Bi177 – Lecture 8 Contrast vs Resolution vs Detection

Review of Kohler Illumination

Tradeoffs in Contrast/Resolution

Phase Contrast

Dark Field

Rheinberg Contrast

Nomarski (Differential Interference)

Techniques for plastic

Measuring Contrast

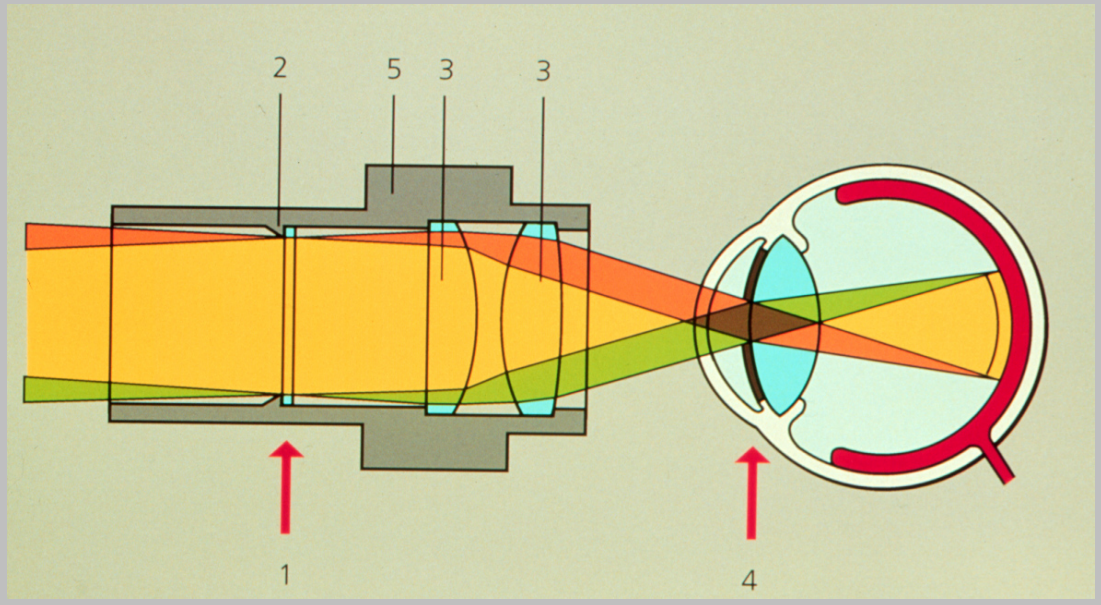
Microscopy as a compromise

Magnification

Resolution

Brightness

Contrast



Contrast

$$\frac{\text{Brightness of Specimen} - \text{Brightness of Background}}{\text{Brightness of Specimen} + \text{Brightness of Background}}$$

50 - 50 / 50 + 50 = 0

50 - 0 / 50 + 0 = 1

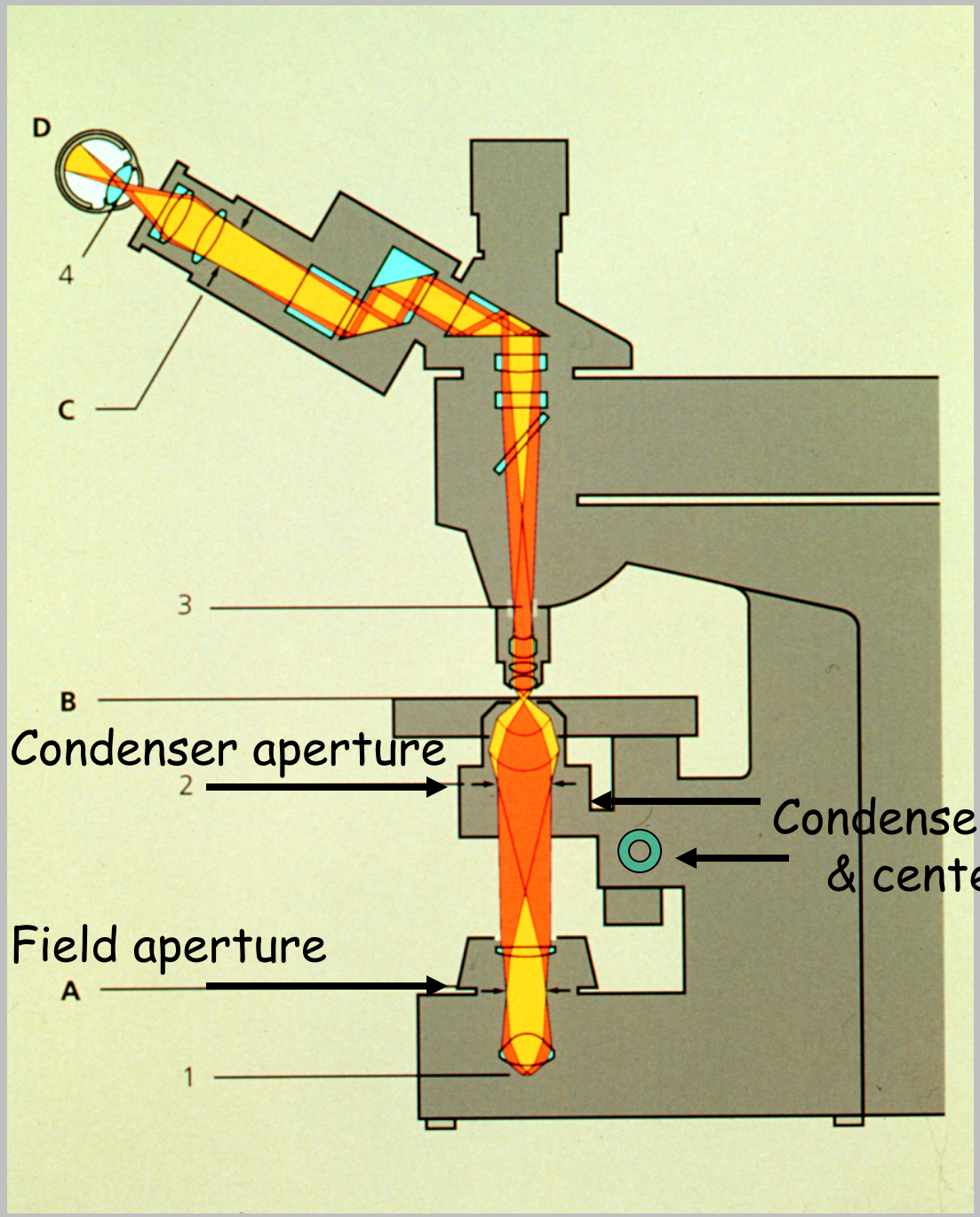
50 - 100 / 50 + 100 = -0.33

"Koehler" Illumination

Prof. August Köhler:

1866-1948

- Provides for most homogenous Illumination
- Highest obtainable Resolution
- Minimizes Straylight and unnecessary Irradiation
- Establishes proper position for condenser elements, for all contrasting techniques


