

High throughput imaging of cell based assays and cellular models using microplate cytometry

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introduction

Microscope-based, high-content instruments are used for many cell based assays in high content screening (HCS). For efficient and rapid analysis most assays require the use of higher resolutions which entail lengthy read times, using single colours. Furthermore, in order to keep plate read times at a minimum, often only a small percentage of the total number of cells are analyzed in a well. acumen[®]eX3 is a laser scanning imaging cytometer with a widefield objective lens and "on-the-fly" laser scanning capabilities. acumen rapidly scans entire wells, enabling statistically robust data to be obtained for a number of cell-based assays relevant to the drug discovery industry such as cell cycle analysis, cell signalling, cell surface marker expression and cell migration.

We present data demonstrating the use of acumen[®]eX3 to assess the effect of compounds on cell cycle and mitotic index. We also highlight the application of laser scanning imaging cytometry for the study of kinase activation determination.

More recent studies demonstrate that acumen[®]eX3 is also capable of rapidly analysing complex cellular or animal models, such as angiogenic tube formation, *C. elegans* or *Drosophila* larvae and Zebrafish embryos.

In addition to acumen's built in software, it offers the flexibility of exporting whole well open source TIFF images for batch processing by third party image analysis software packages.

The combination of image processing methodology and large depth of field cytometric scanning provides the potential for the development of whole organism or tissue-based assay models for high content drug screening to provide information on multi-cellular drug interactions at an early stage.

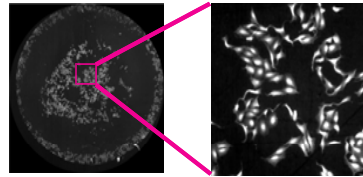
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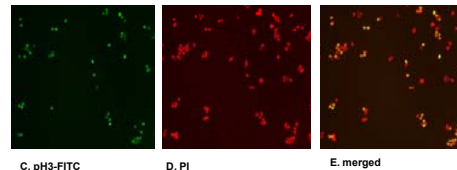
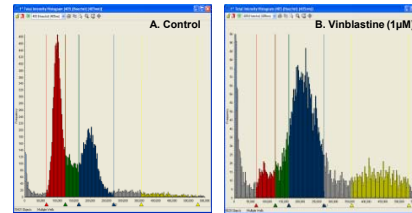
1. TIFF image export

HeLa Cells stained with 1.5µM Calcein-AM



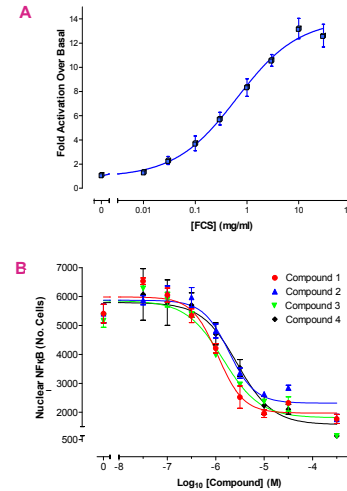
Whole well imaging using acumen[®]eX3 enables total cell enumeration, providing more robust data by eliminating intrawell variability. In addition, whole well cell scanning enables fewer cells to be plated per well thus saving on potentially limited cell culture stock.

2. cell cycle/mitosis analysis



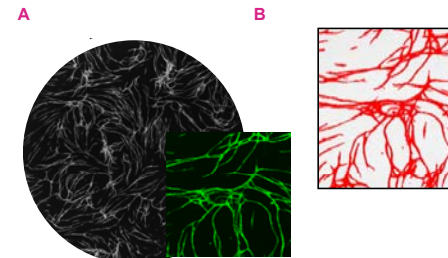
A. Histograms of Propidium Iodide (PI) labelling allowed definition of cell cycle phases. G1=red; G2/M=blue
B. Vinblastine arrested cells in G2/M phase and aneuploidy. Lower panels show: **C.** pH3-FITC staining for mitotic index, **D.** PI staining for cell cycle determination, **E.** Merged images of C and D show the ability to distinguish G2 and M phase cells within the culture.

3. protein kinase profiling



A. HeLa cells were serum starved for 18 hours, then stimulated with FCS for 5 minutes prior to fixation. A standard IF protocol determined ERK phosphorylation. Cells were stained with PI to allow the % of cells with phospho protein to be determined.
B. NFκB translocation. HeLa cells were treated with four different compounds to inhibit NFκB translocation to the nucleus.

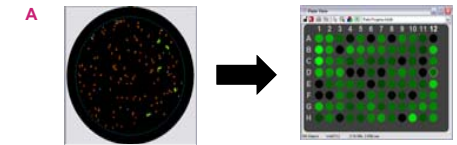
4. angiogenic tube formation and TIFF image export



A. Whole well TIFF image of myotube formation in HUVEC cells stained with calcein-AM. Plates were scanned on an acumen[®]eX3 using 1µm x 8µm resolution (8 minutes per plate read time). The acumen[®]eX3 can generate open-source TIFF files compatible with third party image analysis packages with no detriment to plate scan times.
B. Processed image from inset A showing classified tubes using image analysis software.

5. whole organism assays

Recent advances in experimental approach have resulted in some research groups shifting from using cells to the whole organisms for biological assay models.



GFP-expressing *C. elegans* were treated with an siRNA library. Treated adult worms were then incubated in wells of a 96 well plate to allow production of progeny. acumen software distinguishes between adult and progeny worms based on size and intensity. The effect of the siRNA on reproduction rate can be determined by ratio of number of progeny to adults.



B. Images of whole organisms, Zebrafish and *Drosophila* larva.

conclusion

acumen[®]eX3 is the perfect screening tool for rapid multiplexed cell based imaging. It can be used for a broad range of applications in oncology research and drug discovery including cell cycle and mitotic index analysis, protein kinase activity and whole organism scanning..

acumen's large depth of field and rapid whole well scanning capability (8 minutes per plate) opens up possibilities for the establishment of whole organism models in HCS, providing increased knowledge about tumour development and physiology, potential drug interactions and toxicity at all stages of clinical analysis and drug discovery.

acumen's ability to simultaneously detect multiple fluorescently labelled targets, allows large amounts of information to be obtained quickly in a single assay.