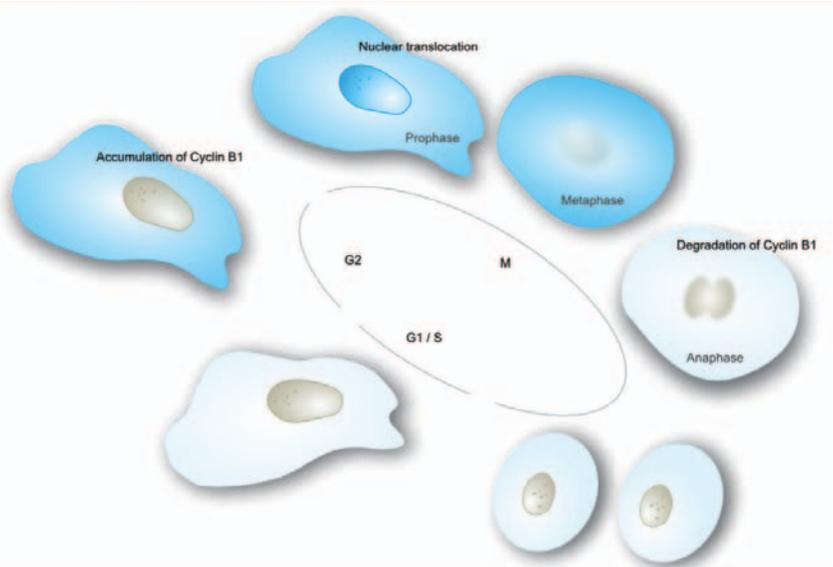


## Image-based Quantification of Cyclin B1 during Cell Cycle using the Opera

### Key Features

- Confocal image acquisition of fixed cells using the Opera™ High Content Screening system
- Quantification of Cyclin B1 during cell cycle
- Image analysis using the Acapella™ CellCycleAnalysis Script



## Cytoplasm-to-nucleus translocation

### Background

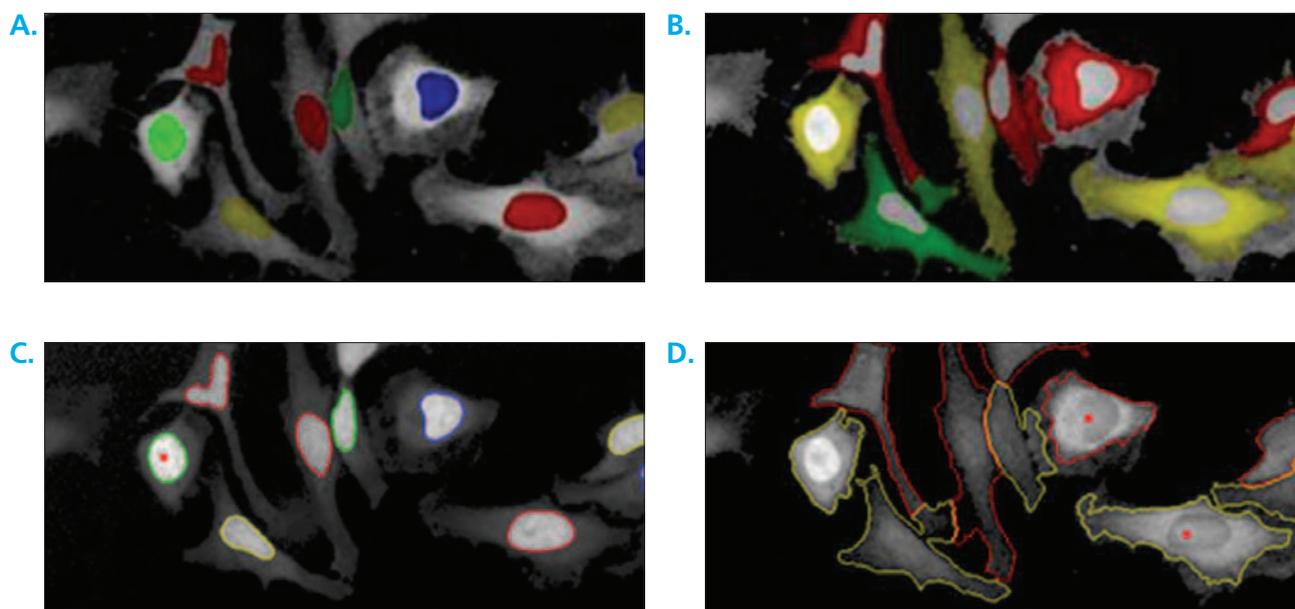
The cell cycle consists of four distinct phases: G1-phase, S-phase, G2-phase (collectively known as interphase) and M-phase. During the M-phase two tightly coupled processes occur: mitosis, in which the cell's chromosomes are divided and distributed to two new daughter cells, and cytokinesis, in which the cell's cytoplasm divides forming two distinct cells. One of the most important tasks in anti-cancer treatment is the inhibition of cell proliferation by interruption of the cell cycle. The cell cycle is controlled by a series of cytoplasmic proteins such as Cyclins. Cyclin B1 is essential for the initiation of mitosis and is concentrated on the outer plate of the kinetochores during pro-metaphase. Deregulation of Cyclin B1 is involved in neoplastic transformation, consequently it is a common target for cytostatic agents.

