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Article

Tips and Tricks for Fully Leveraging Advanced Flow Cytometry

Introduction

Driven by the need for models that allow better predictions of clinical success, drug discovery researchers are turning towards more complex cell-based assays. To assess compound candidates and investigate disease pathophysiology, modern *in vitro* studies now feature a range of cell types (e.g. induced pluripotent stem cells, patient-derived cells from healthy and diseased tissue) and models (e.g. organ-on-a-chip devices and 3D co-cultures), designed to improve physiological relevance over conventional 2D monolayers. However, the success of these systems is dependent on assay technologies and workflows that can support adaptable, high-throughput, and multiplexed analysis.

Flow cytometry remains a powerful tool for cell-based assays, providing information on a cell-by-cell basis. However, traditional flow cytometry systems are not designed to keep up with the high-throughput demands of modern drug discovery, leading to a disjointed and inefficient workflow. To address the urgent need for assay technologies that are compatible with modern drug discovery, flow cytometry design has been transformed in recent years. This article highlights the features of advanced flow cytometry, such as assay miniaturization and automation capabilities, and demonstrates how it can dramatically increase throughput while making it easier to obtain biologically relevant insights.

Sample Cells and Beads in the Same Well for Increased Biological Relevance

Bead-based immunoassays read by flow cytometers have become an industry standard, where uniquely coded beads are used as a solid support matrix for molecular interactions, assessed by fluorescence-based measurements (1). Despite the growing capabilities and sensitivities of bead-based assays, beads and cells are typically analyzed separately; cell processing, followed by bead analysis. With advanced flow cytometry, however, multiple assays can be performed concurrently in a single well of a microtiter plate. As illustrated in Figure 1, multiple assay principles for cells and beads can be combined to assess cytokine secretion, cell health, immunophenotype and proliferation – in a single well. The concurrent analysis of cells and supernatant leads to better data coherence, while allowing for a streamlined workflow. This improved efficiency, achieved using an intuitive platform, makes it easier to identify trends and hits that meet your specific selection criteria.

