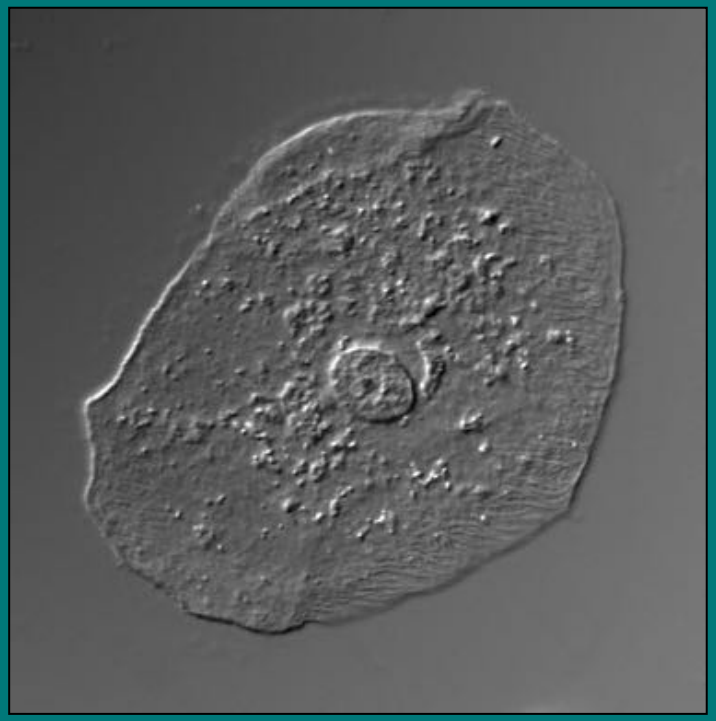
Differential Interference Contrast DIC

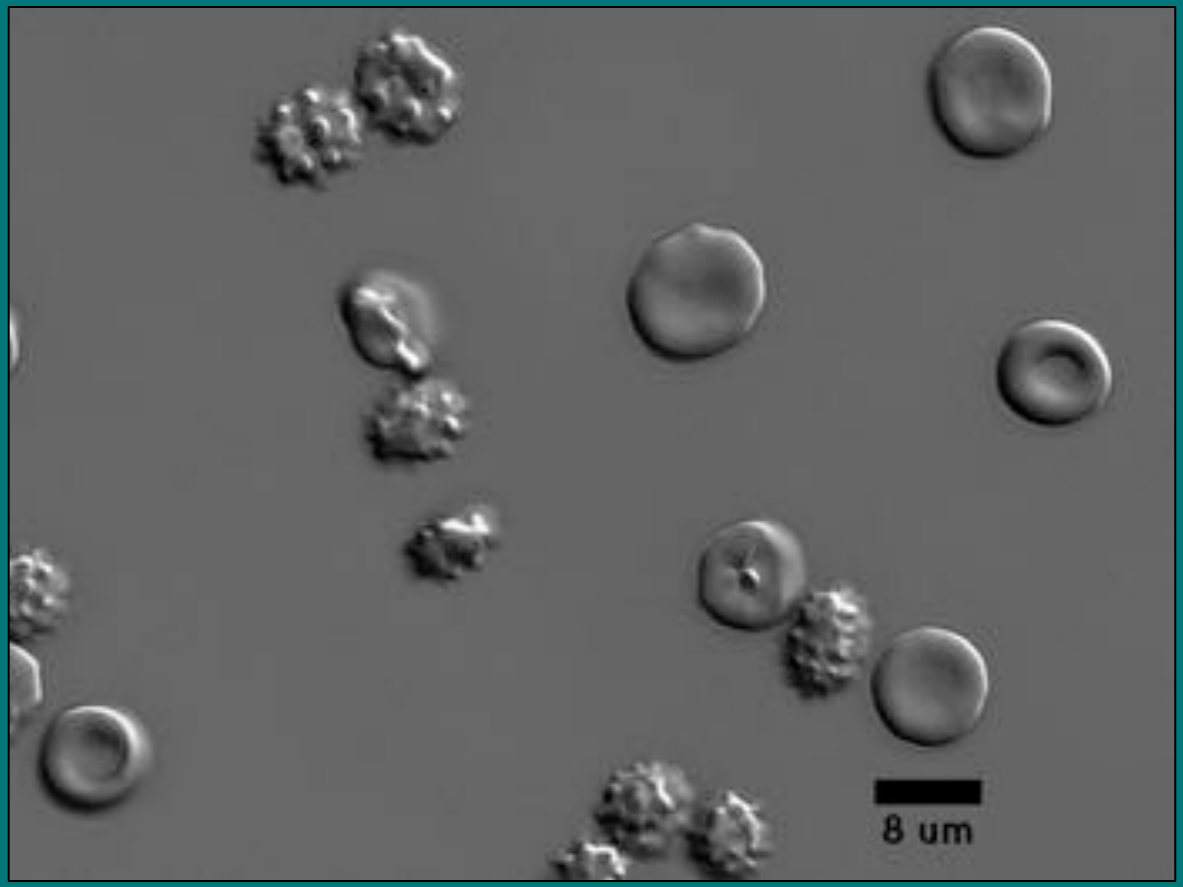


DIC – differential interference contrast

- developed in the mid-1950s by Georges (Jerzy) Nomarski (1919-1997), a Polish optics theoretician working in France at CNRS

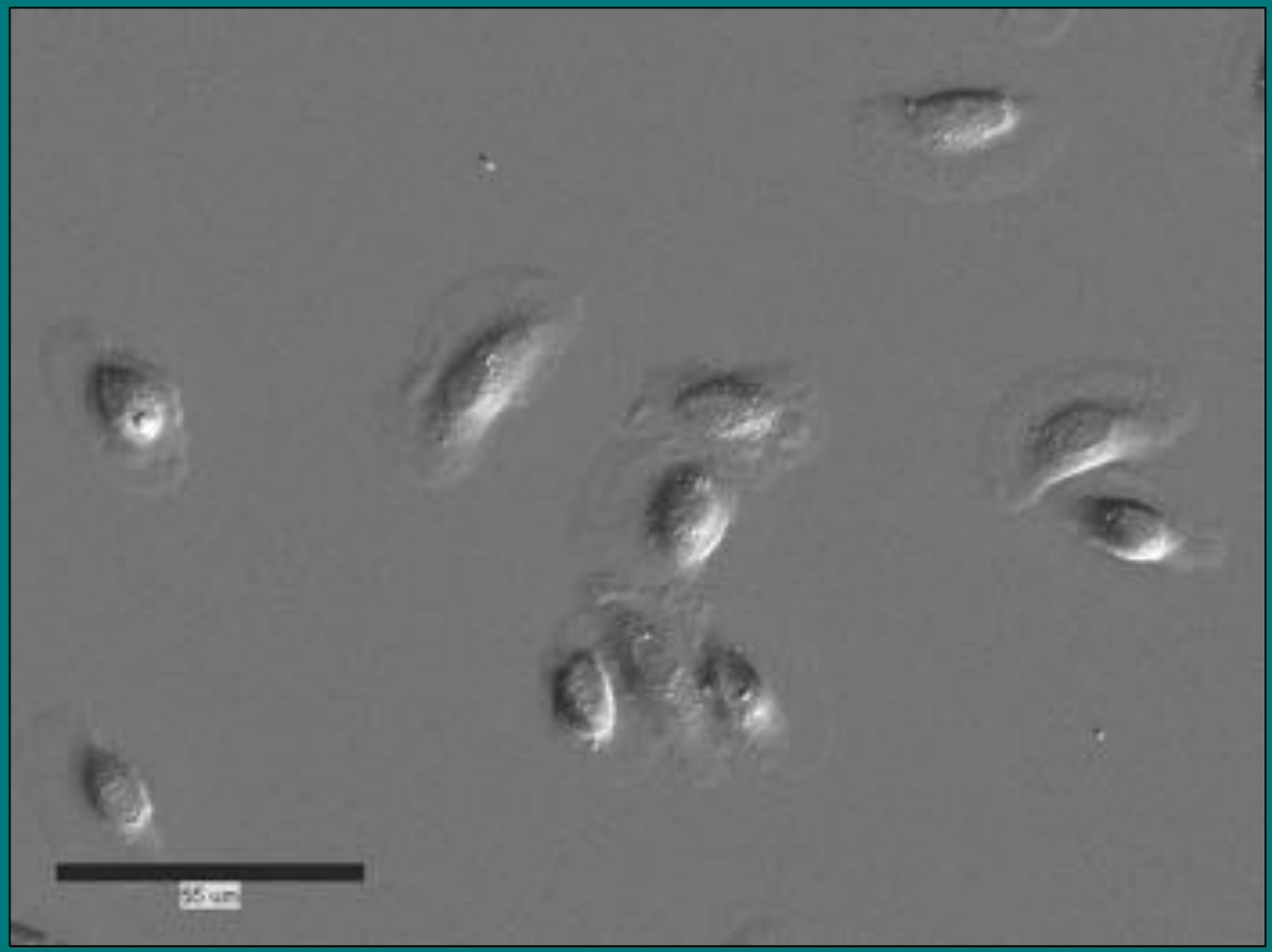
For a detailed biography see: http://micro.magnet.fsu.edu/optics/timeline/people/nomarski.html

Examples of DIC – what it is good for



Unlabeled human RBCs in buffer on uncoated glass cover slip.
Zeiss Axiovert 200M, 100x / 1.4 oil DIC.

