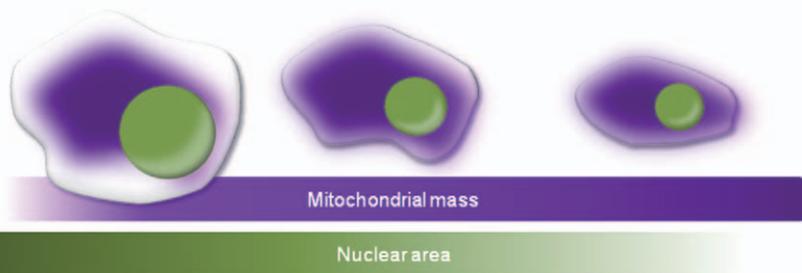


Evaluation of Compound Cytotoxicity using the Opera

Key Features

- Automated confocal image acquisition of live cells using the Opera™ High Content Screening system
- Image analysis using Acapella™ LiveDead Script and CellRegionAnalysis Script
- Quantification of characteristic changes following a toxic insult



Mitochondrial Mass, Cell Count, Membrane Permeability and Nuclear Size

Background

The ability to measure early indicators of toxicity is an essential part of drug discovery. In vitro cytotoxicity assays involving tissue specific cell cultures are considered to be valuable predictors for human drug toxicity. The potential of such in vitro assays lies in the fact that several cytotoxic effects in a variety of relevant pathways can be assessed simultaneously. As a primary organ for drug metabolism, the liver is a target organ for many toxic effects, therefore many in vitro cellular cytotoxicity studies focus on human hepatocytes.

Application

We describe here an image-based study of Tacrine, FCCP and Acetaminophen impact on mitochondrial mass, cell count, membrane permeability and nuclear area in the human hepatocellular carcinoma cell line HepG2, performed on the Opera High Content Screening system.

Using a fluorophore cocktail containing Hoechst 33342, BOBO™-3 and MitoTracker® Deep Red we simultaneously quantify characteristic changes following a toxic insult, i.e. nuclear shrinkage, cell membrane disruption, mitochondrial dysfunction and cell-loss. No washing steps are required after compound treatment, avoiding false read-outs due to cell-loss.

The acquired images of the Hoechst- and BOBO™-3-channels were analyzed with the Acapella LiveDead Script to determine the cell permeability of compound treated cells, followed by the Acapella CellRegionAnalysis Script to calculate the nuclear area, cell number and mitochondrial mass from Hoechst and MitoTracker® Deep Red images. A typical mask generated with the Acapella CellRegionAnalysis Script to convert image-based signals in numerical outputs is shown in Figure 1.

Mitochondrial responses to toxic impact depend on the type of compound, on its concentration and on the specific mitochondrial function that is affected. Mitochondrial dysfunctions can result in different events, as shown in Figure 2. We observed an enhanced biogenesis of mitochondria and an increase in mitochondrial mass after 24 h Tacrine and FCCP treatment caused by increasing mitochondrial respiration. In contrast, loss of mitochondrial ion balance and inhibition of mitochondrial respiration leads to decreasing mitochondrial signal of Acetaminophen treated cells.

The first consequences following a toxic impact on cells are damages in cell structure and function. Therefore, live cell count is a very sensitive indicator of cell stress, as almost all cellular structures and pathways are involved in cell proliferation. All three compounds saw a significant decrease in cell numbers (Figure 3B).

Cell membrane permeability is a common indicator of cell viability and occurs at a late stage of cytotoxicity when the membrane barrier can no longer be maintained. Nuclear shrinkage as a result of toxic impact on cells typically occurs with cell injury. We could show for FCCP and Tacrine a dose-dependant reduction of nuclear size to half of the initial control value. For Acetaminophen treated HepG2 cell, swelling nuclei caused by compound induced necrosis was observed prior to nuclear shrinkage (Figure 3D). Numerical data of compound-generated dose-response curves deduced from mitochondrial mass, nuclei area and cell number are shown in Figure 3.

