Phase contrast 1933

Frits Zernike

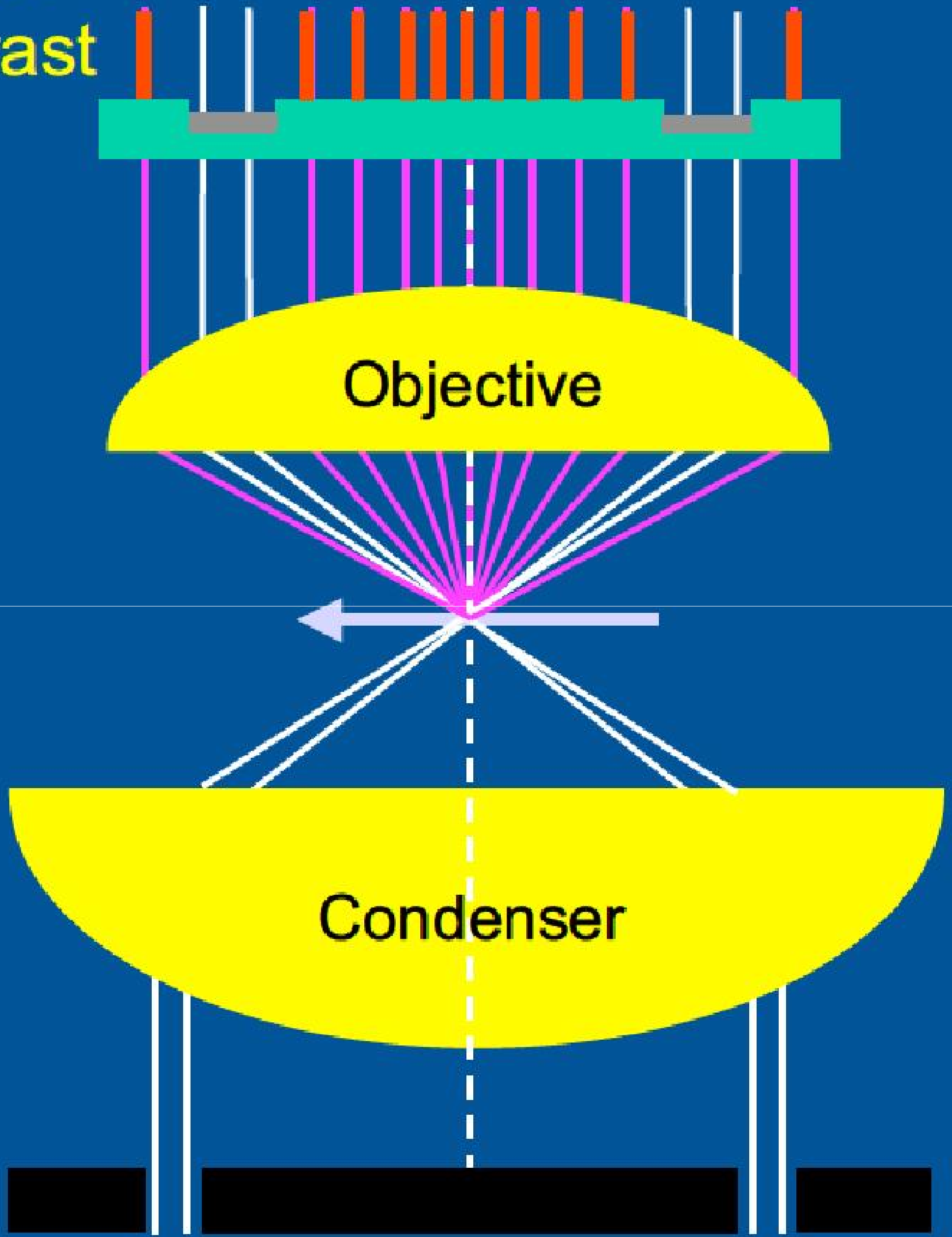
1886 - 1966

Chromosomes of *Chironomus*

Phase contrast Kurt Michel 1942


Phase contrast
© Peter Evennett

Phase contrast



Phase plate retards scattered light another ¼λ, providing ½λ phase difference

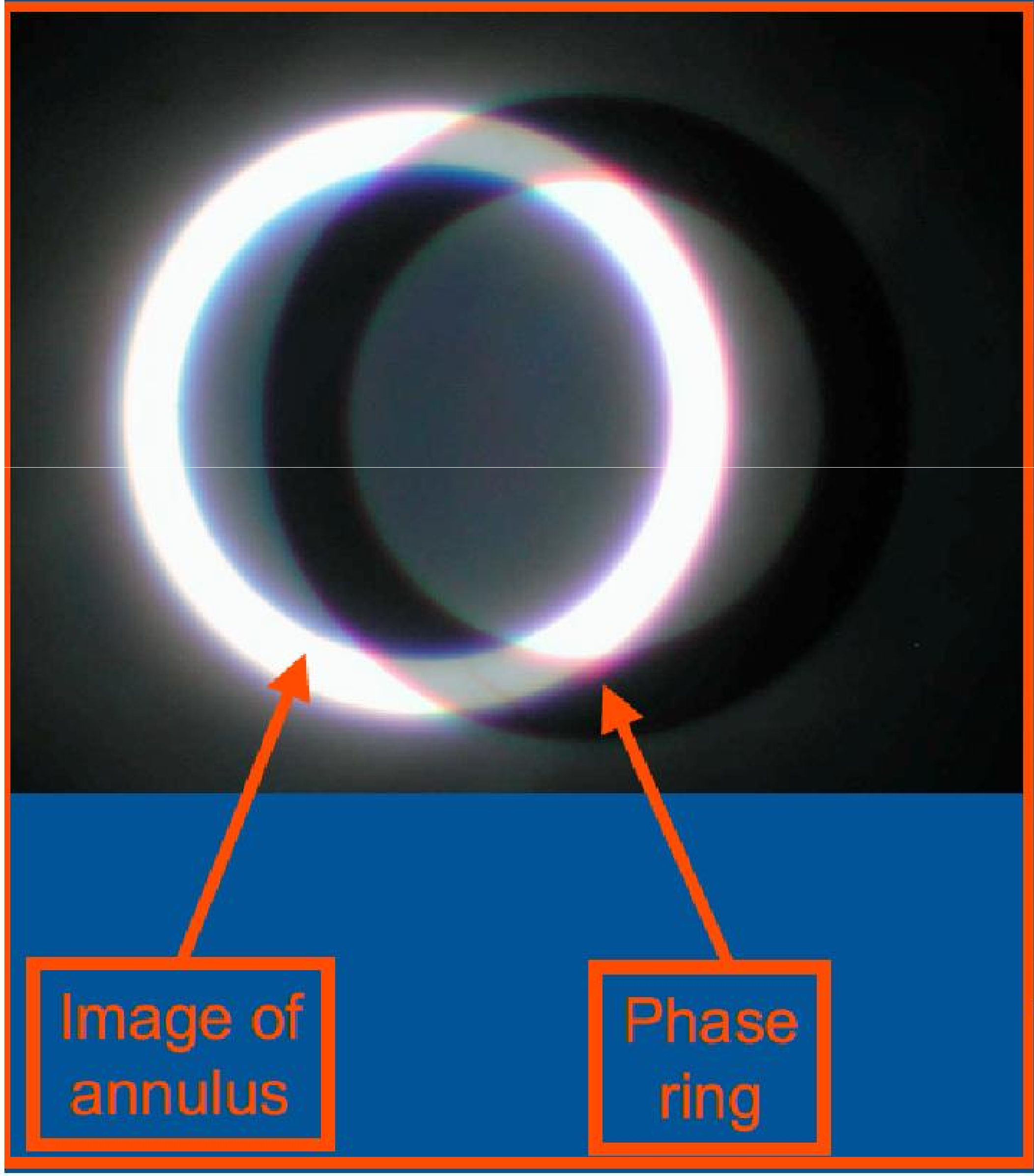
