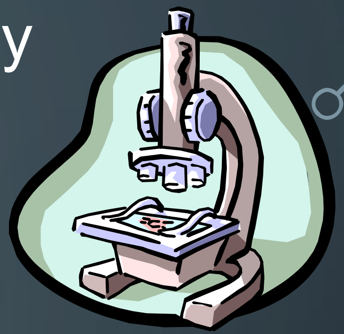
Light microscopy in Cellular Biology

Gabriele Baj
gbaj@units.it

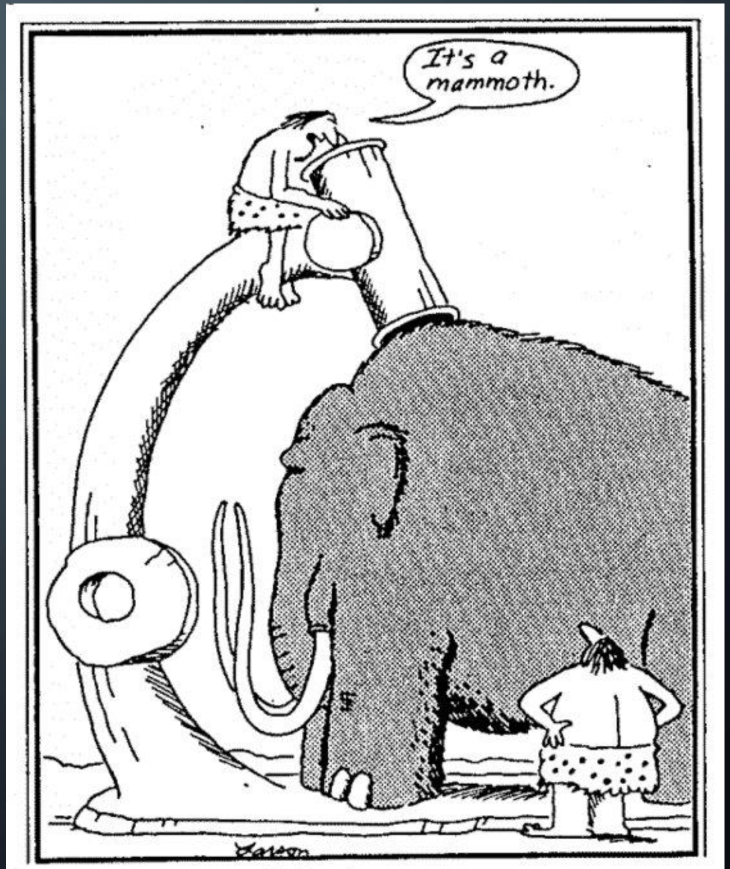


Overview of the Course

1) Introduction
- What Can You Learn with a Light Microscope?
- Early History of Microscopy

2) Image Formation
- Lenses and Image Formation
- Microscope Imaging and Koehler Illumination
- Objectives and Eyepieces
- Diffraction and Point Spread Function

3) Resolution , What is Light?
- How to Focus and setting up Koehler Illumination



Early microscope

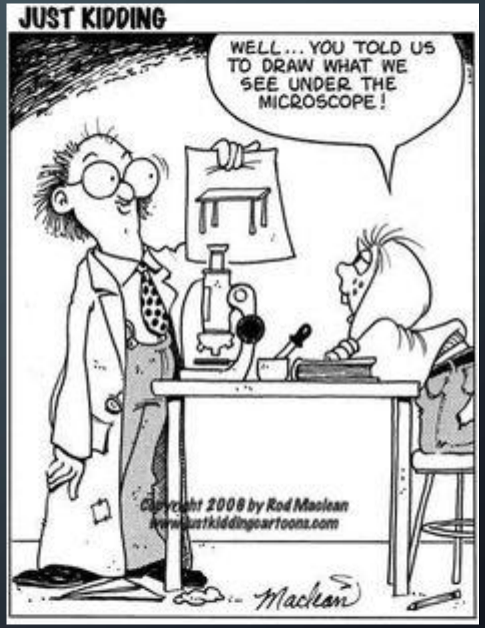
Overview of the Course

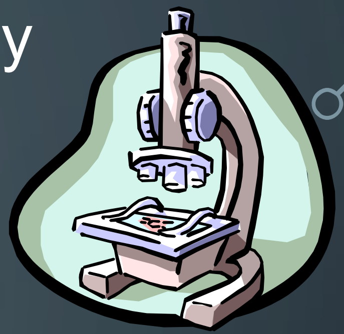
4) Contrast Generation for Transmitted Light

- Darkfield and Phase Contrast Microscopy
- Polarized Light and Polarization Microscopy
- Differential Interference Contrast (DIC) Microscopy

5) Fluorescence Microscopy

- Introduction to Fluorescence Microscopy
- Fluorescent Probes / Fluorescent Proteins
- Optical Sectioning and Confocal Microscopy
- Light Sheet Sectioning





Overview of the Course

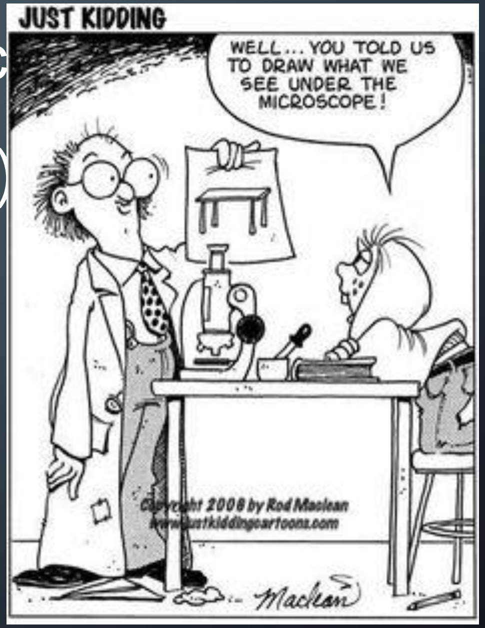
6) Super-Resolution:

Total Internal Reflection Fluorescence (TIRF) Microsc

Overview and Stimulated Emission Depletion (STED)

Localization Microscopy

Structured Illumination Microscopy (SIM)



7) Photobleaching and Photoactivation

Förster Resonance Energy Transfer (FRET) Microscopy

Fluorescence Lifetime Imaging Microscopy

Overview of the Course

8) Designing a Fluorescence Microscopy Experiment

Labeling Proteins with Fluorescent Probes

Correlating Fluorescence with Electron Microscopy

9) Introduction to Digital Images

10) Quantitative Analysis of Biological imaging Microscopy

Cameras and Detectors I: How Do They Work?

11) Image Analysis / Deconvolution Microscopy

Fundamentals of Light Microscopy and Electronic Imaging

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Fundamentals of Light Microscopy and Electronic Imaging, Second Edition

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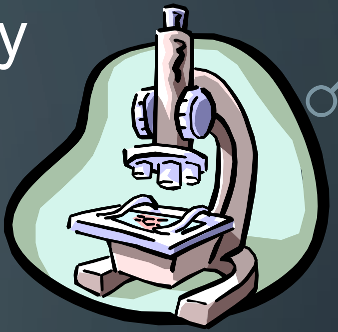
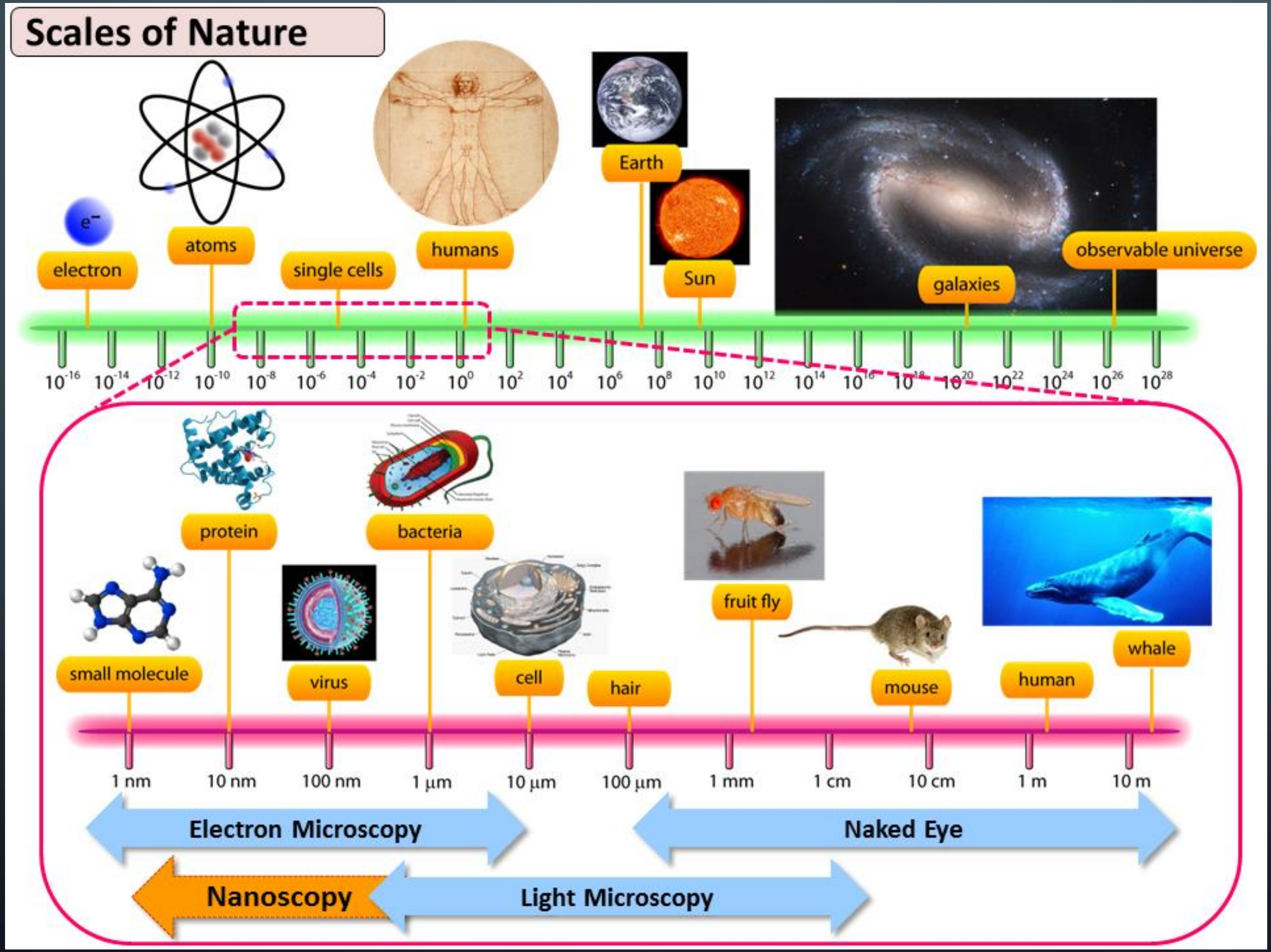
About this Book

Fundamentals of Light Microscopy and Electronic Imaging, Second Edition provides a coherent introduction to the principles and applications of the integrated optical microscope system, covering both theoretical and practical considerations. It expands and updates discussions of multi-spectral imaging, intensified digital cameras, signal colocalization, and uses of objectives, and offers guidance in the selection of microscopes and electronic cameras, as well as appropriate auxiliary optical systems and fluorescent tags.

