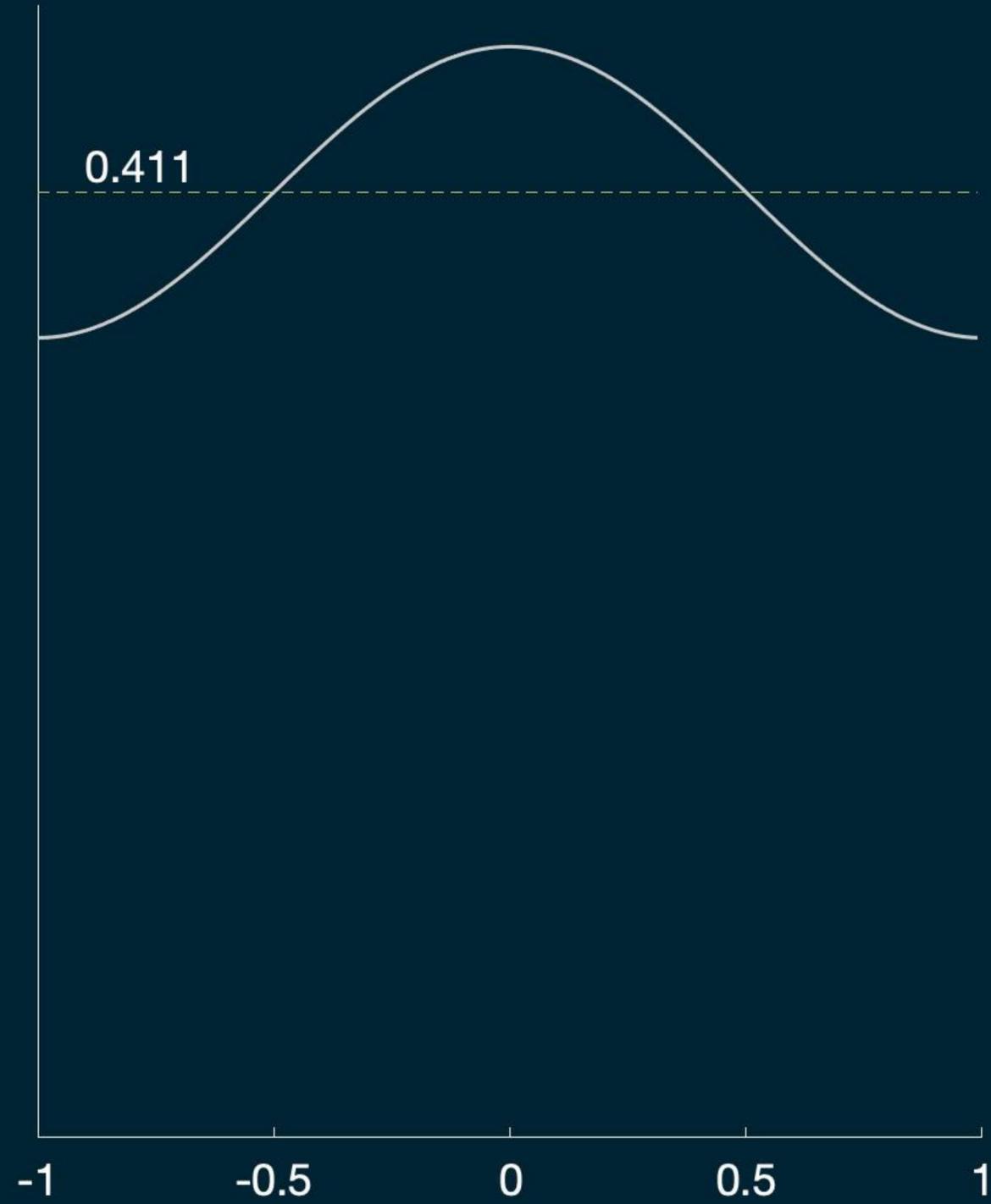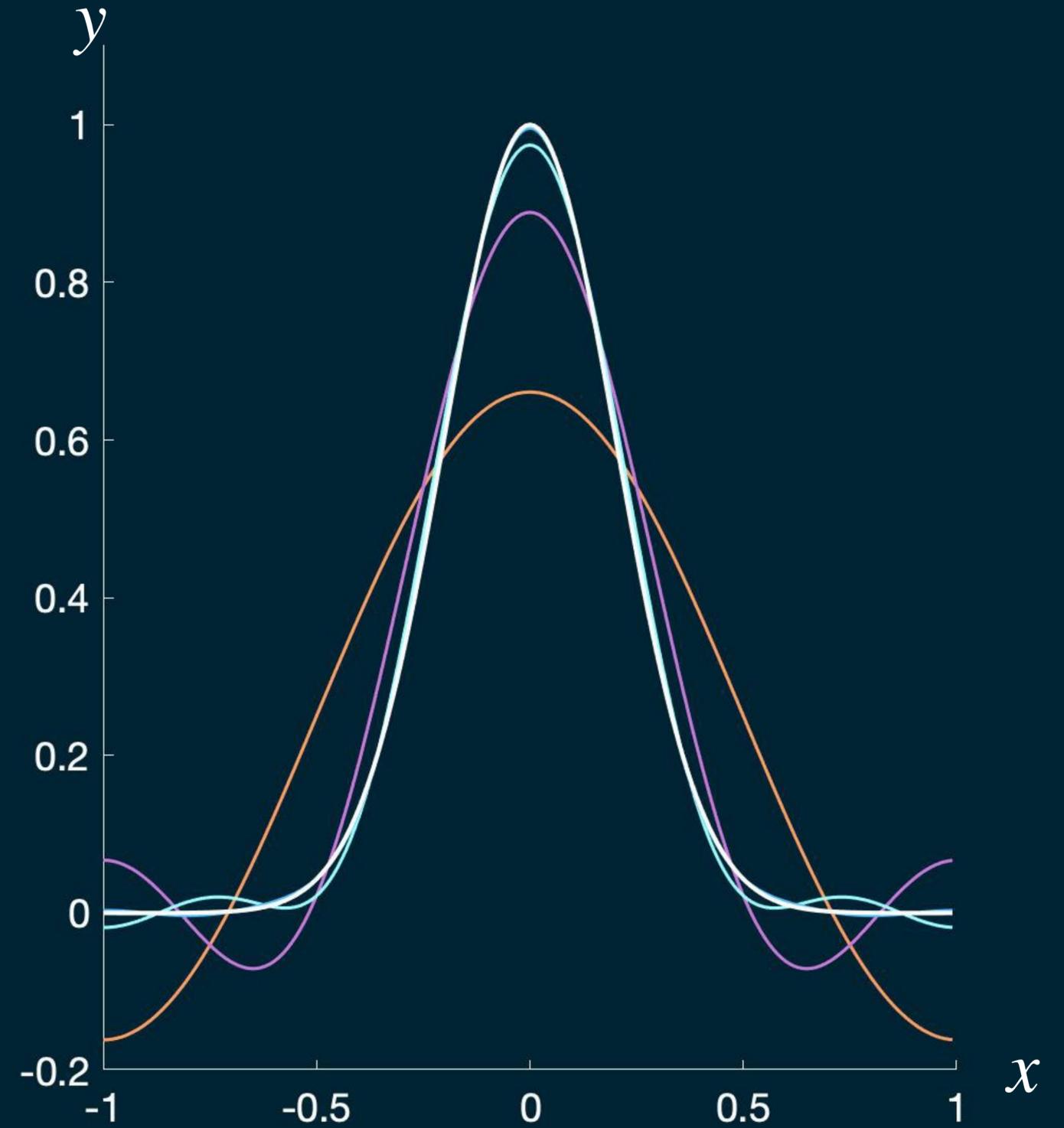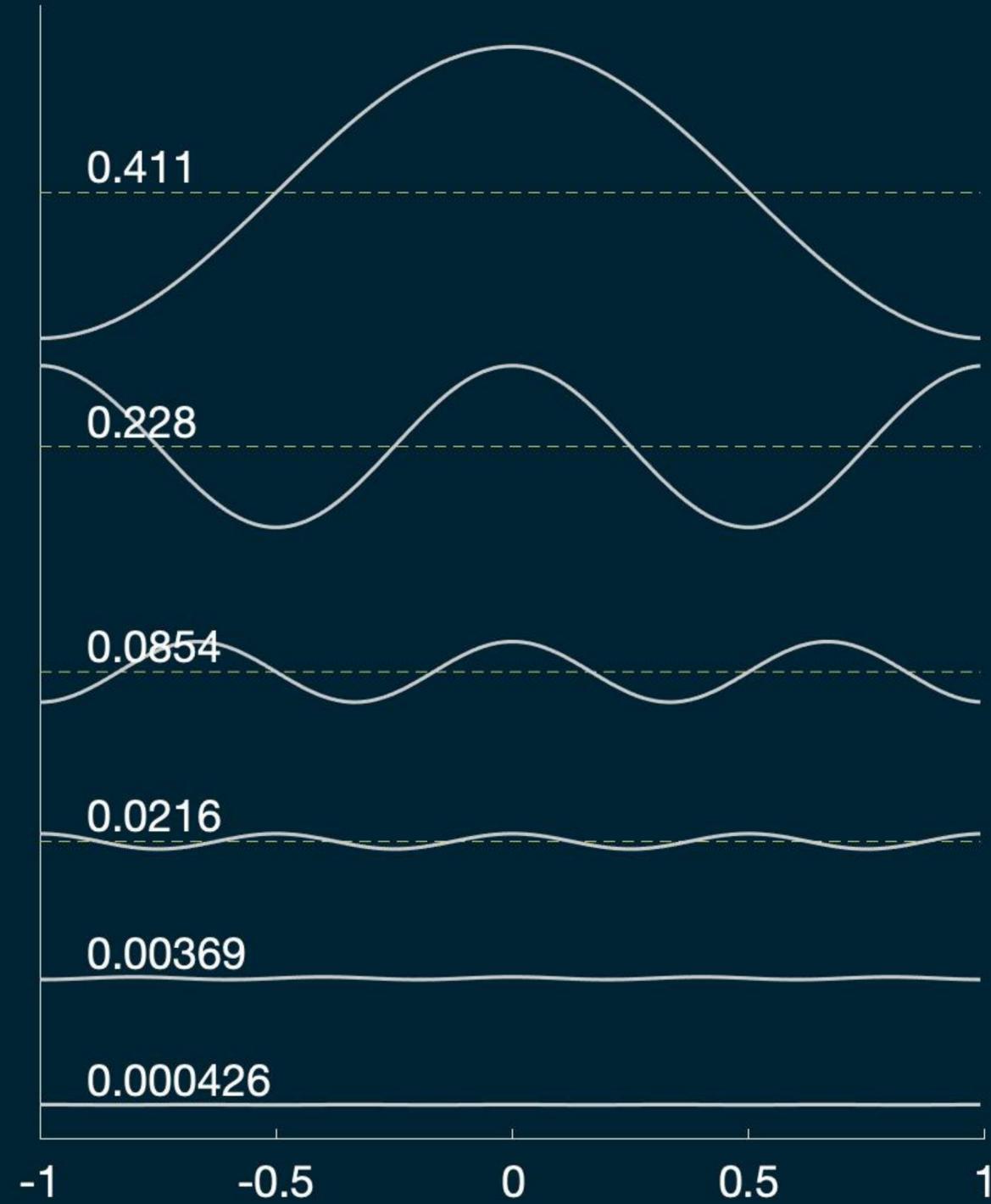
$y = e^{-\frac{\pi}{4}x^2}$

0.411

0.228

0.0854

0.0216

0.00369

0.000426

$y = e^{-\pi x^2}$

$g(x)$ → FT → $G(u)$

y

x

u
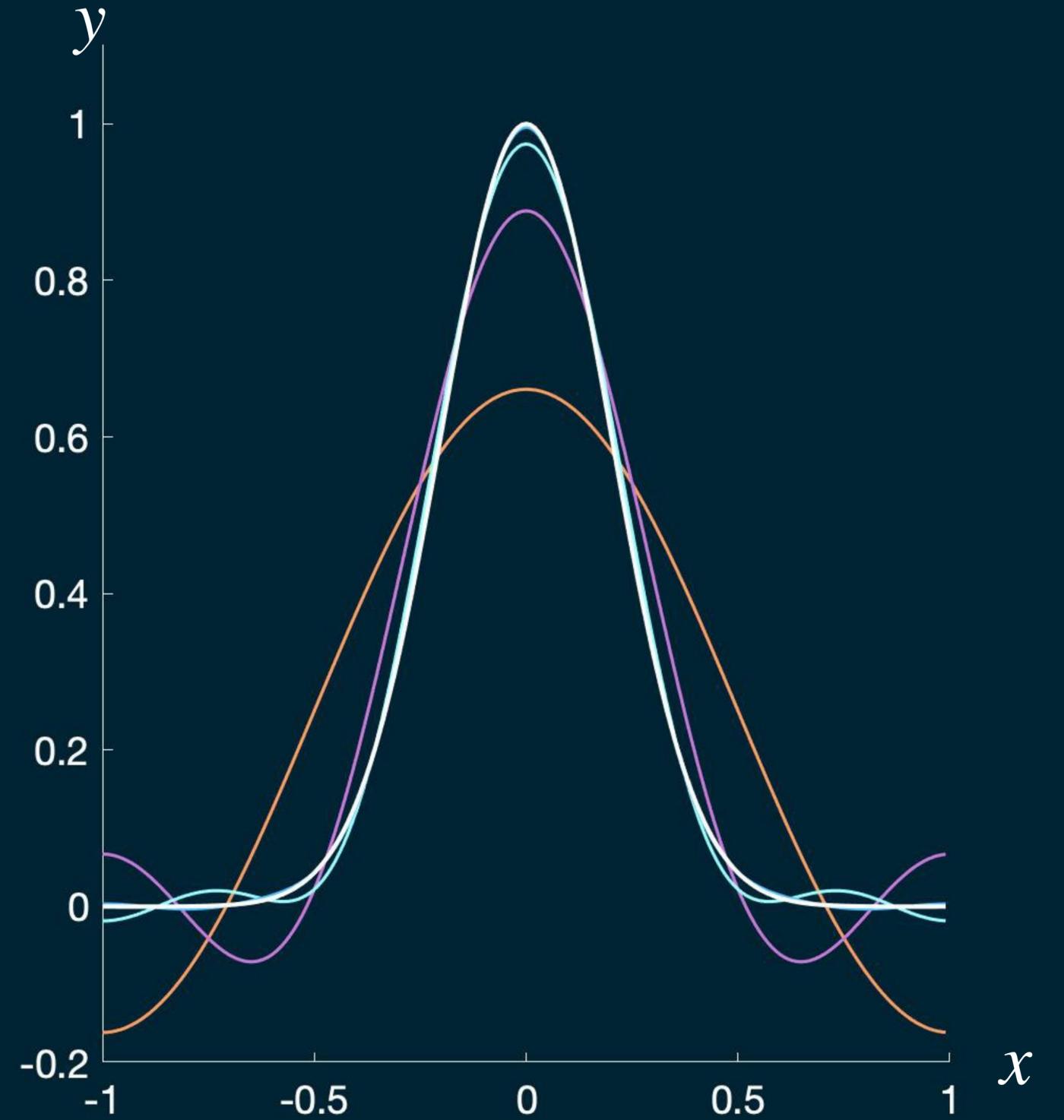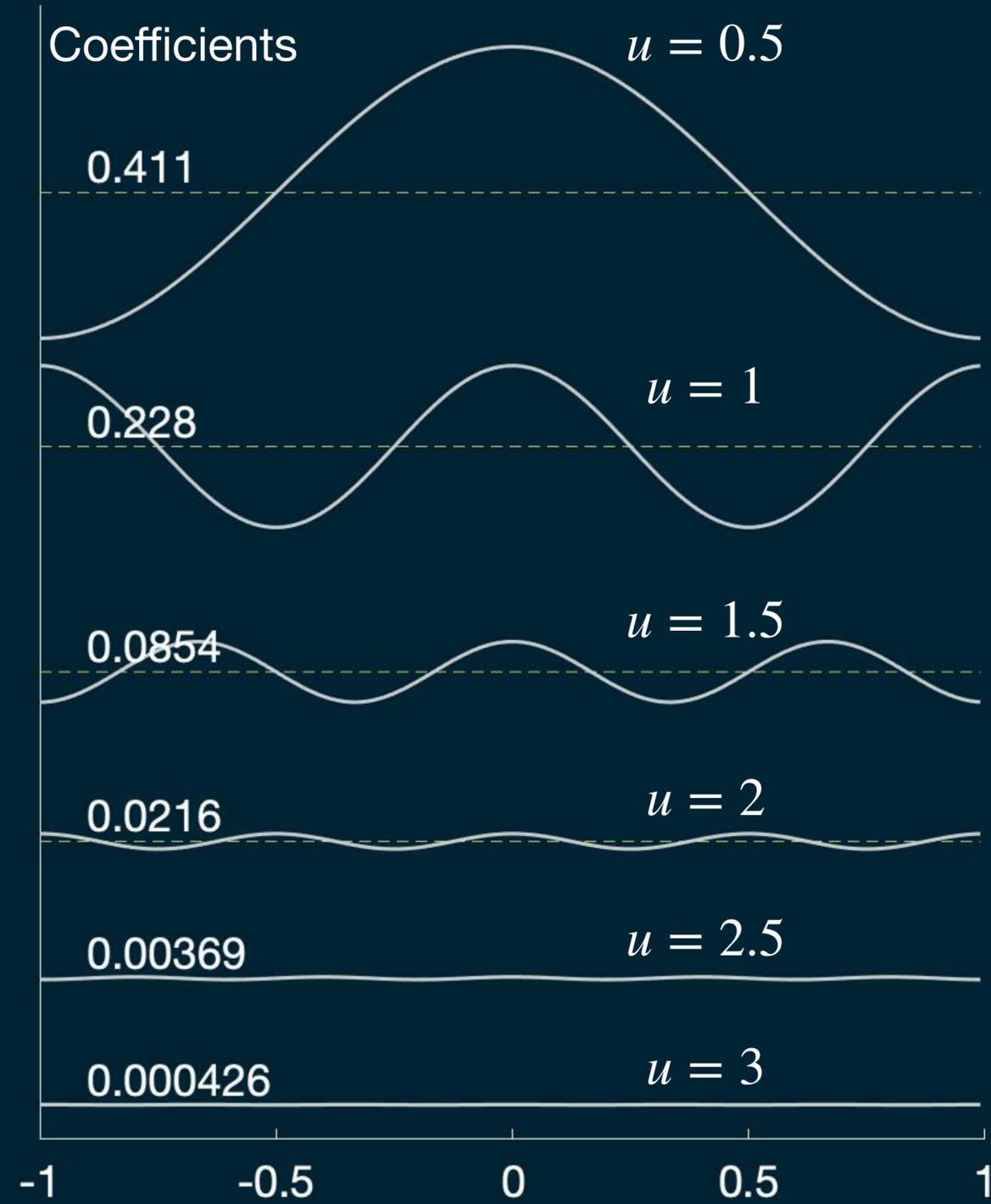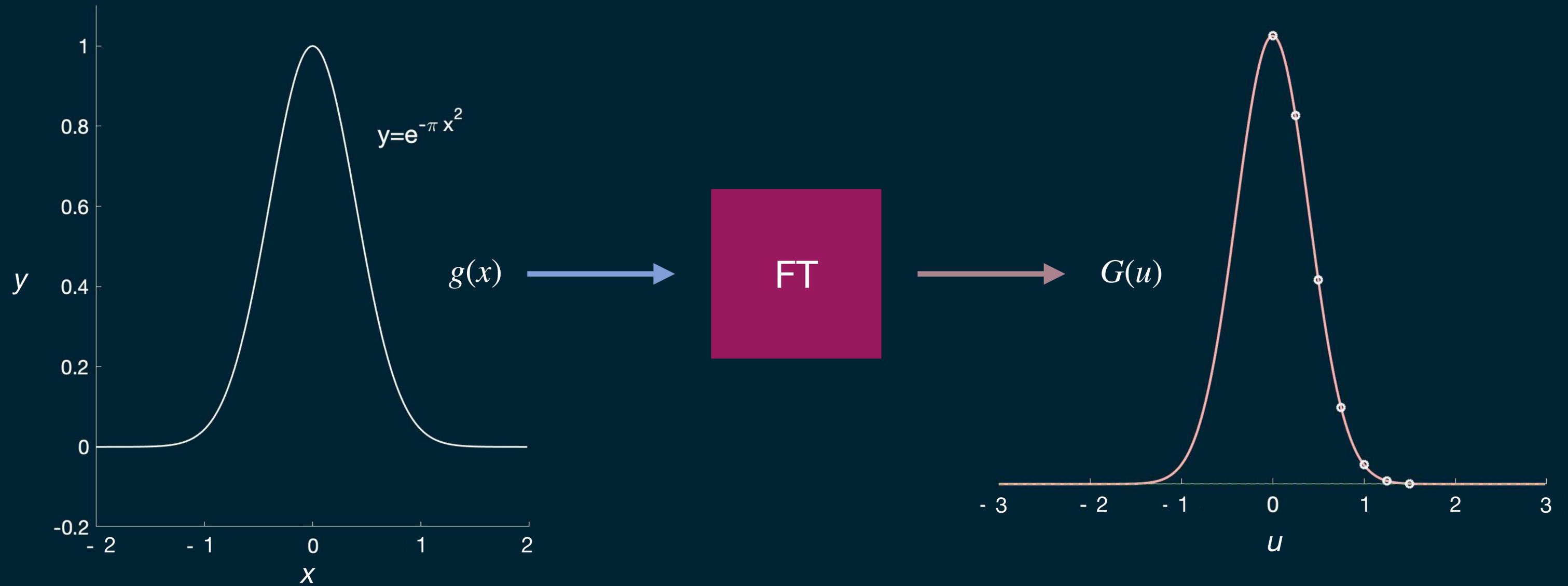
A transform pair $e^{-\pi x^2} \rightarrow e^{-\pi u^2}$

