

Brightfield Contrasting Techniques

Kurt Thorn

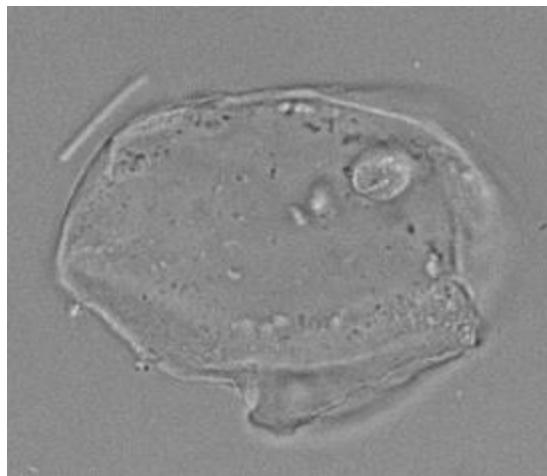
Nikon Imaging Center

University of California, San Francisco

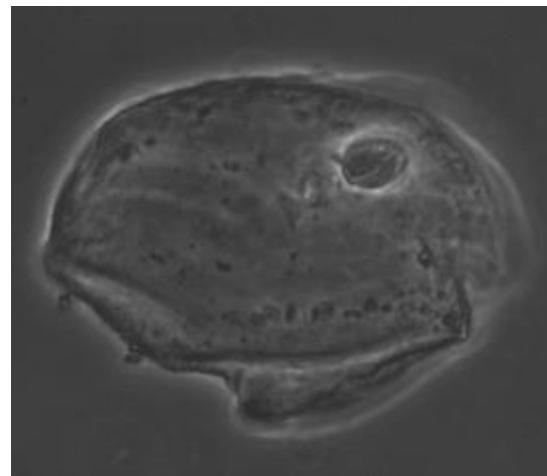
USA

Generating contrast in light microscopy

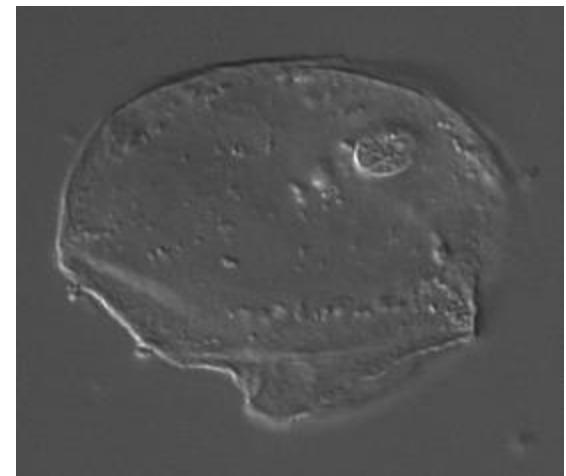
- Problem: Many biological specimens are thin and transparent and difficult to see.
- Solution:
 - Fluorescent staining
 - Brightfield contrasting techniques: DIC, Phase, others



Brightfield

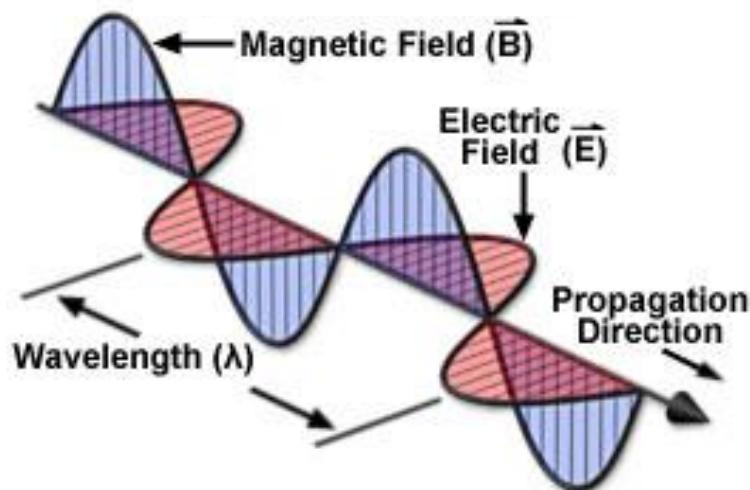


Phase Contrast



DIC

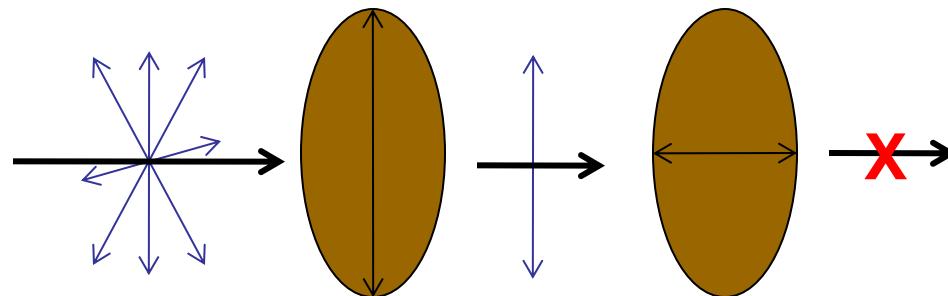
Polarization



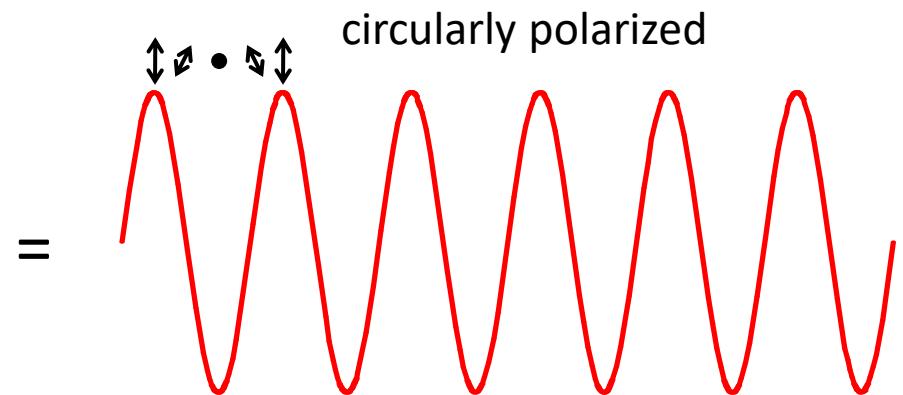
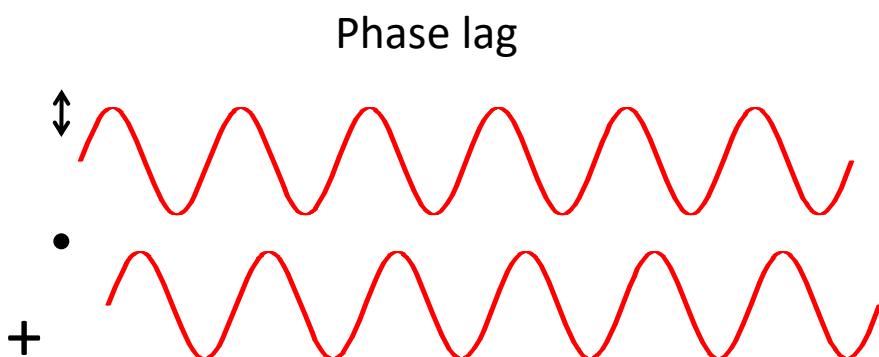
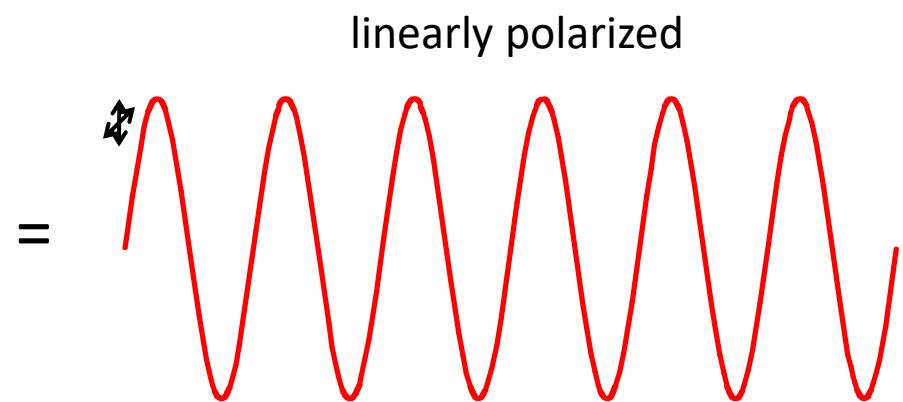
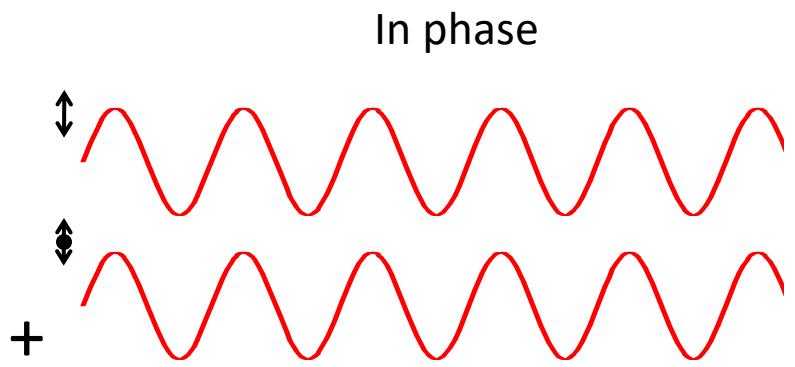
- Polarization: orientation of E-field.
- Most light sources produce unpolarized light – no preferred polarization angle

Polarizers

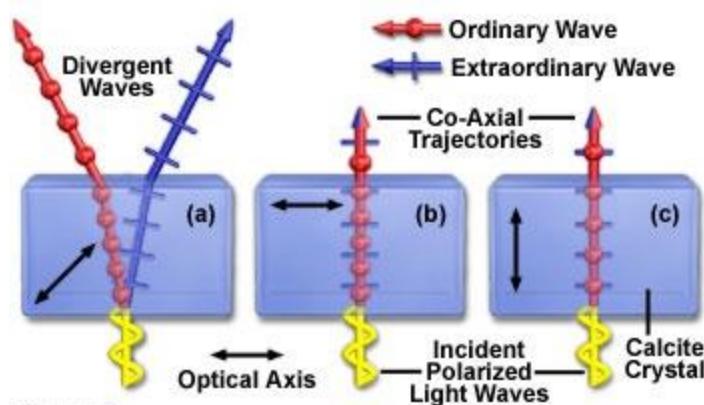
- Polarizers specifically transmit one polarization angle of light
- Crossed polarizers transmit no light