Specimen scatters light into objective

and retards it a little - about ¼λ

Illuminating annulus in front focal plane of condenser

Adjustment of illuminating annulus

as seen in the back focal plane of the objective



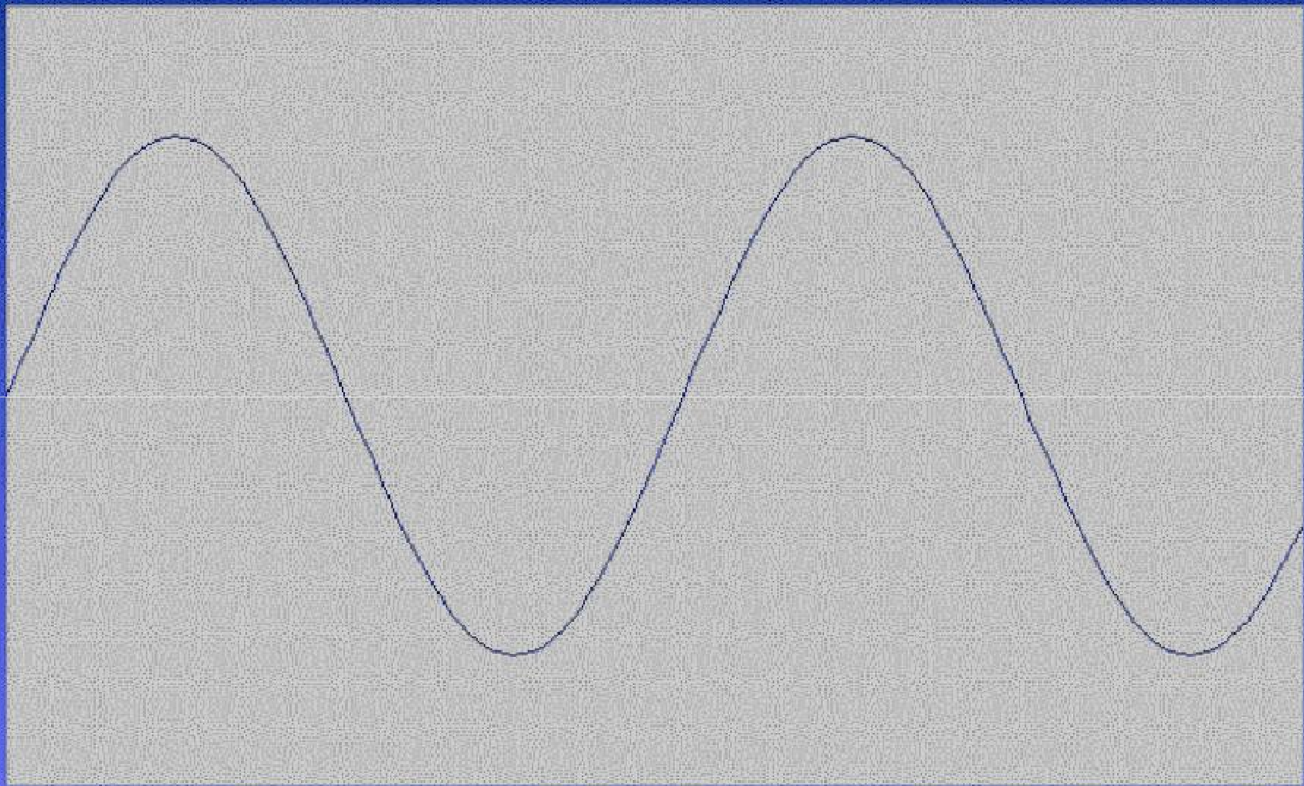
Illuminating annulus adjusted so that its image coincides with phase ring

