



TTP LABTECH

# Multiparameter high-content, high-throughput analysis of the cell cycle: a comparative study using the Acumen <sup>e</sup>X3 and the ArrayScan VTI platforms

Fabio Gasparri<sup>1</sup>, Arturo Galvani<sup>1</sup> and Andrew Goulter<sup>2</sup>

<sup>1</sup>Cell Biology Department, Nerviano Medical Sciences, Milan (Italy) <sup>2</sup>TTP Labtech, Melbourne (UK)



NERVIANO MEDICAL SCIENCES

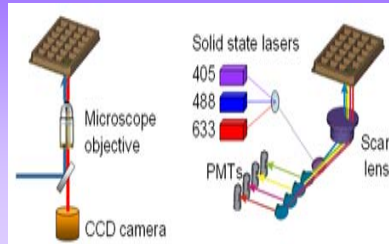
## 1. Introduction

High-content analysis (HCA) defines a series of cell-based approaches usually based on automated fluorescence imaging. When multiplexed, HCA can provide quantitative information on many complex cellular processes, including proliferation and cell cycle progression. Within the field of oncology there is currently great focus on developing compounds that are capable of blocking cell proliferation, ultimately leading to apoptosis through specific inhibition of cell cycle machinery. Historically, screening compound libraries was limited by simple readouts offering little information. While flow cytometry is a good method for cell cycle analysis and offers highly multiplexed data, it is limited to cells in suspension. The majority of cell lines for oncology research are adherent and not amenable to automated screening using flow cytometry. Scientists are looking for new solutions to their screening paradigms, allowing acquisition of more detailed information on larger compound libraries for suspension and adherent cells.

HCA readers can be divided into three categories: flow cytometry, microscope-based imaging systems and laser scanning microplate cytometers. Microscope-based systems can allow high resolution, high quality detailed images useful for identifying sub-cellular structures and in the study of morphological changes. Laser scanning cytometers offer whole well scanning generating data for all cells which improves data robustness, important in assays where there are only a small number of responders and allows normalisation against total cell number. Additionally laser scanning cytometers are capable of scanning up to 300,000 wells of data in a 24 hour period. Importantly both microscope and laser-scanning systems allow *in situ* analysis of adherent cells.

Previously, a four-colour HCA approach was established on the ArrayScan VTI reader (Thermo Fisher Scientific) quantifying the cell number, DNA content, BrdU incorporation, cyclin B1 expression and histone H3 phosphorylation. The aim of this study was to transfer this assay to the Acumen <sup>e</sup>X3 scanner (TTP LabTech) and perform a head-to-head analysis of the phenotypic effects induced in human cancer cell lines treated with cell cycle inhibitors.

## 2. Comparison of optics from a CCD imager and Acumen <sup>e</sup>X3

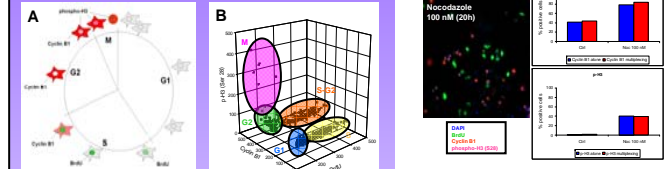


The Acumen <sup>e</sup>X3 can sequentially scan with up to 3 lasers providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Thus reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

## 3. Multiparametric cell cycle high-content analysis

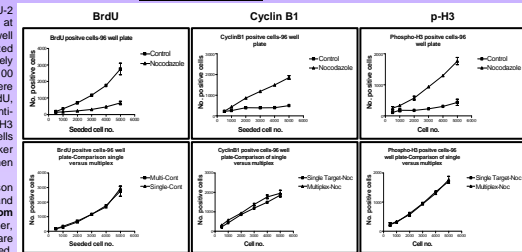
### ArrayScan VTI

The human osteosarcoma cell line U-2 OS was used as an experimental model for cell cycle high-content analysis experiments in 96-well plates. **Figure A:** BrdU incorporation, cyclin B1 expression and histone H3 phosphorylation are markers of specific phases of the cell cycle. **Figure B:** three-parametric ArrayScan analysis reveals distinct cell cycle subpopulations. **Figure C:** representative pictures of U-2 OS cells (untreated or Nocodazole-treated) stained with DAPI (blue), BrdU (green), cyclin B1 (red), and phospho-histone H3 (Ser28) (purple). Histograms show quantification of each biomarker obtained by single staining or in multiplexing with the others. (Scan time for multiplexed data; three hours)



### Acumen <sup>e</sup>X3

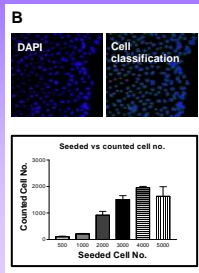
In parallel experiments, U-2 OS cells were seeded at increasing densities in 96-well plates and left untreated (Control) or alternatively treated with Nocodazole 100 nM for 20 hours. Cells were stained with DAPI, anti-BrdU, anti-cyclin B1 and anti-phospho-histone H3 antibodies. Numbers of cells positive for each biomarker were analysed by Acumen <sup>e</sup>X3.



**Upper panels:** comparison between untreated and Nocodazole-treated. **Bottom panels:** for each biomarker, data from single staining are consistent with multiplexed. (Scan time for multiplexed data; 48 minutes, whole well scanning)

## 4. Acumen <sup>e</sup>X3: cell count in 384-well plates

U-2 OS cells seeded in 384-well plates and stained with DAPI were analysed with Acumen <sup>e</sup>X3. **Figure A:** Acumen <sup>e</sup>X3 whole well scanning is well suited where the cells are not evenly distributed. BrdU positive cells can be normalised against total cell number. **Figure B:** agreement between seeded and counted cell numbers (at high cell densities the software is unable to distinguish cells within clumps).

