

# Bi177 - Lecture 9 Contrast, Resolution & AVEC

Review of Nomarski Contrast

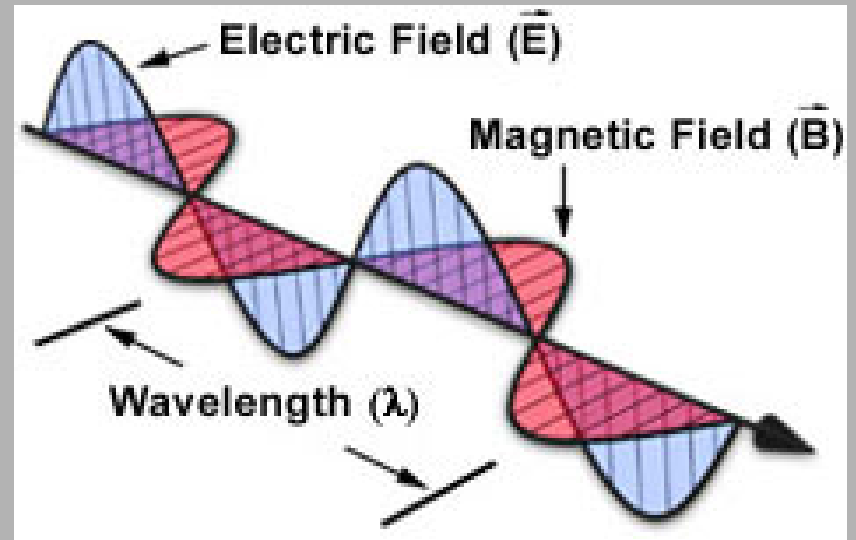
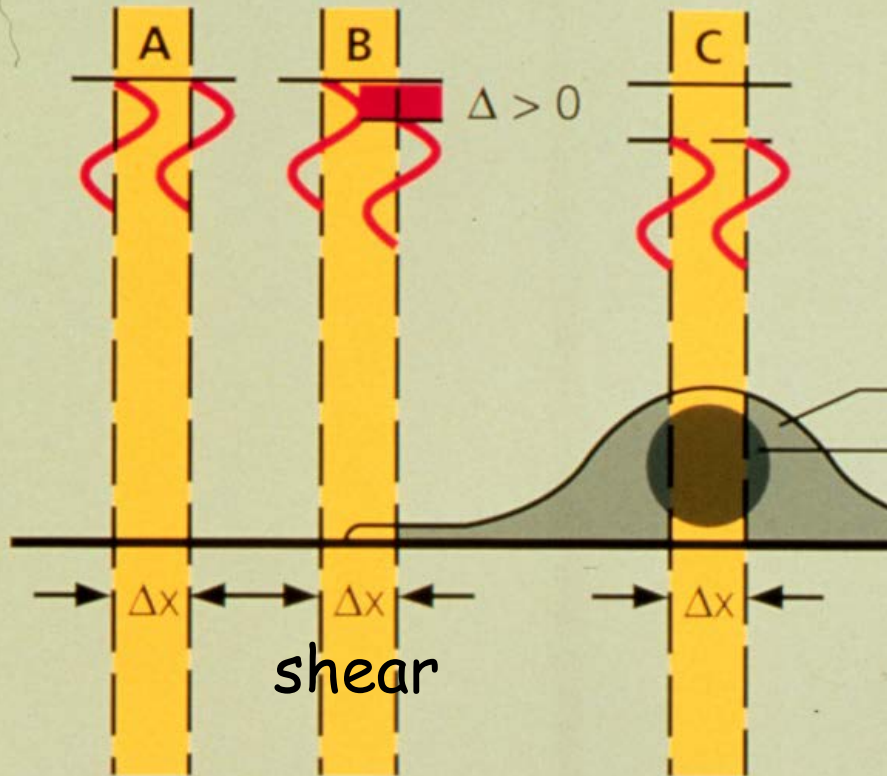
AVEC Microscopy

Structured Illumination

Nomarski thought experiment:

