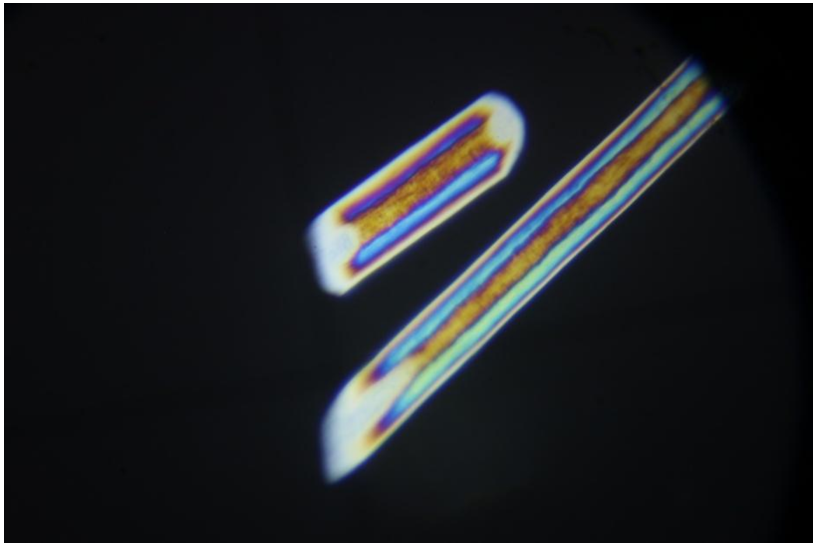
Manchester Microscopical & Natural History Society

Established 1880

www.manchestermicroscopical.org.uk

Polarising Microscopy

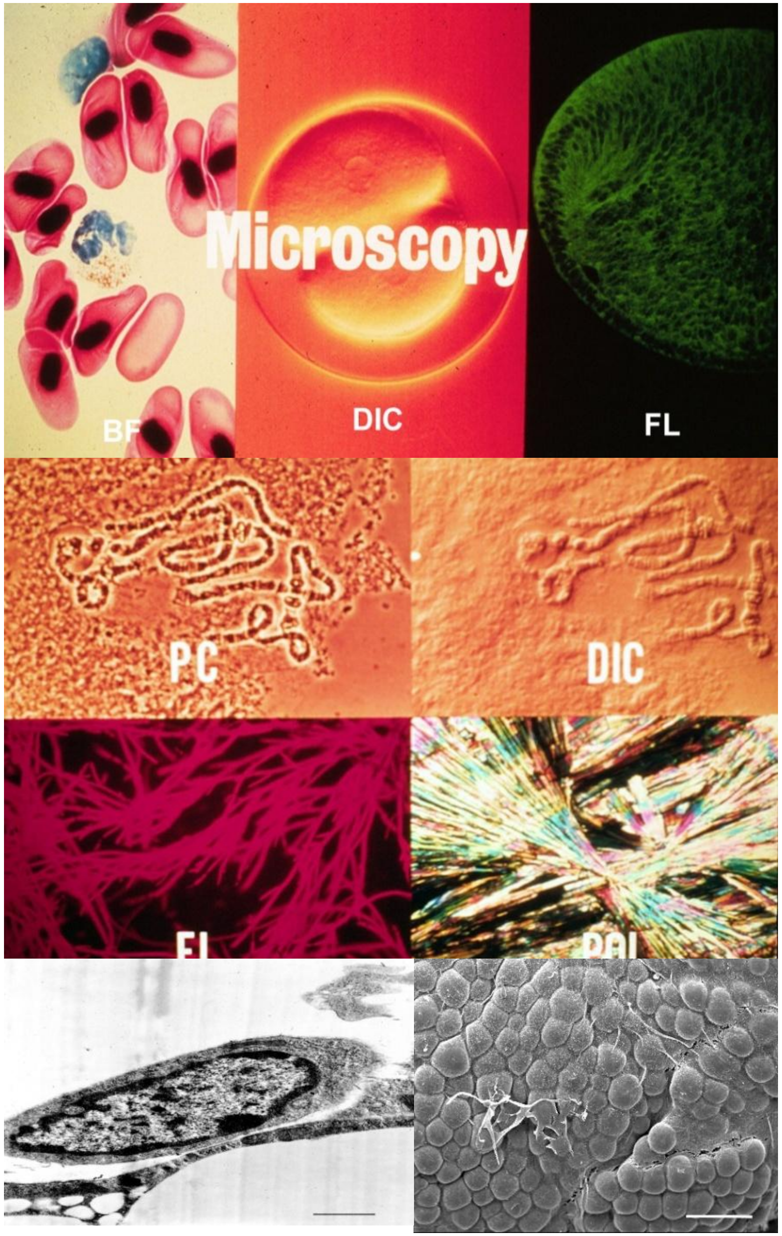
Part of the Microscopist's Armamentarium



Talk &

Demo / Practical

Mike Mahon*, November 22nd 2025*



Aim: which technique to use?

- **Light Microscopy** c1600
  - bright field + stains
  - relectance
  - dark field
  - **polarising**
  - phase contrast
  - Interference / DIC
  - fluorescence
  - computerised / confocal
- **Electron Microscopy**
  - transmission
  - scanning
- **Scanning Probe/Stylus**
- **X-ray, Ion Beam, …**

1800s
Add Visibility
Pretty Colours
Analytical

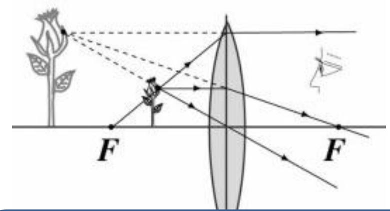
Microscopy Landmarks

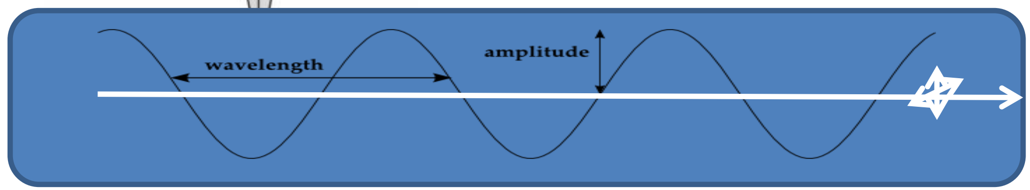
- **<1600s** magnifying lens
- **1600s** simple microscopes
- **1650s** compound microscopes
- **1830s** expansion of light microscopy + **pol**, DF
- **1900-80** UV & fluorescence microscopy
- **1930-1950s** phase contrast & interference
- **1930-60** electron microscopes developed
- **1980s** confocal microscopy
- **1990s-2010s** scanning probe microscopies
- **2010s+** super-res, µCT, X-ray, 3D, AI microscopies,...

Light Microscope Theory

Properties of Light ⇨ Microscopies

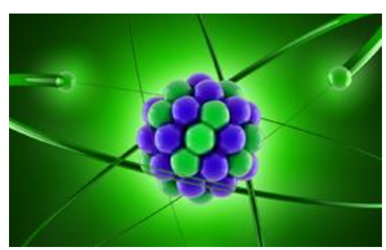
- **Geometric Optics**
  - image location
  - magnification / resolution





- **Wave Optics**
  - direction – reflection,refraction,diffraction / BF / DF
  - amplitude – absorption / BF
  - wavelength – colour
  - phase – phase contrast, interference
  - **vibrational plane – polarisation**
  - velocity – refraction / oil immersion

- **Quantum Optics**
  - formation & destruction
  - fluorescence

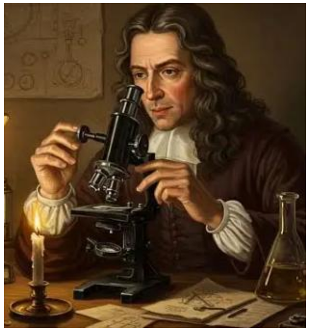


Geometric

Christiaan Huygens 1629-1695 (Dutch)

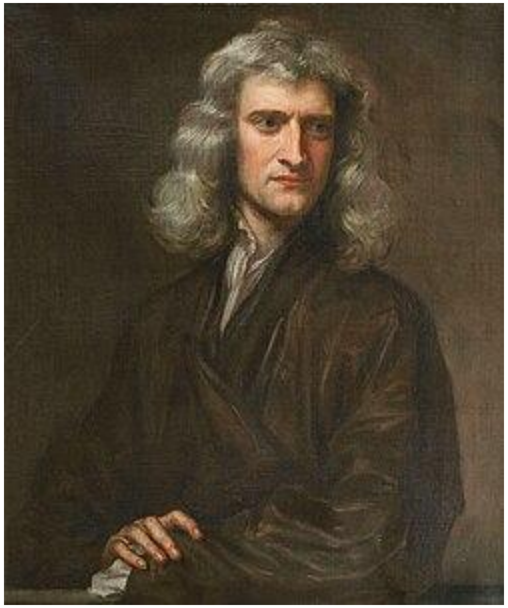
Mathematics, Physics, Engineer, Astronomy, Inventor

Optics .. <u>Wave Theory</u> (p1703), Lenses, Telescopes & Microscopes



Robert Hooke
1635-1703
1665 Micrographia

1689 !



Isaac Newton 1643-1727 (English)

Mathematics, Physics, Astronomy, Alchemy, Inventor

Optics .. Colour Spectrum (1666)
Newton's rings (1671)
Refraction, <u>Corpuscular Theory</u> + Waves (1704)

Etienne Malus
1775-1812

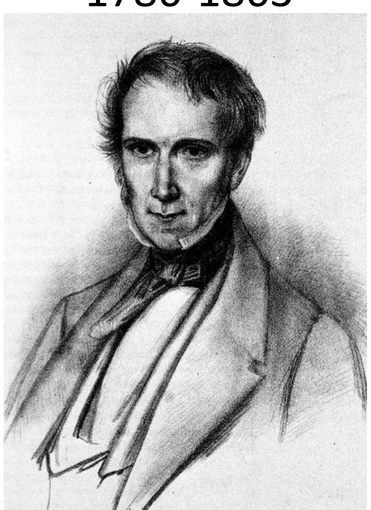
Sir David Brewster
1781-1868

William Nicol
1770-1851

Henry Fox Talbot FRS
1800-1877

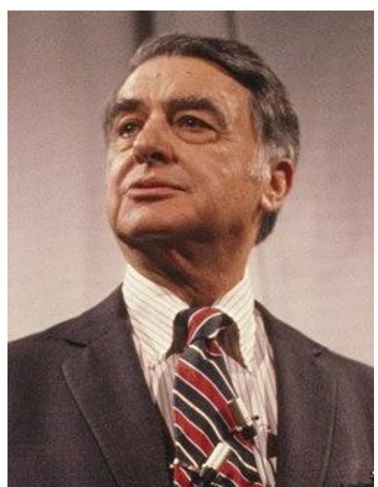
History - People

Giovanni Amici
1786-1863



....

Edwin Land
1909-1991



Walter McCrone
1916-2002

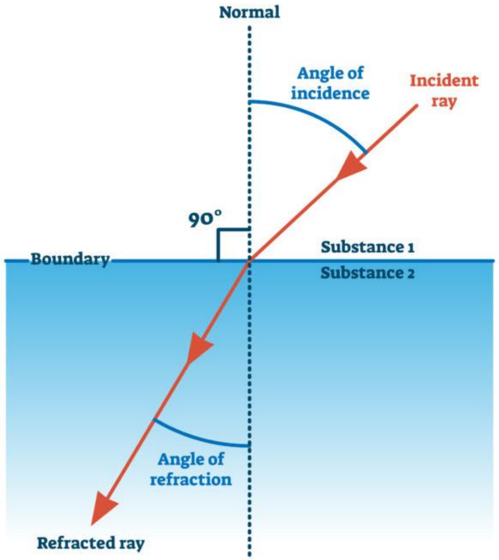
Gustav Delly
1934-2024

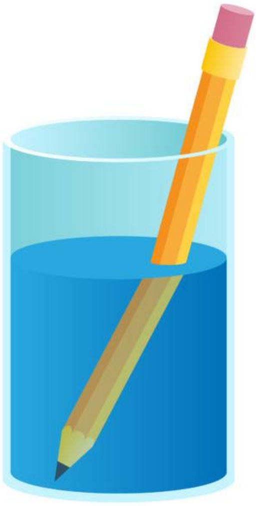
Discovery / Inventions

- **1670s Iceland Spar** (calcium carbonate crystals, calcite),
  Bartholin .. double image, **Huygens** … splits light
- **1808 Malus**
  - rotate Iceland Spar & noted intensity changes 'polarisation'

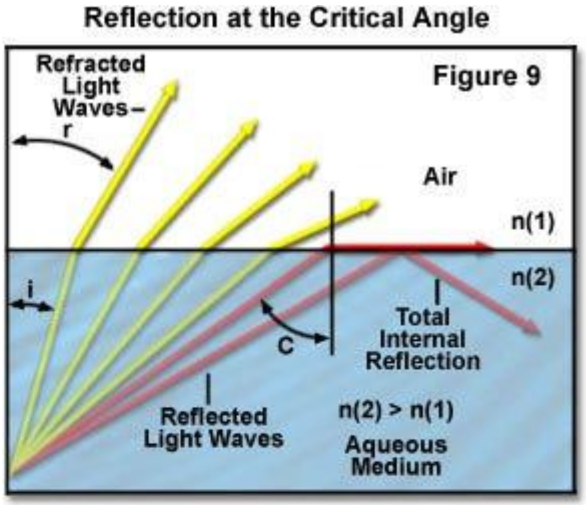
- **Sir David Brewster**
  - 1815-1830s explained theory of light polarisation, refractive index, … **Fresnel**
- **William Nicol *****
  - 1828 created Nicol Prism to polarise light, used thin ground sections
- **Giovanni Amici**
  - 1830 built polarising microscope
- **Henry Fox Talbot**
  - 1834 uses of polarising microscopy
- …….
- **Edwin Land**
  - 1928 synthetic polarising sheet (Polaroid) [iodoquinine sulphate, herapathite] used in sunglasses, microscopes, cameras, LCDs, 3D … [**Herapath 1852**]
- **1950s Smith/Baker, Barer Interference Microscopy**
- **1952 Georges Nomarski DIC, ….**
- **1960s-2000s Walter McCrone, Gustav Delly** … particle analysis, forensics

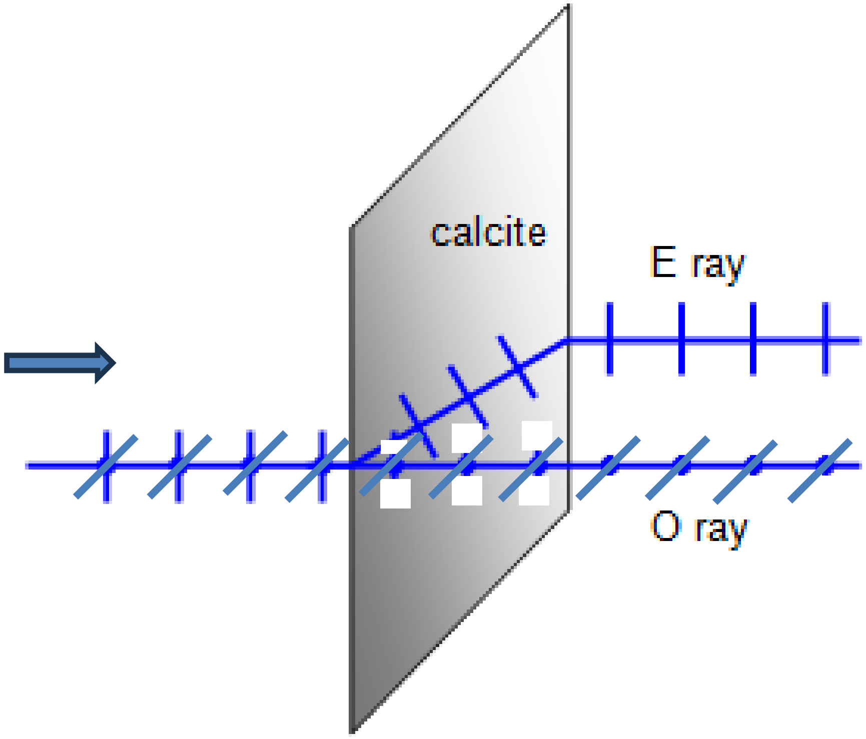
Refraction





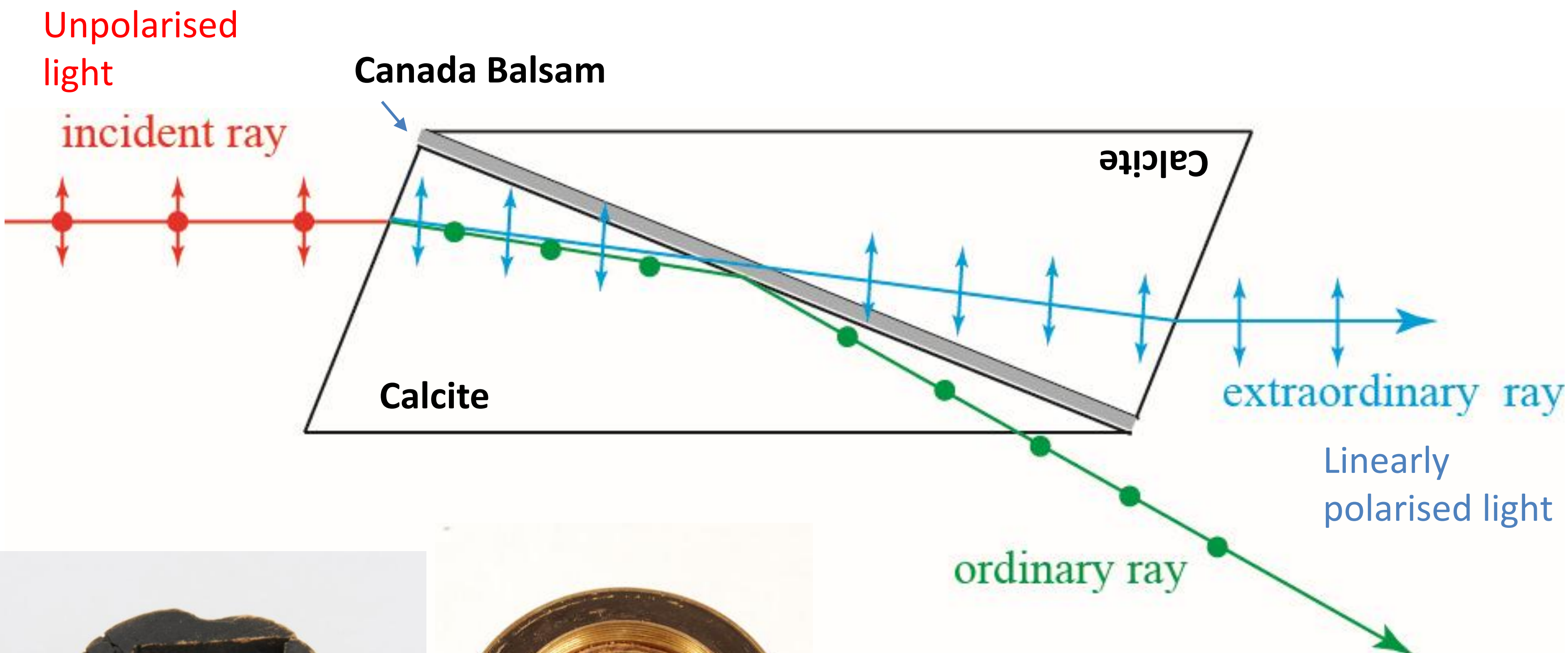
Polarisation



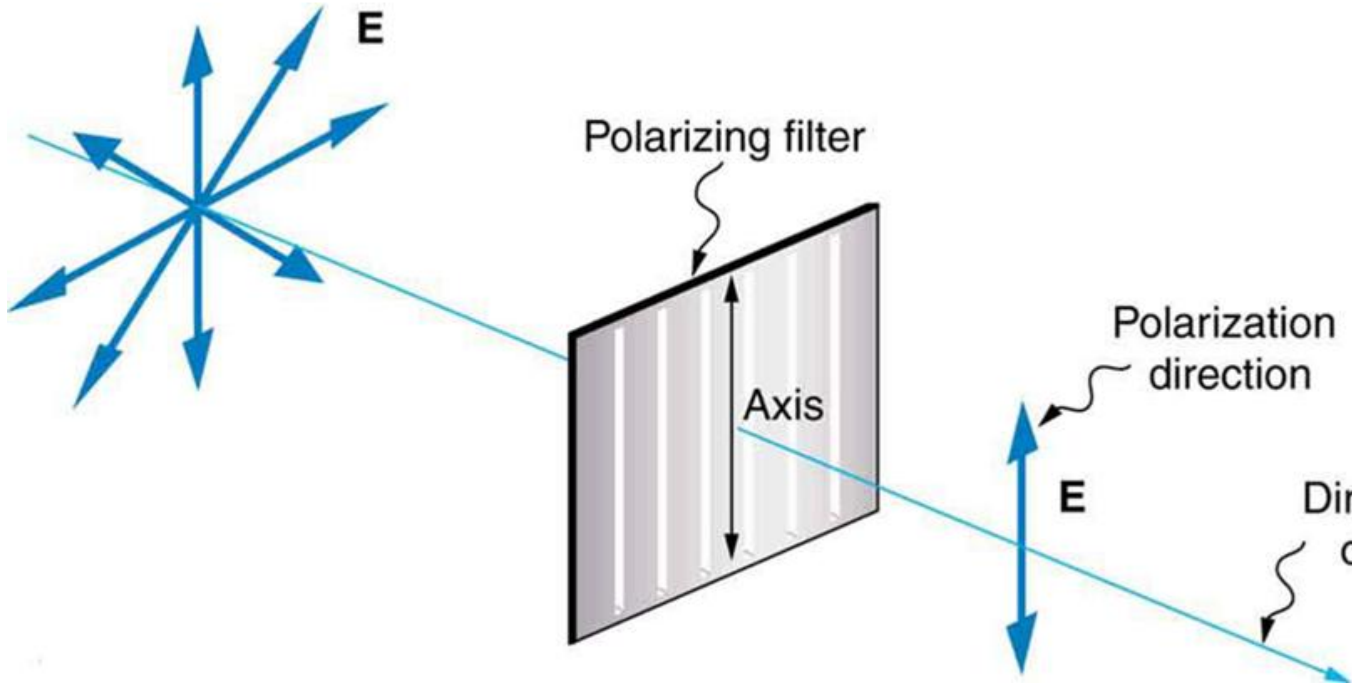


Reflection

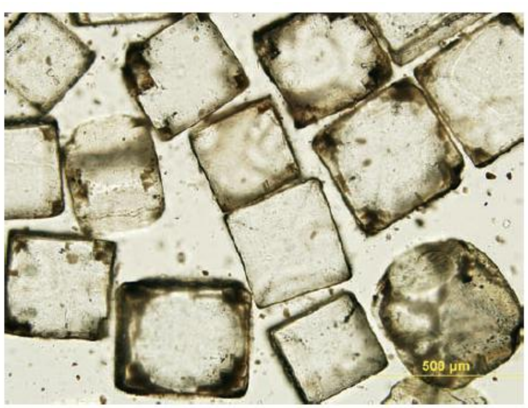
Nicol Prism


Unpolarised light
Canada Balsam
incident ray
Calcite
Calcite
extraordinary ray
Linearly polarised light
ordinary ray

