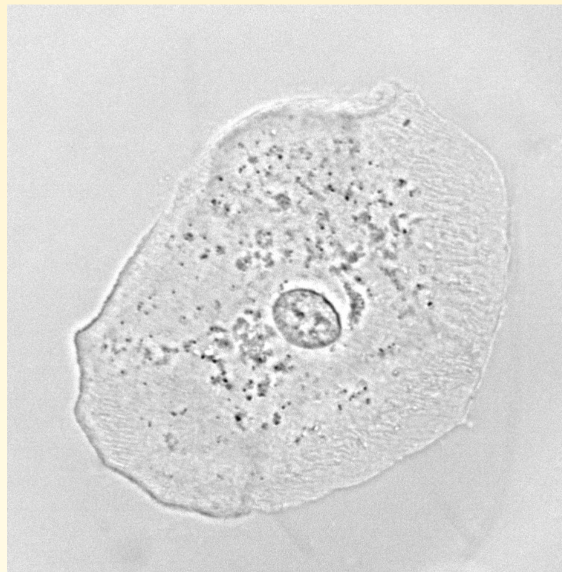
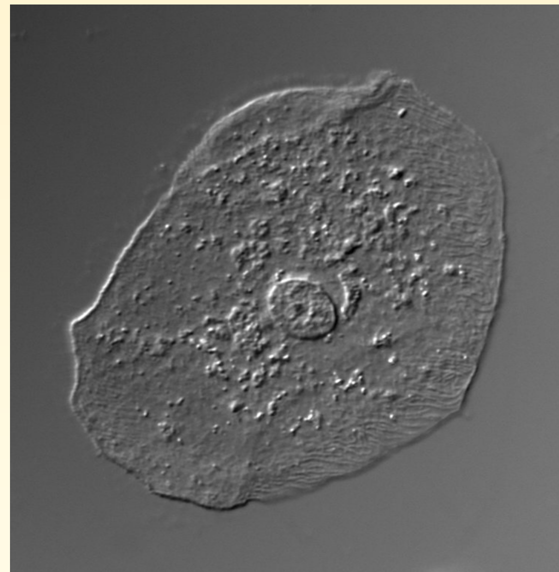
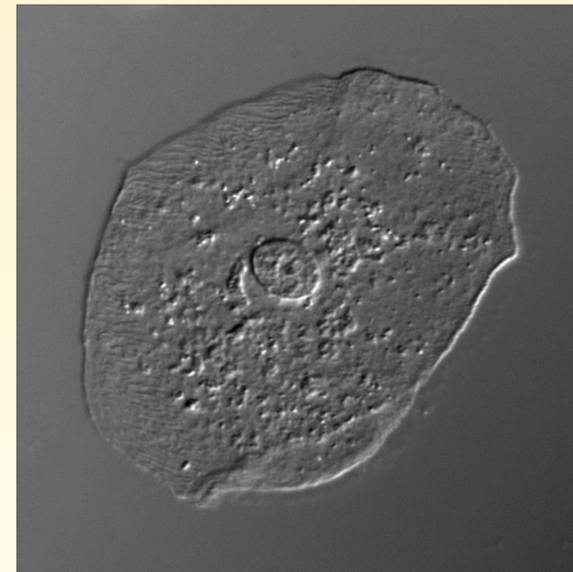
5. Differential Interference Contrast

Bright-field

Differential Interference Contrast (right: image turned)



