The Promise of Regenerative Medicine and AI

I was invited to speak about how these AI-based techniques can be applied to regenerative medicine and cell & gene therapy manufacturing in particular.

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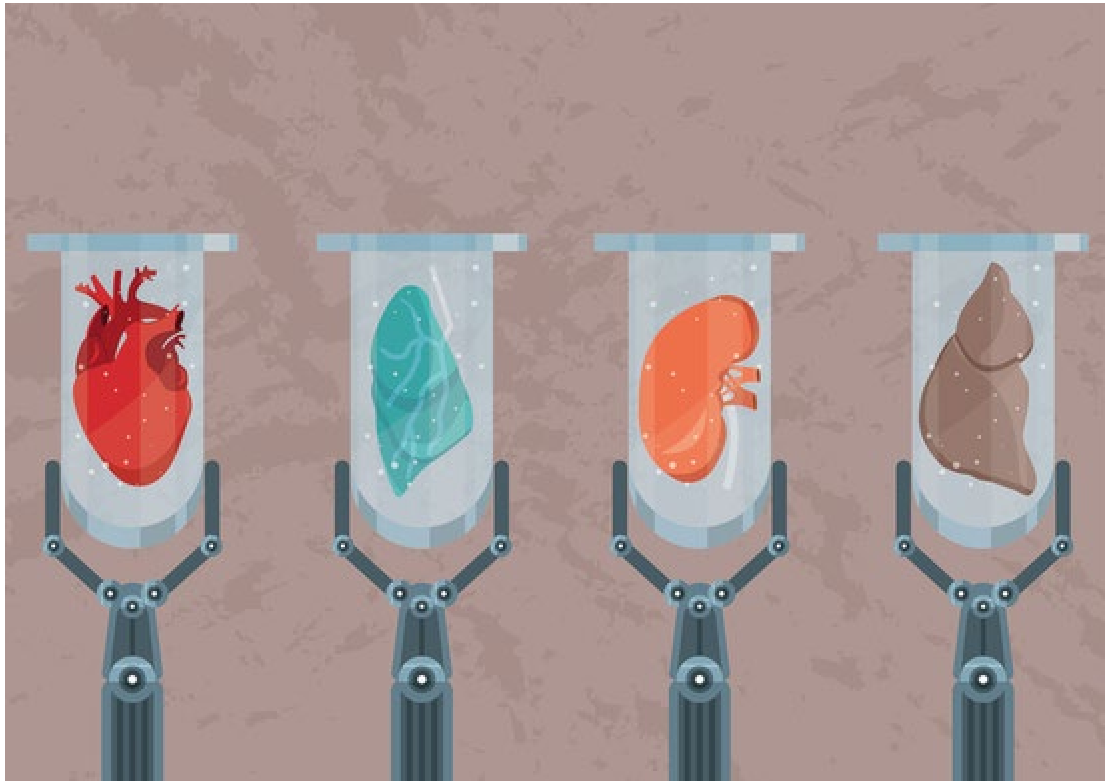
WHAT IS REGENERATIVE MEDICINE?

Regenerative medicine is a branch of medicine that uses tissue engineering and molecular biology to replace, engineer and rejuvenate human cells, tissues or organs.

- Regenerative medicine include:
  - *cell & gene therapies* and
  - *advanced therapy medicinal products* (ATMP).
- The recent successes of regenerative medicine have led to a flood of investments.
- Vaccines and antibiotics have fundamentally changed our society.
- Regenerative medicine is poised to be equally transforming.
- **For the first time in human history, we can envision a future where non-infectious diseases are cured, rather than merely suppressing the symptom.**



My definition of curing: healing in the meaning of making sound and whole.

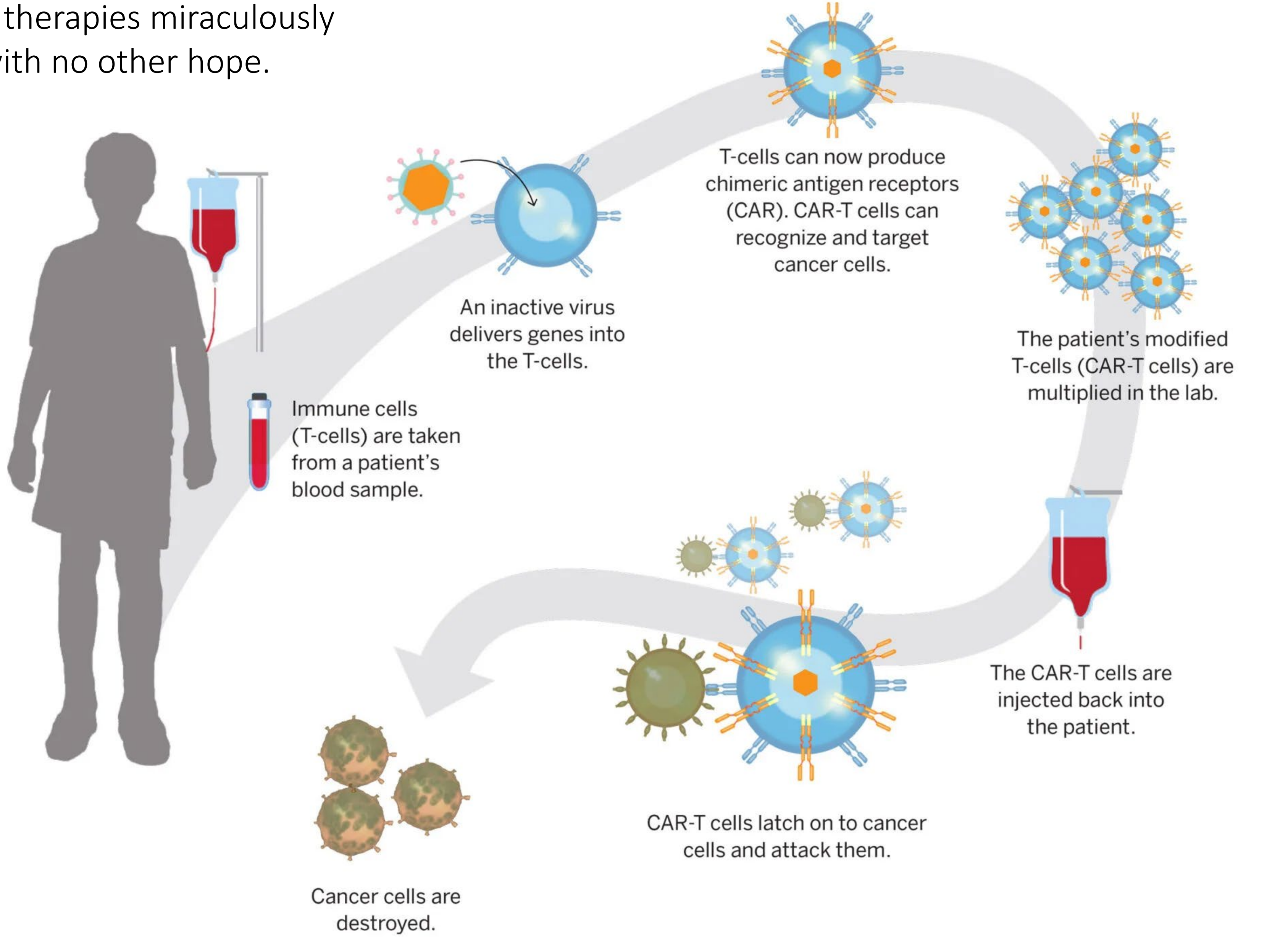
SPINAL MUSCULAR ATROPHY (SMA)

- Untreated infants diagnosed with SMA type 1 are not expected to enjoy their second birthday.
- SMA occurs in one in 10 000 births.
- SMA is genetic disorder that kills nerve cells in the brain and spinal cord, leading to complete paralysis and death.
- Unfortunately, Evelyn's treatment costs $2.1 million — the second most expensive drug in the world.
- The most expensive drug is another one-time gene therapy but for bleeding disorder (hemophilia), priced at $3.5 million per dose when recently approved by the FDA.
- Gene therapies corrects faulty genes using viruses.


Evelyn was one of the first to receive the gene therapy.

