Applications of Laser Scanning Microplate Cytometry in High Content Screening

Paul Wylie, Christopher Lupton, Tristan Cope and Wayne Bowen
TTP LabTech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, UK.

1 Abstract

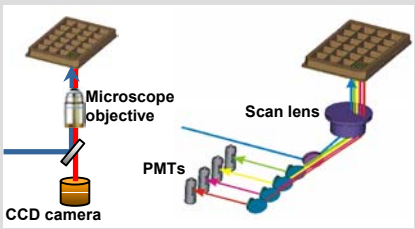
Traditional methods for High Content Analysis (HCA) use technologies such as flow cytometry and microscope-based imaging systems. Laser-scanning microplate cytometry has many advantages over these, and is more amenable for use in High Content Screening (HCS). The advantages include:

- analysing the whole well area of a multiwell plate.
- analysis of live cell assays in both adherent and non-adherent cell lines.
- rapid read and analysis times of plates.
- smaller file sizes by virtue of thresholding algorithms.

Here we demonstrate the utility of fluorescence based microplate cytometry and the advantages that can be gained using the Acumen Explorer in HCS.

2 Optical Comparison with CCD Imager

CCD Imager Acumen Explorer

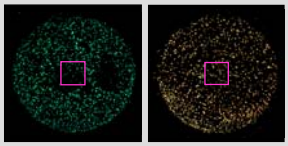


1 mm² (X10)	Field of View	400 mm²
1	Colours / Scan	4

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

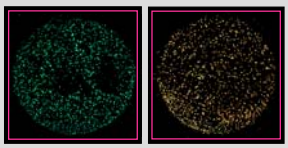
3 Acumen Explorer vs CCD Imager

Active cells All cells



CCD Imager

1 mm² image:
29 active cells
76 total cells
= 38% activation

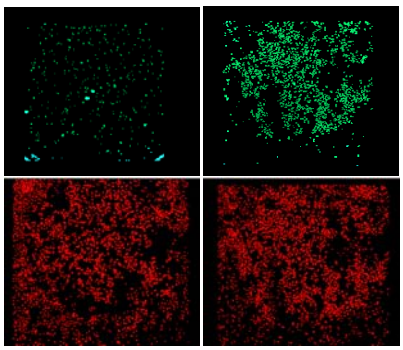


Acumen Explorer

16 mm² scan:
1,995 active cells
2,217 total cells
= 89% activation

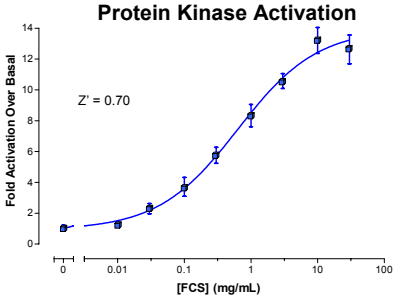
Whole well analysis permits improved normalisation to total cell number, overcoming problems of variable stimulation and random cell distribution.

4 Application in Cellular Imaging: Protein Kinase Activation



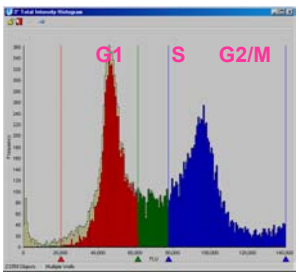
ERK activation: View of control well (left) and treated well (right). Upper panel FITC only showing active cells; lower panel PI showing total cell number.

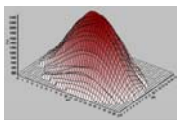
Protein Kinase Activation



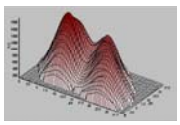
- **CCD Imager**
  - Throughput – 2 hours / plate
  - Partial well imaging
  - File size / plate – 1.7 Gb
- **Acumen Explorer**
  - Throughput – 9 min / plate
  - Whole well scanning
  - File size / plate – 44 Kb
  - 96 & 384 well plate validated

5 Application in Flow Cytometry: Cell Cycle Analysis



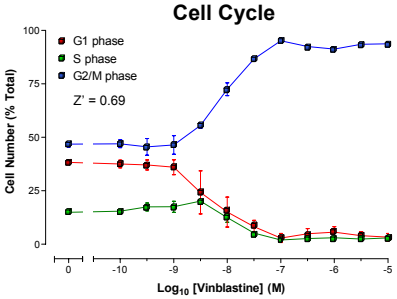


G1 phase nucleus



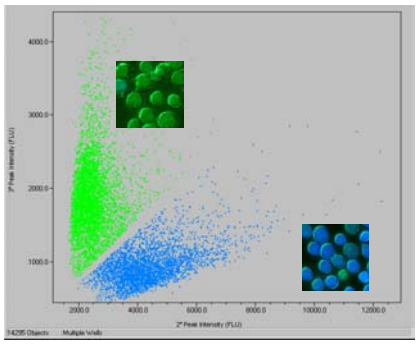
G2/M phase nucleus

Cell Cycle



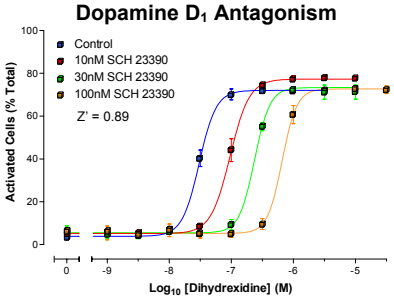
- Scan time: 13 min per plate
- Can be run on adherent or non-adherent cells
- Compatible for multiplexing with morphological readouts

6 Application in Bulk Fluorescence: GeneBLAzer®: β-Lactamase technology



Ratiometric separation of negative and positive cells. High content readout with normalisation on per cell basis

Dopamine D_1 Antagonism



- Typical detection of 30-50-fold activation
- Z' > 0.8 with Acumen Explorer 405
- Throughput – 6 min/plate
- Significant reduction in cell culture: 100-fold less cells / well

Summary

- **Fast plate read times (3 – 13 minutes)**
- **Scans 96, 384 and 1536 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**
- **Enables true High Content Screening**

TTP LABTECH

TTP LabTech Ltd., Melbourn Science Park, Melbourn, Hertfordshire, SG8 6EE, UK. Phone +44 1763 262626

Visit us at www.ttplabtech.com/explorer