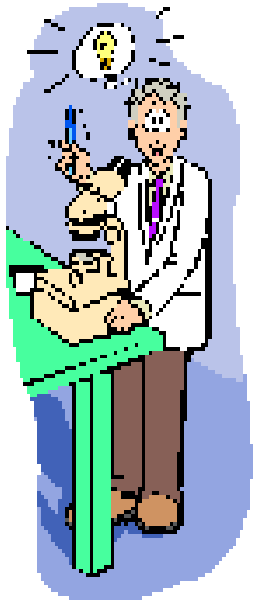
BASICS OF LABORATORY SAFETY

AND

IMPORTANCE OF STREAKING TECHNIQUE

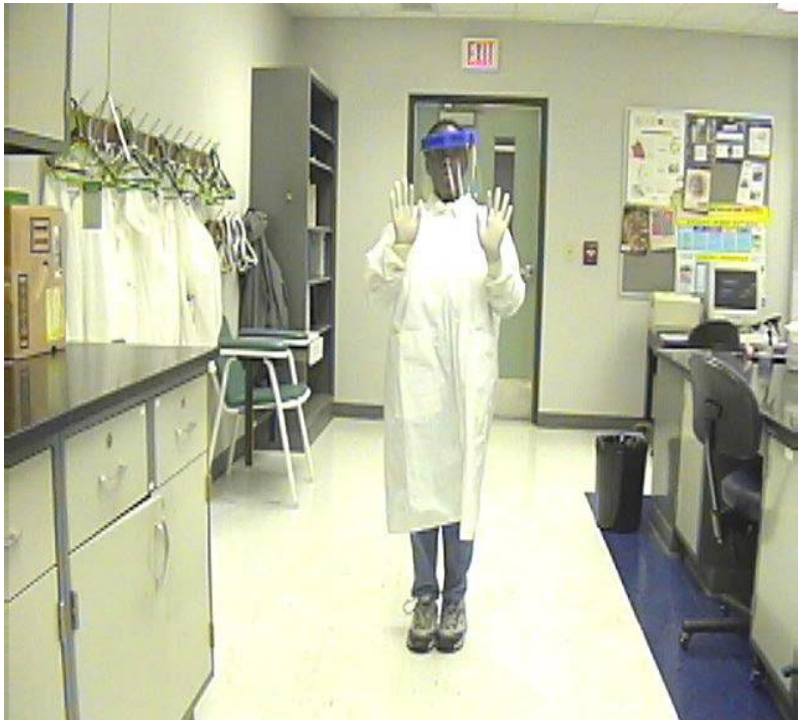
Before Beginning Any Laboratory Exercise

- Know the dangers associated with all materials you will use in this laboratory exercise
- Know the proper way of using all the materials provided for the exercise
- Know all the protective measures you must use during the exercise
- Know the proper procedure how to handle and use the microscope
- Aseptic technique
- Pure culture technique



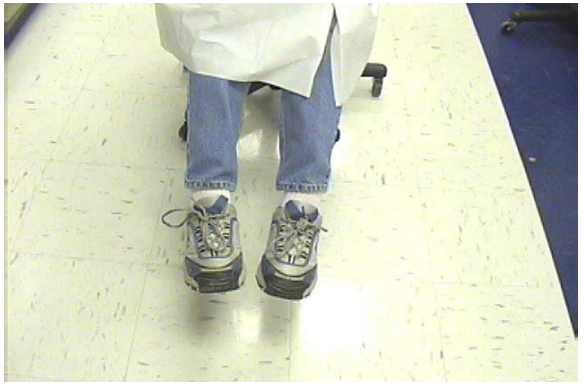
Personal Protection

- Wear a lab coat in the proper manner
- At the beginning and at the end of each lab session clean the benches with disinfectants
- Wash hands thoroughly with 70% Ethanol



Personal Protection

- Tie long hair neatly at the back of the neck
- Wear shoes that enclose your entire feet



Emergency Procedures

- If a reagent spills onto the workbench or floor, notify the instructor immediately for cleanup instructions.
- If a glass container gets broken, notify the instructor immediately for cleanup instructions.
- Report all accidents, injuries and close calls immediately to the instructor.

Good Laboratory Conduct

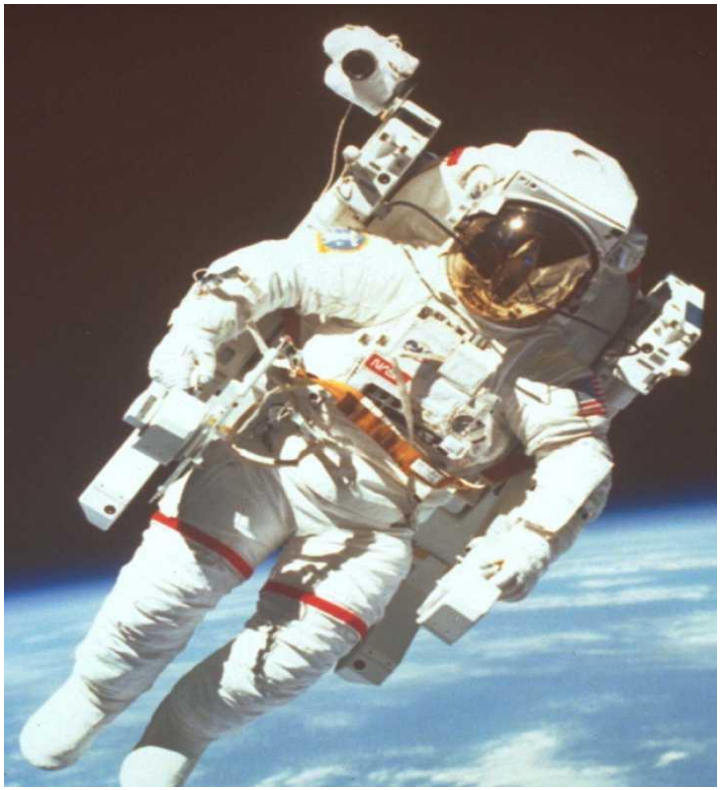
- Follow all instructions
- Do not play in the laboratory
- Do not use any laboratory equipment until you are told to do so
- Do not perform any laboratory procedure until you are told to do so

Good Laboratory Conduct

- Do not bring food, beverages or tobacco products into the laboratory
- Do not eat or drink in the laboratory

Before You Leave The Laboratory

- Clean your workbench with disinfectant at the beginning and at the end of the laboratory exercise
- Leave all equipment, samples and reagents in the lab
- Wash your hands with soap and water



Aseptic Techniques

Refers to a procedure that is performed under sterile conditions

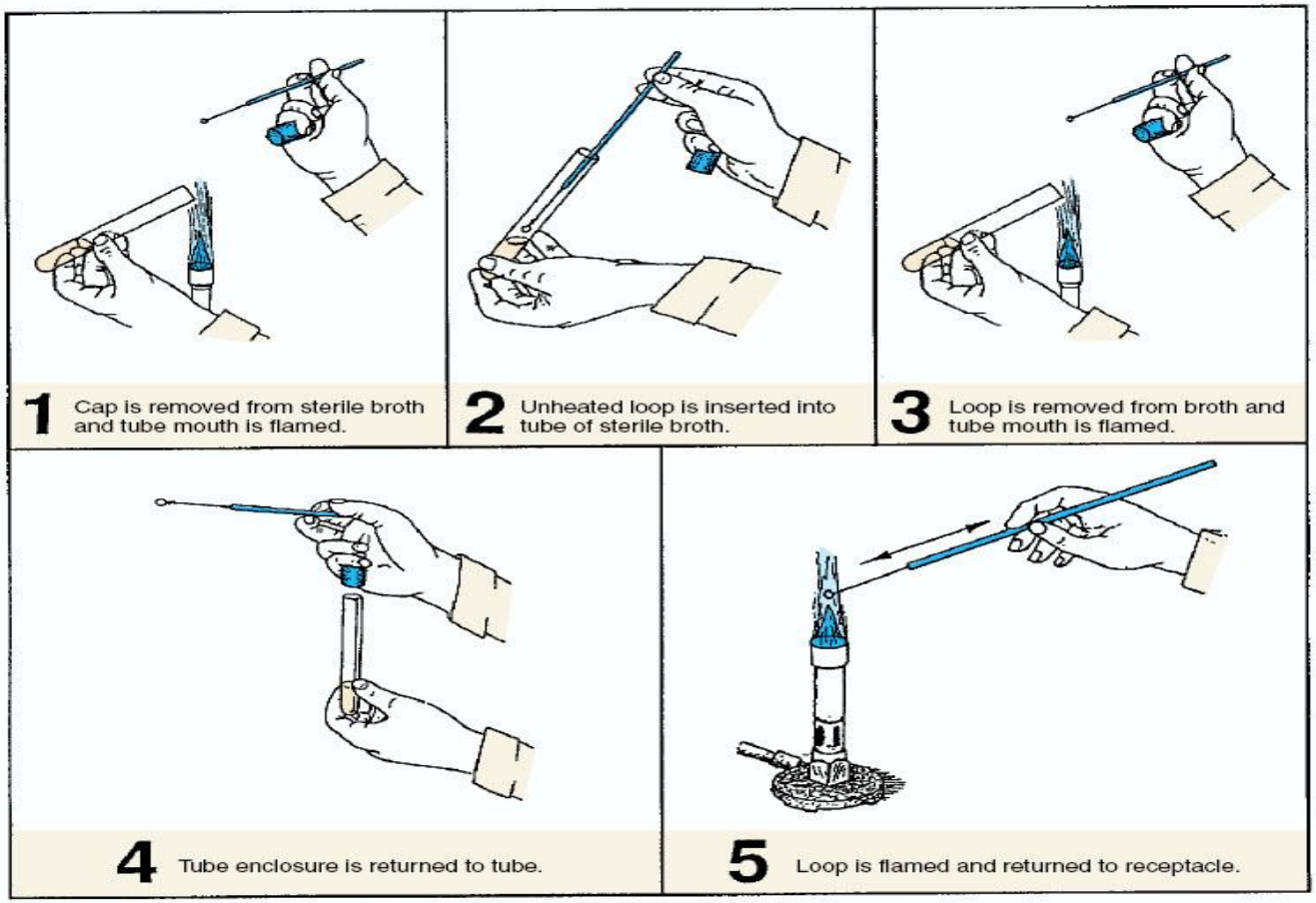
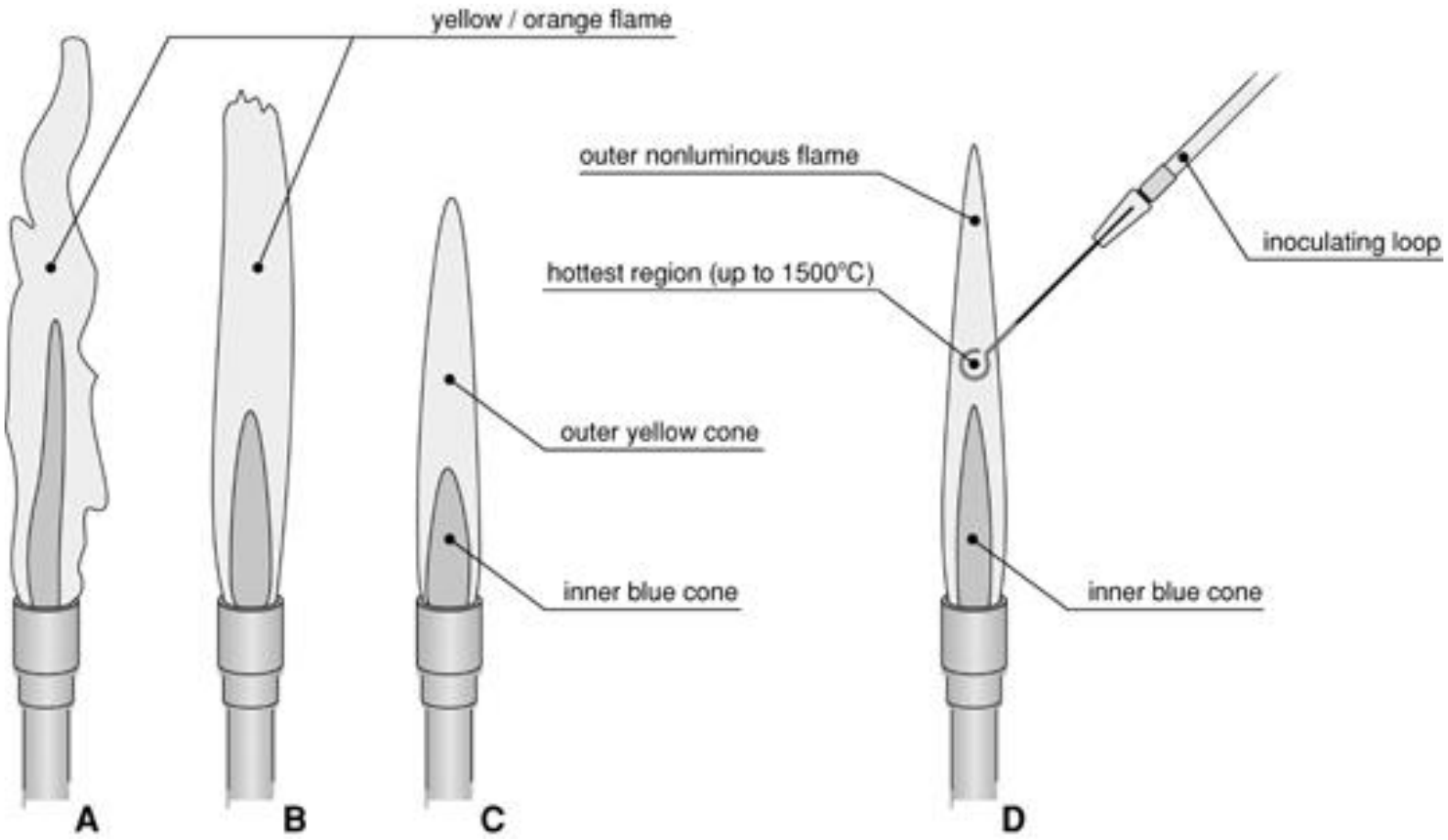


