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Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

Barbara Ghinassi

2D and 3D cellular models for neuronal diseases modelling and drug screening

Università "G. d'Annunzio" di Chieti-Pescara

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Advanced drug delivery systems and in vivo theranostic tools for personalised medicine

The main goal of the WP3 is the design of innovative strategies to reach patient-centered therapies personalized on specific patient needs in terms of efficacy, accuracy, safety, and/or compliance of diagnosis and treatment of diseases. To this end, three specific objectives will be pursued:

T3.1 Development of **personalized pharmaceutical dosage forms** by additive manufacturing technologies;

T3.2 Engineering **bioinspired** and **biomimetic nanomedicines** for precise drug delivery;

T3.3 Optimization of **innovative methods to screen tissues and organs** based on drug loaded iron oxide nanoparticles (SPION) and/or gadolinium synthetic derivatives, embedded into red blood cells that can be magnetically guided and traced by Magnetic Resonance Imaging (MRI) and magnetic particle imaging (MPI).

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

Ud'A Università degli Studi "G. d'Annunzio"

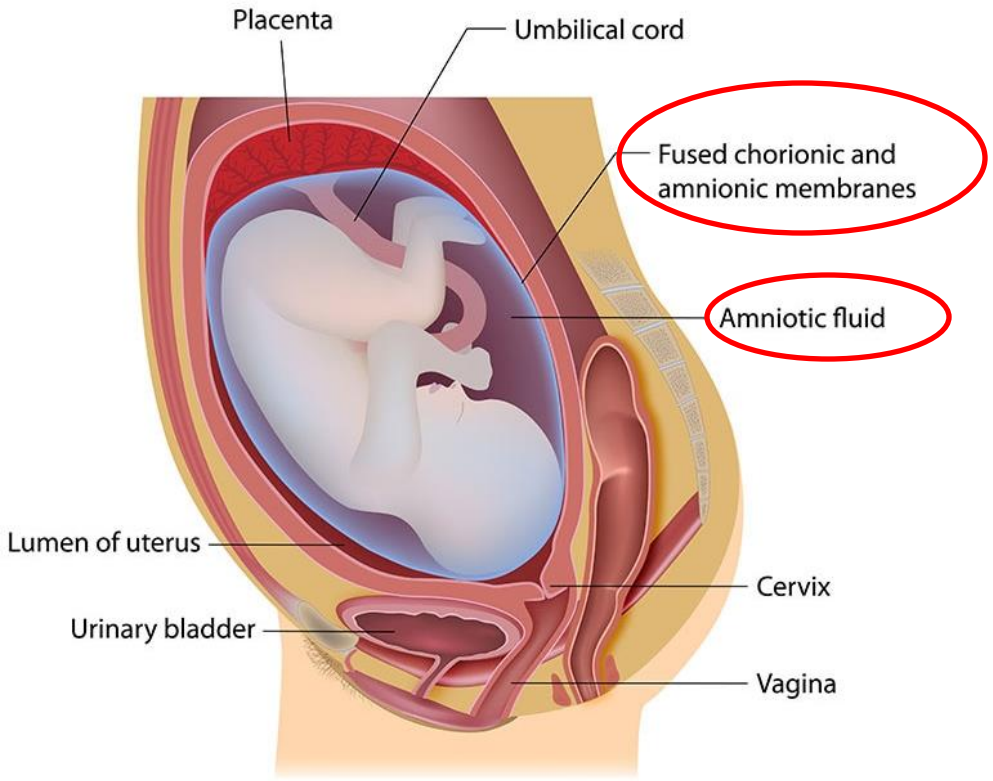
MECCANO human technology for innovation

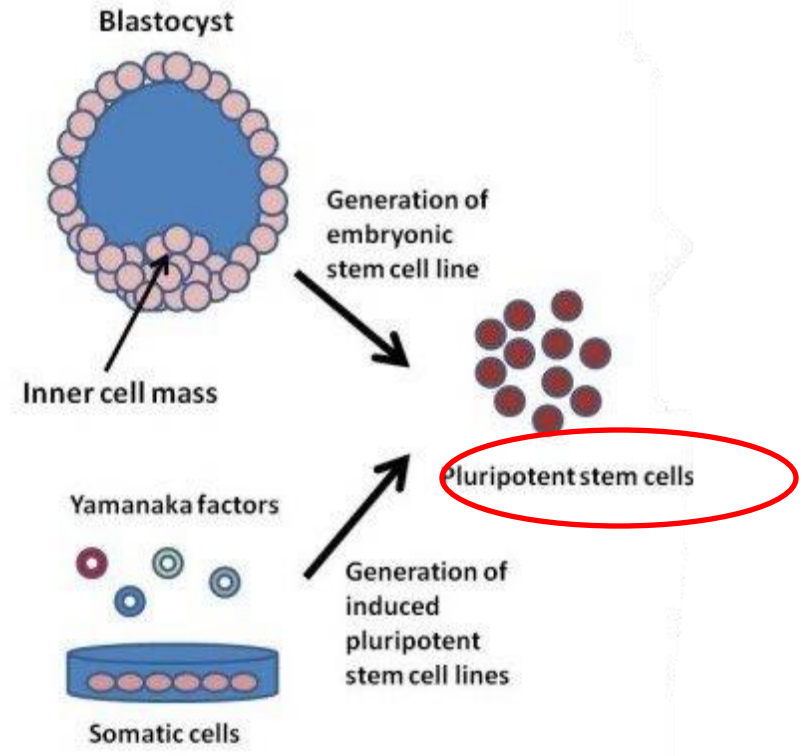
COSMOB

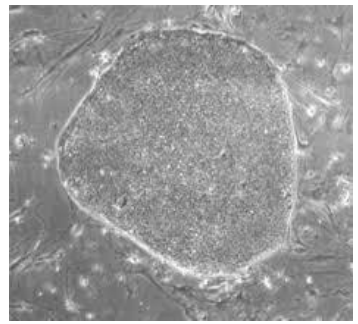
2D and 3D cellular models for neuronal diseases modelling and drug screening

- hiPSCs → Mimic the features of the pluripotent stem cells within the blastocyst
- hAFSCs
- hFM-MSCs

→ Represent the environment where the embryo develops (isolated from fetal annexes)





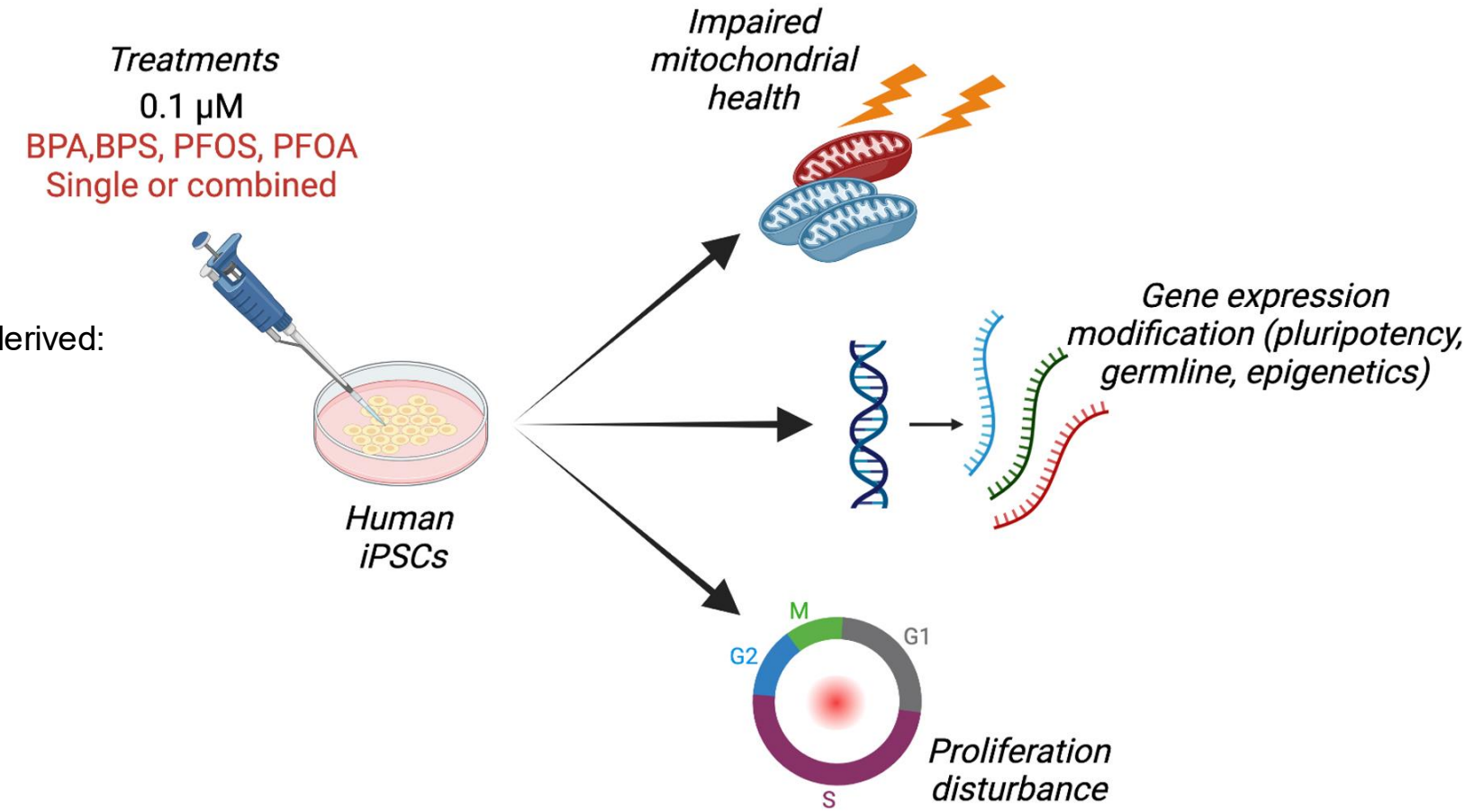


2D and 3D cellular models for neuronal diseases modelling and drug screening

OUR LAB MODELS:

- Human iPSC-derived and perinatal stem cell-derived:

