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

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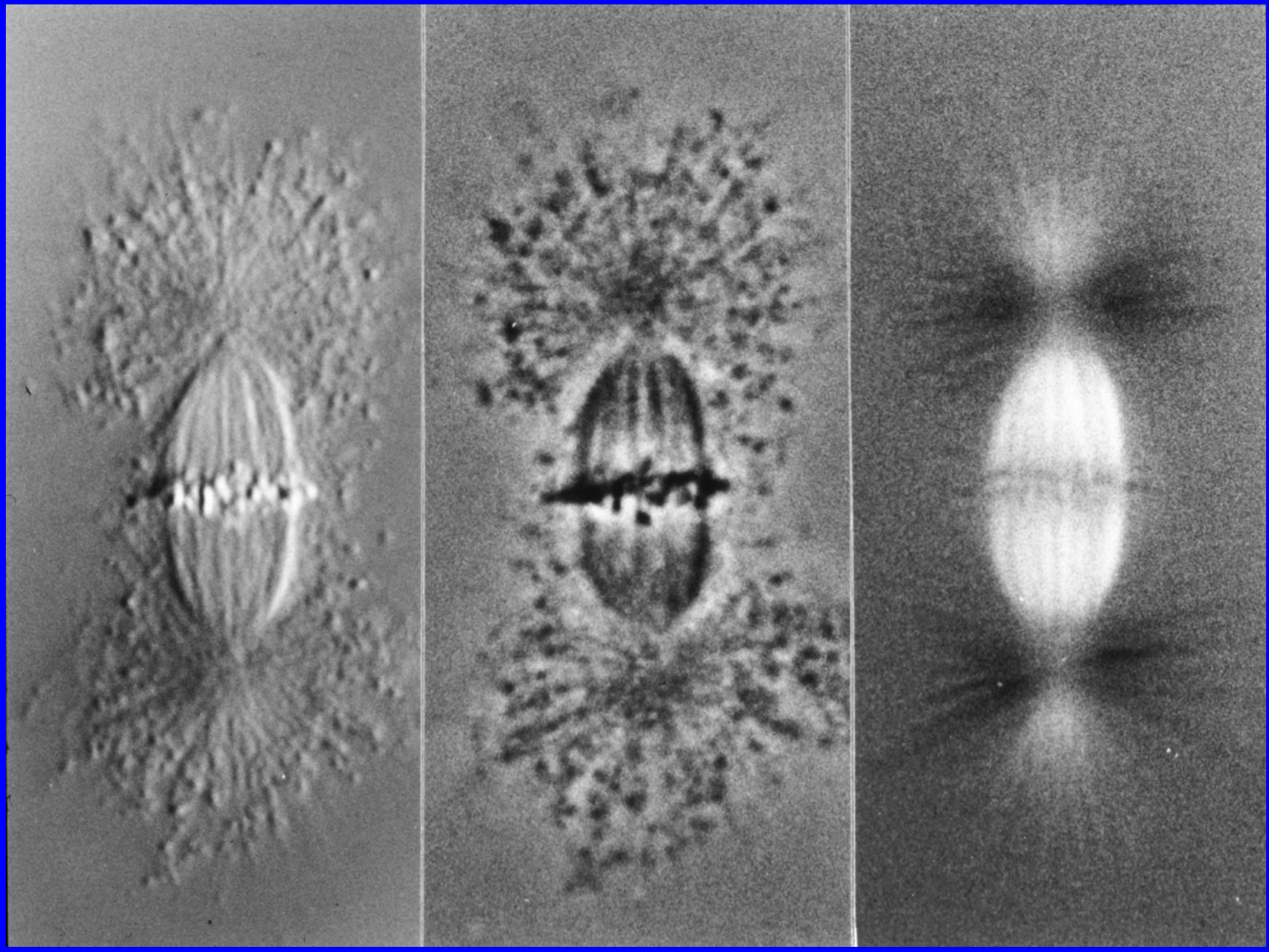
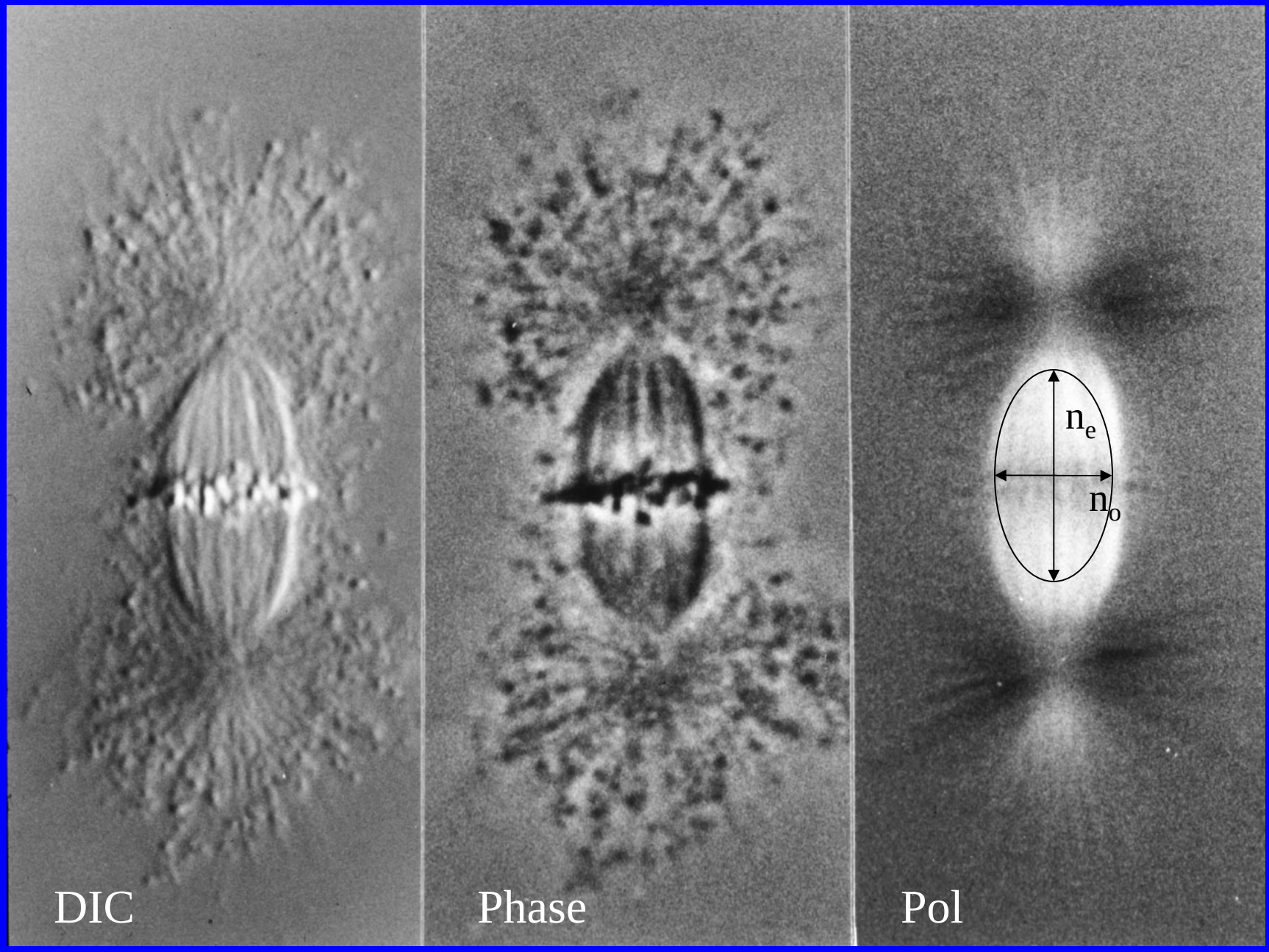
How Does Contrast in DIC Differ from Phase and Pol?




DIC
Phase
Pol
n_e
n_o

General References

- Zernike, Frits. 1955. How I discovered phase contrast. Science 121: 345-349.
- Zernike, F. 1958. The wave theory of microscope image formation. Strong, J. "Concepts in Classical Optics". W. H. Freeman, San Francisco. 525-536.
- Murphy, D. 2001. Fundamentals of Light Microscopy and Electronic Imaging. Wiley-Liss, N.Y.
- Yuste, R. F. Lanni, A. Konnerth, eds, 2000, Imaging Neurons, A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Molecular Expressions, a Microscope Primer at: http://micro.magnet.fsu.edu/primer/index.html

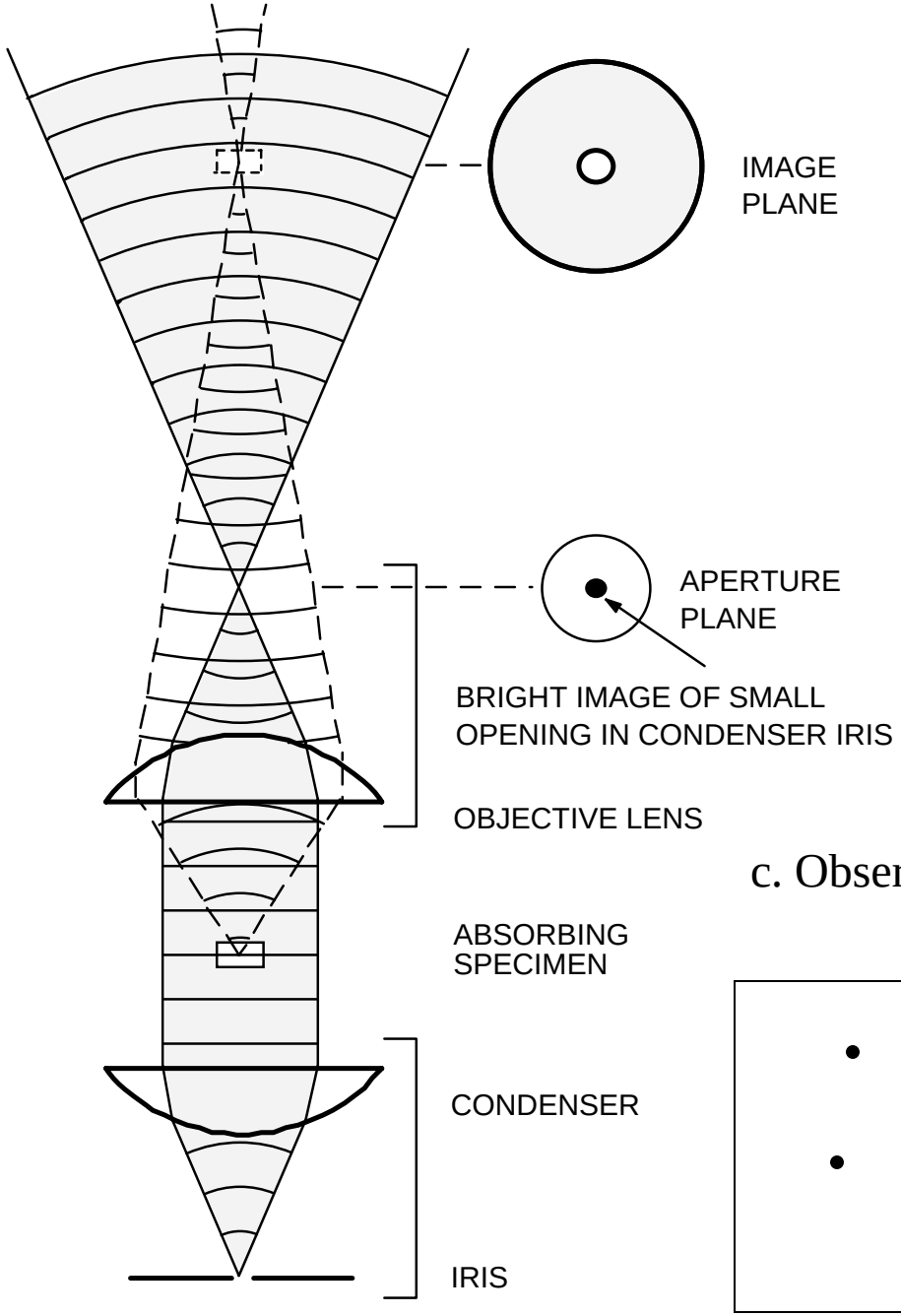
DIC References

- **Salmon ED. 1995. VE-DIC light microscopy and the discovery of kinesin.**
- **Trends Cell Biol. 5:154-8.**
- **Salmon, ED and Tran P. 2003. High-resolution video-enhanced differential interference contrast light microscopy.**
  Methods Cell Biol. 72:289-318.
- **Salmon ED, Shaw SL, Waters J, Waterman-Storer CM, Maddox PS, Yeh E, Bloom K. 2003. A high-resolution multimode digital microscope system. Methods Cell Biol. 72:185-216.**
- **M. Shribak and S. Inoué, "Orientation-independent differential interference contrast microscopy," submitted to *Applied Optics*.**

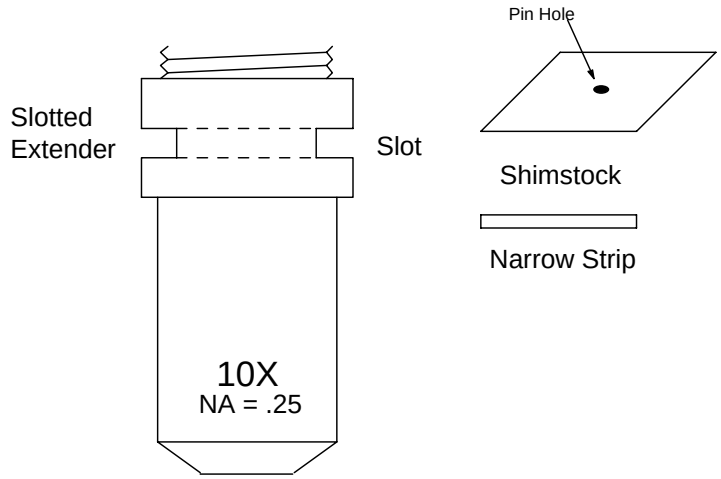
First

- Experiment described by Fritz Zernike in discussion of how he discovered Phase Contrast in 1930’s

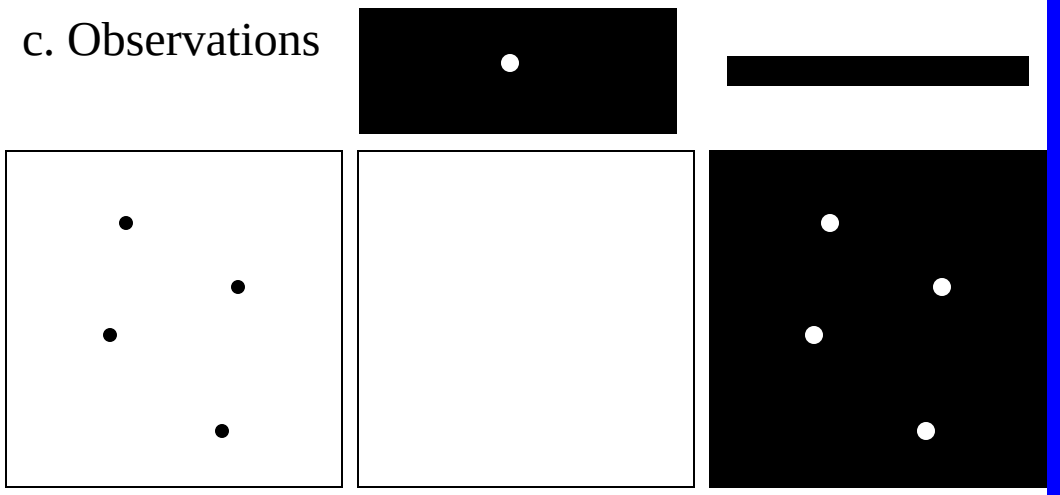
a.



b.



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:

$NA_{cond} > NA_{Obj}$

Objective can have iris diaphragm to limit NA_{obj} and prevent illuminating light from entering objective

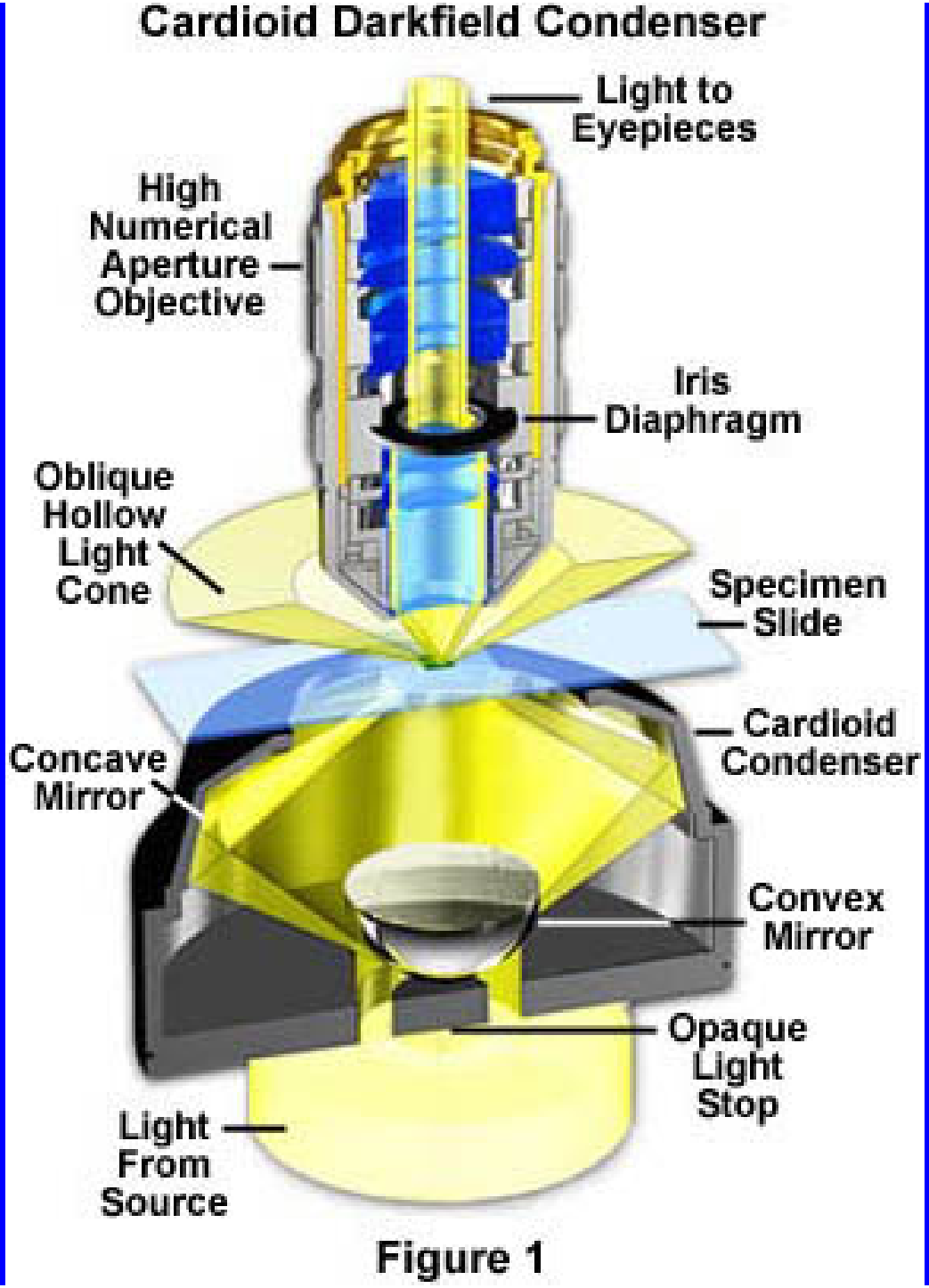
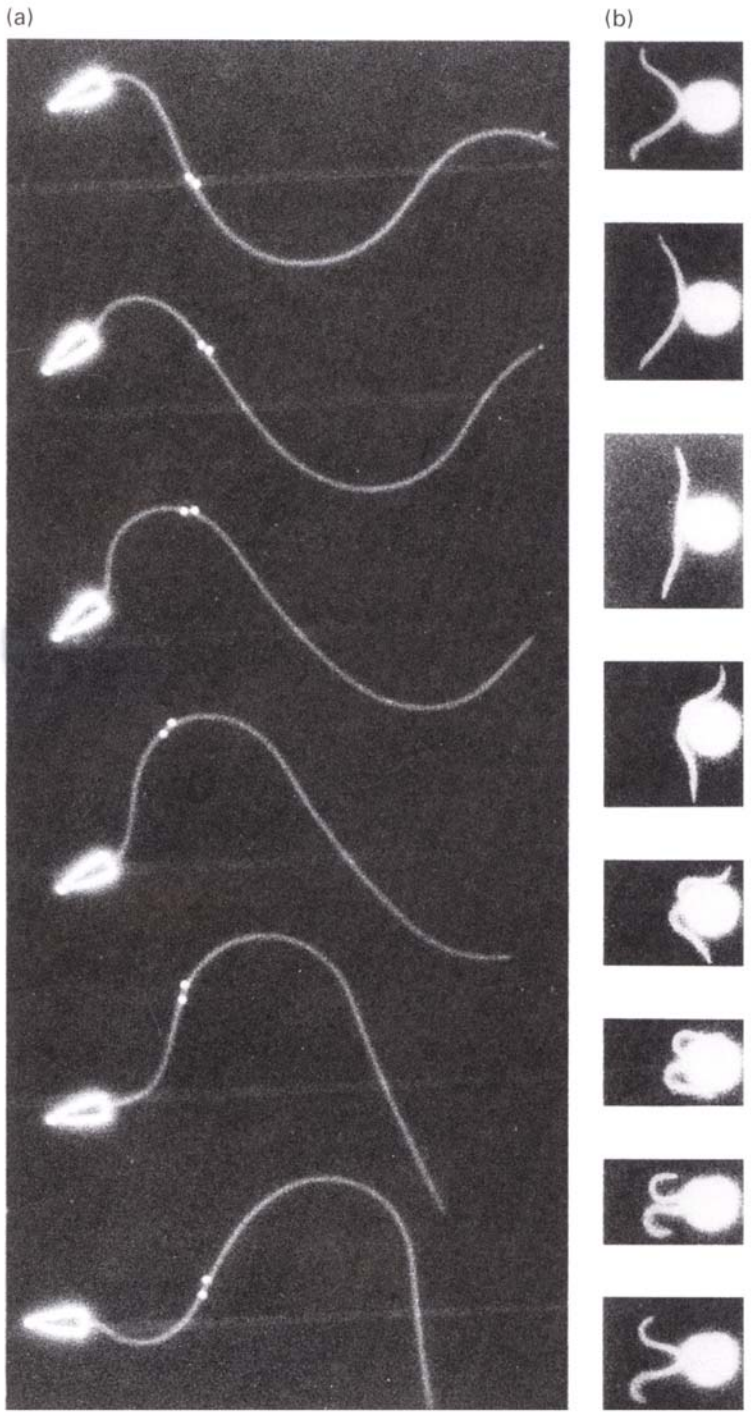
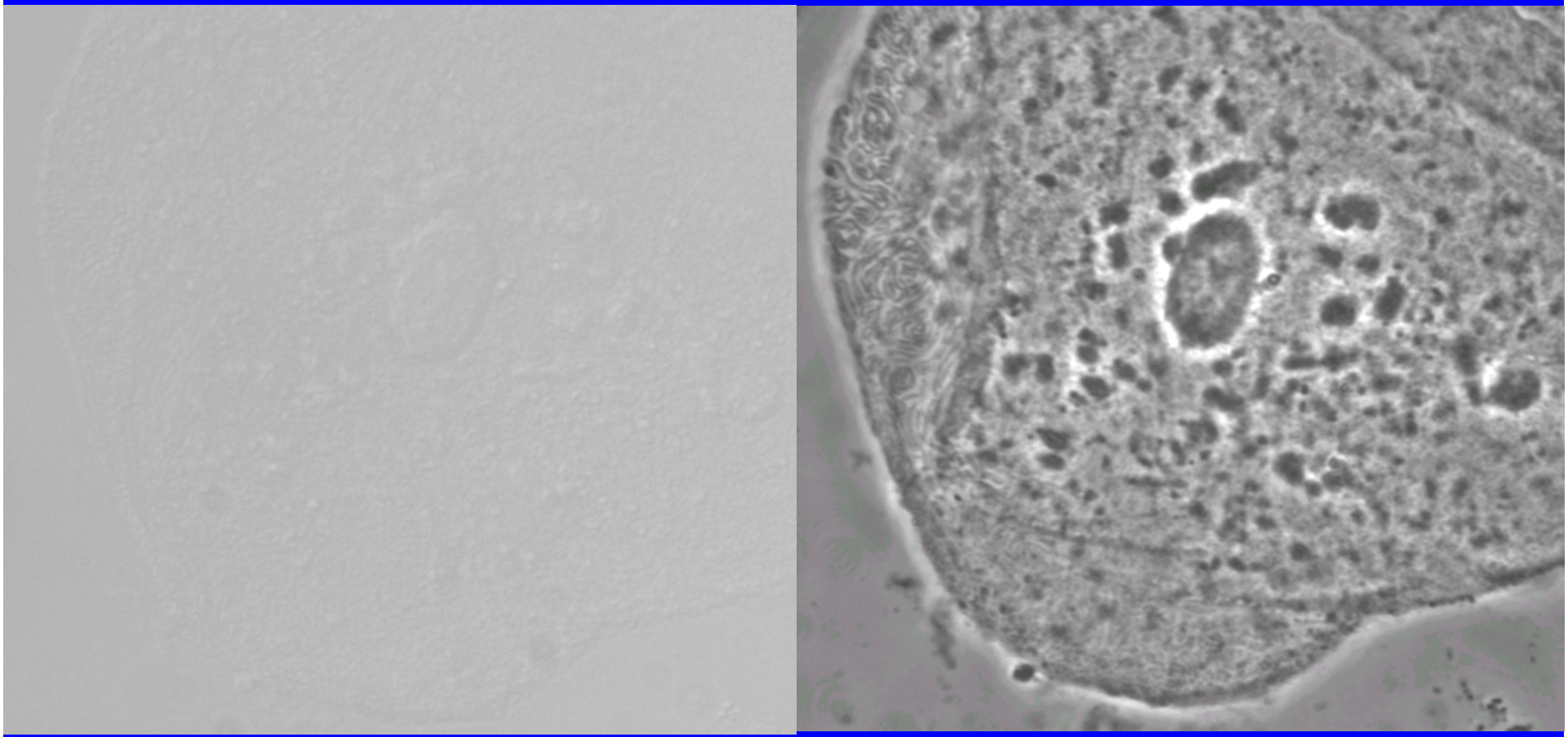