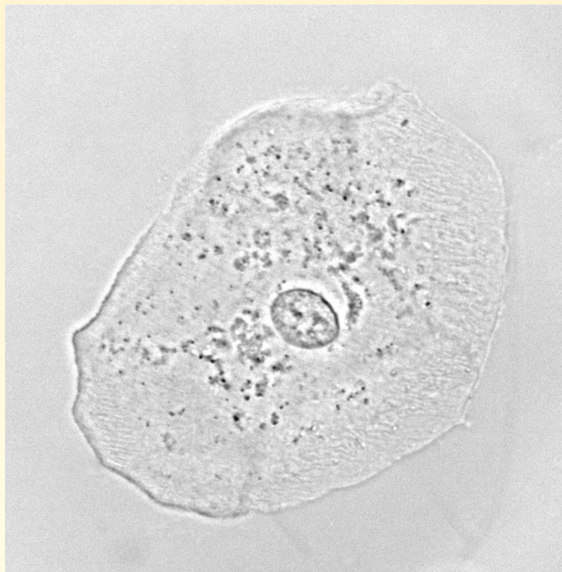




cheek cells, as prepared in the RegBioMed course

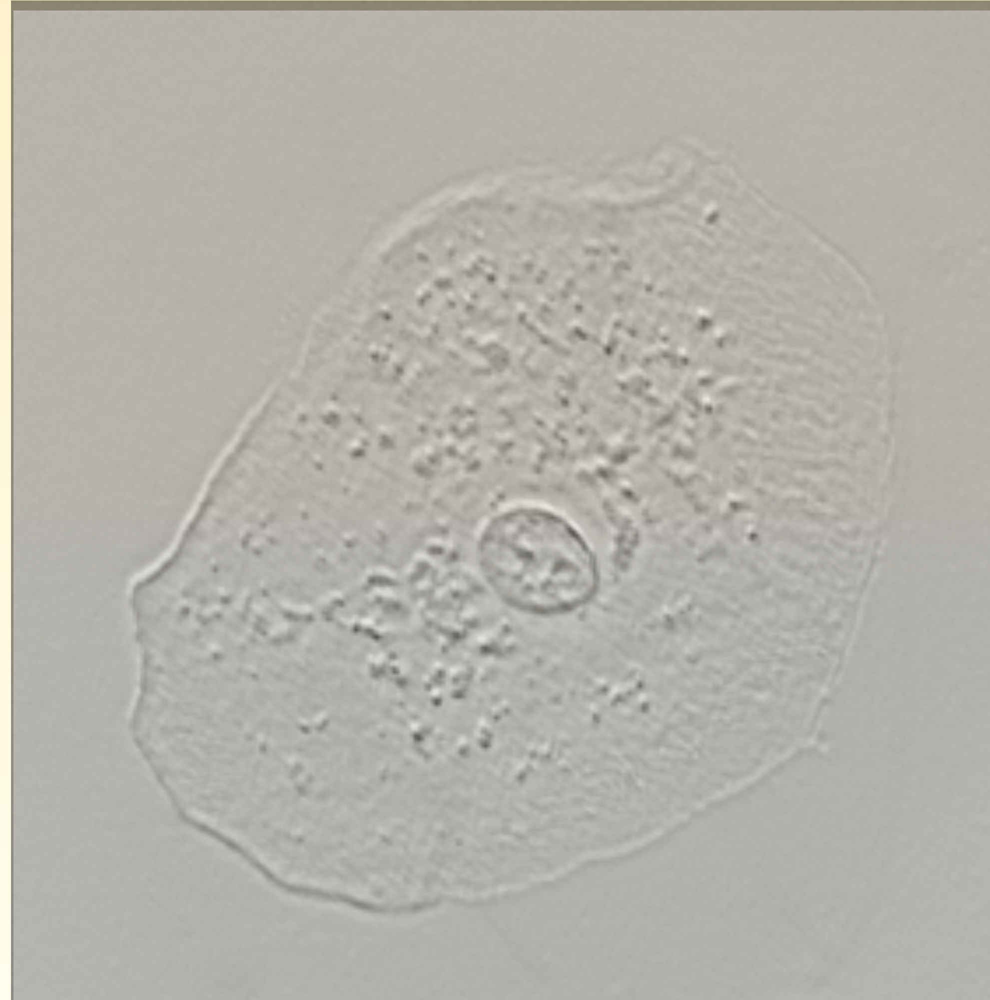
5. 3D-effect done in the computer

Bright-field



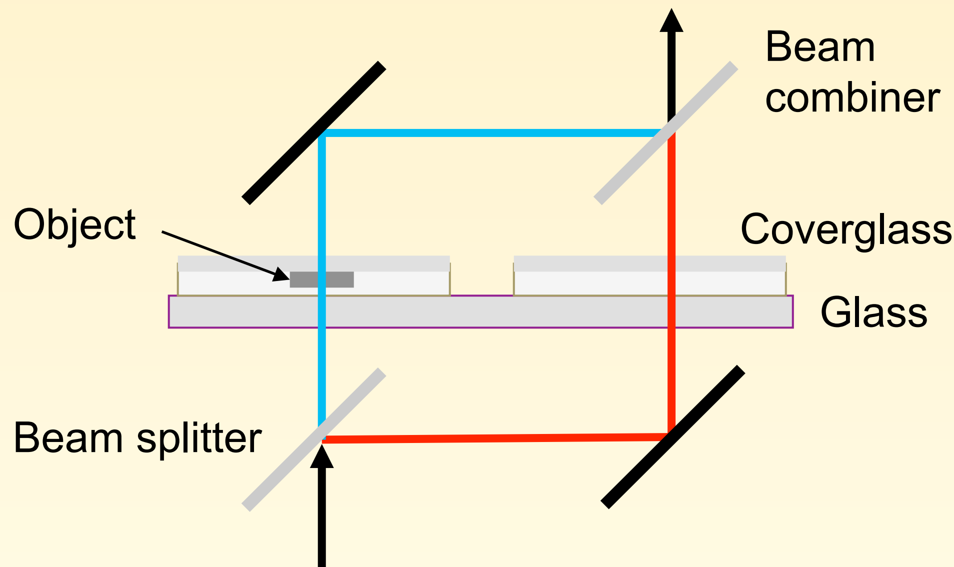
cheek cells, as prepared in the RegBioMed course