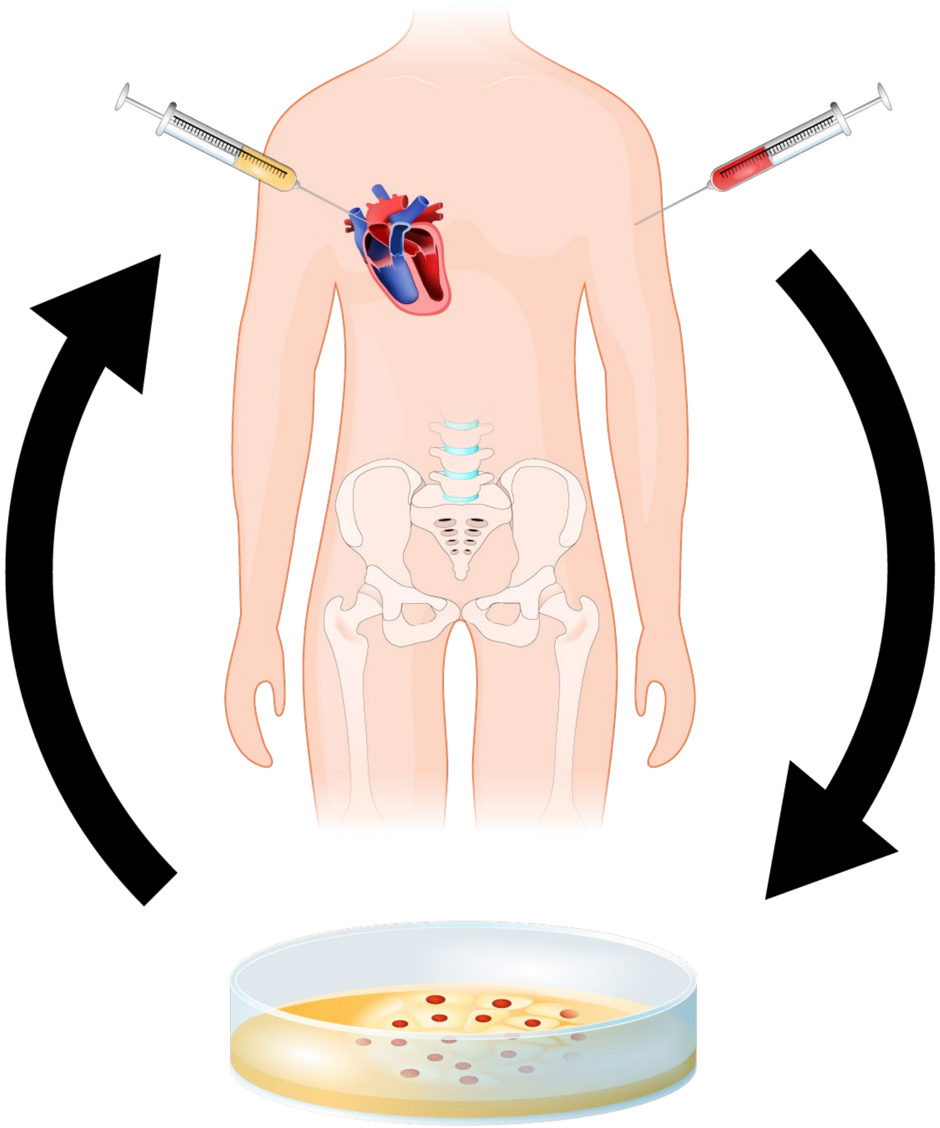
IMMUNE CELL THERAPY

Since 2017, CAR T-cell therapies miraculously cure cancer patients with no other hope.



REGENERATIVE MEDICINE

Basic Principle



- Promise to treat Alzheimer's, Parkinson's, diabetes, heart disease and many cancer forms
- 27 approved treatments as of today
- 2 600 ongoing clinical trials
- Treatments today cost up to $3.5 million
- Unlike traditional drugs, **the cells themselves are the treatment**
- **Must transition from craft to industry** to reduce costs and make treatments available to all

The Manufacturing Problem

- Cell-based therapies are today handcrafted in academic-like labs.
- Regenerative medicine’s biggest challenge is production scale-up.
- Few industrial tools are available, as cell culturing has primarily been an academic activity in the past.

FEDERAL FOOD, DRUG AND COSMETIC ACT

- FDA shall in consultation with the National Institute of Standards and Technology (NIST) and stakeholders
- facilitate an effort to coordinate and prioritize the development of standards to, through regulatory predictability, support the development, evaluation and review of regenerative medicine therapies
- with respect to the manufacturing processes and controls of such products.

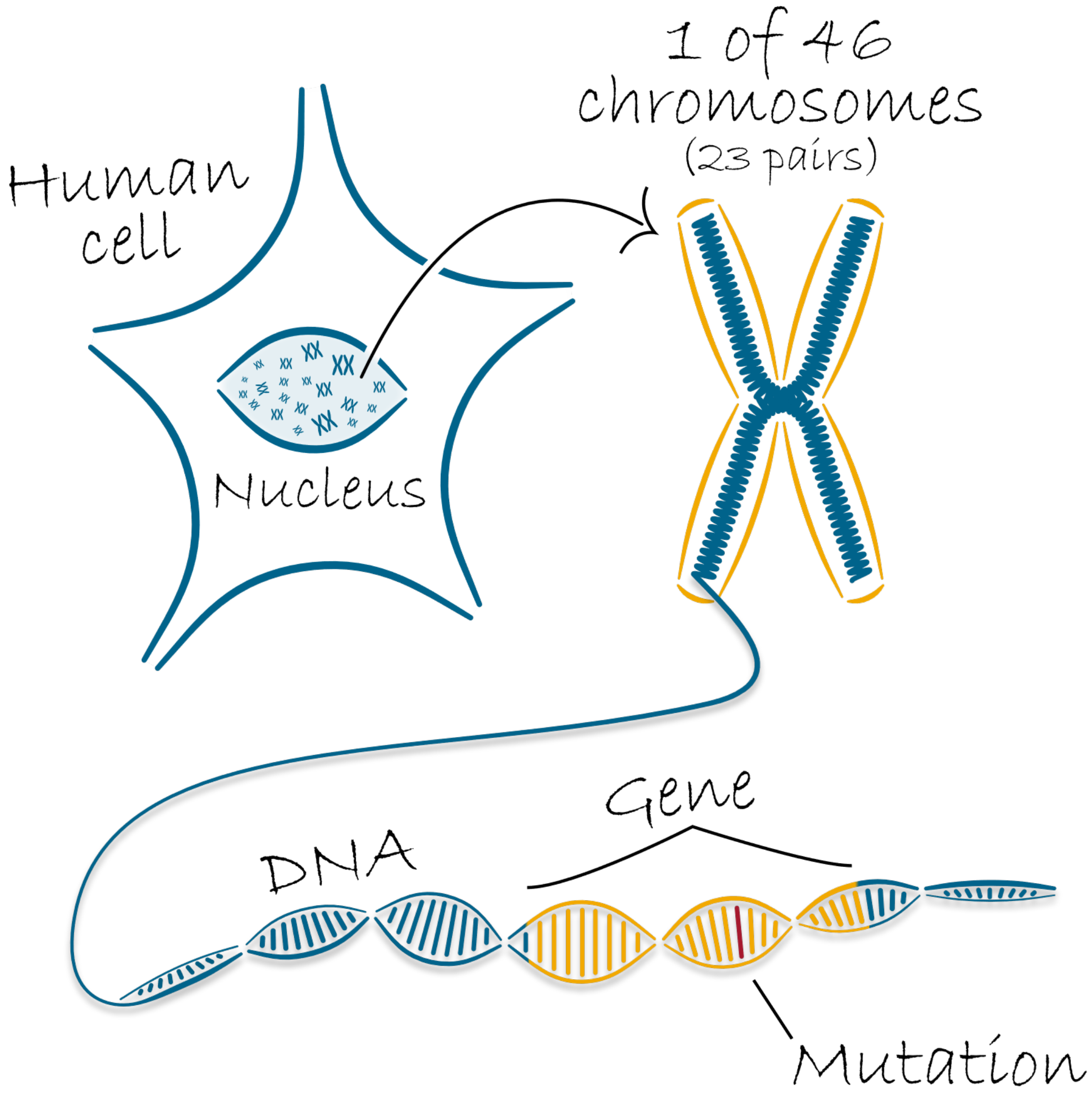
- The stakeholders are
  - regenerative medicine and advanced therapies manufacturers
  - clinical trial sponsors
  - contract manufacturers
  - academic institutions
  - practicing clinicians
  - regenerative medicine and advanced therapies industry organizations and
  - standard setting organizations

Source: 21 USC 356g : Standards for regenerative medicine and regenerative advanced therapies

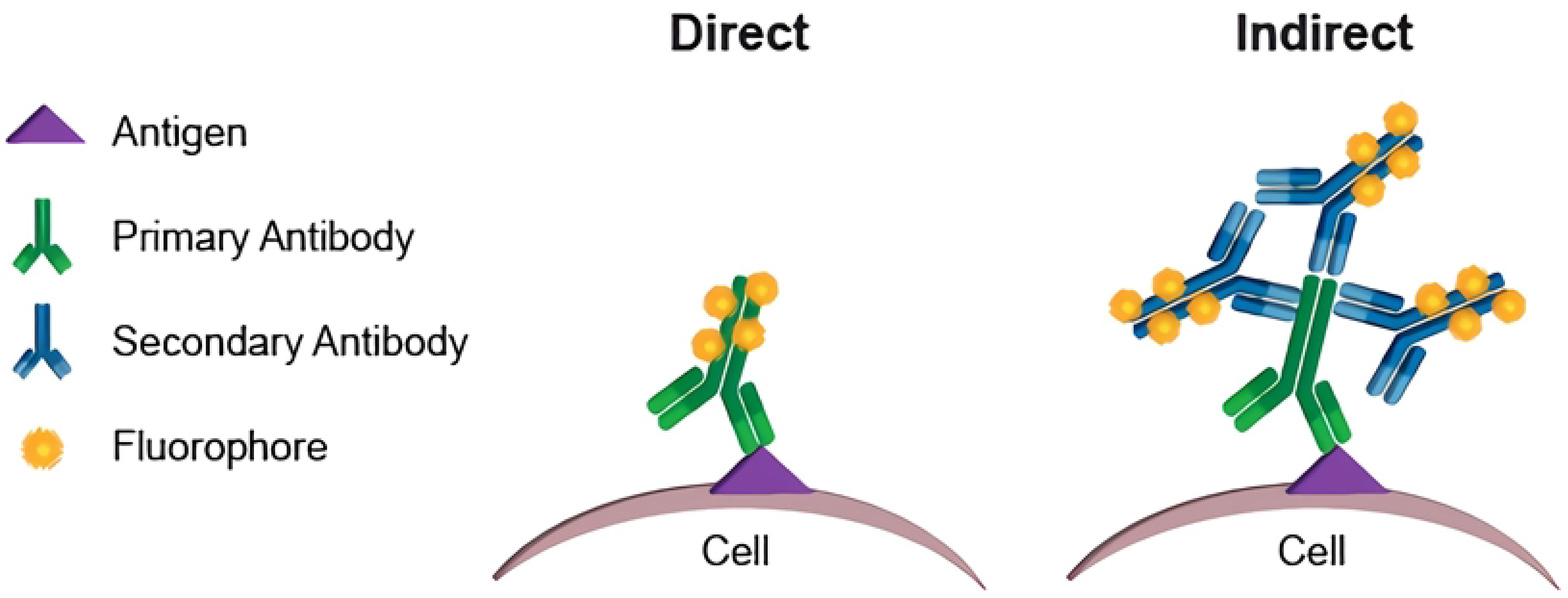
In plain English, the US Congress has essentially ordered FDA to let the regenerative medicine players set the standards for how regenerative therapies shall be manufactured and regulated.

