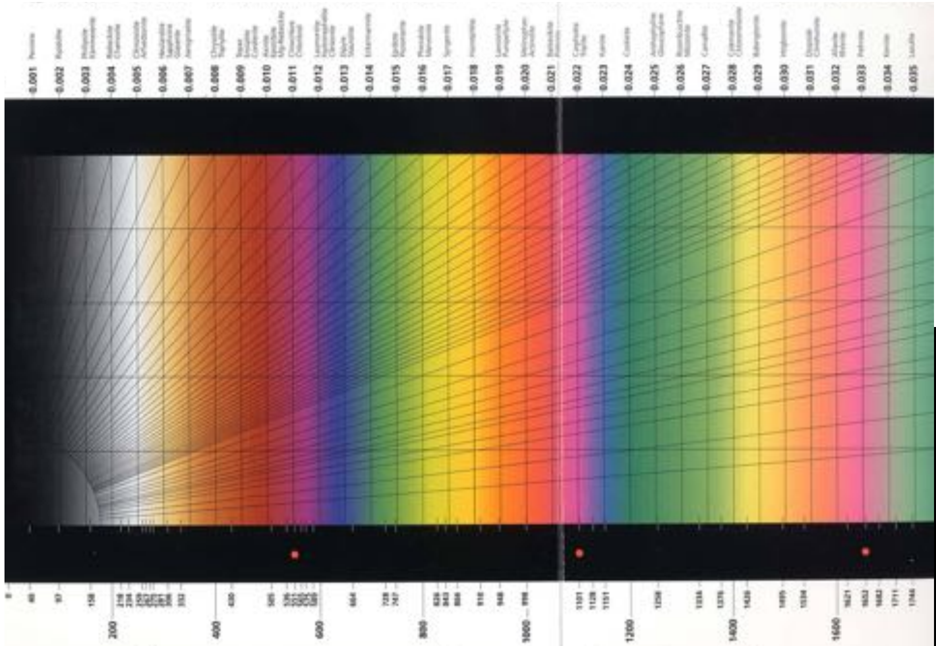
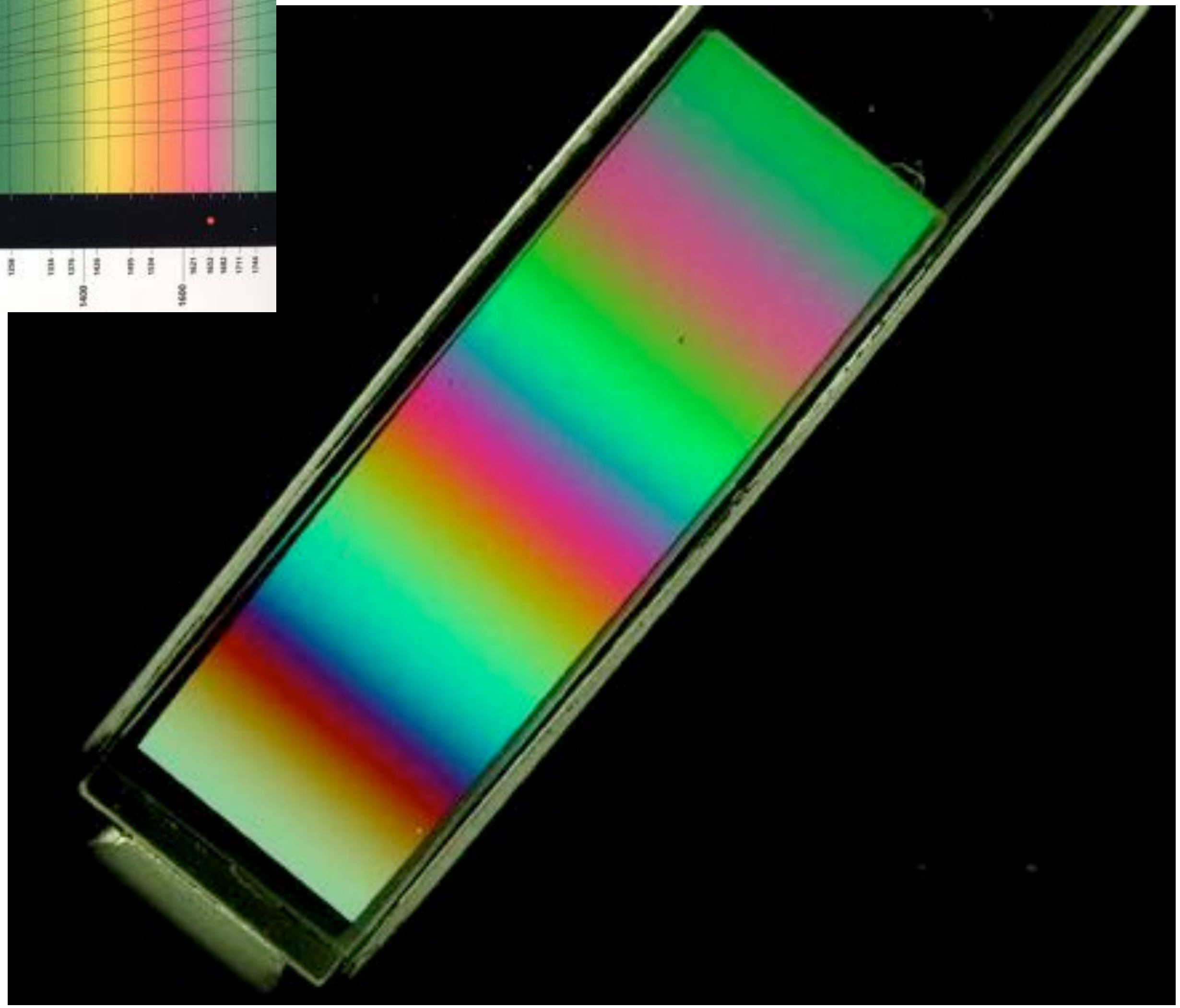
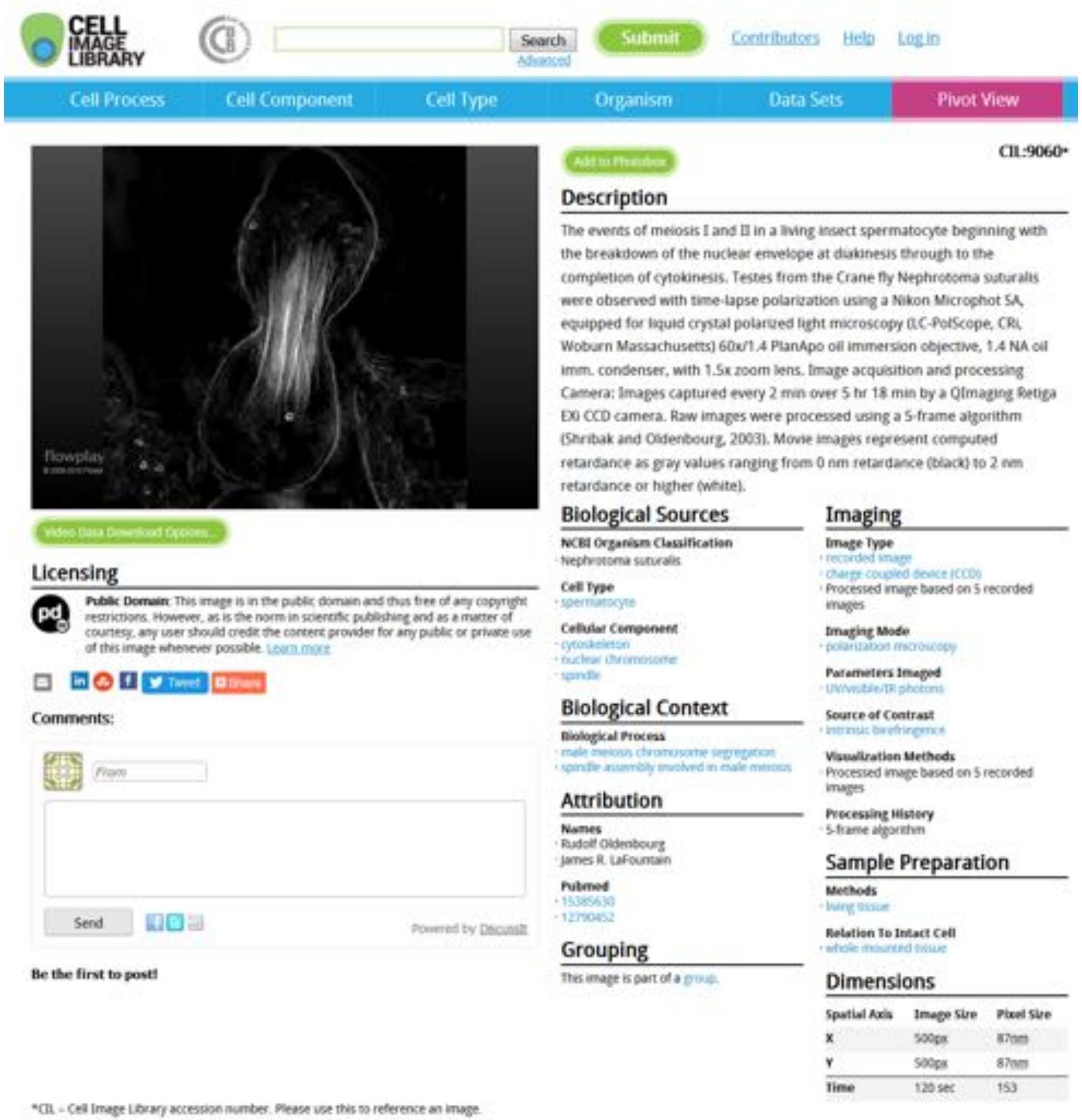
Polarised Light Microscopy



