Lecture 8 - 9
Techniques & Methods

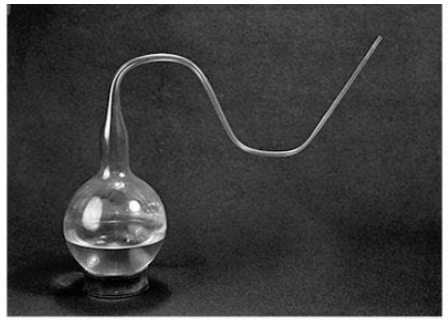
Prof. Sebastian Maerkl

Cell Culturing

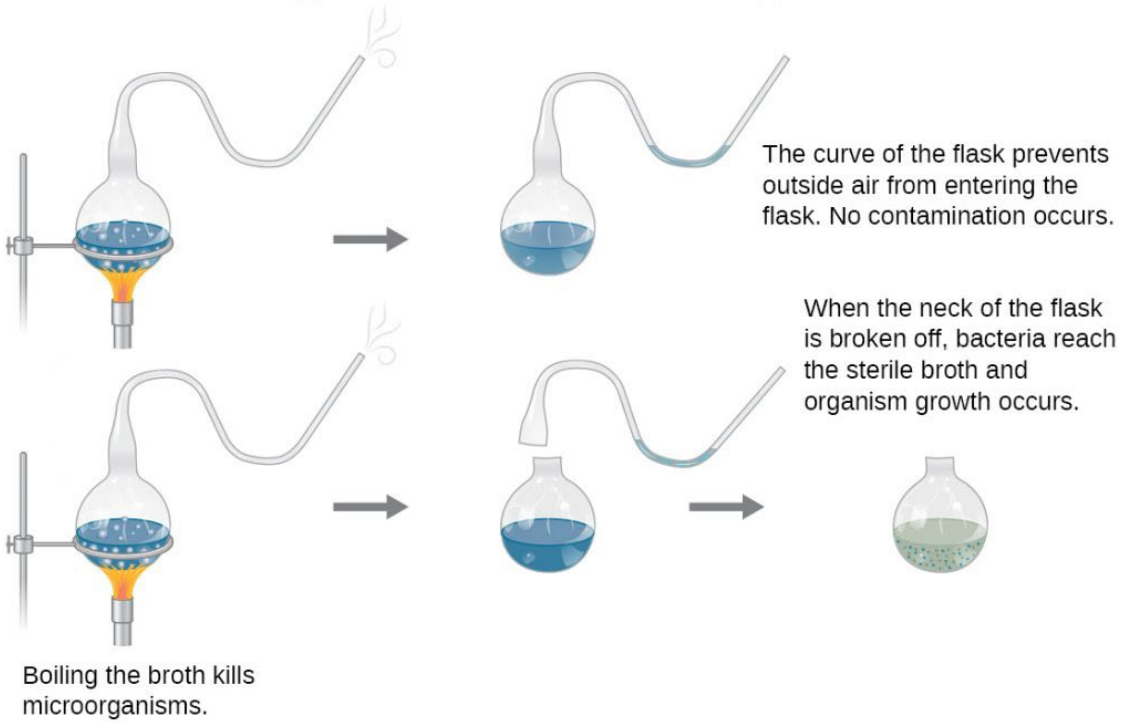
Microbiological Culture Methods

Aseptic Technique

(a)



(b)



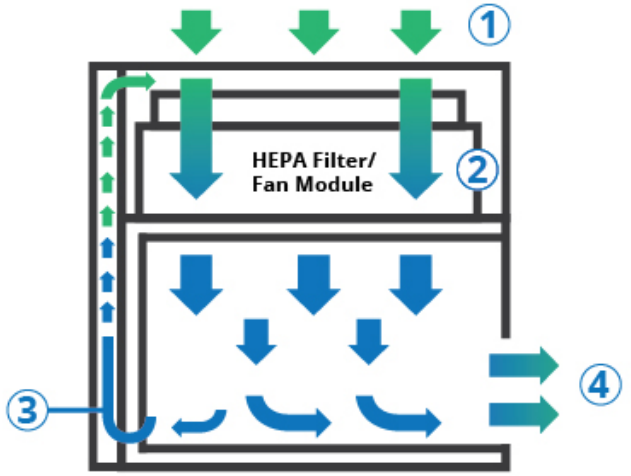
(c)

(a) Louis Pasteur

Aseptic Technique

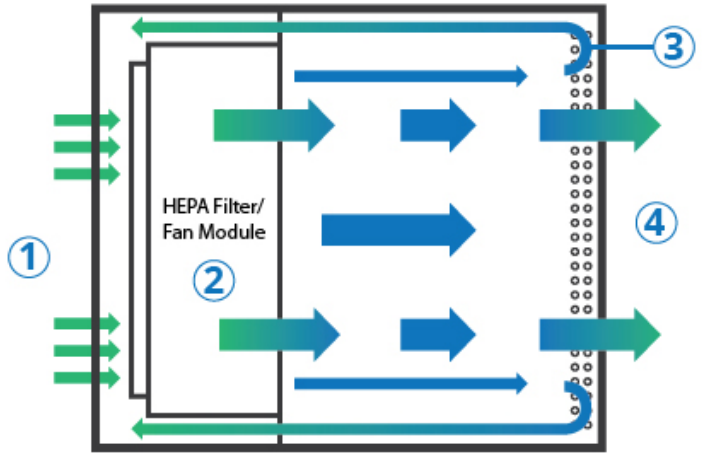
Laminar Flow Hoods

Vertical Laminar Flow Hood Diagram (*Cutaway Side View*)



www.Laboratory-Supply.net

Horizontal Laminar Flow Hood Diagram (*Cutaway Side View*)



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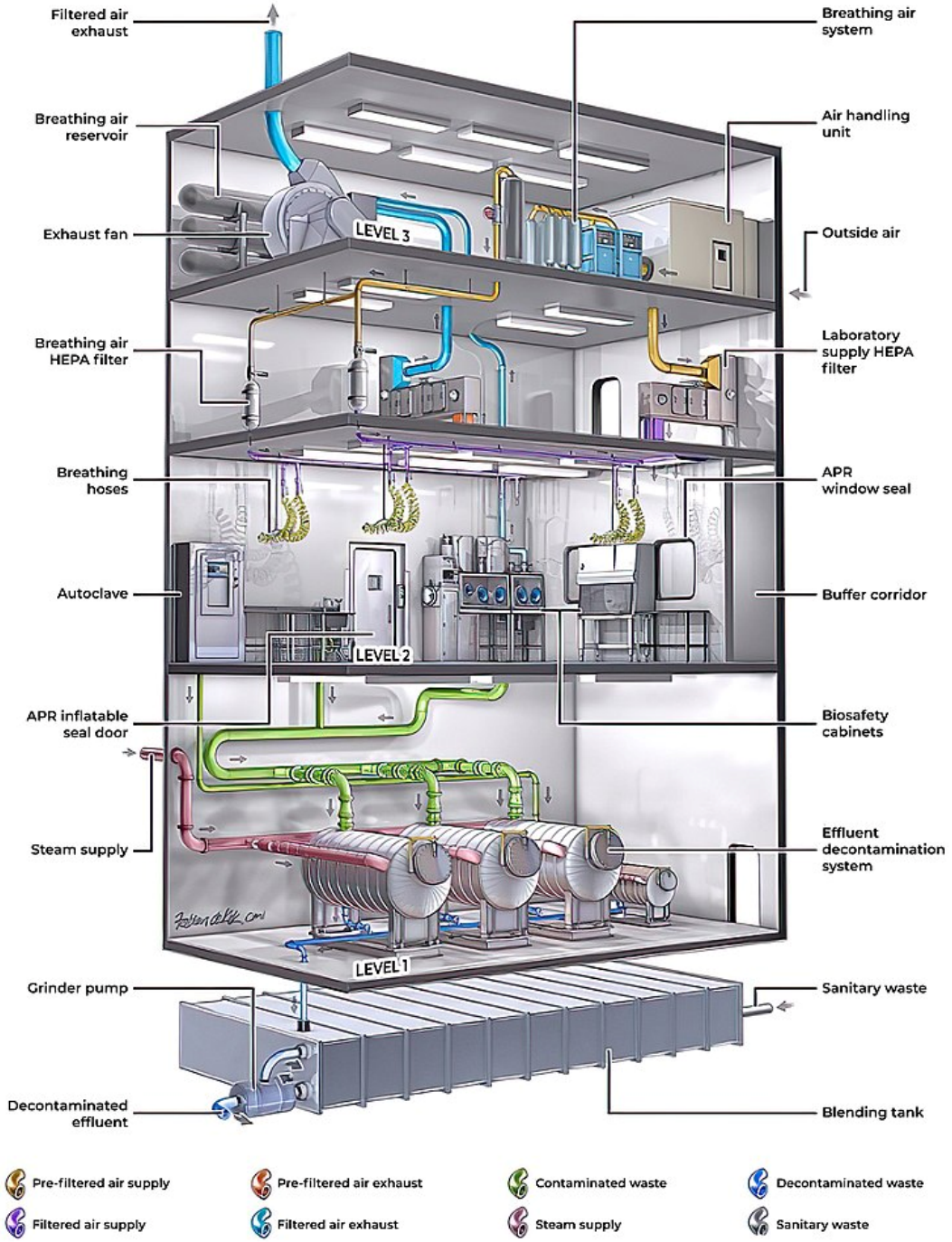
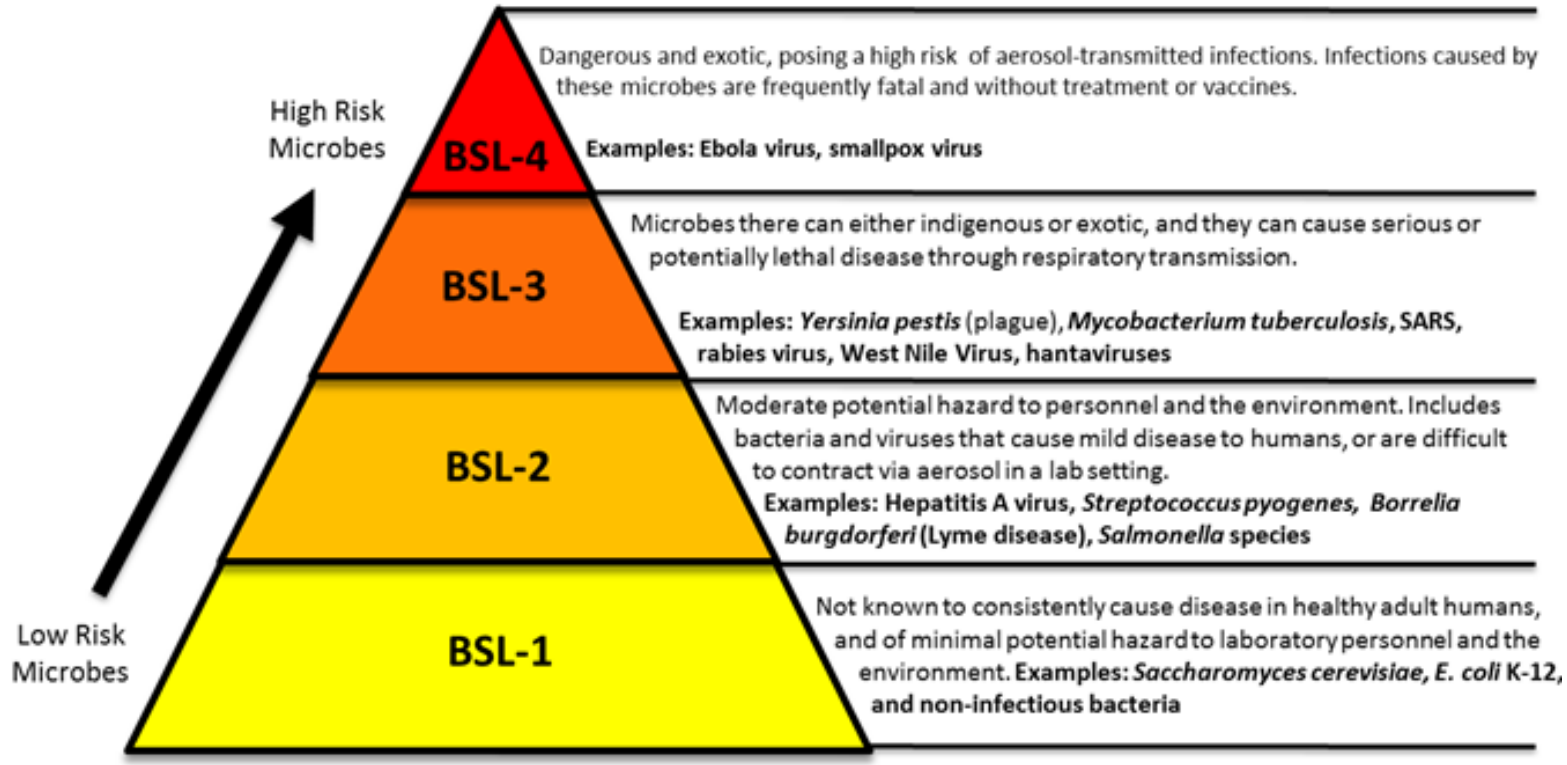
Biosafety Cabinet

Biosafety Cabinet

Outbreak (1995)



Biosafety Levels



Does Switzerland have a BSL-4 Lab?

Does Switzerland have a BSL-4 Lab?

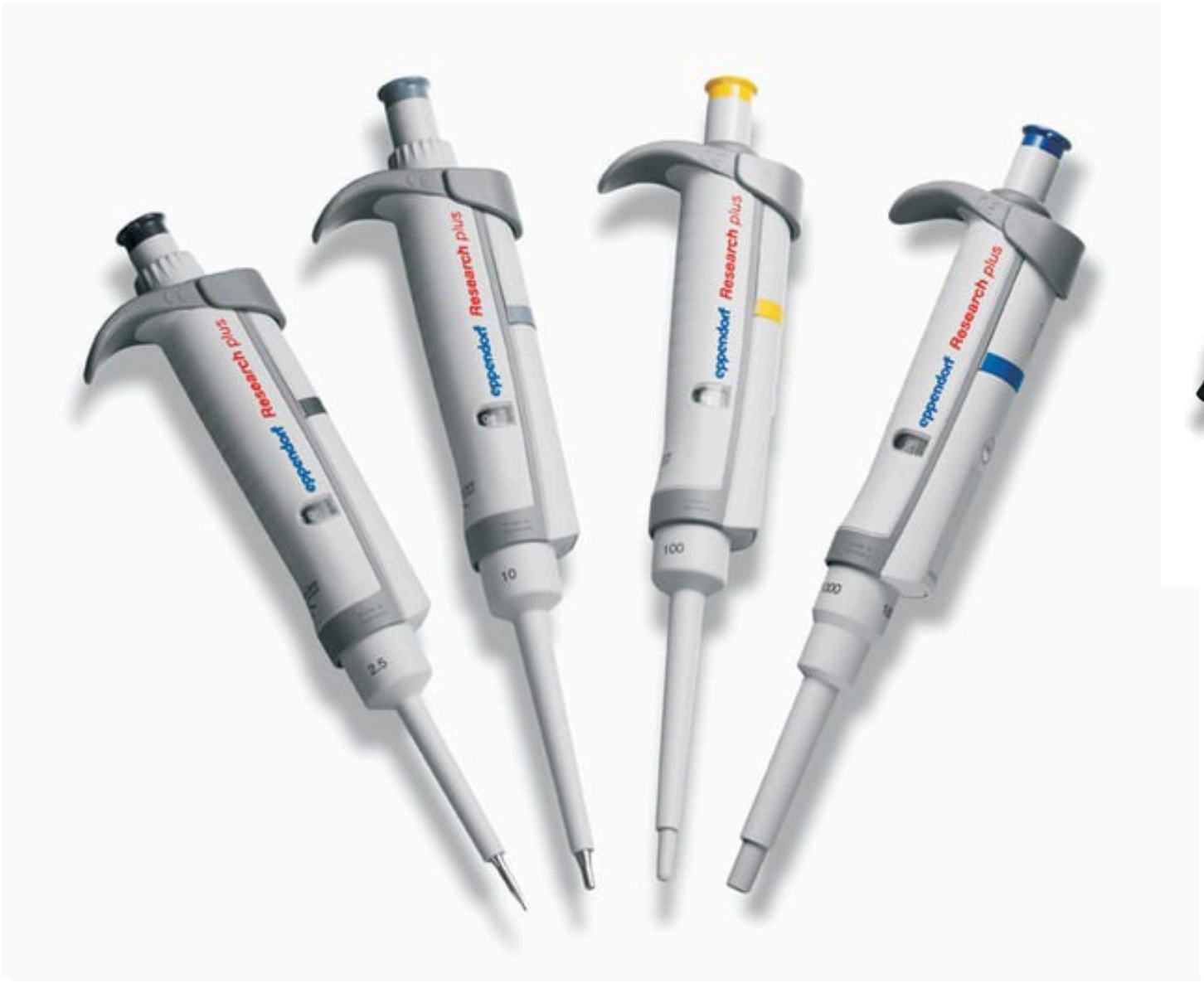
Switzerland	Geneva, Canton of Geneva	University Hospital of Geneva		"Glove box" type laboratory; primarily for handling clinical samples.[73]
	Spiez, Canton of Bern	Spiez Laboratory	2013	Run by the Federal Office for Civil Protection of the Federal Department of Defence, Civil Protection and Sports.[74]
	Mittelhäusern, Canton of Bern	The Institute of Virology and Immunology IVI[75]		Part of the Food Safety and Veterinary Office (FSVO).[76] Primary purpose is diagnostics of highly pathogenic viruses.[74]

Sterilization

Autoclave
Heating to 121-132℃

Filtration
normally with a 0.2µm filter

Pipettes

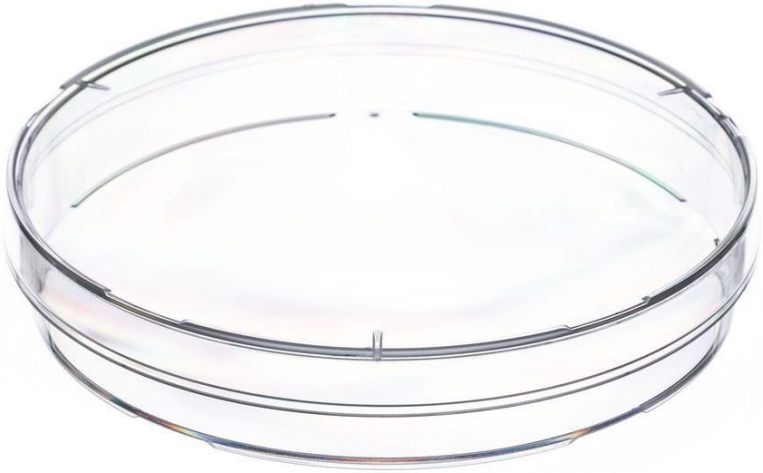

eppendorf Research plus
2.5
10
100


eppendorf Research plus
eppendorf
100


eppendorf
eppendorf
eppendorf

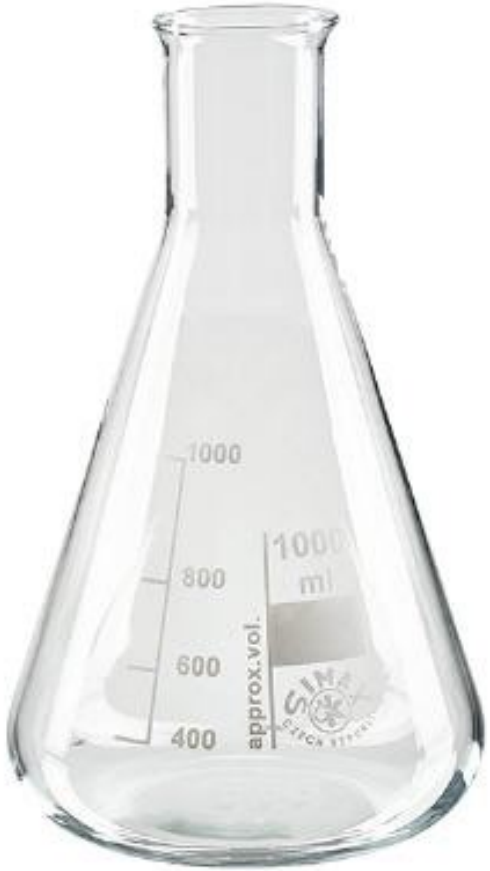
Media types: agar and liquid

Culture Vessels



Petri Dish
(solid media)

Culture Tube
(1- 5mL liquid media)



Erlenmeyer Flask
(10mL – 2L liquid media)

Media

Lysogeny broth (LB)
Luria-Bertani broth

The following is a common method for the preparation of 1 litre of LB:

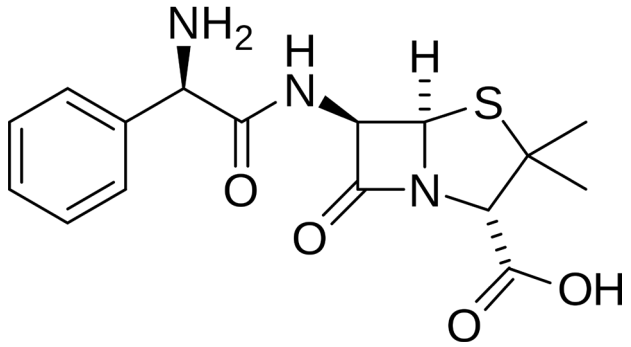
- Measure out the following:
  - 10 g tryptone
  - 5 g yeast extract
  - 10 **or** 5 **or** 0.5 g NaCl as required (see Formulae above; some bacteria are sensitive to NaCl)
- Suspend the solids in ~800 ml of distilled or deionized water.
- Add further distilled water or deionized water, in a measuring cylinder to ensure accuracy, to make a total of 1 liter.
- Autoclave at 121 °C for 20 mins.
- After cooling, swirl the flask to ensure mixing, and the LB is ready for use.[10]

Tryptone is the assortment of peptides formed by the digestion of casein by the protease trypsin.