1. Dopaminergic neurons
2. Motor neurons
3. Brain organoids
4. Cardiomyocytes
5. Myotubes



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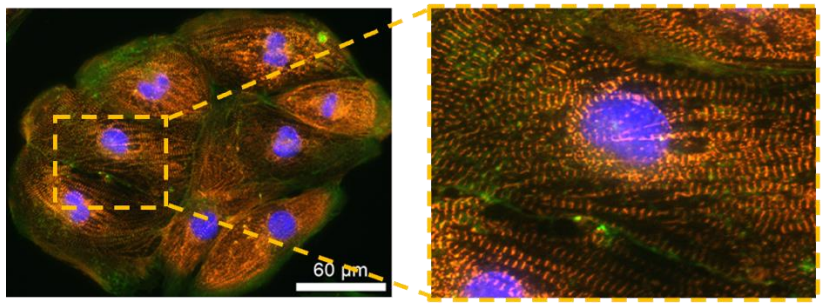
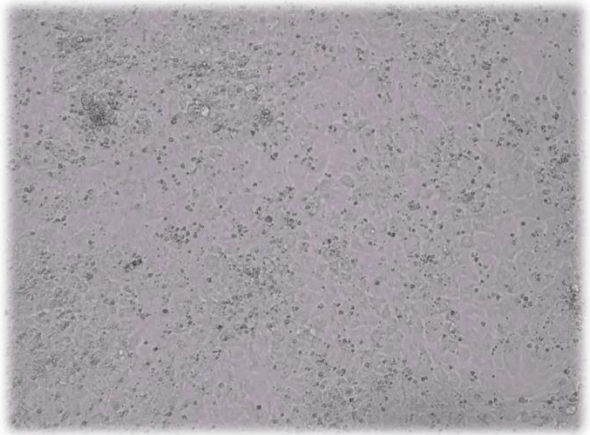
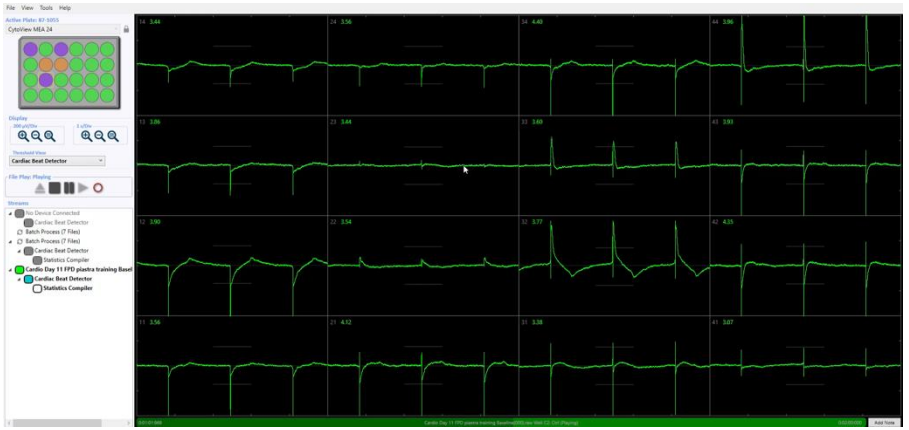
Cardiomyocytes







- Our cardiomyocytes show robust and mature sarcomere
- In addition, their sarcomeres are functional, and they are able to contract

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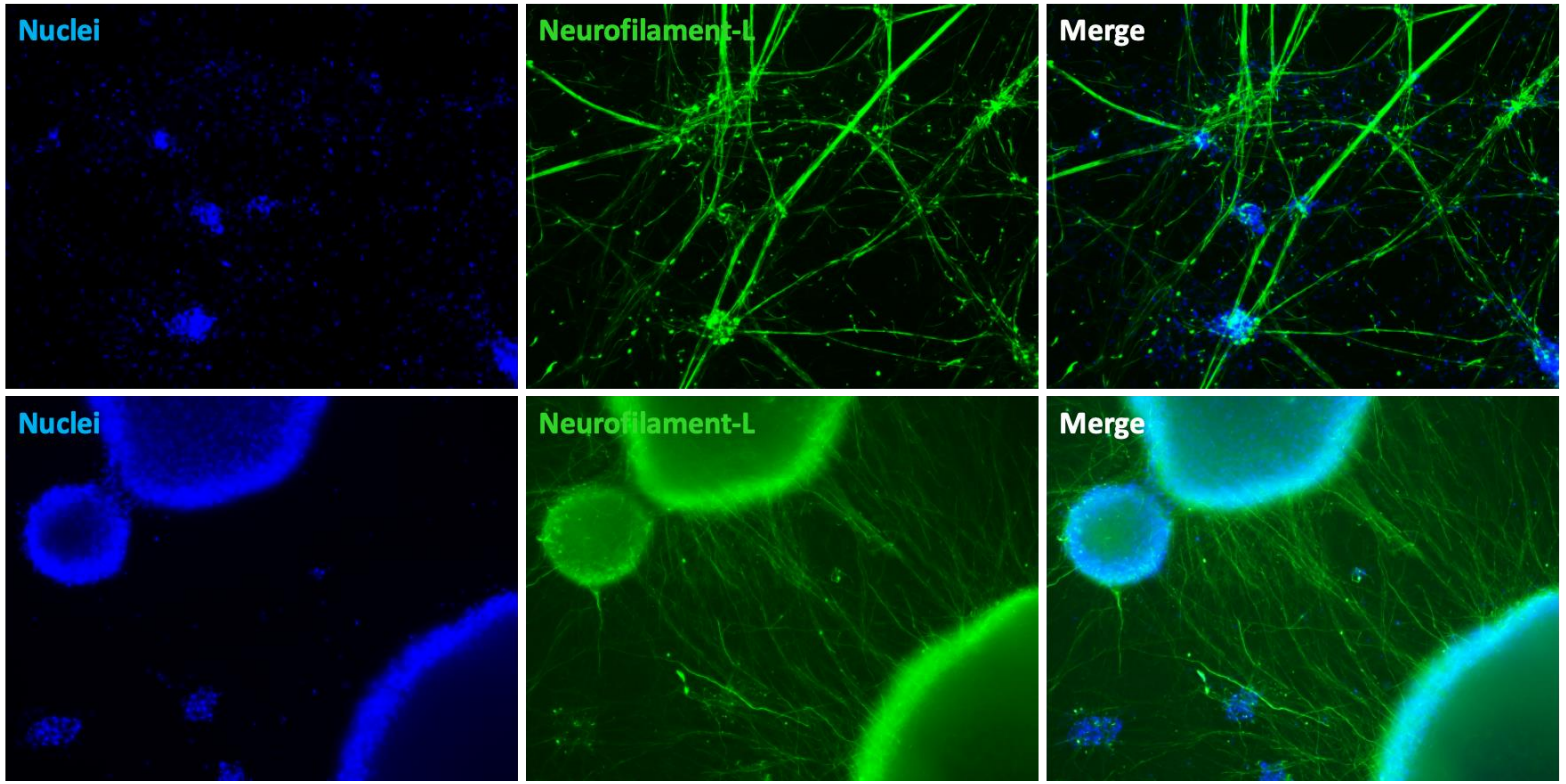
iPSC-derived Dopaminergic Neurons



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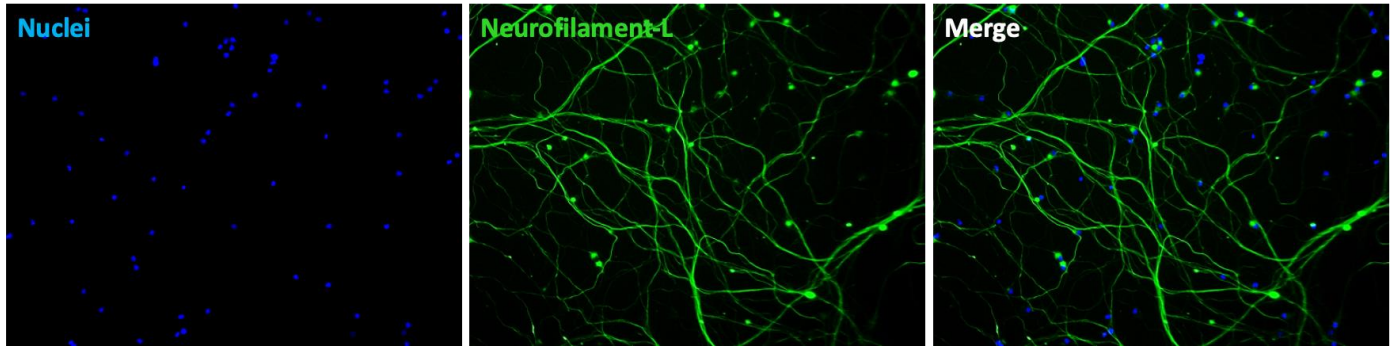
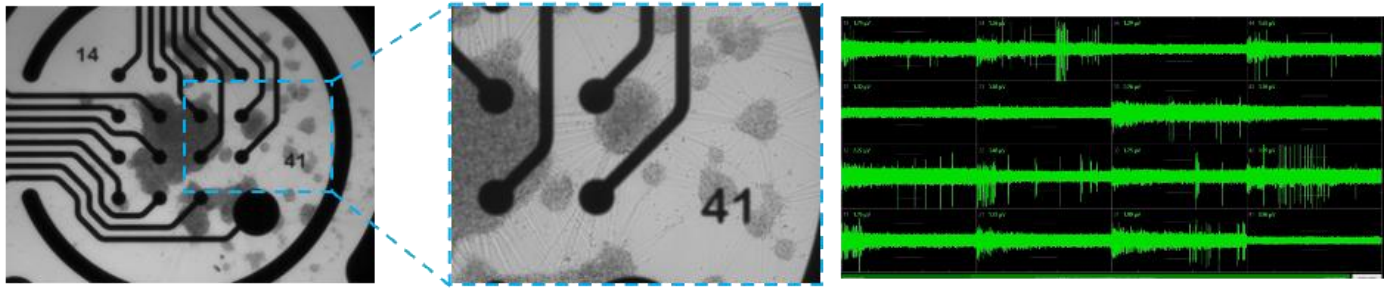
iPSC-derived Motor Neurons



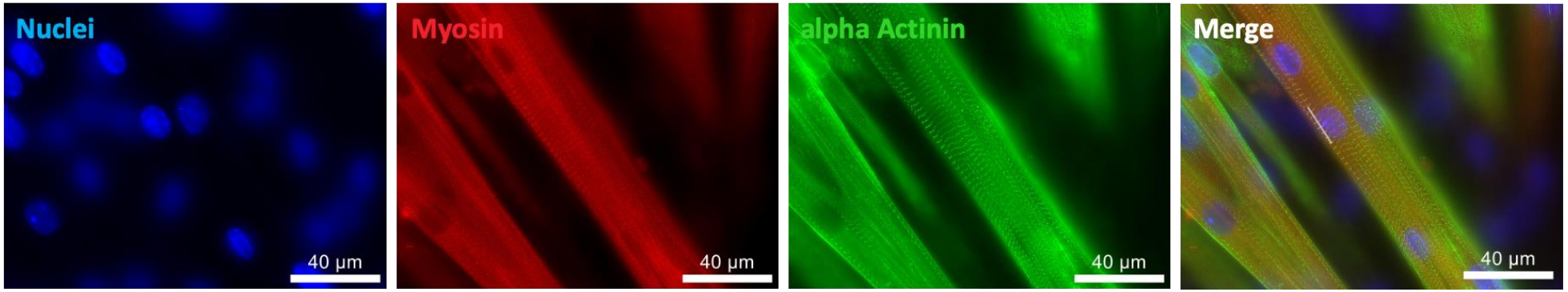
MN on MEA

- Using special plates equipped with electrodes, we can record electrophysiological activity of our neurons in vitro and real time
- To do this, we use our Maestro Edge apparatus



Skeletal Muscle cells

- In addition, we established a robust system to obtain mature myotubes from murine and human myoblasts
- We used myotubes to make a co-colture (motor neurons+skeletal muscle)