Examples of DIC – what it is good for



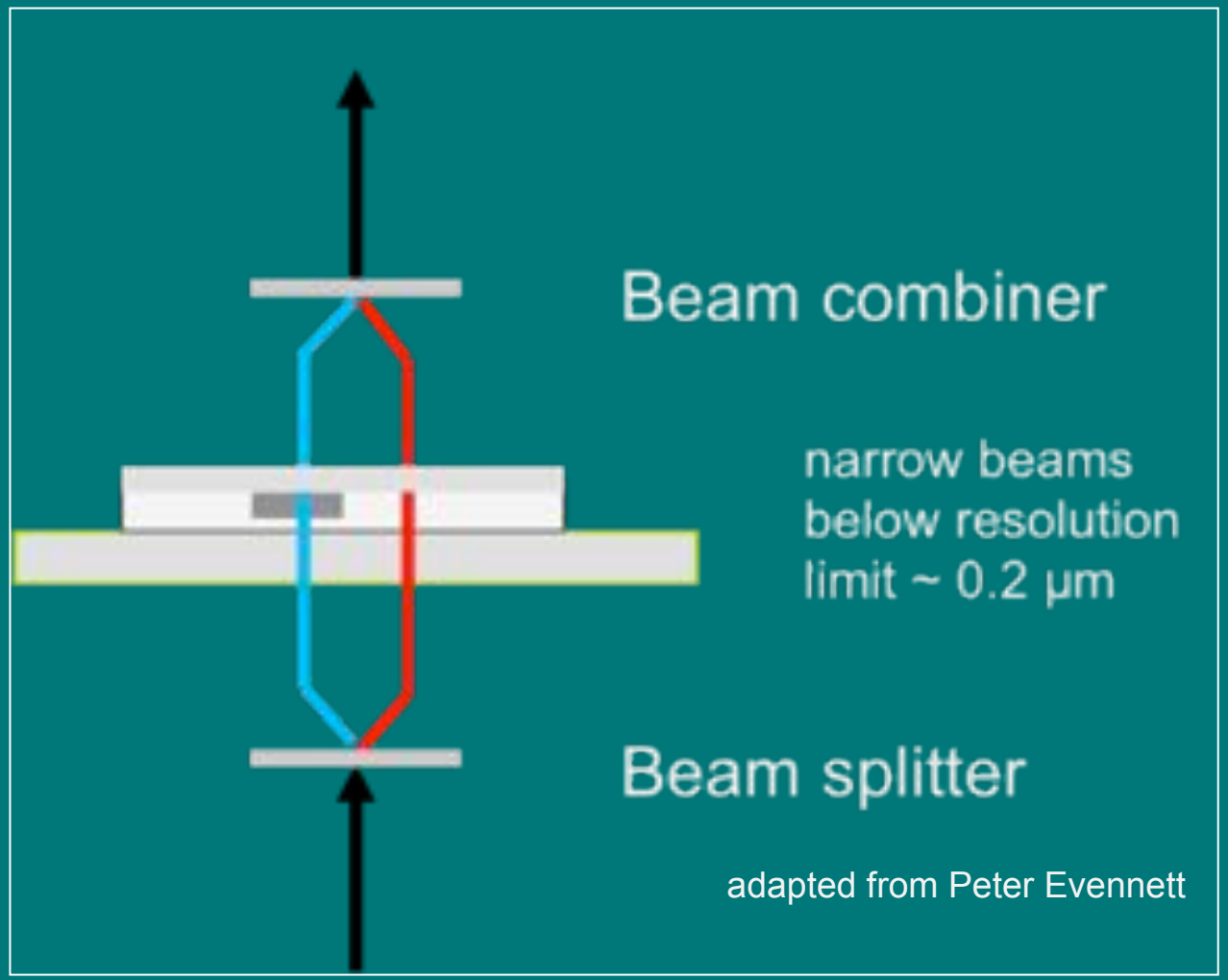
Zebrafish keratocytes

speed of cells: ~ 13.5um/min; 3min 30sec movie

Examples of DIC – what it is good for

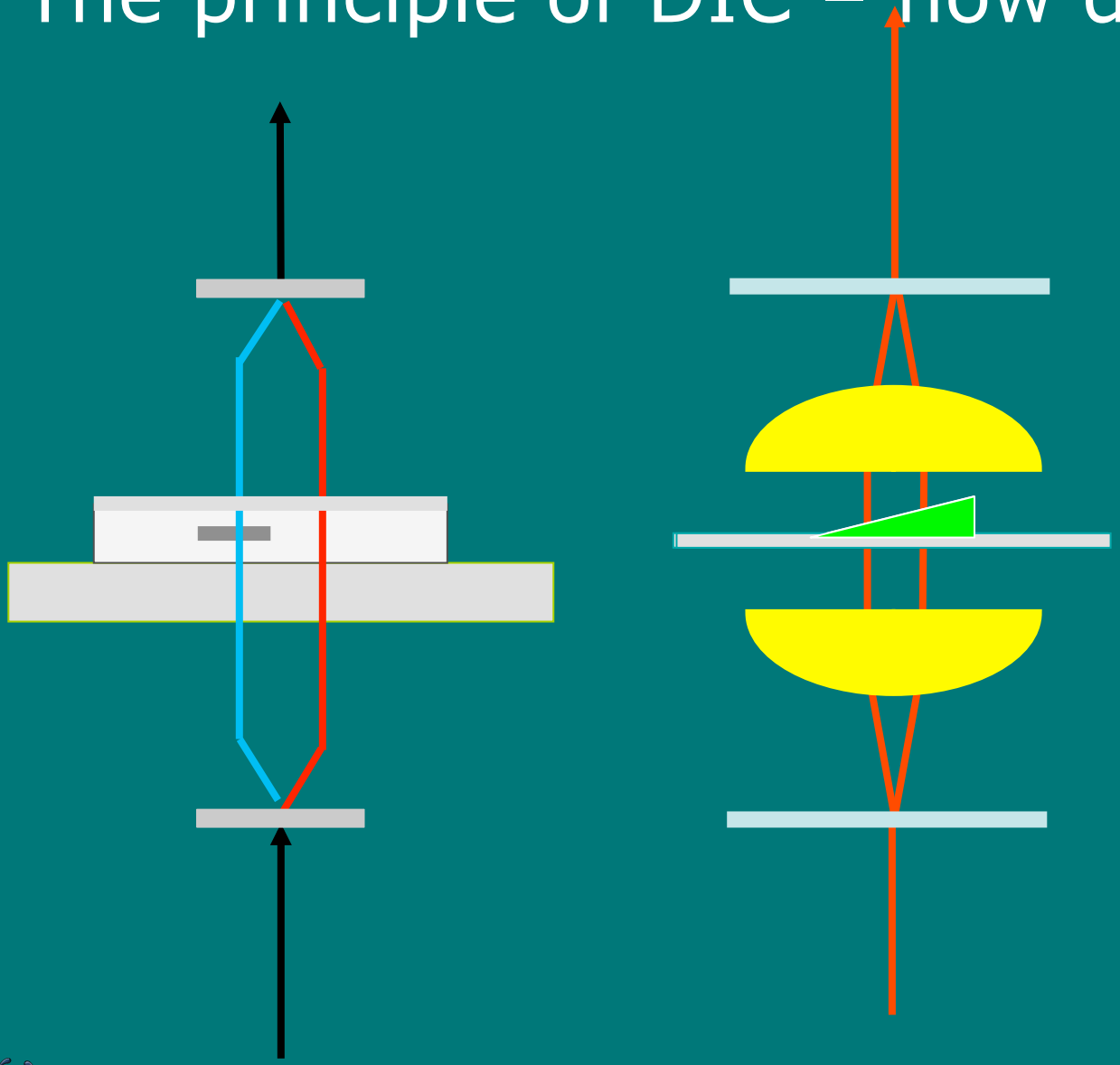
C. elegans embryos
Gunar Fabig, MTZ

The principle of DIC – how does it work



Common path interferometer, lateral shearing interferometer

The principle of DIC – how does it work



