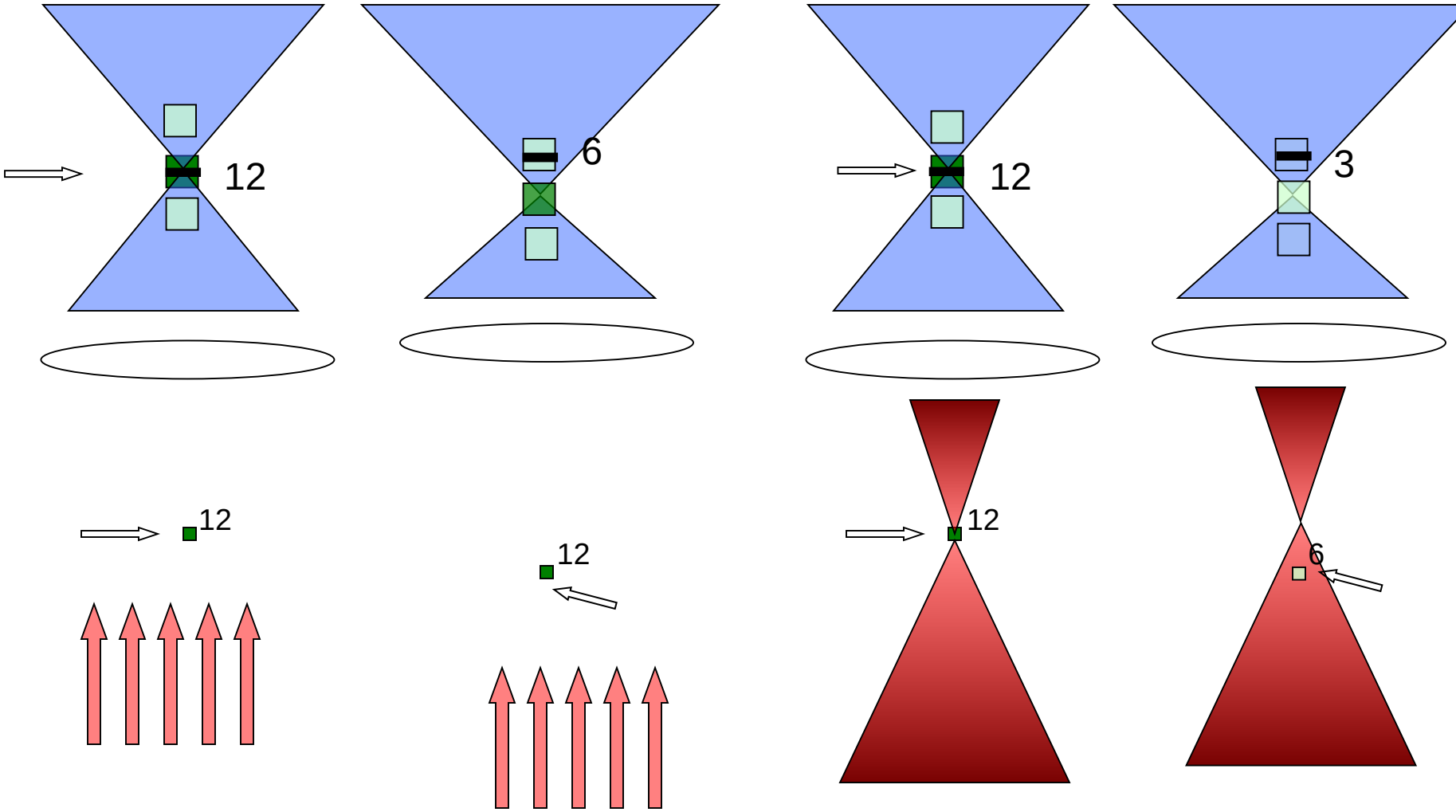
Charles Chevalier's Horizontal Microscope (circa 1834)

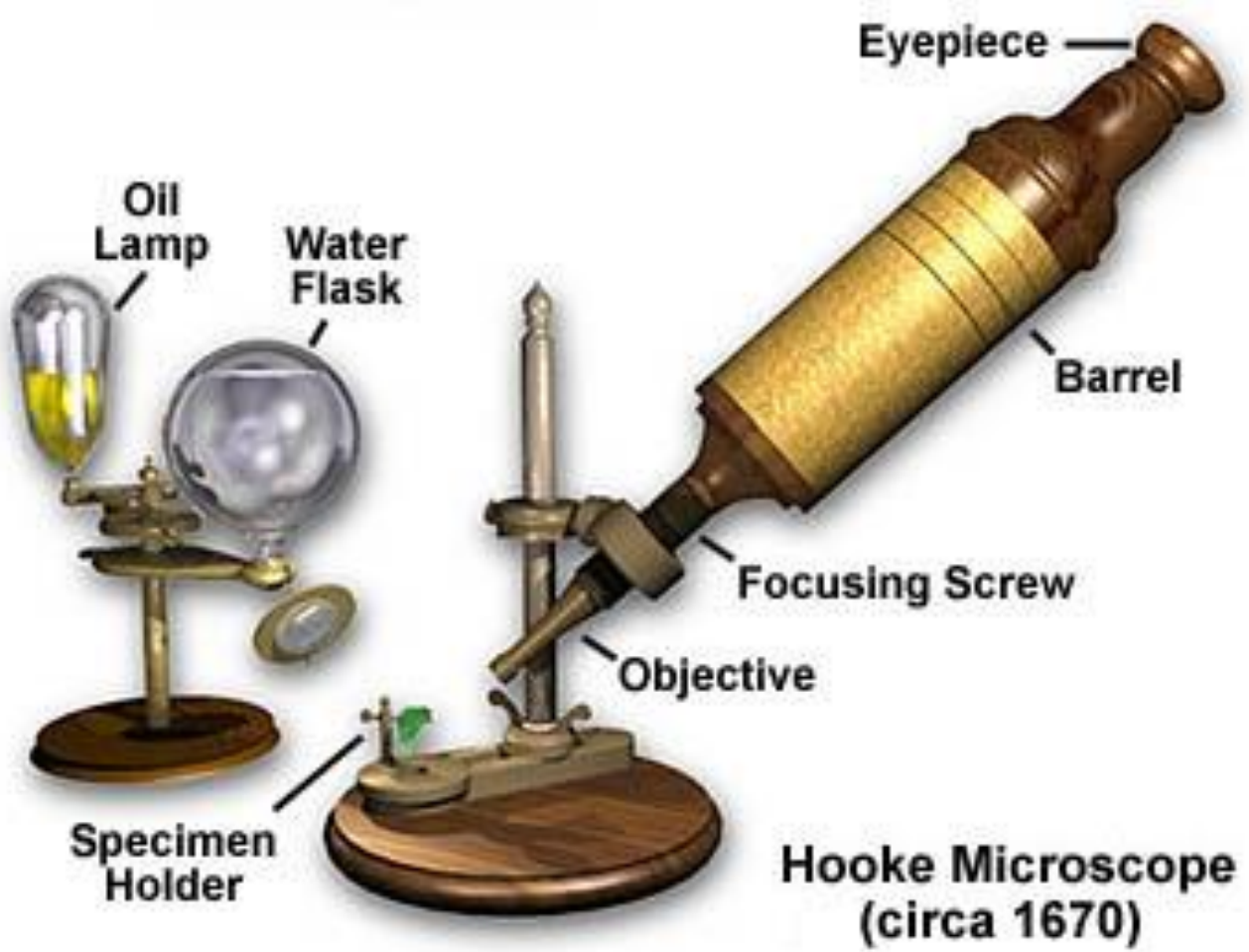
*Visual Microscopy Workshop Series*

# *Kohler Illumination and Conjugate Planes*

*Lai Ding*
*BWH NeuroTechnology Studio*




The two cone structure is the key to increase contrast


Eyepiece
Oil Lamp
Water Flask
Barrel
Focusing Screw
Objective
Specimen Holder
Hooke Microscope
(circa 1670)

