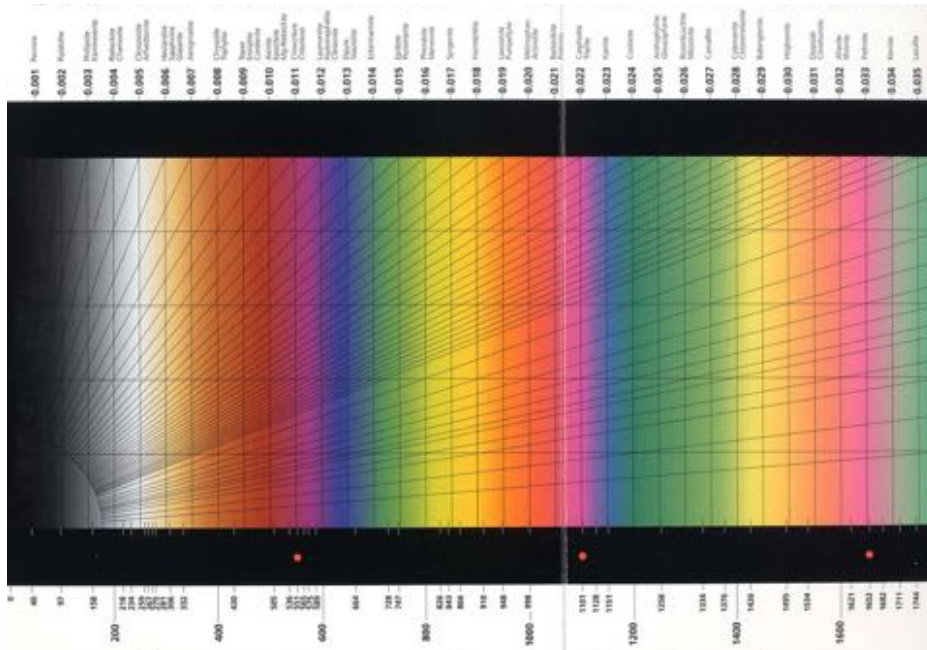
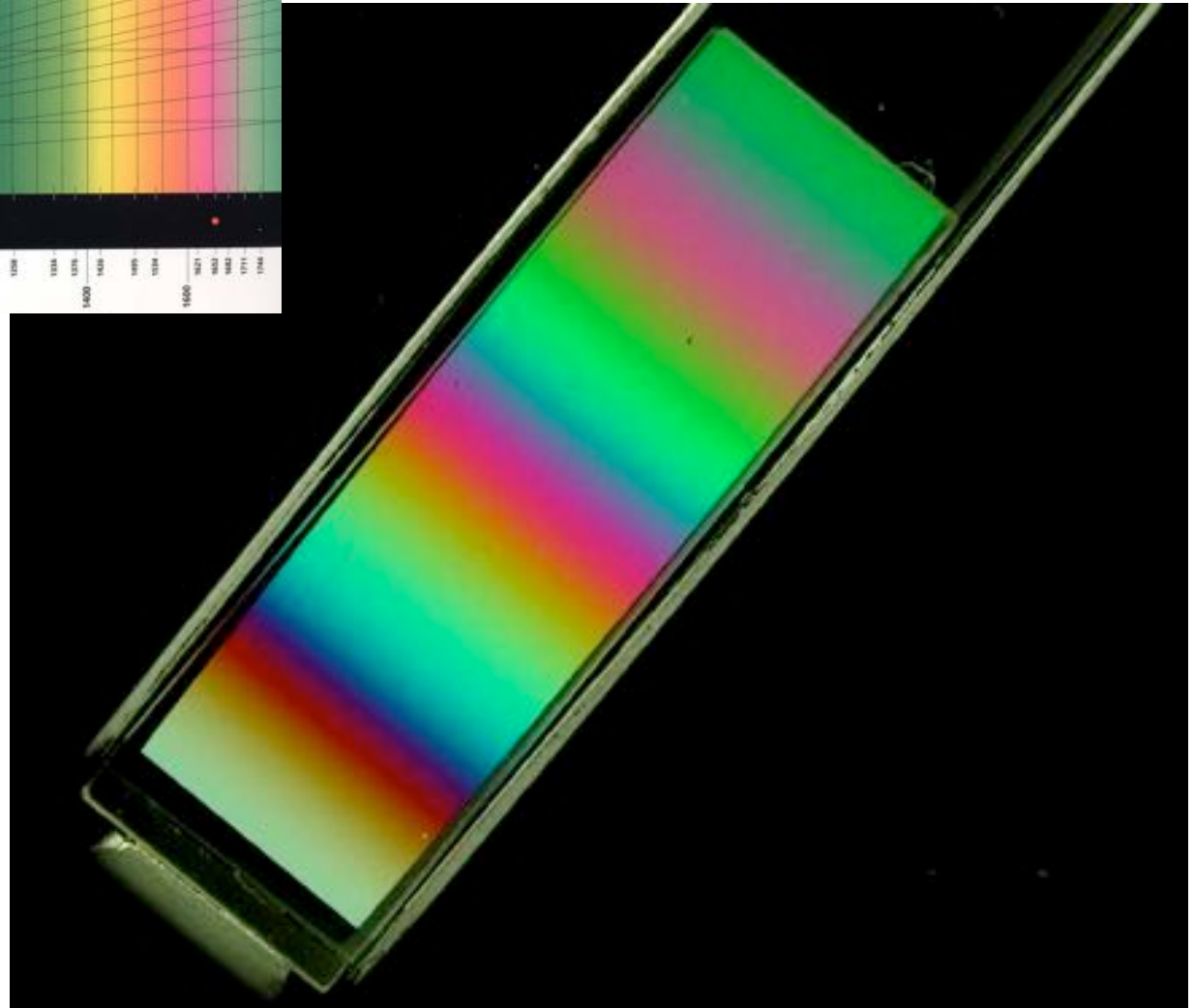




Polarised Light Microscopy




Adapted from
Peter Evennett



Example: Meiosis

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Flowplay
© 2004-2007

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CIL-9060*

Description

The events of meiosis I and II in a living insect spermatocyte beginning with the breakdown of the nuclear envelope at diakinesis through to the completion of cytokinesis. Testes from the Crane fly *Nephrotoma suturalis* were observed with time-lapse polarization using a Nikon Microphot SA, equipped for liquid crystal polarized light microscopy (LC-PolScope, CRI, Woburn Massachusetts) 60x/1.4 PlanApo oil immersion objective, 1.4 NA oil imm. condenser, with 1.5x zoom lens. Image acquisition and processing Camera: Images captured every 2 min over 5 hr 18 min by a QImaging Retiga EX0 CCD camera. Raw images were processed using a 5-frame algorithm (Shribak and Oldenbourg, 2003). Movie images represent computed retardance as gray values ranging from 0 nm retardance (black) to 2 nm retardance or higher (white).

Biological Sources

NCBI Organism Classification
Nephrotoma suturalis

Cell Type
• spermatocyte

Cellular Component
• cytoskeleton
• nuclear chromosome
• spindle

Biological Context

Biological Process
• male meiosis chromosome segregation
• spindle assembly involved in male meiosis

Attribution

Names
• Rudolf Oldenbourg
• James R. LaFountain

Pubmed
• 15385630
• 12790452

Grouping

This image is part of a group.

Imaging

Image Type
• recorded image
• charge coupled device (CCD)
• Processed image based on 5 recorded images

Imaging Mode
• polarization microscopy

Parameters Imaged
• UV/visible/IR photons

Source of Contrast
• intrinsic birefringence

Visualization Methods
• Processed image based on 5 recorded images

Processing History
• 5-frame algorithm

Sample Preparation

Methods
• living tissue


Relation to Intact Cell
• whole mounted tissue


Dimensions

Spatial Axis	Image Size	Pixel Size
X	500px	87nm
Y	500px	87nm
Time	120 sec	153


[Video Data Download Options](#)


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 From

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*CIL - Cell Image Library accession number. Please use this to reference an image.

Ink-spot
on paper

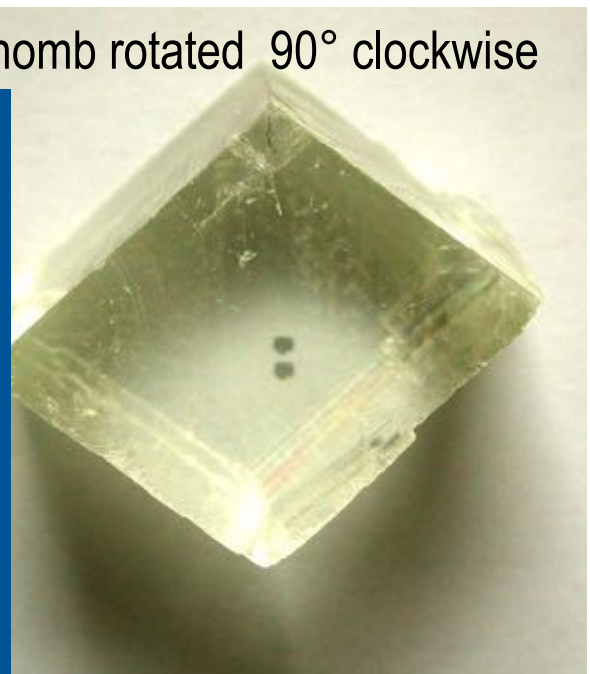


Calcite rhomb

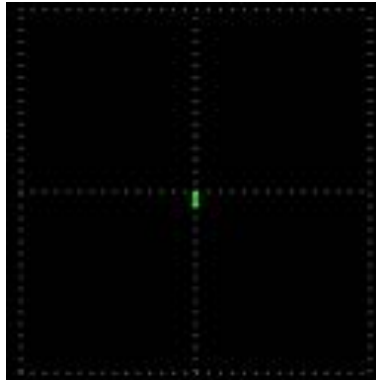
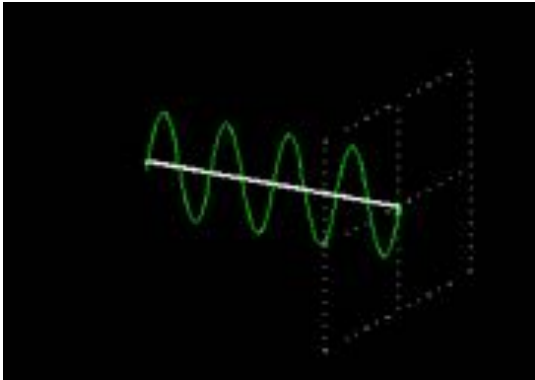


Double
image of
spot,
appearing
in different
positions
and at
different
heights
from
paper

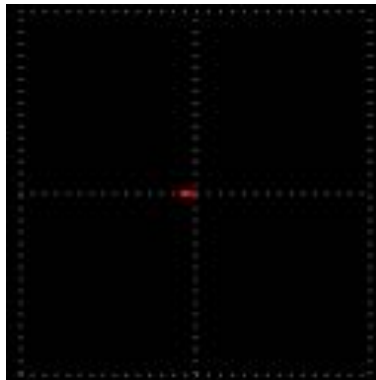
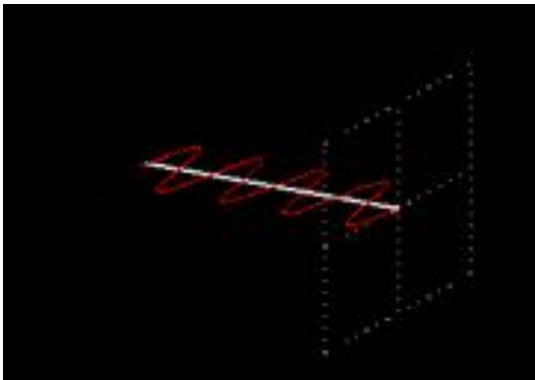
Rhomb rotated 90° clockwise