Fourier transform

$$G(u) = \int g(x)e^{-i2\pi ux}dx$$

Inverse Fourier transform

$$g(x) = \int G(u)e^{+i2\pi ux}du$$

Example:

$$g(x) = e^{-\pi x^2}$$

$$G(u) = e^{-\pi u^2}$$

Fourier reconstruction of a rectangular function

y=rect(x)

0.637

0

-0.212

0

0.127

0

-0.0906

0

0.0703

0
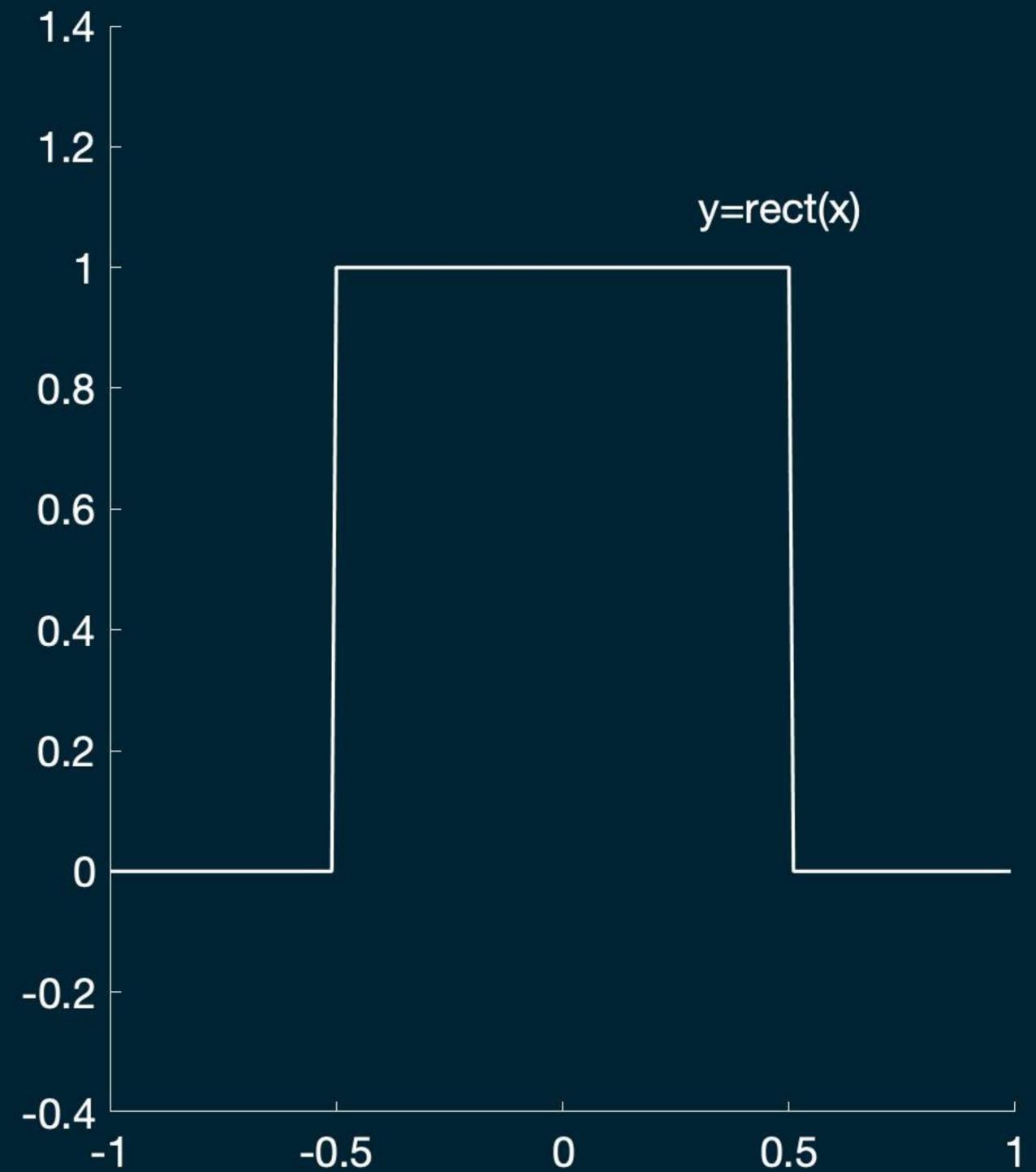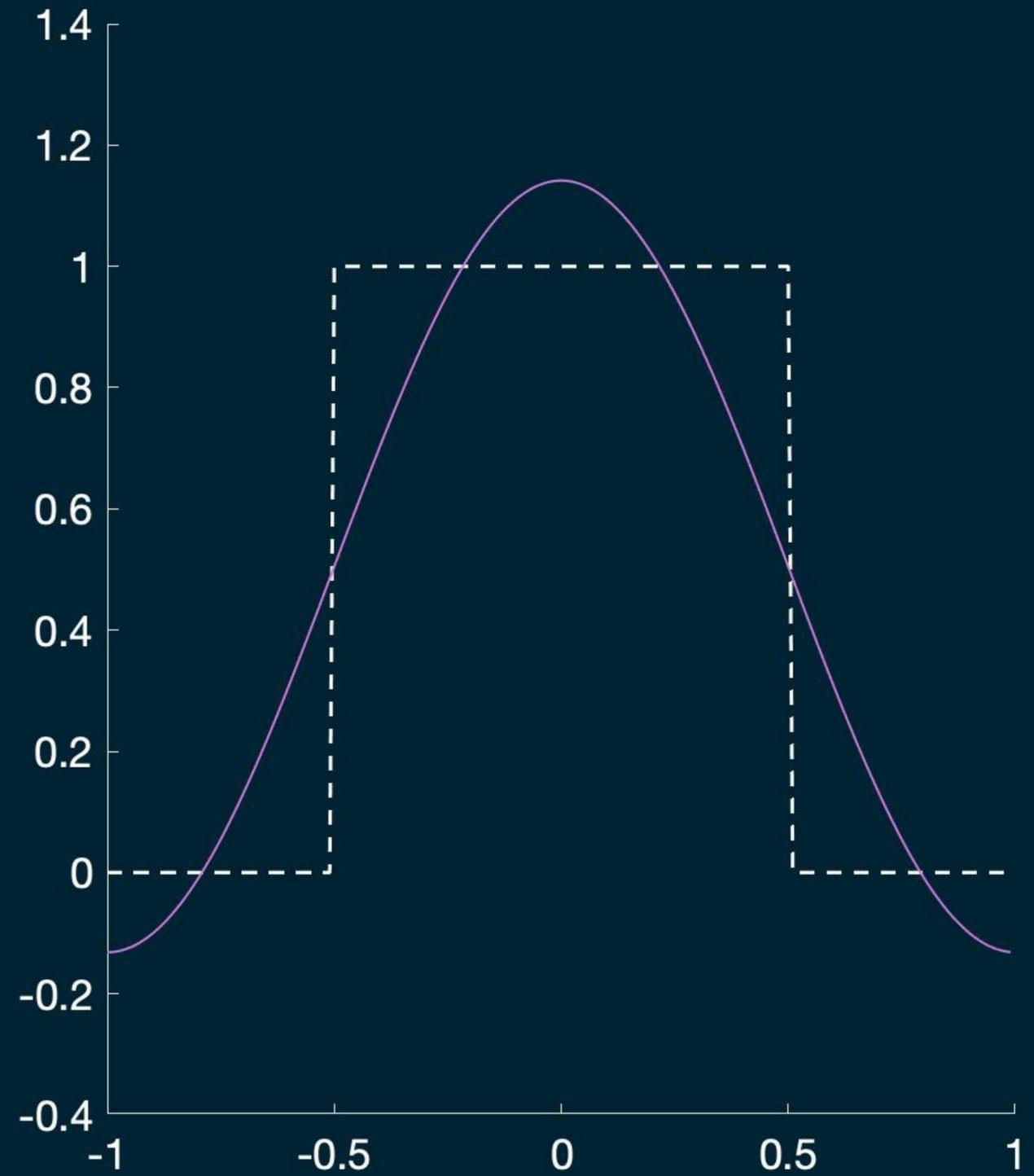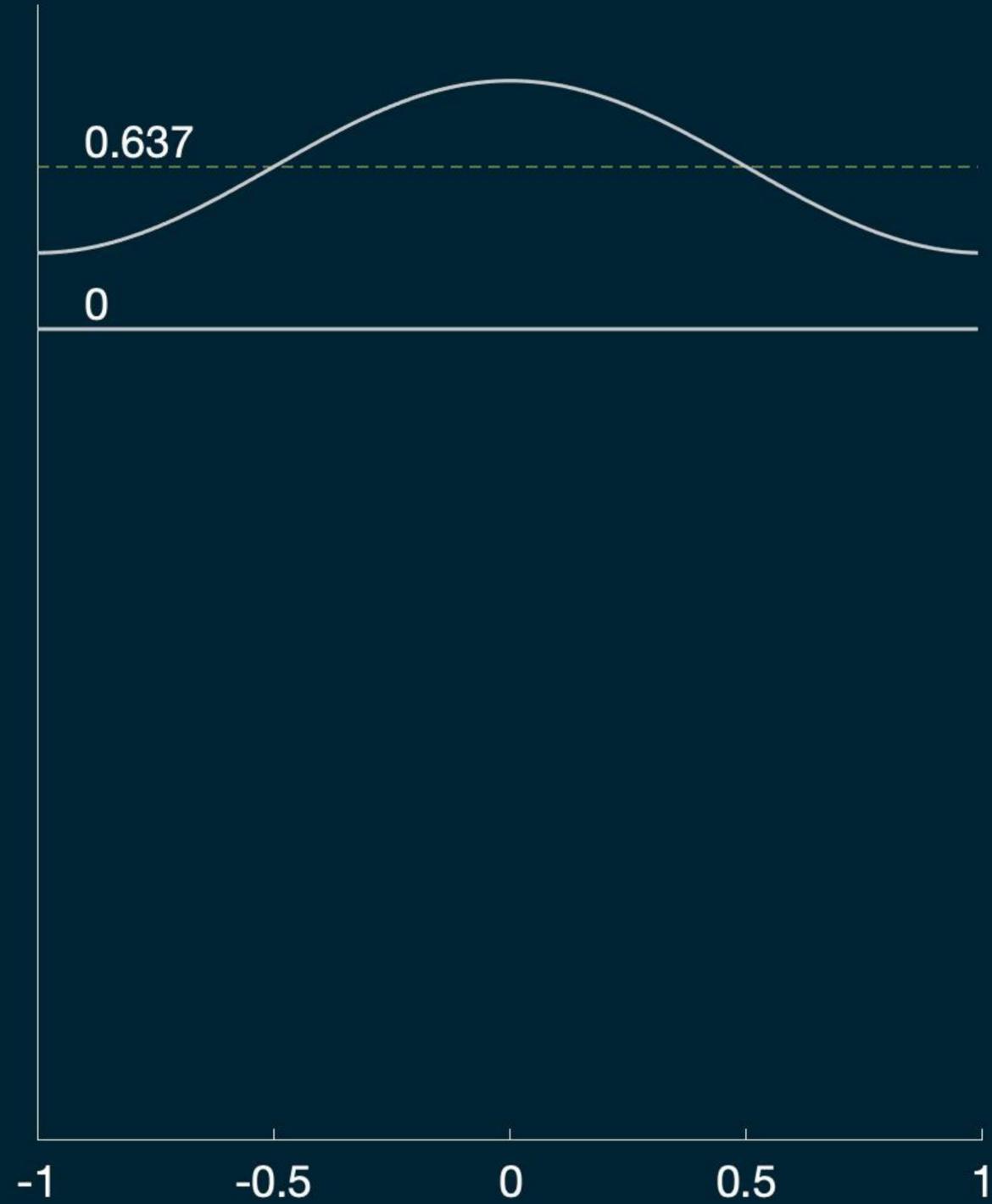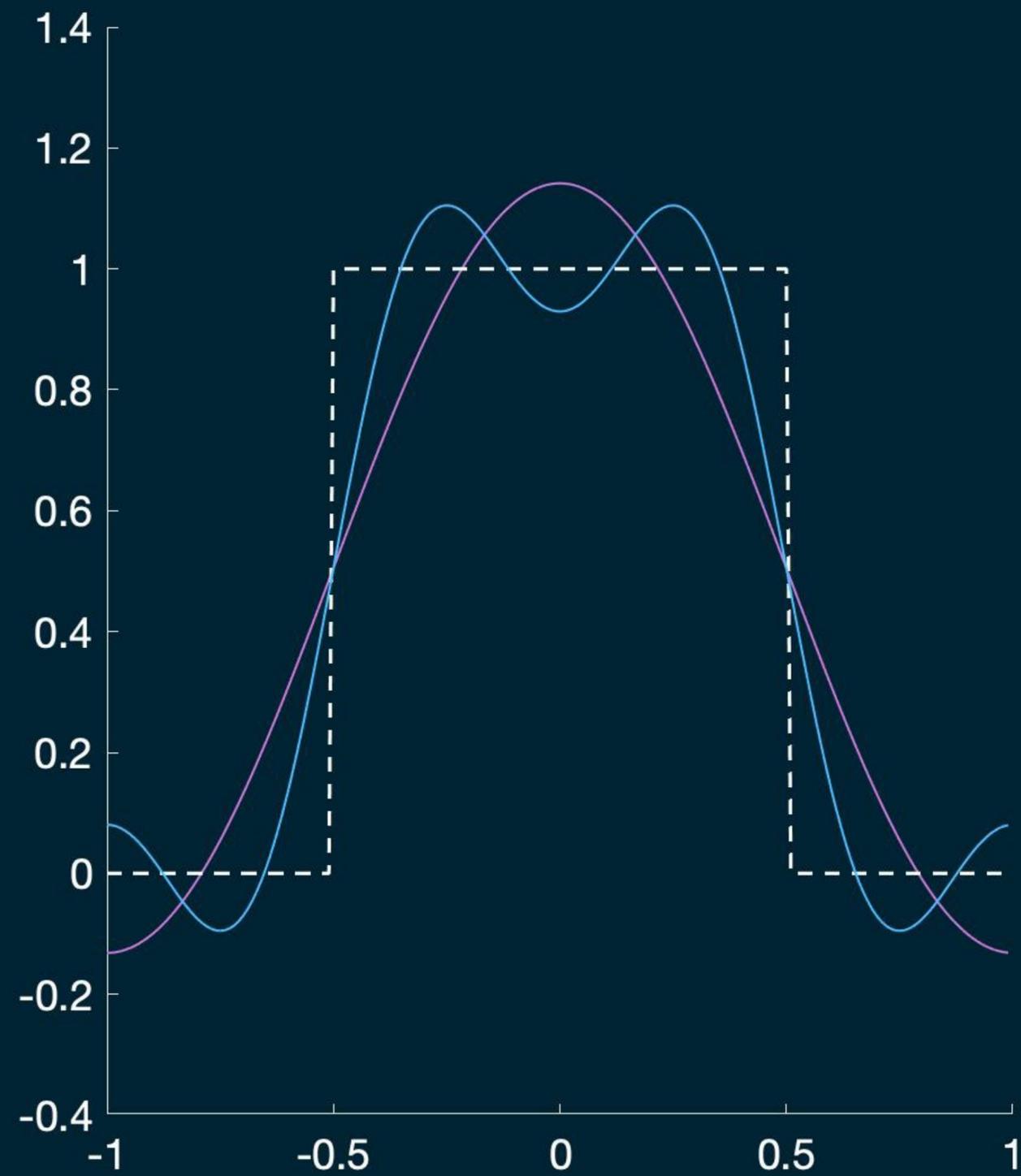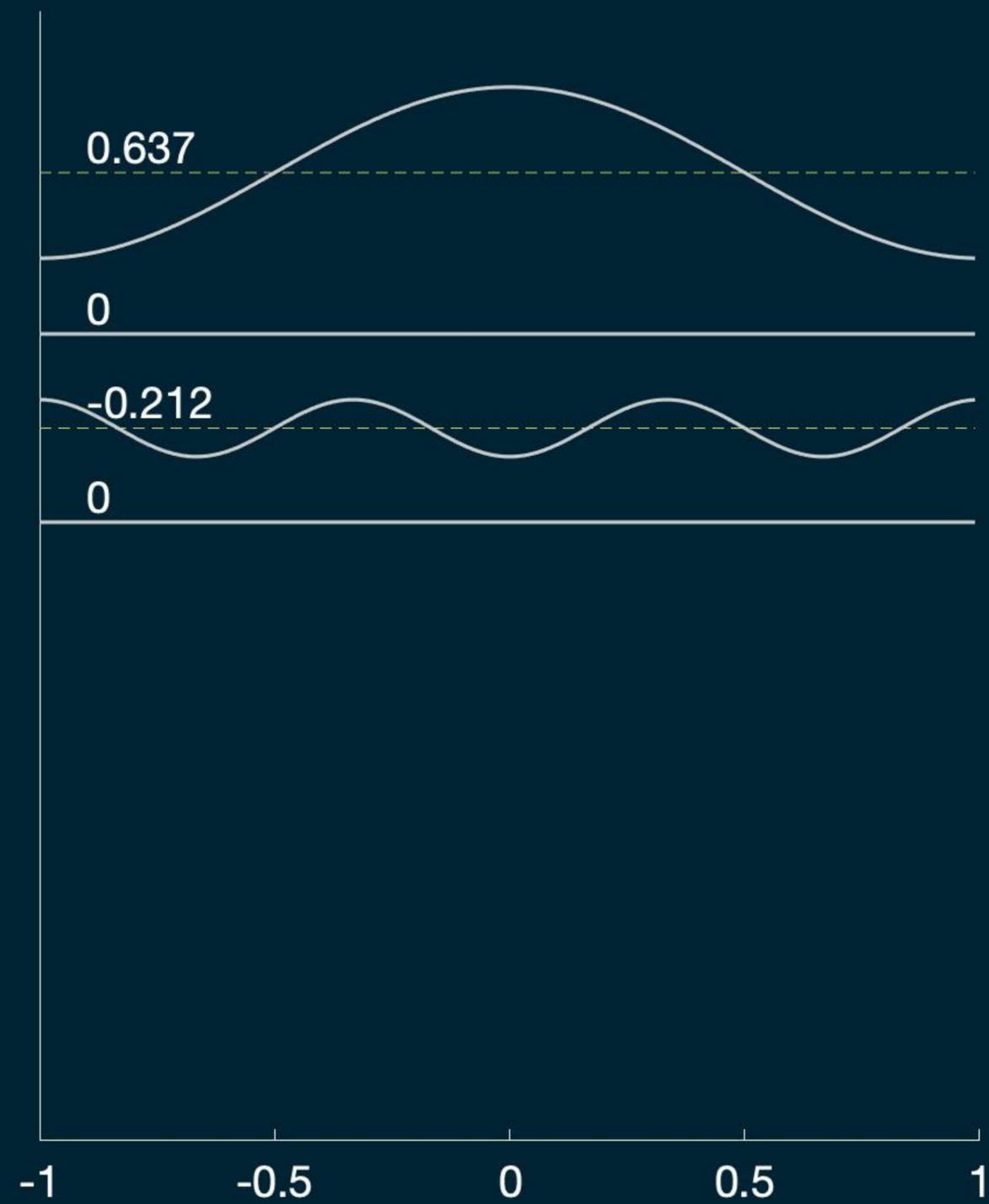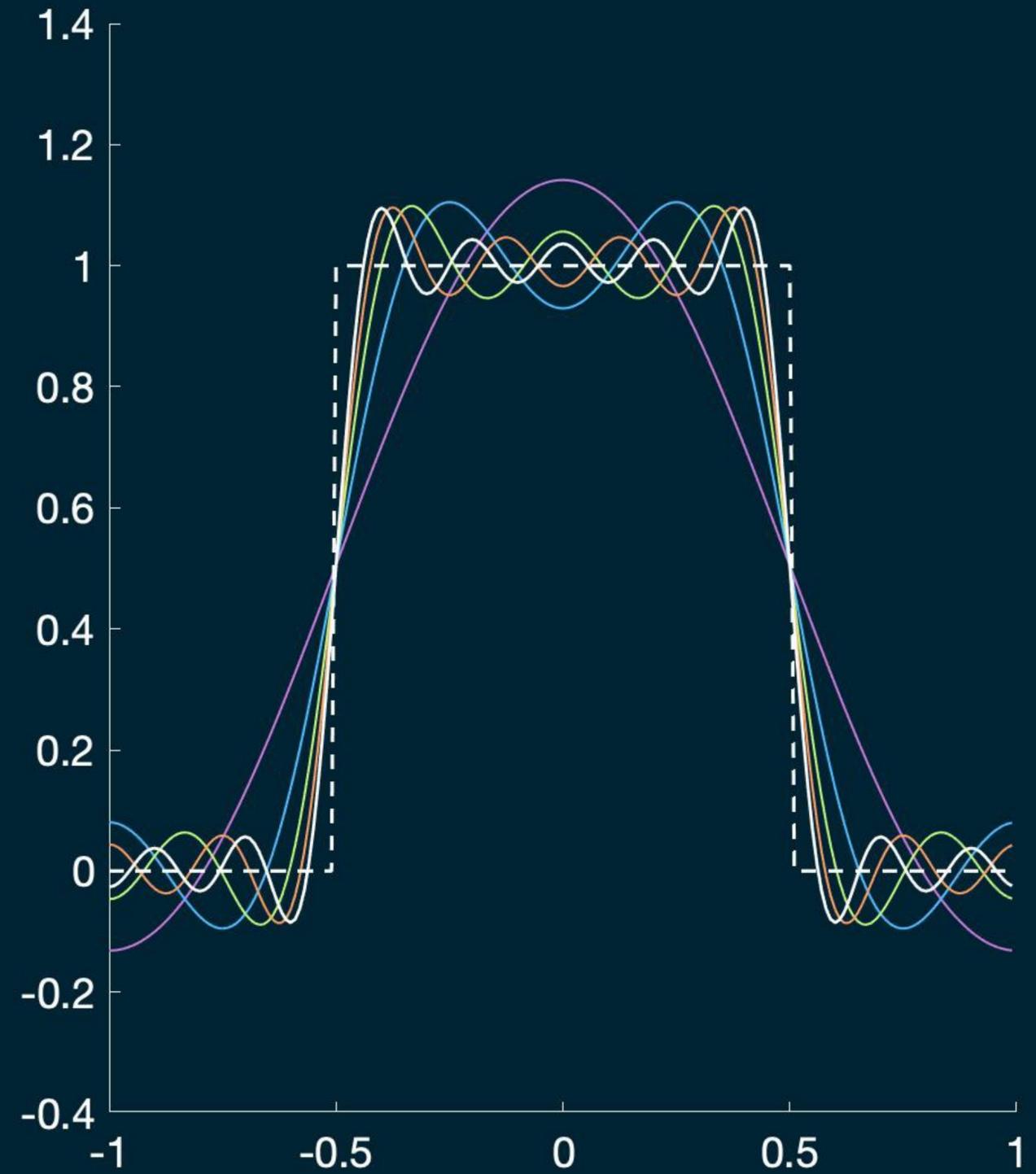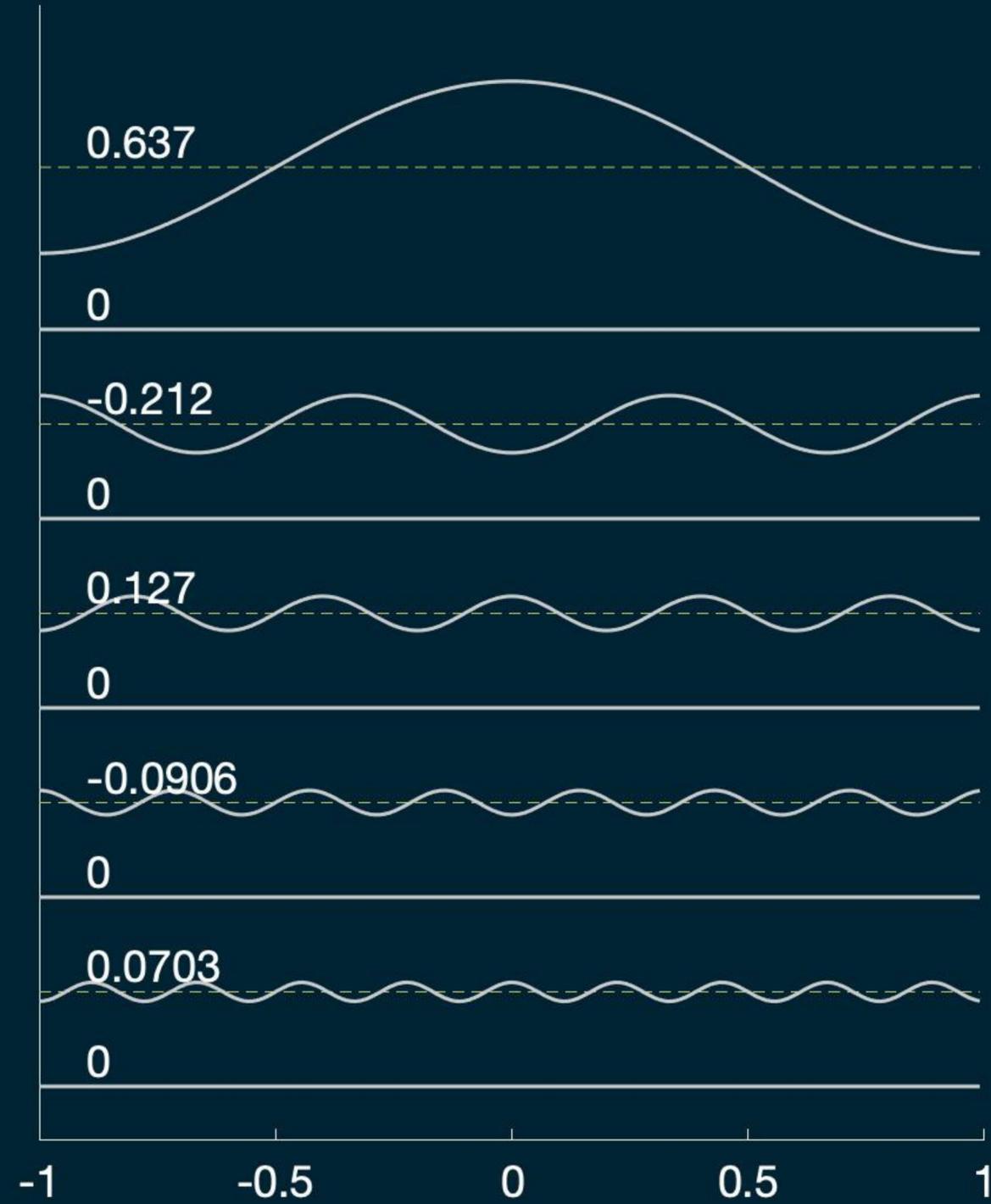
The Fourier Transform of rect(*x*) is sinc(*u*)

