Defocus Contrast
and the Contrast Transfer Function

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Lecture 4a
SPA Short Course
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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

Real part

$$z = a + ib$$

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 $\mathrm{Re}(z) = a$

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

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

Conjugate $z^* = a - ib$

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

The exponential function e^x

$$e = 2.718...$$

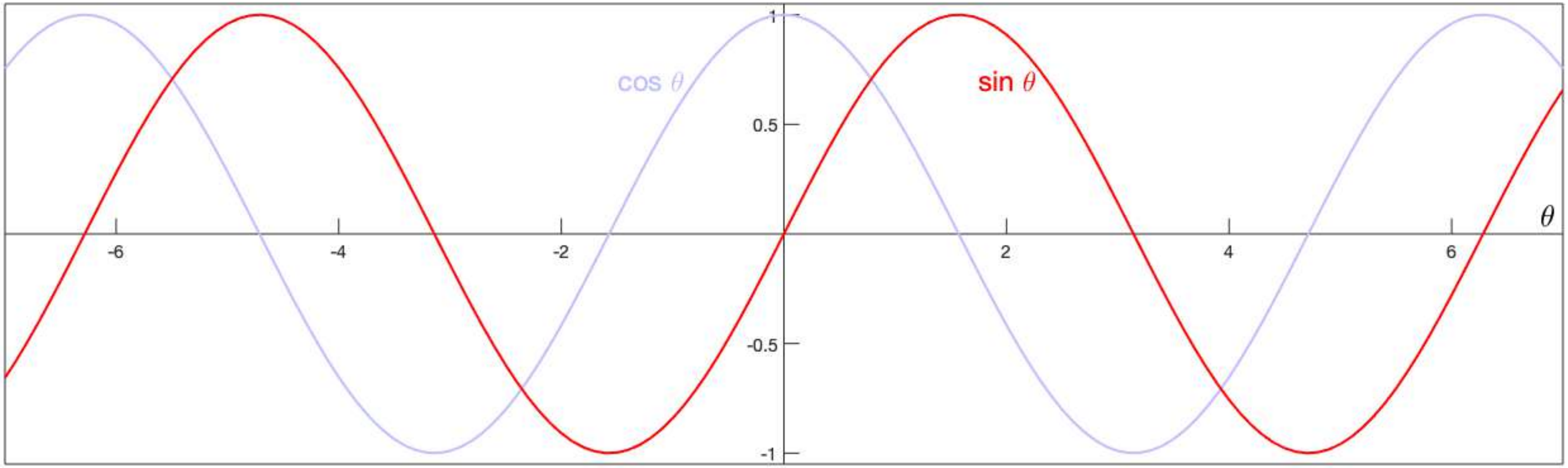
$$e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{2 \times 3} + \ldots$$

A very important approximation

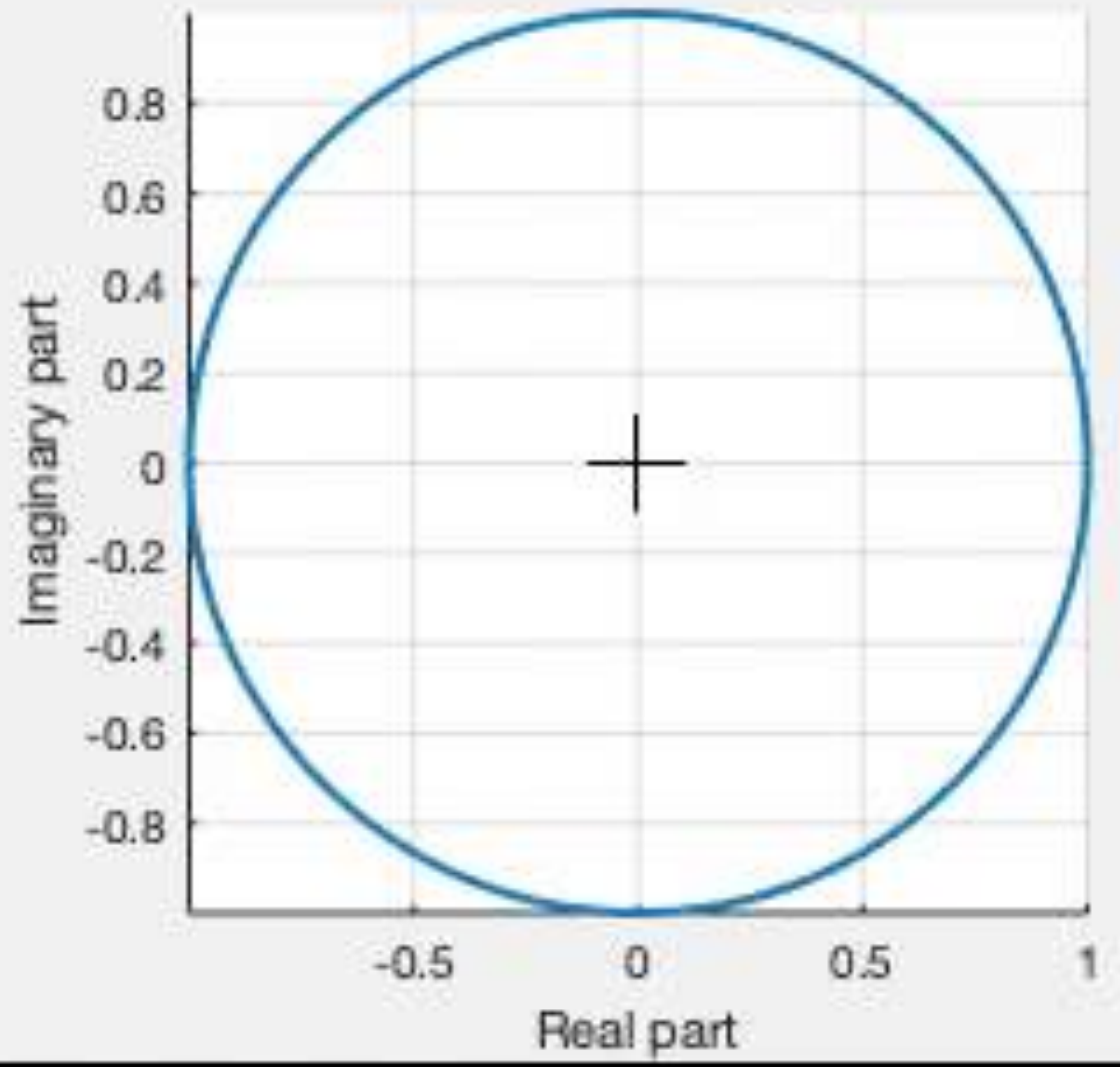
$$e^x \approx 1 + x, \quad x \ll 1$$

The complex exponential

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



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



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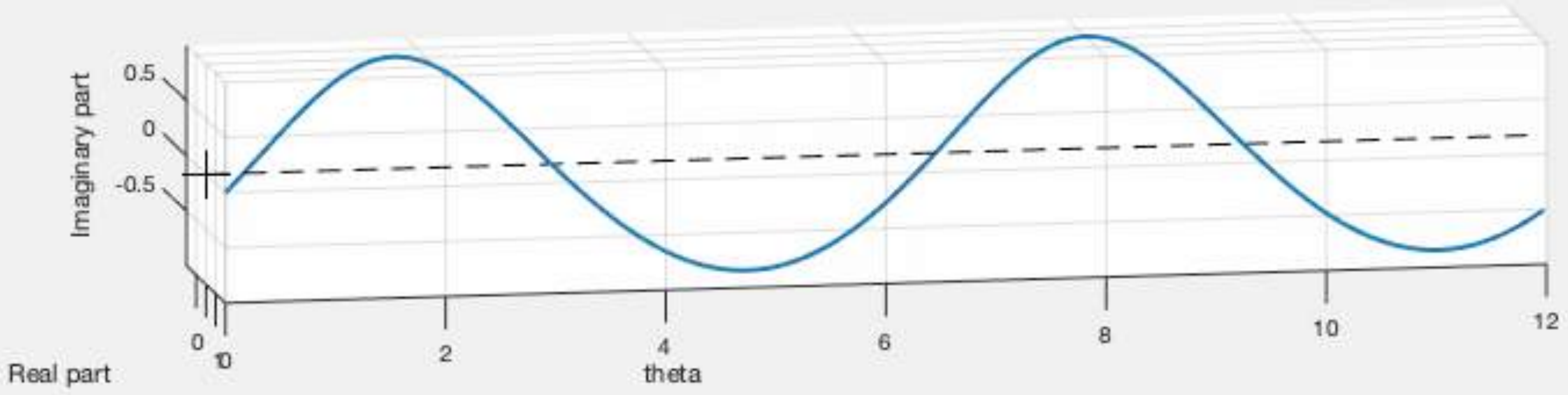


Any z can be represented as (a, b) or as (r, θ)

$z = a + ib$

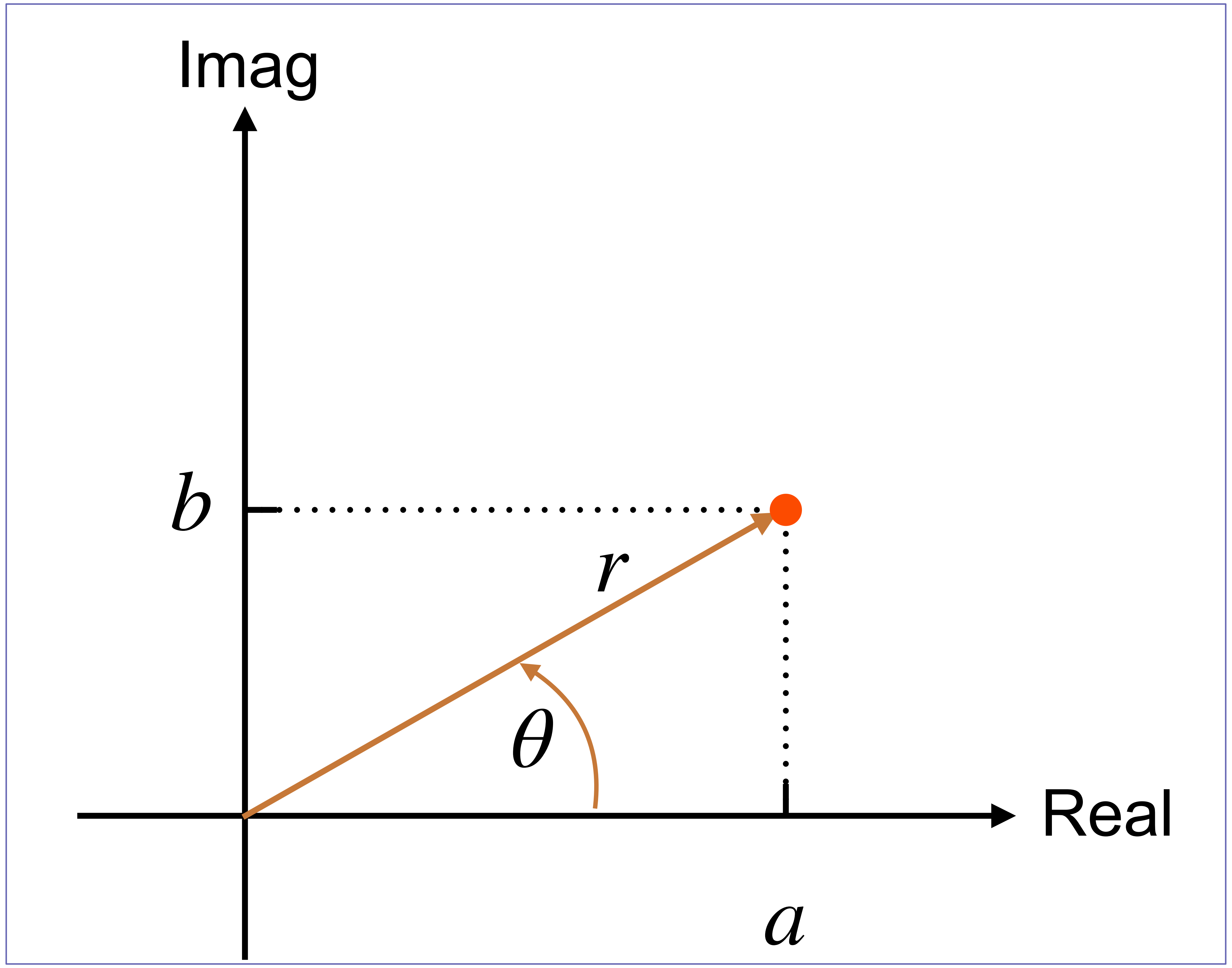
a is the real part

b is the imaginary part

$z = re^{i\theta}$

r is the magnitude

θ is the phase



Recall that

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

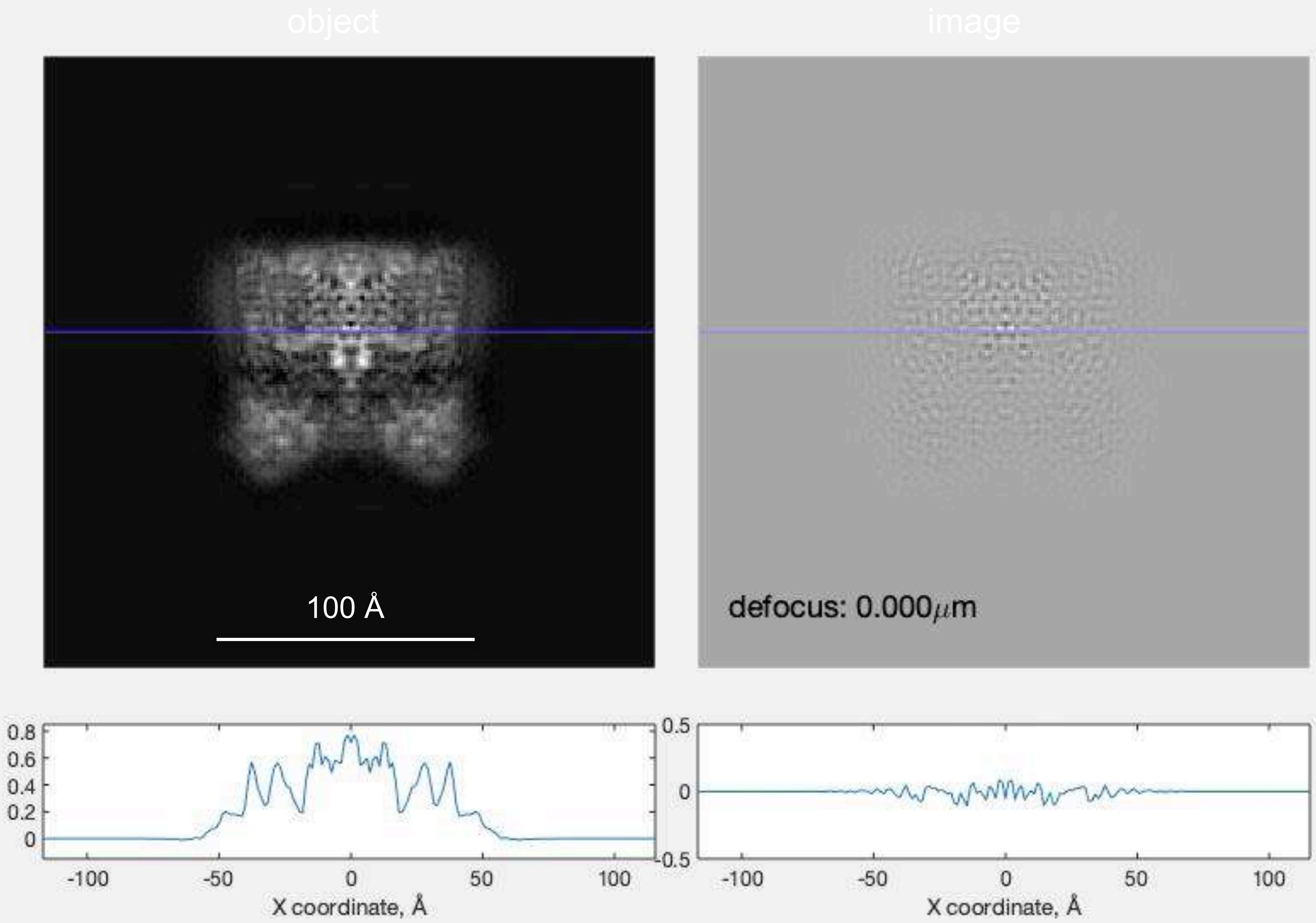
so, when you multiply two complex numbers, the phases add:

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

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



- 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

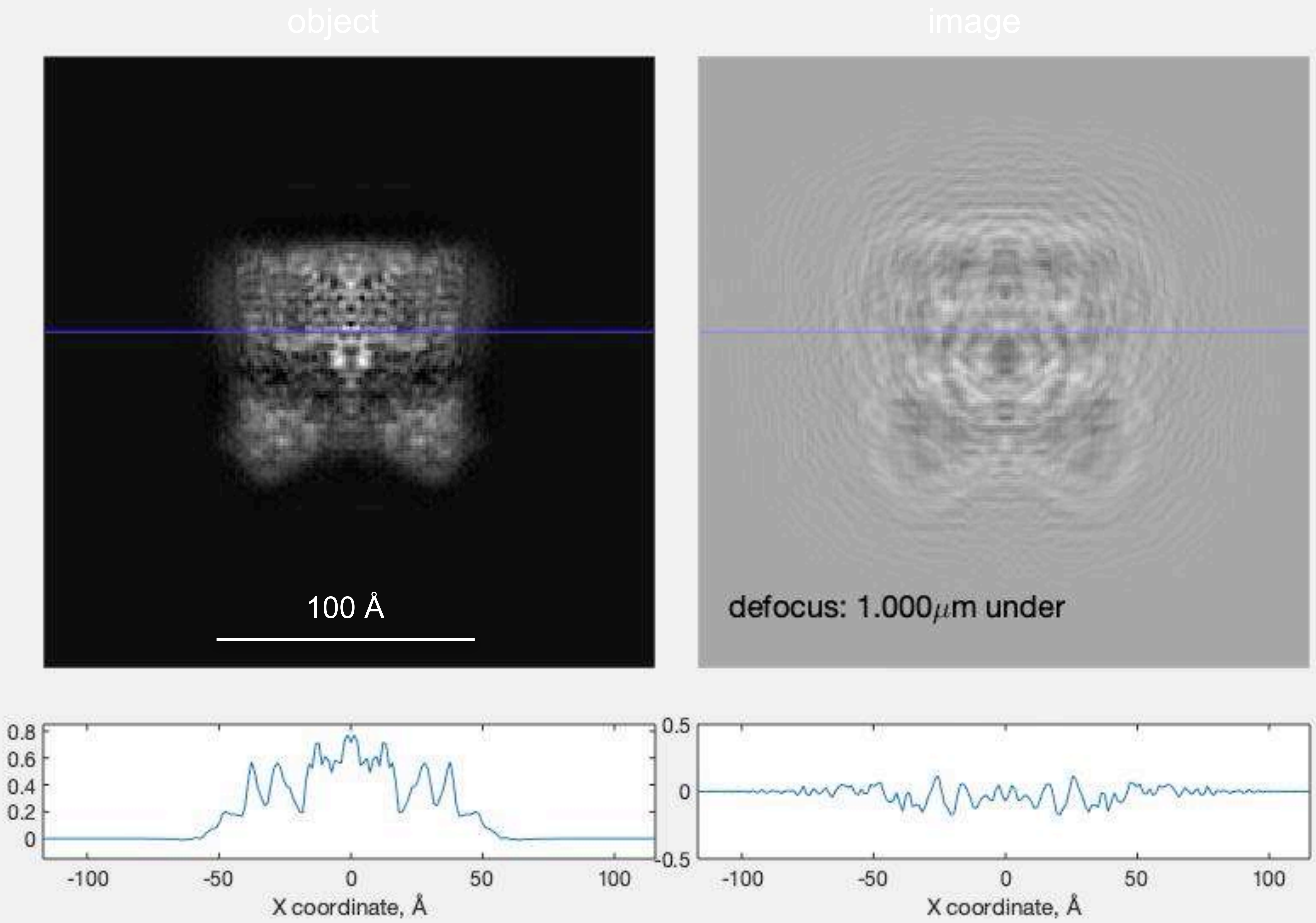


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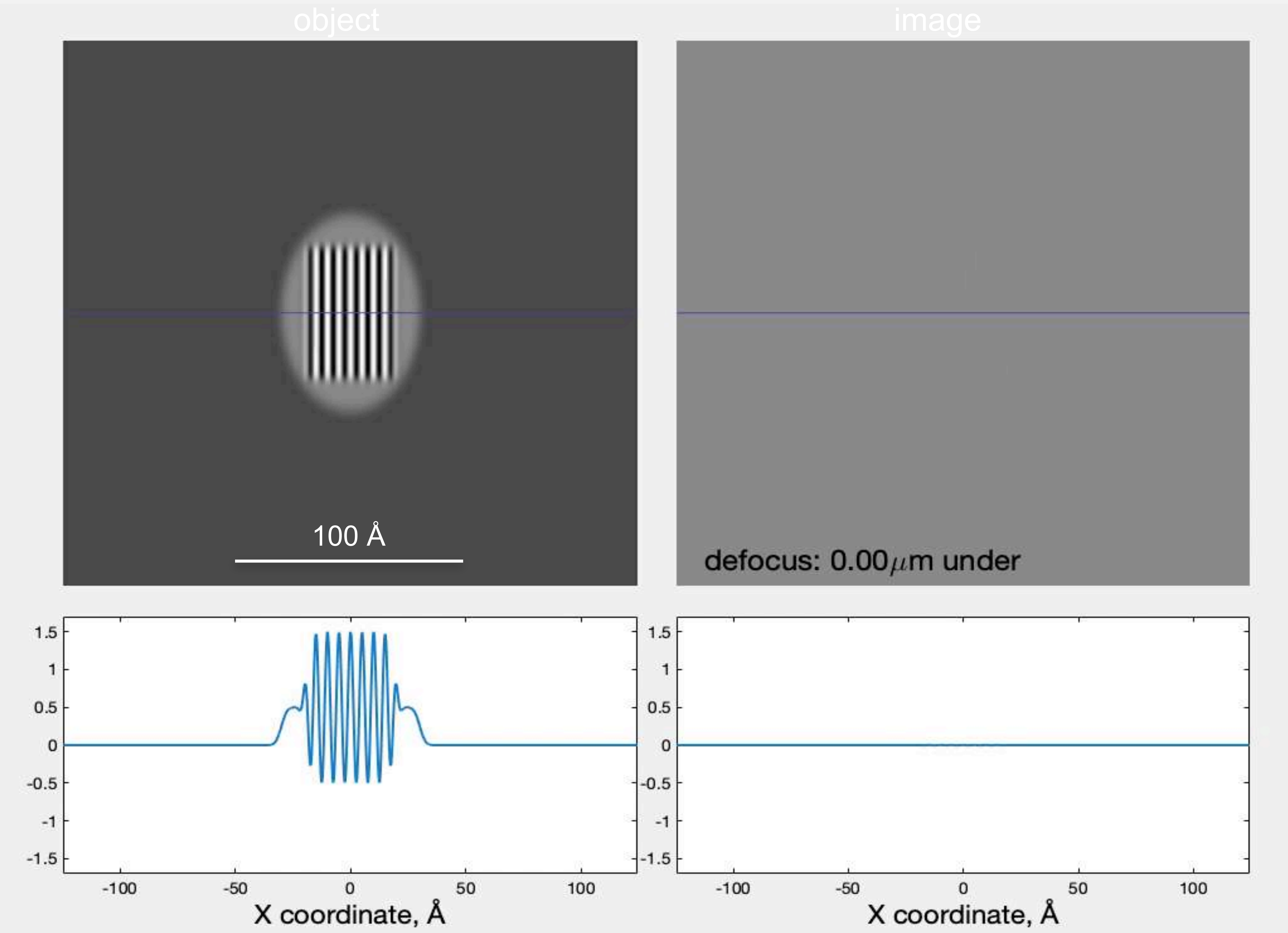
Image of an object with 5Å periodicity

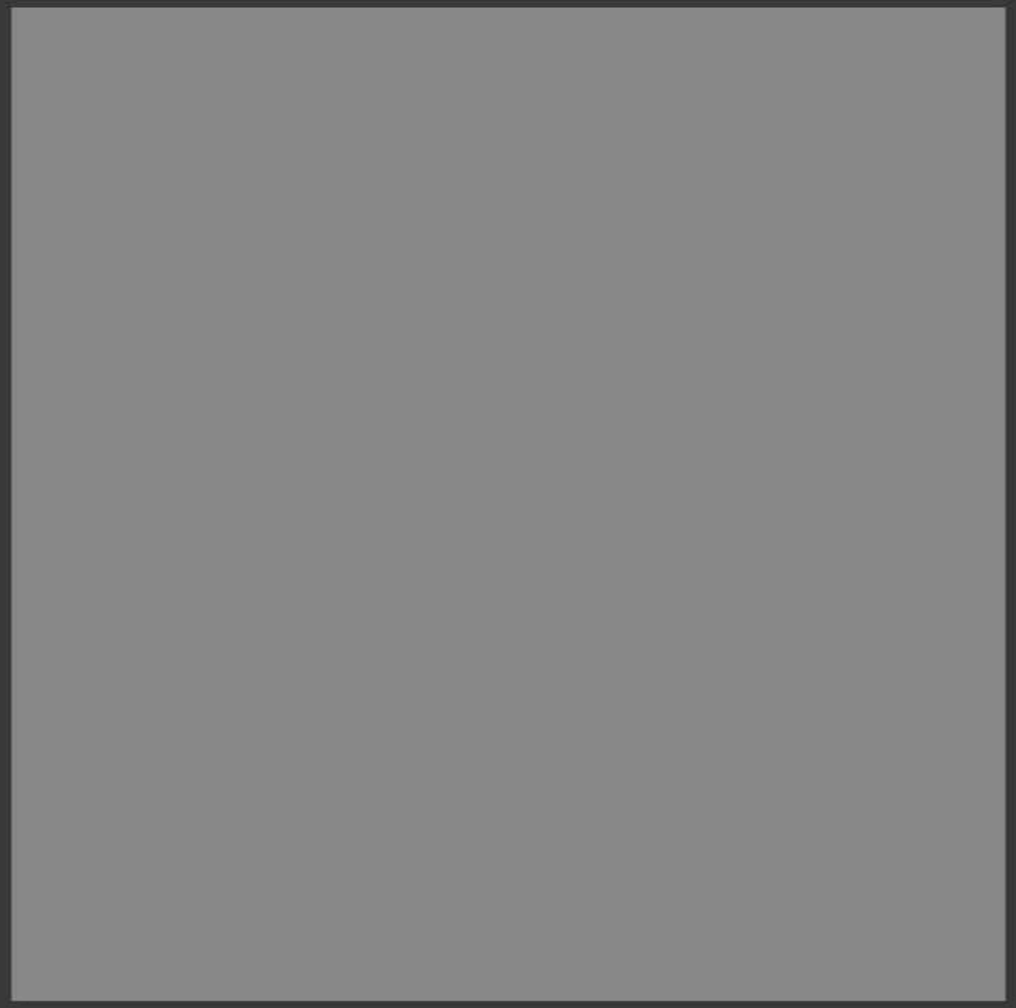


- Defocus values are always “underfocus”. This means decreasing the strength of the objective lens, effectively focusing above the specimen.
- At high defocus, high-resolution information in the image is strongly delocalized.
- Image processing can re-localize the signals, but at most only about half of the theoretical contrast is preserved by defocusing.

