

Cryo-EM Principles

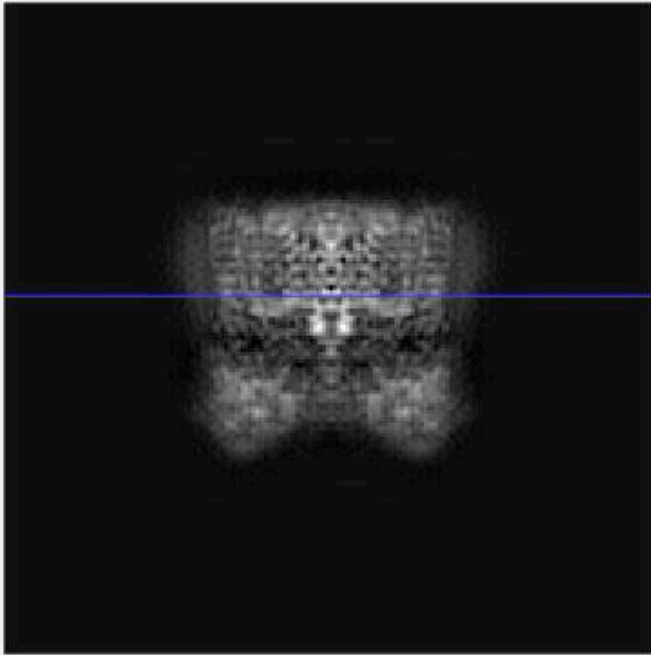
2.2

Defocus phase contrast

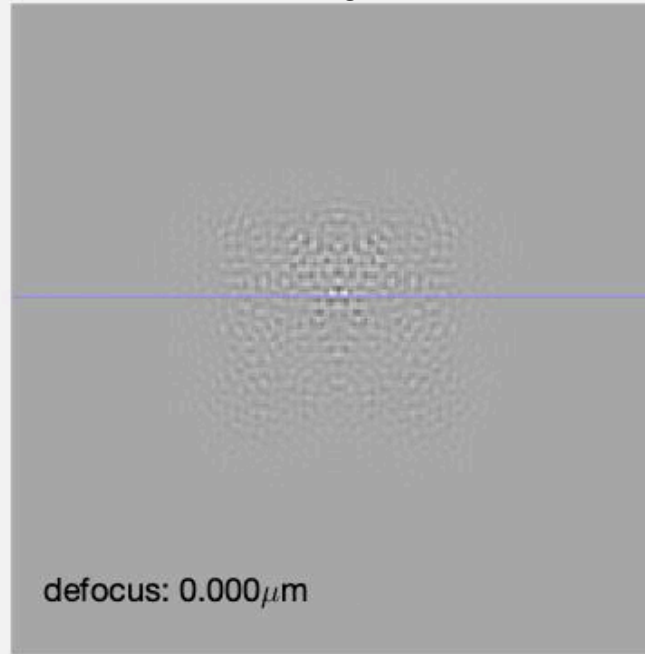
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Most cryo-EM data are acquired using defocus contrast

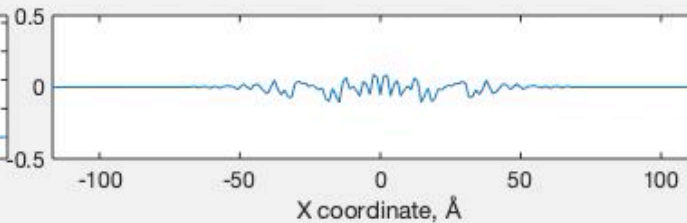
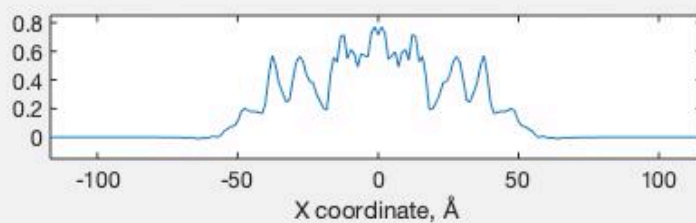
object