Light waves

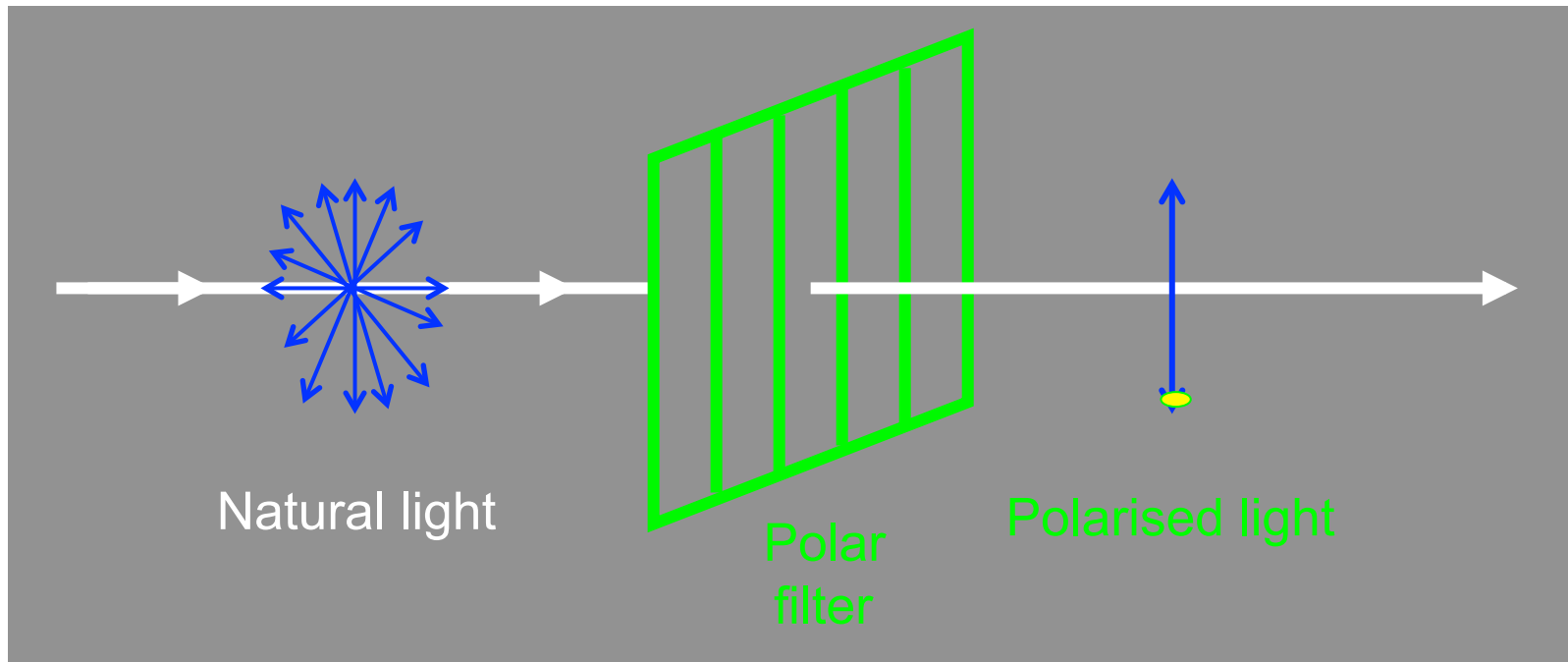


- Light waves vibrate within a plane transverse to the direction of propagation



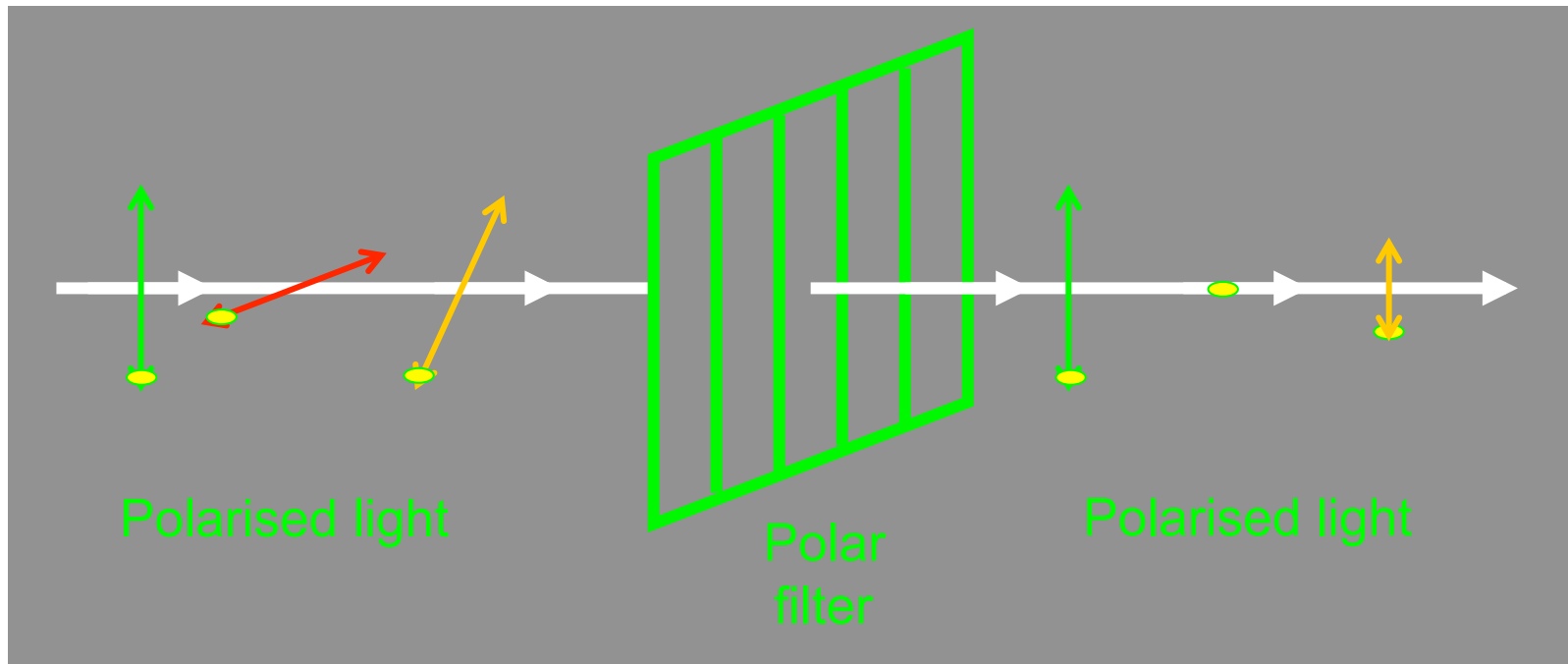
- Orientation of the plane relative to the direction of propagation:
→ **Polarisation**

Polarisation of light



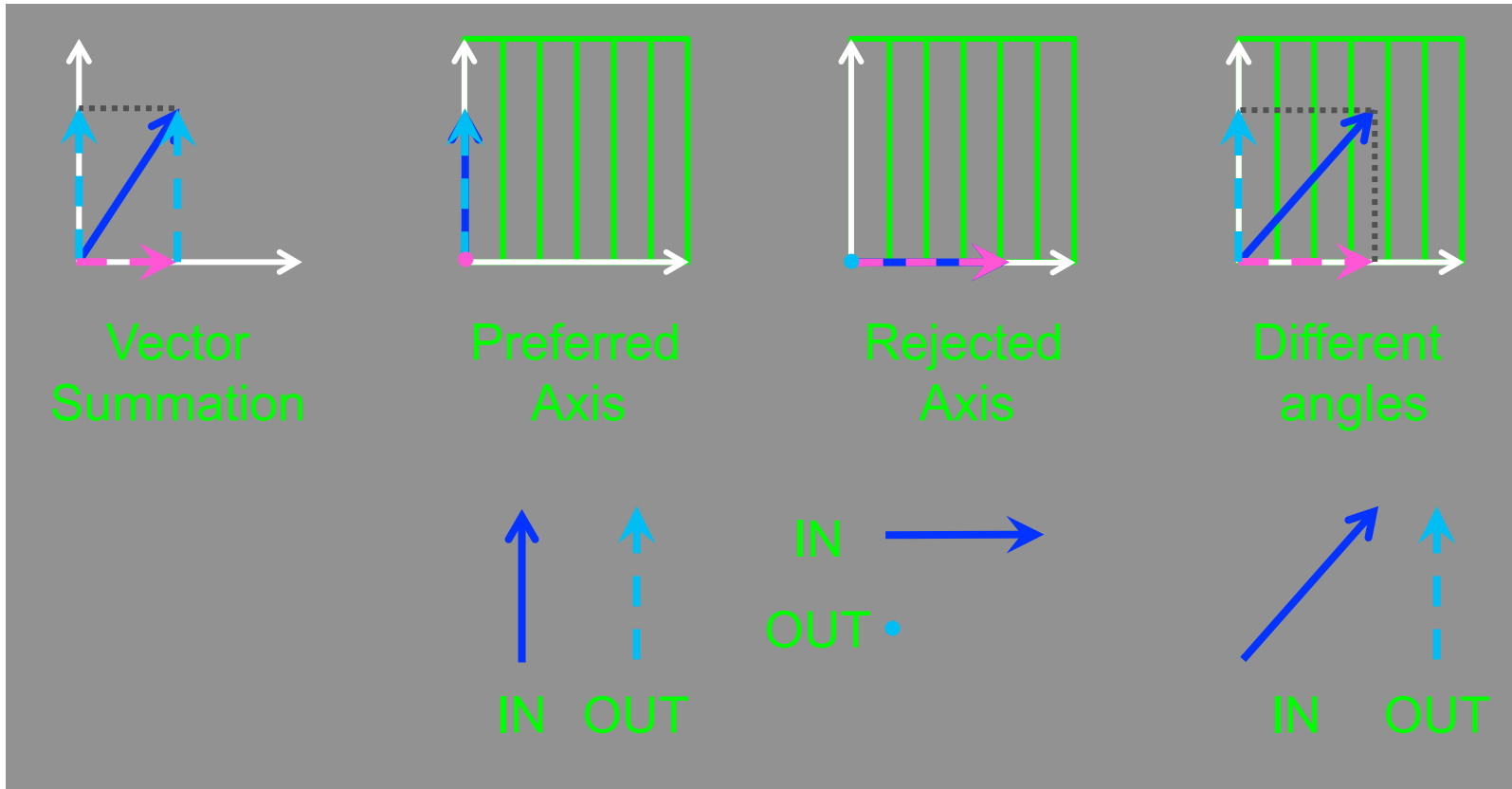
- 'Natural Light': vibrations occur in all directions
- Polarised Light: all but one of these directions have been 'filtered out'

Polarisation Filters

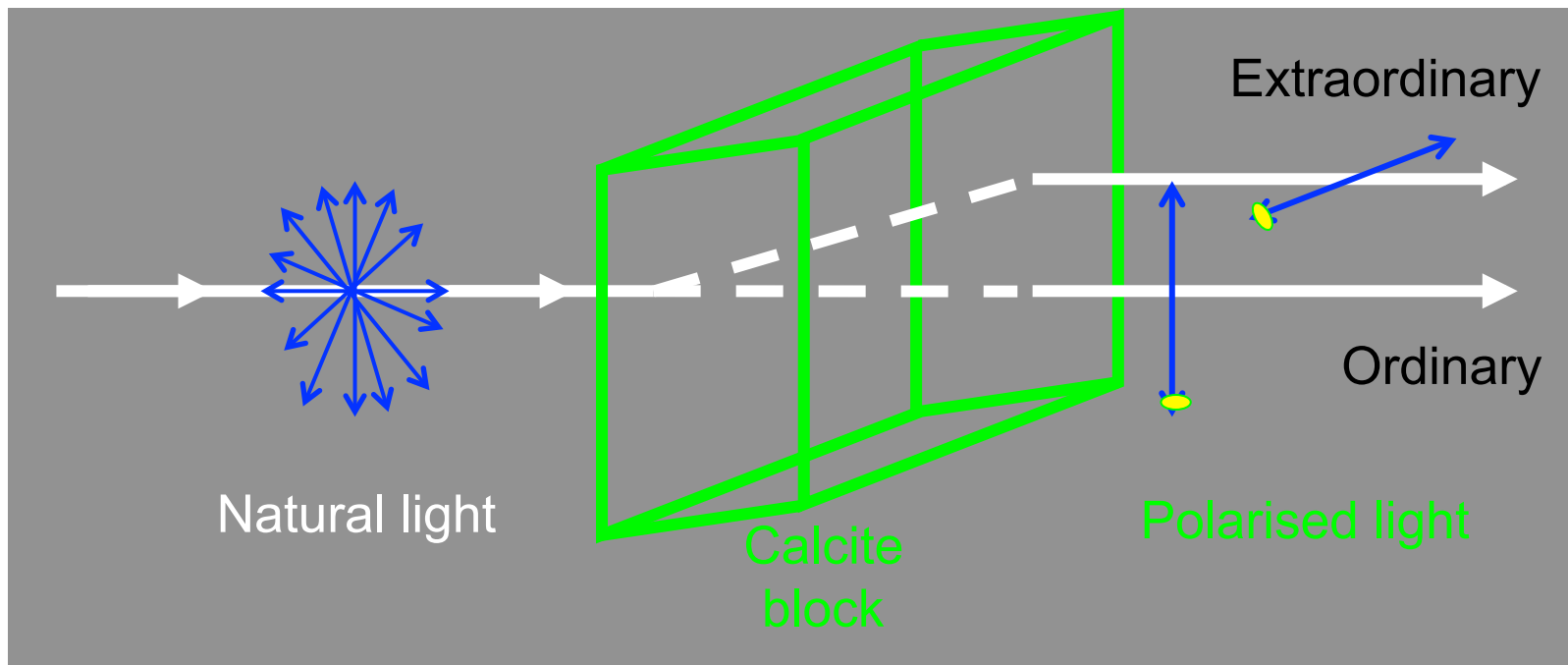


- Preferred angle: all light passes
- Rejected angle: no light passes
- Other angles: some light passes with preferred polarisation angle

Vector Summation



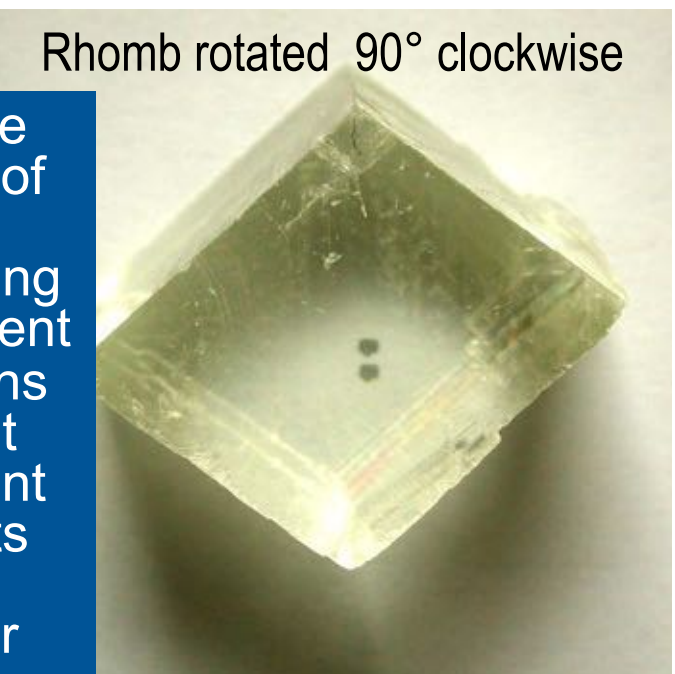
Birefringence



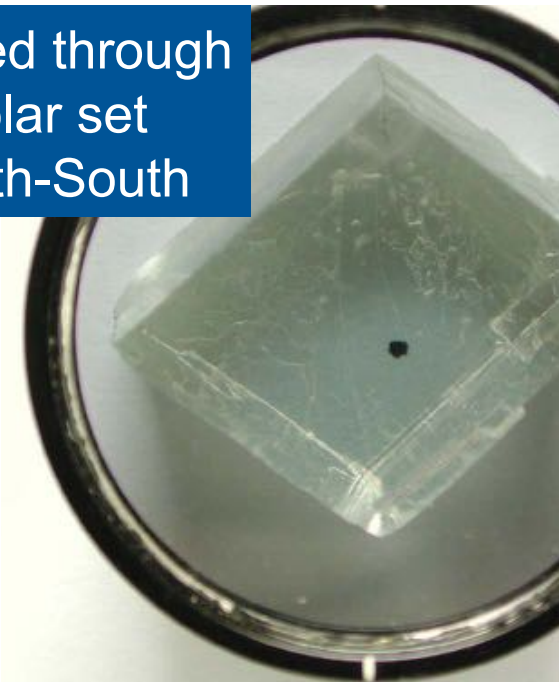
- Separate single beam into two beams/waves
- Refractive index dependent on direction of propagation
 - **Ordinary beam:** refracted normally
 - **Extraordinary beam:** refractive index depends on angle of beam and can exhibit an additional angular shift
- Both waves polarized along orthogonal axes



