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Safety Training

Open Wetlab

Goals of this training

General understanding of:

- Microbiology
- Biosafety jargon like contamination & infection
- Personal Protection Equipment
- Chemicals, MSDS and safety labels
- Handling flames and fire
- Sterile techniques
- Waste disposal
- Documentation

After reading these slides you may obtain an Open Wetlab Safety Certificate after successfully answering all questions of the safety test.


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Open Wetlab Safety Certificate

Has successfully completed the Safety Introduction Certificate training requirements.
Demonstrating sufficient knowledge of:

Personal protection equipment, safe and responsible laboratory behaviour, biological safety, waste management and documentation

July 2014

Pieter van Boheemen
Waag Society Open Wetlab

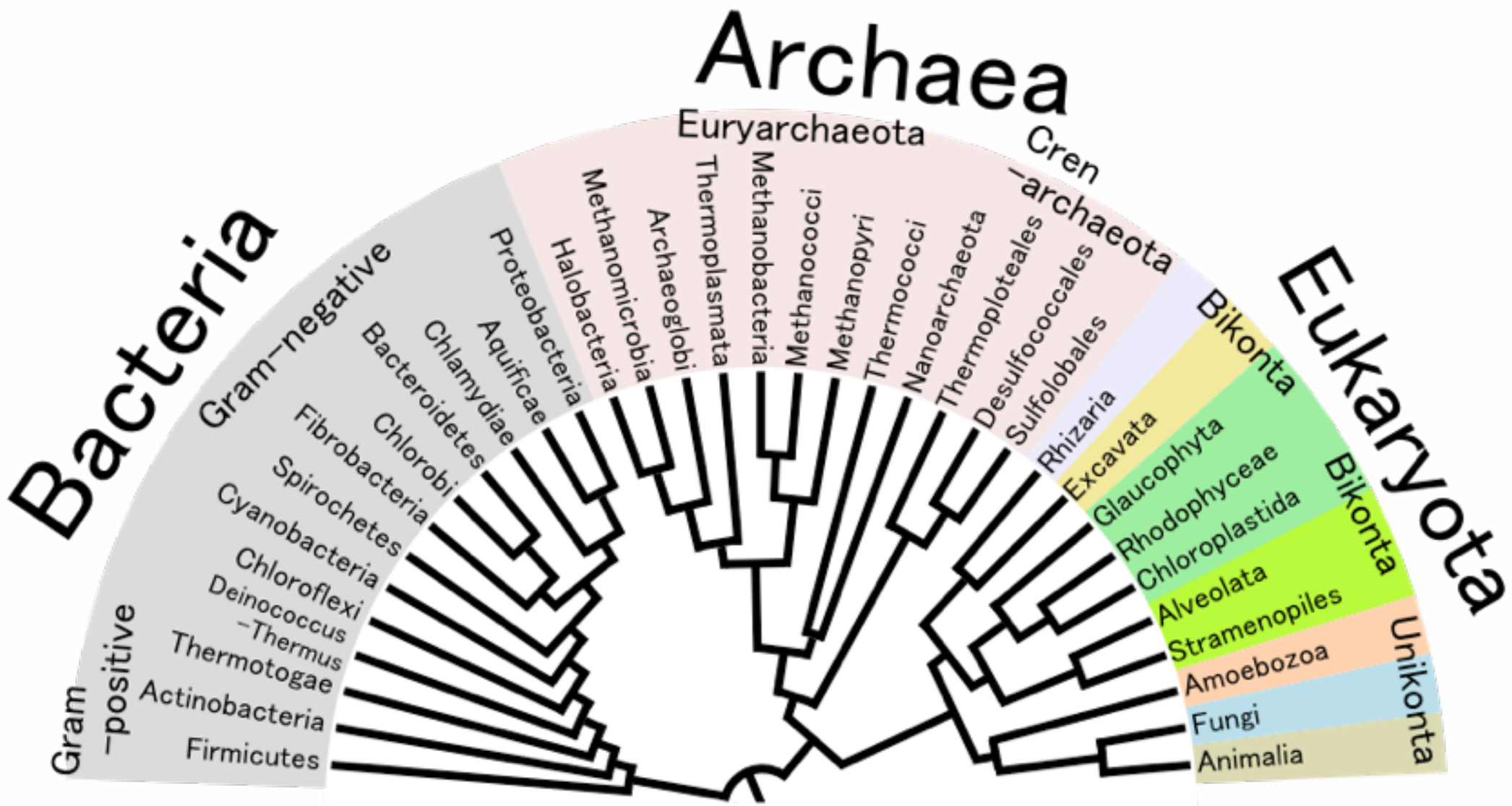
Importance of safety

Safe procedures protect:

1. The environment
2. Your colleagues
3. Yourself

Phylogeny of the living world

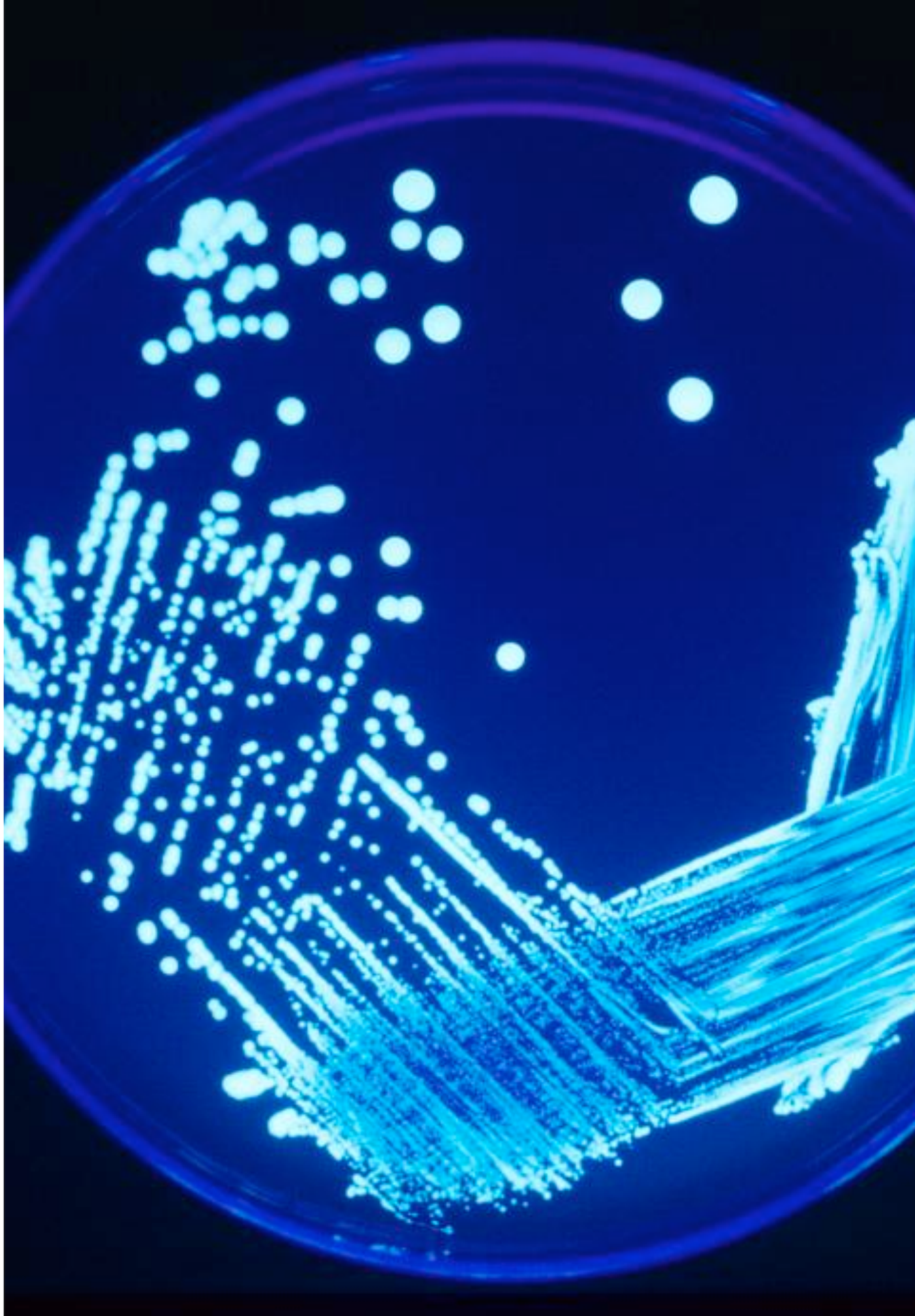
This image represents the classification and relation of living organisms. There is a huge diversity. Especially microbes have evolved the ability to live nearly anywhere. So it's best to assume their presence on anything that has not been sterilized.



