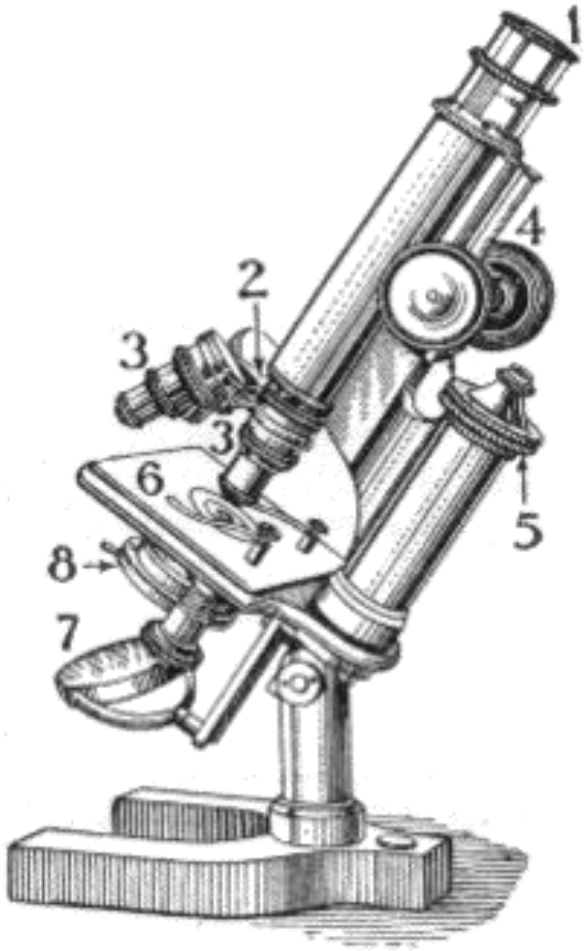


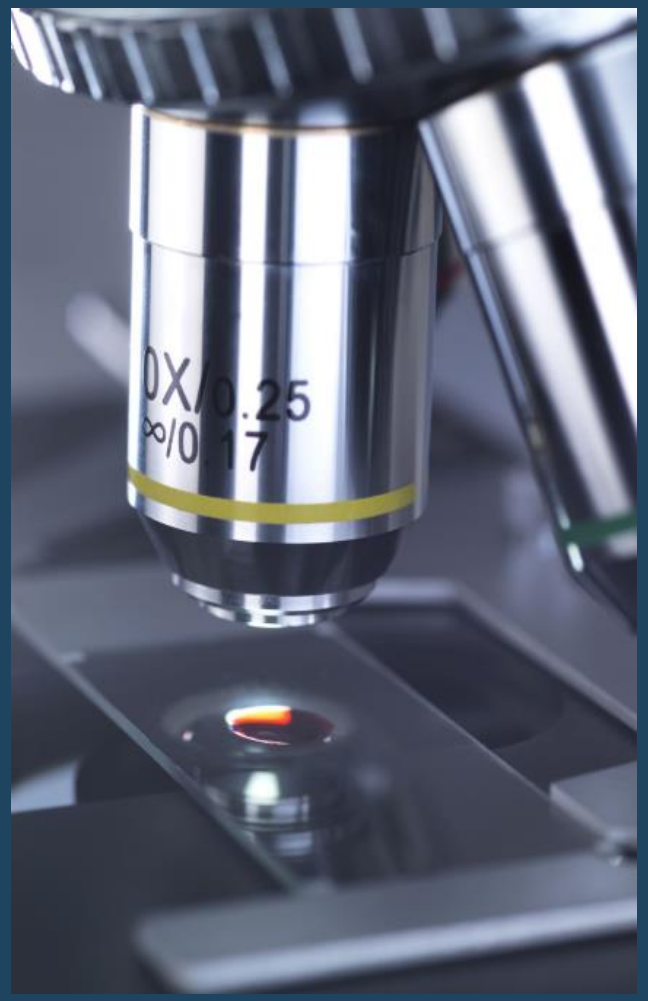
BIOLOGICAL IMAGING AND SUBCELLULAR ORGANISMS

MVDr. Jana Hložková, Ph.D

- ✓ The main method for obtaining new data is observation, for which it is possible to use all our senses (primarily sight), the effectiveness can be multiplied in combination **with devices** (magnifying glass, microscope).

- ✓ Another method of obtaining new data is an experiment,

a research procedure in which the consequences caused by changing one of the factors affecting the object are determined.



MAGNIFYING DEVICE

- Magnifying glass 4x
- Light microscope 1000x

Improvement - Immersion, Dyeing, Phase Contrast (Highlighting Contrast Using Different Sample Rate)

- Electron microscope 1000 000x

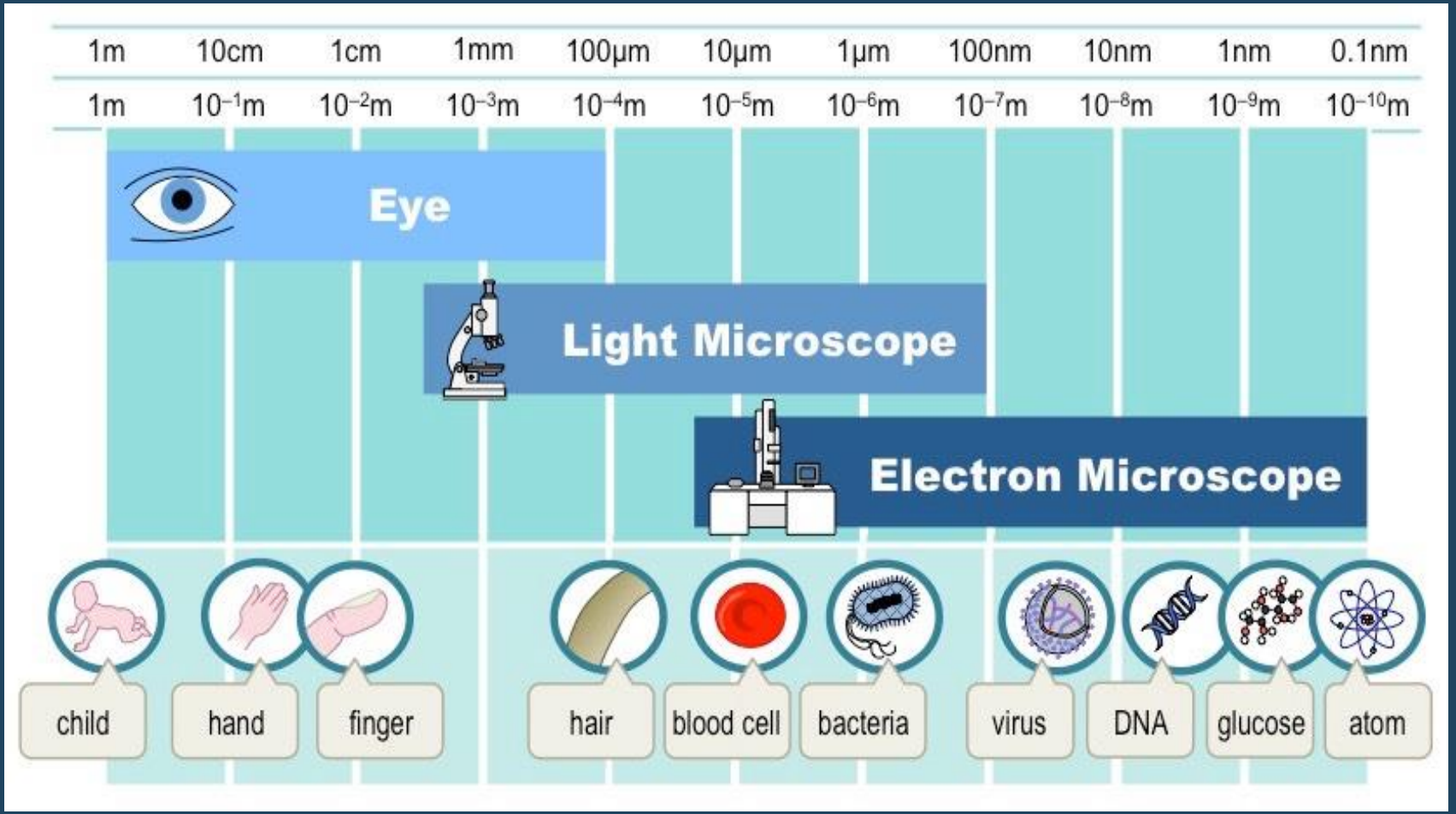
Today - computer, analysis, measurement, calculations, three-dimensional models

ANGULAR RESOLUTION

- The term resolution or minimum resolvable distance is the minimum distance between distinguishable objects in an image,

- Eye 0.2mm
- Light microscope 0.2 µm
- Electron microscope 0.2 nm

Resolution range of the normal eye 0,2mm
Light microscope 0,2 µm
Electron microscope 0,2 nm



Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye)

HISTORY OF MICROSCOPY

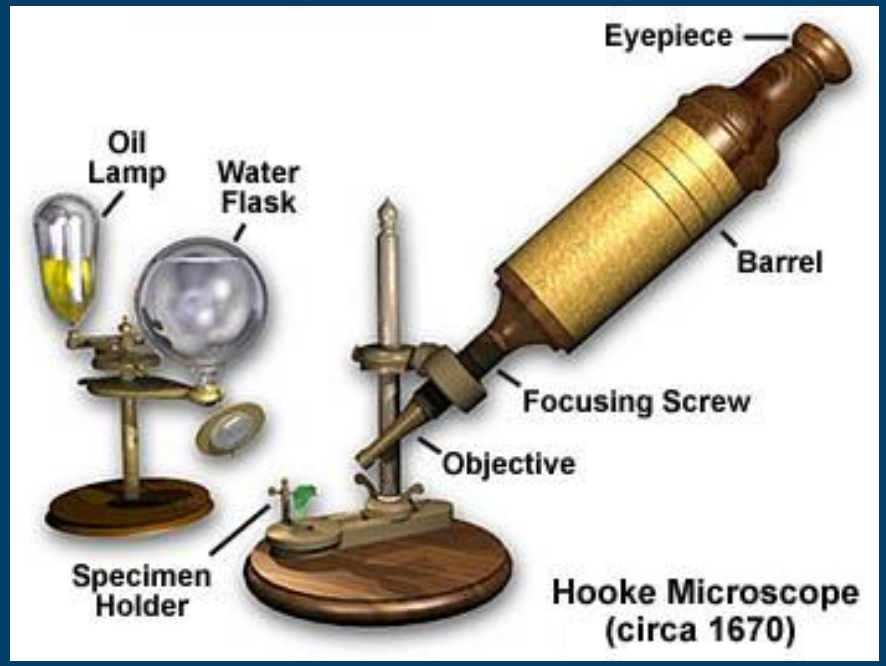
- **Anthoni van Leeuwenhoek.**

best known for his pioneering work in the field of microscopy and for his contributions toward the establishment of microbiology as a scientific discipline.

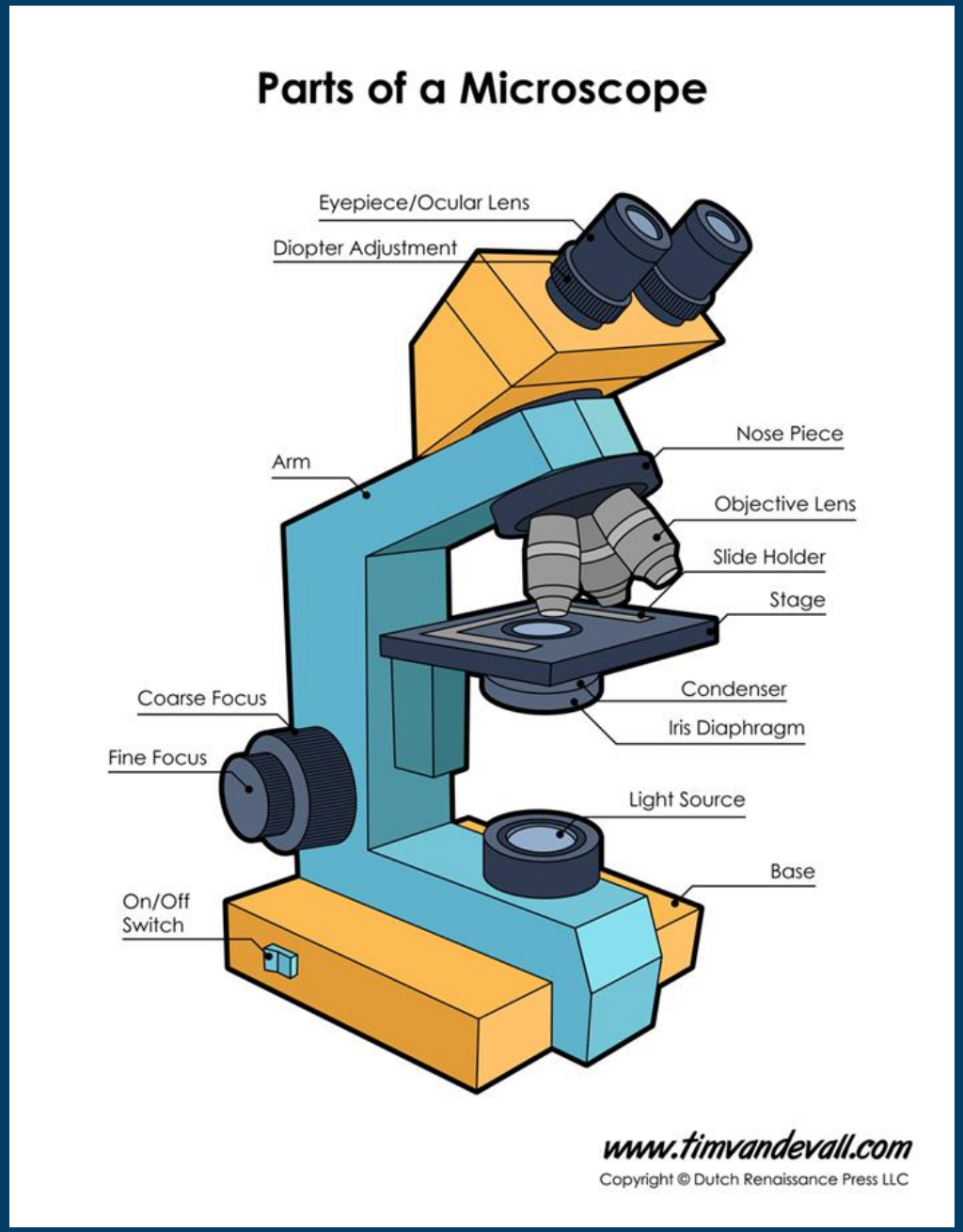
- https://www.youtube.com/watch?v=XgW1HiV9SJs

- **Zacharias Janssen** (1595) magnification 9x

- **Robert Hook** (1665)



https://www.youtube.com/watch?v=TJyOQmdwHhE



Optical part - objectives, eyepieces.

Lighting part - light source, condenser, diaphragma, filters, mirror (in current microscopes built into the base).

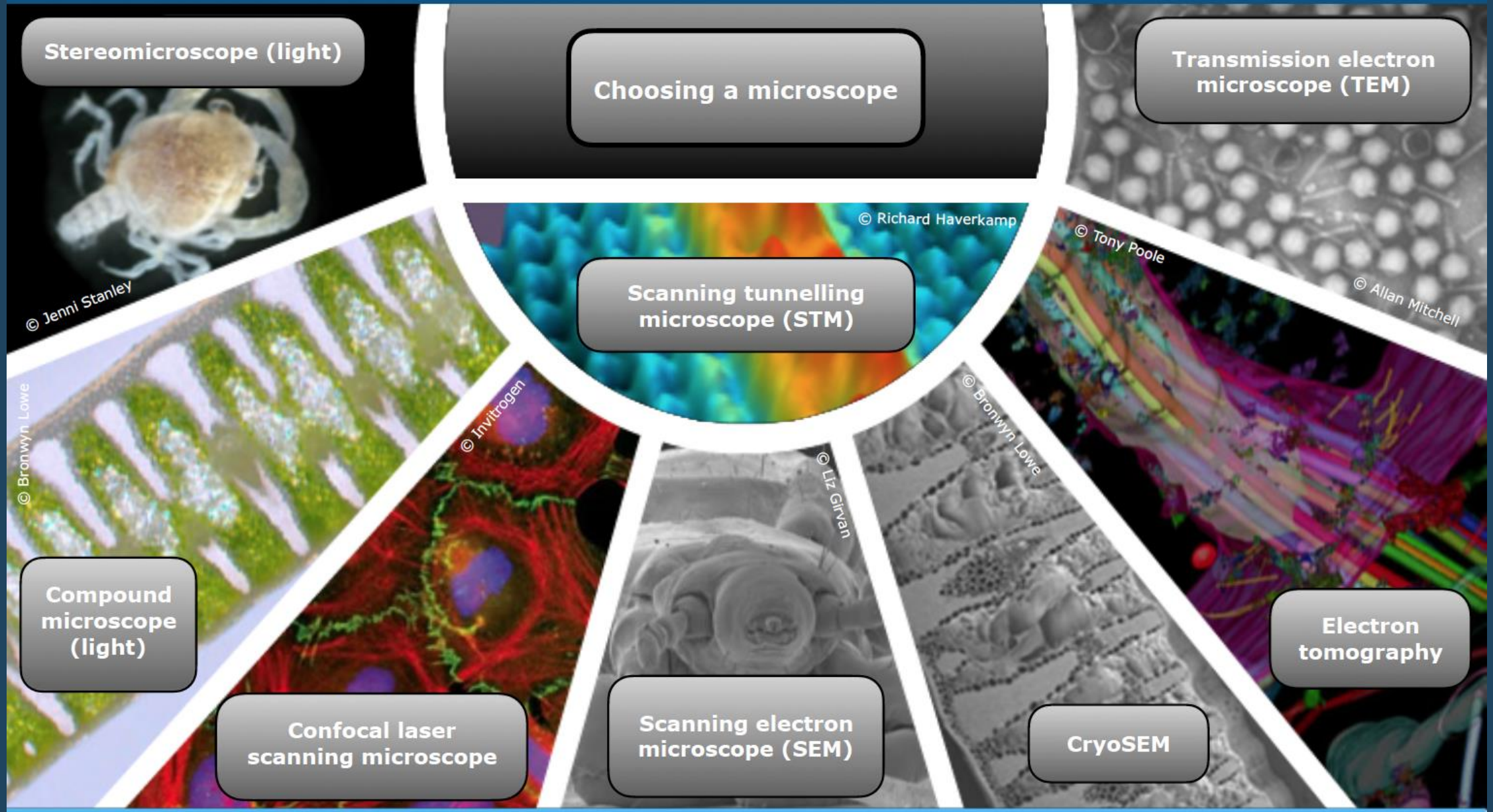
Mechanical part - base, arm, tube, revolver lens changer, stage with clamps and cross-conductor, course adjustment knob and fine adjustment knob

	Magnification			
Ocular	10×	10×	10×	10×
Objective	4×	10×	40×	100×
Total Magnification	**40×**	**100×**	**400×**	**1000×**

Total magnification of the microscope is equal to the product of lens and eyepiece magnification:

Magnification in the drawings (pictures) can be written in two ways: as total magnifications (40 ×, 100 ×, 400 ×, × 1000), or in a fraction of a zoom eyepiece lens used to enlarge (10/4, 10/10, 10/40, 10 / 100).