$g(x)$

FT

$G(u)$
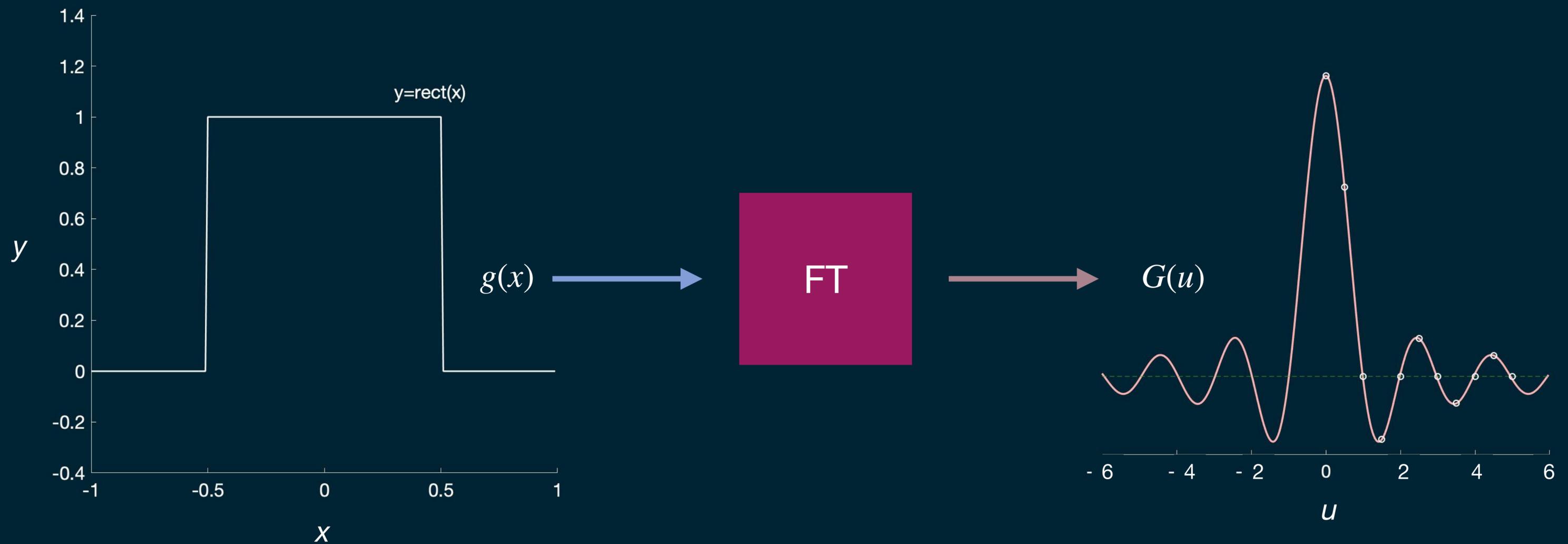
$$\text{rect}(x) \rightarrow \frac{\sin(\pi u)}{\pi u}$$

$\dfrac{\sin(\pi u)}{\pi u}$ is also known as: $\text{sinc}(u)$

$$e^{-\pi x^2} \rightarrow e^{-\pi u^2}$$

$$\text{rect}(x) \rightarrow \frac{\sin(\pi u)}{\pi u}$$

1D Fourier transform properties

$$g(x) + h(x) \rightarrow G(x) + H(x)$$ **Linearity**

$$ag(ax) \rightarrow G(u/a)$$ **Scale**

$$g(x - b) \rightarrow G(u)e^{-i2\pi ub}$$ **Shift**

$$g \star h \rightarrow G(u)H(u)$$ **Convolution**

Convolution

$$f(x) = g \star h \text{ means:}$$

$$f(x) = \int g(x - s)h(s)ds$$

II. Fourier transforms and their properties

Fourier reconstruction of a 2D Gaussian function
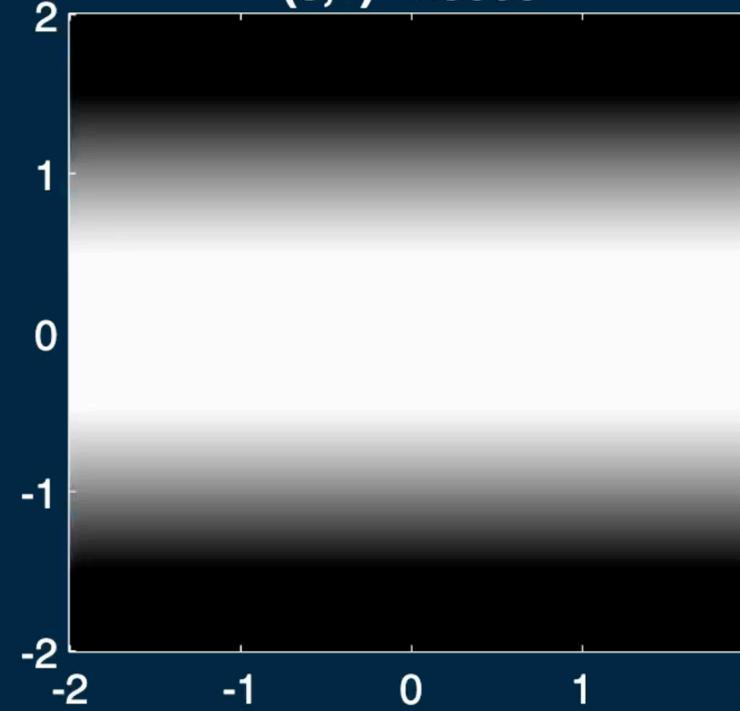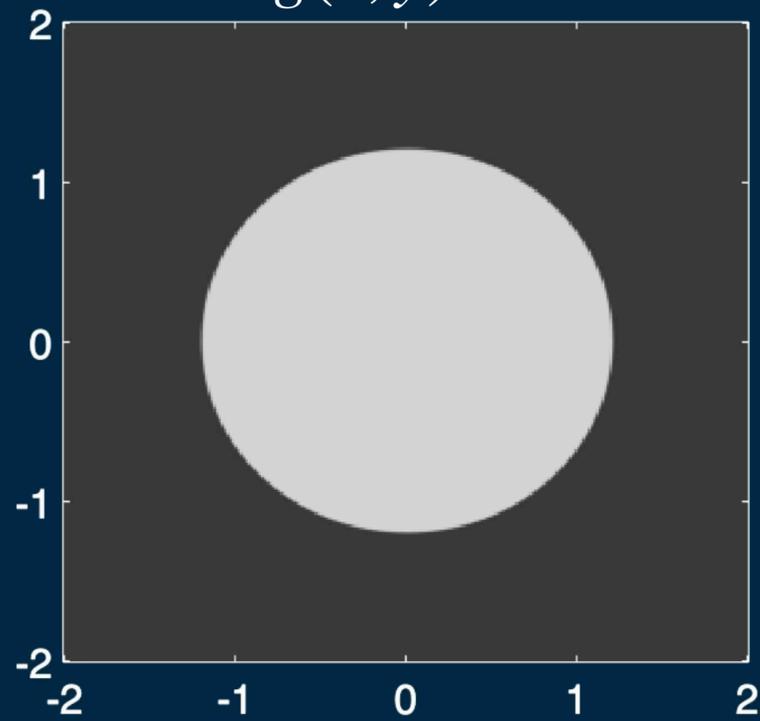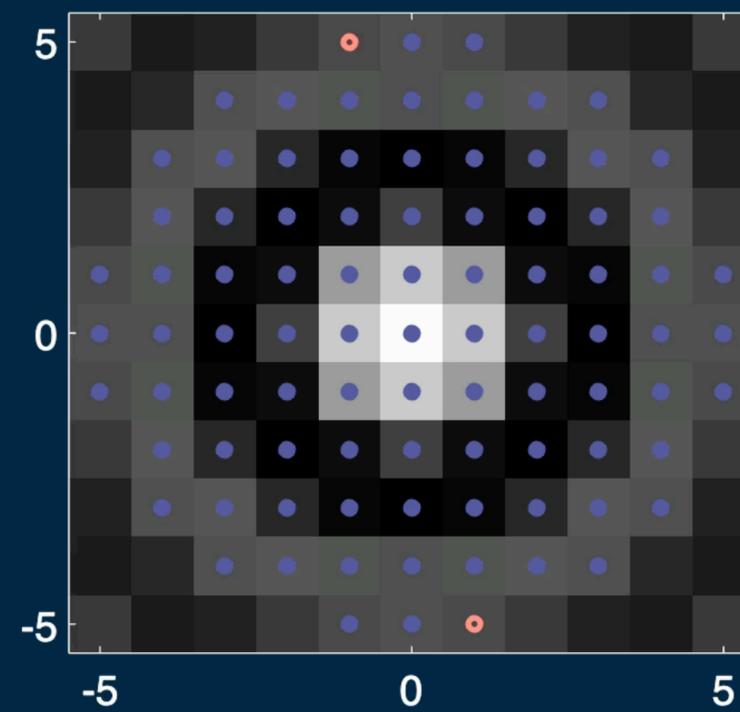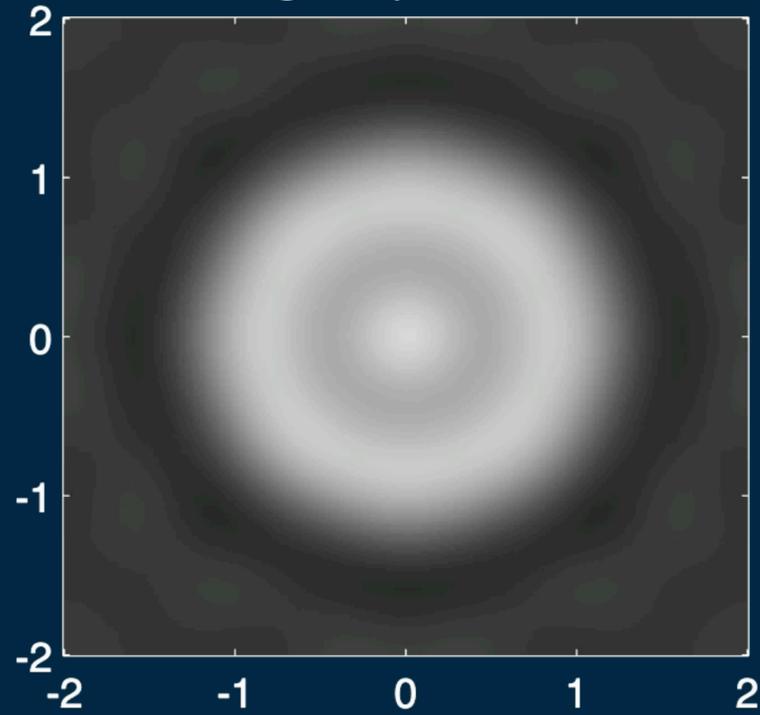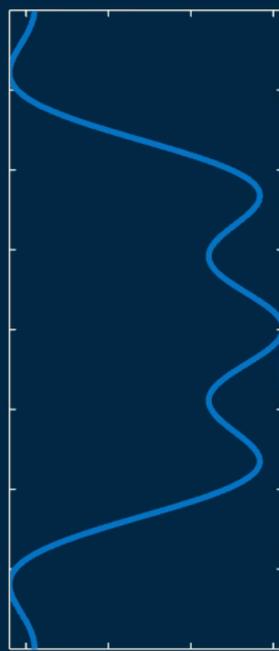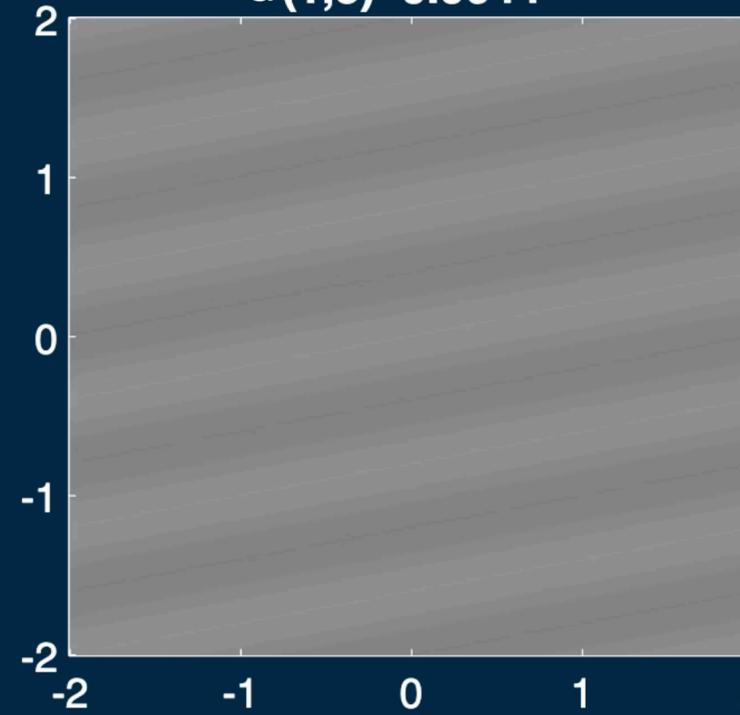
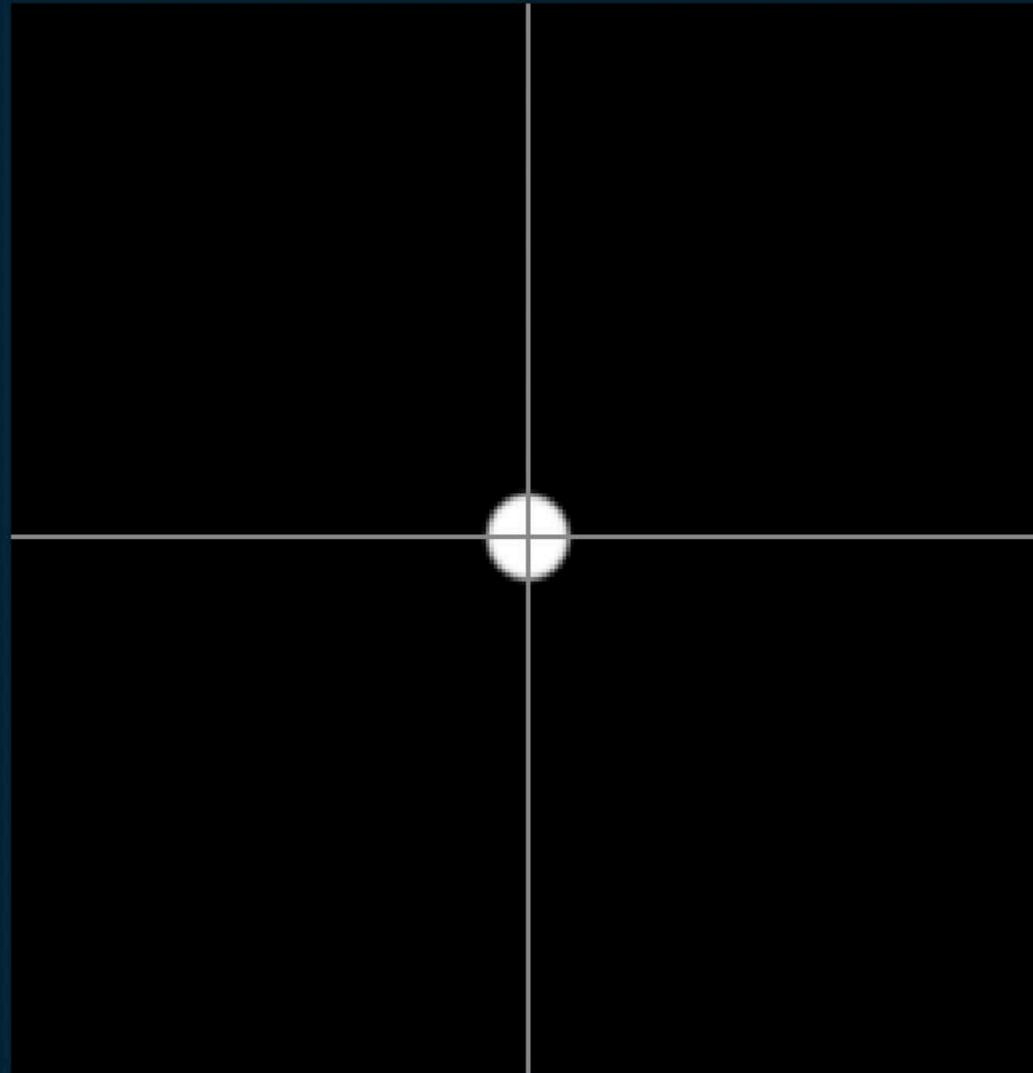# Fourier reconstruction of a 2D Gaussian function

