

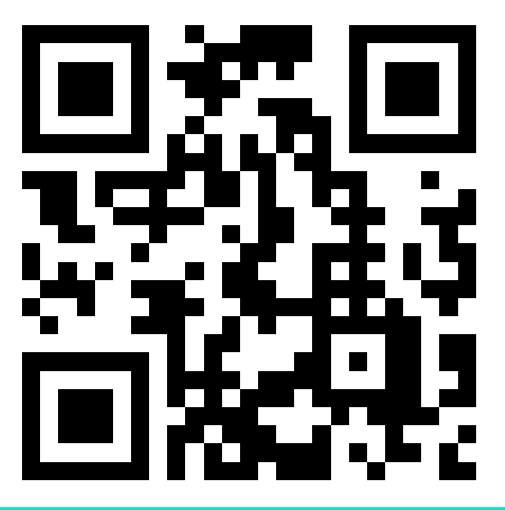
Real-time monitoring of intracellular changes in 3D biology resembling relevant physiological processes in drug discovery

Lab-in-a-cell microchips sensing single living cells in spheroids to study cancer, neurodegeneration, and metabolic diseases

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OVERVIEW

- A4cell has developed proprietary SPACHip® technology for living single-cell analysis.
- SPACHip® technology features absence of cytotoxicity.
- CytoCHECK and SpheroCHECK SPACHip® pH detection kit allows measurement of intracellular and extracellular pH in cell cultures.
- SPACHip® Detection kits are compatible with fluorescence microscopy and flow cytometers.