WHICH MICROSCOPE?



LIGHT MICROSCOPES

- Brightfield
- Darkfield
- Phase contrast
- Nomarski differential contrast (DIC)
- Fluorence
- Confocal

Monocul microscope

Binocular microscope

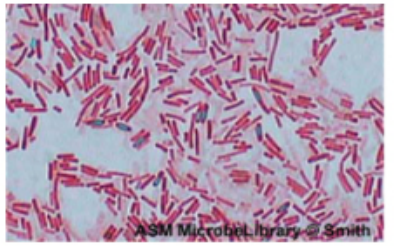
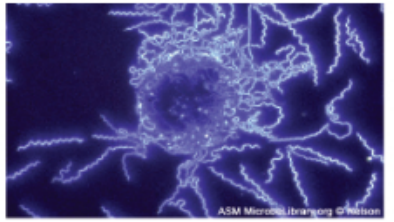
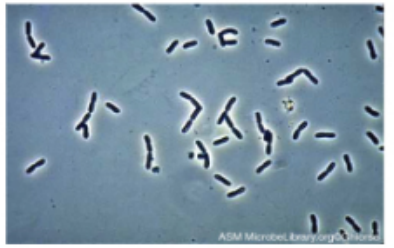
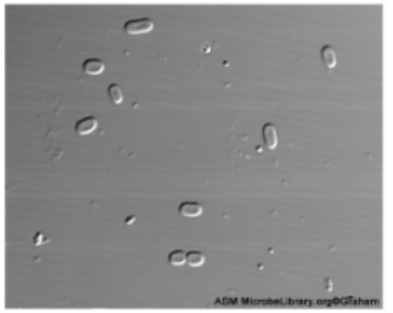
Inverse microscope

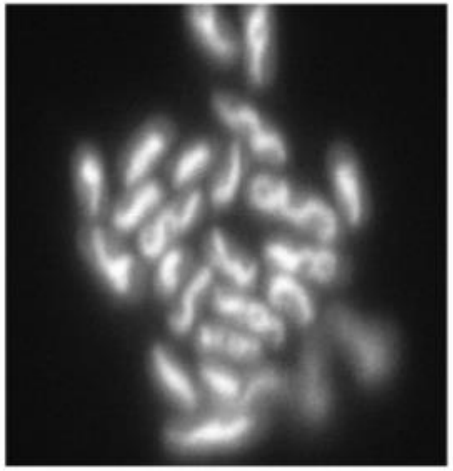
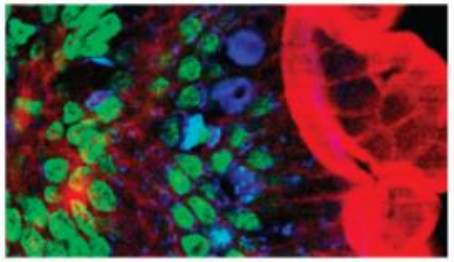
- observations of cultures of **living cells** **and** microorganisms at the bottom of culture dishes or suspended in live soils

- examination of tissue cultures, water quality, sediment analyses, observation of crystalline structures

LIGHT MICROSCOPES Magnification: up to about 1000×

Use visible or ultraviolet light to produce an image.

Microscope Type	Key Uses	Sample Images
Brightfield	Commonly used in a wide variety of laboratory applications as the standard microscope; produces an image on a bright background. **Example:** *Bacillus* sp. showing endospores.	
Darkfield	Increases contrast without staining by producing a bright image on a darker background; especially useful for viewing live specimens. **Example:** *Borrelia burgdorferi*	
Phase contrast	Uses refraction and interference caused by structures in the specimen to create high-contrast, high-resolution images without staining, making it useful for viewing live specimens, and structures such as endospores and organelles. **Example:** *Pseudomonas* sp.	
Differential interference contrast (DIC)	Uses interference patterns to enhance contrast between different features of a specimen to produce high-contrast images of living organisms with a three-dimensional appearance, making it especially useful in distinguishing structures within live, unstained specimens; images viewed reveal detailed structures within cells. **Example:** *Escherichia coli* O157:H7	

Fluorescence	Uses fluorescent stains to produce an image; can be used to identify pathogens, to find particular species, to distinguish living from dead cells, or to find locations of particular molecules within a cell; also used for immunofluorescence. **Example:** *P. putida* stained with fluorescent dyes to visualize the capsule.	
Confocal	Uses a laser to scan mutiple z-planes successively, producing numerous two-dimensional, high-resolution images at various depths that can be constructed into a three-dimensional image by a computer, making this useful for examining thick specimens such as biofilms. **Example:** *Escherichia coli* stained with acridine orange dye to show the nucleoid regions of the cells.	
Two-photon	Uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to penetrate deep into thick specimens such as biofilms. **Example:** Mouse intestine cells stained with fluorescent dye.	

Observation in phase contrast

- observation of uncolored objects, and structures in living cells - nucleus, nucleus, chromosomes and vacuoles

- can significantly increase contrast in poorly stained and poorly distinguishable structures of histological specimens

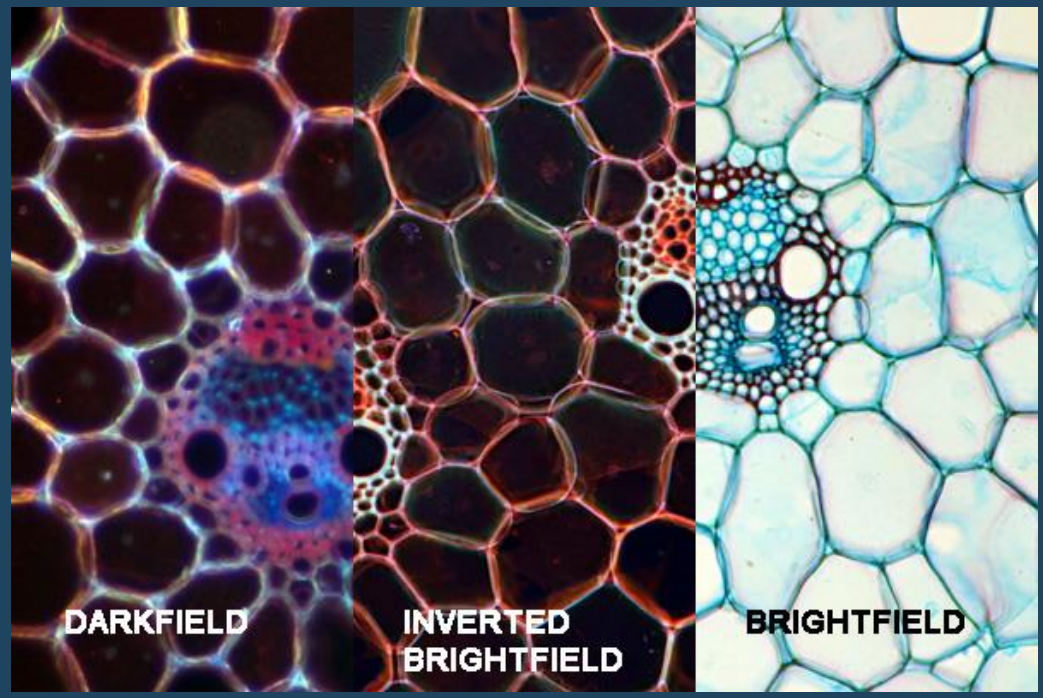
https://www.youtube.com/watch?v=P2teE17zT4I

Observation in dark field

- Microscopes require a special lighting section (**a paraboloid condensor**) that passes only obliquely passing rays

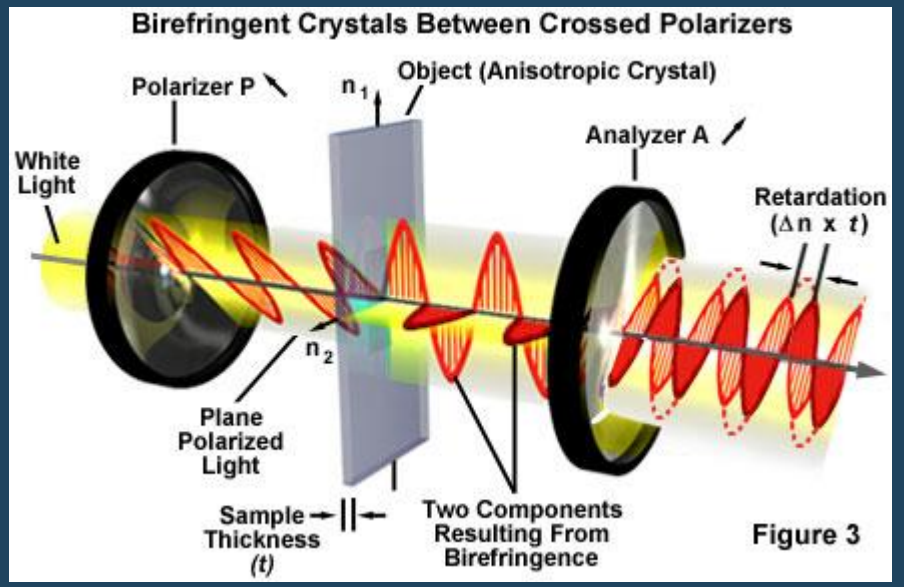
- The subject is illuminated only from the sides and the **field of view is dark**. This makes possible to observe objects much smaller than those that can be observed in the passing light

- for example, to diagnose bacteria (leptospires, Treponema pallidum) that can not be seen in the passing light.



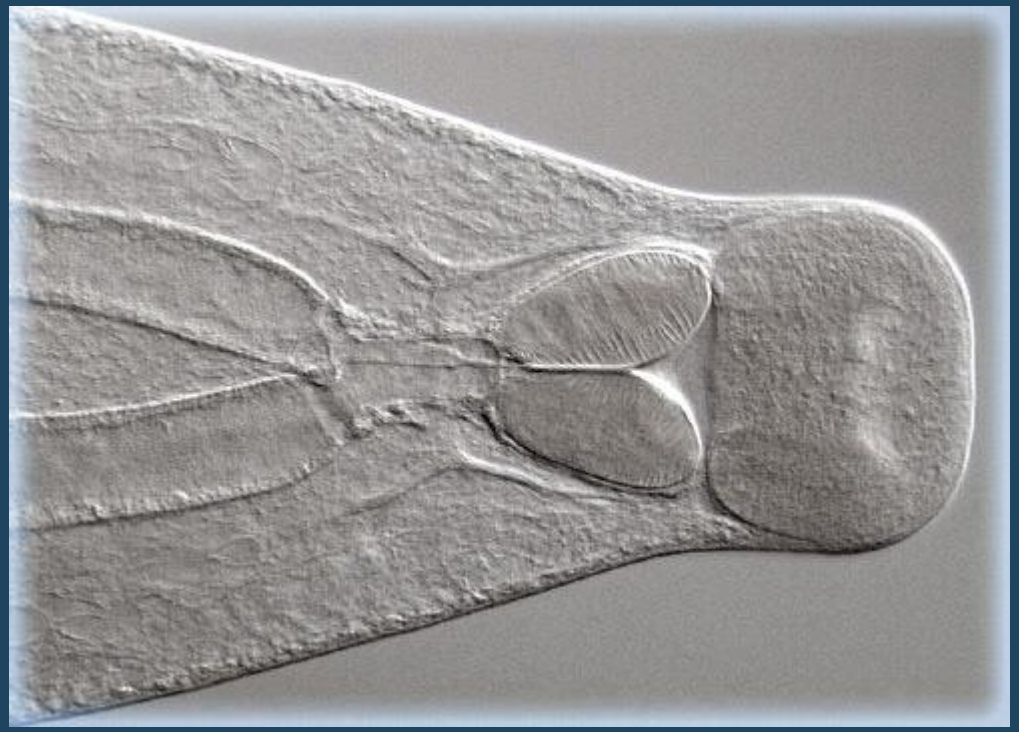
Observation in a polarized field

- In the eyepiece or just below it, is the polarizing filter - the **analyzer** and under the condensor is the **polarizer**.
- inserting object contains an <u>optically active substance</u>
- The object is placed between the two polarization systems



Nomarski differential contrast (DIC)

- Efforts to eliminate "halo" effect around the object details (Georges Nomarski 1960)
- Can differenciate edges of speciments



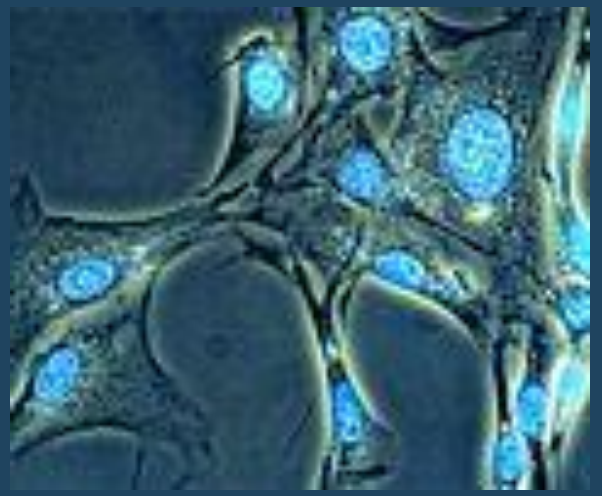
Chinese Liver Fluke (Clonorchis sinensis)

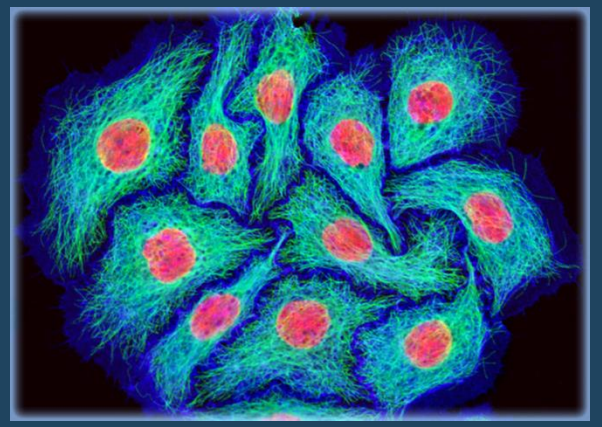
Ancylostoma duodenale

https://www.youtube.com/watch?v=TKTGgAQ2VEs

Fluorescence microscopy

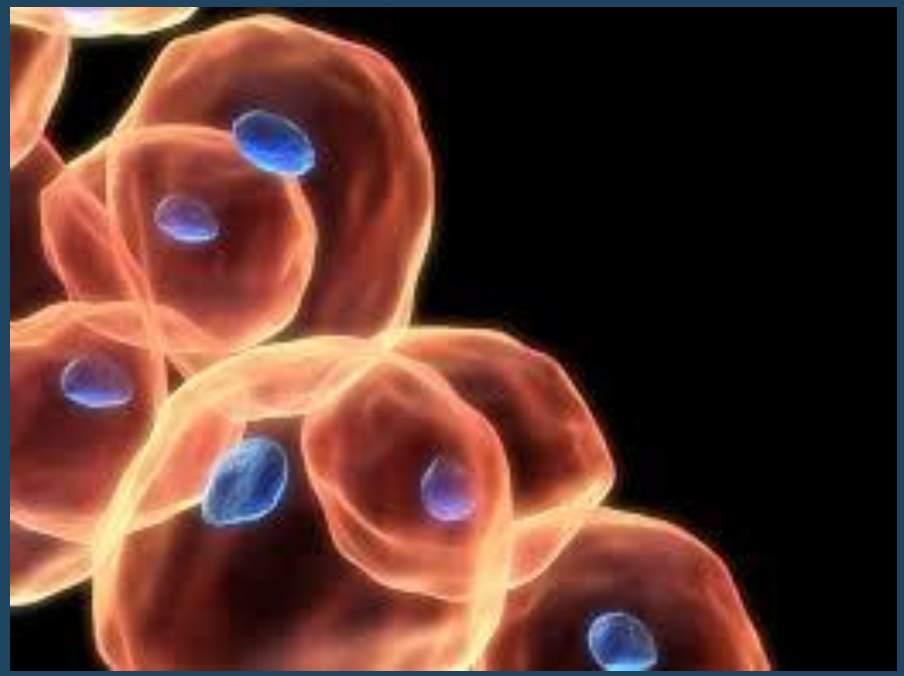
- The sample is exposed to wavelength radiation that can cause fluorescence.
- Areas of the sample in which the fluorescence did not occur remain dark
- Many natural substances, such as **chlorophyll** or some vitamins, shine under the influence of UV rays.
- **Fluorochromes** are fluorescent compounds that are used to visualize different cellular components, such as DNA, proteins (proteins). e.g. in the diagnosis of rabies.





