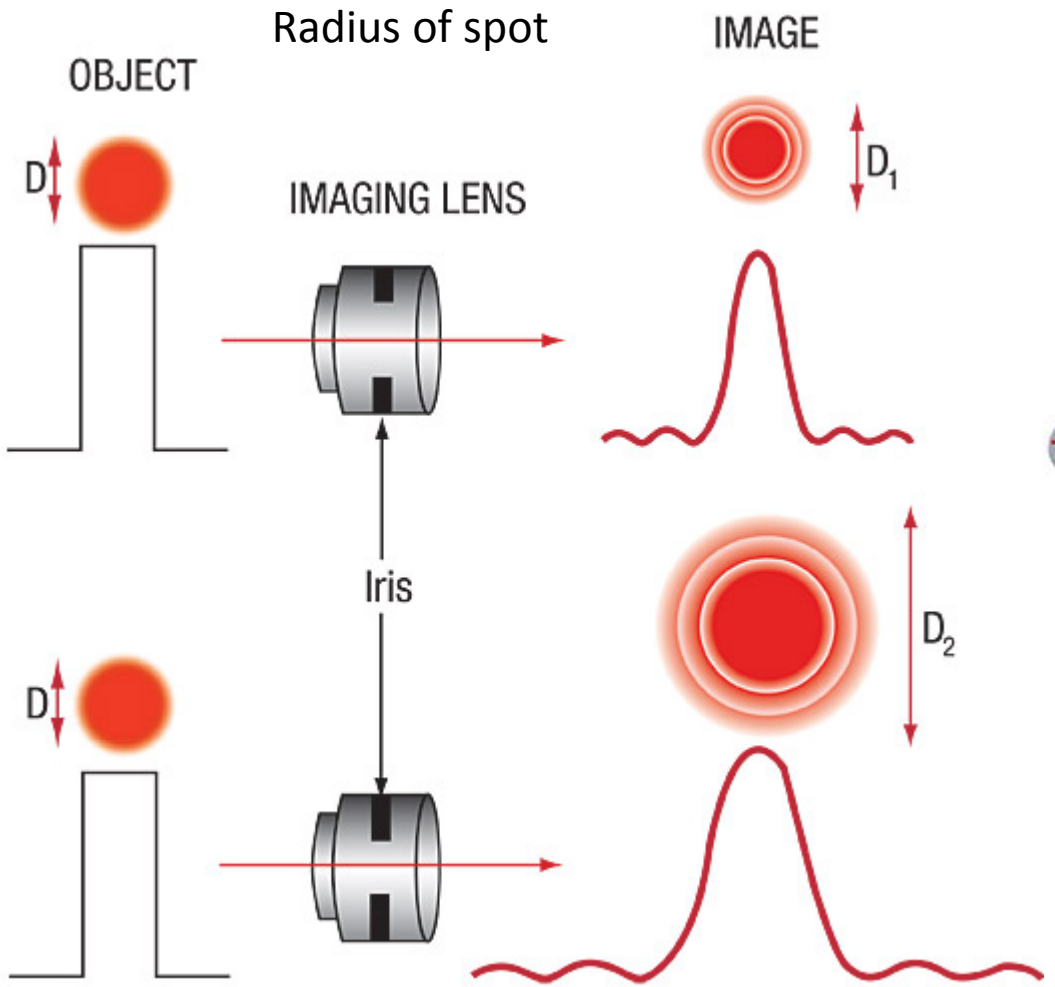
# Polarization, DIC, fluorescence

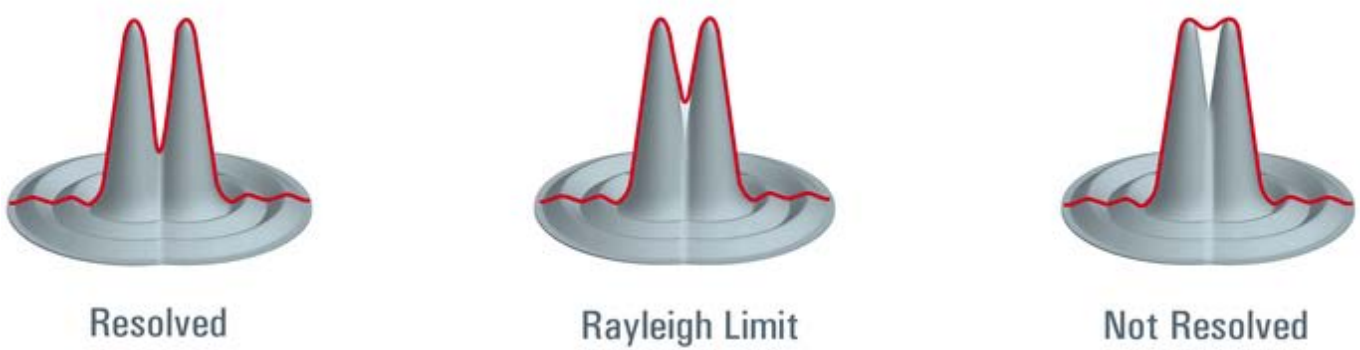
- Last class
  - Morphological operators
  - Matlab morphological image processing
- This class
  - Polarization microscopy
  - DIC
  - Intro to fluorescence