YEPD / YPD
Yeast extract peptone dextrose

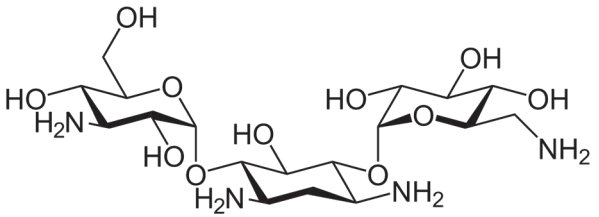
YEPD typically contains:
1% yeast extract
2% peptone
2% glucose
in distilled water.

Antibiotics



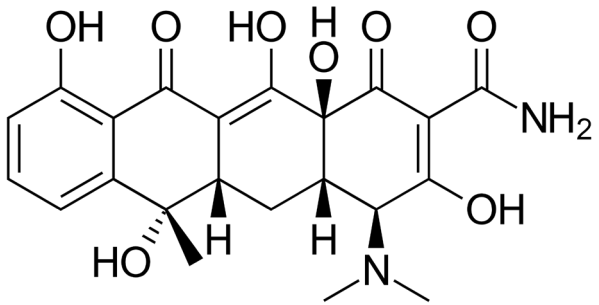
Ampicillin

Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make the cell wall.



Kanamycin A

Kanamycin works by interfering with protein synthesis. It binds to the 30S subunit of the bacterial ribosome. This results in incorrect alignment with the mRNA and eventually leads to a misread that causes the wrong amino acid to be placed into the peptide. This leads to nonfunctional peptide chains.

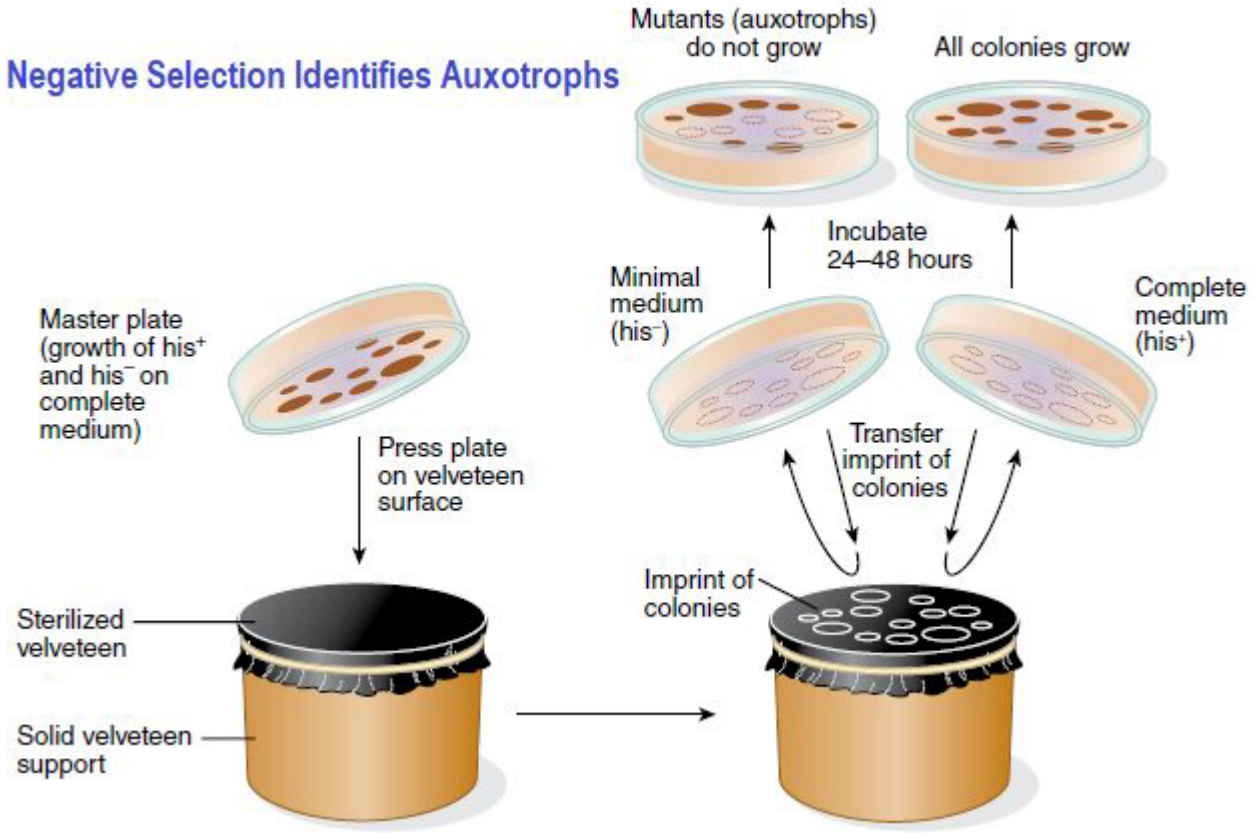


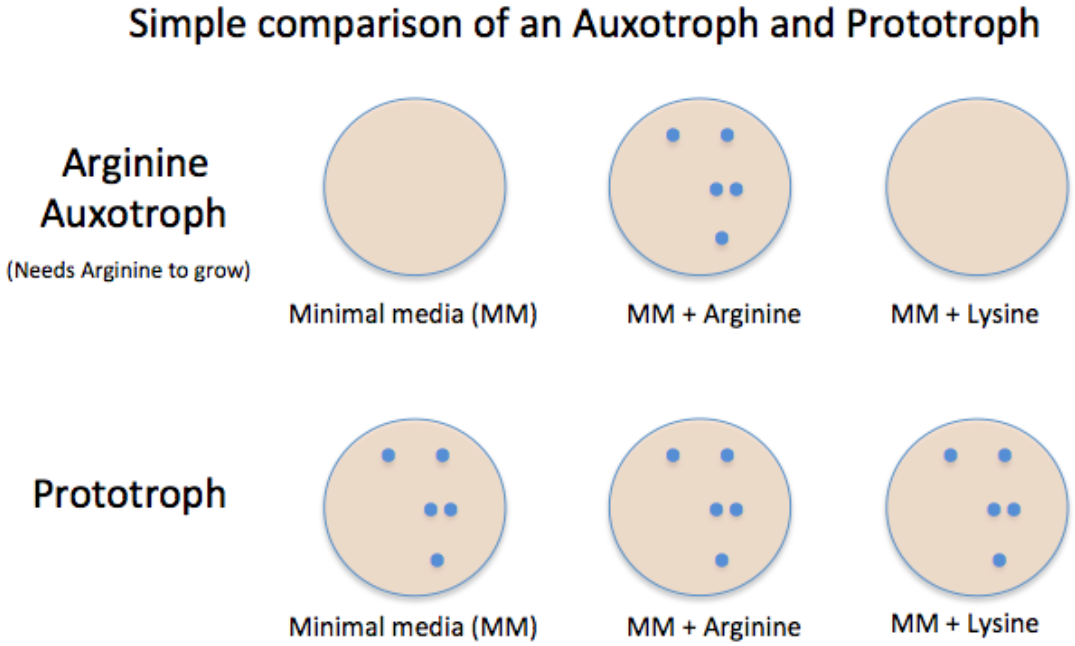
Tetracycline

Tetracycline inhibits protein synthesis by blocking the attachment of charged tRNA at the P site peptide chain.

Selective Media

HIS-selective medium is a type cell culture medium that lacks the amino acid histidine. It can be used with bacteria reliant on the expression of a gene encoding proteins involved in histidine expression in order to survive. Only bacteria expressing such genes (such as hisB in *Escherichia coli* and HIS3 in *Saccharomyces cerevisiae*) will survive on these media.



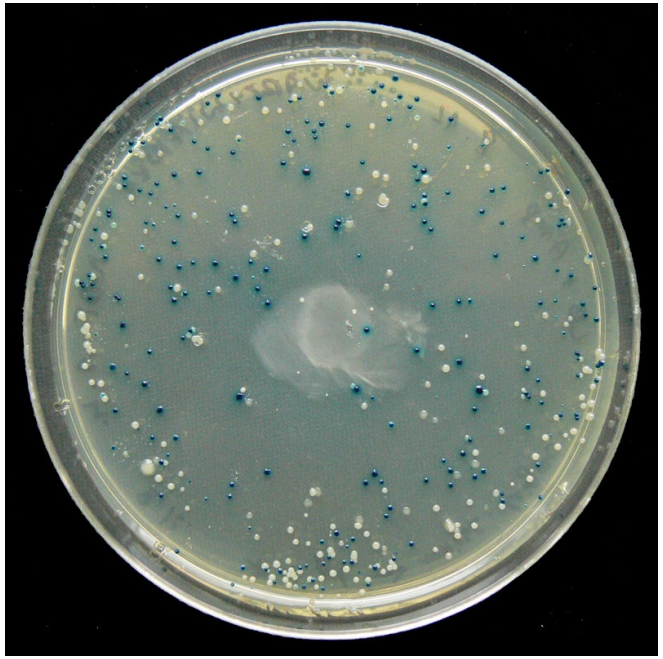


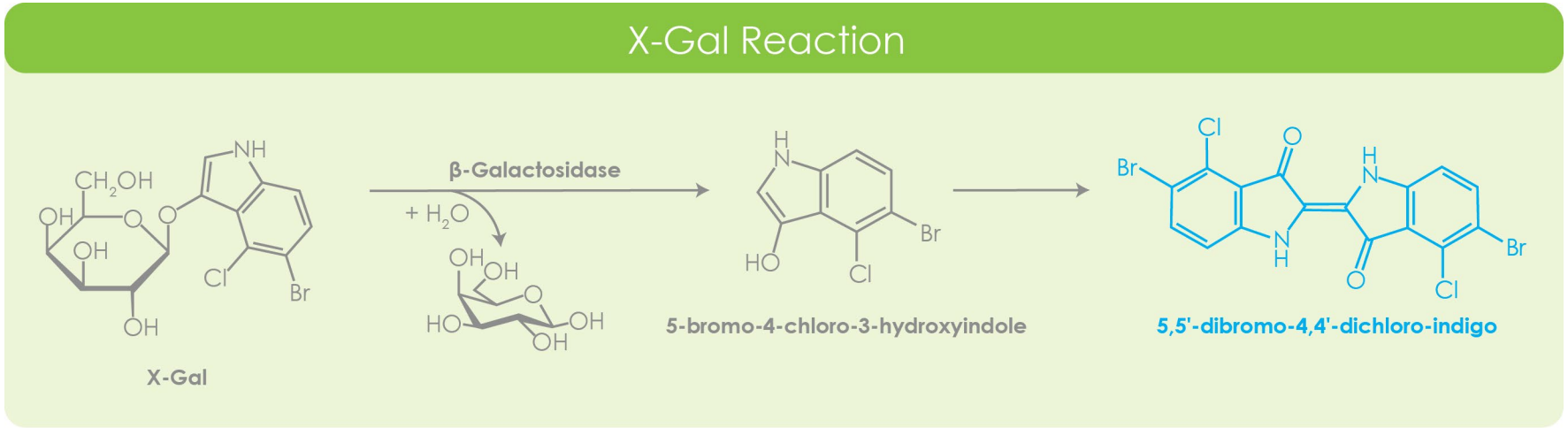
Differential Media

- **MacConkey agar** is a selective and differential culture medium for bacteria. It is designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacteria and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on MacConkey agar, and nonfermenters do not change color. The media inhibits growth of Gram-positive organisms with crystal violet and bile salts, allowing for the selection and isolation of gram-negative bacteria. The media detects lactose fermentation by enteric bacteria with the pH indicator neutral red.

Differential Media

X-gal (also abbreviated **BCIG** for 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) is an organic compound consisting of galactose linked to a substituted indole. The compound was synthesized by Jerome Horwitz and collaborators in 1964. The formal chemical name is often shortened to less accurate but also less cumbersome phrases such as bromochloroindoxyl galactoside. The X from indoxyl may be the source of the X in the X-gal contraction. X-gal is often used in molecular biology to test for the presence of an enzyme, β-galactosidase, in the place of its usual target, a β-galactoside. It is also used to detect activity of this enzyme in histochemistry and bacteriology. X-gal is one of many indoxyl glycosides and esters that yield insoluble blue compounds similar to indigo dye as a result of enzyme-catalyzed hydrolysis.

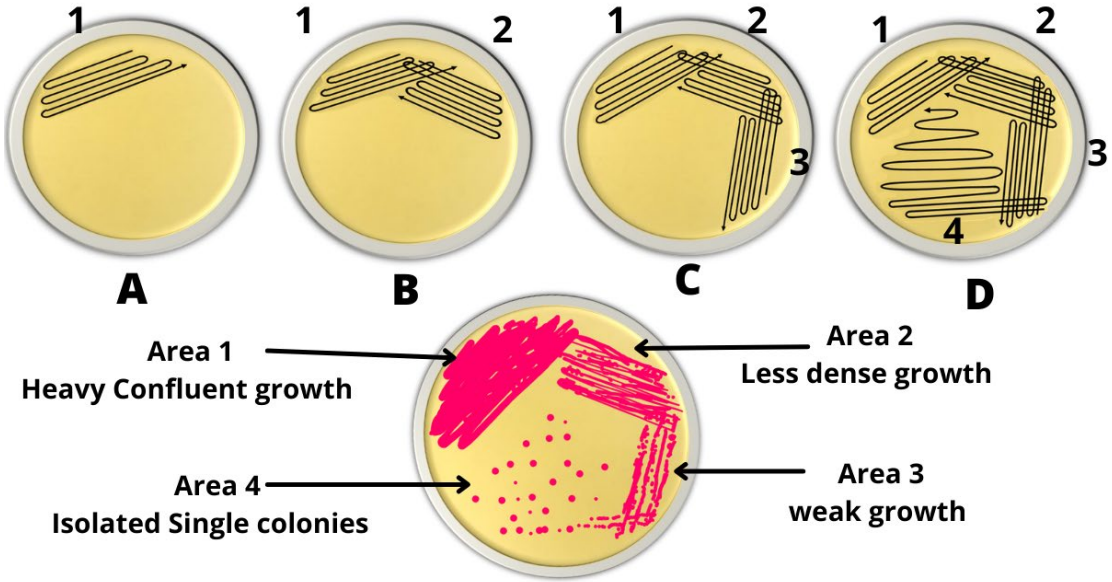




Aseptic Technique for Microbial Cultures

Streak Plating

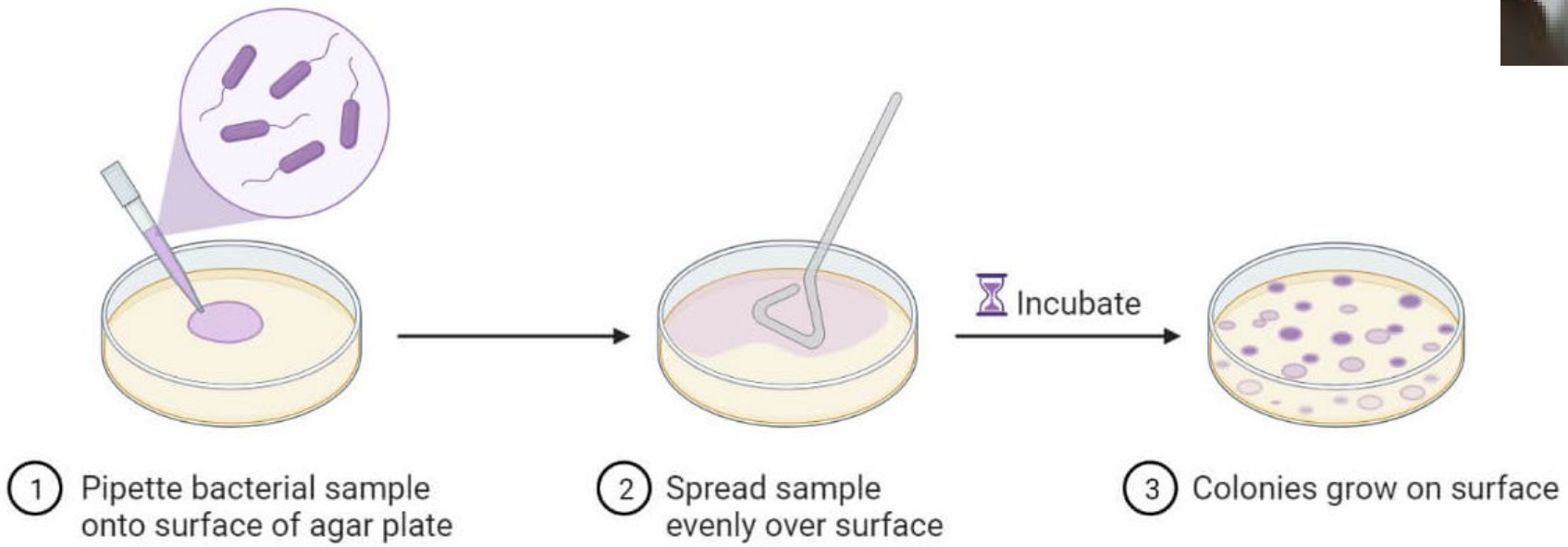
Streak Plating

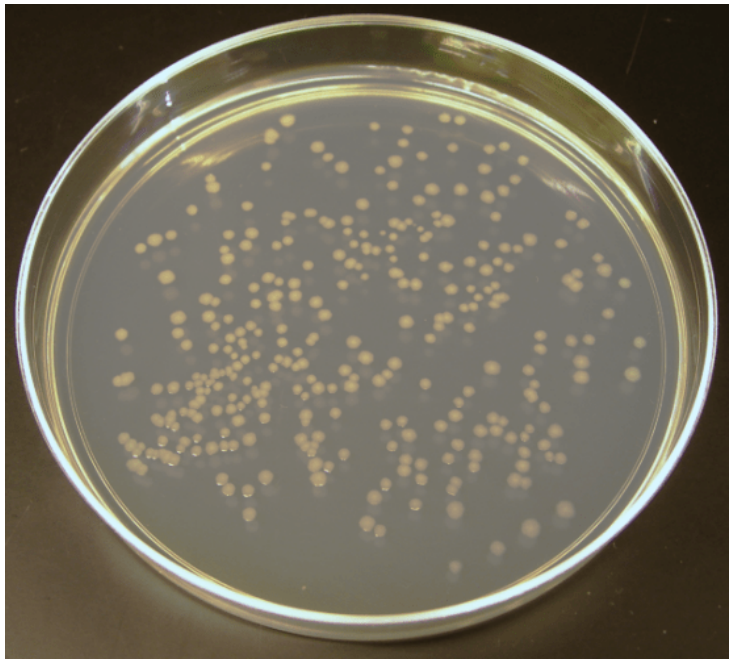


Spread Plating

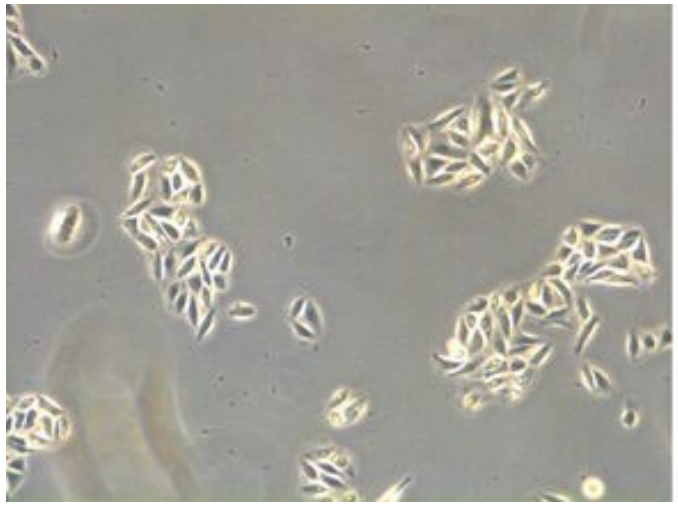
Spread Plating

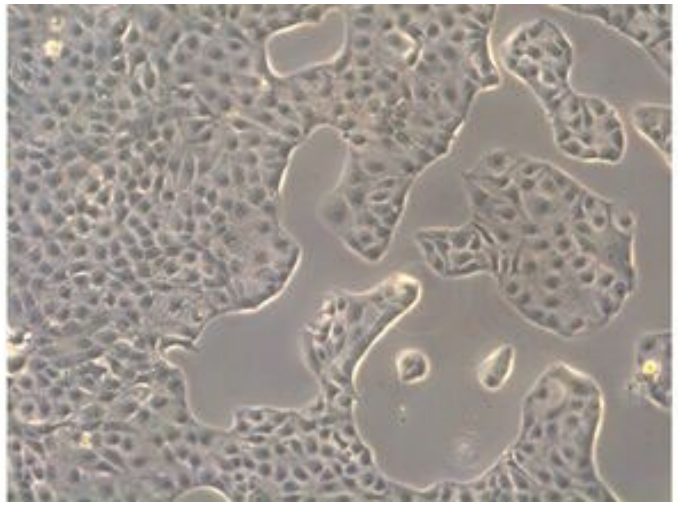
Glass beads can also be used to spread plate…