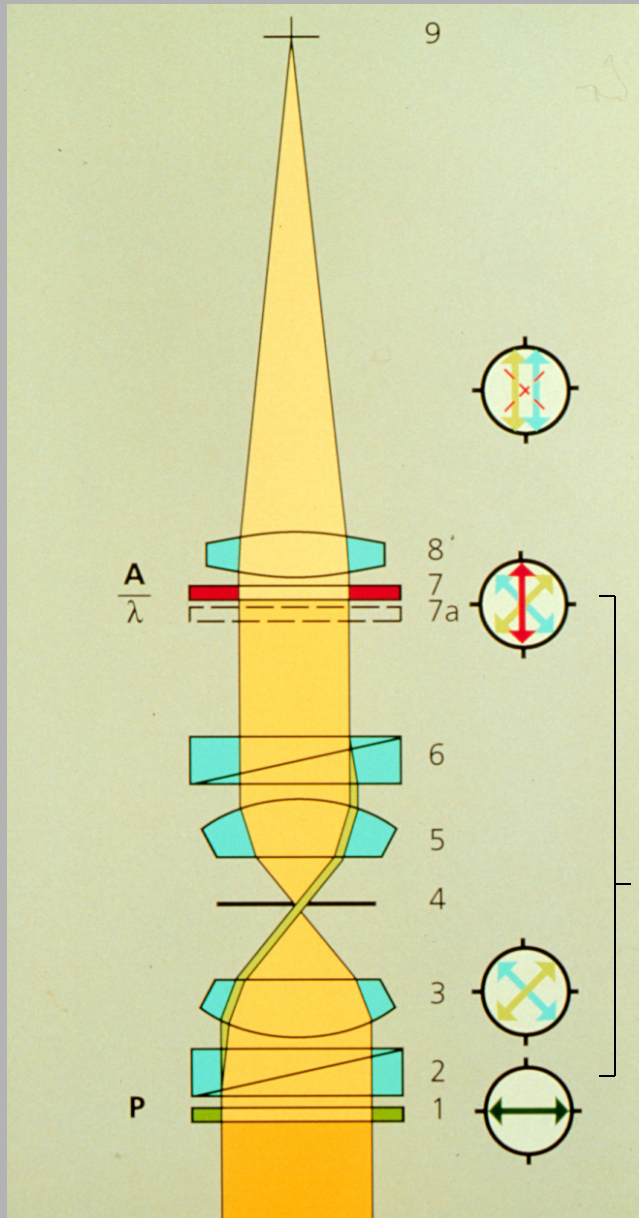
- Need two different labeled light rays
- Pass through specimen independently
- Afterwards, let them interfere with one another

How to label them? How offset them (shear)?



Polarization as the label  
(light must be in same  
plane to interact)