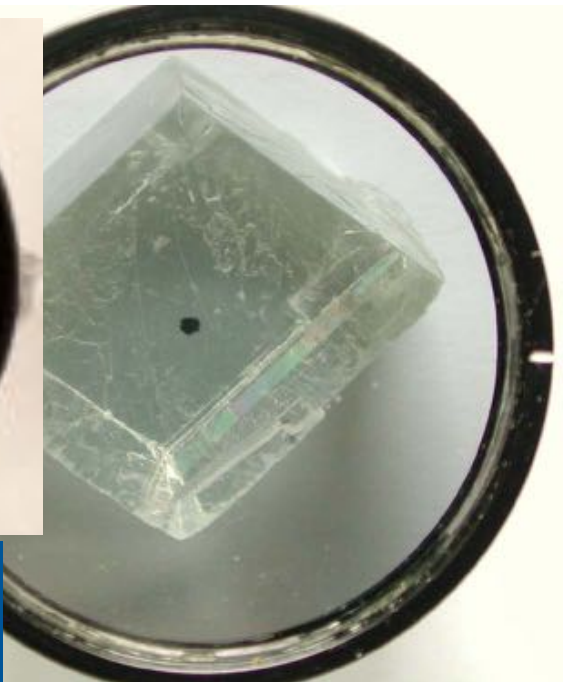
Double
image of
spot,
appearing
in different
positions
and at
different
heights
from
paper



Viewed through
polar set
North-South



Polar rotated
West-East



Adapted from Peter Evennett

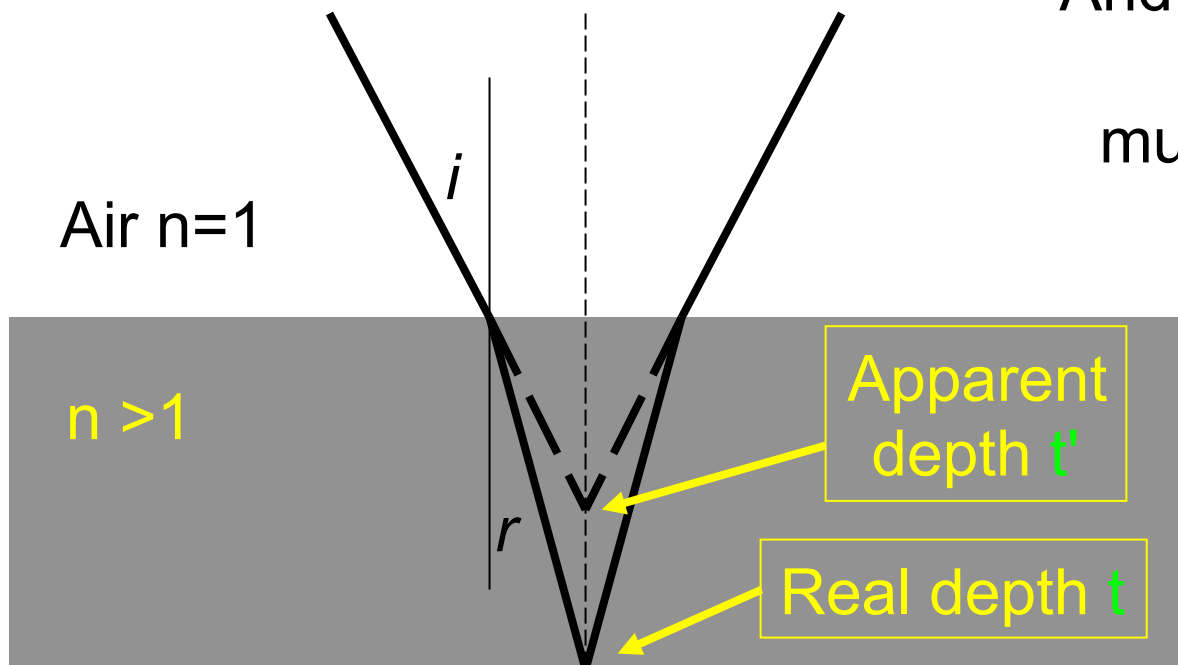
Apparent Depth

Snell's Law:

$$n = \frac{\sin i}{\sin r} = \frac{t}{t'}$$

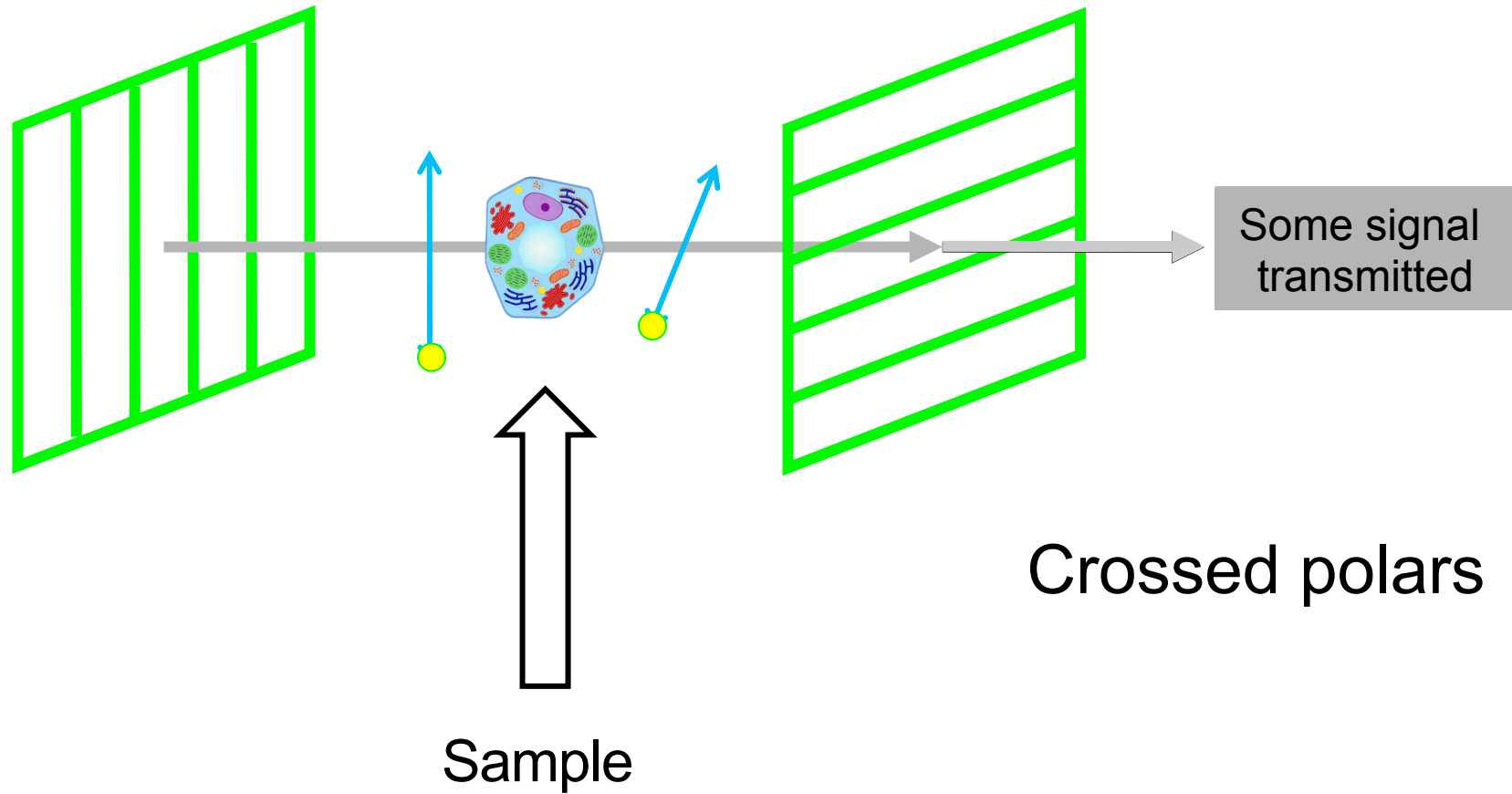
Calcite gives a double image at two apparent depths because it has two refractive indices.

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

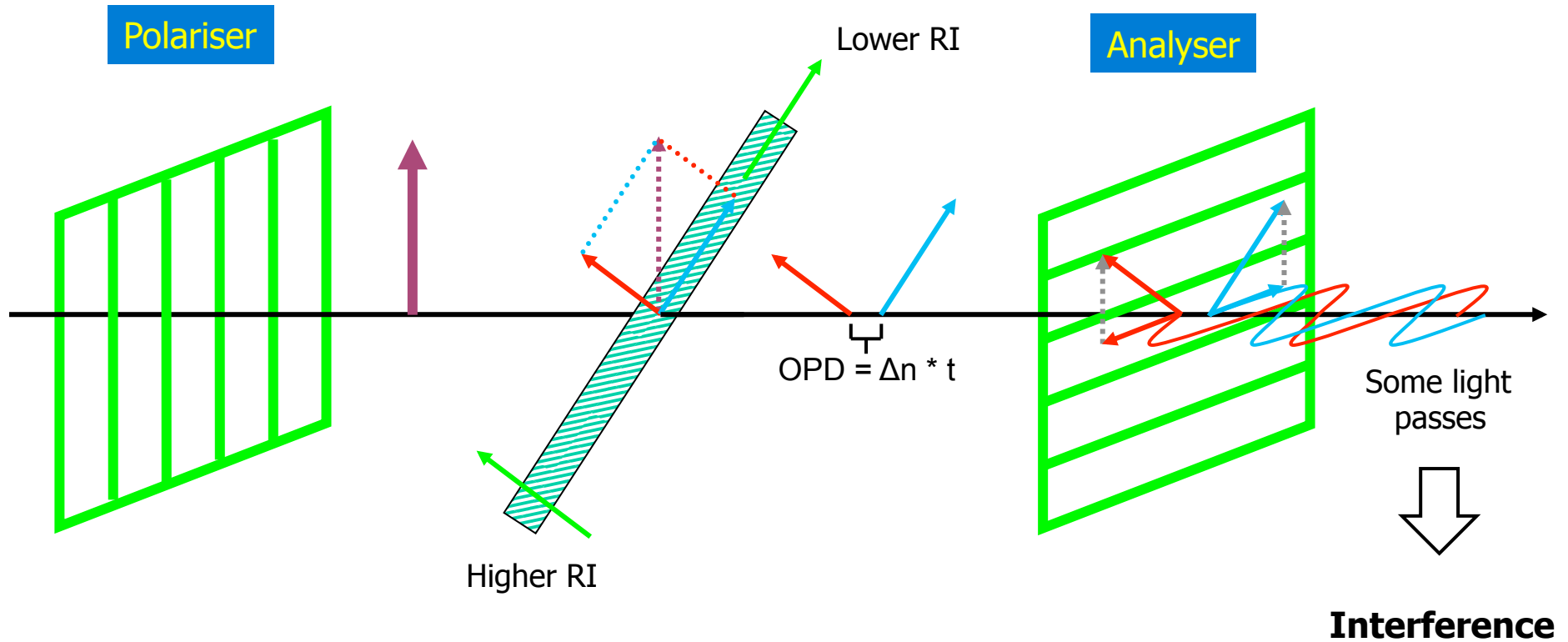


Adapted from Peter Evennett

Basic Principle

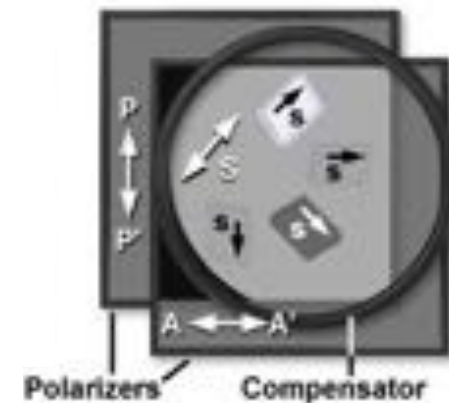
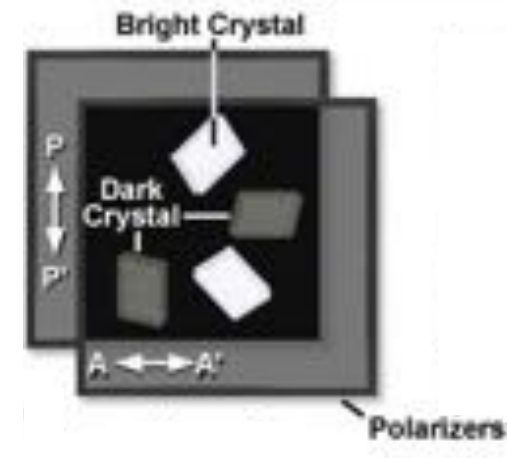


