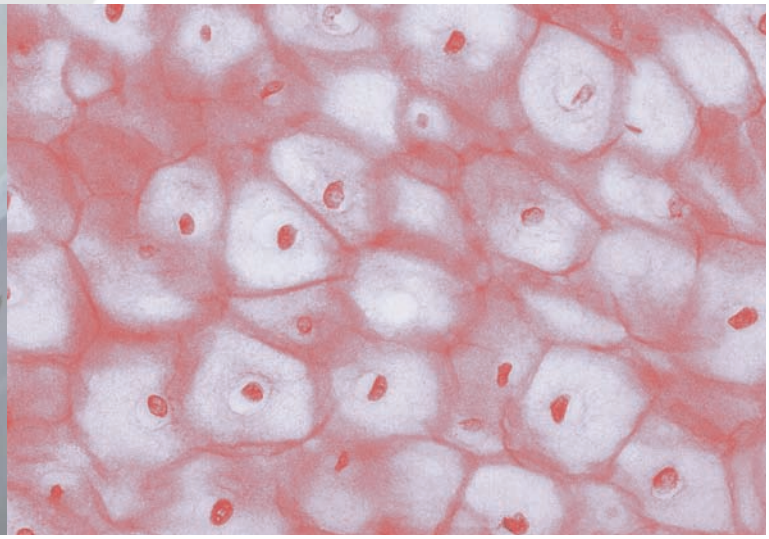

Compound profiling for anti-cancer activity using the NCI-60 cell lines

Easy automation of cell-based assays on the Fluent™ laboratory automation solution

Introduction

The NCI-60 panel represents a wide diversity of common cancer-types and it is one of the most important tools in modern drug discovery for the identification of new anti-cancer compounds. Although, the cell lines are commonly used in phenotypic assays for monitoring cell growth and survival, when combined with bioinformatics tools such as the NCI COMPARE database, NCI panel data can also provide a preliminary indication of the potential mechanisms of action for novel compounds. Typically, large sets of compounds are initially tested at a single concentration. Hits showing growth inhibition or cytotoxic properties are profiled in dose response format over multiple cell lines to establish compound potency and selectivity.

The European ScreeningPort GmbH (ESP) is a public-private partnership which receives project-based funding from governments, industry and academic institutions. ESP offers industry-scale small molecule hit-finding capabilities to academic organizations as well as collaborating closely with the pharmaceutical industry. Approximately half of ESP's hit finding approaches involve cell-based assays and ESP has used the NCI panel successfully in the past to identify novel anti-cancer compounds which have gone on to demonstrate efficacy in *in-vivo* xenograft cancer models.

ESP also provides screening services to multiple academic partners worldwide, screening in excess of >15,000 384-well plates per annum operating comprehensive assay development, cell culture, compound logistics, informatics and screening platforms.

Tecan has re-invented automation with Fluent, a unique instrumentation concept built around the application-specific needs of cell biology and drug discovery laboratories. Fluent breaks new ground, delivering greater capacity and increased speed; the platform provides superior throughput and walkaway time, making it easier to get more done, more effectively. Tecan and ESP have developed protocols that demonstrate the power of this cell-based assay solution, combining expertise in automated liquid handling, detection and cell-based assays to offer a new dimension in fast, reliable, completely automated assays without the need for expert automation personnel. The industry standard method for cell viability determination, measurement of cellular ATP via luciferase detection (CellTiter-Glo®, Promega), was used to demonstrate the performance of the Fluent platform.

Materials and methods

Figure 1: The Fluent cell-based assay workstation. A Fluent 780 is shown, equipped with an eight-channel Flexible Channel Arm, a Multiple Channel Arm 384 and a Robotic Gripper Arm. A Carousel is integrated onto the right hand side of the instrument, along with the latest generation of CO_2 incubator. An Infinite M1000 PRO microplate reader is located below the Dynamic Deck. The dimensions of the compact system are indicated.

Fluent laboratory automation solution

Fluent (Figure 1) is the latest in Tecan's successful family of liquid handling automation platforms. The Fluent cell-based assay solution offers rapid, high definition pipetting for both the eight-channel Flexible Channel Arm (FCA) and the Multiple Channel Arm 384 (MCA384). Its patented Dynamic Deck increases the worktable capacity and boosts productivity by allowing integration of a wide range of Tecan modules – including a Carousel™, for storage of various consumables; a HydroSpeed™ plate washer (not required for this assay); an Infinite® M1000 PRO plate reader; and carriers for troughs, stacked disposable tips and microplates (up to six deep on the worktable, Figure 2) – as well as a third-party high capacity CO_2 incubator.