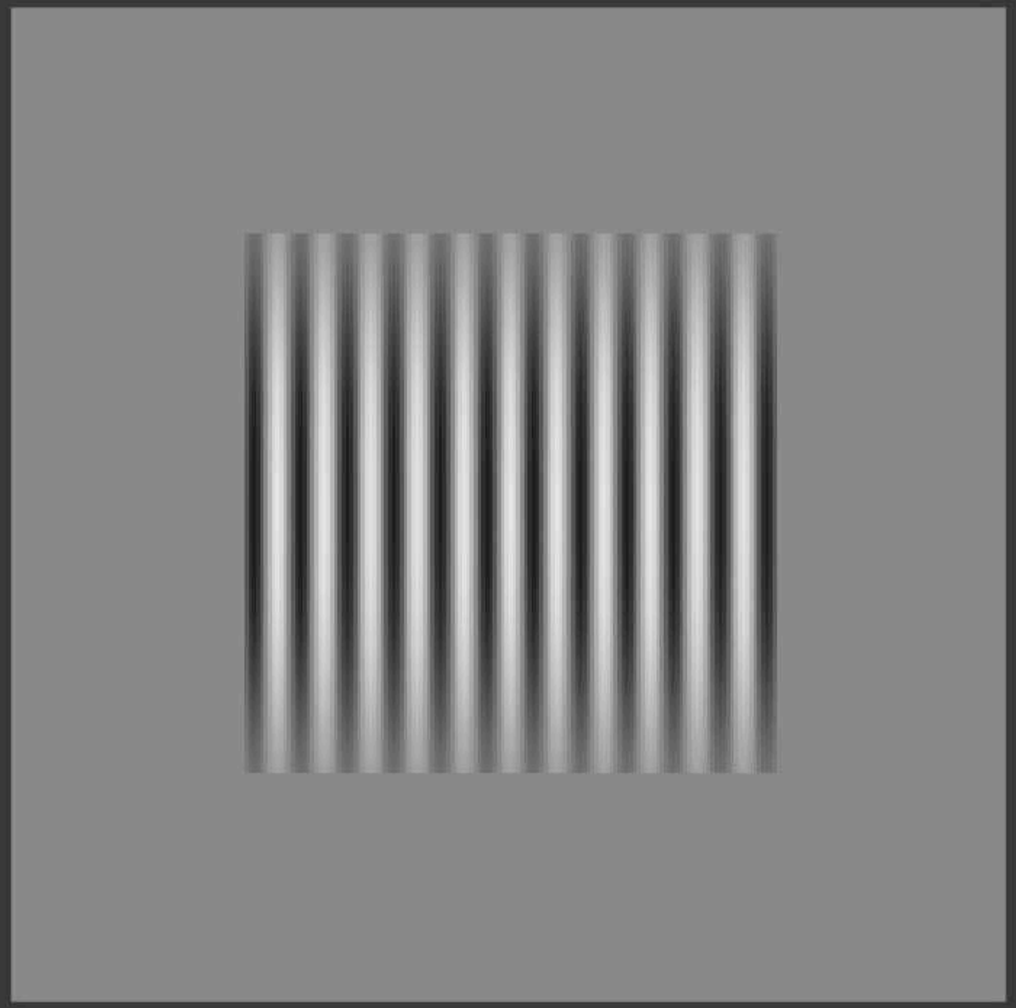
Defocus contrast in a nutshell

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.

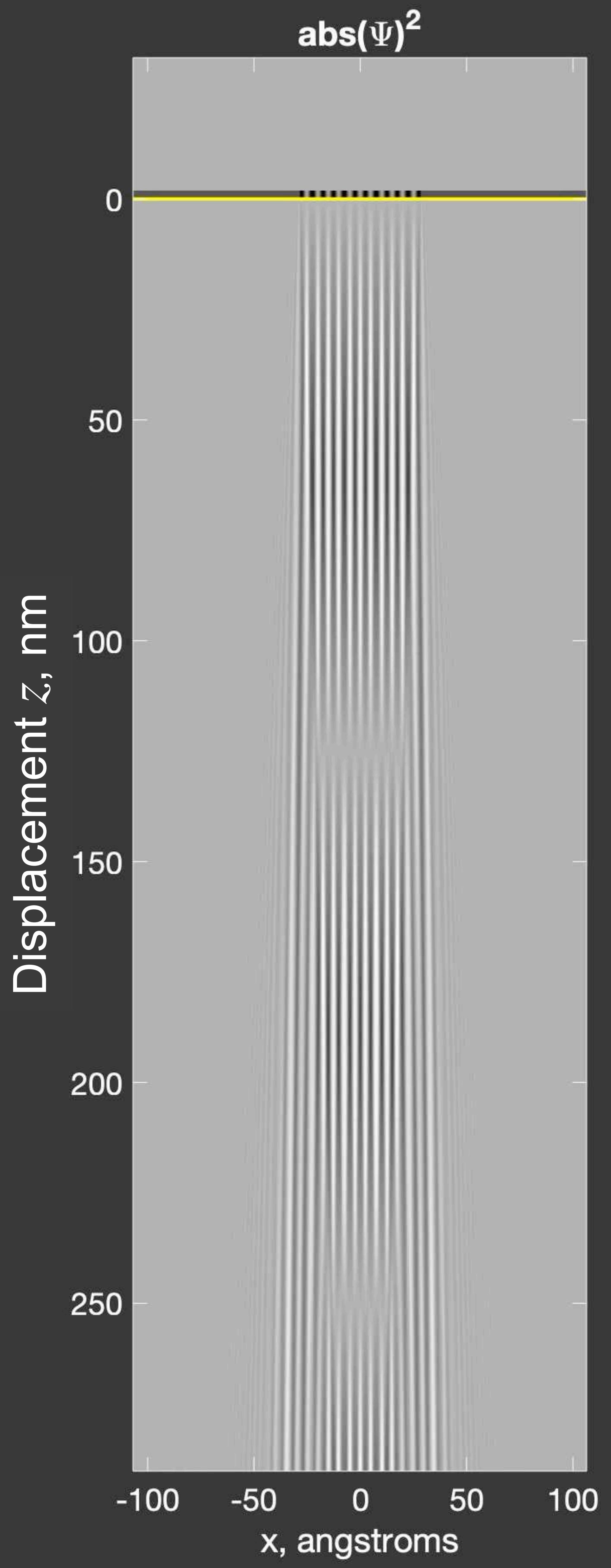
Contrast of a grating object varies with the amount of defocus

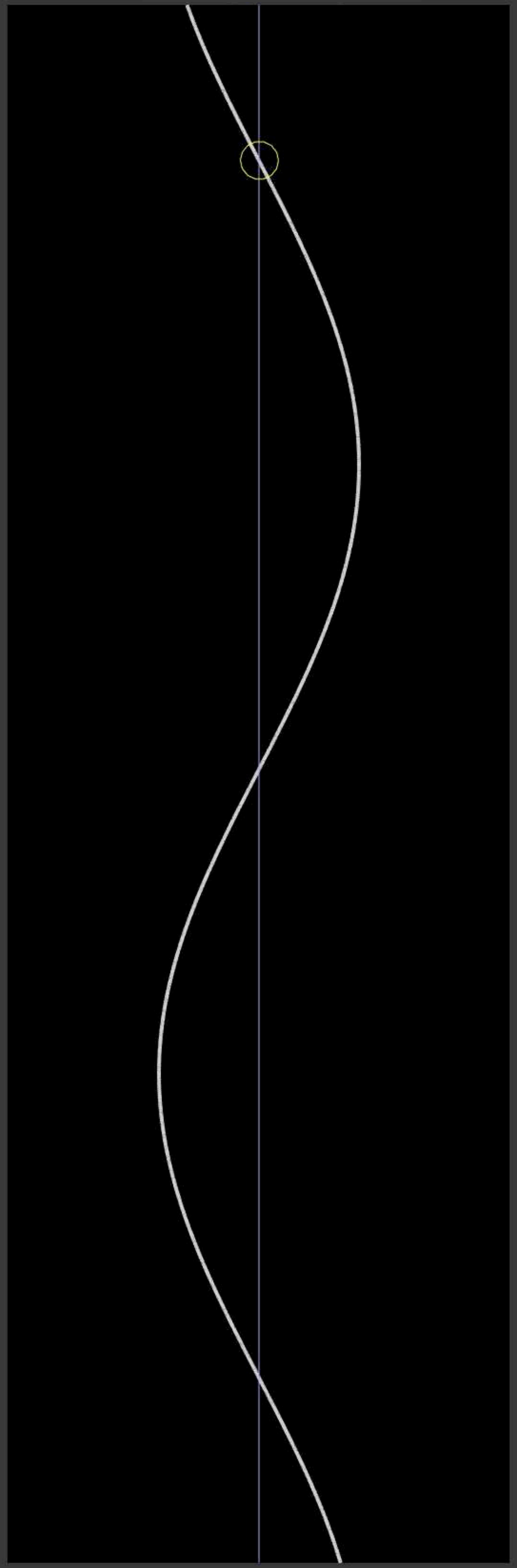


Intensity at z



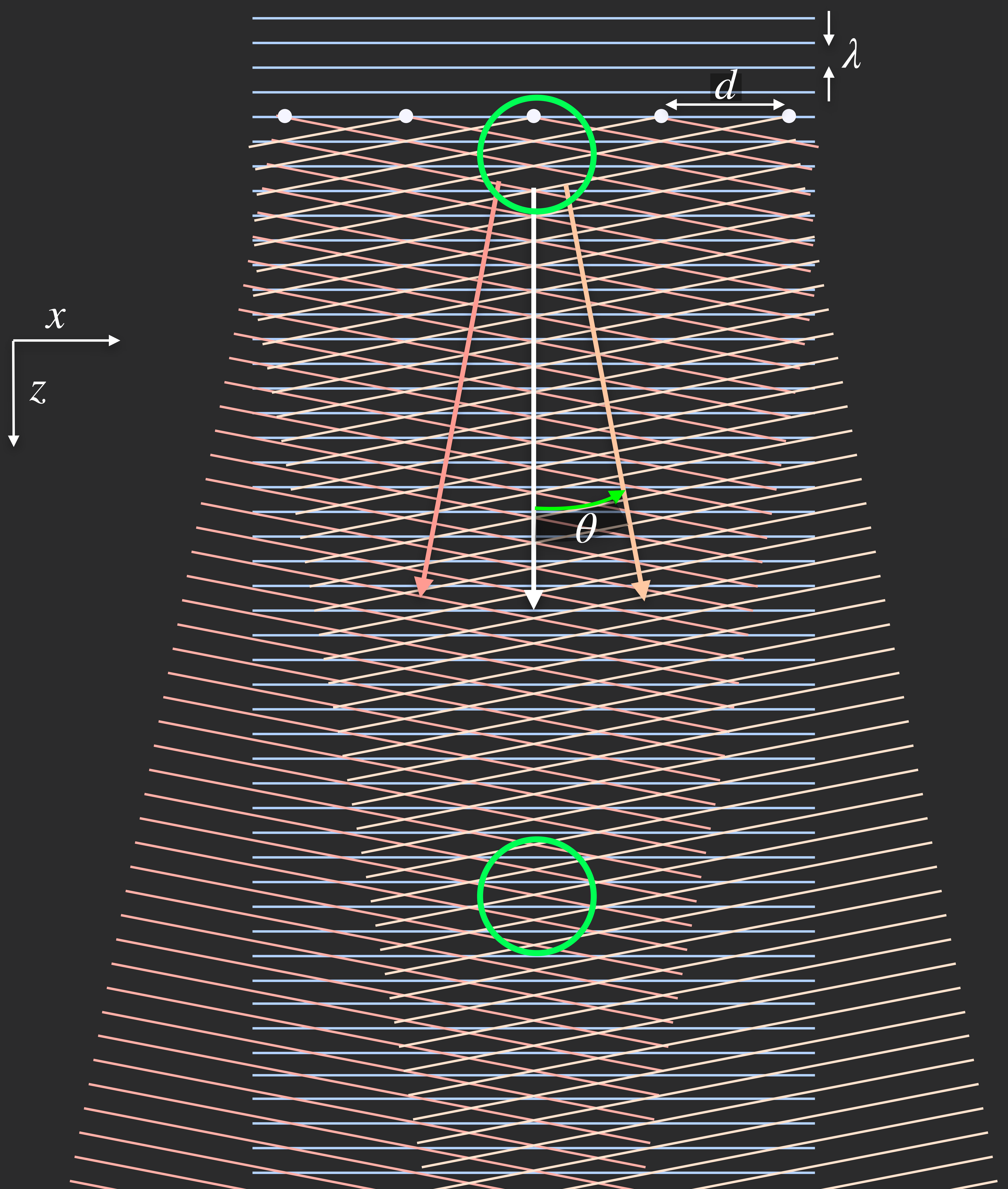
The grating $\phi(x)$



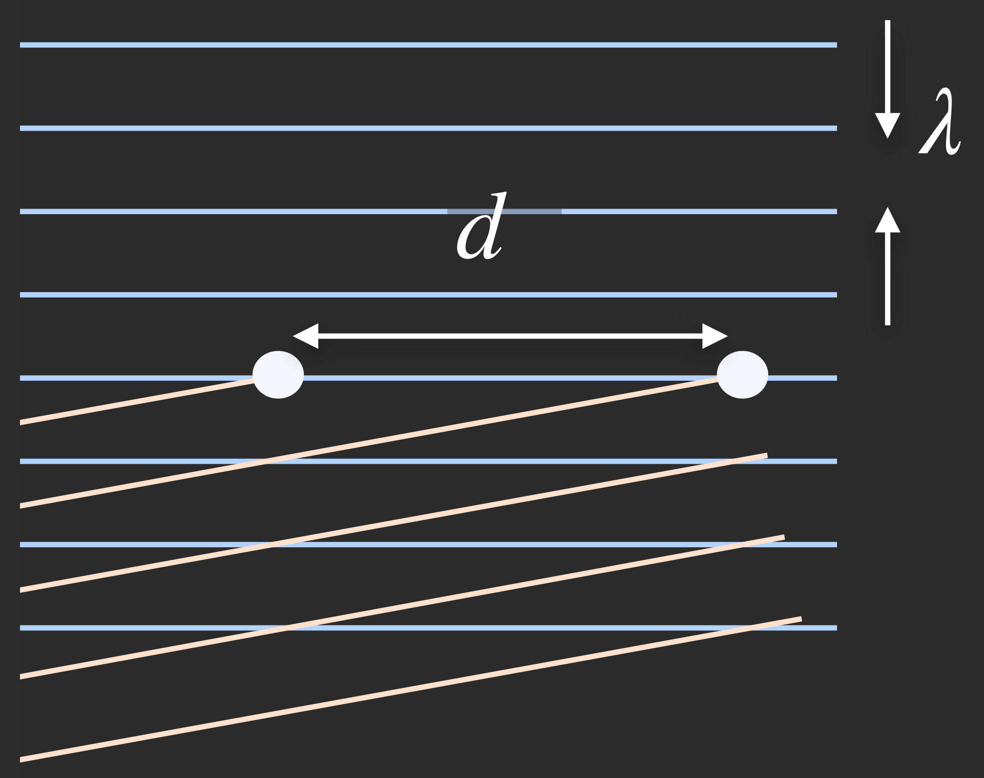


Interference between the unscattered wave and the diffracted waves produces contrast.

The grating object produces diffracted waves

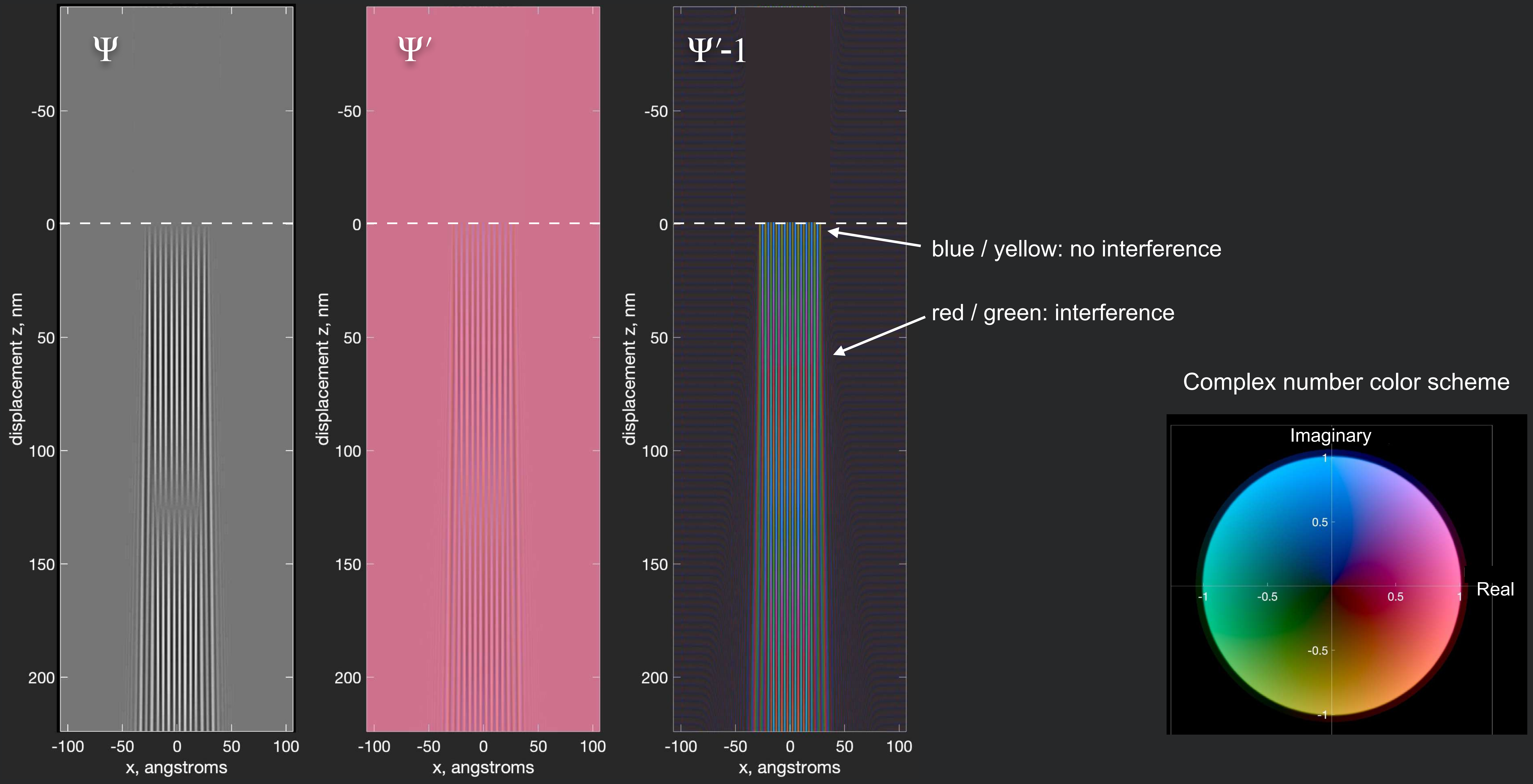


$$\sin\theta = \frac{\lambda}{d}$$



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

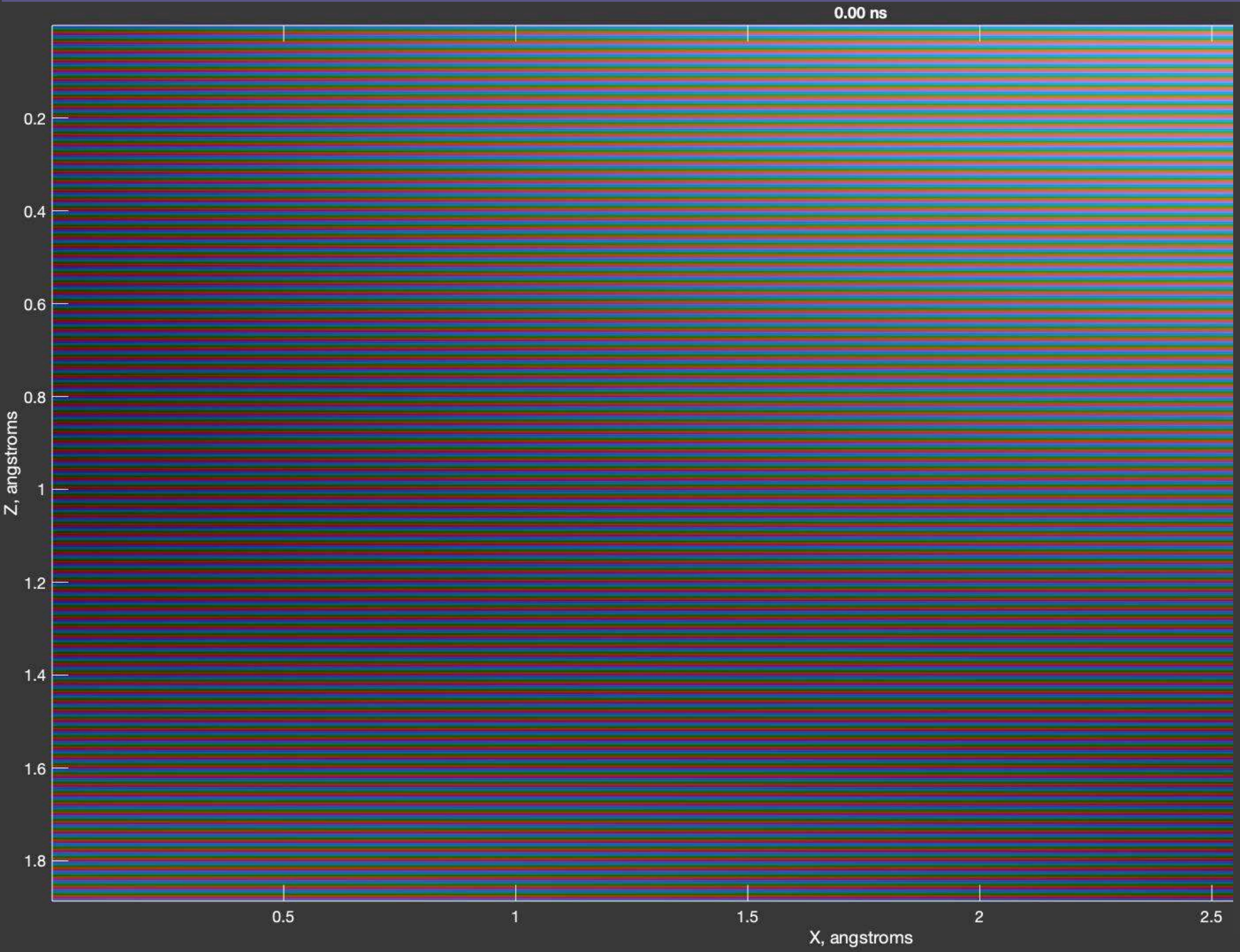
When the phase of the diffracted waves is right, we have contrast.