Figure 1

Stroboscopic Darkfield Imaging of Flagella Motility of Sea Urchin Sperm and Chlamydomonus



Phase Contrast Gives Contrast to Structural Detail in Transparent Specimens

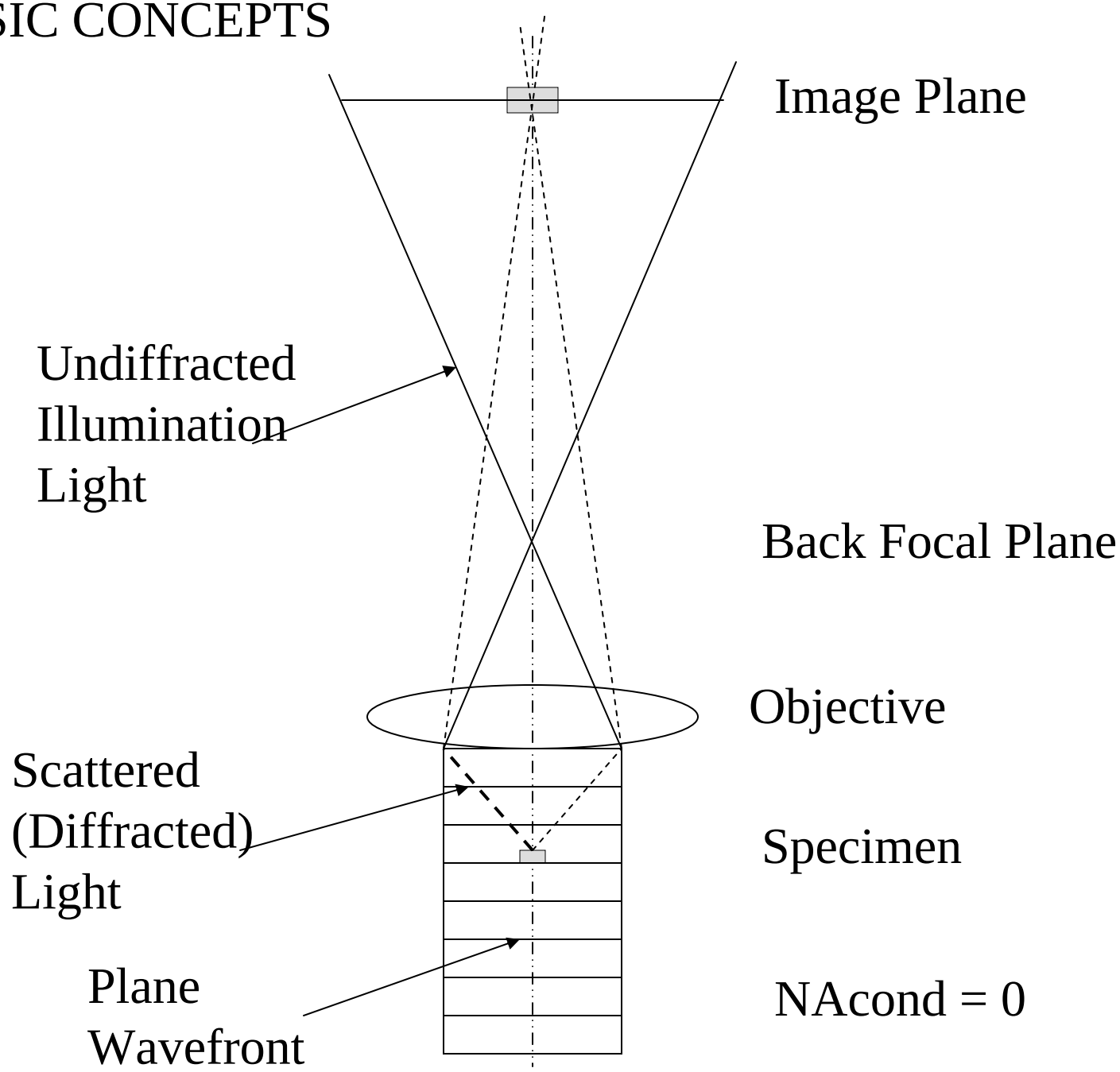


Brightfield

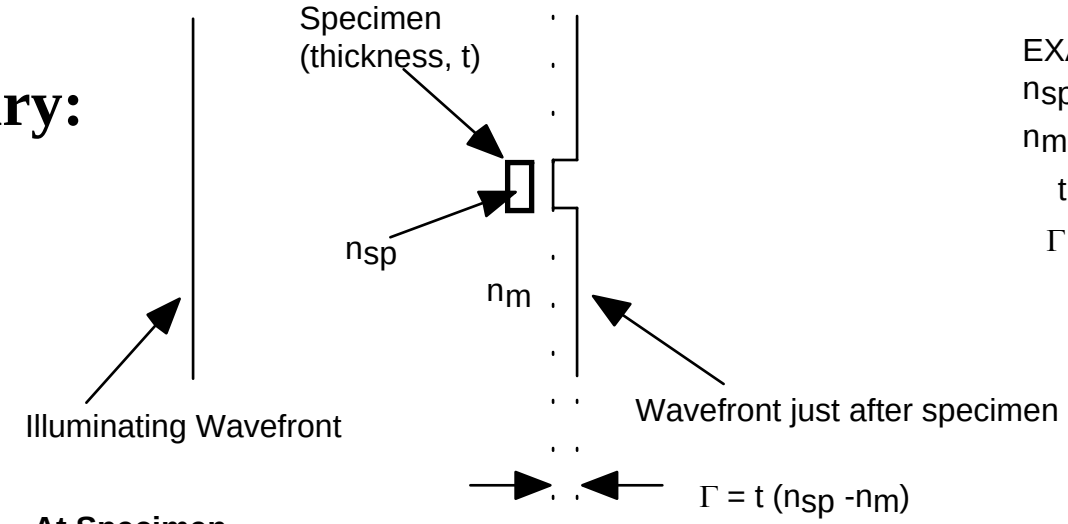
Phase Contrast

(NA_{obj} = 1.4)

BASIC CONCEPTS



Summary:



EXAMPLES:

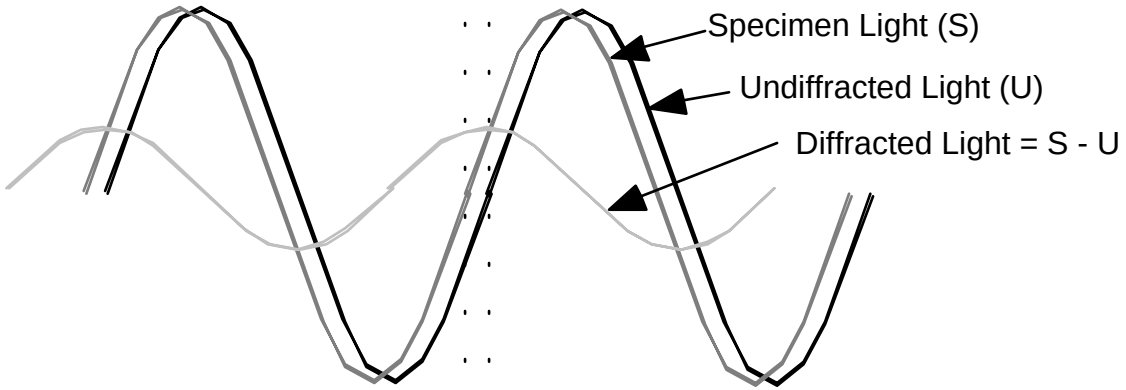
n_{sp} = 1.4 organelle

n_m = 1.36 cell cytosol

t = 1 micron

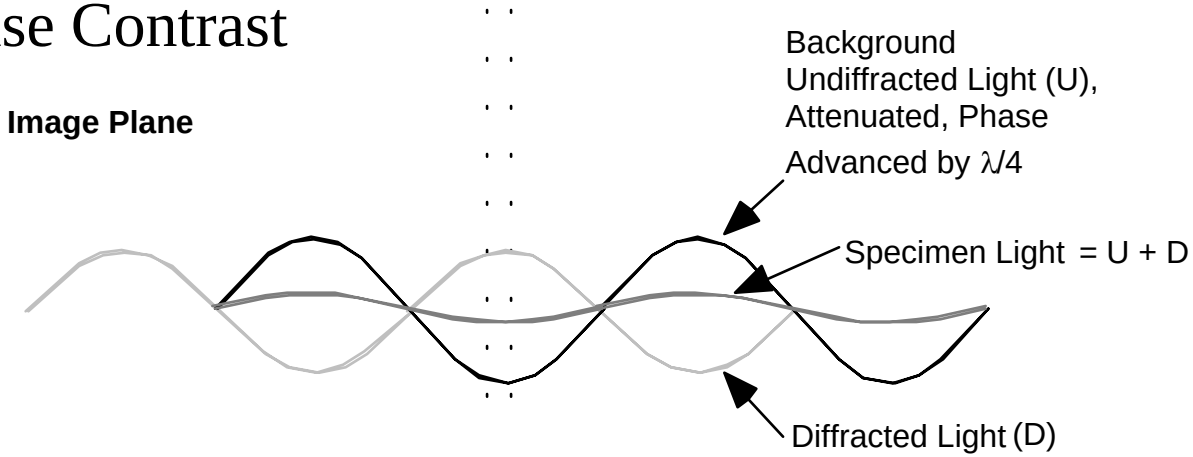
Γ = .04 = 550nm /13

At Specimen

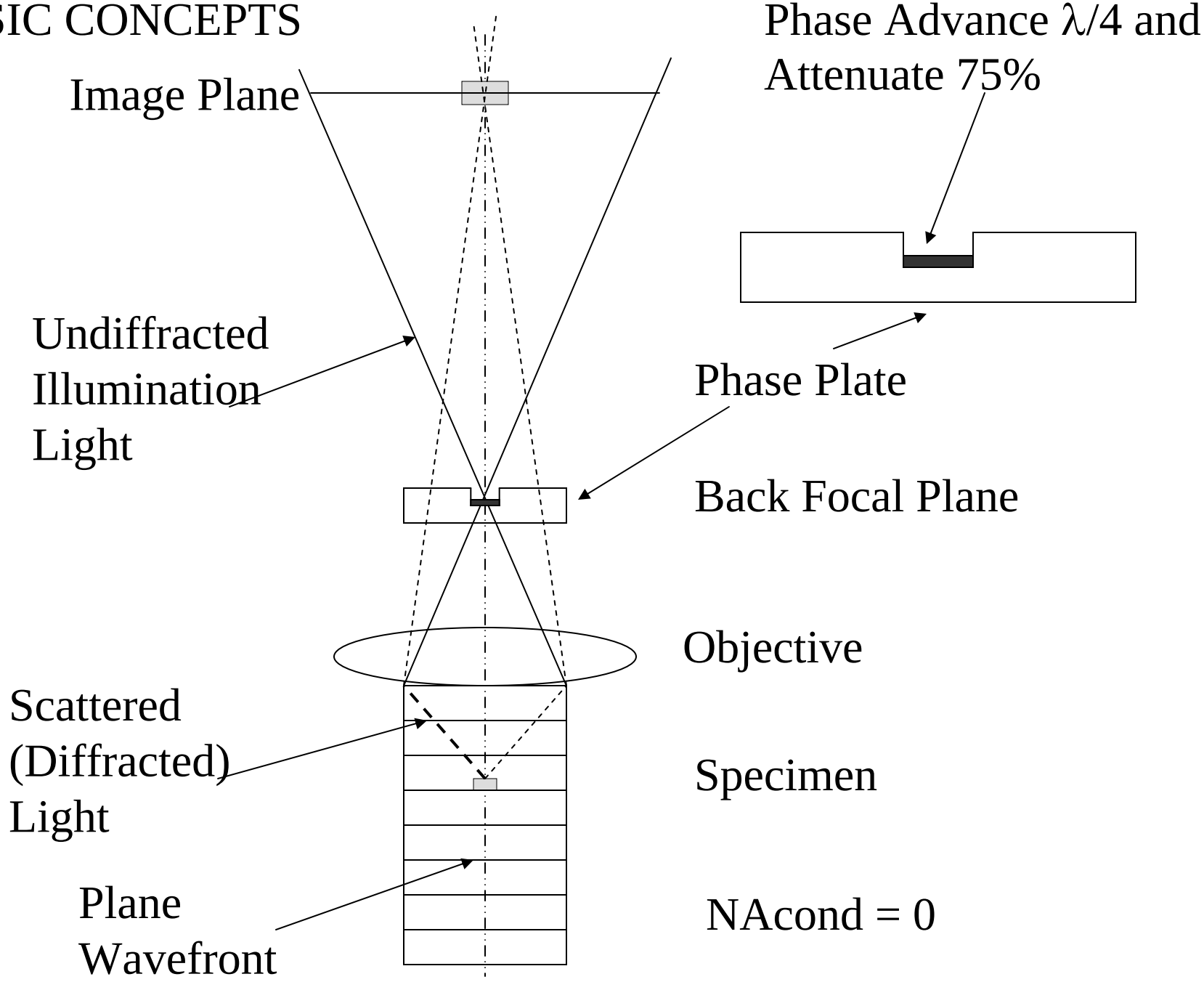


In Dark Phase Contrast

At Image Plane

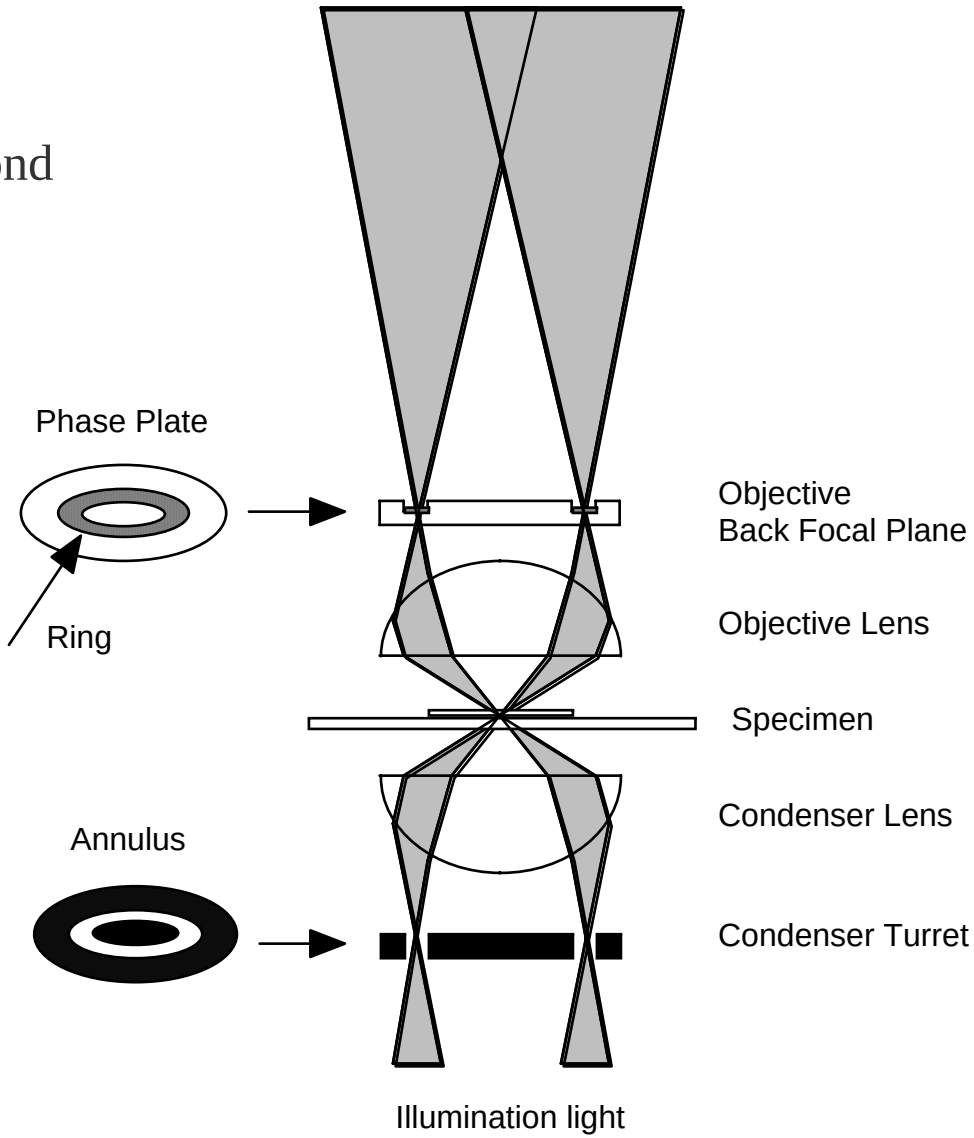


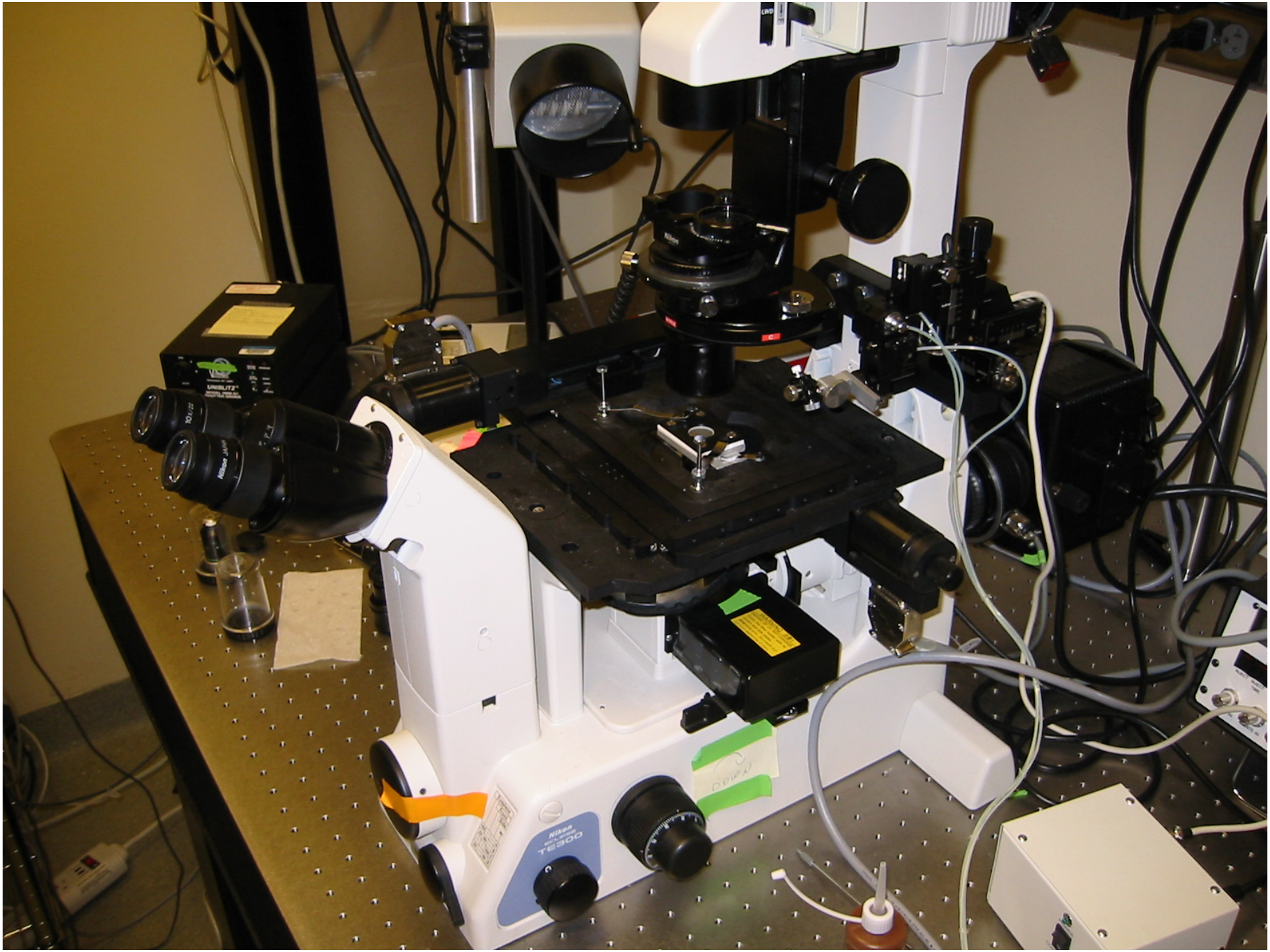
BASIC CONCEPTS



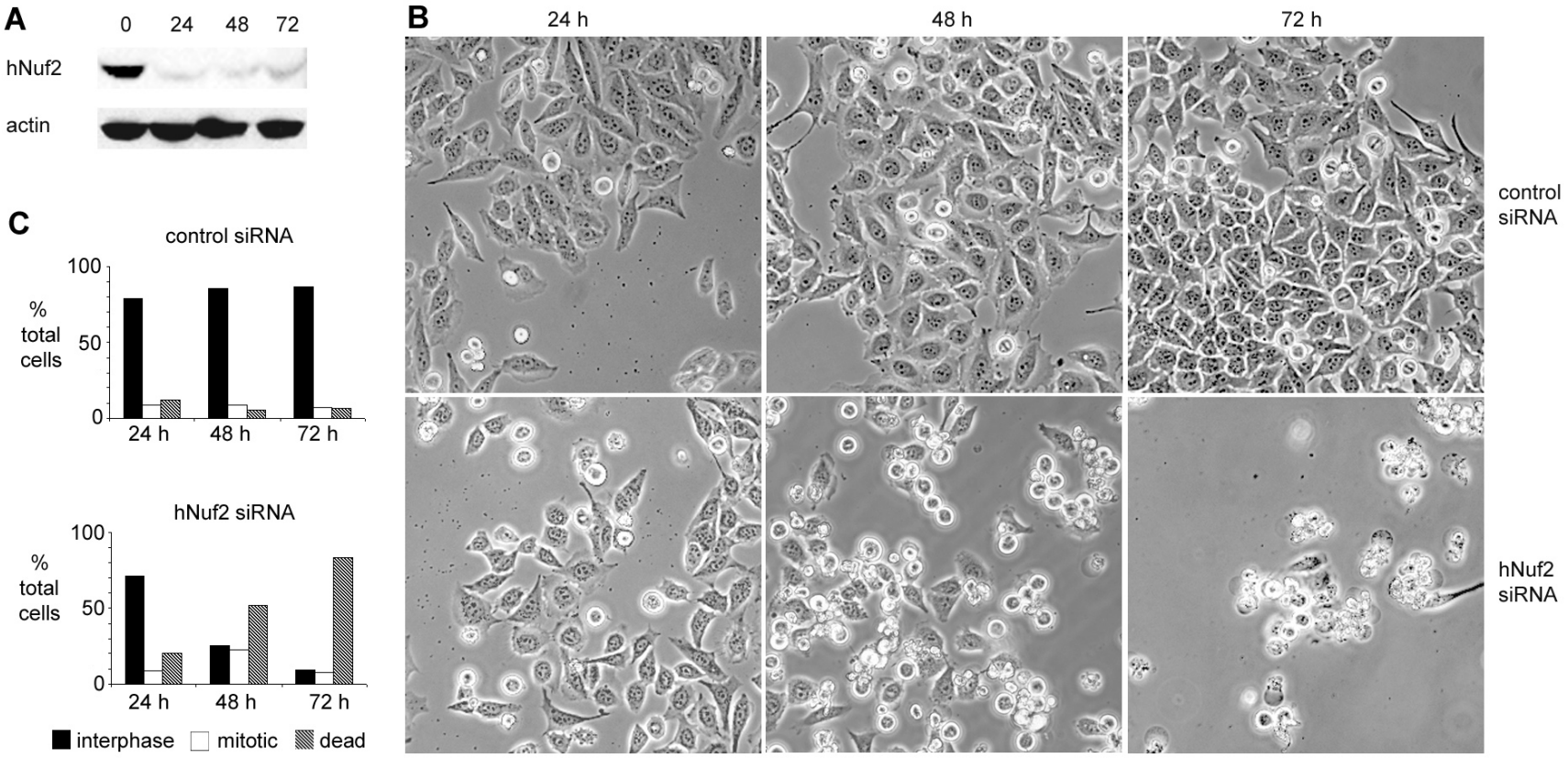
MICROSCOPE ALIGNMENT
FOR
PHASE CONTRAST

To Increase NA_{cond} illumination, Modern Phase Contrast Uses Annular Ring as Condenser Stop and Phase Ring in Objective Back Focal Plane

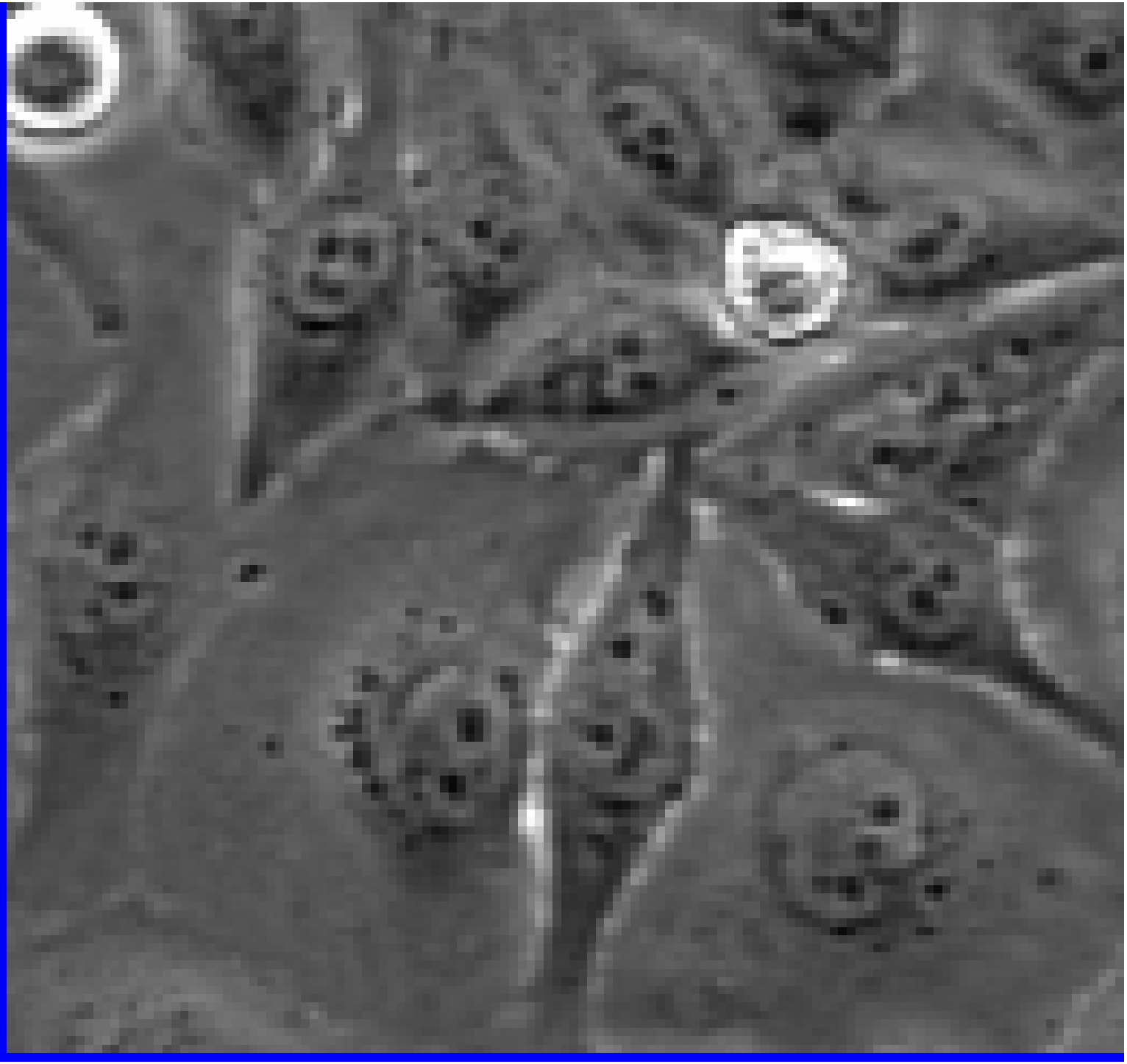



Nikon
TE300

Depletion of hNuf2 from HeLa cells using siRNA.

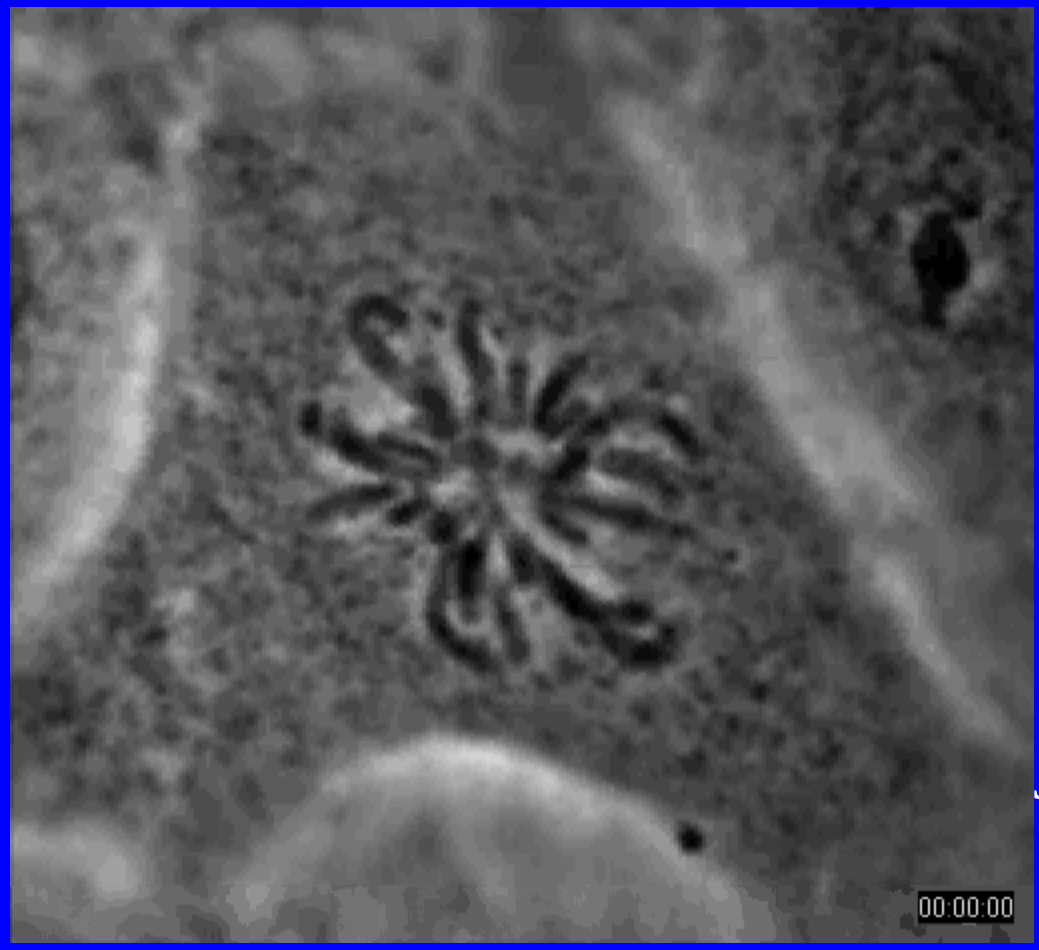


10X Phase Contrast of HeLa Cells: Time-Lapse for 10 hours At 5 min intervals; 1/10 field, 1of 25 fields recorded



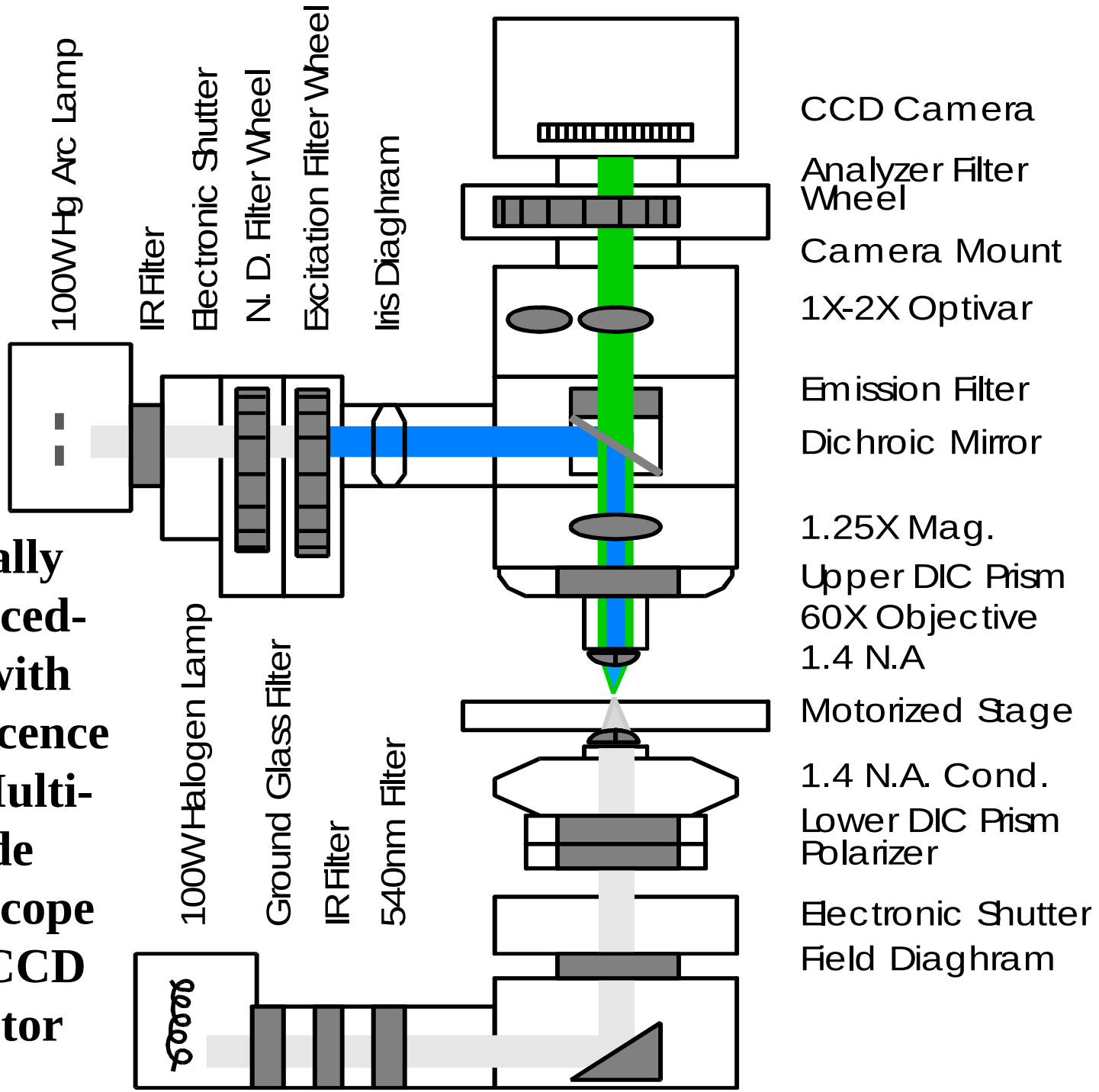
Induce Anaphase in Early Prometaphase by Overcoming the Spindle Checkpoint

Example:
Mad2
Antibody
Injection into
Early
Prometaphase
Ptk1Cells

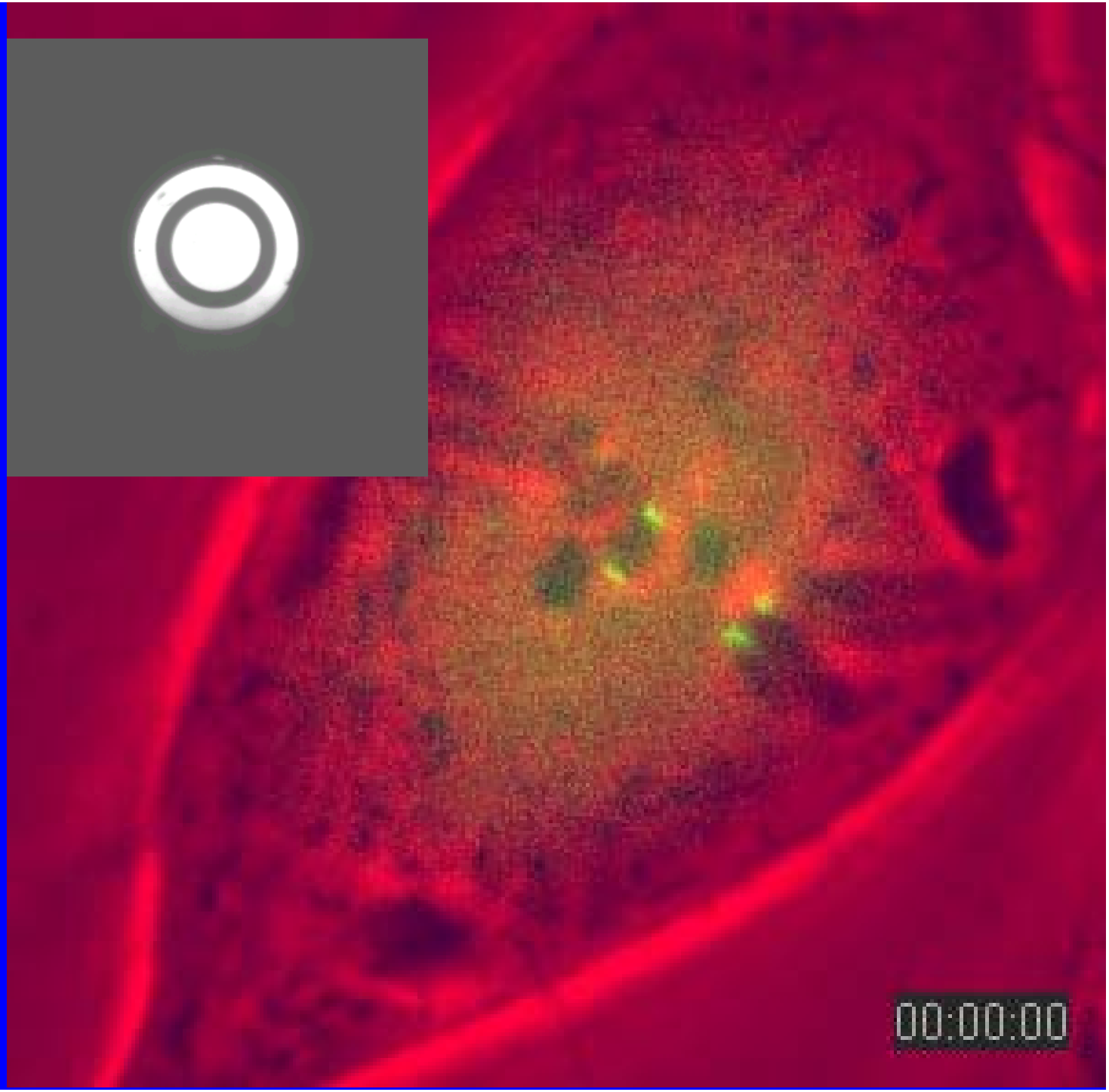


Julie Canman

Digitally Enhanced-DIC with Fluorescence In A Multi-Mode Microscope With CCD Detector



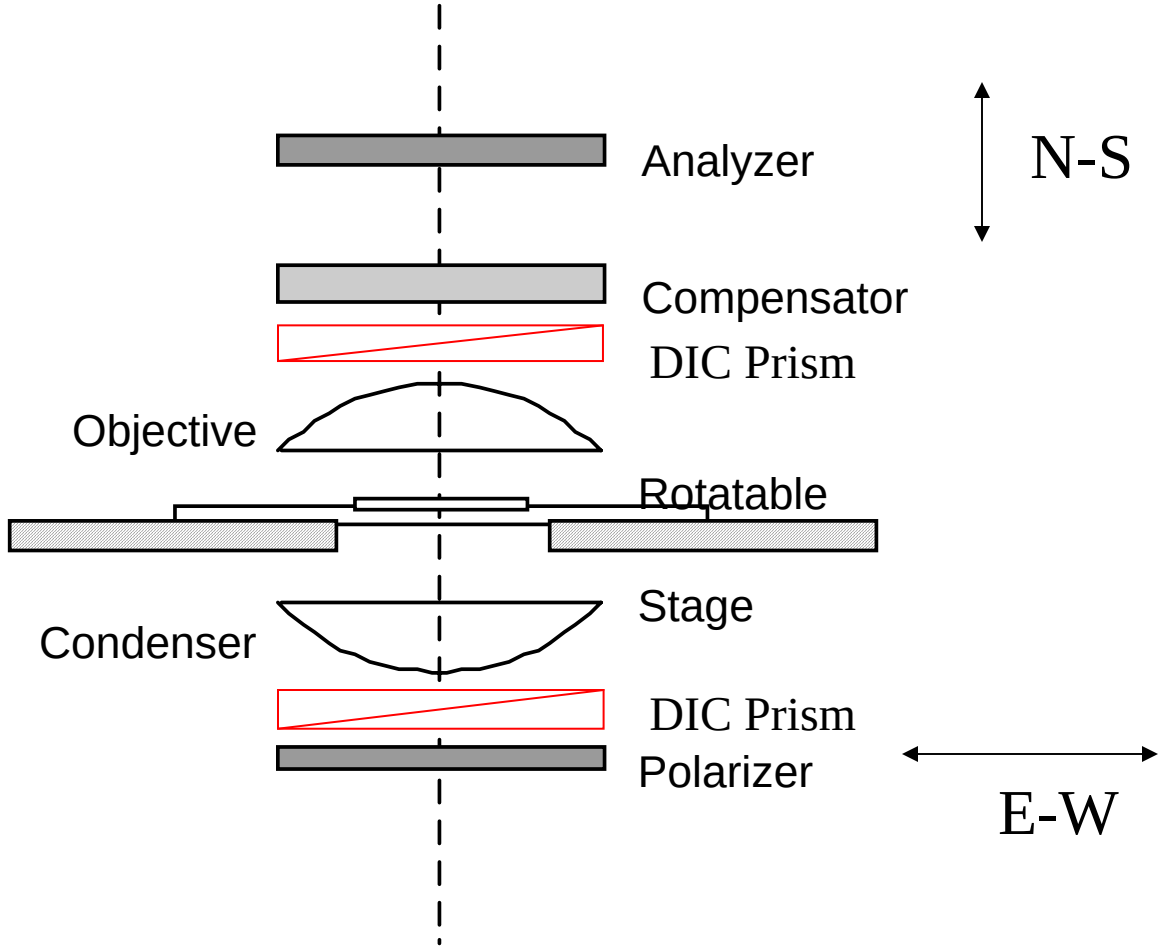
Cdc20 Persists At Kinetochores Throughout Mitosis and Exhibits