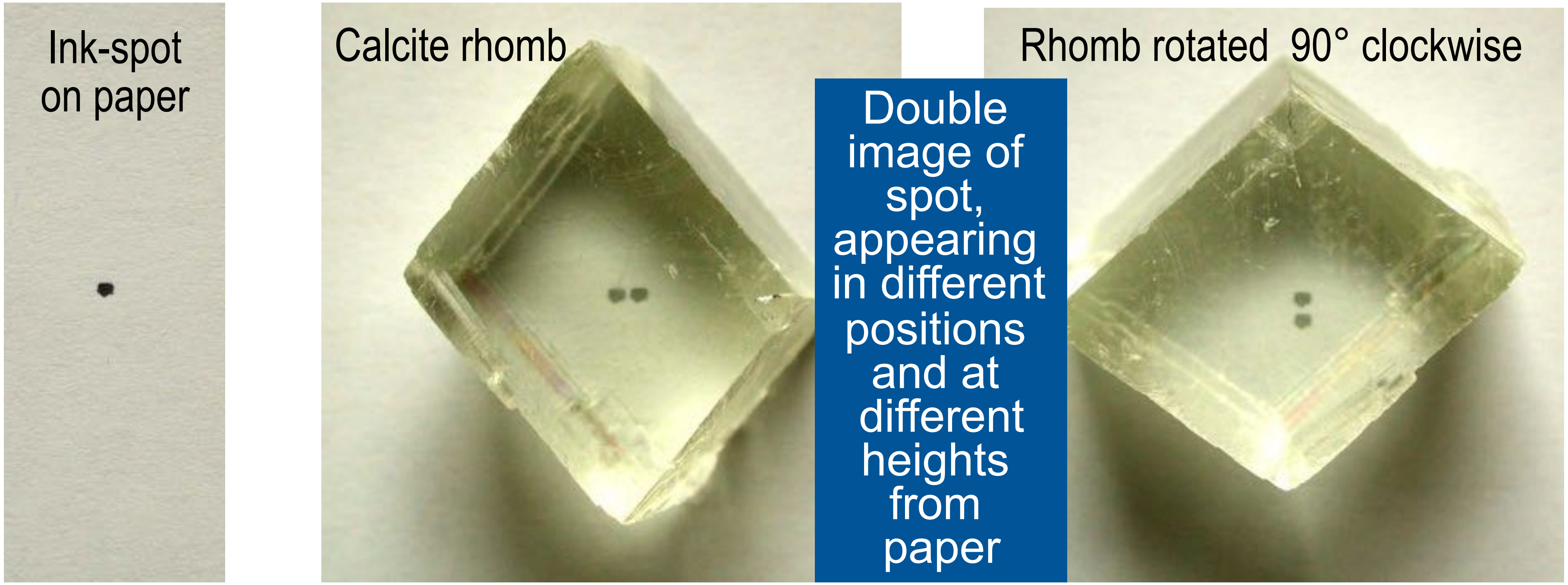


Adapted from Peter Evennett

Example: Meiosis

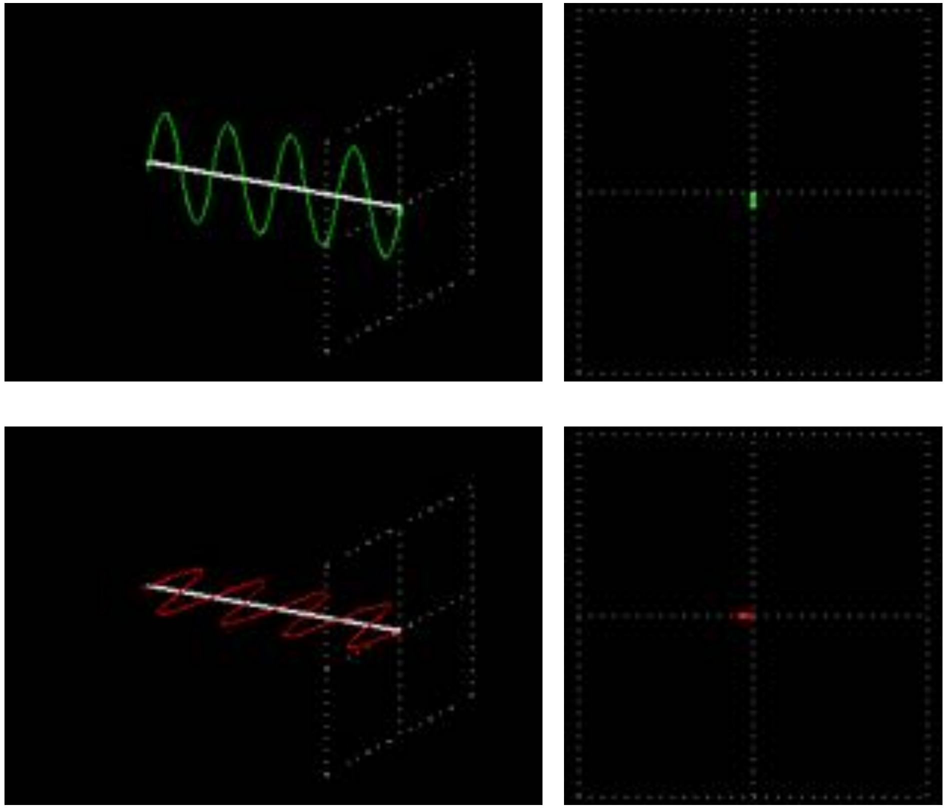


http://www.cellimagelibrary.org/



Adapted from Peter Evennett

Light waves



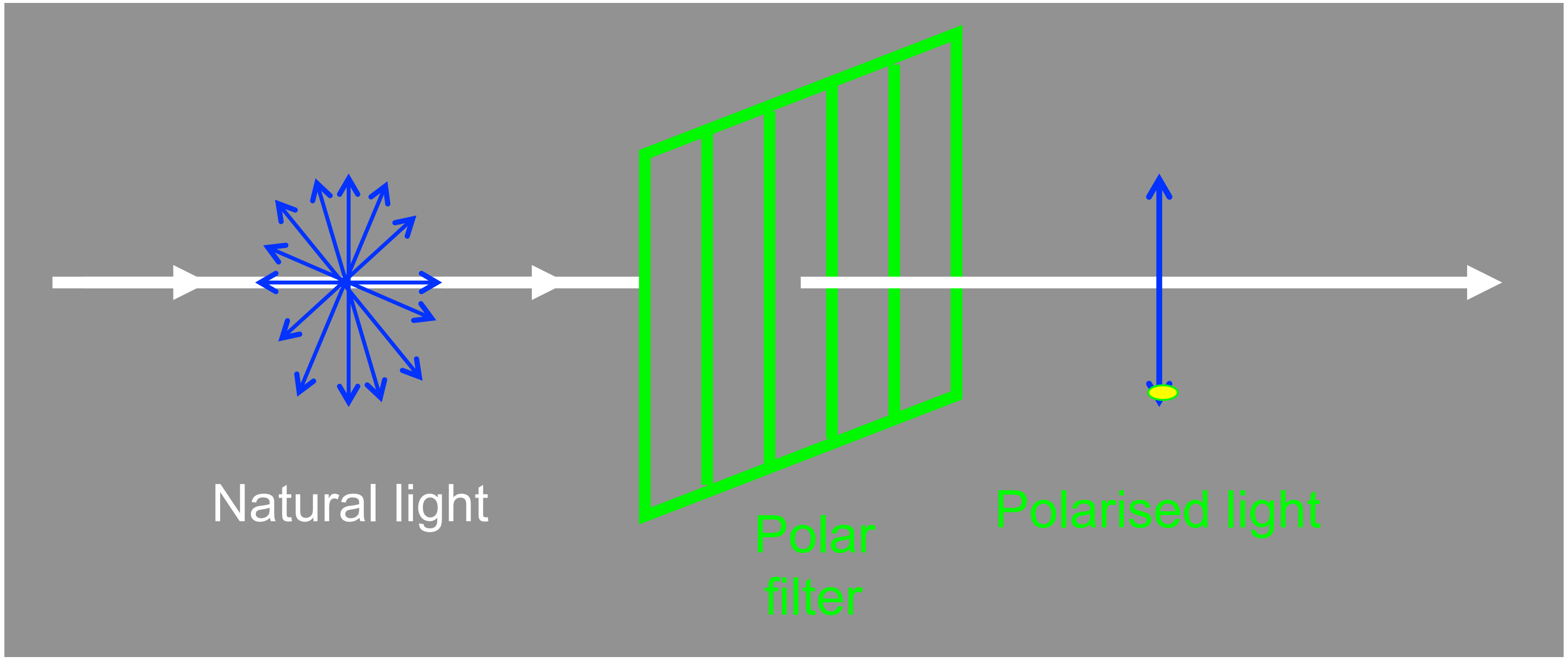
- Light waves vibrate within a plane transverse to the direction of propagation

- Orientation of the plane relative to the direction of propagation:

  → **Polarisation**

http://emanim.szialab.org/

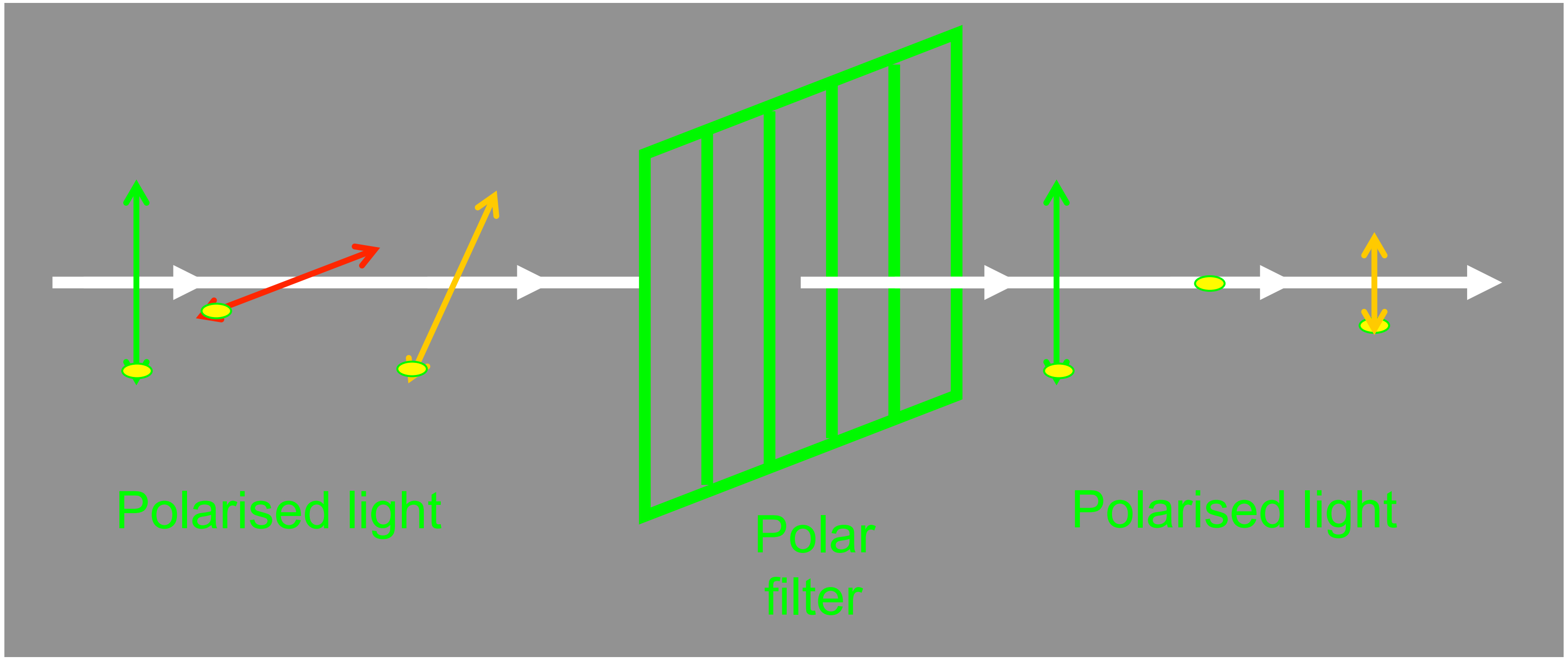
Polarisation of light



- ‘Natural Light’: vibrations occur in all directions
- Polarised Light: all but one of these directions have been ‘filtered out’

Adapted from Peter Evennett

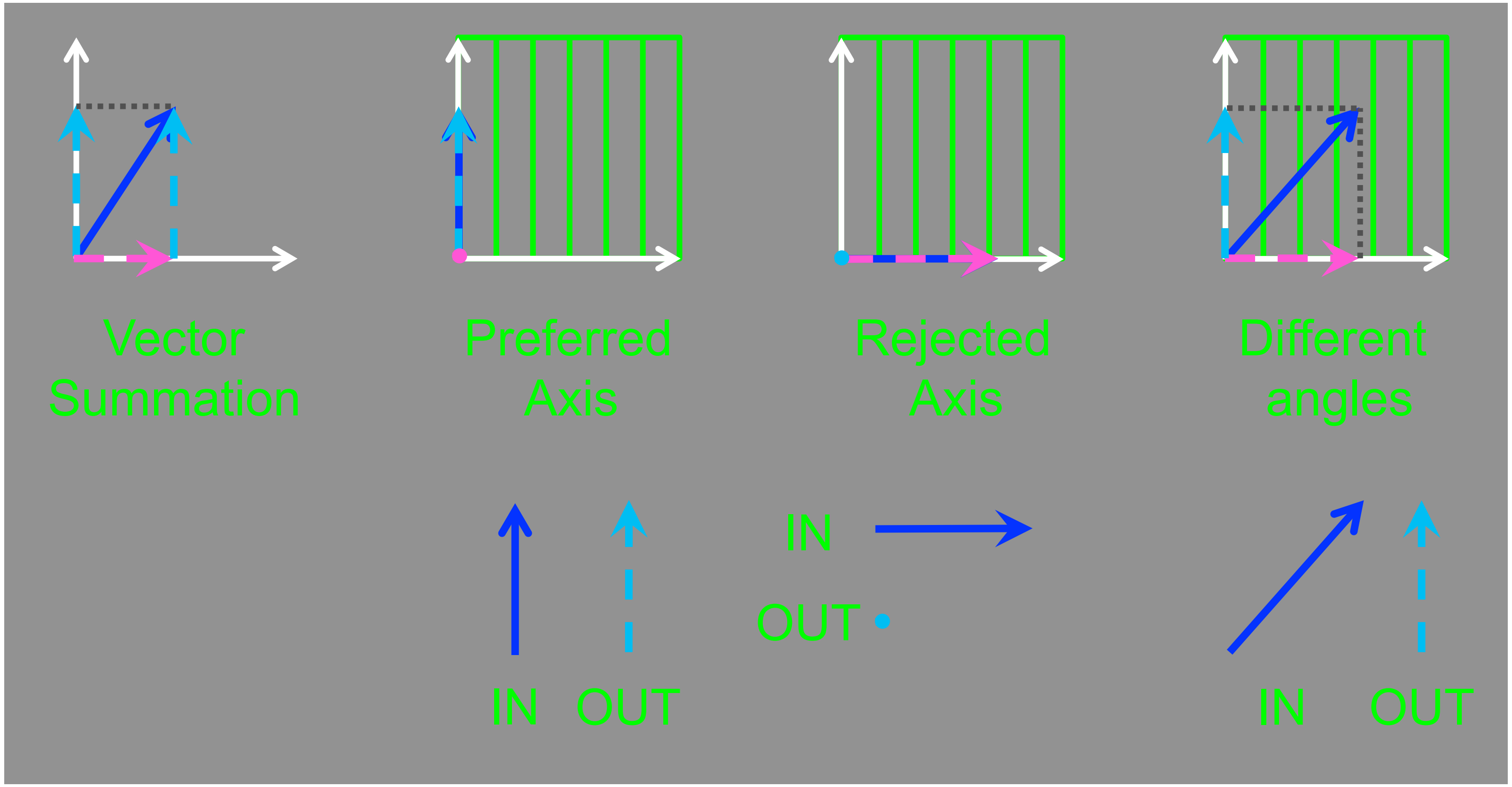
Polarisation Filters



- Preferred angle: all light passes
- Rejected angle: no light passes
- Other angles: some light passes with preferred polarisation angle

