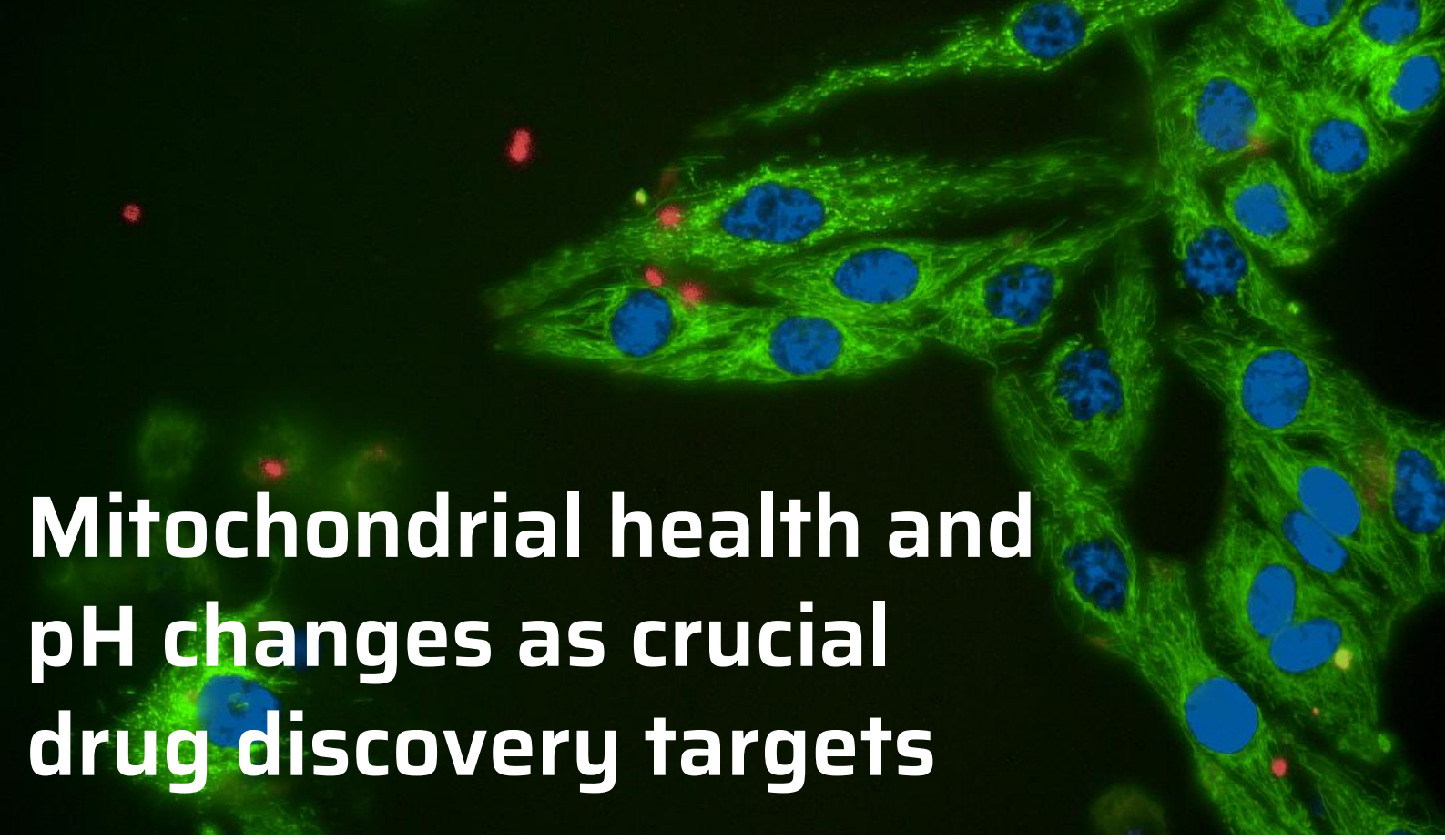

Mitochondrial health and pH changes as crucial drug discovery targets

Several mitochondrial dysfunctions have been linked with multiple human diseases, making mitochondria a crucial pharmacological target for the treatment of a broad range of diseases.

With its ability to specifically target mitochondria, researchers can gain valuable insights into the health and functionality of these vital cellular powerhouses.