# Diffraction limited spot vs resolution limit

d = λ/2*NA
Radius of spot

r = 1.22 λ /2*NA
Rayleigh criterion



# Magnification

Imagine 10x objective
Image on the camera, would be 10x larger

Cell that is 20 um in diameter, would appear 200 um diameter on camera

Conversely, a 10 um pixel on the camera would represent 1 um on the sample

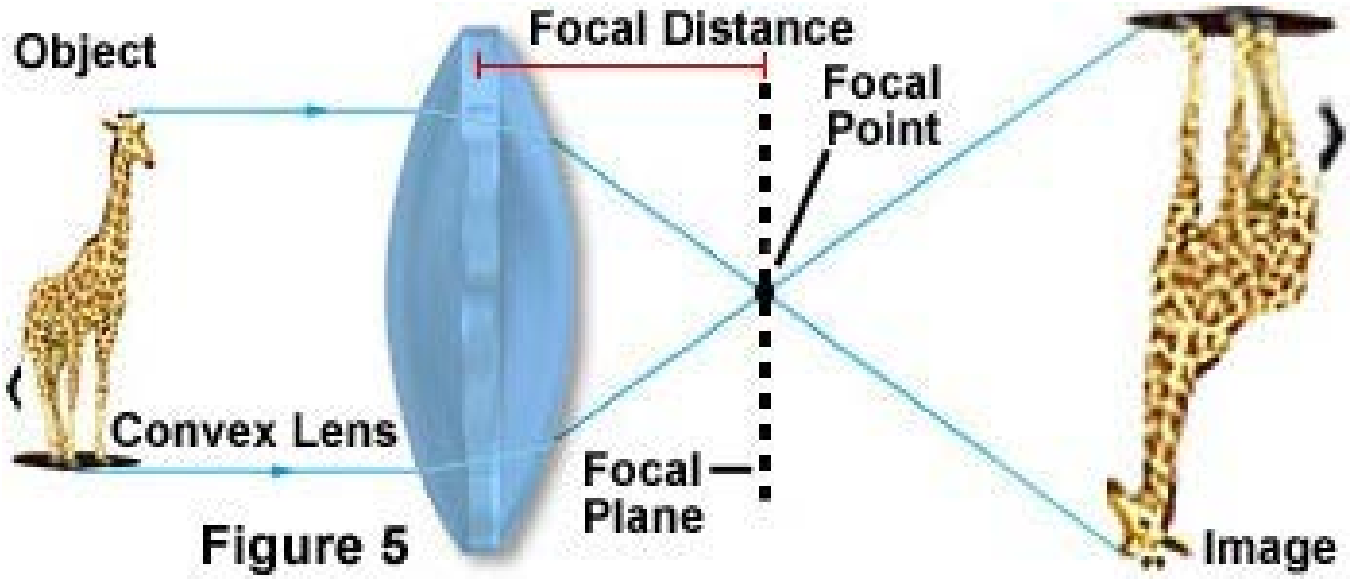


Figure 5

# Polarization microscopy

- Another way to add contrast to samples

# Properties of light

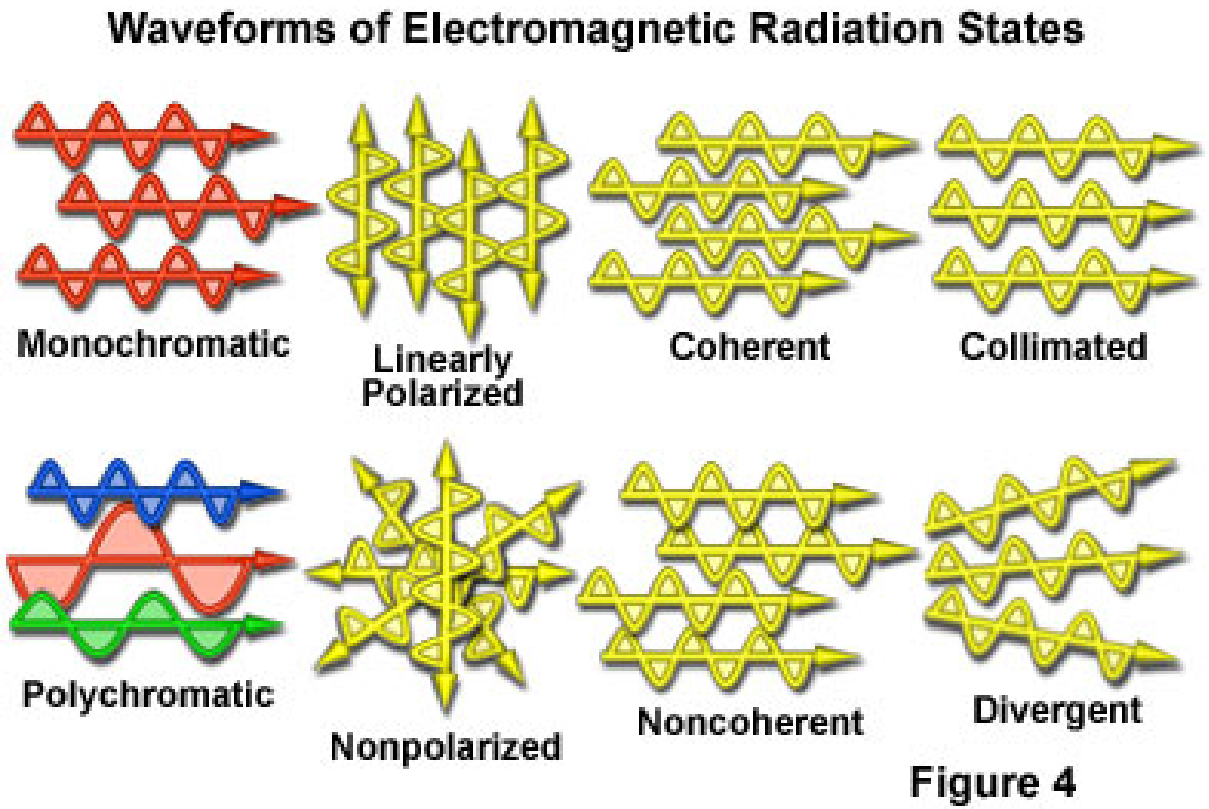
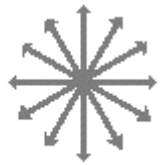


Figure 4

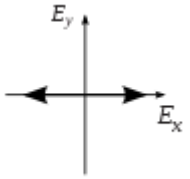
$$y(x,t) = A\cos(kx - \omega t + \varphi)$$

$$y(x,t) = \vec{A}\cos(kx - \omega t + \varphi)$$

# Properties of polarization

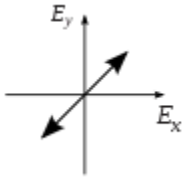


Unpolarized



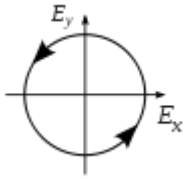

Linear

x



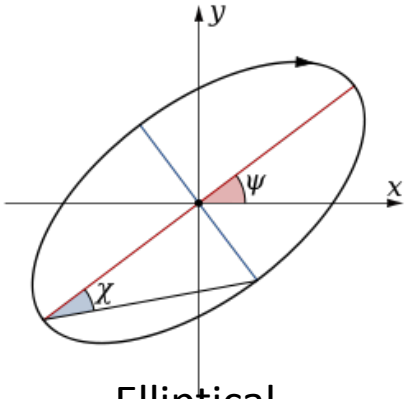

Linear

x + y




Circular

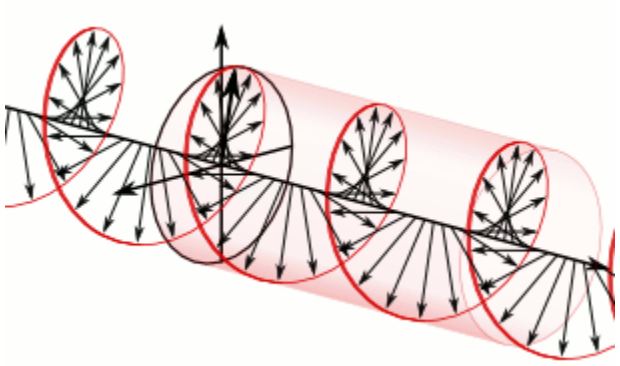
x + (y+ϕ)




Elliptical

ax + b(y+ϕ)

