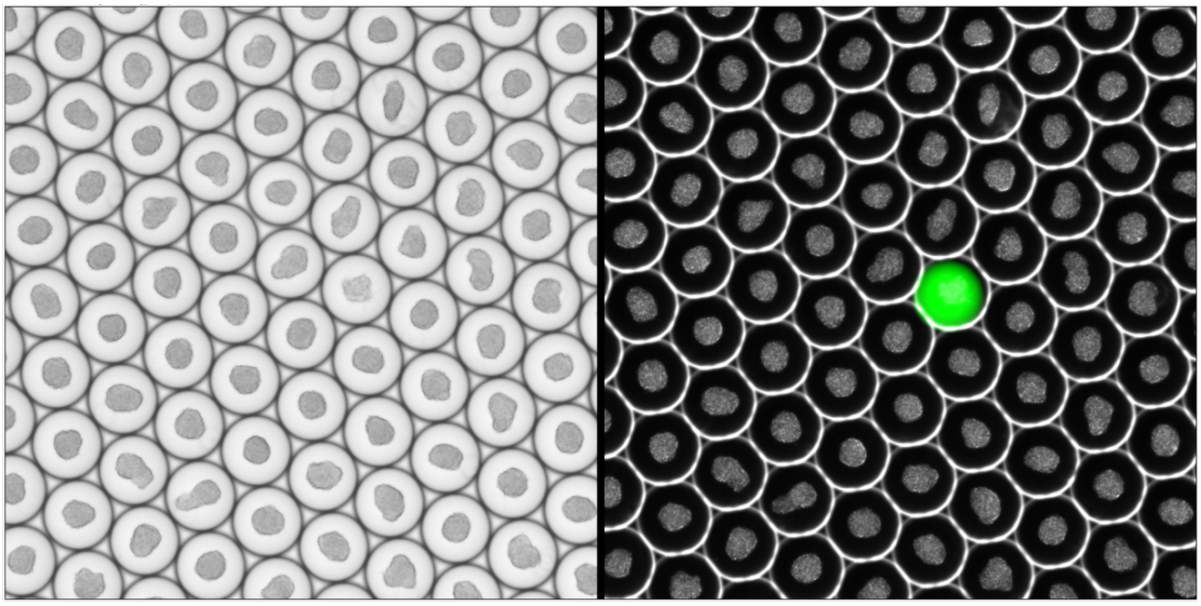
Microfluidics: taking experimentation to the scale and diversity of microorganism

We can't see microbes. How can we find the ones that are special among millions of others?



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Finding the needle in the haystack: millions of genetic variants of a microbial strain were separated in tiny droplets and allowed to grow individually. A light signal was then used to identify the few rare cells that have a particular activity.

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Microfluidics

Storyline

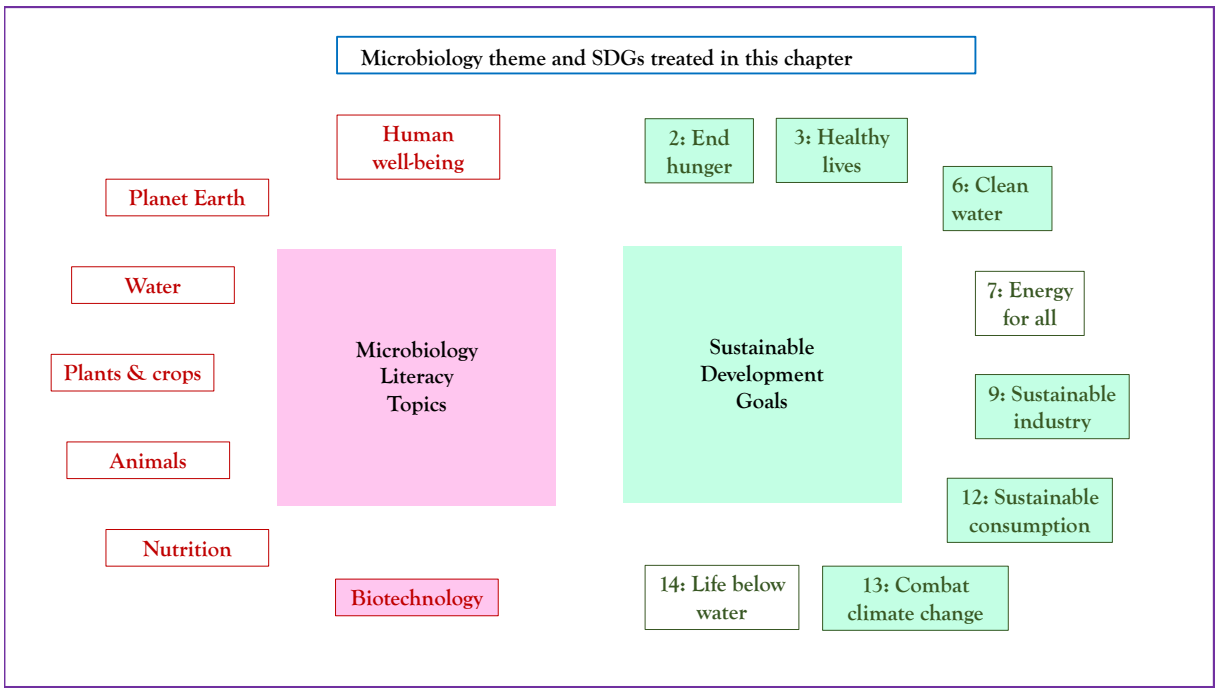
Microfluidics is the study and manipulation of fluids at the sub-millimeter scale. This technology allows for the analysis and control of single microbial cells, which is essential given the vast diversity and importance of microorganisms. Traditional bulk cultivation methods are inadequate for studying individual microbial behavior and discovering new natural products.

