

Defocus Contrast and the Contrast Transfer Function

Fred Sigworth
Yale University



National Center for CryoEM Access and Training

Lecture 4a
SPA Short Course
March 2022

Lecture 4a

1. Complex numbers (quickly)
2. Defocus contrast (the simple version)
3. Defocus contrast (fancy version)
4. Image delocalization
5. The objective lens and the CTF

Why complex numbers?

- Equations are simpler
- Natural for Fourier transforms
- Magnitude and phase of structure factors

i , the imaginary unit

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

A complex number

$$z = a + ib$$

Real part

Imaginary part

$$w = c + id$$

Properties of complex numbers

$$z = a + ib$$

$$w = c + id$$

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

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

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

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

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

Conjugate $z^* = a - ib$

(Exercise: Show that $zz^* = |z|^2$)

The exponential function e^x

$$e = 2.718\dots$$

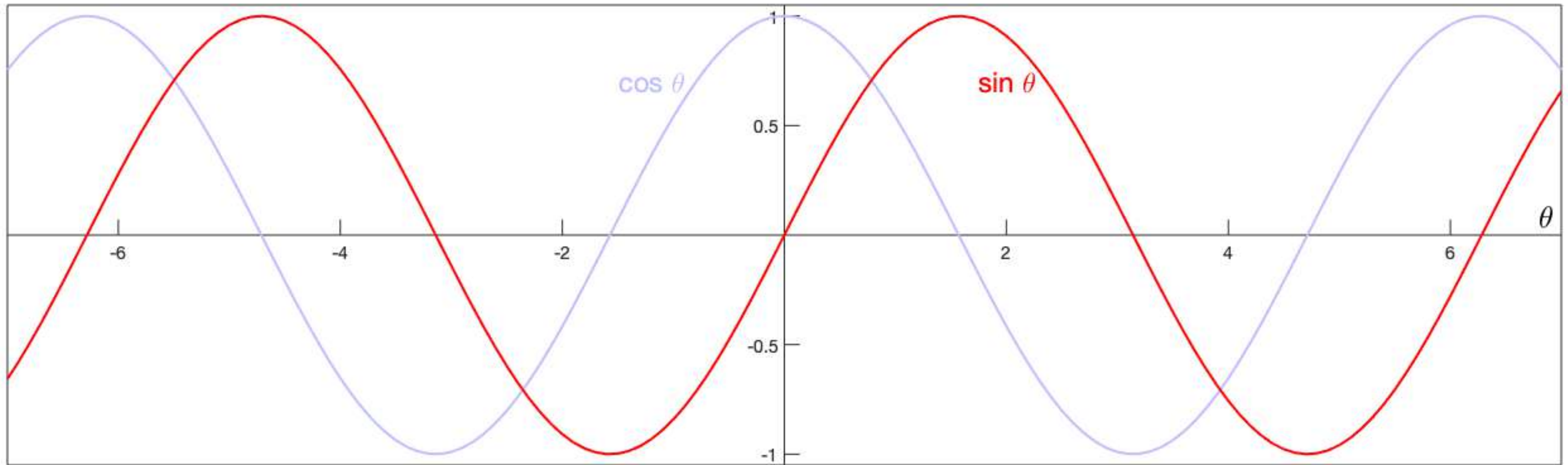
$$e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{2 \times 3} + \dots$$

A very important approximation

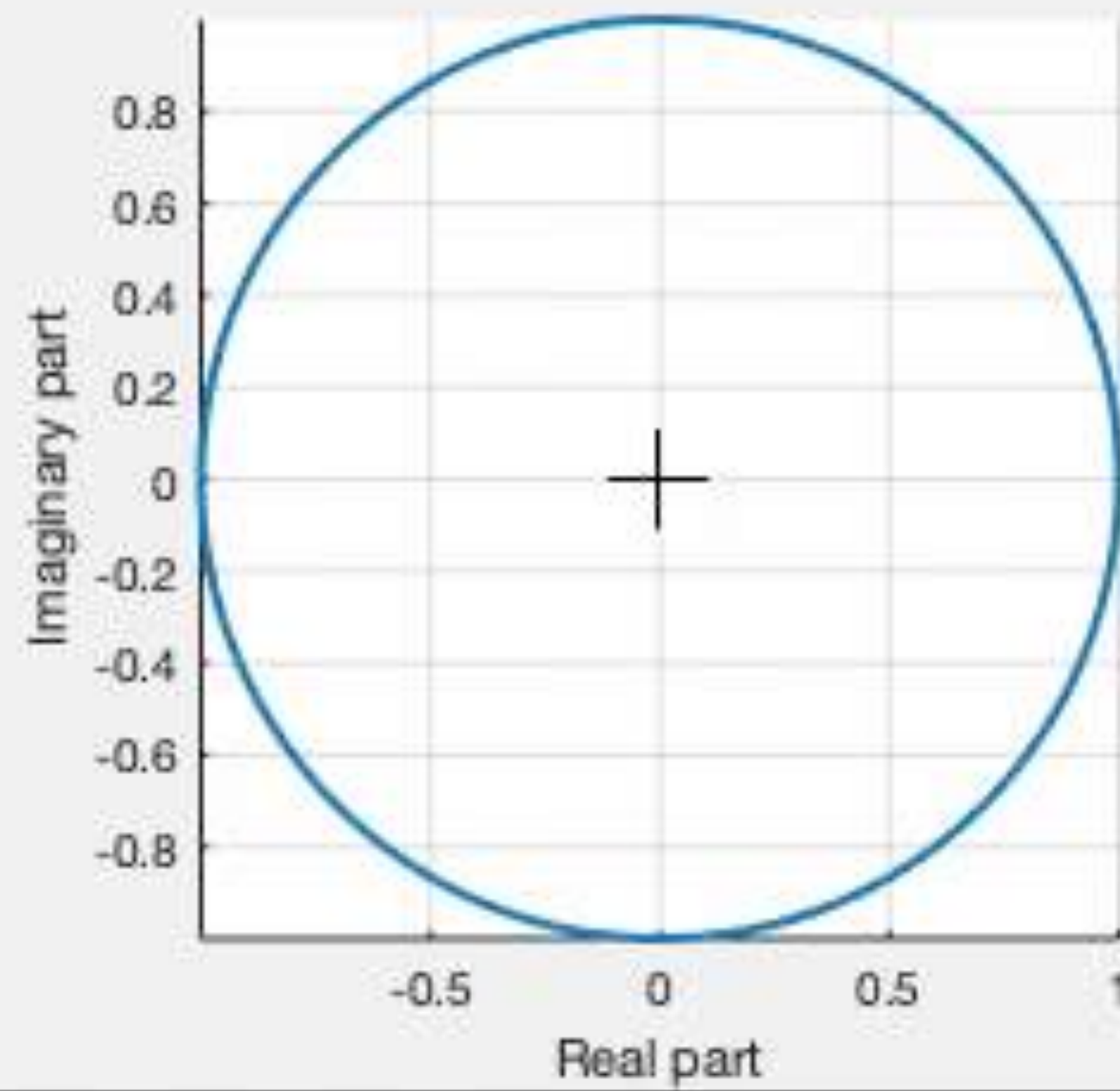
$$e^x \approx 1 + x, \quad x \ll 1$$

The complex exponential

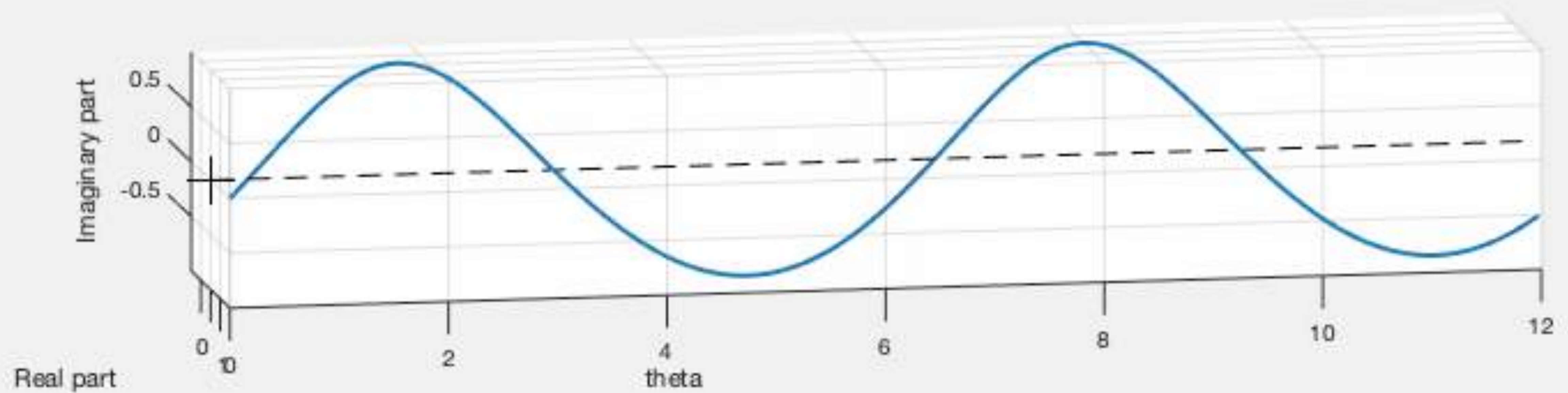
$$e^{i\theta} = \cos \theta + i \sin \theta$$



A plot of $e^{i\theta}$



A plot of $e^{i\theta}$



Any z can be represented as (a, b) or as (r, θ)

$$z = a + ib$$

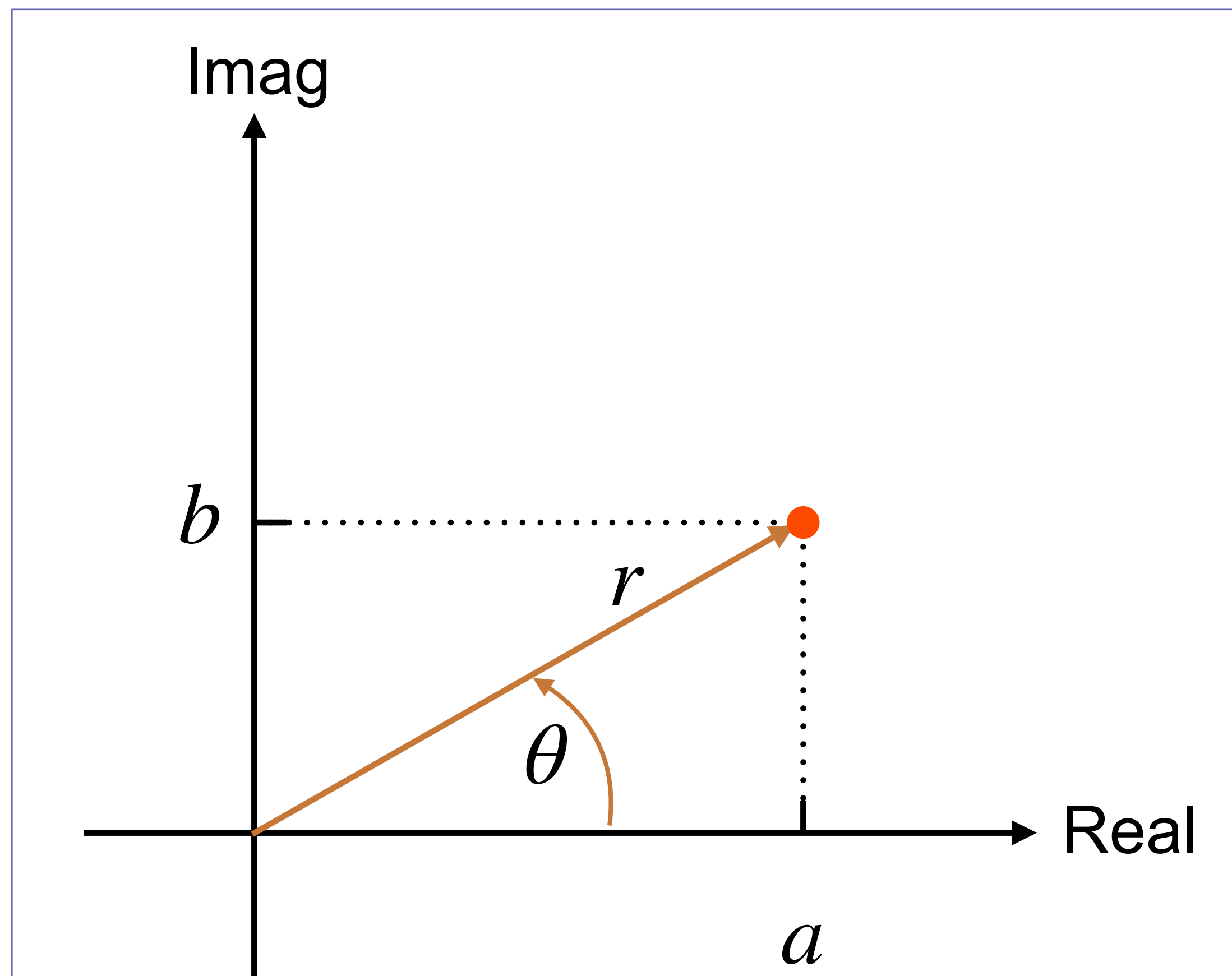
a is the real part

b is the imaginary part

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

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:

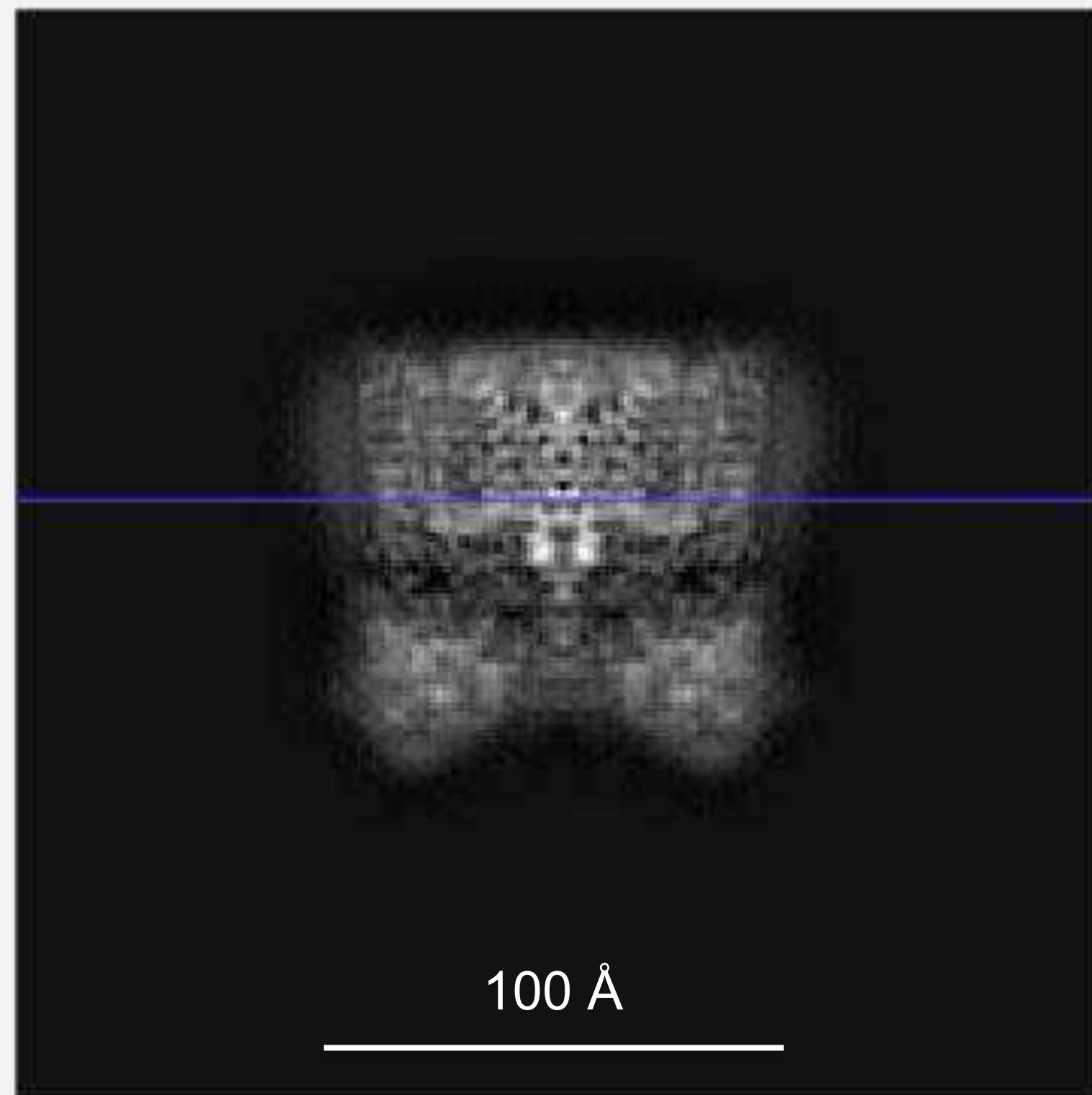
$$e^{i\theta_1} e^{i\theta_2} = e^{i(\theta_1 + \theta_2)}.$$

Lecture 4a

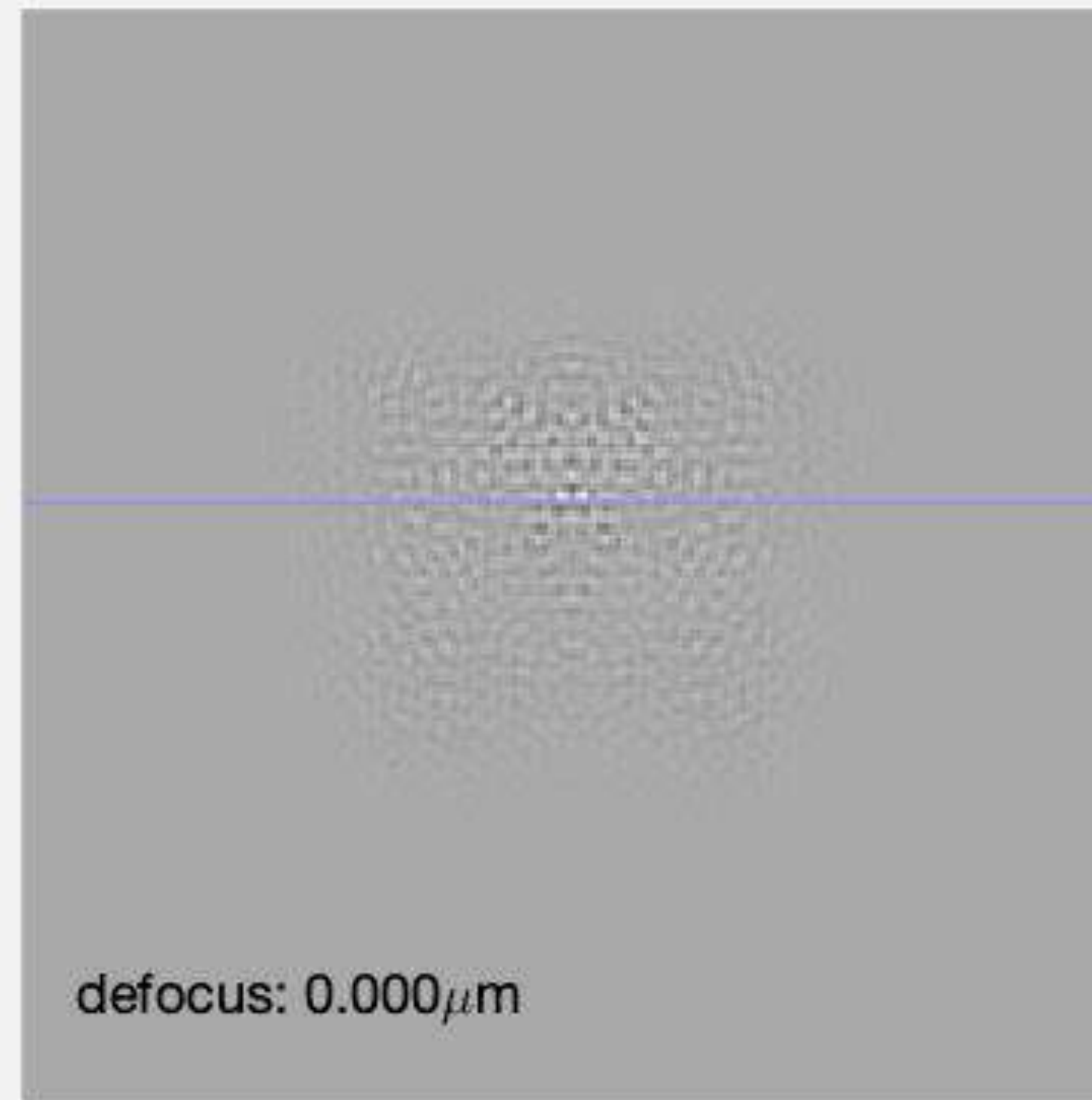
1. Complex numbers (quickly)
2. Defocus contrast in a nutshell
3. Defocus contrast (fancy version)
4. Image delocalization
5. The objective lens and the CTF

Most cryo-EM data are acquired using defocus contrast

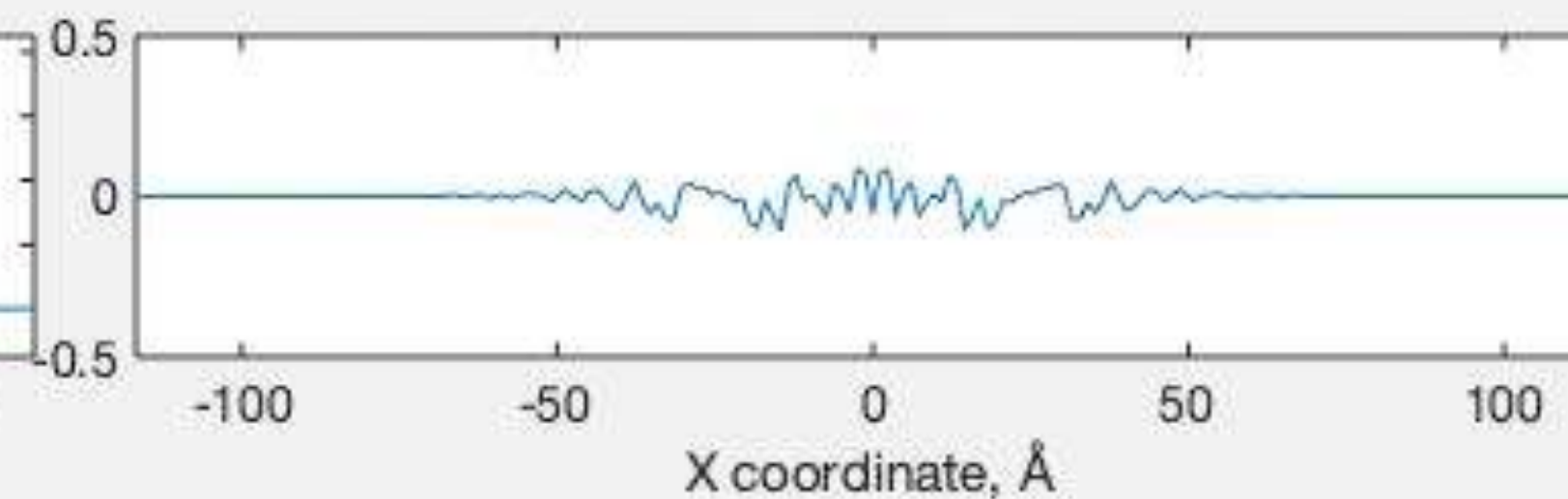
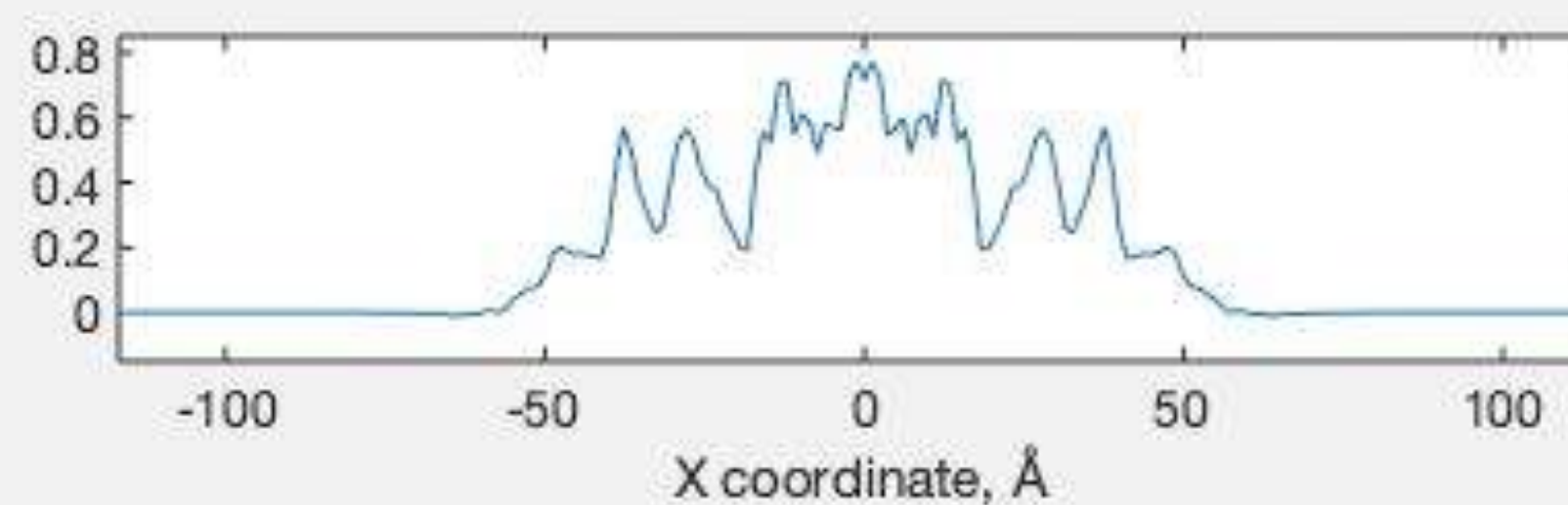
object



image

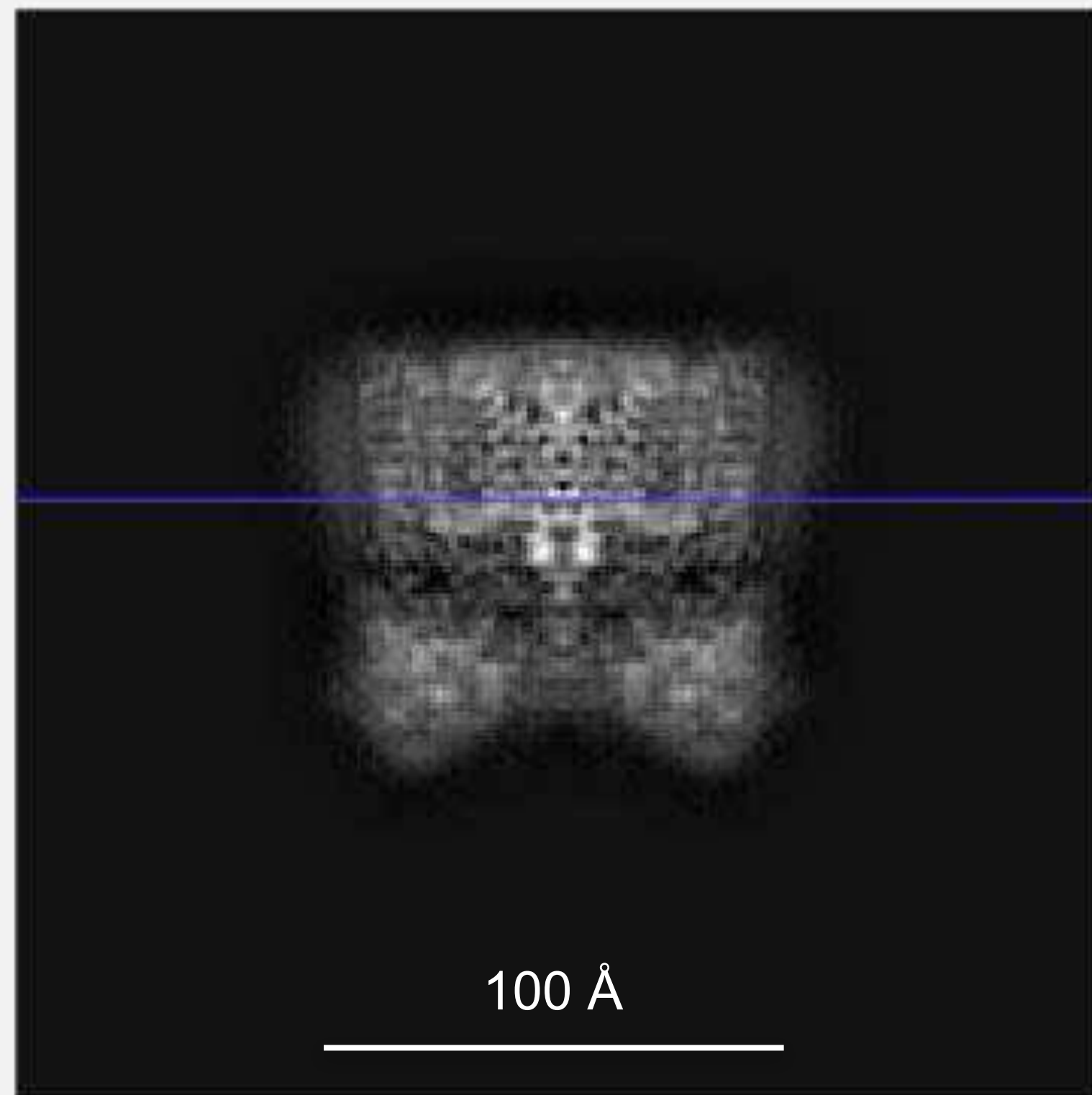


- Defocus values are always “underfocus”. This means decreasing the strength of the objective lens, effectively focusing **above** the specimen.
- At high defocus, high-resolution information in the image is strongly **delocalized**.