D
4
C
3
B
Condenser aperture
2
Condenser focus
& centering
Field aperture
A
1

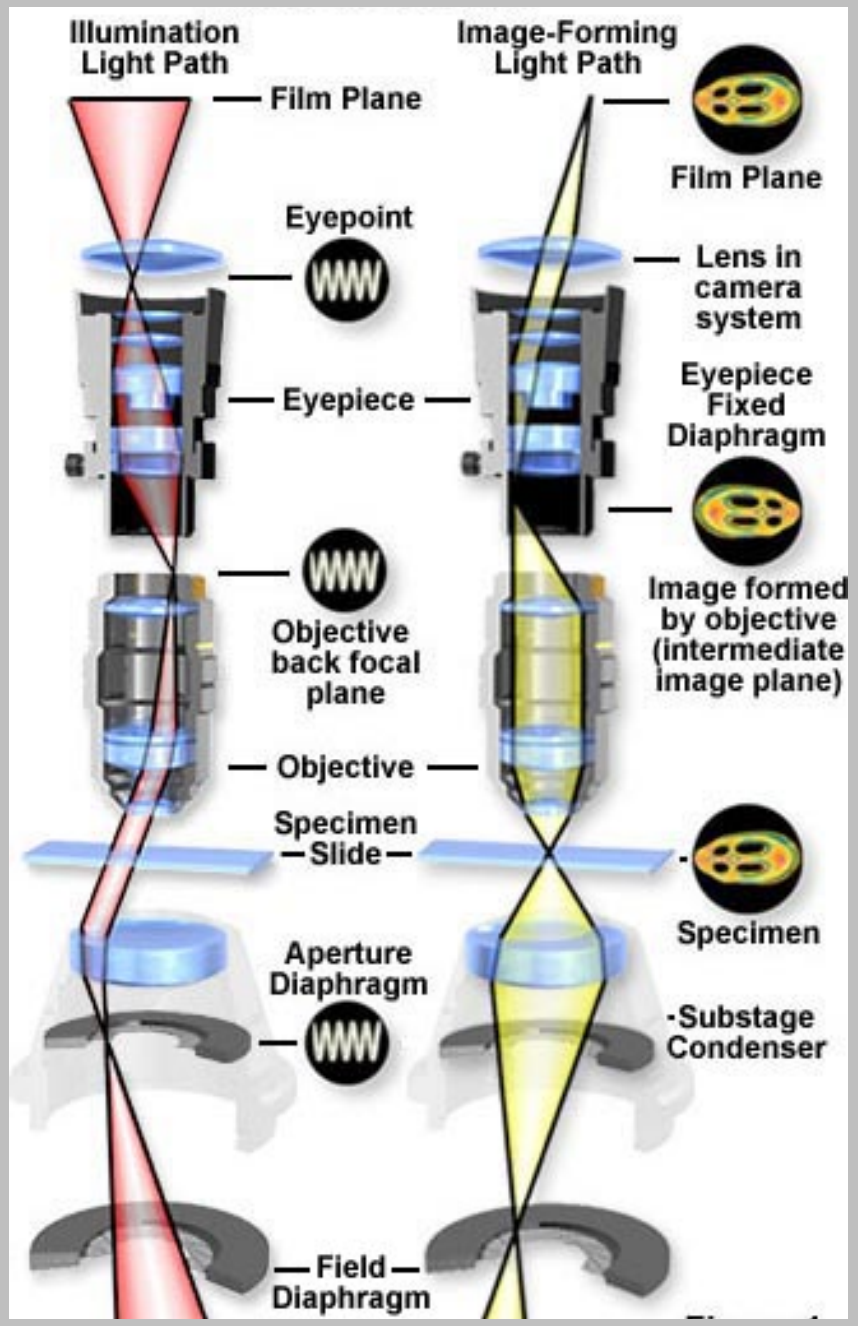
Kohler Rays

Kohler Illumination gives the most uniform illumination

Each part of the light source diverges to whole specimen

Each part of the specimen gets light that converges from the whole light source

Arrows mark conjugate planes



To look at the illumination planes

- Remove eyepiece
- Focus eye at infinity

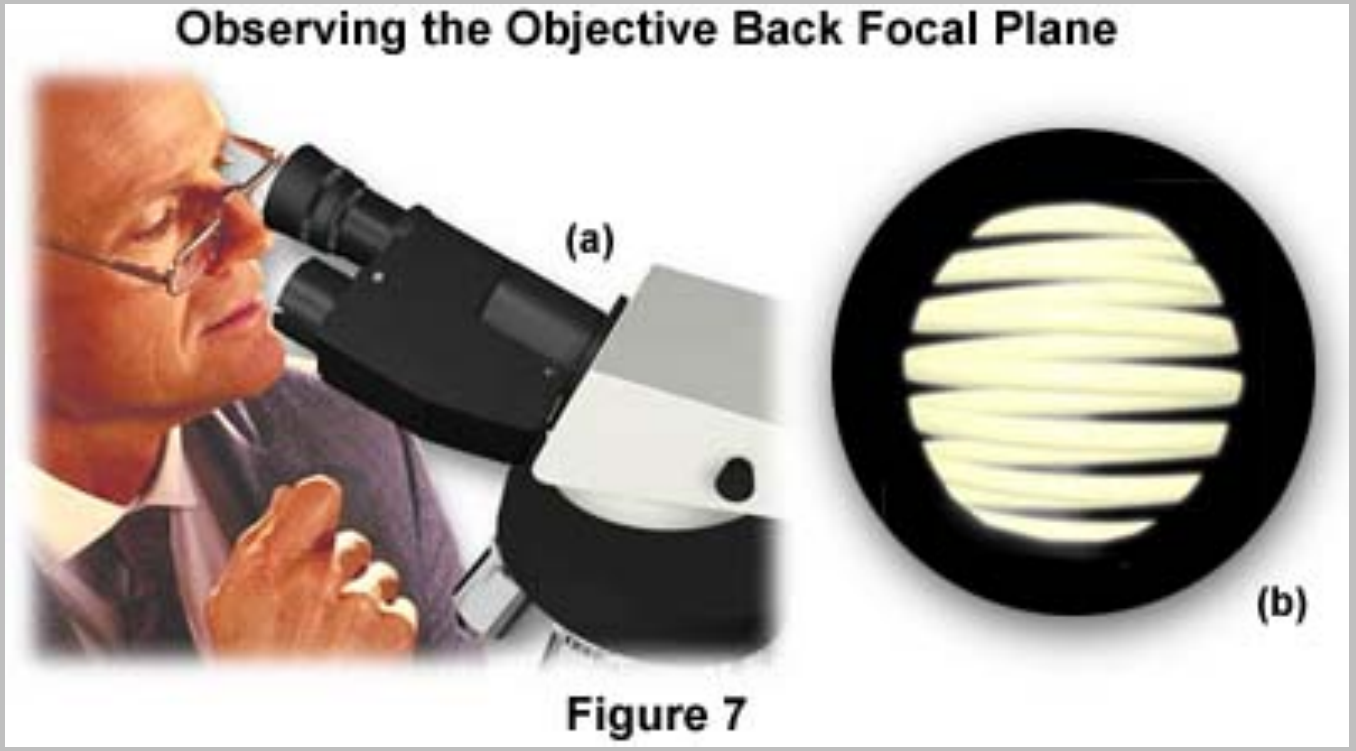
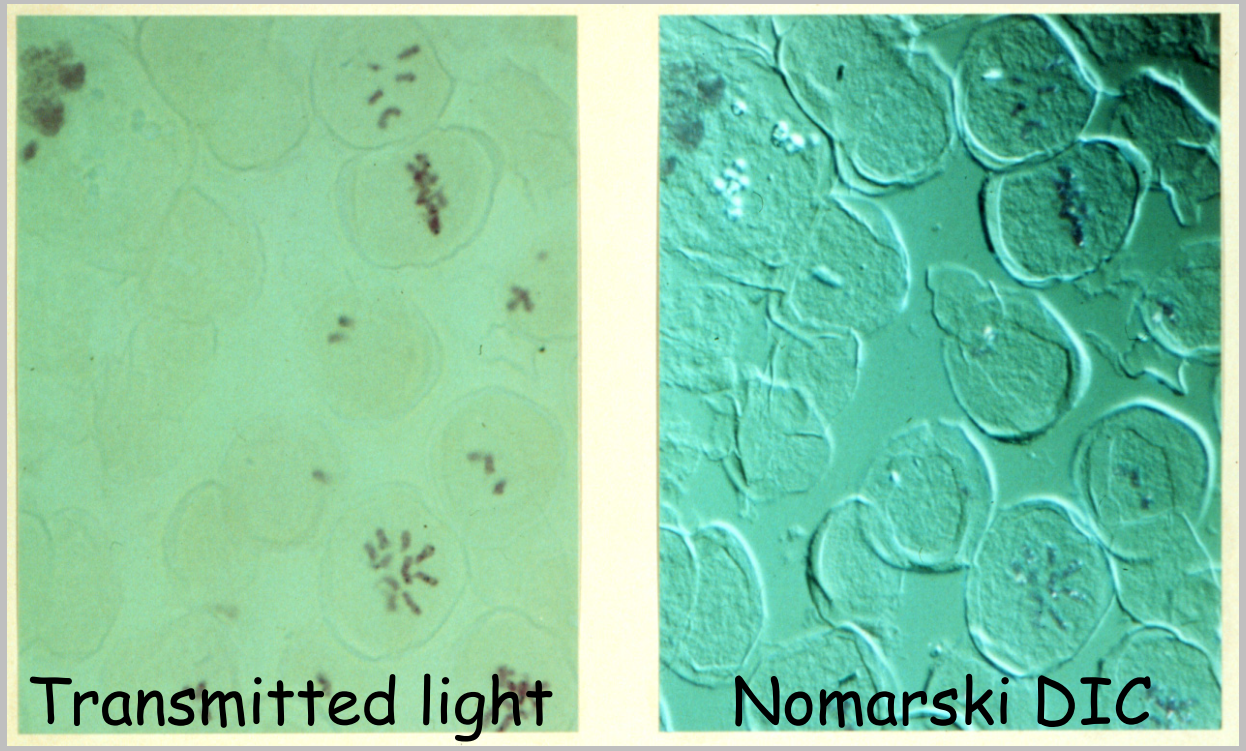
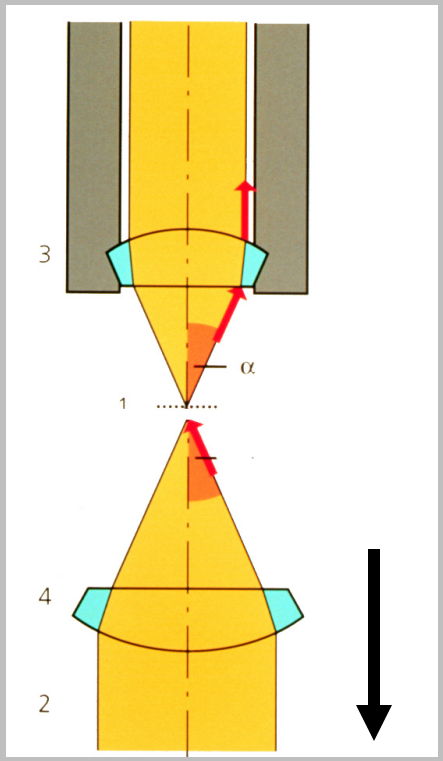


Figure 7

Compromise between Resolution and Contrast

The Big Challenge: The highest resolution is not the highest contrast



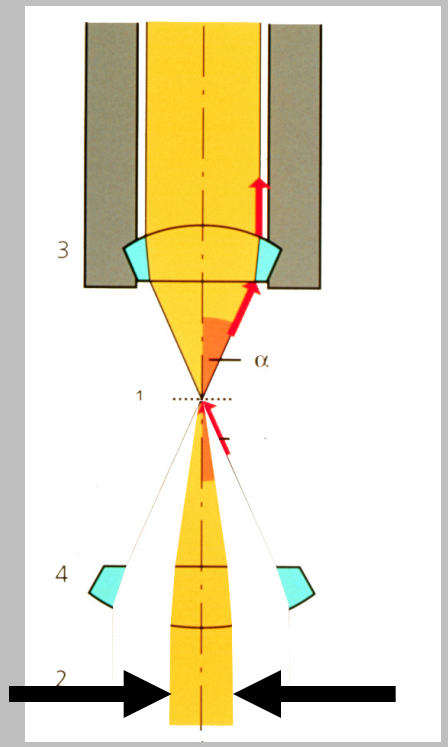


Bad Idea Number 1:
"Dropping" the condenser

Objects scatter light into the objective

Gives contrast, but at the cost of NA

(spherical aberration in condenser)
(bad launch of waves for diffraction)