Microfluidics offers numerous advantages, including reduced sample volumes, faster experimental times, and the ability to conduct complex experiments on a single chip. Techniques such as continuous-flow microfluidics and droplet microfluidics enable precise control and high parallelization of microbial cultures.

Applications of microfluidics in microbiology include studying microbial growth, behavior, and interactions at the single-cell level, screening for new natural products, and optimizing biotechnological processes. The technology is also promising for personalized medicine, point-of-care diagnostics, and rapid detection of antibiotic resistance. Future advancements in microfluidics hold potential for significant contributions to healthcare, environmental monitoring, and sustainable industrial practices.

The Microbiology and Societal Context

The microbiology: understanding microorganisms, finding new natural products, single-cell analysis, solutions to health challenges, point-of-care diagnostics. *Sustainability issues*: resource-saving technology, bio-economy.



Microfluidics: the Microbiology

1. ***Why do we need new methods to study microbes?*** Microorganisms are the most common organisms in the biosphere. They include mostly unicellular organisms from all three domains of life (Archaea, Bacteria and Eukarya) and they are extremely diverse. Genetically speaking, humans are more closely related to other animal species such as cows than even two strains of the same bacterial species sometimes are. Today, we know that these microorganisms are extremely important for us humans. We carry at least as many cells of microorganisms with us in our body as actual human cells. They help us stay healthy and to digest our food.

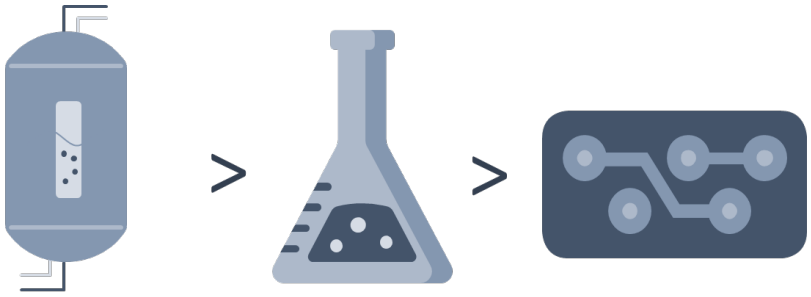
Microorganisms can also produce natural products that are vital for drug development such as antibiotics, anticancer agents, vitamins, and vaccines. Natural products like polymers, surfactants, and bioherbicides are also relevant for industrial processes. In order to understand how microbes can also make us sick, and to find new natural products, we need to study the microbes.

The vast majority of microorganisms are as yet unknown. It is estimated that there are about one trillion species of microbes on earth and 99.999 percent have not yet been discovered and studied in the laboratory. Even the physiological and ecological properties of the few known microbes are mostly poorly understood. The principal reason for this is that microbes are mostly single-celled, so one organism does not provide much material for study. Because of this, microbiologists tend to study not one organism but millions-to-billions they produce by cultivation: allowing a single microbe to reproduce many, many times. This is the standard procedure to amplify microbial material for study. The problem then is that microbiologists tend to study the properties of not one organism but many, on the assumption that they are all the same and have identical properties and behavior, e.g. if they grow well, or what molecules are being produced under which nutrient and oxygen conditions.