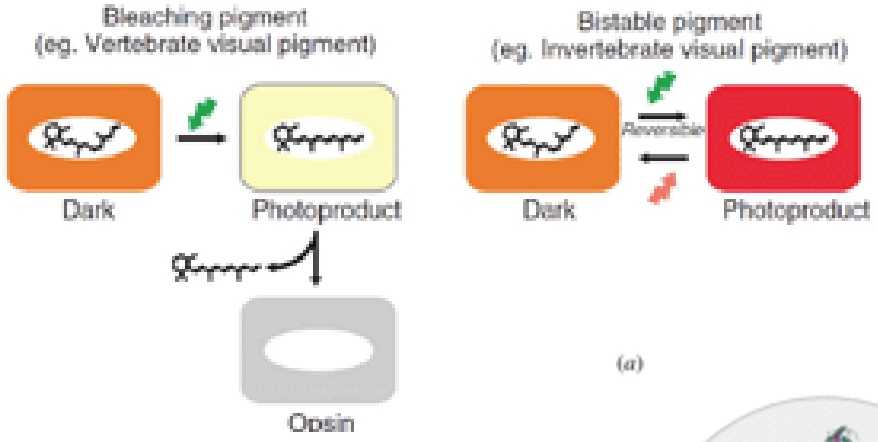
$x^2 + y^2 = 1$

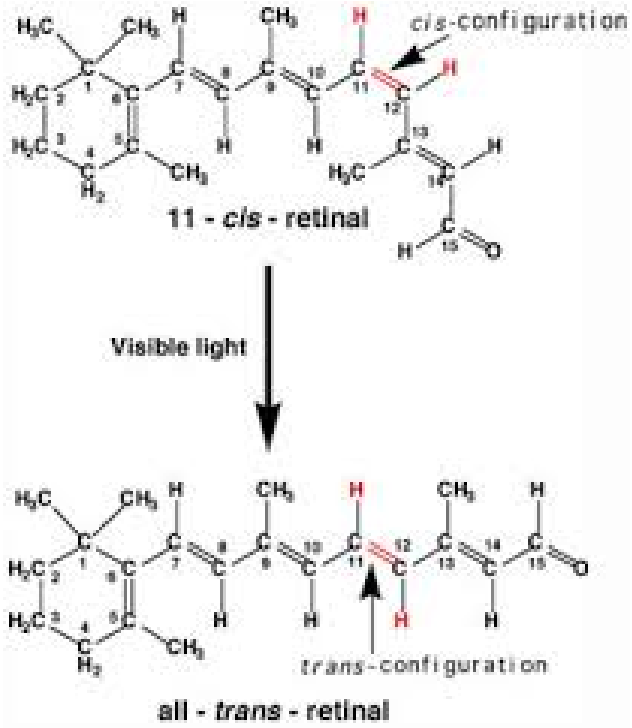


Eyes and cameras can not detect polarization.
Only able to detect intensity.

# Vertebrate vs cephalopod eyes

- Invertebrate eyes are different than our own
- Some invertebrates can detect polarization of light

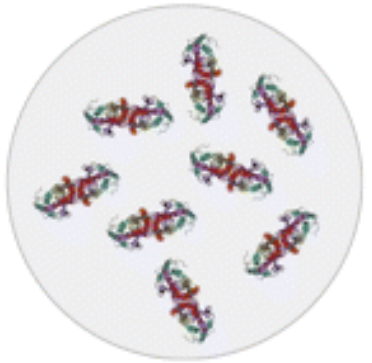



First step is isomerization from 11 cis to all trans

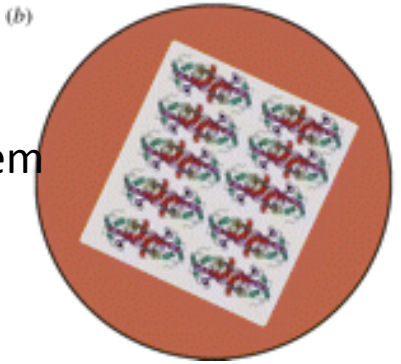
Dipole

Us

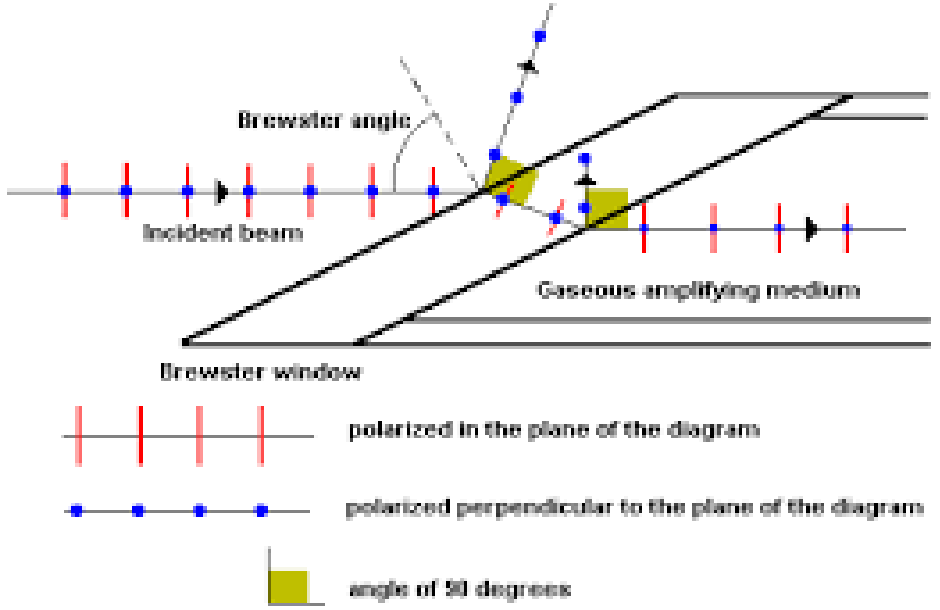


Cephalopods can sense polarization

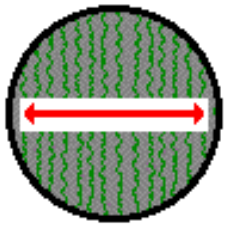
Them



# Manipulating polarization

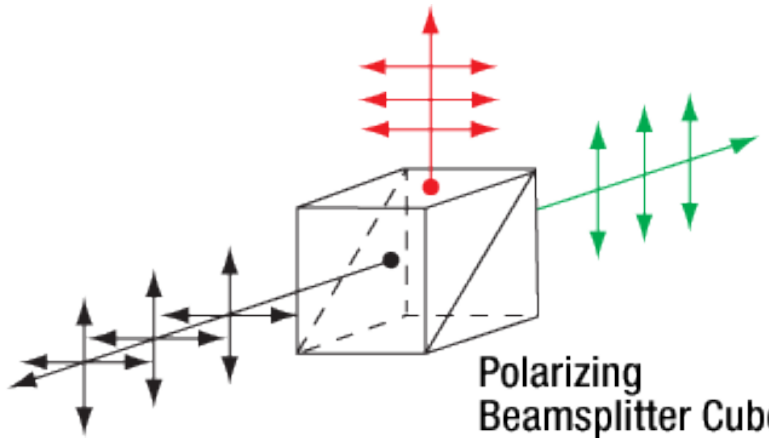



**Relationship Between Long-Chain Molecule Orientation and the Orientation of the Polarization Axis**



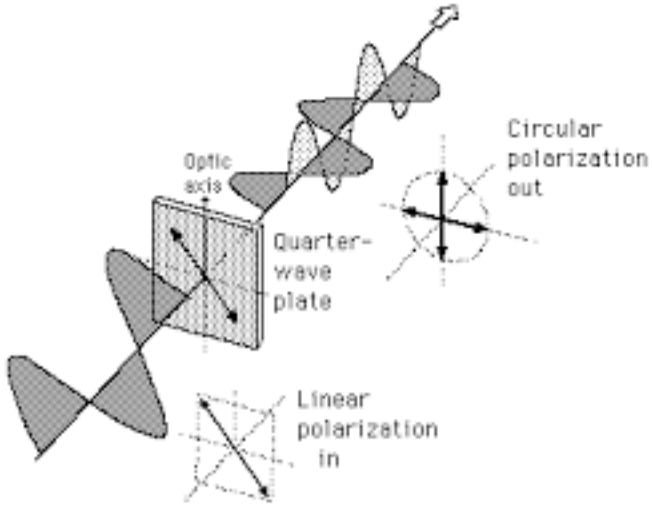
When molecules in the filter are aligned vertically, the polarization axis is horizontal.