Confocal microscopy

- From the second half of the 1980s
- Use the **fluorescence**
- The source of light is a **laser**
- The microscope creates "optical cuts".
- By computerized section processing, three-dimensional models can be created
- „Life cell imaging" allows you to monitor functions of intracellular structures in vivo



ELECTRON MICROSCOPY

- the desire and the need to observe ever-smaller objects
- viruses – in nm of size
- the resolution of the optical microscope is limited by the visible wavelength (400-700 nm)
- technological advancement - the discovery of electrons and their properties
- 19th - 20th century - **J.J. Thompson, Luis de Broglie**
- 1931 **Max Knoll and Ernst Russia** constructed a **transmission electron microscope** - 1986 Nobel Prize
- 1965 **scanning electron microscope** was constructed

ARMIN DELONG

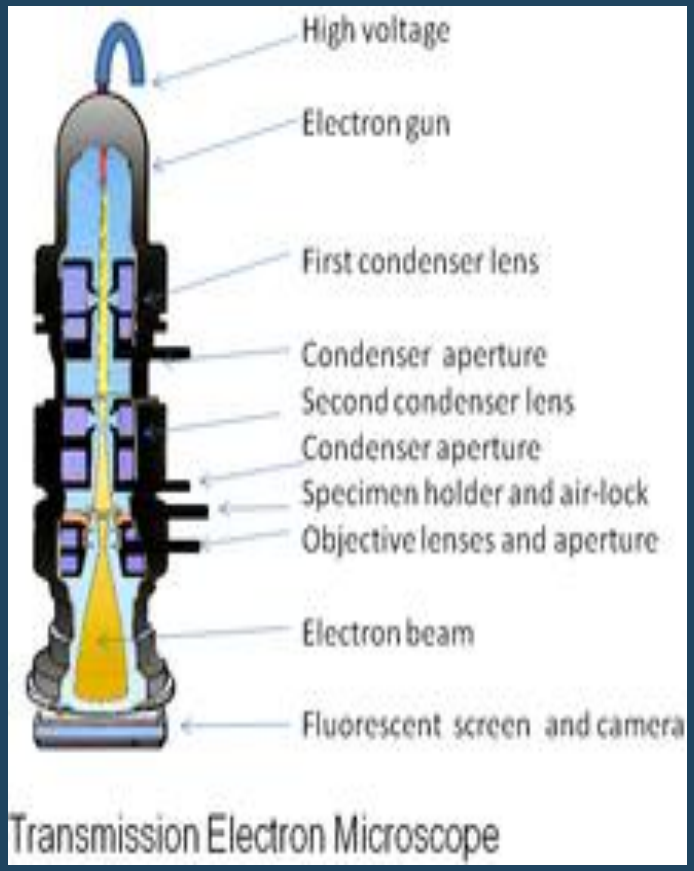
- Brno professor
- LVEM5 (Low Voltage Transmission Electron Microscope) – optical resolution 2 nanometres, 100 more resolution than a high-quality optical microscope.
- His microscope could allow detection of viruses according to their shape
- Rapid diagnosis of the disease (quarantine shortening)
- No sample modification is needed - use in biology
- In 2005, the National Czech Republic Award - Czech Head was awarded

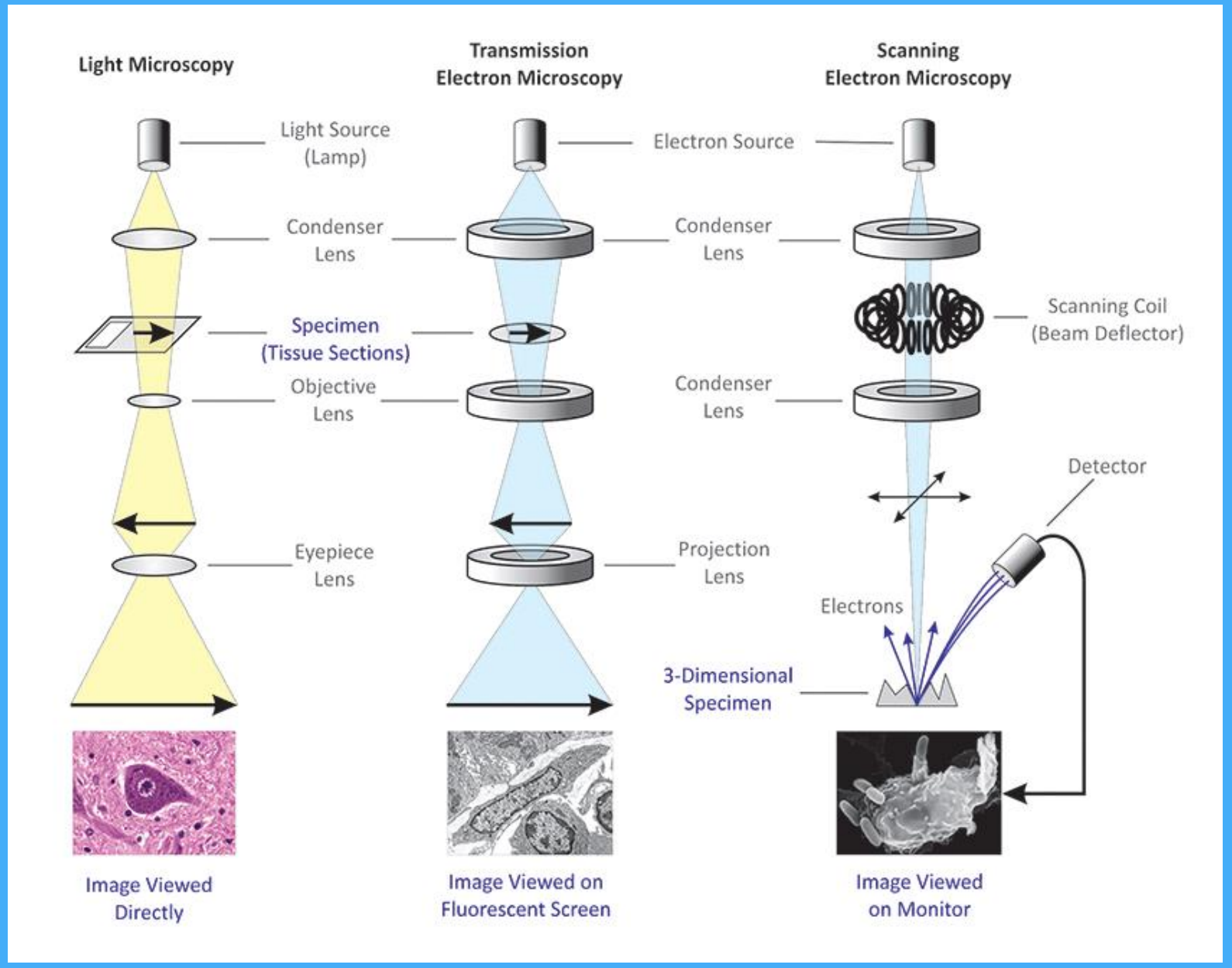
ELECTRON MICROSCOPES
TRANSMISSION (TEM) AND RASTER (REM)

- 1 000 000 magnification
- resolution of 2-20 nm (viruses)
- beam of accelerated **electrons** as a source of illumination
- passage through **electromagnetic lenses**
- the sample must not contain water
- ultra-thin cuts - up to 100nm

THE PRINCIPLE OF AN ELECTRON MICROSCOPE

- an electron gun
- a tube through which the electron passes from the cathode (tungsten fiber) to the anode
- Vacuum - operating temperature 2 500 ° C
- electromagnetic lenses



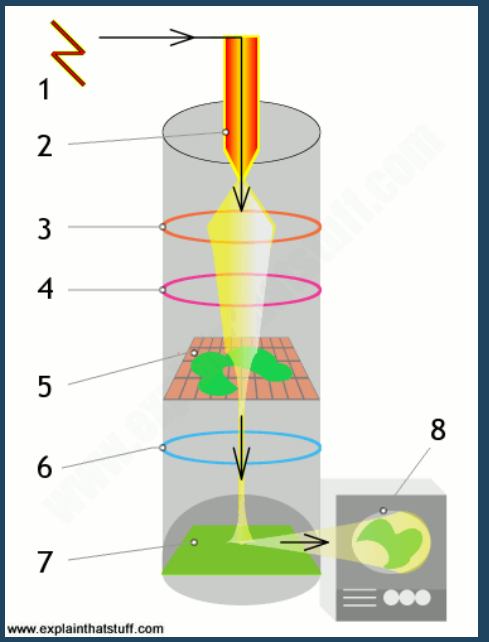


DIFERENCE BETWEEN LIGHT AND ELECTRONIC MICROSCOPY

How a transmission electron microscope (TEM) works

A transmission electron microscope fires a beam of electrons through a specimen to produce a magnified image of an object.

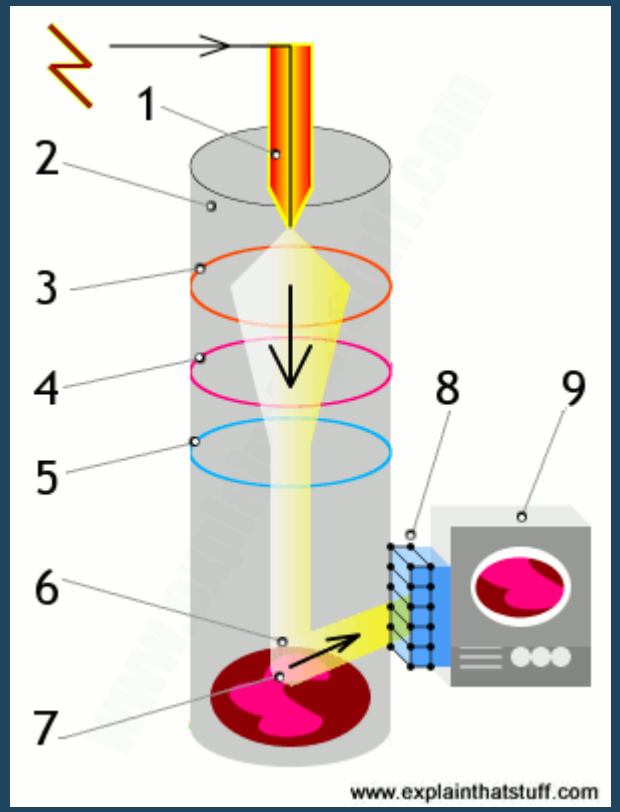
- ✓ A high-voltage electricity supply powers the cathode.
- ✓ The cathode is a heated filament, a bit like the electron gun in an old-fashioned cathode-ray tube (CRT) TV. It generates a beam of electrons that works in an analogous way to the beam of light in an optical microscope.
- ✓ An electromagnetic coil (the first lens) concentrates the electrons into a more powerful beam.
- ✓ Another electromagnetic coil (the second lens) focuses the beam onto a certain part of the specimen.
- ✓ The specimen sits on a copper grid in the middle of the main microscope tube. The beam passes through the specimen and "picks up" an image of it.
- ✓ The projector lens (the third lens) magnifies the image.
- ✓ The image becomes visible when the electron beam hits a fluorescent screen at the base of the machine. This is analogous to the phosphor screen at the front of an old-fashioned TV .
- ✓ The image can be viewed directly (through a viewing portal), through binoculars at the side, or on a TV monitor attached to an image intensifier (which makes weak images easier to see).



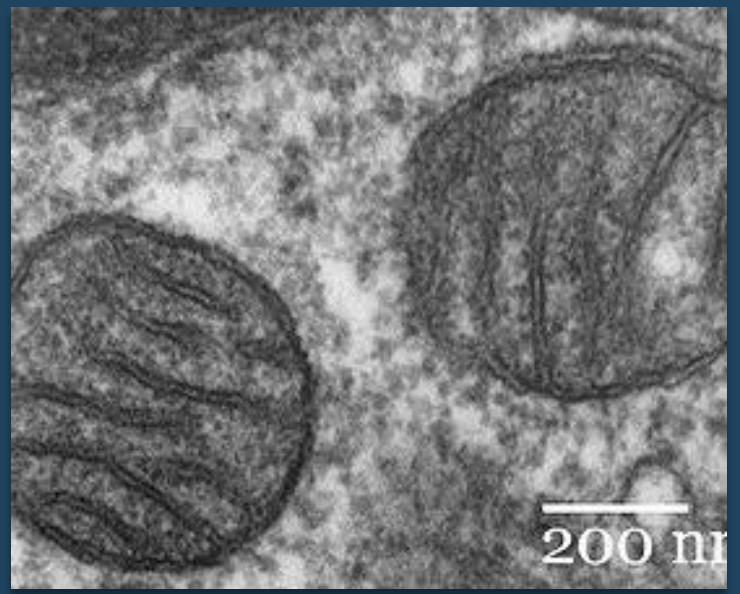
How a scanning electron microscope (SEM) works

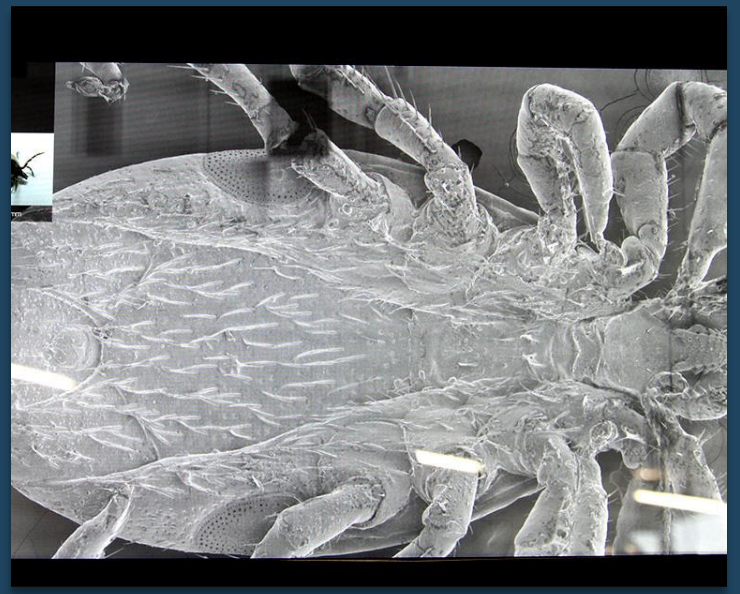
A scanning electron microscope scans a beam of electrons over a specimen to produce a magnified image of an object. That's completely different from a TEM, where the beam of electrons goes right through the specimen.

- ✓ Electrons are fired into the machine.
- ✓ The main part of the machine (where the object is scanned) is contained within a sealed vacuum chamber because precise electron beams can't travel effectively through air.
- ✓ A positively charged electrode (anode) attracts the electrons and accelerates them into an energetic beam.
- ✓ An electromagnetic coil brings the electron beam to a very precise focus, much like a lens.
- ✓ Another coil, lower down, steers the electron beam from side to side.
- ✓ The beam systematically scans across the object being viewed.
- ✓ Electrons from the beam hit the surface of the object and bounce off it.
- ✓ A detector registers these scattered electrons and turns them into a picture.
- ✓ A hugely magnified image of the object is displayed on a TV screen.



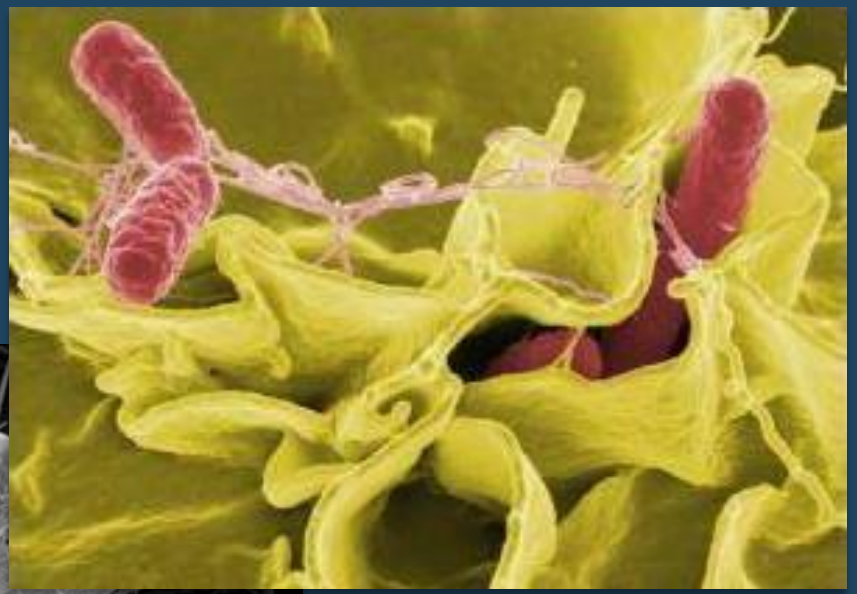
TEM





REM

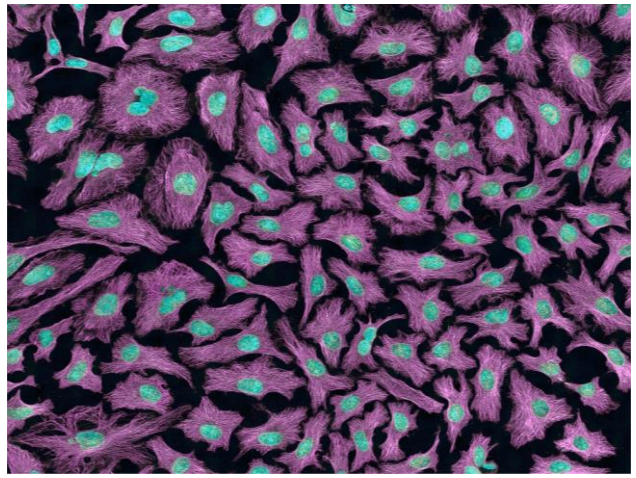
- scanning on the surface
- creates a 3D image



- https://www.youtube.com/watch?v=cQuX8tfwlYA

A FOLDSCOPE

- is an optical microscope that can be assembled from simple components, including a sheet of paper and a lens.
- It was created by Manu Prakash and designed to cost **less than one USD** to build. It is a part of the "frugal science" movement which aims to make cheap and easy tools available for scientific use in the developing world

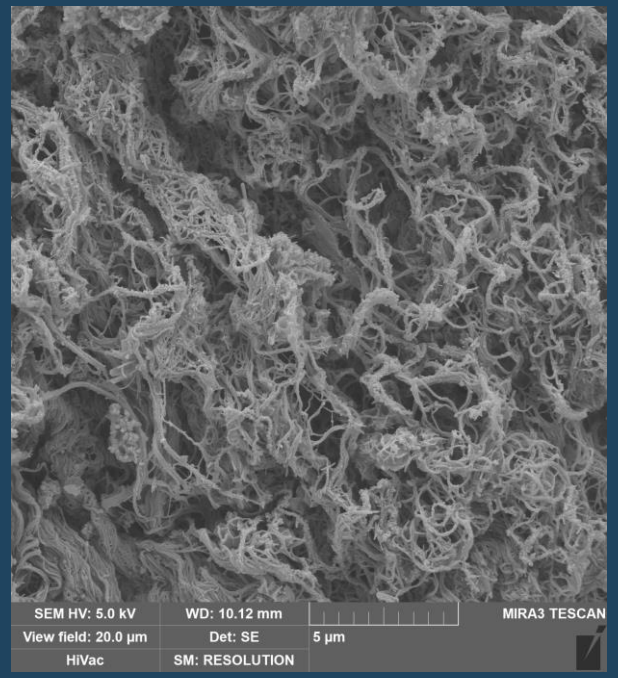


HELA CELLS

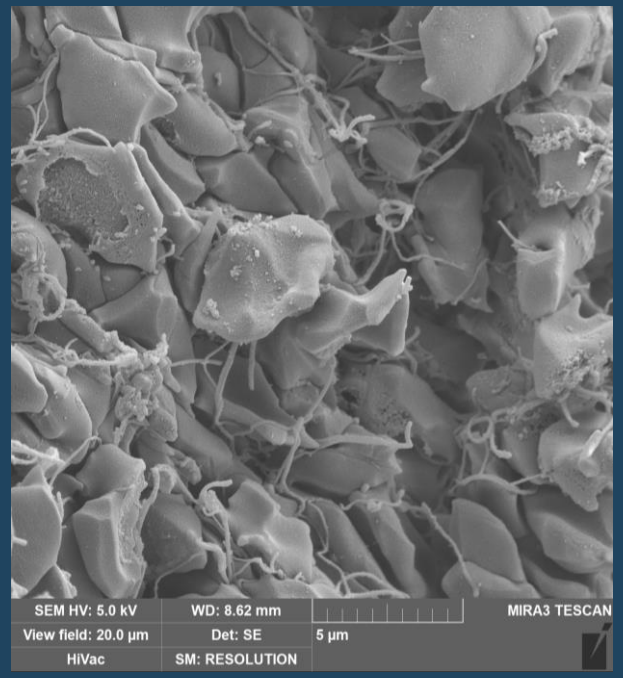
- https://www.youtube.com/watch?v=22lGbAVWhro
- What are HeLa cells?
- HeLa cell | Cancer Research, Immortal Cells & Tissue Culture ...
- The designation HeLa is derived from the name of the patient, Henrietta Lacks.
- HeLa cells were the first human cell line to be established and have been widely used in laboratory studies, especially in research on viruses, cancer, and human genetics