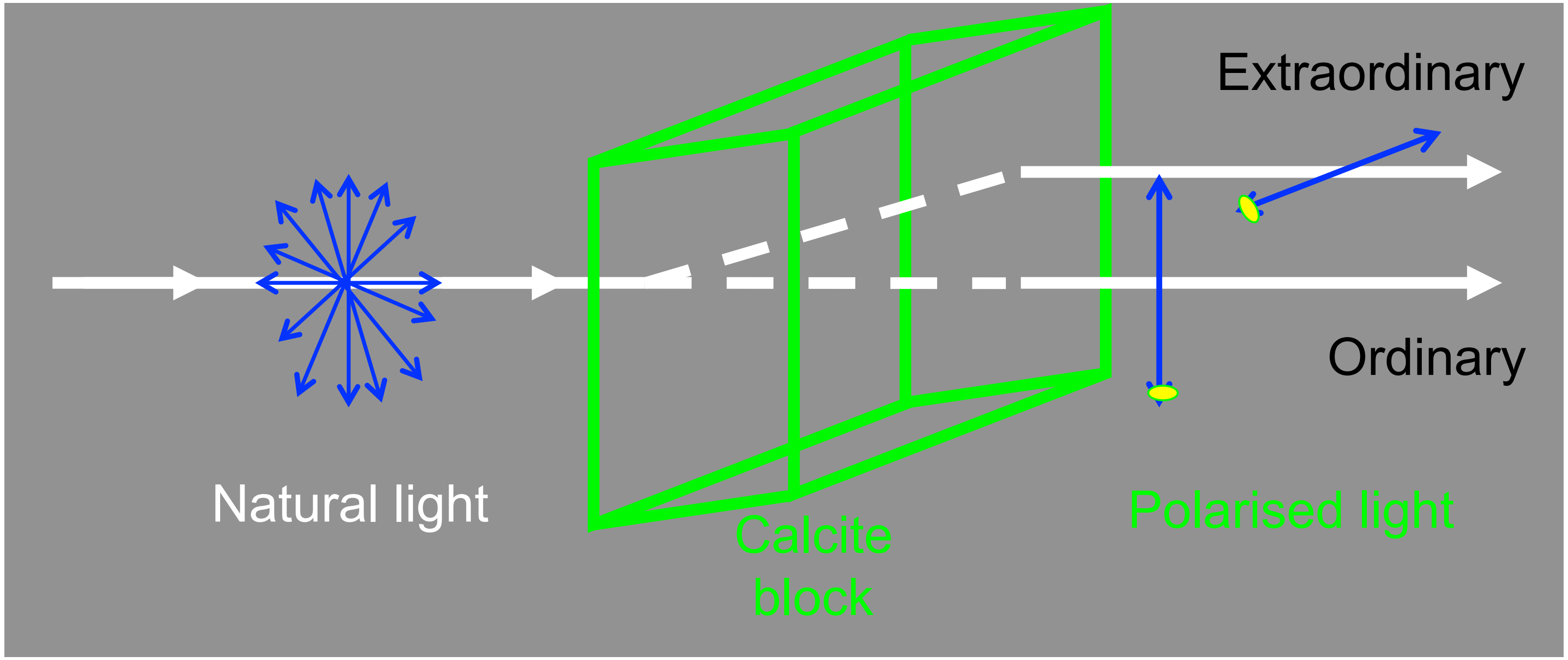
Adapted from Peter Evennett

Vector Summation

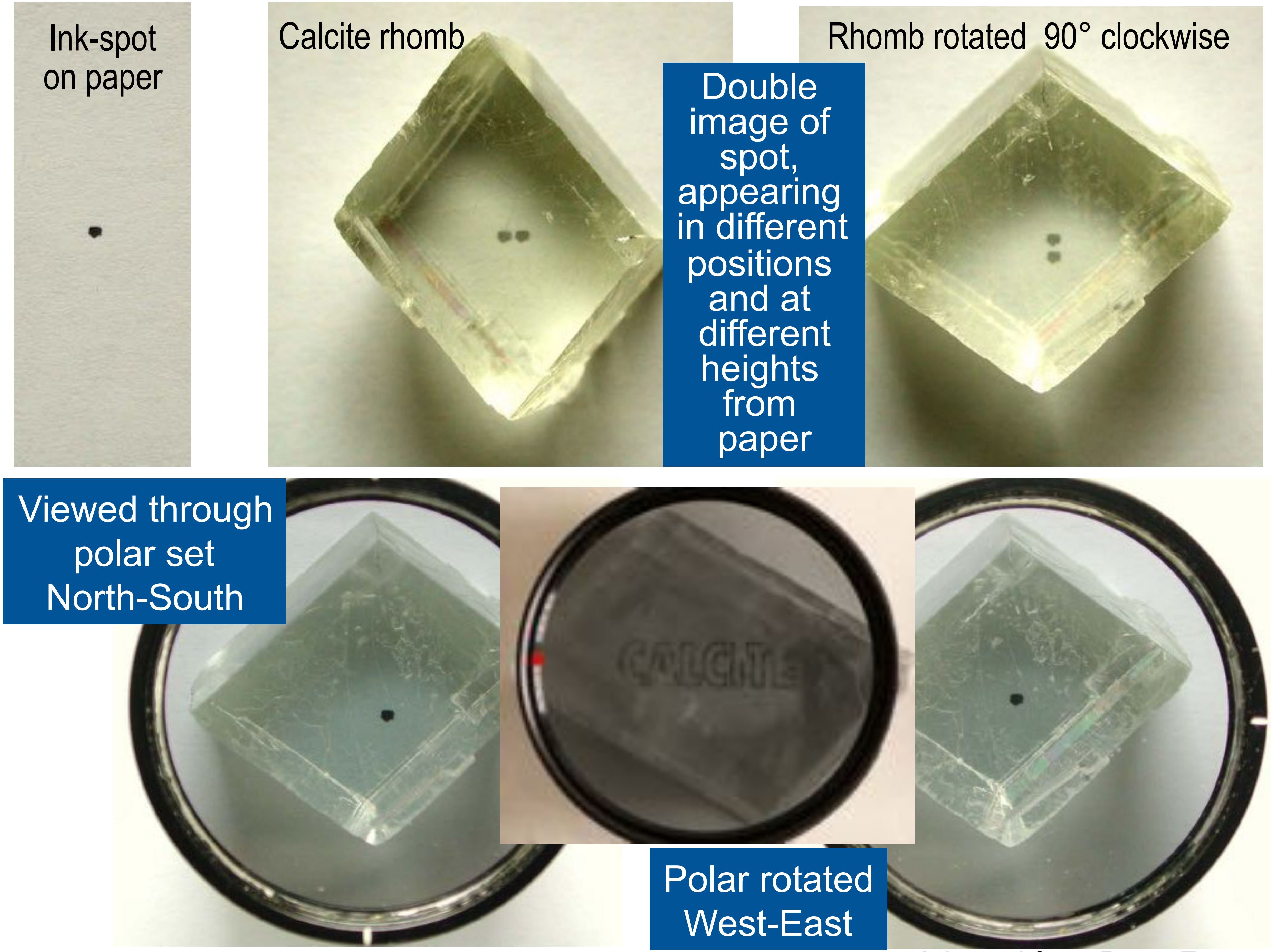


Adapted from Peter Evennett

Birefringence



- Separate single beam into two beams/waves
- Refractive index dependent on direction of propagation
  - **Ordinary beam**: refracted normally
  - **Extraordinary beam**: refractive index depends on angle of beam and can exhibit an additional angular shift
- Both waves polarized along orthogonal axes

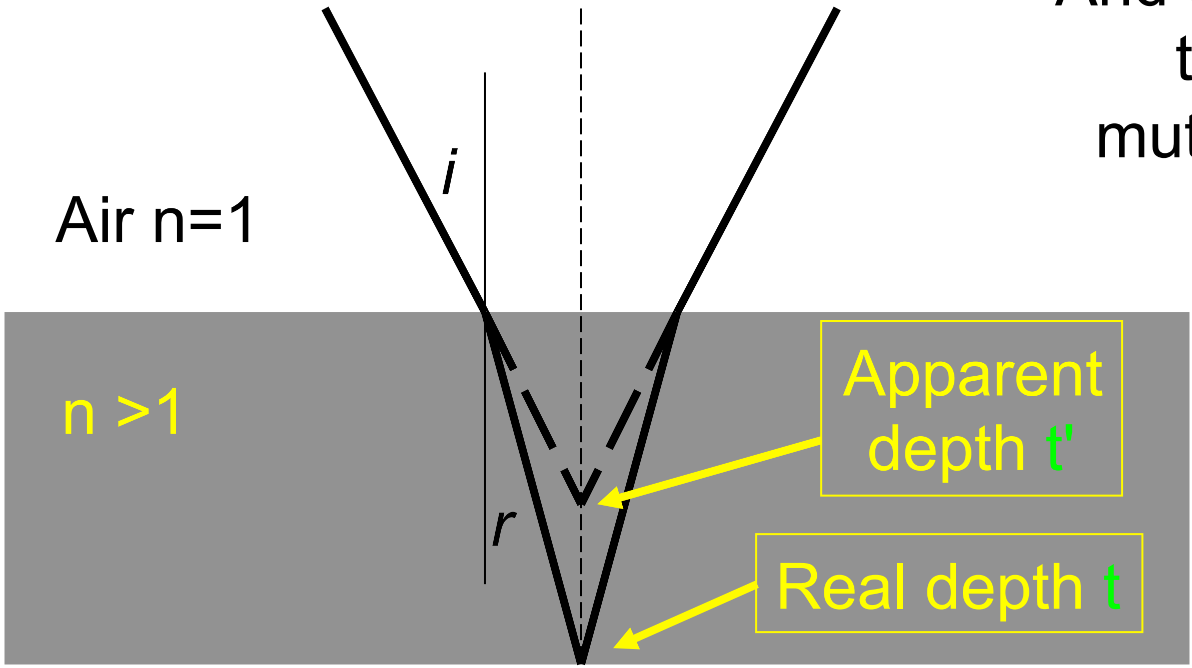


Adapted from Peter Evennett

Apparent Depth

Snell's Law:

$$n = {}^{\sin i}/_{\sin r} = {}^{t}/_{t'}$$

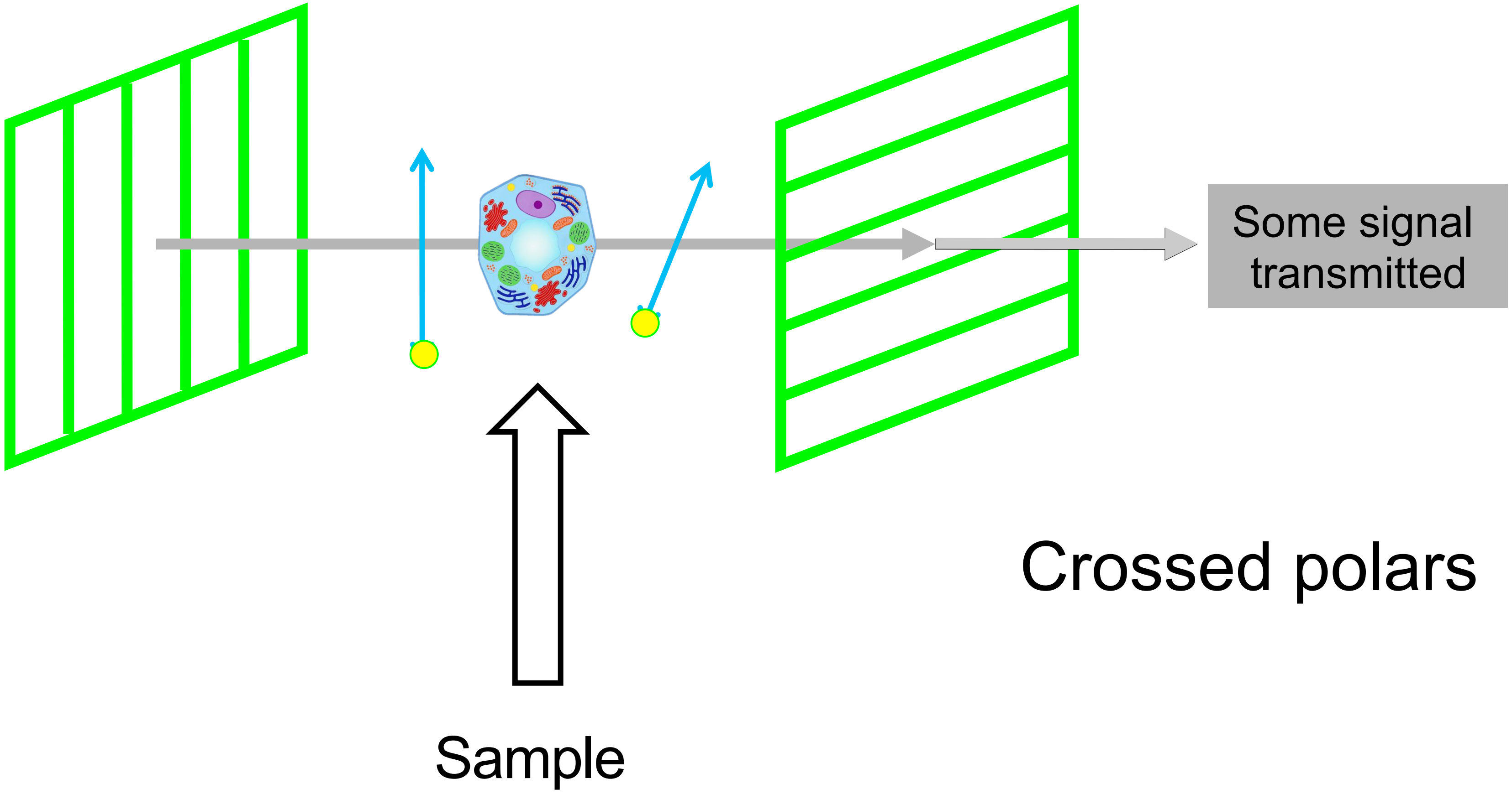


Calcite gives a double image at two apparent depths because it has two refractive indices.

And use of the polar shows that these relate to mutually perpendicularly polarised beams.