We also see this in history: we have used the bulk processes of microbes for millions of years to ferment beer or cheese without even knowing that these are microbial reactions. We only discovered that microbes are behind these reactions, when scientists were able to use a microscope to directly observe these tiny cells.

However, recent studies have shown that different cells in a microbial culture behave differently, and that it is incorrect to assume that all microbial cells descended from one parental cell will have the same or similar behavior at the same time. So: despite the fact that bulk cultivation techniques (e.g. in a flask or bottle) continue to be widely used, they are not suitable for efficiently searching for new natural products, cultivating unknown organisms with very specific needs, or for studying specific properties of microbes. For this, we need to analyze the organisms on an individual, single-cell level, and microfluidics is a great tool to do so.

2. ***What is microfluidics?*** Microfluidics describes the behavior, control, and manipulation of liquids and gases in the sub-millimeter range. Most commonly liquids are pumped through tiny channel structures that are created on a microfluidic chip. This microfluidic chip is usually connected to pumps to flush fluids through the channels and placed under a microscope or sensor to observe the content and to perform analyses. The biggest advantage of microfluidics for microbiology over standard cultivation methods such as a bioreactor is that we can study one single microbial cell and its offspring at a time. Another obvious advantage is the scale down in size, which minimizes the sample, reagents, and chemical volumes needed. But also, many operations can be performed simultaneously due to the compact size, reducing the time required for experiments.



	Bioreactor	Shake flask	Microfluidic chip
Volume	1 - 1000 L	0.01 - 1 L	Smaller than 0.000001 L (10^{-15} – 10^{-6} L)
Cost	high	medium	low
Parallel experiments	few	few dozens	millions
Analysis method	bulk analysis	bulk analysis	single-cell studies

Microfluidic chips can have more than just channels or trapping structures on them. The combination of different functional elements like tiny valves, electrodes or lenses allows for designing complex experiments and investigations on a single chip, just like in a fully equipped laboratory. Therefore, microfluidic chips in which one or multiple analysis processes can be conducted are usually referred to as lab-on-a-chip devices. The name originates from one of the goals of microfluidics: to miniaturize an entire laboratory process to chip size in order to e.g., quickly analyze environmental samples in the field, or human samples in a clinical setting.

In the simplest case, microfluidic chips do not need any external equipment, except for a pipette to introduce the sample into the chip. However, this level of simplification of the operation is still fairly rare. Current microfluidic devices are usually more chip-in-a-lab than lab-on-a-chip devices, since most of the time lots of external equipment is needed such as pumps, connectors, bulky microscopes, and sensors.

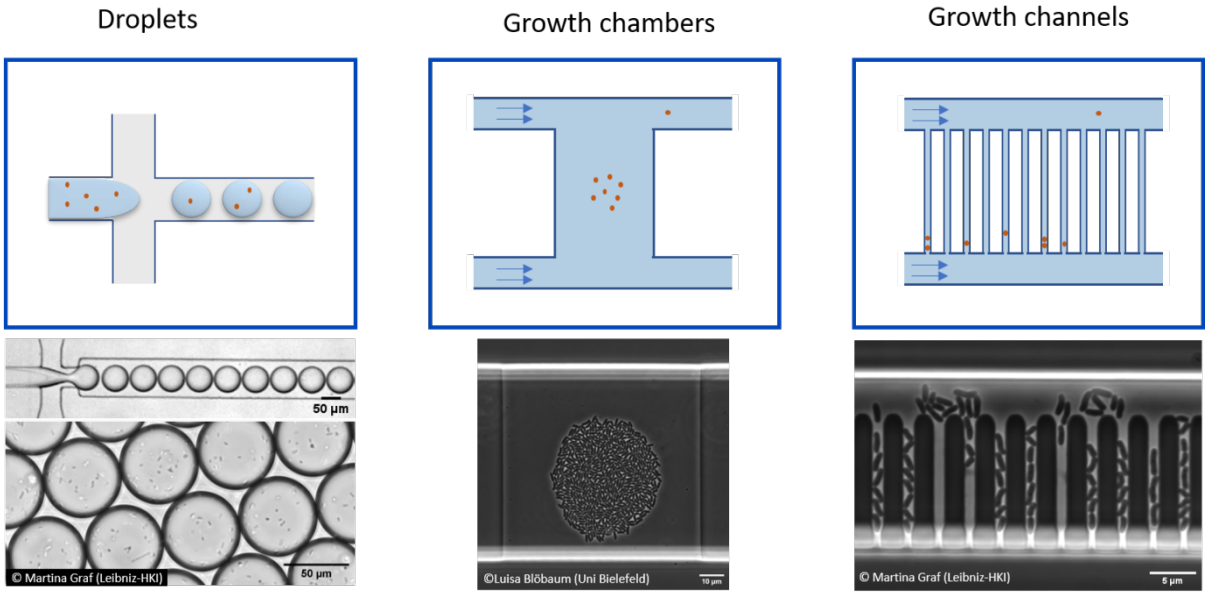
3. ***Hundreds to millions of little bioreactors.*** Single cells can be trapped in microfluidic structures such as chambers or droplets, incubated and visualized through, e.g., microscopic methods. Therefore, single cells and isoclonal colonies derived from a single cell can be studied. This is an enormous advantage since different cells of one strain can exhibit different molecular and physiological behavior, just like human siblings vary. To be able to study such behavioral differences of the cells, they need to be physically separated and this is only possible in such microfluidic structures.