Phase contrast set

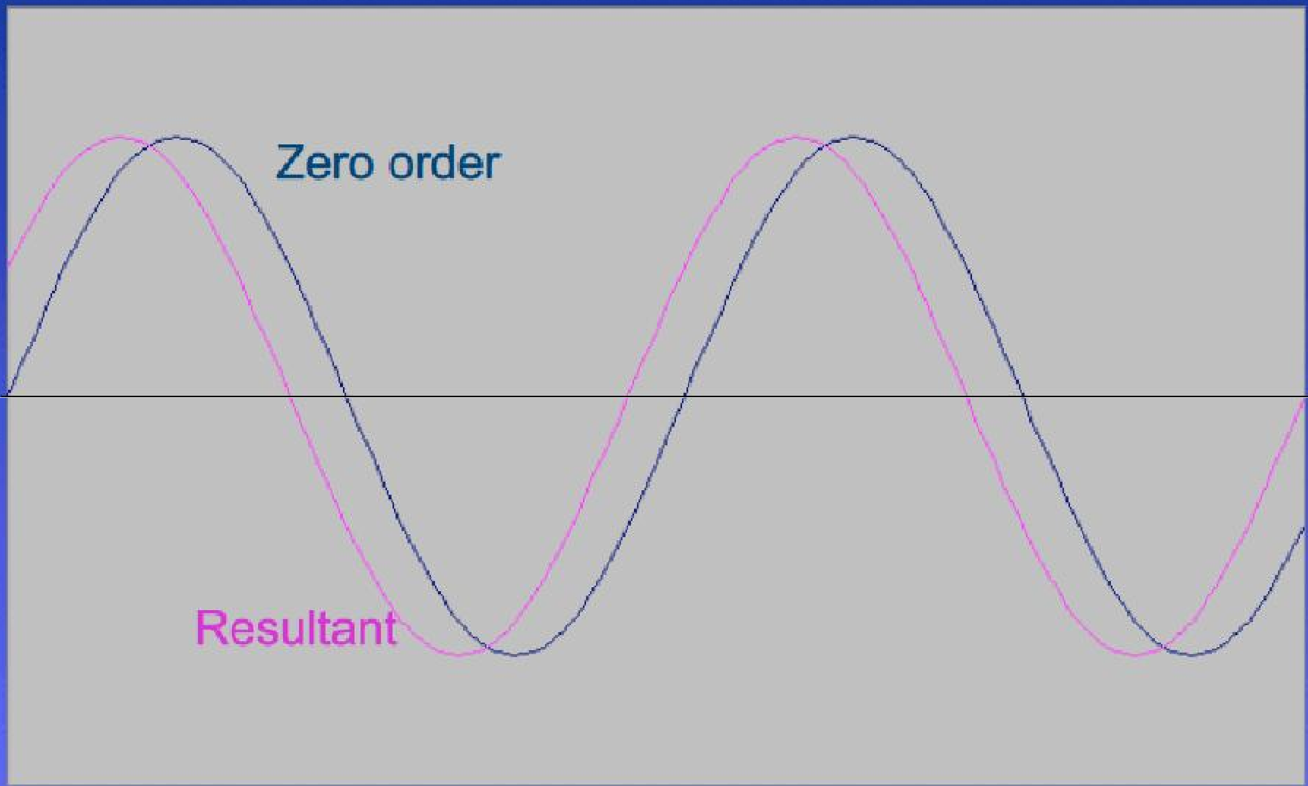
Phase contrast

- Because they consist of areas of different refractive index from their surroundings, transparent, non-absorbing objects produce small differences in the *phase* of light which encounters them, but only small differences in its *amplitude*.
- Thus they are invisible in the microscope image.
- Phase contrast is a technique for converting an *invisible image* into a visible image.