Beam combiner

Objective

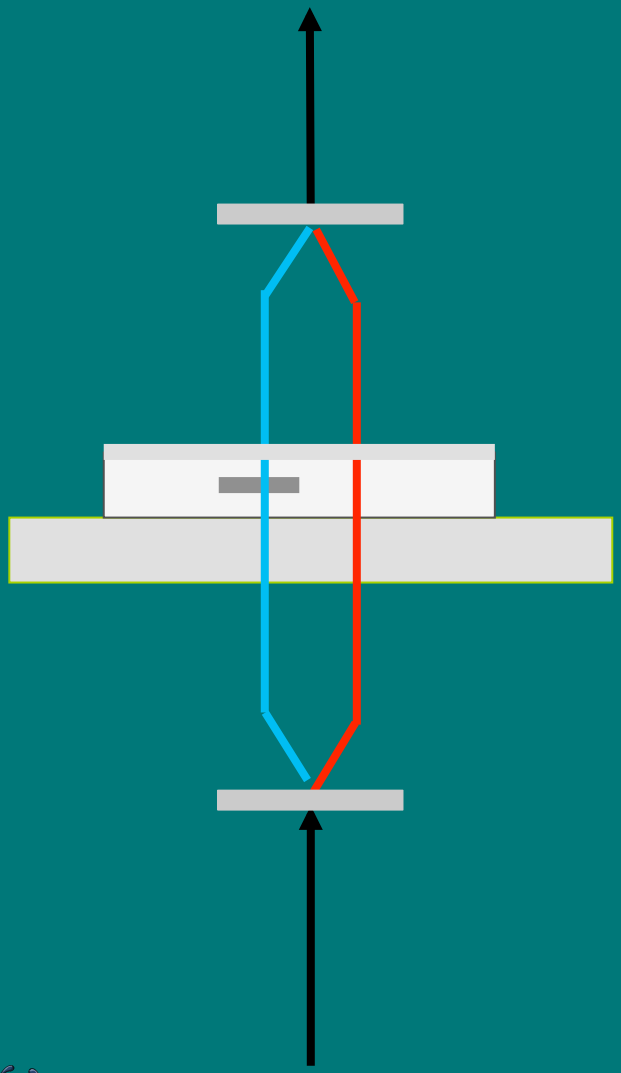
Specimen

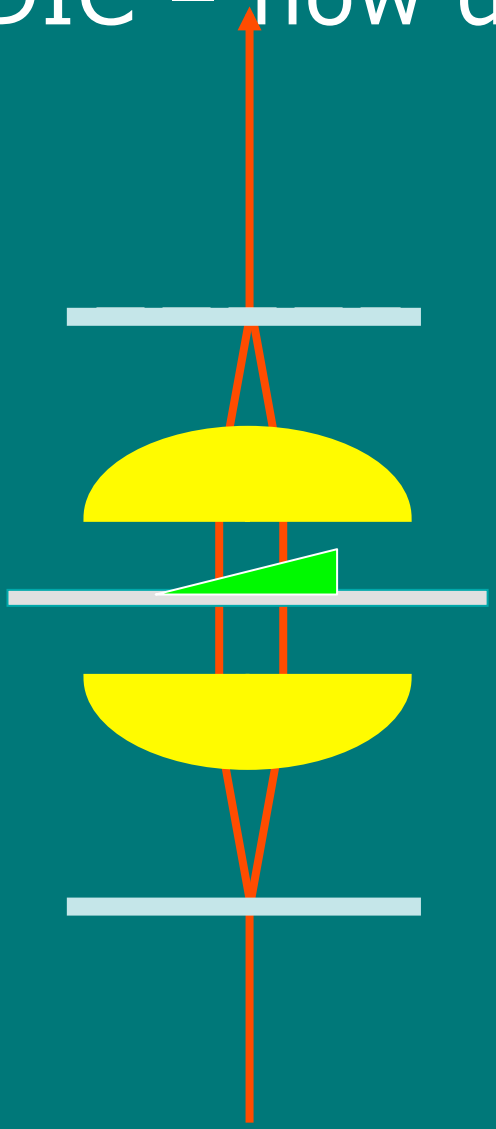
Condenser

Beam splitter

adapted from Peter Evennett

The principle of DIC – how does it work





Beam combiner

Objective

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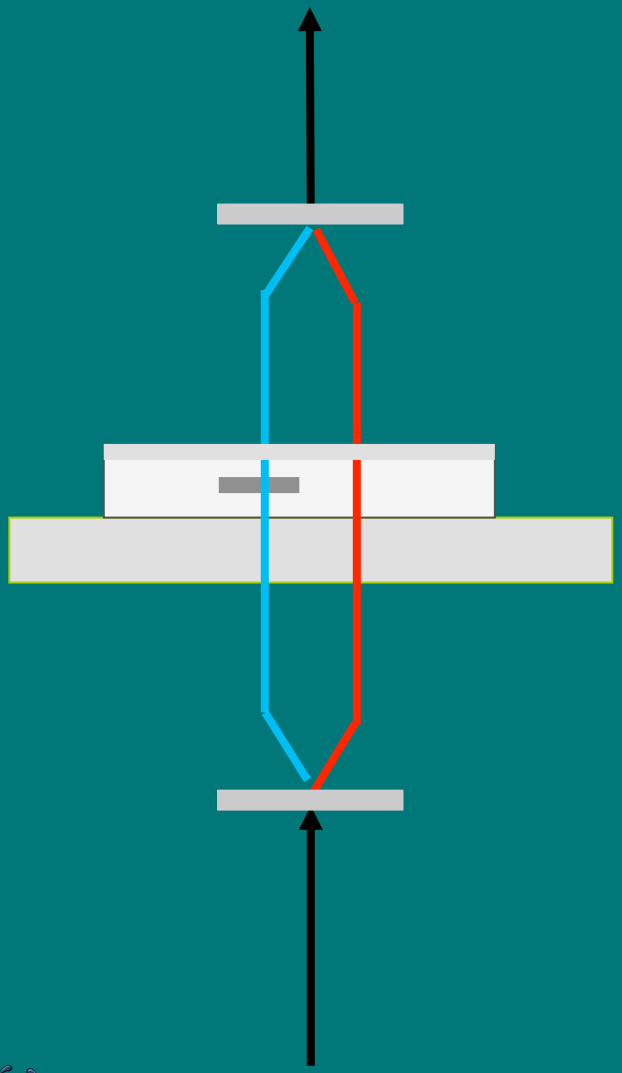
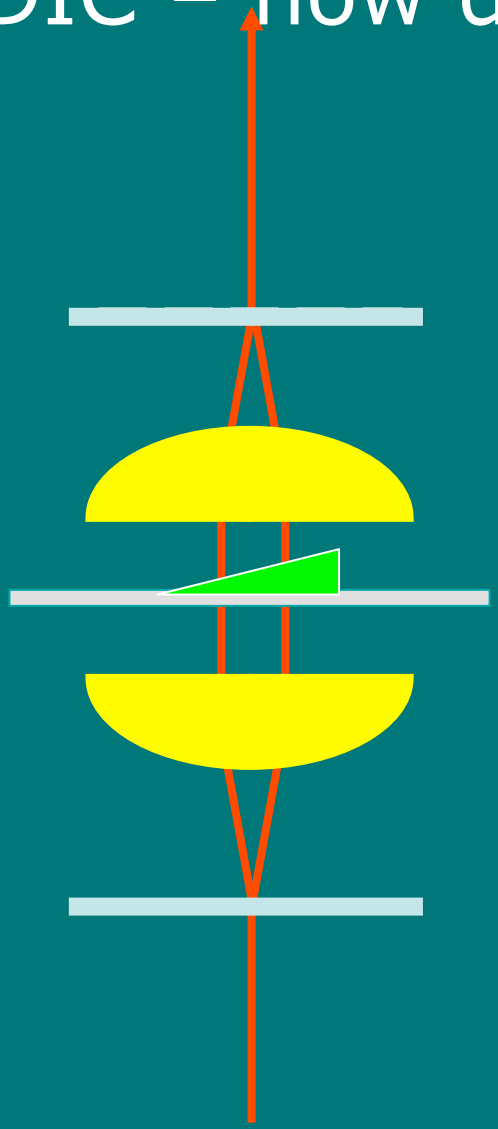
The principle of DIC – how does it work