RESEARCH OF THE NEW THROMBOLYTIC

Trombus
světelný mikroskop, HE



Trombus
Elektronový mikroskop



Trombus
Elektronový mikroskop

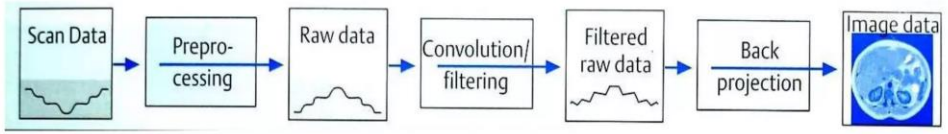
COMPUTED TOMOGRAPHY, CT

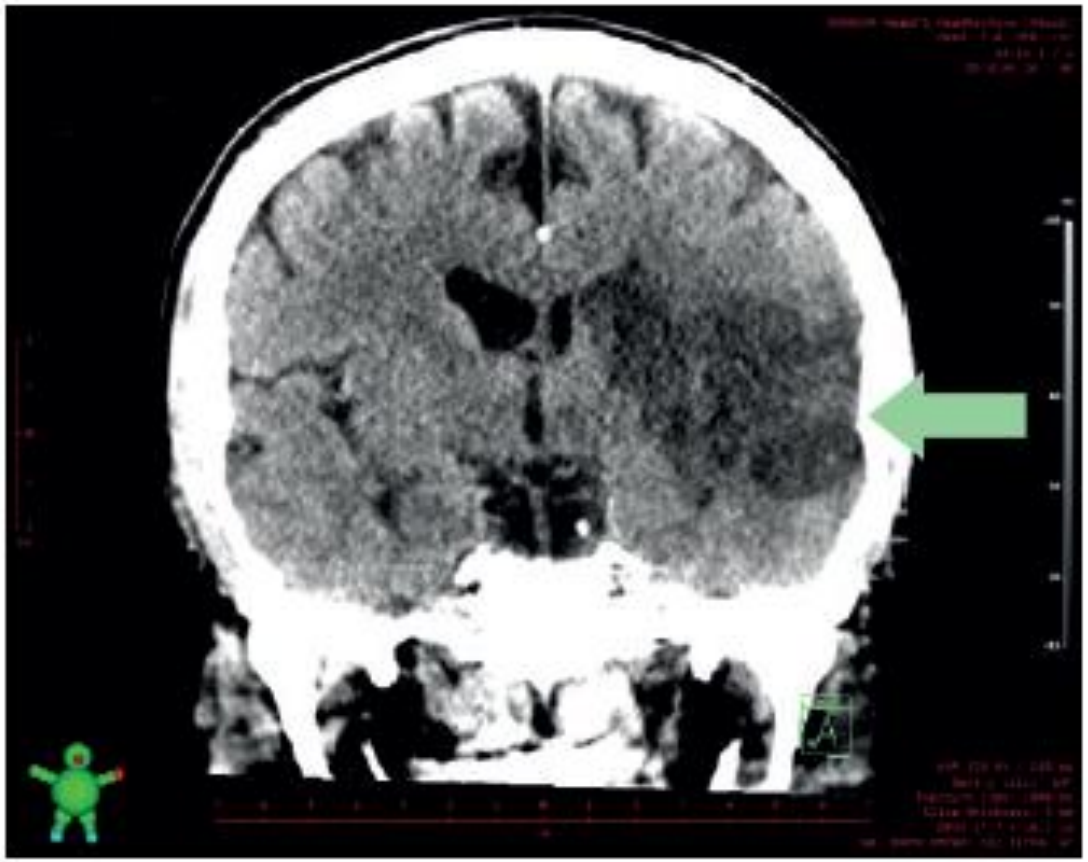
Basics Principle

The basic principle behind CT is that the internal structure of an object can be reconstructed from multiple projections of the object.

The ray projections are formed by scanning a thin cross section of the body with a narrow x-ray beam and measuring the transmitted radiation with a sensitive radiation detector.

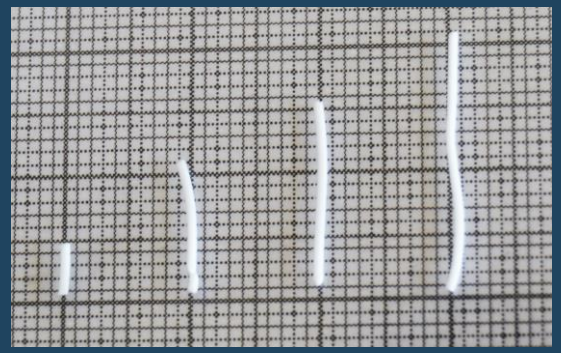
CT scanning is a systematic collection and representation of projection data

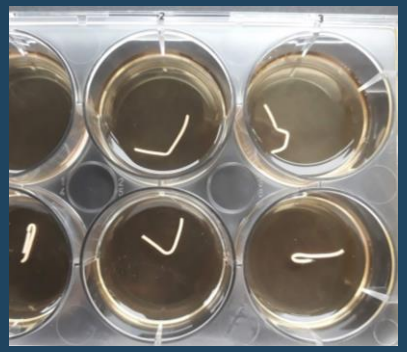


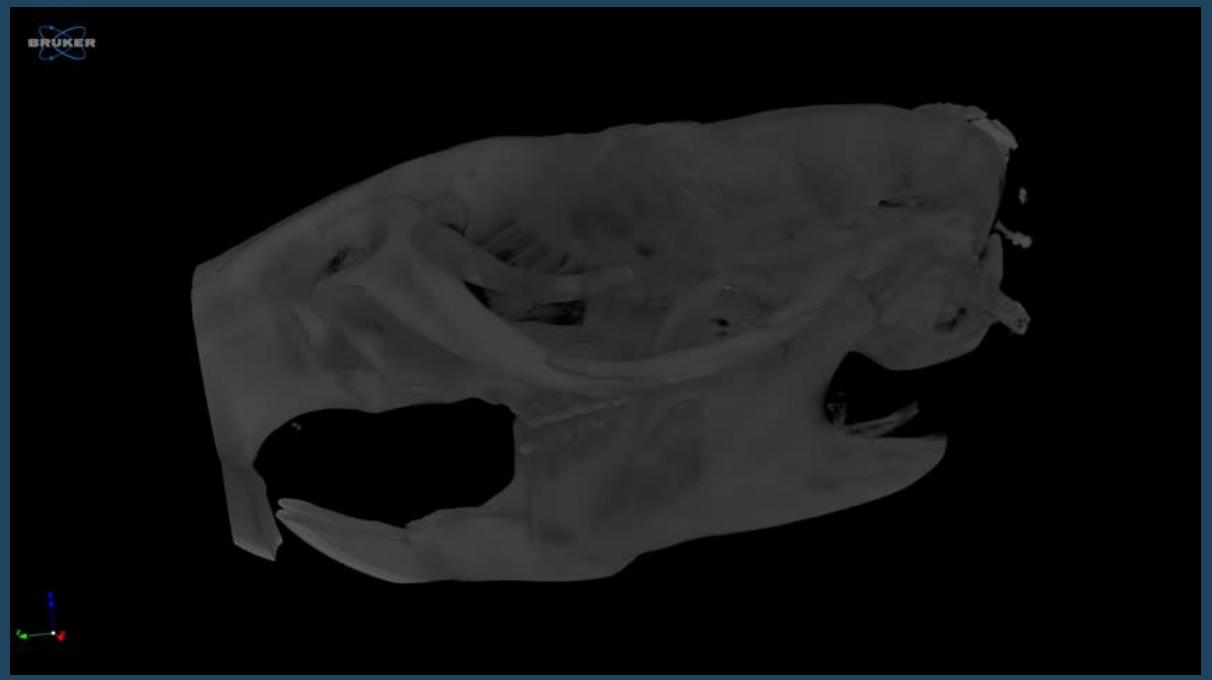


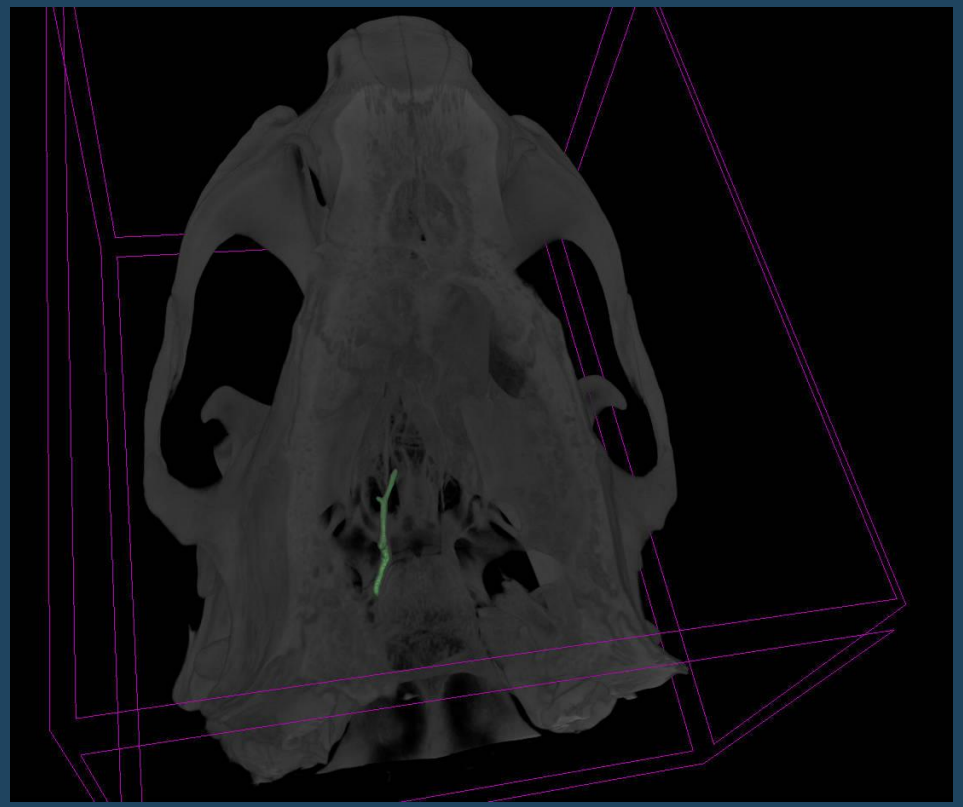
Obr. 1 Nativní CT vyšetření mozku. Teritoriální infarkt zaujímající prakticky celou zadní polovinu oblasti zásobené levou arterií cerebri media (tmavší ložisko v pravé části obrázku).

Novobilsky, Scheer, Hložková

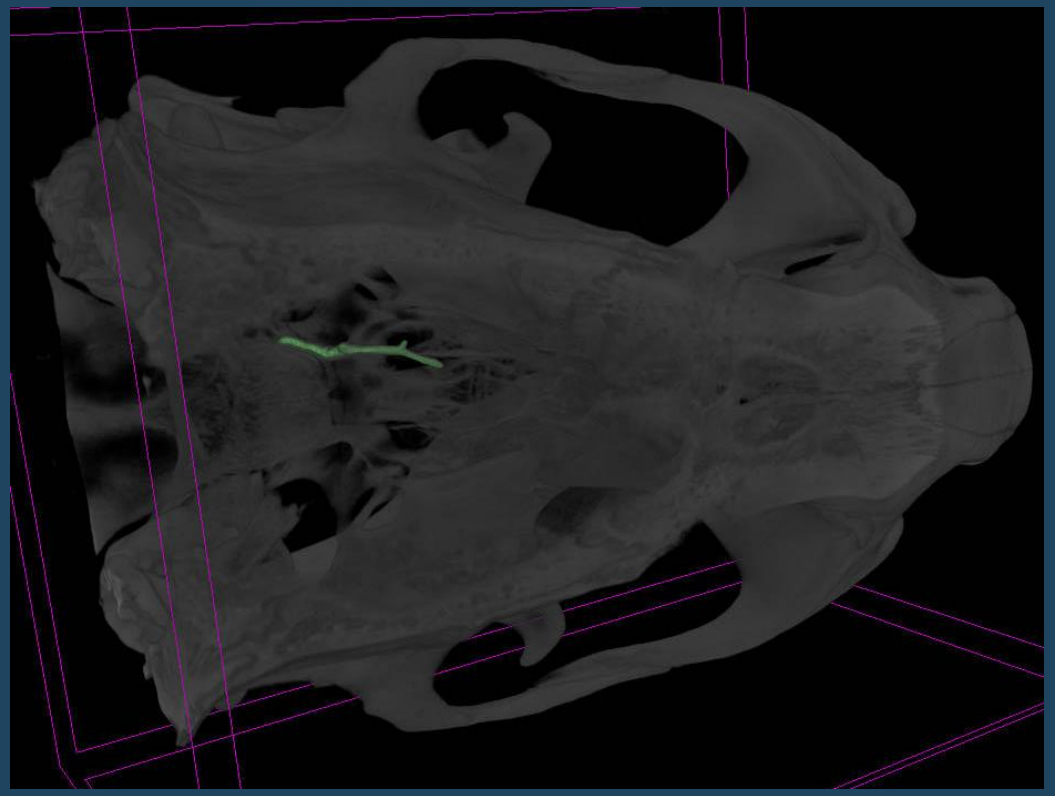








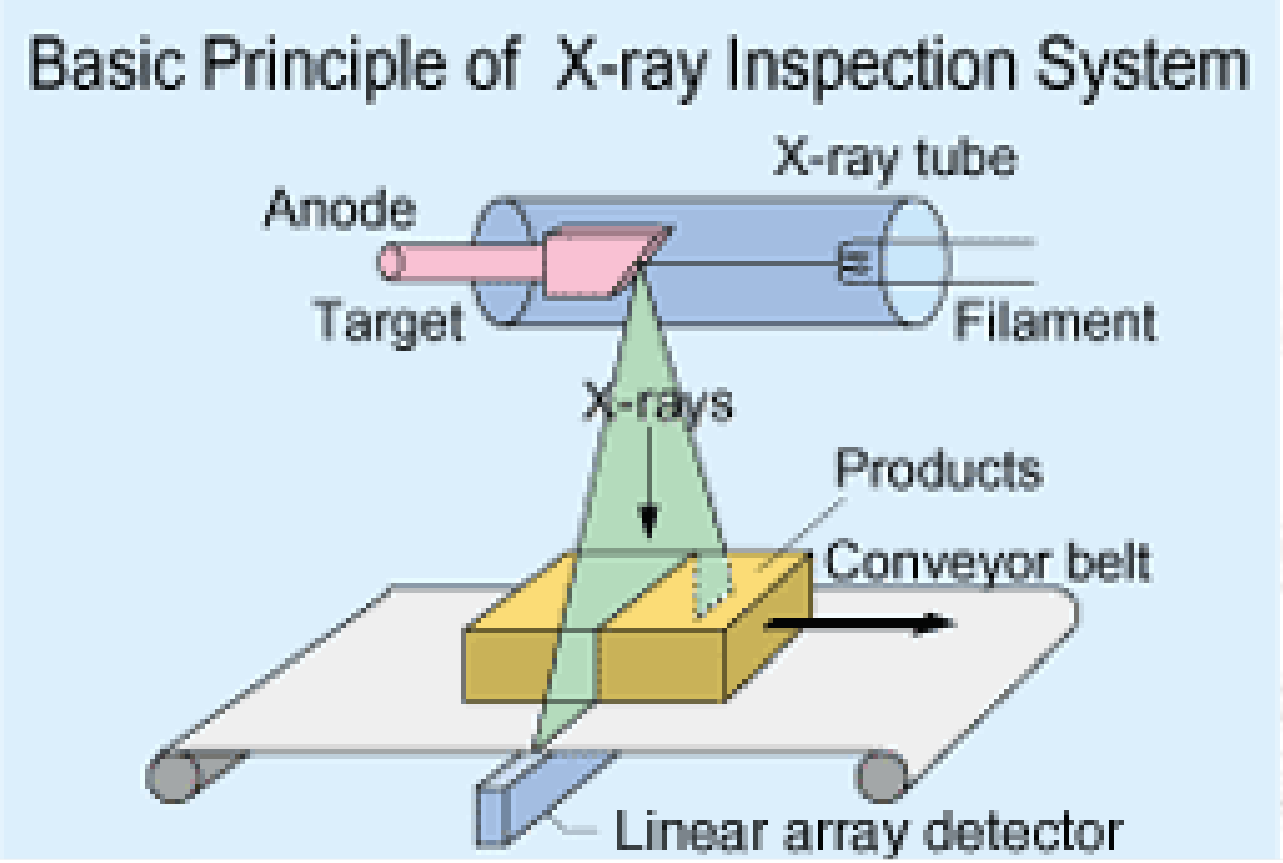
Novobilsky, Scheer, Hložková



Mi CT

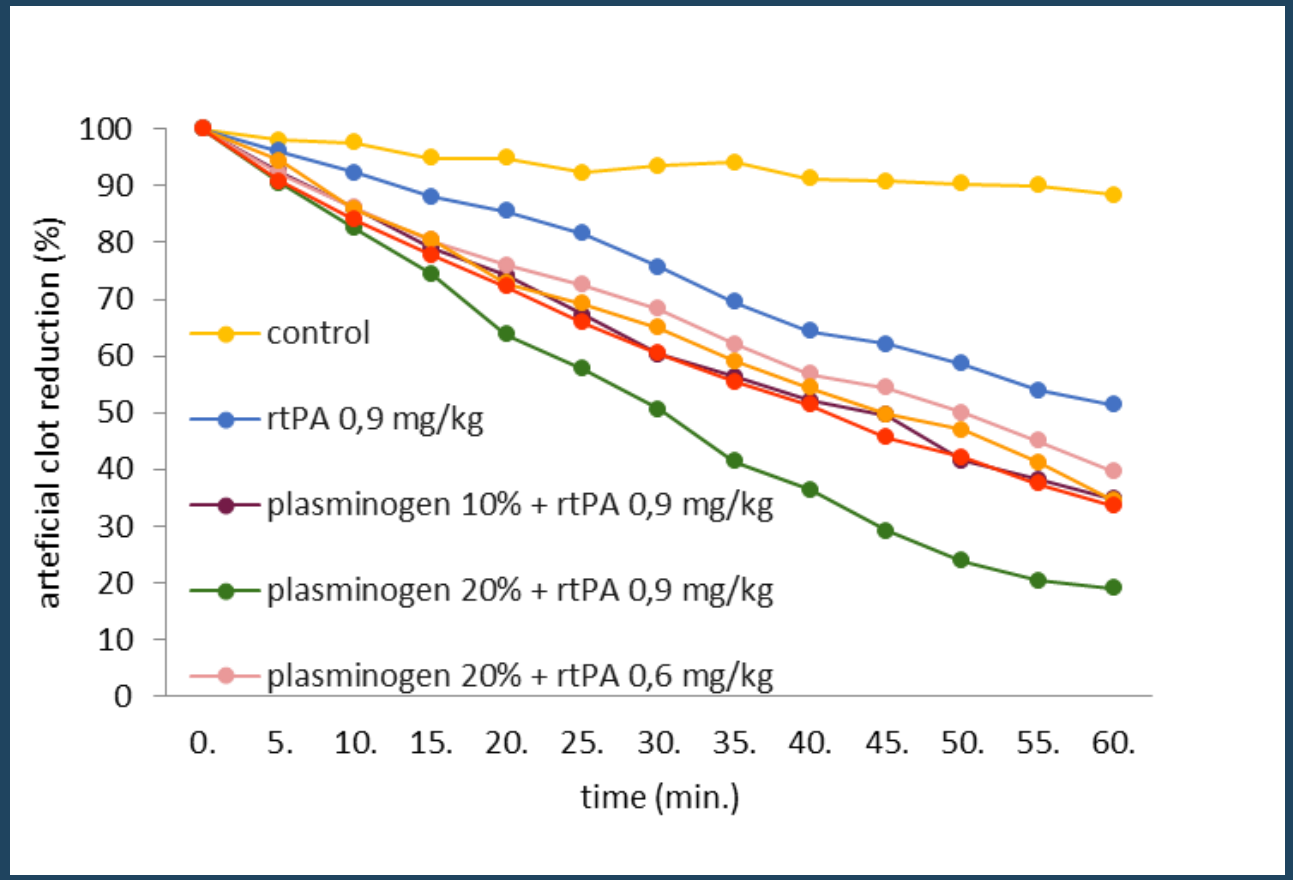
Skiaskopie (RTG)