Projection

$g(x, y)$

$G(u, v)$

$\hat{g}(x, y)$

$G(2,0)=0.4559$
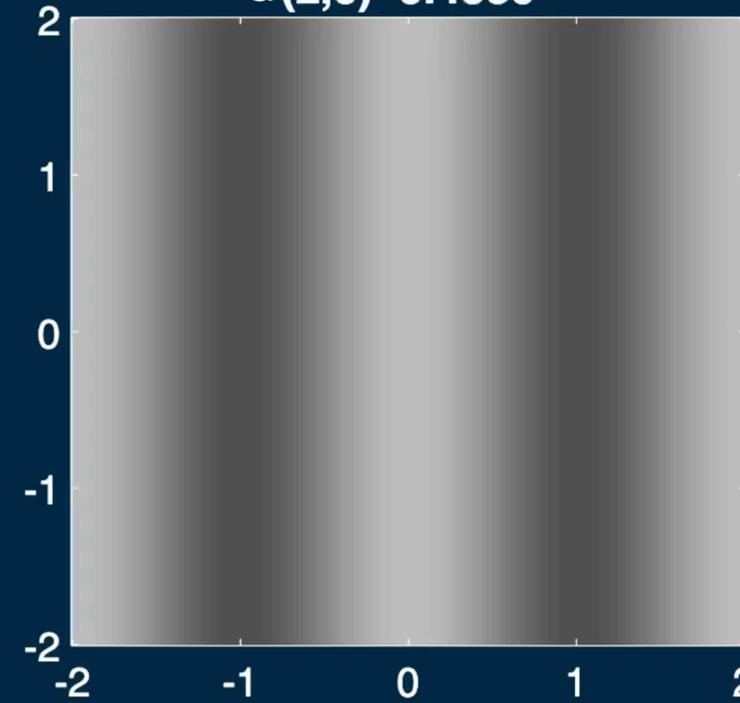
Fourier reconstruction of a 2D Gaussian function
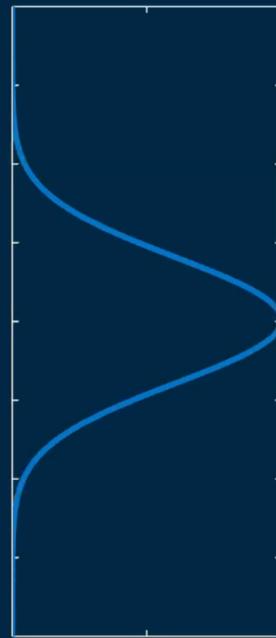
Projection

$g(x, y)$
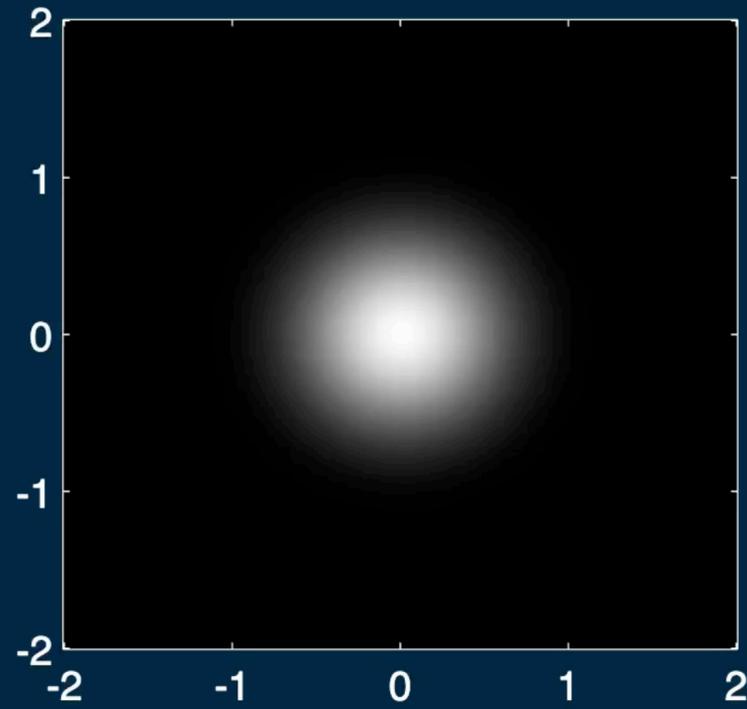
$G(u, v)$
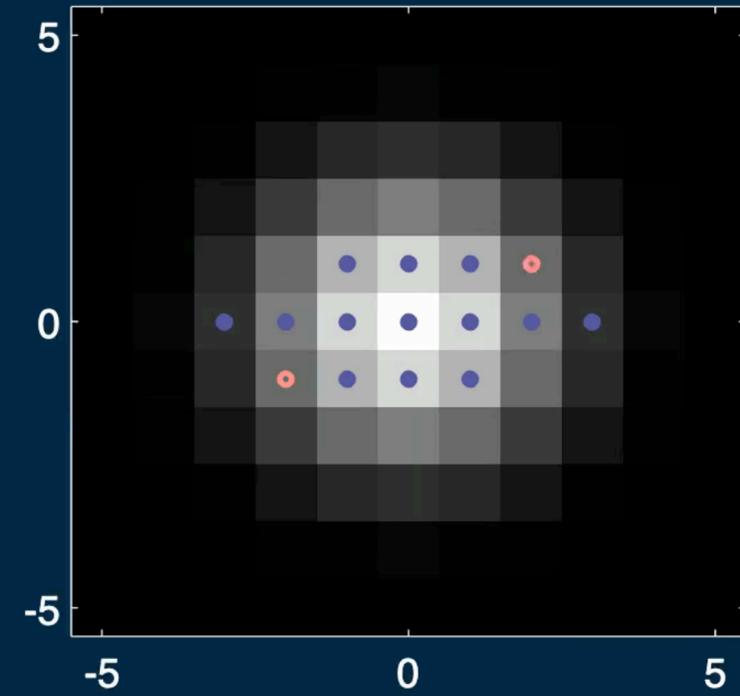
$\hat{g}(x, y)$
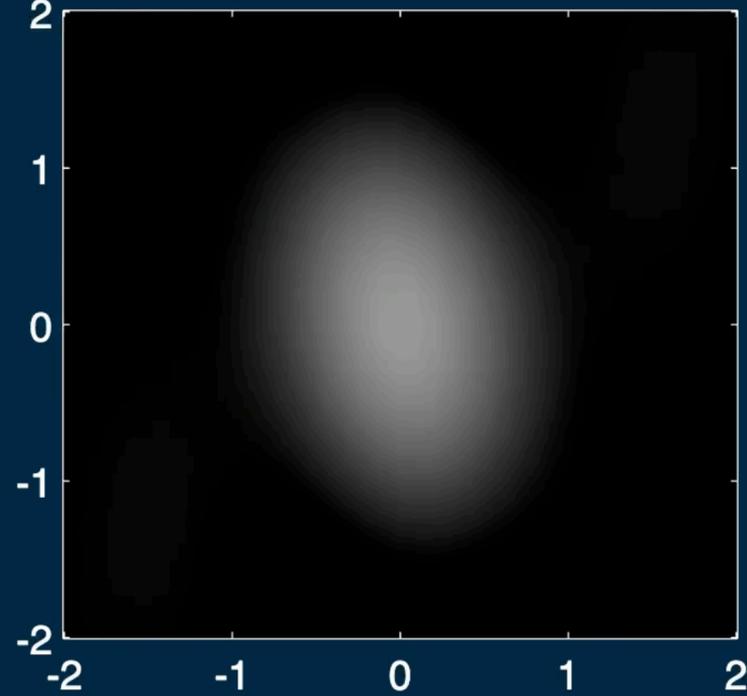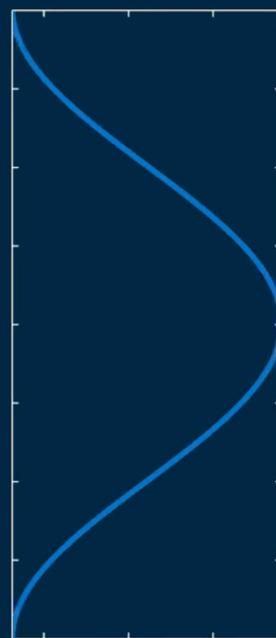
$G(2,1)=0.3747$

Fourier reconstruction of a 2D circ function

Fourier reconstruction of a 2D circ function

Fourier reconstruction of a 2D circ function

(a,b) = (0,0)

$$g(x-a, y-b)$$

$$G(u,v)e^{-i2\pi(au+bv)}$$

Complex number color scheme

Imaginary

1

0.5

-1   -0.5   0.5   1 Real

-0.5

-1

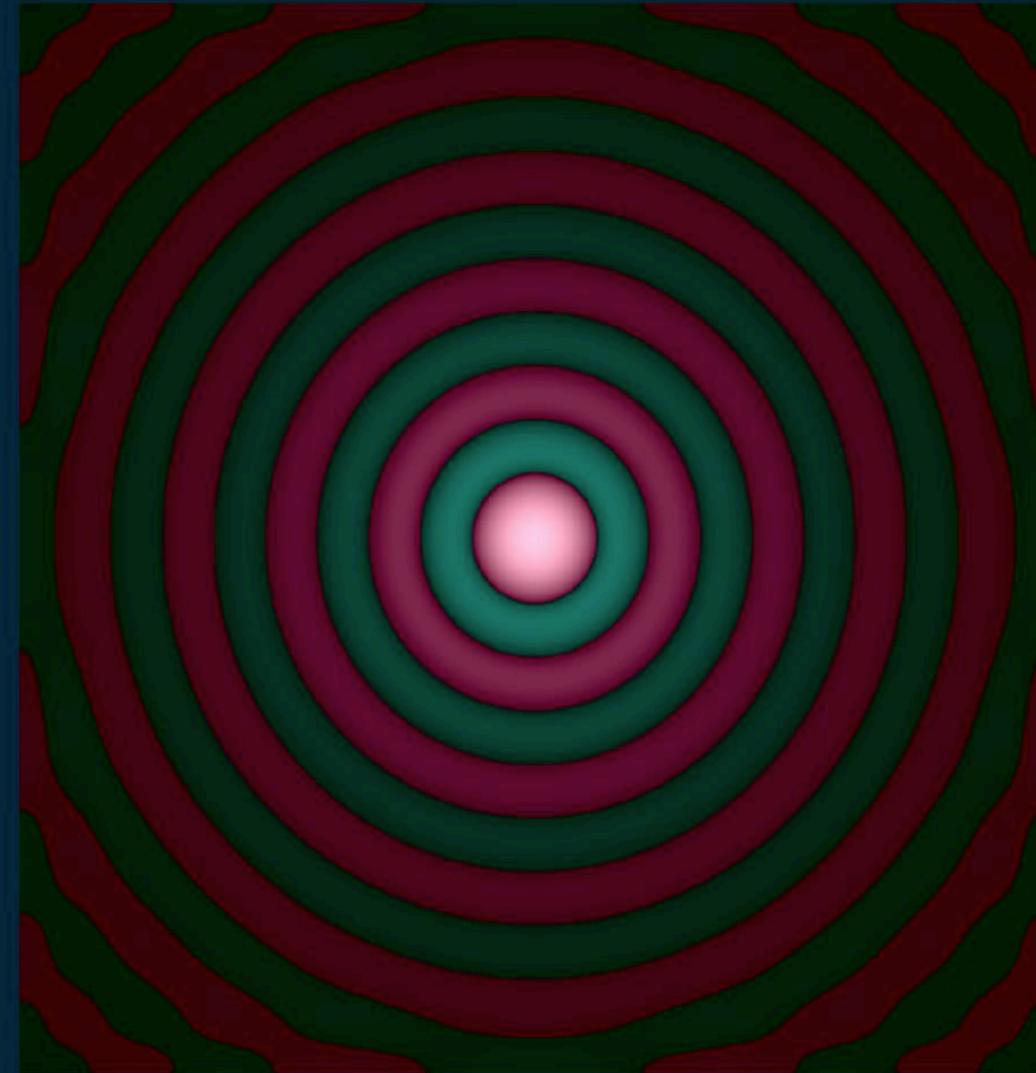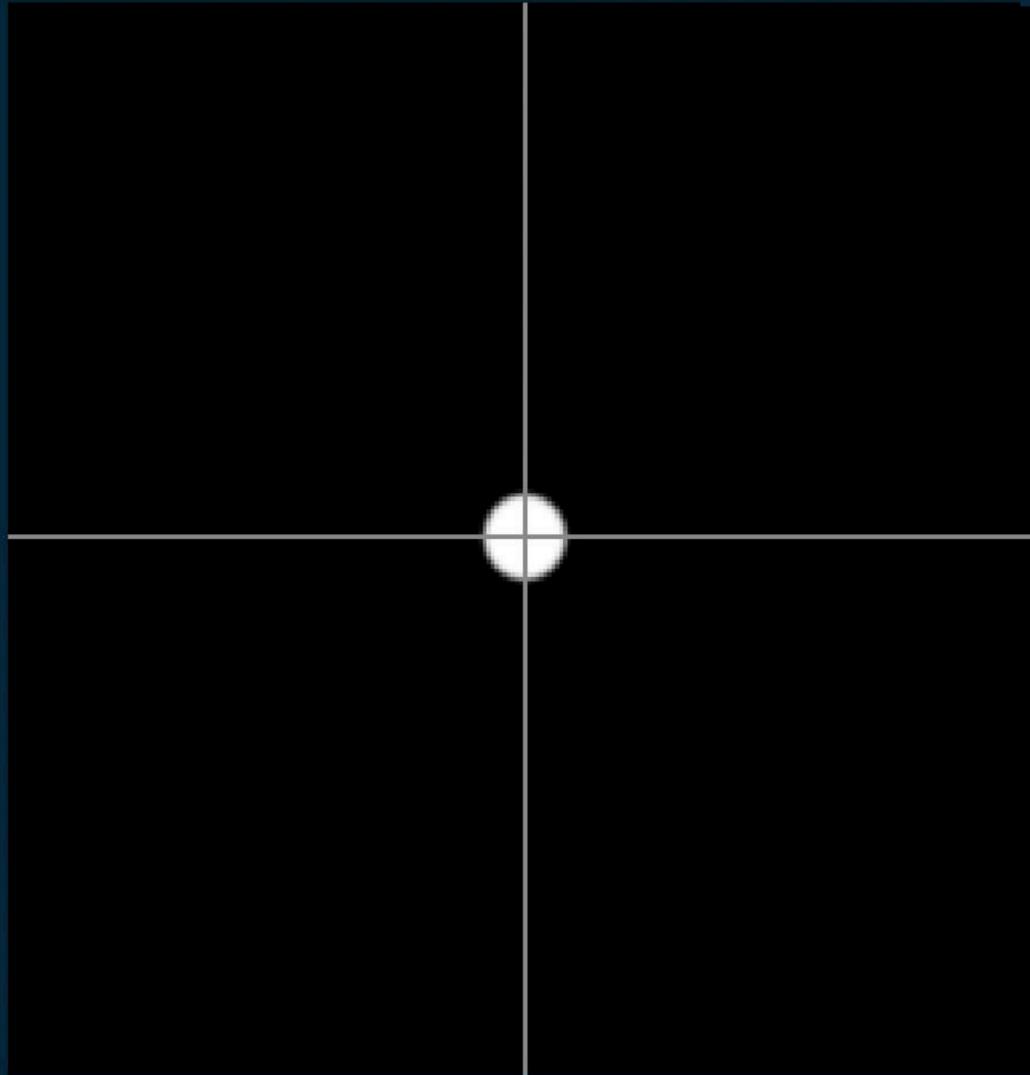**(a,b) = (0,0)**