Interference and polarization



Birefringence



Bi-Refraction in Calcite Crystals

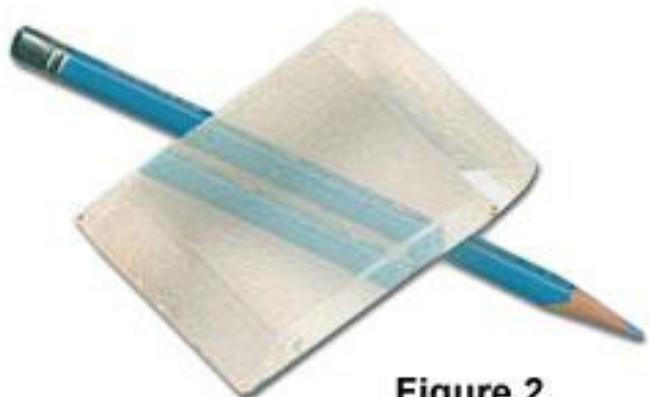
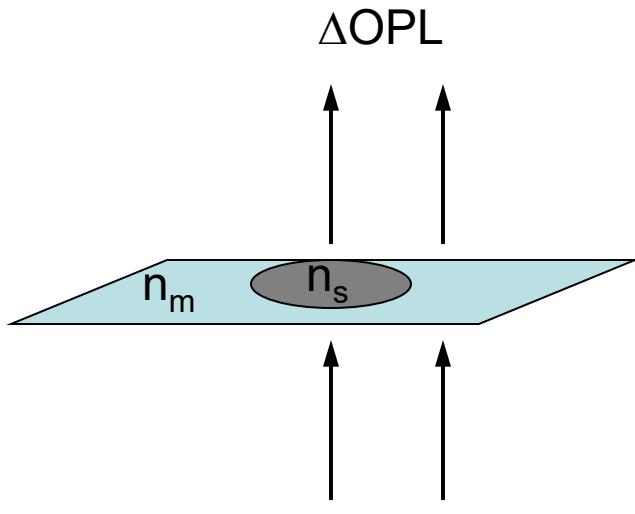


Figure 2

- Birefringent materials have different indices of refraction for light polarized parallel or perpendicular to the optical axis.
- Two beams with orthogonal polarization are produced if illumination is at an angle to optical axis

Differential Interference Contrast (DIC)

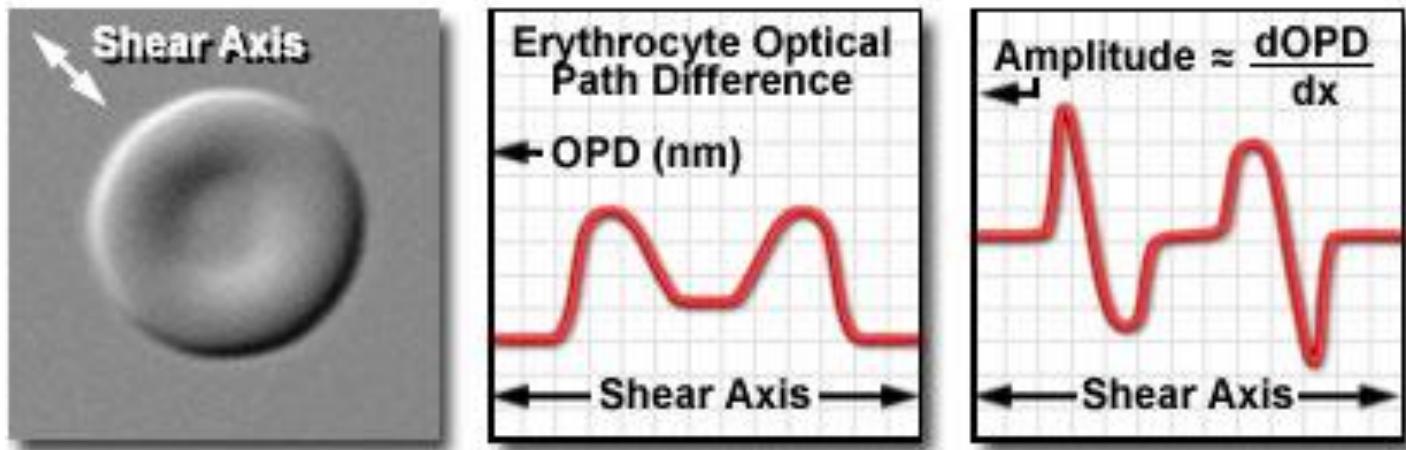


The idea:

Use two beams and interference to measure the path length difference between adjacent points in the sample

What DIC accomplishes

Specimen Optical Path Difference and DIC Amplitude Profile



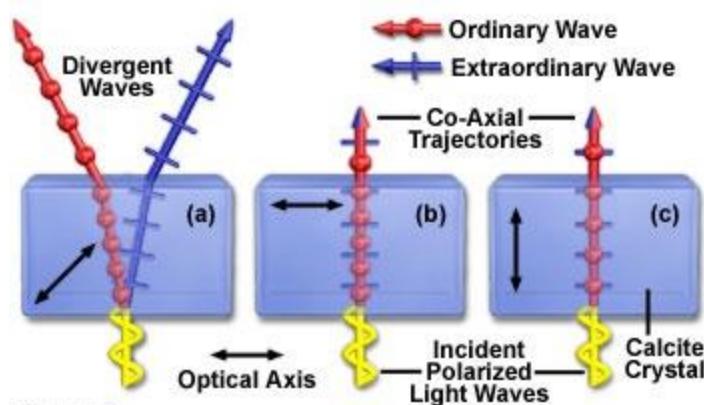
Converts relative differences in optical path length to differences in amplitude

Features of a DIC image



1. Contrast is directional
2. Contrast highlights edges
3. One end brighter, other is dimmer giving a pseudo – 3D image

Birefringence



Bi-Refraction in Calcite Crystals

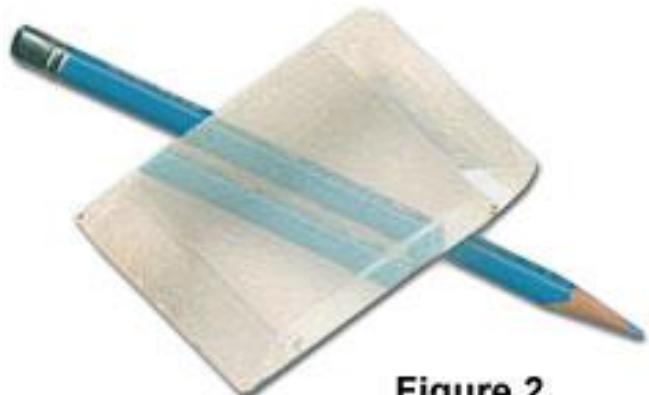
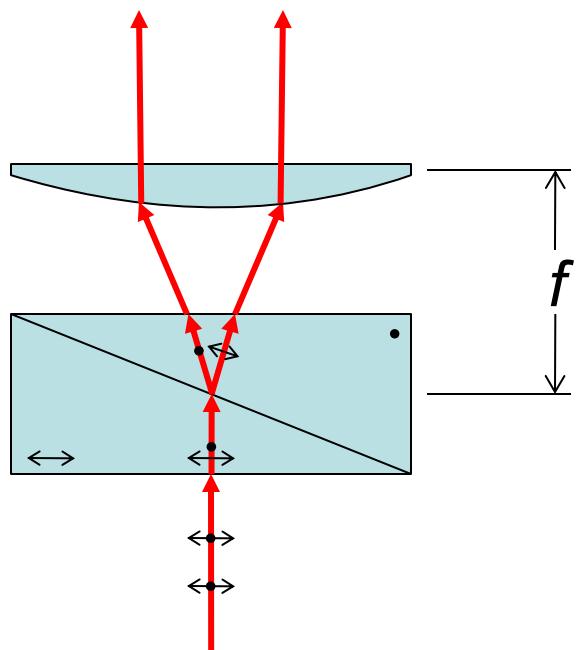