## Halogen lamp

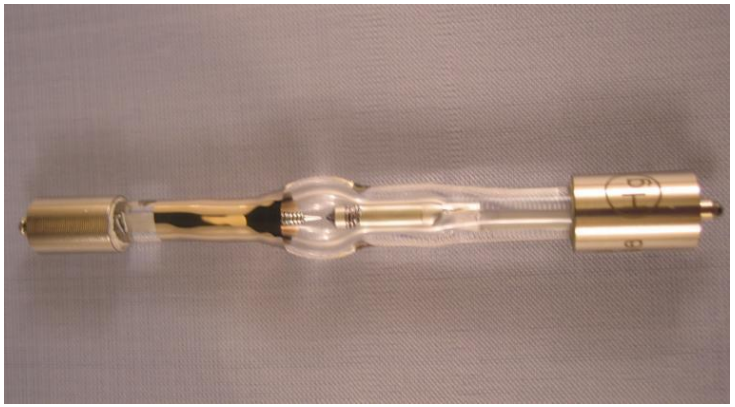

bright

convenient

constant intensity

inexpensive

## Ion arc lamp

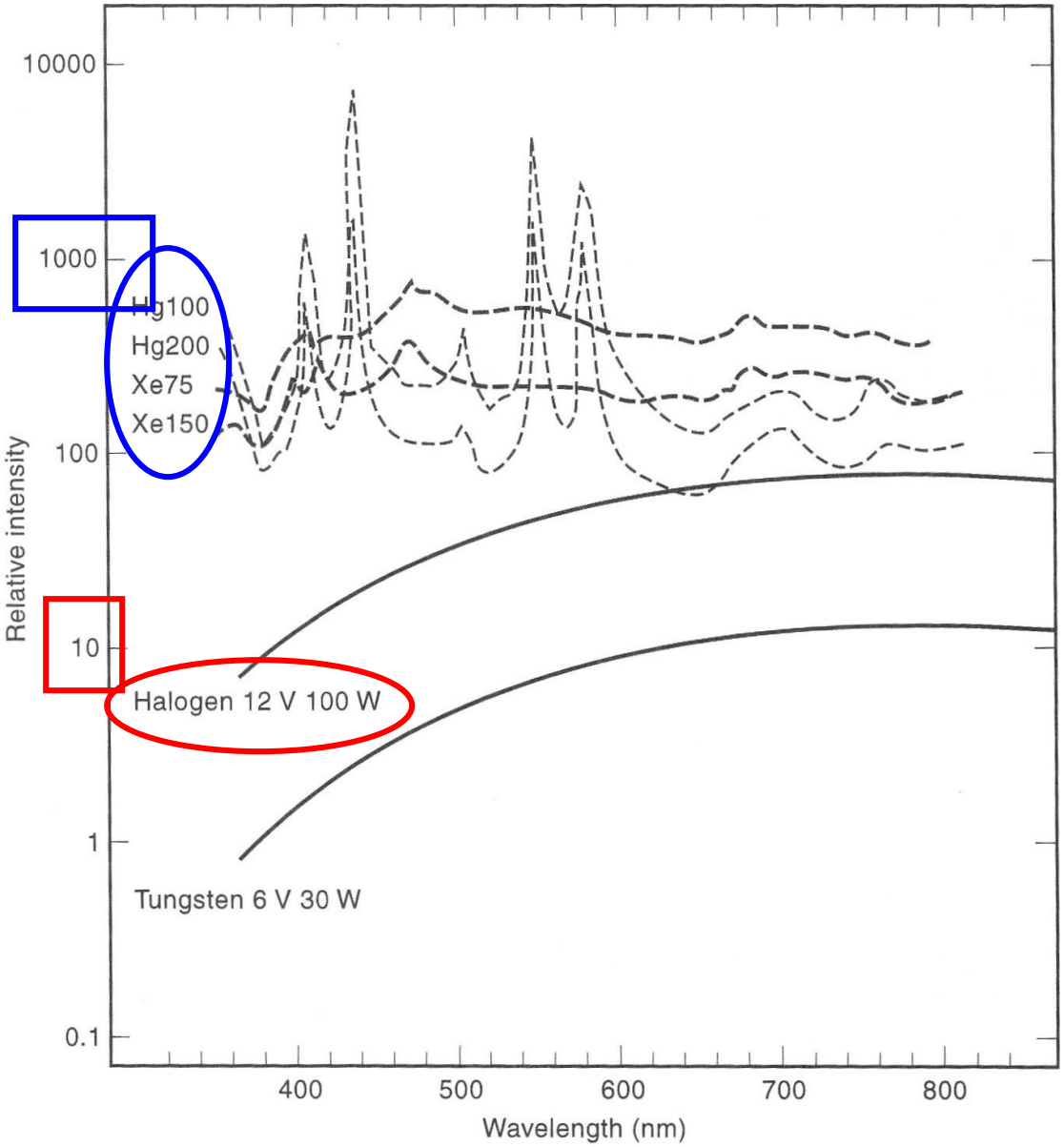


bright

constant intensity

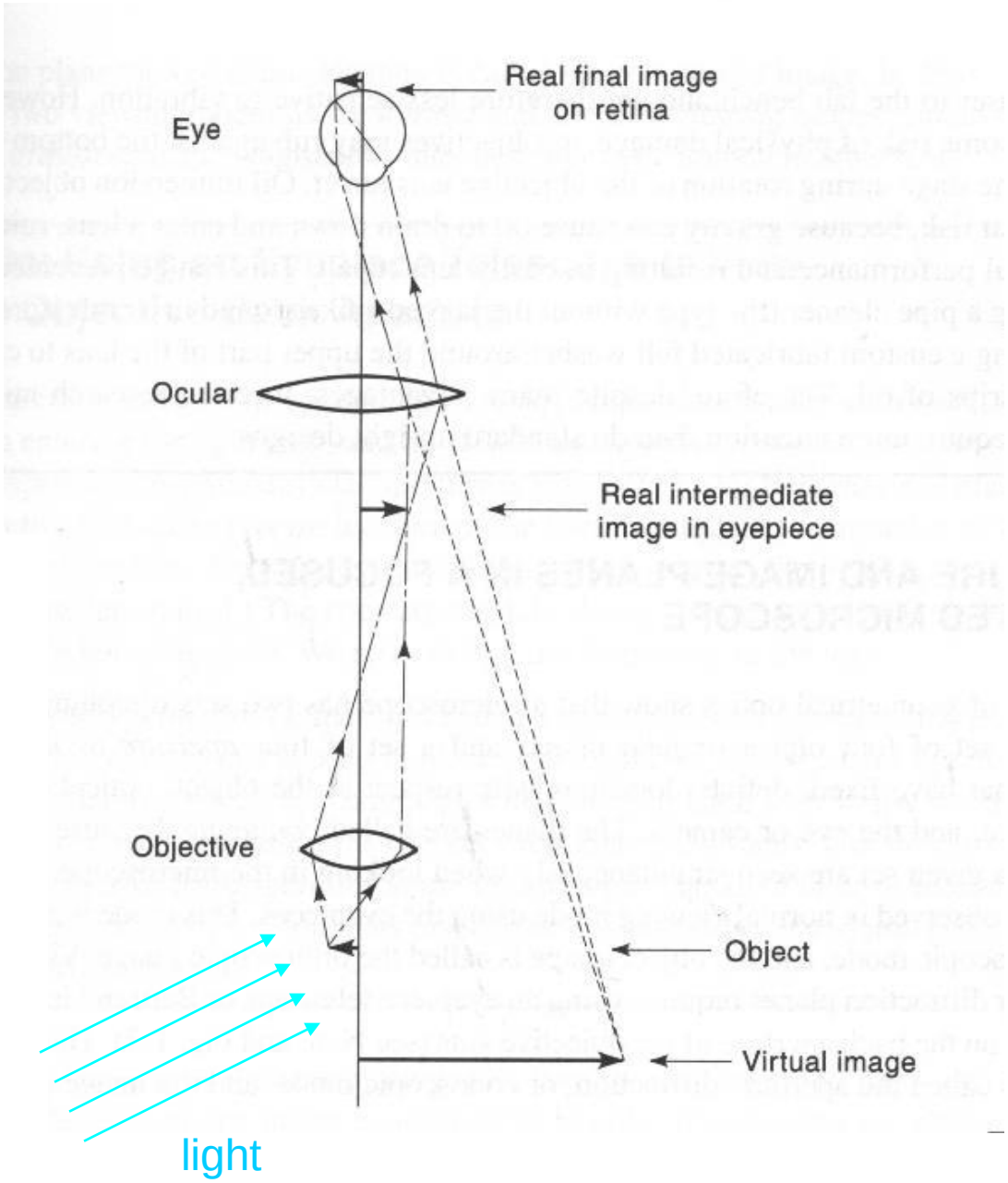
not convenient

expensive (shorter life time ~200 hours)