Back focal plane of objective

Objective

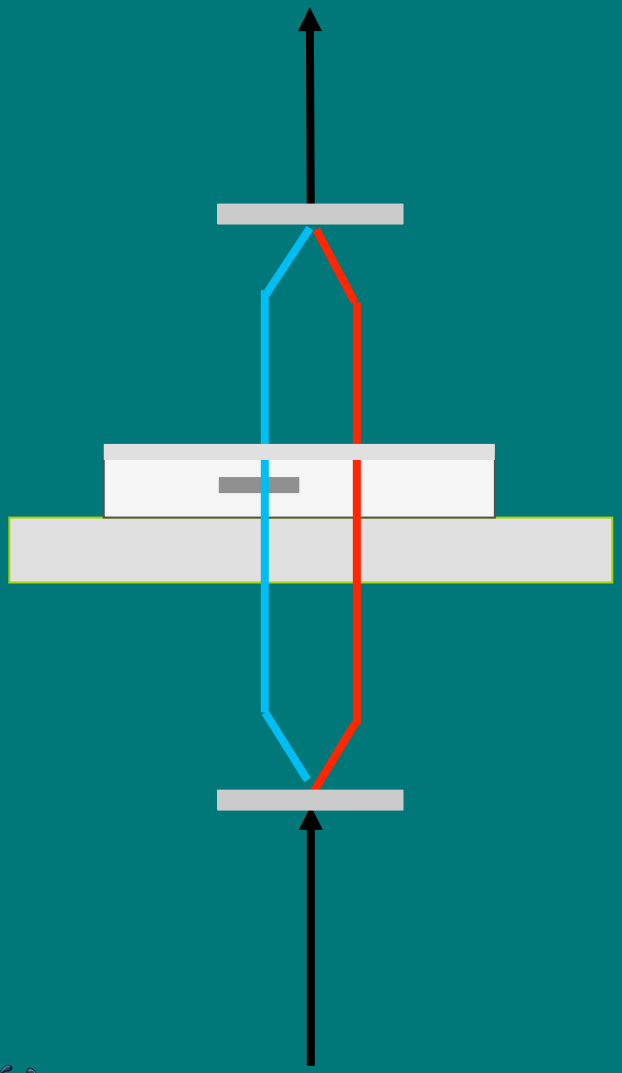
Specimen

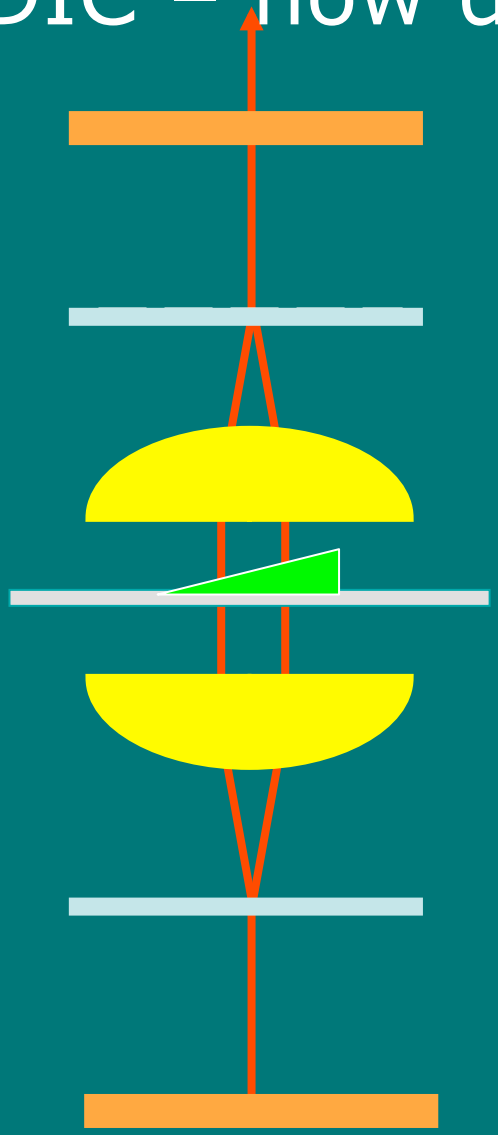
Condenser

Front focal plane of condenser

adapted from Peter Evennett

The principle of DIC – how does it work





Analyser

Nomarski prism

Objective

Specimen

Condenser

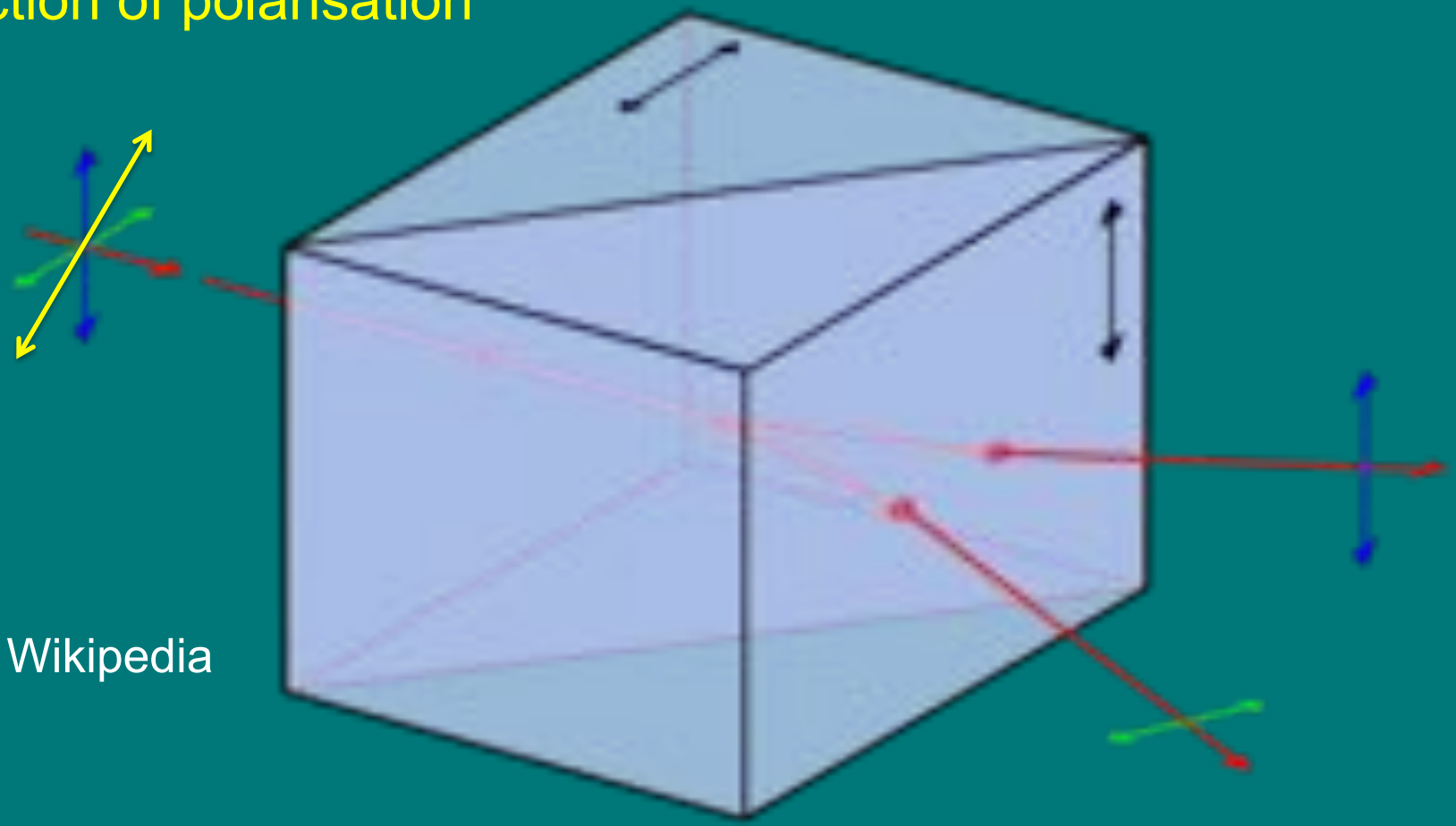
Wollaston prism

Polariser

adapted from Peter Evennett

The Wollaston prism beam splitter – how does it work

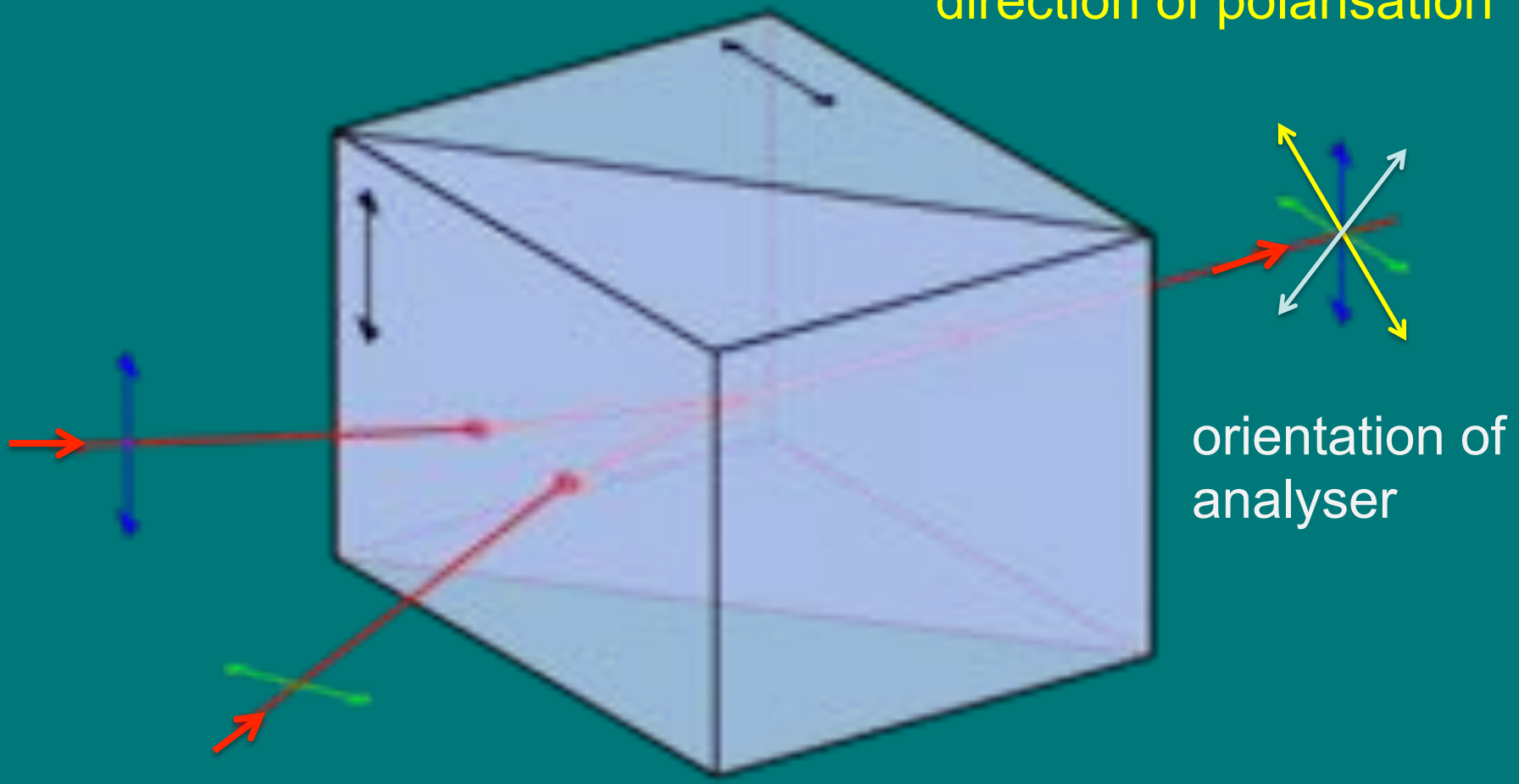
direction of polarisation



Wikipedia

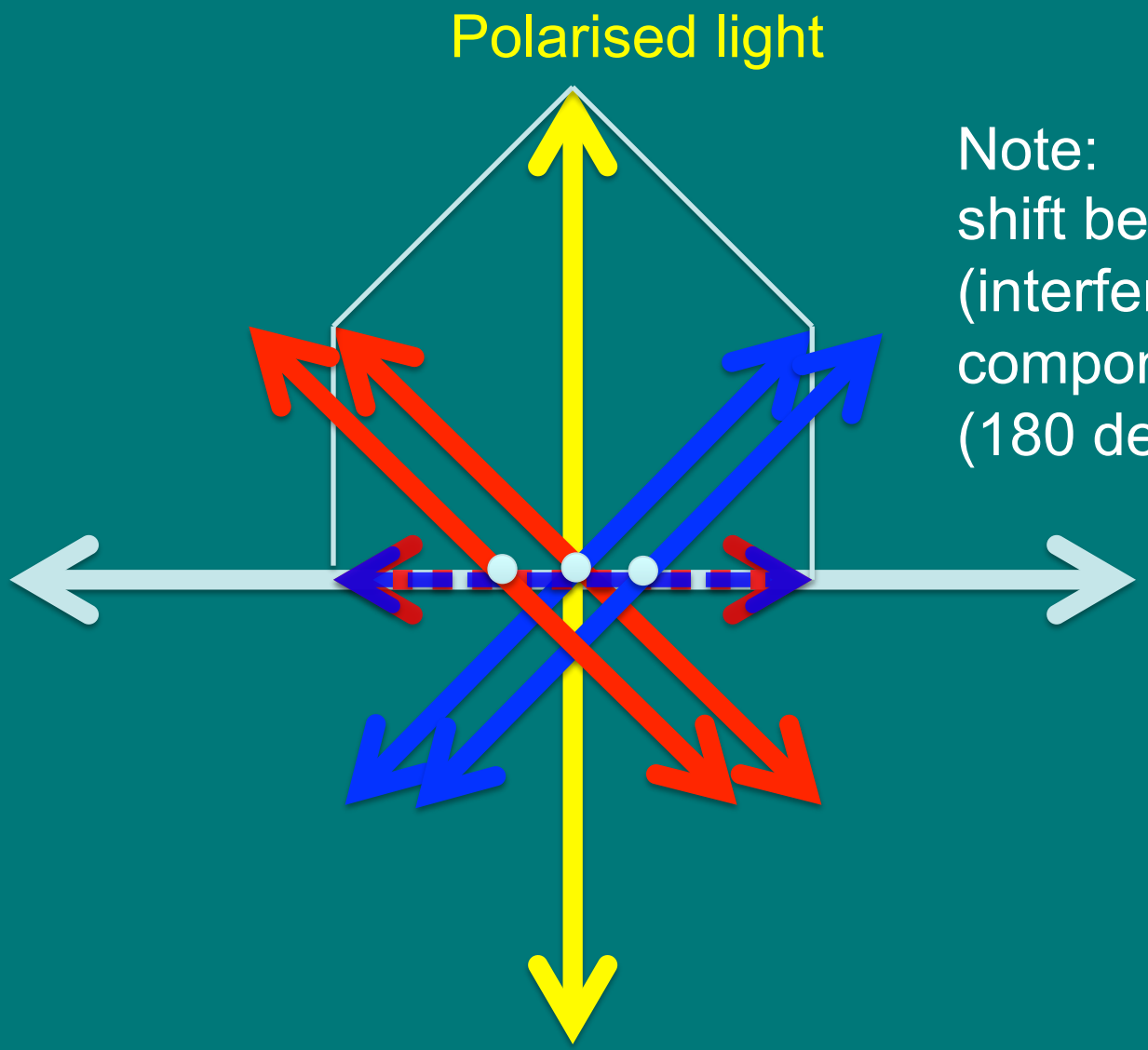
Shear = spatial separation of the two beams

