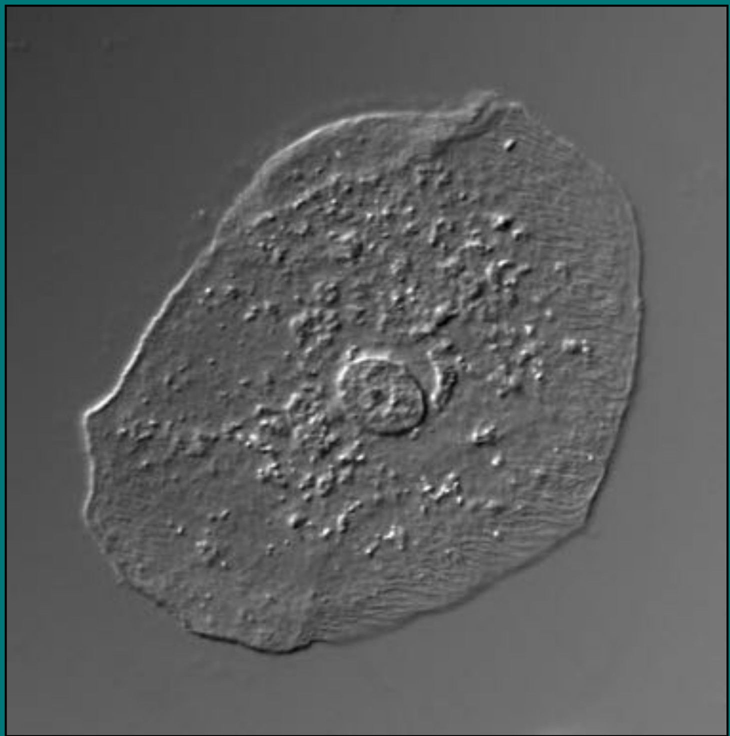
# Light Microscopy course

# 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

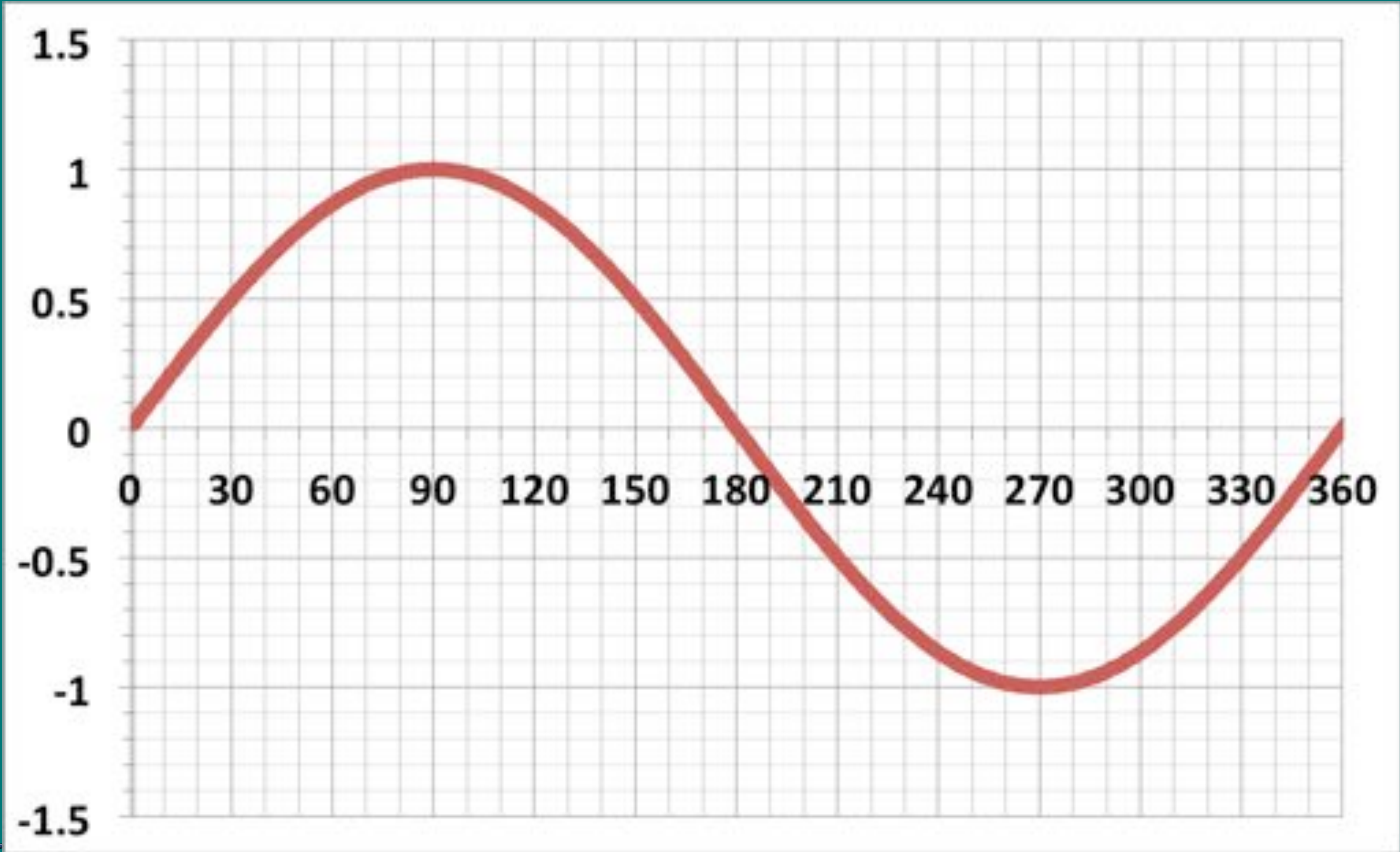
*For a detailed biography see: http://micro.magnet.fsu.edu/optics/timeline/people/nomarski.html*


# DIC – probe **differential** by interference = contrast

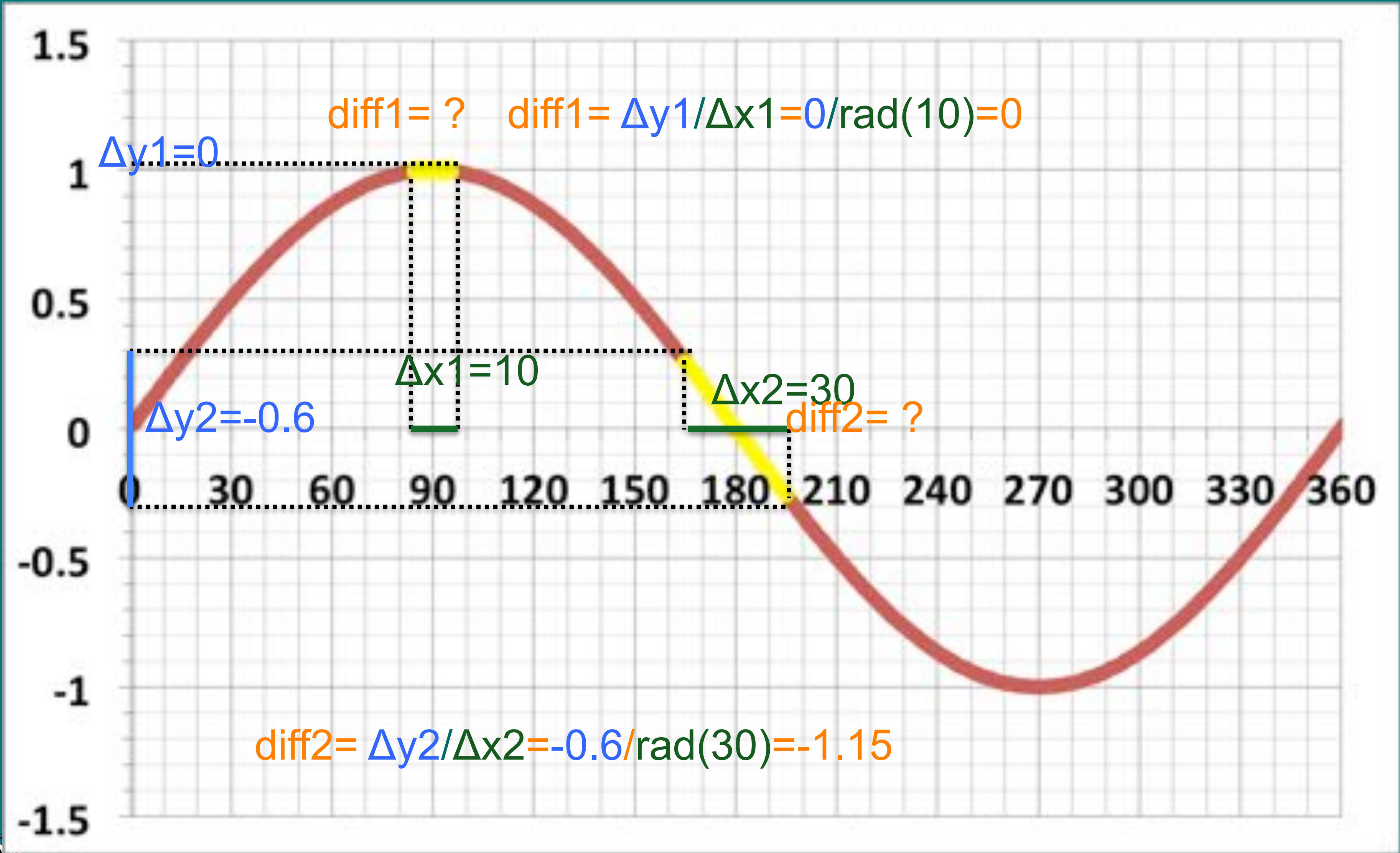
- Differential = (minute) difference between two different values of something (given in Δ or d) over a certain (small) range (gradient or slope =Δy/Δx)

- Used in many different contexts (Math, Physics, Engineering, Biology….)

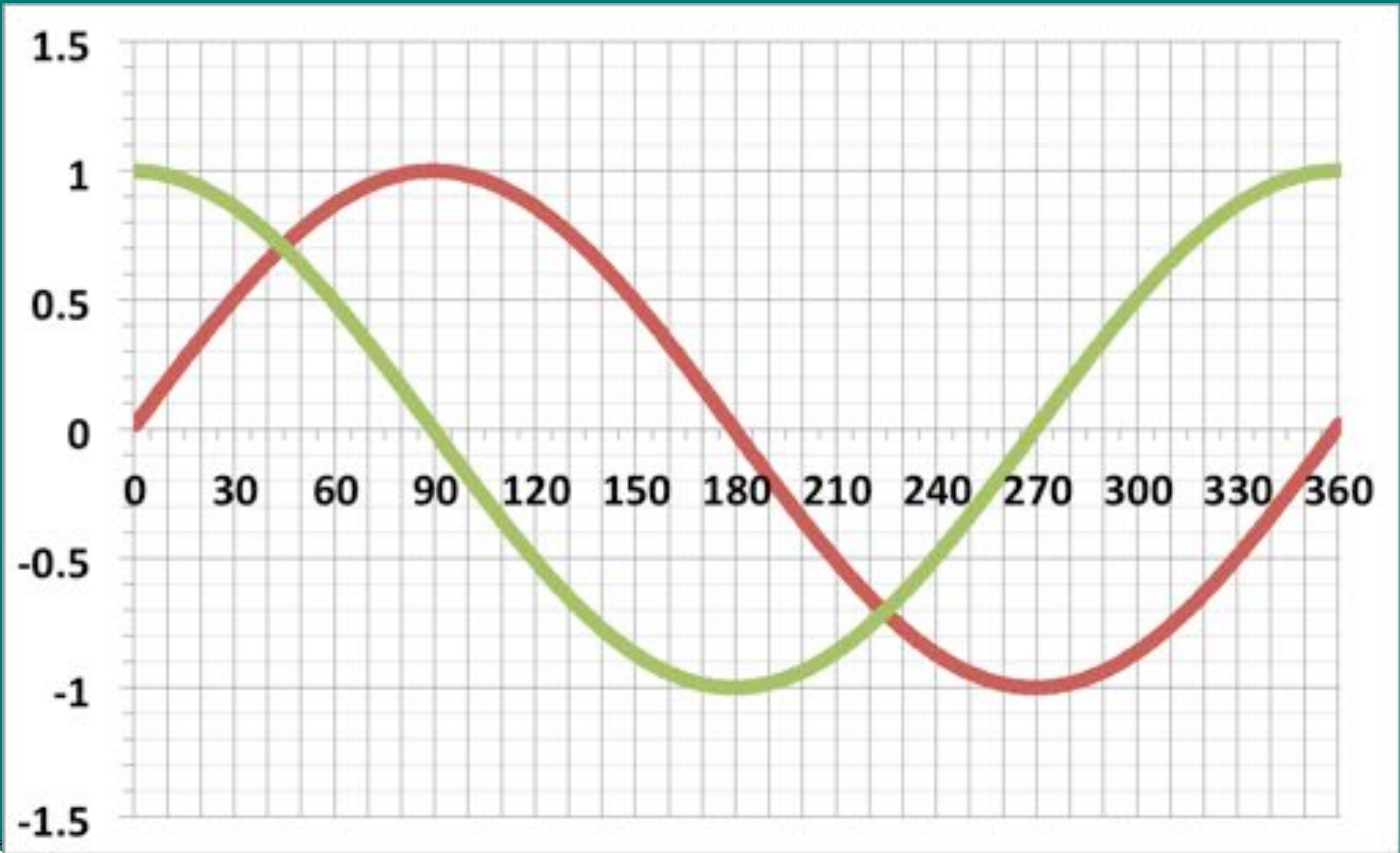
# DIC – probe **differential** by interference = contrast – example for differential



# DIC – probe **differential** by interference = contrast – example for differential

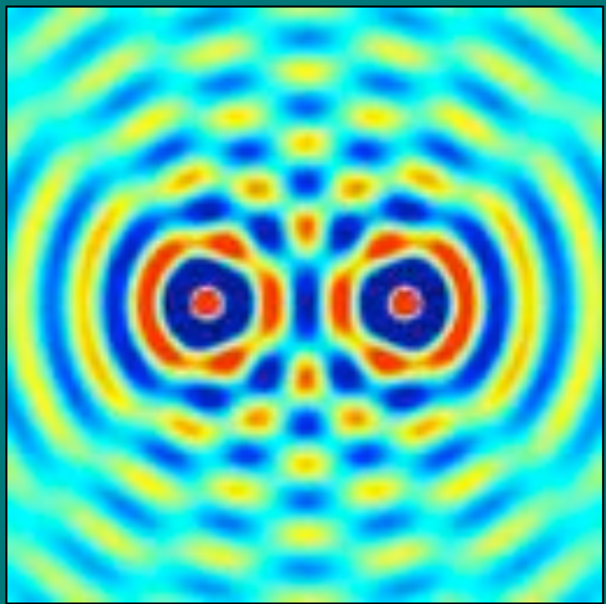


# DIC – probe **differential** by interference = contrast – example for differential

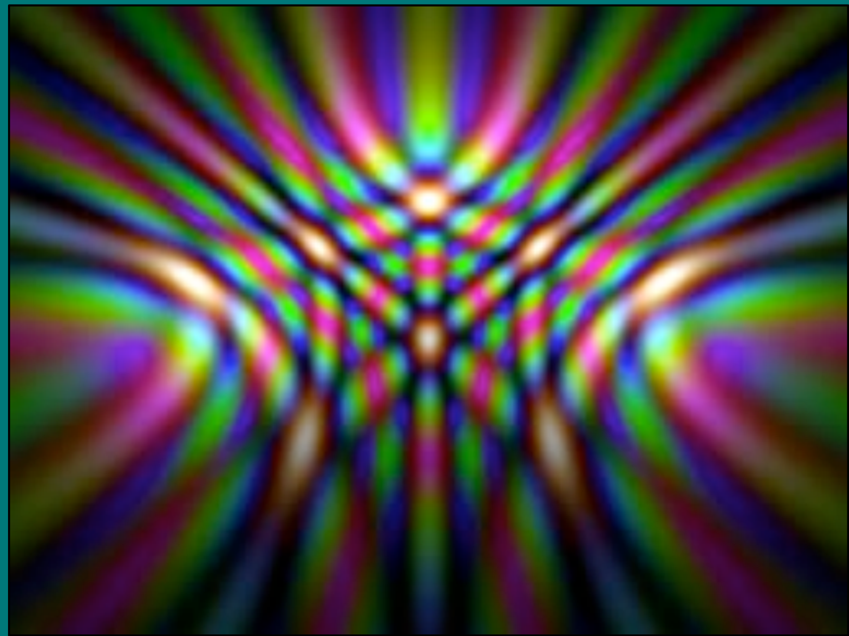


# DIC – differential by **interference** = contrast

Interference is a phenomenon in which two waves superpose to form a resultant wave of greater, lower or same amplitude. (Wikipedia)