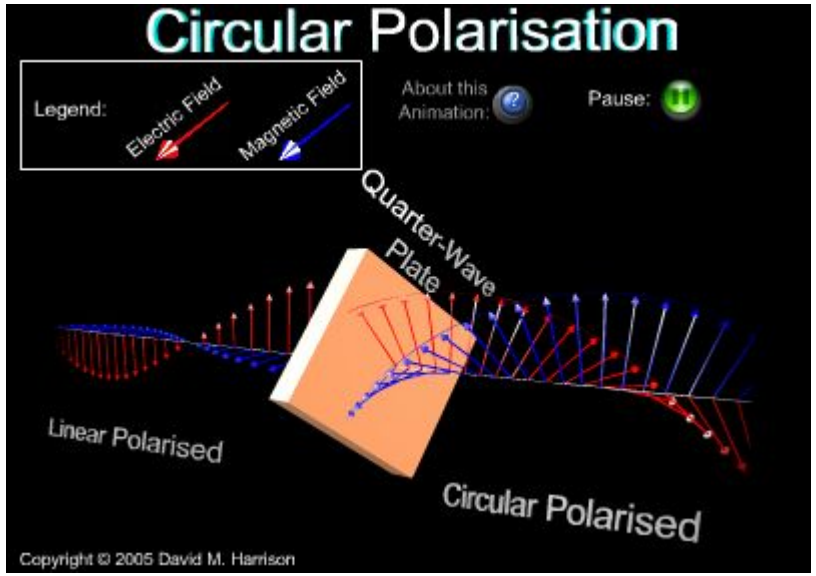
When molecules in the filter are aligned horizontally, the polarization axis is vertical.

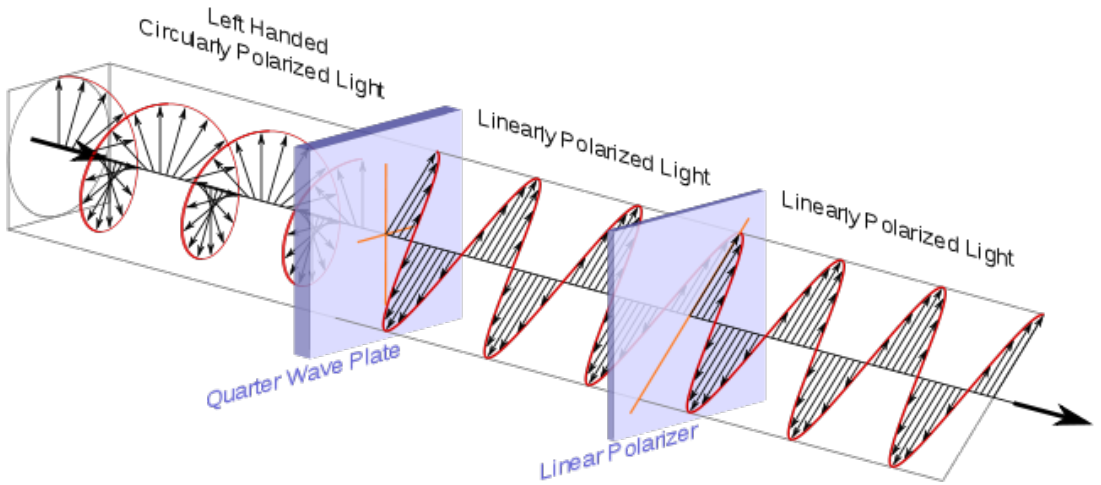


# Manipulating polarization

- Half wave plate, converts to linear polarization
- Quarter wave plate, converts to circular polarization

