Polarization, DIC, fluorescence
• Last class
  • Morphological operators
  • Matlab morphological image processing

• This class
  • Polarization microscopy
  • DIC
  • Intro to fluorescence
Diffraction limited spot vs resolution limit

\[ d = \frac{\lambda}{2} \times NA \]

Radius of spot \( r = 1.22 \frac{\lambda}{2} \times NA \)

Rayleigh criterion

OBJECT

IMAGING LENS

Resolved

Rayleigh Limit

Not Resolved
Magnification

Imagine 10x objective
Image on the camera, would be 10x larger

Cell that is 20 um in diameter, would appear 200 um diameter on camera

Conversely, a 10 um pixel on the camera would represent 1 um on the sample
Polarization microscopy

• Another way to add contrast to samples
Properties of light

\[ y(x, t) = A \cos(kx - \omega t + \varphi) \]

\[ y(x, t) = \mathbf{A} \cos(kx - \omega t + \varphi) \]
Properties of polarization

Unpolarized  Linear  Linear  Circular  Elliptical

Unpolarized  $x$  Linear  $x + y$  Circular  $x + (y + \phi)$  Elliptical  $ax + b(y + \phi)$

$E_x$  $E_y$

$x^2 + y^2 = 1$

Eyes and cameras can not detect polarization.
Only able to detect intensity.
Vertebrate vs cephalopod eyes

• Invertebrate eyes are different than our own
• Some invertebrates can detect polarization of light

First step is isomerization from 11 cis to all trans

Cephalopods can sense polarization
Manipulating polarization

Relationship Between Long-Chain Molecule Orientation and the Orientation of the Polarization Axis

When molecules in the filter are aligned vertically, the polarization axis is horizontal.

When molecules in the filter are aligned horizontally, the polarization axis is vertical.
Manipulating polarization

- Half wave plate, converts to linear polarization
- Quarter wave plate, converts to circular polarization
Linear polarizers

Polaroid filters

Light that comes through is polarized parallel to filter

\[ I = I_0 \cos^2(\theta) \]

Malus’ Law
Cross polarizer puzzle

Forms the basis for polarization microscopy

Have to apply Malus’ law twice

\[ I_2 = I_1 \cos^2(\theta) \]

Then

\[ I_3 = I_2 \cos^2(\theta) \]
Polarization microscopy

Birefringent – refraction is different for different polarizations

Biological samples:
- Mitotic spindles
- Actin filament bundles
- Condensed DNA
- Helical strands of cellulose
- Some lipid bilayers
Strain induced bi-refringence

- Birefringence: refractive index depends on polarization of light
- Useful for detecting strain in extra cellular matrix (ECM)
- Label free technique
Differential Interference Contrast

- Interference technique similar to phase contrast
- The magic starts with a Wollaston prism
- Wollaston prism is birefringent, and rays will exit at different points in space

Wollaston splits polarization
DIC microscope

Beams are split by small amount (nanometers)

If there is a difference in optical path between them, they will negatively interfere at camera

Only get contrast at the edges

Form what looks like a 3d image on the camera
DIC vs phase contrast

**Transparent Specimens in Phase Contrast and DIC**

### Table: DIC vs Phase Contrast

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Phase Contrast</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image Brightness (Brightfield = 100%)</td>
<td>1.3 Percent</td>
<td>0.36 - 2.3 Percent</td>
</tr>
<tr>
<td>Epi-Fluorescence Light Loss (Brightfield = 0%)</td>
<td>28 Percent</td>
<td>73 Percent</td>
</tr>
<tr>
<td>Condenser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restricted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral Resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial Resolution (Depth Discrimination)</td>
<td>Poor</td>
<td>Superior</td>
</tr>
<tr>
<td>Illuminating Aperture</td>
<td>10 Percent of Objective NA</td>
<td>Variable</td>
</tr>
<tr>
<td>Phase Shift Detection Limit</td>
<td>&lt; l/100</td>
<td>&lt; l/100</td>
</tr>
<tr>
<td>Utility at High Phase Shifts</td>
<td>Not Useful</td>
<td>Useful</td>
</tr>
<tr>
<td>Azimuthal Effects</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Halos and Shade-Off</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stained Specimens</td>
<td>Not Useful</td>
<td>Useful</td>
</tr>
<tr>
<td>Birefringent Specimens</td>
<td>Useful</td>
<td>Not Useful</td>
</tr>
<tr>
<td>Birefringent Specimen</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Containers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brightfield Image Deterioration</td>
<td>Slight</td>
<td>None</td>
</tr>
<tr>
<td>Cost</td>
<td>Moderate</td>
<td>High</td>
</tr>
</tbody>
</table>
And on to Matlab...
Optical coherence tomography

- Another interference based technique
- Non destructive, and no labelling
- Limited resolution, but high applicability
OCT contrast

• When light in sample goes through index change, the reflected light interferes with reference arm light – giving rise to bumps in the signal

Particularly useful in ophthalmology.
Also used in the brain (rats)