# Algorithms and Foundational Math

## Part 1a

# Lecture 1a

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

# Why complex numbers?

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

# *i*, the imaginary unit

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

**A complex number**

Real part

$$z = a + ib$$

Imaginary part

# 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** $z^* = a - ib$

# The exponential function $e^x$

$$e = 2.718...$$

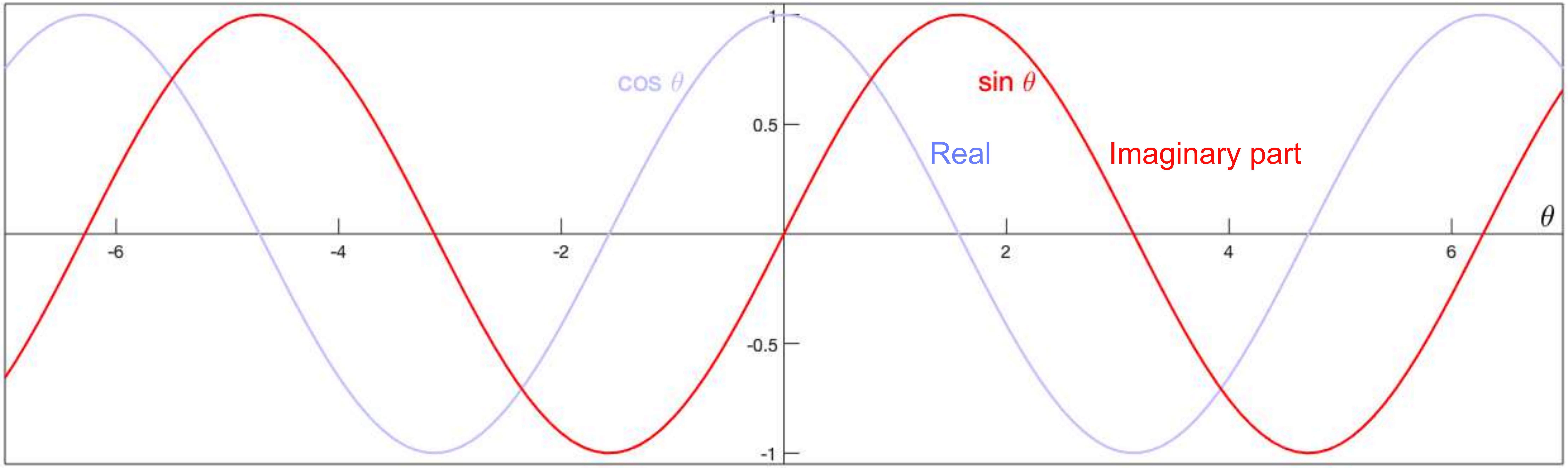
$$e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{2 \cdot 3} + \dots$$

A very important approximation, valid when $x \ll 1$, is

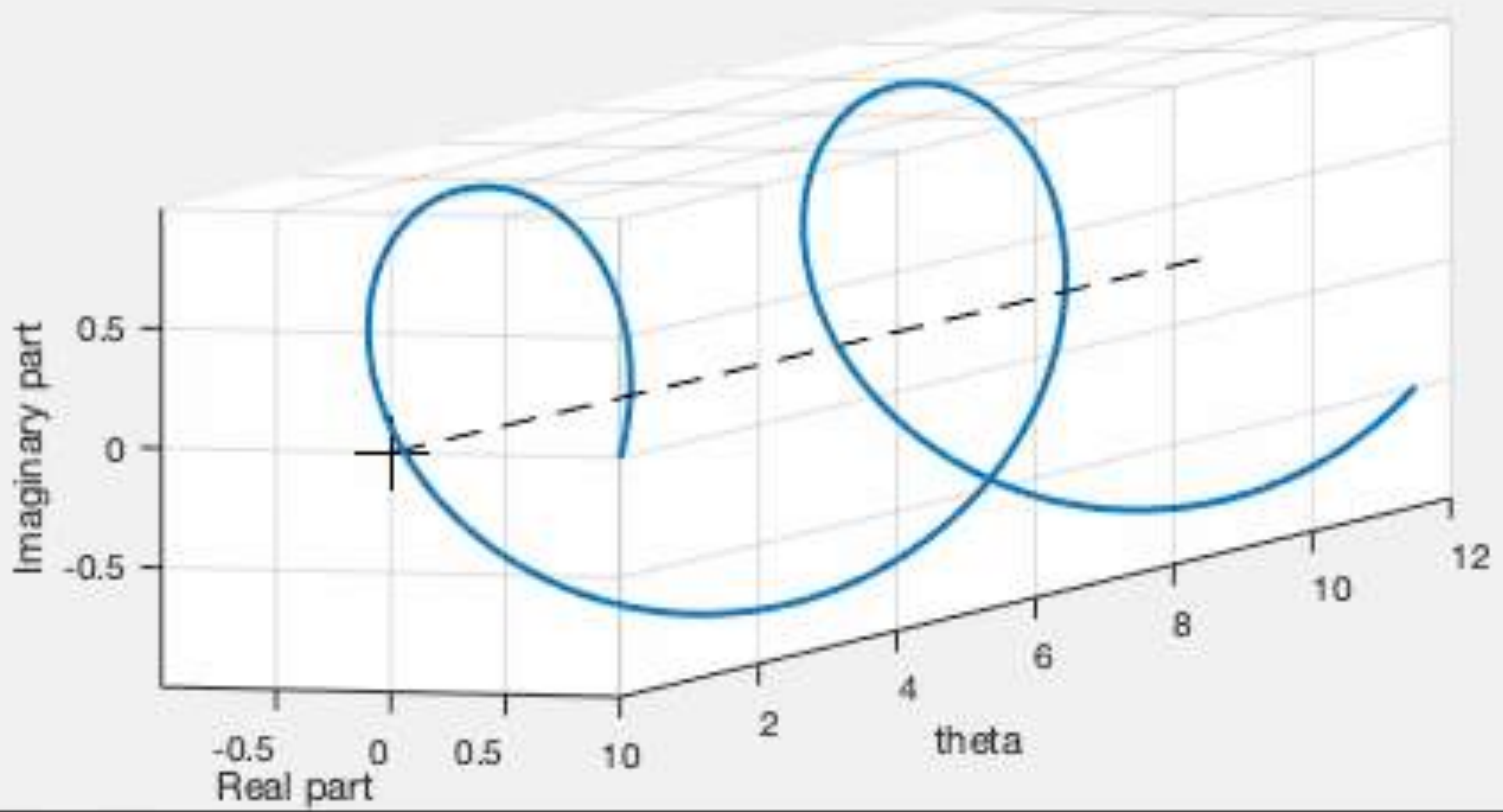
$$e^x \approx 1 + x$$

# The complex exponential $e^{i\theta}$

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



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



# Any $z$ can be represented as $(a, b)$ or as $(r, \theta)$

$$z = a + ib$$

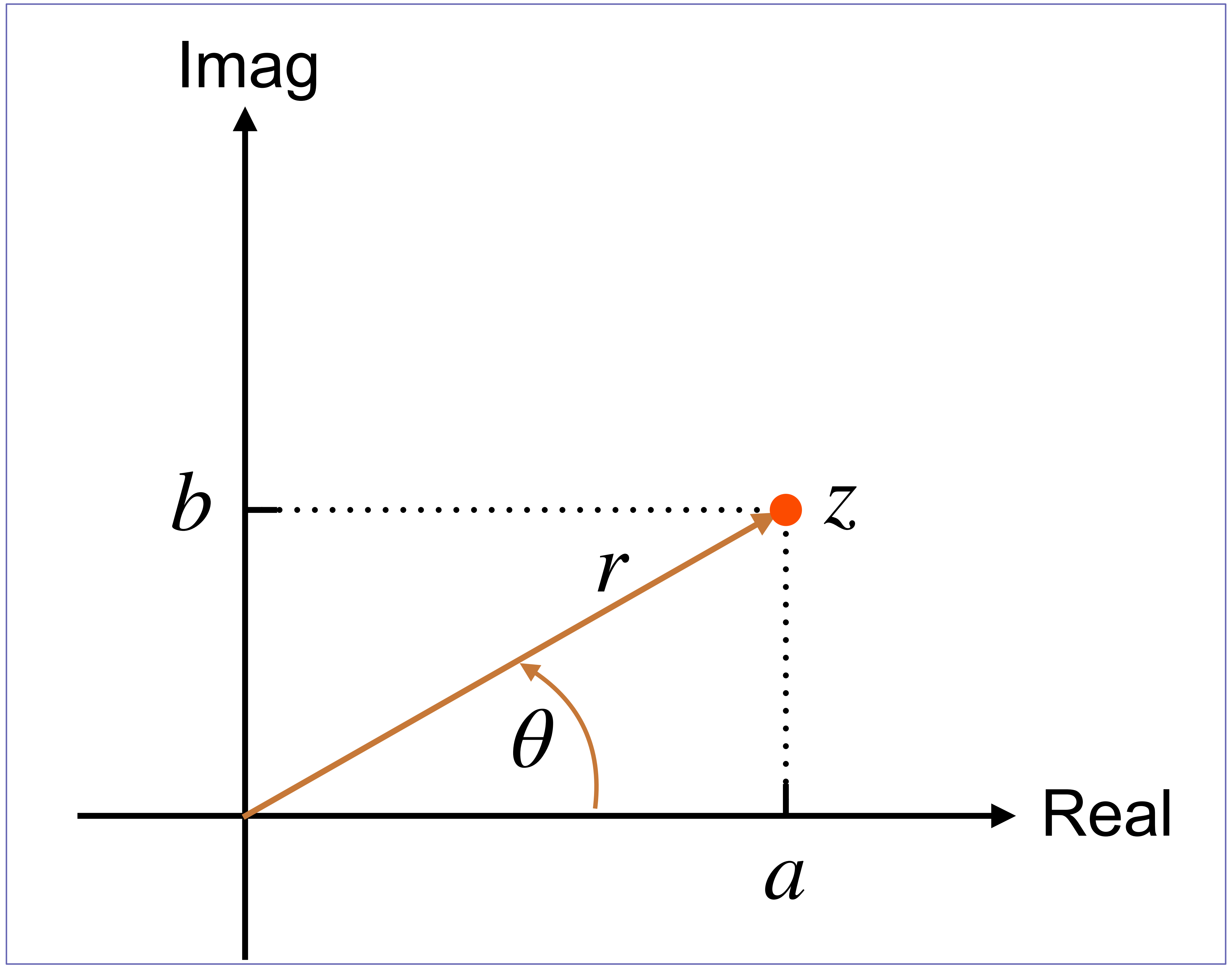
$a$ is the real part

$b$ is the imaginary part

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

$r$ is the magnitude

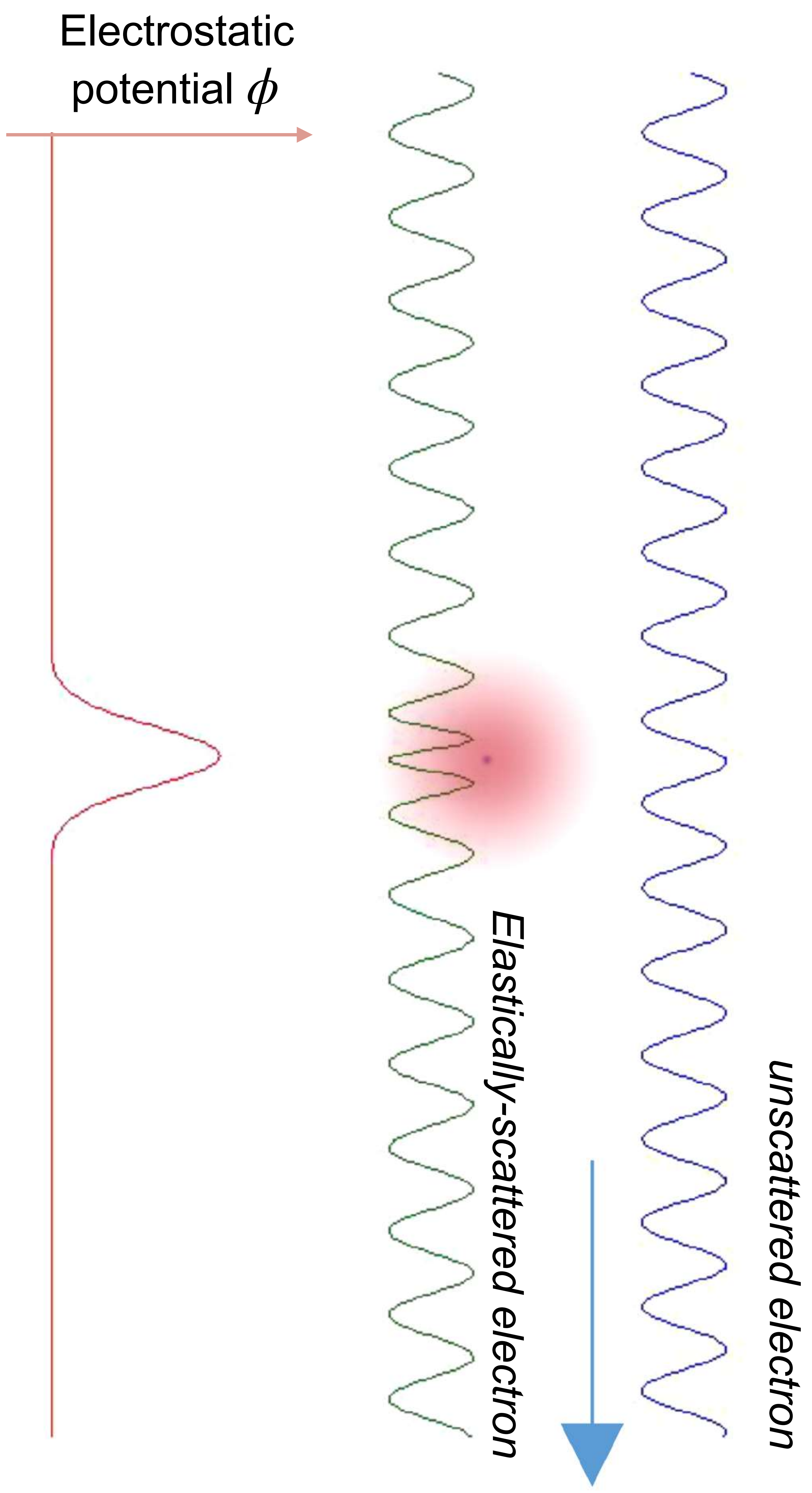
$\theta$ is the phase



# Lecture 4a

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

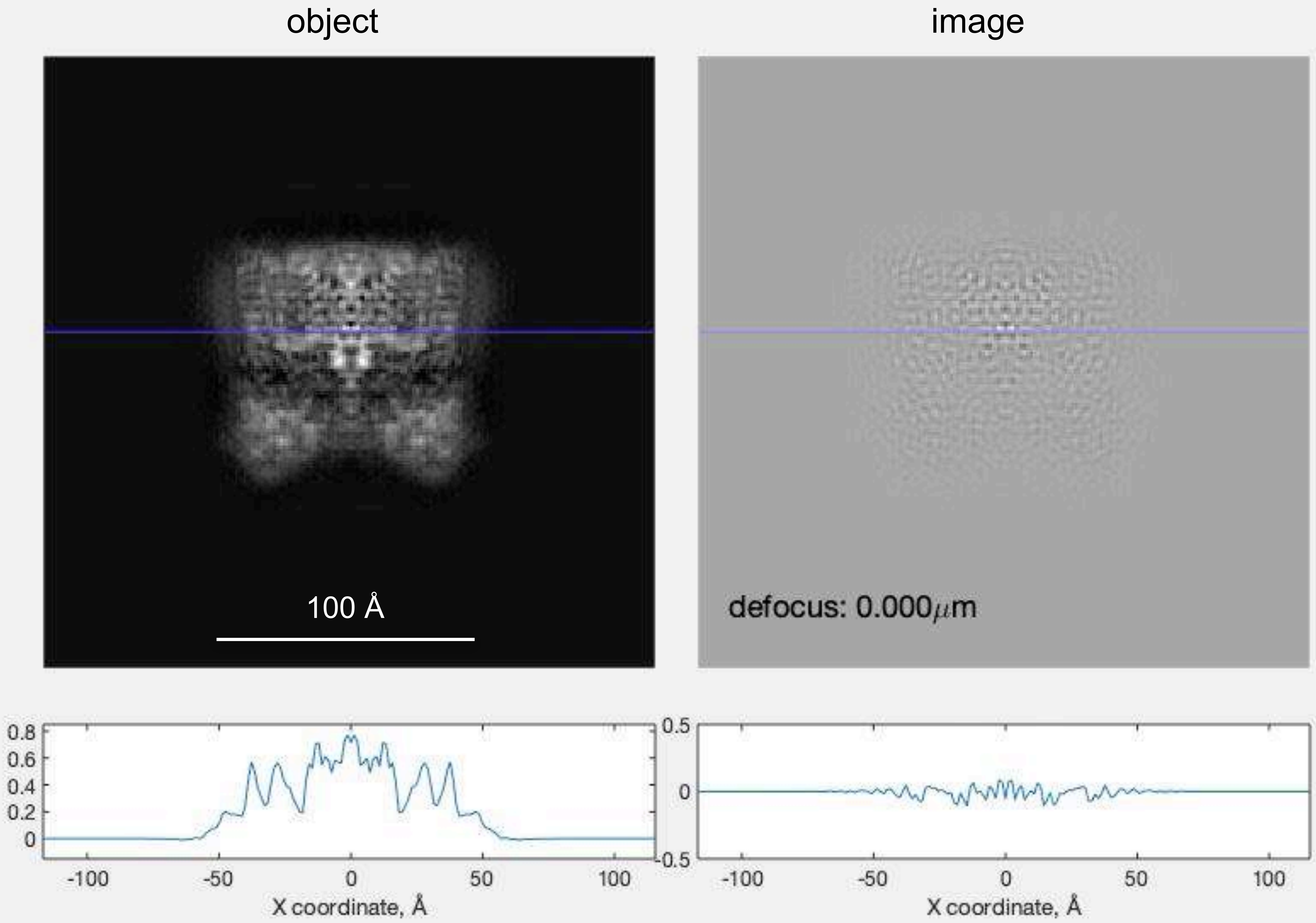
# Cryo-EM specimens are imaged by phase contrast




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

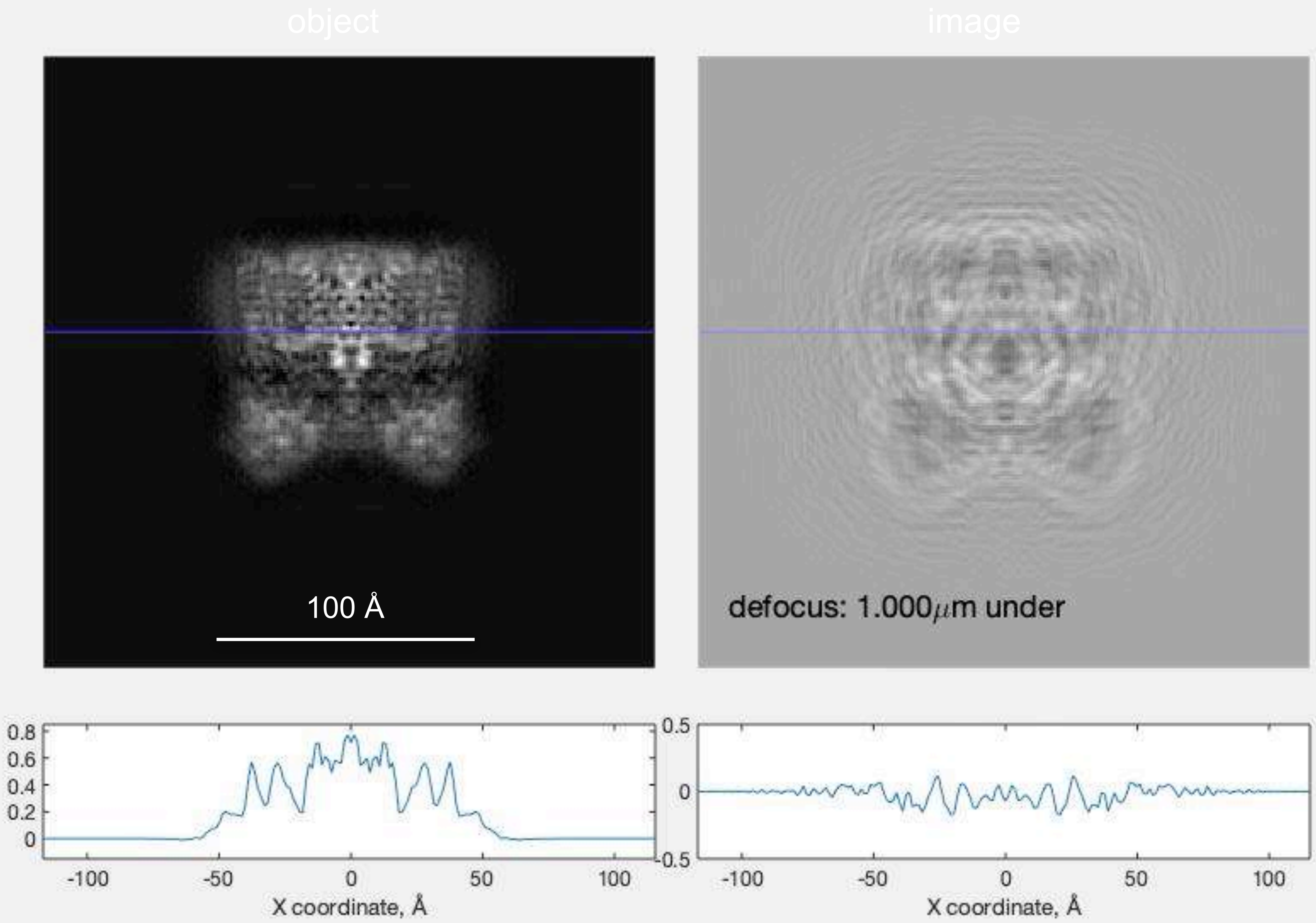
The phase shift coefficient $\sigma$ is about 0.5 milliradian per volt-angstrom of integrated potential.
The phase shift near a single atom is ~1 milliradian.

