Quantitative Analysis of Drug Effects on Cytoskeletal Structure, Cell Morphology and the Cell Cycle by High-Content Assays

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Abstract:

Being fundamental and ubiquitous features of proper cellular function, cytoskeletal rearrangement and cell cycle progression are sensitive indicators of cell growth and cell stress. Previously, we developed a number of multiplexed, quantitative, cell-based, high-content screening (HCS) assays to monitor these key cellular functions using a Thermo Scientific Cellomics ArrayScan® HCS Reader. For example, the Thermo Scientific Cellomics Cytoskeletal Rearrangement HCS Kit simultaneously detects changes in cytoskeletal structures (F-actin and microtubule fibers), nuclear DNA content, and nuclear and cellular morphologies using bright fluorescent probes. The Thermo Scientific Cellomics Cell Cycle I HCS Kit characterizes cell cycle progression by simultaneously quantifying DNA content, BrdU incorporation and Histone H3 phosphorylation. Here we demonstrate the value of using these two multiparameter kits together. By assessing the cell cycle using BrdU and phospho-Histone H3 antibodies while simultaneously monitoring cytoskeletal rearrangements and cell morphological changes, we established a multiparameter profile of different drugs affecting cell cycle, cell growth, cell differentiation and cell death. A variety of cells were treated with different actin or tubulin affecting-drugs or cell cycle inhibitors, and then monitored the drug effect on cellular and nuclear shape, cytoskeletal structure, and cell cycle phases. Evaluation of drugs using these multiplexed cytoskeletal and cell cycle assays is a simple and easy-to-implement method for compound toxicity profiling in high content screening.

Introduction:

Cell stress, cell proliferation and the characterization of agents that either promote or inhibit cell proliferation are particularly of interest in cell biology and drug-discovery research. High content analysis (HCA) involves a fluorescence cell-based assay where the cells are automatically imaged and analyzed using quantitative fluorescence microscopy. HCA can be used to quantify the cell proliferation activity by measuring their DNA content, the state of cell cycle-associated proteins, and morphological and structural changes in individual cells and in cell populations.

We have developed a portfolio of Cellomics HCS kits to identify individual cells' cell cycle phase and proliferation state, using a fixed end-point HCS assay based on immunofluorescence detection in cells grown on standard high-density microplates. These kits all contain the DNA binding dye DAPI which is used to determine the cell cycle phase in individual cells by DNA content, and this is verified by correlating the expression level or phosphorylation state of other cell cycle associated indicators. Cells which have incorporated BrdU into DNA can be quickly detected by using monoclonal antibody directed against BrdU and fluorophore-conjugated secondary antibody. BrdU staining facilitates the identification of cells that have progressed through the S-phase of the cell cycle during the BrdU-labeling period. The Thermo Scientific Cellomics BrdU Cell Proliferation HCS kits have been specifically designed to enable simple BrdU detection in nuclear DNA without extreme treatments to the cell. This enables multiplexed detection of BrdU with other targets in the same cells. We also developed several other cell cycle assay targets, which include Cyclin B1, phosphorylated polo-like kinase 1 (phospho-PLK1), phosphorylated retinoblastoma protein (phospho-Rb) and phosphorylated Histone H3. These kits enable options to directly determine individual cells' cell cycle phase, such as the G1, S phase (DNA content & BrdU) and G2/M phase (Cyclin B1, Phospho-PLK1, Phospho-Rb and mitosis specific Histone H3 phosphorylation).

The intracellular cytoskeletal meshwork is responsible for maintaining cell shape, cell movement, cytokinesis, and the organization of organelles within the cell. The dynamic network of the cytoskeleton also facilitates proper function of other cellular proteins through direct binding, transportation, repositioning and sequestration of these proteins. The structure of the cytoskeleton is controlled by bundling, aligning and repositioning of the filaments through cytoskeleton-associated proteins in response to the external signaling. Alterations of cytoskeletal structure are often associated with pathologies and cell death. Signaling defects in conjunction with cytoskeletal rearrangement can also contribute to increased cell proliferation rate and tumor cell motility which result in metastasis. Thus, the key players controlling cytoskeletal rearrangement in the cell are potential therapeutic targets.

