Kinetic Quantification of Cell Health with Real-time Live Cell Analysis

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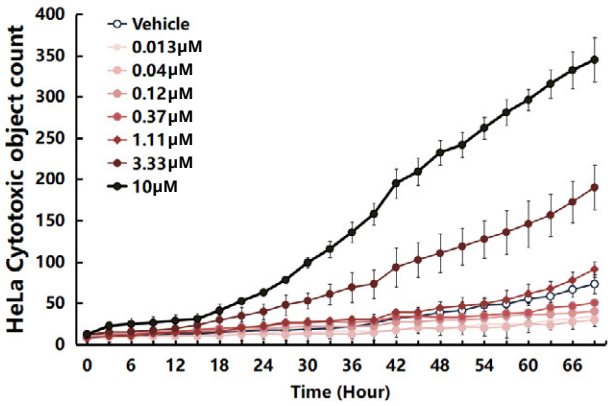
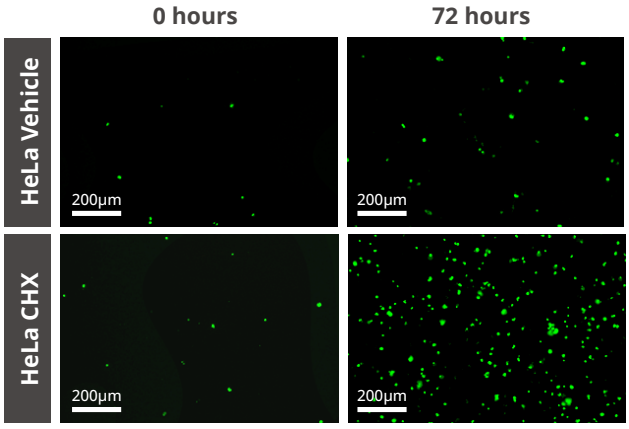
Highlights

1. Spica M1 provides continuous, label-free monitoring of cell proliferation and cytotoxicity, enabling real-time insights under physiologically- relevant conditions.
2. Spica M1's kinetic analysis clearly identifies drug response mechanisms by monitoring confluence, cell death, and morphology across different compounds and cell types.
3. Powered by AI-driven algorithms, Spica M1 ensures precise quantification of confluence, cytotoxicity, and morphology, accelerating high-throughput drug discovery and enhancing phenotypic profiling.

Abstract

Quantifying cell proliferation and cytotoxicity is crucial for understanding drug responses, optimizing dosing strategies, and assessing compound safety. The Countstar® Spica M1 system combines Enhanced Brightfield and fluorescence imaging with AI-driven analysis to provide real-time, label-free evaluation of cellular dynamics under physiological conditions. In this study, we used the Spica M1 to continuously monitor proliferation and cytotoxicity in HeLa and A549 cells treated with Cycloheximide or Staurosporine. Kinetic analysis of confluence and YOYO-1 positivity revealed distinct drug response patterns, emphasizing differences in onset, mechanism, and sensitivity among cell types. The system's improved contrast imaging and reliable object recognition algorithms enabled accurate quantification of morphological changes and viability over time. These findings demonstrate the usefulness of Spica M1 for high-throughput, non-invasive profiling of drug effects, supporting applications in oncology, pharmacology, and cell-based assay development.





Introduction

Cell proliferation and cell death are fundamental aspects of cellular health and serve as critical indicators in drug screening. Traditional endpoint assays—such as measuring DNA synthesis or analyzing proliferation markers like Ki-67 and PCNA—offer only static snapshots of cellular states. These approaches fail to capture the dynamic and often heterogeneous responses of cells to external stimuli, particularly transient or delayed effects. In contrast, long-term, kinetic live-cell imaging enables continuous monitoring of phenotypic changes in real time. This approach allows researchers to observe proliferation, morphological alterations, and cell death as they unfold, uncovering temporal patterns and mechanistic insights that single-time-point assays often miss. Such dynamic monitoring is especially valuable in therapeutic development, where understanding the timing and progression of cellular responses can inform compound selection, dosing strategies, and mechanism-of-action studies.

