### Abstract

Cytotoxicity assays play a critical role in the identification of potential chemotherapeutic agents. Commonly used in vitro assays often give conflicting results, in part due to the release of factor-dengueflavirus (DENV) and glutathione (GSH) following oxidative stress, generation of reactive oxygen species (ROS), cell proliferation, and disruption of mitochondrial transmembrane potential. Critical factors contributing to the unpredictable nature of these assays include compound composition, and more importantly, the time allowed for the compound to elicit an effect. Although these parameters can be simultaneously measured via multivariate analysis of various cytotoxicity assays, they typically assess a single-point in time and are unable to assess the biological activity over the duration of the treatment. As a result, we developed a novel, fully automated, Image-based nuclear count assay using CellPlayer™ with the IncuCyte™ FLR. This system enables the quantitation of mitochondrial, cell membrane integrity, and nuclear stain, offering a quantitative metric of cell viability over time. The objective of this study was to validate the IncuCyte™ FLR as a multi-parametric, fully automated imaging system. The high-throughput, multiday assay provides a statistically valuable method to analyze the kinetics of induction of cytotoxicity in vitro. Specifically, we used HT 1080 fibrosarcoma cells, MDA-MB 231 breast adenocarcinoma cells, and MCF-7 breast adenocarcinoma cells in conjunction with various cytotoxic agents (Doxorubicin, Camptothecin, and Cyclosporin) to illustrate the ability to accurately assess the cell viability over a wide range of determination and cell death. Furthermore, we show that the quantitative data obtained in this study is amenable to analyze throughout screening protocols with z-score values (t/d) without being affected by in vitro and inter- assay reproducibility studies. We also provide evidence that the high-depth phase contrast images provide quantitative verification of cell viability results allowing for the discrimination between cellular and extracellular components.

### Introduction

Cytotoxic response is used as a marker for the development of biochemical pathways, such as resistance or apoptosis, which results in cell death. Individuals of both sexes include the loss of membrane integrity.

**DOS** is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte. The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

### Methodology

- **Cell viability:** The IncuCyte FLR is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte.
- **Cell proliferation:** DOS is capable of encoding a cell in a standard format.
- **Cell death:** The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

### Automated Image Analysis

- **Cell viability:** The IncuCyte FLR is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte.
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- **Cell death:** The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

### Inter-assay Reproducibility

- **Cell viability:** The IncuCyte FLR is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte.
- **Cell proliferation:** DOS is capable of encoding a cell in a standard format.
- **Cell death:** The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

### Intra-assay Reproducibility

- **Cell viability:** The IncuCyte FLR is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte.
- **Cell proliferation:** DOS is capable of encoding a cell in a standard format.
- **Cell death:** The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

### Conclusions

Quantitative, reproducible, kinetic detection of cytotoxicity: Using the IncuCyte™ Live-Cell Imaging System in conjunction with DOX 100 gives the user the ability to monitor morphological changes in parallel with quantification, the combination of which is a powerful and unique tool for detecting pharmacological activity that alters cell viability.

- **MIA, m2, and micromet:** MIA 100 was added as a live-cell neighbor directly to the cultured cells. In complete growth medium, MIA allows for the measurement of cyto-metabolism in a high-precision, high-definition format.
- **Kinetic:** Allows for the detection of both short-term and long-term alterations in cell viability in physiologically relevant conditions. This feature allows for profiling cell-specific and time-dependent biological activity.
- **Automated data acquisition:** IncuCyte™ software and interface allow for automated data acquisition of phase contrast and fluorescent images that can be analyzed to both quantify the amount of and death rate in confirming the fluorescent images.

### Discrimination of Cytotoxic and Cytostatic Compounds

- **Cell viability:** The IncuCyte FLR is a cell-improving system that enhances nuclear and cell stain that leads to DIL. When added to culture medium, DOS is capable of encoding a cell in a non-fibrosarcoma fibrocyte.
- **Cell proliferation:** DOS is capable of encoding a cell in a standard format.
- **Cell death:** The study provides evidence that DOS can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.

**Figure 6:** Anti-oxidative effect and conventional chemotherapeutic agents. The IncuCyte FLR is capable of encoding a cell in a non-fibrosarcoma fibrocyte. In contrast to DIL, DOS is capable of encoding a cell in a standard format. The study provides evidence that DOX can be used as a live cell stain and not be affected by directly to cells in order to measure the kinetics of detection of cytotoxicity in a standard format.