High Throughput High Content Screening using a Multiwavelength Microplate Cytometer

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Abstract

Fluorescence microplate cytometers are designed for a screening environment as they are capable of analysing up to 300,000 high content data points in 24 hours (1536 well format). They deliver the object recognition capabilities of a microscope-based CCD imager with the fast data collection rates and small data file sizes associated with bulk fluorescence readers. Statistical quality of screening data is enhanced by whole well scanning capability; whilst assays requiring larger fields of view than those offered by microscope objectives may be transferred into a higher throughput, automated format.

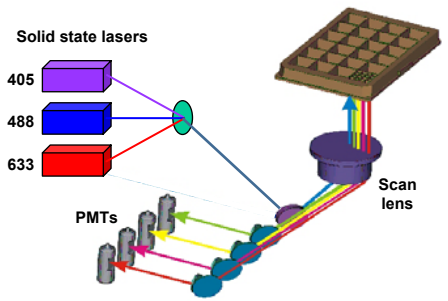
The Acumen eX3 (TTP LabTech Ltd, Melbourn, UK), a multiwavelength microplate cytometer, was launched recently, offering 405, 488 and 633 nm laser excitations in one instrument. This advancement significantly extends the range of fluorescent reagents that can be combined in multicolour, multiplexed assays over a wavelength range for excitation that is similar to that of white light source instrumentation. Thus, it permits seamless transfer of high content assay protocols, developed using CCD-based imaging devices, onto the Acumen eX3 for screening.

Microplate cytometers use scanning lasers to excite fluorescent objects on the bottom of microplates. High content information is derived from the resultant fluorescence emissions without an intermediate imaging processing step. In an Acumen eX3, four photomultiplier tube (PMT) detectors simultaneously monitor four colours per laser, giving a maximum of 12 channels of data for true multiplexing. Multiwavelength microplate cytometers such as the Acumen eX3 represent a new tool for drug discovery research by enabling a broader range of applications and increased assay flexibility.

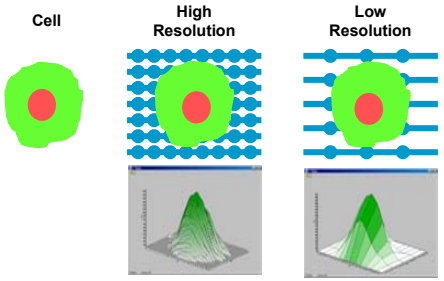
Conclusion

- Microplate cytometry is ideally suited for high-throughput, high-content screening
- Throughputs of > 300,000 wells per day can be achieved without data storage issues
- Multiple laser excitation enhances the range of compatible fluorescent reagents for increased multiplexing
- Applications include cell cycle analysis, multiplex immunoassay and β-lactamase reporter gene analysis

1 Optical Configuration in a Microplate Cytometer



2 Laser Scanning Cytometry



Scanning resolution can be defined in both X and Y directions. Construction of 3D fluorescence intensity profiles permits the calculation of fluorescence and morphological parameters for each object identified.

3 Throughput and Data Storage

	96	384	1536
Plate Read Time (whole well)	9.15	10.24	10.26
Plate Read Time (HTS)	4.13	4.8	6.67
Plates per 24h	350	300	216
Wells per 24h	34,000	115,000	330,000
Total Data for 24h operation	17.5 Mb	15 Mb	11 Mb

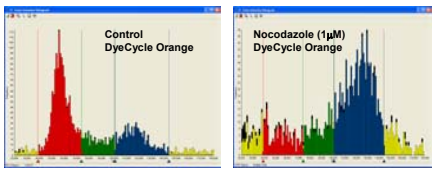
Data for scanning resolution of 1μm x 8μm using a single laser Acumen eX3. Times in minutes.