Bright-field – plus contrast inverted image - overlay

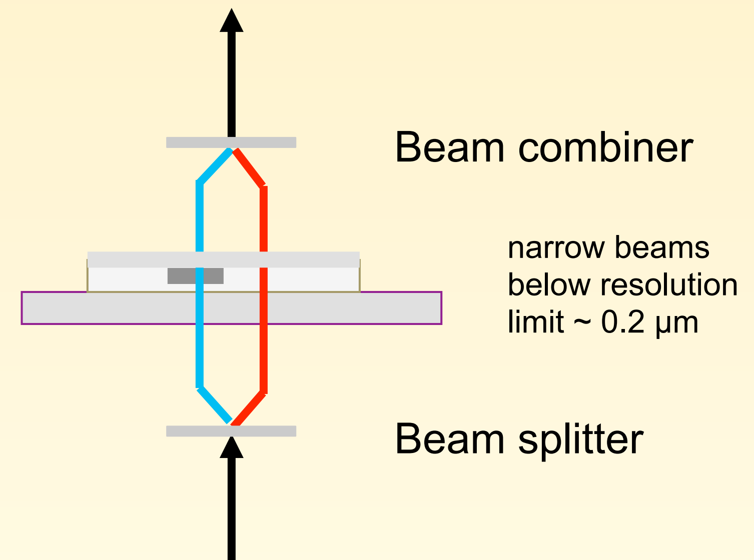


5. DIC - interferometry

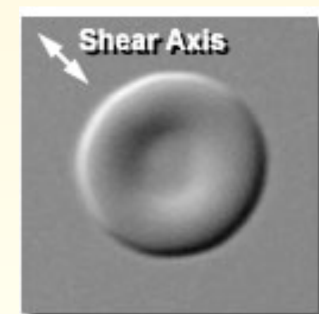
Classical double-path interferometer with a real reference sample