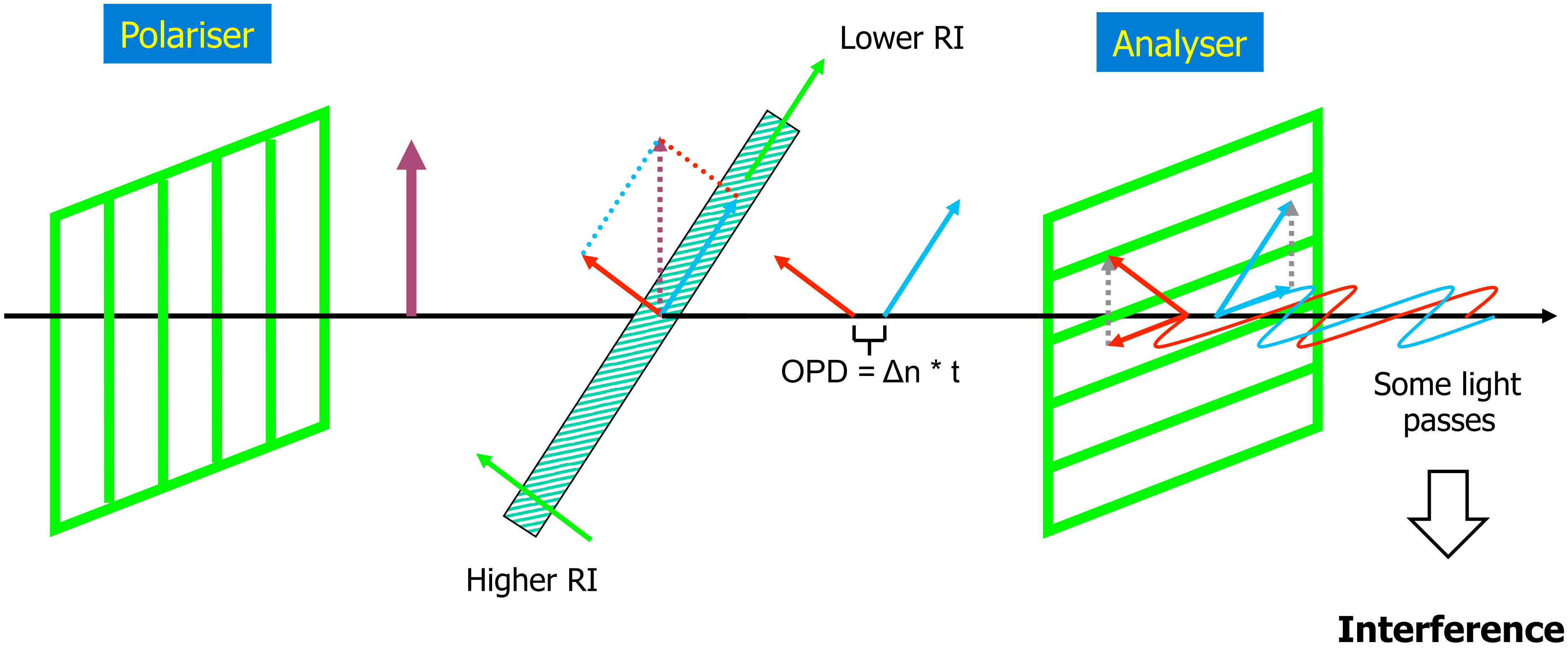
Adapted from Peter Evennett

Basic Principle



Adapted from Peter Evennett

Effect of Birefringent Samples



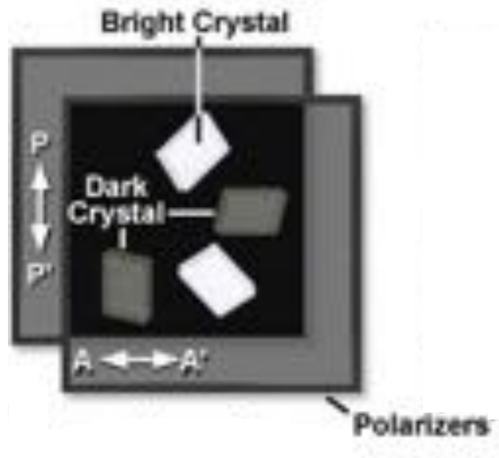
Adapted from Peter Evennett

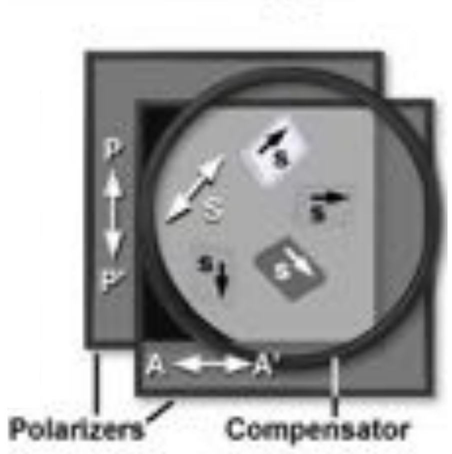
Effects on Contrast

- Optical Path Difference (OPD)
  - Difference in refractive index for polarisation axes
  - Thickness of specimen

- Also depends on orientation of birefringent material relative to polariser/analyser
  - Optimal if diagonal relative to the polariser

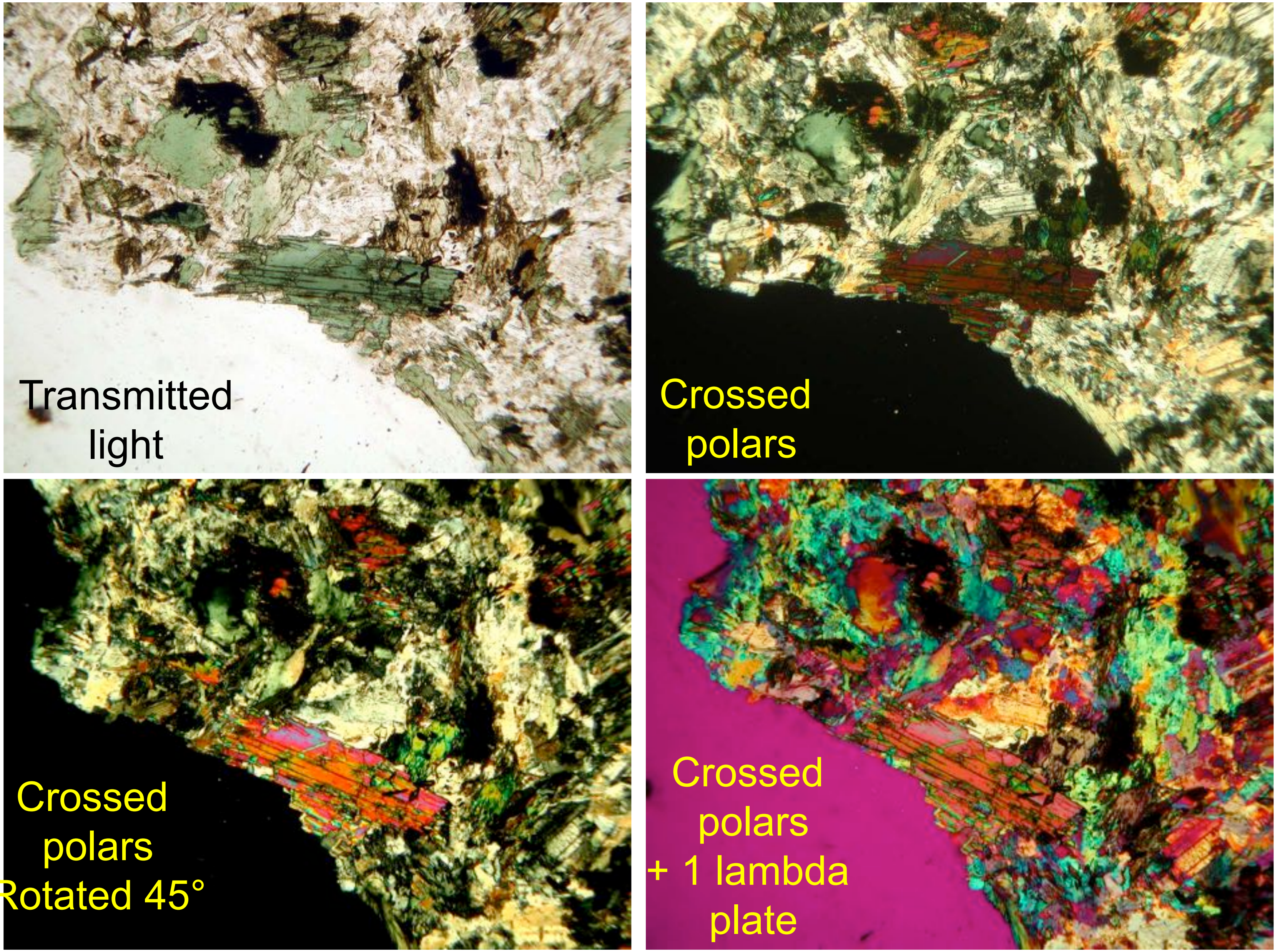
- Additional optical components
  - Retardation plates: introduce fixed optical path difference
  - Compensators: introduce variable optical path length

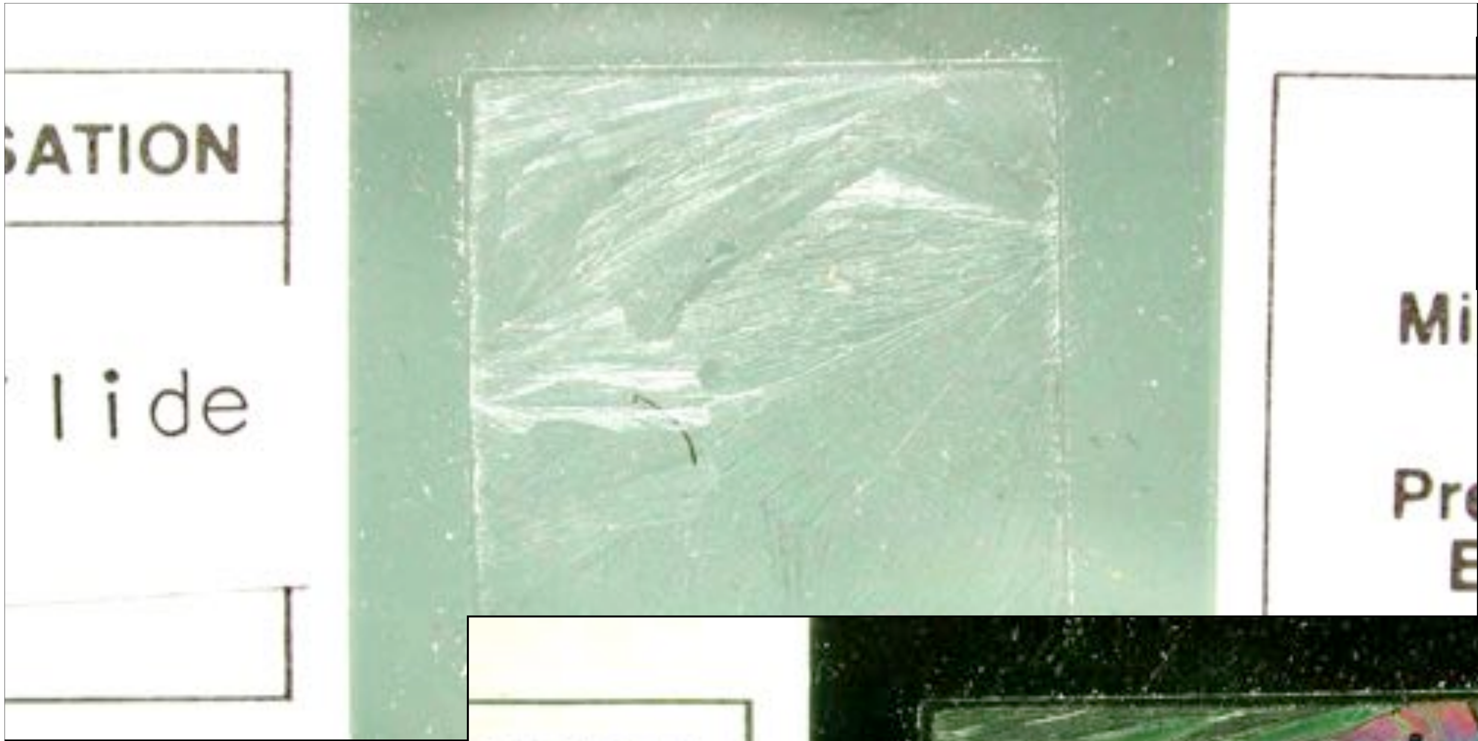




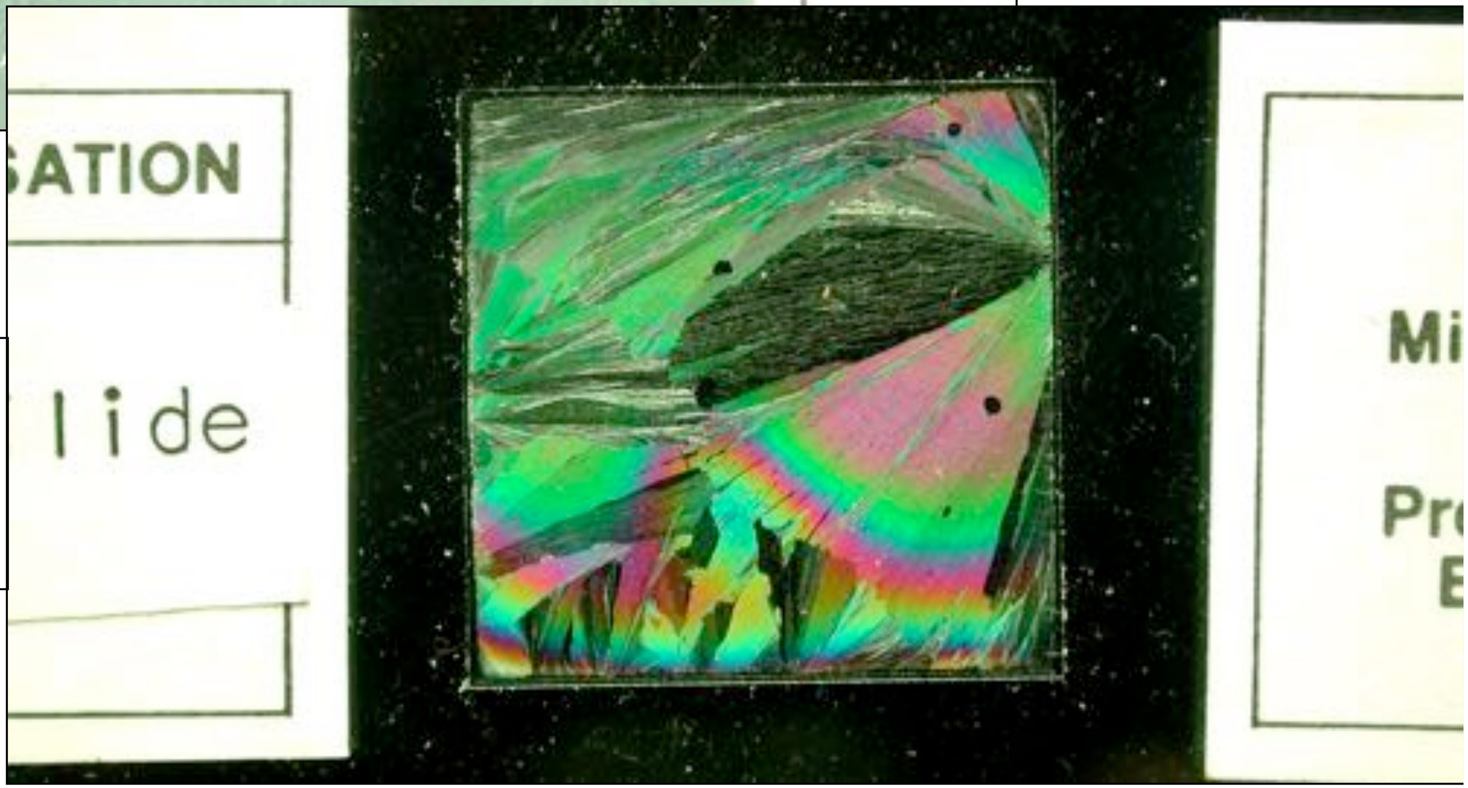
http://micro.magnet.fsu.edu/primer/index.html