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

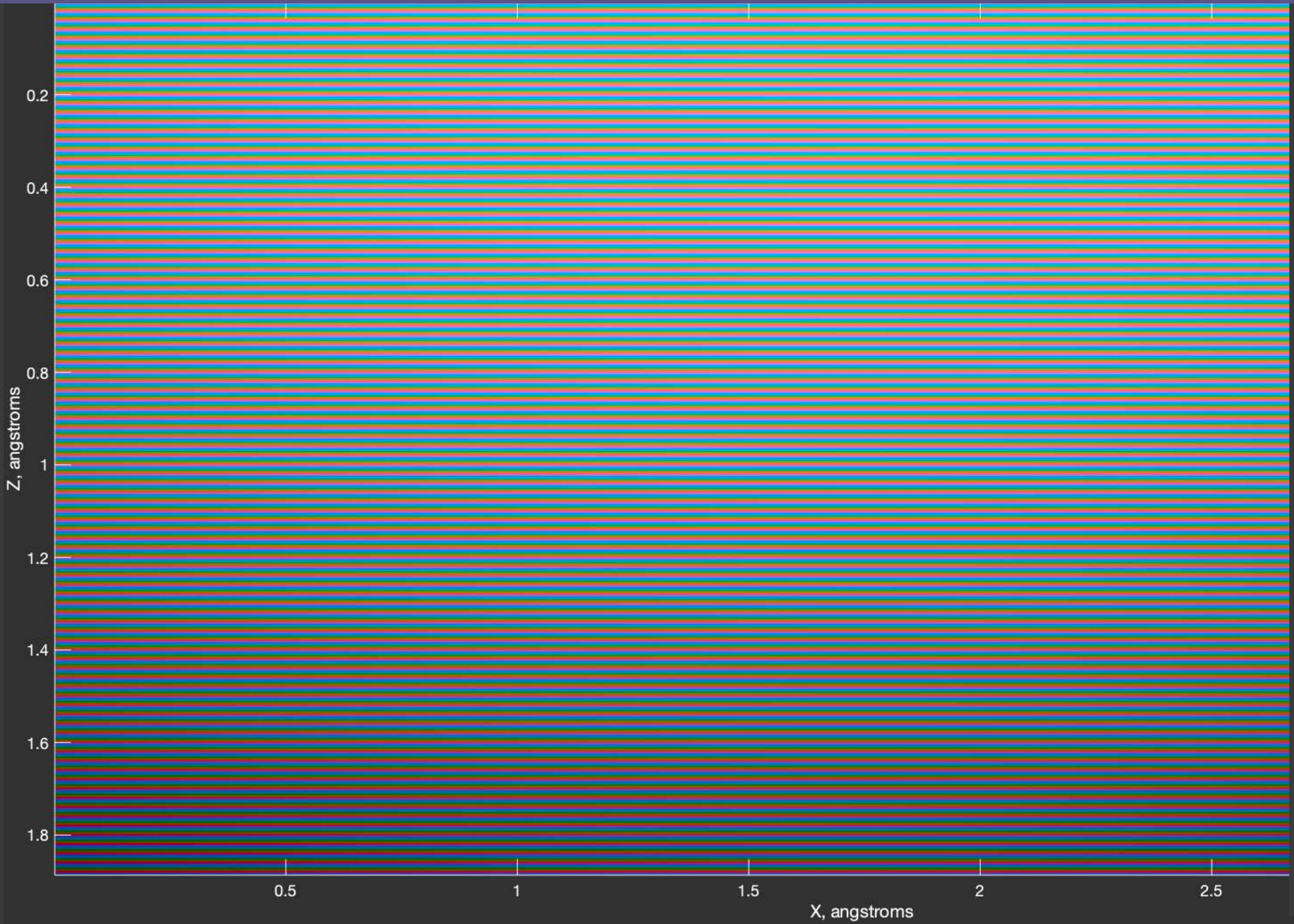


Energy (keV)	Wavelength (Å)	Velocity (fraction of c)
120	0.033	0.59
200	0.025	0.70
300	0.020	0.78

$$\Psi_0 = e^{i(kz - \omega t)}$$

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

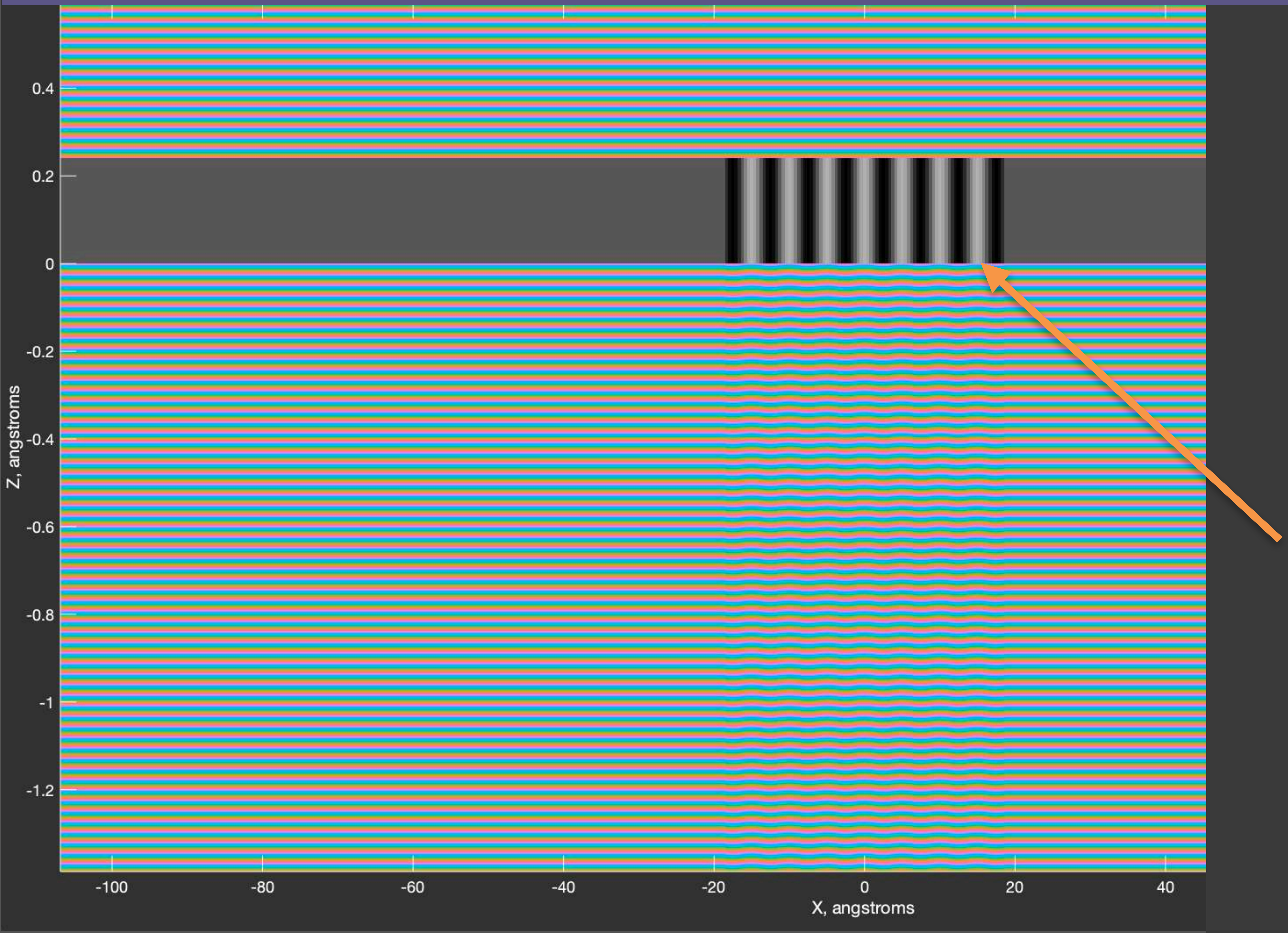
We'll ignore time dependence and take a snapshot of an electron wave



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

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

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



The object is a grating,

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

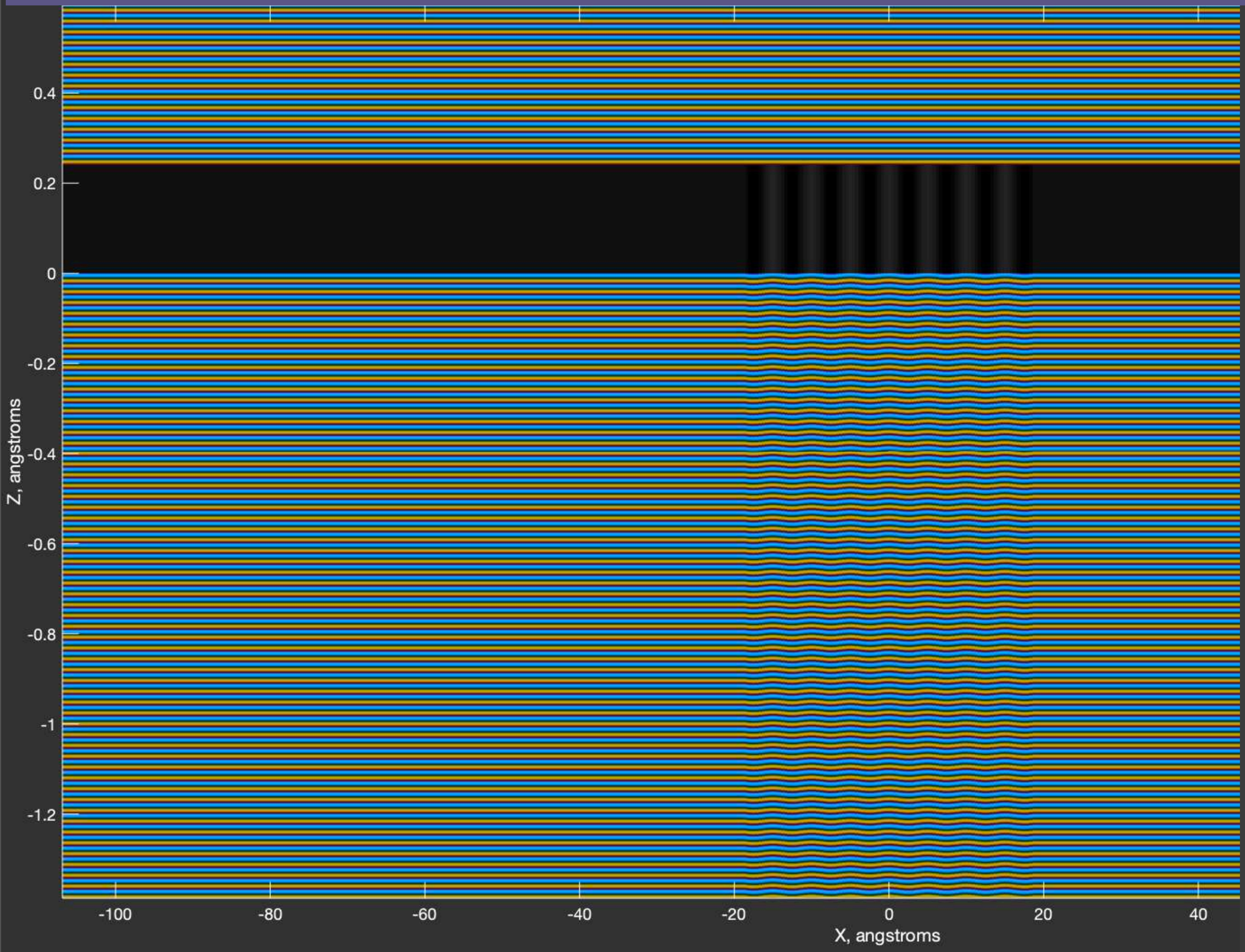
In our example,

$d = 5\text{Å}$ and $\epsilon \ll 1$.

At $z = 0$,

$\Psi = e^{i\epsilon\phi(x)}$

Small ϵ allows the weak-phase approximation



At $z = 0$,

$\Psi = e^{i\epsilon\phi(x)}$

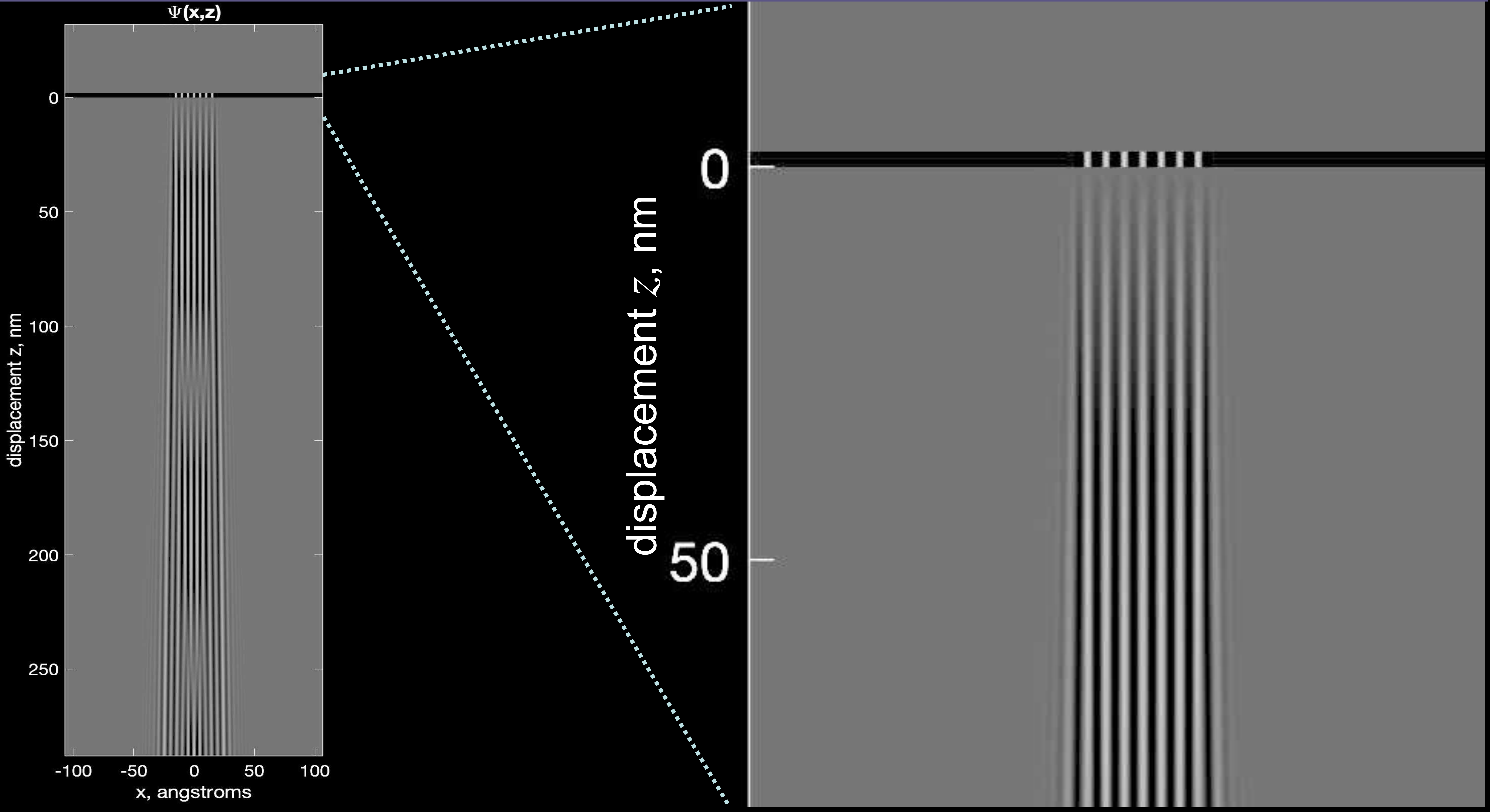
But the weak phase approximation* allows us to decompose Ψ into undiffracted and diffracted waves: at $z = 0$,

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

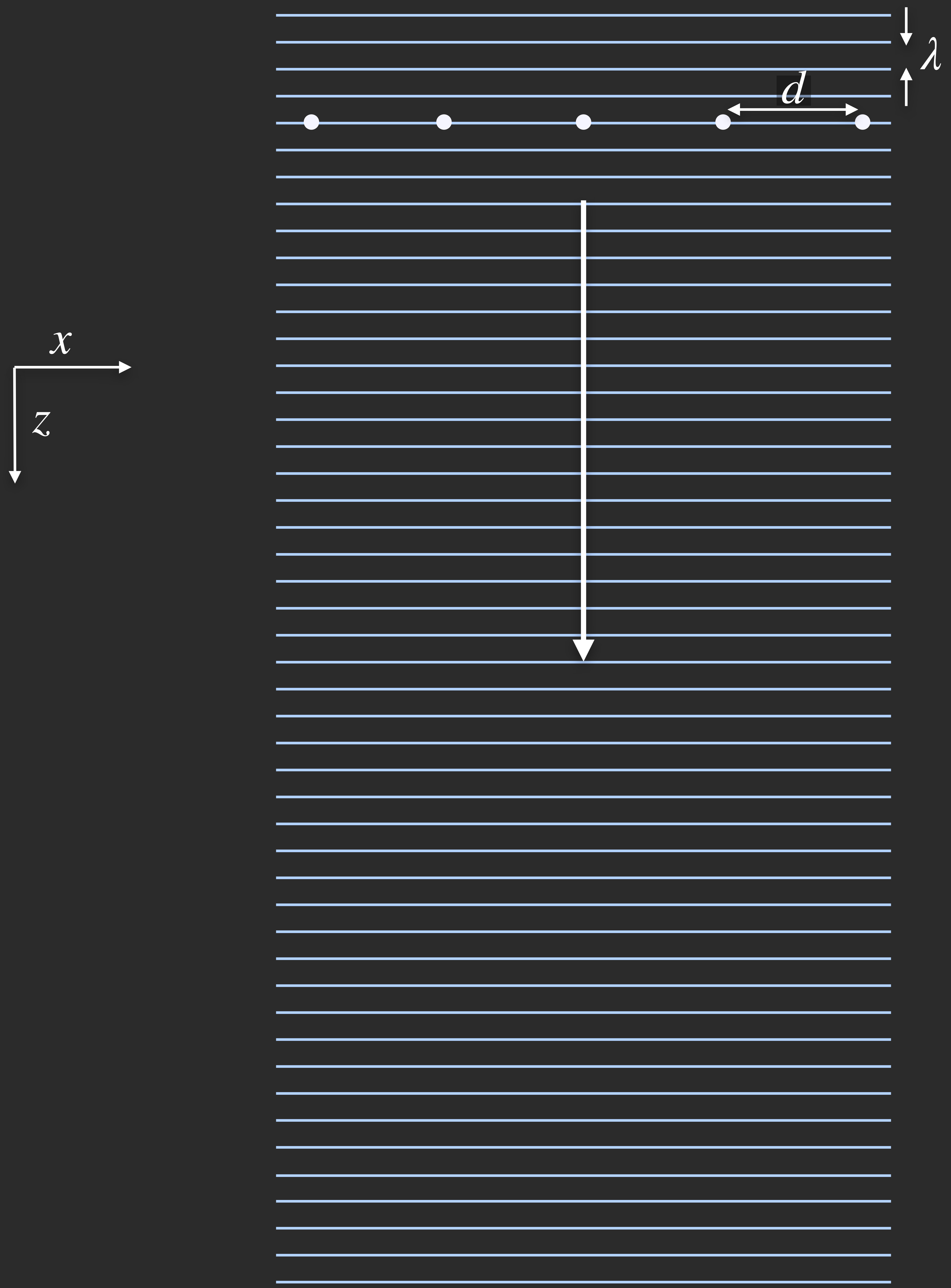
*This comes from the expansion

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

The beam intensity shows variation only away from the specimen

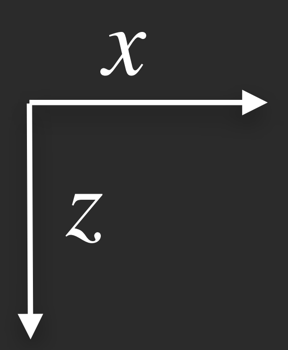


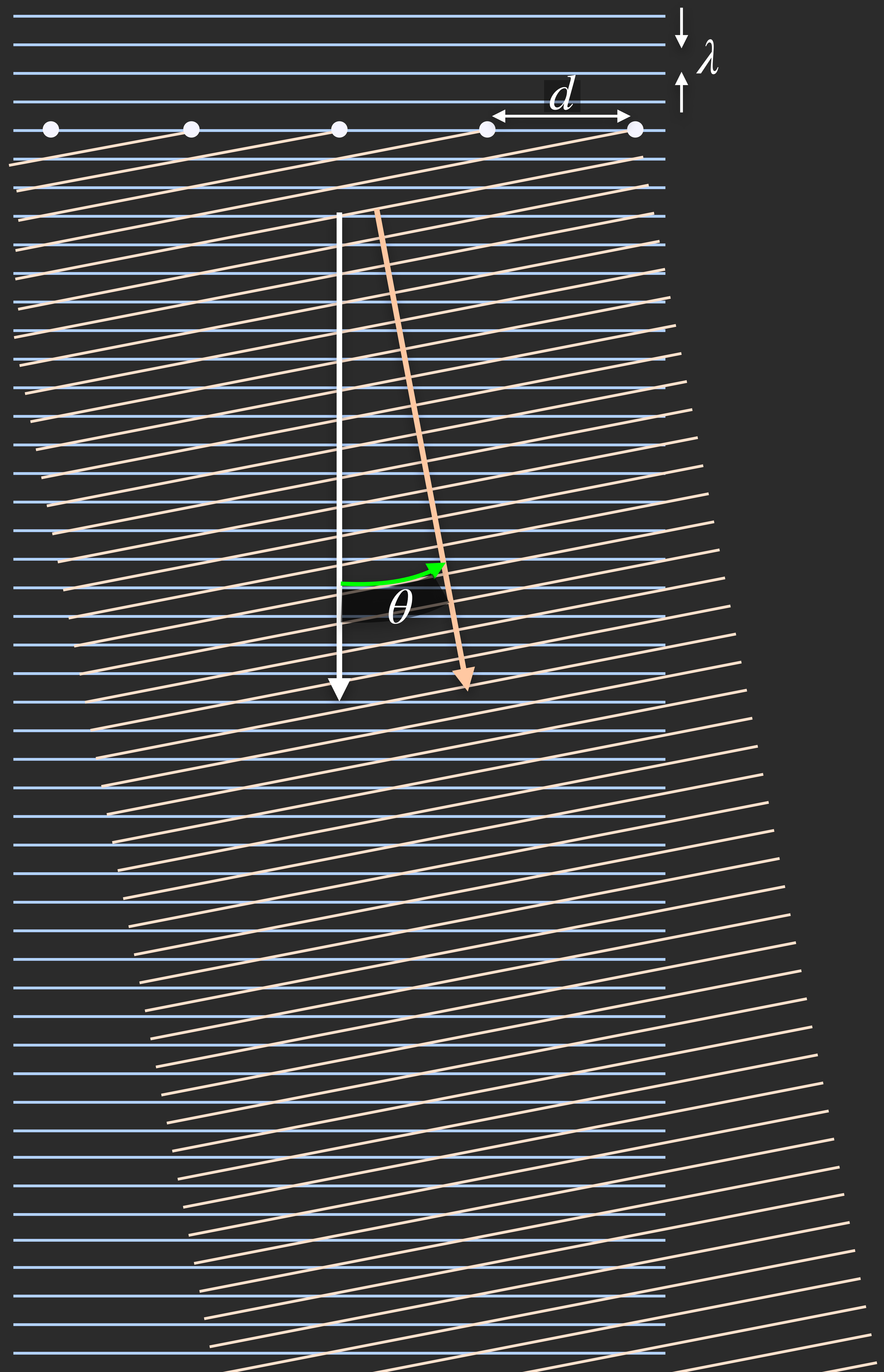
Modeling the components of Ψ: first, the undiffracted wave



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

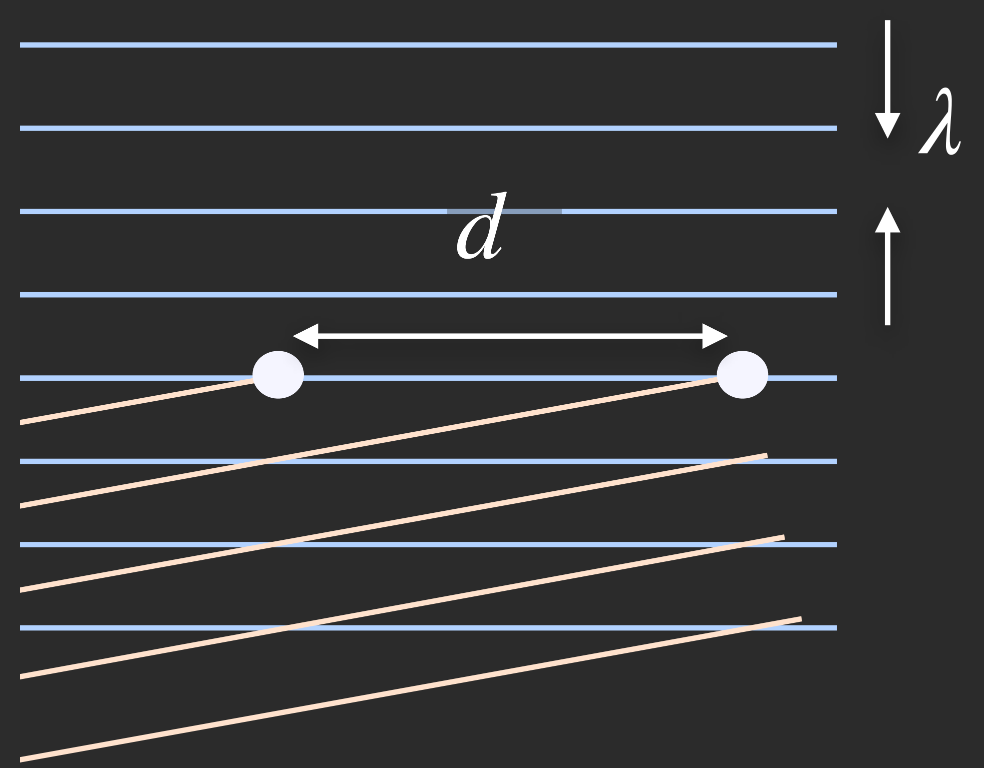
Classical diffraction yields a diffracted wave…