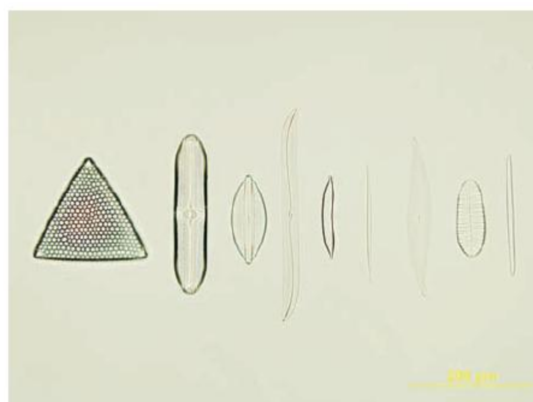
Single Linear Polaroid Filter



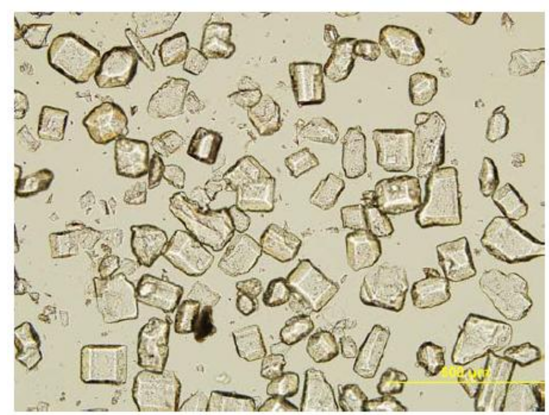
duller



Salt



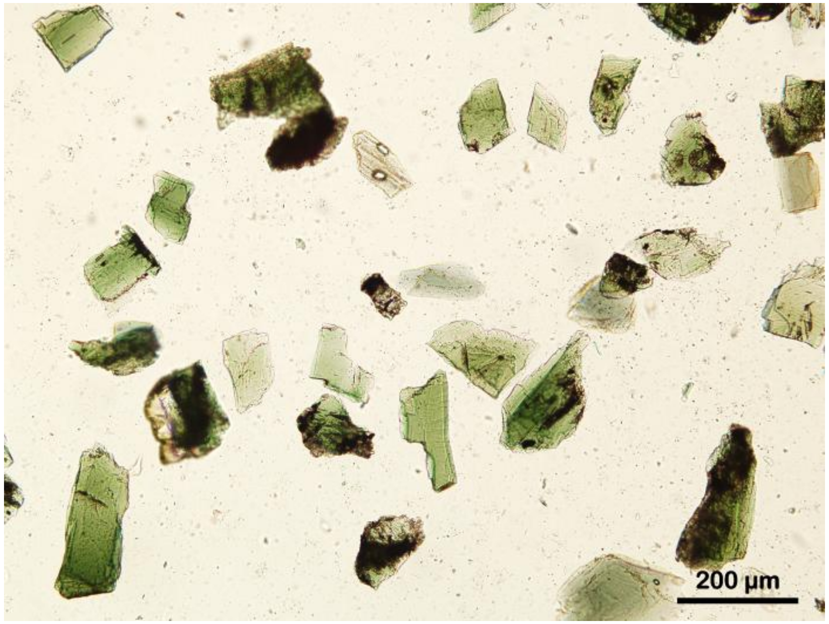
Diatoms



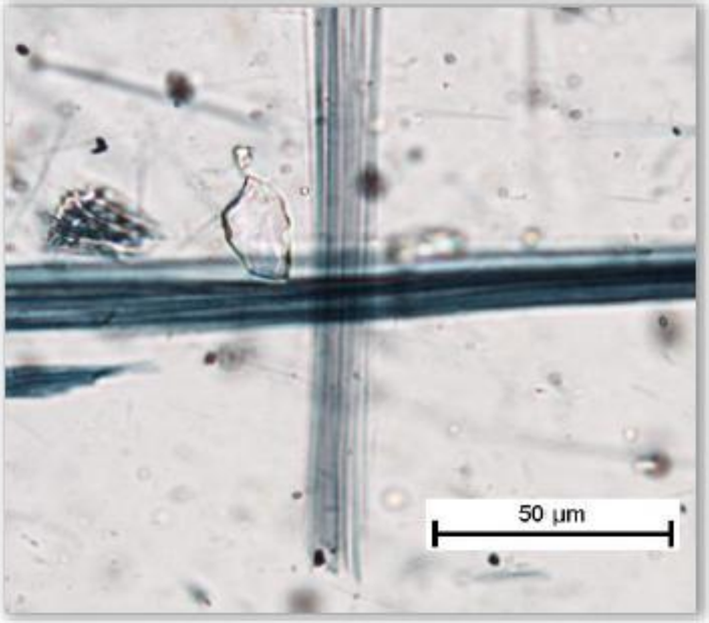
Sucrose

Single Linear Polaroid Filter

Pleochroism



Hornblende



Asbestos

Single Polar – Becke Line Estimation of Refractive Index

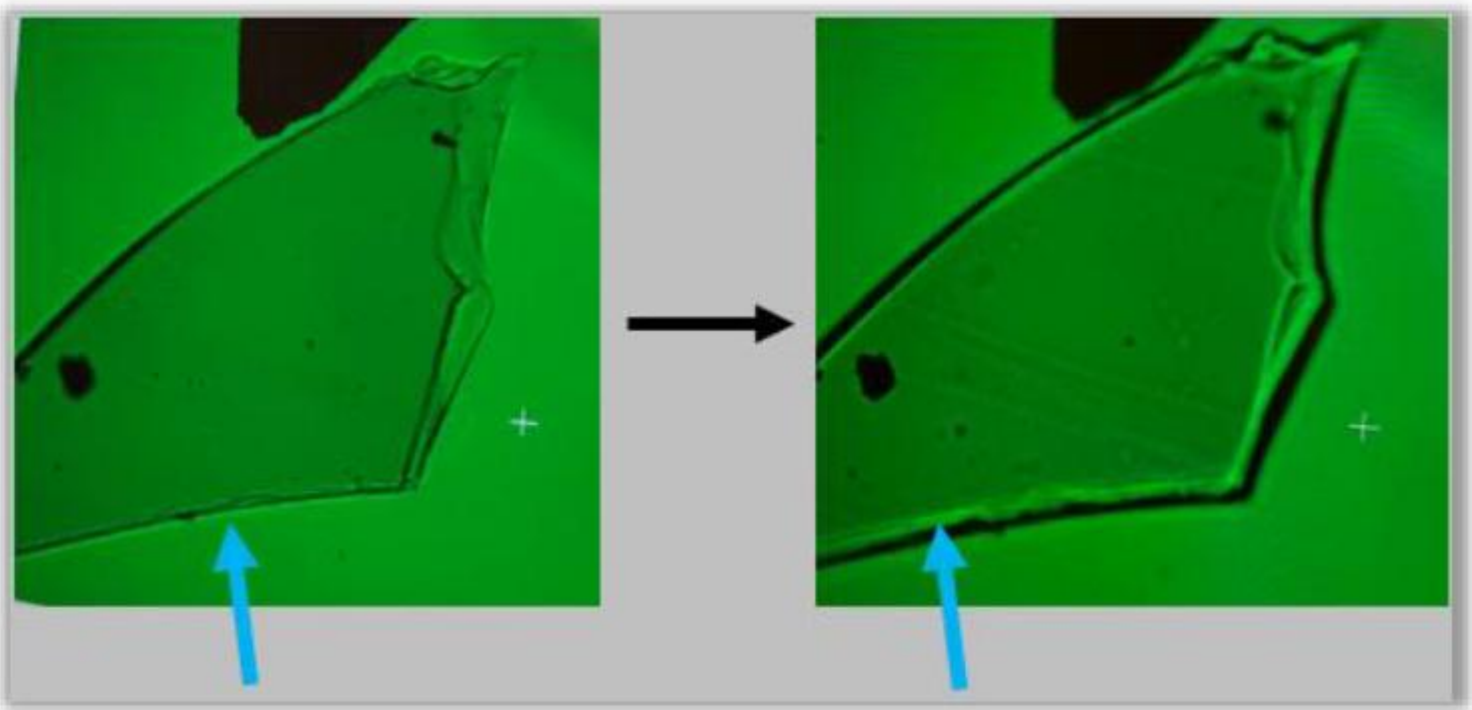


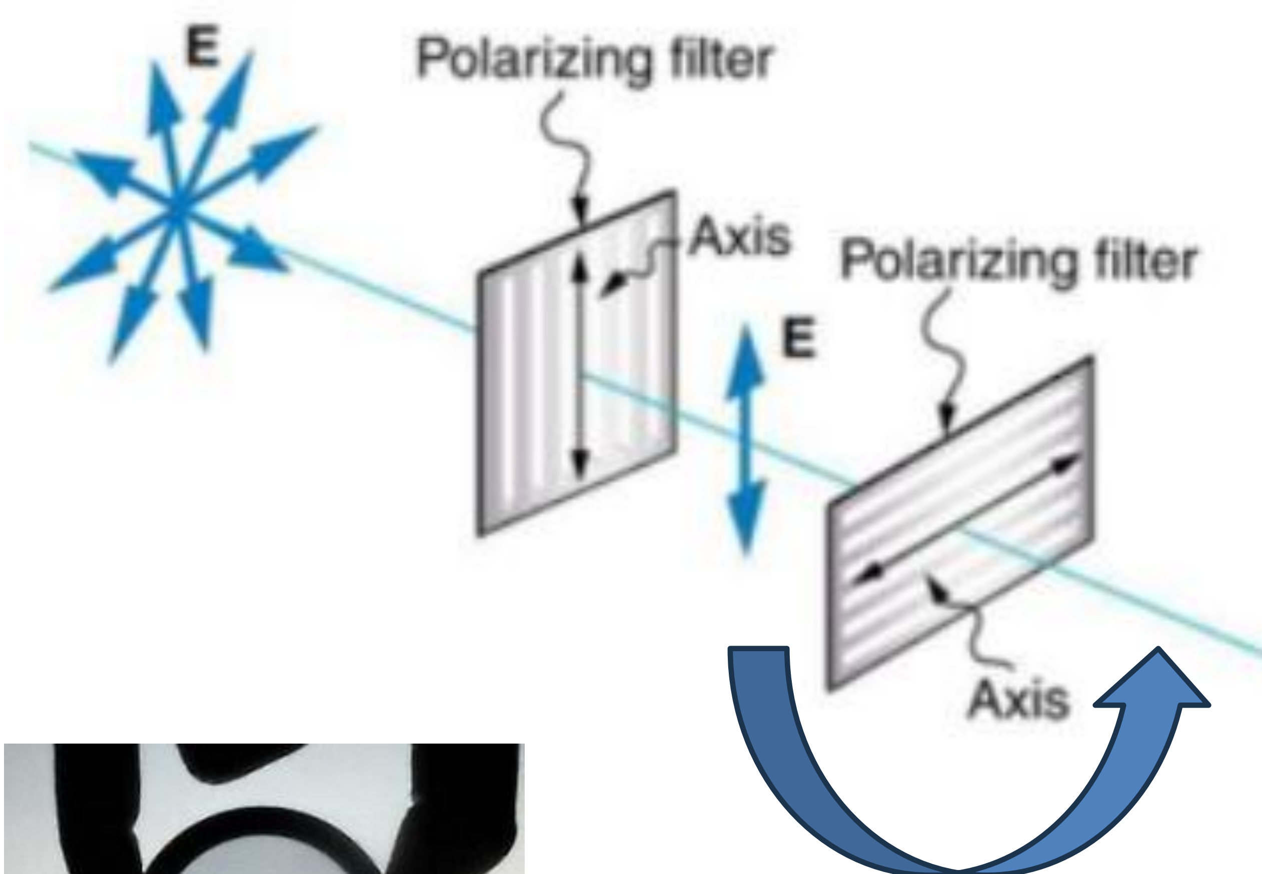
Figure 9. Becke line for isotropic glass. [8,8]

Monochromatic Light

Raise objective .. Becke Line moves into material with HIGHER refractive index

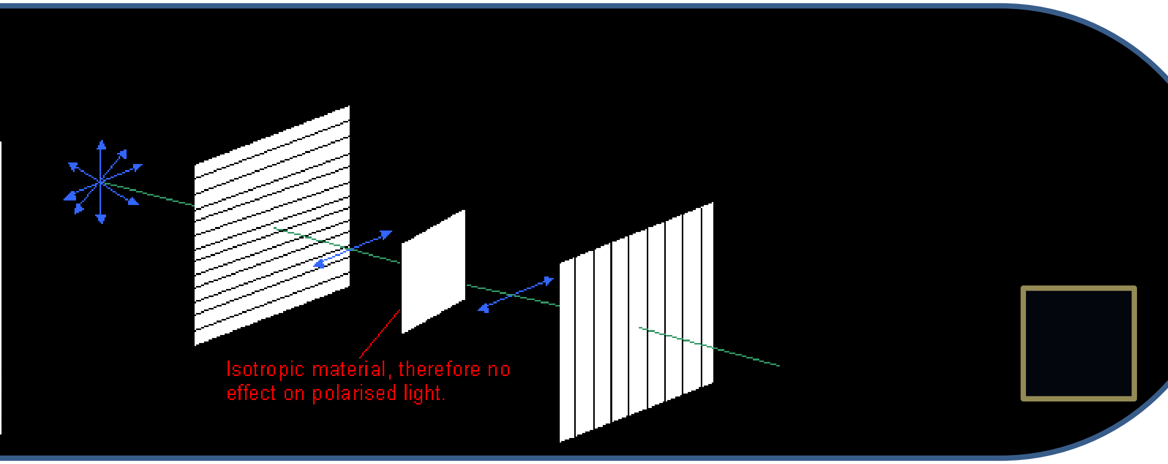
Change Mountants with different RIs

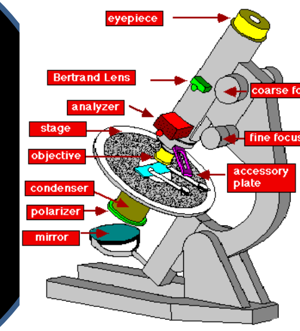
Crossed Linear Polaroid Filters



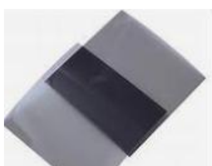
black

Polarising Microscope 1

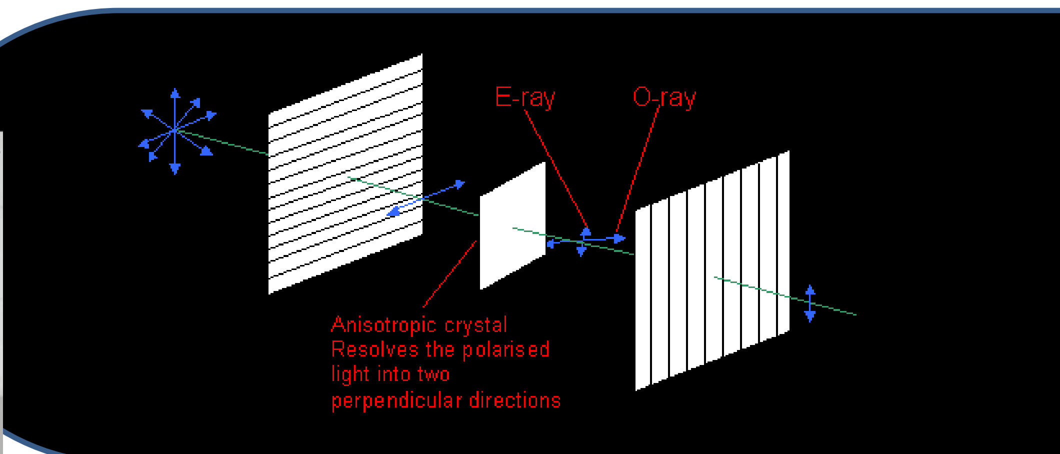


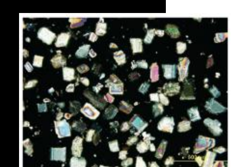


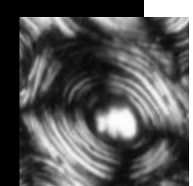
Polariser **Analyser**



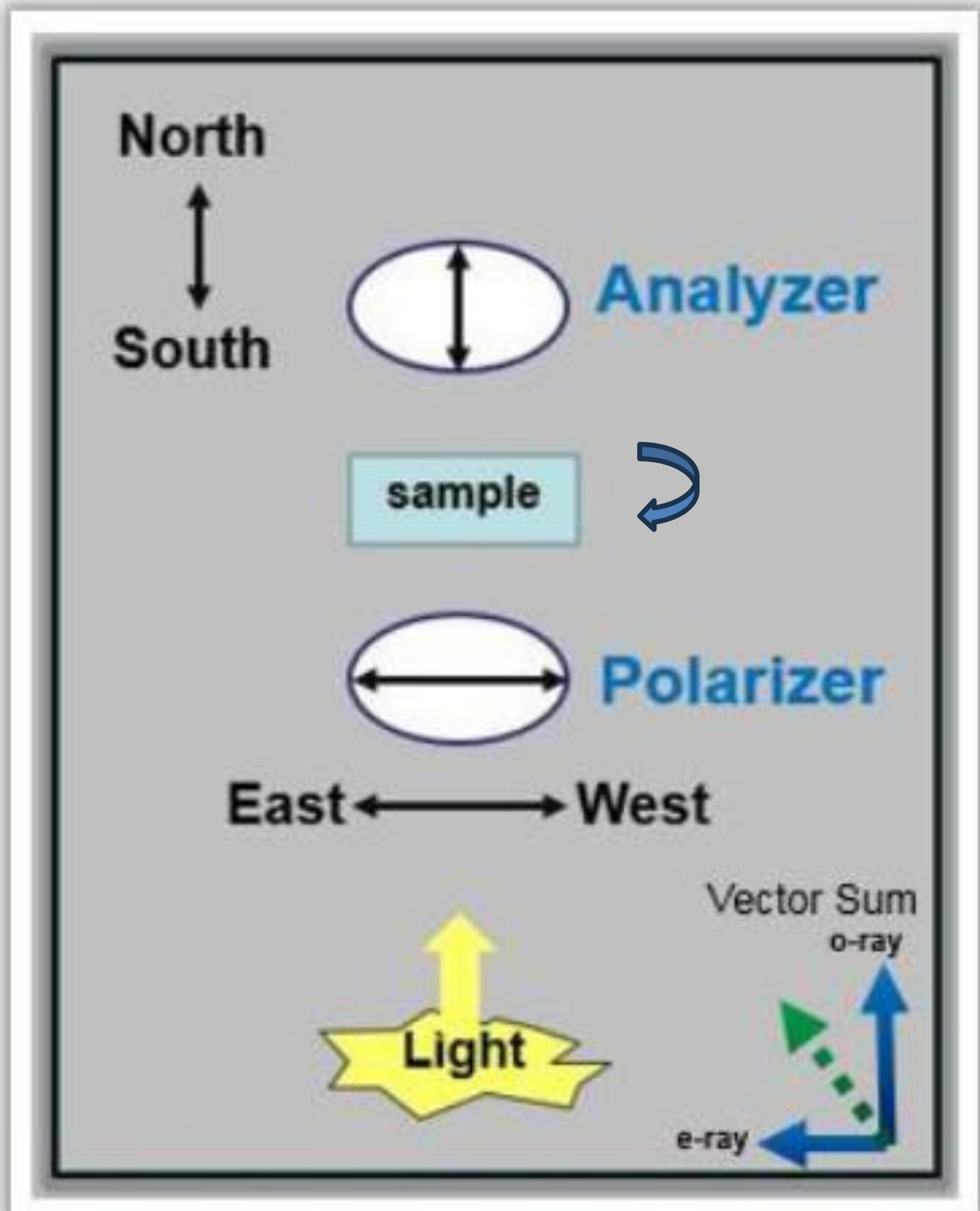
Cheap Version !







In use since 1830s

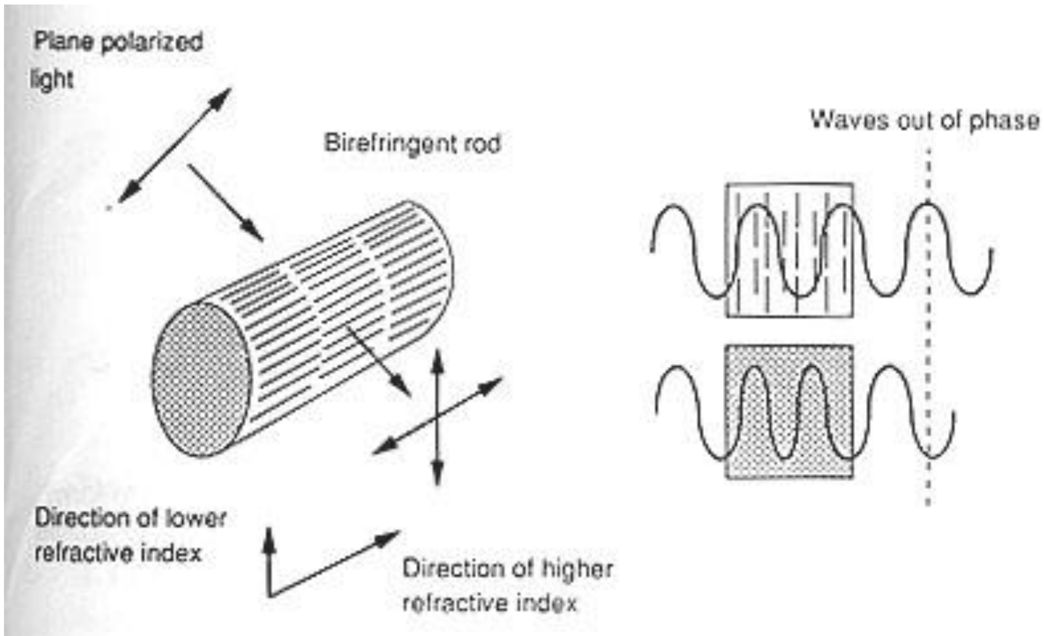


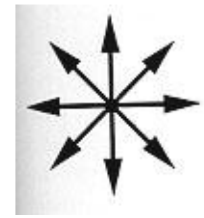
+ Bertrand Lens

+ Compensator

Polarising Microscopy (Specimens) 2

Anisotropy / Birefringence





unpolarised light

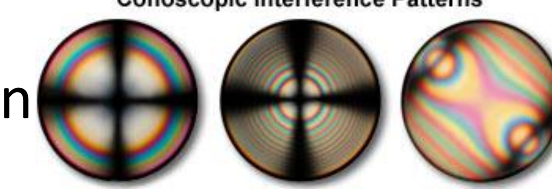
Plane polarised light

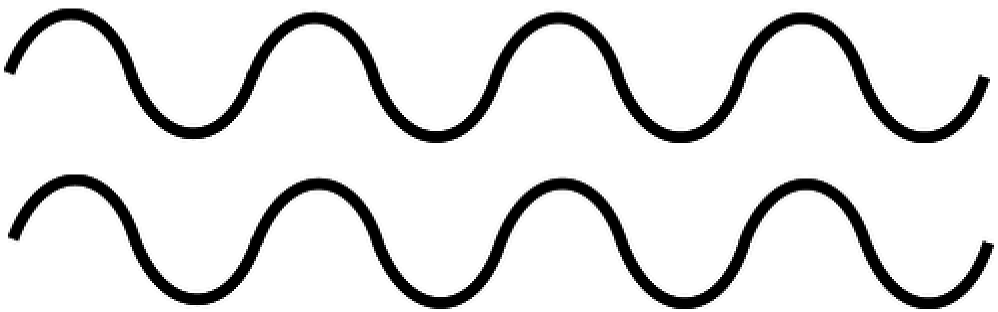
Starch grain

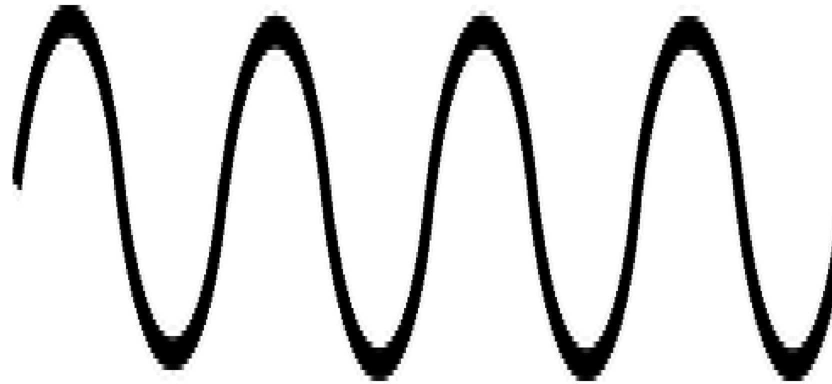
- such objects show two different refractive indices
- dependent on the orientation of plane polarised light
- wavefronts will interfere with each other …
- result ∞ refractive index, thickness, orientation

OPD= (n1-n2) x t

Conoscopic Interference Patterns

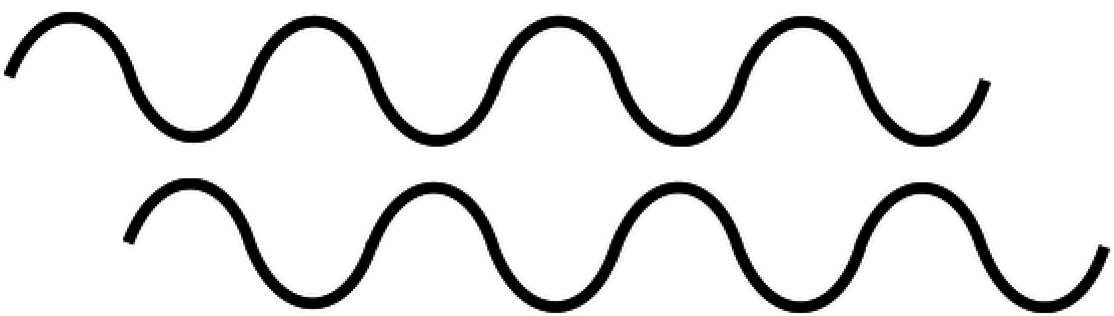






Waves in phase

Constructive Interference



Destructive Interference

Waves out of phase by ½ λ (180°)

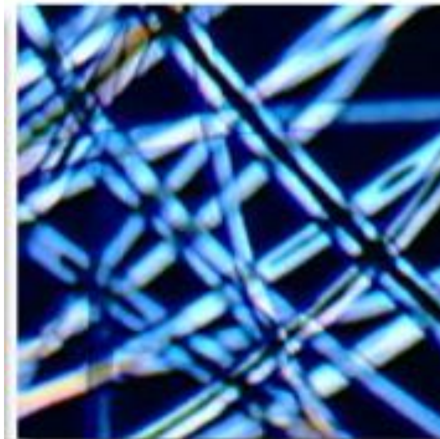
Waves out of phase by ¼ λ (90°) = hardly noticeable change in amplitude !