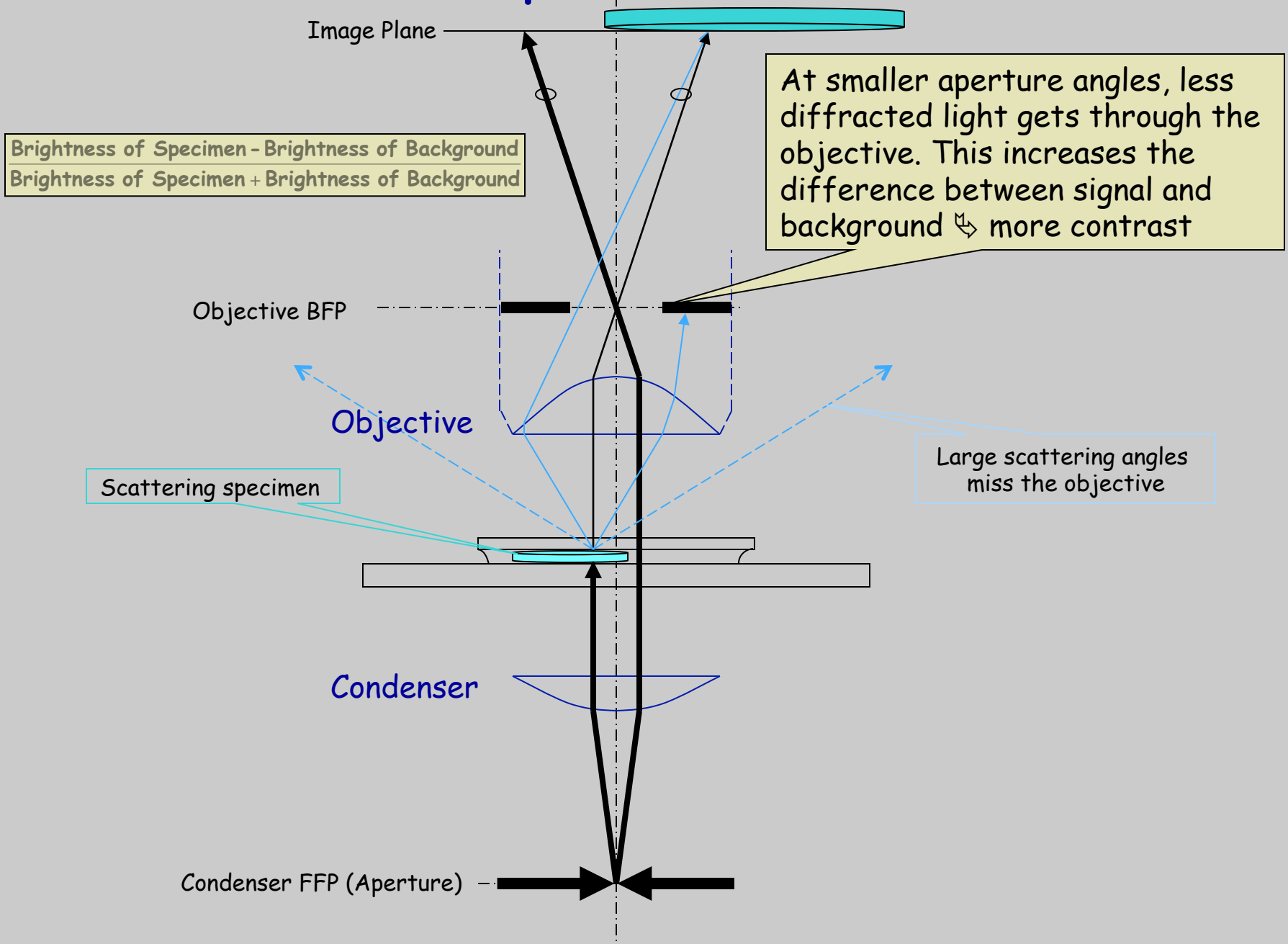


Bad Idea Number 2:
"Stopping down" the condenser

Gives contrast, but at the cost of NA

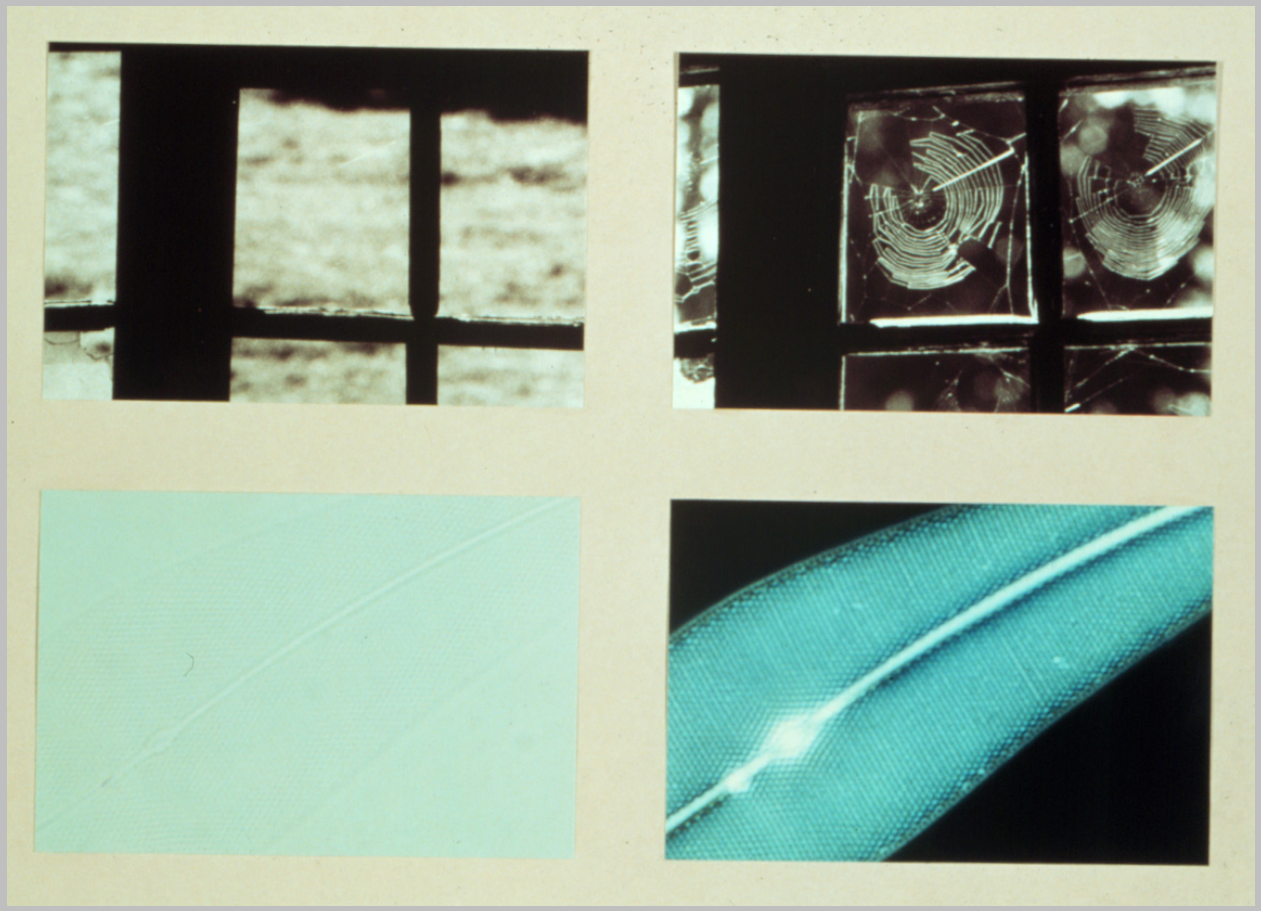
(bad launch of waves for diffraction)

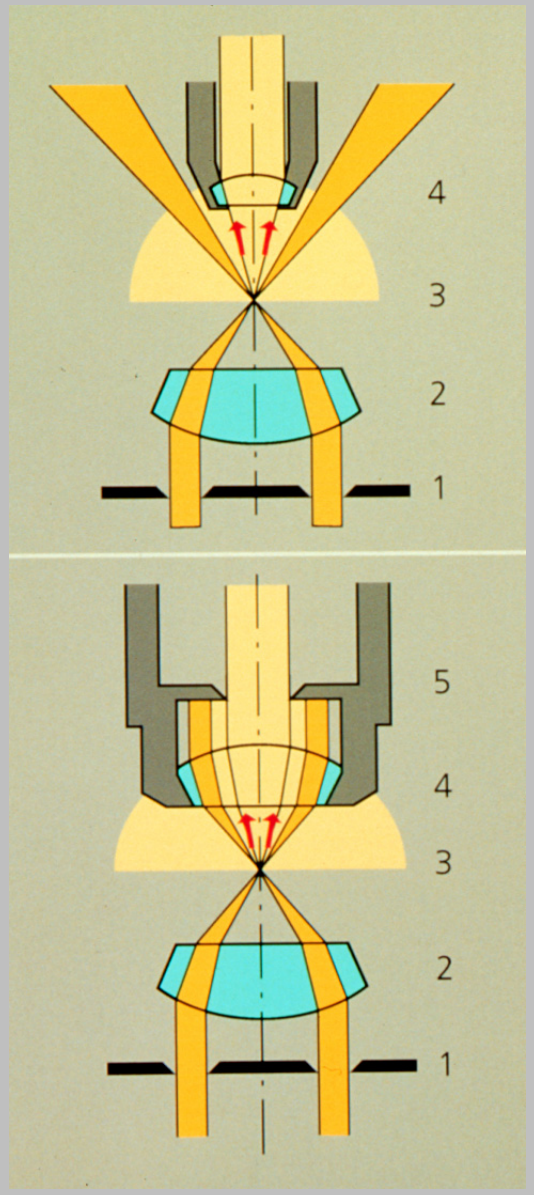
Effect of Aperture on Contrast



Dark-field: Maximizes detectability

Cost in resolution





Dark-field - The GOOD:

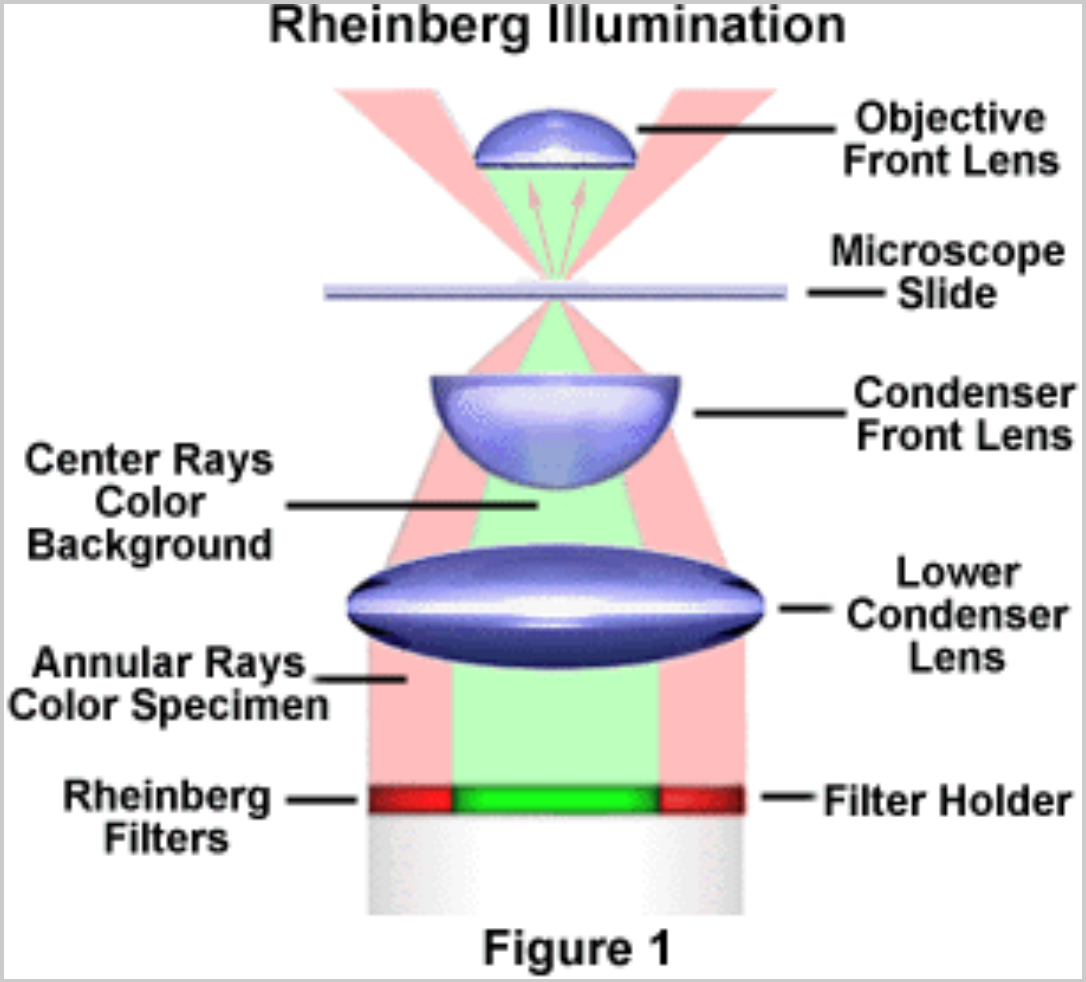
High NA Condenser

"Kohler" Illumination

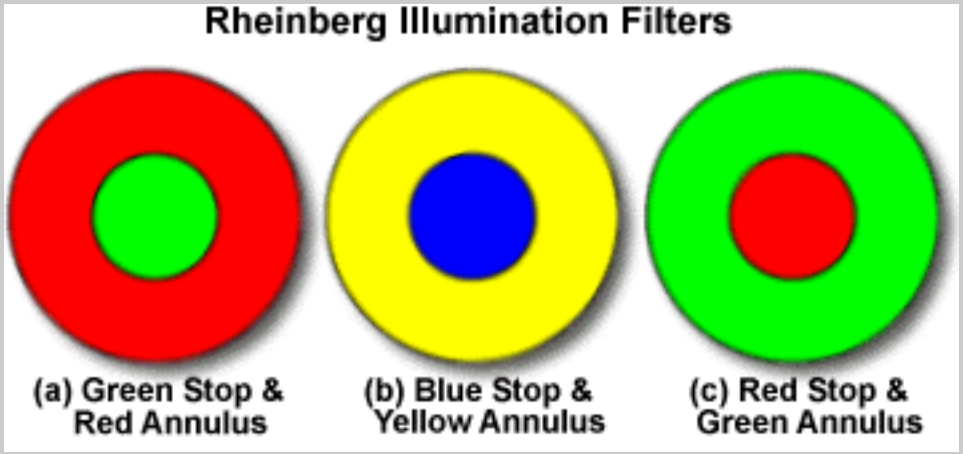
Dark-field - The BAD:

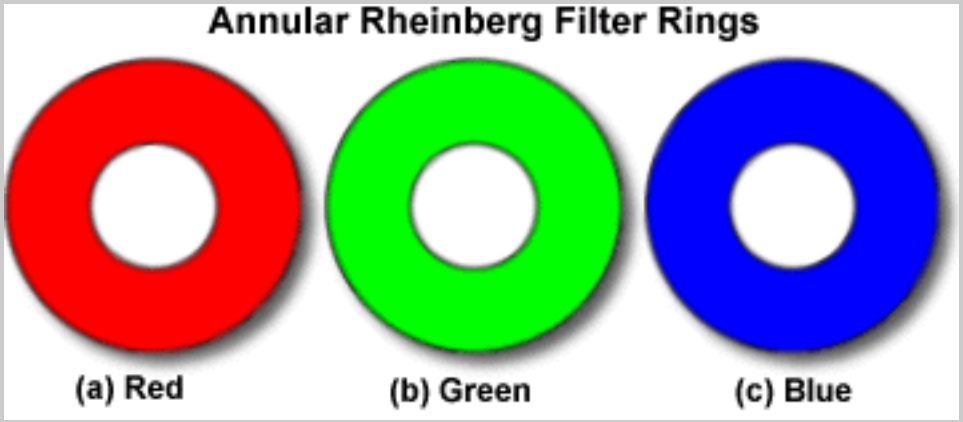
Lower NA light collection

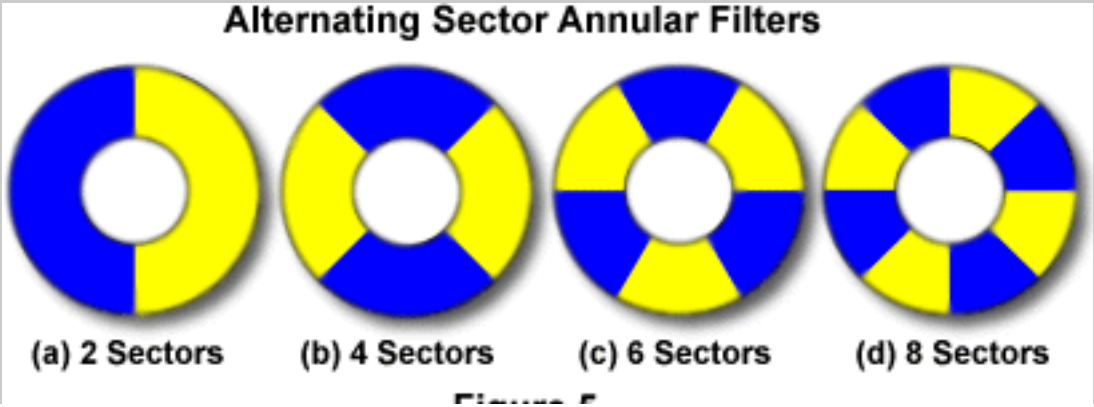
Don't collect 0^{th} order

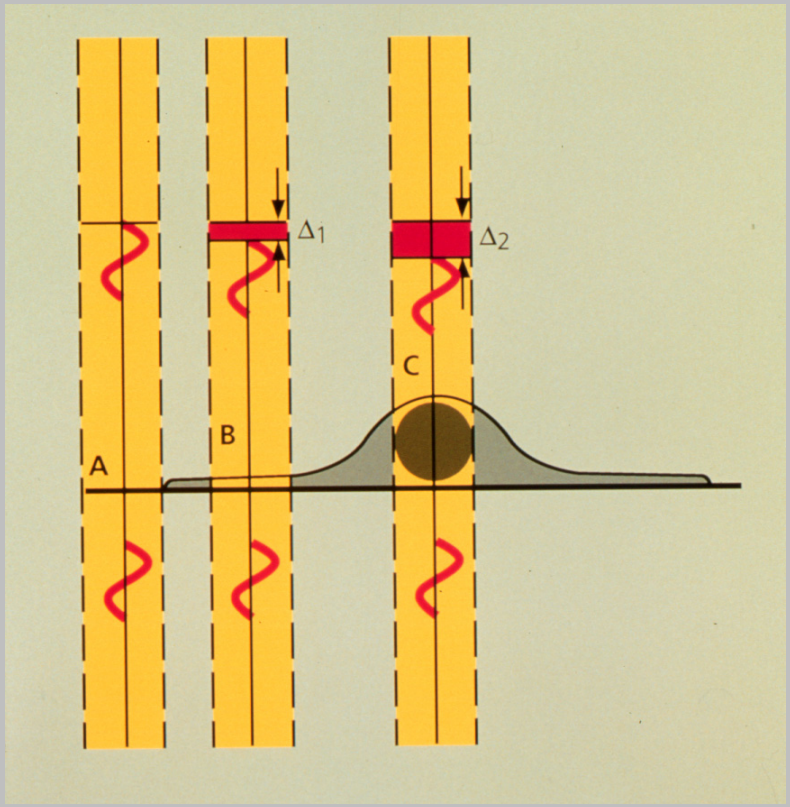


The Good: Striking contrast
The Bad: "dark field" like resolution
(good for seeing things, not as good for measuring)


Rheinberg Illumination Filters
(a) Green Stop & Red Annulus
(b) Blue Stop & Yellow Annulus
(c) Red Stop & Green Annulus


Annular Rheinberg Filter Rings
(a) Red
(b) Green
(c) Blue


Alternating Sector Annular Filters
(a) 2 Sectors
(b) 4 Sectors
(c) 6 Sectors
(d) 8 Sectors



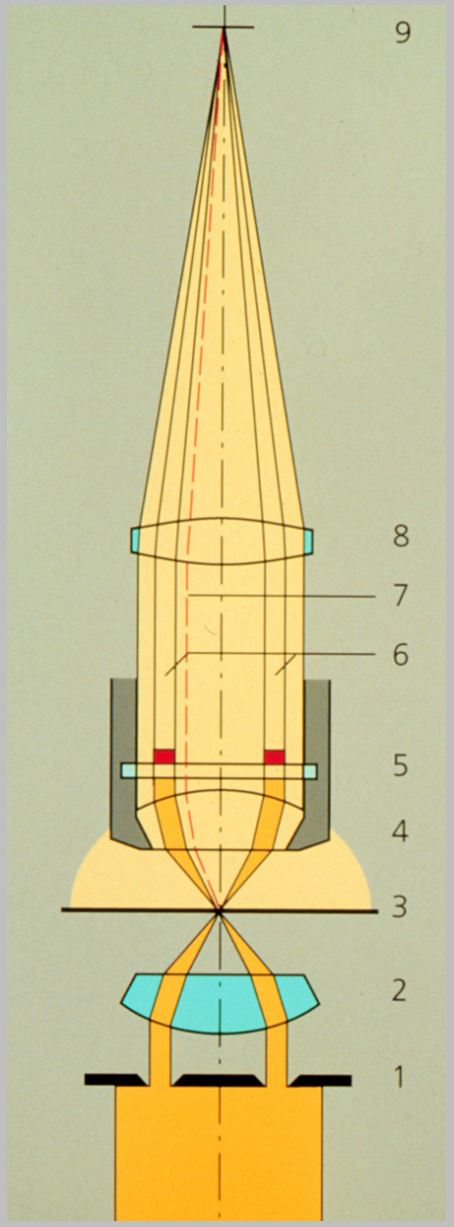
Cells have higher η than water

Light moves slower in higher η

Light has shorter λ

Light will be phase-retarded

How to harvest this?



Phase Contrast:

Illumination from Phase Ring

Defined position of the 0th Order

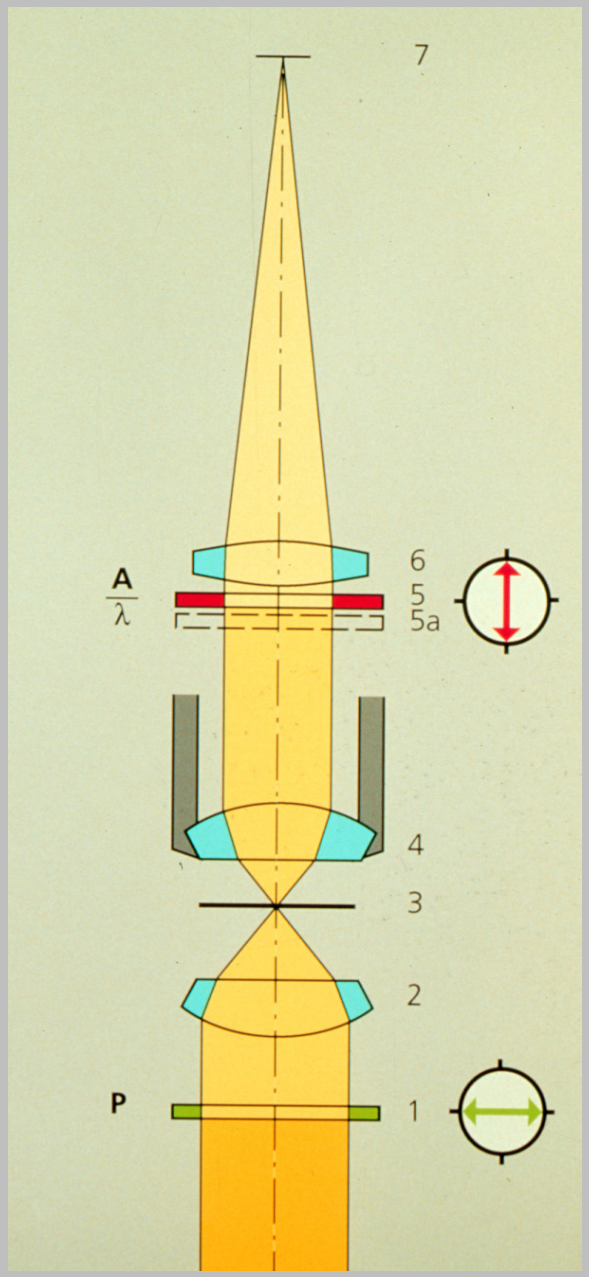
Phase Ring attenuates the 0th Order

(also phase shifts)

Makes image more dependent on subtle changes in 1st Order

Refraction of light by specimen focuses light inside of the phase ring

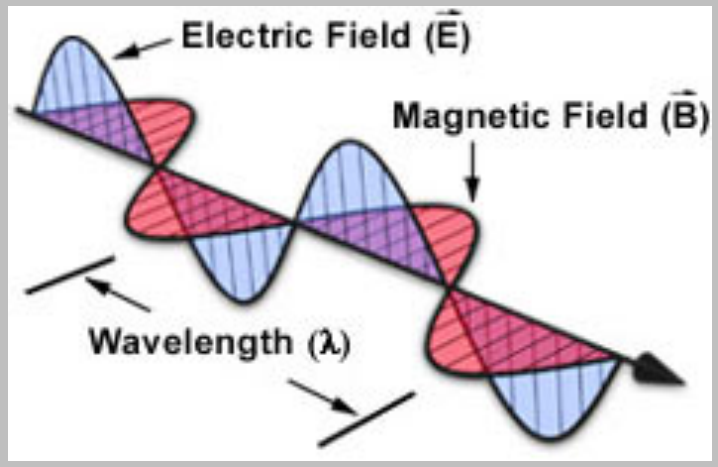
(spherical cells appear "phase bright")