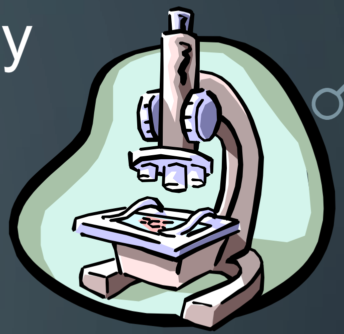
The book is divided into three sections covering optical principles in diffraction and image formation, basic modes of light microscopy, and components of modern electronic imaging systems and image processing operations. Each chapter introduces relevant theory, followed by descriptions of instrument alignment and image interpretation. This revision includes new chapters on live cell imaging, measurement of protein dynamics, deconvolution microscopy, and interference microscopy.



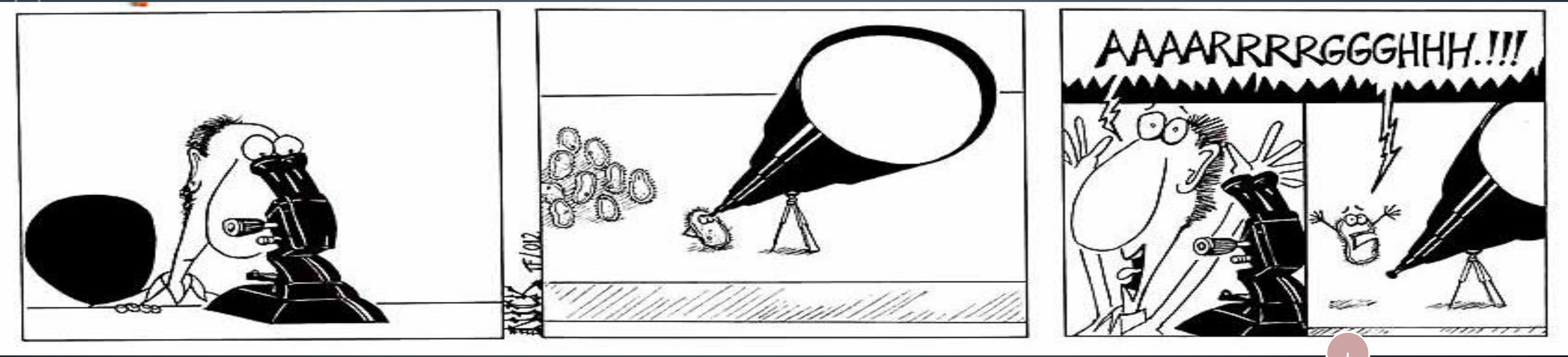
What Can You Learn with a Light Microscope ?



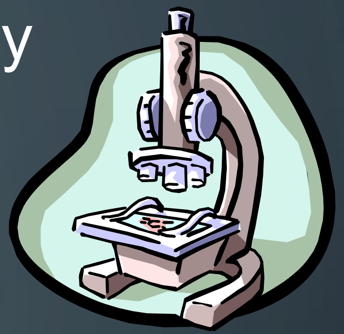
http://www.bates.edu/gould-research-lab/research/



Early History of Microscopy



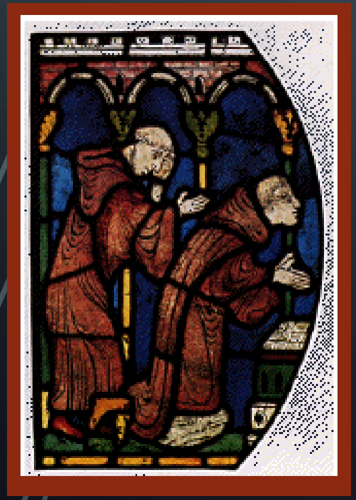
"Microscope" was first coined by members of the first "Academia dei Lincei" (Academy of the Lynx} scientific society which included Galileo. It was not Galileo who came up with the word, it was Johannes Faber, an entomologist and member of the same society that gave the magnifying instrument the name “microscope”

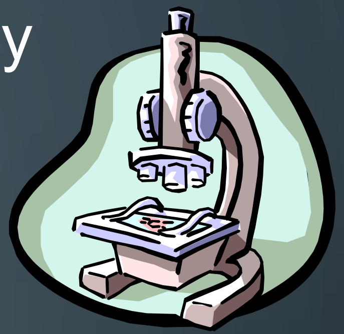


Timeline of the Microscope

- The Greeks & Romans used “lenses” to magnify objects over 2000 years ago
- Circa 1000AD – The first vision aid was invented

(inventor unknown- possibly a monk) called a reading stone. It was a glass sphere that magnified when laid on top of reading materials.

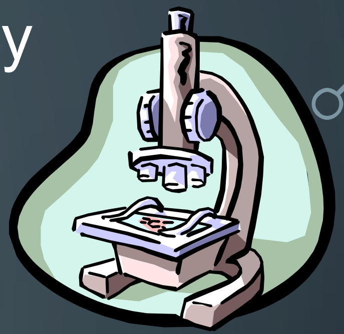




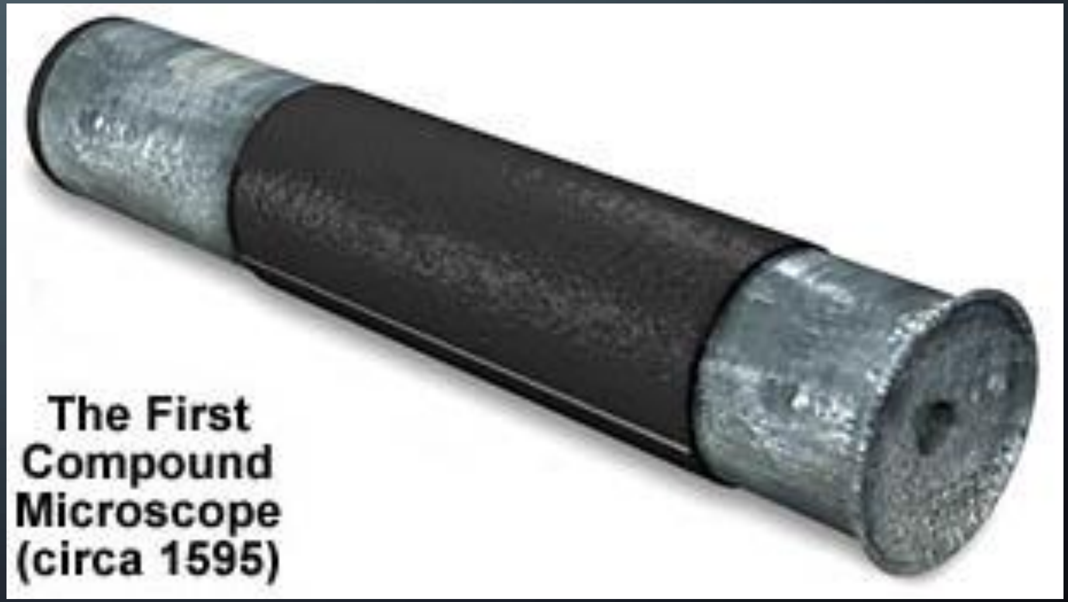
Timeline of the Microscope

13th century: spectacles first made in Italy

Circa 1284 - Italian, Salvino D'Armate is credited with inventing the first wearable eyeglasses



1590: Two Dutch spectacle-makers and father-and-son team, Hans and Zacharias Janssen, create the first microscope.



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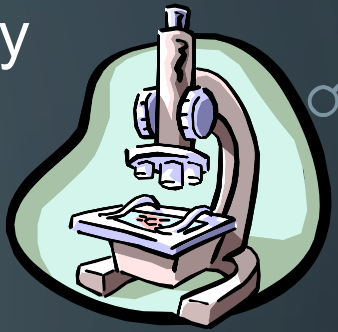
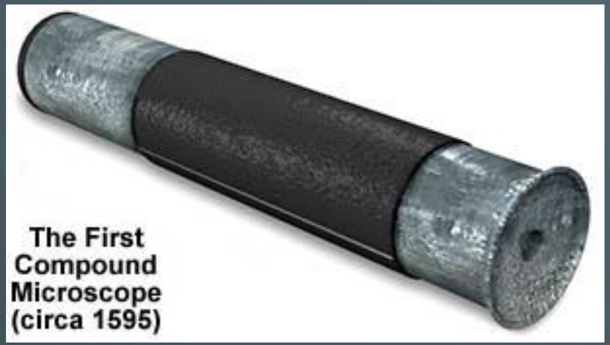