The Multiple Channel Arm 384 uses adapters which can be automatically exchanged during a run, allowing it to act as either a 384- or a 96-channel arm within the same protocol. Fully independent, task-specific arms allow parallel processing and coordinated scheduling, ensuring the runs are completed faster and more efficiently. Each Fluent cell-based assay solution is equipped with:

- Flexible Channel Arm – fitted with eight pipetting channels using disposable that can individually access any well or tube, perfect for explicit sample and control distribution or serial dilutions.

- Multi Channel Arm – instantly swaps between 96- and 384- channel adapters during a run, offering outstanding capabilities for reagent distribution or plate replication.

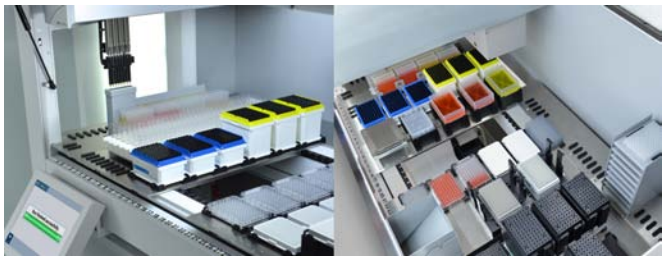

Figure 2: Dynamic Deck with six ANSI/SLAS positions in the depth, equipped with six boxes for disposable tips for the Flexible Channel Arm on the left. Blue colour code for 200µl and yellow colour code for 1000µl disposable tips. Top view of the Dynamic Deck on the right showing nested disposable tips, positions for troughs, active carrier for the Multiple Channel Arm 384 and a hotel in the back.

- Robotic Gripper Arm (RGA) – quickly and smoothly transfers plates and consumables between storage modules, integrated devices and the worktable without interrupting pipetting. Toxicity profile on NCI panel.

Four cell lines were chosen from the NCI-60 panel to determine the toxicity of compounds and demonstrate the capabilities of this experimental set-up for screening anti-cancer agents. The CellTiter-Glo Luminescent Cell Viability Assay (Promega, USA) was used to determine the effect of the compounds on the viability of the cells. Based on ATP quantification, a surrogate measure of the number of metabolically active cells in each well – this assay is widely used in industrial drug discovery. The homogeneous assay involves the addition of a single detection reagent directly to cells cultured in serum-supplemented medium, with no cell washing, medium exchange or other pipetting steps required. This 'add-mix-measure' format results in highly reproducible assay performance and allows measurements to be performed with as few as 500 cells per well. The assay was performed in accordance with the manufacturer's protocol.

Automation process

Overview

The NCI-60 cell viability profile was run in a 384-well plate format. Test compounds are serially diluted to create a dose – response stock plate, from which an intermediate plate was created, with compounds diluted in media to reduce DMSO levels in the final assay. The 384-well cell culture plates, pre-seeded 24 hours previously, are transferred from the CO_2 incubator and compounds added. After 48 hours, the CellTiter-Glo reagent is added to the assay plate, mixed and incubated in the dark for 10 minutes before reading the plate using the integrated Infinite M1000 PRO reader. Baseline control plates were assessed at 24 hours after seeding. The relative growth (at 72 h after seeding)

versus base line control (24 h) was used to calculate cell viability and proliferation parameters (growth inhibition, GI 50, 50 % growth inhibition).

Detailed automation protocol

Lidded 384-well, half deep-well plates containing 20 µl of test compounds in columns 1 and 12, and 10 µl of high and low controls in columns 23 and 24, were loaded into the Carousel, along with boxes of 50 µl Multiple Channel Arm 384 Disposable Tips (DiTi). To simplify the assay, tip boxes were re-arrayed for the DMSO transfer, using the partial tip function of the Multiple Channel Arm 384 head allowing selected columns of tips to be picked up. Here two columns

