

High Throughput Automated High Content Screening using a BioCube™ System and Microplate Cytometry

Wayne Bowen¹, Jason Quarles² and Michael Catalano²

¹TTP LabTech Ltd, Melbourn Science Park, Royston, Hertfordshire, UK

²Proteodyne Corporation, 1000 Day Hill Road, Windsor, Connecticut, USA



Abstract

Many high content analysis (HCA) platforms offer limited capability for primary screening due to their low throughput, large data files and incompatibility with high-density microplates, making it impractical to deploy such systems for high-throughput screening. Laser-scanning microplate cytometers, such as the Acumen Explorer, offer rapid read and analysis times (typically <10 minutes). In addition, 96, 384 and 1,536 well microplates can be analysed at equivalent plate read times since scanning is performed on an area and not well basis.

For example, in measuring protein kinase activation (a common high content assay), the multi-step nature of the protocol is complex and requires much coordinated labour to perform. Here, we model the integration of the Proteodyne BioCube System and the Acumen Explorer for practical high-throughput, high content screening of protein kinases. Integration of cell culture, compound addition, immunodetection and analysis results in capacities up to 40,000 wells/day, with superior process control.

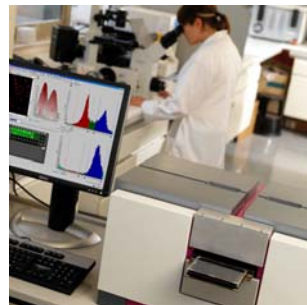
Introduction

The scaling up of cell-based assays used for target identification and validation for primary screening campaigns is not a trivial exercise. Key factors include the culture of large numbers of cells, their plating into microplates, and the complexity of the assay protocol being applied. So-called high content assays are amongst the most demanding since they are involve multiplexing of fluorescent dyes, proteins or probes.

HCA is predominantly performed using CCD Imagers, but for high content screening (HCS) they may be excessively complex, offer limited throughput and create data storage issues. Laser-scanning microplate cytometers, such as the Acumen Explorer, combine the object-recognition capabilities of CCD Imagers with the fast read speeds of bulk fluorescence readers (typically <10 minutes). In addition, 96, 384 and 1,536 well microplates can be analysed at equivalent plate read times since scanning is performed on an area and not well basis.

Protein kinases play a major regulatory role in many cellular pathways, such as those leading to proliferation, differentiation and apoptosis. Consequently, they are an important target for drug discovery research, especially oncology. Measurement of protein kinase activation in cells can be achieved using specific antibodies requiring the application of a multi-step staining protocol. Here, we model the integration of the Proteodyne BioCube System and an Acumen Explorer for practical high-throughput, high content screening of protein kinases. Integration of cell culture, compound addition, immunodetection and analysis results in capacities up to 40,000 wells/day, with superior process control.

1 High Content Screening Using an Acumen Explorer Fluorescence Microplate Cytometer



Key Features

- Fast plate read times (4 – 10 minutes)
- Scans 96, 384 and 1,536 plates in same time
- Small file sizes; down to kb in screening mode
- Multiplexing – up to 4 colours in a single read
- Whole well analysis

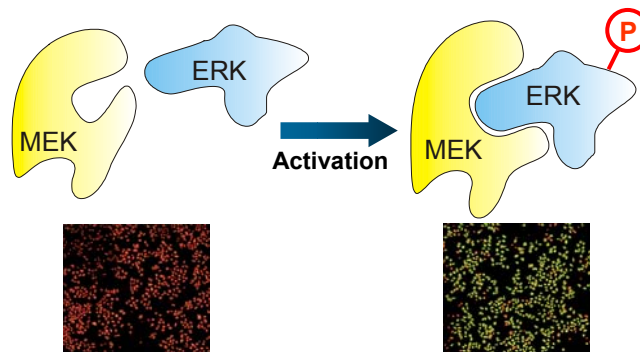
Acumen Explorer is a laser-scanning fluorescence microplate cytometer that offers high content screening at throughputs compatible with primary and secondary screening campaigns. The Acumen Explorer's precision optics deliver unparalleled whole well scanning performance for the execution of multiplexed assays in drug discovery.