The Wollaston prism – beam combiner



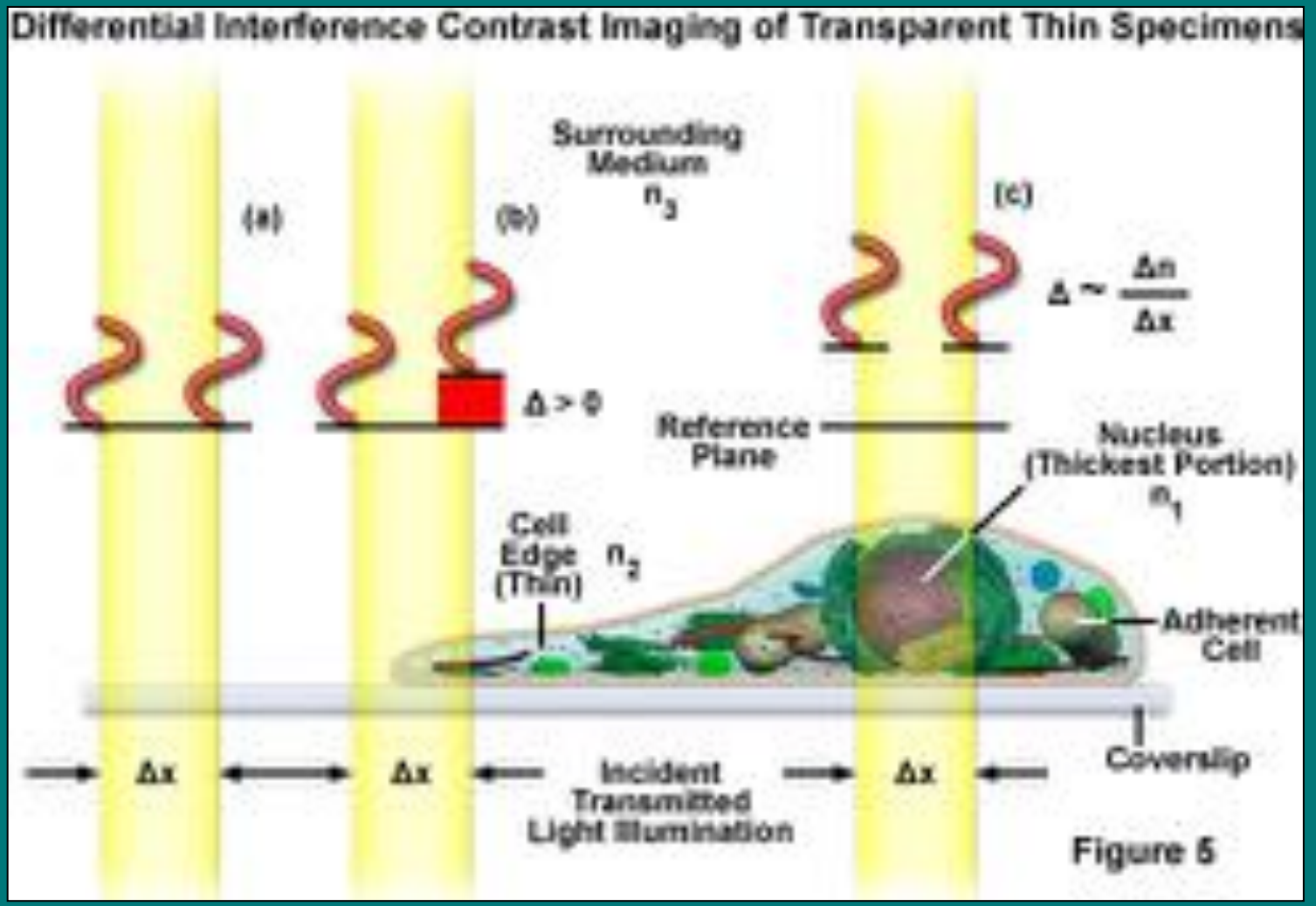
Wikipedia

Interference at analyser



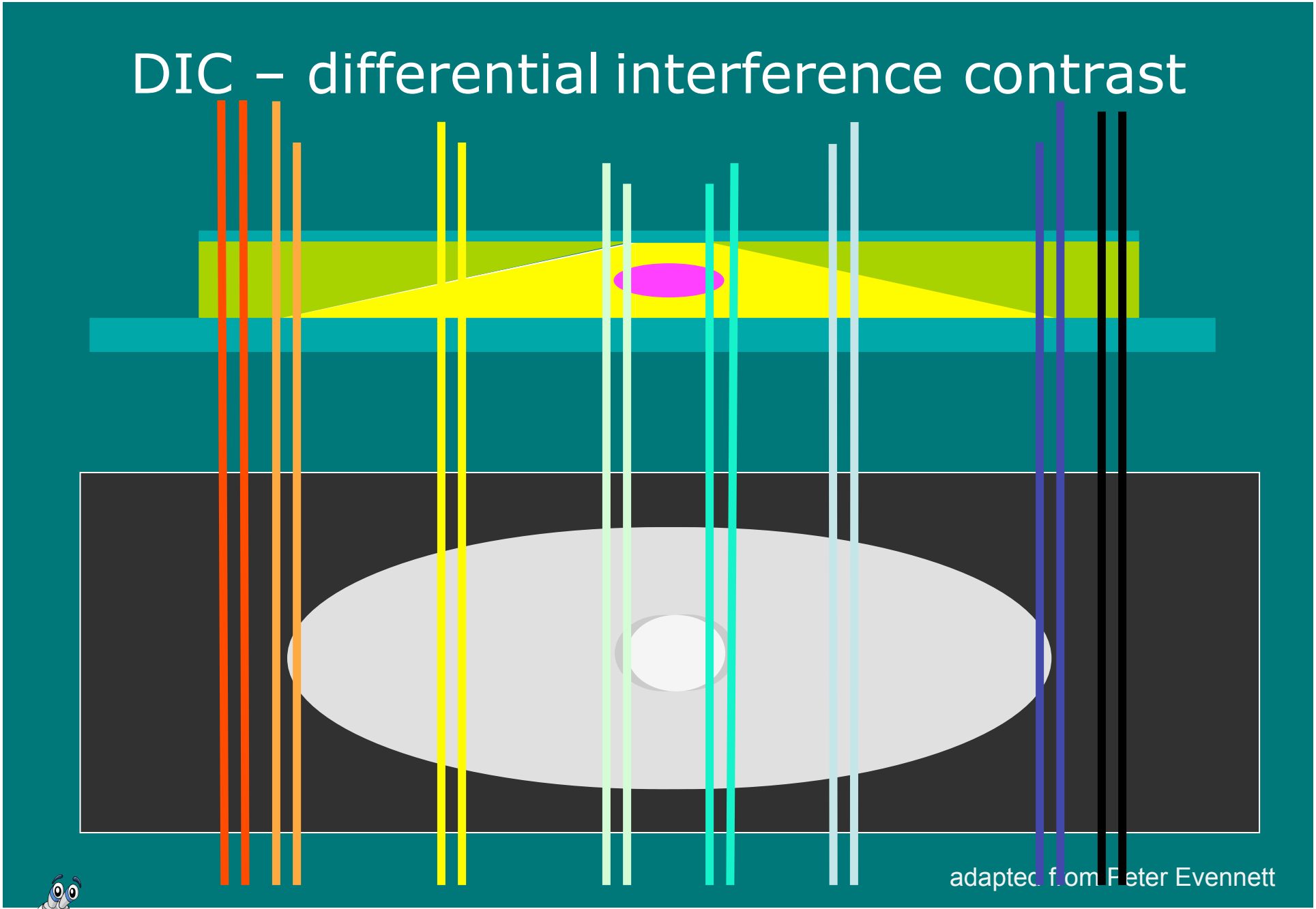
Note:
shift between effective (interfering) beam components by λ/2 (180 degrees)

DIC – differential interference contrast

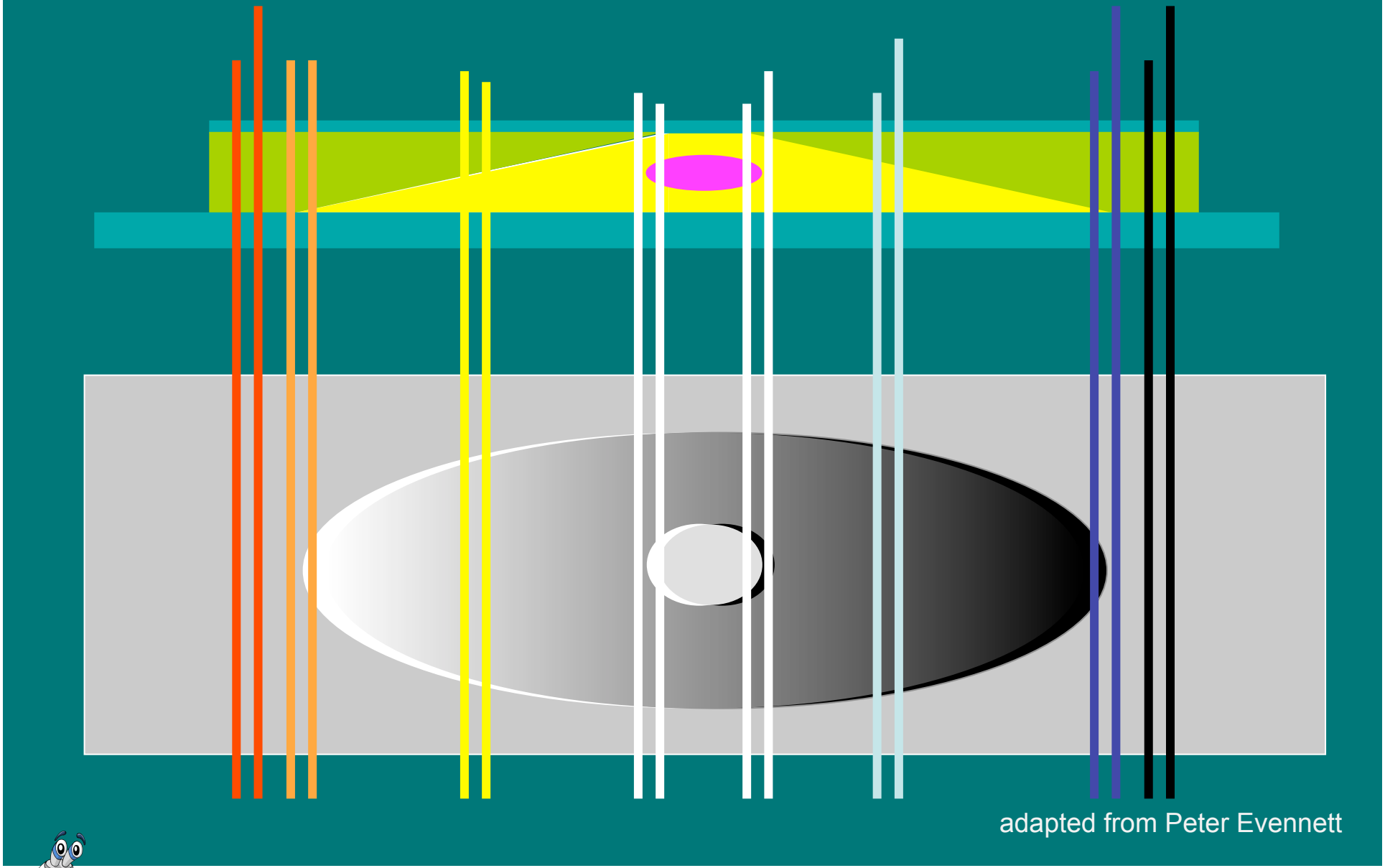


From ZEISS-campus website

DIC – differential interference contrast



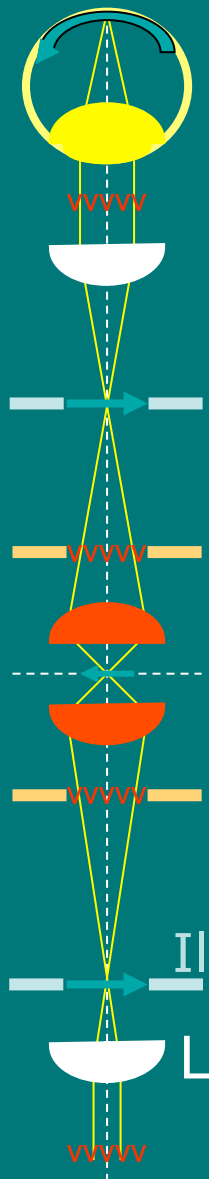
DIC – with retardation bias



adapted from Peter Evennett

The principle of DIC – how to set it up

- Köhler!!!!!



