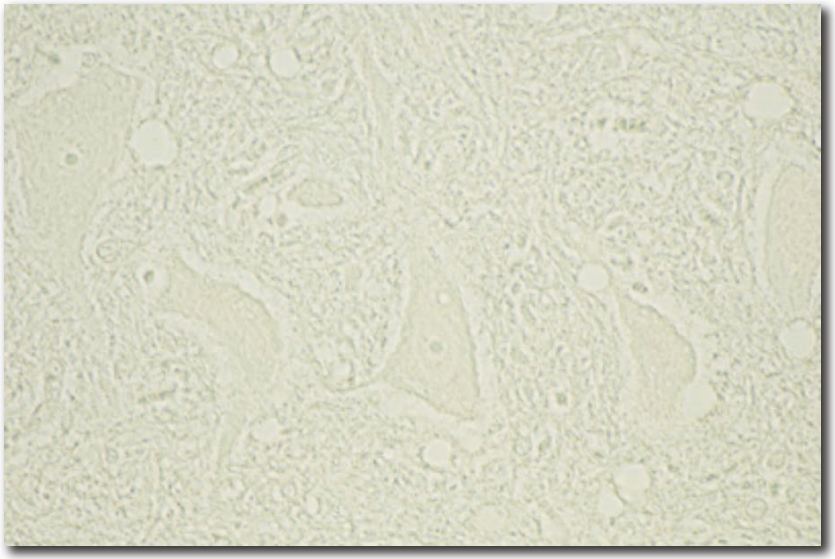
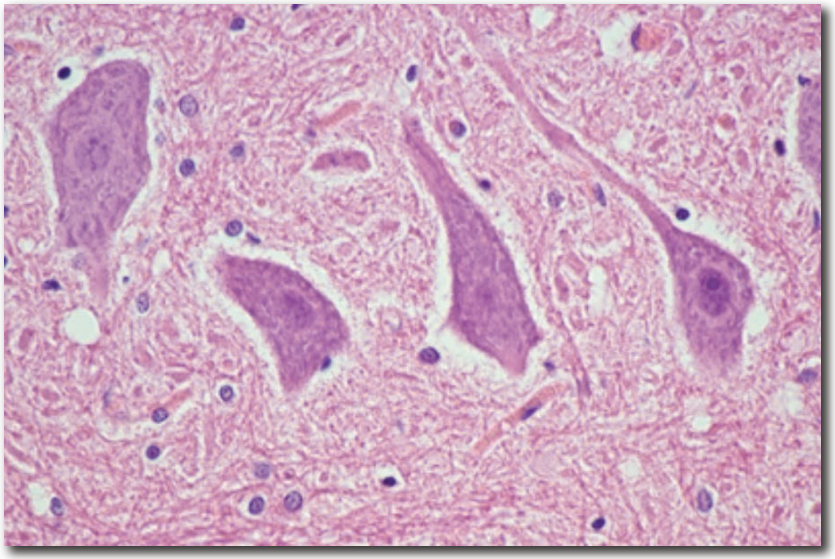
Phase Contrast Technique
&
Creating Image Contrast

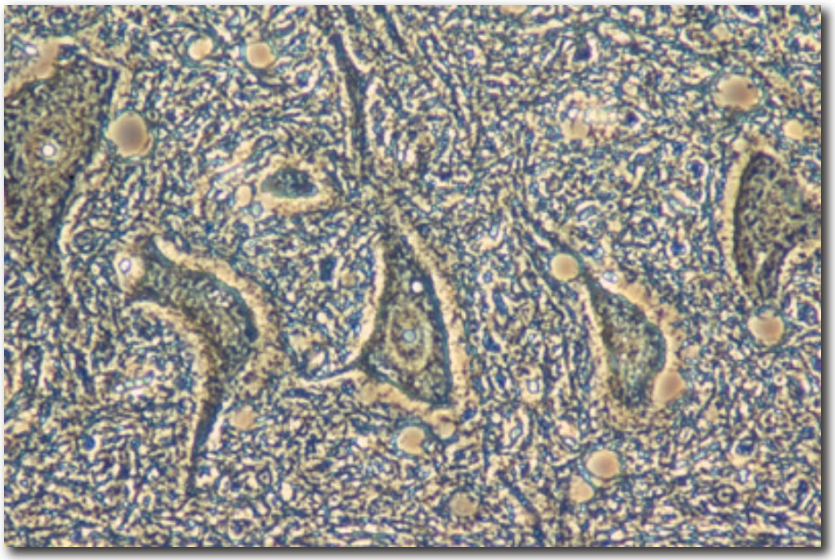
- http://www.microscopyu.com/galleries/phasecontrast/index.html

