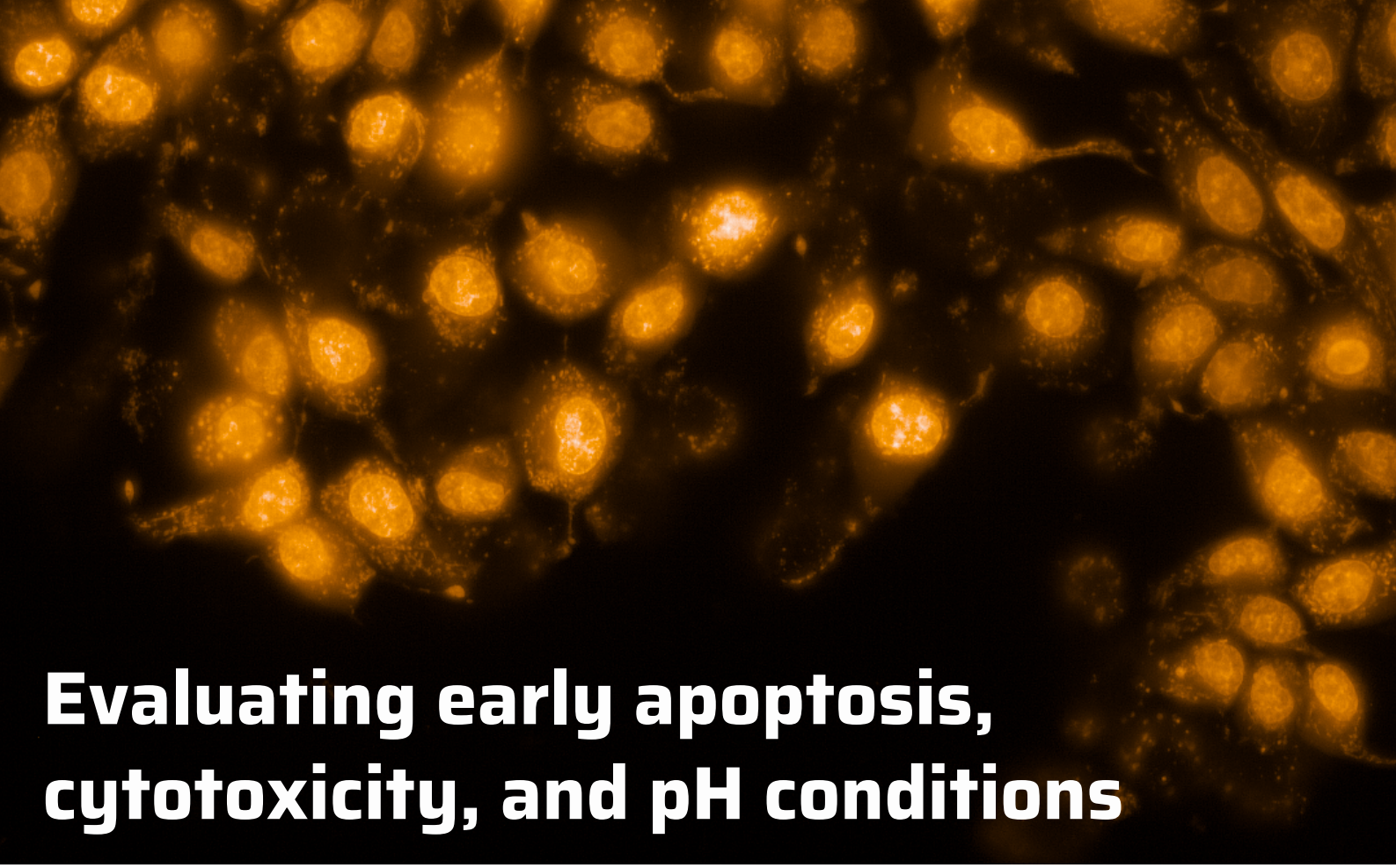

Evaluating early apoptosis, cytotoxicity, and pH conditions

Apoptosis, or programmed cell death, is a crucial event for several biological process in multicellular organisms. In addition, its disruption has been involved in a large number of diseases including cancer, rheumatoid arthritis, Alzheimer's, and Parkinson's diseases.