Thermo Scientific, Inc. provides an integrated set of products that work together to deliver a "total solution" platform for HCS. Integral components of this set are the Thermo Scientific Cellomics HCS Reagent Kits. These kits provide easy-to-use methods and reagents for preparing high-quality samples for automated cell-based imaging assays. Using Thermo Scientific Cellomics Cytoskeleton Rearrangement kit and other Cell Cycle kits, we have developed quantitative HCS assays for assessing the cytoskeletal rearrangement and the cell cycle phases in cells treated with different compounds. The Cellomics HCS kits makes these assays easy to implement, and provides a powerful set of multiplexed parameters for monitoring the response of the cell due to drug treatment in these key functional areas. All images and data shown were acquired and quantitatively analyzed using Thermo Scientific's Cellomics ArrayScan® HCS Reader with associated Cellomics BioApplication image processing software modules.

New HCS Assays for Cyclin B1 Activation and Phospho-PLK1 Activation

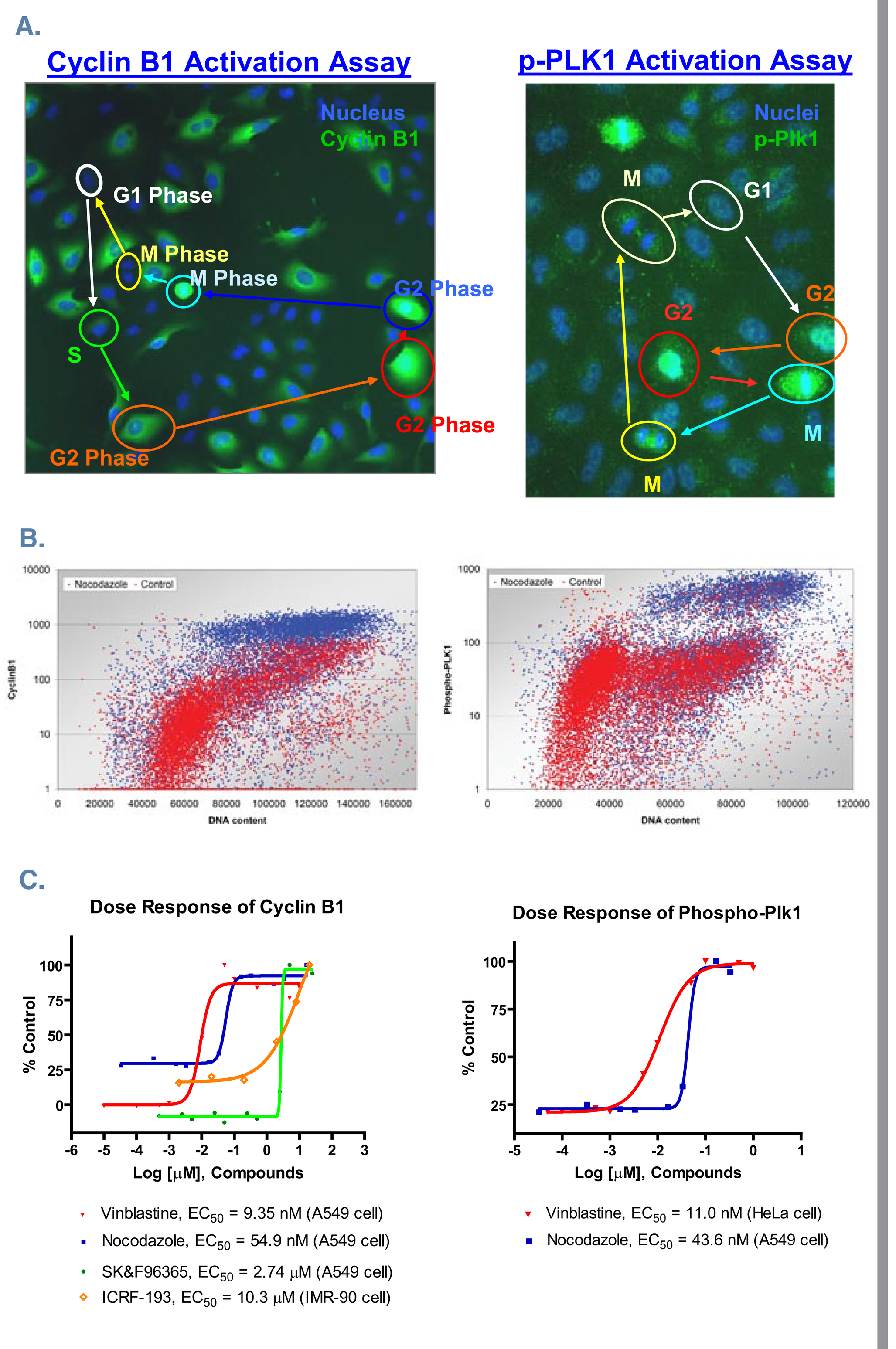

Figure 1: (A) A549 cells stained with the new Thermo Scientific Cellomics HCS kits for Cyclin B1 and phospho-PLK1. The Cyclin B1 and phospho-PLK1 are shown in green, and the DAPI staining is in blue. The individual cells' cell cycle phases are