Micro organisms

A typical micro organism is independently capable of:
- Growth
- Energy production
- Reproduction

But they can also have more interesting features, like bio luminescence (picture)



Sulfur (purple) bacteria bloom

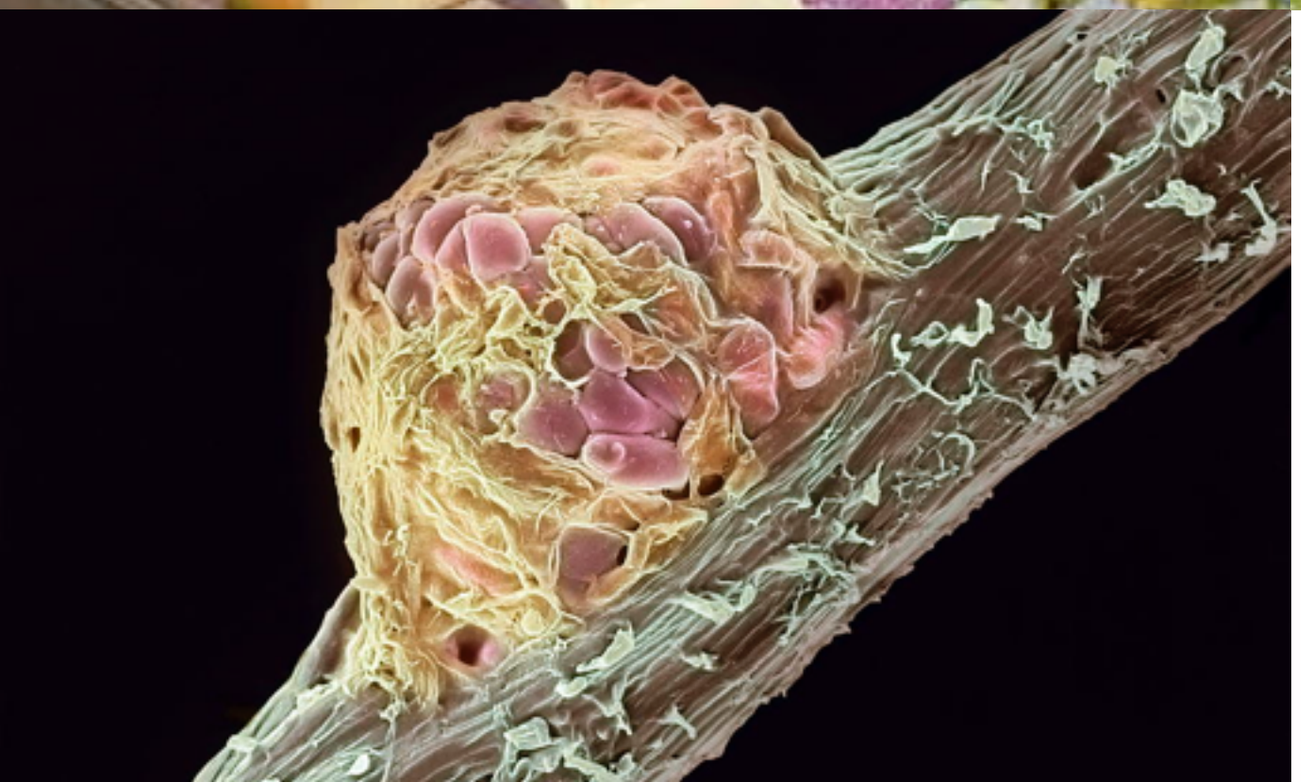
Microbes are capable of turning entire lakes red

Cyanobacteria (algae)