# Most cryo-EM data are acquired using defocus contrast



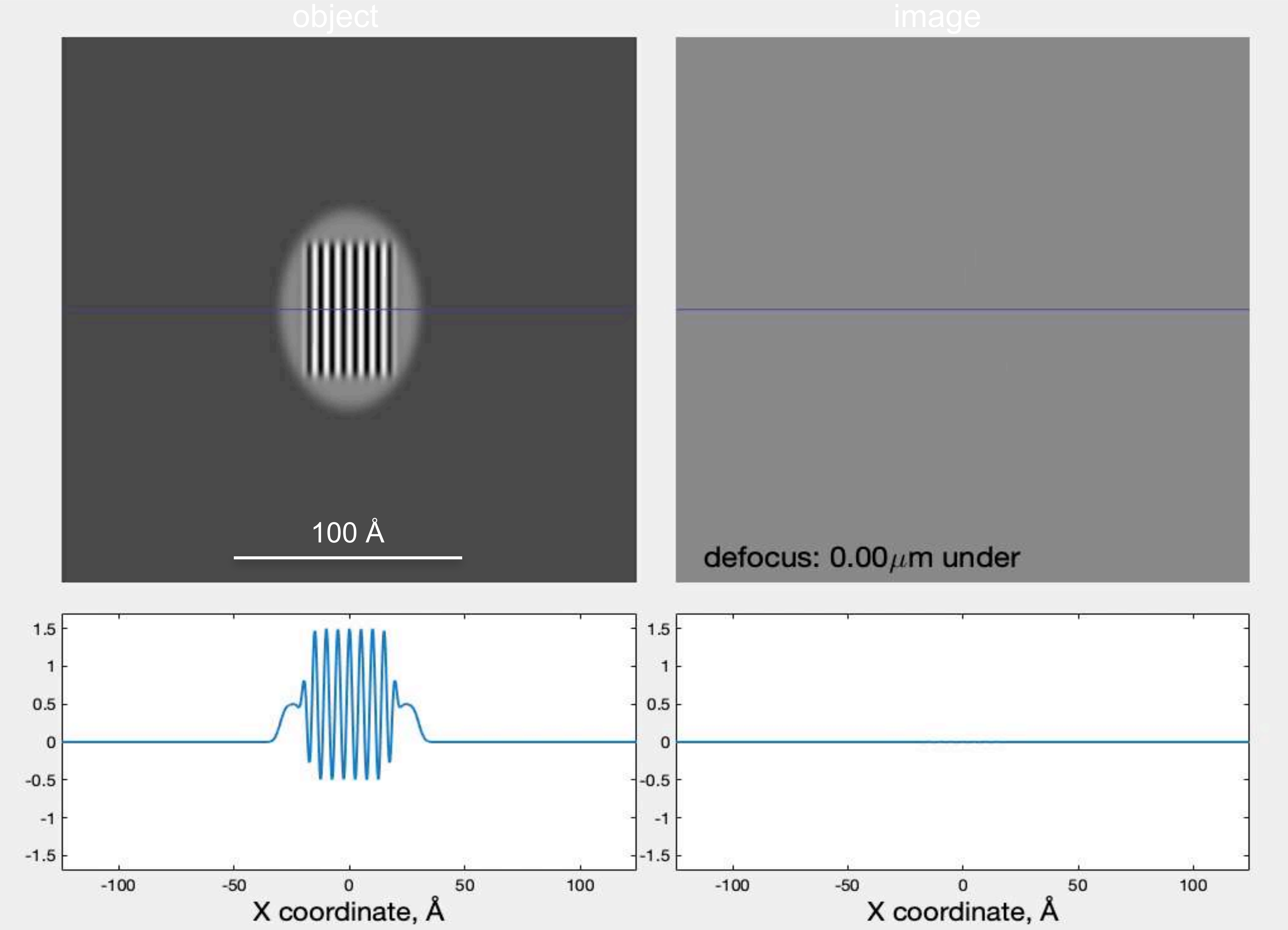

- Defocus values are always "underfocus". This means decreasing the strength of the objective lens, effectively focusing above the specimen.
- At high defocus, high-resolution information in the image is strongly delocalized.

# Most cryo-EM data are acquired using defocus contrast




- 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

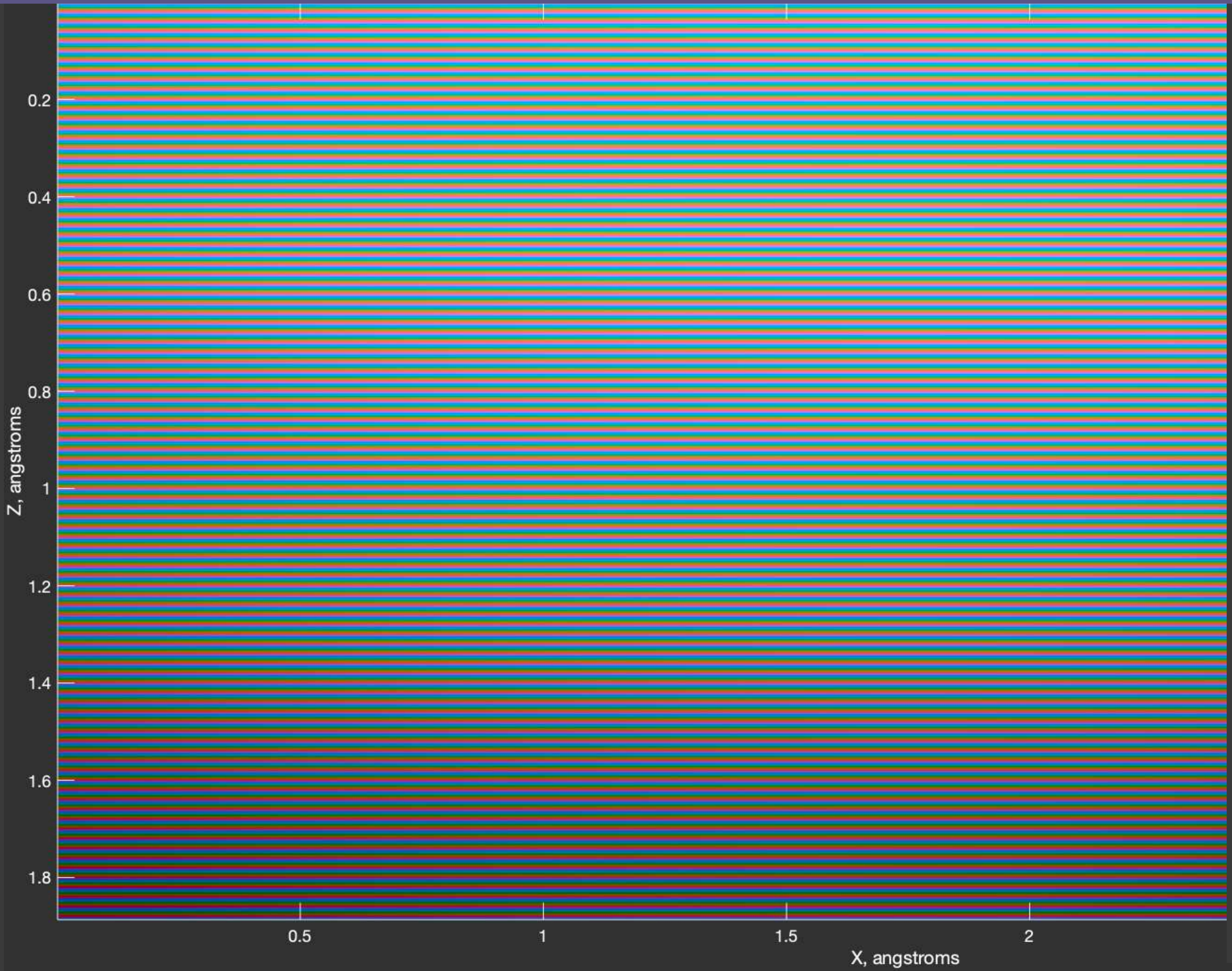



- Defocus values are always "underfocus". This means decreasing the strength of the objective lens, effectively focusing above the specimen.
- At high defocus, high-resolution information in the image is strongly delocalized.
- Image processing can re-localize the signals, but at most only about half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

# 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 |

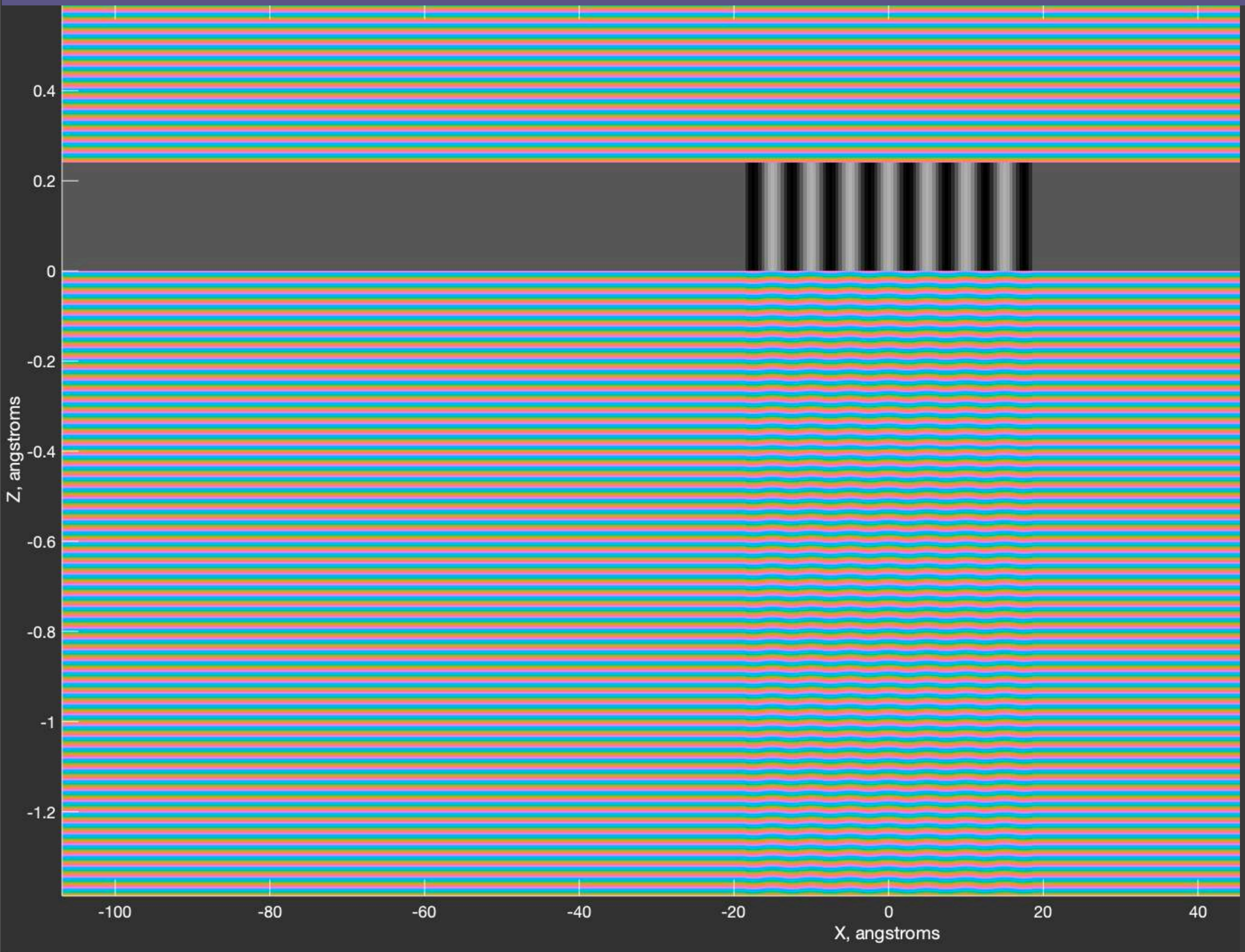
For an electron propagating in the $z$ direction, the time-independent wave function is

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

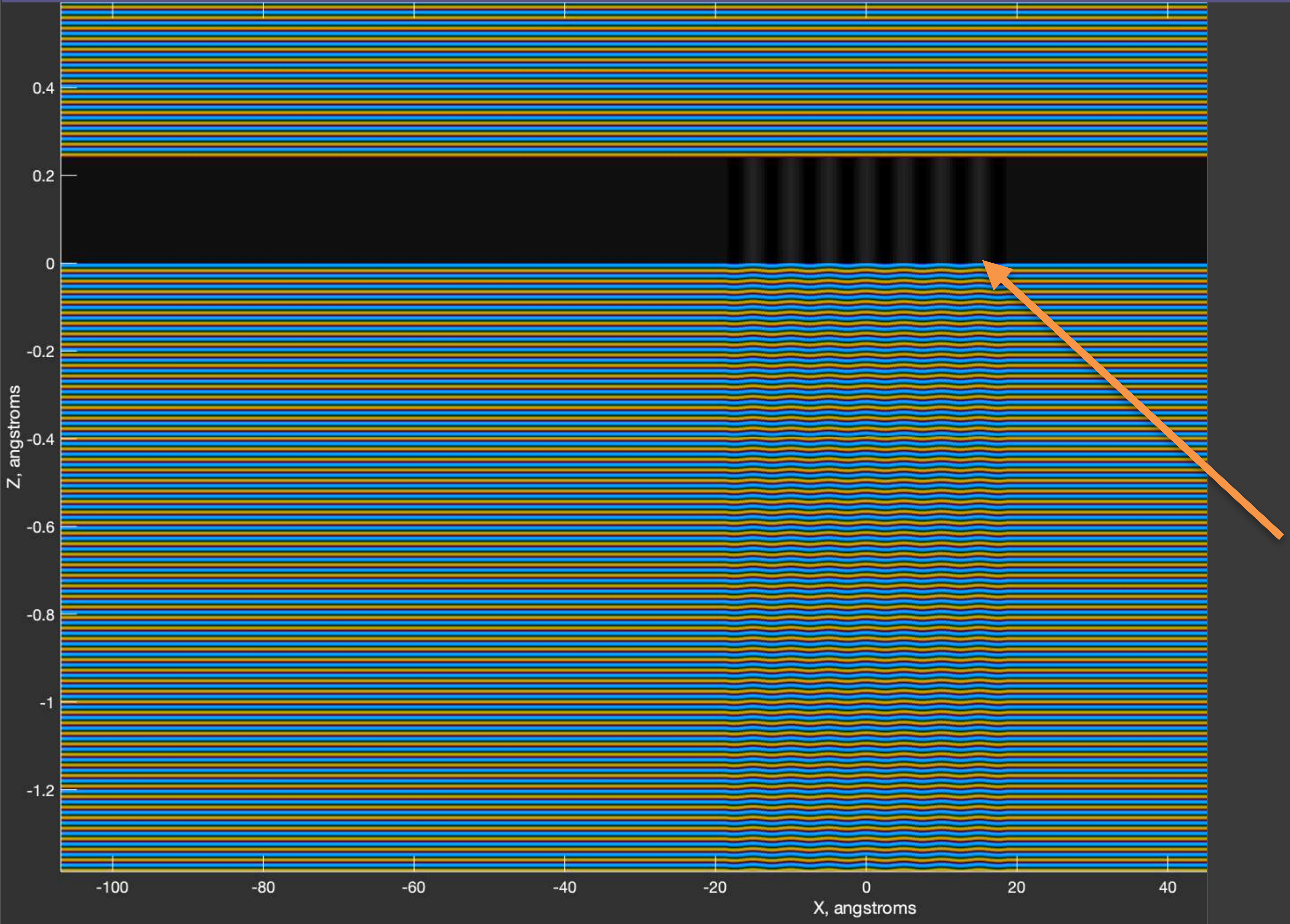
with

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

# Insert a phase-shifting object that perturbs the electron wave function



# Insert a phase-shifting object that perturbs the electron wave function




The object is a grating,

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

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 waves are shifted slightly in phase.
- The size of the perturbation is set by the small number $\epsilon$ which is much smaller than 1.
- The wave function there is $\Psi = e^{i\epsilon\phi(x)}$.
- Then, by the approximation $e^x \approx 1 + x$ we have just after the specimen

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

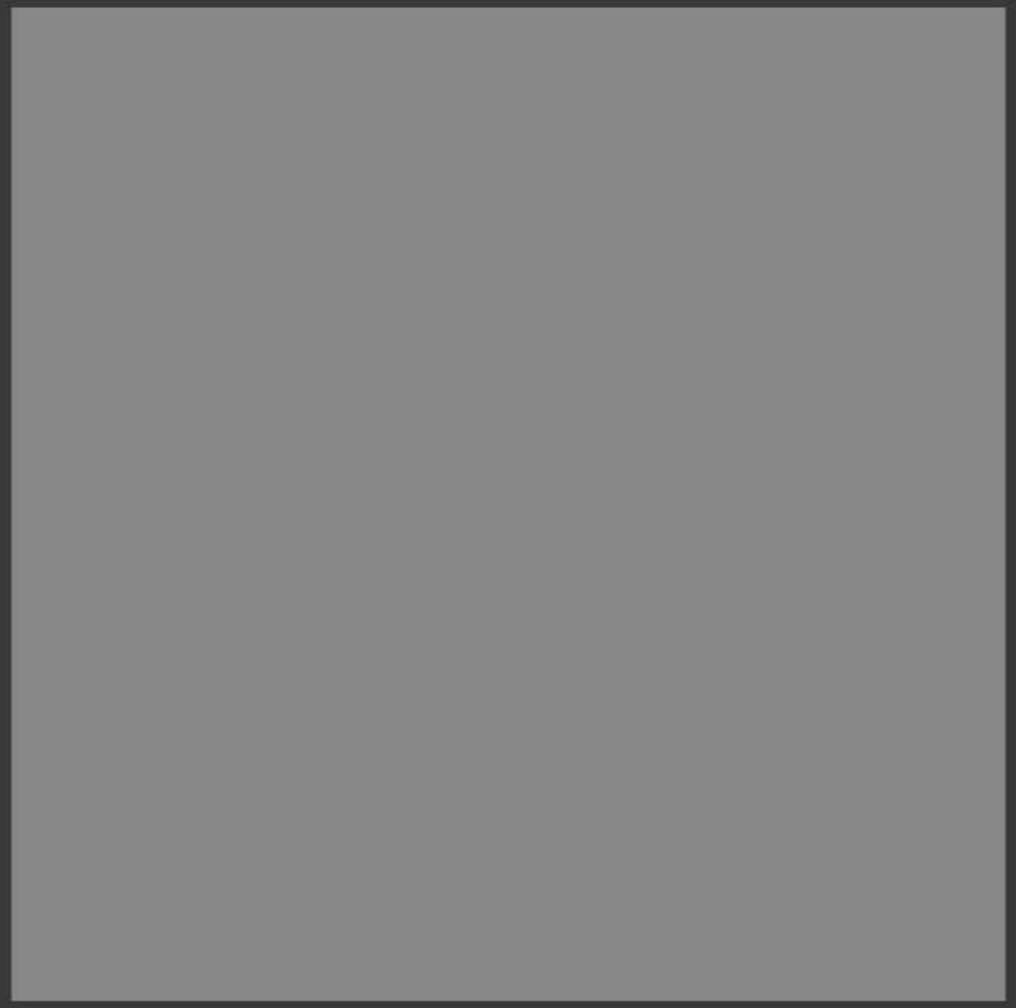
This is the weak phase approximation.

What are the two terms in the approximation?