To capture these dynamic changes, imaging platforms must deliver continuous, long-term and real-time analysis under physiologically relevant conditions. The Countstar® Spica M1 combines Enhanced Brightfield imaging with multichannel fluorescence optics, enabling long-term, label-free analysis alongside fluorescence-based detection. AI-powered confluence analysis provides a non-invasive proxy for proliferation, while cytotoxicity dyes directly quantify cell death through nuclear labeling without compromising cell viability.

In this application note, we demonstrate the use of the Countstar® Spica M1 to evaluate proliferation in adherent cell lines (HeLa, A549, MCF-7) and suspension cells (K562). We further performed kinetic cytotoxicity assays on HeLa and A549 cells exposed to the translation inhibitor Cycloheximide or the pan-kinase inhibitor Staurosporine. By integrating morphological analysis, confluence tracking, and cytotoxic fluorescence readouts, we achieved a comprehensive assessment of drug effects across a wide concentration range.

Materials and Methods

Cell culture and treatments

Proliferation Assay

HeLa, A549, and MCF-7 cells were seeded into flat-bottom 96-well plates (Corning 3599) at 3000, 6000, 12000 cells/well in 200 µL medium and K562 was seeded into the same flat-bottom 96-well plate at 2500, 5000, 10000 cells/well in 200 µL medium. The cells were then allowed to settle at room temperature for 20 minutes and to adhere at 37 °C for 24 h before imaging.

Cytotoxicity Assay

HeLa and A549 were seeded into a flat-bottom 96-well plates (Corning 3599) at seeding density of 3000 cells/well in 100 µL medium. The cells were then allowed to settle at room temperature for 20 minutes and to adhere at 37 °C for 24 h before treatment and imaging. Cycloheximide (MedChemExpress, HY-12320) and Staurosporine (MedChemExpress, HY-15141) were added at 10 µM as starting concentrations and serially diluted 1:3 to yield seven doses. YOYO-1 iodide (AAT Bioquest 17580) was separately added at 1µM in all wells to label dead cell nuclei.

Countstar® Spica M1 imaging and analysis

Proliferation Assay

The Confluence App was selected to set up a 96-hour proliferation experiment with the following parameters: 7.5×, 1FOV, exposure time 2ms/gain 1. The experiment was scheduled to run over 4 days, with scans every 3 hours. Default setting was selected for image analysis.

Cytotoxicity Assay

The Cell Health APP was selected with the corresponding channels. The plate was scanned every 3 hours for 72 hours with two FOV per well. Enhanced Brightfield (BF) channel was set to 2 ms/gain 1, and the green fluorescence channel was set to 15 ms/gain 1. Confluence masks were generated with the AI confluence algorithm, and YOYO-1 positive nuclei were identified and counted using the “BF confluence, G object” AI algorithm. Kinetic data were analyzed and plotted in Excel for cytotoxicity assessment.

Results and Discussion

Baseline Proliferation Profiling of Suspension and Adherent Cells

Cell proliferation is a fundamental biological process necessary for tissue growth, regeneration, and repair. The change in cell proliferation serve as a key indicator of cellular health, transformation, and how cells respond to environmental signals, making it a crucial measure in oncology, immunology, and drug development.[1] Due to proliferation's dynamic nature, continuous monitoring over time provides greater insight into cellular behavior under different conditions. Importantly, abnormal proliferation is a hallmark of malignant transformation and cancer progression. Therefore, accurately assessing proliferative capacity and division potential in response to stimuli is essential for evaluating therapeutic effectiveness and safety in cancer research, stem cell studies, and drug screening.