# Nomarski - two beams labeled by plane of polarization



Analyzer - forces two beams into same plane

Wollaston prism - recombines two beams

Domain of independent paths

Wollaston prism - splits into two beams; adds shear

Polarizer - prepares for Wollaston prism 50-50 split

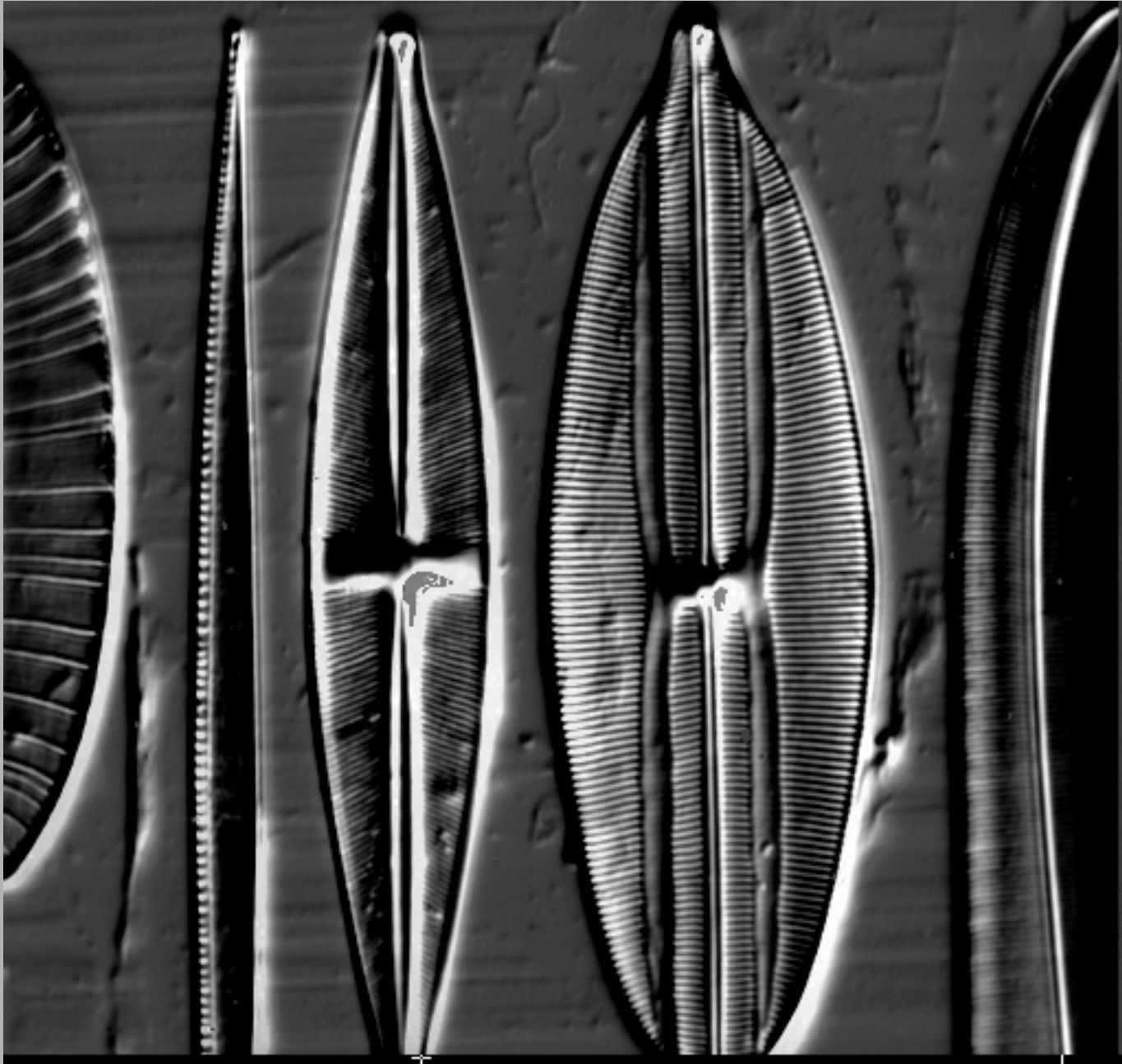
## AVEC - Allen Video Enhanced Microscopy



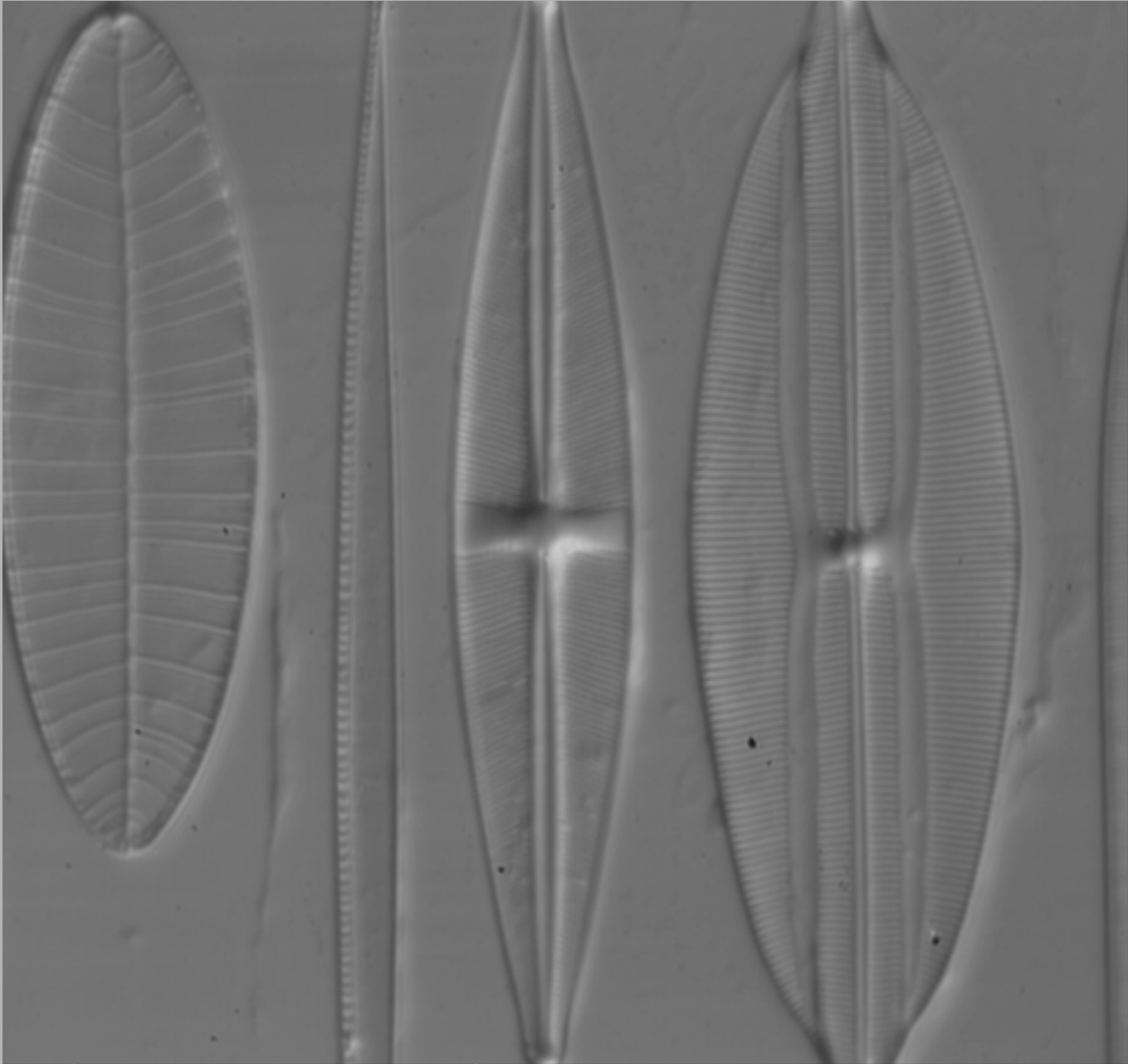
(an attempt to get higher contrast at the highest resolution)