Caspases are cysteinyl aspartate-specific proteases, important signaling molecules with diverse functions highly conserved among multicellular organisms. Caspases can be used as indicators for cellular damage in disorders; thus, they are great markers for detecting apoptotic events and extremely useful for drug research.

Cell-based assays in combination with automated cellular imaging are great tools for evaluating apoptotic events and provide insights into the mechanisms of apoptotic regulation via intrinsic and/or extrinsic pathways.

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01

FUNCTIONAL STUDIES OF APOPTOSIS

To allow visualization and track changes in cell health and viability. Studying apoptotic events provides insight into reponses to diverse stress conditions (e.g. hypoxia, oxidative stress, infection, and mitochondrial malfunctioning).

02

APOPTOTIC EVENTS AS EARLY TOXICITY MARKERS

Analysis of apoptosis intensity and pH changes provides a better understanding of early processes of cell health and cell toxicity in any compound treatment response.

03

AUTOMATED CELL IMAGE ANALYSIS

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

04

CELL VIABILITY, CYTOTOXICITY, AND MITOCHONDRIAL HEALTH

To analyze cell status (e.g. cytotoxicity, viability, mitochondrial damage, and mitochondrial potential measurement) in response to different treatments or compounds. These experiments could be carried out with our SPAchip® technology in combination with other cell-based assays.

Apoptotic events with CytoCHECK SPAchip® pH Single-Detection Kit

Discover a great tool for studying early apoptosis and pH changes in cell-based assays.

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, analysis of apoptosis can be evaluated by using caspase 3 staining. Incubating with both our SPAchip® technology and another commercial dye, caspase 3 staining intensity increases in two different cell lines incubated with commercial dye at 24, 48, and 72 hours after treatment, showing an increase in apoptotic cells. In addition, estimated cells per well significantly decreased in cells incubated with commercial dye at 24, 48, and 72 hours. On the other hand, cells incubated with CytoCHECK SPAchip® pH Single-Detection displayed a caspase 3 fluorescence intensity (indication of number of apoptotic cells) and total cell number similar to control.

Using caspase 3 staining together with our SPAchip® technology, researchers can further investigate apoptosis processes in multiple cell-based assays. By combining these two cutting-edge tools, we can gain a deeper understanding of the impact of treatments and/or compounds on cell viability and cell death.

Cell-based assays and its associated image and statistical analysis are great tool for evaluating apoptotic events and provide insights into the mechanisms of apoptotic regulation and/or apoptotic disruptions in various diseases such as cancer. In conclusion, these cell-based assays are useful for studying and tracking important biological processes as well as evaluating anti-cancer drug treatments.

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
  - Apoptotic cell staining
  - Live/Death cell staining
  - CytoCHECK SPAchip® pH Single-Detection Kit Green or Red
- **Positive and negative controls**
- **Measurements:**
  - Total nuclei count
  - % cell viability
  - % apoptotic cells

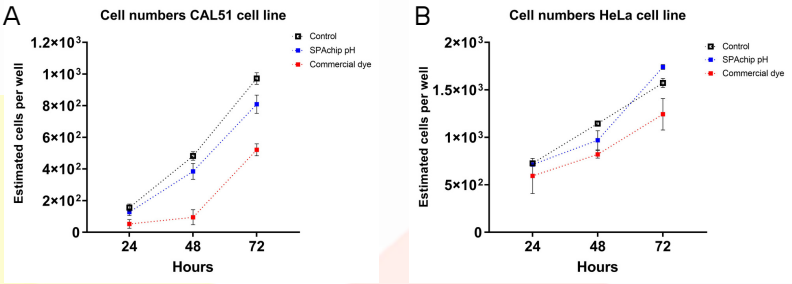

Figure 1: Estimated cells per well in HeLa cells **A)** and CAL51 cell line **B)** after incubation at 24, 48 and 72 hours at basal conditions. Values of estimated cells per well were calculated using nuclei staining. For each measurement, three conditions were evaluated 1) control cells with no treatment, 2) cells incubated with CytoCHECK SPAchip® pH Single-Detection kit and 3) cells incubated with commercial dye. Mean values with SEM error bars were represented for each condition.
Estimated cells per well significantly decreased in cells incubated with commercial dye at 24, 48, and 72 hours while cells incubated with CytoCHECK SPAchip® pH Single-Detection showed similar cell numbers compared with control.

