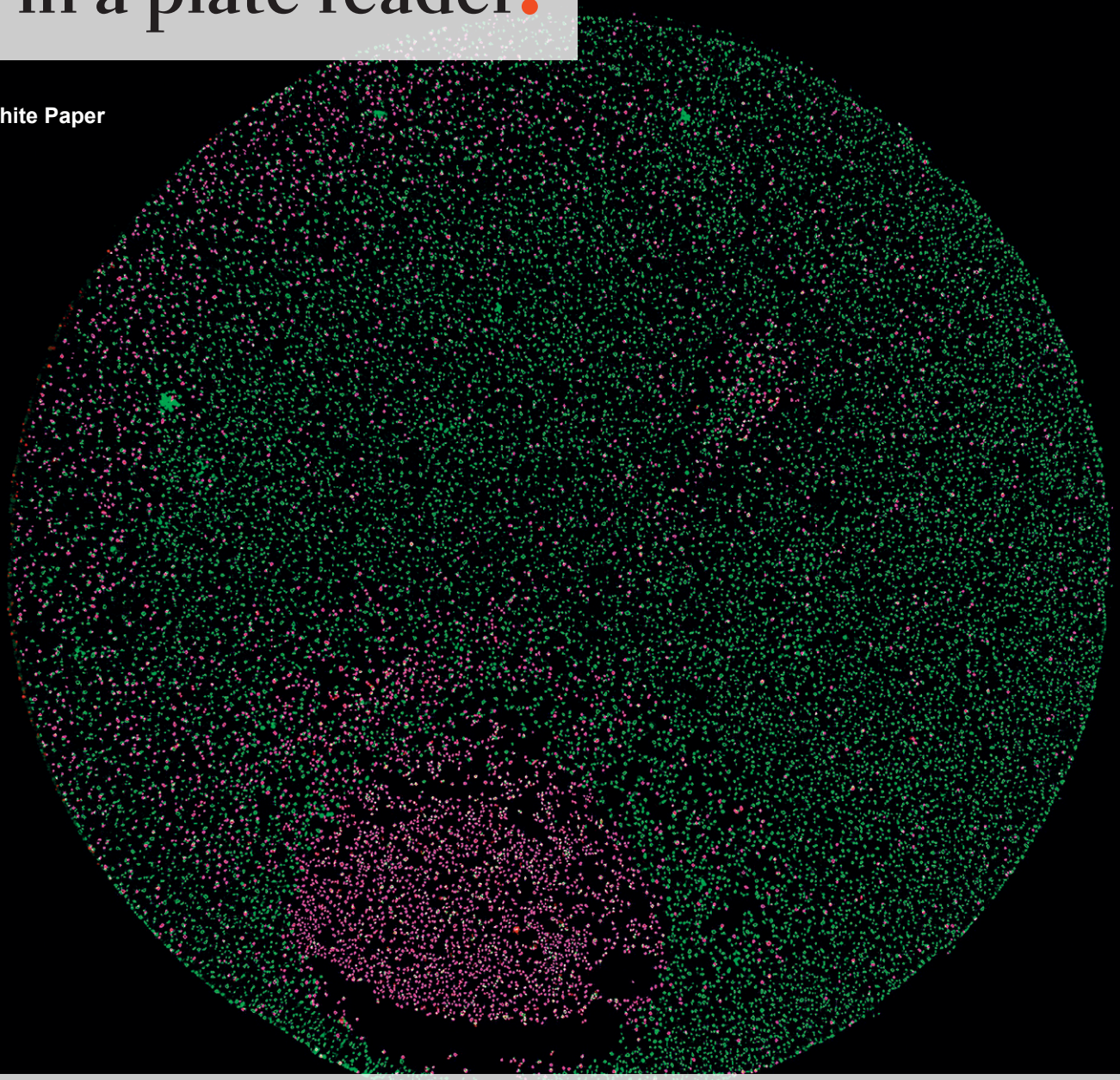
Real-time image cytometry in a plate reader.

White Paper



BRINGING TIME AND COST ADVANTAGES TO LABS.

Cell-based assays make up a large part of the workload for many laboratories. While offering substantial benefits compared to biochemical assays and in vivo animal testing, *in vitro* assays come with their own set of challenges. For instance, dynamics in cell proliferation and other biological characteristics, can vary and be unpredictable in different cell types. Furthermore, unless the cells are monitored continuously, there is the risk of missing critical events, which means that experiments must be repeated, wasting time and incurring cost. This makes it difficult to establish maintenance routines and experimental protocols that fit neatly into standard working hours. As a result, late evening and weekend work is not unusual for cell researchers.