Eyepiece lens

Primary image

Objective

Condenser

Illuminated Field Diaphragm

Lamp collector

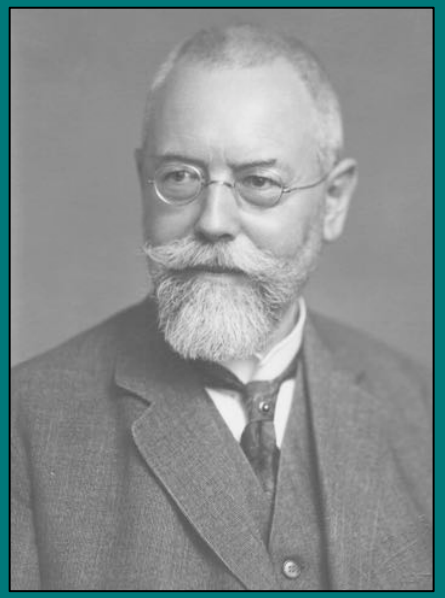
Filament

adapted from Peter Evennett

The principle of DIC – lets practice

1) Köhler your microscope carefully:

- Place sample, switch on light ;-)
- Remove unnecessary components from light path
- Open all diaphragms
- Focus sample
- Close field diaphragm
- Focus condenser
- Center condenser

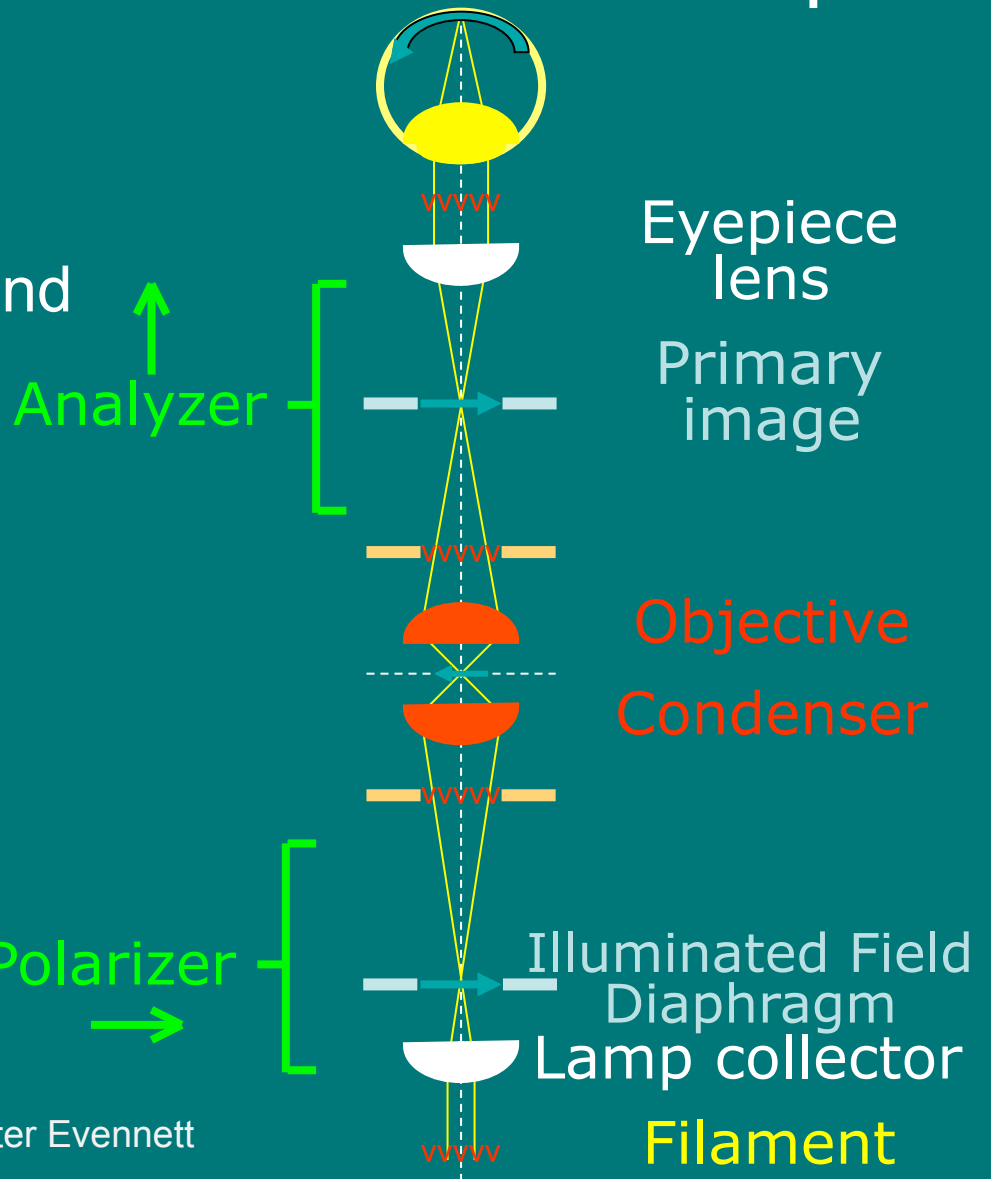


Wikipedia

- Close aperture diaphragm (to ~ 80% pupil of objective)

The principle of DIC – how to set it up

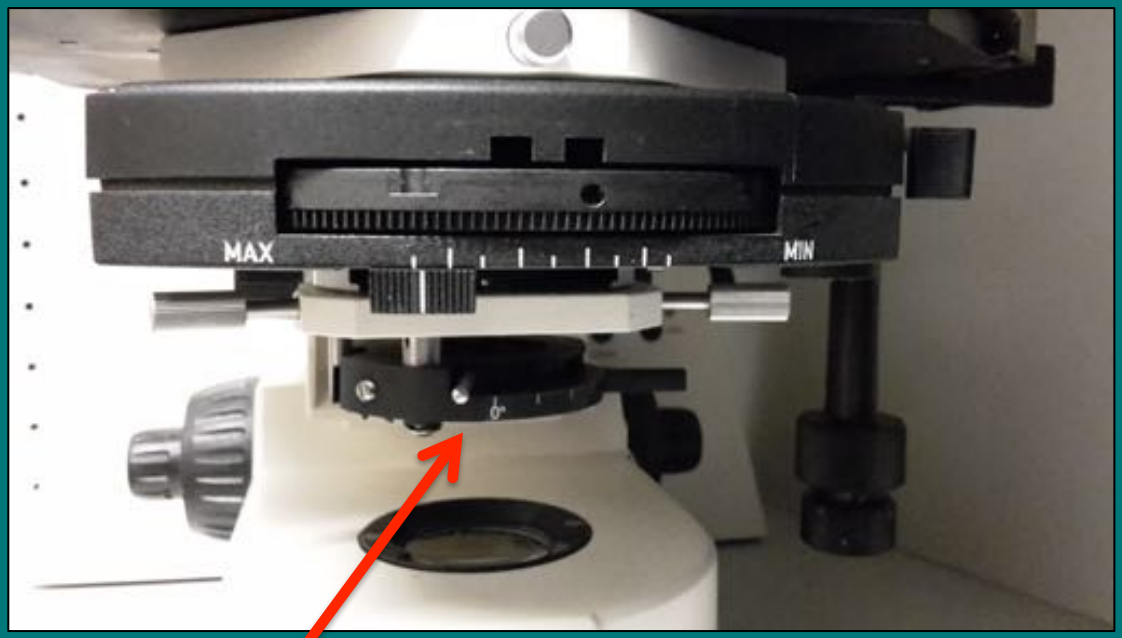
- Köhler!!!!!
- Insert and cross polarizer and analyzer



adapted from Peter Evennett

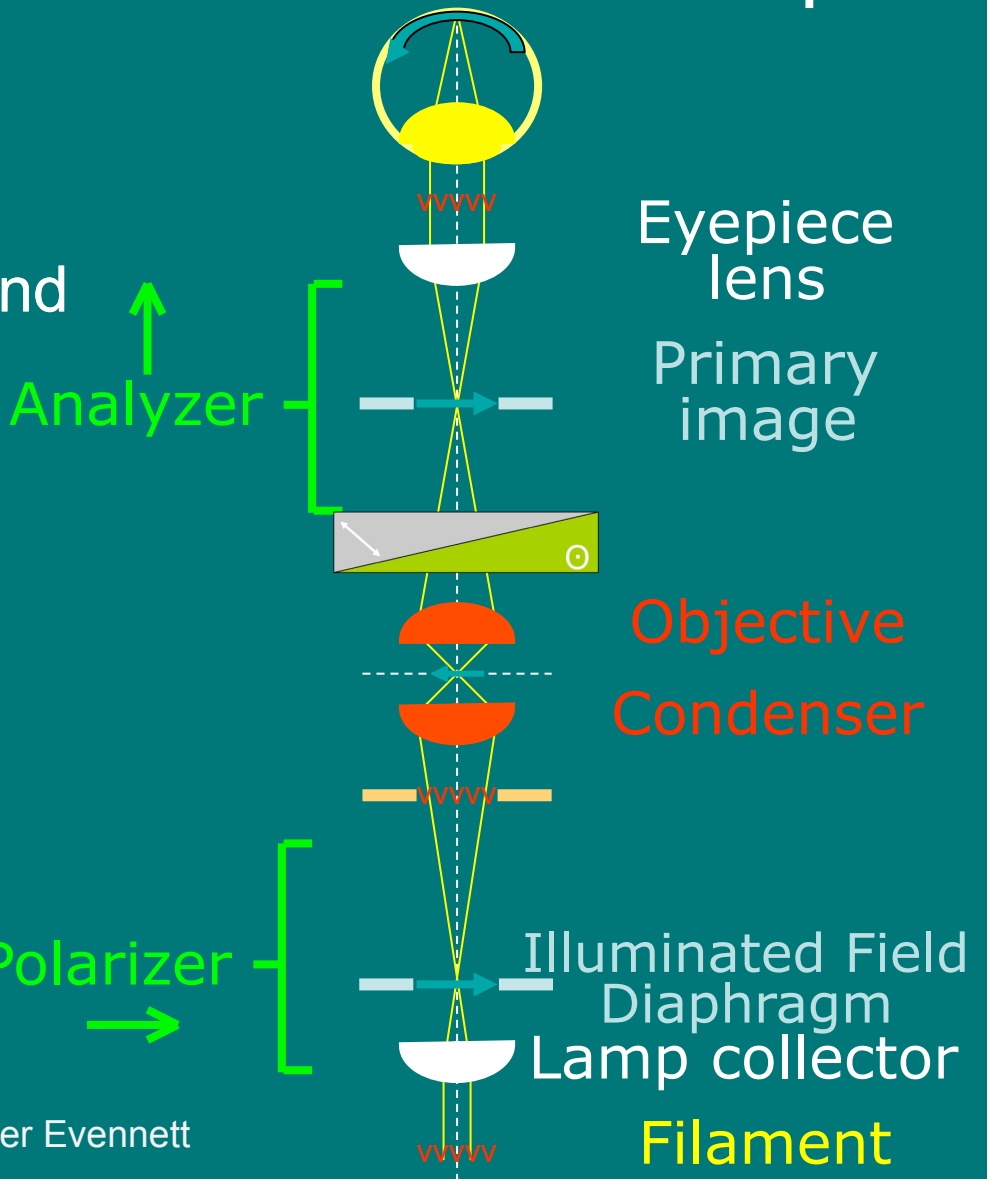
The principle of DIC – lets practice

2) Insert and adjust polarizer and analyzer – crossed polars



The principle of DIC – how to set it up

- Köhler!!!!!
- Insert and cross polarizer and analyzer
- Insert objective Nomarski prism