Effect of Birefringent Samples

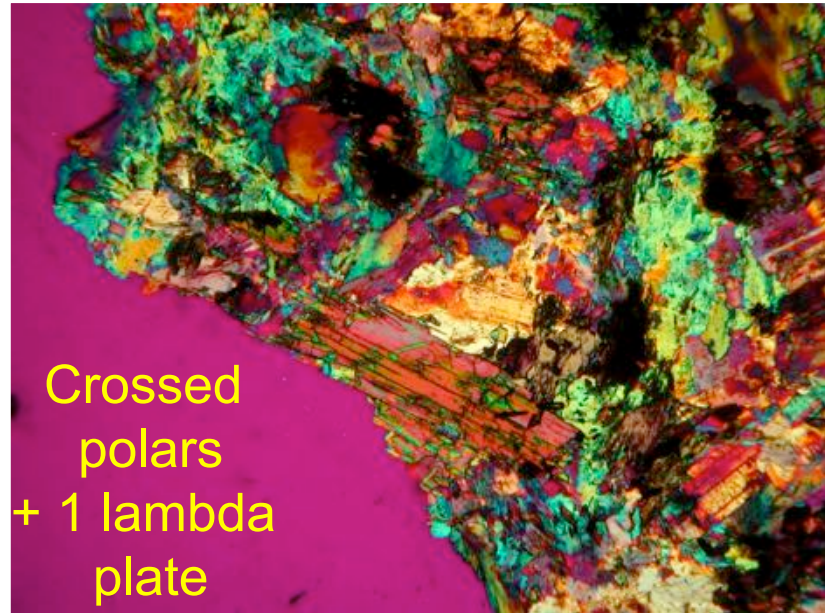
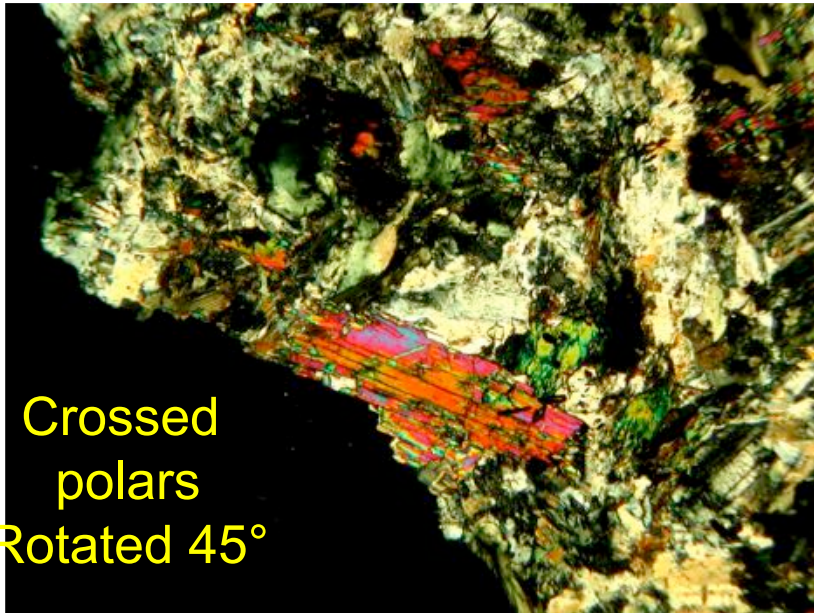
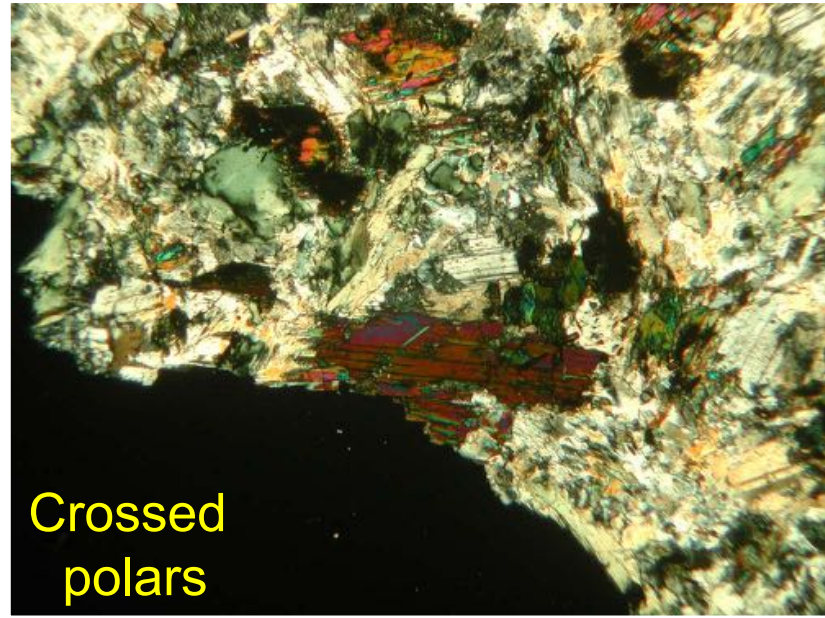
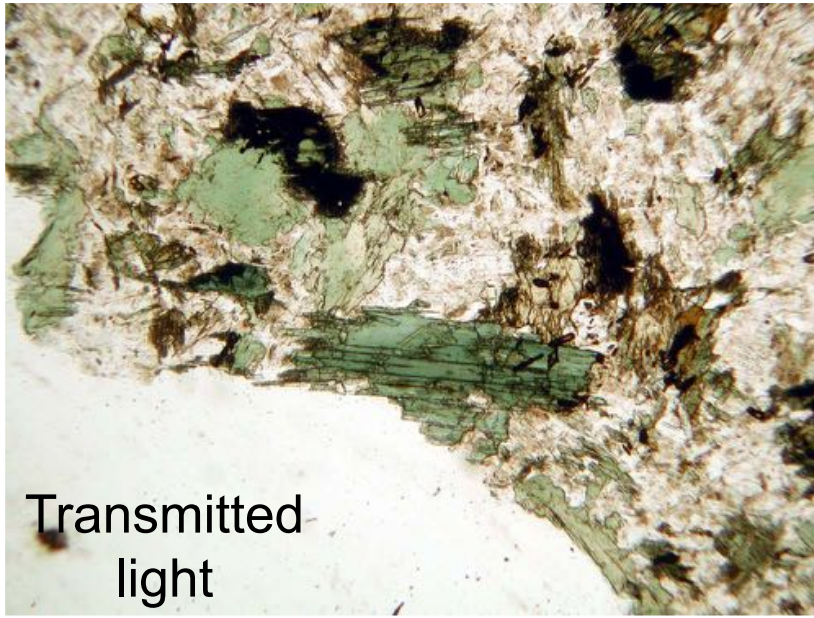