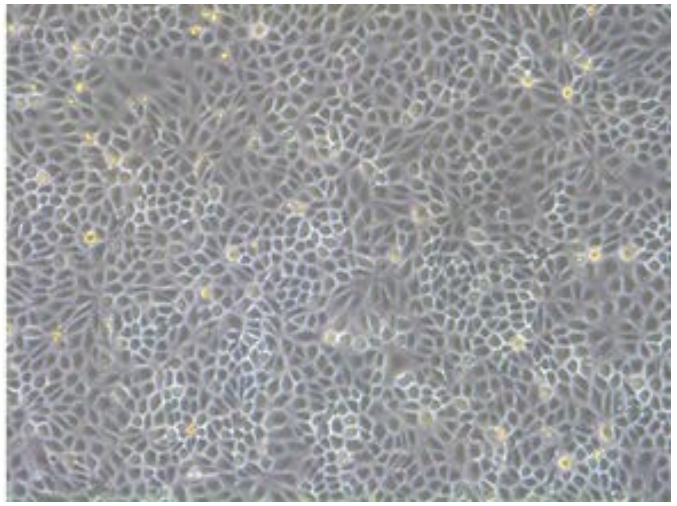




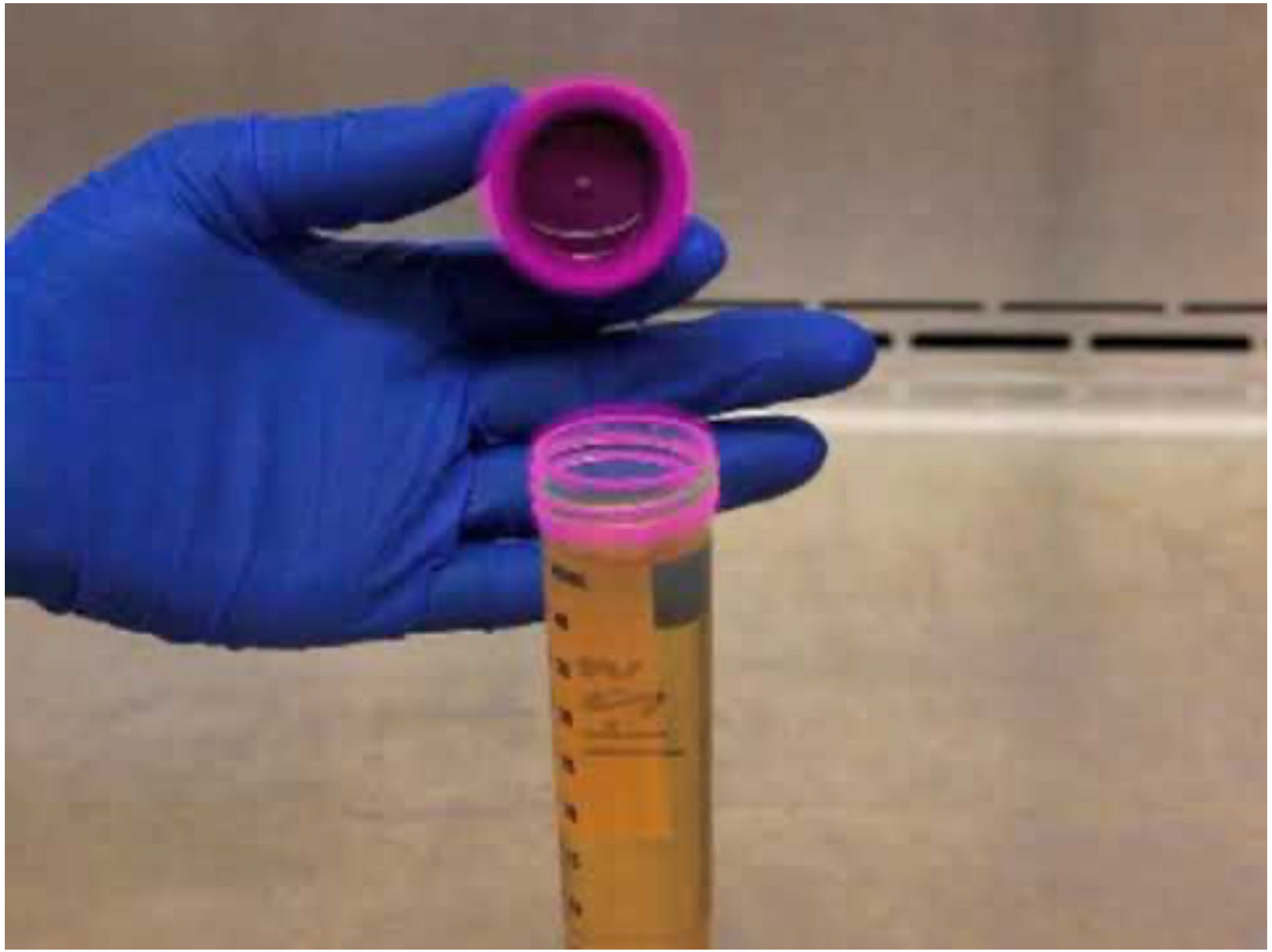
Mammalian cell culturing







Aseptic Technique for Mammalian Cell Culture

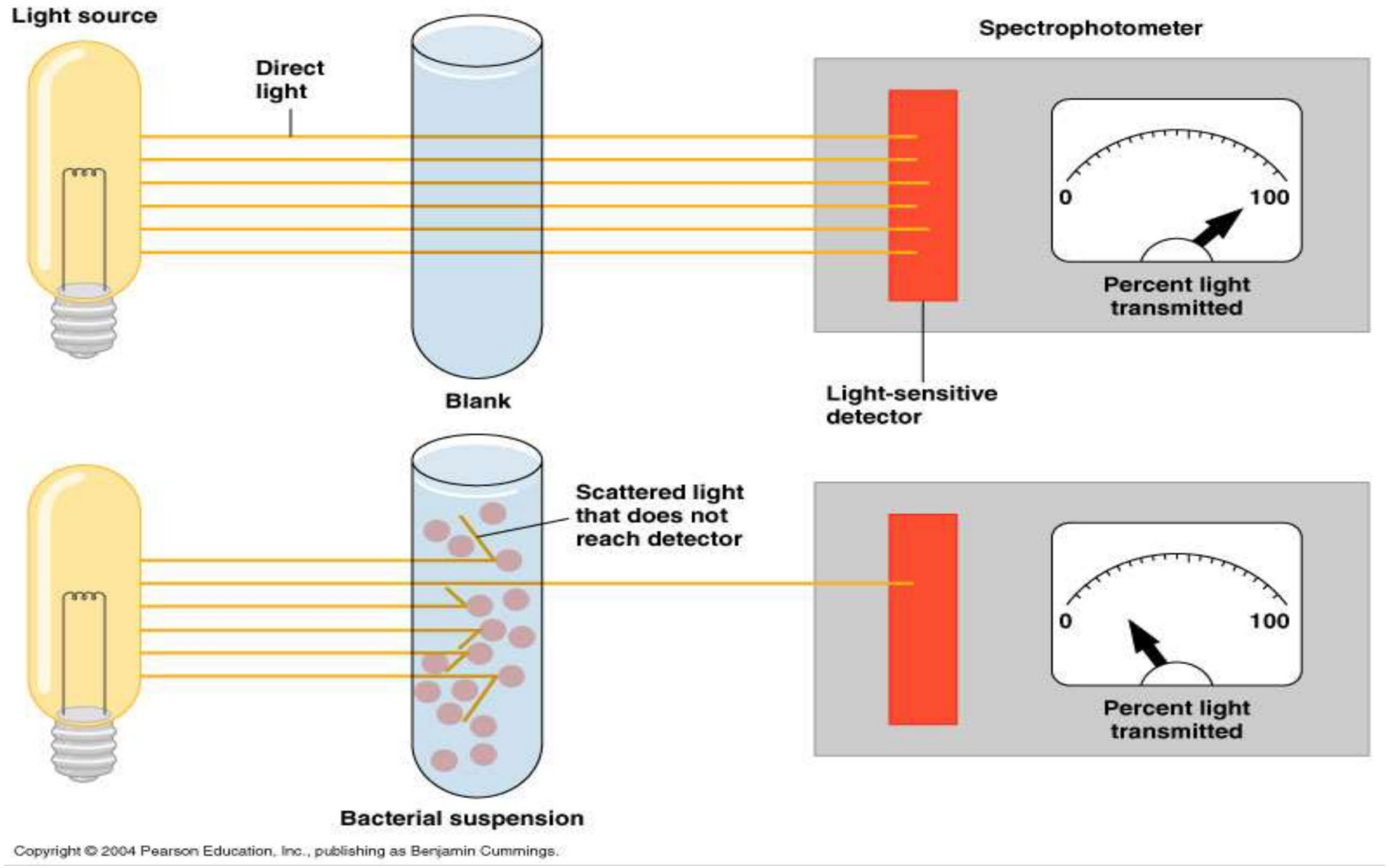


Storage

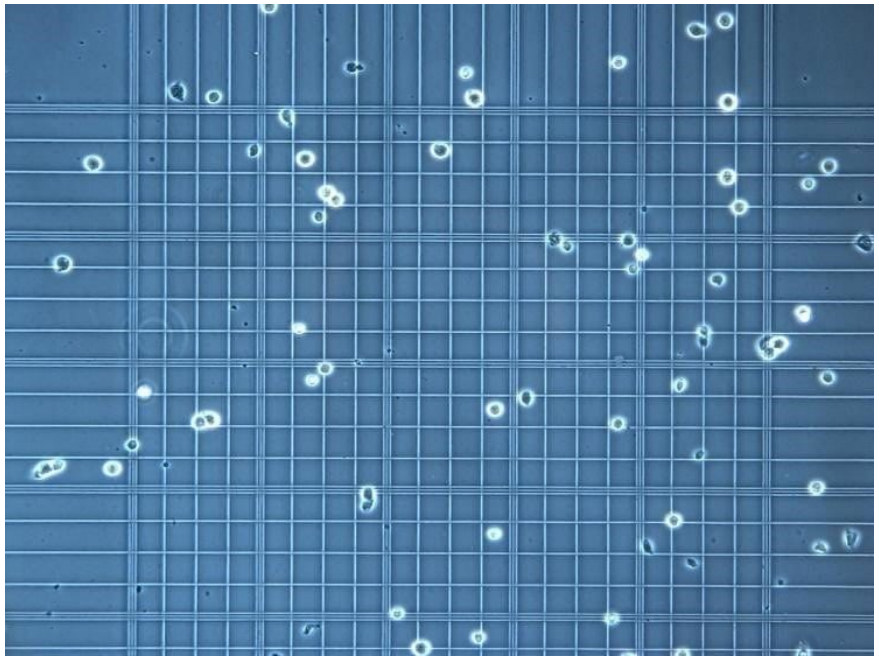
4°C	-20°C	-80°C	-130°C
- Bacterial agar plates (4-6 weeks) - Stab cultures (3 weeks – 1 year) - Short term	- Glycerol stocks (1-3 years) - Medium term	- Glycerol stocks (1-10 years) - Long term	- Mammalian cells

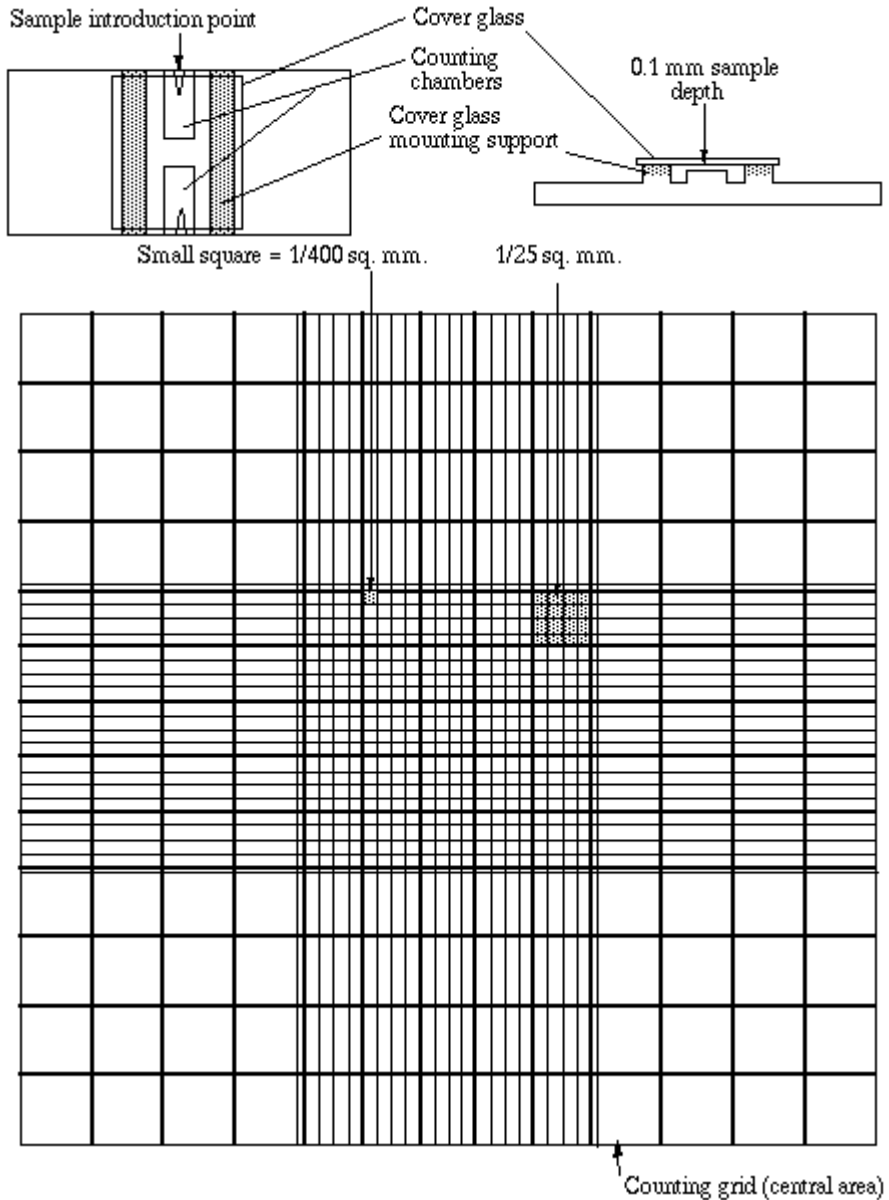
Cell counting
and
growth rate determination