Mitochondrial stainings localize mitochondria of live cells and allows analyses of cell structure, tracing, tracking, cellular imaging, immunofluorescence staining & detection, mitochondria function, mitochondrial structure, and organelle tracing.

a4cell® nanodevices

01 MITOCHONDRIAL FUNCTIONAL ASSAYS

To assess mitochondrial function and activity using different types of assays for measuring mitochondrial membrane potential, oxygen consumption rate (OCR), or reactive oxygen species (ROS) production.

02 MITOCHONDRIAL HEALTH AS EARLY TOXICITY MARKER

Measurement of mitochondrial intensity and pH changes provides insight into early toxicity and cell viability for the study of any compound treatment response.

03 QUANTITATIVE IMAGE ANALYSIS

By quantifying mitochondrial intensity and pH variations during cell studies, researchers can assess how specific drugs affect mitochondrial health and detect mitochondrial dysfunctions.

04 CELL VIABILITY AND CYTOTOXICITY

To evaluate the metabolic activity and viability of cells in response to different treatments or compounds. These assays could be carried out with proprietary SPAchip® cell based assays or with other techniques.

OLS® OMNI Life Science

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Mitochondrial health with CytoCHECK SPAchip® pH Single-Detection Kit

Uncover the optimal tool for advanced mitochondrial cell condition analysis.

CytoCHECK SPAchip® pH Single-Detection Kit allows measurement of intracellular pH levels by changes in fluorescence intensity, which facilitates a more comprehensive study of the living single-cell physiology and maximizes the performance of most of imaging analyzers.

In addition, mitochondrial health can be assessed by using mitochondrial staining. Incubating with both our SPAchip® technology and another commercial dye, mitochondrial green staining intensity decreases in cells incubated with commercial dye at 24 and 48 hours after treatment, whereas cells incubated with SPAchip® displayed a fluorescence intensity similar to control, indicating fully functional mitochondrial structures.

Using mitochondrial staining together with our SPAchip® technology open the door for further research of mitochondrial status, function and activity in a diverse set of cellular assays (e.g. measure of mitochondrial membrane potential, oxigen consumption rate (OCR) and reactive oxygen species (ROS) production). By combining these two cutting-edge tools, researchers can accurately assess mitochondrial health and gain a deeper understanding of the impact of several treatments on these crucial cellular components.

The ability to visualize and track mitochondria using both technologies together opens up new avenues for studying mitochondrial dysfunction and developing targeted therapies for associated diseases. In conclusion, the combination of these powerful tools is a game-changer in the field of mitochondrial research.

Experiment setup:

- **Cell lines:**
  - **HeLa** (human cervical carcinoma cell line)
  - **HEK293** (kidney; embryo)
  - **SH-SY5Y** (ephitelial/neuronal; neuroblastoma cells)
  - **CAL-51** (breast carcinoma)
  - **MDA-MB-231** (epithelial-like cells; breast mammary gland)
  - **ARPE-19** (retinal pigment epithelia)
  - **HL-1** (cardiac muscle cell line)
  - **1095SK** (fibroblast)
  - Any other cell type of your choice
- **Fluorescent dyes:**
  - Nuclei staining
  - Mitochondrial staining
  - Live/Death cell staining
  - CytoCHECK SPAchip® pH Single-Detection Kit Green or Red
- **Positive and negative controls**
- **Measurements:**
  - Nuclei count
  - % cell viability
  - Mitochondrial signal intensity
  - Mitochondrial texture (SER analysis to evaluate the alteration in mitochondria morphology)

