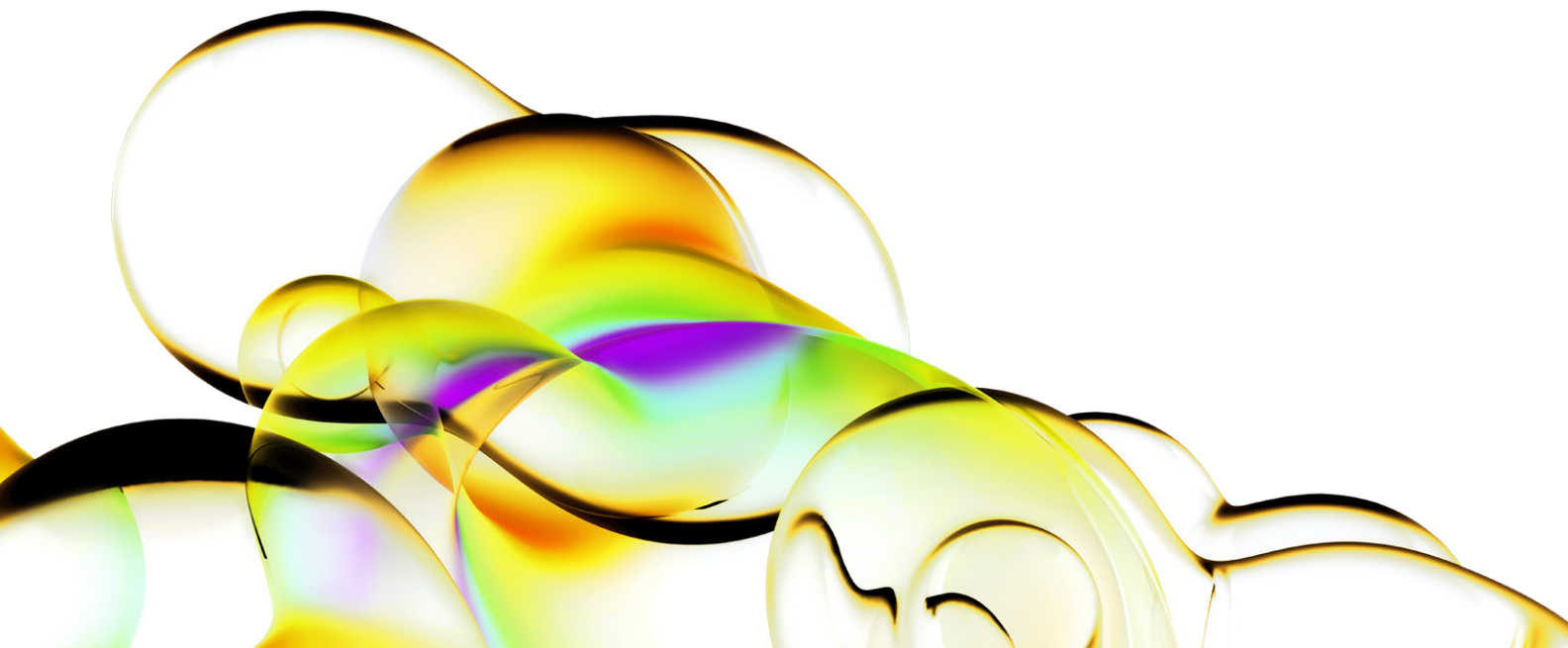


# Cell cycle analysis on Celigo.

## Introduction

Analysis of the cell cycle is often used in drug screening to discover compounds that affect the proliferation and growth of cells. Mitosis is composed of the G0/G1-, S-, and G2-phases, which can be distinguished and quantified by antibody staining and imaging. While this type of assay is usually performed by flow cytometry, we present here the use of the Expression Analysis application on the Celigo® image cytometer to monitor each phase of the cell cycle. The ability to analyze cell cycle progression of adherent cells directly in multi-well plates greatly facilitates the implementation of this assay in compound screens, significantly reduces the number of cells and reagents required for each data point, and eliminates cell physiology impacts caused by trypsinization and suspension of adherent cells.

The Celigo image cytometer is a novel imaging platform that combines brightfield and fluorescent imaging with rapid full-well acquisition of a variety of well formats (1536- to 6-well plates). Celigo optics provide uniquely uniform illumination throughout the entire well with excellent image contrast right to the edge of the well. These capabilities enable the identification of cells anywhere in the well with accurate fluorescence quantification. The cell cycle assay presented in this application note is a 2-color fluorescent assay using the blue and green channels of the Celigo image cytometer.