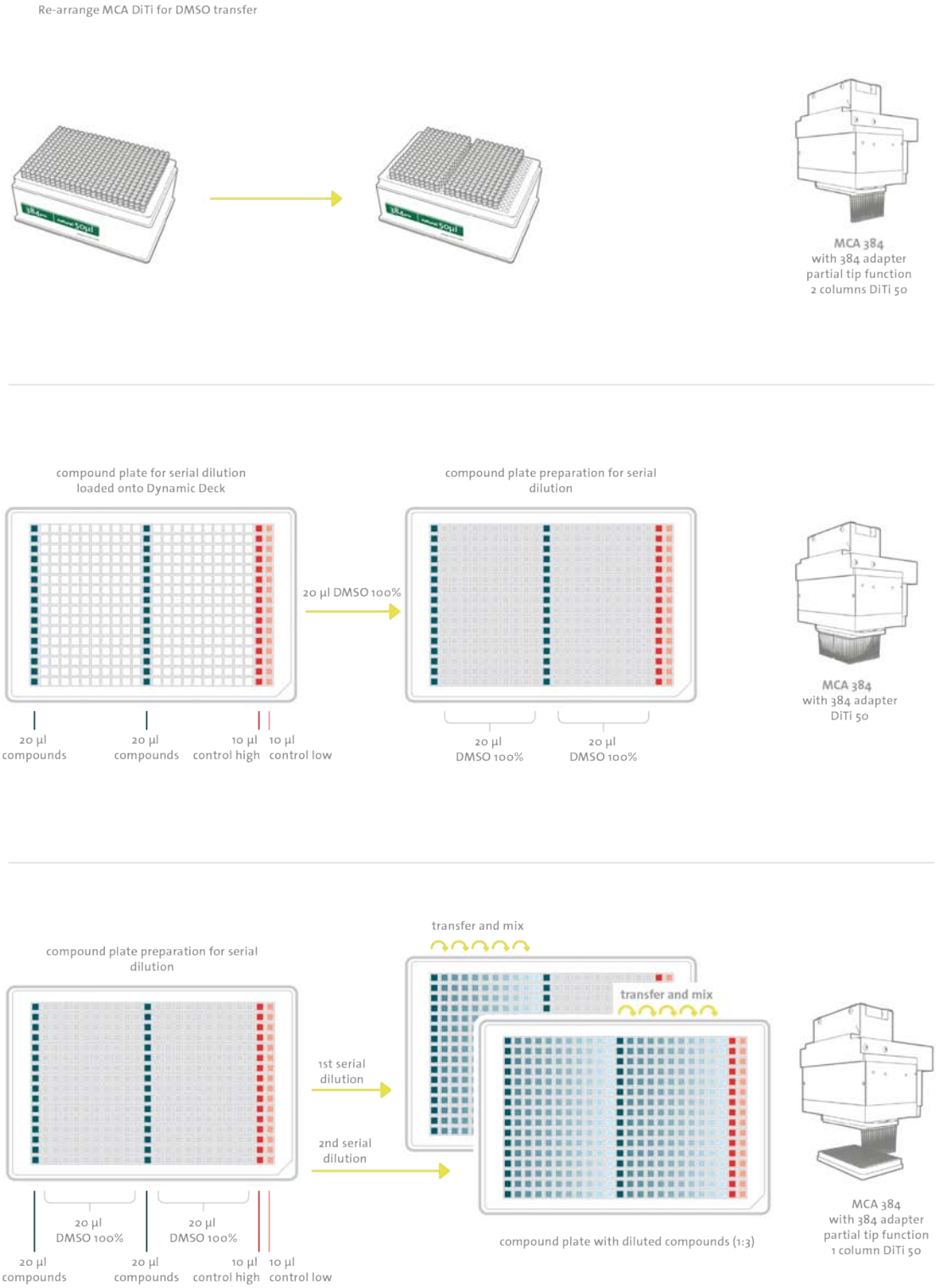

Figure 3: Disposable tips are easily re-arrayed for fast and easy DMSO transfer (top). DMSO is added to columns 2-11 and 13-22 in one dispensing step with the Multiple Channel Arm 384 and the 384-channel adapter. A serial dilution is prepared in two steps, initially from columns 1-11 and thereafter from columns 12-22, generating duplicates of 16 compounds at 11 concentrations.

of tips have been picked-up and then ejected into an empty box in the pattern shown in (Figure 3, top). DMSO and medium were loaded onto the deck in disposable troughs.
The method was started from the touchscreen, using the loading guide to ensure that all necessary reagents were on the worktable. The re-arrayed disposable tips were used to prepare the compound plate for the serial dilution. The MultiChannel Arm using the 384-channel adapter picked up the disposable tips and aspirated DMSO (100 %) from a trough. 20 µl of DMSO was then dispensed into a compound plate delivered to the Dynamic Deck by the Robotic Gripper Arm. Because of the pre-arrayed pattern of the disposable tips, only columns 2-11 and 13-22 of the compound plate were filled. This process can be carried out for multiple plates on the deck at the same time.