Unmodified ‘zero-order’ beam

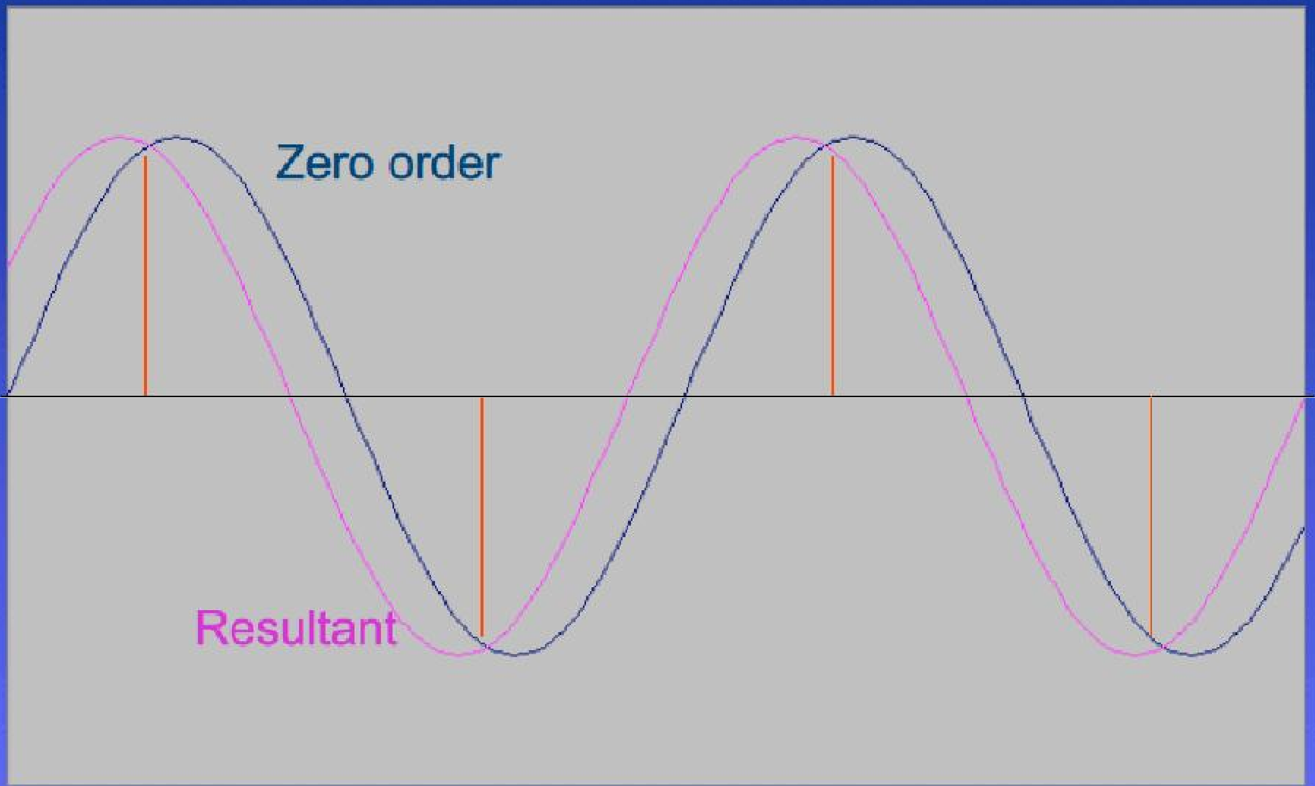


Resultant beam in image of non-absorbing object

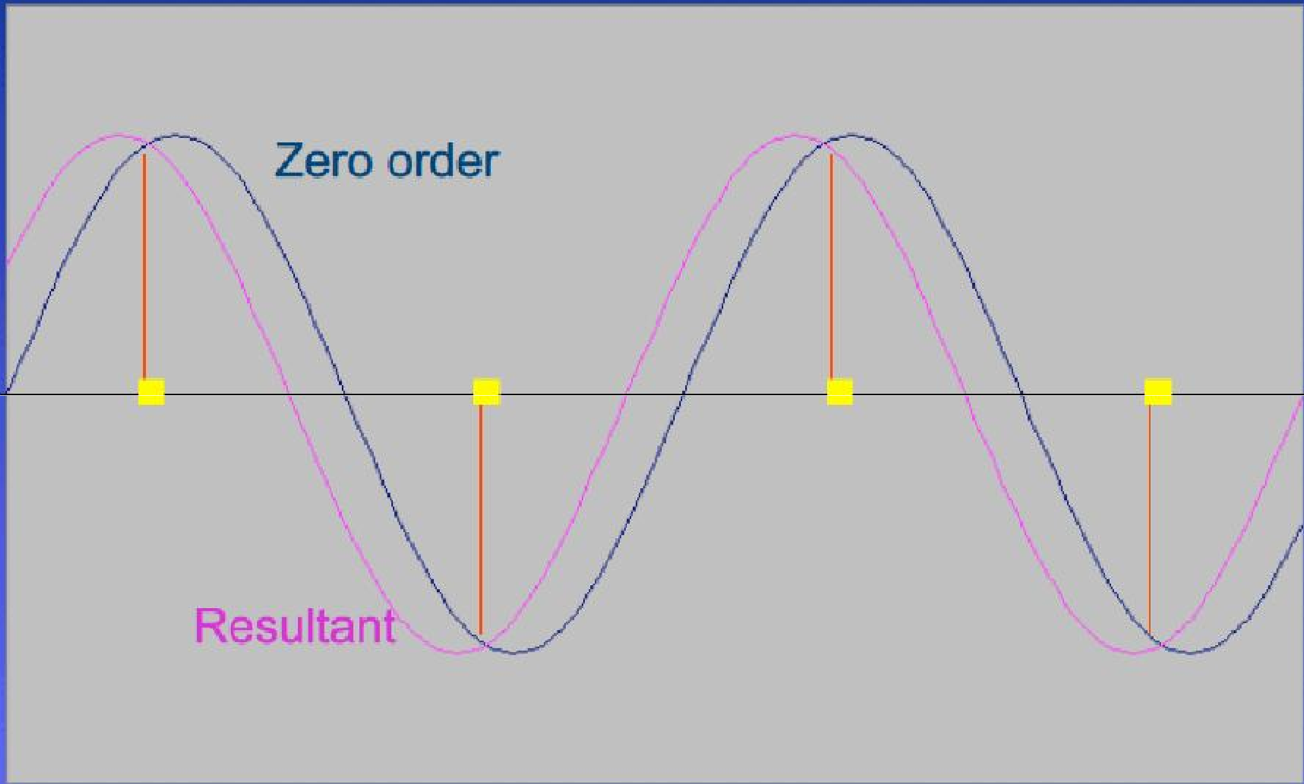


Resultant beam is slightly retarded from zero-order beam

Positions where amplitudes are equal

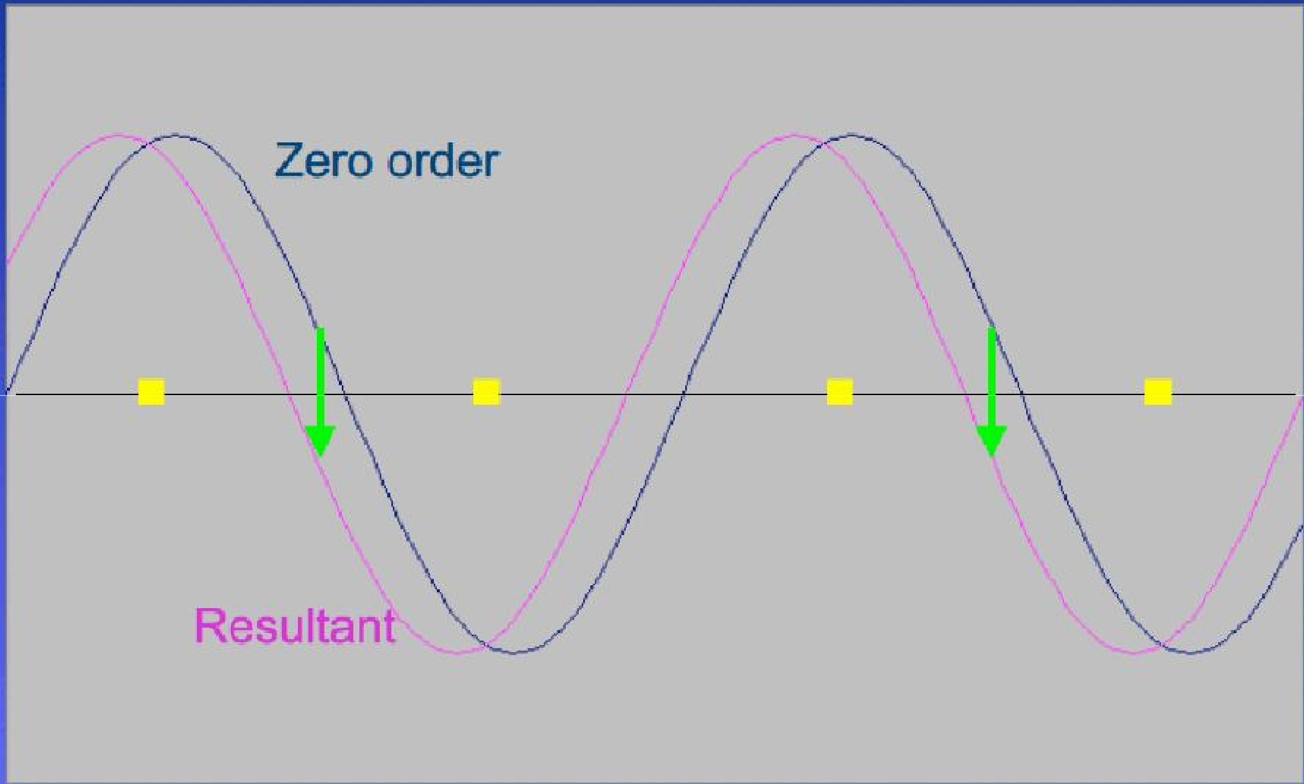


