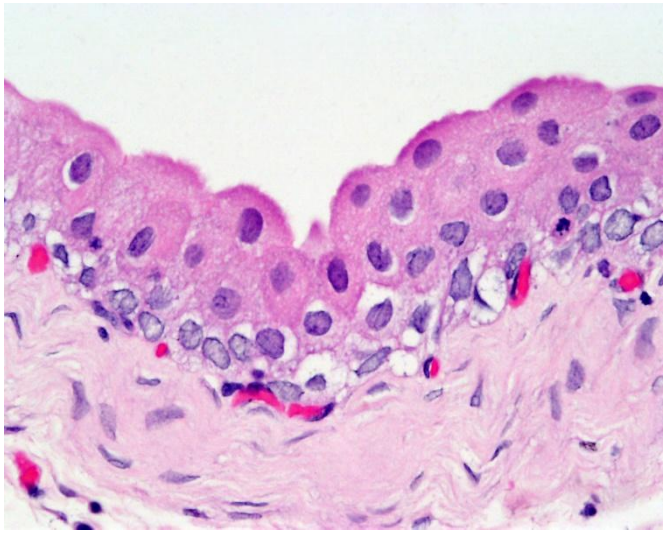
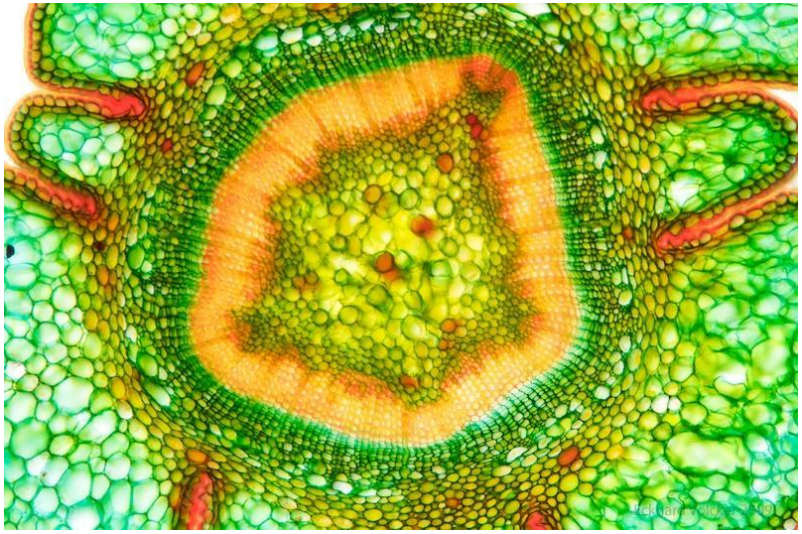
Biology 177: Principles of Modern Microscopy