Cell biology basics

WHAT IS A GENE?



WHAT IS AN IMMUNOASSAY?



- Cell surface antigens reflect the genetic activity.
- Immunoassays are used to determine genetic activity.
- Fluorescent molecules are commonly used.
- However, the signaling molecule can, for instance, be radioactive instead.

WHAT IS A STEM CELL?

- A stem cell is a cell that can ***differentiate*** (transform) into another type of cell through ***asymmetric cell division***, one new stem cell and one differentiated cell.
- A ***somatic cell*** is any cell that is not a stem or sex cell.
- ***Pluripotent stem cells*** can differentiate into all of the cells of the adult body.
- ***Induced pluripotent stem cells*** are normal somatic cells engineered to become pluripotent stem cells.
- ***Embryonic stem cells*** extracted from discarded embryos are the purest form of pluripotent stem cells.
- ***Adult stem cells*** can only differentiate to yield tissue or organ-specific cell types.
- Cell biologists have been tinkering with stem cells since the 1980s.

Induced pluripotent stem cell therapy

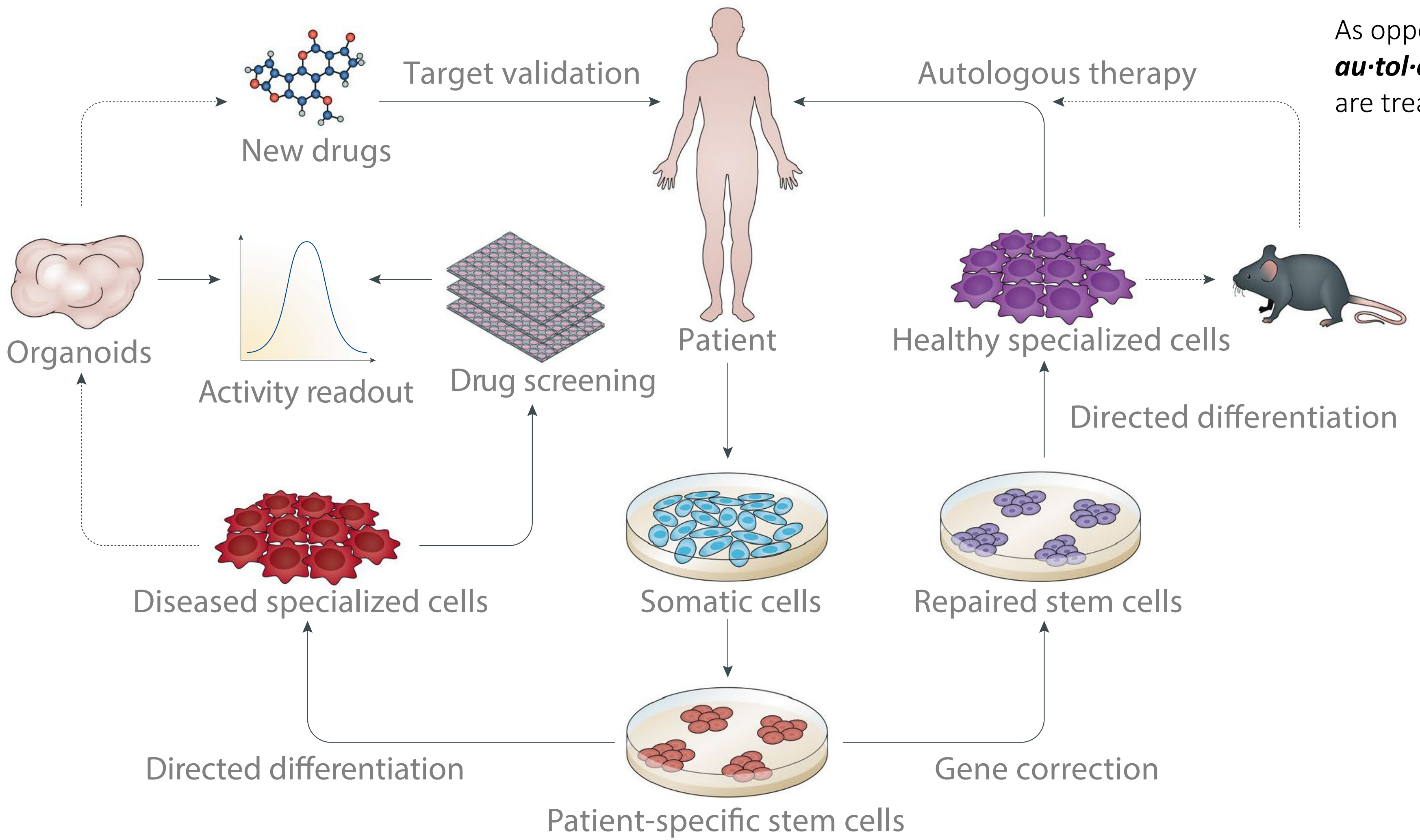
Drug Development
&
Personalized Medicine

STEM CELL APPLICATIONS

Drug development

Therapy

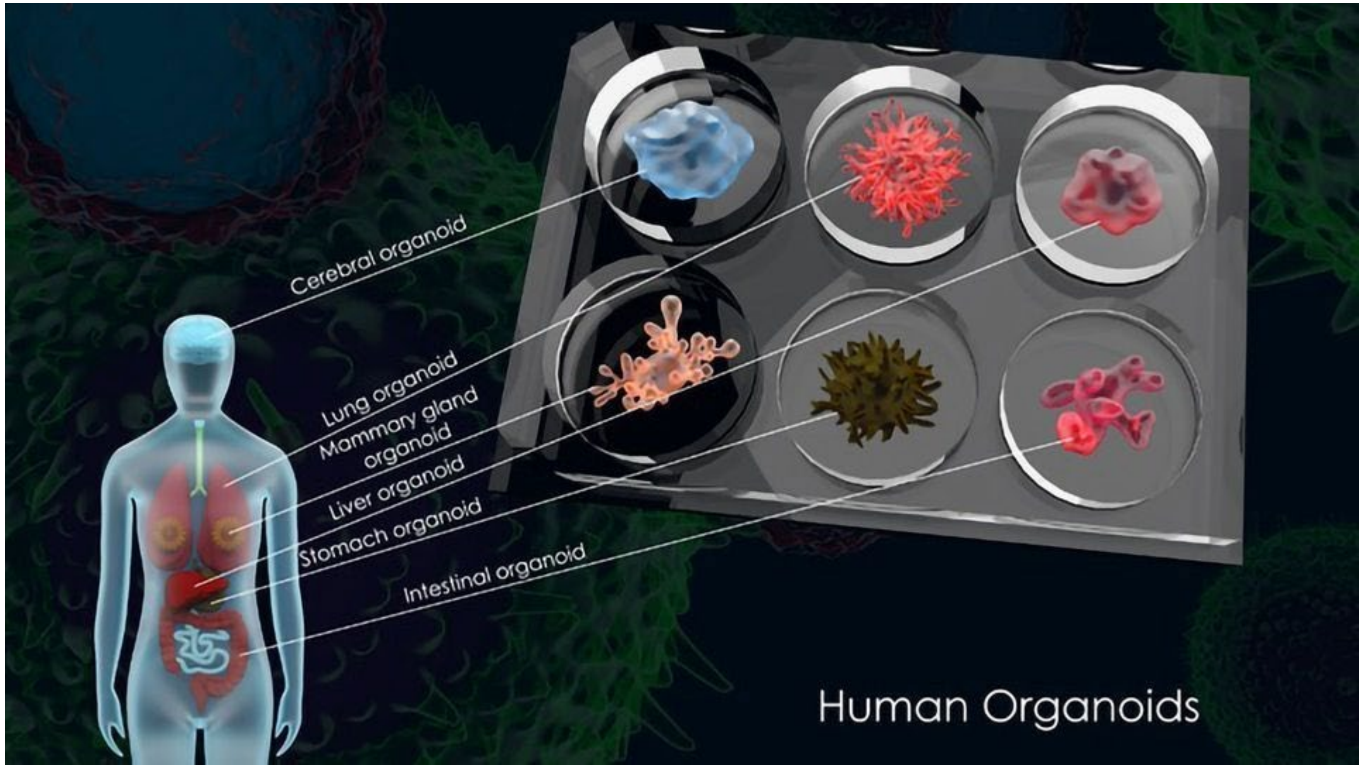
As opposed to ***allogeneic therapy***, ***au·tol·o·gous therapy*** is when patients are treated with their own cells.