In order to prevent precipitation, the DMSO concentration was kept high for as long as possible. For the subsequent serial dilution, the Multiple Channel Arm 384 discarded the disposable tips and picked up a single row of 50 µl tips (partial tip function). A serial dilution (1:3) was then set up across columns 1 to 11 by stepwise transfer of 10 µl of each compound, followed by thorough mixing. A second serial dilution was set up in columns 12 to 22 using new disposable tips (see Figure 3). The compound plate layout was designed to allow testing of either 11 concentrations for 32 different compounds, or duplicate 11-point dose response curves for 16 compounds. The assay included the preparation of an intermediate plate where the Multiple Channel Arm 384 was used to produce a 384-well plate with 8 µl of cell medium and 2 µl of test compound from the serial dilution plate in each well, creating an additional 1:10 dilution (see Figure 4). In the final processing step, up to five 384-well plates with cells, pre-seeded 24 h before the stimulation, were challenged by transferring 0.5 µl of compound from the intermediate plate, giving a final DMSO concentration of 0.5 % in the assay plates.

This method can be performed to treat ten assay plates in one 45 minute in run.
Lids were automatically removed and replaced by the Robotic Gripper Arm, controlled by the FluentControl™ software, to minimize exposure of compounds or cells to the environment. Delivery of plates and disposable tips from the Carousel and third party devices, such as the incubator, was optimized using the system's unique Path Finder™ technology, enabling high speed, coordinated movement of all arms in parallel.
Following a 48 h incubation with the test compounds, the cell viability assay was initiated by loading the CellTiter-Glo reagent into a trough on the Dynamic Deck. The Robotic Gripper Arm retrieved the assay plate from the incubator, moved it to a free deck space and removed the lid. The Multiple Channel Arm, using the 384-channel adapter with 50 µl disposable tips, transferred 10 µl of CellTiter-Glo reagent into every well of the assay plate in free dispense mode. The Robotic Gripper Arm replaced the lid and immediately moved the assay plate into a darkened storage unit for a 10 minute incubation. The plate was then transferred to the Infinite M1000 PRO for the luminescence measurement. Finally, the plate was transferred from the reader to the Carousel, ready for disposal. The schedule for this method is shown in Figure 4 (bottom) and takes 16 min for a single plate, while a batch of four assay plates is completed in 25 min only.

Results

For the toxicity profile, a set of eight compounds was tested on four different cell lines:

- **HT-29:** colon cancer cell line (adenocarcinoma type II)
- **MCF-7:** breast cancer (adenocarcinoma)
- **A549-ATCC:** lung epithelial cancer
- **IGR-OV1:** human ovarian adenocarcinoma

In an exemplary selection, data from three compounds are shown in Figure 5 and 6.