Figure 2

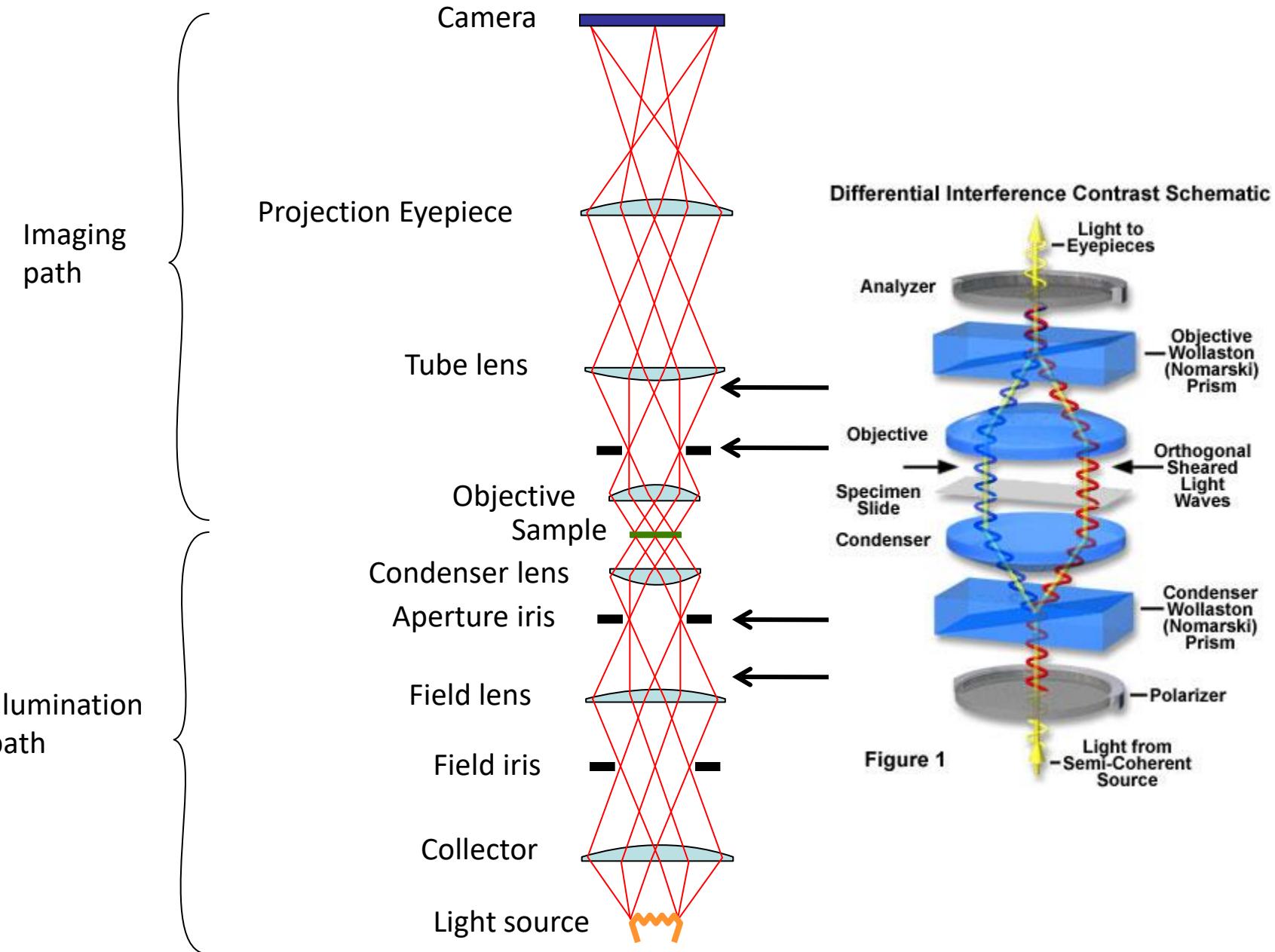
- Birefringent materials have different indices of refraction for light polarized parallel or perpendicular to the optical axis.
- Two beams with orthogonal polarization are produced if illumination is at an angle to optical axis

Wollaston / Nomarski Prisms

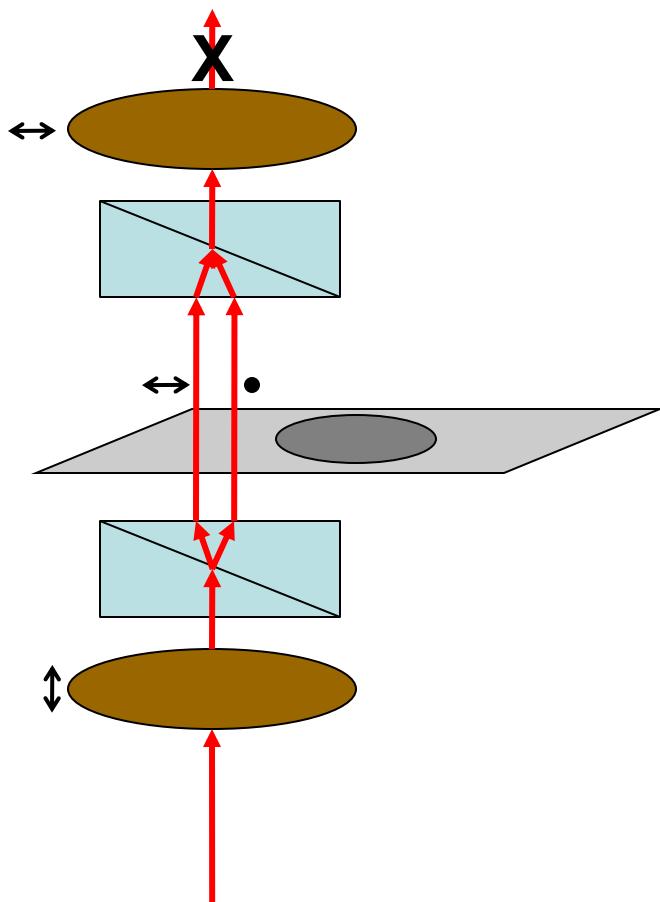


- Two pieces of cemented calcite / quartz
- Produce orthogonally polarized beams propagating at different angles

The differential interference contrast (DIC) microscope

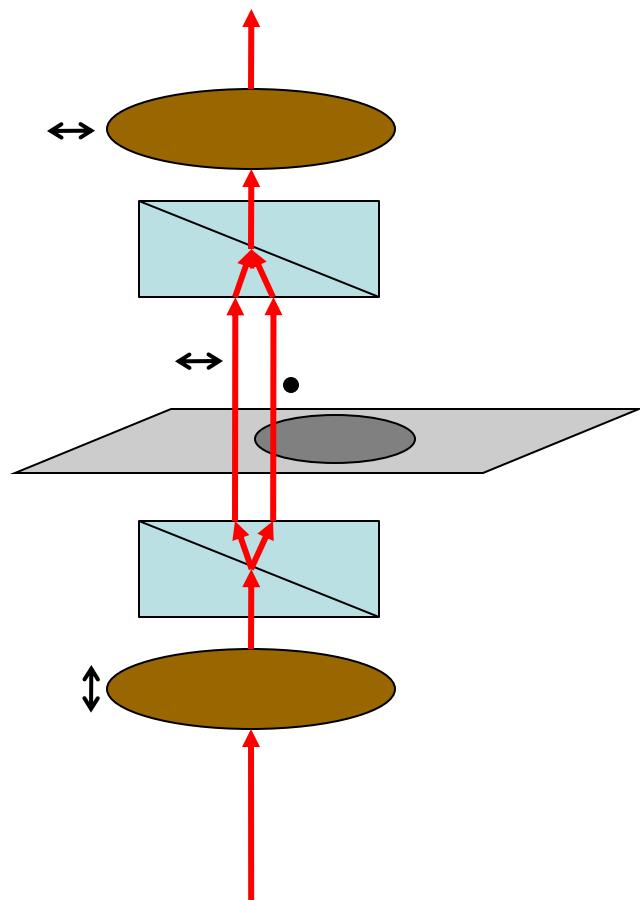


How DIC generates contrast



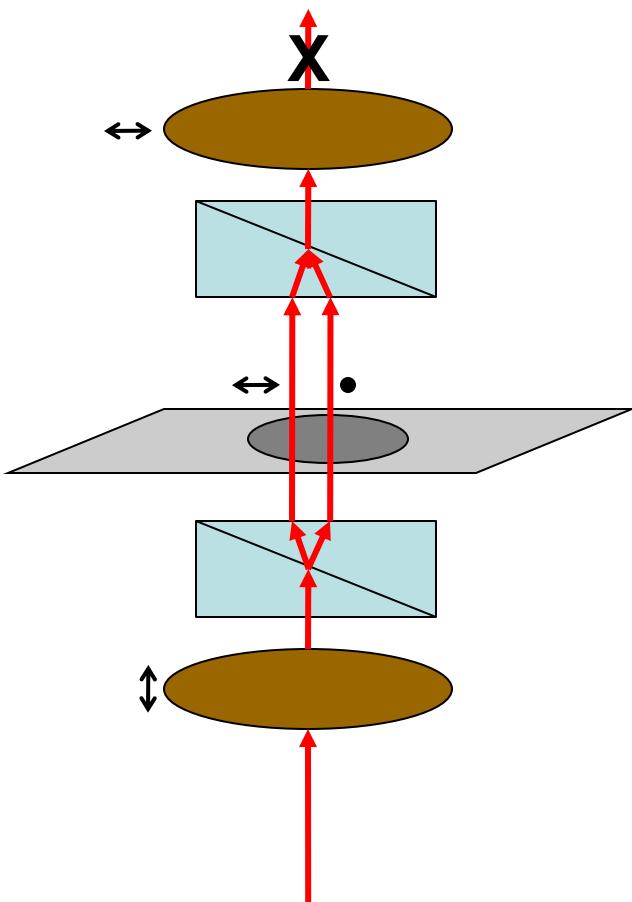
- Both beams see same OPL
- Emerge in phase
- Regenerate initial polarization
- No light makes it through analyzer

How DIC generates contrast



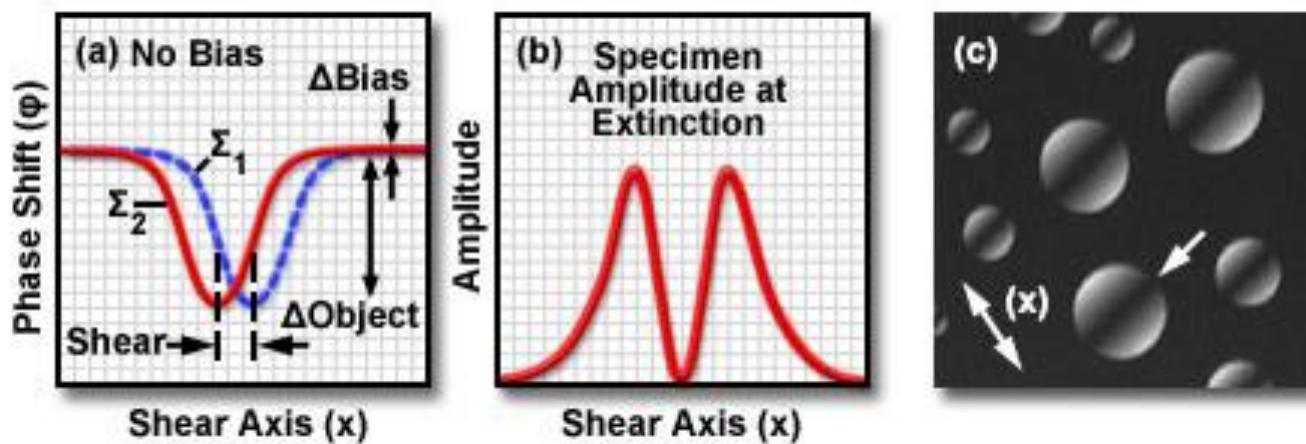
- Beams see different OPL
- Right beam is phase retarded
- Generate elliptical polarization
- Light makes it through analyzer