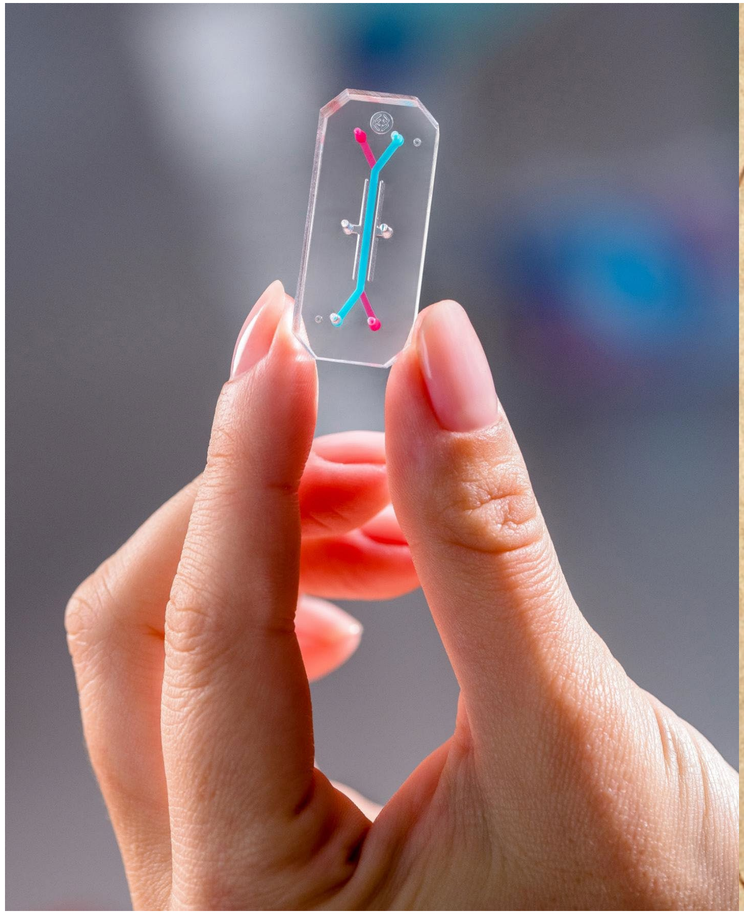
ORGANOIDS

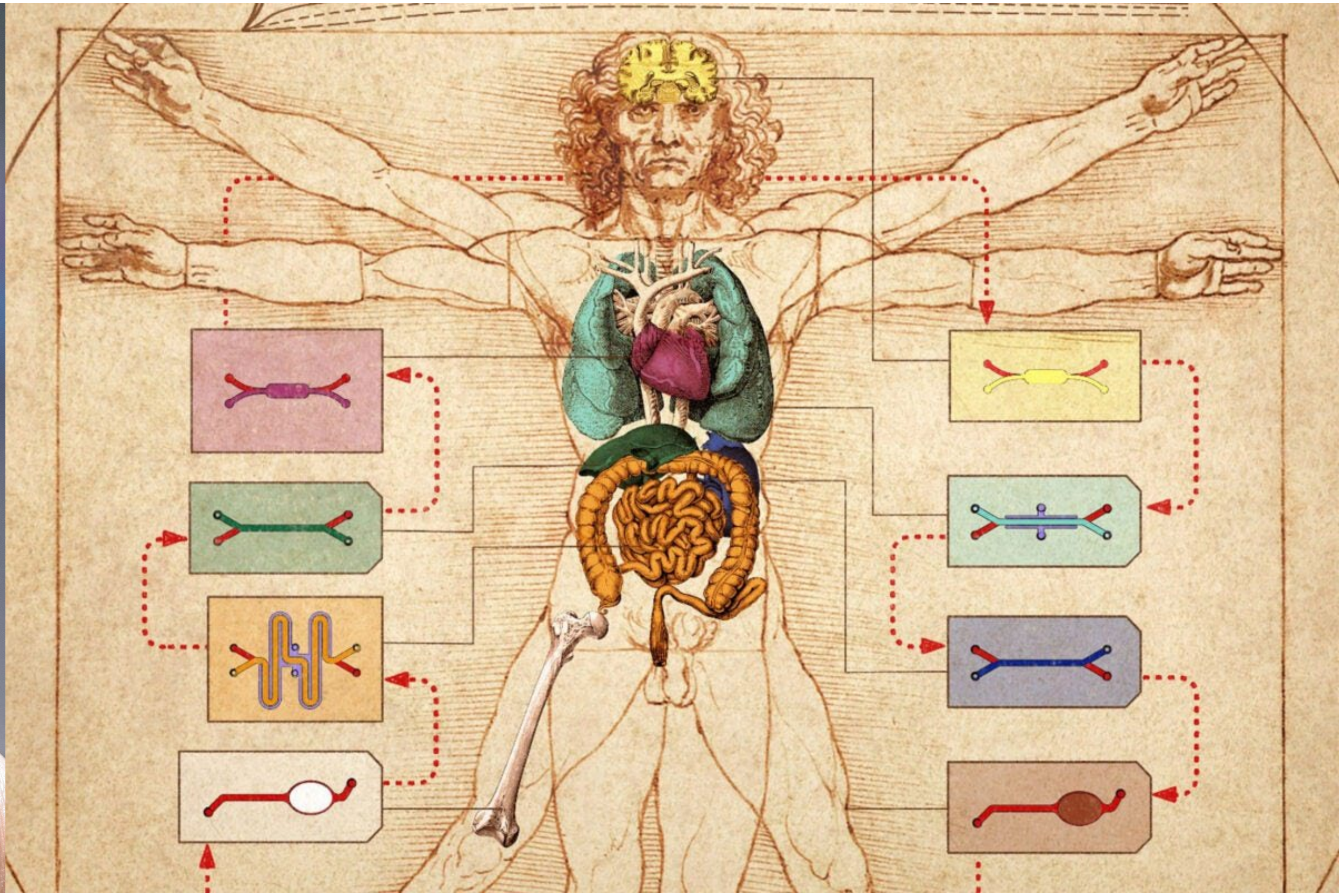
And the Possibility of Radioimmunomicroscopy

- Cells are today identified in 2D samples using immunoassay technology and fluorescence microscopy.
- However, 2D samples are far from the “real thing”.
- Due to light scattering, fluorescence microscopy is of limited use when observing cells in a 3D structure.
- Apparently, it is possible to position radioactive decay within a few µm.
- A typical cell is of the same size, 10 µm.
- Using a sibling to fluorescence immunoassay technology, radioimmunoassay technology, it thus seems possible to build a “radioimmunomicroscope”.
- **Despite some radiotoxicity, such a device would be of immense value to the life sciences as it would allow individual cells to be identified and observed deep within tissue constructs such as organoids.**