In this study, label-free, real-time analysis was used to monitor the growth of both adherent cell lines (HeLa, MCF-7, A549) and suspension cells (K562) over several days. This is made possible by a combination of design and computational features: a fixed-stage imaging system that keeps spatial stability for suspension cell cultures, and AI confluence algorithms that reliably recognize various cell shapes under Enhanced BF imaging. These features enable accurate and consistent measurement of confluence across different cell types.

Using the Spica M1 system, we monitored the growth of adherent cells HeLa (Figure 1A), MCF-7 (Figure 1B), A549 (Figure 1C), and suspension cells K562 (Figure 1D) by measuring confluence in a 96-well plate. The proliferation curves revealed that adherent cells nearly reached 100% confluence within 4 days when seeded at densities over 3000 cells per well, while K562 suspension cells showed increasing confluence levels over time, depending on incubation period and seeding density. Representative images (Figure 1E) illustrate the system's high accuracy in detecting both adherent cell expansion and aggregation behavior of suspension cells from day 0 to day 4. Overall, these findings demonstrate the assay's wide applicability in cancer cell growth profiling, quality control of cell culture processes, and long-term growth monitoring.

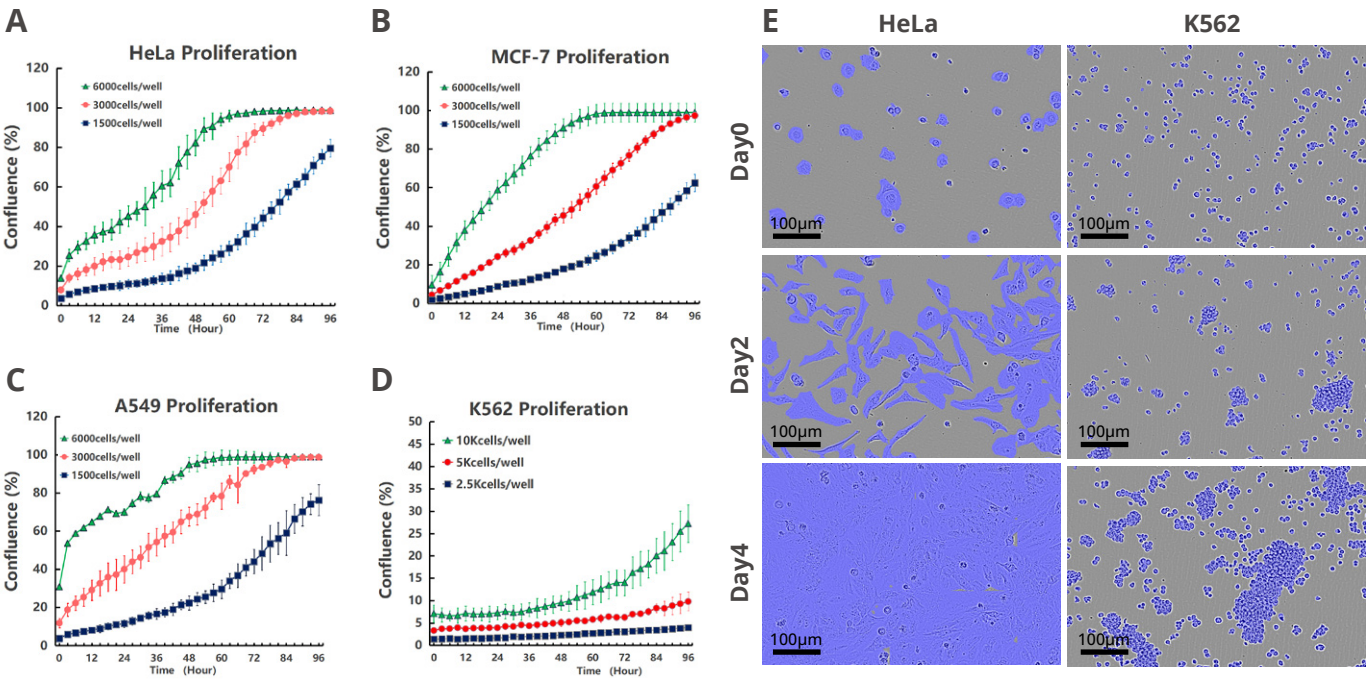


Figure 1. Proliferation curves of Hela (A), MCF-7 (B), A549 (C), K562 (D) in different seeding density. (E) Enhanced BF images of Hela with seeding density of 3000cells/well, and K562 cells of 10K cells/wells at day 0, 2, and 4.