Capable of generating energy from sun light

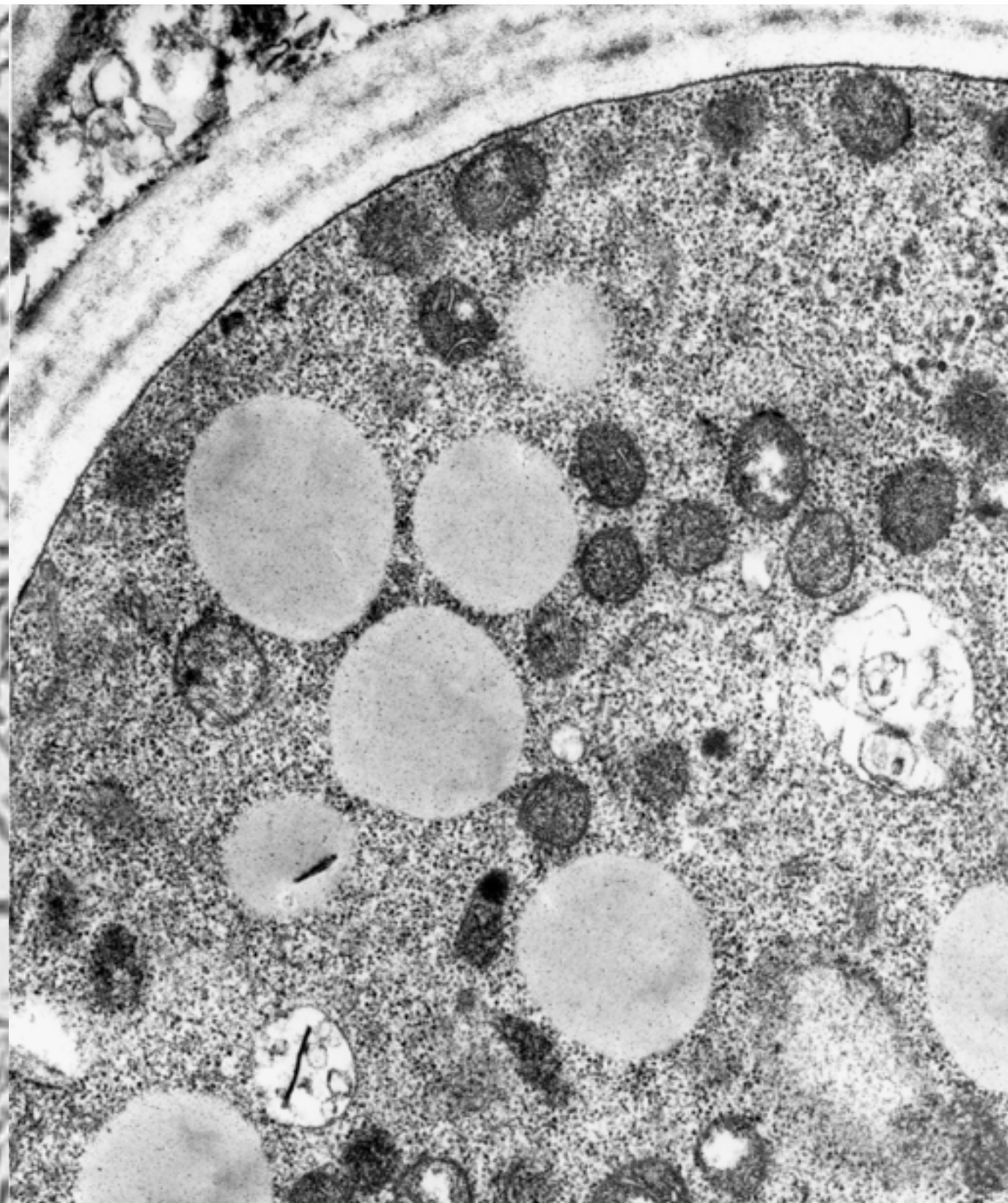
Importance of microbes

Microbes can make food (beer, cheese, etc), oxygen and fixate nitrogen



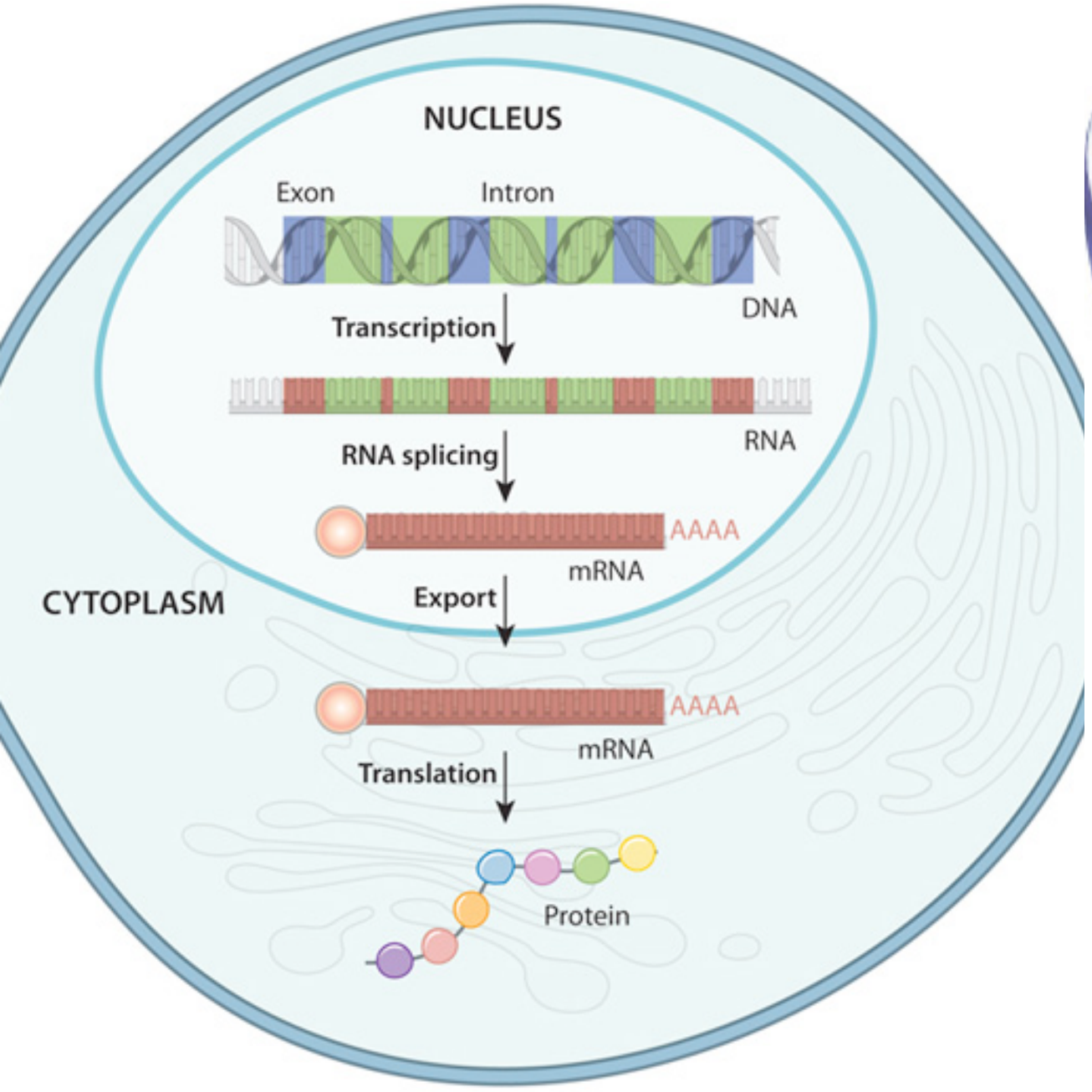
Life is made out of cells

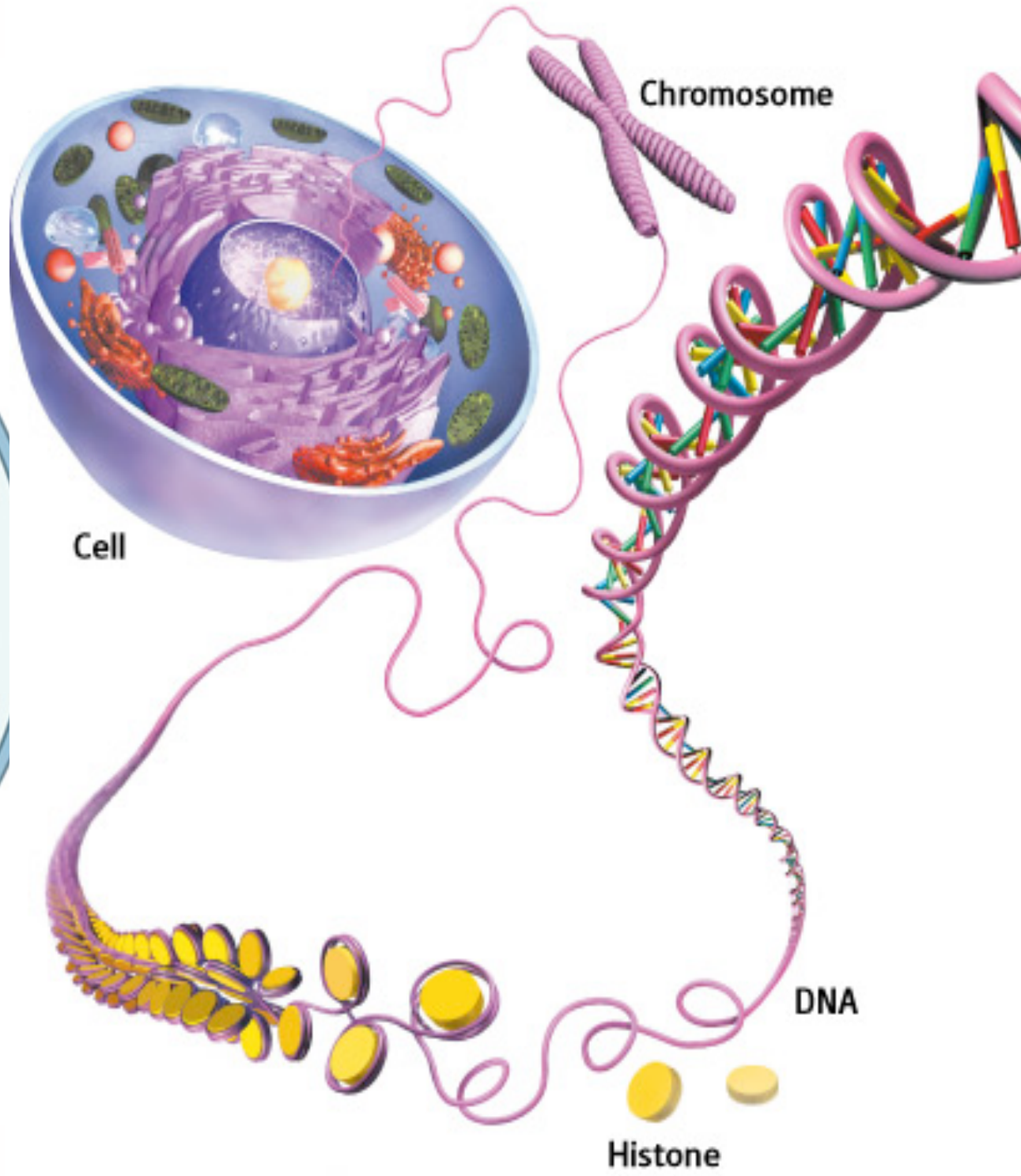
The pictures show low (left) and high (right) magnification of cells