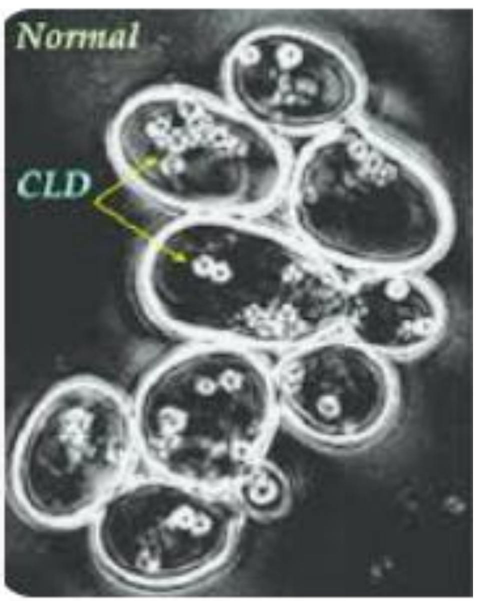
Crossed Polars ...

Starch

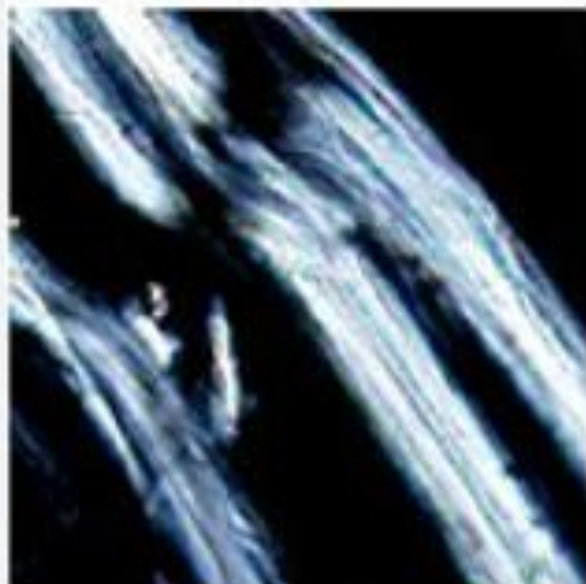
Hairs

Synthetic Fibres



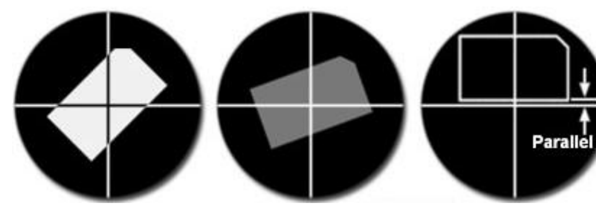


Yeast



Asbestos

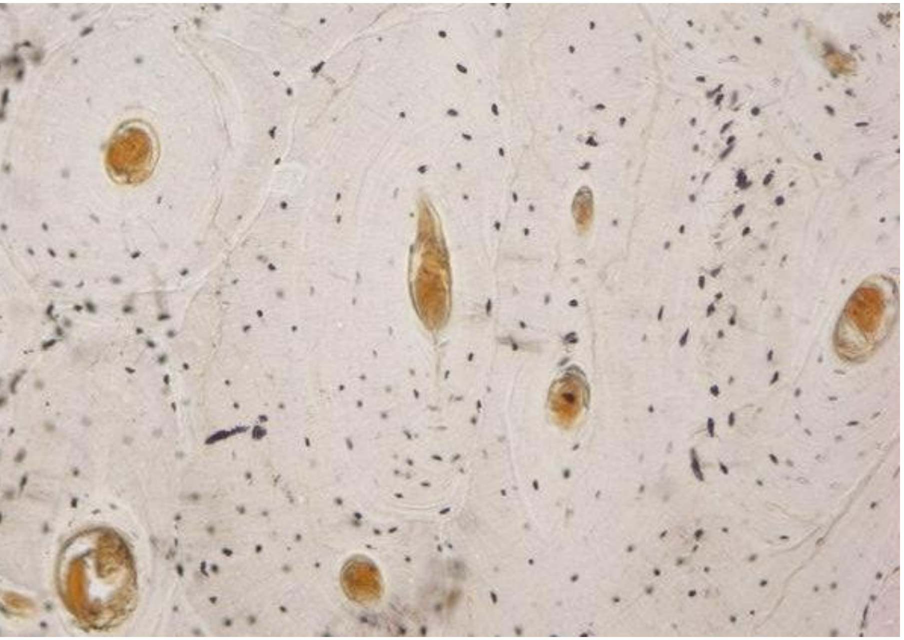
Cross Hair Eyepiece



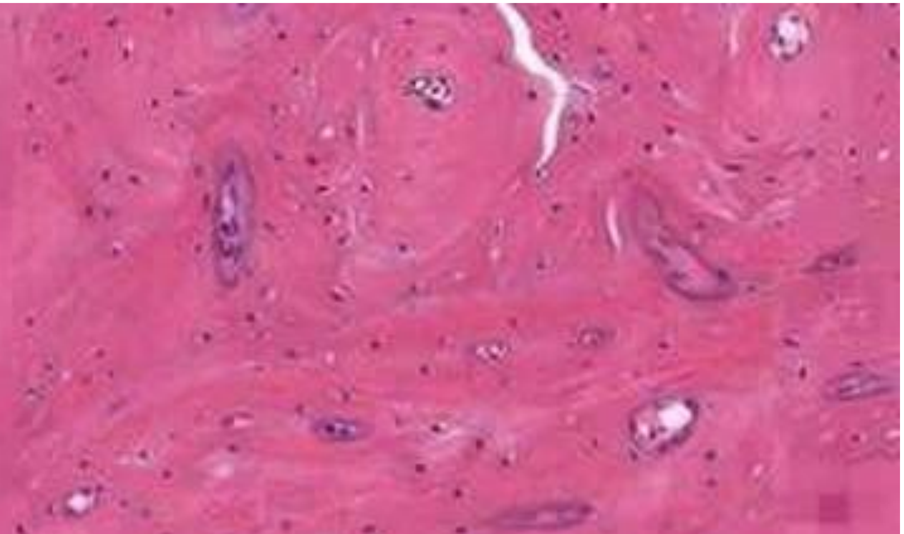
Plants

BONE

Unstained

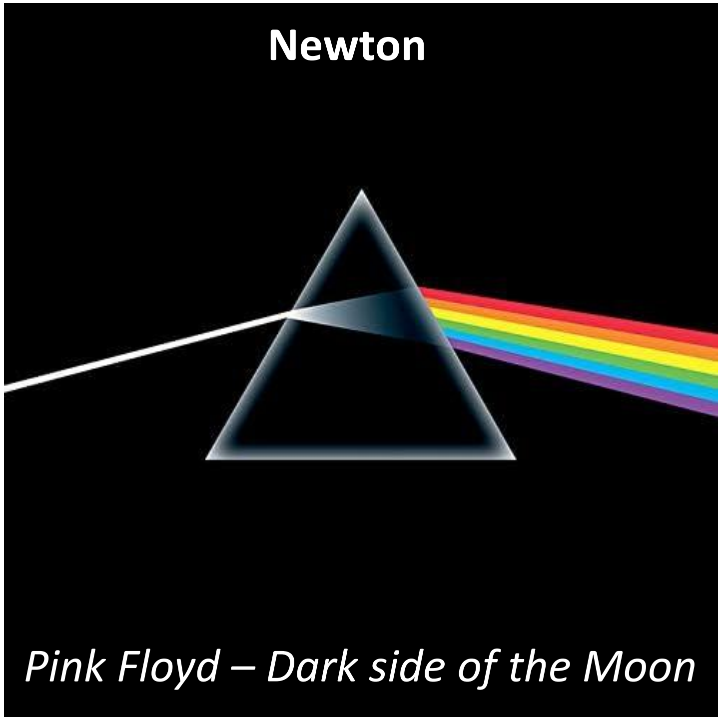


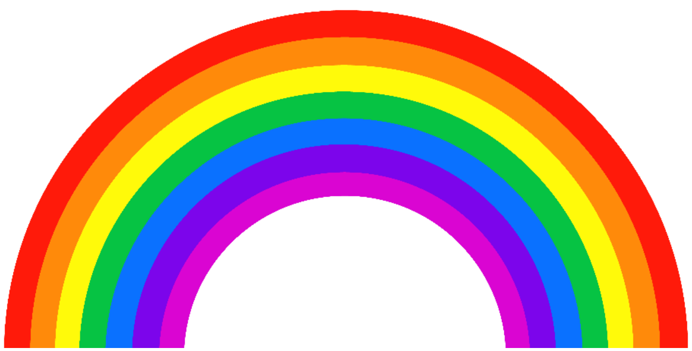
H&E



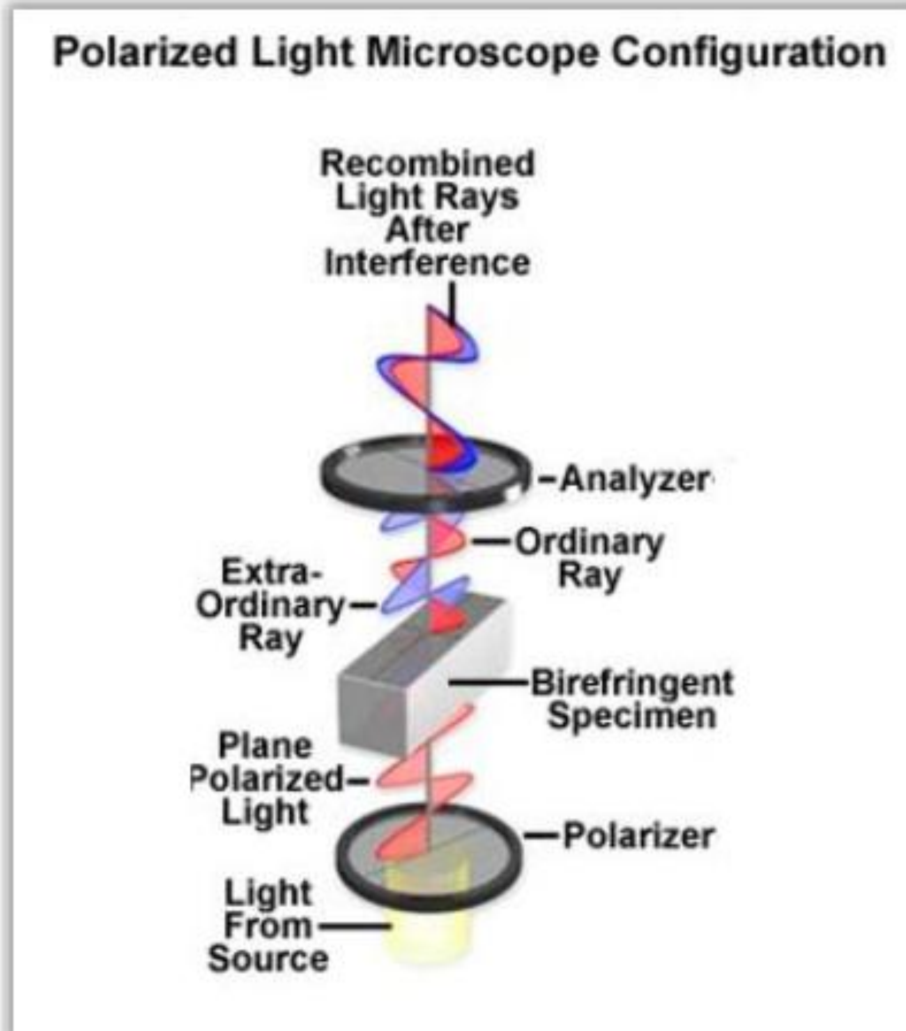
Polarised (Crossed Polars)

What about Polarising Colours?

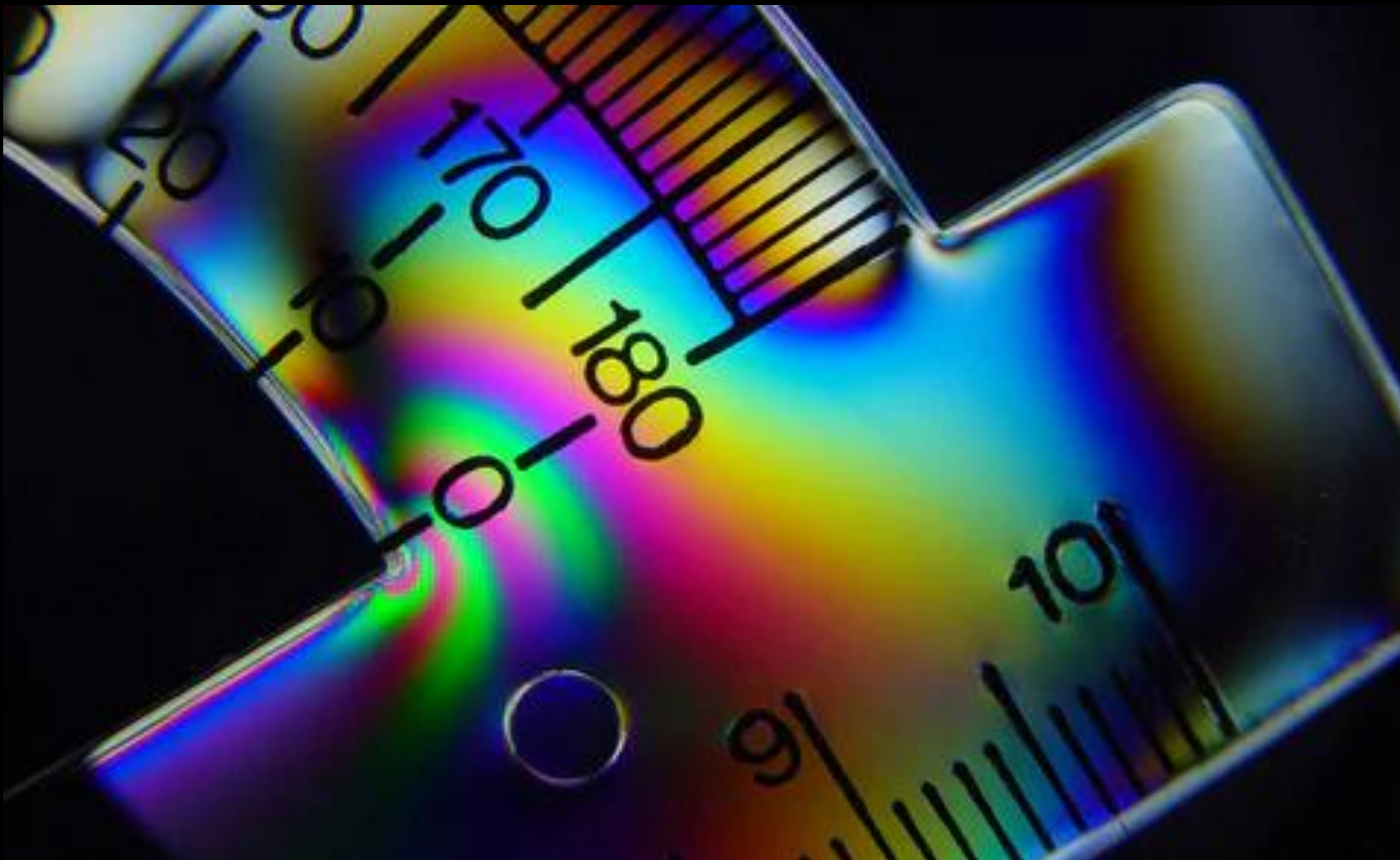




R O Y G B I V … 7



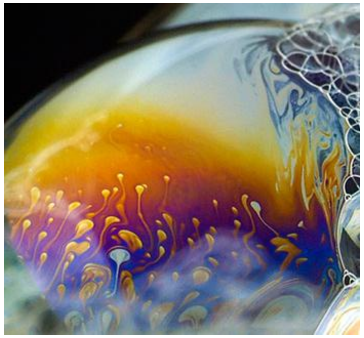
But polarsing colours not in same order !



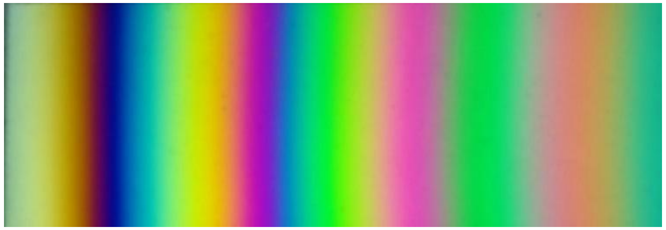
170
180
10

Polarising Microscope 3

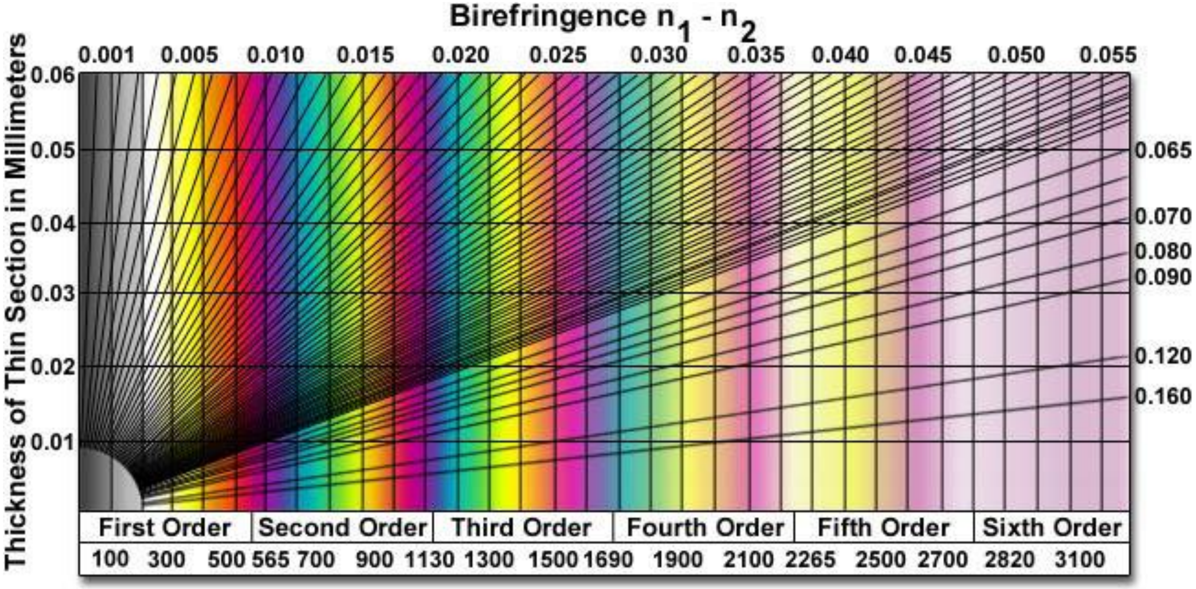
Pool of water on oil



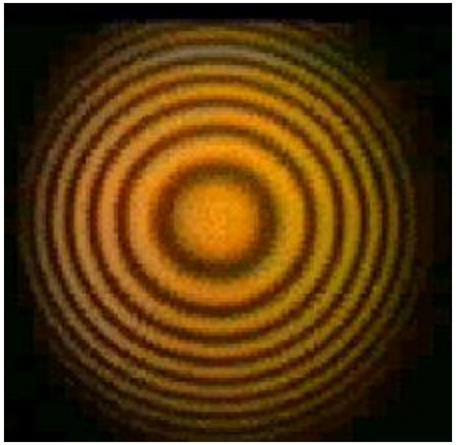
Soap Bubble

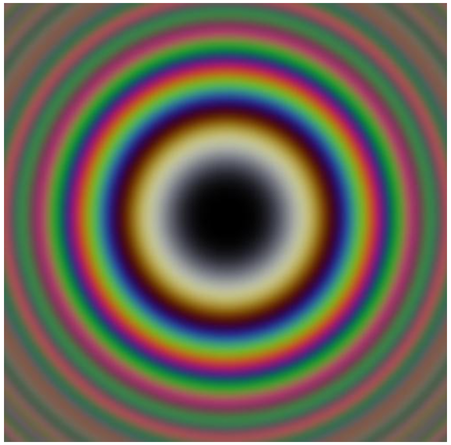


Michel-Levy Colour Chart (1888)



