Phase Contrast Microscopy

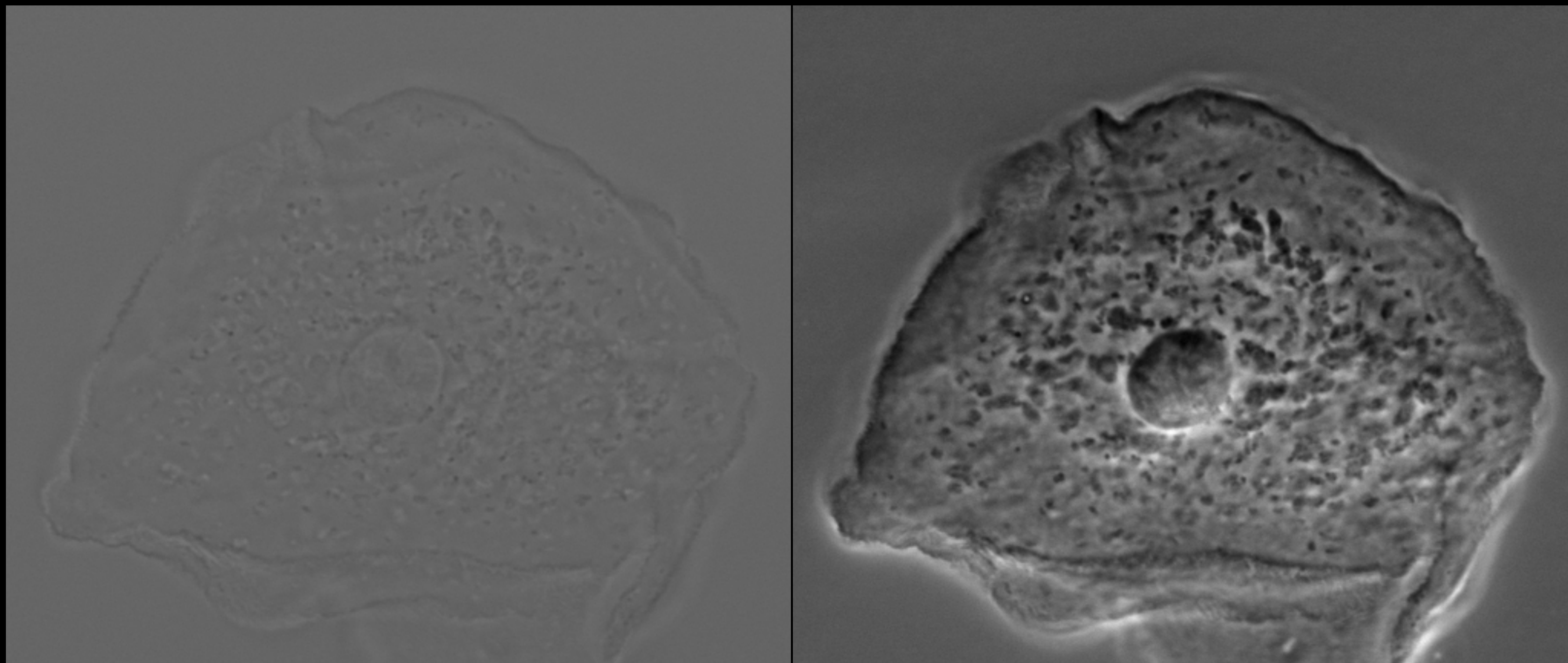
Principles of Light Microscopy Course
MPI-CBG

What is it?

Contrast-enhancing technique described first by Zernike in 1932 - Nobel price for physics in 1953

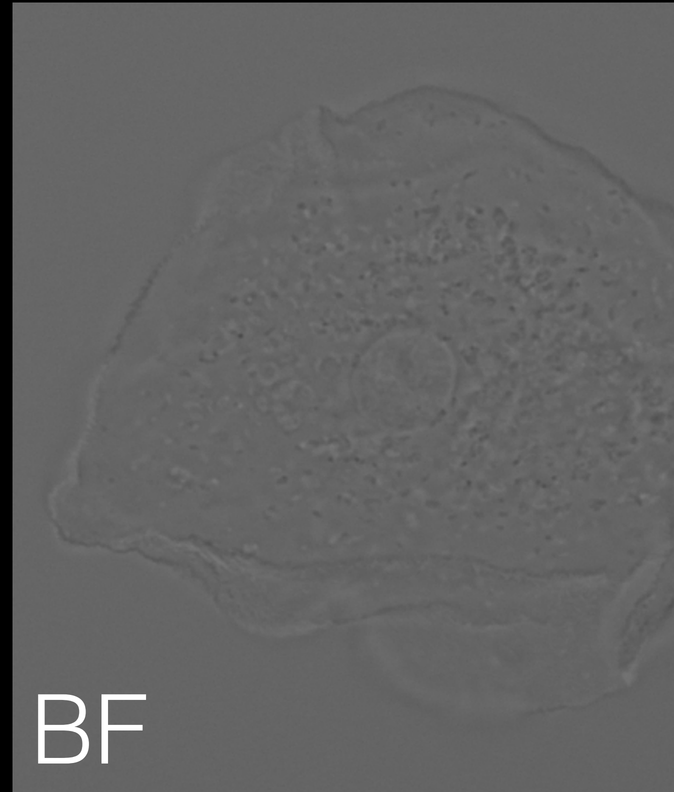
It can be utilised to produce high-contrast images of almost transparent specimens