Spectrophotometric / Turbidimetric

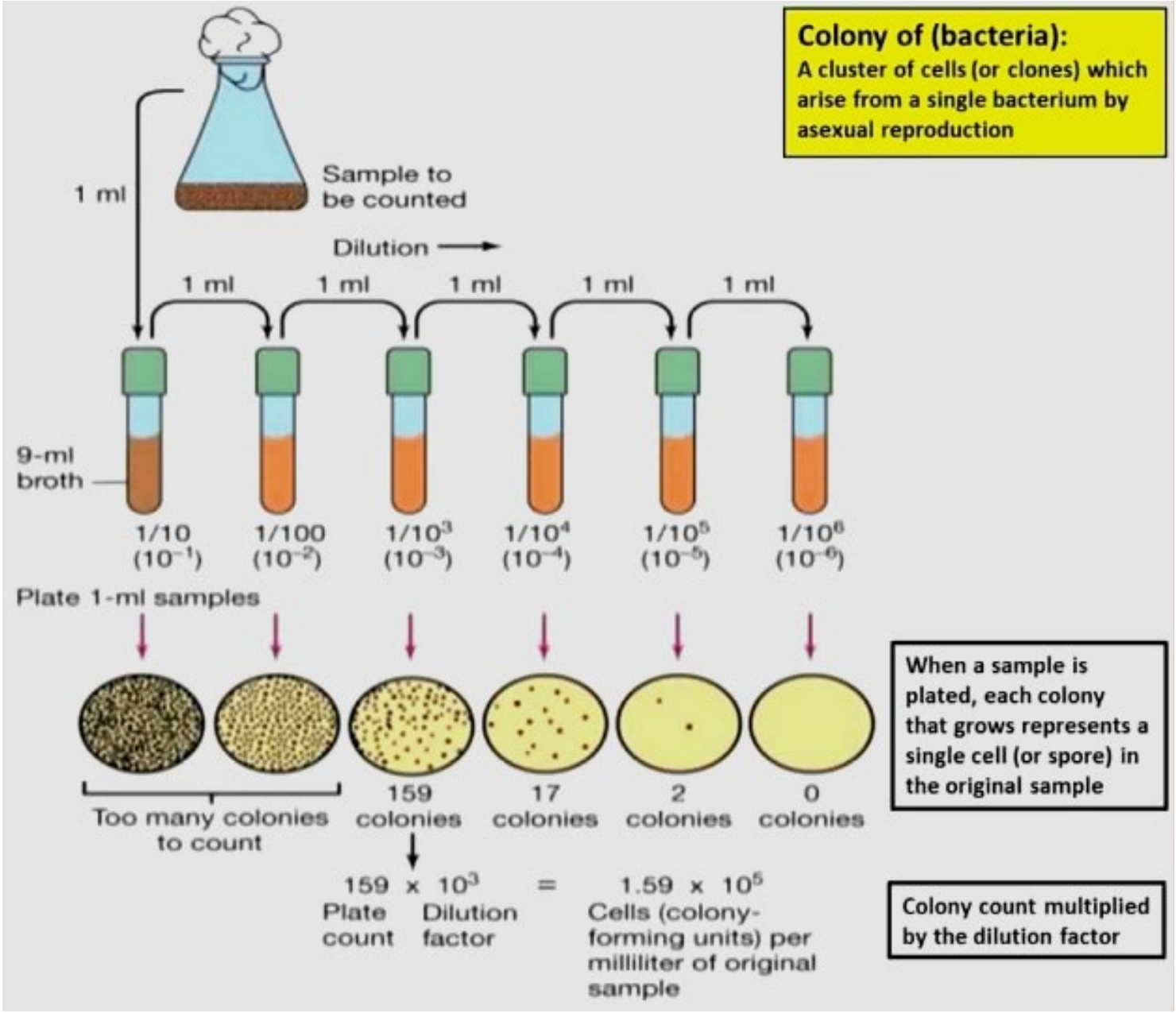


Chamber Counter / Hemocytometer

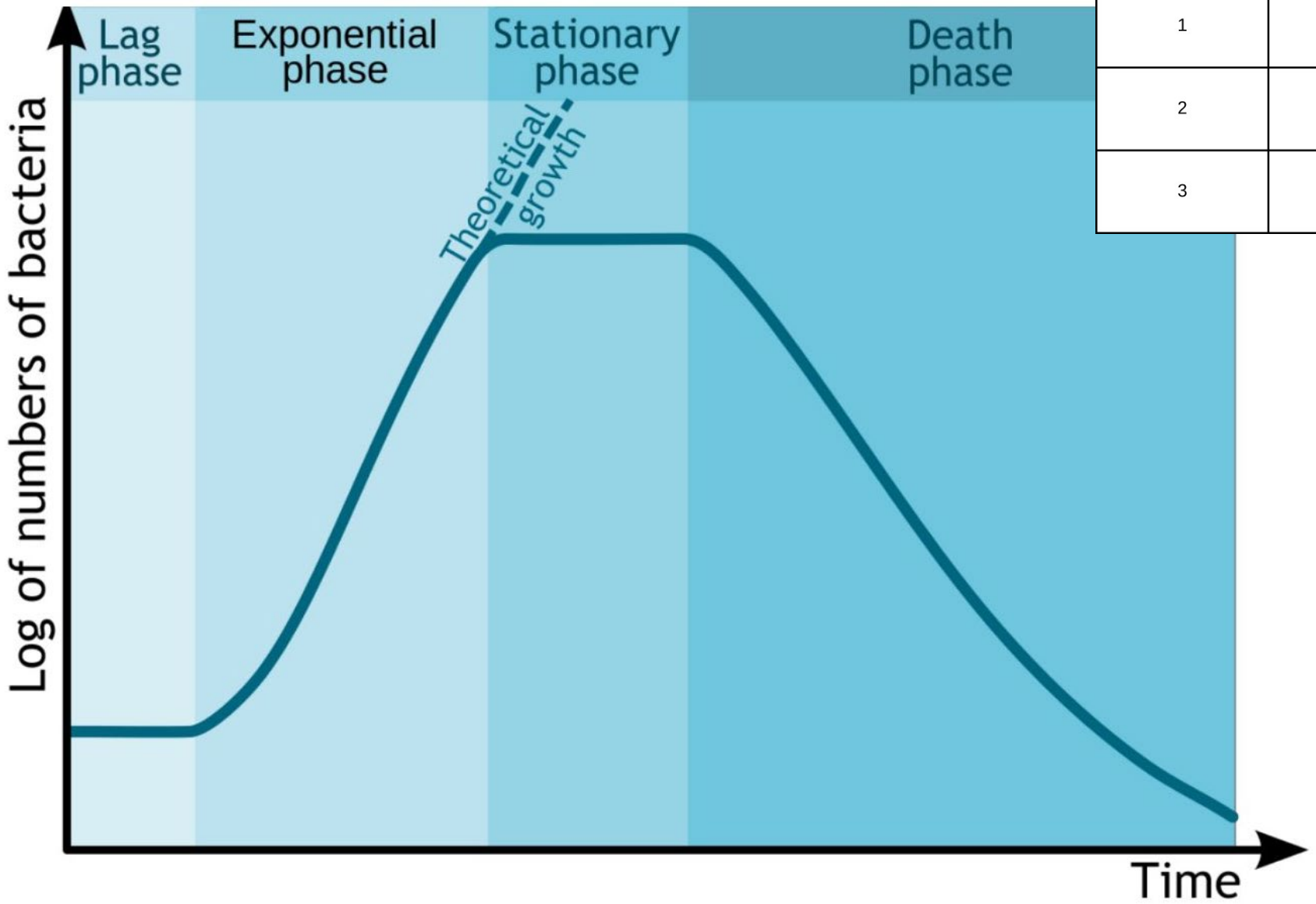


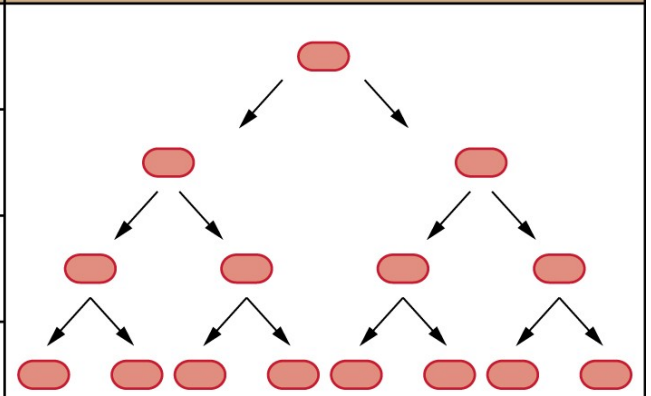


Colony counting

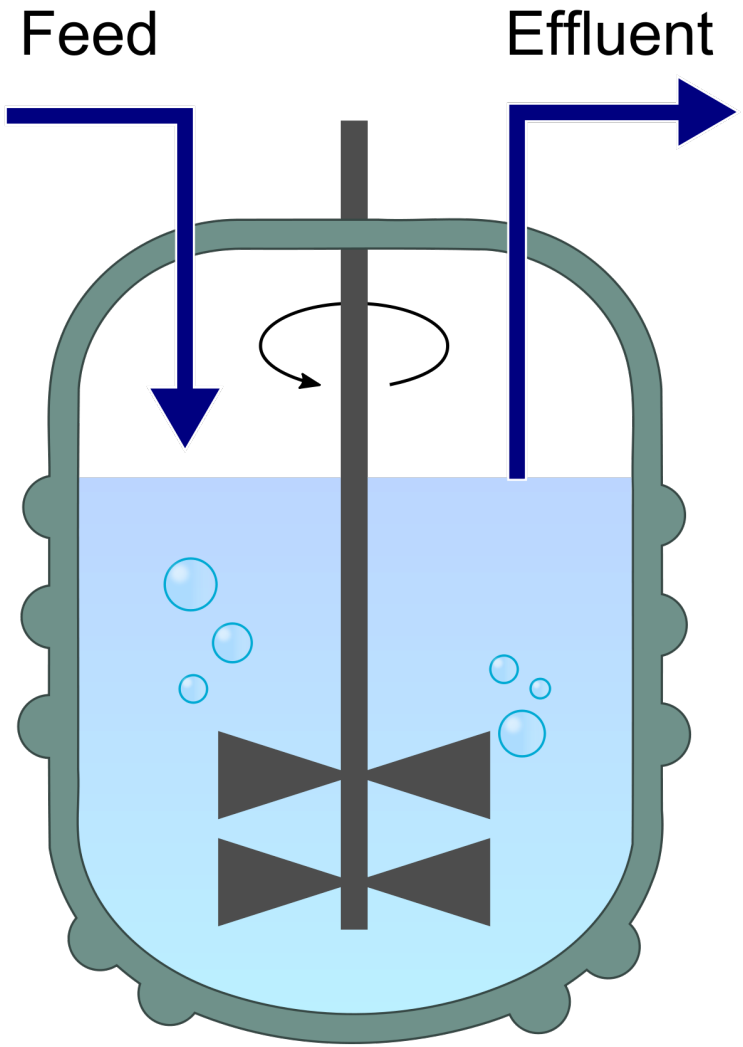


Batch Growth Curves



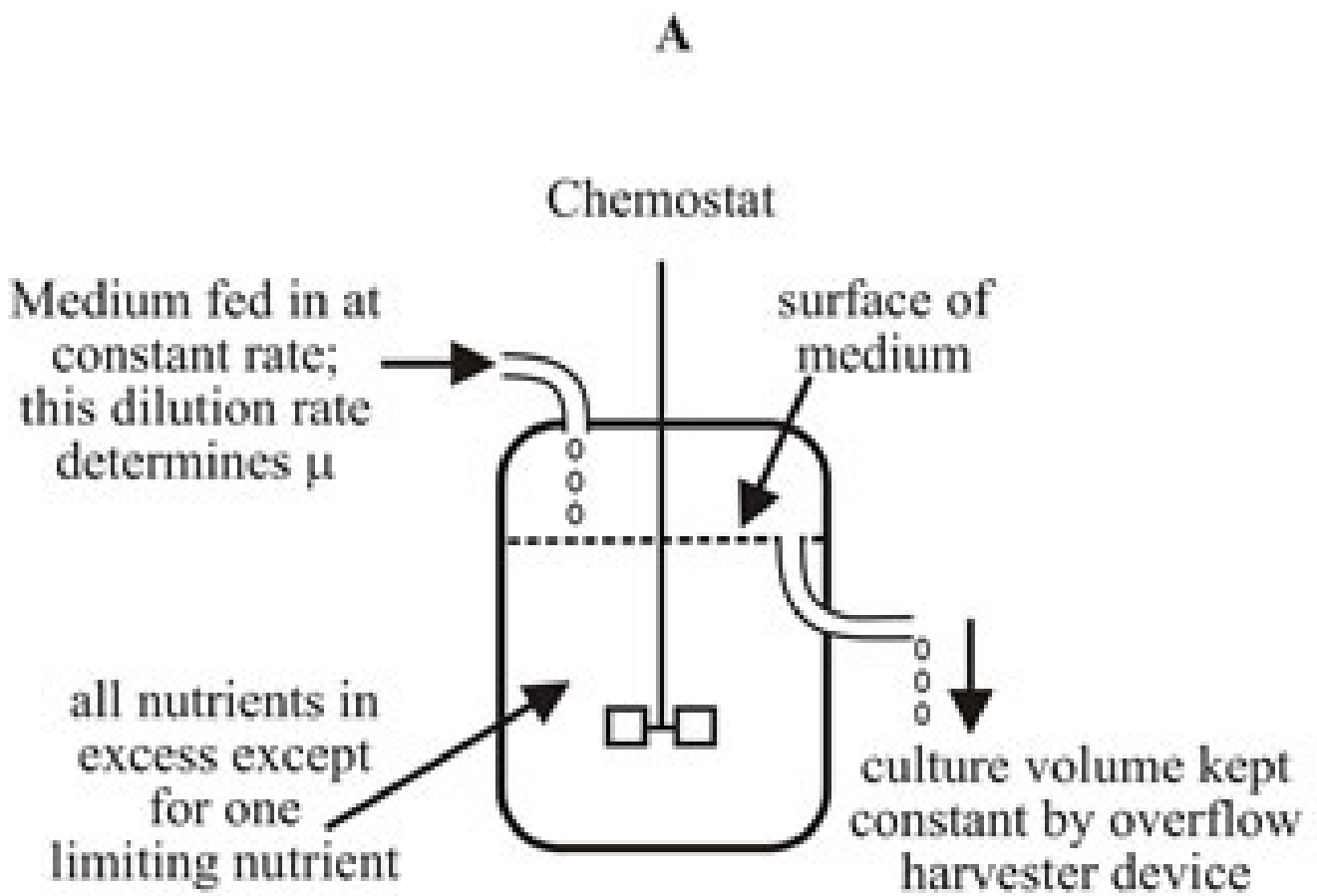
Number of generations (*n*)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

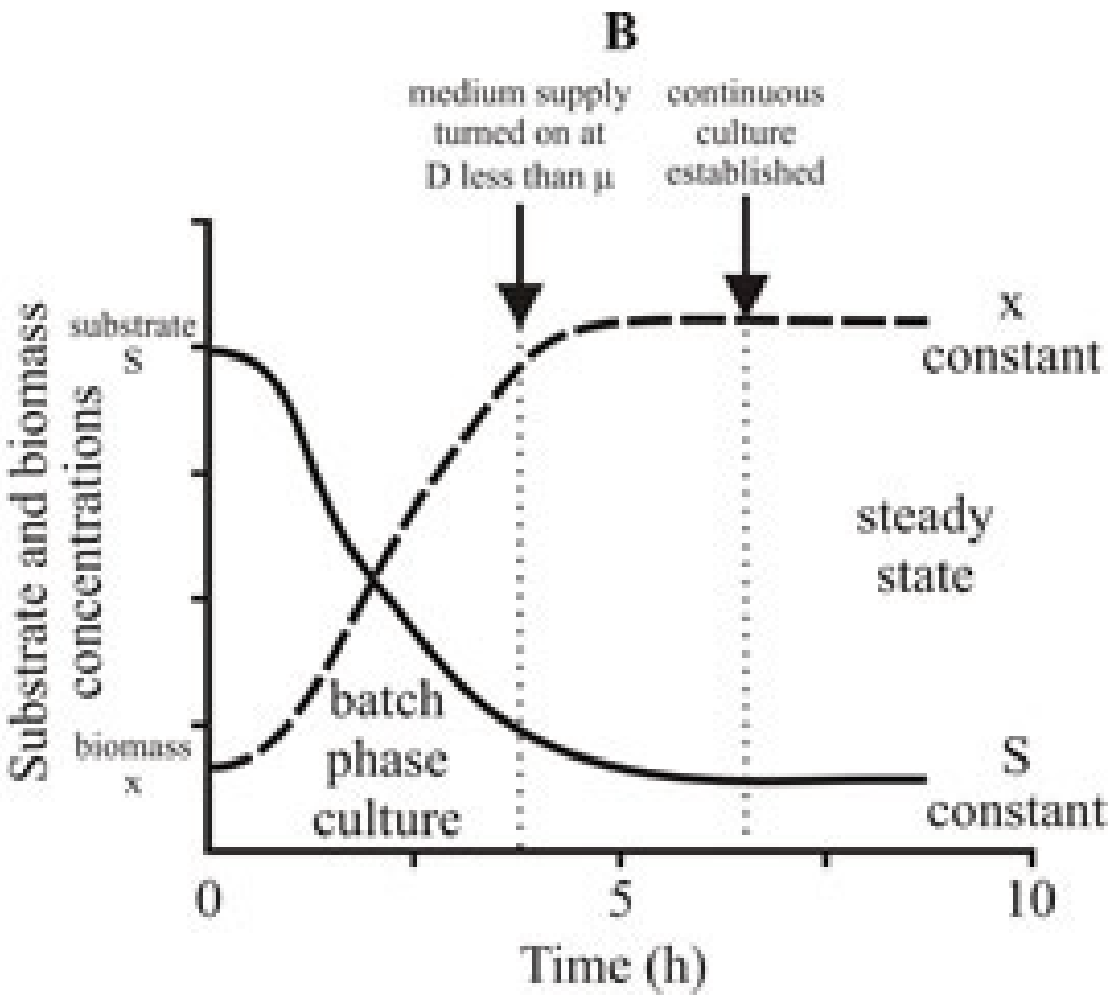
Chemostats and turbidostats



Chemostats and turbidostats

Chemostats and turbidostats

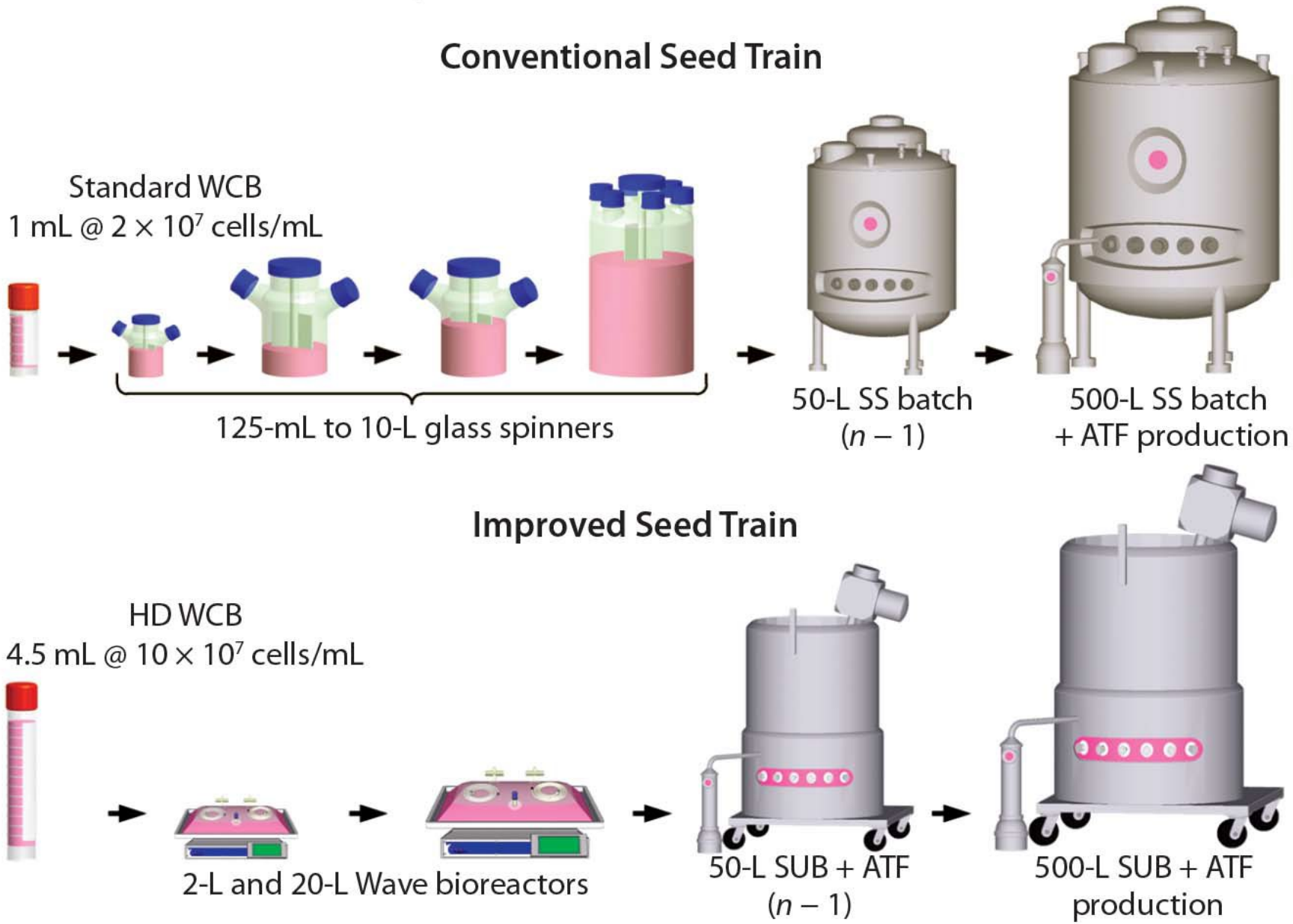




Bioreactors

Bioreactors

Bioreactors



Microscopy

Microscopy

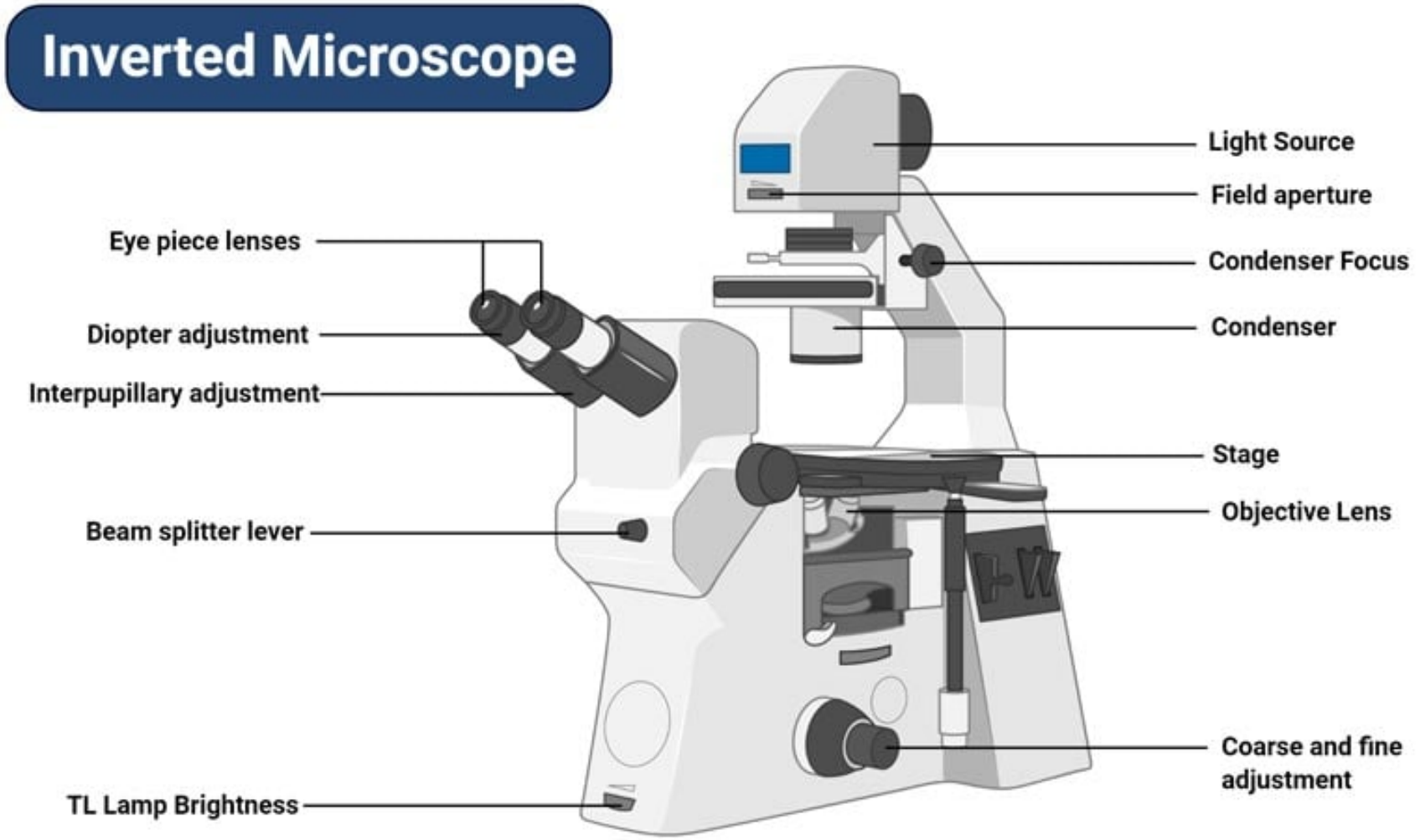
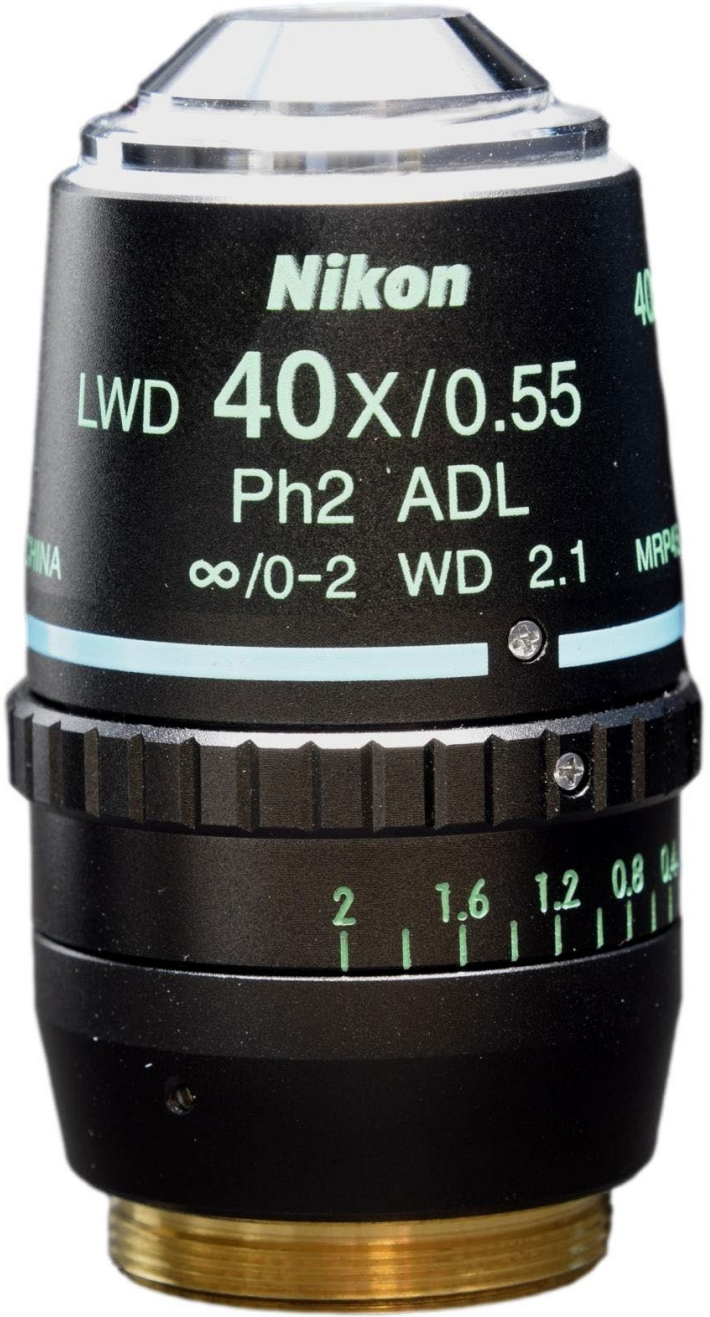