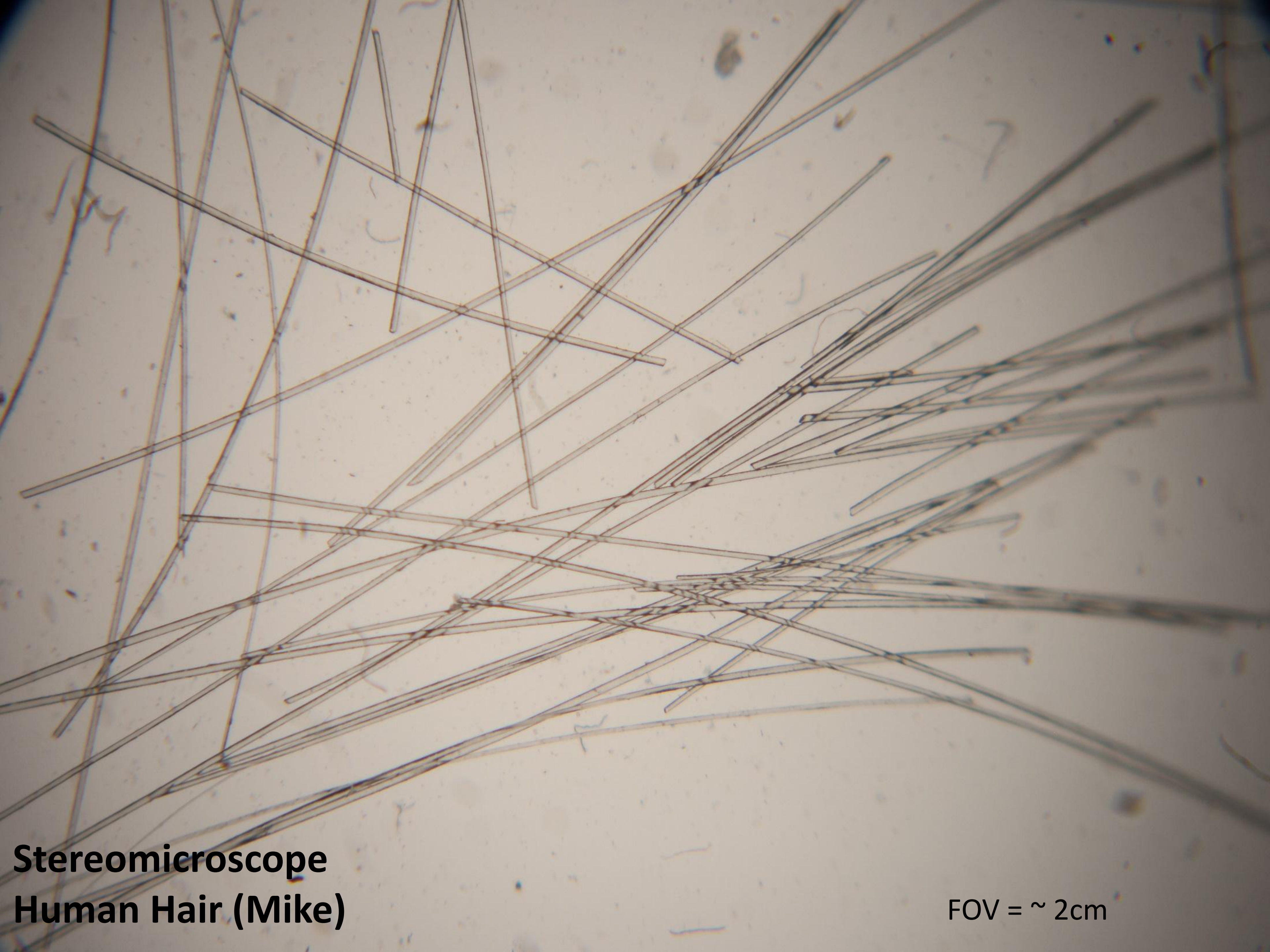
λ nm





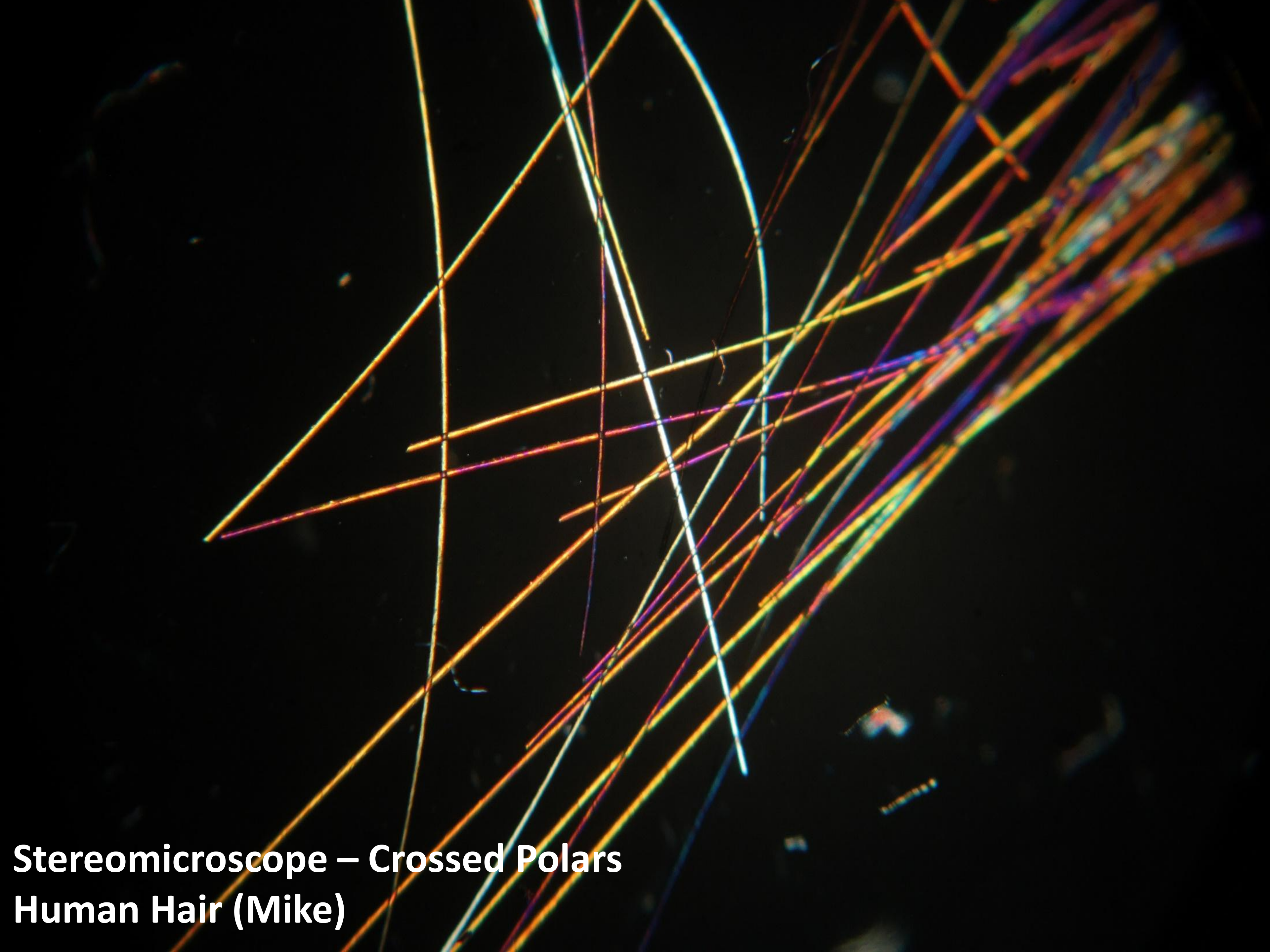
Newton's Rings

Gray White Yellow Orange Red Violet Blue Green

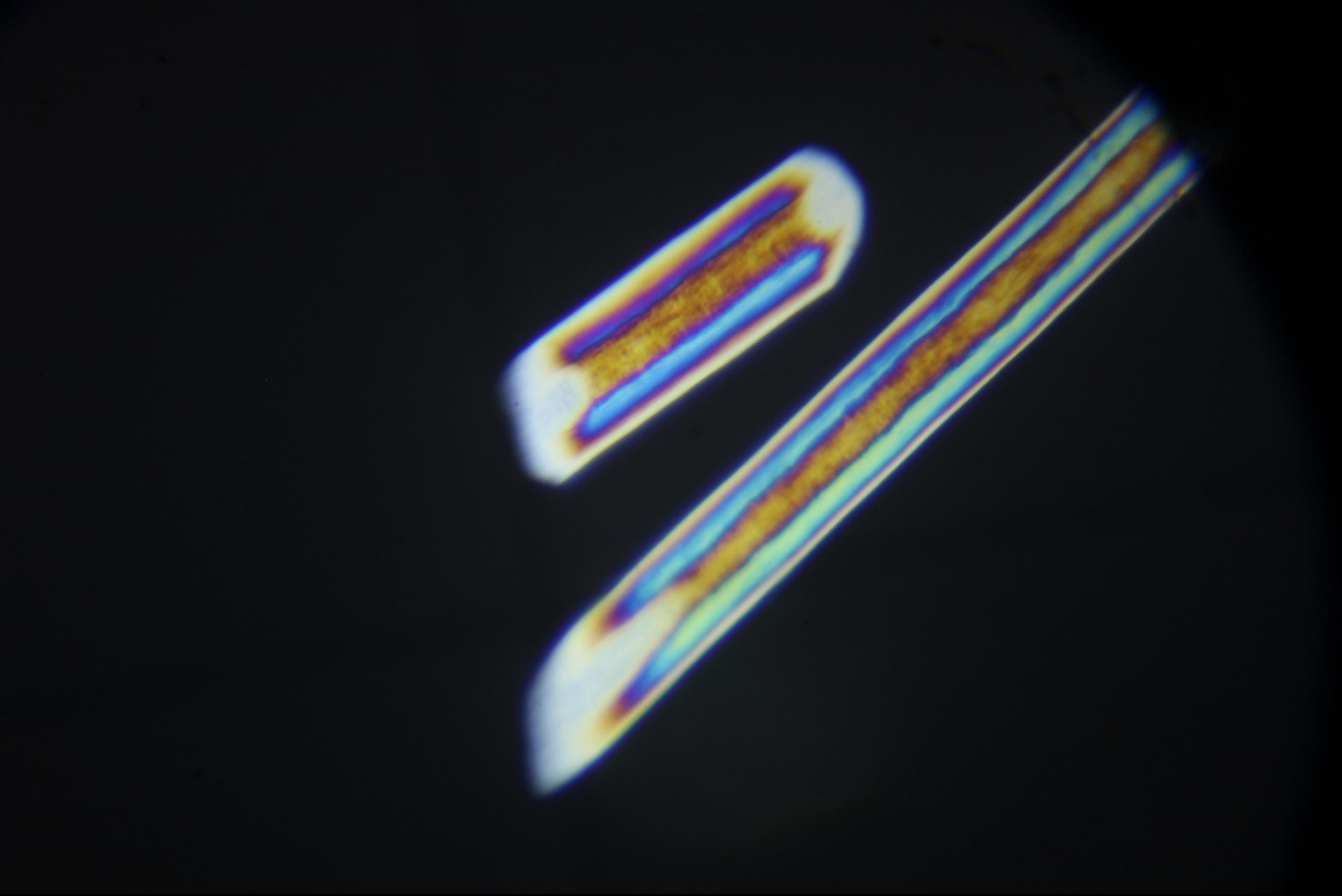


Stereomicroscope
Human Hair (Mike)

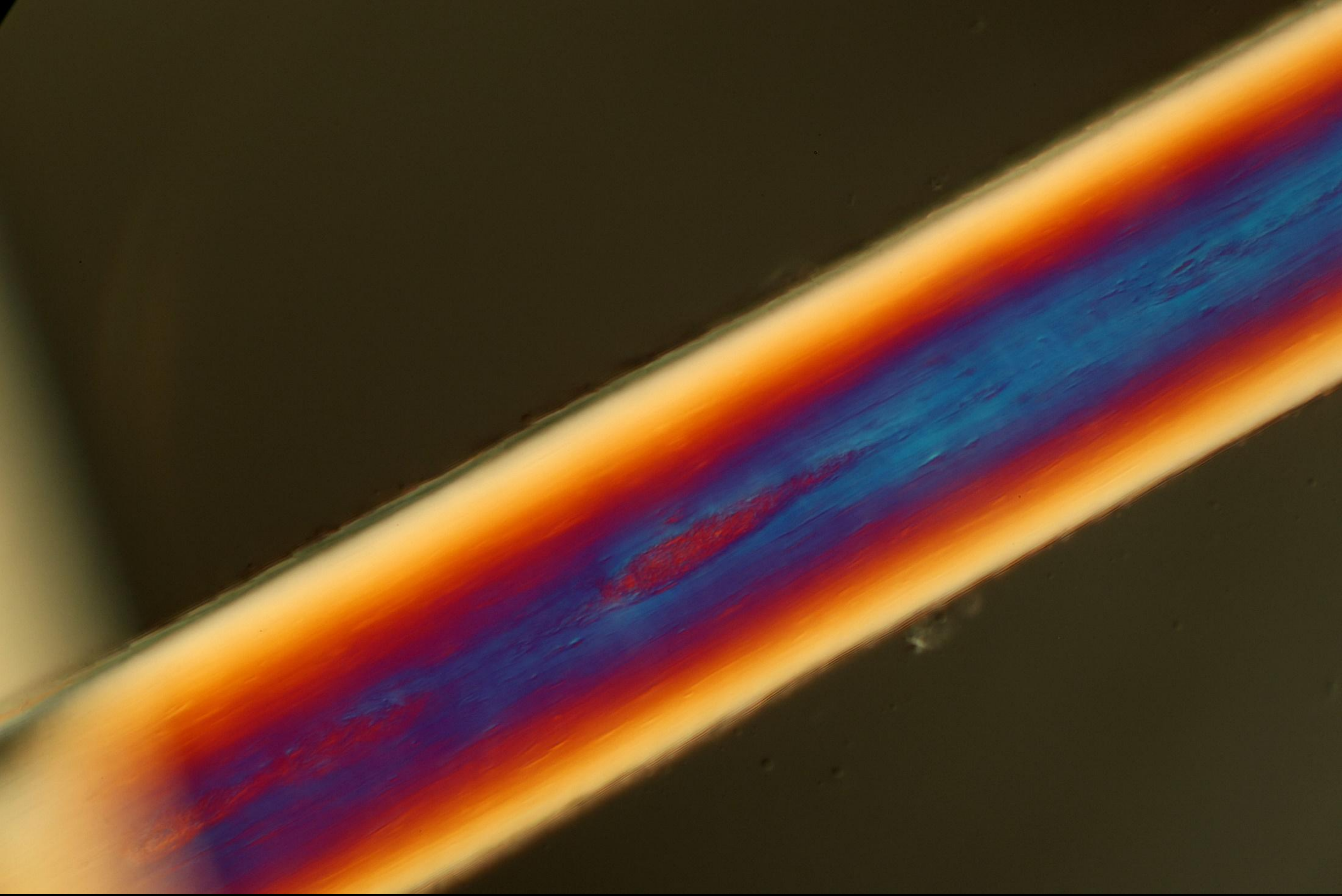
FOV = ~ 2cm



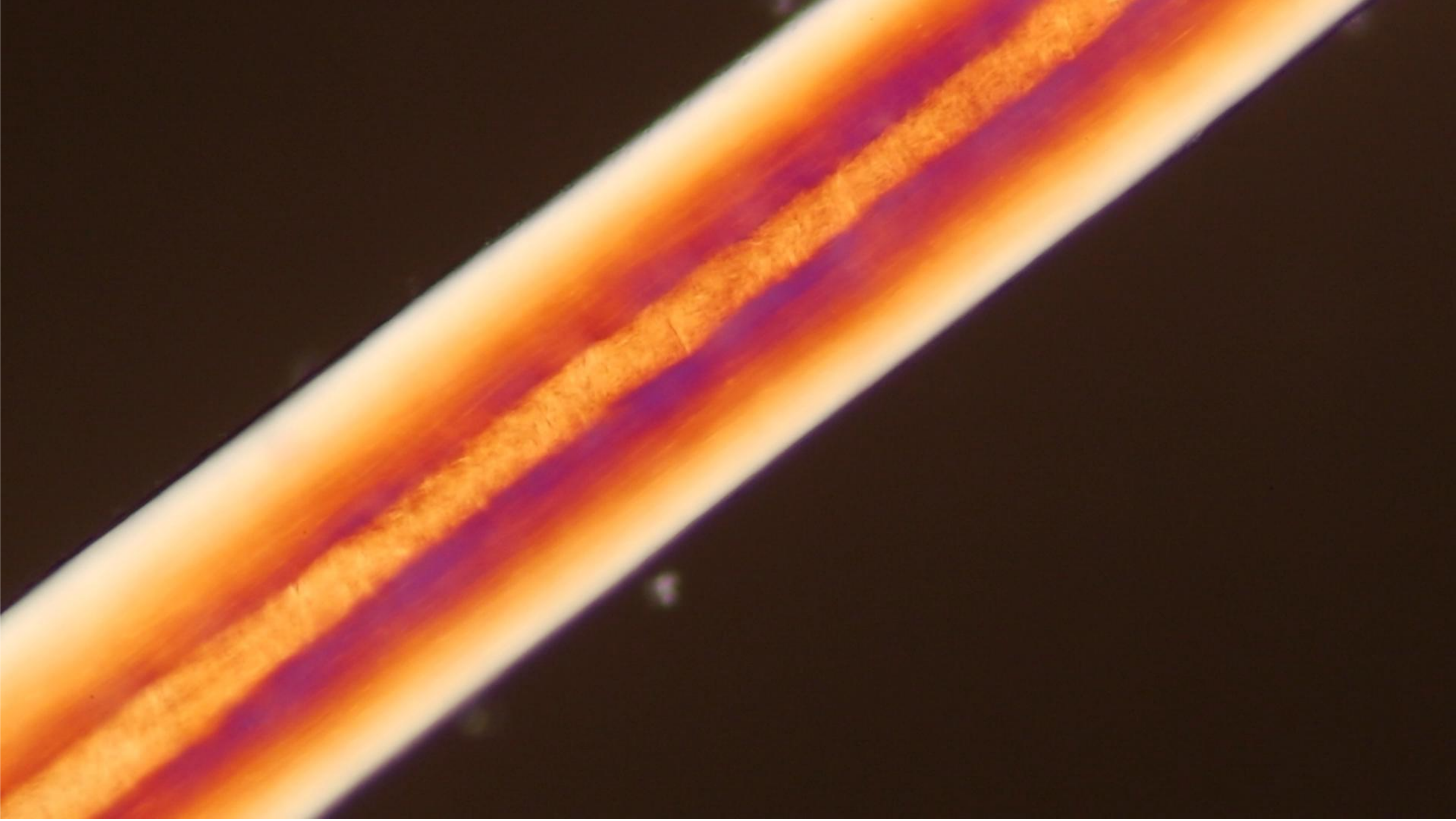
Stereomicroscope – Crossed Polars
Human Hair (Mike)



Compound Microscope – Crossed polars (x40)
Human Hair shavings (Derek Haworth)



Compound Microscope – Crossed polars + DIC (x40)
Human Hair (Mike)
Diameter ~80µm

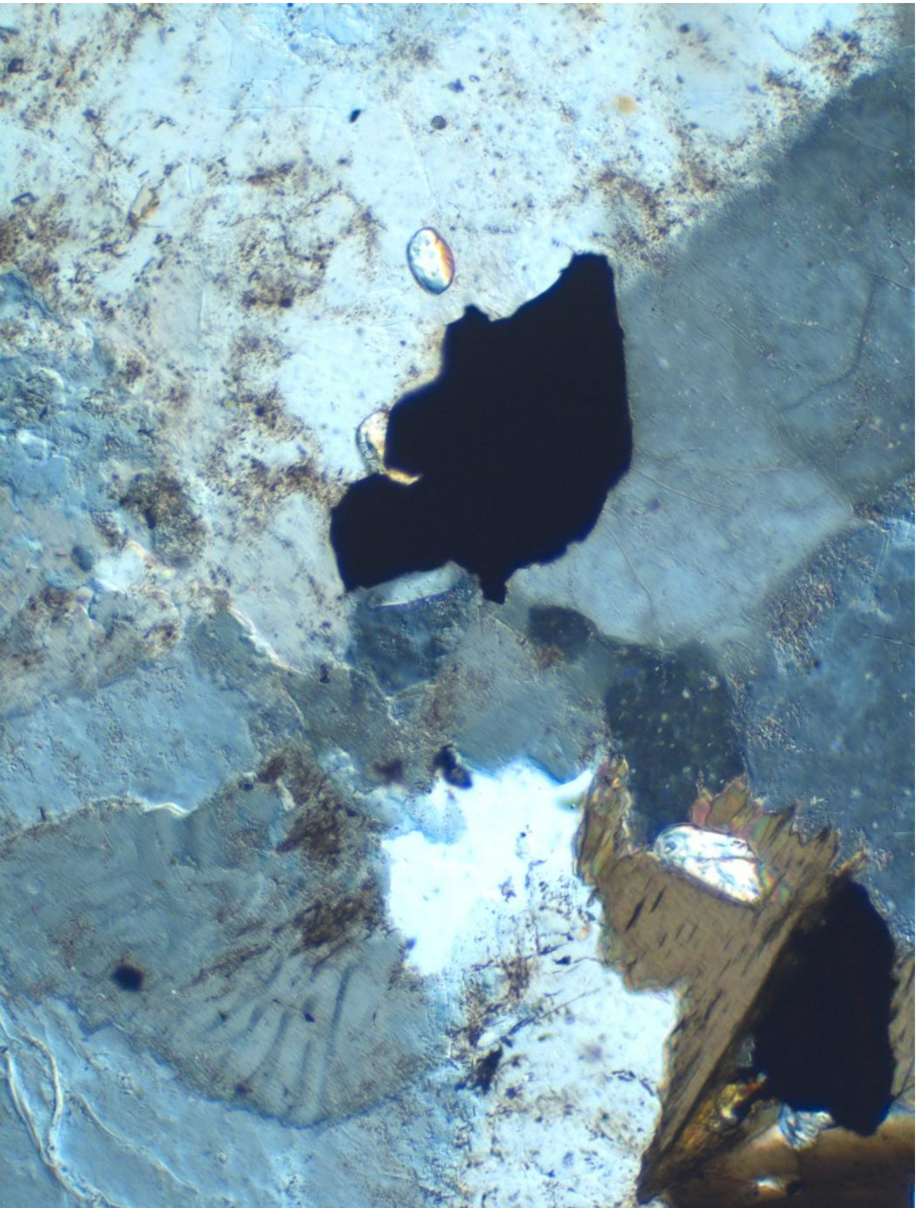


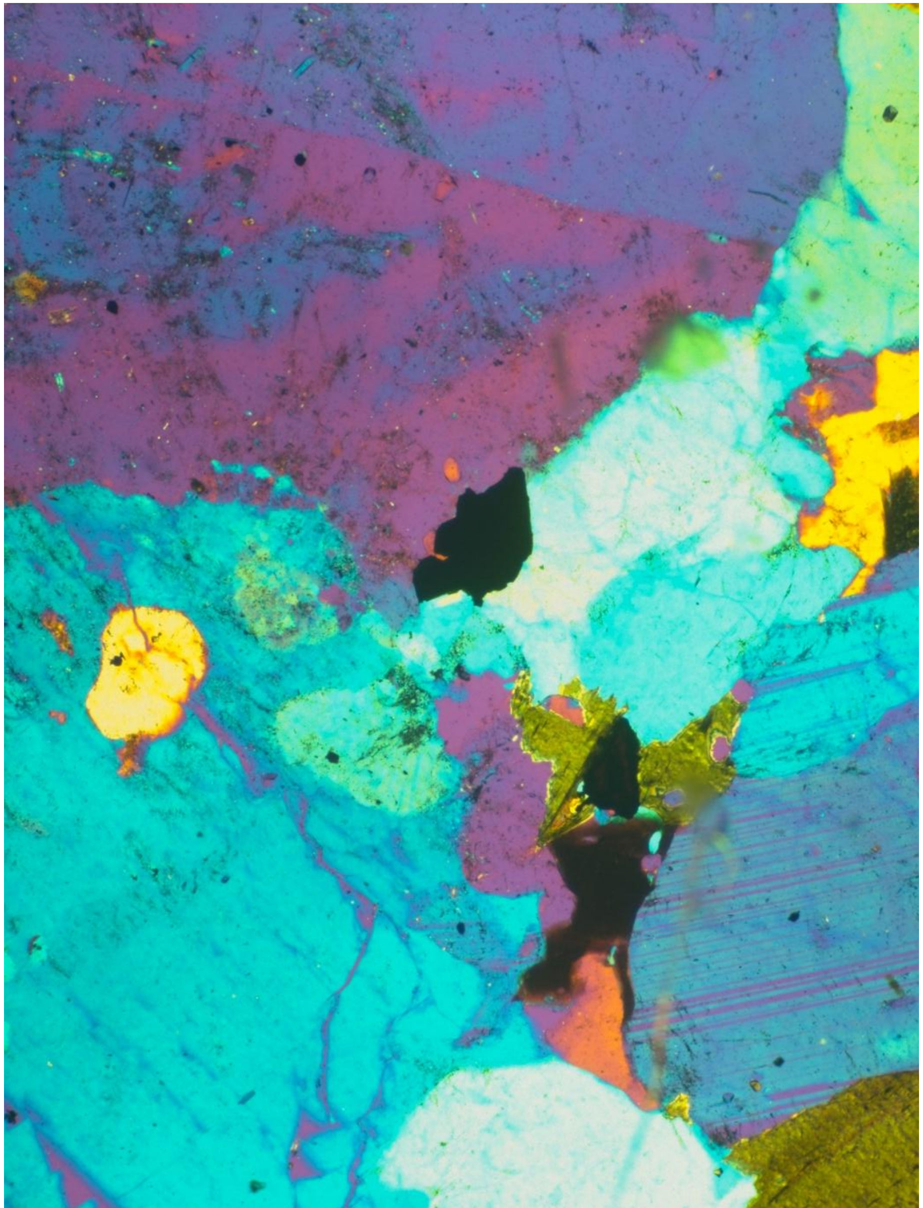
Videos – polarising microscopy in action with rotation of microscope stage