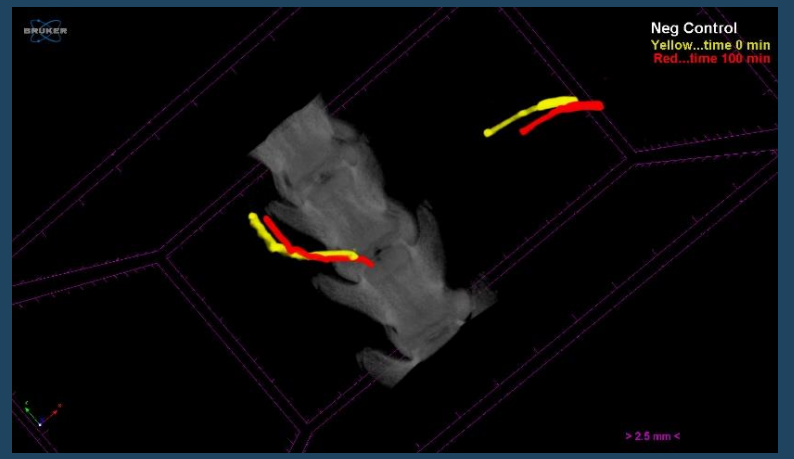
Novobilsky, Scheer, Hložková

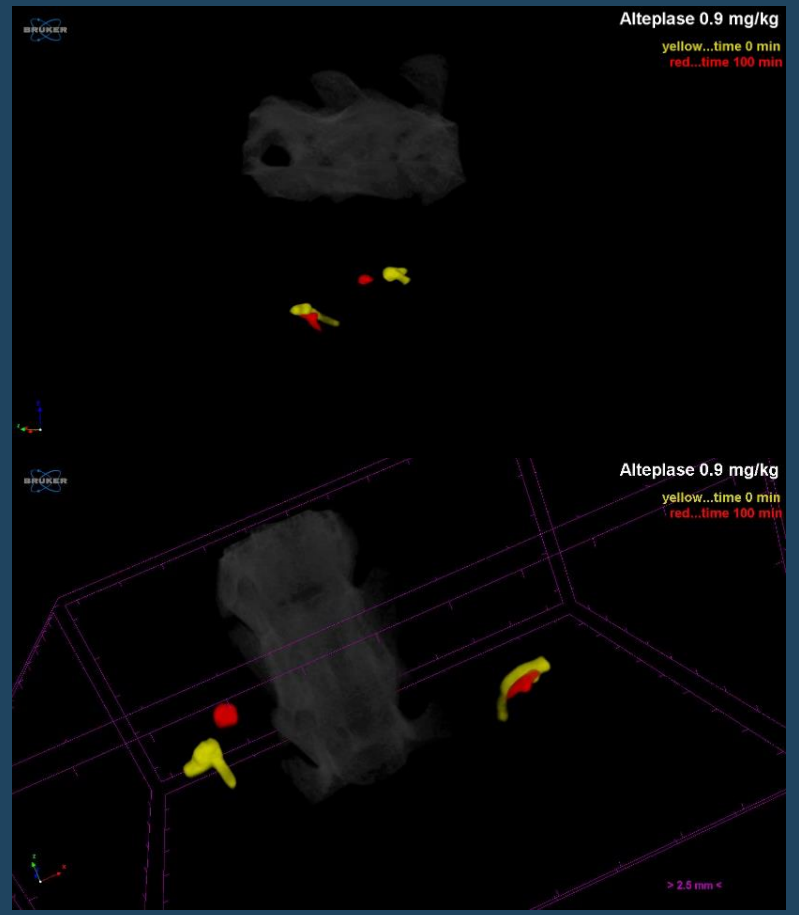

Basic Principle of X-ray Inspection System
X-ray tube
Anode
Target
Filament
X-rays
Products
Conveyor belt
Linear array detector

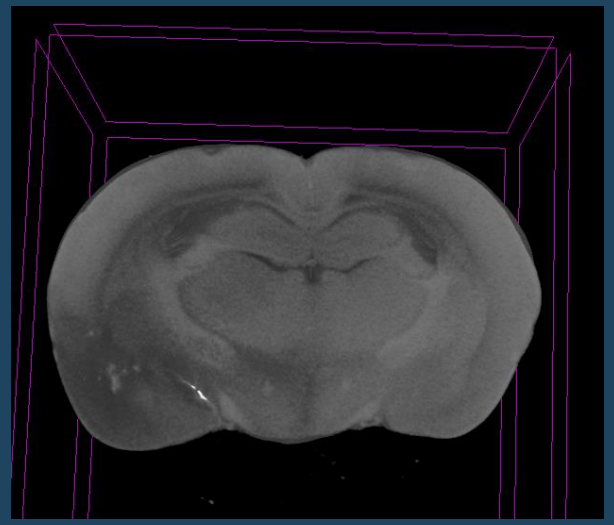

Projectional radiography
X-ray generator
Radiograph
Object
X-ray
detector

X- RAY


arteficial clot reduction (%)
100
90
80
70
60
50
40
30
20
10
0
control
rtPA 0,9 mg/kg
plasminogen 10% + rtPA 0,9 mg/kg
plasminogen 20% + rtPA 0,9 mg/kg
plasminogen 20% + rtPA 0,6 mg/kg
0.
5.
10.
15.
20.
25.
30.
35.
40.
45.
50.
55.
60.
time (min.)


Neg Control
Yellow...time 0 min
Red...time 100 min


Alteplase 0.9 mg/kg
yellow...time 0 min
red...time 100 min
Alteplase 0.9 mg/kg
yellow...time 0 min
red...time 100 min



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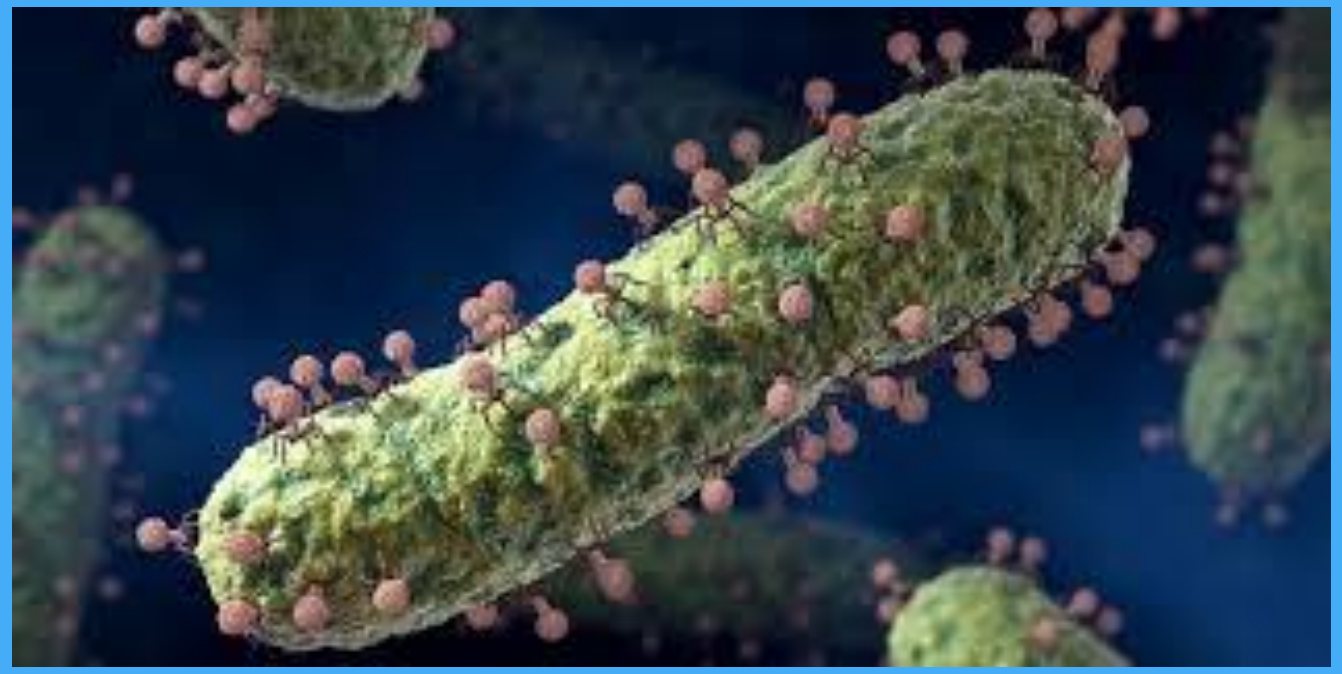


NON-CELLULAR FORMS OF LIFE

NON-CELLULAR FORMS OF LIFE

- Non-cellular life is life that exists without a cellular structure.
- This term presumes the phylogenetic scientific classification of viruses as lifeforms, which is a controversial issue
- Hypothesized artificial life may or may not be considered living.

LIVING ORGANISM

- The definition of life is controversial.

The current definition is that organisms:

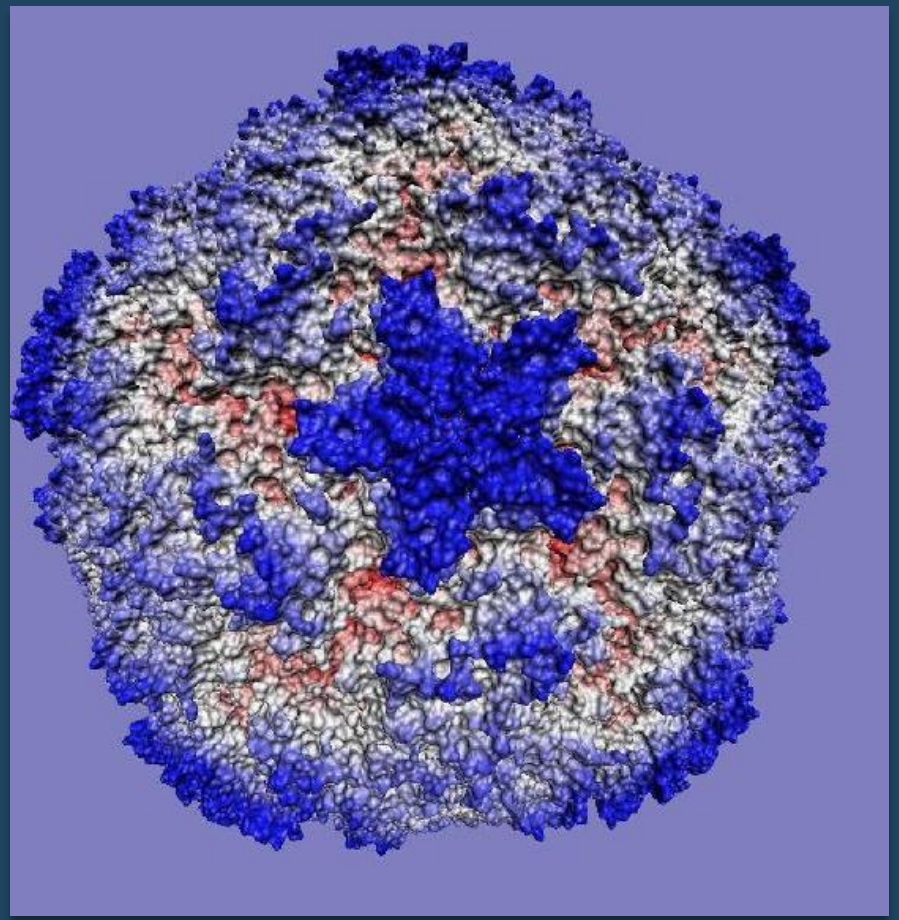
- maintain homeostasis,
- are composed of cells,
- undergo metabolism,
- can grow,
- adapt to their environment,
- respond to stimuli,
- and reproduce.

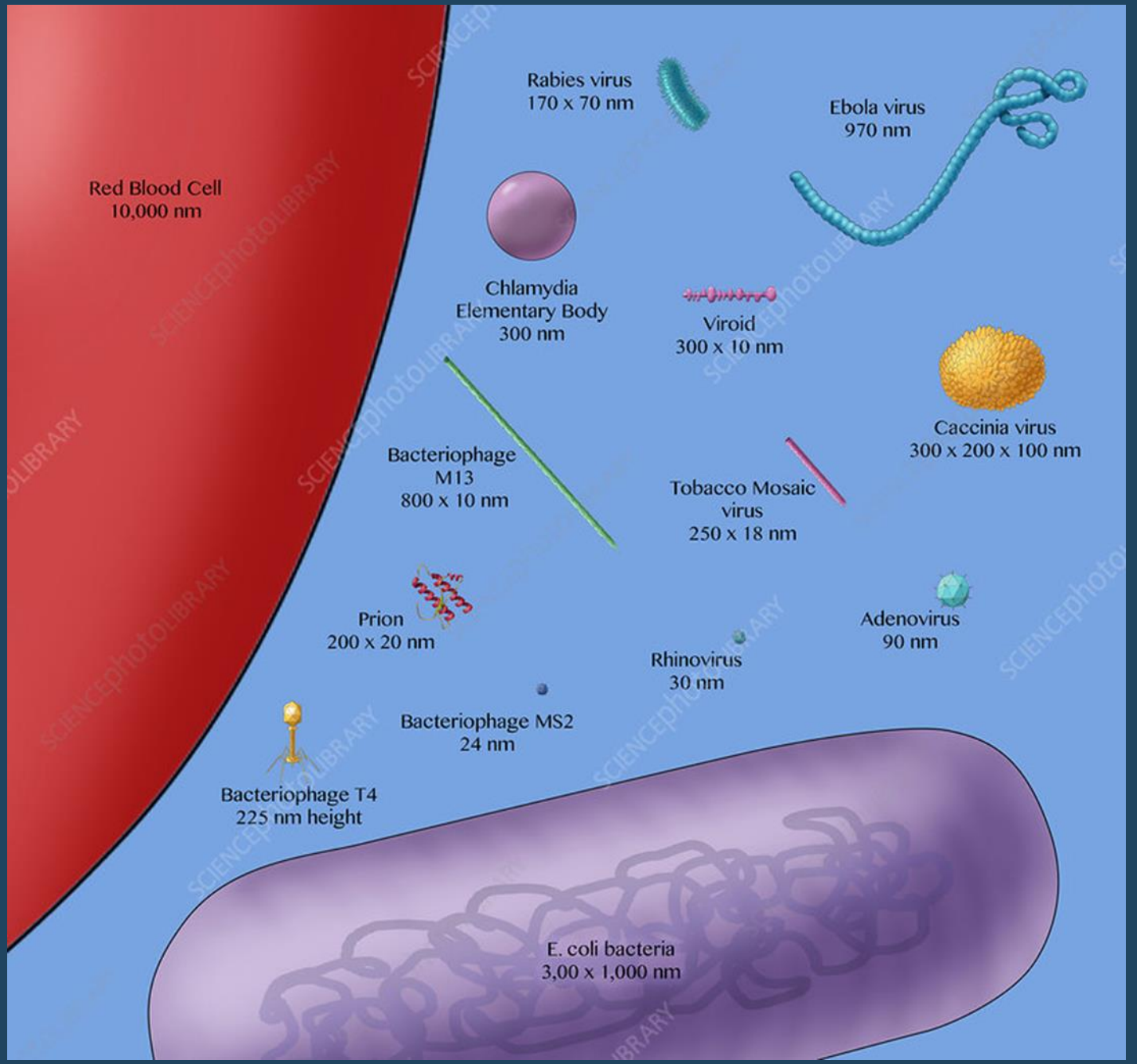
Life on Earth:

Non-cellular life	Viruses
	Viroids
	Satelites
	Prions
Cellular life	Domain Bacteria
	Domain Archaea
	Domain Eukarya

VIRUS

- a small infectious agent that replicates only **inside the living cells** of other organisms **(nm)**
- Viruses can infect all types of life forms
- **Dmitri Ivanovsky** in 1892 described a non-bacterial pathogen infecting tobacco plants
- about 5,000 virus species have been described in detail, although there are millions of types of viruses
- are found in almost every ecosystem on Earth

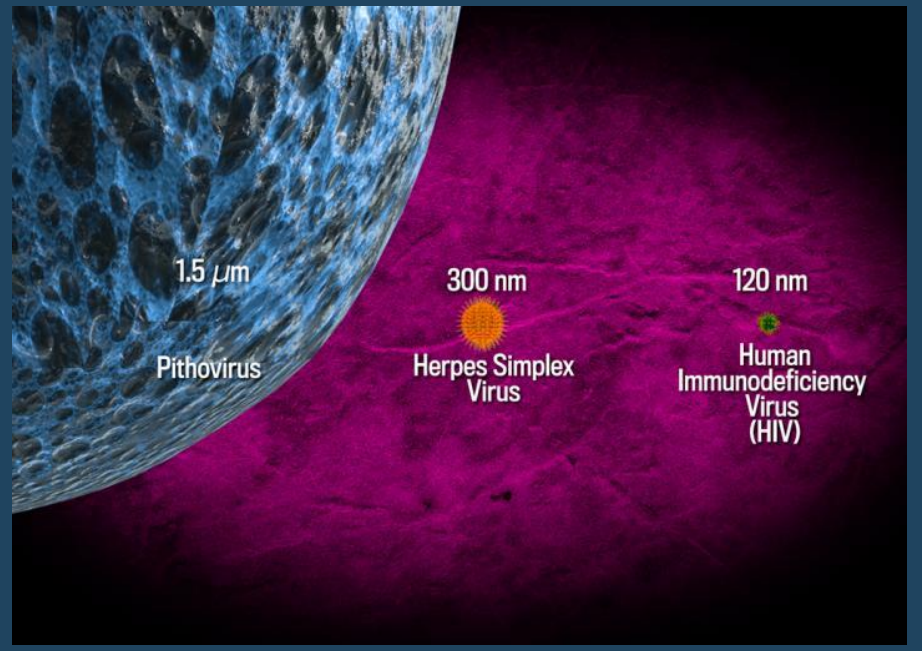



Red Blood Cell
10,000 nm
Rabies virus
170 x 70 nm
Ebola virus
970 nm
Chlamydia
Elementary Body
300 nm
Viroid
300 x 10 nm
Caccinia virus
300 x 200 x 100 nm
Bacteriophage
M13
800 x 10 nm
Tobacco Mosaic
virus
250 x 18 nm
Prion
200 x 20 nm
Adenovirus
90 nm
Rhinovirus
30 nm
Bacteriophage MS2
24 nm
Bacteriophage T4
225 nm height
E. coli bacteria
3,00 x 1,000 nm

- The biggest recently discovered virus is the **Mimivirus** (the bacterium mimics the virus) - found in the cells of the amoebas, measuring 400 nm

- In 2013 and 2014, even more **pandoraviruses and pithoviruses** were discovered - with a capsid of 1000 and 1500 nm.