Figure 2 Procedure for inoculating a nutrient broth

Flaming



Streaking

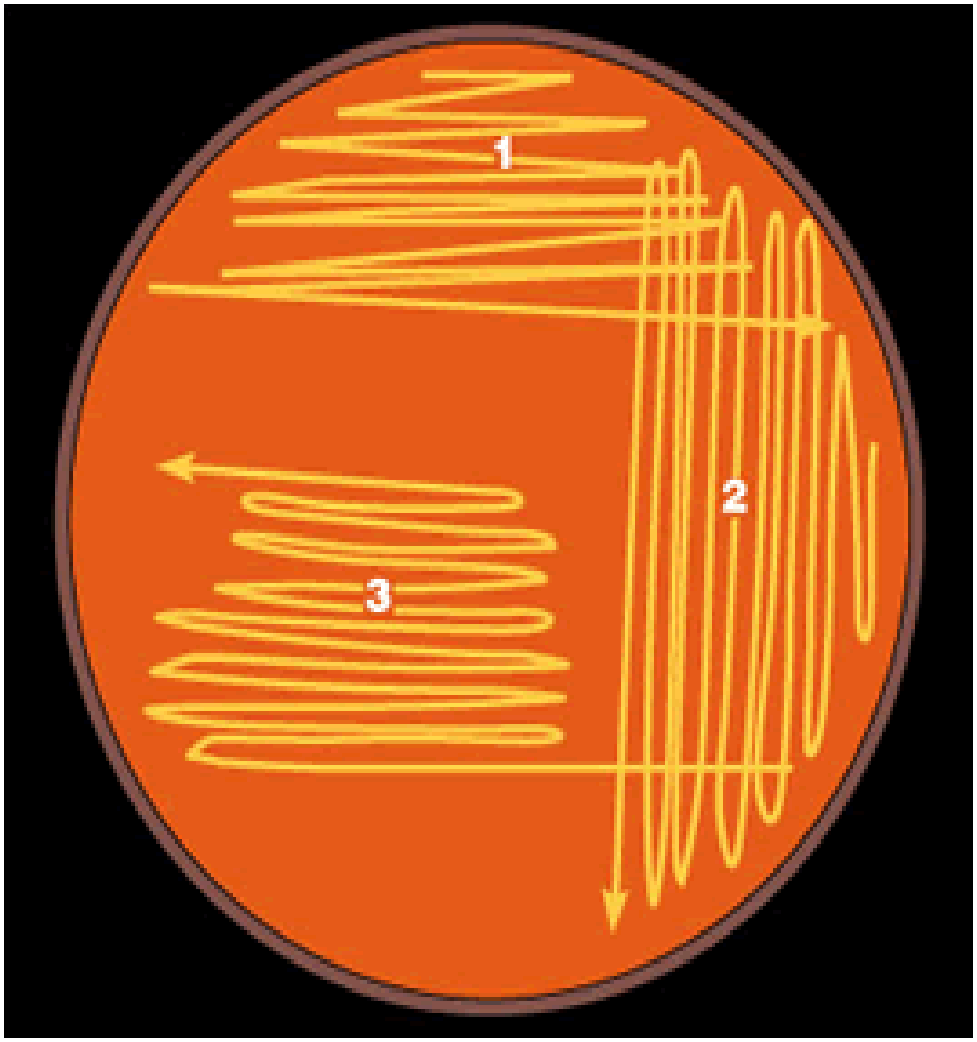
Purpose

The streaking procedure is used in order to check

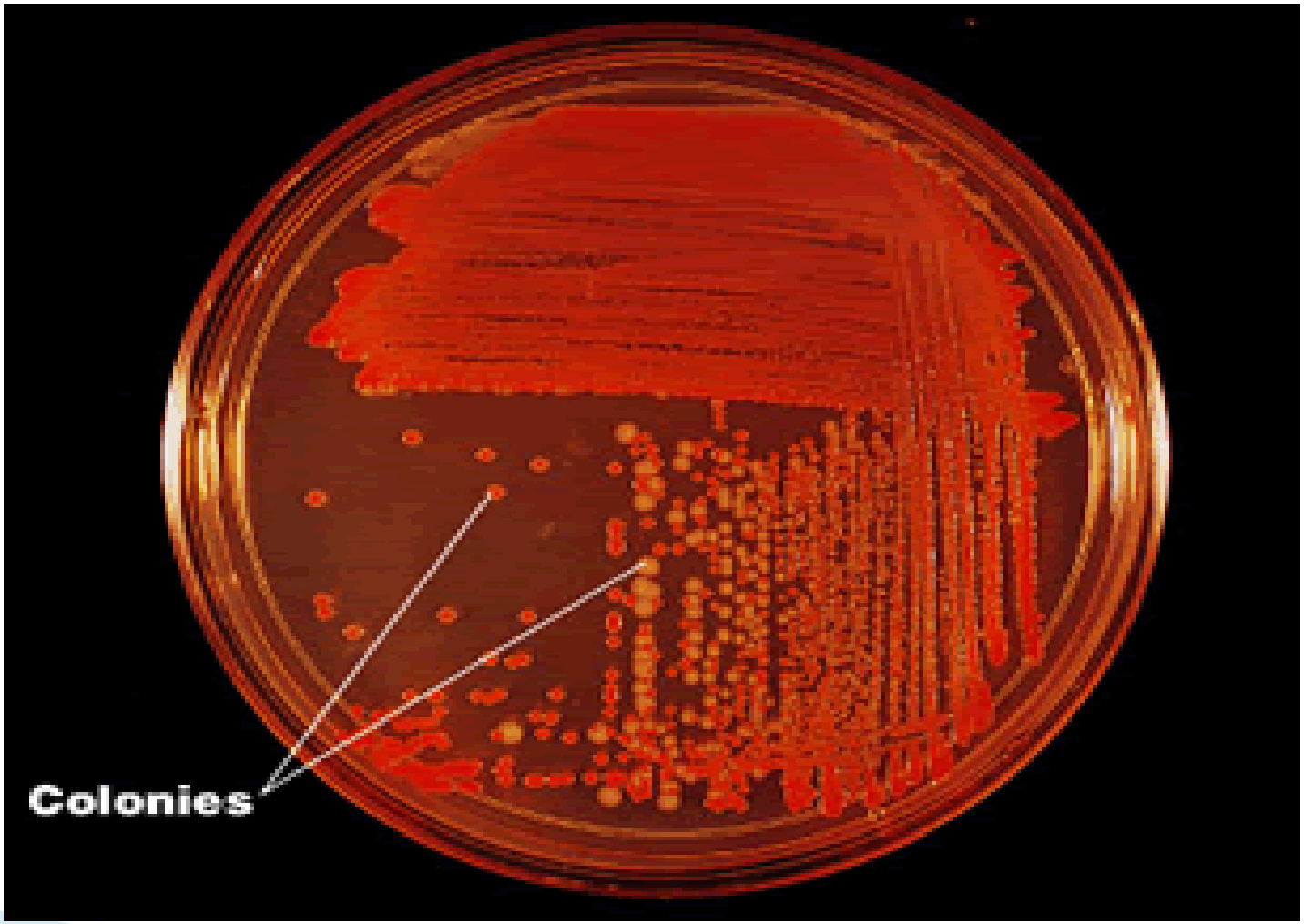
1) whether a culture consists of only one organism (a "pure culture") or if there are more than one organisms in it (contaminated)