Cells as machines

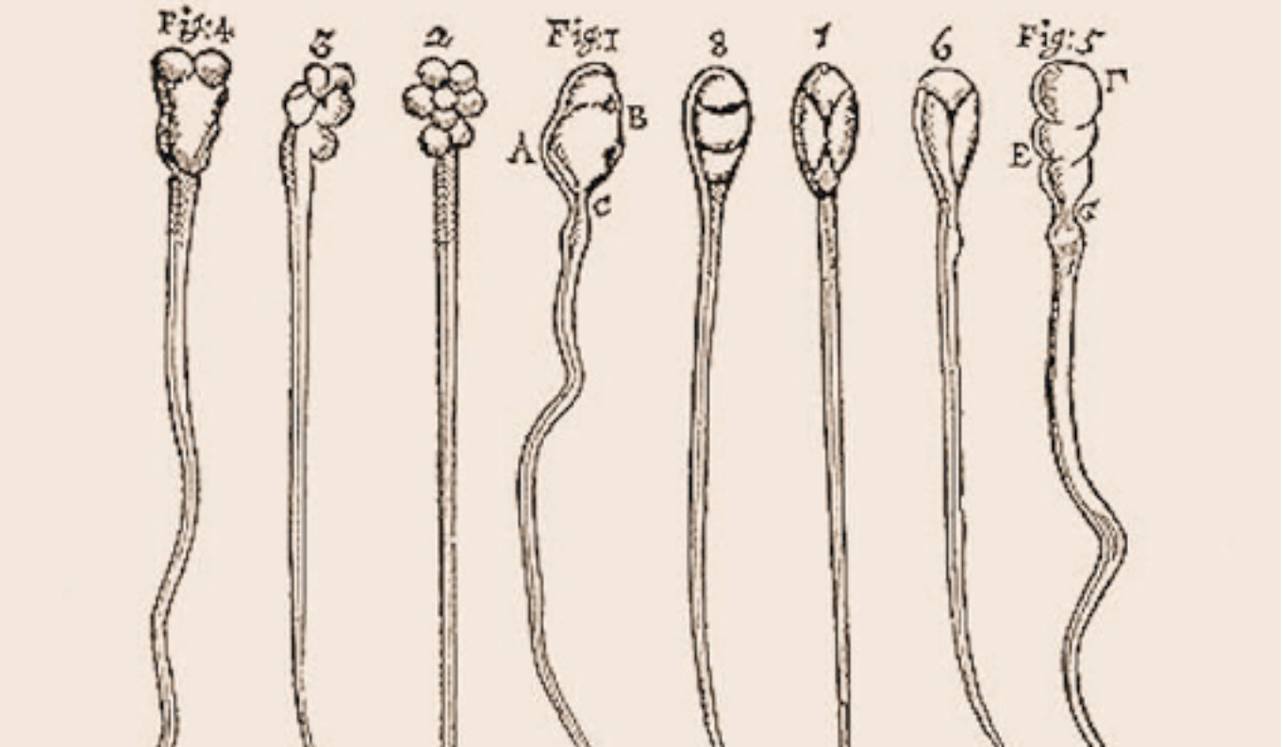
Biotechnology regards cells as molecular programmable machines





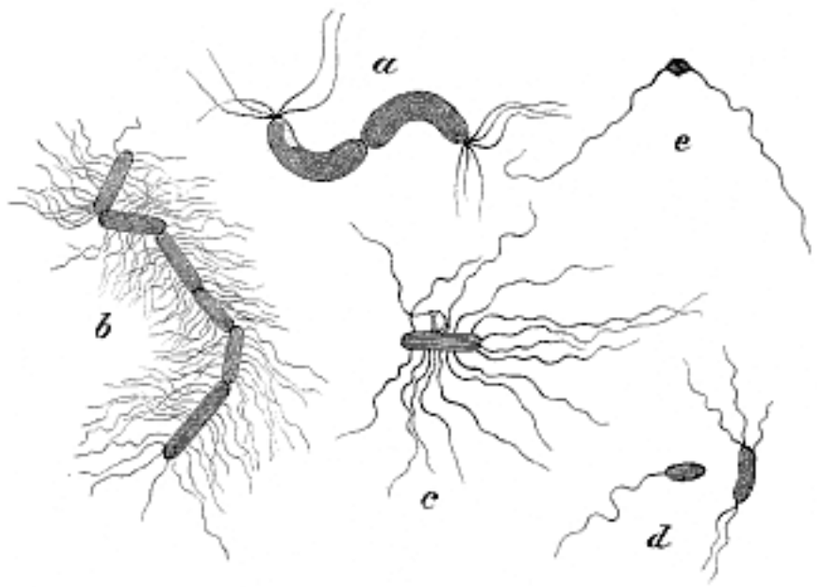
Antoni van Leeuwenhoek 1684

First person to visually observe individual microbes by microscope

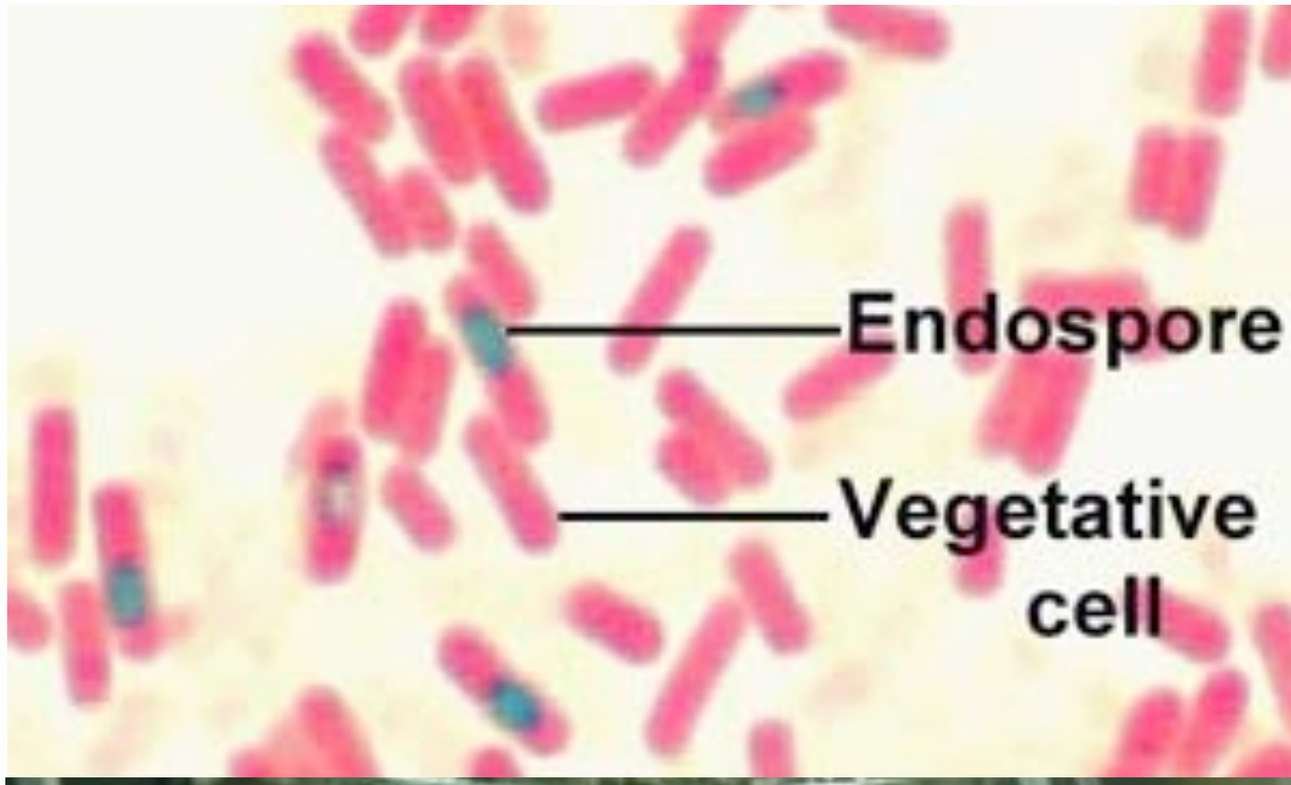


Ferdinand Cohn

Pure Bacillus subtilis cultures, thanks to heat-resistant endospores



Geißeln der Bakterien
a von Spirillum Undula in der Theilung; b vom Heubacillus (Bacillus subtilis), Faden bildend; c einzelnes Stäbchen des Heubacillus; d von Fäulnißbakterien (Ba-

