# PHASE CONTRAST

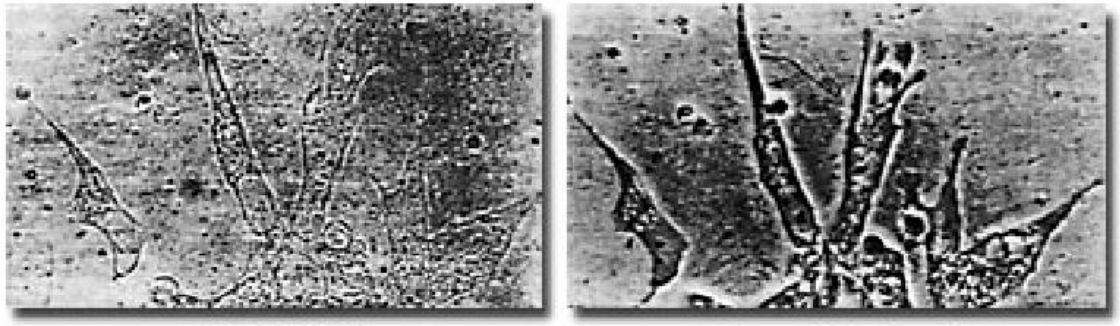
The full name of the microscopy technique could be something like

"phase-strip method for observing phase objects in good contrast, but shortened is phase contrast."

Frits Zernike
(1888-1966)

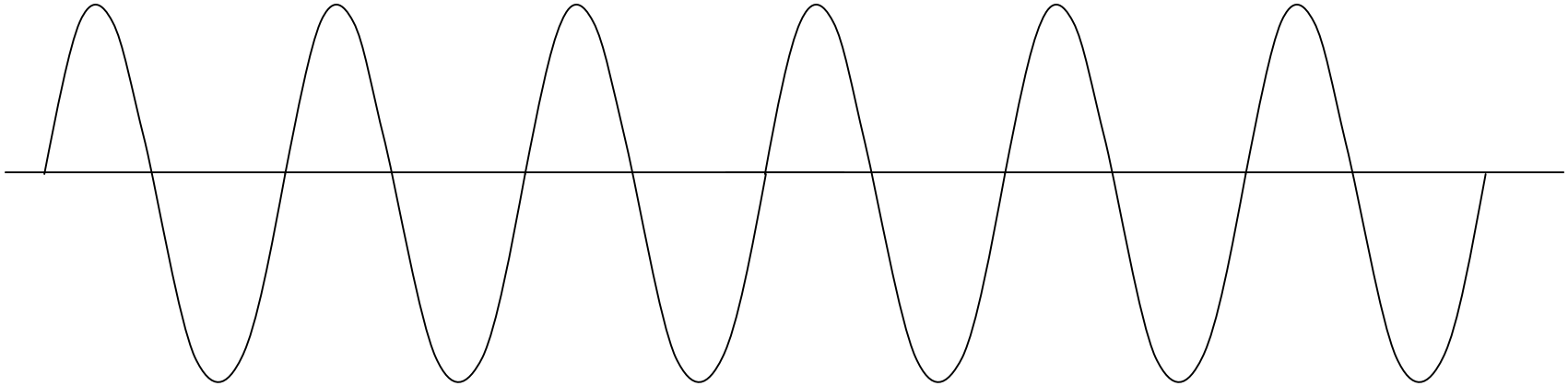
Transforms differences
in relative phase of object waves….
to amplitude differences in the image

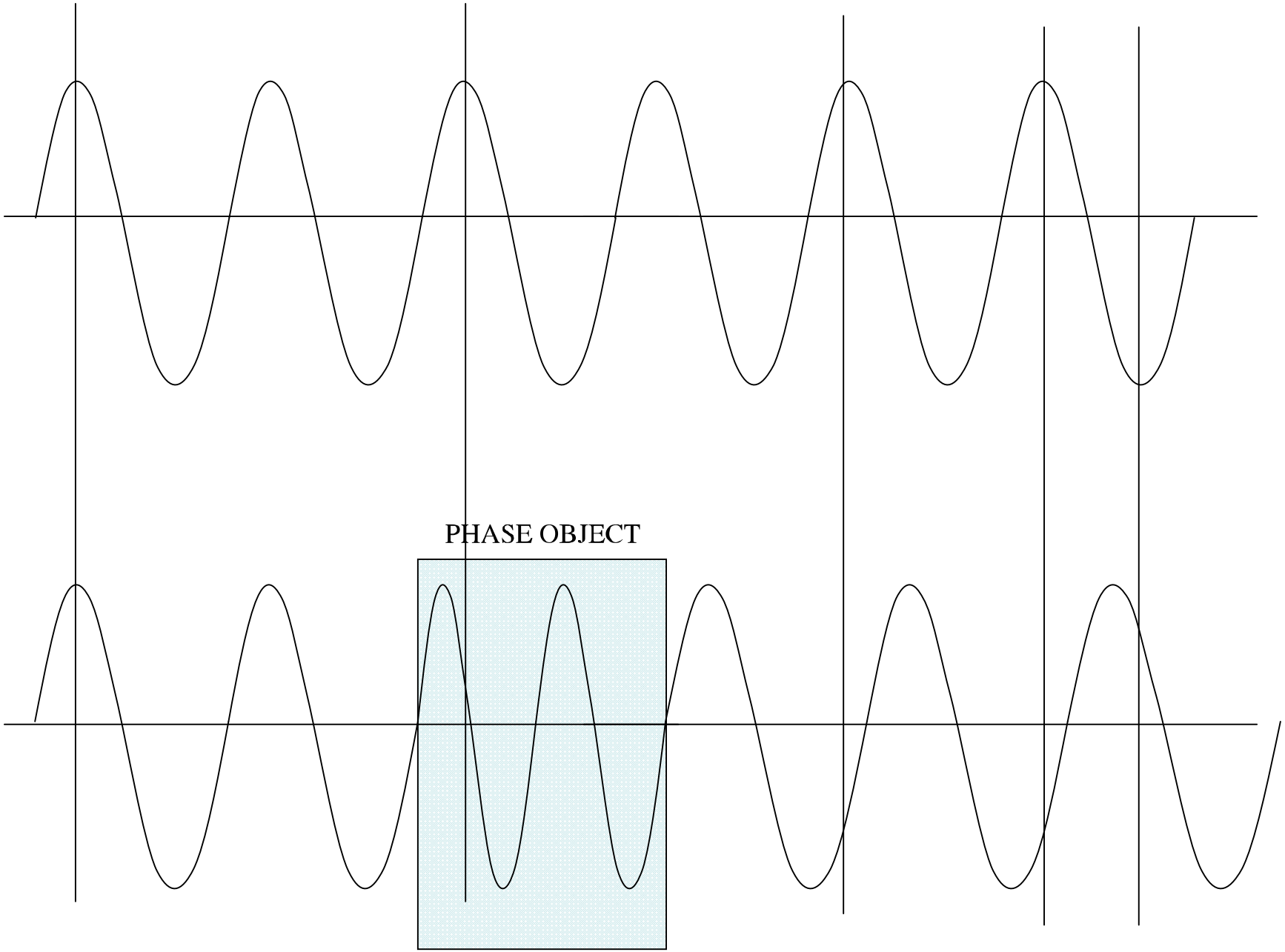
Original Phase Contrast Photomicrographs of Human Cells

