

Eliminating Data Analysis Bottlenecks with iQue® Forecyt® Software Simplifying Progress



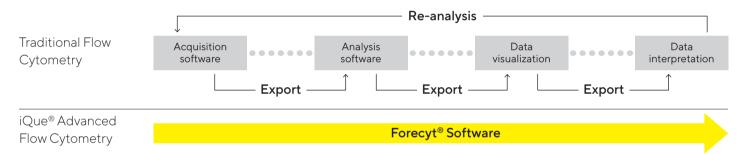
# Interactive Assay Development, Analysis and Multiparametric Data Visualization

Forecyt<sup>®</sup> software, at the heart of the iQue<sup>®</sup> platform, provides a fully integrated yet easy-to-use interactive assay development, analysis and visualization environment, removing the need to manage and learn multiple software packages.

Alongside all the required standard analysis tools required for flow cytometry data, Forecyt<sup>®</sup> software offers plate-level annotation and data visualization tools not found in traditional flow-based software programs. From the linking of data from multiple wells to the combination of several assay outcomes to identify "hits" that meet fully customizable selection criteria, Forecyt® software significantly shortens your time from sample acquisition to the generation of decision-making insights.

Forecyt<sup>®</sup> software seamlessly and intuitively performs tasks from acquisition to analysis without the need to perform any export | import steps, taking you to actionable answers that will progress your research programs faster than any other flow based solution.

### Workflow Comparison



Traditional flow cytometry data analysis workflows versus one unified workflow using Forecyt<sup>®</sup> Software on the iQue<sup>®</sup> 3. Traditionally scientists have been required to export and import data between several software packages in order to go from acquisition to analysis to data visualization and even to statistical packages for interpretation of their data. Forecyt<sup>®</sup> software provides all of this functionality in a single easy-to-follow workflow to get to actionable answers in record time.

## Forecyt<sup>®</sup> Configuration Options–Scalable to Meet Your Growing Needs



#### **Standard Edition**

A standalone solution which comes with each instrument. This can be used to set up experiments, acquire data and analyze results on the PC attached to the instrument.



#### **Offline Edition**

Ideal for users who want to analyze data away from the laboratory. No network connection required. The user's PC hosts its own Forecyt® database, which requires the manual import | export of experiments and templates to | from the instrument. This version has no acquisition capabilities, and is for experiment set-up and analysis purposes only.



#### **Enterprise Edition**

A scalable solution for multi-user or multiinstrument environments. Multiple clients connect directly to the Forecyt® database over your network using floating licenses, eliminating the need to import | export data and allowing multiple users to set up, run and analyze experiments simultaneously from any PC connected to your network. Single or multiple additional floating licenses can be added as your demand increases.

## Simple, Intuitive Experiment and Data Acquisition Set-Up

Forecyt<sup>®</sup> software lets you rapidly customize your sample acquisition, probe rinsing, plate shaking, and sampler cleaning parameters, with user-friendly set-up tools.

Software monitoring of rinse station fluid levels: A new Enhanced Rinse Station (ERS) has been incorporated in the iQue® 3. Combined with Forecyt® software integration, it provides intelligent reagent level monitoring and reporting to help avoid reagent insufficiencies.

Design experiment tab (right): Define sample type | location and additional annotation layers such as dilution series, cell types and other parameters that can be used in downstream analysis.

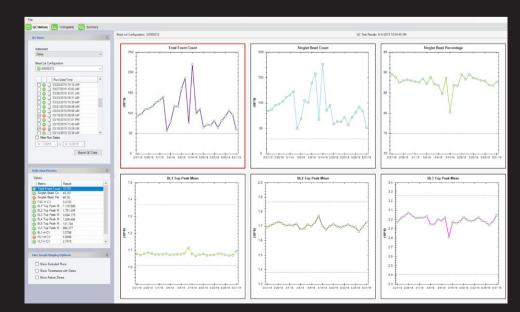




Acquisition settings and worklist (left): Intuitive and user-friendly acquisition panels facilitate quickstart experimentation set-up.



Automated data analysis and tracking of QC functionalities allows identification of potential instrument performance issues before running precious samples.



## Powerful Data Analysis and Visualization Tools

Forecyt<sup>®</sup> software provides all standard flow cytometry analysis tools, and incorporates plate level analysis and visualization features that accelerate and simplify analysis and interpretation of large, multiparametric data sets. Unique analysis tools quickly transform even the most complex experimental data sets into actionable results, and eliminate the need to perform time consuming analysis of data files on a well-by-well basis.

## Advanced Gating Tools

Gating on the cells of interest within data sets can be a tedious and sometimes daunting task. Forecyt<sup>®</sup> has built-in gating tools which help simplify the identification of the correct cells, and can even automatically adjust gates when needed through the use of smart gates.