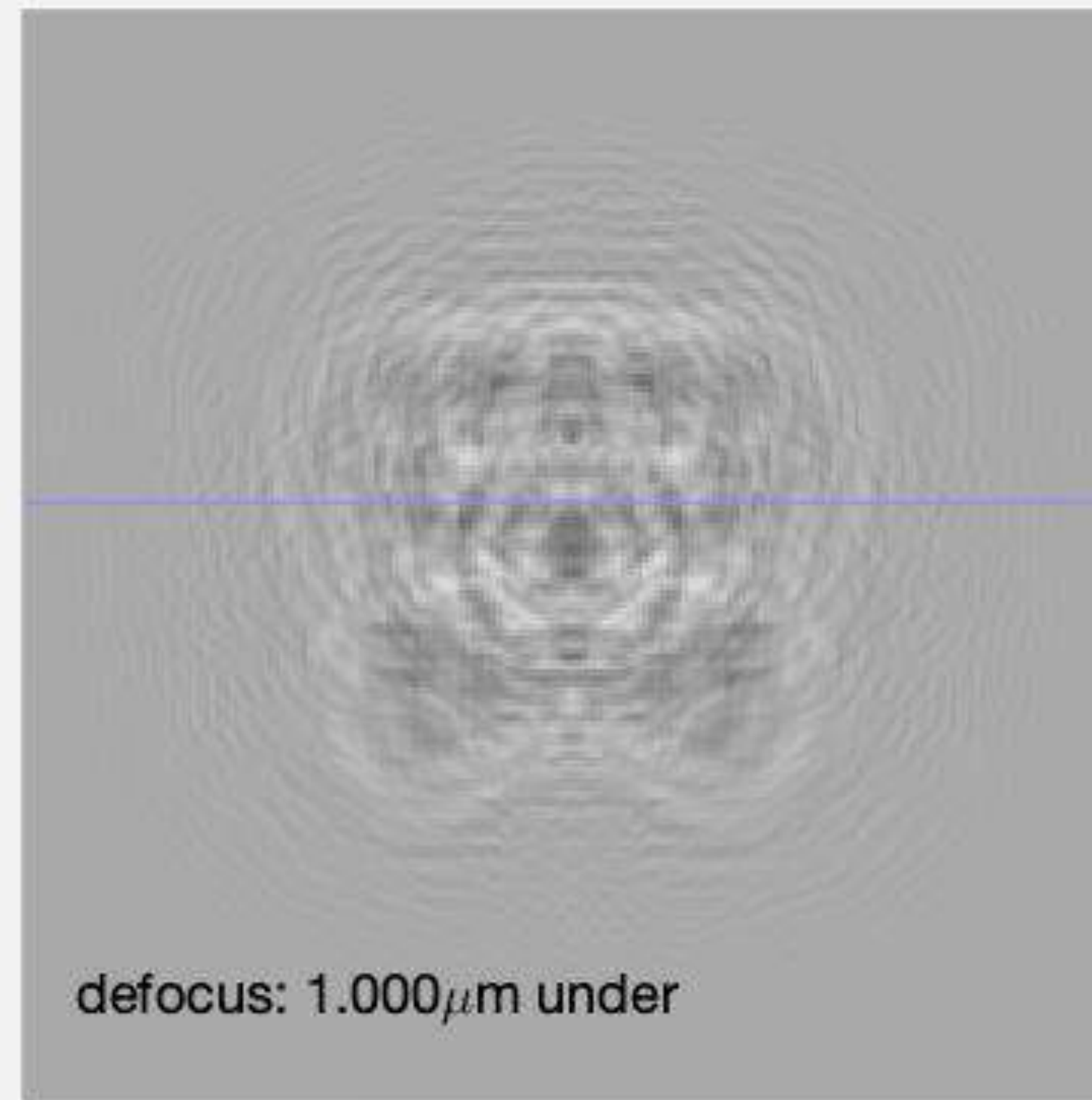


Most cryo-EM data are acquired using defocus contrast

object



image



- Defocus values are always “underfocus”. This means decreasing the strength of the objective lens, effectively focusing **above** the specimen.
- At high defocus, high-resolution information in the image is strongly **delocalized**.

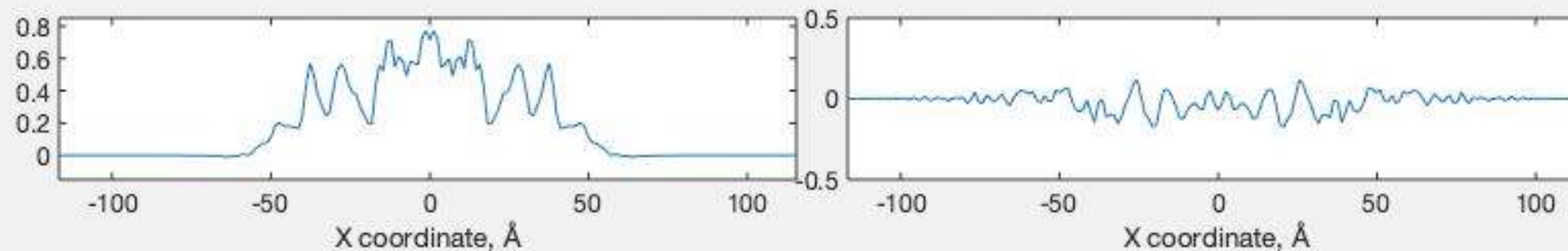
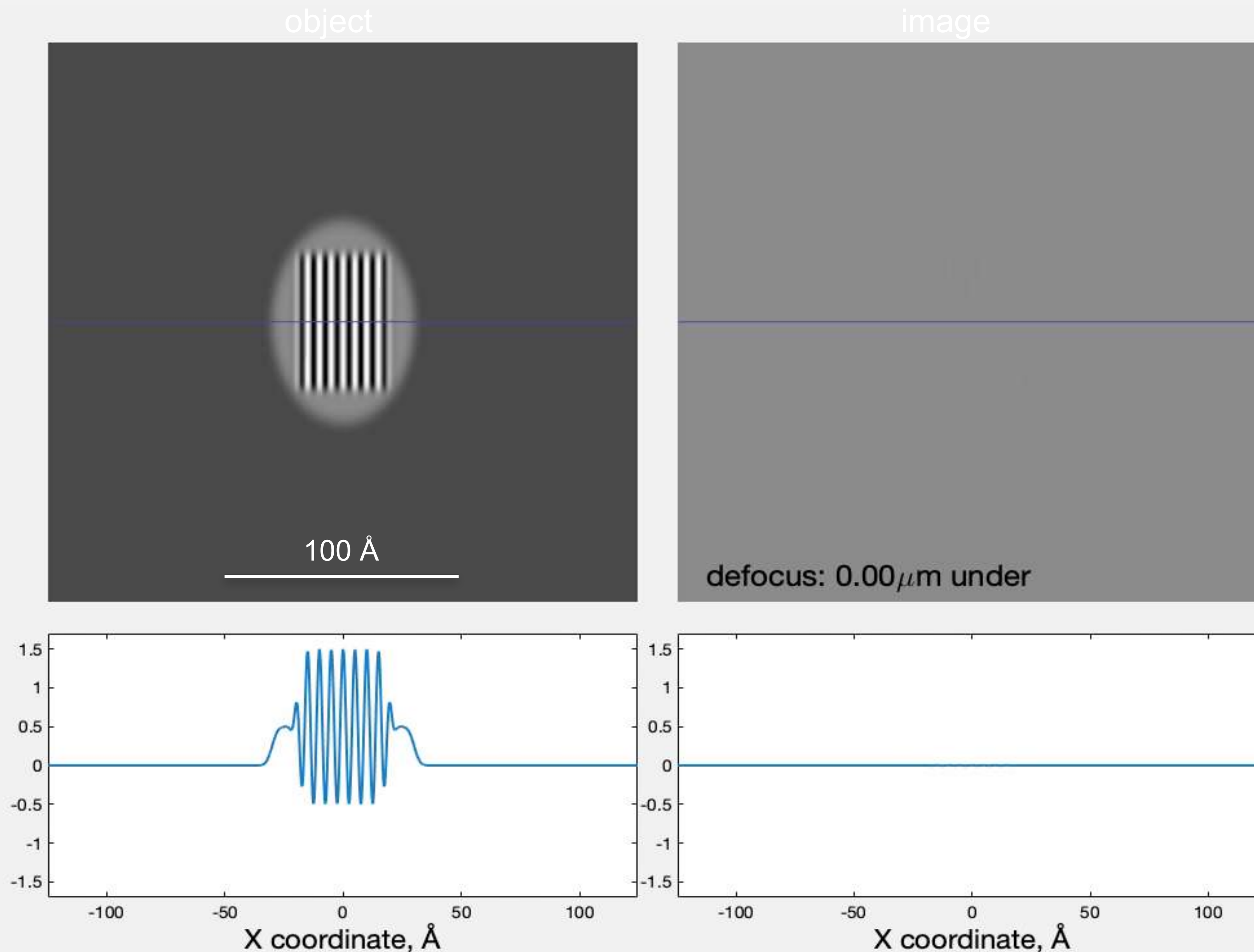


Image of an object with 5Å periodicity



- Defocus values are always “underfocus”. This means decreasing the strength of the objective lens, effectively focusing **above** the specimen.
- 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.

Defocus contrast in a nutshell

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.

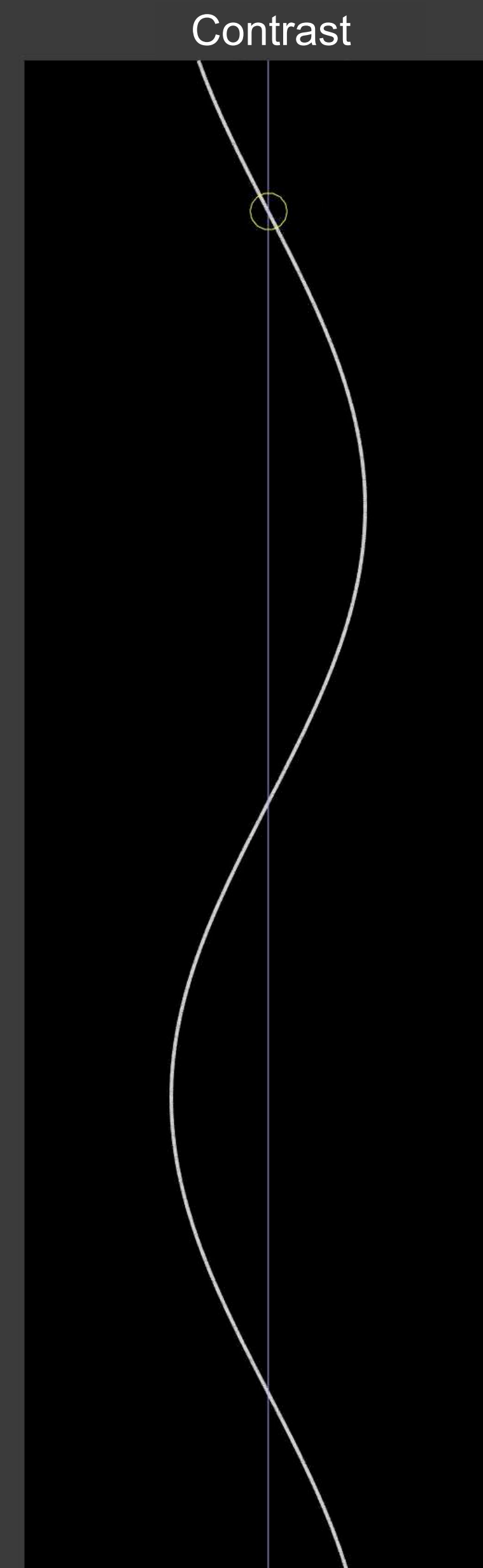
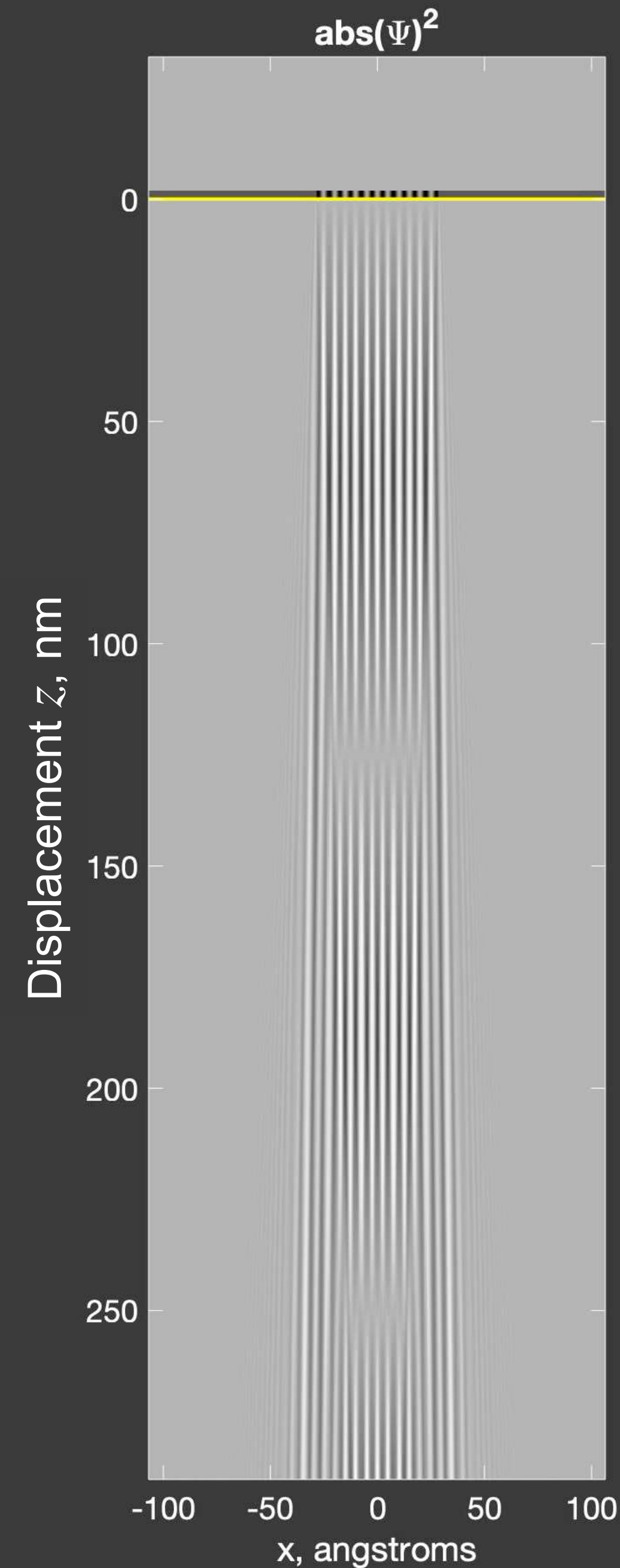
Contrast of a grating object varies with the amount of defocus



Intensity at z

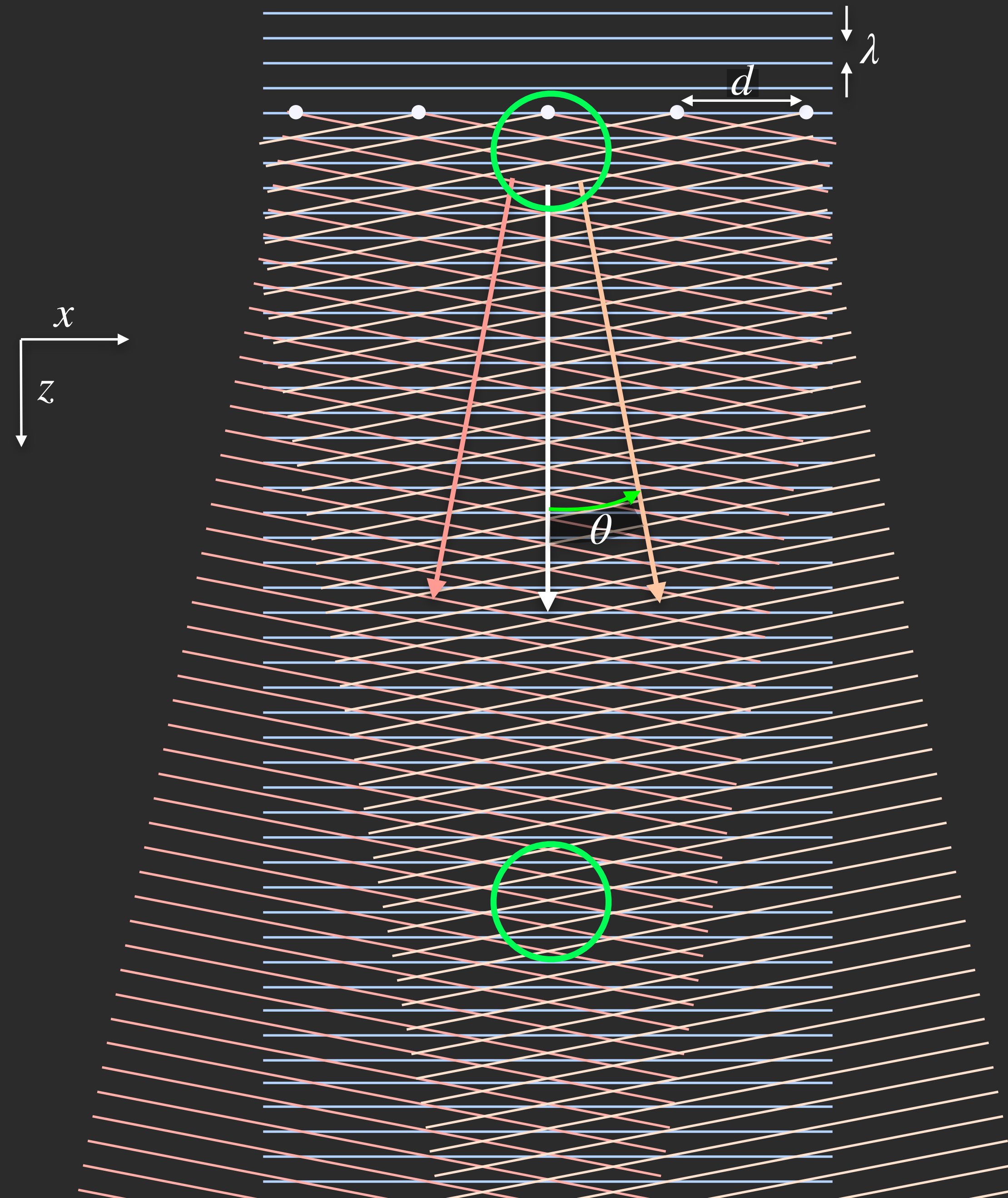


The grating $\phi(x)$

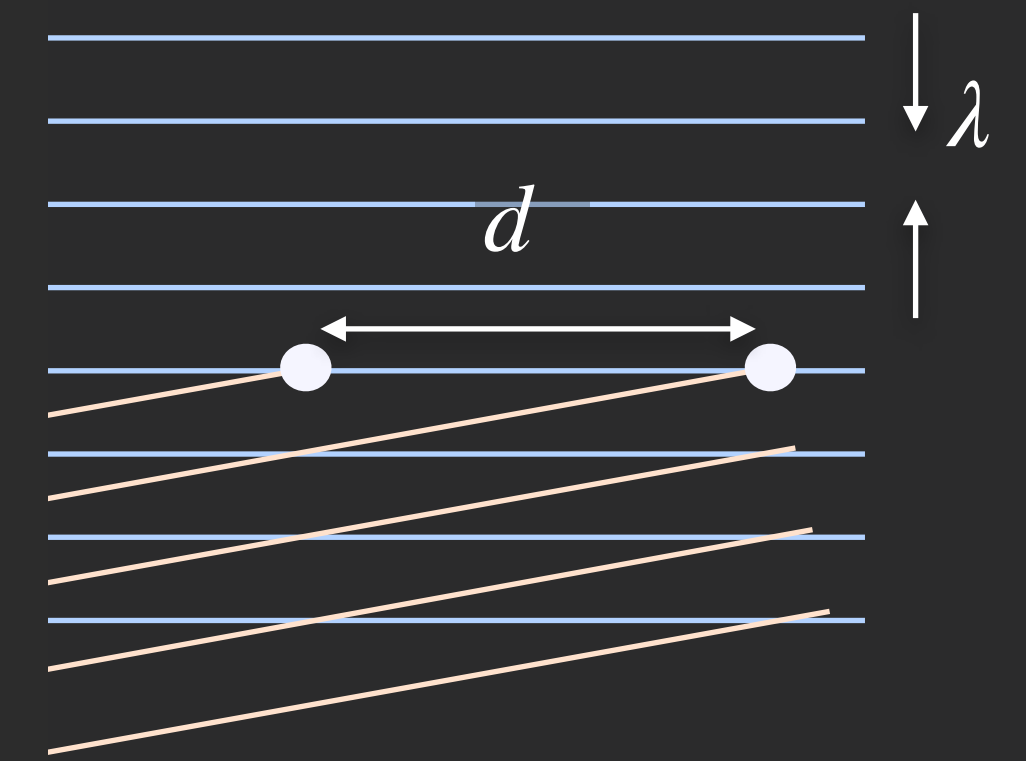


Interference between the unscattered wave and the diffracted waves produces contrast.

The grating object produces diffracted waves

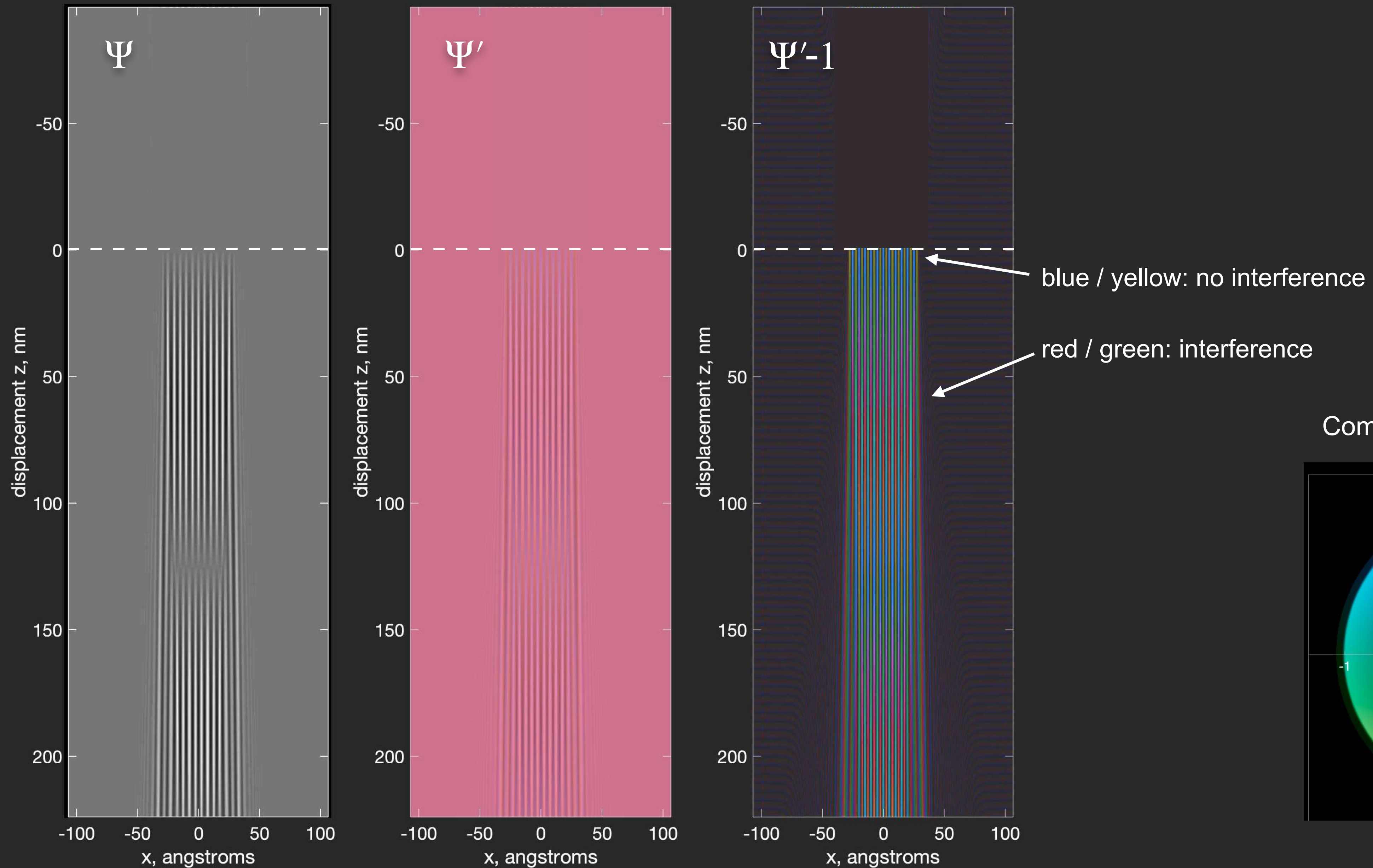


$$\sin \theta = \frac{\lambda}{d}$$

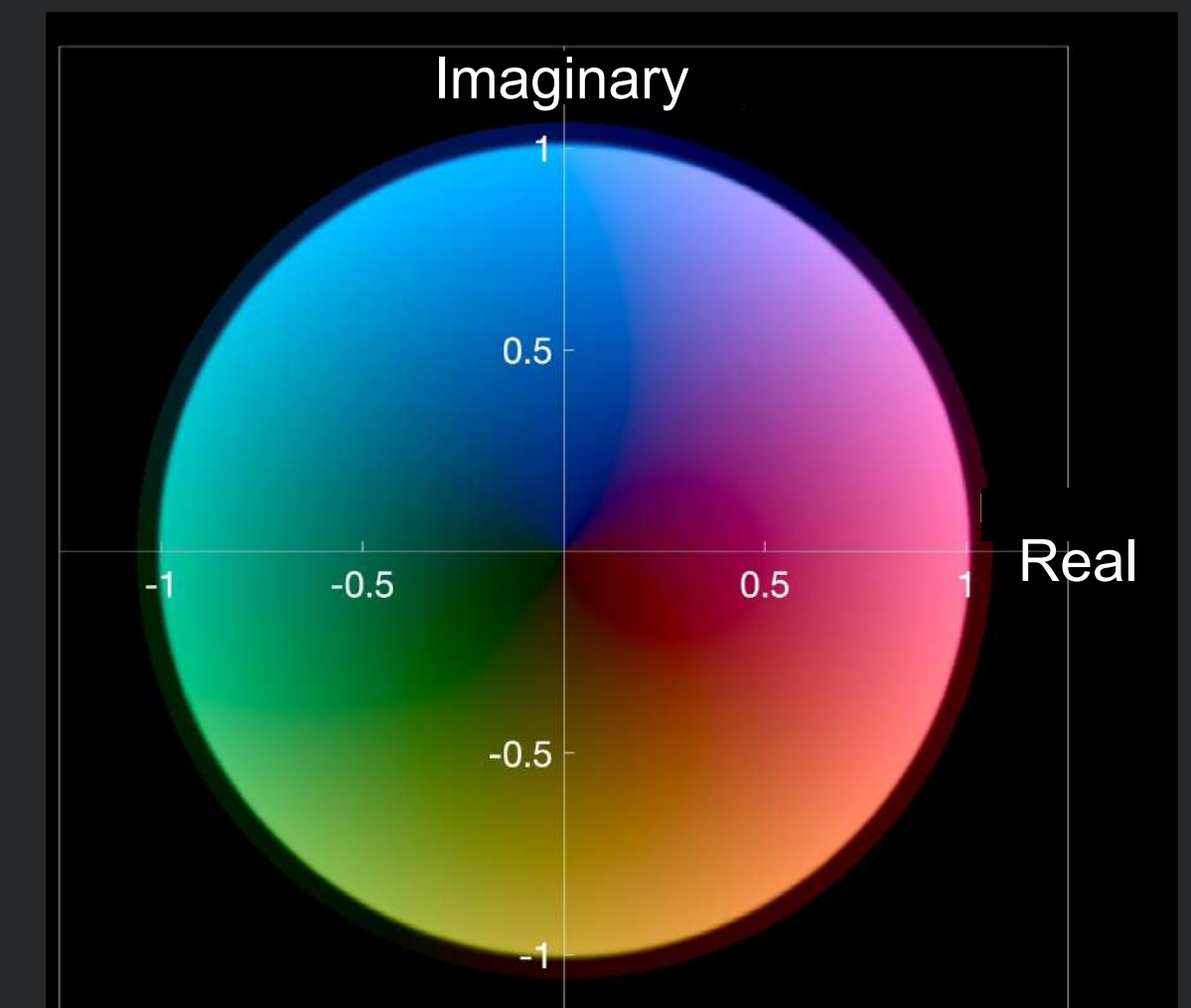


- Note there's a tiny shift of wavefronts, because the diffracted waves follow slightly longer paths.