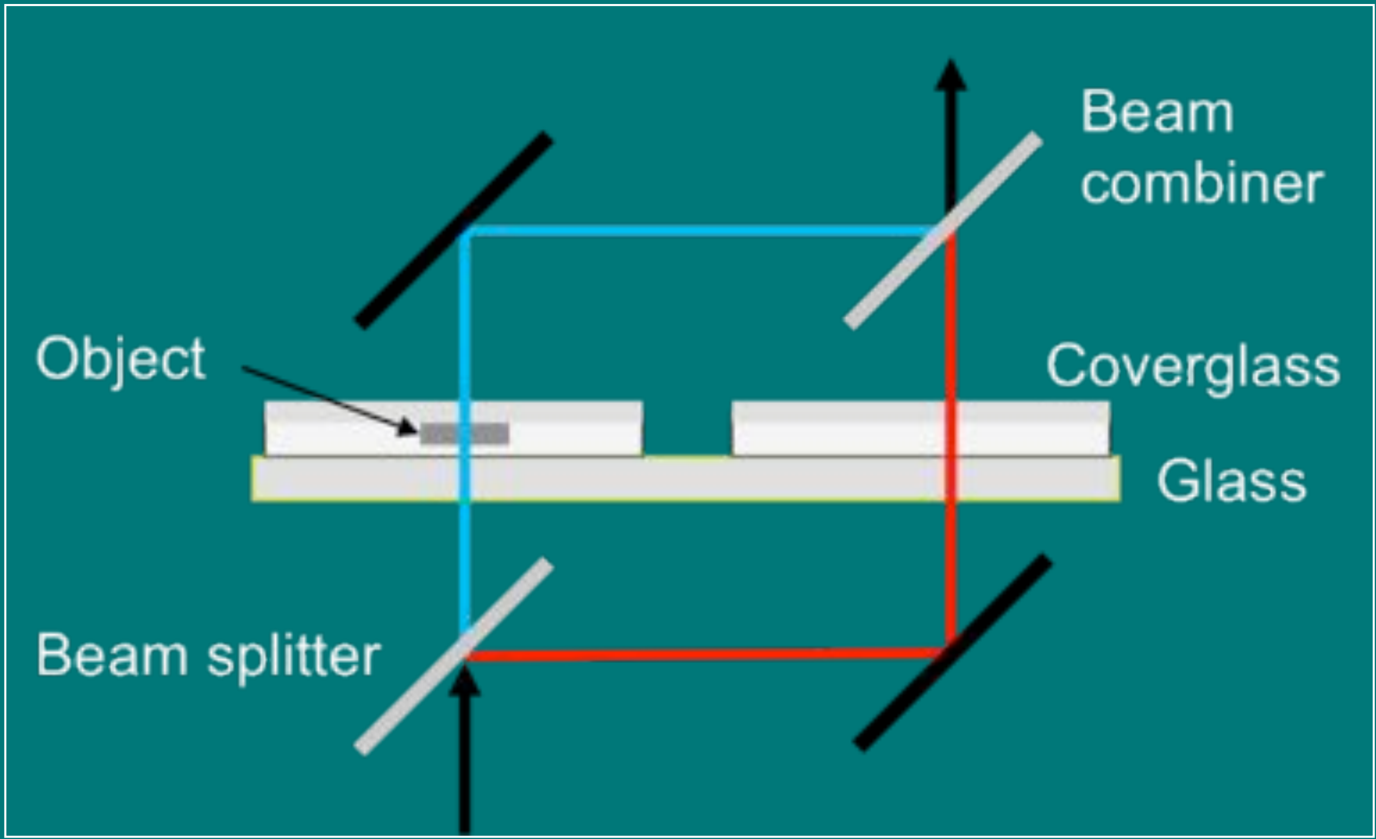
Wikipedia

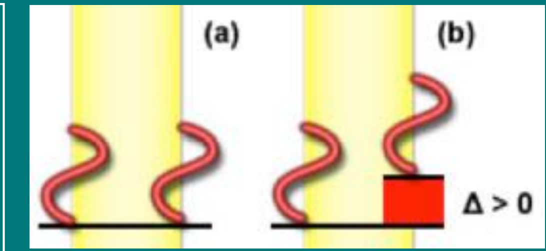


https://machinesdontcare.wordpress.com/2011/02/05/interference-patterns/interference-fringes-04/

# DIC – differential by **interference** = contrast
## The principle of DIC - interferometry

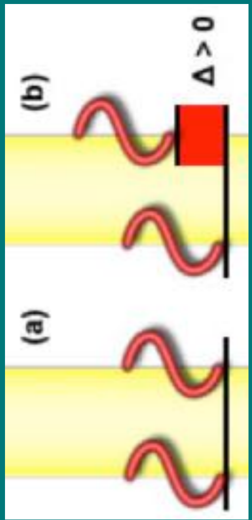
Classical double-path interferometer with a real reference sample

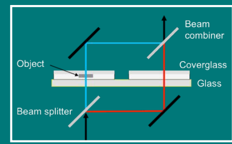


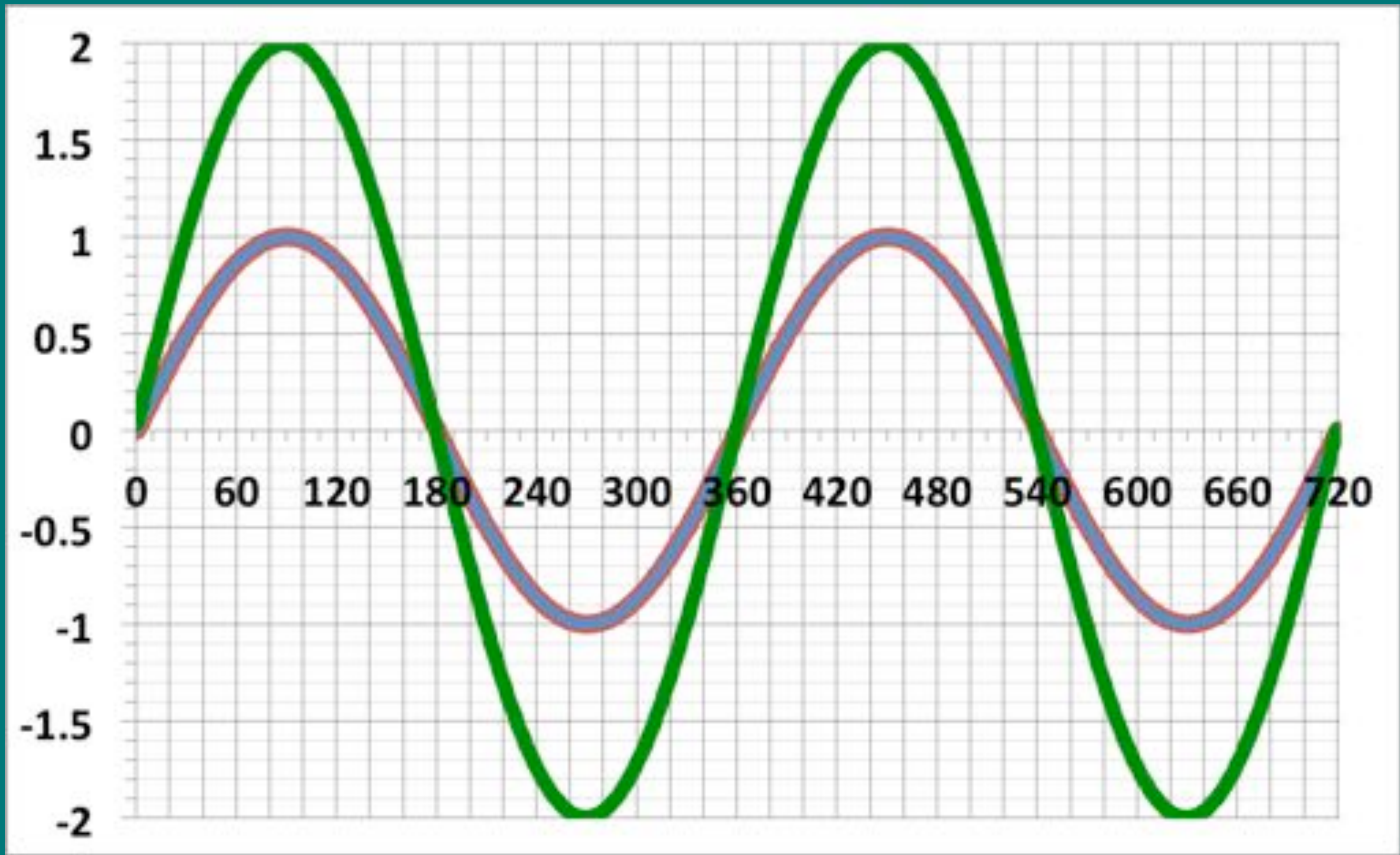




adapted from Peter Evennett

# DIC – differential by **interference** = contrast

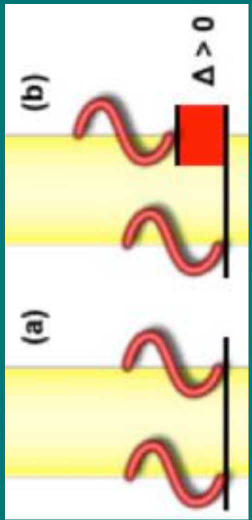


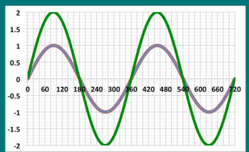



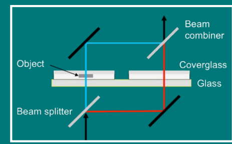



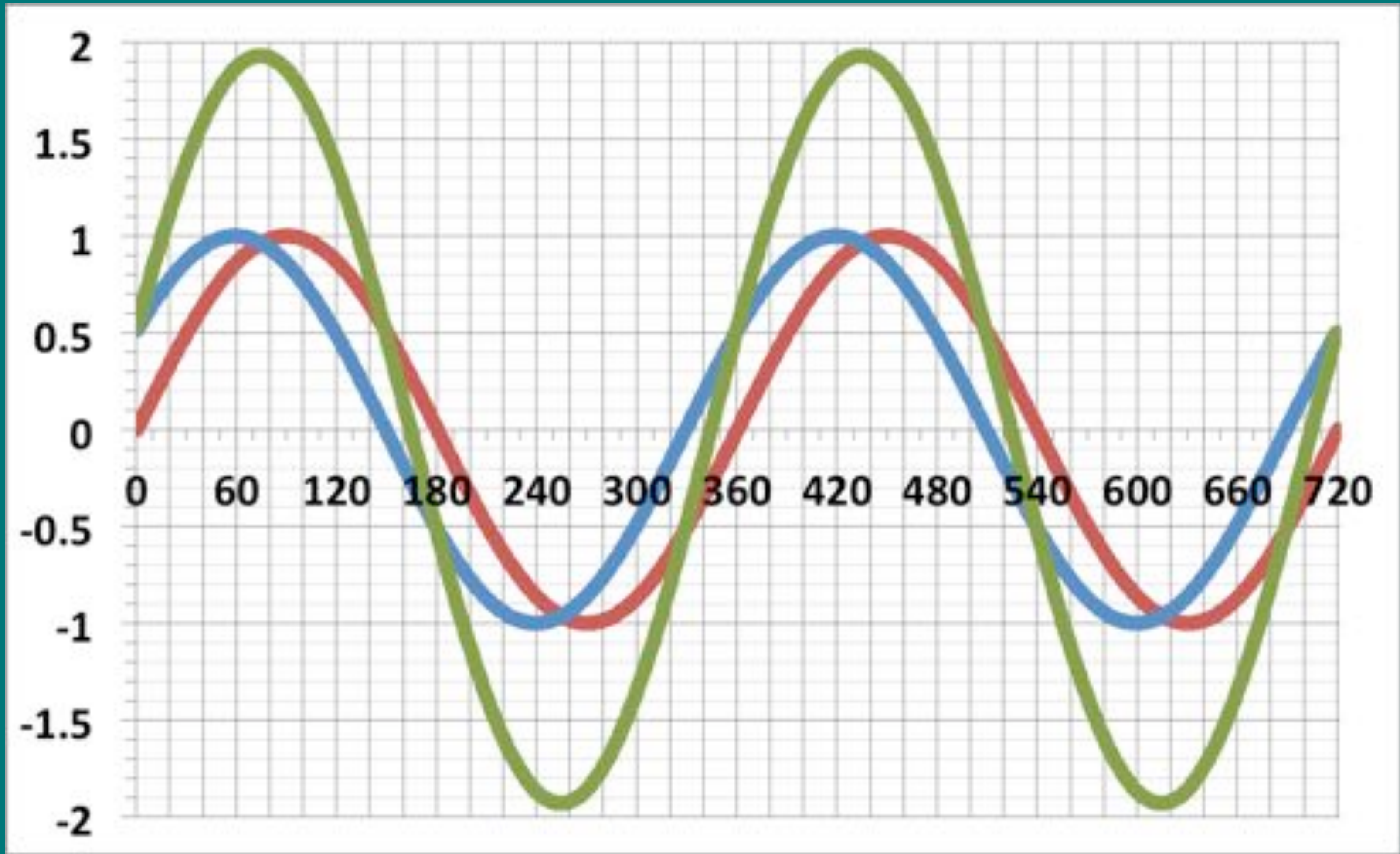

No shift

# DIC – differential by **interference** = contrast



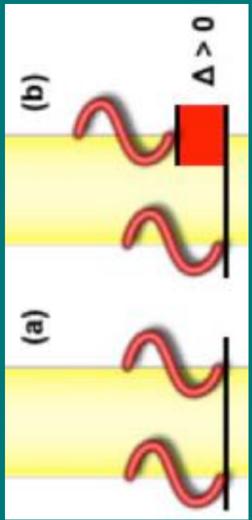



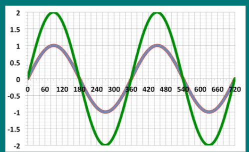


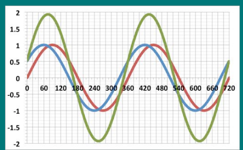



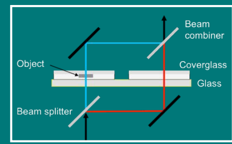

30 degrees slower

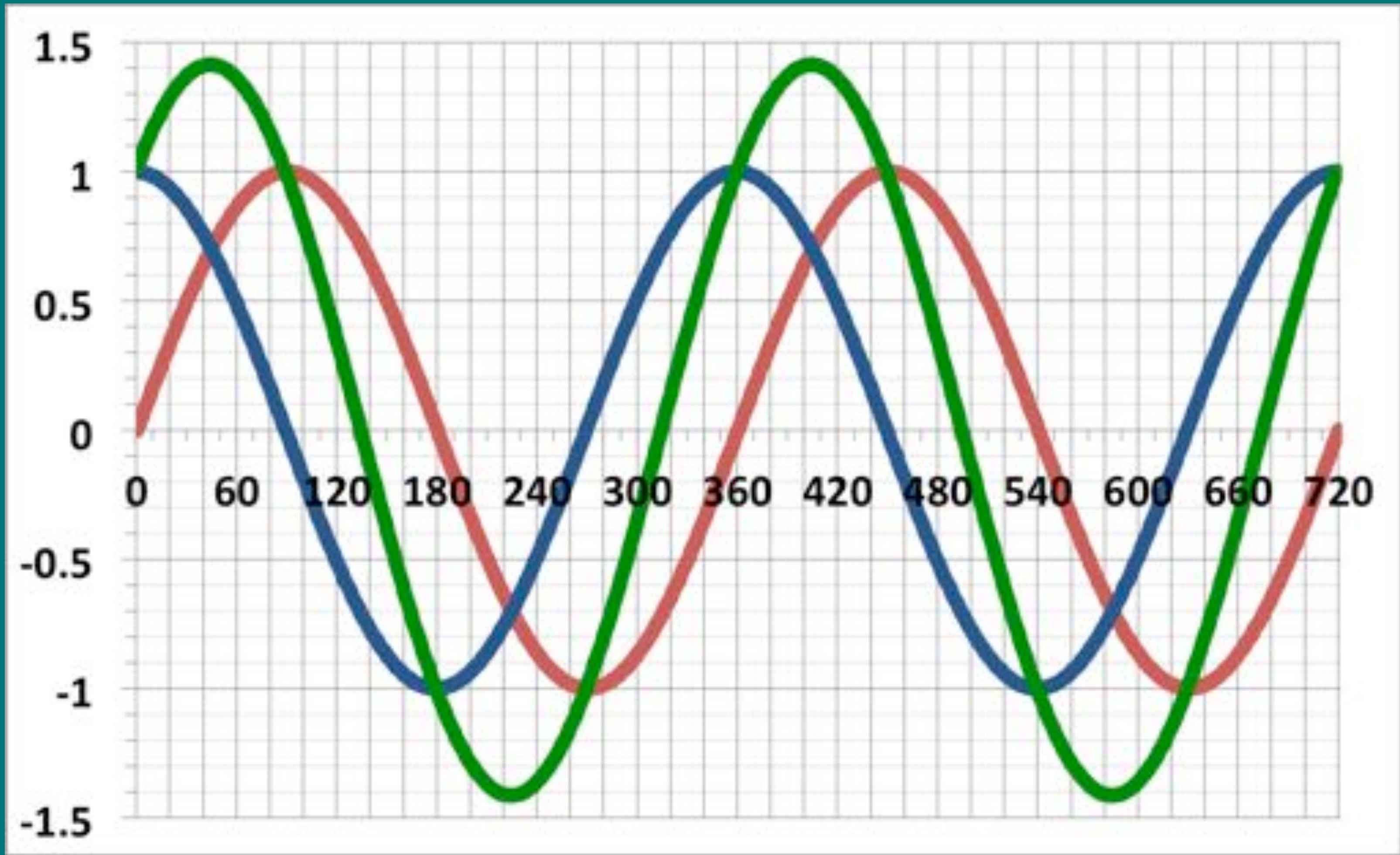
# DIC – differential by **interference** = contrast



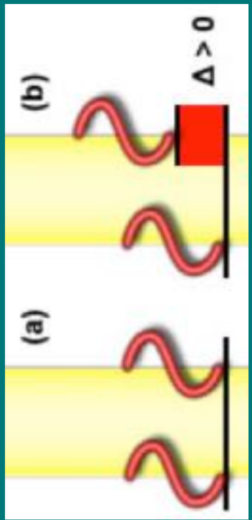


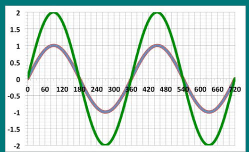


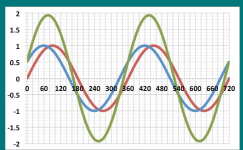



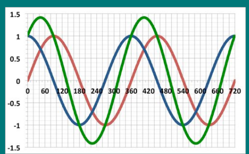

90 degrees slower

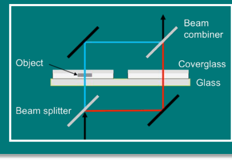
# DIC – differential by **interference** = contrast