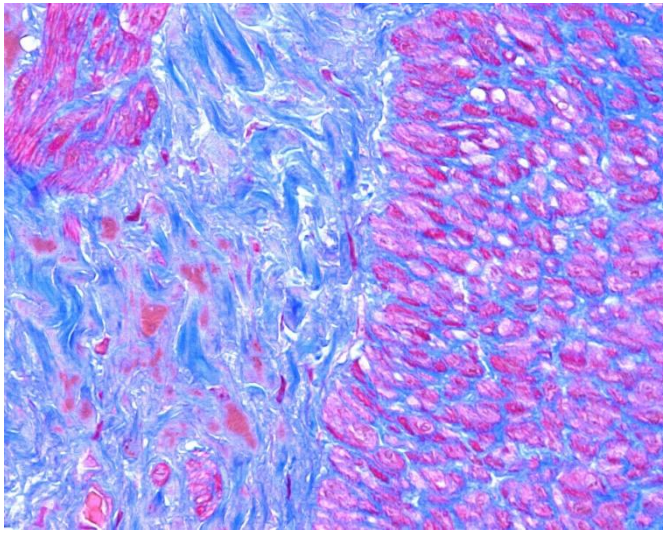
Lecture: Polarization and DIC

The First Contrast

- Histological stains
- Still important today

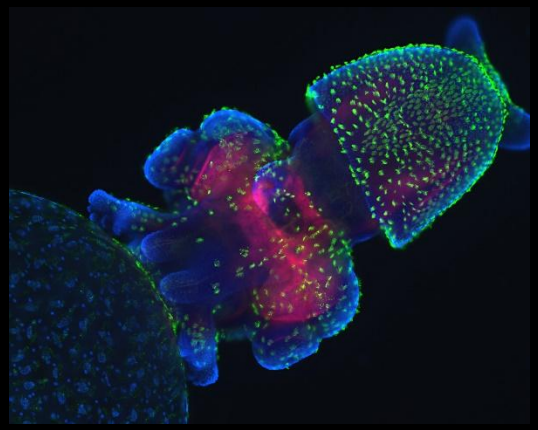






The Ultimate Contrast

- Transparent specimen contrast
  - Bright field 2-5%
  - Phase & DIC 15-20%
  - Stained specimen 25%
  - Dark field 60%
  - Fluorescence 75%

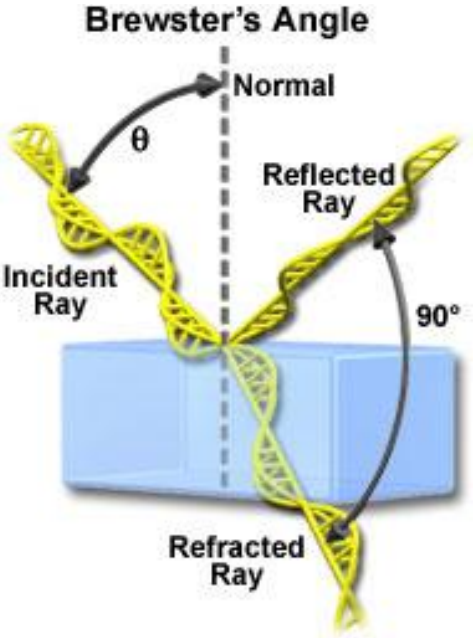


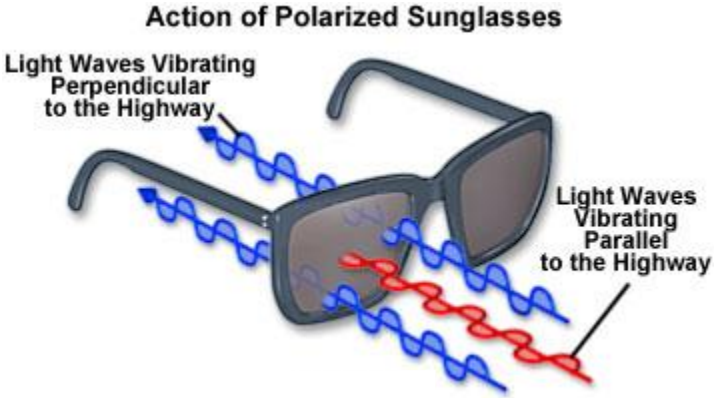
Polarized light

- Circular polarization, rarely produced in nature
- Can see on iridescent scarab beetles and Mantis shrimps
- Mantis shrimps can see circularly polarized light

Polarized light

- Radial light waves becomes polarized when reflected off surface at Brewster's angle
- Brewster's angle ranges from 50° to 70° depending on surface material.
- Used to polarize lasers
- Why sunglasses horizontally polarized