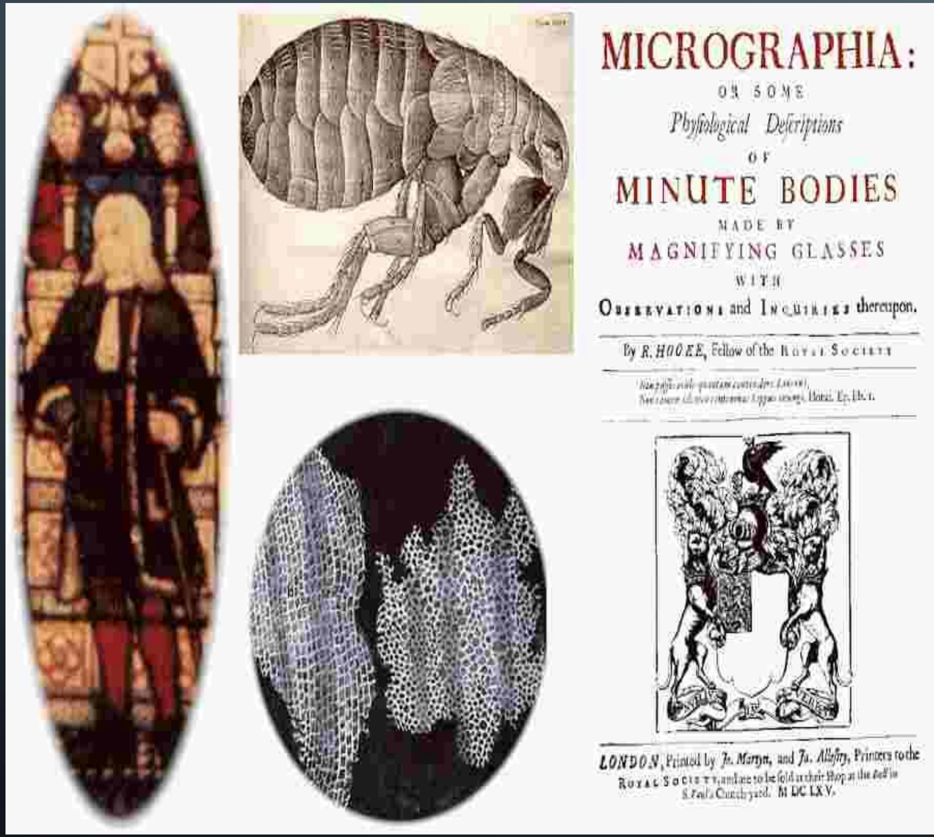
Zacharias Jansen
1588-1631



1660 - Marcello Malpighi **circa 1660,** was one of the first great microscopists, considered the father embryology and early histology - observed capillaries in 1660

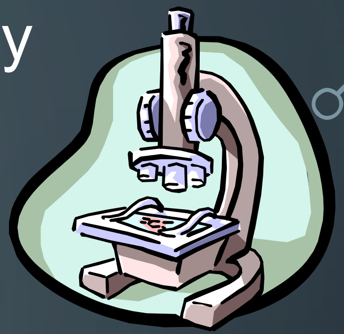
Timeline of the Microscope

1667: Robert Hooke's famous "Micrographia" is published, which outlines Hooke's various studies using the microscope.



Hooke Microscope

Robert Hooke
1635-1703



Timeline of the Microscope

- 1655 – Robert Hooke used a compound microscope to observe pores in cork

  He called them "cells"

- Fruiting structures of molds in 1665 and was the first to describe microorganisms

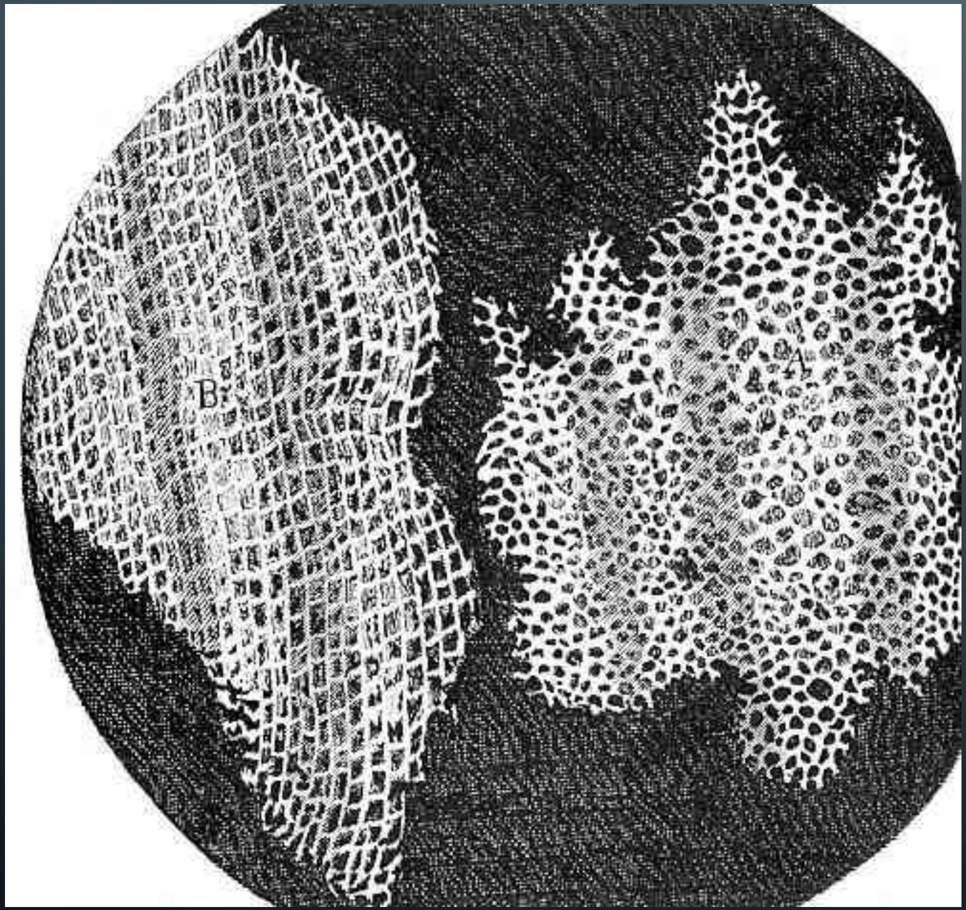
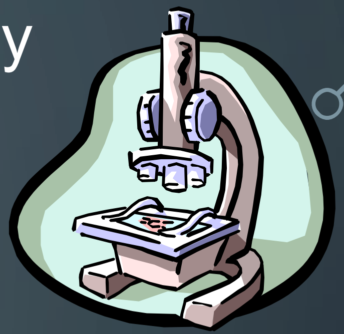
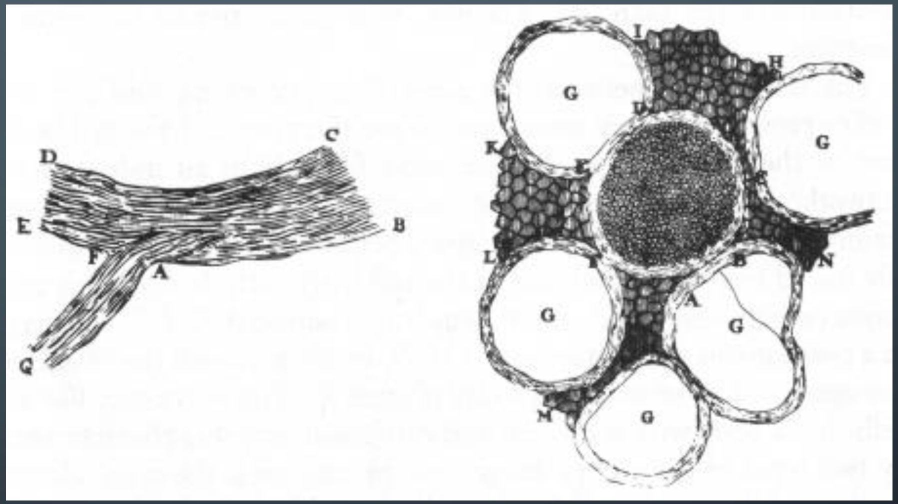


Figure 1-8b Brock Biology of Microorganisms 11/e

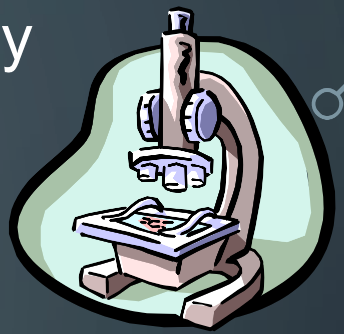


Timeline of the Microscope

1675: Enter Anton van Leeuwenhoek, who used a microscope with one lens to observe insects and other specimen. Leeuwenhoek was the first to observe bacteria



"In the year of 1657 I discovered very small living creatures in rain water."



Timeline of the Microscope

Van Leeuwenhoek's Microscope

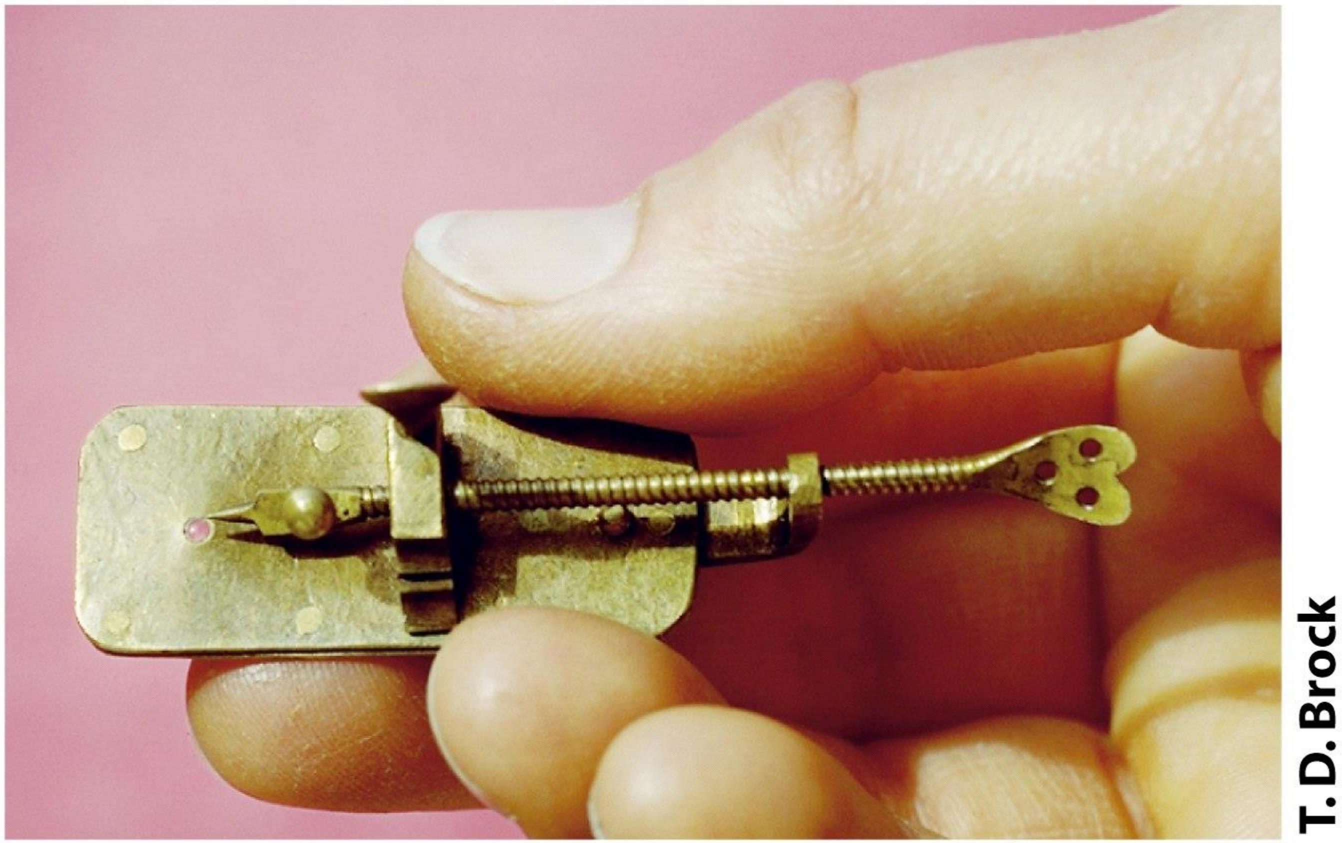
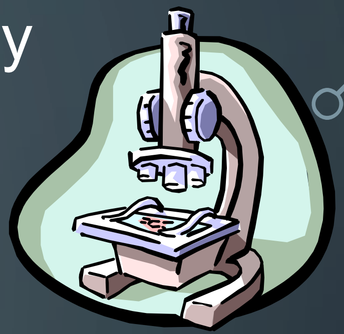