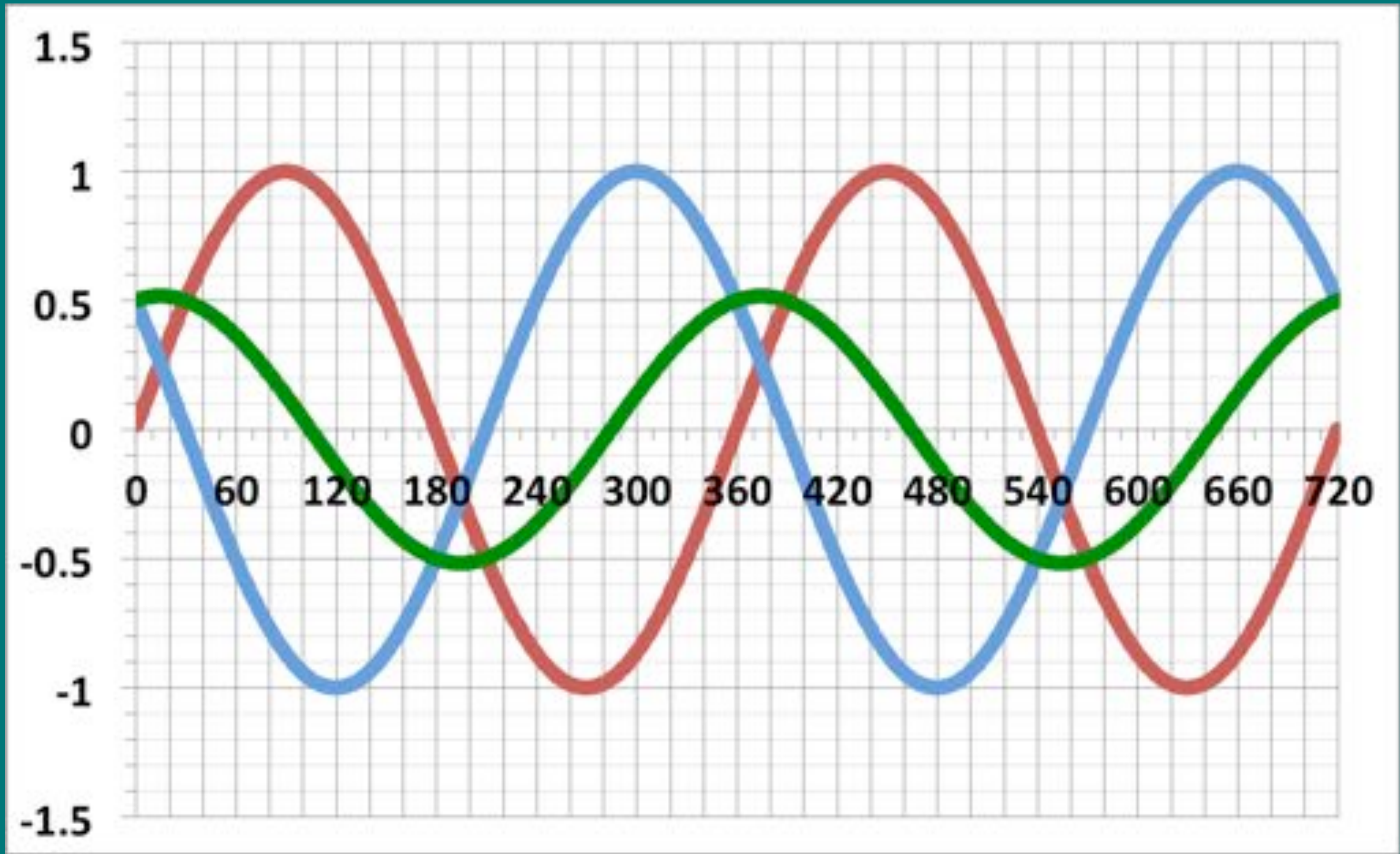




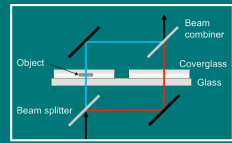


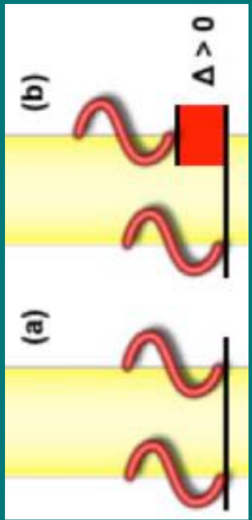


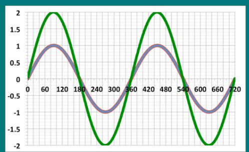



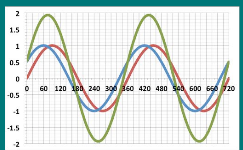

150 degrees slower

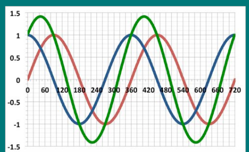
# DIC – differential by **interference** = contrast

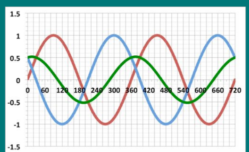


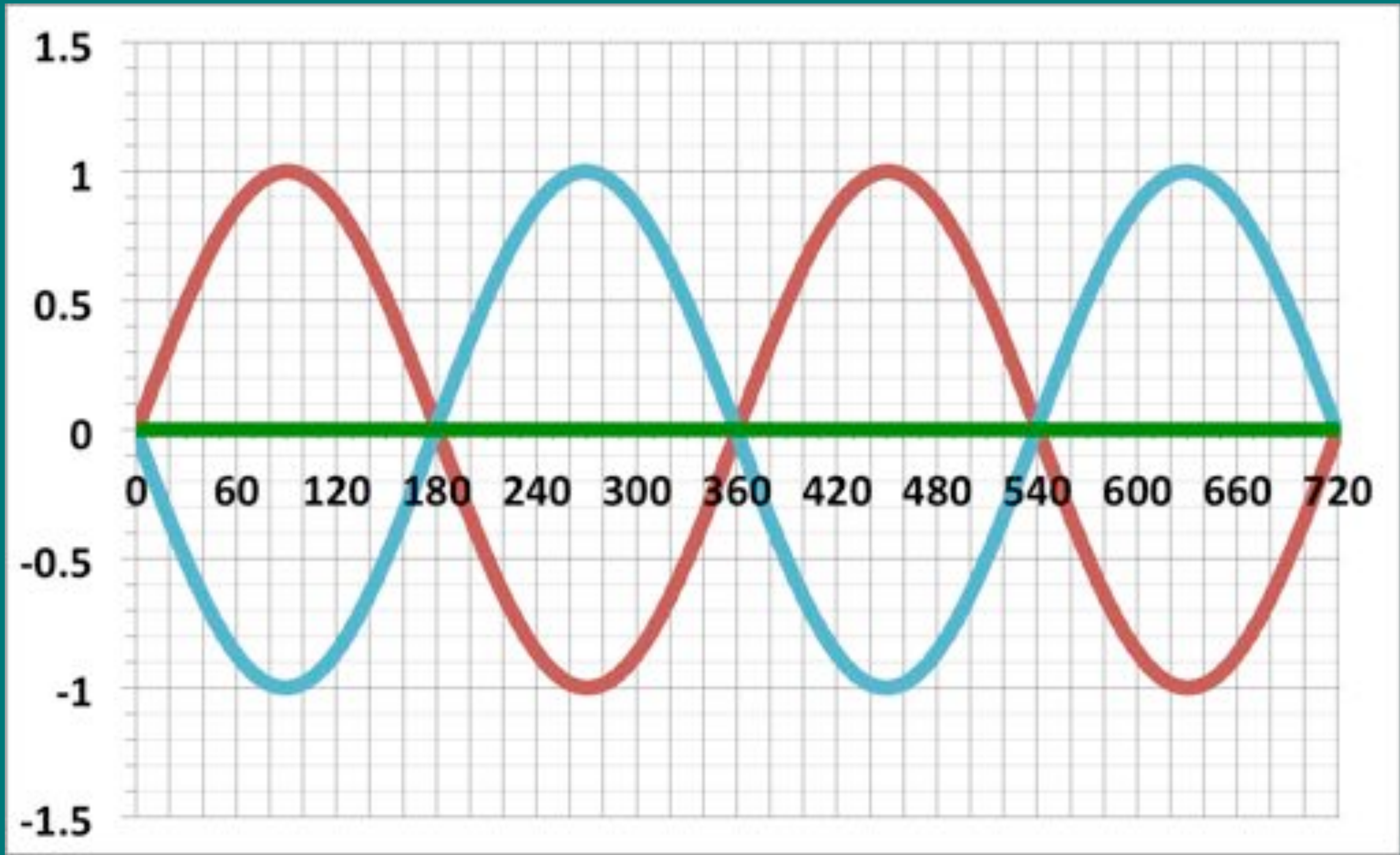







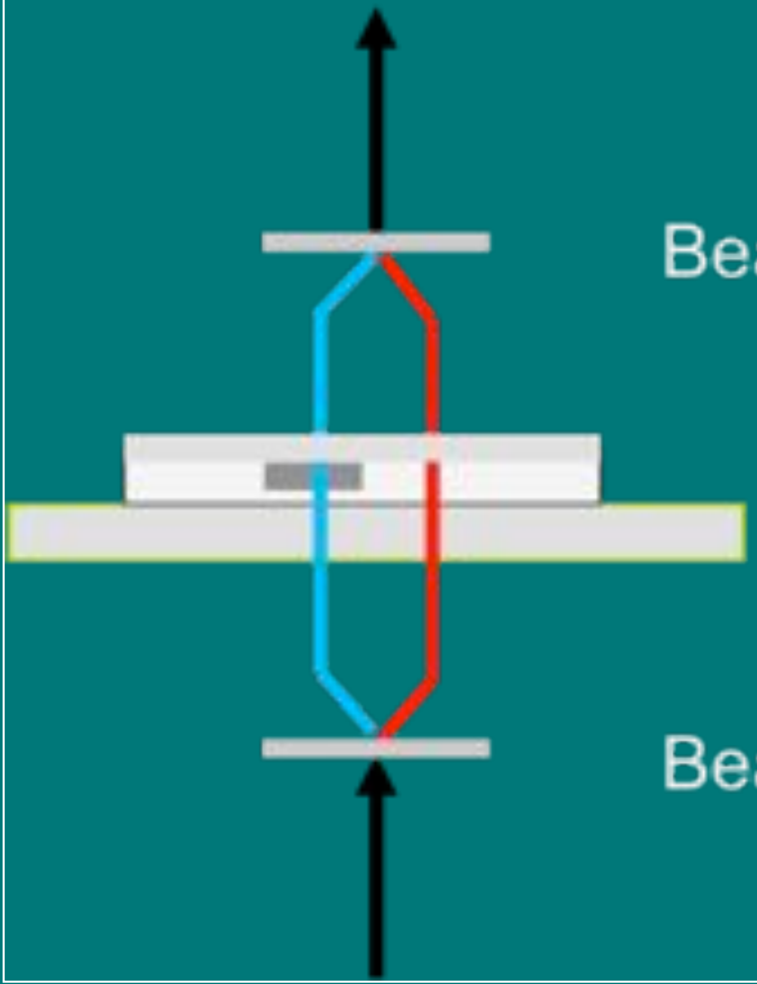





180 degrees slower

# The principle of DIC - interferometry

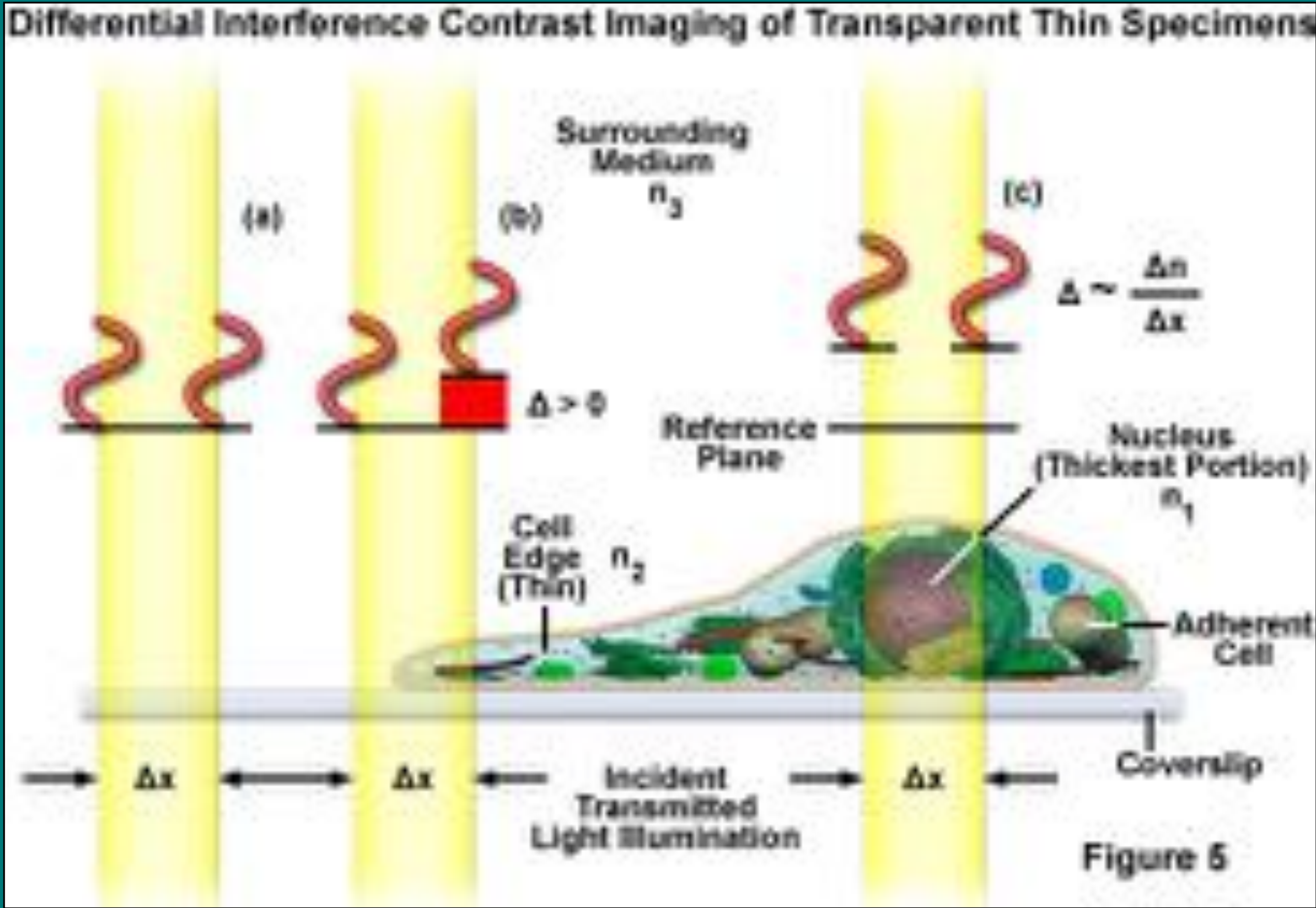
**Common path** interferometer, lateral shearing interferometer




