From Eye to Insight

Leica MICROSYSTEMS

DIFFERENTIAL INTERFERENCE CONTRAST

Relief-like images with polarized light

1

Why do you need DIC?

> Flat and unstained cells are called phase objects that change the phase but not the amplitude of a light wave. They appear inconspicuous and depleted of details in brightfield microscopy.

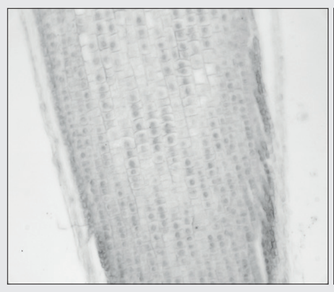
> Stained or naturally colored samples are called amplitude objects and affect the amplitude, but not the phase of light.

> DIC microscopy (by Georges Nomarski) uses refractive index induced gradients in the optical path length to make phase objects visible under the light microscope.

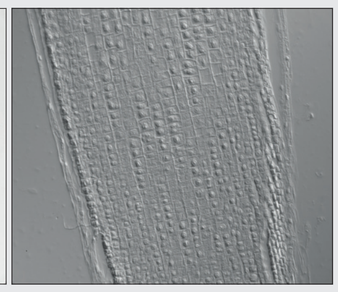
> To obtain the best possible illumination of the specimen, setting up proper Köhler illumination is mandatory!

> Do not use plastic in DIC microscopy! Many polymers are birefringent and would destroy the contrast. For plastic containers please use Integrated Modulation Contrast (IMC).

Brightfield



DIC



2

The wave character of light

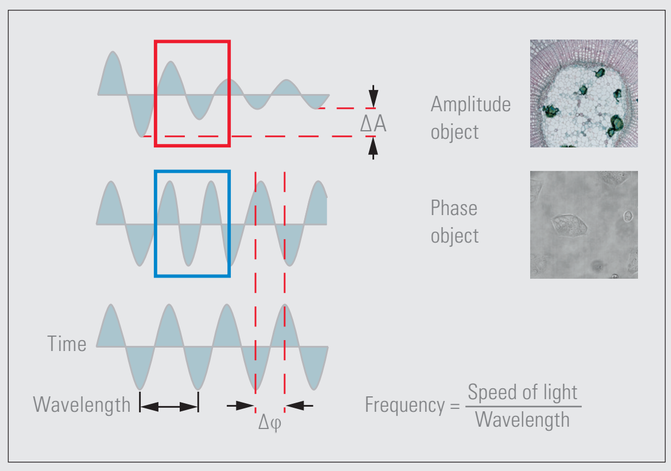
> The amplitude depicts the brightness. The frequency defines the color.

> Amplitude objects lower the amplitudes of passing light waves (ΔA). The human eye detects this as a loss of brightness.

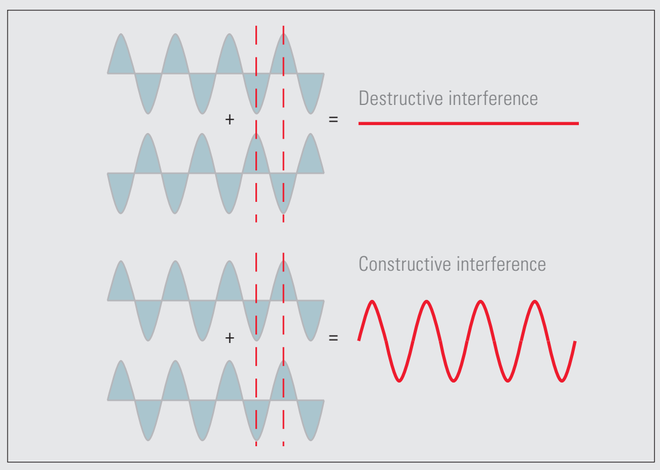
> Phase objects induce phase shifts of passing light waves (Δφ), which cannot be detected by the human eye.

> Interference describes the interaction of two waves with each other and the resulting formation of a new wave pattern following the principle of superposition.

Amplitude and phase objects



Interference



3

How does DIC work?

> The first polarizer (a) produces linearly polarized light (0°).