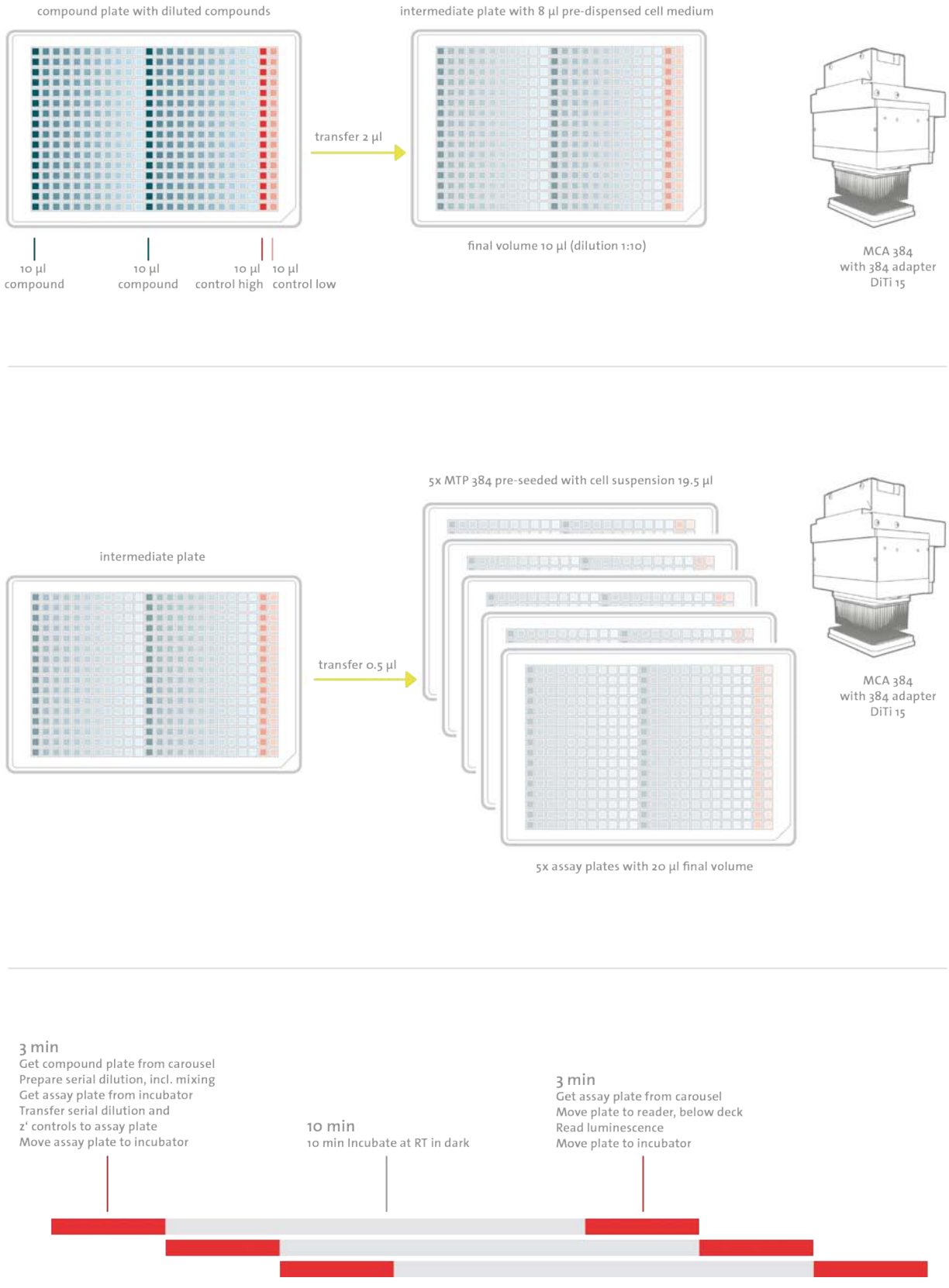

Figure 4: Creating an intermediate dilution (top) initially, to achieve a final concentration of 0.5 % DMSO in the assay plates (center). CellTiter-Glo schedule for a batch of four assay plates (bottom). Running the assay after a 48 h incubation time takes 16 min with the compounds in a single plate, while a batch of four plates takes just 25 min.

