TTP LABTECH

Multiple-laser microplate cytometers: adding a third dimension to high-content screening

The Acumen® eX3 microplate cytometer is the next generation of the Acumen Explorer® high-content screening system. It is equipped with multiple lasers, offering a wavelength range for excitation that greatly increases the variety of fluorescent reagents compatible with the system. The Acumen eX3 simplifies assay transfer by combining the powerful object recognition of charge-coupled device (CCD) imagers with the fast reads of bulk fluorescence readers.

Microplate cytometry is widely applied in primary screening owing to its unique ability to perform high-content cell-based assays requiring subcellular resolution at high throughput. The Acumen eX3 microplate cytometer uses scanning lasers to excite fluorescent objects on the bottom of microplates[1] (**Fig. 1**). The instrument offers a wavelength range for excitation that is similar to that of white-light-source instrumentation by using up to three lasers at 405, 488 and 633 nm. Four photomultiplier tube (PMT) detectors simultaneously monitor four colors per laser, giving a maximum of 12 channels of data for true multiplexing.

Data acquisition and analysis

Acumen eX3 generates high-content information from the fluorescence detected by each PMT, which is displayed as three-dimensional intensity profiles of objects (**Fig. 2**). This does not require image generation and analysis, and thus gives very fast results. The three-dimensional intensity profiles allow the calculation of various morphological and fluorescence parameters. Cell subpopulations can be defined using parameters such as width, depth and area. Biological responses from cells are detected using a range of fluorescence-intensity measurements for up to four colors, or by ratiometric analysis using any of these parameters. The resulting data are reported in CSV or FCS file formats, giving reduced file sizes (up to 99% reduction or around 50 kb per plate) that alleviate issues of data-server implementation and maintenance. This approach to data extraction lends itself to use within a high-throughput screening environment[2].

Figure 1 | The Acumen eX3 microplate cytometer from TTP LabTech.

Whole-well analysis

Acumen eX3's optics allow it to analyze every cell in every well on a microplate at high throughput. This has many advantages over restricted reporting, which results from only capturing a few images from a small well area. First, whole-well analysis reports high-content data for every cell, thus generating statistically robust data from a truly representative cell population. This is key for rare-event-detection assays such as mitotic index and stem-cell differentiation. Second, whole-well analysis can overcome problems of variable stimulation and random cell distribution often observed in screening plates. Third, it allows normalization of biological responses to total cell number, offering a simple toxicity or proliferation readout with every test. Finally, whole-well analysis removes the need for image stitching steps before image analysis on large objects such as *Caenorhabditis elegans* or cell colonies.

Image-export capability

Where throughput is not an issue, microscope-based CCD imagers have predominated within the high-content field because of the breadth of biological assays that can be addressed by image analysis techniques. The Acumen eX3 offers this same breadth, as its enhanced software gives the flexibility of exporting TIFF images for subsequent batch analysis by third-party image analysis software (**Fig. 3**). Notably, images generated

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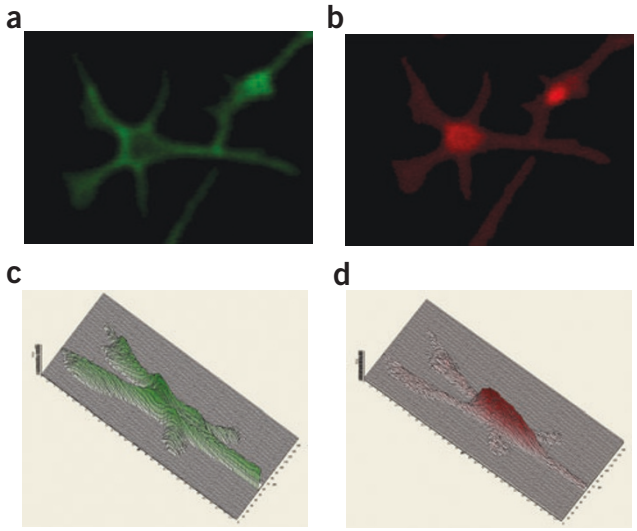

Figure 2 | Multicolor cytometric analysis using an Acumen ^{e}X3. (**a**–**d**) Cytoplasmic staining for tubulin using a fluorescein isothiocyanate (FITC) conjugate (**a**,**c**) and nuclear counterstaining using propidium iodide (**b**,**d**). Well views (**a**,**b**). Three-dimensional fluorescence intensity profiles (**c**,**d**).

by the Acumen ^{e}X3 closely match those captured using a 20× microscope objective (**Fig. 3**). The whole-well imaging capability increases the utility of the Acumen ^{e}X3 in areas in which cytometric analysis is inappropriate, for example, cell segmentation within monolayers.

