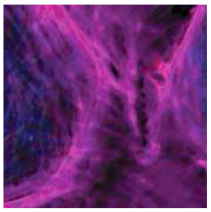
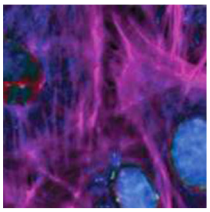
acumen®
application note

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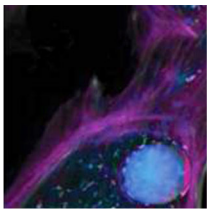
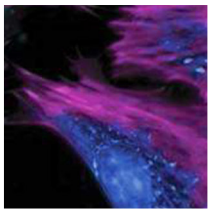
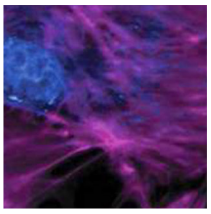
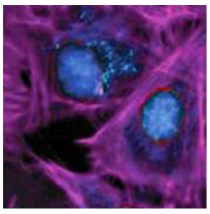
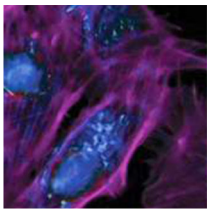
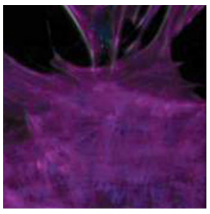

High throughput quantification of HepG2 cell colony formation in soft agar

introduction

Research to identify new anticancer drugs is currently facing significant challenges, as only 5% of compounds that show efficacy in pre-clinical development go on to become licensed drugs. Traditionally 2D cell culture models have been employed to evaluate drug candidates in the early phases of the drug discovery process, however, there is increasing evidence that cells grown in 2D monolayers do not accurately reflect the biological complexity of tumours. The requirement for better *in vitro* tumour models has led to the development of 3D cell culture models, which retain many of the morphological and genetic traits of tumours.

Enumeration of colonies has been traditionally carried out using a semi-solid agarose bilayer system in Petri dishes and involves manual counting on a microscope. High content analysis can be used to determine additional parameters such as colony size, shape, or fluorescence intensity, however, this approach has proved to be complicated due to the technical limitations of many CCD-based imaging systems:

1) such systems typically capture only a small area of the well (< 1 mm^2). In order to analyse all colonies in the well, several images must be taken and stitched together post acquisition.

2) most CCD-based imaging systems have a limited depth of field and require the acquisition of a Z-stack of images to estimate the volume of colonies. Overall, this process is slow and cumbersome.

TTP Labtech's acumen® can rapidly and accurately quantify cell colony formation in soft agar. This laser scanning imaging cytometer allows the operator to scan whole wells to enumerate fluorescent colonies and can also be used to determine the size of colonies through the application of a spherical volume algorithm. This allows the differentiation of small cell clusters (< 20 cells) from cell colonies.

materials & methods

cell culture

HepG2 cells were grown in a 3-layer soft agar format in 96-well plates. Briefly, the wells of the plate were covered with a base layer of 0.6% agarose in complete growth medium (25 µL). The middle layer comprised a single cell suspension of HepG2 cells in 0.4% agarose (500 cells per well in 50 µL agarose). The agarose layers were covered with a top layer of complete growth medium (75 µL). After 24 h, doxorubicin stocks were added to the wells (5 µL, final concentrations 1 nM - 1 µM). The colonies were then cultured for a further 4 days, with an overlaying media/doxorubicin change 3 days after seeding. Prior to imaging, the colonies were stained by adding calcein-AM (5 µM final) to the wells, followed by incubation for 1 h at 37°C.

key points

TTP Labtech's acumen® Cellista provides the fastest way to perform high-content cell colony formation assays in soft agar

- **all colonies are counted by rapid whole well imaging**
- **colony volumes are determined without Z-stacking**
- **easily distinguish between colonies and small clusters of cells**