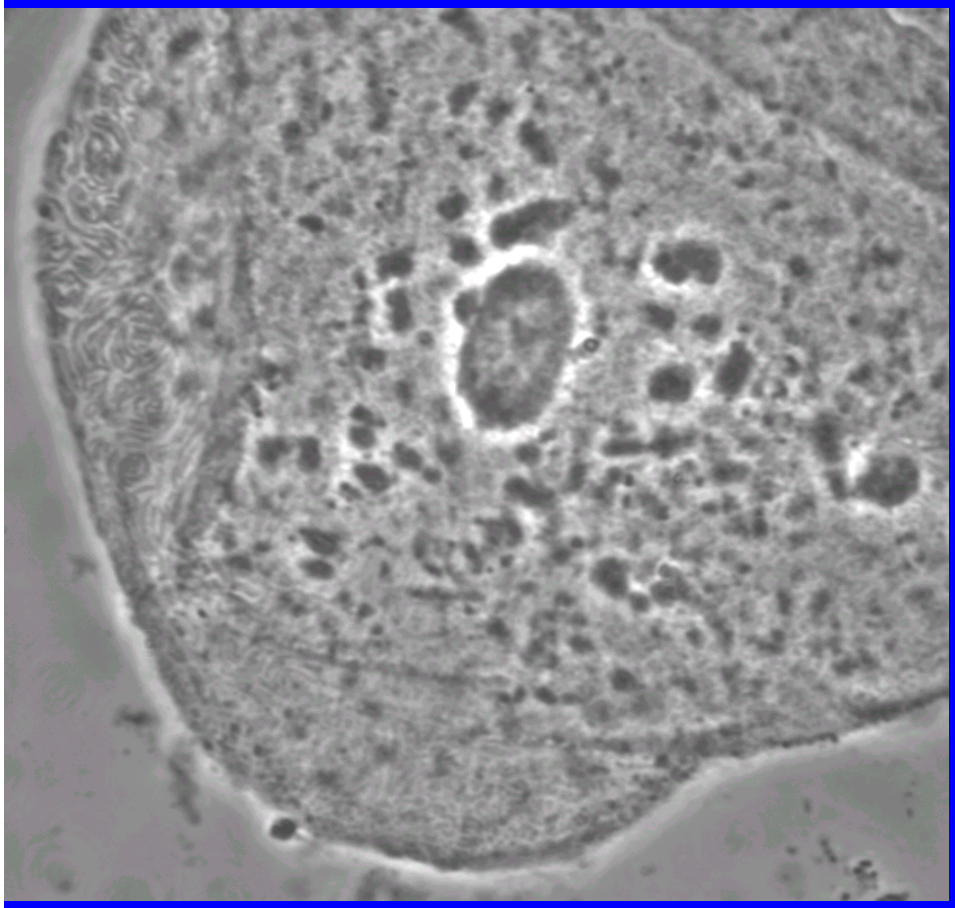
Green:
GFP-Cdc20
At
Kinetochores

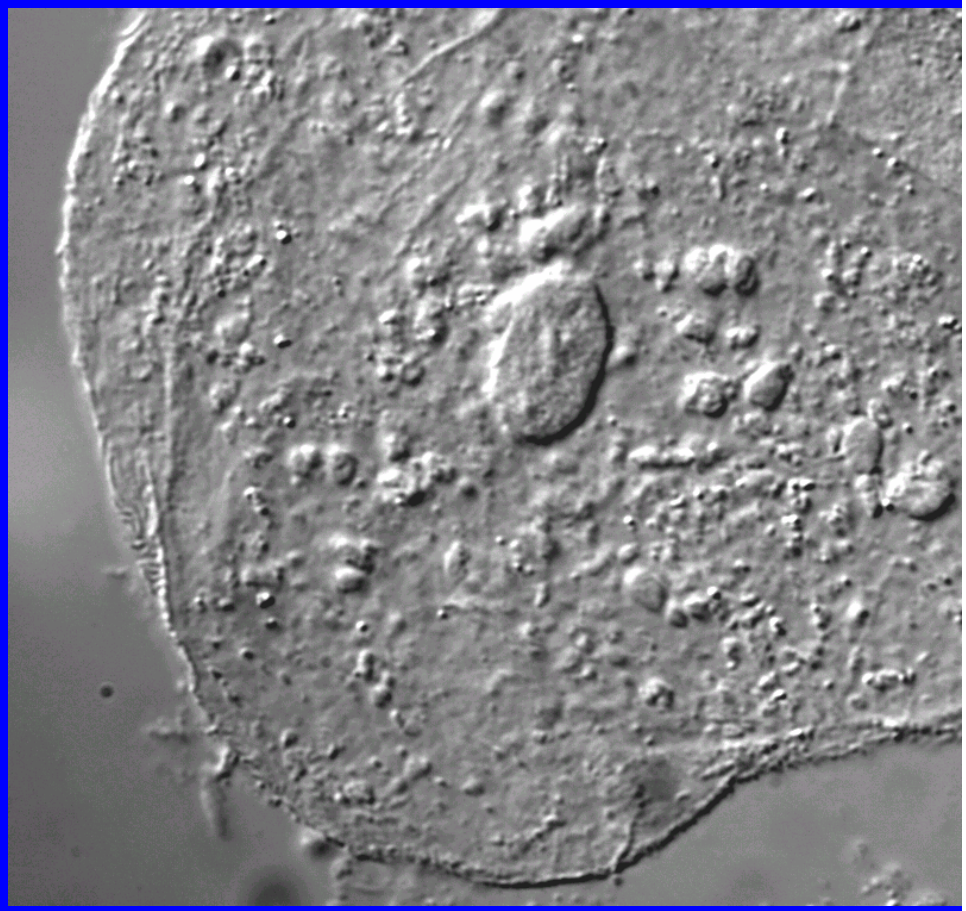
Red:
Phase Contrast
Images of PtK1
Tissue Cells

A DIC Microscope is a Polarizing Microscope with Condenser an Objective DIC Prisms

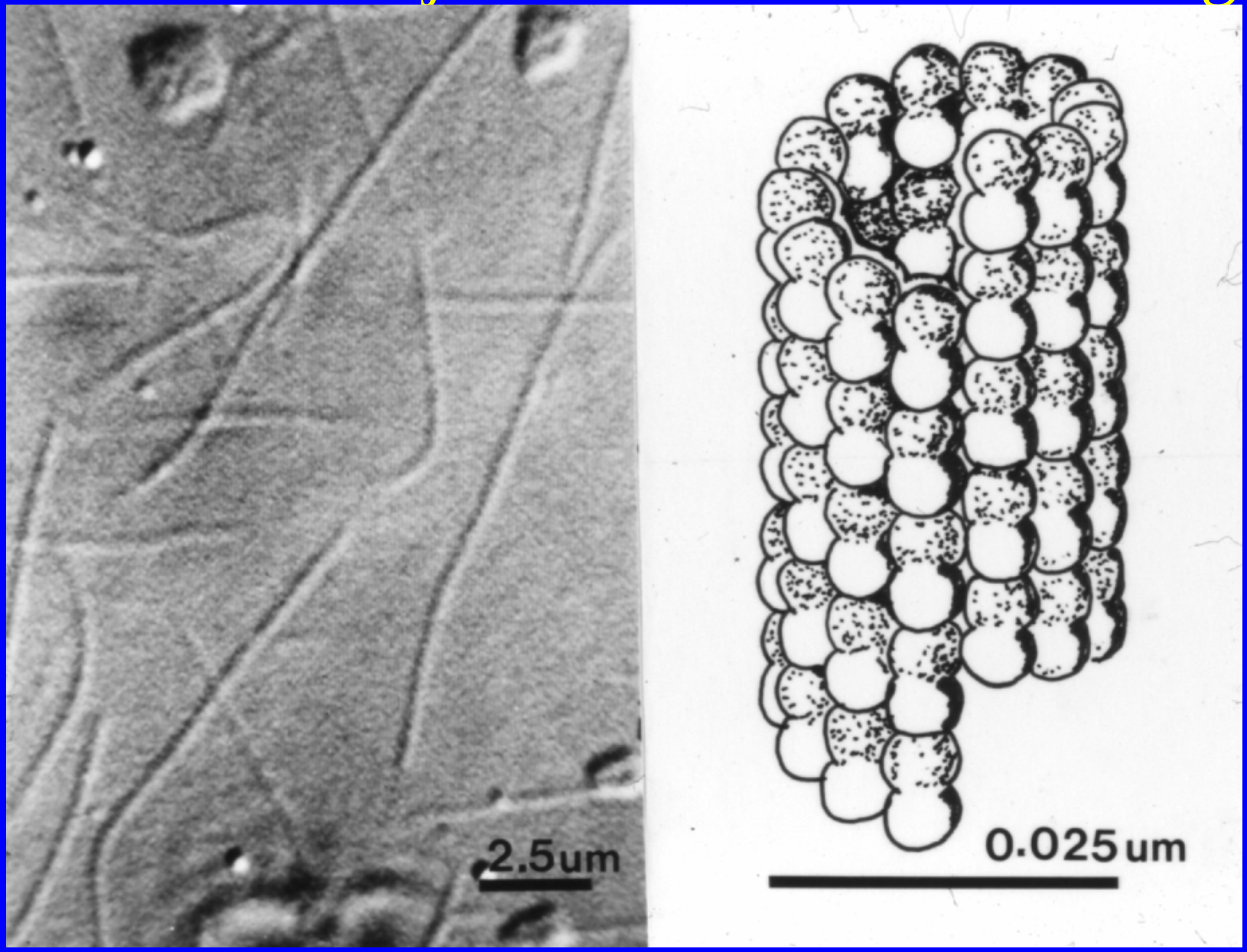


Comparison of Phase Contrast to DIC for Cheek Cell





What Are 5 Major Features of A DIC Image?

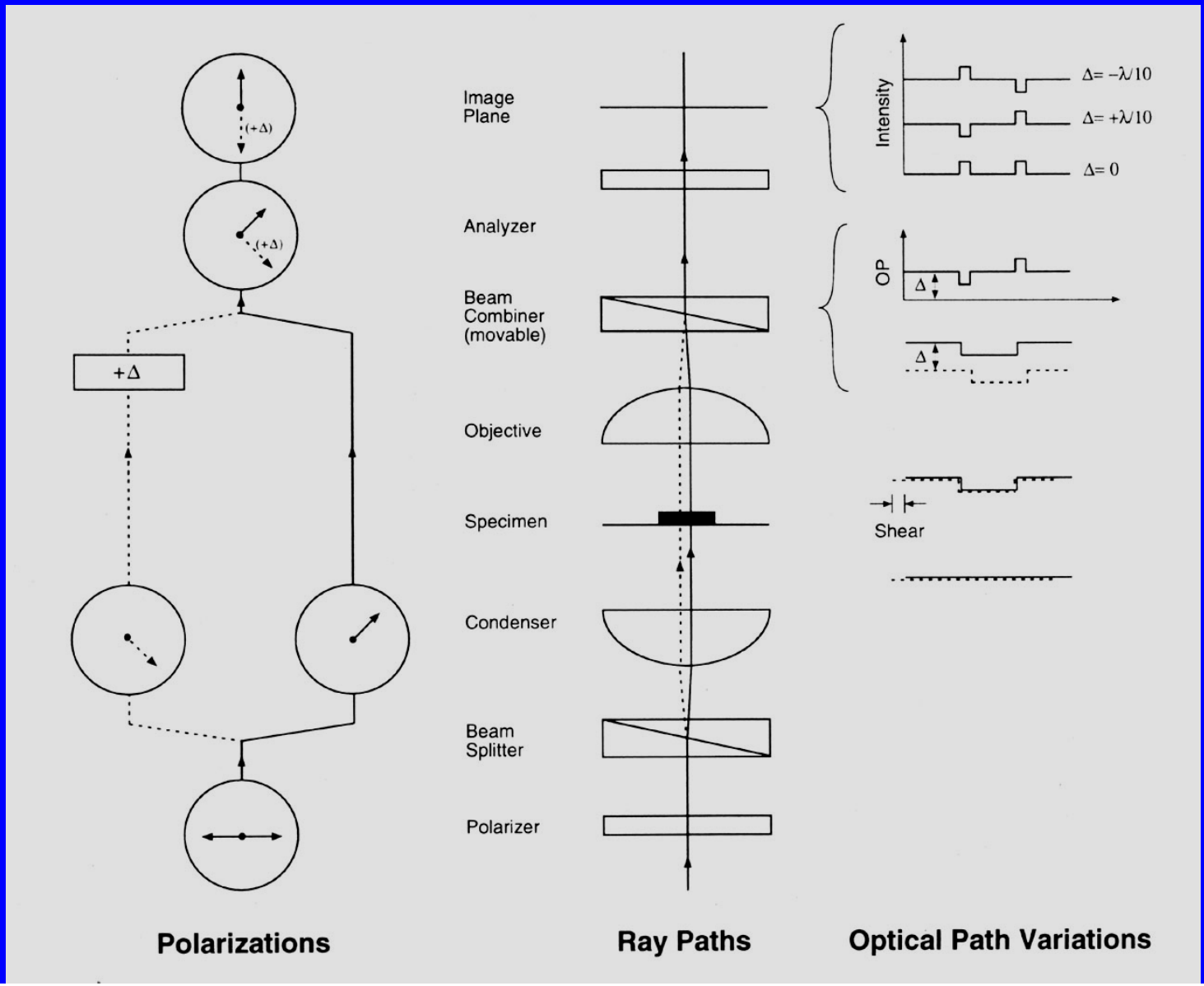


High Resolution VE-DIC Image of Microtubules

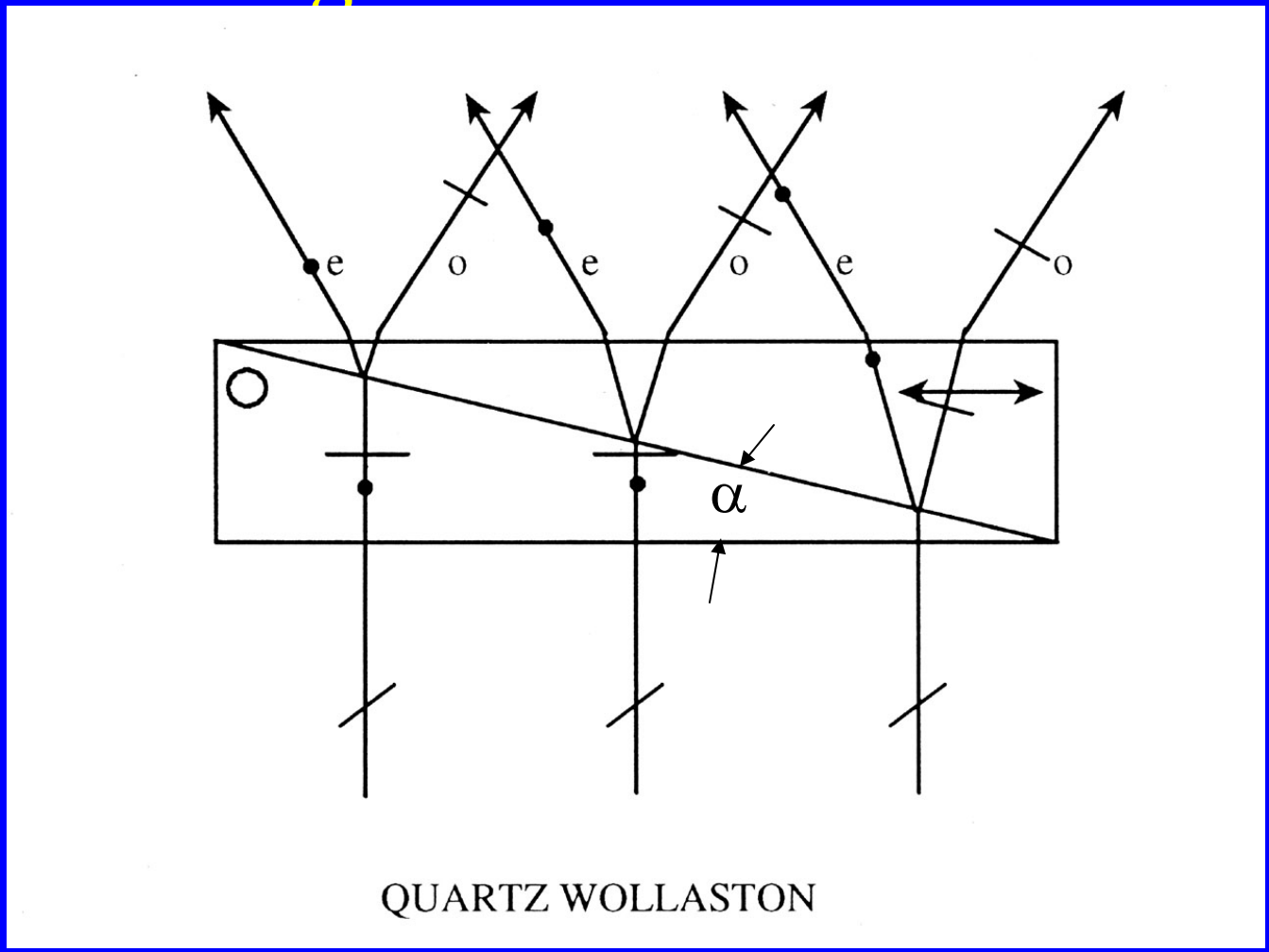
What Are 6 Major Features of A DIC Image?

- 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



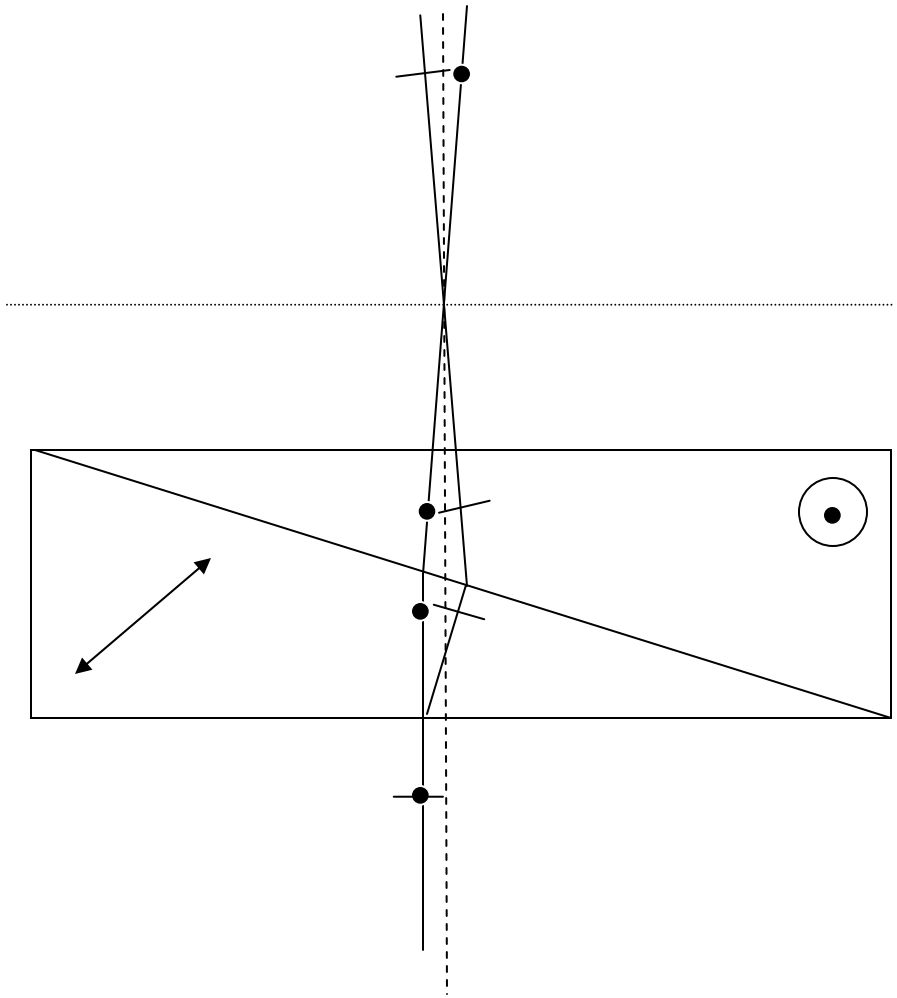
The Condenser DIC Prism Splits Illumination Light into 2 Divergent Orthogonal Polarized Beams



Prism is Oriented with the Optic Axes at 45^{o} to Polarizer.Why?

A Modified-Wollaston Prism is Used To Place Prism Above Objective or Below Condenser Diaphragm

Effective Beam Splitting Plane



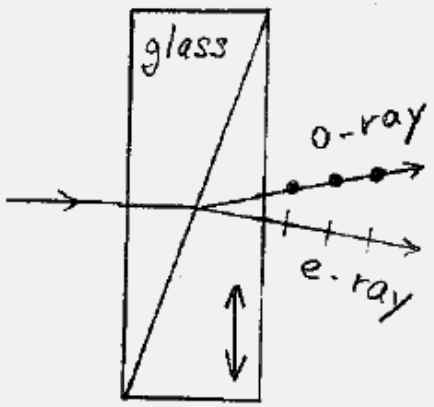
Nikon's New DIC System Uses One Birefringent Prism Combined With A Glass Wedge (courtisy of Mr. Toshimitsu)

4/05/06

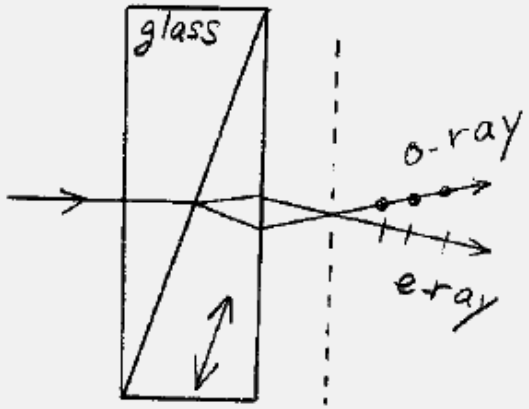
DIC Prism

Two linearly polarized light components

Single quartz plate

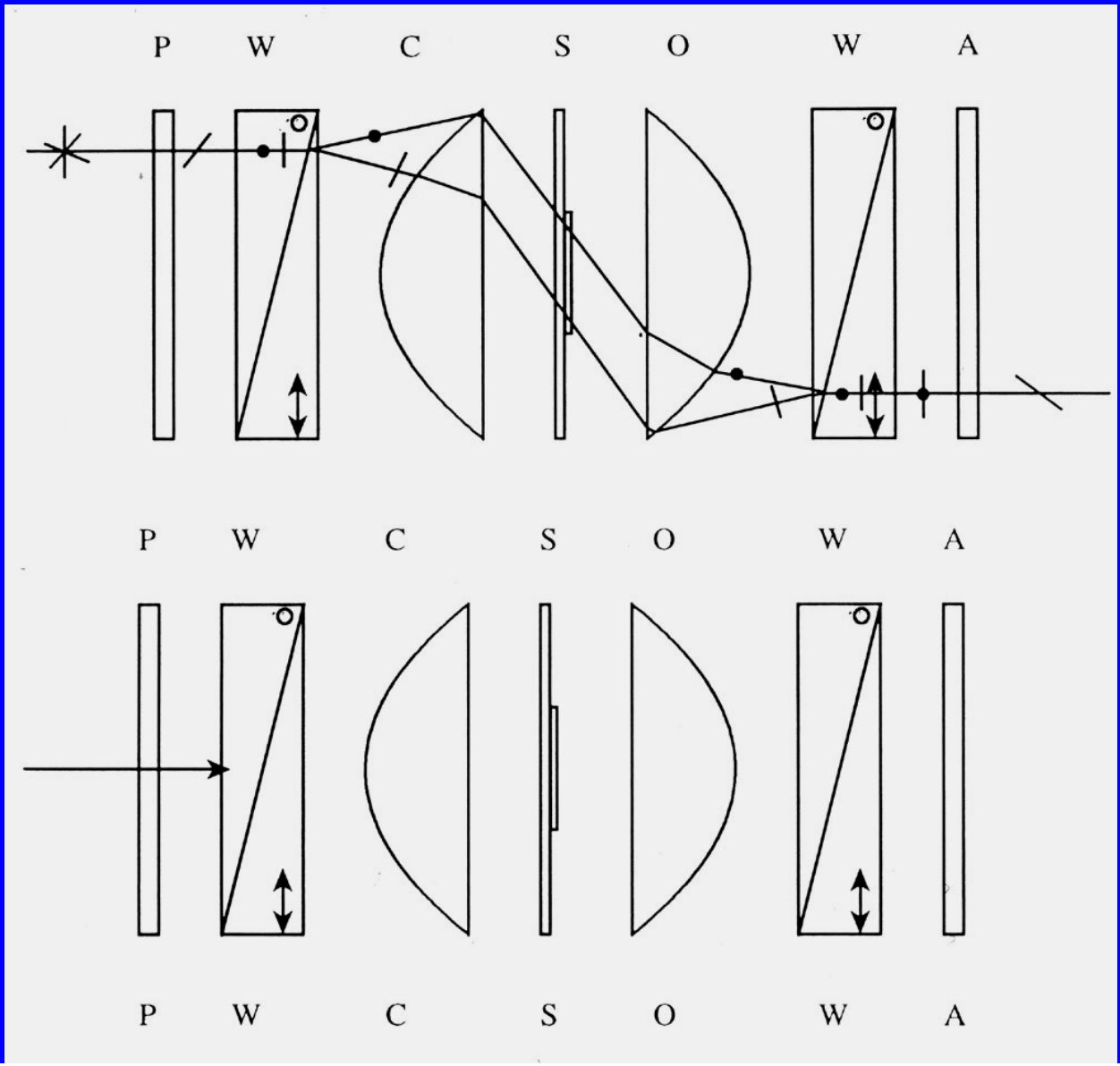


Wallaston Prism



Nomarski Prism

Divergent Beams from Condenser Prism Pass through Specimen as Parallel Beams



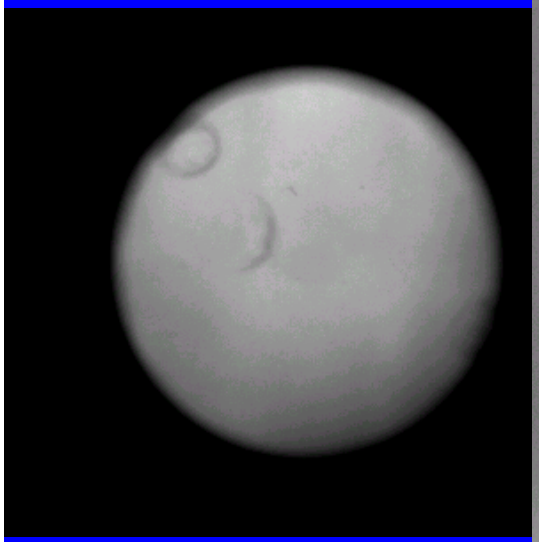
Microscope Alignment For DIC

- 1. Achieve Koehler illumination
- 2. Align for Polarization Microscopy: Polarier 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