Dynamic Proliferation Suppression Revealed by Confluence Analysis

Label-free drug screening allows researchers to evaluate cellular responses in a natural physiological environment without interference from fluorescent dyes or chemical indicators.[2] This approach is especially important in cancer biology and therapeutic assessment, where subtle differences in drug sensitivity and timing can greatly impact treatment decisions. By continuously monitoring confluence and cell morphology over time, researchers can observe drug responses from early to late stages, enabling kinetic profiling of drug effectiveness and mechanisms of action. This supports such as personalized therapy testing, resistance analysis, and dose adjustment.[3] Based on the calibration of label-free analysis of cell confluence above, we then explored how real-time confluence monitoring reveals drug-induced responses.

The following study examined A549 cells exposed to Cycloheximide and Staurosporine, showing dose-dependent growth inhibition. Cycloheximide, a protein synthesis inhibitor, suppresses translation elongation, causing gradual stress and growth delay. Doses above 0.37μM limits growth, while lower doses below 0.04μM allowed partial recovery after 40 hours (Figure 2A), indicating reversible stress or adaptation. Conversely, Staurosporine, a broad-spectrum kinase inhibitor, caused rapid and sustained growth arrest at concentrations as low as 0.004 μM, the confluence was just increased from 14.8±0.93% at day 0 to 23.3±1.1% at day 3, while the vehicle group showed about 4X growth rate that increased from 18.9±2.6% to 87.2±6.3% of confluence (Figure 2B). These responses highlight two mechanisms: Cycloheximide gradually inhibits growth by disrupting protein synthesis, while Staurosporine induces quick cytotoxicity via apoptosis.

These biological insights are made possible through the Spica M1's Confluence Algorithm: AI-driven image analysis capable of recognizing a wide range of drug-induced morphological changes, including membrane blebbing, shrinkage, and cell death. Together with the system's Enhanced BF imaging and fixed-stage platform, this algorithm allows accurate, label-free quantification of dynamic cellular responses across diverse cell types and treatment conditions. These tools make Spica M1 a powerful, non-invasive tool for real-time drug assessment in both research and translational settings.