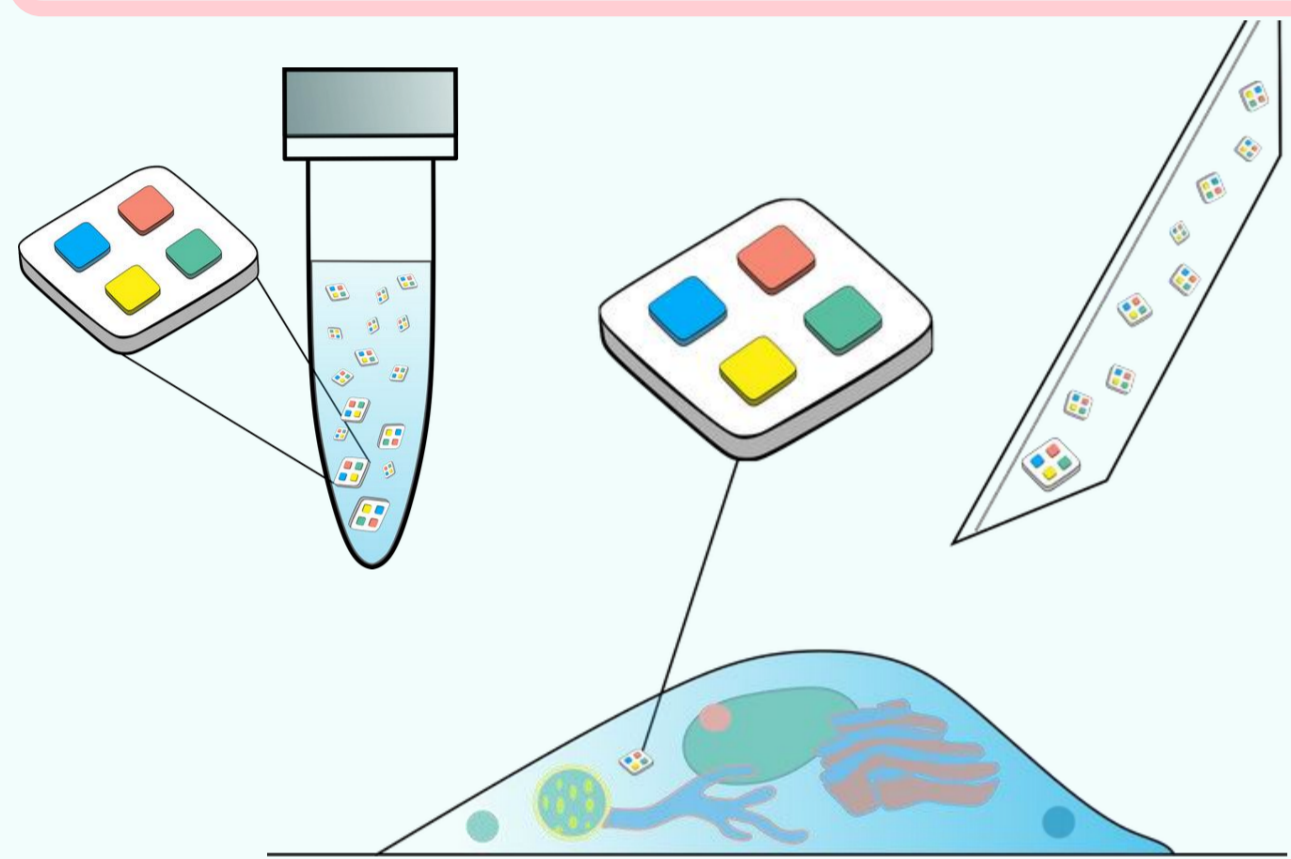
INTRODUCTION

Imagine having an eye inside your living cells



Spheroids are simple 3D biological structures, exhibit extensive cell-cell adhesion and have properties that mimic *in vivo* tissues and tumour behaviour more effectively than 2D cell cultures. Spheroids contain both cells at the surface readily exposed to the media and oxygen and buried cells at the hypoxic centre of the structure, proliferating and non-proliferating cells. **Spheroid's morphology and physiology are greatly affected by pH during its formation.** 3D biology in drug discovery fills the gap between 2D cell cultures and *in vivo* models, enabling a more reliable and relevant assessment of safety and therapeutics in cancer and neurodegenerative and metabolic diseases.

SPACHIP® TECHNOLOGY



SPACHIP®: An intracellular silicon device on which multiple highly concentrated fluorescent probes can be printed to provide intracellular readouts over long culture periods

- **SpheroCHECK SPACHIP® pH Green Single-Detection Kit** measures intracellular and extracellular pH levels on spheroids and 3D-biology. pH changes can be tracked in living single cells over long periods of time, which allows for a more comprehensive study of spheroid's establishment, metabolism, and viability.
- SPACHIPS are **internalised by cells** after an overnight incubation.
- Fluorescence intensity is measured from individual SPACHIPS harboring a pH-sensitive chemical probe covalently attached. **SpheroCHECK SPACHIP®** kits are optimised for its use with **confocal microscopes and HCS/HCA analysers** with 20X or over magnification objectives, yet widefield fluorescence microscopes and flow cytometers have also been validated.