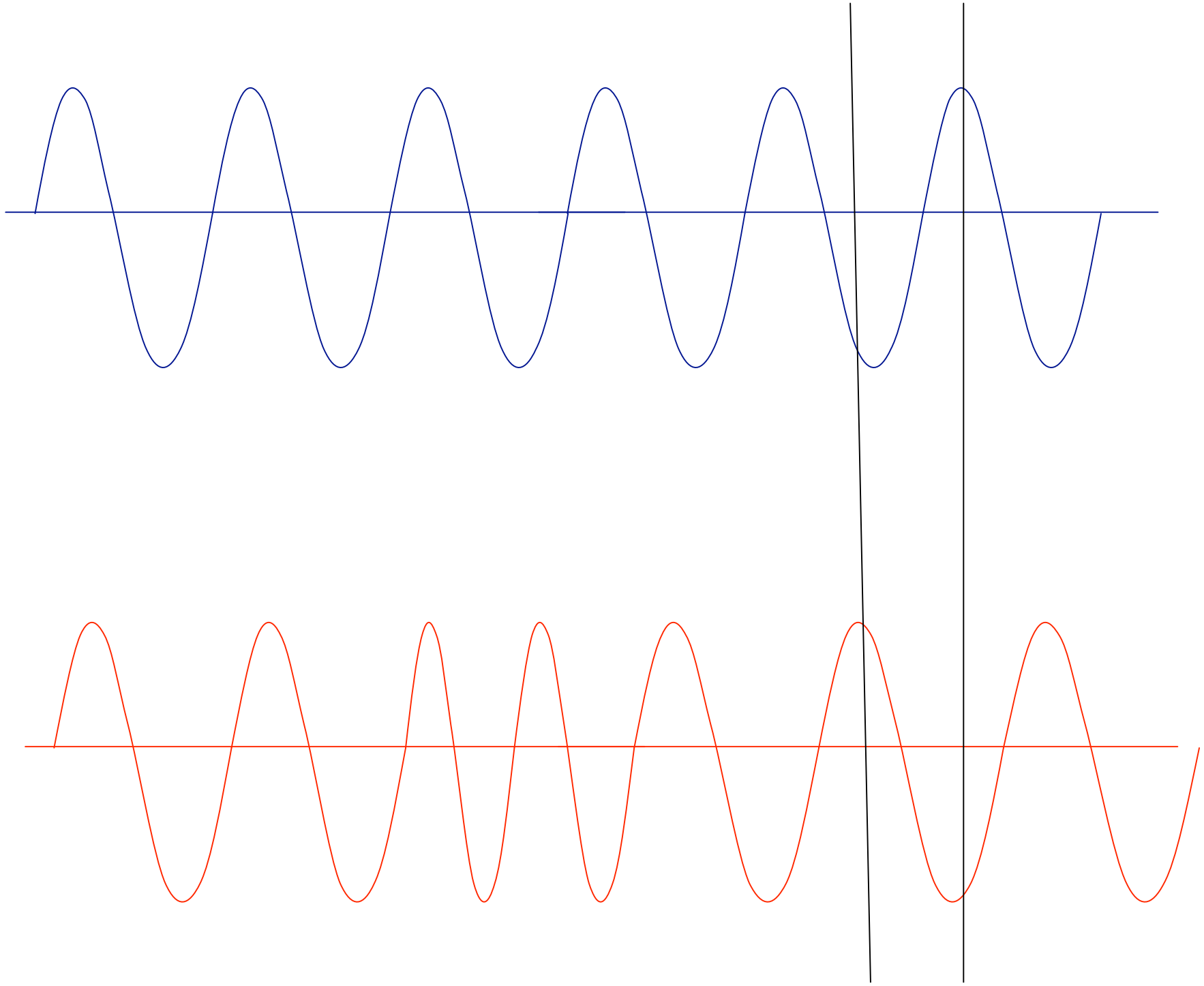


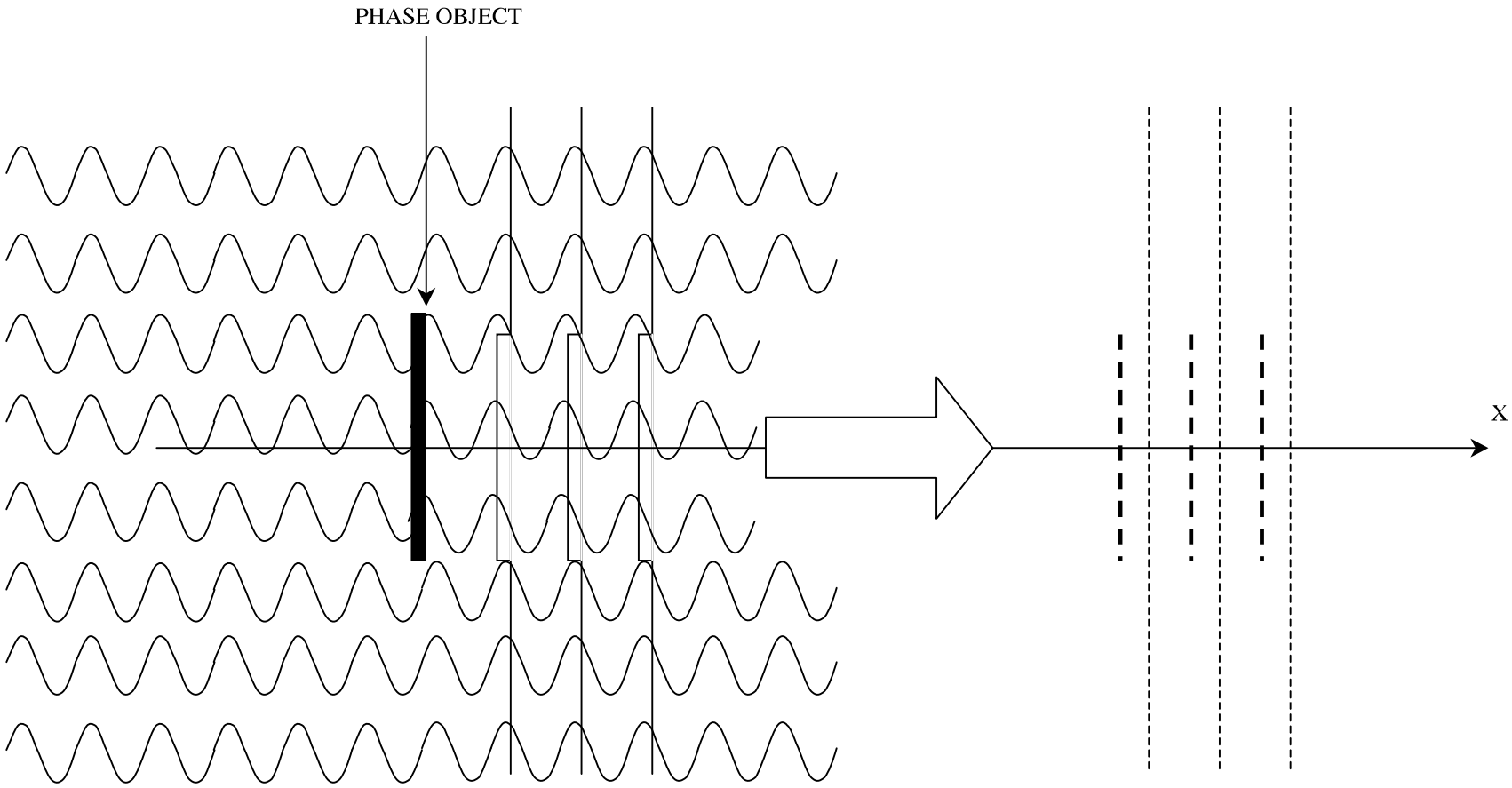
Brightfield

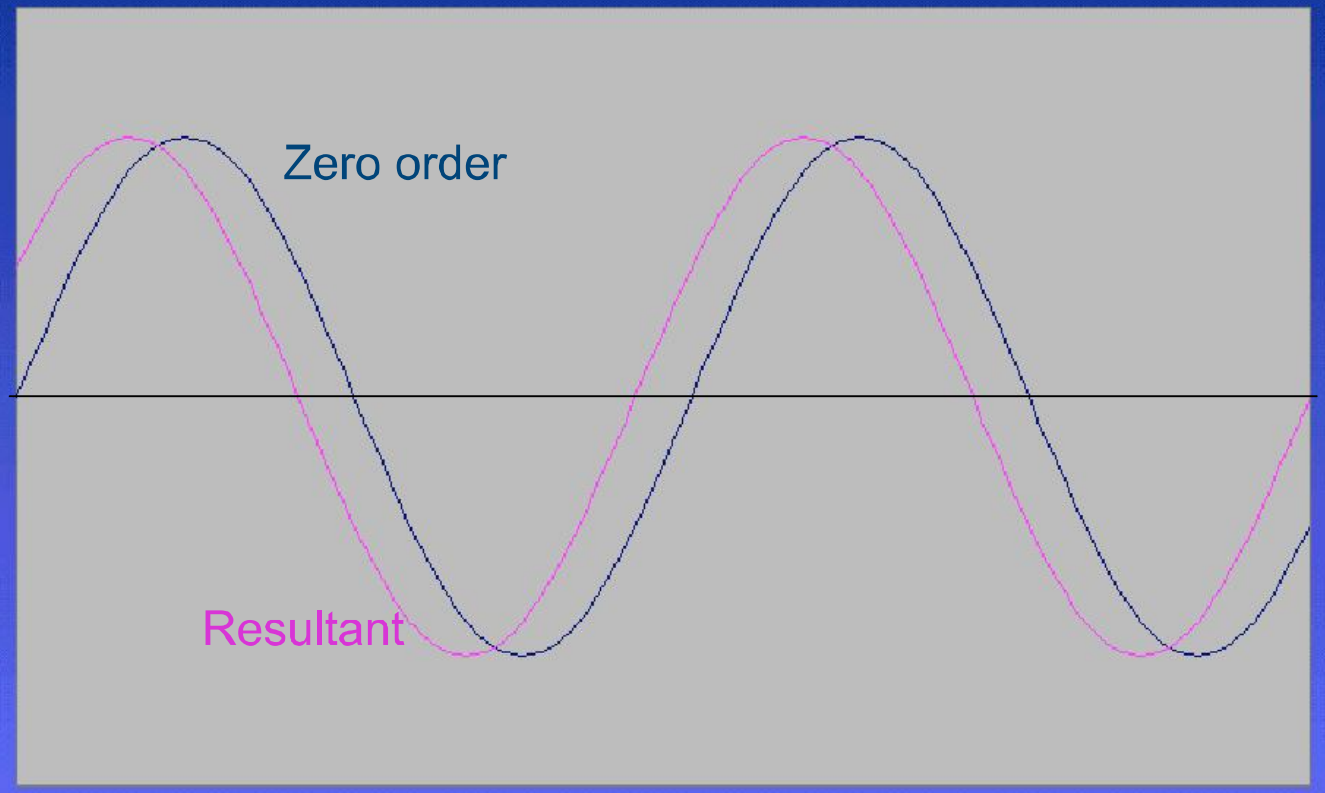
Phase Contrast



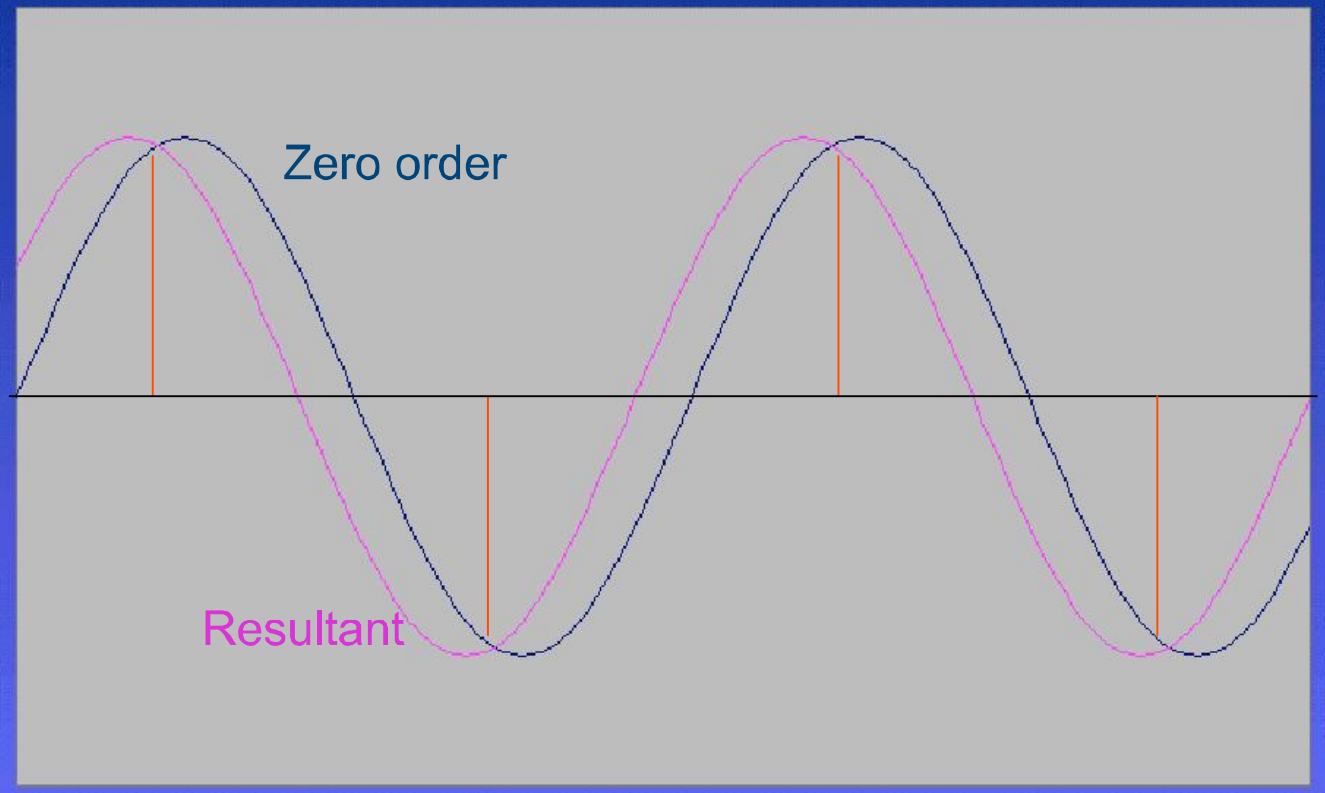

PHASE OBJECT



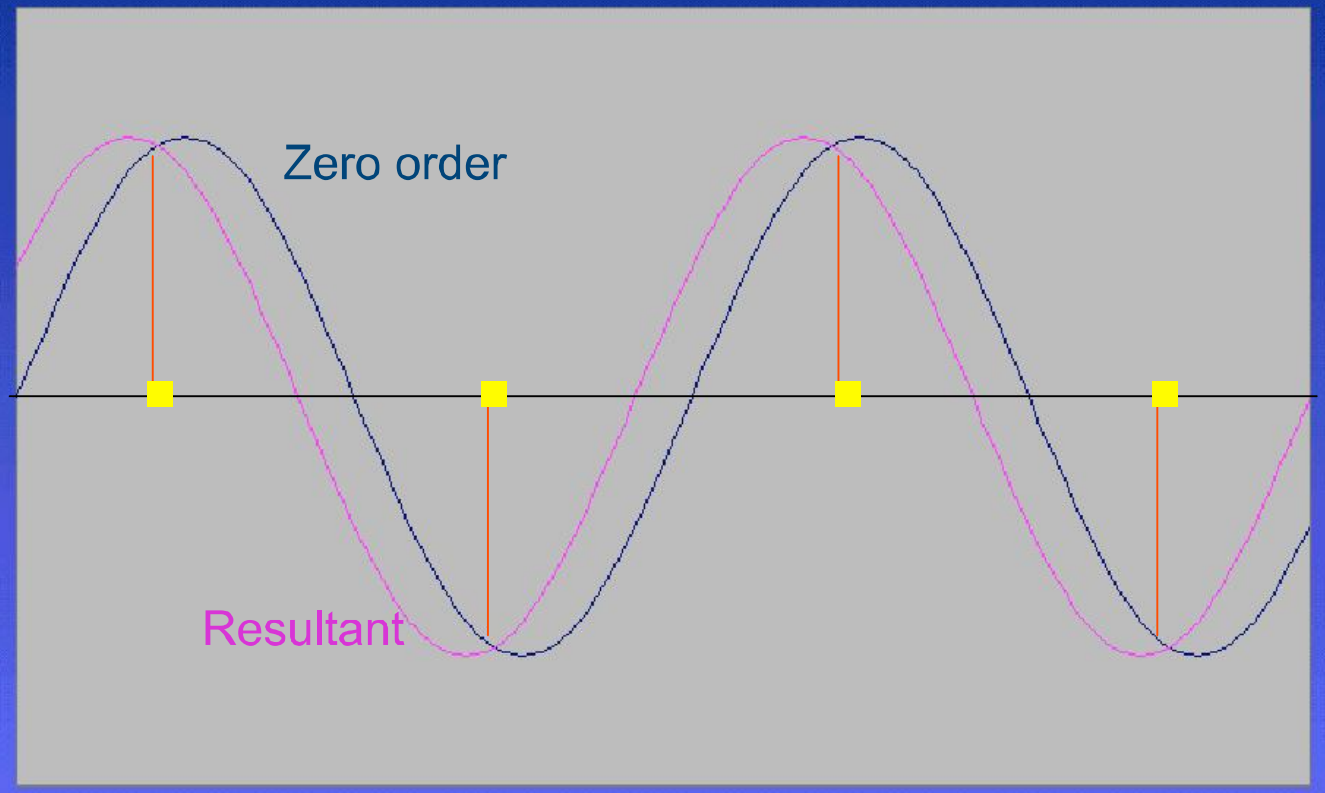

PHASE OBJECT
X