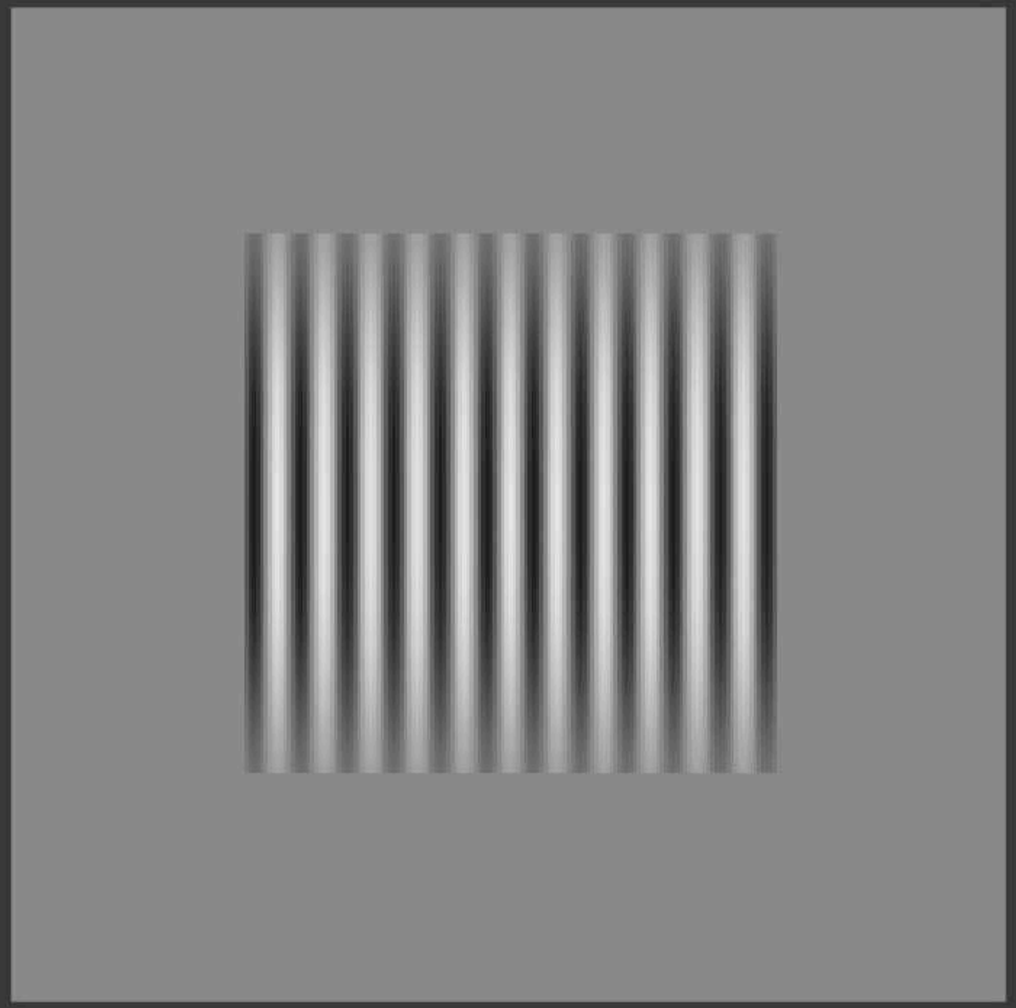
- Given a wave of amplitude 1 incident on our weak-phase object, there is an undiffracted wave —essentially unchanged—of amplitude 1. We'll call this $\Psi_0$.
- And there is a new wave combination of amplitude $\epsilon$. In this example of a grating there are actually two small diffracted waves, $\Psi_+$ and $\Psi_-$.
  - The full wavefunction is

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

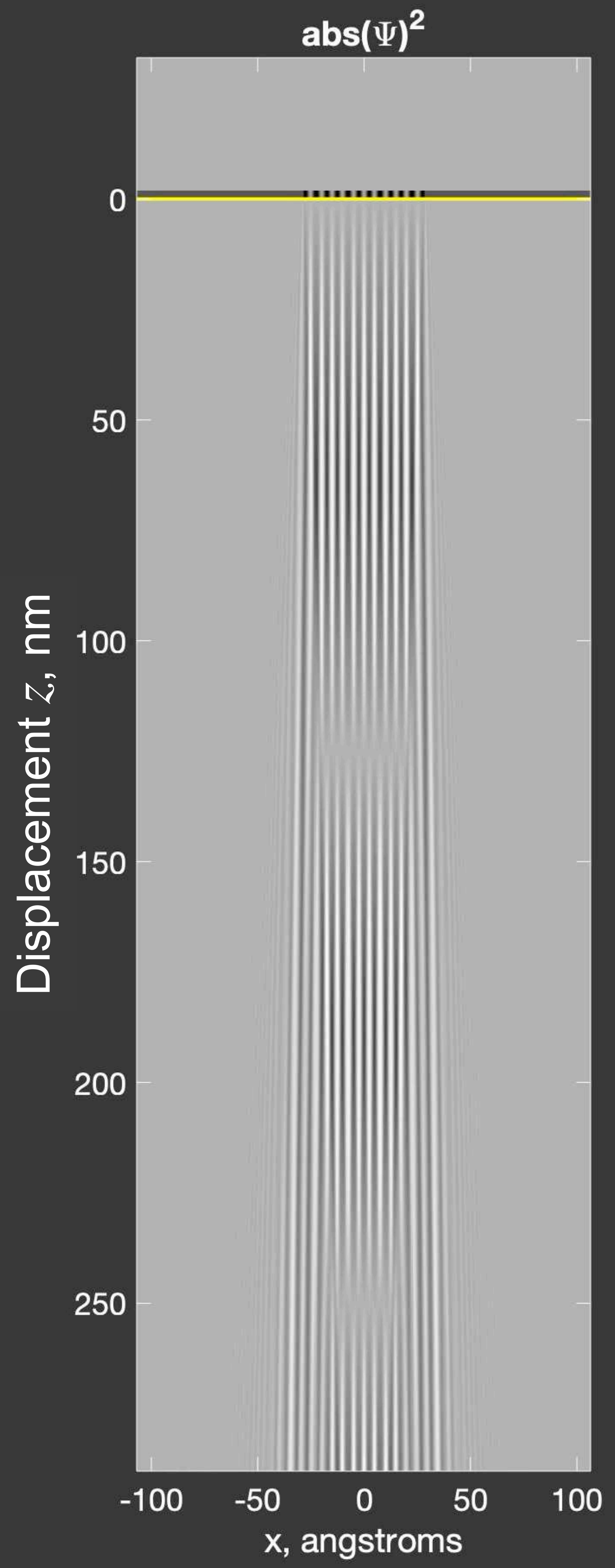
# The contrast of a grating object varies with the distance below the object

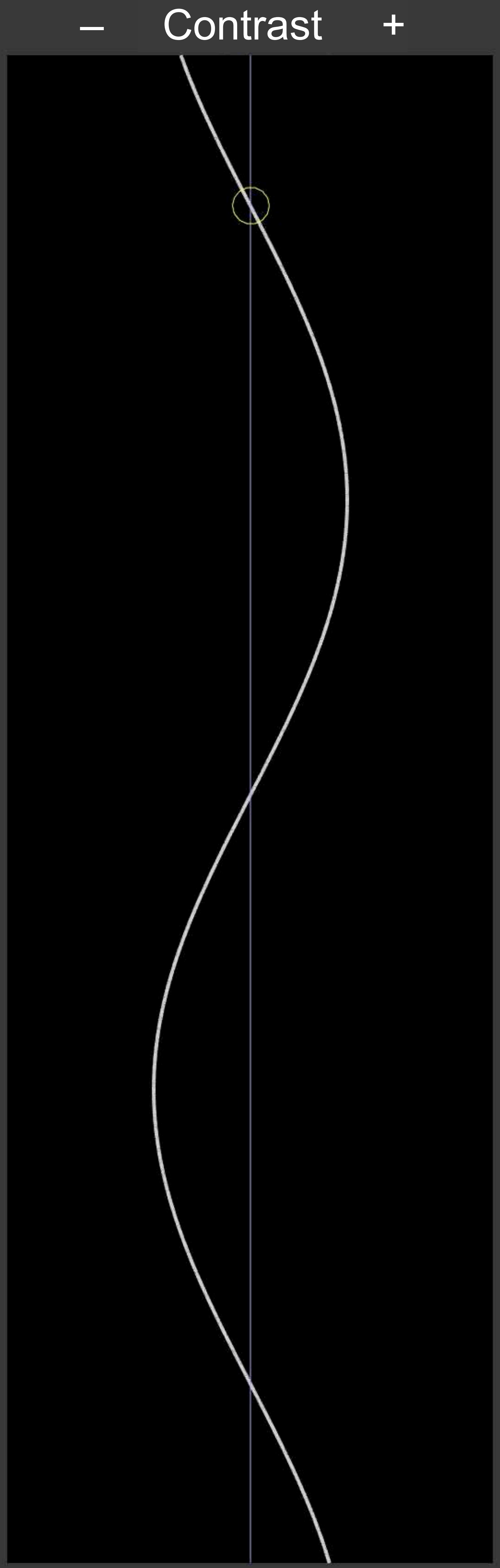


Intensity at $z$



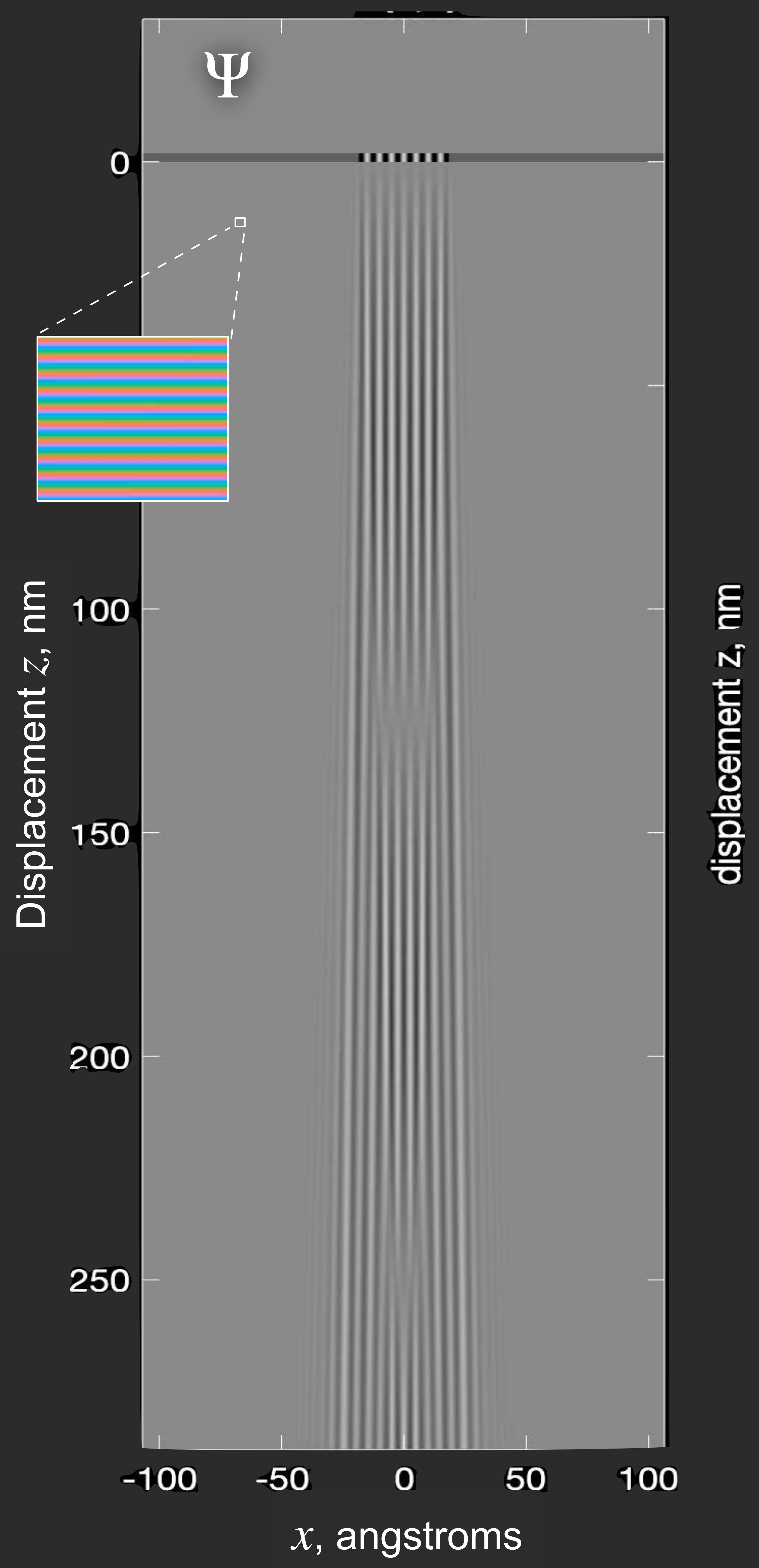
The grating $\phi(x)$

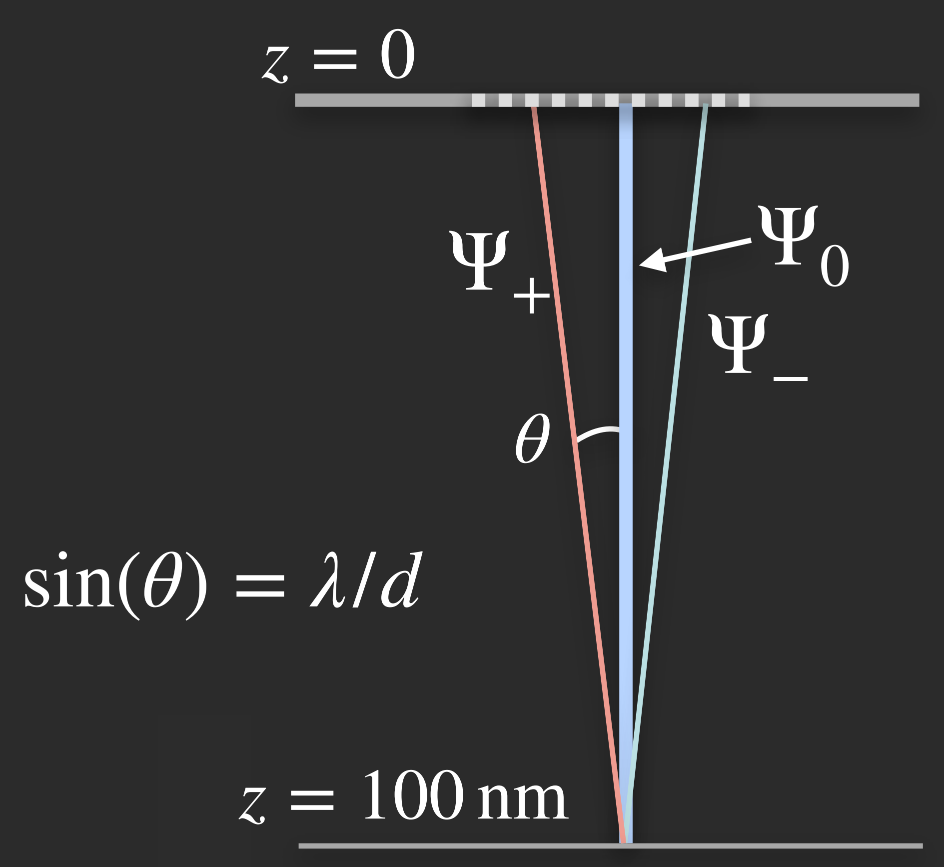



Interference between the undiffracted wave and diffracted waves produces contrast.

# Waves interfere to make contrast



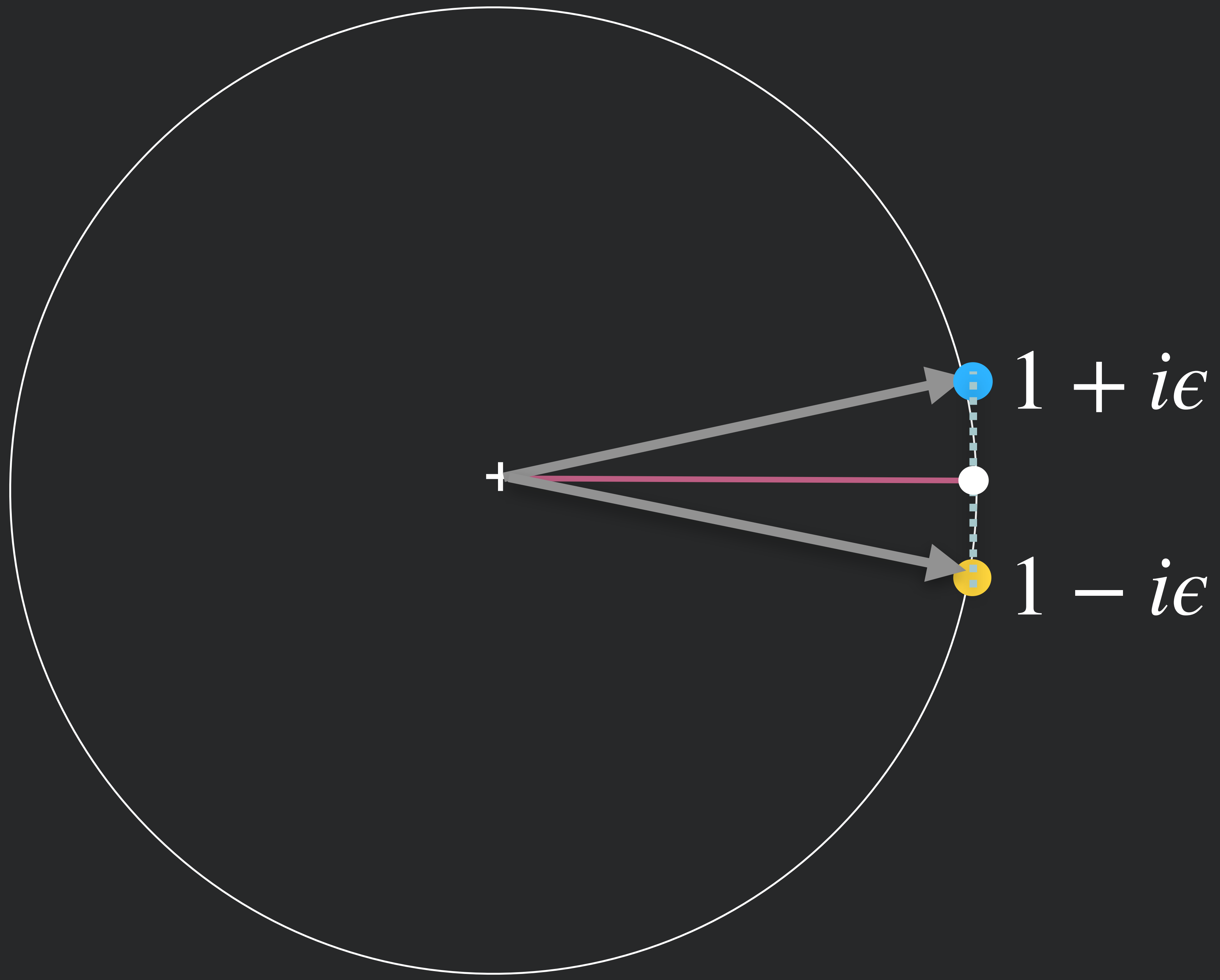

- The two diffracted waves $\Psi_+$ and $\Psi_-$ travel at very small angles $+\theta$ and $-\theta$ to the undiffracted wave.
- To reach the same point below the specimen, the path length traveled by each of the two diffracted waves $\Psi_+$ and $\Psi_-$ is longer by a small amount, we'll call $\zeta$.