Human Hair (Mike)

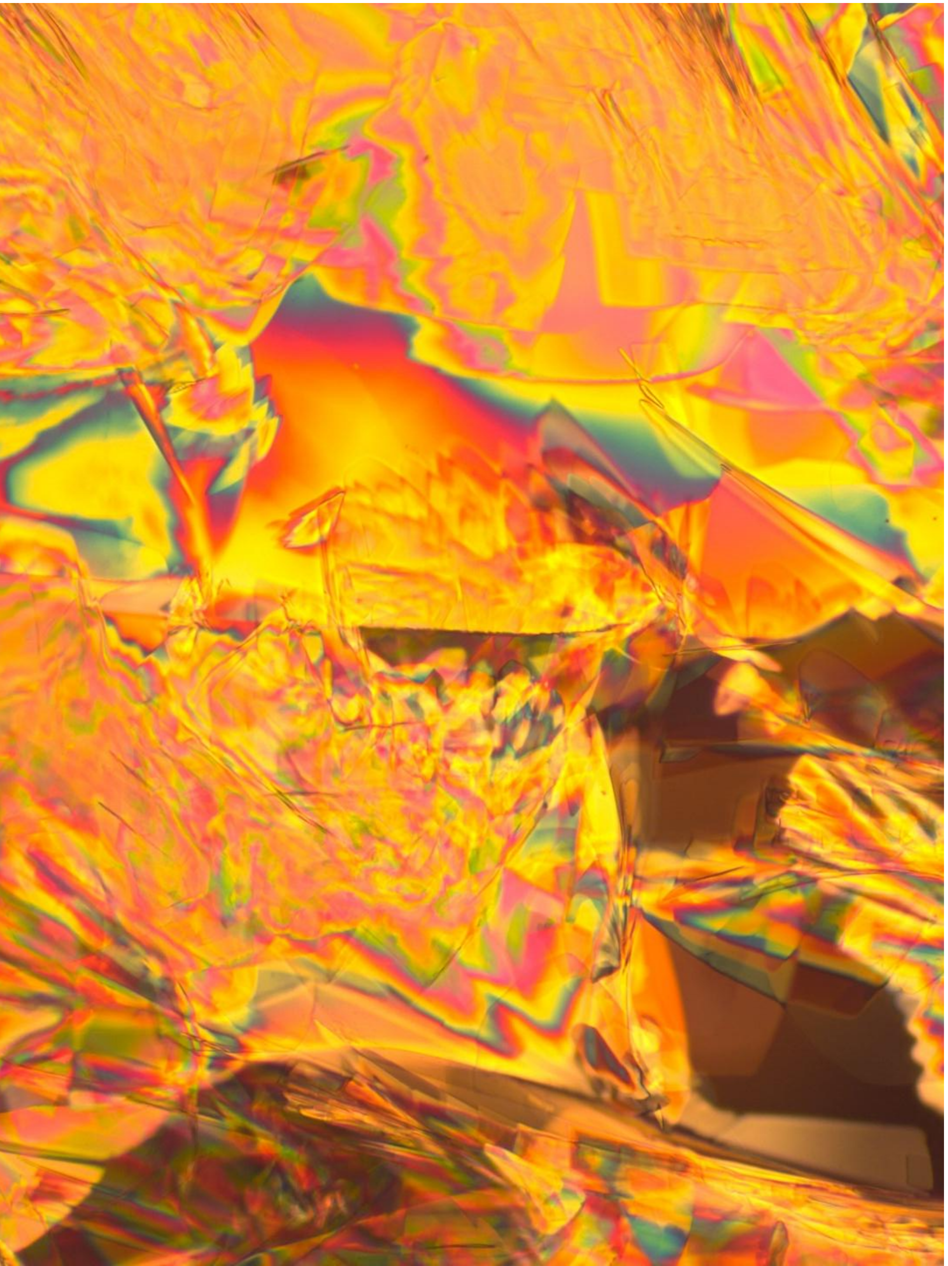
Maximum brightness when sample main RI is at 45^0 to polars

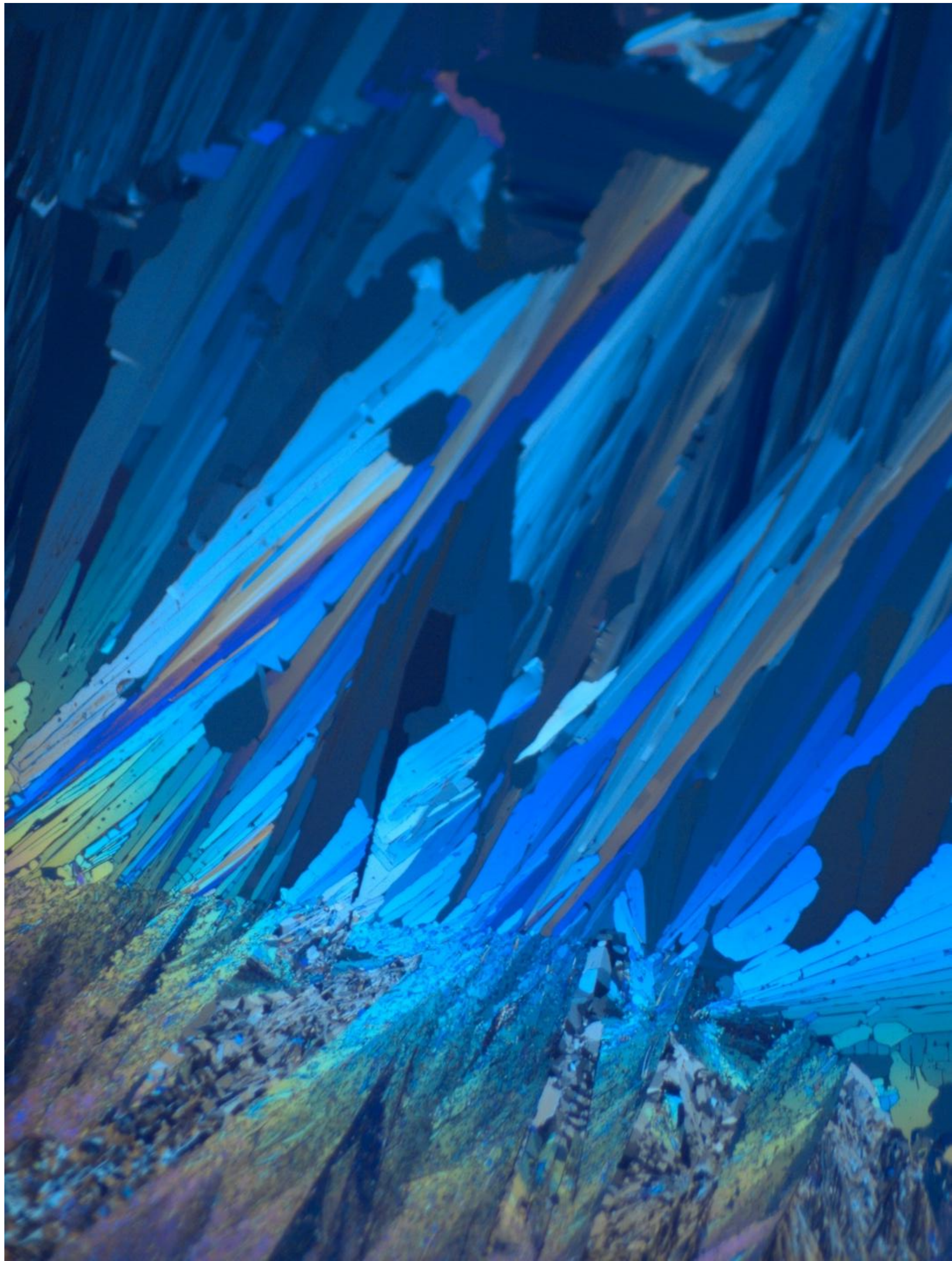
Rocks





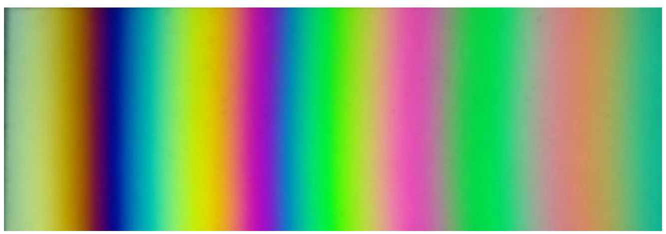
Crystals



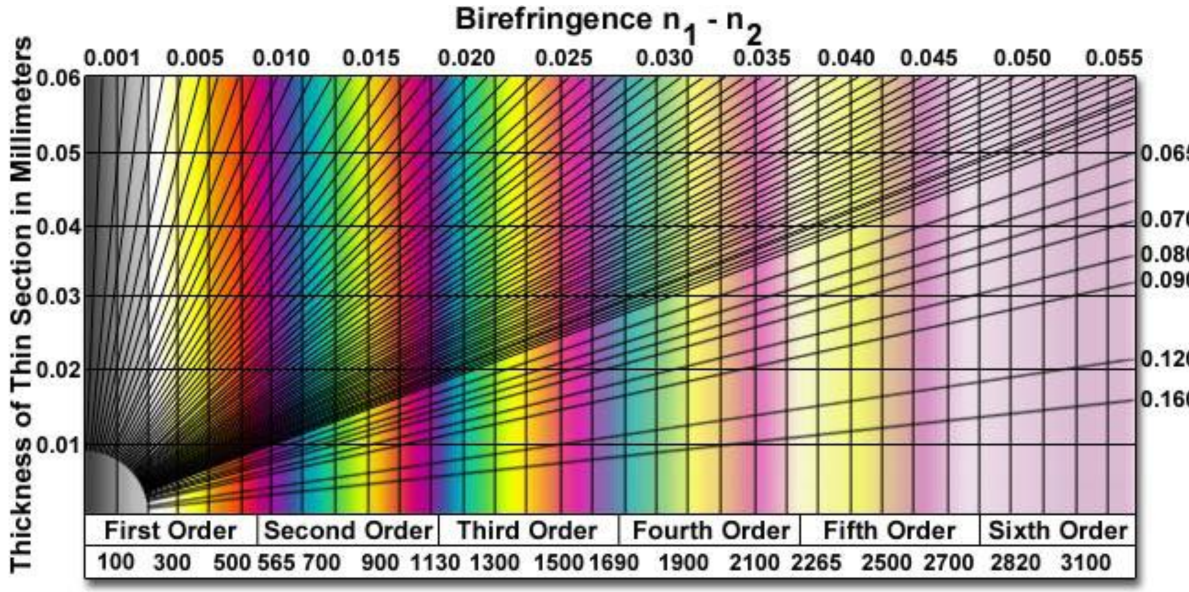


Polarising Microscope 4

Pool of water on oil



Michel-Levy Colour Chart

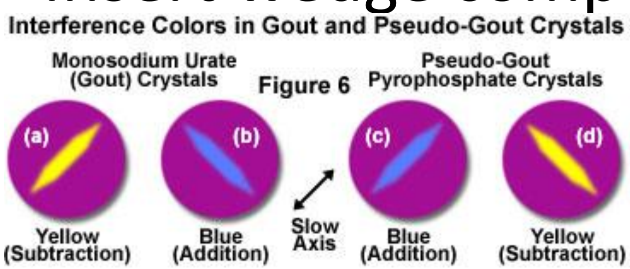


ʎ nm

- Insert Full λ, ½ ʎ, ¼ λ retardation plate to change colours

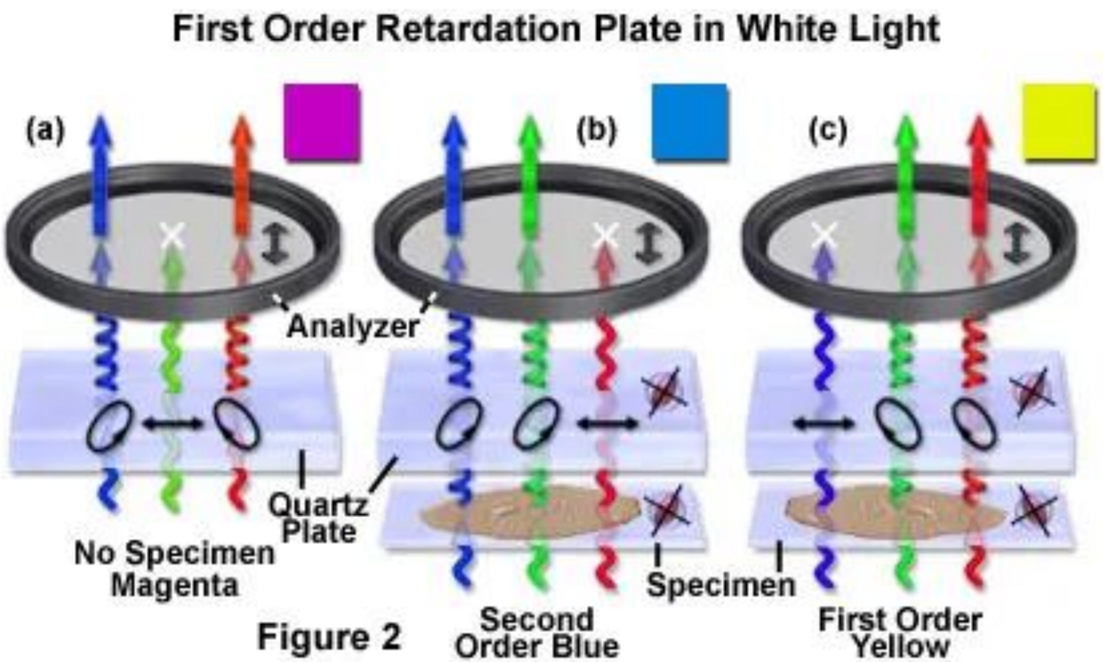
  550, 275, 137 nm

- Insert wedge compensator to measure thickness

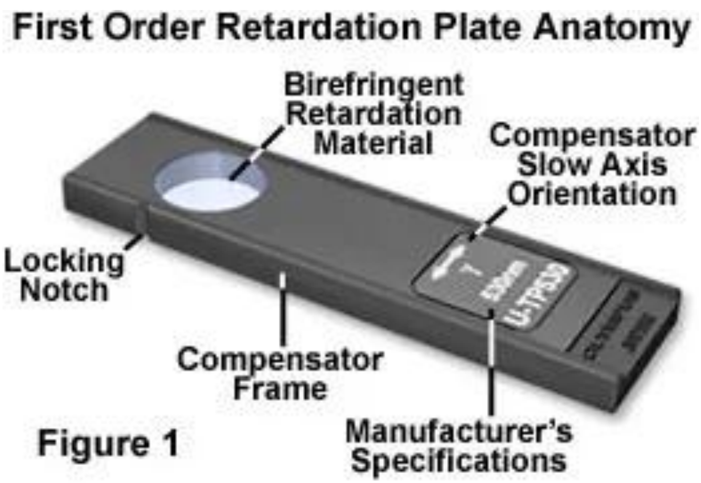


.. at 45^0 to Analyser

Full λ, red plate



Primary Colours

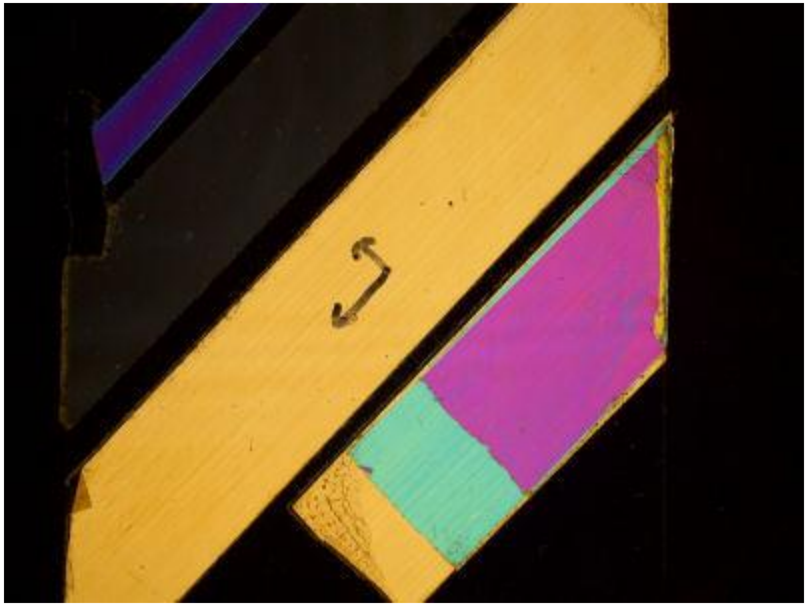


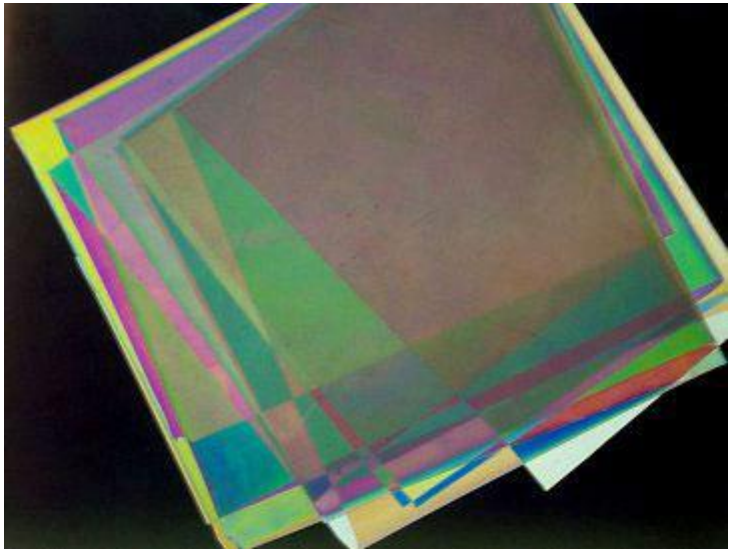
.. at 45^0 to Analyser

Cheap version !