Figure: Inverted Microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Objectives

Magnification:
- 2x – 100x

Numerical Aperture (NA):
- Light acceptance angle
- Determines brightness and resolution
- Higher is better



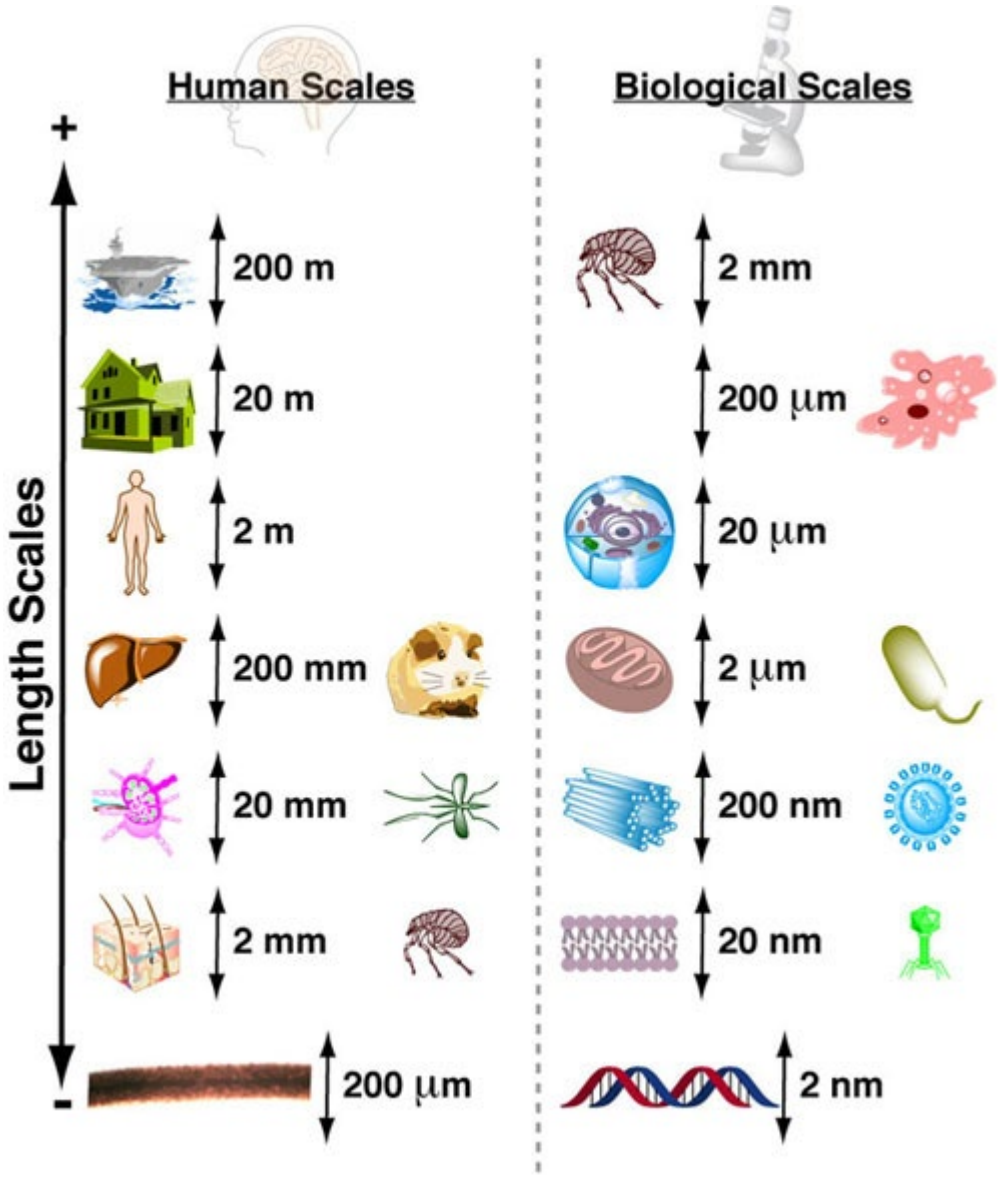
Diffraction Limit

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

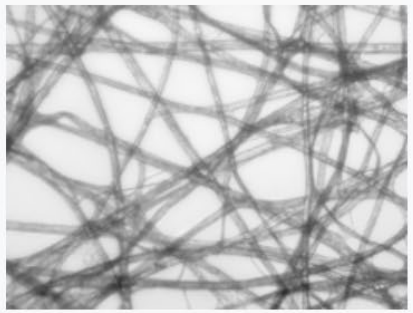
NA = 1.4

Green light = 500nm

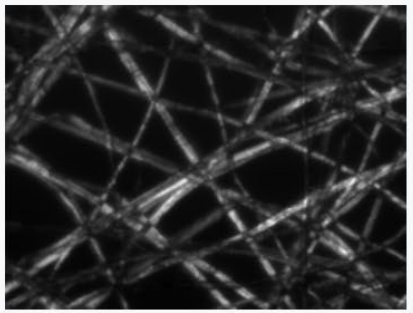
d = 250nm



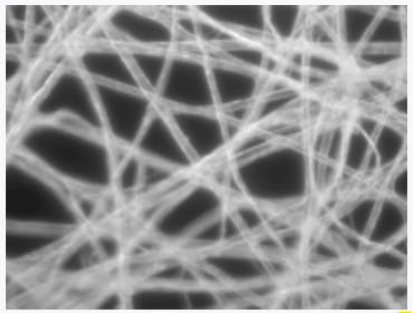
Brightfield microscopy



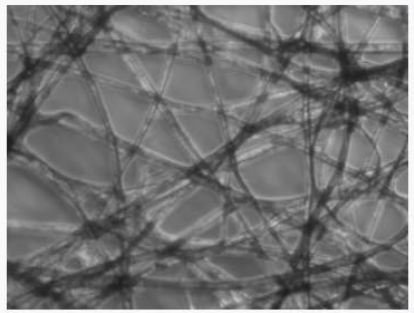
Bright field illumination, sample contrast comes from absorbance of light in the sample



Cross-polarized light illumination, sample contrast comes from rotation of polarized light through the sample

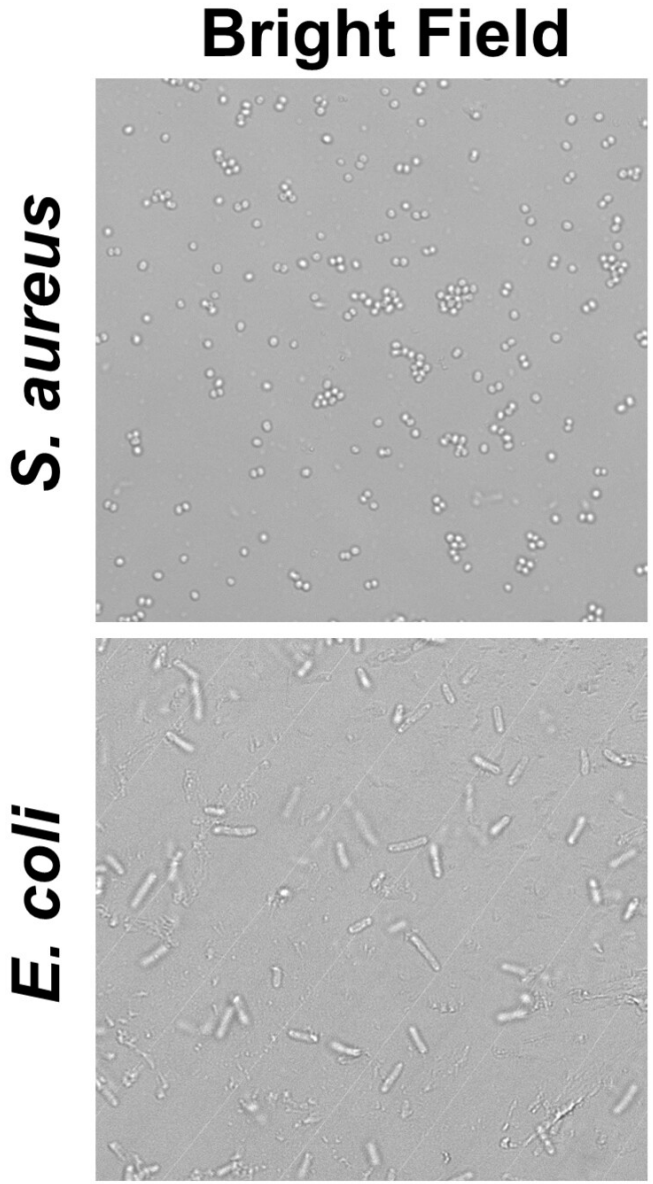


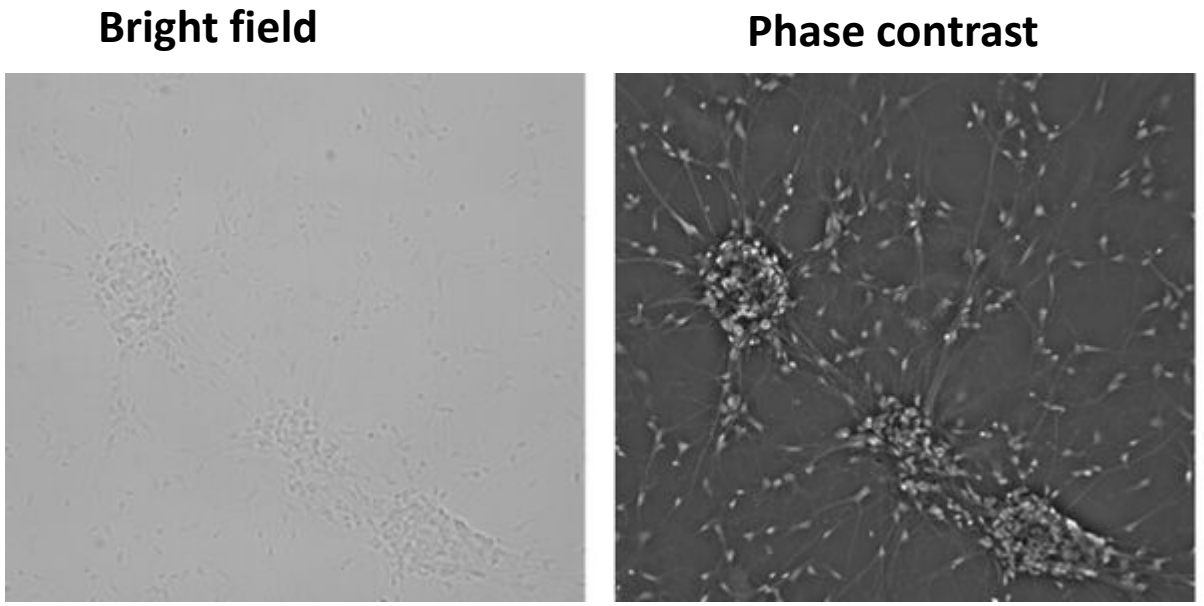
Dark field illumination, sample contrast comes from light scattered by the sample



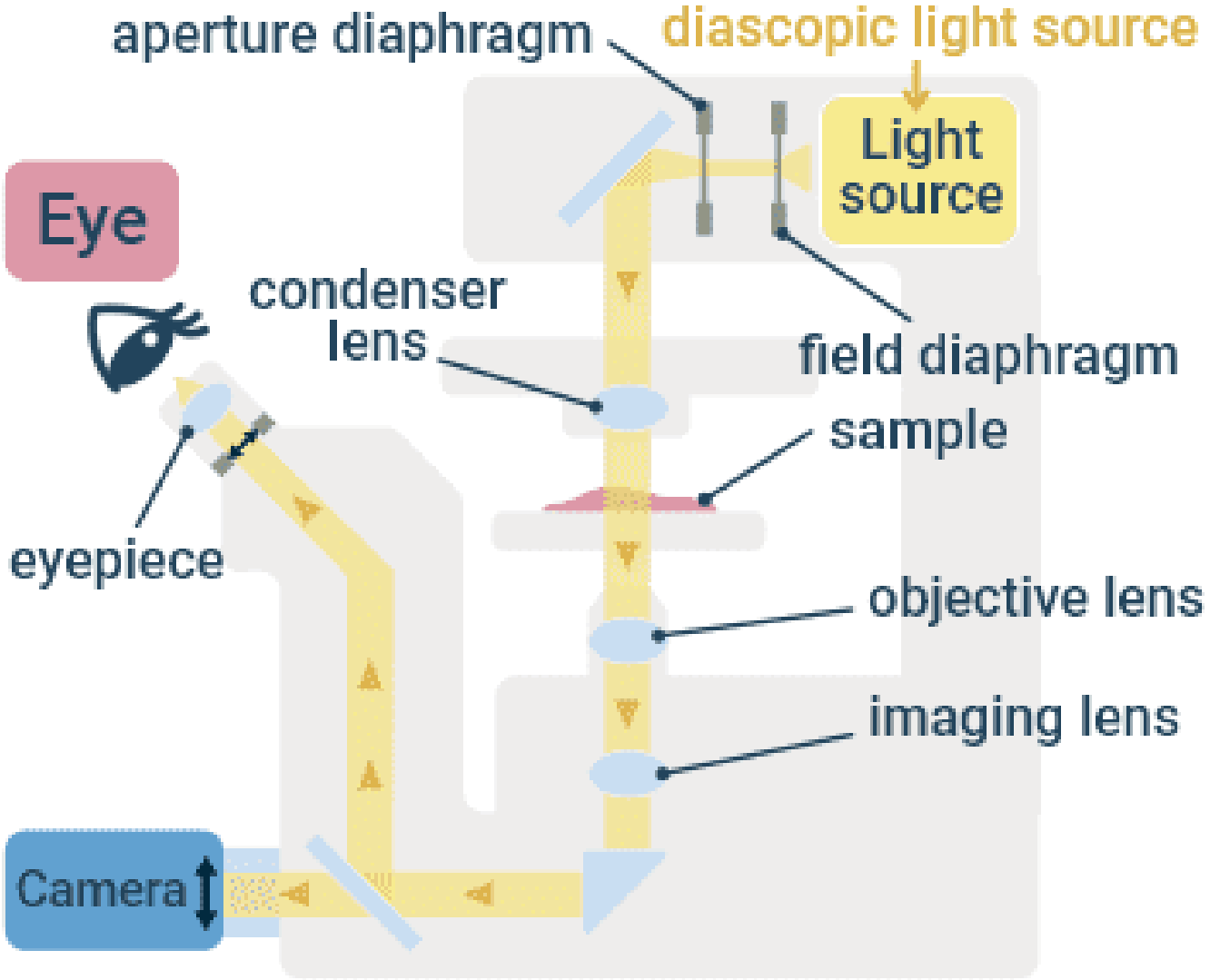
Phase contrast illumination, sample contrast comes from interference of different path lengths of light through the sample