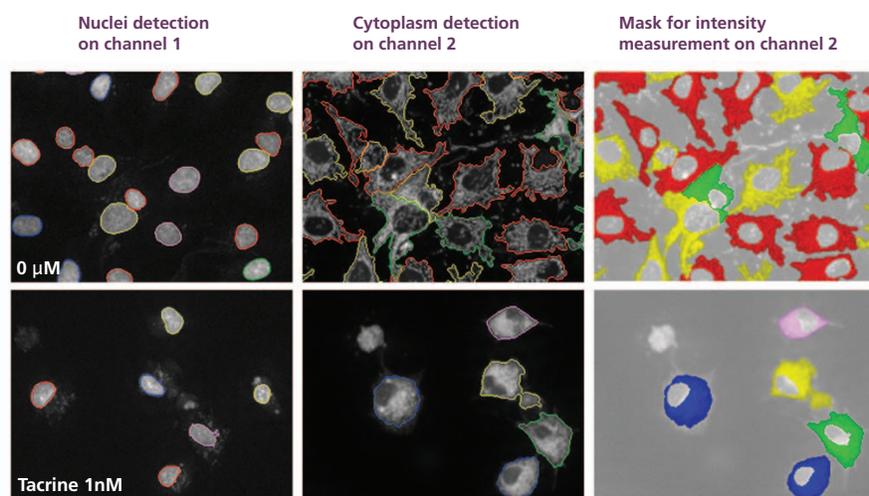


Figure 1: Image analysis. Images were taken of untreated and 1 mM Tacrine treated HepG2 cells using the Acapella CellRegionAnalysis Script. Left: Channel 1, which represents the Hoechst stained nuclei, was used for the nuclei detection and for the calculation of the read-out parameters nuclear area and cell number. Middle: Channel 2, the MitoTracker® Deep Red stained mitochondria, was used for cytoplasm detection and thereafter for analysis of mitochondrial mass. Right: Based on the results of the cytoplasm detection a mask, excluding the nuclei, was generated for the intensity calculation of stained mitochondria.

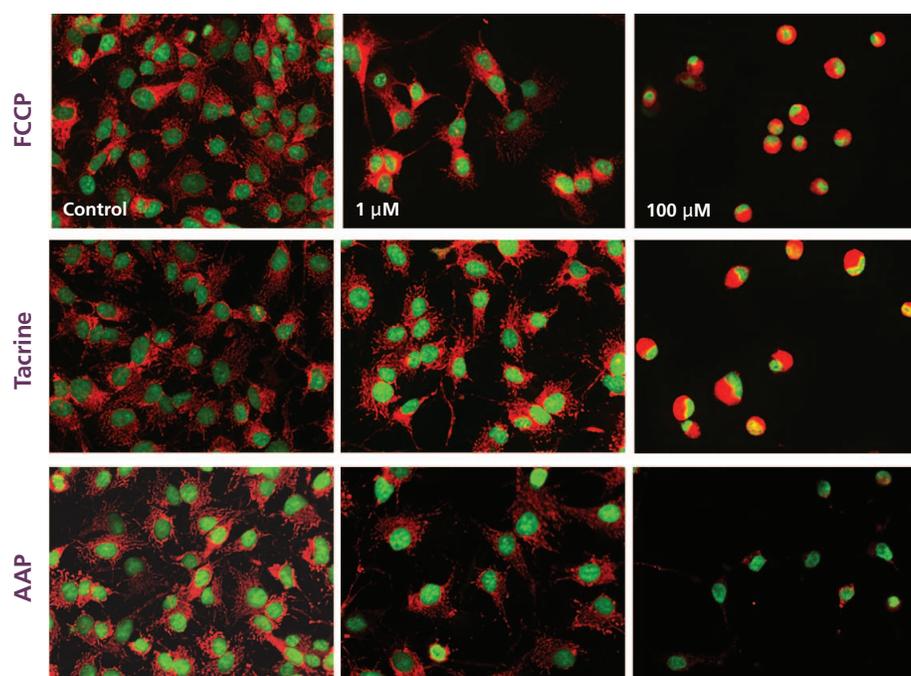


Figure 2: Opera images. Confocal images of Hoechst stained nuclei, shown in green and MitoTracker® Deep Red stained mitochondria, shown in red. The images show significant changes of mitochondrial signal as well as loss of cells in FCCP, Tacrine and Acetaminophen treated cells. 24 h FCCP and Tacrine treatment resulted in an enhanced biogenesis of mitochondria and an increase in mitochondrial mass. The mitochondrial signal of Acetaminophen treated cells clearly decreases. The loss of cells due to toxic impact of the compounds can be observed after treatment with all three test compounds. Images were acquired on the Opera using the 20X water immersion objective.