Used for DIC:
Common path interferometer, lateral shearing interferometer

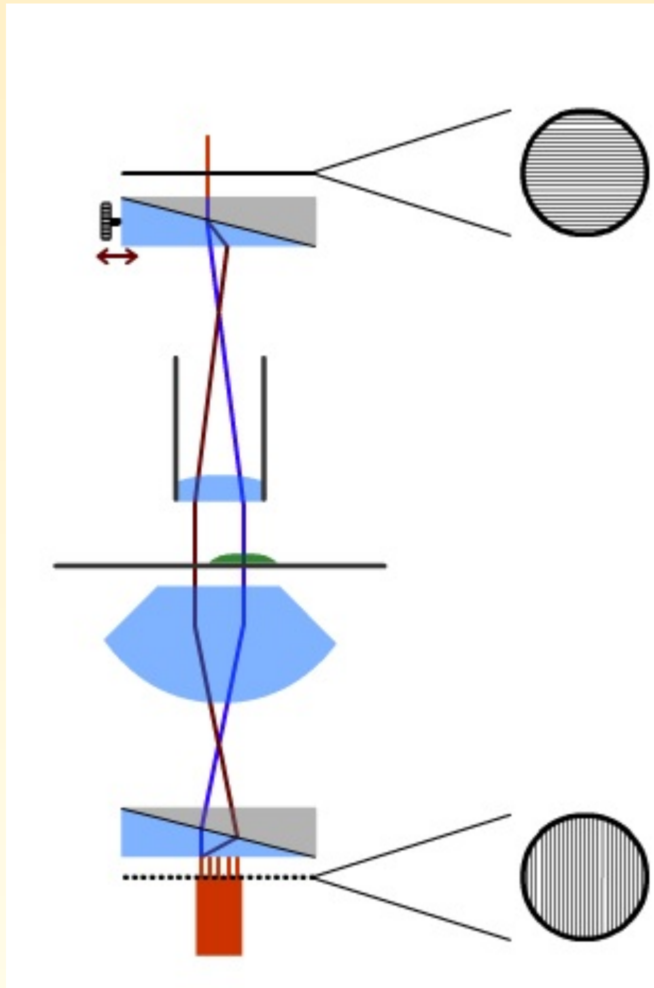


- superimposed waves interfere
- information about optical path difference (caused by Δx or Δn)
- DIC: contrast is proportional to the path length gradient (i.e. differential) along the shear direction (i.e. edge contrast)



http://www.olympusmicro.com/primer/techniques/dic/dicintro.html

5. DIC – optical setup overview



analyzer: beams interfere

objective prism reunites the beams

prism can be shifted horizontally to tune phase shift

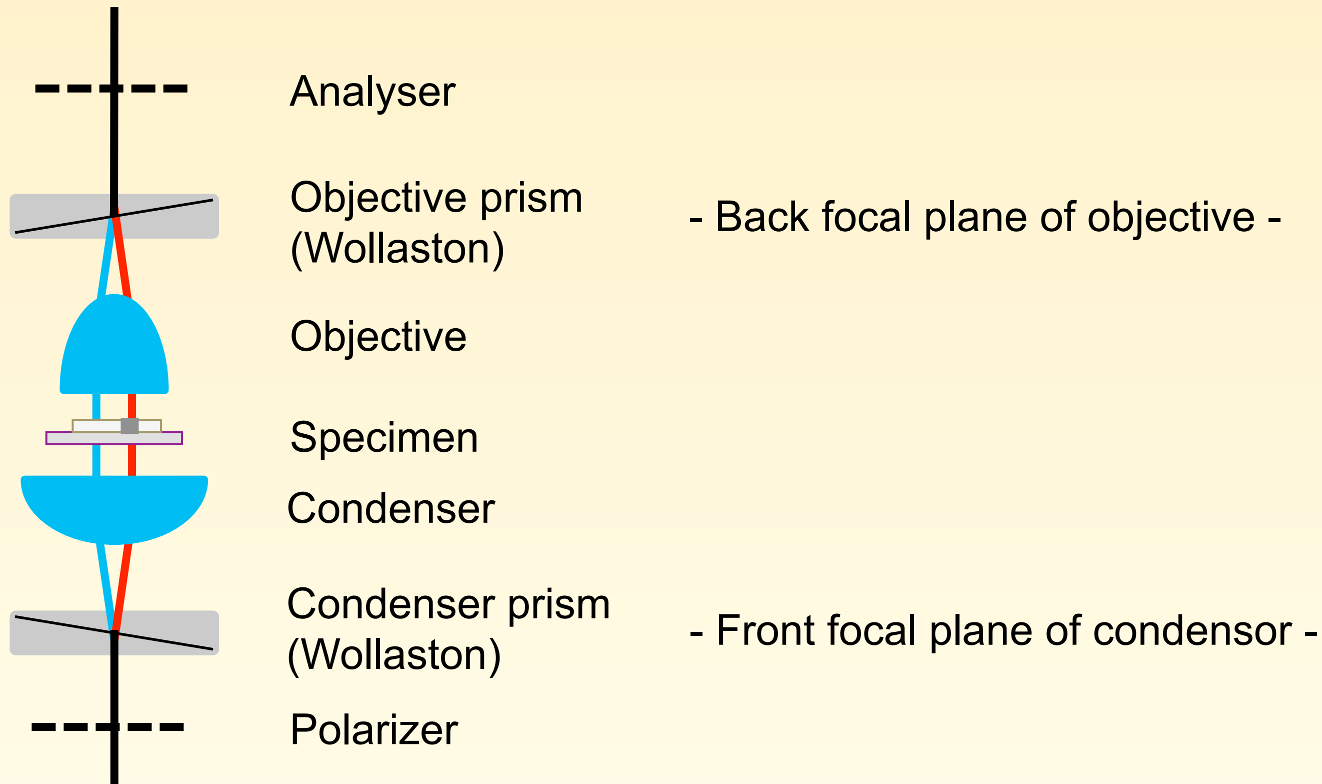
two narrow beams below resolution limit: phase shifts at edges of sample or structures