adapted from Peter Evennett

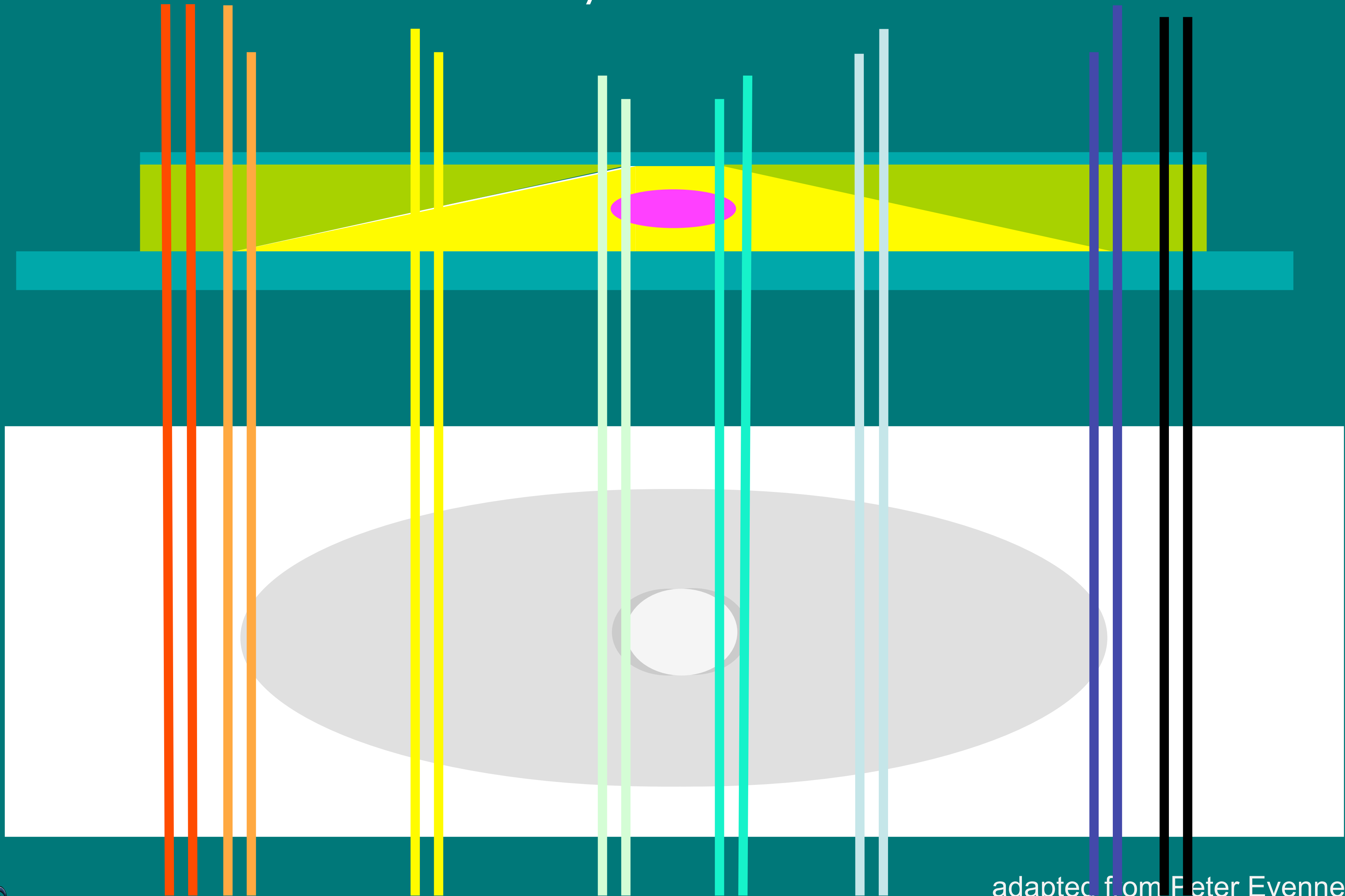
Used for DIC

# DIC – differential by interference = contrast



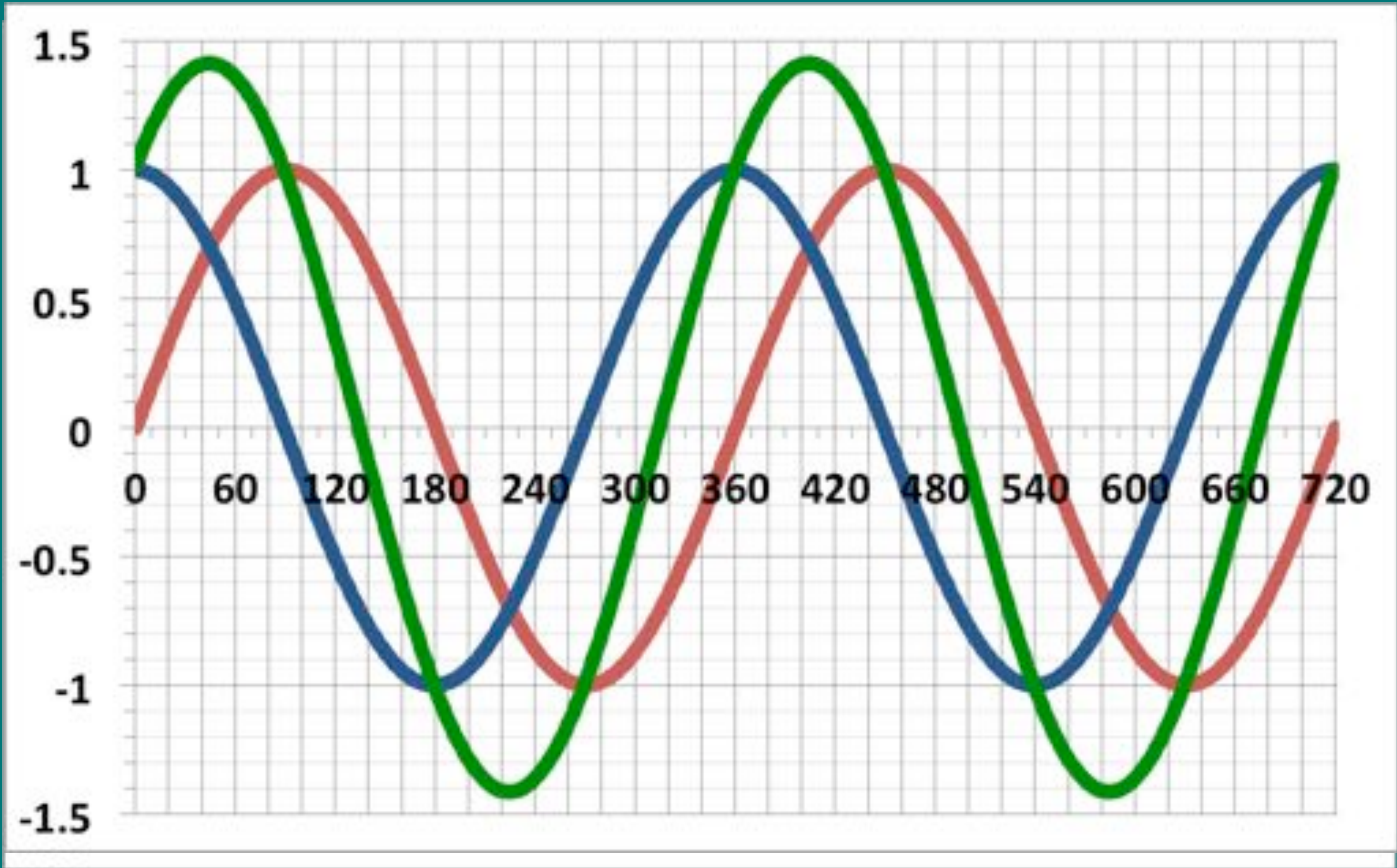

From ZEISS-campus website

# DIC – differential by interference = contrast



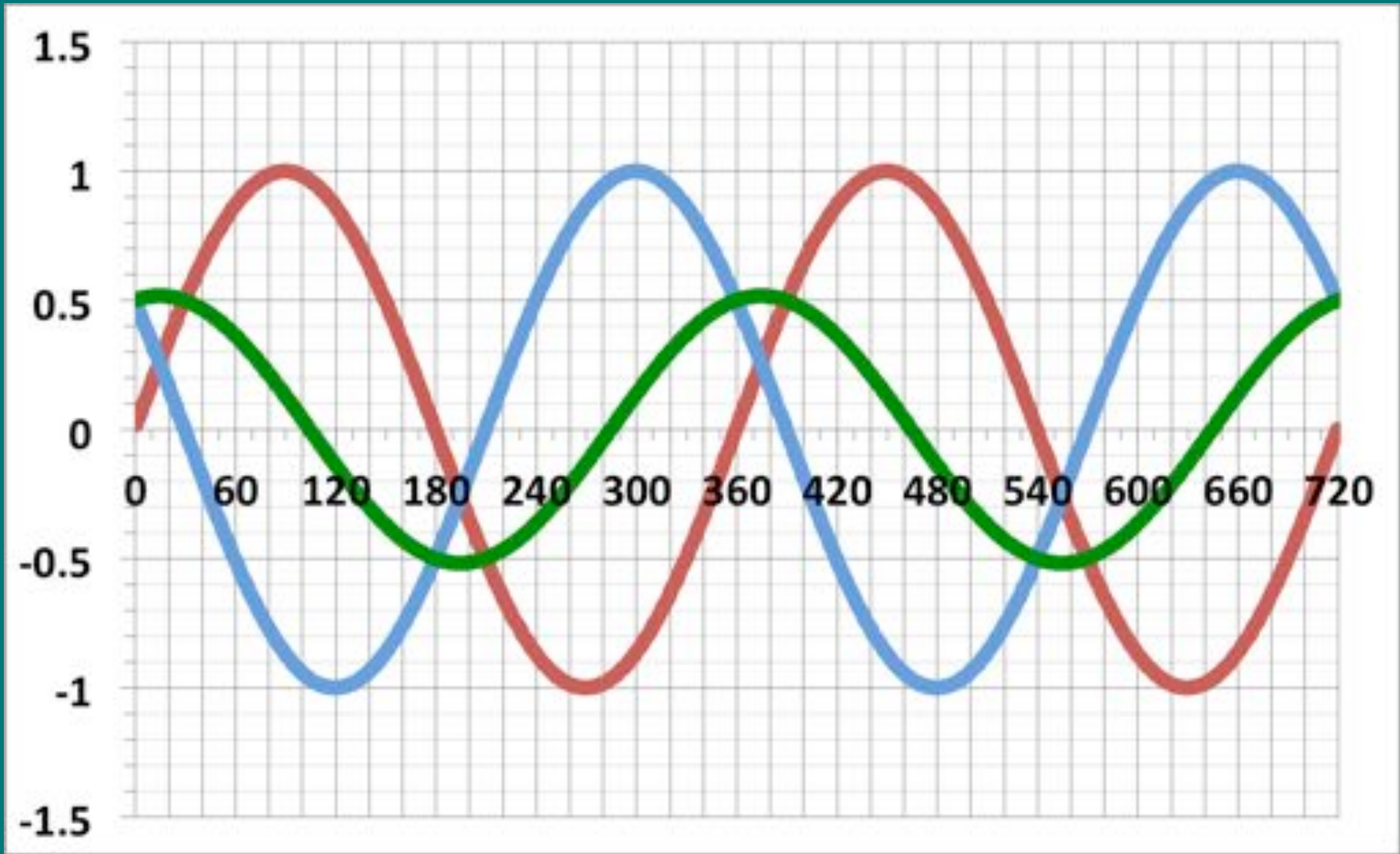
adapted from Peter Evennett

# DIC – introducing retardation bias

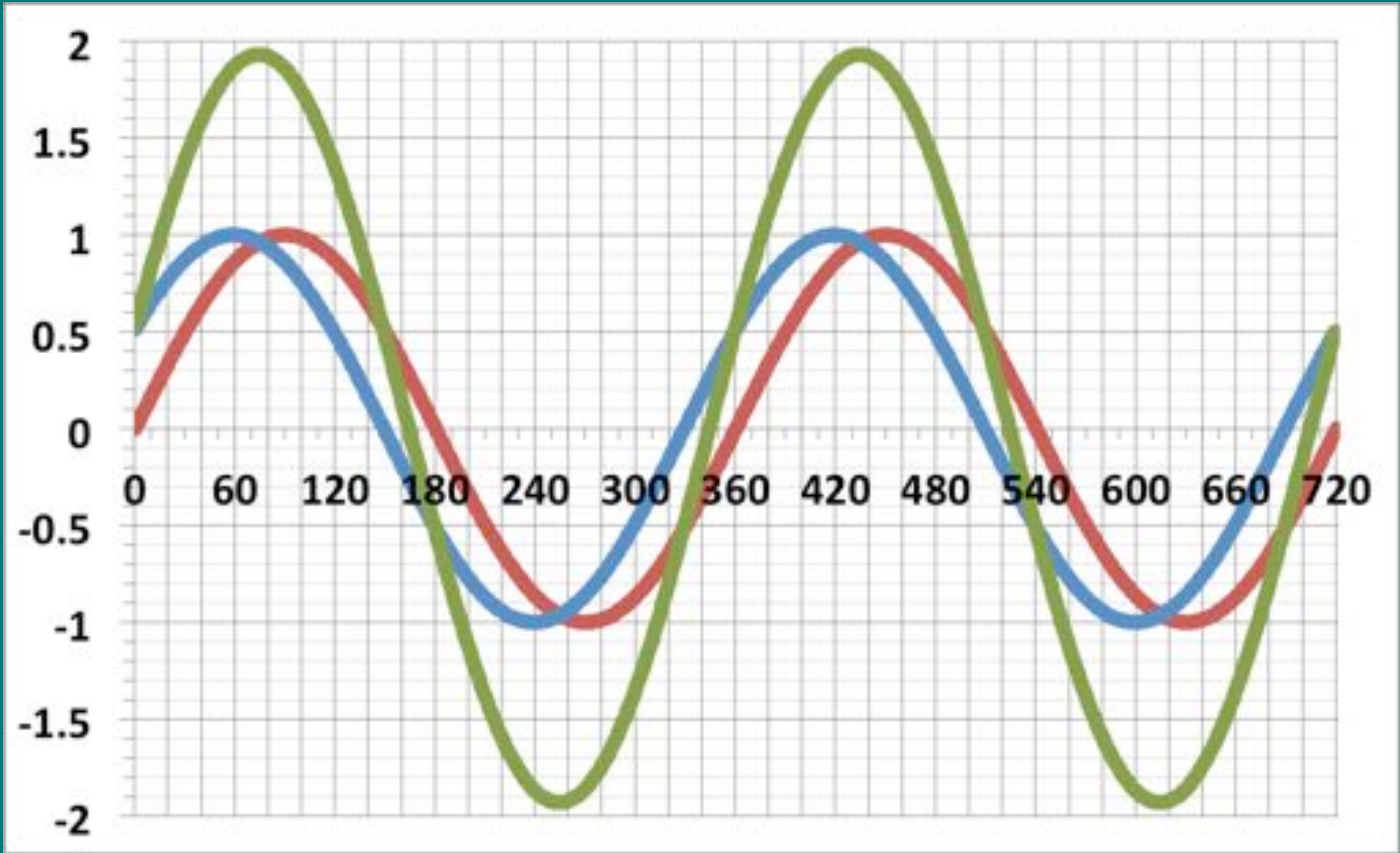



Blue beam retarded by λ/4 – 90 degrees, no further retardation

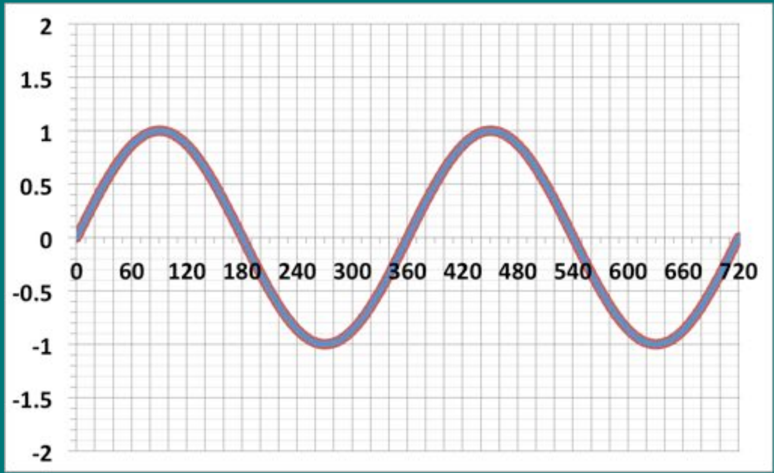
# λ/4 bias + further λ/6 retardation of blue beam



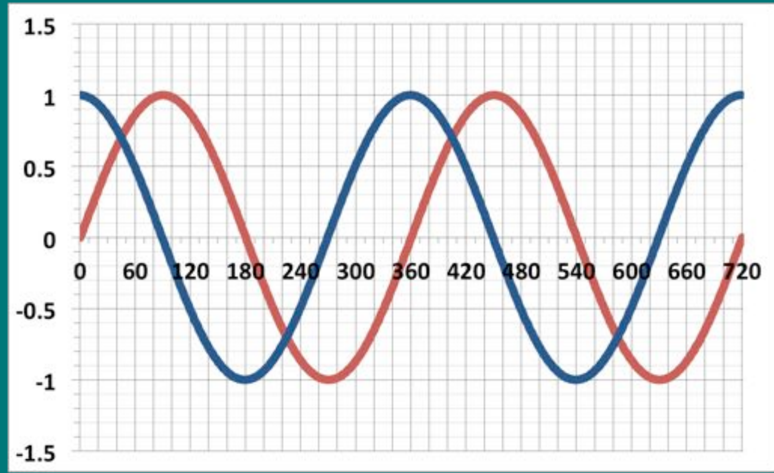
# λ/4 bias of blue + λ/6 retardation of red beam



