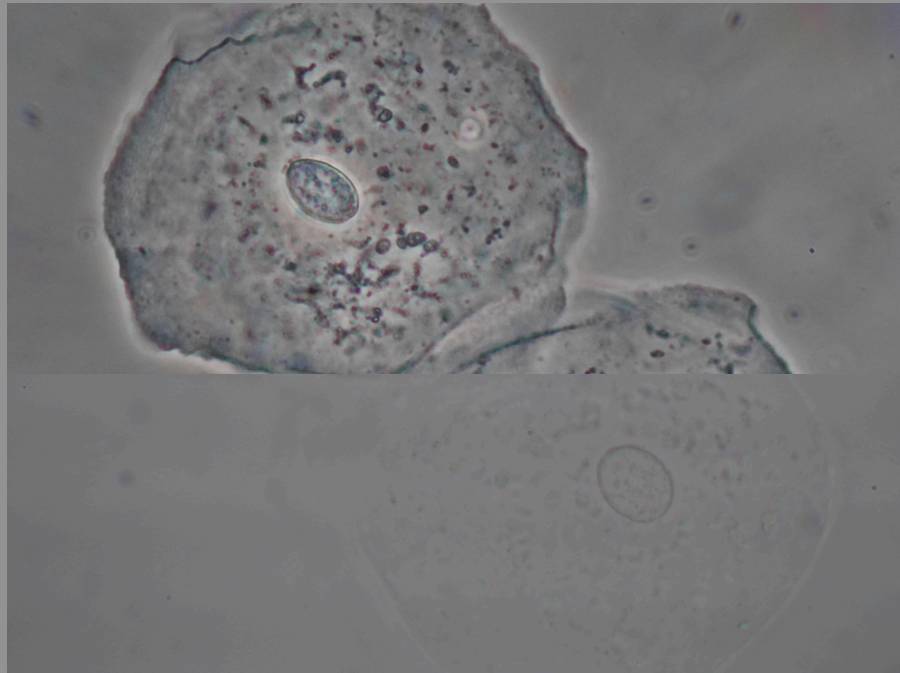


# In phase contrast microscopy phase shift in translated into brightness contrast

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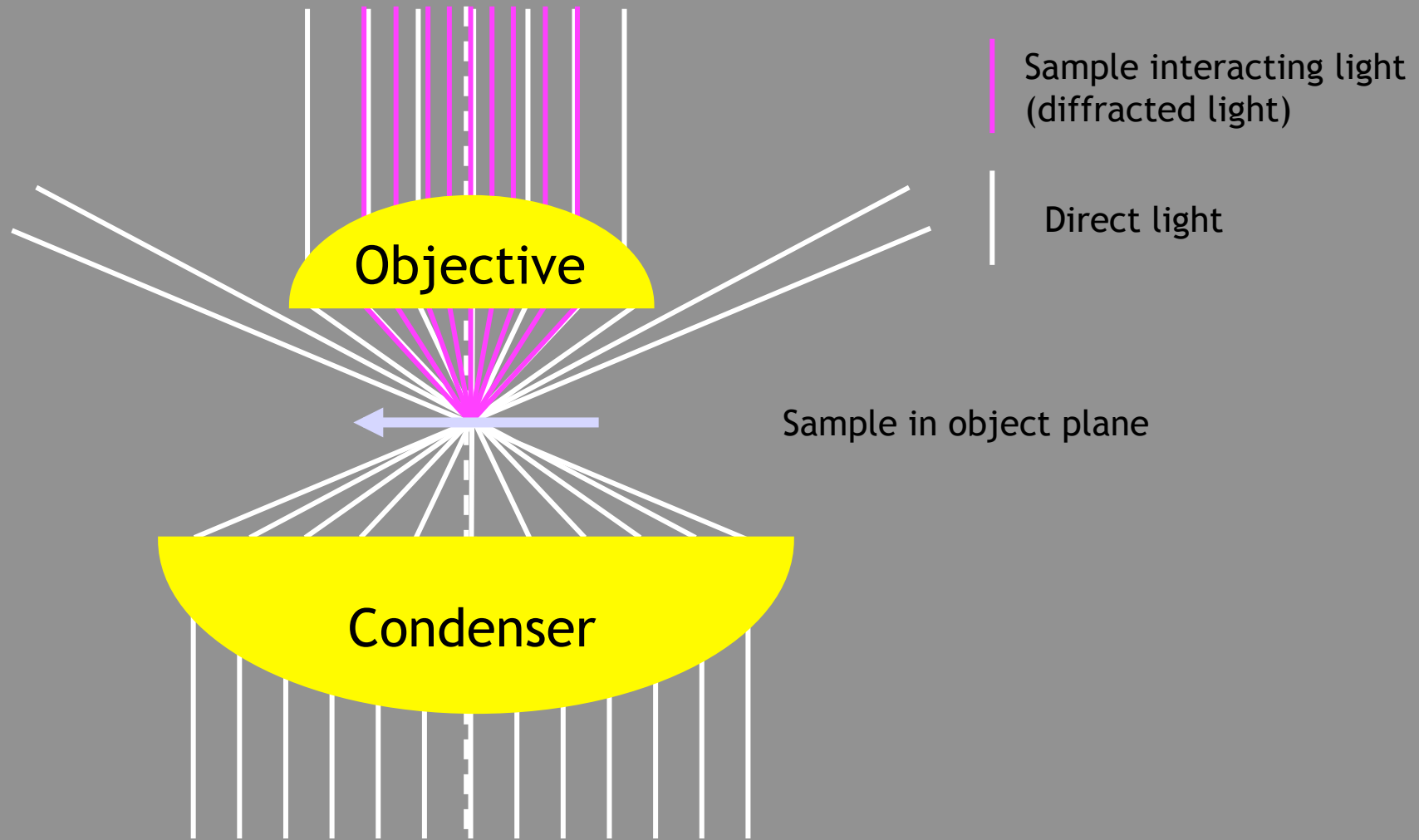