## Approach and results

### Cell staining

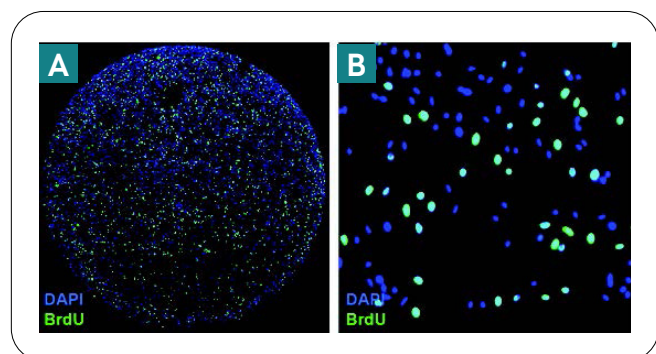


Figure 1: Image of entire well of a 96-well plate with A549 cells stained with DAPI and anti-BrdU antibody. Celigo's unique optics rapidly image entire wells of microplates of various sizes (1536- to 6-well plates). (A) Overlay image of cells stained for BrdU (green) and DAPI (blue). (B) Zoomed-in image shows all the cell nuclei stained with DAPI and the cells in the S-phase stained green.

5-bromo-2'-deoxyuridine (BrdU), a thymidine analog, was used to detect DNA replication in actively proliferating cells. A549 cells in 96-well plates were treated with aphidicolin or nocodazole and pulse-labeled with BrdU. After fixation, cells were stained with a monoclonal antibody against BrdU and a fluorescently-conjugated secondary antibody and counterstained with DAPI.

The Celigo Expression Analysis (Target 1 + Mask) application was used to quantify fluorescent signals and analyze data. Cells were identified using the DAPI nuclear stain and DNA synthesis was quantified by measuring BrdU incorporation. Fig. 1 shows a full well acquisition (A) and zoomed-in view (B) of a two-color stain for BrdU (green) and DAPI (blue).

### Data visualization using histograms

Following image analysis, the distribution of DAPI integrated intensity was visualized in a histogram plot (Fig. 2A). A double peak distribution was seen where the left and right peaks

correspond to cells in the G<sub>0</sub>/G<sub>1</sub>- and G<sub>2</sub>/M-phases, respectively. Cells in the S-phase are located between the two peaks. In order to quantify these 3 cell populations, the Celigo gating tool was used to divide the histogram in 3 areas in which counts and percentages of the total cell population were measured (Fig. 2A).

Cells were treated with nocodazole, a compound that interferes with the polymerization of microtubules and prevents the formation of mitotic spindles, causing cells to arrest in prometaphase. A549 cells were exposed for 18 h to nocodazole at concentrations up to 50  $\mu$ M. At intermediate concentrations (up to 1  $\mu$ M), the proportion of cells in the S- and G<sub>2</sub>/M-phases increased because the cells could not undergo mitosis. Concomitantly, we observed a reduction of cell numbers in the G<sub>0</sub>/G<sub>1</sub>-phase (Fig. 2B). Cells were also treated with a high concentration of nocodazole (50  $\mu$ M). This triggered apoptosis, seen as nuclear condensation, and caused the appearance of a new population of cells with low DAPI intensity ("Apo." region in Fig. 2C).

### Data visualization using scatter plots

Alternatively, the distribution of cells along the cell cycle can be quantified using a scatter plot by plotting the DAPI integrated intensity vs. the BrdU stain integrated intensity using a log scale for both axes. Fig. 3A shows the typical "horse shoe" shape where cells are distributed in three populations corresponding to the G<sub>0</sub>/G<sub>1</sub>-, S- and G<sub>2</sub>/M-phases. This representation benefits from a better separation between cell populations and is considered more accurate<sup>1</sup>.

A549 cells were treated with aphidicolin, a well-known DNA synthesis inhibitor for 18 h at concentrations up to 100  $\mu$ M.