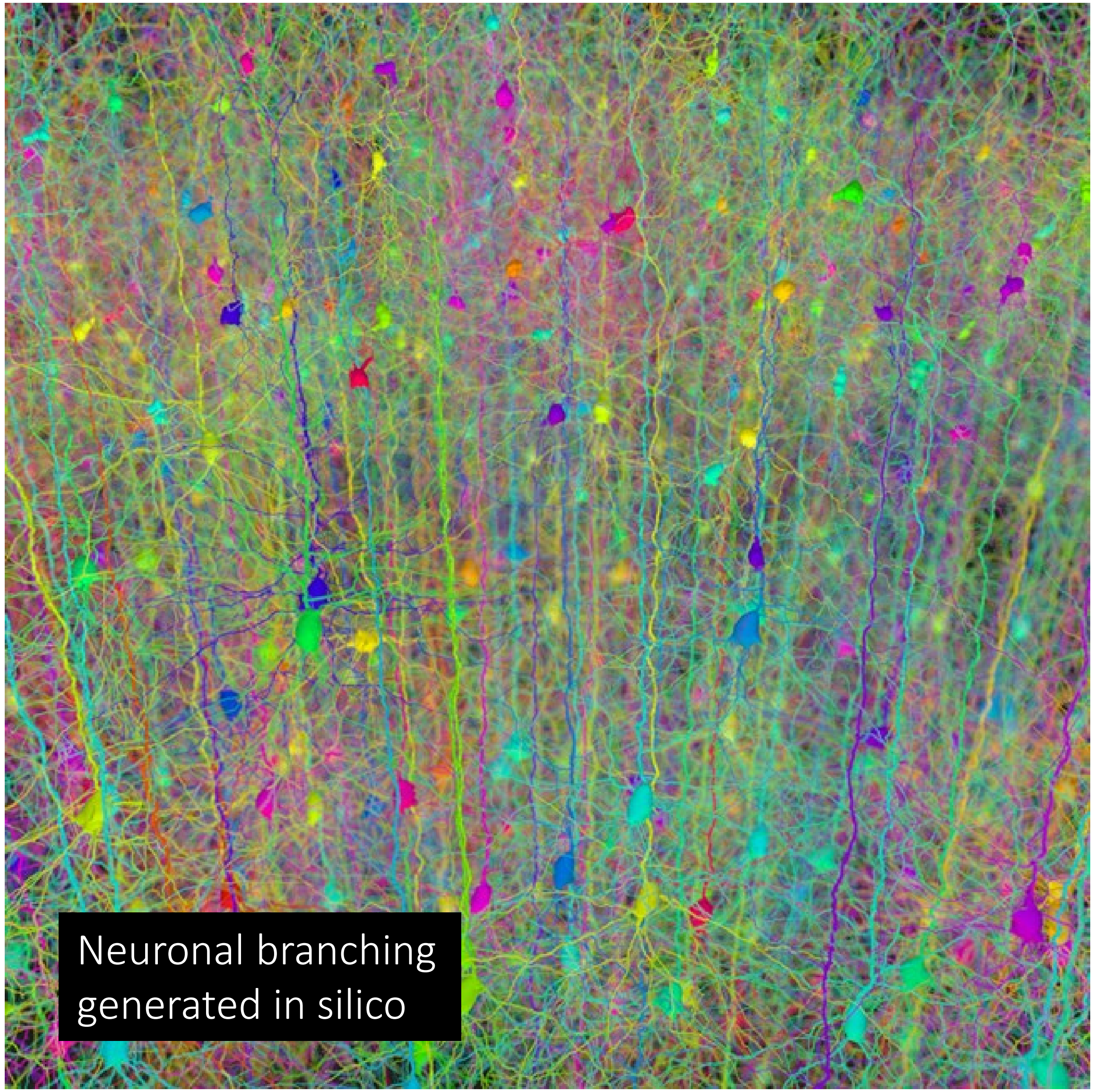


Beyond Organoids





- Multicellular organisms (like you) are systems of individuals, much like an anthill or beehive.
- The somatic worker cells interact and live their lives to maximize the propagation of your or related sex genes.
- You are an incredibly complex and seemingly chaotic community of 37 000 billion cells, interacting within and with countless bacteria and viruses.
- **Computers have today reached a capacity level allowing the modeling of small cellular systems "in silico".**
- **The first success in this direction is modern AI and artificial neural networks.**

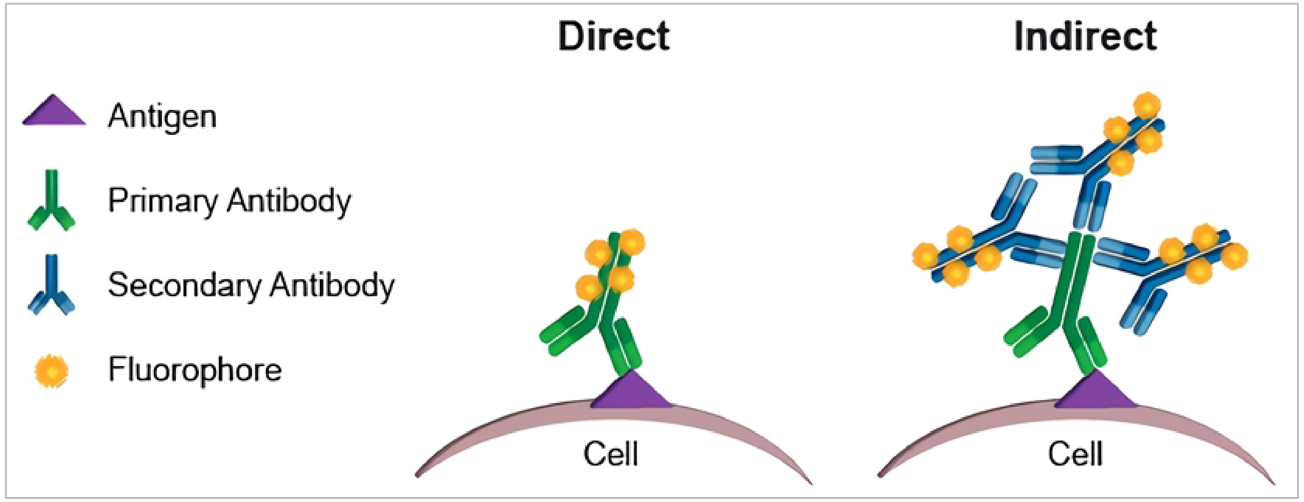


Neuronal branching generated in silico

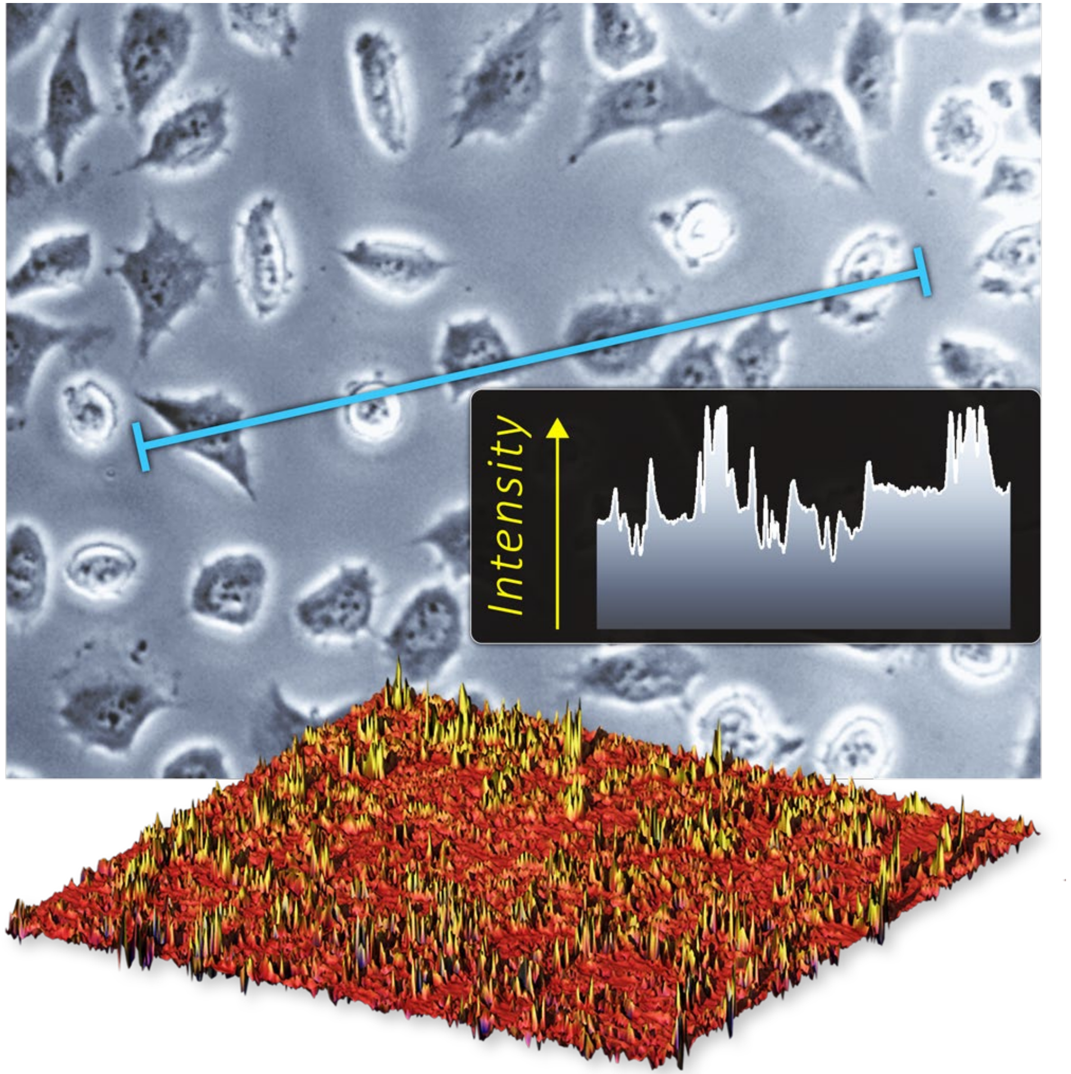
Quantitative Phase Imaging

THE PROBLEM OF FLUORESCENCE MICROSCOPY

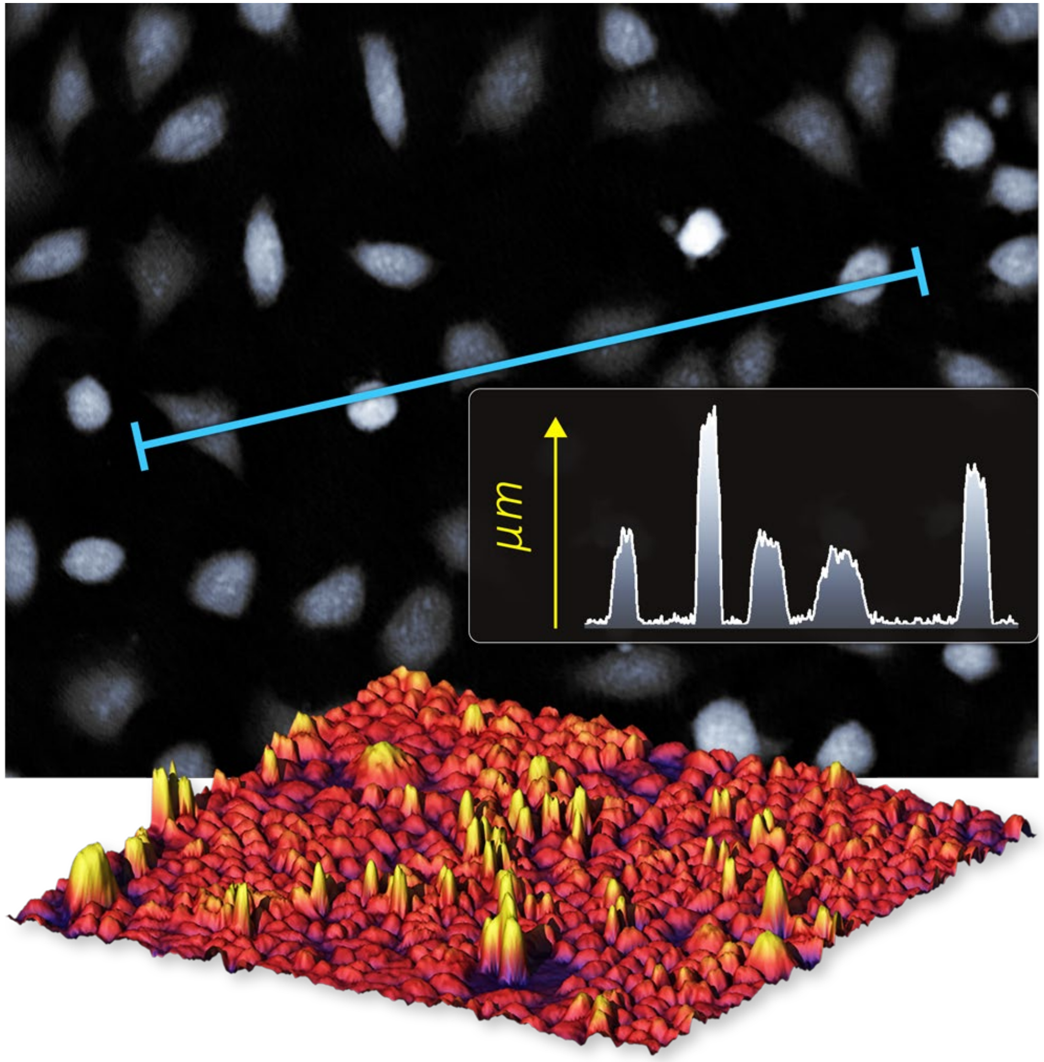
- Fluorescence microscopy is invasive.
- The phototoxicity of the fluorescent molecules alters cell behavior or kills the cells.