image



defocus: 0.000 μm



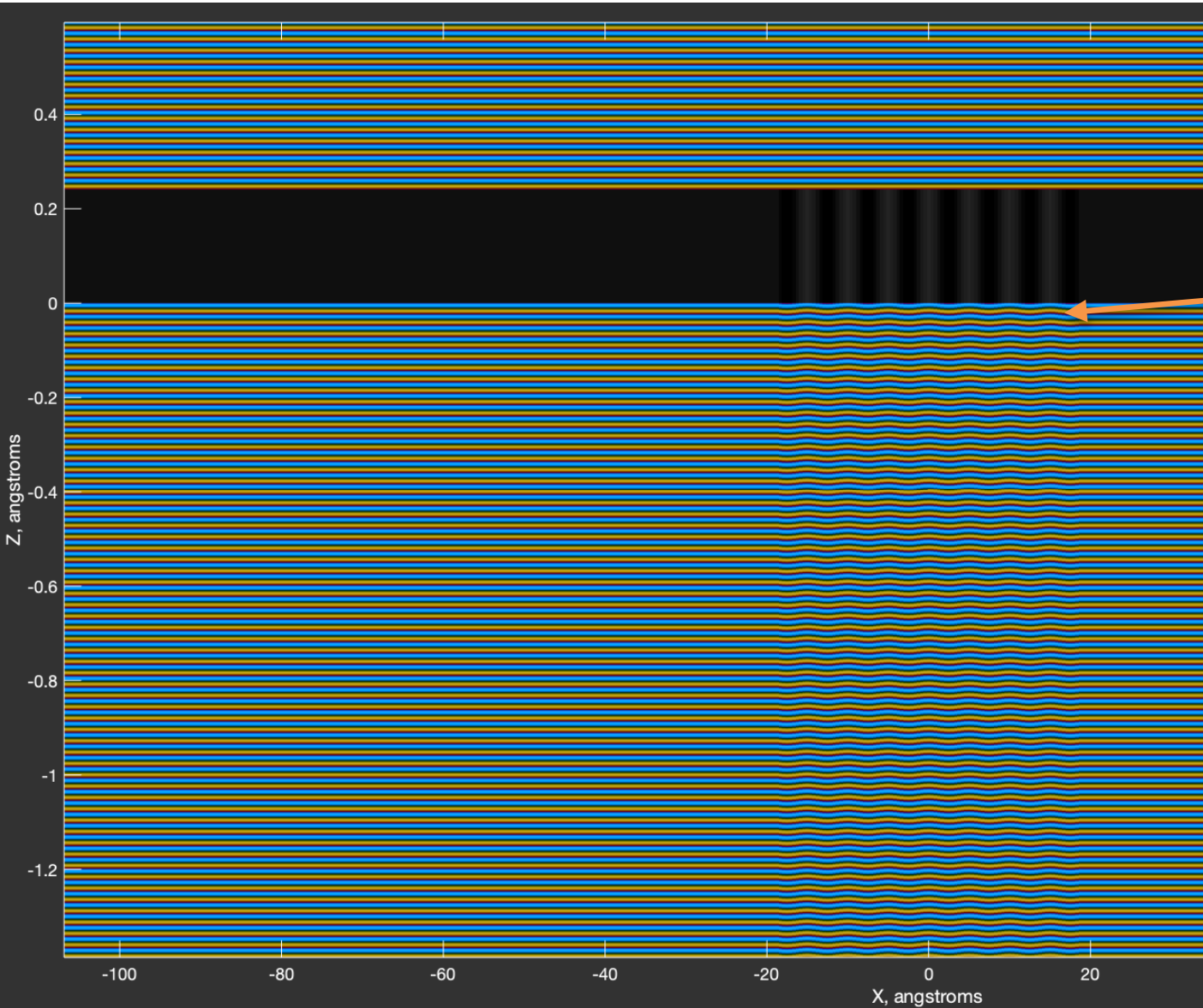
- At high defocus, high-resolution information in the image is strongly **delocalized**.
- Image processing can re-localize the signals, but at most **only about half of the theoretical contrast** is preserved by defocusing.
- “Underfocus” means decreasing the strength of the objective lens, effectively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