[Olympus]

Quekett, Don Thompson – Experiments with Sellotape

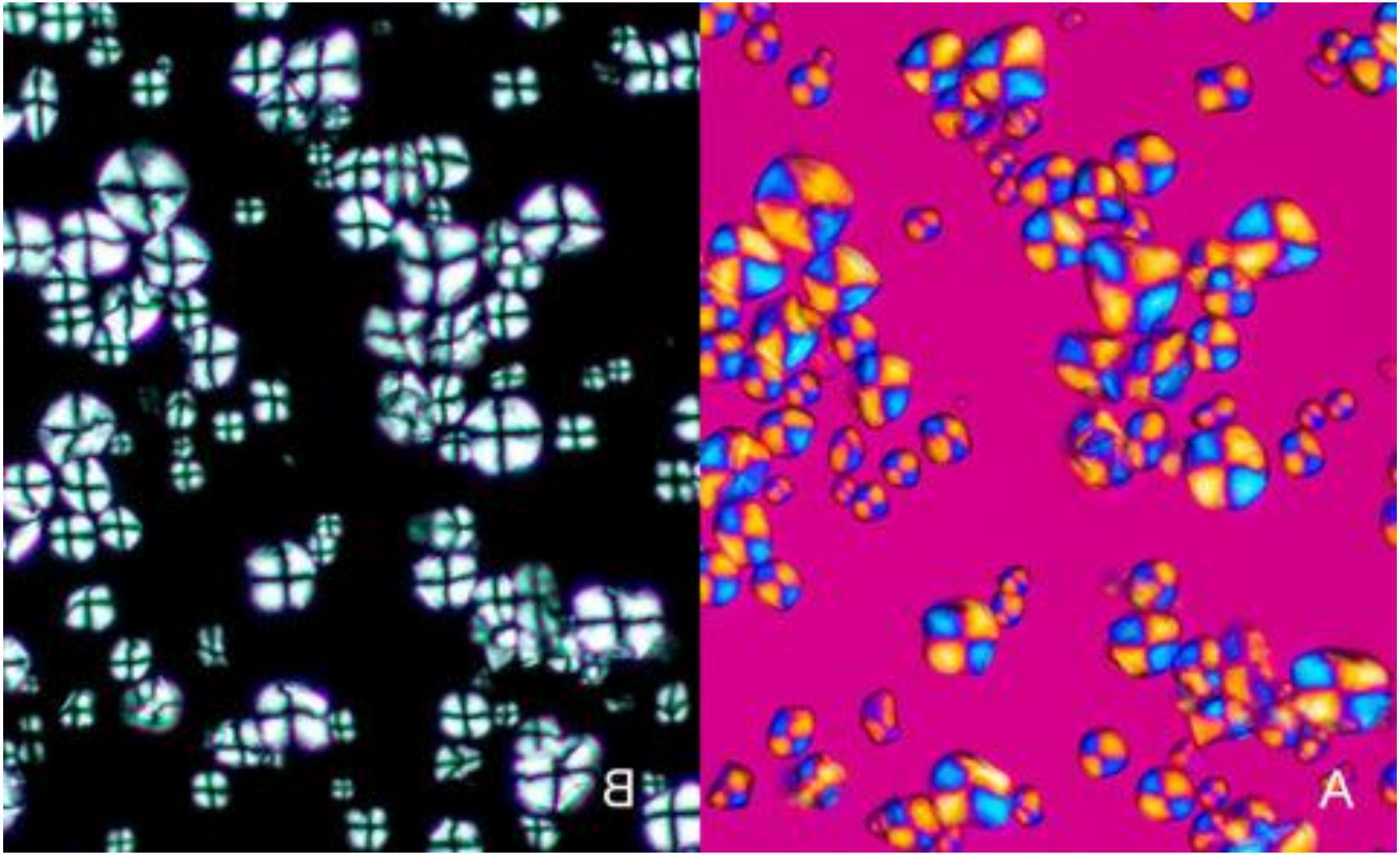


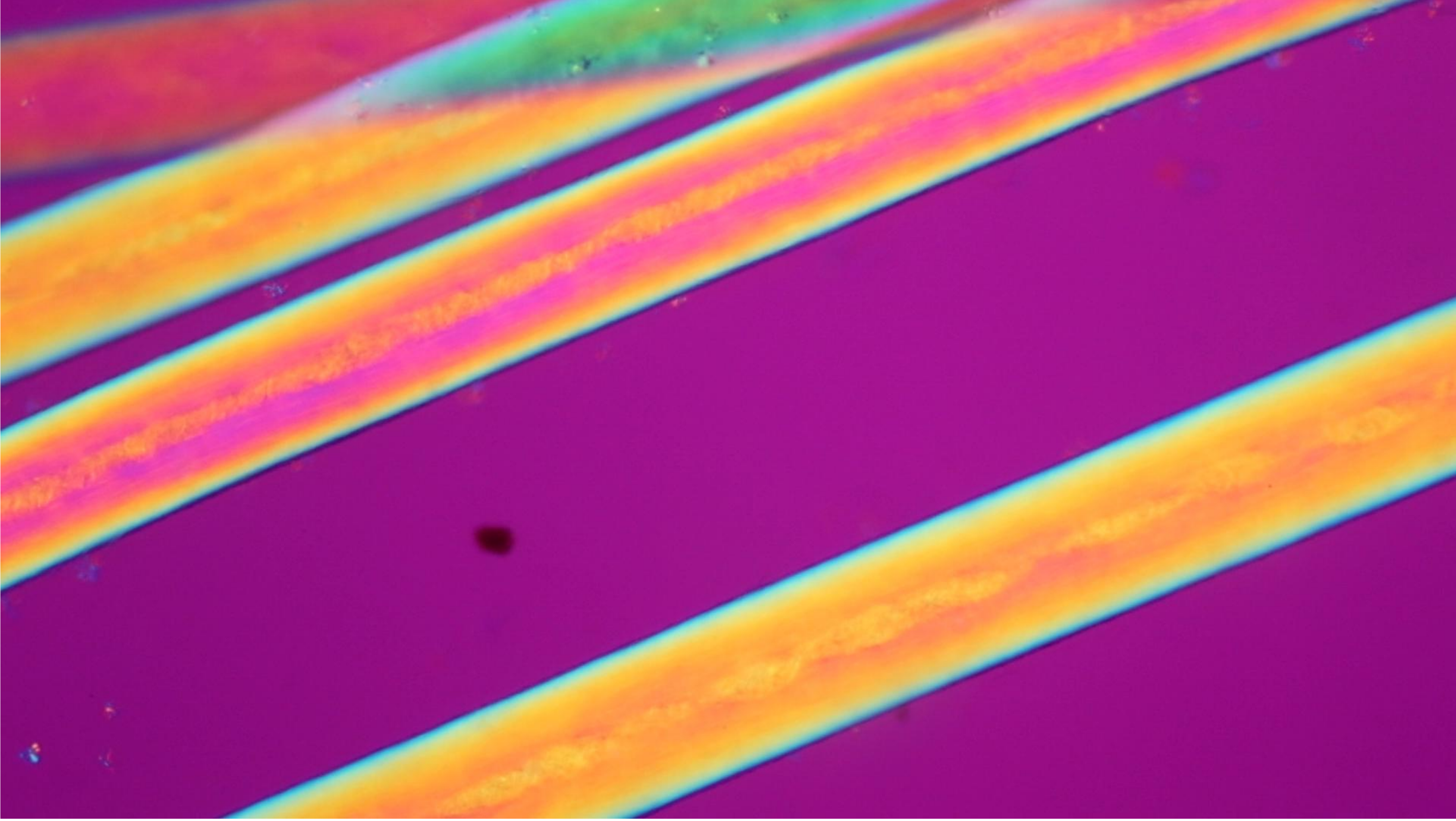


Cellophane

Starch

Crossed Polars **Crossed polars + Full ʎ Compensator**





Videos – polarising microscopy in action with rotation of microscope stage

+ 530nm Full Wave plate

Human Hair (Mike)

Tardigrade – Crossed Polars + Full λ Compensator (Motic)

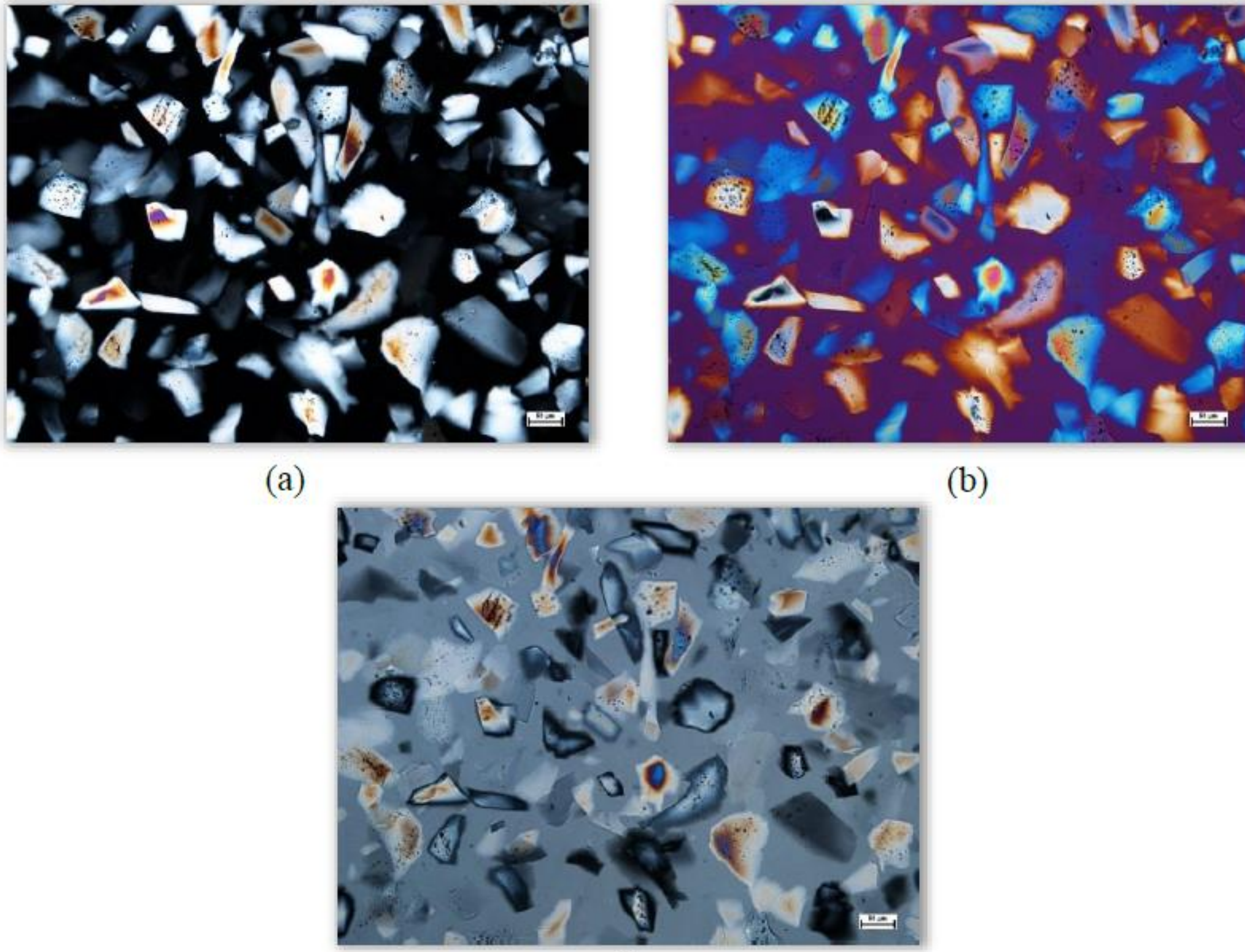


Figure 16. (a) CP with no waveplate. (b) CP with red waveplate. (c) CP with quarter-wave plate. [8,8,8]

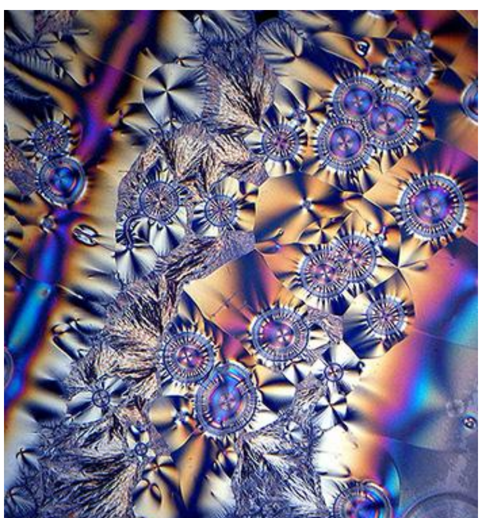
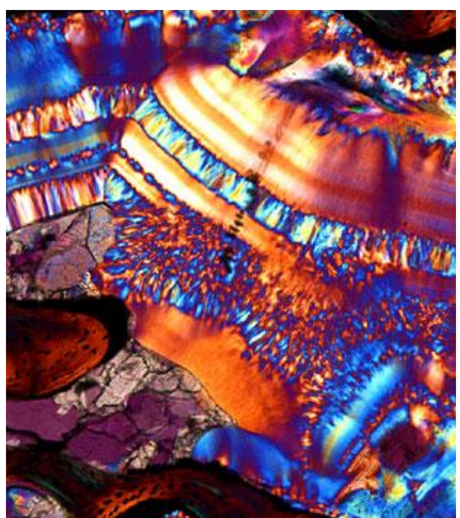
Polarising Microscopy - Uses

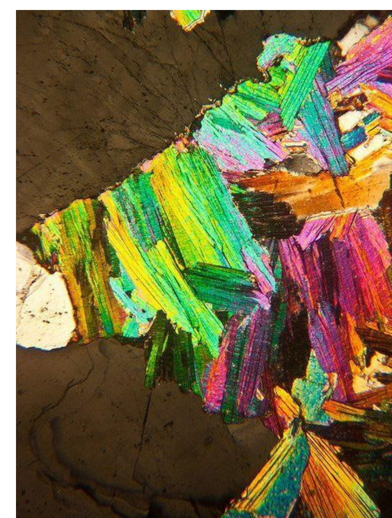
- **Micro/Macro** – images with pretty colours !
- **Histology** - identifying orientation (bone, tendon, teeth, ligaments, muscle, …)
- **Pathology** - identifying deposits (amyloid, urate, …)
- **Forensics** - fibres, glass, plastics, particle analysis
- **Chemistry** – crystals
- **Geology** – rock, oils, composition
- **Metallurgy** – metal organisation, stress
- **Ceramics** – clays, provenance
- **Astrophysics** – meteorites, planets
- **IT** – semiconductor analysis, LCD screens, 3D, …

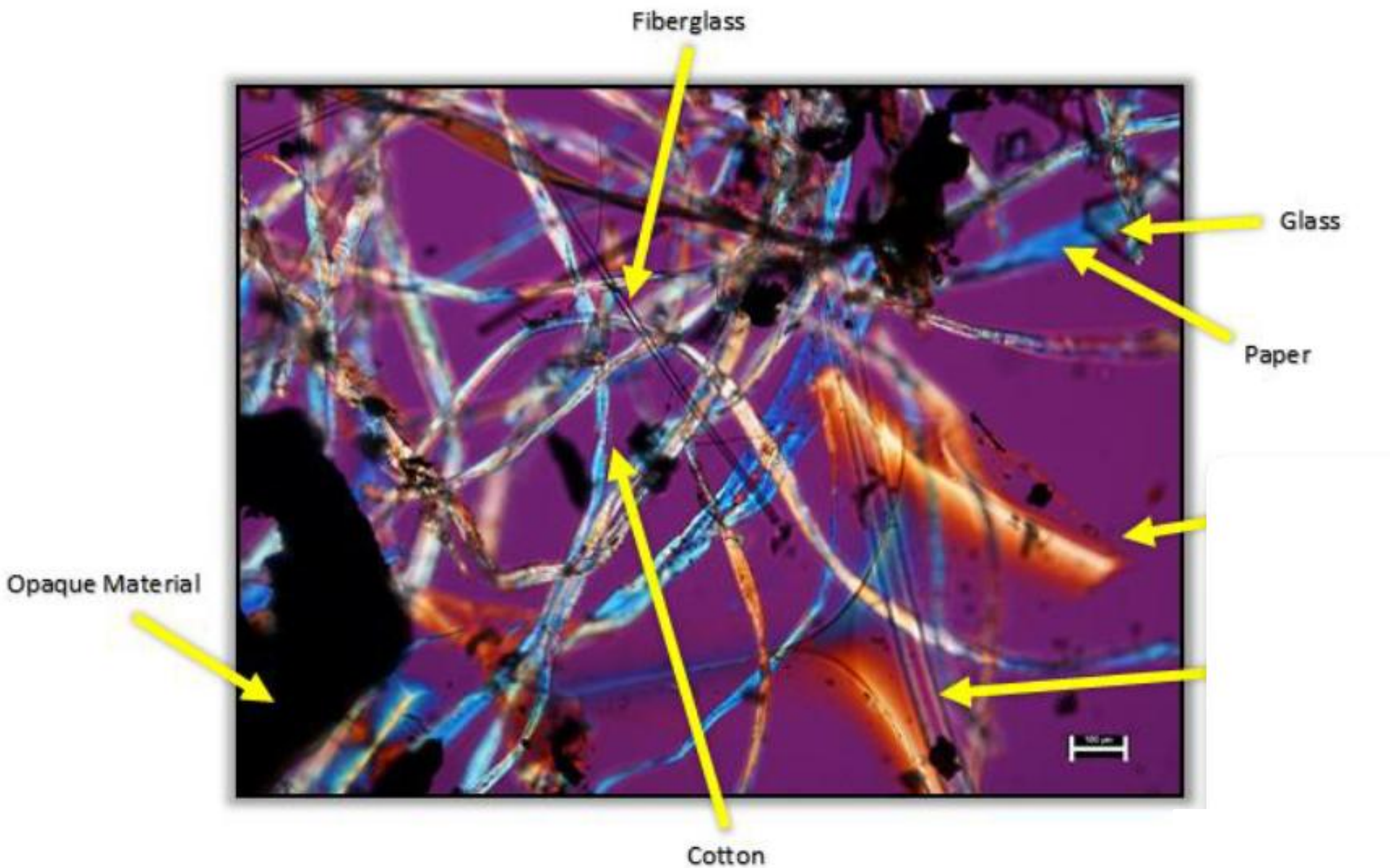
.. also **Reflectance Polarising mode, Conoscopic mode**

Nikon

Demo




Fiberglass
Glass
Paper
Opaque Material
Cotton

Particle / Fibre Analysis

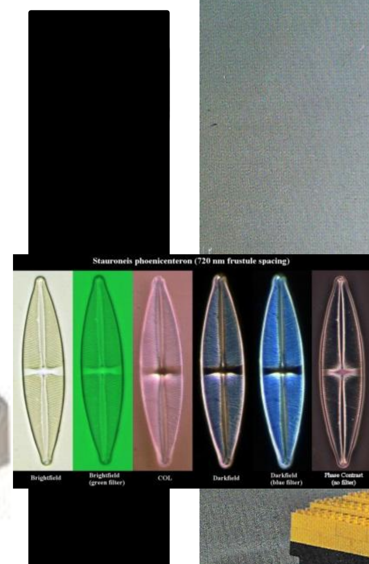
No Polars	Single Polars	Crossed Polars
• Shape/Morphology • Size • Color • Surface features • Transparency/Opacity • Number of phases • Homogeneity • Aggregation • Presence of associated substances	• Pleochroism • Individual refractive indices ▪ Becke line • Dispersion staining	• Isotropy vs. Anisotropy • Extinction • Retardation • Birefringence • Degree of Birefringence • Sign of Elongation • Anomalous polarization colors
		• Optical axial angle • Optic sign