Classical Zernike Phase Contrast Microscopy
(Nobel Prize in Physics in 1953)



Quantitative Phase Microscopy
(No Nobel Prize, Yet)



How does it work?

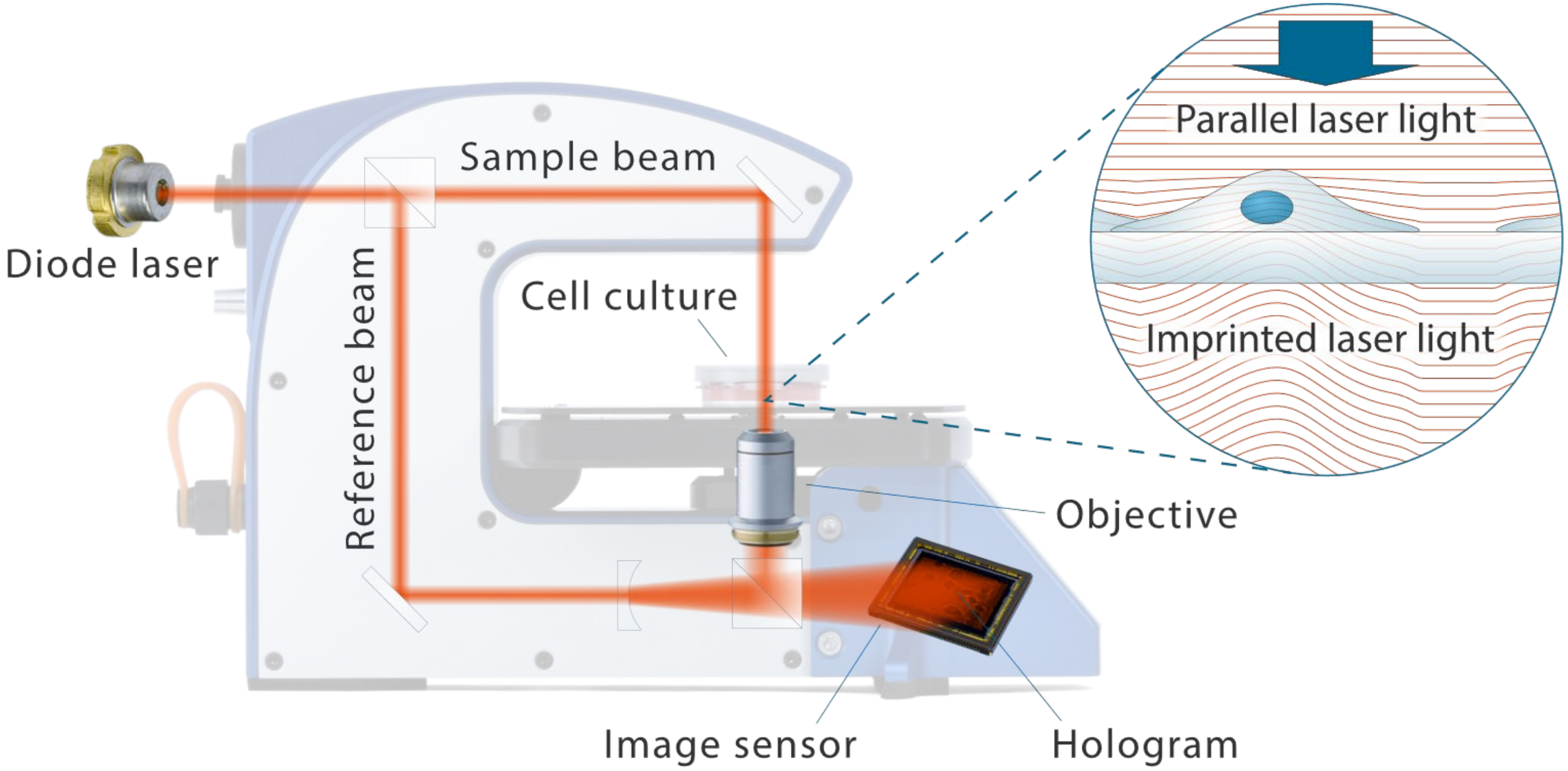
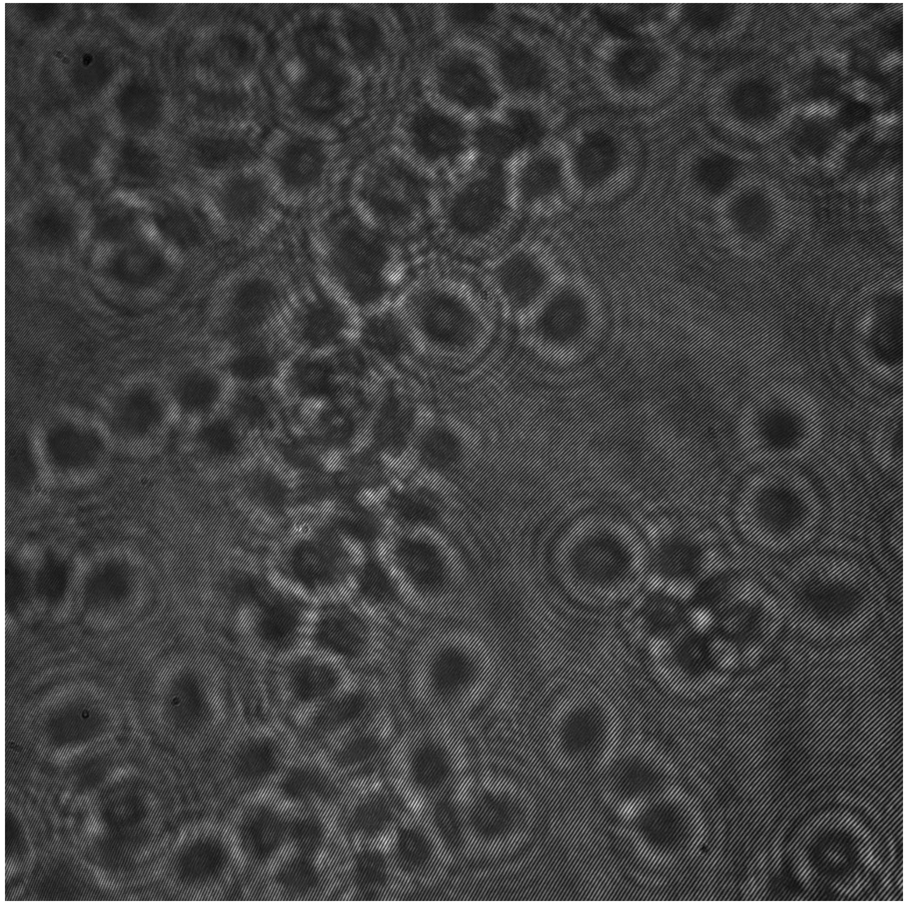
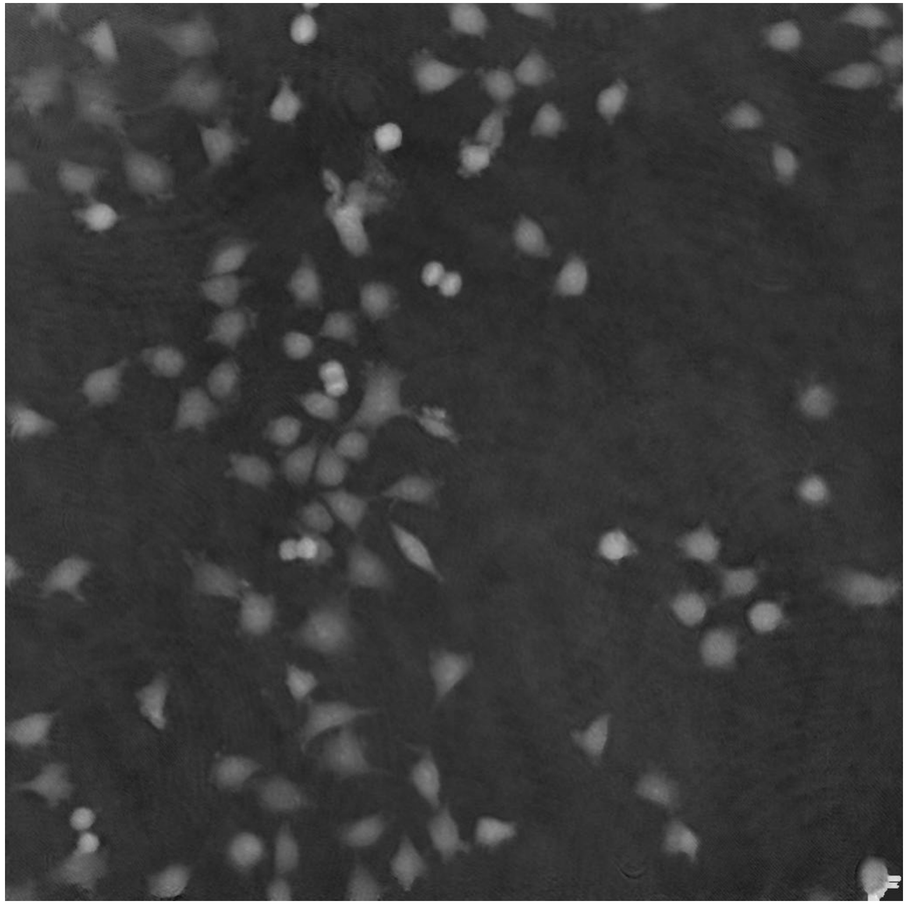


