

Cell Proliferation and Cell Cycle Analysis using a Multiple Laser Microplate Cytometer

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Abstract

There is an increasing demand for multiplexing in cell based assays to obtain multiple readouts from single samples, thereby reducing labour and consumable costs whilst increasing throughput. Currently the degree of multiplexing can be limited by the available reagents, with the majority of fluorescent probes optimised for excitation at 488 nm. The use of fluorescent probes with spectral profiles that overlap hinders their use in multiplexing assays. Another limitation is that many fluorescence detection systems excite and detect over a narrow wavelength range, making them incompatible with certain probes. Both reagent and instrument developers alike have increased the range of products available which enable the possibility of assessing multiple probes with distinct spectral profiles for enhanced multiplexing.

Laser-scanning fluorescent microplate cytometers, such as the Acumen® eX3 (TTP LabTech Ltd, Melbourn, UK), offer 405, 488 and 633 nm laser excitation in a single instrument. This coupled with the ability to acquire up to four channels of fluorescent data per laser 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. The Acumen eX3 also combines the powerful object-recognition capabilities of CCD imagers with fast read speeds, across entire wells in 96, 384 and 1536-well format at throughputs of up to 300,000 data points in 24 hours.

Fluorescence microplate cytometers are heavily used in oncology research including cell proliferation and cell cycle analysis using the DNA stain propidium iodide (488 nm excitation). Here, we describe the extension of this capability through the use of DNA stains, Hoechst 34580 (405 nm) and TO-PRO®-3 (633 nm) on an Acumen eX3, and discuss the potential for multiplexing in immunodetection assays.

Conclusion

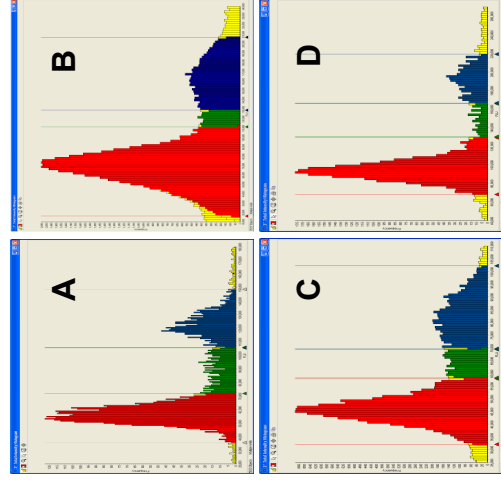
- Good correlation of cell number is observed using nuclear stains excited at 405, 488 and 633 nm
- Hoechst 34580, propidium iodide and TO-PRO-3 can be multiplexed in a single well
- Cell cycle analysis can be assessed in live and fixed cells, with 405, 488 and 633 nm laser excitation using an Acumen eX3
- Microplate cytometry is ideally suited for high-throughput, high-content screening, with throughputs of > 300,000 wells per day can be achieved without data storage issues.

1 Acumen eX3 Microplate Cytometer



The Acumen eX3 laser scanning fluorescence microplate cytometer offers triple laser excitation in a compact bench top unit. This design enables a wide range of high content assays to be performed at high throughput, especially when the instrument is fully integrated. Patented signal thresholding methods enable on-the-fly, cytometric analysis and dramatically reduce file sizes to around 50Kb in HTS screening mode.

4 Cell Cycle Analysis: Comparison of DNA Stains Excited at 405, 488 and 633 nm



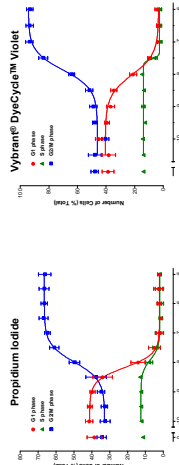
HeLa cells (2,000 per well) were labelled *in situ* with, A, propidium iodide (10 µM); B, Hoechst 34580 (10 µM); C, Vybrant® DyeCycle™ Orange (5 µM); D, TO-PRO-3 (0.5 µM). Analysis was performed on an Acumen eX3 microplate cytometer using 405, 488 and 633 nm excitation.

2 Table of Common Excitable Fluorescent Reagents

405 nm	488 nm	633 nm
Hoechst	Propidium iodide	TO-PRO-3
DyeCycle™ Violet	DyeCycle™ Orange	DRAQ5
Alexa 405	Calcein-AM	VITA Blue
Quantum Dots	Alexa 488	Alexa 633
FuraRedH1	FITC	Allophycocyanin
Pacific Blue	Phycocerythrin	Cy5
AmCyan	eGFP	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.

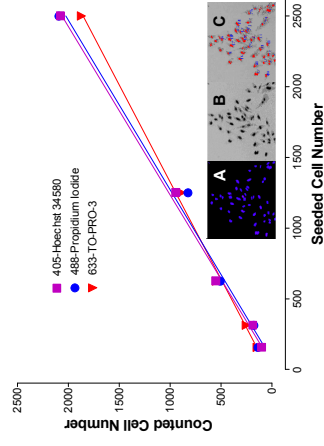
5 Cell Cycle Analysis in Live and Fixed Cells



DNA Stain	G1 Phase (pEC50)	G2/M Phase (pEC50)	n
Propidium iodide	8.19 ± 0.06	8.04 ± 0.06	5
Vybrant® DyeCycle™ Violet	8.15 ± 0.07	8.00 ± 0.05	3

Live cells were stained with Vybrant® DyeCycle™ Violet and analysed on an Acumen eX3 microplate cytometer using 405 nm excitation. Fixed cells were stained with propidium iodide and scanned at 488 nm. The pEC50 values obtained for both the G1 and G2/M phases are comparable across the two nuclear stains used.

3 Multiplexed Cell Quantification using Multiple Laser Excitation



HeLa cells, seeded at different cell number (156-2500 per well) were fixed *in situ* using ethanol, and stained with Hoechst 34580 (10 µM; 405 nm), propidium iodide (1.5 µM; 488 nm) and TO-PRO-3 (0.5 µM; 633 nm). Analysis was performed on an Acumen eX3 microplate cytometer. In this experiment we sought to investigate the ability to determine different cell numbers with multiple dyes excited at 405, 488 and 633 nm in a multiplexed assay. The three DNA stains were added to the same well. The data demonstrates close correlation between each of the three nuclear stains in determining cell number, and secondly the ability to multiplex all three stains in one well. Inset, Image A is a well view image of Hoechst stained cells, B is a TIFF export of the same well section as seen in A, C is the TIFF image with the cells numbered (Image-Pro Plus; Media Cybernetics).

6 Throughput and Data Storage

Plate Read Time (whole well)	96	384	1536
Plate Read Time (HTS)	9.15	10.24	10.26
Plates per 24h	4.13	4.8	6.67
Wells per 24h	350	300	216
Total Data for 24h operation	34,000	115,000	330,000
	17.5 Mb	60 Mb	170 Mb

An Acumen eX3 scans on an area and not well basis, thus scan times are virtually identical for any SBS format microplate. Typically plate cycle times of 10 minutes are achievable but these can be further cut by scanning reduced well areas. Several hundred plates can be scanned per day with minimal requirements for data storage. Data for scanning resolution of 1 µm x 8 µm using a single laser Acumen eX3. Plate read times given in minutes.