Example

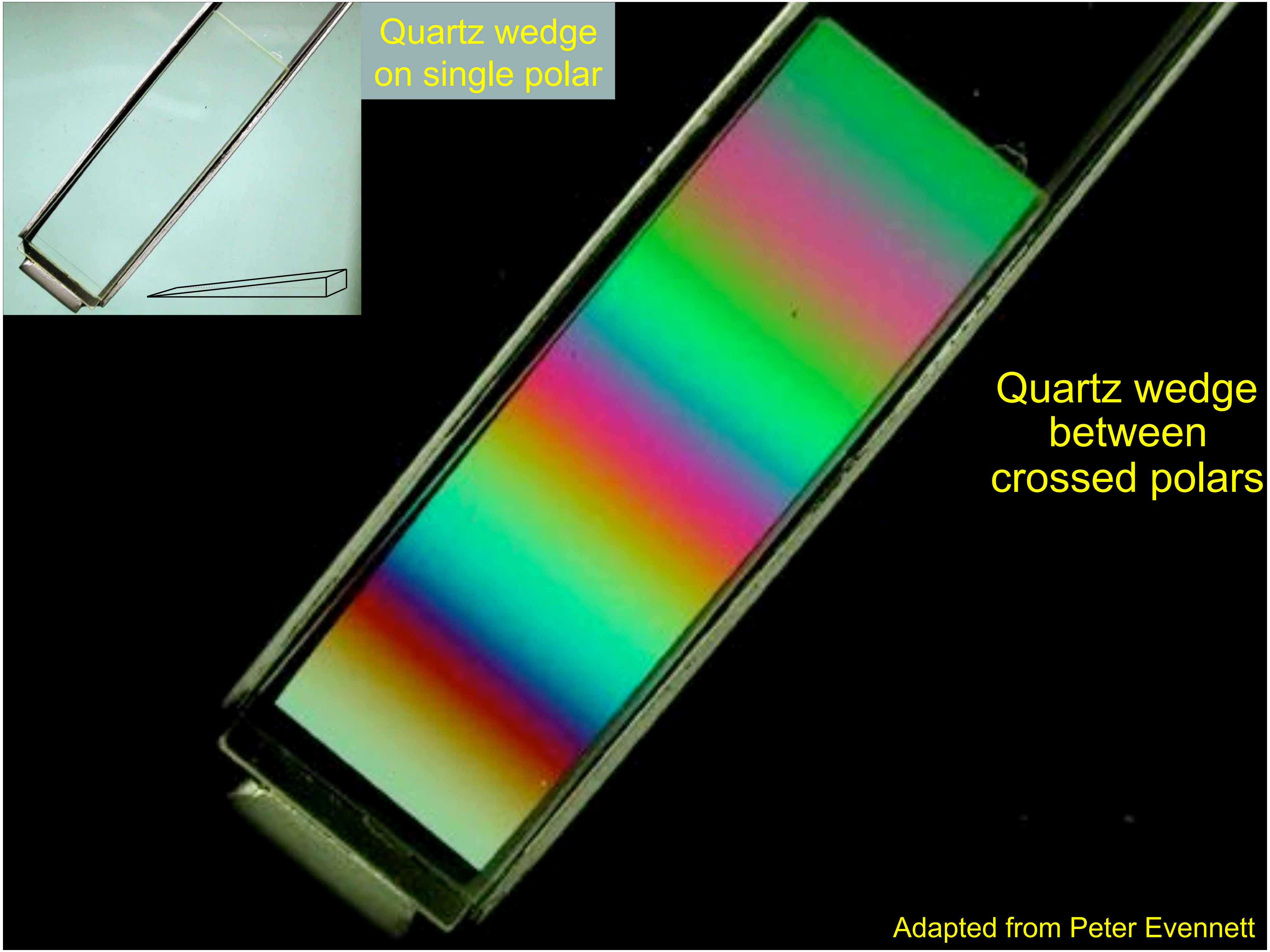

Transmitted light
Crossed polars
Crossed polars Rotated 45°
Crossed polars + 1 lambda plate



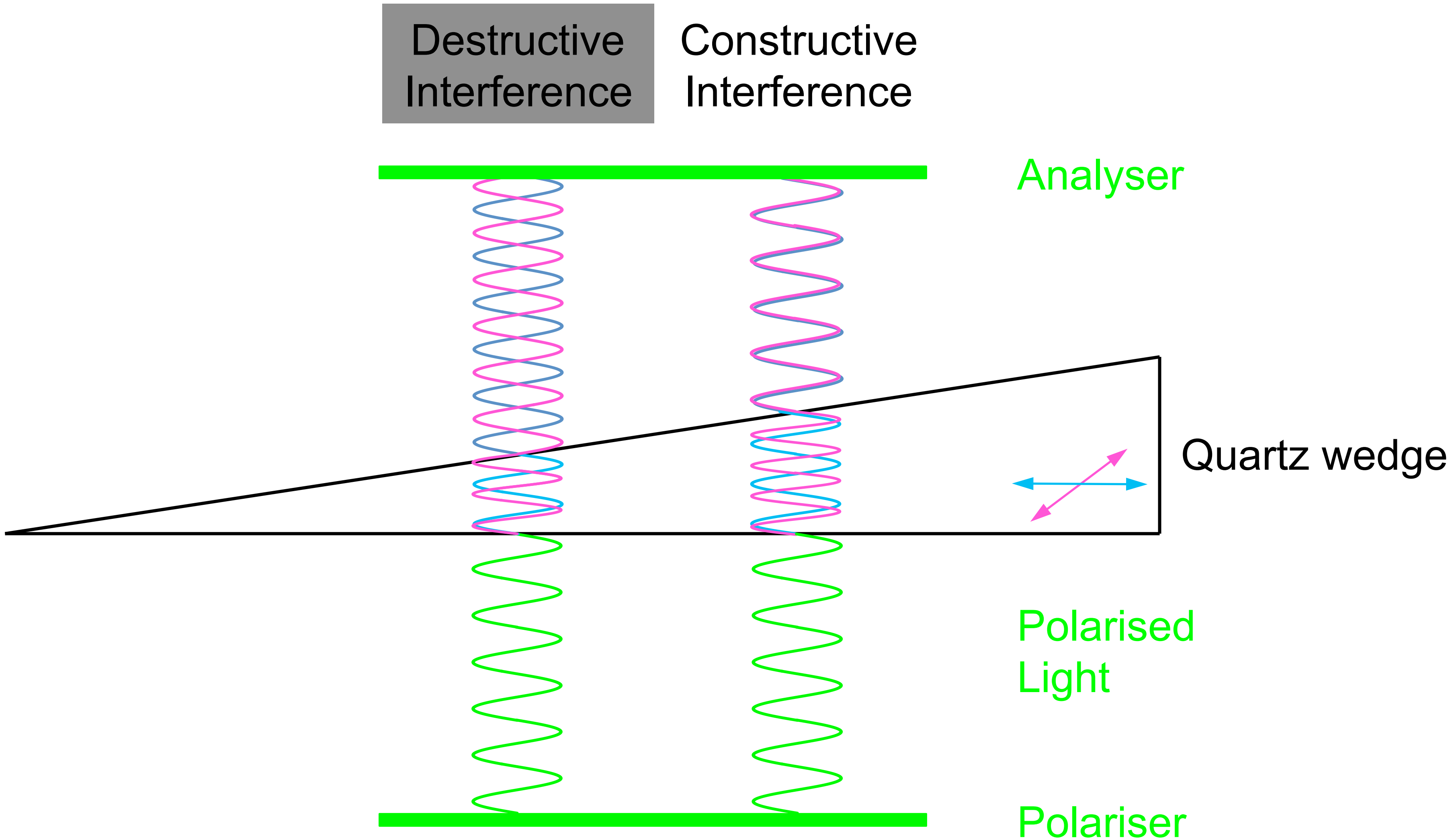
Crystals of Acetanilide. Natural light

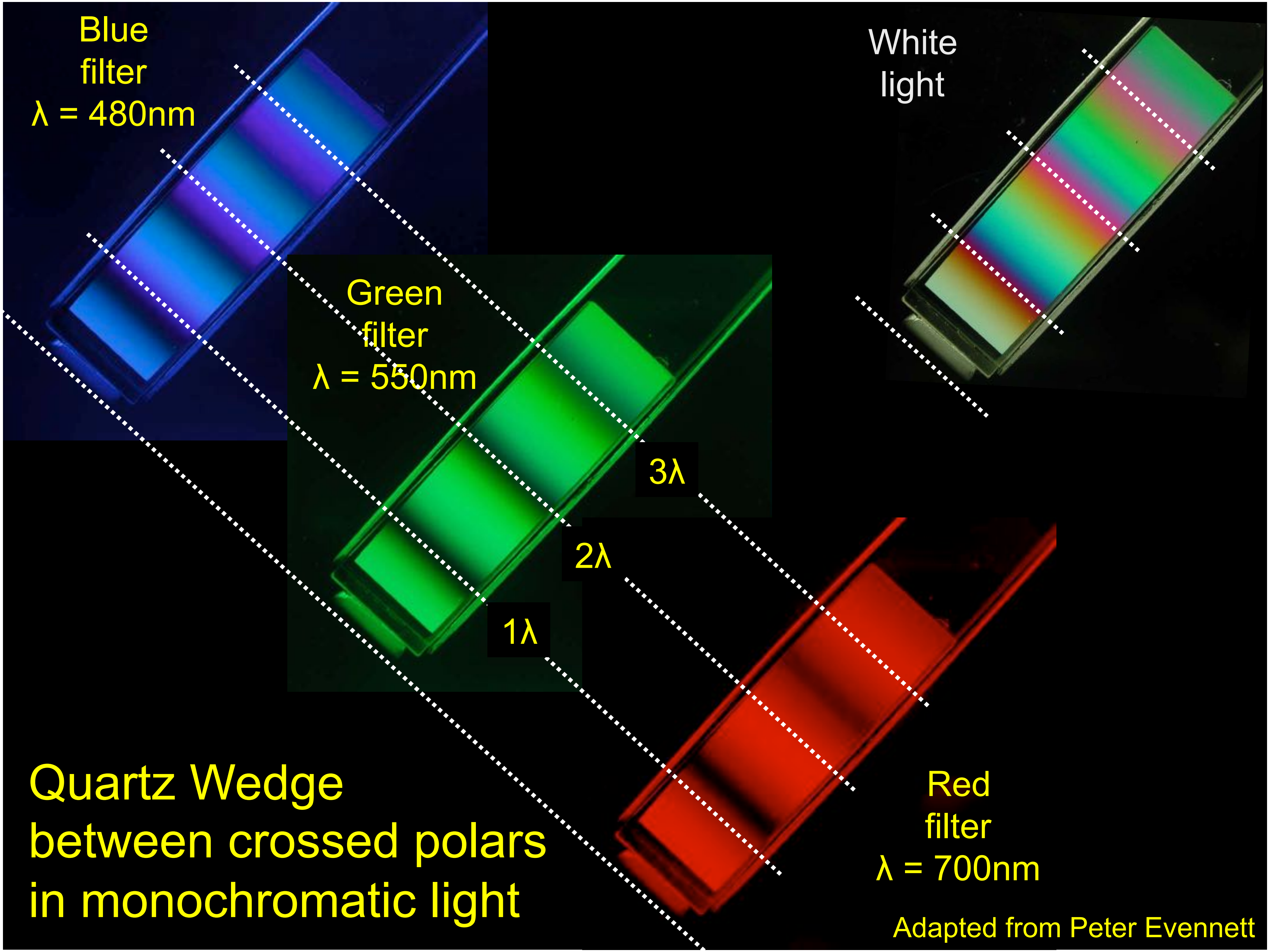


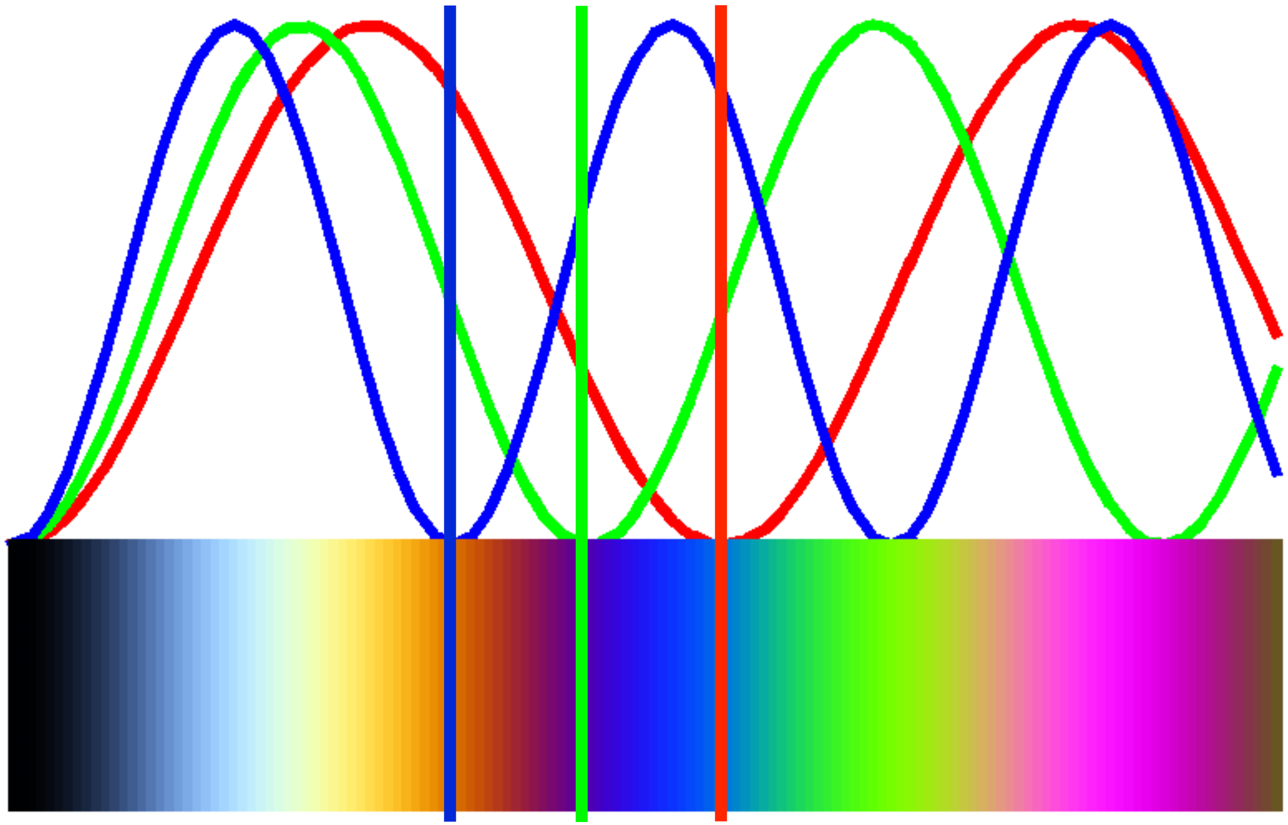
Between crossed polars


Quartz wedge on single polar
Quartz wedge between crossed polars
Adapted from Peter Evennett