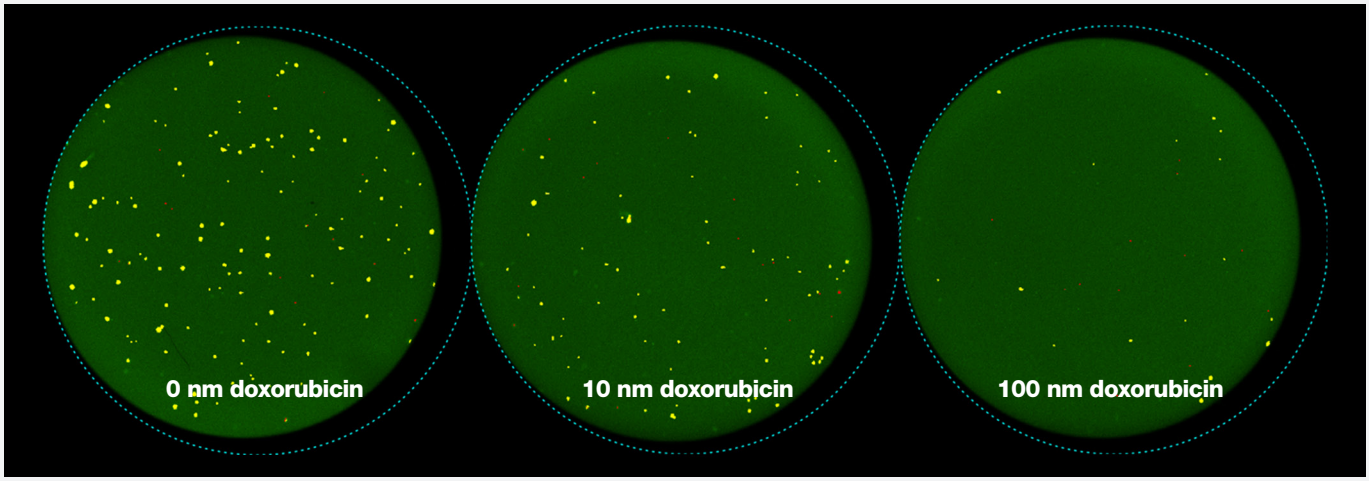

Fig 1. HepG2 tumour spheroids grown in soft agar on 96-well plates.

image acquisition

Whole well TIFF images were acquired using the acumen's 488 nm laser excitation with the FL-2 detection channel (500-530 nm) for calcein-AM. The acumen's unique optics provide a large depth of field, allowing colonies to be imaged even in multiple agar layers, without the need to acquire Z-stacks (Fig 1).

results

Colonies of HepG2 cells were identified as having a spherical volume of > 5,000 μm^3; cell clusters were identified as having a spherical volume of 10 - 50,000 μm^3. The total area and spherical volume of colonies in the well was determined and the number of colonies per well was also counted (Fig 2).

conclusions

These data demonstrate the ability of TTP Labtech's acumen to rapidly analyse and assess cell colony formation assays:

- compared to manual counting under a microscope, this automated approach offers a rapid means of enumerating fluorescent colonies
- the spherical volume algorithm to determine the colony volume does not require a Z-stack of images for analysis
- this method can be used to unambiguously distinguish small cell clusters from colonies of cells

about acumen

TTP Labtech's acumen is a laser scanning imaging cytometer designed to provide single-shot, 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. Its 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 (as little as 5 mins/plate)
- can export whole well, OME-compliant TIFF files in the same scan times
- choice of 405, 488, 561 or 640 nm lasers
- PMT detectors simultaneously acquire up to four channels of fluorescence data per laser
- acumen complements your existing imaging and analysis assets to optimise workflows and maximise throughput

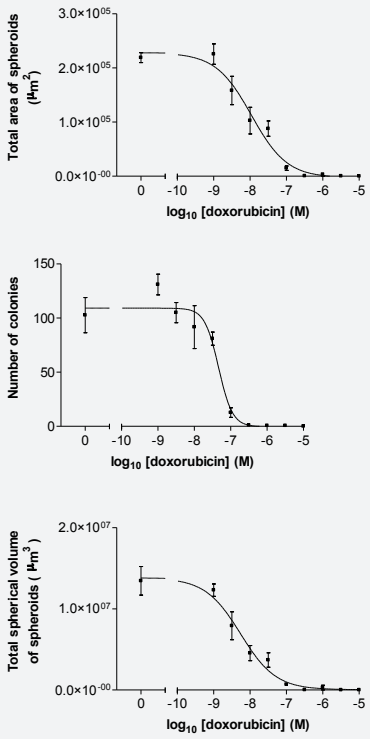

Fig 2. Drug concentration-response curves (mean ± SEM, n = 3)

get in touch

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