Multiplexing

Developments in fluorescence reagents and assay methodologies have markedly increased the opportunity to multiplex within biochemical screens. Acumen ^{e}X3's multi-laser excitation and ability to acquire up to 12 channels of fluorescence data per scan complement these innovations well. As nuclear staining is not required to locate the cells, all probes may be used for reporting biological responses. The upshot is that the Acumen ^{e}X3's possible performance exceeds the limits of current multicolor, multiplexed assay protocols, giving an assurance of future-proofing.

High-content screening

Given its accepted benefits, researchers are now seeking to expand the use of high-content analysis into high-throughput screening. Unfortunately, the marked increase in compound throughput required to achieve this goal does not merely involve replication of existing experimental workflows and instrumentation. The Acumen ^{e}X3's ability to scan entire wells offers improved data quality, plus its high-speed laser scanning and data capture allow throughputs of >300,000 wells per day without creating data-storage issues. Because the Acumen ^{e}X3 can include a 405 nm laser, it permits running of β-lactamase reporter gene assays for G protein–coupled receptor screening.

Oncology research

Oncology research has been quick to adopt high-content analysis because of the direct relevance of several applications. Acumen ^{e}X3

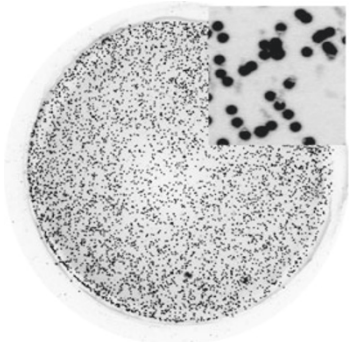

Figure 3 | Whole-well TIFF image of THP-1 cells treated with a fluorescent whole-cell stain. Scan resolution was 1 × 1 μm. Inset shows a 20× objective equivalent image from within the well.

offers unparalleled throughput for cell-cycle screening over traditional flow-cytometry methods. Standard protocols are capable of reading an entire 384-well microplate in less than 10 min, and this includes multiplexing the assay with mitotic-index determination. Acumen ^{e}X3 can analyze adherent cells *in situ*, unlike flow cytometry, which requires cell suspensions for processing.

Acumen ^{e}X3 provides a high-content approach to identifying kinase modulators using either phospho-specific antibodies, which recognize only the active form, or antibodies against proteins to determine kinase translocation from the cytoplasm into the nucleus (indicating activation). Alternatively, GFP-tagged proteins can be monitored to provide simplified and more easily automated protocols for screening.

Cell colony–formation assays are extensively used to assess the functional integrity of cells after *in vitro* manipulations. Colony enumeration traditionally involves laborious and subjective manual counting using a microscope. Acumen ^{e}X3 can visualize whole wells, identifying and enumerating colonies of any size or shape.

Summary

Microplate cytometry combines the power of rapid, whole-well data collection with the ability to classify objects within cells or subcellular events. By offering a comparable range of dyes to that of white-light-source instrumentation, the Acumen ^{e}X3 represents a major breakthrough for microplate cytometers in the assay-development-to-screening process.

Additional information about the Acumen microplate cytometer product family and its high-content screening performance is available on the TTP LabTech website (http://www.ttplabtech.com).

1. Bowen, W.P. & Wylie, P.G. Application of laser-scanning fluorescence microplate cytometry in high content screening. *Assay Drug Dev. Technol.* **4**, 209–221 (2006).
2. Auld, D.S. *et al.* Fluorescence protein-based cellular assays analyzed by laser scanning microplate cytometry in 1536-well format. *Meth. Enzymol.* (in the press).