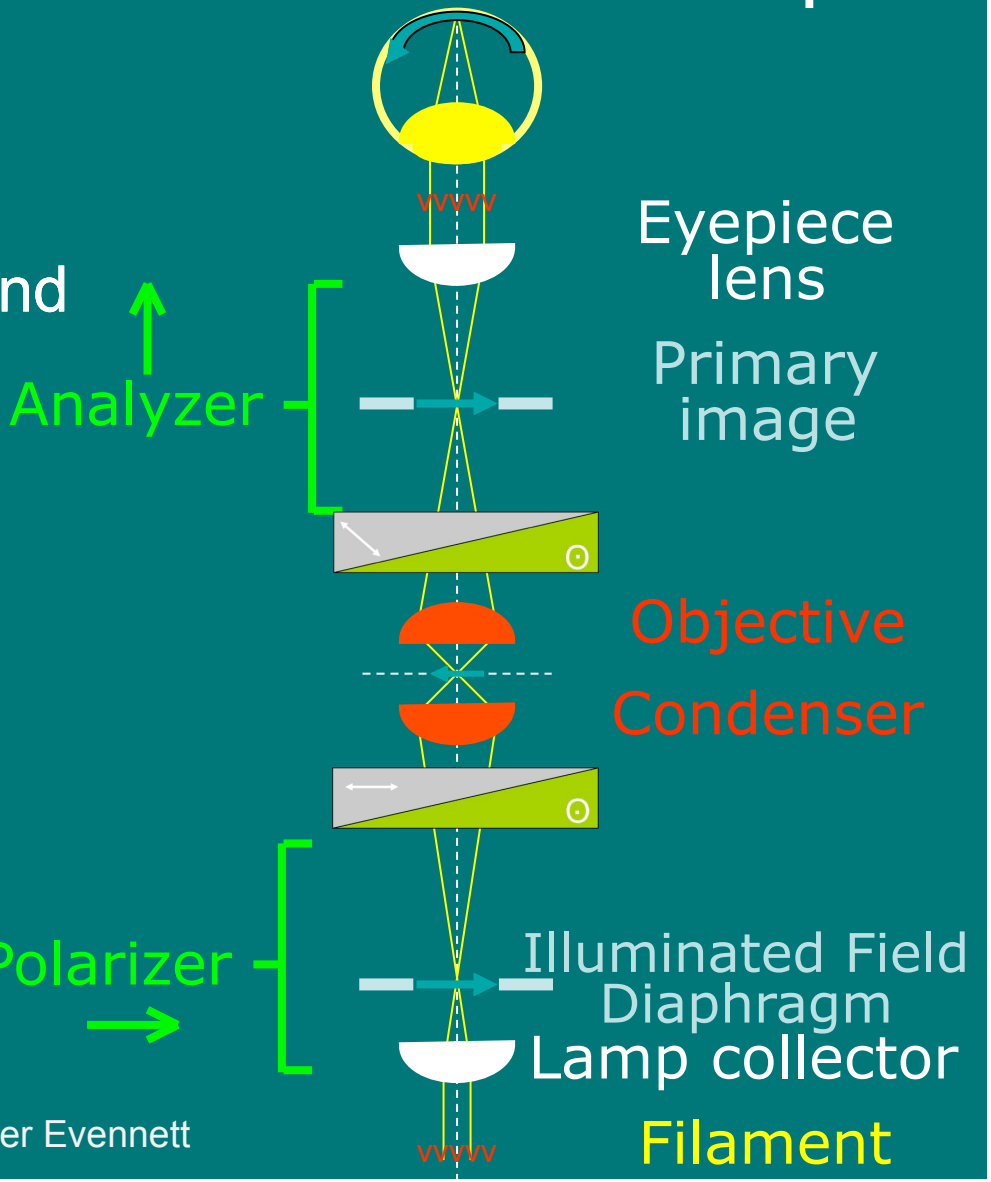
adapted from Peter Evennett

The principle of DIC – lets practice

3) Put in correct objective prism into back focal plane (BFP) - related position of objective

The principle of DIC – how to set it up

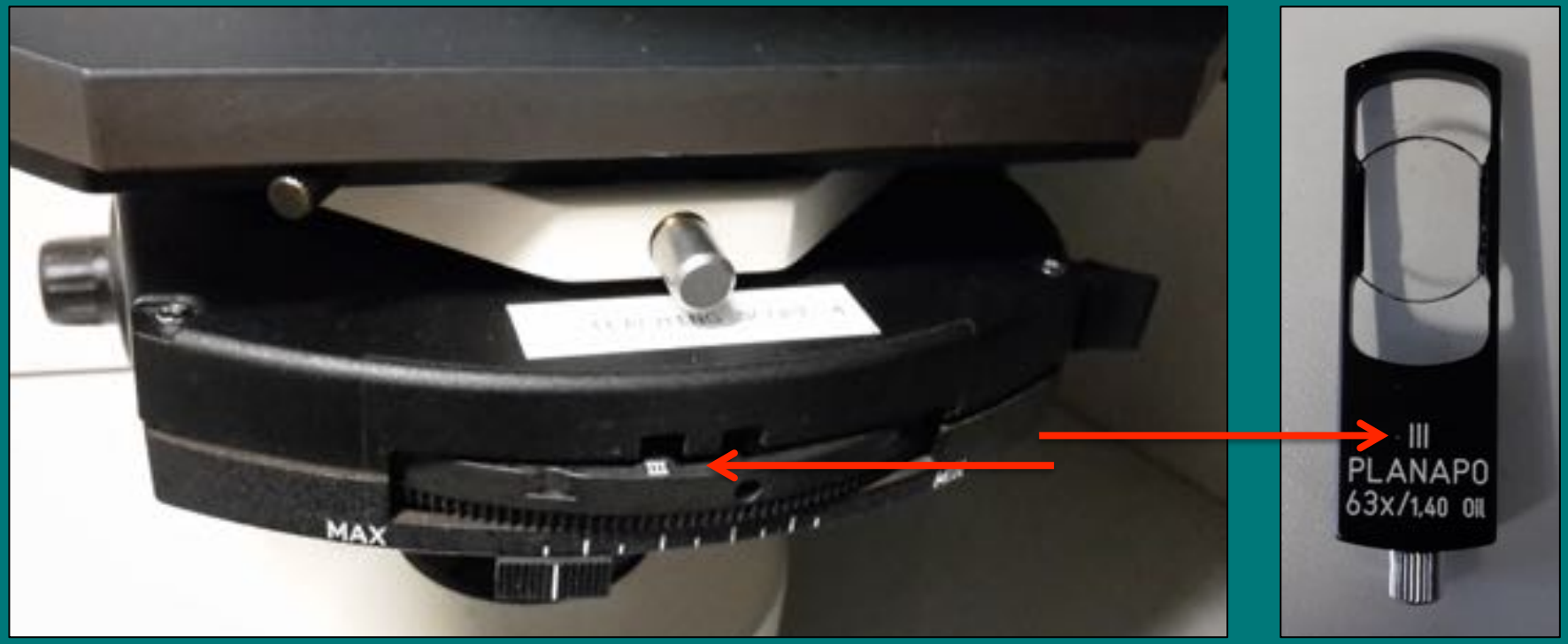
- Köhler!!!!!
- Insert and cross polarizer and analyzer
- Insert objective Nomarski prism
- Swing in corresponding condenser prism