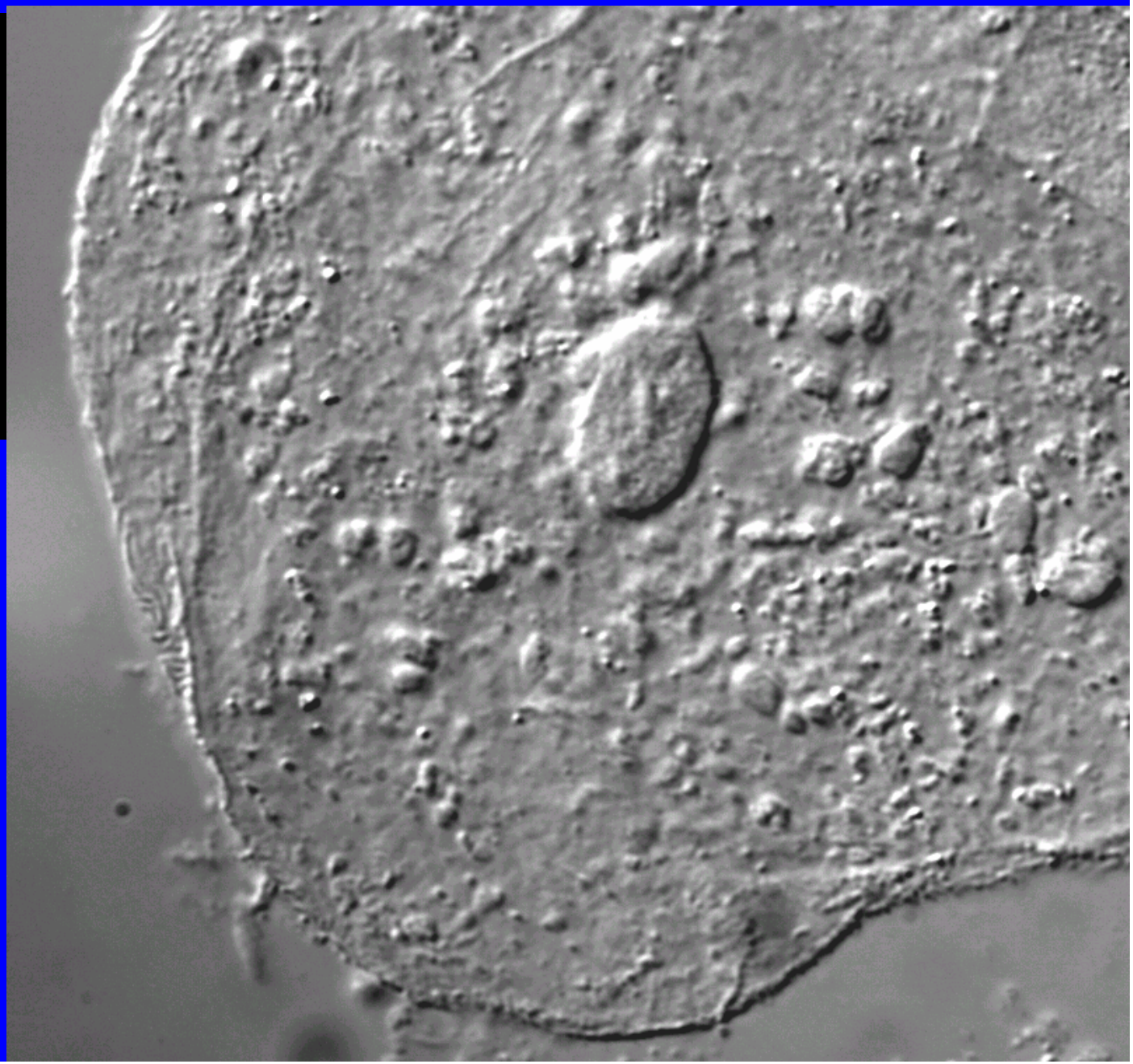

DIC Accessories for Transmitted Light
Swing-Out Top Lens
Wollaston Prism Turret
Universal Condenser
Polarizer
Analyzer
DIC Slider
Wollaston Prisms
Oil Immersion Top Lens

Figure 4

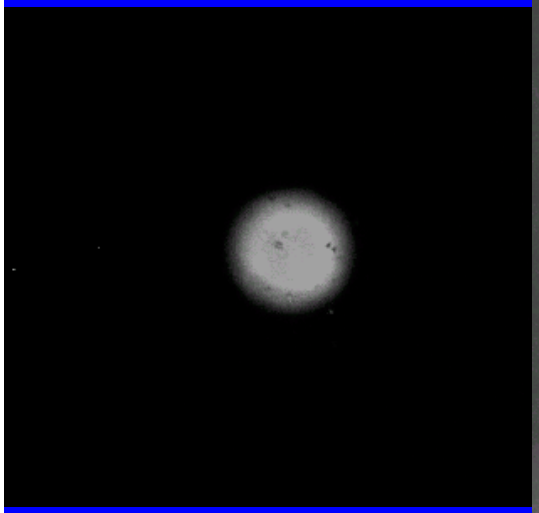
Microscope Alignment for DIC



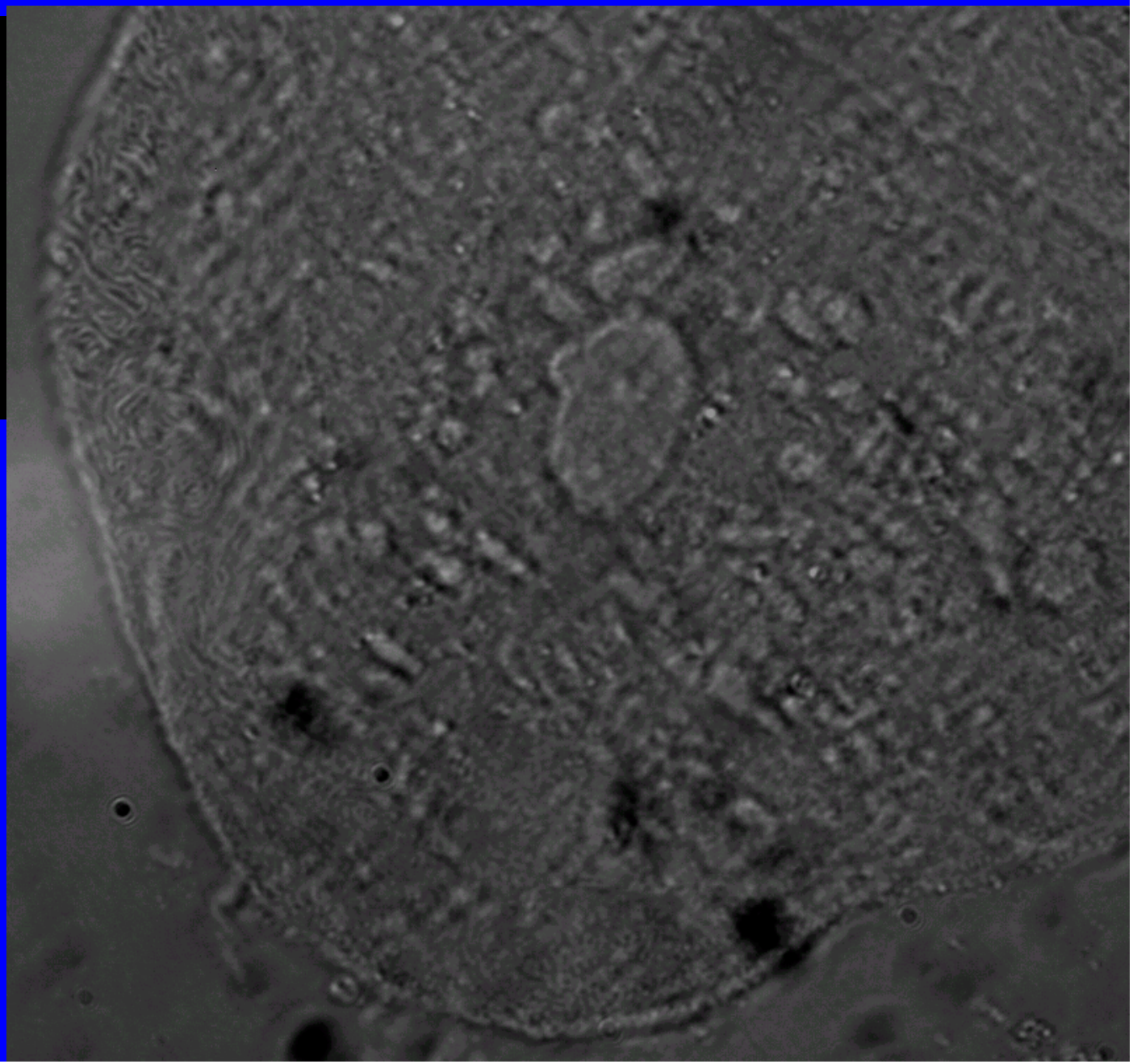
Objective Back Aperture: Full Aperture Illumination



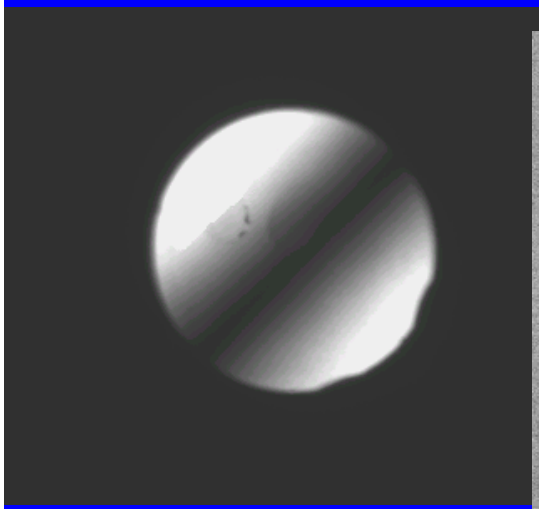
Poor Condenser Illumination



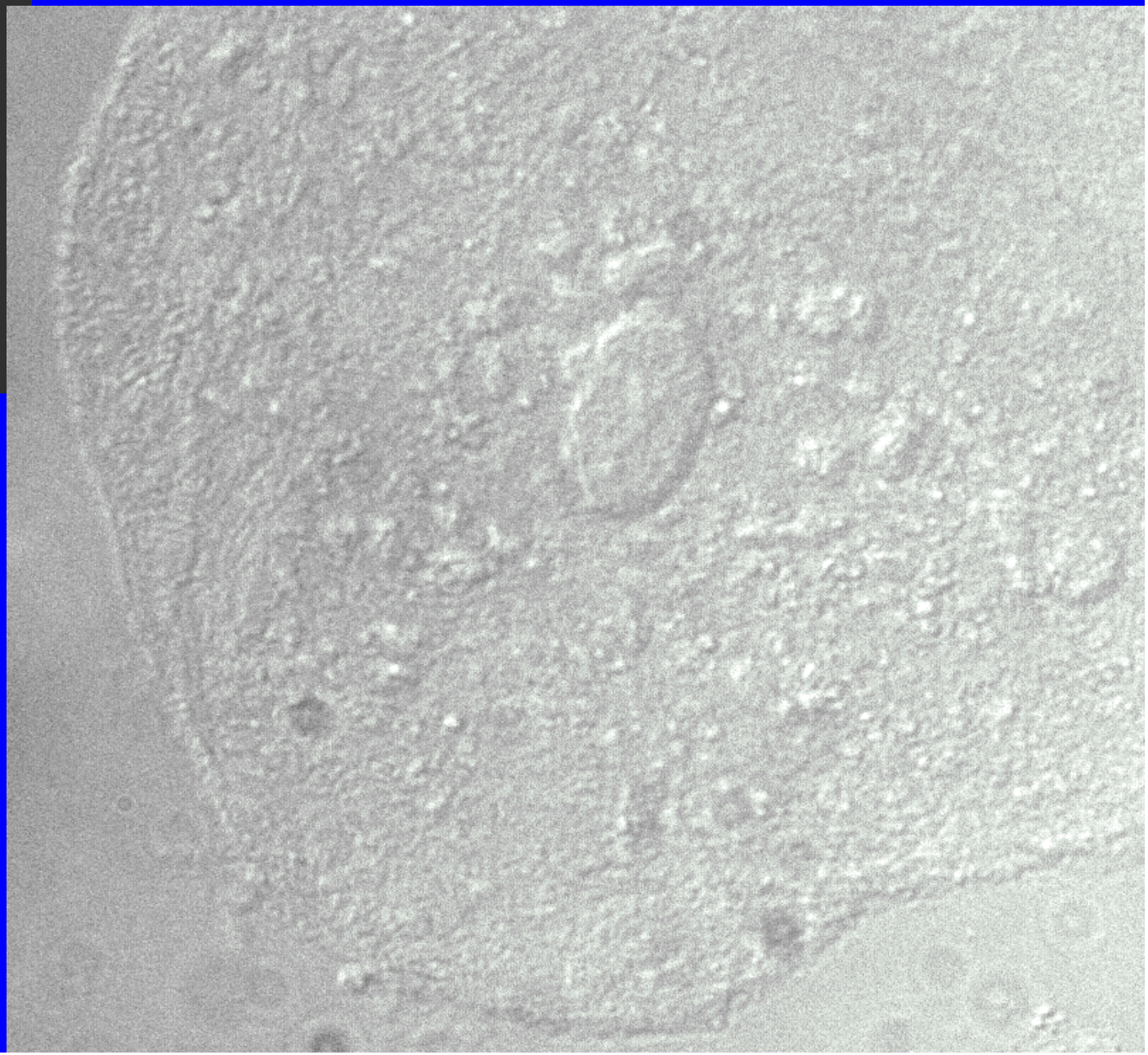
Objective
Back Aperture:
1/3 Aperture
Illumination



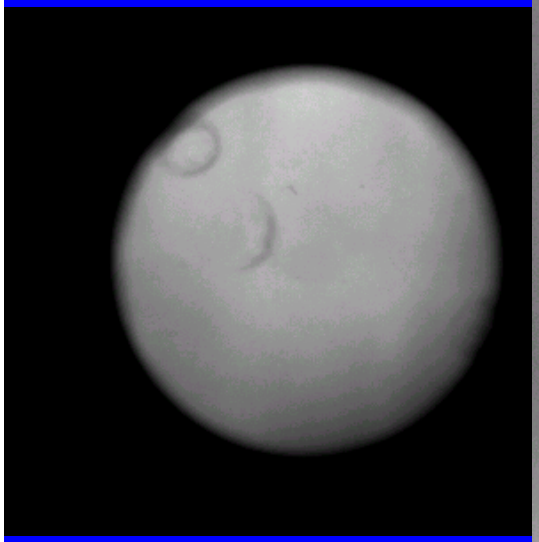
Miss-Matched Prisms, or a Missing DIC Prism