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



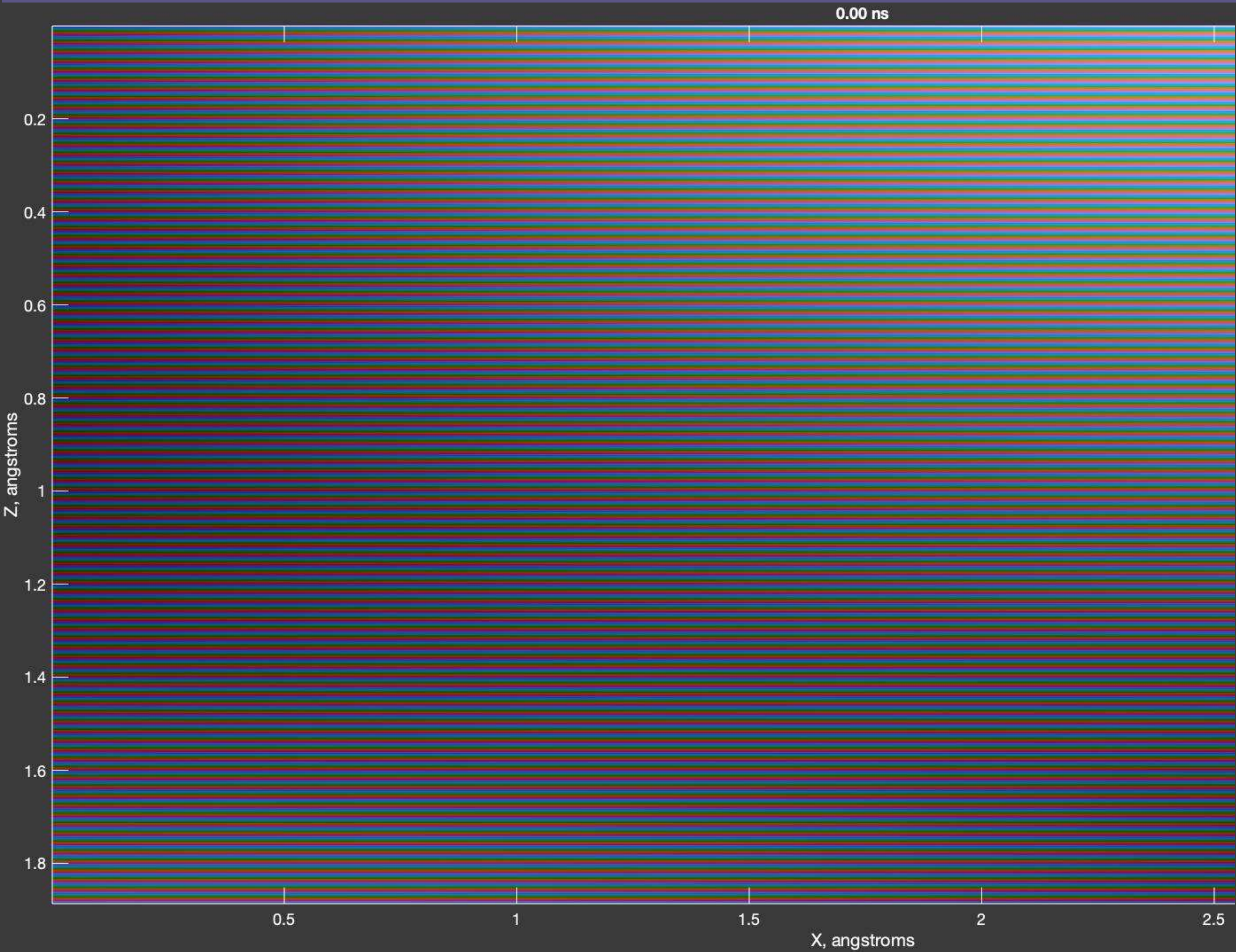
Complex number color scheme



Now for the theory in all its beauty...

1. Electrons have really short wavelengths, and they travel through the column one by one.
2. The contrast in the image of a grating object varies with the amount of defocus
3. The grating object produces diffracted waves with shifting phase
4. When the phase of the diffracted waves is right, we have contrast.
5. A lens reproduces the wavefronts at the image plane.
6. Spherical aberration and amplitude contrast introduce new terms in the CTF.
7. A phase plate changes the wavefronts before they reach the camera.

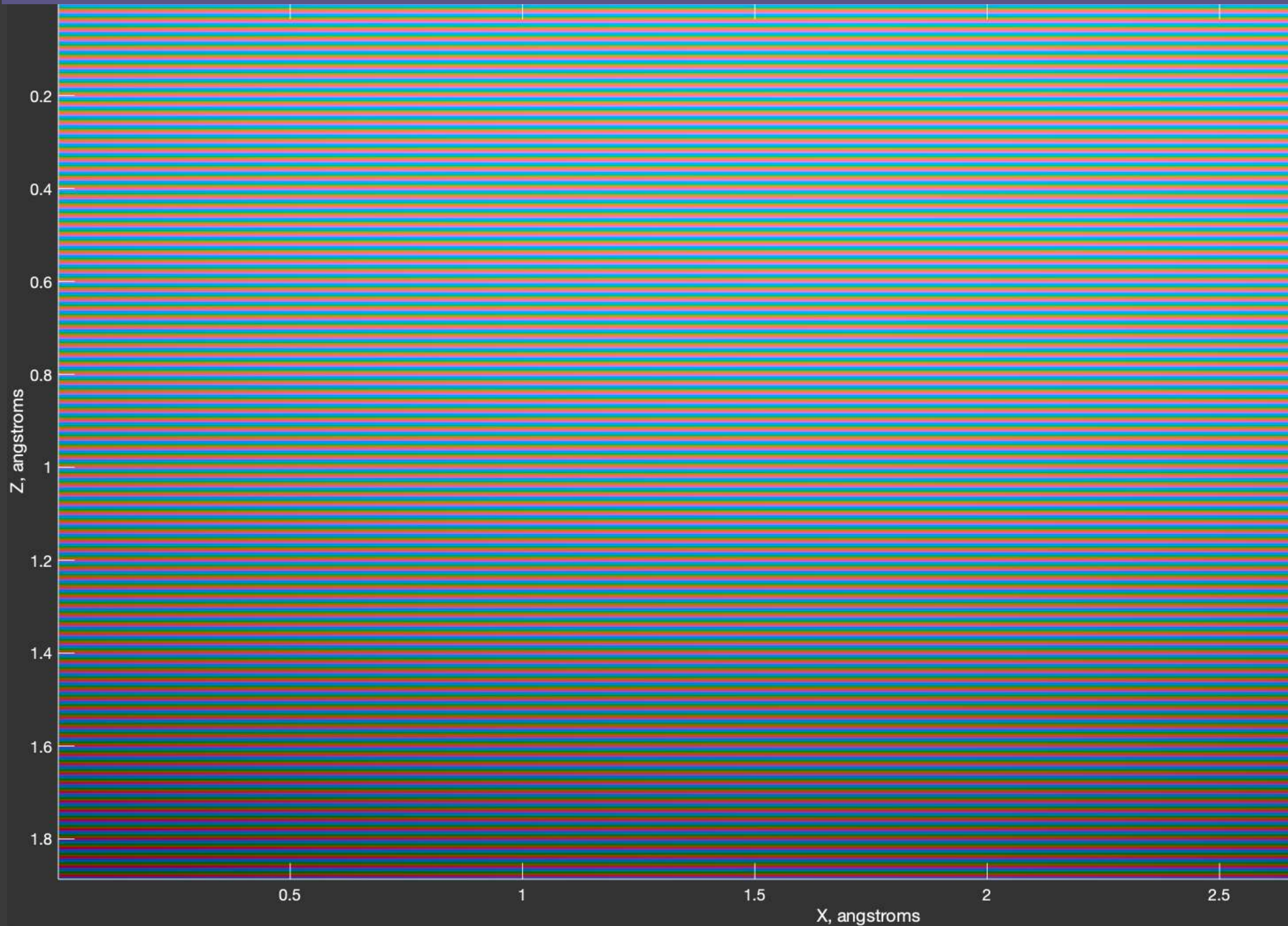
Electrons pass through the column one at a time



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

$$\Psi_0 = e^{i(kz - \omega t)}$$
$$k = 2\pi/\lambda$$

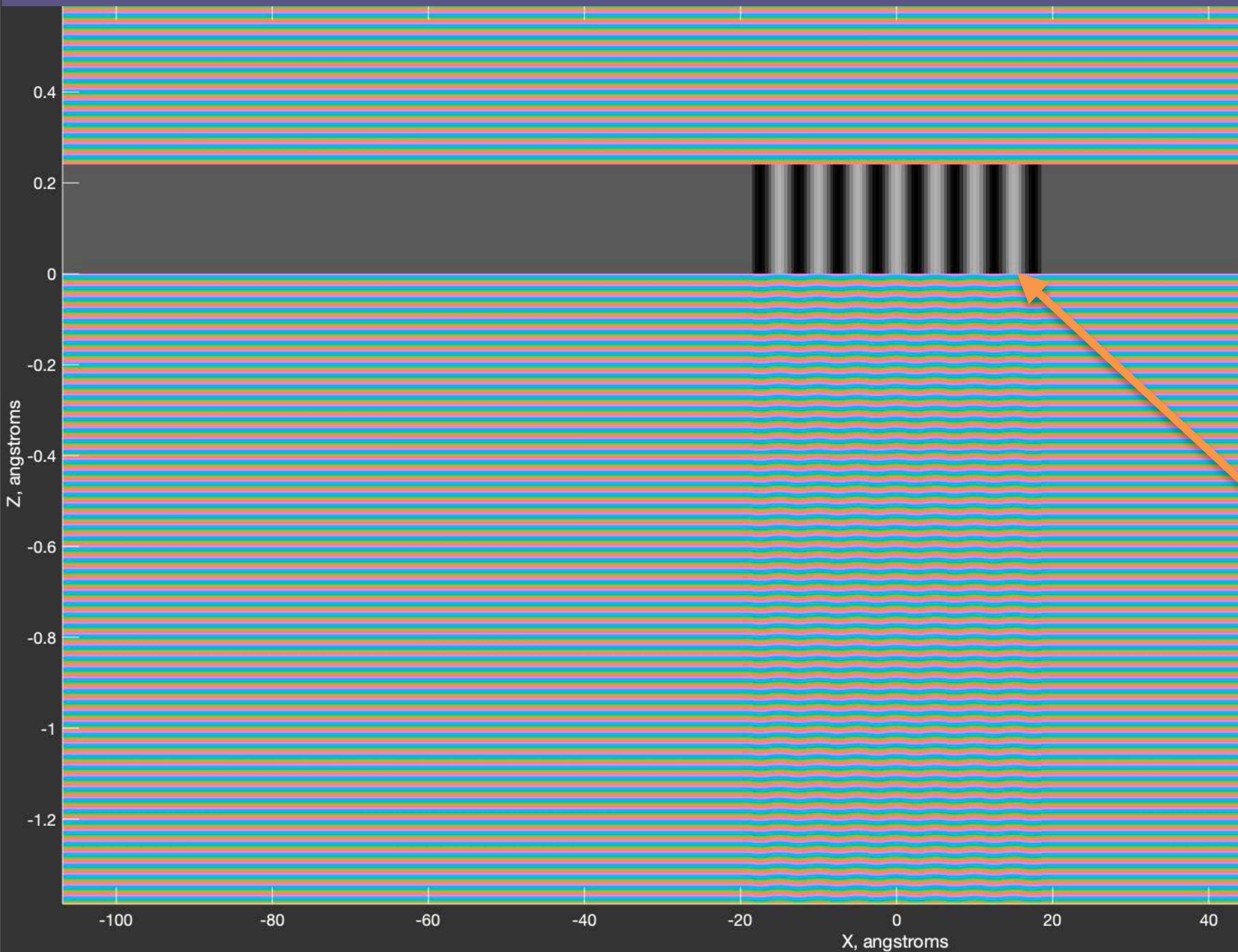
We'll ignore time dependence and take a snapshot of an electron wave



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

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

...and insert a phase-shifting object that perturbs the electron wave function

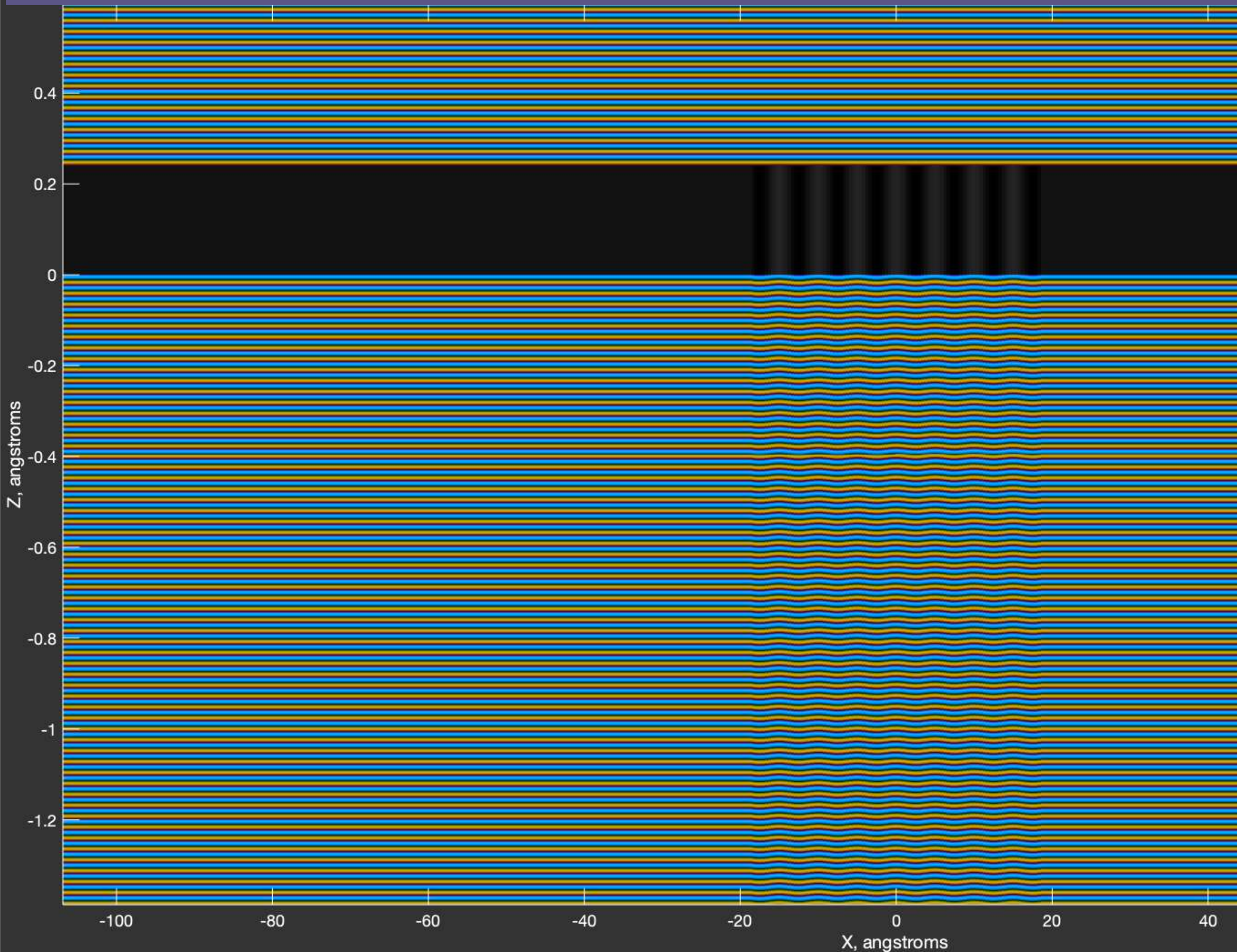


The object is a grating,
 $\epsilon\phi(x) = \epsilon \cos(2\pi x/d)$.

In our example,
 $d = 5\text{\AA}$ and $\epsilon \ll 1$.

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

Small ϵ allows the weak-phase approximation



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

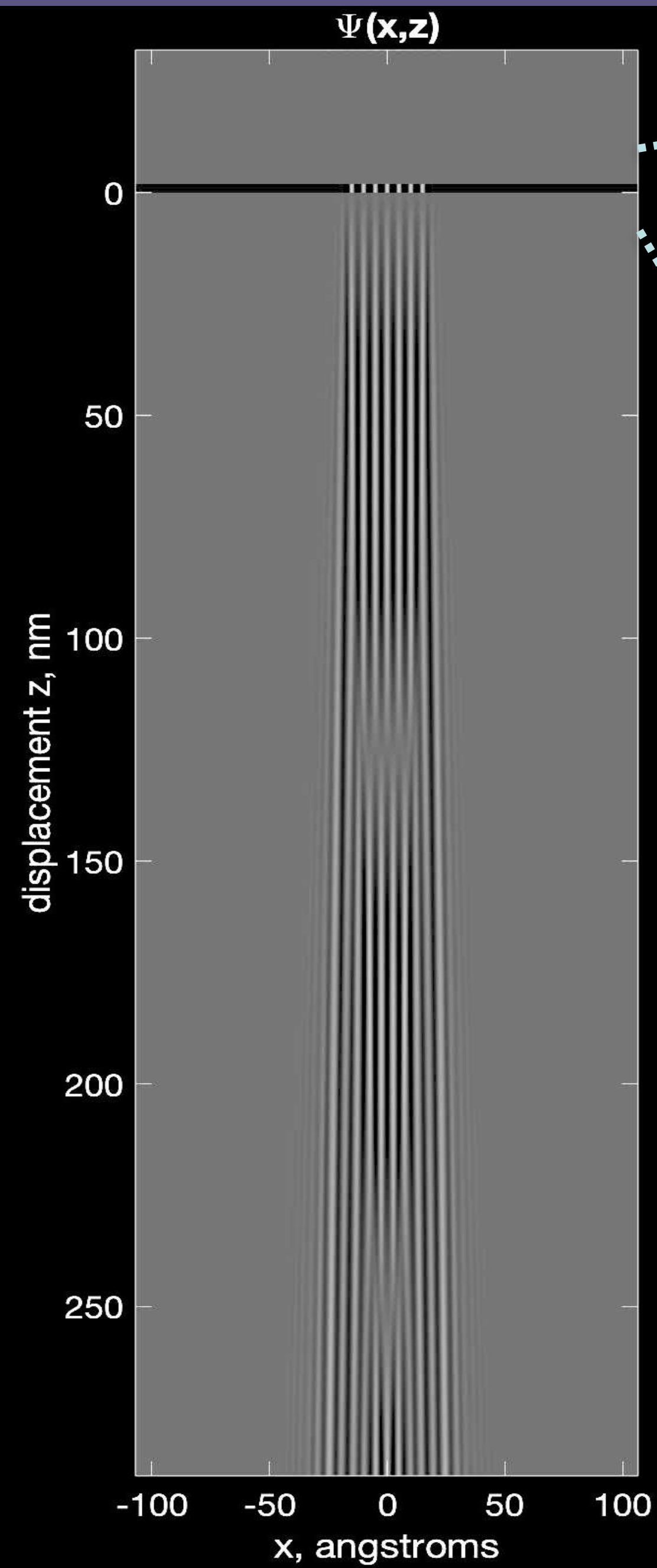
But the **weak phase approximation***
allows us to decompose Ψ into
undiffracted and diffracted waves:
at $z = 0$,

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

*This comes from the expansion

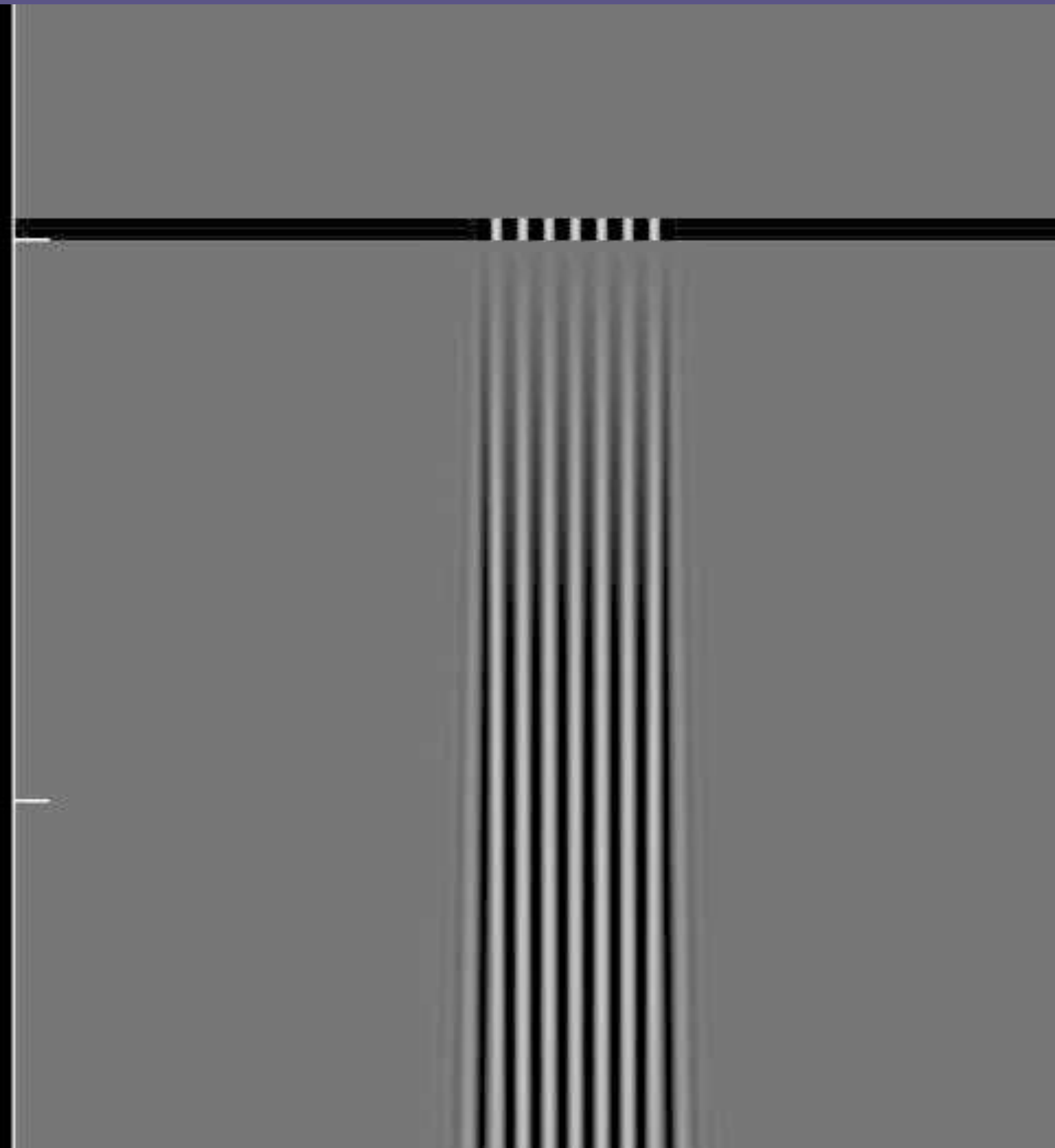
$$e^{iy} = 1 + iy - \frac{y^2}{2} + \dots$$

The beam intensity shows variation only away from the specimen

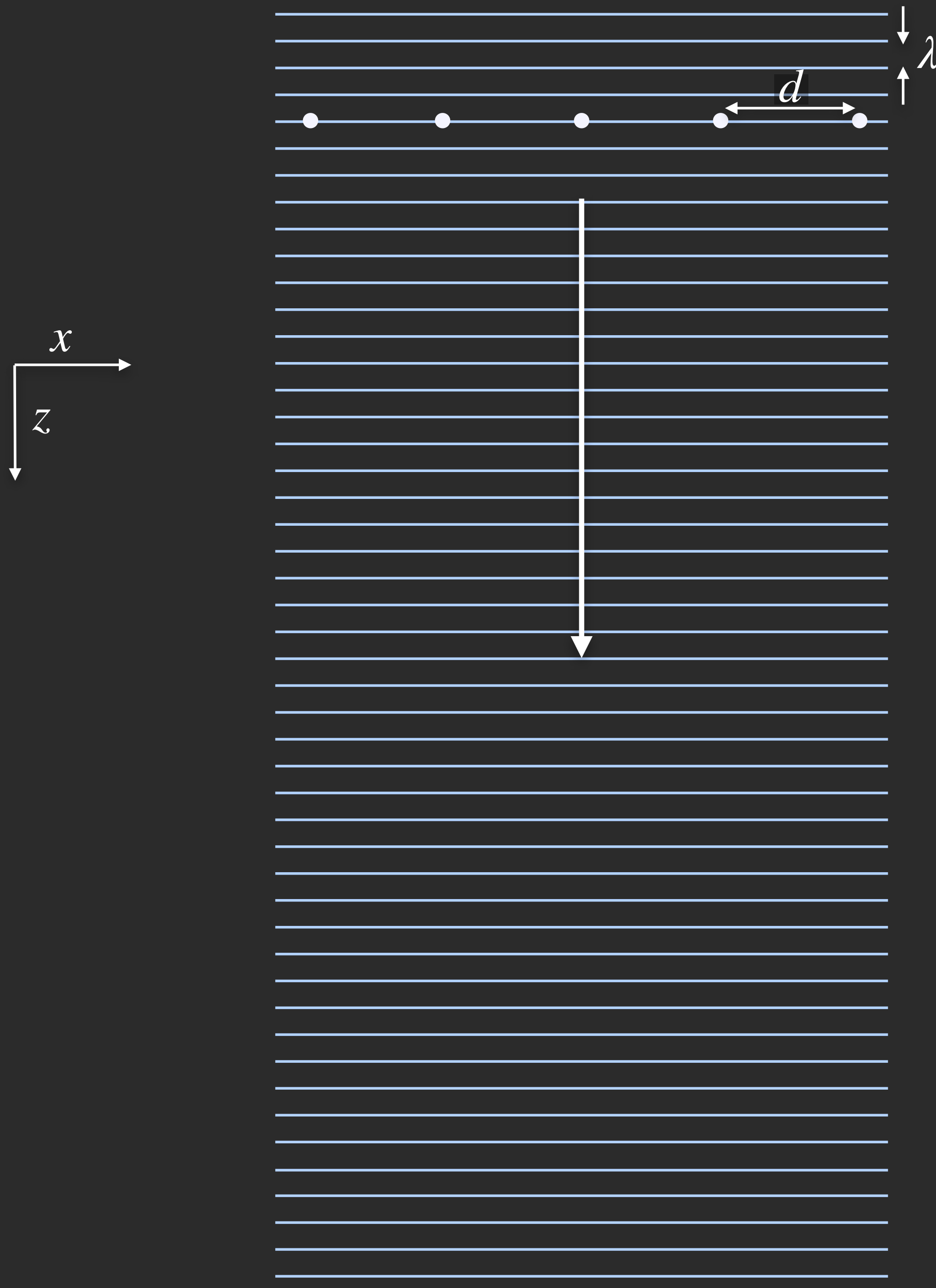


displacement z , nm

0
50

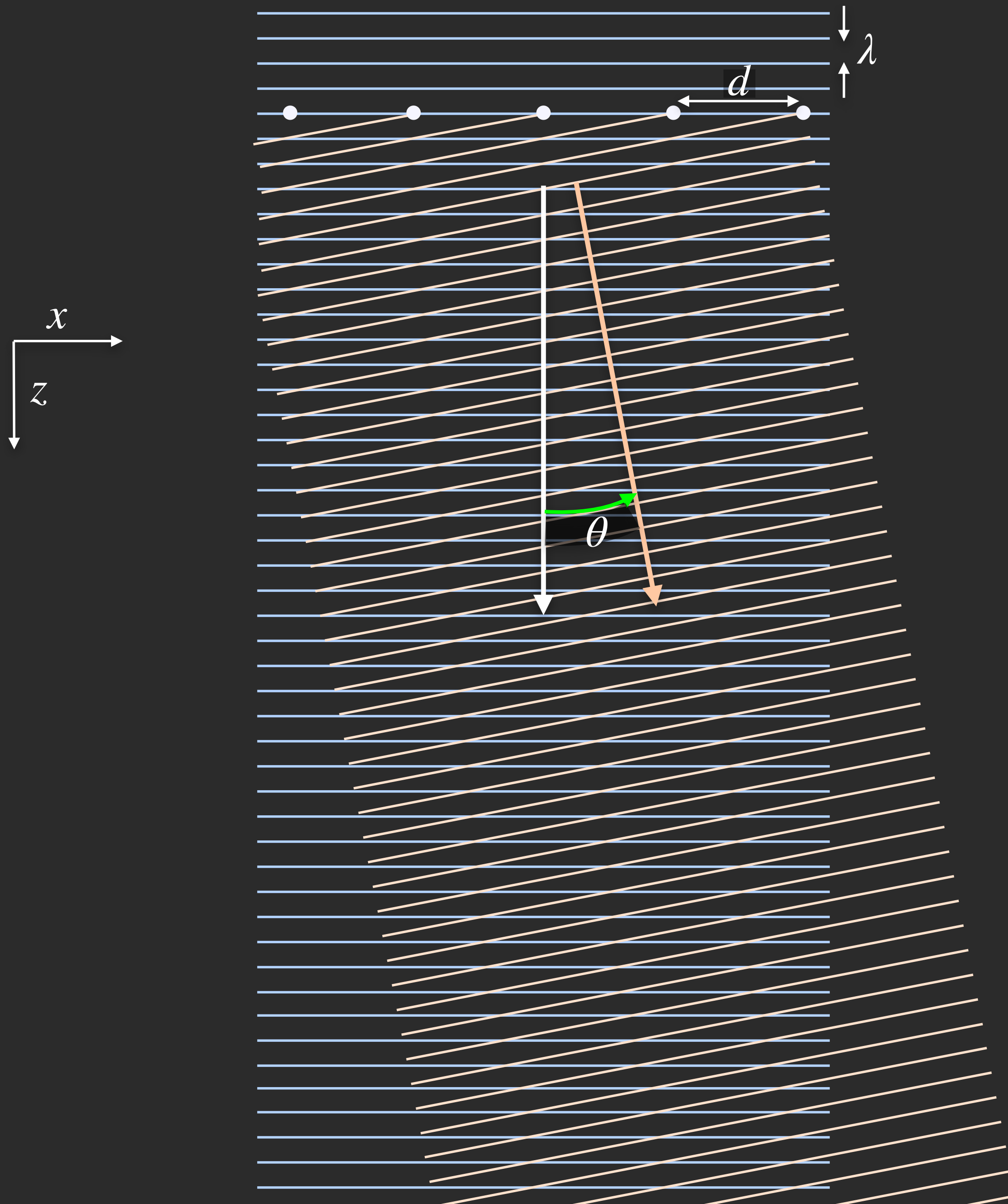


Modeling the components of Ψ : first, the undiffracted wave



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

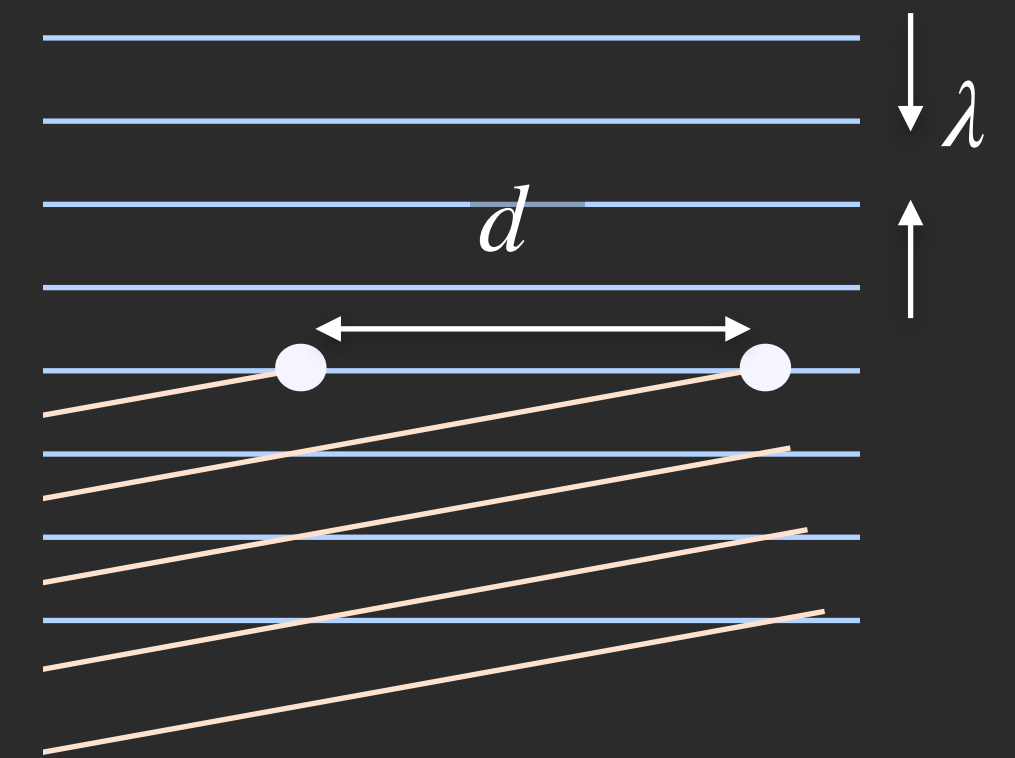
Classical diffraction yields a diffracted wave...