adapted from Peter Evennett

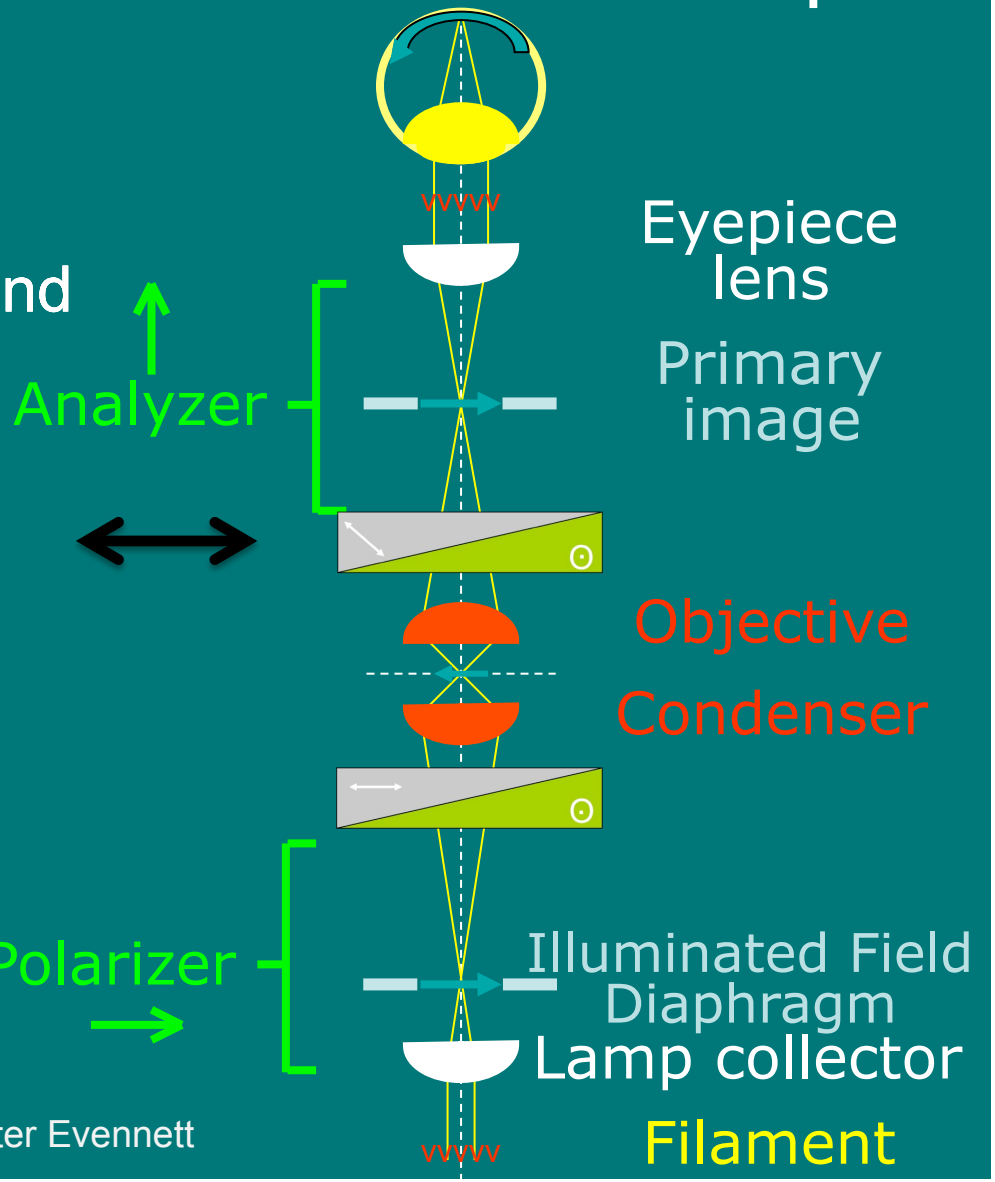
The principle of DIC – lets practice

4) Swing in correct condenser prism in front focal plane
   - Roman number (I, II, or III) has to correspond to the number on objective prism used



The principle of DIC – how to set it up

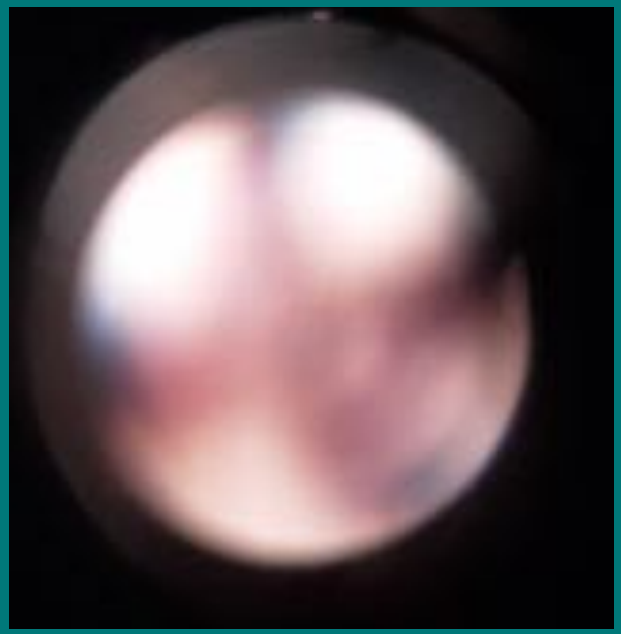
- Köhler!!!!!
- Insert and cross polarizer and analyzer
- Insert objective Nomarski prism
- Swing in corresponding condenser prism
- move objective prism to maximum extinction



adapted from Peter Evennett

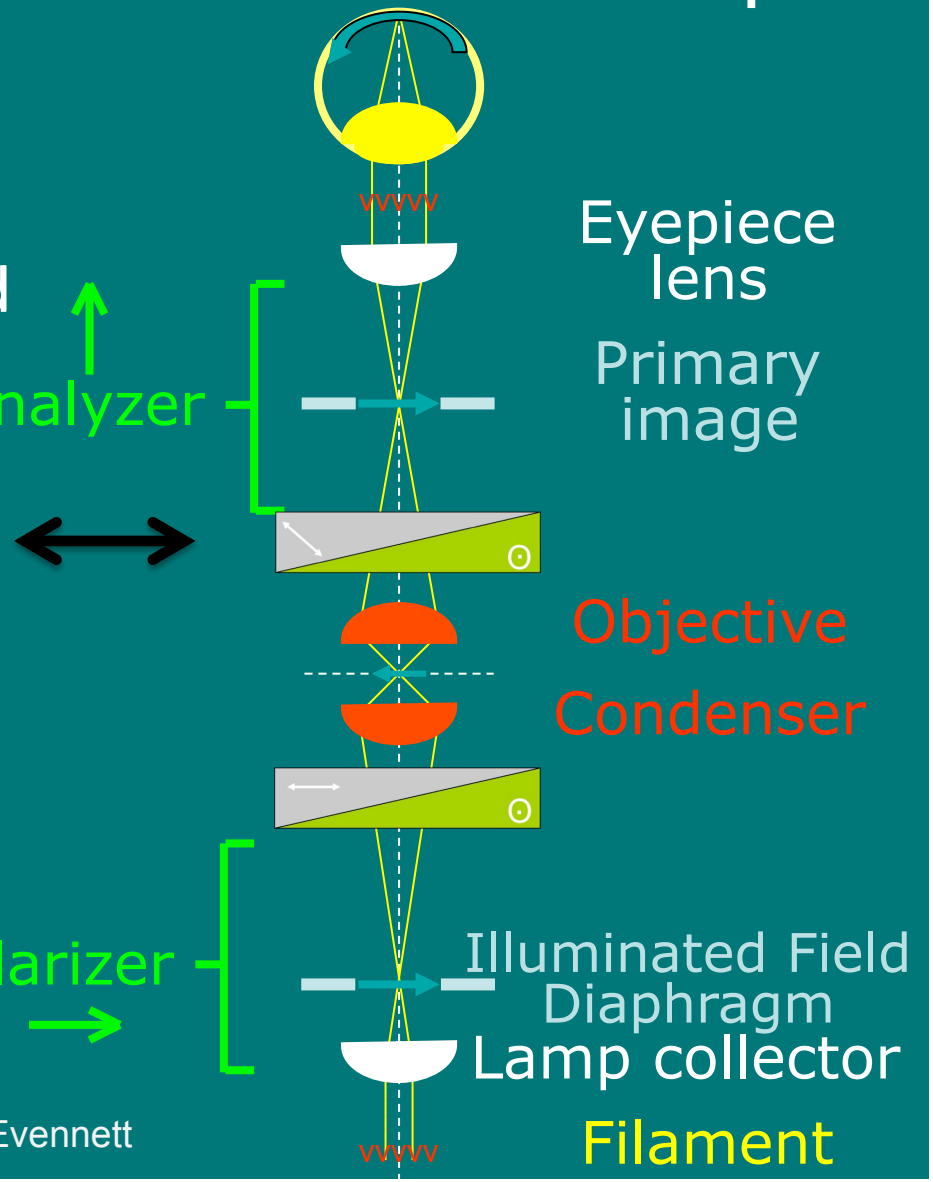
The principle of DIC – lets practice

5) check BFP (using Betrand lens or telescope) for image of blurred cross or move objective prism into position were background is darkest (maximum extinction)



The principle of DIC – how to set it up

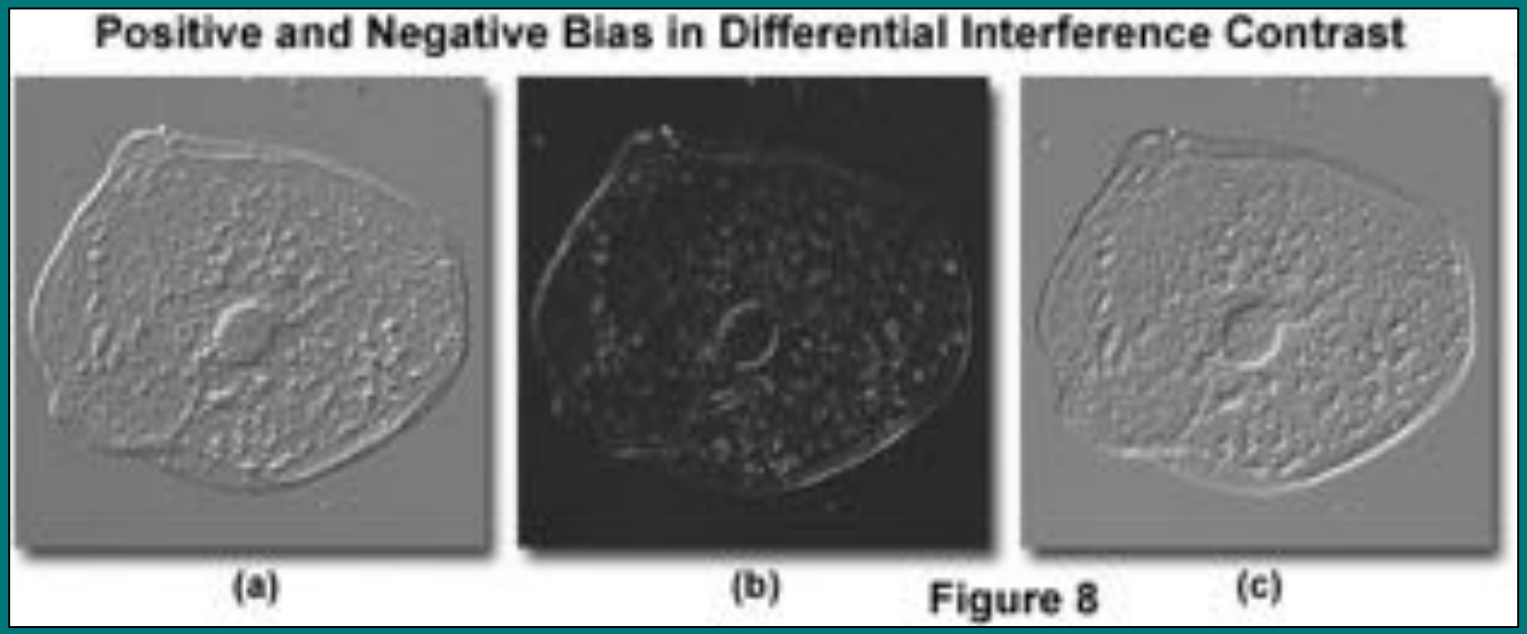
- Köhler!!!!!
- Insert and cross polarizer and analyzer
- Insert objective Nomarski prism
- Swing in corresponding condenser prism
- move objective prism to maximum extinction
- move objective prism for optimal bias



adapted from Peter Evennett

The principle of DIC – lets practice

7) turn objective prism or compensator for optimal bias



from olympusmicro.com