

An Ultra-High Throughput Approach to High Content Screening in 1536-Well Format

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Abstract

Ultra-high throughput screening (uHTS) using Kalypsys' technology has enabled unprecedented levels of efficiency and economy in primary screening, and has proved especially useful at rapidly profiling screening hits in selectivity and safety assays. Combining throughput of over 1 million assay wells per day with on-line storage capacity of over 2 million compounds, the Kalypsys® Integrated Screening System provides unmatched screening efficiency. To effectively utilize whole cell assay formats in primary and secondary screening, image-based high content readers will be required to have multi-channel capability, rapid read times and compatibility with high-density plate formats. Laser-scanning fluorescence microplate cytometers, such as the Acumen Explorer, offer whole-well, high content analysis of 1,536-well microplates in less than 10 minutes per plate, while collecting data for up to four colours in multiplex protocols. The Acumen Explorer thus combines the object-recognition capabilities of image-based systems with read speeds similar to that of bulk readers. Here, we demonstrate the powerful integration of an Acumen Explorer with the Kalypsys® Integrated Screening System, with capability to screen > 300,000 wells per day of high content data.

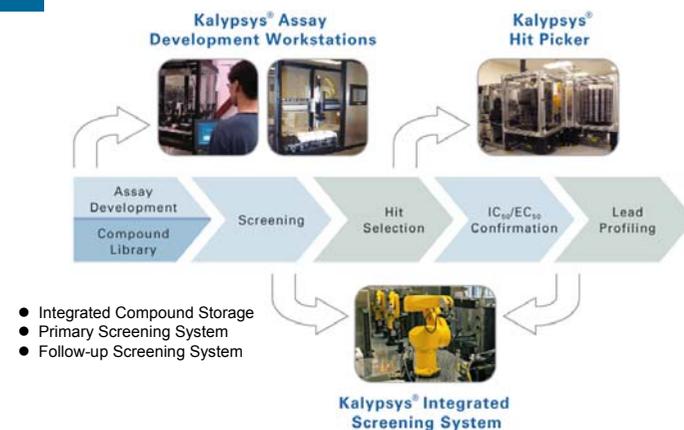
Introduction

To remain competitive, pharmaceutical organizations must continually seek and apply the most beneficial new technology and screening paradigms. The development of ultra-high throughput screening platforms capable of profiling entire compound inventories in a single day has enabled unparalleled primary screening capability. In addition, flexible uHTS technology has permitted rapid profiling of screening hits in selectivity and safety assays.

The emerging area of High Content Analysis (HCA) permits derivation of multi-parametric information from cells using microscopic analysis. Most high content applications to date have been run using CCD imagers for small numbers of compounds. Laboratories, however, are now beginning to realize that HCA instrumentation used for assay development to understand the underlying biology, does not necessarily meet the very different requirements of High Content Screening (HCS). Laser-scanning fluorescence microplate cytometers, such as the Acumen Explorer, offer whole-well, high content analysis of 1,536-well microplates in less than 10 minutes per plate, while collecting data for up to four colours in multiplex protocols. The absence of an image processing stage means that in screening mode, the software reports only population statistics, reducing file sizes down to 50 KB and alleviates informatics-related issues associated with CCD Imagers.

Here, we show the integration of a single Acumen Explorer with the Kalypsys® Integrated Screening System, with capability to generate > 300,000 wells per day of high content data.

1 Ultra-High Throughput Technology



- Integrated Compound Storage
- Primary Screening System
- Follow-up Screening System

Now in its third generation, the Kalypsys® Integrated Screening System has a throughput of over 1 million assay wells per day, and an on-line storage capacity of over 2.2 million compounds. An ultra-reliable, highly miniaturized 1536-well format enables multiple screens per drug target including multi-day cellular assays, making the system ideal for high content screening.

3 Combination of Kalypsys® Integrated Screening System and Acumen Explorer



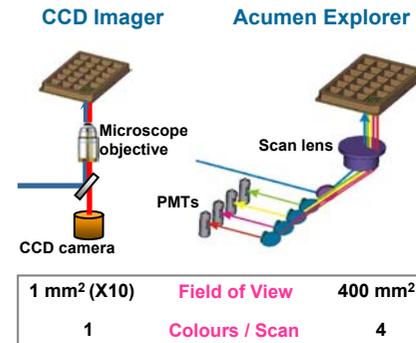
Acumen Explorer in a Kalypsys® Integrated Screening System

2 High Content Screening Using an Acumen Explorer Fluorescence Microplate Cytometer

Traditional methods for High Content Analysis (HCA) use technologies such as flow cytometry and microscope-based imaging systems. Laser-scanning microplate cytometry has many advantages over these, and is more amenable for use in High Content Screening (HCS). The advantages include:

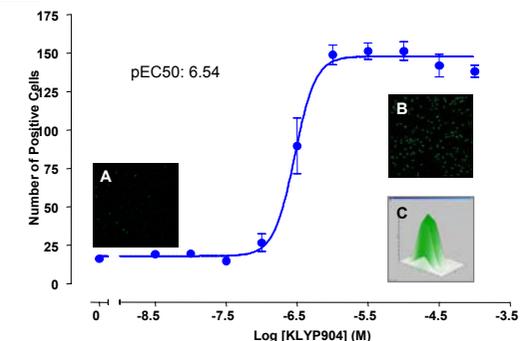
- Fast plate read times (4 – 10 minutes)
- Scans 96, 384 and 1,536 plates in same time
- Small file sizes; down to kb in screening mode
- Multiplexing – up to 4 colours in a single read
- Whole well analysis for improved data quality and normalisation of intra-well assay artefacts.

The Acumen Explorer is widely applied within the pharmaceutical industry. 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.



Using a scan lens rather than a microscope objective permits rapid whole well analysis, and the PMT detection system collects up to 4 colours simultaneously.

4 High Content Readout in 1,536 Well Format – Mitotic Index



Mitotic Index was determined in human lung carcinoma cells following treatment with a proprietary compound, KLYP904. Cells were fixed, immunostained for a mitotic index marker, and scanned on an Acumen Explorer. Robust stimulation was observed with KLYP904 (pEC50 – 6.54). Well Views from Explorer software: A, Control; B, 30 µM KLYP904; C, 3D fluorescence intensity profile of a positive nucleus.