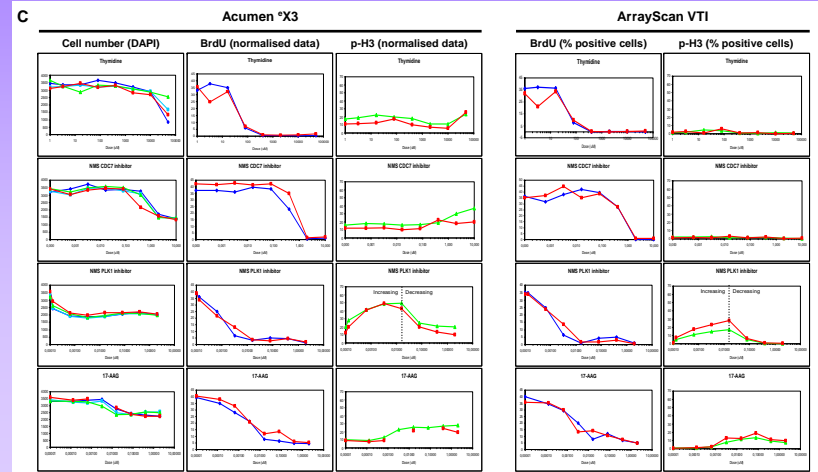
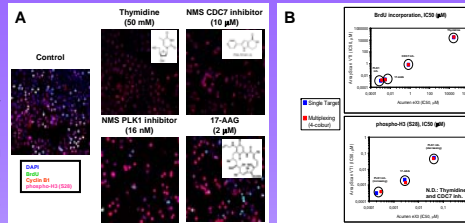


## 6. Multiparametric analysis of selected cell cycle inhibitors: high concordance of ArrayScan VTI Vs. Acumen <sup>e</sup>X3 data

U-2 OS cells seeded in 384-well plates were treated with increasing doses of four selected cell cycle inhibitors:

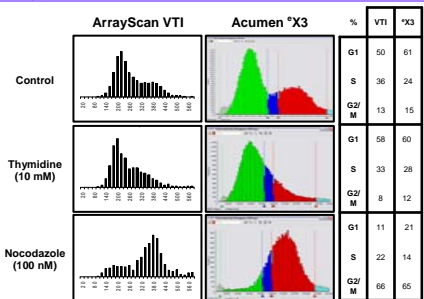
- **Thymidine** (DNA synthesis inhibitor)
- **NMS CDC7 inhibitor** (PHA-767491, Montagnoli et al. Nat Chem Biol. 2008)
- **NMS PLK1 inhibitor** (unpublished)
- **17-AAG** (Hsp90 inhibitor)

**Figure A:** representative pictures (from ArrayScan VTI) of control or treated cells stained with DAPI (blue), BrdU (green), cyclin B1 (red), and phospho-histone H3 (Ser28) (purple). Inserts show compound structures. **Figure B:** Agreement between the IC<sub>50</sub> values (μM) obtained for BrdU and phospho-H3 markers with the Acumen <sup>e</sup>X3 and the ArrayScan VTI in parallel as single marker or in multiplexing with the others (4-colour). **Figure C:** Concentration-response curves of two cell cycle markers (BrdU and phospho-H3) after 20 hour treatment with the selected inhibitors obtained by parallel analysis with Acumen <sup>e</sup>X3 and ArrayScan VTI.



## 5. DNA content distribution: ArrayScan VTI Vs. Acumen <sup>e</sup>X3

U-2 OS cells seeded in 384-well plates were treated with Thymidine or Nocodazole for 20 hours, fixed and stained with DAPI. DNA content histograms were obtained by analysis with Acumen <sup>e</sup>X3 and ArrayScan VTI (% of cell cycle subpopulations from histograms are indicated).



## 7. Conclusions

In this study we established a robust four-colour high-content cell cycle assay for the Acumen <sup>e</sup>X3, capable of detecting changes in cell cycle markers in the presence of cell cycle inhibitors.

In the field of oncology where compounds of interest can have anti-proliferative effects and be potentially cytotoxic it is important to be able to determine total cell number to enable normalisation of a specific response. The Acumen <sup>e</sup>X3 offers whole well scanning allowing normalisation of responses leading to the generation of robust data.

The data sets obtained from the head-to-head analysis between two HCS platforms (Acumen <sup>e</sup>X3 and ArrayScan VTI) revealed that the data is in concordance, both as single markers and in multiplexing. In this case the choice of HCA technology used does not affect the consistency and reliability of data and allows the user to exploit the strengths of each technology.

## 8. References

1. Gasparri F, Ciavolella A, Galvani A. Cell-cycle inhibitor profiling by high-content analysis. *Adv Exp Med Biol.* 2007;604:137-48. Review.
2. Gasparri F, Cappella P, Galvani A. Multiparametric cell cycle analysis by automated microscopy. *J Biomol Screen.* 2006 Sep;11(6):586-98.
3. Montagnoli A, Vatasina B, Croci V, Manichcheri M, Rainoldi S, Marchesi V, Tibolla M, Tenca P, Brotherton D, Albanese C, Patton V, Azzari R, Ciavolella A, Sola F, Molinari A, Volpi D, Avanzi N, Fiorenzini F, Cattori M, Healy S, Ballinari D, Piseni E, Sica A, Moll J, Benismou A, Vanotti E, Santocanale C. A Cdc7 kinase inhibitor restricts initiation of DNA replication and has antitumor activity. *Nat Chem Biol.* 2008 Jun;4(6):357-6.