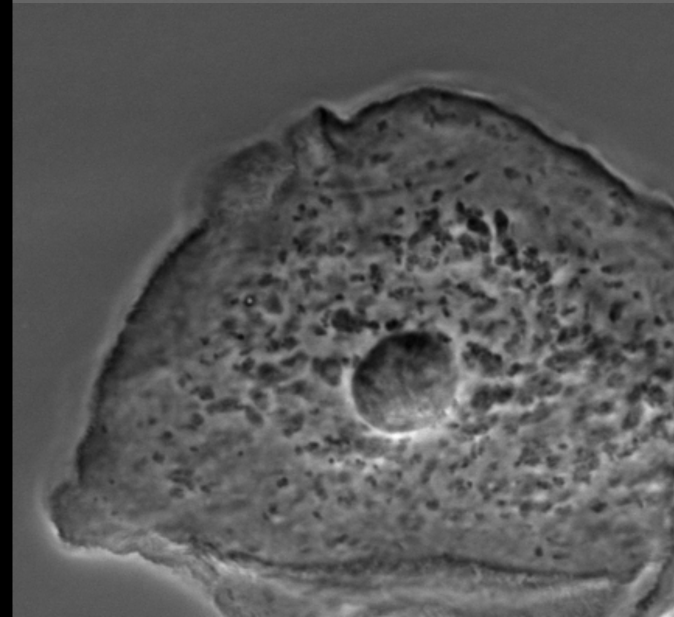
Frits Zer



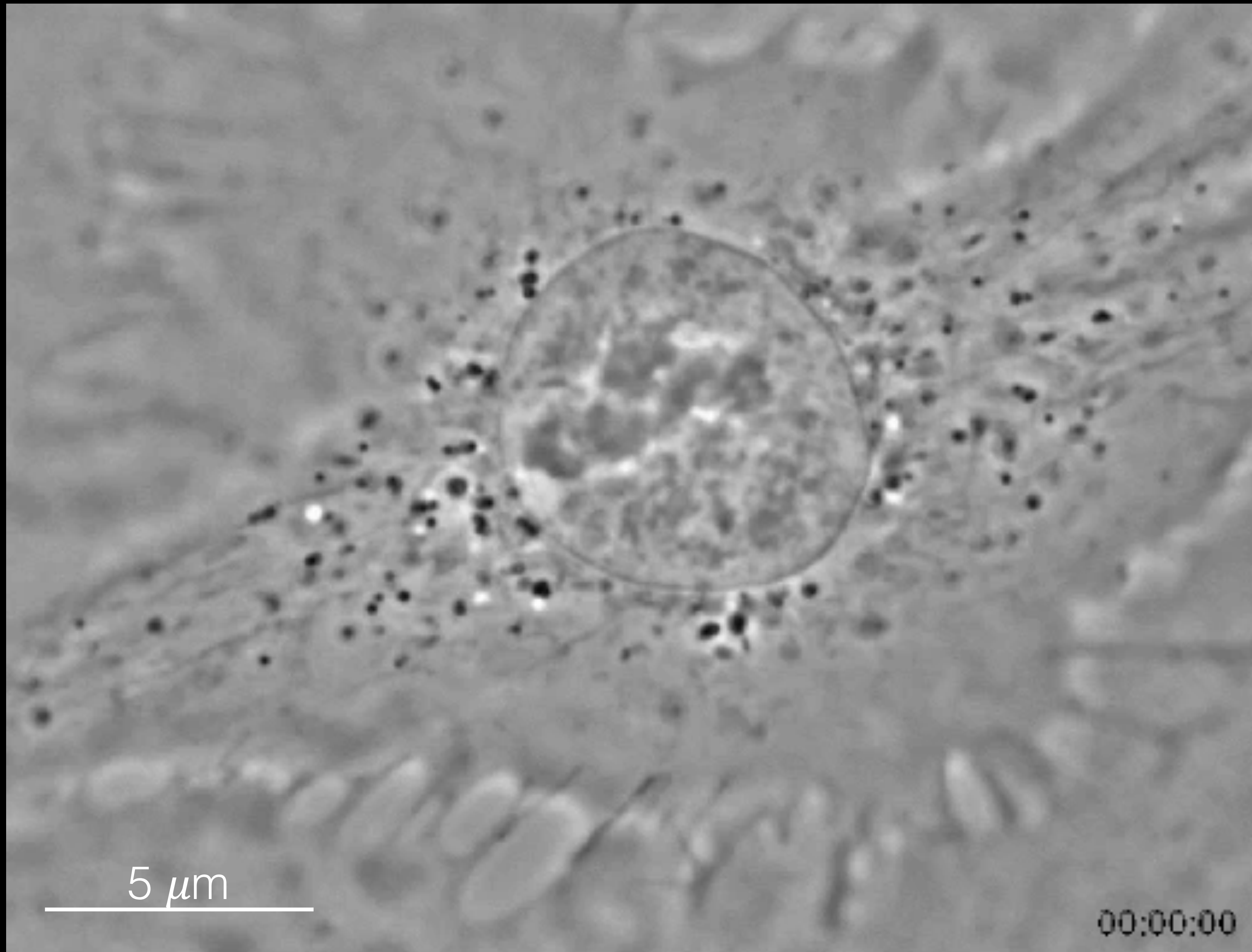
What is it good for?

- Living cells (usually in culture)
- Microorganisms
- Subcellular particles
- Thin tissue slices
- Lithographic patterns
- Fibers