How DIC generates contrast



- Both beams see same OPL
- Emerge in phase
- Regenerate initial polarization
- No light makes it through analyzer

Role of Bias in DIC



Role of Bias in DIC

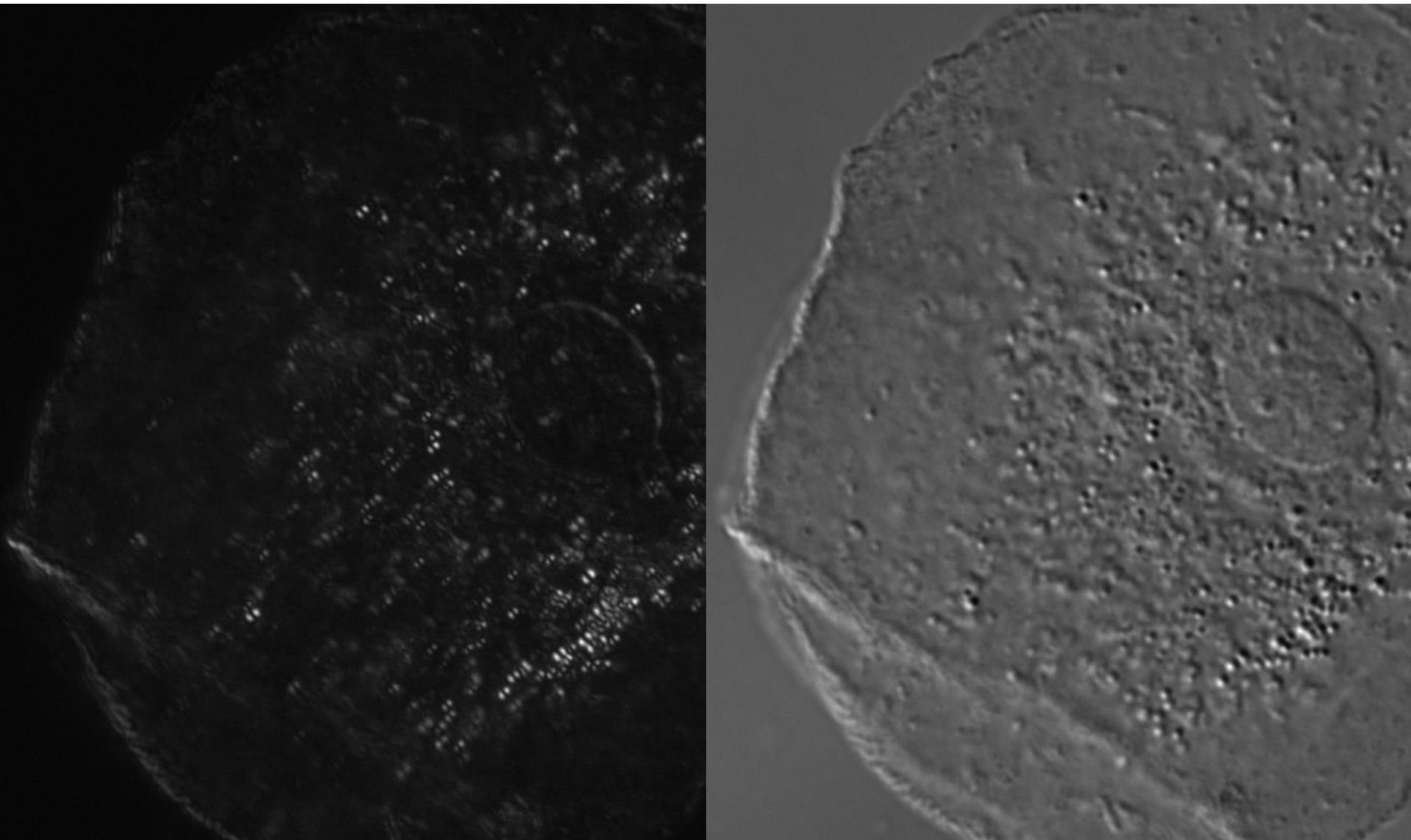
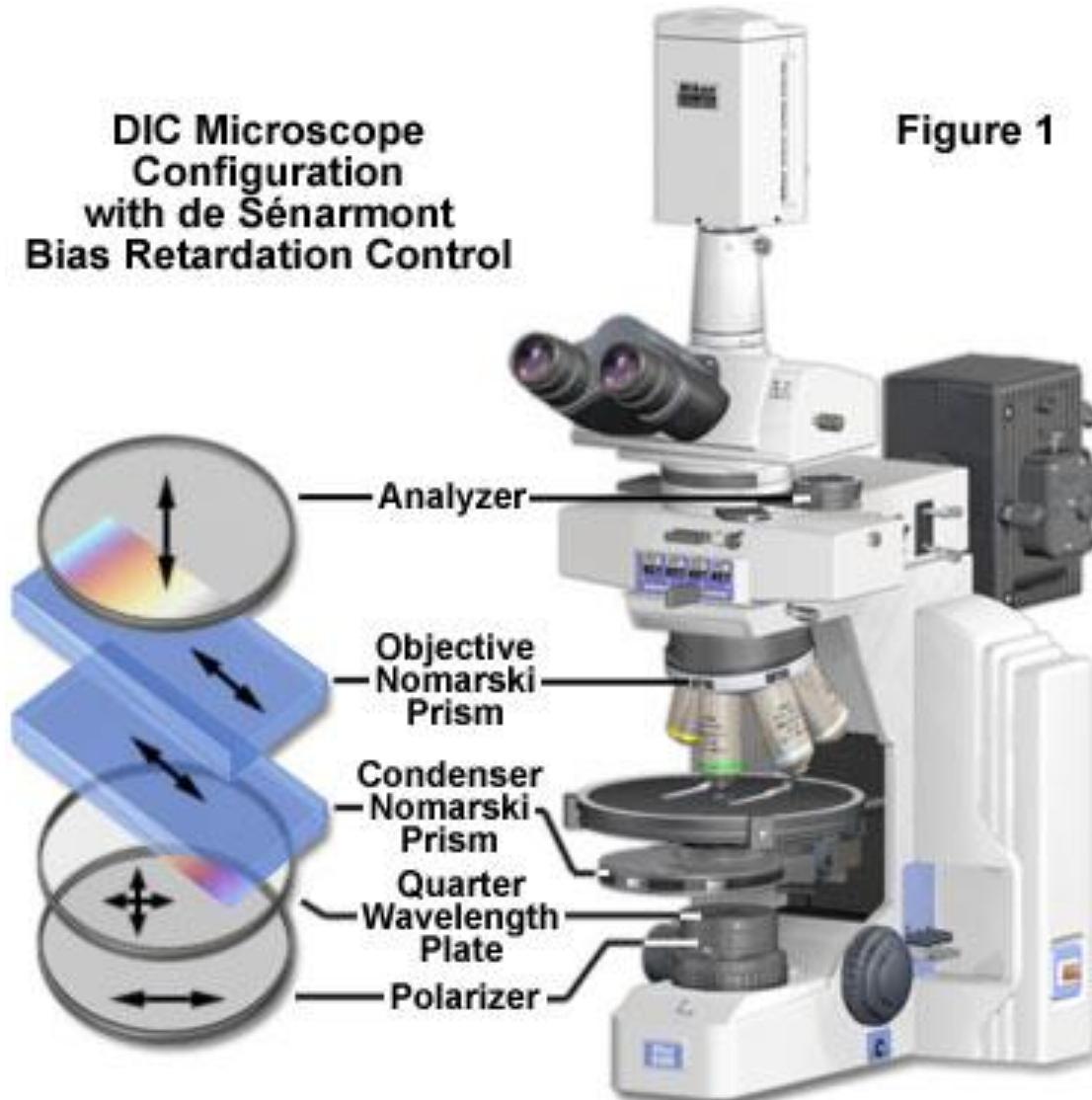