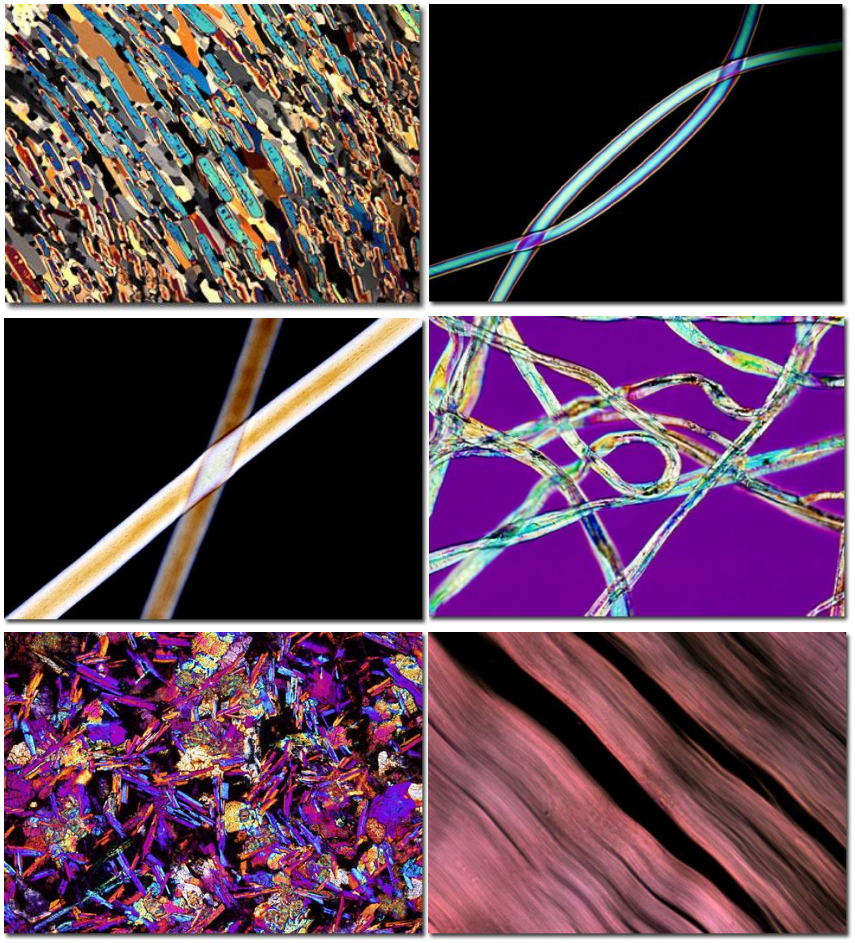


Polarized light

- We cannot detect the polarization of light very well
- But some animals can see polarized light
- Many insects, octopi and mantis shrimps

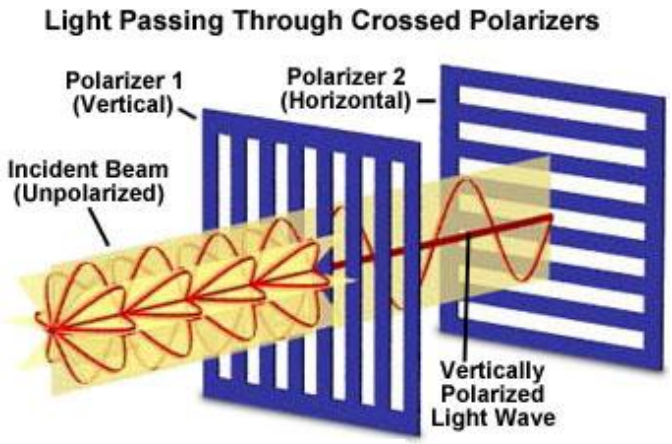
Polarized light microscopy

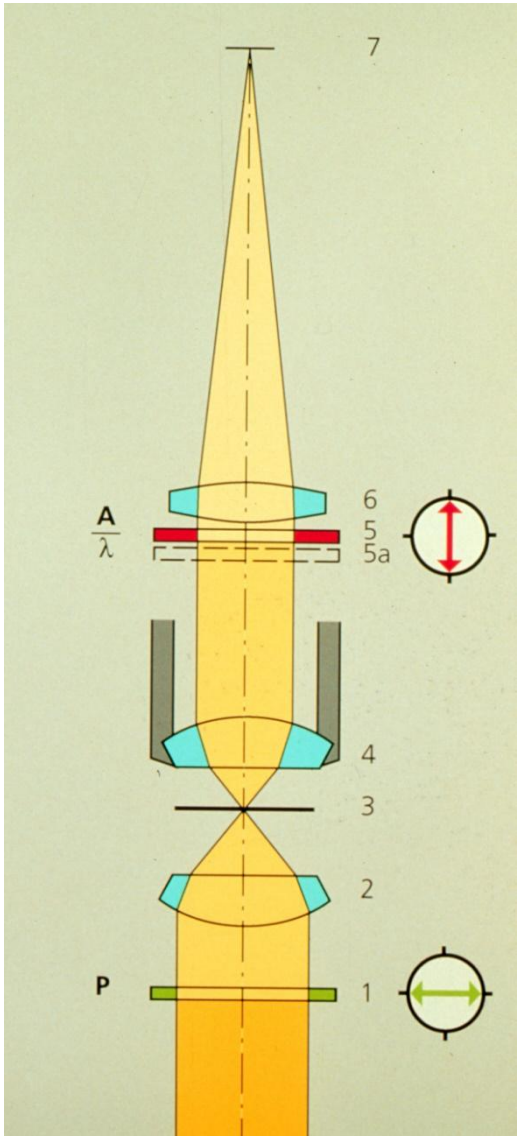
- Highly specific detection of birefringent components
- Orientation-specific
- Less radiation than through other techniques such as fluorescence
- Linear / circular Polarized Light
- Differential Interference Contrast (DIC) uses polarized light