$$g(x - a, y - b)$$

$$G(u, v)e^{-i2\pi(au+bv)}$$

Complex number color scheme

Imaginary

Real

2D Shift property

$$g(x-a, y-b)$$

$$G(u,v)e^{-i2\pi(au+bv)}$$

Complex number color scheme

Imaginary

1

0.5

-1   -0.5   0.5   1 Real

-0.5

-1
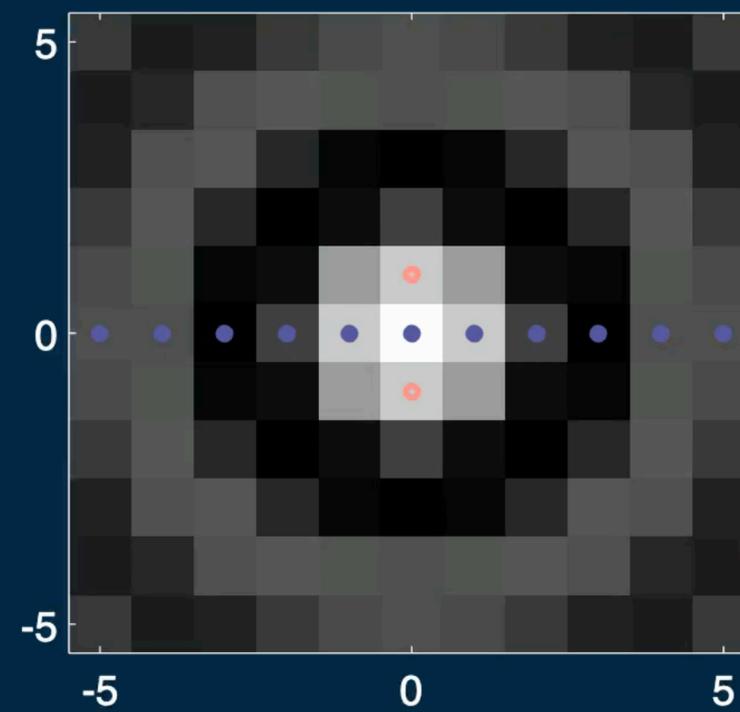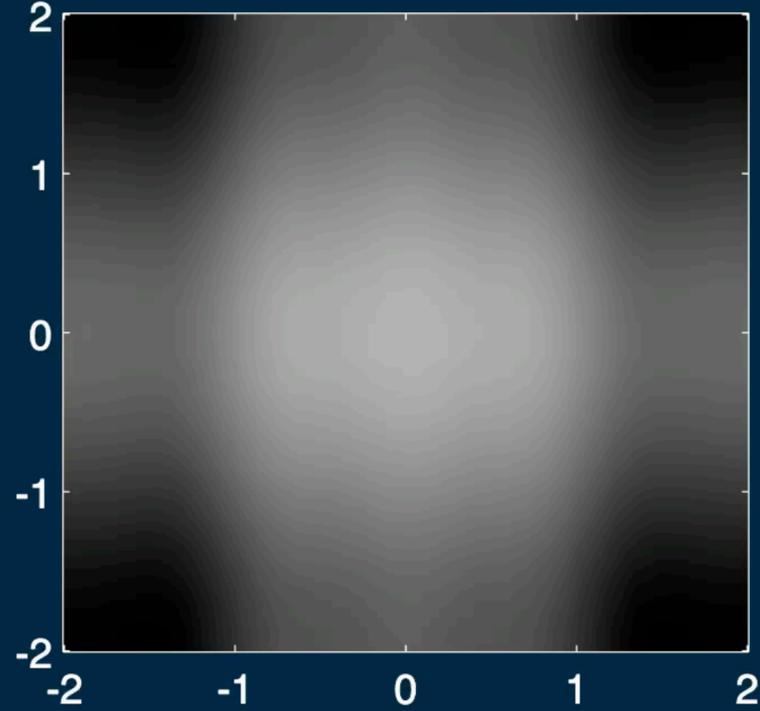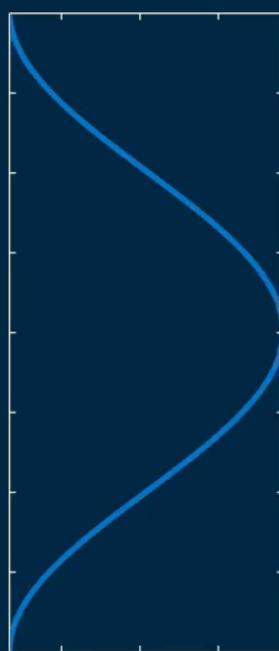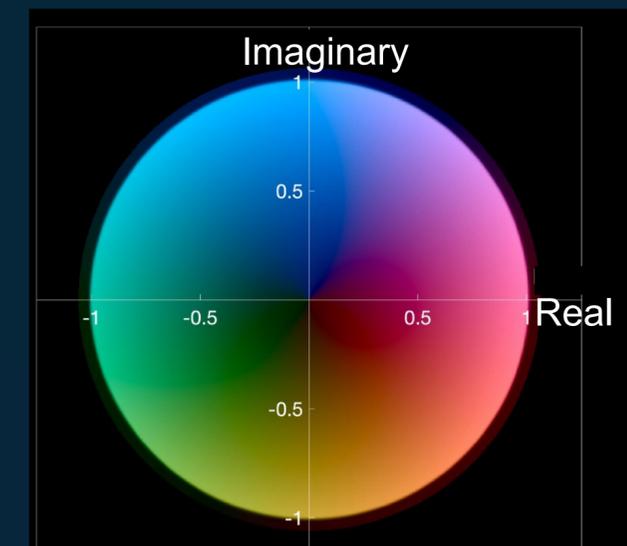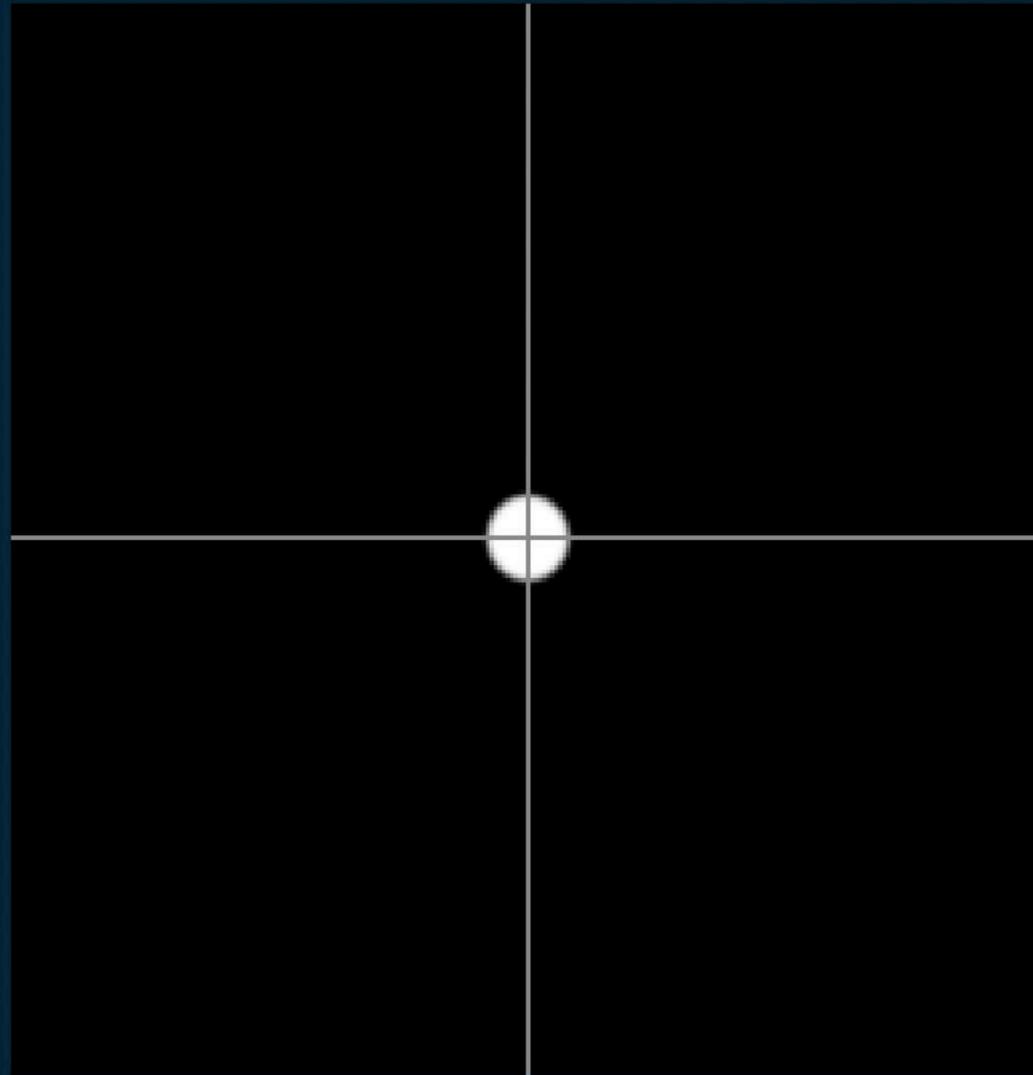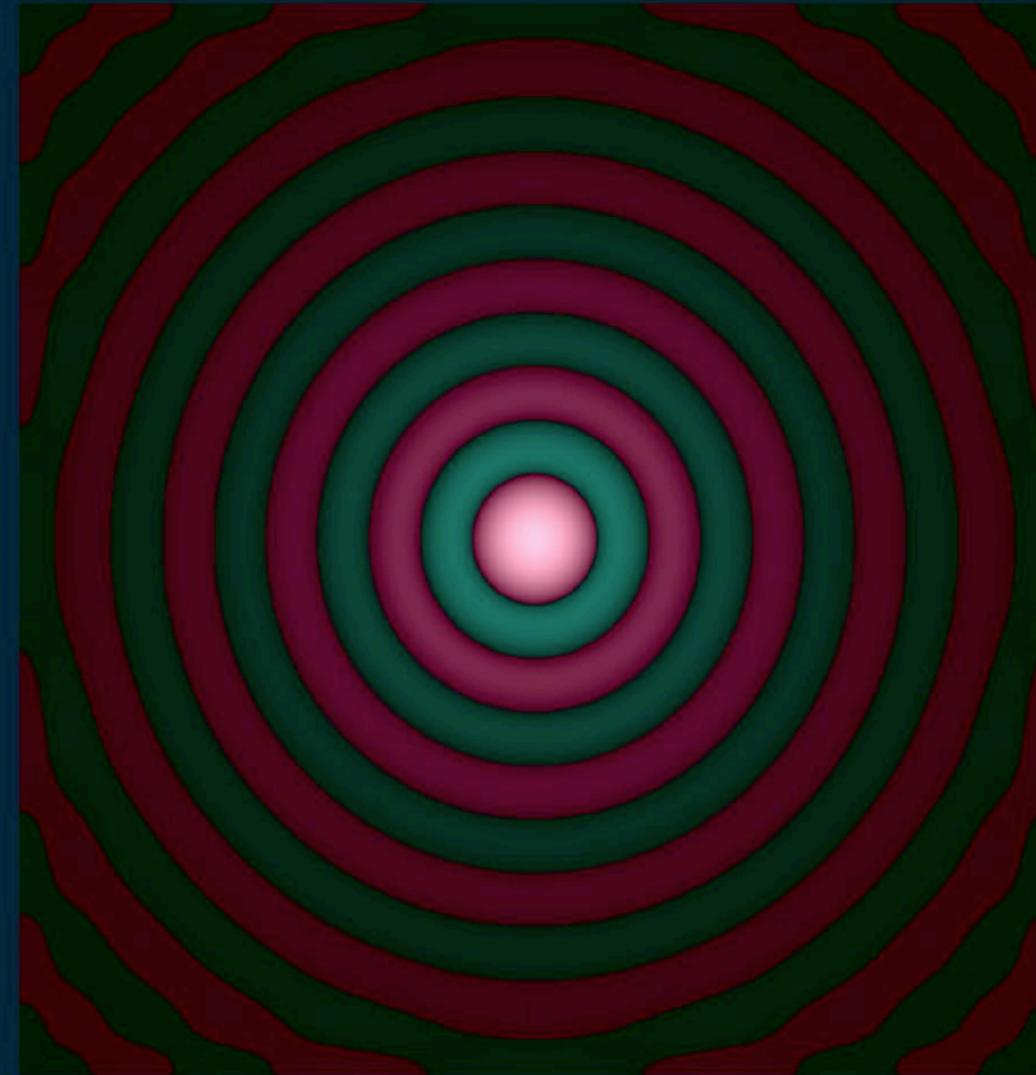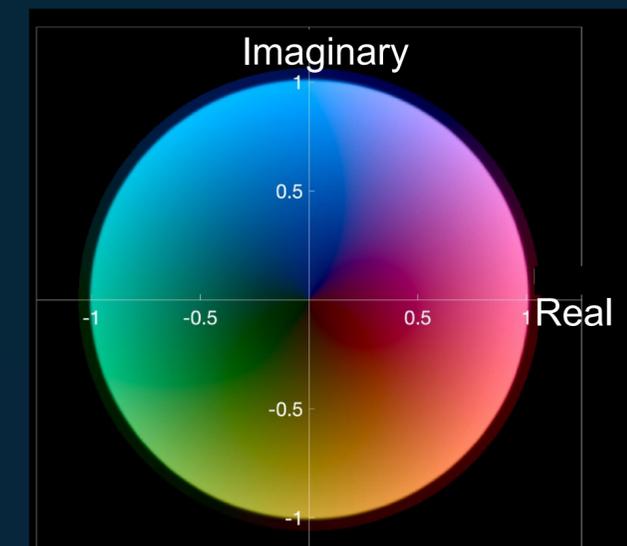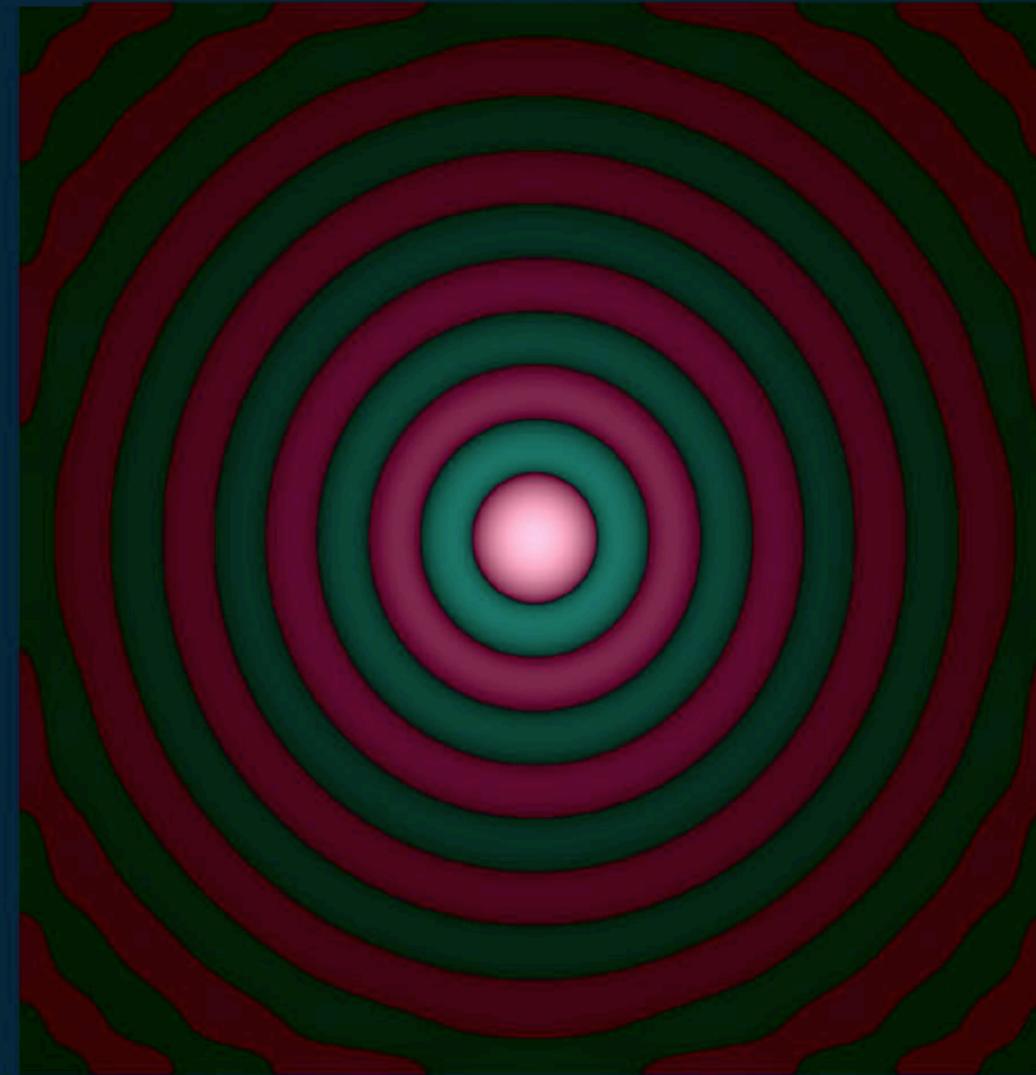
$$e^{-\pi(x^2+y^2)} \rightarrow e^{-\pi(u^2+v^2)}$$

$$\text{circ}(x, y) \rightarrow \frac{J_1(2\pi\rho)}{\pi\rho}, \quad \rho = \sqrt{u^2 + v^2}$$

2D Fourier transform properties

$$ab\,g(ax, by) \rightarrow G(u/a, v/b)$$ **Scale**

$$g(x - a, y - b) \rightarrow G(u, v)e^{-i2\pi(au+bv)}$$ **Shift**

$$g * h \rightarrow GH$$ **Convolution**

$$g(x', y') \rightarrow G(u', v')$$ **Rotation**
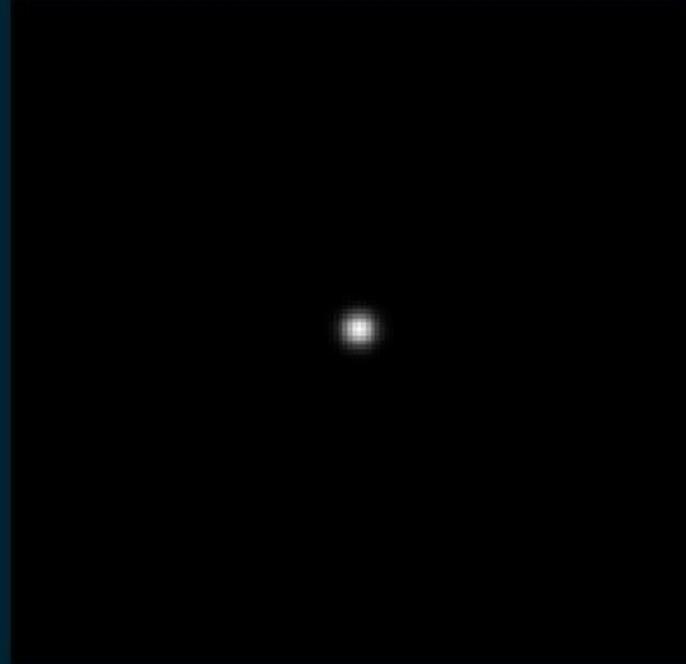
$$P_y\,g(x, y) \rightarrow G(u, 0)$$ **Projection**

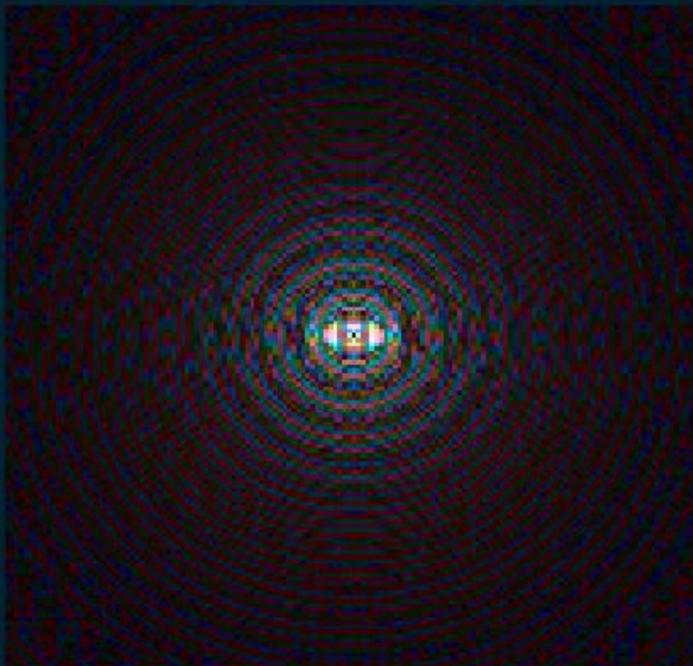$$g * h = \iint g(x - s, y - t)\, h(s, t)\, ds\, dt$$
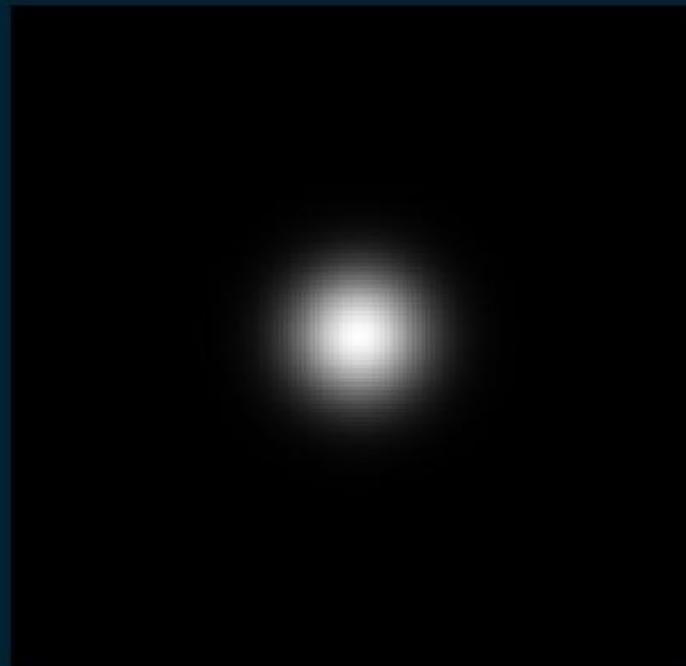
Convolution with a Gaussian
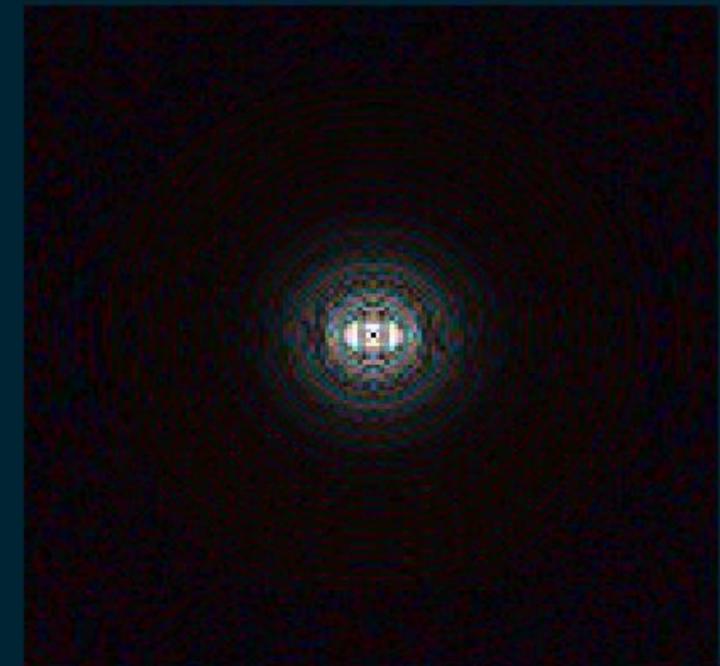
Visualizing the contrast transfer function