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

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

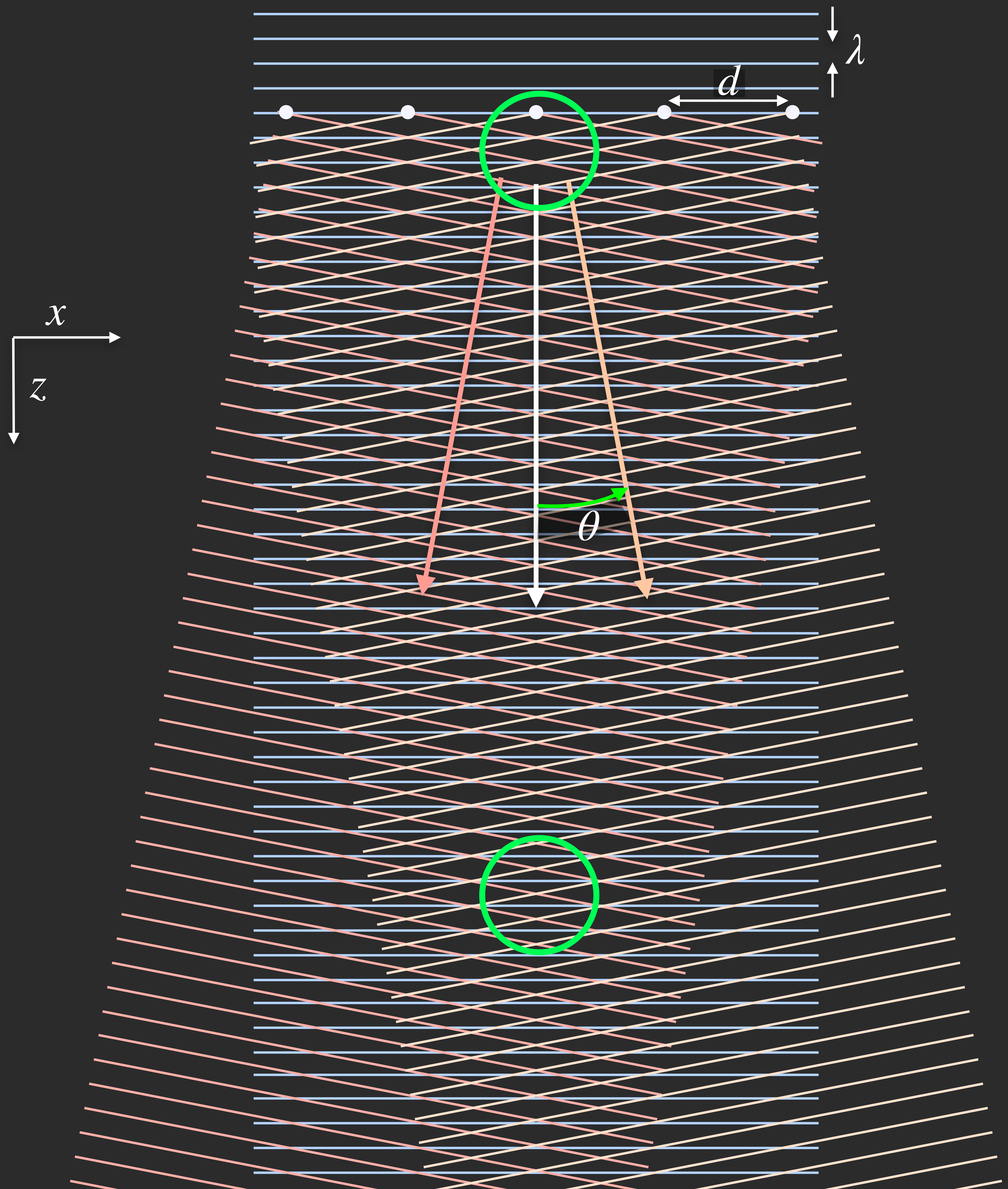


$$\Psi_0 = e^{ikz}$$

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

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

…and the other diffracted wave



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

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

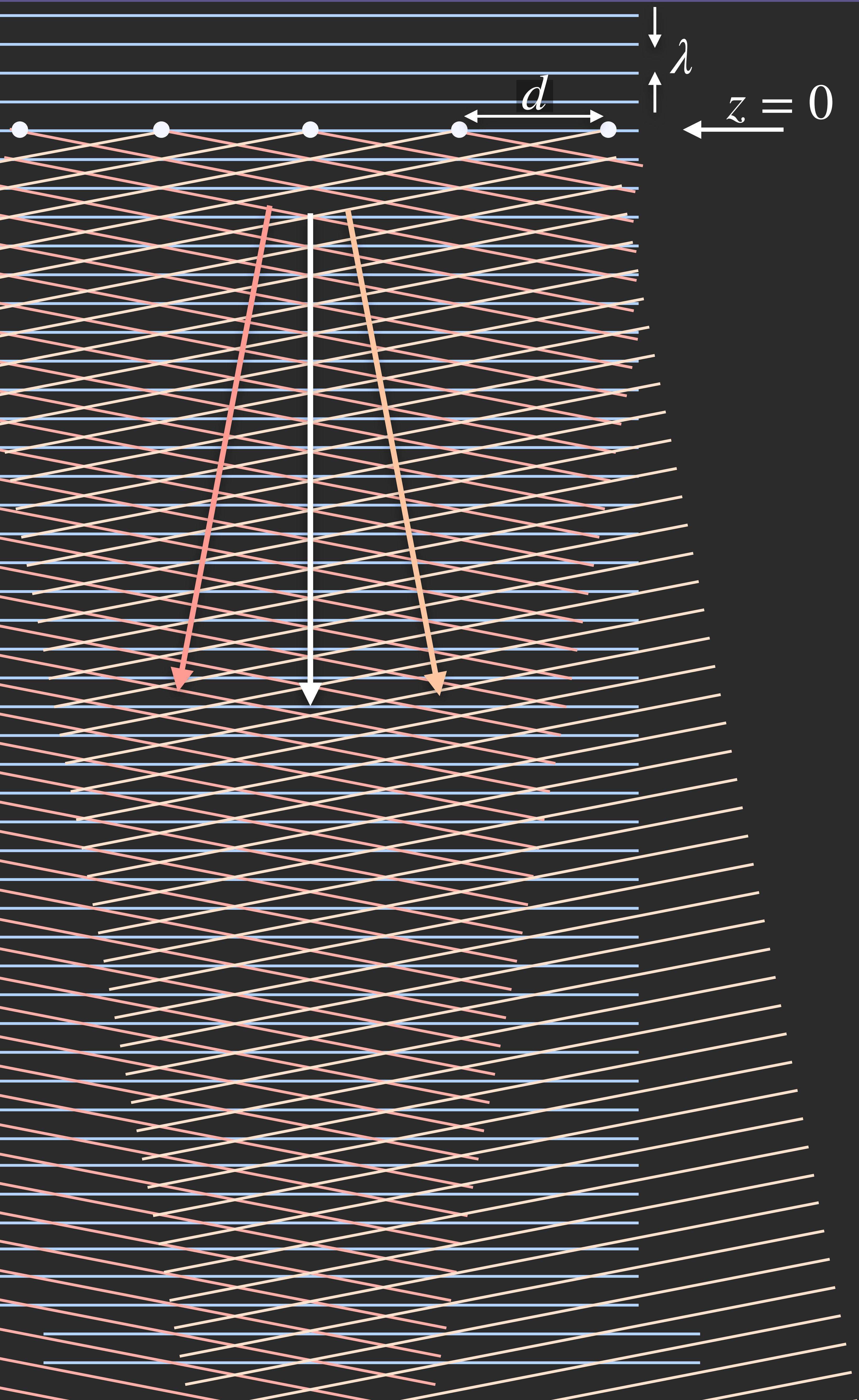
$$\Psi_0 = e^{ikz}$$

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

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

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 $z = 0$.



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

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

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

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

At $z = 0$ we have

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

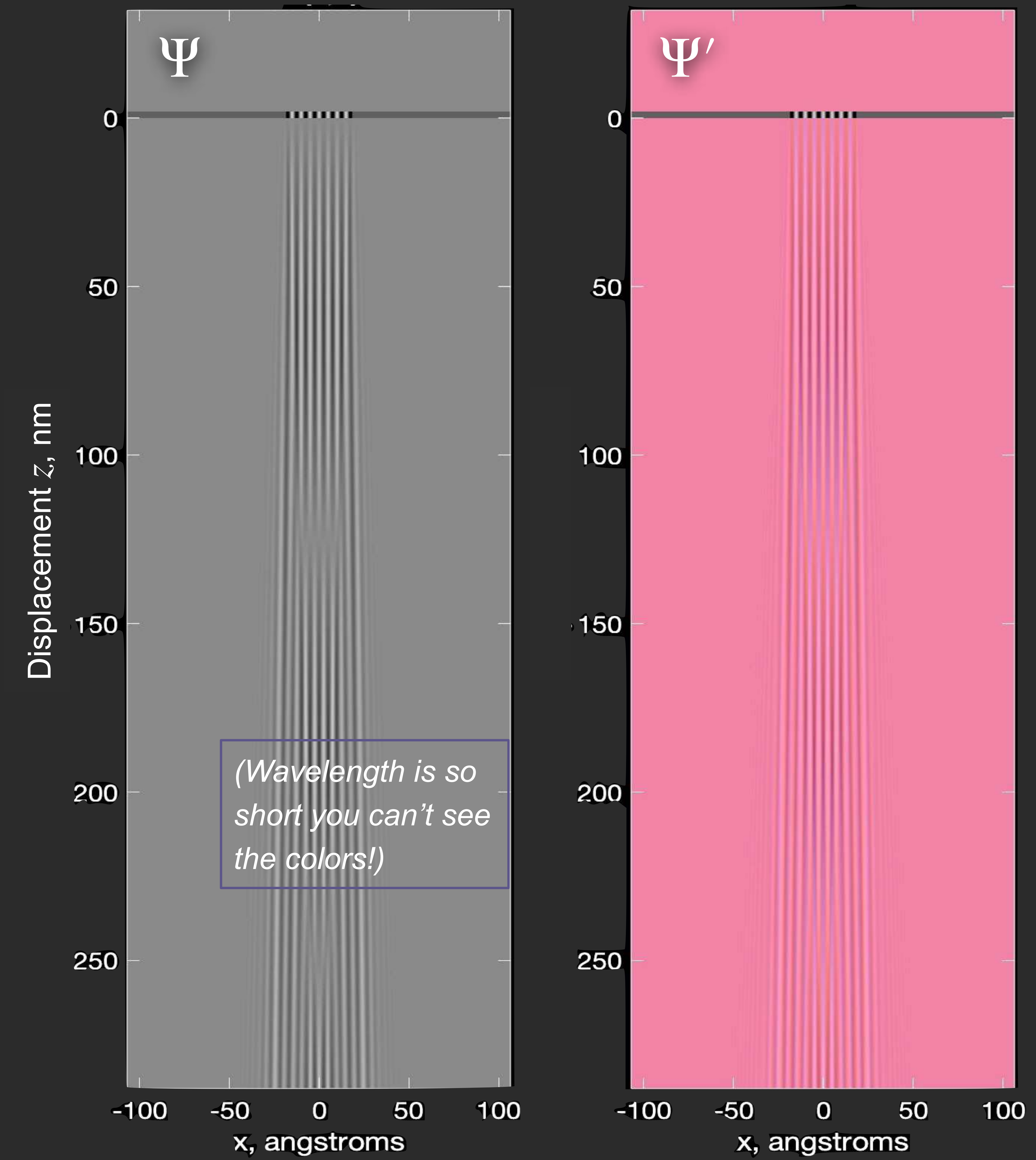
…because at $z = 0$

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

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

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

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

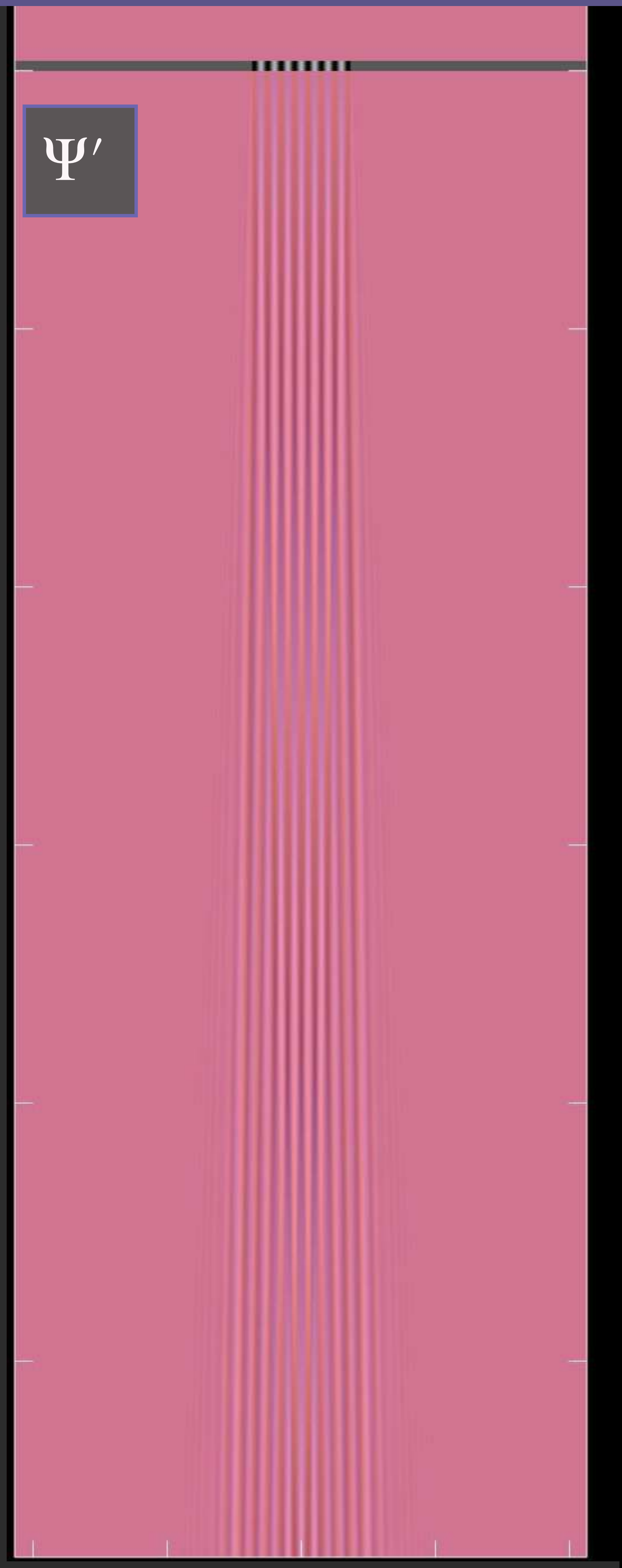
$$\Psi = \Psi_0 + \Psi_+ + \Psi_-$$

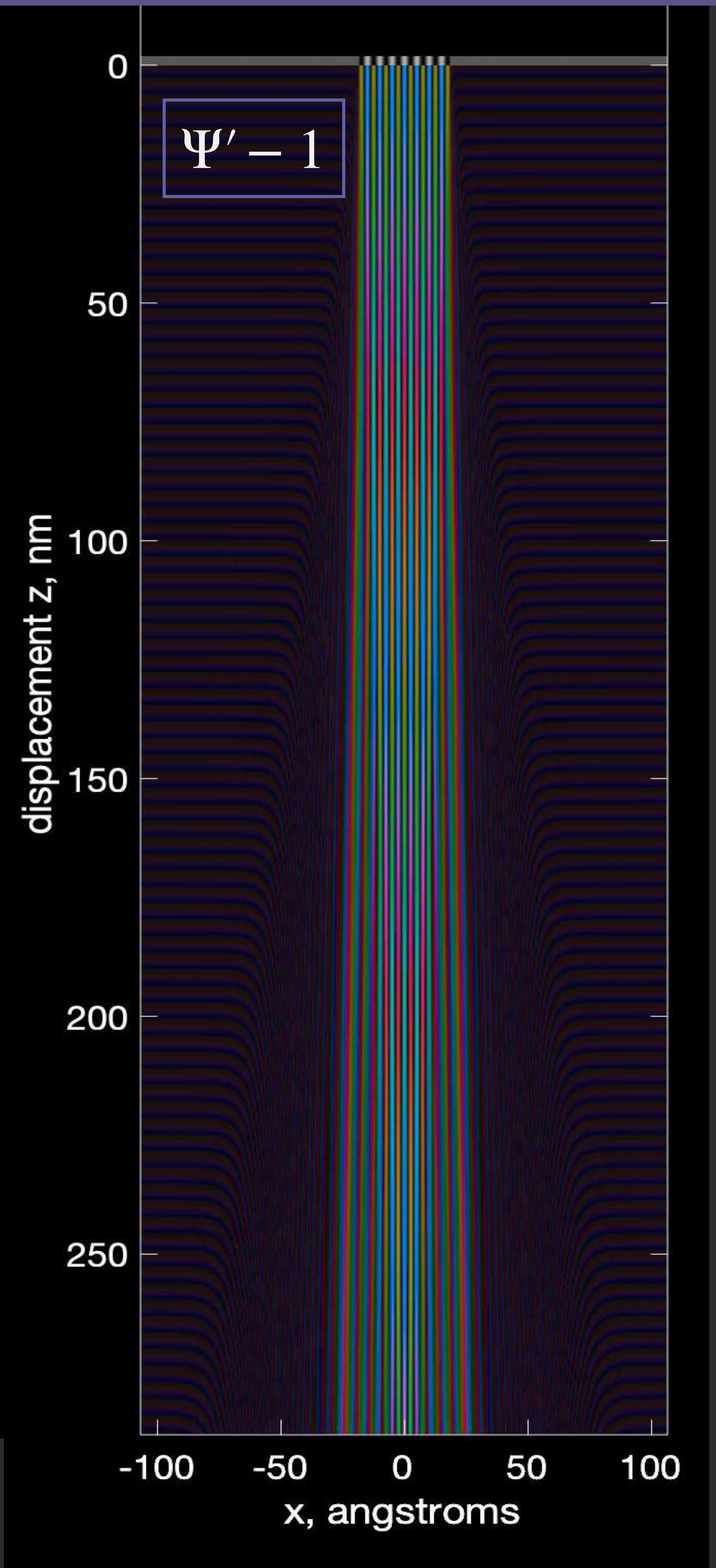
For simplicity, we'll define Ψ': $\Psi = \Psi' e^{ikz}$

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

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

The diffracted-wave phases change relative to the unscattered wave





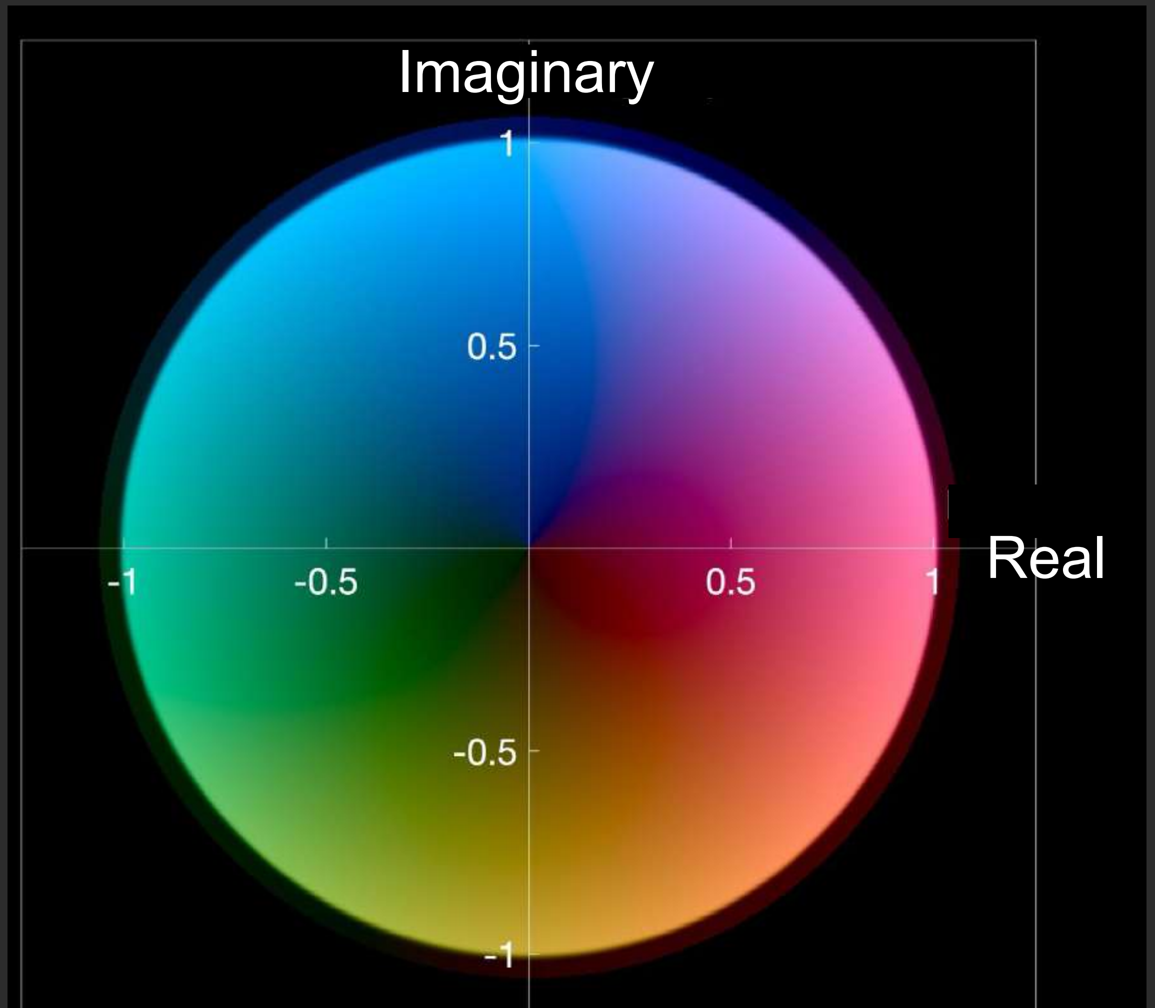
Let's remove the undiffracted wave (constant part of Ψ').