Figure 1-9a Brock Biology of Microorganisms 11/e

Van Leeuwenhoek's Microscope… today

https://www.foldscope.com/our-story

Timeline of the Microscope



Van Leeuwenhoek's drawing
on various organsisms

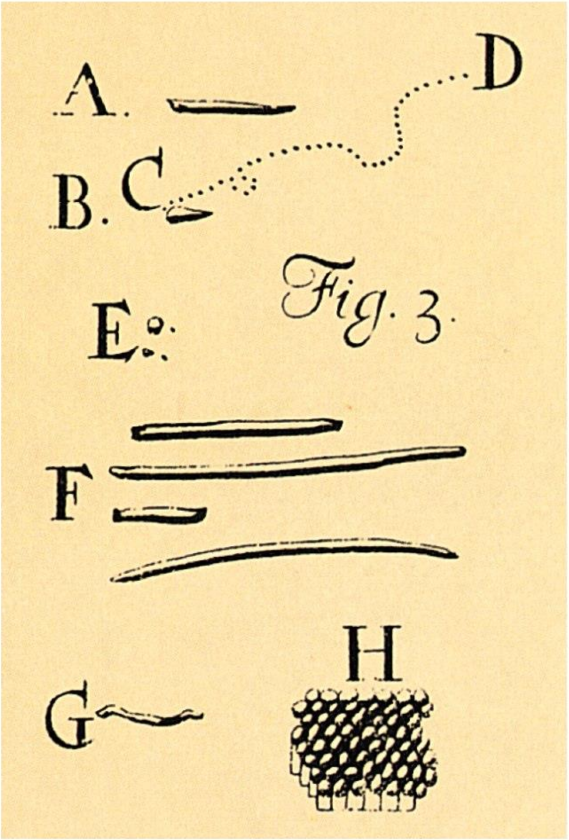


Figure 1-9b Brock Biology of Microorganisms 11/e

blood smear

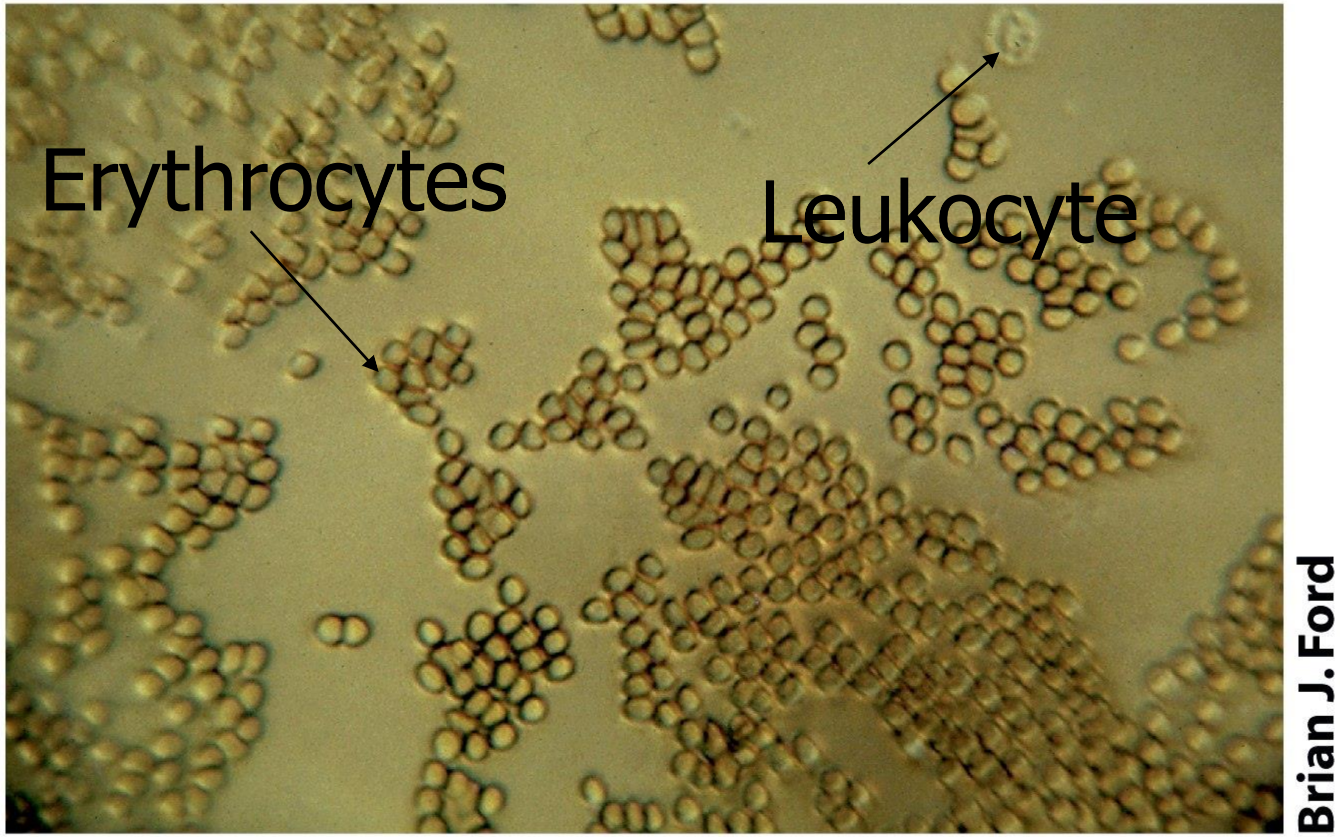
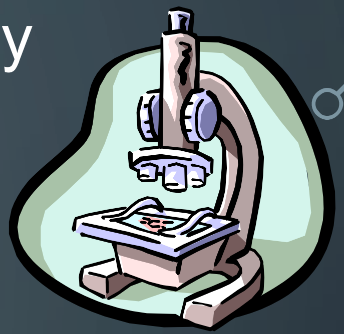


Figure 1-9c Brock Biology of Microorganisms 11/e

The field of microbiology was unable to develop until Leeuwenhoek constructed microscopes that allowed scientists to see organisms too small to be seen with the naked eye



1st result using microscope:

The Concept of Biogenesis Replaces Spontaneous Generation Theory

- **Spontaneous generation** claims that life can originate from non-living matter.
- **Biogenesis** states that living cells originate from living cells.
- Louis Pasteur's disproved spontaneous generation.
- His work led to the development of methods for controlling the growth of microorganisms.

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1690 - Campani was the leading Italian telescope and microscope maker in the late `17th century - he probably invented the screw focusing mechanism shown on this scope - the slide holder in the base allows transparent and opaque objects to be viewed

1720Screw barrel Microscope - Made by Charles Culpeper

1730s a barrister names **Chester More Hall** observed that flint glass (newly made glass) dispersed colors much more than “crown glass” (older glass). He designed a system that used a concave lens next to a convex lens which could realign all the colors (*chromatic aberration*). This was the first *achromatic lens*. **George Bass** was the lens-maker that actually made the lenses, but he did not divulge the secret until over 20 years later to **John Dollond** who copied the idea in 1759 and patented the achromatic lens.

HOW A MICROSCOPE WORKS

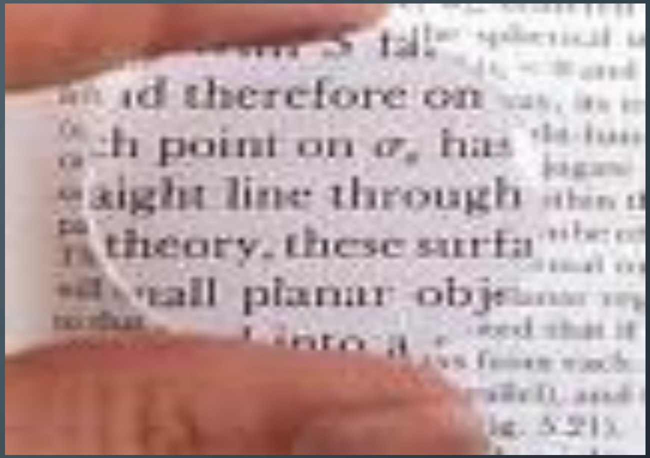
Ocular Lens
(Magnifies Image)

Objective Lens
(Gathers Light,
Magnifies
And Focuses Image
Inside Body Tube)

Body Tube
(Image Focuses)