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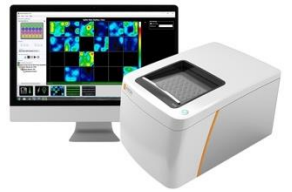
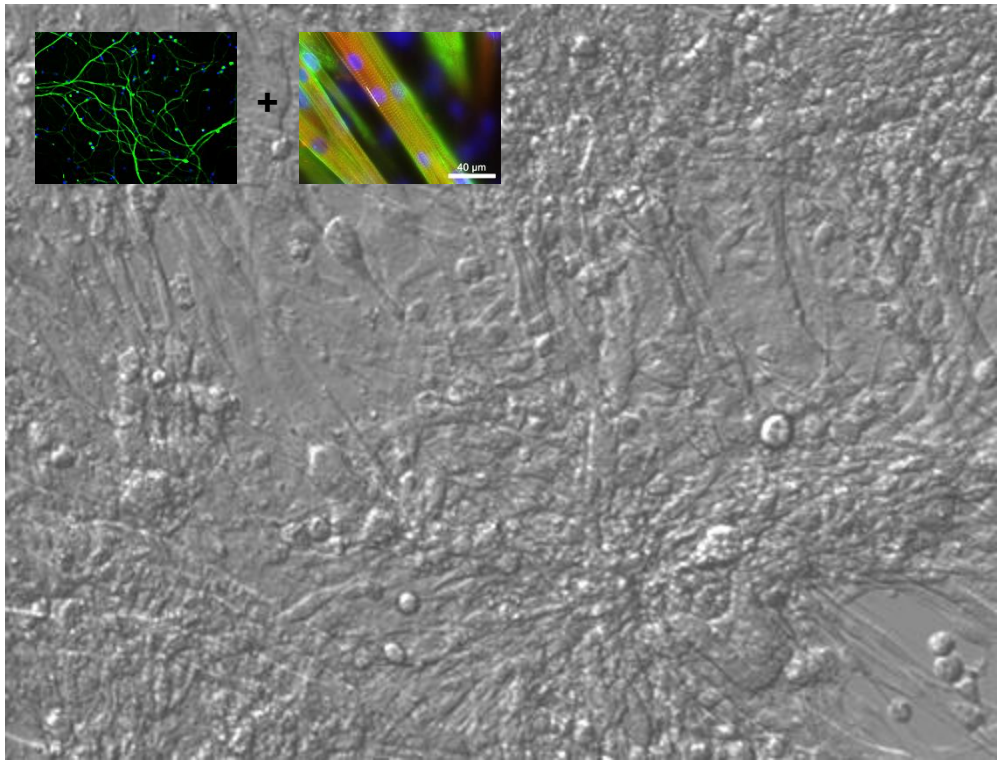
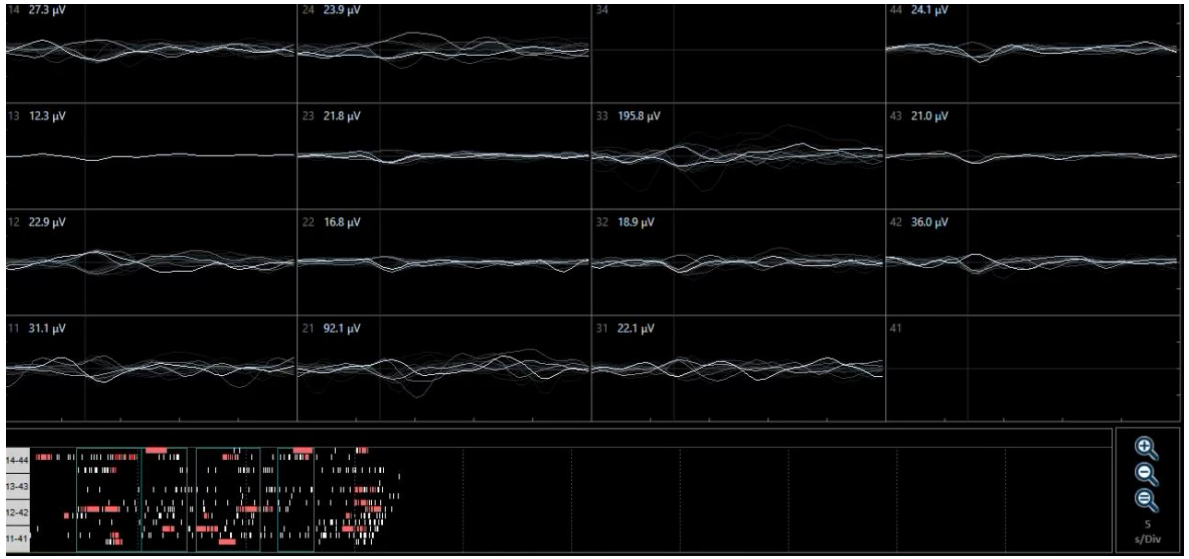
Functional Co-colture: Motor Neurons and Skeletal Muscle







- We were able to obtain a functional co-colture when iPSC-derived motor neurons lead to myotubes contraction (as showed in the video on the left)
- Our MEA technology allows us to record real-time neural activity (video on the right)

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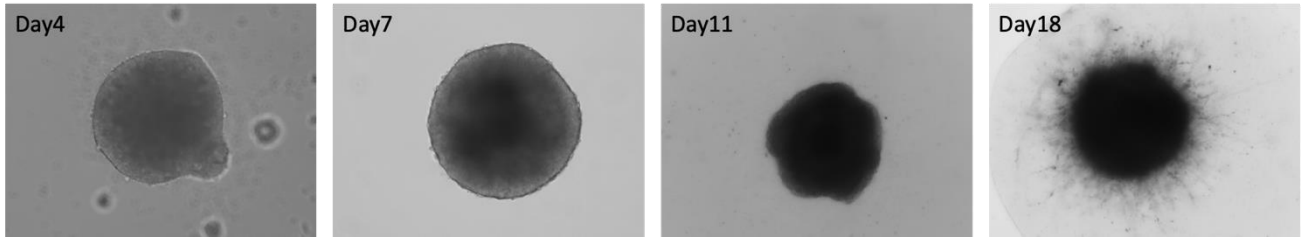
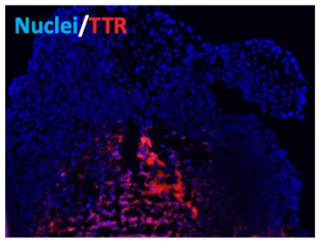
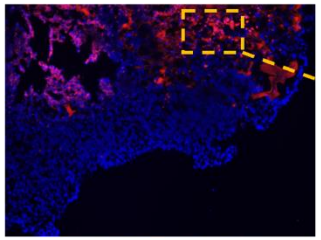
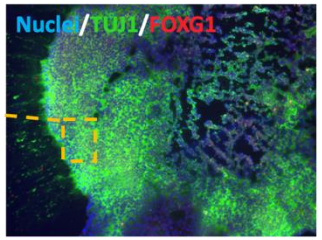
Development of Brain Organoids



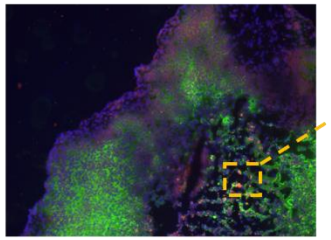




Transthyretin (TTR) expression in the brain localizes in the choroid plexus

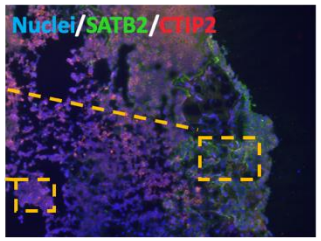


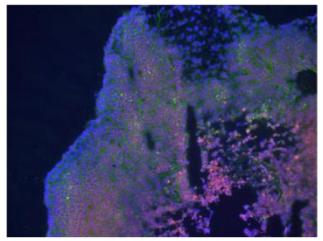
TUJ1 is expressed in neurons of the PNS and CNS: involved in microtubule stability and axonal transport



FOXG1 is a transcription repression factor. role in the regional subdivision of the developing brain and in the development of the telencephalon

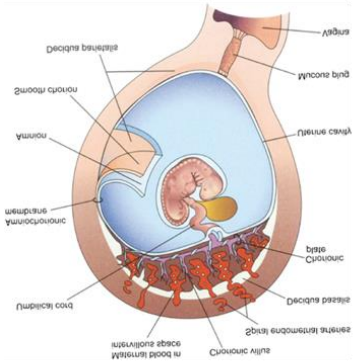
SATB2 regulates chromatin organization and gene expression. In the developing brain, it is expressed in cortical neurons in the corpus callosum

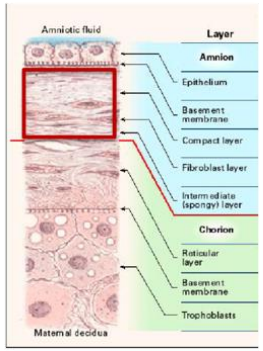




CTIP2 is a transcription factor involved in the extension of subcortical projections by cortical neurons

Perinatal Stem cells





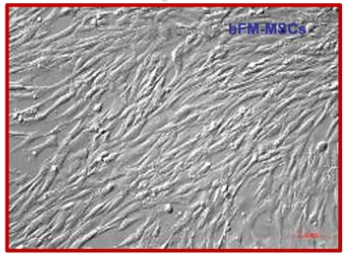
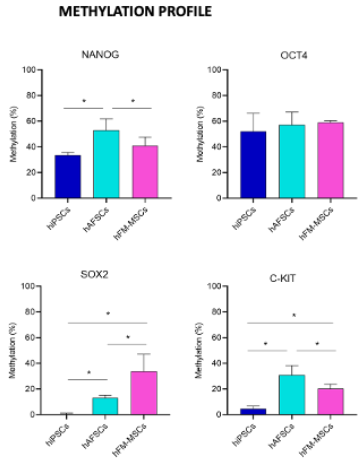
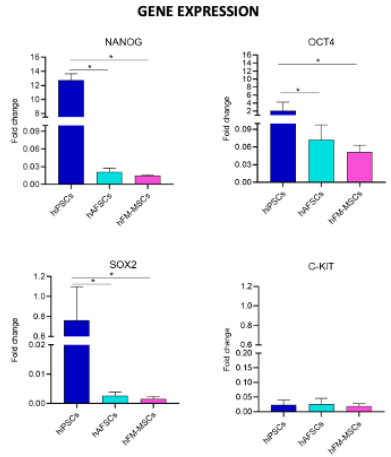


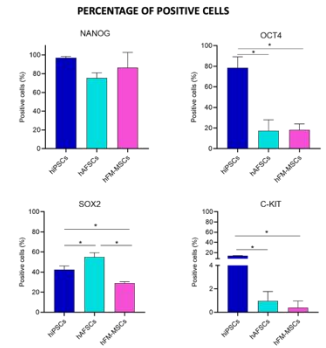
Table 1. hFM-MSC phenotypic characterization

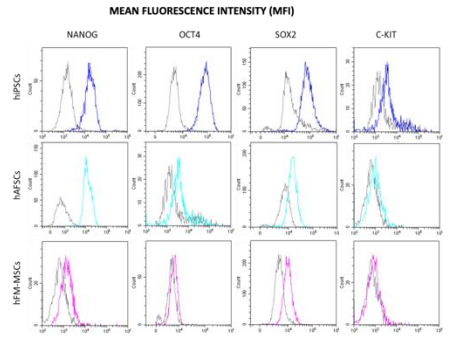
Marker	Positive cells (%)
Pluripotent stem cell markers	
OCT4	25.2 ± 8.3
SSEA4	18.3 ± 4.1
SOX2	28.9 ± 4.6
NANOG	88.1 ± 5.7
TRA1-60	4.1 ± 1.6
C-KIT	1.9 ± 1.2
Mesenchymal stem cell markers	
CD90	88.7 ± 6.4
CD73	92.1 ± 4.5
CD105	84.2 ± 8.5
CD44	94.4 ± 2.3
Hematopoietic cell markers	
CD45	negative
CD14	negative