# Positions where amplitudes are equal

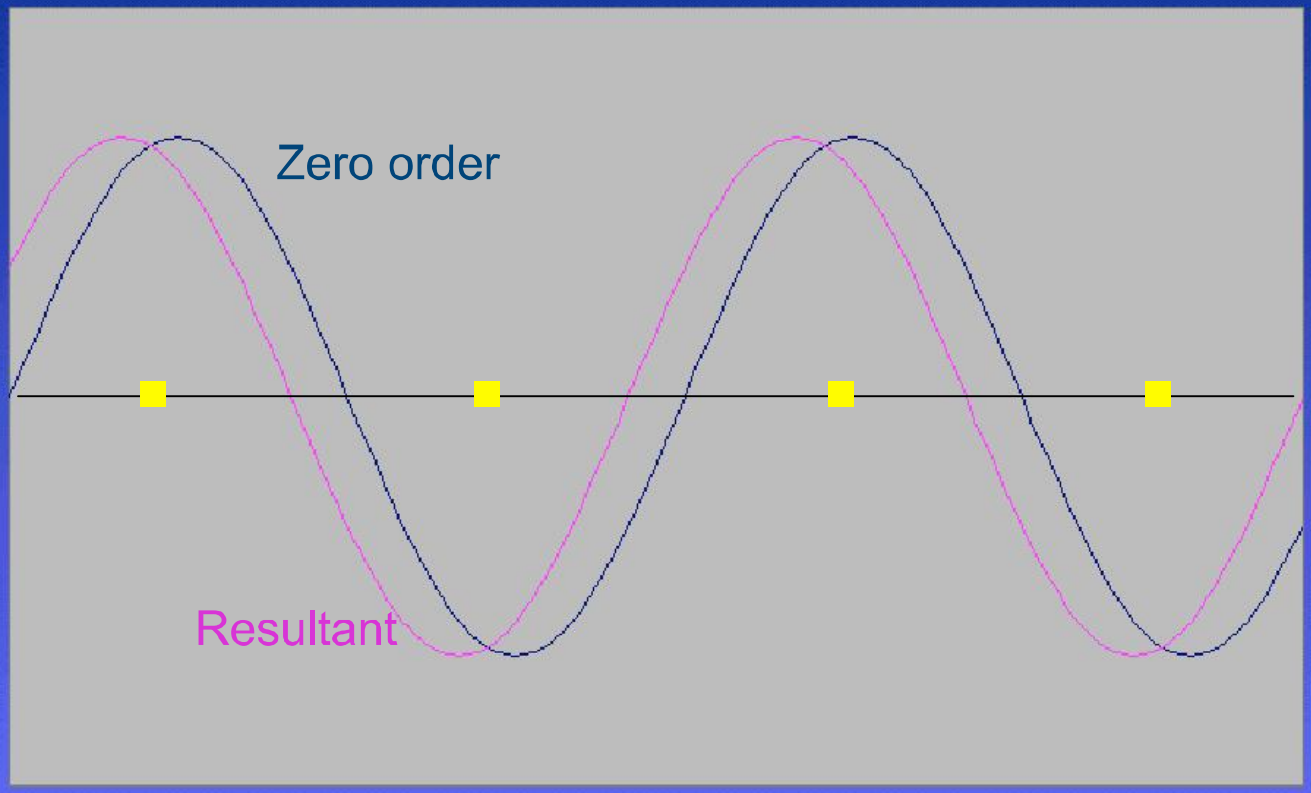


# Positions where amplitudes are equal



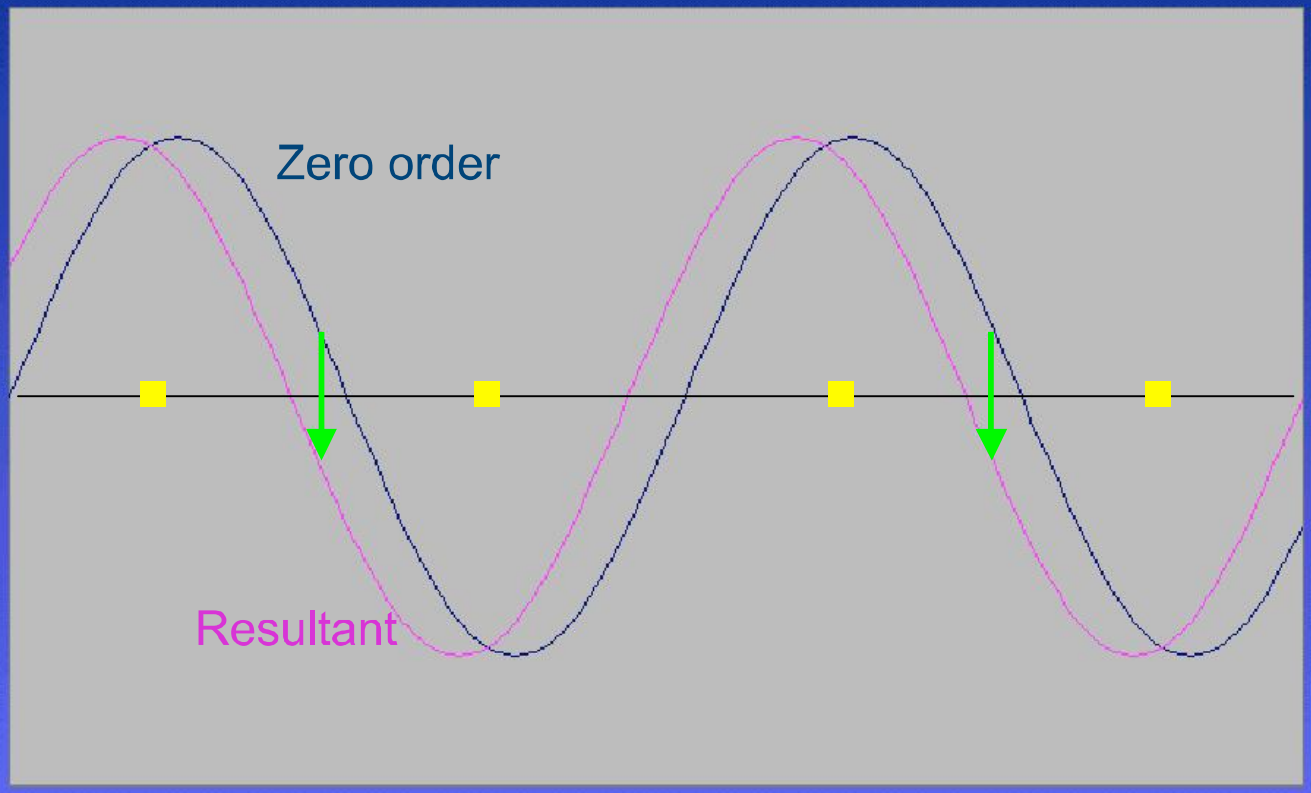

In these positions the diffracted ray must have a value of zero

# Positions where amplitudes are equal



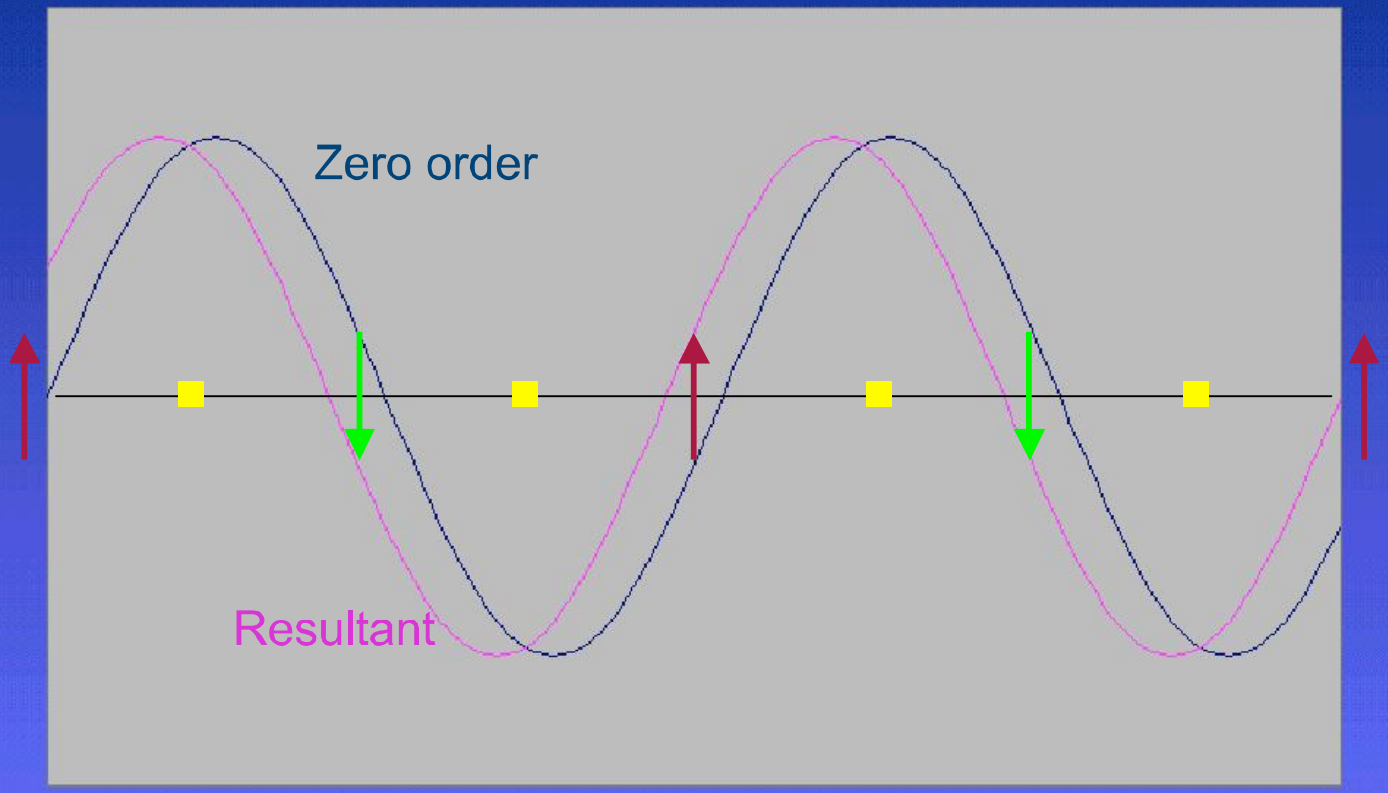

In these positions the diffracted ray must have a value of zero