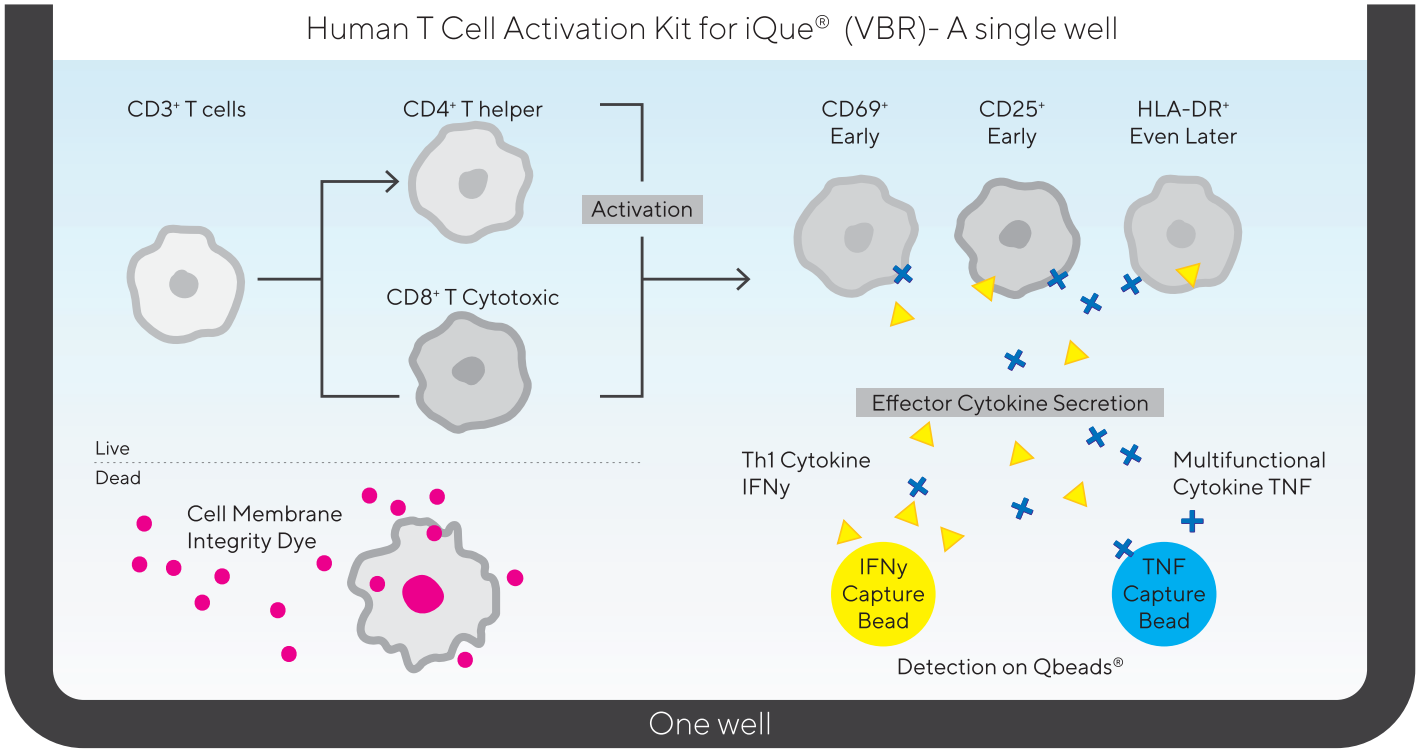


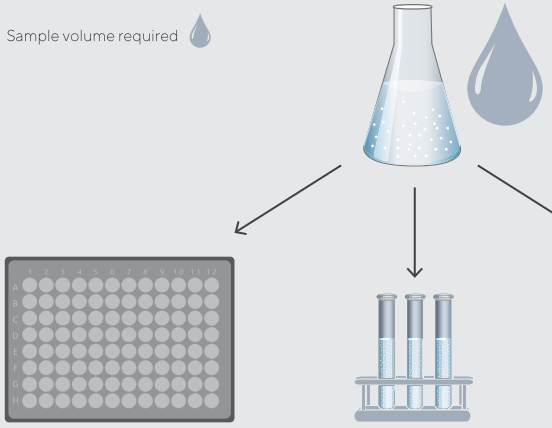
Figure 1: Illustration of Human T Cell Activation Cell and Cytokine Profiling Kit assay principles. Different T cell phenotypes are profiled for the expression of three early/late activation markers: CD69 (early), CD25 (late), and HLA-DR (even later). The two effector cytokines (IFN and TNF) are also quantified using 2-plex QBeads in a sandwich immunoassay format in the same well. Simultaneous measurement of T cell proliferation or encoded target cells is possible but is not included in this illustration.

Collapse the Workflow to Streamline Analysis

Like many other conventional techniques, standard flow cytometry has a limited ability to handle large sample numbers, and multiple analytical instruments are often used to obtain data related to cell health, function and type (2). While immunophenotyping and quantification of secreted protein can be achieved through the use of plate readers, traditional flow cytometry and ELISAs, stitching data together from separate instruments and samples is tedious and inefficient. Advanced flow cytometry platforms remove the need to export data for analysis, visualization and interpretation – as is required in traditional flow cytometry. Combining data from multiple analytical methods also presents challenges related to accuracy and confidence; each technique has its own limitations and error margins, while multi-step operations require efforts from multiple laboratory personnel and samples, further increasing the opportunities for error.

Traditional Workflows

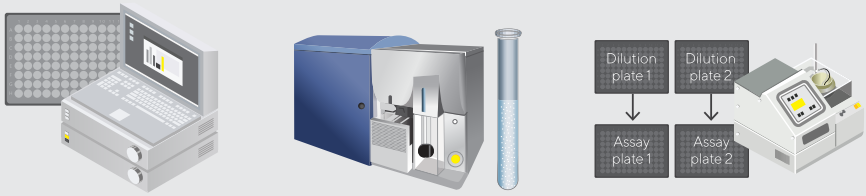
Sample volume required



Transfer cells to microtiter plate

Transfer cells for immunotyping

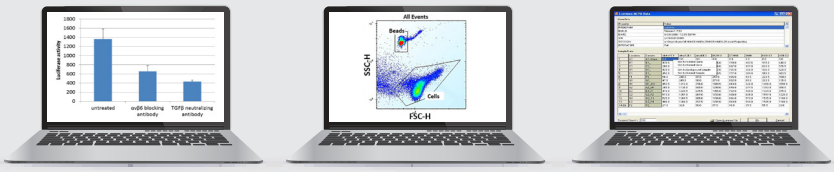
Centrifuge cells and transfer supernatant to dilution plates