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

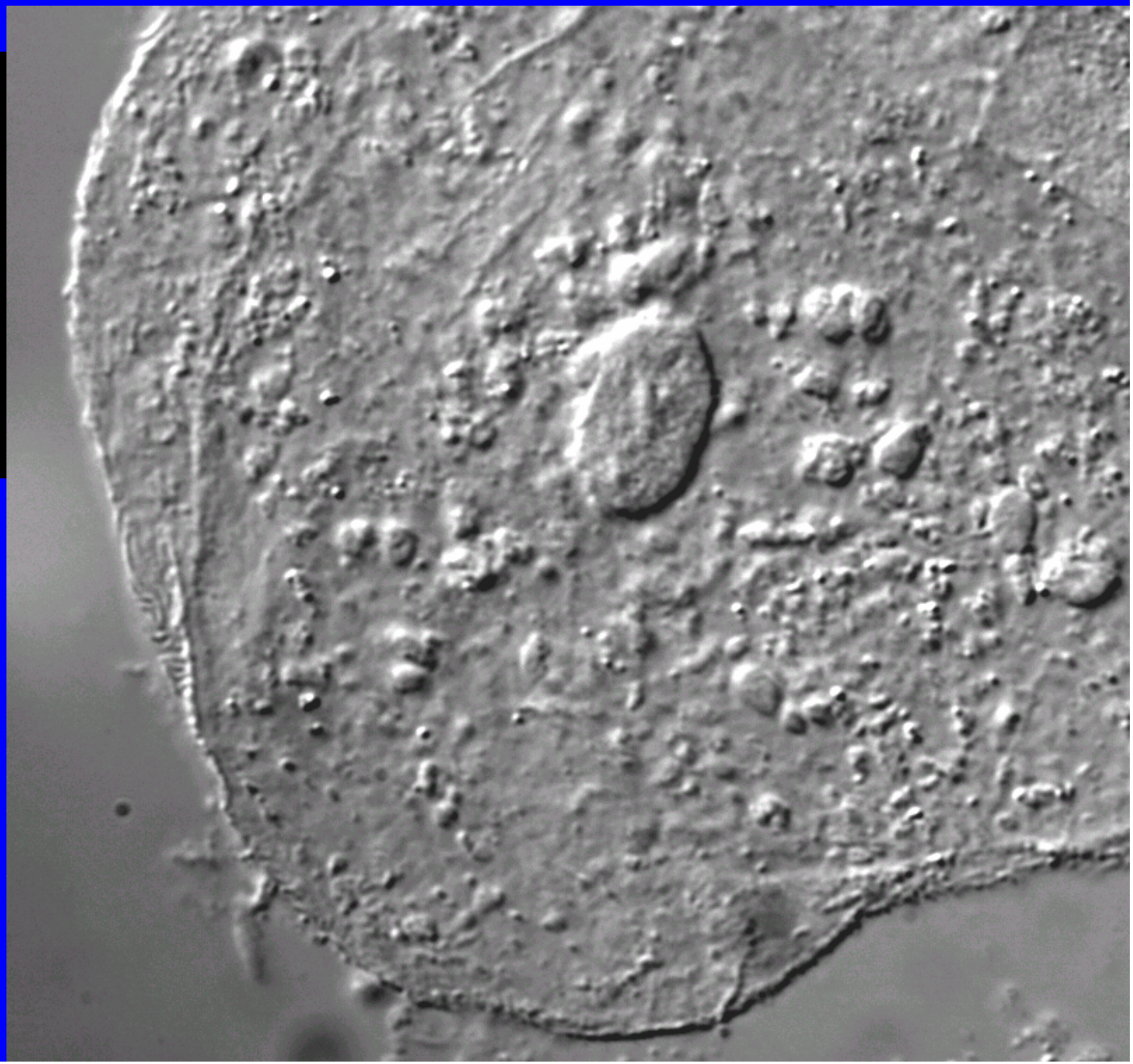


Image Intensity for Test Specimen With No Compensation

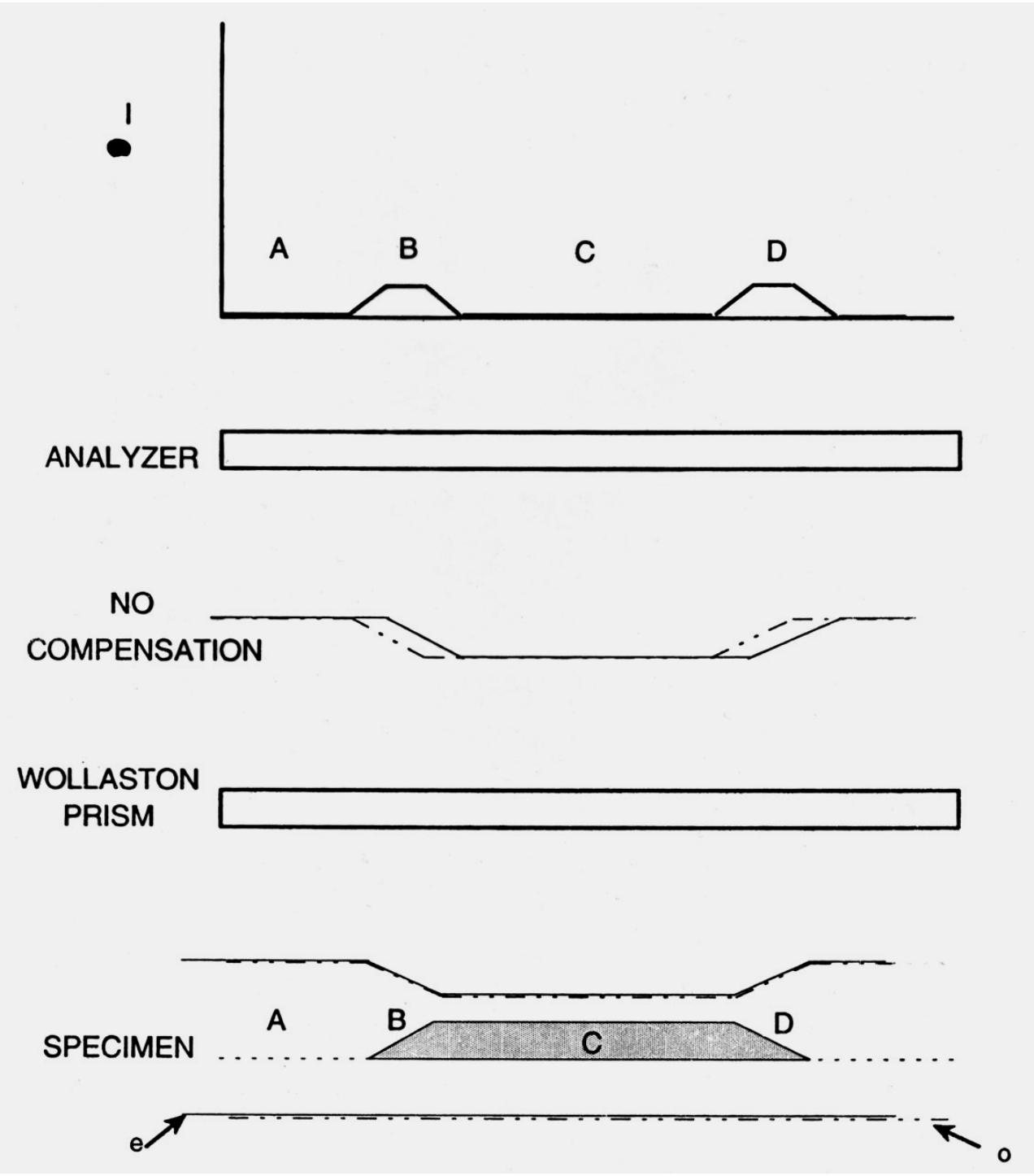


Image Intensity for Test Specimen With Plus Compensation

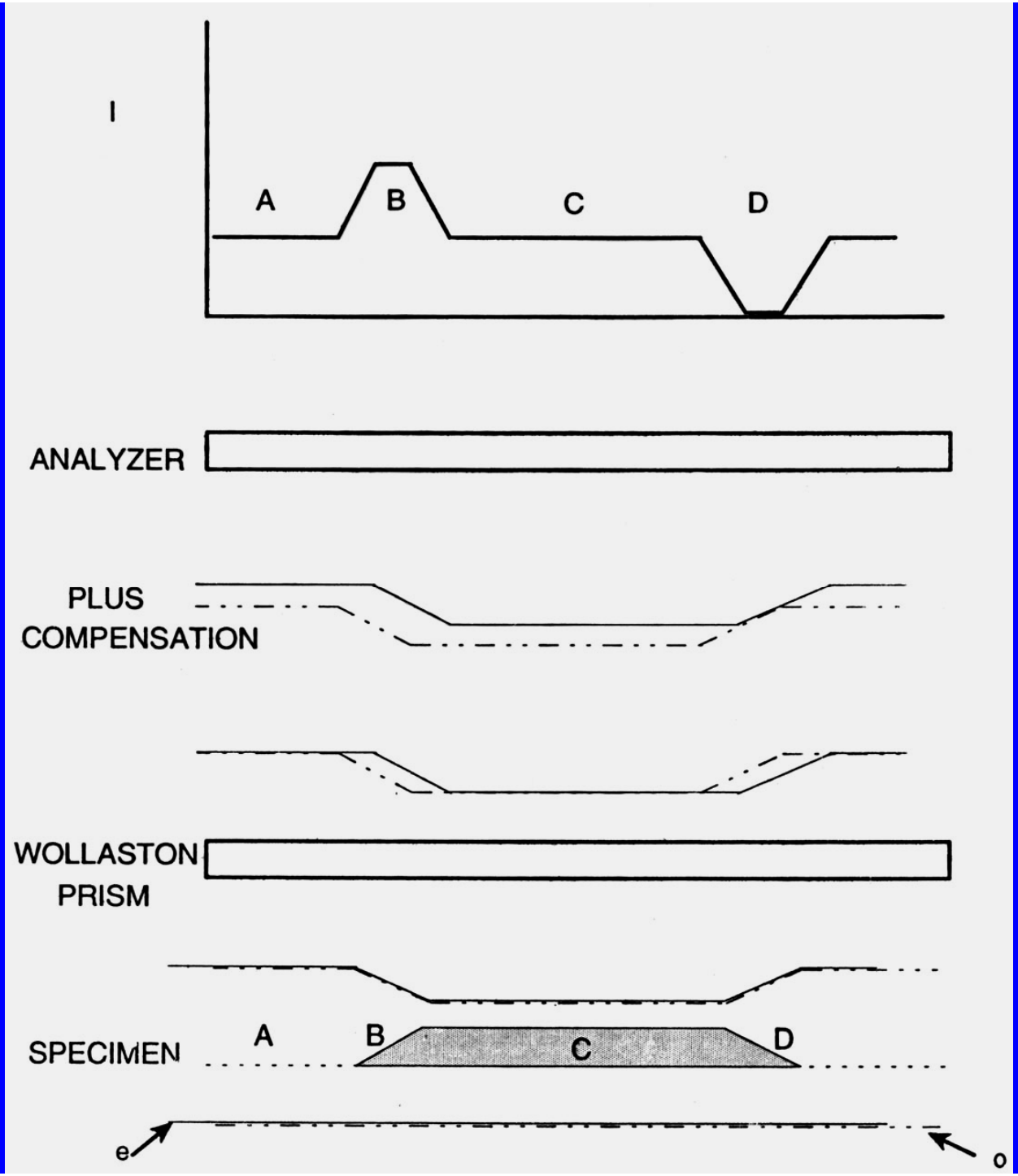
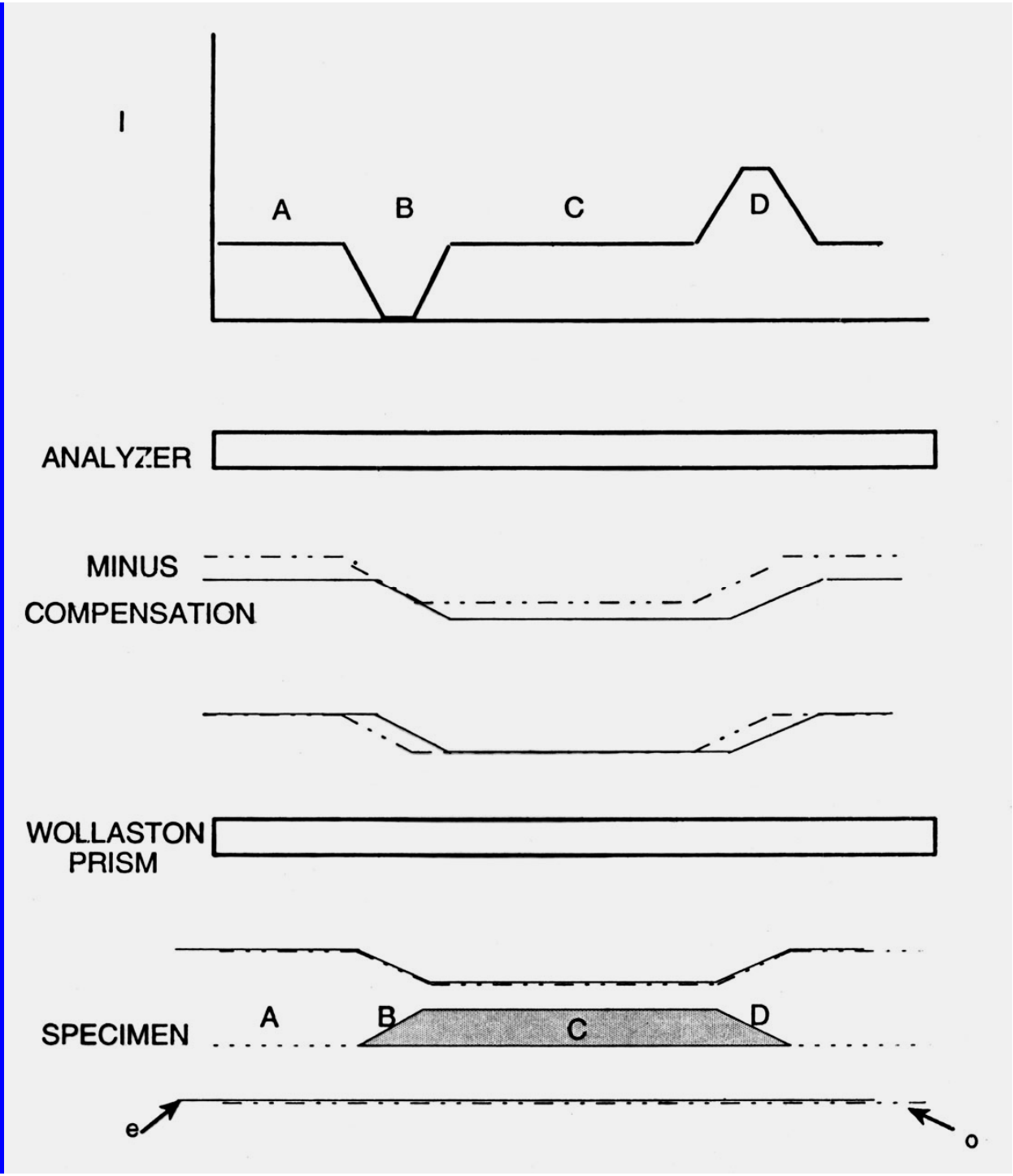
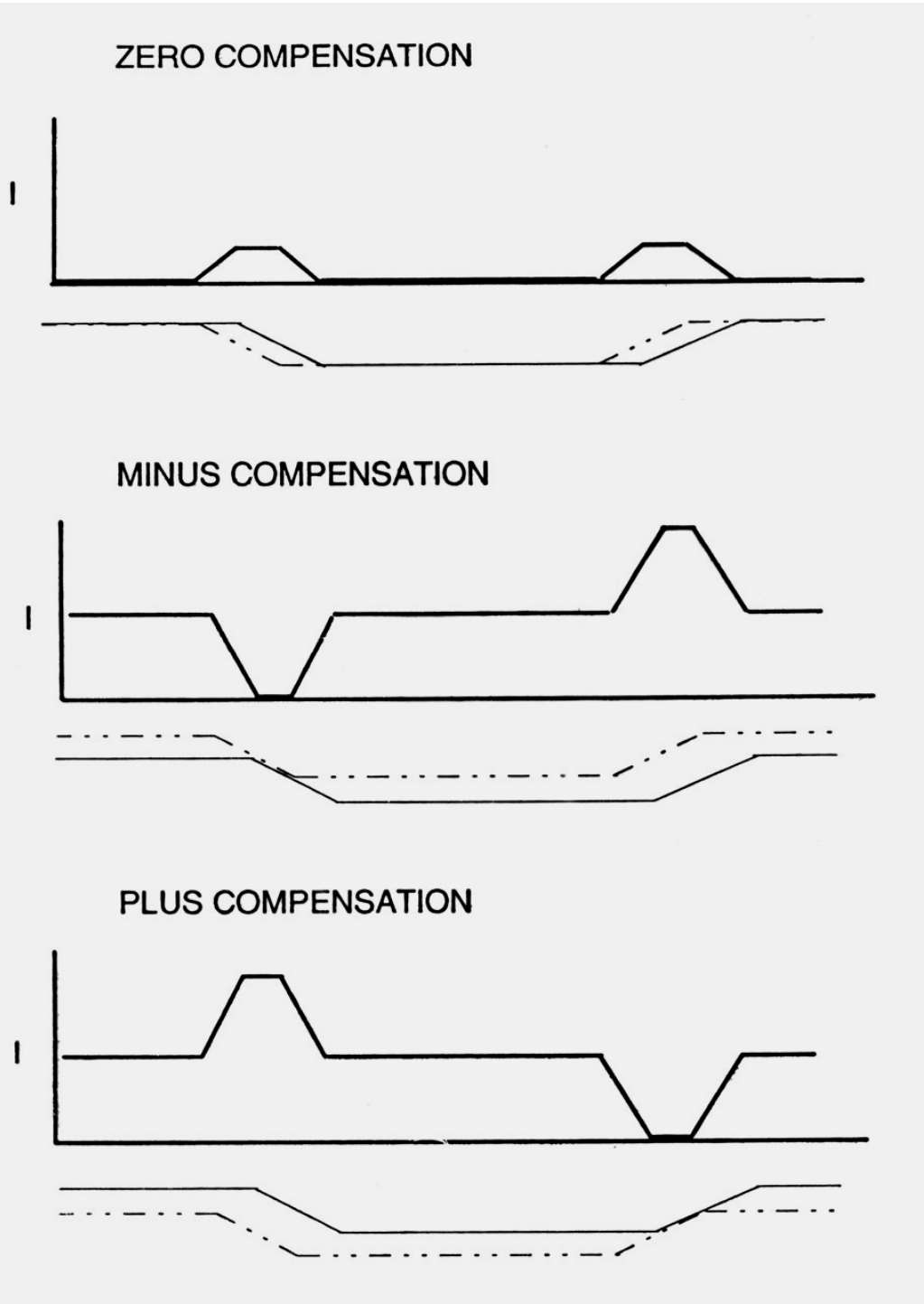


Image Intensity for Test Specimen With Minus Compensation

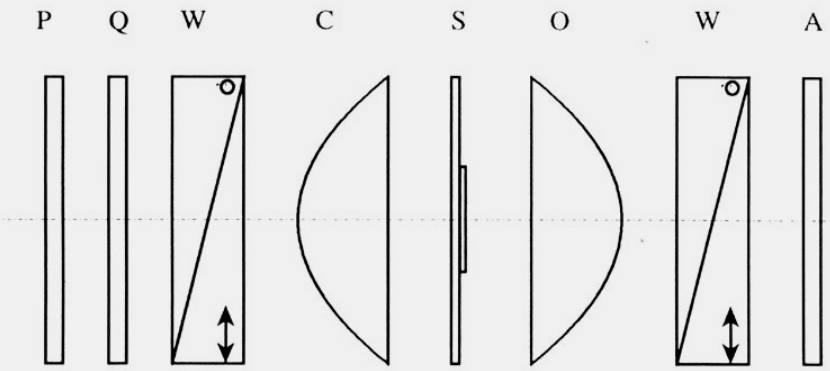


Comparison of DIC Image Intensity for Test Specimen With No, Plus and Minus Compensation



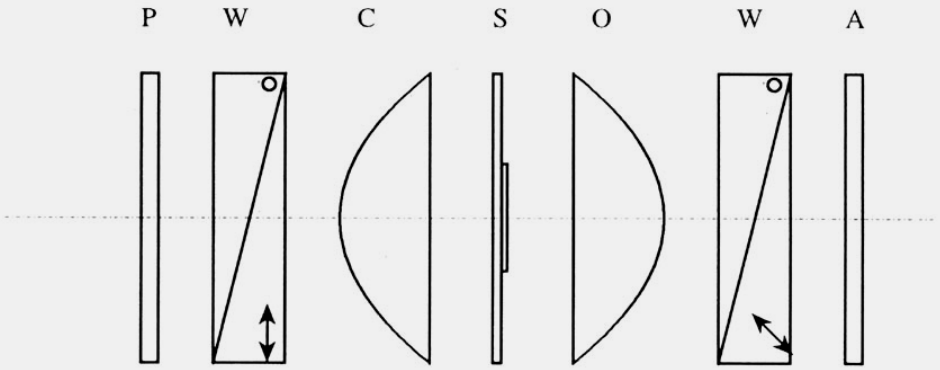
Two Types of Compensation Are Used For DIC Microscopes

SMITH DIC



ROTATE POLARIZER FOR COMPENSATION

NOMARSKI DIC

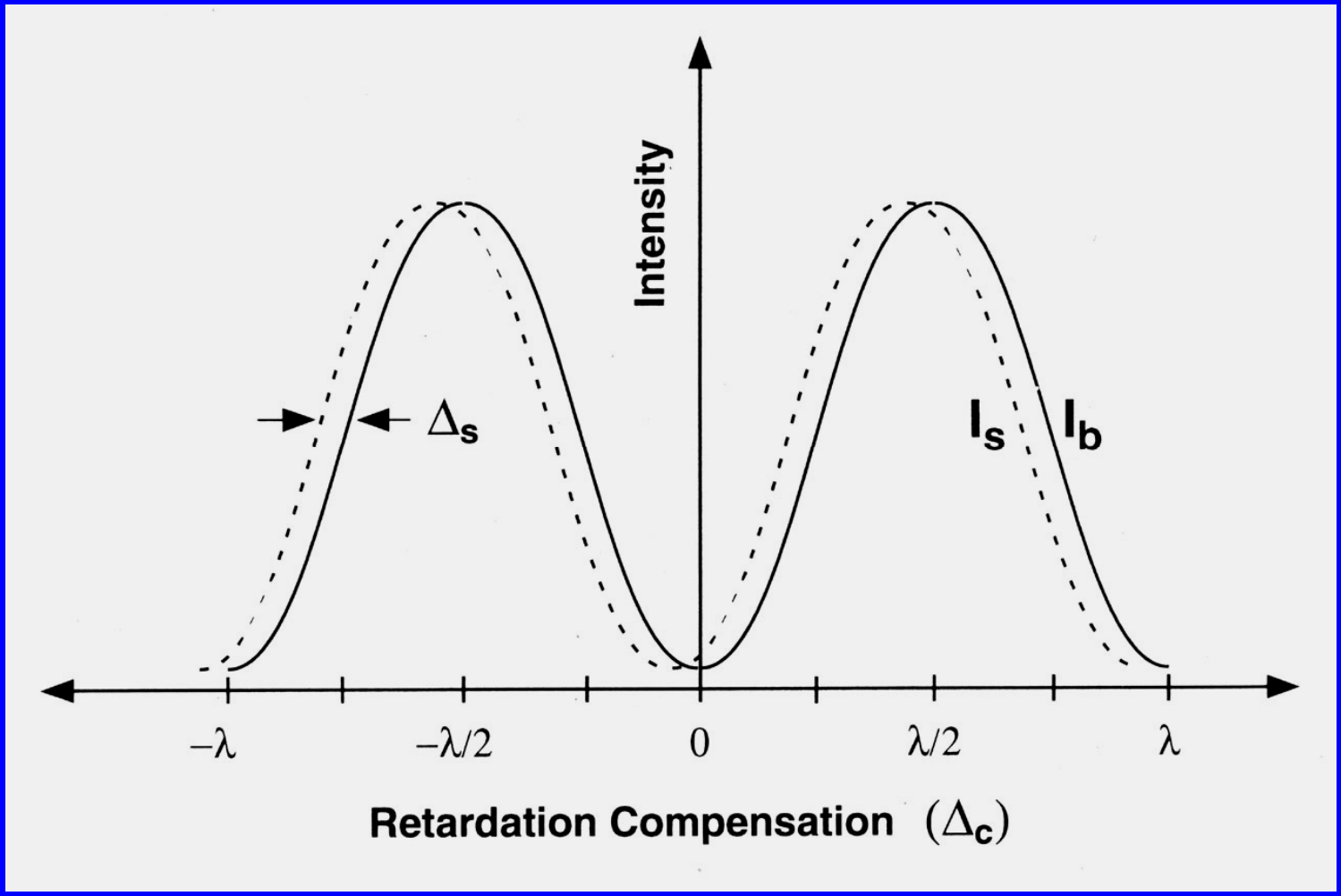


TRANSLATE OBJECTIVE WOLLASTON
FOR COMPENSATION

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

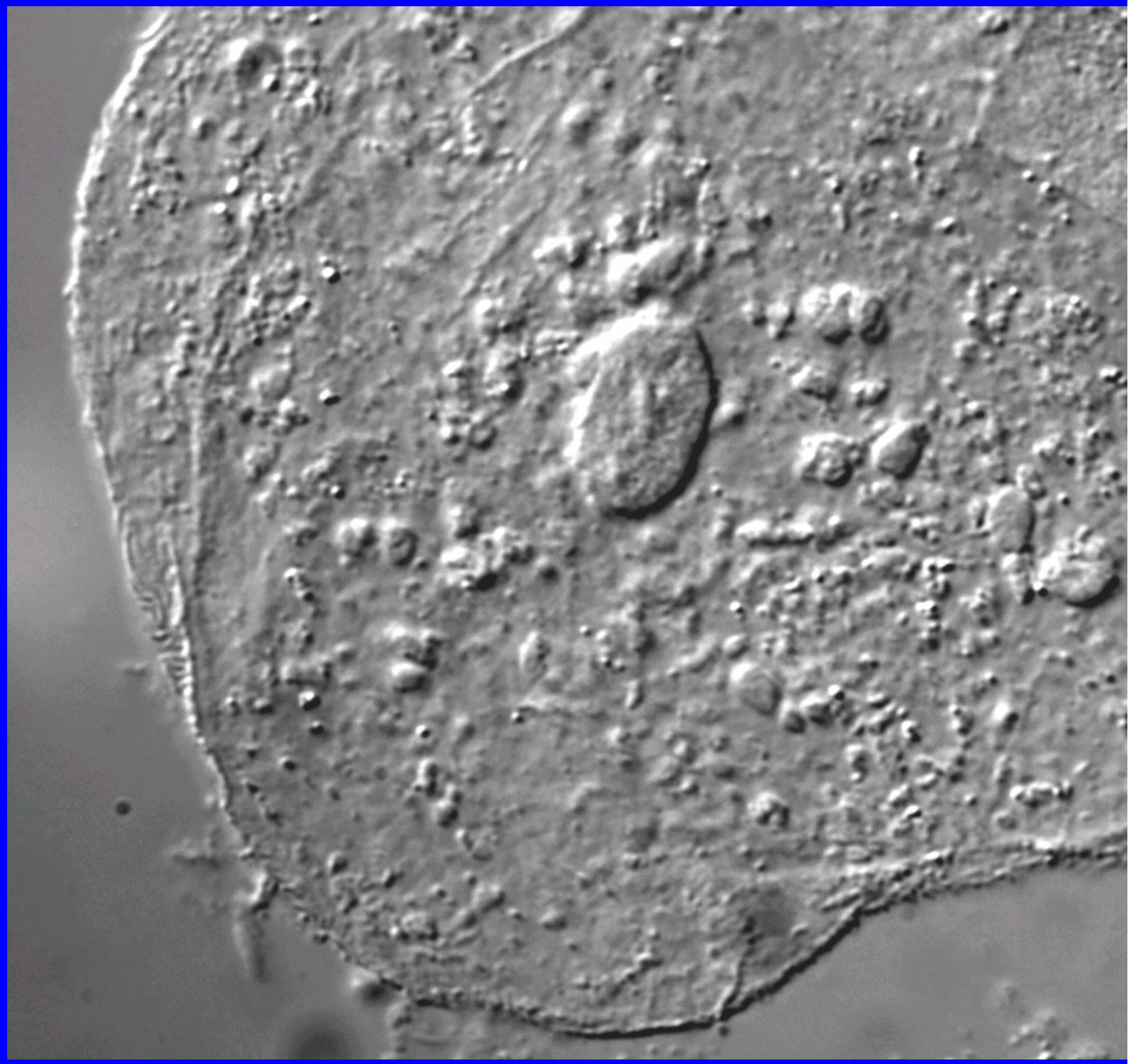


$$I_{sp} = I_c + I_p \sin^2((\Delta_{comp} + \Delta_{sp})/2) \quad I_{bg} = I_c + I_p \sin^2(\Delta_{comp}/2)$$

For Maximum Contrast:

1) Adjust Compensation So One Edge of Specimen Detail is Near Extinction; or

2) Use About λ/10- λ/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:

  $r = \lambda_o/(NA_{obj} + NA_{cond}) = 0.5\ \lambda_o/NA_{obj}$

  when $NA_{cond} = NA_{obj}$

- For $NA_{obj} = 1.4$ and $\lambda_o = 550$ nm:

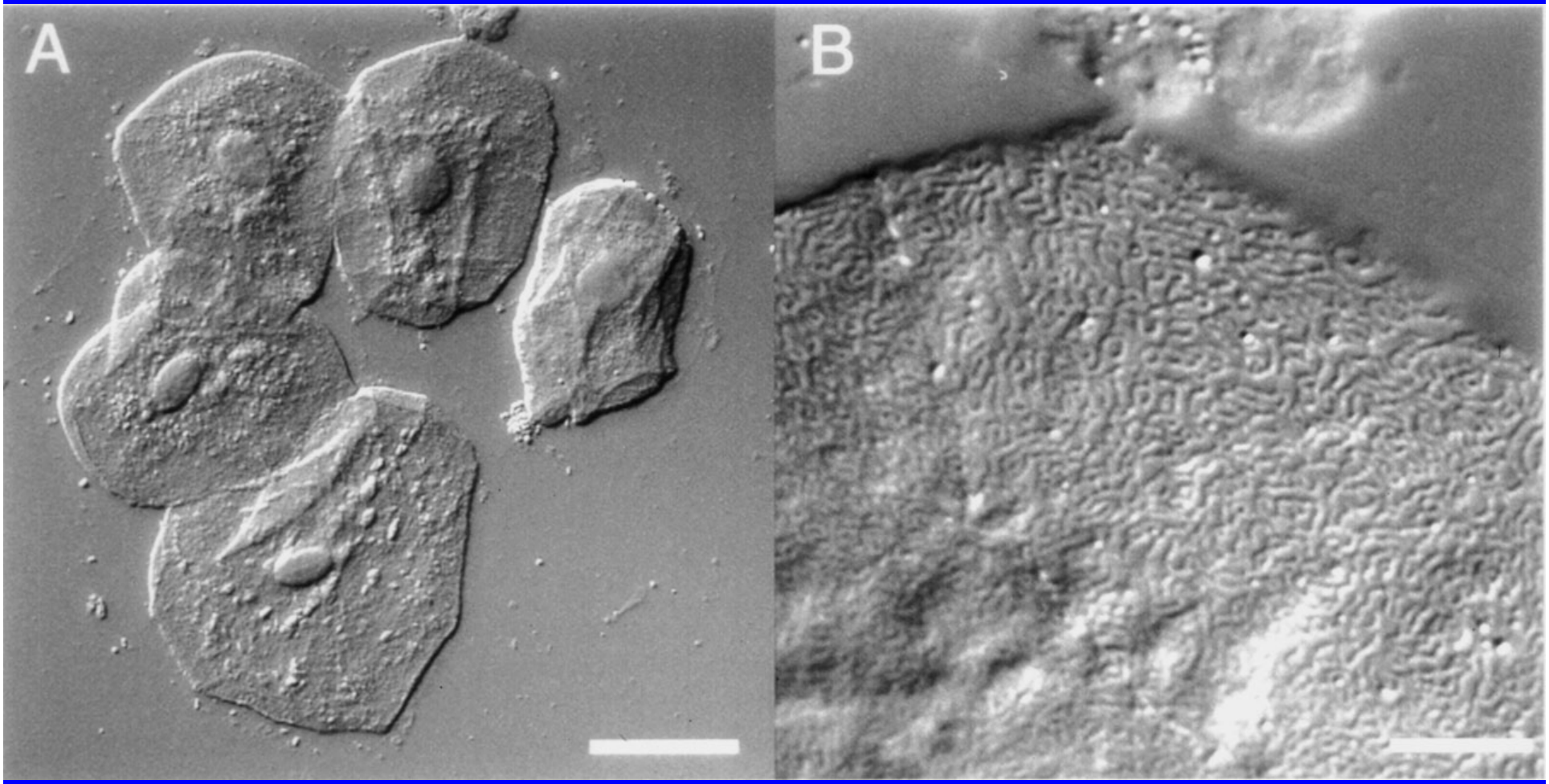
  $r = 190$ nm

- This resolution limit corresponds to a maximal resolvable spatial frequency:

  $fsmax = 1/r = 5.1$ cycles/μm

- A shear of ~r/2 will give the maximum retardation (and contrast) between the e and o wavefronts at fsmax; let's see how……

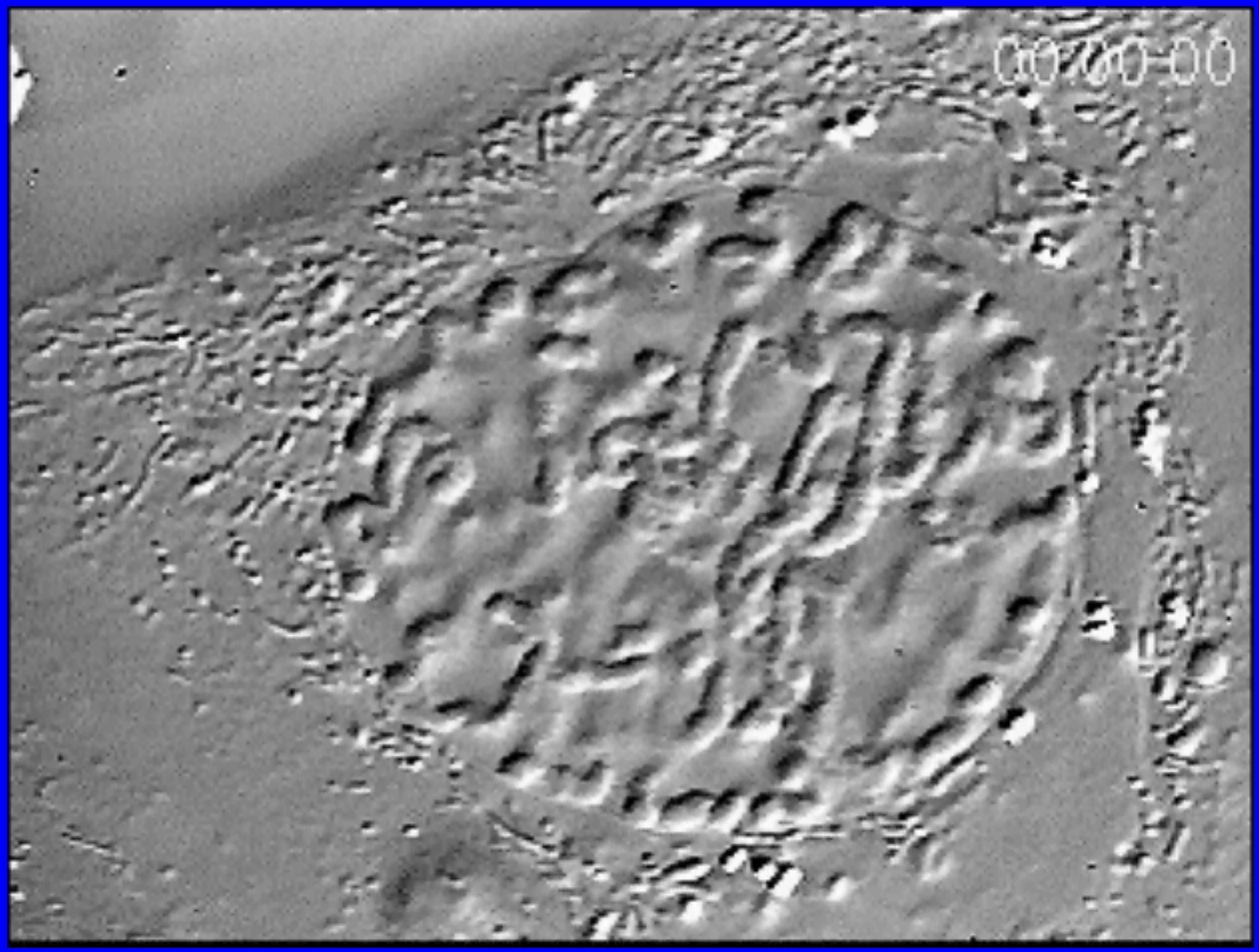
Use Cheek Cells for Contrast and Resolution Test



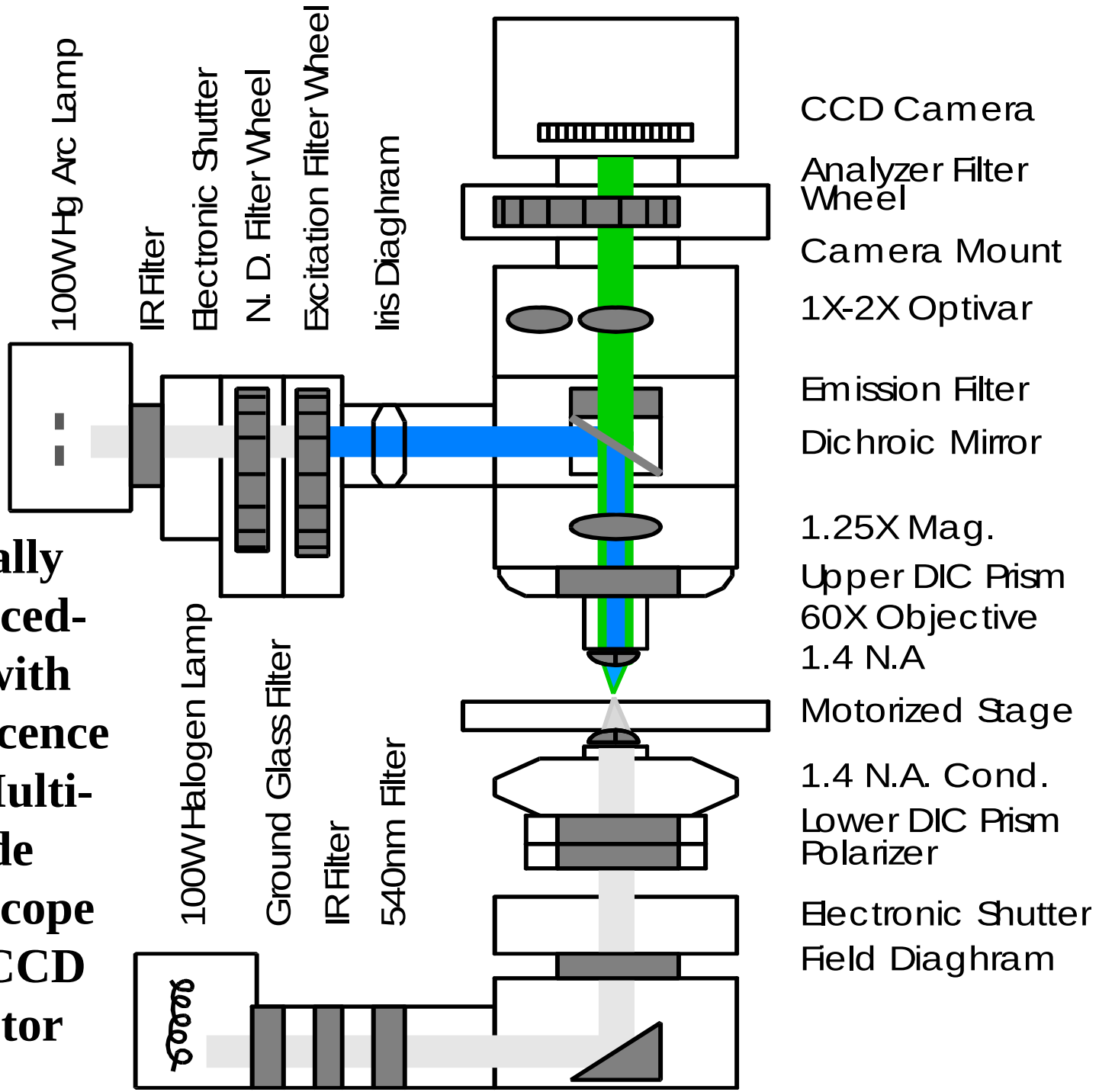
A. 20X/NA = .45 Objective

B. 100X/NA = 1.4 DIC Objective

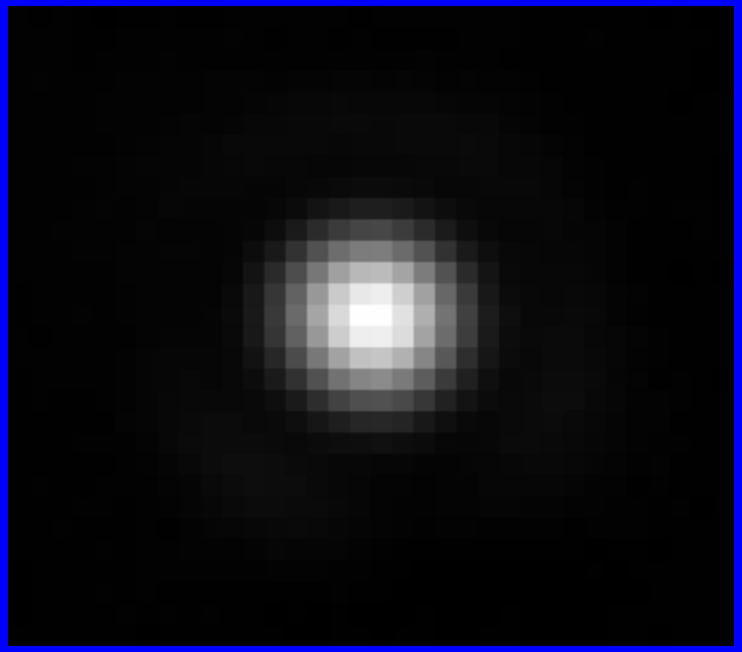
Example DIC: Mitosis in Mitotic Newt Lung Cells



Digitally Enhanced-DIC with Fluorescence In A Multi-Mode Microscope With CCD Detector

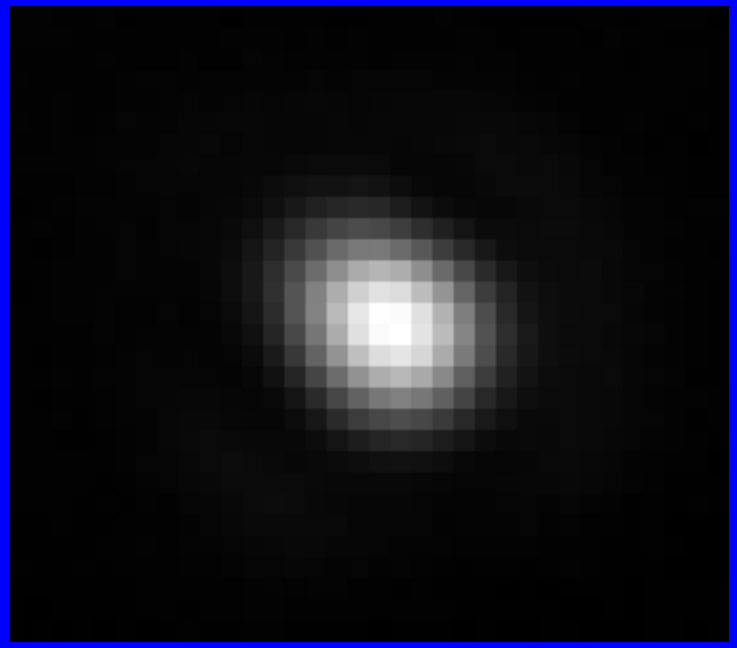


Fluorescent Images of 200nm bead: 100x/NA=1.4, detector pixel scale = .065 nm



No DIC Prism

Peak = 3650

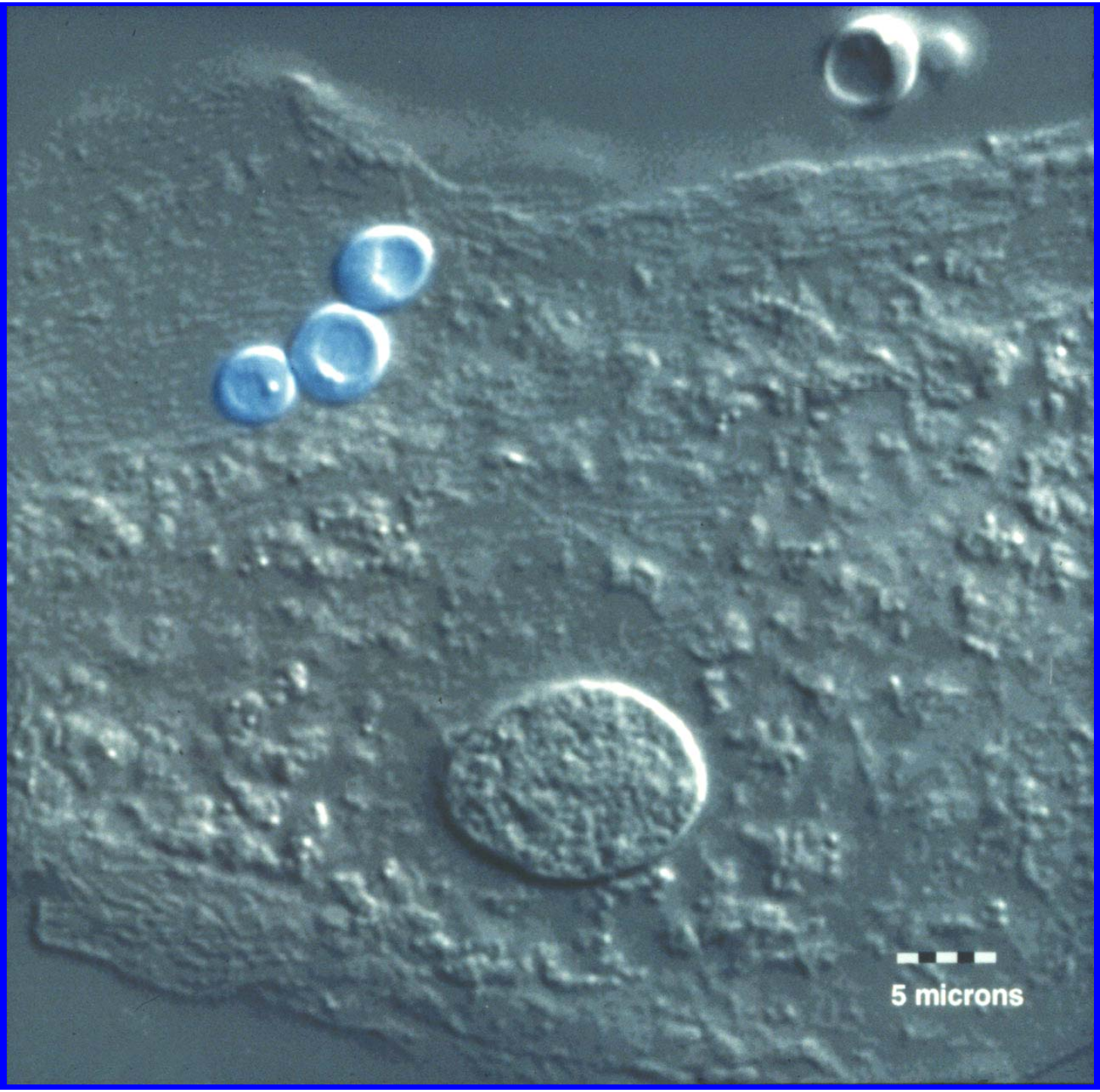


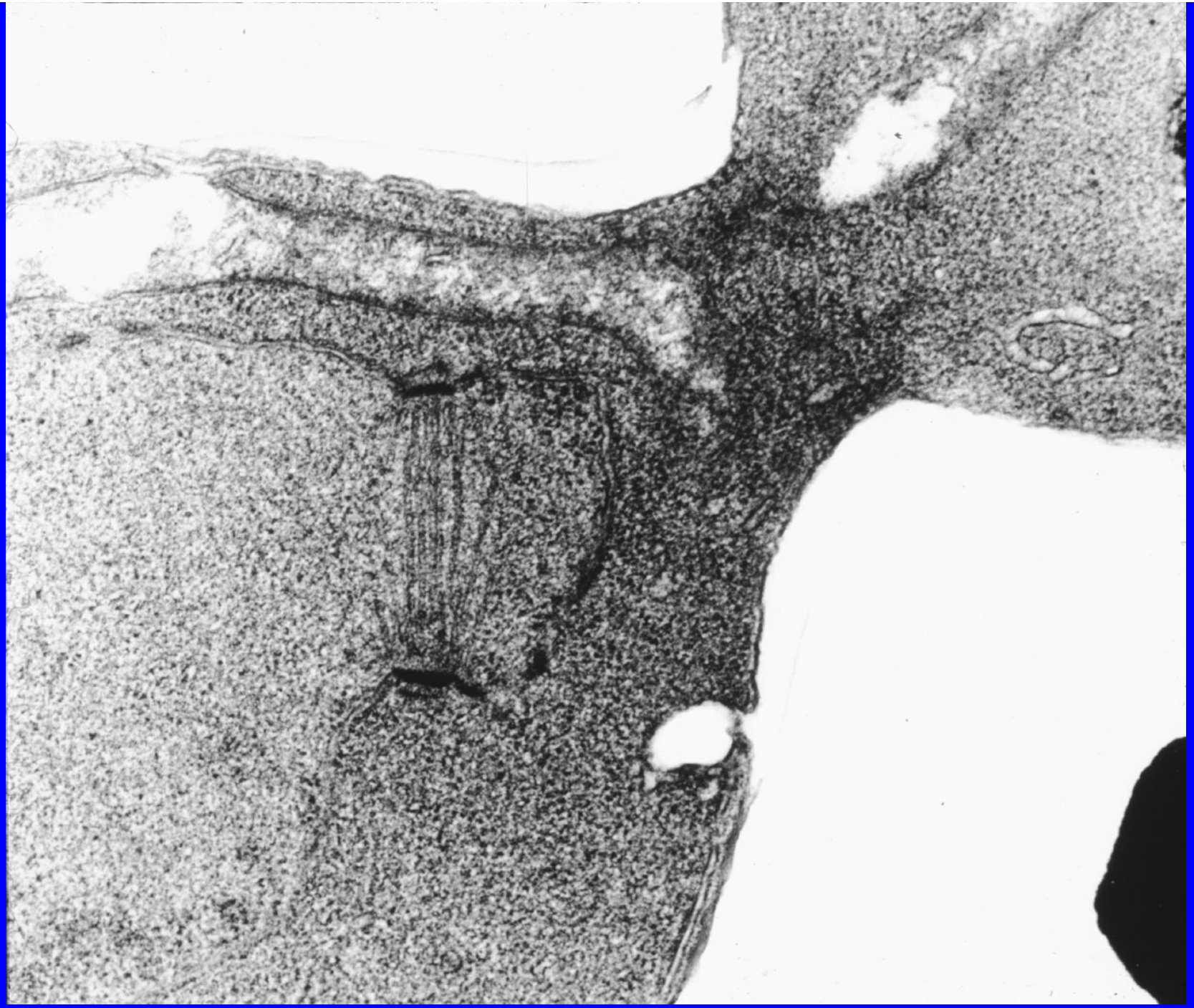
With DIC Prism

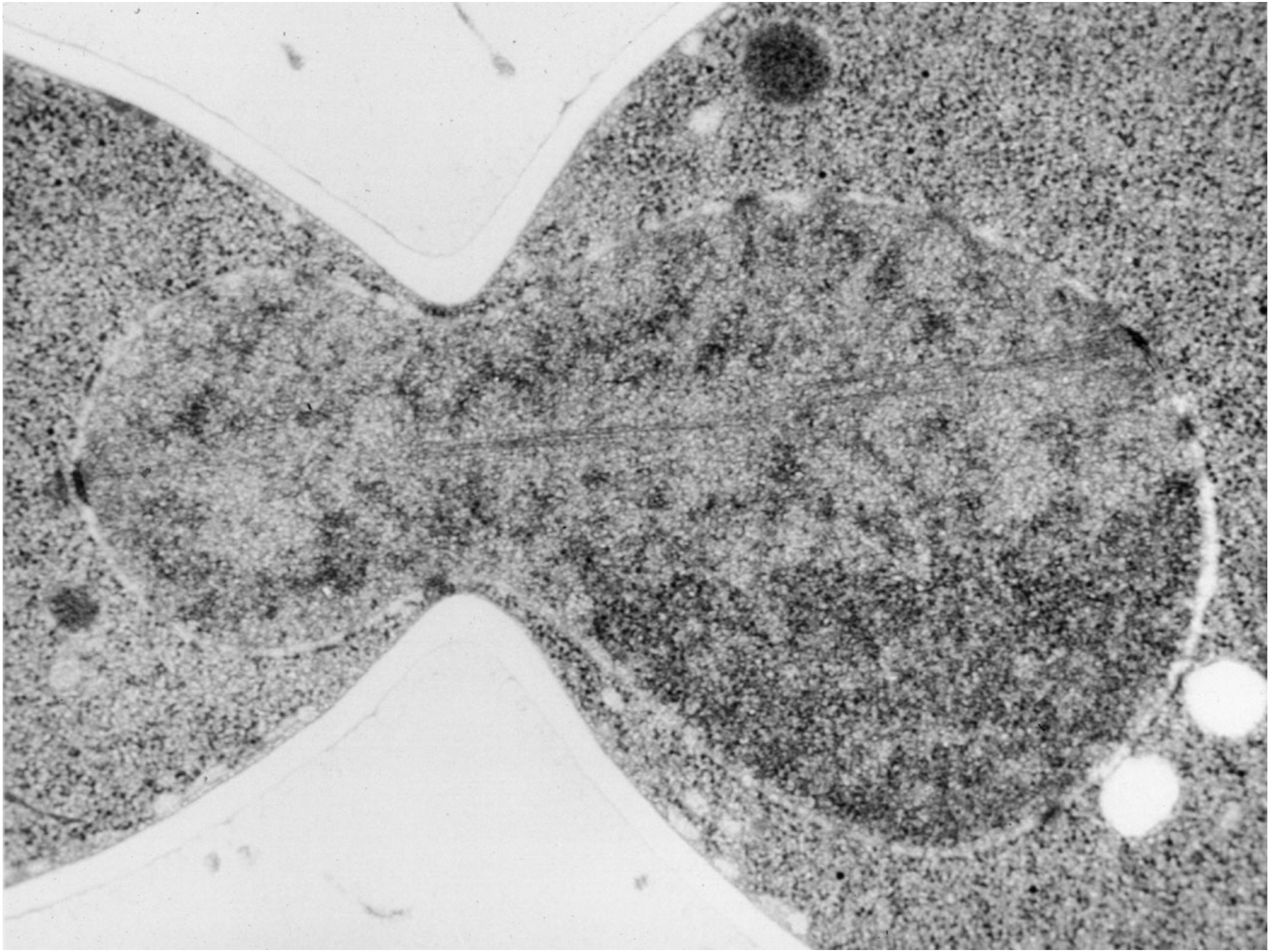
Peak = 2710 (75%)