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



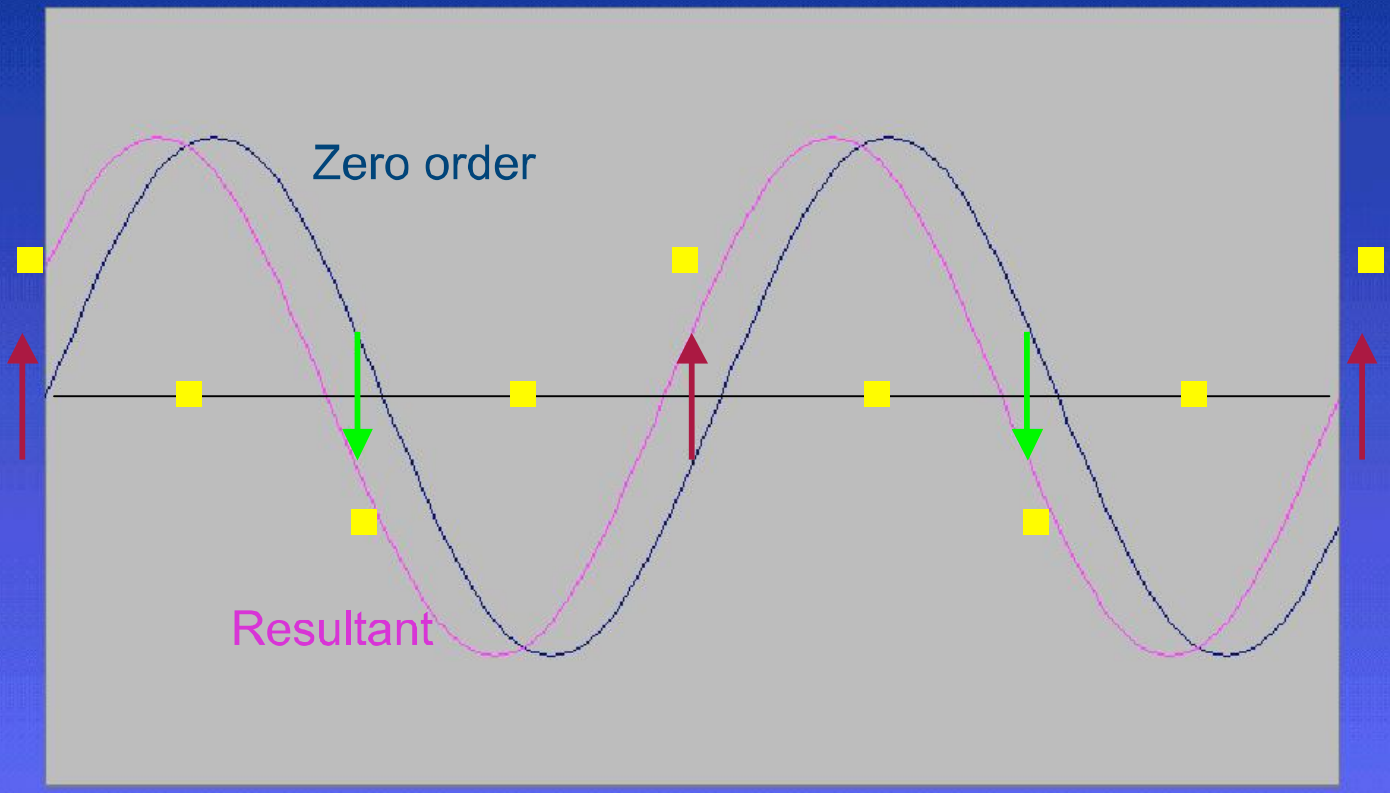

In these positions the diffracted ray must have a negative value

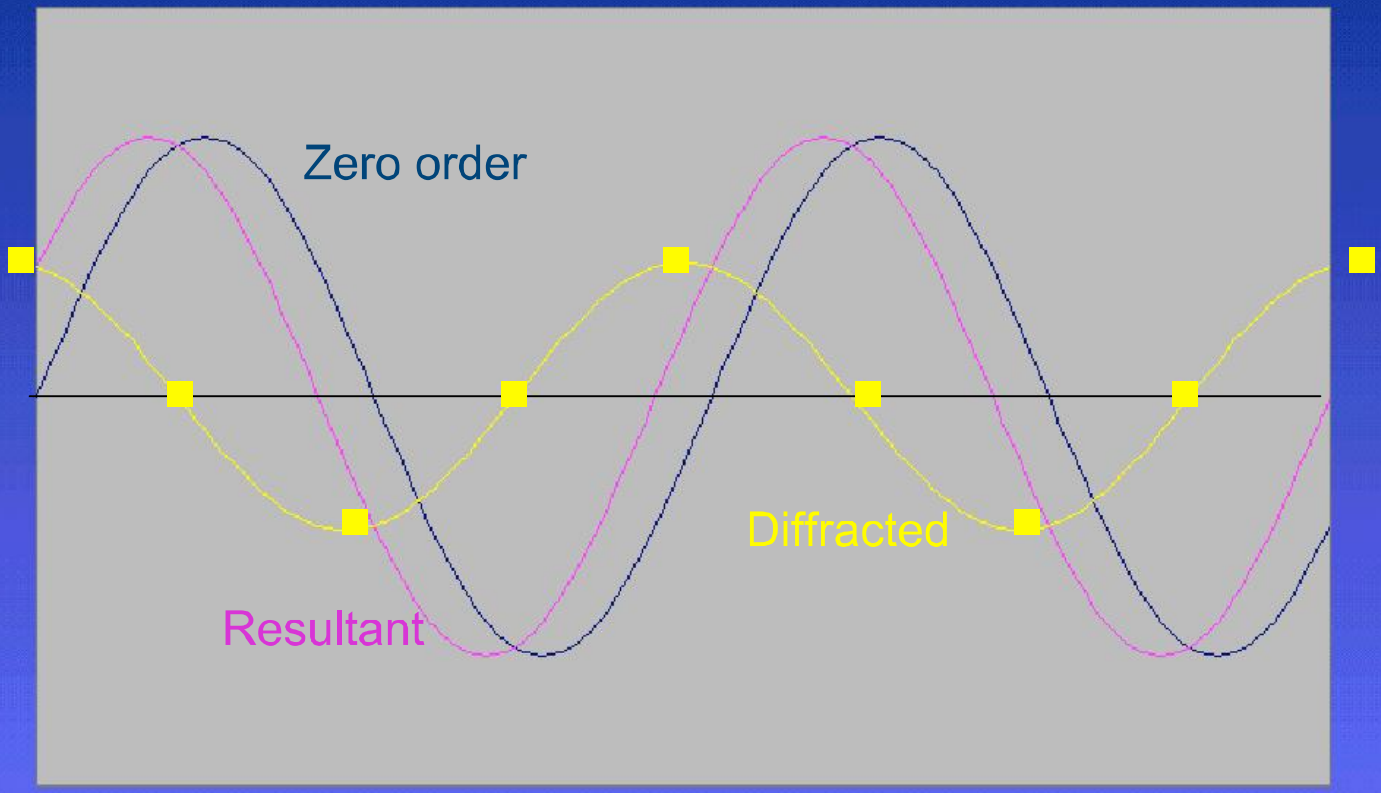
# Positions where amplitude of resultant is *greater* than that of zero order

