Universitas Esa Unggul

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INTRUMENTASI BIOTEKNOLOGI

Program Studi Bioteknologi

Oleh: Seprianto, S.Pi, M.Si

Meeting 10

IMAGING : Confocal Microscope , Scanning Electron Micrograph, Transmission Electron Micrograph

Universitas Esa Unggul

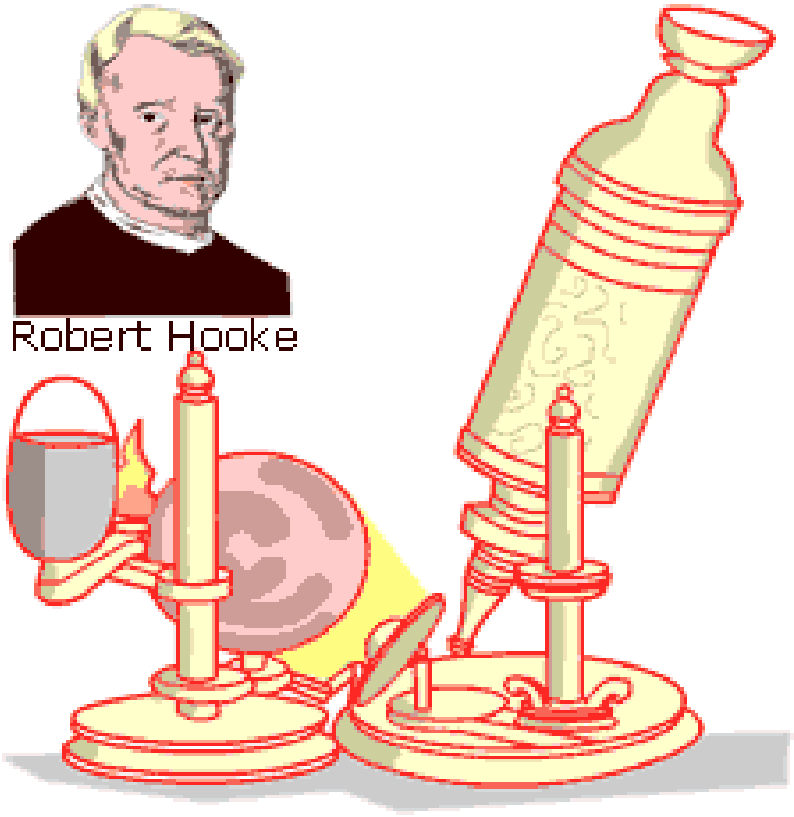
Tujuan Perkuliahan

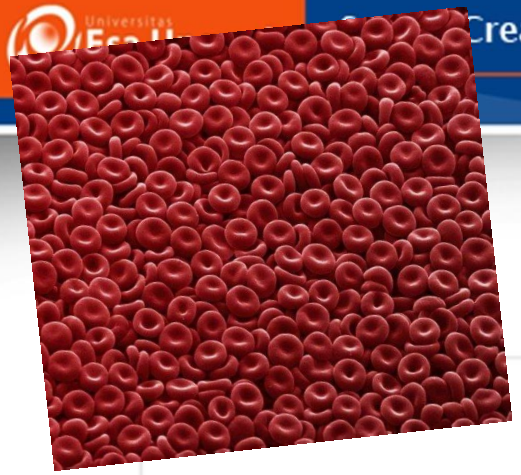
- Mengidentfikasi alat *imaging*: Mikroskop, TEM, SEM
- Mengetahui prinsip bekerjanya alat-alat tersebut

Imaging Laboratory

Microscope History

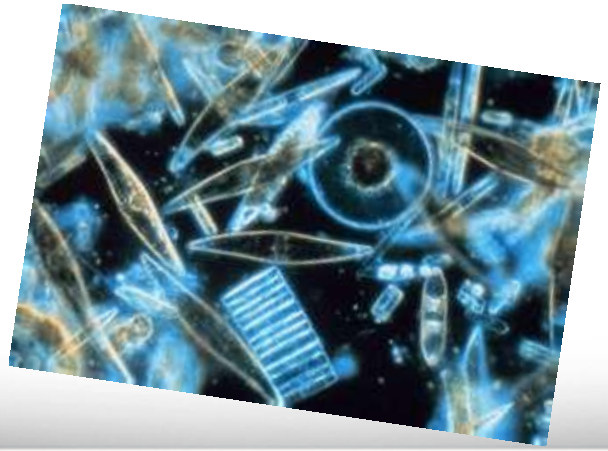
1665 – English physicist, Robert Hooke looked at a sliver of cork through a microscope lens and noticed some "pores" or "cells" in it.





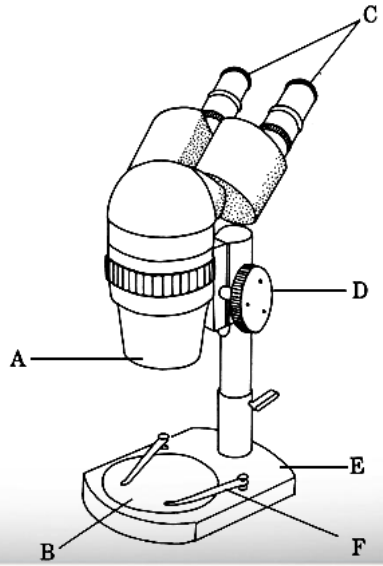
MICROSCOPES

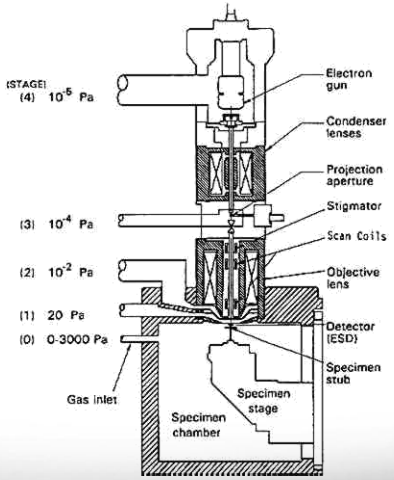
- magnifies objects (makes objects look bigger)
- help scientists study objects & living things too small to see with the naked eye



Types of Microscope

- Compound Microscope
- Dissection Microscope
- Scanning Electron Microscope (SEM)
- Transmission Electron Microscope (TEM)