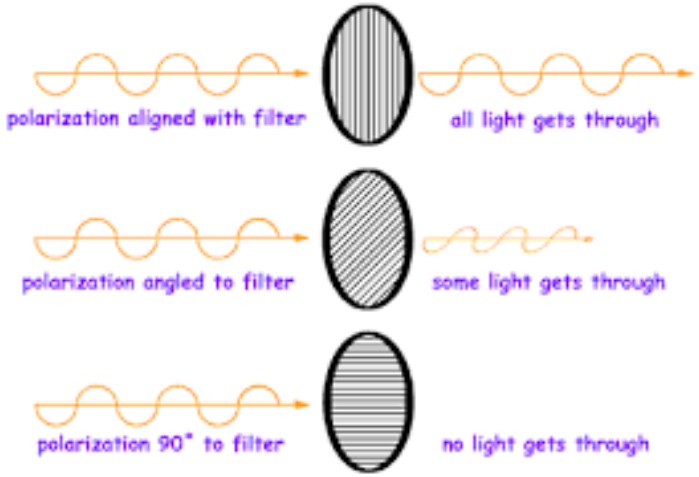


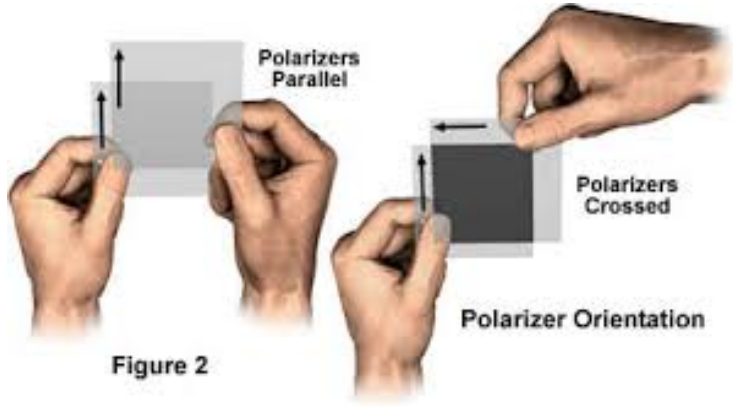
# Linear polarizers

Polaroid filters

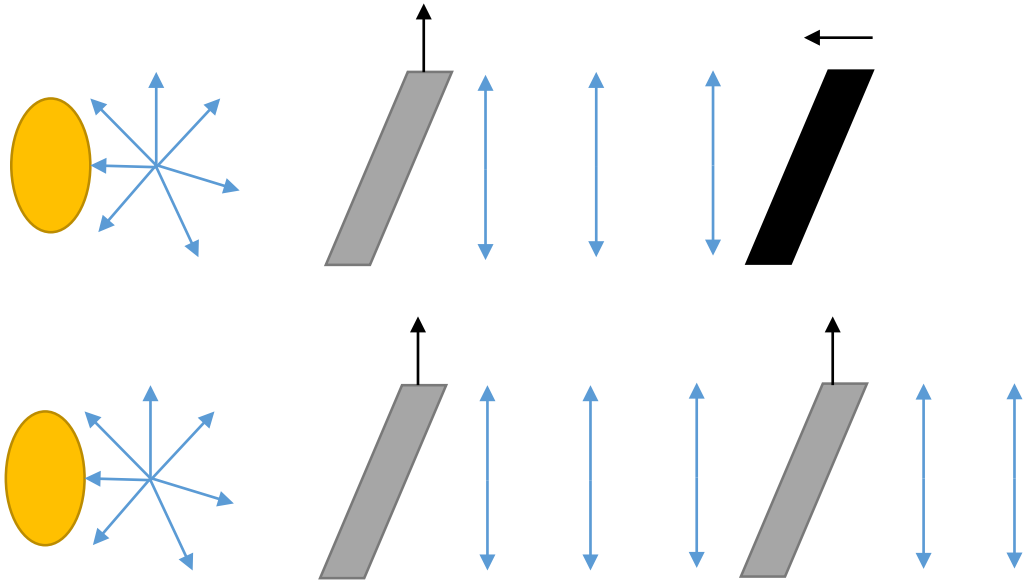



$I = I_0 \cos^2(\theta)$

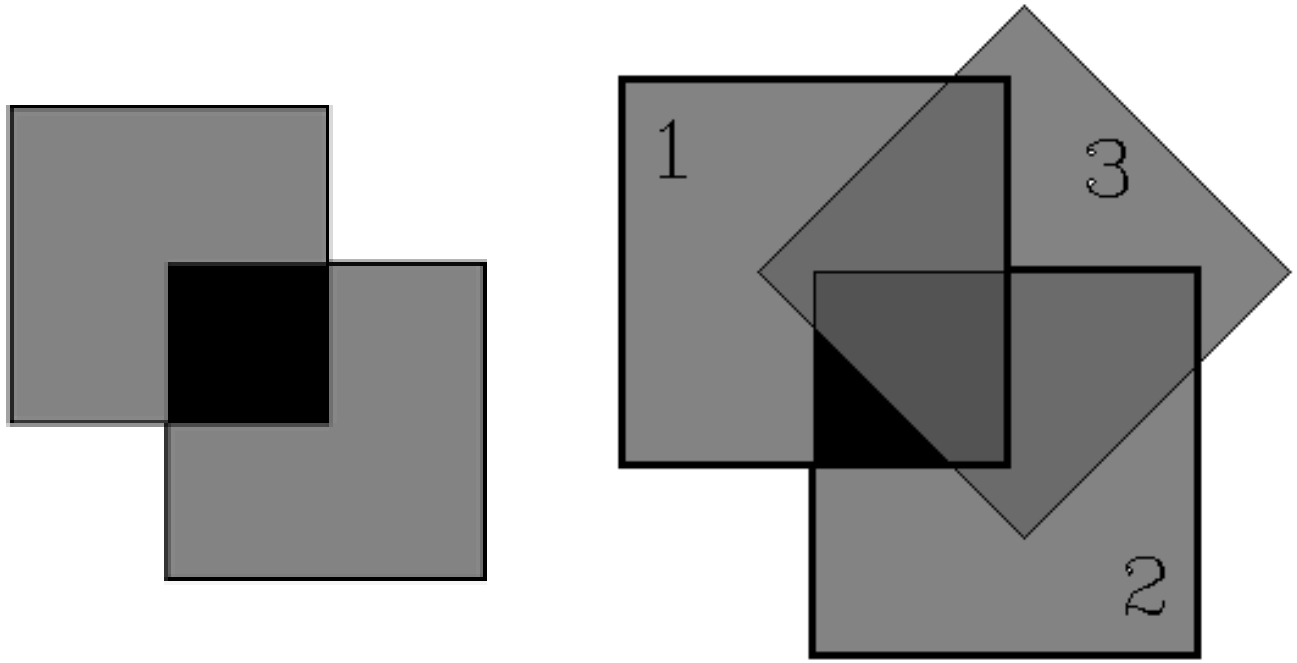
Malus' Law




Light that comes through is polarized parallel to filter



# Cross polarizer puzzle




Forms the basis for polarization microscopy

Have to apply Malus' law twice

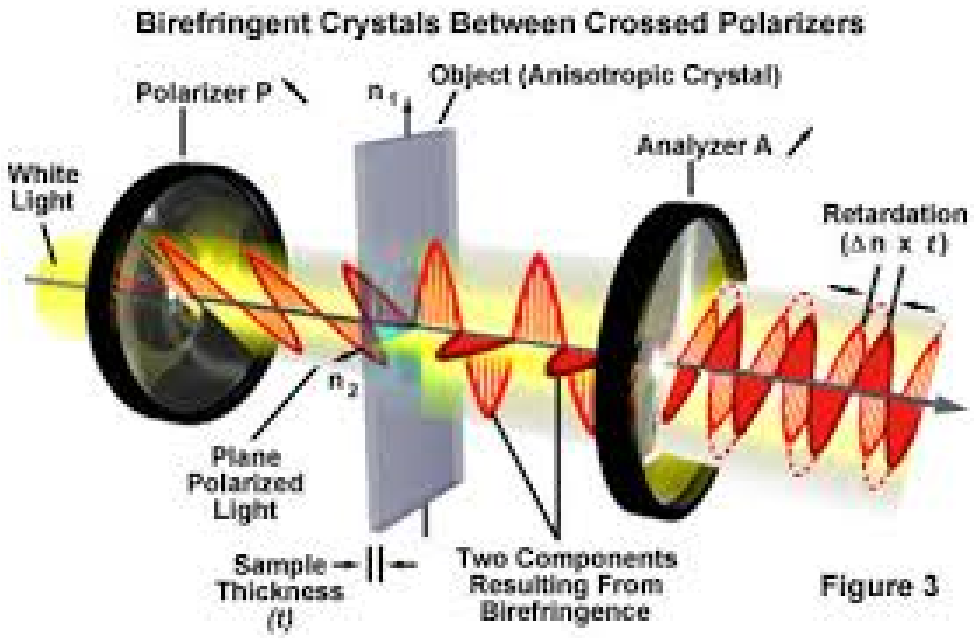
$I_2 = I_1\cos^2(\theta)$

Then

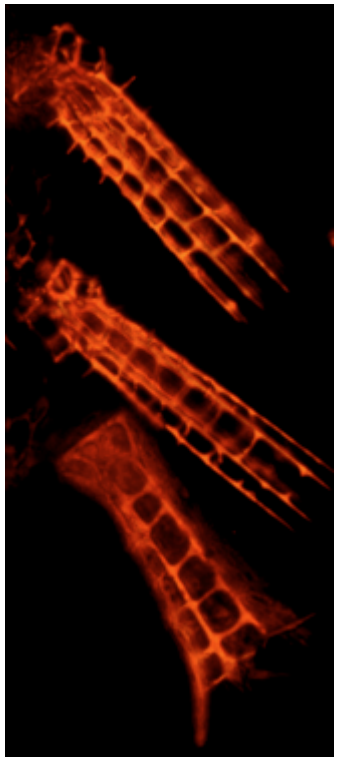
$I_3 = I_2\cos^2(\theta)$

# Polarization microscopy

Biological samples:
-Mitotic spindles
-Actin filament bundles
-Condensed DNA
-Helical strands of cellulose
-Some lipid bilayers



