

Utility of 405nm-excitable dyes in High Content Screening using an Acumen Explorer Microplate Cytometer

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Introduction

Shorter-wavelength amine-reactive fluorophores are infrequently used for preparing bioconjugates because dyes excited with longer wavelengths, and therefore lower energy, are widely available and less likely to cause photodamage to labeled biomolecules. Moreover, many cells and tissues autofluoresce when excited with ultraviolet (UV) light and thus preclude the use of blue-fluorescent conjugates in a number of applications. However, for certain multicolour fluorescence applications, including immunofluorescence, a blue-fluorescent probe provides a contrasting colour that is easily distinguished from the green, yellow, orange or red fluorescence of the longer-wavelength probes.

The Acumen Explorer is a fluorescent microplate cytometer equipped with either a 405nm or 488 nm laser. The instrument offers rapid read and analysis times of individual cells within 96-384 well plates (typically 5-10 minutes), making it compatible with the sustained use of high content methods in primary screens. In addition, small file sizes (>50kB in screening mode) are produced, therefore removing the requirement for expensive data storage solutions in compound screening programs. The system does not require image processing for the output of biologically relevant fluorescence readings on a cellular or sub-cellular level, relieving the need for specialist operators. These features have made the Acumen Explorer an easily integrated and widely used High Content Screening platform.

Within the high content screening industry, the availability of compatible reagents for use in multiplexed assays has been described as limiting. Despite this, the shorter wavelength area of the visible spectrum is underutilized and often limited to the excitation of probes such as Hoechst and DAPI for cell nuclei staining. In this study, we have used an Acumen Explorer equipped with a violet 405nm laser in conjunction with a selection of shorter-wavelength amine-reactive fluorophores (Invitrogen, Molecular Probes) to demonstrate a greater utility for blue fluorescent probes within high content assays.

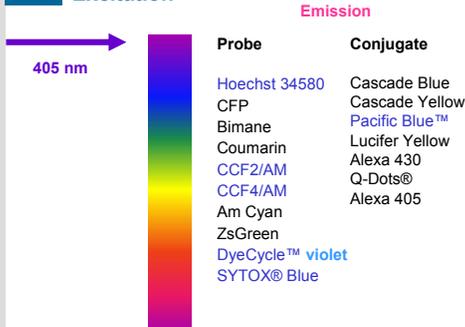
The following commonly multiplexed targets were chosen for analysis: Pacific Blue™ Annexin V conjugate for apoptosis assays, SYTOX® Blue for vitality assays and DyeCycle™ violet for cell cycle analysis.

In addition, a GPCR activation assay widely used in primary screening has been included. The GeneBLazer β-lactamase-based assay is ideally suited for use with the Acumen Explorer 405 nm platform. Ratiometric data are reported from individual cells, increasing assay sensitivity and robustness over bulk fluorescence readouts, plus an indication of toxicity is provided by means of a total cell count/well. The use of high density plates (1536-, 3456-well plates) ensures that scanning speeds of 5-10 minutes per plate are not limiting factors for implementation of this technology.

Conclusions

- Short wavelength fluorophores are compatible with an Acumen Explorer equipped with a 405nm laser, enhancing multiplexing capability in high content screening.
- High content analysis of β-lactamase reporter gene assays reports data on a per-cell basis suitable for antagonist profiling.
- Vybrant® DyeCycle™ violet DNA stain offers comparable cell cycle analysis to propidium iodide without the requirement for RNase treatment.
- SYTOX® Blue gives comparable results to propidium iodide, the standard 488nm dye in the identification of necrotic cells.

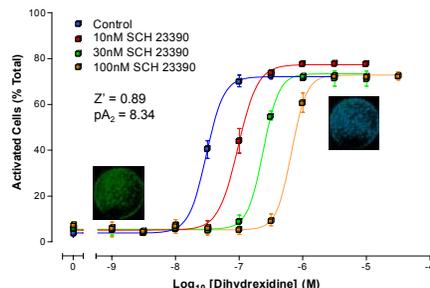
1 Fluorescent Dyes suitable for 405 nm Excitation



The availability of an Acumen Explorer equipped with a 405nm (violet) solid-state laser, creates opportunities for utilising new dyes as shown above and enhances the multiplexing capability for High Content Screening.

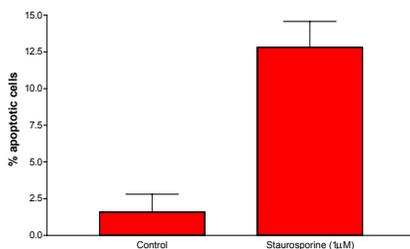
2 Detecting the FRET Response of the GeneBLazer® Technology.

Acumen Explorer equipped with 405nm laser line can simultaneously scan both the blue (β-lactamase expressing) and green spectrum (β-lactamase negative) to discriminate active from inactive cells.



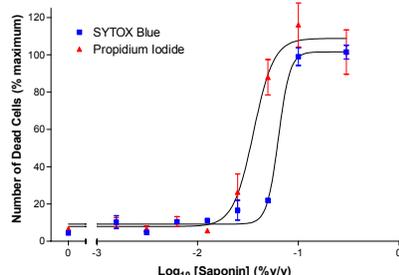
SCH-23390-concentration dependent shift of dihydrodextridine curves (data represent means ± S.D. of 4 replicates and are representative of results obtained from 3 separate experiments). Insets are well views of inactive (green) and active (blue) cells.

3 Pacific Blue™ Annexin V Conjugate Analysis of Apoptosis



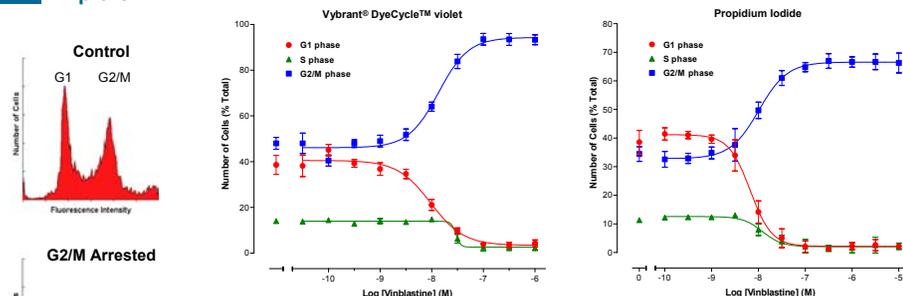
Cells were treated with staurosporine as shown, and apoptotic cells detected with Pacific Blue on an Acumen Explorer using 405 nm laser.

4 Comparison of PI and SYTOX® Blue in Analysing Cell Death



Cells were treated with saponin as shown, and stained with either Sytox® Blue or Propidium Iodide and analysed on an Acumen Explorer using 405nm or 488nm laser excitation respectively.

5 Comparison of PI and Vybrant® DyeCycle™ on Cell Cycle Analysis using an Acumen Explorer



Live cells were stained with Vybrant® DyeCycle™ violet and analysed on an Acumen Explorer using 405nm laser excitation. Fixed cells were stained with Propidium Iodide and scanned on a 488nm Acumen Explorer as a control.

| DNA Stain | G1 Phase (pEC ₅₀) | G2/M Phase (pEC ₅₀) | n |
|---------------------------|-------------------------------|---------------------------------|---|
| Propidium Iodide | 8.19 ± 0.06 | 8.04 ± 0.06 | 5 |
| Vybrant® DyeCycle™ violet | 8.15 ± 0.07 | 8.00 ± 0.05 | 3 |

DNA Histograms of Cells Labelled with Propidium Iodide