- This means that while the smallest virions resemble their ribosome size, giant viruses are larger than the smallest bacteria.



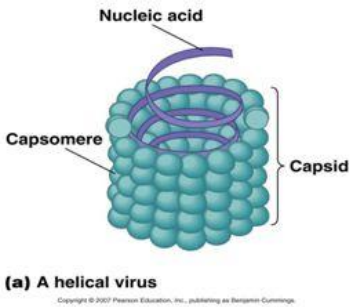
TAXONOMY

- Size and morphology – capsid symmetry
- **Presence (absence) of the surface coat:** enveloped or naked viruses
- **Type and molecular structure of genomic NK:**
- **Type NA: rna-viruses, dna-viruses**
- **NA:** single-stranded ssRNA**, dsRNA –** double-stranded, **ssDNA, dsDNA,** linear (mostly) or circular NK
- **NA polarity**: (+) RNA (plus RNA, positive RNA, same polarity as mRNA), **(-) RNA (+ RNA), (+) DNA, (-) DNA.**

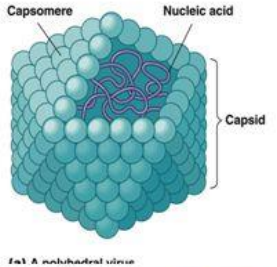
➢ Capside symmetry:

Virus structure

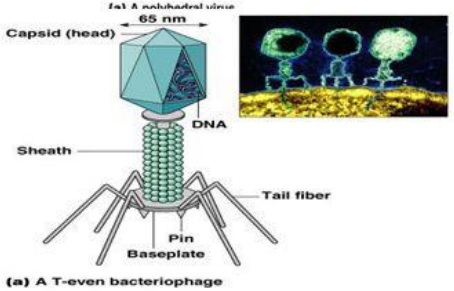
- **Helical viruses**
  - The protein subunits and the nucleic acid are arranged in a helix.



- **Polyhedral viruses**
  - The protein subunits assemble into a symmetric shell that covers the nucleic acid-containing core.



- **Complex Viruses**
  - Often have architecture consisting of both **helical** and **polyhedral** parts confined to different structural components

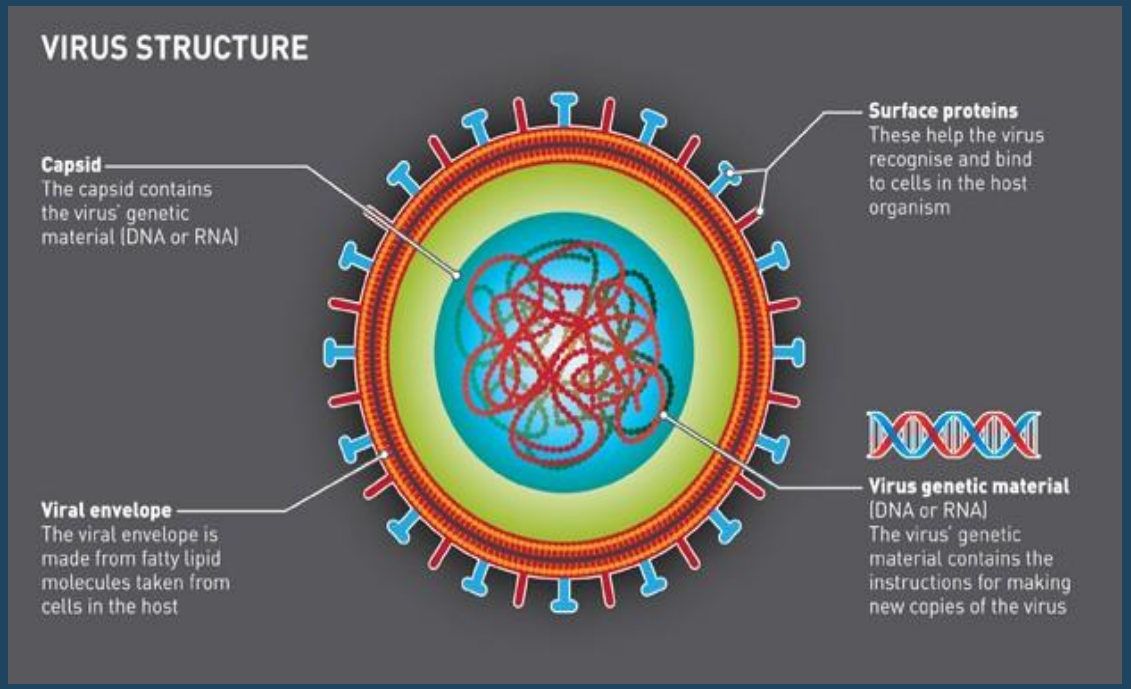


Host Type: animal viruses
human viruses
plant viruses,
fungi viruses,
algae and protozoa,
bacterial viruses (bacteriophages, phages)
phototrophic bacteria (cyanophages)

Affinity to tissues: respiratory, enteral, sexually transmitted, hepatic

VIRION

- Virion the individual particles capable of infecting host cells and multiply therein.

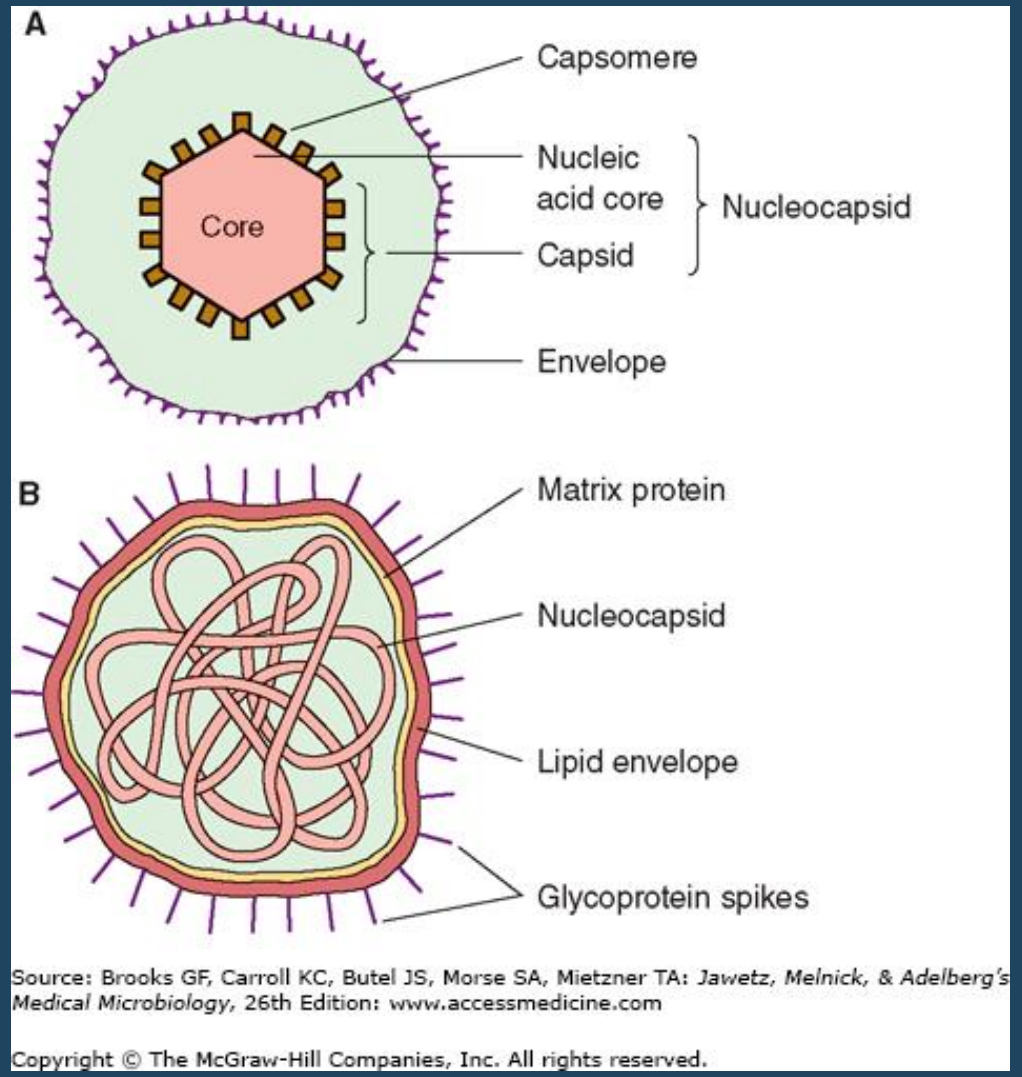


Capsid: The protein shell, or coat, that encloses the nucleic acid genome.

Capsomeres: Morphologic units seen in the electron microscope on the surface of icosahedral virus particles.

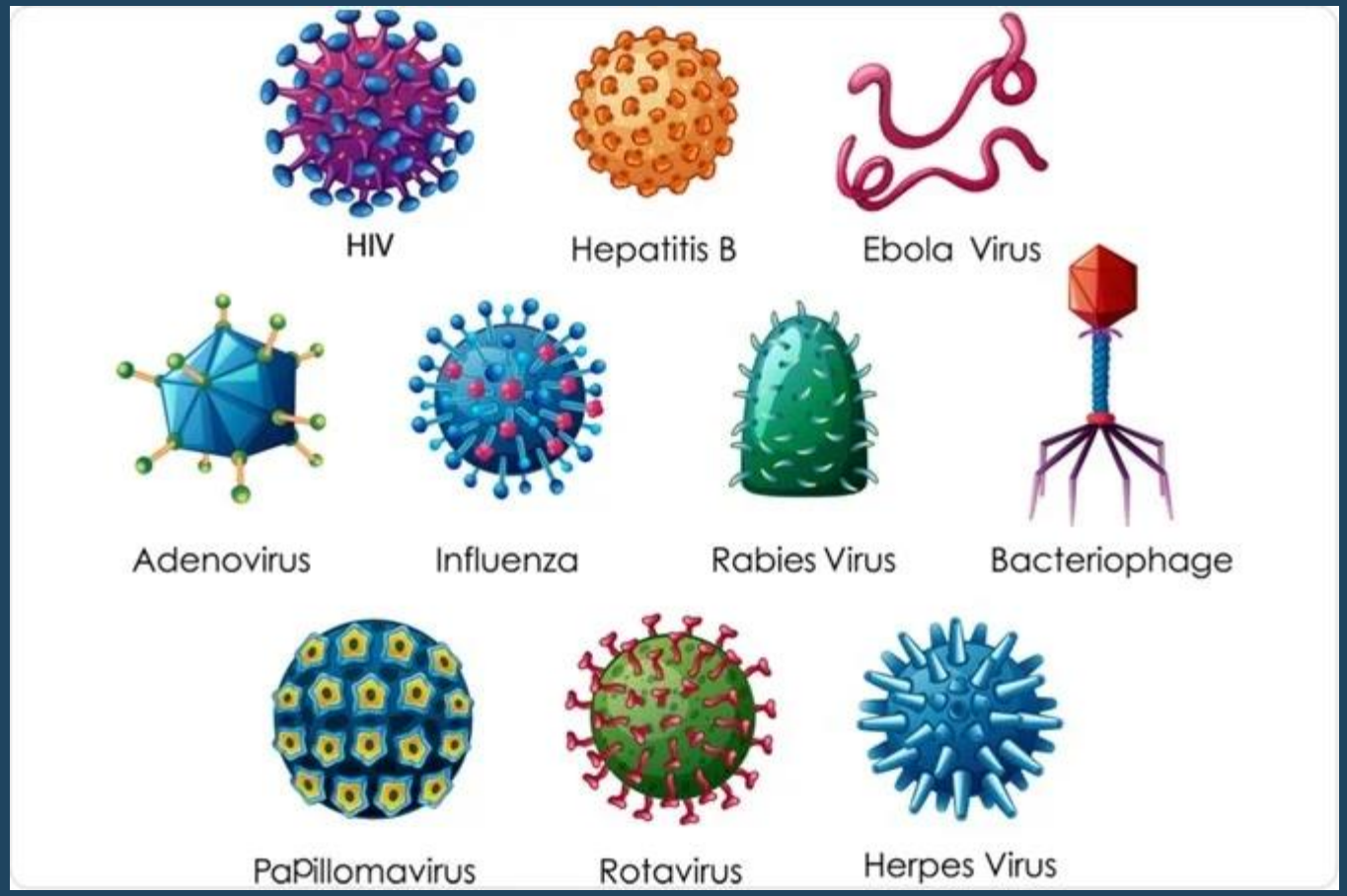
Envelope: A lipid-containing membrane that surrounds some virus particles. It is acquired during viral maturation by a budding process through a cellular membrane.

Nucleocapsid: The protein–nucleic acid complex representing the packaged form of the viral genome.



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology*, 26th Edition: www.accessmedicine.com

human viruses



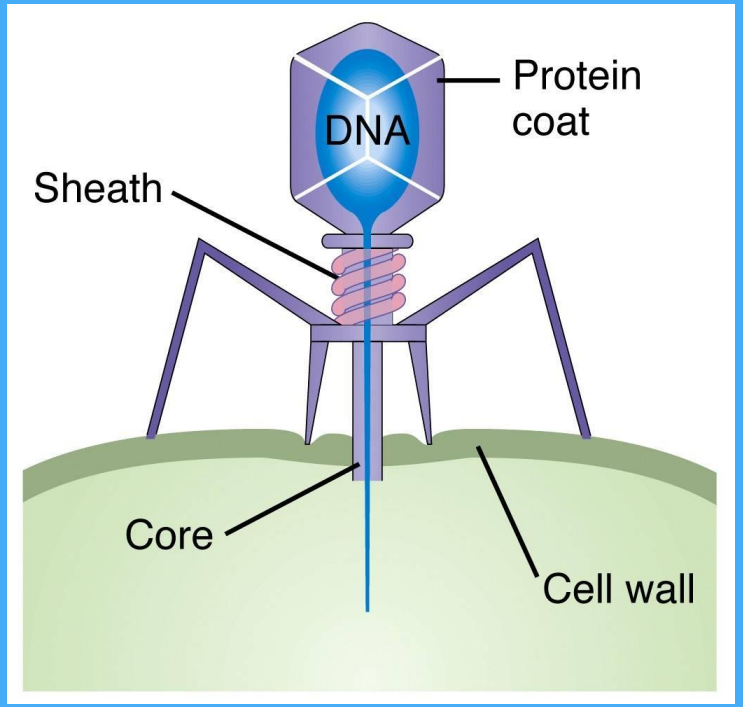
AIDS.
Common cold.
Ebola.
Genital herpes.
Influenza.
Measles.
Chickenpox and shingles.
Coronavirus disease 2019 (COVID-19)

ANIMAL VIRAL DISEASE:

- African horse sickness. ...
- African swine fever (ASF) ...
- Bluetongue. ...
- BRSV. ...
- Coronavirus. ...
- Crimean Congo haemorrhagic fever. ...
- Equine infectious anaemia. ...
- Equine viral arteritis.

BACTERIOPHAGES

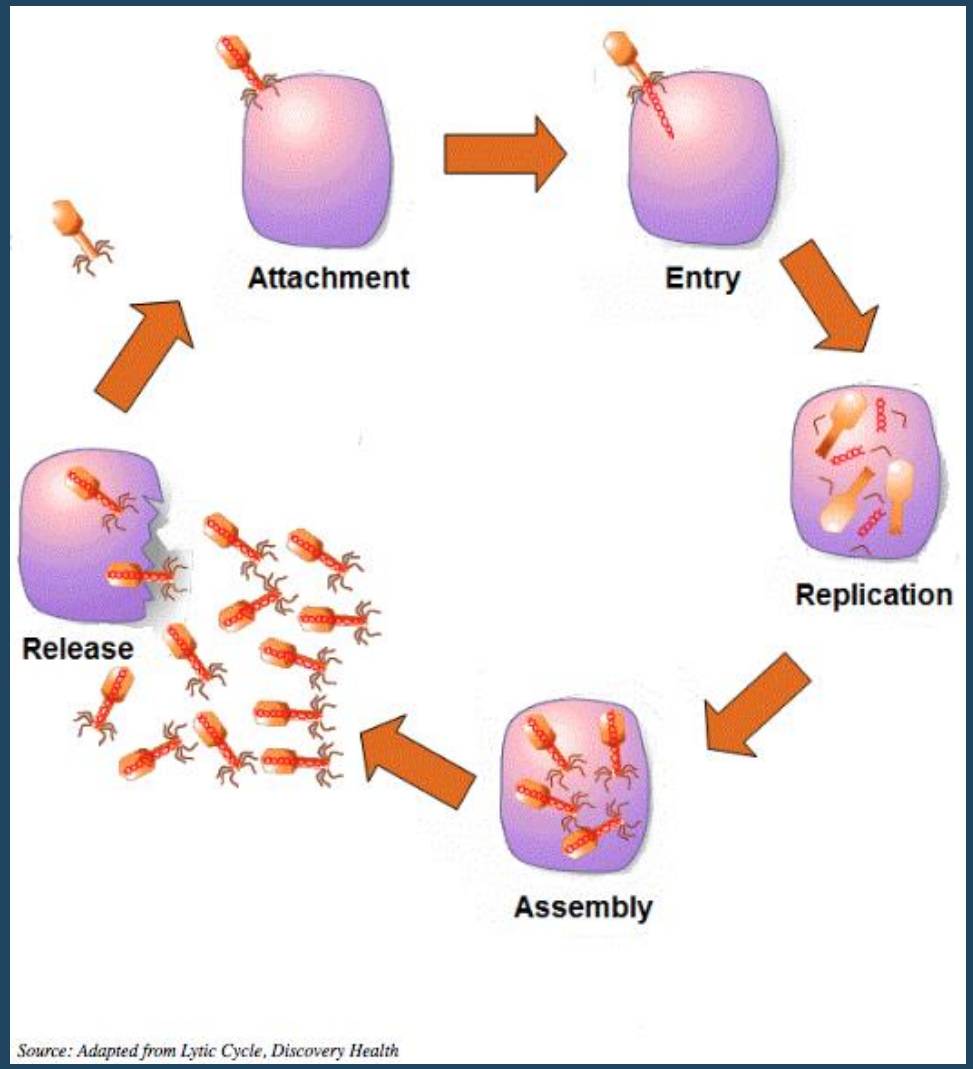
- Bacteriophages are viruses that attack bacteria.
- The name means "bacteria eaters" and is commonly shortened to just "phage".
- Phage particles comprise a **"head"** that contains DNA packaged within a protein coat, and a hollow **"tail"** by which they attach to the outside membrane of bacteria.
- The phage DNA is injected into the bacterium, where it uses the cell's replication machinery to reproduce itself.
- Production of new phage particles causes the host cell to **rupture** ("lyse") and release the phage, which goes on to infect other bacteria.