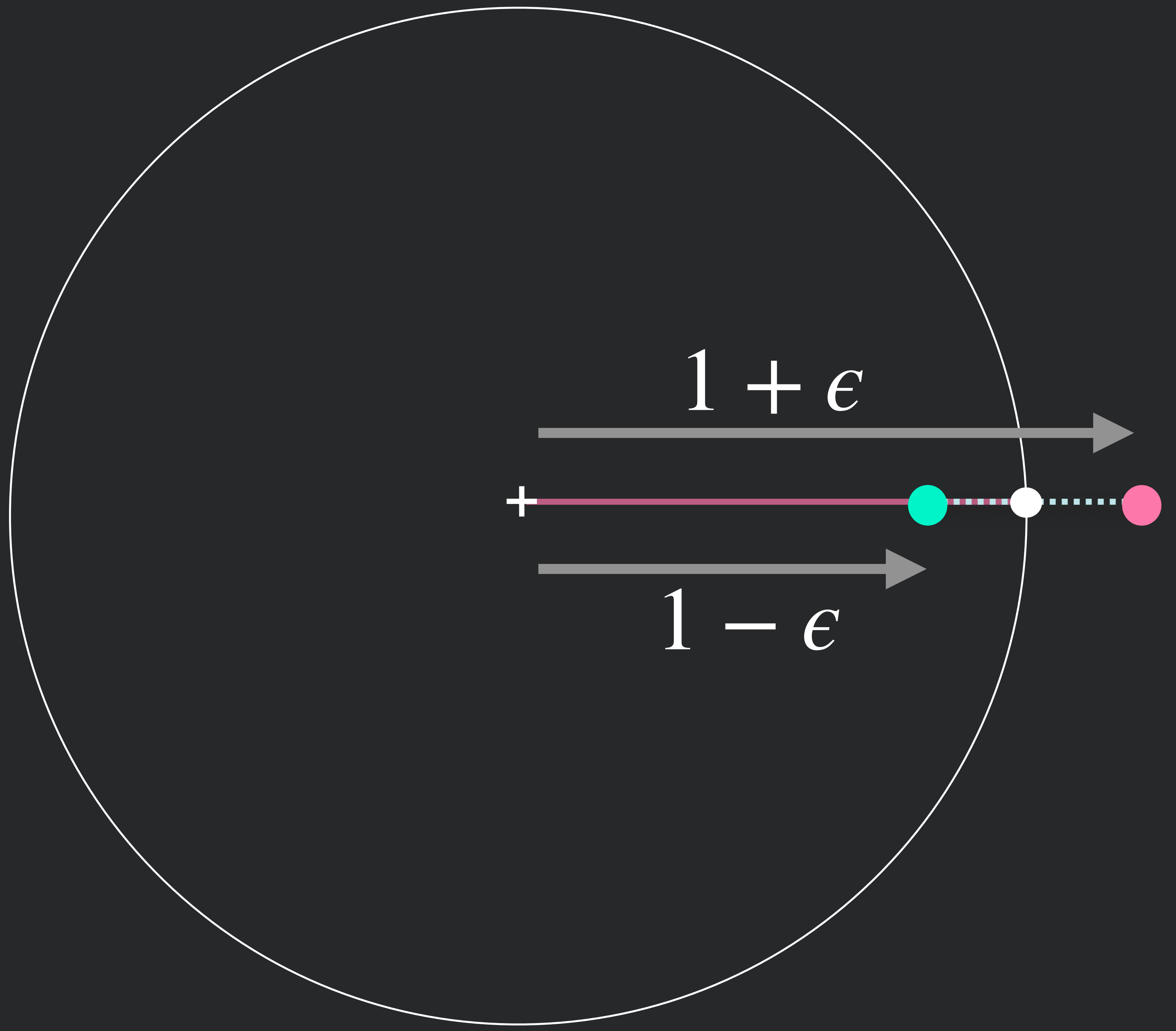
$$\zeta = \frac{z}{\cos\theta} - z \;\approx\; z\lambda^2/2d^2.$$

- In our example $\lambda = .02\text{Å}$ and the grating $d = 5\text{Å}$. At the level $z = 100\,\text{nm}$, then $\zeta = .008\text{Å}$, about half a wavelength.
- Define $\chi$ = the phase difference between the undiffracted and diffracted waves. $\chi = 2\pi\zeta/\lambda$.
- In this example $\chi = 0.8\pi$

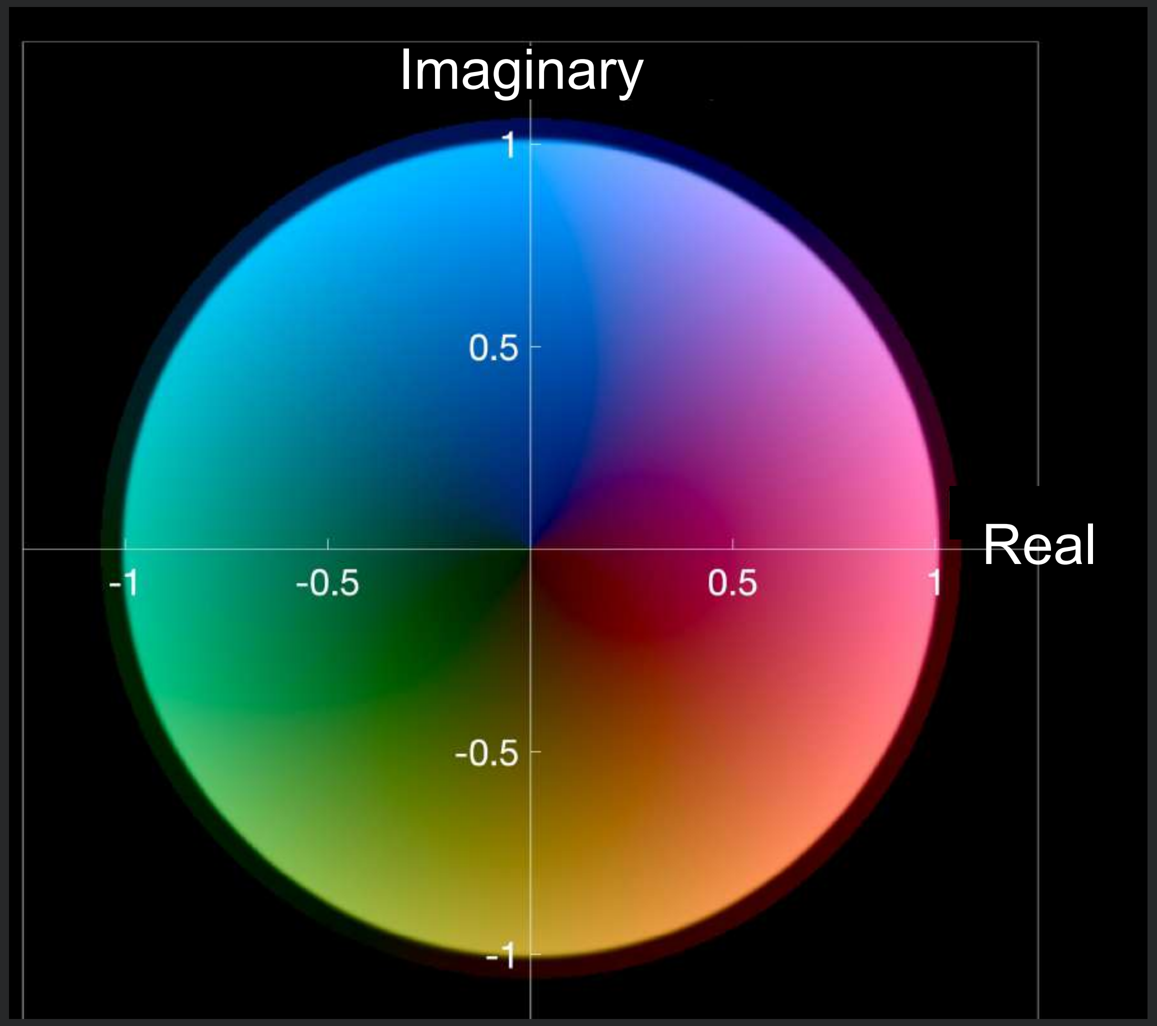
# Why the phase of a perturbation is a big deal

We care about the intensity (the magnitude squared) of the overall electron wave function. Imaginary (out of phase) perturbations make negligible changes in intensity; real (in phase) perturbations make substantial changes.

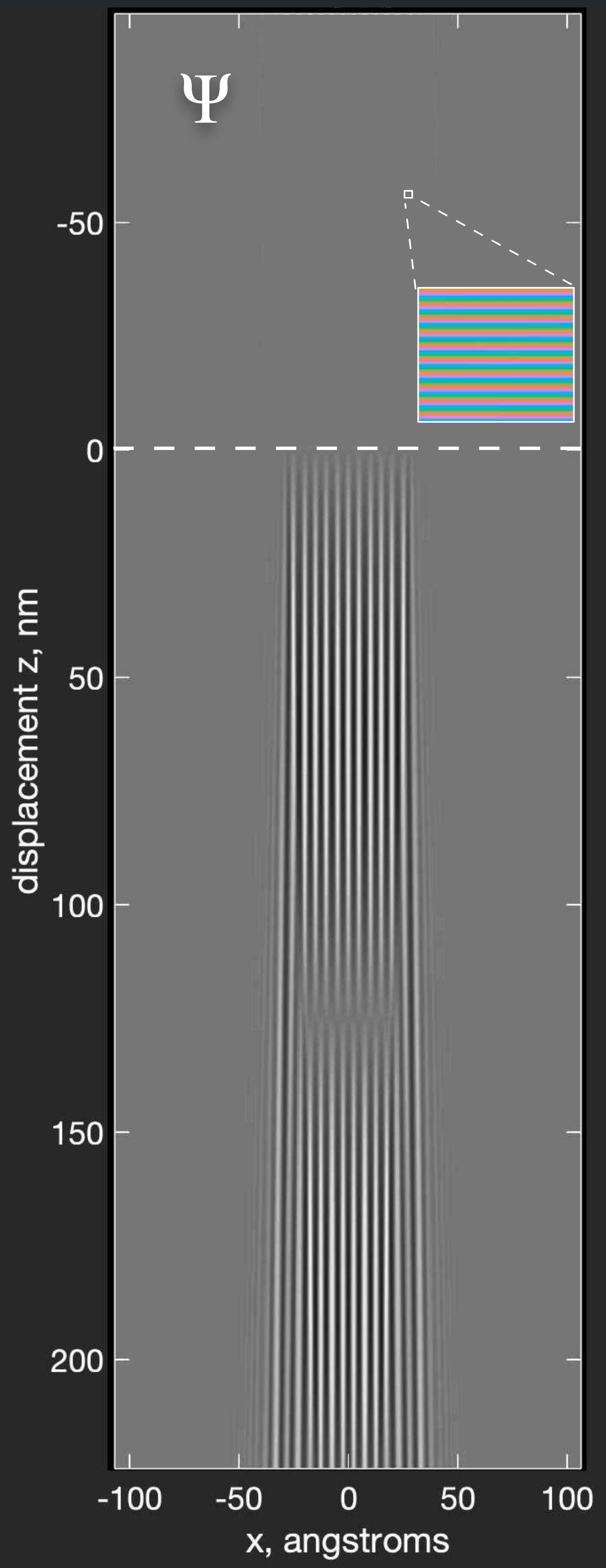


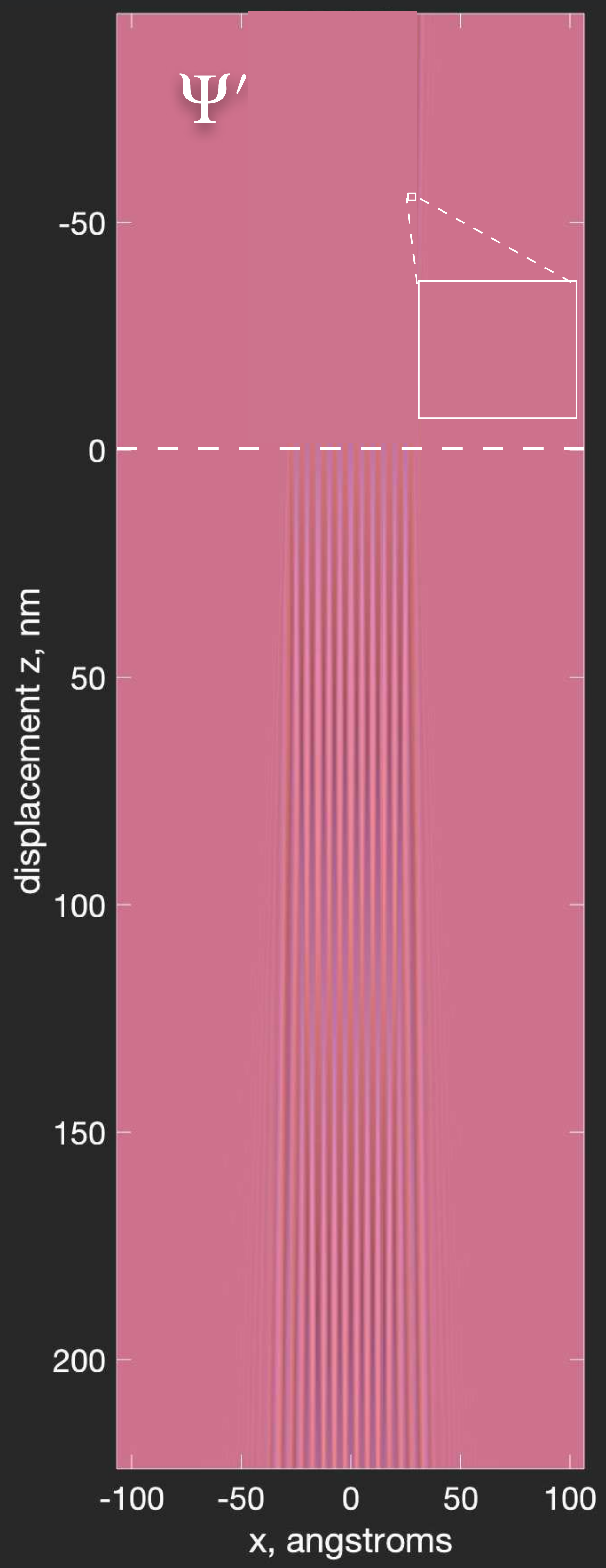




Complex number color scheme



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

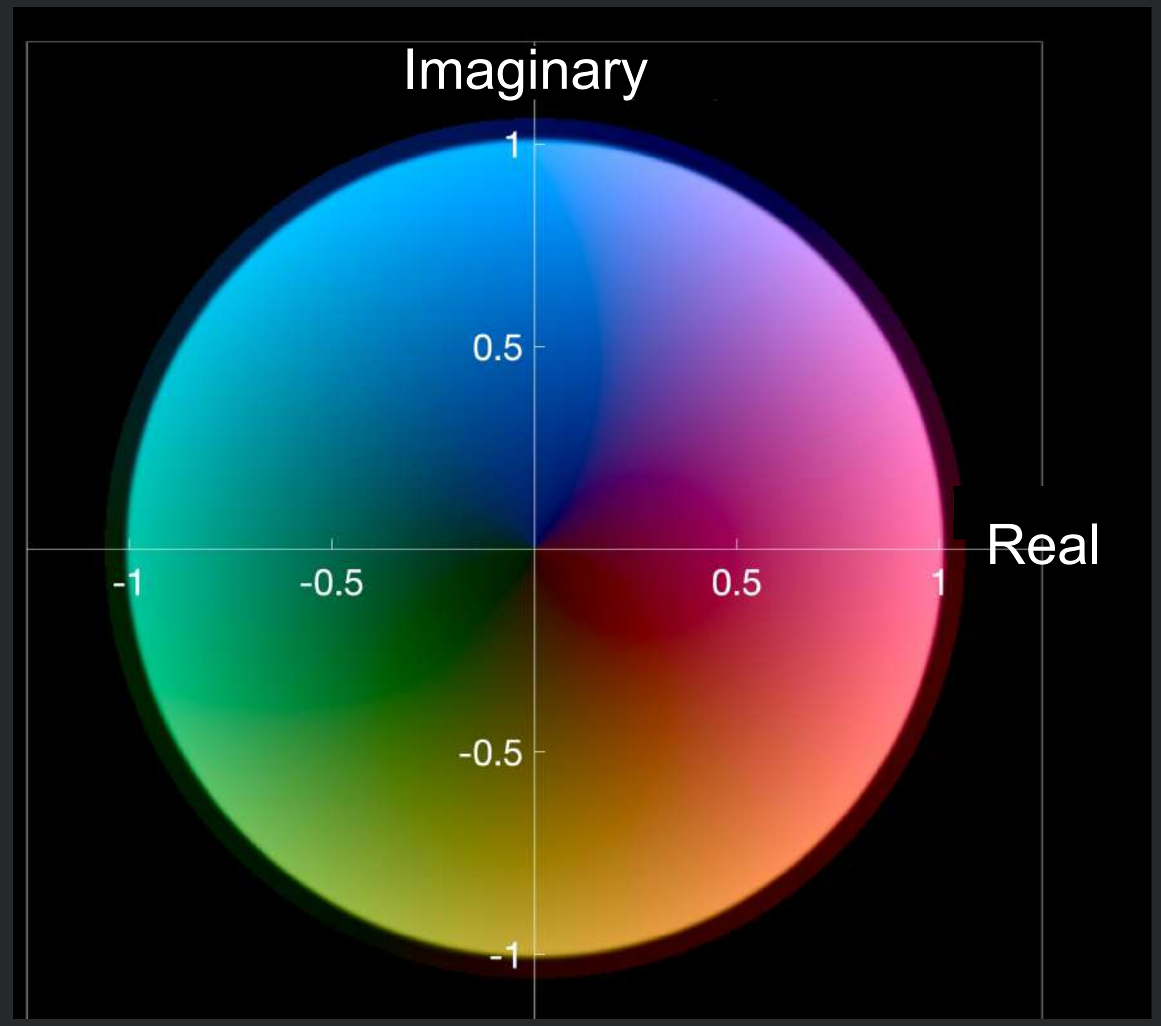


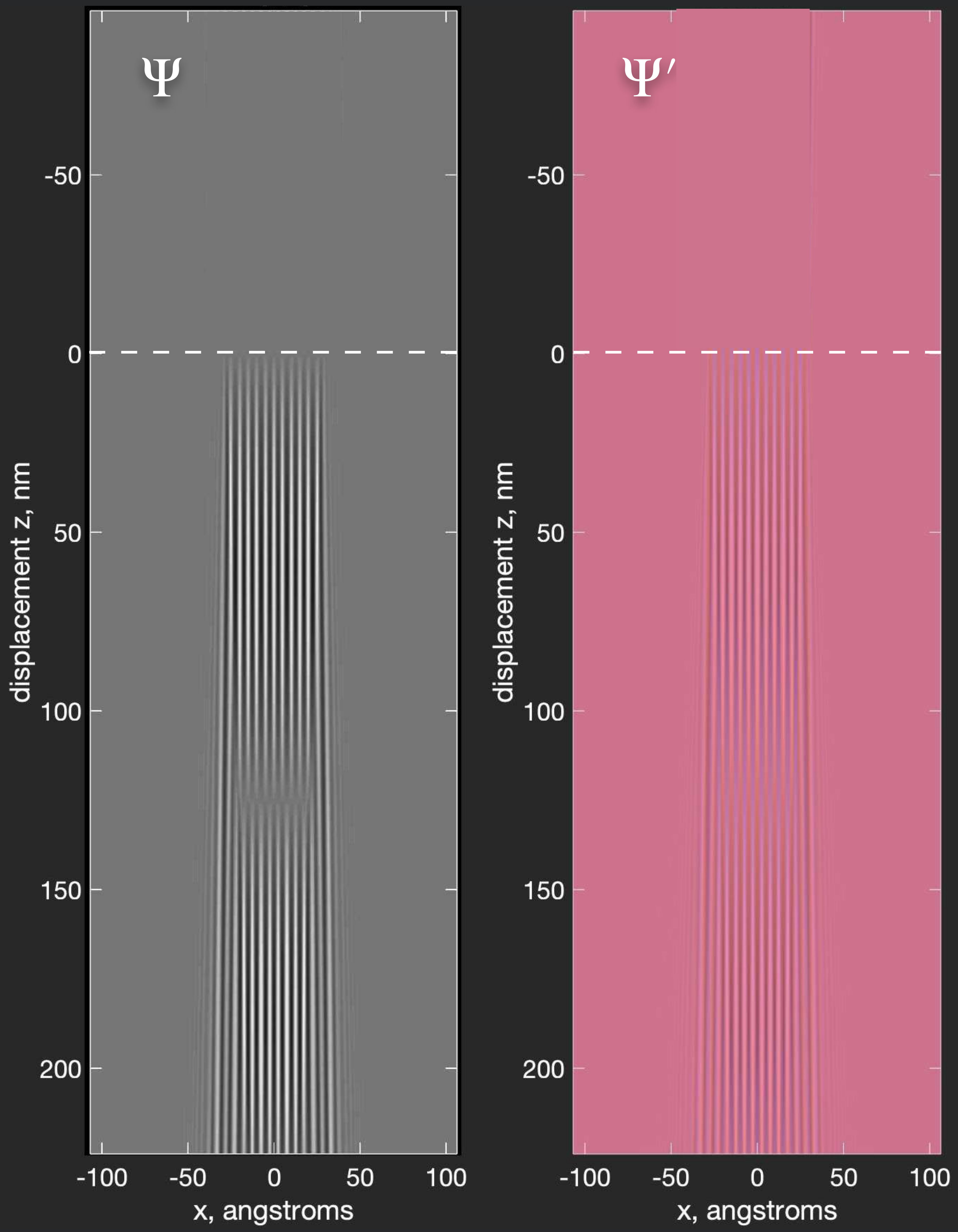

Let's unwrap the oscillations in $\Psi$:

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

Complex number color scheme



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



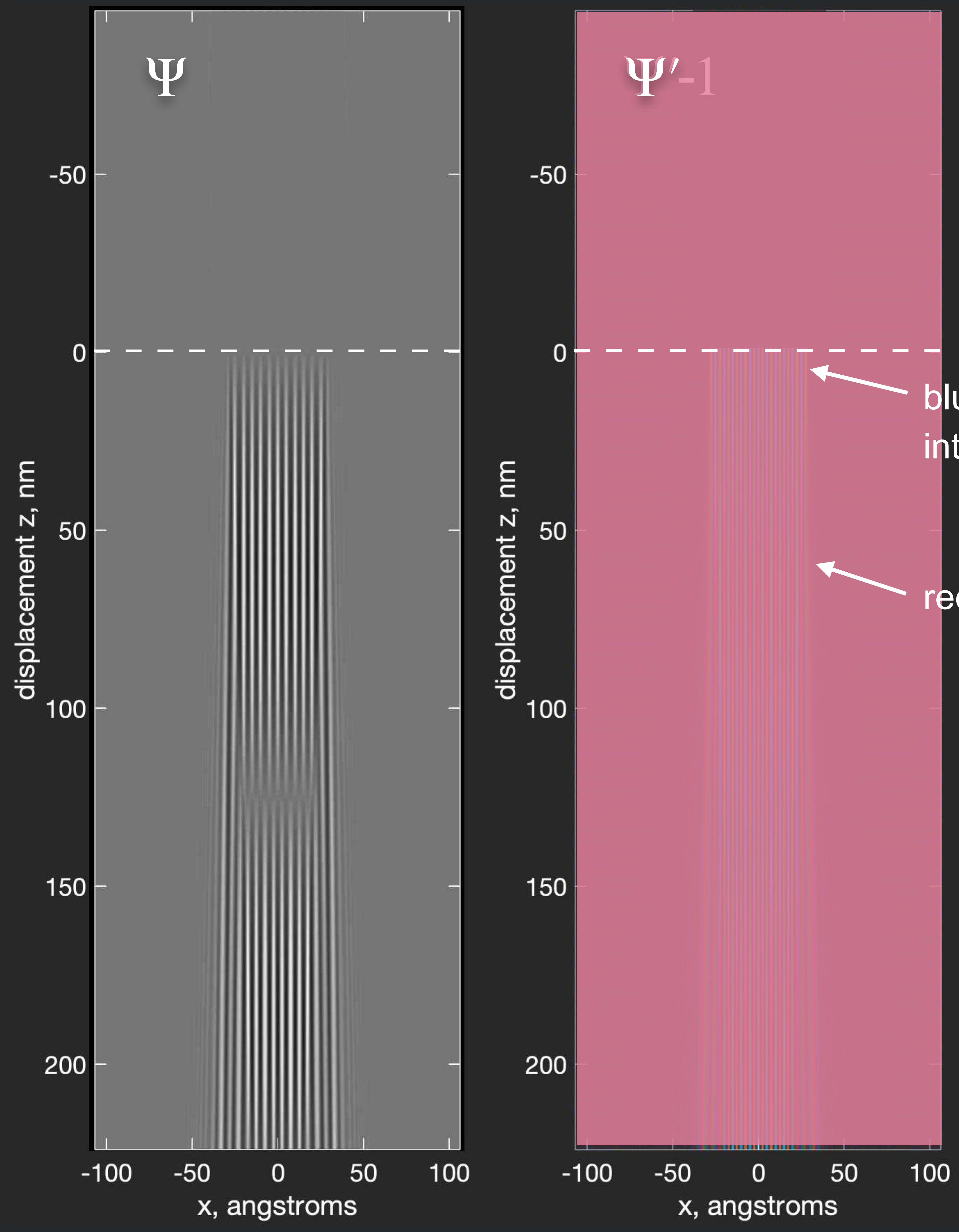

Let's unwrap the oscillations in $\Psi$:

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

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

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

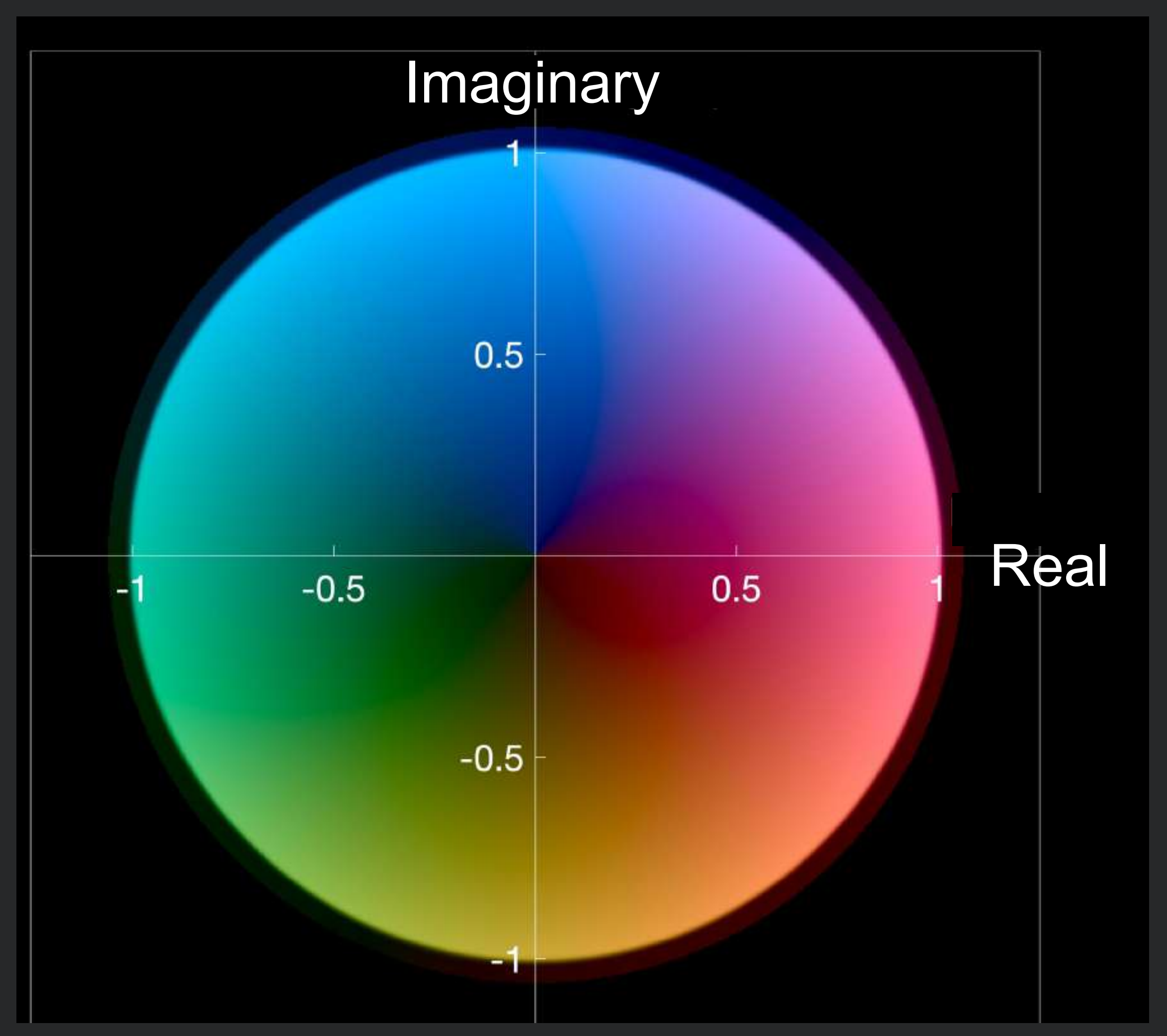
# When the phase of the diffracted waves is right, we have contrast.



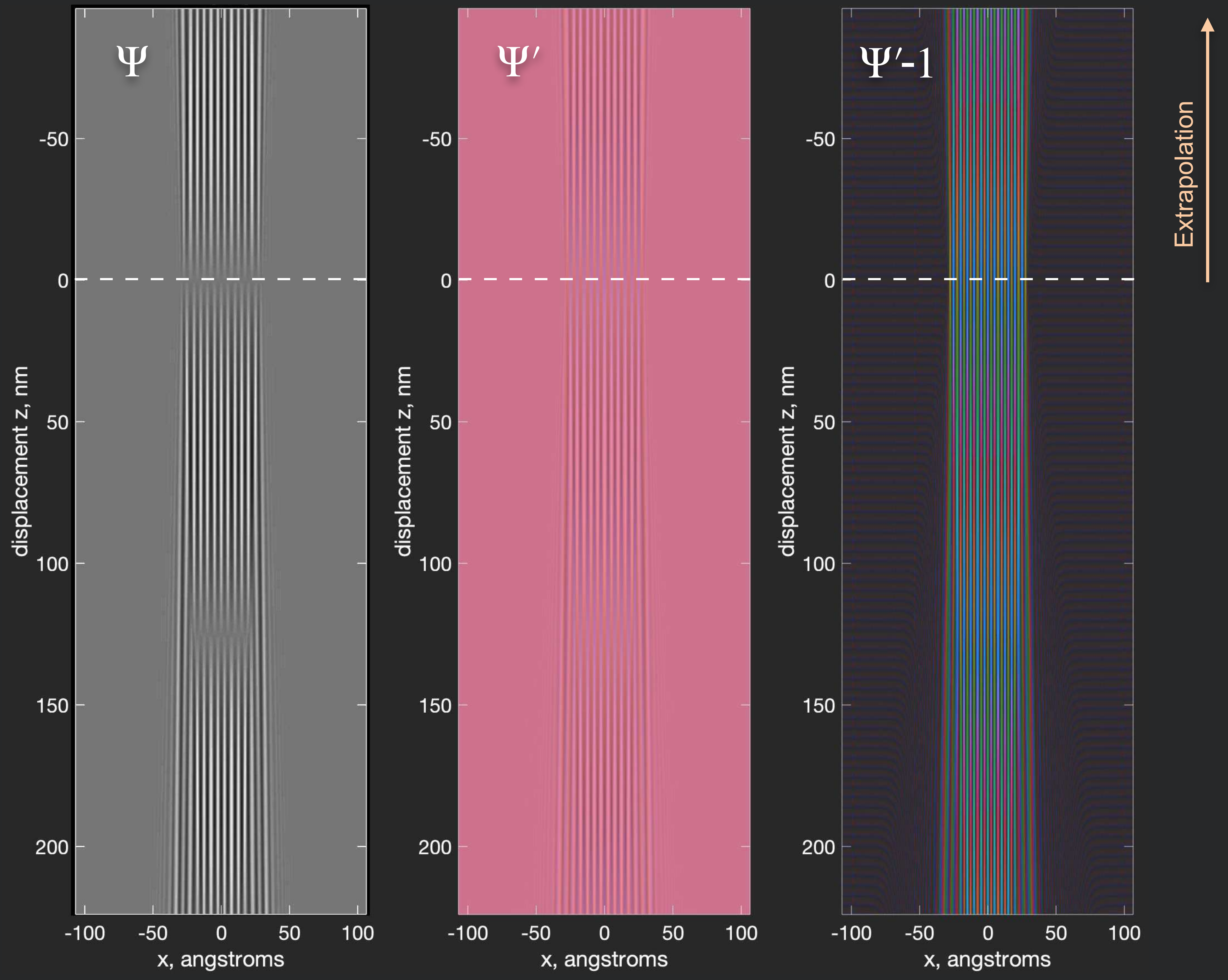

blue / yellow: no interference, no contrast

red / green: interference

Complex number color scheme



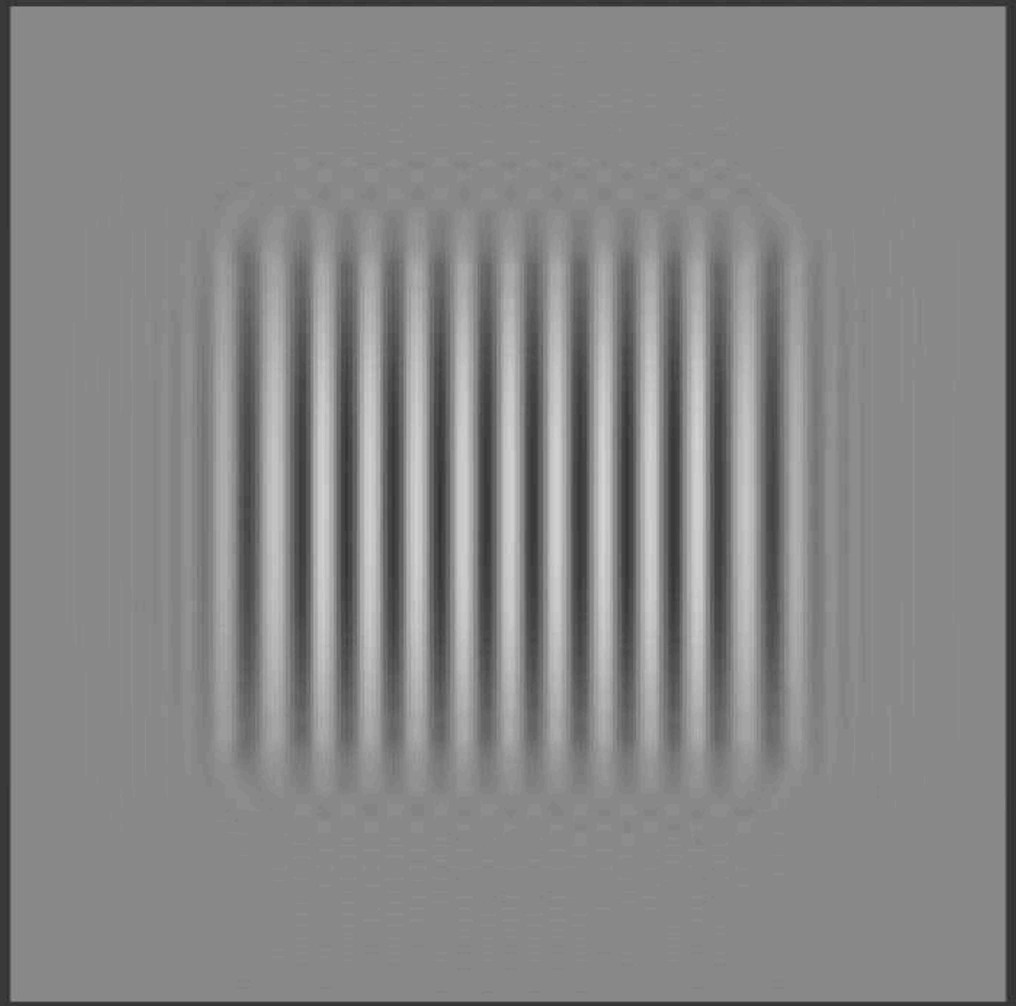
# What happens when the objective lens is focused *above* the specimen?



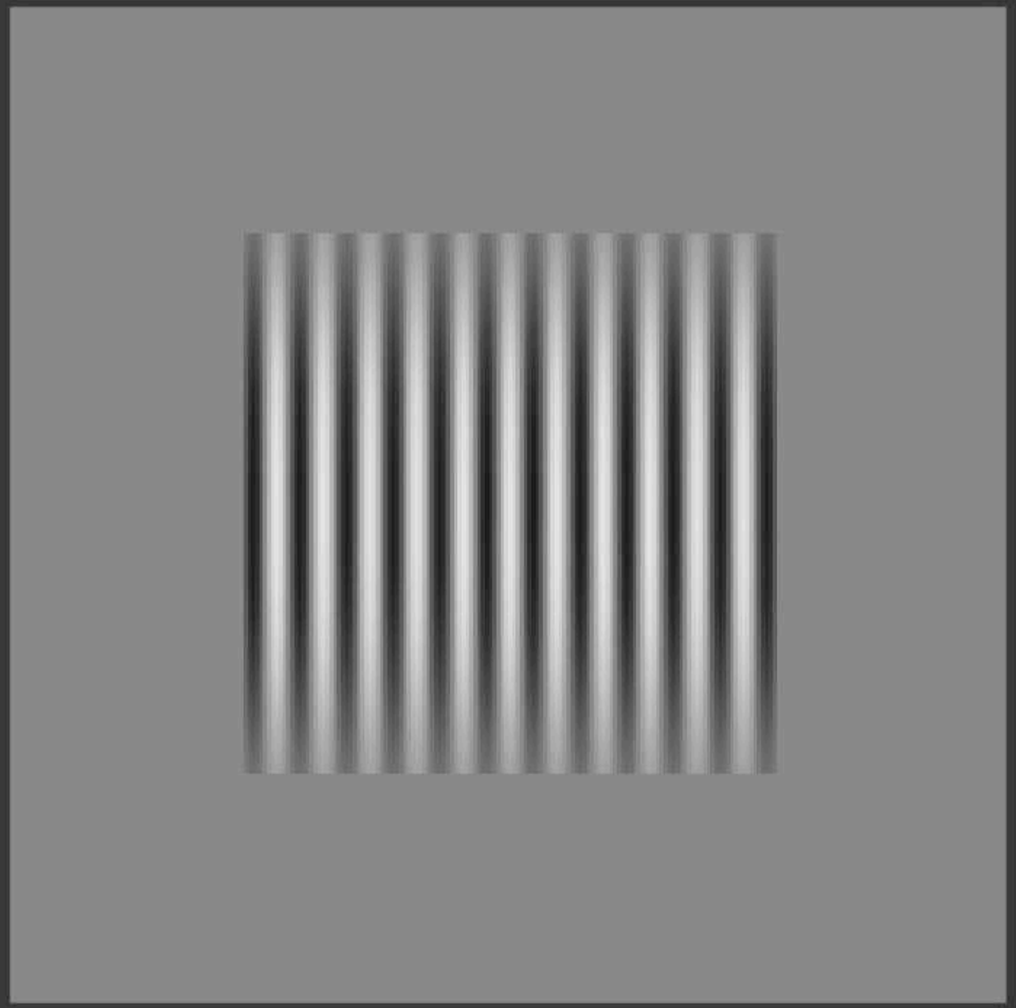

What wavefunction above the specimen would give rise to what we see below it?

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

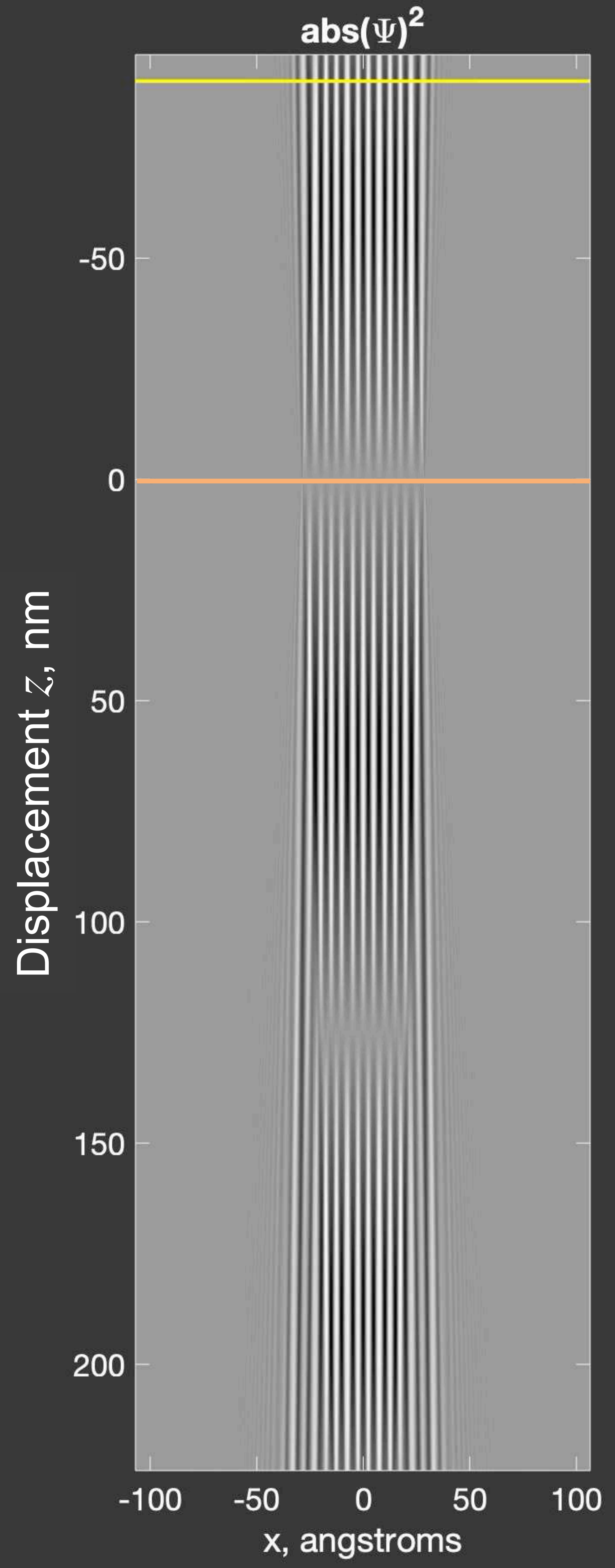
# What happens when the objective lens is focused *above* the specimen?