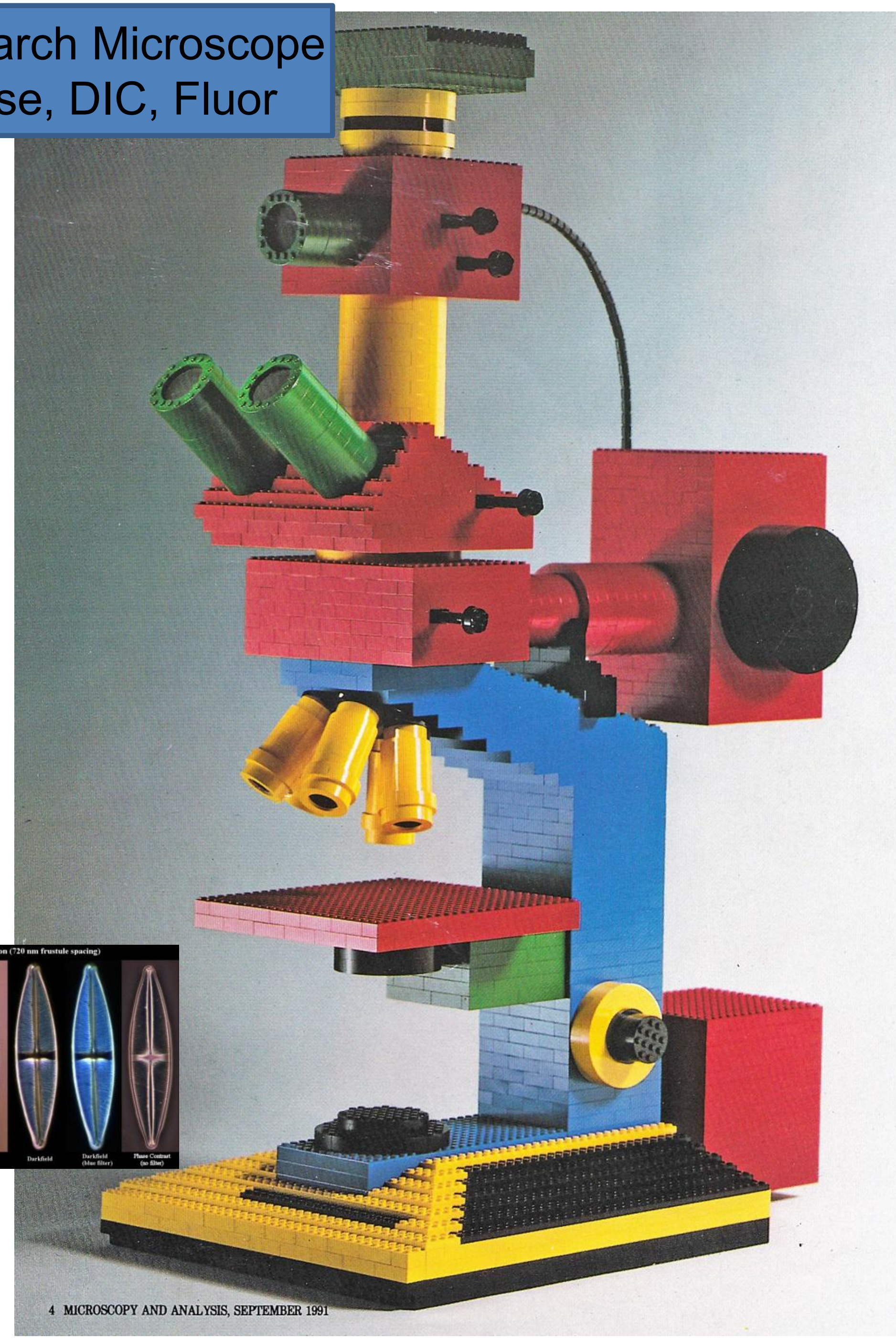
See McCrone, Delly, NASA

Polarized Light Microscope Configuration

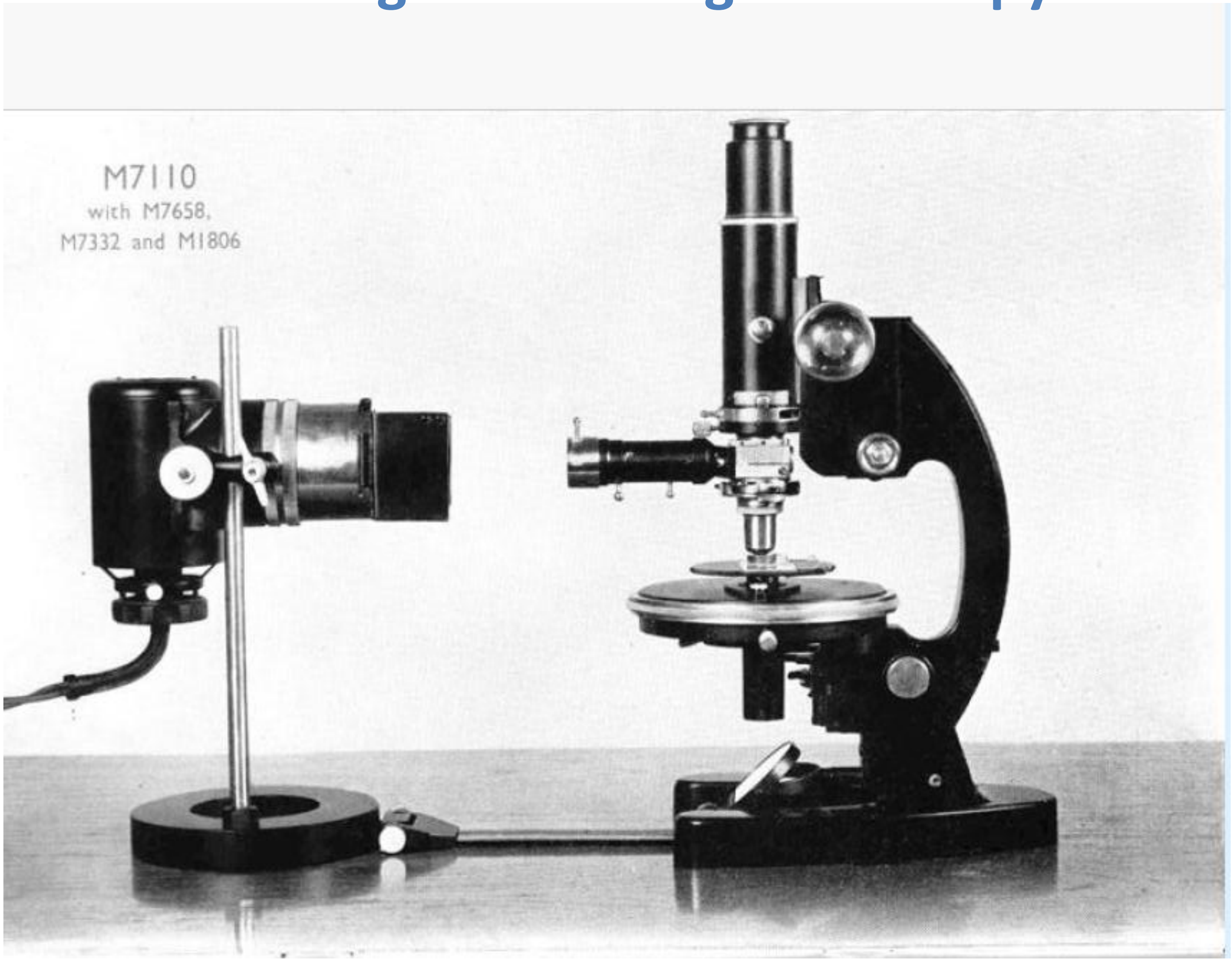
Figure 1

Modular Research Microscope
BF, DF, Phase, DIC, Fluor



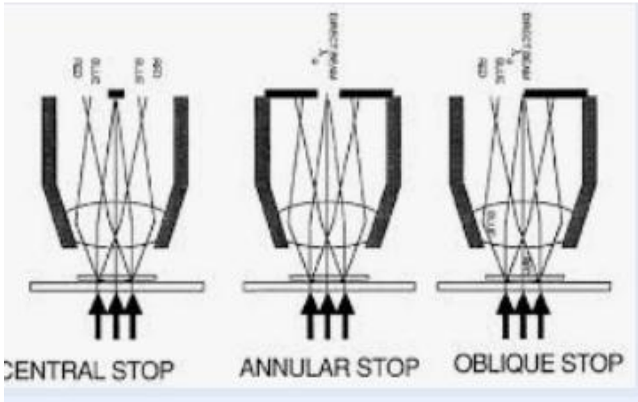


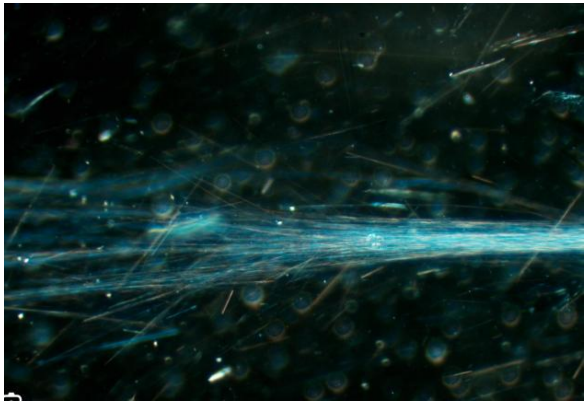
+ Reflected Light Polarising Microscopy

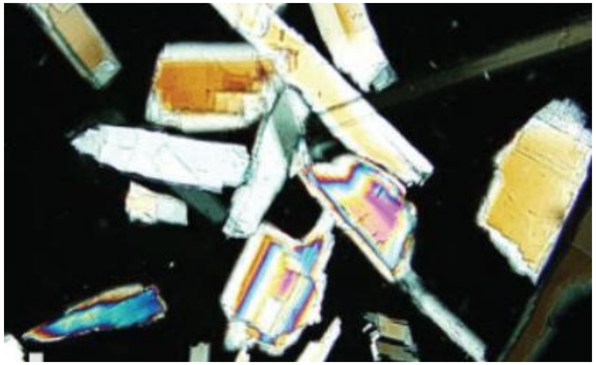


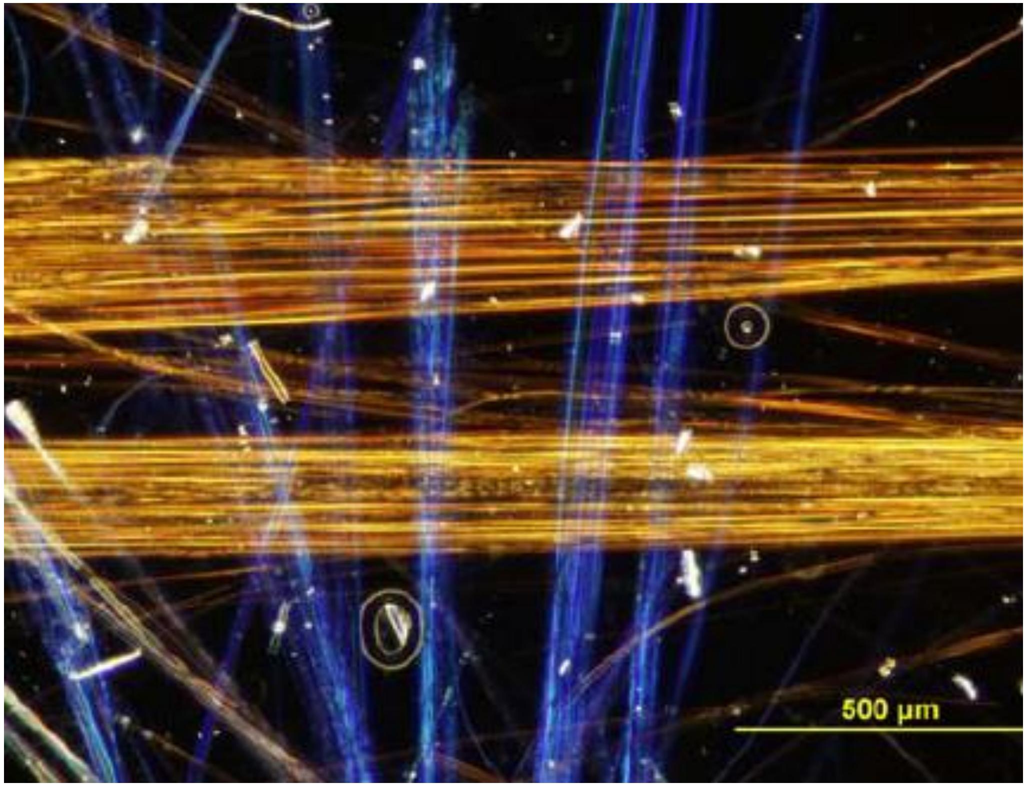
Addendum

Single Polar – Dispersion Staining Objective



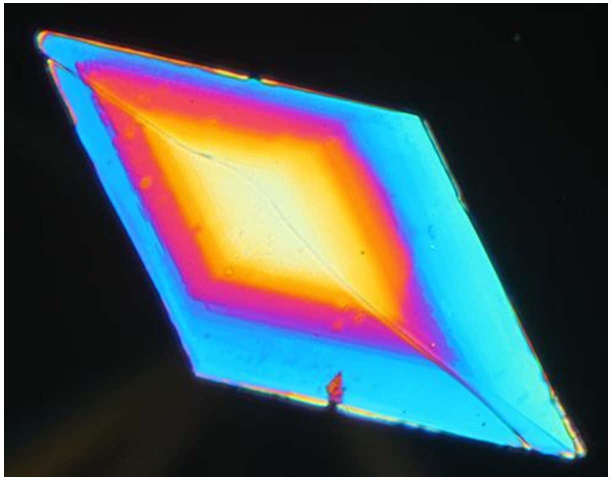
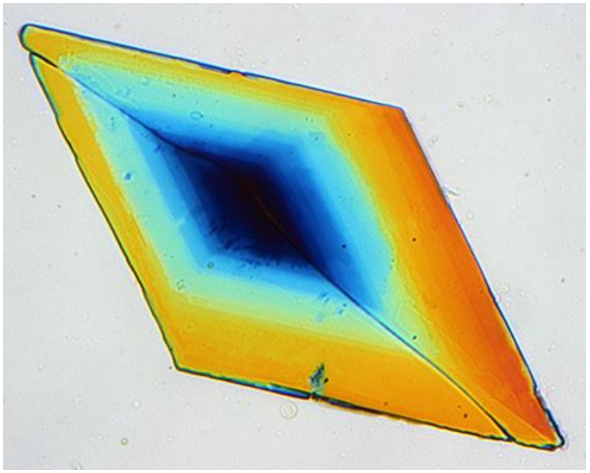




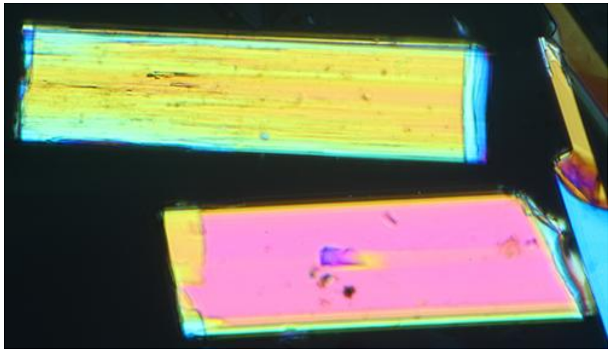
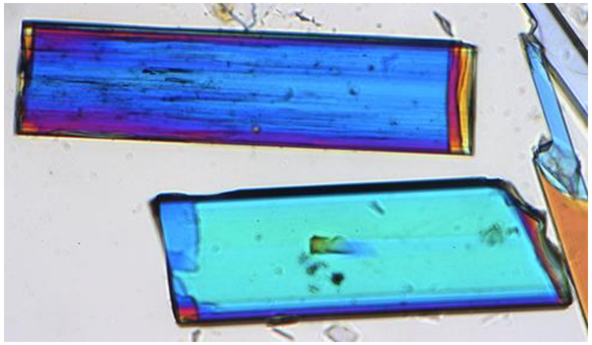


Crossed Polars

Parallel Polars – David Walker, Micscape, 2020

Aspartic acid

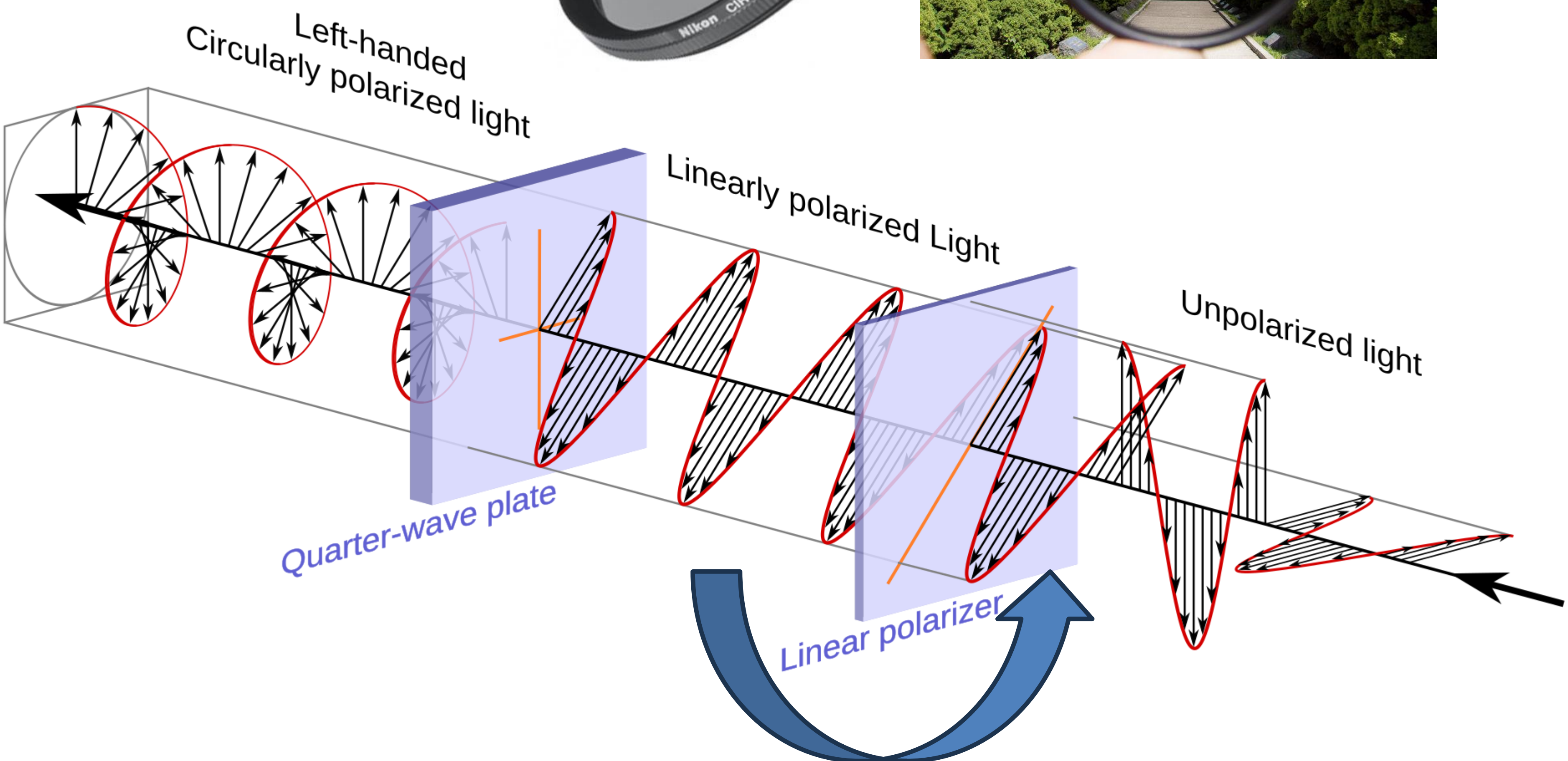
Zeolite


Barium Platinocyanide

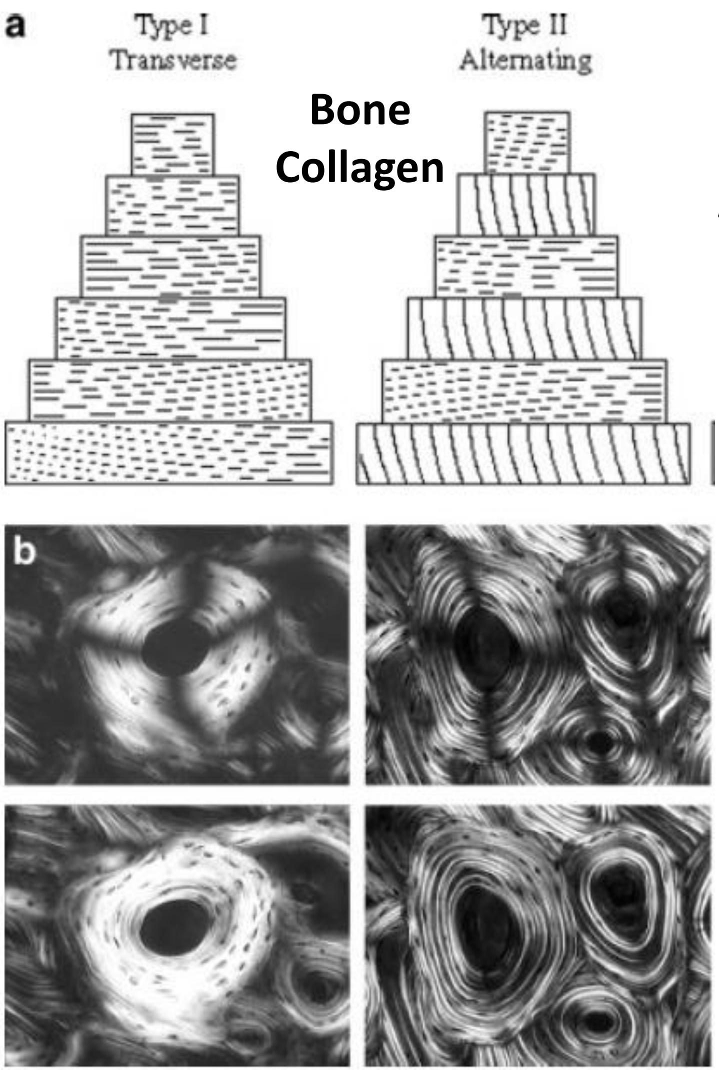
Circular Polarising Filters – used on Cameras

Not usually for polarising microscopy !!

Elliptical / Helical !



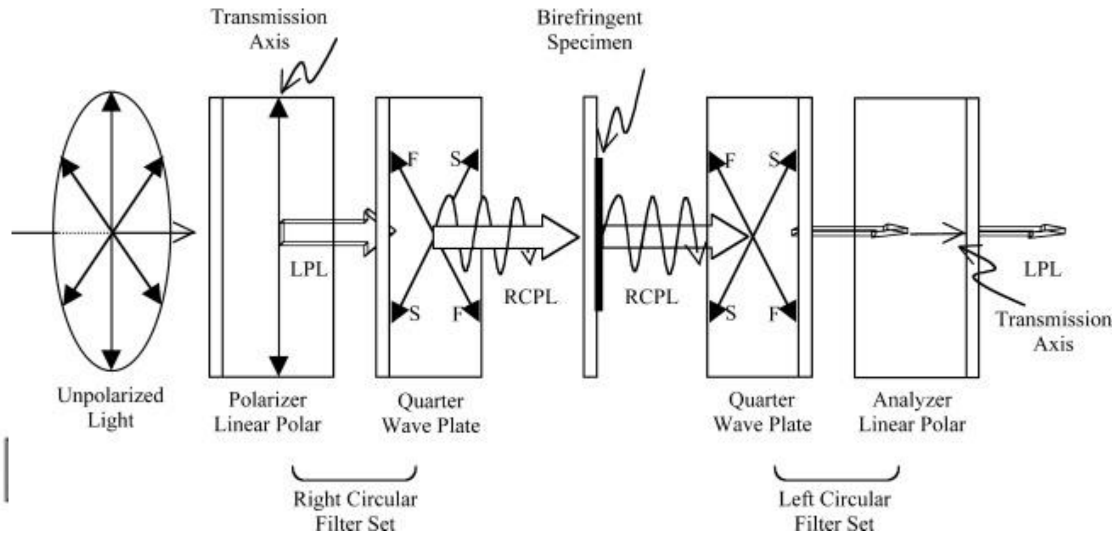
Circular Polarisation Microscopy – Bromage … Boyde 2003

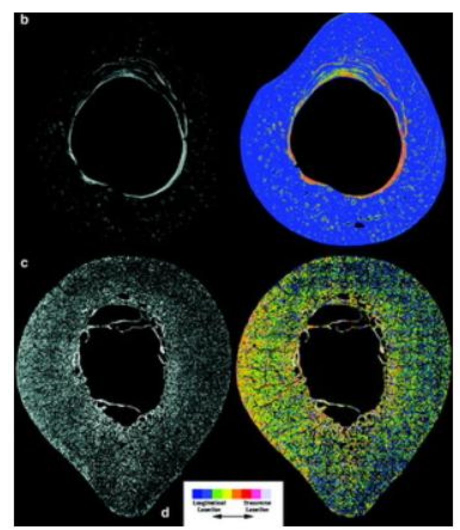


Bone
Collagen

LPL

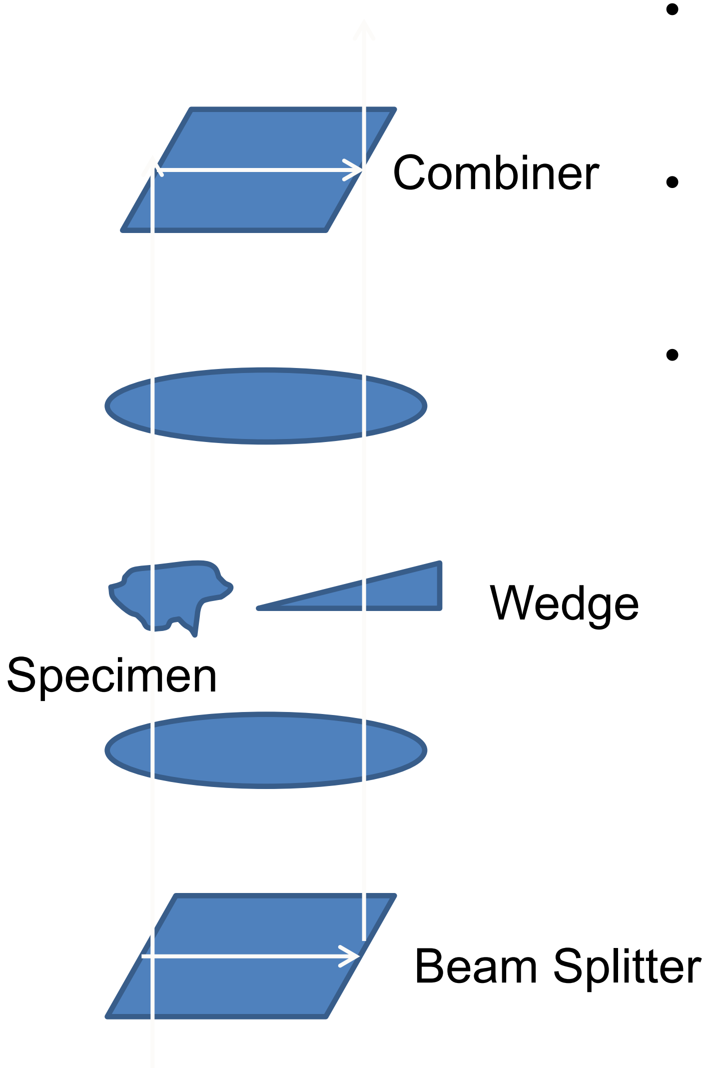
CPL





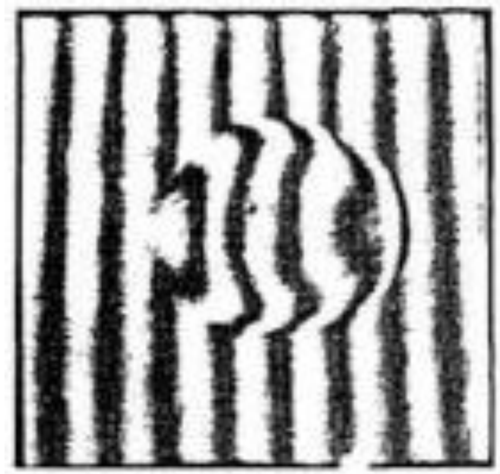
Ref1 Ref2

Interference Microscopy



- 1950s Jamin-Lebedeff / Smith / Baker
- Quantify section thickness (Barer, Ross)
- Mass ∞ OPD x Area