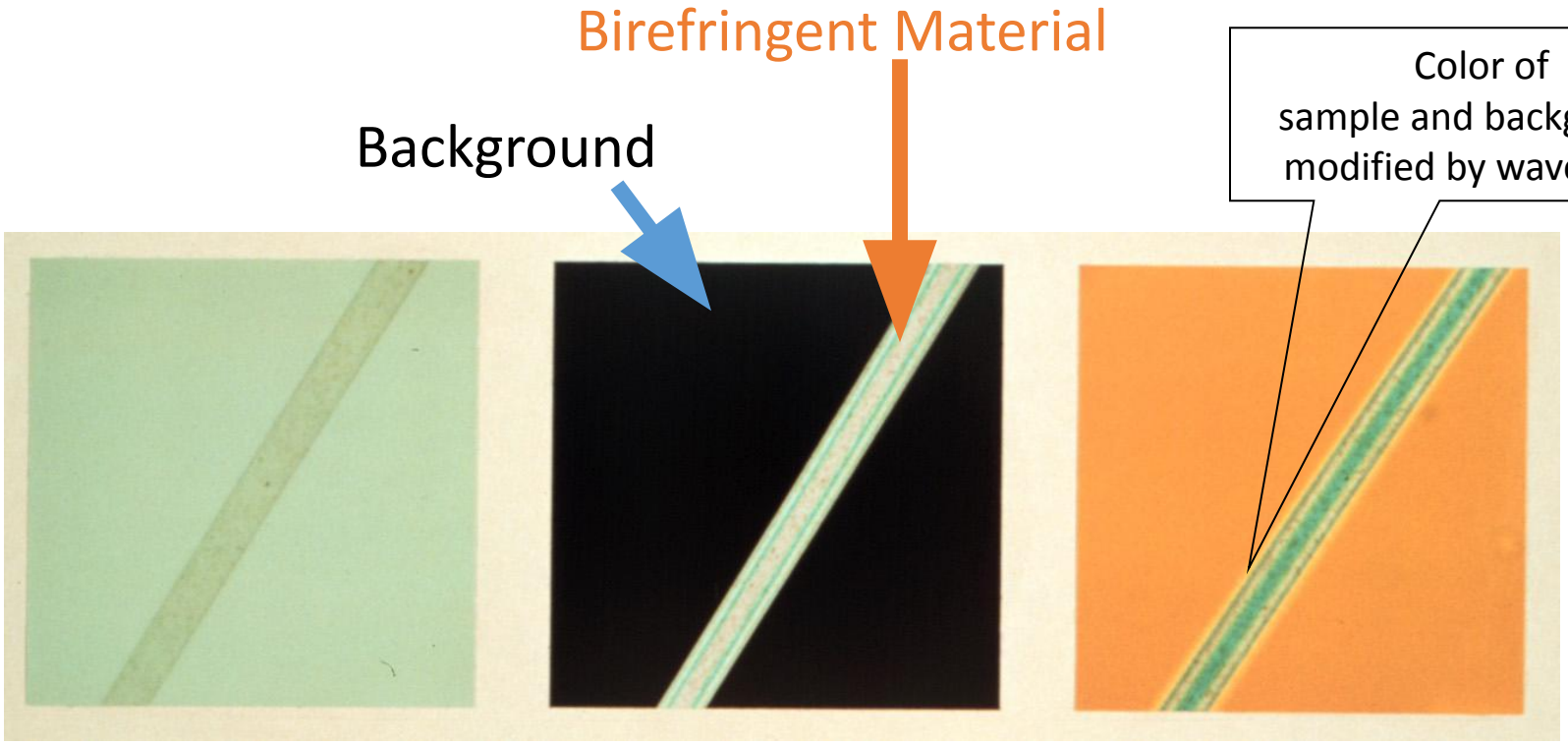
Polarized light microscopy

- With crossed polarizers:
  - Only items that rotate the plane of polarization reach the detector
- Retardation plate optional
  - Converts contrast to color