determined by the expression level (i.e. fluorescence intensity) and the localization of the cell cycle target (Cyclin B1 or phospho-PLK1) correlated with the DNA content. (B) Population analysis of Cyclin B1 and phospho-PLK1 fluorescence intensity vs DNA content in individual cell levels after Nocodazole treatment. Nocodazole treatment causes cells to be blocked at G2/M, and have double the DNA content and high levels of Cyclin B1 and phospho-PLK1. (C) Dose response analyses of Cyclin B1 and phospho-PLK1 for various drugs that cause inhibiton at G2/M phase.

Multiparameter Quantification of Cell Structures and Cell Cycle Markers

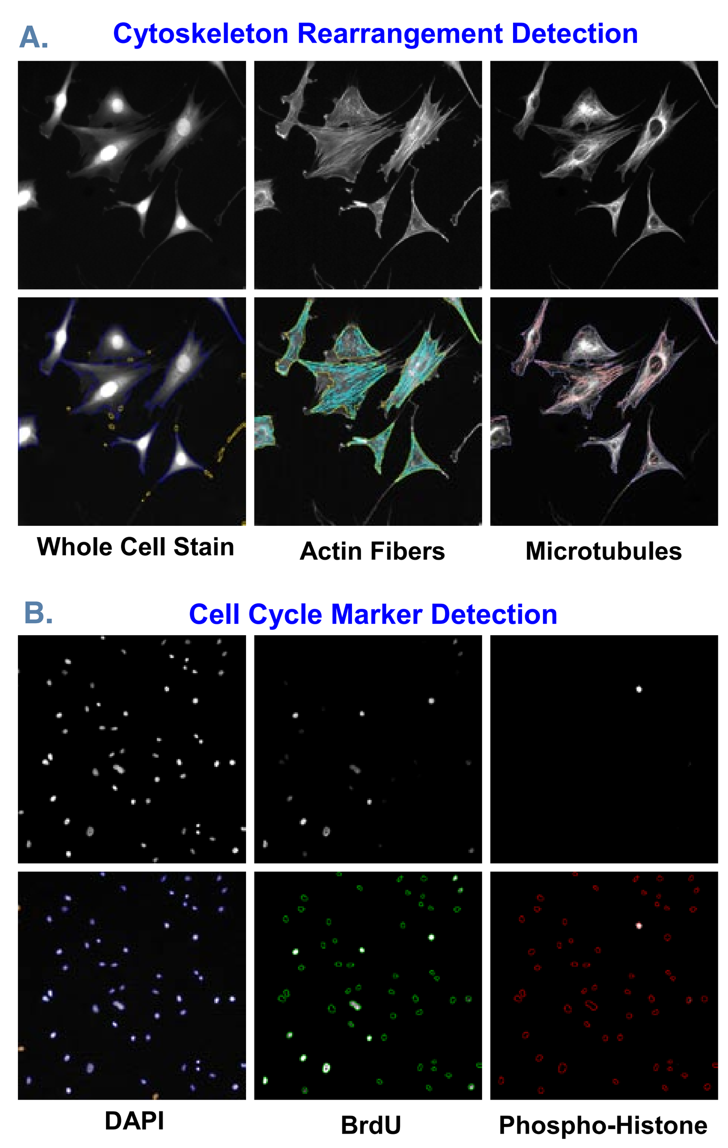

Figure 2: (A) Cell morphology, F-actin fibers and microtubule fibers in NIH 3T3 cells are visualized with the Cytoskeleton Rearrangement kit, imaged with the ArrayScan V[TI] HCS Reader using a 20X objective lens, and analyzed using the Morphology Explorer BioApplication. (B) DNA content, BrdU and phospho-Histone H3 in A549 cells stained with the Cell Cycle Kit I, imaged with the ArrayScan V[TI] HCS Reader using a 10X objective lens, and analyzed by the Target Activation BioApplication. For both panels, the top row shows the acquired fluorescence image, and the colored traces in the bottom row shows the features identified and analyzed by the respective BioApplication.

