The Difference Between Efficiency, Recovery, And Yield

In flow cytometry cell sorting, the statistics of efficiency, recovery, and yield are often used to describe the performance of the cell sorting instrument, in varying terms, relating to the number of target events(1) obtained after a cell sort compared to some pre-sort starting number of events.

The definitions of these terms can vary depending on the context, which can be confusing when attempting to utilize these statistics for cell sorter assessments and comparative studies between instruments. Here, standard definitions are proposed to facilitate a more thorough understanding of these terms when they appear in literature and marketing material.

**Efficiency**
**Efficiency is defined as the number of target events marked for sorting divided by the number of target events detected (expressed as a percentage).

Efficiency = (Number of target events marked for sorting)/(Number of target events detected) × 100**

The disparity between the total target cells detected and target cells marked for sorting is due to sort coincidences, which may also be called sort aborts, soft aborts, or conflicts, depending on the instrument.

Coincidences occur when a target particle does not fulfil the sort mode (2) criteria, which ensures that the required purity and/or counting constraints have been met. For example, when strict purity criteria are set, a target event will not be sorted if a non-target event is predicted by the instrument electronics to be located close to the target event. This ensures that the non-target event doesn’t get sorted along with the target event.

The multiple factors that dictate the coincidence abort rate are complicated and beyond the scope of this article, but the abort rate does directly depend on the event rate – the faster that droplets are populated by events (remember, the droplet formation rate stays constant), the more chance than coincidences  will occur. The higher the coincident rate, the lower the efficiency of the sort will be.

The efficiency is never calculated manually – all sorters calculate this value for you. It is a statistic that will be displayed during and after sort, allowing for real-time monitoring of the event rate.

**Recovery**
**Recovery is another important parameter. This statistic measures the number of target particles in the collection tube compared to the number of particles reported to have been sorted by the instrument, as a percentage.

Recovery = (Total number of particles in the collection tube × Purity)/ (Number of target particles reported to have been sorted by the instrument) × 100**

The recovery must be calculated by counting the total number of particles in the collection tube using a particle counter. Cell sorters are not particle counters, so an absolute particle counter must be used to obtain this value.

This statistic can provide two useful pieces of information. **Firstly**, and more importantly, it indicates how accurate the instrument’s timing was between interrogation and droplet charging – in other words, the drop delay. When the drop delay is inaccurate, the target particle may not be in the breakoff period during droplet charging and thus will not be sorted appropriately. Additionally, timing inaccuracy between the laser interrogation point and the break-off may also result from sticky or clumpy cells that affect the transit time of cells between this crucial region in the sorting path.
 **Secondly**, recovery can be affected by the destruction of cells during the sorting process. Fragile cells may die or burst when depressurized at the nozzle. Additionally, if the side streams are not set properly or if they are fanning (3), cells may be deposited on the sides of the tubes, rather than in the capture medium on the bottom of the tubes, and dry out. These will not be counted during the recovery assessment, resulting in a decreased recovery statistic.
Recovery is a useful parameter, but it may not take into account events lost at the interrogation point due to electronic aborts(4) or events that are simply lost in the system

**YIELD**
**However, the yield parameter does take these factors into account. It is defined as the number of target events recovered compared with the total number of target events in the sample tube before sorting, expressed as a percent.

Yield = (Number of particles in the collection tube × Purity)/(Total number of particles in the sample tube before sorting × Percent of target events) × 100**

Like the recovery, yield must be calculated by counting (using a particle counter) both the number of particles in the sample tube and the number of particles in the collection tube. The purity and percent positive can be accessed via the original sort file and the purity check.

The yield parameter is a very powerful and all-encompassing statistic. It will account for any cell loss during the entire process of sorting – from the sample tube to the collection tube. Unlike the recovery, the cell loss estimated by the yield accounts for electronic aborts, as well as sort aborts. However, some care must be taken when utilizing this parameter to assess the electronic performance of a cell sorter; the yield is also impacted by the dead volume(5) in the sample line and particles that remain in the sample line or sample tube, especially when sorting sticky cells.

In general, it is impossible to remove every single particle from the sample tube – some will always remain behind, and these lost events will cause a lower yield statistic than would be generated based on sorter electronic performance alone.
**Another way of assessing the yield, which is termed the effective yield, accounts for the total number of target events detected compared to target events in the collection tube.

Effective Yield = (Number of particles in the collection tube × Purity)/ (Total number of particles acquired during the sort × Percent of target particles)**

The effective yield is not impacted by cells lost in the sample tube or sample line, so it can more directly assess the electronic performance of the system. However, be aware that this statistic may not account for electronic aborts, depending on the system and how the electronic aborts are reported.

The effective yield is affected by factors that impact both the efficiency and recovery, so this statistic can be understood as a compound of the efficiency and recovery.

(1) Target events are those that fulfil the optical (scatter and fluorescence) sort criteria.
(2) Sort modes are sets of logical instructions that tell the instrument how to sort particles in ambiguous situations so that the output is in-line with what is desired for experimental purposes (i.e. purity, yield, or accuracy in counting).
(3) Fanning refers widening side streams and results from inconsistency in charge magnitude on droplets. This inconsistency can be caused by improper droplet setup, cells that are too large for the nozzle, or debris in the sample.
(4) Electronic aborts occur when two particles arrive at the interrogation point too close together to resolve individually. The events are thus not included in the data set for parameter and sort processing.
(5) The remaining volume in the sample line that can never be introduced to the nozzle without also introducing an air bubble.