Tissue Culture Cell



Principle of Phase Contrast

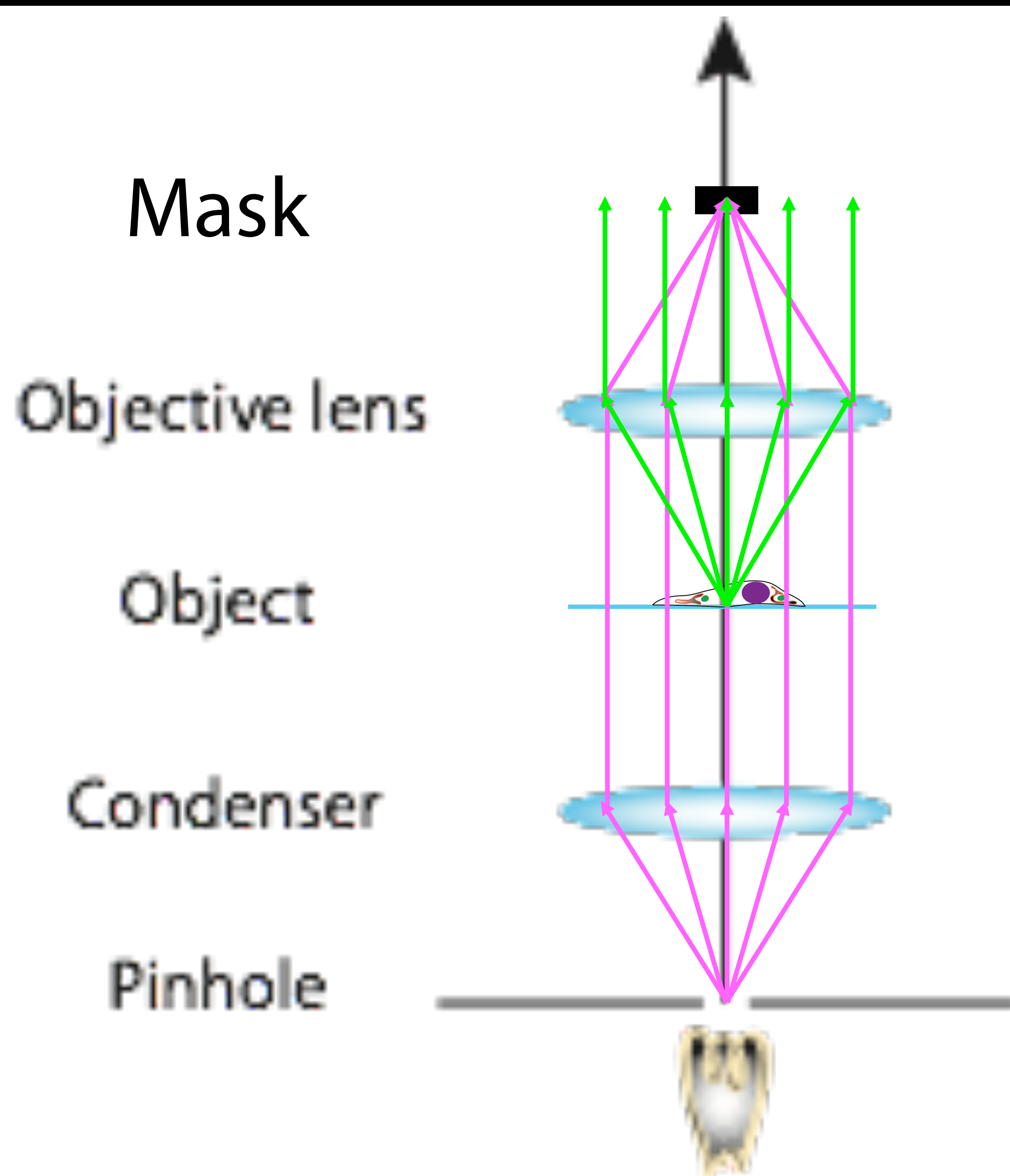
- Modifying the light diffracted by the object and the undiffracted light in such a way that an amplitude contrast occurs

Separation of diffracted and undiffracted light

Mask
ck focal plane)
jective lens
Object
ondenser
Pinhole

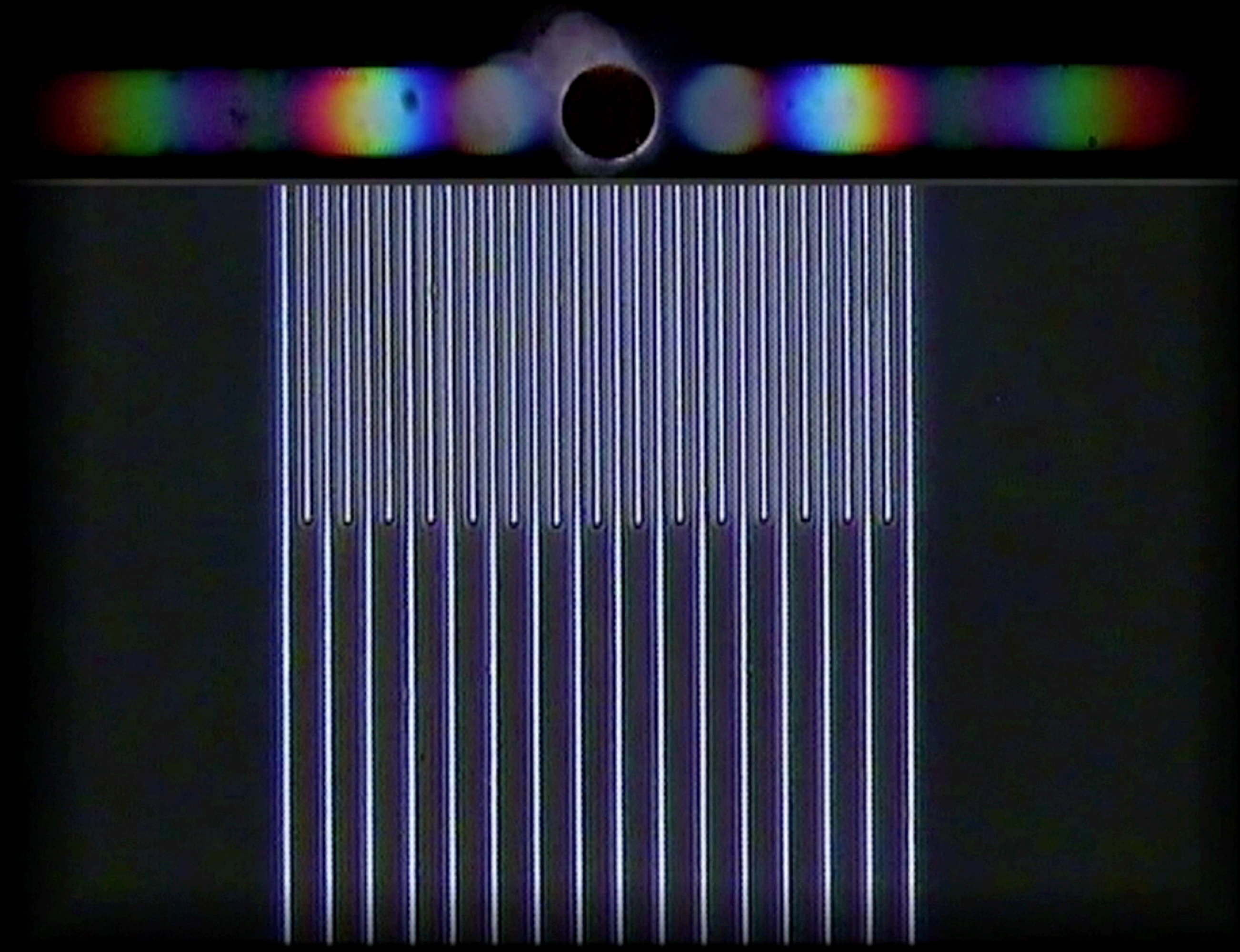
Mask
(back focal plane)
Objective lens
Object
Condenser
Pinhole