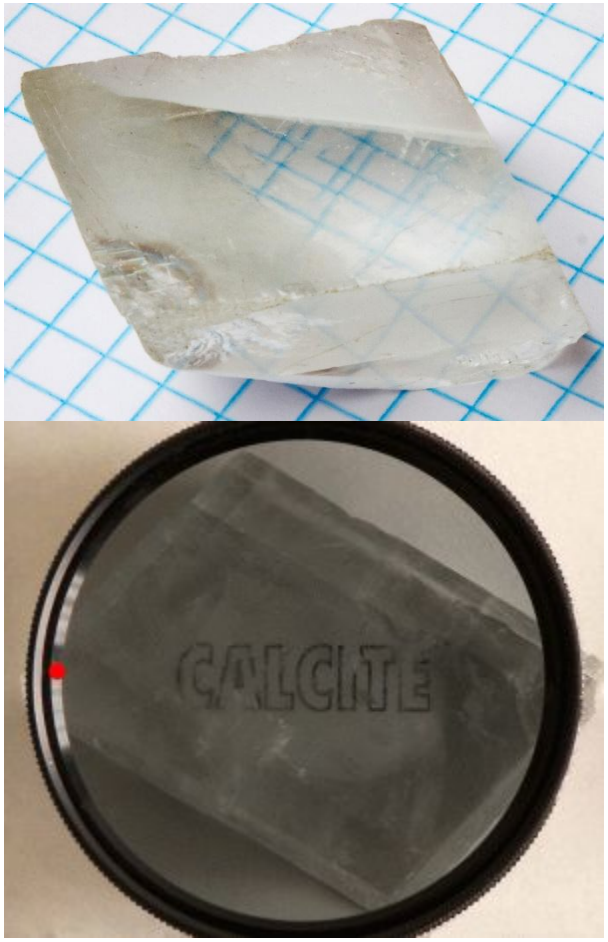
Polarized light microscopy images



Brightfield Polarized Light Pol + Red I

Birefringence

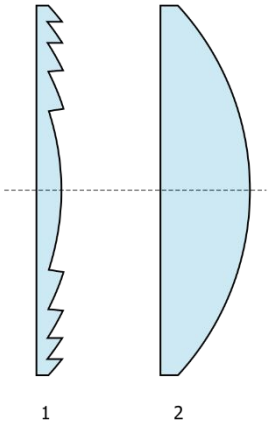
- Material having a refractive index (η) dependent on polarization
- Responsible for DOUBLE REFRACTION, splitting of a ray of light into two with differing polarization

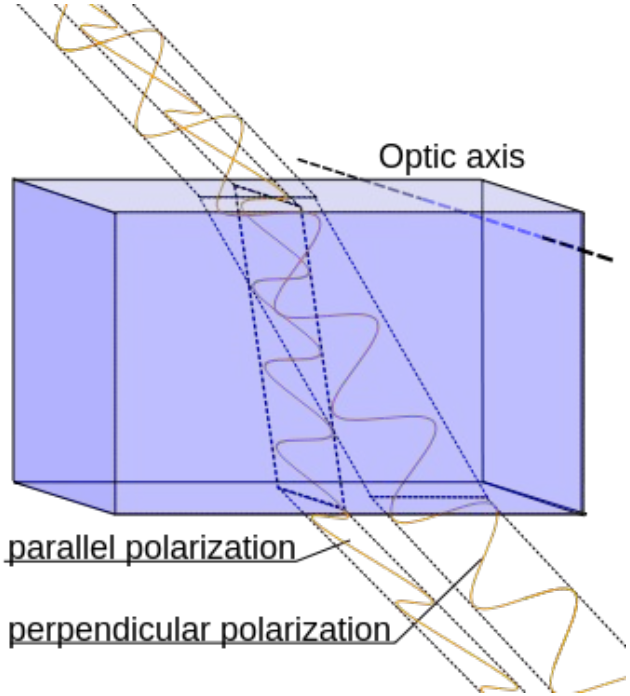


Birefringence

- Augustin-Jean Fresnel first described in terms of polarized light
- Isotropic solids are not birefringent (glass)
- Anisotropic solids are birefringent (calcite, plastic dishes)
- Splits light into two rays with perpendicular polarization