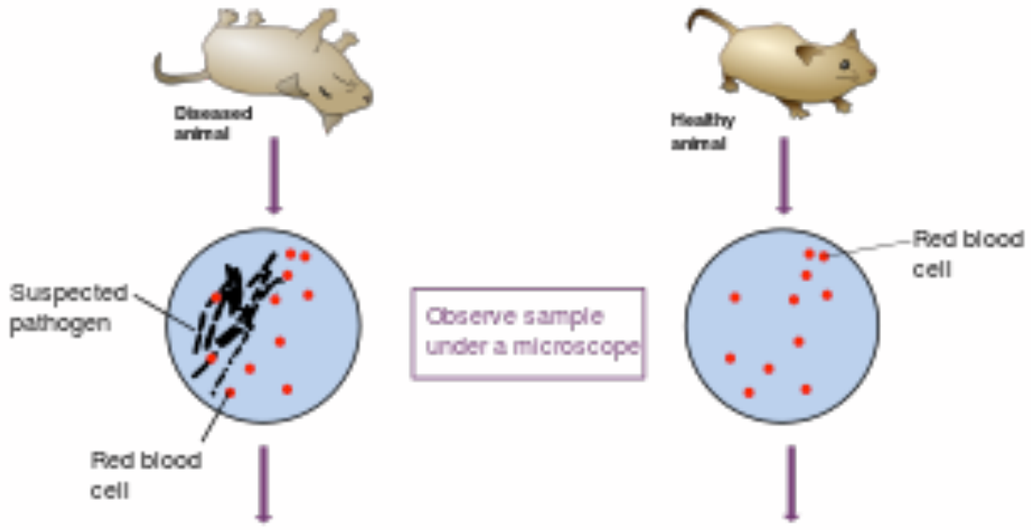


Koch Postulates

Methodology to proof microbes are causing illness

Koch's Postulates:

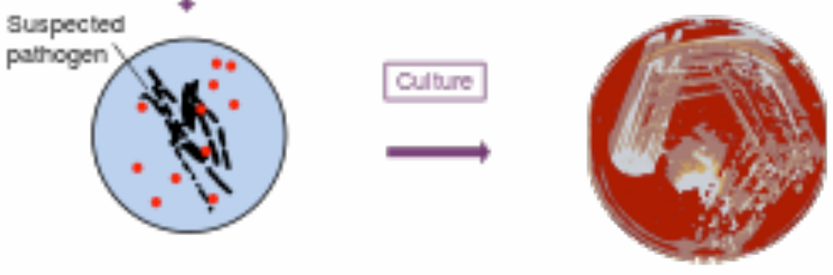
① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.



② The microorganism must be isolated from a diseased organism and grown in pure culture.

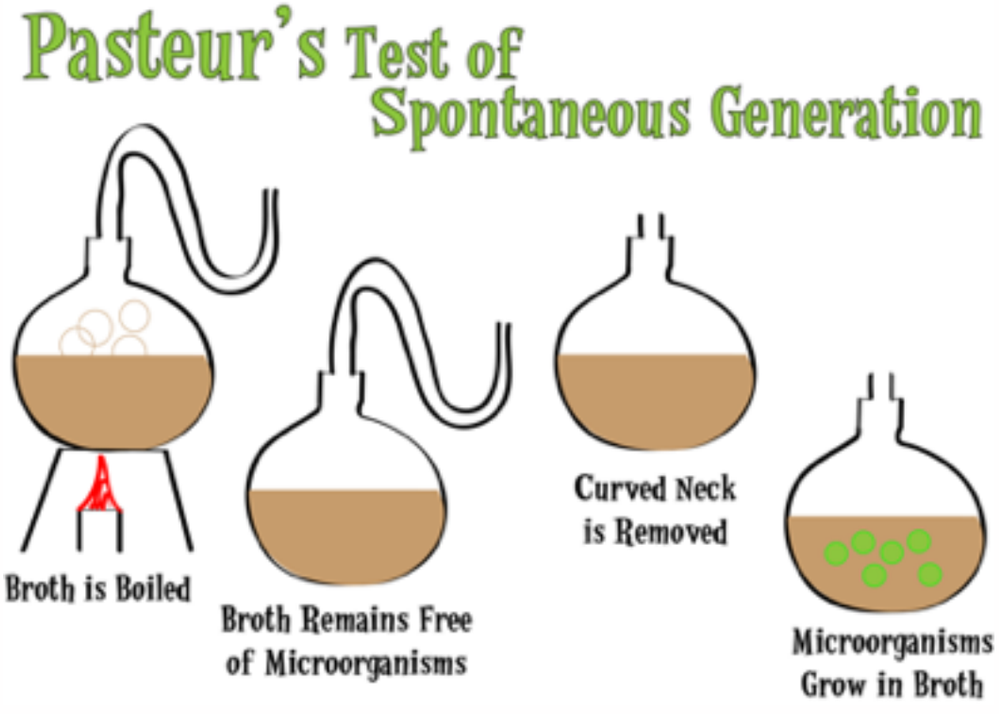
③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



Louis Pasteur

Proof that microbes do not “spontaneously appear”