Also, a precise control of the immediate environment can be achieved when cells are trapped in tiny chambers and medium is passed continuously through the chip (continuous-flow microfluidics). Thereby the growth, division and fate of one single cell can be directly observed. In this case, even the exposure time of the cells to different chemicals that are pumped through can be controlled and one can study the direct response of microorganisms to chemicals, be it stimulants or toxins. One example application is to find the best media composition for a specific cellular response (e.g., growth or substance production). Hundreds of these little trapping structures can be operated in parallel on one microfluidic chip.

In a different mode of operation, cells can be trapped inside of small femto- to microliter (10^{-15} – 10^{-6} liter) droplets that flow with the fluidic stream (droplet microfluidics). Droplets are created when two immiscible fluids – fluids that do not mix – come into contact, usually water and oil. This enables a much higher parallelization of experimental conditions, because every aqueous droplet can be a miniaturized bioreactor with its own environmental condition or a

different microbial content: in other words, every microbe gets their own house to grow in – they don't have to share resources with others, only their own offspring.

For most microorganisms, the ideal conditions at which they will grow best are unknown. With the high parallelization of the droplets, thousands of conditions and various strains can be analyzed in one experiment. While droplets mimic bioreactor conditions, the environment inside each droplet cannot be controlled as precisely as with continuous-flow microfluidics.



Performance parameter	Droplets	Growth chambers	Growth channels
• Single cell studies possible	+	++	+++
• Parallelization/ Capacity for screening experiments	+++	0	+
• Environmental control for each cell	+	+++	+++
• Time resolution	0	+++	+++
• Recovery of interesting cells	+++	-	-

Grading scheme: - not possible, 0 – base level, comparable to bulk cultivation, +/++/+++ degree of improved feature compared to bulk cultivation

4. ***Applications of microfluidics in microbiology.*** Microfluidic chips are used to address many different kinds of microbiological research questions. We can choose between operations that either trap a single cell and enable its direct continuous observation, or operations of millions of parallel cultivations in droplets that allow us to screen a large number of cells for a certain function.

In single-cell-based microfluidics, the growth behavior of a cell, when and how a cell divides, aging processes, and cell-cell interactions can be studied directly by looking at the cell through a microscope. Thus, we can learn many details about the life and interactions of a certain microorganism. Since many microorganisms can live under very extreme conditions, like high salt, or low pH or with toxic compounds, we are interested to understand their lifestyles in more detail. And even if they seem very different from us humans, we can learn about basic physiological and biochemical processes from these tiny cells that are also important to understand biological functions in general (as Jacques Monod once said: 'What is true for *E. coli* is true for the elephant'. Thus, these microbial cells represent great models for biological studies in physiology or genetics.

On the other hand, we have this gigantic diversity in properties and potential metabolites in the microbial world and there is a huge interest in screening processes, where cells with a specific behavior are searched for among millions of other cells. Such screening can be effectively performed in microfluidic systems such as droplets due to the high parallelization and high

throughput: millions of individual microbes can be cultured and analyzed in just a few hours. A specific behavior can be the formation of a so far unknown natural product.