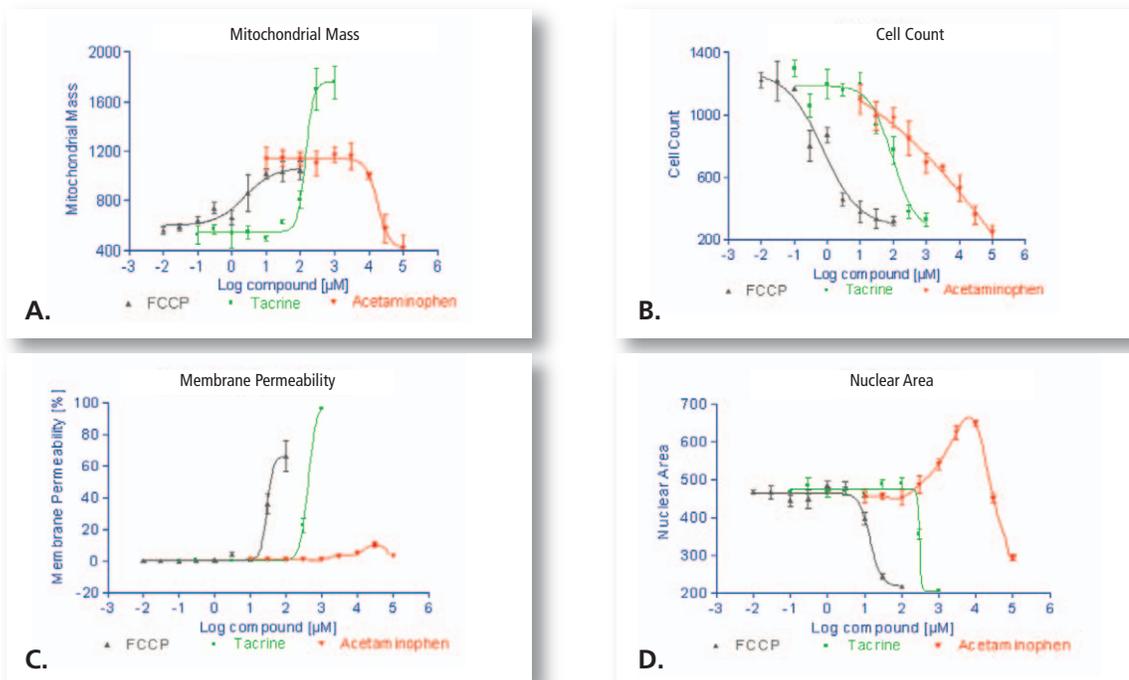


Figure 3: Quantification of compound effects on cytotoxicity. FCCP, Tacrine and Acetaminophen-generated dose-response curves deduced from mitochondrial mass, cell count, membrane permeability and nuclear area (N = 4 wells each).

A. EC_{50} value calculated from mitochondrial mass measurement of FCCP is $2.4 \mu\text{M}$, of Tacrine $140.1 \mu\text{M}$ and of Acetaminophen is 17.9mM .

B. The toxic effects on cell proliferation produced similar results; all three compounds led to a significant loss of cells with increasing concentrations. EC_{50} values calculated from cell count are $0.7 \mu\text{M}$ for FCCP and $95.5 \mu\text{M}$ for Tacrine.

C. Numerical data of dose-response curves deduced from membrane permeability show for FCCP and Tacrine treated cells a significant increase, whereas the Acetaminophen treated cells show marginal cell membrane disruption with escalating concentrations. Calculated EC_{50} value of membrane permeability measurement of FCCP is $29.1 \mu\text{M}$ and of Tacrine is $413 \mu\text{M}$.

D. The cytotoxic effect on nuclear size show for FCCP and Tacrine treated cells nuclear shrinkage, whereas Acetaminophen initially caused nuclear swelling prior to nuclear shrinkage. EC_{50} values calculated from measurement of nuclear area of cells are $14.9 \mu\text{M}$ for FCCP and $304.3 \mu\text{M}$ for Tacrine.

Conclusions

We demonstrated that this rapid, time saving and easy-to-use HCS cytotoxicity live cell application using the Opera platform in combination with the Acapella image processing software is well suited to providing reliable results of compound toxicity on HepG2 cells. By applying a multi-labeling dye-cocktail combined with measuring multiple parameters this application can detect early as well as late-stage occurrences of cytotoxicity.

Furthermore, this high content analysis (HCA) approach can be easily modified by replacing one organelle dye with another or it can be multiplexed easily.

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