Differential Interference Contrast (DIC) Versus Dark Field and Phase Contrast Microscopy

E. D. Salmon
University of North Carolina at Chapel Hill
How Does Contrast in DIC Differ from Phase and Pol?
General References

• Molecular Expressions, a Microscope Primer at: http://micro.magnet.fsu.edu/primer/index.html
DIC References

First

• Experiment described by Fritz Zernike in discussion of how he discovered Phase Contrast in 1930’s
c. Observations

Tiny carbon particles
Conclusions:

• 1. Image is formed by interference of direct (undiffracted) and diffracted (scattered) light.
• 2. Blocking diffracted light results in uniform illumination of image by direct light.
• 3. Blocking direct light results in darkfield image generated by interference of diffraction orders at image plane.
• 4. Darkfield image emphasizes higher spatial frequencies like those of edges, but does not accurately reproduce object because of absence of direct light.
• 5. Absorbing objects behave like transparent objects that make $\lambda/2$ retardation relative to direct light.
For Darkfield imaging, specimen is illuminated with a hollow cone of light with:
\[ \text{NA}_{\text{cond}} > \text{NA}_{\text{Obj}} \]

Objective can have iris diaphragm to limit \( \text{NA}_{\text{obj}} \) and prevent illuminating light from entering objective.
Stroboscopic Darkfield Imaging of Flagella Motility of Sea Urchin Sperm and Chlamydomonous
Phase Contrast Gives Contrast to Structural Detail in Transparent Specimens

Brightfield

Phase Contrast

\( (\text{NA}_{\text{obj}} = 1.4) \)
BASIC CONCEPTS

Objective

Specimen

Image Plane

Back Focal Plane

Undiffracted Illumination Light

Scattered (Diffracted) Light

Plane Wavefront

NAcond = 0
Summary:

Illuminating Wavefront

Specimen (thickness, t)

Wavefront just after specimen

Γ = t (n_{sp} - n_{m})

At Specimen

Undiffracted Light (U)

Specimen Light (S)

Diffracted Light = S - U

At Image Plane

Background
Undiffracted Light (U), Attenuated, Phase Advanced by \( \lambda/4 \)

Specimen Light = U + D

Diffracted Light (D)

EXAMPLES:

n_{sp} = 1.4  organelle
n_{m} = 1.36  cell cytosol
t = 1 micron
Γ = 0.04 = 550 nm/13

In Dark Phase Contrast
BASIC CONCEPTS

Image Plane

Undiffracted Illumination Light

Scattered (Diffracted) Light

Plane Wavefront

Phase Advance $\lambda/4$ and Attenuate 75%

Phase Plate

Back Focal Plane

Objective

Specimen

NAcond = 0
To Increase $N_{\text{cond}}$
illumination, Modern Phase Contrast Uses Annular Ring as Condenser Stop and Phase Ring in Objective Back Focal Plane
Depletion of hNuf2 from HeLa cells using siRNA.
10X Phase Contrast of HeLa Cells: Time-Lapse for 10 hours At 5 min intervals; 1/10 field, 1 of 25 fields recorded
Induce Anaphase in Early Prometaphase by Overcoming the Spindle Checkpoint

Example:
Mad2 Antibody Injection into Early Prometaphase Ptk1Cells

Julie Canman
Digitally Enhanced-DIC with Fluorescence In A Multi-Mode Microscope With CCD Detector
Cdc20 Persists At Kinetochores Throughout Mitosis and Exhibits

Green: GFP-Cdc20 At Kinetochores

Red: Phase Contrast Images of PtK1 Tissue Cells
A DIC Microscope is a Polarizing Microscope with Condenser an Objective DIC Prisms
Comparison of Phase Contrast to DIC for Cheek Cell
What Are 5 Major Features of A DIC Image?

High Resolution VE-DIC Image of Microtubules
What Are 6 Major Features of A DIC Image?

• Contrast is directional: maximum in one direction and minimum in the orthogonal direction
• Contrast highlights edges; uniform areas have brightness of background
• In direction of contrast, one edge is brighter, the other darker than the background
• Each point in object is represented by two overlapping Airy disks in the image, one brighter and one darker than background
• The Direction of Airy disk separation is the “Shear” direction and direction of maximum contrast
• Peak-to-Peak separation of Airy Disks is amount of Shear, typically ½ to 2/3 radius of Airy Disk
The DIC Microscope is a Dual-Beam Interferometer Made with Polarization Optics
The Condenser DIC Prism Splits Illumination Light into 2 Divergent Orthogonal Polarized Beams

Prism is Oriented with the Optic Axes at 45° to Polarizer. Why?
A Modified-Wollaston Prism is Used To Place Prism Above Objective or Below Condenser Diaphragm
Nikon’s New DIC System Uses One Birefringent Prism Combined With A Glass Wedge (courtesy of Mr. Toshimitsu)