REPRODUCTION OF VIRUSES

LYTIC CYCLE

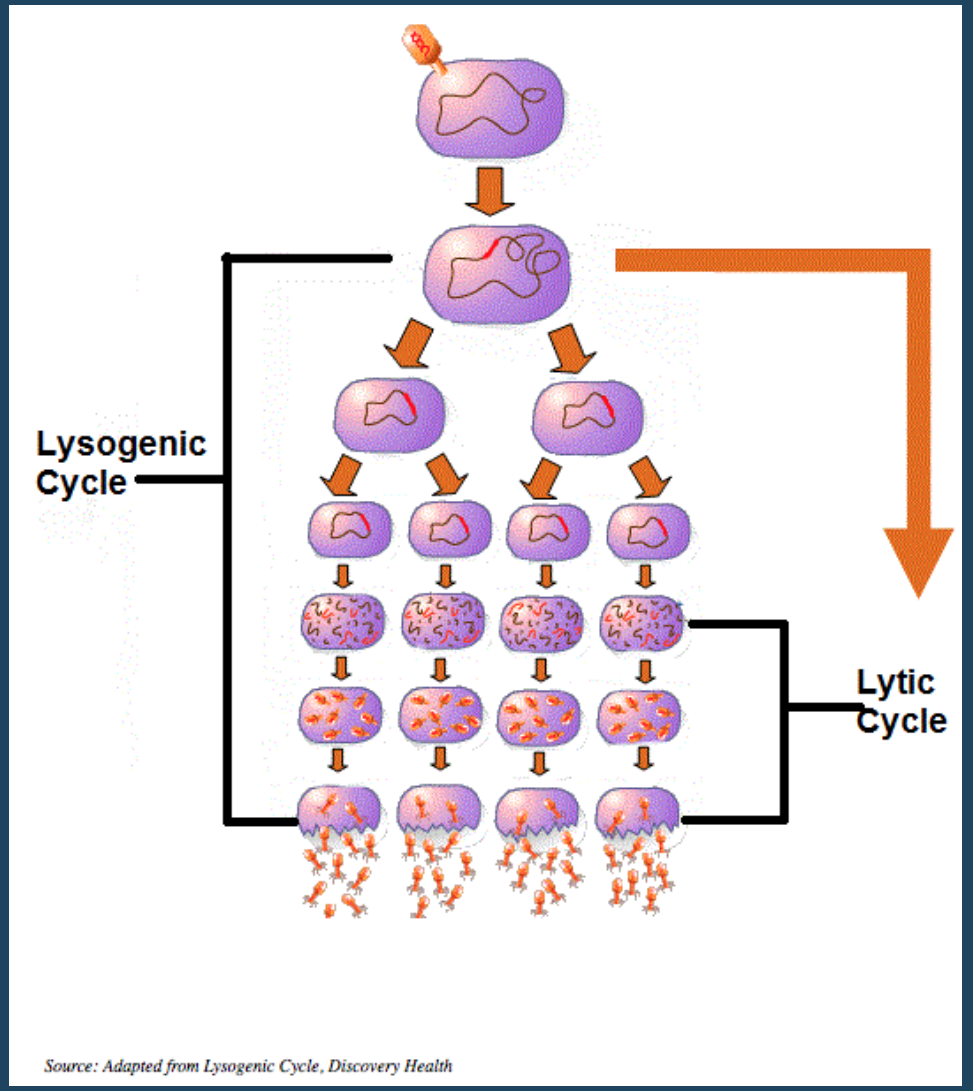
- **Attachment:** Virus attaches to the host cell.
- **Entry:** Genetic material is injected into the host cell.
- **Replication:** The virus takes over the cell's metabolism, causing the creation of new proteins and nucleic acids by the host cell's organelles.
- **Assembly:** Proteins and nucleic acids are assembled into new viruses.
- **Release:** Virus enzymes cause the cell to burst, and viruses are released from the host cell. These new viruses can infect other cells.



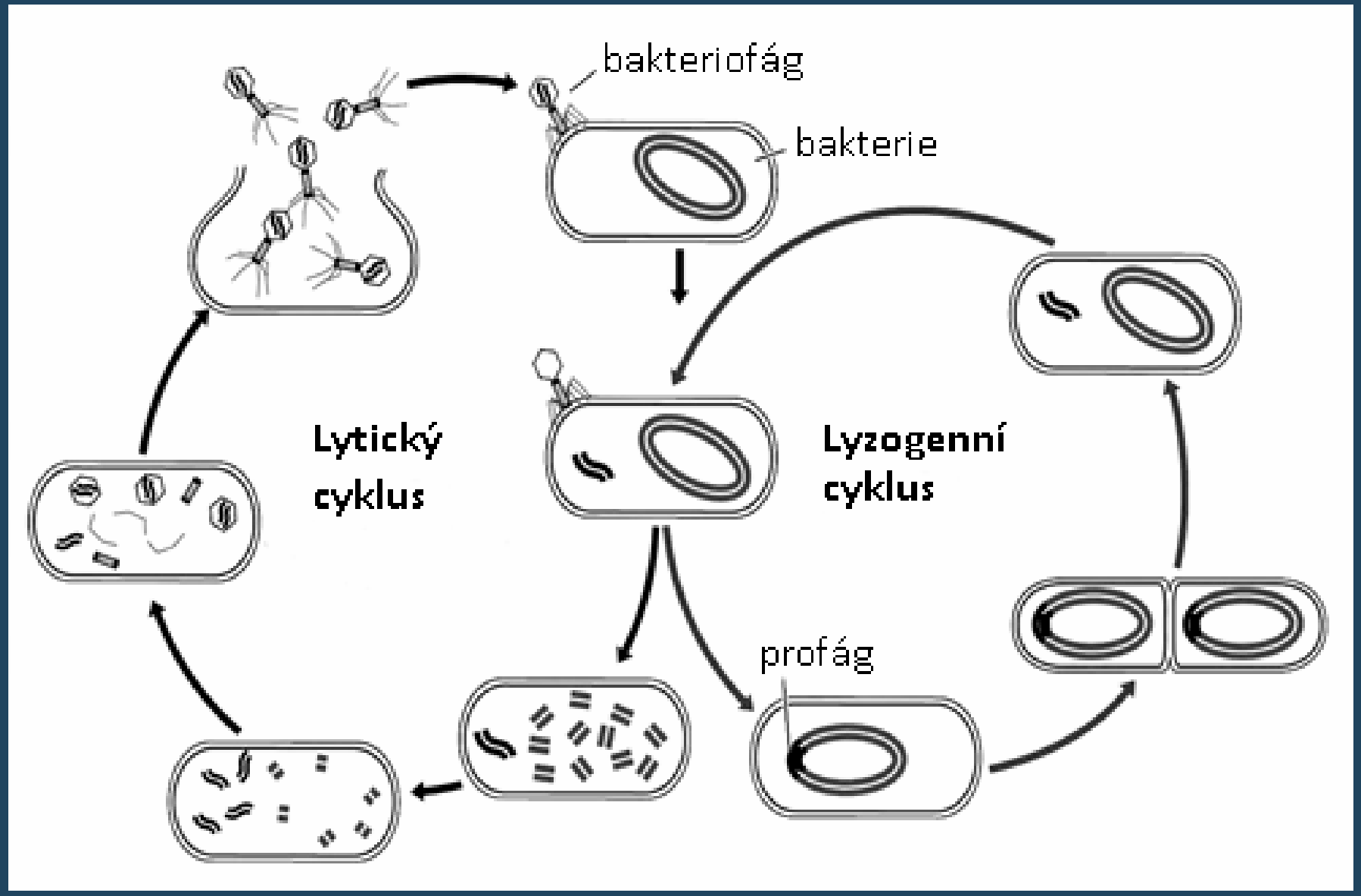
Source: Adapted from Lytic Cycle, Discovery Health

LYSOGENIC INFECTION

- **Attachment:** The virus attaches to the host cell.
- **Entry:** Genetic material is injected into the host cell.
- **Integration:** Viral DNA integrates into the host cell's genome.
- **Replication** (lysogenic cycle): When the host cell replicates, viral DNA is copied along with host cell DNA. Each new daughter cell is infected with the virus.
- **Induction:** When the infected cells are exposed to certain environmental conditions, viral DNA is activated and enters the lytic cycle.
- **Replication** (lytic cycle): The virus takes over the cell's metabolism, causing the creation of new proteins and nucleic acids by the host cell's organelles.
- **Assembly:** Proteins and nucleic acids are assembled into new viruses.
- **Release:** Virus enzymes cause the cell to burst and viruses are released from the host cell. These new viruses can infect other cells.



Source: Adapted from Lysogenic Cycle, Discovery Health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Endonuclease fingerprint indicates a synthetic origin of SARS-CoV-2

Valentin Bruttel, Alex Washburne, Antonius VanDongen

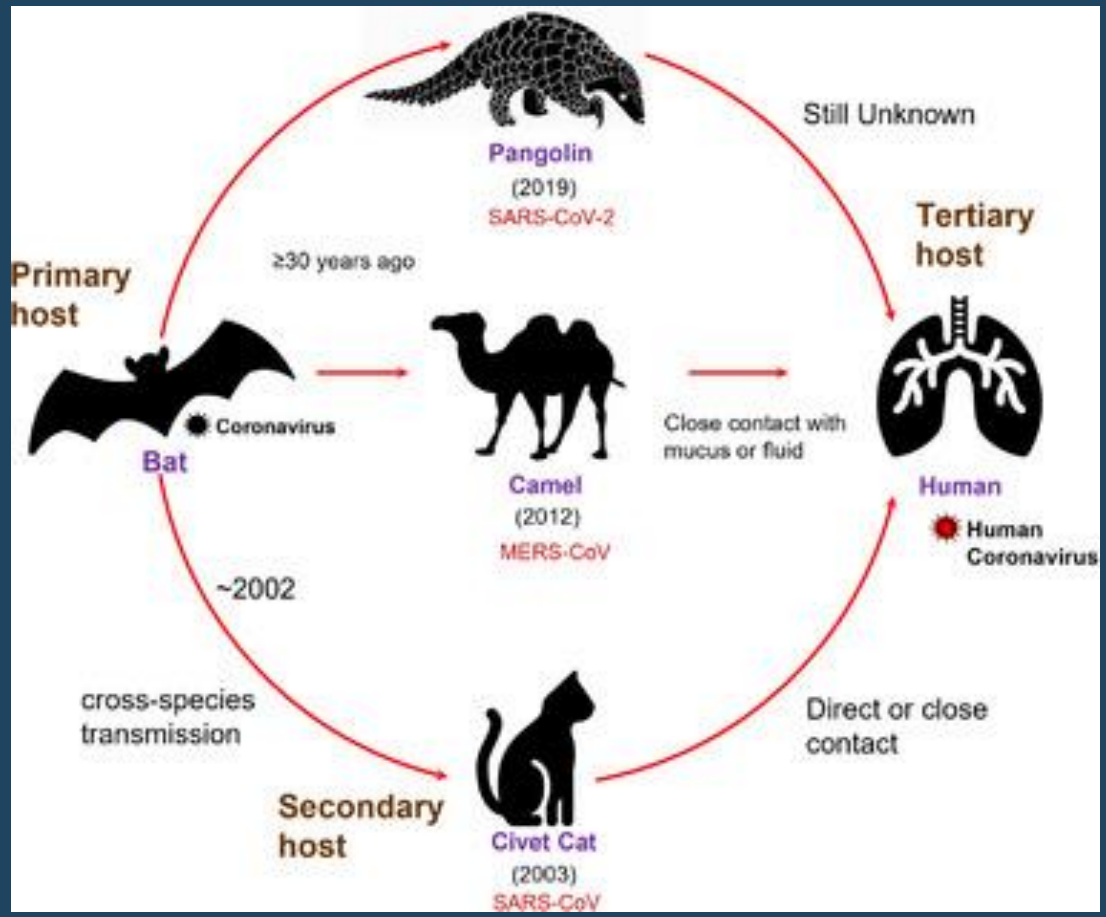
doi: https://doi.org/10.1101/2022.10.18.512756

Abstract

To prevent future pandemics, it is important that we understand whether SARS-CoV-2 spilled over directly from animals to people, or indirectly in a laboratory accident. The genome of SARS-COV-2 contains a peculiar pattern of unique restriction endonuclease recognition sites allowing efficient dis- and re-assembly of the viral genome characteristic of synthetic viruses. Here, we report the likelihood of observing such a pattern in coronaviruses with no history of bioengineering. We find that SARS-CoV-2 is an anomaly, more likely a product of synthetic genome assembly than natural evolution. The restriction map of SARS-CoV-2 is consistent with many previously reported synthetic coronavirus genomes, meets all the criteria required for an efficient reverse genetic system, differs from closest relatives by a significantly higher rate of synonymous mutations in these synthetic-looking recognitions sites, and has a synthetic fingerprint unlikely to have evolved from its close relatives. We report a high likelihood that SARS-CoV-2 may have originated as an infectious clone assembled *in vitro*.

Lay Summary To construct synthetic variants of natural coronaviruses in the lab, researchers often use a method called *in vitro* genome assembly. This method utilizes

SCHEMATIC DIAGRAM OF THE TRANSMISSION PROCESS OF THREE HCOVS. HUMANS ACQUIRED SARS-COV AND MERS-COV FROM BATS THROUGH CIVET CATS AND DROMEDARY CAMELS, RESPECTIVELY. IT IS UNCLEAR HOW SARS-COV-2 SPREAD TO HUMANS.



The origins of COVID-19 pandemic: A brief overview
Ying-Jian Hao, 1 Yu-Lan Wang, 1 Mei-Yue Wang, 1 Lan Zhou, 1 Jian-Yun Shi, 1 Ji-Min Cao,corresponding author 1 and De-Ping Wangcorresponding author 1

Coronavirus

Virus classification

(unranked): Virus

Realm: Riboviria

Kingdom: Orthornavirae

Phylum: Pisuviricota

Class: Pisoniviricetes

Order: Nidovirales

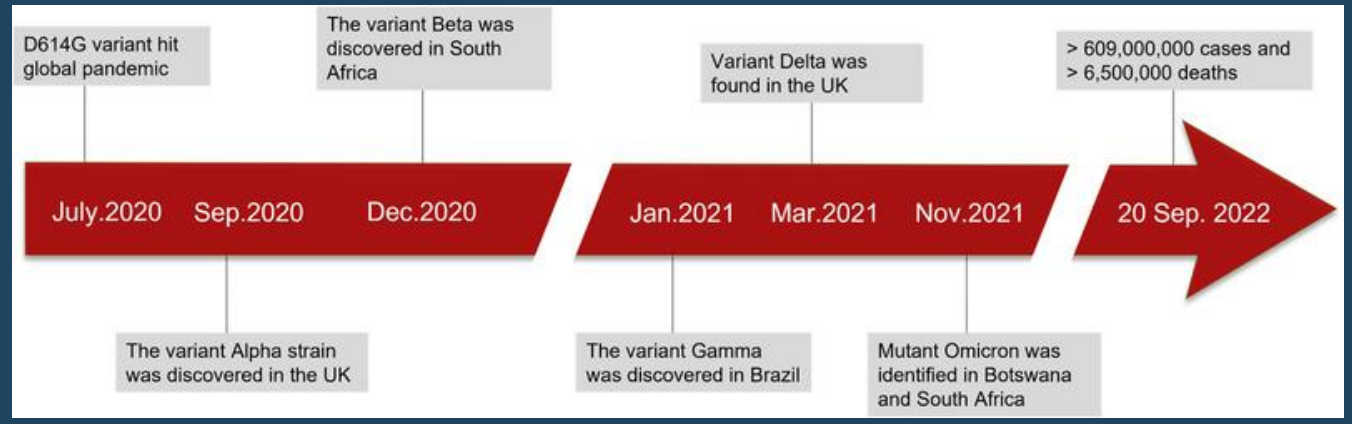
Family: Coronaviridae

Genus: Betacoronavirus

Subgenus: Sarbecovirus

Species: Severe acute respiratory syndrome–related coronavirus

Virus: **Severe acute respiratory syndrome coronavirus 2**



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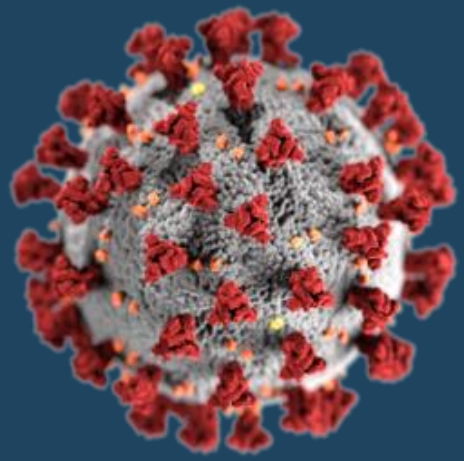
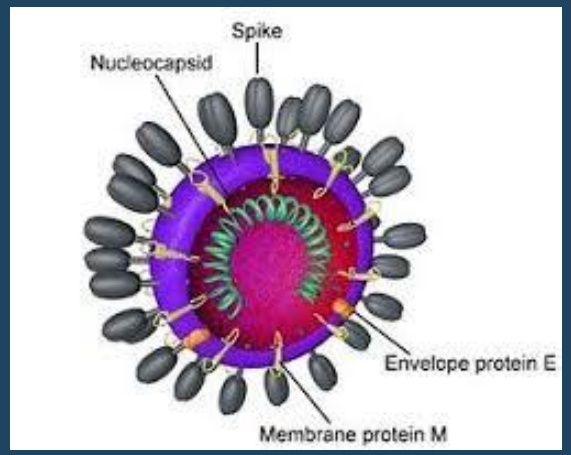
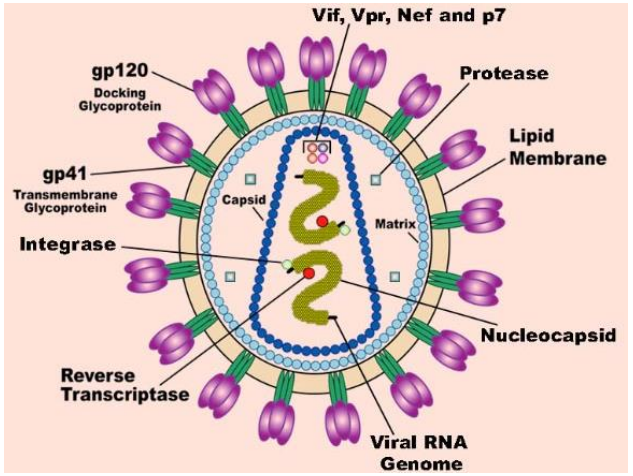




HIV - HUMAN IMMUNODEFICIENCY VIRUS

- AIDS from English Acquired Immune Deficiency Syndrome, also Acquired Immunodeficiency Syndrome), also acquired immune failure syndrome.
- an enveloped RNA virus from the lentivirus genus, belonging to the retroviridae family.
- AIDS is one of the most serious infectious diseases today.
- It occurs en masse, affects entire continents = pandemic.
- Around 1981, the first cases of AIDS in the United States were observed in gay men, therefore the disease also received the temporary name GRID (Gay-Related Immune Deficiency).