condenser prism: splits in two beams, mutually orthogonal linearly polarized

polarizer: creates linearly polarized light

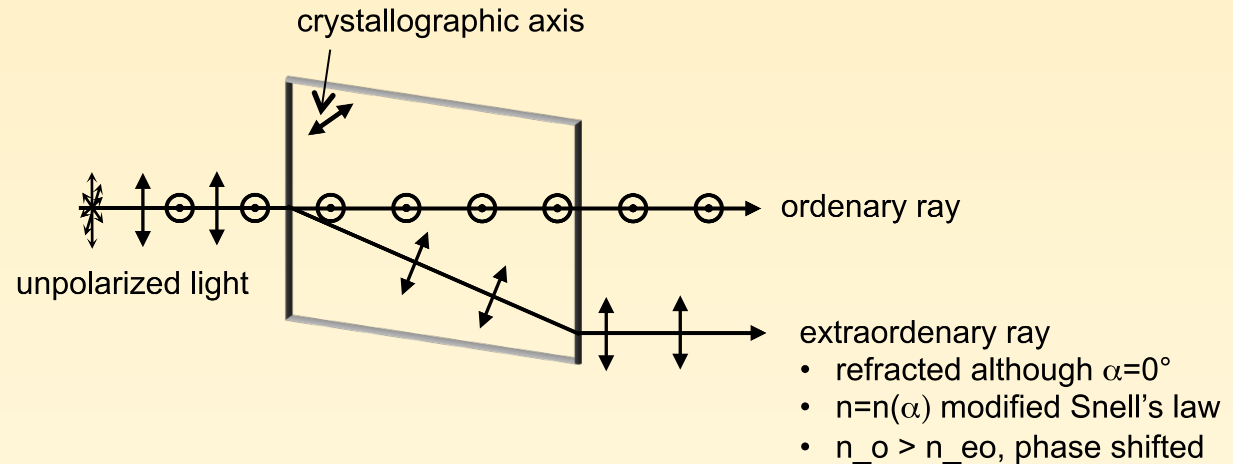
http://www.univie.ac.at/mikroskopie/2_kontraste/interferenz/2_prinzip.htm

5. DIC – beam splitting, where?

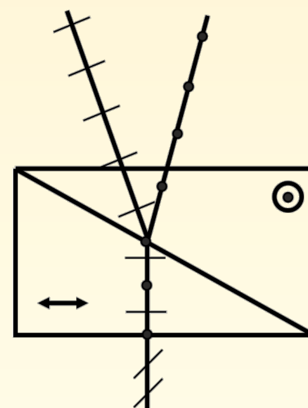


5. DIC – beam splitting, how?

Calcite ($CaCO_3$) crystal:
double refractive material



Condensor prism
(type Wollaston)



- made out of two glued calcite wedges
- perpendicular crystallographic axes
- axis of first prism 90° to beam direction
- 45° linearly polarized incoming beam

5. DIC – Smith vs. Nomarski

Interference microscope after ...