2) whether a culture is viable, i.e. able to grow

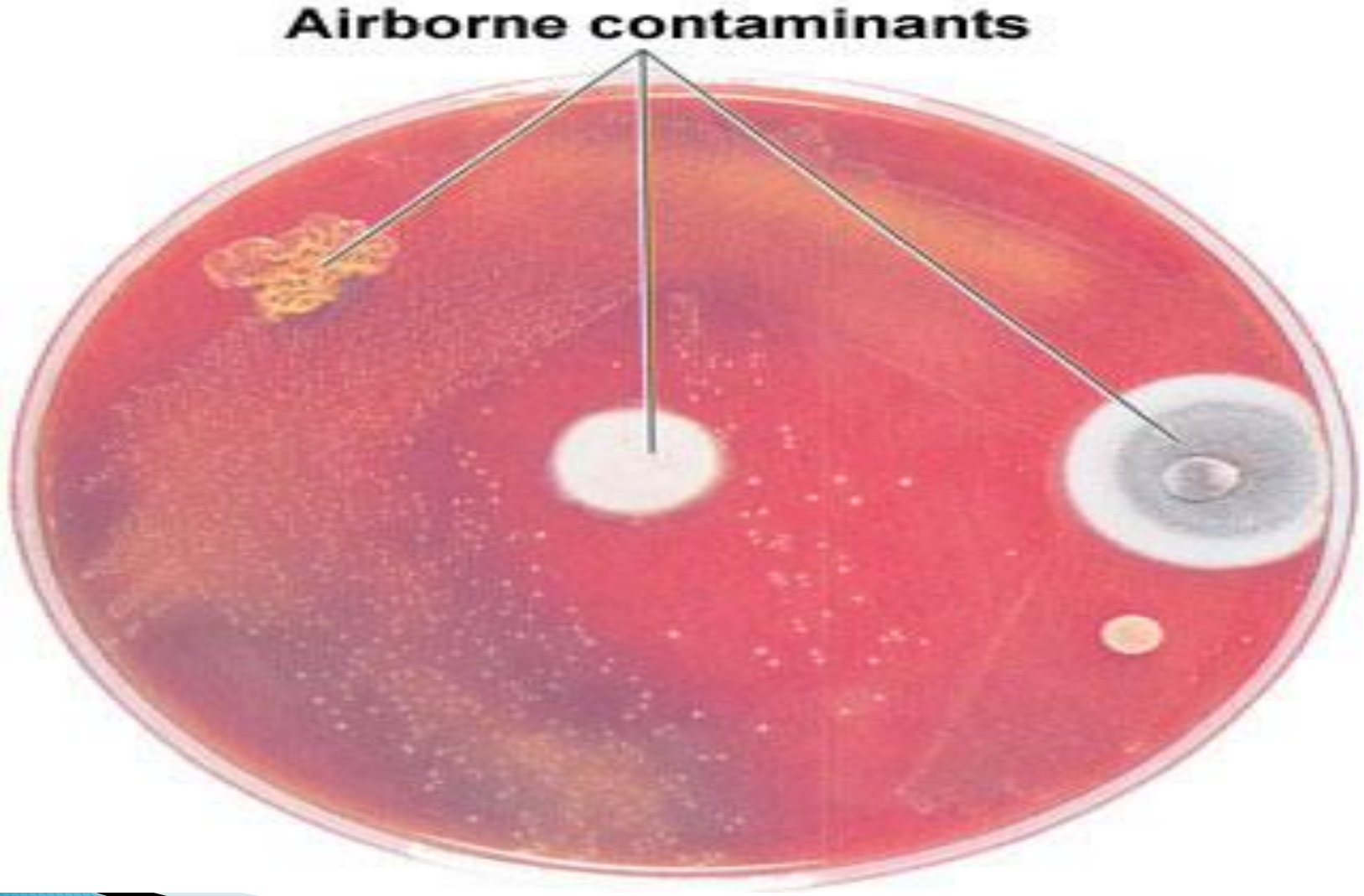
3) Describe colony morphology



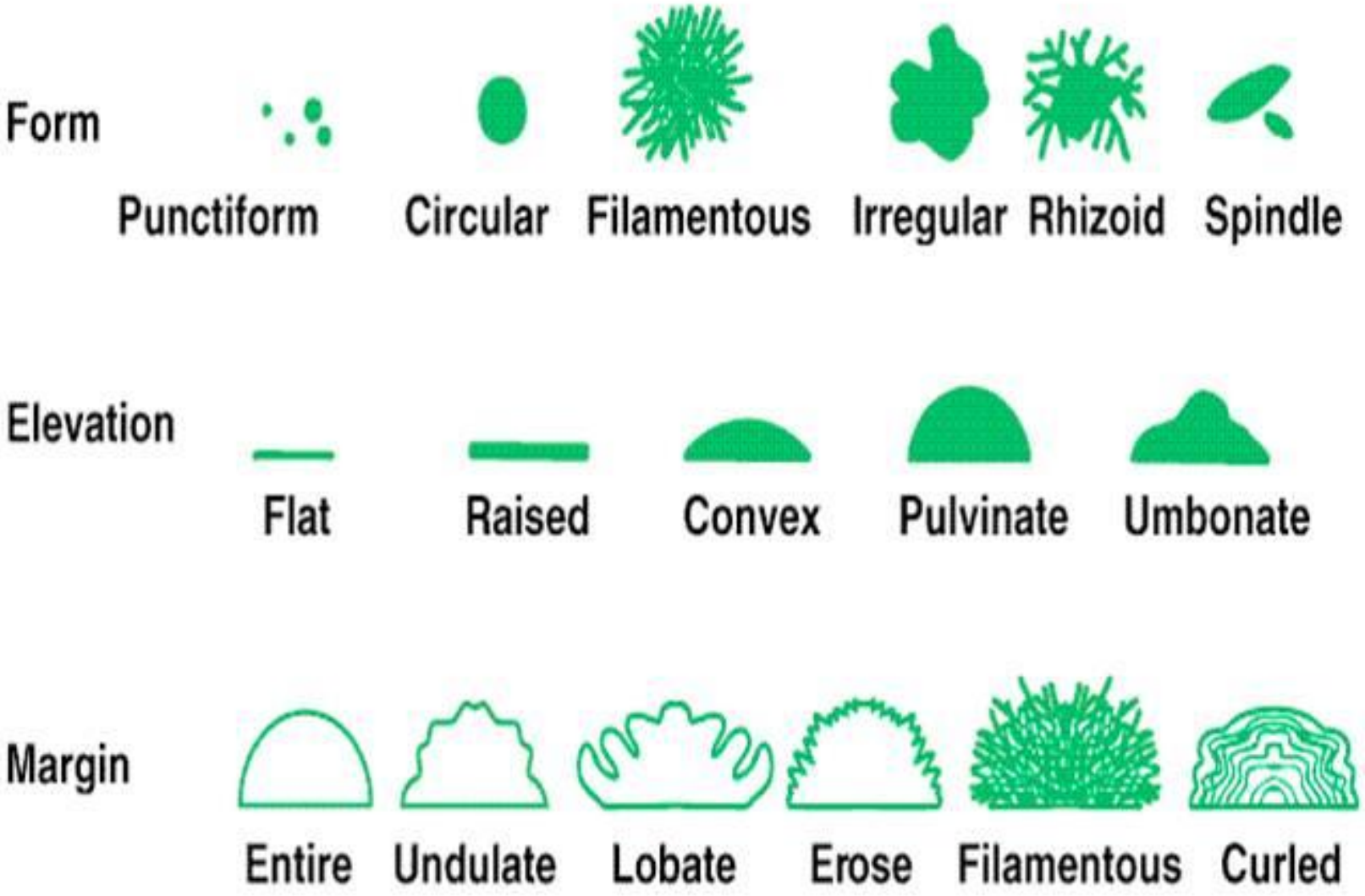
Streaking



Streaking



Colony Morphology

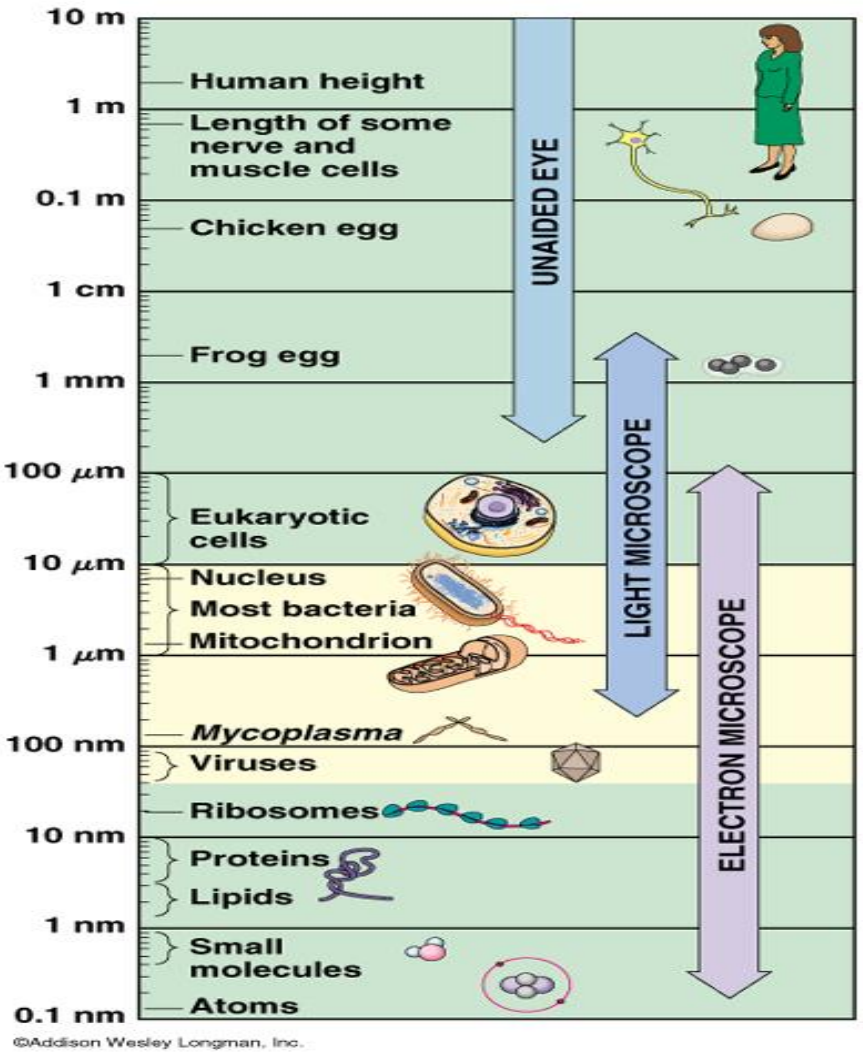


(a)

Hans N.