#### Smart Gates

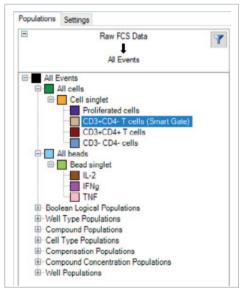
Smart Gates automatically center themselves on the closest dense area on the graph, and are an invaluable update if you have a multiparametric experiment with a cell population that may shift over time. Any time the data updates, changes, is cleared, or more data are added, the Smart Gate will calculate its new position.

#### **Back Gating**

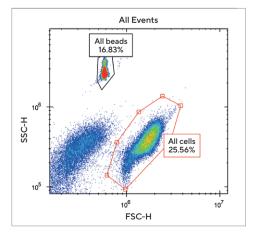
Back Gating is a feature that identifies where the cells within a gate are located in the all events plot. Any gate can be used as a Back Gate, and multiple Back Gates can be used simultaneously to visualize distinct populations of interest all on a single plot.

### Opti-Gate: Interactive Gating with Data Updates in Real-Time

Simple and rapid analysis optimization by gating on an entire plate of data at once using control wells to guide desired gating strategy. Changing gate position results in data being updated in real-time, allowing gating strategy effects to be visualized immediately across the entire plate.

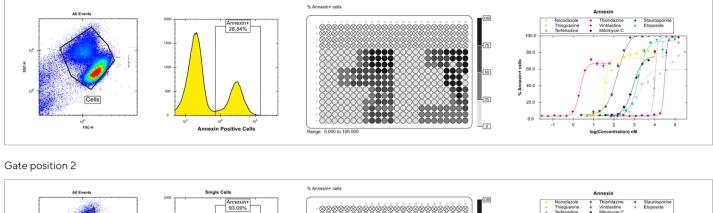


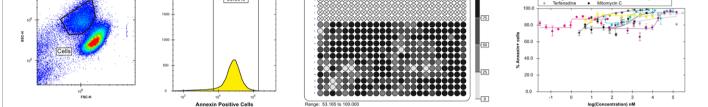
Smart Gates can be applied to any gate from the plot itself or within the population tree.



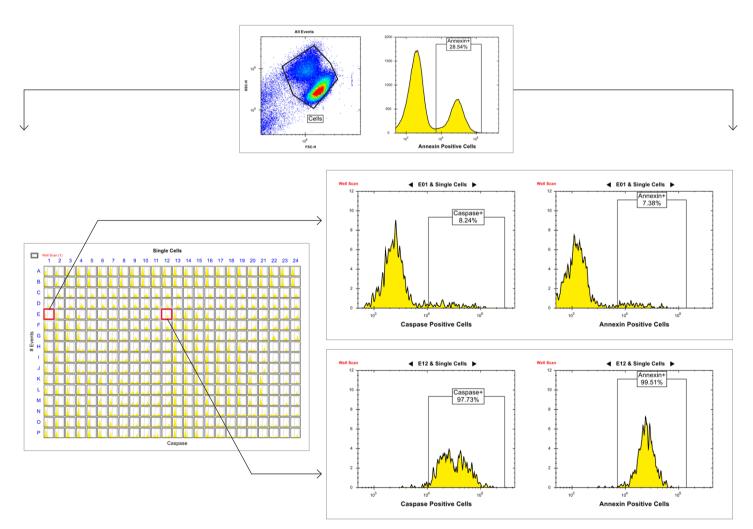
Single step population verification: Identify where the cells within your gates fall in the all events plot.

Gate position 1





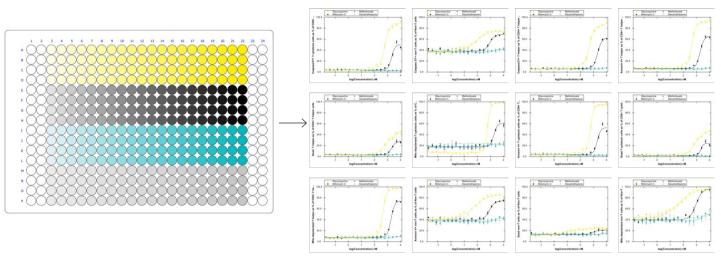
Interactive gating with real-time results: Simply "drag and drop" or adjust gate shape, size, or position, and data updates in real-time according to changes in gate position.



Data visualization linked by well: A unique tool that allows the linkage of multiple data views to rapidly verify active wells from large data sets. Data can be viewed at the plate level using dot plots and histograms. Well scan allows users to easily drill down to the individual well level data by simply clicking on the wells of interest.

## Simple Dose-Response Curve Generation

Forecyt<sup>®</sup> software is the only flow cytometry analysis package that can automatically generate dose-response curves, providing a faster time to result by eliminating the need to export your raw data into a separate software package. When linked together with scatter plots or histograms, the impact of changes to gating strategies on dose response curves can be instantly visualized.



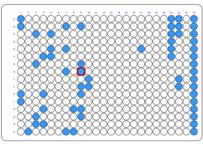
Dose-response curves for four compounds which induce apoptosis in PBMCs: PBMCs were immunophenotyped into three subpopulations and dose-response curves generated for each population for caspase positive, Annexin V positive, membrane permeability and mitochondrial depolarization using a curve fitting algorithm. Quickly generate dose response curves for all experiments across an entire plate.