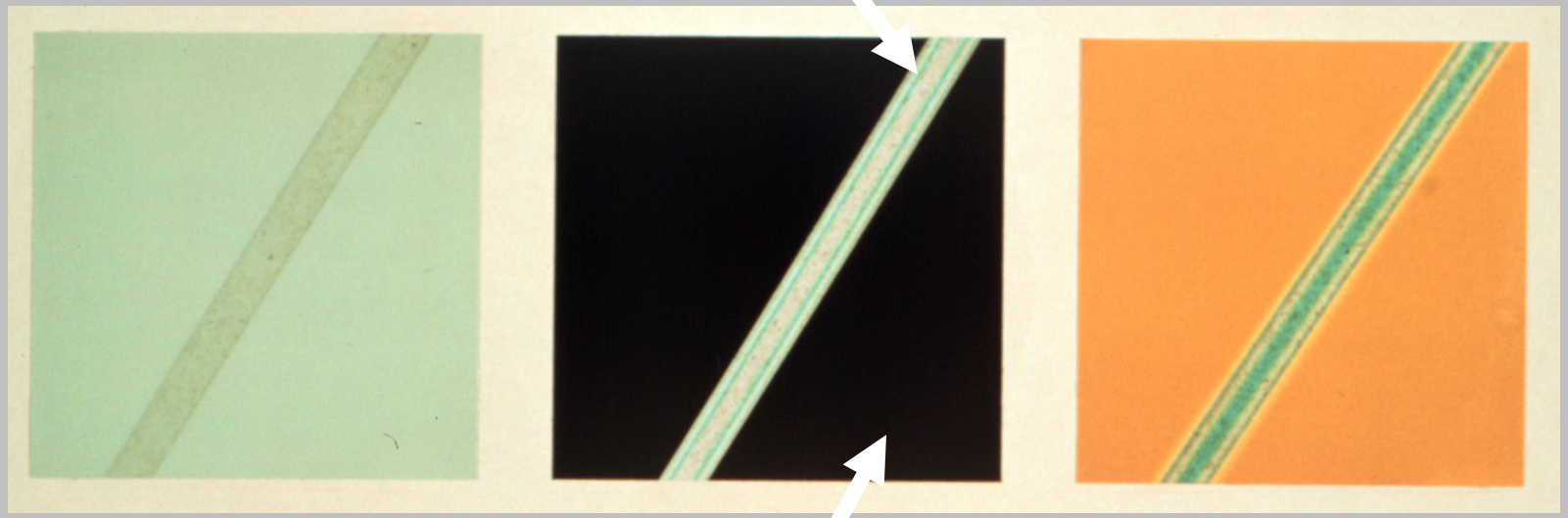
Crossed Polarizers:

Only items that rotate the plane of Polarization reaches the detector.

(quarter wave plate adds color)

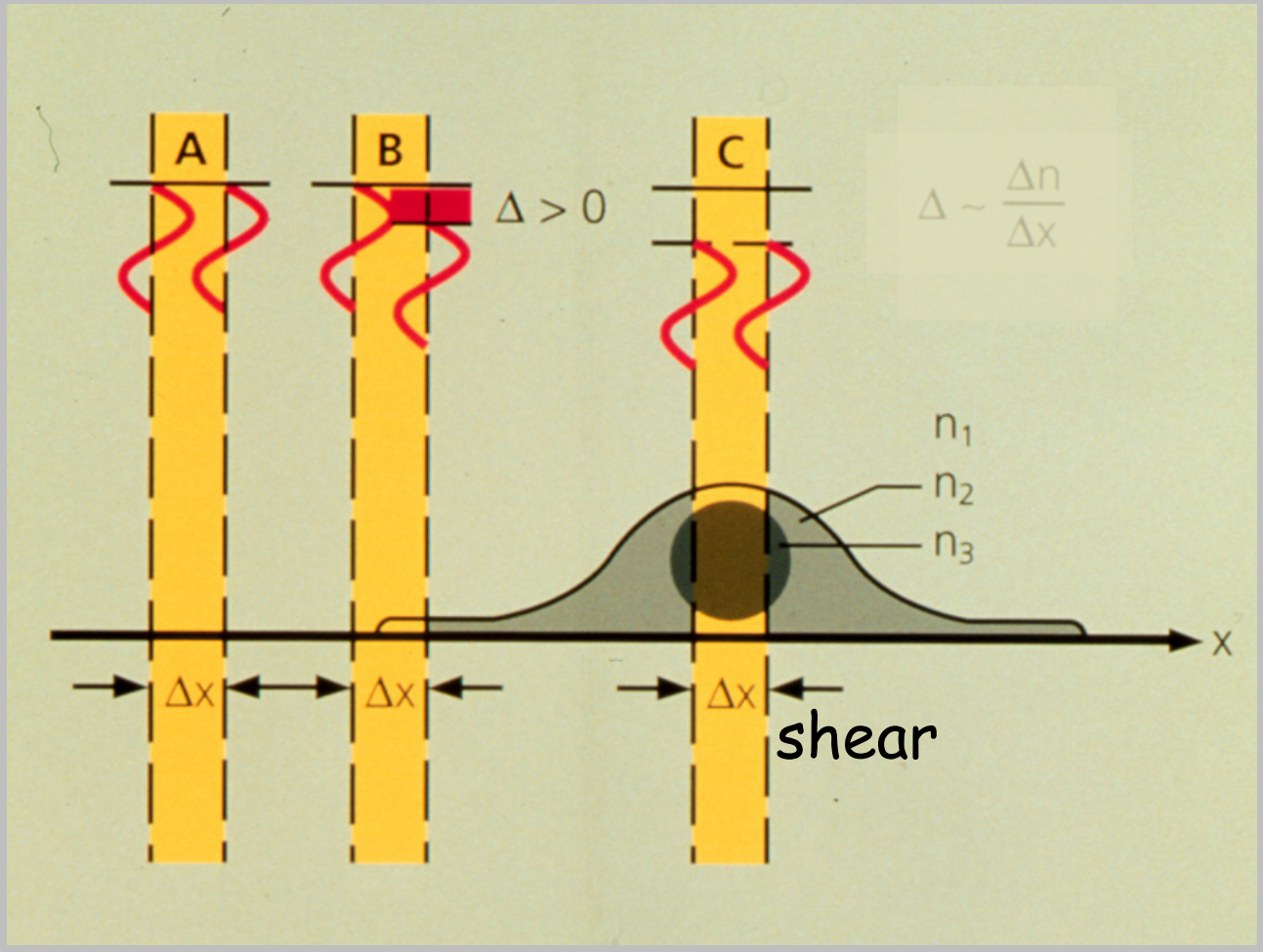


Rotation, so light

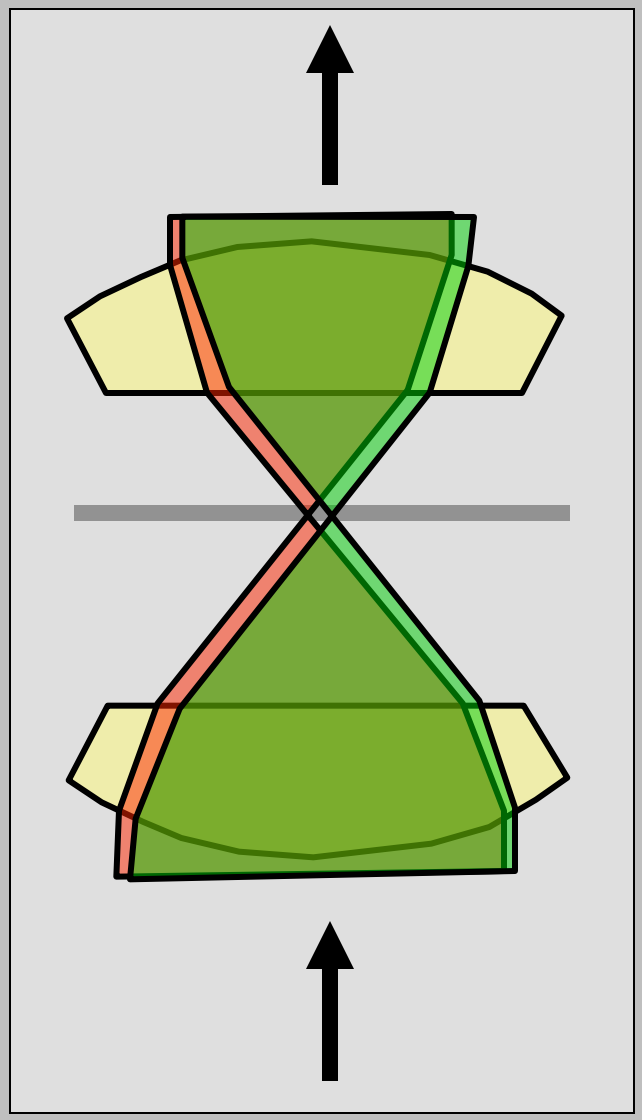


No rotation, so black

Nomarski thought experiment: need two different light rays
Pass through specimen independently
Afterwards, let them interfere with one another
How to label them? How offset them (shear)?



Thought experiment: Color code two paths offset



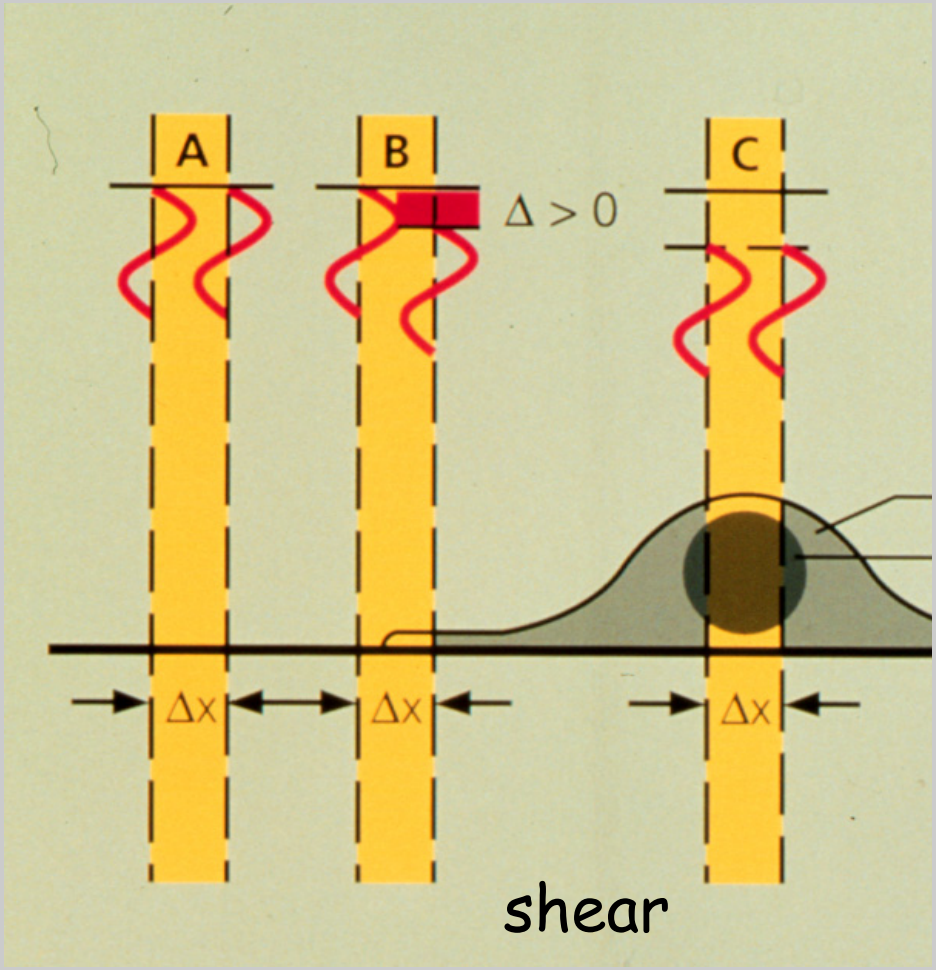
Problem: red and green light don't interfere with each other