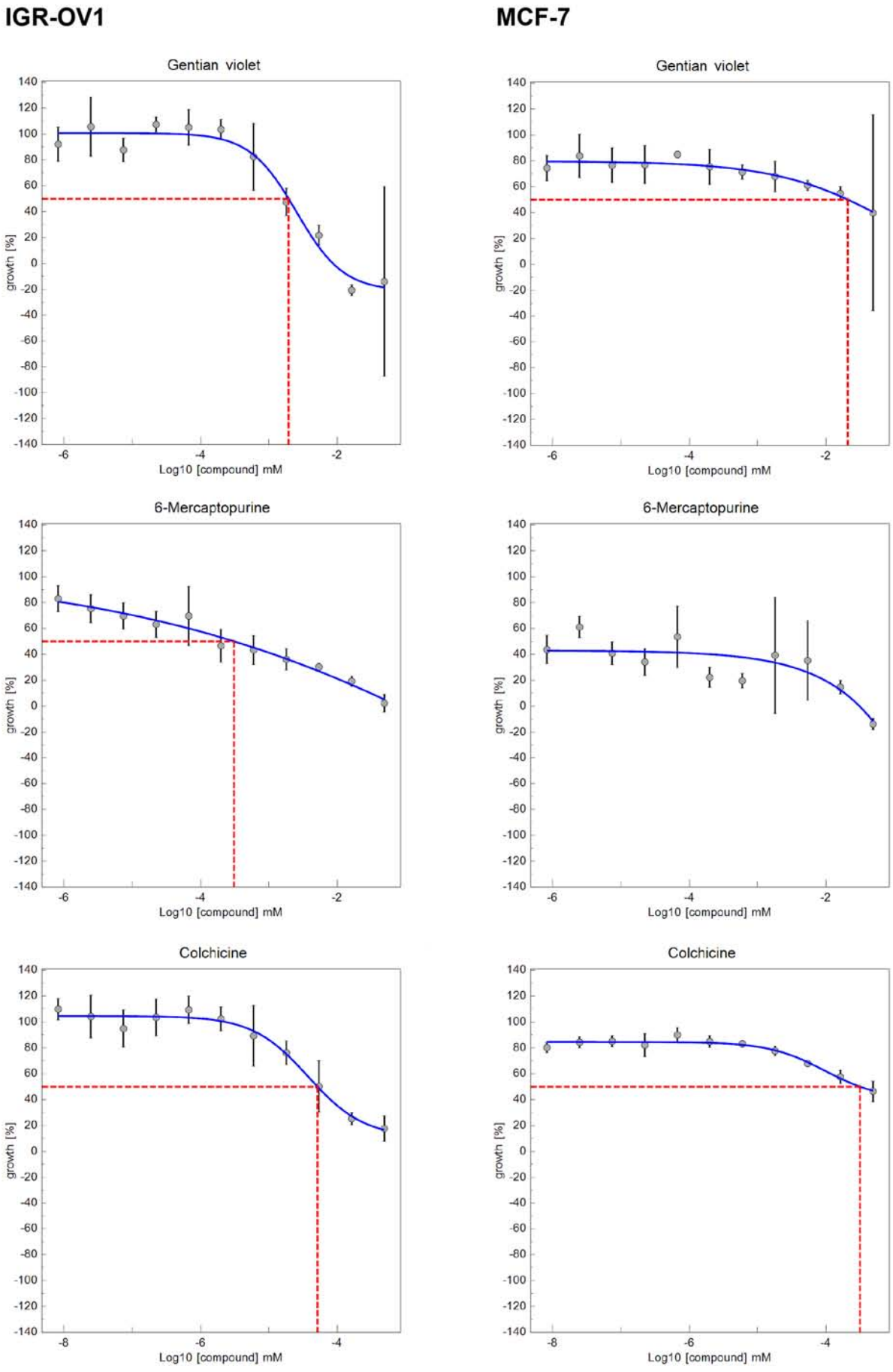

Figure 5: Relative cell growth (%) versus compound concentration for IGR-OV1 and MCF-7 cell lines. Cells were exposed to compounds for 48 hours.