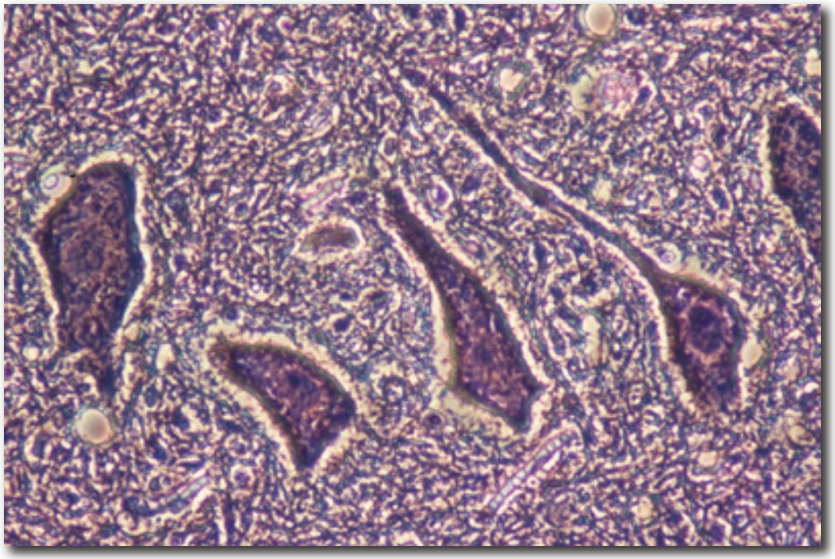
Inverted Phase Microscopy

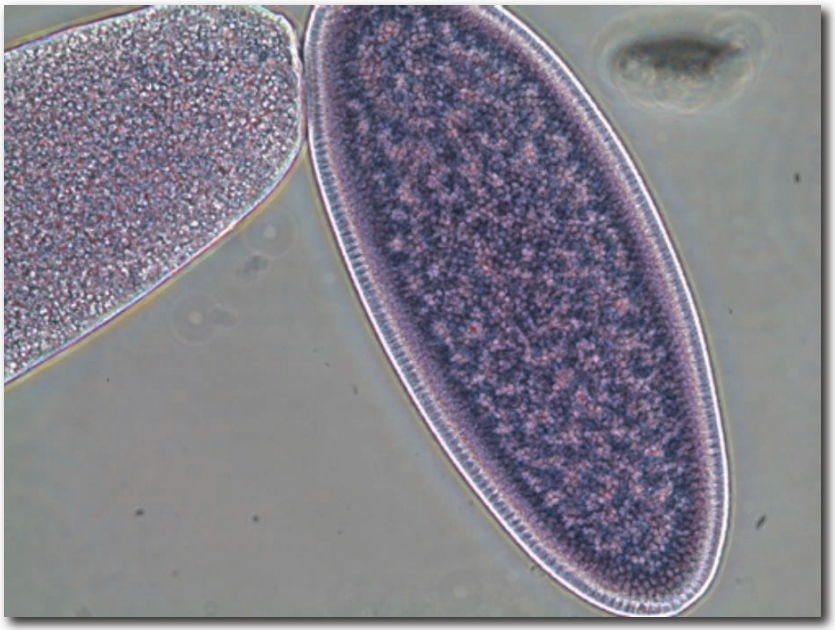
- Live cell imaging

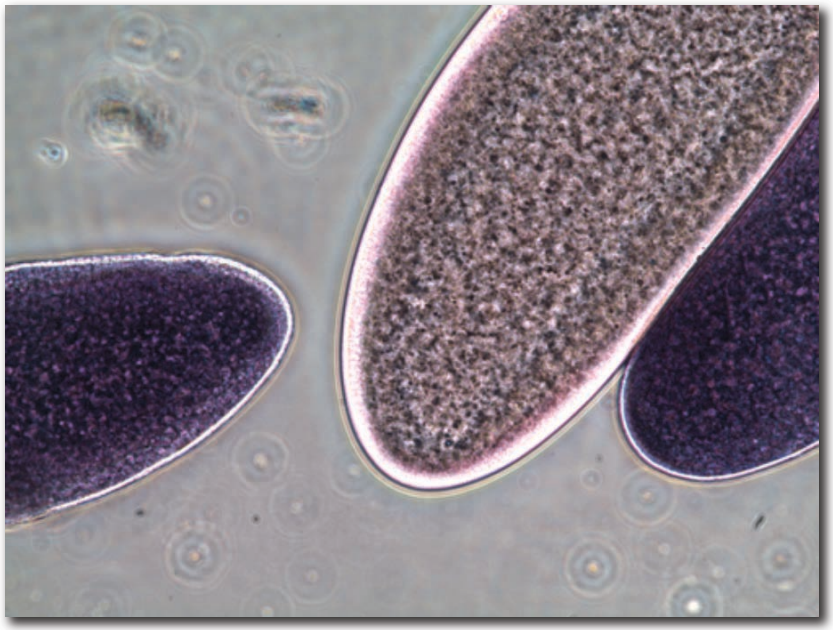












Diptera (sp)

Equipment & Optics

Phase Objectives

Phase Condenser

Optional - phase telescope

Frits Zernike, Nobel Prize for Physics, 1953.
The Dutch physicist, when experimenting with reflection gratings in 1930, discovered that he could observe the phase position of each ray, and sought to utilize the effect for microscopy. Together with Zeiss he developed the first phase-contrast microscope, the prototype of which was made in 1936. It allowed the examination of living cells without harmful chemical staining.

