Microscope Basics

NGSSS:
SC.912.N.2.1
through N.4.2

I. The First Microscopes

A. About 1590, two Dutch spectacle makers, Zaccharias Janssen and his son Hans, while experimenting with several lenses in a tube, discovered that nearby objects appeared greatly enlarged.

B. Anton van Leeuwenhoek (1632-1723)

1. Also made his own microscopes.
2. He was the first to see and describe bacteria, yeast(fungi), plants, the teeming life in a drop of water, and the circulation of blood corpuscles in capillaries

C. Robert Hooke (1635-1703)

1. Invented universal joint and iris diaphragm
   - amazing scientist
2. His book ***Micrographia*** published in 1665
   - Also observed cork cells (same year)
3. Perfected the best microscope of his time
   - Compound microscope

II. The Bright Field Microscope

A. The Compound Microscope

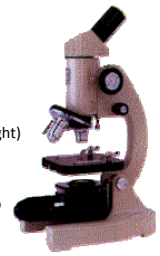
1. Most common microscope.
2. Light passes through a condenser lens, then through two more lenses.
3. Magnification up to 1000x
4. Limitations:
   a. Resolution, illumination and contrast
   -Resolution can be improved using oil immersion lenses, and lighting and contrast can be dramatically improved using modifications such as dark field, phase contrast, and differential interference contrast.
5. Specialized types
   a. Fluorescence and confocal microscopes

Confocal Laser Scanning Microscopy

- A technique for obtaining high-resolution optical images with depth selectivity
- Key feature
  - ability to acquire in-focus images from selected depths
  - process known as optical sectioning
- Images are acquired point-by-point and reconstructed with a computer
  - 3-D reconstructions of topologically-complex objects

Microscope

- **Compound Light Microscope**
  - Ocular lens (eyepiece)
  - Objective lens
  - Stage
  - Glass slide
  - Coverslip
  - Diaphragm (regulates light)
  - Base
  - Fine adjustment knob
  - Coarse adjustment knob
  - Stage clips
  - Arm



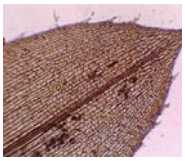
Ocular lens: magnification 10x

Objective lens: magnification up to 100x

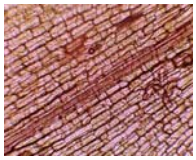
Gives up to 1000x total magnification

100 x 10 = 1000

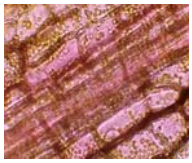
Elodea



40 X



100 X



400 X

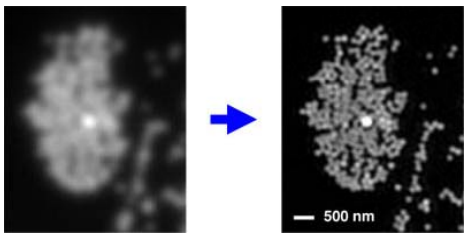
Microscopes Magnify and Resolve

two very different things

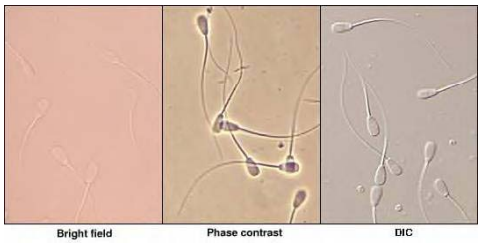
Magnification \mag-ne-fe-'ka-shen\ *n*
- Apparent **enlargement** of an object
- The ratio of image size to actual size
  - Magnification of "100x" means the image is *100 times* bigger than the actual object

Resolution \rez-e-loo-shen\ *n*
- **Clarity, sharpness**
- The ability of a microscope to show two very close points separately

Resolution



Examples



Differential Interference Contrast

III. Electron Microscopes

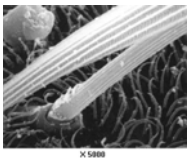
A. History

1. Electron microscopes were developed because of the limitations of compound microscopes.

2. First developed in the 1930's.

More on Microscopes

- **Electron Microscopes**
  - overcomes resolution restrictions
    - Transmission electron microscopes (TEM's)
    - Scanning electron microscopes (SEM's)
    - ESEM link Environmental SEM



Photos: http://www.mos.org/sln/sem/tour04.html

B. **Transmission Electron Microscopes (TEM)**

1. Operates on the same principle as a light microscope, but uses a stream of electrons instead of light.
2. Increases magnification to **200,000 x**.
3. 2-D images of
4. Can not observe living organisms.