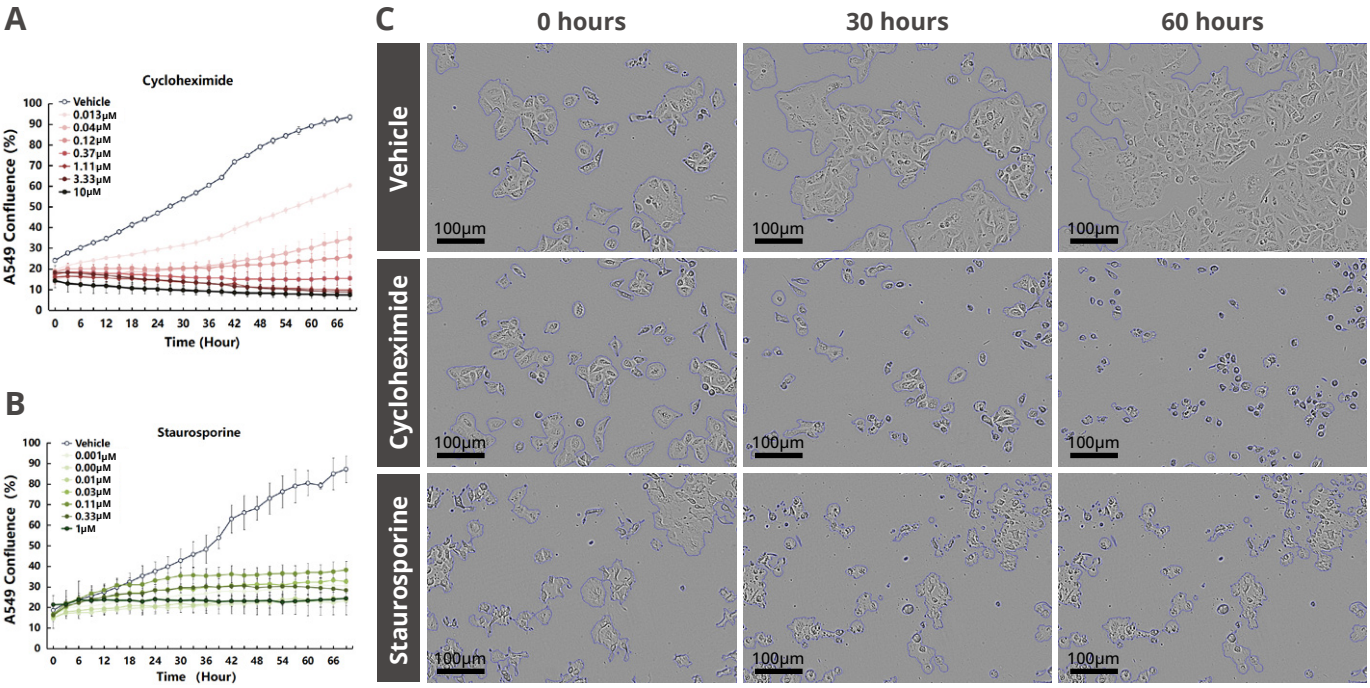


Figure 2. Confluence plots of A549 under the Cycloheximide (A) and Staurosporine (B), and the representative images of A549 exposed to 10μM Cycloheximide and 1μM Staurosporine (C)

Direct measurement of dose-dependent cytotoxicity

Cytotoxicity—the process of cell injury or death in response to chemical or biological agents—is a crucial parameter in assessing drug safety and efficacy [4]. Traditional endpoint assays such as MTT, LDH release, and ATP detection offer only static snapshots that often overlook early or temporary effects.[5] In contrast, the Spica M1 allows for continuous, direct measurement of cytotoxicity by detecting YOYO-1–positive nuclei—cells with compromised membranes—under physiologically relevant conditions. The AI-driven object detection enables precise analysis of dying cells over time and across various morphological states, supporting drug efficacy studies and cytotoxicity profiling with real-time monitoring.

Using Countstar® Spica M1, we evaluated the cytotoxic responses of HeLa and A549 cells to Cycloheximide over 72 hours. HeLa cells showed early YOYO-1 positivity at concentrations as low as 1.11 μM, with signal counts increasing steadily over time (Figure 3A). In contrast, A549 cells demonstrated a slower onset and lower overall counts, indicating decreased drug sensitivity (Figure 3B). By the 72-hour mark, HeLa reached 344±26.7 YOYO-1–positive nuclei per field, while A549 only reached 195±20.9 (Figure 3C). These findings highlight cell line-specific differences in both the timing and magnitude of the cytotoxic response, emphasizing the importance of kinetic measurements for distinguishing between sensitive and resistant drug responses. The heightened sensitivity of HeLa cells could be due to their higher baseline protein synthesis activity and lower stress adaptation capacity, making them more susceptible to translation inhibition. Conversely, A549 cells display stronger stress resilience and slower proliferation, which likely contributes to their reduced response.[6] Notably, the HeLa vehicle group itself showed background cell death with the higher cytotoxic count than low doses (≤0.37 μM) (Figure 3A), suggesting the vehicle itself induces low-level cytotoxicity, and sub-0.37 μM cycloheximide exerts minimal additional toxicity.

