Quantitative Live-Cell Analysis Using Automated Long-Term Imaging

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Overview

- **BioSpa™ 8 Automated Incubator provides complete environmental control for kinetic live-cell assays spanning days to weeks.**
- **Cytation™ 5 Cell Imaging Multi-Mode Reader enables brightfield, high contrast brightfield, phase contrast, and fluorescence image capture for monitoring cells in real time.**
- **96- to 384-well plate format for robust assays and increased productivity.**
- **Gen5™ Cell Imaging Multi-Mode Reader Software offers powerful tools for automated image analysis with meaningful quantitative results.**

Introduction

Characterizing cell proliferation is a crucial aspect of biological research and therapeutic drug development. Most current cell proliferation assays rely on indirect biochemical metrics that are limited by artifacts or imaging-based endpoint measures. Here we describe a continuous live-cell assay for determining cell proliferation profiles using the BioSpa Automated Live Cell Imaging System, consisting of BioSpa 8 and Cytation 5. This fully automated method enables quantitative and phenotypic long-term analysis of cell growth using non-invasive measures of confluence or direct cell count.

To demonstrate the abilities of this system to conduct robust and reproducible kinetic proliferation assays, NIH3T3, HeLa, and HCT116 cell growth was followed for five days. All three cell types exhibited robust logarithmic growth up to full confluence with doubling times consistent with literature values. Additionally, to demonstrate the ability of this system to screen pharmacological agents, cell proliferation profiles for cells cultured with eight concentrations of two literature-standard inhibitory compounds were generated. Calculated IC_{50} values were used to measure drug response for each compound and cell type.

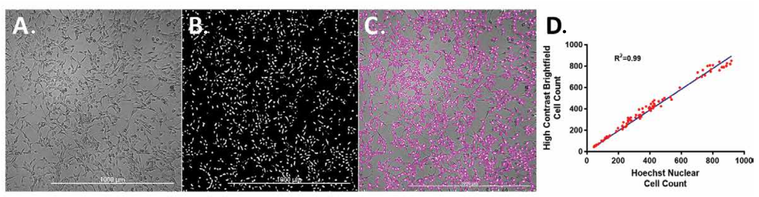
BioTek Instrumentation

BioSpa™ 8 Automated Incubator: The BioSpa8 Automated Incubator features temperature, gas, and humidity control for up to eight plates and integration with liquid handling and imaging for full workflow automation.

Cytation™ 5 Cell Imaging Multi-Mode Reader: Cytation 5 Cell Imaging Multi Mode Reader provides high-quality cellular and sub-cellular imaging in brightfield, high contrast brightfield, phase contrast, and fluorescence. Live cell imaging is optimized with temperature and CO_2/O_2 control, shaking, and dual reagent injectors.

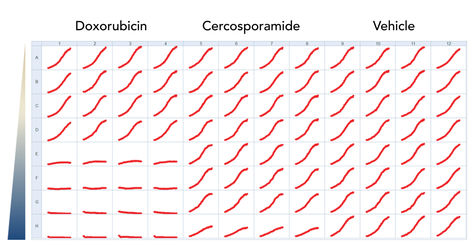
Methods

High contrast brightfield label-free cell counting



The High Contrast Brightfield Kit enables accurate label-free cell counting from a brightfield image using Gen5™ Software. **(A)** In focus image of NIH3T3 cells. **(B)** Defocused image produces a single bright spot corresponding to each cell. **(C)** Gen 5 object masking tool can readily identify each bright spot to generate cell counts. **(D)** High contrast brightfield cell counts are comparable to those achieved using Hoechst labeled nuclei across a range of cell densities.