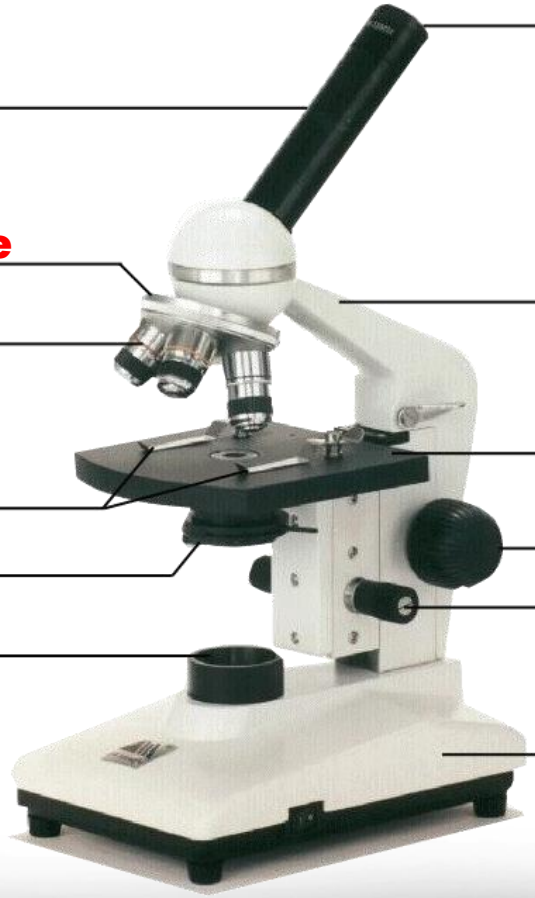




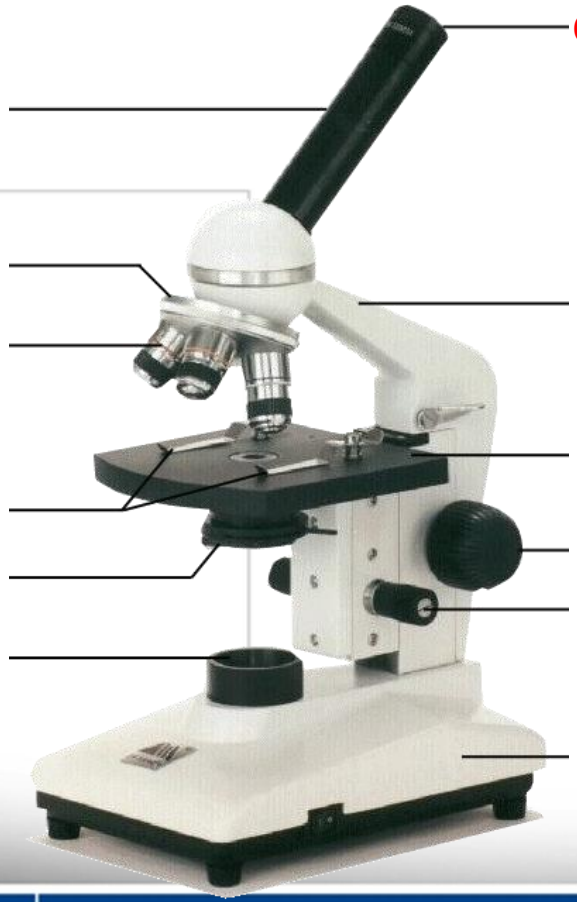
Microscope Care

- Always carry with 2 hands
- Never touch the lenses with your fingers.
- Only use lens paper for cleaning
- Keep objects clear of desk and cords
- When you are finished with your "scope", rotate the nosepiece so that it's on the low power objective, roll the stage down to lowest level, rubber band the cord, then replace the dust cover.

Microscope Parts



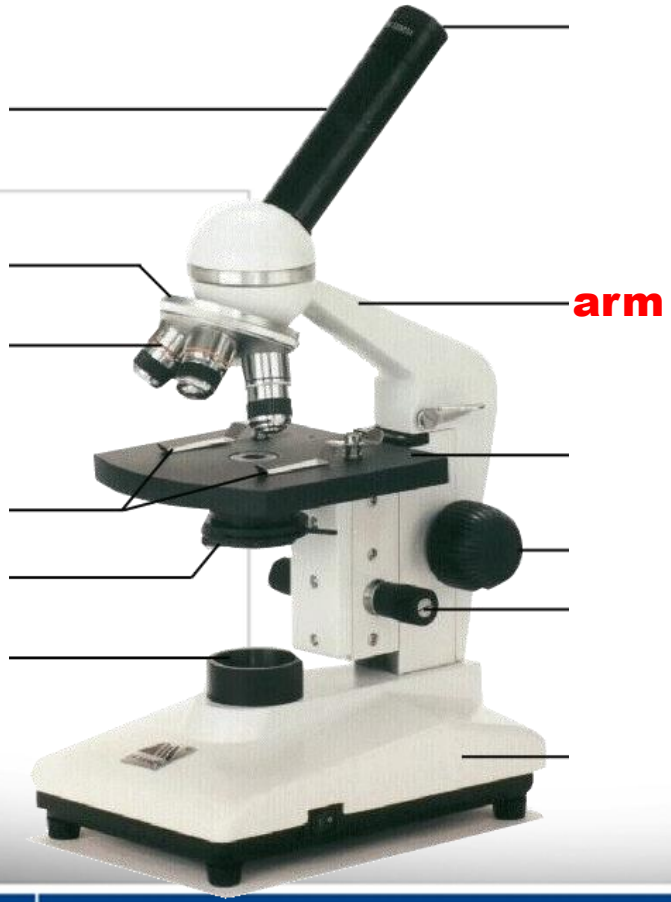
Ocular Lens



magnifies; where you look through to see the image of your specimen.

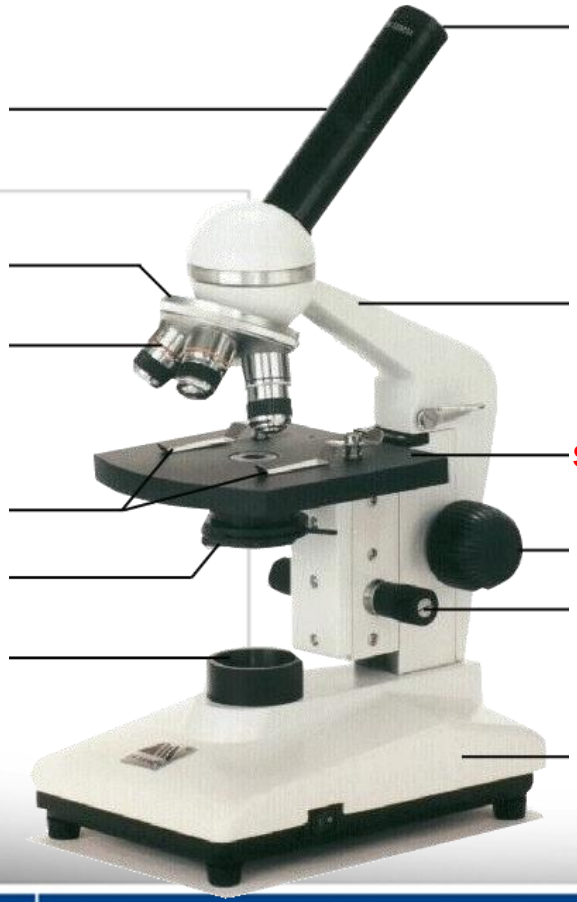
They are usually 10X or 15X power. Our microscopes have an ocular lens power of 10x.

Arm



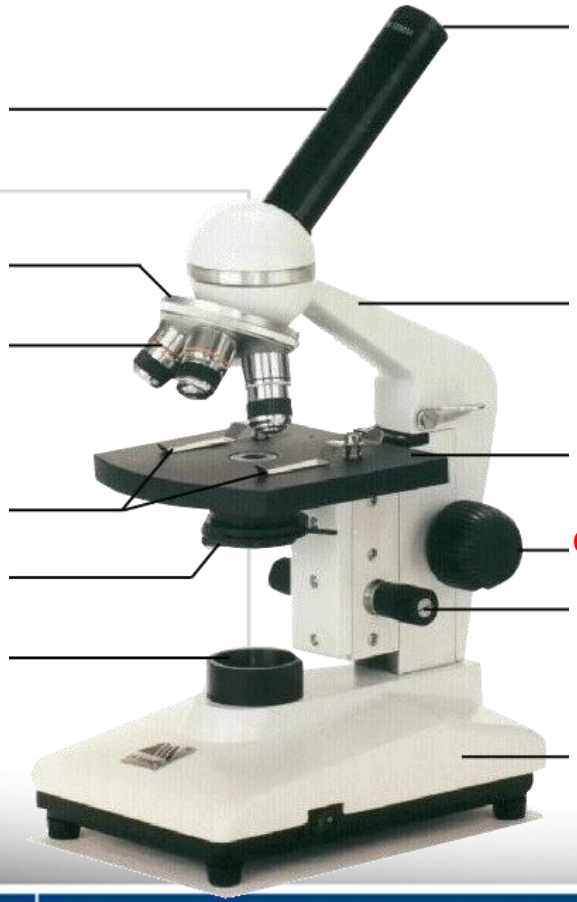
supports the tube and connects it to the base