Interference Colours

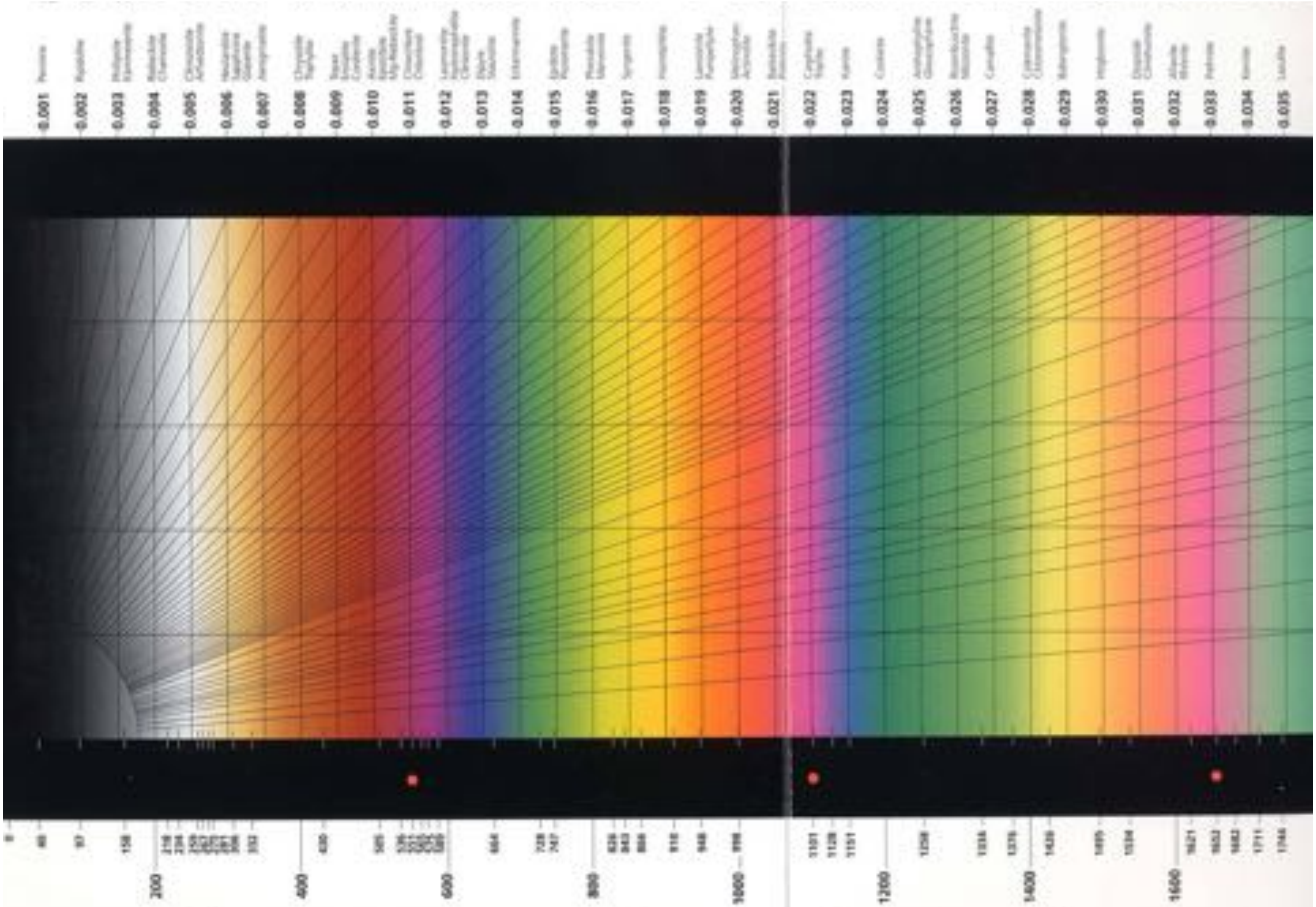



Blue filter λ = 480nm
White light
Green filter λ = 550nm
3λ
2λ
1λ
Red filter λ = 700nm
Quartz Wedge between crossed polars in monochromatic light
Adapted from Peter Evennett

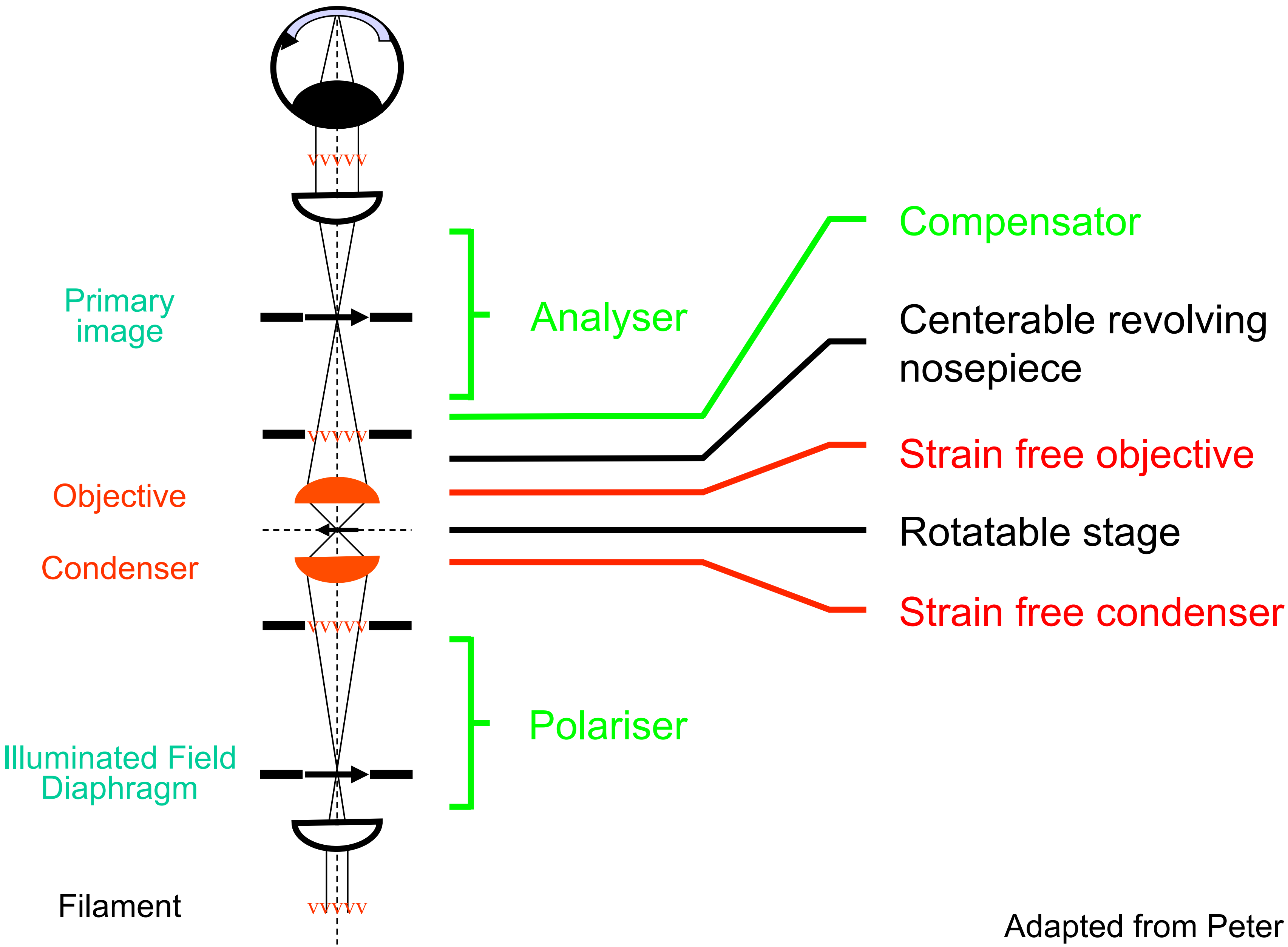


It is possible to guess the OPD from the interference colour using the Michel Lévy chart. If the thickness of the object is known, the birefringence can be calculated.

Michel-Lévy Interference Colour Chart



How to set it up



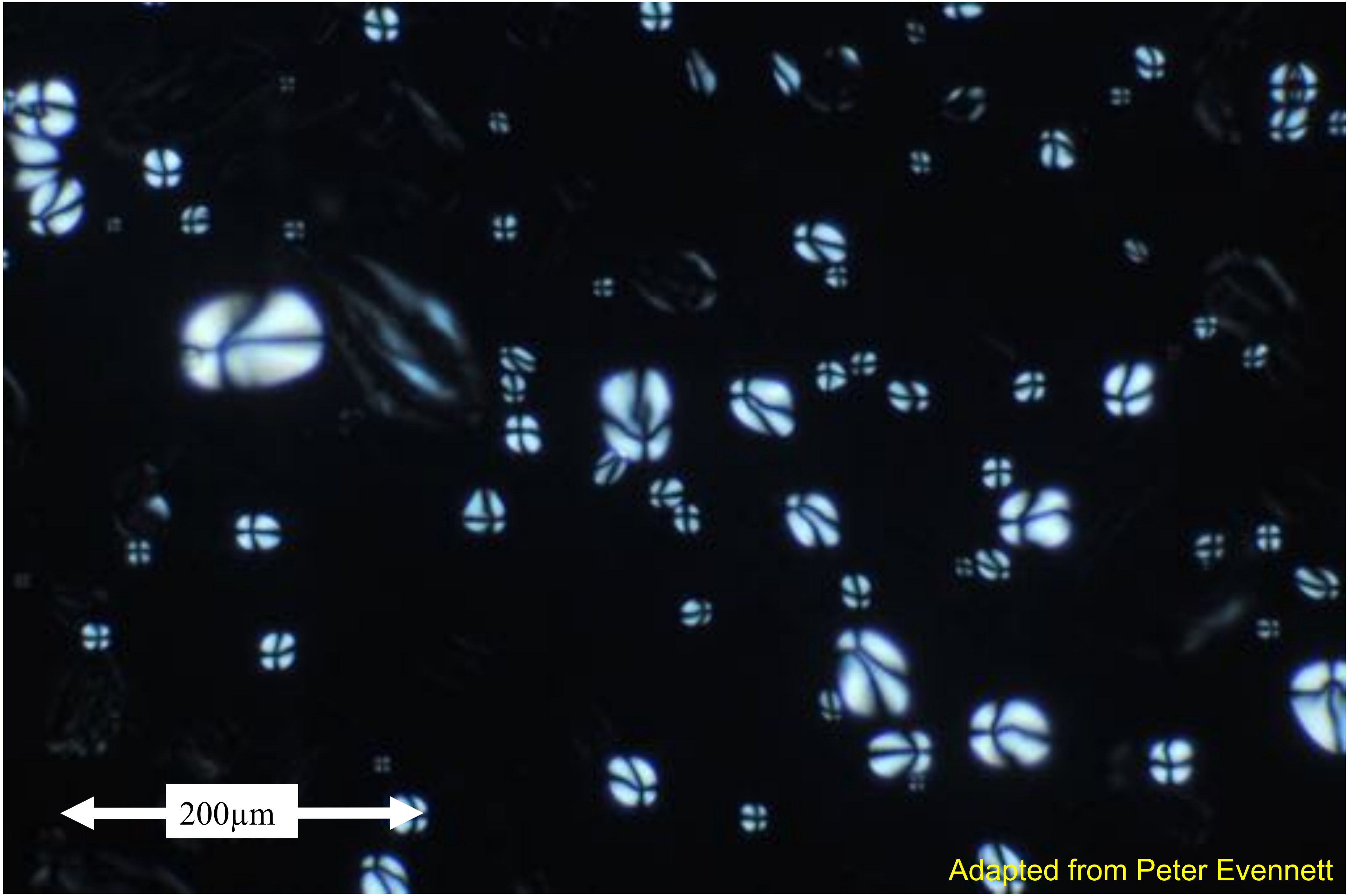
Adapted from Peter Evennett

What is it good for

- Imaging of birefringent materials
  - Microtubuli (e.g. spindle apparatus)
  - Actin (e.g. muscle cells)
  - Cellulose (e.g. starch)

Potato Starch

Potato Starch – Crossed Polars



What is it good for

- Imaging of birefringent structures
    - Microtubuli (e.g. spindle apparatus)
    - Actin (e.g. muscle cells)
    - Cellulose (e.g. starch)
    - Crystals
- Visualization of sample properties
    - Differences in refractive indices in birefringent structures
    - Composition of materials
    - Thickness of sample
    - Molecular structure
    - Orientation of molecules (e.g. stretching/stress)
- **BUT:** limited to birefringent materials!