Analysis of Cellular Structures Followed by Cytochalasin D (F-actin Drug) Treatment

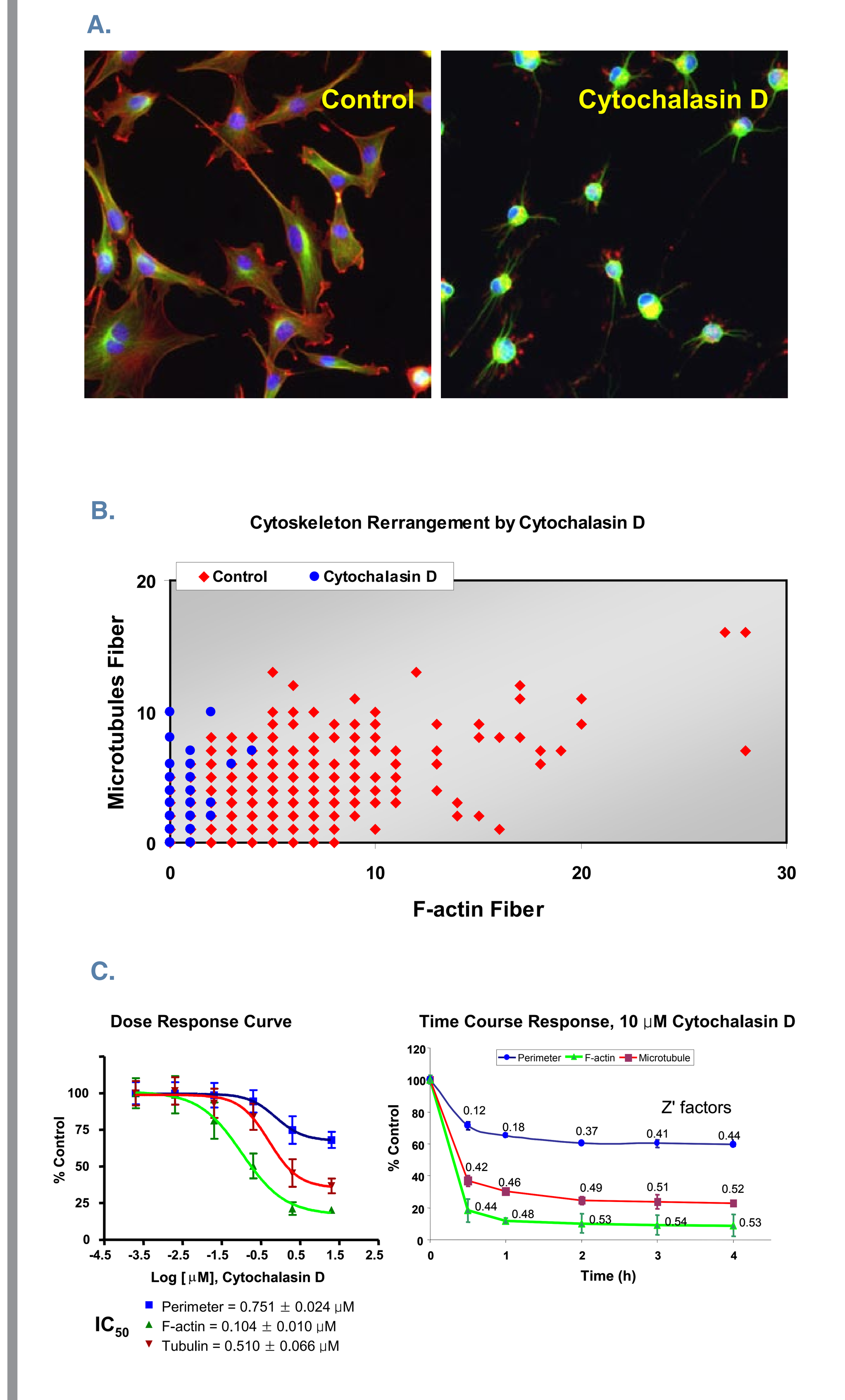

Figure 3: (A) Cell images of F-actin (green), microtubules (red), and nuclei (blue) in NIH 3T3 cells with or without Cytochalasin D treatment (20 µM, 3 hrs). (B) The numbers of F-actin fibers and microtubule fibers are decreased by Cytochalasin D treatment (20 µM, 3 hrs) – individual cell are plotted. (C) Dose response and time course response of Cytochalasin D on the cell morphology, and the number of F-actin and microtubule fibers.