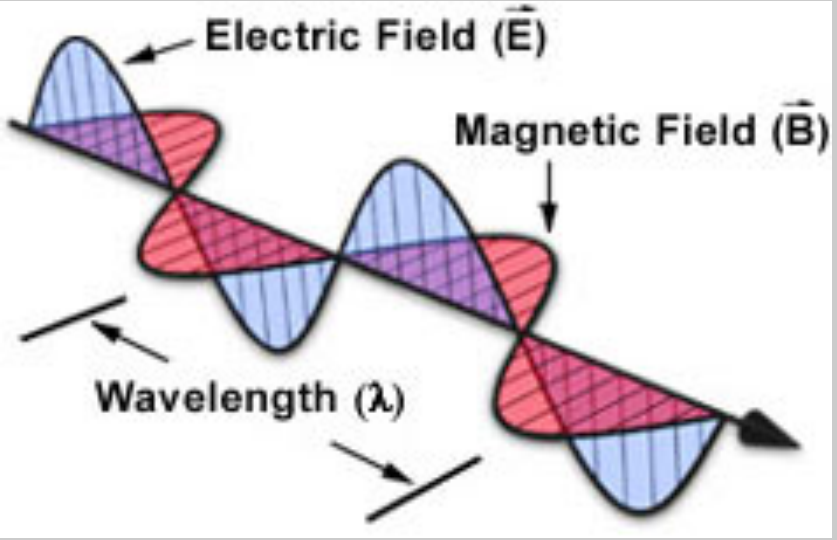
Nomarski thought experiment: need two different light rays

Pass through specimen independently

Afterwards, let them interfere with one another

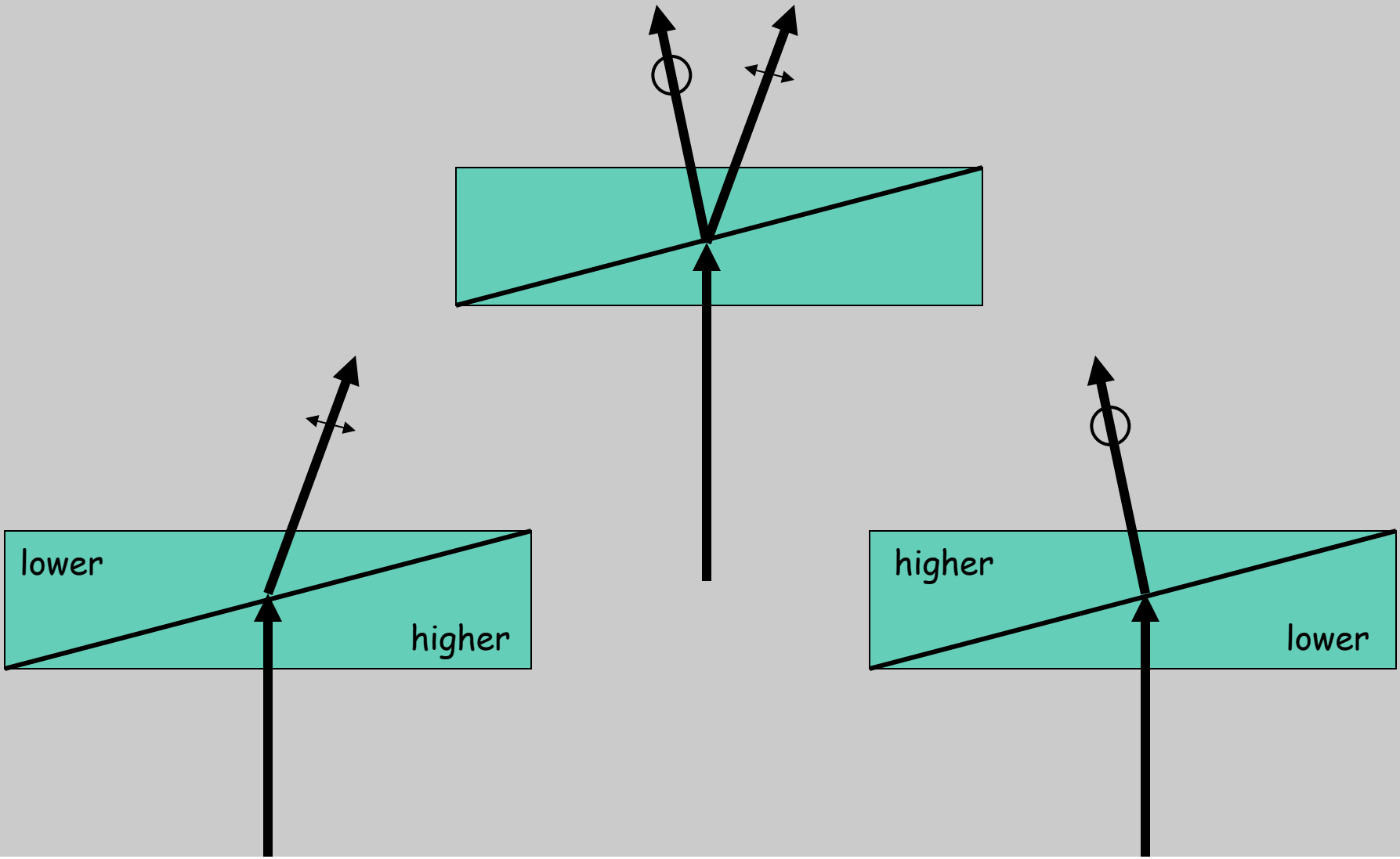
How to label them? How offset them (shear)?





Polarization as the label (light must be in same plane to interact)

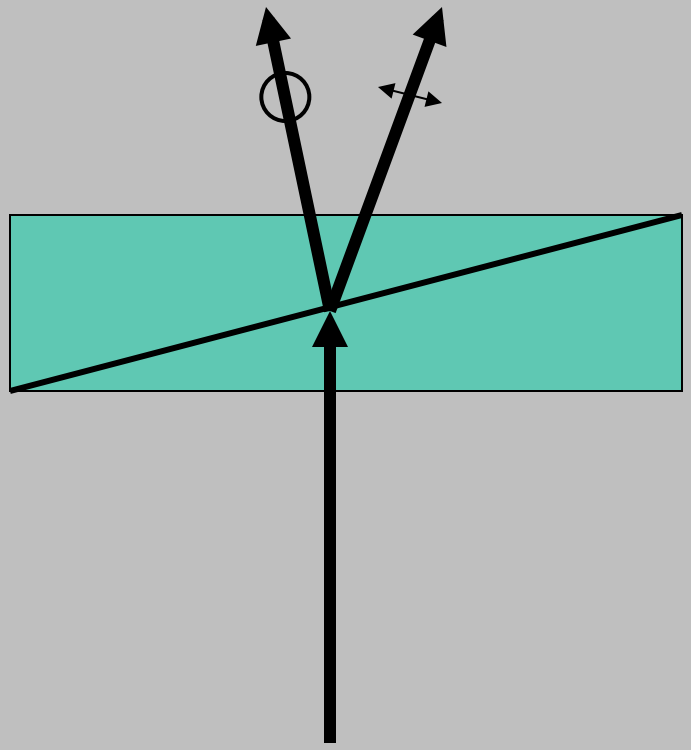
Wollaston Prism
Birefringent material
Different η for different polarizations

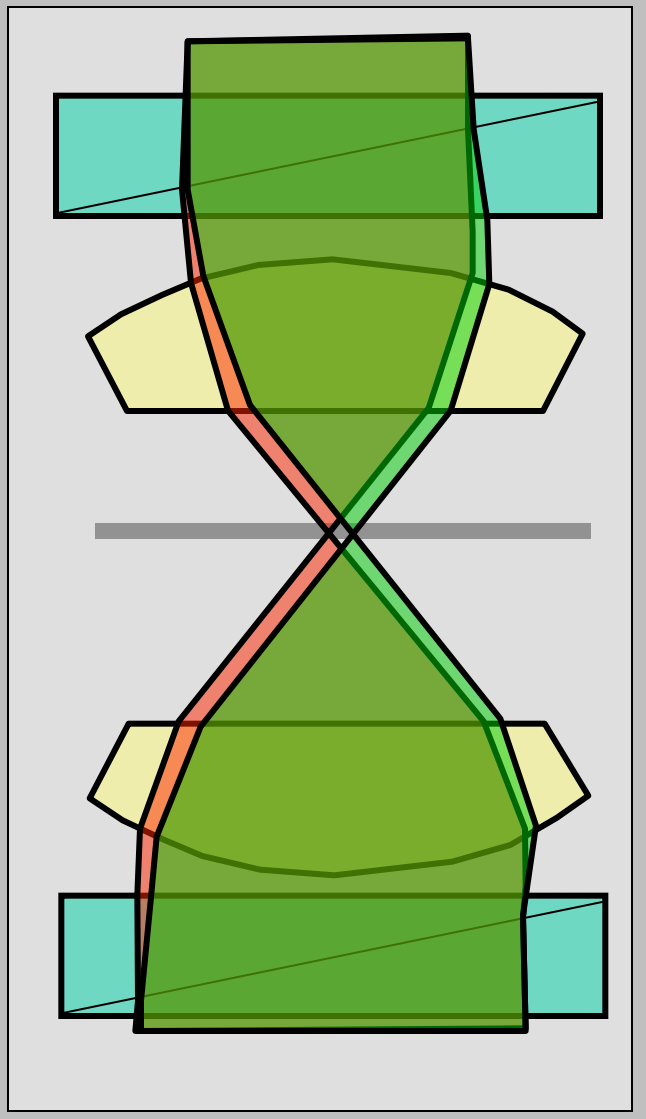


Wollaston Prism

Birefringent material

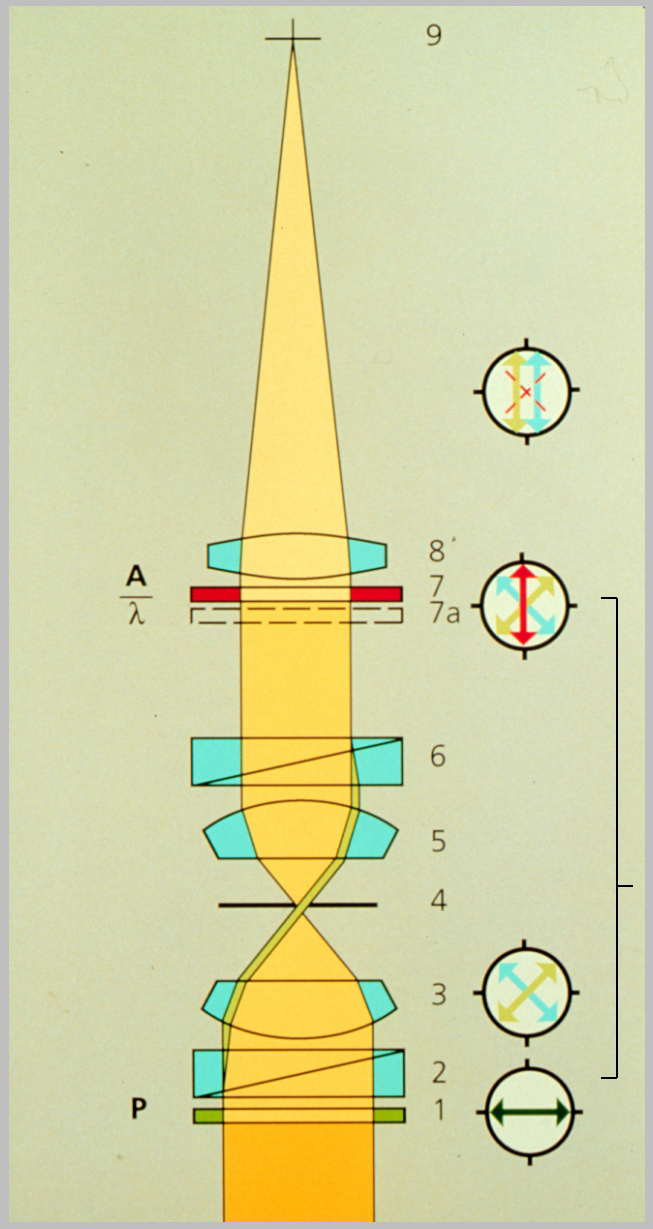
Different η for different polarizations





Problem: light in different planes of polarization don't interfere with each other (need an analyzer)