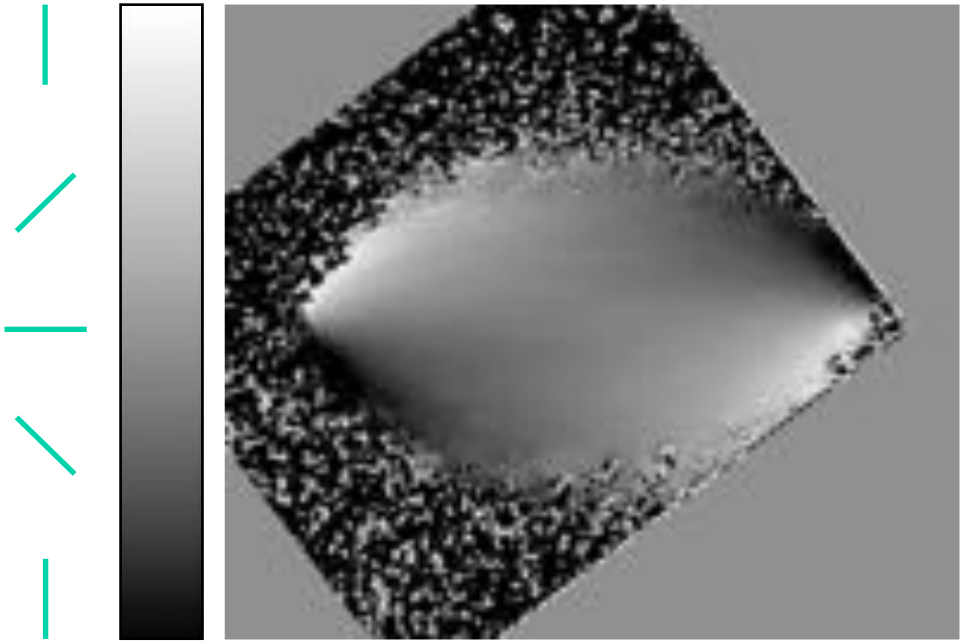
PolScope

Brugues Lab

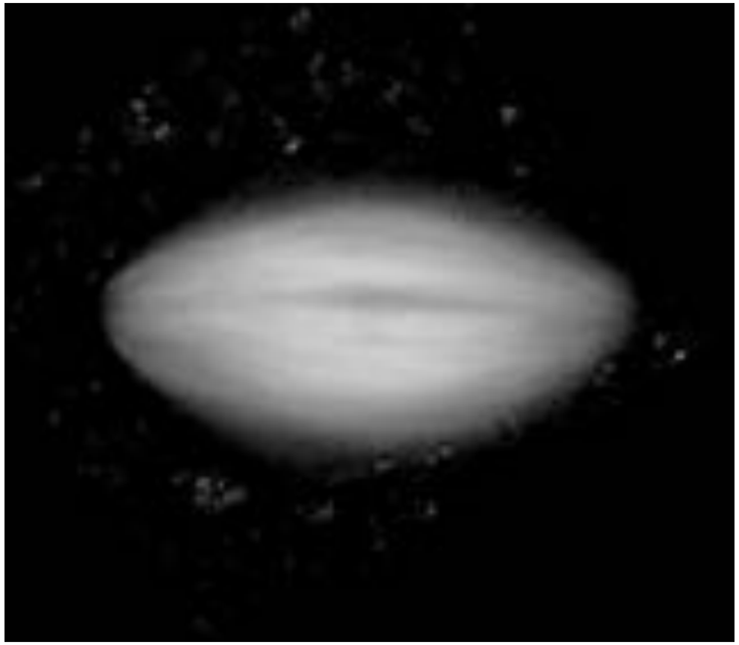
Primary Spermatocyte (nephrotoma suturalis)