Separation of undiffracted and diffracted light



Optical setup of Zernike's second experiment

diffracted light



DARK
FIELD

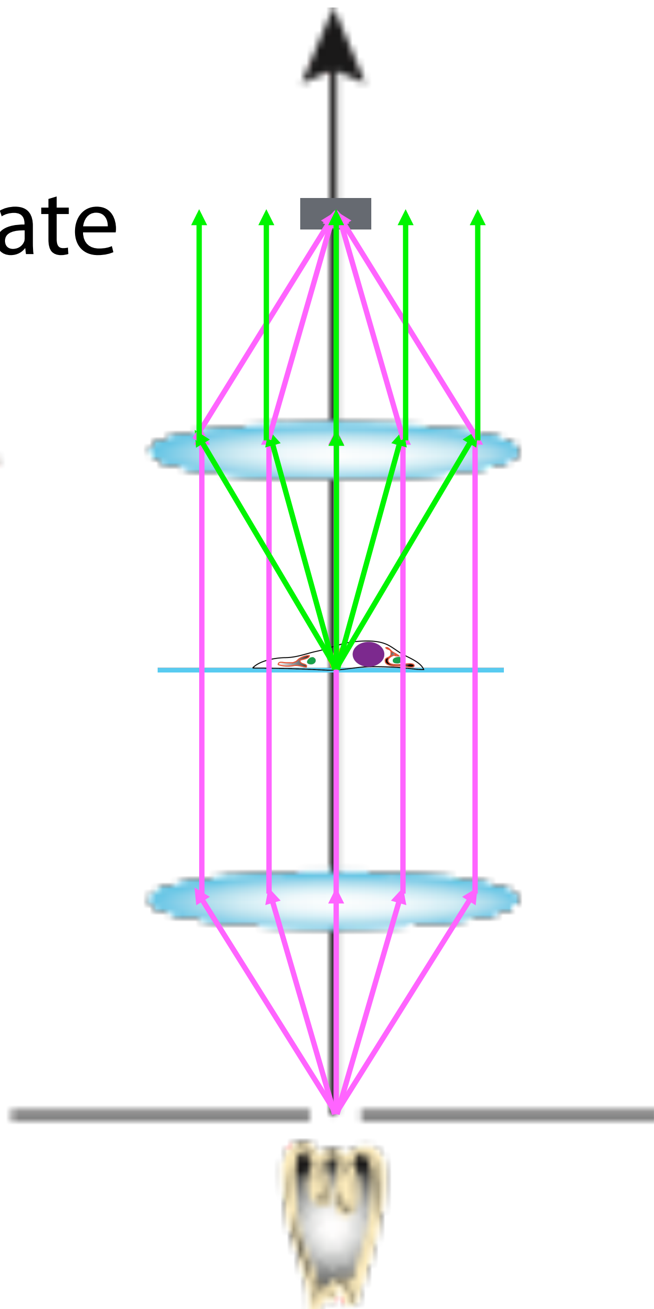
λ/4 Phase plate

Objective lens

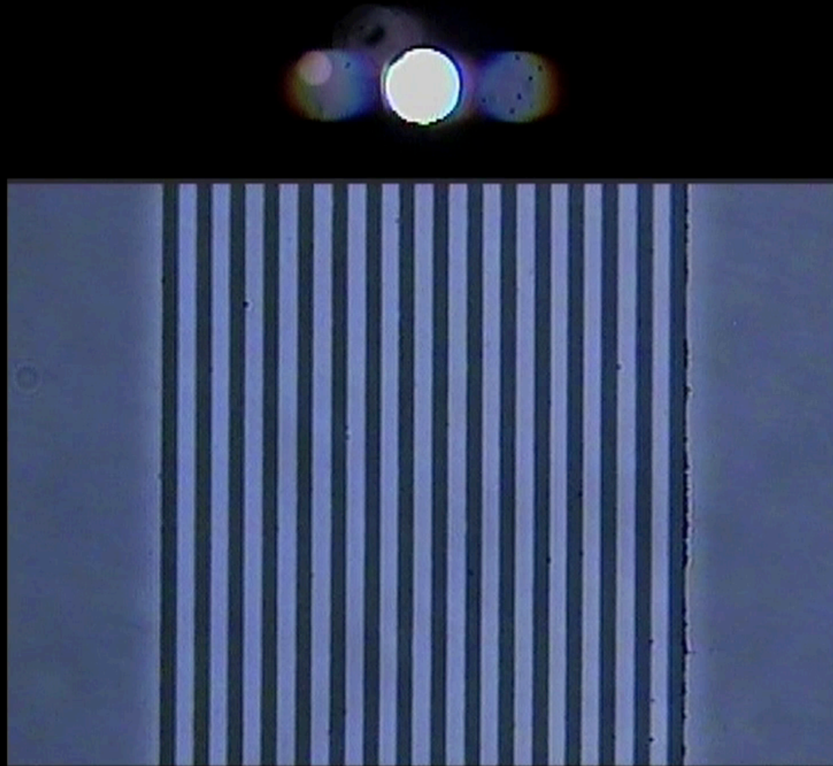
Object

Condenser

Pinhole

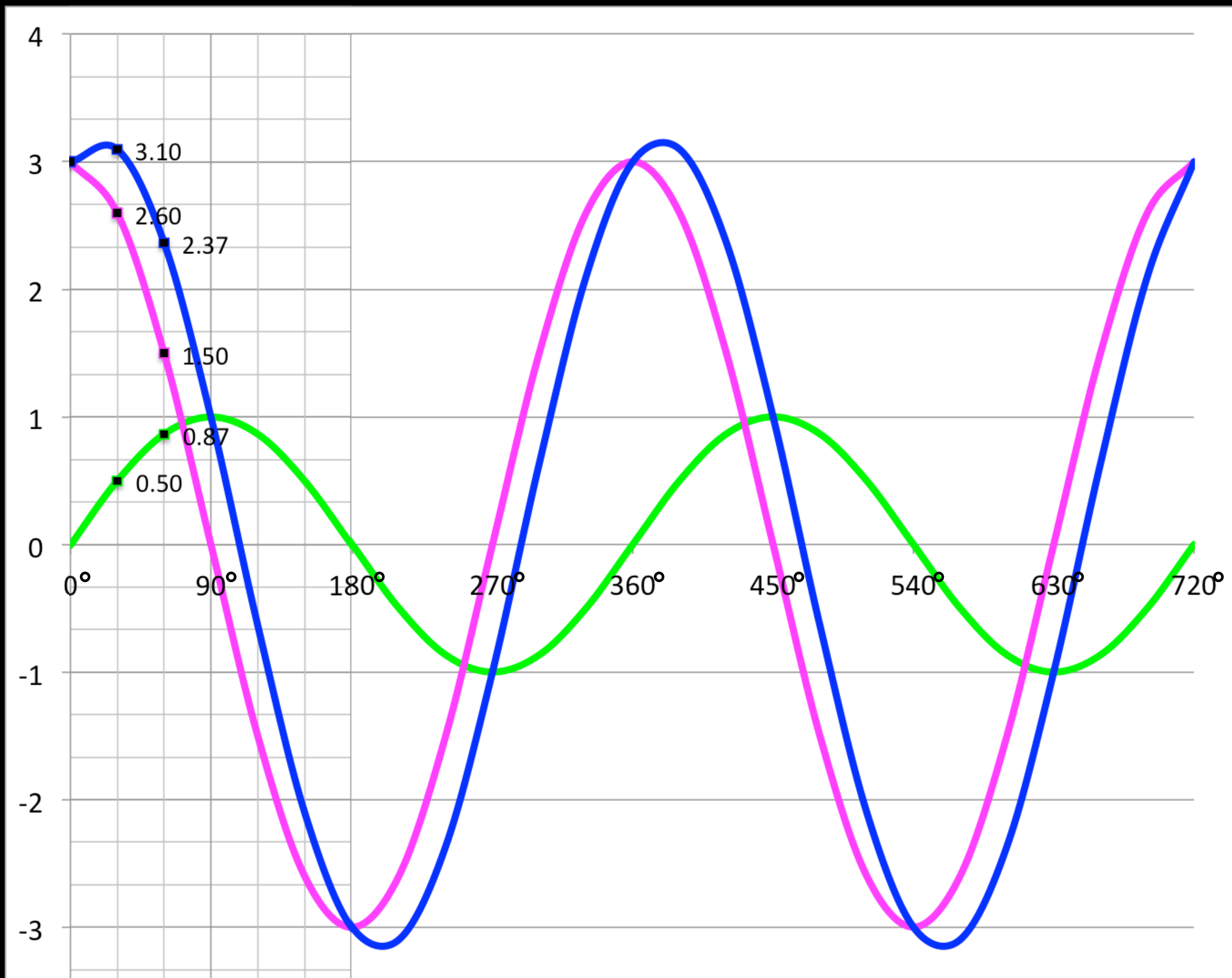


Optical setup of Zernike second experiment



PHASE CONTRAST

hase contrast optical accessori



hase contrast optical accessori

Undiffracte
Diffracted
Resultant

Interaction of Light Waves with Phase Specimens

An incident wave front becomes divided in to two components upon passing through a phase specimen:

- Undiffracted light (U) - Planar wavefront - Primary component