Figure 1

**DIC Microscope
Configuration
with de Séenarmont
Bias Retardation Control**



Bias adjustment in de Sénarmont DIC

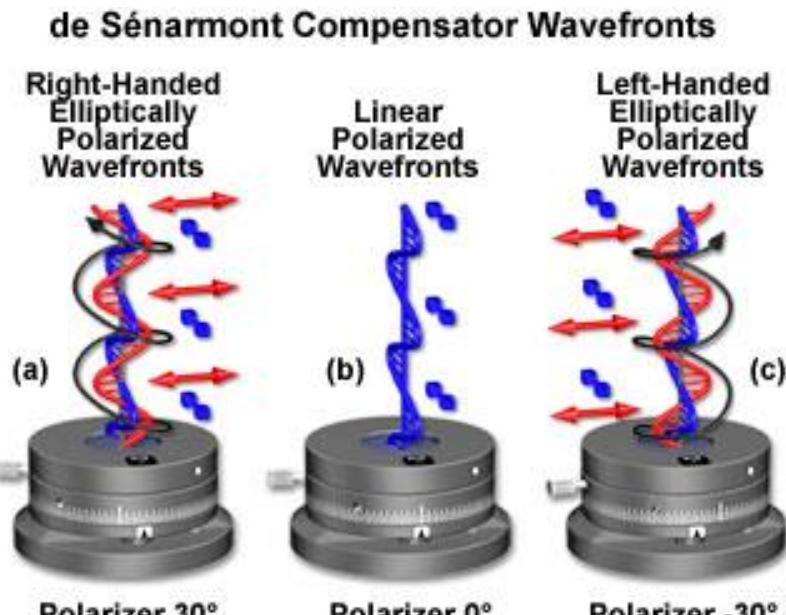


Figure 4

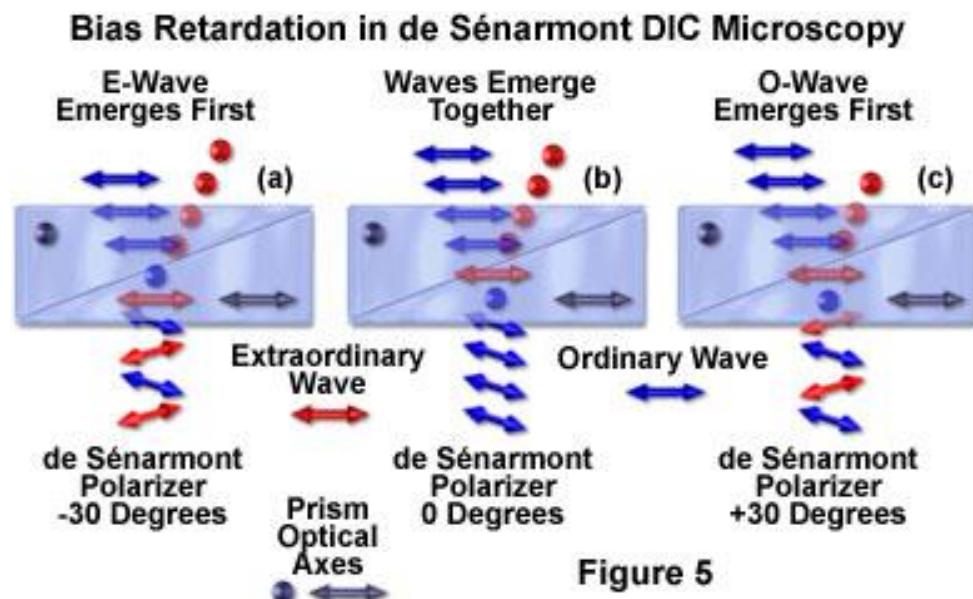
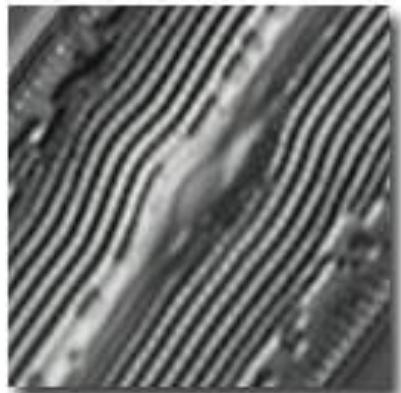
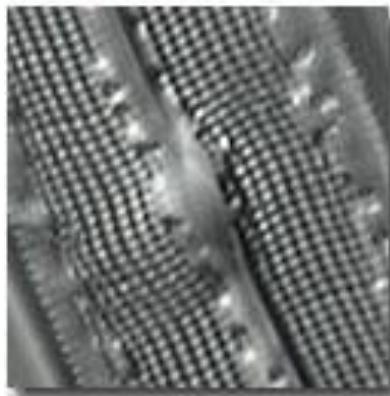


Figure 5

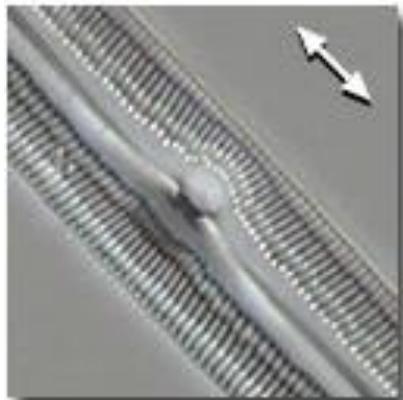
DIC is sensitive to specimen orientation



(a)



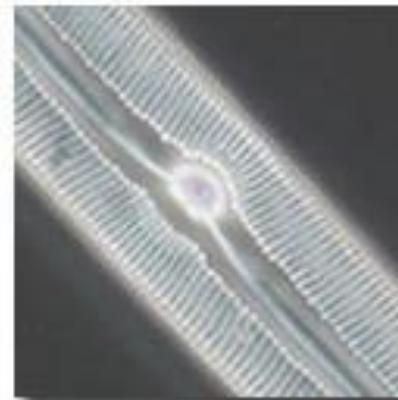
(b)



(a)



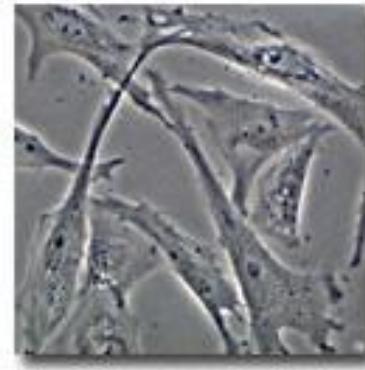
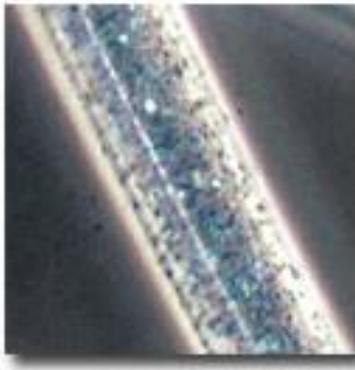
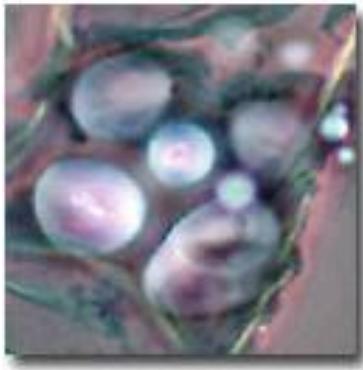
(b)



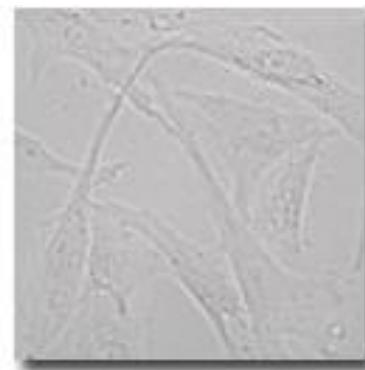
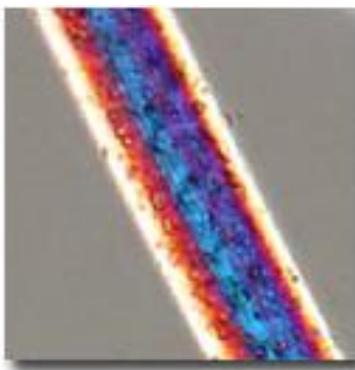
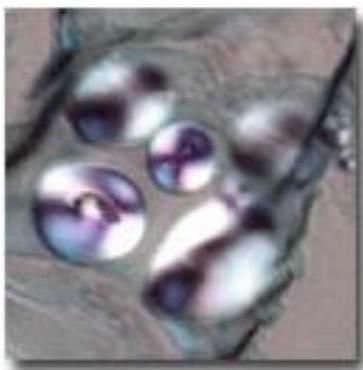
(c)

DIC doesn't work on birefringent samples

Phase



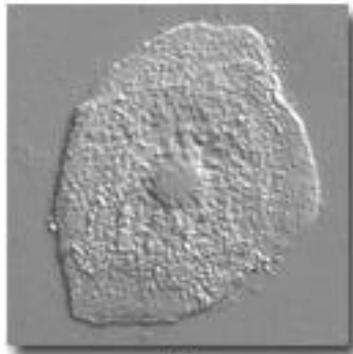
DIC



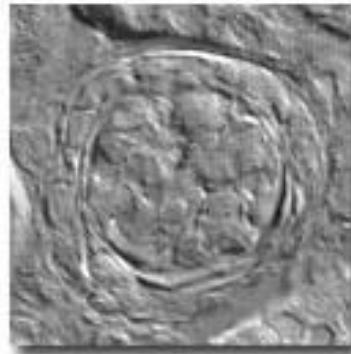
Can't plate cells on or
or cover cells with
plastic.

DIC is higher resolution than phase contrast

DIC

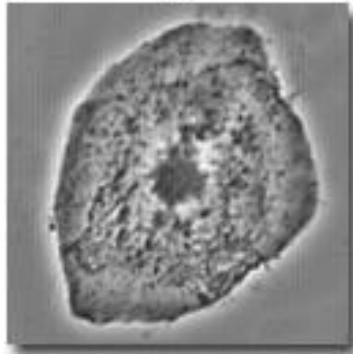


(a)



(c)

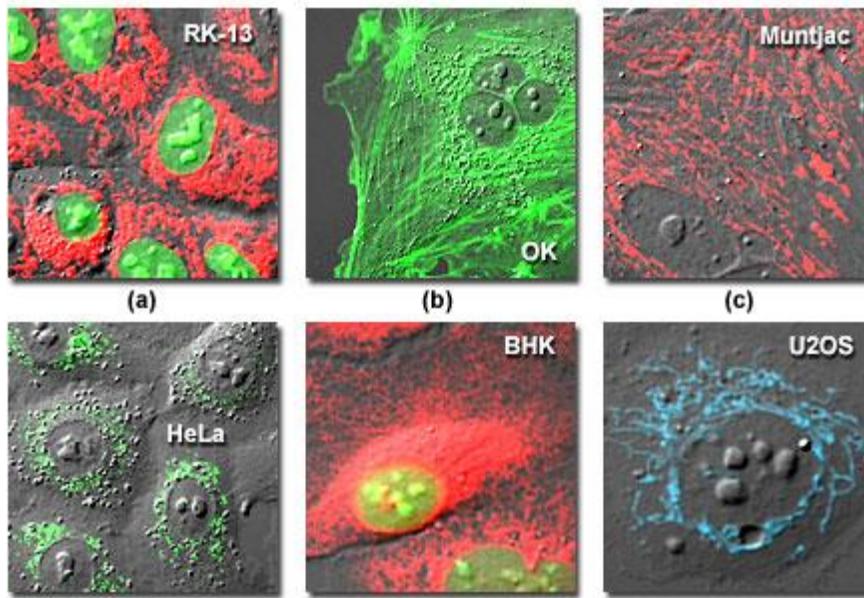
Phase



Microscope Apertures in DIC and Phase Contrast



Combining Phase / DIC with fluorescence



To provide cellular or organismal reference.
Phase and DIC are more general (and less toxic) than fluorescence.

