BME 2200: Biostatistics and Research Methods

Lecture 9: Microscopy

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Content, Schedule

1. Scientific literature:
   - Literature search
   - Structure biomedical papers, engineering papers, technical reports
   - Experimental design, correlation, causality.

2. Presentation skills:
   - Report – Written report on literature search (individual)
   - Talk – Oral presentation on biomedical implant (individual and group)

3. Graphical representation of data:
   - Introduction to MATLAB
   - Plot formats: line, scatter, polar, surface, contour, bar-graph, error bars. etc.
   - Labeling: title, label, grid, legend, etc.
   - Statistics: histogram, percentile, mean, variance, standard error, box plot

4. Biostatistics:
   - Basics of probability
   - t-Test, ANOVA
   - Linear regression, Least-squares curve fit
   - Error analysis
   - Test power, sensitivity, specificity, ROC analysis

5. Microscopy
Modern Microscope Types

- Conventional Microscope (bright and dark field)
- Phase Contrast Microscope
- Fluorescence Microscope
- Confocal Microscope
- Transmission Electron Microscope (TEM)
- Scanning Electron Microscope (SEM)
- Scanning Tunneling Microscope (STM)
- Atomic Force Microscope (AFM)
- Fixation and sectioning (cross-linking, cryofixation)
- Staining (dyes, enzymes, fluorescence)
- Fluorescent antibodies and proteins

Most material in these slides is from:
- http://micro.magnet.fsu.edu/primer/
- http://nobelprize.org/physics/educational/microscopes/
# Wavelength and resolution

<table>
<thead>
<tr>
<th>1 m</th>
<th>1 dm</th>
<th>1 cm</th>
<th>1 mm</th>
<th>100 μm</th>
<th>10 μm</th>
<th>1 μm</th>
<th>100 nm</th>
<th>10 nm</th>
<th>1 nm</th>
<th>1 Å</th>
<th>0.1 Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 m</td>
<td>10⁻¹ m</td>
<td>10⁻² m</td>
<td>10⁻³ m</td>
<td>10⁻⁴ m</td>
<td>10⁻⁵ m</td>
<td>10⁻⁶ m</td>
<td>10⁻⁷ m</td>
<td>10⁻⁸ m</td>
<td>10⁻⁹ m</td>
<td>10⁻¹⁰ m</td>
<td>10⁻¹¹ m</td>
</tr>
</tbody>
</table>

- **Eye**
- **Light microscope**
- **Electron microscope**

- man height
- hand
- finger
- thickness of hair
- cell
- bacterium
- virus
- macro molecule
- small molecule
- atom
Magnification with lenses

Figure 4
Conventional Light Microscope
Conventional Light Microscope

Eye

Eyepiece

Stage

Objective

Filter Holder

Condenser

Darkfield

Brightfield
Photomicrographs of Chlamydomonas are shown in Figure 2A-C and a buccal cell in Figure 2D. The difference in refractive indices of the media with the cell wall and other components of the algae are visualized in brightfield (Fig. 2A & B). Closing the condenser aperture diaphragm increases contrast allowing the flagella to be barely visible (Fig. 2A). Darkfield clearly shows many intracellular details as well as the flagella (Fig. 2C). Because these are unmounted living cells, they have moved a bit between the different exposures. Buccal cells are so similar in refractive index to the medium that photos of them could not be obtained in brightfield. Figure 2D shows buccal cells in darkfield; nucleus and other intracellular structures are clearly visible. Samples were photographed using CH2 student-grade Olympus microscope with 40X objective and 3.3X photo eyepiece on Kodak TMAX 400 film.
Phase Contrast Microscope

Zernike, Nobel price 1953

The phase-plate increases the phase difference to half a wavelength. Destructive interference between the two sorts of light when the image is projected results in the specimen appearing as a dark object.
Phase Contrast Microscope

Bright field image

phase contrast
Confocal Microscope
Confocal Microscope

Figure 6
Fluorescence Microscopy

Excitation and Emission Spectral Profiles

Figure 2

- Absorption (Excitation)
- Fluorescence Emission
- Absolute Shift
- Spectral Overlap

Wavelength (Nanometers)

300 400 500 600 700

Relative Intensity

filters
beam splitting mirror
specimen
Fluorescence Microscopy

Figure 4

Photobleaching Rates in Multiply Stained Specimens

(a)  (b)  (c)

(d)  (e)  (f)
Fluorescent tagging
Scanning Electron Microscope

- Rock vs. Mount Everest
  - Rock image enlarged x100,000
  - Mount Everest image enlarged x100,000

- Electron Beam Characteristics
  - Wavelength of accelerated electrons (6 pm)
  - Wavelength of light (600 nm)

- Electron Source
- Electron Beam
- Specimen
- Electromagnetic Lens
- Viewing Screen
Scanning Electron Microscope

Golgi apparatus

Salmonella bacteria
Assignment

Assignment 10:

Pick one of the topics in slide #3 and prepare 3-4 slides explaining:

1) basic operation principle
2) spatial resolution
3) specimen preparation
4) examples of what can be imaged.

Make sure you address in particular item 3) which we have not covered in class.