- Diffracted light (D) - Spherical wave front

U and D combine in the image plane through interference producing a Resultant wave (R)

- R = U + D

etection of the Specimen Imag

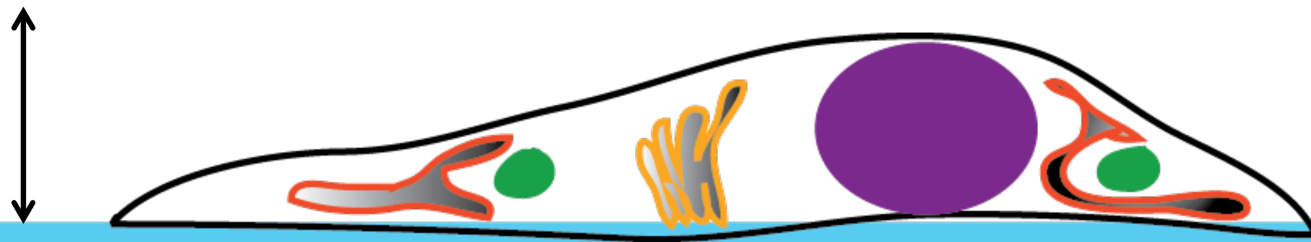
Considering R = U + D

- It depends on the relative intensity differences (amplitudes) of R and U
  - If R is significantly different in amplitude from U → **Contrast**
  - If R is NOT significantly different in amplitude from U → **No Contrast**

Optical Path Length (OPL)

$$OPL = n \times t$$

- Where “n” is the refractive index of the specimen and “t” its thickness



Phase shift $\delta = 2\pi\mathbf{\Delta}/\lambda$

Optical Path Length (OPL)