http://nobelprize.org/educational_games/physics/microscopes/tem/index.html

Electron Micrographs

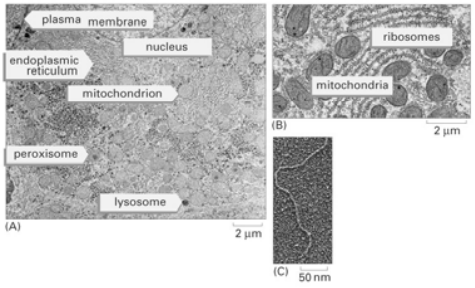
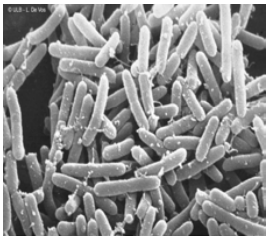
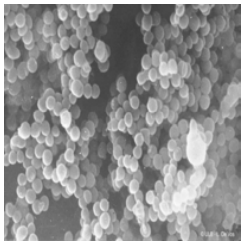


Figure 1-8 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Electron Micrographs



E.coli



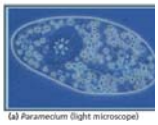
S. auerus

C. Scanning Electron Microscope(SEM)

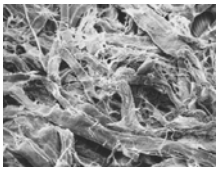
1. Specimen coated with fine metal coating
2. Magnifies to **100,000x**
3. 3-D images of surface/topography

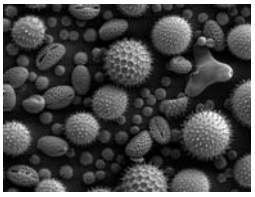
http://www.mos.org/sln/SEM/seminfo.html

Image Comparison



SEM micrographs

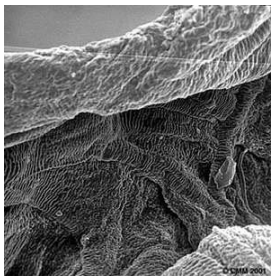




Scanning Electron Microscopy

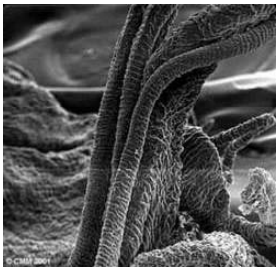
- **Heart - papillary muscle** Heart - papillary muscle (small muscles within the heart which anchor the heart valves)

website



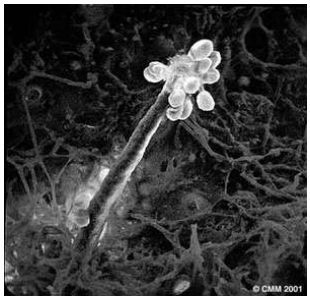
(Coppermine Photo Gallery, 2009)

- **Heart - collagen bundles**



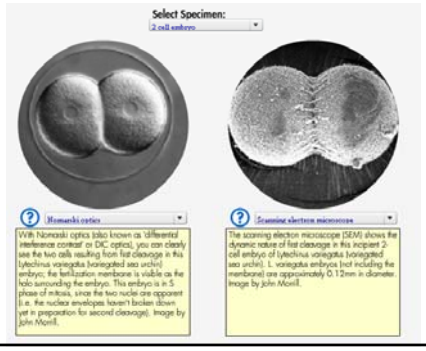
(Coppermine Photo Gallery, 2009)

- **Stigmatella**
- Gram-negative, rod-shaped
- Saprophytic bacteria of the phylum myxobacteria
- Typically found embedded in slime

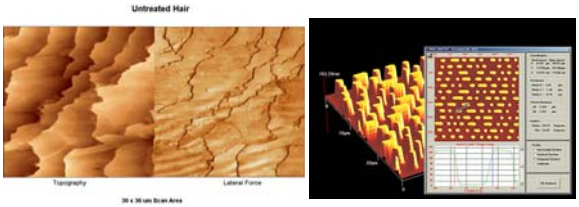


(Coppermine Photo Gallery, 2009)

Compound vs. SEM



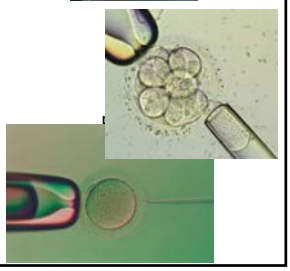
- **Probe Microscopes**
  - Don't use lenses to produce images
  - Ability to see atoms
  - Moves a probe across the surface of specimen
  - Records surface shape info on computer



- **Types of Microscopes:**
  - http://www.cas.muohio.edu/~mbi-ws/microscopes/types.html

- **Centrifuge**
  - Spins down and separates culture
    - Heavy parts settle on bottom
    - Light parts rise to top

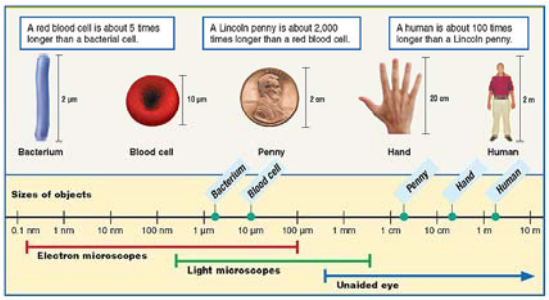
- **Micromanipulation**
  - Technique used to dissect, insert or manipulate
    - Invitro



