Contrast – A Summary

If you can read this,
you are sitting in a
microscopy course

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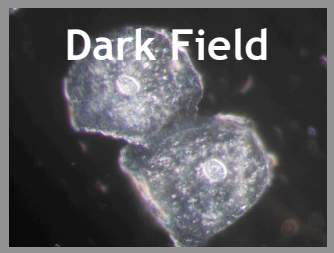
AND…

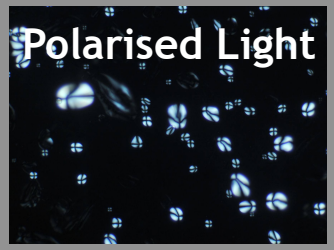
Resolution is still nothing without contrast

Principles of selected contrast enhancing techniques in a nutshell



Background of undiffracted light (zero order) removed to increase contrast

Phase shift converted into intensity information to generate contrast based on optical path length in sample

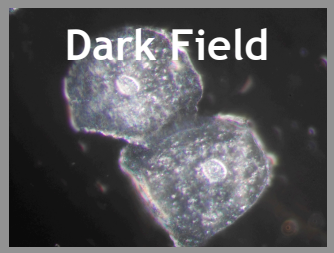


Material property, namely birefringence, made visible as intensity information

Interferometric imaging, which translates optical path gradients into intensity information

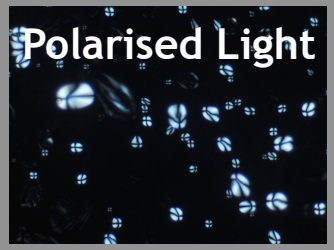
Images from Peter Evennett

Typical applications of selected contrast enhancing techniques



Blood cells, cilia/flagella, small scattering structures like microtubules or nanoparticles

Thin Samples with small phase shifts, e.g. flat monolayers of cells in culture, microoganisms, thin tissue sections



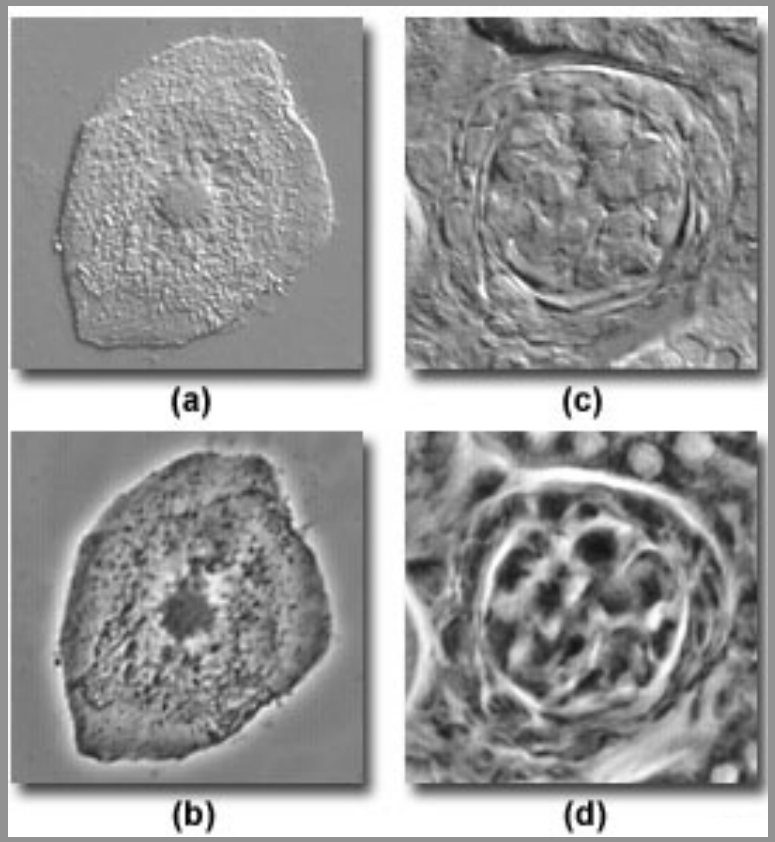
Fibrous structures like muscles, collagen or microtubule bundles

Thin and thick samples (also with large phase shifts), e.g. lamellipodia, c. elegans embryos, blood cells

Images from Peter Evennett

Phase Contast or DIC – that's the question

Buccal cell **Kidney tissue**



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

Phase Contast or DIC – that's the question

Characteristic	Phase Contrast	DIC
Image Brightness (Brightfield = 100 Percent)	1.3 Percent	0.36 - 2.3 Percent
Epi-Fluorescence Light Loss (Brightfield = 0 Percent)	28 Percent	73 Percent
Lateral Resolution	Condenser Annulus Restricted	Superior
Axial Resolution (Depth Discrimination)	Poor	Superior
Illuminating Aperture	10 Percent of Objective NA	Variable
Phase Shift Detection Limit	< λ/100	< λ/100
Utility at High Phase Shifts	Not Useful	Useful
Azimuthal Effects	No	Yes
Halos and Shade-Off	Yes	No
Stained Specimens	Not Useful	Useful
Birefringent Specimens	Useful	Not Useful
Birefringent Specimen Containers	Yes	No
Brightfield Image Deterioration	Slight	None
Cost	Moderate	High

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