Stage



the flat platform where you place your slides

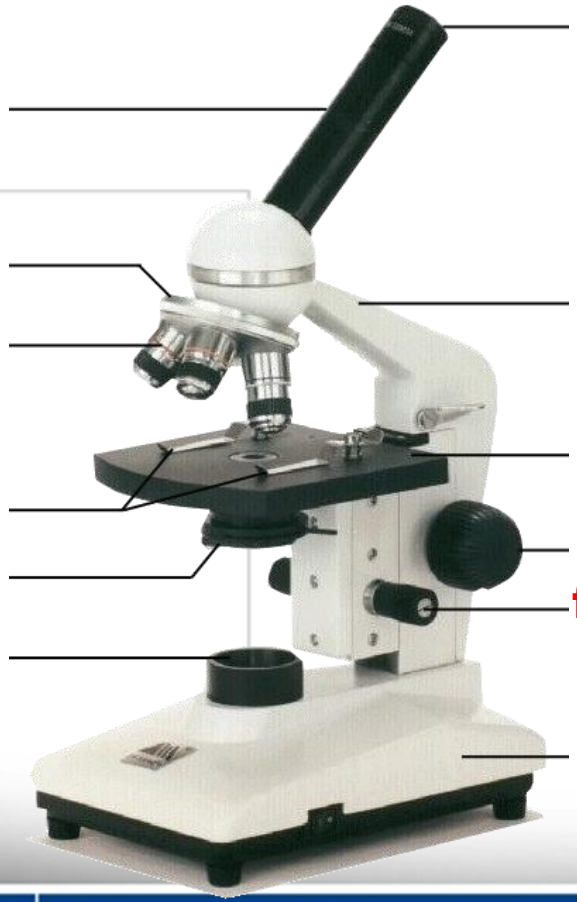
coarse adjustment knob



moves stage (or body tube) up and down

coarse adjustment knob

fine adjustment knob

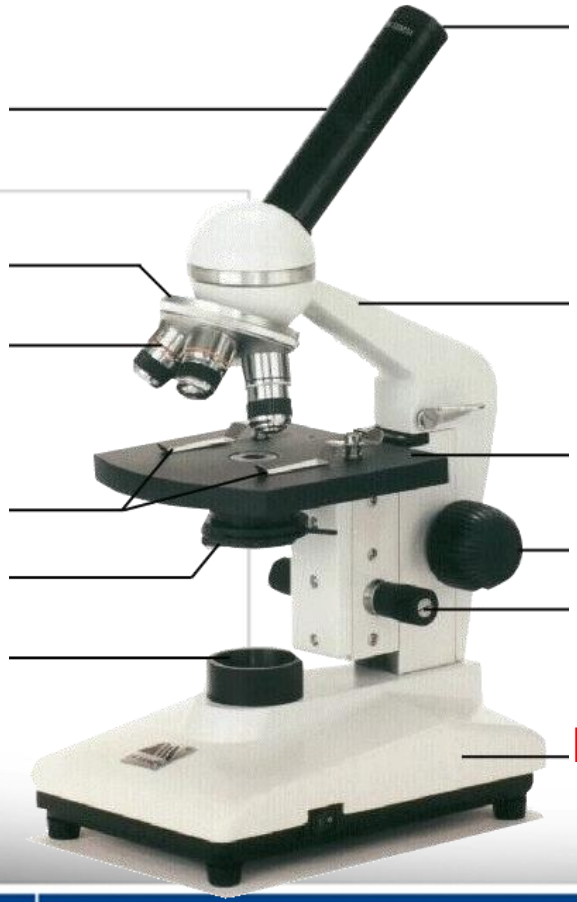


small, round knob on the side of the microscope used to fine-tune the focus of your specimen

fine adjustment knob

after using the coarse adjustment knob

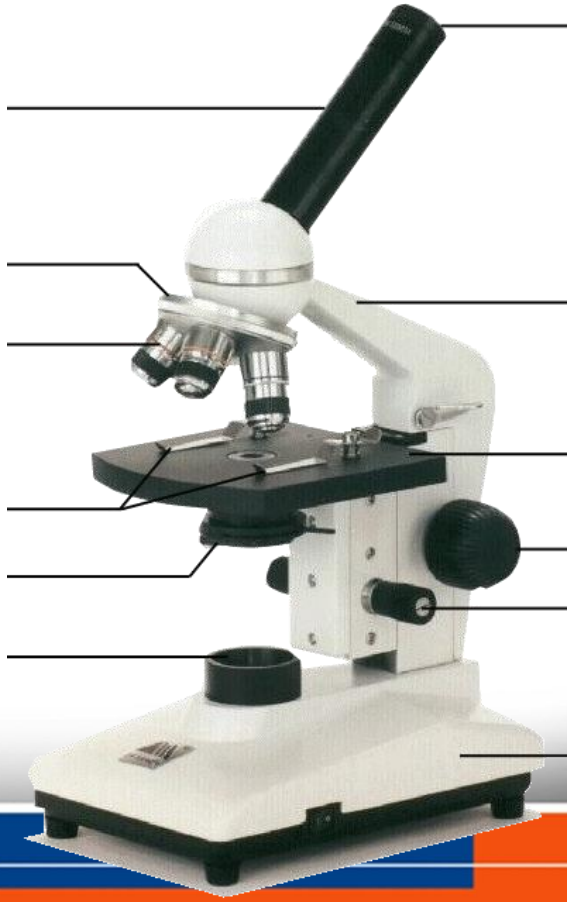
base



the bottom of the microscope, used for support

body tube

connects the eyepiece to the objective lenses

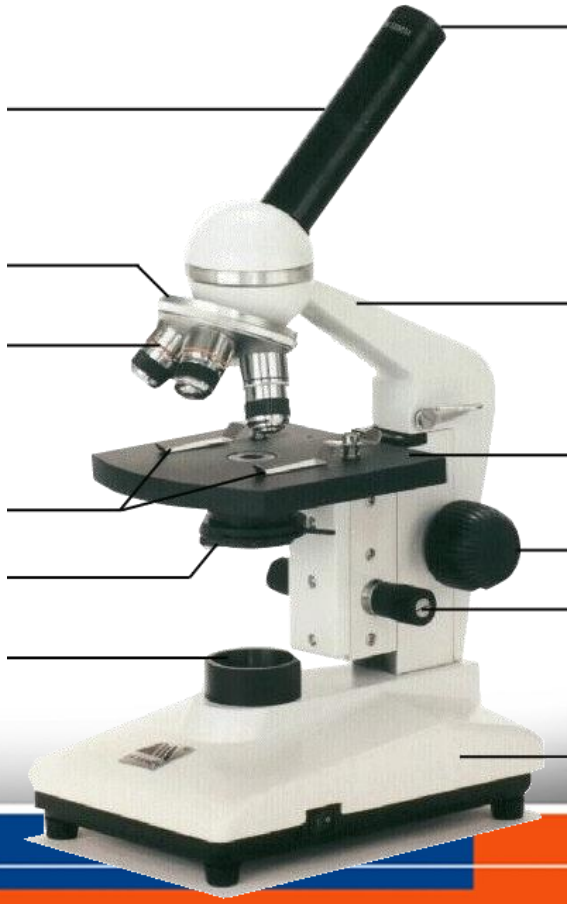


revolving nosepiece

the part that holds two or more objective lenses

revolving nosepiece

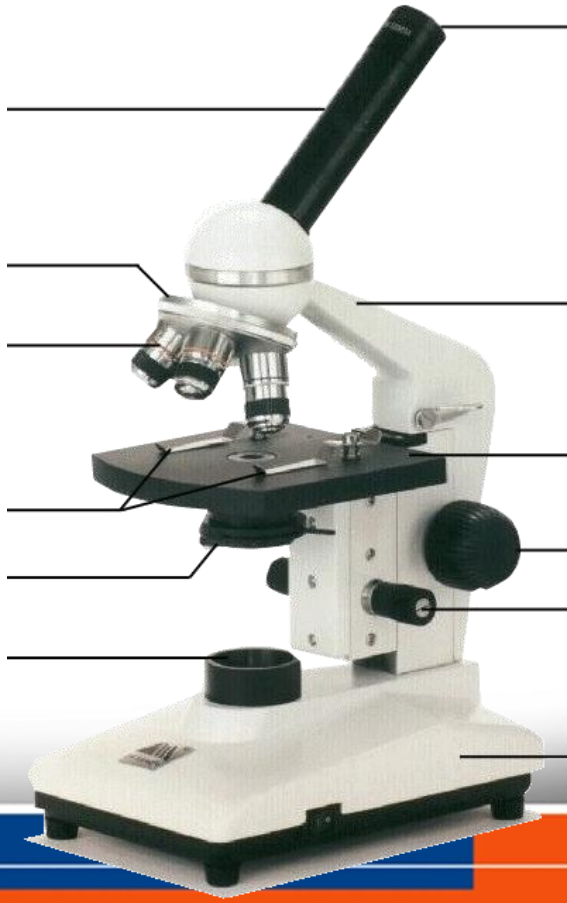
and can be rotated to easily change power