Phase Theory

Shift zero order - 1/4 λ from the diffracted rays

ⅅ

Phase ring - objective exit pupil

Phase Annulus- aperture diaphragm

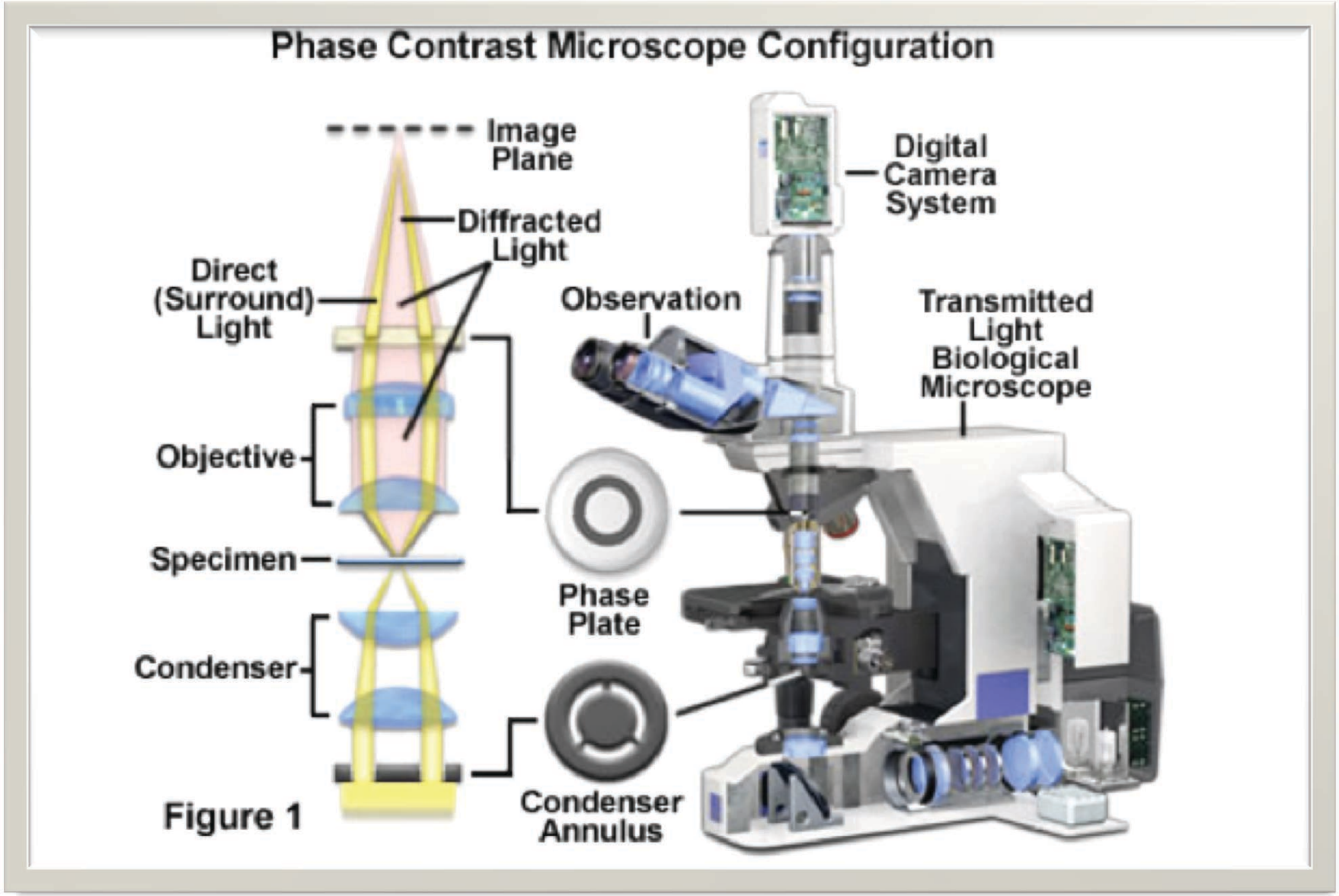
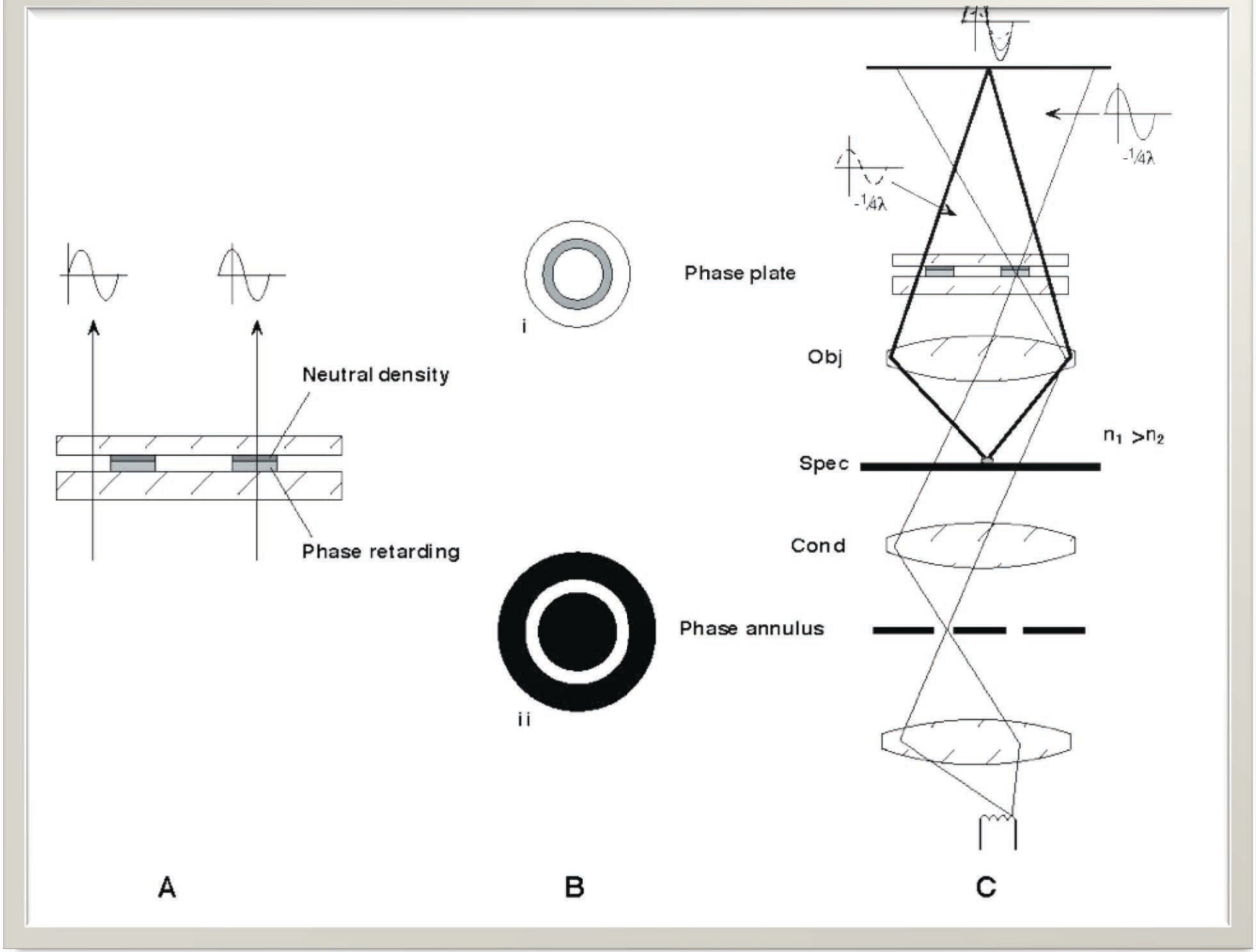