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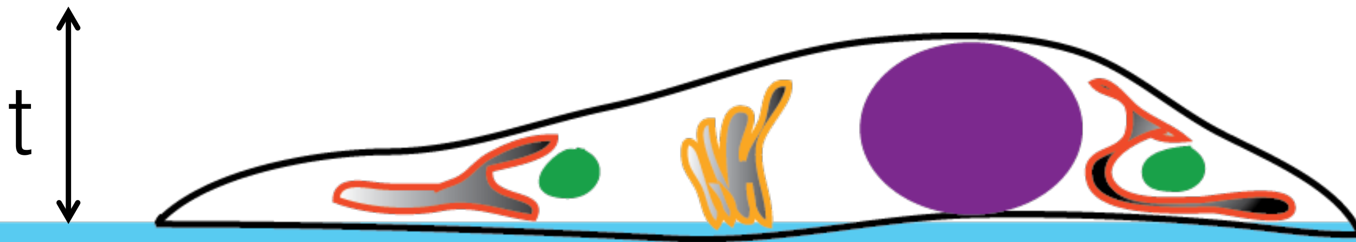
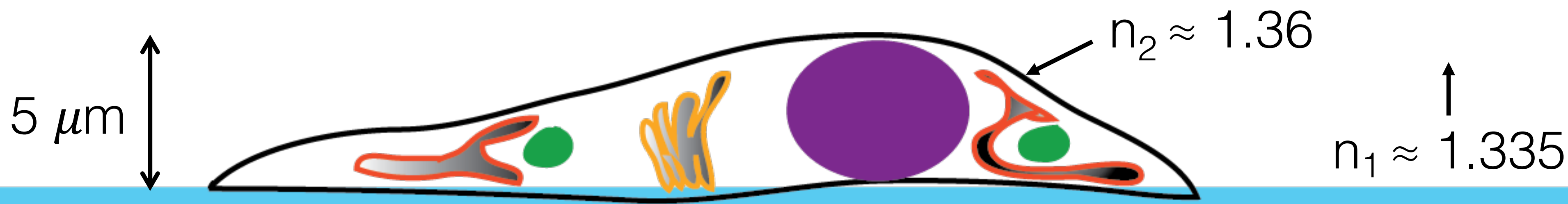


Optical Path Difference (OPD)

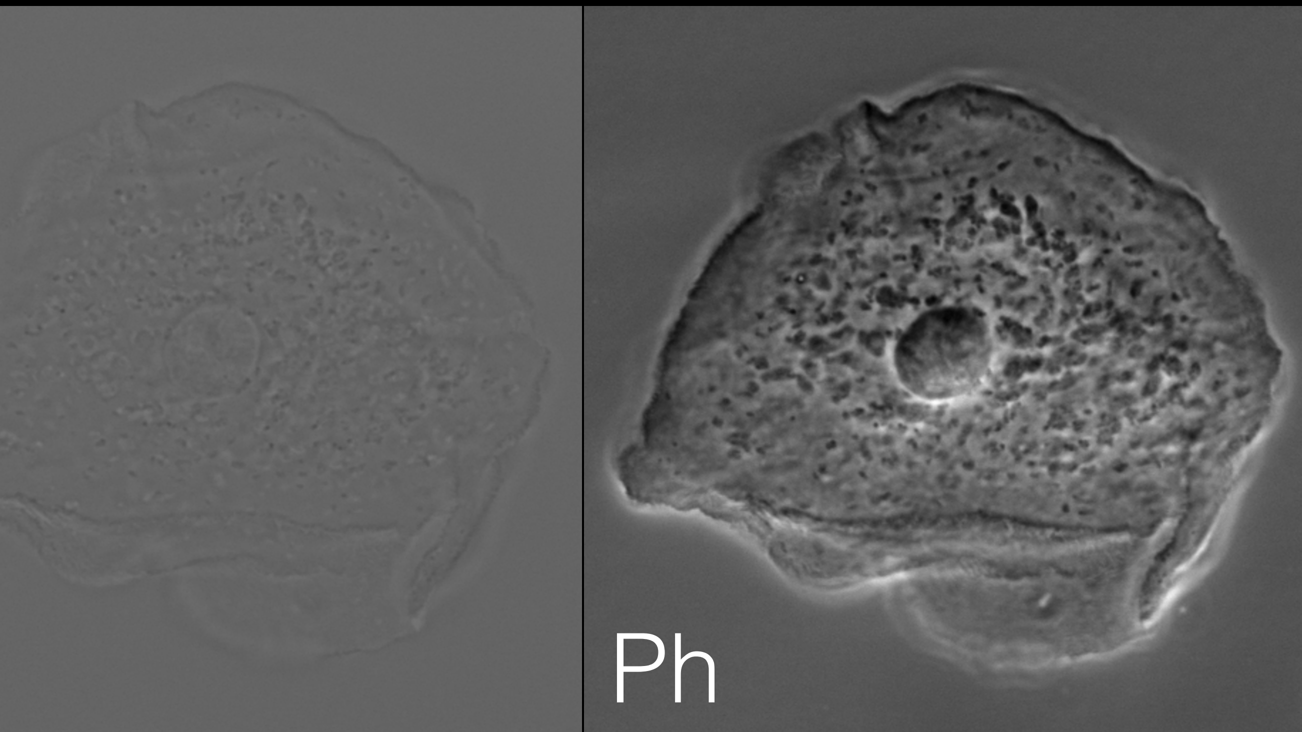
$\mathbf{\Delta}$ = Optical Path Difference (OPD) = $(n_2 - n_1) \times t$