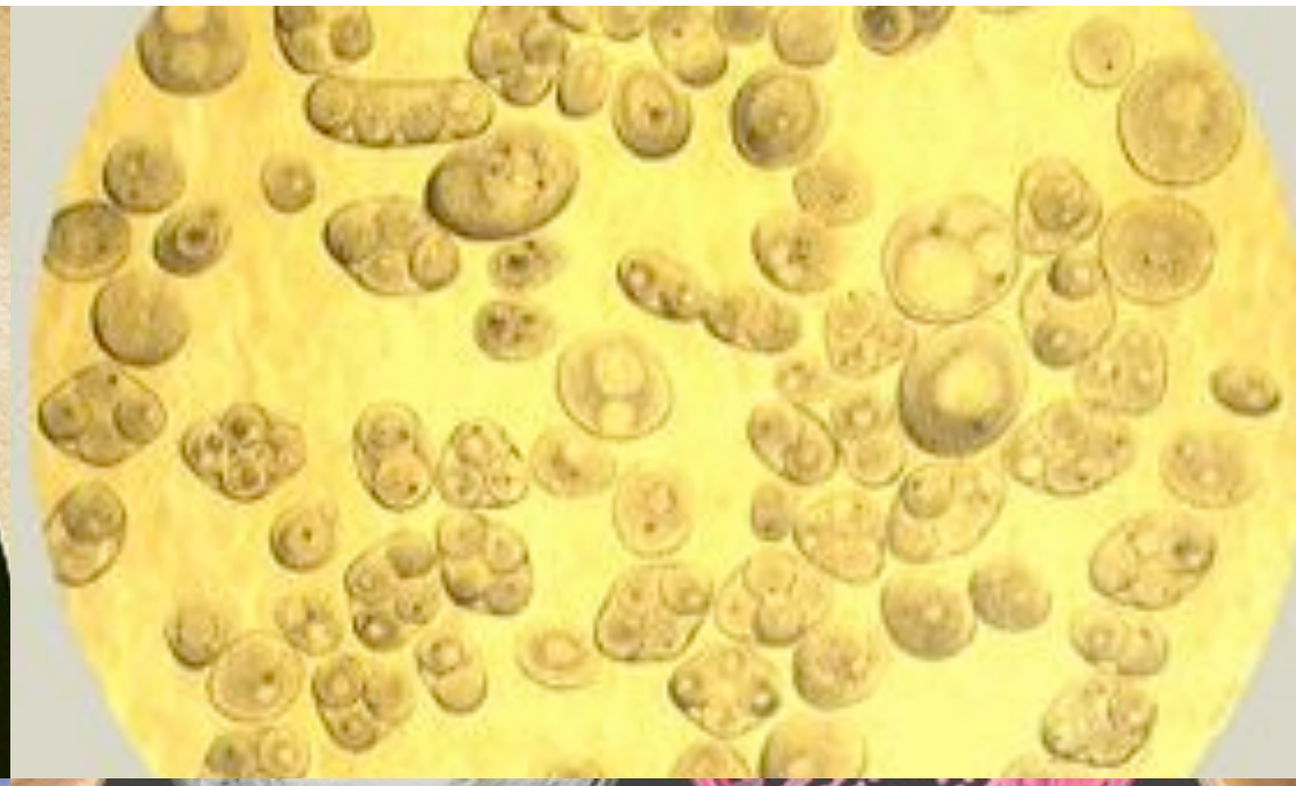


Richard Petri and agar agar

Invented agar dishes as a substitute growth medium for potato slices

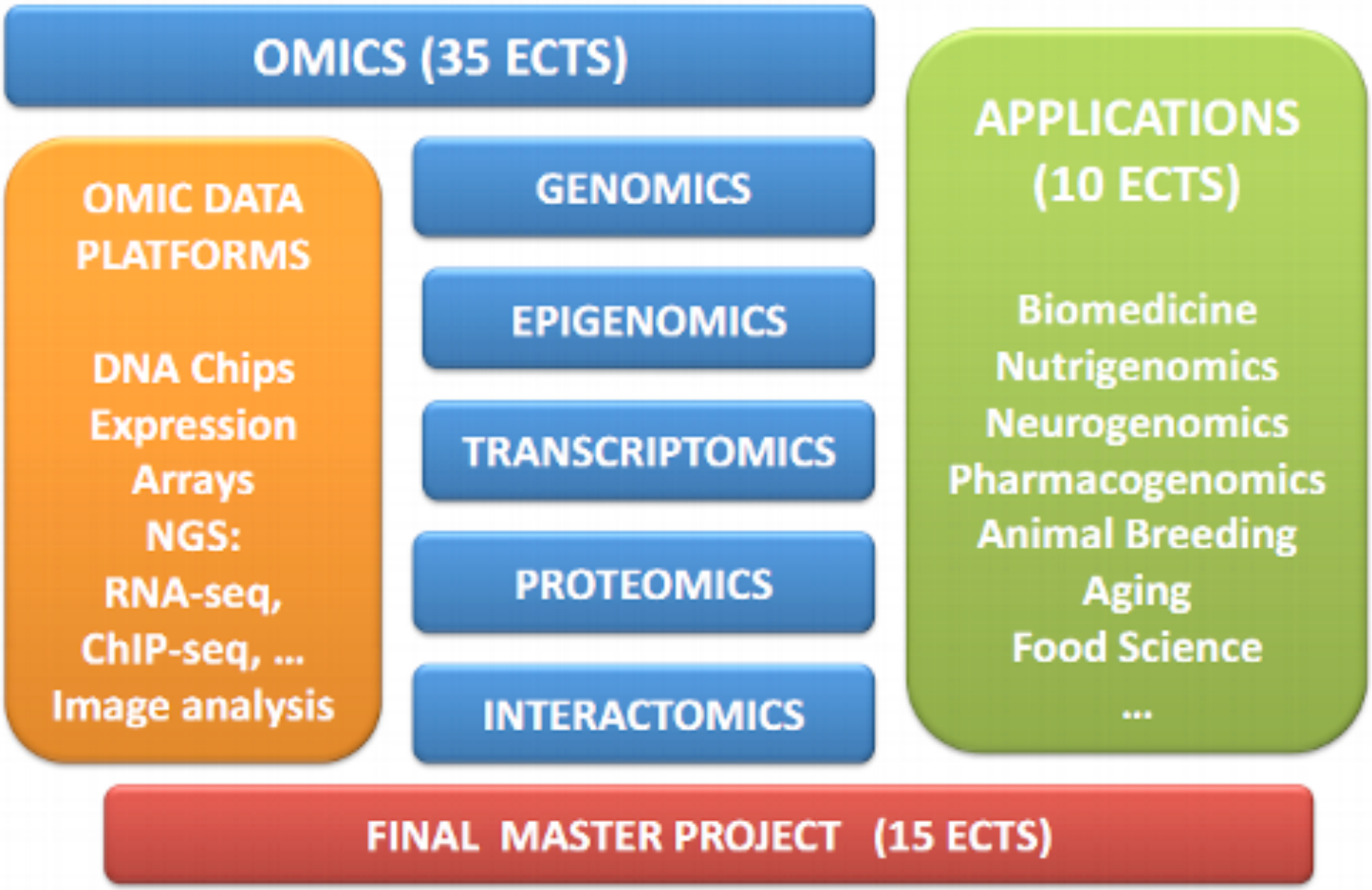
Beijerinck

Discovery of the “virus”



Modern microbiology

Has become a data science due to data acquisition “-omics” techniques



Understanding contamination

- Pasteur already said “Bacteria are everywhere”
- There are 3 types micro organisms:
  - Saprophytes – live on inanimate material
  - Commensal – live on other living organisms
  - Pathogens – cause disease in living organisms

- Contamination means:
  - “in contact with micro organisms”
- Infection means:
  - Illness due to micro organisms that are able to grow in the host
  - F.e. measles virus, cholerabacter, chickenpox virus, etc
- Pathogen means:
  - Illness causing micro organisms
  - There is always an interaction between pathogen and host

Understanding contamination (2)

- Virulence is:
  - Measure of pathogenity
- Infection doses are defined as:
  - ID50 = doses at which 50% of test animals get sick
  - LD50 = doses at which 50% of test animals die
- Most common pathways of infection are:
  - Respirator infections via contact between mouth and hand
  - Droplets infection via sneezing
  - Aerosols and dust (most effective indoors)
  - Toilet bacteria hardly survive on the door handle, but love your food!