$$s = \sin \theta = \frac{\lambda}{d}$$
$$c = \cos \theta \approx 1 - \frac{\lambda^2}{2d^2}$$

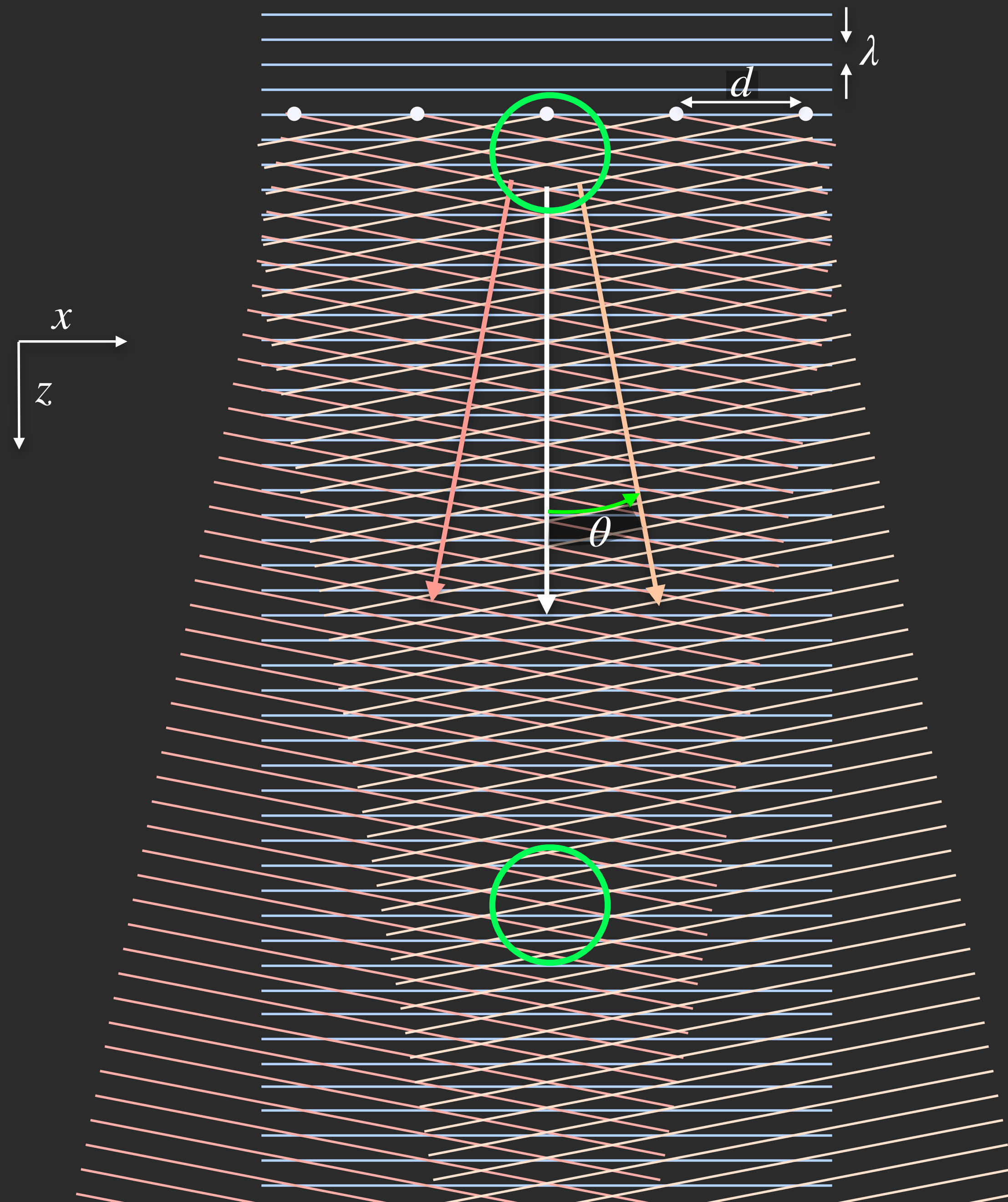
$$\Psi_0 = e^{ikz}$$

$$\Psi_+ = \frac{\epsilon}{2} e^{ik(cz+sx)}$$



With $\lambda = .02\text{\AA}$ and $d = 5\text{\AA}$,
 θ is only 4 milliradians, or 0.23°

...and the other diffracted wave



$$s = \sin \theta = \frac{\lambda}{d}$$
$$c = \cos \theta \approx 1 - \frac{\lambda^2}{2d^2}$$

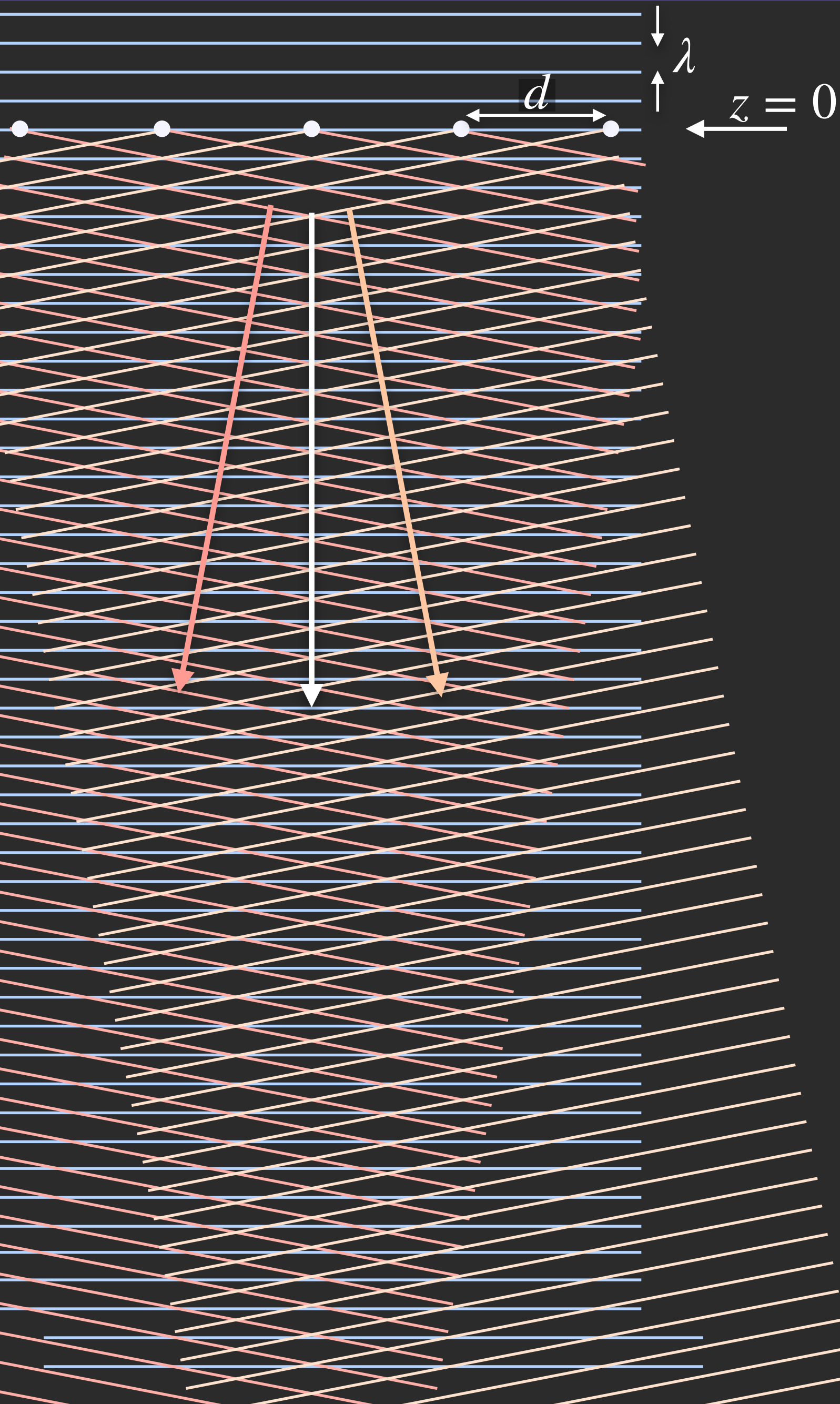
$$\Psi_0 = e^{ikz}$$

$$\Psi_+ = \frac{\epsilon}{2} e^{ik(cz+sx)}$$

$$\Psi_- = \frac{\epsilon}{2} e^{ik(cz-sx)}$$

- Note there's a tiny shift of wavefronts, because the diffracted waves follow slightly longer paths.

The sum of these three waves gives a perfect match at $z = 0$.



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

$$\Psi_+ = \frac{\epsilon}{2} e^{ik(cz+sx)}$$

$$\Psi_- = \frac{\epsilon}{2} e^{ik(cz-sx)}$$

← The sum of these match the weak-phase wave function we wanted.

At $z = 0$ we have

$$\Psi_0 + \Psi_+ + \Psi_- = 1 + i\epsilon\phi(x).$$

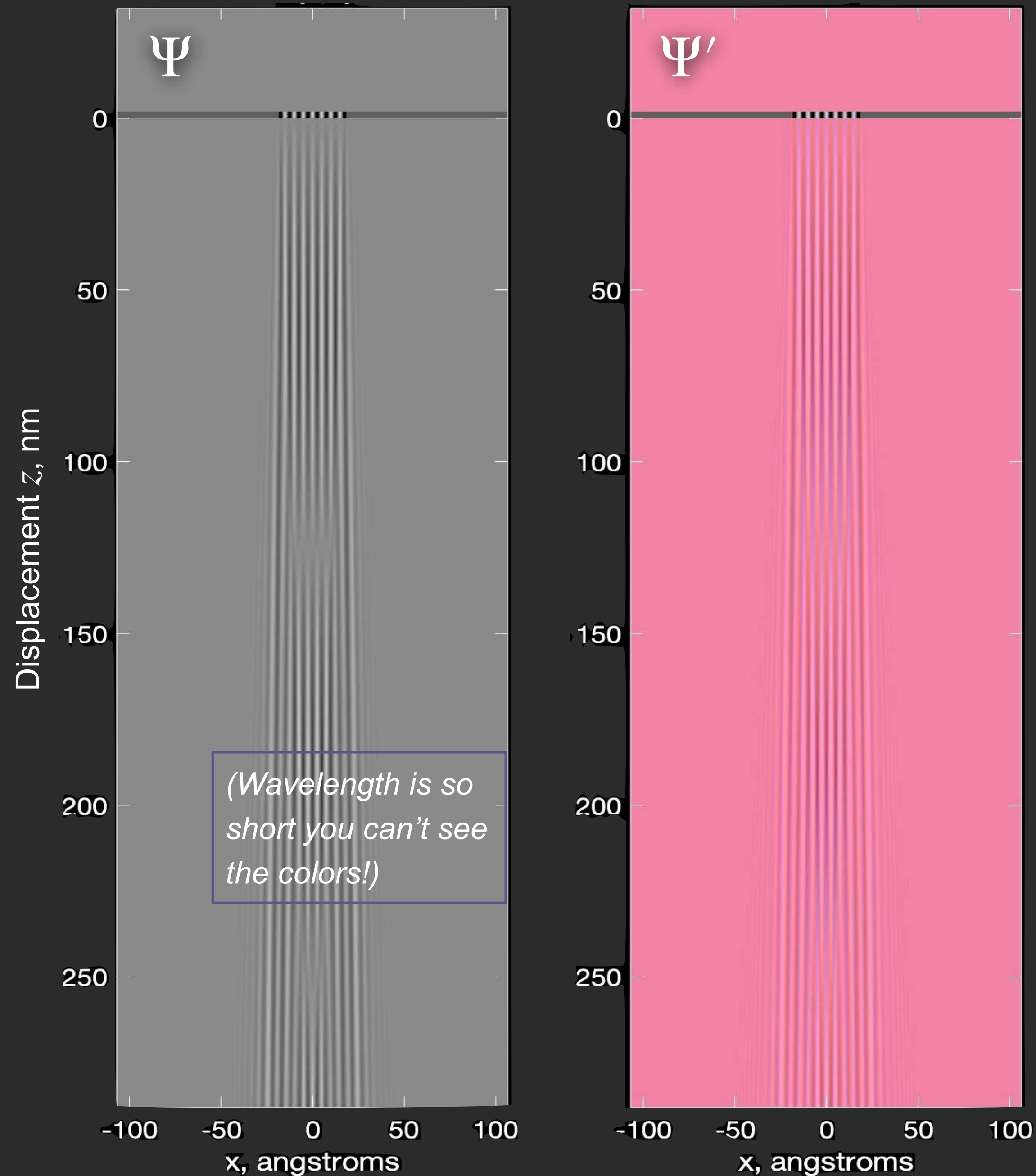
...because at $z = 0$

$$\Psi_+ + \Psi_- = i\epsilon(e^{iksx} + e^{-iksx}) = i\epsilon \cos(ksx)$$

and since $k = \frac{2\pi}{\lambda}$ and $s = \frac{\lambda}{d}$,

$$\Psi_+ + \Psi_- = i\epsilon \cos(2\pi x/d) = i\epsilon\phi(x)$$

Given the boundary condition, we know $\Psi(z)$ for all $z \geq 0$



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

$$\Psi_+ = \frac{i\epsilon}{2} e^{ik(cz+sx)}$$

$$\Psi_- = \frac{i\epsilon}{2} e^{ik(cz-sx)}$$

The net wavefunction is

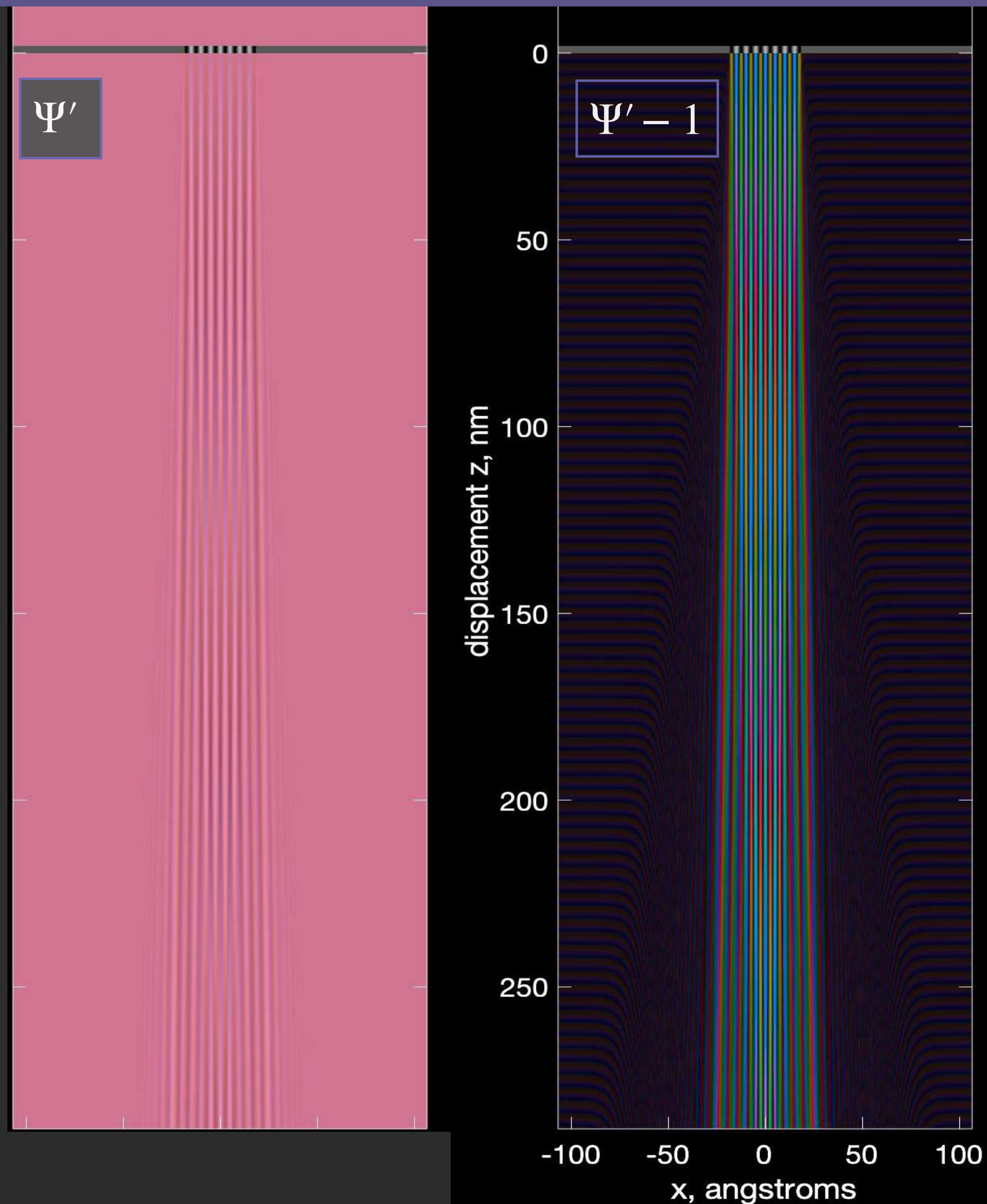
$$\Psi = \Psi_0 + \Psi_+ + \Psi_-$$

For simplicity, we'll define Ψ' : $\Psi = \Psi' e^{ikz}$

$$\begin{aligned} \Psi' = 1 &+ \frac{i\epsilon}{2} e^{ik(c-1)z} e^{iksx} \\ &+ \frac{i\epsilon}{2} e^{ik(c-1)z} e^{-iksx} \end{aligned}$$

$$\Psi' = 1 + i\epsilon e^{ik(c-1)z} \cos(ksx)$$

The diffracted-wave phases change relative to the unscattered wave



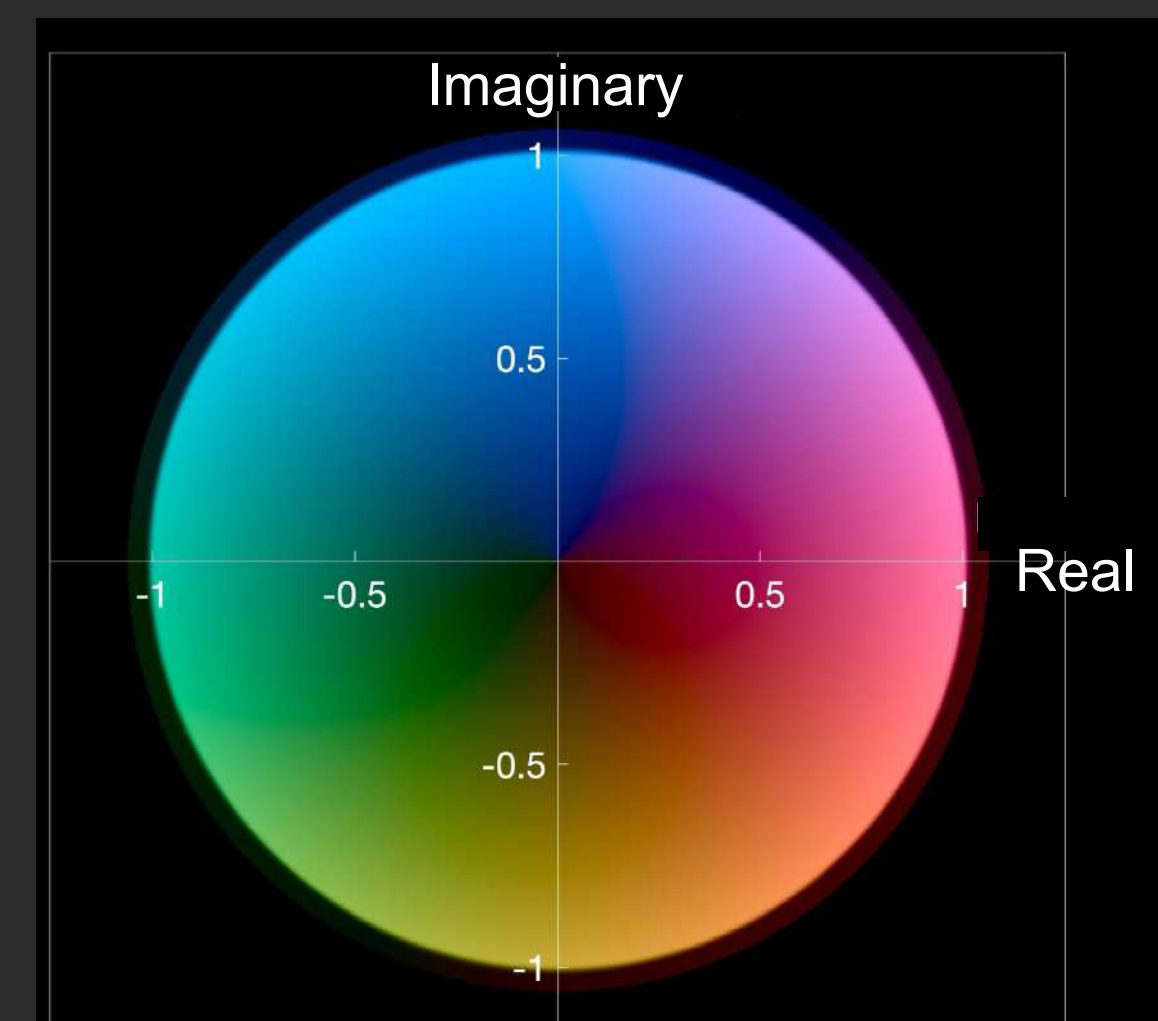
Let's remove the undiffracted wave
(constant part of Ψ').

The two diffracted waves interfere to
show the grating signal. Their overall
phase changes with z .

$$\Psi' - 1 = e^{ik(c-1)z} \cdot i\epsilon \cos(ksx)$$

$$\begin{aligned} k &= 2\pi/\lambda \\ s &= \sin(\theta) = \lambda/d \\ c &= \cos(\theta) \end{aligned}$$

Our original phase object
 $\phi(x) = \epsilon \cos(2\pi x/d)$



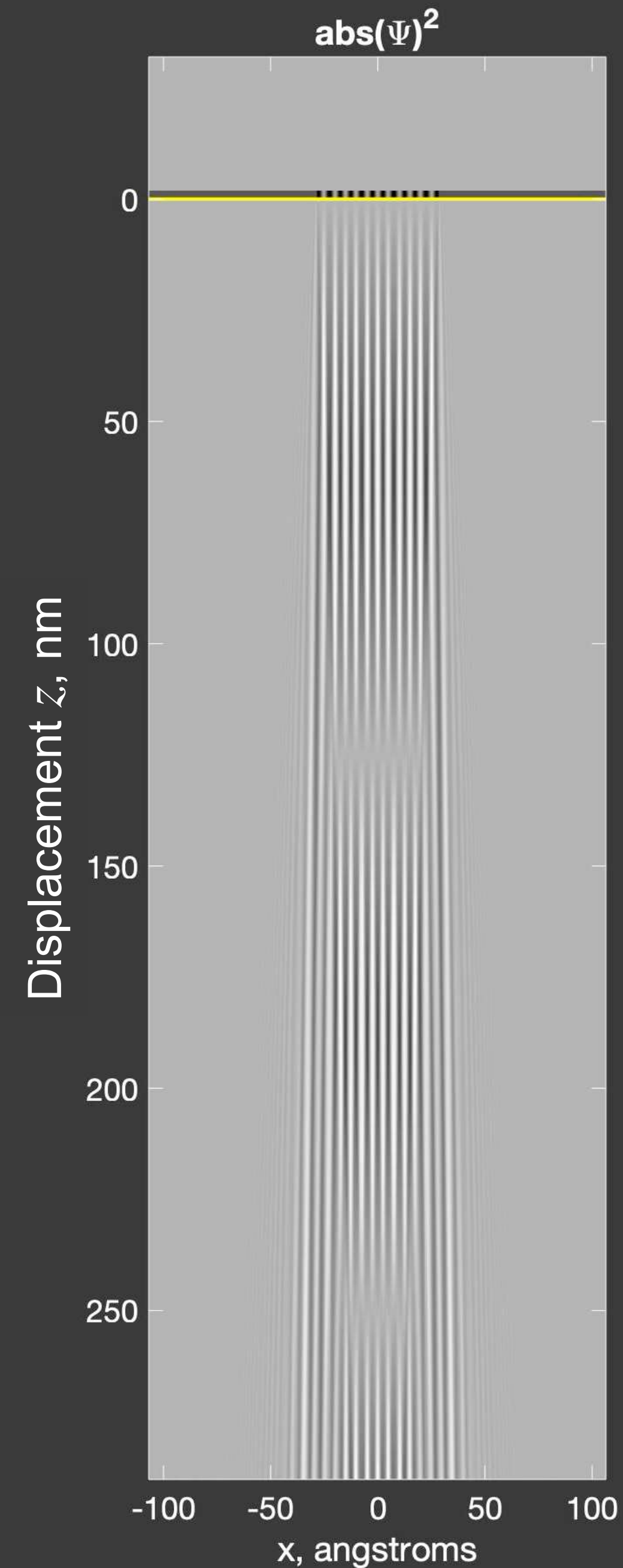
Contrast varies with the amount of defocus



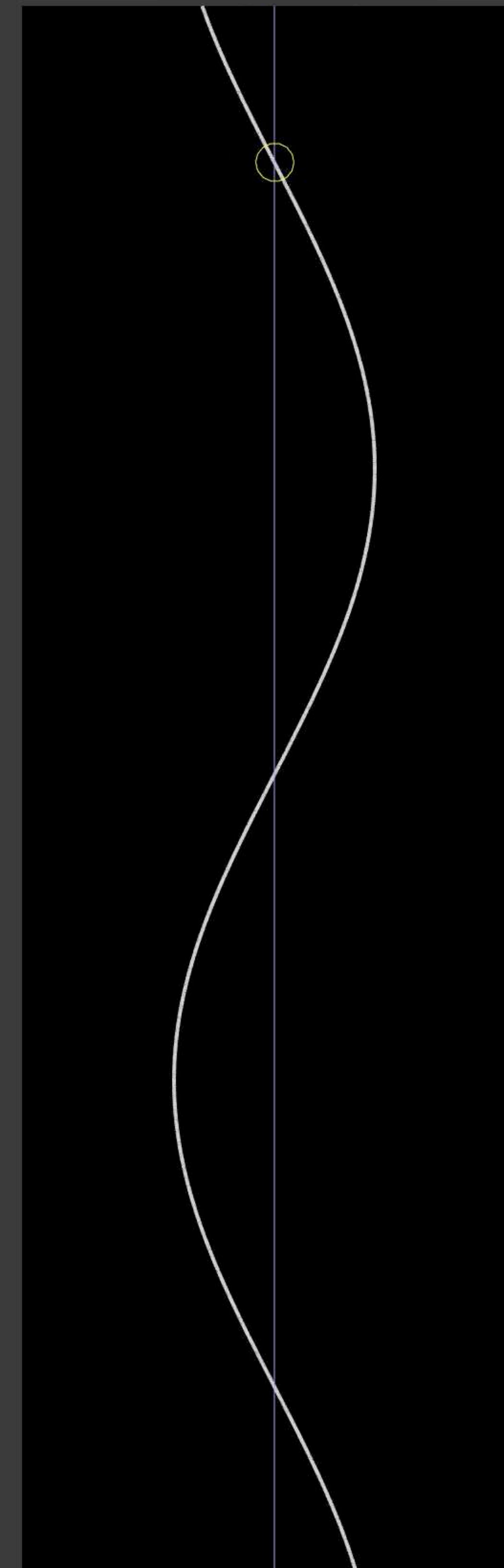
Intensity at z



The grating $\phi(x)$



Contrast



Interference between the unscattered wave and the diffracted waves produces contrast.