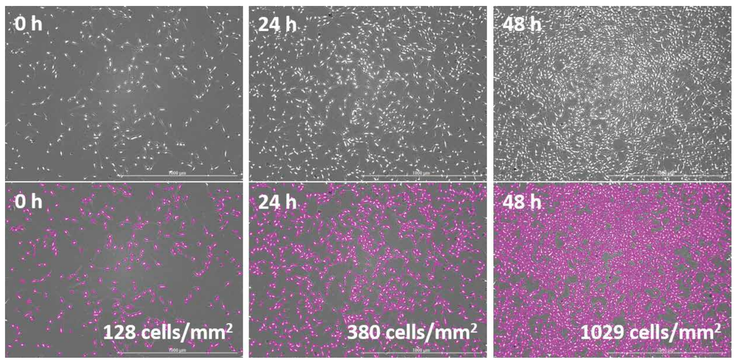
Proliferation assay



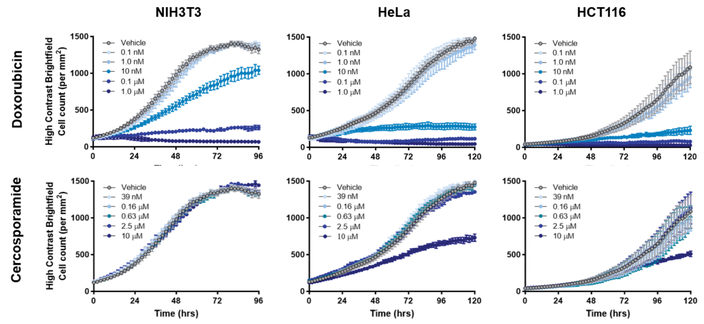
Matrix view of HeLa cell count profiles over five days with anti-proliferation drug treatment. HeLa, HCT116, and NIH3T3 cells were seeded in 96 well plates at low density and followed over five days. Cells were cultured in BioSpa 8 which routinely transferred plates to Cytation 5 for imaging every two hours. Kinetic cell growth profiles were generated in Gen5 from high contrast brightfield cell counts, fluorescently labeled nuclei counts, or percent confluence. Time and dose dependent drug responses to doxorubicin and cercosporamide were measured for each cell type. Cells were treated with serial drug dilutions at time = 0.

Results

Direct cell counts over time: Label-free high contrast brightfield

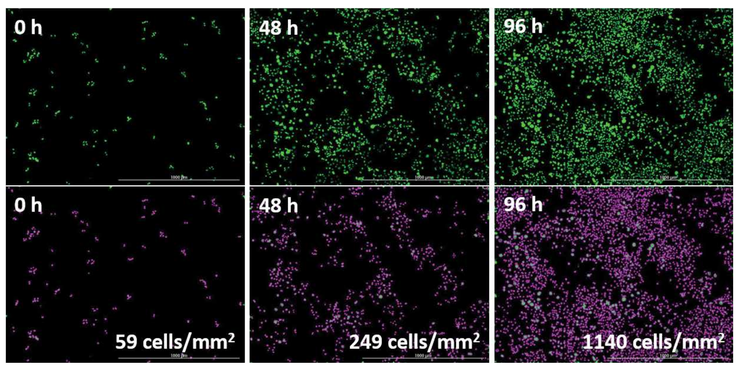


High contrast brightfield images enable accurate label-free cell counting using Gen5. NIH3T3 cultures at three time points with cell counts per mm². Each bright spot corresponding to a cell is identified among the dark background by the Gen5 Software. High contrast brightfield settings can be readily adjusted to account for differences in cell type morphology.

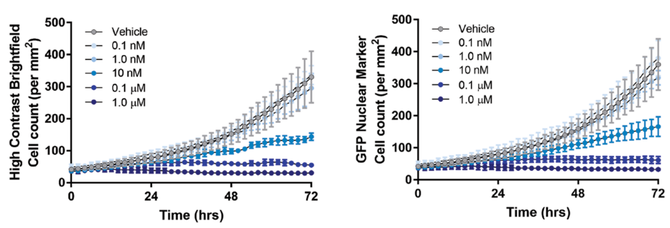


NIH3T3, HeLa and HCT116 kinetic cell proliferation profiles generated from high contrast brightfield cell counts enable quantitative analysis of drug response. Cell counts per mm² were calculated every two hours for five days or until cells reached full confluence. Profiles from five concentrations of doxorubicin and cercosporamide demonstrate a cell type-dependent differential dose response.