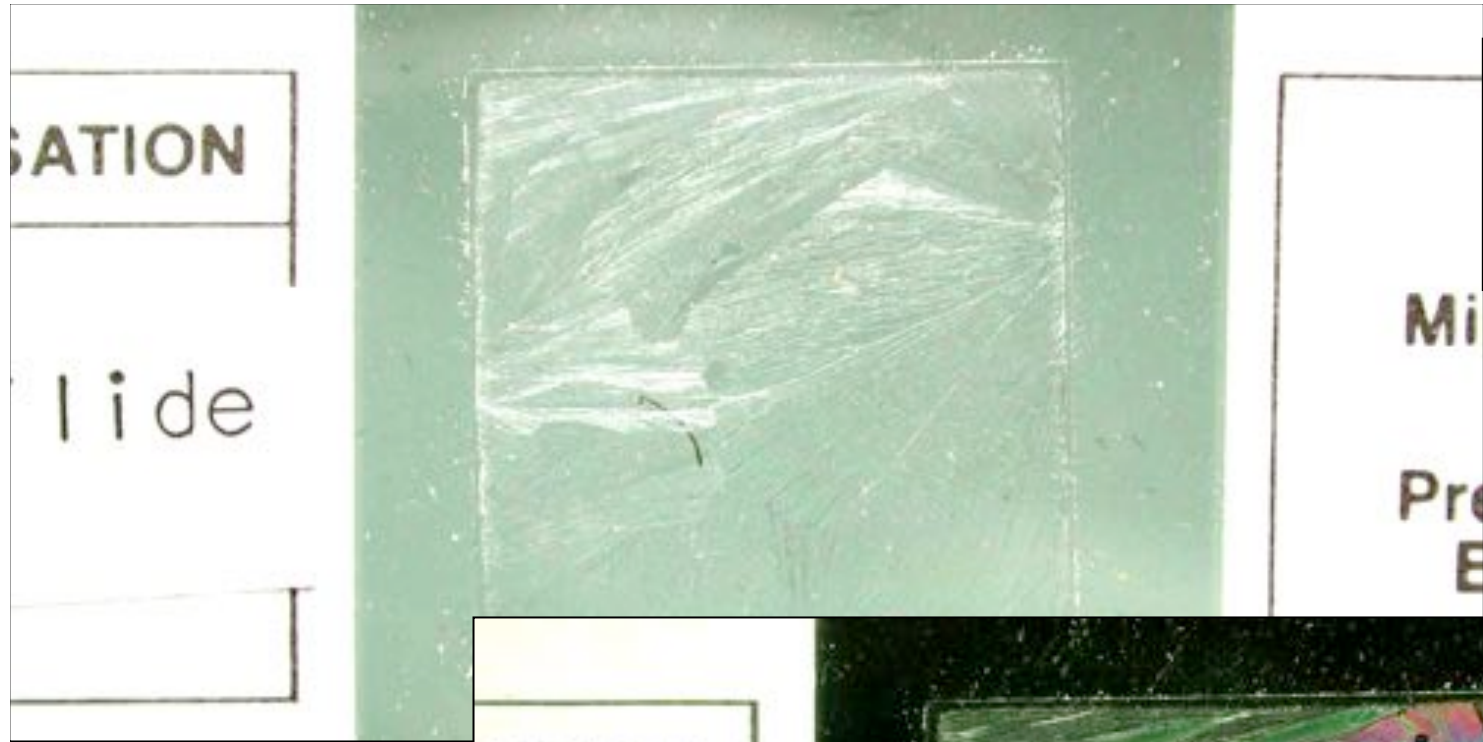
Effects on Contrast

- 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

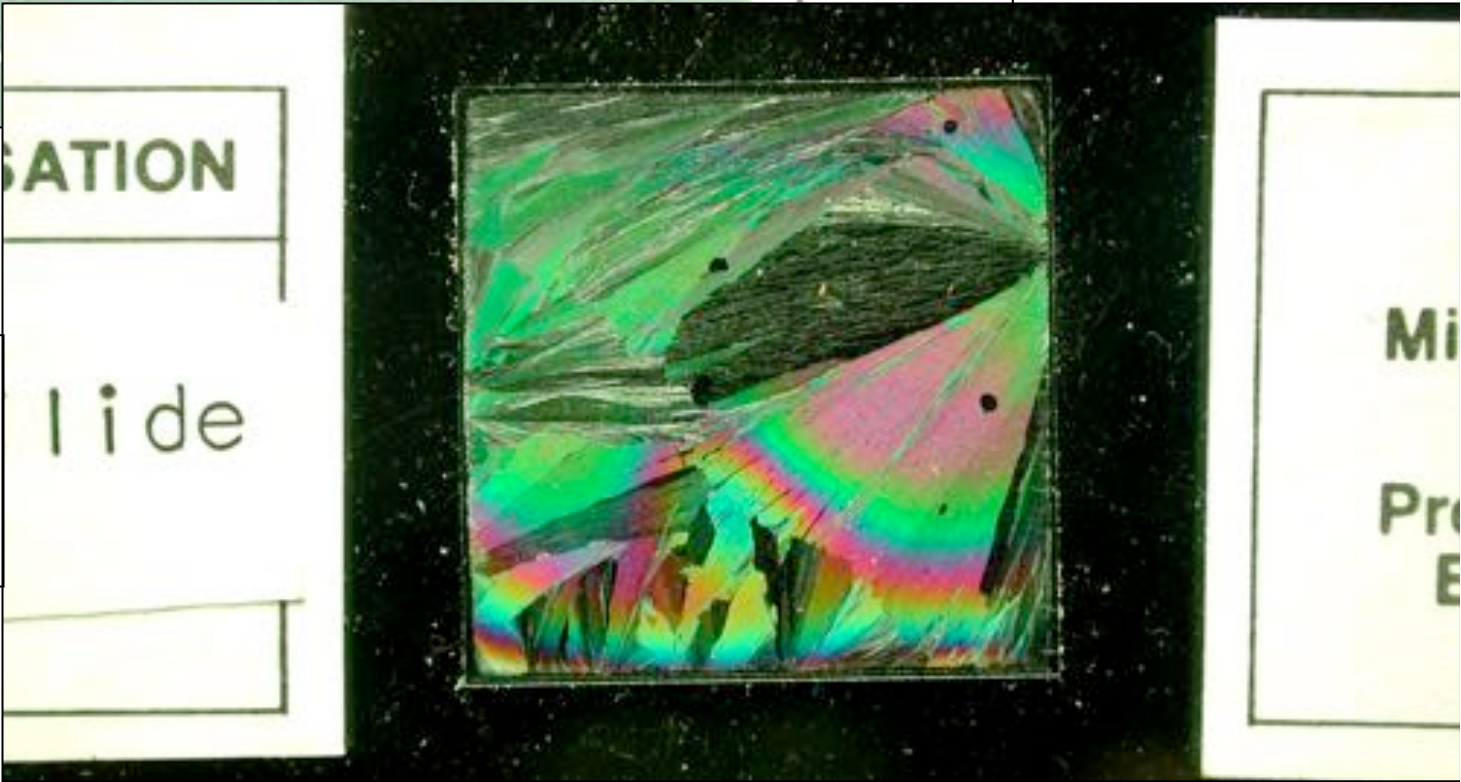


Example



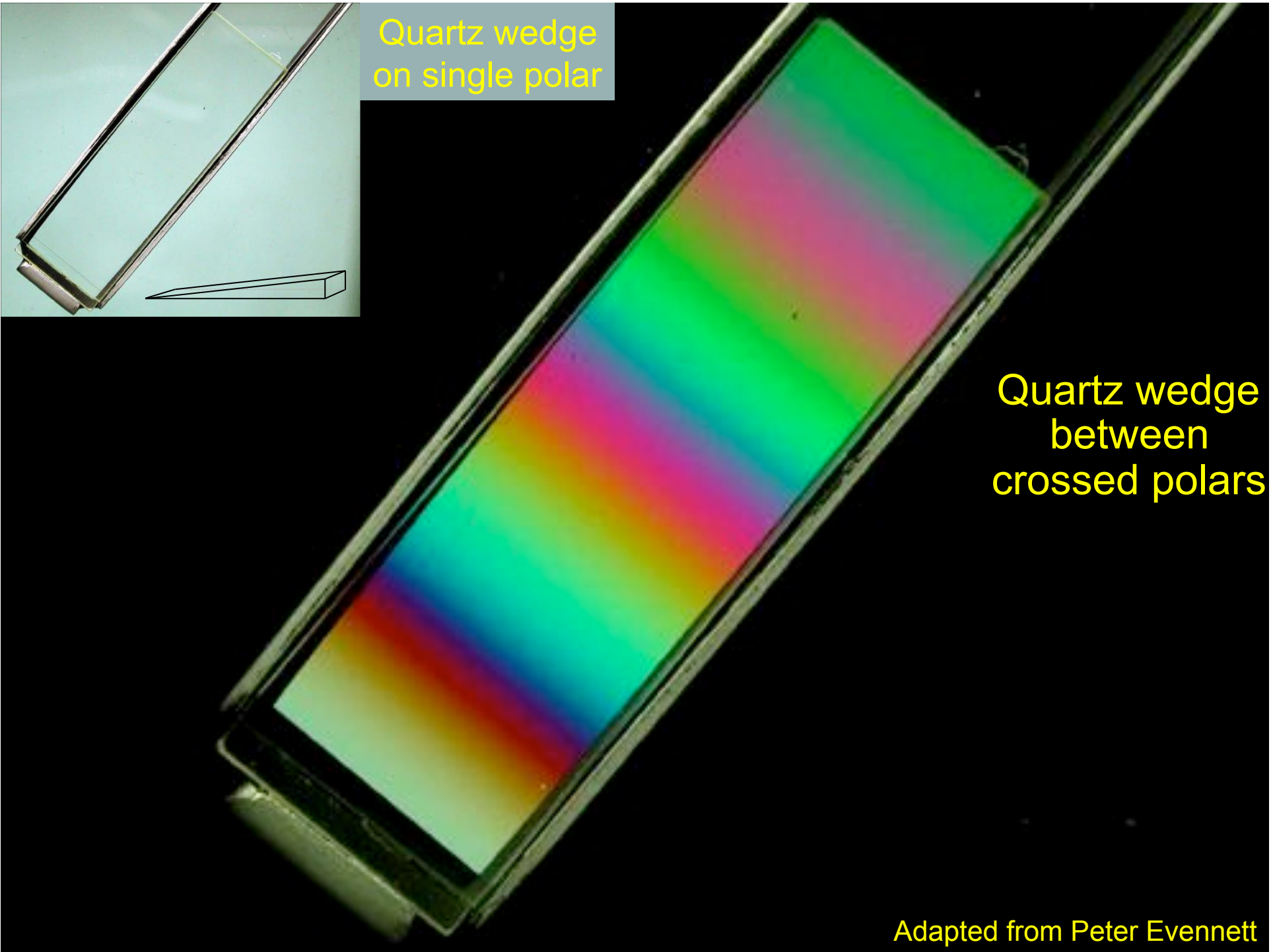
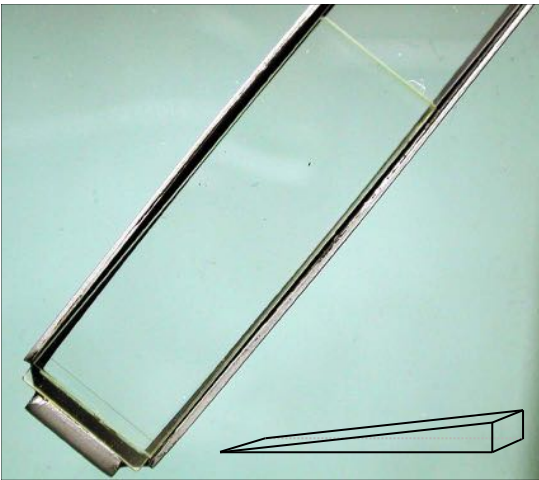