Frits Zernike

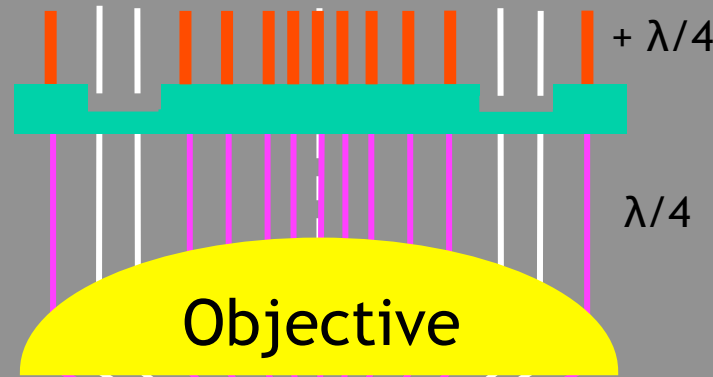
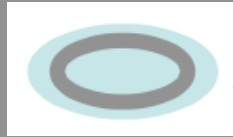
- Discovered principle of phase contrast in early 1930ies
  - Nobel price for physics in 1953
-

For thin non-absorbing samples the phase of the diffracted light gets retarded by  $\lambda/4$

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In phase contrast diffracted light gets retarded by another  $\lambda/4$  resulting in  $\lambda/2$  phase shift



Phase plate in back focal plane of objective

Sample in object plane

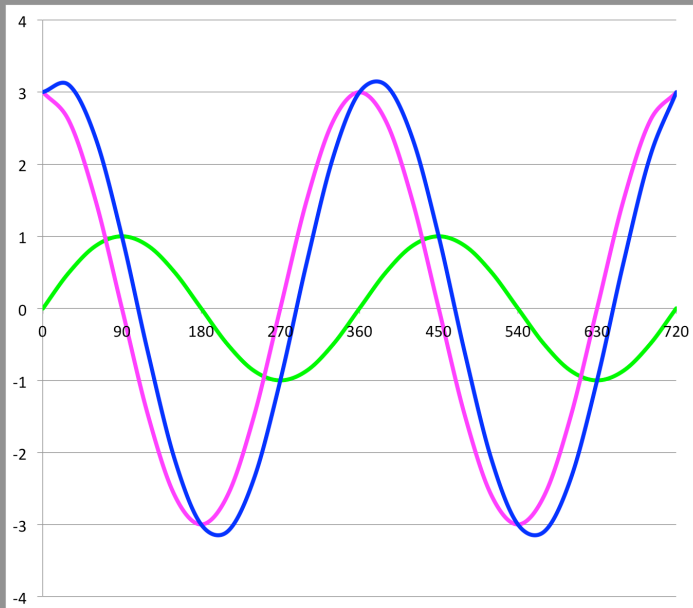
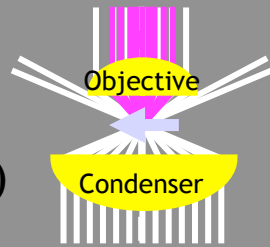