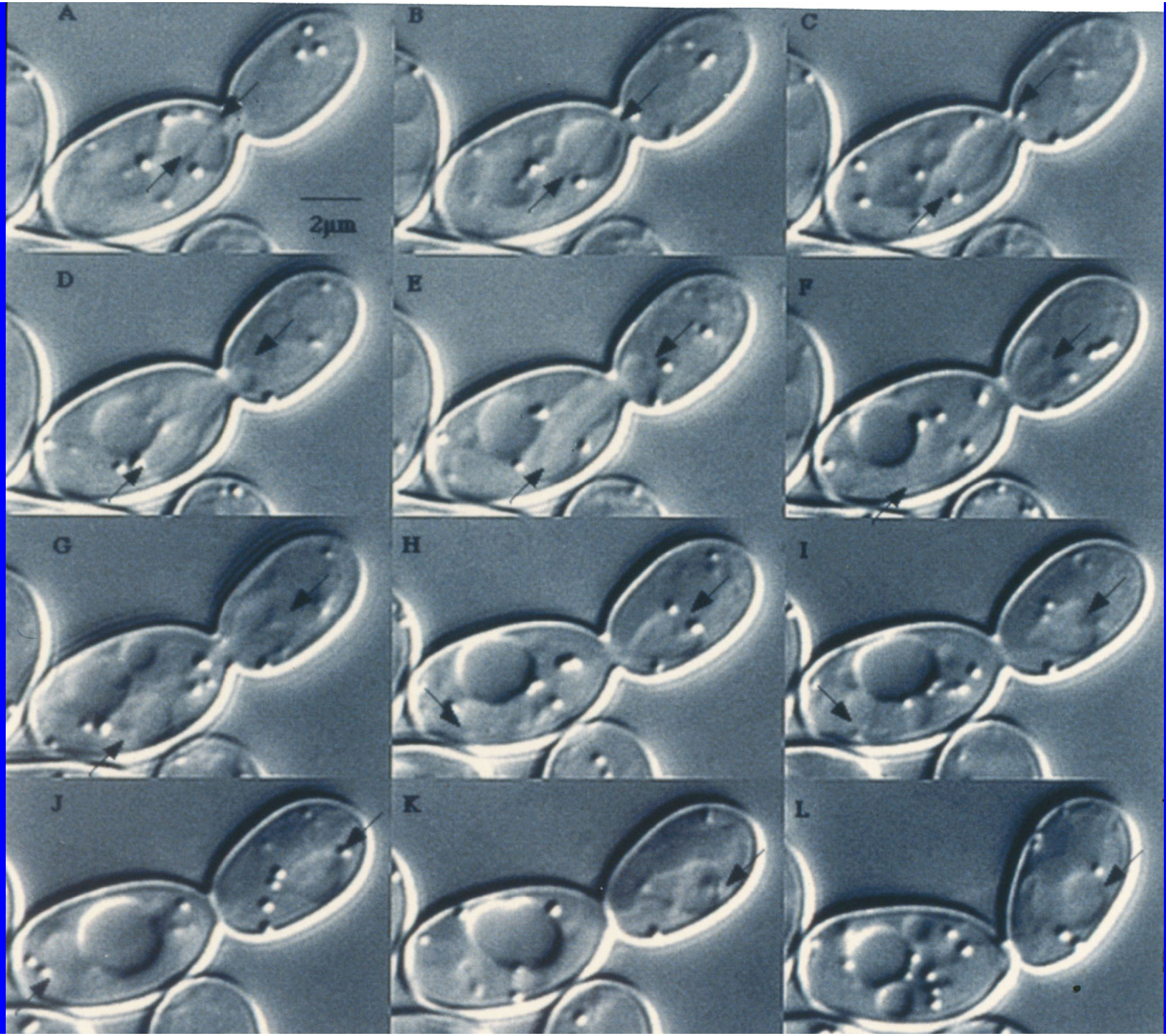
Yeast Digital Imaging System(s)

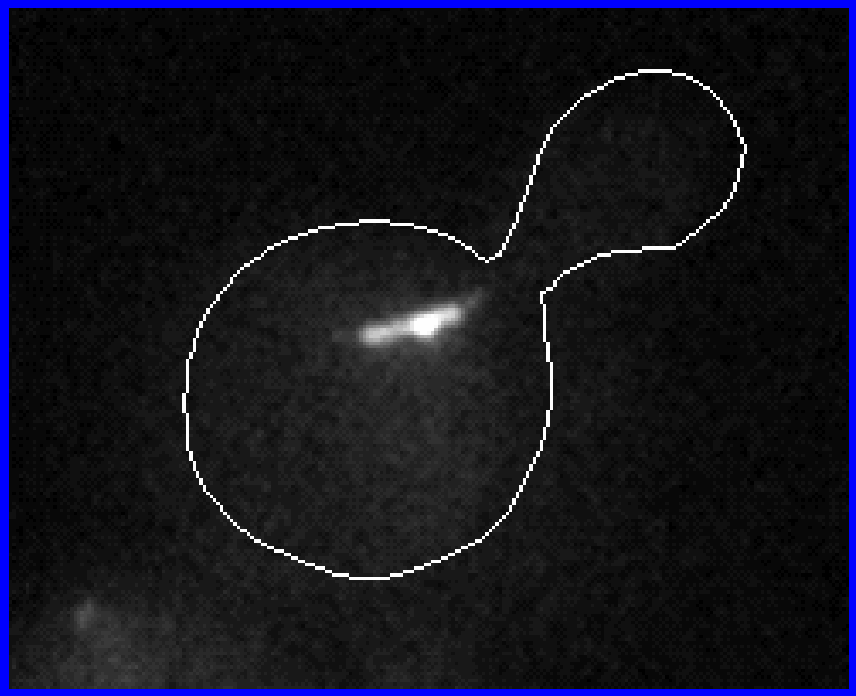
Kerry Bloom Lab, UNC-CH


5 microns



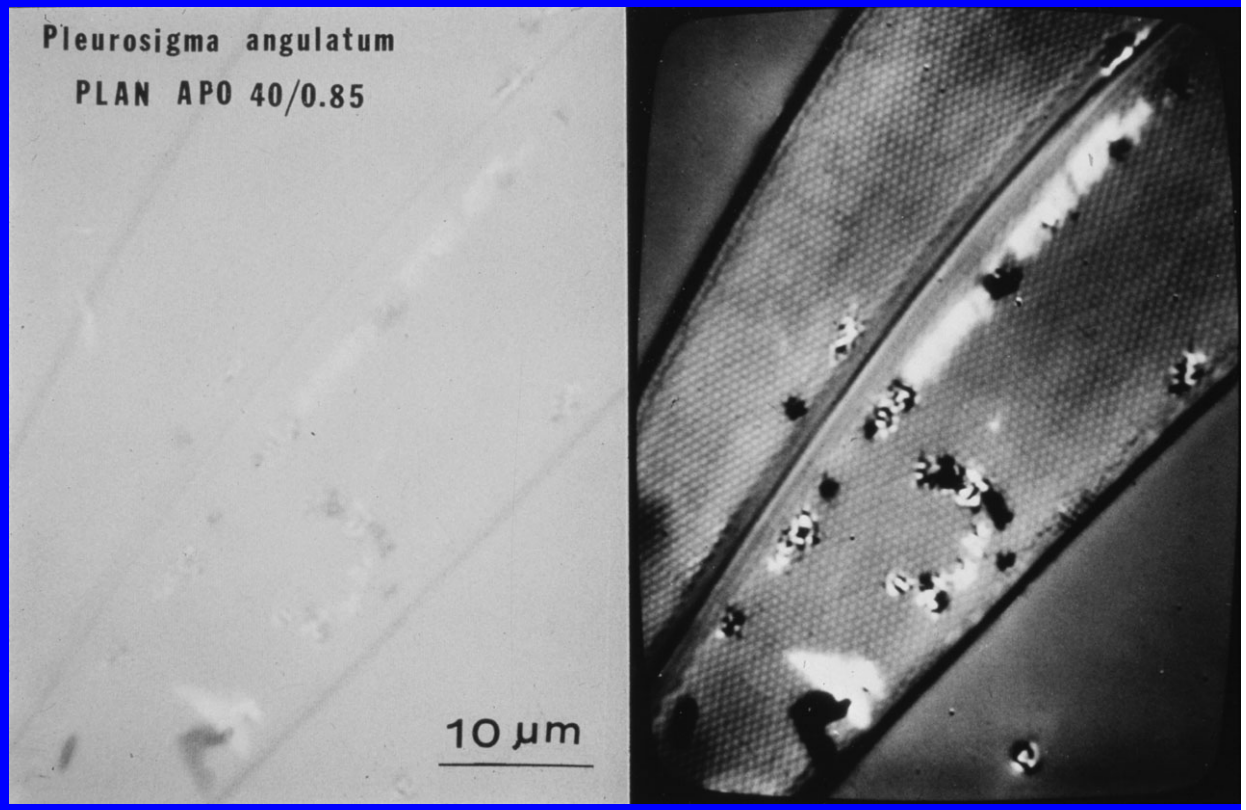



A
B
C
2µm
D
E
F
G
H
I
J
K
L

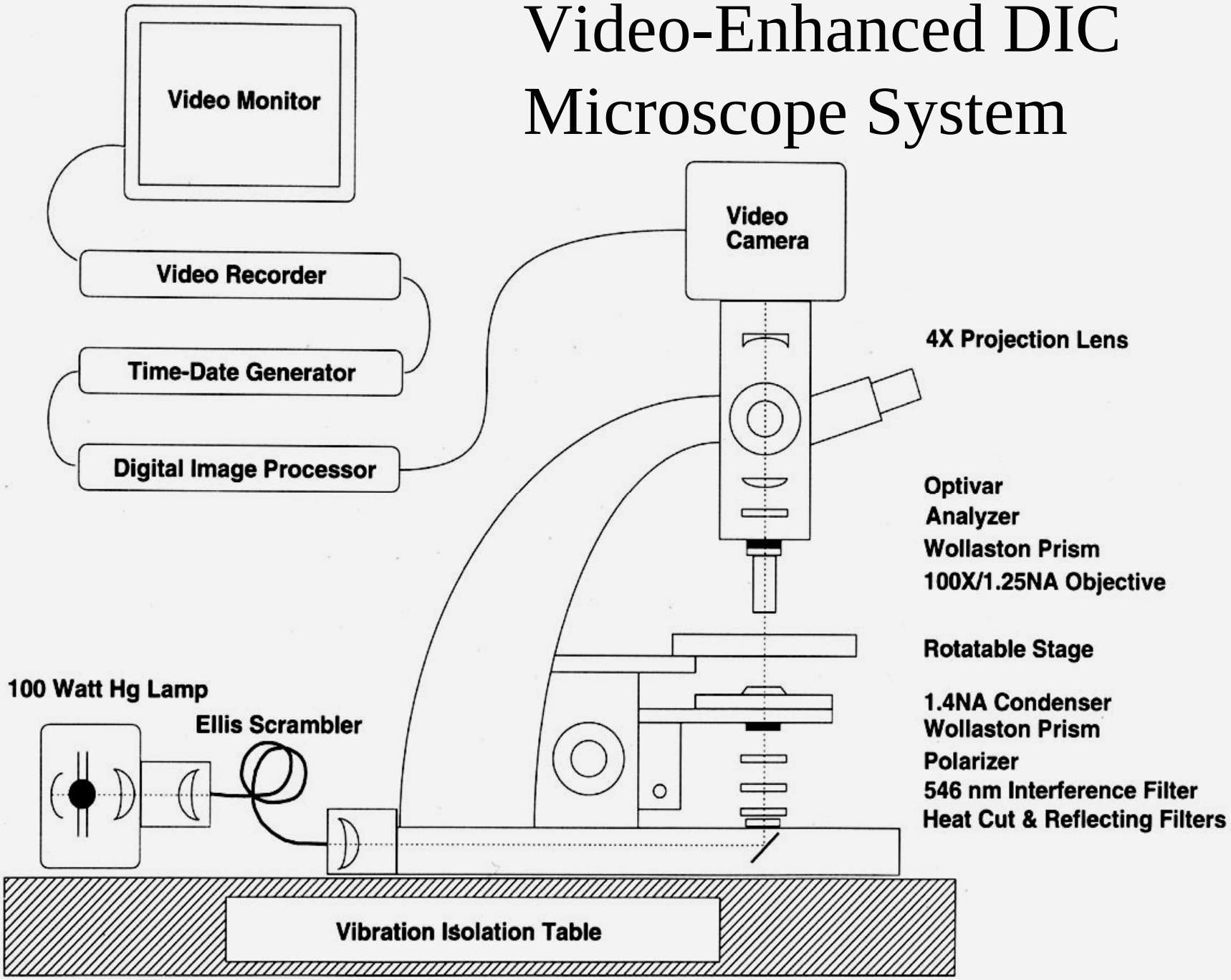


Pearson et al., 2001, JCB

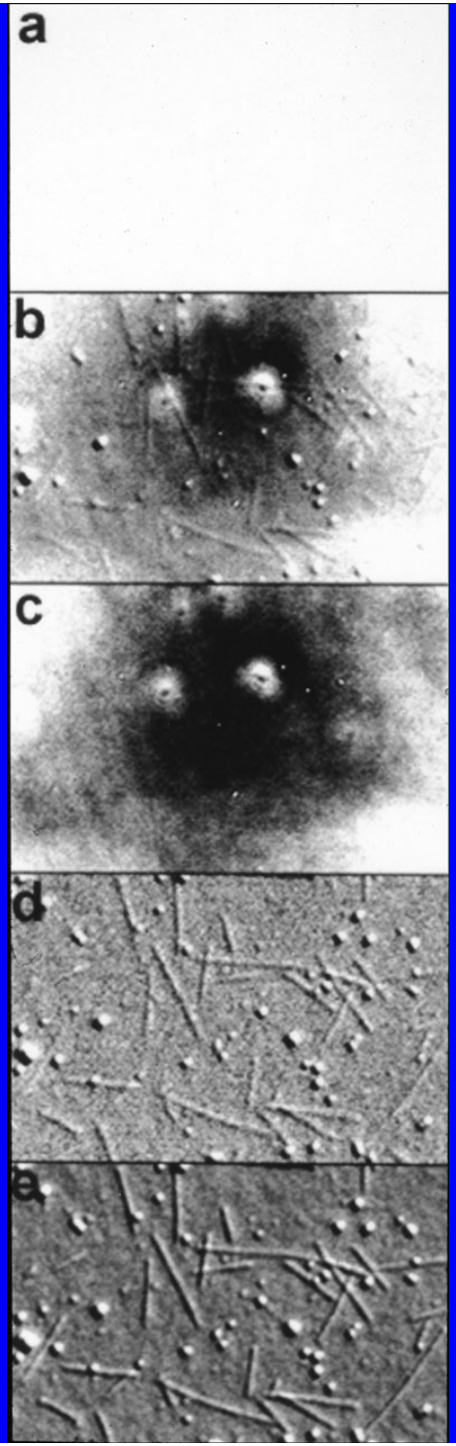
Video-Enhanced Contrast Methods Developed in Early 1980's by Inoue and Allen Revealed Cellular Structures and Macromolecular Complexes Invisible by Eye or Film



Video-Enhanced DIC Microscope System



Practical Example: VE-DIC of Isolated Microtubules



View by eye

Analog Contrast Enhancement Live Image

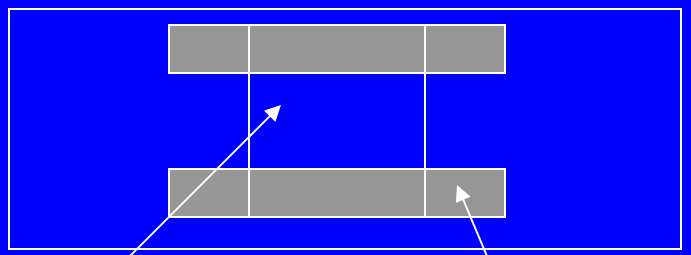
De-focus Slightly; Acquire Background Image and Store into Frame Buffer

Subtract Background from Live Image at Video Rates

Increase Contrast Digitally

Preparations for Motility Assays

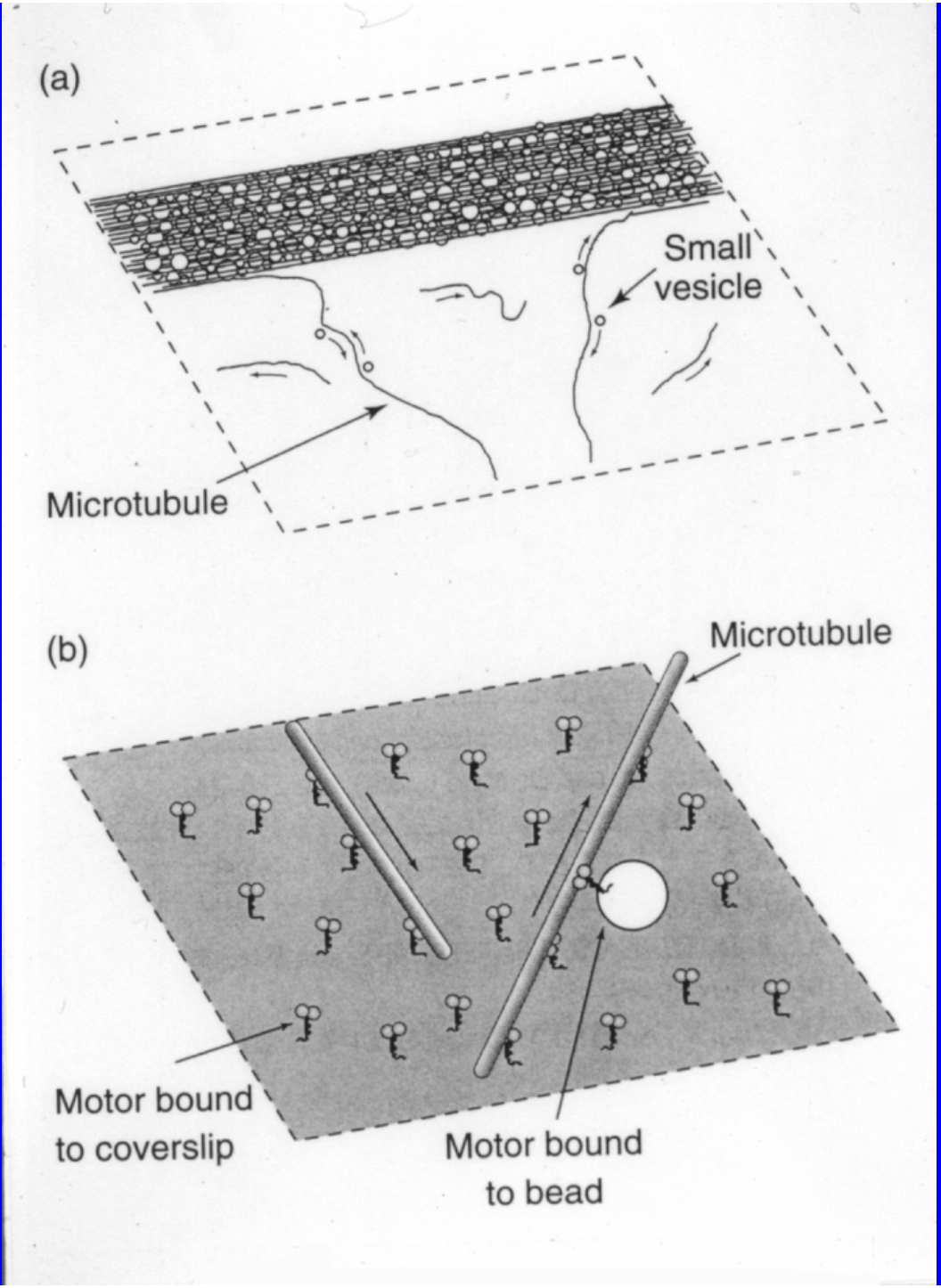
Slide



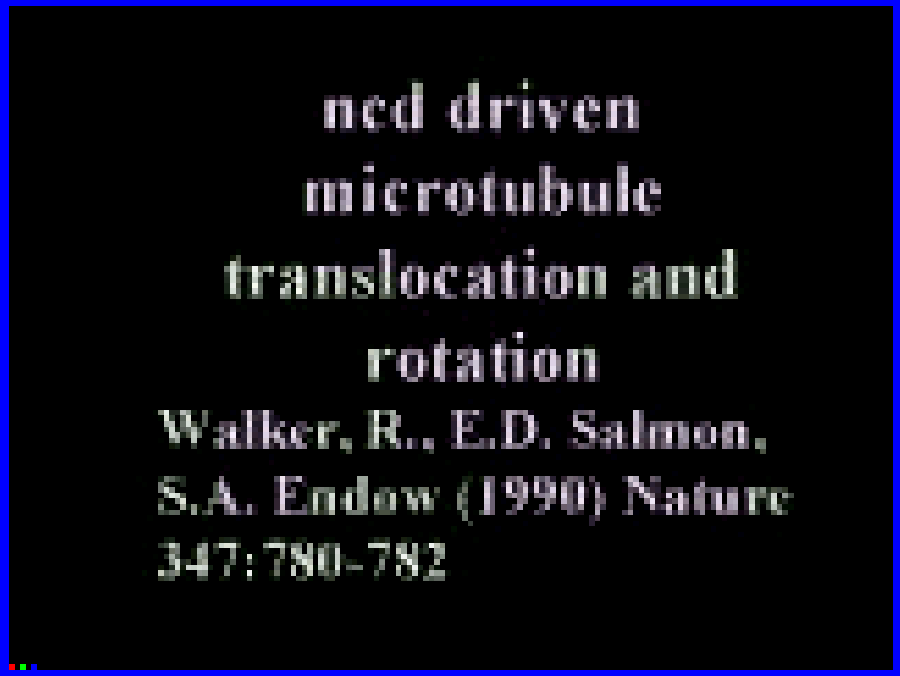
#1.5 Coverslip

70mm Thick Double-Stick Tape

Perfusion Chamber



VE-DIC Microtubule Motility Assay for Minus-Kinesin ncd



(3.3 rotations/um forward movement)

Color DIC with Full Wave (RED) Plate