Bi177 - Lecture 8  Contrast vs Resolution vs Detection

Review of Kohler Illumination

Tradeoffs in Contrast/Resolution

Phase Contrast

Dark Field

Rheinberg Contrast

Nomarski (Differential Interference)

Techniques for plastic

Measuring Contrast
Microscopy as a compromise
Magnification
Resolution
Brightness
**Contrast**
Contrast

\[
\begin{align*}
50 - 0 / 50 + 0 &= 1 \\
50 - 50 / 50 + 50 &= 0 \\
50 - 100 / 50 + 100 &= -0.33
\end{align*}
\]

Brightness of Specimen - Brightness of Background

Brightness of Specimen + Brightness of Background
“Koehler” Illumination

Prof. August Köhler:
1866-1948

• Provides for most homogenous Illumination
• Highest obtainable Resolution
• Minimizes Straylight and unnecessary Irradiation
• Establishes proper position for condenser elements, for all contrasting techniques
Kohler Rays

Kohler Illumination gives the most uniform illumination

Each part of the light source diverges to whole specimen

Each part of the specimen gets light that converges from the whole light source

Arrows mark conjugate planes
To look at the illumination planes
- Remove eyepiece
- Focus eye at infinity
Compromise between Resolution and Contrast

The Big Challenge: The highest resolution is not the highest contrast

Transmitted light  Nomarski DIC
Bad Idea Number 1: “Dropping” the condenser

Objects scatter light into the objective

Gives contrast, but at the cost of NA

(spherical aberration in condenser)
(bad launch of waves for diffraction)
Bad Idea Number 2:
“Stopping down” the condenser

Gives contrast, but at the cost of NA
(bad launch of waves for diffraction)
Effect of Aperture on Contrast

At smaller aperture angles, less diffracted light gets through the objective. This increases the difference between signal and background and more contrast.

Large scattering angles miss the objective.

Brightness of Specimen - Brightness of Background
Brightness of Specimen + Brightness of Background

Effect of Aperture on Contrast

At smaller aperture angles, less diffracted light gets through the objective. This increases the difference between signal and background and more contrast.

Large scattering angles miss the objective.
Dark-field:  Maximizes detectability
Cost in resolution
Dark-field - The GOOD:
High NA Condenser
“Kohler” Illumination

Dark-field - The BAD:
Lower NA light collection
Don’t collect 0th order
The Good: Striking contrast
The Bad: “dark field” like resolution
(good for seeing things, not as good for measuring)
Rheinberg Illumination Filters

(a) Green Stop & Red Annulus  (b) Blue Stop & Yellow Annulus  (c) Red Stop & Green Annulus

Annular Rheinberg Filter Rings

(a) Red  (b) Green  (c) Blue

Alternating Sector Annular Filters

(a) 2 Sectors  (b) 4 Sectors  (c) 6 Sectors  (d) 8 Sectors

Figure 5
Cells have higher $\eta$ than water

Light moves slower in higher $\eta$

Light has shorter $\lambda$

Light will be phase-retarded

How to harvest this?
Phase Contrast:

Illumination from Phase Ring

Defined position of the 0th Order

Phase Ring attenuates the 0th Order

(also phase shifts)

Makes image more dependent on subtle changes in 1st Order

Refraction of light by specimen focuses light inside of the phase ring

(spherical cells appear “phase bright”)
**Crossed Polarizers:**
Only items that rotate the plane of Polarization reaches the detector.

(quarter wave plate adds color)
Rotation, so light

No rotation, so black
Nomarski thought experiment: need two different light rays Pass through specimen independently Afterwards, let them interfere with one another How to label them? How offset them (shear)?
Thought experiment: Color code two paths offset

Problem: red and green light don’t interfere with each other
Nomarski thought experiment: need two different 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)
Wollaston Prism
Birefringent material
Different $\eta$ for different polarizations

lower

higher

higher

lower
Problem: light in different planes of polarization don’t interfere with each other (need an analyzer)
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
Nomarski Optics

Good -
• Contrast at full aperture
• Optical sectioning (to \(\sim 0.3 \text{um}\))
  (two beams mostly overlap)

Bad -
• Expense
• Very sensitive to polarization
  Plastic
  Glass with stress
Modulation Contrast (Hoffman)

- For unstained (live) specimens
- Combination of oblique illumination and attenuation of non-diffracted light
- Simulated 3-D image (similar to DIC)
- Less resolution, not as specific as DIC
- No “Halo”-effect
- Usable with plastic, birefringent dishes
Hoffman Modulation Contrast

Required Components:

- Specially Modified Objective (With Built-in Modulator)
- Modified Condenser with off-axis slit (double slit with polarizer)
Varel Contrast (1996 - Zeiss)

- For unstained (live) specimens
- Combination of oblique illumination and attenuation of non-diffracted light
- No “Halo”-effect
- Complementary technique to Phase (easy switchover)
- Simulated 3-D image (similar to DIC)
- Less resolution than DIC
- Works with plastic dishes
Varel Contrast (1996 - Zeiss)

**Objective**

Required Components for Varel:
1. Objective with Varel- and Ph ring
2. Slider or Condenser with specific Varel 1, 2 and Phase rings

**Condenser**

Movable Ring Sector (Varel Ring)
Resolution: smallest distance between two points on a specimen that can still be distinguished as two separate entities.

\[ R = 0.61 \frac{\lambda}{NA} \]
\[ R = 1.22 \frac{\lambda}{(NA(\text{obj}) + NA(\text{cond}))} \]
Resolution

- Light from points of specimen passes through the objective, forms image,
- Points of the specimen appear in the image as small patterns: **Airy patterns**.
  - caused by diffraction or scattering of the light passing through specimen
- Central maximum of the Airy patterns: **Airy disk**, region enclosed by the first minimum
  - contains 84 percent of the luminous energy.
Resolution

Numerical Aperture and Airy Disc Size

Figure 4
MTF

• The resolution and performance of an optical microscope can be characterized by the modulation transfer function (MTF)
• The MTF is a measurement of the microscope's ability to transfer contrast from the specimen to the image plane at a specific resolution.
MTF

- The effect of increasing spatial frequency on image contrast

Modulation (M) = (I(max) - I(min))/ (I(max) + I(min))

MTF = Image Modulation/Object Modulation
• The effect of increasing spatial frequency on image contrast