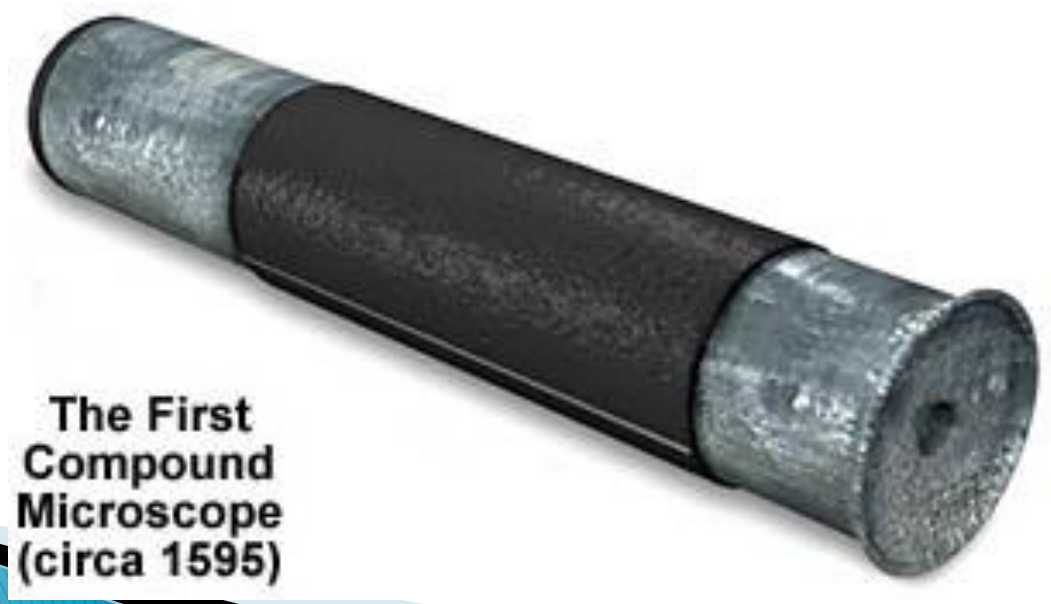
Microscopes

- Microorganisms range in size from the smallest viruses which are measured in nanometers (nm), to the largest protists and bacteria, which can be about 200 micrometers (μm).



History of the Microscope

- 1590 –first compound microscope

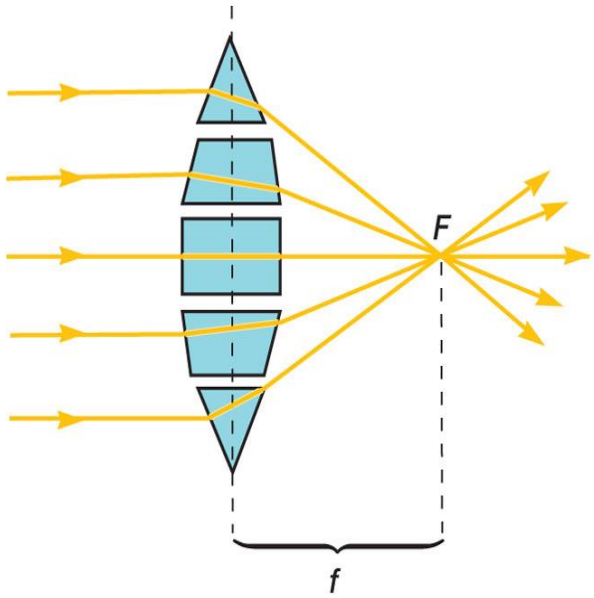


The First Compound Microscope (circa 1595)

History of the Microscope

- 1655 – Robert Hooke used a compound microscope to observe pores in cork
  - He called them “cells”

Microscopes



- Important tool in microbiology.
- We need to distinguish between
  - Magnification: a function of lenses
  - Resolution: a function of light
- Resolution depends on the **wavelength** of light. Visible light has a wavelength that ranges between 0.4 to 0.7 μm. The best possible resolution is 0.2 μm.

Microscopes

- What do we mean by resolution?
  - Definition: the capability of an optical system, or other imaging **system to distinguish two adjacent objects as distinct and separate**
- What do we mean by Magnification ??
  - Magnification means the act of making something bigger or increasing the size of something. This can be done by the of optical lenses

Microscope

- Many varieties
  - bright-field microscope
  - dark-field microscope
  - phase-contrast microscope
  - fluorescence microscope
  - confocal microscope
- Compound microscopes
  - image formed by action of ≥2 lenses

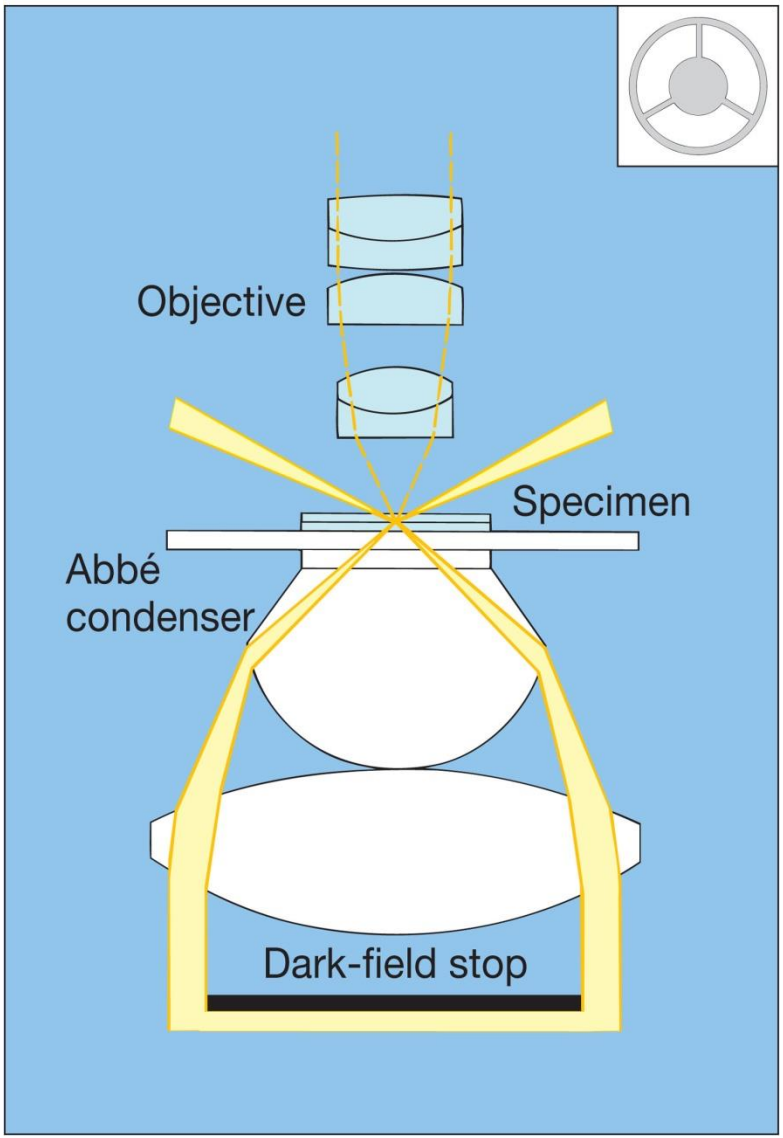
The Bright-Field Microscope

Courtesy of Leica, Inc.

- Produces a dark image against a brighter background
- Has several objective lenses
  - parfocal microscopes remain in focus when objectives are changed
- Total magnification
  - product of the magnifications of the ocular lenses and the objective lenses

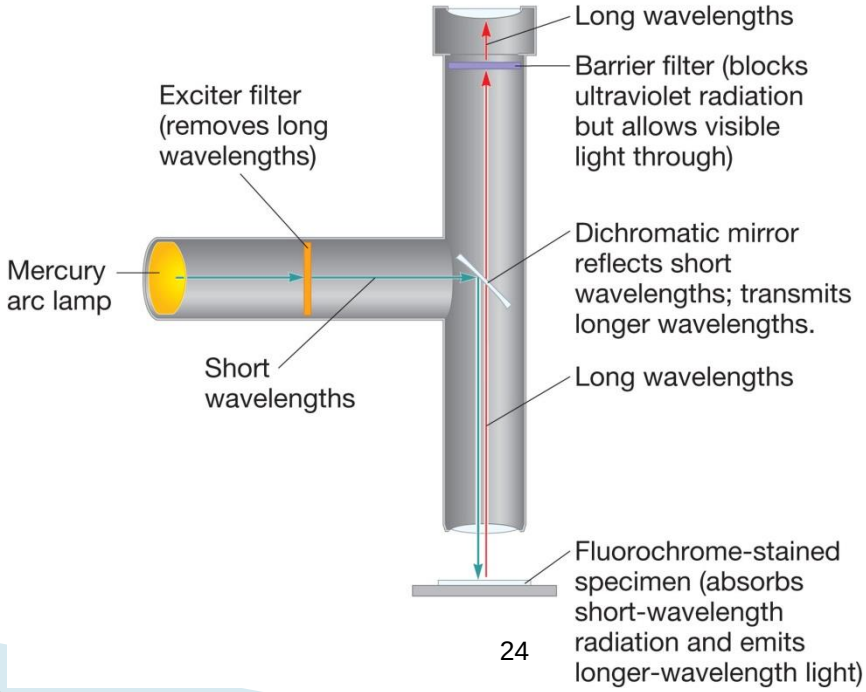
The Dark-Field Microscope

- Image is formed by light reflected or refracted by specimen
- Produces a bright image of the object against a dark background
- Used to observe living, unstained preparations
  - used to observe internal structures in eukaryotic microorganisms
  - used to identify bacteria such as *Treponema pallidum*, the causative agent of syphilis



The Fluorescence Microscope

- Exposes specimen to ultraviolet, violet, or blue light
- Specimens usually stained with fluorochromes
- Shows a bright image of the object resulting from the fluorescent light emitted by the specimen
- Has applications in medical microbiology and microbial ecology studies

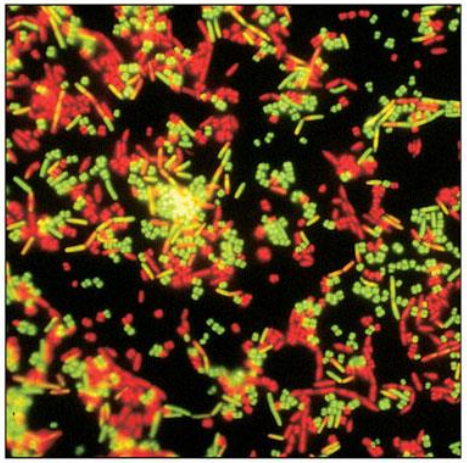


Fluorescence Microscopy

- Essential tool in microbiology
  - fluorochrome-labeled probes, such as antibodies, or fluorochrome dyes tag specific cell constituents for identification of unknown pathogens
  - localization of specific proteins in cells

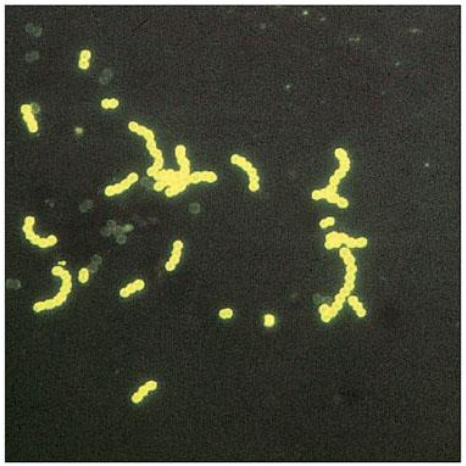
Table 2.3 Commonly Used Fluorochromes

Fluorochrome	Uses
Acridine orange	Stains DNA
Diamidino-2-phenyl indole (DAPI)	Stains DNA
Fluorescein isothiocyanate (FITC)	Often attached to DNA probes or to antibodies that bind specific cellular components
Tetramethyl rhodamine isothiocyanate (TRITC or rhodamine)	Often attached to antibodies that bind specific cellular components



(a)

10 µm



(b)

