

# How and When Markers Can Make a Better Assay

## SUMMARY

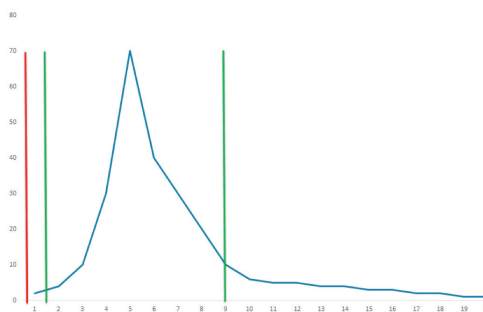
The use of IntelliCyt Markers ensures positive well/sample identification, even when samples have no objects.

- Demonstrated utility with compound solubility
- Is applicable to low cell count assays such as stem cells and high toxicity conditions
- Ensures that all data is included in “long sip” assays
- Does not affect time-to-result

## PROBLEM

The first step in analyzing data from a high throughput flow system such as the iQue Screener is to properly identify each well sample as a distinct unit. The presence of cells or beads in a well typically provide this information in a positive way, but there are certain scenarios and applications that will, by their nature, have few or no discernable objects present. Examples include:

- Screening of **low cell count samples** such as when using stem cells or other cells that are difficult to obtain
- Assays in which **highly toxic compounds may drastically reduce cell number**. These conditions with low object numbers mean that accurately identifying and separating samples is tenuous.
- Assays which have **long sip times**. In this case, the well ID algorithm often truncates the sample set after the initial sample front has passed the detector, but well before the sample finishes passing it (Figure 1). The consequence is that a significant portion of each well is not included in the analysis and the accurate identification of downstream wells is at risk.



**Figure 1.** A hypothetical sample (blue trace) from a sip time of 20 seconds. Under standard instrument use, data will be obtained from between the green marks. Addition of “between well markers” will define the limits of that same sample to include all data between 0 and 20 seconds (red marks). This adds 15% more objects to the analysis for this example data.

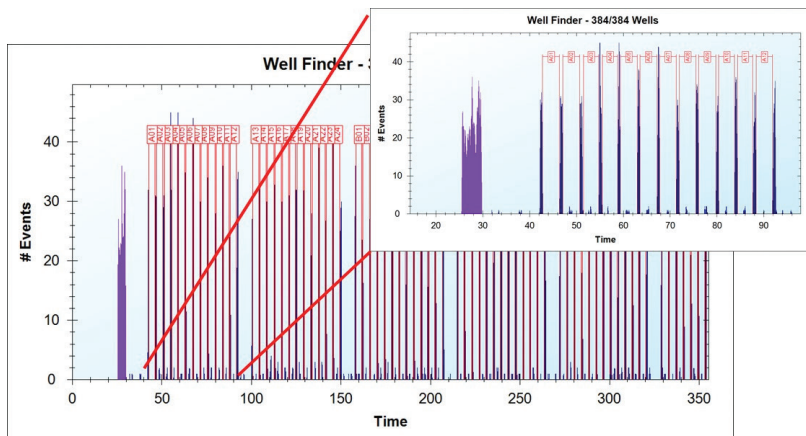
- Applications in which no objects are anticipated, such as **testing compound samples for solubility**. Compound solubility is an important chemical screen that is done before the biological screen. Compounds that are not in solution can produce false negatives because the actual concentration in the assay is less than expected. They can also cause false positives by many artifactual means, such as interfering with the detection of photons or by causing clumping of tissue/cells.

## RESULTS

A feasibility study was performed on compounds with known solubility by assaying them for the formation of precipitates on the iQue Screener. Forward scatter provides information on relative particle size; side scatter informs about the density of the object, so there is the potential for rich data on the compound's behavior.

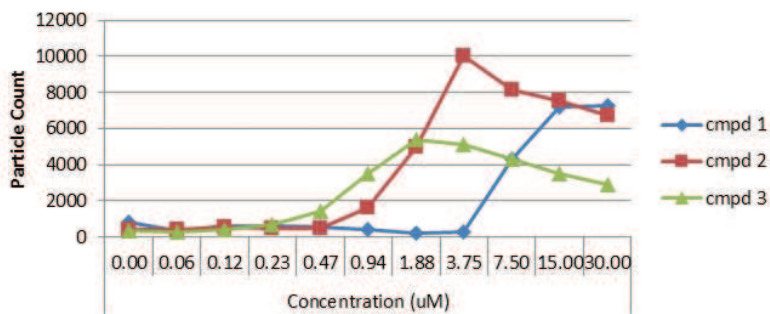
## THE INTELLICYT SOLUTION

- In-well markers** - Inert beads that can be added to every well (or a subset of wells) to ensure an adequate number of objects in the analyzed sample. In-well markers allow the iQue to analyze samples with no effect on time to results. The in well markers should be tested prior to use for any effect on the biology being assayed.
- Between well marker** - This bead-based product is offered as a cartridge that sits on the deck of an iQue. A small sample of these beads are taken between every well, avoiding any possible interaction with the well components, at the expense of slightly longer time to results.
- Robust data analysis package** - The use of between well markers allowed ForeCyt control and analysis software to successfully identify more than 3000 wells that contained no objects and was then applied to the challenge of compound solubility screening.



**Figure 2.** Between well markers (noted as dark blue spikes) allowed ForeCyt to properly identify over 3000 wells without error that contained no objects (area between spikes). This approach was used to successfully screen a chemical library for precipitates (indicating insolubility).

## Compound Solubility Dose Response



**Figure 3.** Three compounds with known solubility were diluted serially and analyzed on an iQue for particle number. Particle count increased with dose for each compound at different concentration minimums (7.5, 0.94, 0.47  $\mu$ M, respectively).

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