First accomplished with video cameras in which the gain and black level can be adjusted (contrast & brightness)

Lovely electronically enhanced DIC image



Best standard DIC image by eye



## AVEC

Step 1: subtract baseline electronically (before ADC)

Must adjust before digitization, because

a dim stored image can't be rescued

(too few different digitized brightness levels)

Step 2: increase contrast to fill dynamic range

But this also emphasizes dirt and defects (Mottle)

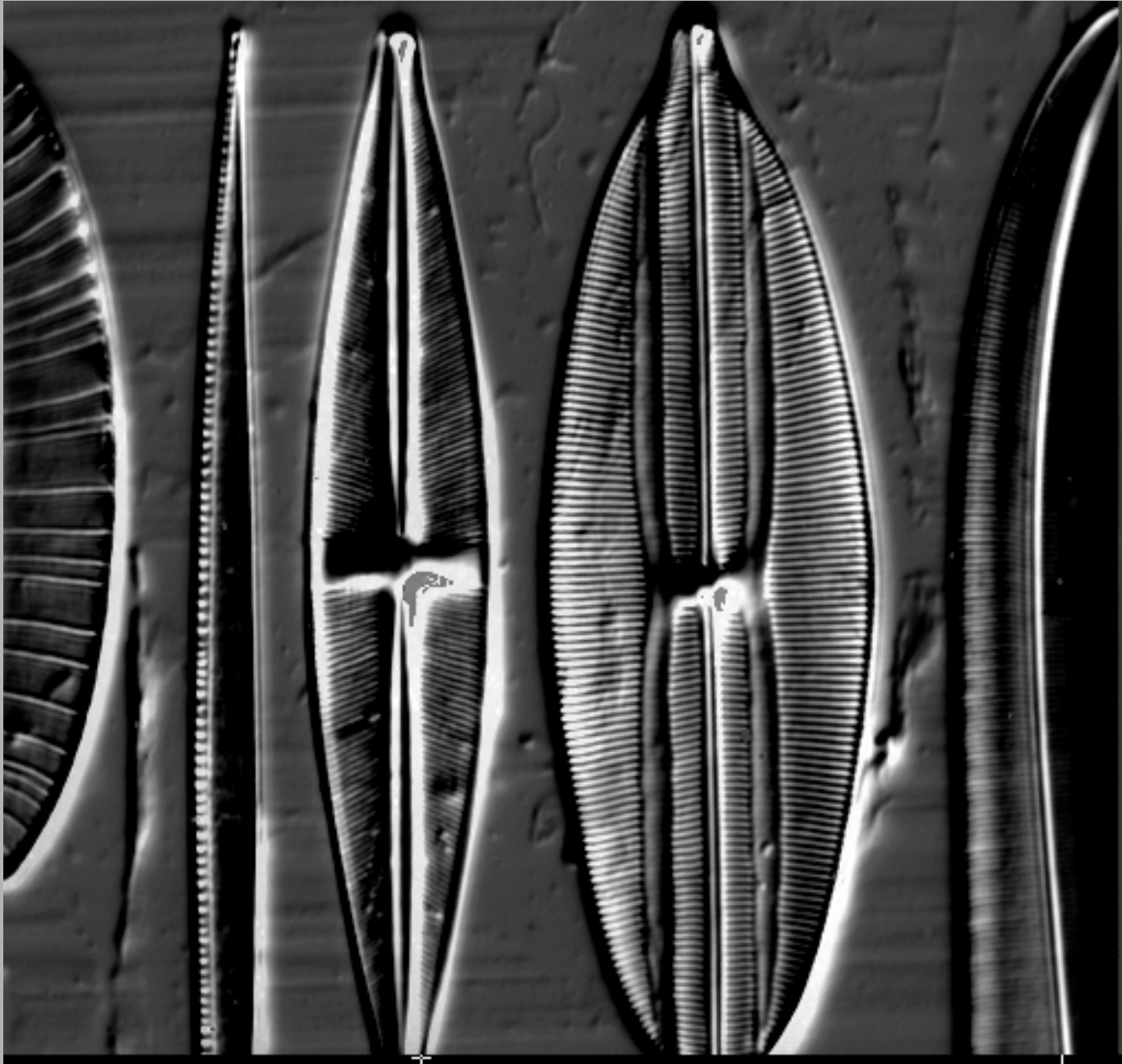
Change focus: dirt gone, mottle remains

Noise and irregularities in sensor emphasized

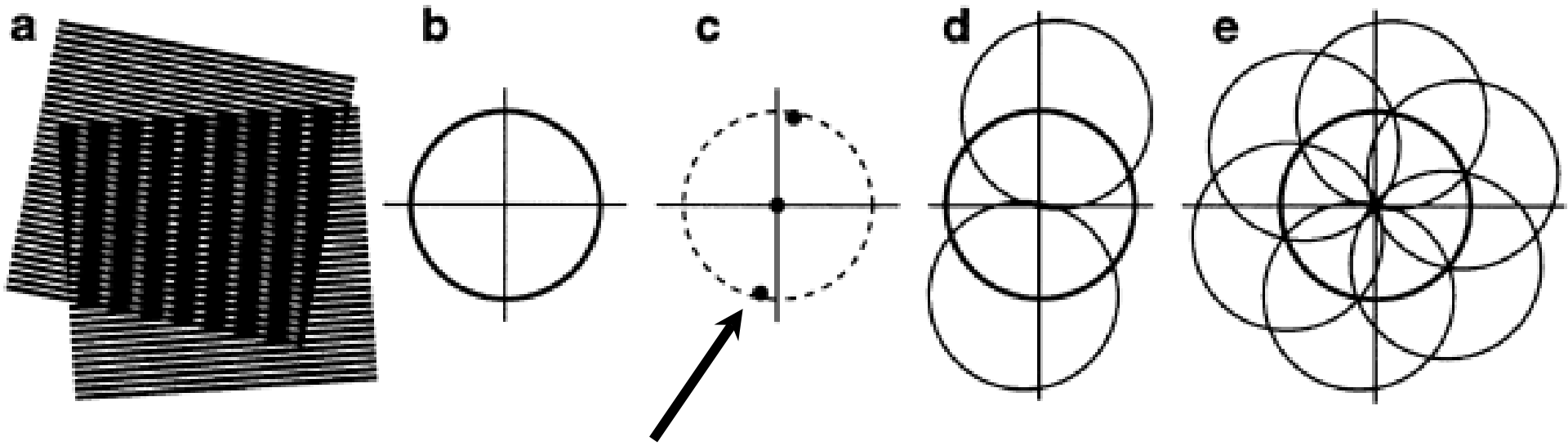
Step 3: Subtract averaged image of mottle and defects