Gaggi et a. Cells **2020**, *9*, 1304; doi:10.3390/cells9051304



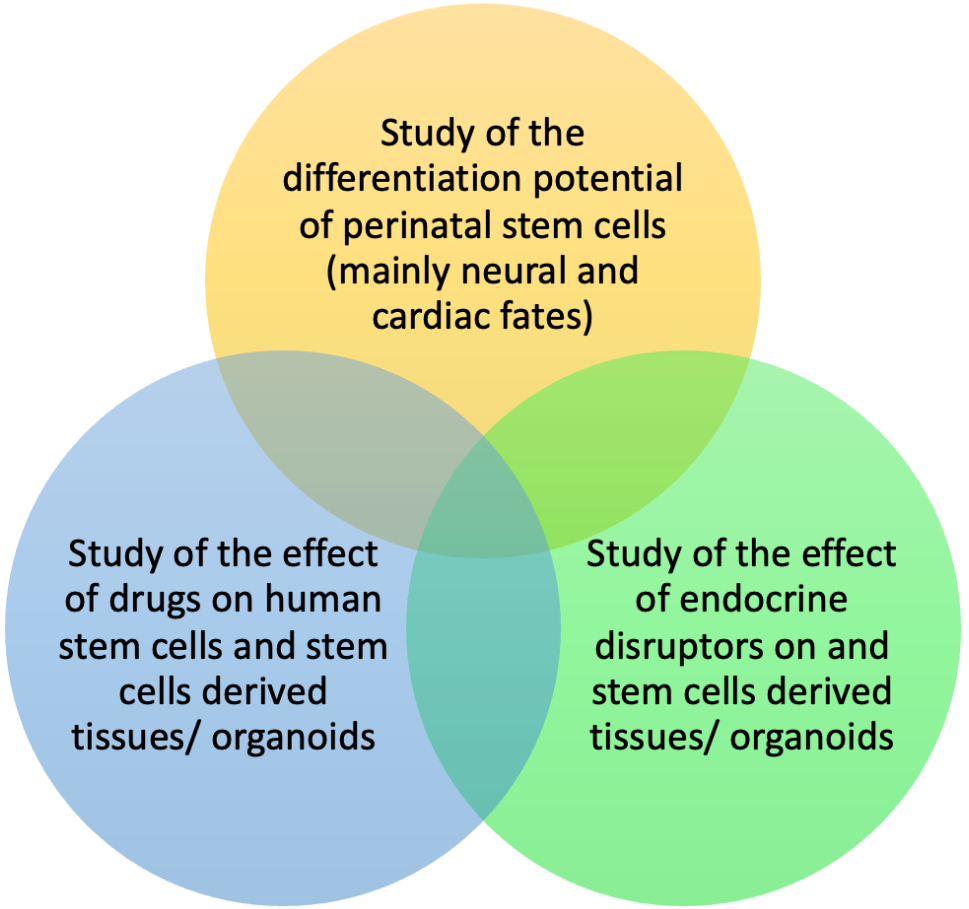






- Genetic, epigenetic and biological characterization (using a line of hiPSCs as positive control)
- Differentiating potential towards the cardiac lineage (mesodermal), and towards two different neuronal lineages (ectodermal), since the ability to differentiate towards different embryonic lineages is a feature of pluripotency.

Research Areas



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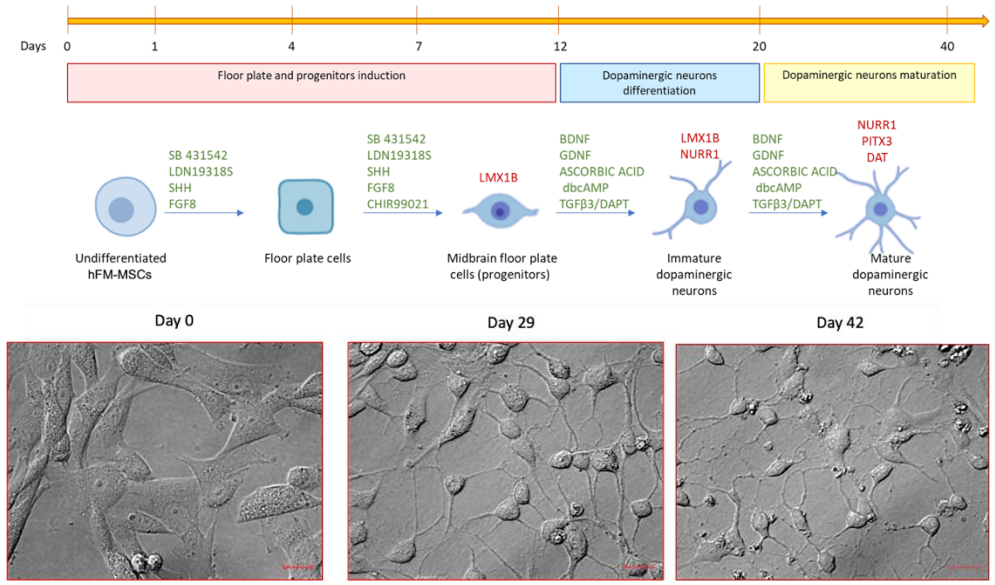
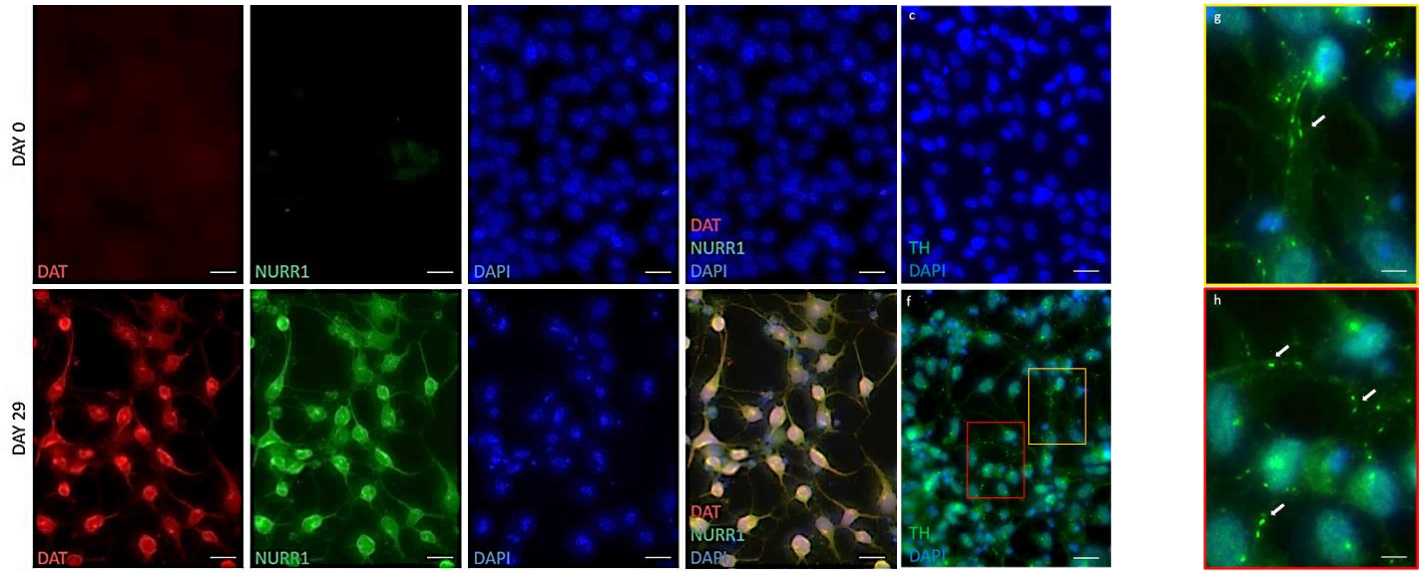
2D culture of human DOPAMINERGIC neurons

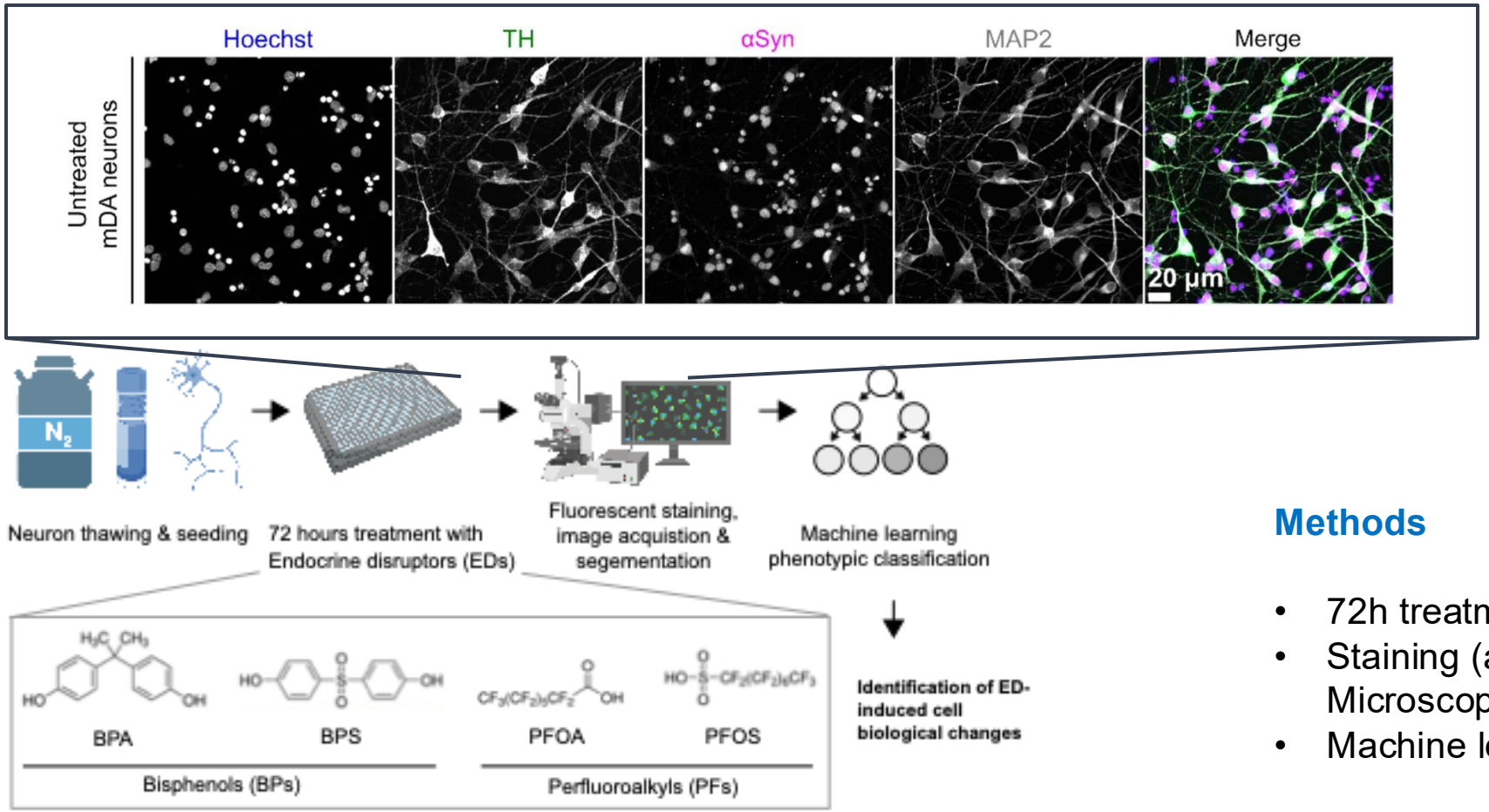
- Stem cells underwent a multistep differentiation process, consisting in the sequential exposure to different small molecules, mimicking the steps of the dopaminergic neurons development in vivo.
- The immunofluorescence analysis revealed that NURR1, DAT and Tyrosine hydroxylase (TH, the enzyme responsible for conversion of the L-tyrosine to L-DOPA), undetectable in undifferentiated hFM-MSCs, was dramatically upregulated after 29 day of differentiation





Di Credico et al.Int J Mol Sci. 2023 doi: 10.3390/ijms241915018.

