High Throughput Imaging of Cellular Models using an Acumen eX3

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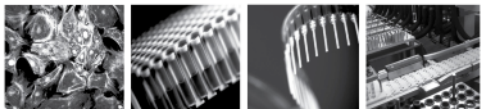


Introduction

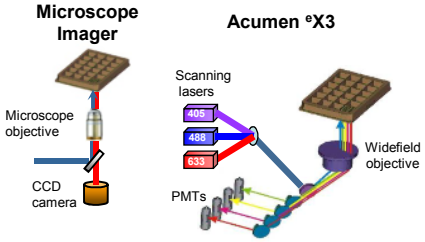
Microscope-based, high-content instruments are used for many cell based assays. However, these instruments require a trade-off to be made between using high optical resolution over a small area, and the robust data sets gained from viewing larger areas of the well but at the price of using low power objectives. Most assays require the use of higher resolutions which entail lengthy read times, even without using multiple colours. Wherever possible, users are constrained to analyse only a small percentage of the total number of cells in a well to keep plate read times at a minimum.

The Acumen eX3 is the fastest imaging system available, collecting and simultaneously analysing over 40 images/second, covering the entire well, without the trade off of having to use lower resolution. Acumen is well established for cell-based high-content screening, but researchers have recently applied its large field of view to rapidly analyse complex cellular or animal models, such as angiogenic tube formation, *C. elegans* or drosophila larvae.

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. This new screening paradigm represents a major breakthrough in how microplate cytometers can be applied to complex cellular models since rapid cytometric analysis can now be combined with image-processing methodology.

Here we demonstrate flexibility of the Acumen eX3 high throughput imaging platform in both cellular and whole organism assays.

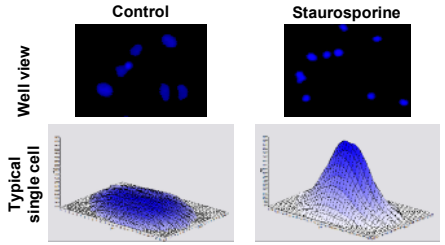
1. Comparison of High Content Instrumentation Optics



The Acumen eX3 can sequentially scan with up to 3 lasers providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Thus reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

2. Cytometric Analysis

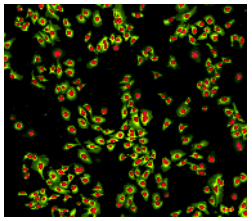
During apoptosis, chromatin condenses and is subsequently fragmented and packaged into apoptotic bodies. When cells undergo nuclear condensation, their nuclear staining becomes smaller and brighter. The Acumen eX3 has built-in cytometric analysis functionality allowing the simultaneous scanning and quantification of chromatin condensation using the mean half-width intensity characteristic.



Nuclear Condensation. HeLa cells stained with Hoechst 34580. Images show the effect of nuclear condensation on the partial well view and 3-D fluorescence profile for typical cells.

3. Cellular Assays

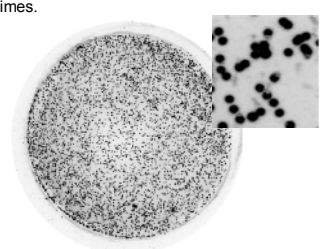
The Acumen eX3 allows rapid scanning and analysis of high content cellular assays, with typical screening scan times from 6 minutes per plate



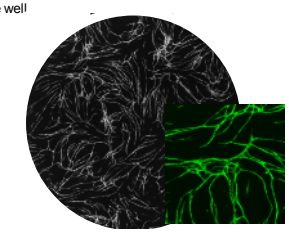
Fluorescein-stained HeLa cells with propidium iodide co-staining for DNA

4. TIFF Image Export

The Acumen eX3 can generate open-source TIFF files compatible with third party image analysis packages with no detriment to plate scan times.



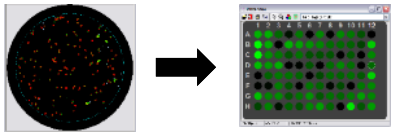
Single whole well TIFF image of THP-1 cells treated with a fluorescent whole cell stain scanned on an Acumen eX3. Plate type, 96 well; Scan resolution, 1µm x 1µm. Inset shows a 20x objective equivalent image enlarged from within the well



Angiogenesis. Whole well TIFF image of myotube formation in HUVEC cells stained with calcein-AM. Plate scanned on an Acumen eX3 using 1µm x 4µm resolution.

5. Whole Organism Assays

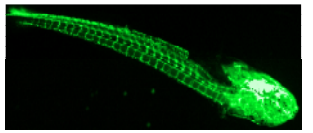
Recent advances in experimental approach have resulted in some research groups shifting from the cell to the whole organism for biological assay models.



Adults: Yellow
Progeny: Red

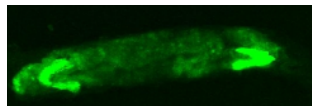
Ratio number of Progeny to Adults

GFP-expressing *C. elegans* were treated with an siRNA library. Treated adult worms were then incubated in wells of 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.



Zebrafish

Drosophila larva



Conclusion

- Simultaneous data capture and analysis in 6 minutes per plate
- Can generate open-source TIFF files for 3rd party software analysis
- Whole well scanning
- Amenable to both cell based and whole organism screening

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