These biological differences are captured in real-time by Spica's high-throughput kinetic imaging and AI-powered analysis, which enable the simultaneous analysis of confluence and cell death markers without labeling artifacts. This gives researchers a deeper understanding of drug action dynamics, supporting mechanism studies and precise drug screening across various cell models.

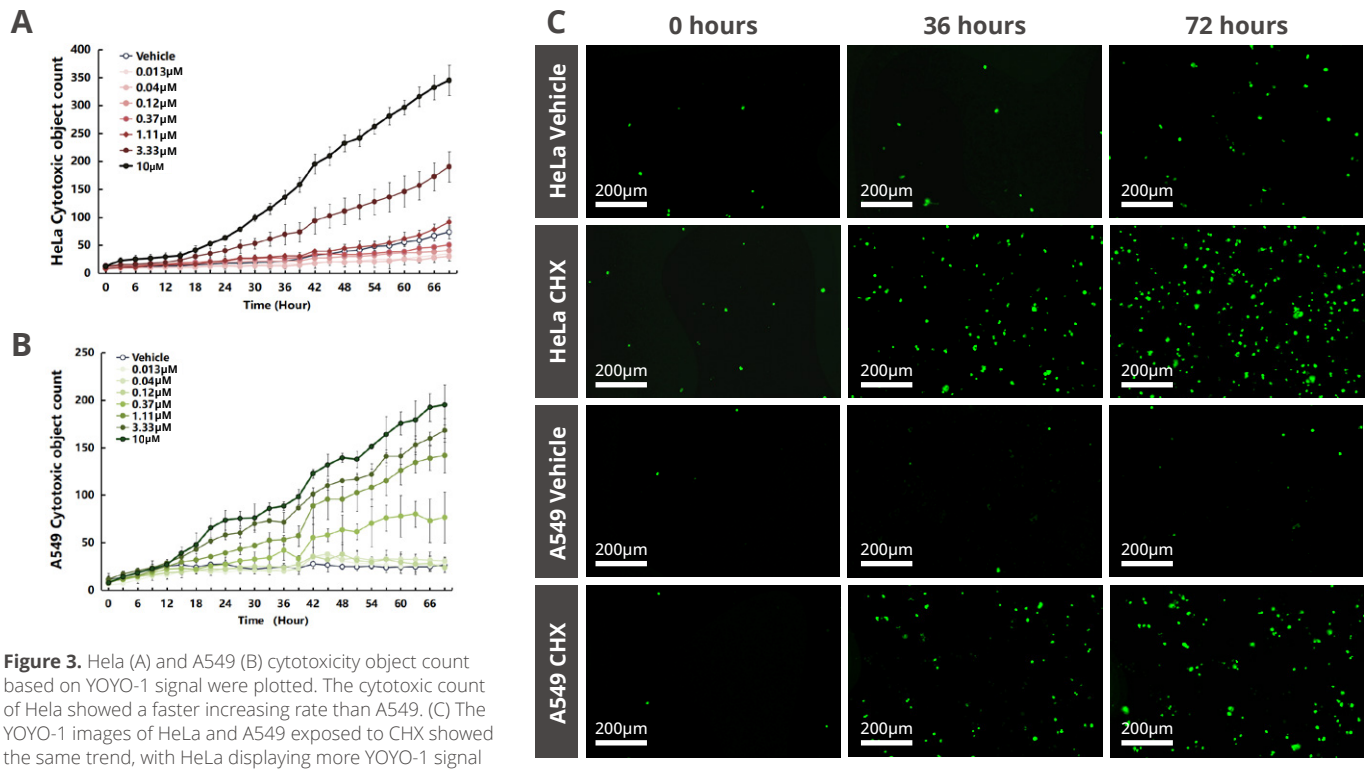


Figure 3. Hela (A) and A549 (B) cytotoxicity object count based on YOYO-1 signal were plotted. The cytotoxic count of Hela showed a faster increasing rate than A549. (C) The YOYO-1 images of HeLa and A549 exposed to CHX showed the same trend, with HeLa displaying more YOYO-1 signal than A549.