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

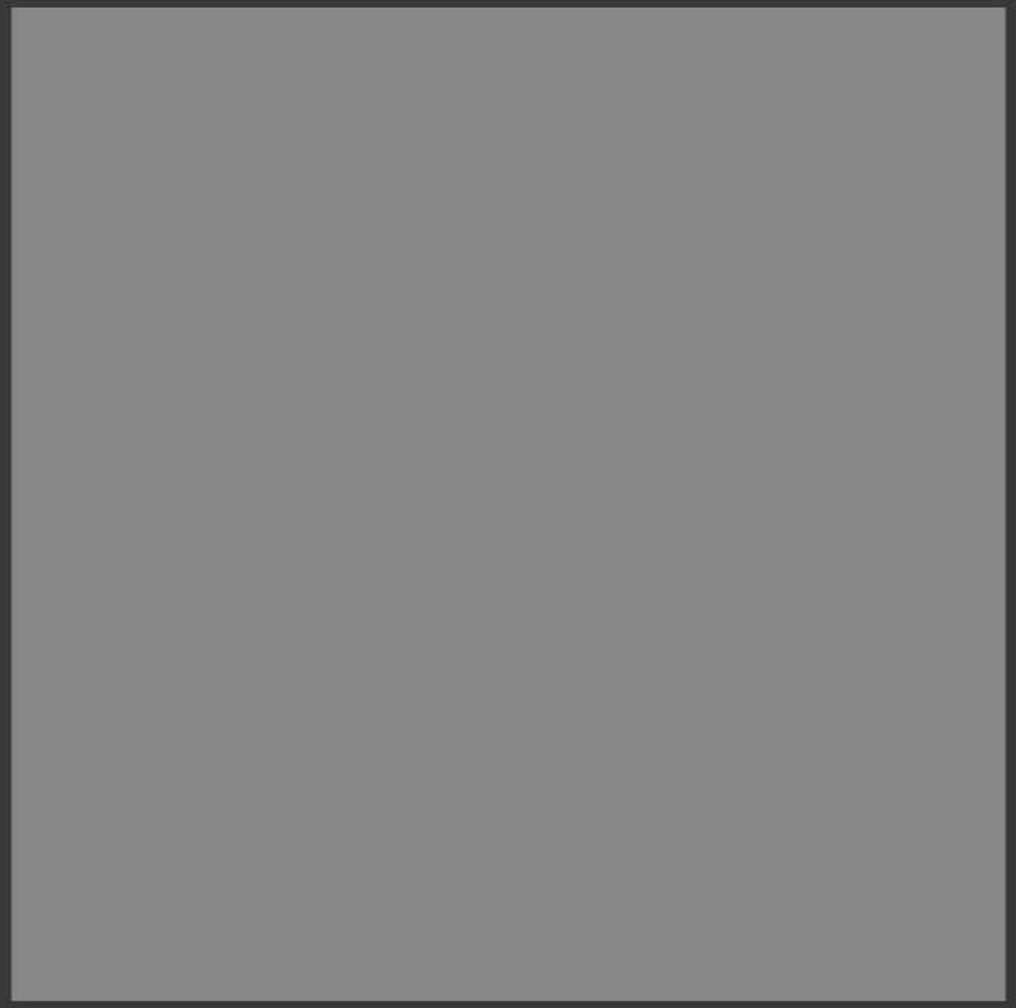
$$\Psi' - 1 = e^{ik(c-1)z} \cdot i\epsilon \cos(ksx)$$

$k = 2\pi/\lambda$
$s = \sin(\theta) = \lambda/d$
$c = \cos(\theta)$

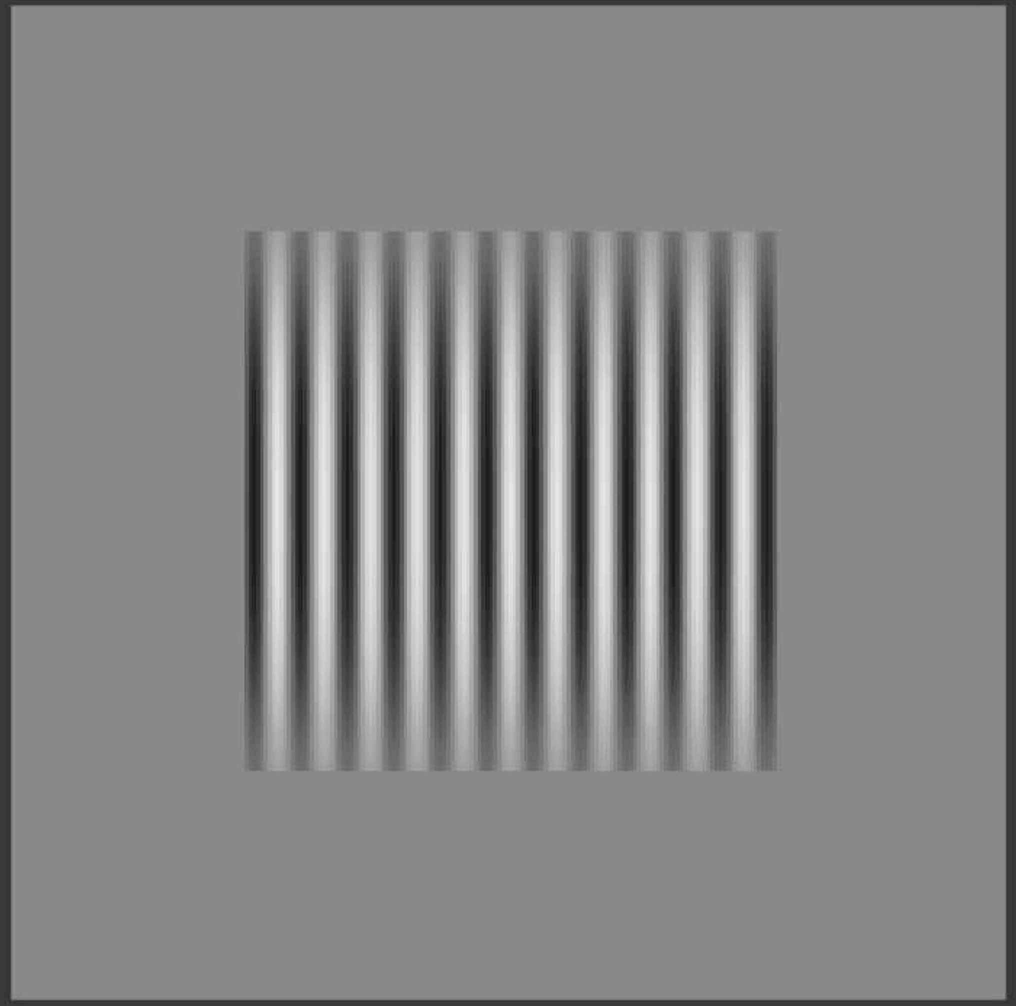
Our original phase object
$\phi(x) = \epsilon \cos(2\pi x/d)$



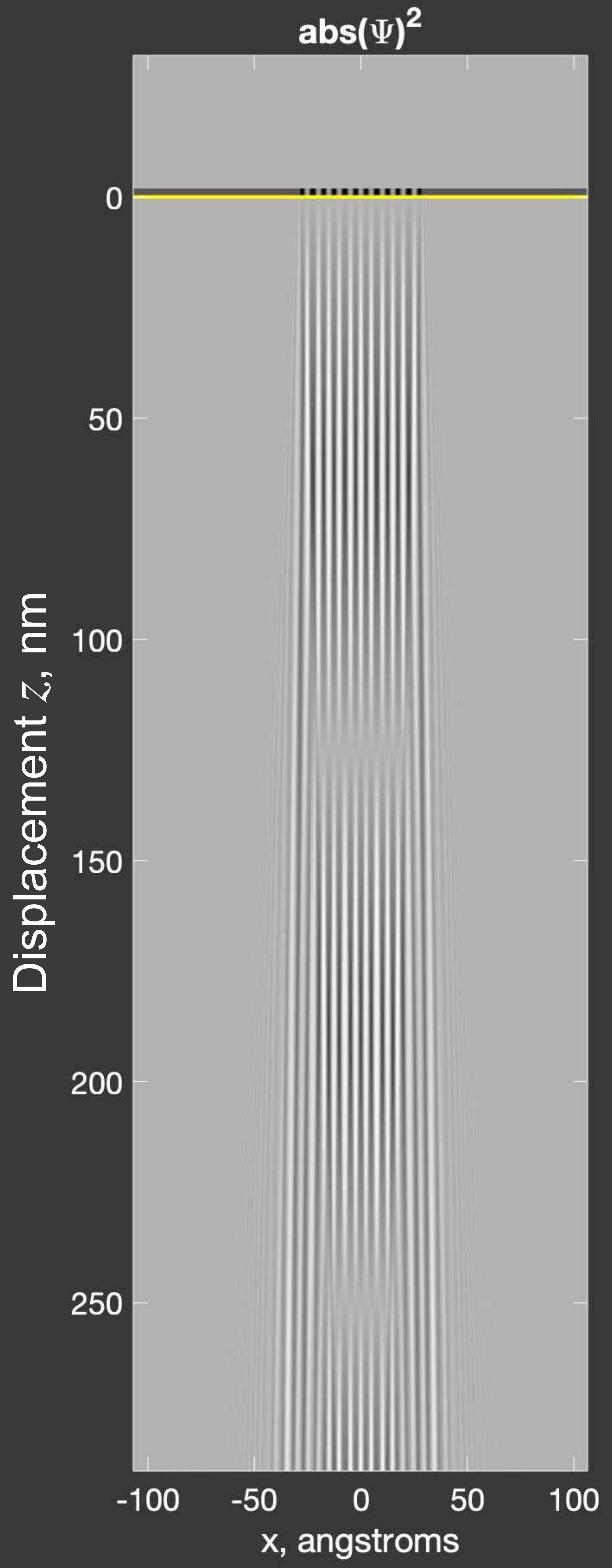
Contrast varies with the amount of defocus

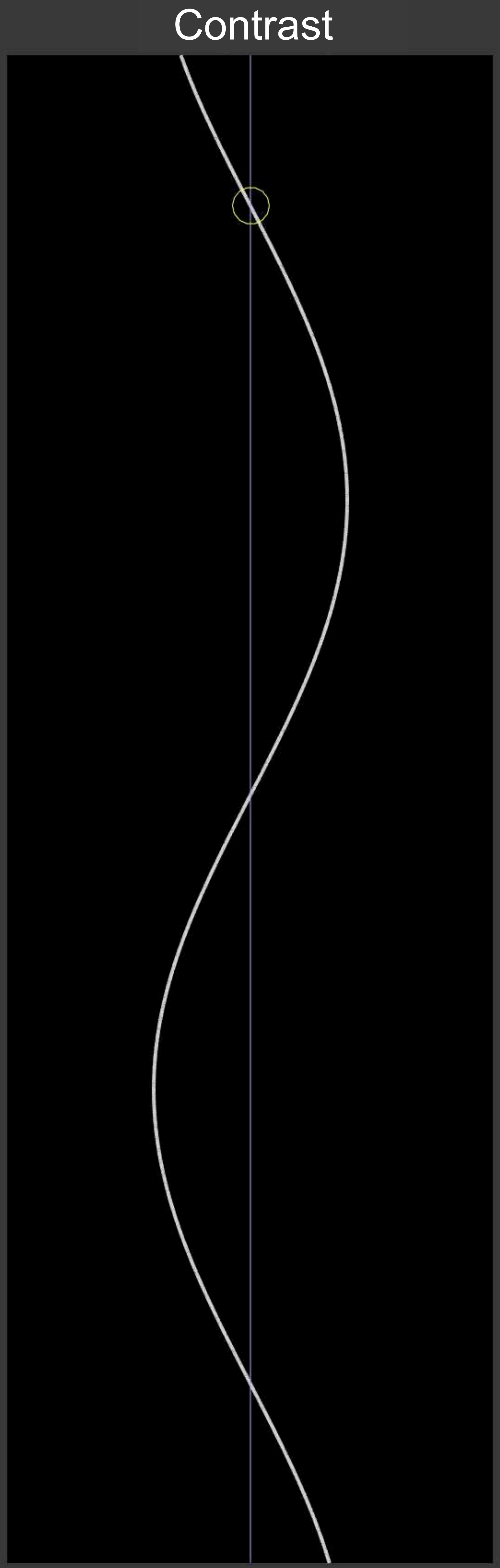


Intensity at z



The grating $\phi(x)$





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

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

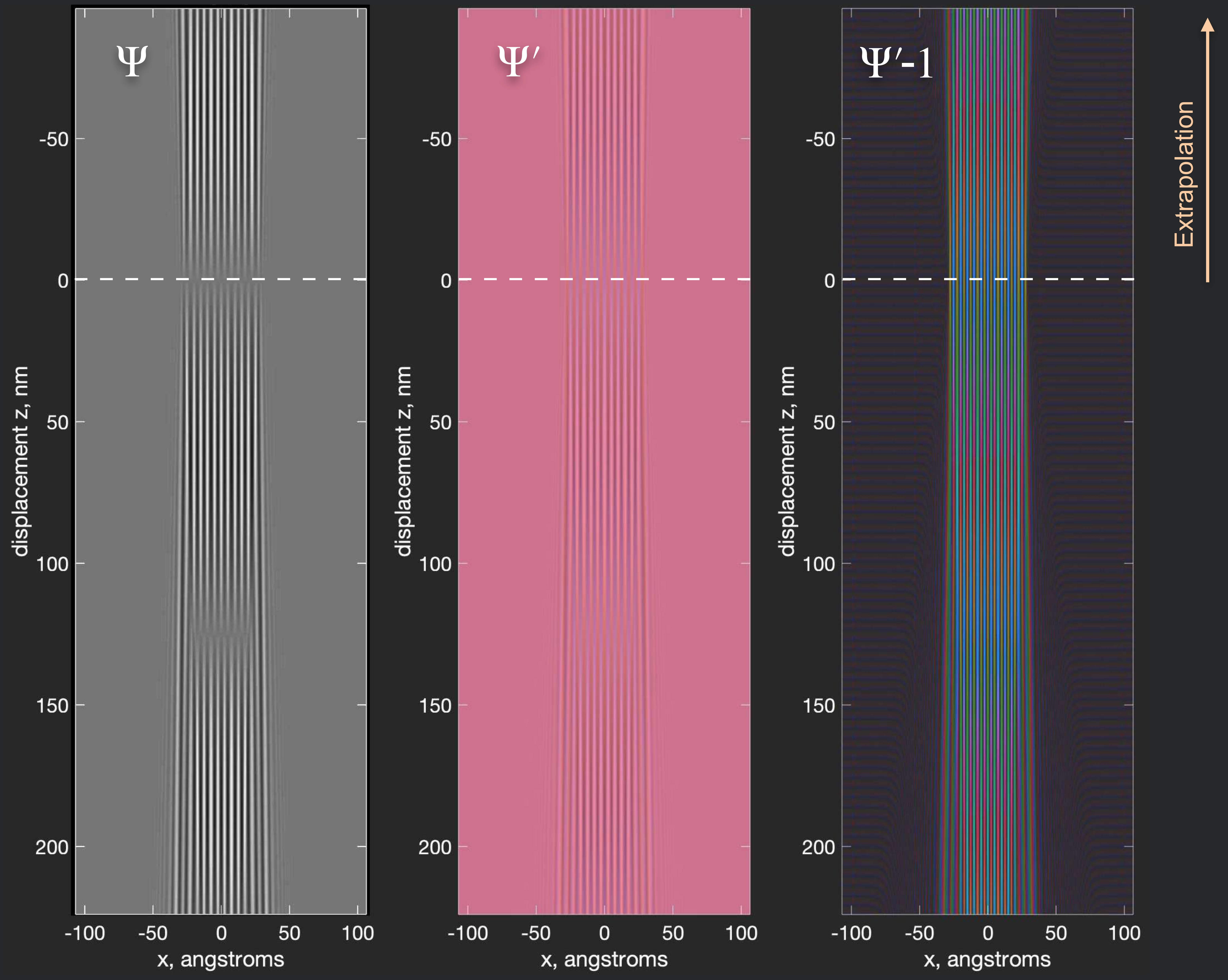
$\epsilon\phi(x) = \epsilon\cos(2\pi x/d)$

$k = 2\pi/\lambda$

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

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

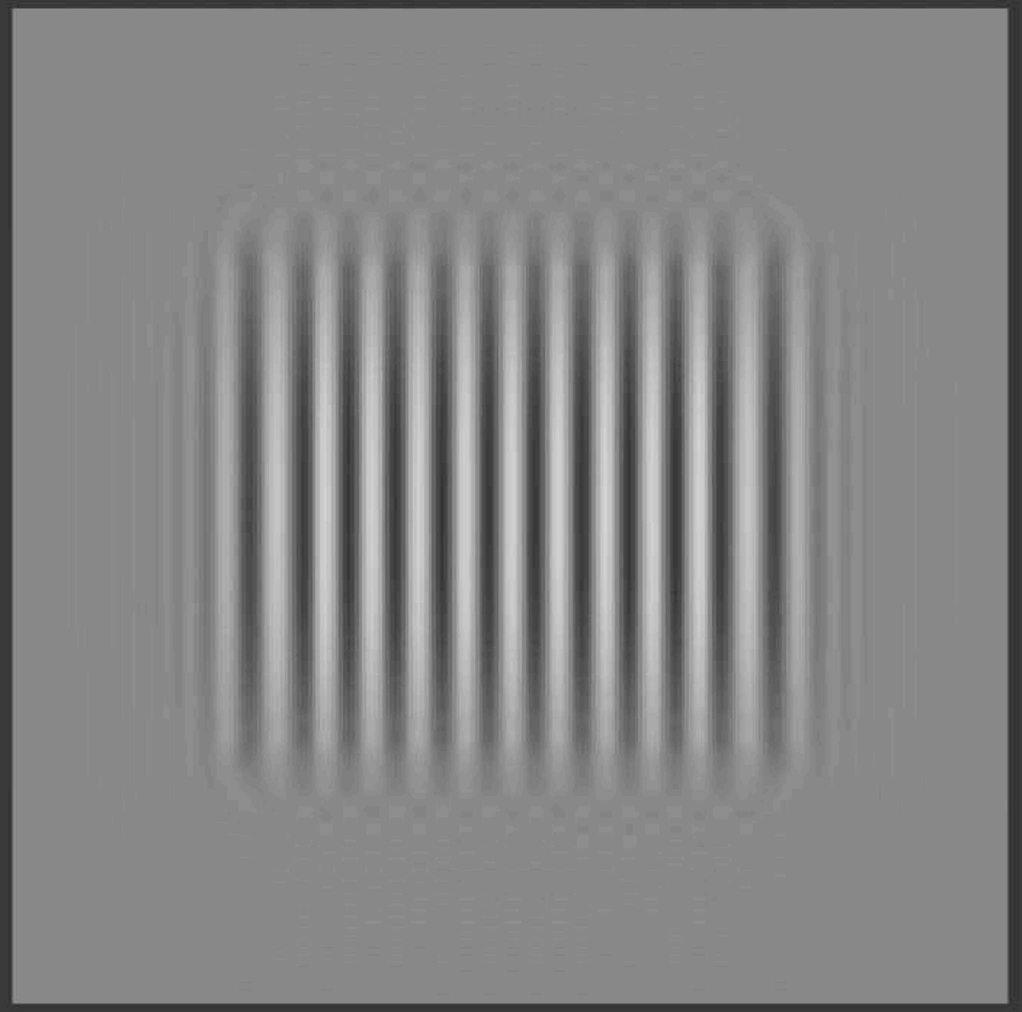
What happens when the objective lens is focused *above* the specimen?



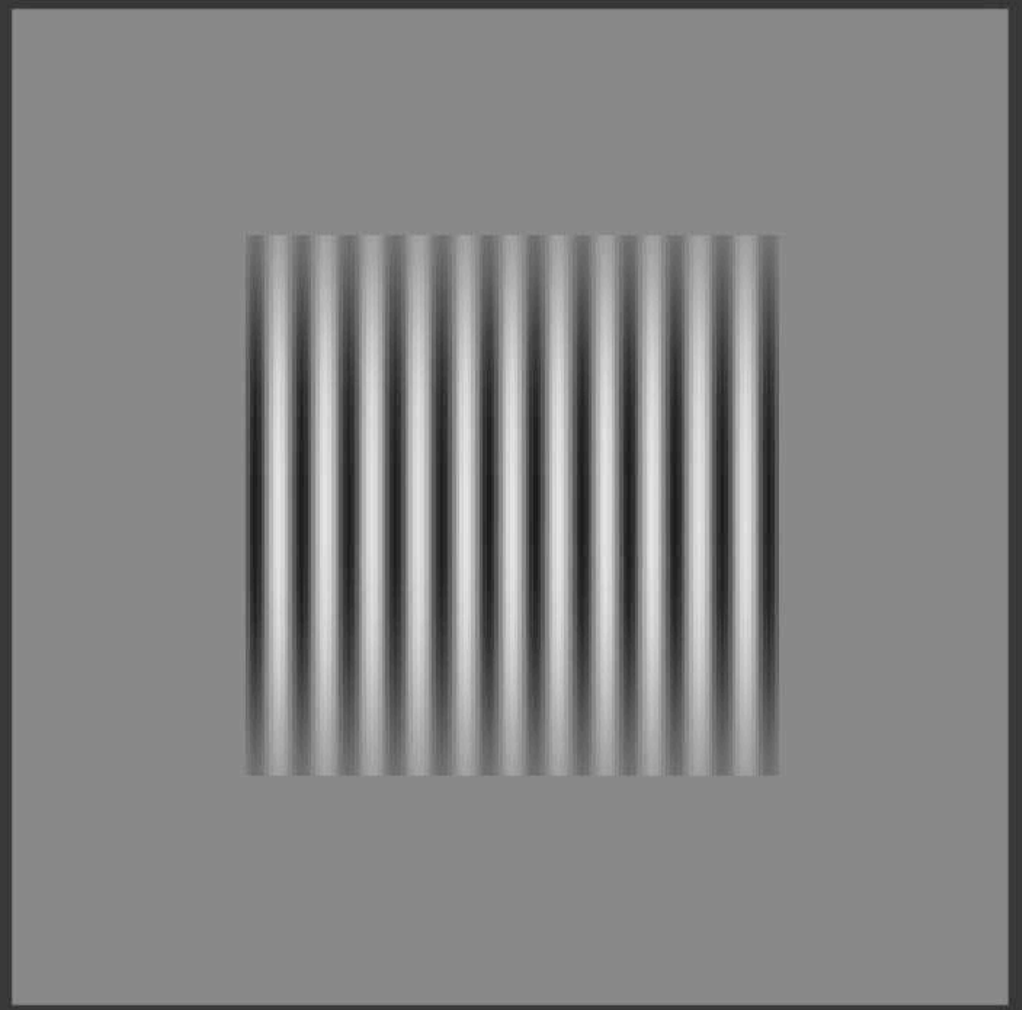
What wavefunction above the specimen would give rise to what we see below it?

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

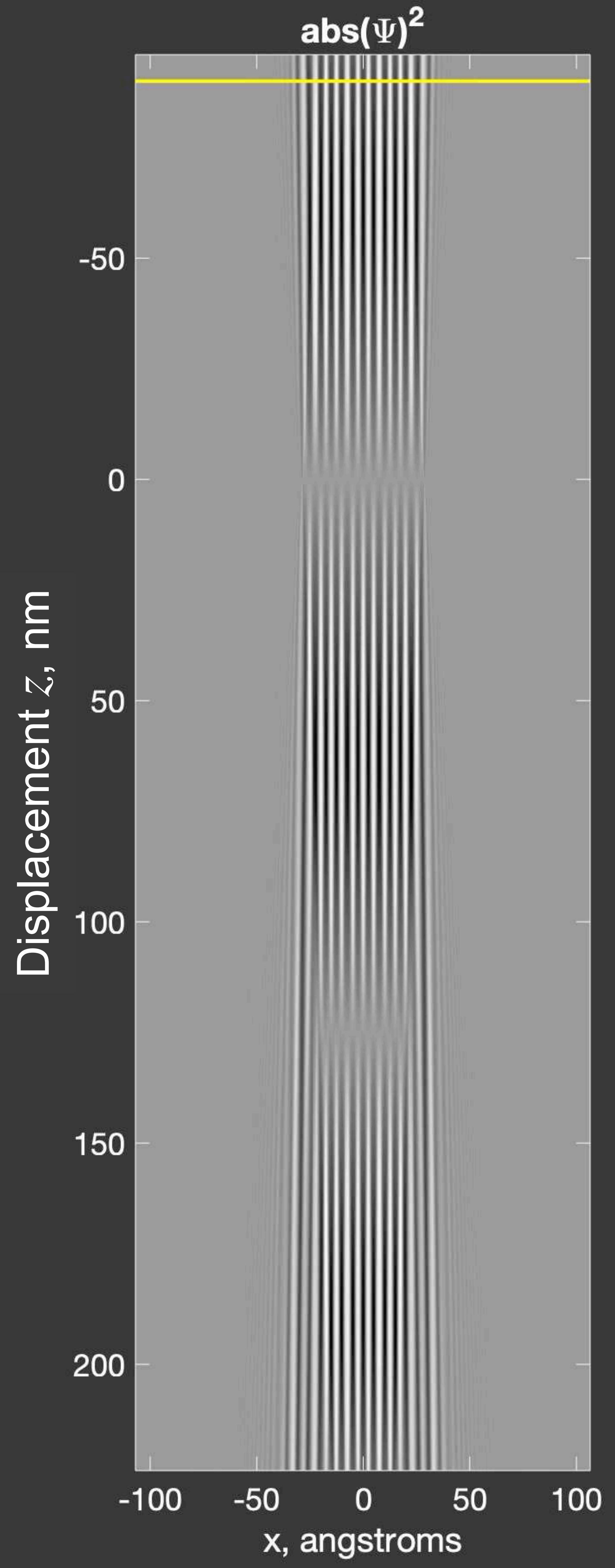
What happens when the objective lens is focused *above* the specimen?