The lack of a cell-friendly environment during signal detection and image acquisition can alter cell behavior, compromising experimental validity and reproducibility. When manual handling steps are involved in the assay workflow, there is an increased risk of error, ranging from a missing data point or the need to repeat an experiment to major problems, like the loss of an expensive primary cell line, or the need to shut down the entire lab for decontamination.

While most labs cannot afford to compromise on quality, the market is competitive, and budgets are limited. The ideal situation is, of course, to be able to increase the quality of cell-based assays while at the same time maintaining cost efficiency. This might seem like a contradiction, but new advances in cell-based technology like real-time imaging and cytometry bring higher levels of automation, reproducibility, and flexibility to cell-based research. The result is improved quality and productivity at reduced costs. Below is a review of some of the new opportunities available in live-cell imaging and cytometry.

WHY LIVE CELL ANALYSIS HAS BECOME SO IMPORTANT

Cell-based assays are an effective way to mimic the natural behavior of cells in a controlled, scalable and reproducible environment. They can offer more physiologically relevant information compared to destructive *in vitro* tests, at a dramatically lower cost than animal testing. Data collection in cell-based assays can be either at a single, defined time point (end-point assays) or at multiple time points throughout the course of the experiment (kinetic assays).

The advantage of kinetic cell assays is obvious; you can compare them to recording a video of a process instead of just taking a random snapshot of the outcome at the end. That means it is possible to get a more thorough understanding of the complex development of your cells and their responses, allowing you to observe critical events as they take place. Kinetic assays also give you the ability to make real-time decisions by better discovering the mode of action. Live-cell analysis, kinetic signal detection and image acquisition of living cells in a non-destructive way add an important dimension to cell-based assays. This enables the capture of dynamic events and sensitive processes in real-time without the artifacts that can be introduced when the cells are fixed or lysed for more conventional cell-based assays.

HOW THE ENVIRONMENT POSES CHALLENGES FOR LIVE CELL ANALYSIS

One of the main challenges when conducting assays with living cells is to keep the physiological conditions optimal; otherwise the assay may not give you a reliable or meaningful result. A common problem when working with live cells in a standard academic laboratory is the need to use many different instruments for their culture, handling and analysis. Moving plates to and from different instruments exposes the cells to changing environmental conditions that can impact their behavior in unpredictable and irreproducible ways. Fluctuations in the environment can lead to reduced cell viability or more subtle responses that may go undetected but nonetheless compromise the outcome of your experiment. In addition, moving the plates to and from different instruments critically increases the risk of contamination and is also time-consuming. Besides the practical issues associated with using multiple instruments, it can be difficult to correlate the information collected from one type of instrument with the data that comes from another.

If you are running an assay for an extended time period in one instrument, such as a multimode plate reader, integrated environmental control functions inside the detection device are beneficial in order to maintain optimal O_2, pH level (via CO_2 control), temperature and humidity levels.

HOW IMAGING TECHNOLOGY CAN EXTEND PLATE READER APPLICATIONS

Visual inspection - taking a look through the microscope - is a fundamental skill for all cell researchers. Traditionally, cell biologists use a manual microscope to quickly assess cell morphology in a qualitative way, or they run basic cell counting and viability assays to determine the quantitative and qualitative state of their cell culture. However, inspection and cell counting assays with a manual microscope are not fully reliable because they tend to be subjective and error-prone, as described in the paragraph above. Incorporating brightfield imaging and cell counting capability into a standard plate reader is thus a very useful option to increase efficiency in the lab.
While brightfield imaging can extend the functionality of your plate reader to a certain degree, multicolor fluorescence imaging capability brings significant additional advantages.

FROM WELLULAR TO CELLULAR INFORMATION: THE ADVANTAGES OF IMAGE-BASED CYTOMETRY

Fluorescent cell-based assays are typically conducted using a plate reader to collect an average fluorescence intensity measurement from the well, yielding a single data point for each sample. With this method, differences in intensity among various subpopulations within the sample entity will be missed because the signal is averaged across the whole cell population.