To examine the potential **neurotoxicity** of BPs and PFs at **real-exposure concentration** on **human iPSC-derived mDANs** by a **ML- approach applied to high content microscopy**



Methods

- 72h treatments (BPA, BPS, PFOS, PFOA)
- Staining (aSyn, TH, MAP2) and High-Content Microscopy
- Machine learning training and classification

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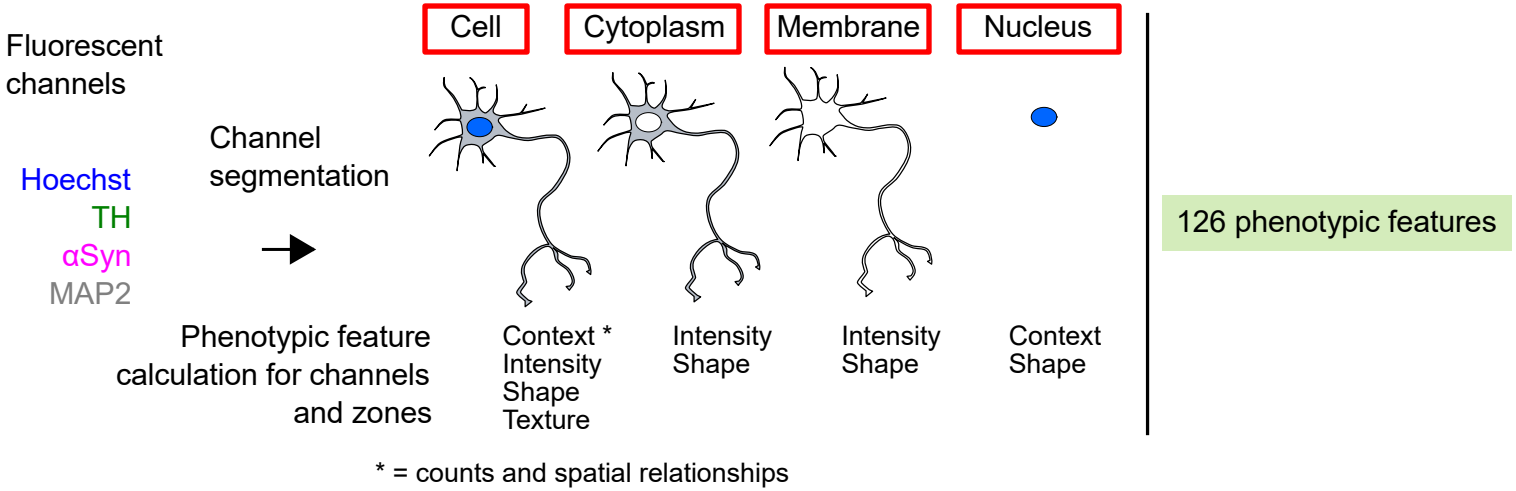
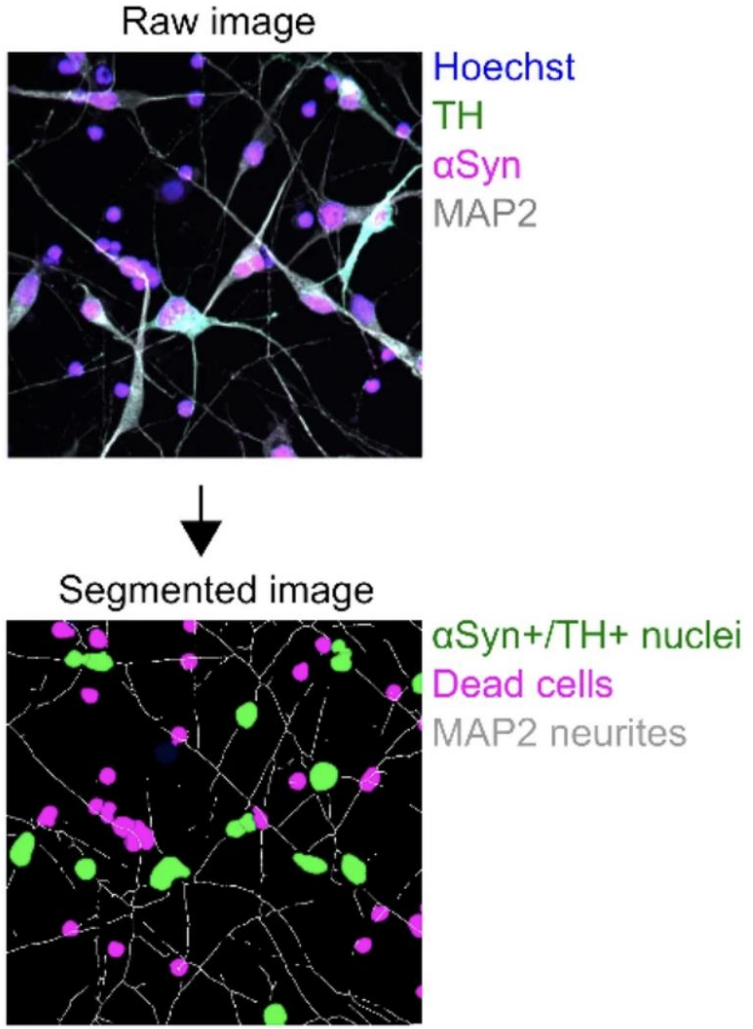
Human iPSC-derived midbrain dopa neurons: BPs and PFs induce some hallmarks of PD

Image segmentation and features extraction





- For high-content screening, cells were segmented into **four compartments (cell, cytoplasm, membrane, nucleus)**
- Based on the segmentations, in total 126 quantitative image features were calculated
- The resulting quantitative data was then used to construct **median phenotypic profiles** per treatment condition and to compare phenotypic profiles

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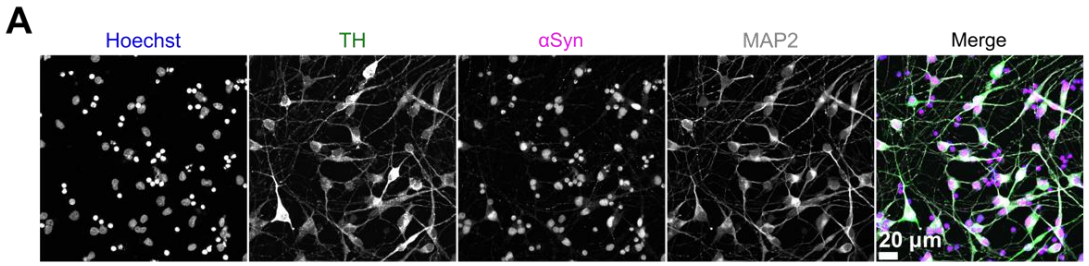
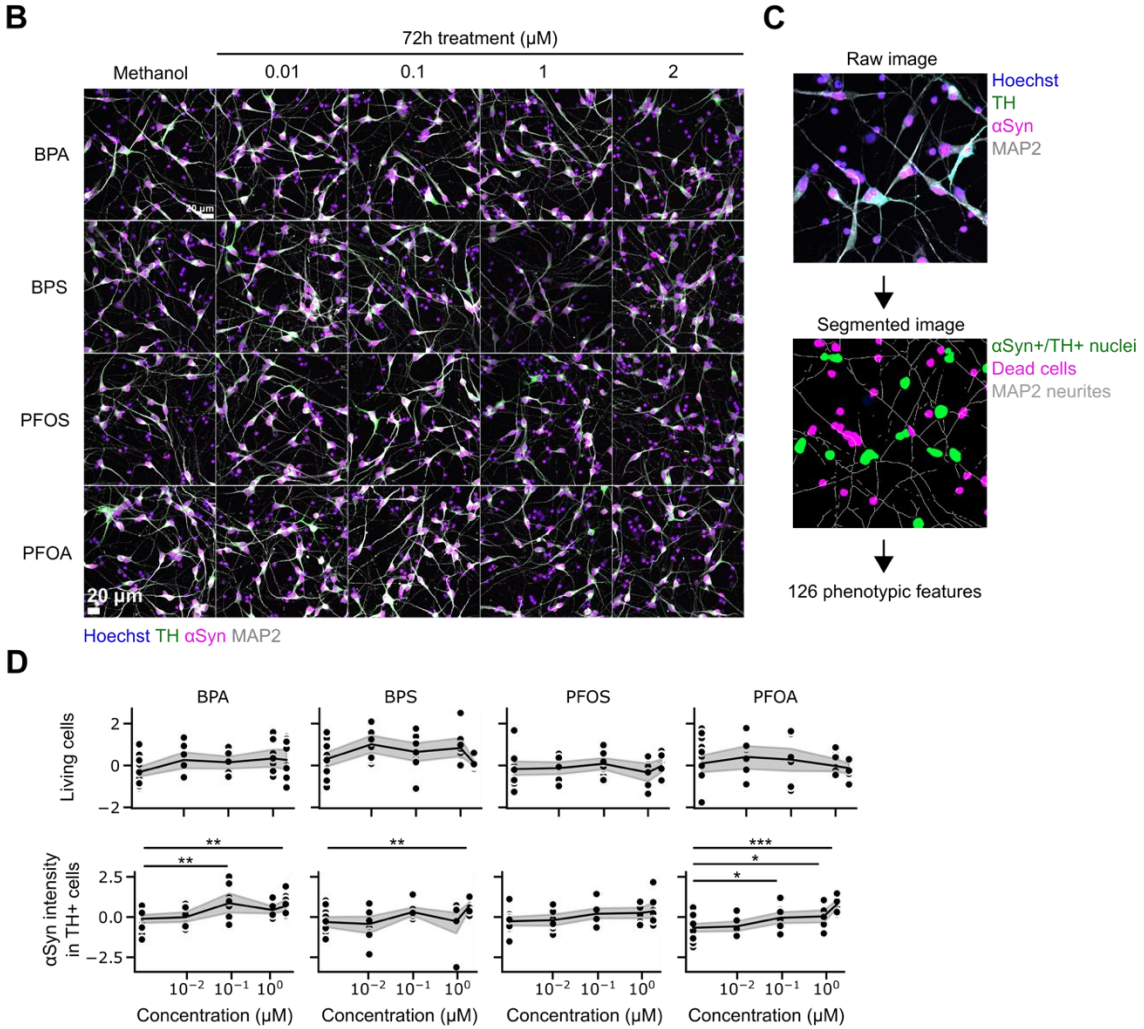
Human iPSC-derived midbrain dopa neurons: BPs and PFs induce some hallmarks of PD





- No differences in cell viability
- Significant difference in αSyn intensity in TH+ cells

Di Credico et al. Sci Rep. 2023. doi: 10.1038/s41598-023-49364-y.