The contrast transfer comes from interference in the real part of Ψ

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

can be written as

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

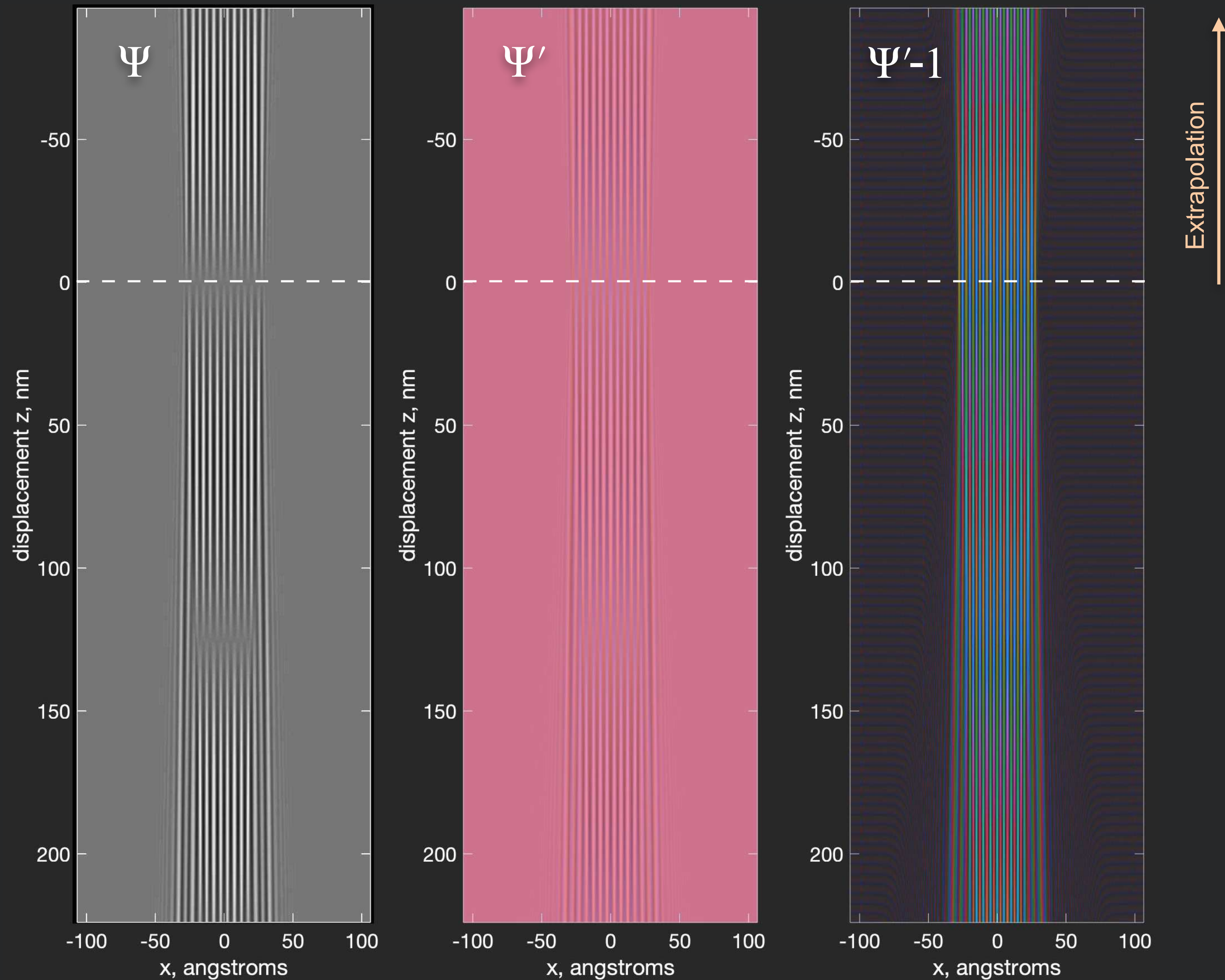
$$\epsilon \phi(x) = \epsilon \cos(2\pi x/d)$$

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

$$c = \cos(\theta) \approx 1 - \lambda^2/d^2$$

$$\chi = k(1 - c)z = \pi\lambda z/d^2$$

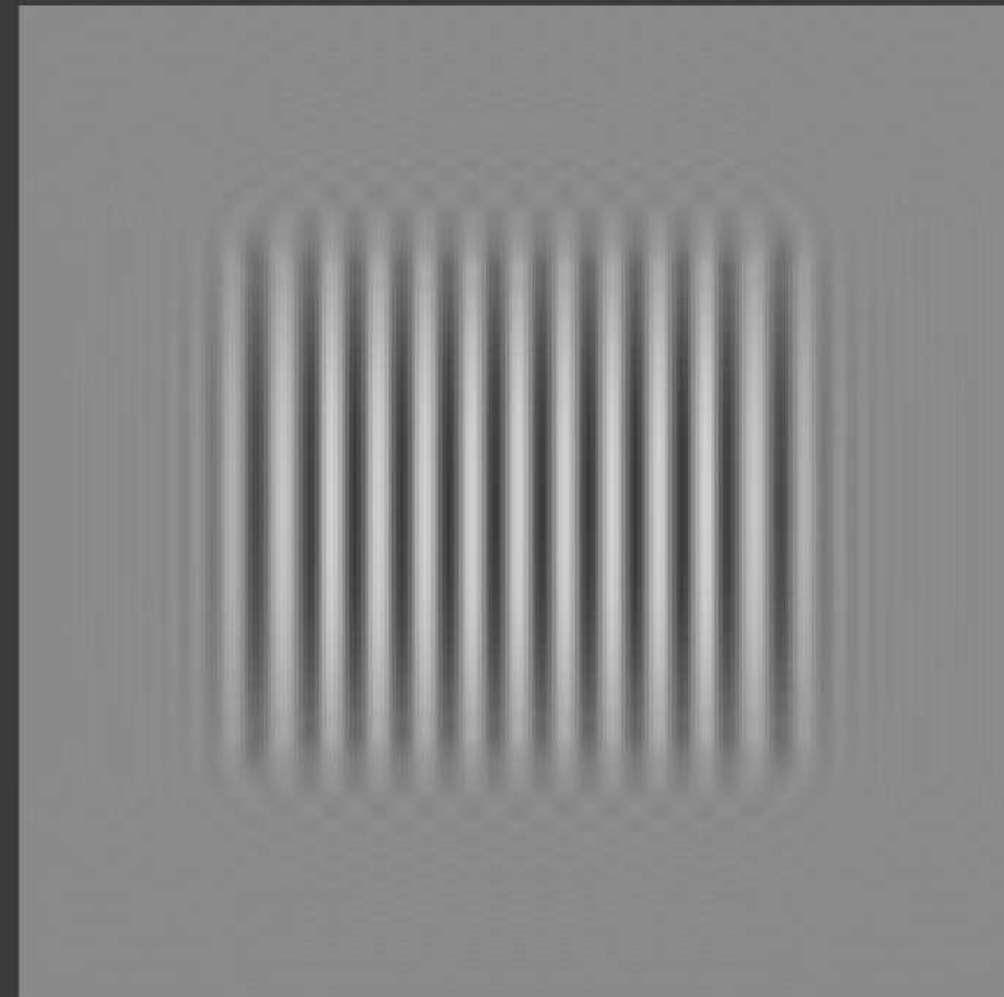
What happens when the objective lens is focused *above* the specimen?



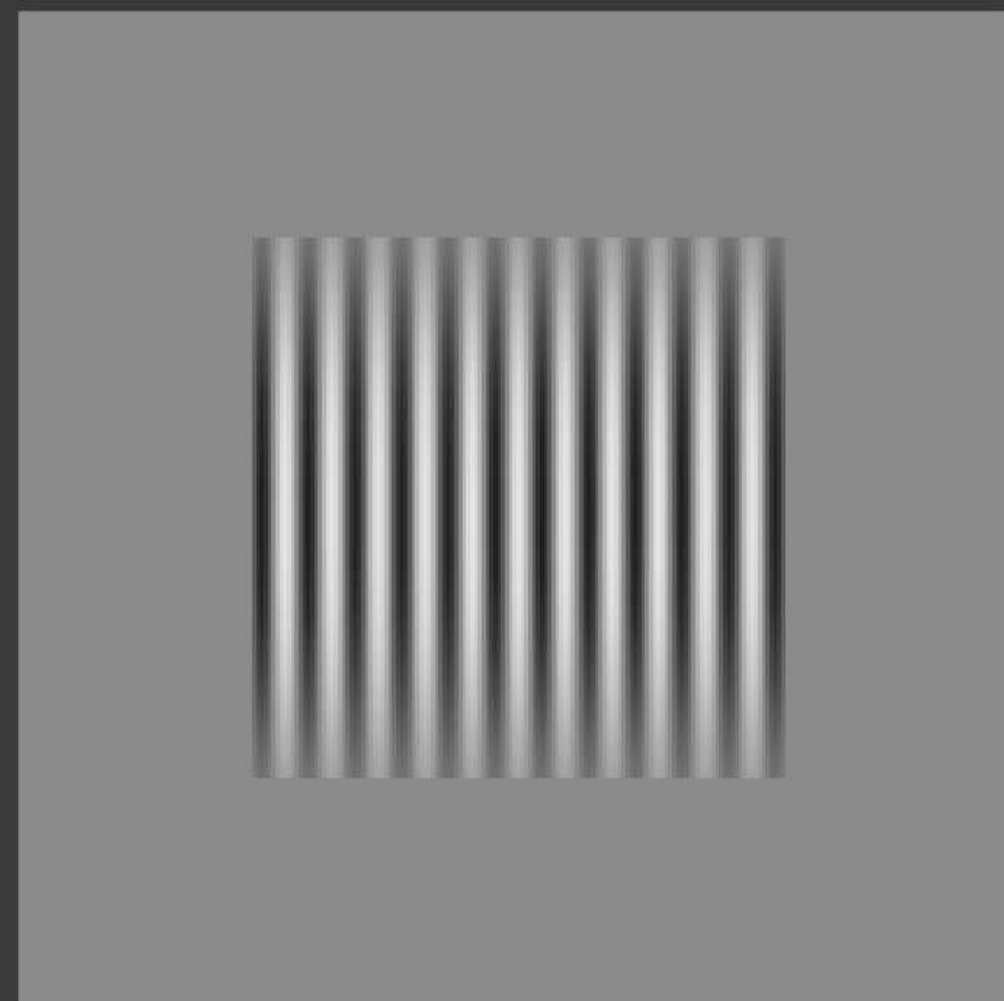
What wavefunction **above** the specimen would give rise to what we see below it?

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

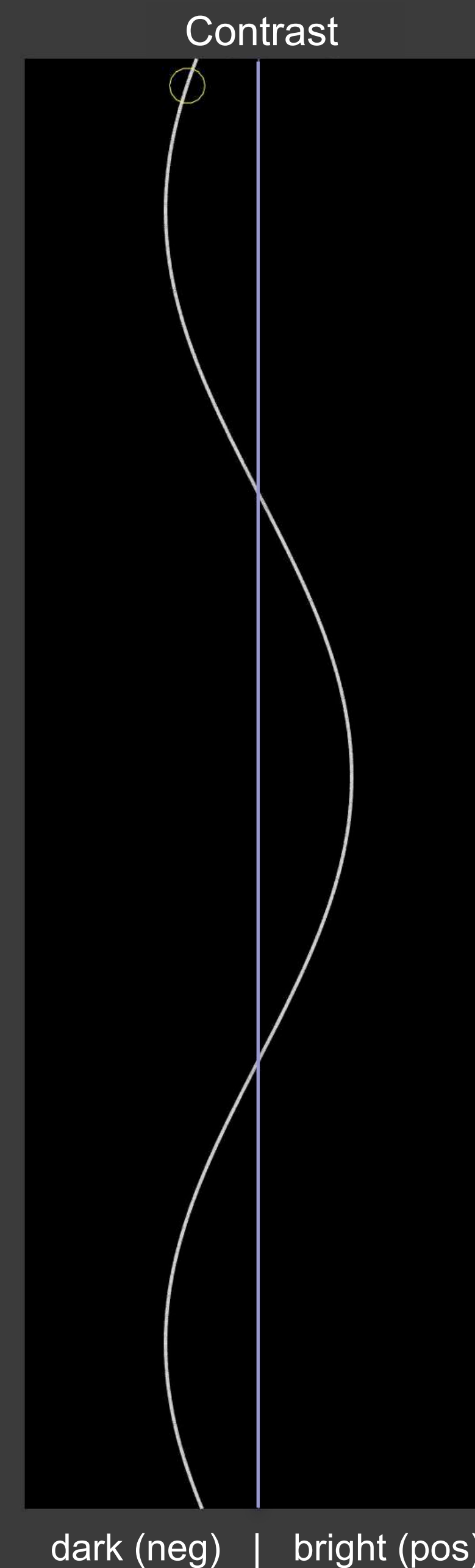
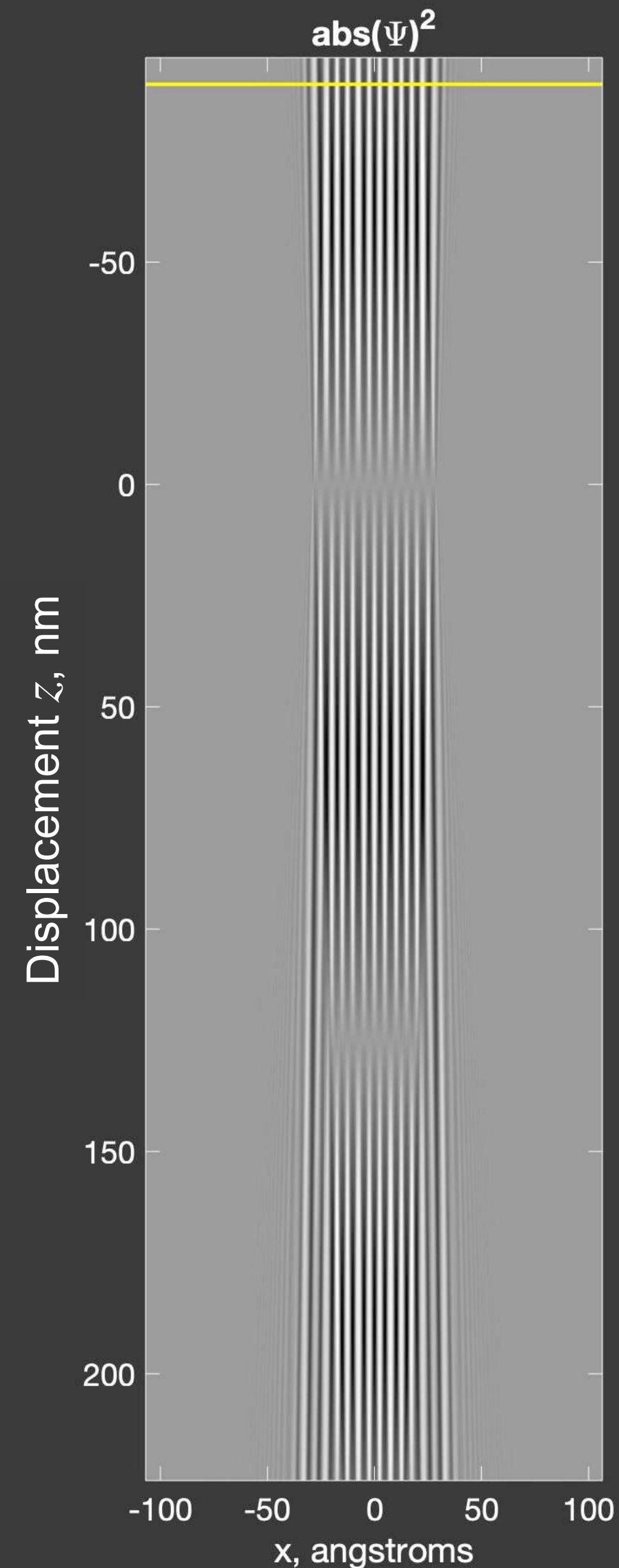
What happens when the objective lens is focused *above* the specimen?



Intensity at z



The grating $\phi(x)$



“Underfocus” is focusing the objective lens above the specimen.

Standard terminology

- Defocus values δ are positive for underfocus,

$$\delta = -z$$

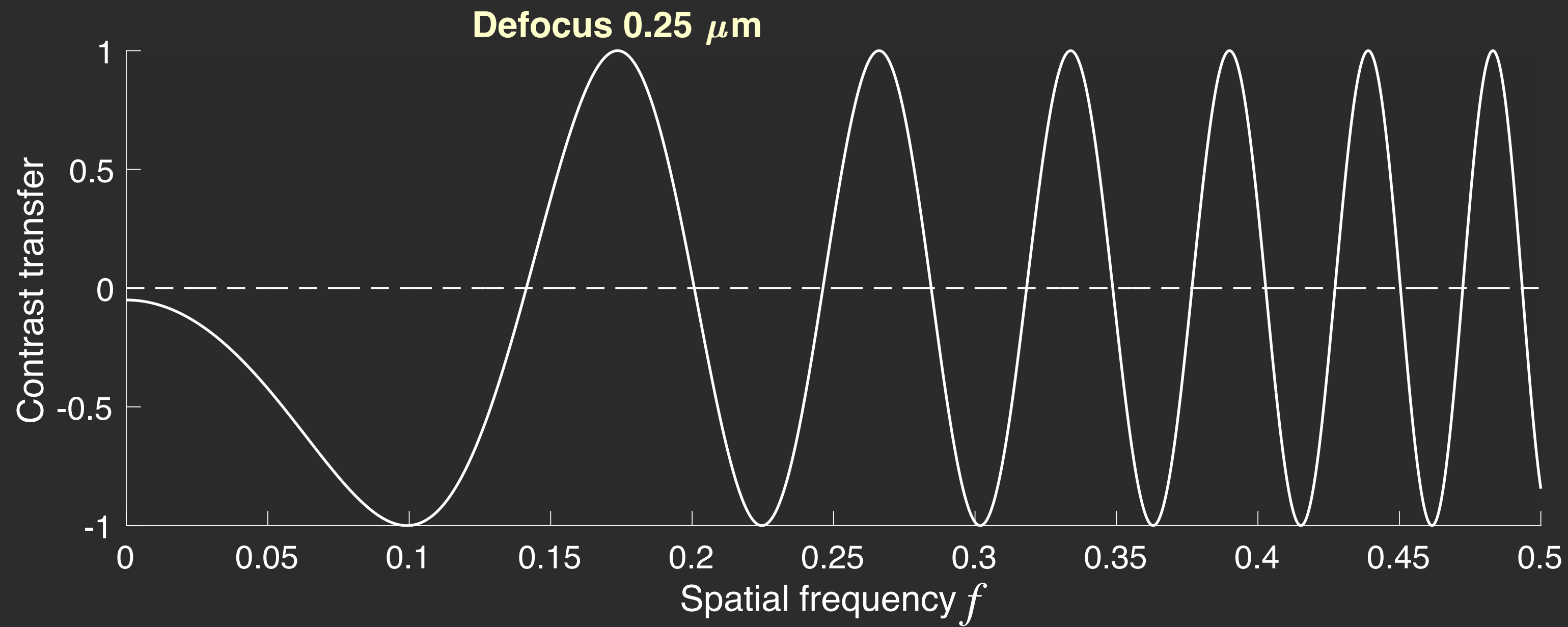
- Spatial frequency is

$$f = 1/d$$

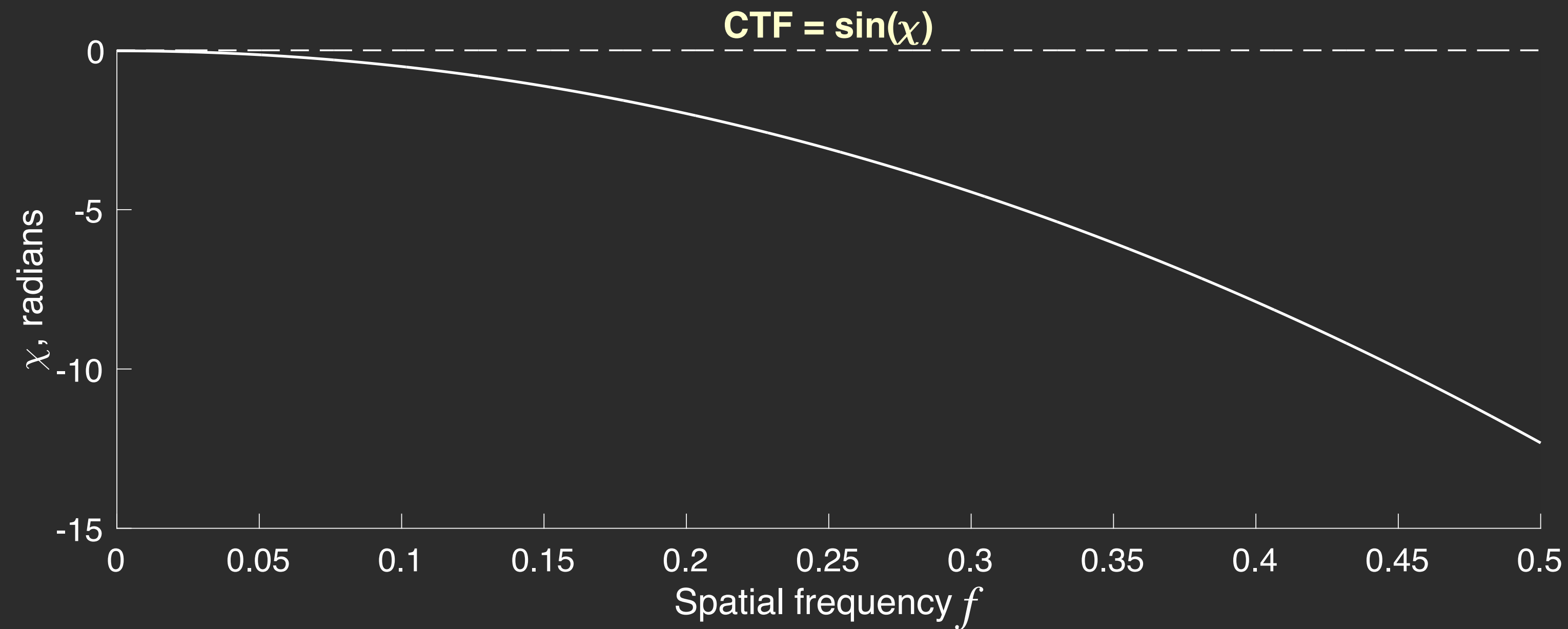
So we can write the defocus phase contrast as:

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

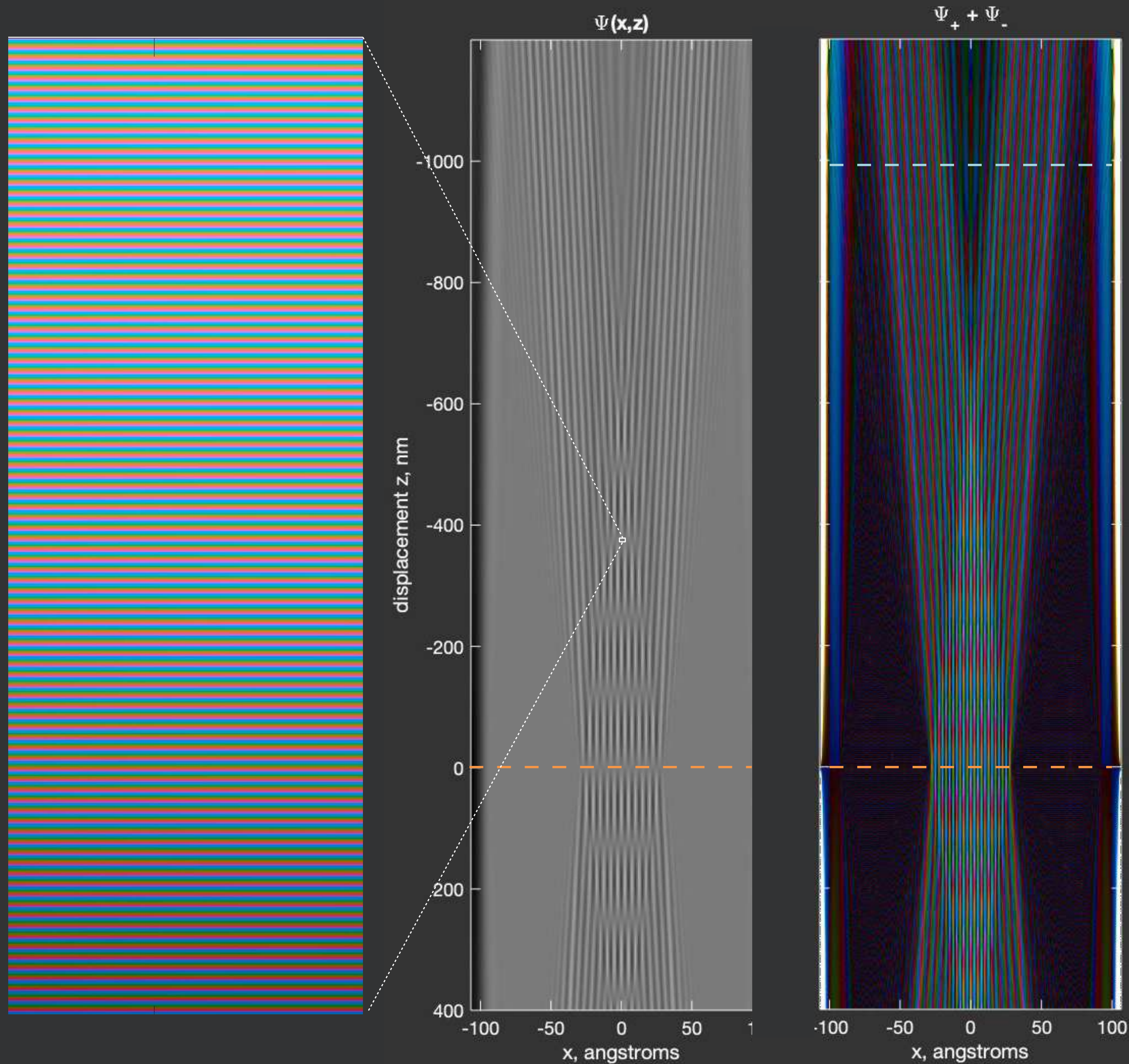
The contrast-transfer function as a function of f



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



A little defocus is actually a long distance

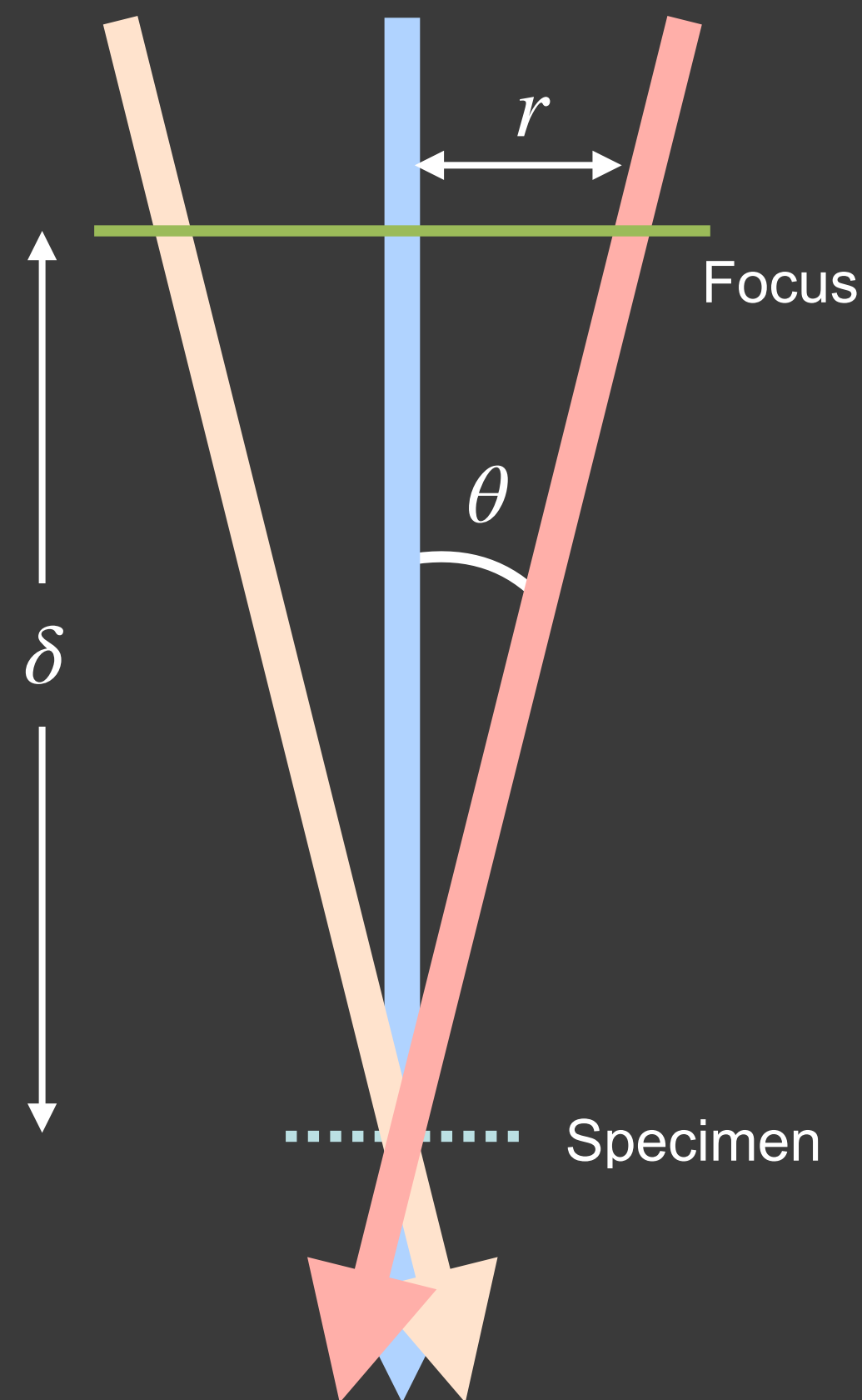


1 μm —a small defocus for cryo-EM imaging is 500,000 wavelengths!