http://www.openpolscope.org

Spindle fluctuations

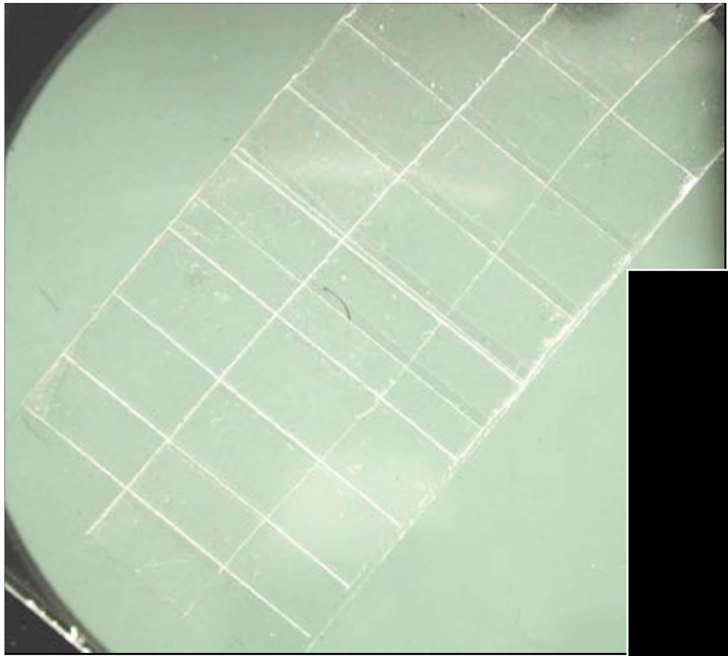


Orientation field n(x,t)
Microtubule orientation at every pixel

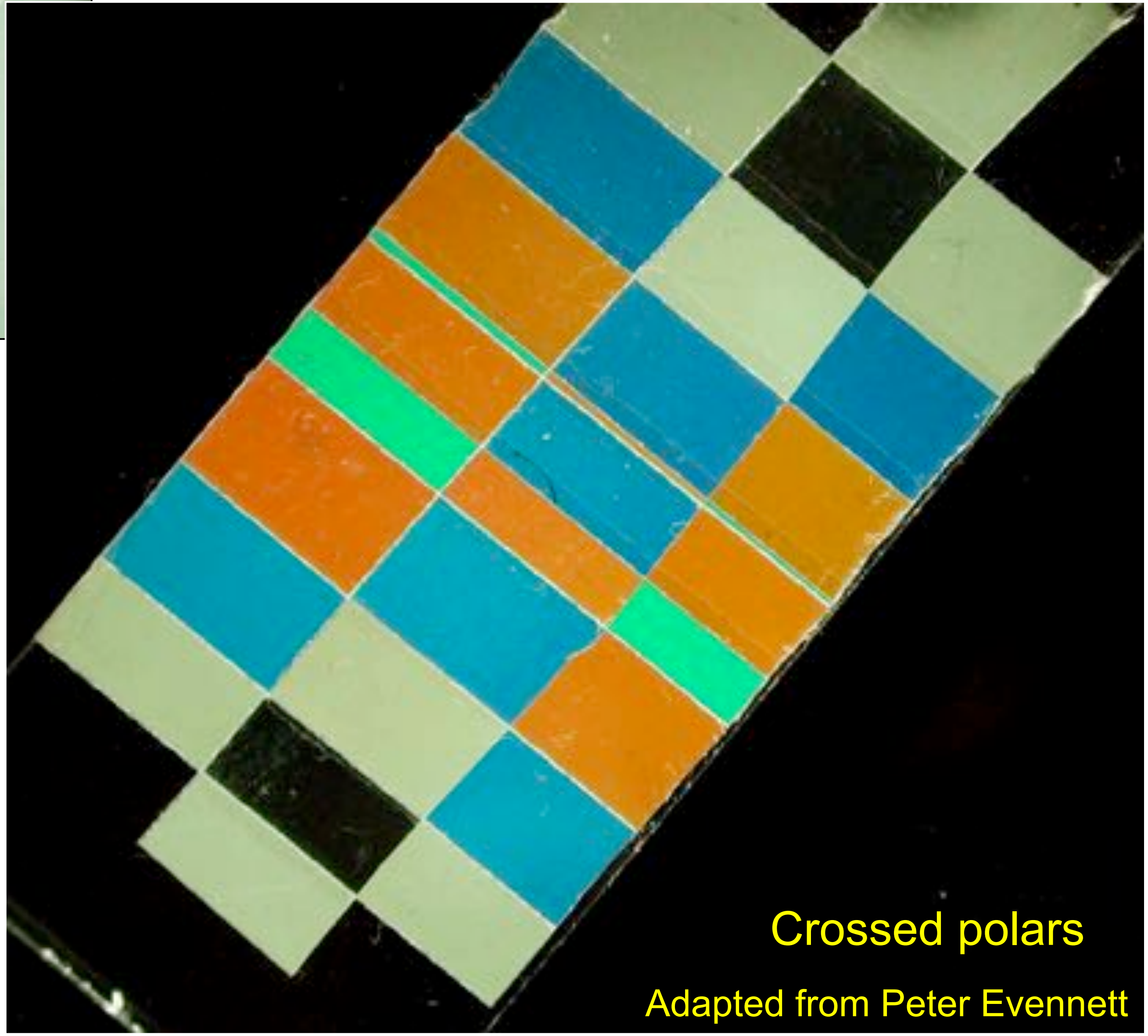


Density field c(x,t)
Retardance: microtubule density x degree of alignment

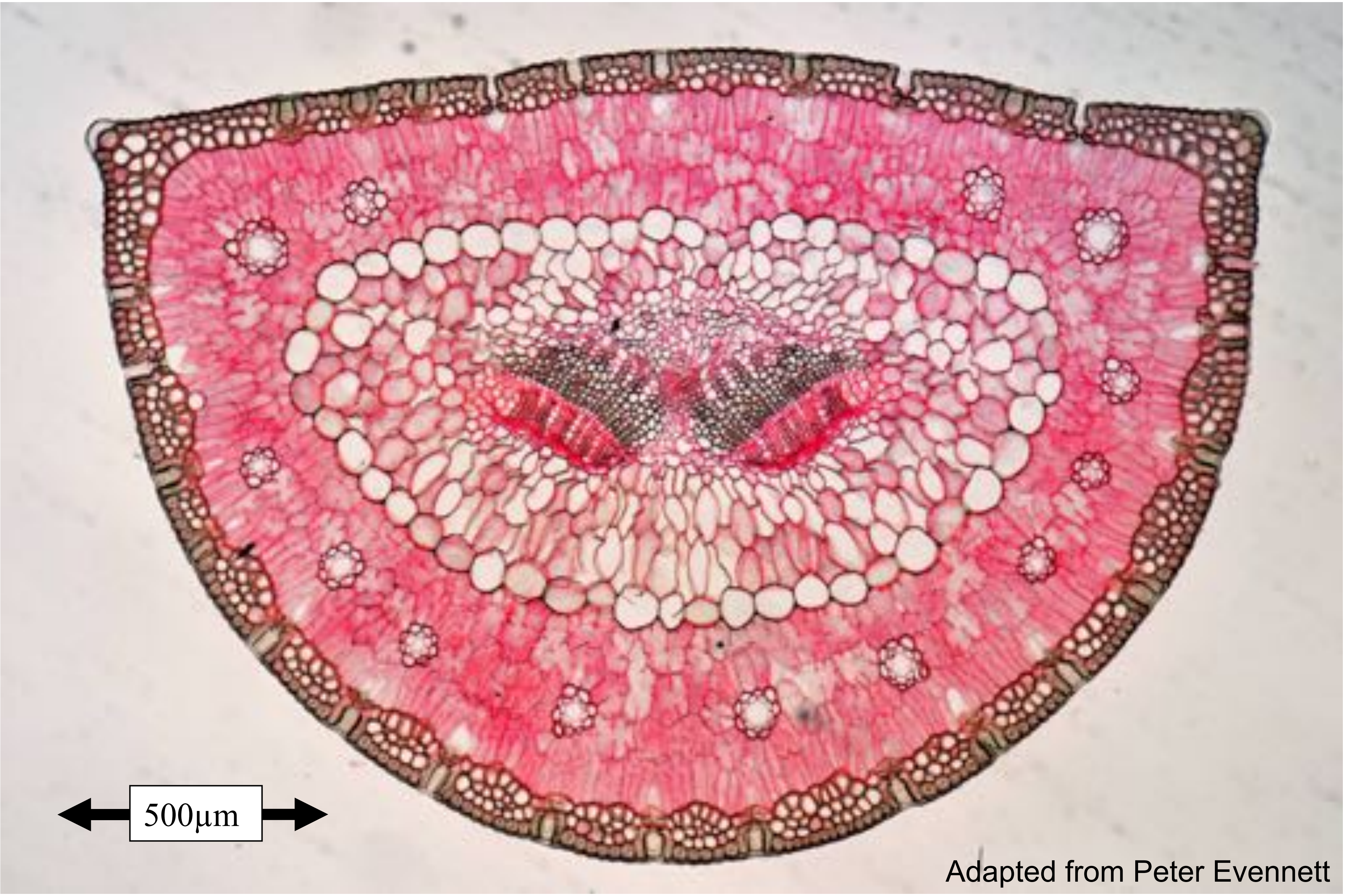
Brugues Lab



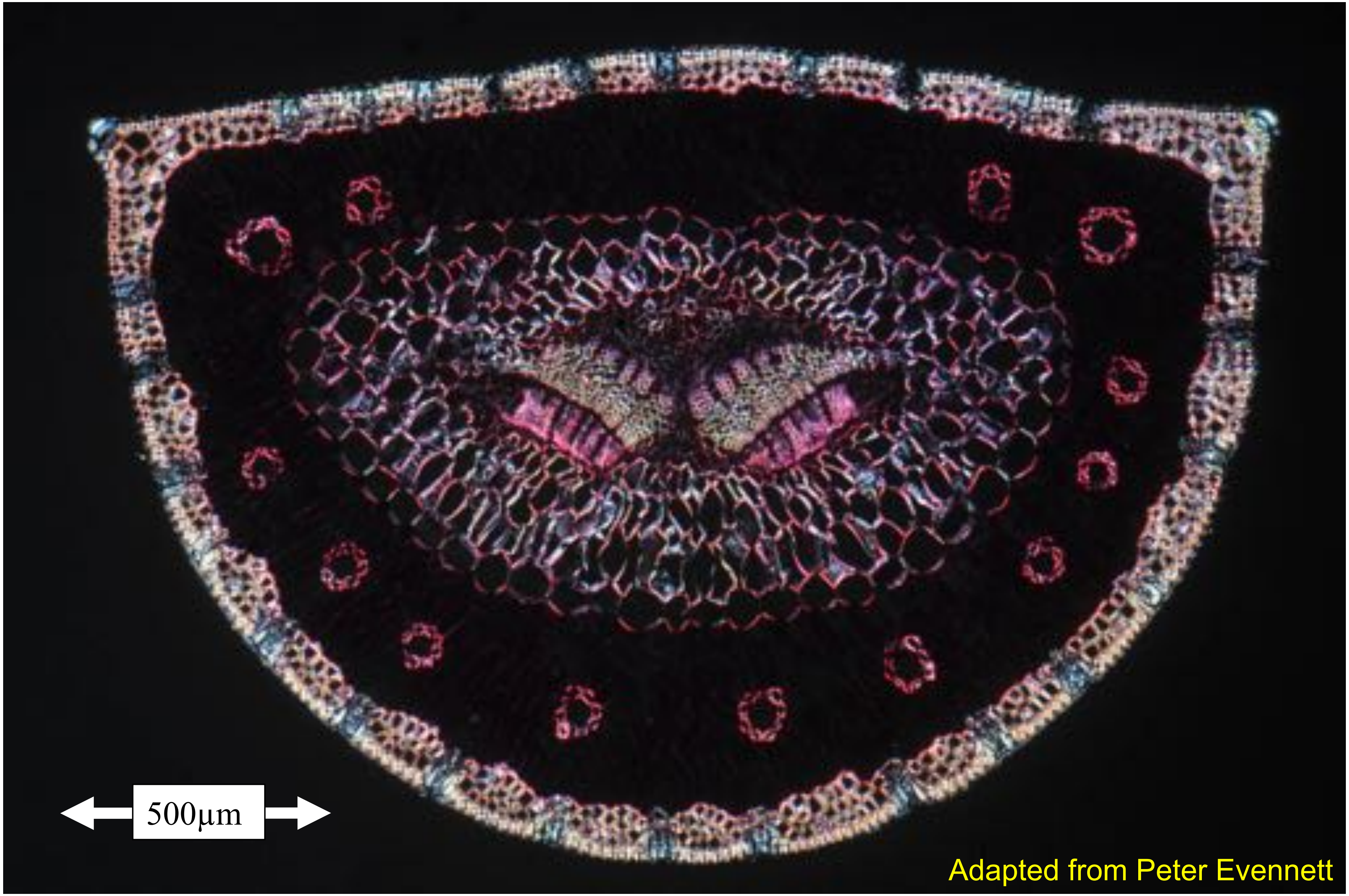
Overlapping pieces of Sellotape



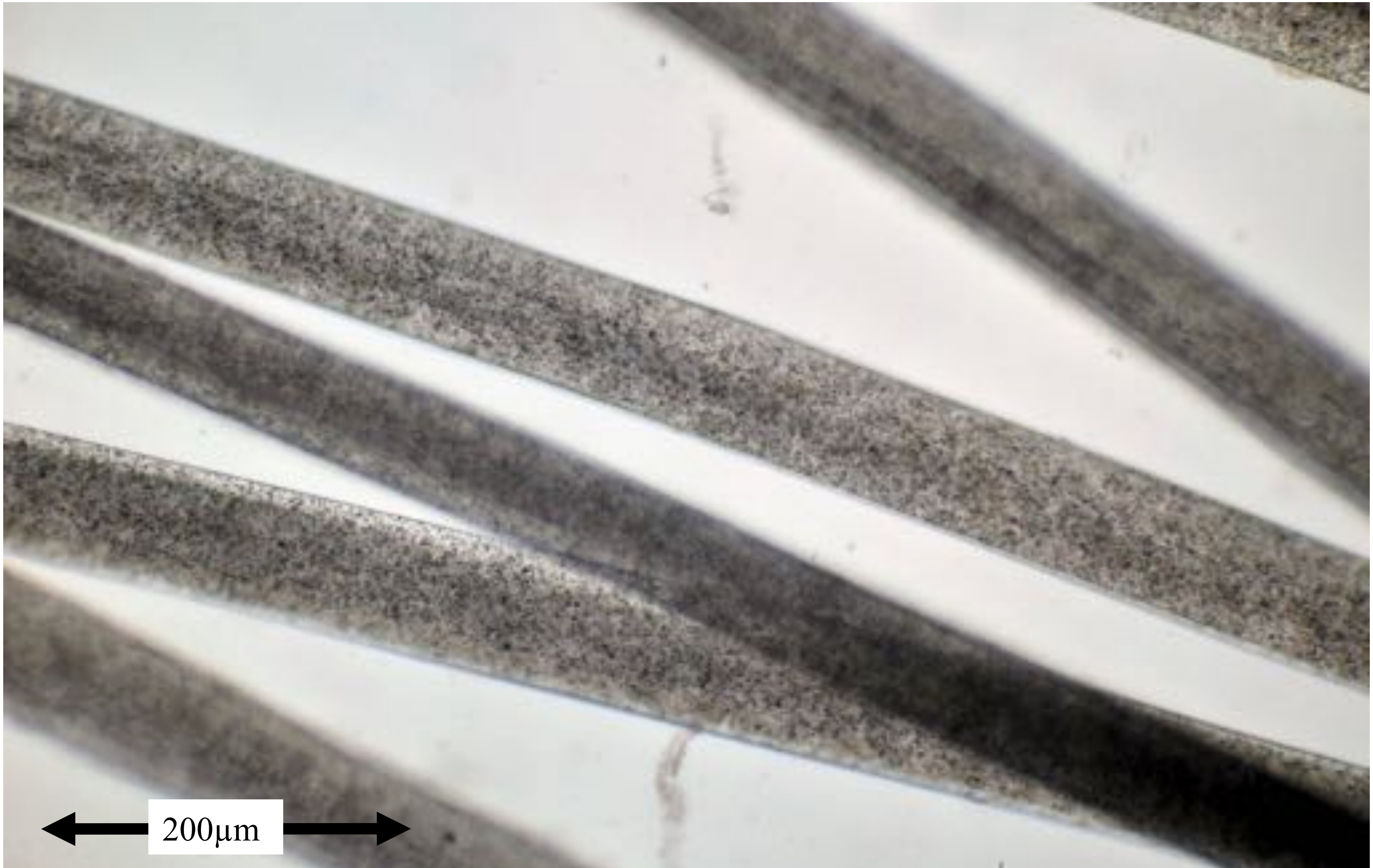
Pine needle



Pine needle – Crossed polars

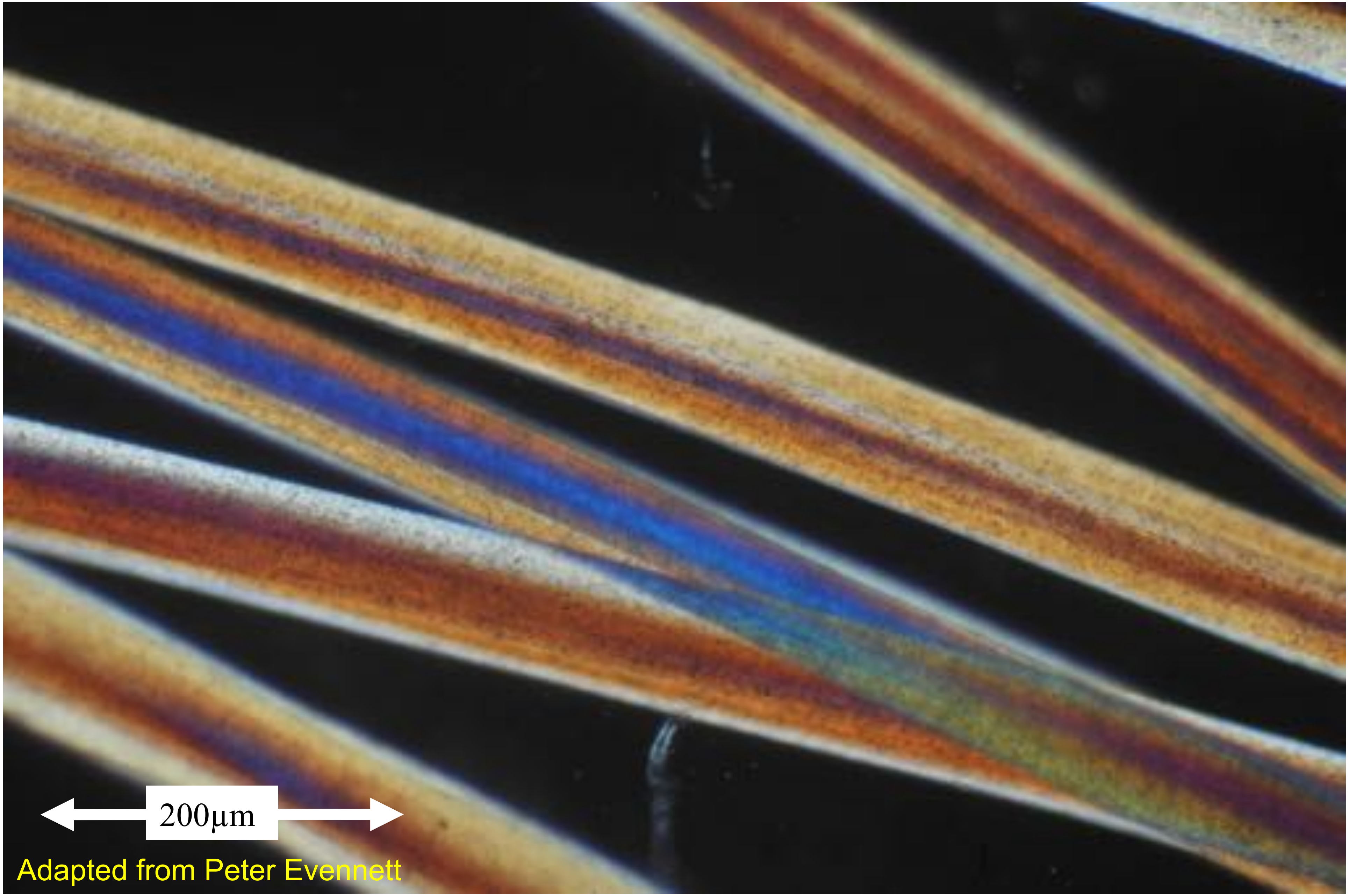


Viscose fibres

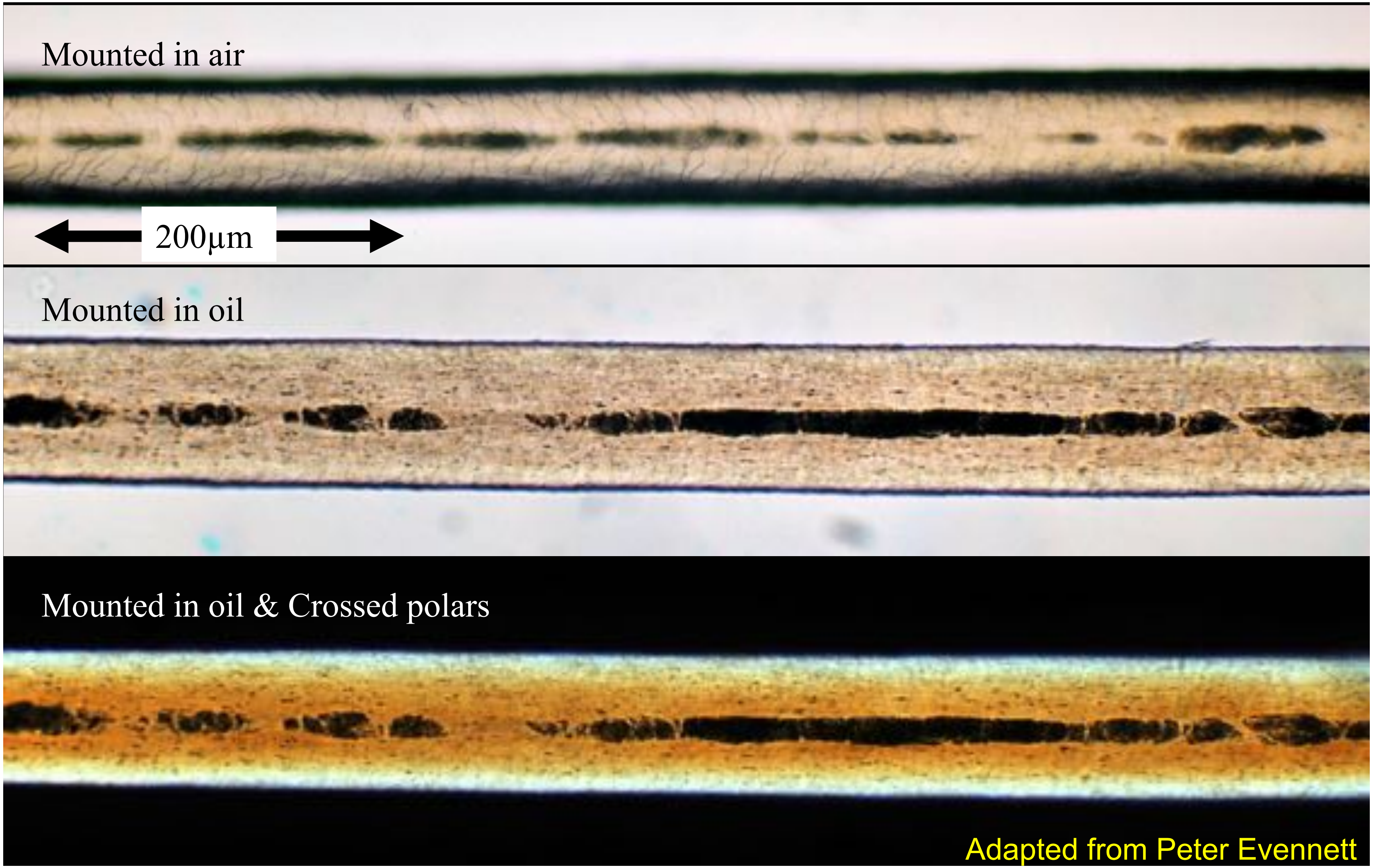


Adapted from Peter Evennett

Viscose fibres – Crossed polars



Human hair


Mounted in air
200µm
Mounted in oil
Mounted in oil & Crossed polars
Adapted from Peter Evennett