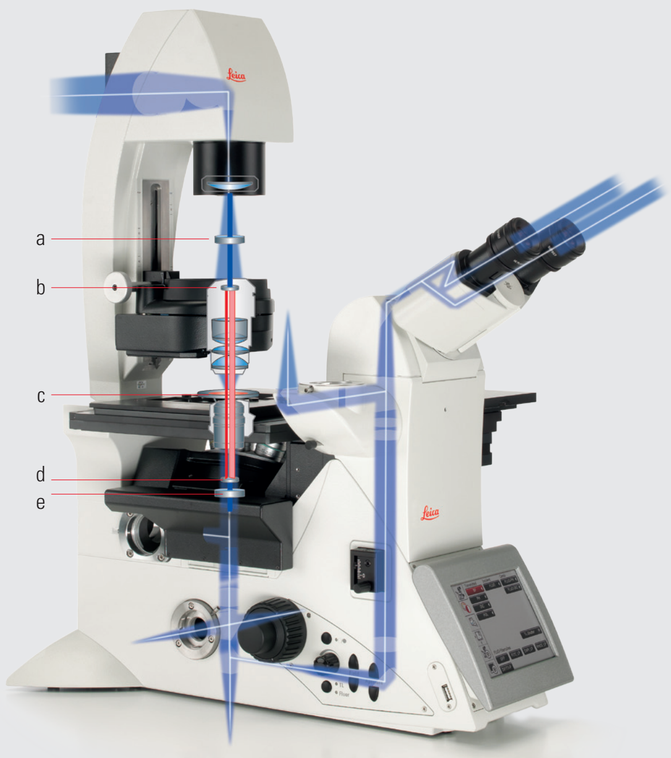
> The first Wollaston prism (b) disperses light into two light rays with a certain distance and orthogonal plane of polarization.

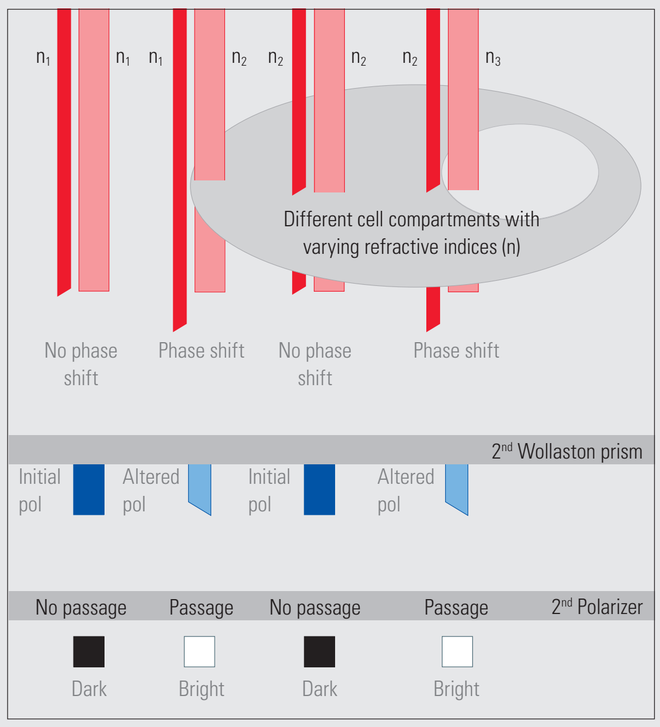
> When passing the specimen (c), the two light rays can experience differential refractive indices, resulting in a phase shift.

> The second Wollaston prism (d) reunites the two sister rays into one combined ray.

> Interference will occur, if there was a phase shift. In this case the resulting light is not polarized to 0° anymore.

> Only light that is not polarized to 0° passes the second polarizer (e). Thus the edges of phase objects appear bright and give a contrast to areas without phase shift.



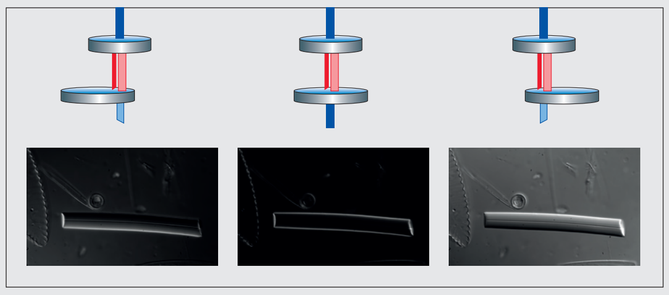


4

Bias retardation

> DIC can be modified by moving the second Wollaston prism laterally. This adds a phase shift to one of the sister rays, resulting in changes in the amplitude of the combined waveform.

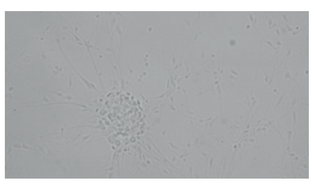
> The effect is a bright impression on one edge of the object and a dark impression on the other edge.



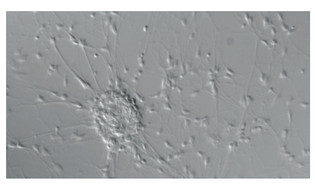
See also the interactive tutorial on Science Lab "Differential Interference Contrast – Step by Step Guide to Optimal DIC Setup":

www.leica-microsystems.com/science-lab/differential-interference-contrast

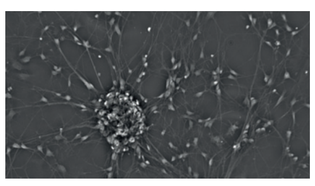
Brightfield



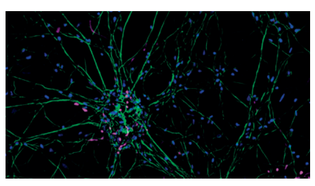
DIC



Phase contrast



Fluorescence



Science Lab

www.leica-microsystems.com/science-lab