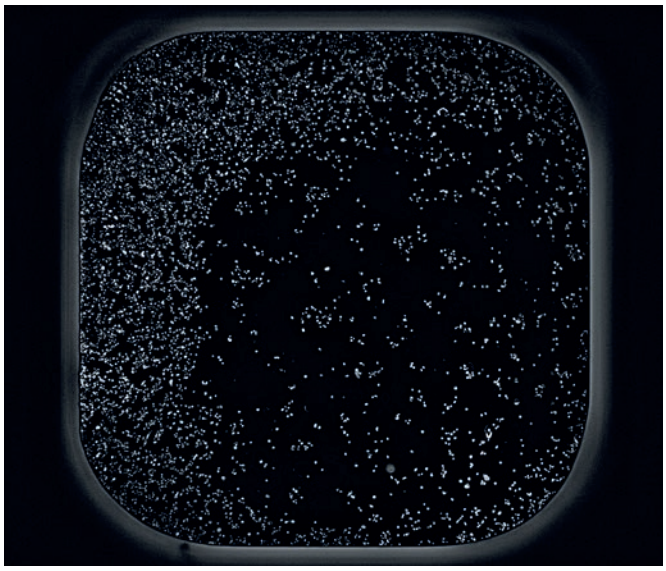


Figure 1: Whole-well view of HeLa cells stained with Hoechst 33342 cultured in a 384-well plate.

However, when working with an imaging cytometer it is possible to capture information on a “cellular level” instead of the “wellular level”. This powerful approach means that you are able to get a fluorescence intensity signal from every single cell in the well (see figure 1), yielding results similar to those obtained with flow cytometry. For example, you could evaluate fluorescent reporter activity by calculating the percentage of cells that are highly expressing a fluorescent protein such as GFP, versus the percentage that are expressing it at medium or low levels. Because an image can capture data from hundreds of cells at once, it is possible to substantially increase throughput compared to a standard flow cytometer.

Imaging might also detect events that might be missed in a plate reader or a flow cytometer and provides additional information that can be very insightful. For example, imaging can reveal information about the distribution of the cells in the well, revealing potential edge-well effects or uneven seeding. Moreover, with image-based cytometry it is possible to quantify adherent cells directly, rather than having to subject them to the harsh treatments like trypsinization, centrifugation and wash steps needed prior to flow cytometry. You can often avoid other destructive methods like toxic DNA intercalating agents or other reagents for cell mass determination or proliferation assays by using other imaging modes like brightfield or phase contrast. Additionally, you can monitor all your cells in real time, and the images can be stored for future reference and re-analysis to answer new questions.

A BROADER AND MORE FLEXIBLE VIEW

An important feature of image-based cytometry applications is the high quality of the images. The system should preferably be able to capture whole-well images so that you can get high-quality information from all cells inside the well. Since the 96-well plate is the most commonly used microplate format in academic laboratories, the ideal is to image a 96-well plate in one single photo (see figure 2).
If images need to be composed, it is critical that the system includes precise and accurate x-y positioning for image acquisition. Furthermore, most imaging systems are used for many different types of assays and cells. Hence, the system must be able to identify cells of varying sizes, densities and contrast levels.

In order to do that, the system needs intelligent detection algorithms that are easy to optimize for each cell type and application, to give you consistent and reliable results from all of your experiments.

Figure 2: Whole-well view of a 96-well in brightfield, recorded in one field of view – shadow effects resulting from the liquid meniscus are compensated for.

Up until now, many standard academic laboratories have relied on both a microplate reader and an imager or flow cytometer to monitor cell cultures and run cell-based assays. For the reasons discussed, some instrument providers are pioneering the integration of these functionalities into single-instrument solutions. Microplate reading paired with good fluorescent cell imaging capabilities is a relatively novel combination that can improve cell-based analysis considerably.

REAL-TIME IMAGING AND AUTOMATED EXPERIMENTAL CONTROL: THE FUTURE OF CYTOMETRY

Endpoint assays come with the disadvantage that critical events might be missed because the measurement instrument is being used by someone else, or they have taken place overnight when you were not in the lab, reducing the overall informative value of the experiment. Real-time experimental control, in contrast, gives you the full picture: you know when your cells have responded to a particular treatment, or when they are ready for certain stimuli or reagent additions. Combining real-time experimental control with real-time imaging technology is a convenient way to solve this challenge.

The importance of real-time experimental assessment (see figure 3) for the accurate characterisation of cells and cellular events cannot be overestimated. Real-time information and decision-making can give you much greater control over your cell assays.