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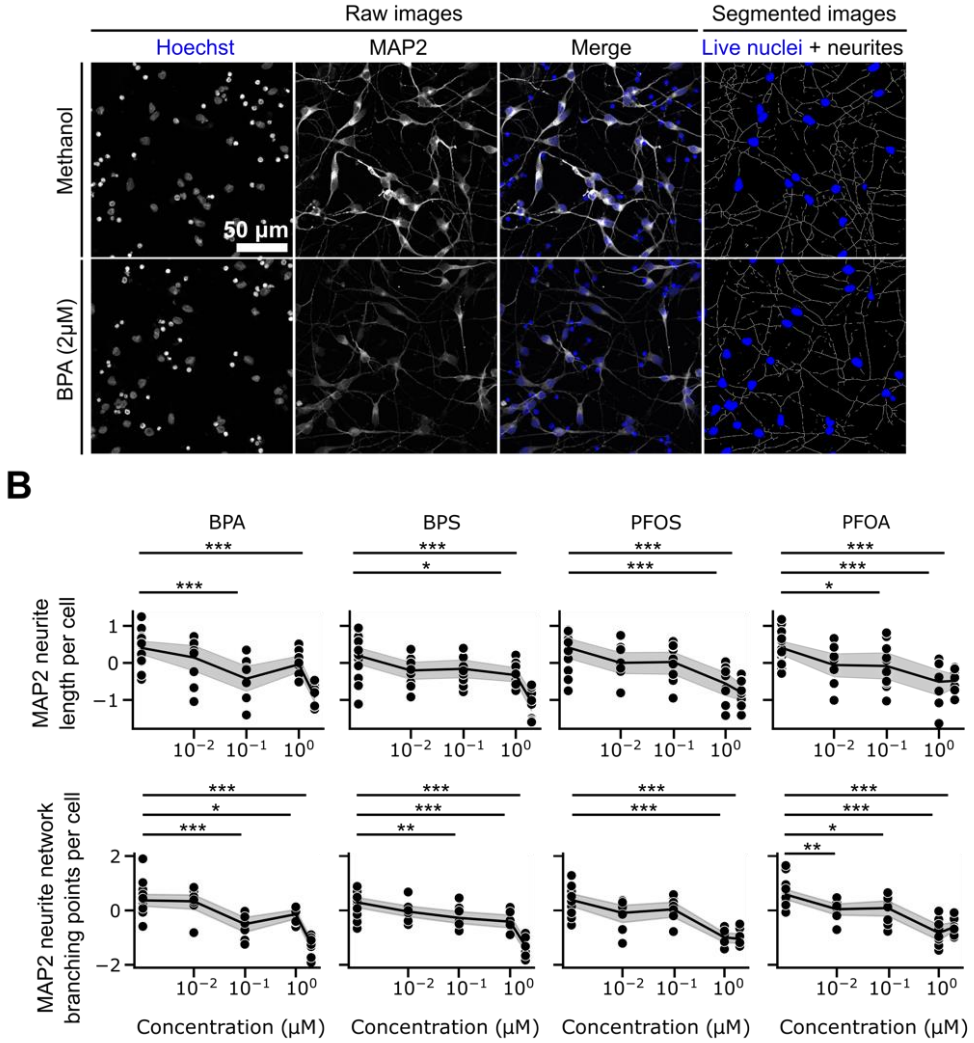
Human iPSC-derived midbrain dopa neurons: BPs and PFs induce some hallmarks of PD

- All compounds have dose-dependent negative effect of neurite length
- In addition to reduced neurite length, the treatments also resulted in a significant decrease in the number of branching points, that are critical for the formation of complex neuronal networks and communication



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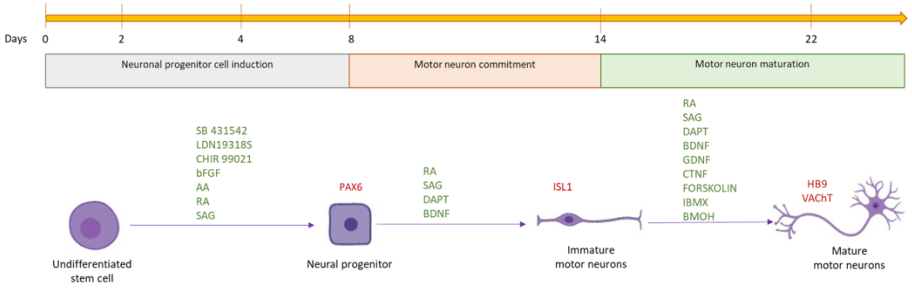
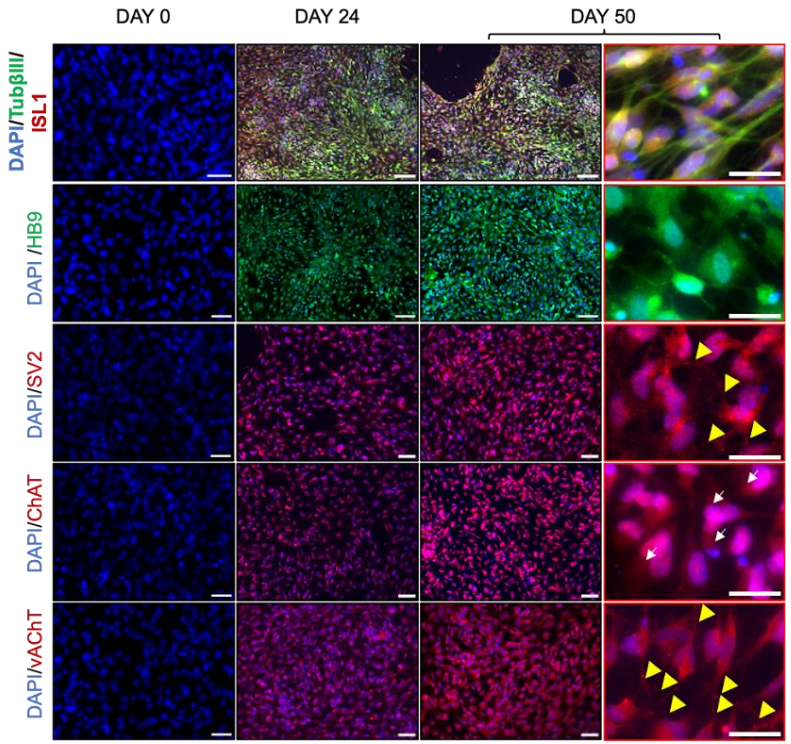
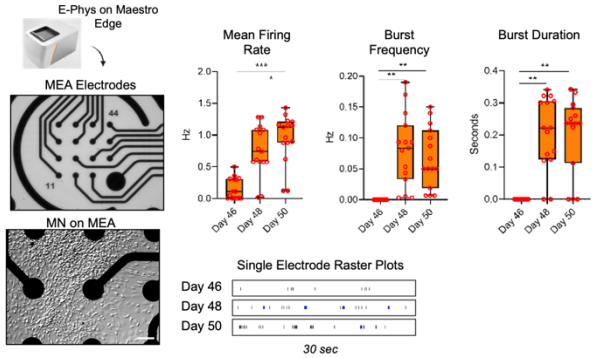
2D culture of human MOTOR neurons





Marker	Positive cells (% of the total)		Fluorescence intensity (MFI)	
	Day 24	Day 50	Day 24	Day 50
Tubulin βIII	77.3 ± 3.2	73.0 ± 2.2	1016.7 ± 70.1	1002.4 ± 66.4
ISL1	81.3 ± 5.4	80.1 ± 4.1	1116.5 ± 83.2	1222.8 ± 74.3
NF-L	87.6 ± 2.8	89.5 ± 5.1	14387 ± 87.1	14464.8 ± 79.9
HB9	70.8 ± 5.7	73.3 ± 4.5	11393.7 ± 74.2	9775.8 ± 91.0
ChAT	78.5 ± 6.2	80.1 ± 5.8	881.74 ± 69.2	1212.1 ± 70.0*
vAChT	80.2 ± 3.3	84.4 ± 4.6	851.5 ± 75.5	1247.0 ± 68.4*
SV2	81.4 ±4.2	83.4 ± 3.3	854.6 ± 73.3	1110.4 ± 70.3*

MNs show spontaneous electrical activity



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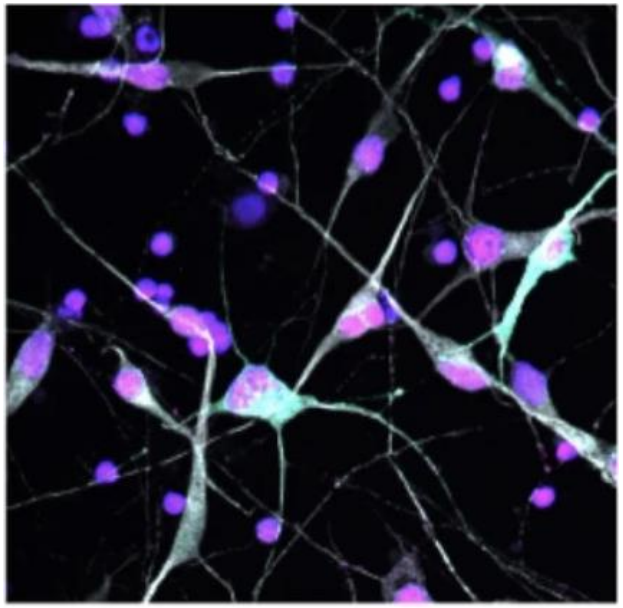
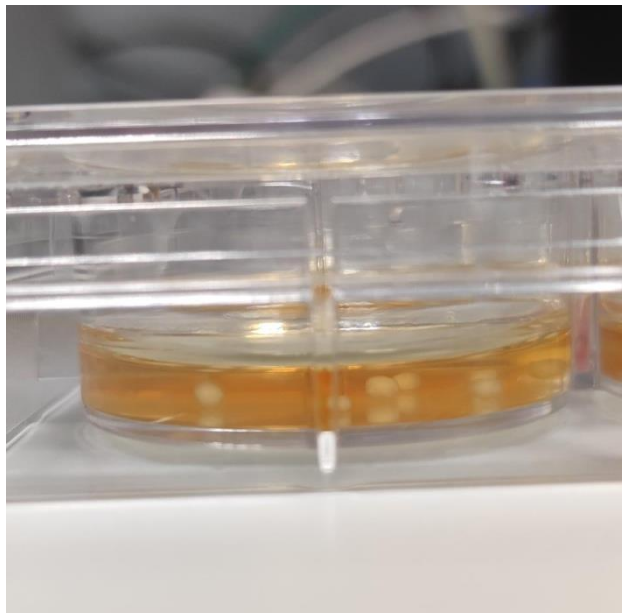
From Cell to Systems: 3Dmodels

2D model



3D model



To make a step further, we developed human IPSCs-derived **brain organoids** to study the effect of long-term chronic exposure to endocrine disruptors using real-life exposure doses.