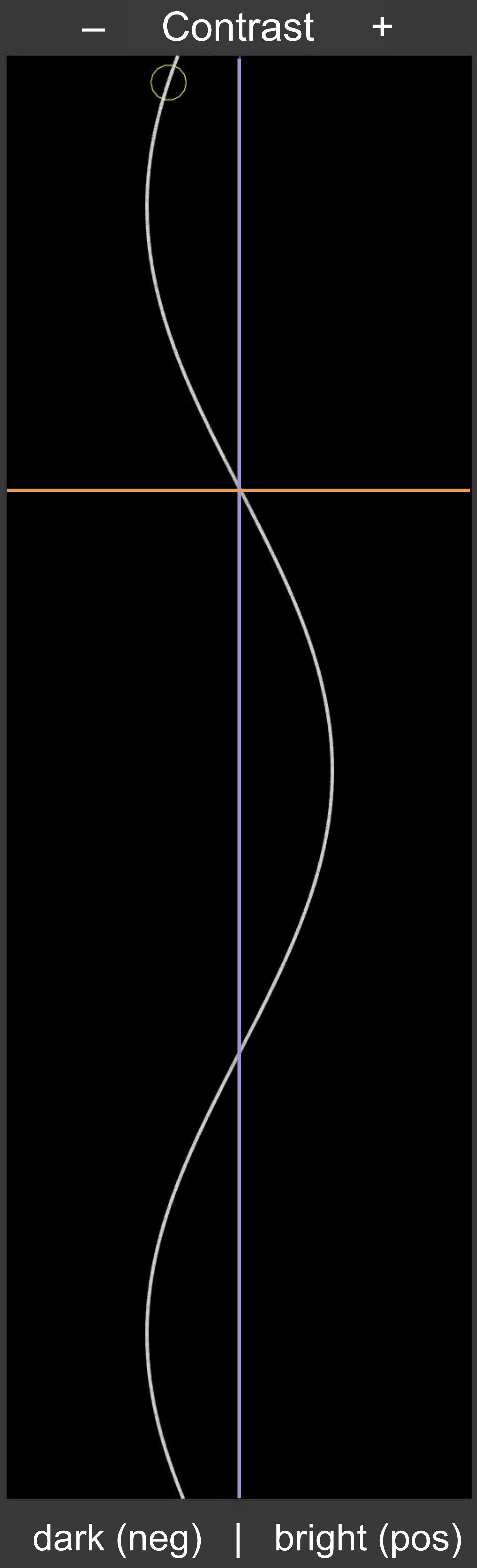


Intensity at $z$



The grating $\phi(x)$



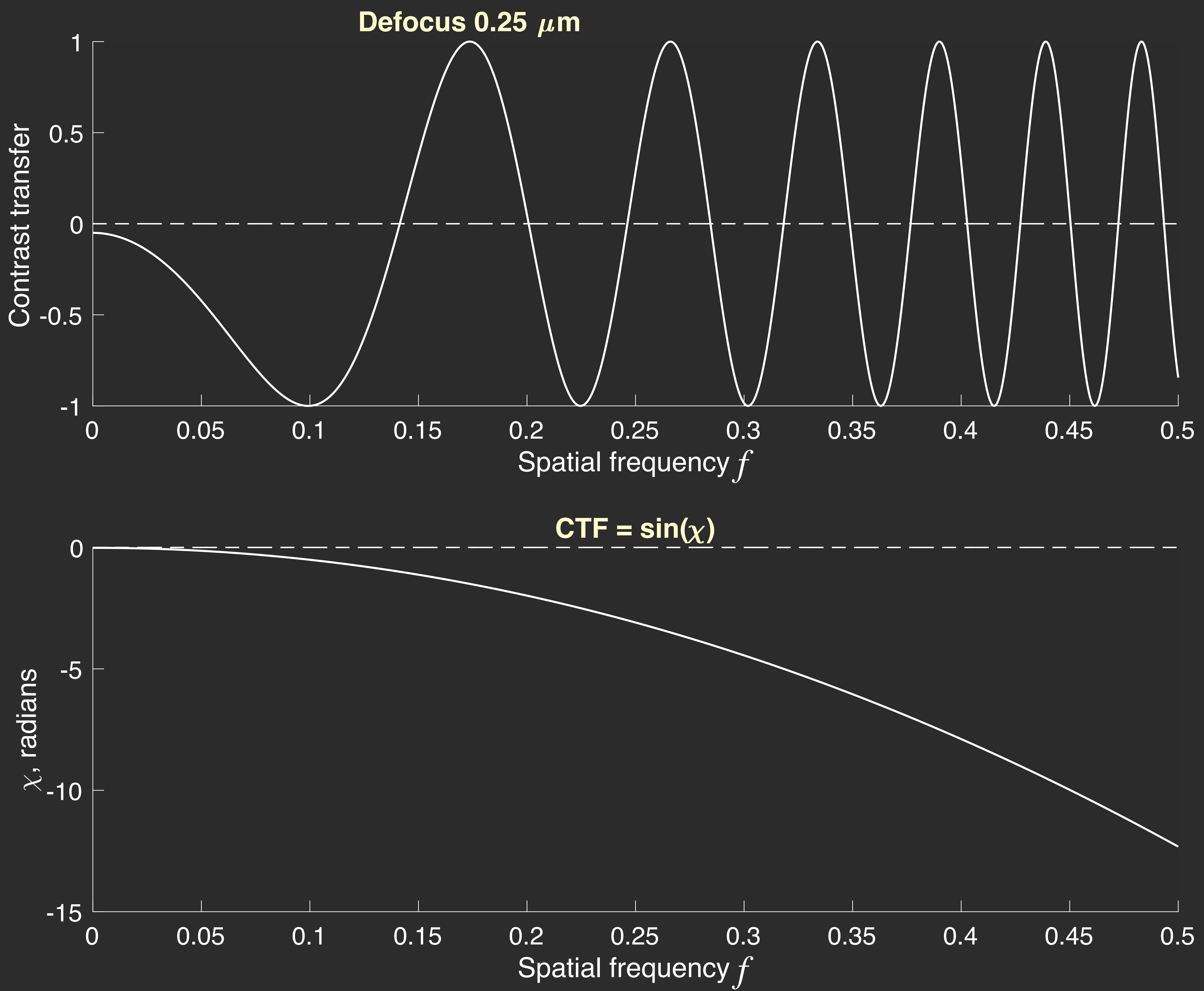




## Terminology

- "Underfocus" is focusing the objective lens above the specimen.
- Defocus values $\delta$ are positive for underfocus: $\delta = -z$
- Spatial frequency is $u = 1/d$
- The phase shift $\chi$ is proportional to $\delta$.
- The contrast transfer function is given by

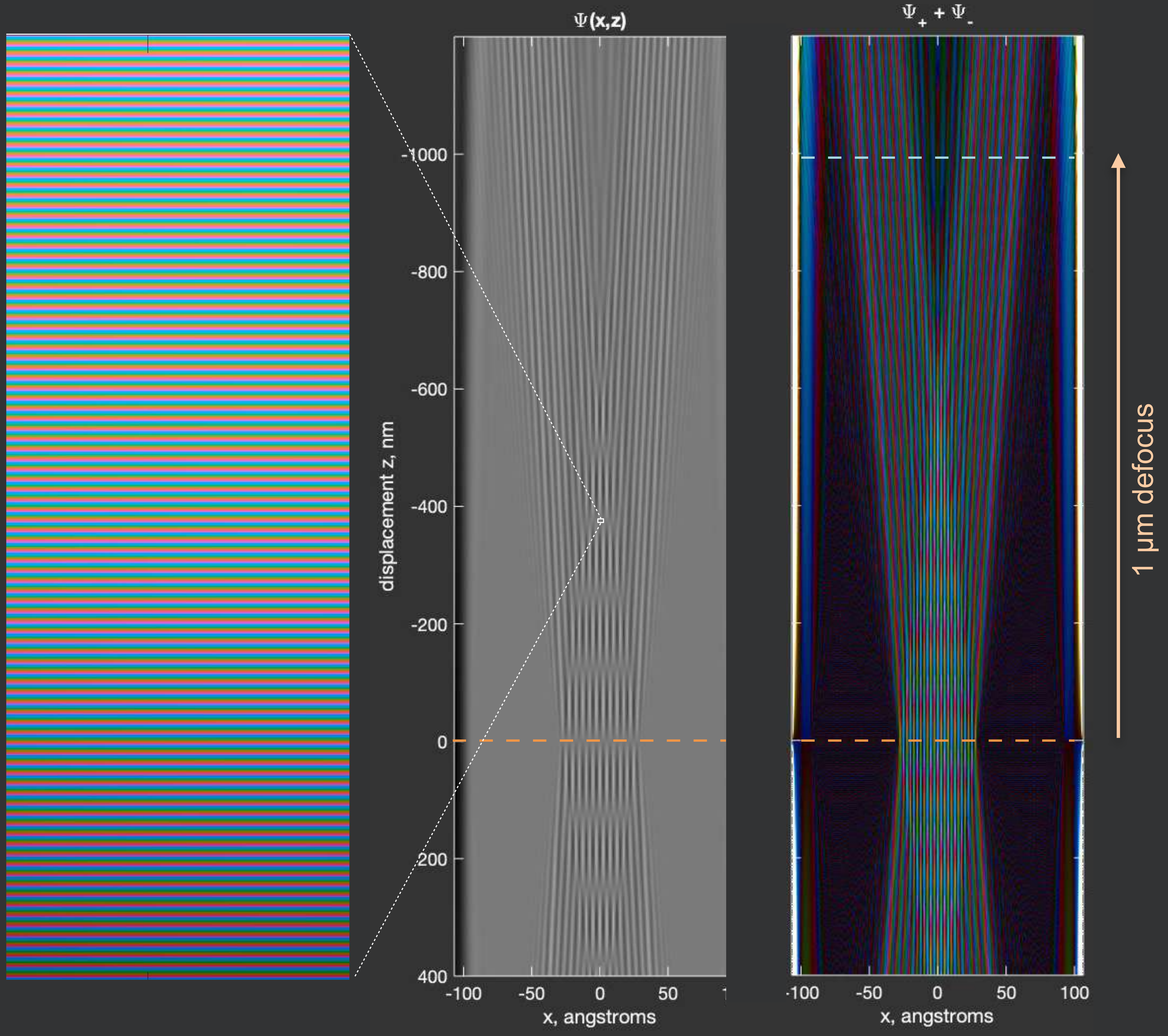
$$\begin{aligned} \mathrm{CTF} &= \sin(\chi) \\ &\approx \sin(-\pi\lambda\delta u^2) \end{aligned}$$

# The contrast-transfer function as a function of $f$




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

# A little defocus is actually a long distance

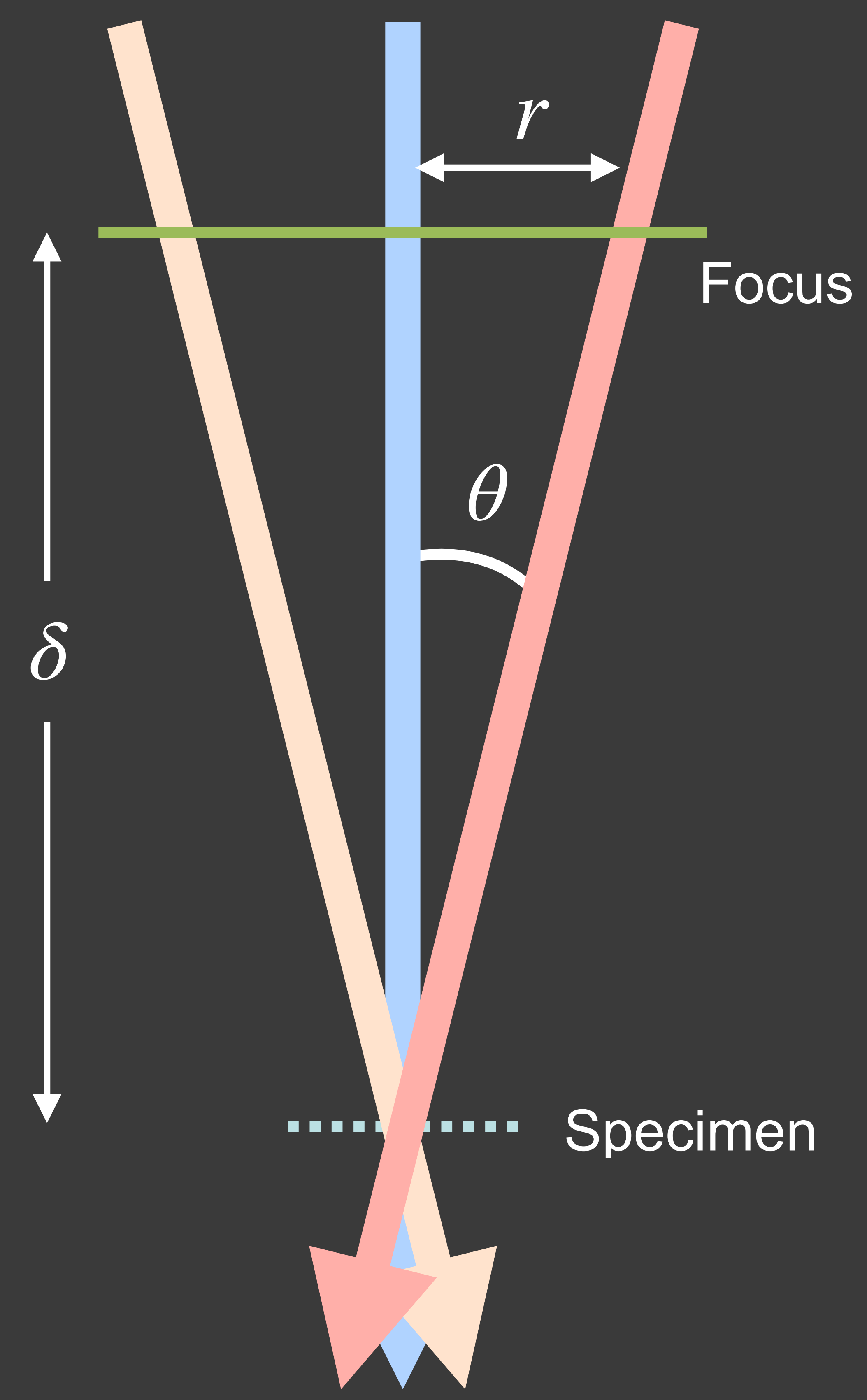



1 μm—a moderate defocus for cryo-EM imaging–is 500,000 wavelengths!

This has ramifications regarding

- beam coherence
- specimen charging
- delocalization

# With large defocus, how bad is the image delocalization?




The dispersion radius is given by

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

For example at 3μm defocus and 3Å resolution

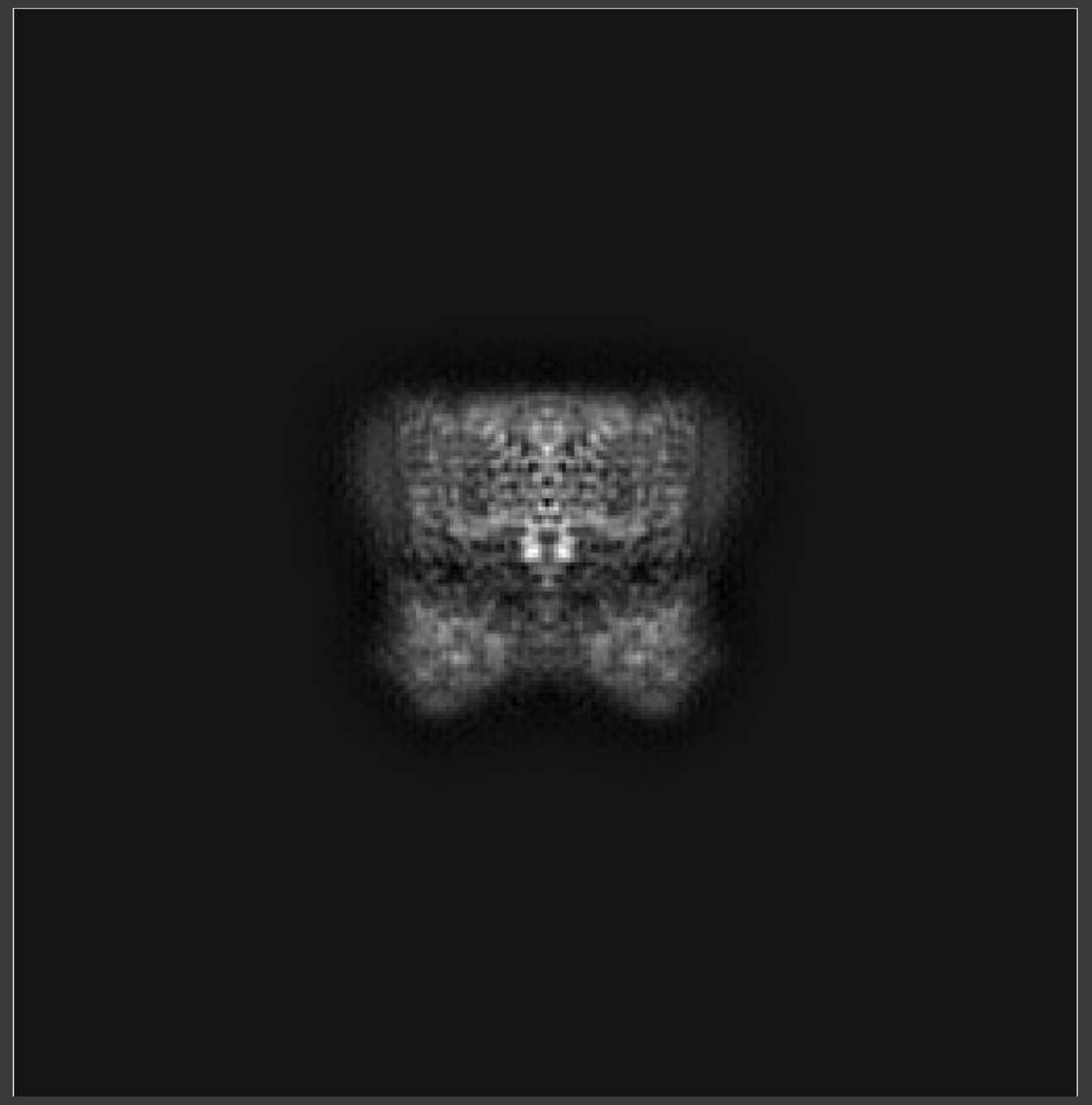
$$\delta = 3 \times 10^4 \text{Å}$$
$$\lambda = .02\text{Å}$$
$$f = 0.33\text{Å}^{-1}$$