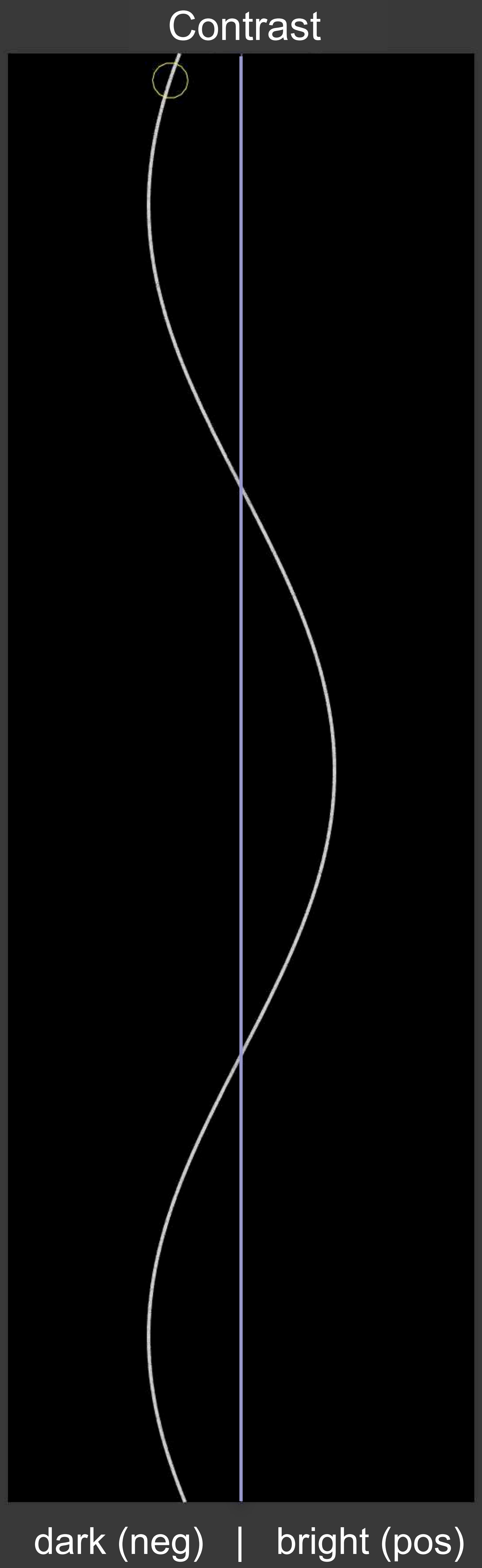


Intensity at z



The grating $\phi(x)$





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

Standard terminology

- Defocus values δ are positive for underfocus,

$$\delta = -z$$

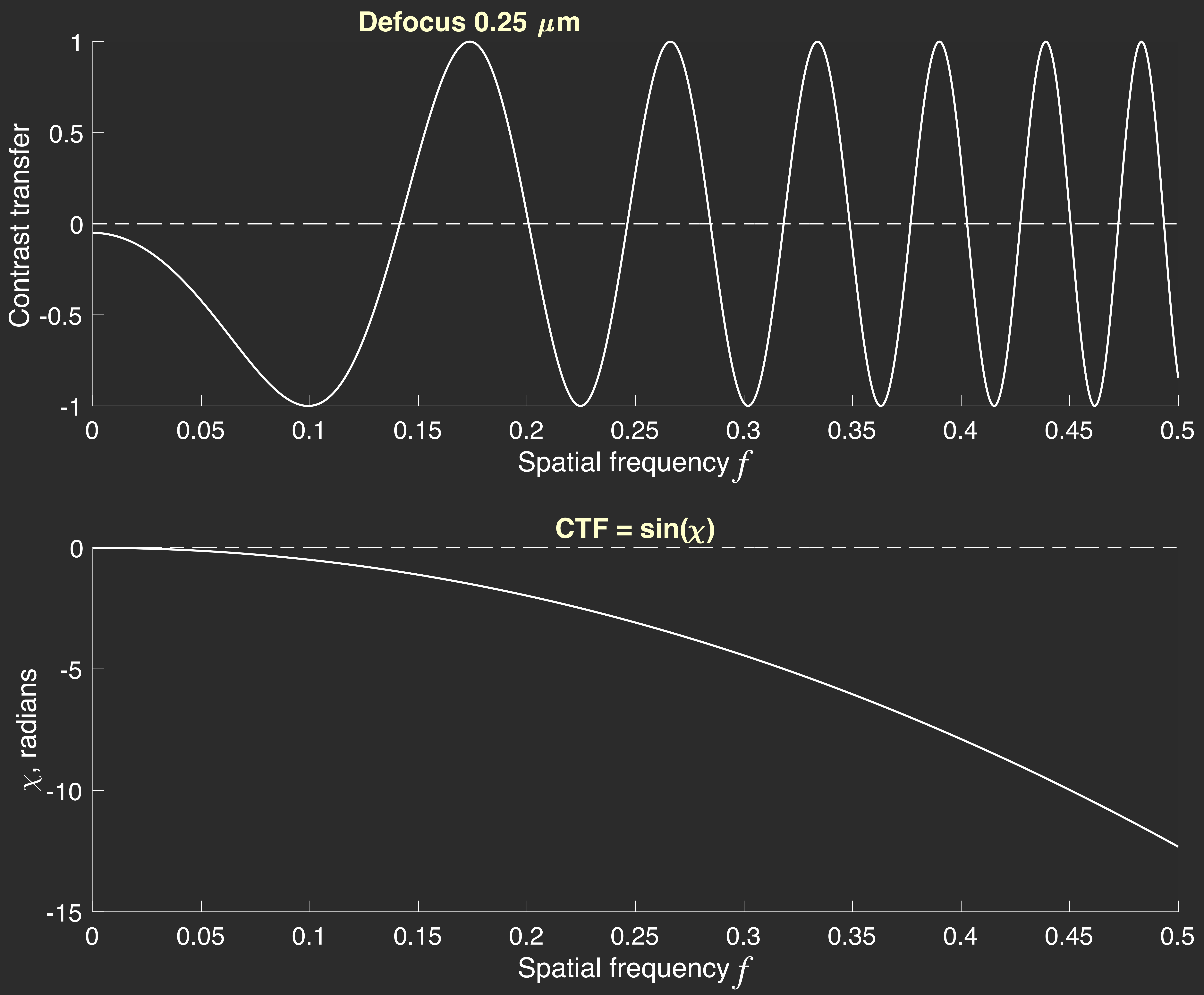
- Spatial frequency is

$$f = 1/d$$

So we can write the defocus phase contrast as:

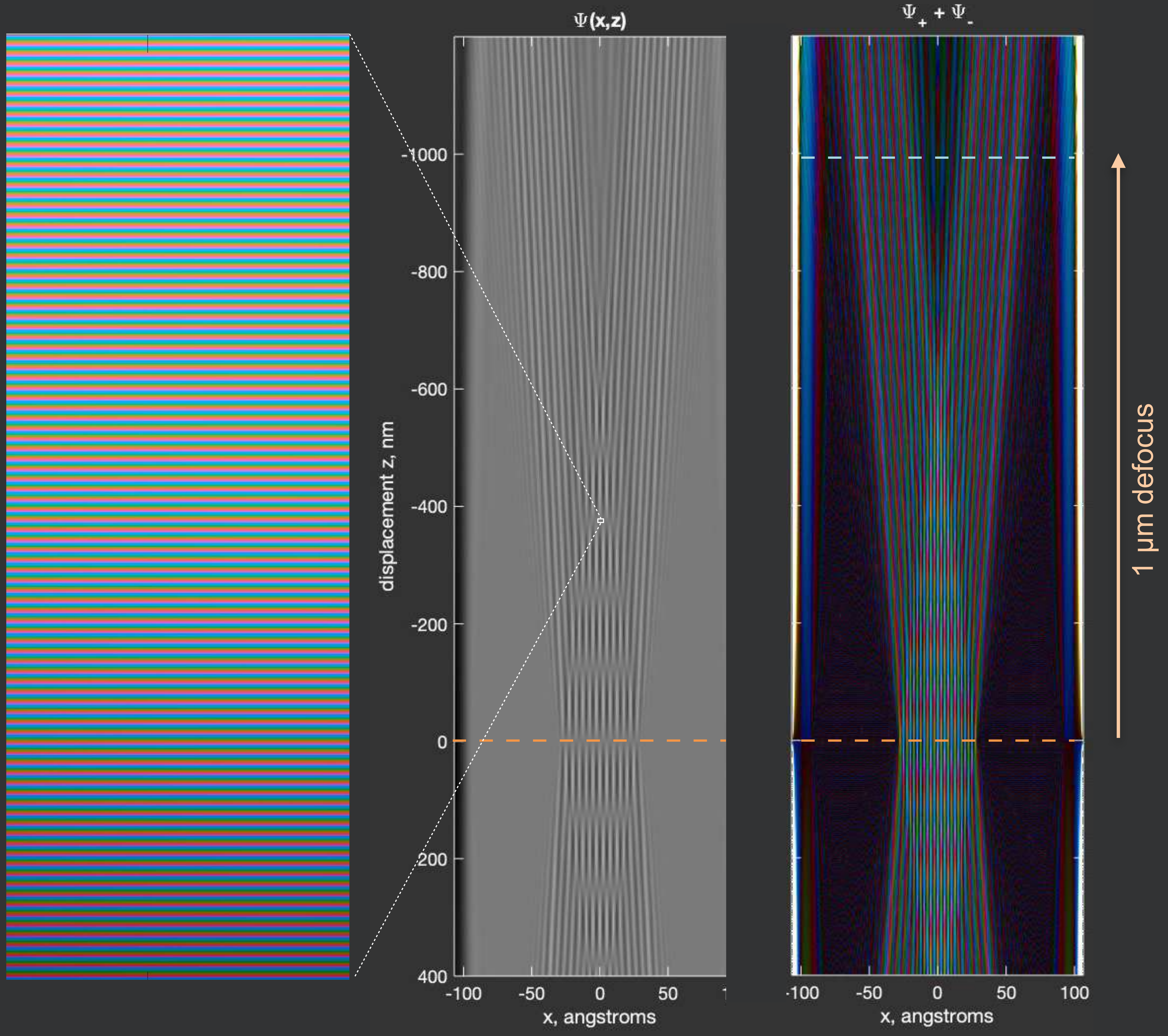
$$\text{CTF} = \sin(-\pi\lambda\delta f^2)$$

The contrast-transfer function as a function of f



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

A little defocus is actually a long distance

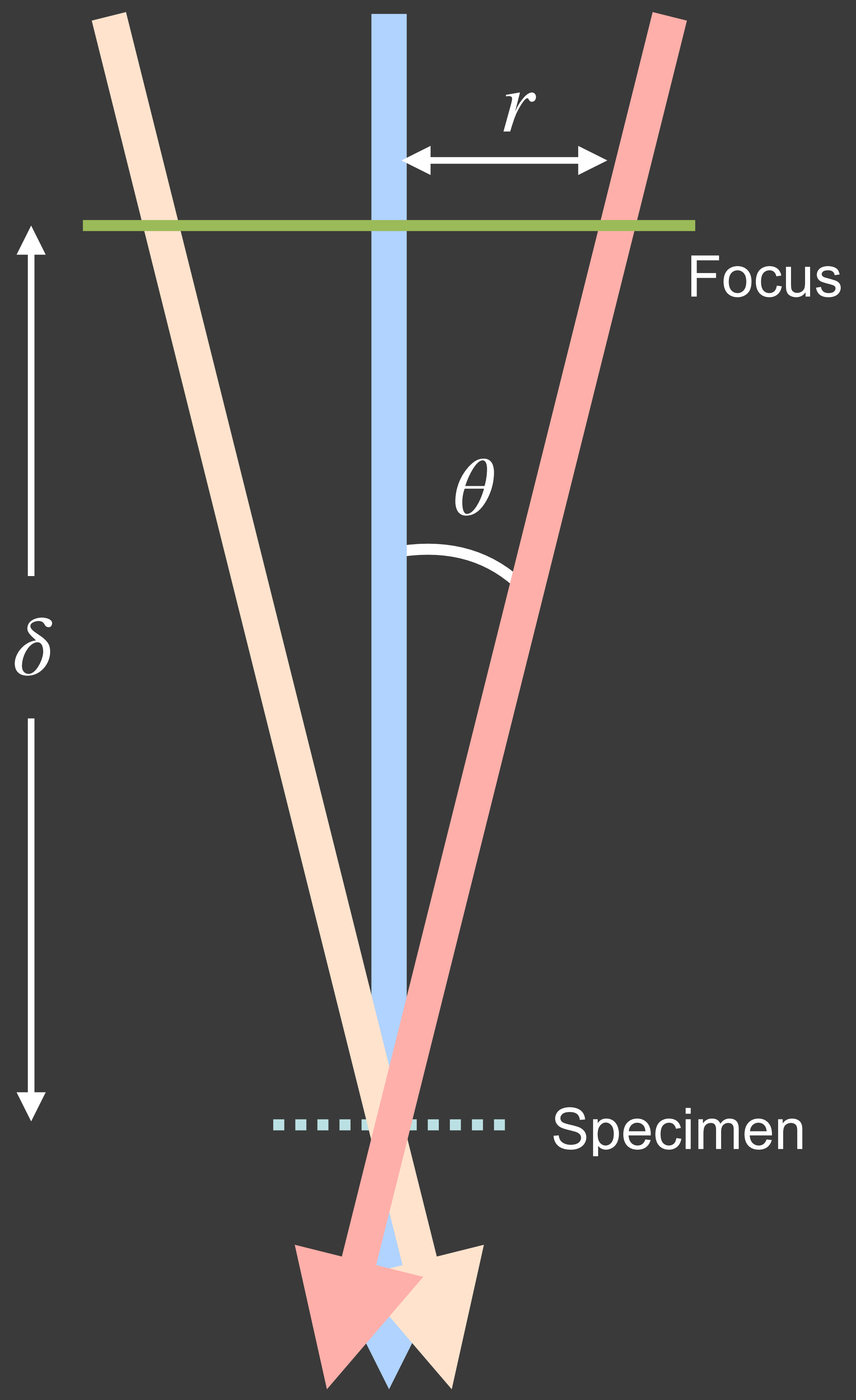


1 µm—a small defocus for cryo-EM imaging is 500,000 wavelengths!

This has ramifications regarding

- beam coherence
- specimen charging
- delocalization

With large defocus, how bad is the image delocalization?

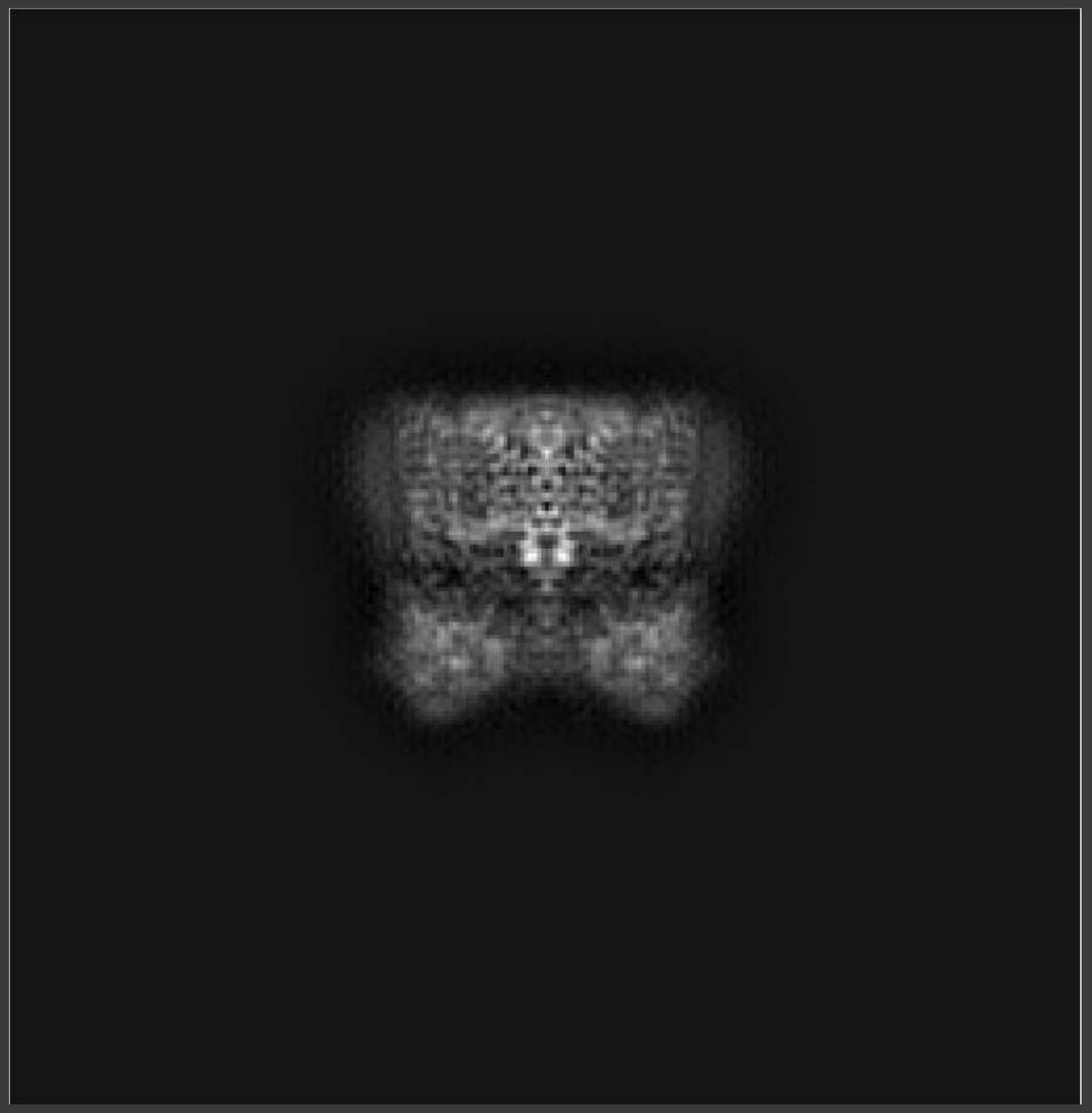


The dispersion radius is given by

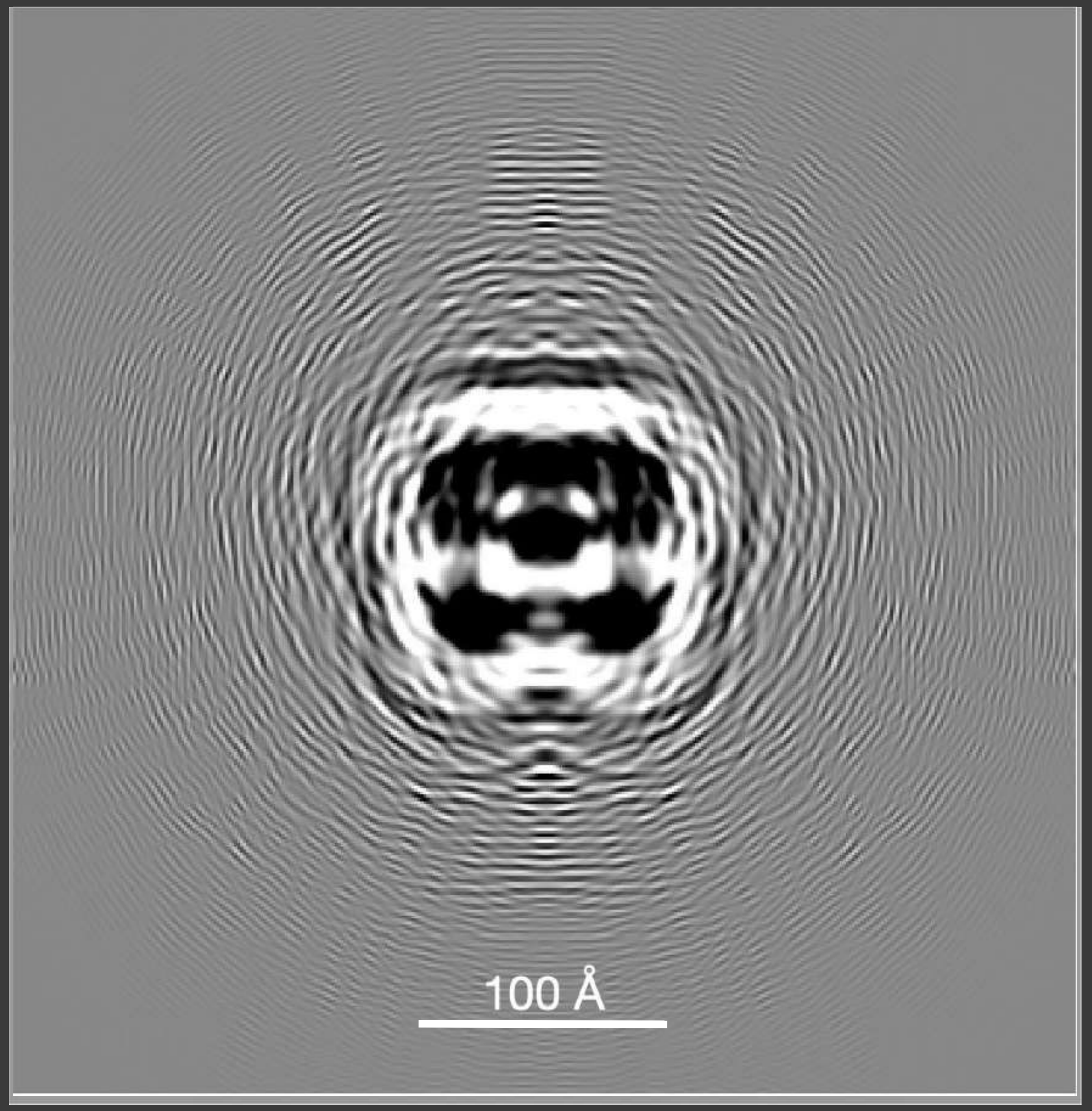
$$r = \delta \tan\theta$$
$$= \delta\lambda f \quad \text{(small angle approx.)}$$

Homework problem:

- How big a box do I need around my particle to include all the information up to 3Å, if I use 3μm of defocus?
- How big a box would I need for 1.5μm of defocus?

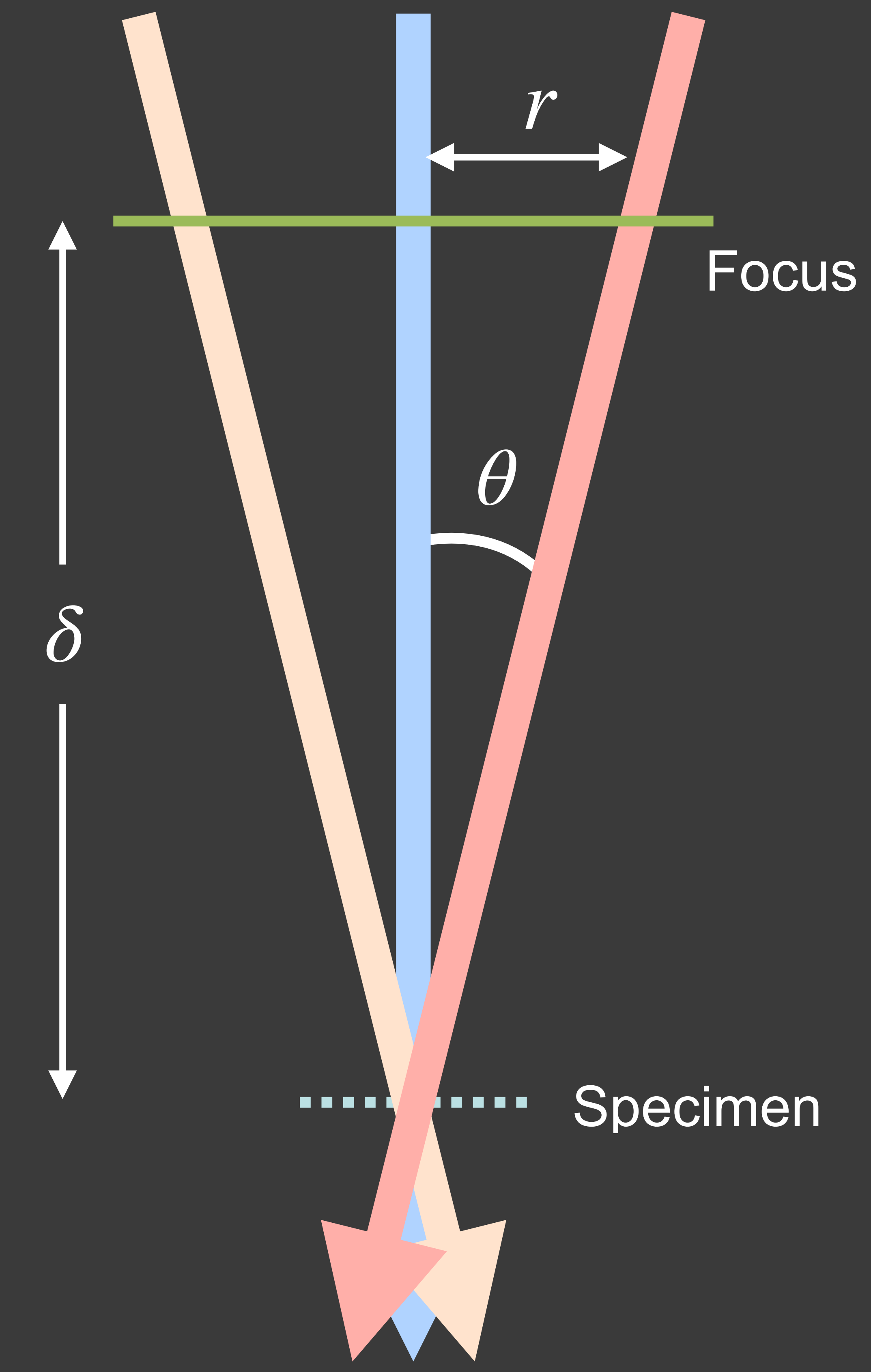


Object



3 μm defocus

With large defocus, how bad is the image delocalization?