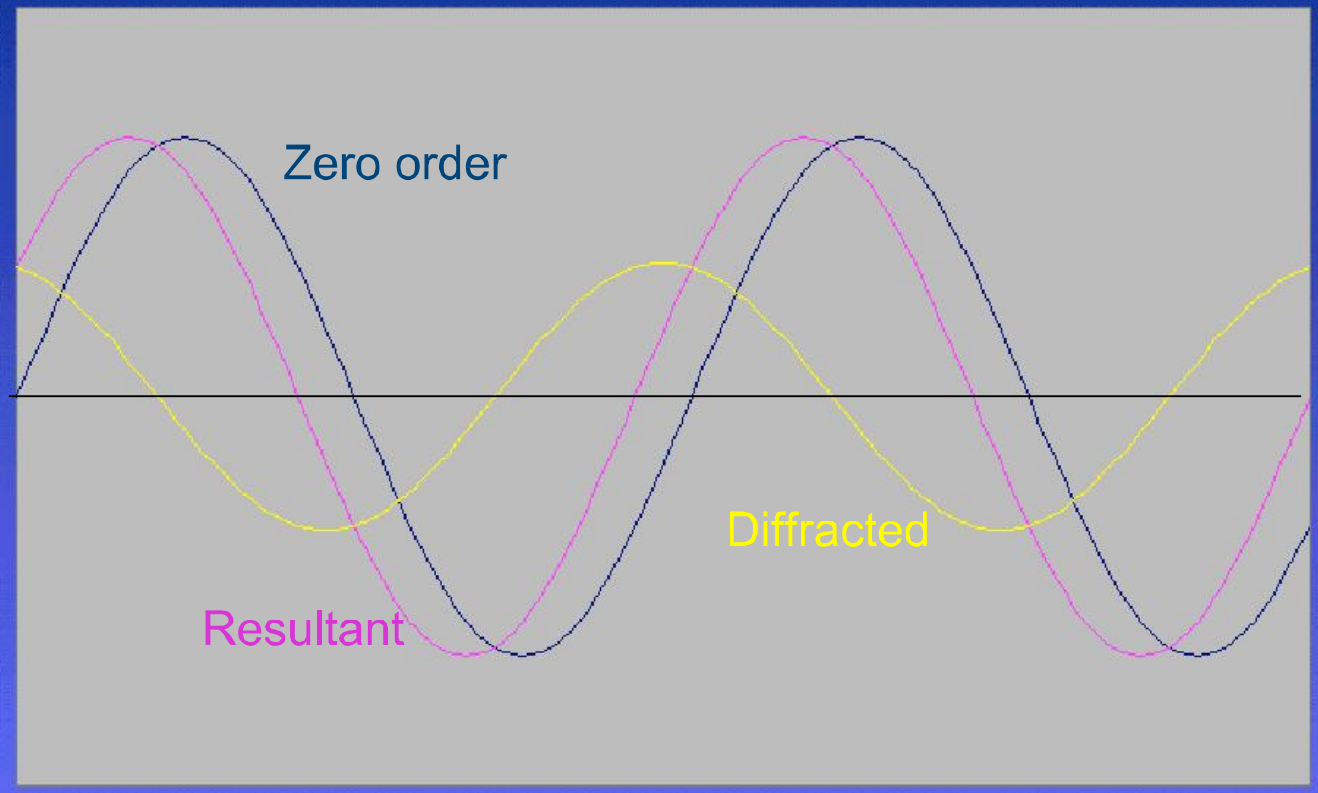


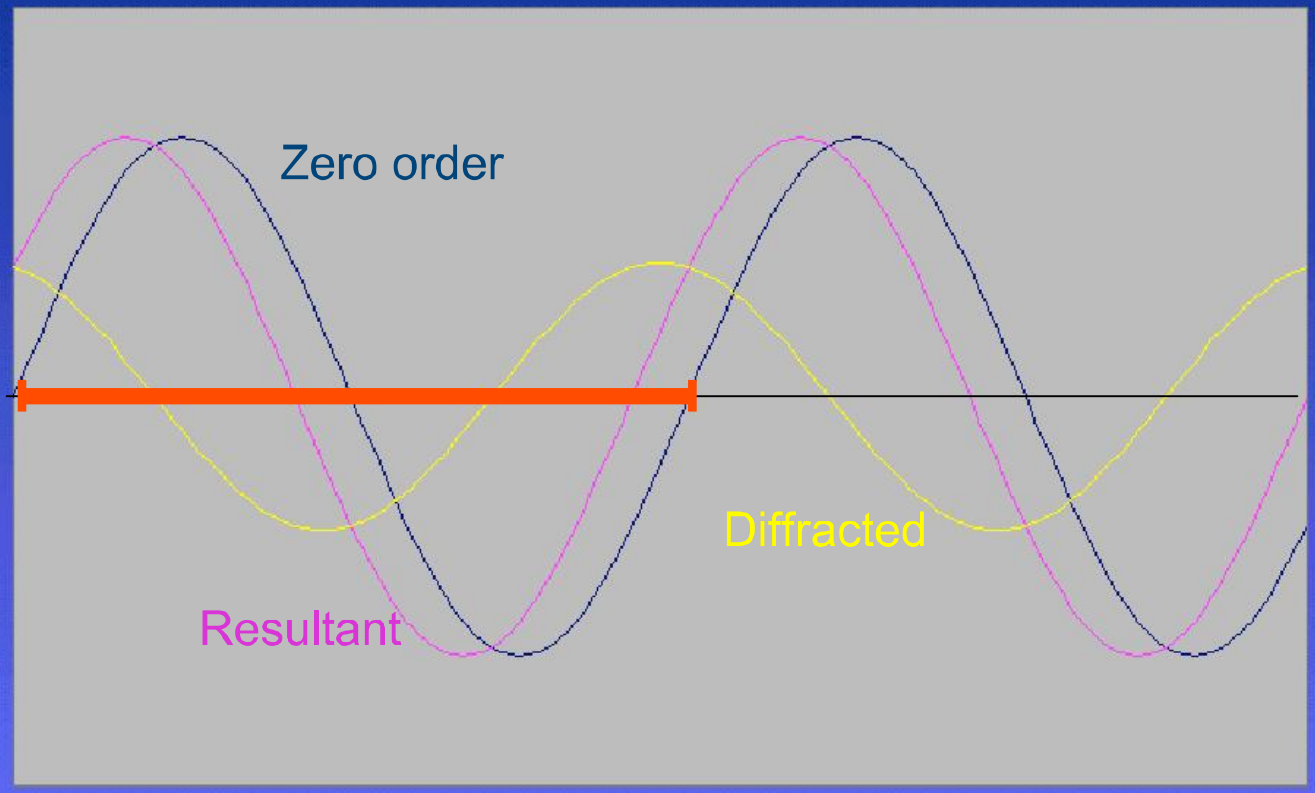

In these positions the diffracted ray must have a positive value

# Points for plotting the diffracted ray

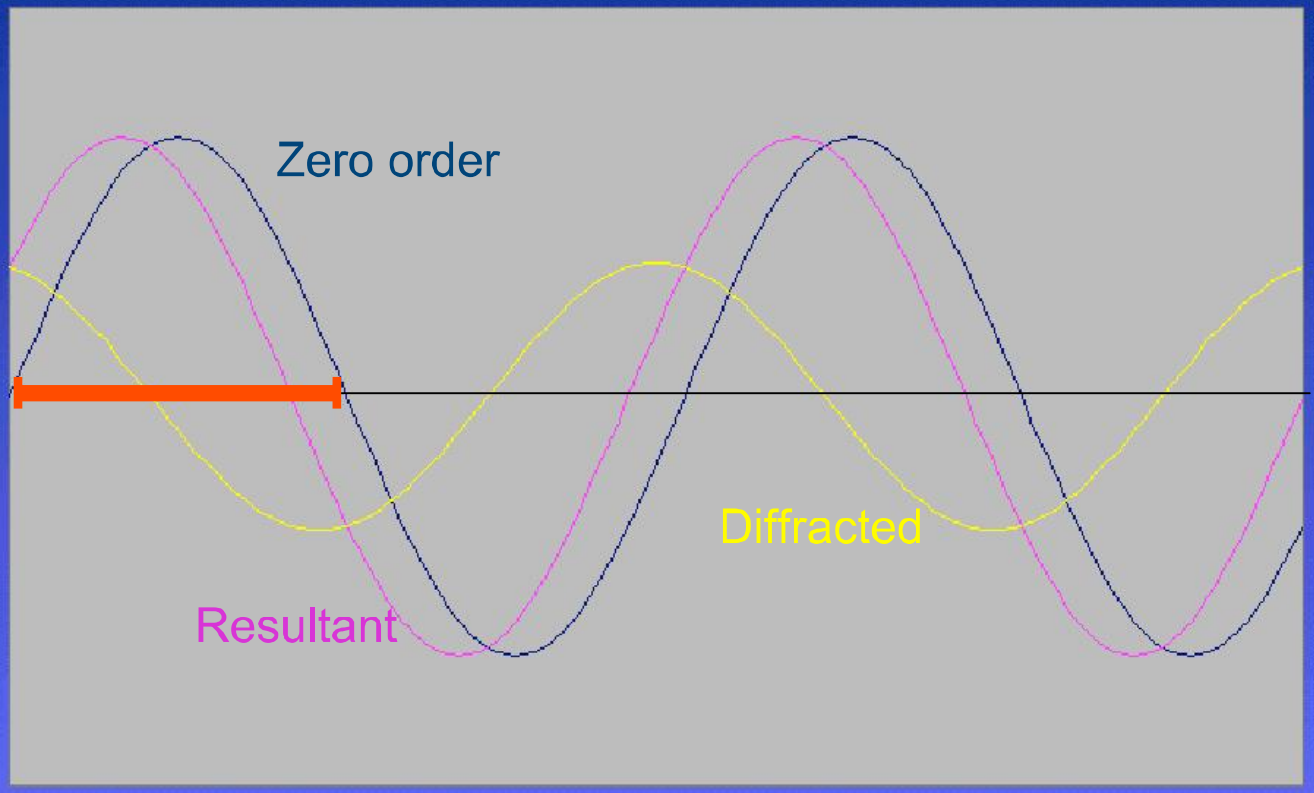




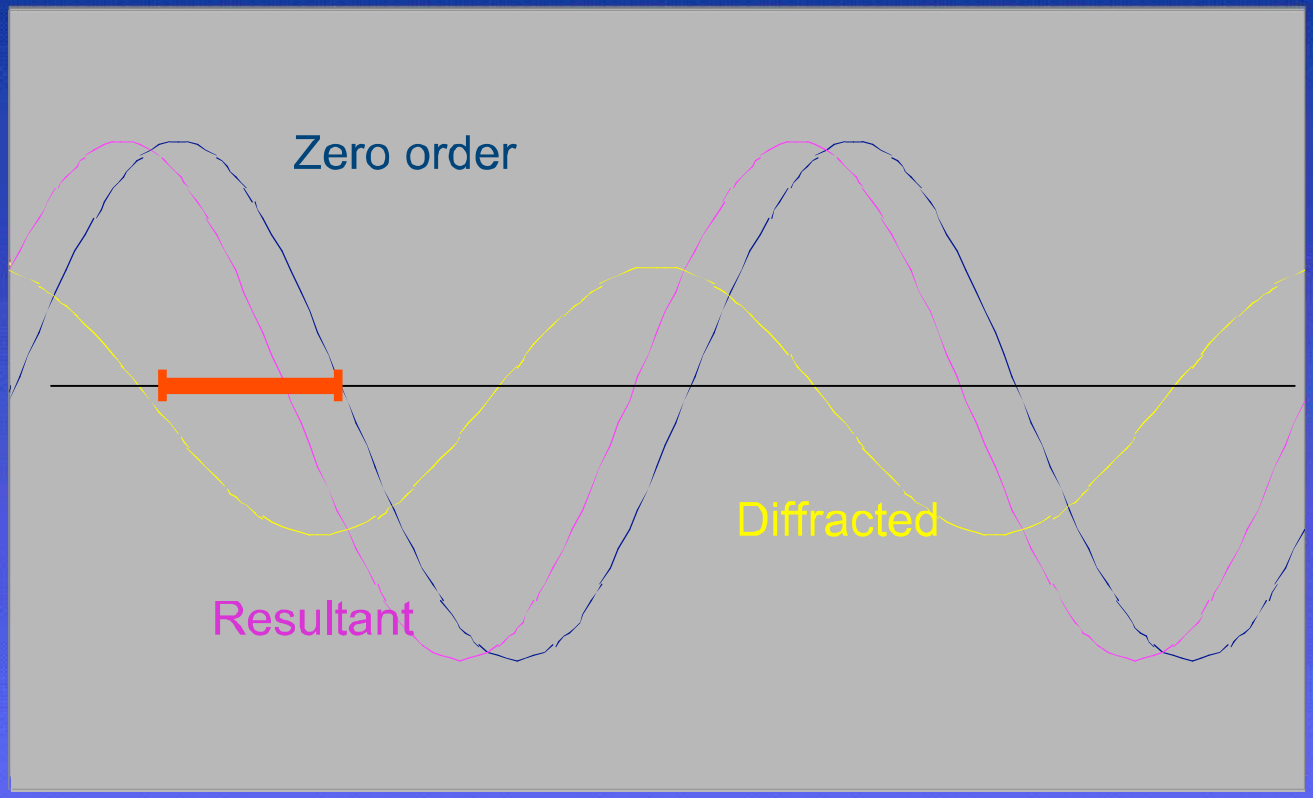




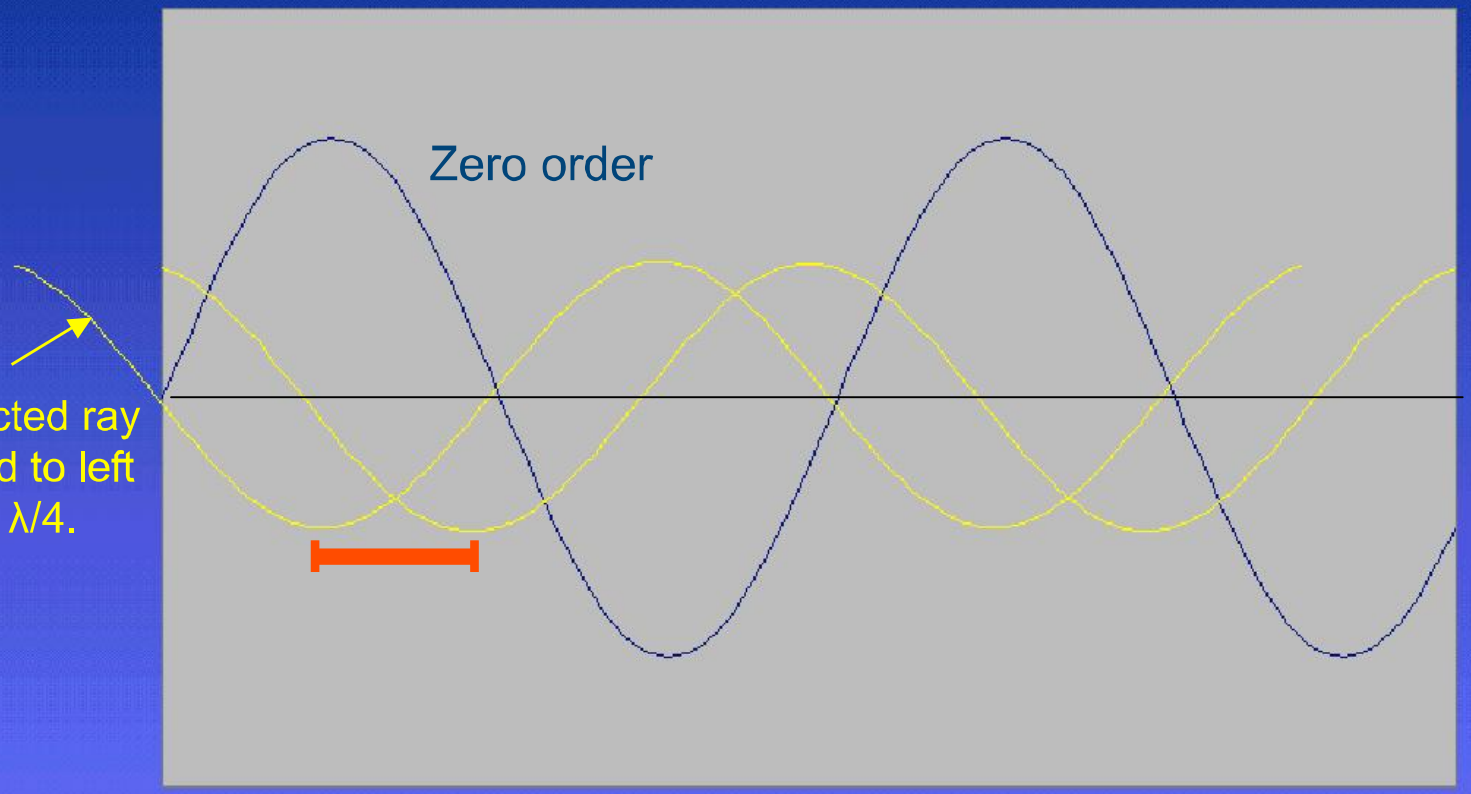

One wavelength




Half a wavelength

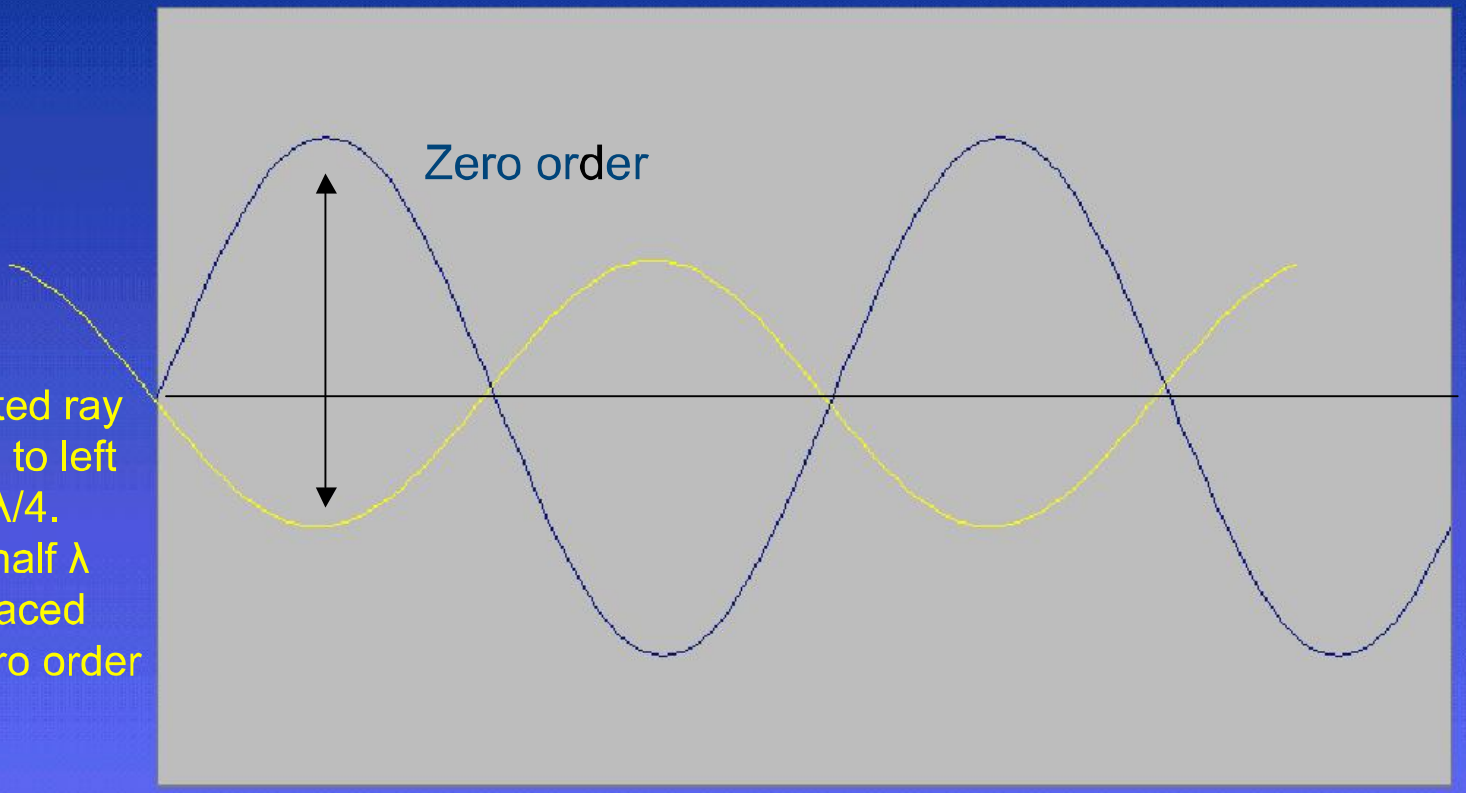



Quarter of a wavelength



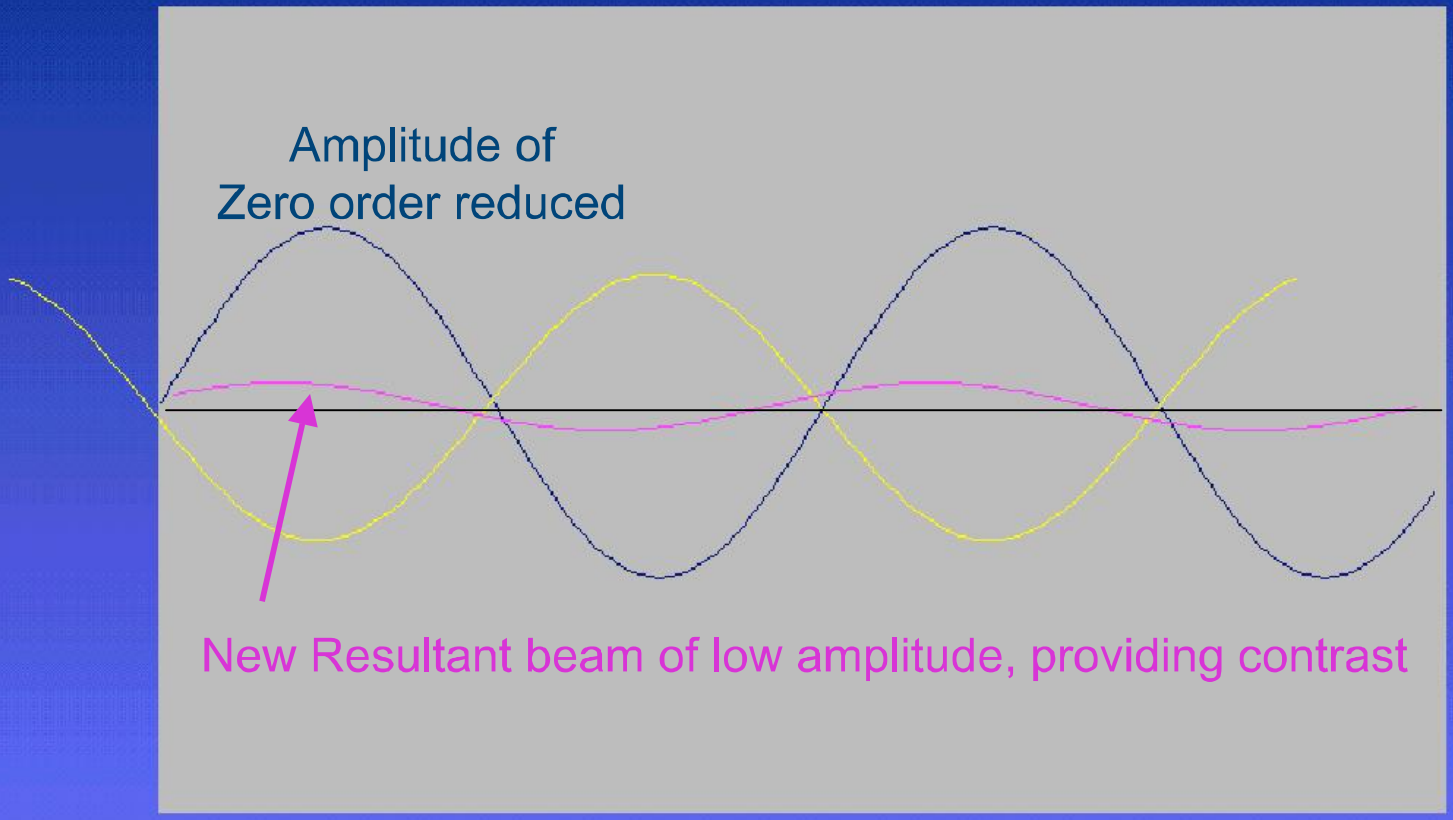

Diffracted beam now approximately half a wavelength behind zero order