Whole well scanning

When screening, intrawell effects such as patchy stimulation or variable cell growth can markedly affect the stability of assay responses. Acumen Explorer can scan 100% of each well at speeds compatible with primary screening thanks to its specialised scan lens with a field of view of 400mm² (20 x 20mm). This equates to the simultaneous scanning of 4, 16 or 64 wells for 96, 384 or 1536 plates, respectively. This performance cannot be achieved on CCD-based imaging systems as these have much smaller fields of view - typically <1mm² for a x10 objective.

Key biological applications include determination of protein kinase activation, cell cycle analysis, and - following the introduction of Acumen Explorer 405 - beta-lactamase reporter gene analysis.

3 High Content Analysis of Protein Kinase Activation Using Anti-Phosphokinase Antibodies



Activation of ERK through phosphorylation by its upstream activator MEK in CHO cells. FCS-activated ERK was detected using phospho-p44/42 MAP kinase antibody (CST #9101) and goat anti-rabbit FITC antibody (Jackson; 111-096-045). Cells were counter-stained with propidium iodide. Well Views from the Acumen Explorer software show control well (left) and FCS-treated well (right). The ability of Acumen Explorer to analyse the entire well permits normalisation of responses to the total cell number, and compensate for variable cell growth or kinase stimulation within the well.

2 Automation of High Content Screening on a BioCube™ System



Key Features

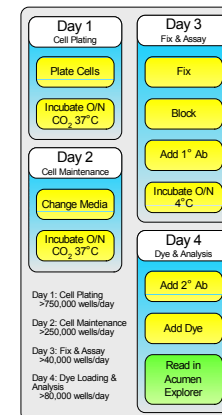
- Highly integrated, advanced process control & compact package
- Fully enclosed w/ class 100 HEPA filtering for sterility
- High walk-away capacity (>60,000 wells)
- Sophisticated real-time hierarchical data tracking, data driving and LIMS integration with LILY SQL-based data management
- Interchangeable SmartTools and integrated peripherals for flexibility

The BioCube System is an advanced laboratory automation platform that brings industrial automation practices to the research laboratory. The BioCube System is based around an industrial-design 4-axis cartesian robot with modular, interchangeable SmartTools and accessories, and a flexible database-driven software architecture. This allows the system to be optimally configured for a variety of applications in addition to high content screening.

Advanced Process Control

The BioCube System is designed to deliver superior process control. The enclosed system and positive ID barcoding system catalogs and protects the integrity of all samples and materials in the system. The sophisticated LILY data management software tracks all operations and samples along with their relationships as they are processed through the system. Key assay data can be supplied, and resultant information can be queried and retrieved, through files or a direct LIMS connection. This enables output data from the Acumen Explorer to be fed-back and utilized in a closed loop fashion for secondary sample hit picking or in-line real-time quality control.

4 Automated Protein Kinase Screening using a BioCube System and an Acumen Explorer



Estimated Performance (per 24 hours)

Combined: >40,000 samples / day

High Content Screening Configurations

Highly Integrated Solution:

Performs all steps of 4-day assay on-line except for day 3 overnight 4°C incubator and Acumen Explorer analysis.

- BioCube System SX 1400 w/16 deck positions
- Gripper and 200 µL and 20 µL SmartTool Pipettors
- Ambient Carousel and 37°C CO₂ Incubated Carousel
- 5 Heated and 1 Chilled Deck Positions
- Integrated Biotek ELx405HT Plate Washer

Fully Integrated Solution (above items plus the following): Performs all steps of 4-day assay on-line.

- BioCube System EX300 (6-Axis Robot)
- 4°C Chilled Carousel
- Directly Integrated Acumen Explorer