SMITH

NOMARSKI

Wollaston Prisms

Nomarski Prisms

Analyser

Back focal plane

special objective

normal objective

Specimen

Condenser

Front focal plane

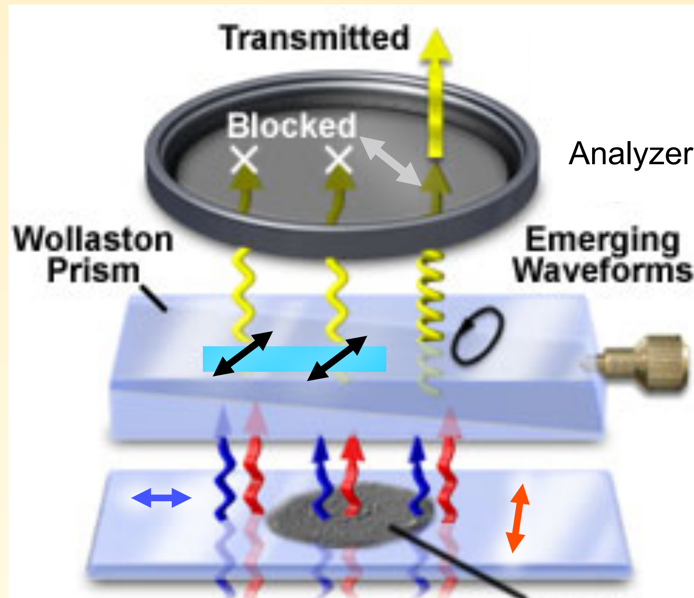
Polarizer

Nomarski modification:
crystallographic axis turned

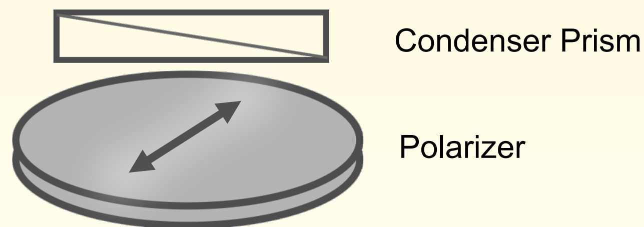
→ preferred configuration

5. DIC – phase shifts

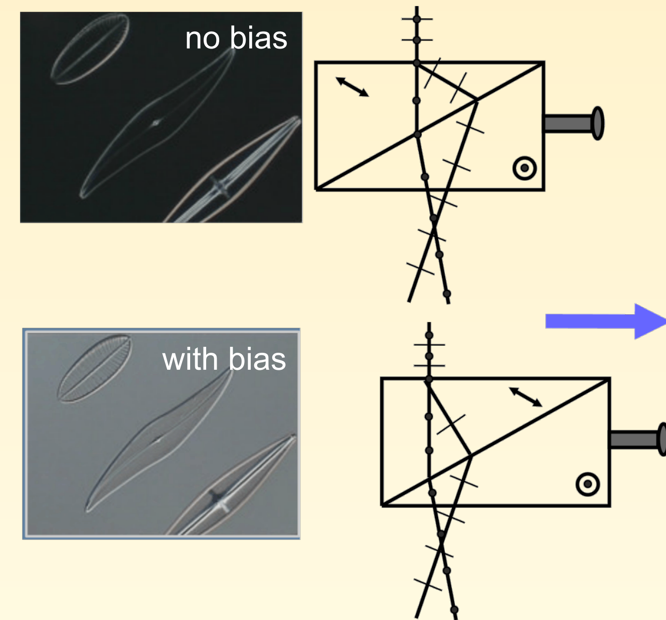
by the phase SAMPLE



phase specimen: phase shift between both beams



by BIAS retardation



- lateral shift of the objective prism (Nomarski)
- bias, i.e. offset phase shift, grey background
- features bright and dark on different sides