The dispersion radius is given by

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

For example at 3μm defocus and 3Å resolution

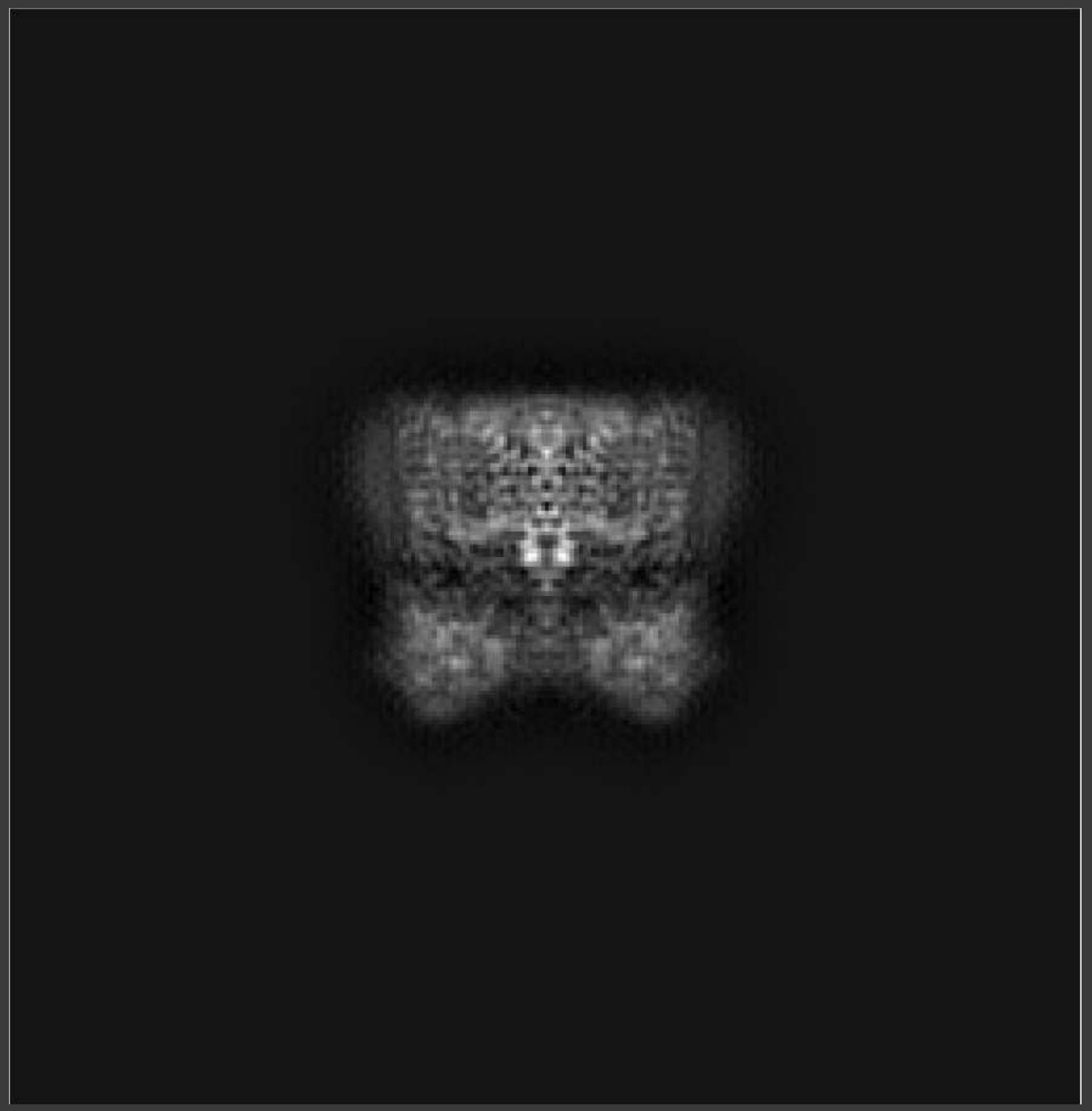
$$\delta = 3 \times 10^4 \text{Å}$$
$$\lambda = .02\text{Å}$$
$$f = 0.33\text{Å}^{-1}$$

then

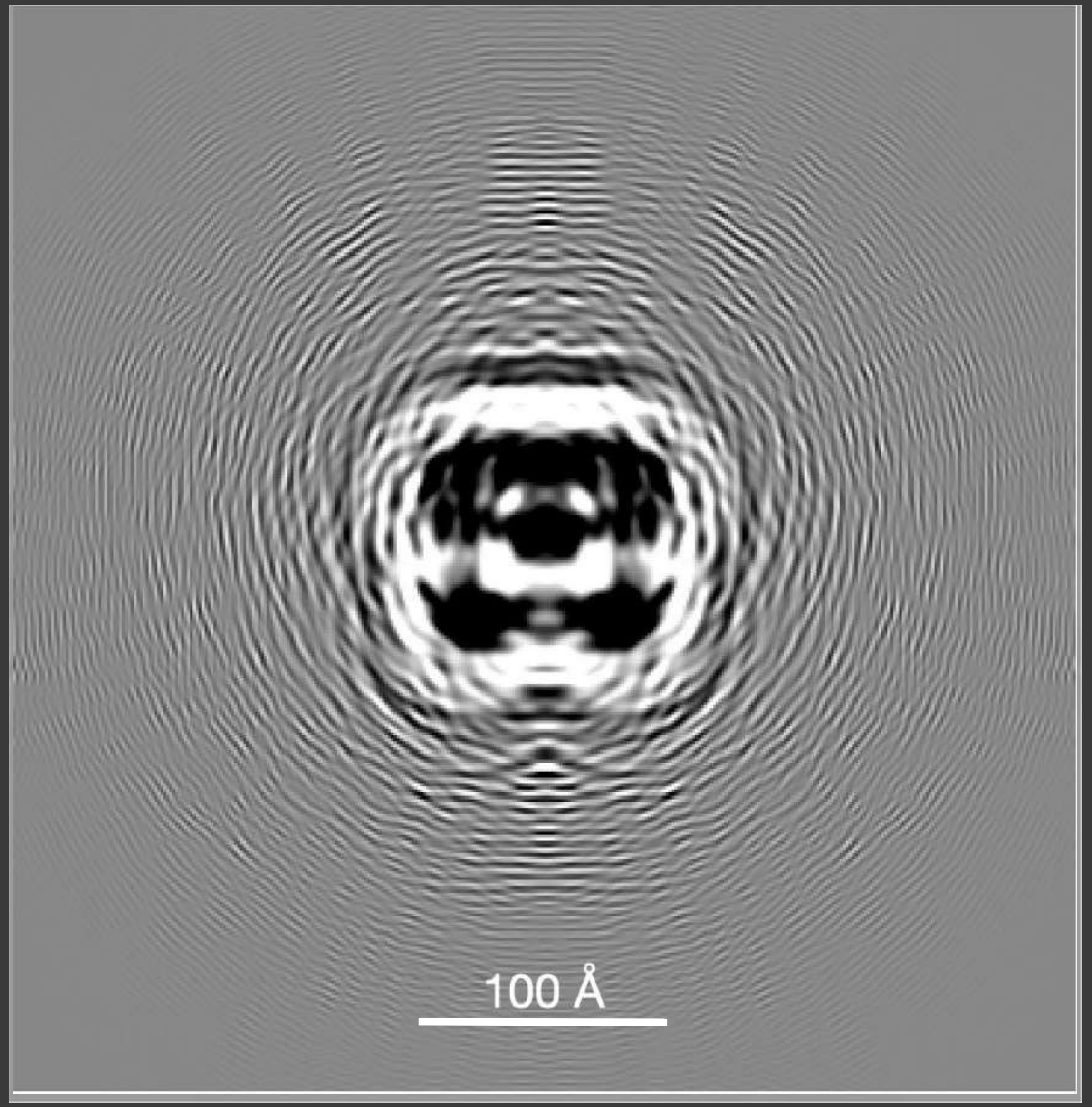
$$r = 200\text{Å}$$

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

*Note: beyond about 3Å, spherical aberration needs to be taken into account too.



Object



3 μm defocus

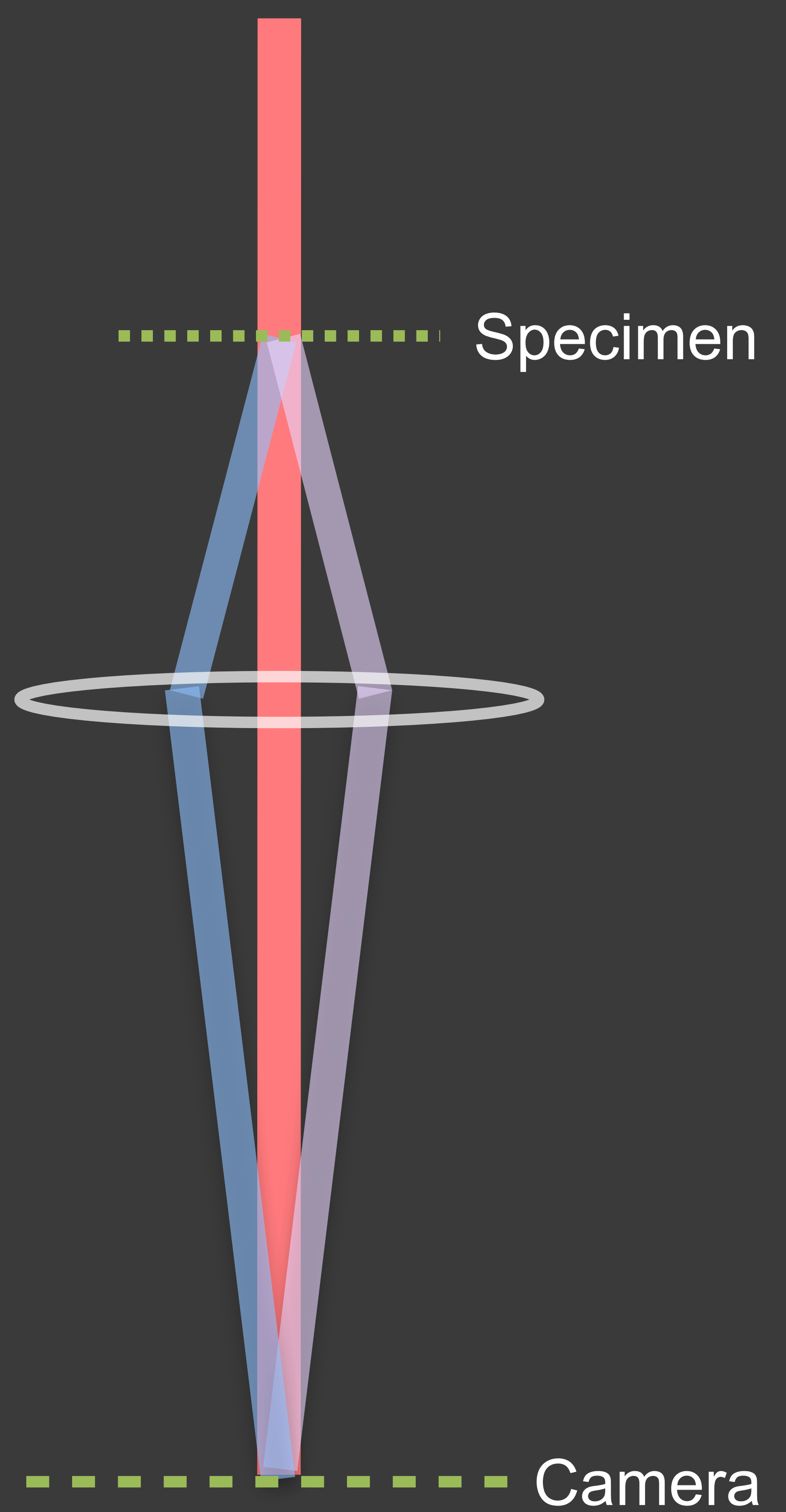
More details about the CTF

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

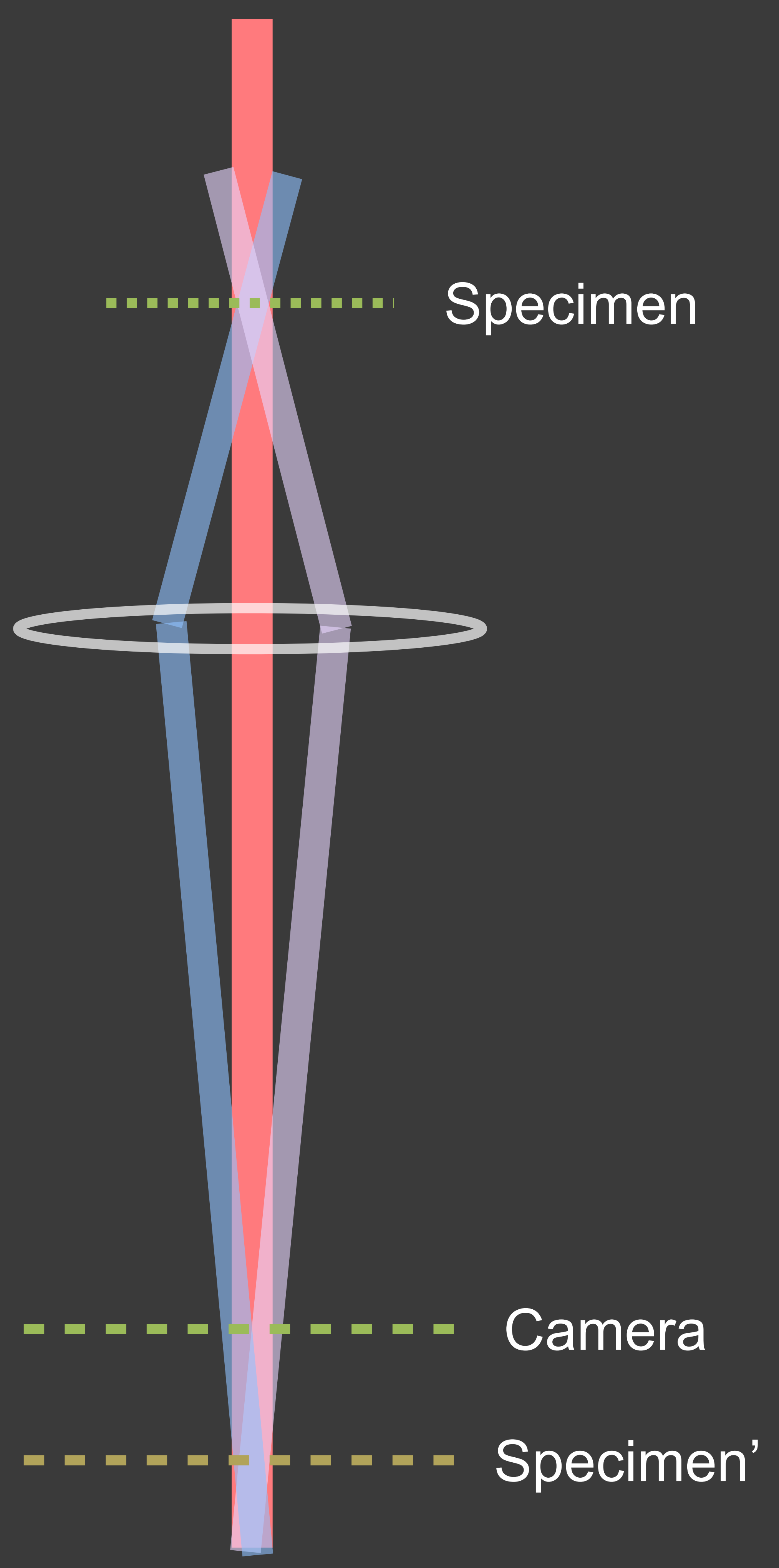
In focus

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

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

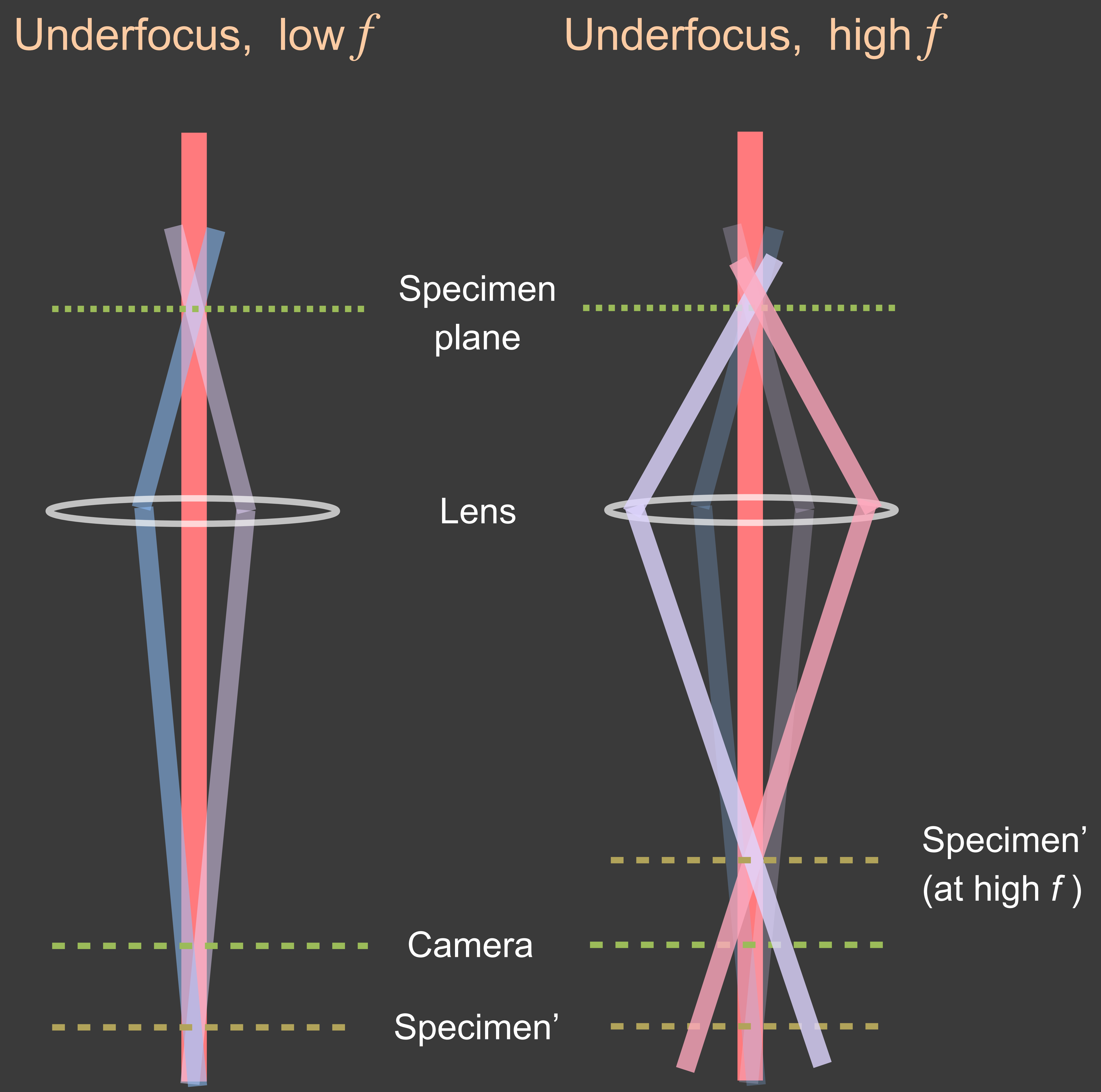
The contrast transfer function has a new term,

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

or, expanded,

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

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

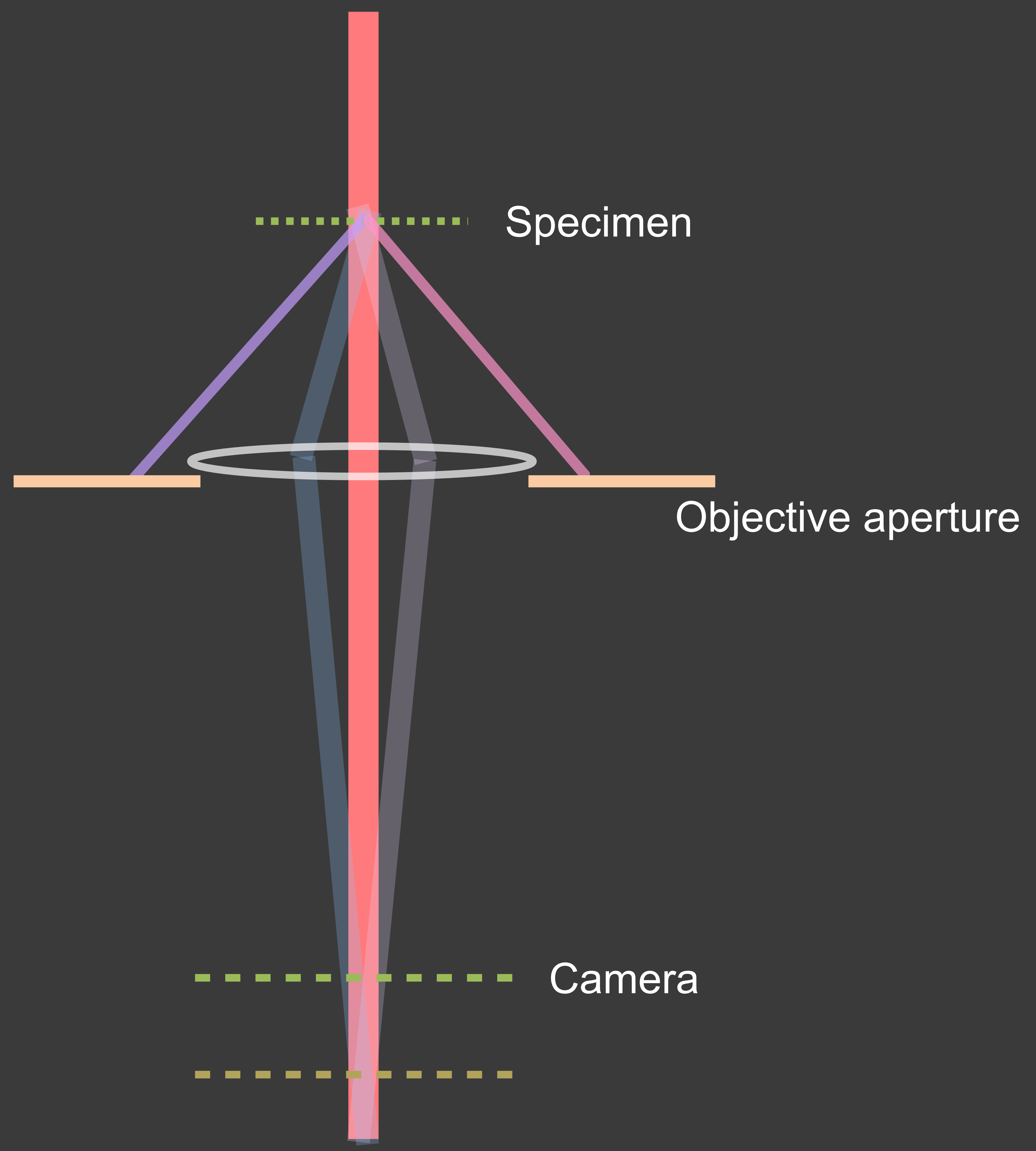


Also, high-angle scattering yields a small amplitude contrast

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.

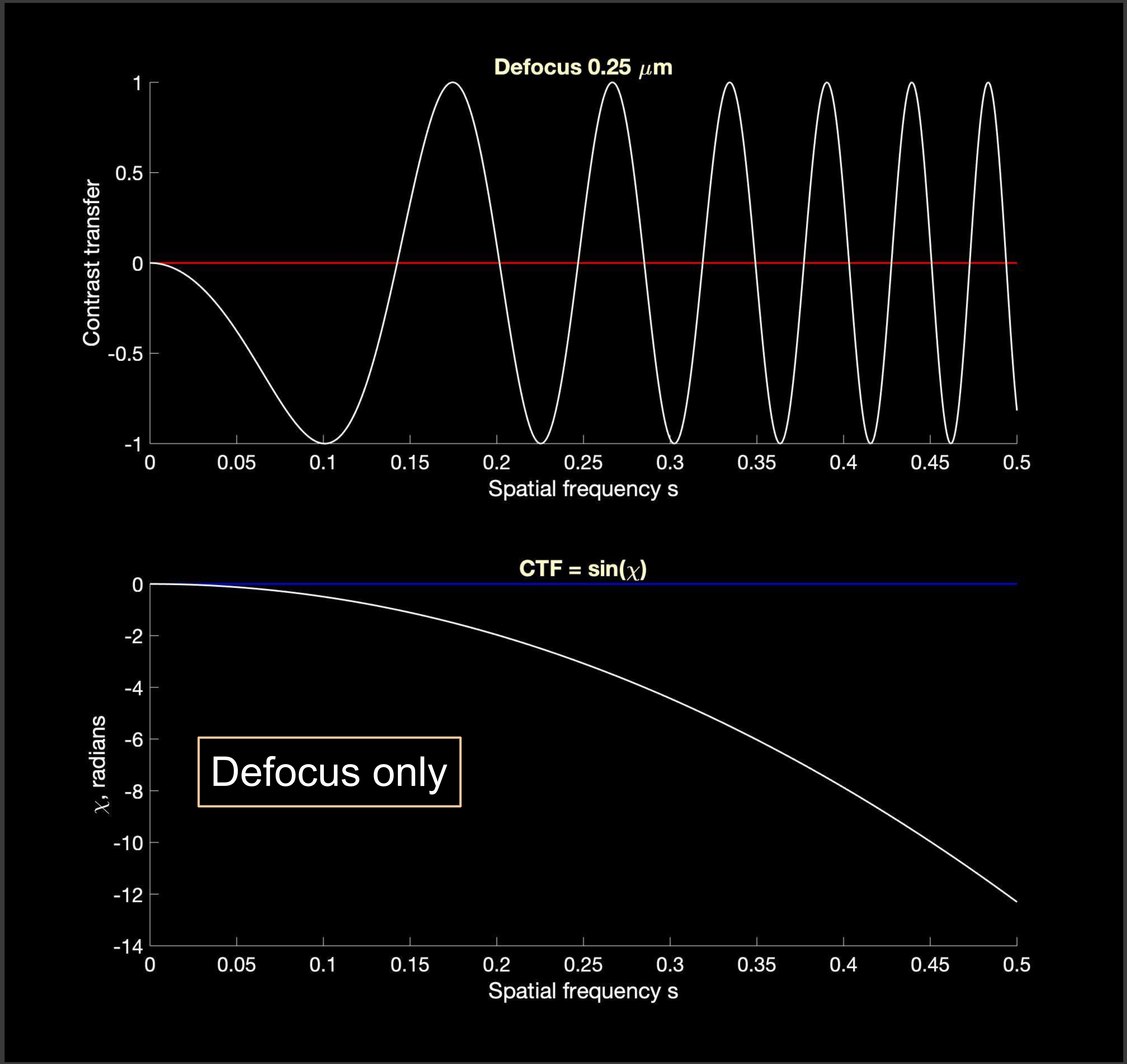


The simple defocus contrast is what we've seen before

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

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

defocus sphere abb. amplitude



Now adding in spherical aberration and amplitude contrast

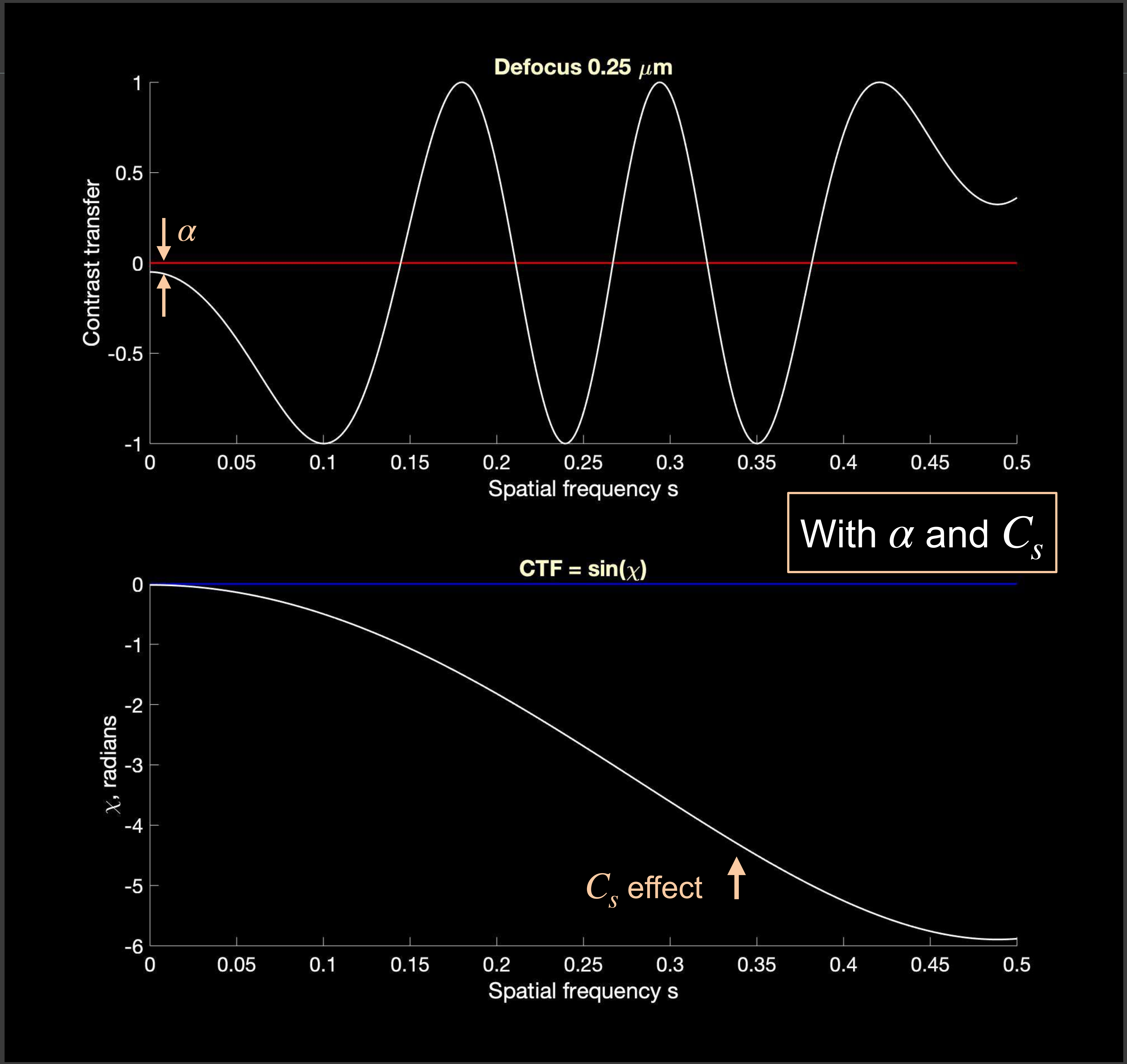
Here you can see why everyone uses underfocus: the amplitude contrast and defocus contrast are additive in this case.