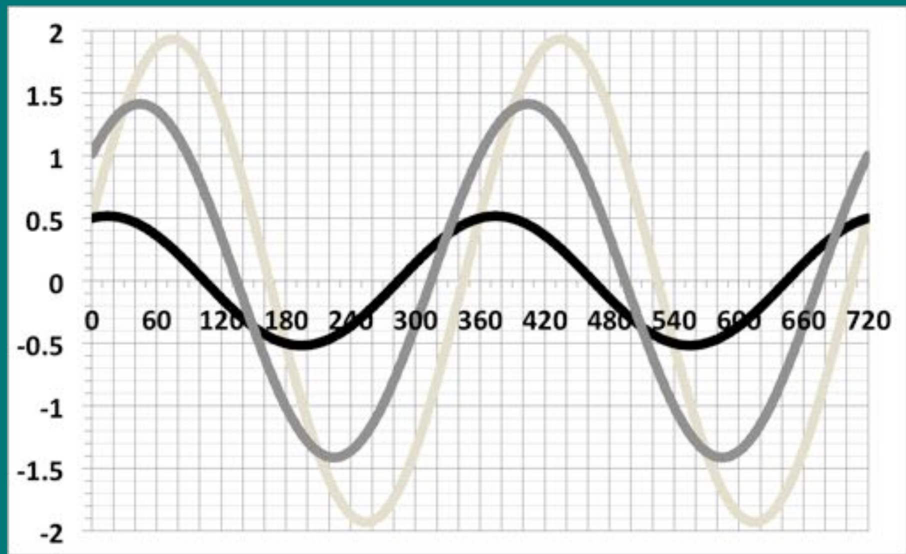
## Without bias




## With λ/4 bias of blue beam

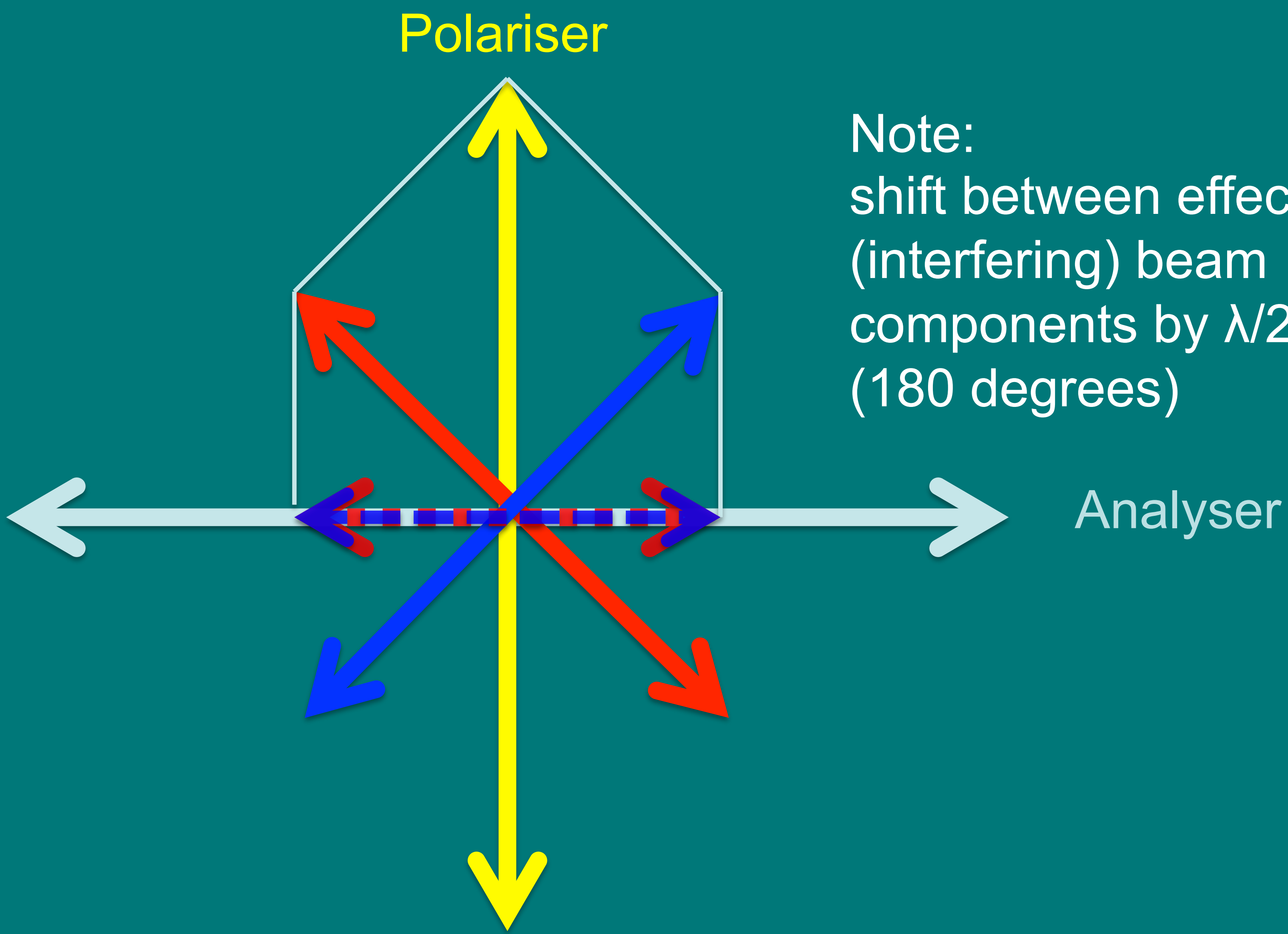



## Resultants of retardation of beam by 60 deg (λ/6)

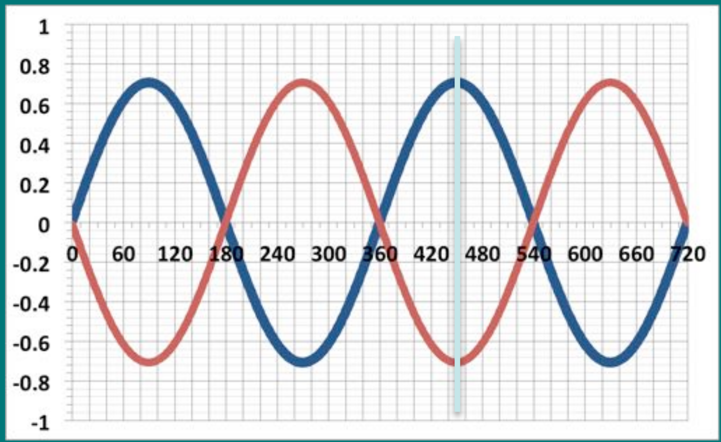

grey: no retardation, dark: blue retarded, light: red retarded

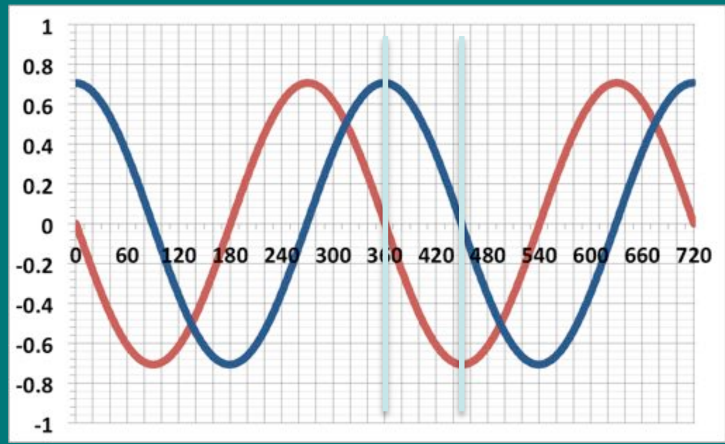
# Interference at analyzer



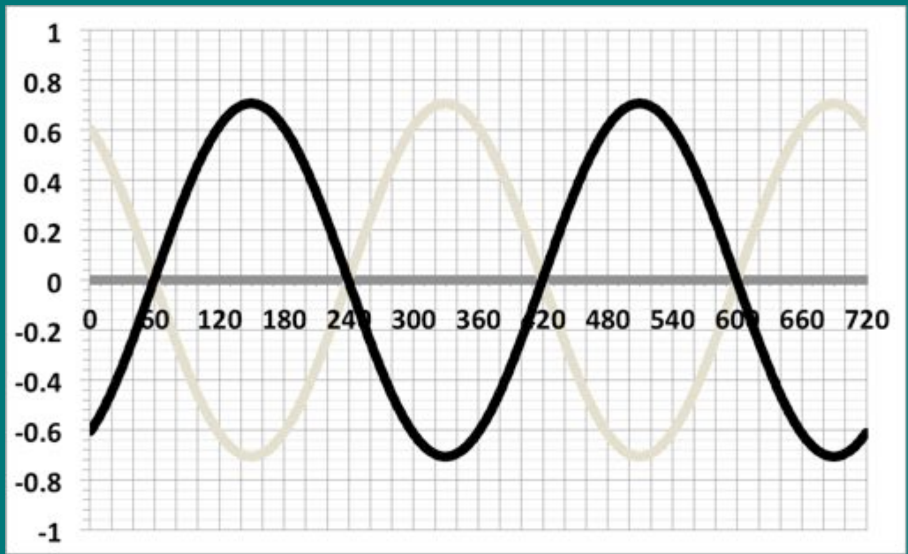

Note:
shift between effective (interfering) beam components by λ/2 (180 degrees)

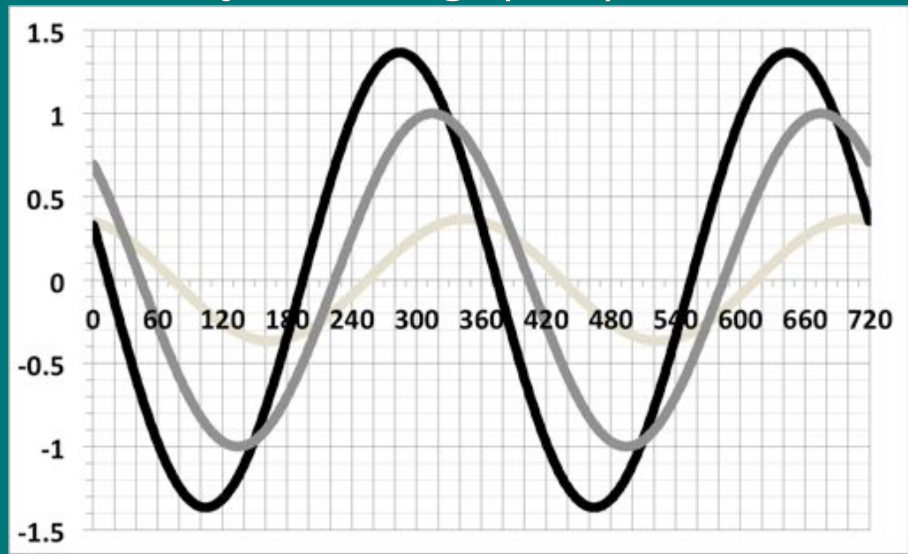
## Without bias λ/2 shift at analyser With λ/4 bias



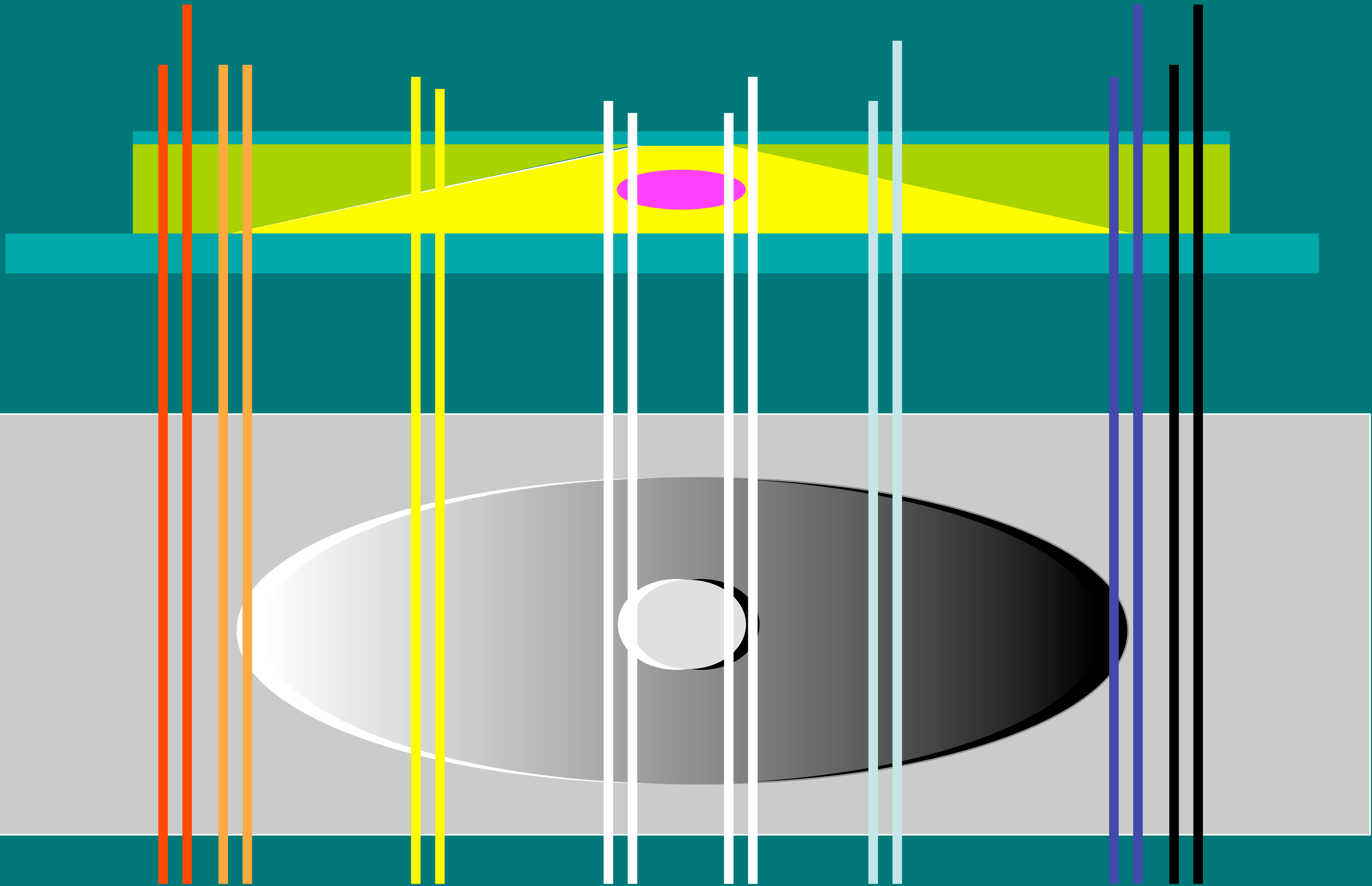

## Resultants of retardation of beam by 60 deg (λ/6)



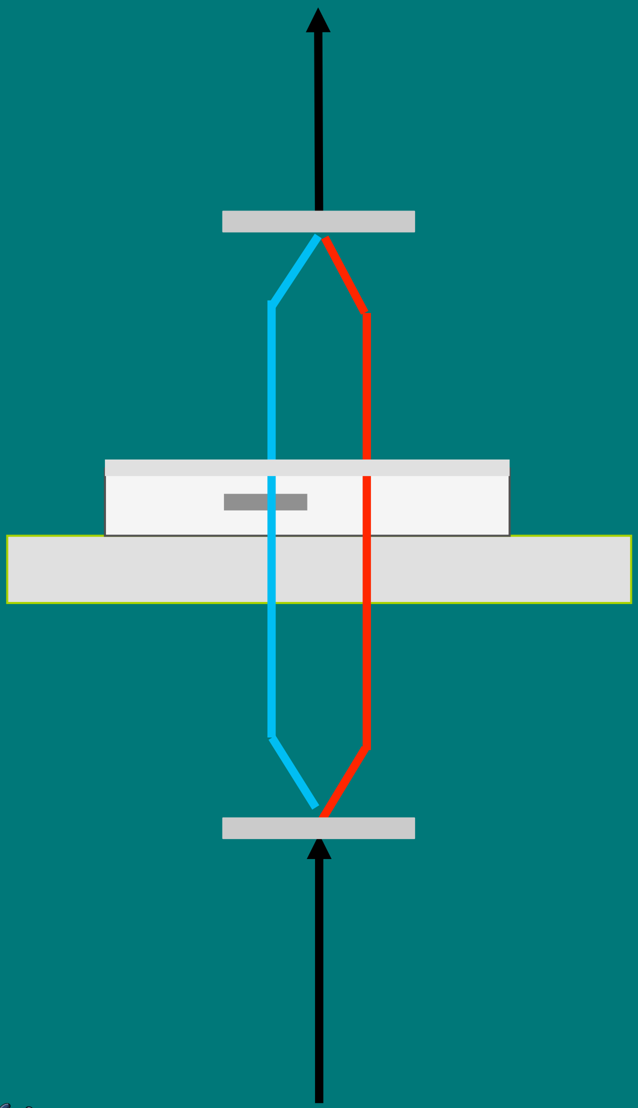

grey: no retardation, dark: blue retarded, light: red retarded

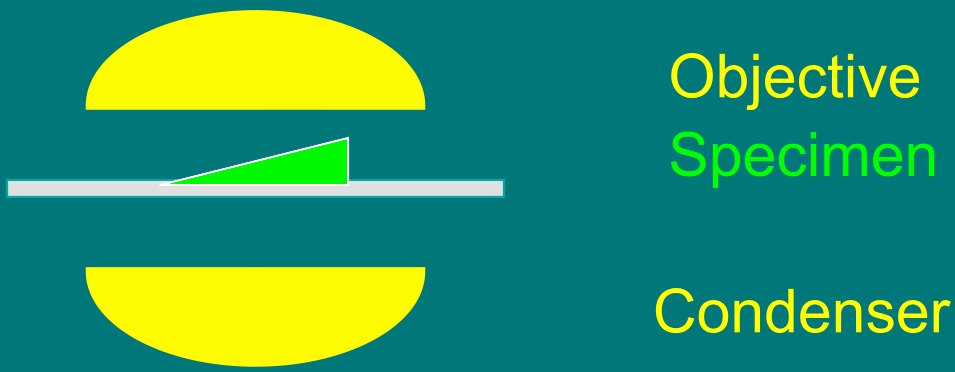
# DIC – with retardation bias



adapted from Peter Evennett

# The principle of DIC – how does it work



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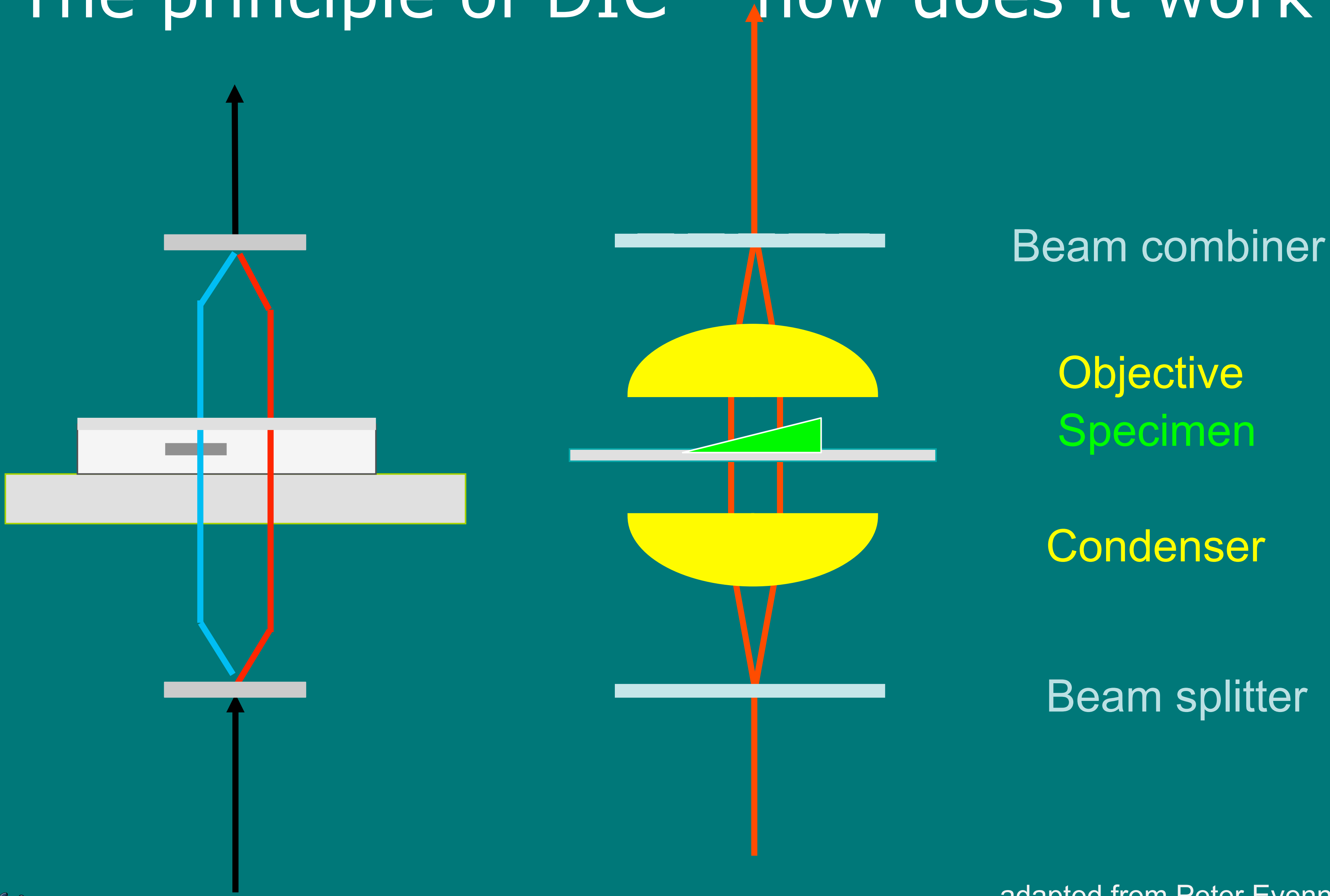