## Instant Active Identification with Information Profile Maps

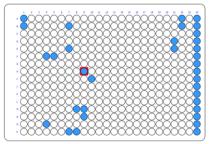
Make sense of high content data and identify samples with desirable activity fingerprints faster. Forecyt® combines assay outcomes (profiles) with Boolean logic to instantly locate specific wells that meet your multiplexed criteria using Profile Maps. User-definable profiles can be easily created by selecting from any metric created during upstream analysis. Dynamic adjustment of activity ranges within the selected metrics enable real-time hypothesis testing or optimization of activity ranges to align unknown samples with positive and negative controls.

Profile map: By changing the slider bar specifying the acceptable limit of a chosen parameter, different wells with corresponding characteristics can be rapidly identified. Here, the selected well remains constant, but as the slider bars are adjusted, different wells of interest across the plate are highlighted based on the expression of each parameter.

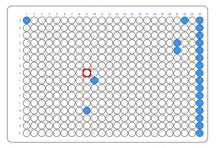
### Immunosuppressing Compounds



59 hits out of 384 wells meeting all criteria (15.36% hit rate)



34 hits out of 384 wells meeting all criteria (8.85% hit rate)



22 hits out of 384 wells meeting all criteria (5.73% hit rate)

Median BL2-H of IL-2 ≤ 125,580.5940

Median BL2-H of TNF ≤ 178,235.6016

Proliferated CD3+CD4+T cells as % of CD3+CD4+T cells ≤ 70.69%

Median BL2-H of IFNg ≤ 121,161.2585

Proliferated cells-% of cell singlet ≤ 65.9%

Proliferated CD3+CD4-T cells as % of CD3+CD4-T cells ≤ 72.76%

#### Median BL2-H of IL-2 ≤ 103,040.0281

Median BL2-H of TNF ≤ 120,043.1120

Proliferated CD3+CD4+T cells as % of CD3+CD4+T cells ≤ 21.60%

Median BL2-H of IFNg ≤ 77,255.9415

Proliferated cells-% of cell singlet ≤ 32.08%

Proliferated CD3+CD4-T cells as % of CD3+CD4-T cells  $\leq$  36.21%

Median	BI 2-H of	-2 < 103	040.0281

Median BL2-H of TNF ≤ 61.850.6576

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Proliferated CD3+CD4+T cells as % of CD3+CD4+T cells ≤ 22.58%

Median BL2-H of IFNg ≤ 77,255.9415

Proliferated cells-% of cell singlet ≤ 32.39%

Proliferated CD3+CD4-T cells as % of CD3+CD4-T cells ≤ 18.28%

### Combined Analysis of Multi-Plate Experiments with Panorama

The Panorama feature in Forecyt<sup>®</sup> allows you to see a snapshot of your entire experiment. View multiple plate data simultaneously to compare heat maps, identify hits across profile maps, and rank wells by any metric with user-definable line graphs.



Panorama view: Easily visualize "hits" across multiple plates according to different metrics. In this example 10 plates were analyzed simultaneously using the Cyclone Plus kit which correlates IgG titer-per-cell with cell health information and cell growth readouts. The wells which meet the user-identified selection criteria are highlighted instantly for each plate.

### Custom Metric Creation and Report Generation

#### **Custom Metric Creation**

Create custom metrics derived from basic metric building blocks using a simple wizard. Whether standard or custom, all metrics are available for additional analysis, visualization and | or exporting.

#### **Report Generation**

A report generation wizard makes it easy to document your work in electronic notebooks and share it with your team. Forecyt<sup>®</sup> makes your data presentations quick and easy with high quality image exporting options up to 1200 DPI. Images can be drag-and-dropped directly into any Microsoft Office application, ensuring your presentations or reports truly reflect the data observed in Forecyt<sup>®</sup>.

Customize metric output using Forecyt®'s software wizards.



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Count of	proliferated CD3+CD4+ T cells
Count of	as a percentage of CD3+CD4+ T cells

## Ordering Information

iQue® Forecyt® Software and Tools	Cat. No.
iQue® Forecyt® Enterprise Floating Client License (1)	60028
iQue® Forecyt® Enterprise Floating Client Licenses (5 Pk)	60029
iQue® 21 CFR Part 11 Software Tool License	60035
iQue® Forecyt® Offline Edition for single PC	90272
iQue® Forecyt® Enterprise Including 5 Floating Client Licenses	90212
iQue® Robotic Integration API License (1) for iQue® 3, iQue® Screener; iQue® Screener Plus; iQue® Screener HD; HTFC®	90208

iQue® 3 Advanced Flow Cytometry Configurations	Lasers	Channels	Plate Formats	Cat. No.
iQue® 3 110V Blue-Red	2	8	96, 384	91375
iQue® 3 110V HD Blue-Red	2	8	384, 1536	91381
iQue® 3 110V Violet-Blue-Red	3	15	96, 384	91377
iQue® 3 110V Violet-Yellow-Blue	3	15	96, 384	91379

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