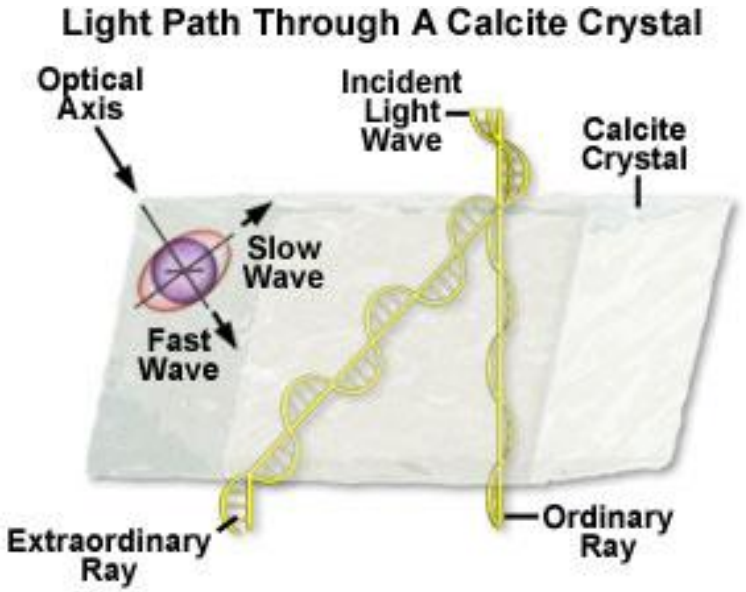
Augustin-Jean Fresnel
1788-1827





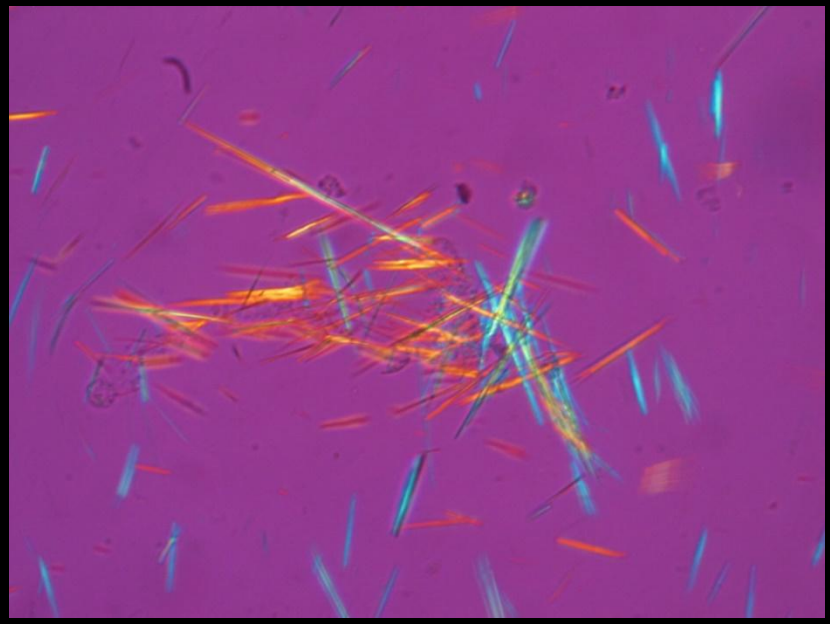
Birefringence

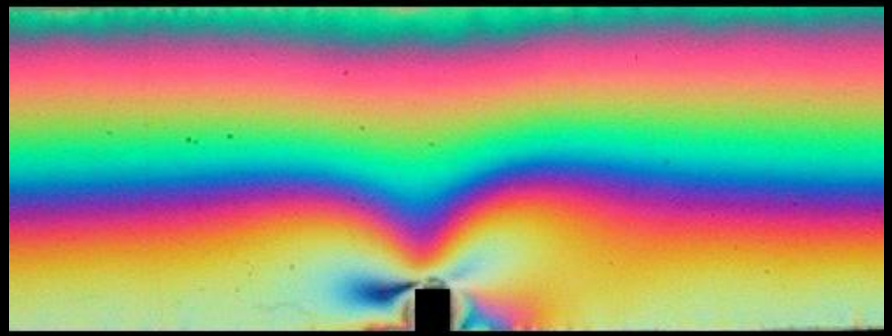
- Light split into extraordinary and ordinary rays
- Birefringence difference between refractive index of extraordinary ray (η_e) and ordinary ray (η_o)