adapted from Peter Evennett

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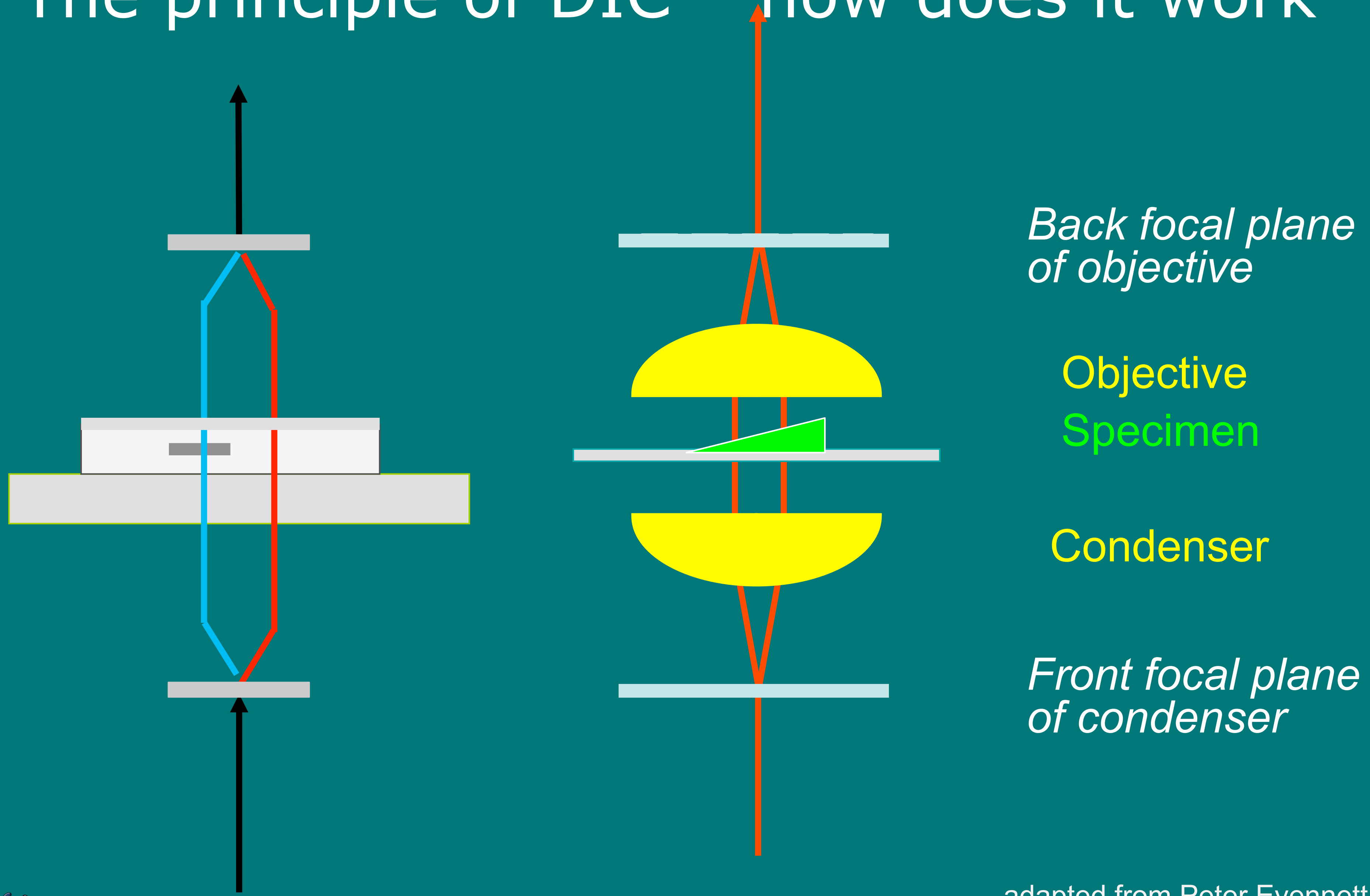
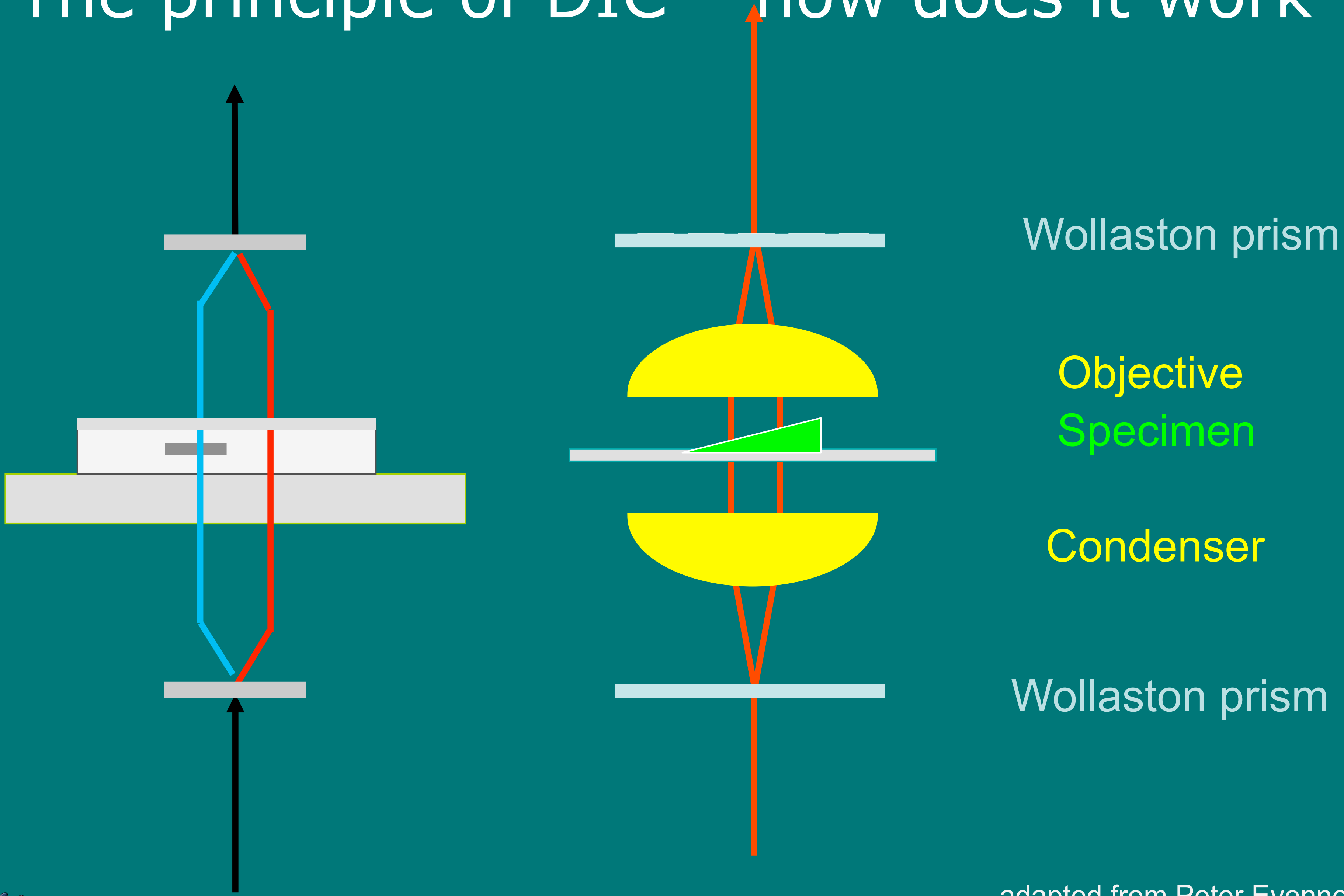


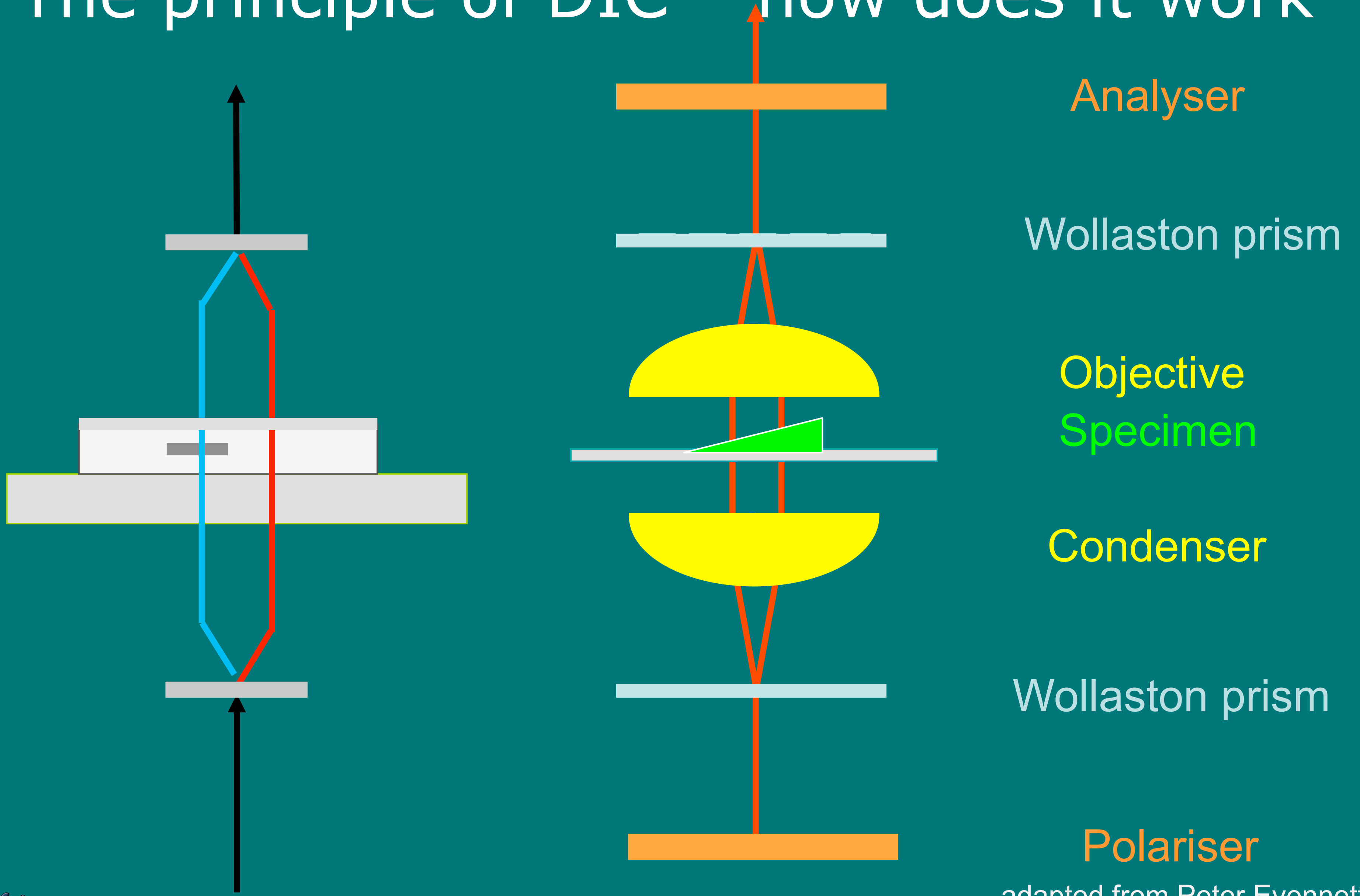

adapted from Peter Evennett

# The principle of DIC – how does it work




adapted from Peter Evennett

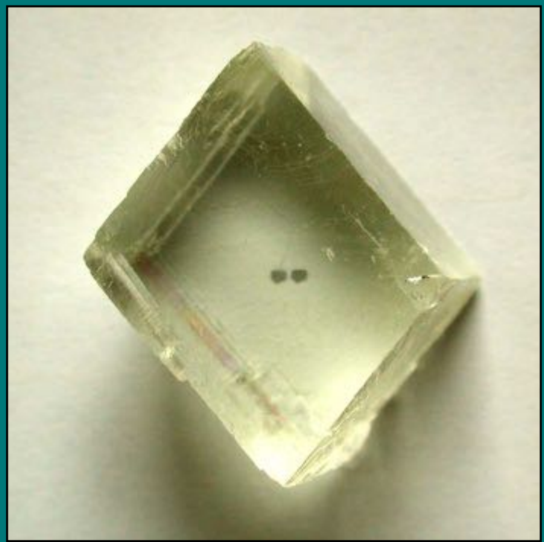
# The principle of DIC – how does it work




adapted from Peter Evennett

# The Wollaston prism – how does it work

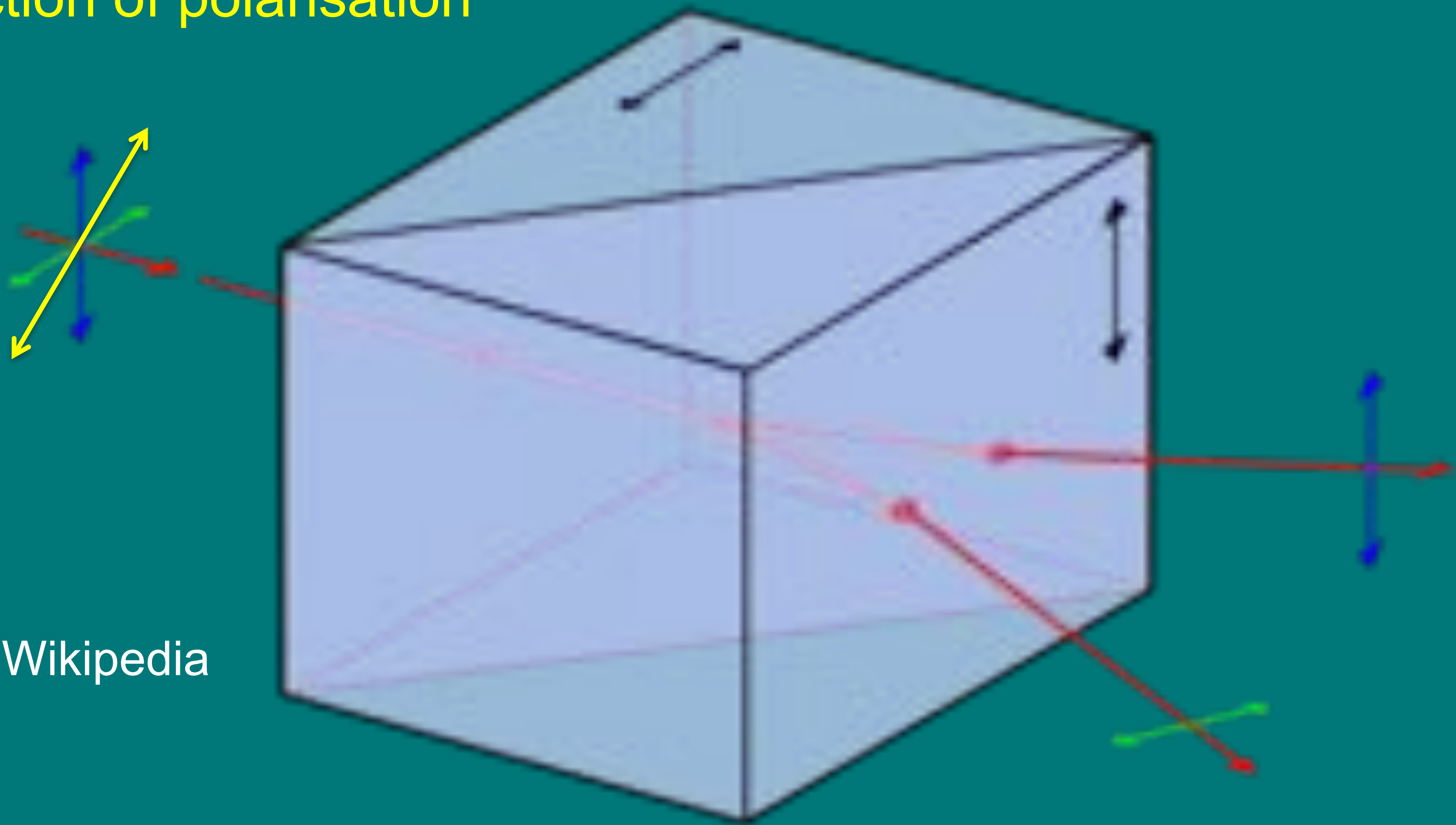
- Remember birefringence in a Calcite crystal:



- two spots out of one
- But: one looks to be further away (ie had a retardation introduced in its path)