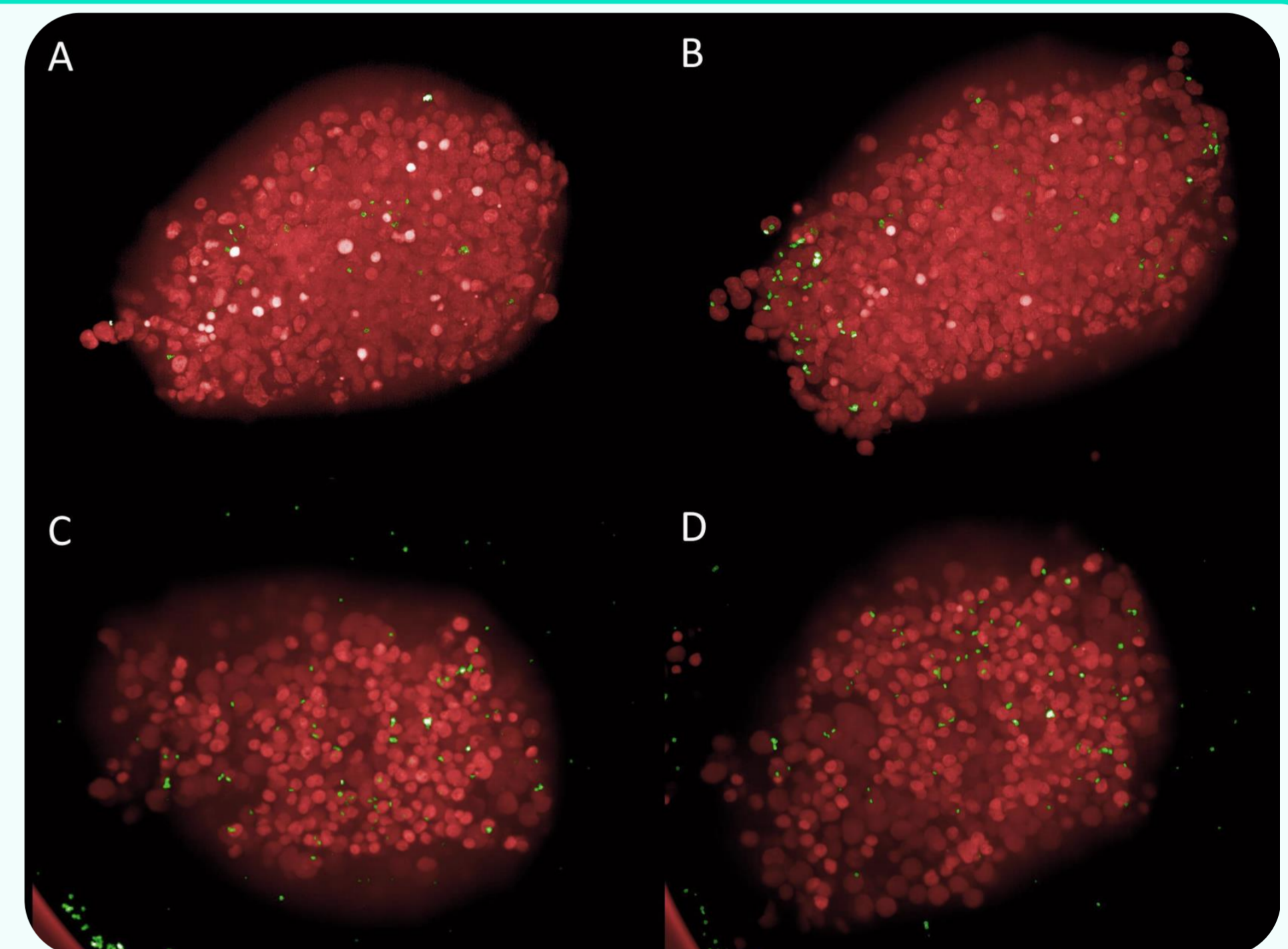
METHODOLOGY

Time course of spheroid formation

Spheroids formation takes long. Some cells will readily form spheroids within hours, while other cells will require several days. Monitoring spheroid formation over time will help in determining the ideal culture period for each application. SPACHIPS remain stable in the cytosol over long periods of time (more than a month) without causing cytotoxicity.

- SPACHIP® technology enables dynamic, real-time assays monitoring intracellular pH inside 3D cell aggregates over a period of minutes, hours, or even days.
- SPACHIPS can be internalised by cells prior to, along or after formation of spheroids.
- SPACHIPS remain in the cytosol reluctant to exocytosis during spheroid handling.

Hours	Spheroid Diameter (µm)	Number of cells / well	SPACHIP® Number / well (2:1 ratio)
0	N/A	400	-
24	~200	800	1,600
48	~500	1,600	3,200