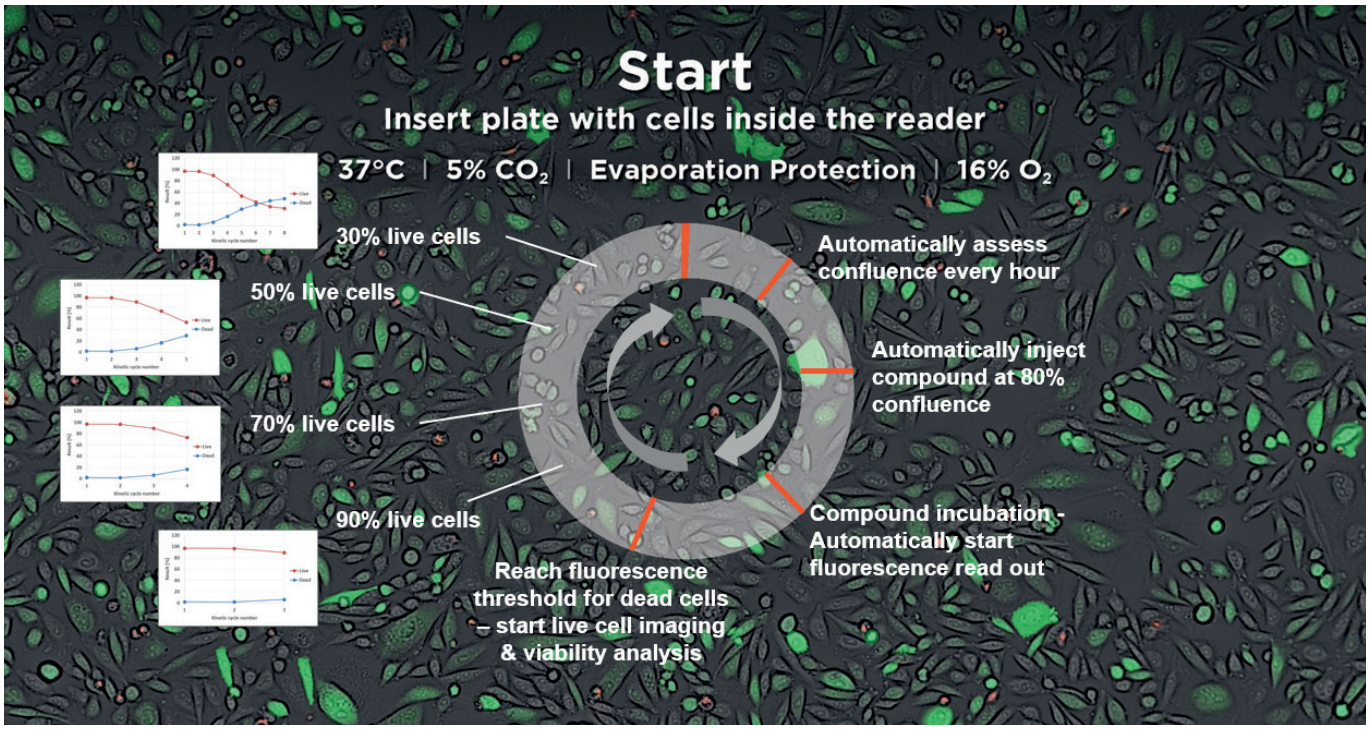


Figure 3: Real-time experimental control - a fully automated workflow in the instrument's control software allows hands-off kinetic studies, for example, cell viability (Calcein-AM / Propidium Iodide stain).

For example, you may want to interrupt or stop an experiment because it isn't developing as expected. Instead of recording images over two or three days and then doing the analysis at the very end of the process, you can analyse cellular responses as they happen and take action immediately.

Standardizing the workflow in a controlled environment is also vital for reliable experimental results. Features like automated lid-lifting and dispensing systems, gas and temperature control, and evaporation protection, help you decrease the risk of having to repeat an experiment due to external environmental fluctuations.

An important advance in cytometry, workflow automation and live cell analysis is *kinetic scheduling or kinetic conditioning*. It means that you can allow certain actions, e.g. reagent additions, to be performed as soon as a particular data value is reached. This enables full walkaway automation of workflows, even when you are not in the lab. For example, it is possible to define a criterion for a reagent to be added automatically when the culture has reached a certain confluence or to record data only within a certain range of interest. This way you can automate almost all your workflows.

All labs have limitations in terms of time and resources, but today labs have access to technology and equipment that have not previously been available. By combining multiple cell analysis capabilities into a single platform, labs can increase both quality of their data and the overall productivity in their lab. Paramount to realizing these benefits is the ability to automate the workflow and avoid unnecessary mistakes and repeated experiments. New advances in real-time image-based cytometry and experimental control, combined with fully integrated automation and multiplexing capability, bring great benefits to the understanding of cells – and ultimately of life.

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