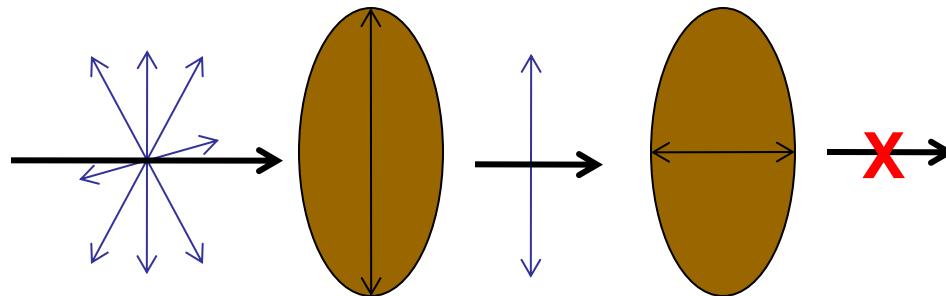
Phase and DIC do degrade fluorescence performance slightly

Birefringence in Biological Materials

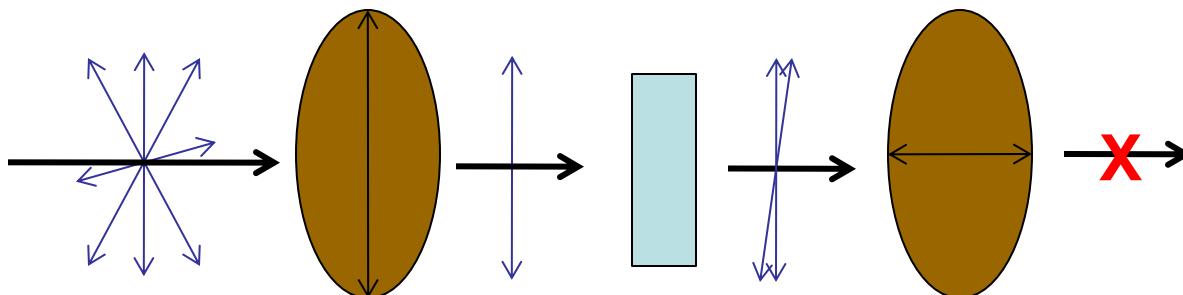
- Anisotropic materials will generally be birefringent
- What's anisotropic in the cell?
 - Polymers: DNA, actin, microtubules
 - Membranes

How to detect a birefringent material?