# Diffracted ray now one half wavelength behind zero order

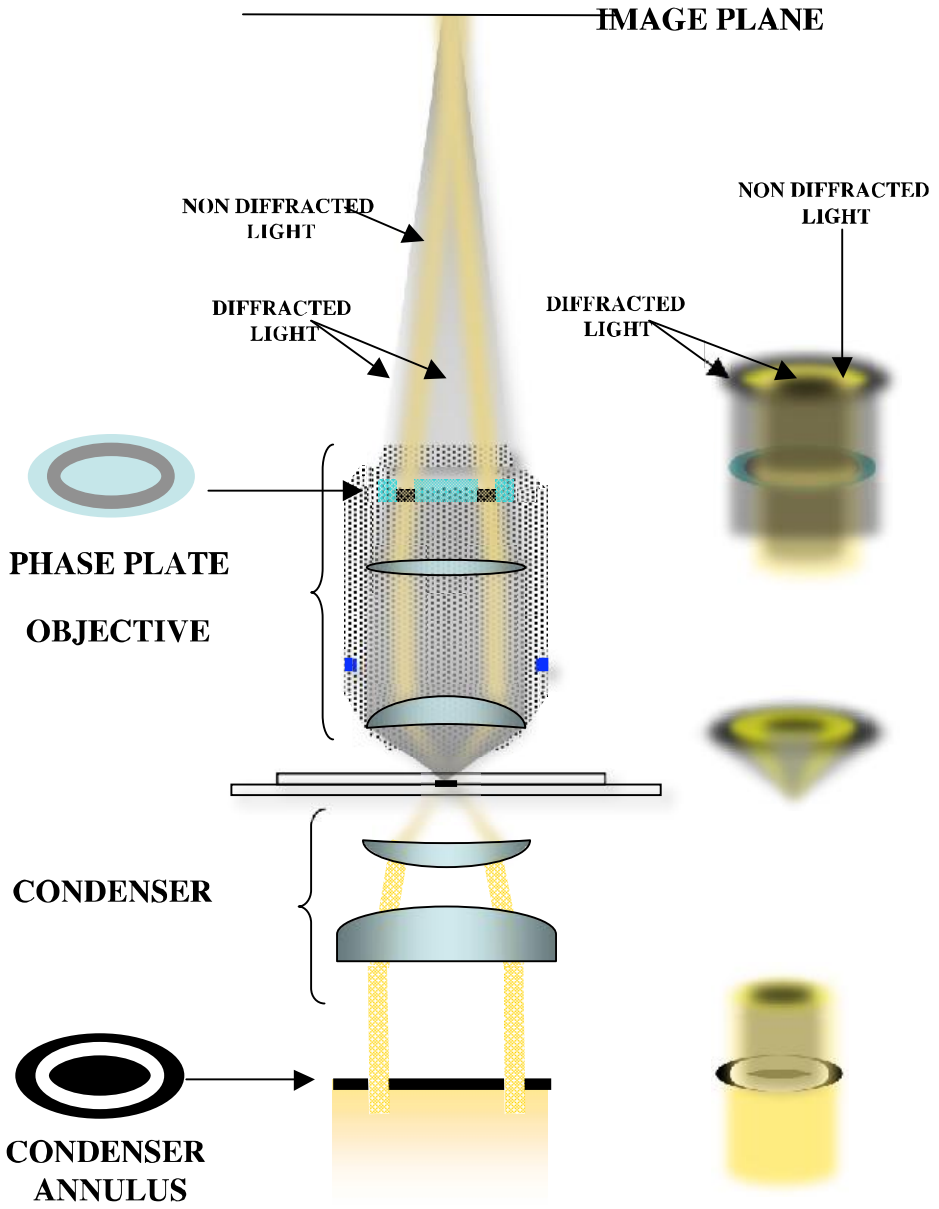



The diffracted ray is now in a position to interfere destructively with the zero order, but it is of lower amplitude

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



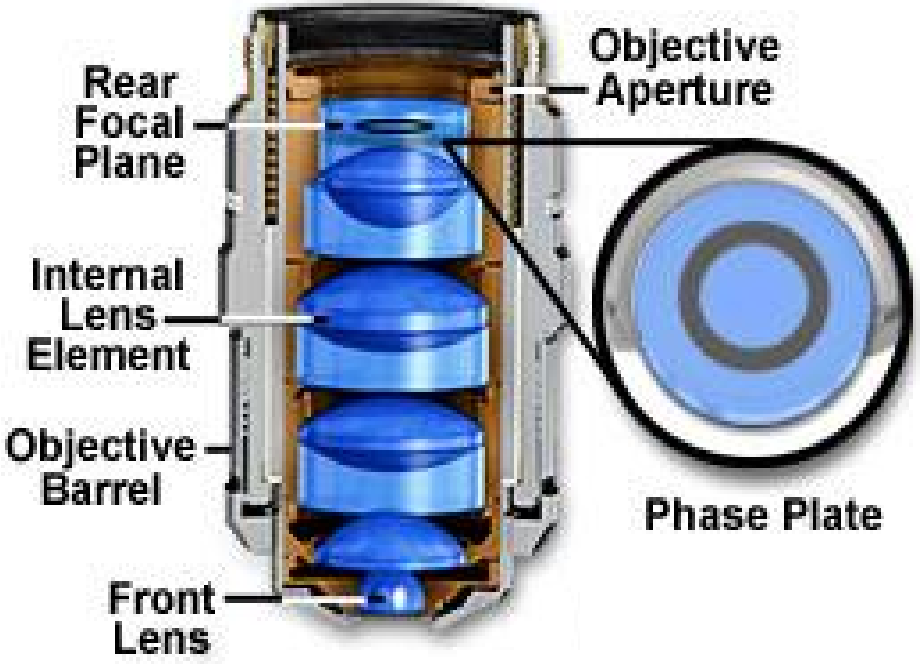
# PHASE CONTRAST



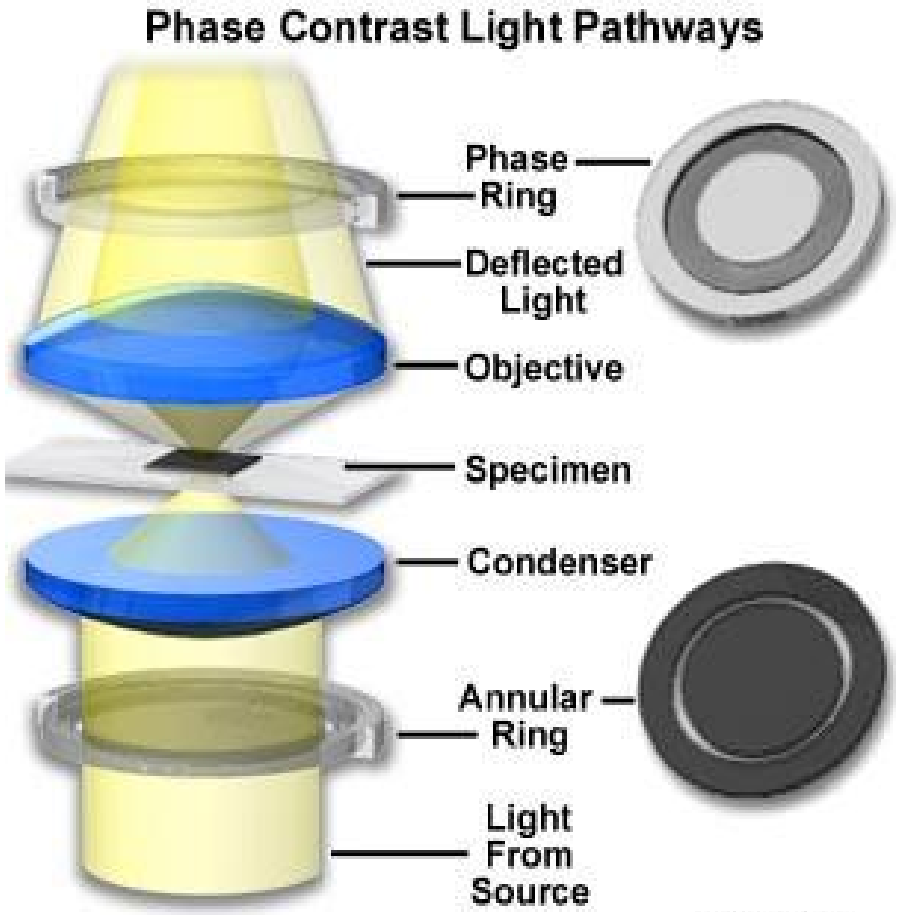

Phase Contrast Optical Components
Condenser Top Lens
Lens Swing Arm
Phase Contrast Objectives
Universal Swing-Lens Condenser
Condenser Annulus
Phase Telescope
Universal 7-Position Turret
Figure 1