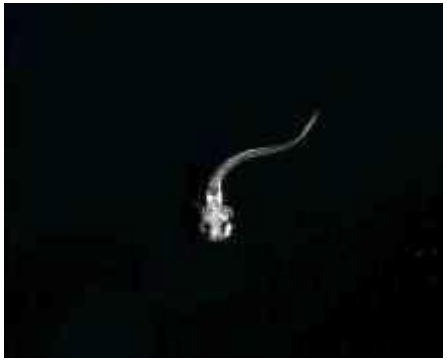


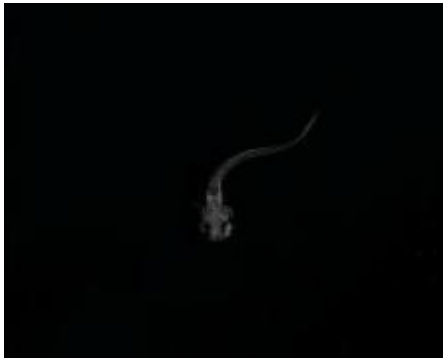

10-100 times brighter than halogen lamp

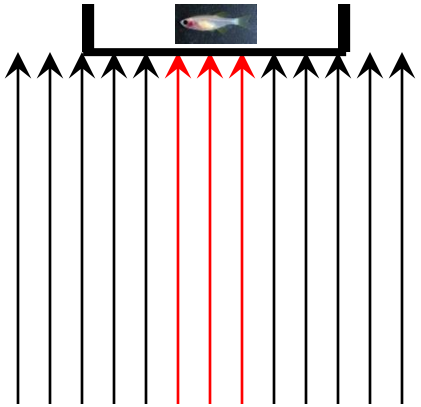
Microscope
Image Formation

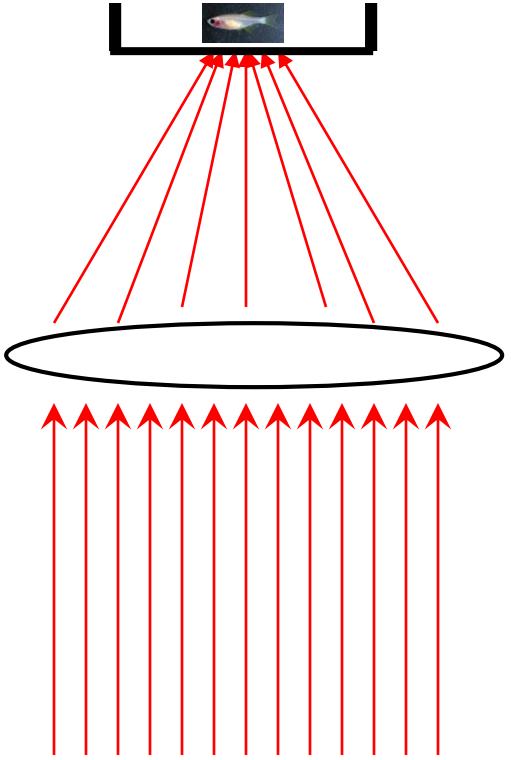


Zebra-fish

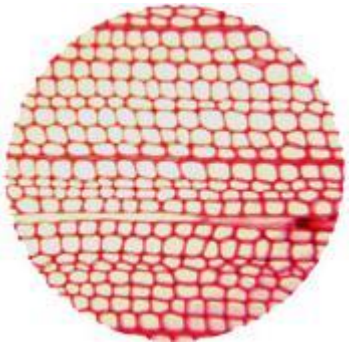


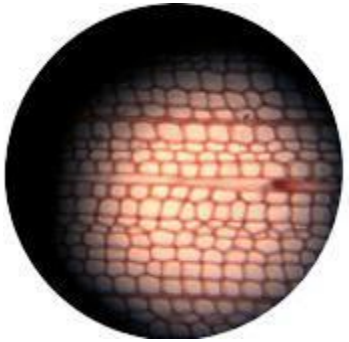






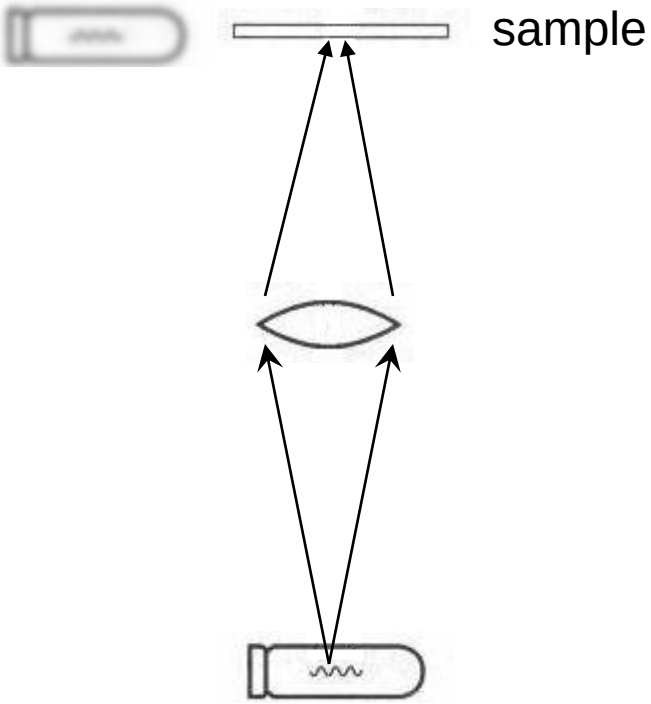
not just focus light onto the sample



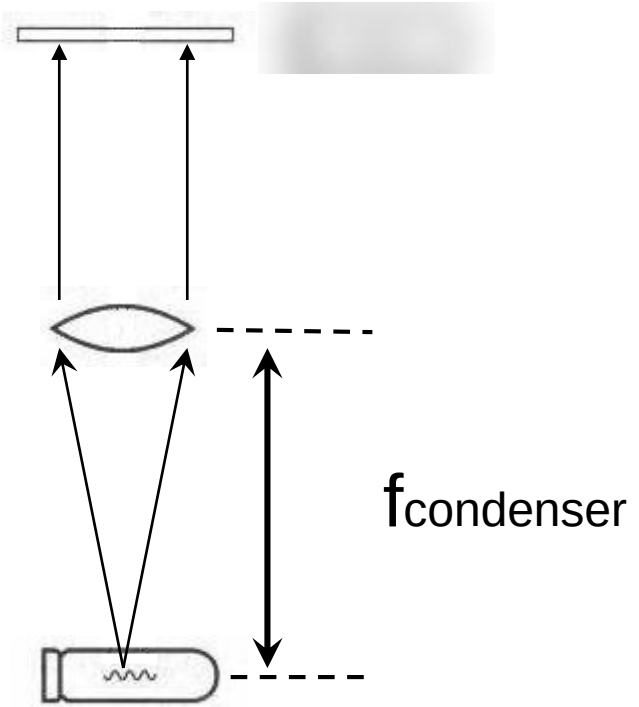


the field should be homogeneously bright

# position of the lamp

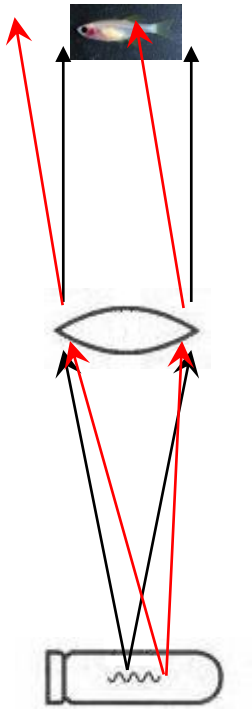


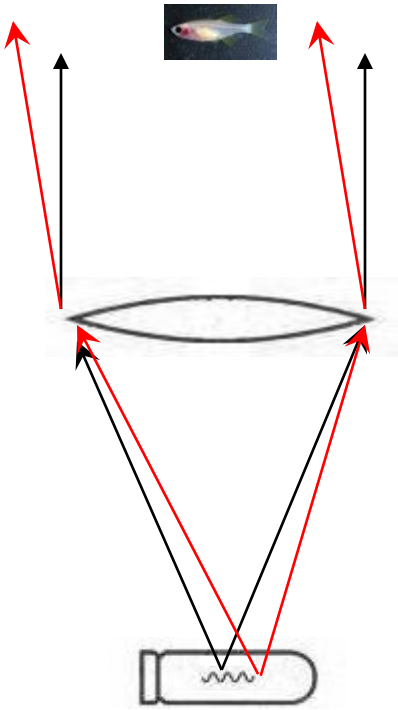

focus lamp on sample

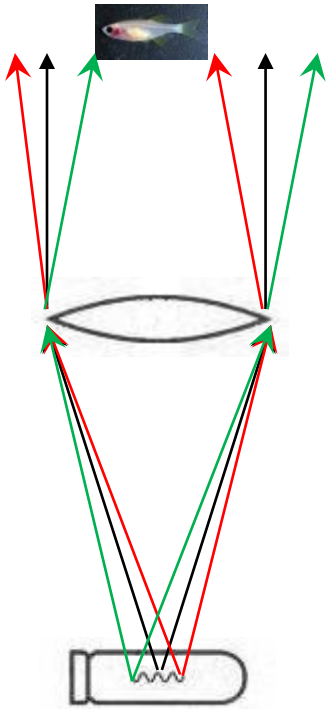


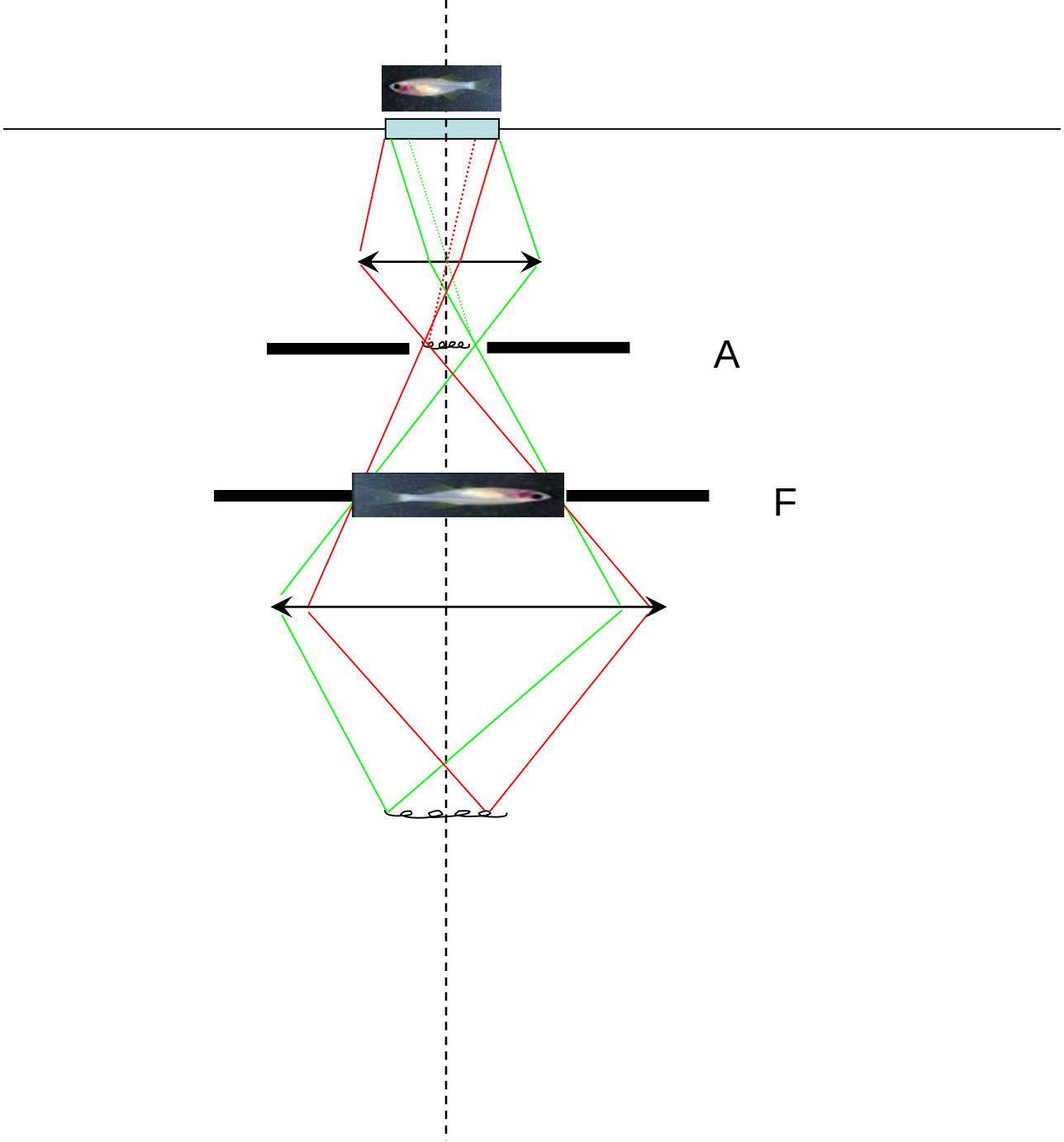

lamp on condenser (front) focal point

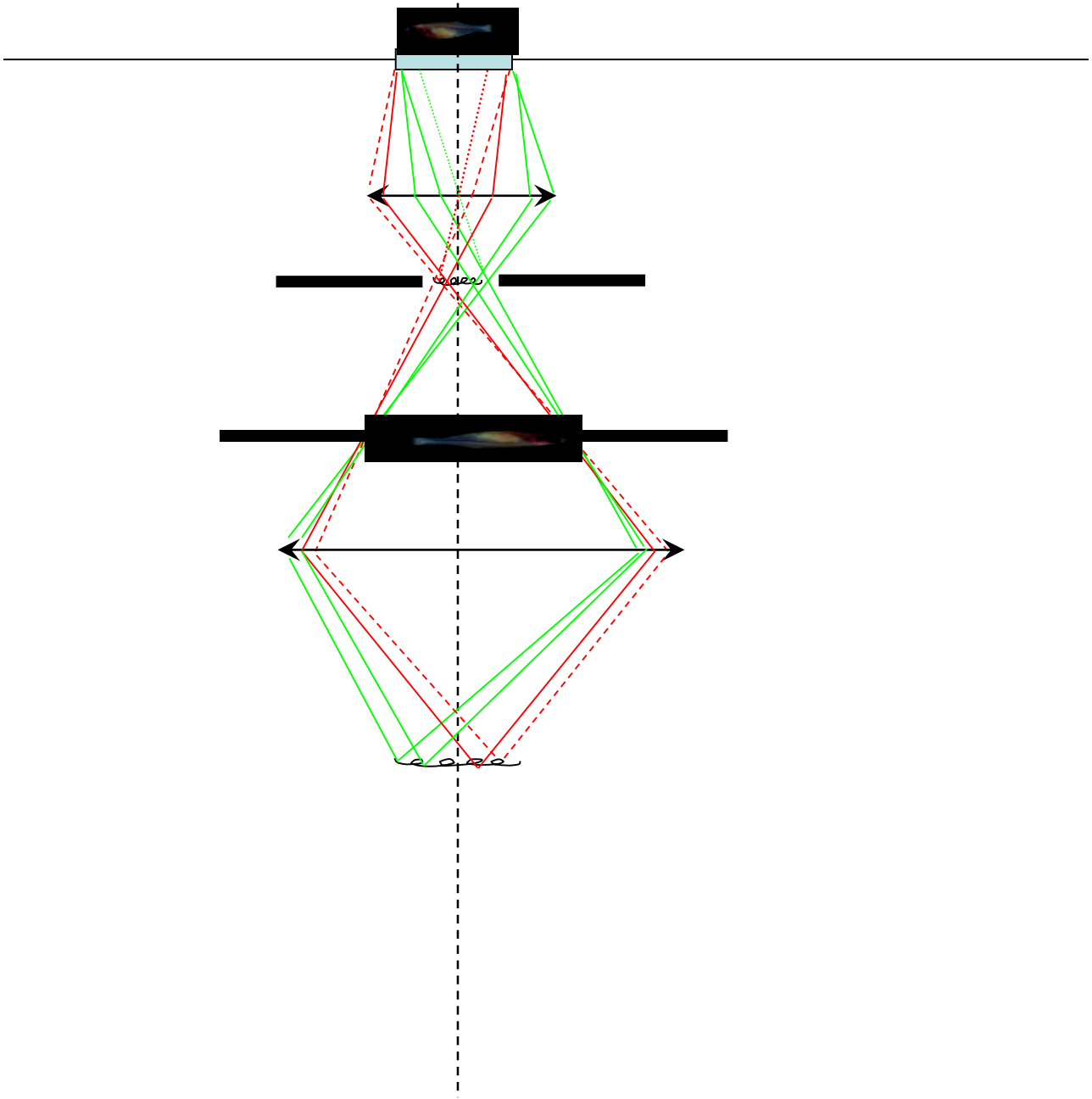
Lamp Filament







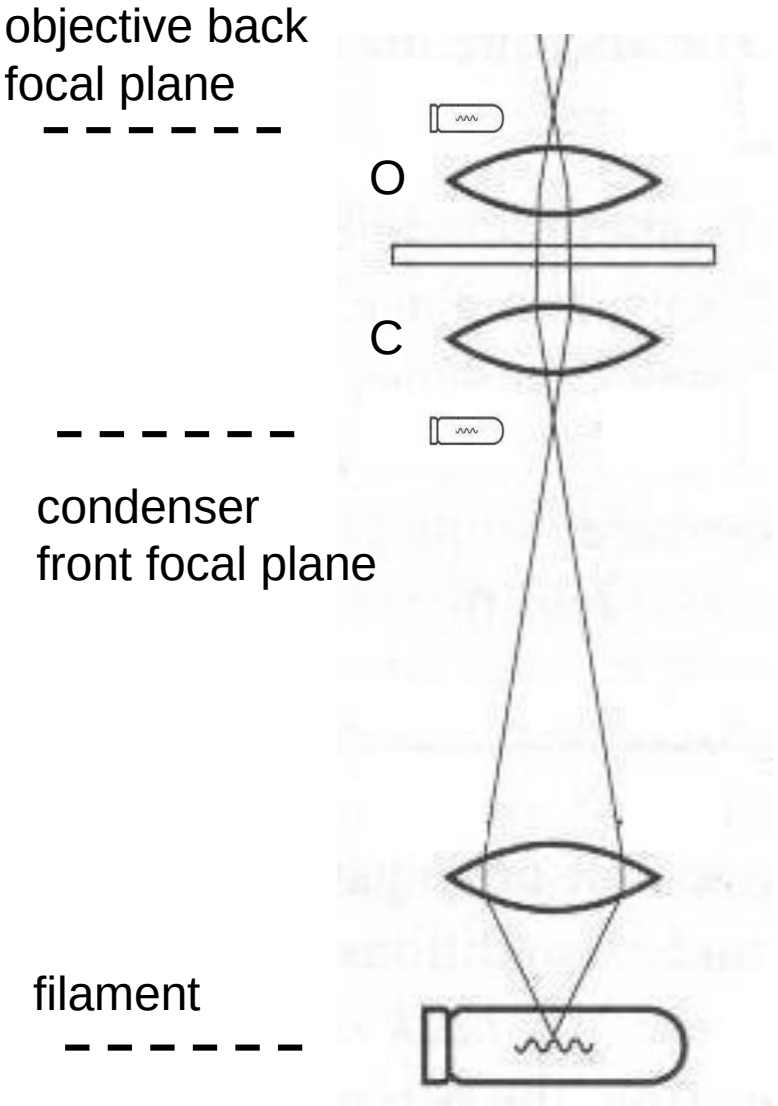

A
F



uniform illumination