DIC Prism

Two linearly polarized light components

Single quartz plate

Wallaston Prism

Nomarski Prism

4/05/06
Divergent Beams from Condenser Prism Pass through Specimen as Parallel Beams
Microscope Alignment For DIC

1. Achieve Koehler illumination
2. Align for Polarization Microscopy: Polarizer E-W, Analyzer Crossed
3. Rotate Condenser Turret to Select DIC Prism to Match Objective
4. Use Correct Objective DIC Prism
5. Add Bias Retardation to Brighten Image
6. Adjust Compensation for maximum Contrast of Specimen Detail of Interest
DIC Accessories for Transmitted Light

- Swing-Out Top Lens
- Universal Condenser
- Wollaston Prism Turret
- Polarizer
- Analyzer
- Wollaston Prisms
- DIC Slider
- Oil Immersion Top Lens

Figure 4
Microscope Alignment for DIC

Objective
Back Aperture:
Full Aperture Illumination
Poor Condenser Illumination

Objective
Back Aperture:
1/3 Aperture
Illumination
Miss-Matched Prisms, or a Missing DIC Prism

Extinction Fringe Not Spread Across Aperture; This is View When Objective or Condenser prism Removed
Matched DIC Prisms; Full Objective Aperture Illumination

Extinction Fringe Spread Across Aperture
Image Intensity for Test Specimen With No Compensation
Image
Intensity for Test Specimen With Plus Compensation
Image Intensity for Test Specimen With Minus Compensation
Comparison of DIC Image Intensity for Test Specimen With No, Plus and Minus Compensation
Two Types of Compensation Are Used For DIC Microscopes

- **SMITH DIC**
  - Rotate polarizer for compensation

- **NOMARSKI DIC**
  - Translate objective Wollaston for compensation

**COLOR:** Insert red plate just above polarizer or just below analyzer oriented at 45 degrees to polarizer azimuth for either Smith or Nomarski systems
How Intensity Changes With Compensation

\[ I_{sp} = I_c + I_p \sin^2\left(\frac{(\Delta_{comp} + \Delta_{sp})}{2}\right) \quad I_{bg} = I_c + I_p \sin^2\left(\frac{\Delta_{comp}}{2}\right) \]
For Maximum Contrast:
1) Adjust Compensation So One Edge of Specimen Detail is Near Extinction; or
2) Use About $\lambda/10 - \lambda/20$ for Video/Digital-Enhanced Contrast
Why is shear chosen to be 0.5 to 0.6 of radius of Airy Disk?

• The Abbe limit of resolution is:
  \[ r = \frac{\lambda_o}{(NA_{obj} + NA_{cond})} = 0.5 \frac{\lambda_o}{NA_{obj}} \]
  when \( NA_{cond} = NA_{obj} \)

• For \( NA_{obj} = 1.4 \) and \( \lambda_o = 550 \text{ nm} \):
  \[ r = 190 \text{ nm} \]

• This resolution limit corresponds to a maximal resolvable spatial frequency:
  \[ fs_{max} = \frac{1}{r} = 5.1 \text{ cycles/\mu m} \]

• A shear of \( \sim r/2 \) will give the maximum retardation (and contrast) between the e and o wavefronts at \( fs_{max} \); let’s see how…….
Use Cheek Cells for Contrast and Resolution Test

A. 20X/NA = .45 Objective
B. 100X/NA = 1.4 DIC Objective
Example DIC: Mitosis in Mitotic Newt Lung Cells
Digitally Enhanced-DIC with Fluorescence In A Multi-Mode Microscope With CCD Detector
Fluorescent Images of 200nm bead:
100x/NA=1.4, detector pixel scale = .065 nm

No DIC Prism
Peak = 3650

With DIC Prism
Peak = 2710 (75%)
Pearson et al., 2001, JCB
Video-Enhanced Contrast Methods
Developed in Early 1980’s by Inoue and Allen Revealed Cellular Structures and Macromolecular Complexes Invisible by Eye or Film
Practical Example: VE-DIC of Isolated Microtubules

View by eye

Analog Contrast Enhancement Live Image

De-focus Slightly; Acquire Background Image and Store into Frame Buffer

Subtract Background from Live Image at Video Rates

Increase Contrast Digitally
Preparations for Motility Assays

Slide

- #1.5 Coverslip
- 70mm Thick Double-Stick Tape
- Perfusion Chamber
VE-DIC Microtubule Motility Assay for Minus-Kinesin ncd

ncd driven microtubule translocation and rotation

(3.3 rotations/um forward movement)
Color DIC with Full Wave (RED) Plate

Transmitted and Reflected DIC Photomicrographs

(a)  

Figure 3  

(b)