then

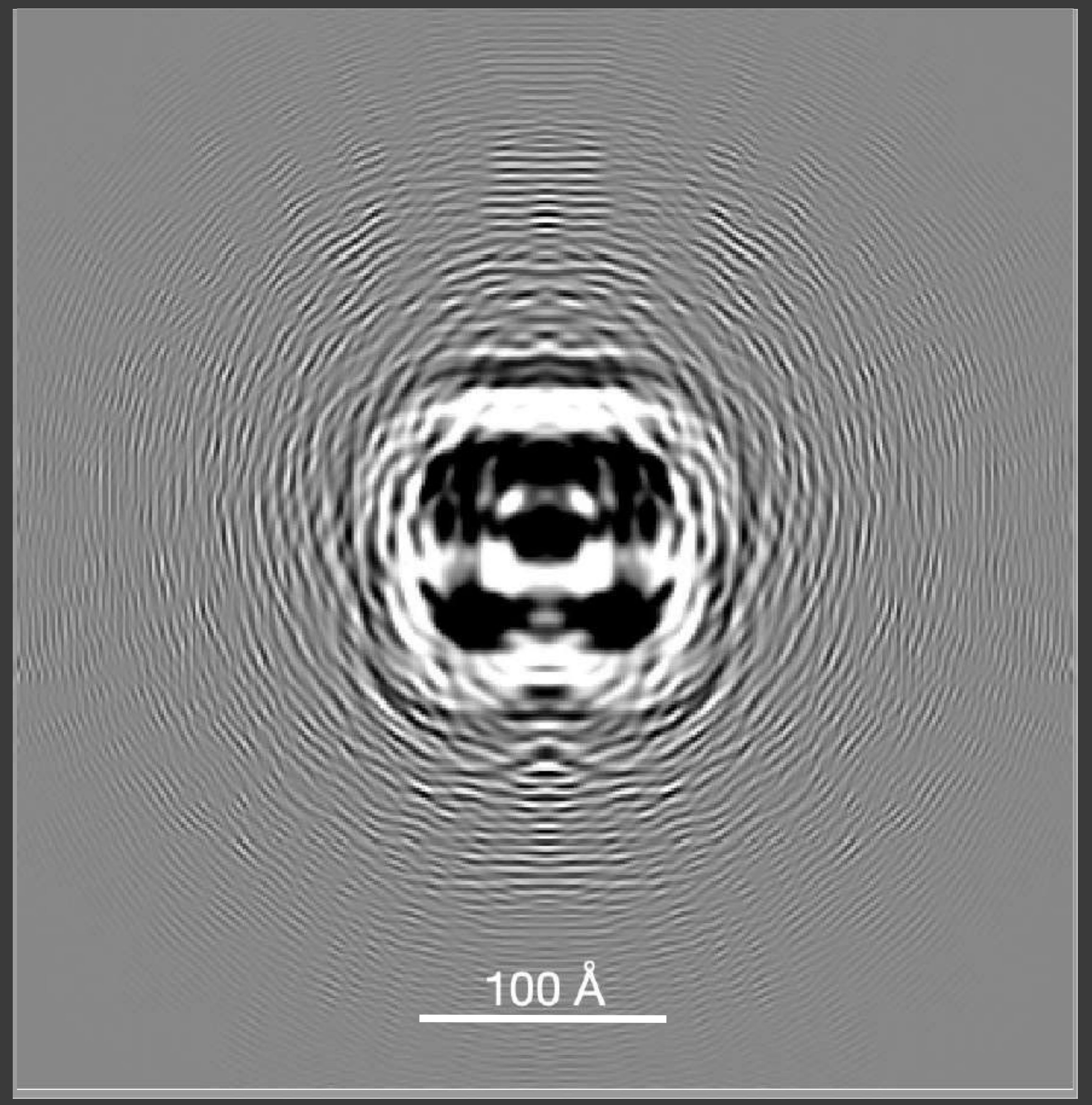
$$r = 200\text{Å}$$

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

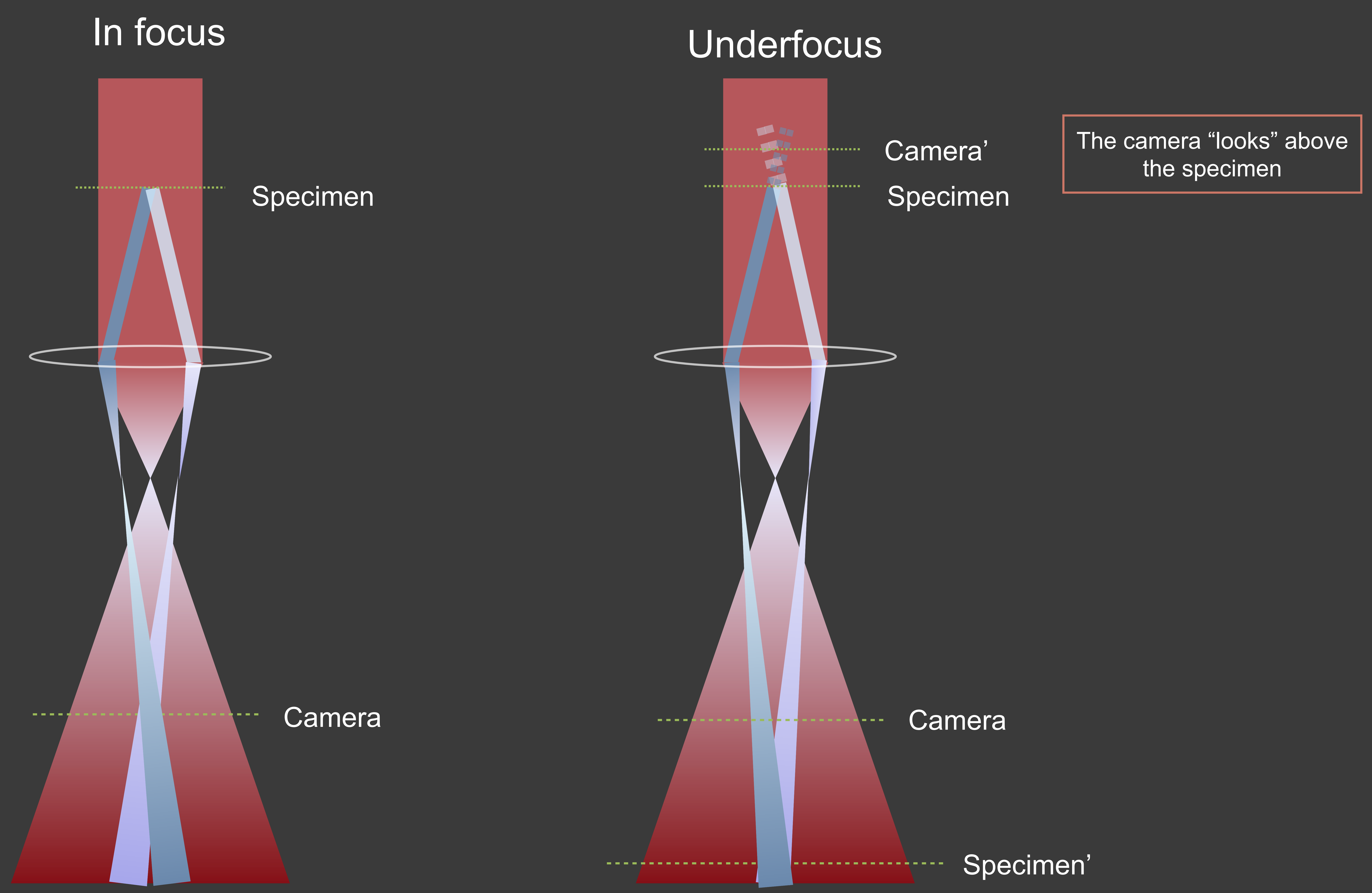



3 μm defocus

# 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.

# An objective lens reproduces interference patterns at the camera



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

Spherical aberration changes the defocus by

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

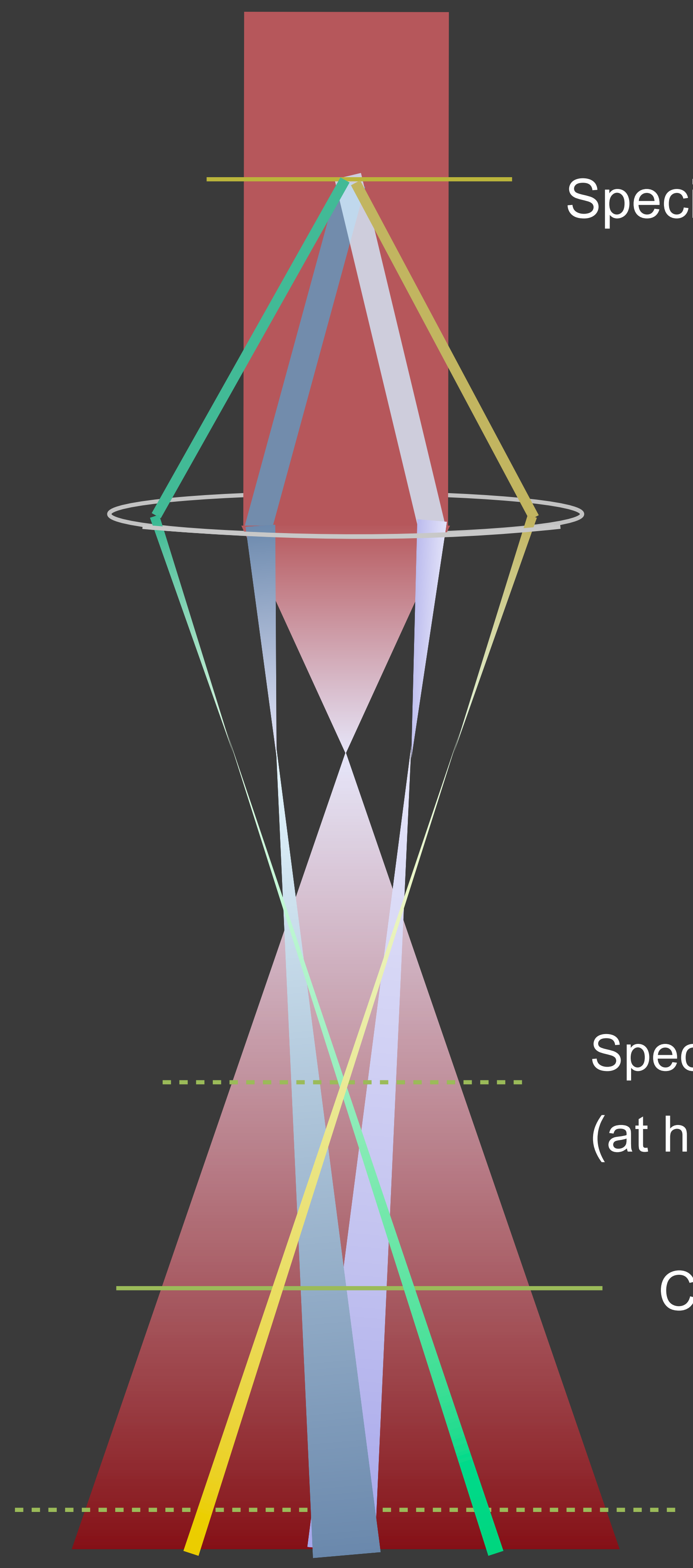
The contrast transfer function has a new term,

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

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. This makes spherical aberration important only for $s \gtrsim 0.25\text{Å}^{-1}$, or about 4Å resolution.



# Very high-angle scattering yields some contrast

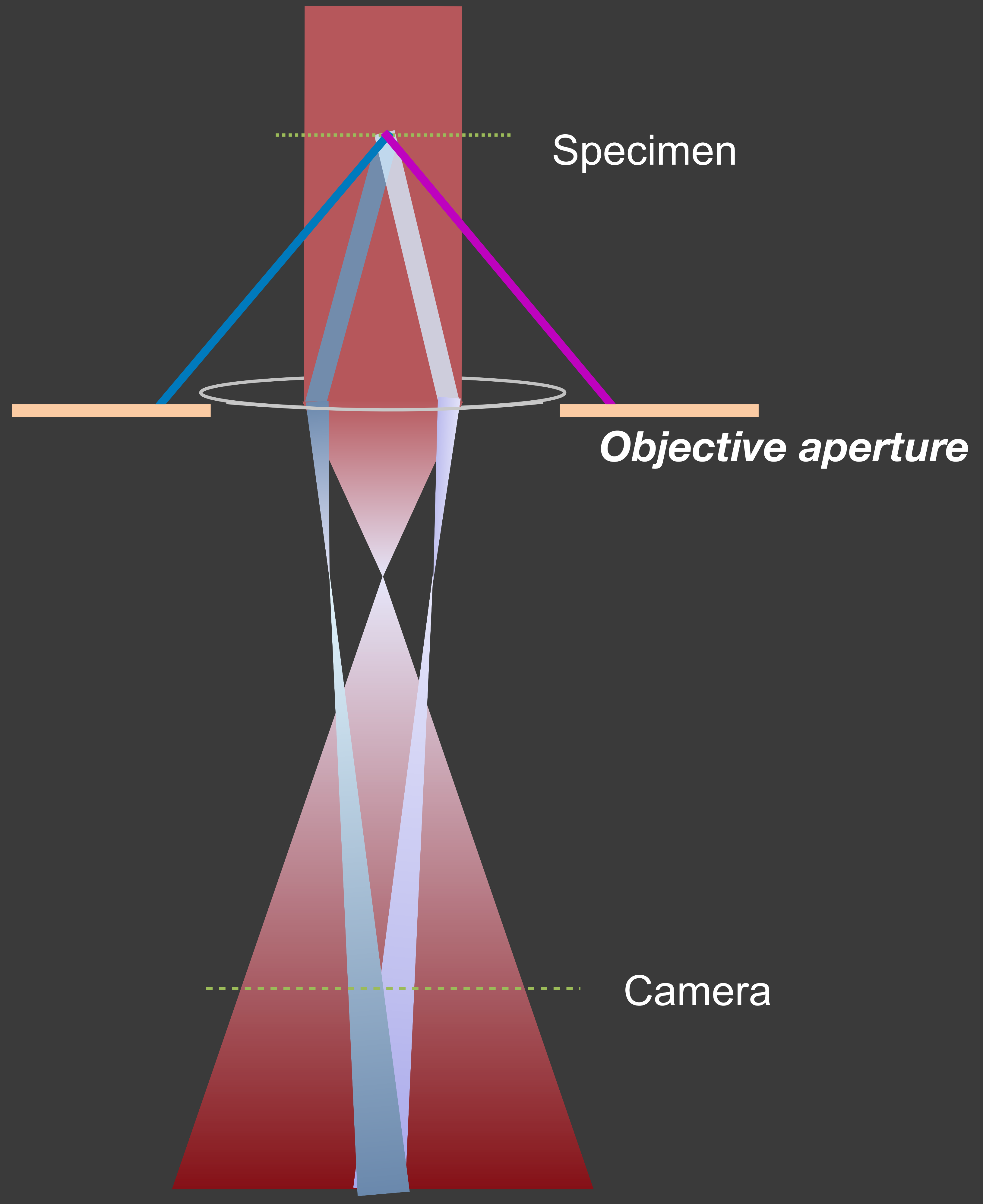
Electrons that pass very close to an atomic nucleus are scattered at high angles, and are caught by the objective aperture.

- The loss of these electrons results in a small amount of negative amplitude contrast.
- Its small magnitude $\alpha$ 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

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

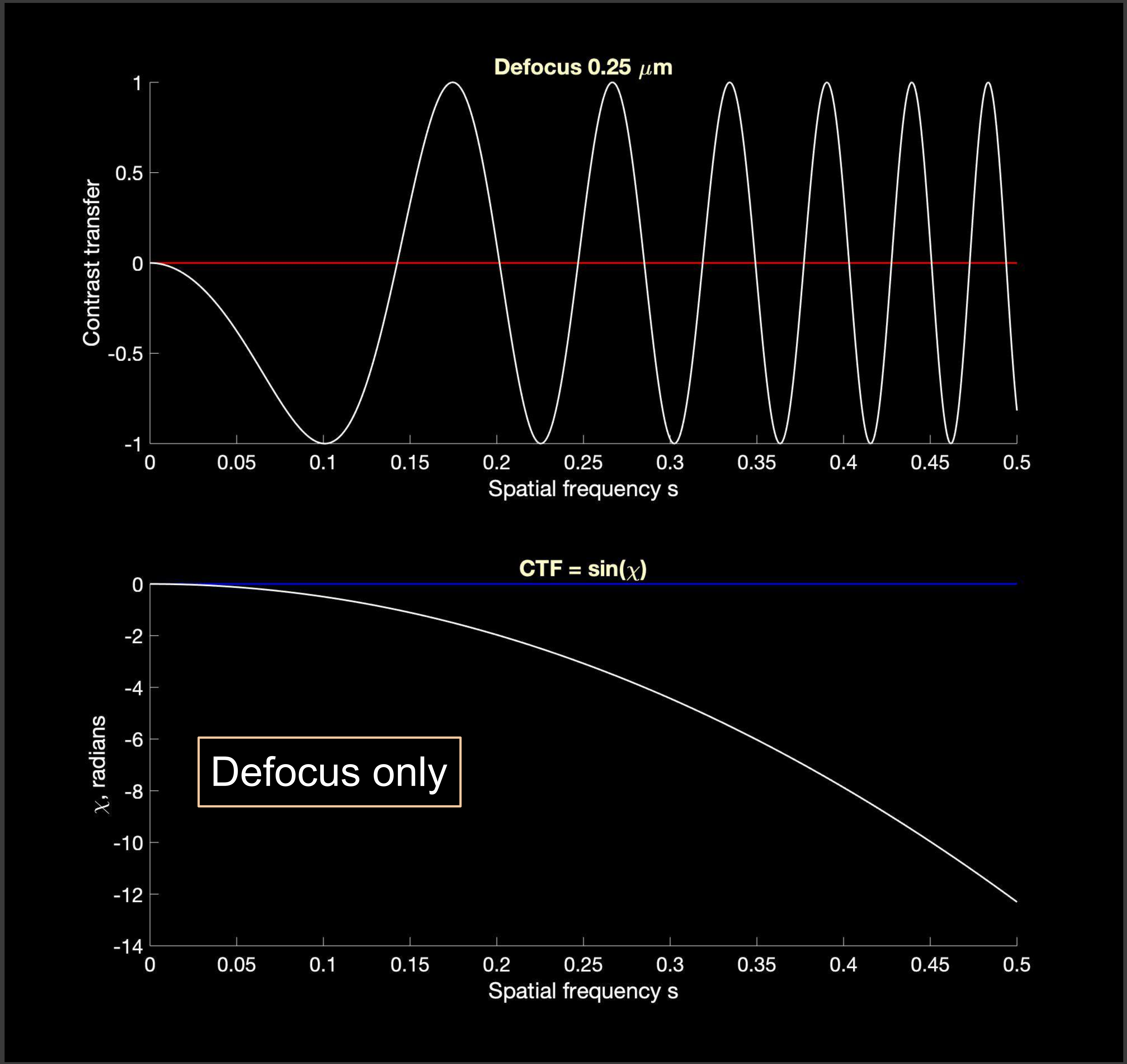
*defocus* *sphere abb.* *amplitude*



# The simple defocus contrast is what we've seen before

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

defocus sphere abb. amplitude



# Now adding in spherical aberration and amplitude contrast

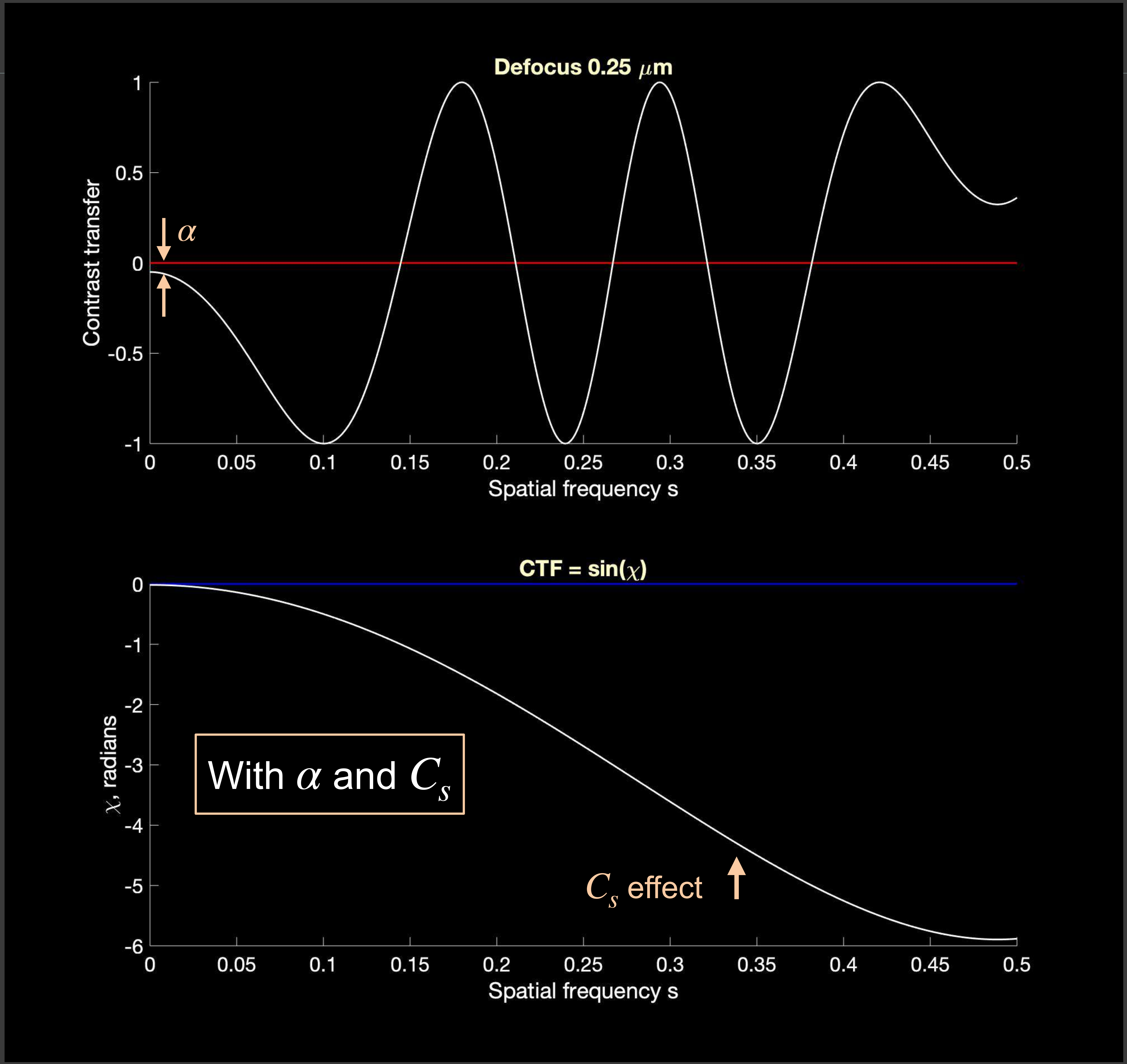
Here you can see why everyone uses underfocus: the amplitude contrast and defocus contrast are additive in this case.

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

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

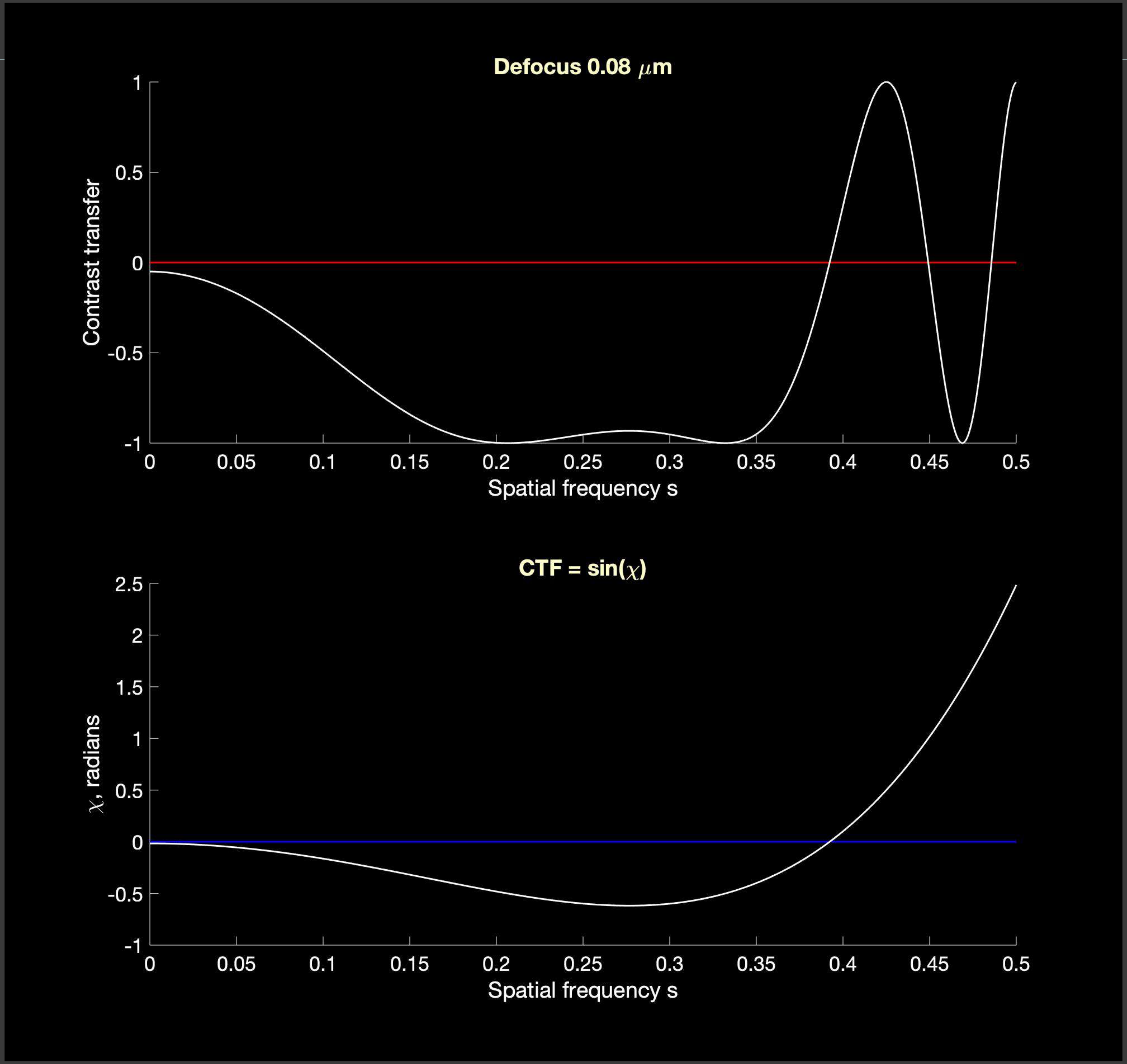
defocus sphere abb. amplitude



# 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.



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# The contrast transfer function for a grating of spacing $d$