Positions where amplitudes are equal



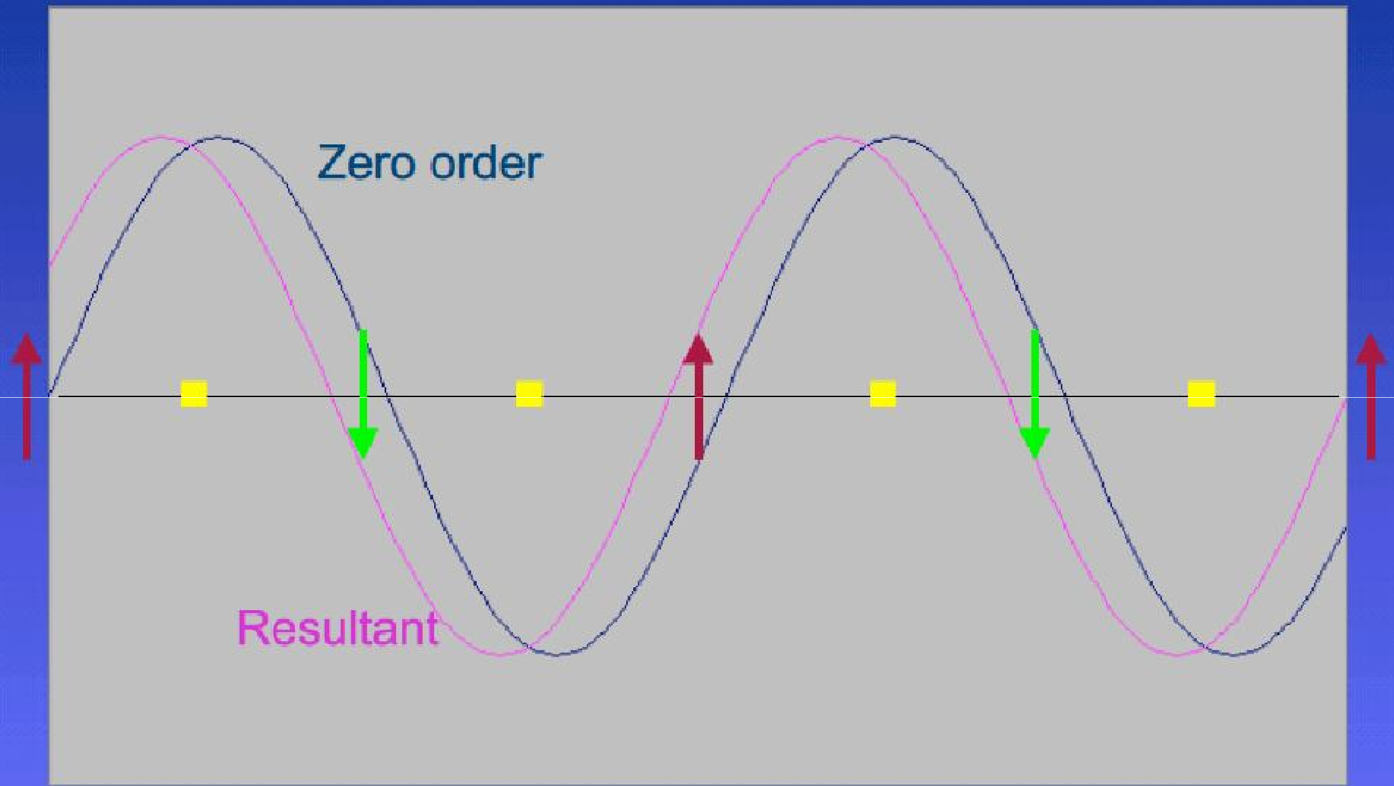
In these positions the diffracted ray must have a value of zero

Positions where amplitude of resultant is *less* than that of zero order



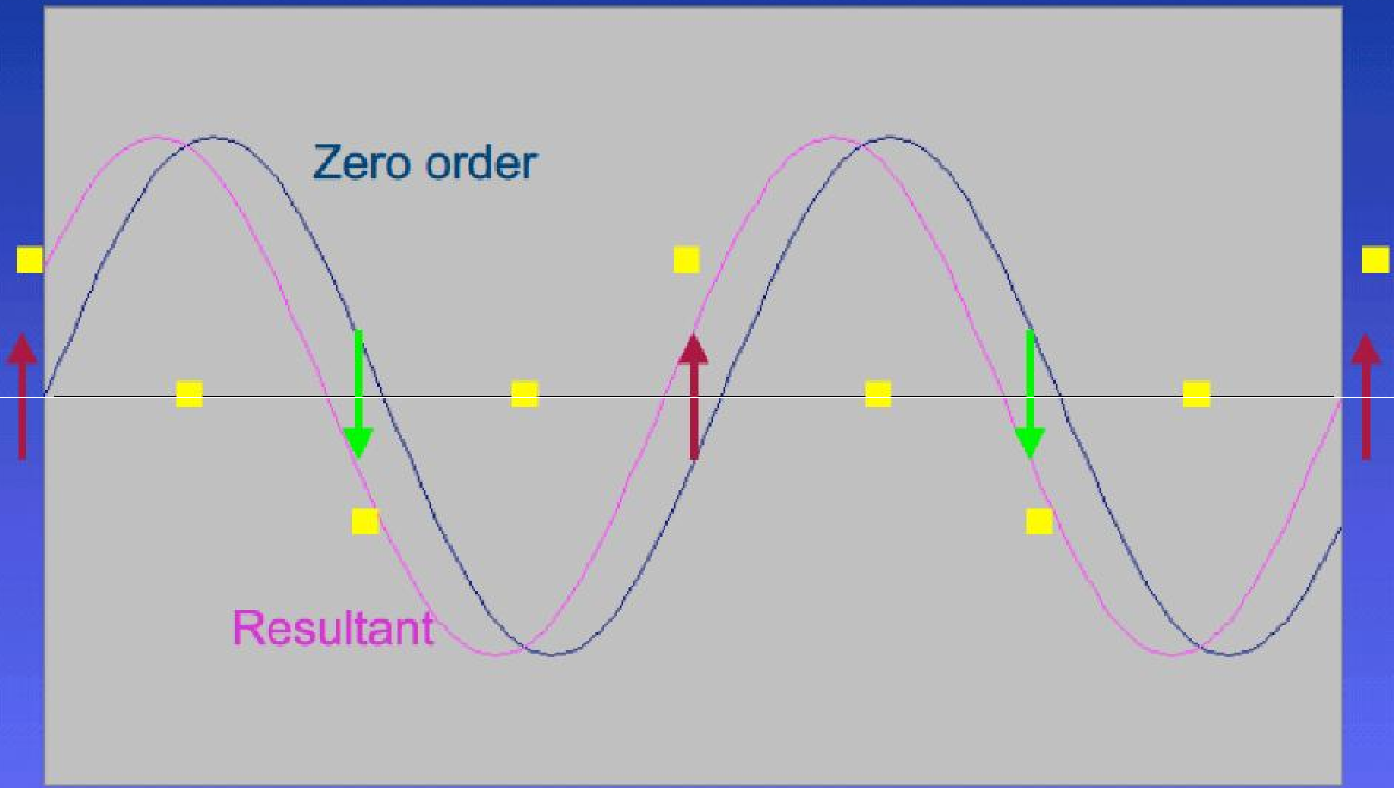
In these positions the diffracted ray must have a negative value