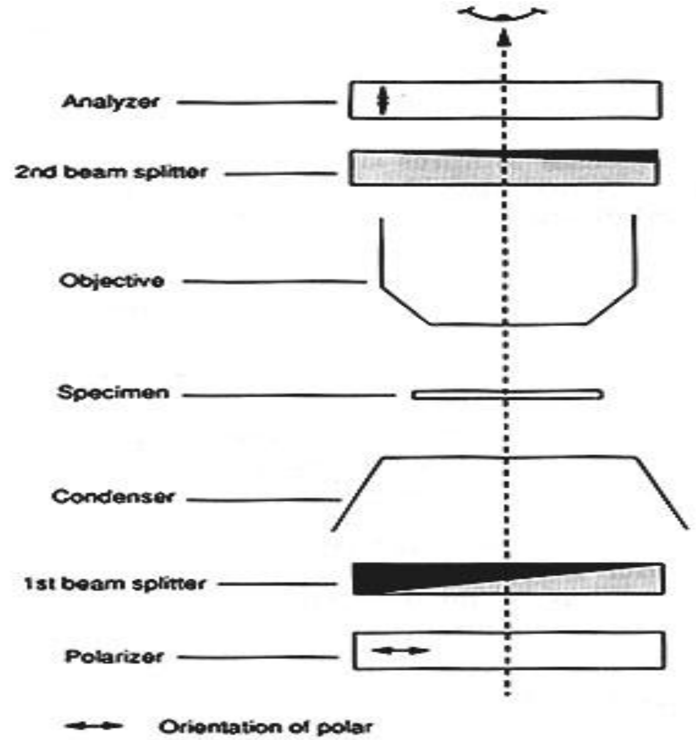
OPD= (n1-n2) x t

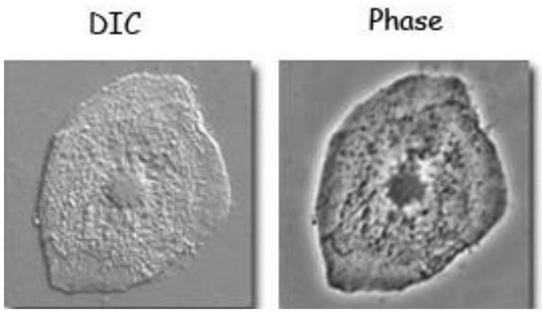


Differential Interference Contrast (Nomarski) 1

1960s

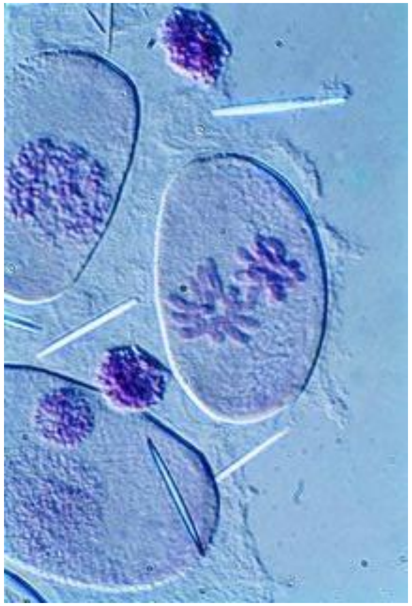
- Crossed polarisers
- Beams splitters introduces shear
- Beams polarised differently
- Gives relief effect
- Better than phase for thick specimens

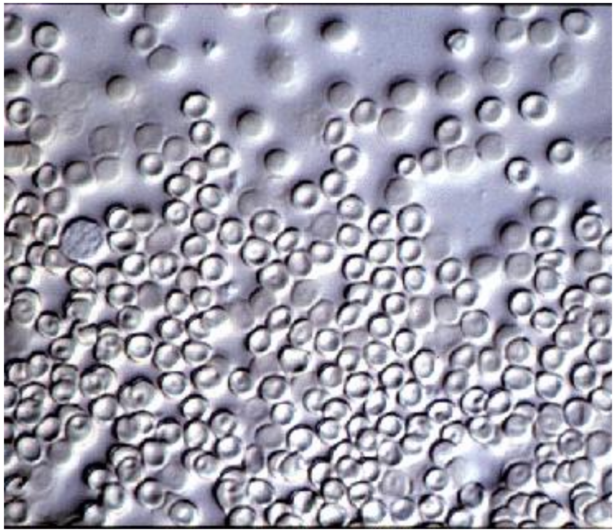
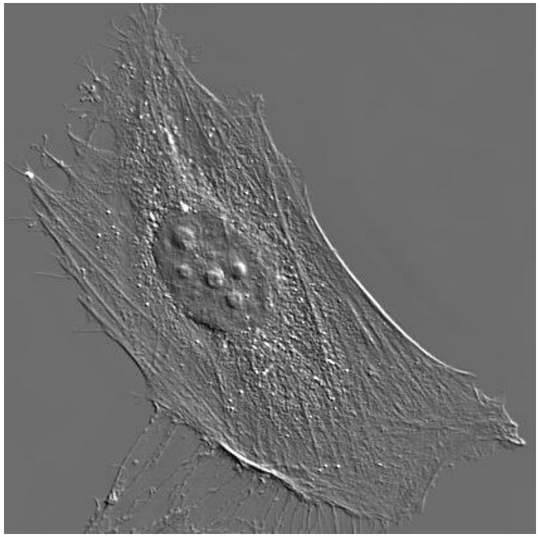




DIC 2

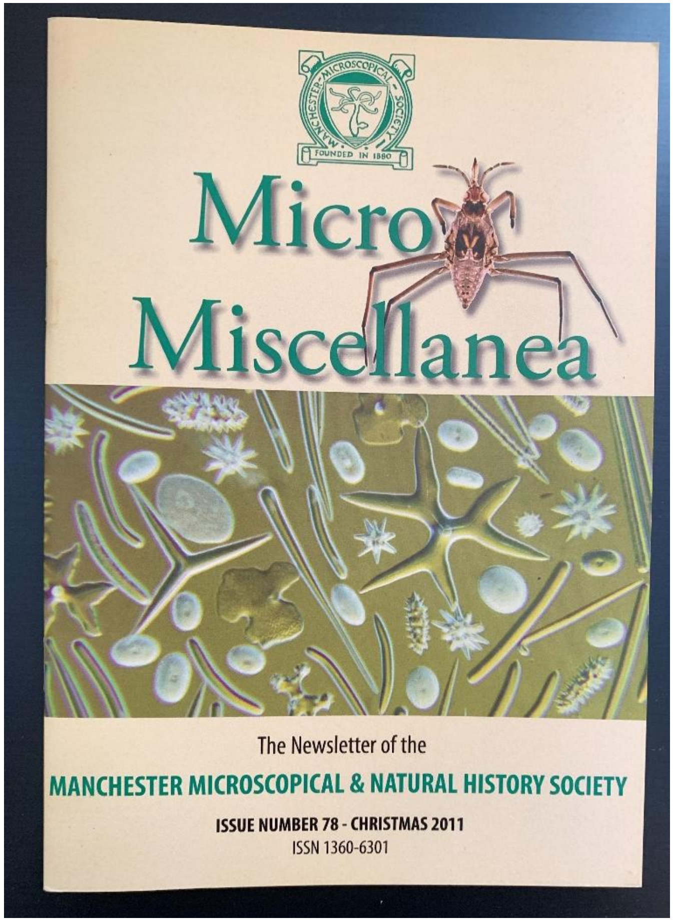
Use to study and manipulate unstained living cells + in vitro fertilisation, stem cell engineering, … !

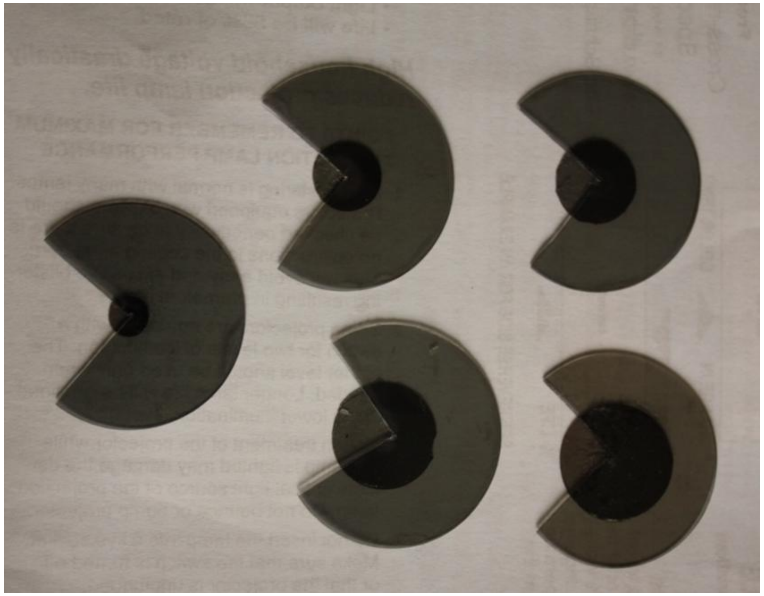


VAC – Variable Amplitude Contrast Microscopy

? DIC on the Cheap





Refs

Arthur Strange (1989) Microscope, Brian Wilkinson (2011) Balsam Post, … also see Barer (1949) Nature

Concentric Colour Contrast – Spikeberg Illumination –

(Phase Contrast or Rheinberg + Polarising) **Spike Walker 2007, 2019, 2023 … Barer 1949**

Bicolour Polarising Filters

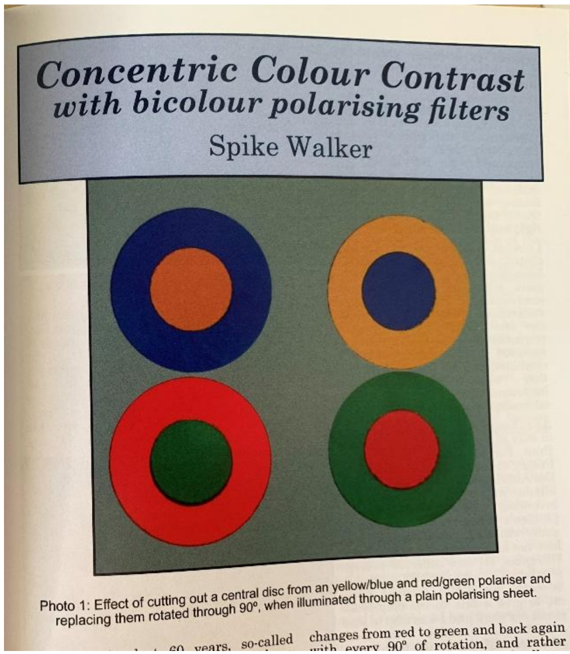
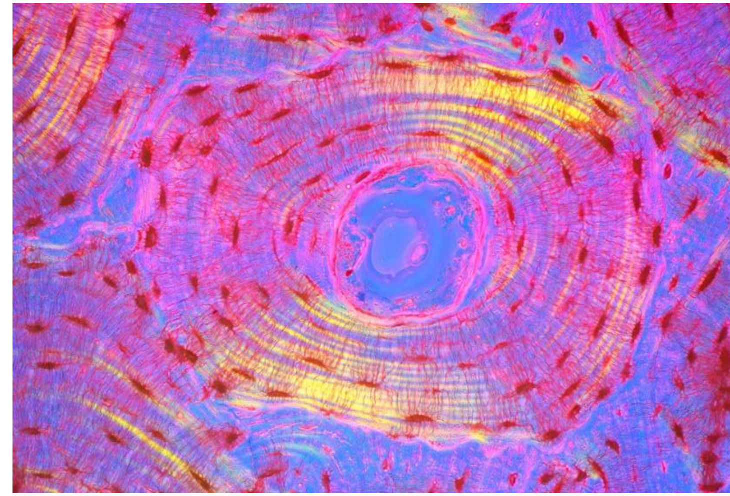
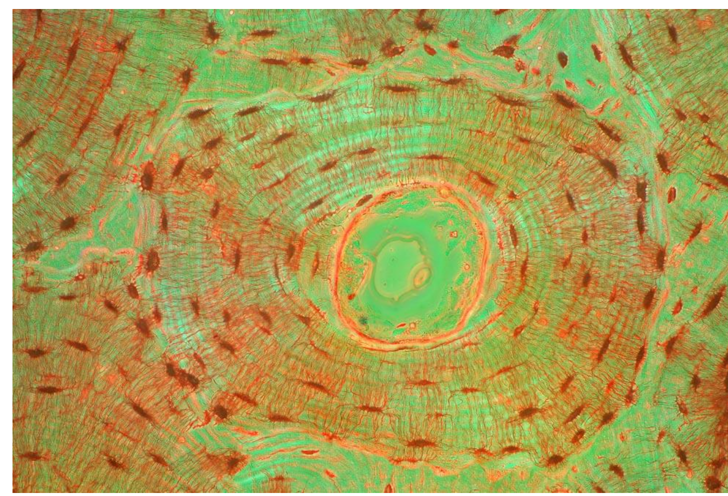

Concentric Colour Contrast
with bicolour polarising filters
Spike Walker

Photo 1: Effect of cutting out a central disc from an yellow/blue and red/green polariser and replacing them rotated through 90°, when illuminated through a plain polarising sheet.

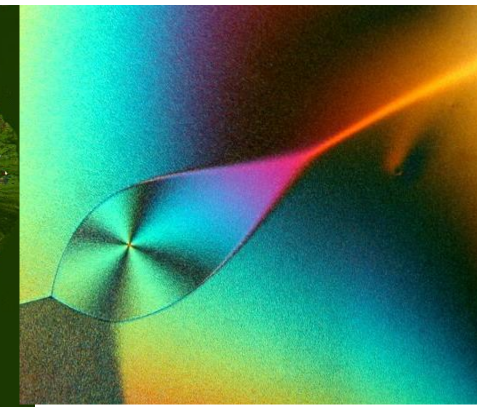
changes from red to green and back again





Richard L Howey, Wyoming, USA 1937-2023

Polarising Microscopy as Art & Philosophy



36 articles for MMS in Micro Miscellanea

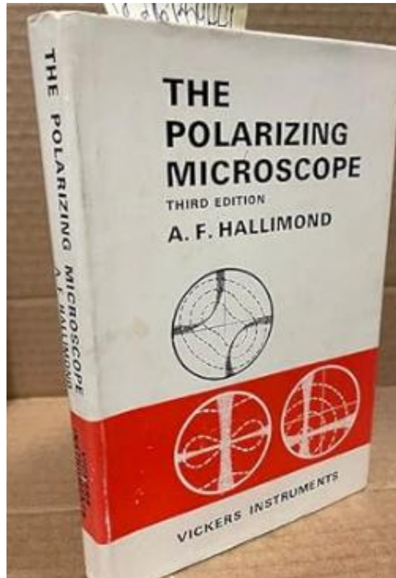
Micscape Tribute

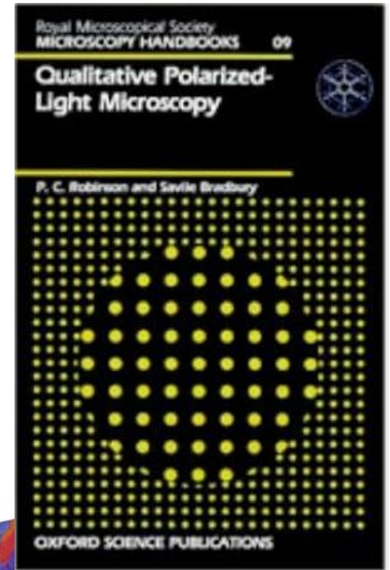
Micscape Articles

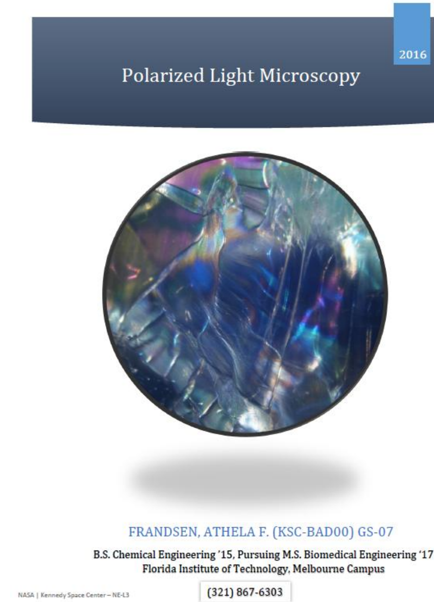
Micscape Memory

Thankyou from MMS Members!!

References

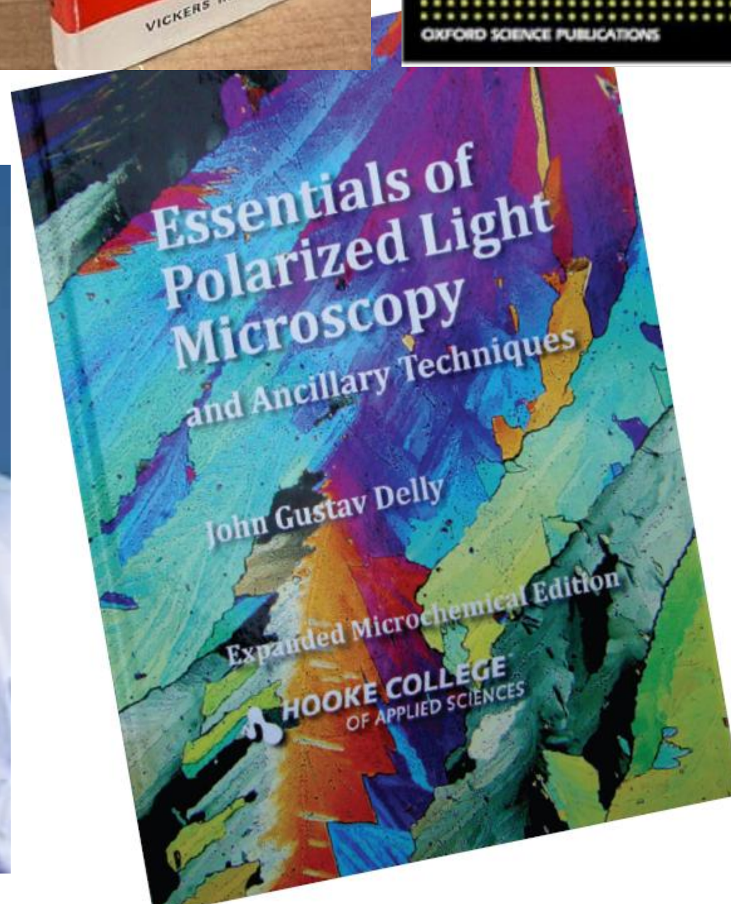






[Selected topics from Essentials… Delly]()

[NASA]()



- [Motic]()
- [Nikon]()
- [Olympus]()
- [Leitz]()
- [Zeiss]()

Practical

1. **Create Polarising Microscope Cheaply**
   … cut polaroid film, pringle lids, cellophane

2. **Examples of Polarising Microscopy & Examples of Polarisation (Macro)**

3. **Polarising Microscopes**
   (Some or all of the following…)
   - Eyepiece crosshair
   - Bertrand Lens (In/Out)
   - Analyser Polar (In/Out)
   - Compensator (In/Out) + Waveplates ʎ, ¼ ʎ , wedge
   - Strain Free Objectives, centrable, ? Dispersion Objectives
   - Rotatable stage
   - Polariser (In/Out, Rotatable)

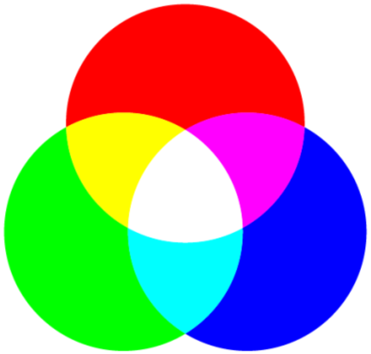
   + Michel-Levy Colour Chart
   + Delly's book !!

4. …… Geologist's Uses, Manufacturing, Food Industry, Health & Safety, ..

Primary Colours

X

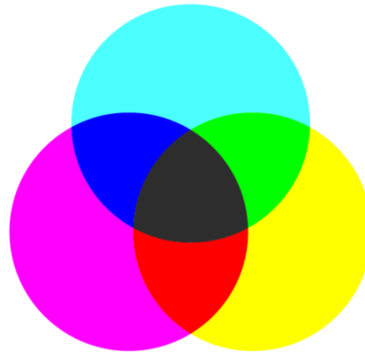
Additive colour



RGB

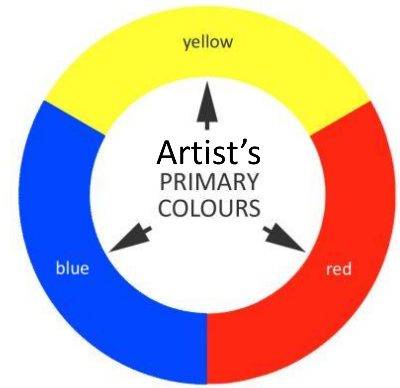
Light

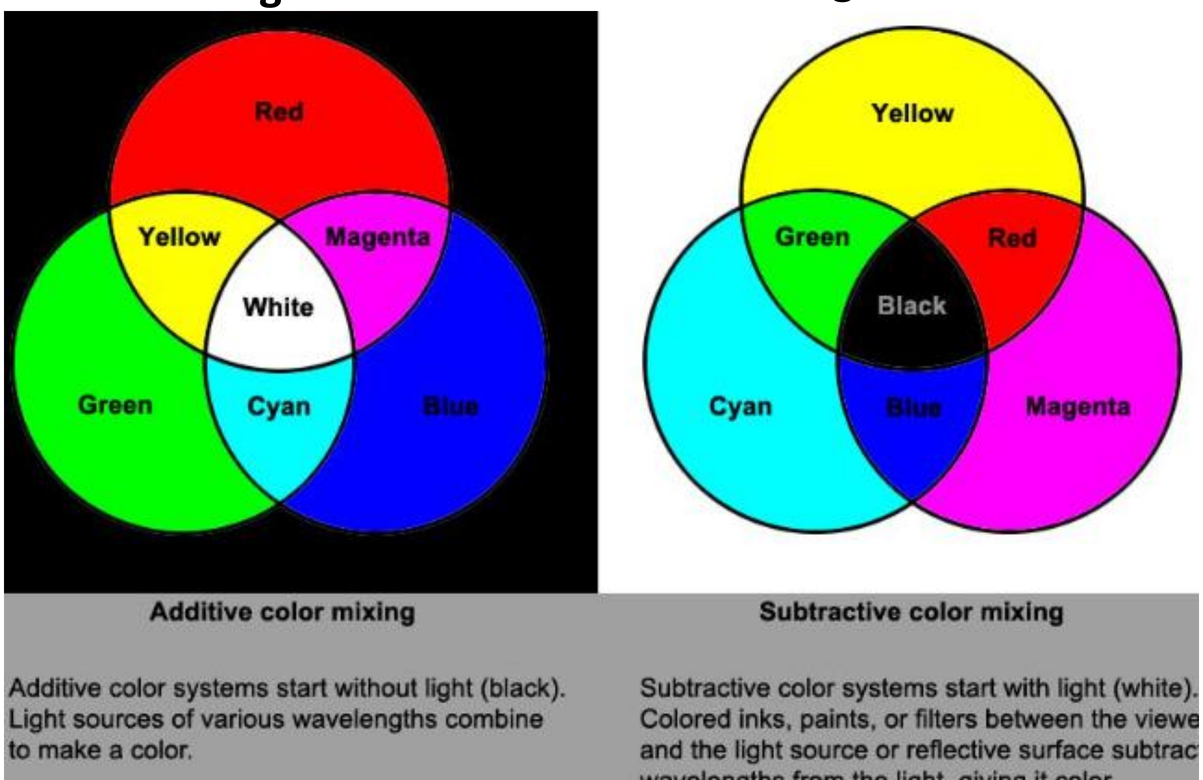
Subtractive colour



CMY (K)

Pigments





Pigments

Y absorbs B light

M absorbs G light

C absorbs R light