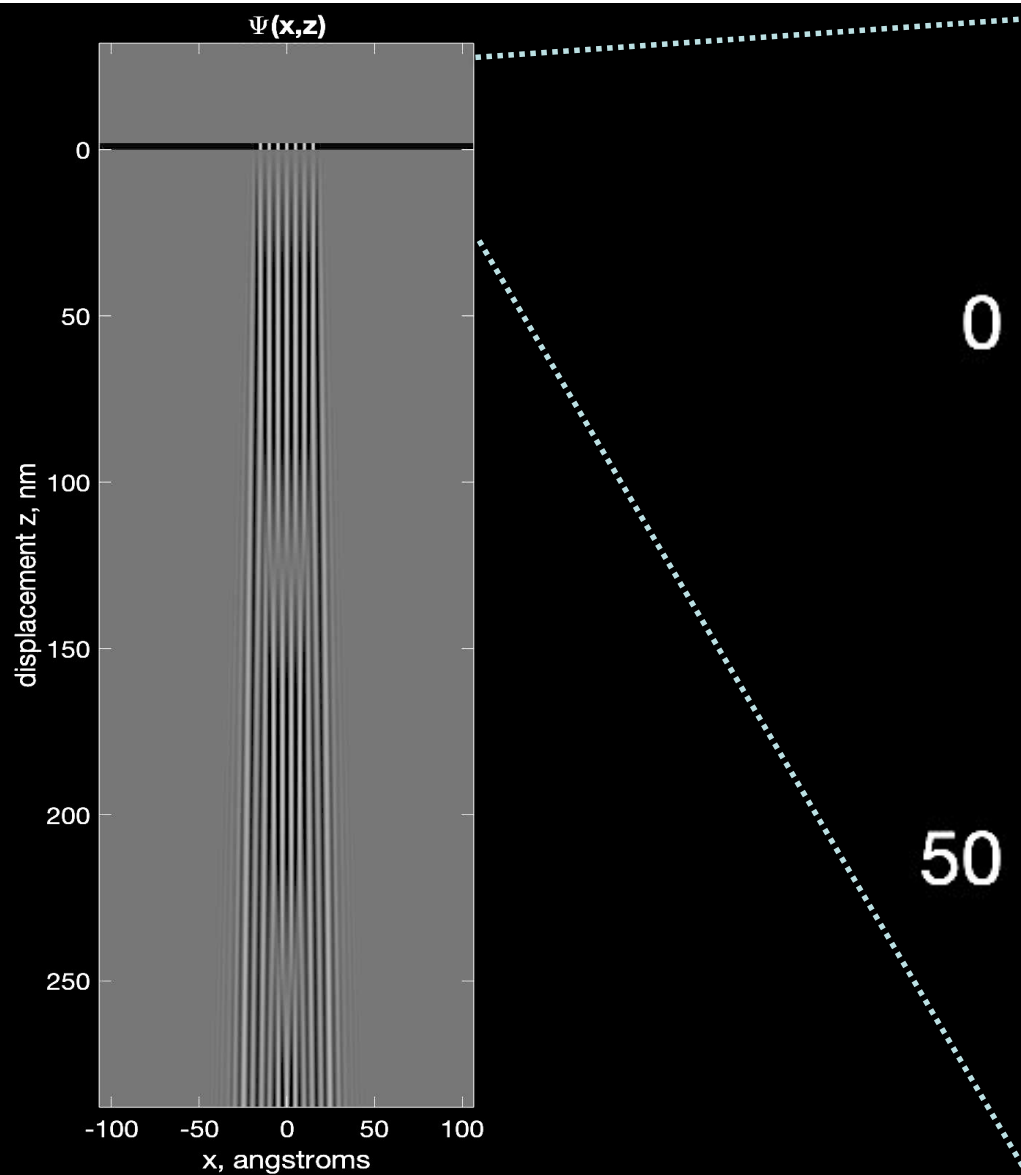
Effect of a phase object

at $z = 0$,
$$\Psi = \Psi_0 e^{i\epsilon\phi(x)}$$

But the weak phase approximation says

$$\Psi = \Psi_0(1 + i\epsilon\phi(x))$$