Crystals of Acetanilide.
Natural light



Between
crossed
polars

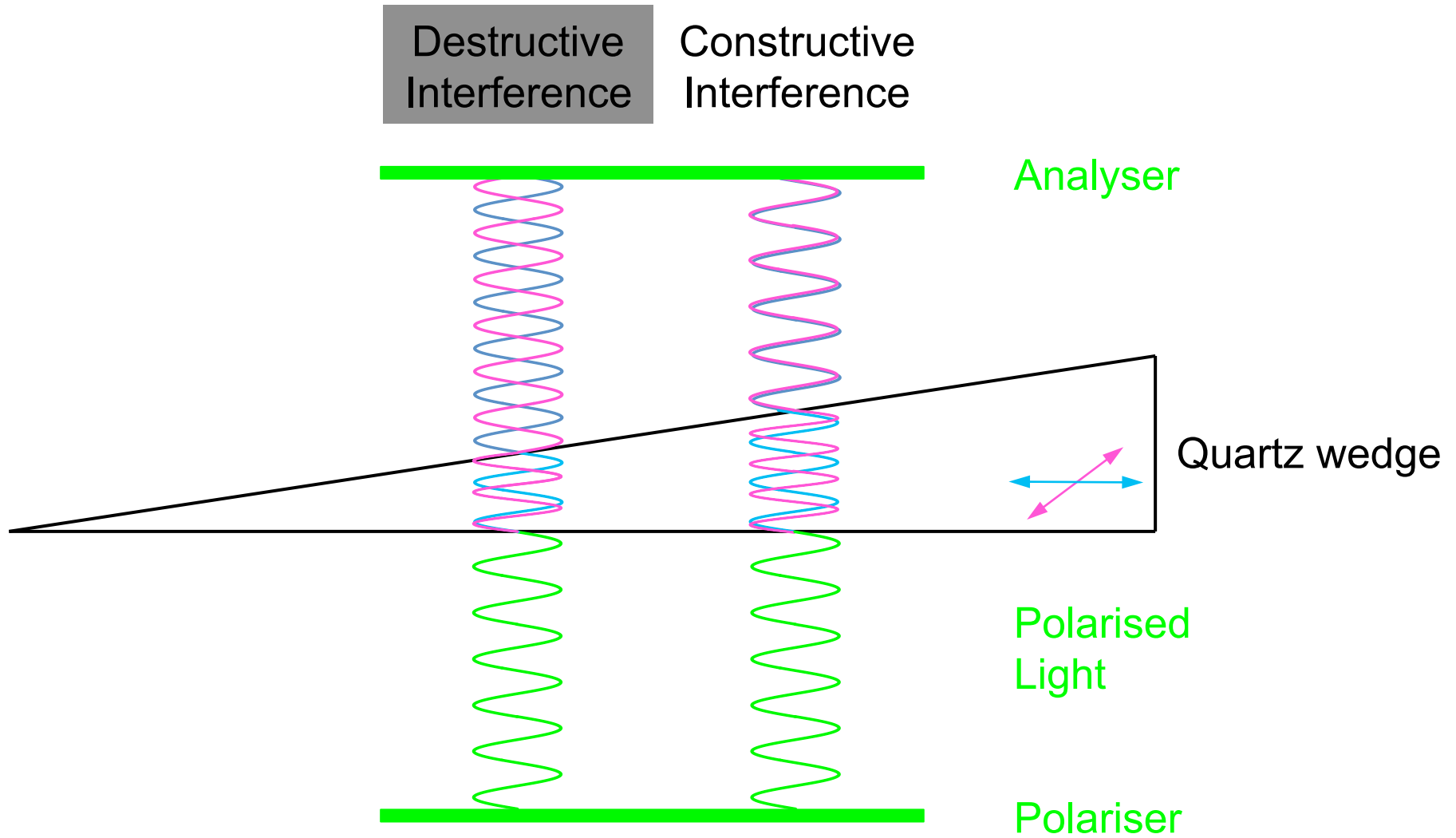
Quartz wedge
on single polar



Quartz wedge
between
crossed polars

Adapted from Peter Evennett

Interference Colours



Blue filter
 $\lambda = 480\text{nm}$

Green filter
 $\lambda = 550\text{nm}$

White light

3λ

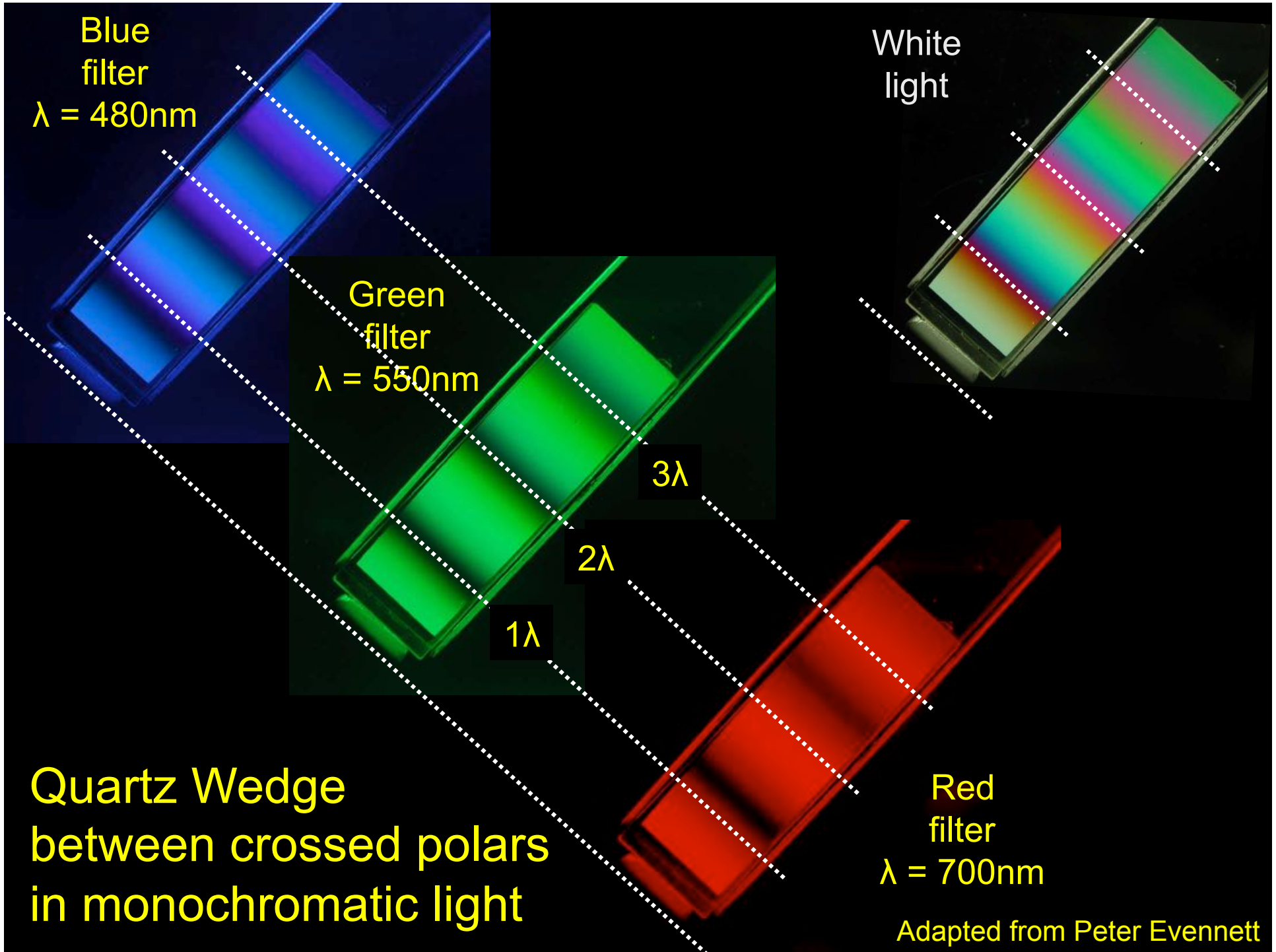
2λ

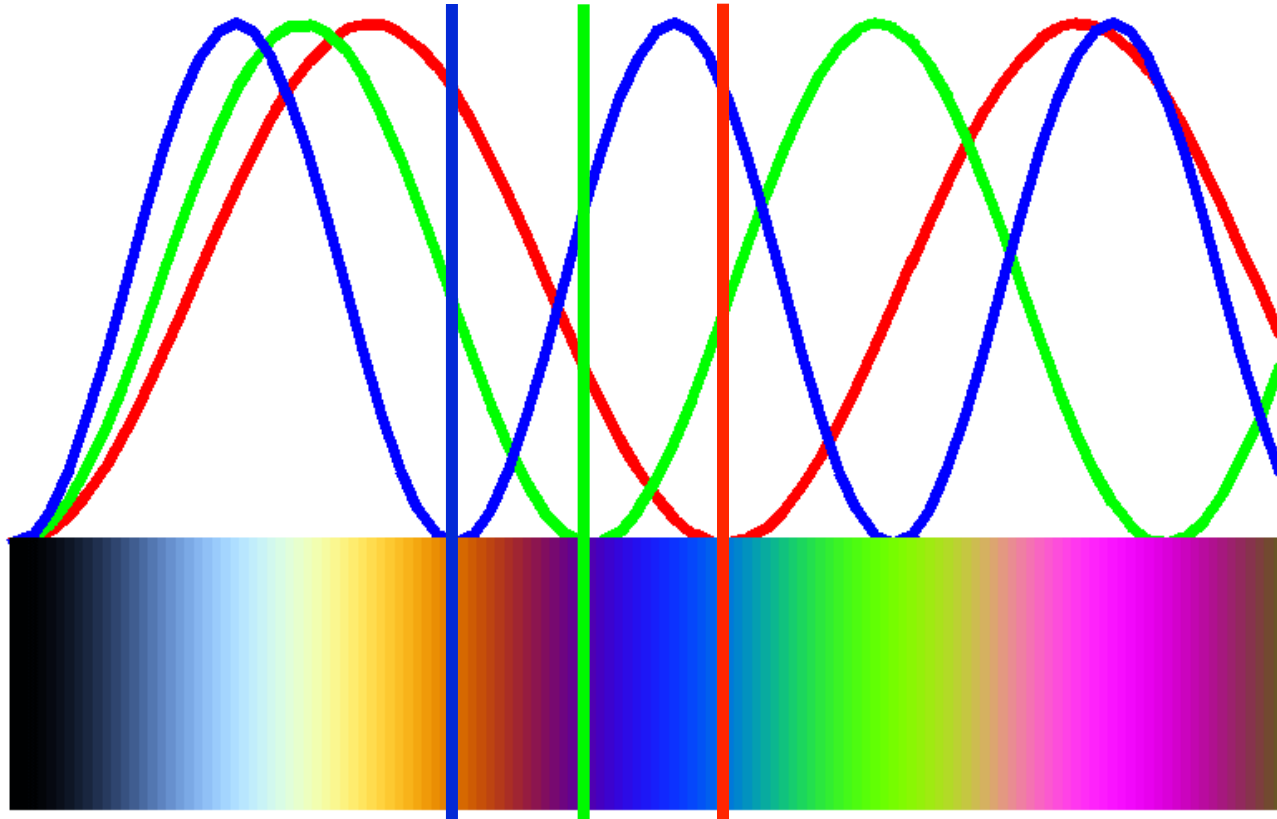
1λ

Red filter
 $\lambda = 700\text{nm}$

Quartz Wedge
between crossed polars
in monochromatic light

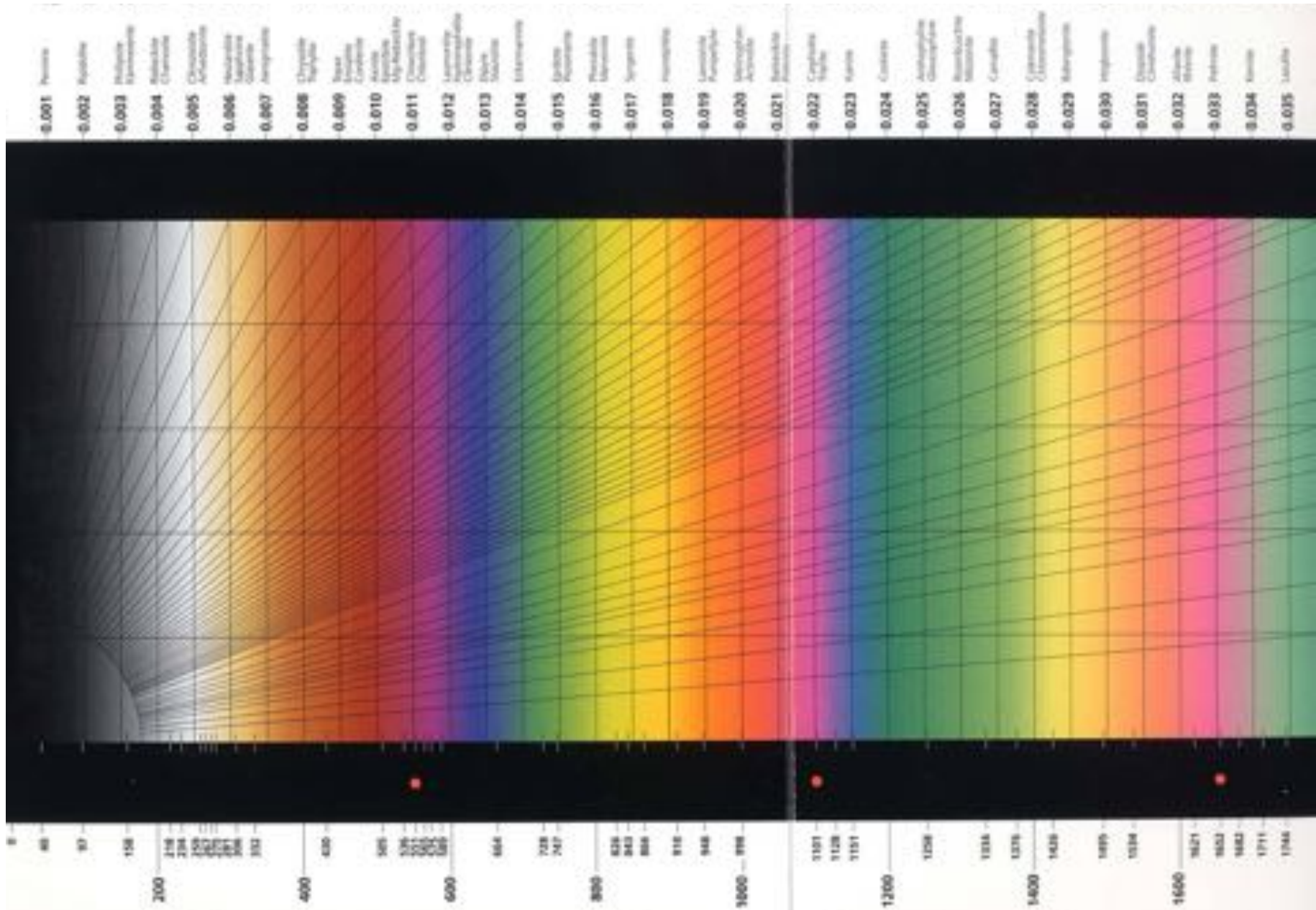
Adapted from Peter Evennett



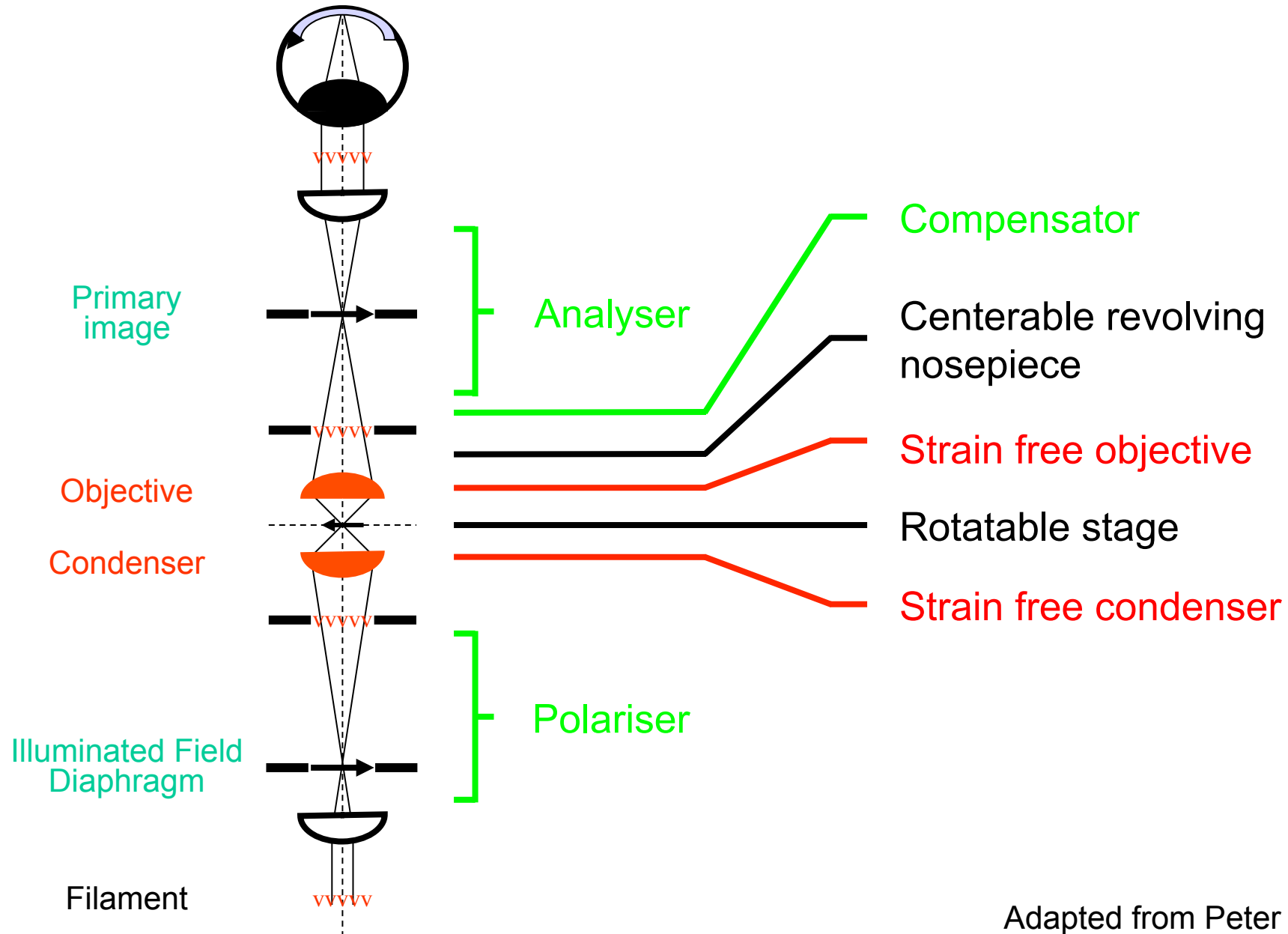


It is possible to guess the OPD from the interference colour using the Michel Lévy chart. If the thickness of the object is known, the birefringence can be calculated.

Michel-Lévy Interference Colour Chart



How to set it up



Adapted from Peter Evennett

What is it good for

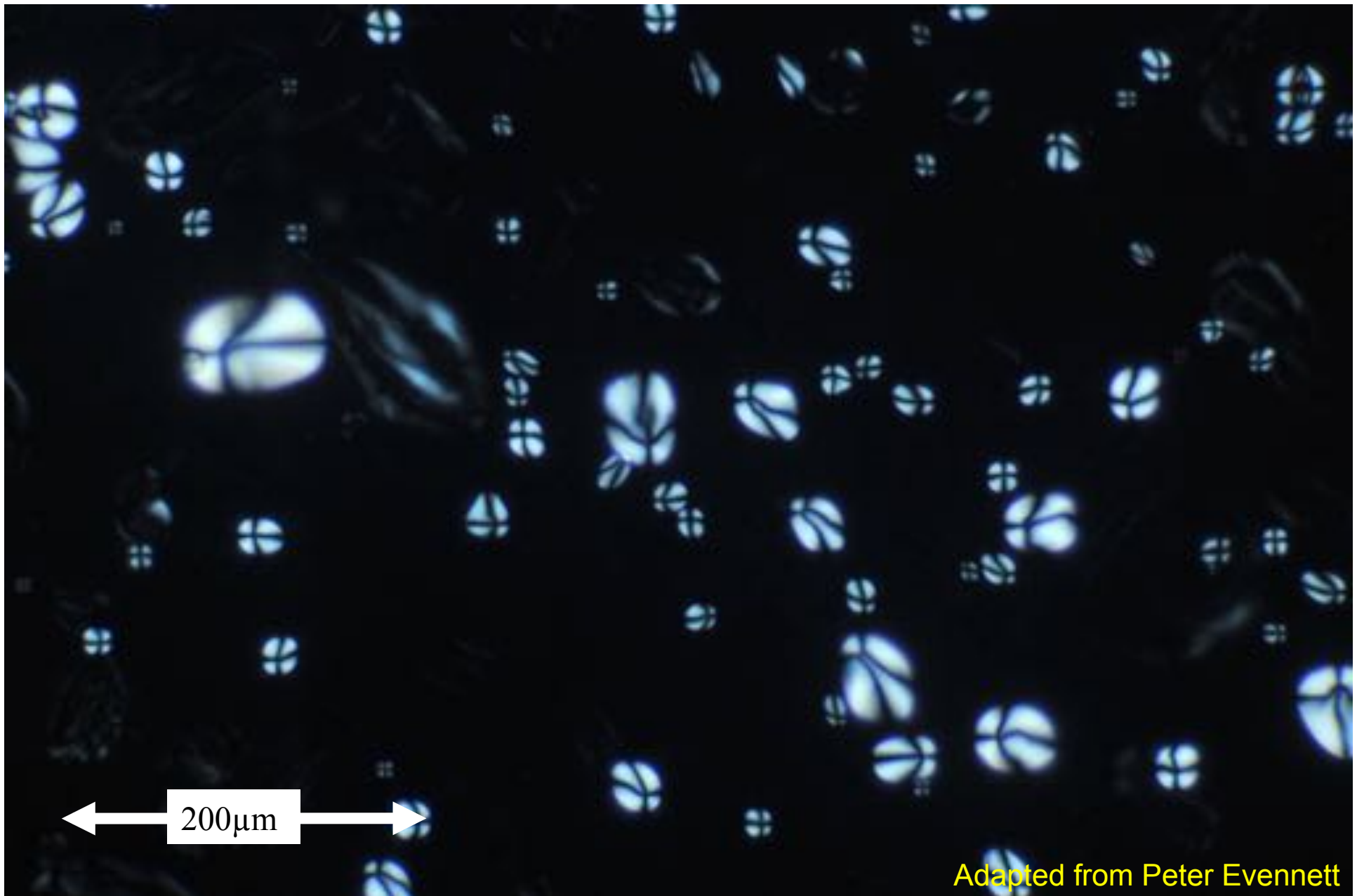
- Imaging of birefringent materials
 - Microtubuli (e.g. spindle apparatus)
 - Actin (e.g. muscle cells)
 - Cellulose (e.g. starch)