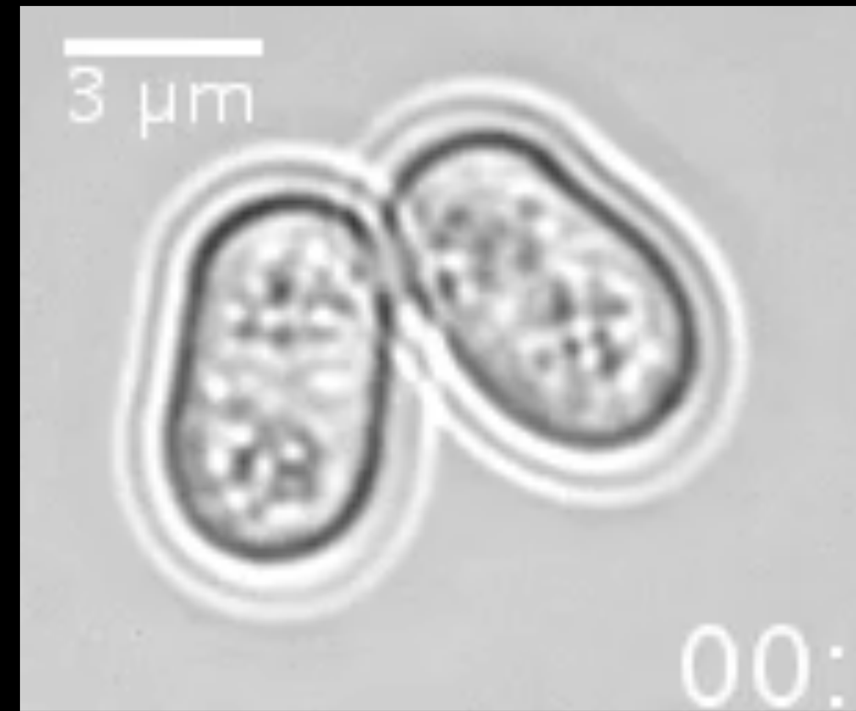
Where n_2 is the refractive index of the specimen and n_1 is the refractive index of the surrounding medium

a typical cell in monolayer culture:



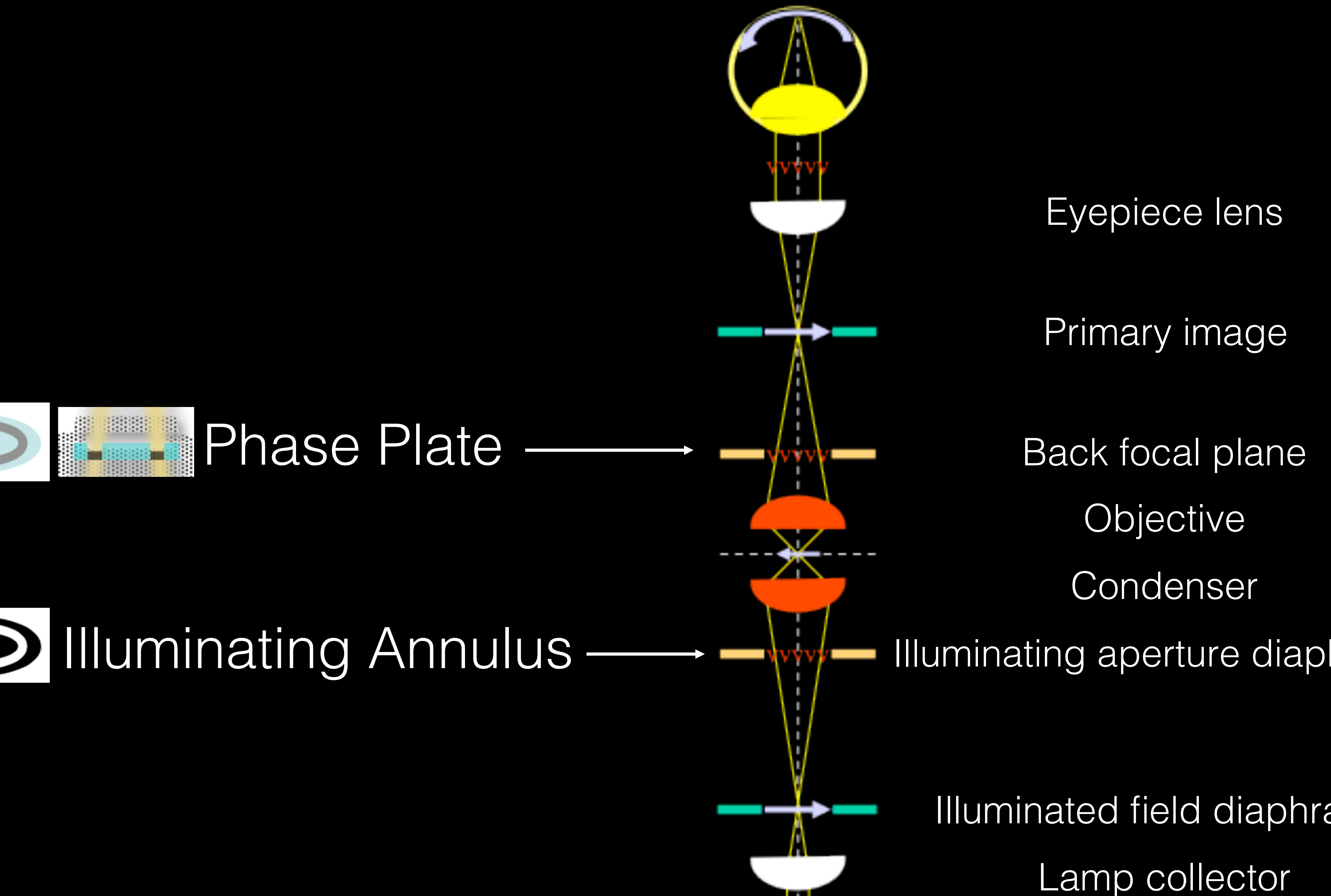
$\mathbf{\Delta} = 0.125\ \mu m$



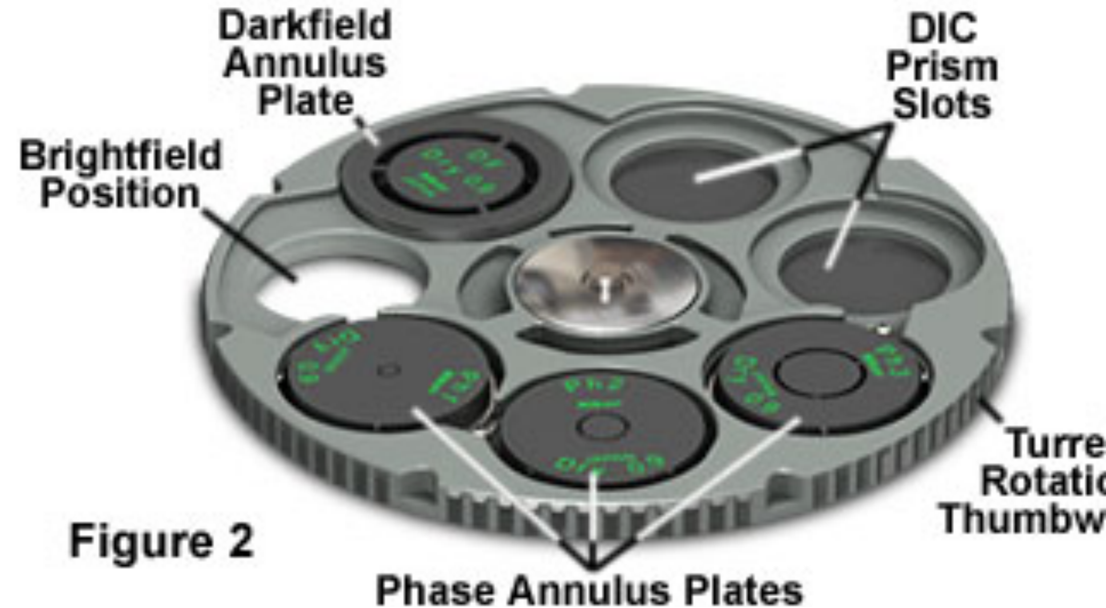


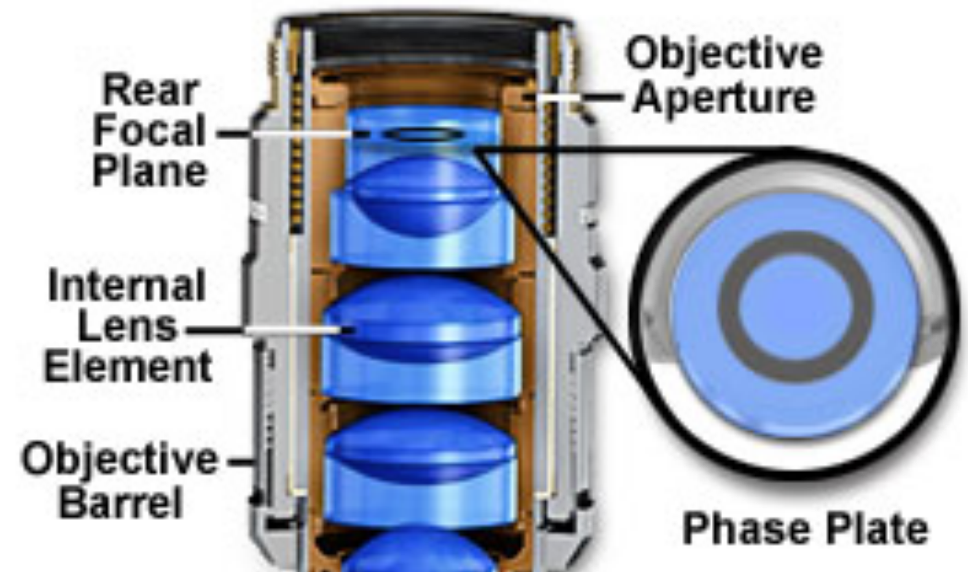
ack of homogeneity due to the different OPL given b
ifferent portion of the sample

Halo artefact caused by incomplete separation of th
iffracted light from the undiffracted light


Eyepiece lens
Primary image
Phase Plate
Back focal plane
Objective
Condenser
Illuminating Annulus
Illuminating aperture
Illuminated field
Lamp collector

Needed Tools



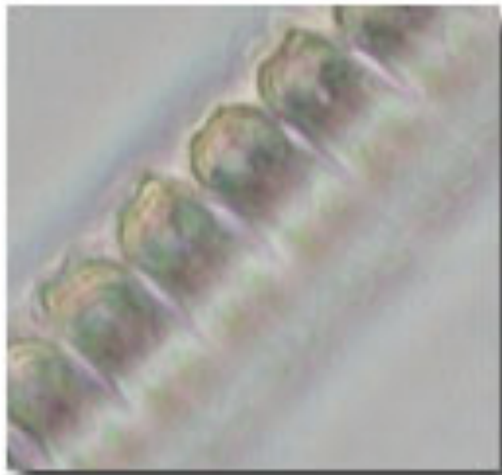


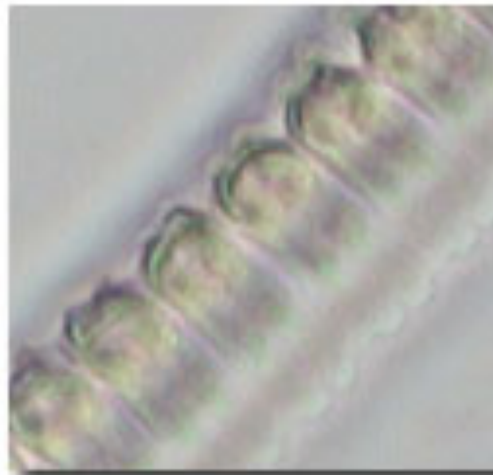
and its Effect on Contrast

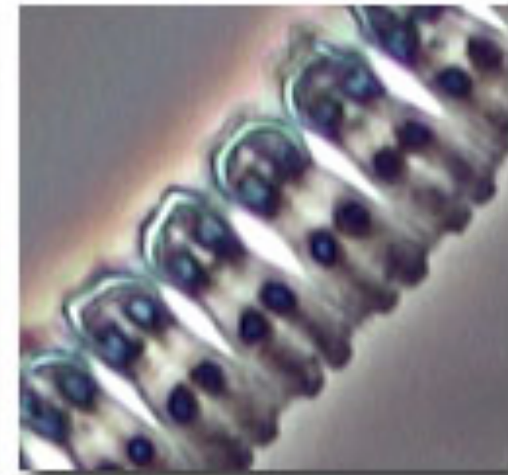
(a)

(c)

(e)


(b)


(d)


(f)