Nomarski - two beams labeled by plane of polarization



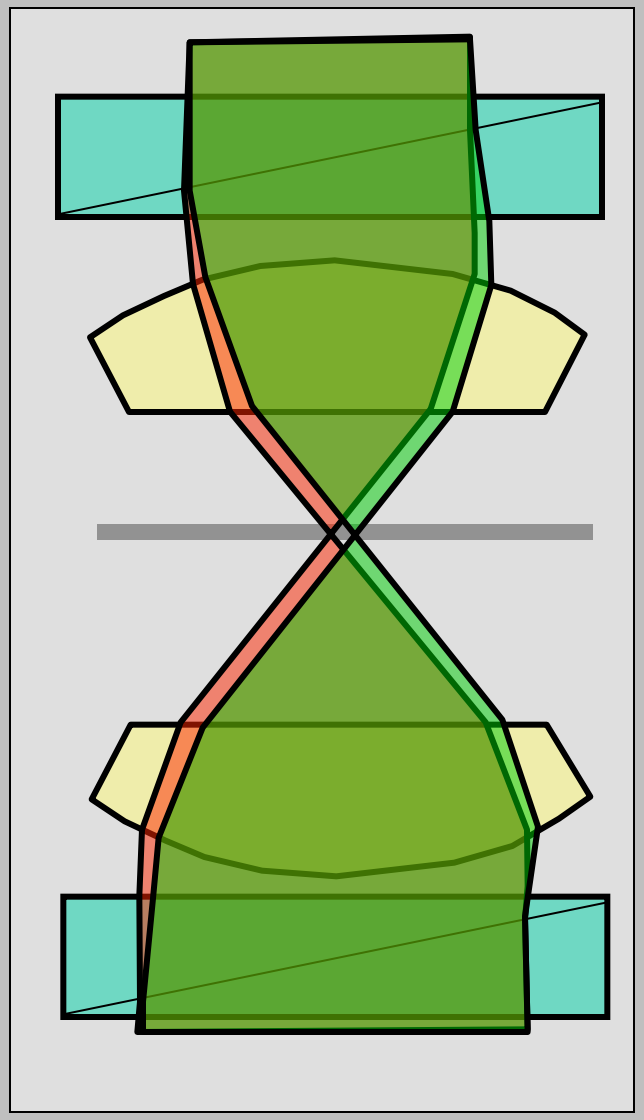
Analyzer - forces two beams into same plane

Wollaston prism - recombines two beams

Domain of independent paths

Wollaston prism - splits into two beams; adds shear

Polarizer - prepares for Wollaston prism 50-50 split



Nomarski Optics

Good -

- Contrast at full aperture
- Optical sectioning (to ~0.3um)
  (two beams mostly overlap)

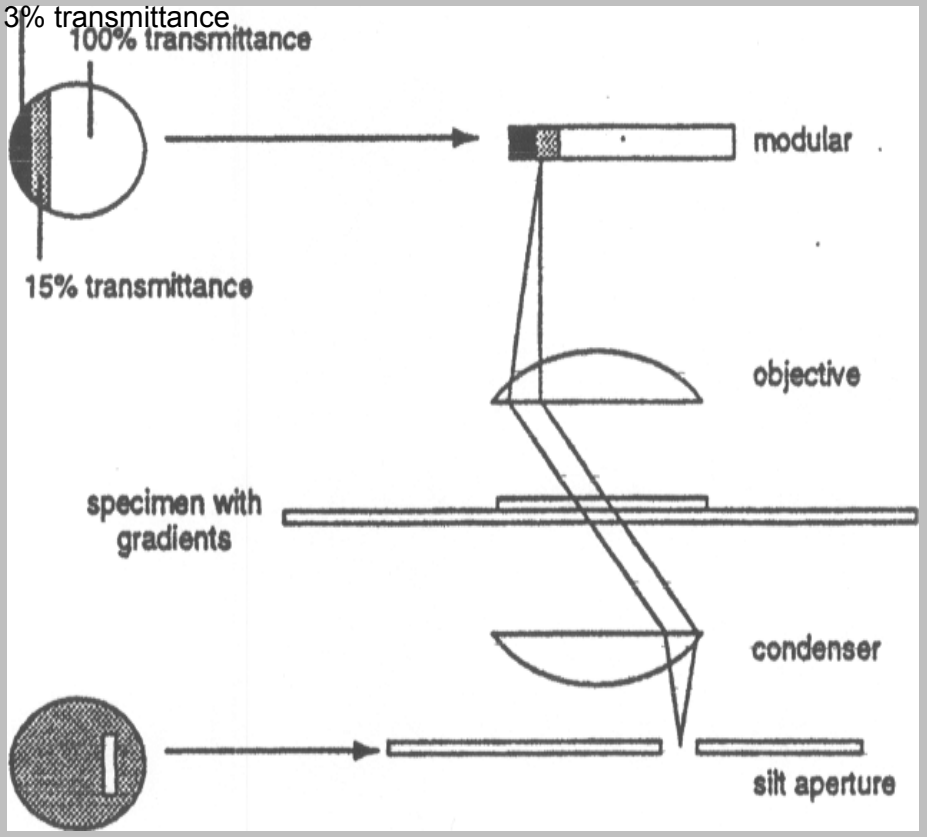
Bad -

- Expense
- Very sensitive to polarization
  Plastic
  Glass with stress

Modulation Contrast (Hoffman)

- For unstained (live) specimens
- Combination of oblique illumination and attenuation of non-diffracted light
- Simulated 3-D image (similar to DIC)
- Less resolution, not as specific as DIC
- No “Halo”-effect
- Usable with plastic, birefringent dishes

Hoffman Modulation Contrast



Required Components :

Specially Modified Objective (With Built-in Modulator)

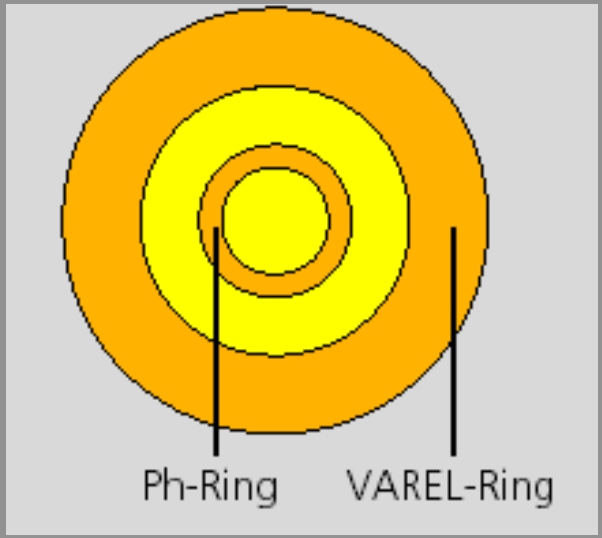
Modified Condenser with off-axis slit (double slit with polarizer)

Varel Contrast (1996 - Zeiss)

- For unstained (live) specimens
- Combination of oblique illumination and attenuation of non-diffracted light
- No "Halo"-effect
- Complementary technique to Phase (easy switchover)
- Simulated 3-D image (similar to DIC)
- Less resolution than DIC
- Works with plastic dishes

Varel Contrast (1996 - Zeiss)

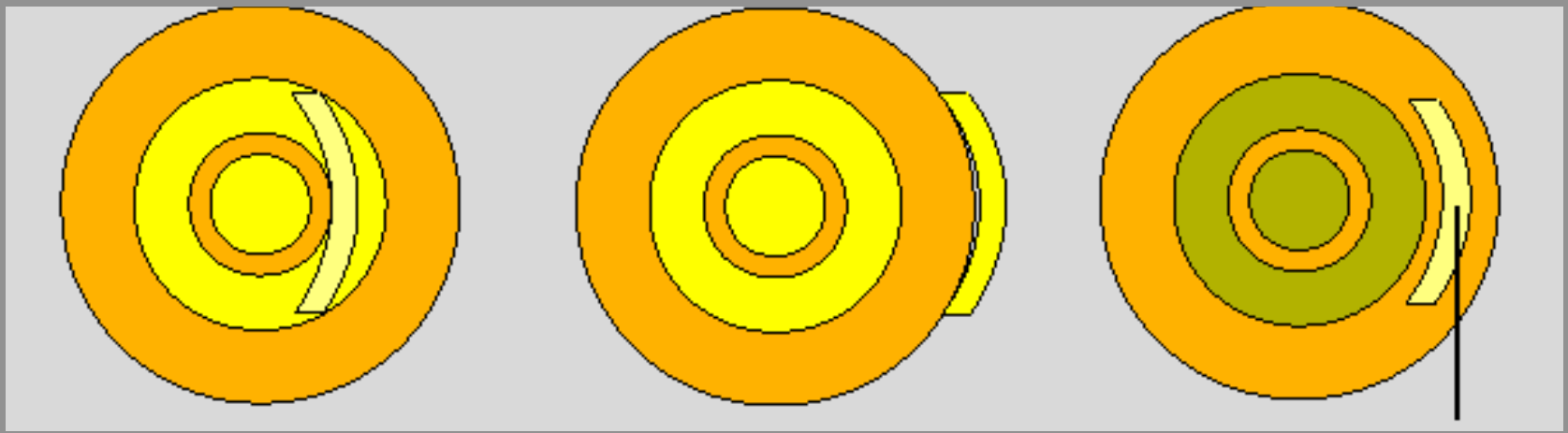
Objective



Required Components for Varel:

1. Objective with Varel- and Ph ring
2. Slider or Condenser with specific Varel 1, 2 and Phase rings

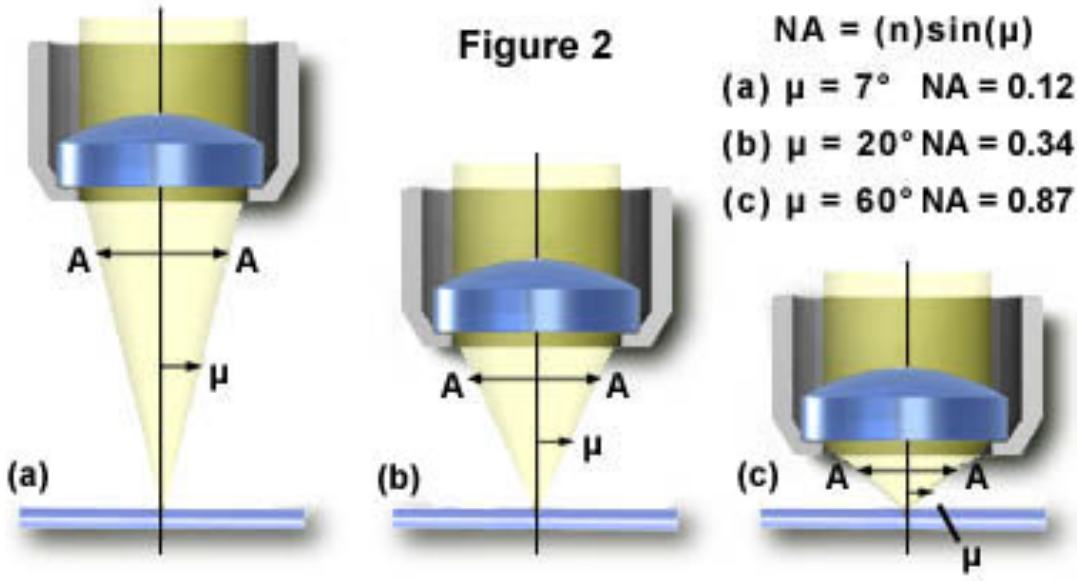
Brightfield – oblique **Darkfield - single band** **Varel**