Cytoskeletal Structures are Affected by Cell Cycle Inhibitory Drugs

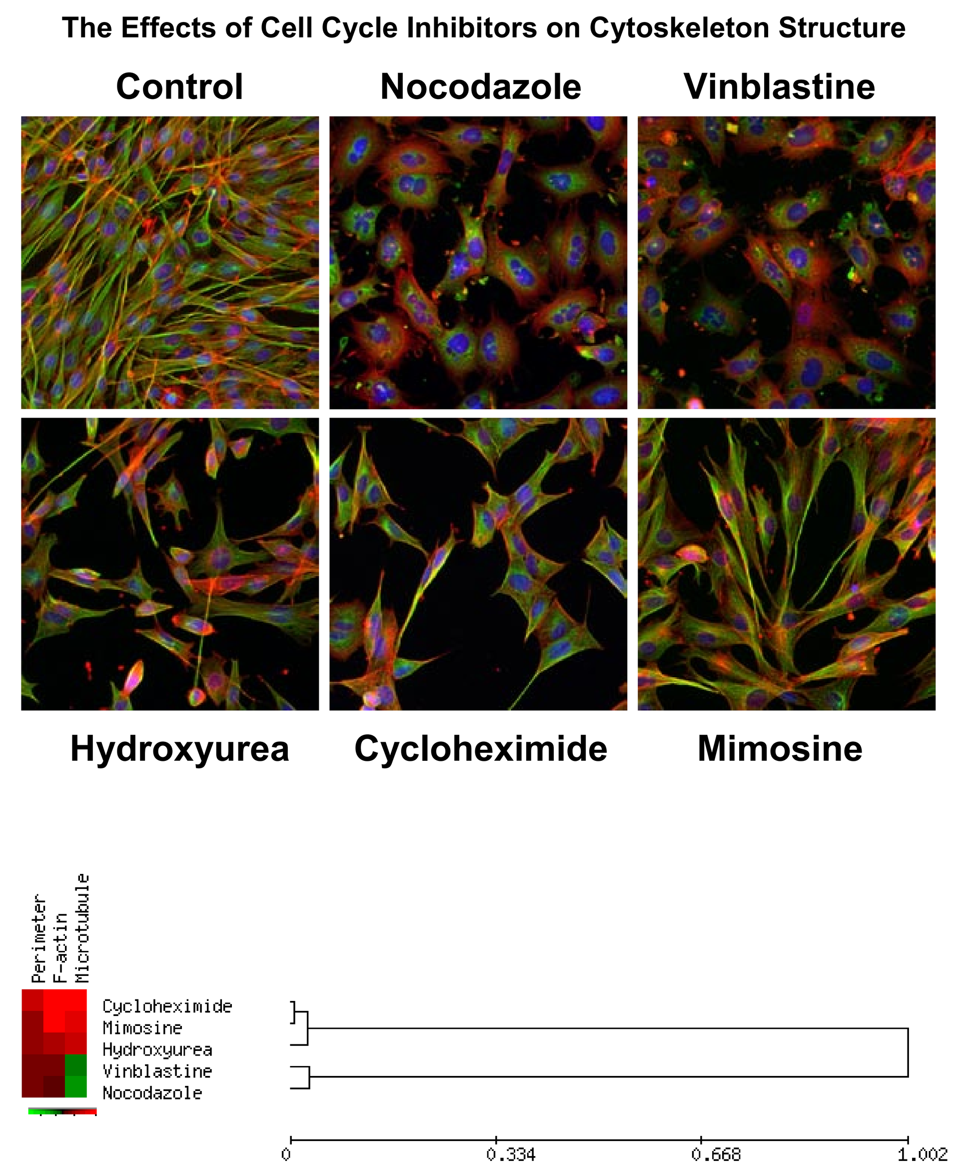

Figure 4: NIH 3T3 cells were labeled with the Cytoskeleton Rearrangement kit (F-actin = green, microtubules = red, and nuclei = blue). Hierarchical clustering analysis was performed with the data of drug effect on microtubules, F-actin and cell morphology, which indicates that Nocodazole and Vinblastine disrupt microtubule structure but Hydroxyurea, Cycloheximide and Mimosine increase F-actin fibers (stress fibers).