Brightfield microscopy



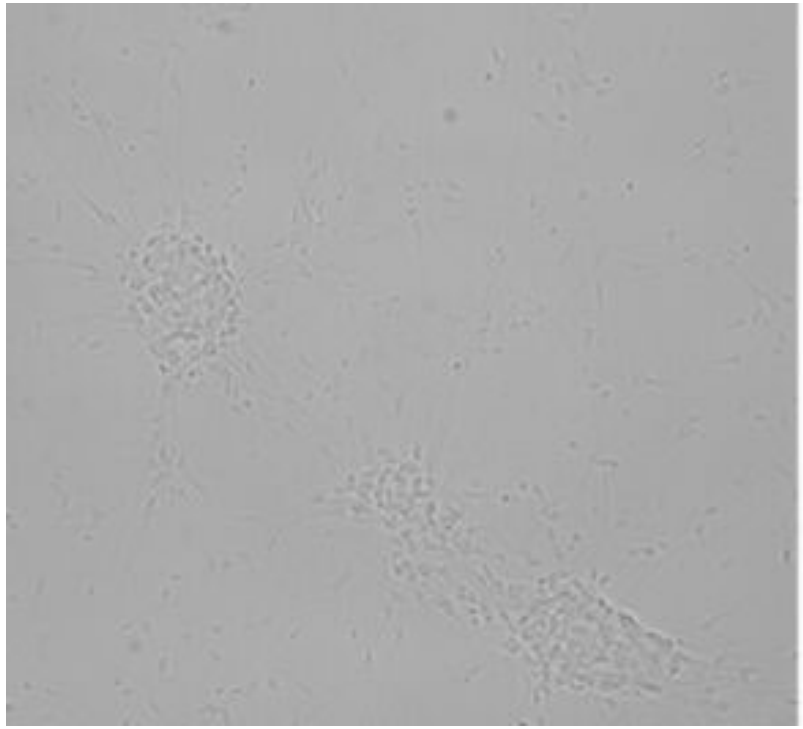


Brightfield light path

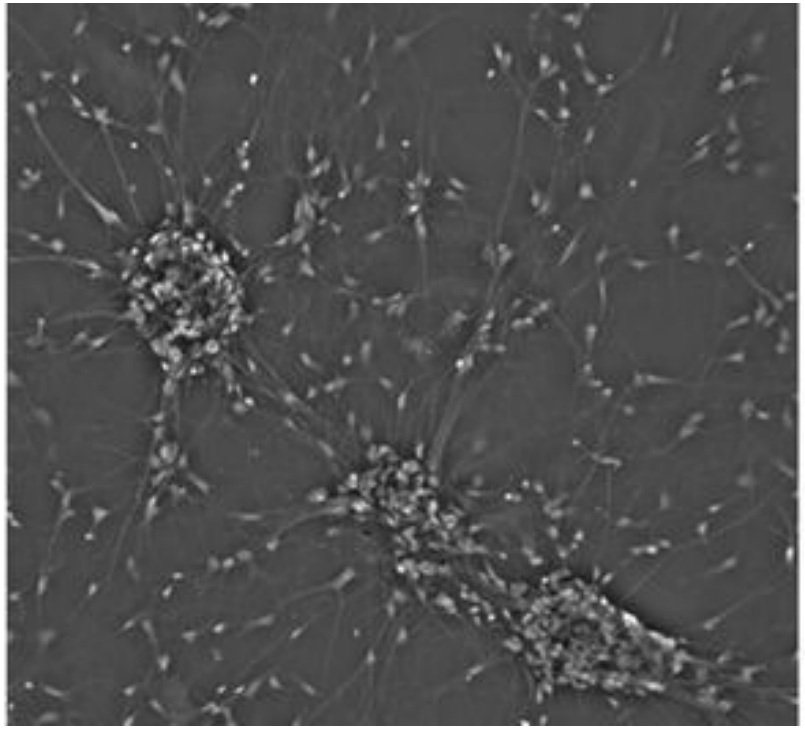


Fluorescence Microscopy

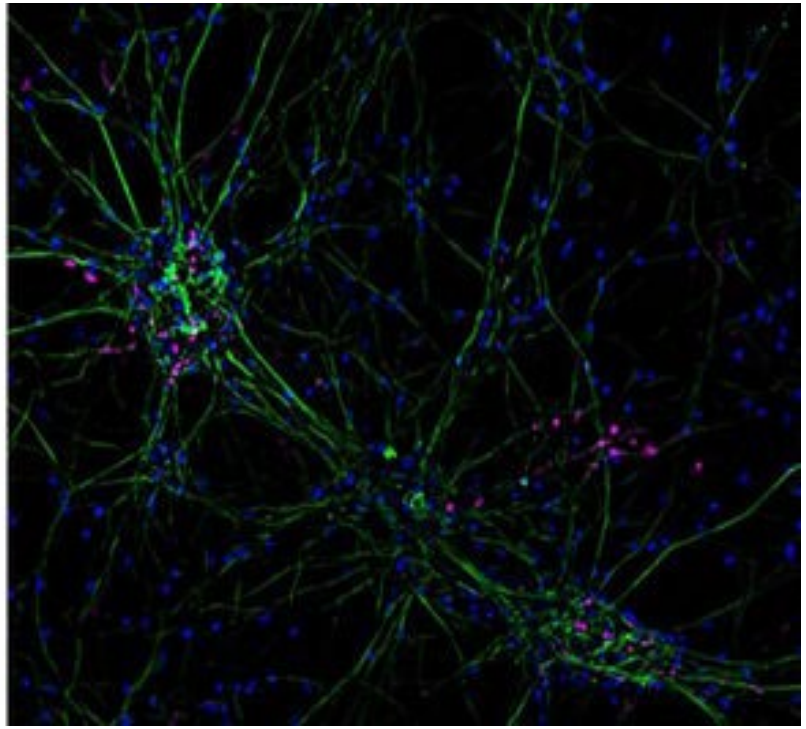
Bright field



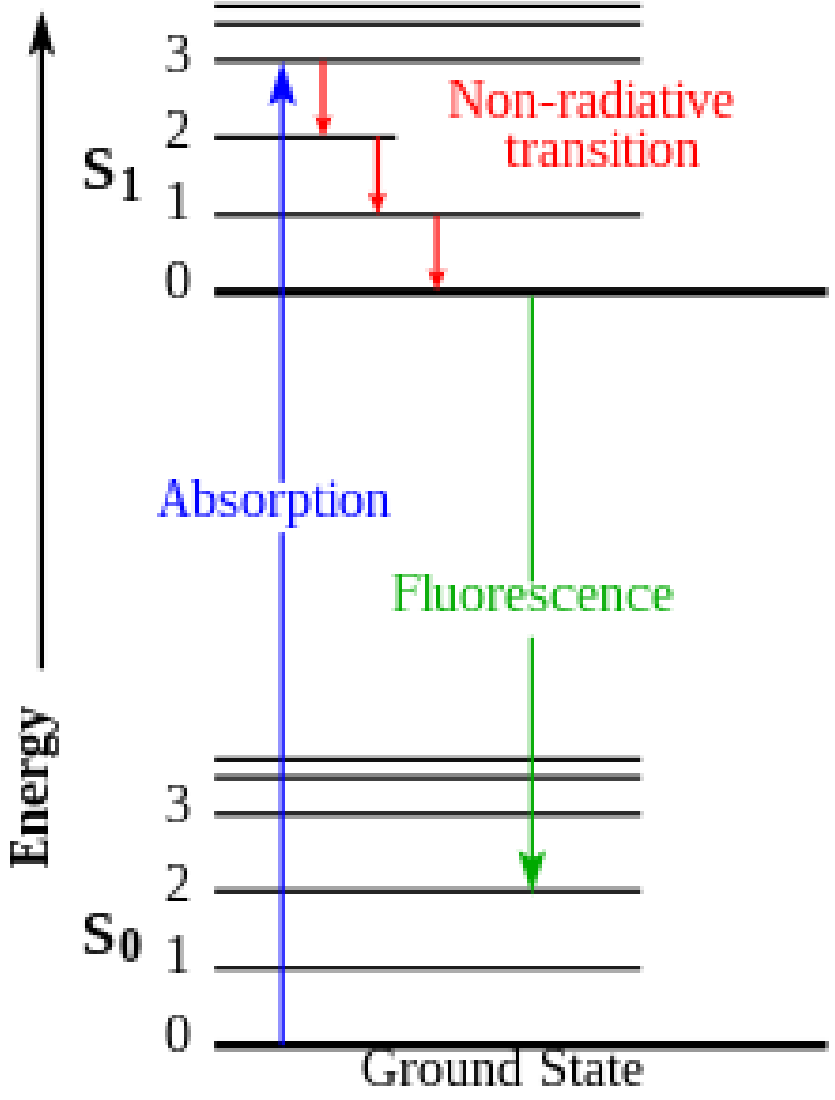
Phase contrast



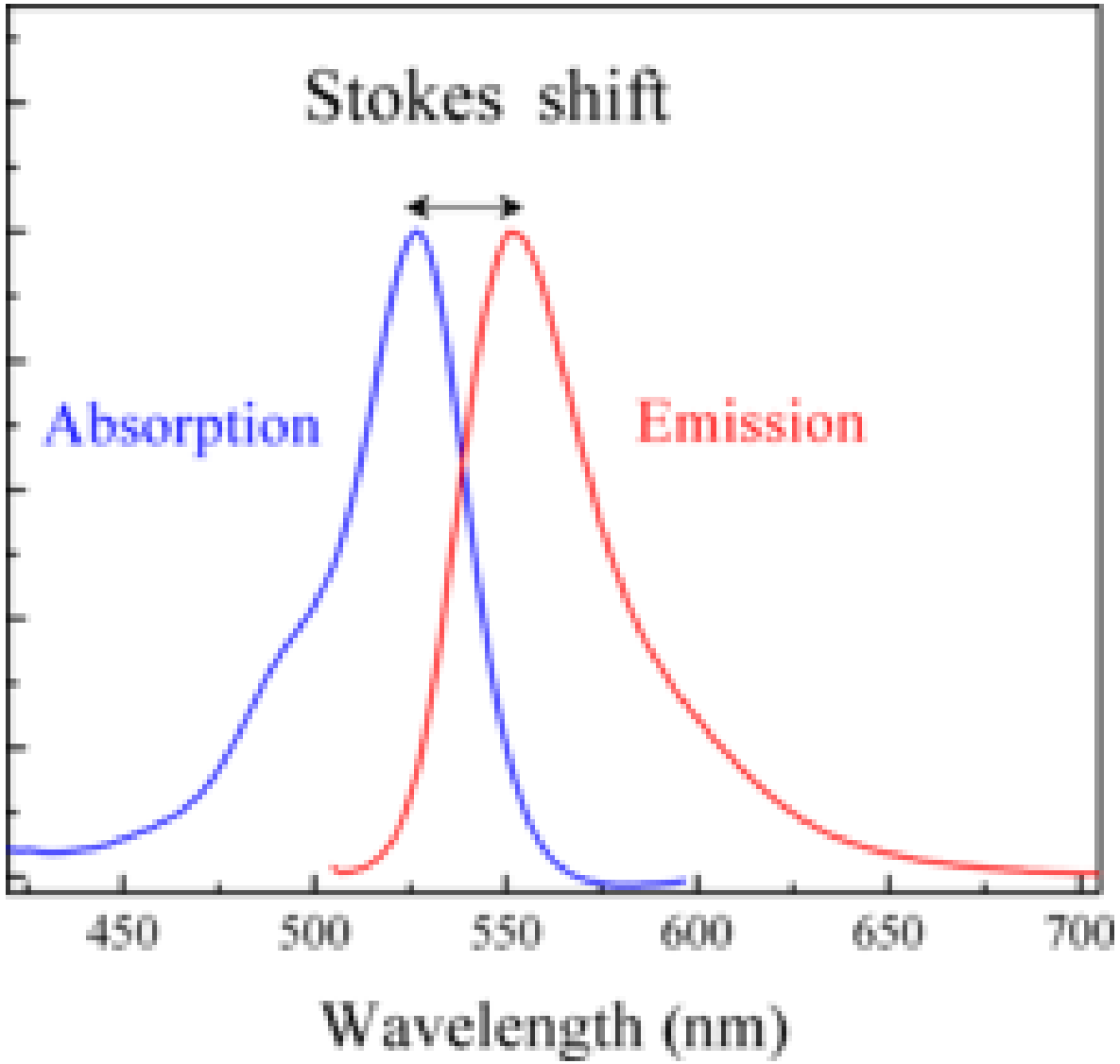
Fluroescence



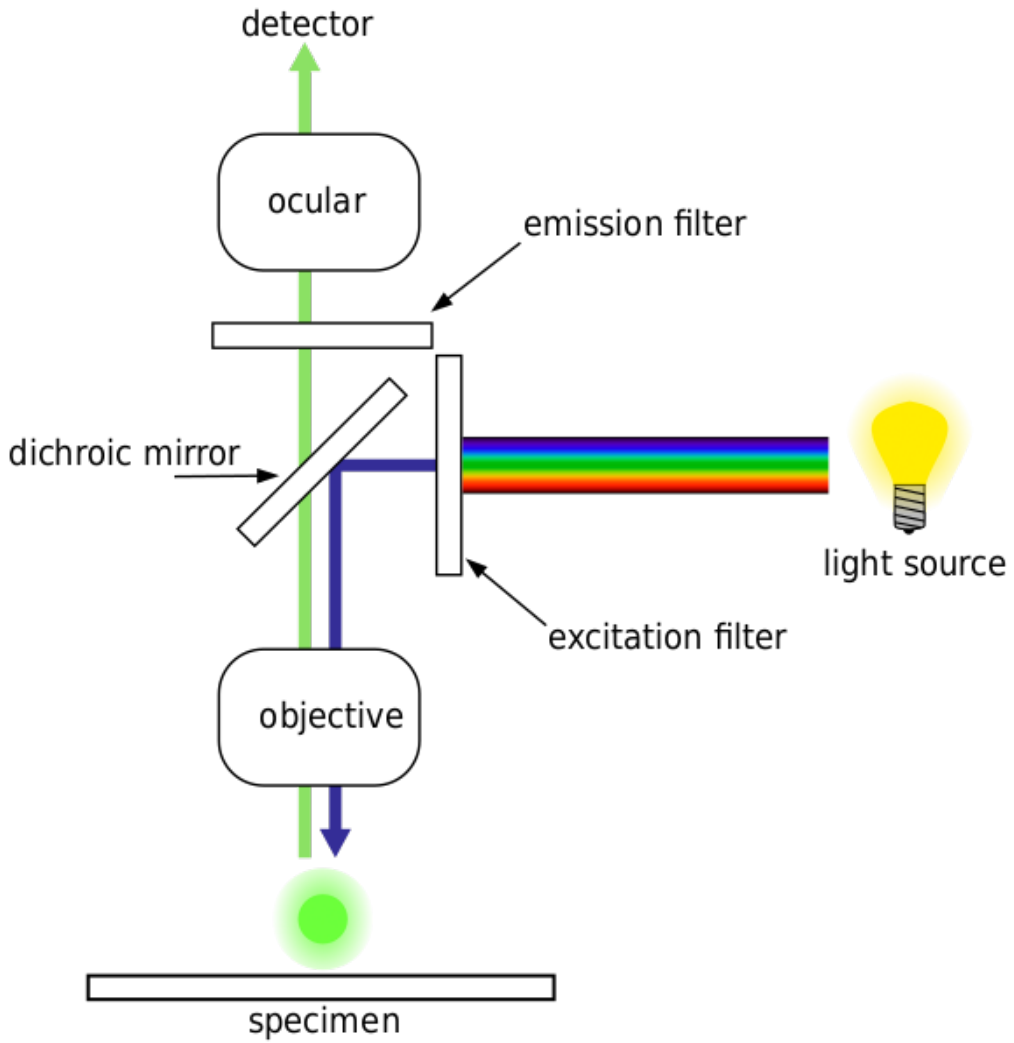
Fluorescence Microscopy

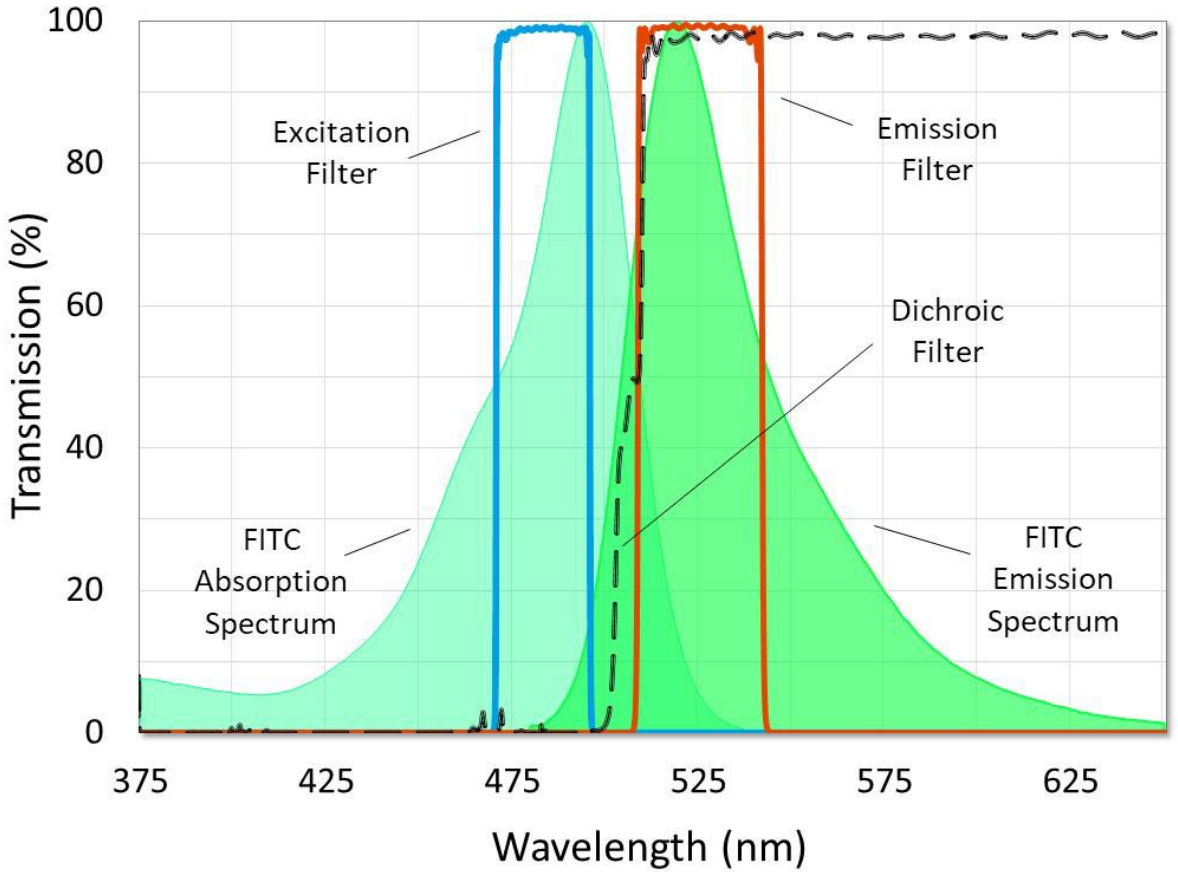


Rhodamine 6g

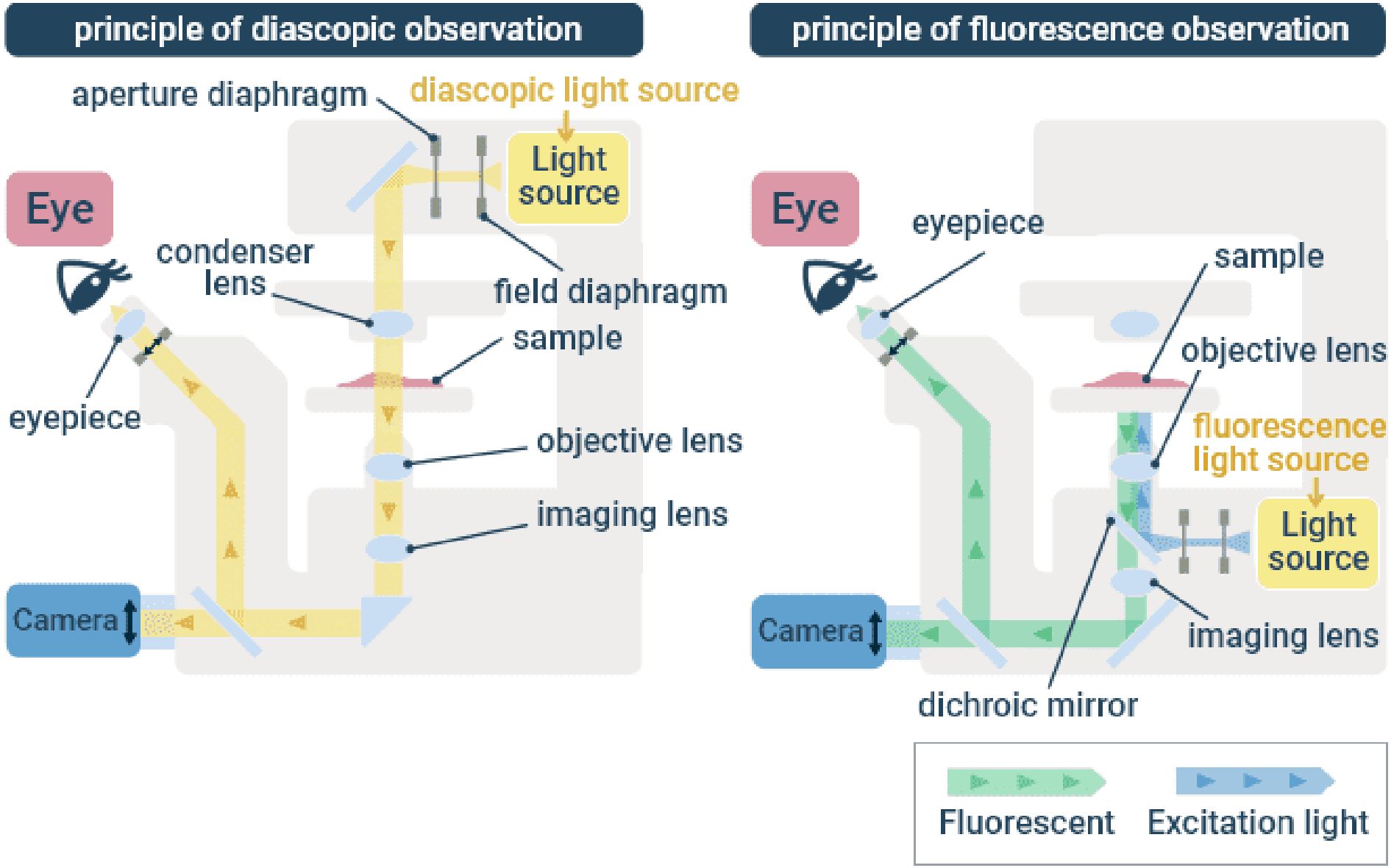


Fluorescence filter cubes

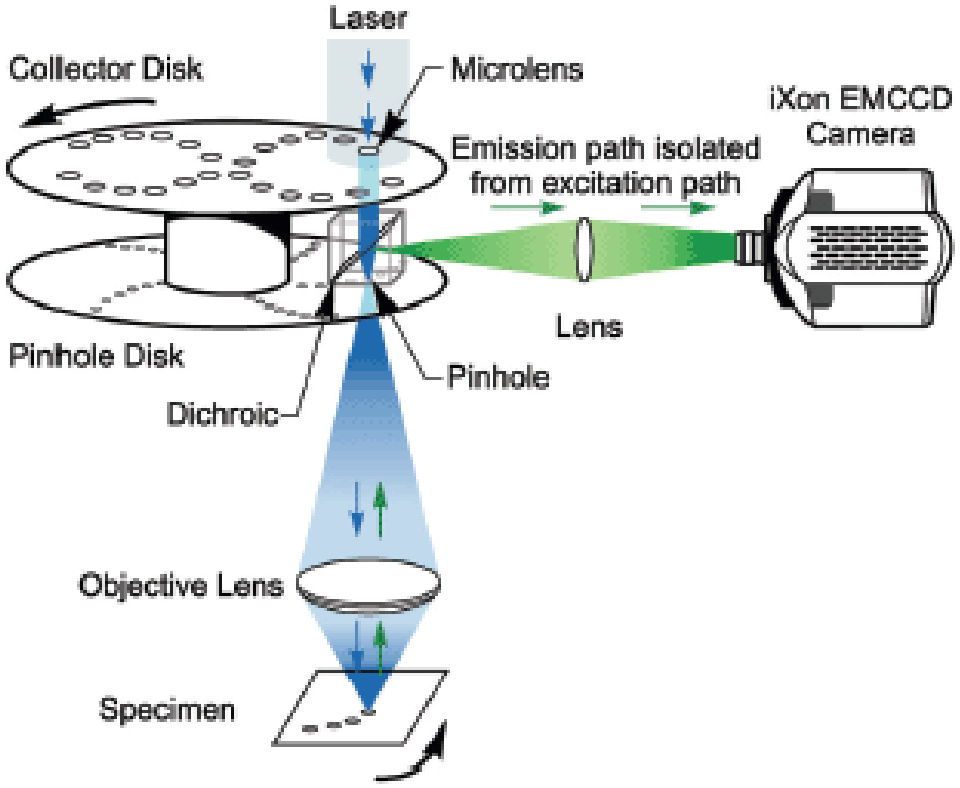


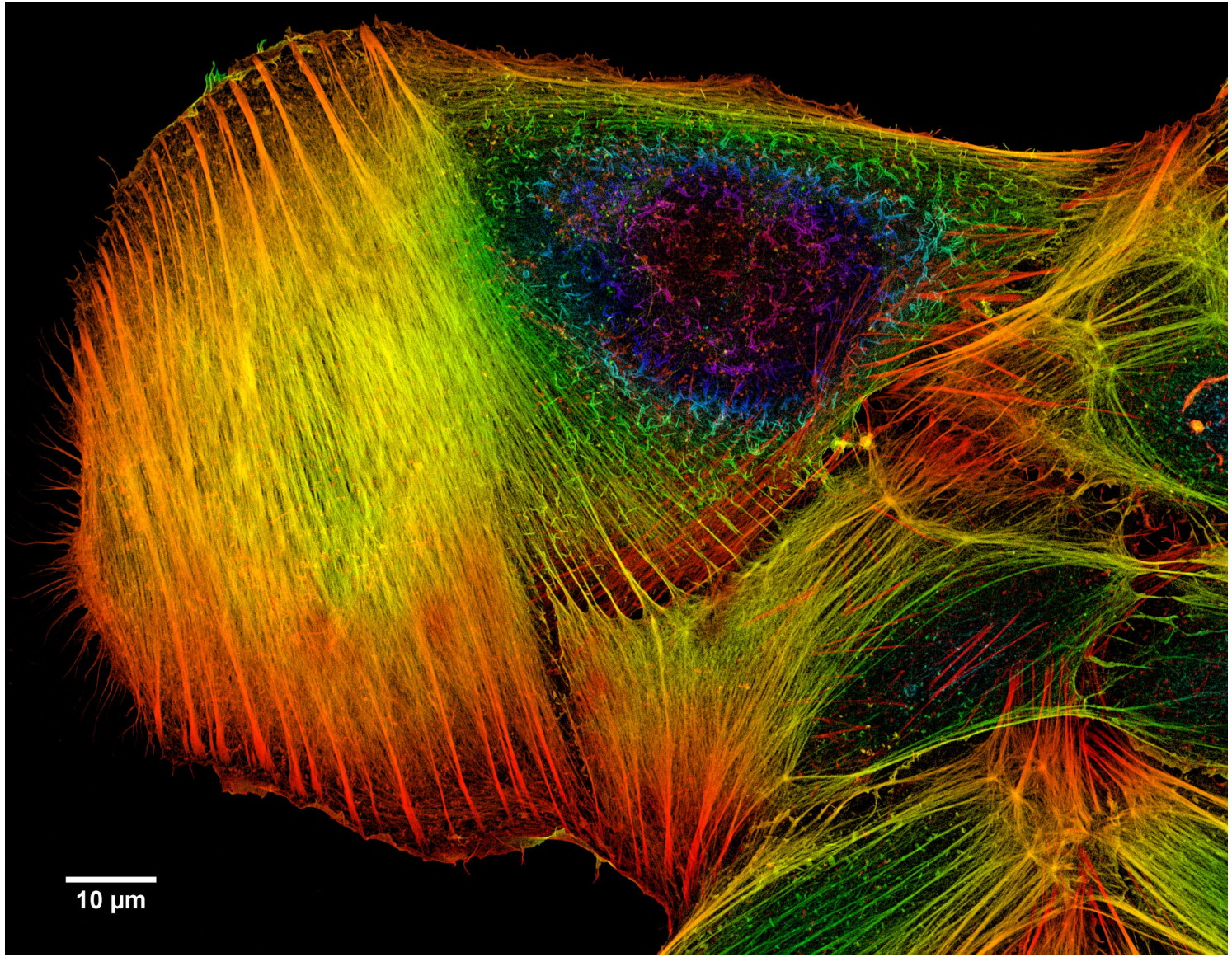


Fluorescence light path



Confocal Microscopy