*light from each point of the filament uniformly distributed on sample stage*

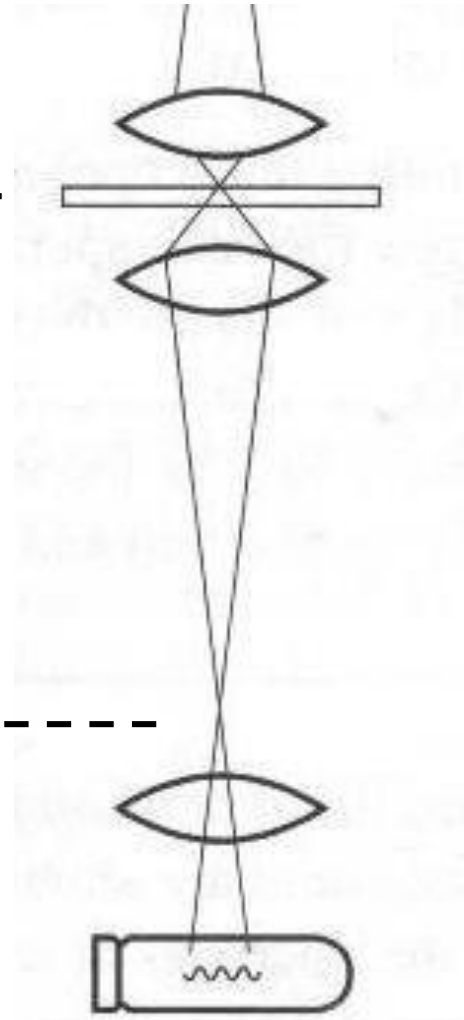
– – – – – – conjugated planes

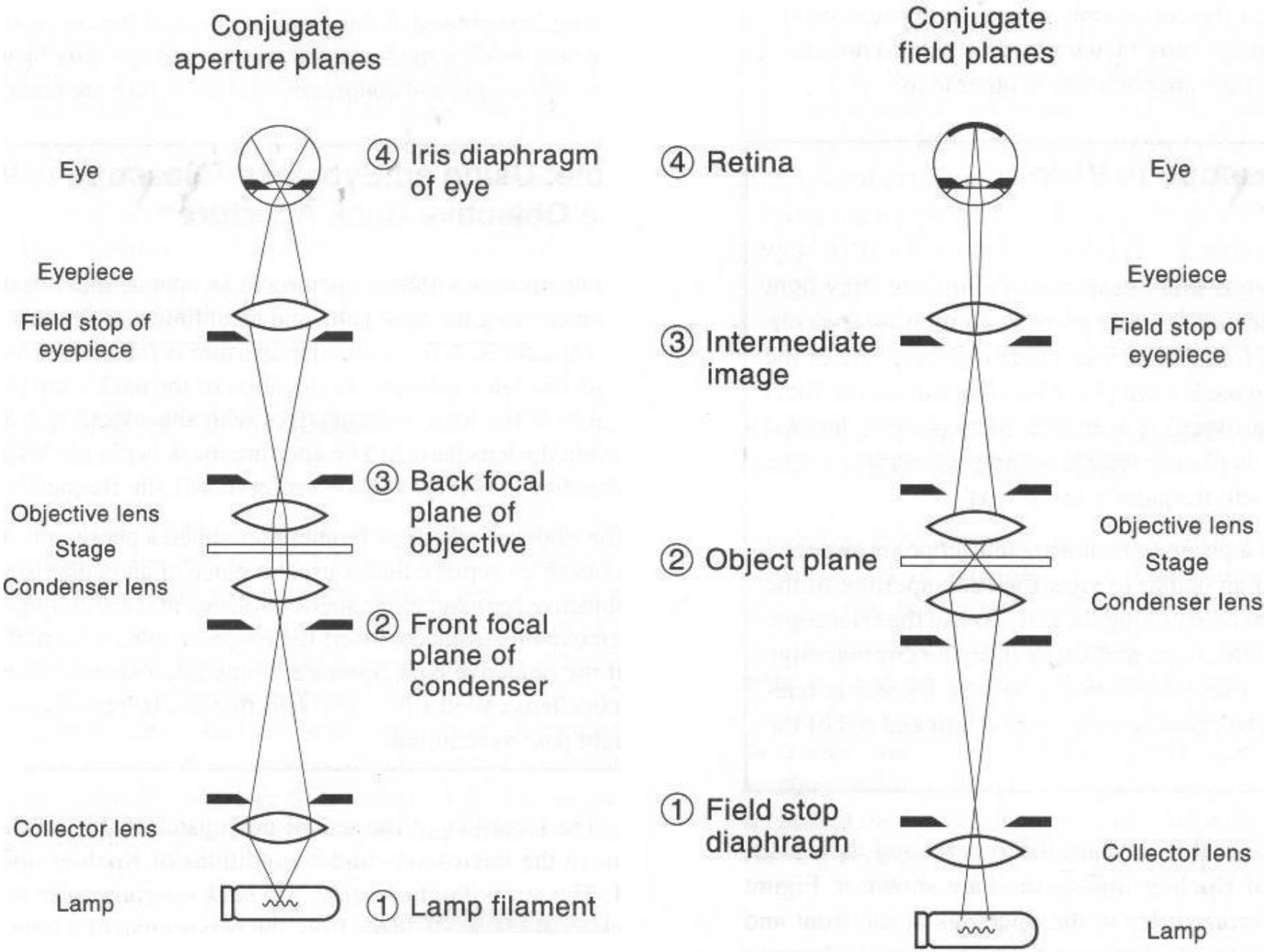


uniform illumination

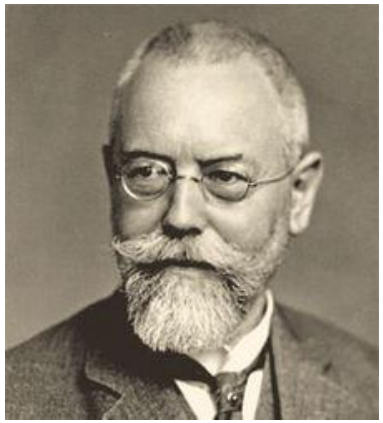
*each point of the sample is illuminated by whole (or same) filament region*

– – – – – – conjugated planes

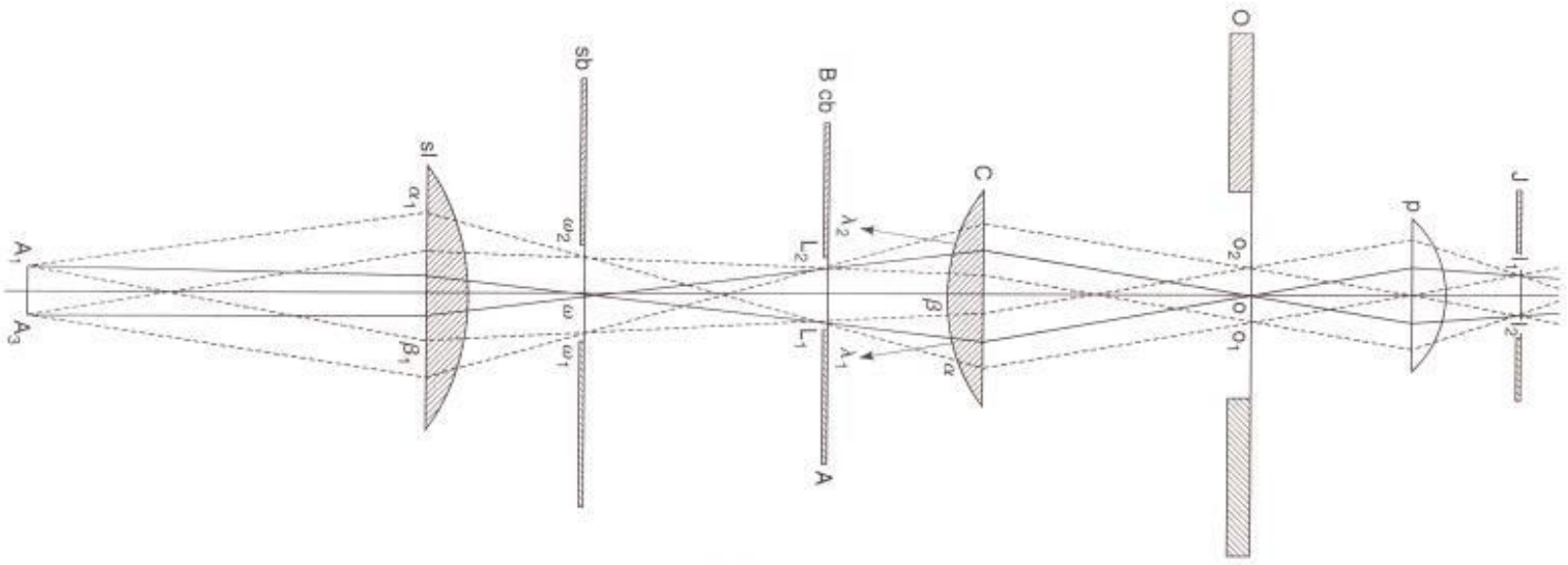


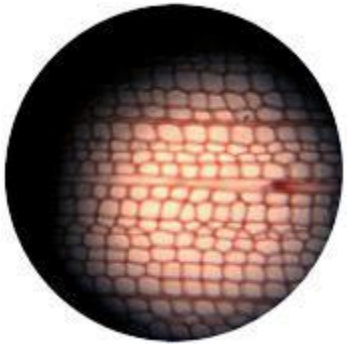

Conjugate aperture planes
Eye
④ Iris diaphragm of eye
Eyepiece
Field stop of eyepiece
③ Back focal plane of objective
Objective lens
Stage
Condenser lens
② Front focal plane of condenser
Collector lens
Lamp
① Lamp filament
Conjugate field planes
④ Retina
Eye
Eyepiece
③ Intermediate image
Field stop of eyepiece
Objective lens
② Object plane
Stage
Condenser lens
① Field stop diaphragm
Collector lens
Lamp

Kohler illumination.