Potato Starch



Adapted from Peter Evennett

Potato Starch – Crossed Polars



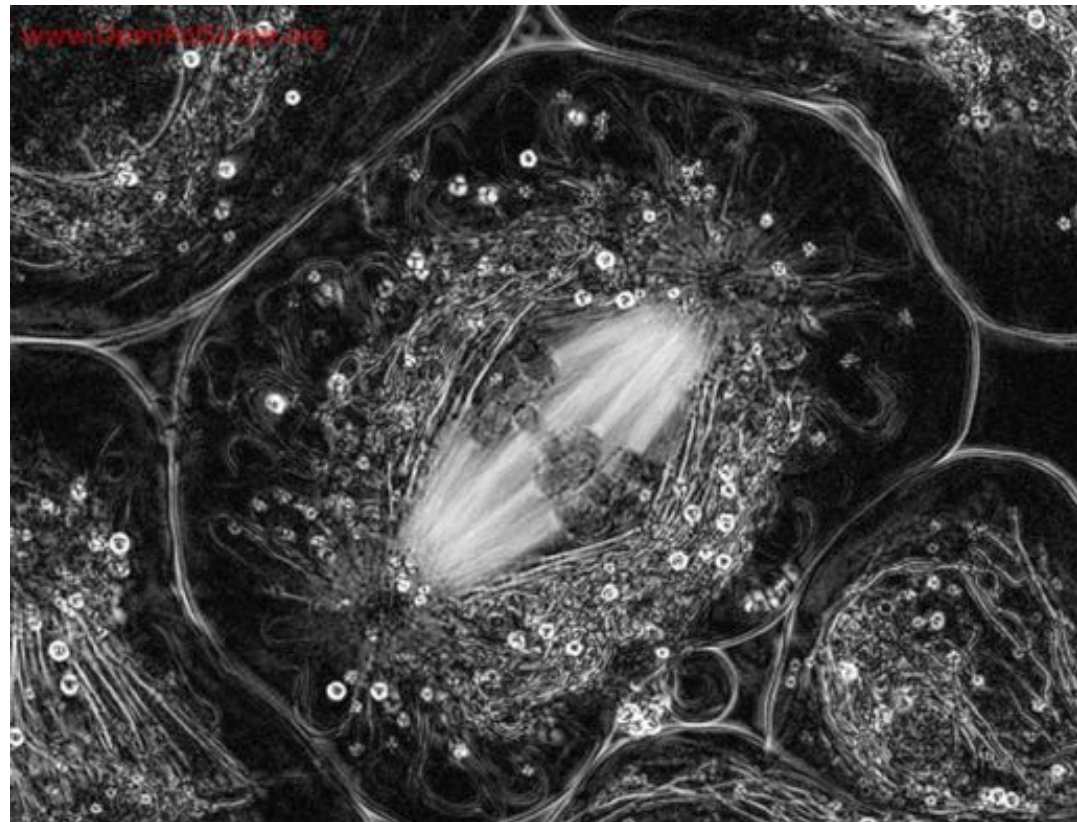
What is it good for

- 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



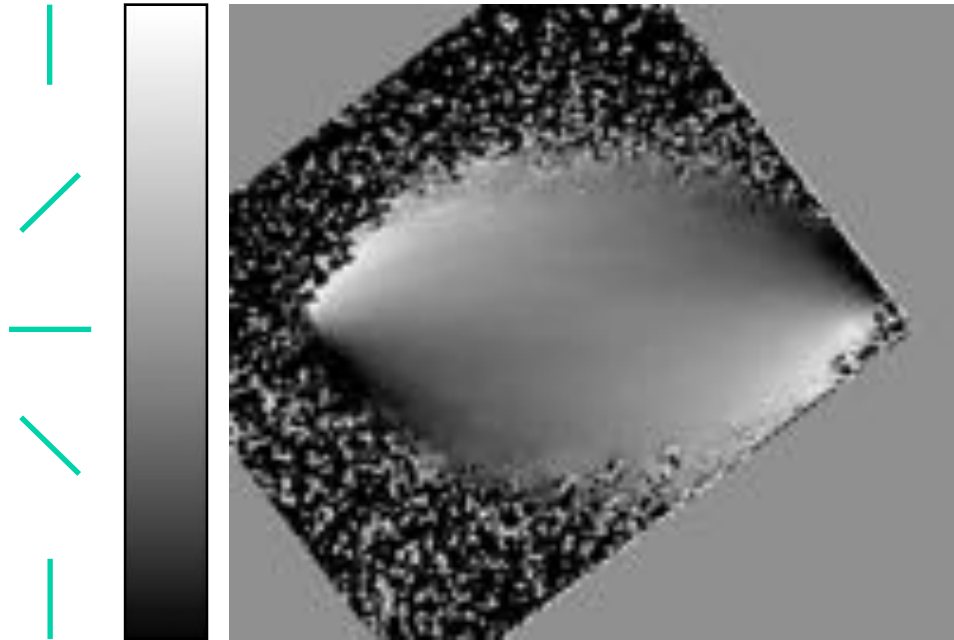
Liquid Crystal
Compensator



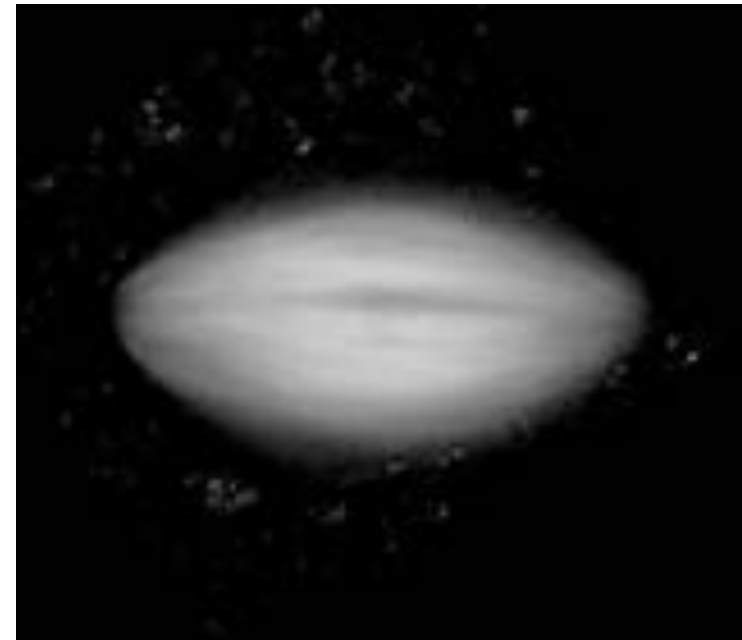
Brugues Lab

Primary Spermatocyte (*nephrotoma suturalis*)

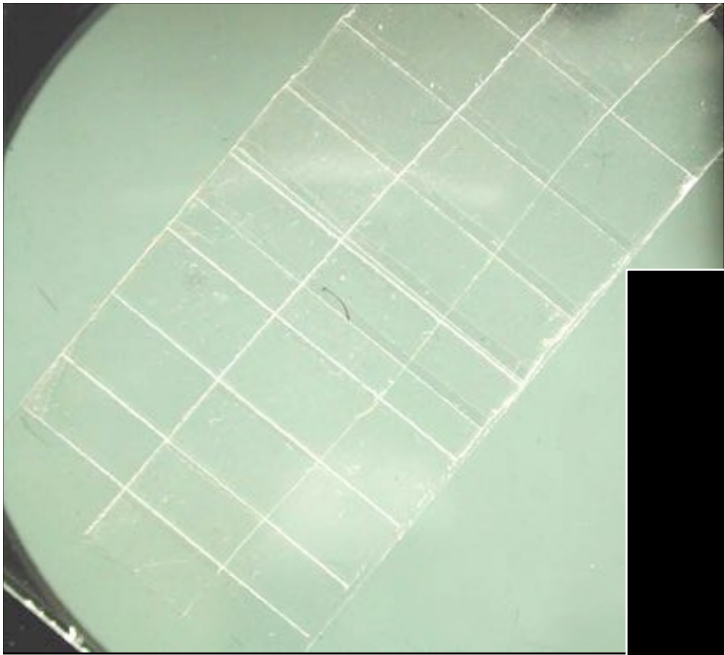
Spindle fluctuations



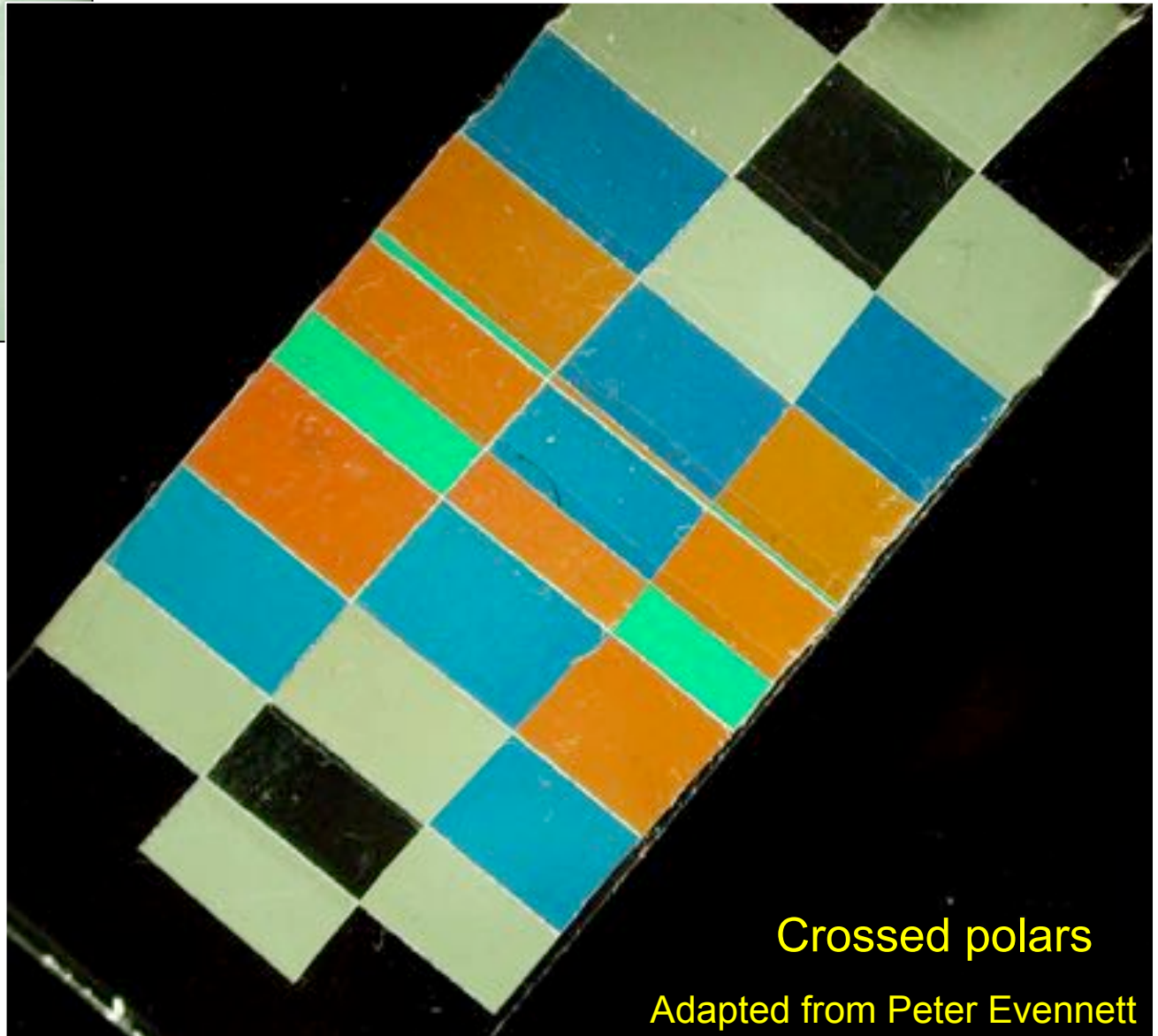
Orientation field $n(x,t)$
Microtubule orientation at
every pixel