Cell viability and proliferation

Cell phenotyping

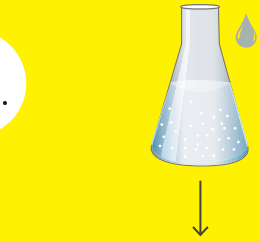
Cytokine analysis

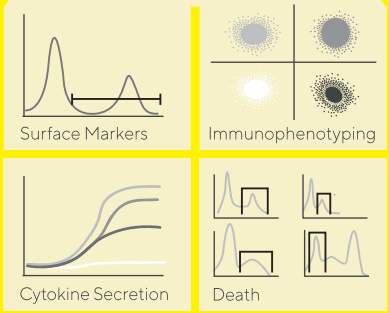


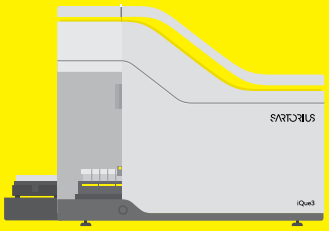
Data merging and analysis with multiple software packages

Vs.

Advanced Flow Cytometry Workflow







Cytokine profiling, Cell health and proliferation, and Immunophenotyping

Reduce Sample Volume for Greater Experimental Flexibility

Reducing the sample volume required for analysis in flow cytometry systems enables a greater number of samples to be analyzed in each run, decreasing overall processing times and the cost of reagents. However, the benefits of assay miniaturization run deeper, and significant design efforts have been directed at advancing sampling technology to maximize these benefits. Advanced flow cytometry systems can now analyze samples in volumes as small as 4 µL (in 1536 well plates) and 10 µL (in 96 and 384 well plates), with no requirement for dead volume. Furthermore, volumes as low as 1 µL can be taken from 10 µL samples if required. This is in stark contrast to traditional flow cytometers, which are largely designed for tube-based sampling – requiring larger sample volumes and allowances for dead volume.

Microvolume sampling is particularly beneficial where sample volume is inherently limited, such as in advanced cell models (e.g. microphysiological systems), or in precious patient-derived tissues. The drastic reduction in volume requirement creates room to analyze samples at different concentrations, obtain triplicate measurements, include more controls, or generate broader EC50/IC50 curves. This greater flexibility allows researchers to have more confidence in their data and decision making. For example, profiling mediators of T cell receptor activity is critical to the development of vaccines, cancer therapies, and treatments for autoimmune disorders – requiring the evaluation of multiple parameters. Figure 2 provides an overview of a simple workflow used to detect antibodies and assess T cell proliferation and membrane integrity in a single experiment. When using reagent kits that are optimized for your assay on the iQue®3 Advanced Flow Cytometry platform, there is no need for labeling, color compensation or reagent optimization steps, further reducing sample and reagent use.

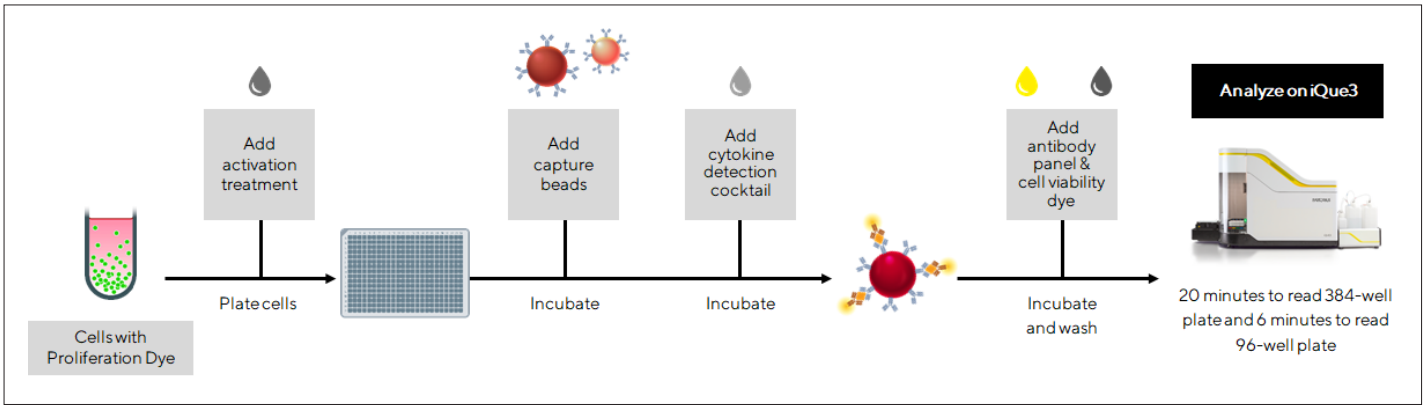


Figure 2: An overview of the steps involved in sample analysis using the iQue3.

