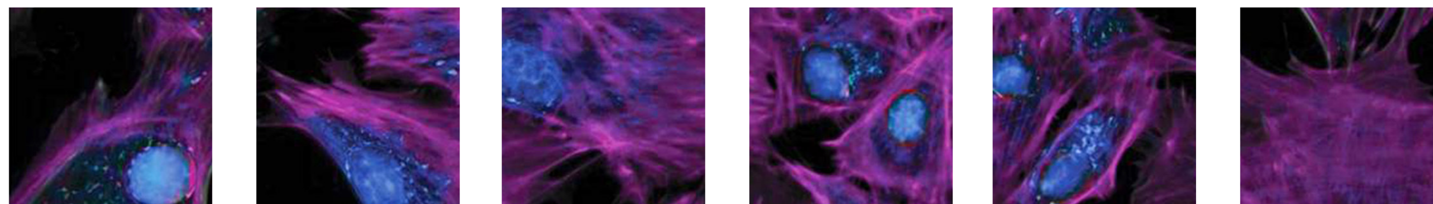
acumen® CELLISTA
application note

ttplabtech
natural innovators



Multiplex measurement of toxicity indicators

introduction

A major problem for the pharmaceutical industry is the failure of promising drug candidates very late in the testing phases. Often, there are unpredicted side-effects and toxicity issues which limit or prevent a candidate molecule being taken to market. Such failures very late in the product development pipeline result in substantial increases in time and cost to the whole pharmaceutical and healthcare industries, which need additional drugs to increase their product portfolios and address a wider range of clinical conditions. Any testing regimes that can identify toxic molecules and exclude them from the screening programmes as early as possible will lead to significant time and cost savings.

Previous studies using imaging-based high content analysis have shown the possibility of its usage in toxicity screening (1). Traditional image-based systems have limited throughput capability which has prevented their use in primary toxicity screening campaigns. Here we describe a method for high content, high throughput toxicity screening in 384-well microplates for whole well scans on the acumen® Cellista, with a scan time of 10 minutes per plate which includes both data acquisition and analysis time.

Three fluorophores were used to monitor the hepatotoxic effects of a small panel of commercially available compounds on HepG2 cells. The 405 nm laser-excited Hoechst 34580 stains all nuclei and facilitates enumeration of total cell number (giving a measure of cellular proliferation). Additionally, Hoechst nuclear half-width measurements give a measure of nuclear condensation which occurs during apoptosis. The 488 nm laser-excited TMRM is a cell-permeant, cationic, red-orange fluorescent dye that is readily sequestered by active mitochondria. TOTO-3 is a 640 nm laser-excitable fluorophore which stains the nucleus of dead/dying cells where the cell membrane has been compromised, thus providing a number of total dead cells per well.

materials & methods

cell treatment

HepG2 cells were seeded at 1000 cells/well in 384-well plates in 50 µL culture medium per well. After overnight incubation at 37ºC, 5% CO_2, compounds or vehicle control in culture medium were added (10 µL per well), to give the desired working concentration. Cells were then incubated at 37ºC, 5% CO_2 until the appropriate timepoint when 10 µL per well of detection mix was added (Life Technologies products TOTO-3 [4 µM, cat# T-3604], TMRM [2 µM, cat# T-668] and Hoechst 34580 [4 µM, cat# H21486] in culture medium). Cells were incubated with detection mix in the dark for 1 hour at 37ºC, 5% CO_2, prior to scanning on the acumen Cellista imaging cytometer.

data collection

acumen Cellista is a laser scanning imaging cytometer, and its typical work mode generates small files of high content cytometric data. Its unique optics perform simultaneous scanning and analysis to give very fast results for the entire well. Here, acumen excites with three lasers to capture three dyes in order to report out four parameters per cell, for every cell in the well.

results

By analysing staining from the three different fluorophores (Hoechst, TMRM and TOTO-3) that are present in all wells, acumen Cellista is able to determine multiple measures of cell health. Obvious visual differences can be observed from the well views (Fig 1). If tested over a range of timepoints and drug concentration, this can demonstrate how different drugs cause toxic effects via alternative pathways (Fig 2).

key points

TTP Labtech's acumen® Cellista provides a way to incorporate toxicity screening during the earliest phases of the drug discovery pipeline:

- **rapid scan and analysis time**
- **multiple toxicity readouts per single test well**
- **whole well analysis provides robust data confidence**
- **fail drugs with off-target toxicity effects early in the pipeline**