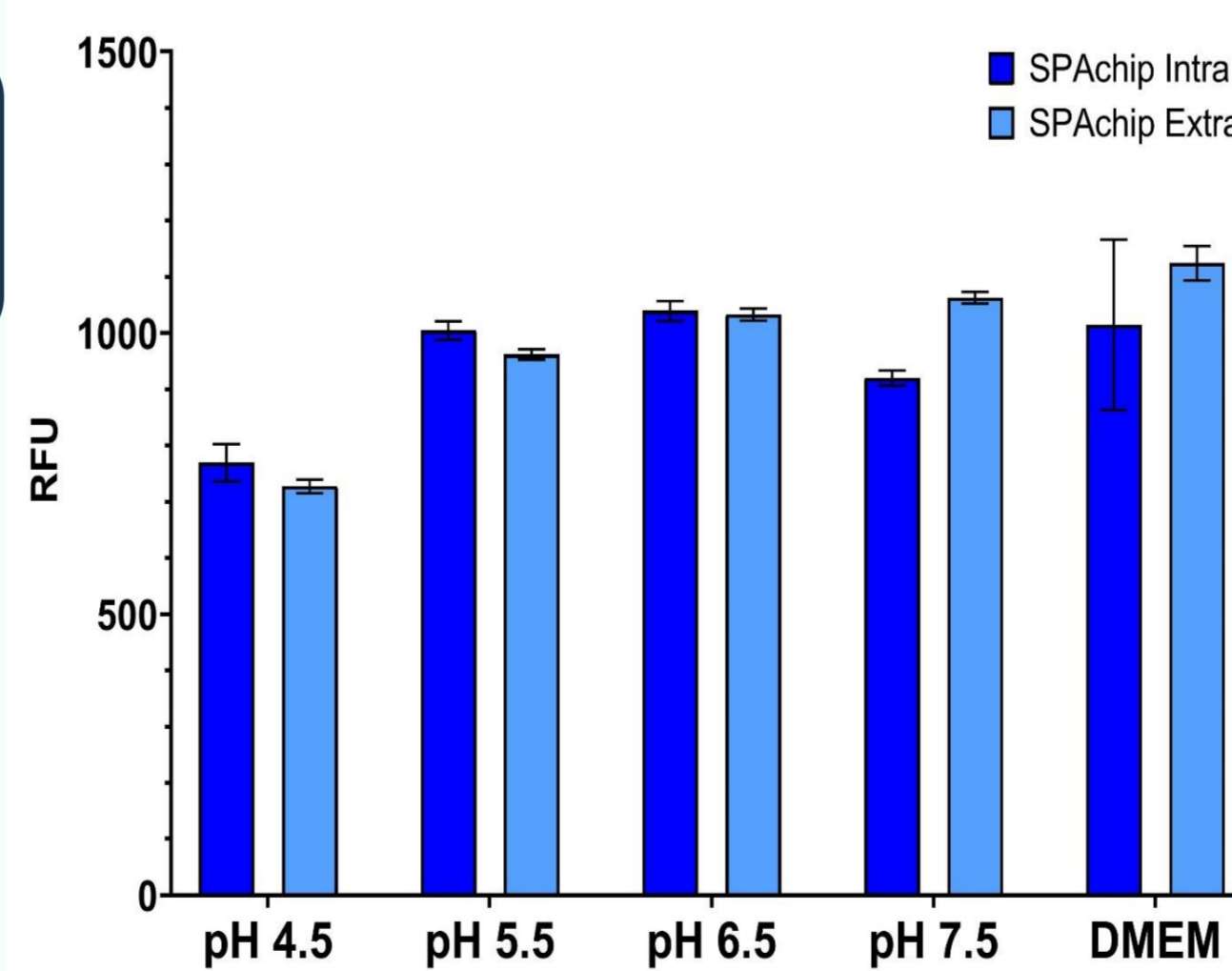


HEK293 spheroid formation. Timelapse of HEK293 cell line with internalisation of SpheroCHECK SPACHIP® pH Single-Detection Kit Green. DRAQ5™ in red stained live nuclei. HEK293 cells were incubated with SpheroCHECK SPACHIP® pH Green Single-Detection Kit for 24 (A), 28 (B), 48 (C), and 52 hours (D).