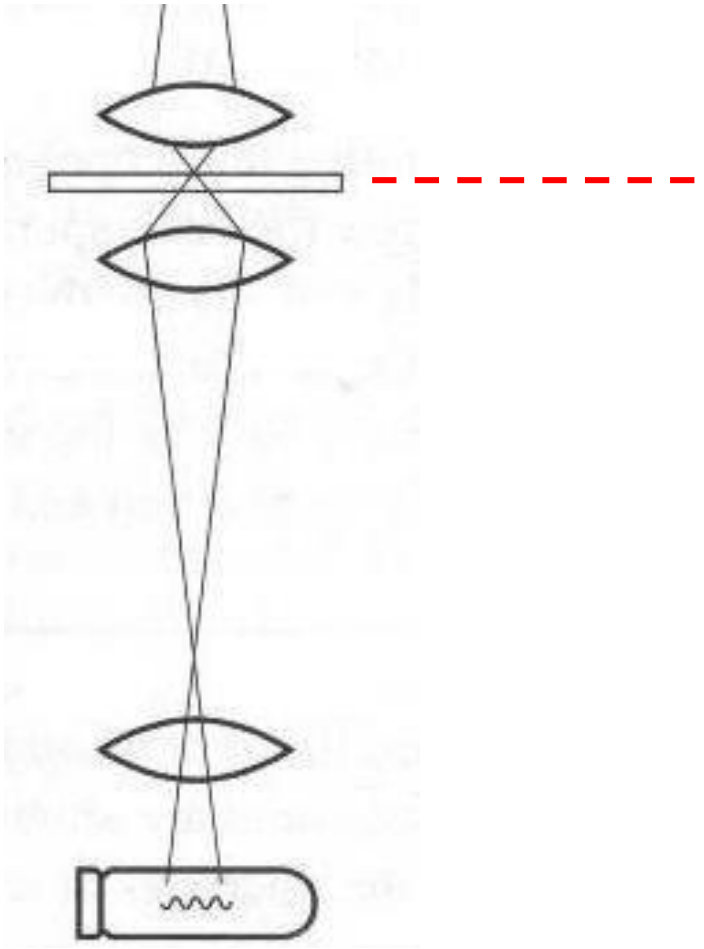


August Köhler
1866-1948



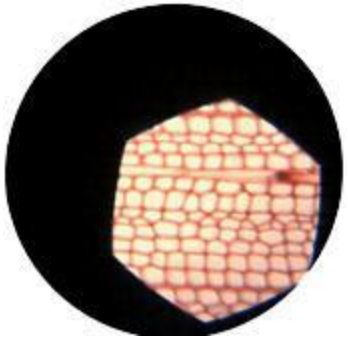


step1: focus sample by 10x objective

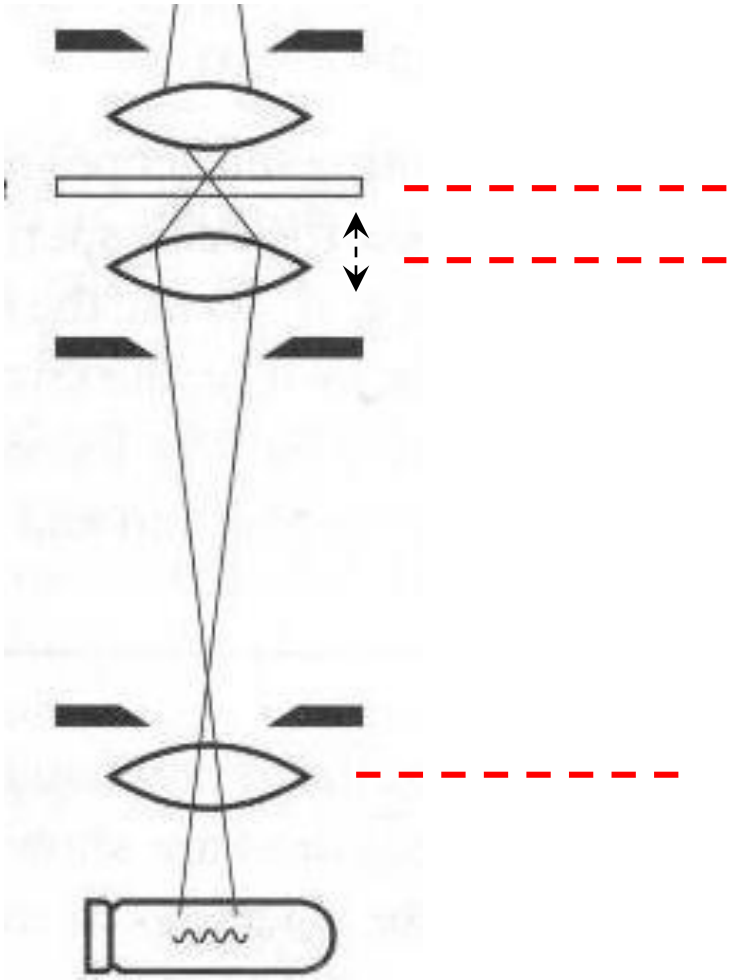


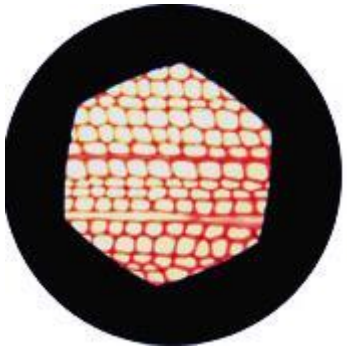
fixed plane

fix stage position

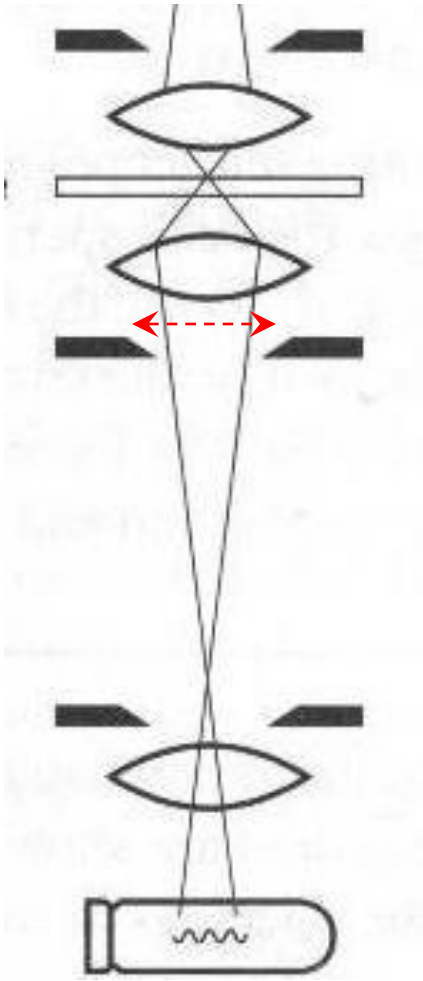


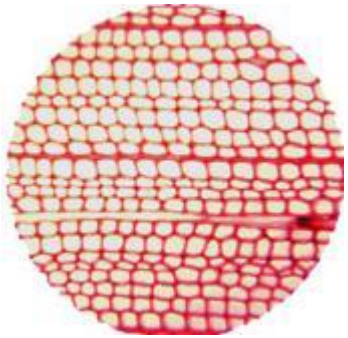
Step2 : focus condenser



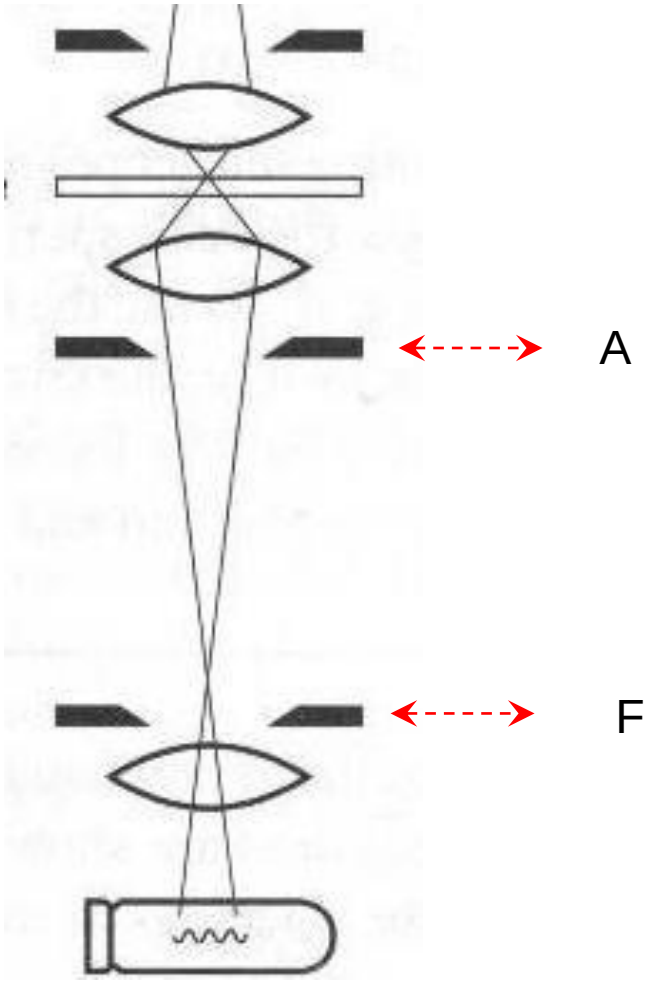


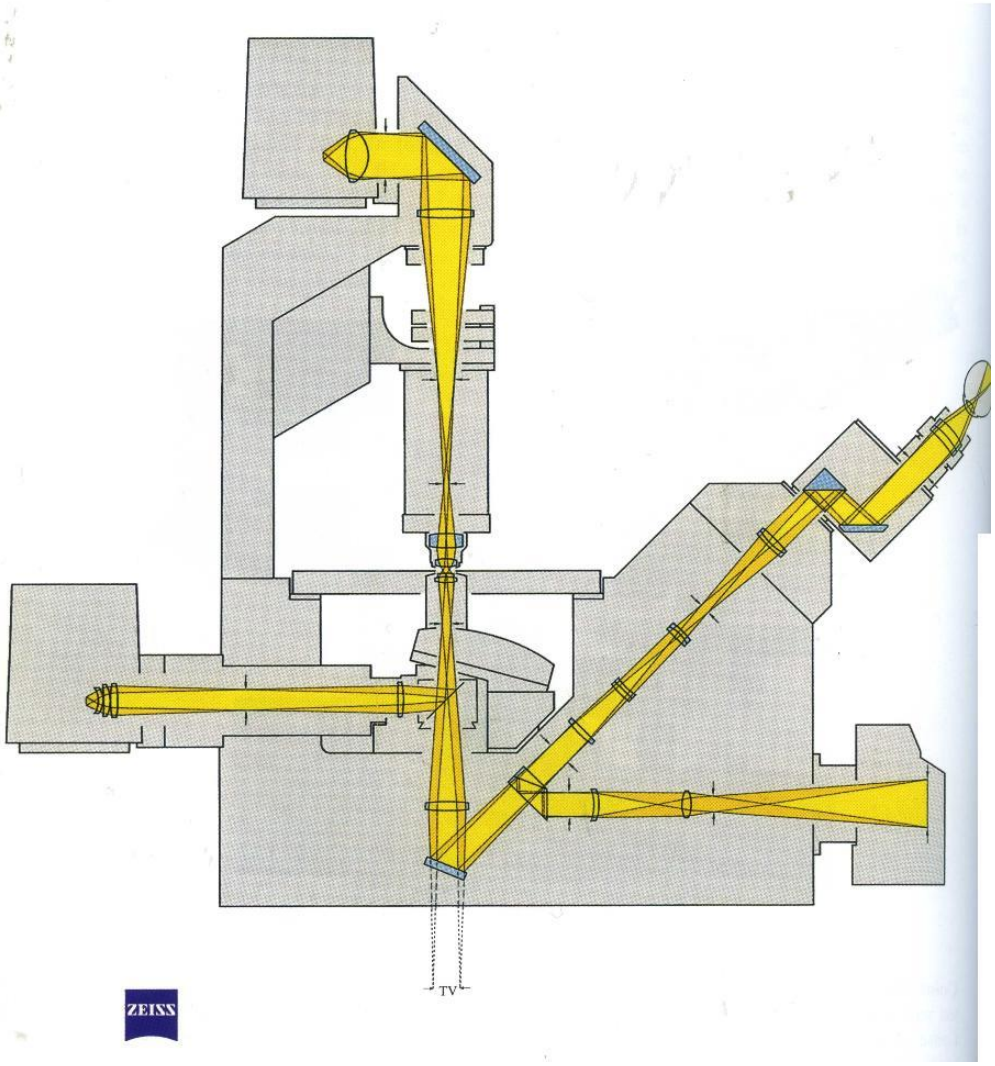
Step3: centering condenser diaphragm





Step5: open field diaphragm
adjust aperture diaphragm

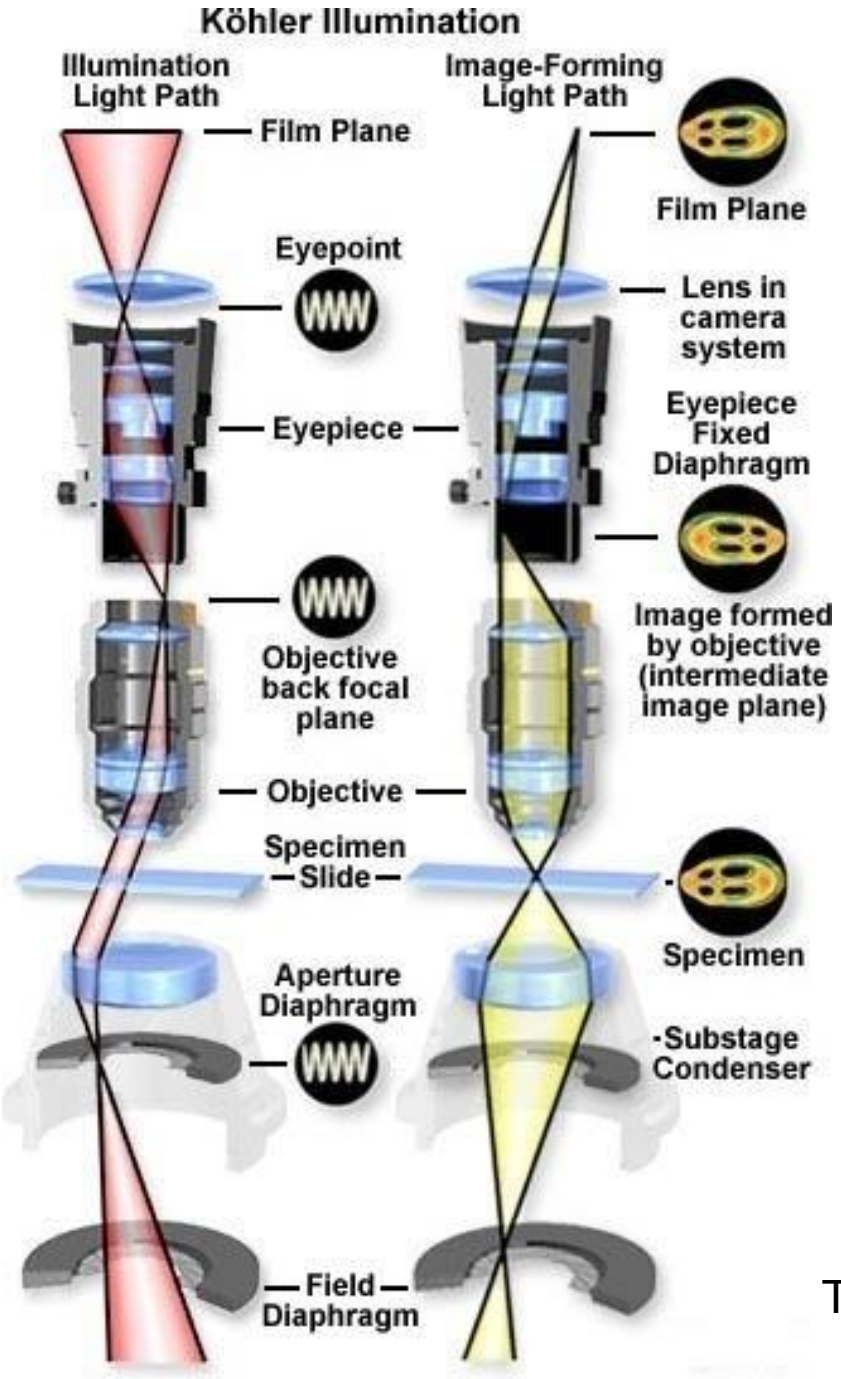





Conjugated Planes

“Fundamentals of Light Microscopy and Electronic Imaging”