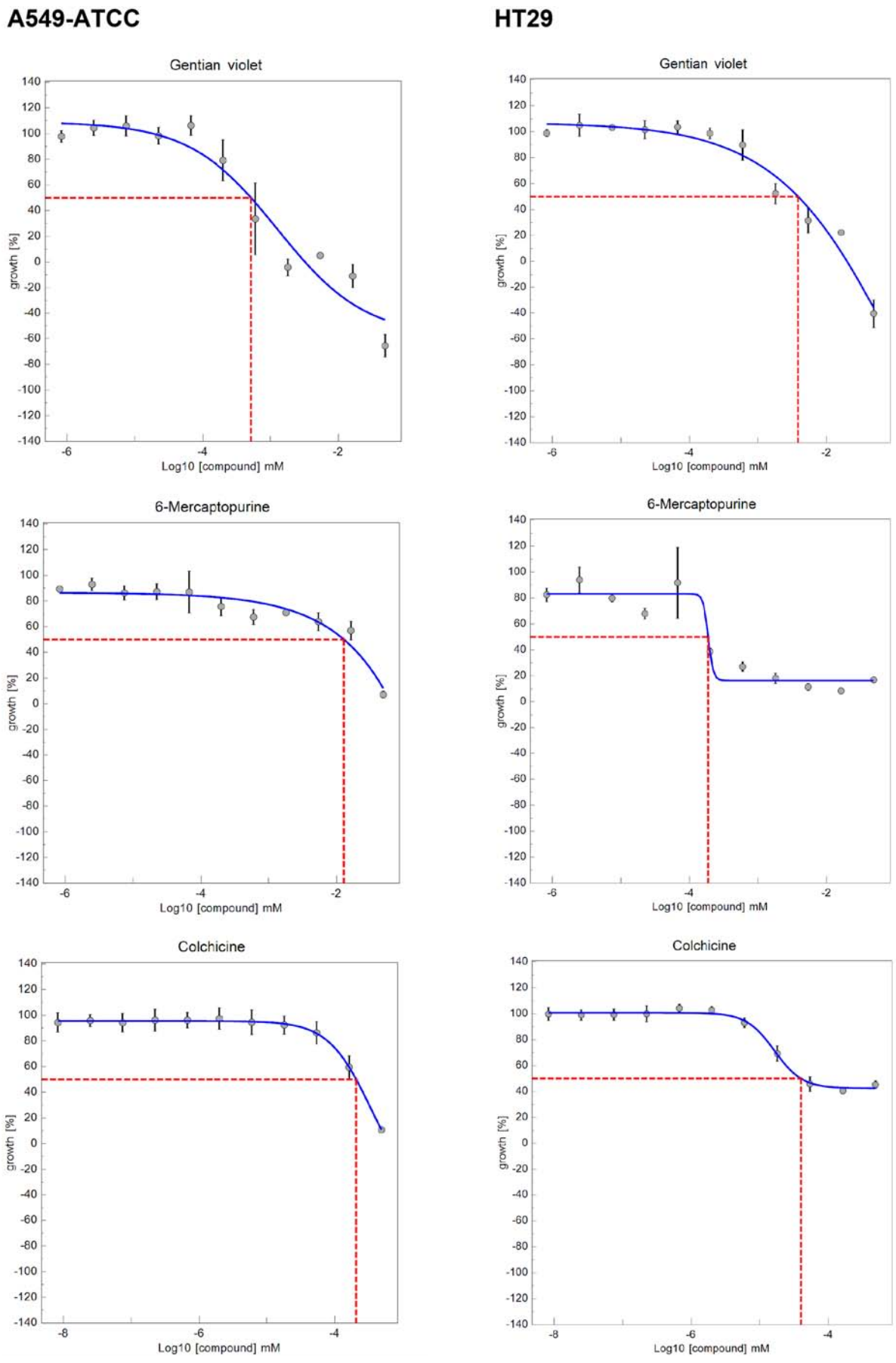

Figure 6: Relative cell growth (%) versus compound concentration for A549-ATCC and HT-29 cell lines. Cells were exposed to compounds for 48 hours.

Cell line		Test compound: Colchicine	Gentian violet	Mercapto-purine
HT-29	TGI	-	20	-
	GI50	0,04	3,9	0,19
A549	TGI	0,63	3,2	-
	GI50	0,21	0,52	13
MCF-7	TGI	-	1900	31
	GI50	0,32	21	-
IGR-OV1	TGI	-	8,7	78
	GI50	0,052	2	0,31

Table 1: Test compound concentrations at 50% growth inhibition (GI50) and 100% growth inhibition (TGI). All values are given in µM.

Summary

This application notes describes the successful automation of a cell viability assay using a selection of NCI cell lines which are commonly employed in cancer drug discovery. Although in this investigation four cell lines were studied in parallel, the flexibility and throughput capabilities of the Fluent laboratory automation solution provides researchers with the opportunity to further expand their protocols to profile many more cell lines in parallel. In principle, up to 100 compounds could be screened in dose – response (11 point in triplicate) against 20 cell lines within a single unattended experimental run. An industry-standard assay format, CellTiter-Glo, was used for the cell viability measurement. Using the Fluent to perform cell-based assays in 384-well plate format is a straightforward exercise. Its rapid speed and low volume liquid handling capability are perfectly aligned with modern throughput and miniaturization requirements. Tecan engineers have integrated liquid handling, incubation and detection processes into one flexible platform, with a small footprint and enormous capabilities.

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