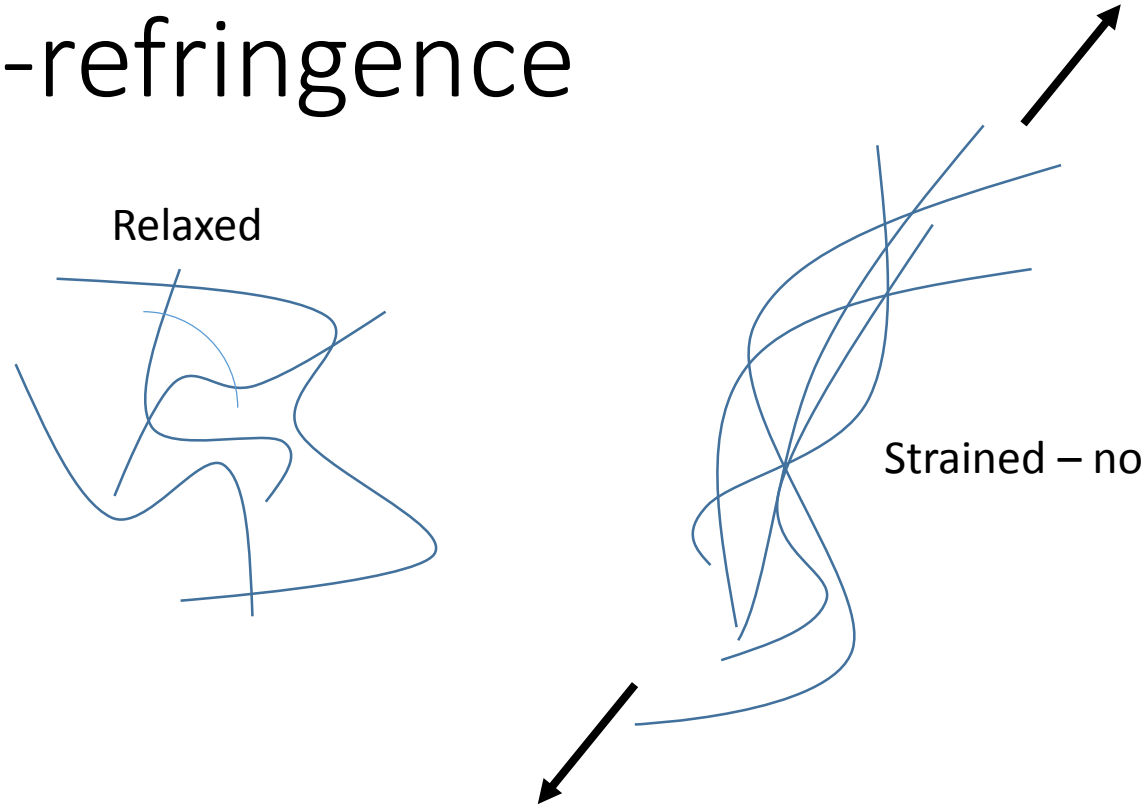


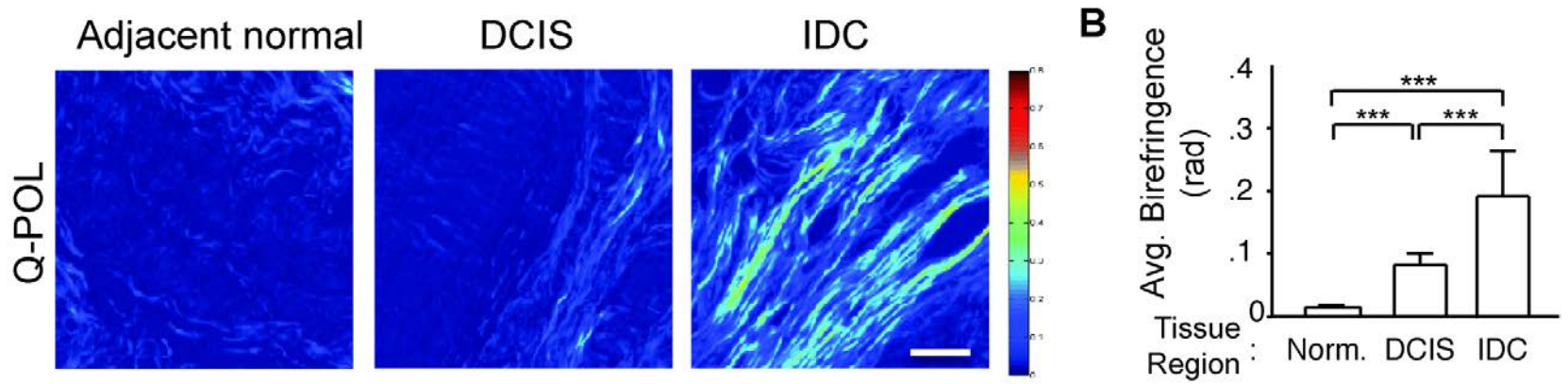


Birefringent – refraction is different for different polarizations

# Strain induced bi-refringence

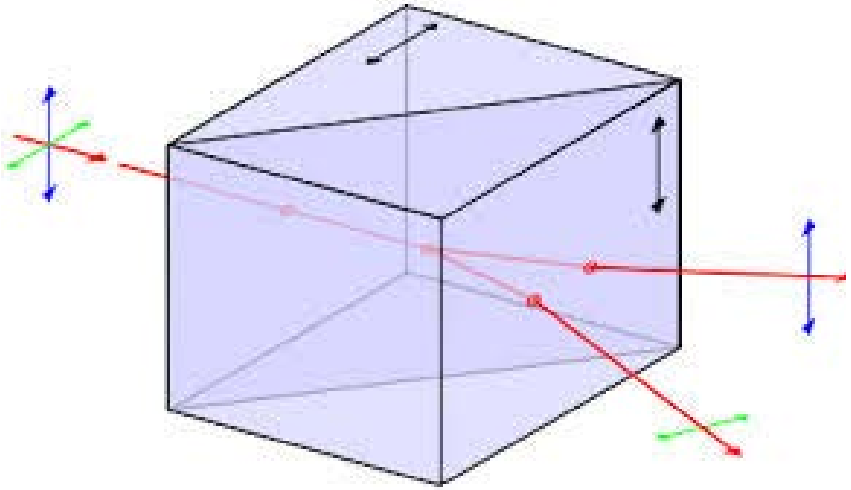
- Birefringence: refractive index depends on polarization of light
- Useful for detecting strain in extra cellular matrix (ECM)
- Label free technique