Step 4: increase contrast to fill dynamic range

Lovely electronically enhanced DIC image

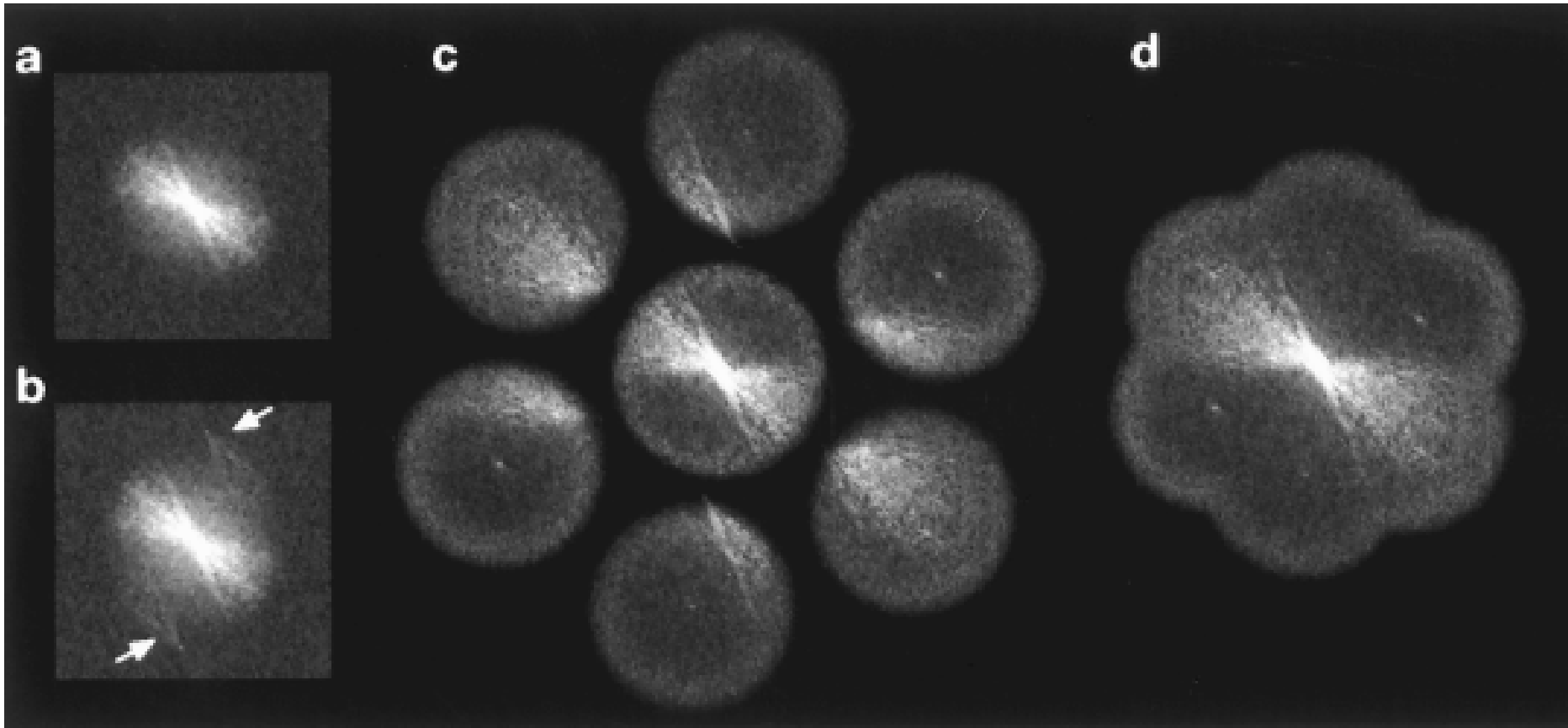


Structured illumination to collect light from outside of the objective exit pupil