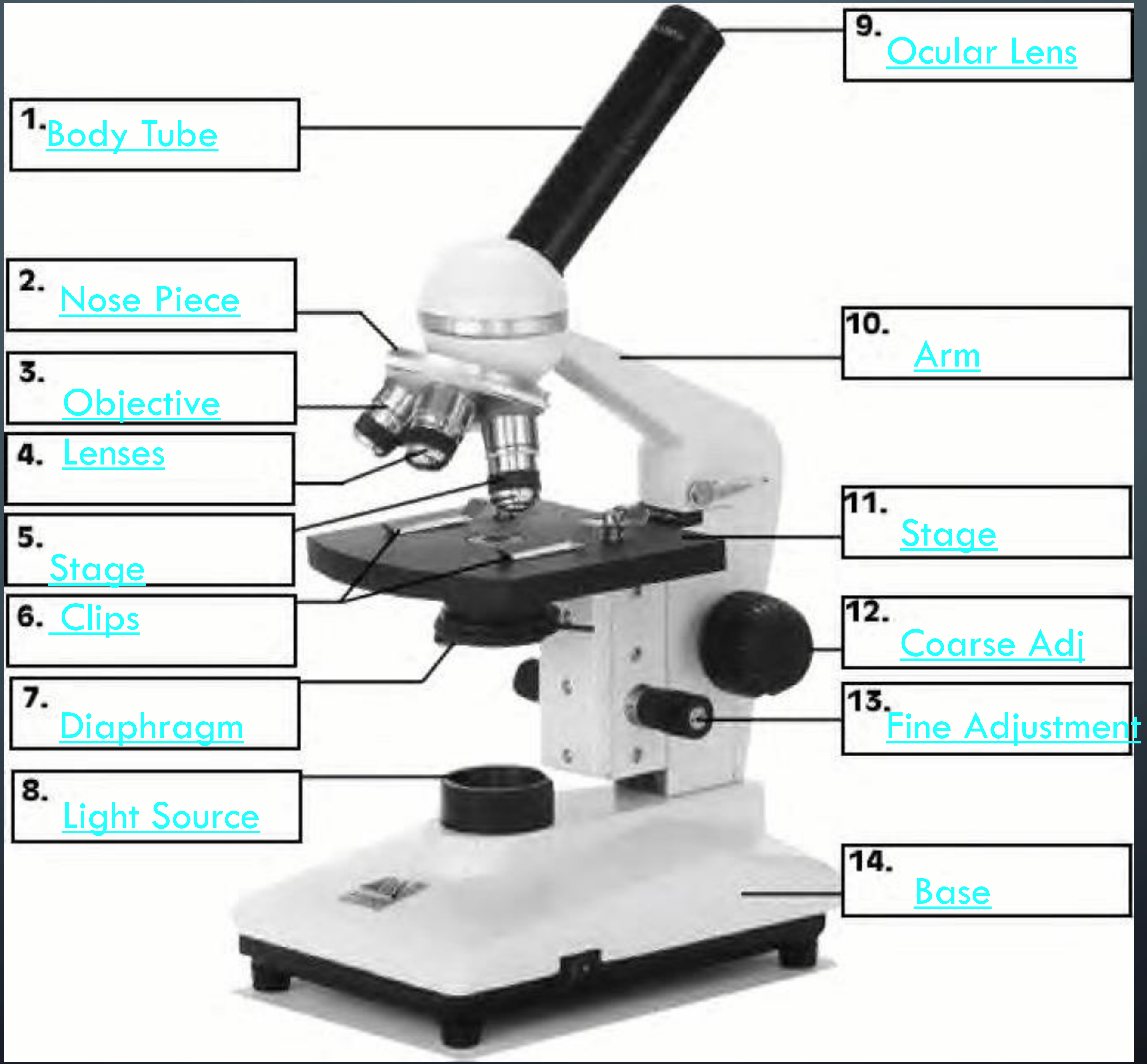
- **Bending Light**: The objective (bottom) convex lens magnifies and focuses (bends) the image inside the body tube and the ocular convex (top) lens of a microscope magnifies it (again).

HOW A MICROSCOPE WORKS

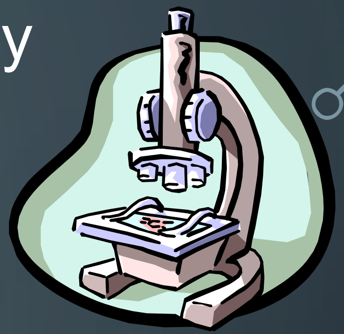


Convex Lenses are curved glass used to make microscopes (and glasses etc.)

Convex Lenses bend light and focus it in one spot.



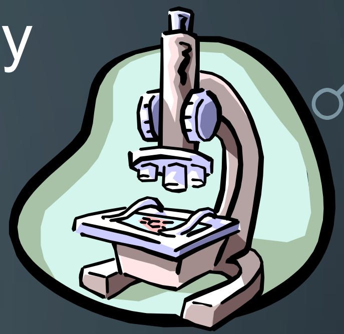
1. Body Tube
2. Nose Piece
3. Objective
4. Lenses
5. Stage
6. Clips
7. Diaphragm
8. Light Source
9. Ocular Lens
10. Arm
11. Stage
12. Coarse Adj
13. Fine Adjustment
14. Base



Timeline of the Microscope

1830: Joseph Jackson Lister discovers that using weak lenses together at various distances provided clear magnification. *Spherical Aberration*

1878: A mathematical theory linking resolution to light wavelength is invented by Ernst Abbe with **Carl Zeiss**. (paper in 1877 defining the physical laws that determined resolving distance of an objective. Known as Abbe's Law

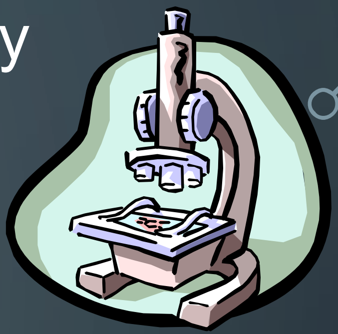


Timeline of the Microscope

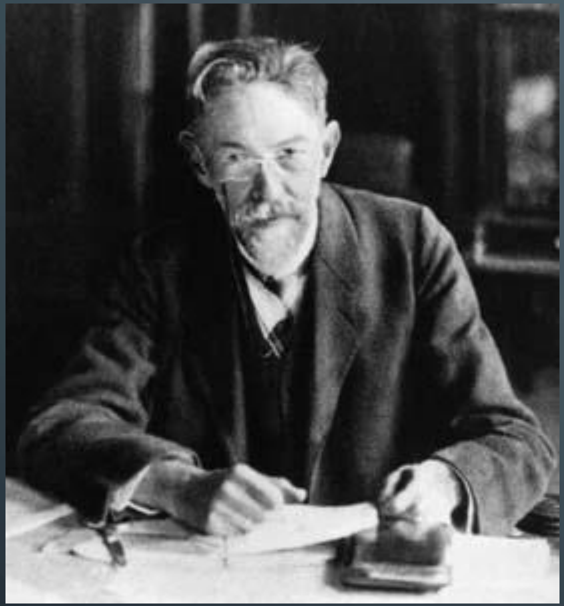
Abbe's Law

$$d = \frac{\lambda}{2\, n \sin \theta}$$

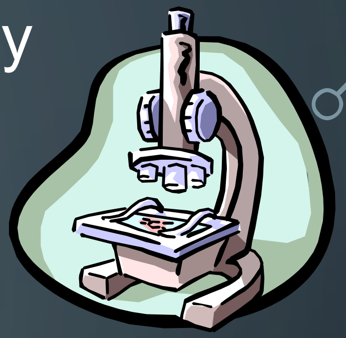
"minimum resolving distance (d) is related to the wavelength of light (lambda) divided by the Numeric Aperture, which is proportional to the angle of the light cone (theta) formed by a point on the object, to the objective".



Timeline of the Microscope



- **1903** – Richard Zsigmondy developed the ultramicroscope that could study objects below the wavelength of light. He won the Nobel Prize in Chemistry in 1925.



Timeline of the Microscope

Frits Zernike
(1888-1966)

- **1932** – Frits Zernike invented the phase-contrast microscope that allowed for the study of colorless and transparent biological materials for which he won the Nobel Prize in Physics in 1953.

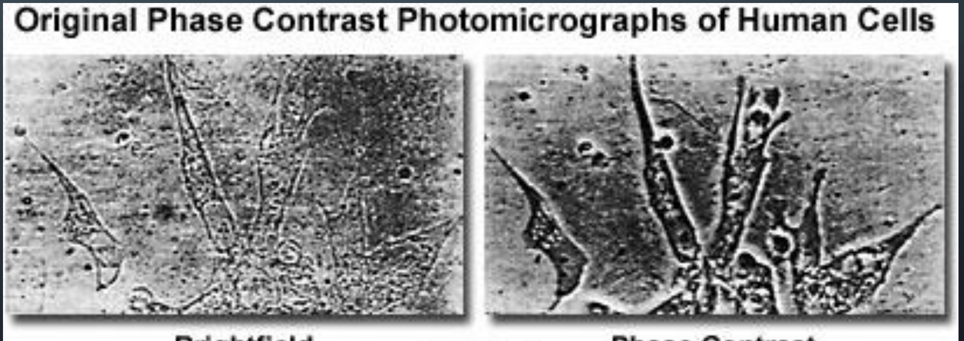
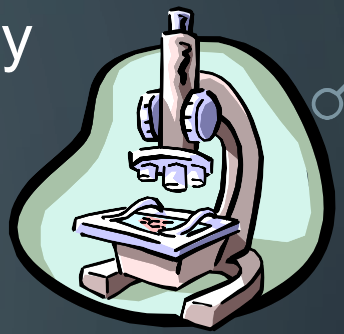


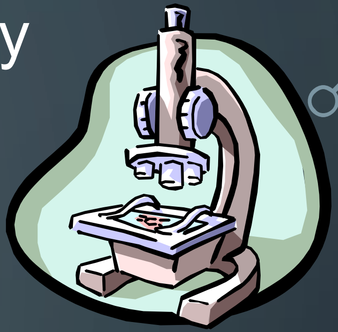
Figure 1

Timeline of the Microscope



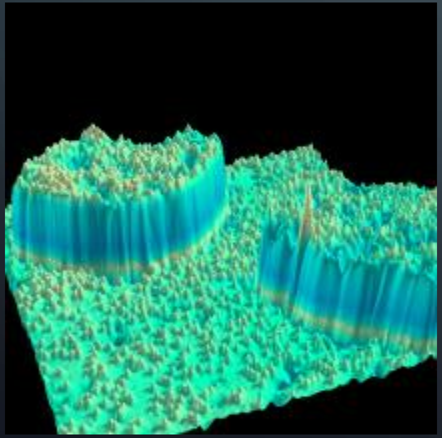
Ernst Ruska
(1906-1988)

- **1931** – Ernst Ruska co-invented the electron microscope for which he won the Nobel Prize in Physics in 1986. An electron microscope depends on electrons rather than light to view an object, electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light. Electron microscopes make it possible to view objects as small as the diameter of an atom.