Condenser

Movable Ring Sector (Varel Ring)

Numerical Aperture and Resolution



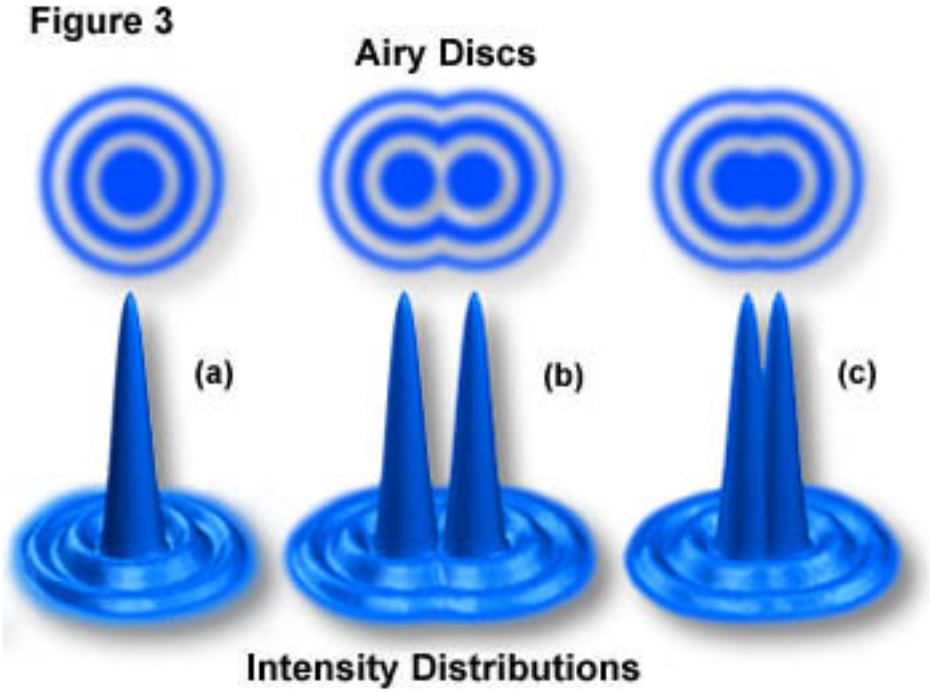
Resolution: smallest distance between two points on a specimen that can still be distinguished as two separate entities.

R = 0.61λ/NA

R = 1.22λ/(NA(obj) + NA(cond))

Resolution

zeroth order maximum) surrounded by concentric 1st, 2nd, 3rd, etc., order maxima of sequentially decreasing brightness that make up the intensity distribution.



•Light from points of specimen passes through the objective, forms image,
•Points of the specimen appear in the image as small patterns: ***Airy patterns***.
-caused by diffraction or scattering of the light passing through specimen
•Central maximum of the Airy patterns: **Airy disk**, region enclosed by the first minimum
-contains 84 percent of the luminous energy.

Resolution

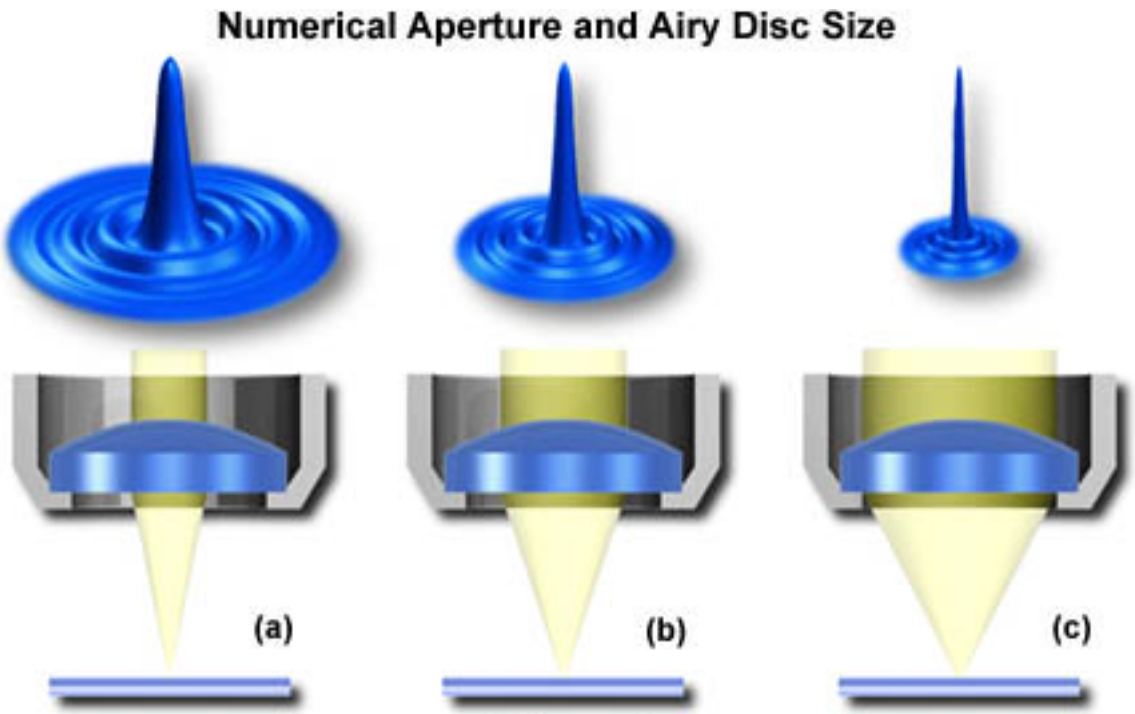


Figure 4

MTF

- The resolution and performance of an optical microscope can be characterized by the modulation transfer function (MTF)
- The MTF is a measurement of the microscope's ability to transfer contrast from the specimen to the image plane at a specific resolution.

MTF

- The effect of increasing spatial frequency on image contrast

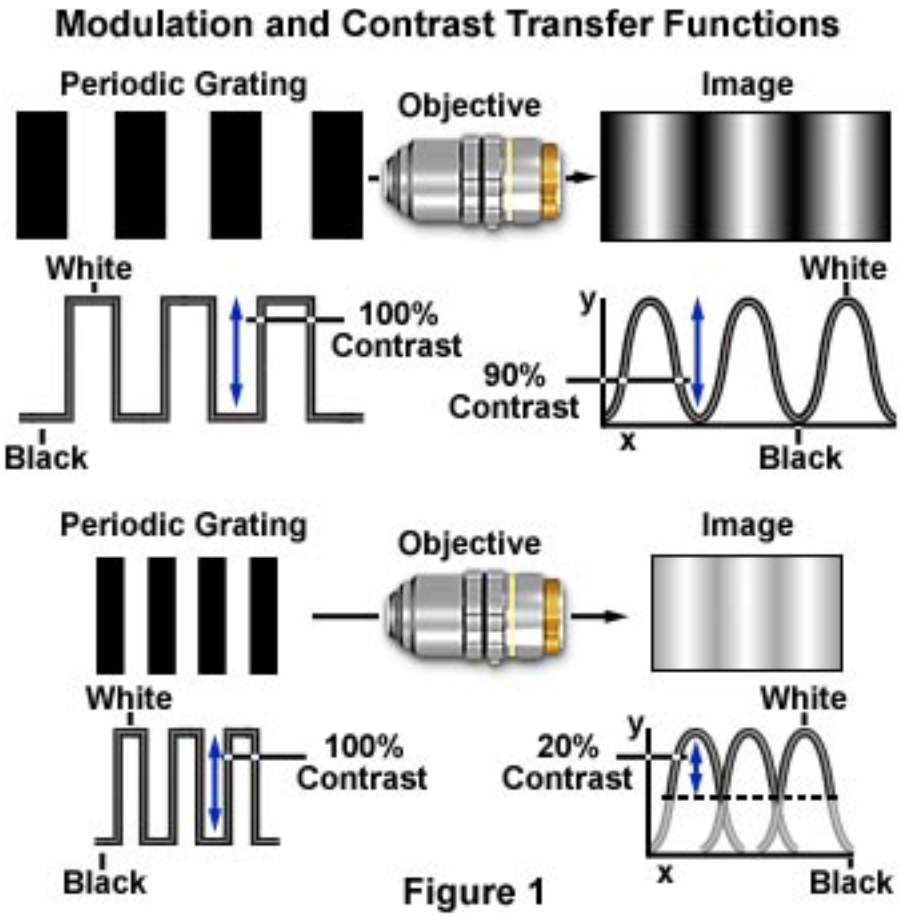


Figure 1

Modulation (M) = (I(max) - I(min))/ (I(max) + I(min))

MTF = Image Modulation/Object Modulation

MTF

- The effect of increasing spatial frequency on image contrast

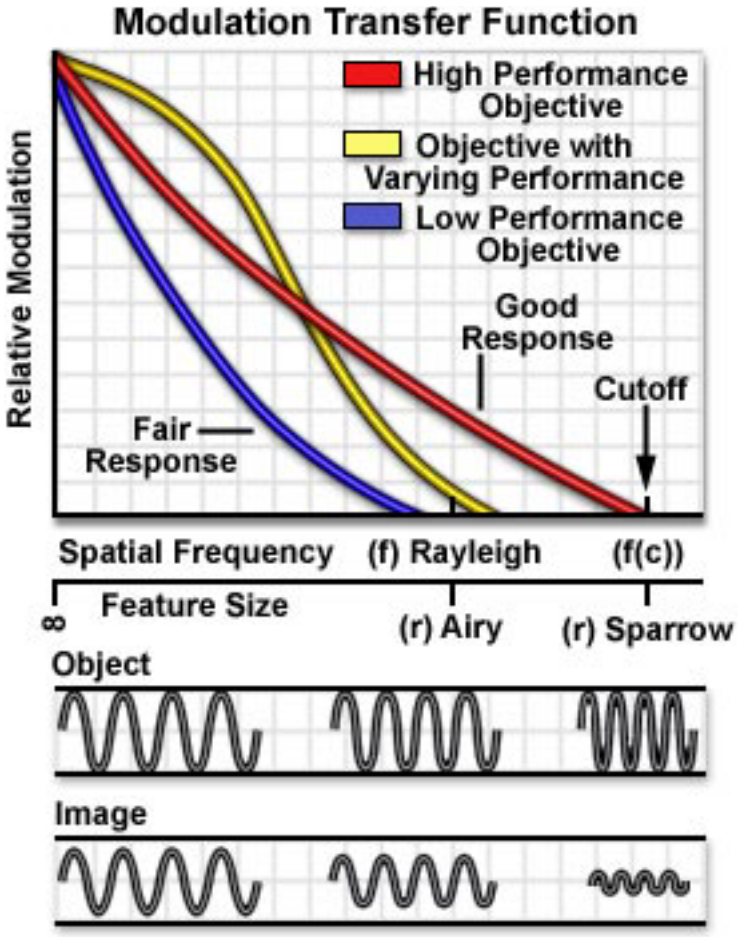


Figure 2