# Introduction to live-cell analysis for cytotoxicity

**Live-cell analysis offers a powerful technique for monitoring cell proliferation and death. By acquiring images at regular intervals, cell proliferation can be measured in real-time for days or even weeks, and overall cell health can be evaluated based on morphology and growth. This information is vital for robust, reliable assay development, and allows experimental conditions to be easily optimized prior to cell treatments. The non-perturbing nature of live-cell imaging also allows these assays to be used alongside other techniques to maximize the information gained from precious samples. The Incucyte® Live Cell Analysis System offers a range of tools to visualize and quantify cell proliferation, identify specific subpopulations, and measure cell death.**

*Immuno-Oncology Insights* 2021; 2(2), 83
DOI: 10.18609/ioi.2021.017

## UNDERSTANDING CELL BEHAVIOR AND FUNCTION

The Incucyte Live-cell imaging system acquires Phase HD, brightfield and fluorescent images of cells from within an incubator, so the entire process is non-perturbing. Cells can be quantified and imaged in real-time across the entire workflow. During culture, cell health, morphology and plate uniformity can be examined. Kinetic assays can then be performed to monitor the effects of treatment – for example, cytotoxicity. Images can be acquired regularly for as long as is required, with no need to define an endpoint. After every image acquisition, images are analyzed using integrated software.

## LABEL-FREE MONITORING

Three main label-free methods are available for measuring cell growth and proliferation **(Figure 1)**. Confluence provides a percentage of the field of view that is covered by cells. Brightfield analysis allows for measurement of the area of 3D objects such as spheroids and organoids. Using cell-by-cell analysis, individual cells can be identified in 2D phase images, allowing cell count to be accurately measured over time.

## FLUORESCENCE LABELLING

Fluorescent imaging can provide even more information on cell-subpopulations and cytotoxicity **(Figure 2)**. Again, three main approaches to fluorescent labelling of cells are available. The first is to generate a stable cell line that expresses a fluorescent reporter. Secondly, a range of rapid dyes and reagents are available, which have been validated as non-perturbing, making them ideal for use in live-cell assays. These include rapid nuclear dyes, as well as reagents for detection of surface markers using live-cell immunocytochemistry. In particular, rapid dyes and reagents are useful in systems where generating a stable cell line is too time-consuming, or not possible. Lastly, a suite of cell health reagents can be used to measure cell viability and apoptosis. By combining these readouts, complex and translational assays can be developed to provide meaningful insights into cell behavior and function.

## VALUABLE INSIGHTS IN REAL TIME

Label-free imaging and quantification using the Incucyte® Live Cell Analysis System enables kinetic, non-perturbing analysis of cells in monolayer culture, along with analysis of more complex cultures, such as spheroids and organoids. Fluorescent labelling adds insight into cell subpopulations including analysis of cell cycle stage, identification of cells in co-culture, and real-time apoptosis measurements. Together, these tools provide valuable real-time insight into kinetic cell behaviors in both simple and complex multi-cellular cultures, with applications across the whole cell analysis workflow.

**Figure 1. Label-free proliferation.**

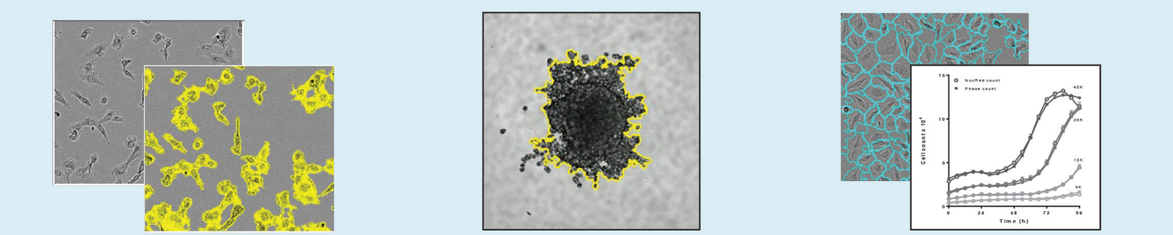
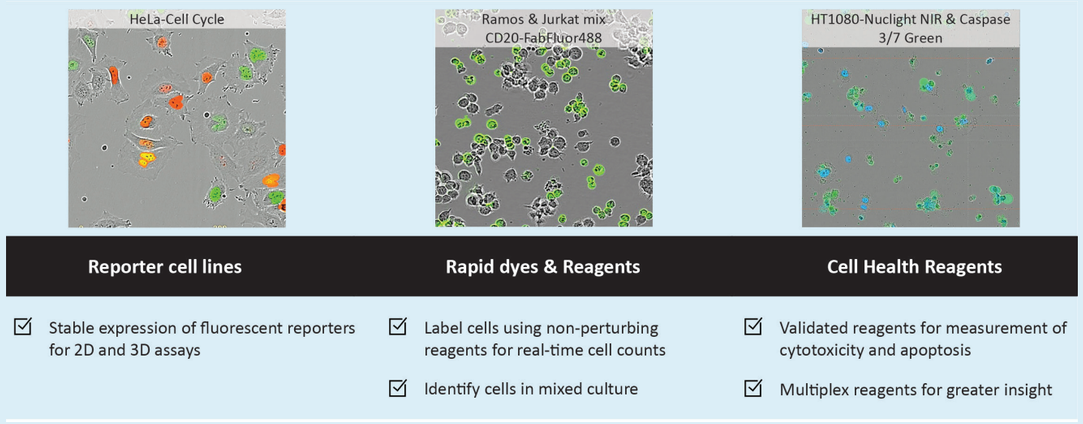



**Figure 2. Fluorescence labelling.**




IMMUNO-ONCOLOGY INSIGHTS

In partnership with: SARTORIUS