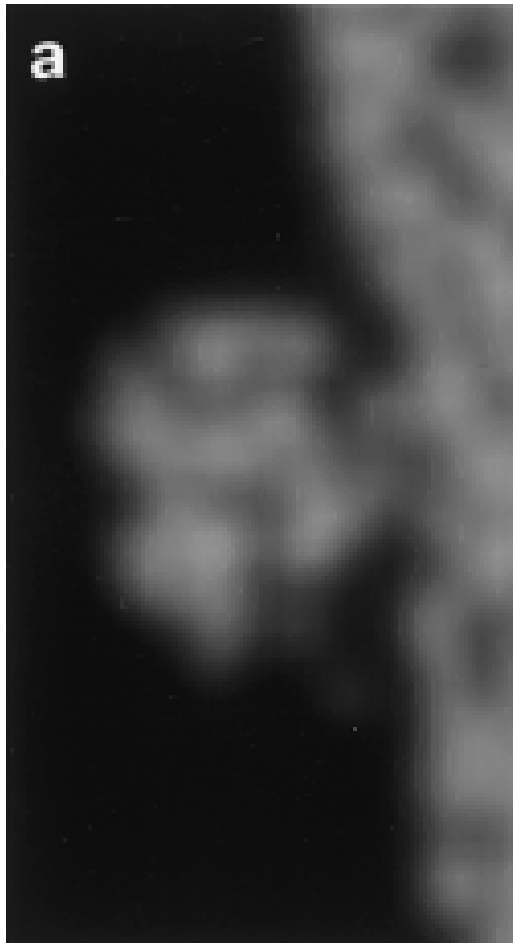
Grating diffraction just missed by objective

Structured illumination to get extra diffraction orders  
from six extra exposures

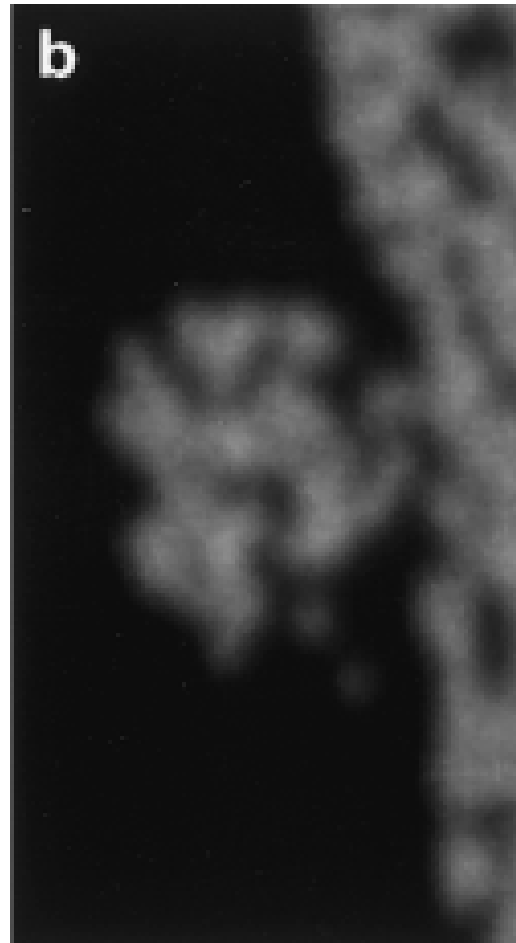


Gustaffson et al, UCSF

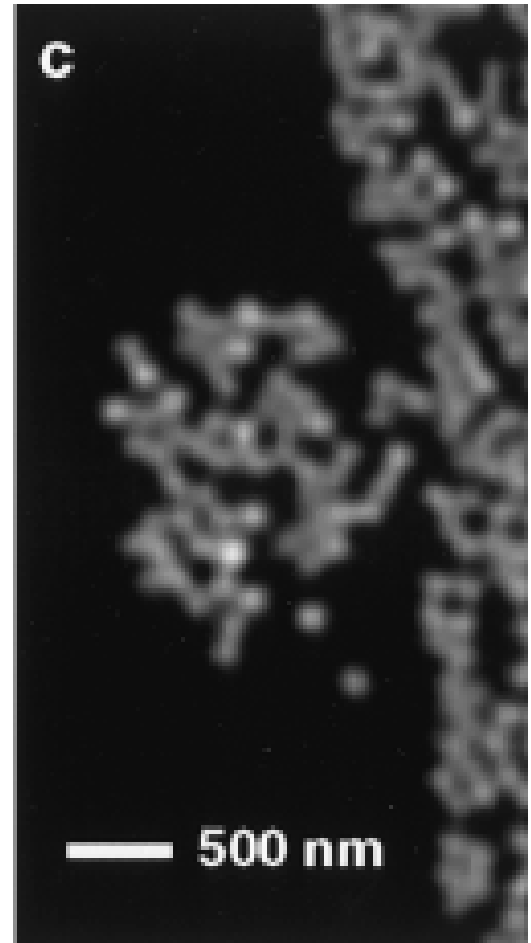
# High resolution images of beads as a test



Conventional



Confocal



Structured

# Cytoskeleton as a test case