It can spread through

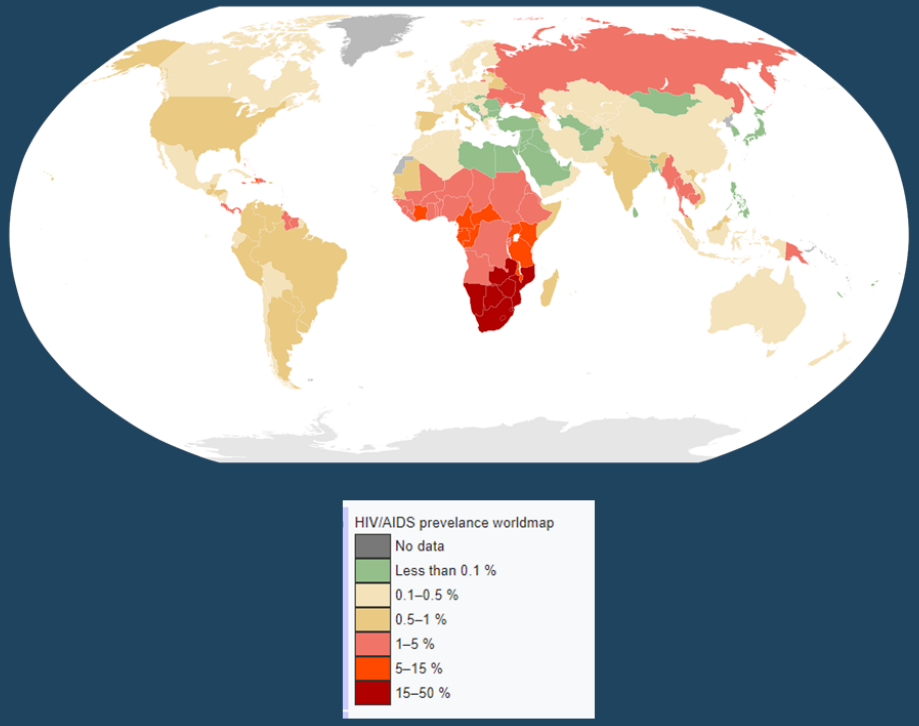
- sexual contact,
- illicit injection drug use or sharing needles,
- contact with infected blood,
- or from mother to child during pregnancy,
- childbirth or breastfeeding.
- HIV destroys CD4 T cells — white blood cells that play a large role in helping your body fight disease.

Key facts

- HIV remains a major global public health issue, having claimed 40.4 million [32.9–51.3 million] lives so far with ongoing transmission in all countries globally; with some countries reporting increasing trends in new infections when previously on the decline.

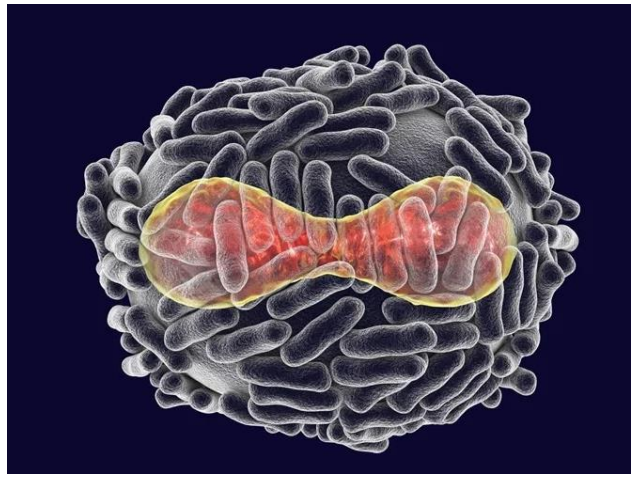
- There were an estimated 39.0 million [33.1–45.7 million] people living with HIV at the end of 2022, two thirds of whom (25.6 million) are in the WHO African Region.

- In 2022, 630 000 [480 000–880 000] people died from HIV-related causes and 1.3 million [1.0–1.7 million] people acquired HIV.



HIV. (2023, June 11). In Wikipedia. https://cs.wikipedia.org/wiki/HIV

40 mil of victomes



SMALLPOX

- is considered one of the greatest epidemic disease scourges in human history.
- Smallpox is an acute infectious disease caused by the variola virus. The term "smallpox" was initially introduced in Europe in the 15th century and is also known by the Latin names "Variola" or "Variola vera,"
- Edward Jenner
- Karel Raška
- 1980 eradicated

VIROIDS

- the smallest infectious pathogens.
- They are composed solely of a short strand of circular, single-stranded RNA without protein coat. All known viroids are inhabitants of **higher plants**

- The first recognized viroid, the pathogenic agent of the potato spindle tuber disease, was discovered, initially molecularly characterized, and named by Theodor Otto Diener,

SATELITS (VIRUSOIDS)

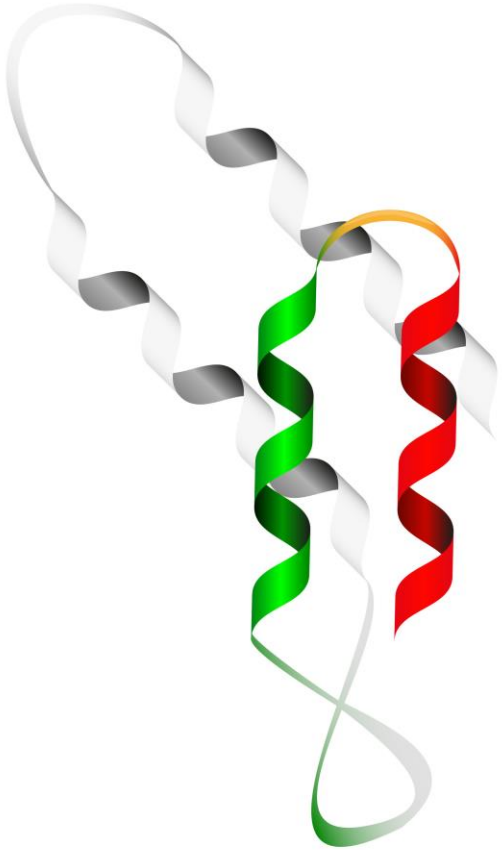
- are separate short nucleic acid molecules (DNA or RNA)
- cause diseases in plants
- discovered only in 1981
- they can not replicate independently but require a **helper virus** in which they are closed (they are, therefore, some "**parasites of other viruses**")
- do not encode any protein, replicate in the cytoplasm.

PRIONS

- infectious proteins (without NK) are encoded by the structural hosts of the host organism
- They result from a conformational change of PrPC prion protein with a spatial structure of? -Helix (helix) on PrPSc with β-structure (folded leaf)
- Prions are agents of transmissible spongiform encephalopathy (spongiform appearance of degenerated central nervous system tissue)
- In humans: Creutzfeldt-Jakob disease and Kuru,
- Sheep and goat scrapie,
- Bovine spongiform encephalopathy (BSE)
- Feline spongiform encephalopathy (FSE)

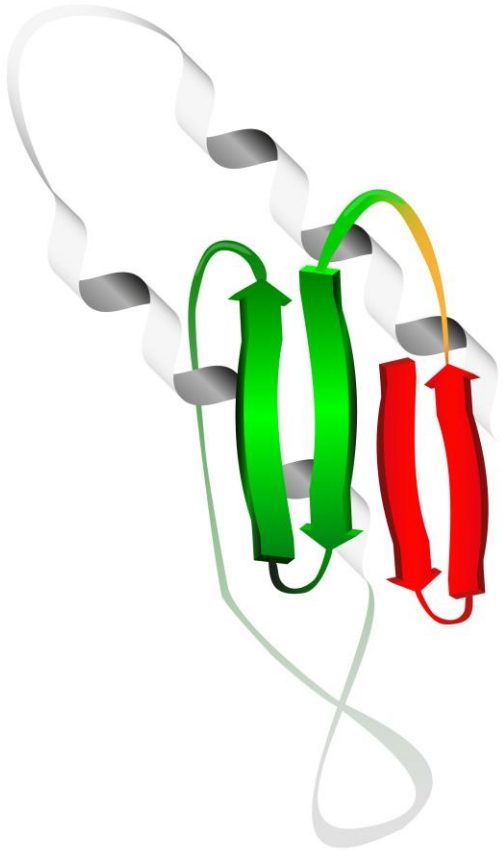
PrP^{C}

is a normal protein



PrP ^{Sc}

the disease-causing form of the prion protein



PRIONS

- Prions, named for their description as "proteinaceous and infectious particles", lack any detectable nucleic acids or virus-like particles. They resist inactivation procedures that normally affect nucleic acids.

- Mammalian prions: Agents of spongiform encephalopathies
- Fungal prions

Affected animal(s)	Disease
sheep, goat	Scrapie[42]
cattle	Bovine spongiform encephalopathy (BSE), mad cow disease[42]
mink	Transmissible mink encephalopathy (TME)
white-tailed deer, elk, mule deer, moose	Chronic wasting disease (CWD)
cat	Feline spongiform encephalopathy (FSE)
nyala, oryx, greater kudu	Exotic ungulate encephalopathy (EUE)
ostrich	Spongiform encephalopathy (Has not been shown to be transmissible.)
human	Creutzfeldt–Jakob disease (CJD)[42]
	Iatrogenic Creutzfeldt–Jakob disease (iCJD)
	Variant Creutzfeldt–Jakob disease (vCJD)
	Familial Creutzfeldt–Jakob disease (fCJD)
	Sporadic Creutzfeldt–Jakob disease (sCJD)
	Gerstmann–Sträussler–Scheinker syndrome(GSS)[42]
	Fatal familial insomnia (FFI)[44]
	Kuru[42]
	Familial spongiform encephalopathy[45]
	Multiple System Atrophy (MSA): Not a TSE and is not by typical prions Prp/PrP^{Sc} but by a misfolded α-Synuclein.[46]

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) MAD COW DISEASE

- transmissible spongiform encephalopathy and fatal neurodegenerative disease in cattle
- causes a spongiform degeneration of the brain and spinal cord.
- has a long incubation period, of 2.5 to 5 years
- Transmission of prions - cannibalism - MBM

- vertically (mother-fetus) - no

- horizontally (excretions) – no

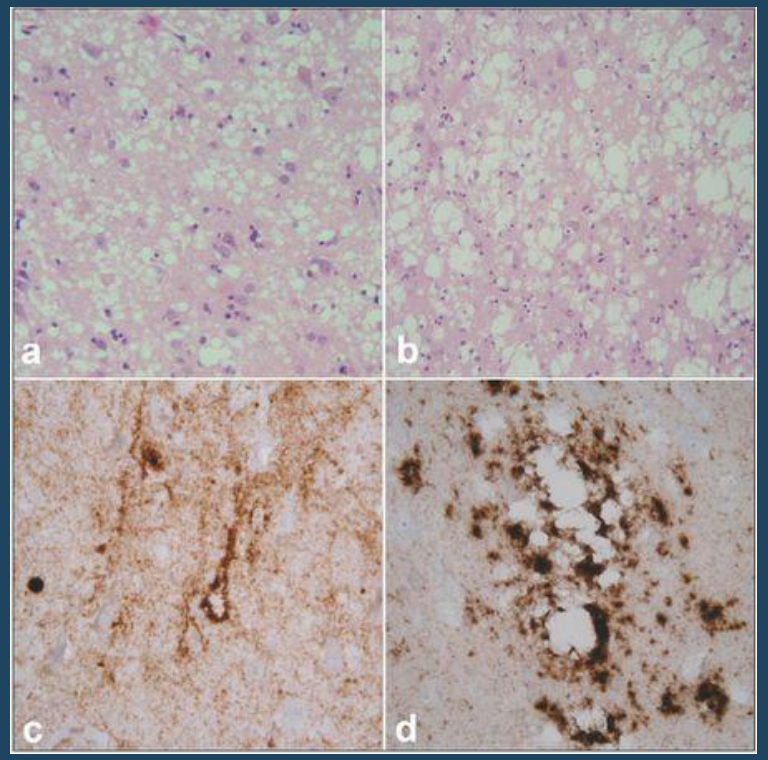
- Transmission to humans – new Variant of Creutzfeldt–Jakob disease (vCJD or nvCJD), and by June 2014 it had killed 177 people in the United Kingdom

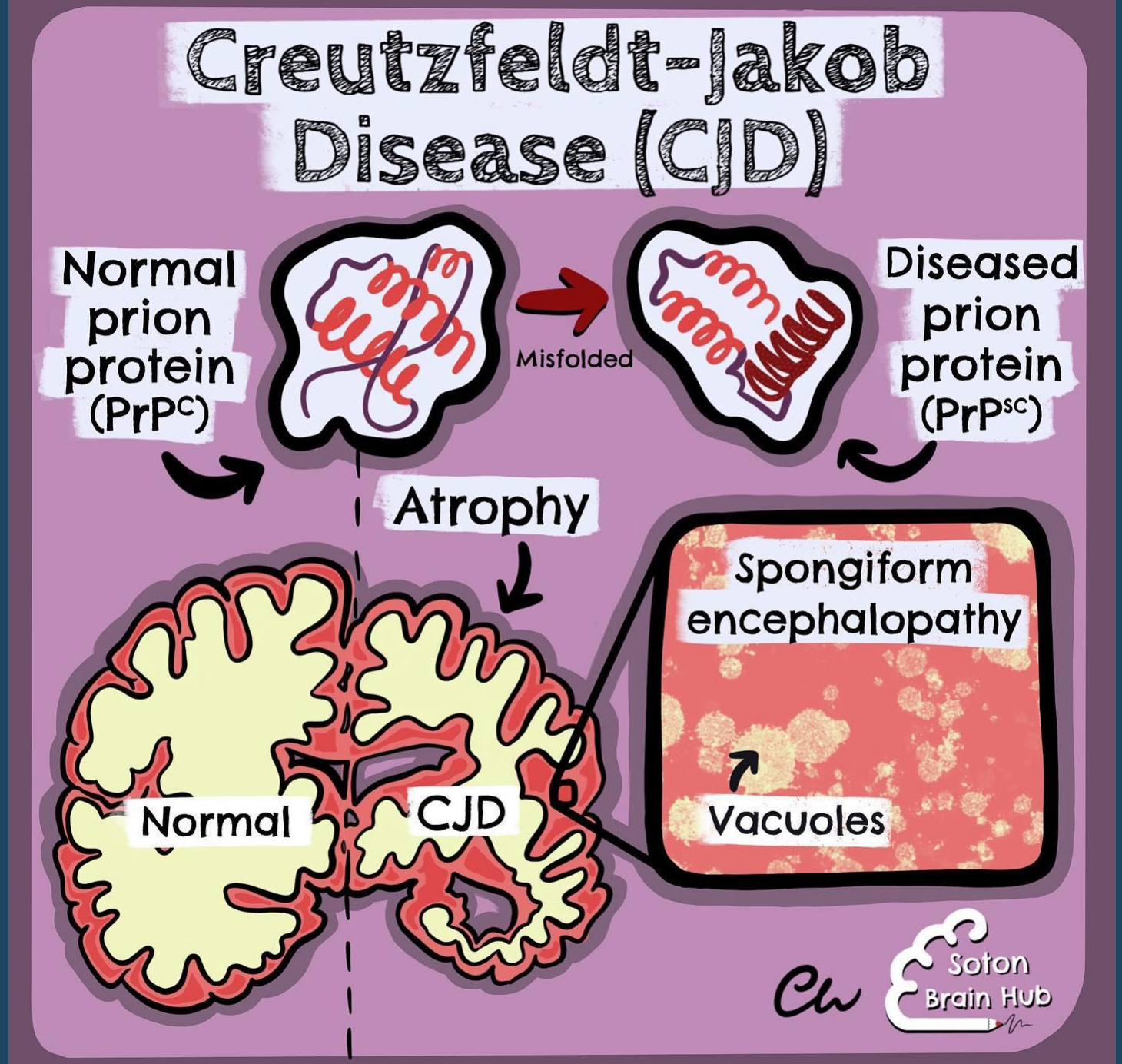
BSE

- Precautions - Meat-bone meal must not be fed to ruminants

Confiscated - the skull, brain, spinal cord, eyes, intestines, lymph node, spleen

MBM (meal bone meals) - pieces up to 50 mm, 130-140C at 3 bars 20min.


a
b
c
d



Creutzfeldt-Jakob disease (CJD) is a very rare and fatal degenerative brain disorder. CJD is a type of prion disease, which is caused by an infectious protein.

KURU DISEASE

- The name kuru means “to shiver” or “trembling in fear.”
- The symptoms of the disease include muscle twitching and loss of coordination.
- Other symptoms include difficulty walking, involuntary movements, behavioral and mood changes, dementia, and difficulty eating.
- The latter can cause malnutrition.

- Thank you for your attention