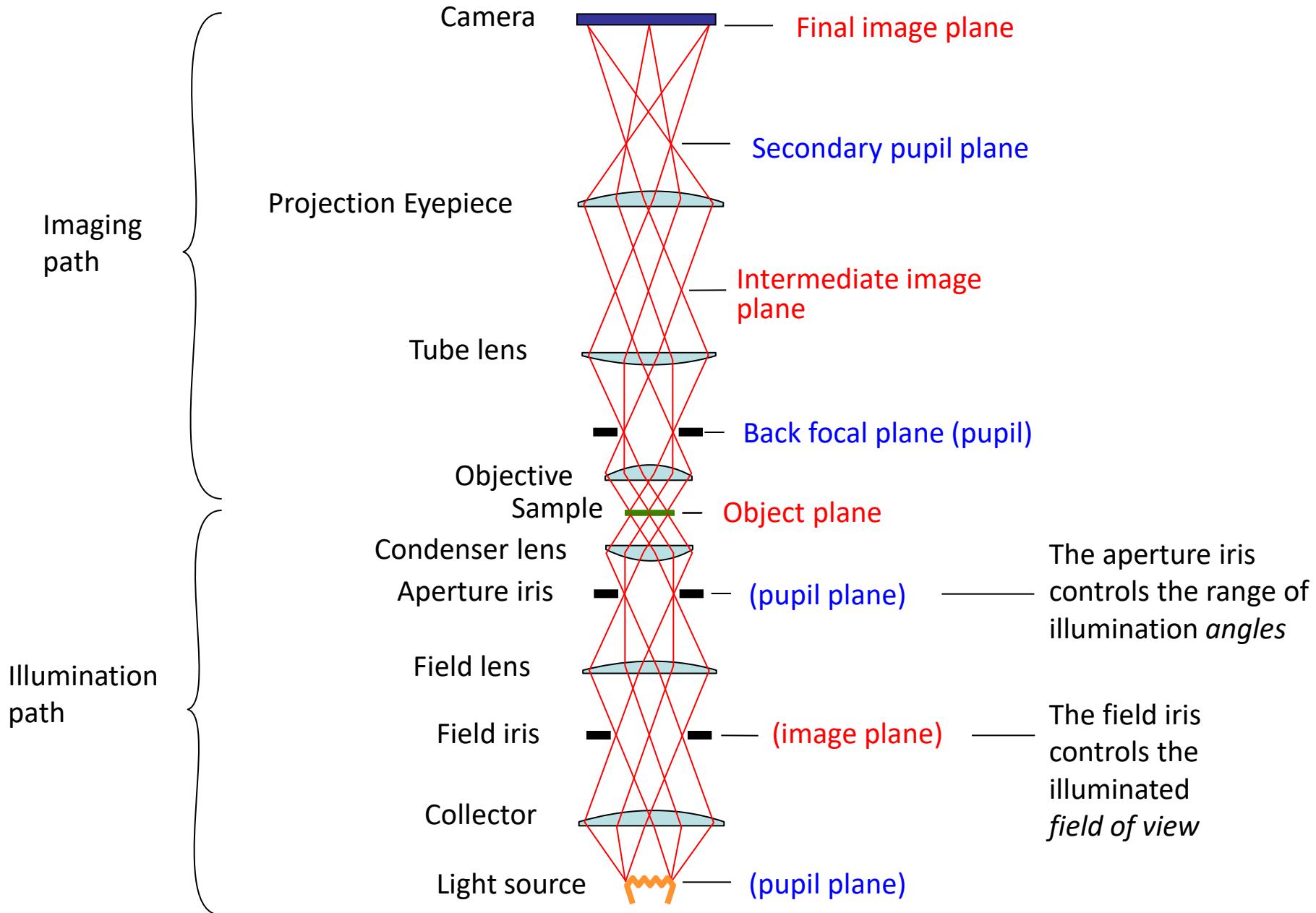
- Start with crossed polarizers



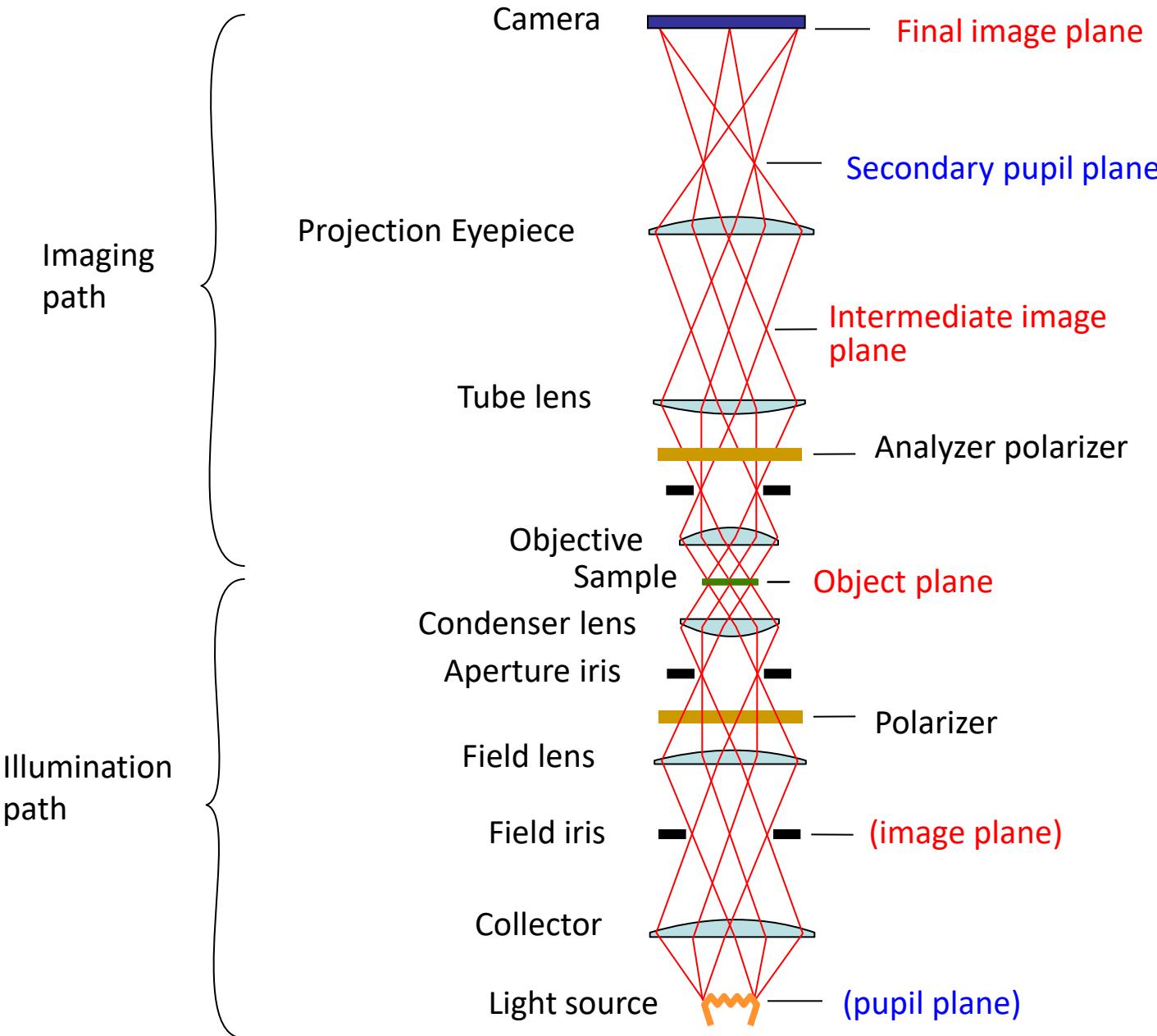
- Insert a birefringent material



Review: The Trans-illumination Microscope

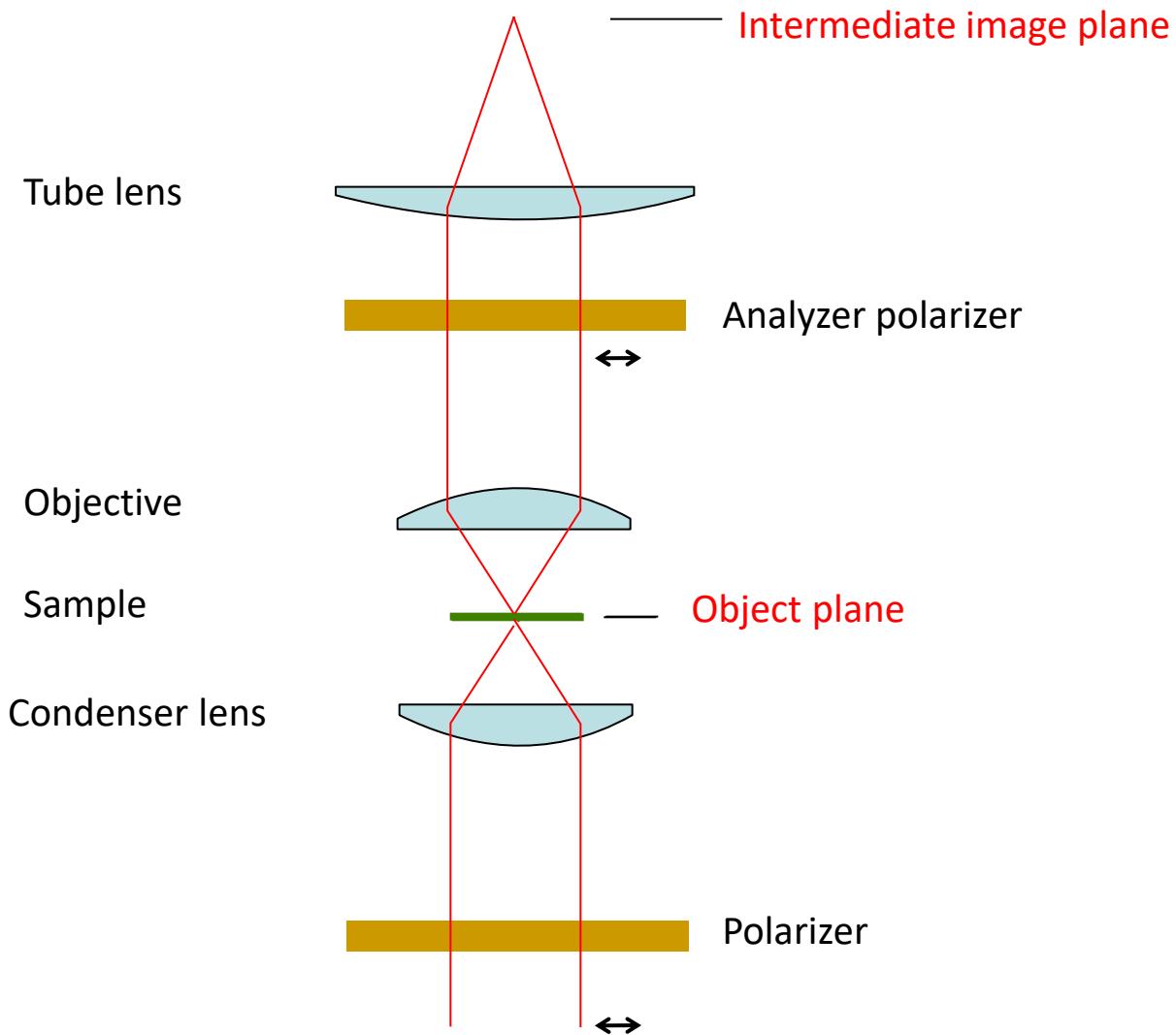


The Polarized Light Microscope



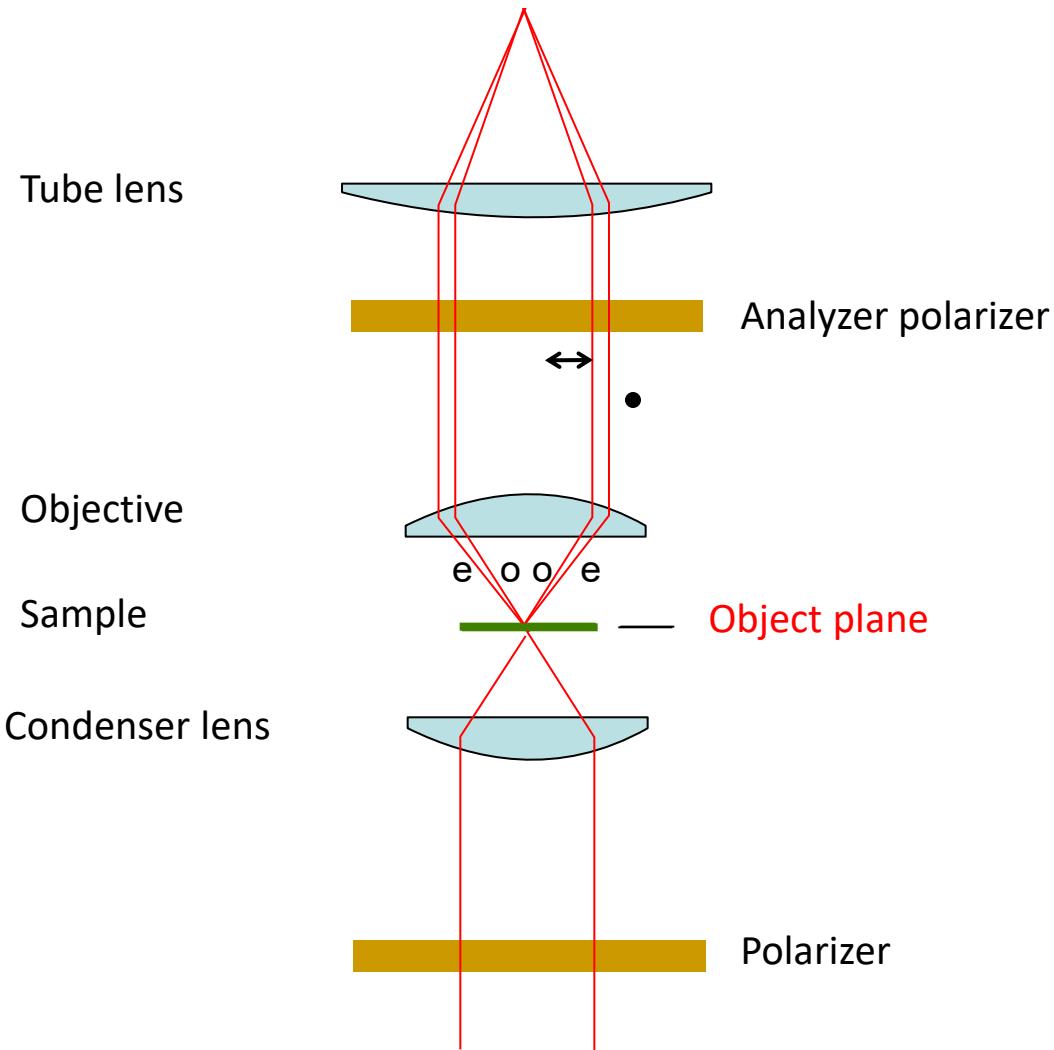
The Polarized Light Microscope

Imaging a normal sample



The Polarized Light Microscope

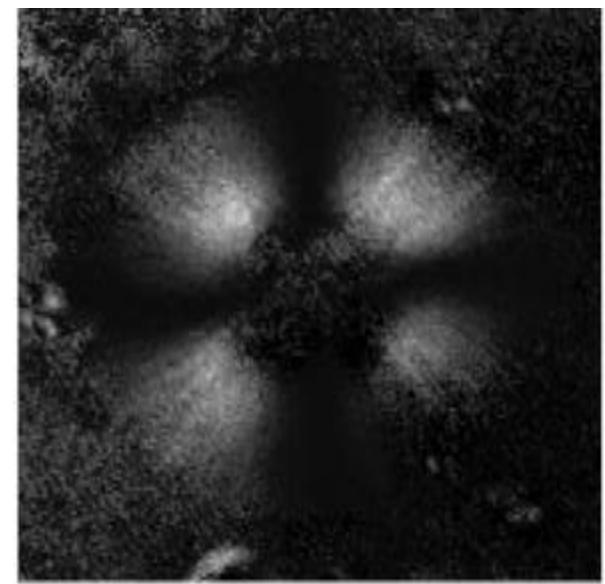
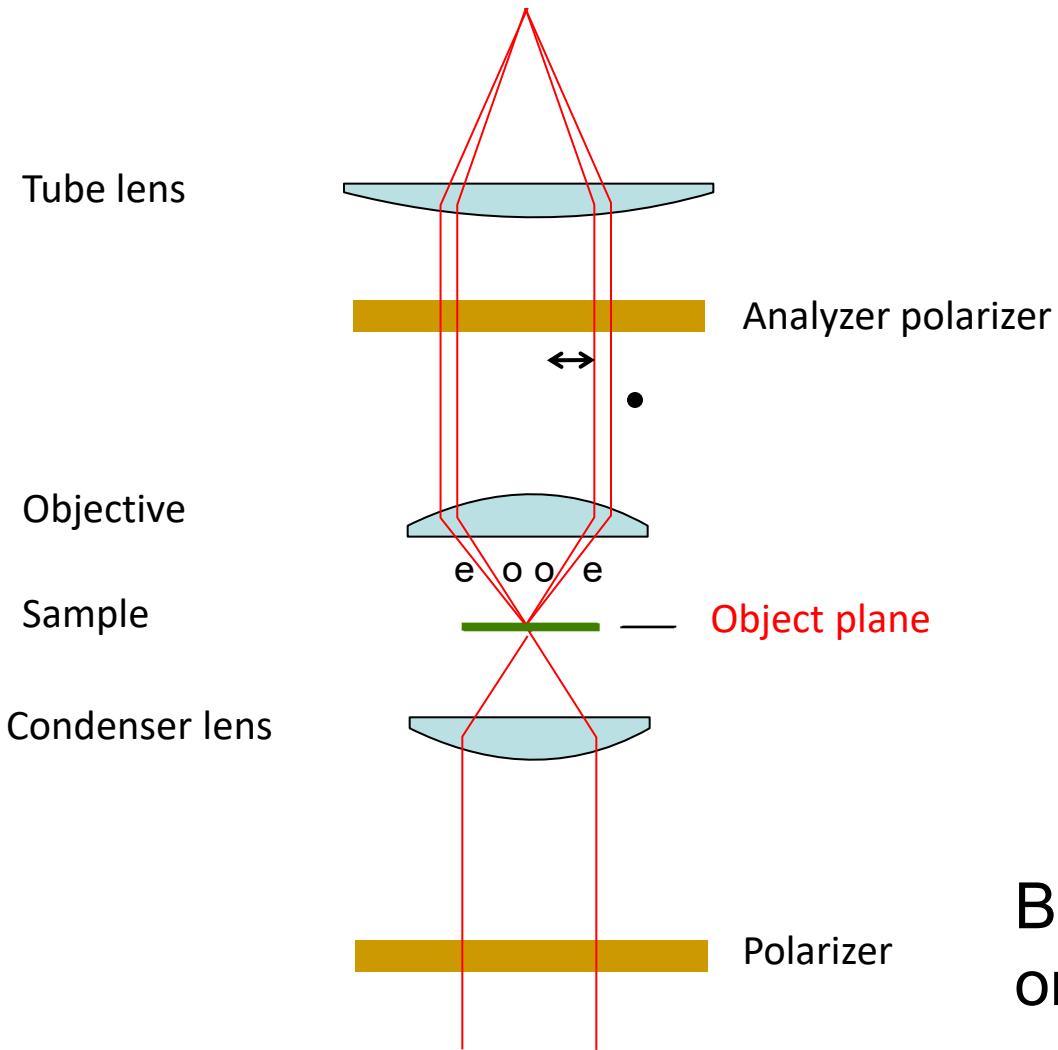
Imaging a birefringent sample