This has ramifications regarding

- beam coherence
- specimen charging
- delocalization

With large defocus, how bad is the image delocalization?

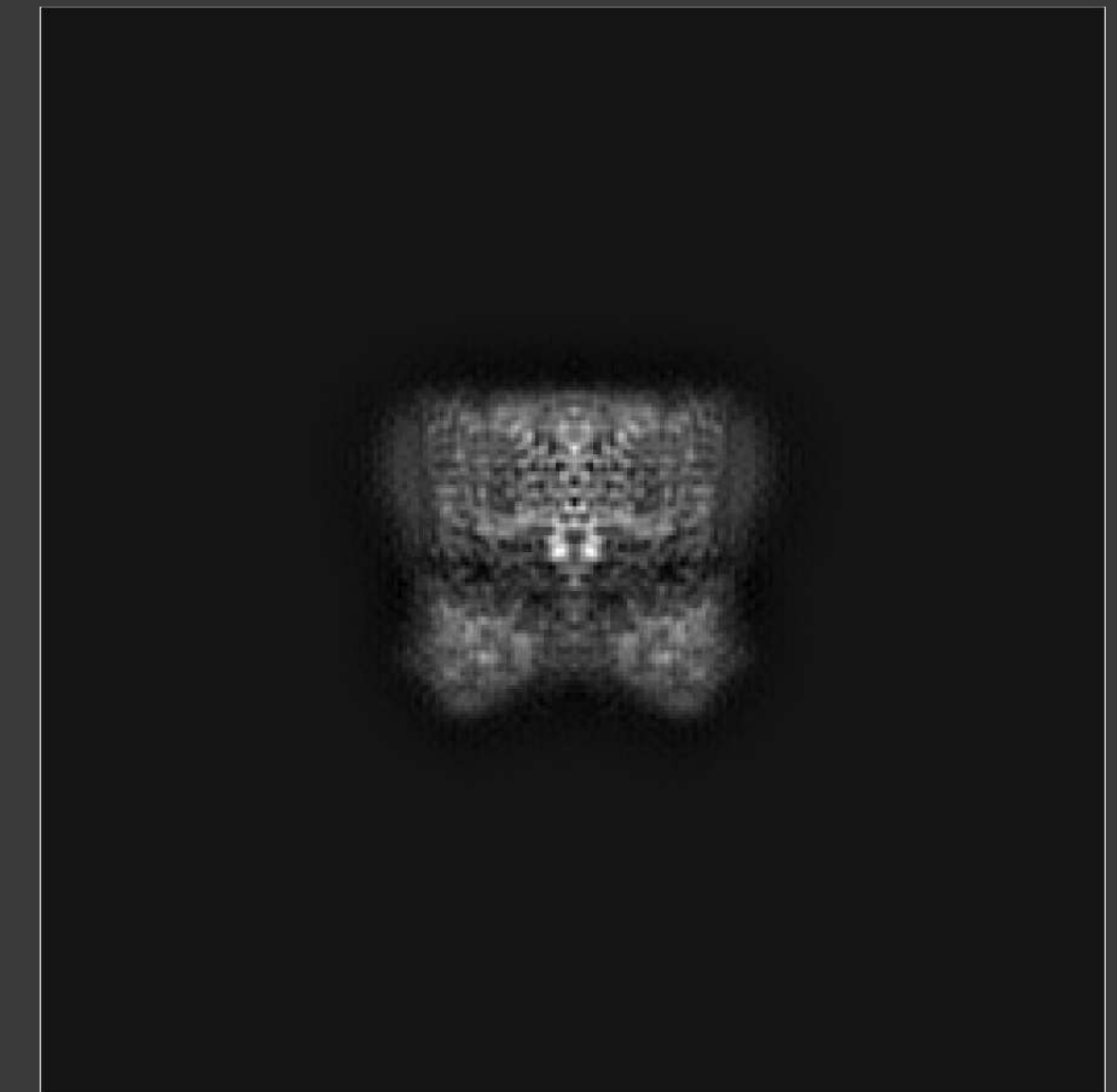


The dispersion radius is given by

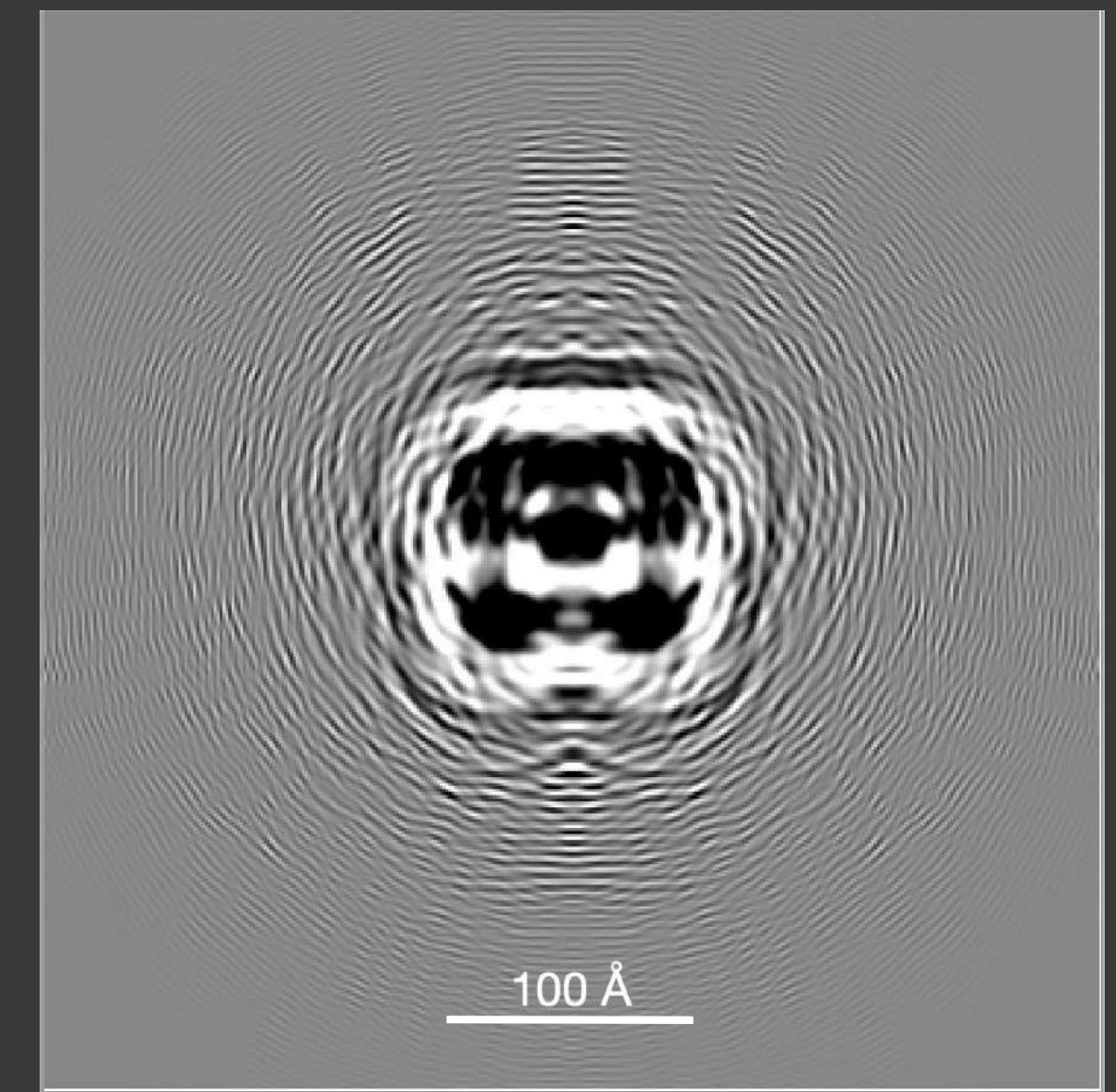
$$r = \delta \tan \theta$$
$$= \delta \lambda f \text{ (small angle approx.)}$$

Homework problem:

- How big a box do I need around my particle to include all the information up to $3\text{\AA}$, if I use $3\mu\text{m}$ of defocus?
- How big a box would I need for $1.5\mu\text{m}$ of defocus?

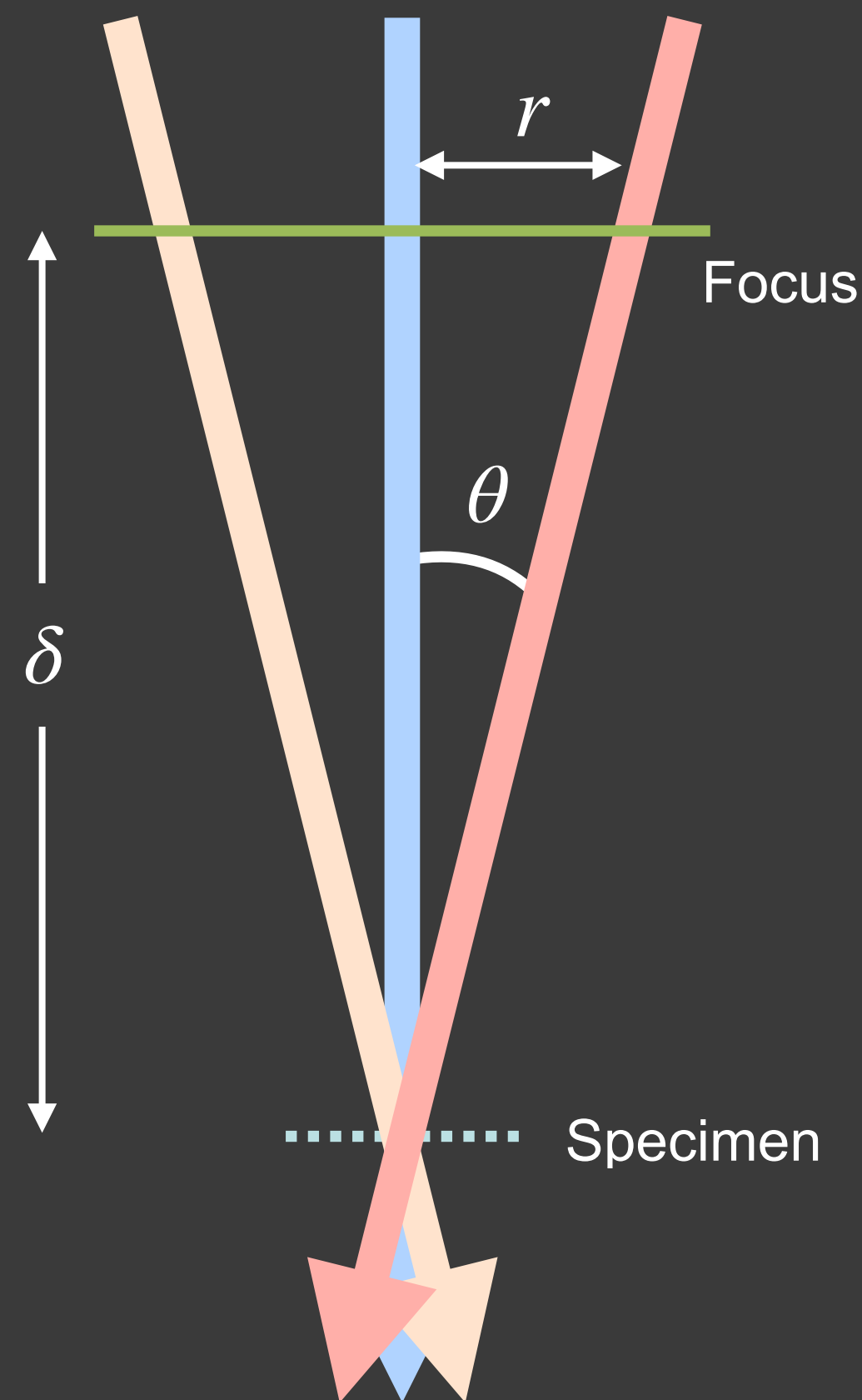


Object



3 μm
defocus

With large defocus, how bad is the image delocalization?



The dispersion radius is given by

$$r = \delta \tan \theta$$
$$= \delta \lambda f \text{ (small angle approx*)}$$

For example at 3 μm defocus and 3 $\text{\AA}$ resolution

$$\delta = 3 \times 10^4 \text{\AA}$$

$$\lambda = .02 \text{\AA}$$

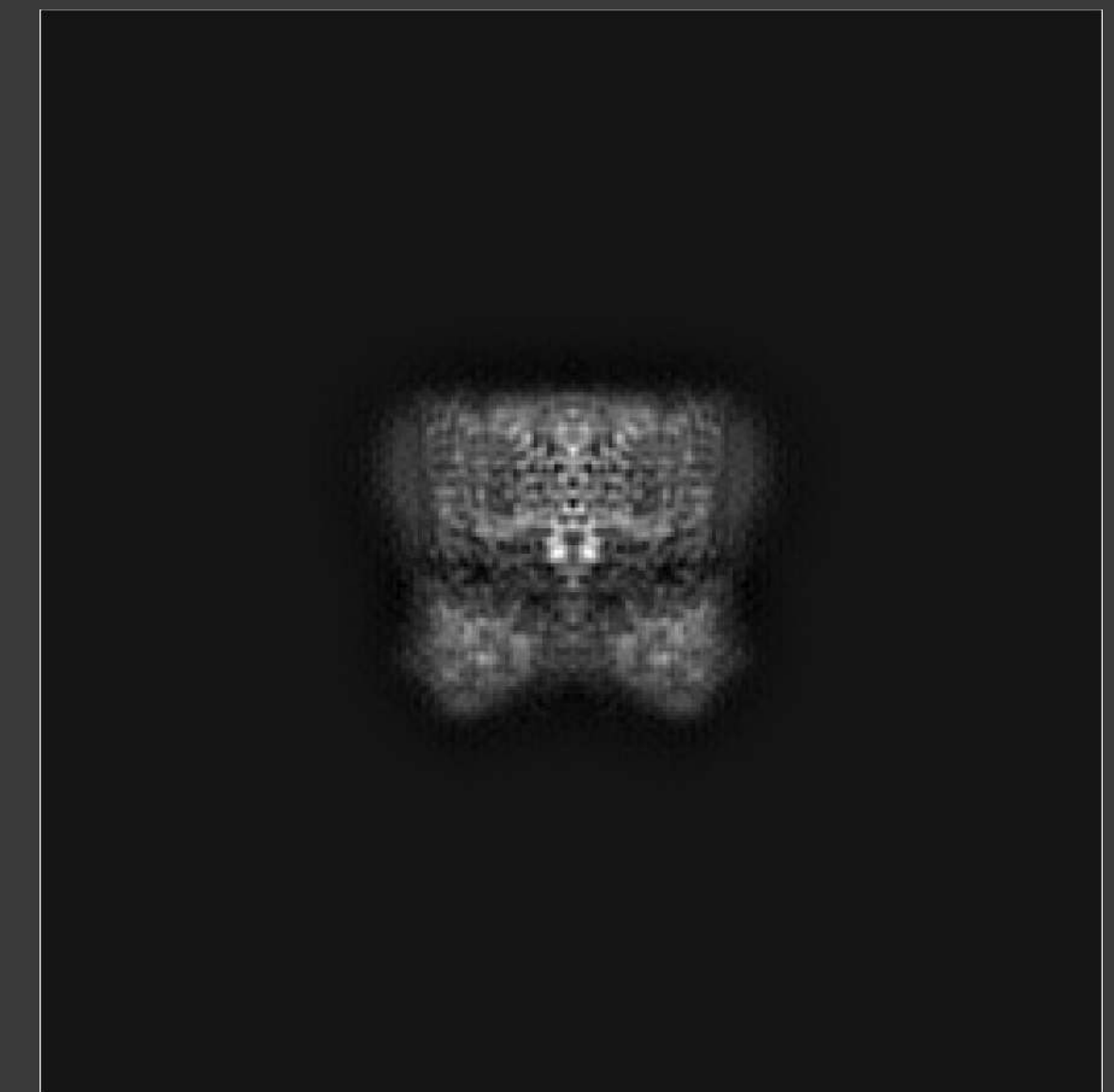
$$f = 0.33 \text{\AA}^{-1}$$

then

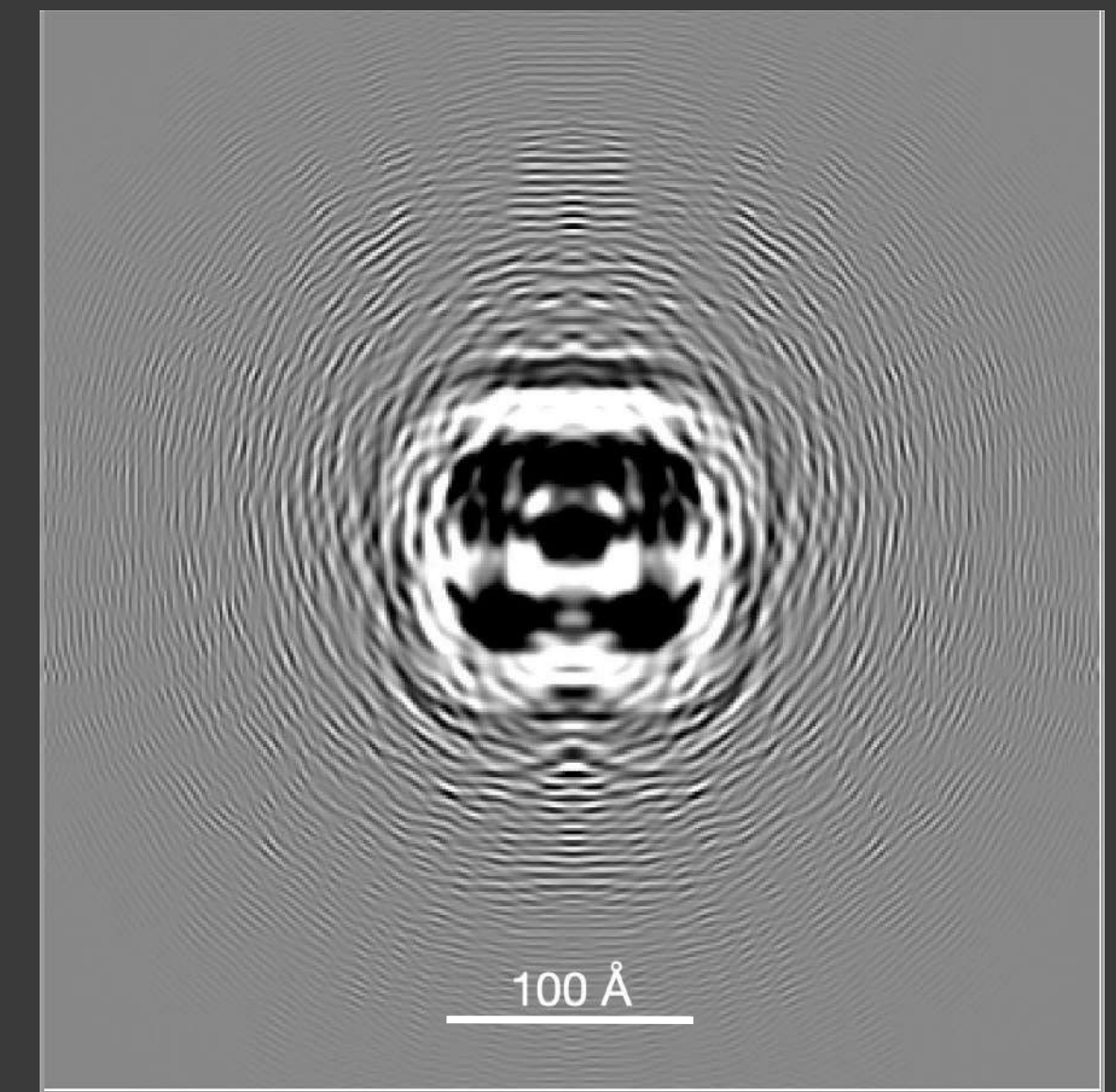
$$r = 200 \text{\AA}$$

In this case one would want 200 $\text{\AA}$ of space in the box around each particle image.

*Note: beyond about 3 $\text{\AA}$, spherical aberration needs to be taken into account too.



Object



3 μm
defocus

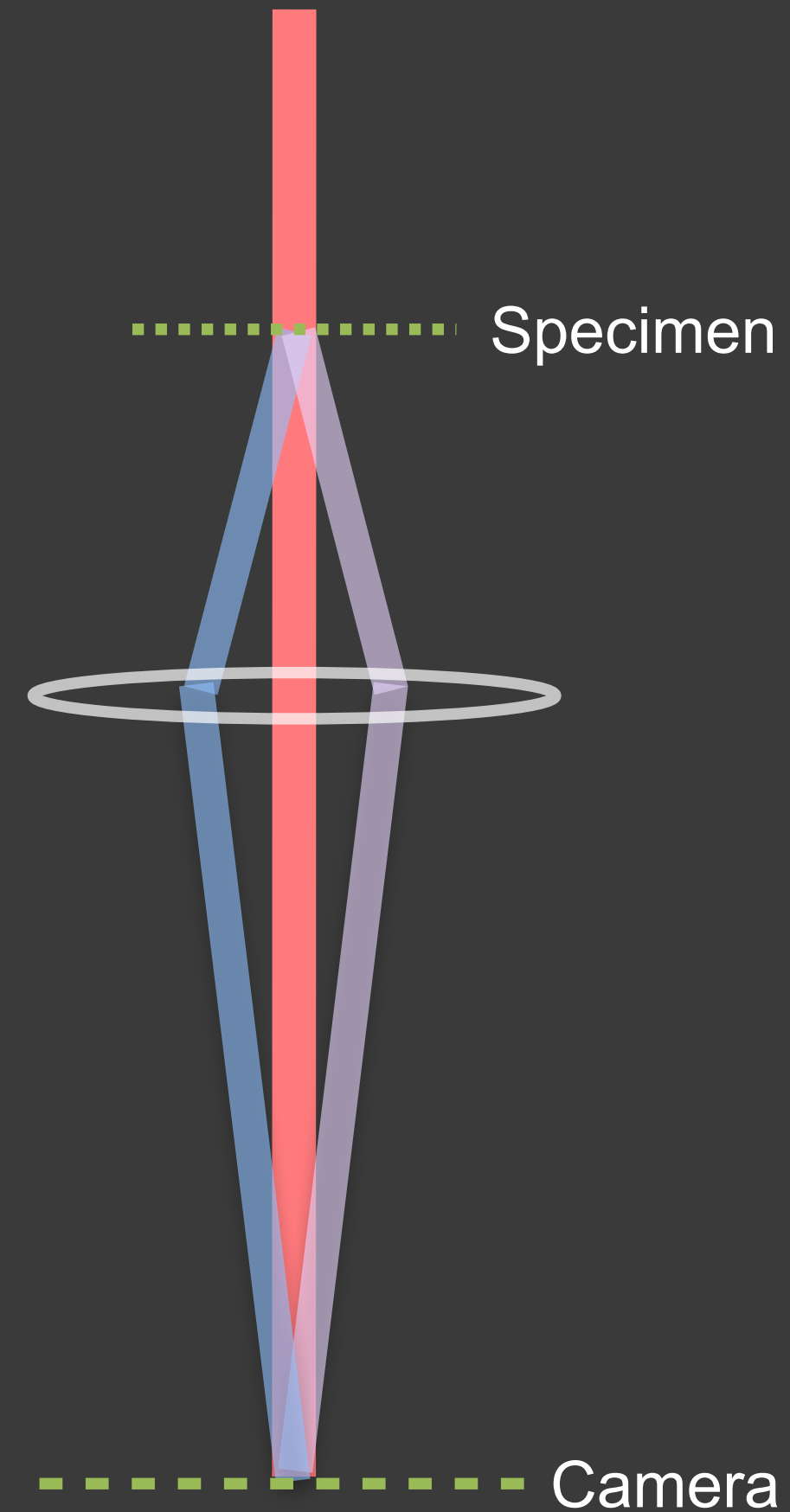
More details about the CTF

1. Electrons have really short wavelengths, and they travel through the column one by one.
2. The contrast in the image of a grating object varies with the amount of defocus
3. The grating object produces diffracted waves with shifting phase
4. When the phase of the diffracted waves is right, we have contrast.
5. A lens reproduces the wavefronts at the image plane.
6. Spherical aberration and amplitude contrast introduce new terms in the CTF.
7. A phase plate alters the wavefronts after they've passed through the lens.