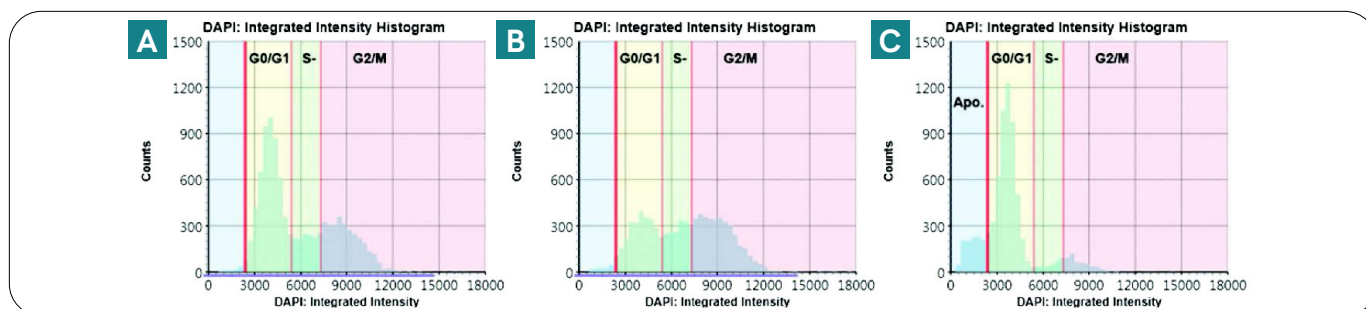


Figure 2: Treatment of A549 cells with nocodazole. (A) Histogram representation of the integrated DAPI stain intensities shows a two-peak distribution of A549 cells along the cell cycle. The regions corresponding to the G0/G1-, S- and G2/M-phases are marked accordingly. (B) Cells were treated with 150 nM nocodazole, resulting in a decrease of the number of cells in the G0/G1-phase of the cell cycle. At high concentration of nocodazole (50 μM), DNA fragmentation was detected with low DAPI integrated intensities (“Apo.” region in C).

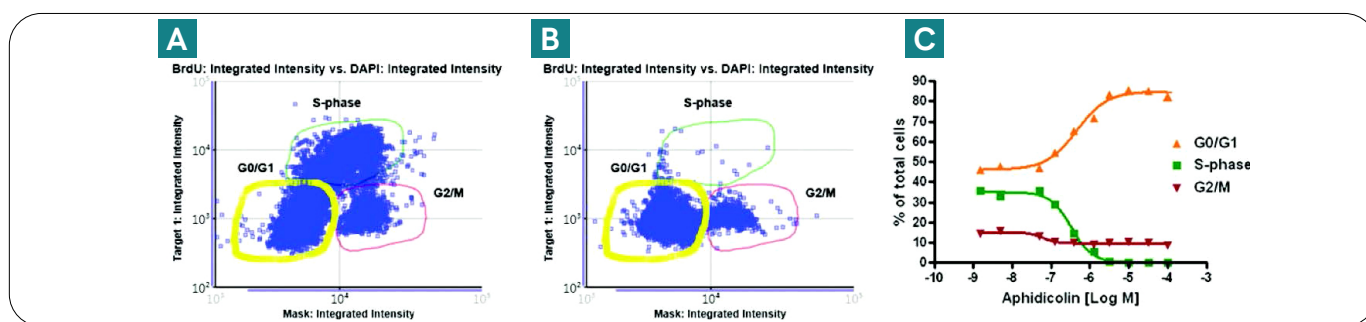


Figure 3: Treatment of A549 cells with aphidicolin. (A) Scatter plot representation of the integrated intensities of the DAPI vs. BrdU stain exhibited the typical “horse shoe” shape of the cell cycle. The regions corresponding to the G0/G1-, S- and G2/M-phases are marked accordingly. (B) When treated with 100 μM aphidicolin, the number of cells in the S-phase was dramatically reduced. (C) Concentration-response curve of aphidicolin for each phase of the cell cycle.

After treatment, we observed very few cells in the S-phase (Fig. 3B). Consistent with the fact that cells cannot go through the S-phase, they accumulate in the G0/G1-phase. Fig. 3C shows a concentration-response curve of aphidicolin for each phase of the cell cycle, measured using the “horse shoe” scatter plot (Figs. 3A and 3B). The data shows that increasing the concentration of aphidicolin increases the proportion of cells in the G0/G1-phase and decreases the number of cells in the S-phase. EC50 values of aphidicolin for G0/G1- and S-phase were 463 nM and 335 nM, respectively.

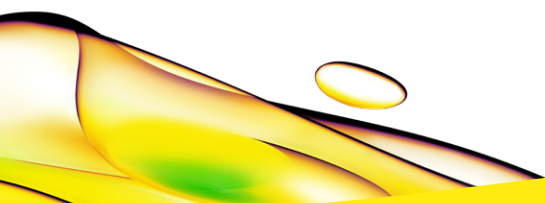
## Conclusion

These data demonstrate the ability of the Celigo image cytometer to accurately determine the proportion of cells in each phase of the cell cycle. The ability to run cell cycle assays in a wide range of plate formats, including 1536- and 384-well plates, using adherent as well as non-adherent cells, coupled with short scan times (6 minutes per 96-well plate), makes cell cycle analysis on the Celigo cytometer highly suitable for high-throughput applications.

## References

1. Sasaki et al. Flow cytometric estimation of cell cycle parameters using a monoclonal antibody to bromodeoxyuridine. *Cytometry*. 1986; 7(4):391-5.

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