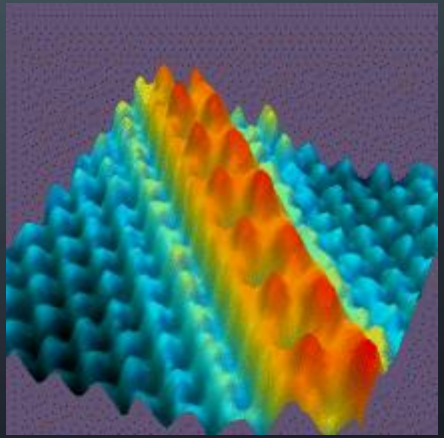


Timeline of the Microscope

- **1981** – Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level. Binnig and Rohrer won the Nobel Prize in Physics in 1986.



STM image, 35 nm x 35 nm, of single substitutional Cr impurities (small bumps) in the Fe(001) surface.



STM image, 7 nm x 7 nm, of a single zig-zag chain of Cs atoms (red) on the GaAs(110) surface (blue).

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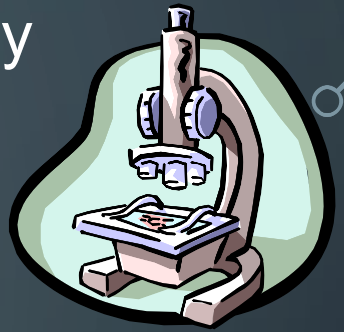


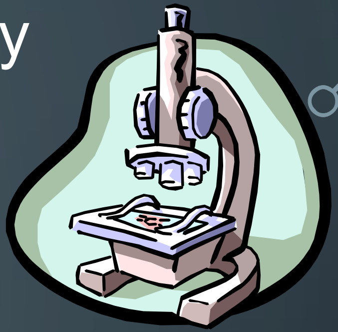
Timeline of the Microscope

Erik Betzig

StefanW.Hell

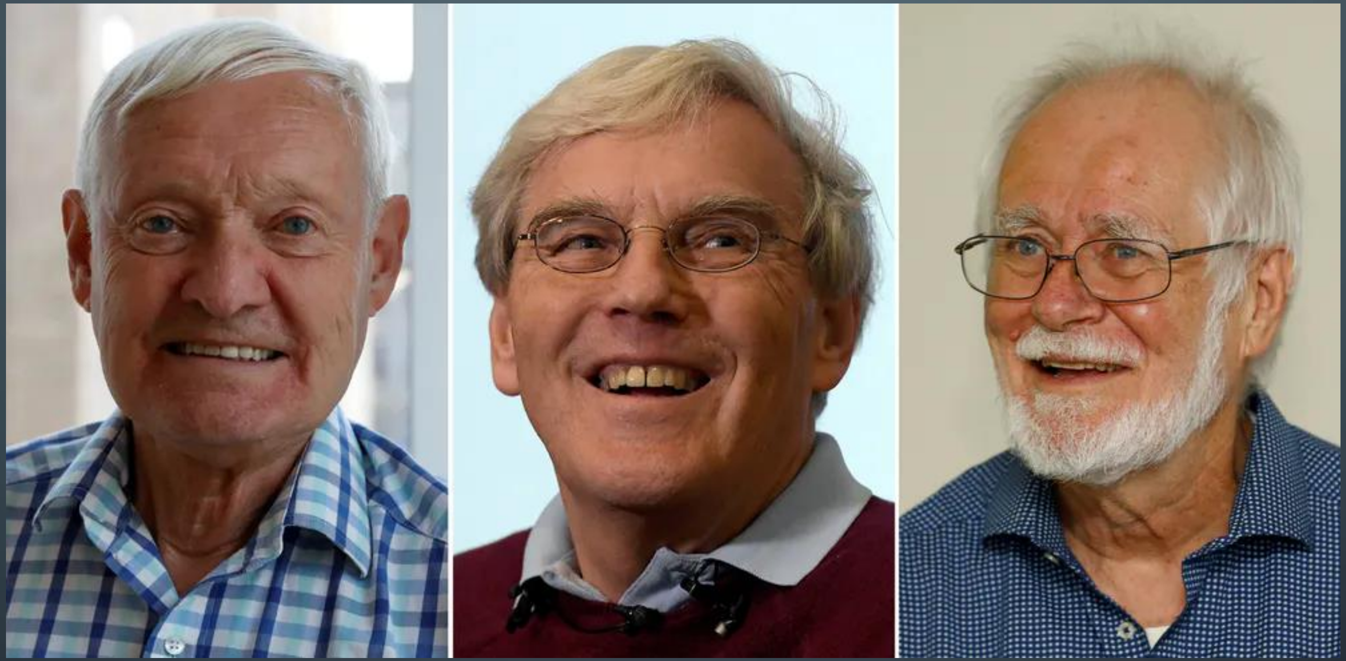
W. E.Moerner

The Royal Swedish Academy of Sciences has decided to award Erik Betzig, Stefan W. Hell and W. E. Moerner the Nobel Prize in Chemistry 2014 for the development of super-resolution fluorescence microscopy.

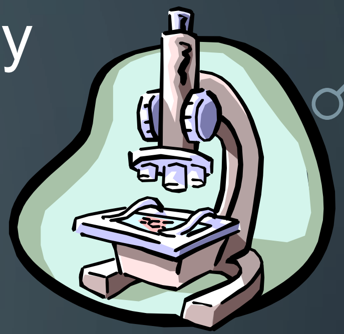


Timeline of the Microscope

Joachim Frank Richard Henderson Jacques Dubochet



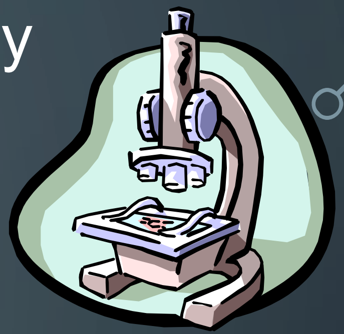
The Royal Swedish Academy of Sciences has decided to award Joachim Frank, Richard Henderson, Jacques Dubochet the Nobel Prize in Chemistry 2017 for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution.



Microscopes are essential for biological studies

- Light microscopes: cellular resolution
  - bright-field (stains)
  - dark-field
  - phase contrast
  - fluorescence (stains)

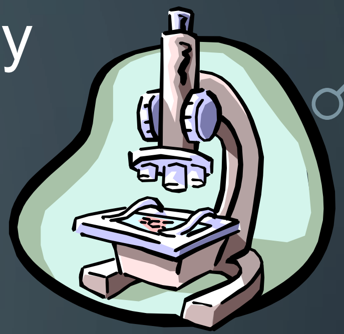
- Super resolution microscopy: subcellular resolution



Some Definitions

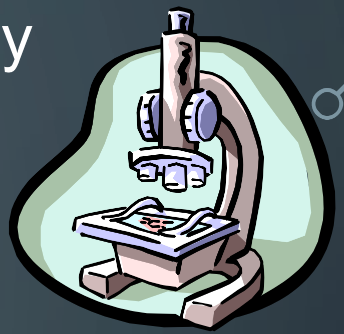
- Magnification:

- Resolution:



Some Definitions

- Magnification: increase of an object's apparent size
- Resolution: power to show details clearly
- Both are needed to see a clear image



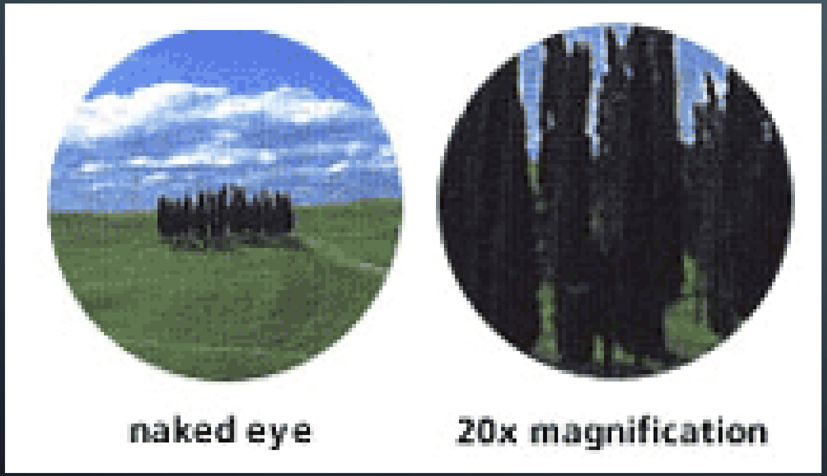
Magnification

enlargement of an object

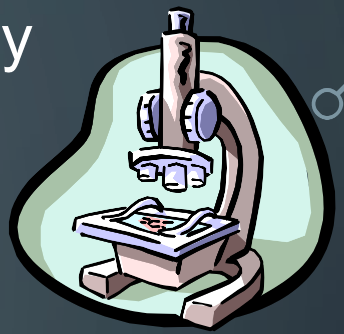
compare size of image to actual size of object

total magnification

ocular power x objective power = total magnification

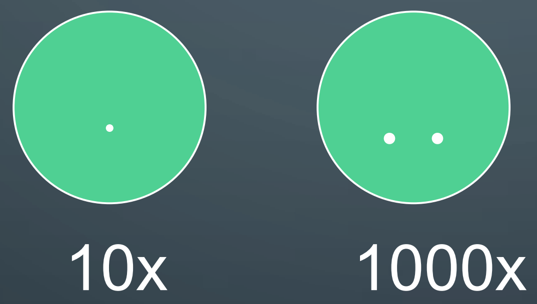


Magnification is NOT ALWAYS related with resolution

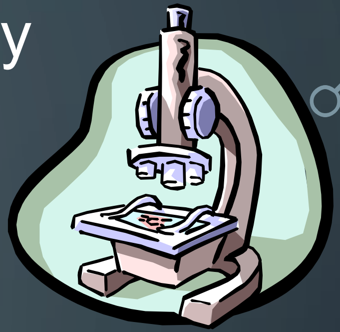


Resolution power to show details clearly

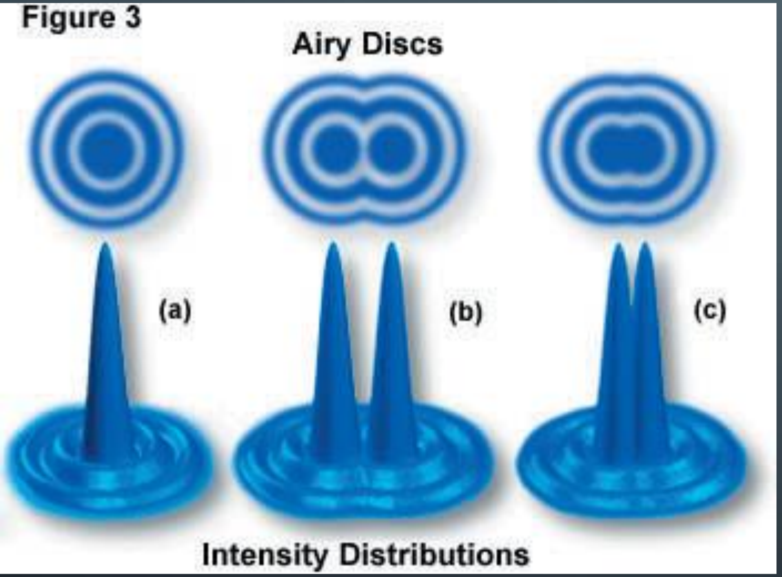
- **Resolution** – capacity to show 2 points that are close together as separate