10 µm

a: Courtesy of Molecular Probes, Eugene, OR; b: © Evans Roberts

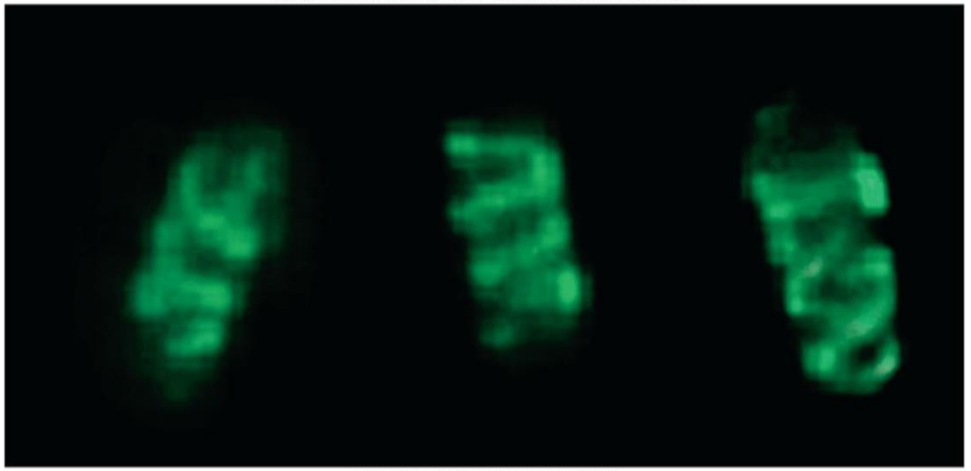
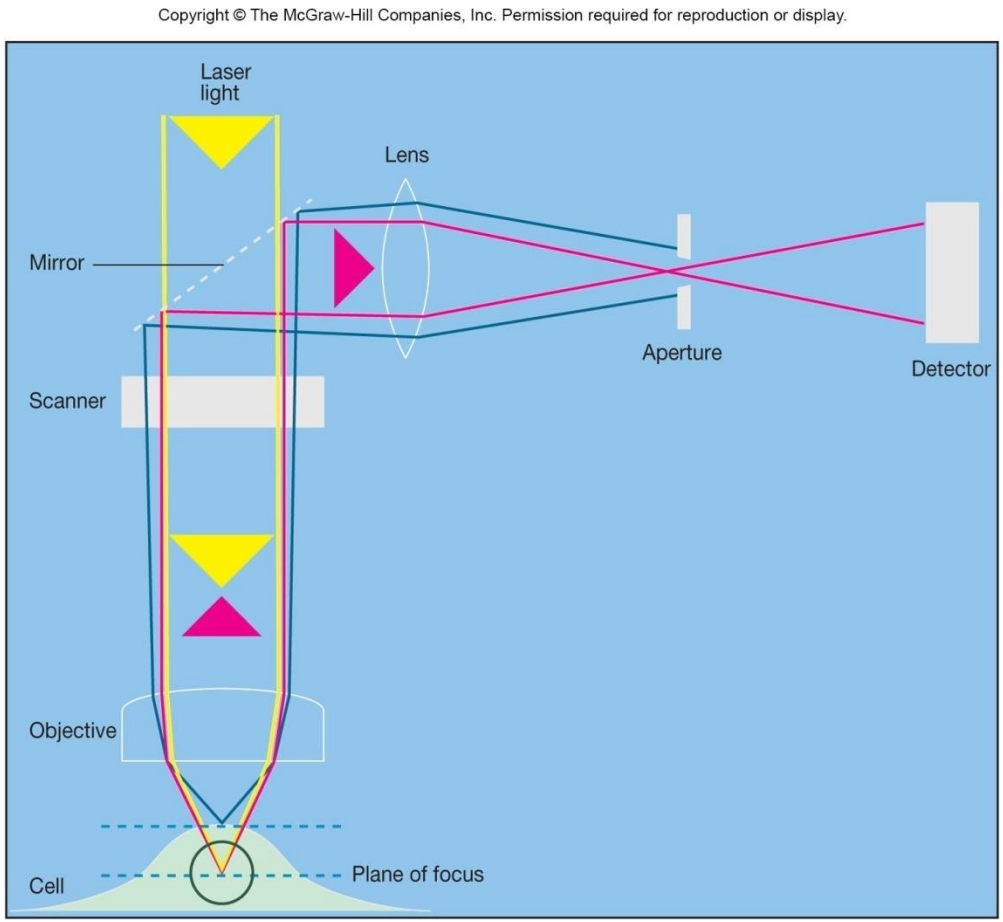


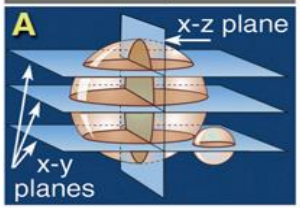
Image courtesy of Rut Carballido-Lo'pez and Jeff Errington

Confocal Microscopy

- Confocal scanning laser microscopy (CLSM) creates sharp, composite 3D image of specimens by using laser beam, aperture to eliminate stray light, and computer interface
- Numerous applications including study of biofilms



Schematic diagram of CSLM planes

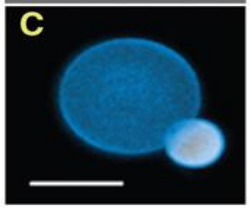


(a) All three-dimensional objects are defined by three axes: x, y, and z. The confocal scanning laser microscope (CSLM) is able to create images of planes formed by the x and y axes (x-y planes) and planes formed by the x and z axes (x-z planes).

Light microscopy

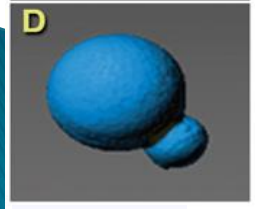
(b) The light microscope image of the two beads shown in **(a)**. Note that neither bead is clear and that the smaller bead is difficult to recognize as a bead. This is because the image is generated from light emanating from multiple planes of focus.

CSLM composite of all sections



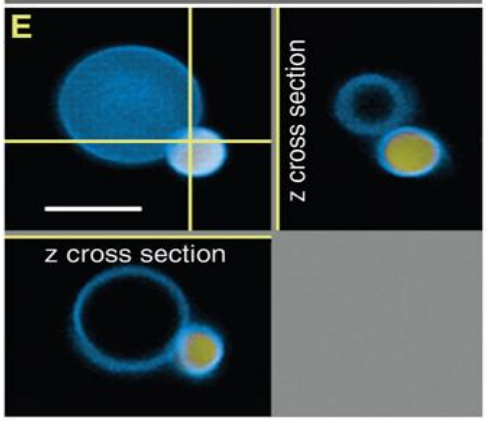
(c) A CSLM uses light from a single plane of focus to generate an image. A computer connected to a CSLM can make a composite image of the two beads using digitized information collected from multiple planes within the beads. The result is a much clearer and more detailed image.

CSLM 3-D reconstruction



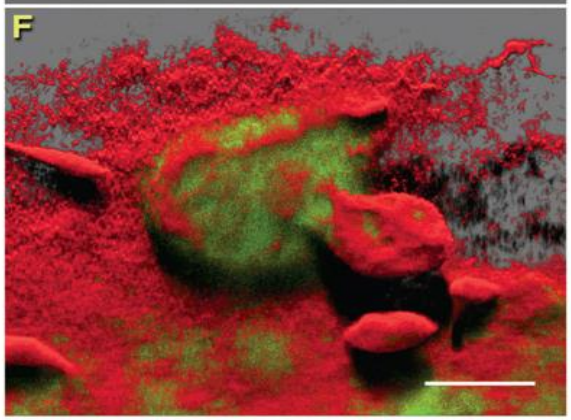
(d) The computer can also use digitized information collected from multiple planes within the beads to generate a three-dimensional reconstruction of the beads.

CSLM: Three different views



(e) The computer can also generate views of the specimen using different planes. The top left panel is the image of a single x-y plane (i.e., looking down from the top of the beads). The two lines represent the two x-z planes imaged in the other two panels. The vertical line indicates the x-z plane shown in the top right panel (i.e., a view from the right side of the beads) and the horizontal line indicates the x-z plane shown in the bottom panel (i.e., a view from the front face of the beads).

CSLM 3-D reconstruction of *P. a.* biofilm

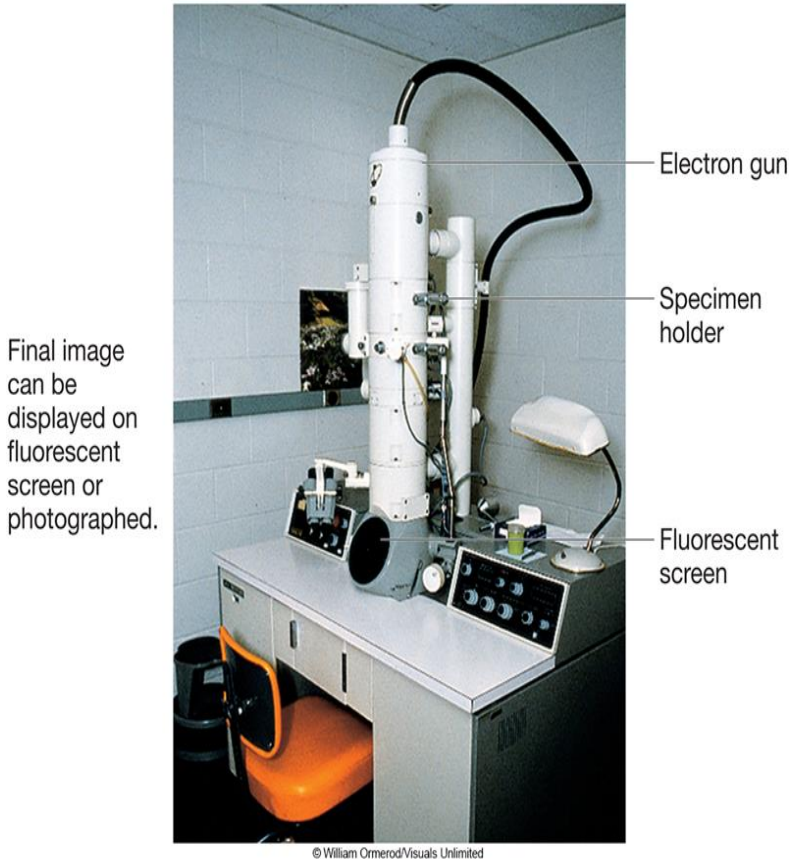


(f) A three-dimensional reconstruction of a *Pseudomonas aeruginosa* biofilm. The biofilm was exposed to an antibacterial agent and then stained with dyes that distinguish living (green) from dead (red) cells. The cells on the surface of the biofilm have been killed, but those in the lower layers are still alive. This image clearly demonstrates the difficulty of killing all the cells in a biofilm.