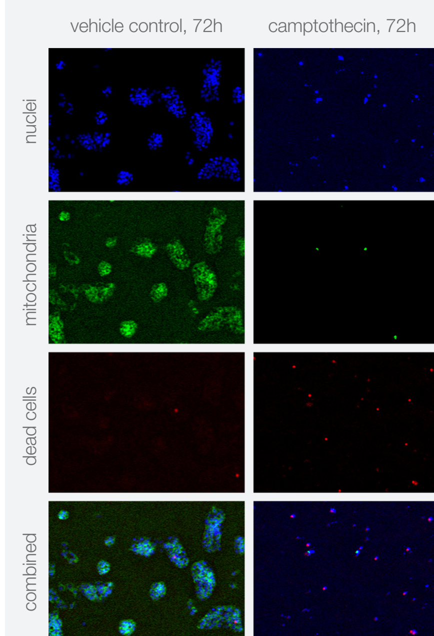


Fig 1. Treatment of HepG2 cells with Camptothecin (2 µM) for 72 hours significantly reduces cell proliferation, decreases mitochondrial health and increases the proportion of dead cells. Open source TIFF files generated by the acumen Cellista were false-coloured using ImageJ (2).

conclusions

These data demonstrate the ability of TTP Labtech's acumen Cellista to rapidly analyse multiple parameters for cell health.

- acumen Cellista facilitates a three colour hepatotoxicity screen in 384-well plates at 10 minutes per plate (including analysis time).
- Whole well scanning capability of acumen Cellista ensures robust data performance.
- The ability to rapidly obtain multiple measures of cell health facilitates the generation of a more informed risk profile for drug candidates.

about acumen

TTP Labtech's acumen Cellista is a microplate-based laser scanning cytometer designed to provide whole well, content-rich cytometric and image-based analysis. Its F-theta lens gives a uniform illumination across the field of view with a large focussed depth of field, which enables high throughput, whole well image acquisition across a range of plate types. acumen enables a wide range of fluorescent reagents to be combined in multicolour, multiplexed assays. It's easy-to-use, template-driven software offers an industry-proven route for quick adoption across a wide range of applications.

key capabilities:

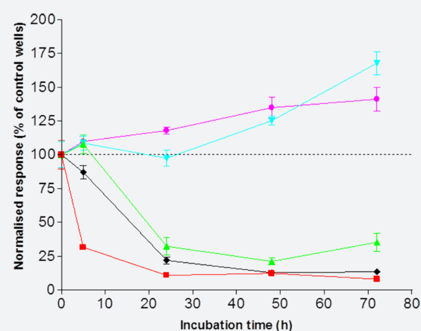
- scans and analyses 96- to 1536-well plates in the same time (in as little as 5 minutes/plate)
- can export whole well, OME-compliant TIFF files in the same scan times
- choice of 405, 488, 561 and 640 nm lasers
- PMT detectors simultaneously acquire up to four channels of fluorescence data per laser
- multimodal workflows complement existing imaging solutions

references

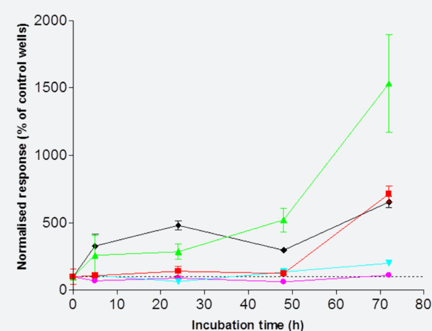
(1) O'Brien, P.J. *et al. Arch Toxicol* (2006) 80: 580-604

(2) Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011

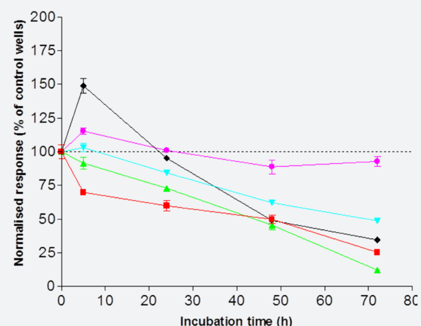
mitochondrial health (TMRM staining per cell)



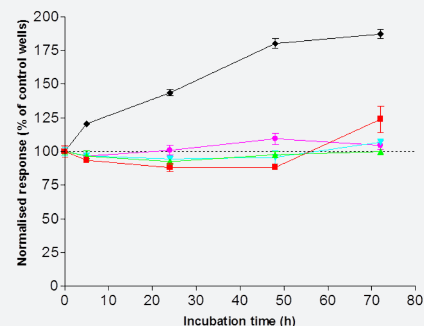
dead cell staining (TOTO-3 staining per cell)



total cell number (Hoechst 34580 staining)



nuclear condensation (Hoechst 34580 staining)



key: Anisomycin, Camptothecin, Etoposide, Staurosporine, Epinephrine

Fig 2. Timecourse of toxicity measurements in HepG2 cells on treatment with 2 μM of compound. Dotted line indicates no change relative to control wells. Data are from quadruplicate wells. Error bars indicate S.E.M.

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