Contamination in the lab

- Bio safety level number indicates the level of regulations that are in place to prevent contamination.
- Types of organisms allowed per level:
  - 1) Well characterized non pathogenic organisms on humans
  - 2) Micro organisms with high infection doses, and known cures
  - 3) Micro organisms with low infection doses, and known cures
  - 4) Micro organisms with extremely low infection doses, severe disease and no cure

General Rules in the lab

The following general rules apply to working in the Open Wetlab:

- Wash your hands before entering and when leaving
- Always wear a lab coat
- Clean up any spills immediately
- Label everything!
- Request help in case of an (small) accident from an assistant
- In case of doubt: ask for help
- Be careful with hot plates, glass ware and the microwave. Others might not suspect that the item is hot.
- Keep an close eye on flames. Never leave it unattended.
- Keep chemicals away from flames
- Only use machines after instructions

- Do not eat or drink during experiments

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Personal Protection

Equipment to protect yourself

Personal Protection

These items are recommended in the lab

Eye protection by safety goggles

- Wear safety goggles, especially when handling liquids

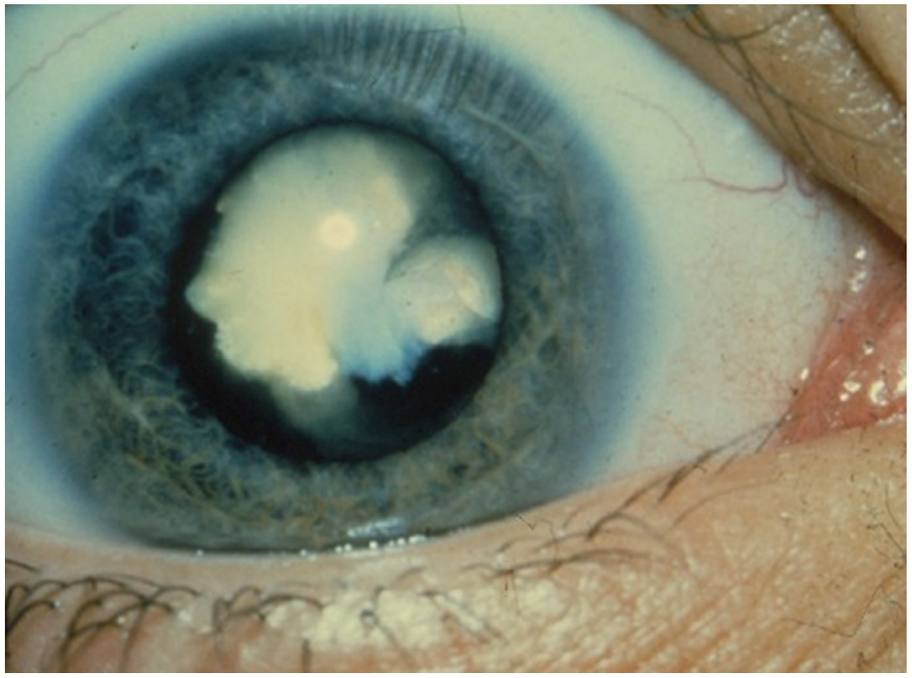
- Contact lenses
  - OK, as long as goggles are worn

- Normal glasses
  - Wear goggles over them

UV Goggles

- Wear when you are exposing yourself to anything light source below 400 nm

- UV is dangerous to skin and eyes!
  - Causes DNA damage that leads to cancer
  - Causes cataract in the eye



How to wear your hear and jewelry

- Tie back long hair
  - Hair is flammable
  - Gets tangled up easily

- Same goes for jewelry
  - No watches
  - No rings
  - No necklaces

Shoes

- Not allowed:
  - Sandals
  - Flip flops
  - Crocs
  - Open toe
  - Open top
  - Canvas

- Wear leather closed shoes instead

Hand protection

- **<u>Wear gloves only when necessary</u>**

- When you spill on your glove
  - Change it immediately

- Do NOT reuse gloves
  - Throw them in the bin after use

- Do NOT use latex gloves
  - Not protective against all chemicals
  - Use nitrile instead

- Nitrile and latex are highly flammable!
  - Never wear gloves next to a flame
  - Plastic burns into your skin

One Glove Rule

- Always keep one hand ungloved
- This is your “clean” hand

- With this hand you touch:
  - Door handle
  - Telephones
  - Pens
  - Keyboards
  - pH meter
  - Buttons

Wash your hands!

Remember, before and after experiments:

- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Even after wearing gloves

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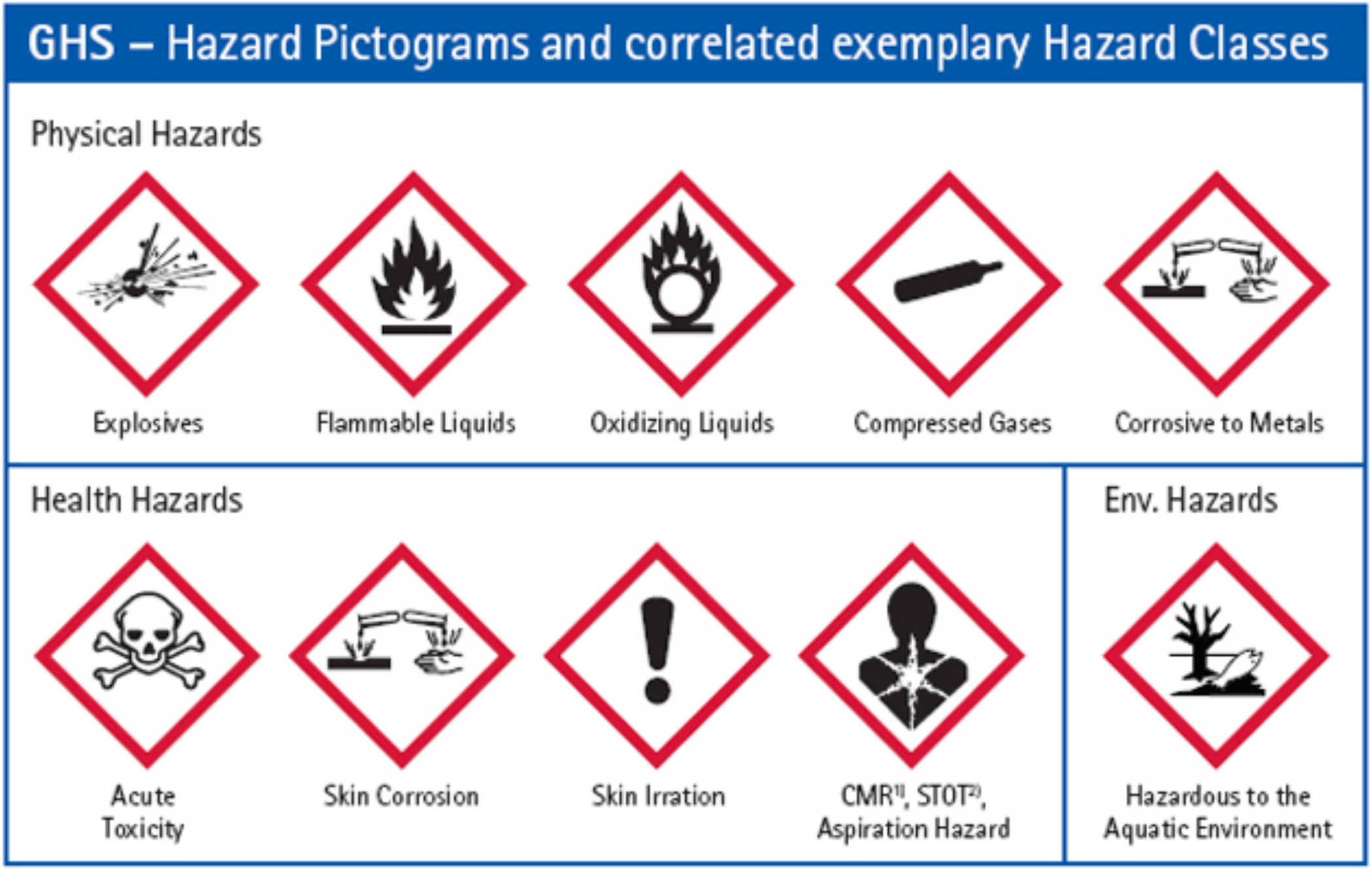
Chemicals

Label everything

- Use labels on everything!!
- You are the only one who knows what is in the container
- Labels must consist of:
  - Content
  - Date
  - Name