Positions where amplitude of resultant is *greater* than that of zero order

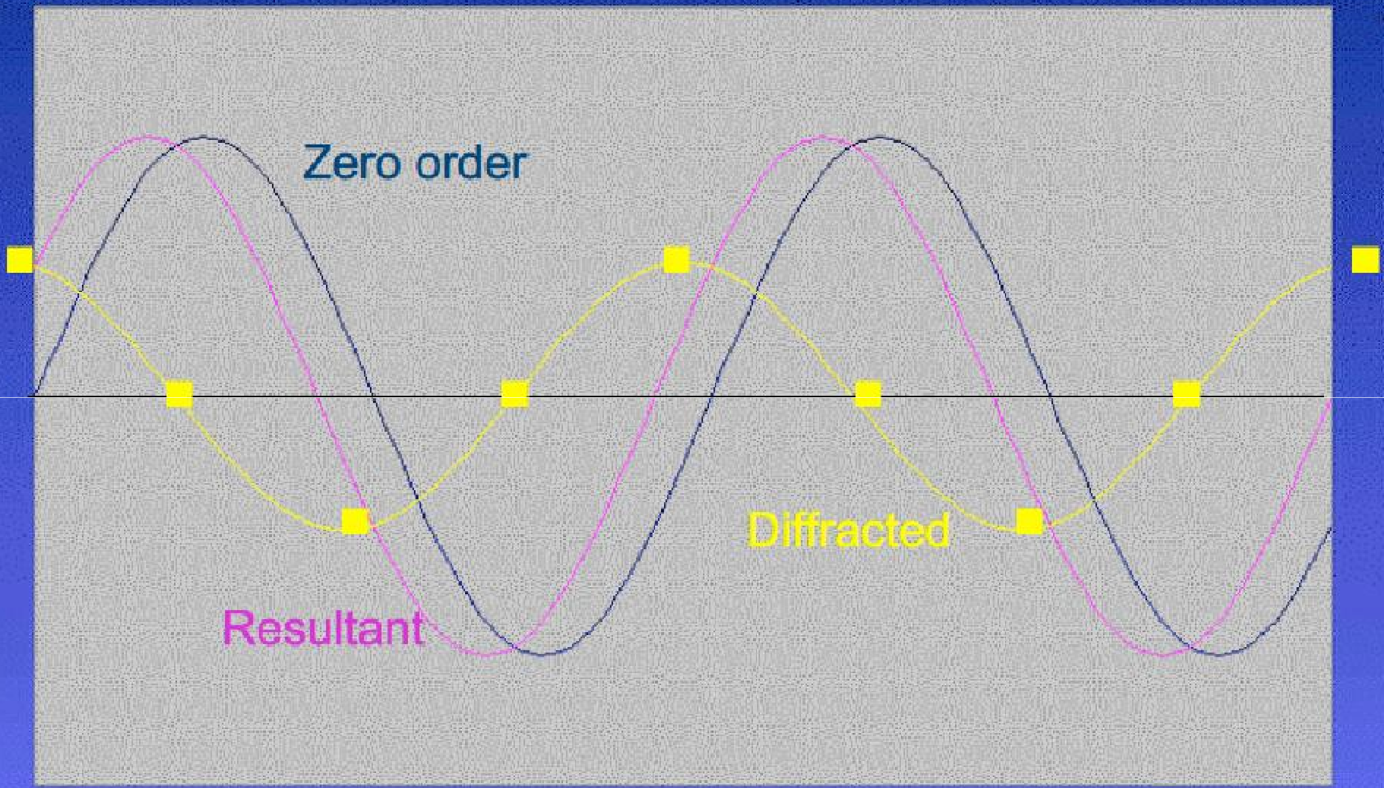


In these positions the diffracted ray must have a positive value

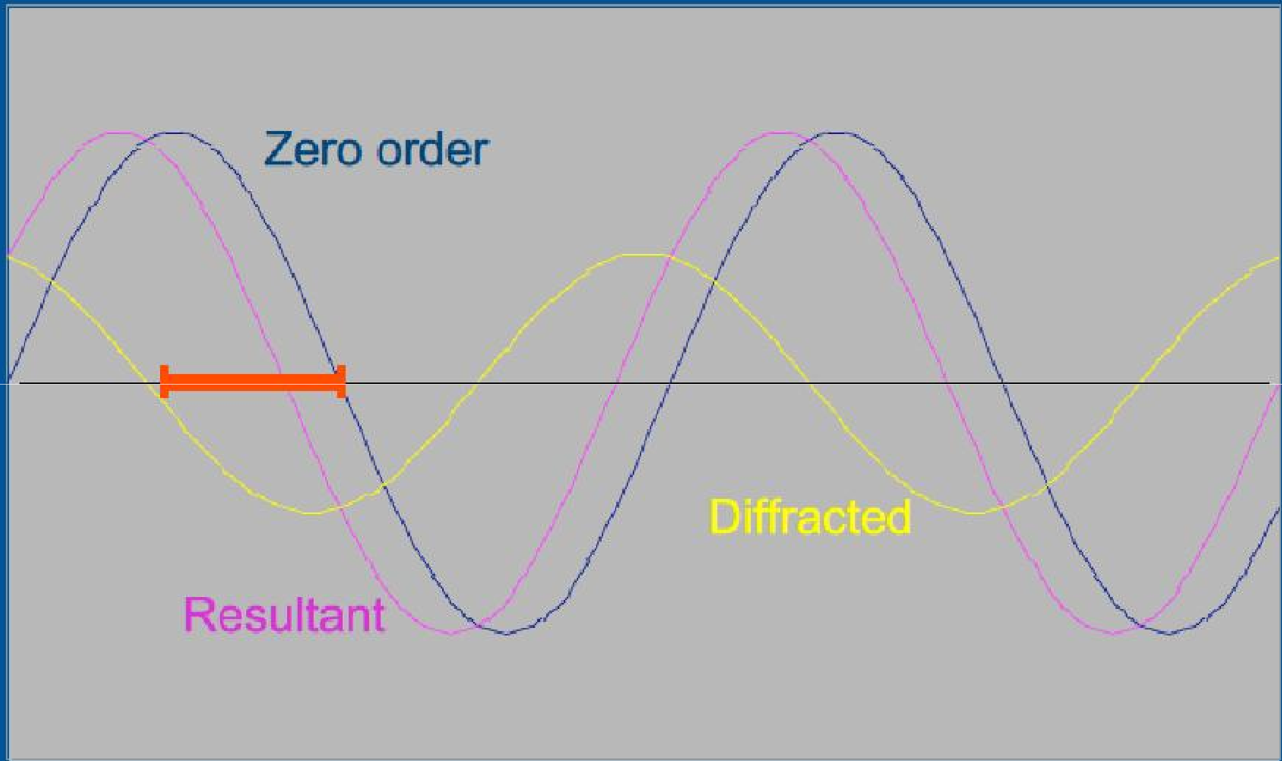
Points for plotting the diffracted ray



Diffracted ray required to convert zero order into resultant