Birefringence

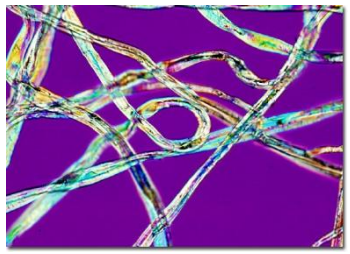
- Structural
  - Anisotropic
- Stress or strain
  - Isotropic



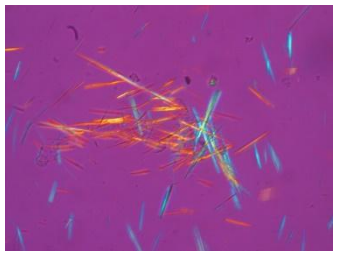


Full Wave (First Order) Retardation Plate

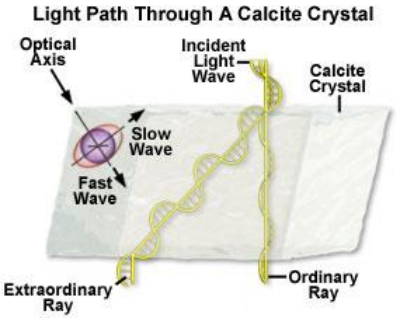
- Also known as:
  - Lambda plate
  - Red plate
  - Red-I plate
  - Gypsum plate
  - Selenite plate
- Retard one wavelength in the green (550 nm) between extraordinary ray and ordinary ray