B. Pitts/P. Dirckx, MSU Center for Biofilm Engineering

Electron Microscopy

- Electrons replace light as the ‘illuminating’ beam
- Wavelength of electron beam is much shorter than light, resulting in much higher resolution
- Allows for study of microbial morphology in great detail



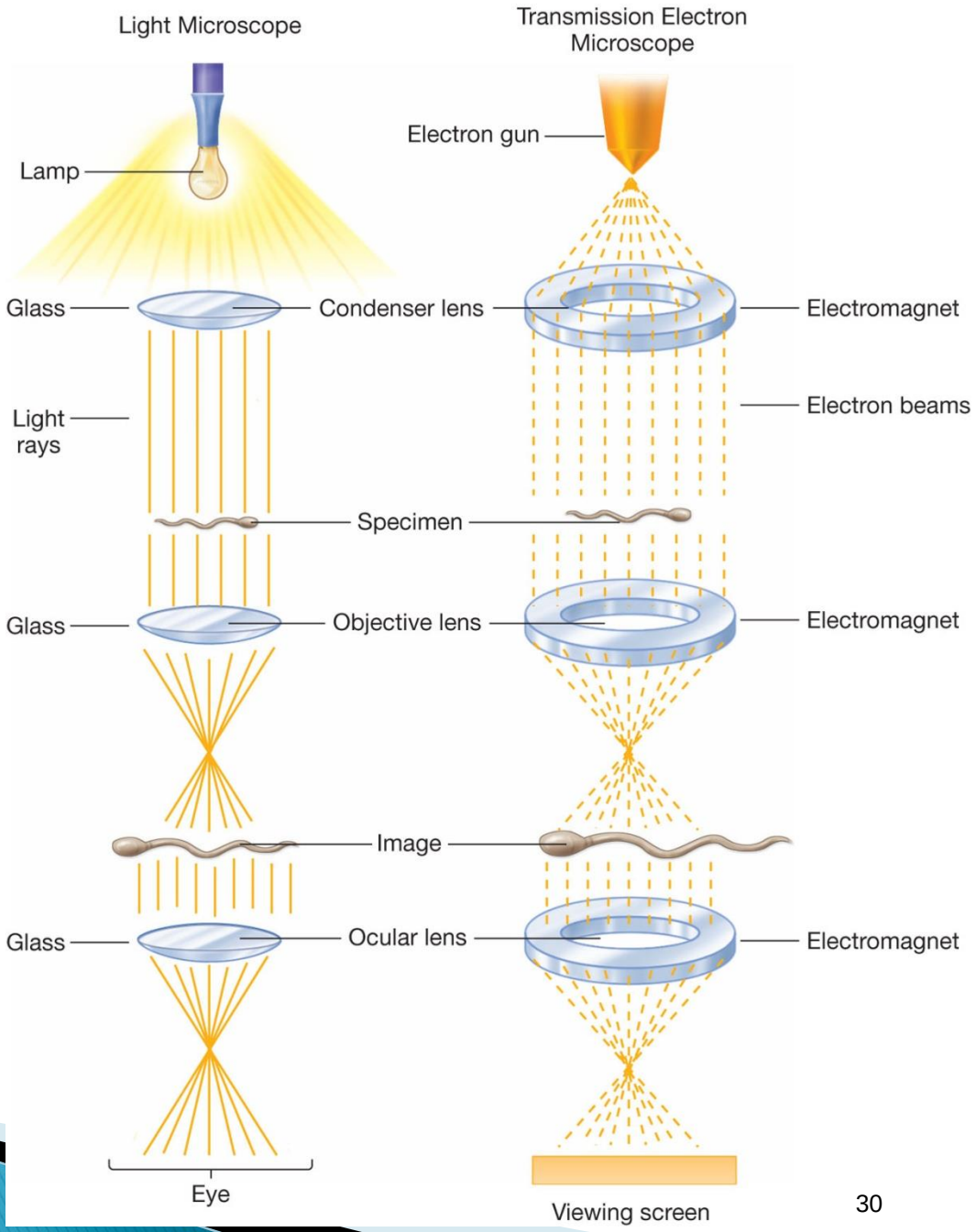


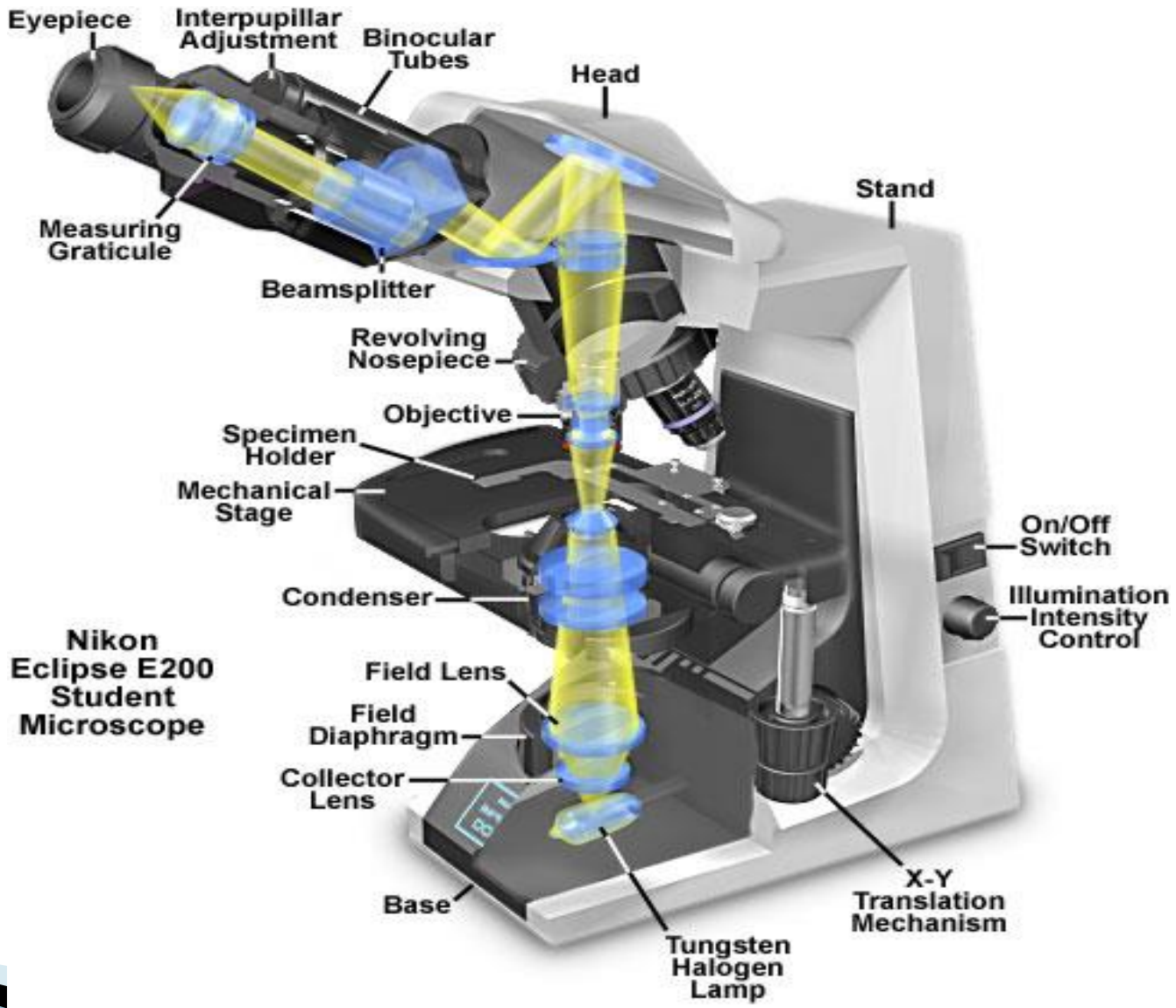
Table 2.5 Characteristics of Light and Transmission Electron Microscopes

Feature	Light Microscope	Transmission Electron Microscope
Highest practical magnification	About 1,000–1,500	Over 100,000
Best resolution[1]	0.2 µm	0.5 nm
Radiation source	Visible light	Electron beam
Medium of travel	Air	High vacuum
Type of lens	Glass	Electromagnet
Source of contrast	Differential light absorption	Scattering of electrons
Focusing mechanism	Adjust lens position mechanically	Adjust current to the magnetic lens
Method of changing magnification	Switch the objective lens or eyepiece	Adjust current to the magnetic lens
Specimen mount	Glass slide	Metal grid (usually copper)