Illuminating annulus in front focal plane of condenser

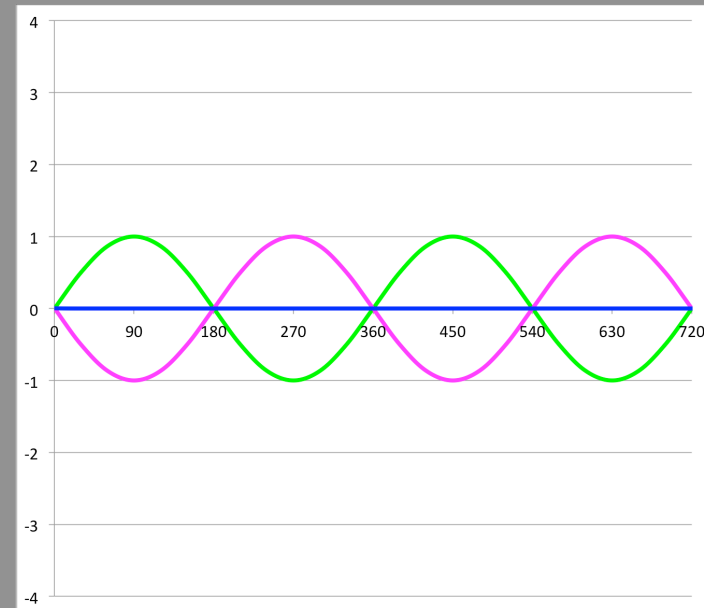
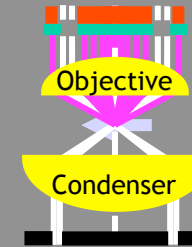


# In phase contrast diffracted light gets retarded by another $\lambda/4$ resulting in $\lambda/2$ phase shift

Bright field  
( $\lambda/4$  phase shift)



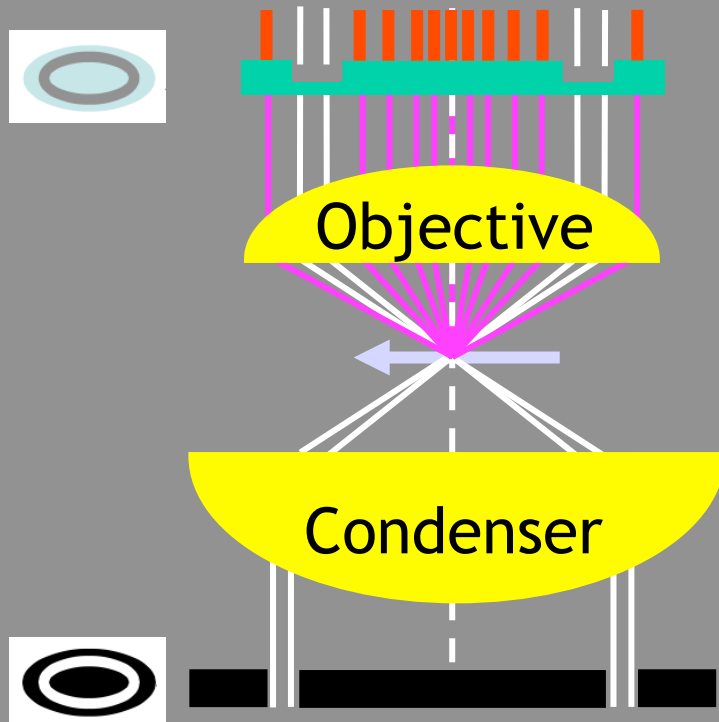
Phase contrast  
( $\lambda/2$  phase shift)



— Direct Light    — Diffracted Light    — Resultant

# Three steps to adjust phase contrast illumination

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1. Set up Köhler illumination

2. Swing in illuminating annulus  
("1", "2" or "3" in condenser; must  
match number on objective)

3. While looking into the BFP of the  
objective, adjust position of illuminating  
annulus until its concentric with phase plate

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