Random object

$g(x,y)$

Point-spread

h

Image

$f = g * h$

FT of object

$G(u,v)$

CTF

H

FT of image

$F = GH$

Visualizing the contrast transfer function
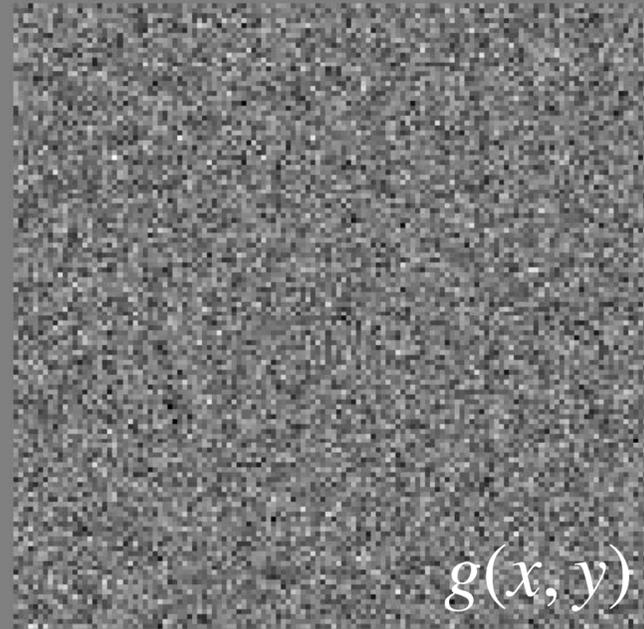
Random object

$g(x, y)$

Point-spread

h

Image

$f = g * h$

Autocorrelation

$f \otimes f$

FT of object

$G(u, v)$

CTF

H

FT of image

$F = GH$

Power spectrum

$|F|^2$
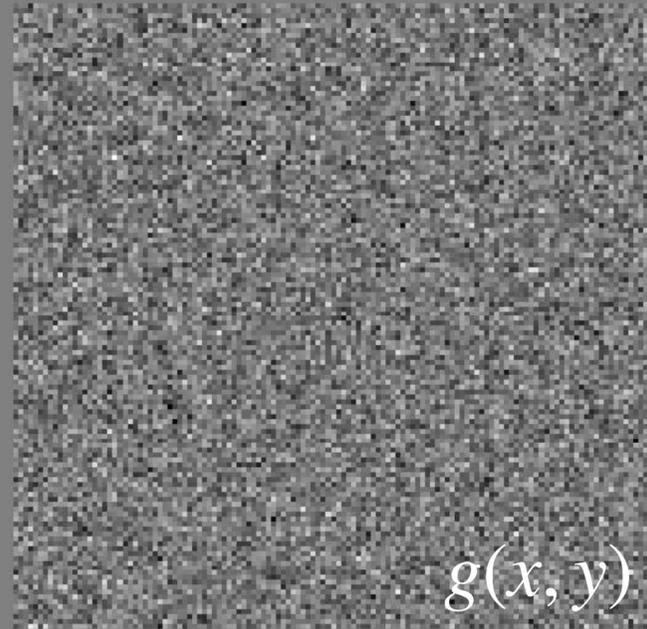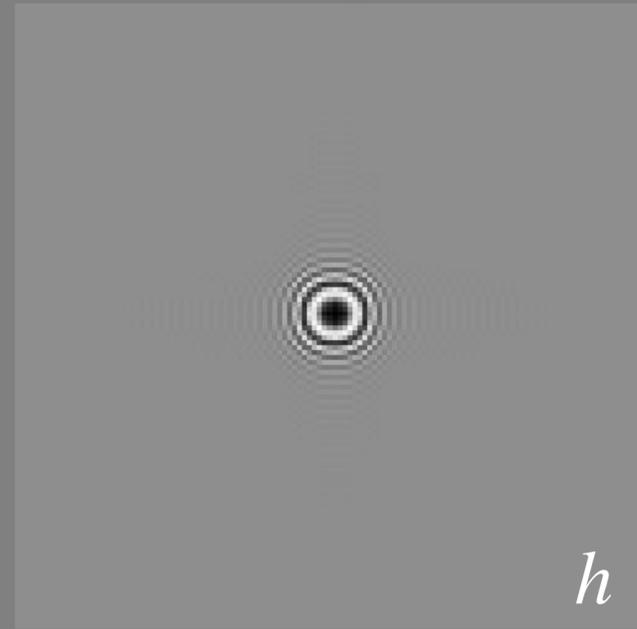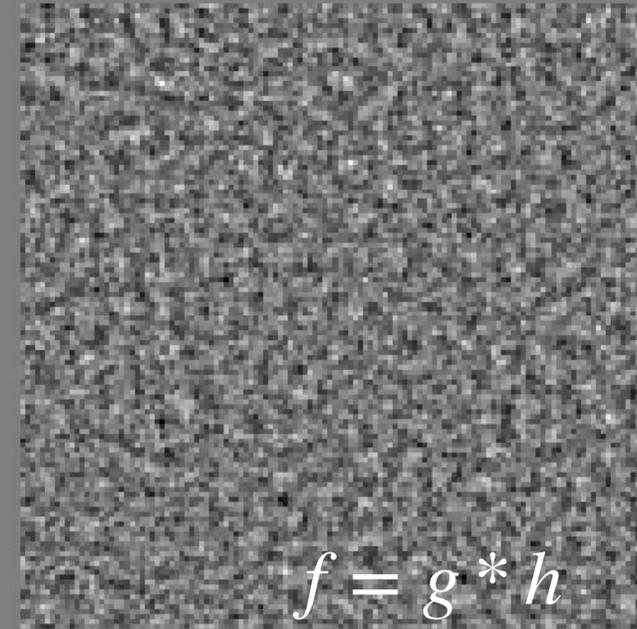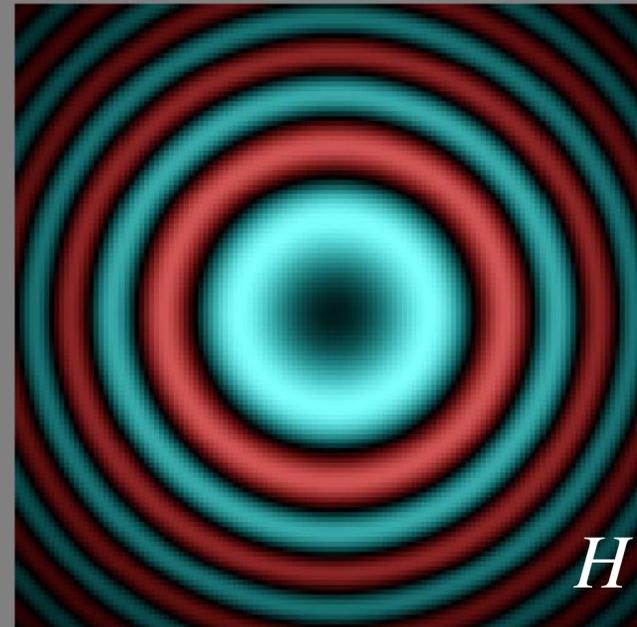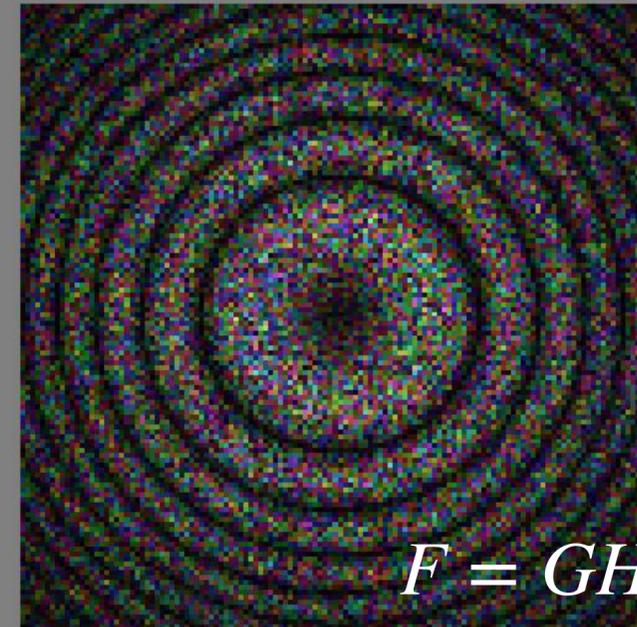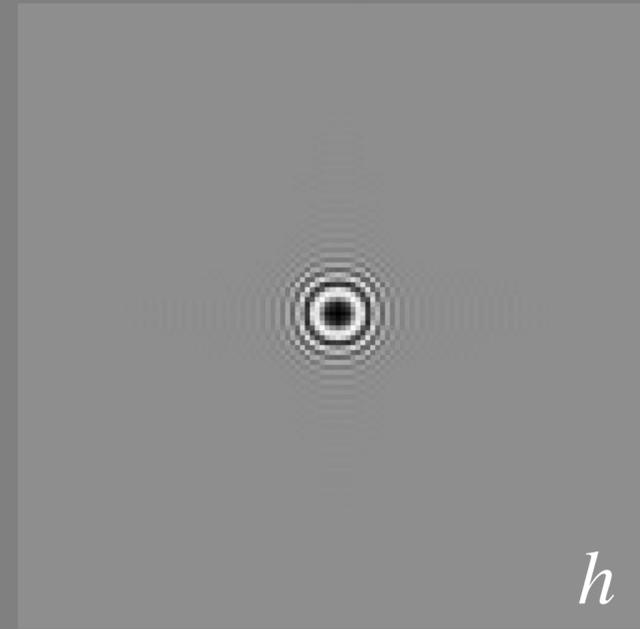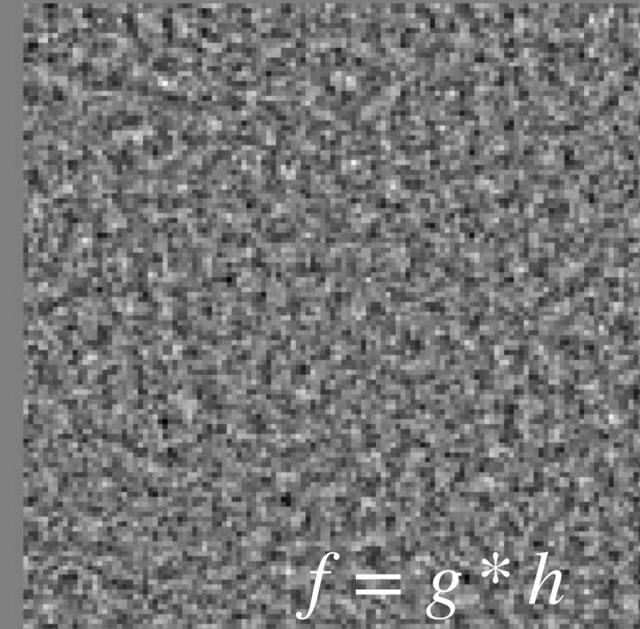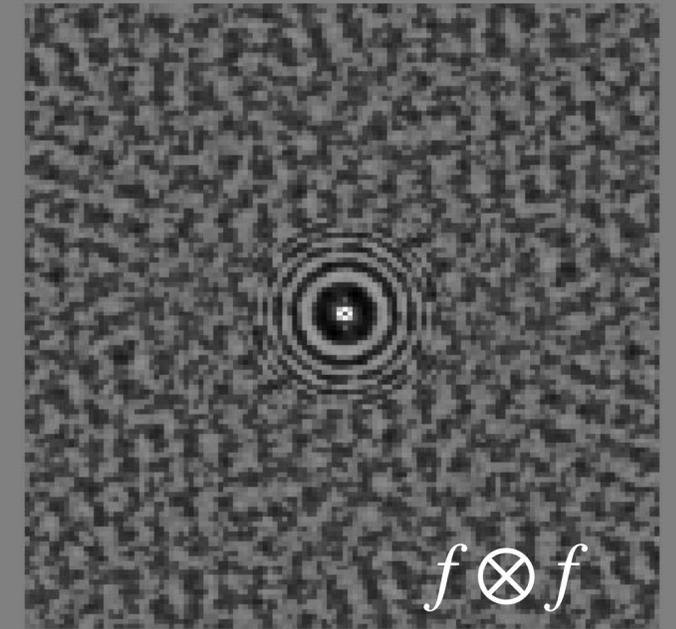
II. Fourier transforms and their properties

The rotation property

2D Fourier Transform

$$G(u, v) = \iint g(x, y) e^{-i2\pi(ux+vy)} dx\, dy$$

FT using 2D vectors

$$G(\mathbf{u}) = \iint g(\mathbf{x}) e^{-i2\pi(\mathbf{u}\cdot\mathbf{x})} d^2\mathbf{x}$$

The dot-product is invariant under rotations!
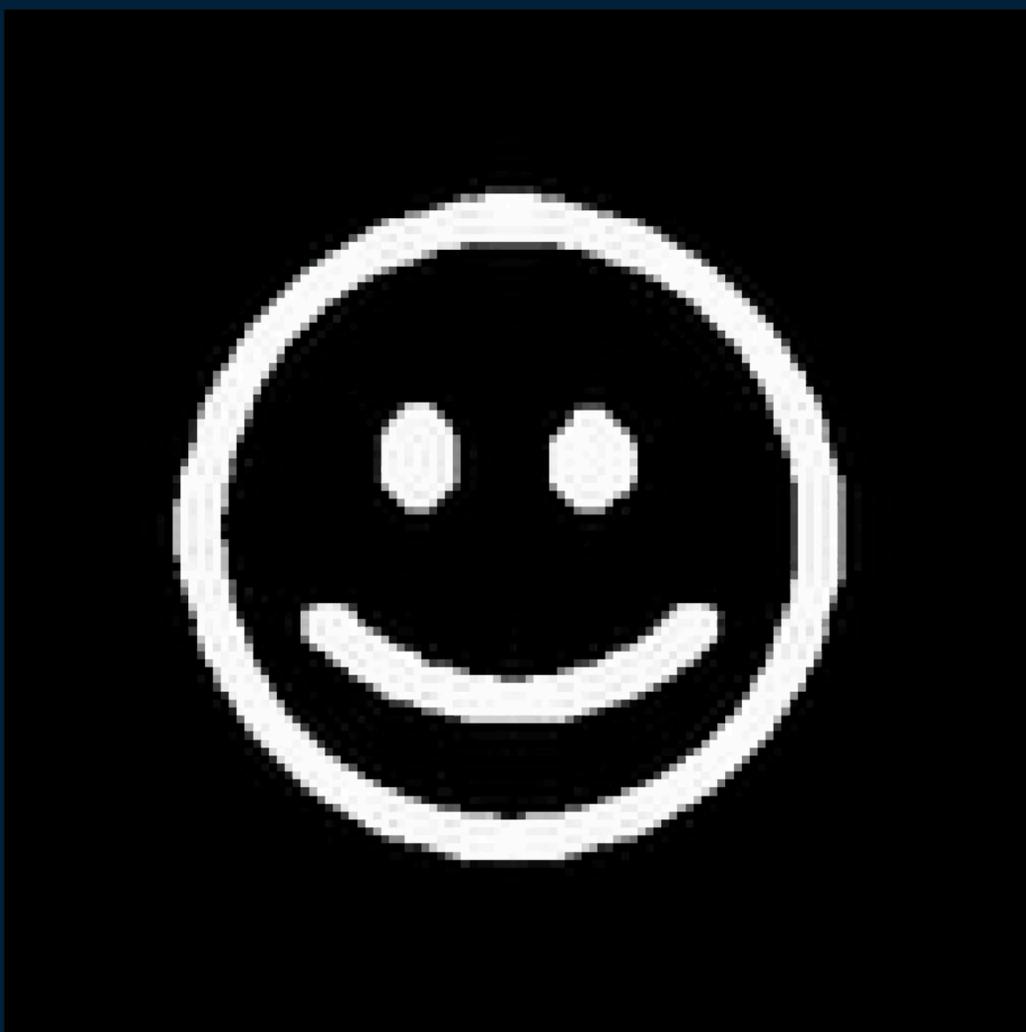
FT
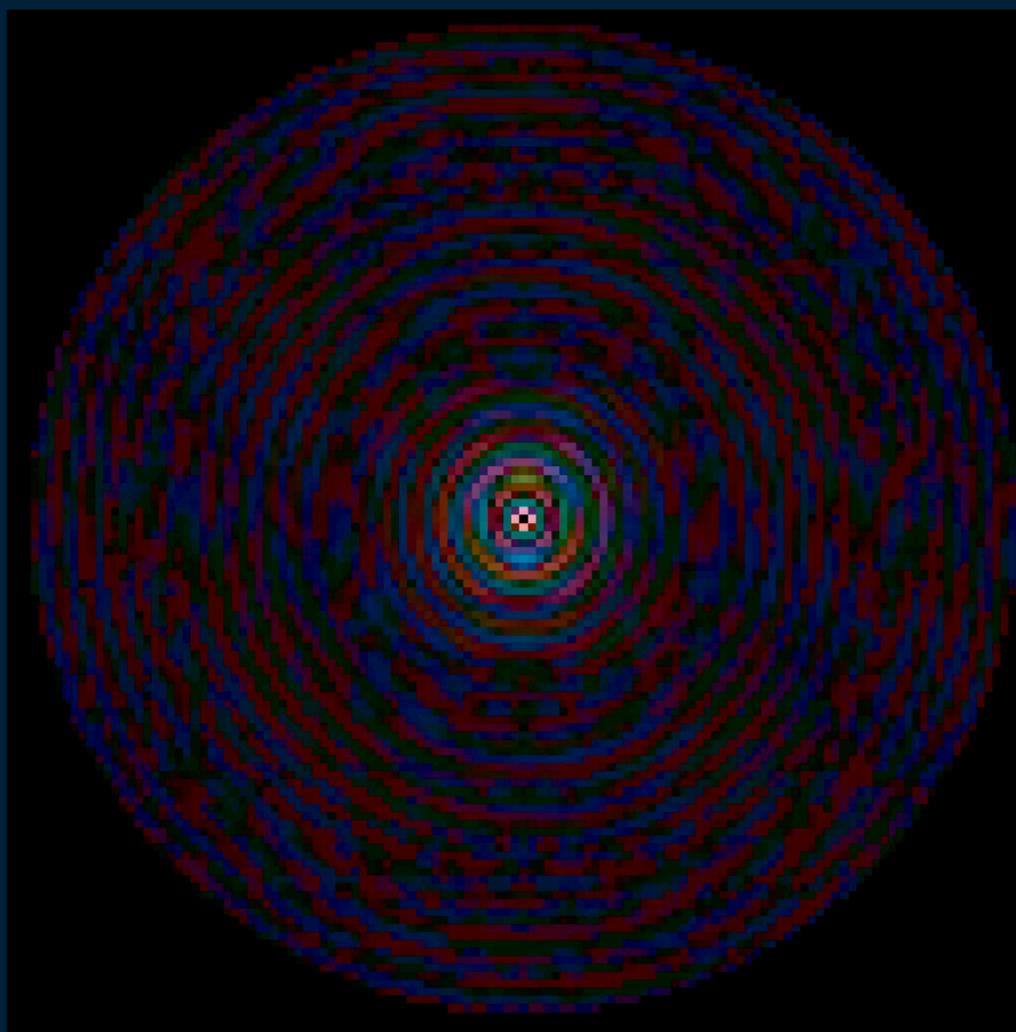
The Fourier Slice Theorem in 2D
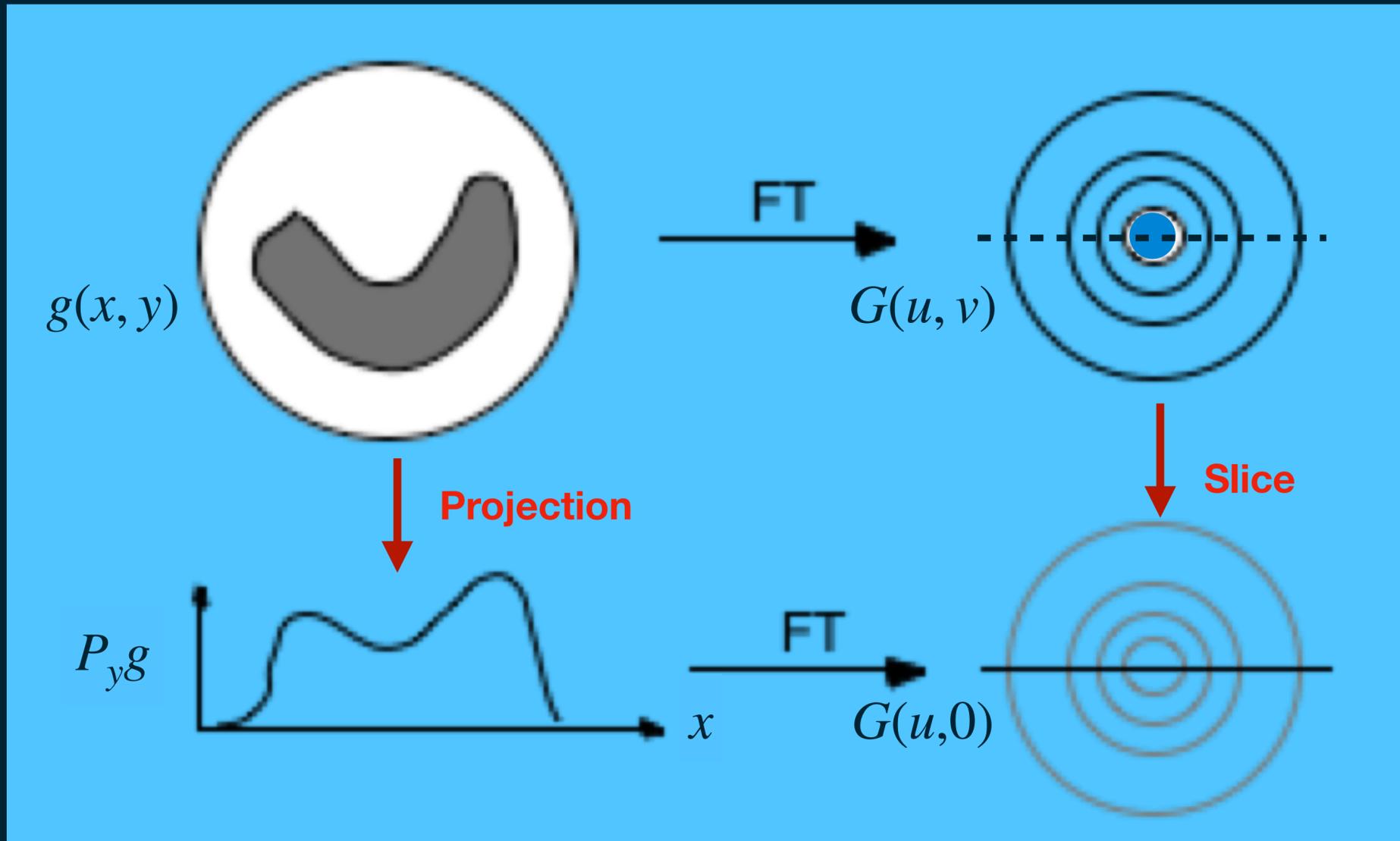
$$G(u, v) = \iint g(x, y) e^{-i2\pi(ux+vy)} dx dy$$

