Lab-in-a-cell SPAchip® sensing and filming live-cell physiology of relevance for drug discovery

Marina Martínez-Bartolomé, Irene Palacios-Doiztúa, Alberto Garcia-Nieto, Alberto Hernández-Pinto, Rubén Miguez, Antonio Quílez-Álvarez, and Julio Martin*

Arrays for Cell Nanodevices (A4cell), Madrid, Spain

** Presenting author:* ***julio.martin@a4cell.com***

SITUATION

- **Live-cell continuous monitoring** of hallmarks for signalling, metabolism, and cell health (e.g. pH, calcium, ROS, oxygen) over long periods of time is priceless.
- Biological samples are **precious and unique**.
- Outputs obtained from the same single cell diminish **variability** and establishes more **reliable correlations and robust statistics**.
- Live-cell imaging in drug discovery and cell biology research is hampered by **drawbacks of traditional fluorescent chemical probes in solution**:
  - cytotoxicity over time,
  - difficulty of multiplexing,
  - metabolization and expelling of molecules from inside the cell,
  - poor cell penetration.

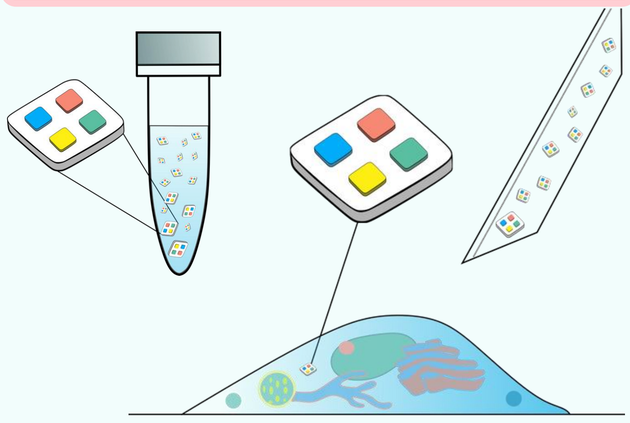
TARGET

There is a demand for **new technologies advancing high-content live cell imaging assays** from morphological snapshots to physiological feature films.

PROPOSAL

Lab-in-a-cell-microchips sensing and filming live cell physiology for drug discovery and cell biology research

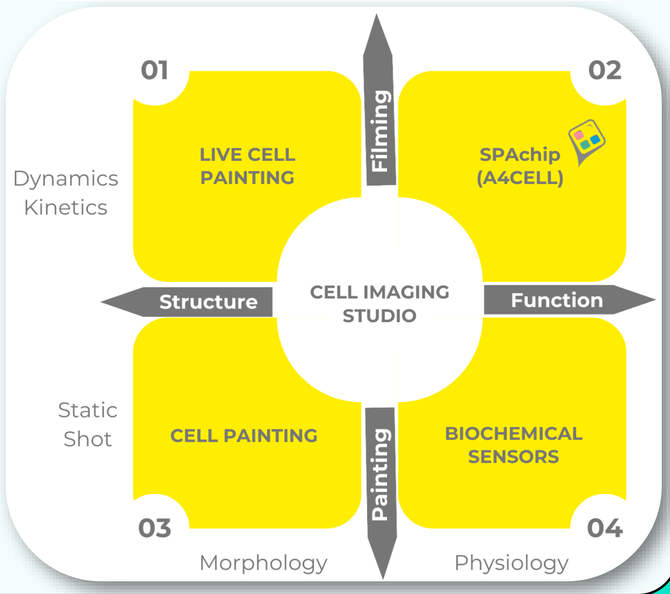
SPAchip® TECHNOLOGY



SPAchip®: *A silicon device on which multiple highly concentrated fluorescent probes can be covalently printed to provide intracellular readouts over long culture periods*