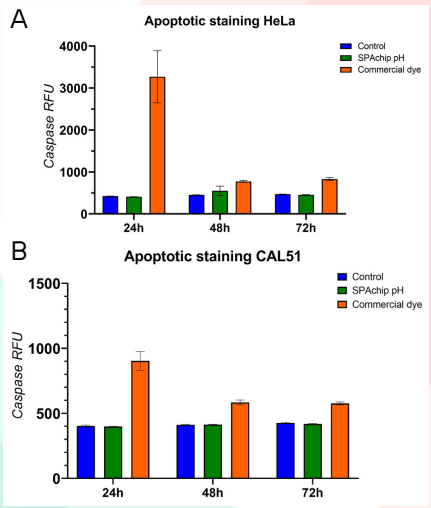

Figure 2: Caspase staining intensity in HeLa cells A) and CAL51 B) incubated 24, 48 and 72 hours after treatment at basal conditions. For each time measurement, three conditions were evaluated 1) control cells with no treatment, 2) cells incubated with CytoCHECK SPAchip® pH Single-Detection kit and 3) cells incubated with commercial dye. Bars represent mean values for each condition and error bars correspond to SEM values.
Caspase staining intensity increased in cells incubated with commercial dye at 24, 48, and 72 hours, showing an increase in apoptotic cells. Cells incubated with CytoCHECK SPAchip® pH Single-Detection showed almost identical caspase staining intensity compared with control.

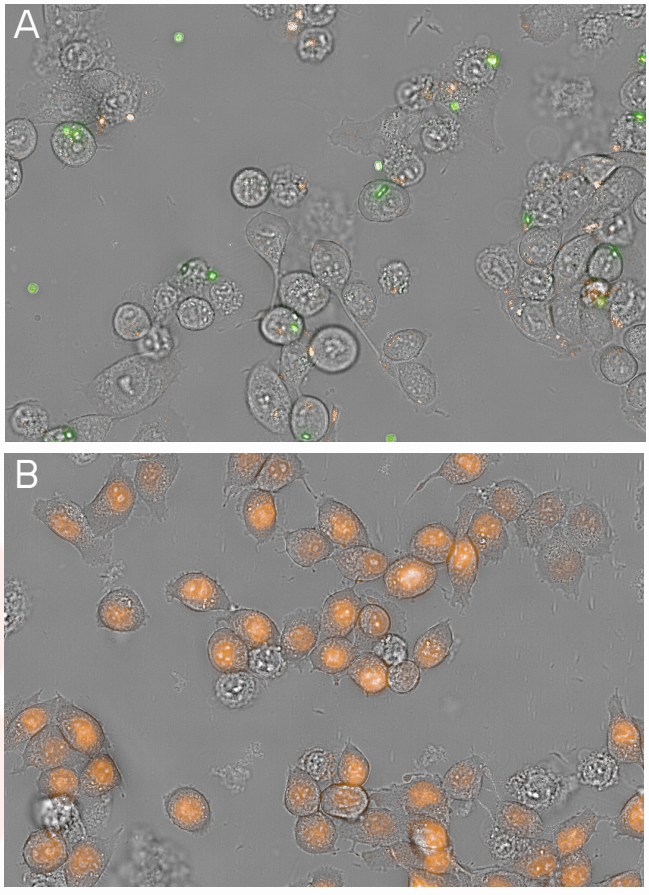

Figure 3: HeLa cell line stained with caspase 3 staining in red and CytoCHECK SPAchip® pH Single-Detection kit in green. Apoptotic events were tested in HeLa cells incubated with both SPAchip® technology **A)** and another commercial dye **B)**.
Commercial dye increased caspase 3 intensity and; therefore, showed an increment in apoptotic cells. Cells incubated with CytoCHECK SPAchip® pH Single-Detection showed a number of apoptotic cells similar to control.

Dynamic Film about cell sensing

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