Synergistic Assessment of Growth and Death Enhances Mechanistic Interpretation

Drug-induced cellular responses often involve multiple overlapping processes, such as proliferation arrest, cytotoxicity, and morphological changes.[7] Relying on a single readout may lead to misinterpretation—for example, reduced confluence may indicate either cell shrinkage or death. By integrating confluence dynamics, cytotoxicity markers, and morphological features, the Spica M1 enables multidimensional profiling for more reliable interpretation.

In this study, HeLa cells treated with 10 μM Cycloheximide showed a rapid decline in confluence at around 12 hours, along with a marked increase in YOYO-1–positive nuclei (Figure 4A). Enhanced BF and fluorescence images further revealed cytotoxic morphology, including inhibition of proliferation and increased cell death (Figure 4B), confirming a clear drug-induced cytotoxic response. By enabling high-resolution, time-resolved analysis of proliferation, death, and morphology, Spica M1 provides confident, mechanism-relevant insights into drug action. This combined evaluation of cell health is especially useful in phenotypic screening, compound comparison, and mechanistic profiling, where certainty in interpreting cellular outcomes is essential.

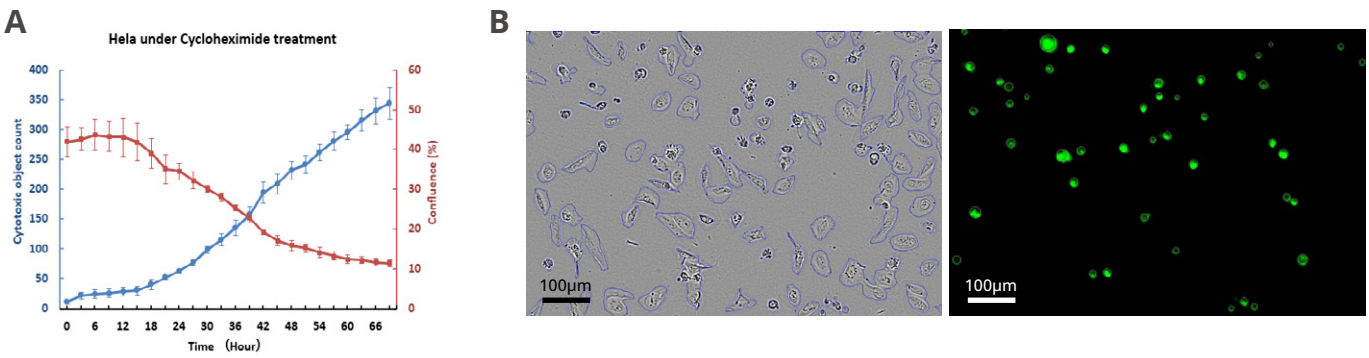


Figure 4. Combined plot of confluence and cytotoxic count of HeLa under 10μM Cycloheximide (A). The digital phase image and YOYO-1 fluorescent image of HeLa after 36h treatment.

Conclusions

This application note shows that the Countstar® Spica M1 system is an effective high-throughput platform for monitoring cell proliferation and cytotoxicity in real-time. Using label-free imaging, fluorescence-based viability detection, and AI analysis, it allows continuous assessment of cell health in various cell lines. Spica M1 allows synergistic analysis of confluence, cytotoxic objects, and cell morphology, revealing differences in drug response and mechanisms of action. It helps distinguish between growth inhibition and cell death, enabling early detection and better dosing strategies. As a non-invasive, automated, and scalable tool, it supports drug screening, compound profiling, and mechanistic research.

References

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