Use Templates and Automation to Fast-Track Data Acquisition

Data management is one of the major challenges of modern research in the life sciences; running high-throughput experiments is of little benefit if there is a tight bottleneck at the data acquisition/analysis end of the workflow. The ability to quickly set up an experiment is an important foundation to achieve streamlined data acquisition, yet traditional flow cytometry requires a high level of expertise and attentiveness to do so. Dynamic ranges built into advanced flow cytometry systems remove the need for photomultiplier adjustments, while the ability to select a desired order of sampling enables optimal use of the microtiter plate. Experiments can be saved as templates for future use, which saves time at the beginning of subsequent experiments and helps maintain consistency around parameters such as gates, color compensation and data metrics. With this function, researchers can open their saved template and "plug and play" their data acquisition and analysis, rather than tediously selecting the appropriate parameters every time.

Compatibility with automation is a key consideration for modern laboratories seeking improved reproducibility and traceability. Therefore, the iQue®3 Advanced Flow Cytometry platform has been designed to allow connection with automated plate loaders and other sample preparation instruments, allowing assays to be set up and analyzed without the need for human intervention. The iQue®3 can be integrated within larger Biosafety enclosures in laboratories that exceed Biosafety Level 2 and is purpose-built for drug discovery. Automated tracking of quality control (QC) measures helps ensure high quality data is obtained, as potential instrument performance issues can be identified prior to running precious samples. To further streamline the workflow, third party automation controllers can access Forecyt® software remotely to perform a range of tasks related to:

- Instrument maintenance
- Experimental setup
- Data acquisition
- Data management

Harness Novel Visualization Tools to Expedite Data Analysis

The automated resolution of cells and beads in the same well streamlines data collection, which is further complemented by a comprehensive data analytics program. In an age where remote working capabilities are essential, the ability to access and analyze laboratory data remotely is critical to maintaining collaborations and momentum in the laboratory. Advanced flow cytometry systems feature multi-license options, so that reports and publications can be generated from the convenience of the home or office. Forecyt, the system's software and automated, template-based analysis, is one of the greatest strengths of the iQue®3 advanced flow cytometry workflow. The Forecyt® Enterprise edition enables remote access and secure data sharing for multiple users, so that users can set up, run and analyze experiments from any PC connected to the network. To fast-track data downloads, lightweight modes can be selected for data intensive files.

Novel visualization and analytical tools have been created to ease the traditionally daunting task of cell gating Complex gating strategies are invaluable to data analysis, as they make it easier to visualize distinct cell populations of interest while removing the tedious aspect of the job. Forecyt's novel visualization tools include:

- **Plate View:** visualize a plate in its entirety to identify sample characteristics more quickly
- **Heat Maps:** visualize a single metric across multiple plates
- **Profile Map:** identify wells with specific user-defined characterizations (based on multiple metrics), and follow-up with additional tools for further analysis (Figure 3)
  - **Slider Bars** (a Profile Map feature)**:** allow for real-time adjustment and visualization of wells meeting newly created criteria

Screening small molecules, antibodies, and other biologics often requires many plates, which easily add up in screening studies designed to assess a large number of compounds or biologics, and in profiling experiments which often require analysis over a number of days. The efficient comparison of flow cytometry data acquired on different days can present logistical challenges. Therefore, advanced visualization tools have been developed to make this process easier and faster. Using the Panorama feature on Forecyt Software is an effective way to quickly visualize large amounts of multiplate data. Near real-time interpretation of individual well and plate data can be achieved through the use of profile maps, heat maps and line graphs, viewed with the Panorama software feature.