Cell Cycle Markers, BrdU and Phospho-Histone H3 are Affected by Cell Cycle Inhibitory Drugs

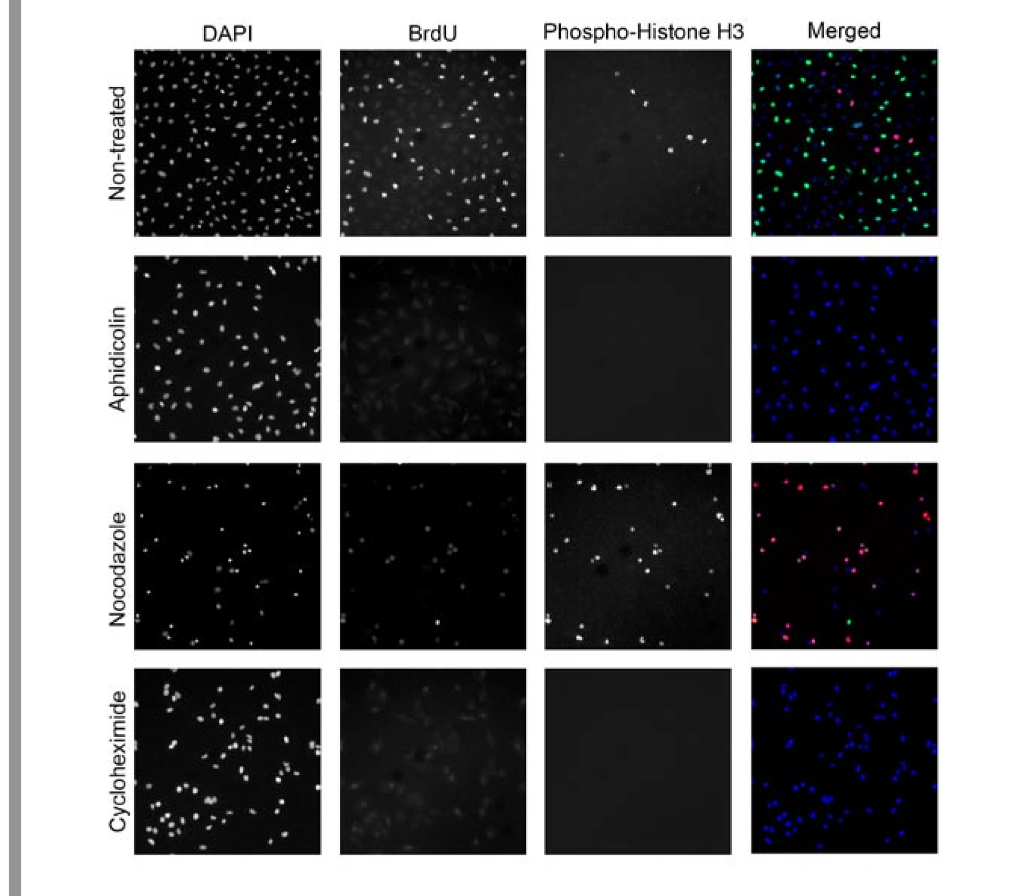

Figure 5: Aphidicolin and Cycloheximide treatment inhibit both BrdU and phospho-Histone H3. Nocodazole increases phospho-Histone H3 positive cells, which indicates the cells are in G2/M phase.

Population Analysis of the Changes in Cell Cycle Phase After Cell Cycle Inhibitory Drug Treatments