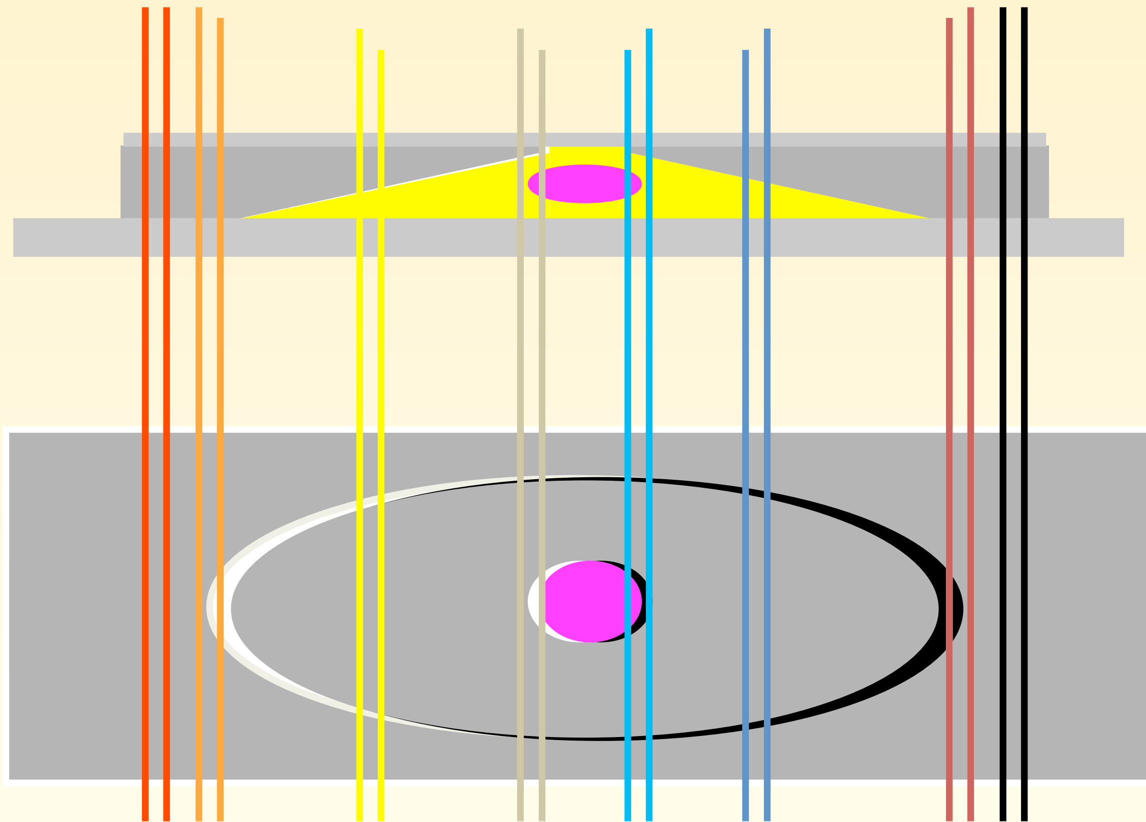
modified from:
http://www.olympusmicro.com/primer/techniques/dic/dicintro.html

modified from:
http://www.mikroskopie.de/pfad/pol_interferenz/acht.html

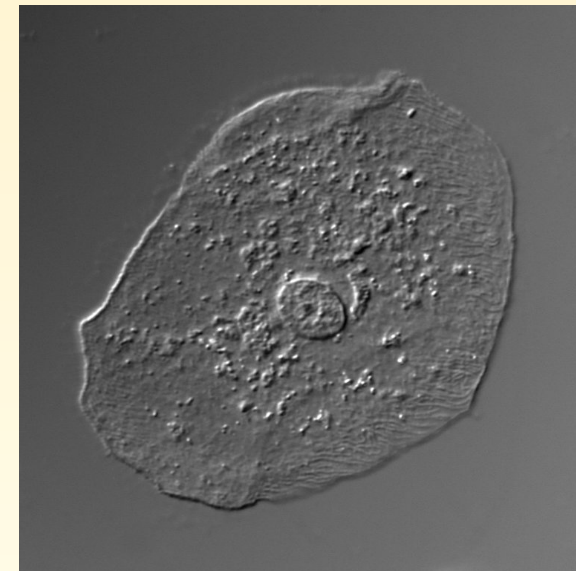
5. DIC – phase shifts in the sample

... for example a cell: phase shifts occur at:

- edges of the cell
- edges of subcellular components

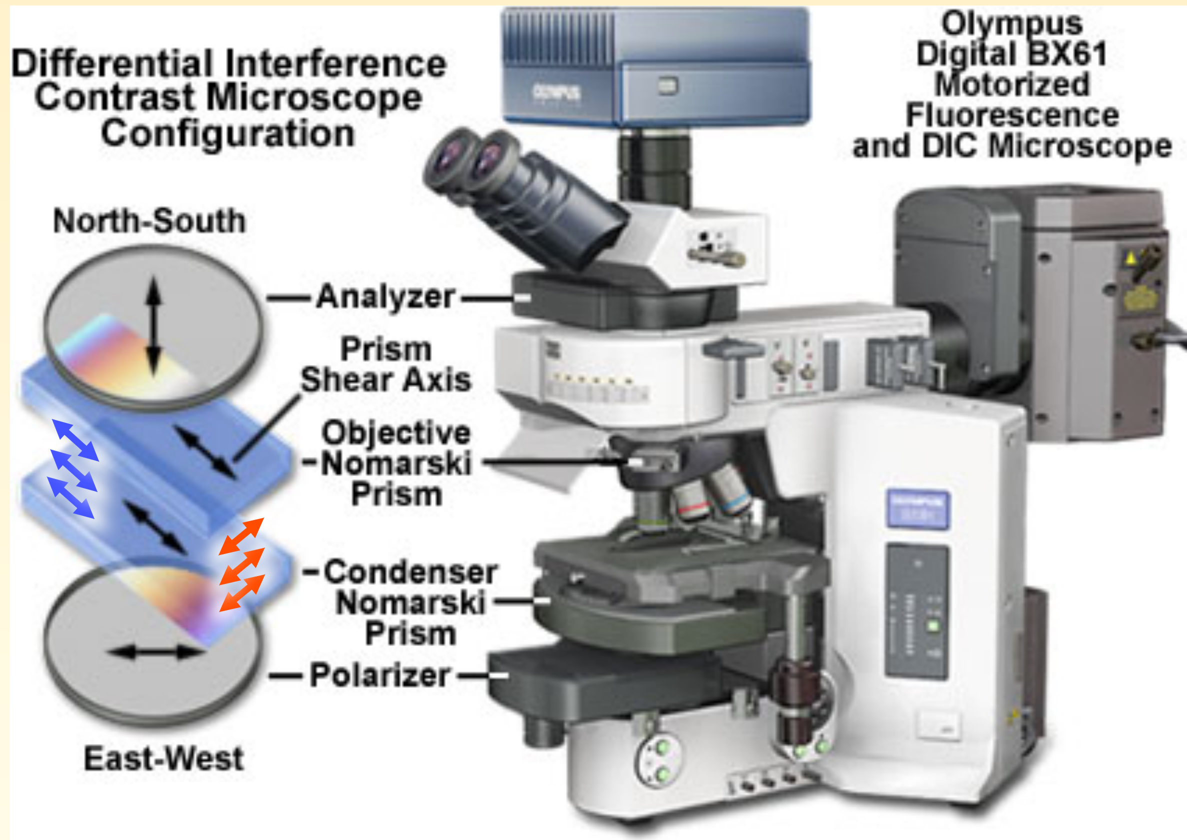


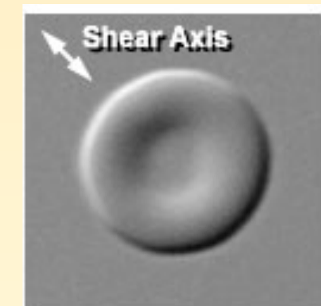
modified from © Peter Evennett

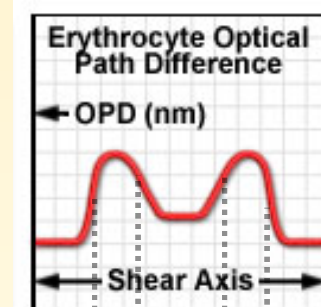


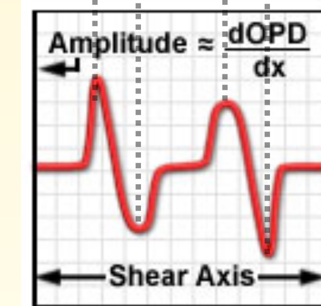
cheek cell

5. DIC – microscope setup









d OPD / dx is the differential of the optical path difference (thickness or Δn) along the shear axis x

modified from:
http://www.olympusmicro.com/primer/techniques/dic/dicintro.html

5. Differential Interference Contrast

Try yourself

Setup:

- Köhler the microscope
- adjust polarizer and analyzer – crossed polars (observe BFP as well)
- put in the correct condensor prism (observe BFP as well)
- put in correct objective prism, first separately, then both prisms
- tune the objective prism for the best DIC effect (best “shadows”, colors)

Specimen:

- diatomes (observe which substructures give strongest effect, then turn the sample)
- cheek cells

Additional information:

- no plastic dishes, as it is birefringent. Only use glass, or glass bottom dishes (no lid).
- some objectives are especially suited for polarisation and DIC. They are labeled with “DIC” or labels are in red letters. All other objectives still work.

DIC Example