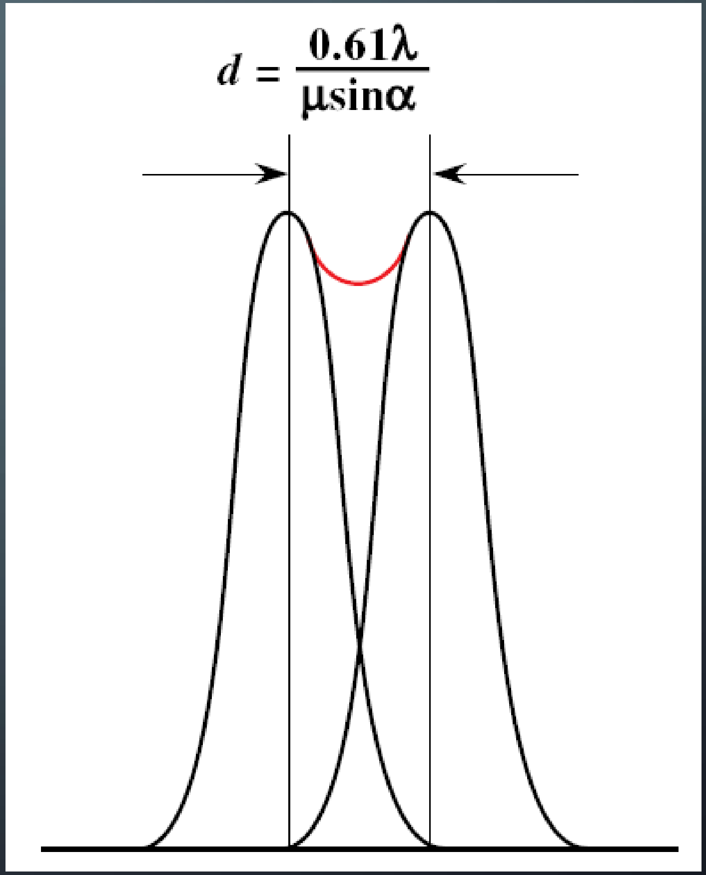


Poor Resolution = Blurry Image
Good Resolution = Clear Image

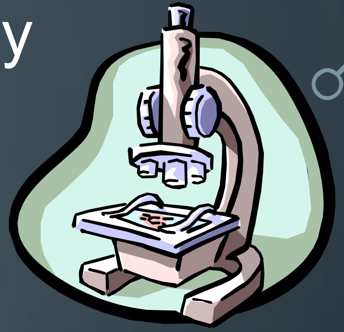


Abbe's Criterion





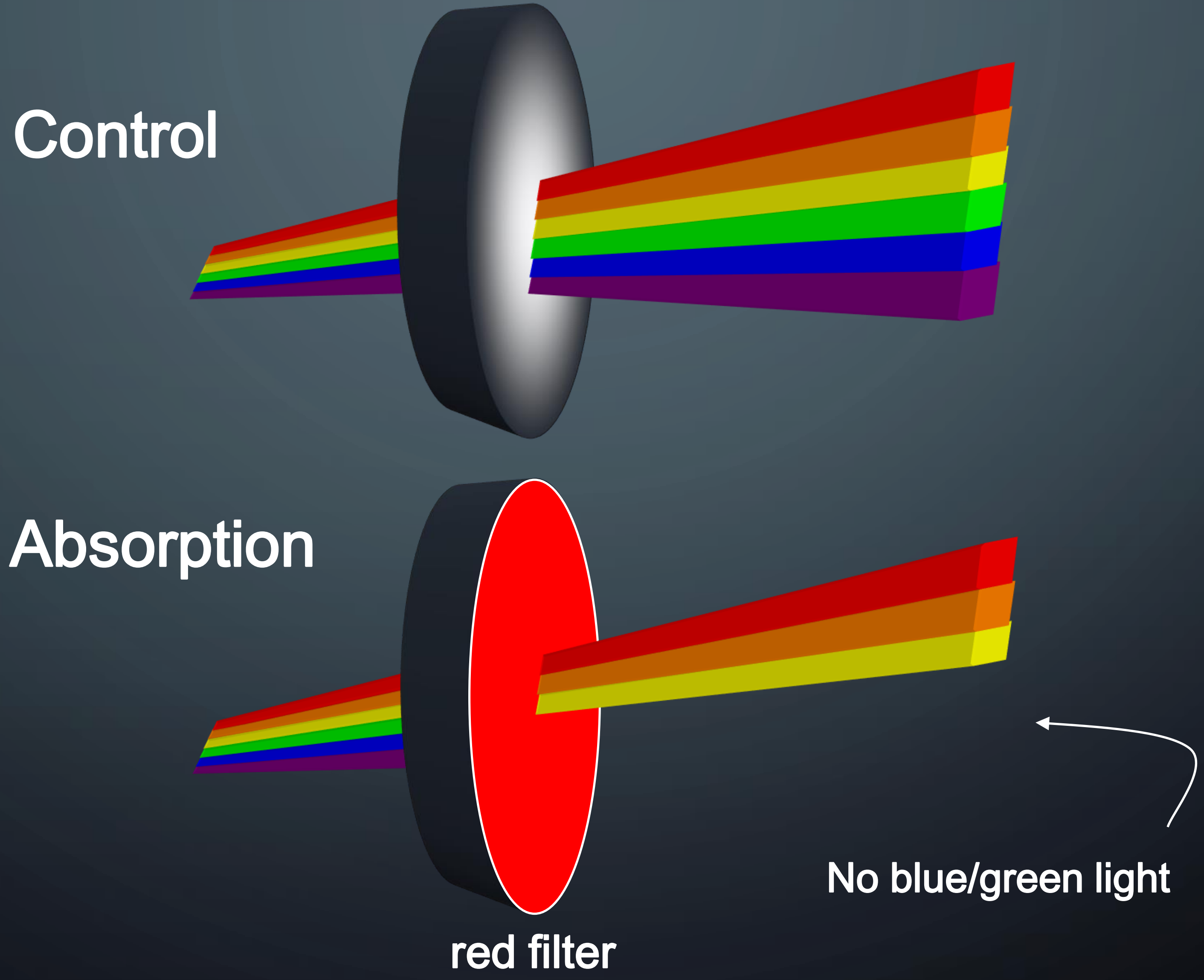
Resolution

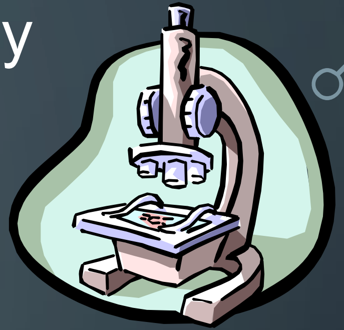


Some Definitions

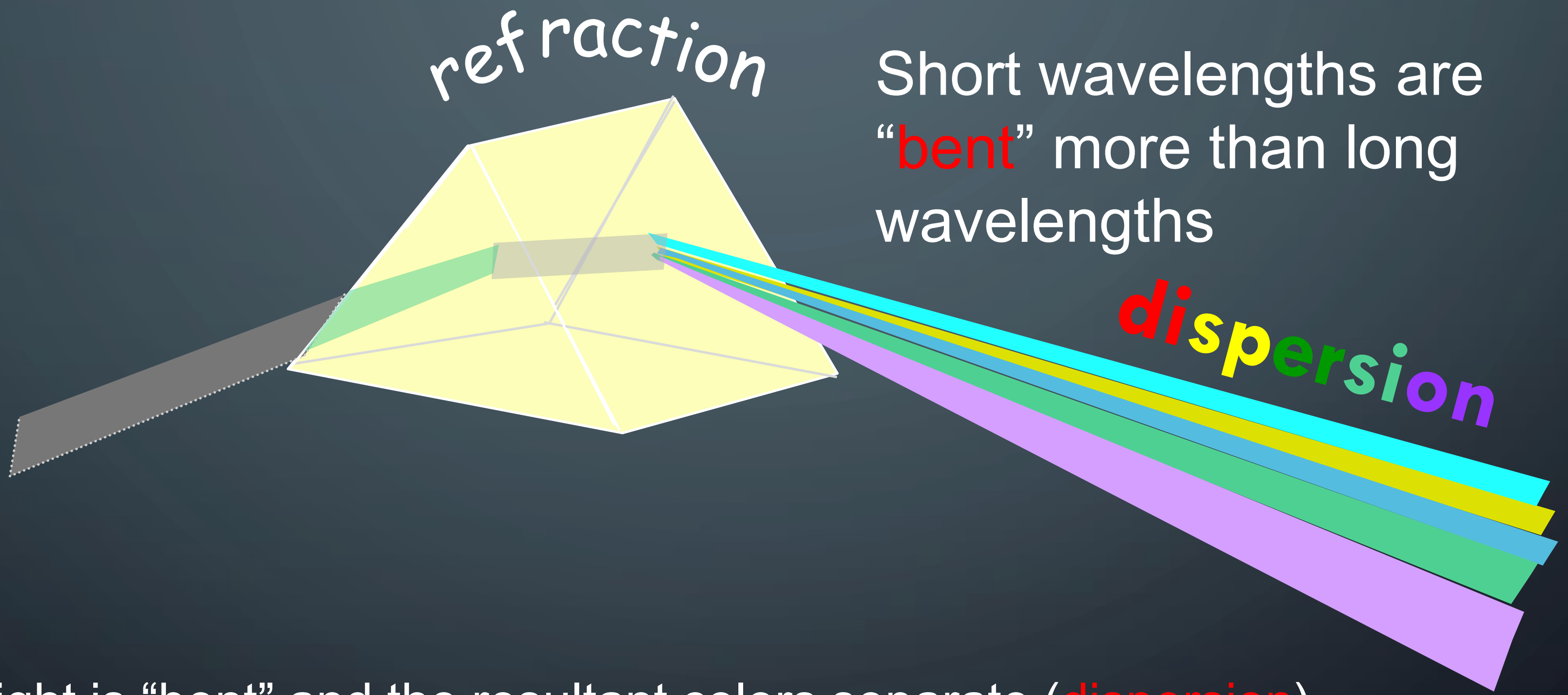
- **Absorption**
  - When light passes through an object the intensity is reduced depending upon the color absorbed. Thus the selective absorption of white light produces colored light.
- **Refraction**
  - Direction change of a ray of light passing from one transparent medium to another with different optical density. A ray from less to more dense medium is bent perpendicular to the surface, with greater deviation for shorter wavelengths
- **Diffraction**
  - Light rays bend around edges - new wavefronts are generated at sharp edges - the smaller the aperture the lower the definition
- **Dispersion**
  - Separation of light into its constituent wavelengths when entering a transparent medium - the change of refractive index with wavelength, such as the spectrum produced by a prism or a rainbow