Cell Photos: http://www.ivf.com/insem.html

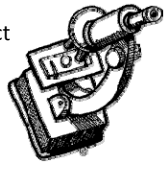
(Leica Microsystems GmbH, 2009)

Object Size and Magnifying Power of Microscopes



Using the Microscope

- Place the Slide on the Microscope
- Use Stage Clips
- Click Nosepiece to the lowest (shortest) setting
- Look into the Eyepiece
- Use the Coarse Focus to find object



High Power Focusing

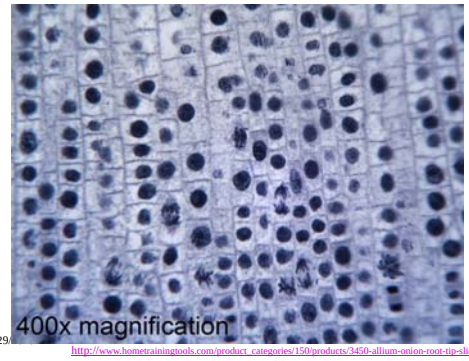
- Find the specimen using low power, then…
- Click the nosepiece to the longest objective
- Do **NOT** use the Coarse Focusing Knob
- Use the Fine Focus Knob to bring the slide into focus
  - without breaking the slide or ruining the sample

Prepared Slides

Ficus (dicot) leaf slide

http://www.hometrainingtools.com/products/5583-ficus-dicot-leaf-slide-c-s

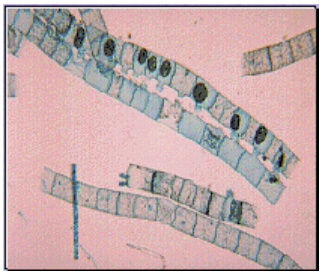
Allium (onion) root tip slide



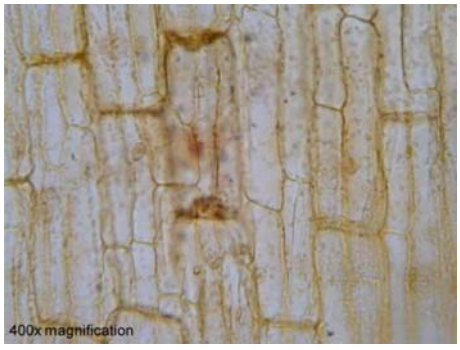
8/29/

http://www.hometrainingtools.com/product_categories/150/products/3450-allium-onion-root-tip-slide-l-s-

Spirogrya – Scalariform Conjugation

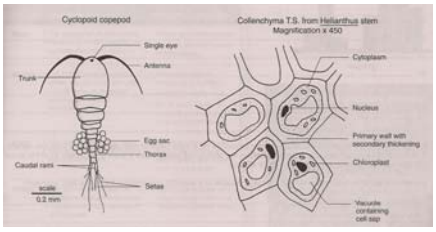


Elodea

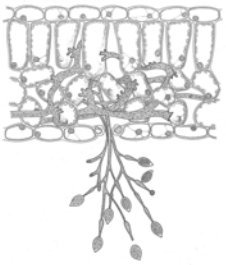


Biological Drawings

- Refer to BioZone workbook for proper drawing and labeling techniques

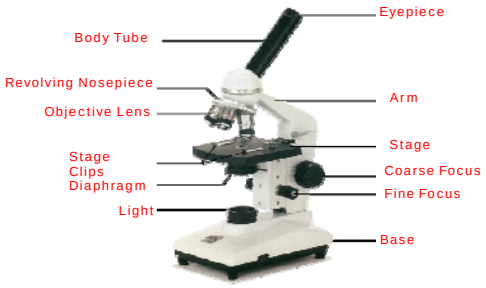


- Drawing the Field of View
  - link



Let's Review

Anatomy



Virtual Sites

- Optical Microscopy Primer
  http://micro.magnet.fsu.edu/primer/virtual/virtual.html
- Histology: Human Anatomy Slides
  http://www.dccc.edu/departments/biology/virtuals/virtualmicroscope/VMPage/very1st.htm
- Microscope Tutorial:
  http://virtualurchin.stanford.edu/microtutorial.htm

Resources

Coppermine Photo Gallery, (2009). Xtalent image gallery. Retrieved August 10, 2009, from Scanning Electron Microscopy Web site: http://www.xtalent.com.au/gallery/thumbnails.php?album=2&page=1

Davidson, M. (2010). Optical microscopy primer. Retrieved August 23, 2010 from Molecular Expressions website http://www.microscopy.fsu.edu/primer/museum/hooke.html

Epel, D. (2010). Virtual urchin. Stanford University. Retrieved August 24, 2010 from website http://virtualurchin.stanford.edu/microcompare.htm

Leica Microsystems GmbH, (2009). Surgical microscopes. Retrieved August 11, 2009, from Leica Web site: http://www.leica-microsystems.com/products/surgical-microscopes/

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