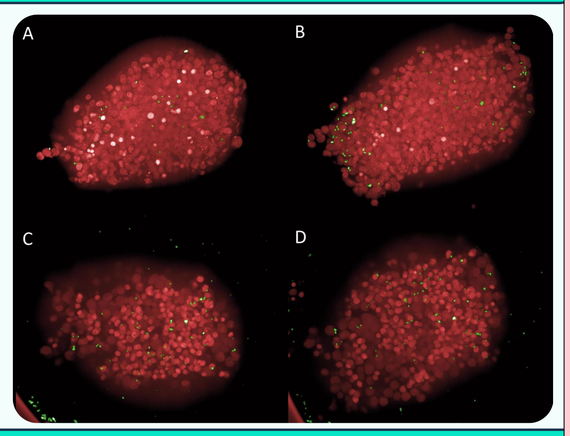
- **SPAchip® detects pH, Calcium ion and ROS** in living single cells over long periods of time, both **in 2D and 3D cultures.** *Upcoming O_2 kit.*
- SPAchips are **internalised by cells** after an overnight incubation.
- SPAchip® technology is **harmless, non-cytotoxic**.
- **Intracellular and extracellular** on the same sample.
- Amenable to **multiplexing**: 2-sensors-in-one SPAchip.
- Fluorescence intensity is measured from individual SPAchips. **Confocal fluorescence microscopes and HCS/HCA analysers** with at least 20X, yet widefield fluorescence microscopes and flow cytometers as well.

Cell Imaging Studio



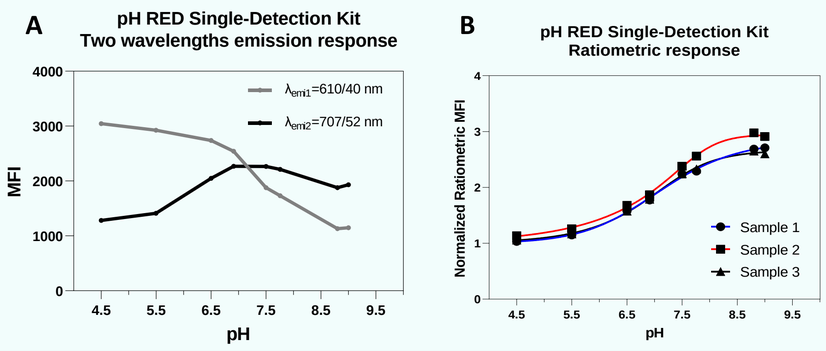
3D-Biology: Spheroids

- SPAchip® technology enables dynamic, real-time assays monitoring intracellular pH inside 3D cell aggregates over a period of minutes, hours, or even days.
- SPAchips remain in the cytosol reluctant to exocytosis during spheroid handling.



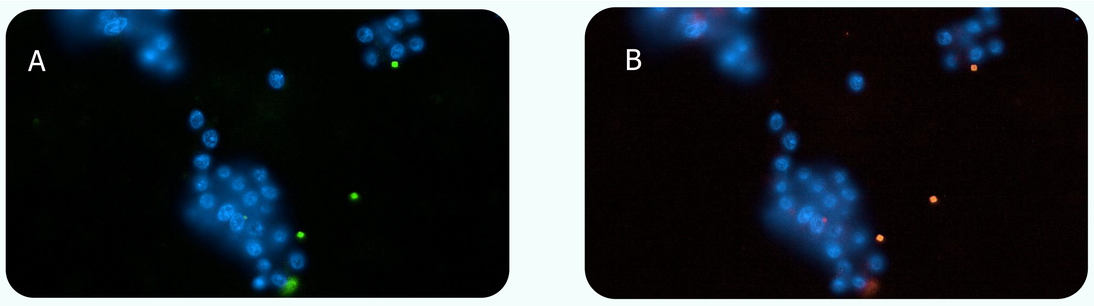
HEK293 spheroids. Timelapse with SpheroCHECK SPAchip® pH Single-Detection Kit Green for 24 (A), 28 (B), 48 (C), and 52 hours (D). DRAQ5 in red stained live nuclei.

(#3) Precise intra- and extra-cellular pH



CytoCHECK SPAchip® pH RED Single Detection at different pHs using commercial calibrators. Ratiometric values were obtained by dividing λemi2/ λemi1 emission signals in HCS-Operetta with λexc=545/15 nm.