Douglas B. Murphy




Function of Diaphragm

Field Diaphragm

*limit field of view*

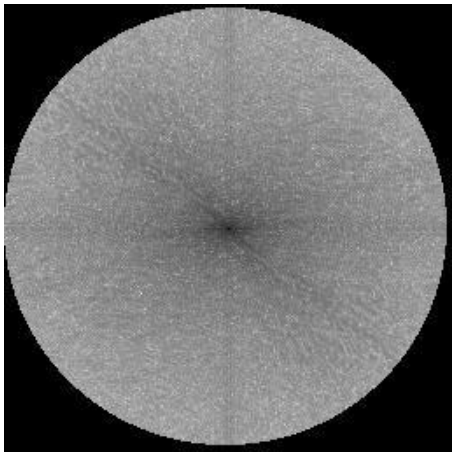
Aperture Diaphragm

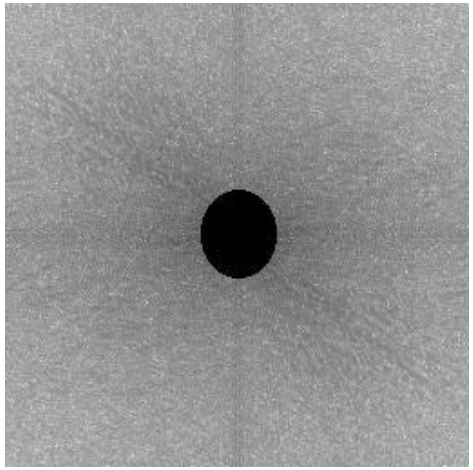
*change light intensity*

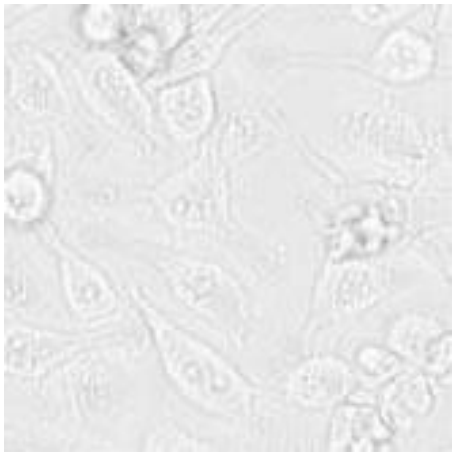
*resolution*

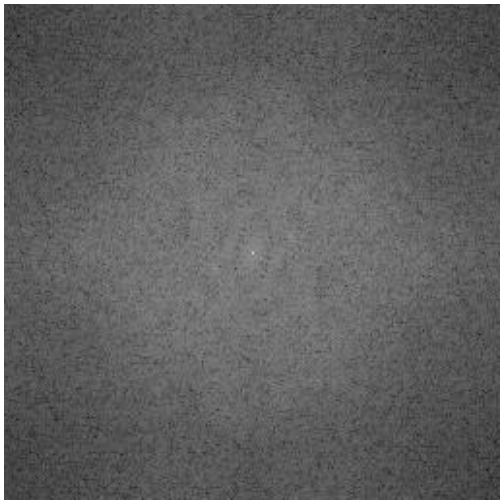
Take off the eyepiece, what will you see?

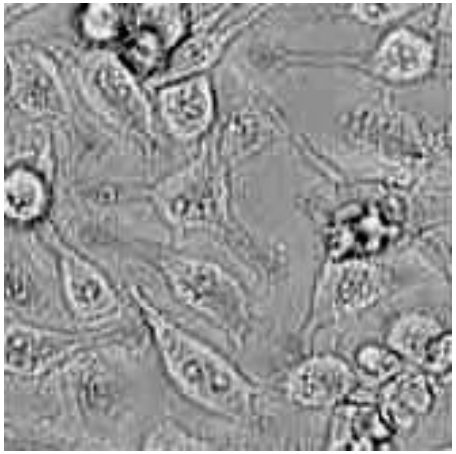
Remember?