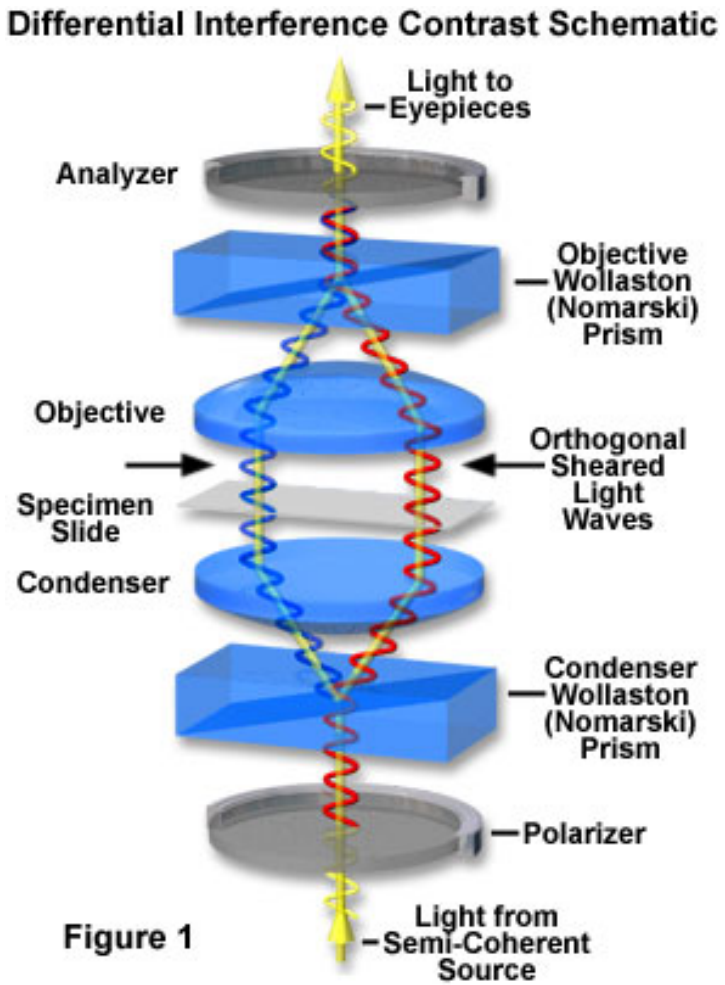
# Differential Interference Contrast

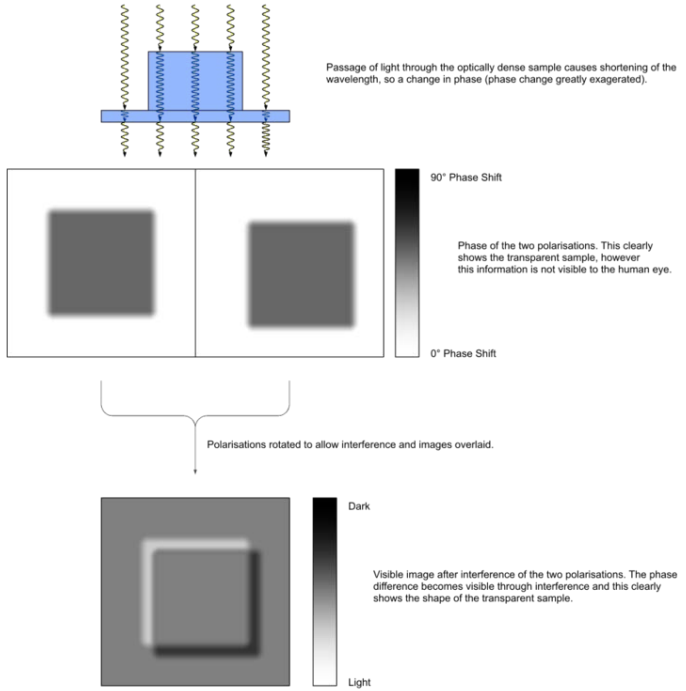
- Interference technique similar to phase contrast
- The magic starts with a Wollaston prism
- Wollaston prism is birefringent, and rays will exit at different points in space

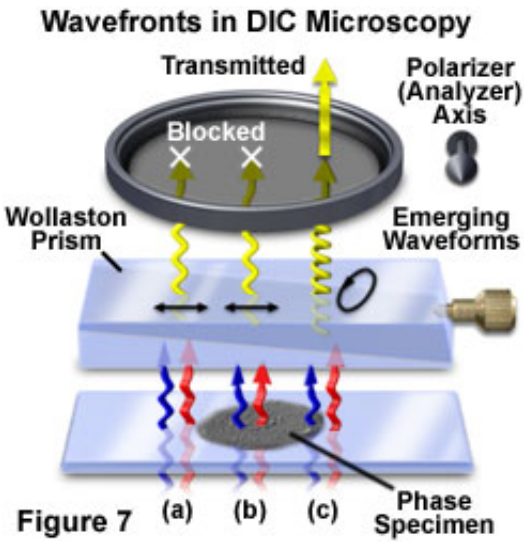


Wollaston splits polarization

# DIC microscope




Beams are split by small amount (nanometers)

If there is a difference in optical path between them, they will negatively interfere at camera