objective lenses

Adds to the magnification

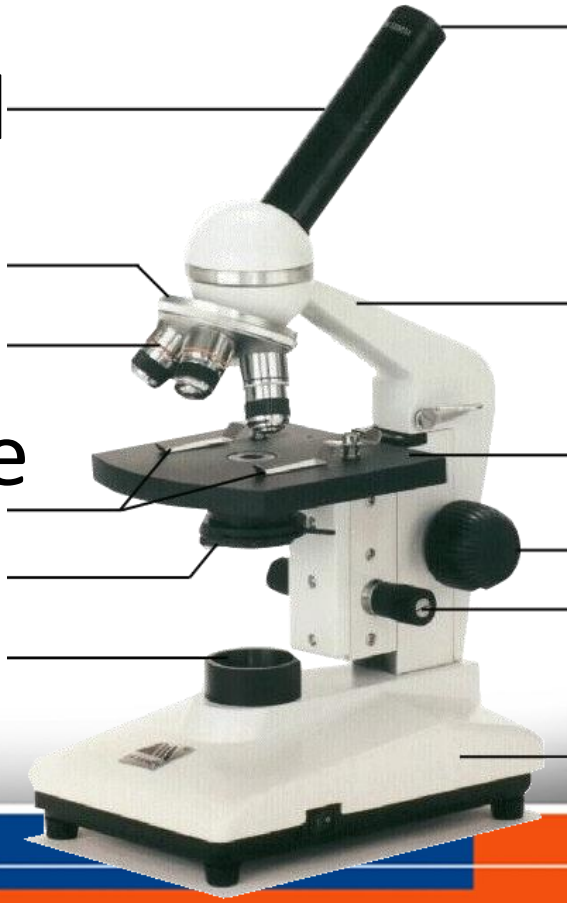
Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common)



objective lenses

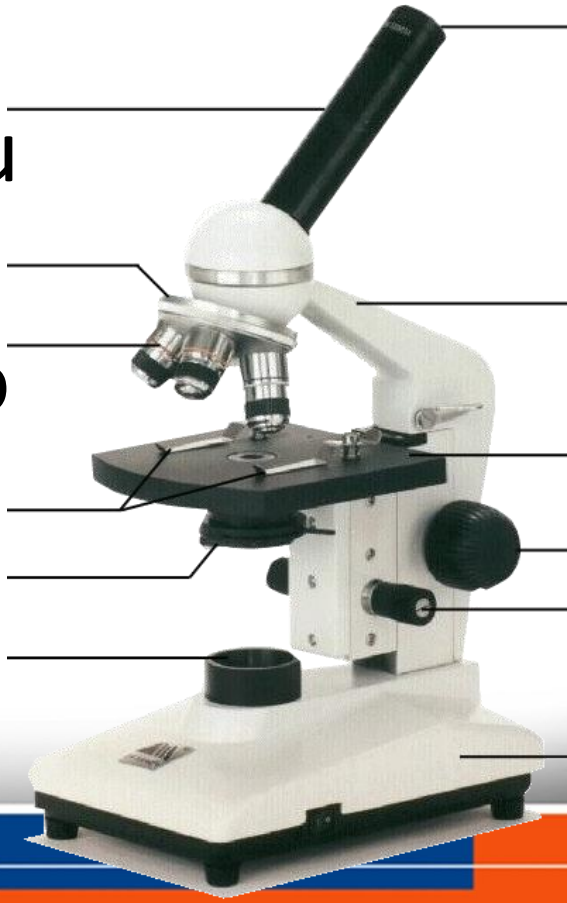
eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X , 400X and 1000X.

The shortest lens is the lowest power, the longest one is the lens with the greatest power. Lenses are color coded.



stage clips

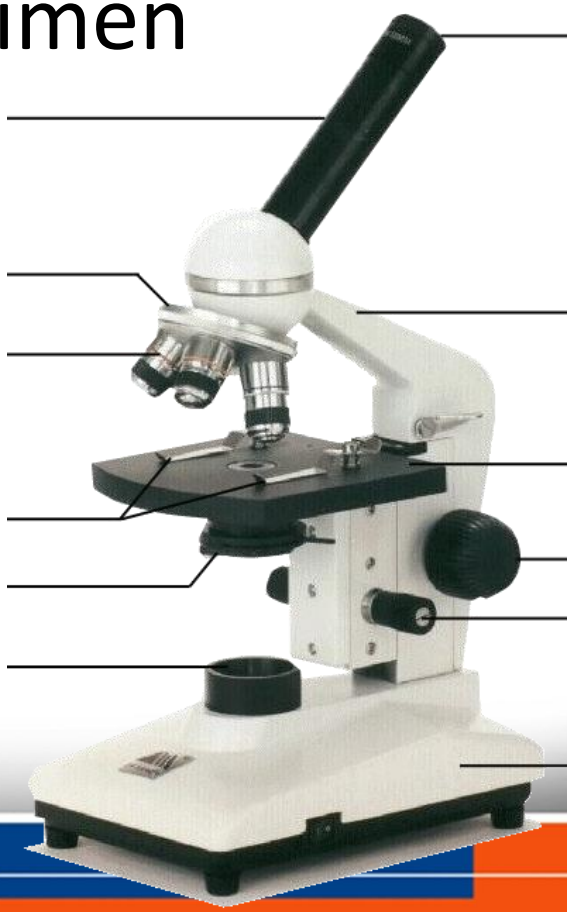
Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.



diaphragm

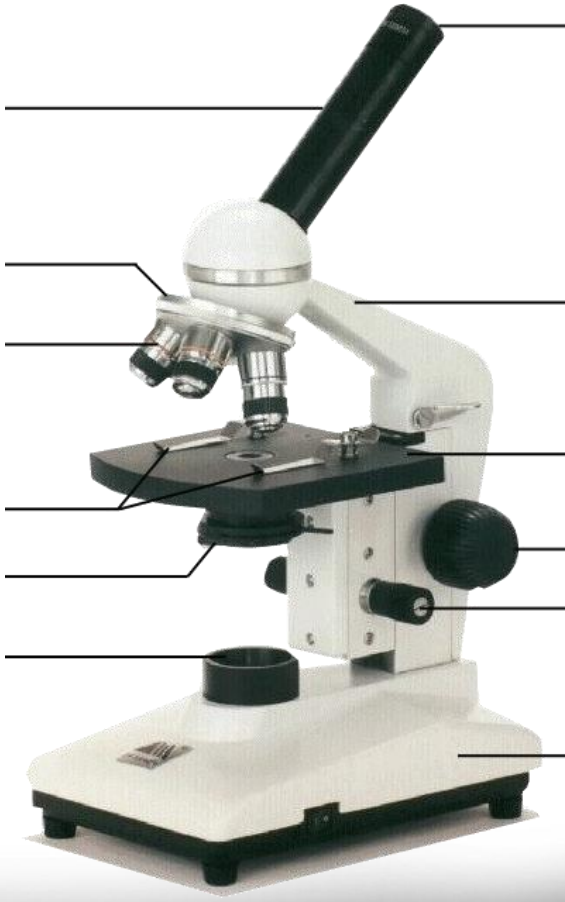
controls the amount of light going through the specimen

Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light



light

makes the specimen easier to see



light

Simple Microscope

A Simple microscope is a microscope that uses only one lens for magnification.

Electron Microscopes

The electron microscope is a type of microscope that uses a beam of electrons to create an image of the specimen.

Phase-Contrast Microscope

A microscope that utilizes the phase differences of light rays transmitted by different portions of an object to create an image in which the details of the object are distinct despite their near-uniformity of refractive index.