$$G(u,0) = \int \left(\int g(x, y) dy \right) e^{-i2\pi(ux)} dx$$
$$= \mathscr{F}\{P_y g\}$$

$$P_y g(x, y) = \int g(x, y) dy$$
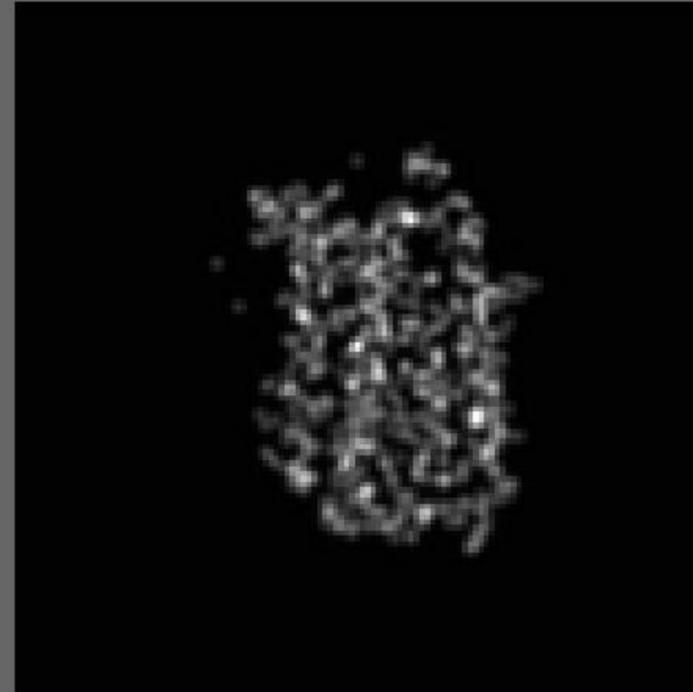
Here we'll demonstrate building up a 2D structure from 1D projections.

Fourier transform
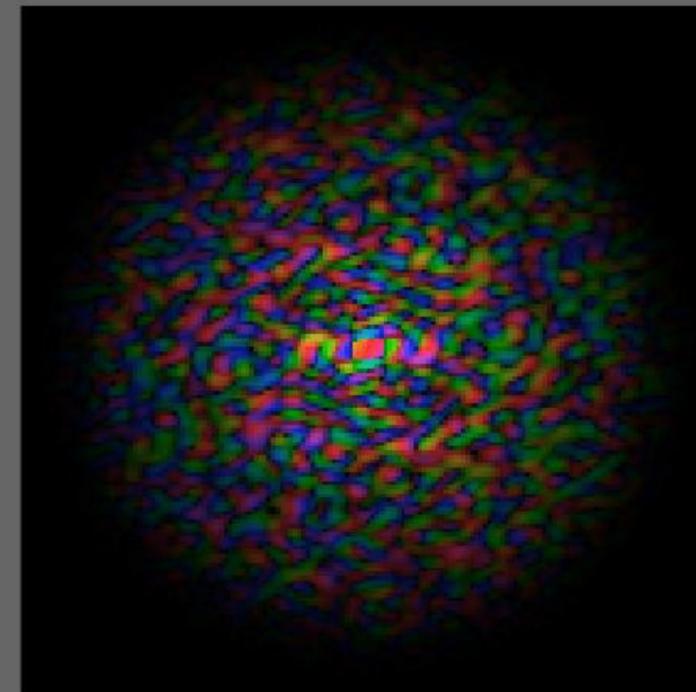
Tomographic reconstruction: 2D image from 1D projections

Tomographic reconstruction: 2D image from 1D projections
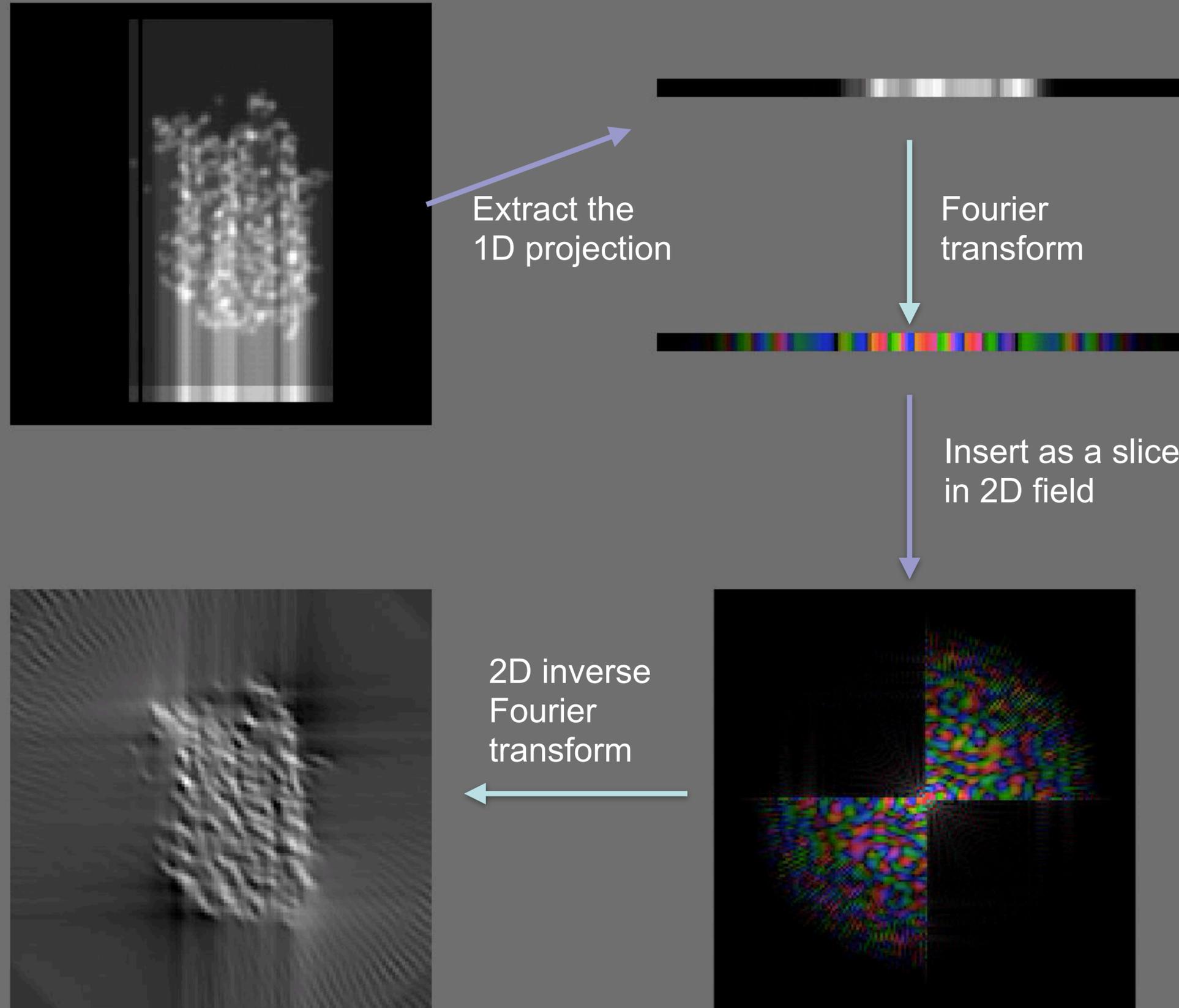
Extract the
1D projection

Fourier
transform

Insert as a slice
in 2D field

2D inverse
Fourier
transform

2D Fourier transform

$$G(u, v) = \iint g(x, y) \, e^{-i2\pi(ux+vy)} dx \, dy$$

u, v are real numbers

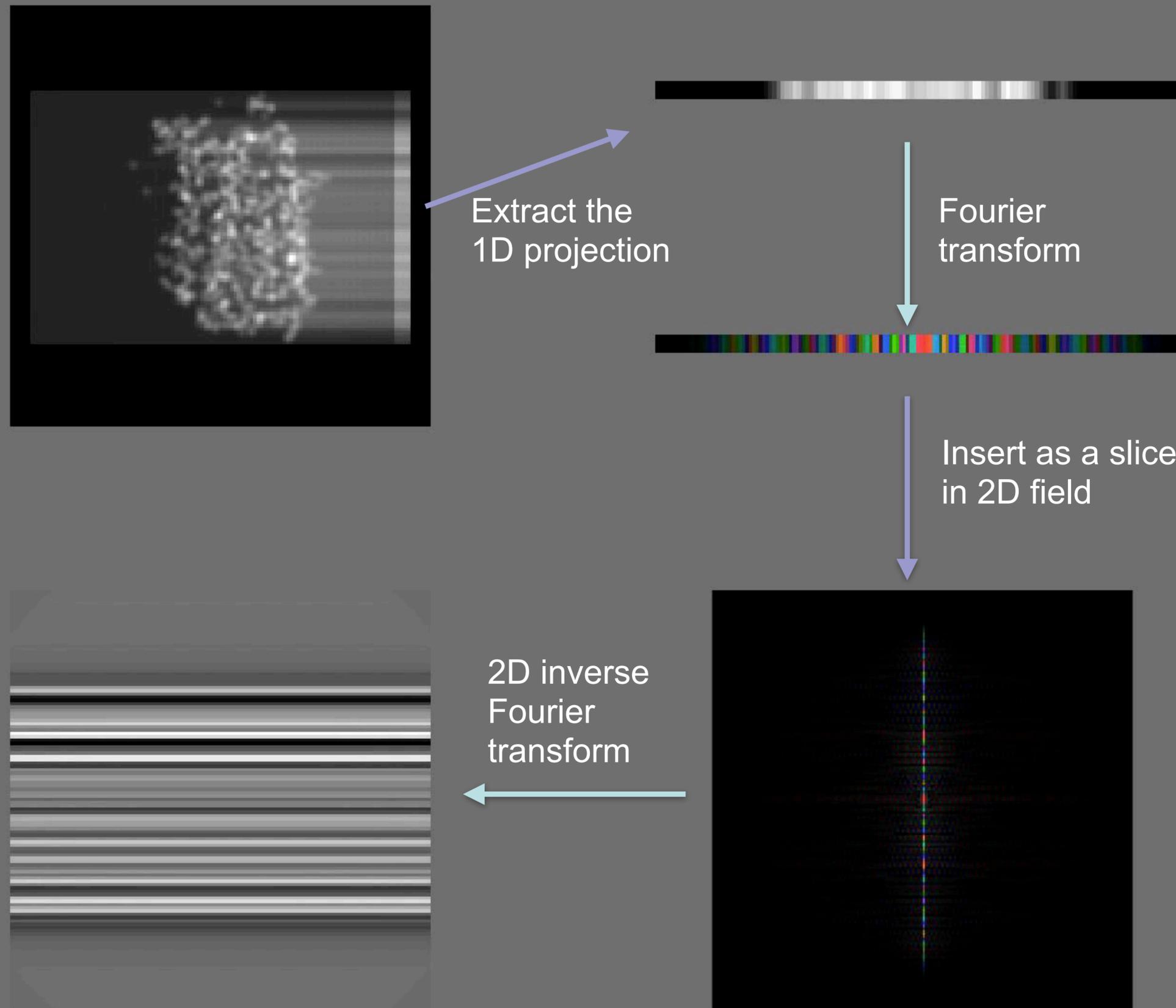## 2D discrete Fourier transform

$$G(k, l) = \frac{1}{N} \sum_{i,j=-N/2}^{N/2-1} g(i,j) \, e^{-i2\pi(ik+jl)\,/N}$$