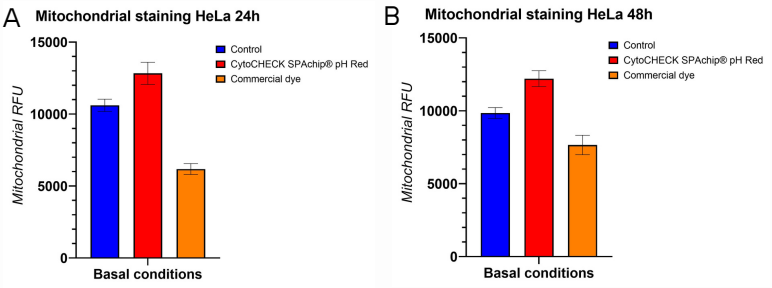


Figure 1: Mitochondrial green staining intensity in HeLa cells incubated 24 hours **A)** and 48 hours **B)** after treatment at basal conditions. For each time measurement, three conditions were evaluated 1) control cells with no treatment, 2) cells incubated with CytoCHECK SPAchip® pH Red Single-Detection kit and 3) cells incubated with commercial dye. Bars represent mean values for each condition and error bars correspond to SD.
Mitochondrial green intensity decreased in cells incubated with commercial dye at both 24 and 48 hours, showing affected mitochondrial health. Cells incubated with CytoCHECK SPAchip® pH Red Single-Detection showed similar mitochondrial green intensity compared with control.

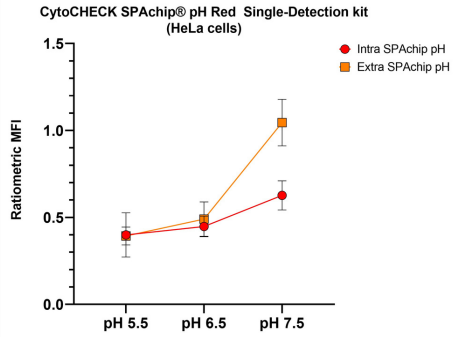
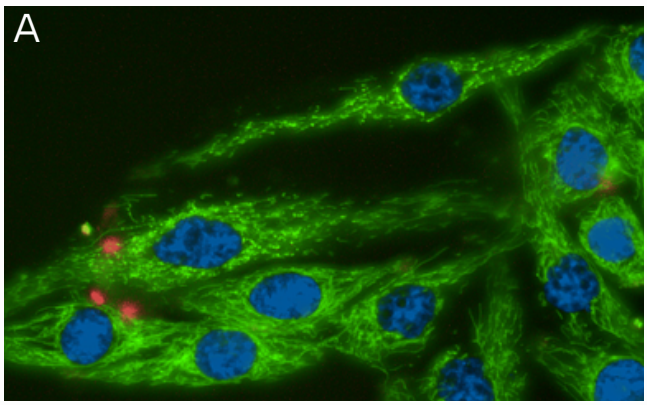


Figure 2: CytoCHECK SPAchip® pH RED Single-Detection at different pH conditions using commercial calibrators inside cells. Graph showing ratiometric normalized fluorescence intensity values of intracellular CytoTRACK SPAchip® pH RED Single-Detection at different pH conditions using intracellular calibrators in HeLa cells. Ratiometric values were obtained by dividing λemi2=707/ λemi1=610emission signals in HCS-Operetta equipment with the excitation in the range λexc=546/15 nm. Red line shows intracellular SPAchip® pH values and orange line represents extracellular SPAchip® pH measurements. Mean values for each condition were represented and error bars correspond to SD.



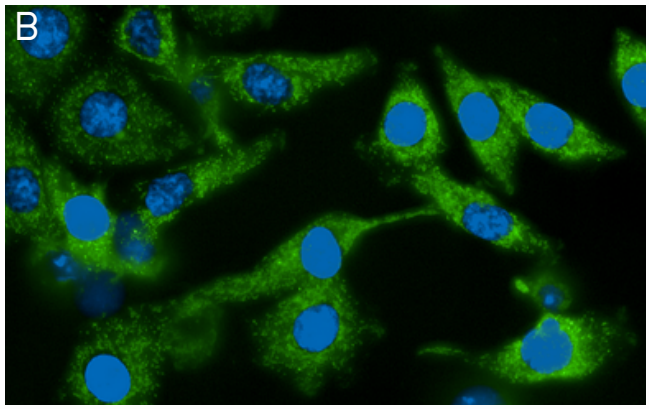


Figure 3: HeLa cell line stained with mitochondrial green staining and nuclei staining in blue. Mitochondrial health tested in HeLa cells incubated with both SPAchip® technology and another commercial dye at 24 and 48 hours after treatment. **A)** Hela cells incubated 24 hours after treatment with CytoCHECK SPAchip® pH Red Single-Detection kit at basal conditions in HeLa cells. **B)** HeLa cells incubated 24 hours after treatment with commercial dye at basal conditions.
Commercial dye affected mitochondrial structure and health versus cells incubated with CytoCHECK SPAchip® pH Red Single-Detection showed healthy mitochondrial structures.

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