Mikroskop Flouresen

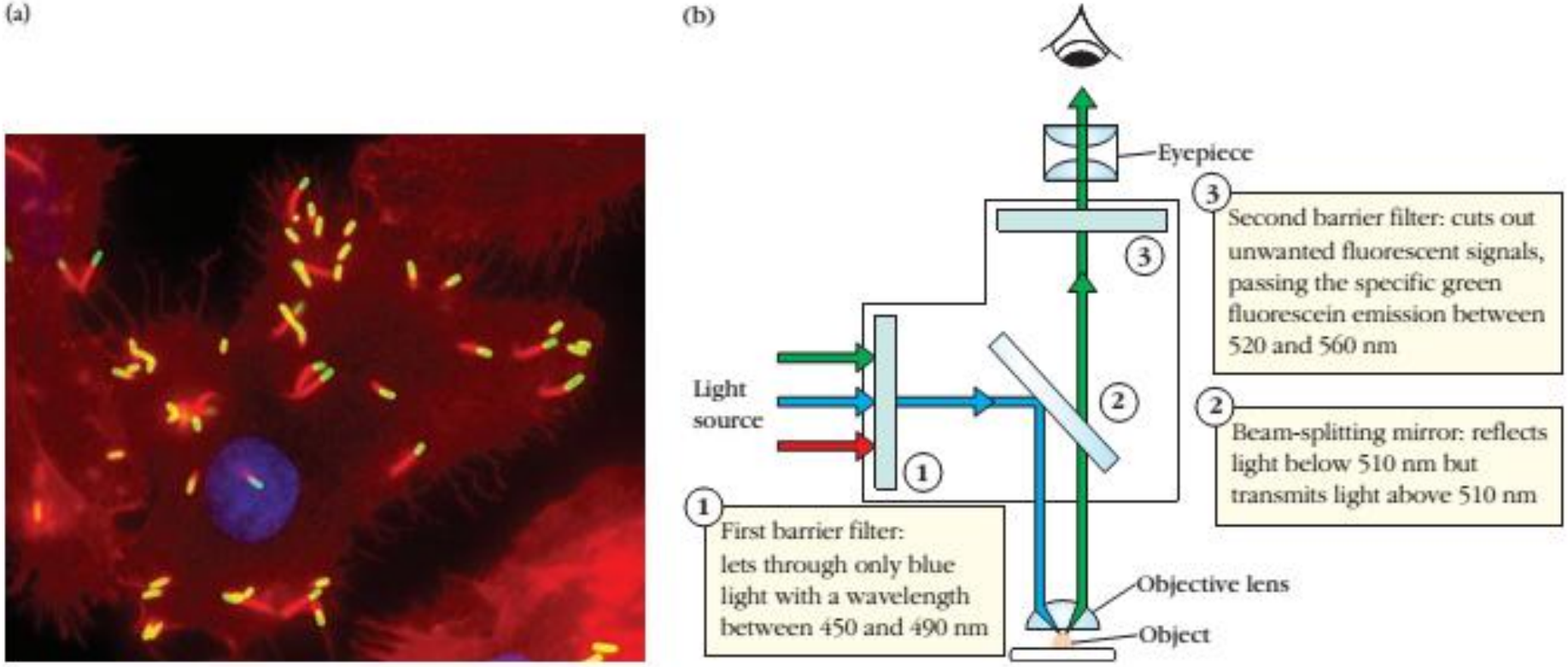
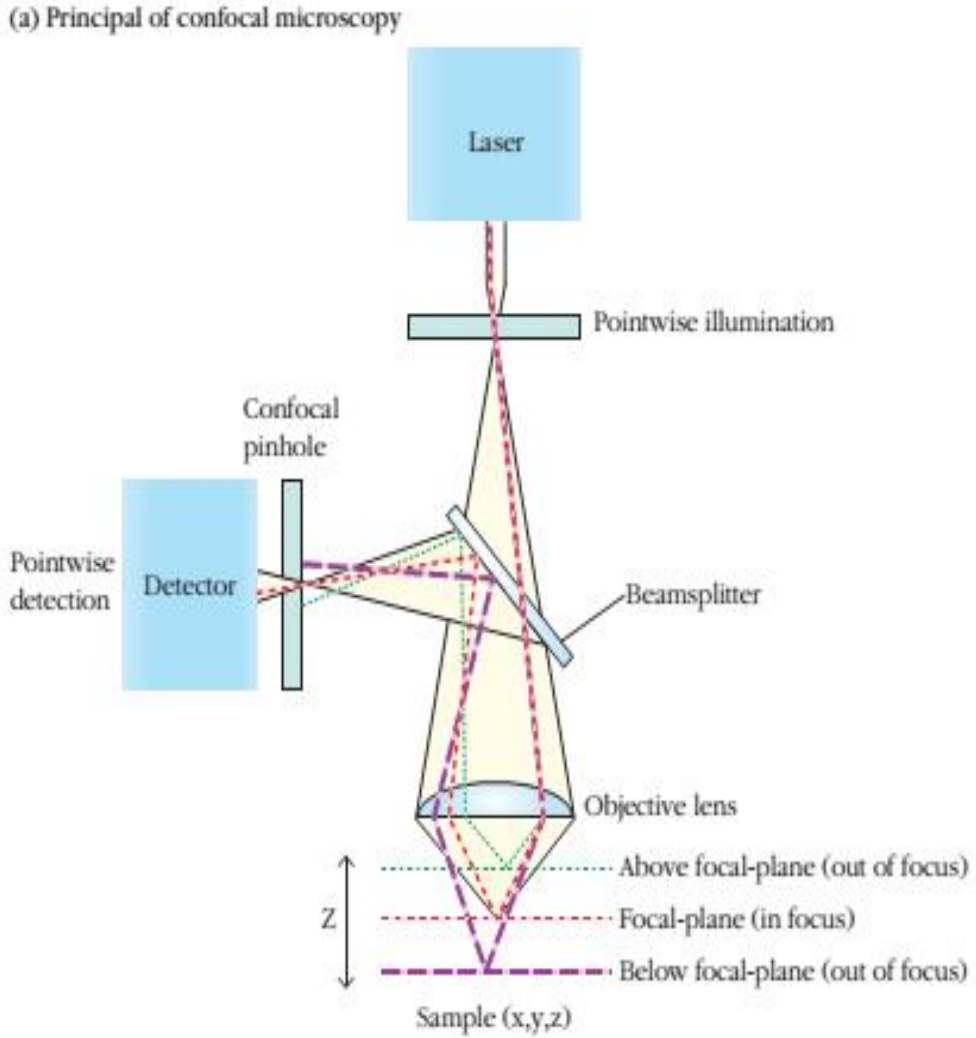


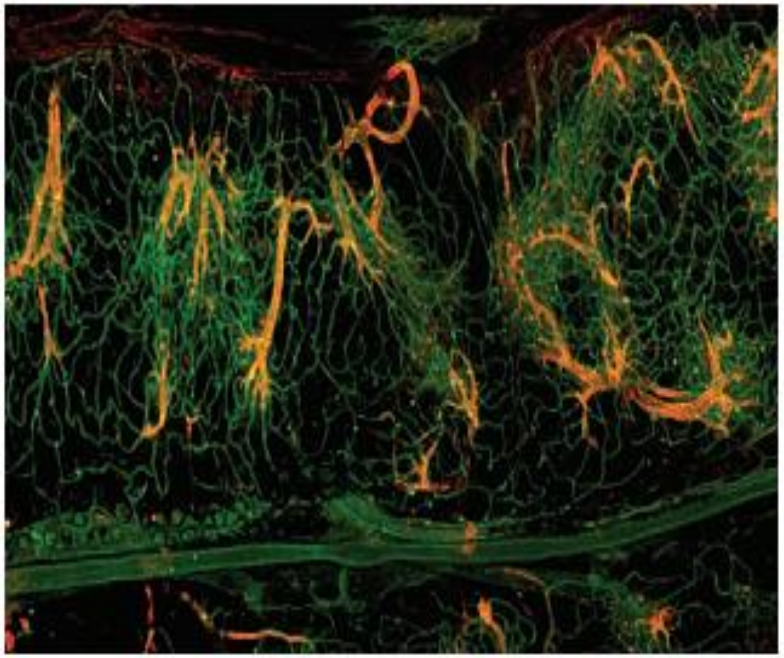
FIGURE 20-16 Fluorescently-labeled cells and the passage of light through a fluorescence microscope. (a) Fluorescence

particular wavelengths. The light is then directed onto the sample by a dichroic mirror that reflects light of short wavelengths (below

Mikroskop Konfokal



(b)