Only get contrast at the edges

Form what looks like a 3d image on the camera

# DIC vs phase contrast

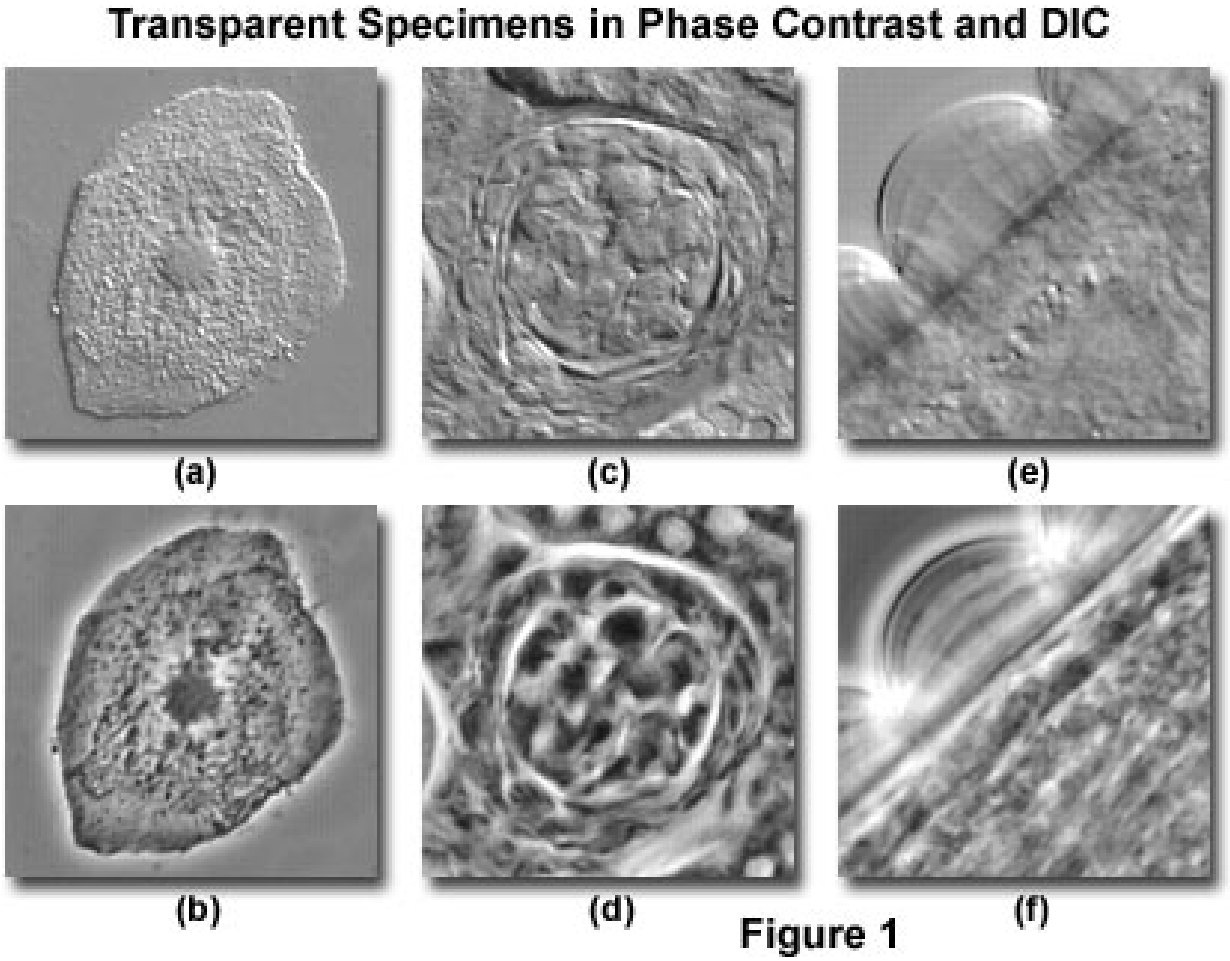


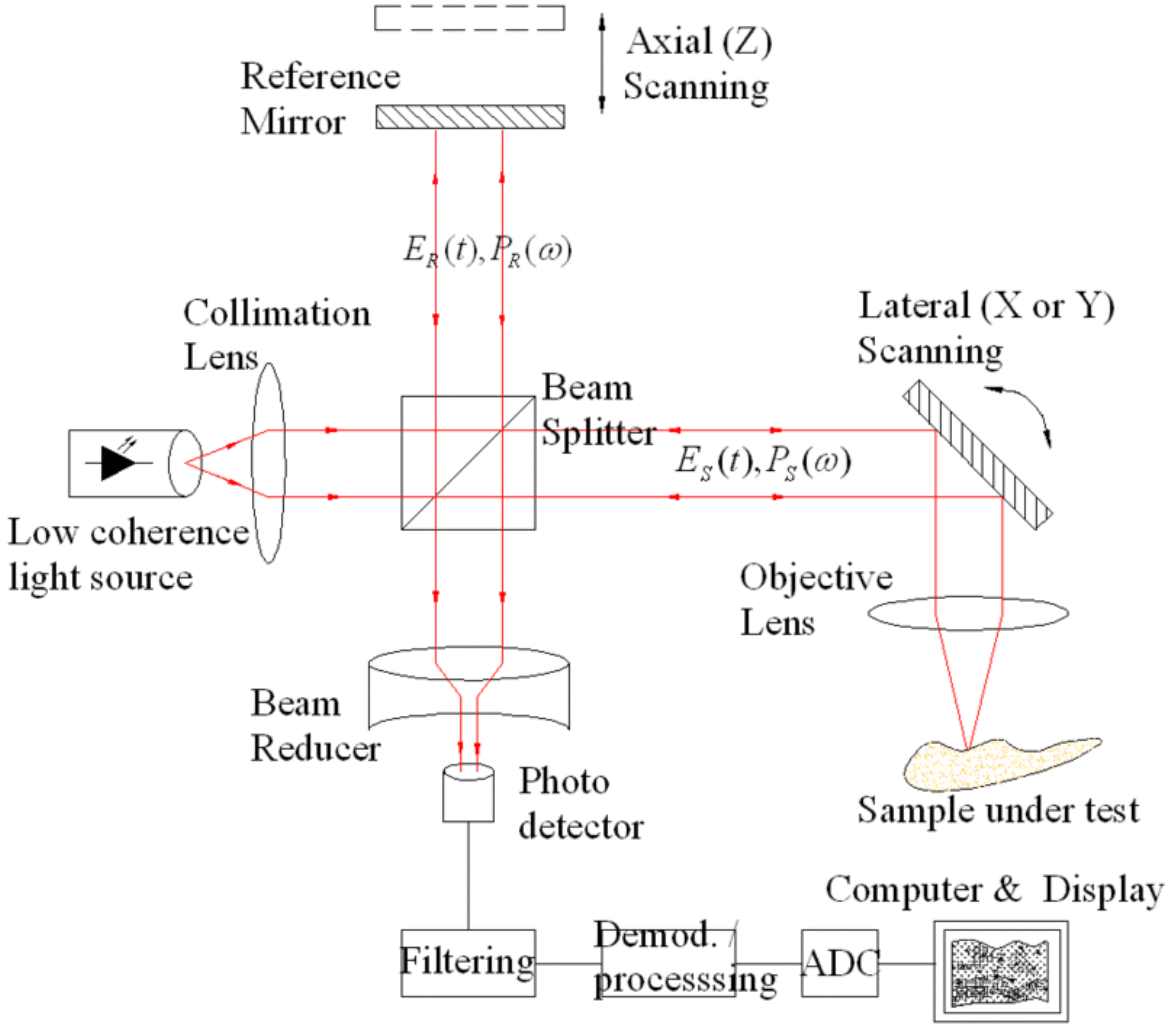
Figure 1

| Characteristic | Phase Contrast | DIC |
|---|---|---|
| Image Brightness (Brightfield = 100 Percent) | 1.3 Percent | 0.36 - 2.3 Percent |
| Epi-Fluorescence Light Loss (Brightfield = 0 Percent) | 28 Percent | 73 Percent |
| Lateral Resolution | Condenser Annulus Restricted | Superior |
| Axial Resolution (Depth Discrimination) | Poor | Superior |
| Illuminating Aperture | 10 Percent of Objective NA | Variable |
| Phase Shift Detection Limit | < l/100 | < l/100 |
| Utility at High Phase Shifts | Not Useful | Useful |
| Azimuthal Effects | No | Yes |
| Halos and Shade-Off | Yes | No |
| Stained Specimens | Not Useful | Useful |
| Birefringent Specimens | Useful | Not Useful |
| Birefringent Specimen Containers | Yes | No |
| Brightfield Image Deterioration | Slight | None |
| Cost | Moderate | High |

# And on to Matlab…

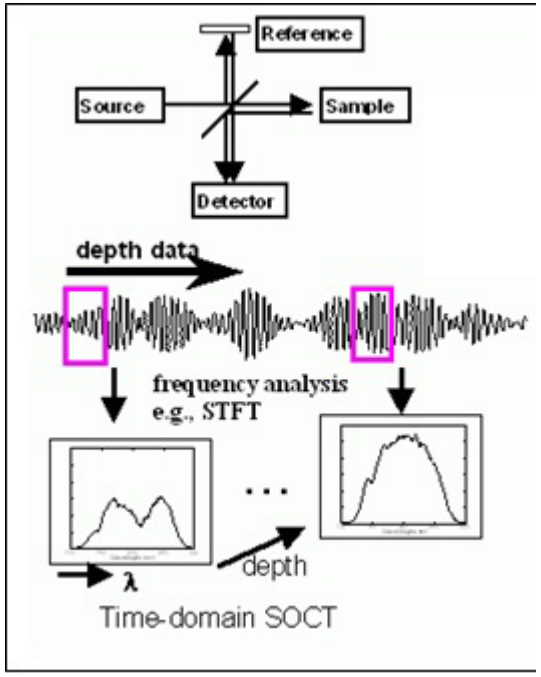
# Optical coherence tomography

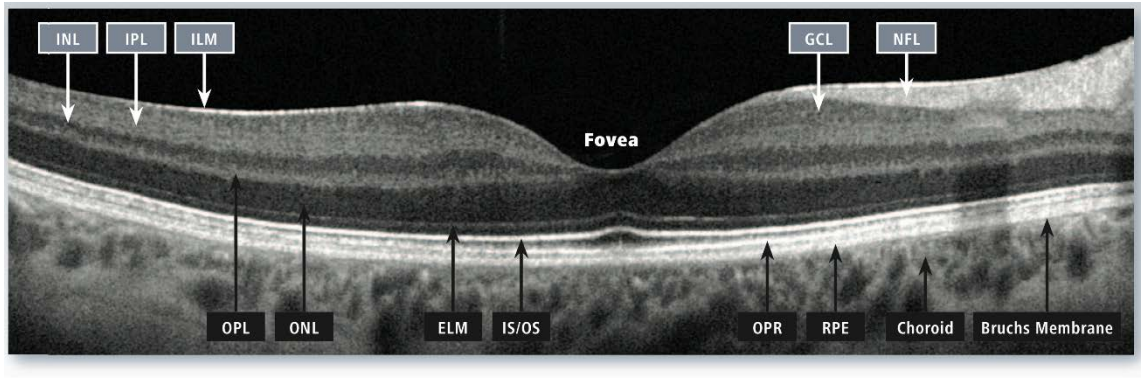
- Another interference based technique
- Non destructive, and no labelling
- Limited resolution, but high applicability



# OCT contrast

- When light in sample goes through index change, the reflected light interferes with reference arm light – giving rise to bumps in the signal




*ILM:* Inner limiting membrane
*IPL:* Inner plexiform layer
*INL:* Inner nuclear layer
*OPL:* Outer plexiform layer
*ONL:* Outer nuclear layer

*ELM:* External limiting membrane
*IS/OS:* Junction of inner and outer photoreceptor segments
*OPR:* Outer segment PR/RPE complex

*NFL:* Nerve fiber layer
*GCL:* Ganglion cell layer

Particularly useful in ophthalmology.
Also used in the brain (rats)