Underfocus means weakening the field in the objective lens

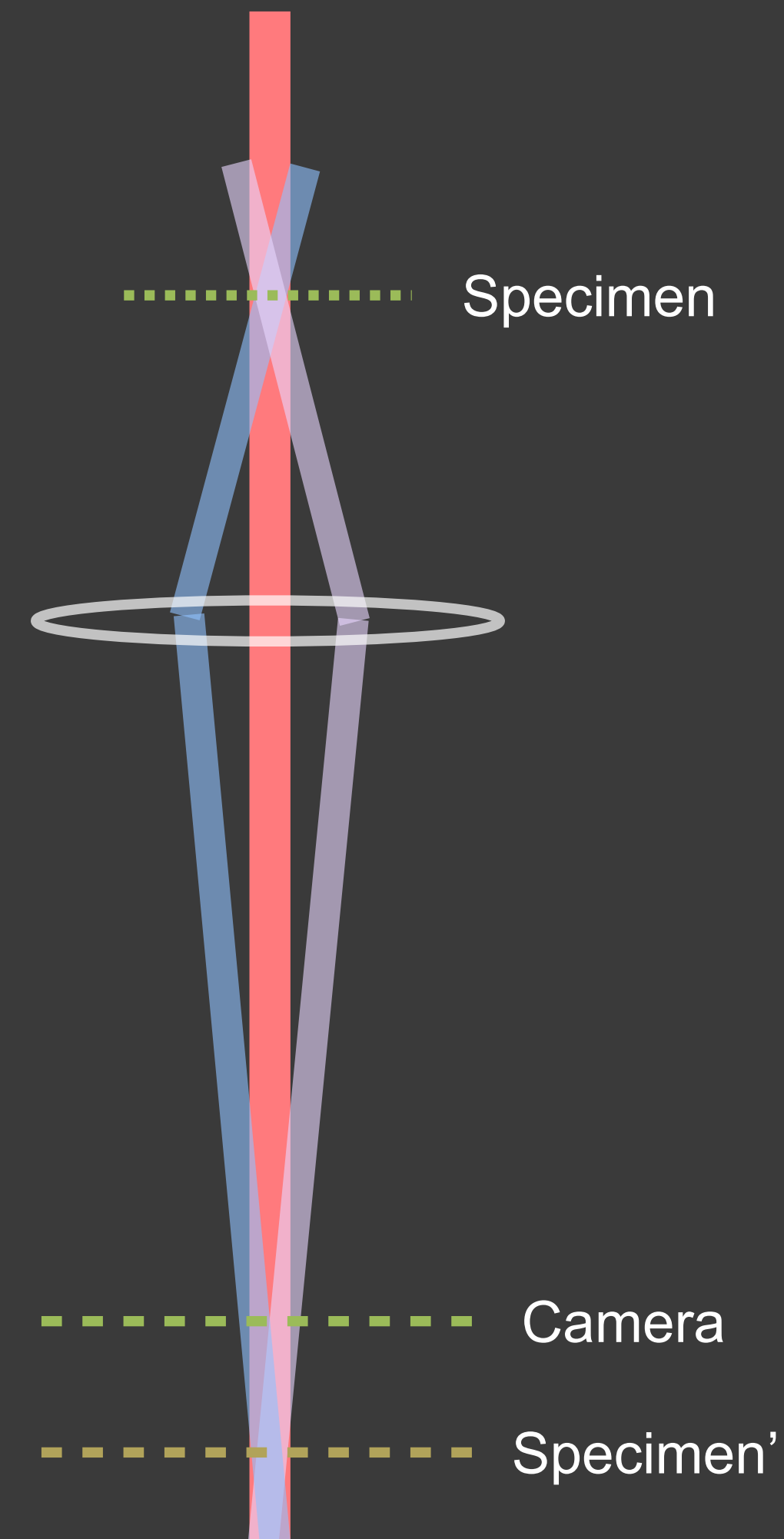
In focus

Specimen is imaged
onto the camera



Underfocus

The specimen image
is below the camera



With spherical aberration a lens bends high-angle rays more strongly

Spherical aberration changes the defocus by

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

The contrast transfer function has a new term,

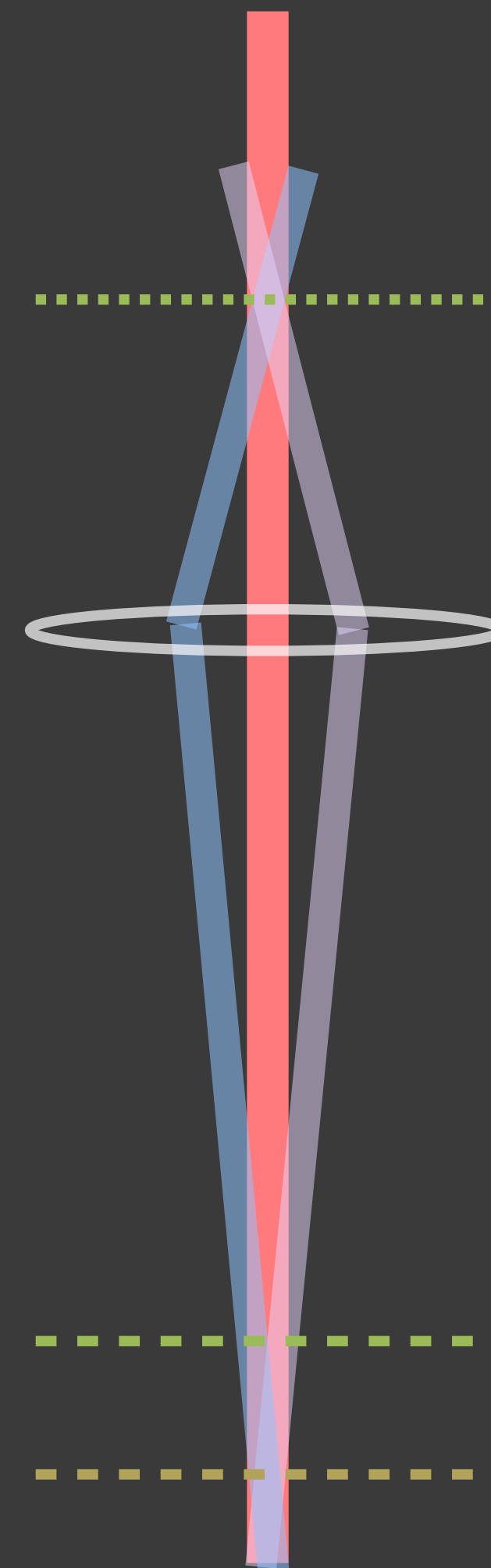
$$\text{CTF} = \sin(-\pi\lambda\delta f^2 - \pi\lambda\delta' f^2)$$

or, expanded,

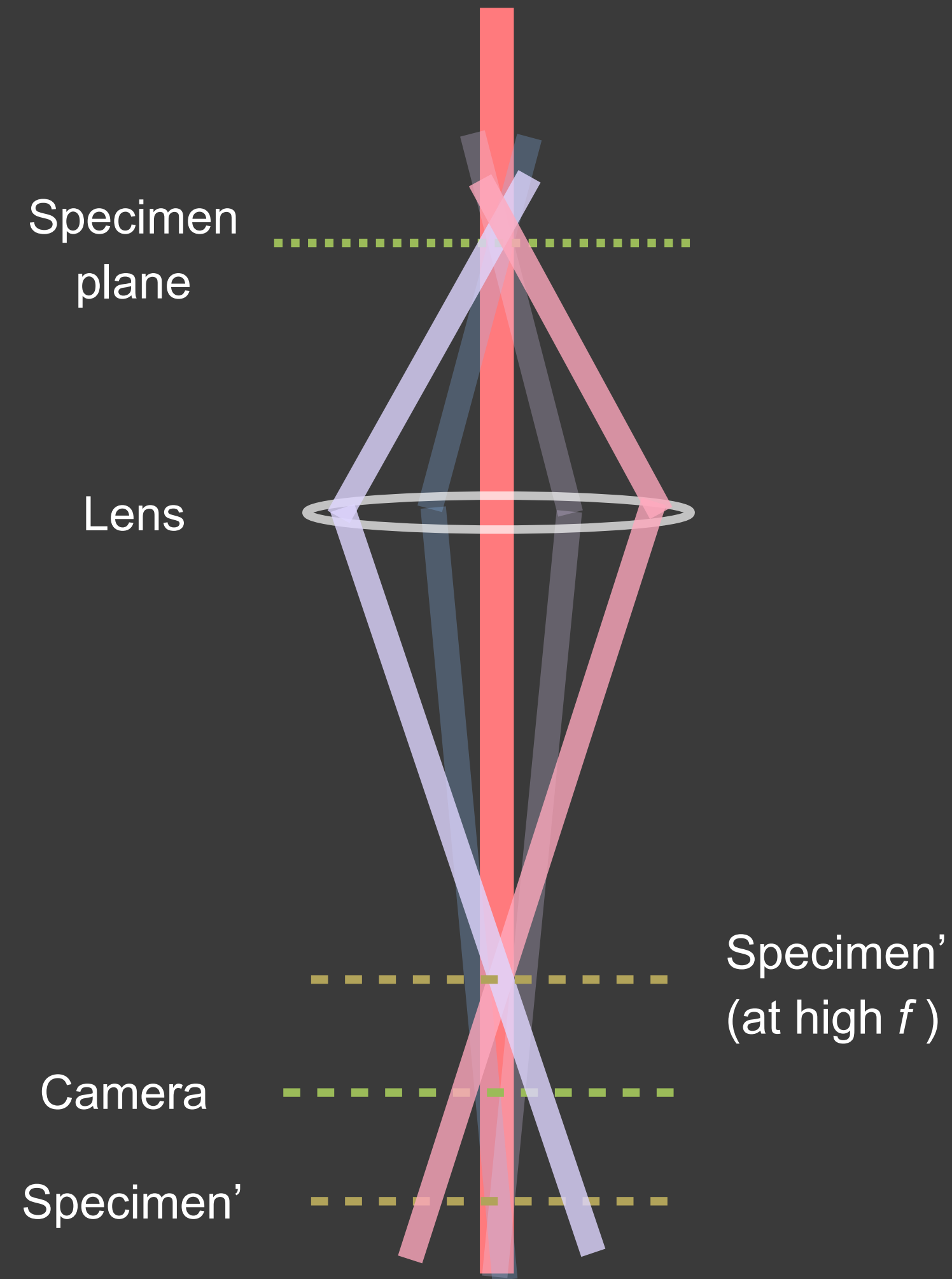
$$\text{CTF} = \sin\left(-\pi\lambda\delta s^2 + \frac{\pi}{2}C_s\lambda^3 f^4\right)$$

The coefficient C_s is typically ~2mm. This makes spherical aberration important only for $f > 0.25\text{\AA}^{-1}$, or about 4 $\text{\AA}$ resolution.

Underfocus, low f



Underfocus, high f



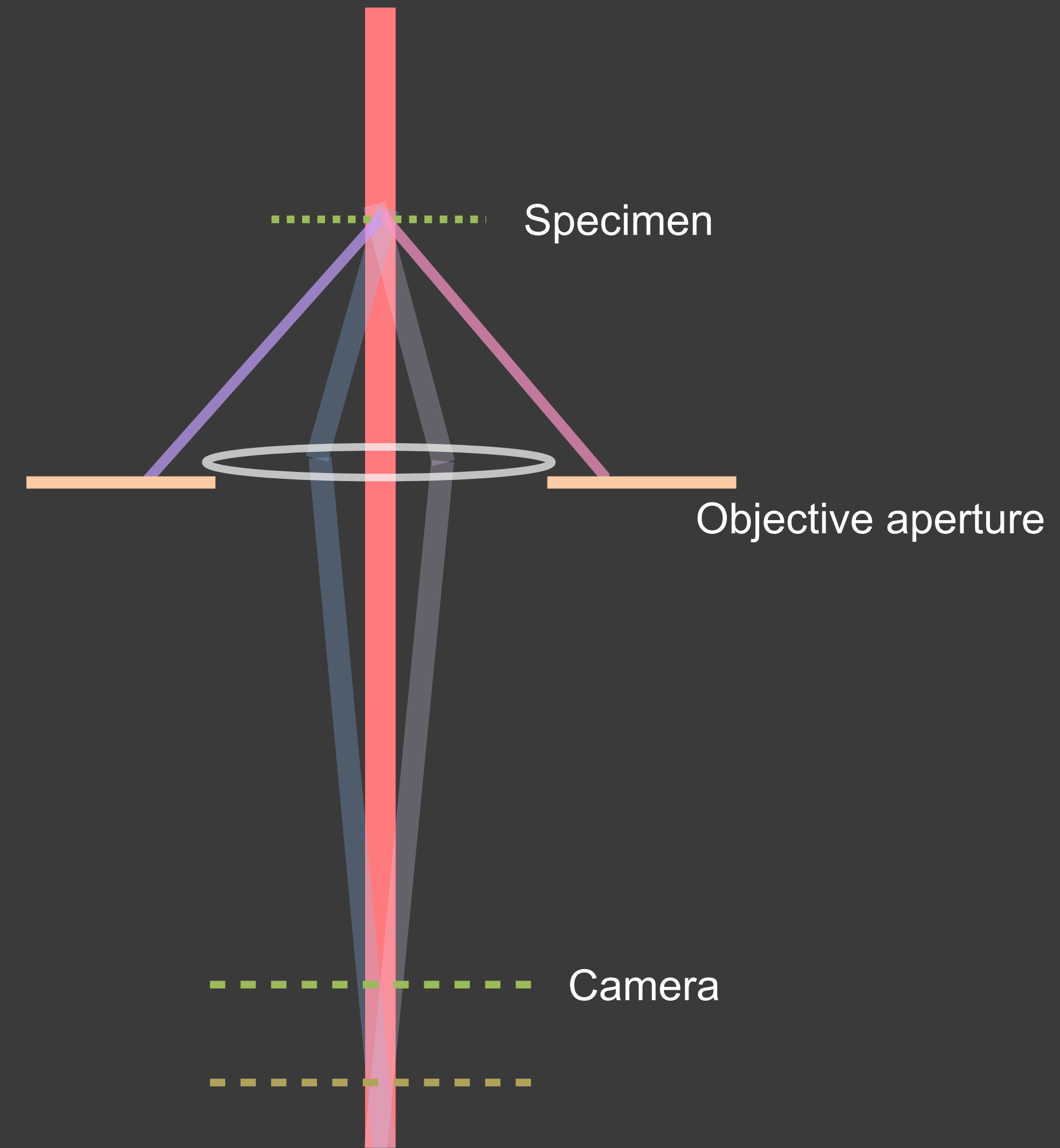
Also, high-angle scattering yields a small amplitude contrast

Electrons that pass very close to an atomic nucleus are scattered at very high angles, but are stopped by the objective aperture.

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

Its small magnitude, $\sin(-\alpha)$, is typically around -0.07.

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

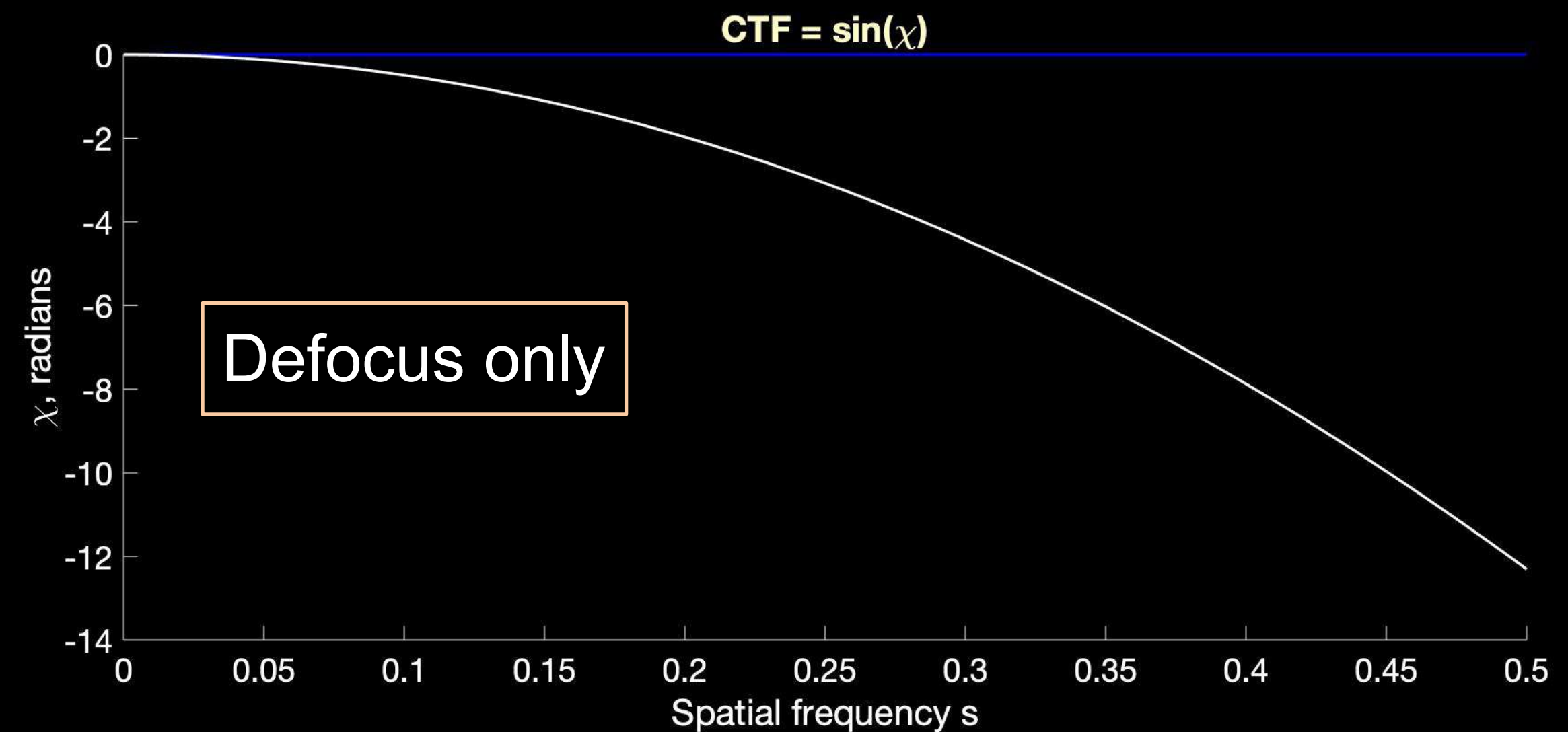
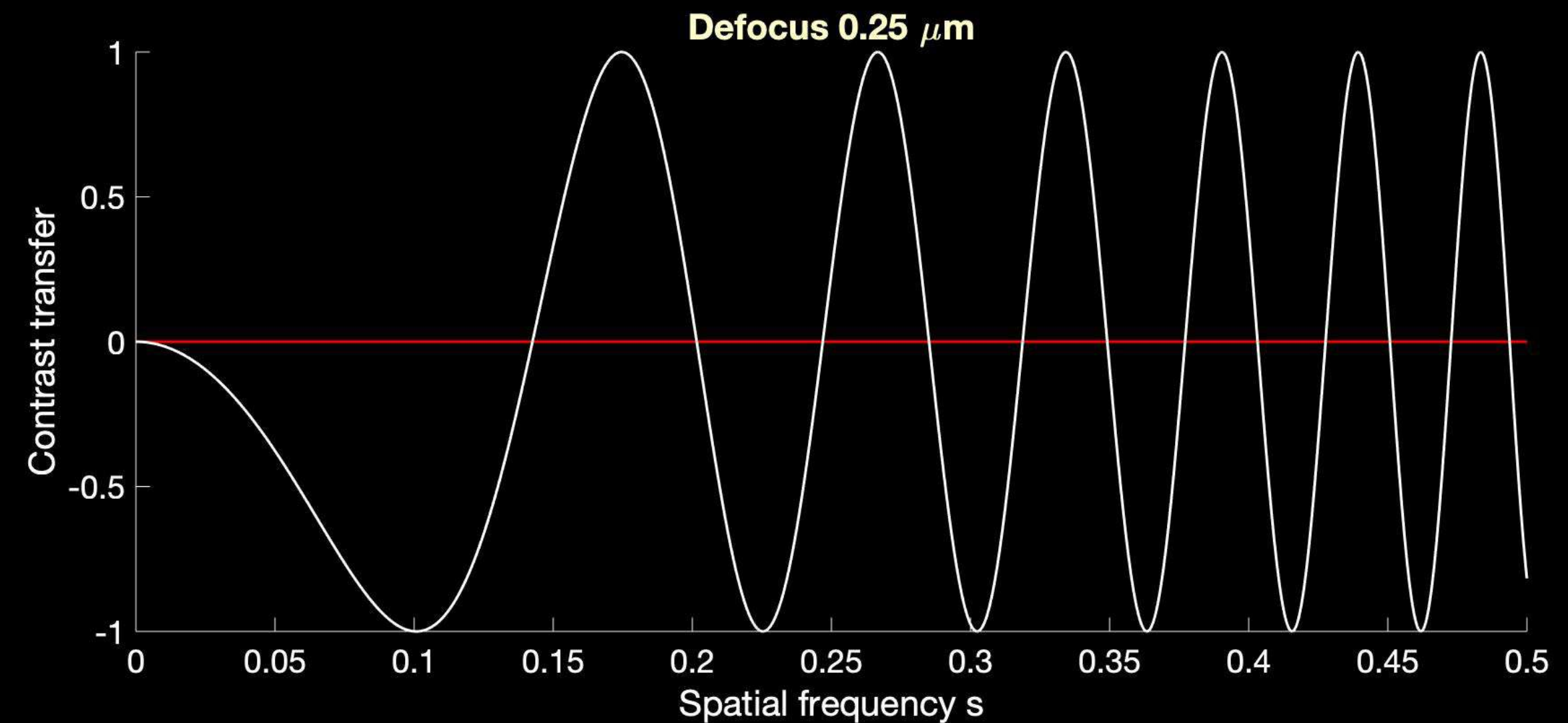


The simple defocus contrast is what we've seen before

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

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

defocus sphere abb. amplitude



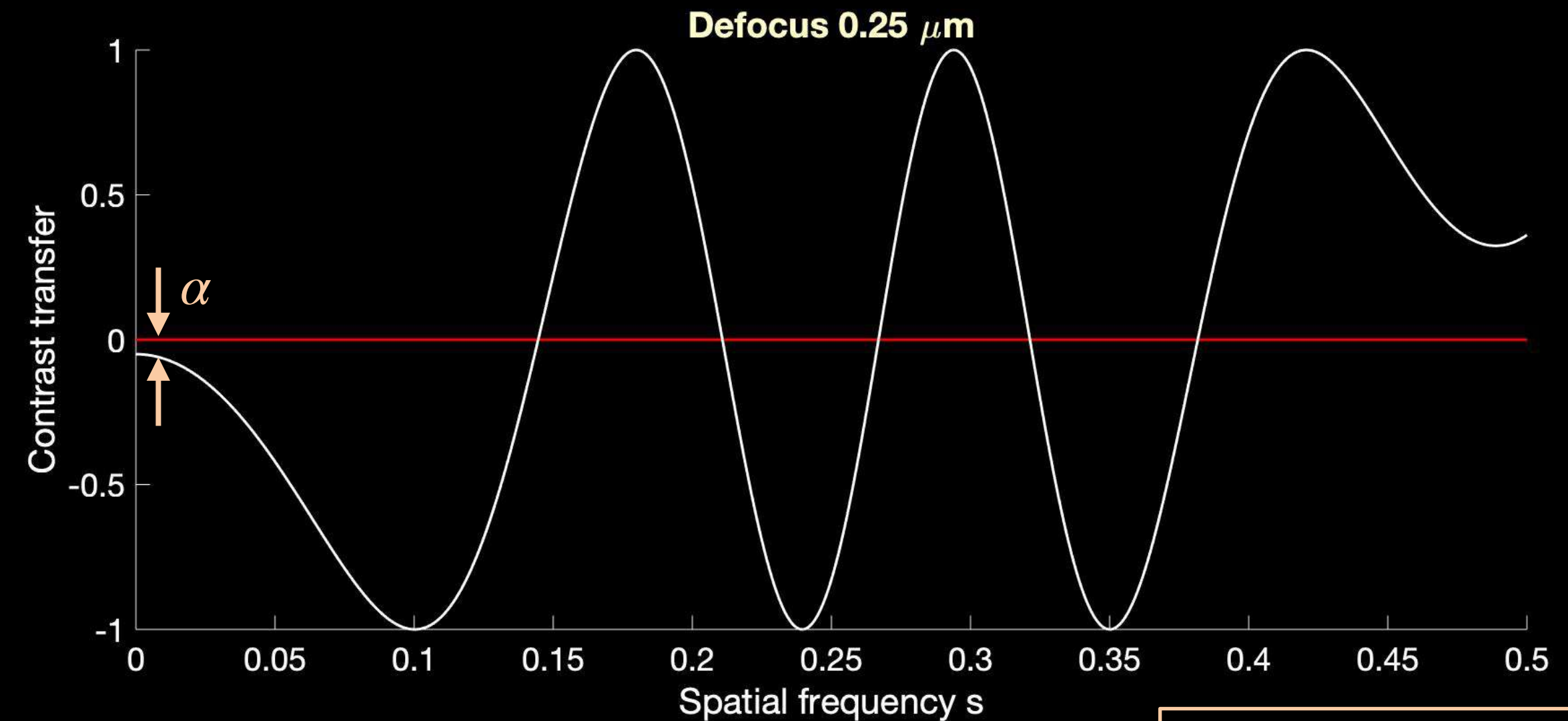
Now adding in spherical aberration and amplitude contrast

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

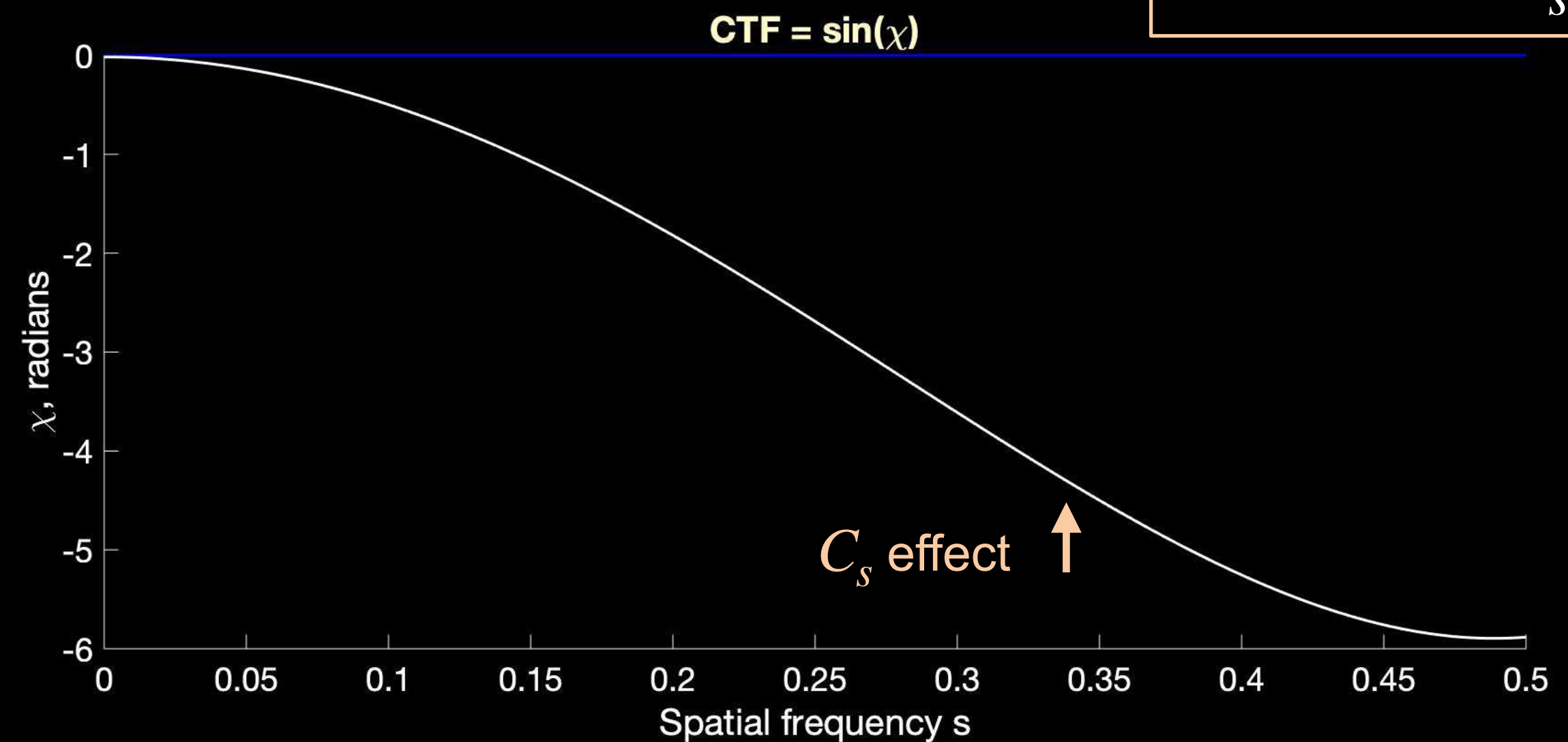
Also, C_s has the effect of reversing some of the oscillations in the CTF.