## Application

The activity of Cyclin B1 is closely associated with its intracellular level and sub-cellular distribution. HeLa (human cervix carcinoma) cells were treated with Nocodazole, Aphidicolin and Demecolcin. Cyclin B1 distribution patterns in these cells were imaged on the Opera High Content Screening system through the cell cycle using an anti-Cyclin B1 antibody. Nocodazole is an antineoplastic agent which exerts its effect by depolymerizing microtubules (arrest in G2/M-phase). Aphidicolin inhibits the growth of eukaryotic cells by selectively inhibiting the cellular replication of DNA polymerase II (arrest in late G1/S-phase) and Demecolcin is a phytogetic antineoplastic agent (arrest in M-phase).

Cell cycle perturbation induced by compound treatment was precisely detected by our CellCycleAnalysis Script approach (Figure 1). The percentage of cells in G1/S-phase vs. the ones in G2/M-phase were quantified. The G2/M-cell population is considered to include all cells with an increased Cyclin B1 intensity in the nucleus and/or cytoplasm. All other cells are classified as G1/S cells.

The mitotic inhibitors Demecolcin and Nocodazole both induced G2/M arrest but no effect could be observed with Aphidicolin, a G1/S blocker, as expected. Dose-response curves gave an  $EC_{50}$  of 16.81 ng/ml for Nocodazole and 10.95 ng/ml for Demecolcin (Figure 2).



*Figure 1:* Image analysis. Images were analyzed using the CellRegionAnalysis Script of the Acapella Script Collection. The amount of Cyclin B1 is determined in Alexa Fluor® 488 channel images based on nuclei detection (A) and cytoplasm detection (B) using the DRAQ5™ channel images (upper). Two subpopulations of cells with Cyclin B1 intensity in the nucleus or cytoplasm exceeding a defined threshold are identified; Detection of Cyclin B1 positive nuclei (Panel C, cell with red dot); Detection of Cyclin B1 positive cytoplasm (Panel D, cells with red dot). The G2/M-cell population is considered to include all cells with an increased Cyclin B1 intensity in either the nucleus and/or cytoplasm.

## Conclusions

In summary, this application allows accurate quantification of cell cycle phases in asynchronous HeLa cell populations with as little as 2000 cells per data point. Therefore, it is ideally suited for high-throughput primary and secondary cellular screening to uncover novel anticancer drugs.

Furthermore, this two-channel approach can be easily multiplexed with other biomarkers and so functions as a core assay building block for the addition of further dyes, antibodies and readout parameters.

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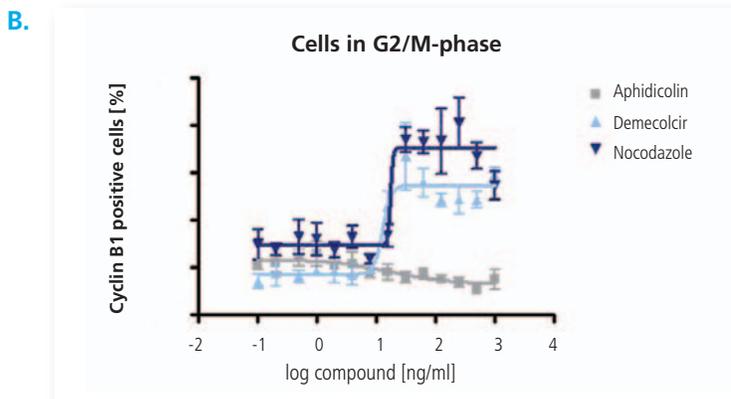
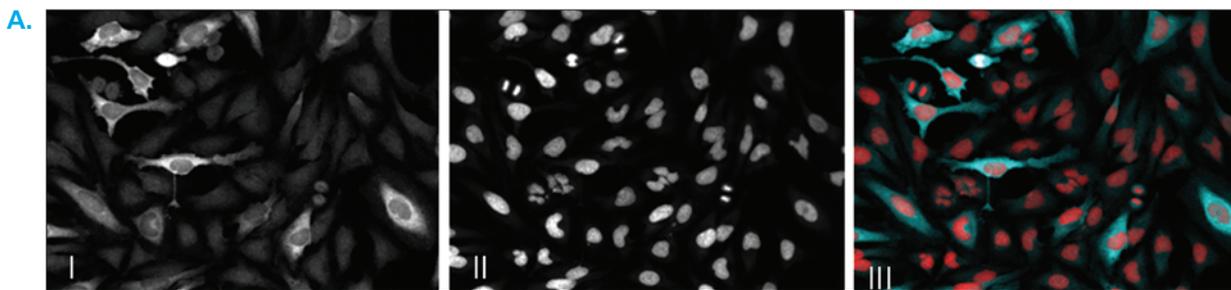


Figure 2: A. Opera images. Confocal images of HeLa cells stained with Cyclin B1 rabbit polyclonal IgG, Alexa Fluor® 488 goat anti-rabbit IgG and DRAQ5™ using the 20X water immersion objective. I, shows cells positive for Cyclin B1; II, shows nuclei; III, shows an overlay of channel 1 (Cyclin B1, blue) and channel 3 (DNA stain, red).

B. Quantification of compound effects on cell cycle. Dose-response curves for Aphidicolin, Demecolcin and Nocodazole on the expression-level of Cyclin.

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