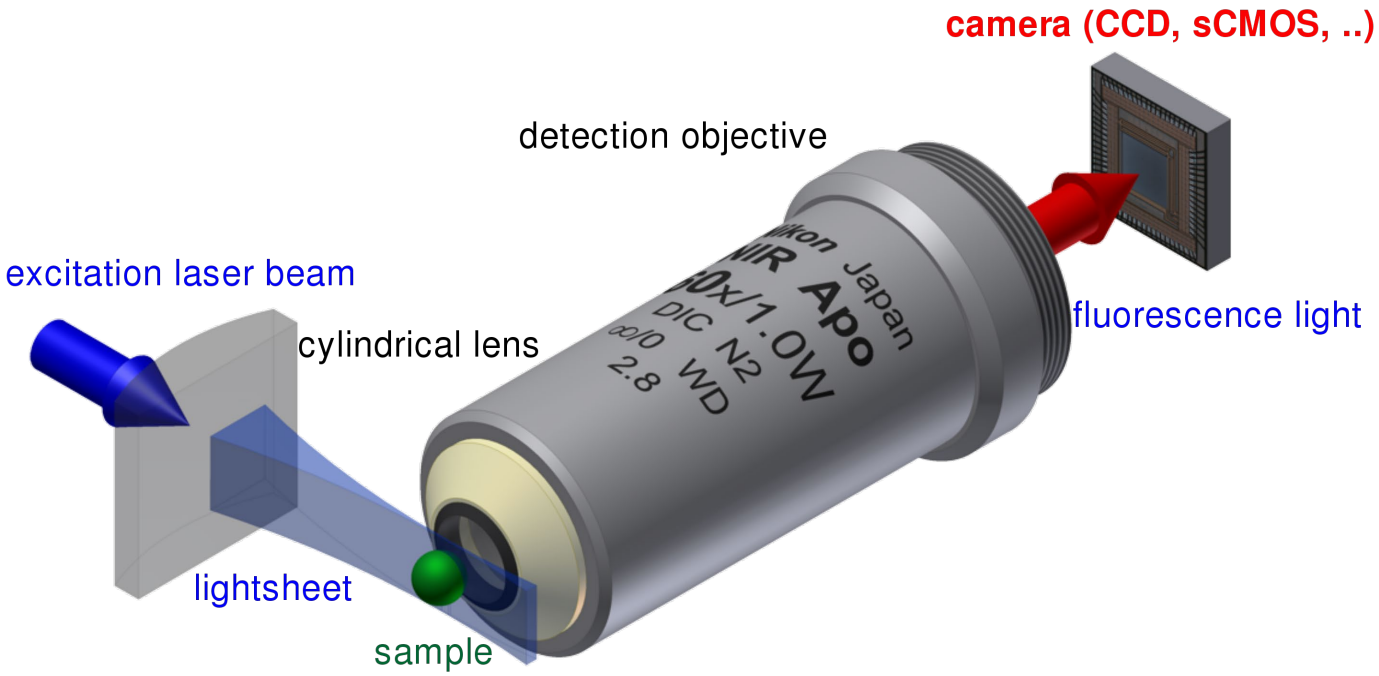




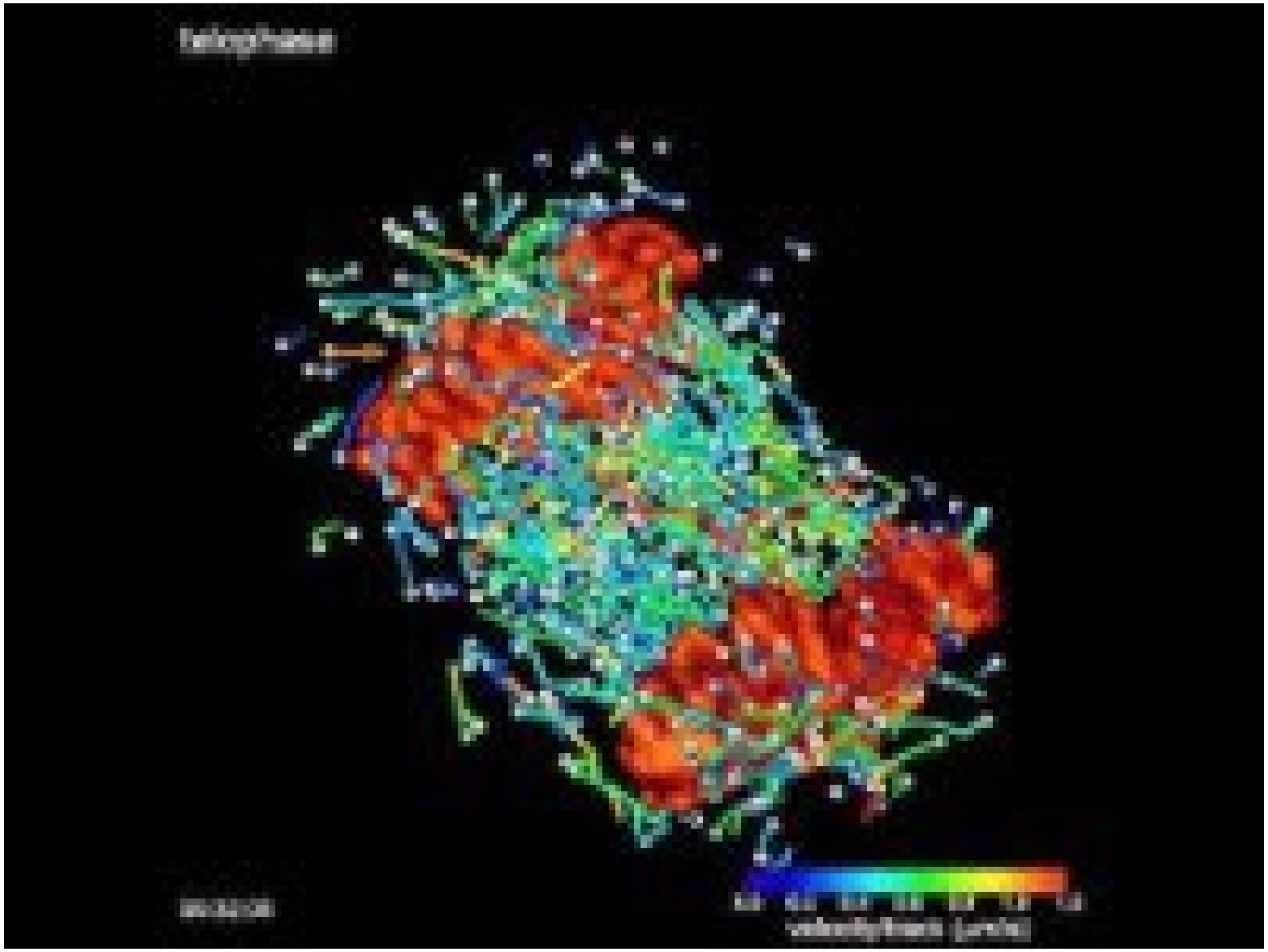
Confocal Microscopy

https://en.wikipedia.org/wiki/Confocal_microscopy

Light-sheet microscopy



Light-sheet microscopy



Super Resolution Microscopy (Nobel Chem 2017)

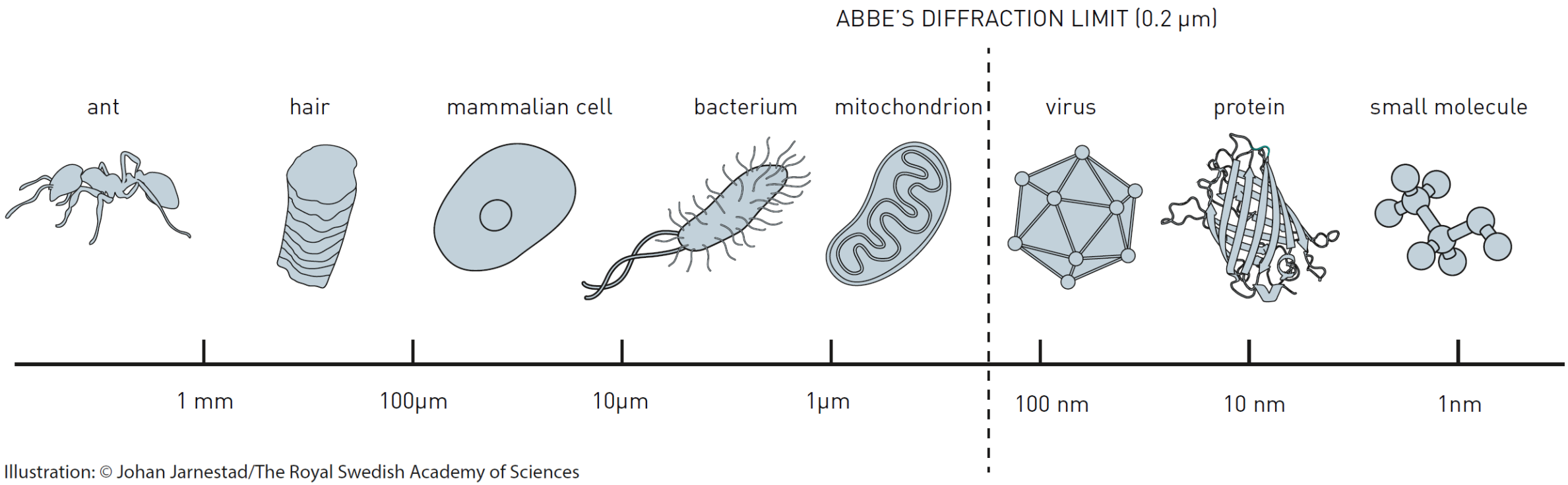
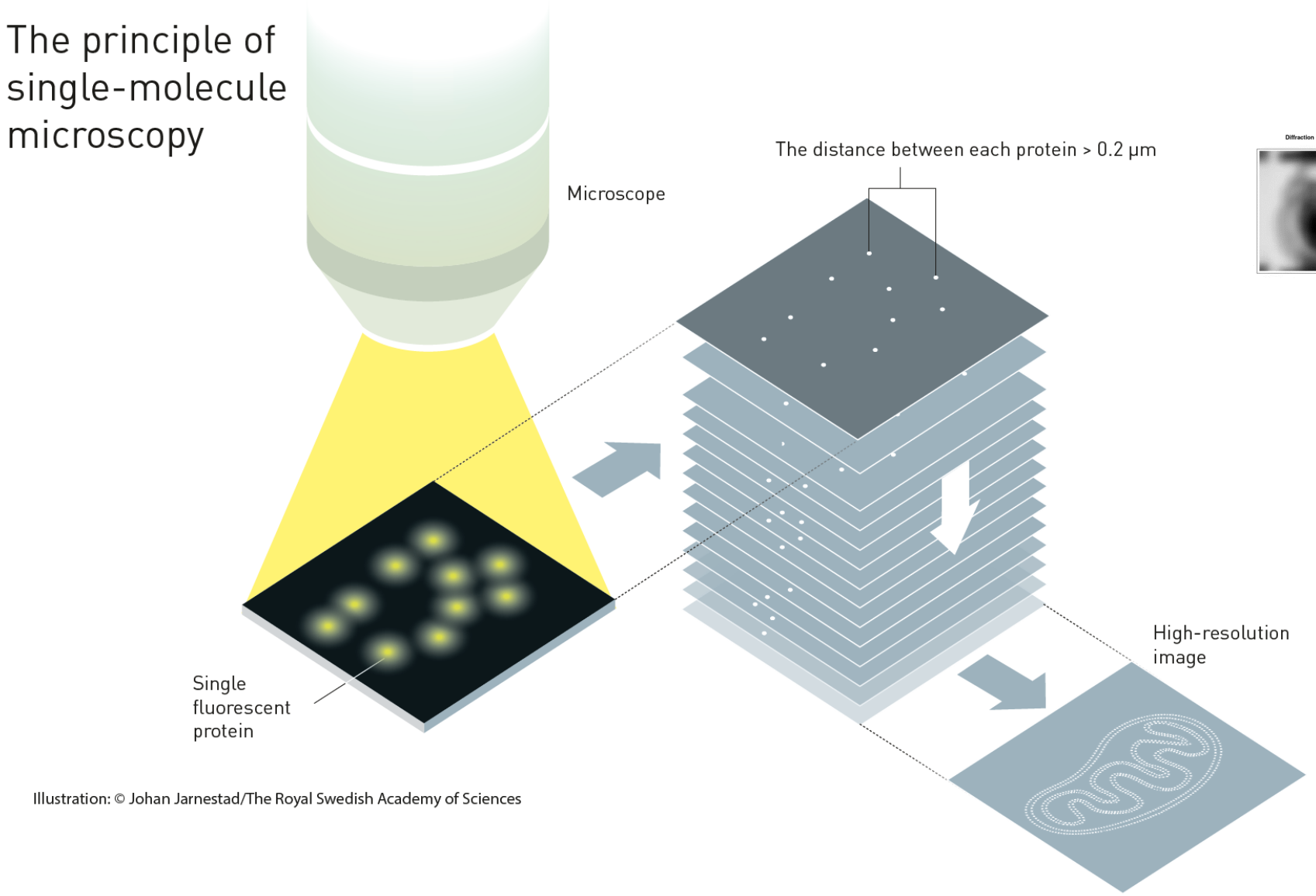
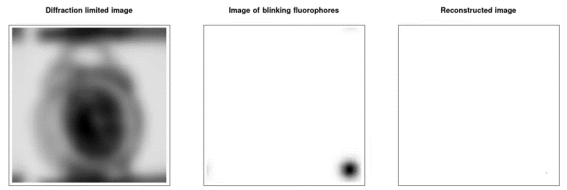


Illustration: © Johan Jarnestad/The Royal Swedish Academy of Sciences

Super Resolution Microscopy (Nobel Chem 2017)





Super Resolution Microscopy (Nobel Chem 2017)

Diffraction limited image

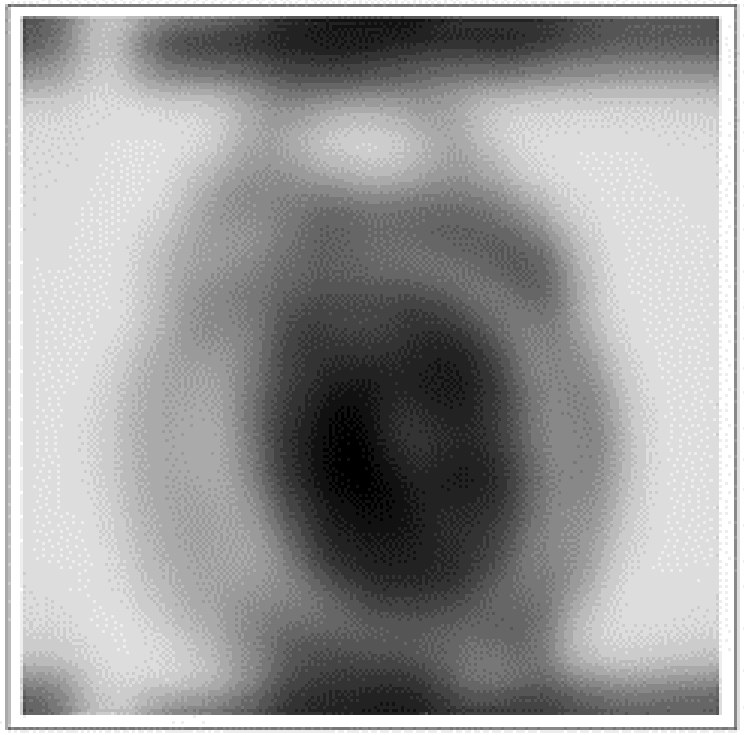
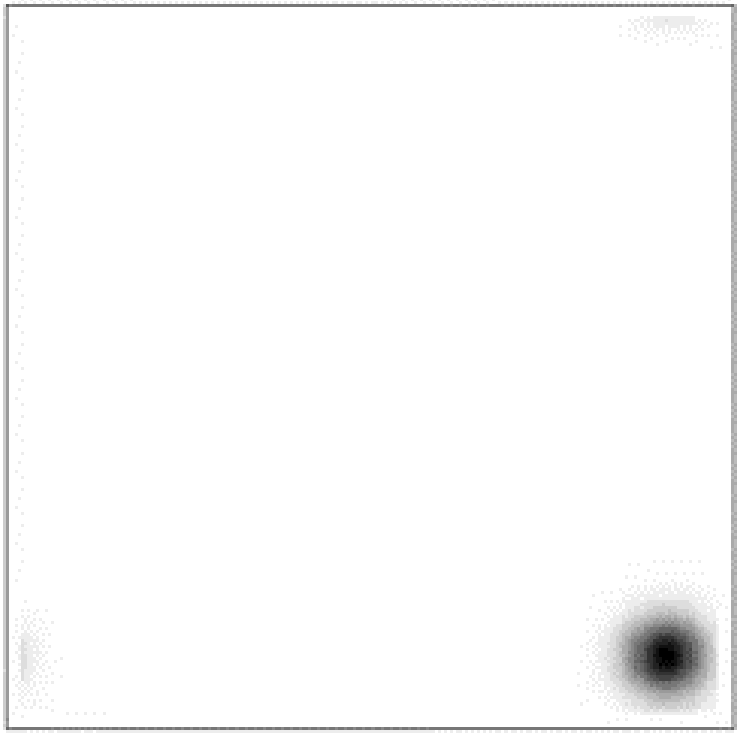
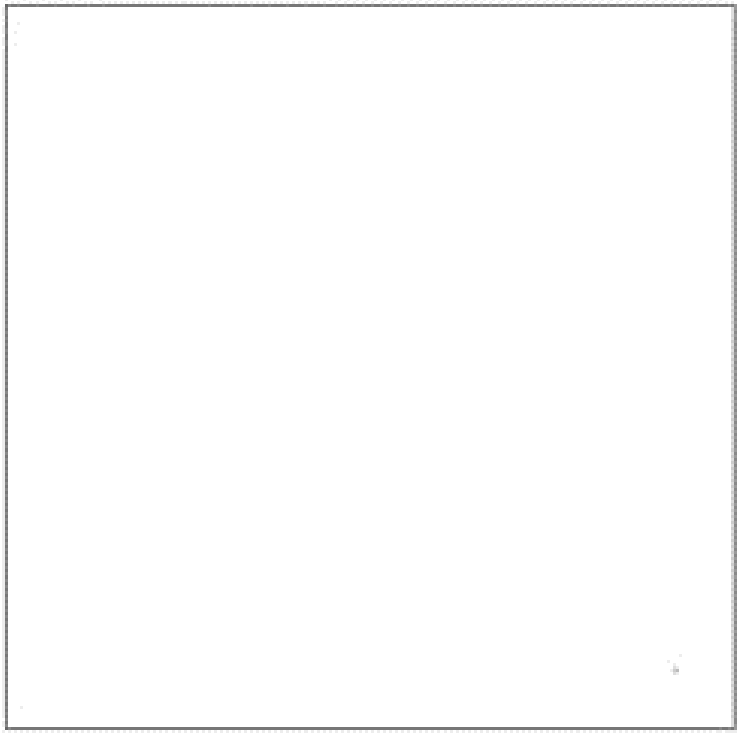