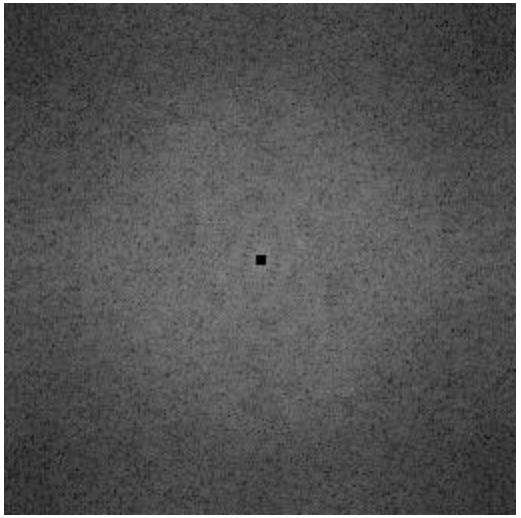


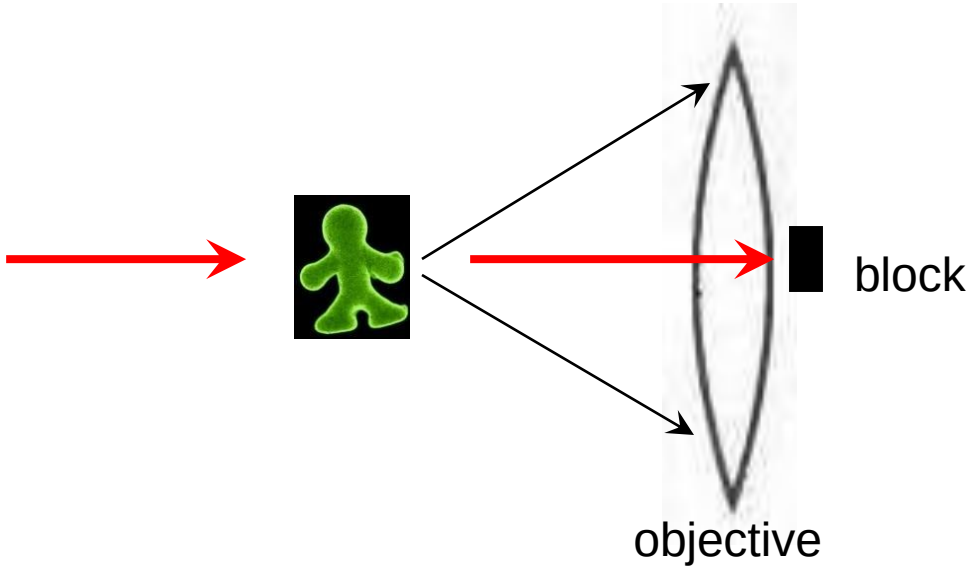


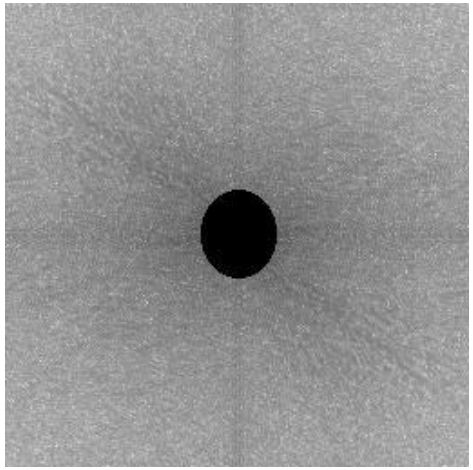








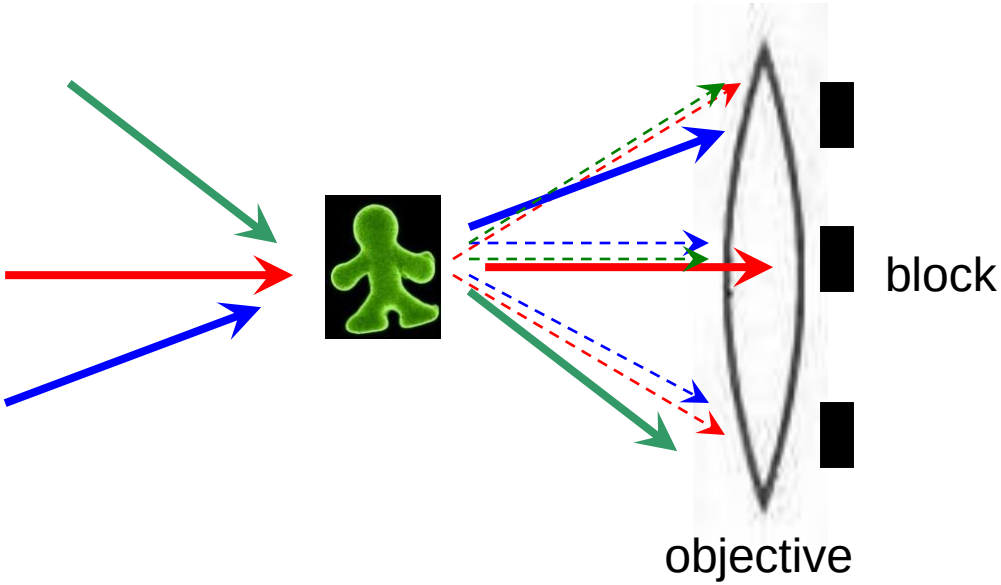


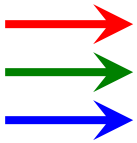



"0th " order light

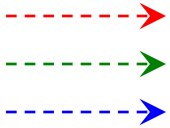
diffracted light

*Will this work?*

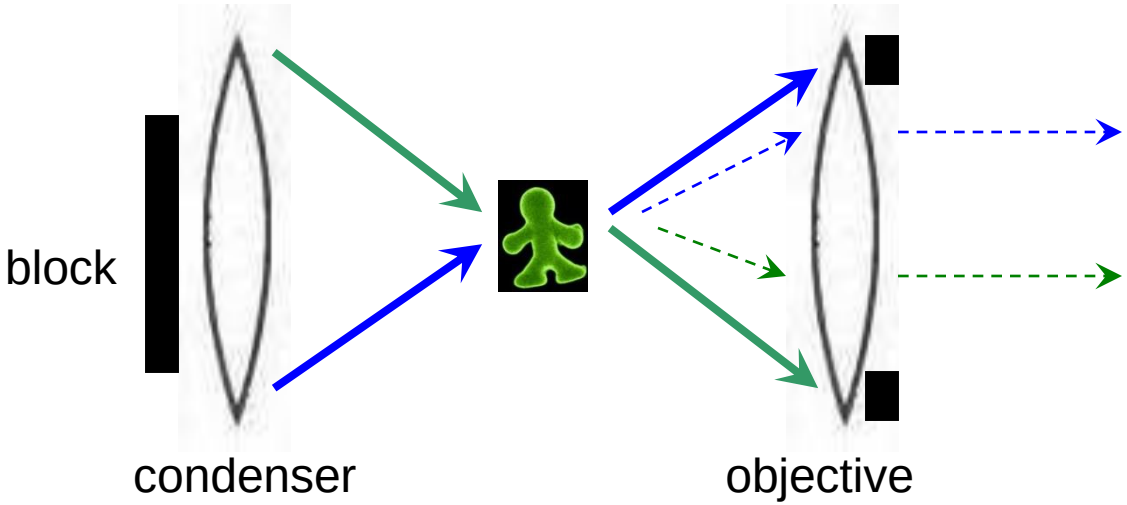


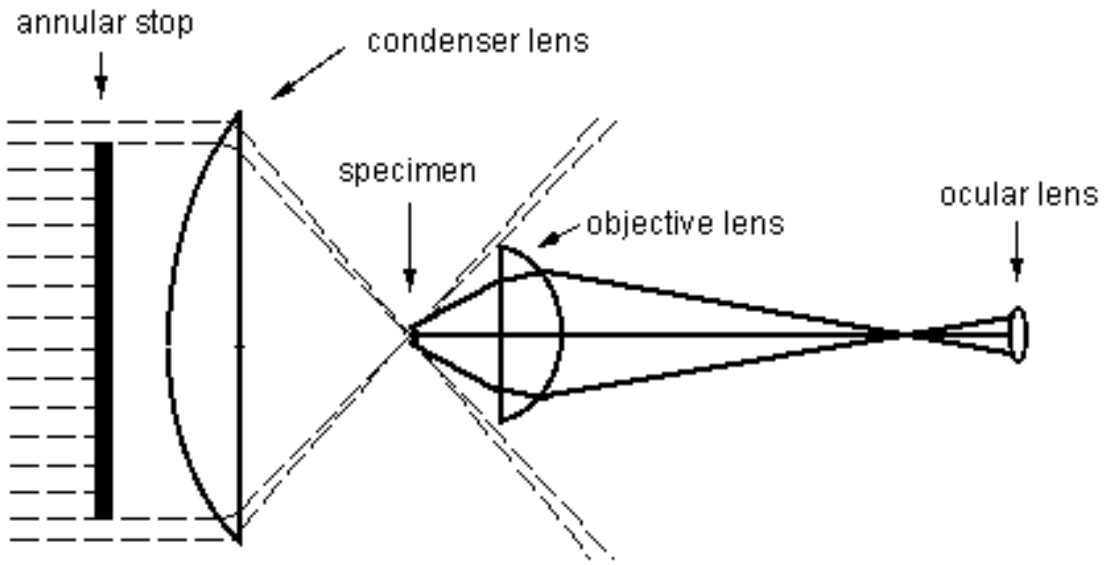



0th order light



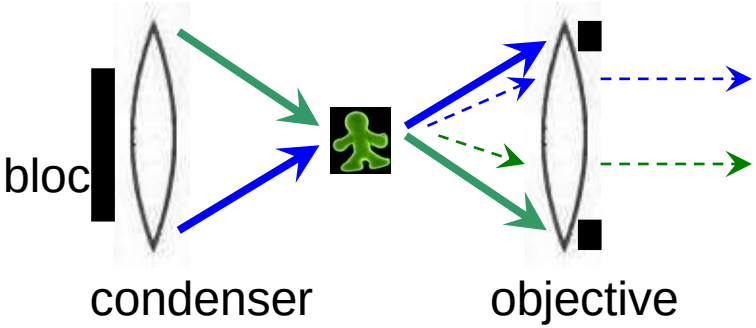
Diffracted light

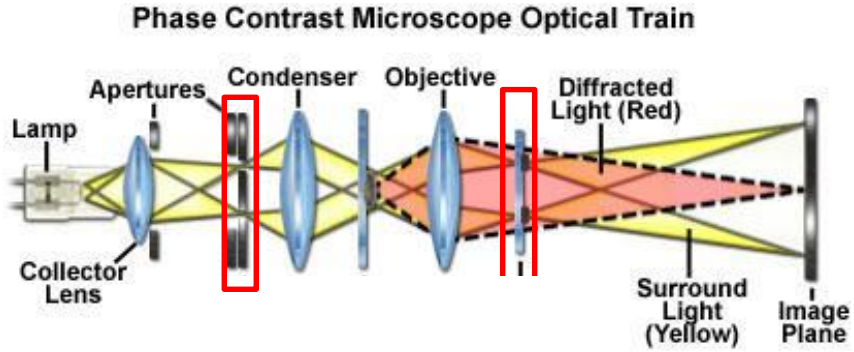

block
condenser
objective

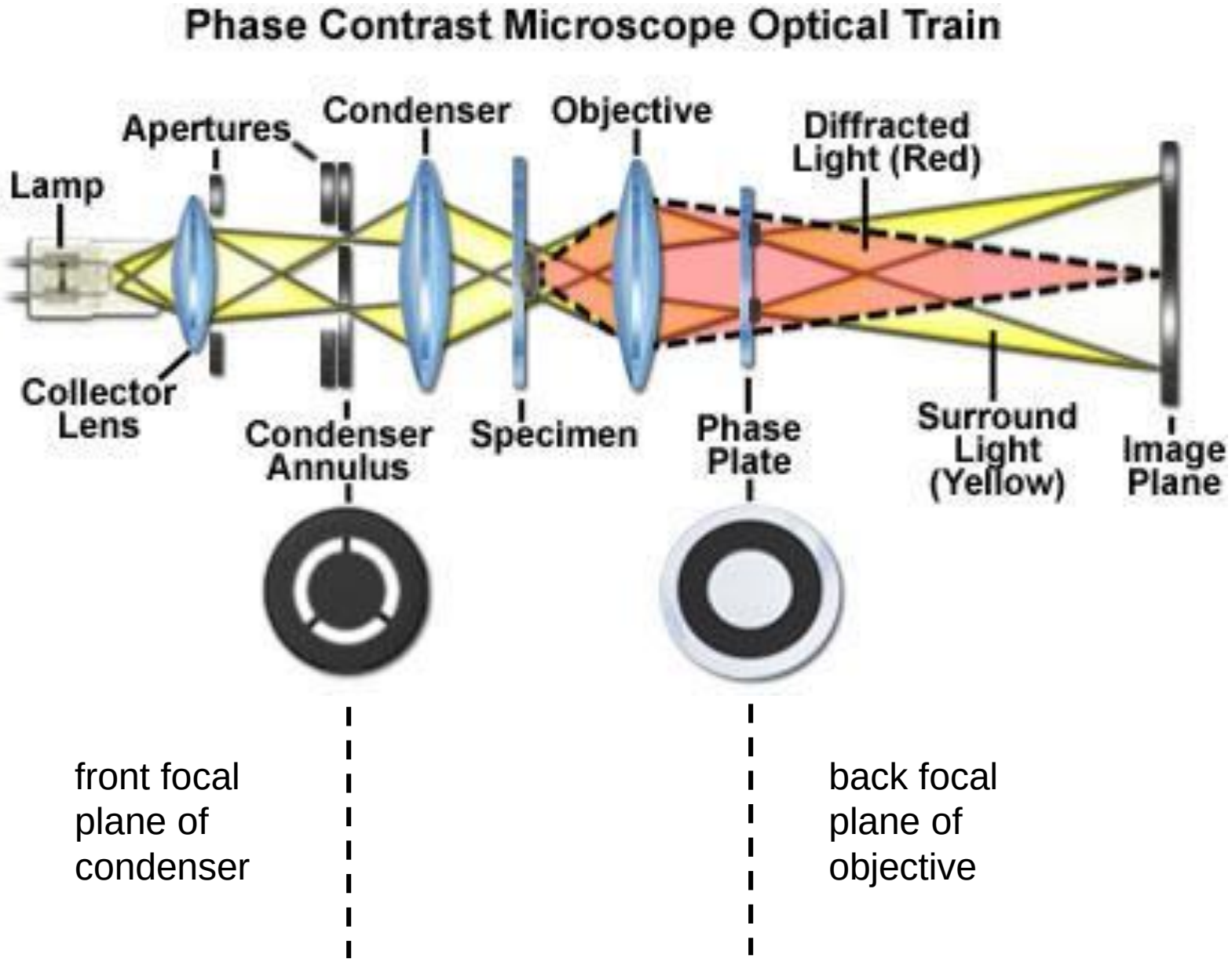

annular stop
condenser lens
specimen
objective lens
ocular lens

# Phase Contrast

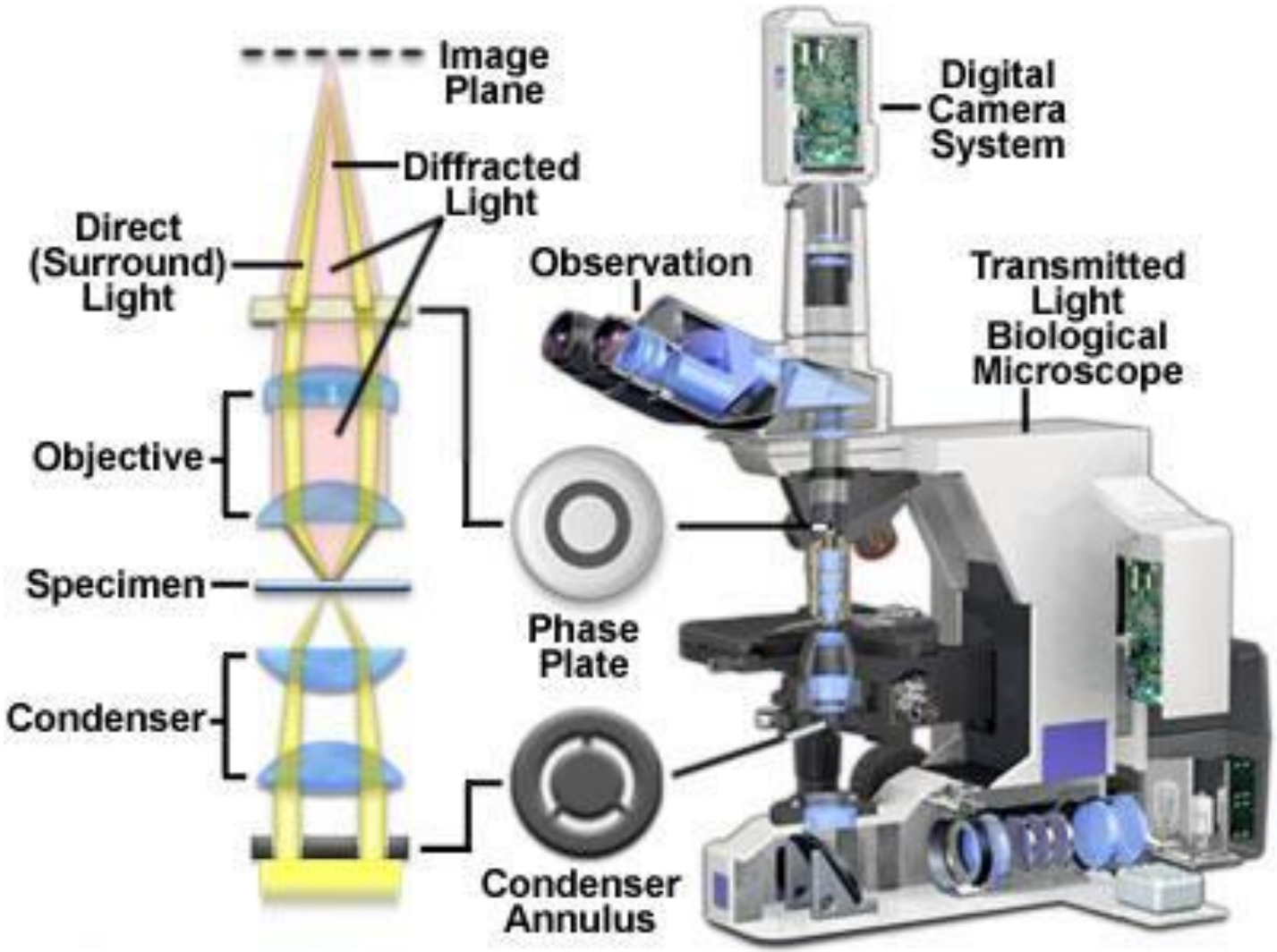
Only block the 0$^{th}$ order with ~70%



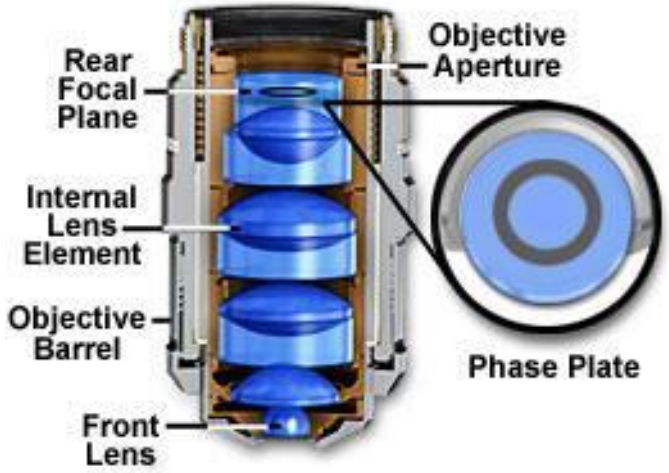

Phase Contrast Microscope Optical Train
Lamp
Apertures
Condenser
Objective
Diffracted Light (Red)
Collector Lens
Condenser Annulus
Specimen
Phase Plate
Surround Light (Yellow)
Image Plane
front focal plane of condenser
back focal plane of objective

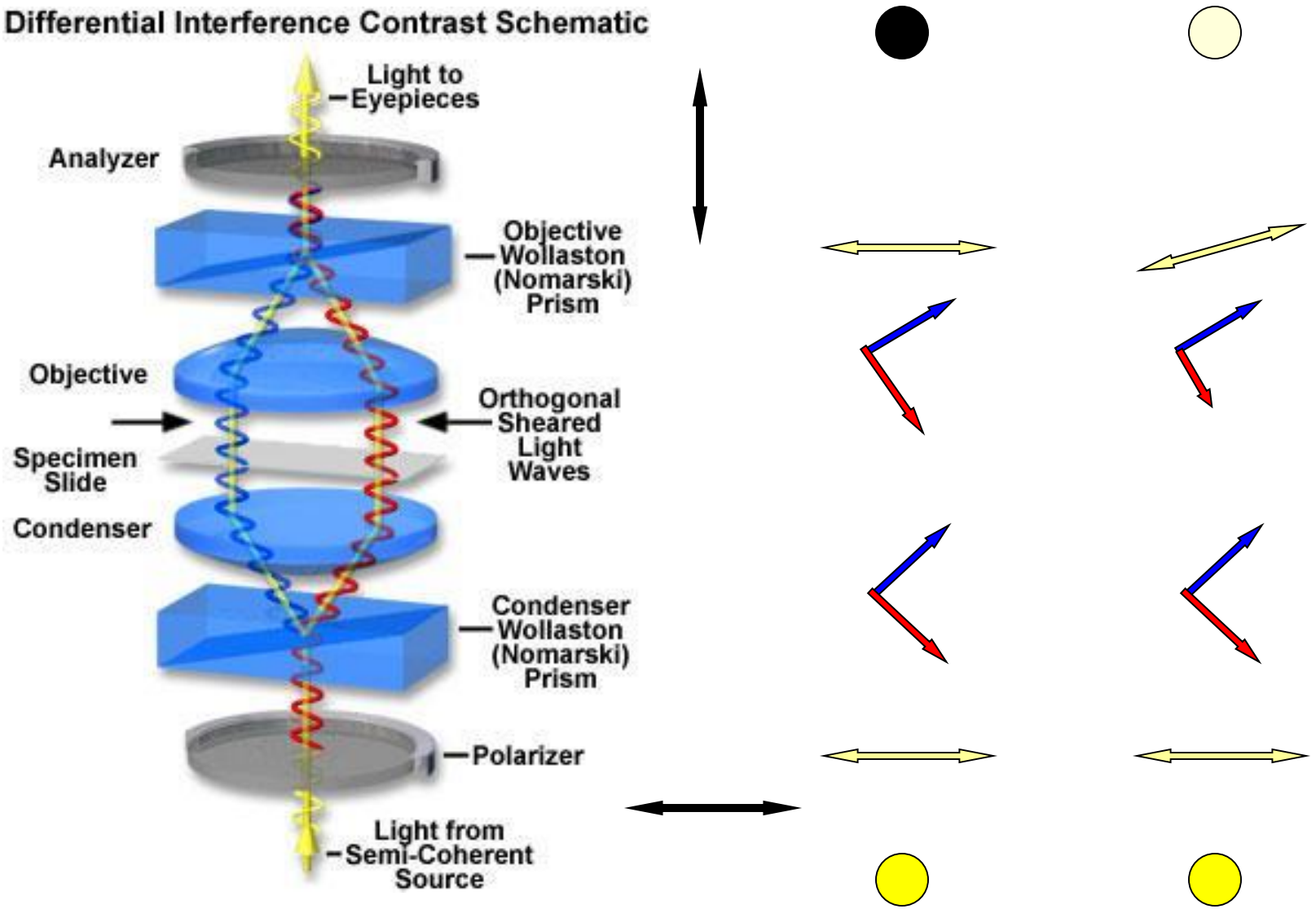
# Phase Contrast Microscope Configuration