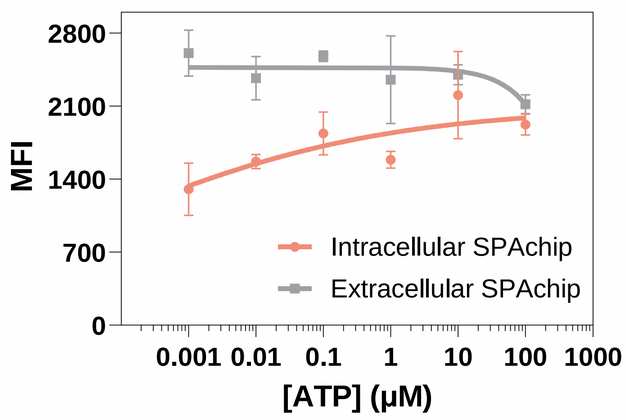
Multiplexing pH & Calcium detection



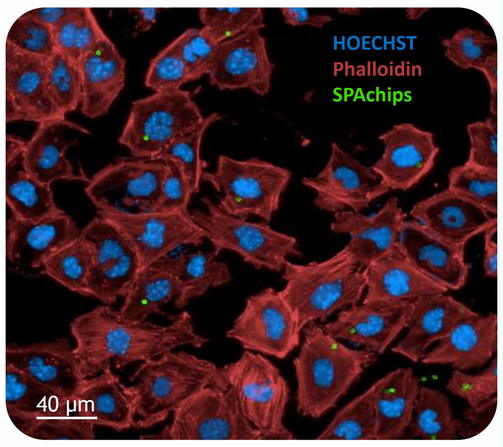
SH-SY5Y cell line (neuroblastoma cells) with nuclei stained in blue and CytoCHECK SPAchip® Multi-detection kit in (A) green channel (Calcium) and (B) red channel (pH). NB: Both pH and calcium probes are attached to each chip.

USE CASES

Calcium in cell health and receptor signaling



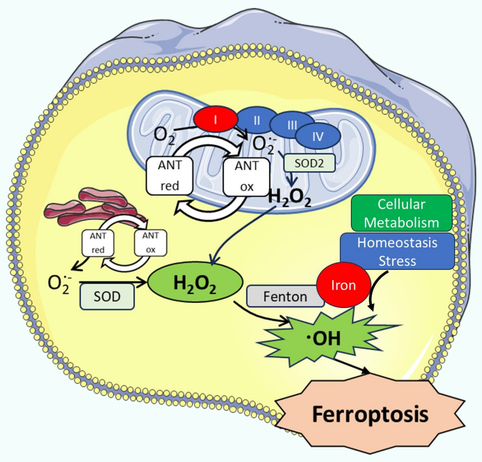
Calcium green SPAchip® in HL1 cells responding to the addition of ATP, agonist of purinergic receptors, in the culture media



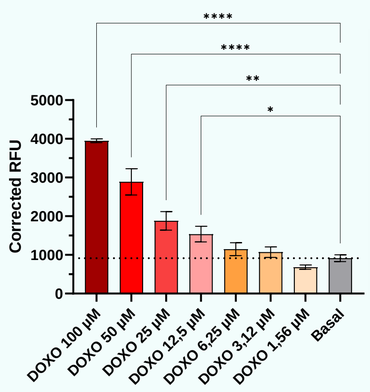
HL1 cells micrograph at 40X with calcium green detection SPAchip® internalized.

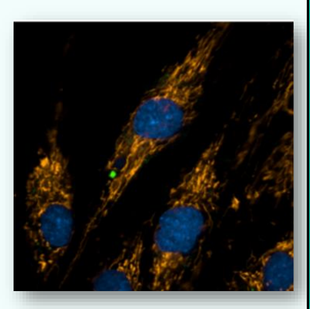
ROS (Reactive Oxygen Species) detection

OHrad ROS SPAchip®



- **KEY FEATURES:**
  - **Stability:** active only when processed by intracellular cytosolic esterases
  - **Specificity:** hydroxyl radical (•OH)
  - **Robustness**: pH-insensitive





Take Home Messages

- ***SPAchip® enables real-time monitoring of physiological hallmarks on living single-cells over long periods of time*** *to gain a deeper understanding of cell biology, cancer and metabolic diseases, and accelerate drug discovery.*
- ***SPAchip®*** *product line comprises* ***pH, Calcium*** *(Single- and Multi-detection kits) and* ***ROS****, and it will be expanded with the* ***upcoming molecular oxygen kit.***

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