k, l are integers

G(k,l)
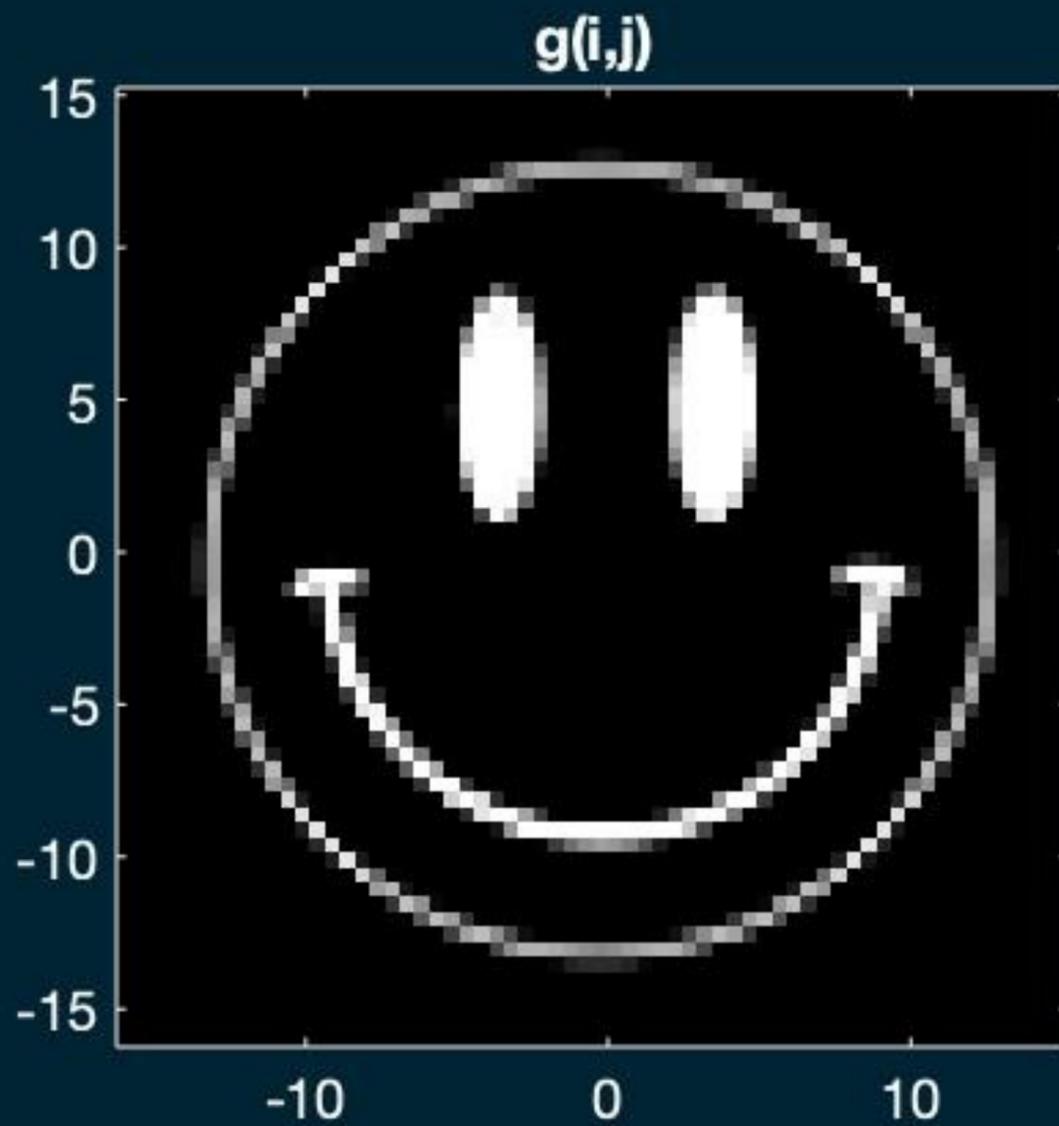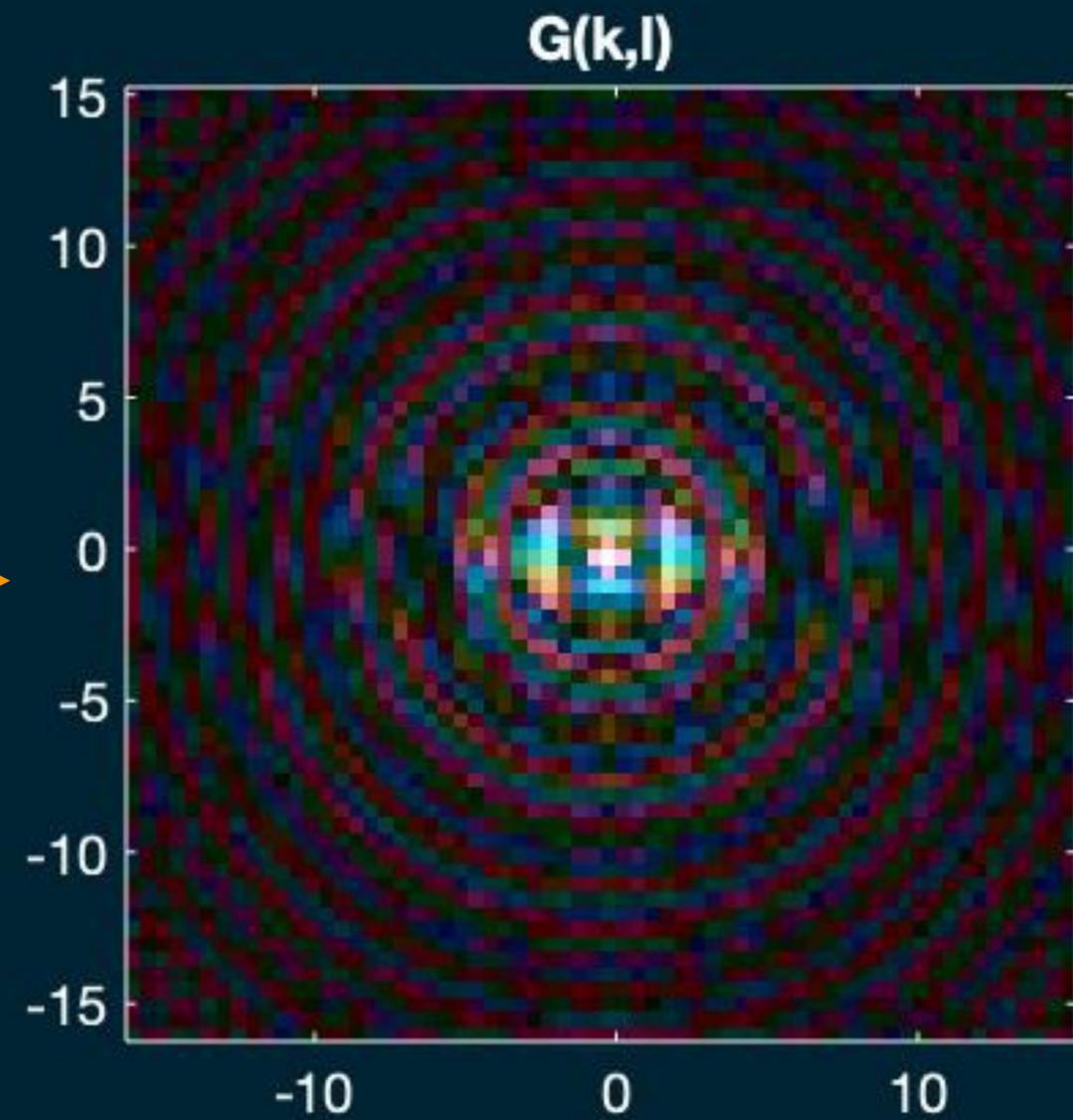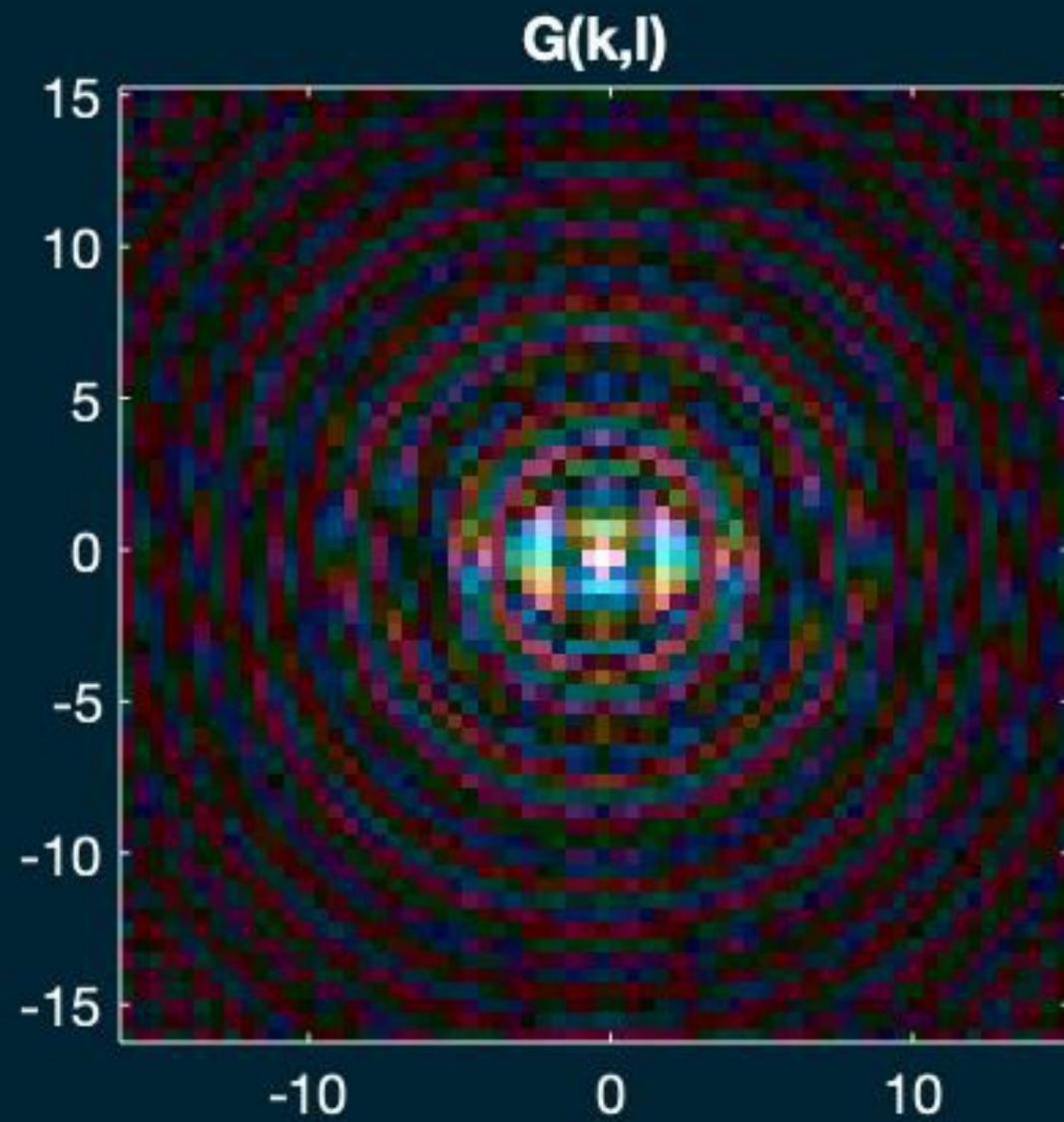
Real part
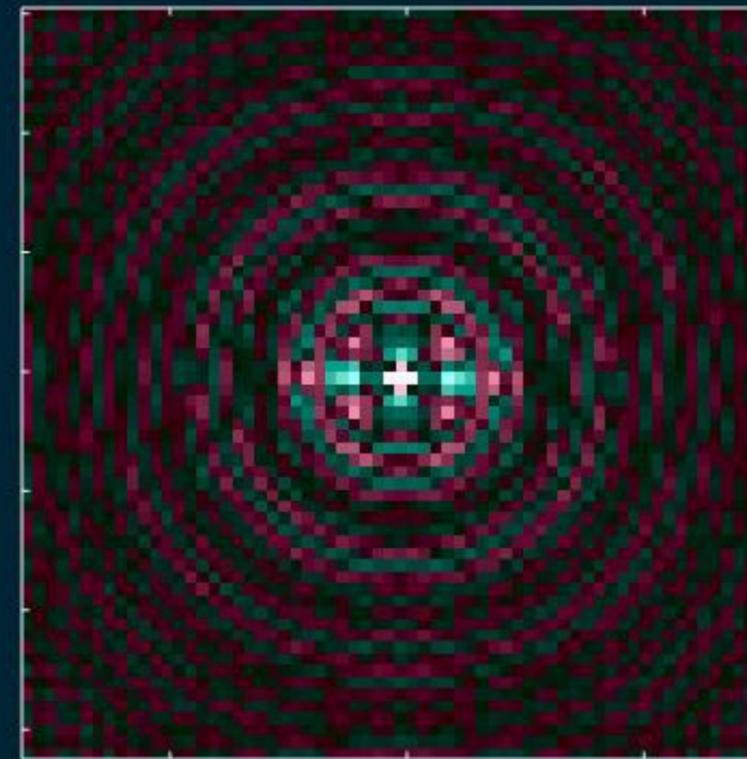
Imaginary part
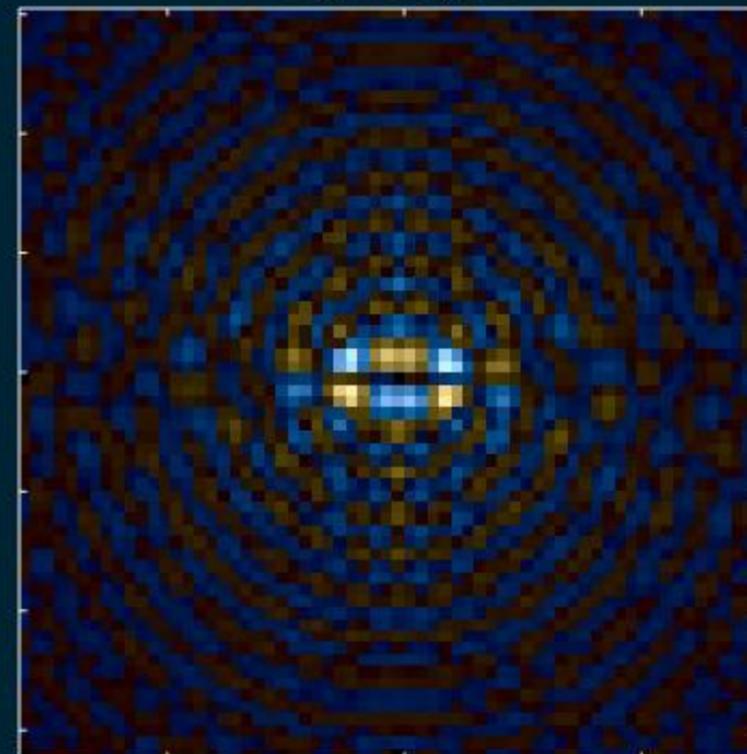
What is the pixel size of the transformed image?

$$dx = \frac{1}{n\,du}$$

$$du = \frac{1}{n\,dx}$$
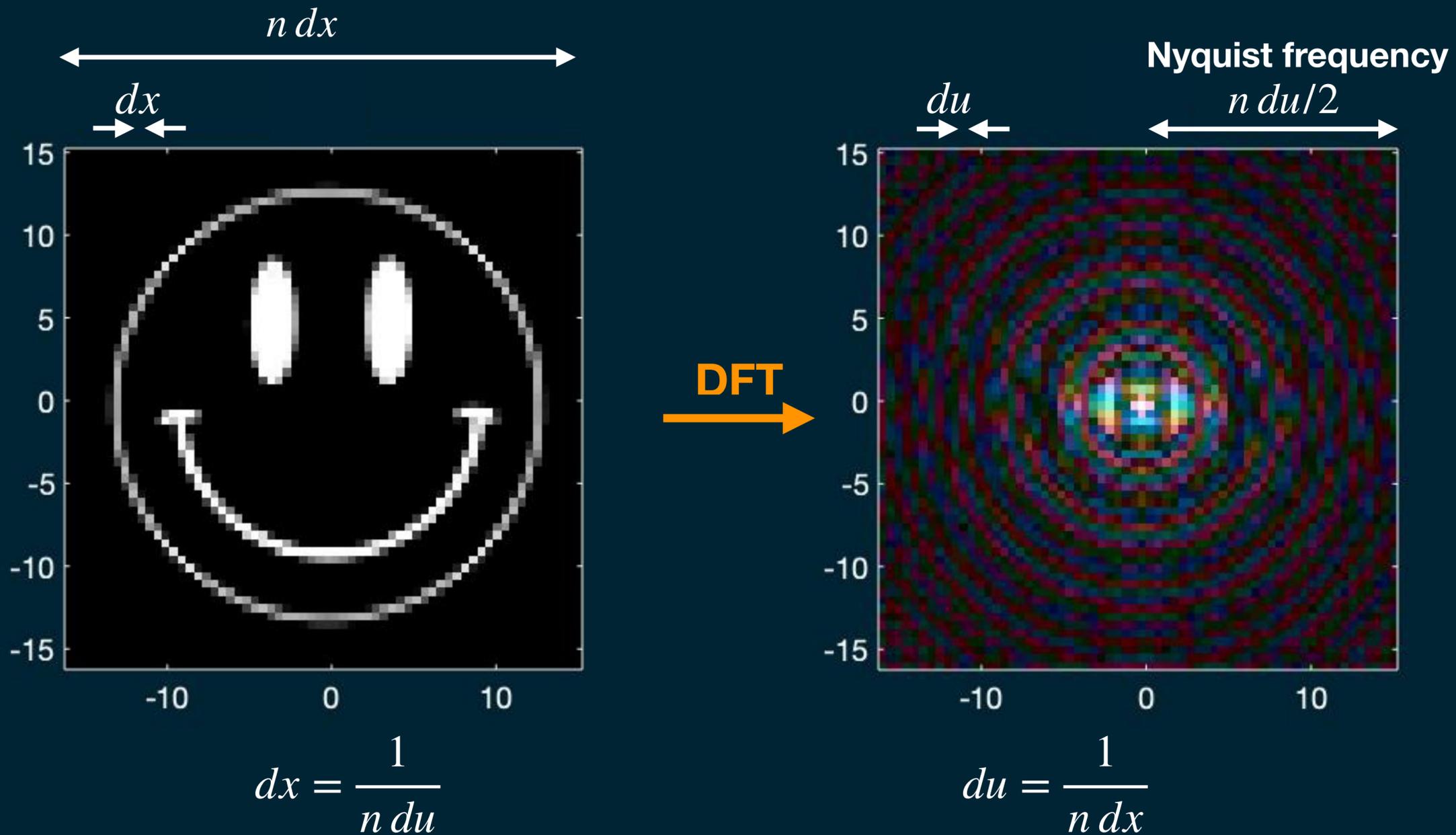
Note that the maximum accessible frequency $n\,du/2$ (the Nyquist frequency) corresponds to $2dx$, twice the sampling period.

The Fourier transform in three dimensions

3D Fourier transform

$$G(u, v, w) = \iiint g(x, y, z) e^{-i2\pi(ux+vy+wz)} dx \, dy \, dz$$

3D Inverse Fourier transform

$$g(x, y, z) = \iiint G(u, v, w) e^{+i2\pi(ux+vy+wz)} du \, dv \, dw$$

3D Fourier transform properties

$$abc\, g(ax, by, cz) \rightarrow G(u/a, v/b, w/c)$$

Scale

$$g(x - a, y - b, z - c) \rightarrow G(u, v, w)e^{-i2\pi(au+bv+cw)}$$

Shift

$$g * h \rightarrow GH$$

Convolution

$$g(x', y', z') \rightarrow G(u', v', w')$$

Rotation

$$P_z\, g(x, y, z) \rightarrow G(u, v, 0)$$
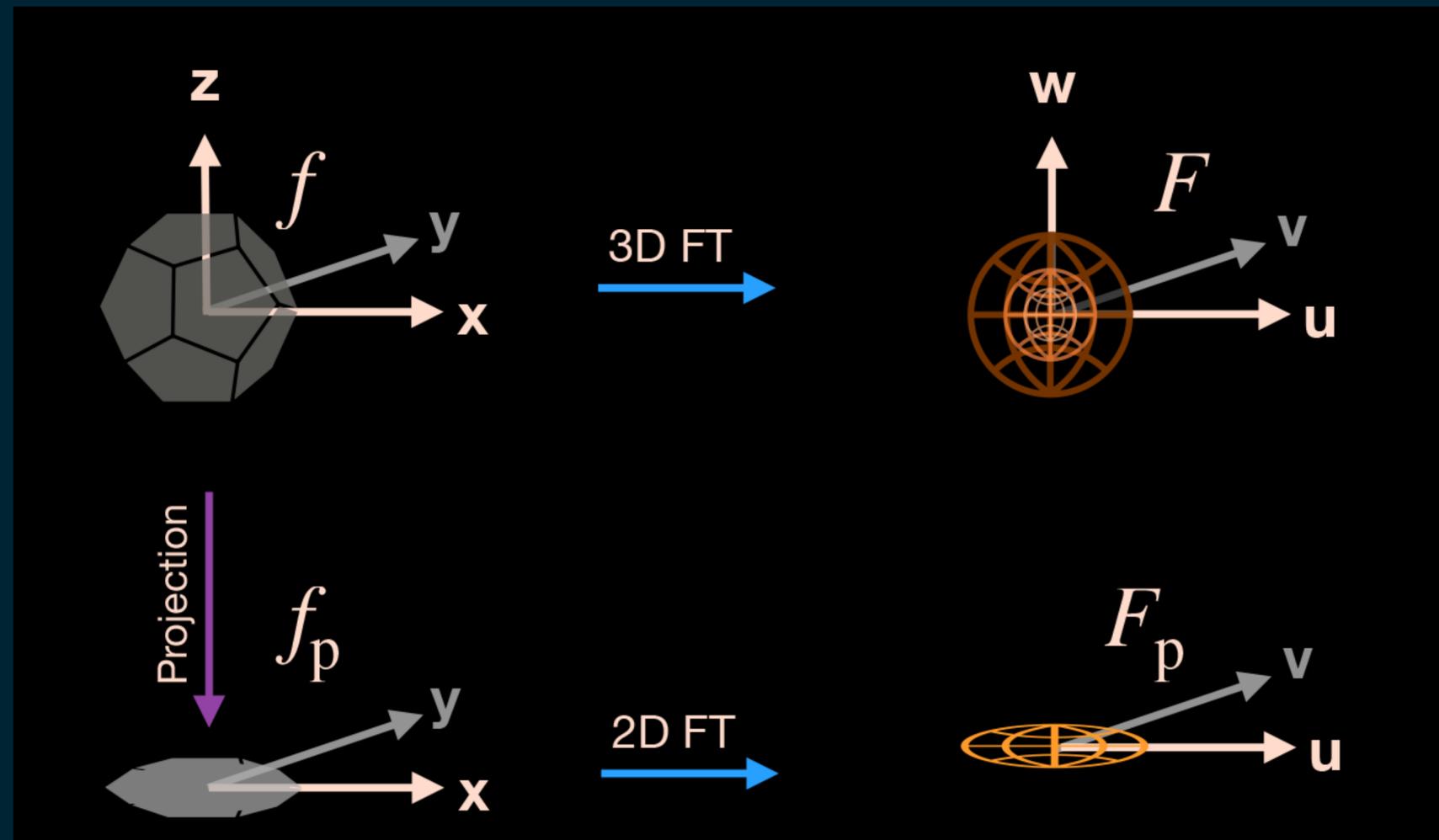
Projection

Suppose we have a 3D density $f(x, y, z)$. The projection along z is a 2D image we'll call $f_p(x, y)$:

$$f_p(x, y) = \int f(x, y, z)\, dz$$

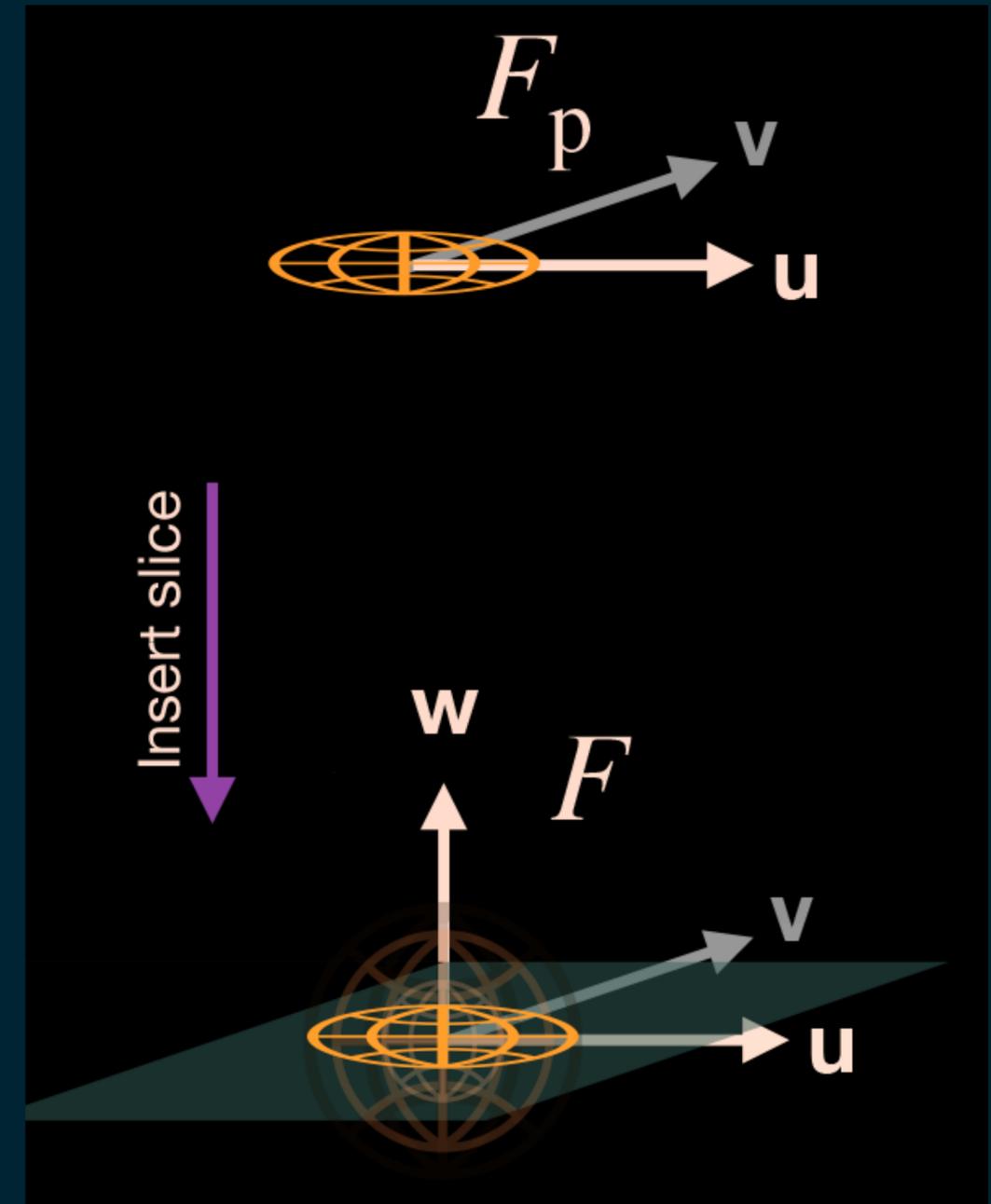Let its Fourier transform be $F_p(u, v)$.

$F_p(u, v)$ is actually the same as the FT of the 3D volume, when we evaluate it at $w = 0$. In other words,

$F_p(u, v) = F(u, v, 0)$.

That's because we can separate out the integral over z to get

$$F(u, v, 0) = \iint \underbrace{\left(\int f(x, y, z) \, dz \right)}_{f_p(x, y)} e^{-i2\pi(ux+vy)} dx \, dy$$

We can make use of the rotation property of the 3D FT to compute projections in many different directions, and insert the planes at the corresponding angles into the 3D Fourier volume.

If we've covered the volume completely then we can transform back to recover the complete 3D volume.