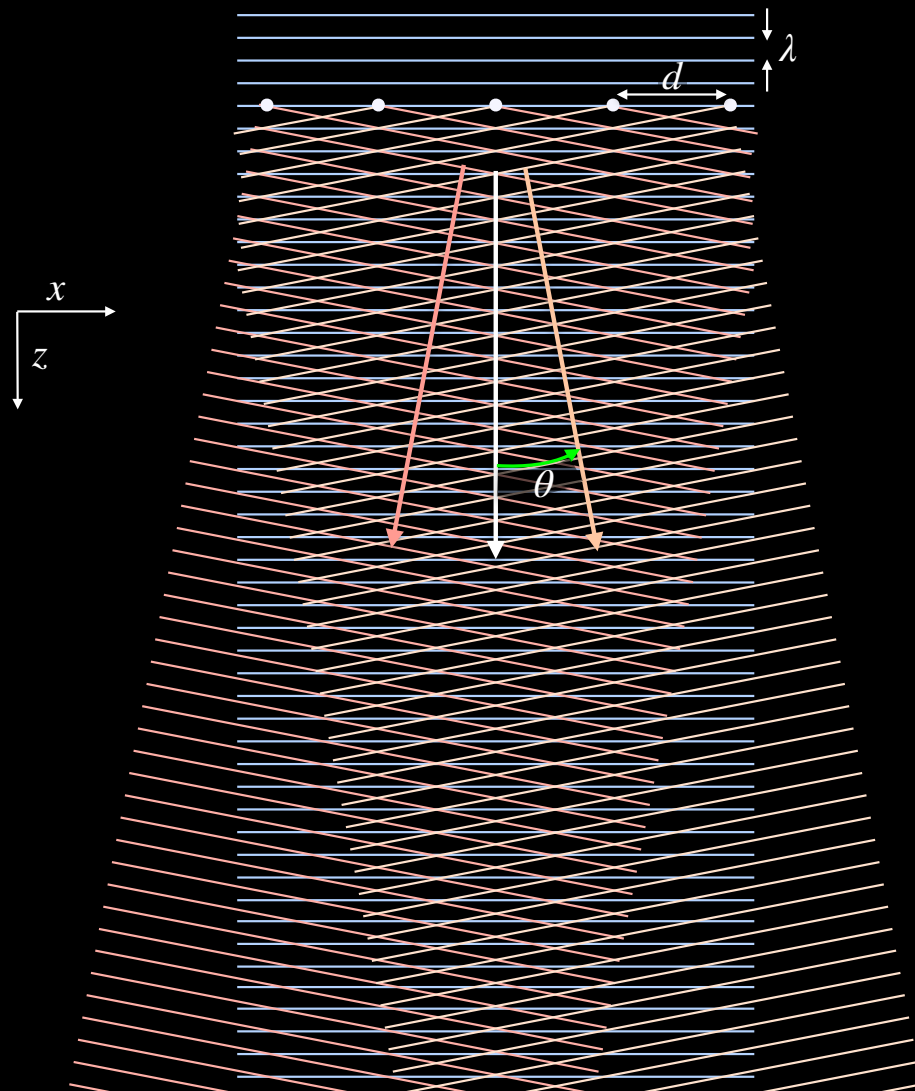


Effect of a phase object

0

50

Classical diffraction from a grating



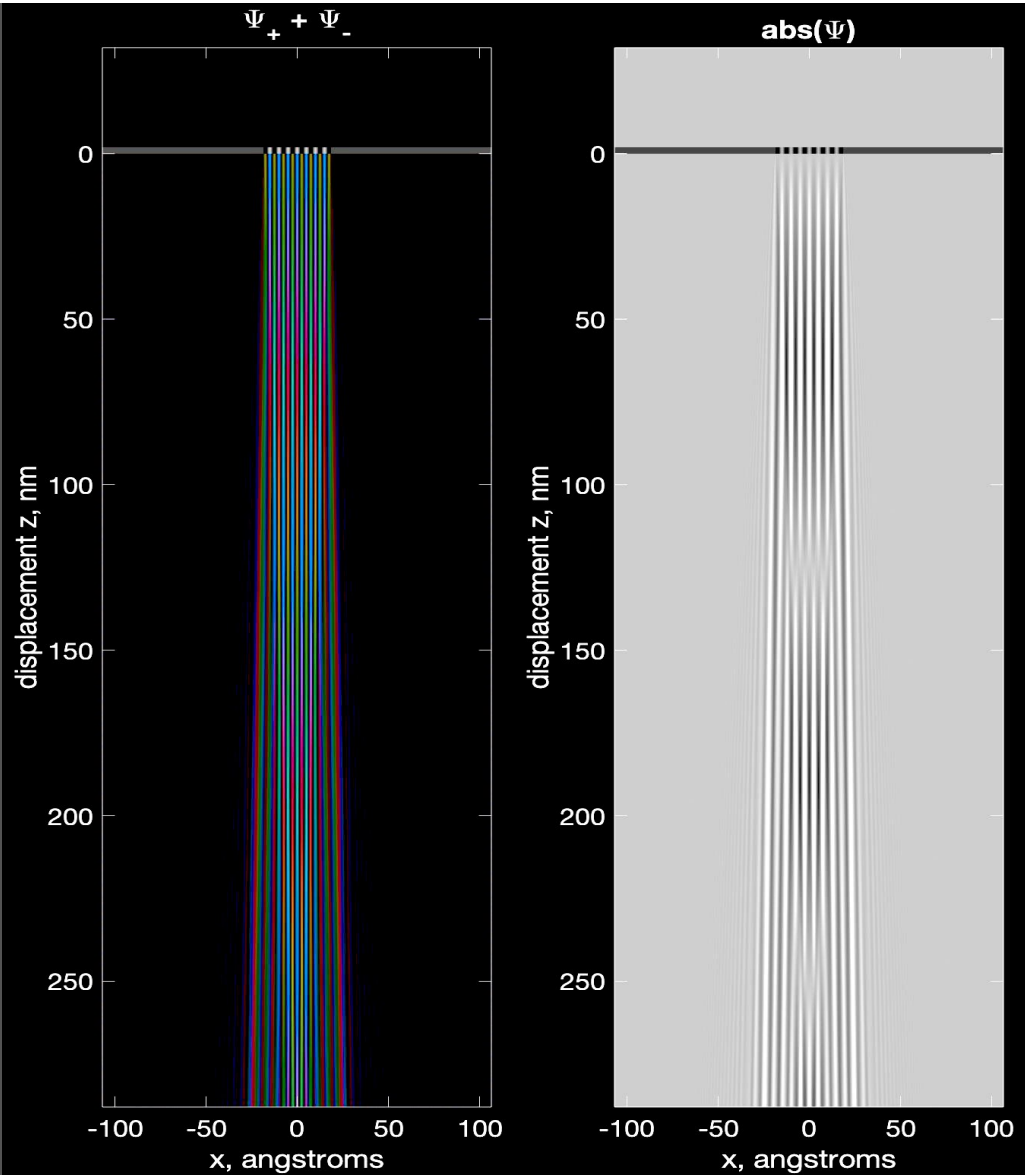
$$s = \sin \theta = \frac{\lambda}{d}$$

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

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

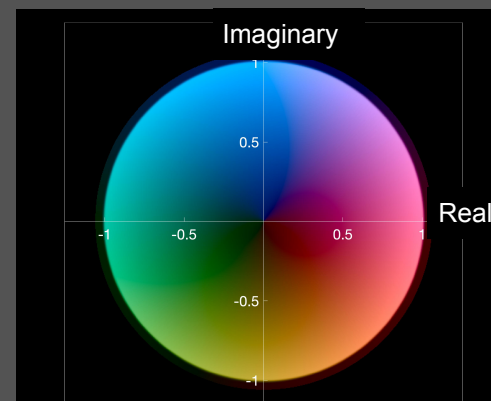
$$\Psi_+ = \frac{\epsilon}{2} e^{ik(cz+sx)}$$