Diffracted ray is one quarter wavelength behind zero order



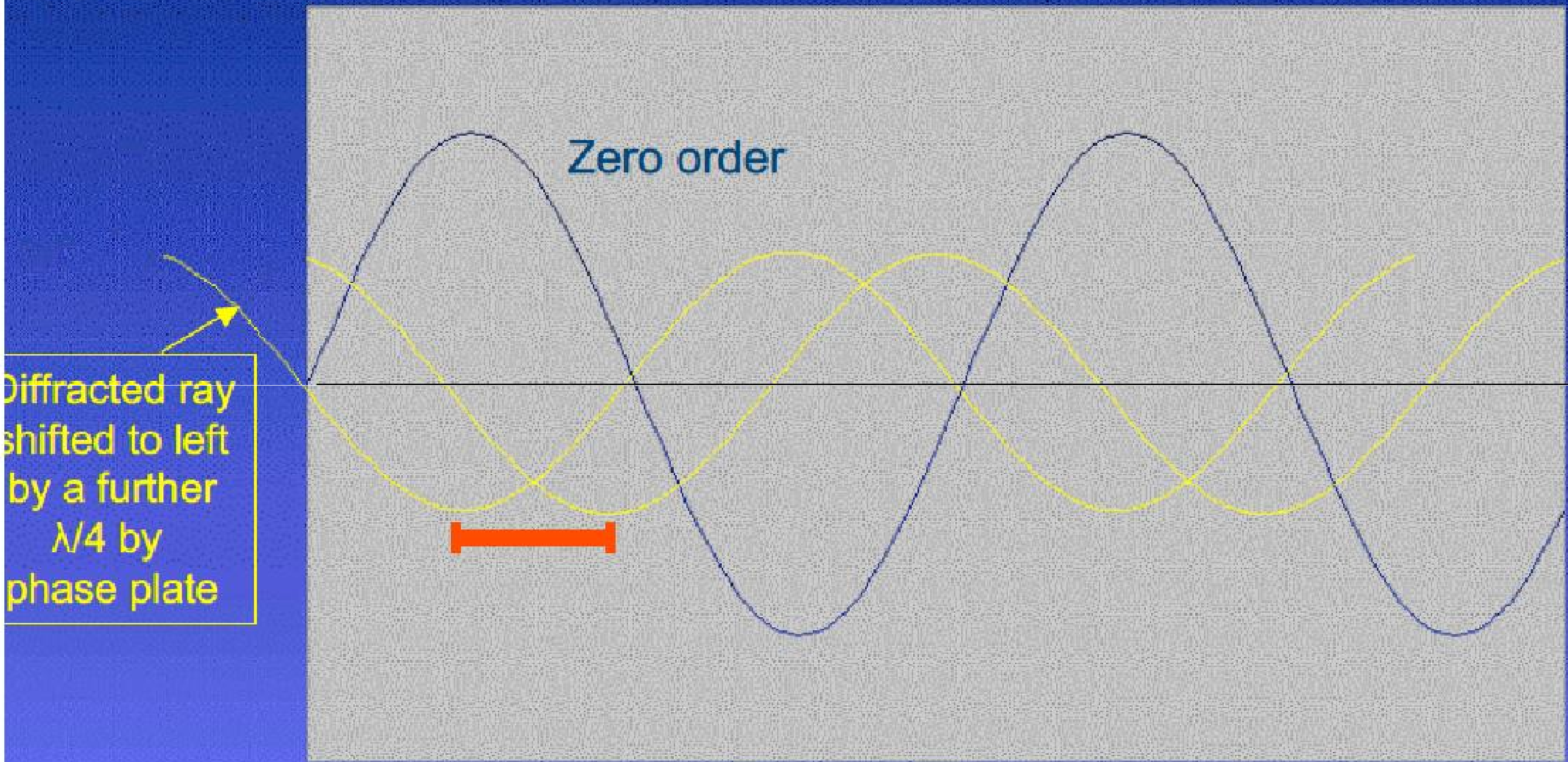
Quarter of a wavelength

The diffracted ray differs from the zero-order by one quarter of a wavelength

…and we know that this leads to an invisible image because of lack of contrast.