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

$$\text{CTF} = \sin(\underbrace{-\pi\lambda\delta f^2}_{\text{defocus}} + \underbrace{\frac{\pi}{2}C_s\lambda^3 f^4}_{\text{sphere abb.}} - \underbrace{\alpha}_{\text{amplitude}})$$



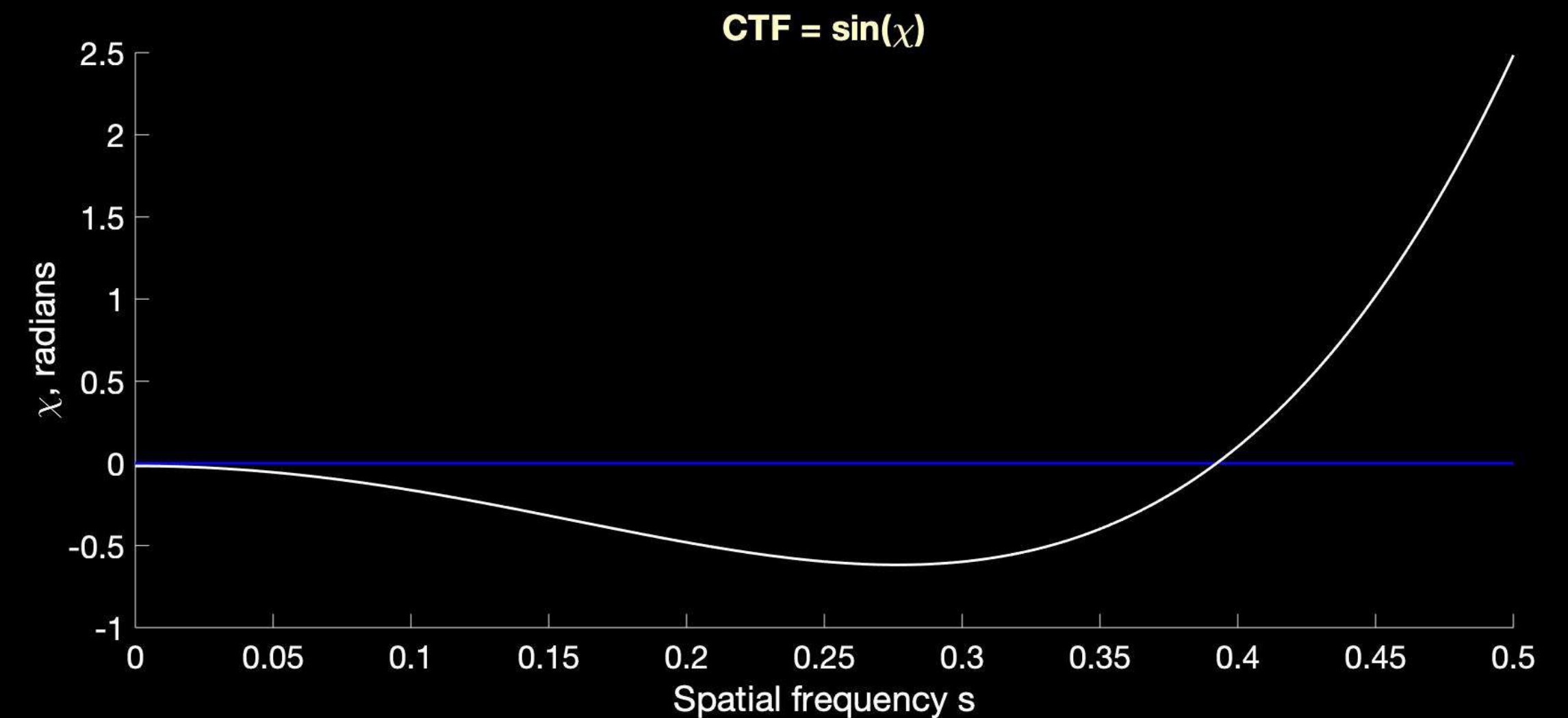
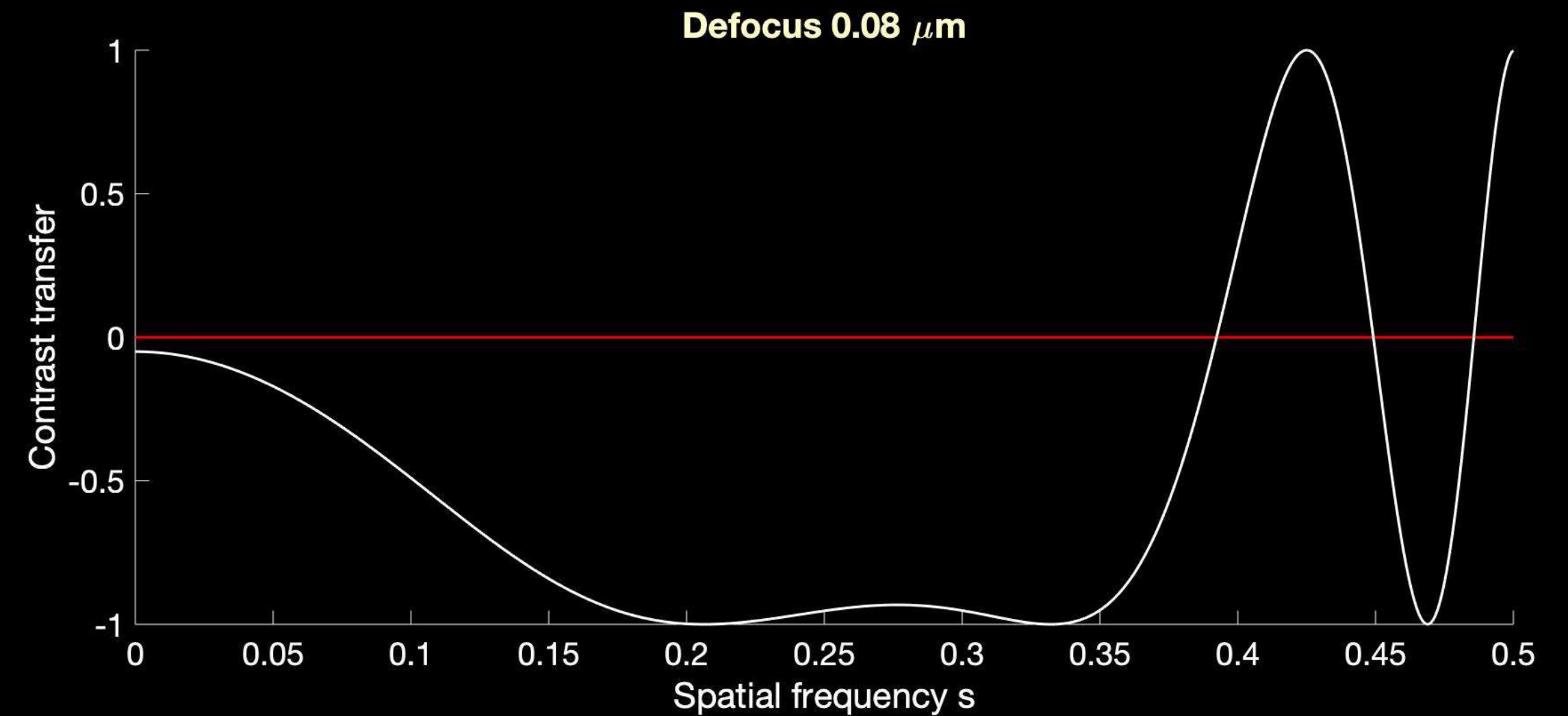
With α and C_s



Spherical aberration can be our friend

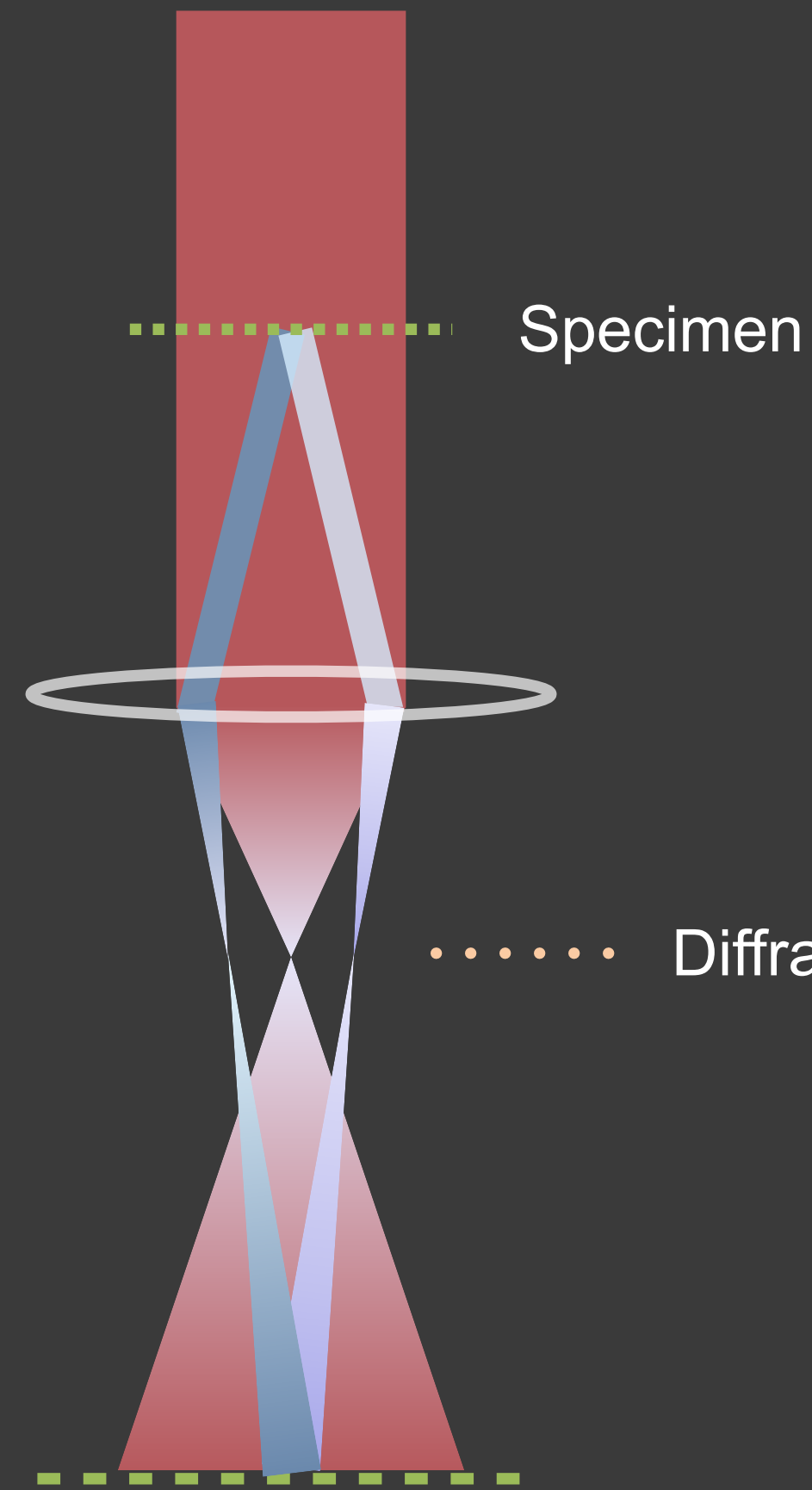
If we're not using image processing to remove CTF effects, Scherzer defocus is a good solution: just enough defocus to give signal over a broad range of spatial frequencies.

It's popular in materials science but not much for cryoEM: the signal transfer at low frequencies is poor.

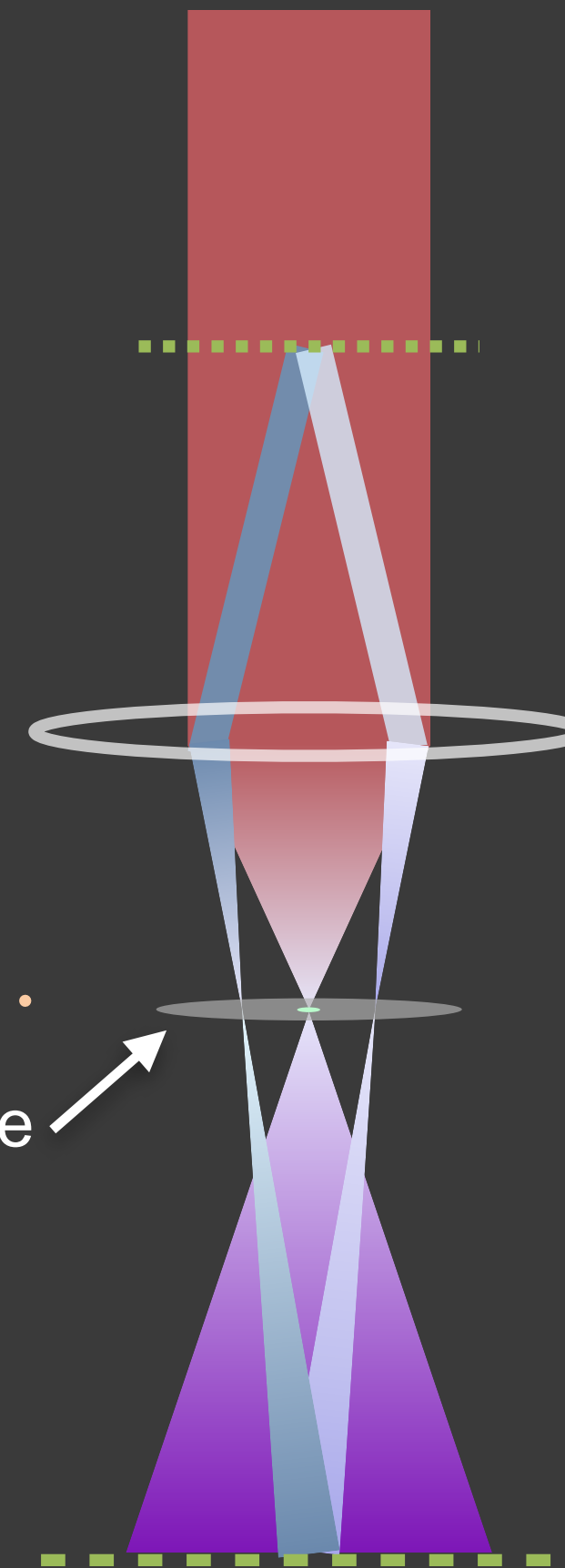


A phase plate modifies the interference of electron waves at the camera

In focus



Phase plate



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

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

Homework: See if you can derive this.

If $\phi = 90^\circ$ then the CTF at $f = 0$ becomes 1.

The contrast transfer comes from interference in the real part of Ψ

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

can be written as

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

The measured intensity is

$$\begin{aligned} |\Psi|^2 &= |\Psi'|^2 = (\text{real part})^2 + (\text{imag part})^2 \\ &= [1 + \sin(\chi) \epsilon \phi(x)]^2 + [\cos(\chi) \epsilon \phi(x)]^2 \\ &= [1 + 2 \sin(\chi) \epsilon \phi(x) + \mathcal{O}(\epsilon^2)] + [\mathcal{O}(\epsilon^2)]. \end{aligned}$$

So, ignoring the factor of 2, we say the transfer from phase shift to intensity change is

$$\begin{aligned} \text{CTF} &= \sin(\chi) \\ &= \sin(\pi \lambda z / d^2) \end{aligned}$$

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

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

$$c = \cos(\theta) \approx 1 - \lambda^2/d^2$$

$$\chi = k(1 - c)z = \pi \lambda z / d^2$$

The phase plate allows in-focus imaging, but precise focusing is necessary.

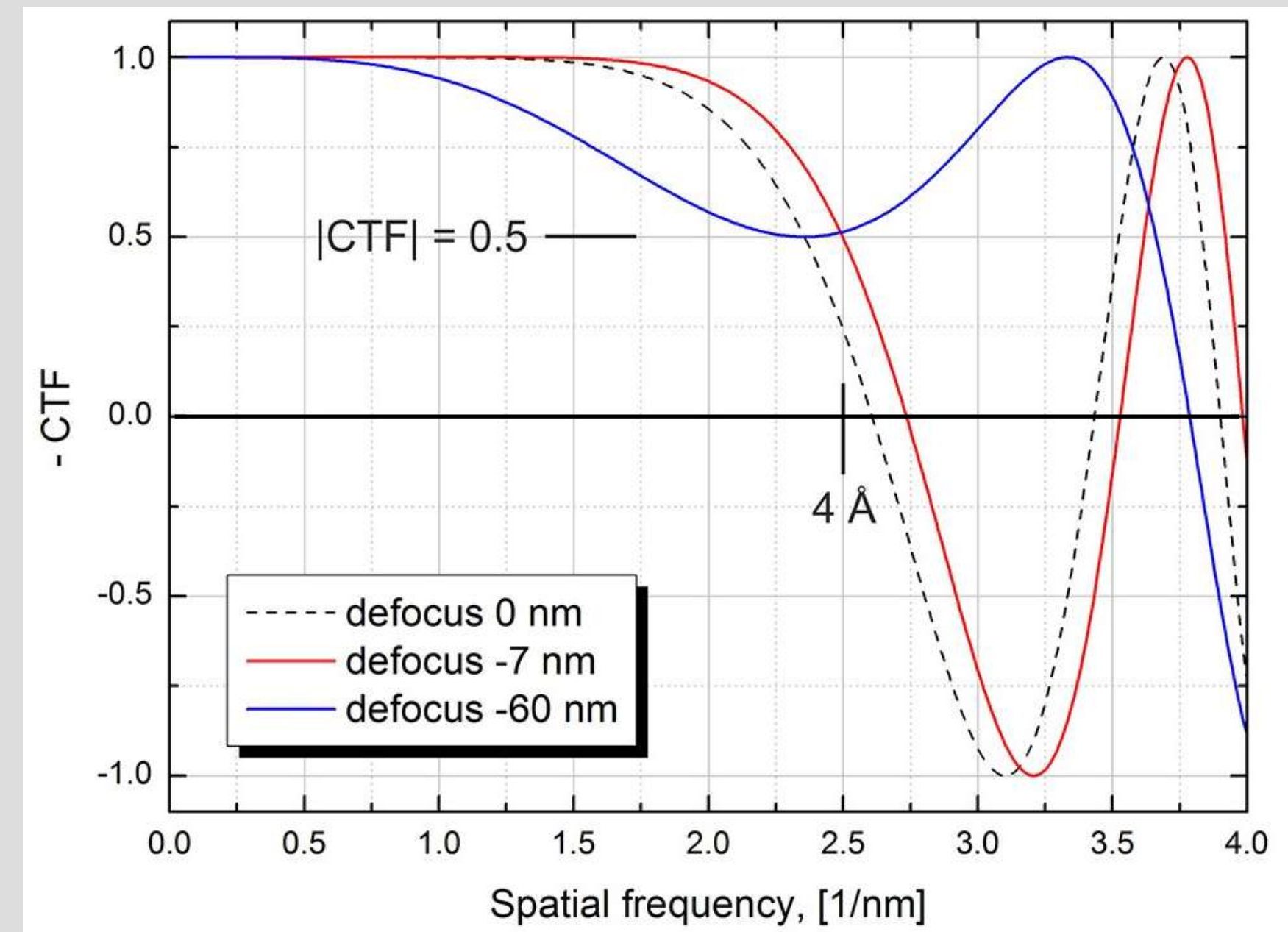
Cryo-EM single particle analysis with the Volta phase plate

Radostin Danev*, Wolfgang Baumeister

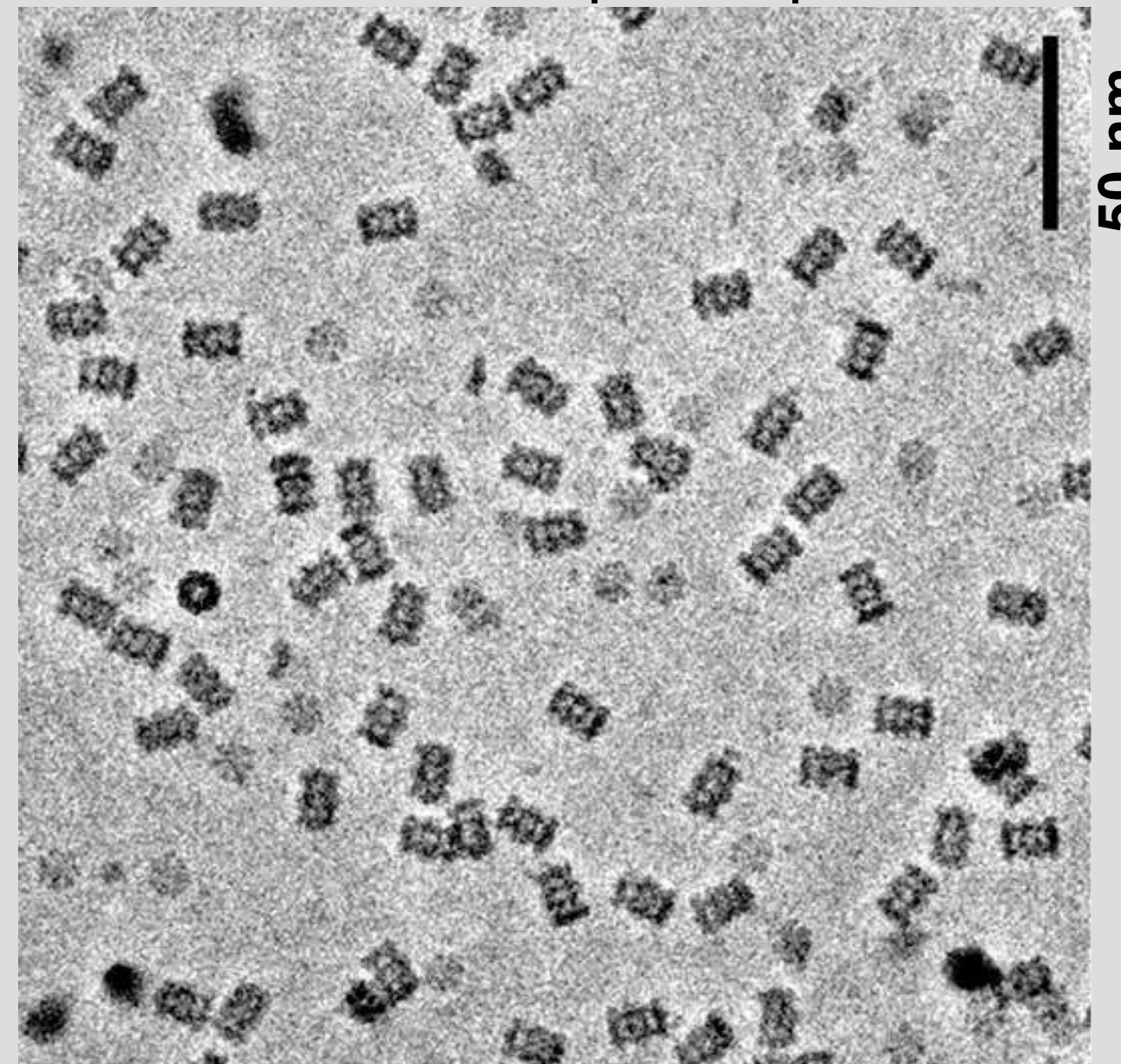
Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany

eLife 2016

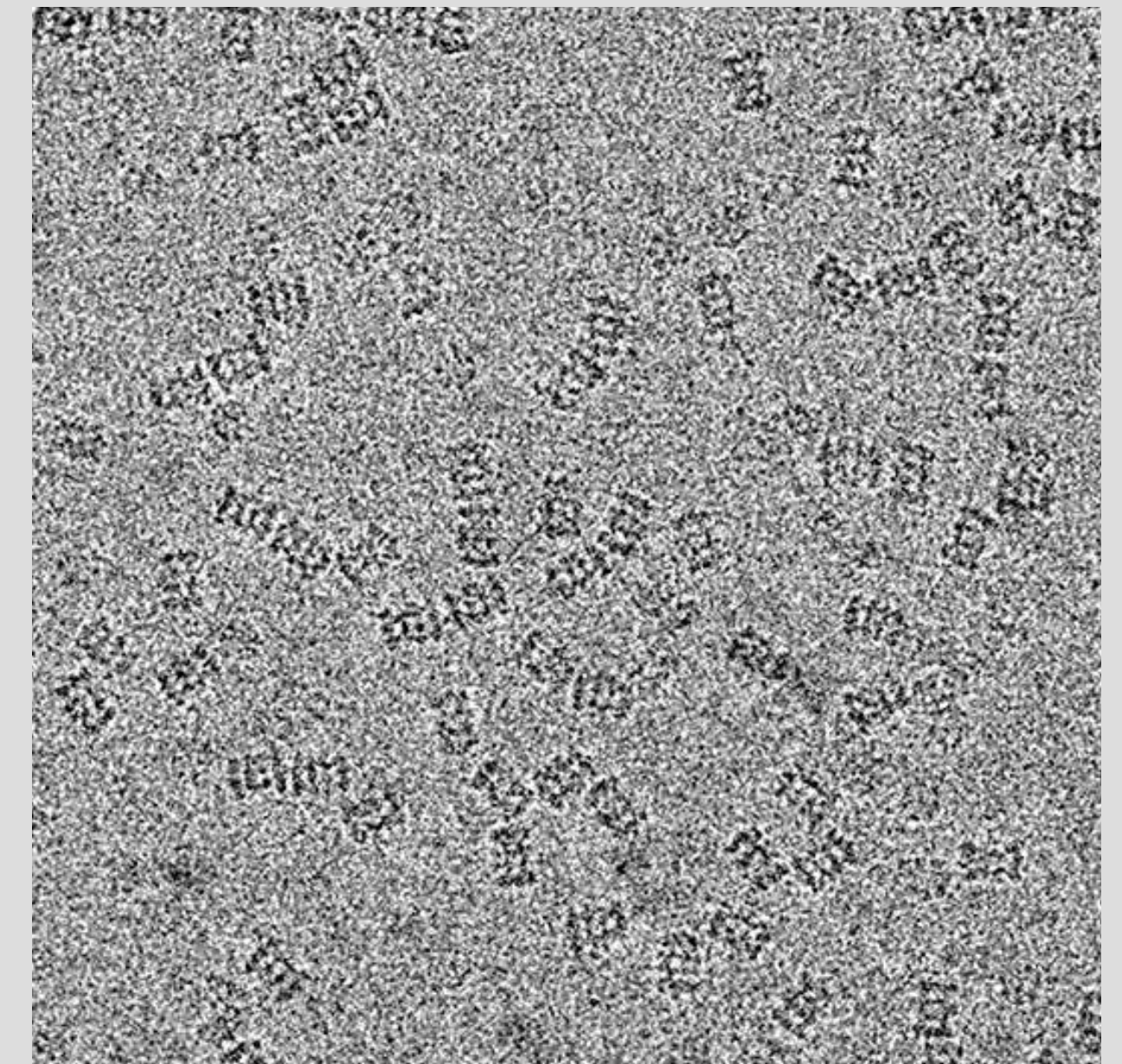
- The defocus value must be precise within 60 nm in order to get 4 Å resolution.
- The better low-frequency contrast makes particles much more visible.



In-focus phase plate

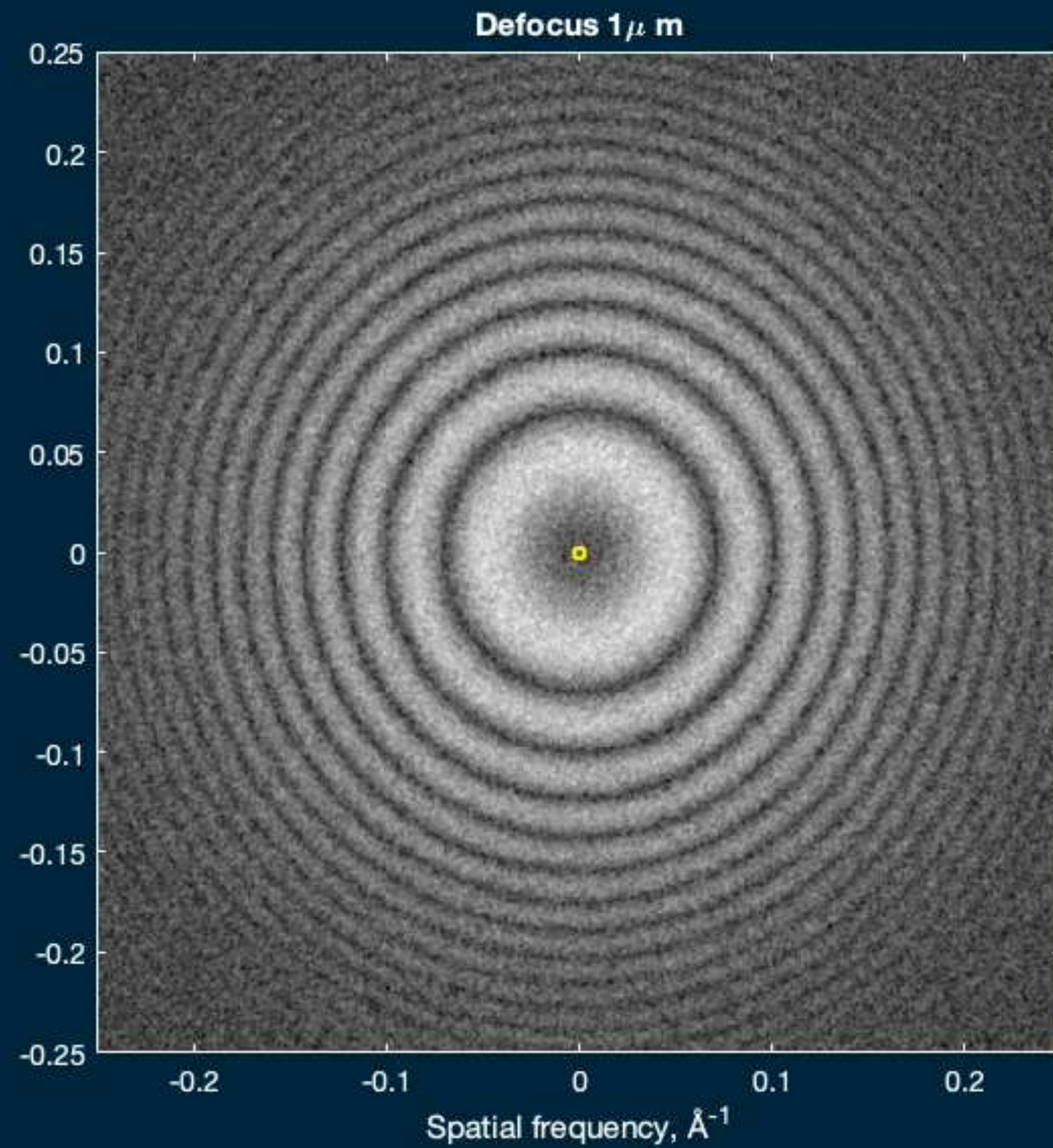


Defocus contrast



The power spectrum describes the magnitude of Fourier components

Power spectrum



Grating at the spatial frequency