# The Wollaston prism beam splitter – how does it work

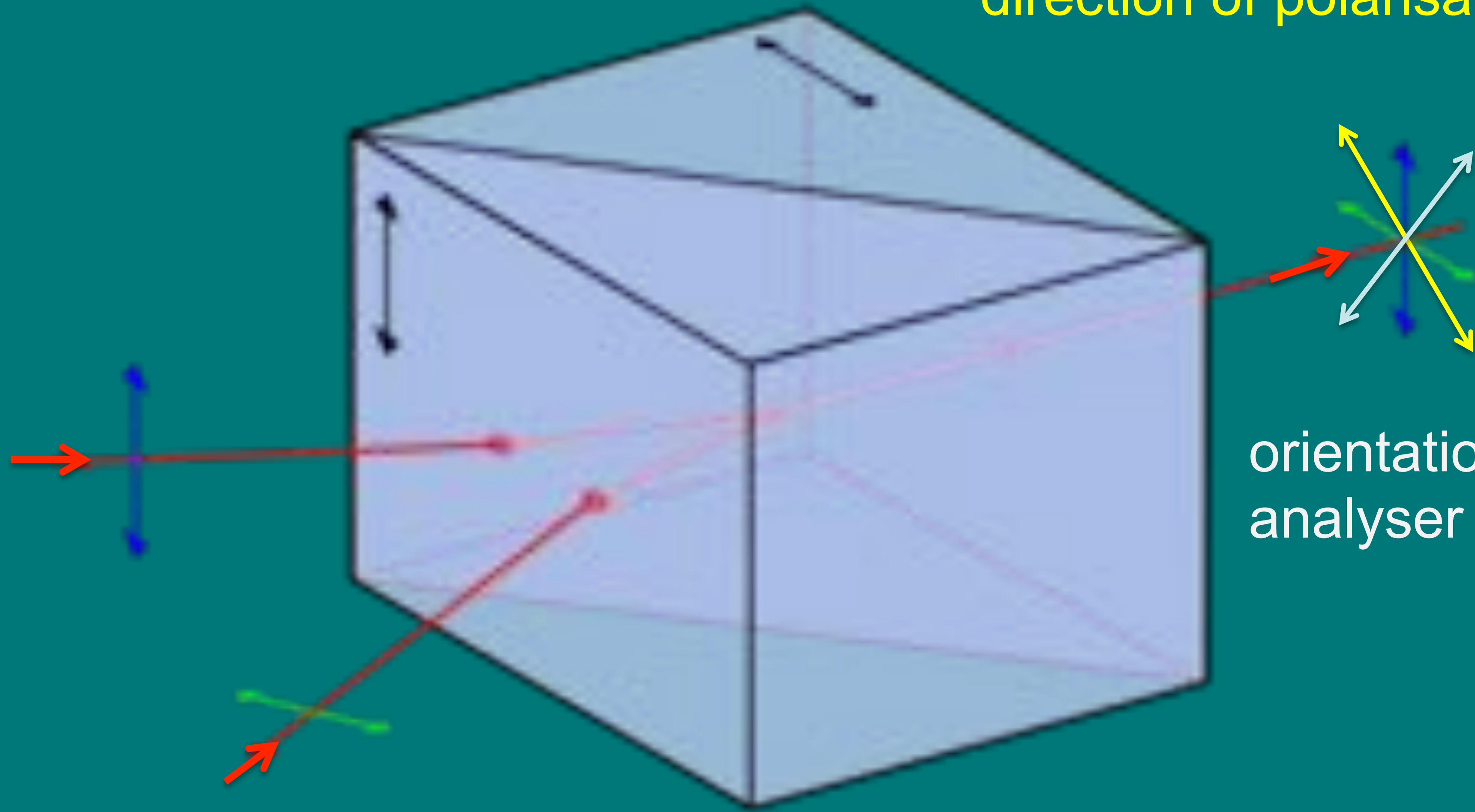
direction of polarisation



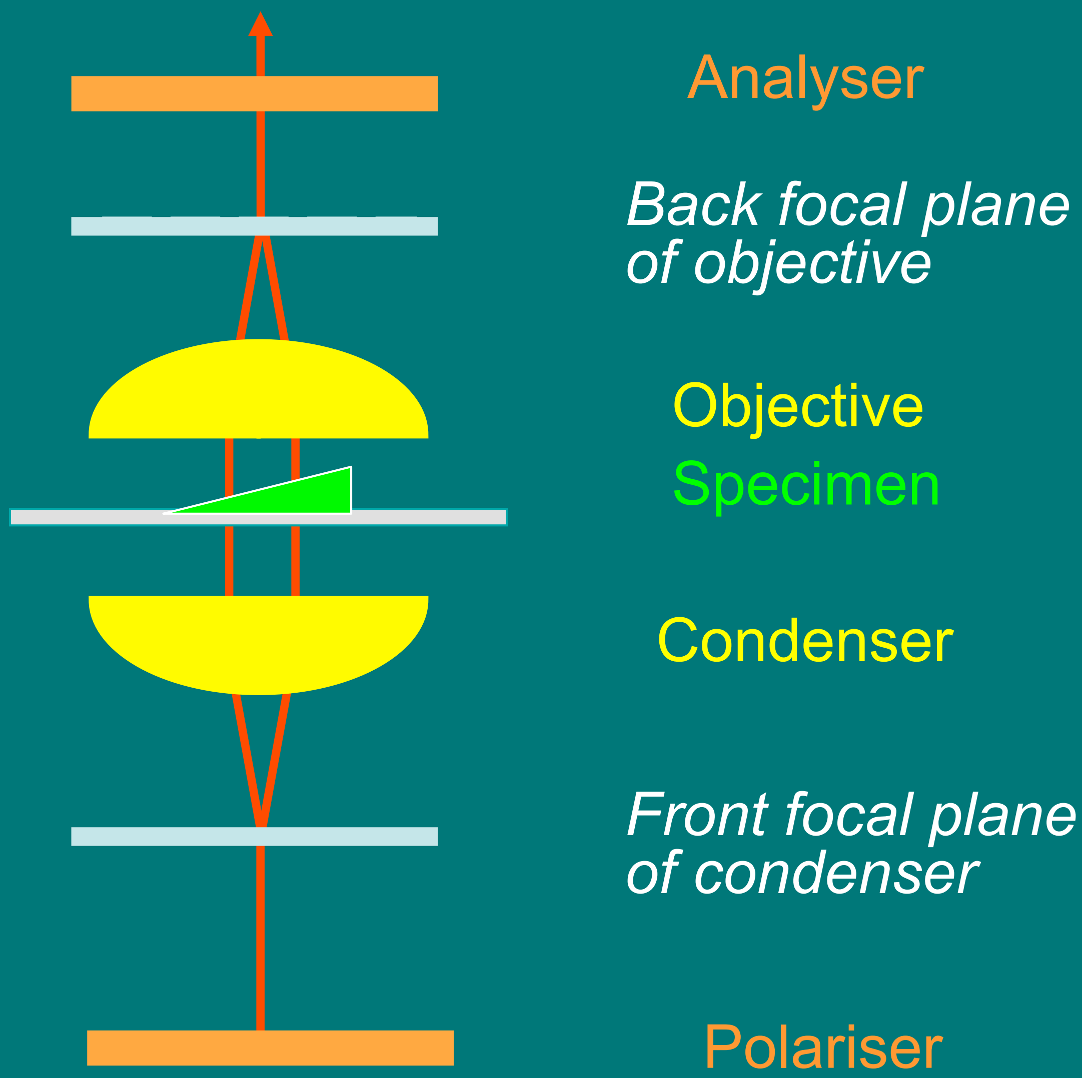
Wikipedia

Shear = spatial separation of the two beams

# The Wollaston prism – beam combiner

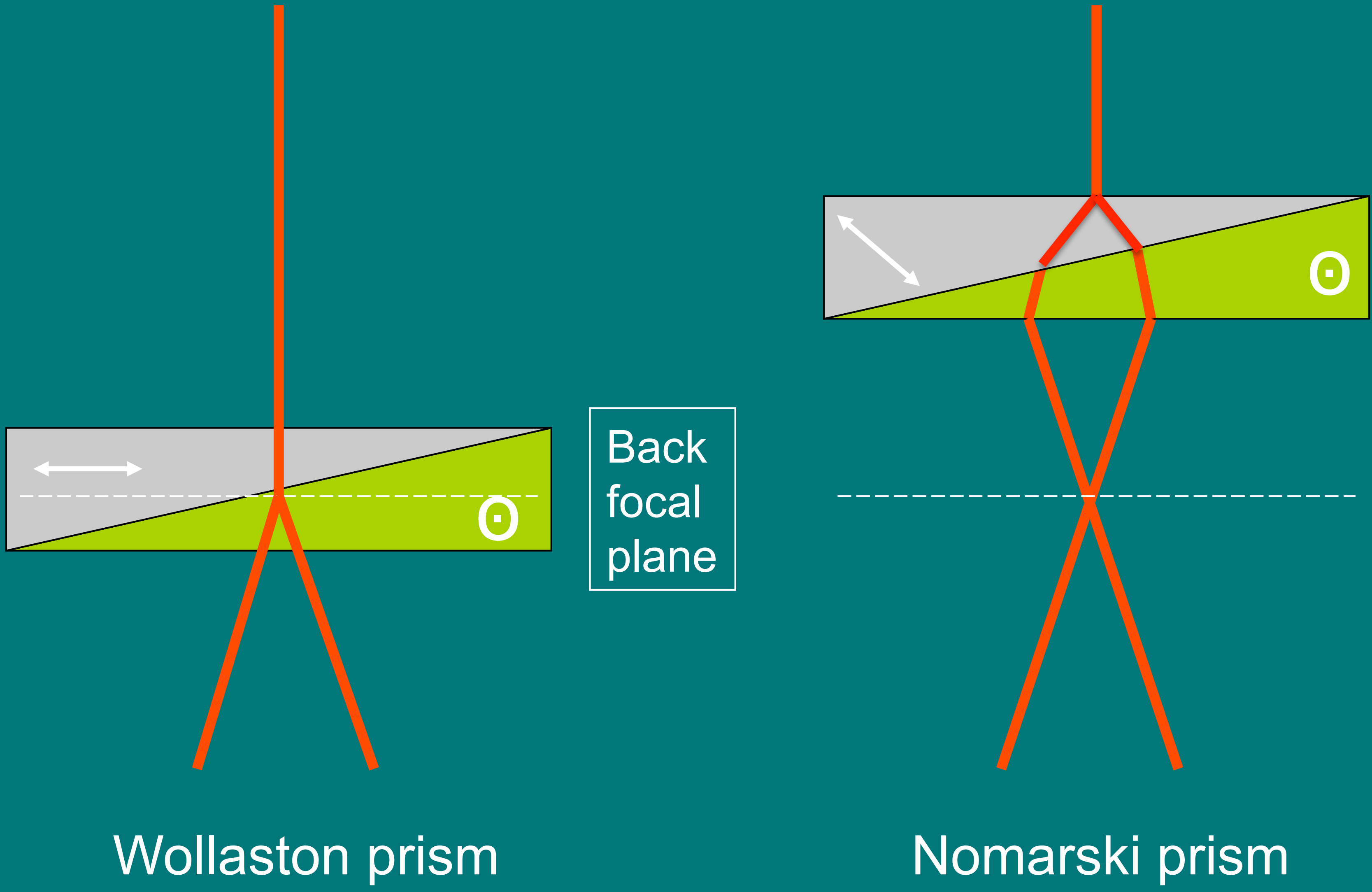


# Problem with objective Wollaston prism




adapted from Peter Evennett

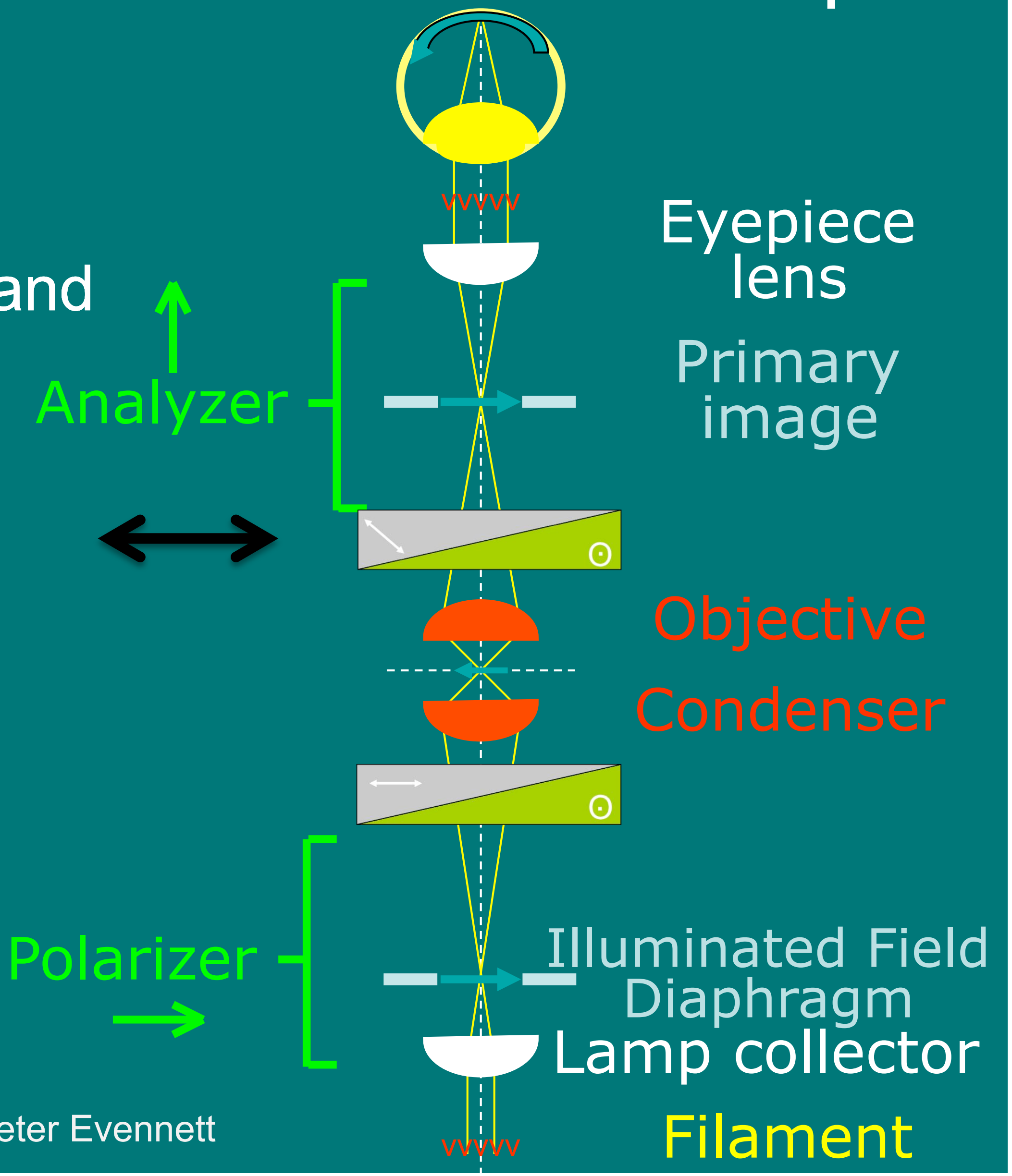
# Wollaston versus Nomarski-modified prism




adapted from Peter Evennett

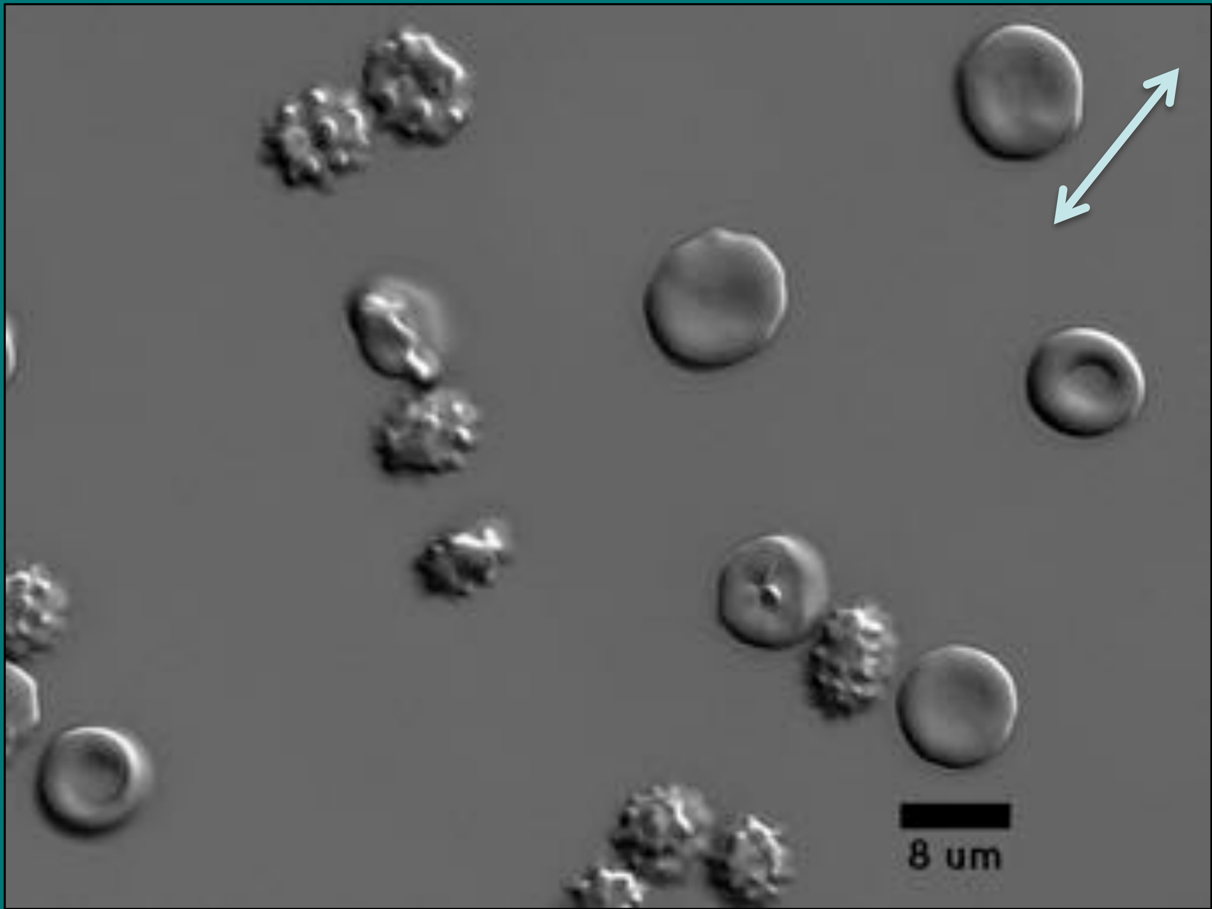
# The principle of DIC – how to set it up

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




adapted from Peter Evennett

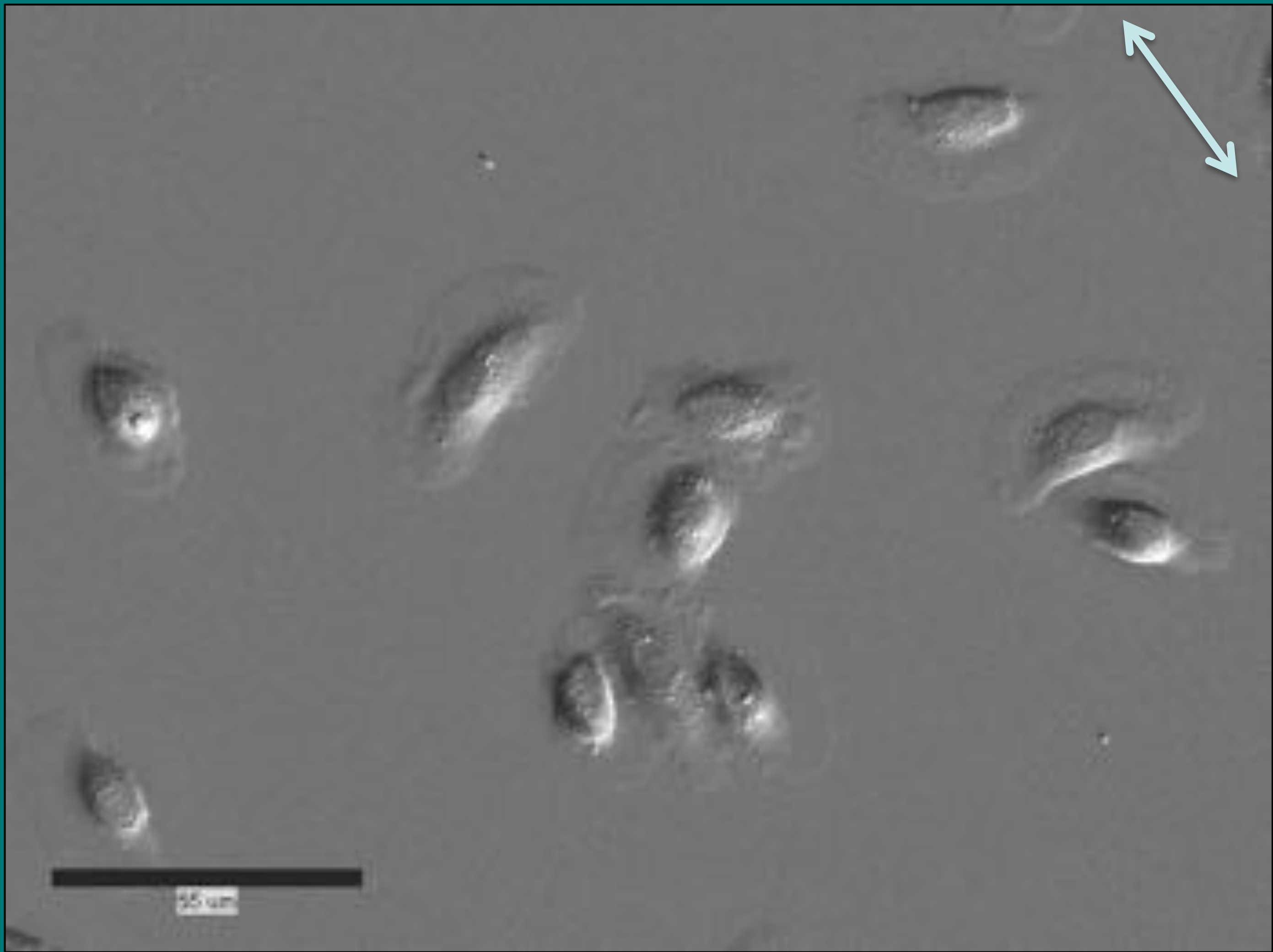
# Examples of DIC – what it is good for




axis of shear

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



axis of shear

Zebrafish keratocytes

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

Zeiss Axiovert 200M, 100x 1.4 oil DIC

# Examples of DIC – what it is good for

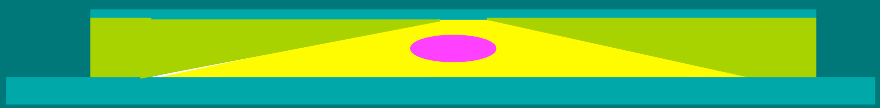
C. elegans embryos
Gunar Fabig, MTZ

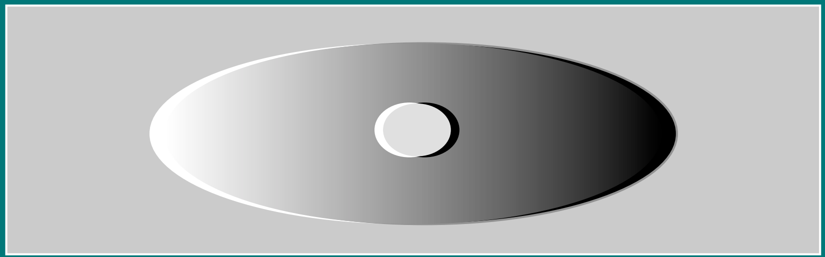
# Examples of DIC - summary

- Good for thin or thicker specimen
- labeled or unlabeled specimen

- Highlights **gradients** in optical path differences, not **absolute** optical path values