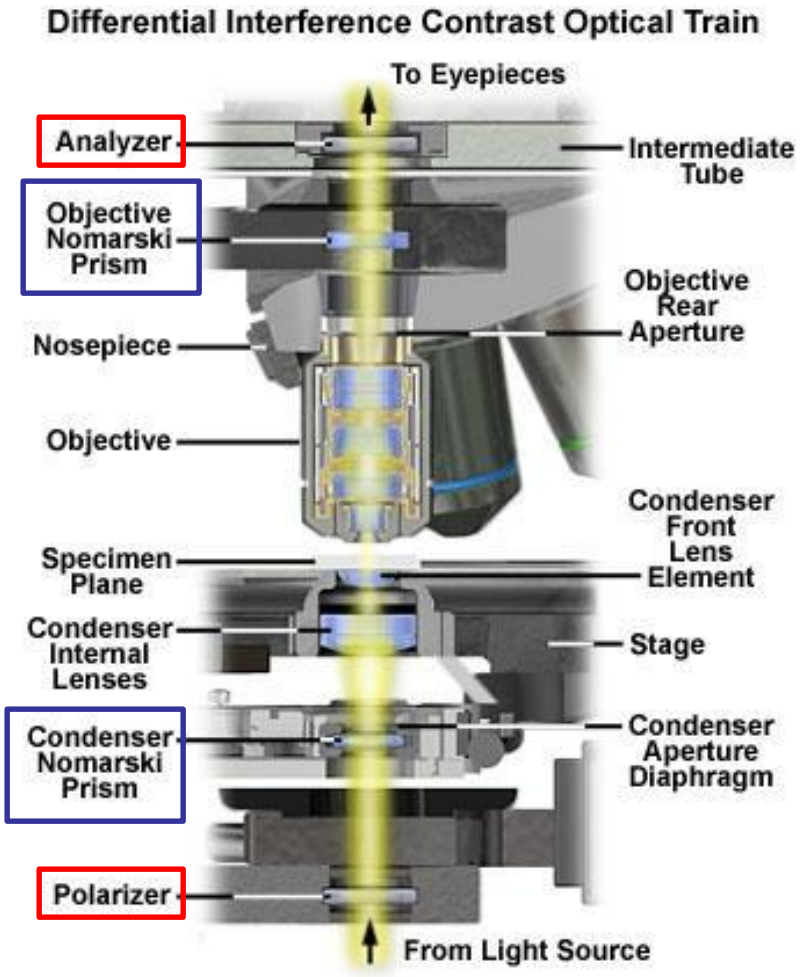


## Phase Contrast Optical Components


## Phase Contrast Objective



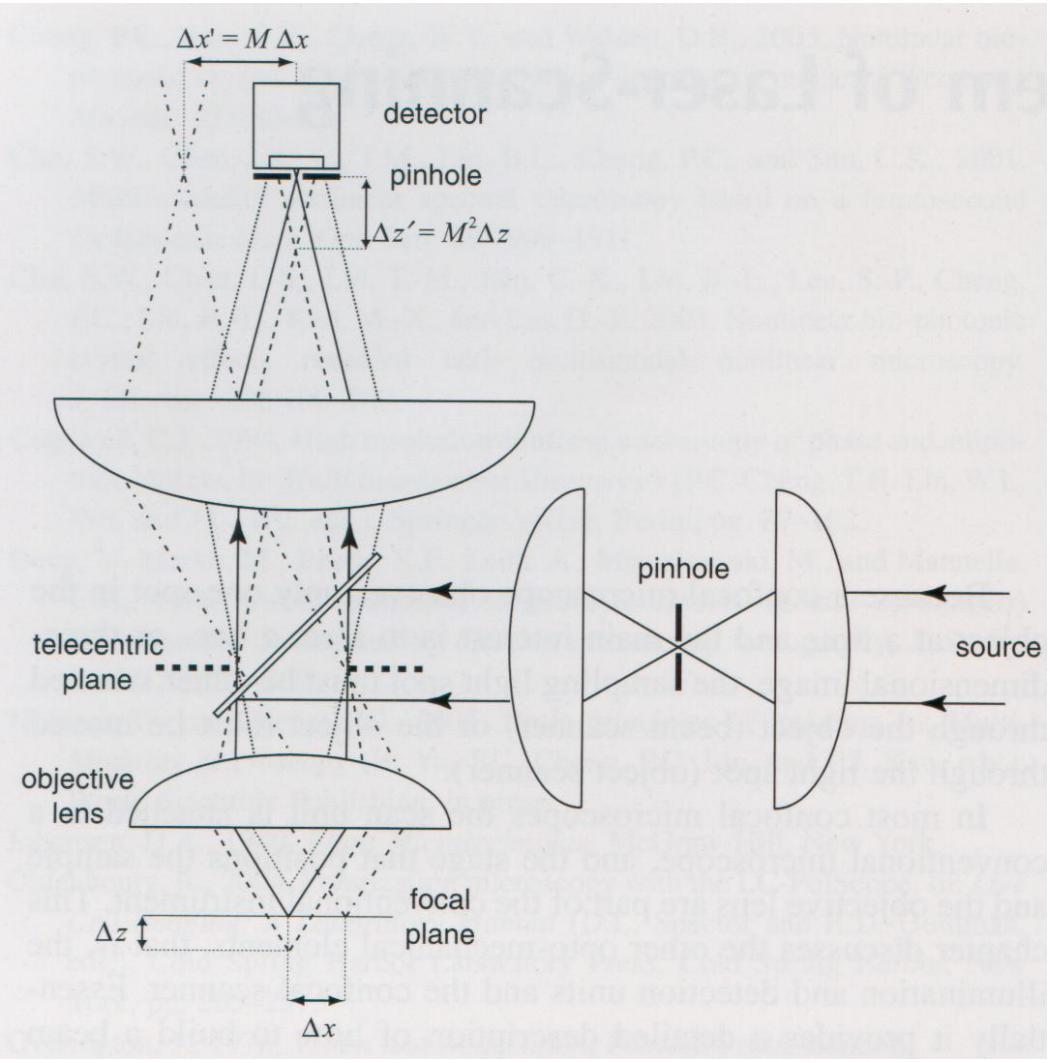

Differential Interference Contrast Schematic
Light to Eyepieces
Analyzer
Objective Wollaston (Nomarski) Prism
Objective
Orthogonal Sheared Light Waves
Specimen Slide
Condenser
Condenser Wollaston (Nomarski) Prism
Polarizer
Light from Semi-Coherent Source



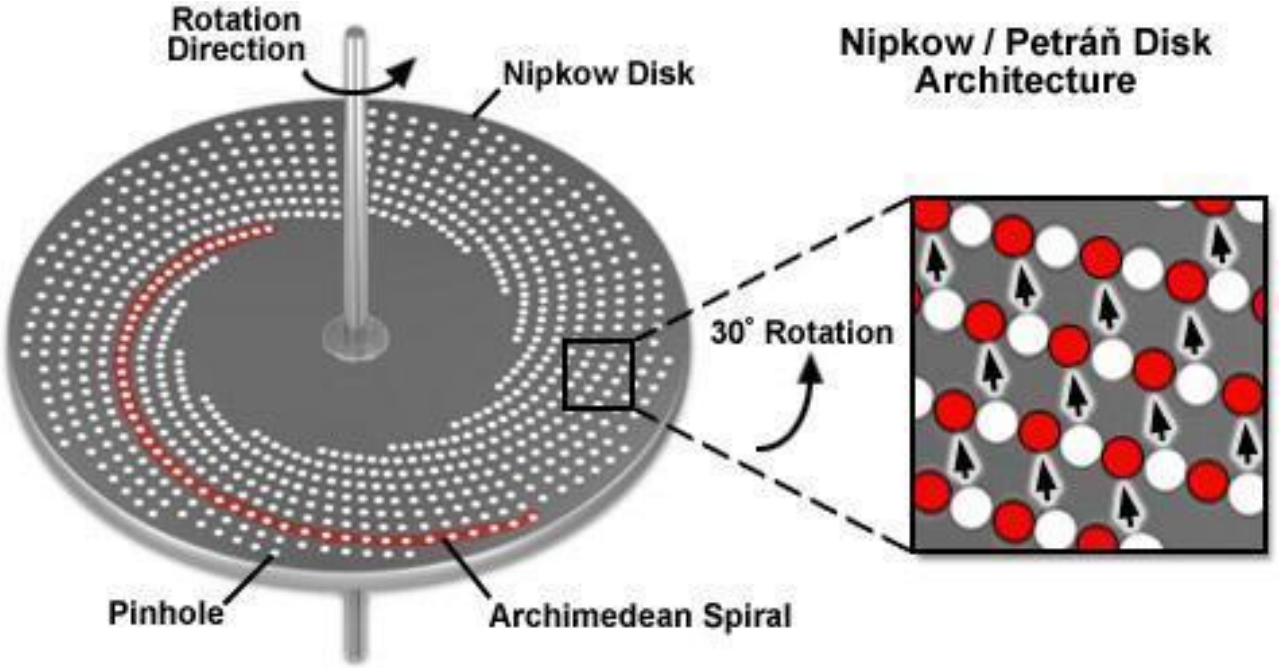


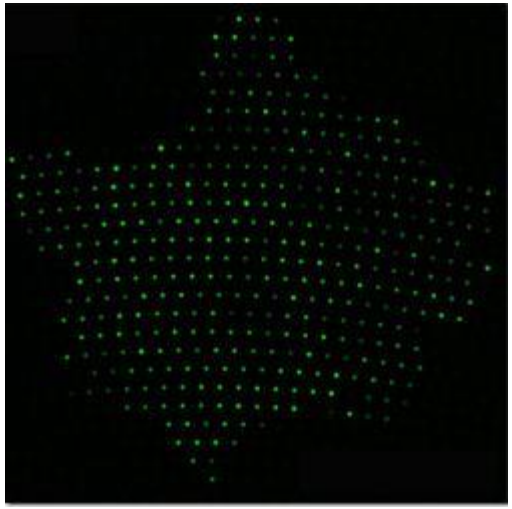
polarizer

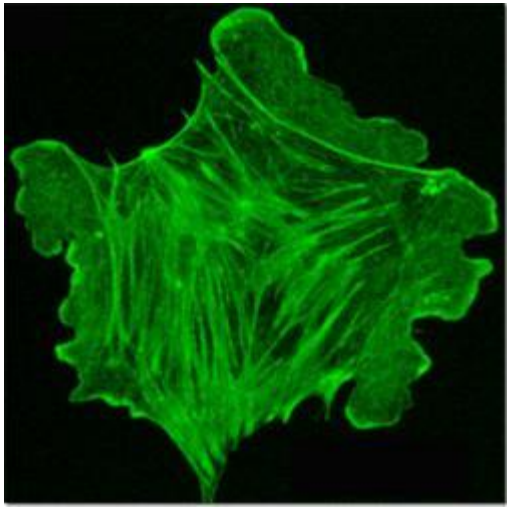
Birefringence crystal




Confocal Pinhole Plane


Rotation Direction
Nipkow Disk
Nipkow / Petráň Disk Architecture
30˚ Rotation
Pinhole
Archimedean Spiral





Insert many pinholes here