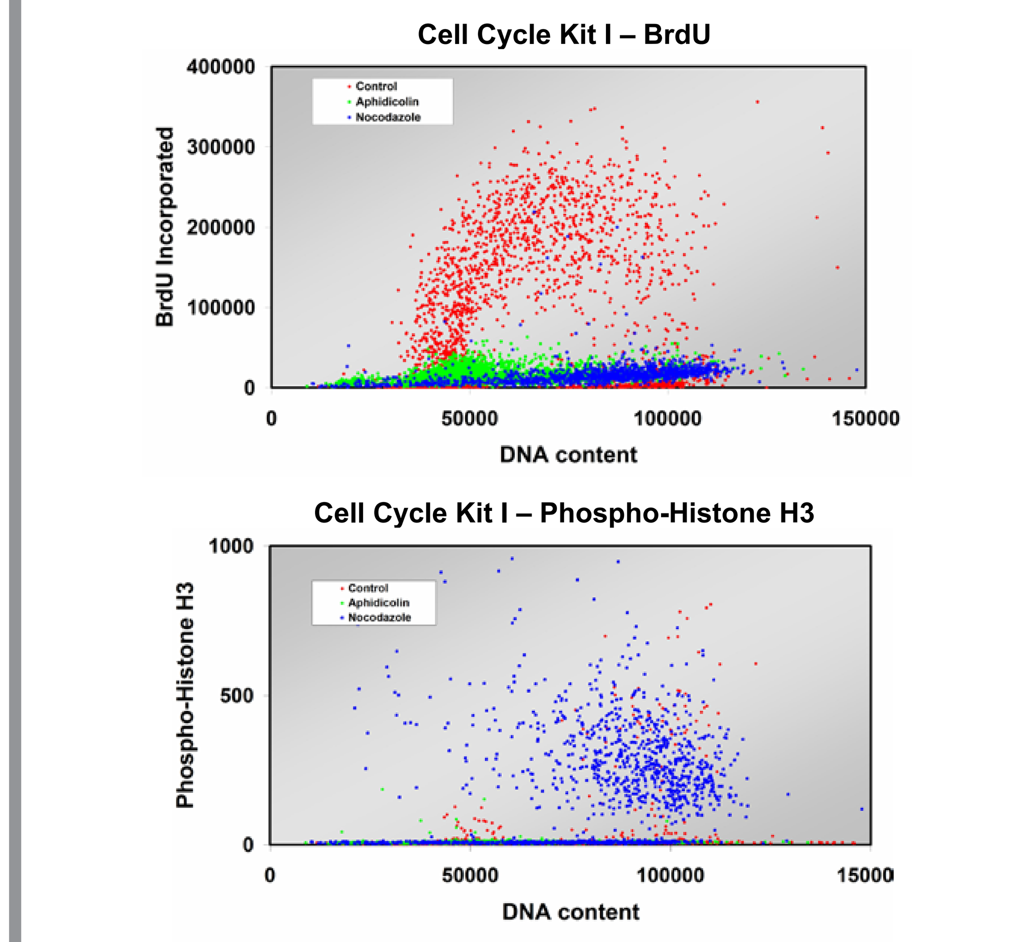

Figure 6: Inhibition of BrdU incorporation in cells by the G1/S blocker, Aphidicolin (strong inhibition) and the G2/M blocker, nocodazole (weak inhibition) after 2 hours and 16 hrs treatment, respectively. Nocodazole causes a strong increase in cells with phospho-Histone H3.

Combined Analysis of Cytoskeleton Rearrangement and Cell Cycle Markers

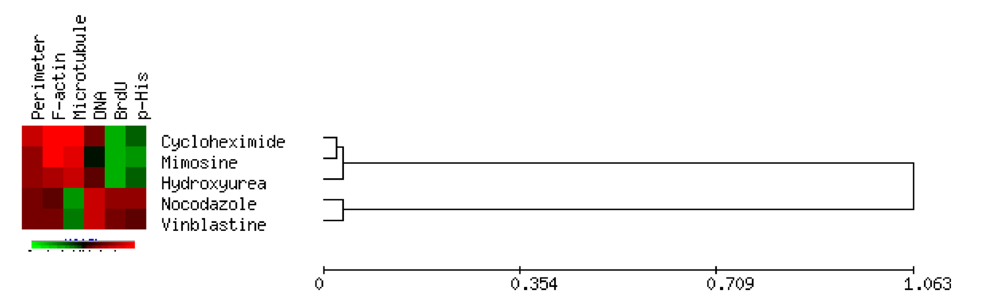

Figure 7: Hierarchical clustering of the quantitative results from the previous figures shows that the changes in cell structures and cell cycle markers are closely related to each other when treated with cell cycle inhibitory drugs. The combined parameters of these two assays are good indicators of the cell's response to a drug treatment.

Summary:

- New Cell Cycle Marker analysis provide comprehensive information on cell cycle phases (Kits: Cyclin B1:#8404401&2, p-PLK1:#8404801&2, p-Rb:#8404501&2 and BrdU/p-Histone H3:#8404601&2).
- Cytoskeletal rearrangement (#8402401&2) and cell cycle marker detection allow to evaluate and profile the multiparameter cellular effect of cell cycle inhibitory drugs.
- Thermo Scientific Cellomics HCS Reagent Kits and ArrayScan HCS Reader offer the powerful tools for High Content Cell-Based Screening and Analysis