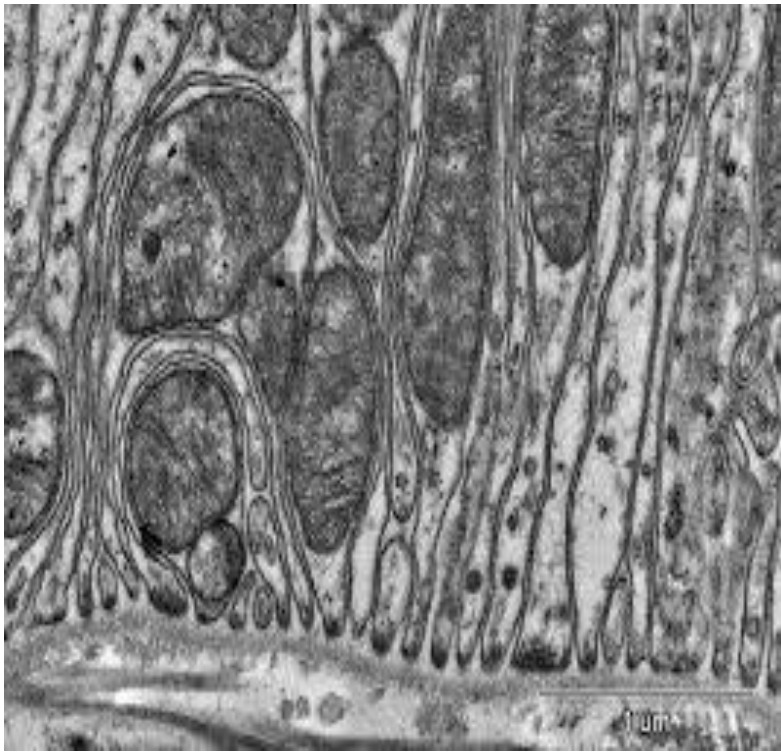
(Owen, Punt et al. 2013)

Mikroskop tipe lain

1. Mikroskop cahaya:
   - Bright field
   - Dark Field
   - Phase Contrast
   - Nomarski

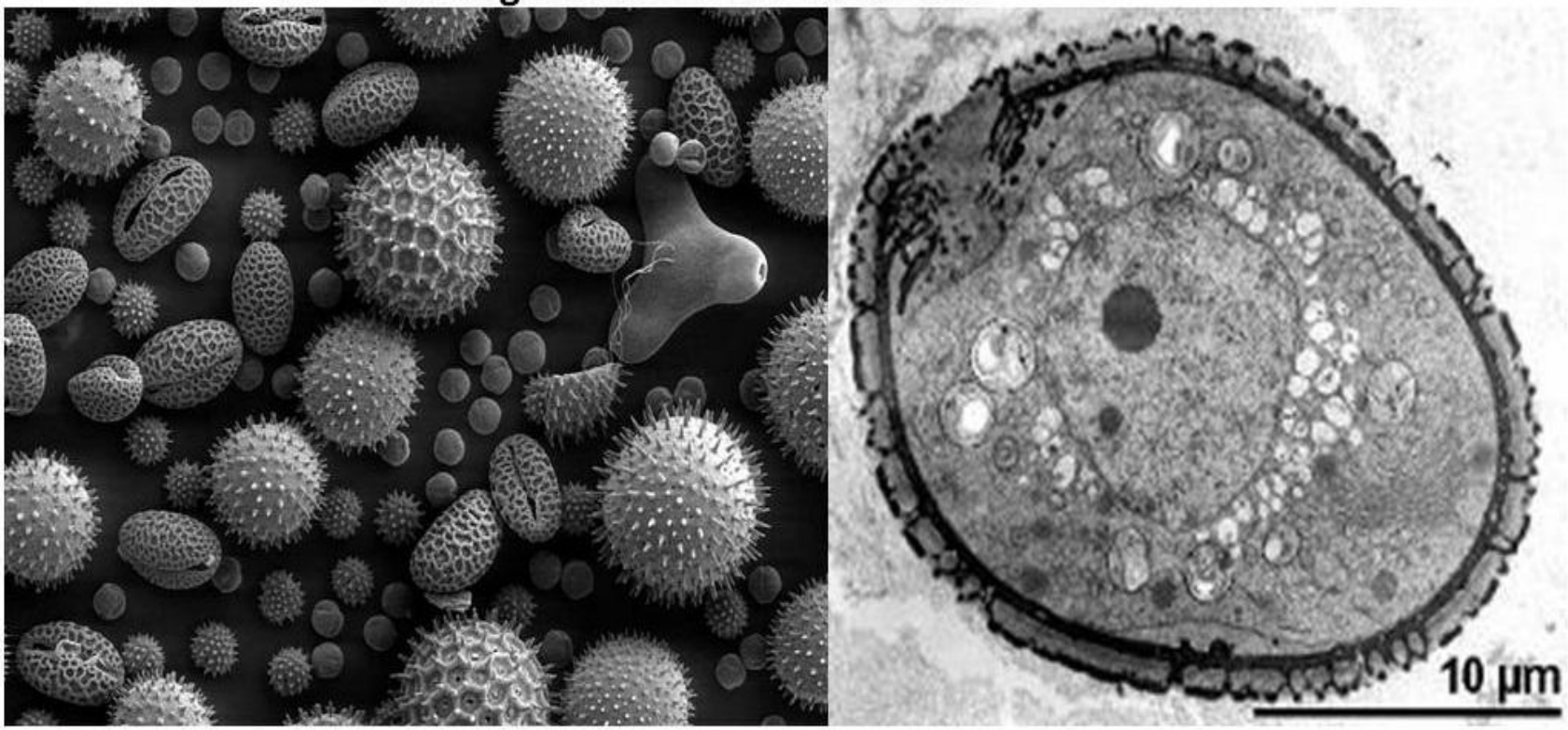
2. Electron microscope:
   - Transmission Electron Microscopy (TEM),
   - Scanning Electron Microscopy (SEM),
   - Reflection Electron Microscopy (REM),
   - Scanning Transmission Electron Microscopy (STEM)

Transmission Electron Microscopy (TEM)



Transmission Electron Microscopy (TEM)