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

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

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

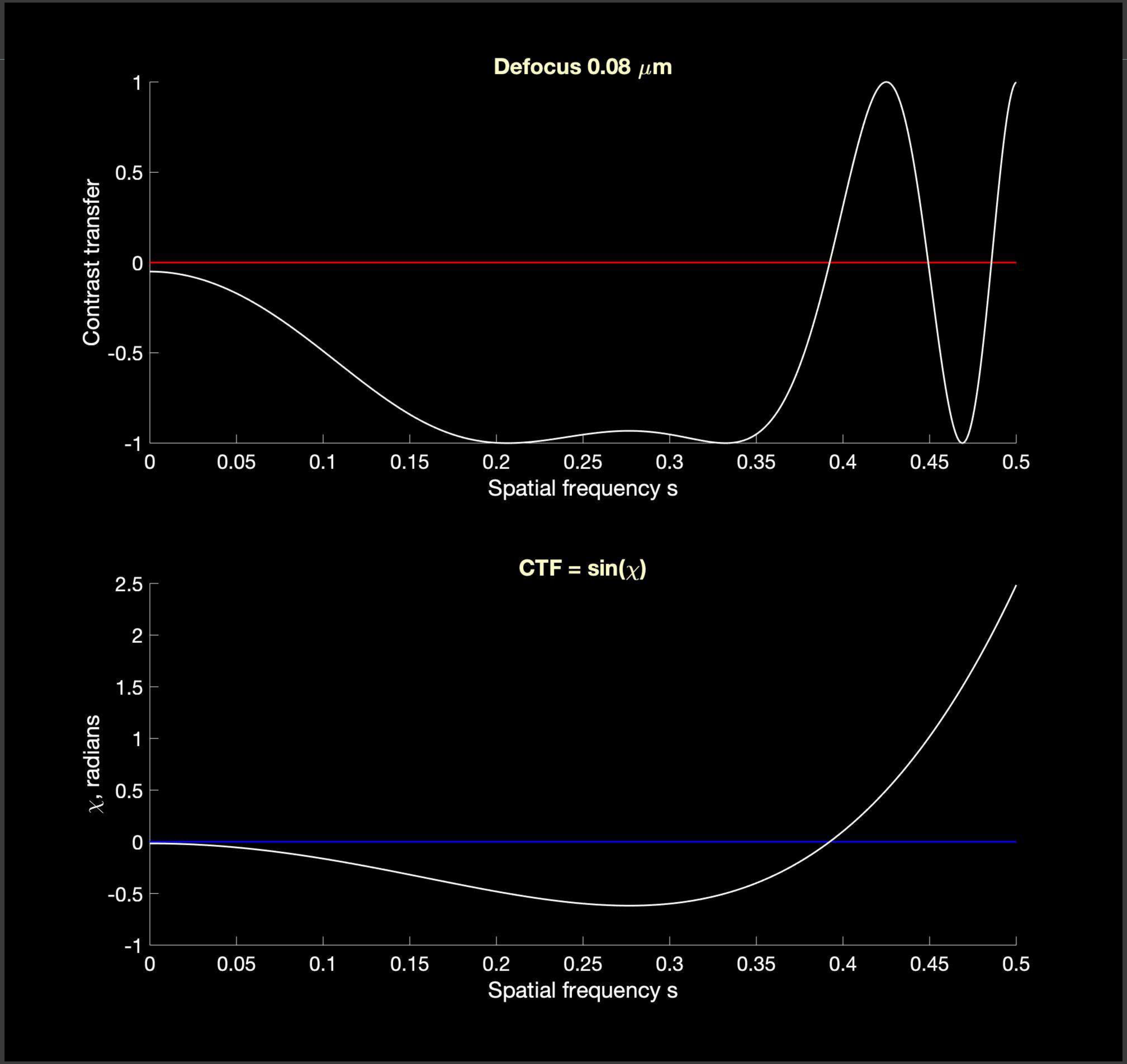
defocus sphere abb. amplitude



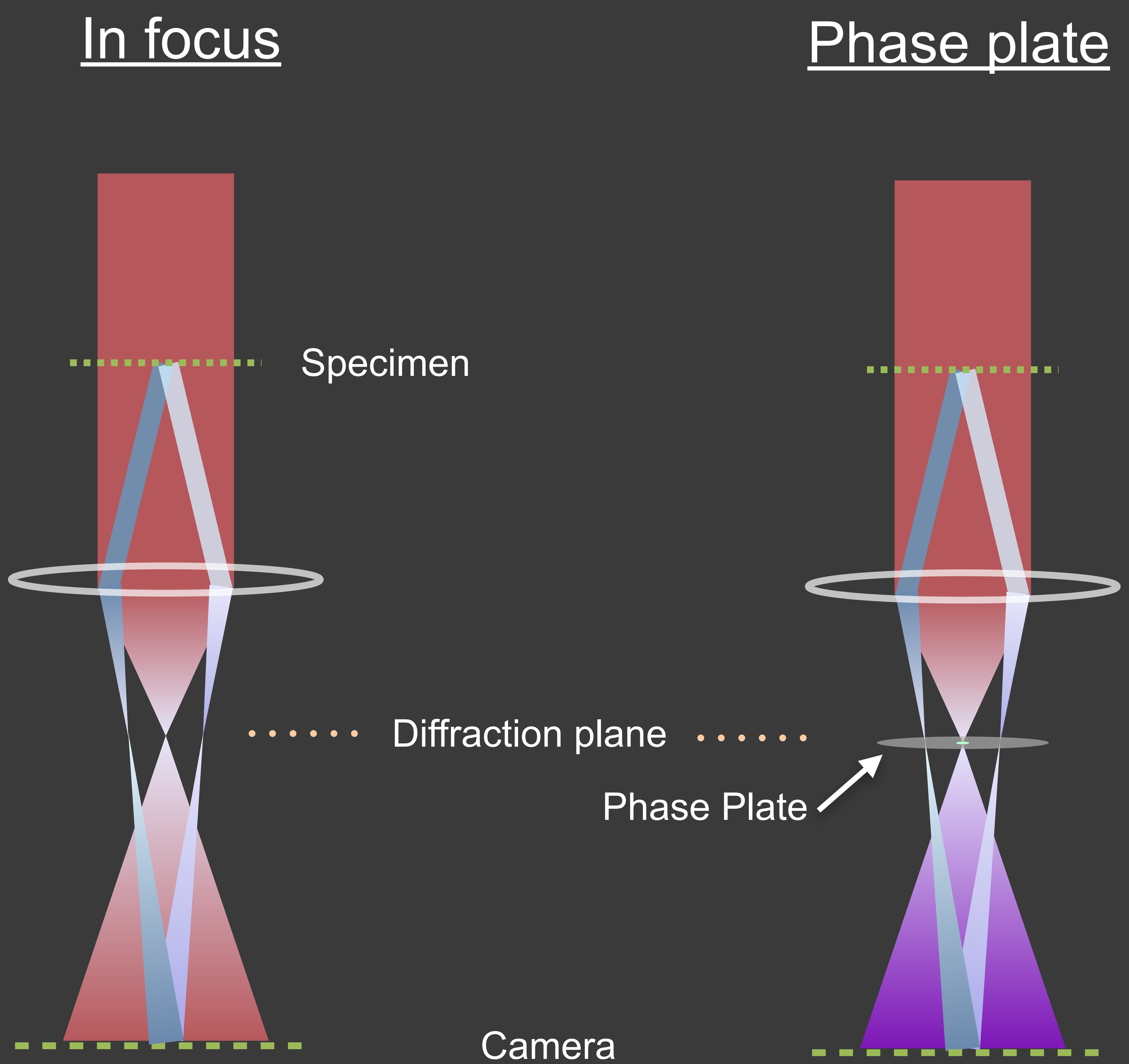
Spherical aberration can be our friend

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



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

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

Homework: See if you can derive this.

If $\phi = 90^o$ 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

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

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

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

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

$$\text{CTF} = \sin(\chi)$$

$$= \sin(\pi\lambda z/d^2)$$

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

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

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

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

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

Cryo-EM single particle analysis with the Volta phase plate

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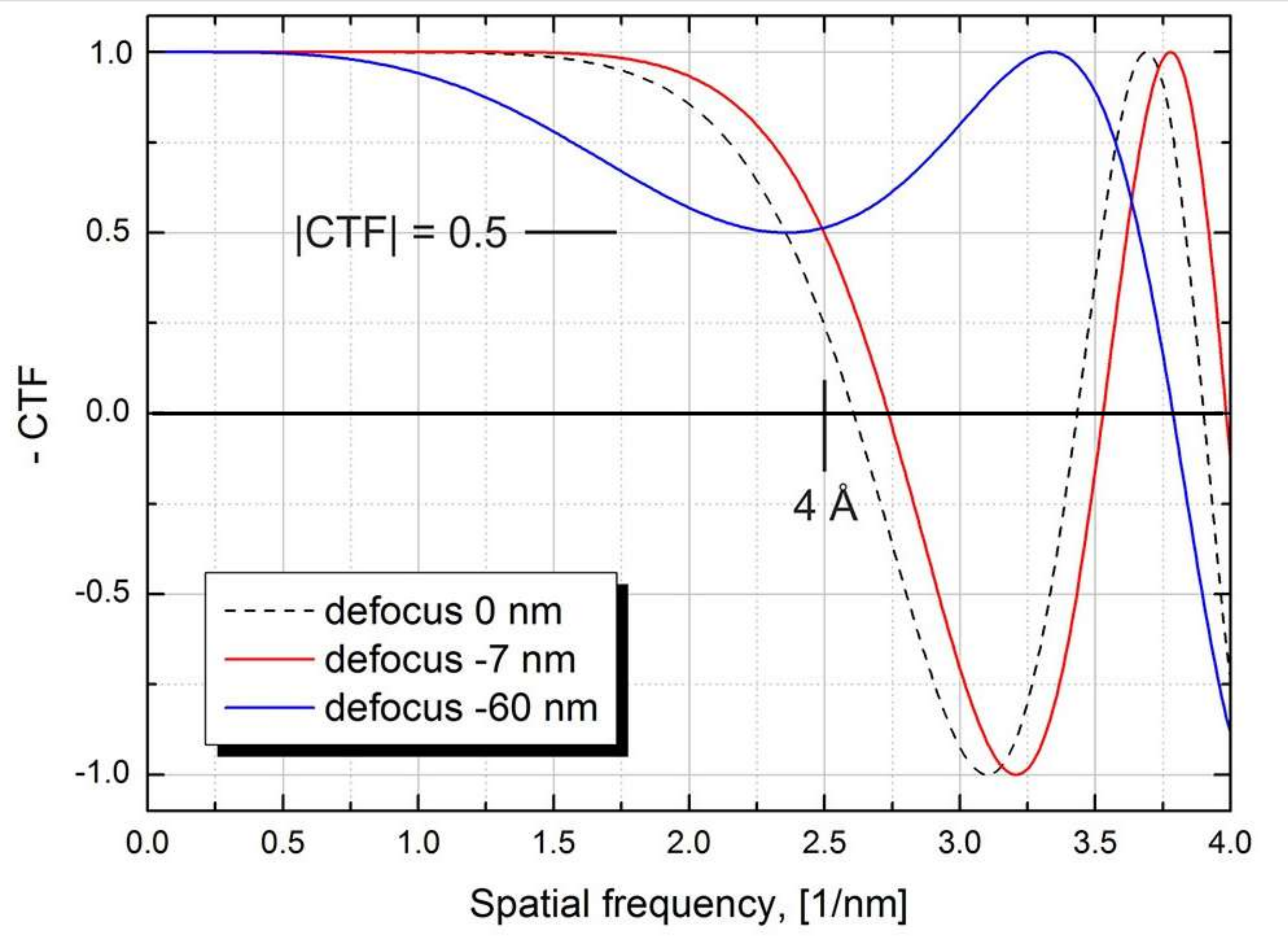
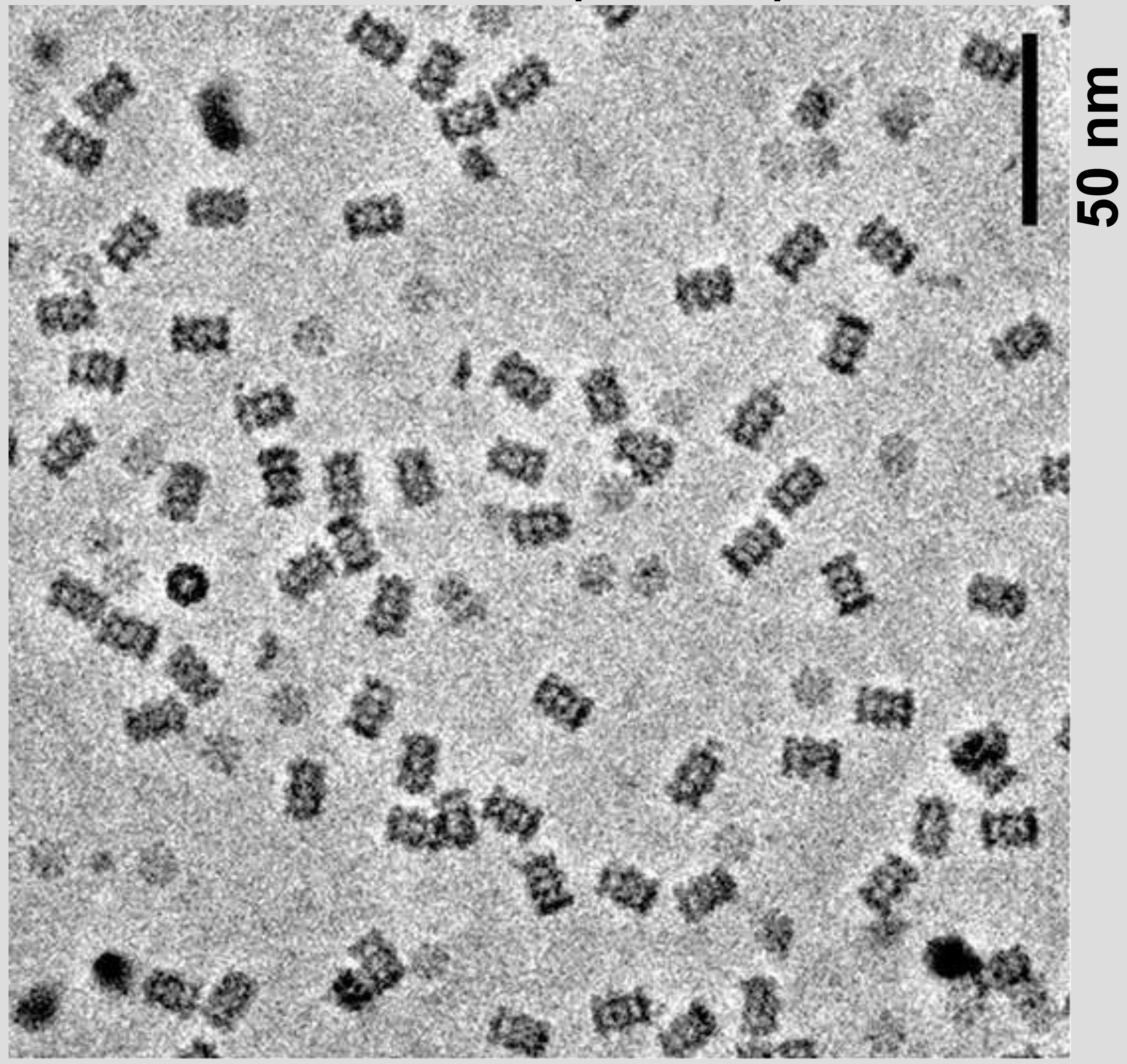
eLife 2016

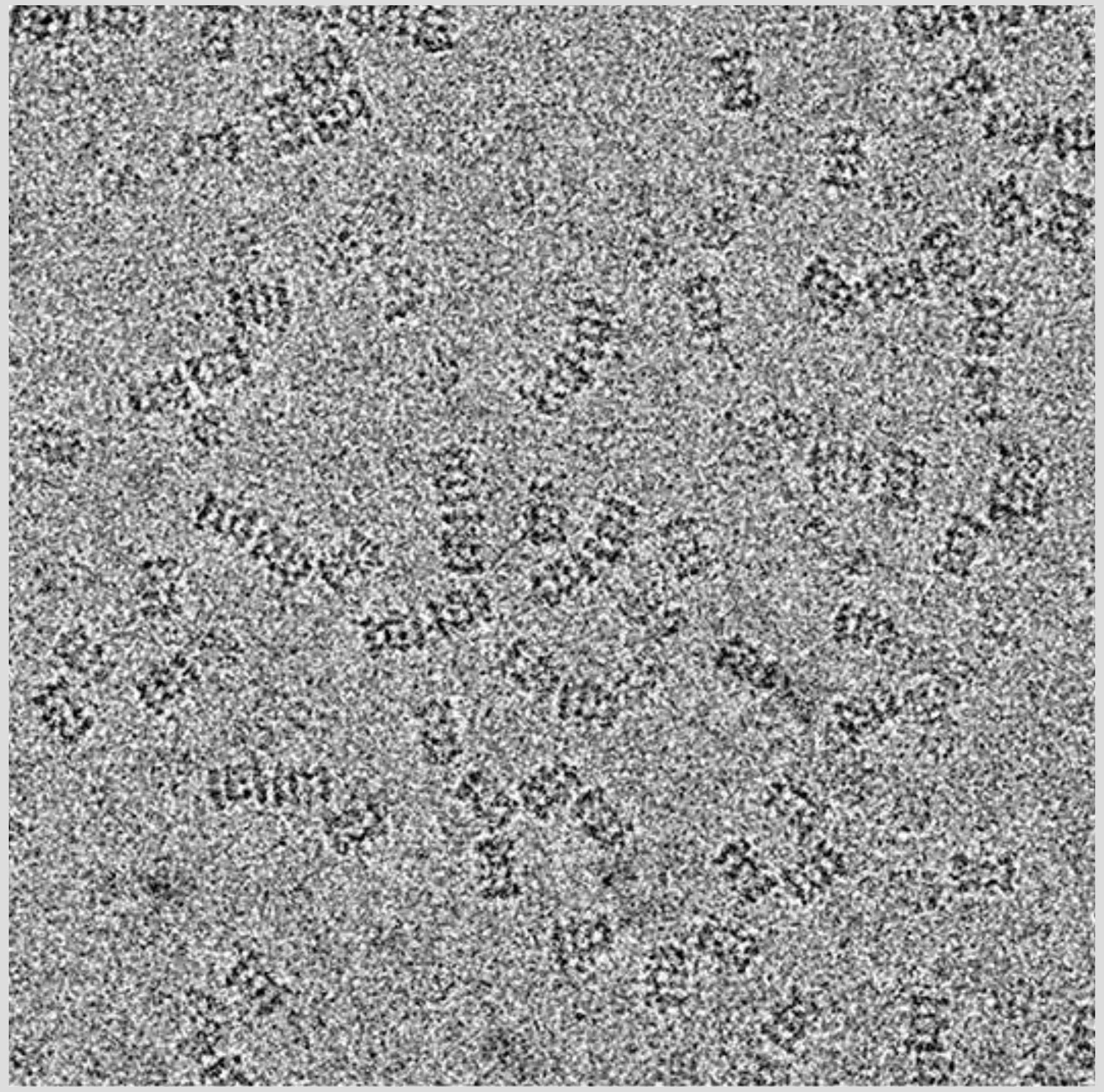


- The defocus value must be precise within 60 nm in order to get 4 Å resolution.
- The better low-frequency contrast makes particles much more visible.

In-focus phase plate

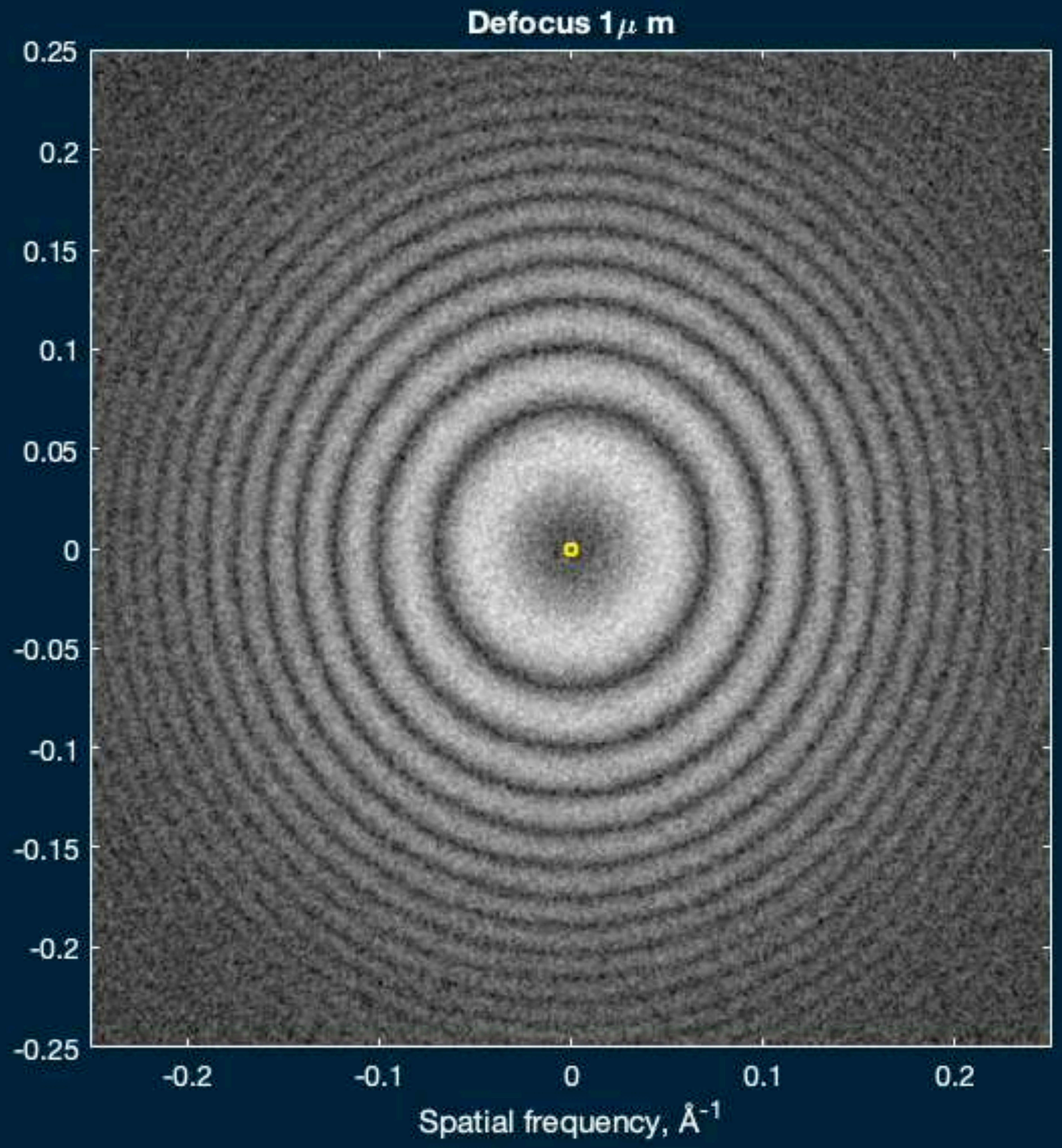


Defocus contrast



The power spectrum describes the magnitude of Fourier components

Power spectrum



Grating at the spatial frequency