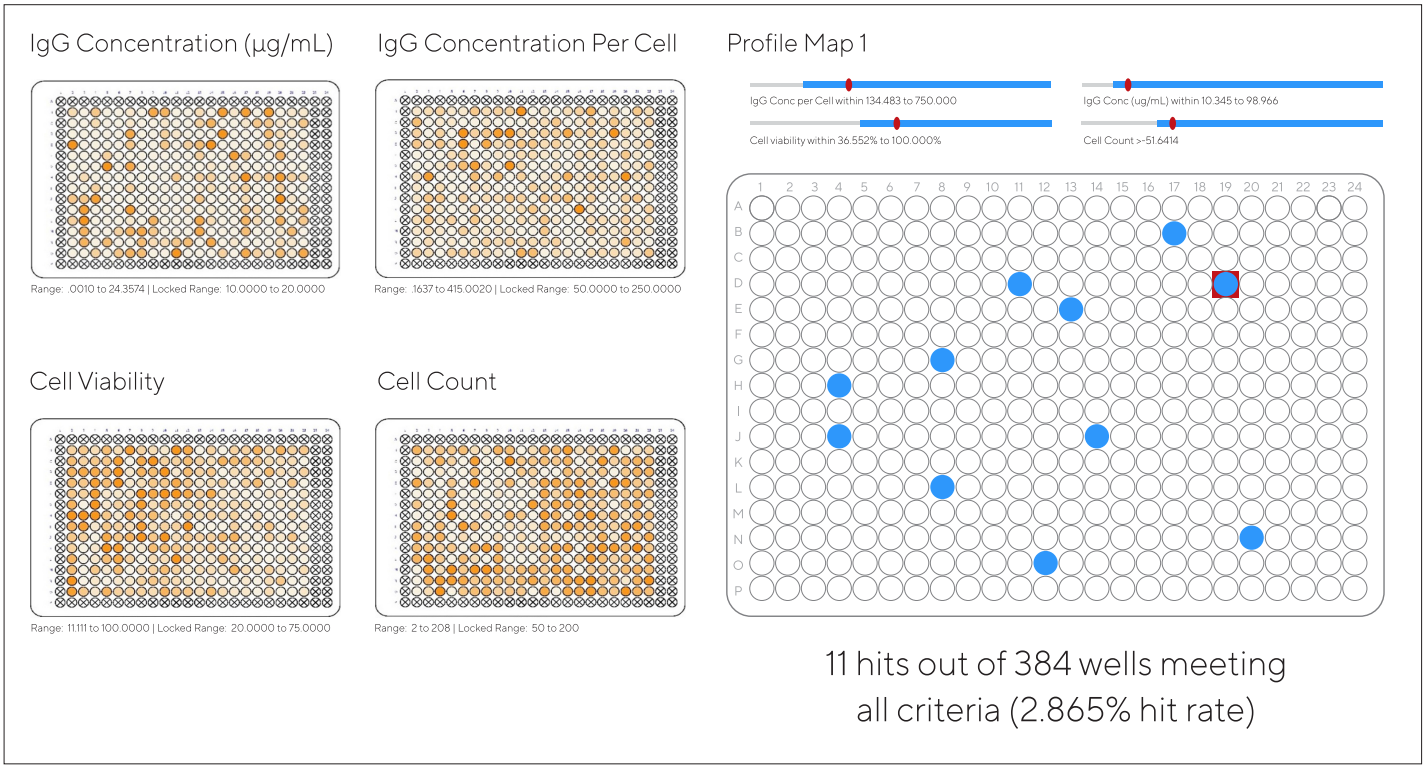


Figure 3: Profile Maps provide a single, easy-to-interpret visualization tool by combining multiparametric data to identify samples that meet user-defined criteria.

A Simpler Workflow with More Insightful Data Analysis

Modern drug discovery research laboratories are under increasing pressure to produce biological insights that translate to success in the clinic, yet conventional flow cytometry methods are not designed to provide the high-throughput support that is needed. Advanced flow cytometry dramatically collapses the workflow, which reduces variability, saves time and money, and provides the flexibility to create better experiments that generate more biologically relevant data. Critically, the simultaneous assessment of phenotypes and cytokines in the same well removes the need to stitch data together from multiple instruments.

This streamlined approach and novel visualization tools decrease the time to actionable results and allows researchers to have greater confidence in their data.

References

1. Surviladze, Z., Young, S. M., & Sklar, L. A. (2012). High-Throughput Flow Cytometry Bead-Based Multiplex Assay for Identification of Rho GTPase Inhibitors. In F. Rivero (Ed.), Rho GTPases (Vol. 827, pp. 253–270). Springer New York. https://doi.org/10.1007/978-1-61779-442-1_17
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