Phase Contrast Microscope Configuration
Image Plane
Diffracted Light
Direct (Surround) Light
Objective
Specimen
Condenser
Phase Plate
Condenser Annulus
Digital Camera System
Observation
Transmitted Light Biological Microscope

Figure 1

http://www.microscopyu.com/articles/phasecontrast/index.html

http://www.microscopyu.com/tutorials/java/phasecontrast/microscopealignment/index.html


Neutral density
Phase retarding
i
ii
Phase plate
Obj
Spec
Cond
Phase annulus
-1/4λ
-1/4λ
n1 >n2
A
B
C

Set Up

Uses Köhler

No AD – replaced by Phase annulus

Select proper objective & condenser

Focus sample

Focus field stop properly

Pull out eyepiece and in body tube

Superimpose the phase ring on the phase annulus

Viewed at exit pupil of objective

Annulus of Condenser
must be matched to ring
located at the Objective exit pupil

Diffracted Rays go through the sample

Zero order goes through background

Sample displaces pathway

Interference Image

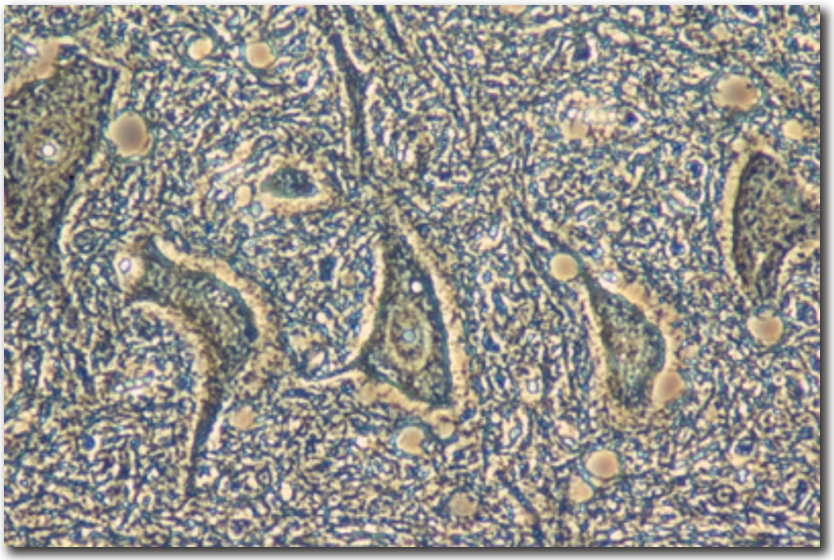
Interference occurs at

Intermediate Image Plane

A result of sample

and its inherent characteristics

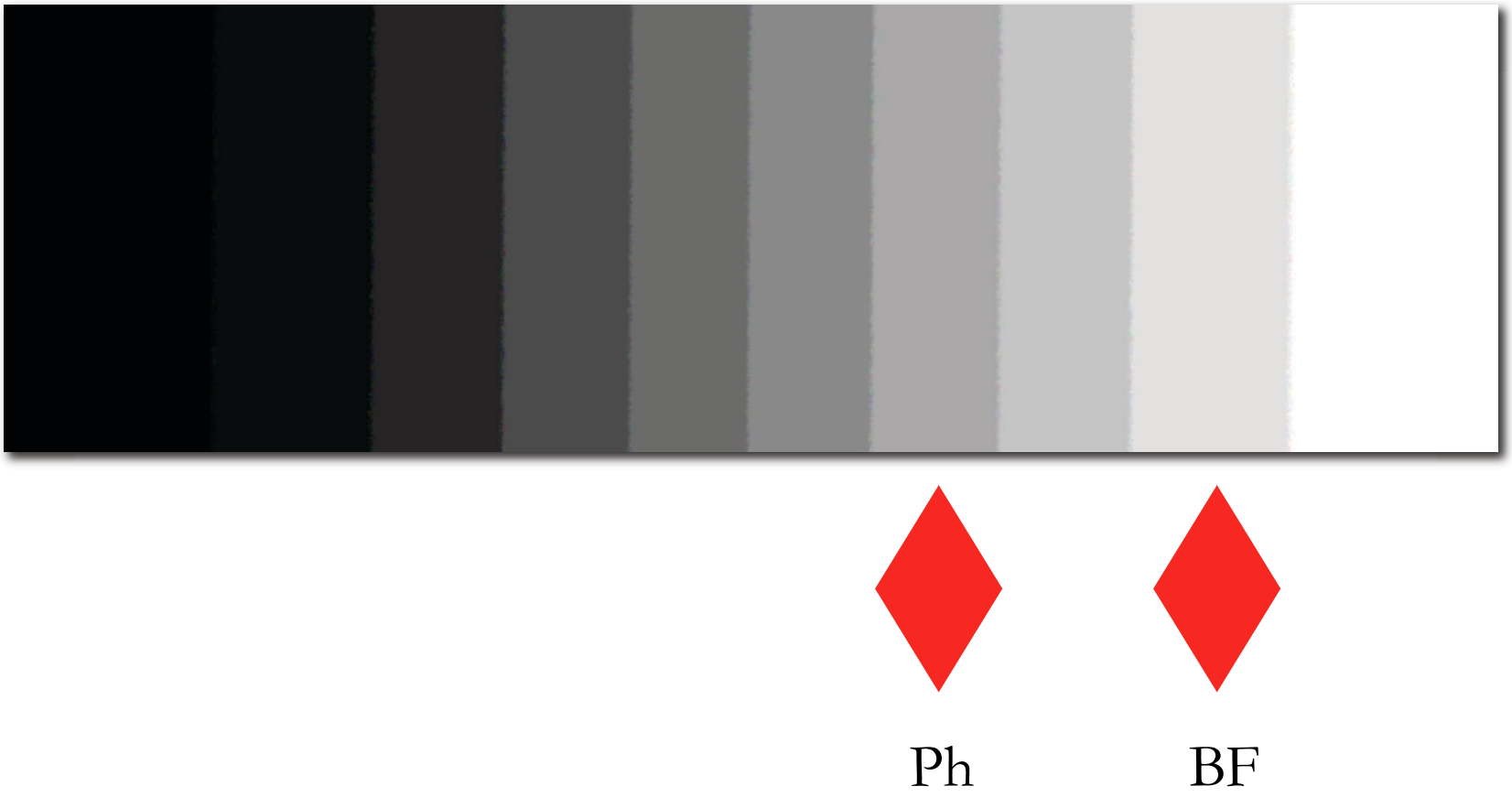
Imaging Problems



-Monochromatic

-HALOS

Exposure Placement



Popular technique

- Live cell imaging
- In vitro fertilization