- For contrast in DIC shape of an object is more important than the absolute phase shift produced by the specimen



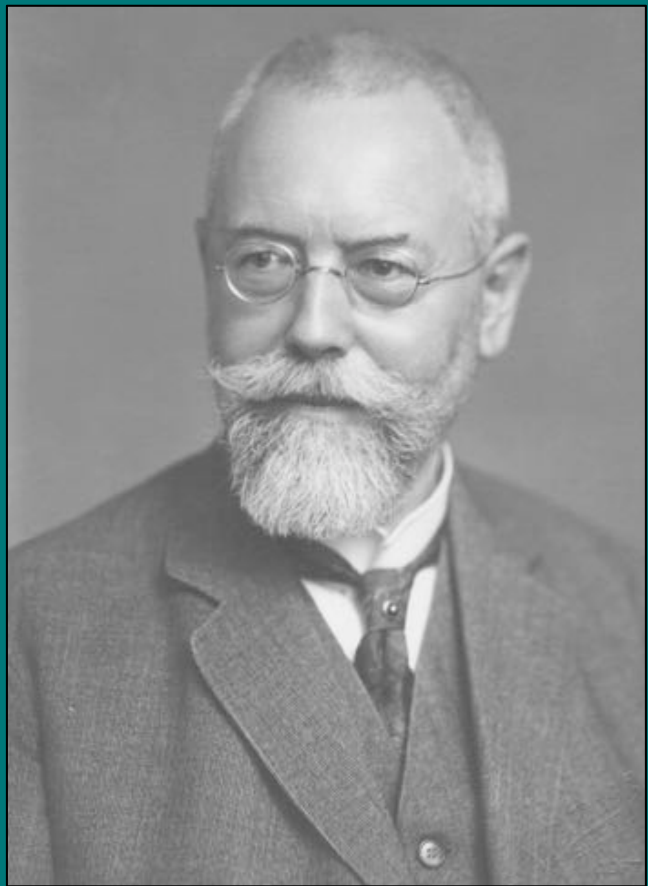


# Examples of DIC – limitations

- Samples must be placed in an non-bi-refringent environment (no plastic dishes, no plastic lids!)
- dependent on perfectly set up Koehler illumination and strain free optics
- some objectives are especially suited for polarization and DIC:
  - labeled with “DIC” or labels in red letters.
  - all other objectives still work too, but there might be quality problems
- contrast only achieved in direction of shear between the two beams!

# The principle of DIC – lets practice

1) Köhler your microscope carefully!



Wikipedia

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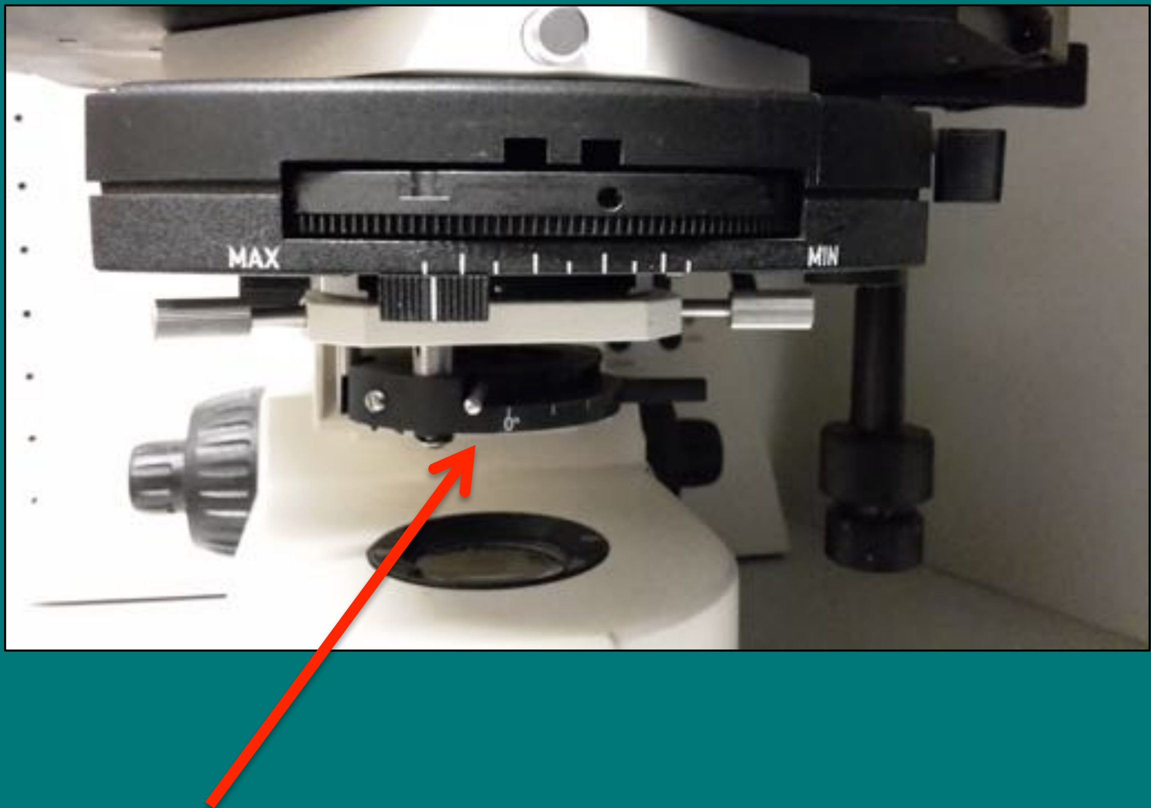
2) Insert and adjust polarizer and analyzer – crossed polars

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3) If microscope has a De Senarmont compensator, put it into its position of zero bias

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

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5) 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


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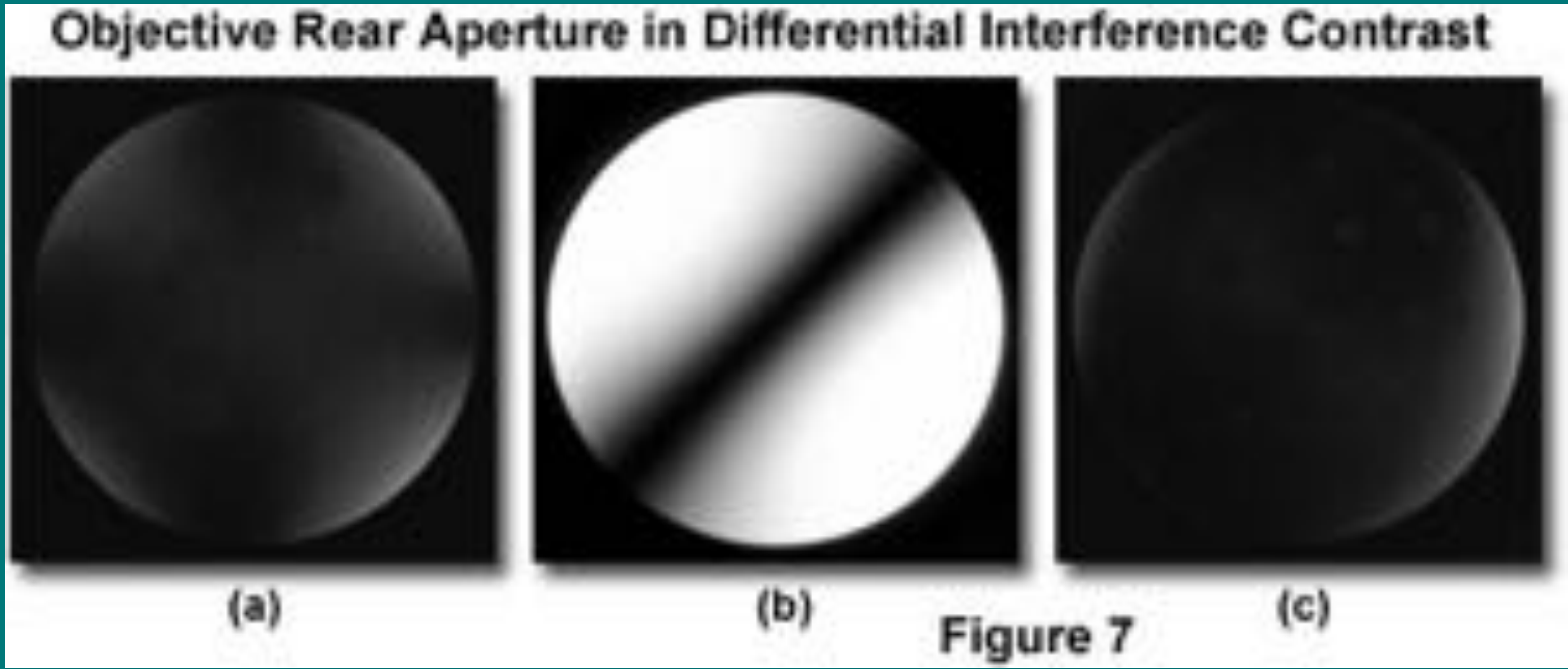
6) check BFP (using Betrand lens or telescope) for image of blurred cross or move objective prism into position were background is darkest (maximum extinction)




from olympusmicro.com

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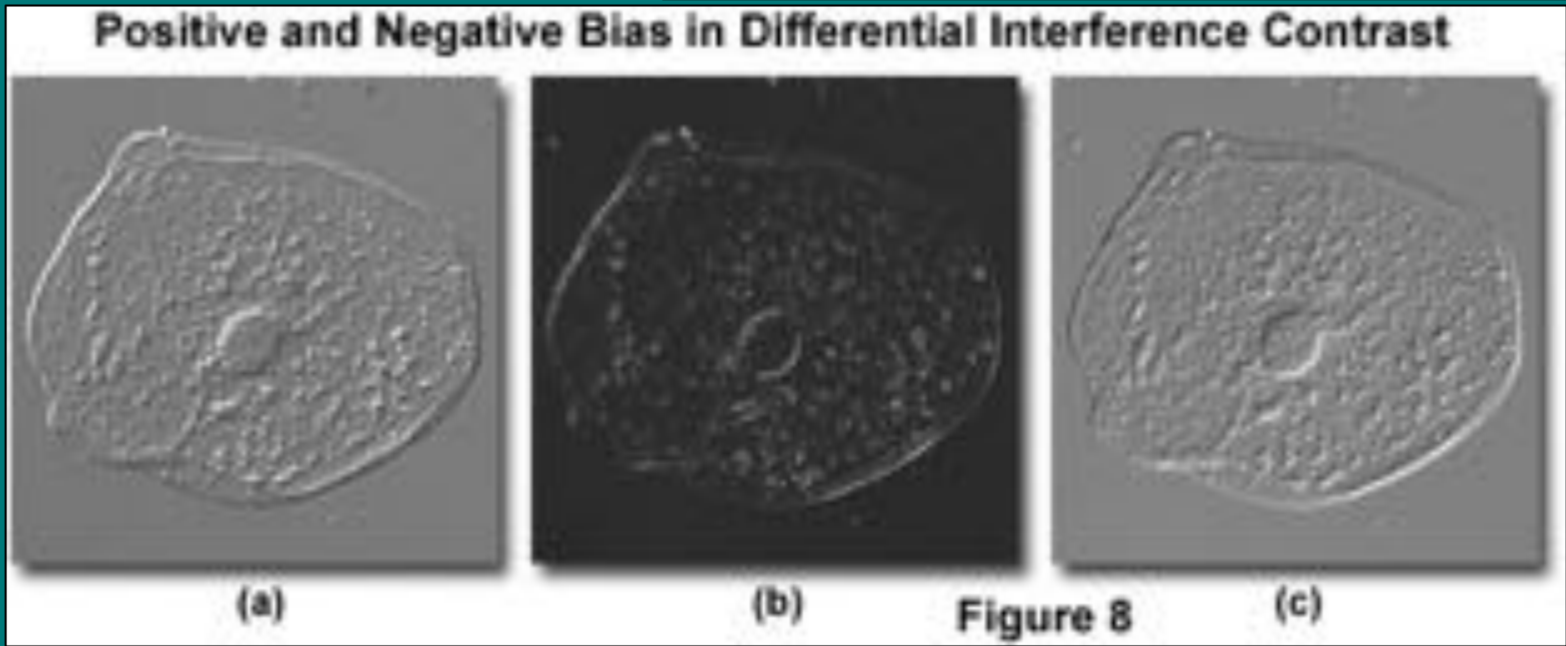
7) turn objective prism or compensator for optimal bias



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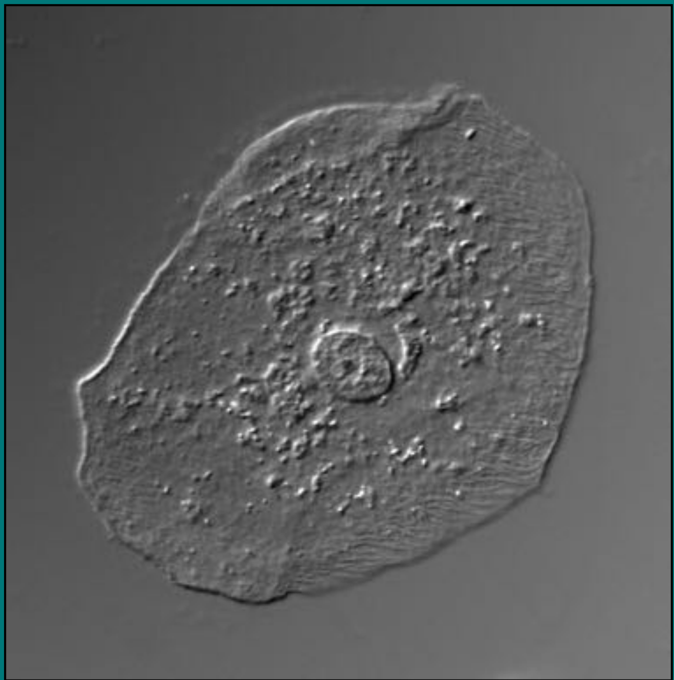
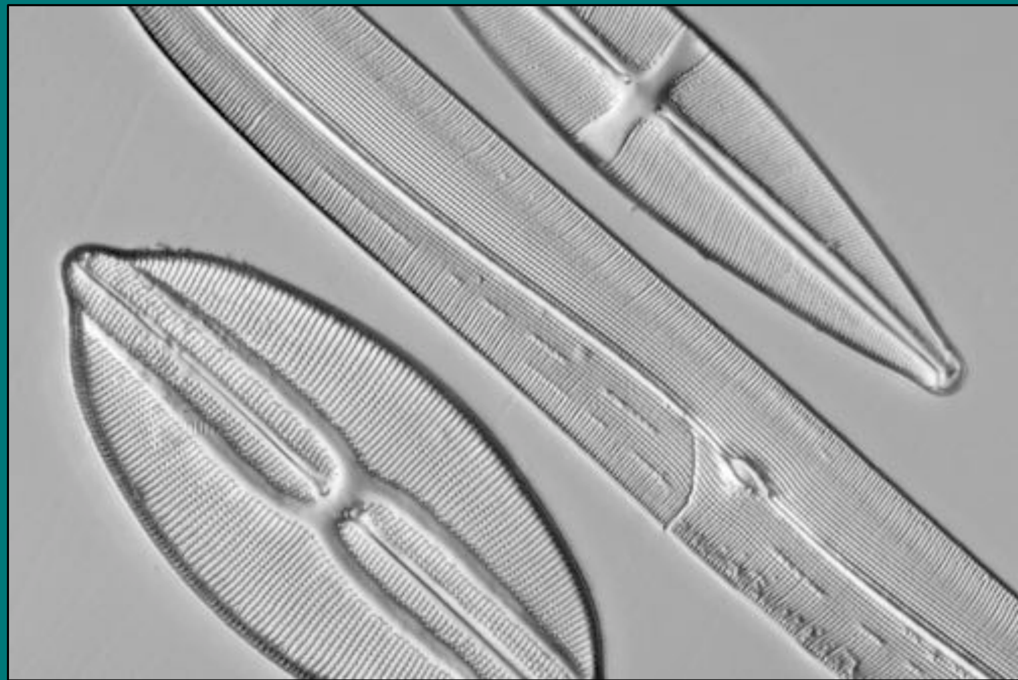
Use either cheek cells or diatoms





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