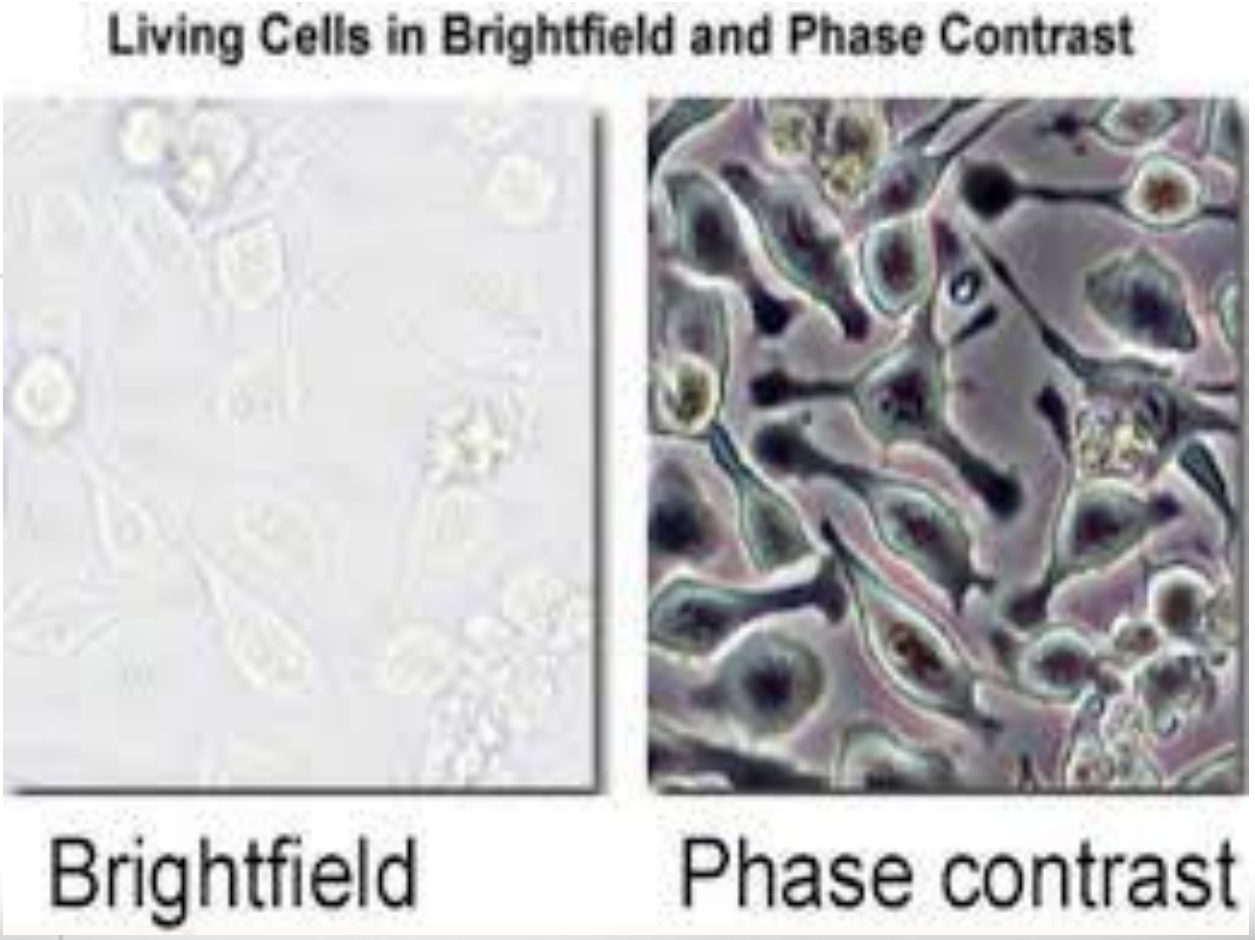
Pollen grain under SEM and TEM



Scanning Electron Microscope (SEM) vs Transmission Electron Microscope(TEM)

www.majordifferences.com

Brightfield vs Phase Contrast



4 Steps in Bioimaging

1. Manipulate

Molecules , cells, tissues modification

- Fixation (using formaldehyde, ethanol, aceton etc)

Stabilize cell morphology and tissue architecture
Disable proteolytic enzymes
Strengthen samples to withstand further processing and staining
Protect samples against microbial contamination and decomposition

- Permeabilization (using tween, triton x, saponin etc)
- Staining (fluorophore, antibody conjugated fluorophore)
- Mounting (Mouviol, antifade..)

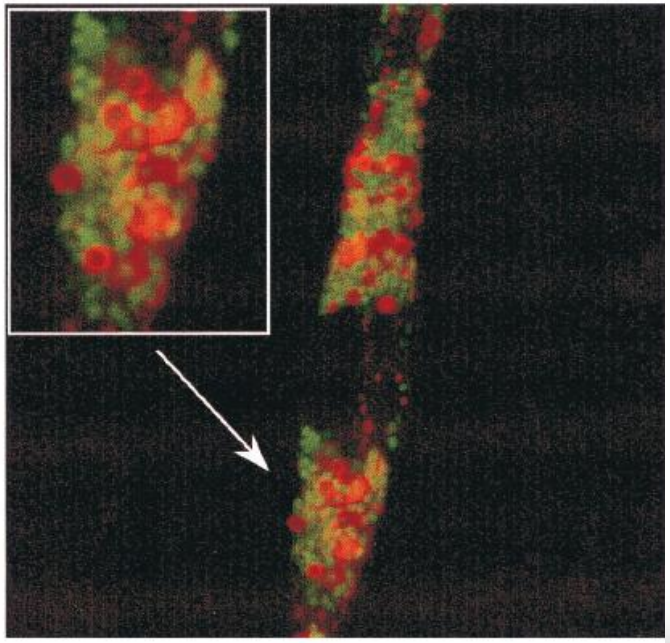
2. Measure

Acquisition of Images with various methods by managing technique

- Minimizing Noise
- Reducing unwanted room light
- Reducing unwanted light
- Other : vibration electromagnetic interference, contamination

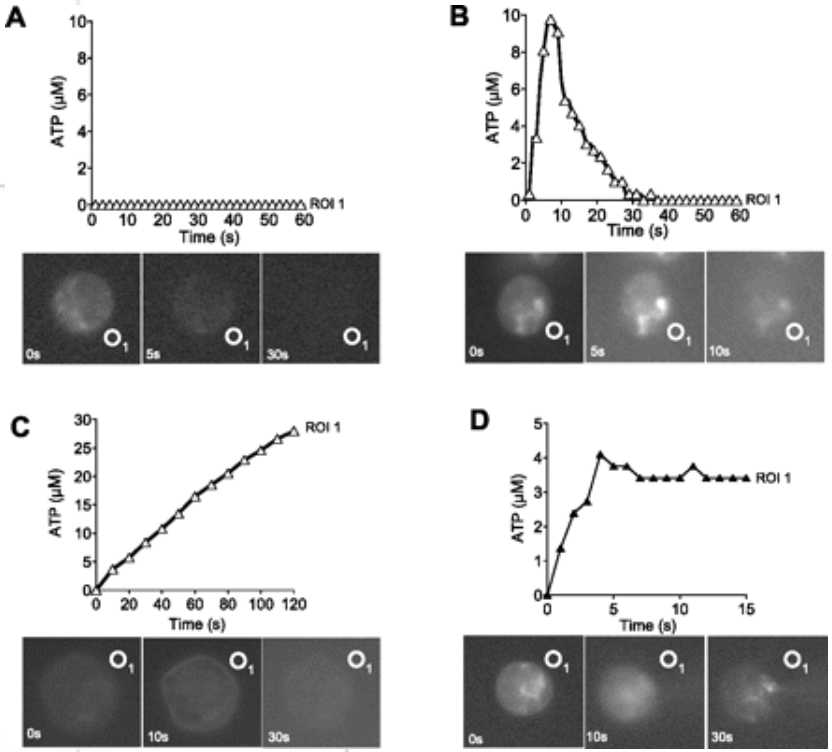
3. Mine

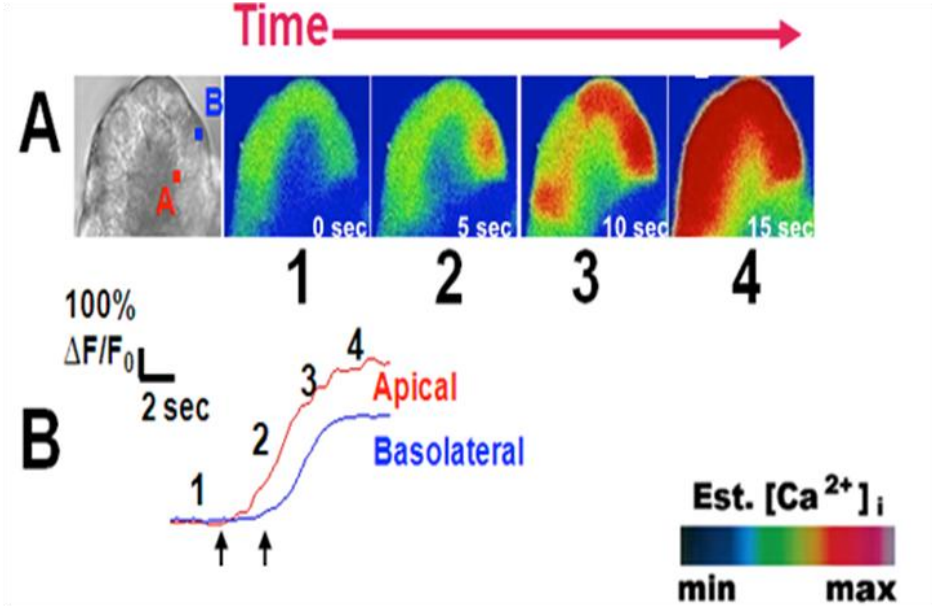
Analysis of acquired images by focusing on the point of interest



4. Model

Developing algorithms to model and
Assemble the knowledge gained from the imaging initiative