IMAGE RECONSTRUCTION



Hologram

1. Filter in the hologram Fourier space to isolate the sample beam from the reference beam.
2. Remove the sample beam field curvature.
3. Apply a numerical lens to the image.
4. Propagate the aperture to focus the image using the Fresnel transfer function or similar.
5. If the image is out of focus, go to step 3 and refocus using a different focal (propagation) distance.
6. Else, do phase unwrapping.
7. Done!



Reconstructed Phase Image

Can AI be used to accelerate and improve reconstruction?

AI & Non-invasive Imaging
in
Biomanufacturing

CELL-BASED THERAPY

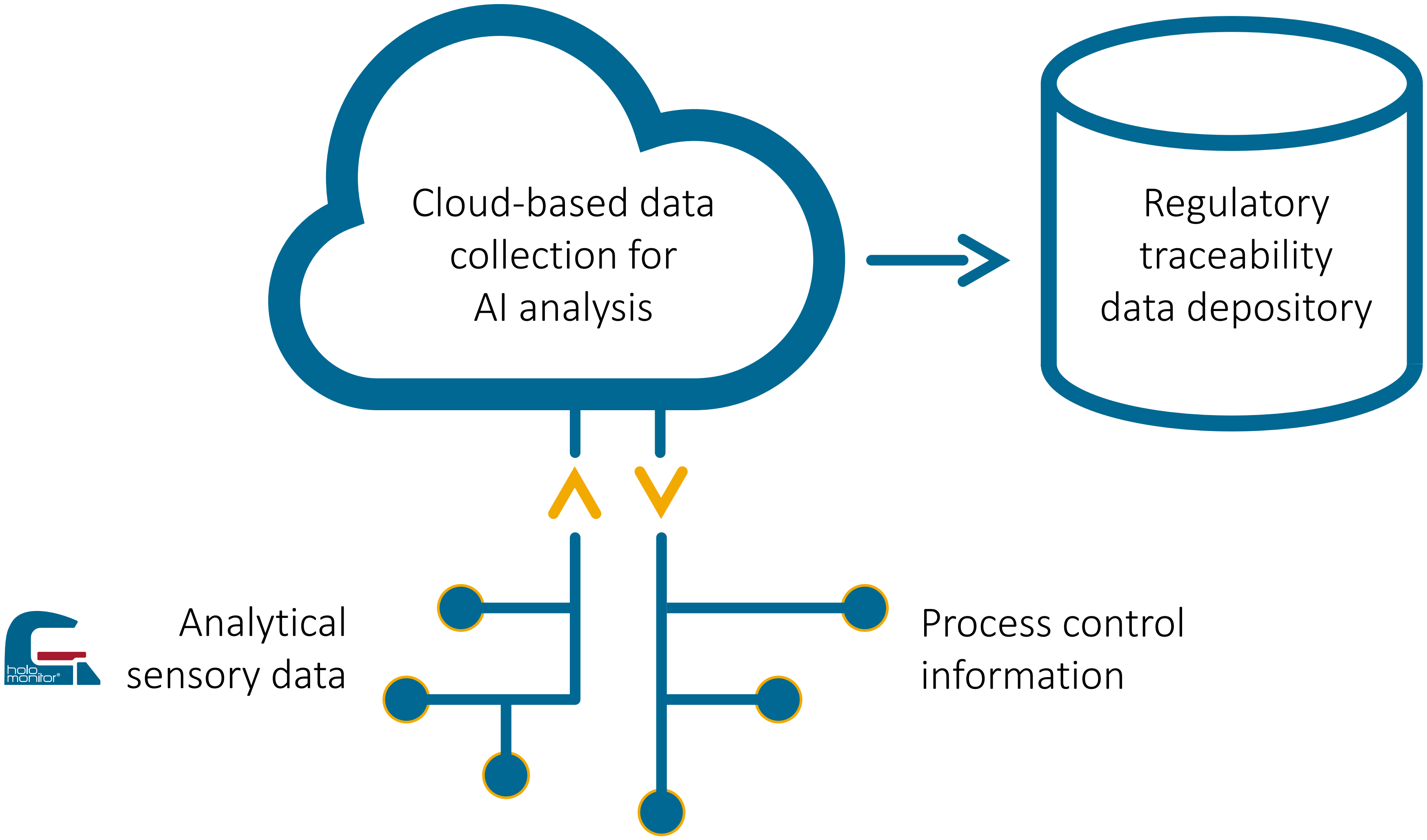
The Manufacturing Opportunity

- Traditionally, cell culturing is a small-scale research activity
- Accordingly, available tools are not suitable for large-scale biomanufacturing (right image)
- **This has created an unprecedented opportunity in providing these much-needed large-scale manufacturing tools**

Cell culture quality control at a leading contract manufacturer of cell therapies