- Birefringent sample splits light into e- and o-rays, which see different refractive indices
- The phase retardation of one ray with respect to the other gives rise to elliptically polarized light, which is transmitted by the polarizer

The Polarized Light Microscope

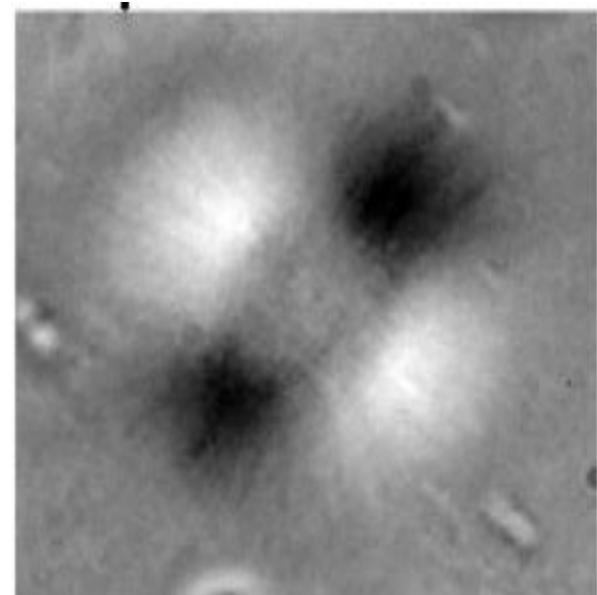
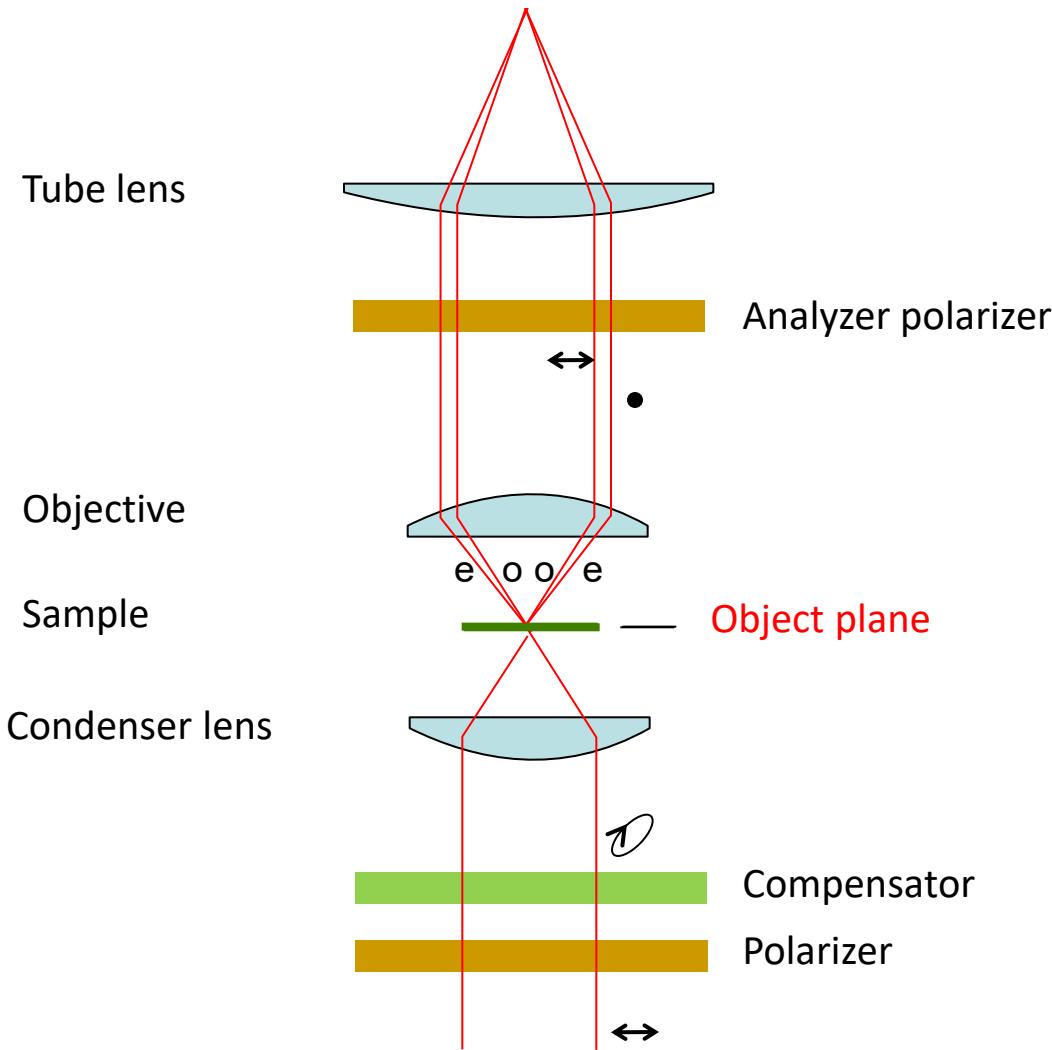
Imaging a birefringent sample



Birefringent sample is bright
on dark background

The Polarized Light Microscope

Add a compensator (wave plate) for better contrast



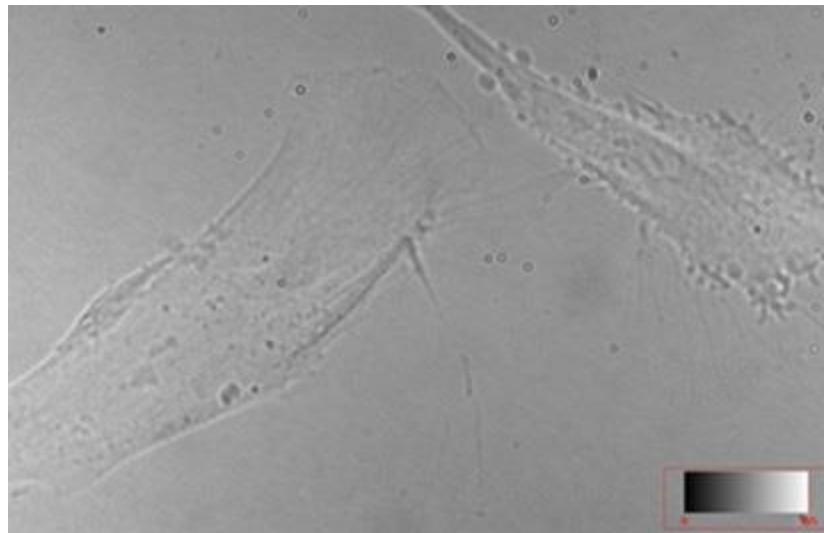
Commercial implementation: LC-Polscope (Abrio)

- Uses a circular polarizer analyzer and variable liquid crystal retarders to measure orientation independent polarization.

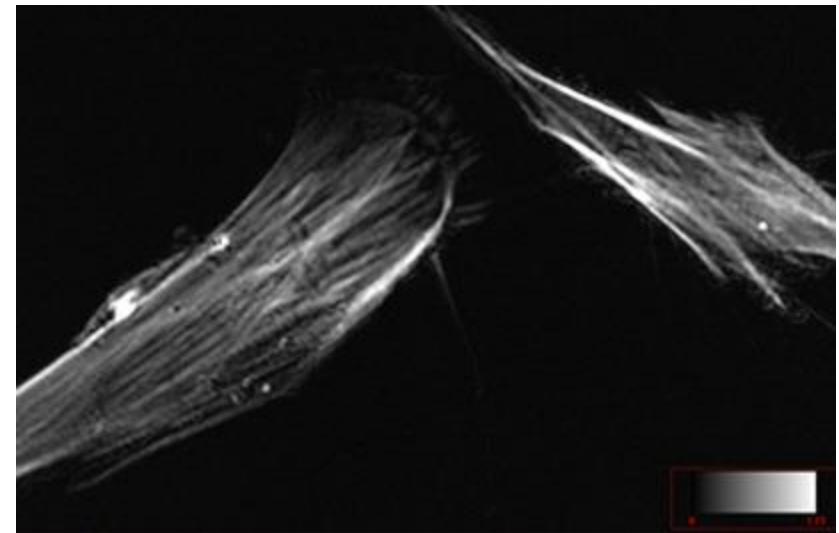
Polarized light microscopy

- Good for
 - Seeing ordered structures in the cell:
 - Spindles
 - Other cytoskeletal structures
 - Membranes
 - Collagen
- No staining required!

Examples – astrocyte (from CRI)



Brightfield



Polarization

Crane Fly Spermatocytes



Rudolf Oldenbourg and James LaFountain

Further reading

www.microscopyu.com

micro.magnet.fsu.edu

Douglas B. Murphy, “Fundamentals of Light Microscopy and Electronic Imaging”

Hecht, “Optics”

Slides available:

<http://nic.ucsf.edu/dokuwiki/doku.php?id=presentations>

Acknowledgements

Orion Weiner / Mats Gustafsson / Rudolf Oldenbourg