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



Overlapping
pieces of
Sellotape



Crossed polars

Adapted from Peter Evennett

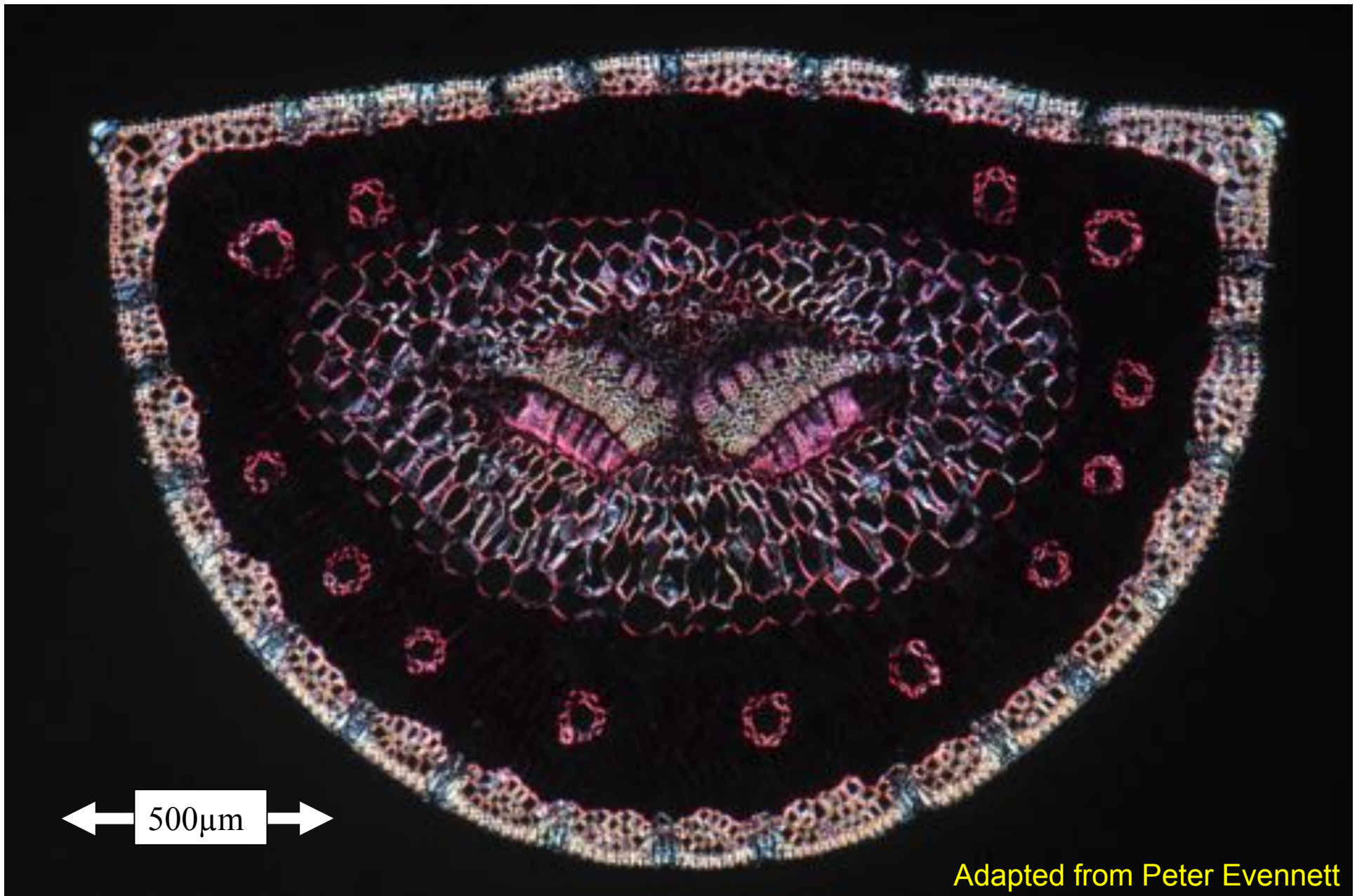
Pine needle



500 μ m

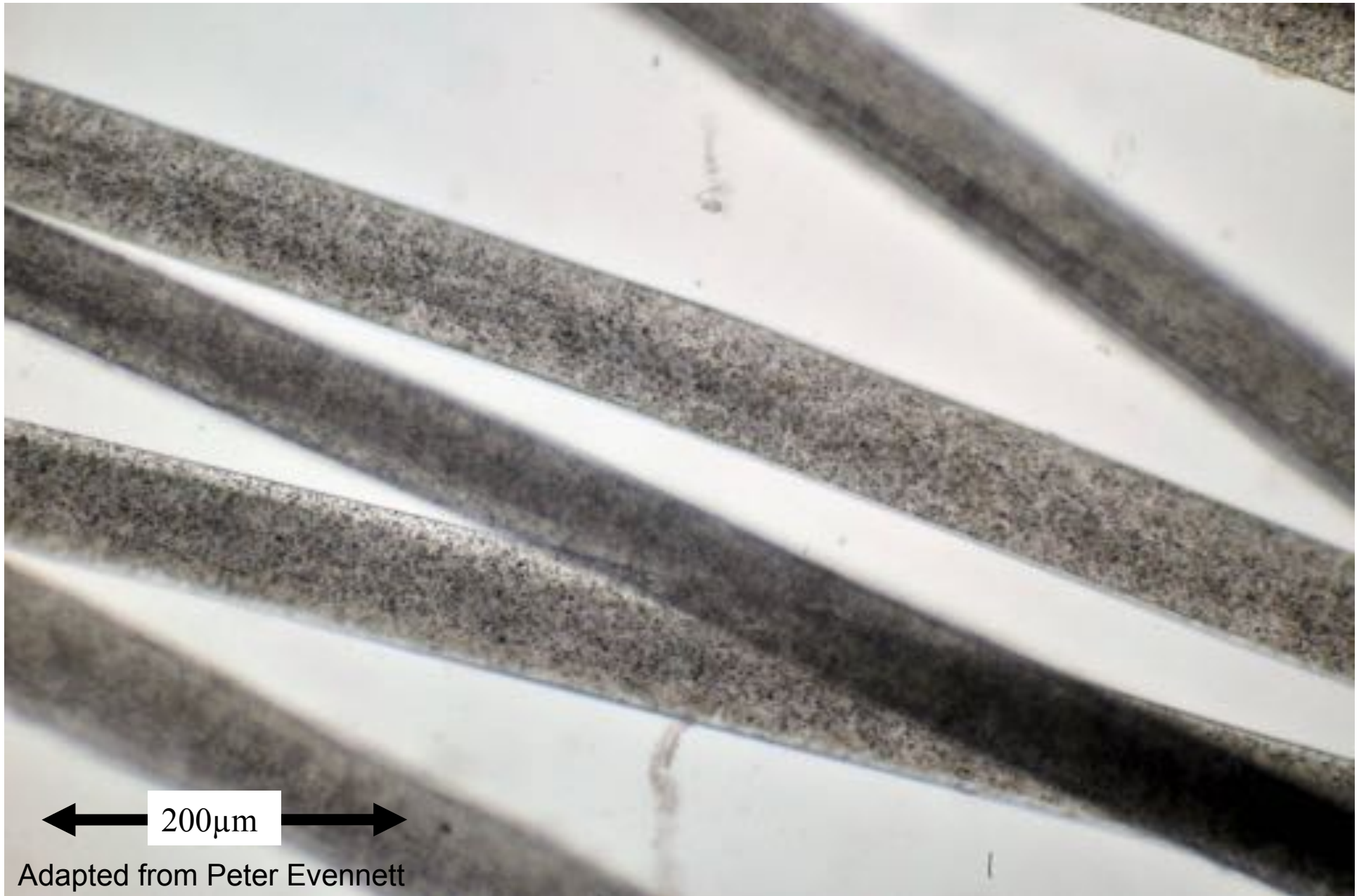
Adapted from Peter Evennett

Pine needle – Crossed polars

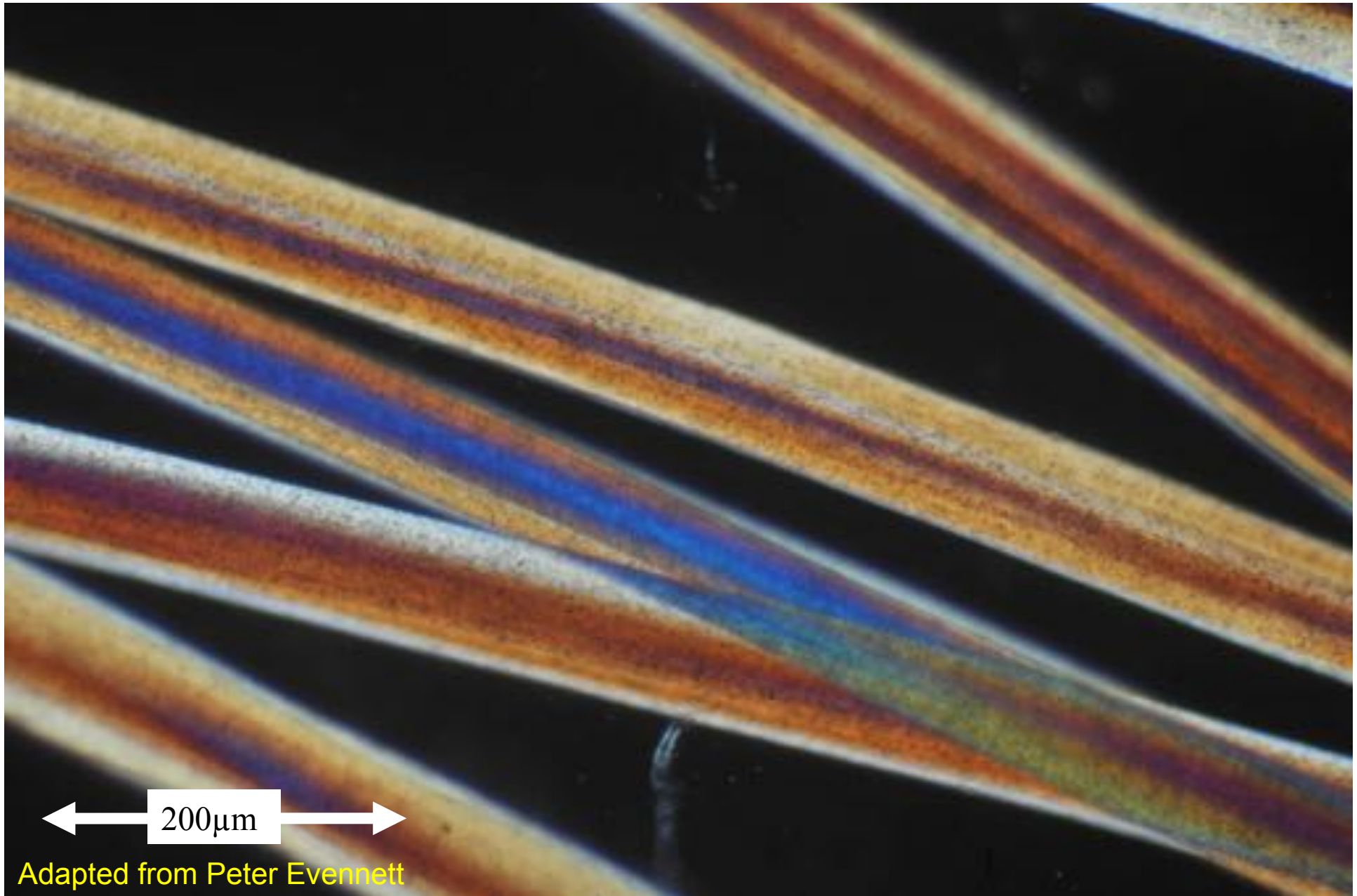


Adapted from Peter Evennett

Viscose fibres

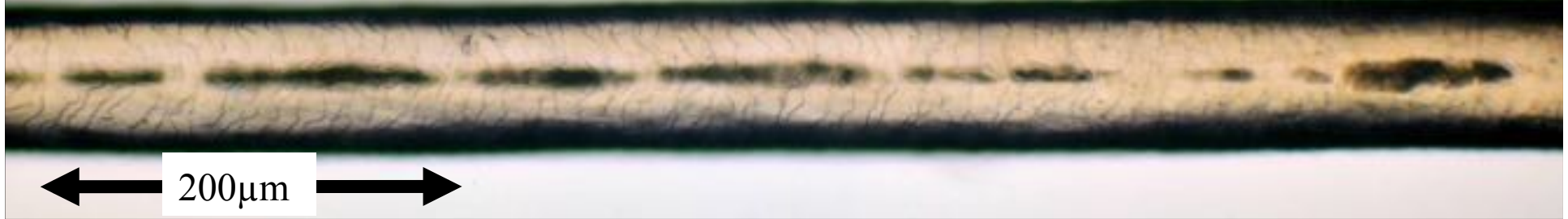


Viscose fibres – Crossed polars



Human hair

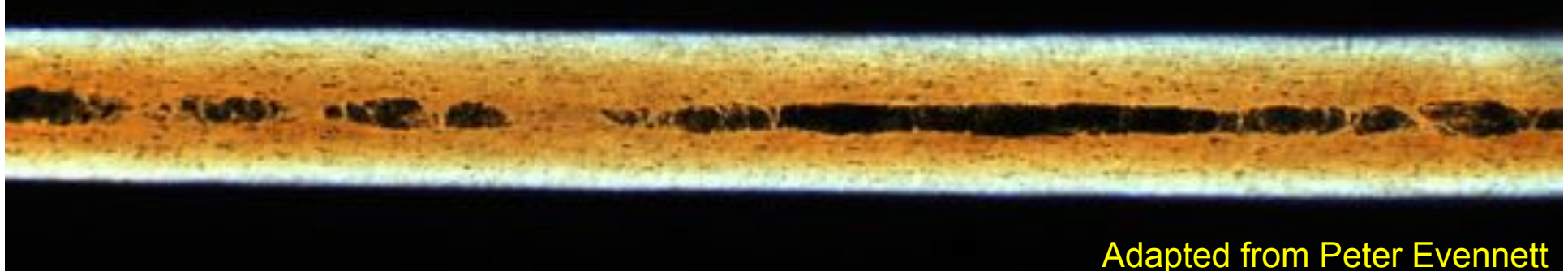
Mounted in air



Mounted in oil



Mounted in oil & Crossed polars



Adapted from Peter Evennett