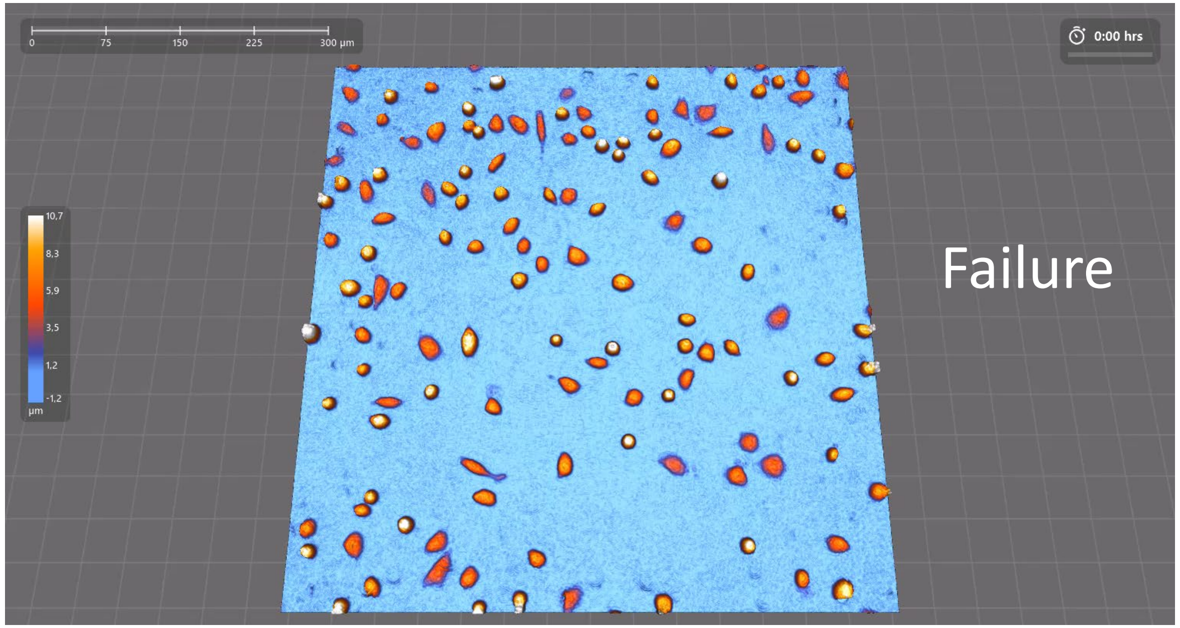
THE "BRAIN" OF BIOMANUFACTURING

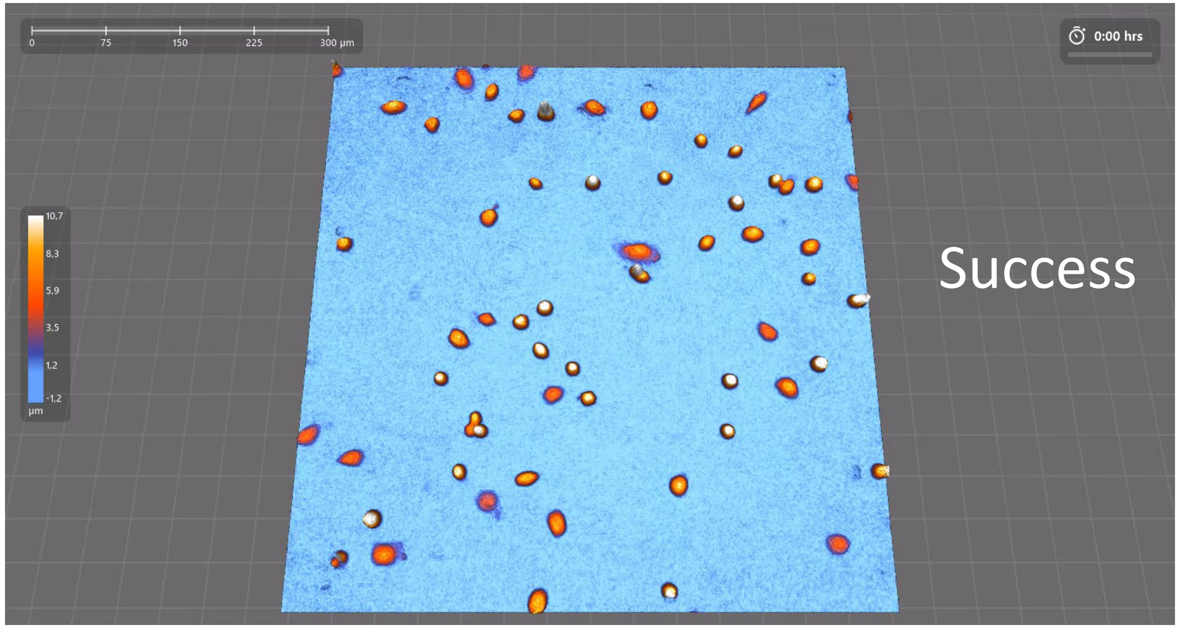
Advanced cell therapy manufacturing



PREDICTING BIOMANUFACTURING

- The manufacturing of cell-based therapies can take up to several weeks
- Predicting the outcome at an early stage before cell death occurs is possible by quantifying cell morphology and failed cell divisions
- It would be an invaluable tool for biomanufacturing!

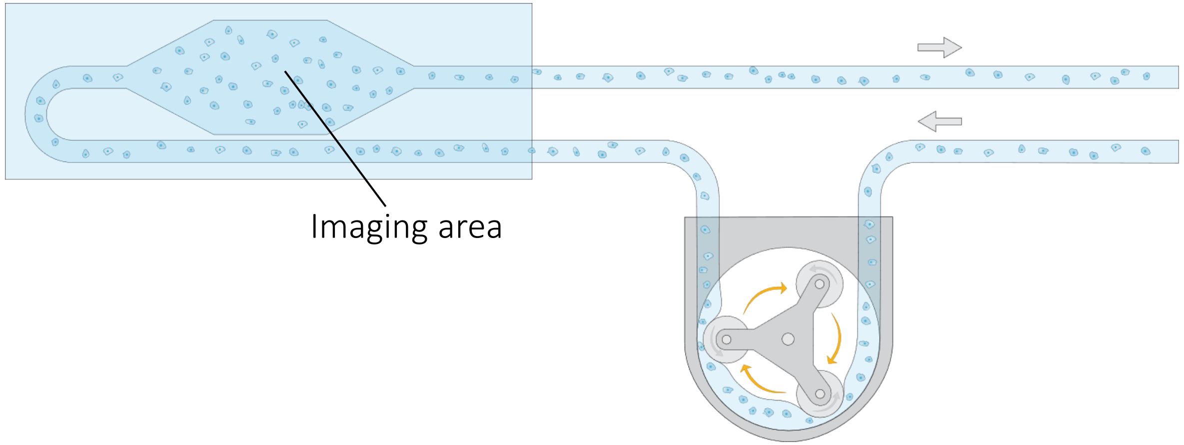




Non-invasive time-lapse videos of proliferating cell cultures created using HoloMonitor.
Watch the above and other cell videos here on YouTube.

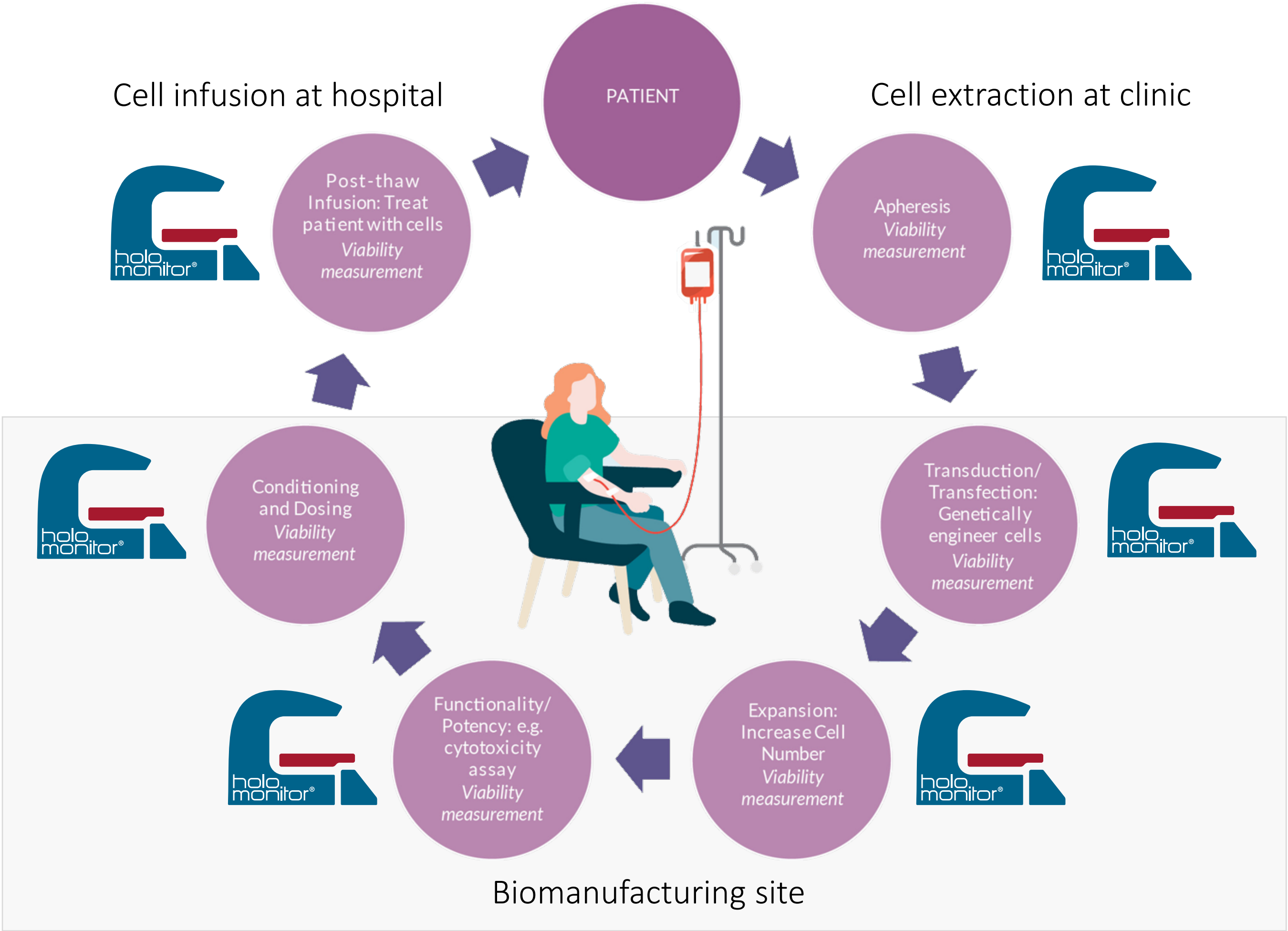
CELL ANALYSIS IN BIOREACTORS

- T-cells and other immune cells are cultured in disposable bioreactors.
- Today there are probes for analyzing the culturing media.
- A small probe or device that non-invasively image and analyze the cells is needed.
- For regulatory traceability, images of abnormal cells should be saved.



CELL VIABILITY

- Cell viability is the number of dead cells divided by the total number.
- NIST is leading the ISO standardization group to create a cell viability standard.
- However, NIST has concluded that available cell visibility assays are inadequate for cell therapy manufacturing.
- Currently, all cell viability assays on the market are invasive.



Treatment cycle image by courtesy of NIST

NON-INVASIVE CELL VIABILITY

Live/Dead Cell Proof-Of-Concept

- Fluorescence microscopy is commonly used to determine cell whether a cell is dead or not.
- The task was to replicate the fluorescence results by only using morphological cell data, which was acquired non-invasively.
- Erastin, Shikonin and Staurosporine are treatments that induce different types of cell death.
- **Similar results using fluorescence microscopy would require four invasive fluorescent reagents!**

	F1-score	Precision	Recall	Support
Living Cell	0.99	0.98	0.99	4457
Expert is Uncertain	0.51	0.76	0.38	103
Dead by Erastin	0.94	0.92	0.96	440
Dead by Shikonin	0.94	0.94	0.93	414
Dead by Staurosporine	0.82	0.82	0.82	152

Modeling and results by Vladimir Loncar, CERN and MIT

Thank You