ABSORPTION





REFRACTION & DISPERSION

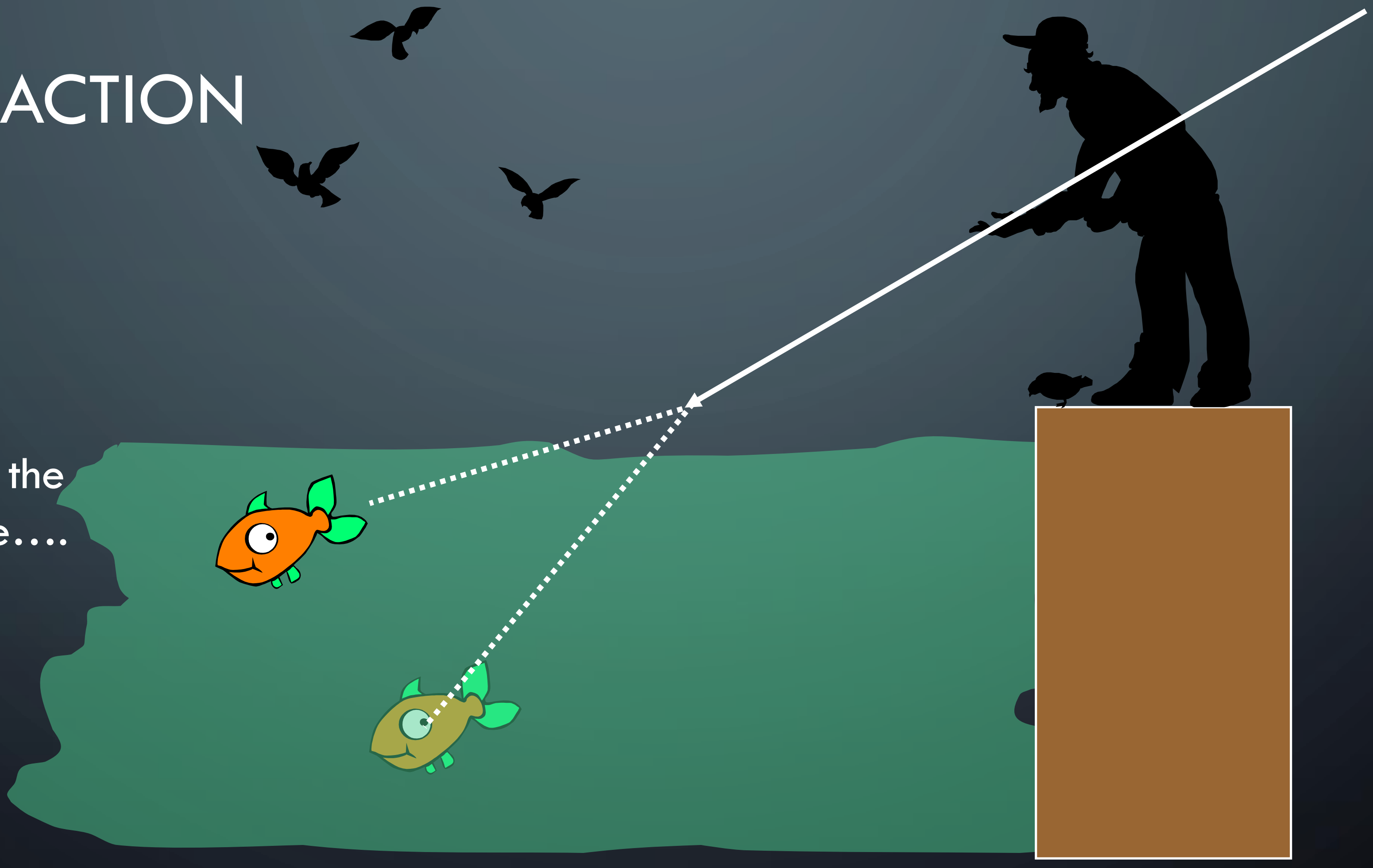


Short wavelengths are "bent" more than long wavelengths

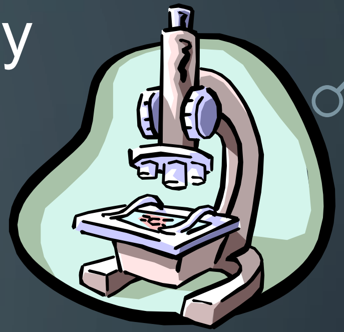
Light is "bent" and the resultant colors separate (dispersion).
Red is least refracted, violet most refracted.

REFRACTION

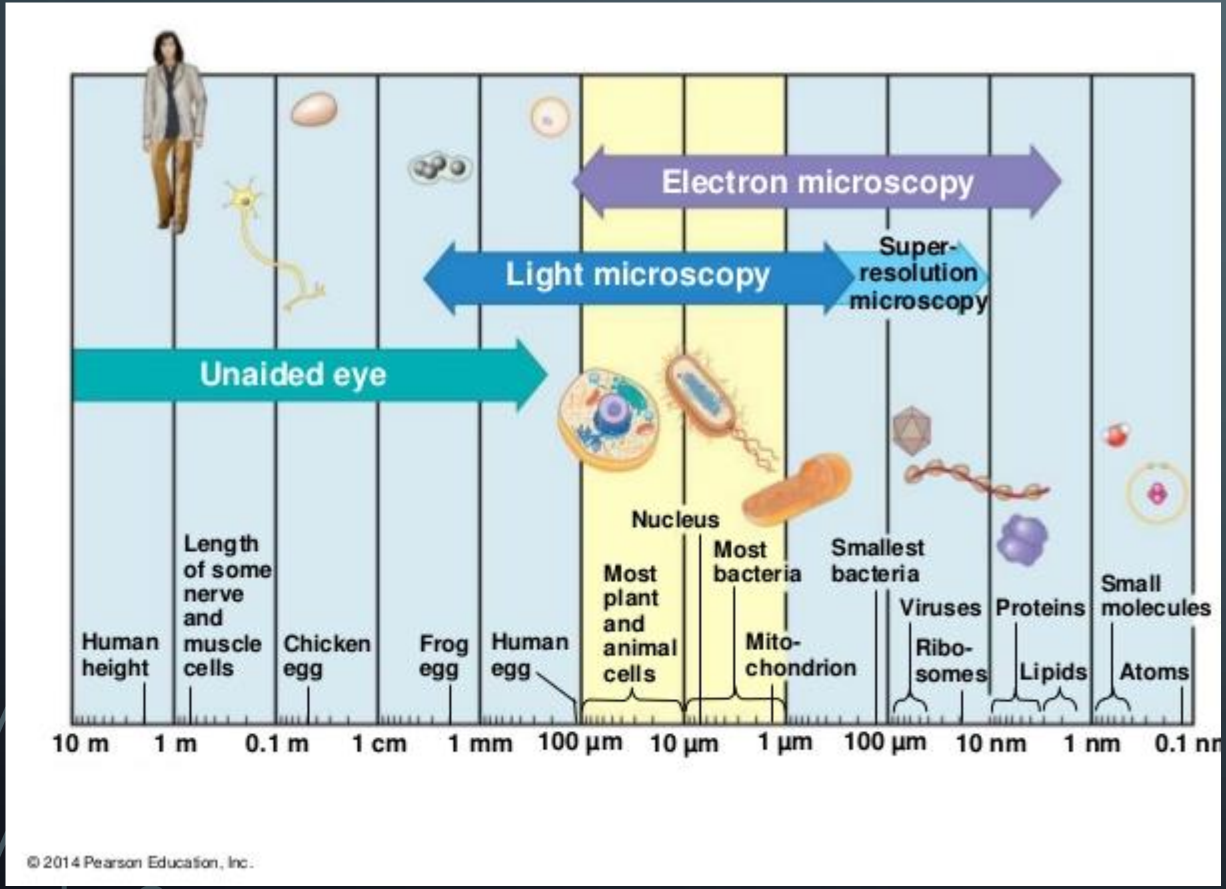
He sees the
fish here….



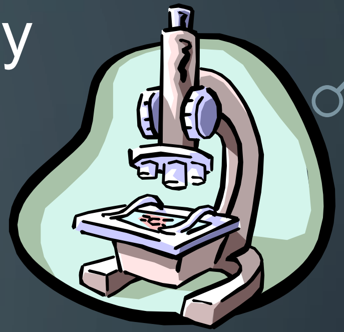
But it is really here!!



WHY MICROSCOPY ?



- Microscopes are essential for biological studies
- Light microscopes: cellular resolution
  - bright-field (stains)
  - dark-field
  - phase contrast
  - fluorescence (stains)
- Super resolution microscopy: subcellular resolution



Question?

See You next week !

Gabriele Baj
gbaj@units.it