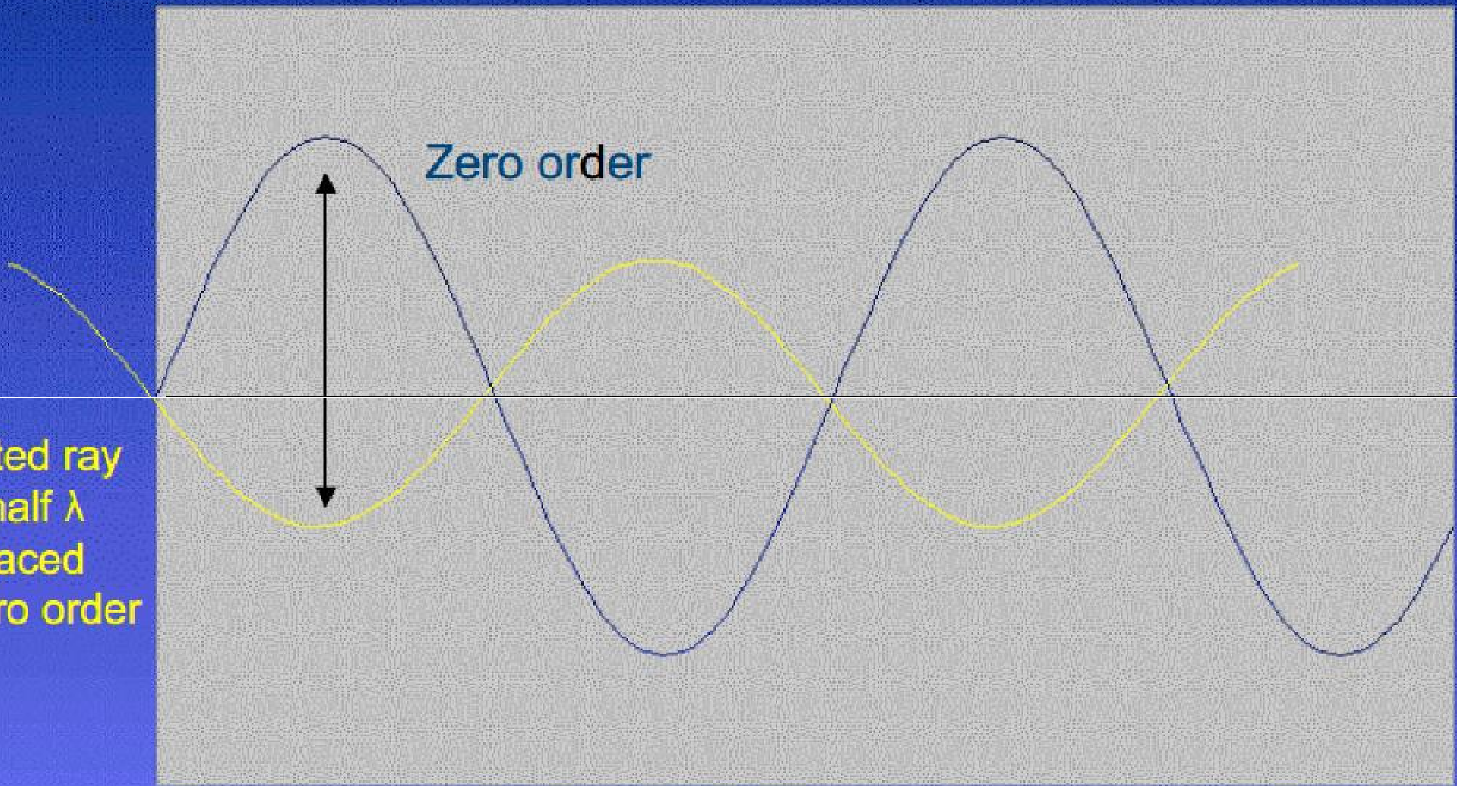
- We know too that with an *absorbing* object, there is one-half a wavelength difference, and this leads to good contrast.
- If we were able to convert the quarter-wavelength difference into a half-wavelength difference, the non-absorbing object would appear in the image as if it were an absorbing object.

Diffracted ray retarded by another one quarter wavelength



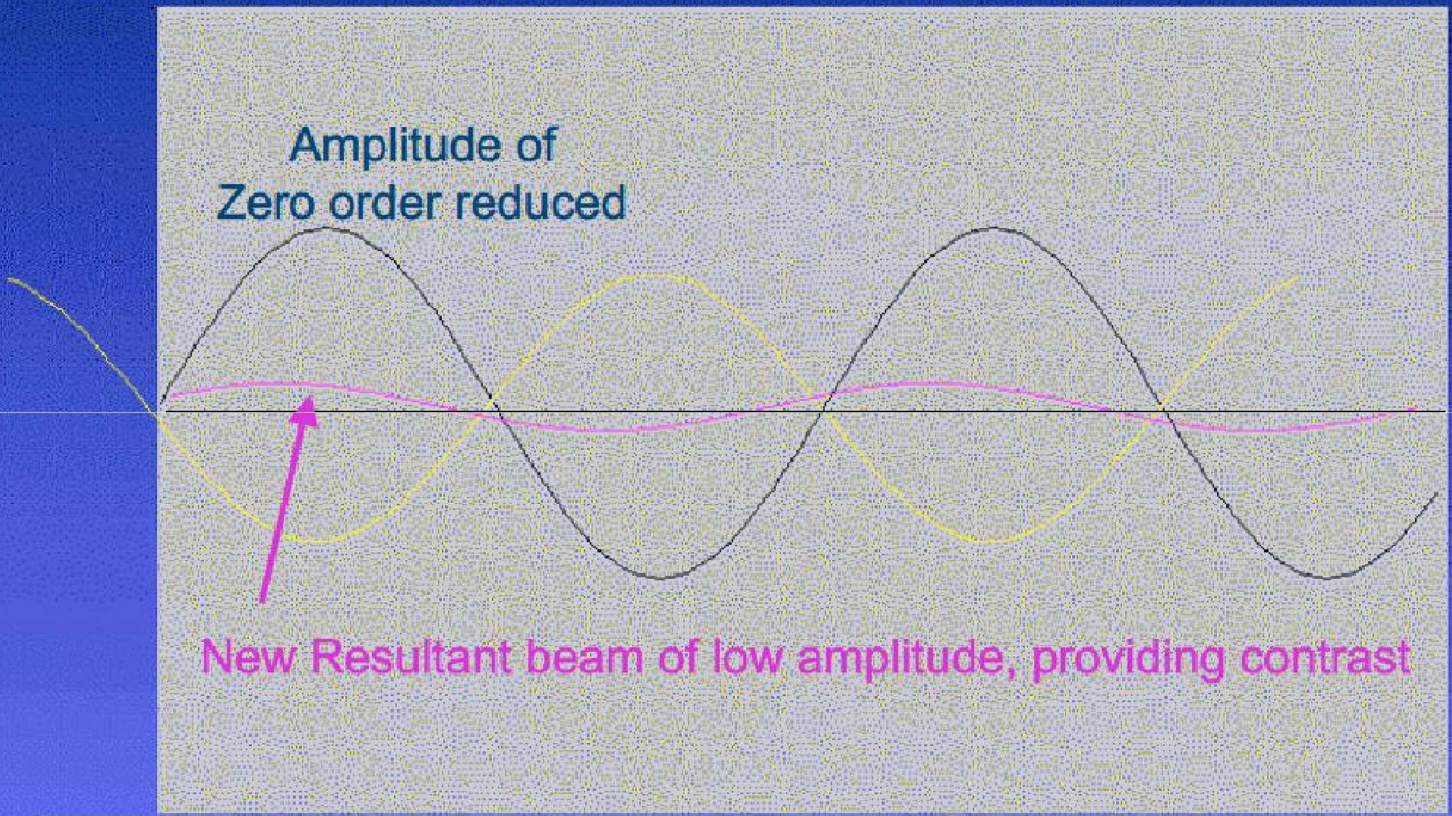
Diffracted beam now approximately half a wavelength behind zero order

Diffracted ray now one half wavelength behind zero order



The diffracted ray is now in a position to interfere destructively with the zero order, but it is of lower amplitude

Diffracted ray now one half wavelength behind zero order *and* amplitude of zero order reduced



The diffraction pattern of our non-absorbing object has been converted into a diffraction pattern similar to that of an absorbing object - so the image looks like an image of an absorbing object.