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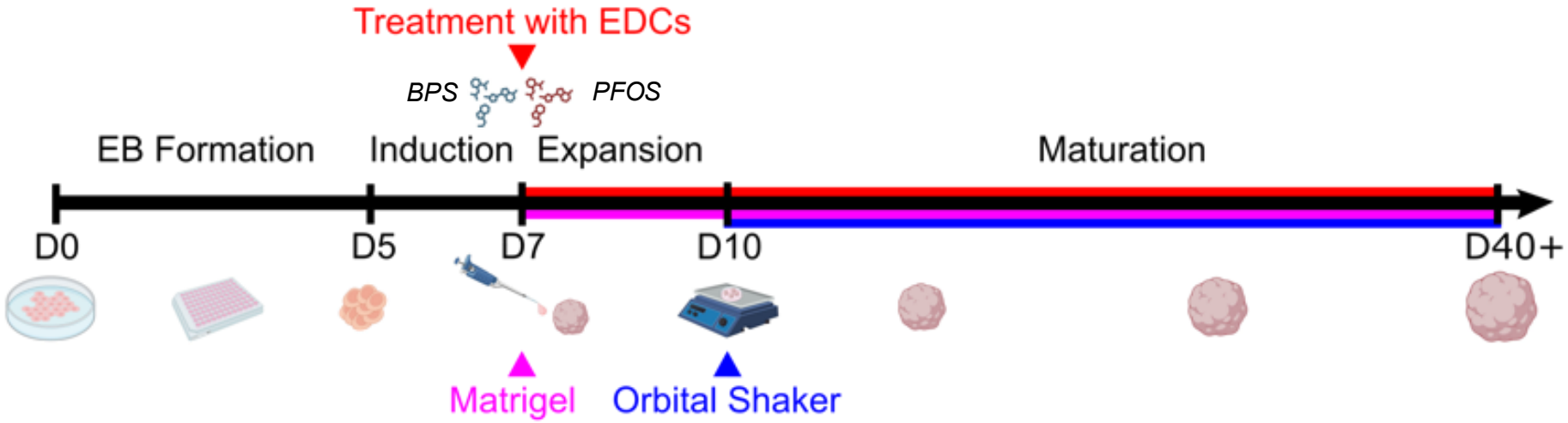
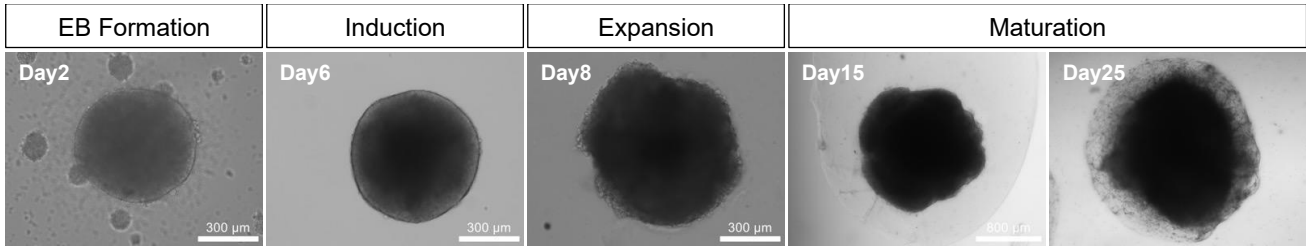
Main objectives

To examine the impact of chronic exposure to BPS and PFOS at **real-exposure concentration** on **human iPSC-derived BRAIN ORGANOIDS** studying:

1. The influence on maturation/proliferative capacity
2. Morphological development of cerebral structures





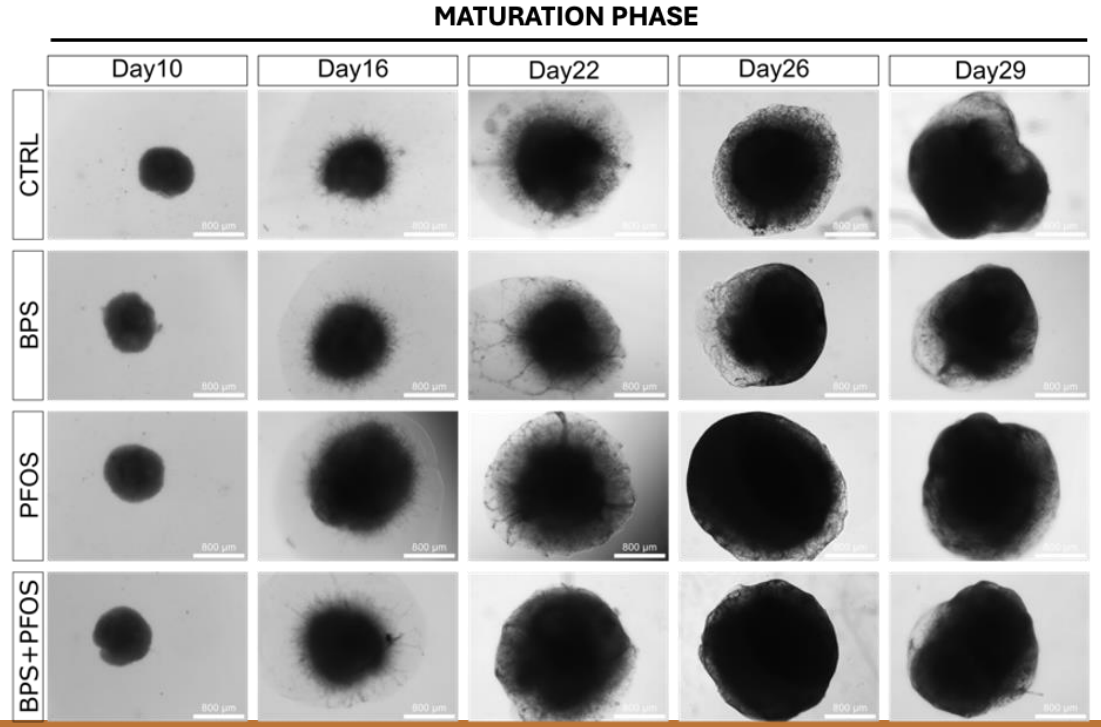
**Representative images of organoids during the different phases*

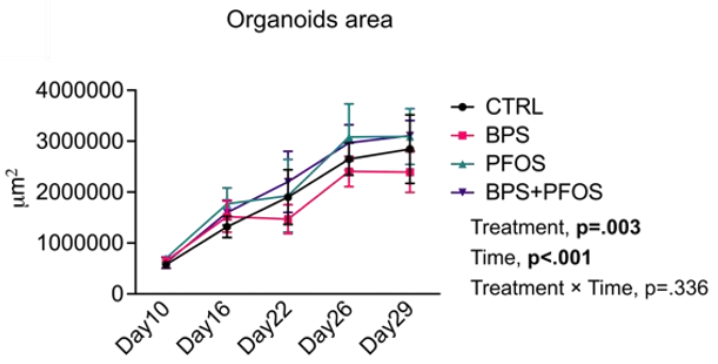
Methods

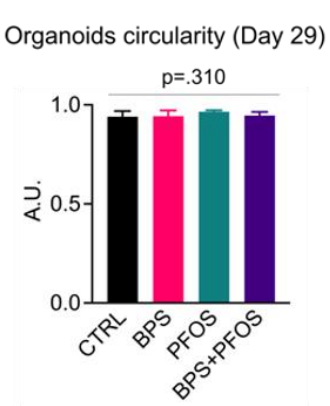
- Treatments with BPS and PFOS from day 7 to day 40
- Morphometry, proliferation index, neuronal subtype characterization

Organoid area and shape modifications

- Effect of treatments on organoid growth during different phases of maturation period: the whole organoid area was measured at day 10, 16, 22, 26, and 29 using brightfield images.
- Mixed-effect analysis showed that both time (p<.001) and treatments (p=.003) had significant effects on the organoids area. No interaction between time and treatment was detected (p=.336).
- Circularity (representing the external organization) was not affected by the EDs.







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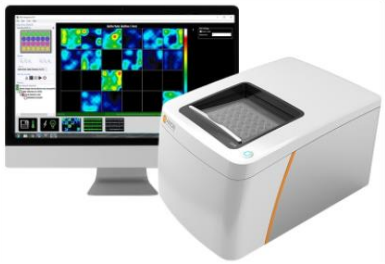
The equipment

Operetta CLS

EVOS M7000

High Content Microscopy

MAESTRO Edge



Electrical activity measurement

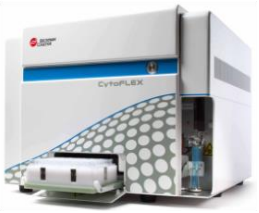
4D Nucleofector

Cell transfection

QuantStudio3 **iBright**

Gene and protein expression

CytoFLEX



Flow cytometry

Varioskan LUX

Multimode microplate reader

Fluorescence microscopy

EVOS M7000

The EVOS M7000 Imaging System from Invitrogen is a high-performance, fully integrated digital fluorescence and transmitted light imaging platform. Designed for advanced cellular imaging and analysis, the EVOS M7000 combines speed, precision, and versatility, making it an ideal tool for demanding research applications in cell biology, immunology, and drug discovery.

Key Features

High-Resolution Imaging and Fully Automated Operation: Automated stage movement, focus, and imaging settings streamline workflows and enhance reproducibility. Ideal for high-throughput screening and analysis.

Applications Examples

Live-Cell Imaging, Immunofluorescence, Transfection Efficiency Analysis, Organoid and 3D Culture Studies, Cell Viability and Proliferation Assays

High Content Imaging

Operetta CLS

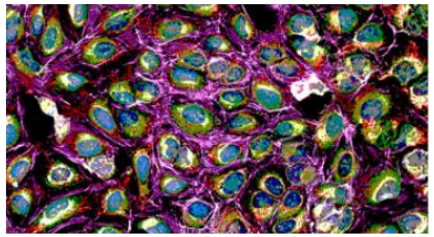
The Operetta CLS system is an advanced confocal microscope designed for high-content quantitative cellular analysis. It is an ideal tool for scientific studies that require in-depth evaluation of the effects of drugs, supplements or health products using representative cellular models. With its confocal imaging, fluorescence and phenotypic analysis capabilities, it provides precise and detailed data for product characterization in preclinical settings.

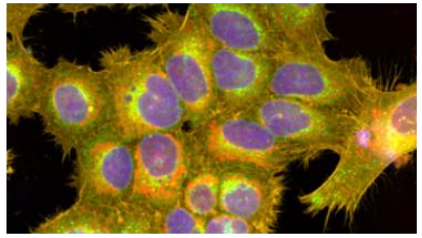
Applications

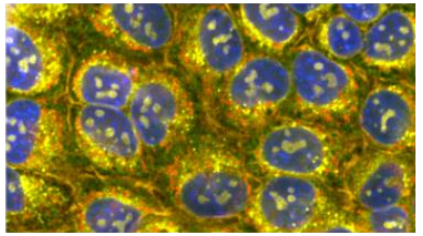
The Operetta CLS system is ideal for a wide range of assays, from simple to complex, thanks to its combination of flexible excitation, sensitive optics, and advanced software capabilities.

Fixed-cell assays, Live-cell assays, Brightfield or digital phase contrast imaging, Complex cellular models.
Advanced software to address the challenges of imaging and analyzing complex cellular models (i.e., Machine Learning for phenotypic classification).