Global Harmonized System Labels

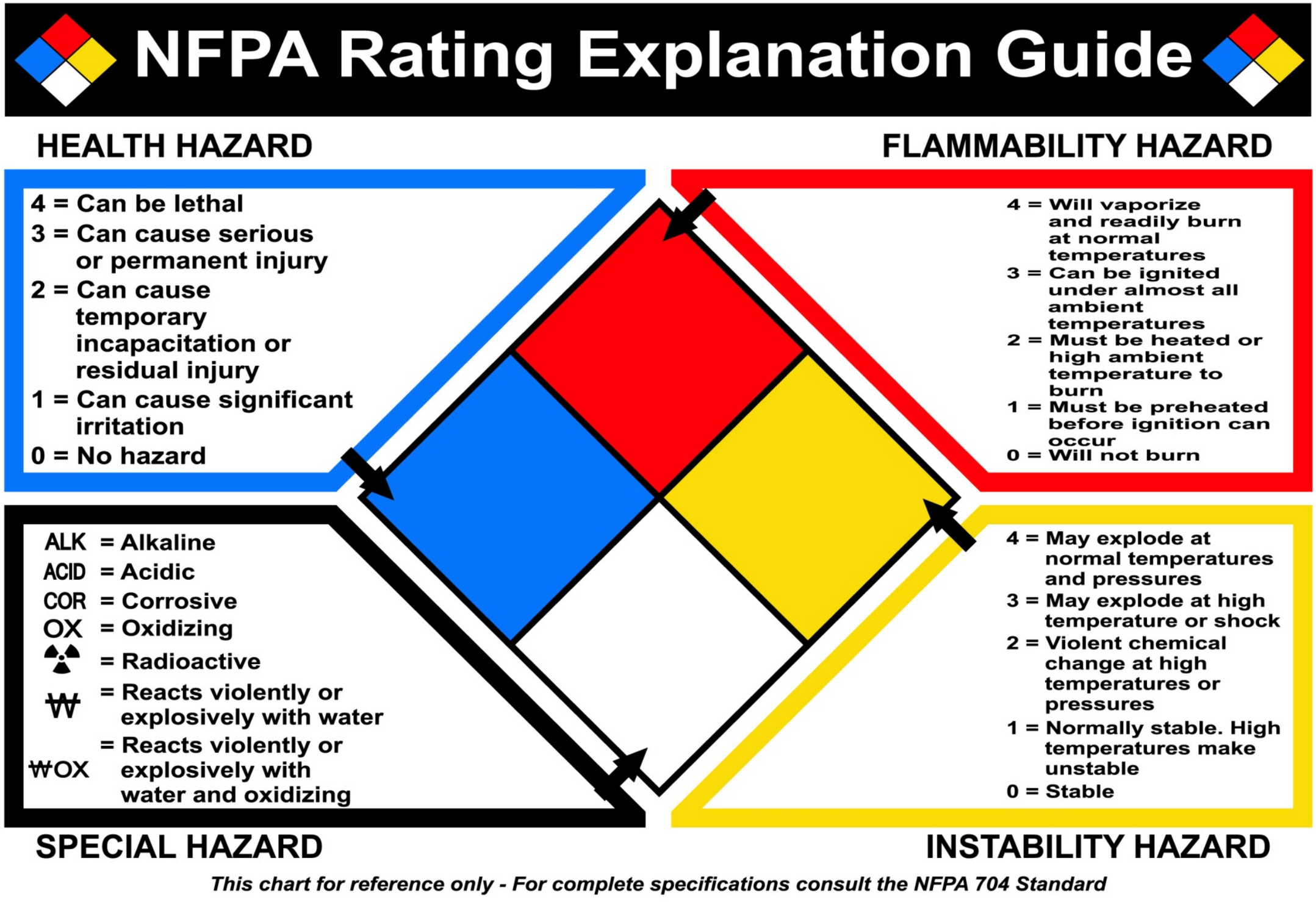
Familiarize yourself with the meaning of these symbols:



1) carcinogenic, germ cell mutagenic, toxic to reproduction / 2) specific target organ toxicity

NFPA safety diamond

NFPA diamonds are often used as well



Special labels

Do not bring anything with such label to the Open Wetlab

MSDS

- Material Safety Data Sheets come with every chemical and contain information about all safety aspects such as:
  - Procedures for safe handling
  - Physical Data
    - Melting point
    - Boiling point
    - Toxicity
    - Reactivity
  - Storage
  - First aid procedure

- Read the MSDS before you use any chemical!

Chemicals in the Open Wetlab

- Mostly house hold / super market chemicals
- Ask for permission before introducing new chemicals
- All chemicals can be found in the Inventory Folder
  - Please add new chemicals to the list

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Flames and Fire

Gas Burner

- Do NOT use when close to flammable gases or liquids

- Alert others in the lab before lighting a flame

- NEVER leave a flame unattended
  - NEVER
  - NEVER

In case of fire

- Notify everyone

- If the fire is small:
  - Take a fire extinguisher and try to extinguish the fire

- When the fire is too big or extinguishing fails:
  - Follow the exit lights to the nearest exit
  - Pull fire alarm

- Evacuate the building

- Call the emergency number 112

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Biosafety

Pathogens

- It is prohibited to bring pathogens to the Open Wetlab
- Do NOT incubate environmental samples at body temperature: 37 C
- To check if an organism is a pathogen, search for it on the internet or look it up in this list of organisms:
  - http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

Wash your hands

Remember, the best protection against infection:

- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Wash your hands
- Even after wearing gloves

Working sterile

Working sterile is important for safety:

- Definition: aseptic techniques in prevention of contamination
- Dutch: “Veilige Microbiologische Technieken” (VMT)

- Common techniques to sterilize are:
  - Heating: glowing in a flame, boiling, pasteurization
  - Physical sterilization: UV, Roentgen or gamma radiation
  - Chemical: ethanol, chloride, etc
  - Filtration

- Most common solid and liquids:
  - Steam boiling
    - 20 minutes at 120 degree Celsius
- Glass sterilization:
  - 2 – 4 hours at 180 degrees Celsius

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Waste Disposal

Waste disposal

- Think of how to dispose of things **before** you bring it into the lab

Biological Waste

You are responsible for killing anything you grow:

- Kill of any culture with 50% bleach, 50% water solution
  - Incubate for 24h before disposal

- Clean any used surface and object with 70% ethanol (red capped bottles)

Broken glassware

- Do NOT dispose in the normal trash bin

- Special “broken glass” container
- Use broom to clean up, because you can easily cut yourself

- Glass container is on the Nieuwmarkt

Chemical waste

- Check what is allowed to store in the lab with the labmanager
- Check what is allowed to go down the sink with the labmanager
- Do NOT mix:
  - Concentrated Acids and Bases
  - Oxidizers and Flammables
  - Water reactive substances and aqueous solutions
  - Cyanides and acids => cyanide gas
  - Bleach and acids => chloride gas

- Search for reactivity on the internet!
- Read the MSDS before using a chemical!

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Documentation

Projects

All work in the Open Wetlab must be documented

- fablab.waag.org
  Register yourself
  Document your project
  Update with progress
  You can also find documentation of machines on this website
  There also is a reservation system

- meetup.com
  There is a forum for questions to the community
  Feel free to start your own event

Stock keeping

- Report low stock before it runs out
- Fill out the form in the pink “Lab Map”
- Request new orders at the lab manager, wetlab@waag.org

Incident report form

Always report incidents to the labmanager

- Let’s learn from mistakes
- Now matter how small or seemingly insignificant

- Form in the pink “Lab Map”
- Write down what you were doing, when, what went wrong and how it could be prevented

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Wrap up

And finally… Wetlab House Rules

1. Wash your hands!
2. No drinking or eating in the lab during experiments
3. Sign your name on the attendance form when you come in and out of the lab
4. Tie back long hair & wear a lab coat
5. Label everything you experiment with (name owner, date, content)
6. Clean up! No paperwork, dirty glasses, left-overs etc.

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Safety Practical

Practical

Now it is time for some hands-on instructions:

- Washing hands
- Signing in and out
- Personal protection equipment
- Clean workspace
- Pipetting
- Emergence exits and fire extinguishers
- Cleaning up, especially glassware
- Label tour
- Machine tour
- Stock keeping
- Brewing coffee / making tea