ZEISS

## Phase Contrast Objective

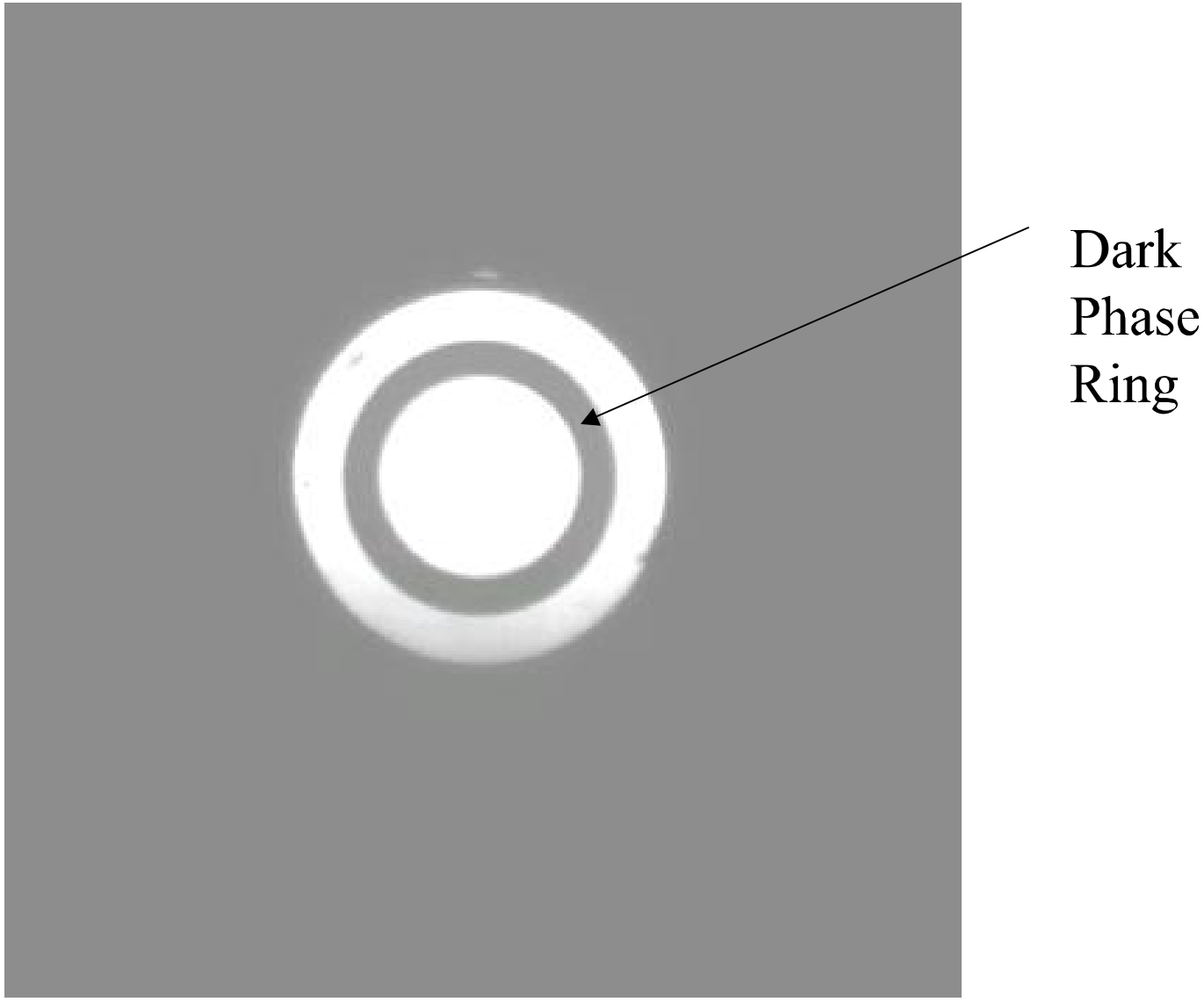



## Phase Condenser Annulus Plate Alignment

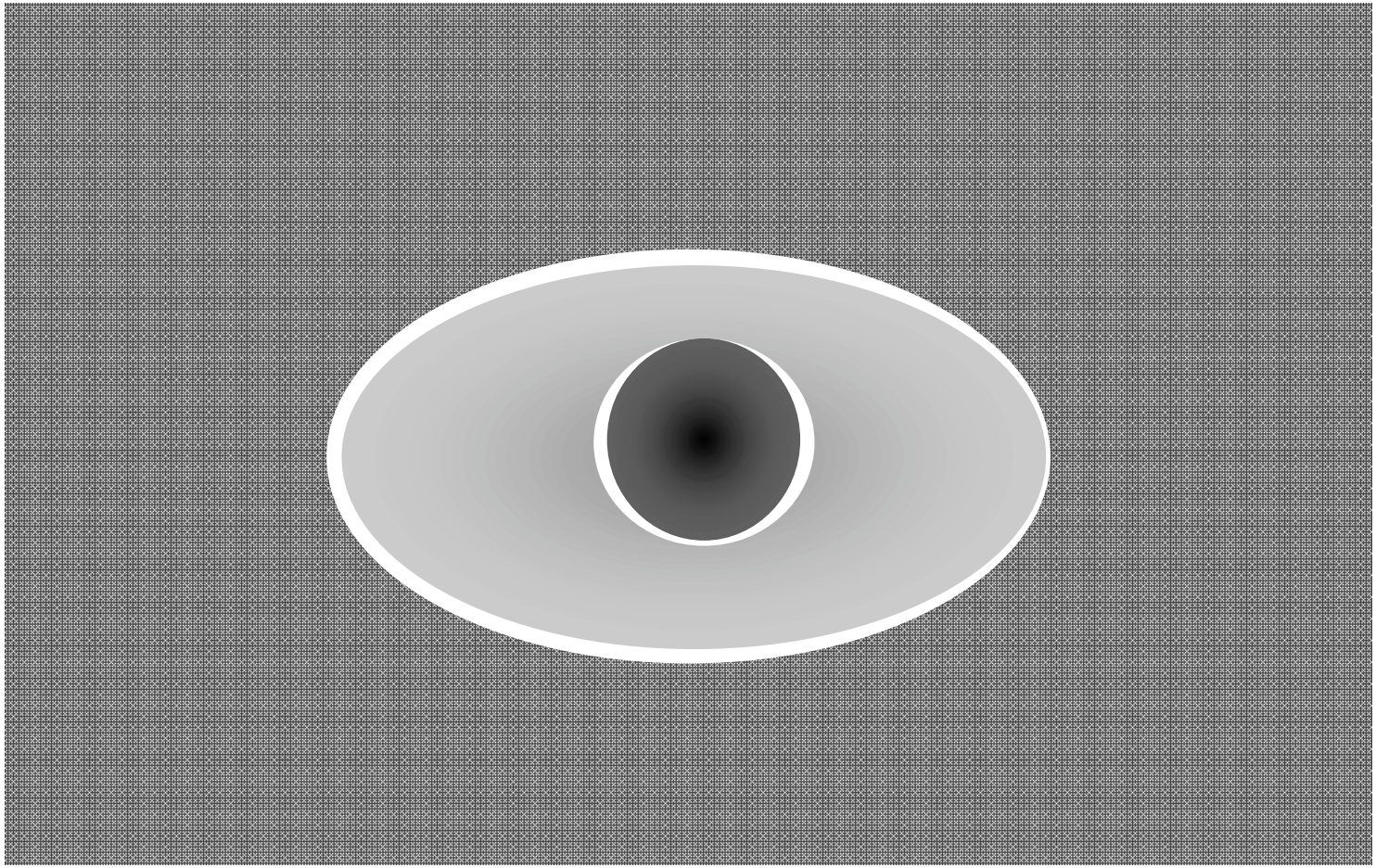

Phase Contrast Light Pathways
Phase Ring
Deflected Light
Objective
Specimen
Condenser
Annular Ring
Light From Source

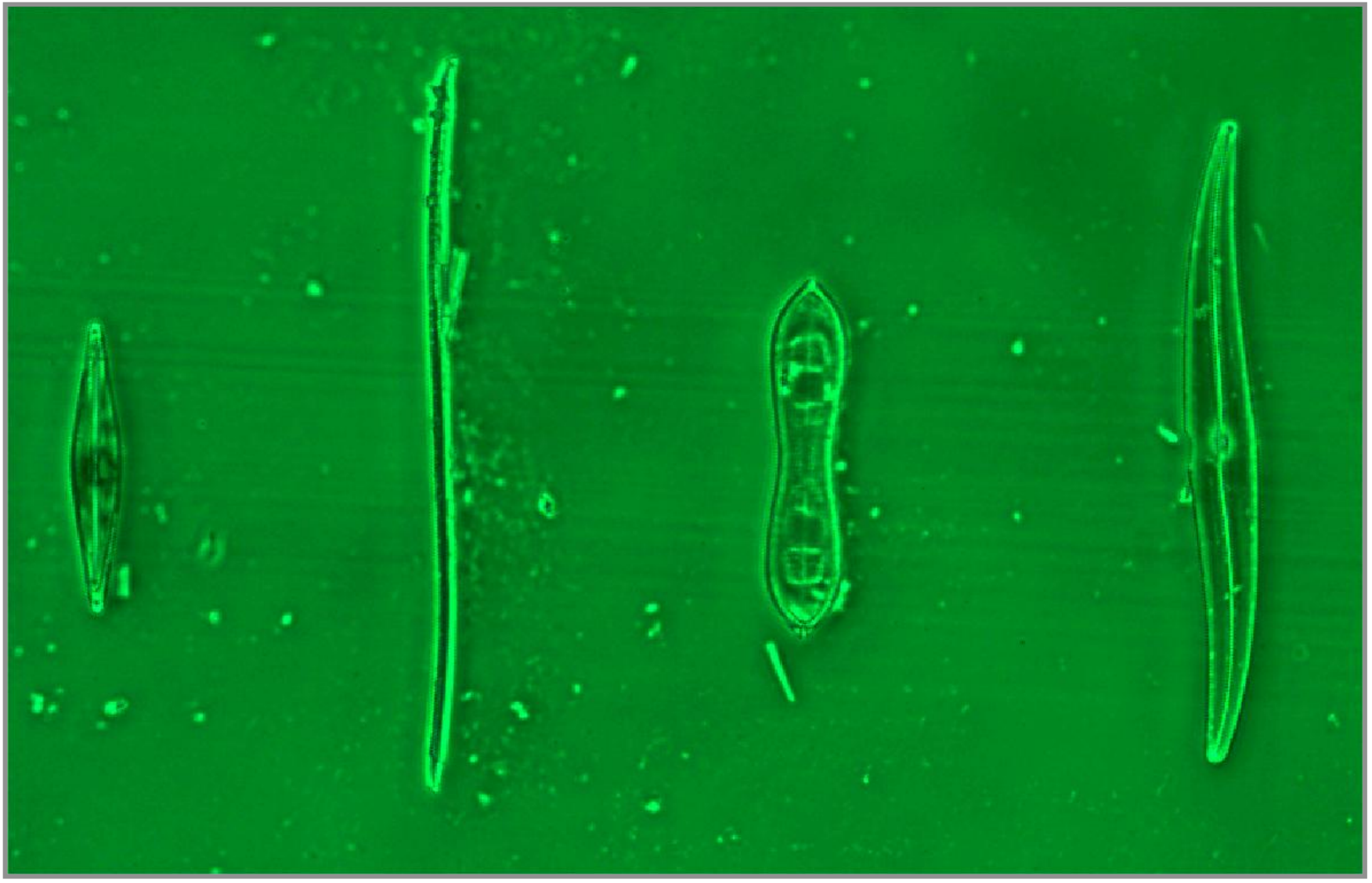

ZEISS

# View of Objective Back Focal Plane for Dark Contrast Phase Objective



# The final result -





# Phase Contrast Gives Contrast to Structural Detail in Transparent Specimens

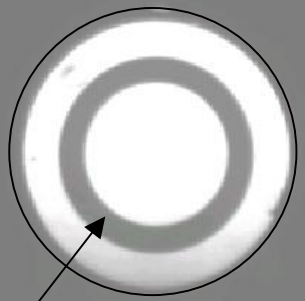
Brightfield

Phase Contrast

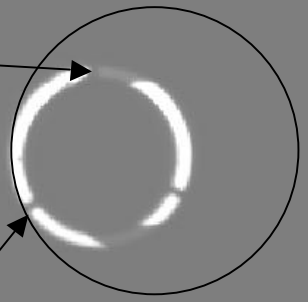

No Annulus
Phase II (Highest NA)
Phase II
PHASE CONTRAST
JAPAN
Phase I (Lowest NA)