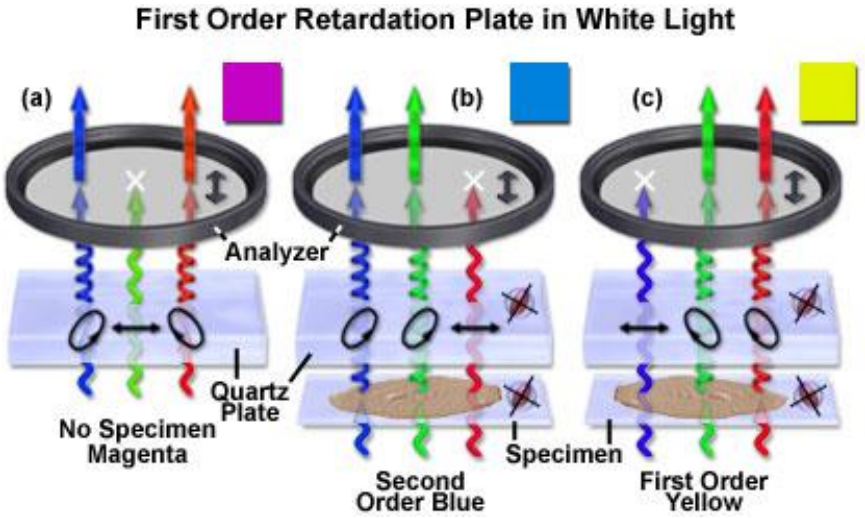


Cotton



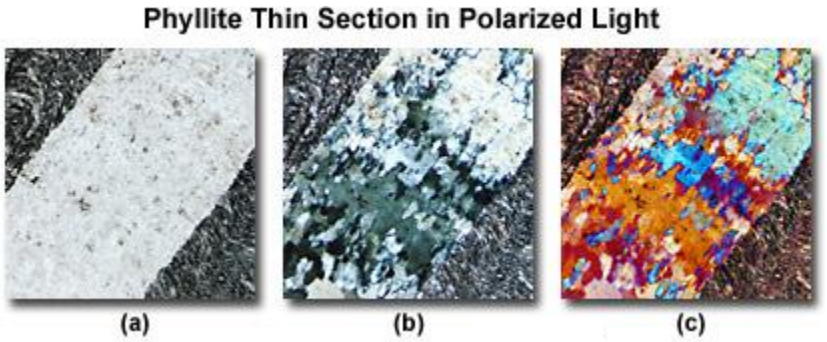
Uric Acid



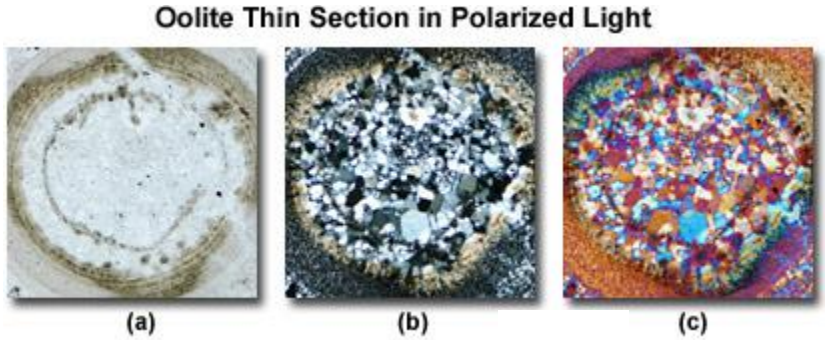


Polarized light microscopy
Using full wave retardation plate

- Phyllite
  - Metamorphic rock aligned under hear and stress



- Oolite
  - Sedimentary rock of cemented sand grains



Plane-Polarized

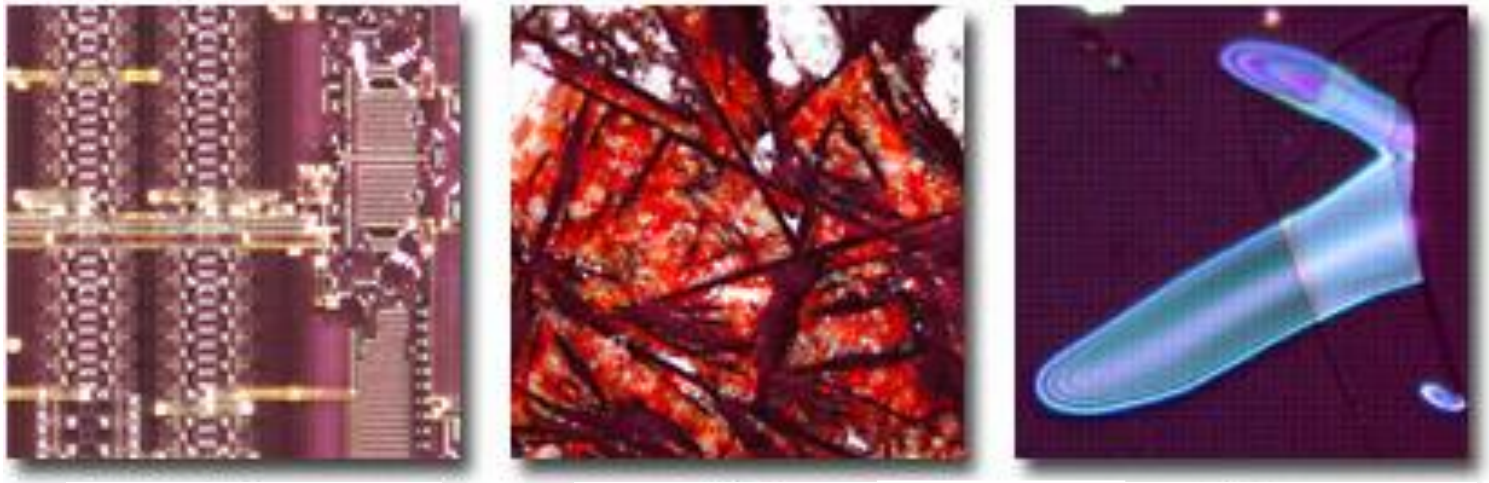
Cross-Polarized

Full wave retardation plate

Reflected polarized light microscopy

- Requires special objective
- Not corrected for viewing through cover glass
- Strain free

Reflected Polarized Light Microscopy



Integrated circuit | Ceramic crystal | Copper imperfections