Operetta CLS High Content Analysis System







Intelligent image acquisition

Machine learning

Easily quantify cellular phenotypes in complex 3D models

Powerfully simple analysis capabilities

High efficiency excitation design

fps

Fast Frame Rate Imaging

Automated water-immersion objectives

Confocal spinning-disk technology

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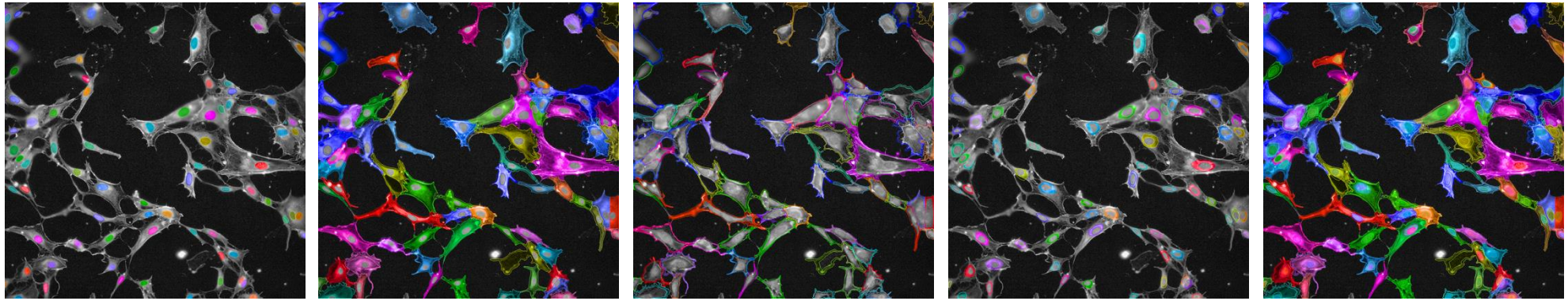
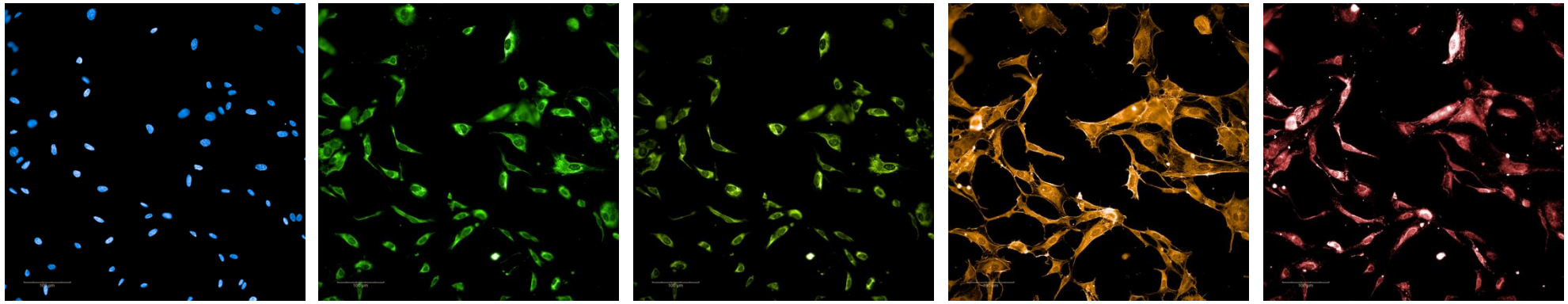
Cell Painting

Segmentation (zone localization into the cell)



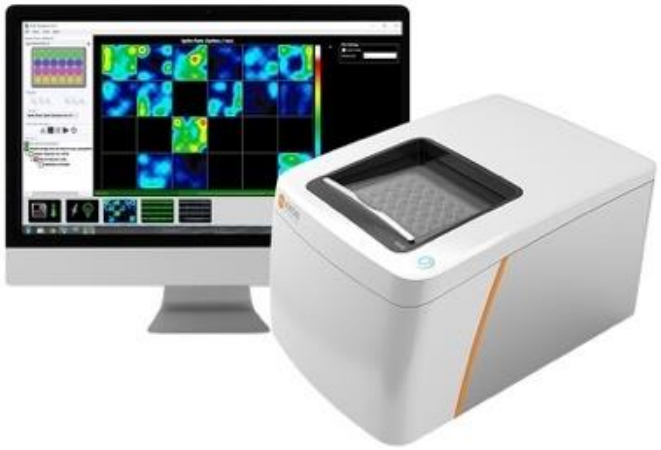
Nuclei | Cytoplasm | Membrane | Peri-nuclear ring region | Merge

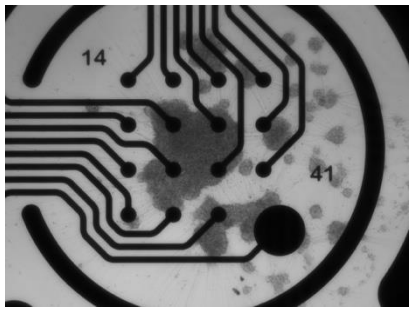
Channels (relevant compartments)



Hoechst (nuclei) | 488 (marks RER) | 512 (RNA) | 555 (membrane-Golgi) | 641 (mitochondria)

Maestro EDGE



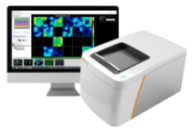


Multielectrode Array (MEA) technology

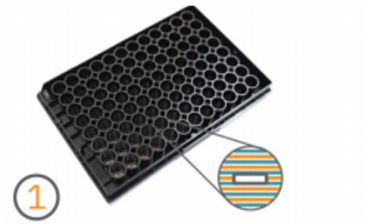
The Maestro Edge is a state-of-the-art, multiwell MEA system designed to non-invasively evaluate cellular function in vitro. It combines versatility and precision, offering robust insights into excitable cell activity and cellular viability through real-time, label-free assays. The platform supports 6-, 24-, and 96-well formats, making it adaptable for various experimental scales and applications.

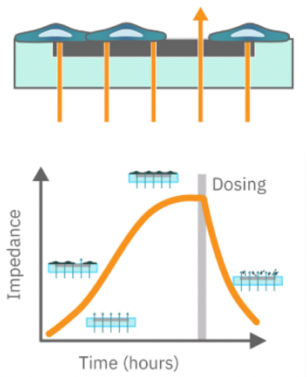
Key Features

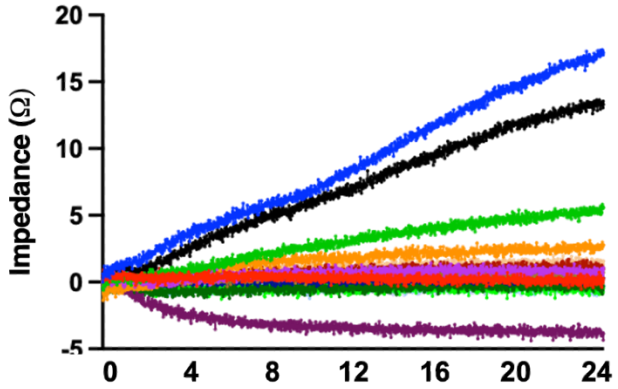
Electrophysiological Activity: Monitors intricate electrical activity in excitable cells such as neurons and cardiomyocytes.
Viability and Impedance: Tracks cell proliferation, morphology, and viability in real-time without the need for labels.
High-Quality Data: Provides detailed and reproducible data across all assay types, facilitating rigorous analysis for research and development.

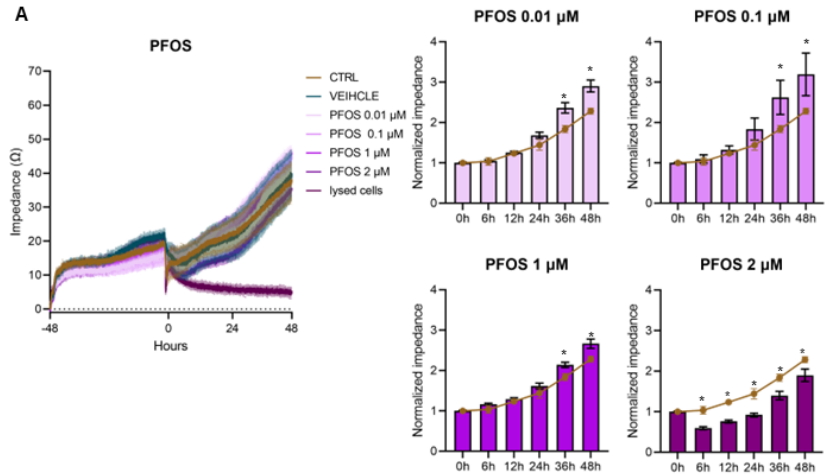


Real-time cell proliferation









- Our Maestro EDGE with MEA technology can also record real-time impedance to monitoring cell proliferation

Nucleofector

4D-Nucleofector

The 4D-Nucleofector® is a cutting-edge transfection system designed for efficient and reliable delivery of genetic material into a wide range of cell types, including primary cells, stem cells, and hard-to-transfect cell lines. This technology uses a combination of optimized electrical parameters and tailored chemical solutions to enable precise transfer of nucleic acids and proteins directly into the nucleus and cytoplasm, bypassing the need for cellular proliferation.

Key Feature

Versatile Configurations: Compatible with multiple cell types, including adherent and suspension cultures.

Efficient Transfection: Achieves high transfection efficiency (up to 99% for certain oligonucleotides) while maintaining cell viability and physiological function.

Cell-Specific Protocols: Offers optimized protocols for various cell types, providing tailored guidance for transfection conditions.

Gene and protein expression

QuantStudio3 **iBright**

The QuantStudio 3 Real-Time PCR System is a versatile and user-friendly platform for quantitative gene expression analysis. It is designed to meet the needs of researchers seeking precise, reproducible, and high-sensitivity results in real-time PCR experiments.

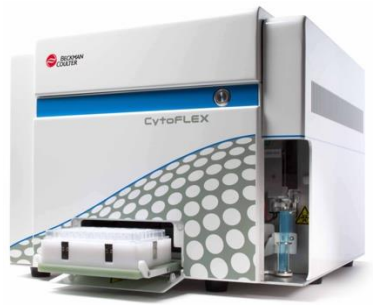
The QuantStudio 3 provides reliable and reproducible data, making it ideal for applications in molecular biology, drug development, and translational research.

The iBright Imaging Systems from Invitrogen are advanced platforms for imaging and analyzing western blots, gels, and other luminescent, fluorescent, or colorimetric applications. These systems are designed to deliver high-resolution imaging with a user-friendly interface, enabling researchers to generate publication-ready results efficiently.

The iBright Imaging Systems enable precise and reproducible analysis of proteins and nucleic acids, making them indispensable for labs conducting molecular biology, proteomics, and translational research.

Flow cytometry

CytoFLEX



The CytoFLEX Flow Cytometer by Beckman Coulter is a highly sensitive, compact, and modular system designed for advanced flow cytometry applications. Renowned for its ease of use and exceptional performance, it delivers high-quality data for diverse research and clinical needs. Its customizable configuration allows users to expand capabilitie, accommodating the most complex multicolor panels.

Application examples

- Immunophenotyping:
- Apoptosis and Cell Viability:
- Cell Cycle Analysis:
- Extracellular Vesicle and Exosome Analysis:
- Multi-Color Immunoassays
- Stem Cell Research

Multimode microplate reader

Varioskan LUX

The **Varioskan LUX** is a versatile multimode microplate reader designed to support a wide range of assays by combining multiple detection technologies in a single platform. With its precision, flexibility, and user-friendly design, it is widely used in biomedical and pharmaceutical research, clinical diagnostics, and high-throughput screening.

Applications

- Detection of biomarkers in ELISA or bead-based assays.
- Measure cell viability, proliferation, or apoptosis using fluorescence or luminescence.
- Track kinetic changes in metabolic activity, ion flux, or signal transduction pathways.
- Quantify protein concentrations and analyze binding interactions in real time.
- Evaluate enzymatic reactions and inhibition profiles.
- Perform time-course experiments to monitor changes in absorbance or fluorescence over time, such as enzyme kinetics or reaction rates.

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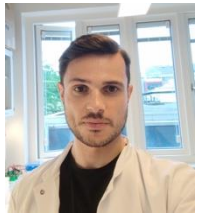
Cell Reprogramming and Differentiation Lab

Angela Di Baldassarre

Barbara Ghinassi

Giulia Gaggi



Andrea Di Credico

Sandra Bibbò

Grazie per l'attenzione