Direct cell count over time: Fluorescent nuclear label

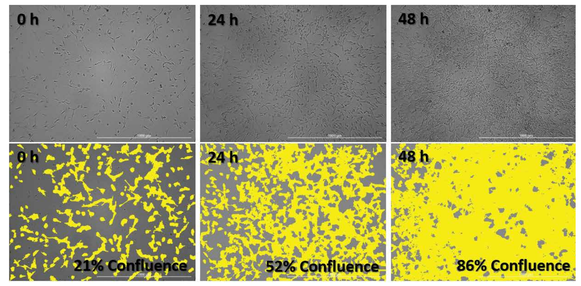


Fluorescent nuclear markers provide a robust method for identifying and counting cells for kinetic proliferation assays. HCT116 cells expressing integrated H2B-GFP were monitored for five days and imaged every two hours. Three representative time points with cell counts per mm² illustrate the ability of this method to accurately identify fluorescently labeled cells.

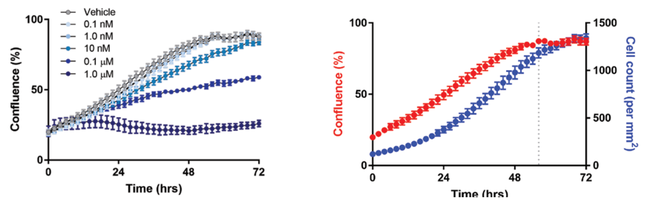


A comparison of HCT116 H2B-GFP kinetic proliferation profiles derived from high contrast brightfield cell counts and GFP labeled nuclei counts. The two cell counting methods produce highly comparable results over a range of population sizes.

Cell confluence over time: Label-free brightfield



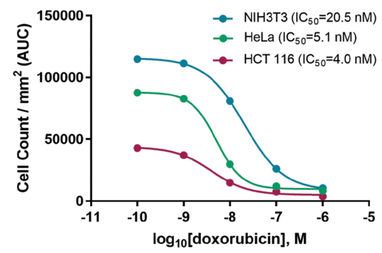
Label-free confluence measurements using brightfield images and Gen5 Software provide a direct and straightforward metric for measuring cell population size. NIH3T3 cultures at three time points with percent confluence measurements. Gen5 cellular analysis features enable accurate identification of cell outlines from preprocessed brightfield images.



Kinetic profiles of NIH3T3 confluence at five concentrations of doxorubicin. Percent confluence values were determined in Gen5 by dividing the area occupied by cells by the total area and multiplying by 100.

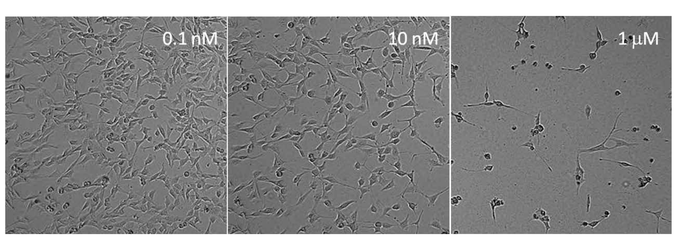
Percent confluence relative to cell counts over time. A comparison of NIH3T3 confluence and high contrast brightfield cell counts demonstrate the different characteristics of these two cell growth metrics. Cells exhibit robust cell growth up to full confluence (dashed line), at which point cell numbers continue to increase, although at a slower rate.

Quantifying dose-dependent inhibition of cell proliferation with calculated IC_{50} values



Measuring dose-dependent inhibition of cell proliferation by doxorubicin. Area under the curve (AUC) of cell count per mm² were used to calculate IC_{50} values for NIH3T3, HeLa and HCT116. HCT116 had the highest sensitivity to doxorubicin.

Kinetic phenotypic analysis of cellular response to anti-proliferation drugs



Qualitative analysis of kinetic cell proliferation provides valuable insight into phenotypic response to drug treatment. NIH3T3 proliferation images 36 hours after treatment with indicated concentration of doxorubicin. At 10 nM doxorubicin cell division is inhibited without causing overt cytotoxicity. At 100 nM and higher concentration,s signs of cytotoxicity are evident.

Conclusions

Coupling the fully automated cell-handling abilities of the BioSpa 8 with the imaging and image analysis capabilities of the Cytation 5 provides a simple and powerful system to conduct long-term proliferation assays. This method enables accurate, label-free or fluorescence label-based cell growth measurements that can be readily scaled-up to meet the needs of high-throughput applications.