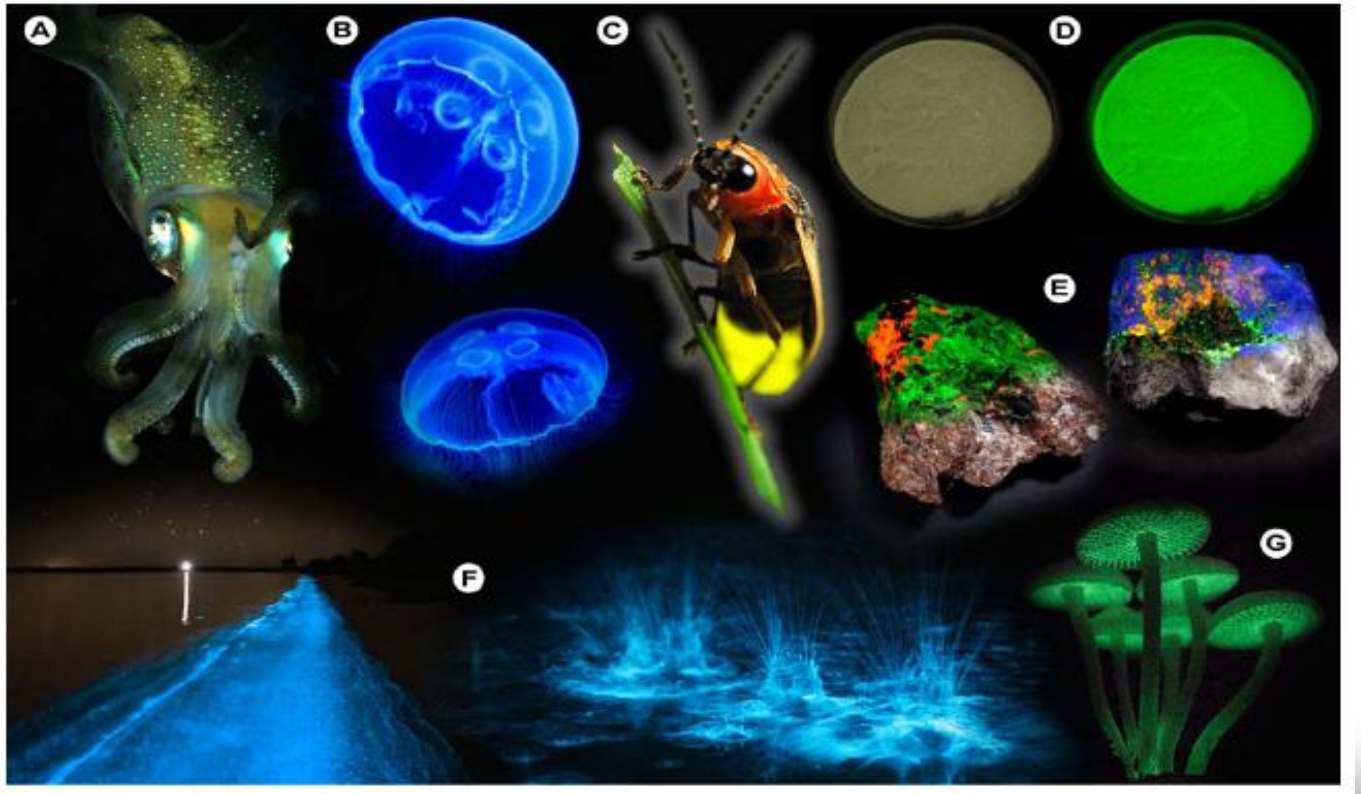
A novel method using fluorescence microscopy for real-time assessment of ATP release from individual cells

Ross Corriden , Paul A. Insel , Wolfgang G. Junger

American Journal of Physiology - Cell PhysiologyPublished 1 October 2007Vol. 293no. C1420-C1425DOI: 10.1152/ajpcell.00271.2007

Fluorescence and Fluorophore

Fluorescence, the absorption and re-emission of photons with longer wavelengths

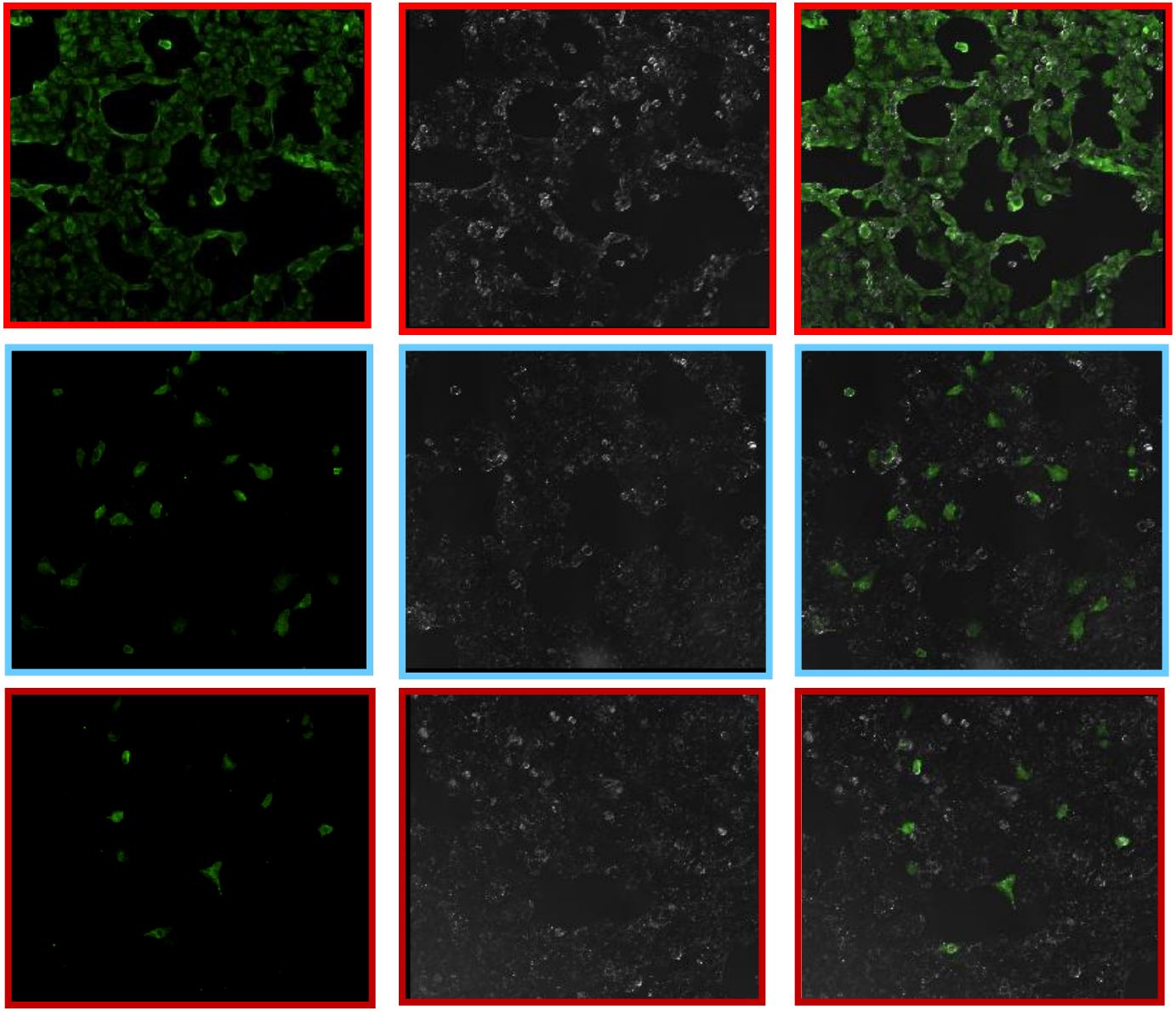


Concofal Microscope Application

1. Colocalization of proteins
2. Cell structure analysis
3. Host-pathogen interaction
4. Gen transfection
5. Fluorescence Resonance Energy Transfer (FRET)
6. etc

Virus detection : Immunofluorescence

H1N1 (green) | H1N1 (green) | Merged)



Aerosolized virus

Cells infected by original virus

Cells infected by aerosolized vrus (PCI OFF)

Cells infected by aerosolized virus (PCI ON)

Thank You!!!