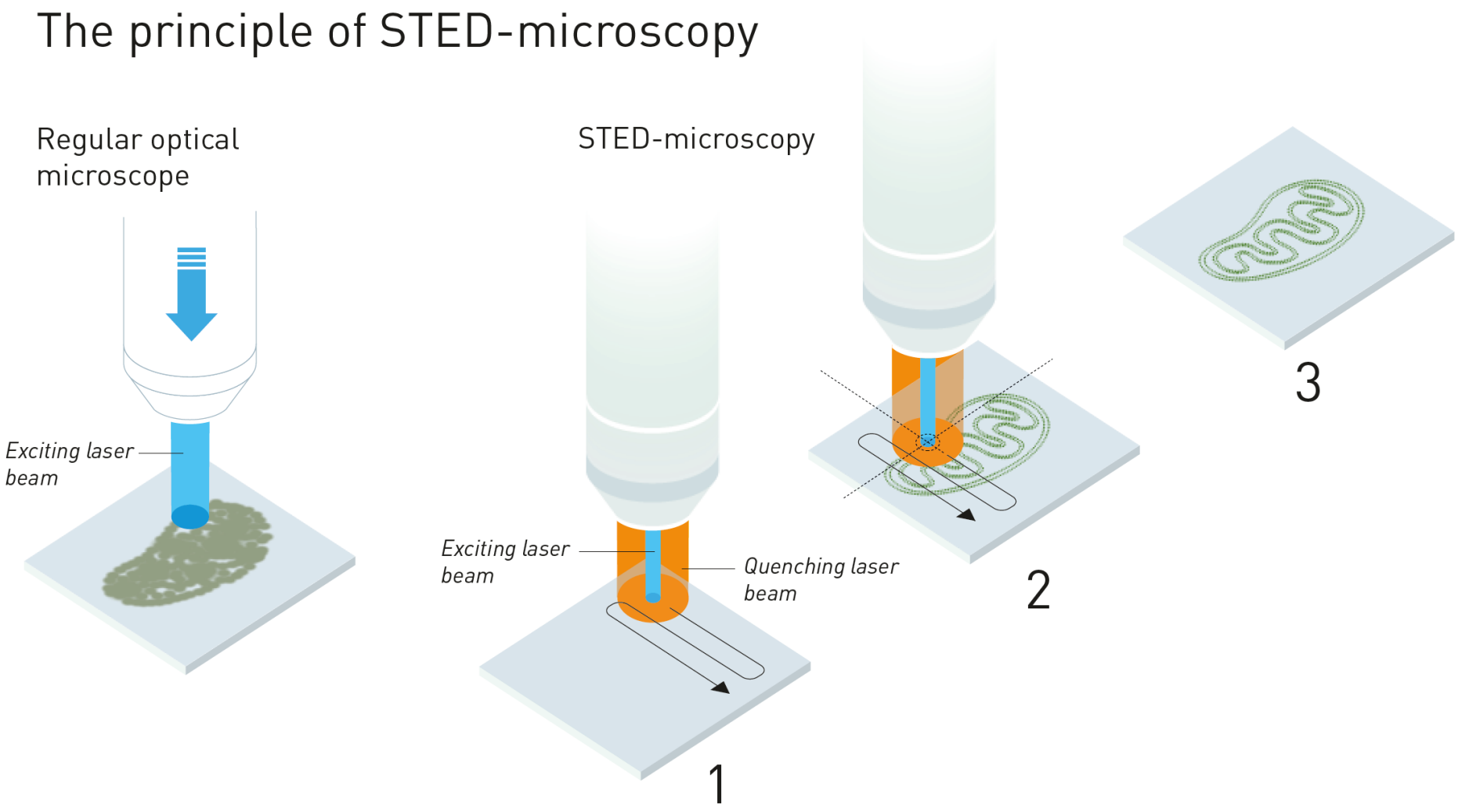
Image of blinking fluorophores



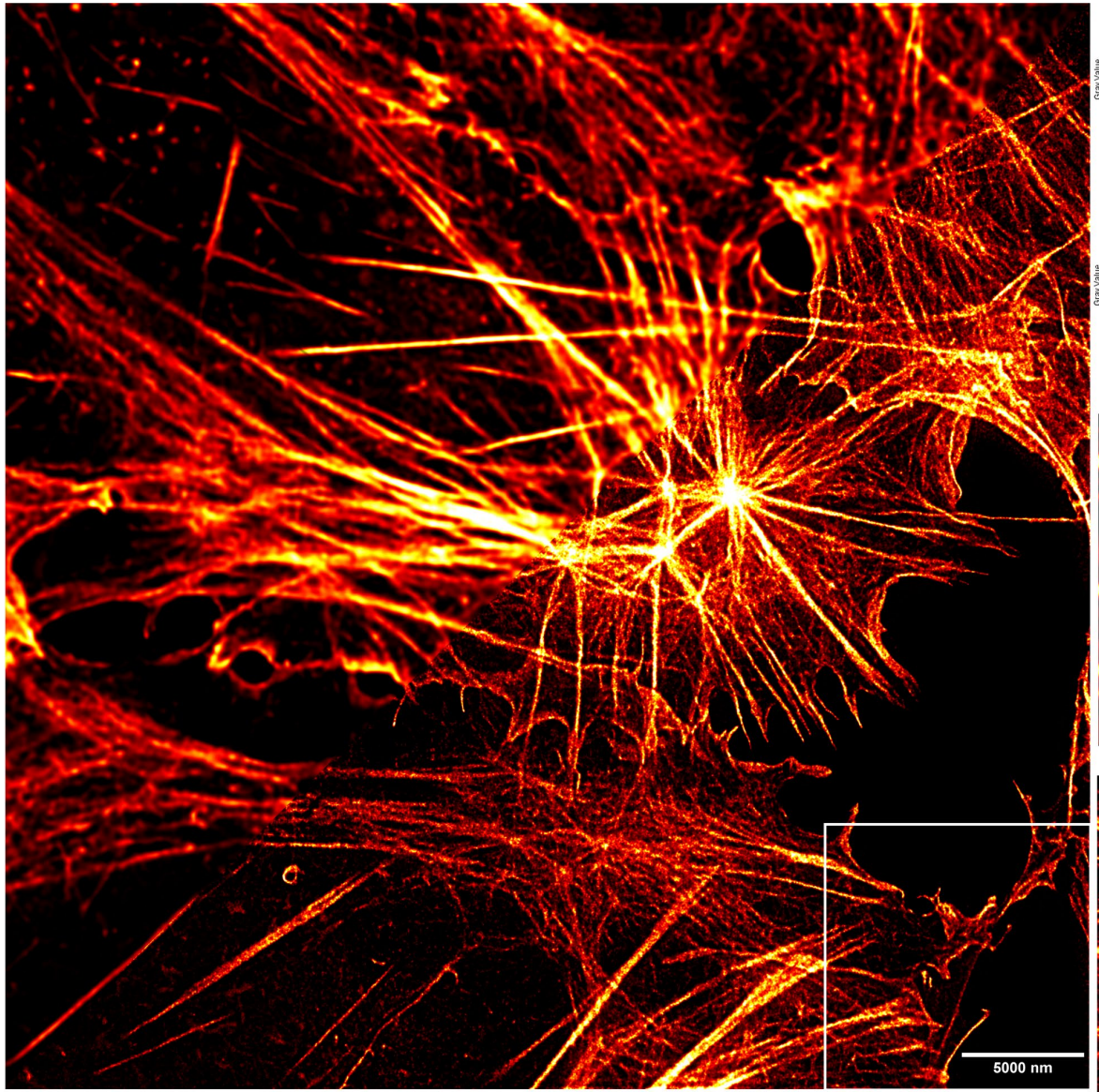
Reconstructed image

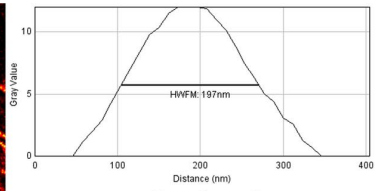


Super Resolution Microscopy: STED
Stimulated emission depletion

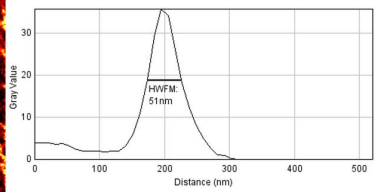


Super Resolution Microscopy





Confocal



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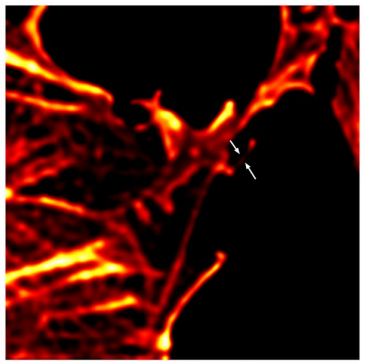
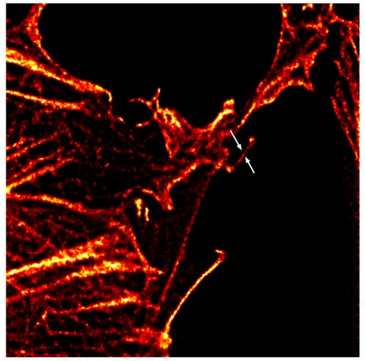
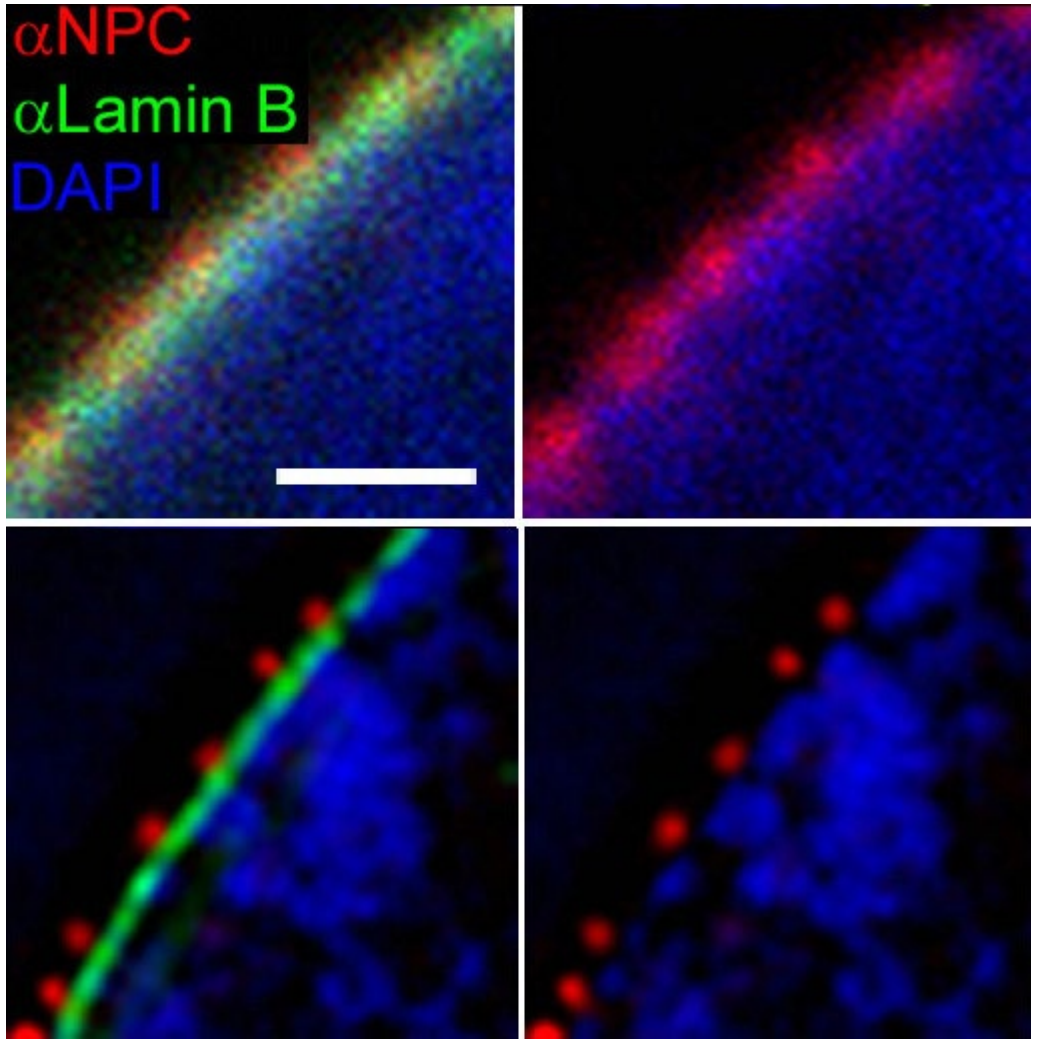


Confocal



STED

Super Resolution Microscopy

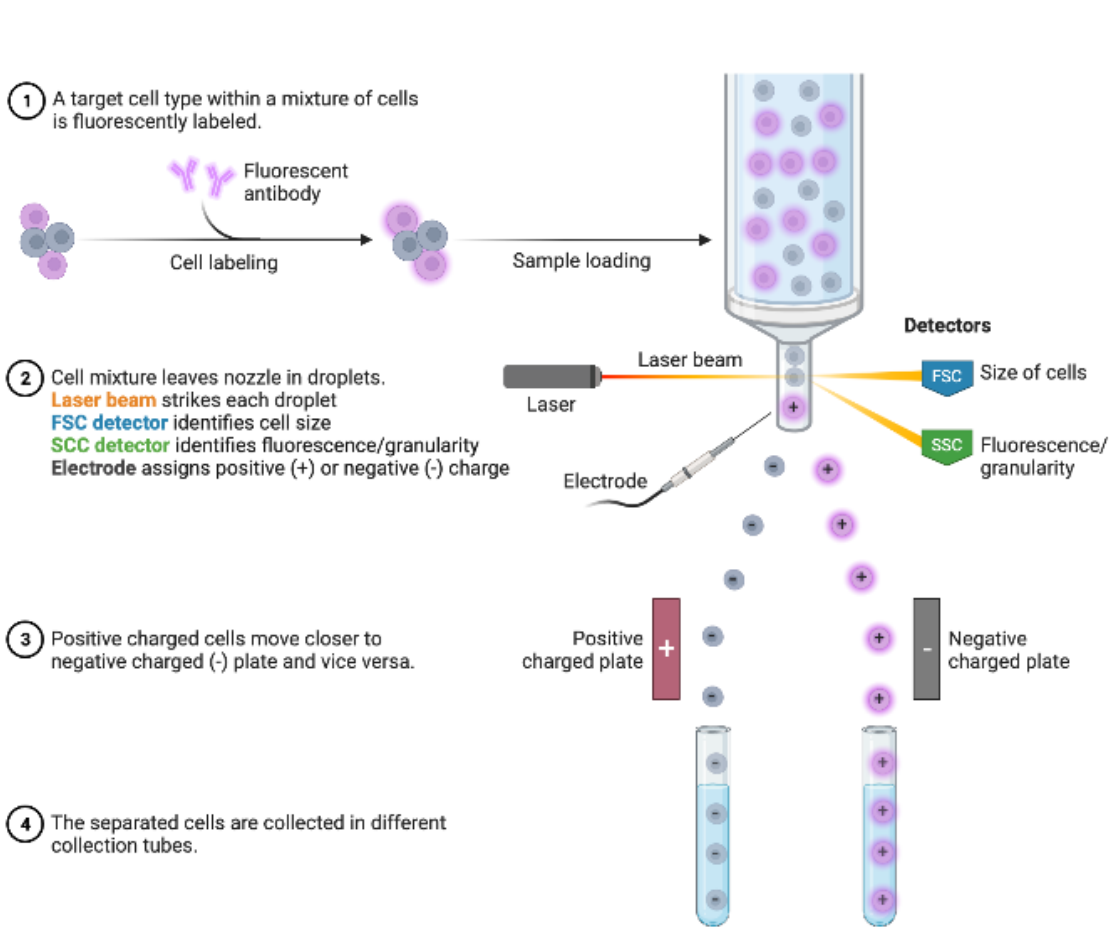


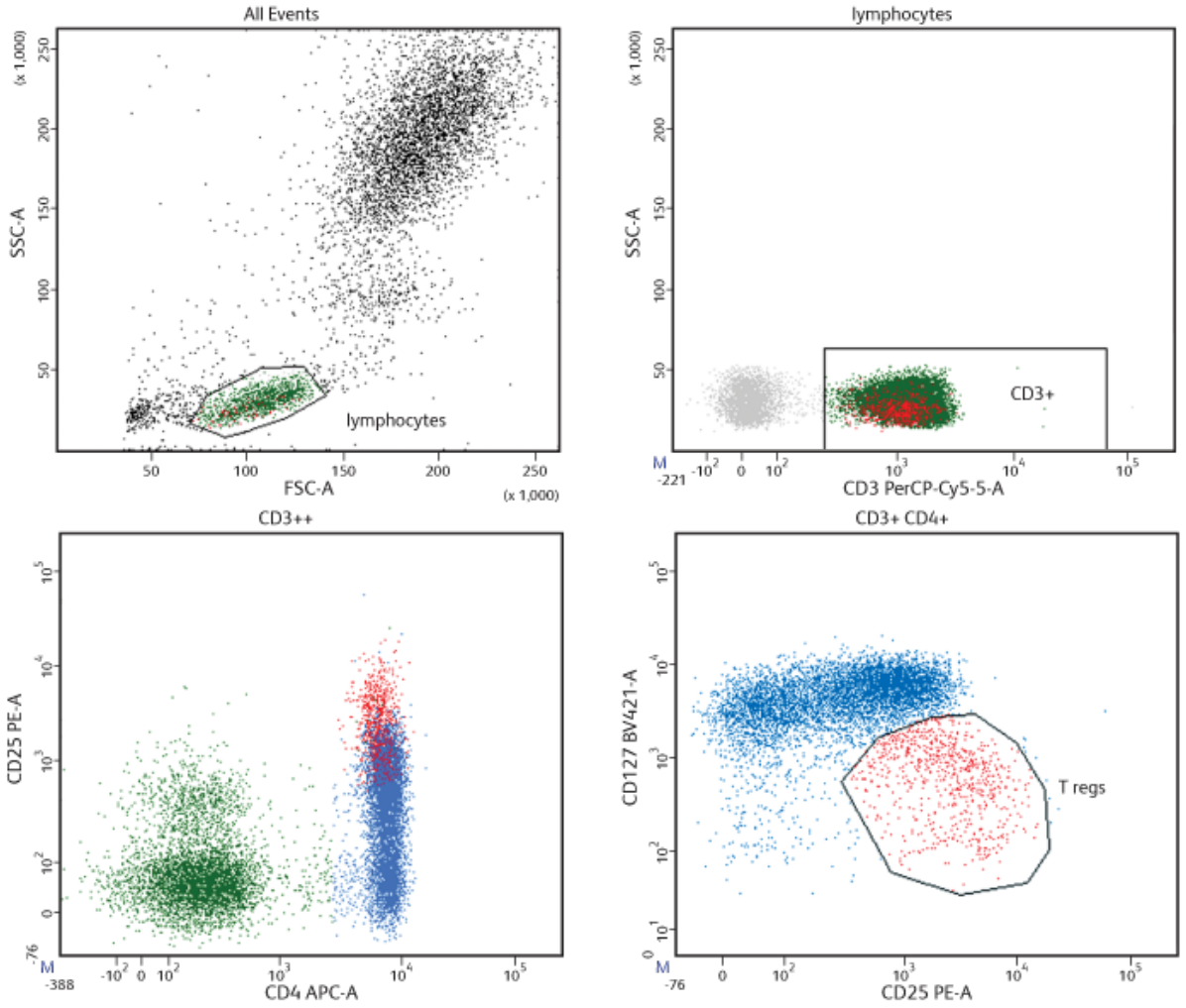
Comparison of the resolution obtained by confocal laser scanning microscopy (top) and 3D structured illumination microscopy (3D-SIM-Microscopy, bottom). Shown are details of a nuclear envelope. Nuclear pores (anti-NPC) red, nuclear envelope (anti-Lamin) green, chromatin (DAPI-staining) blue. Scale bar: 1µm.

FACS

Fluorescence-activated cell sorting (FACS)

Fluorescence-activated cell sorting (FACS)





Fluorescence-activated cell sorting (FACS)

Flow cytometry and FACS (fluorescence-activated cell sorting) have several critical clinical applications.

Immunophenotyping - Immunophenotyping is the most common application of flow cytometry and FACS. This technique allows researchers to identify and quantify multiple populations of cells in a heterogeneous sample such as bone marrow, peripheral blood and lymph material. Immunophenotyping is widely used in hematological labs to diagnose hematological malignancies such as lymphomas and leukemia.

Cell Sorting - Researchers use flow cytometry and FACS to select specific cells of interest from a mixed population and physically isolate them into separate collection tubes. This technique is widely used to isolate stem cells, tumor cells, transfected cells, and lymphocyte subpopulations for further analysis. This application is useful for noninvasive patient monitoring, clinical studies, personalization medicine, and non-clinical research.

Cell Cycle Analysis - Flow cytometry and FACS can be used to determine proliferation, viability and cell death mechanisms as well as the cell cycle phase. Researchers use these cell-based assays to identify cell anomalies with the help of certain fluorescent dyes. Cell cycle analysis provides crucial information in many different research areas.

Cell Proliferation Assays - In cell biology, cell proliferation assays are used as a tool for measuring cellular metabolic activity in response to specific stimuli such as cytokines, growth factors, and assorted media components. On being activated, the cells begin to proliferate and undergo mitosis. As the cells divide, each of the daughter cells inherits half of the original dye. Researchers are able to calculate cellular activation and proliferation by measuring the reduction of the fluorescence signal.

Serological Testing - Flow cytometry and FACS is more sensitive than traditional immunoassays in serological testing. They can measure multiple antibodies at the same time and allow for higher throughput.

Intracellular Calcium Flux - Researchers use flow cytometry and FACS to detect and measure the flux of calcium into the cell.

Apoptosis - Flow cytometry and FACS is also used to determine the mechanisms of cell death. Identifying the morphological, molecular, and biochemical changes occurring in dying cells allows researchers to differentiate between necrosis and early apoptosis.

Drug Development - Flow cytometry and FACS are widely used as an invaluable tool in drug development research.