RESULTS

Accurate and precise measurement of both extracellular and intracellular pH

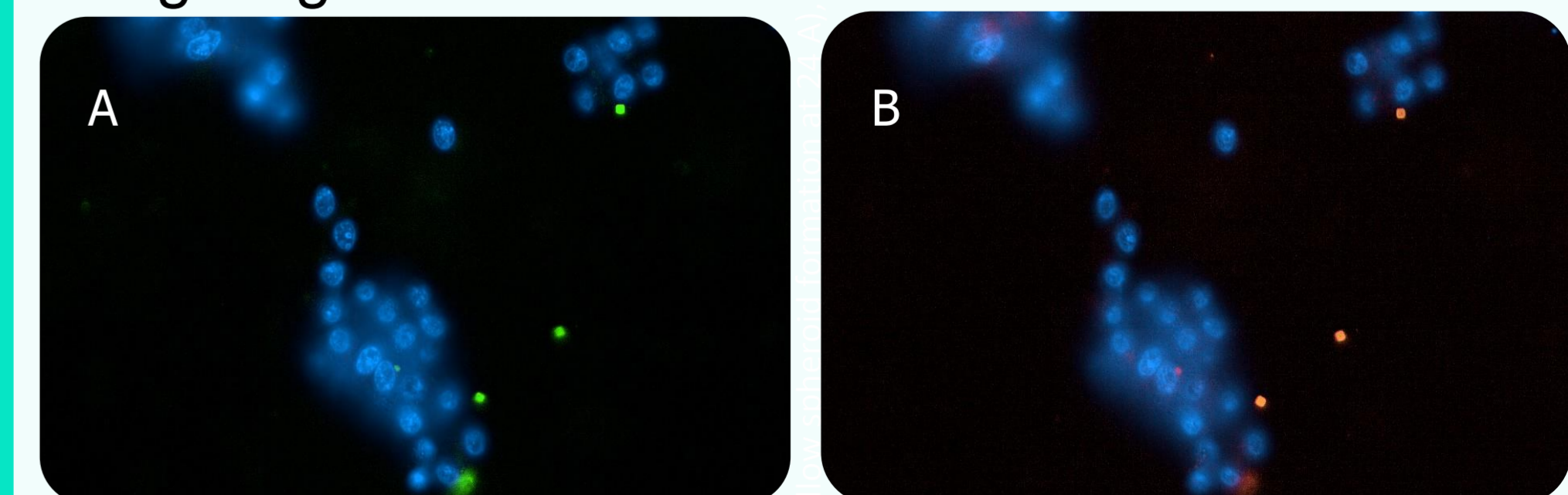
pH fluctuations directly affect morphology, cell viability, proliferation rates, and metabolic activities of spheroids; ultimately impacting their formation and physiological status. It is essential to maintain an optimal pH environment to ensure reproducibility and reliability as well as to accurately assess the efficacy and toxicity during drug testing in 3D cell culture. Furthermore, pH may become a valuable QC marker of scaled-up spheroid cultures.



SpheroCHECK SPACHIP® pH Green Single-Detection Kit at different pH conditions using commercial intracellular calibrators in HEK293 cell line (from Human Embryonic Kidney) spheroids. Images and fluorescence intensity values (RFU) were obtained in HCS-Operetta equipment.

Future Directions: Multiplexing pH & Calcium signals in live spheroids

CytoCHECK SPACHIP® CALCIUM and pH Multi-detection kit enables continuous, simultaneous, and precise monitoring of both pH and calcium in living single cells.



2D-cultures of SH-SY5Y cell line (neuroblastoma cells) with nuclei stained in blue and CytoCHECK SPACHIP® Multi-detection kit in (A) green channel (Calcium) and (B) red channel (pH). Both pH and calcium probes are attached to each chip.

CONCLUSIONS

- **Combining novel SPACHIP® technology with spheroids** allows researchers to gain a deeper understanding of cancer and metabolic diseases, accelerate drug discovery, and develop more targeted and personalised treatment approaches.
- **SpheroCHECK SPACHIP® product line**, started with pH Green Single-detection kit, may be extended to other SPACHIPS, such as calcium and upcoming ROS and molecular oxygen (e.g. for hypoxia studies).