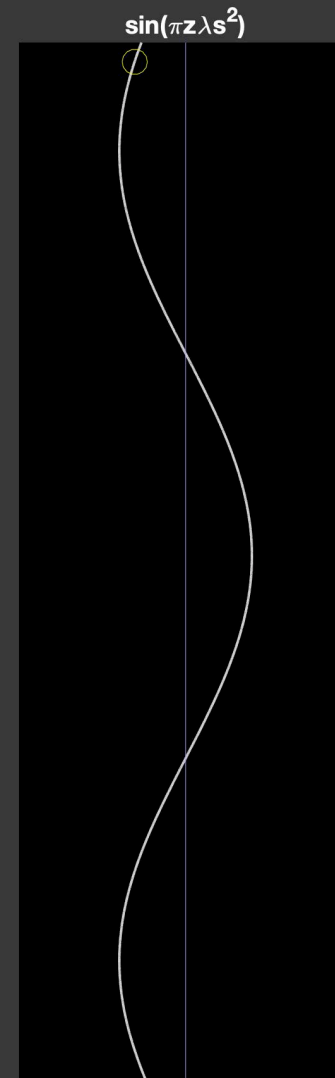
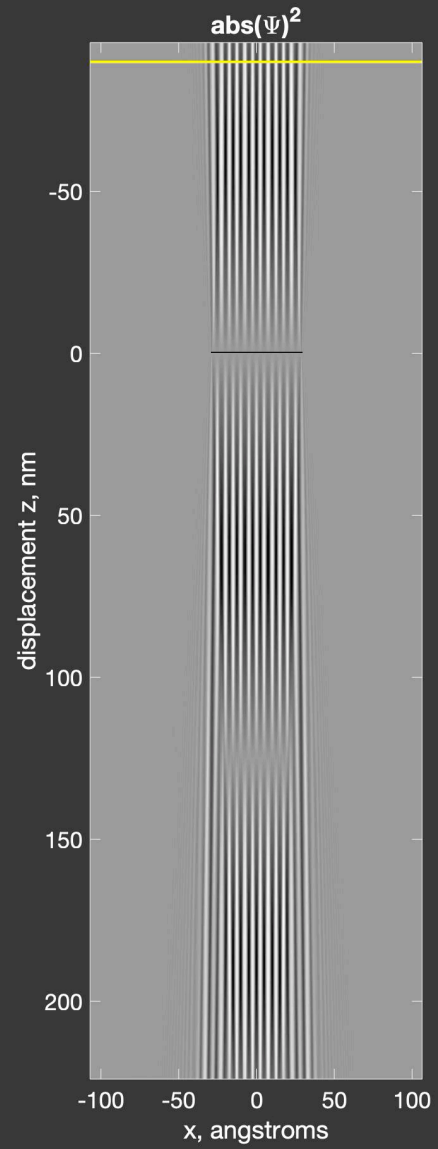
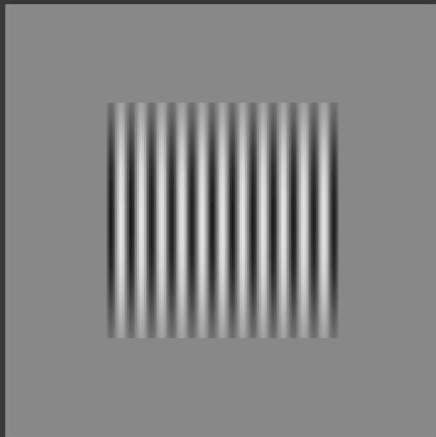
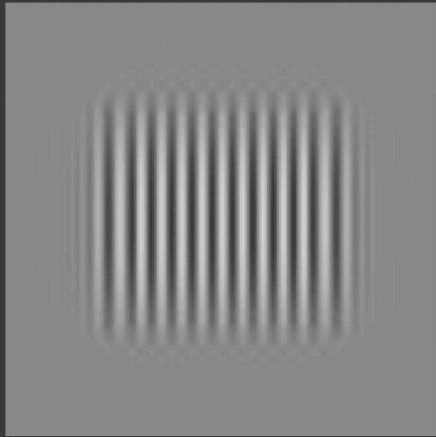
$$\Psi_- = \frac{\epsilon}{2} e^{ik(cz+sx)}$$



$$\begin{aligned}
 & (\Psi_+ + \Psi_-)/\Psi_0 \\
 &= \underbrace{ie^{i(c-1)kz}}_{ie^{-i\pi\lambda z/d^2}} \underbrace{\epsilon \cos(2\pi x/d)}_{\epsilon\phi(x)}
 \end{aligned}$$

$$\begin{aligned}
 & |\Psi_0 + \Psi_+ + \Psi_-|^2 \\
 &= 1 + 2 \sin(\pi\lambda z/d^2) \cdot \epsilon\phi(x)
 \end{aligned}$$





“Underfocus” is focusing the objective lens above the specimen