We have an urgent need to find new natural products, especially antibiotics, since existing antibiotics are losing their efficacy. Most common antibiotics originate from microorganisms. But pathogenic microorganisms are very active in developing resistances against these common antibiotics leaving us with fewer and fewer defense strategies against those microbial diseases like pneumonia or tuberculosis. With microfluidics, we can be more effective in screening the vast majority of the unknown microbial world for potential new drug candidates.

Further, we might want to screen a microbial culture for the best producers of a highly active enzyme, which is of interest for biotechnology, e.g. in laundry detergents, or chemical manufacturing to replace unsustainable reactions. Or we can extract the entire community of microorganisms from certain environments like soil, water or an animal gut and use droplet microfluidics to separate and grow them in the lab for future studies. Traditional cultivation techniques are not very successful at this task and only cultivate 3-5 % of the different community members. With droplet microfluidics, every cell gets its own environment and has a better chance to grow because it has no competitors.

Evolutionary studies can be performed as well. Microfluidic chambers with different growth conditions are connected over corridors mimicking different environmental landscapes. Bacterial populations in these habitats interact with each other through extinction and colonization processes. Therefore, bacterial adaptation to new ecological niches can be studied.

5. ***Outlook (Future Applications).*** The field of microfluidics has become a rapidly growing field since its start in the early 1980s. Systems without living cells are already a central part of many analytical processes, e.g. blood sugar measurement devices or when blood and urine is analyzed in a laboratory.

Due to the simple handling, cost efficiency and rapid execution, microfluidic systems have emerged with a huge potential for point-of-care diagnostics. With the development of methods to capture individual cells, combined with the precise manipulation of fluids, new possibilities of single-cell studies have emerged. Potential applications of cell-based point-of-care diagnostics would be in the field of personalized medicine. Tumor cells could be treated with different drugs and combinations of them in microfluidic devices, to analyze which drug has the highest potential for treatment before it is administered to the patient. Similarly, microfluidics could be also used for fast detection of sepsis and antibiotic resistance. Samples of patients could be tested for bacterial resistances against all available antibiotics in a single experiment. A fast and effective treatment could be chosen, decreasing mortality of the patients as well as the spread of antibiotic resistances.

Another way to battle the threat of antibiotic resistances is to find new antibiotics by screening known and unknown organisms. With the possibility of extremely high parallelization, microfluidics has the potential to play a major role in the discovery of new natural products.

There is great hope that the above-mentioned applications and many more will advance science, revolutionize diagnostics, and reach the market in the next decade.

Relevance for Sustainable Development Goals and Grand Challenges

The need for new methods to study microbes and the advancements in microfluidics technology have significant relevance to several Sustainable Development Goals (SDGs):

- **Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture**. Microfluidics can enhance our understanding of soil

microbiomes, improving agricultural productivity by optimizing crop health and soil fertility. Additionally, identifying microbes that produce natural bioherbicides or biofertilizers can reduce reliance on chemical inputs, promoting sustainable agriculture.

- **Goal 3: Ensure healthy lives and promote well-being for all at all ages**. Microfluidics is instrumental in discovering new antibiotics and other drugs. The technology also aids in personalized medicine by enabling precise drug testing on single cells, leading to more effective treatments for diseases such as cancer and sepsis.
- **Goal 6: Ensure availability and sustainable management of water and sanitation for all**. Understanding microbial communities in water through microfluidic techniques can improve water purification processes and wastewater treatment, ensuring better water quality and reducing pollution.
- **Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation**. Microfluidics represents a significant technological innovation, offering cost-effective and rapid analysis tools for various industries. This fosters sustainable industrial practices by enabling more efficient production processes and reducing resource consumption.
- **Goal 12: Ensure sustainable consumption and production patterns**. By enabling the efficient screening of microorganisms for industrial applications, microfluidics can identify microbial processes that replace chemical processes, leading to more sustainable production methods and reducing environmental pollutants.
- **Goal 13: Take urgent action to combat climate change and its impacts**. Microfluidics can enable the discovery of biocatalyst producing microorganisms. These biocatalysts can be utilized in bioeconomy applications, reducing the reliance on oil-based products and thereby mitigating greenhouse gas emissions.