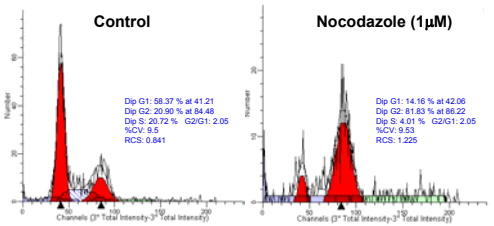
4 Table of Common Excitable Fluorescent Reagents

405 nm	488 nm	633 nm
Hoechst	Propidium Iodide	DRAQ5
DyeCycle™ Violet	Calcein-AM	VITA Blue
Alexa 405	Alexa 488	Alexa 633
Quantum Dots	FITC	Allophycocyanin
FuraRedHI	Phycoerythrin	TP-PRO1
Pacific Blue	eGFP	Cy5
AmCyan	DsRed	HcRed1

Acumen eX3's multi-laser excitation and ability to acquire up to 12 channels of fluorescent data per scan enables use of a broad range of fluorescent dyes, probes and proteins for enhanced multiplexing within assays. Since nuclear staining is not required to locate the cells, all probes may be used for reporting biological responses. By offering a comparable range of dyes to that of white light source instrumentation, an Acumen eX3 simplifies transfer of assays from microscope-based CCD Imagers onto the instrument for primary screening.

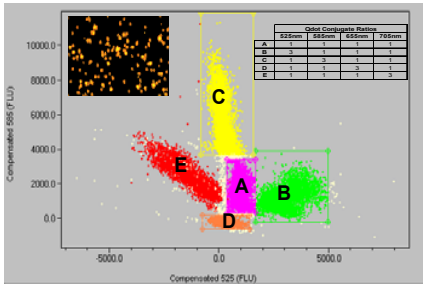
5 Cell Cycle Analysis in HeLa Cells using Microplate Cytometry





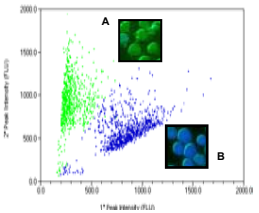
Day-old HeLa cells (2,000 per well) were treated with nocodazole for 22 hours. Cultures were labelled *in situ* with Vybrant® DyeCycle™ Orange (5 μM for 30 min at RT). Analysis was performed on an Acumen eX3 microplate cytometer using 488 nm excitation. Data was exported in FCS 3.0 format and analyzed using ModFit 3.1 LT SP3 (Verity House Software). Multiplex protocols are available for simultaneous mitotic index (anti-pH3) and cell cycle determination.

6 Multiplex Quantum Dot Analysis

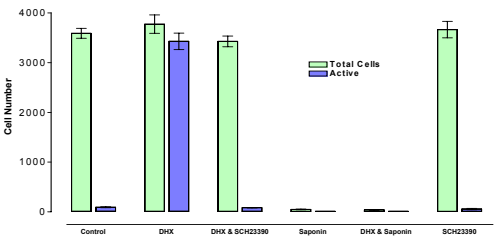


Samples of human monocytes were incubated with biotinylated anti-CD45 antibody and then labelled the 5 different ratios of Streptavidin Qdot® Bioconjugates as described in the embedded table. When the fluorescence emissions from each Qdot conjugate were determined simultaneously using an Acumen eX3 , all 5 cell populations could be discriminated. This demonstrates the combination of QDot-labelling with an Acumen eX3 microplate cytometer for multiplex immunodetection experiments.

7 GPCR Screening: β-Lactamase Reporter Gene Analysis



Plot showing Inactive (A, green) and Active (B, blue) cells expressing β-lactamase reporter gene. Data were generated using 405 nm laser excitation and CCF4-AM substrate. Note the heterogeneity in green and blue fluorescence for each population. The reporting of ratiometric fluorescence data on a per cell basis dramatically reduces cell requirements from those used in bulk fluorescence assays without compromising assay performance



Dopamine D1-receptor activity. By simultaneously measuring both β-lactamase reporter gene activity and total cell number in each well, microplate cytometers enable correlation of compound activity with cytotoxicity in a single read. This cannot be achieved using bulk fluorescence readers. DHX, dihydrexidine agonist.

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