The non-oscillating wavefunction

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

can be written as

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

The measured intensity is

$$|\Psi|^2 = |\Psi'|^2 = (\text{real part})^2 + (\text{imag part})^2$$
$$= \left[1 + \sin(\chi)\, \epsilon\phi(x)\right]^2 + \left[\cos(-\chi)\, \epsilon\phi(x)\right]^2$$
$$= \left[\boxed{1 + 2\sin(\chi)\, \epsilon\phi(x)} + \mathcal{O}\epsilon^2\right] + \left[\mathcal{O}\epsilon^2\right].$$

<u>In practice</u>

- We ignore the constant background intensity.
- Everyone ignores the factor of 2 also.
- So we say the transfer from phase shift to intensity change is

$$\boxed{\text{CTF} = \frac{\Delta\text{Intensity}}{\Delta\text{Electron phase}} = \sin(\chi)}$$

Grating object:

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

Electron propagation:

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

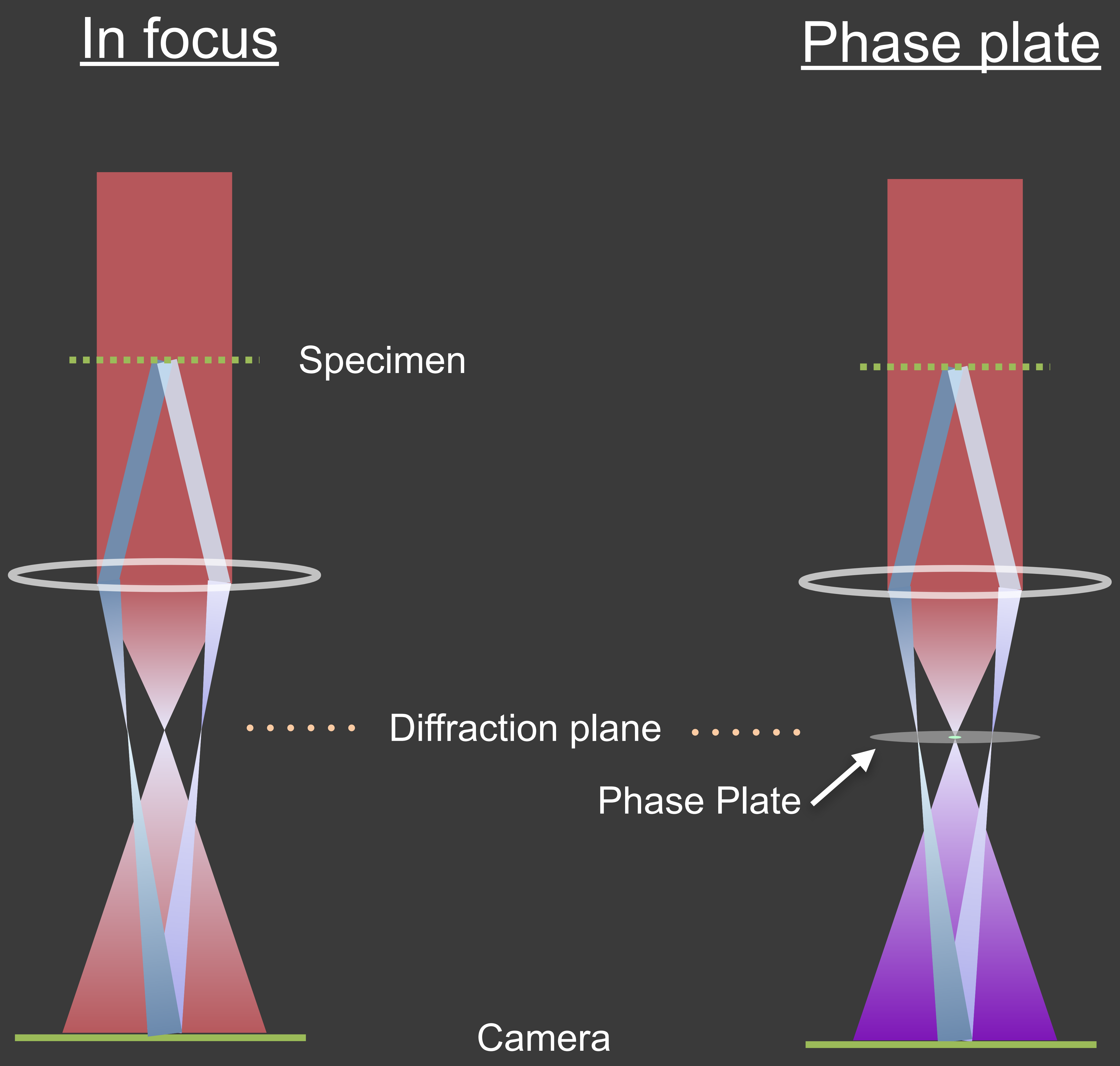
Diffracted wave path difference:

$$\zeta \approx z\lambda^2/2d^2$$

Wave aberration function:

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

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




The phase plate shifts the phase of the undiffracted beam $\Psi_0$ by some angle $\phi$.

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

If $\phi = 90^{\mathrm{o}}$ then

$$\mathrm{CTF} = -\cos(\chi)$$

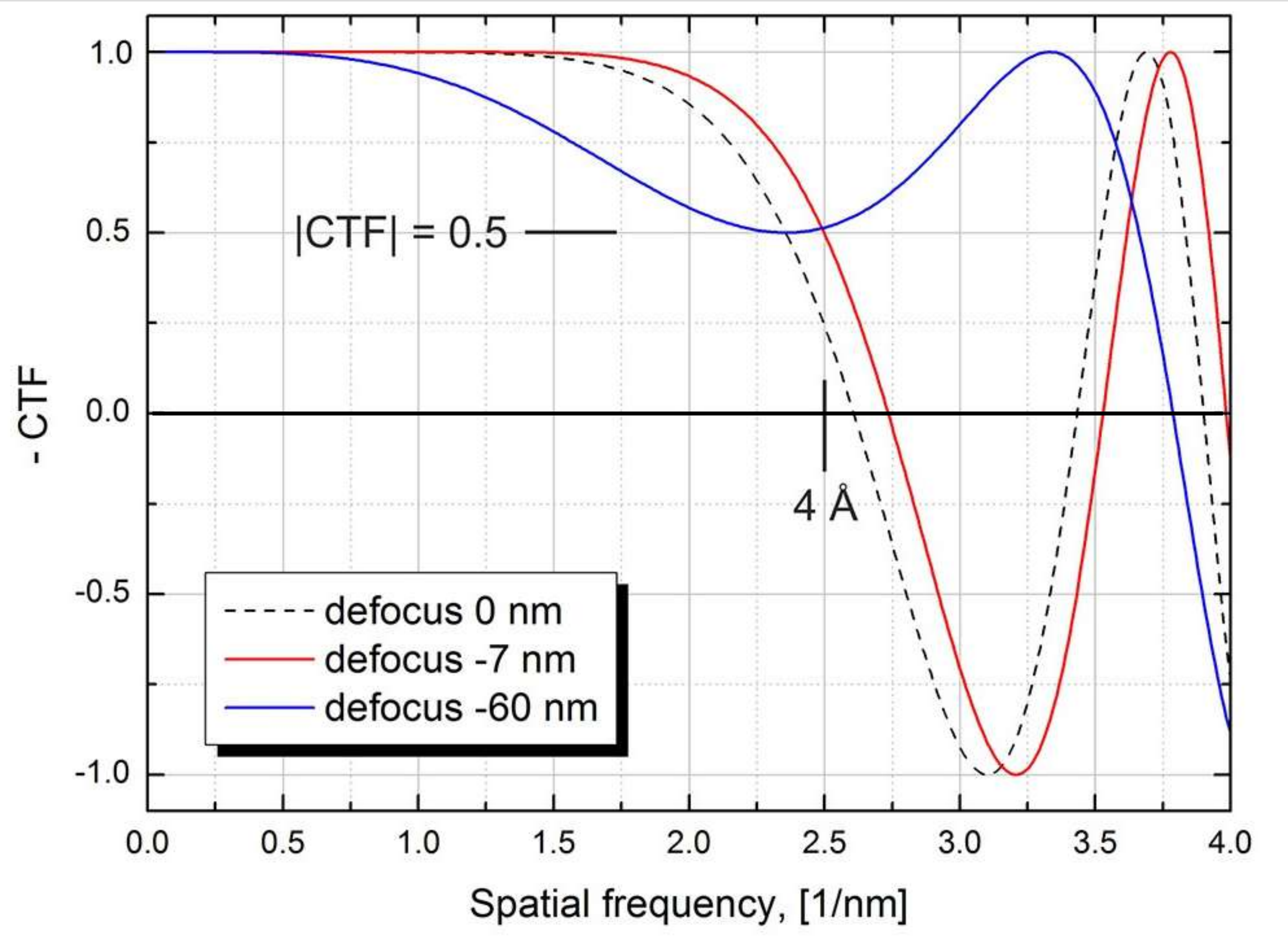
# The phase plate allows in-focus imaging, given precise focusing.

**Cryo-EM single particle analysis with the Volta phase plate**

**Radostin Danev*, Wolfgang Baumeister**

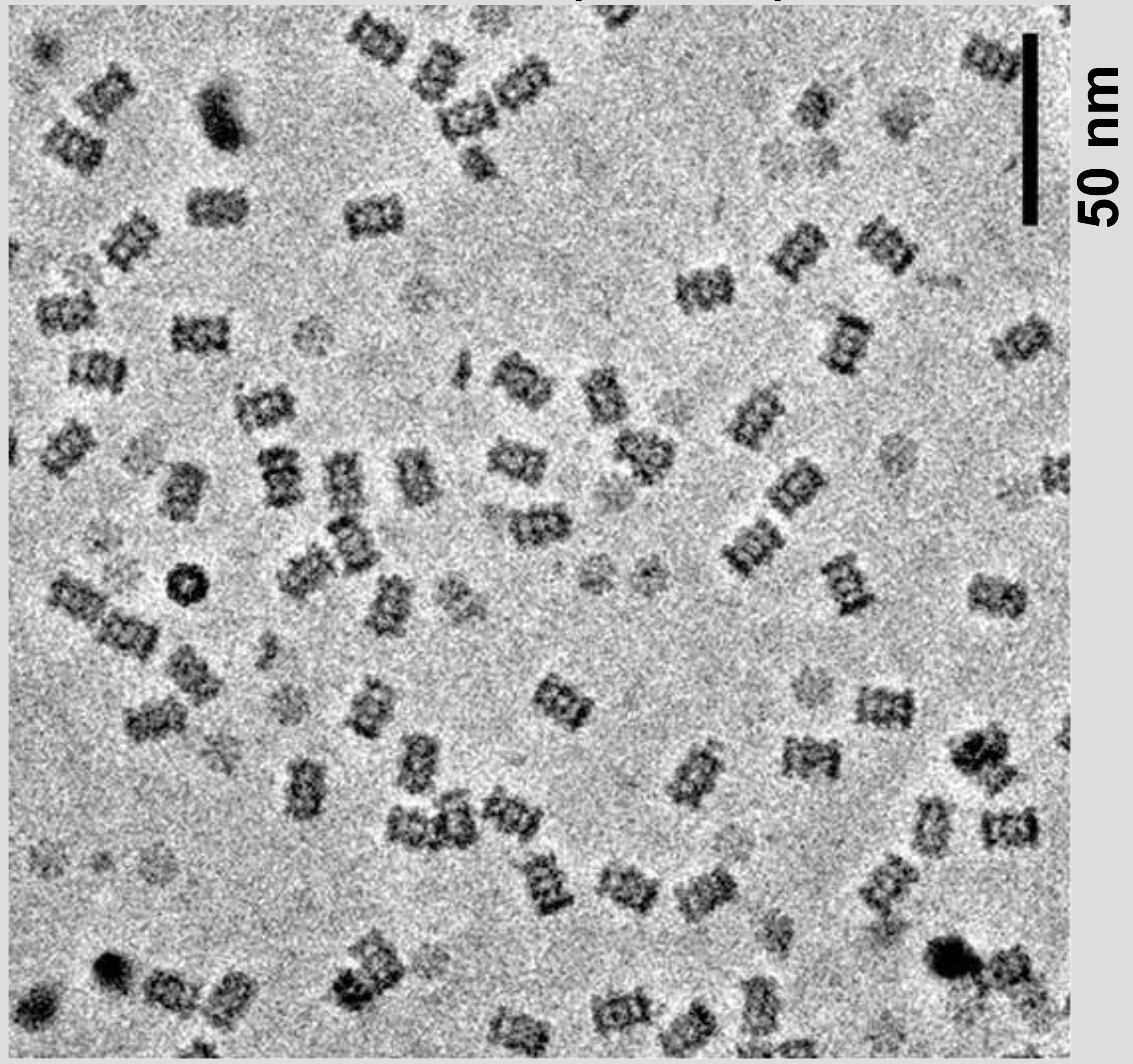
Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany

*eLife* 2016

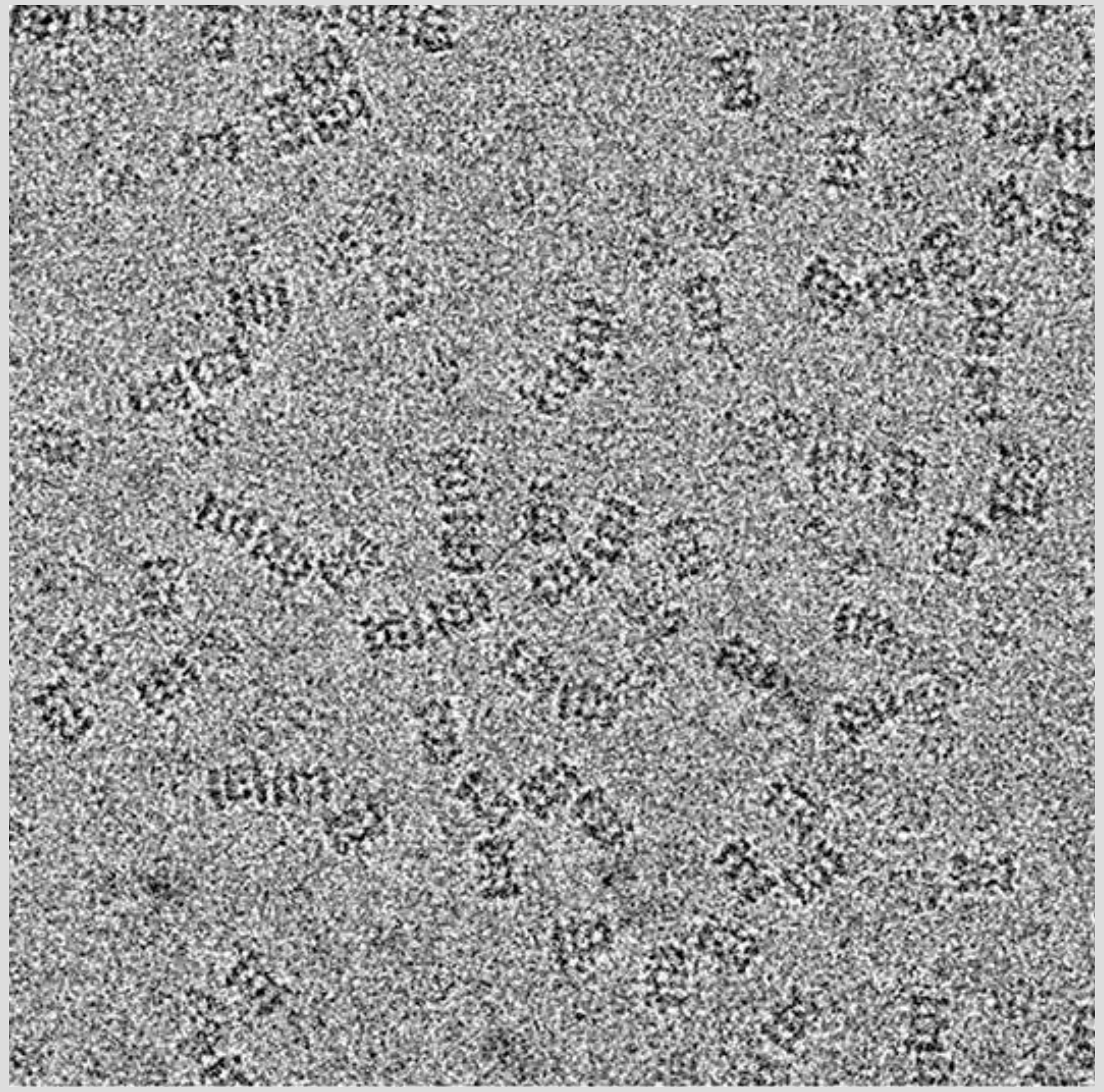



- The better low-frequency contrast makes particles much more visible.
- The defocus value must be precise within 60 nm in order to get 4 Å resolution.

In-focus phase plate

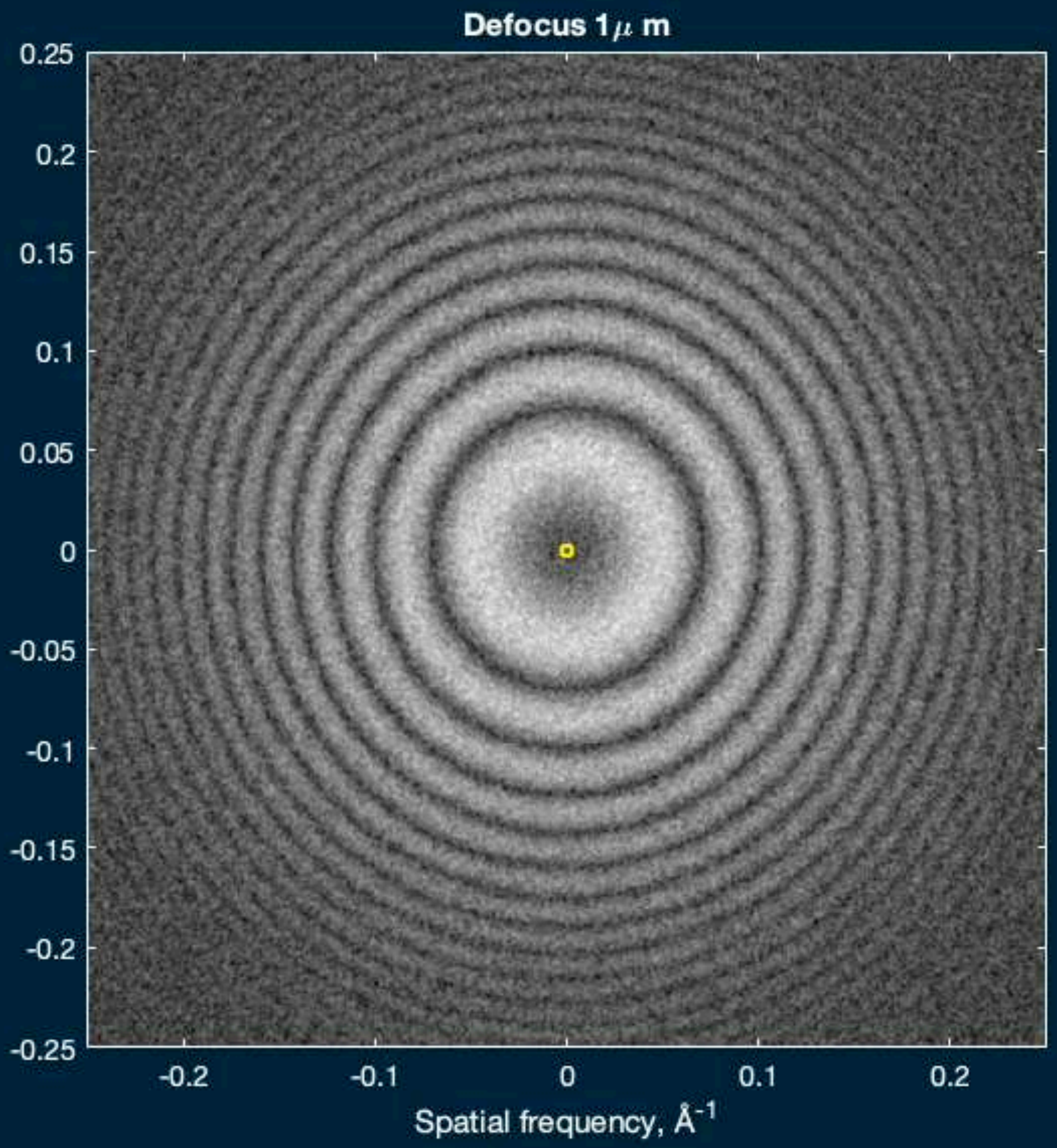



Defocus contrast



# The power spectrum describes the magnitude of Fourier components

Power spectrum




Grating at the spatial frequency