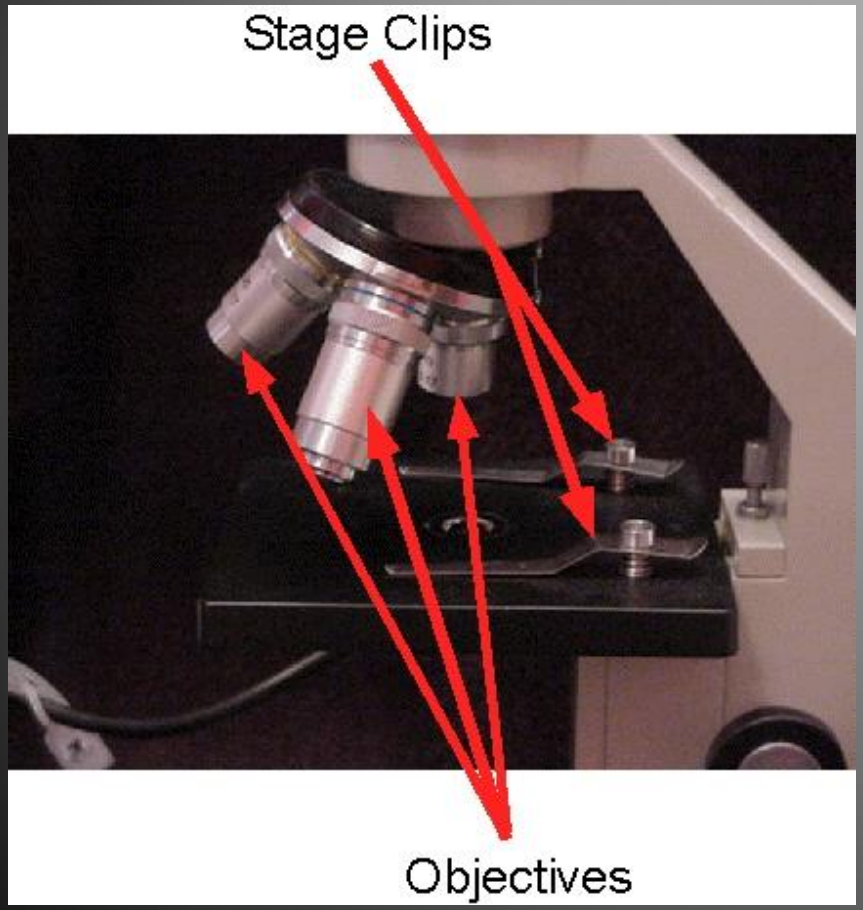
Using the Microscope

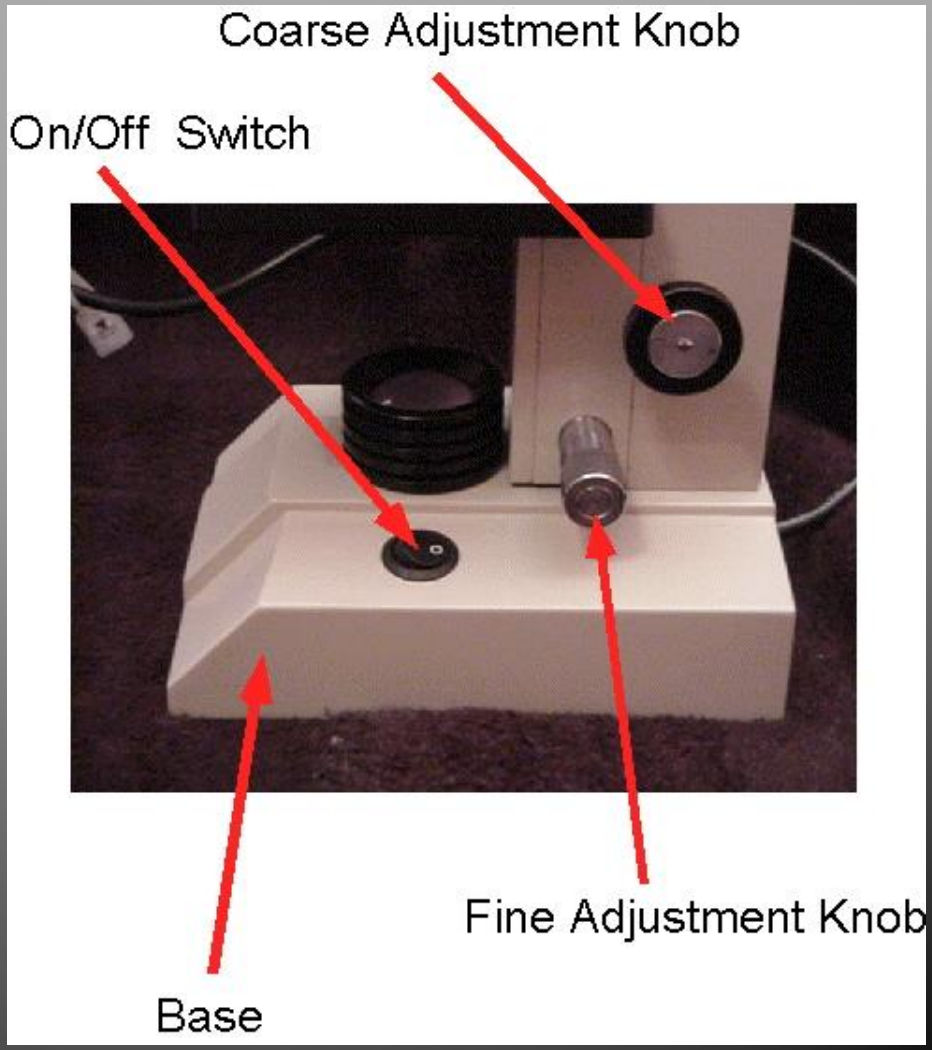
- To carry the microscope grasp the microscopes arm with one hand. Place your other hand under the base.

Light microscope

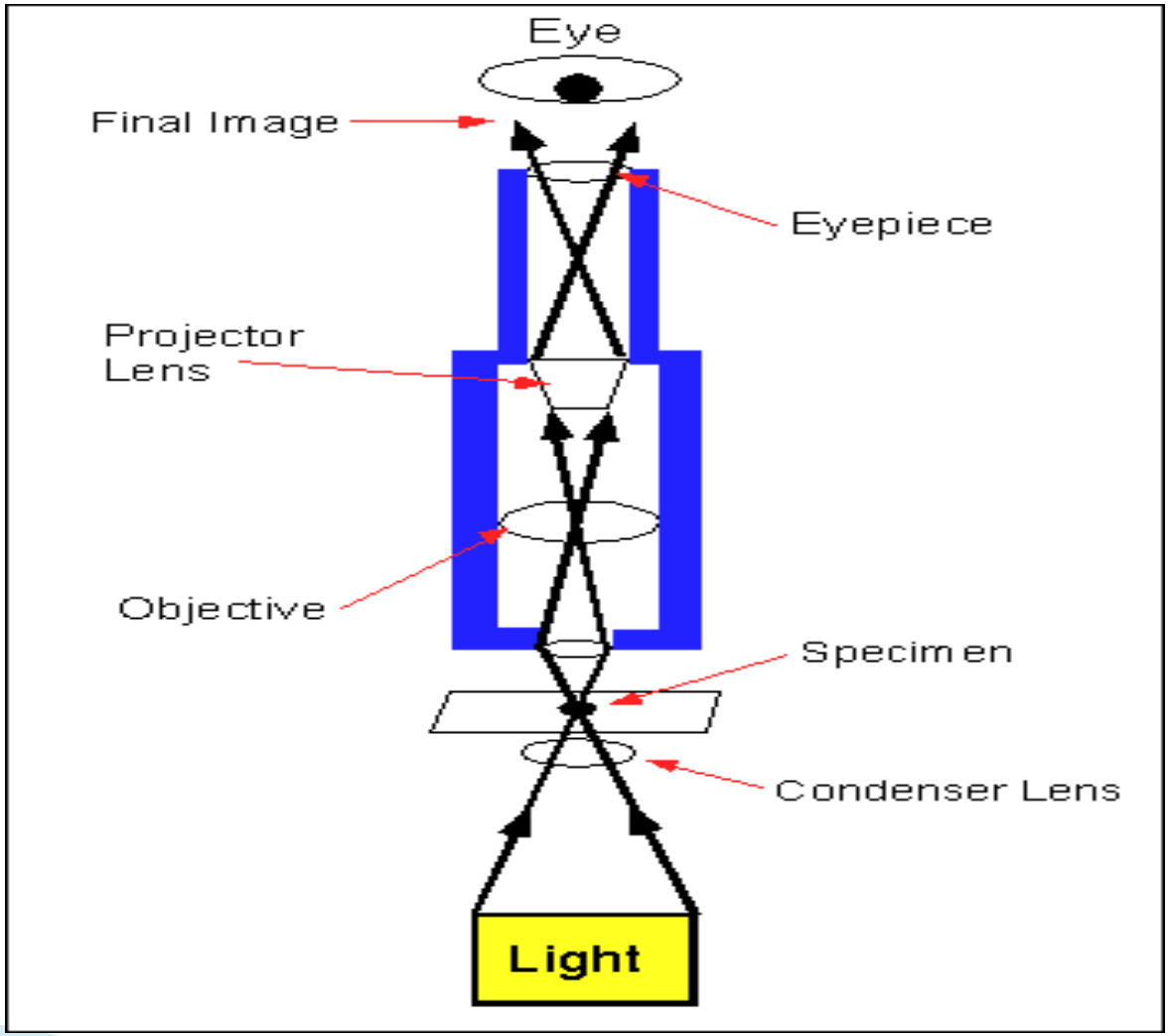


COMPOUND MICROSCOPE





Compound Light Microscope



Total Magnification

Total Magnification:

X

= 40 X

4X Scanning Objective 10X Eyepiece

X

= 100 X

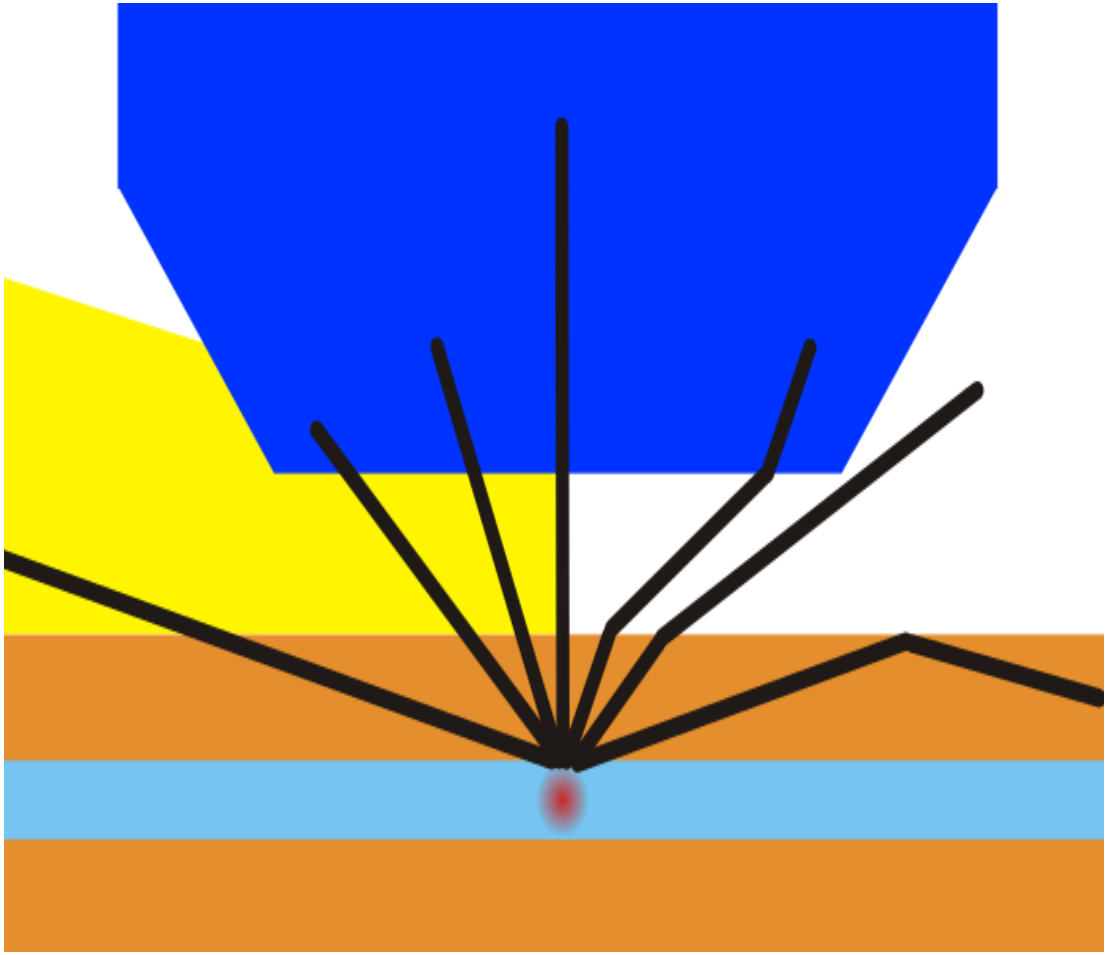
10X Objective 10X Eyepiece

X

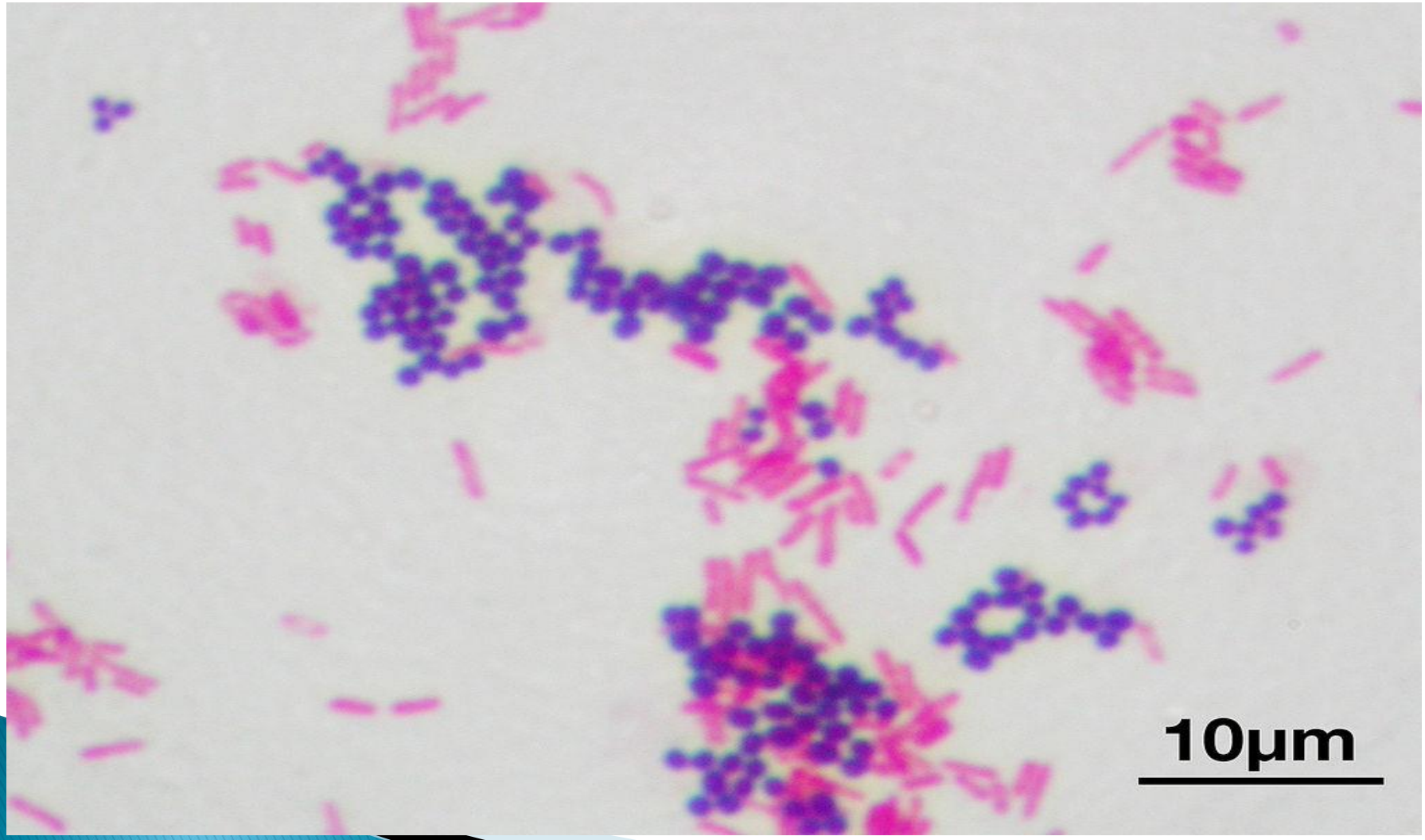
= 400X

40X Objective 10X Eyepiece

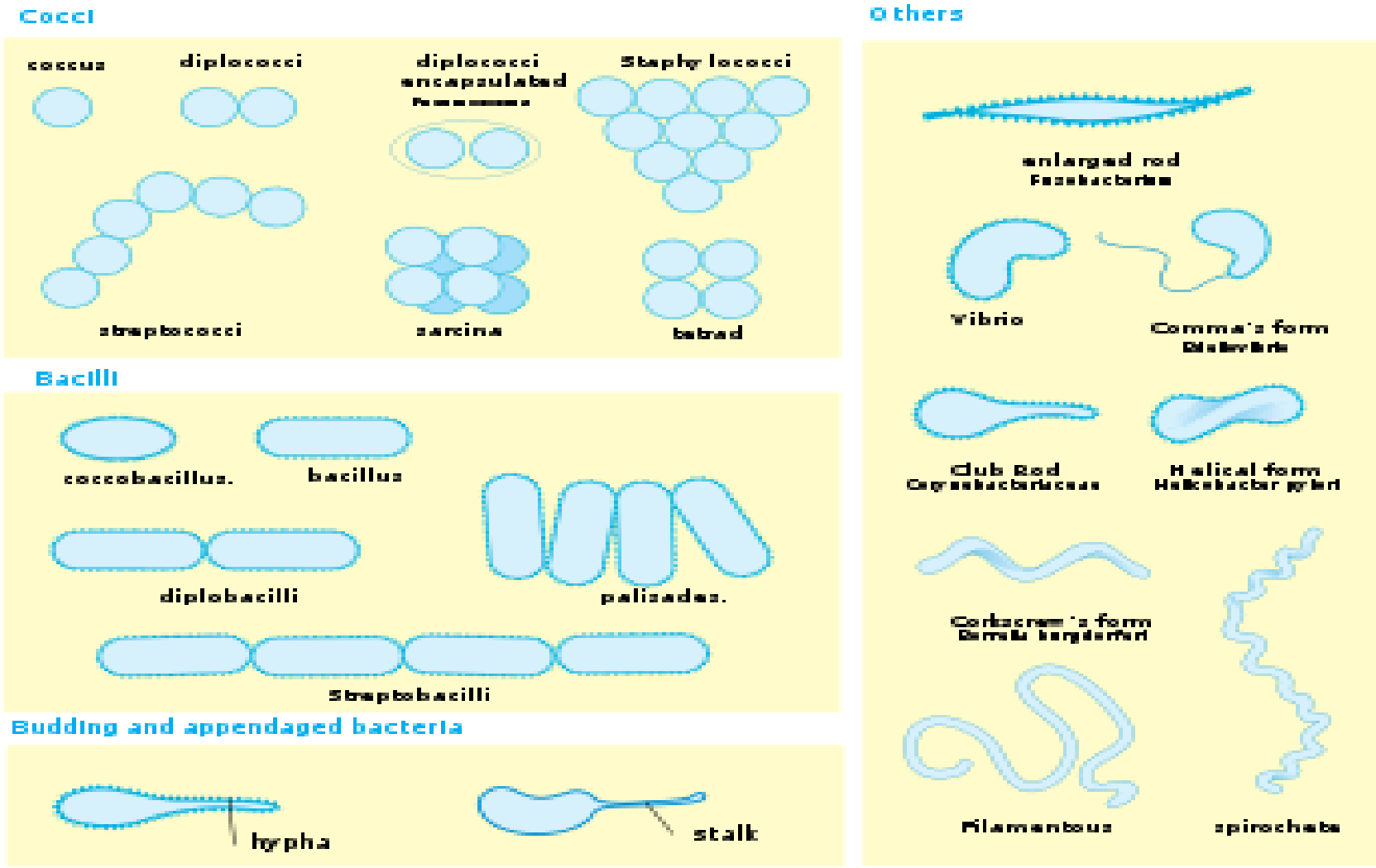
Immersion Oil lens



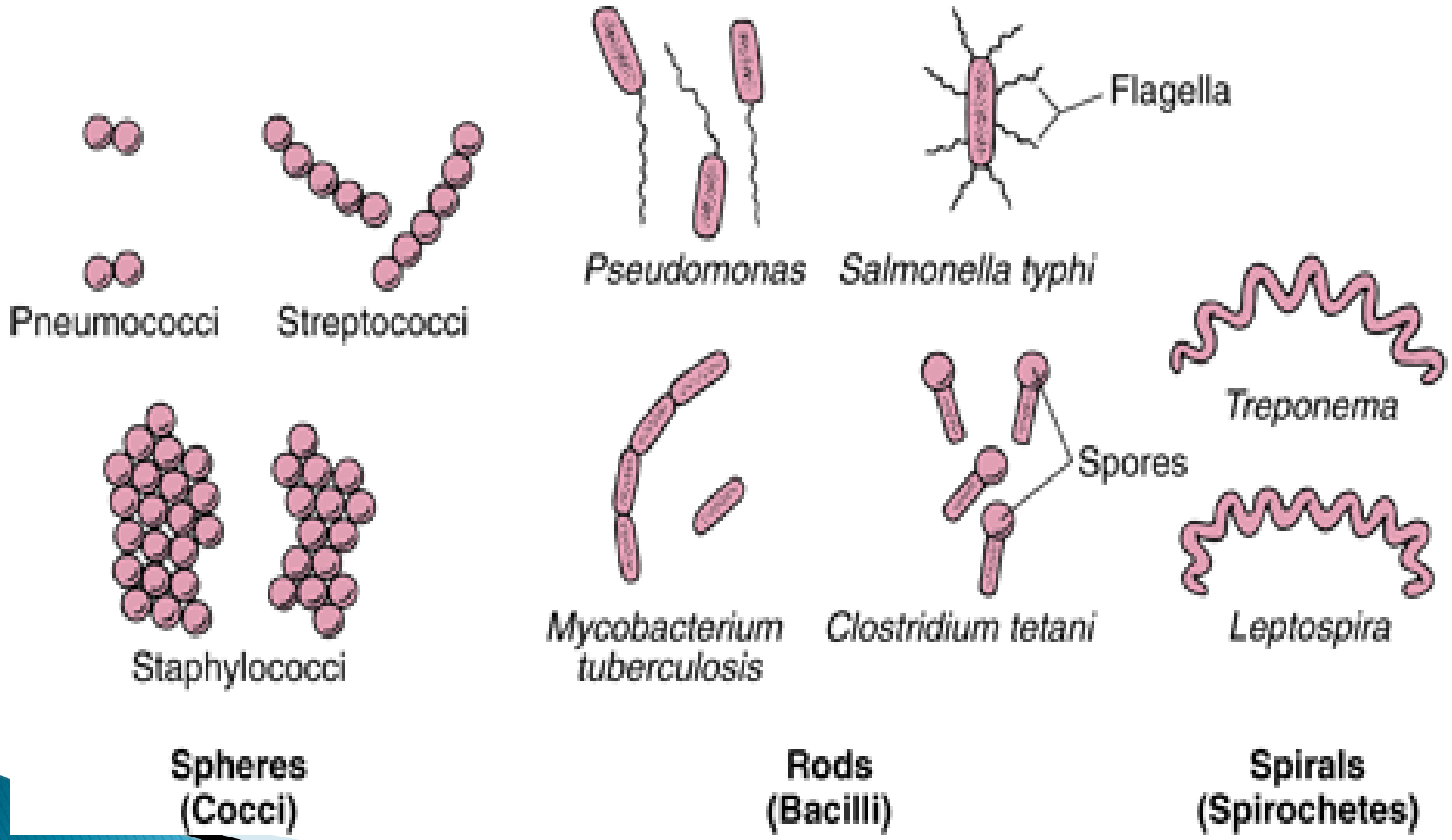
Light microscopy bacteria



Bacterial shapes



Bacterial shapes



Different types of bacteria in this lab

- *S.aureus*
- *M.luteus*
- *S. epidermidis*
- *E.faecalis*
- *E.coli*
- *K.pneumoniae*
- *P.mirabilis*
- *P.aeruginosa*
- *B.subtilis*

- *Staphylococcus aureus*
- *Micrococcus luteus*
- *Staphylococcus epidermidis*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Bacillus subtilis*