Image-based quantification of HCS cell-based assays using the Operetta / Harmony imaging platform

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1 Introduction

As a creator of outstanding solutions for High Content Screening (HCS), PerkinElmer Inc. introduces the Operetta™, a compact bench-top approach to HCA research and screening. This imaging platform is designed to accelerate and simplify the High Content Analysis (HCA) process for every laboratory. A complete range of ready-made solutions (RMSs) for all key image analysis tasks and assays are pre-installed and ready to use.

We have applied the Operetta to: 1) Quantify cell cycle phases of human cells treated with compounds that induce either G1/S or G2/M cell cycle arrest, 2) Quantify changes in different parameters of human cells undergoing apoptosis: caspase-3 activation and nuclear fragmentation, and 3) Quantify the cytotoxicity of compounds using a live cell approach.

Fig. 1 Operetta

Key Features

- High quality images and very large field of view. Wide range of objectives for automated change: 10x, 20x high NA and long WD; 40x, 60x and 100x long WD. Very large sample area covered (see Fig. 2)
- Short exposure time. High power xenon fibre-optic light source, 360-640 nm continuous spectrum
- High sensitivity camera with great dynamic range. 14 bit Peltier-cooled camera
- Maximum flexibility. Fully automated eight position excitation and emission filter wheel with a wide range of filters available, fluorescence and brightfield imaging
- Harmony software included, comprising image acquisition, image analysis, and both image and metadata management
- Ready-made modular image analysis solutions included, consisting of easy to combine building blocks
- Automated parameter tuning for convenient assay development
- On-line image analysis

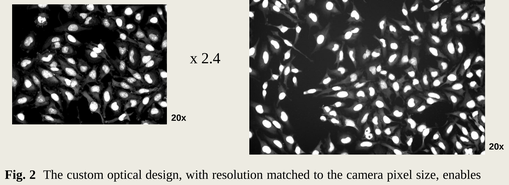

Fig. 2 The custom optical design, with resolution matched to the camera pixel size, enables you to image a 2.4 fold larger area (right) with every objective than compared to a standard microscope (left).

2 Harmony for accurate image analysis

Harmony™ is designed to streamline and simplify the HCS process by utilizing an intuitive user interface that navigates the user through their HCS experiment. Harmony suggests logical progressive steps where appropriate, simplifying the process for both new and experienced HCS users.

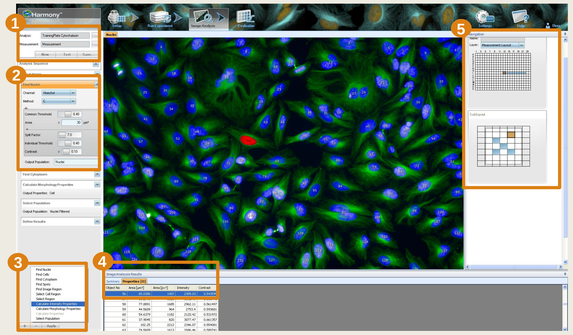

Fig. 3 Ready-made solutions / applications are stored within the Harmony database (1) but can be easily extended by choosing a building block from the drop-down menu (3). Adjustment of the image analysis according to your needs is easy with the tuning parameter pane (2). Detailed information about a cell can be obtained by selecting the desired object (red). The corresponding data is then shown in the results table (4). Simple navigation through the plate dataset can be done by clicking on the well and the field (5).

A full range of ready-made solutions and applications (RMSs/RMAs) for frequently used image analysis tasks and assays is pre-installed. Harmony provides the flexibility to change and modify your experiments utilizing image analysis building blocks. Each clearly defined block has primary tuning parameters with optimized values that can be automatically or manually adjusted with real-time visual feedback to facilitate the set-up process.

The RMAs of all three HCA applications described below can be found in the database (DB).

3 Determination of cell cycle phases

The characterization of agents that inhibit cell proliferation and division is particularly important for drug discovery research. Both events can be analyzed using HCS approaches by multiplexing cell cycle-specific cellular targets. One technique is EdU staining, which detects the S-phase of the cell cycle through incorporation of the nucleoside analog Uridine into newly synthesized DNA strands. Furthermore, there are well validated protein markers available that are associated with certain cell cycle phases. An example is the phosphorylated histone H3 (pHH3), which is a common M-phase marker.

By combining these two specific cell cycle markers we present a user-friendly solution for the precise classification and quantification of cells in S- and M-phase.

The results presented in Fig. 4 were obtained using the *CellCycle-1* RMA by tuning the parameters in view of the read-outs.