# View Objective Back Focal Plane

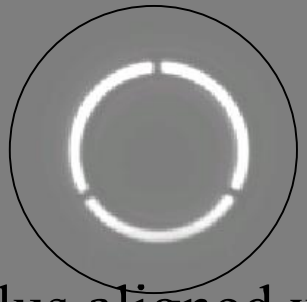
No Annulus



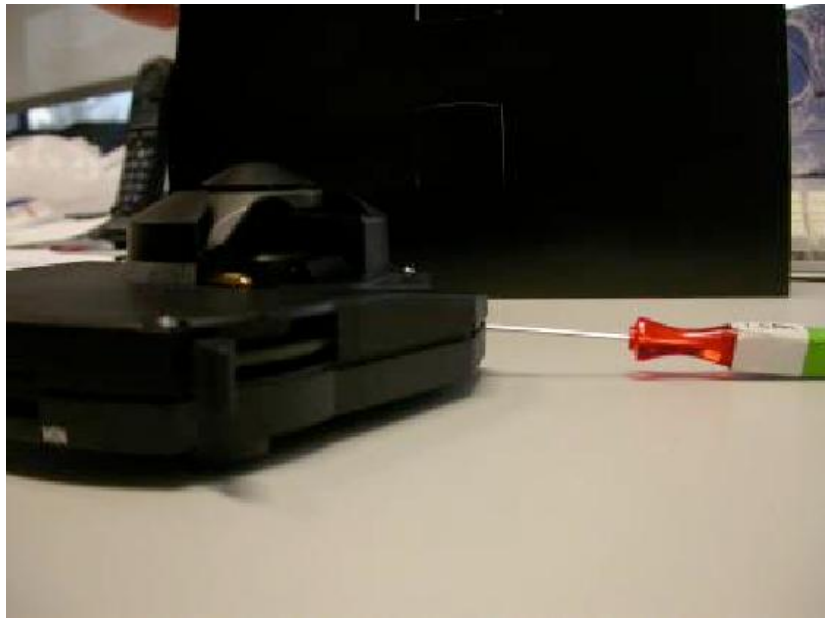
Objective Phase Ring

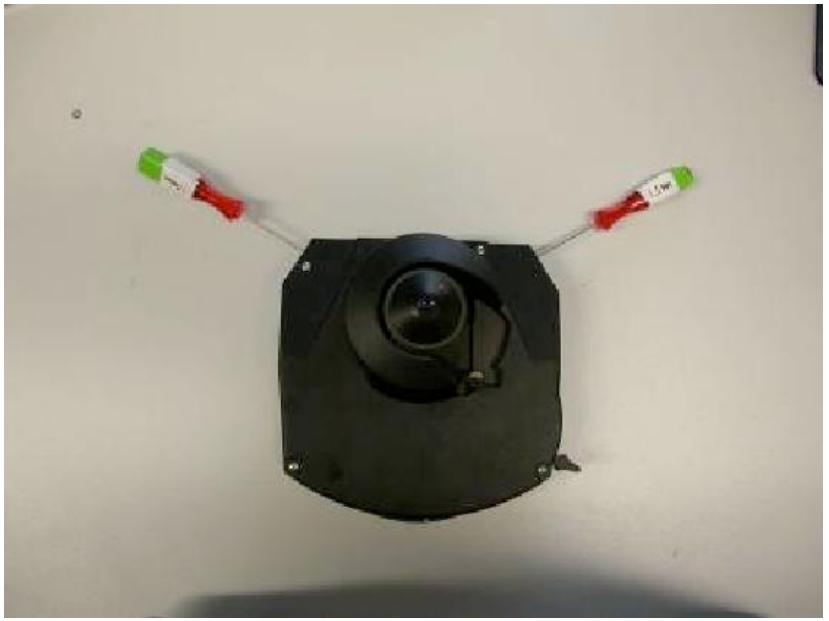


Miss-Aligned Annular Illumination

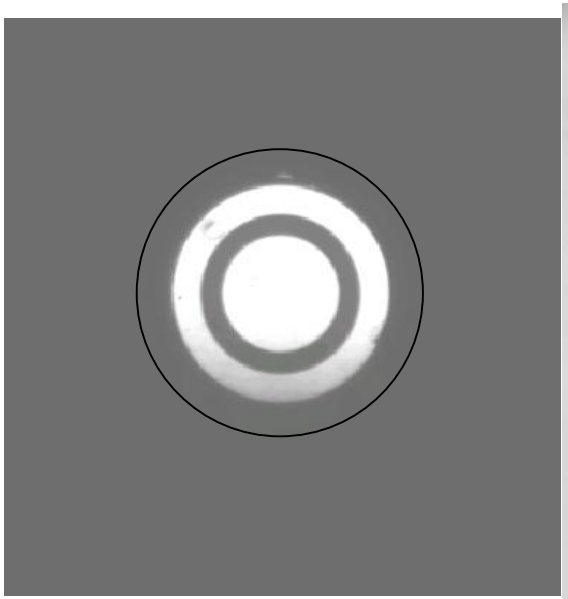


Annulus aligned with Phase Ring in Objective

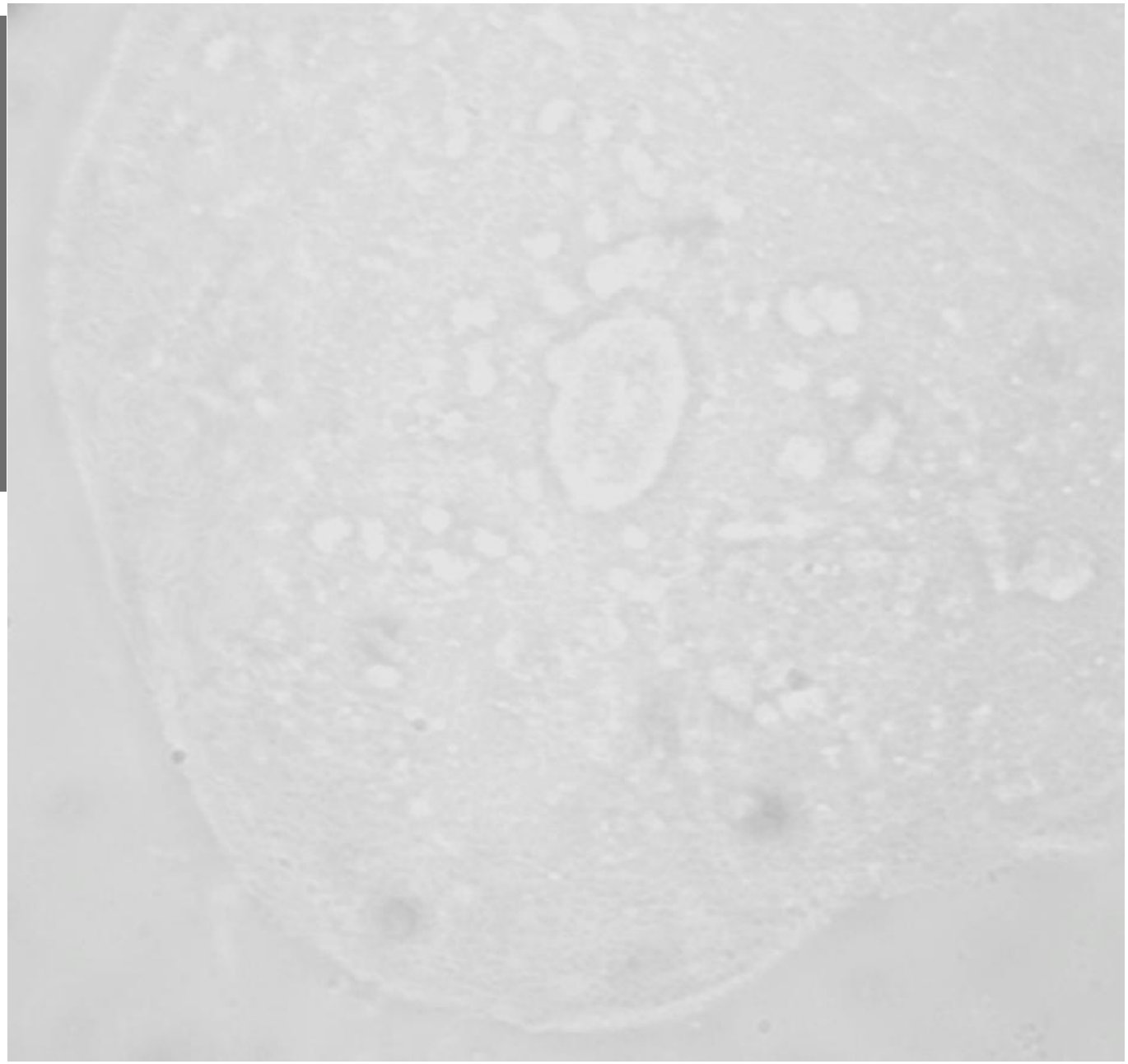


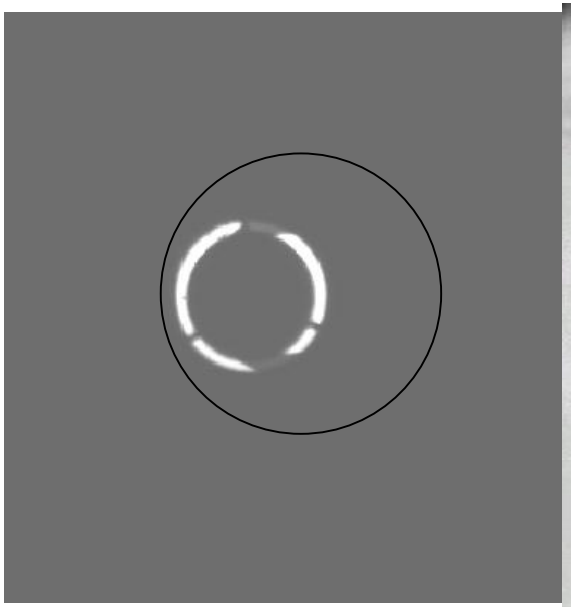



ZEISS
1

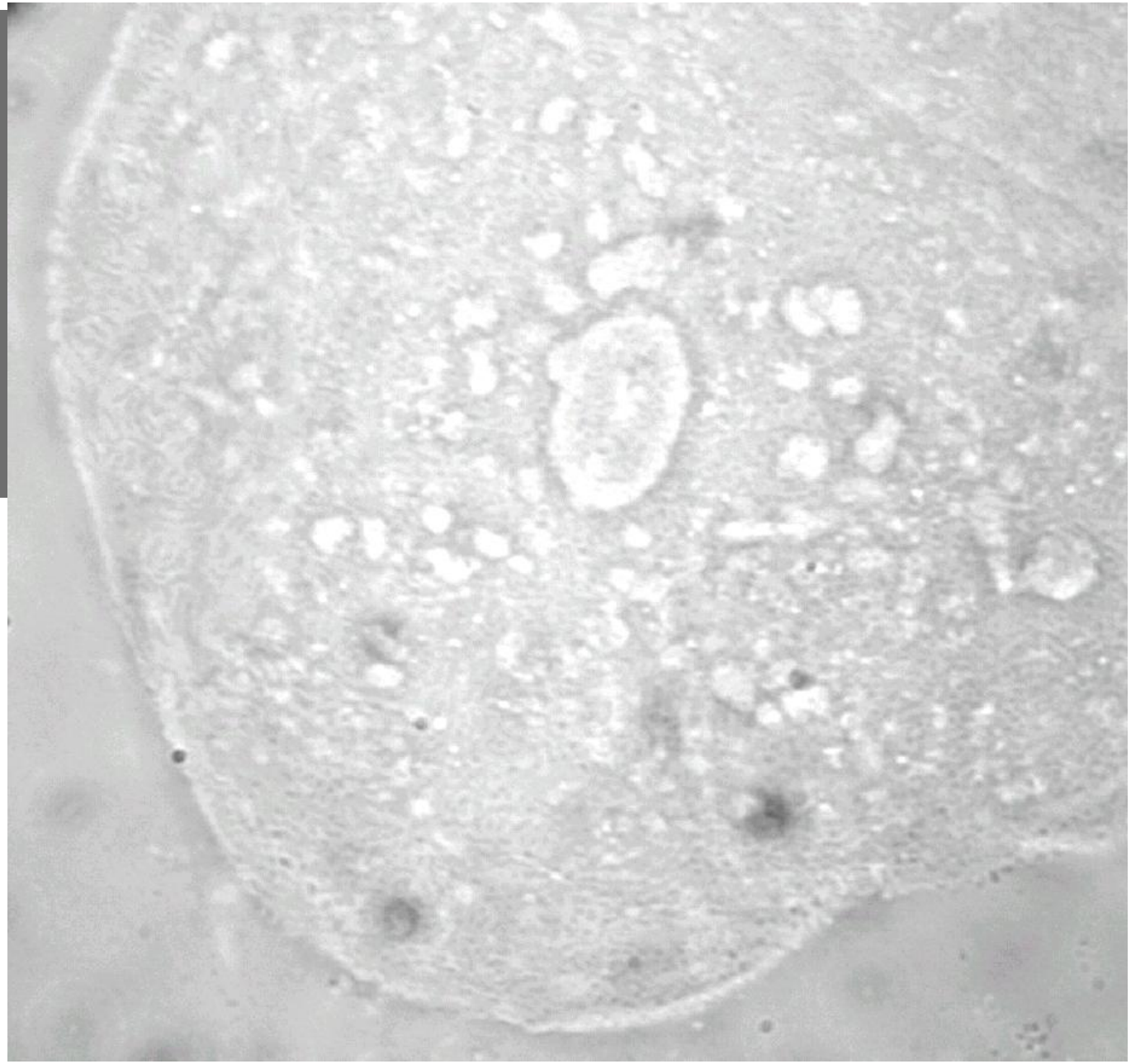


Cheek Cell, No Condenser Annulus





Cheek Cell, Miss-Aligned Condenser Annulus



Cheek cell,
Properly Aligned
Condenser Annulus