Potential Implications for Decisions

1. ***Research Institutes and Industry***
    a. Initial investment cost for microfluidic equipment needed
    b. Microfluidic experiments are very cheap in comparison to bulk experiments
    c. Special know-how needed: Investing in education and training programs to equip scientists/ laboratory staff with skills in microfluidic technology
    d. Microfluidics is a cutting-edge technology which might help with allocating funding
    e. Microfluidics could accelerate drug discovery

2. ***Global Implications***
    a. Microfluidics enables cost-effective personalized medicine and point-of-care diagnostics which can reduce the overuse and misuse of drugs such as antibiotics. This could help for example to defuse the global antibiotic resistance crisis.
    b. The high-throughput screening of microbial strains could lead to new environmentally friendly natural products, such as bioherbicides or biodegradable polymers. Also new antibiotics and other therapeutics could be discovered.
    c. Enhanced ability to study microbial communities in various environments leads to better understanding and possibly management of ecosystems.

Pupil Participation

- What challenge do you see for studying a specific microorganism?
- Why is it so difficult to grow most microorganisms in the laboratory?
- What would you like to know about a microorganism?
- How would you attempt to study this microorganism?
- Why do we need to search for new natural products, like antibiotics, urgently?
- Do you know other products of microorganisms that we could look for using microfluidics?
- What other applications of microfluidics can you envision?

The Evidence Base, Further Reading and Teaching Aids

Droplet microfluidics:

Shang, Luoran, Yao Cheng, and Yuanjin Zhao. 2017. "Emerging Droplet Microfluidics." *Chemical Reviews* 117 (12): 7964–8040. https://doi.org/10.1021/acs.chemrev.6b00848.

Joensson, Haakan N., and Helene Andersson Svahn. 2012. "Droplet Microfluidics-A Tool for Single-Cell Analysis." *Angewandte Chemie - International Edition* 51 (49): 12176–92. https://doi.org/10.1002/anie.201200460.

Single-cell microbiology:

Rosenthal, Katrin, Verena Oehling, Christian Dusny, and Andreas Schmid. 2017. "Beyond the Bulk: Disclosing the Life of Single Microbial Cells." *FEMS Microbiology Reviews* 41 (6): 751–80. https://doi.org/10.1093/femsre/fux044.

Lab-on-a-chip devices:

Roszek, S.A.B. Hermsen B., and A.W. van Drongelen R.E. Geertsma. 2013. "Lab-on-a-Chip Devices for Clinical Diagnostics." *National Institute for Public Health and the Environment RIVM Report*.

Youtube:

- World of Microfluidics European Micro Cup: Microfluidics and Microbioreactor: https://www.youtube.com/watch?v=zI0ZjKdZle4
- The Lutetium Project: Microfluidics Adventures: https://www.youtube.com/watch?v=EYuyRUjnTgc
- Programmable Droplets: https://www.youtube.com/watch?v=z0NBsyhApvU

Glossary

Unicellular organism:	Also known as a single-celled organism. Organism that consists only of a single cell. Opposite of multicellular organism.
Archaea:	Prokaryotic domain of life, no nuclear membrane. Biochemistry and RNA markers differ from bacteria
Bacteria:	Prokaryotic domain of life, no nuclear membrane, cells with bacterial rRNA
Eukarya:	Membrane-bound nucleus. Animals, fungi, plants but also single-celled organism belong to this domain

Natural products:	Chemical compound or substance produced by a living organism. In medicinal chemistry, only secondary metabolites (metabolites that are not essential for survival but produce some evolutionary advantage) are meant.
Lab-on-a-chip:	A combination of multiple functions for treatment and analysis of a sample on a microfluidic chip to conduct an entire experiment
Isoclonal:	Relating to the same clone, i.e. a cell with the same genetic identity.
Cell-cell interaction:	Refers to the direct interaction between cells. They allow cells to communicate with each other when changes in the microenvironment occur.
Ecological niche:	Describes how a species interacts within an ecosystem. The niche of a species depends on the availability of resources and conditions, allowing it to survive.
Point-of-care diagnostic:	Any kind of diagnostic test that does not need to be performed in laboratory. Examples: blood glucose monitoring and pregnancy tests.
Bioeconomy	Transformation from an oil-based economy to an economy in which fossil resources are replaced by various renewable raw materials.