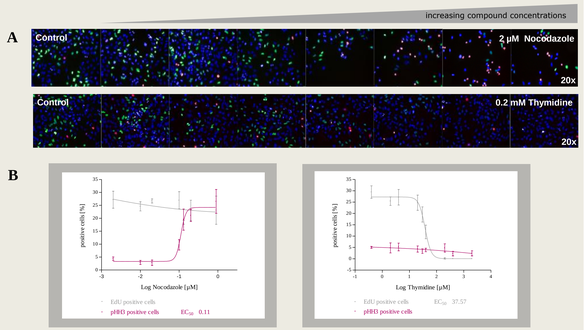

Fig. 4 A | False color overlay (green - EdU, red - pHH3 and blue - DNA) of either Nocodazole or Thymidine treated HeLa cells after an 18 h incubation at various compound concentrations. The images (presented as a well montage) show a significant dose-dependent increase of the pHH3 signal in response to Nocodazole treatment and a significant dose-dependent decrease of the EdU signal in response to treatment with Thymidine. **B |** The corresponding Harmony data analysis for Nocodazole (left) and Thymidine (right) shows the percentage of cells positive for EdU (representing cells in S-phase) in grey, and the percentage of cells positive for pHH3 (equal to the mitotic index) in magenta. The excess of Thymidine arrests the cells in G1/S-phase, and Nocodazole inhibits the cell division (G2/M blocker). N = 3 wells.

4 Determination of apoptosis

Apoptosis, or programmed cell death, is the most common form of eukaryotic cell death and occurs normally during development and ageing, and as a homeostatic mechanism to maintain cell populations in tissues. Events that occur during apoptosis comprise structural changes such as nuclear condensation and fragmentation as well as activation of caspases. Here we combine a nuclear morphological read-out (fragmentation; a late apoptotic event) with a specific apoptotic marker (caspase-3 activation; an early apoptotic event) to determine programmed cell death on the Operetta/Harmony platform.

The results presented in Fig. 5 have been obtained using the *Apoptosis-1* RMA, which is stored in the Harmony DB.

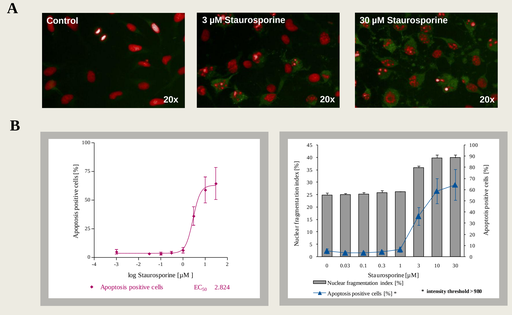

Fig. 5 A | False color overlay (red - nuclei, green - caspase-3) of staurosporine treated HeLa cells. Apoptosis manifests itself by an increasing fluorescence intensity of labeled activated caspase-3, as well as by the increasing appearance of smaller, pyknotic nuclei. **B |** The intensity of caspase-3 was converted into the "percentage of apoptotic cells" by applying a threshold, thus classifying a subpopulation of apoptotic cells. The resulting values were the basis for determining the EC_{50} (left). The morphological marker (nuclear fragmentation) confirmed these results by giving a specific read-out of caspase-3 (right). N = 3 wells.

5 Quantification of compound toxicity

In vitro cytotoxicity assays are an essential part of drug discovery, considered as valuable predictors for human drug toxicity. The HCA assay shown here is a rapid and flexible dye-based, live cell approach performed with HepG2 (human hepatocellular carcinoma) cells. Five cellular parameters are analyzed simultaneously, providing reliable results of compound toxicity.

The results presented in Fig. 6 have been obtained using the *Cytotoxicity-1* RMA.

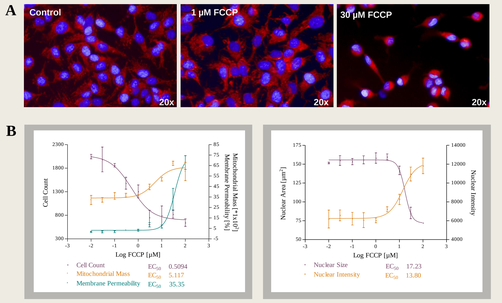

Fig. 6 A | False color overlay (blue - nuclei and red - mitochondria) of FCCP treated HepG2 cells at various compound concentrations. Cells were plated in triplicate on 384 Collagen I coated CellCarrier plates over night, and were treated with various FCCP concentrations for 24 h. The images show a significant dose-dependent increase of mitochondrial signal as well as a loss of cells accompanied by phenotypic changes, e.g. nuclear condensation and shrinkage. **B |** Compound-generated dose-response curves deduced from the cell count, mitochondrial mass and membrane permeability (left) and from the nuclear area and nuclear intensity (right). Increasing compound concentrations lead to a loss of cells as almost all cellular structures and pathways are involved in cell proliferation. The effect of FCCP on the Mitochondria of HepG2 cells results in an increase in mitochondrial respiration due to a decrease in mitochondrial transmembrane potential. Cell membrane disruption usually occurs in the later stages of cytotoxicity, as the EC_{50} values demonstrate. Nuclear condensation and shrinkage are characteristic morphological changes follow toxicity and cell injury. N = 3 wells.

6 Summary

Using the Operetta HCS system for fully automated fluorescent microscopy, we have measured and quantified a broad range of parameters: nuclear fragmentation, nuclear condensation, caspase-3 activation, DNA content, mitotic-index, cell-loss, mitochondrial mass and membrane permeability.

This technology enabled us to perform HCS studies for: cell cycle check point controls, apoptosis inducers and the cytotoxicity of selected compounds.

The assays presented here are well characterised for optimum performance and work robustly with reproducible EC_{50} values. Every assay was analysed using one of the image analysis solution approaches (RMA) pre-stored in the Harmony database to enable a ready to use implementation of the assay.

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