Rmax: Cell Sorter Performance

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In order to be confident in your sorting results, cell sorter performance must be evaluated through the appropriate QC methods. **Rmax** is a method that calculates the **maximum recovery** of the sort sample by looking at how much is lost in the unsorted fraction. Deviations from maximum recovery indicate a problem in sorter performance.

Checking post-sort purity is the gold standard to QC sorting performance. However, any issue affecting sorter performance, such as the drop-charge delay timing being off, will affect recovery before affecting purity. Therefore, to assess cell sorter performance, it's important to use a method that measures recovery, rather than purity.

Rmax

During sort, the absolute number of target & non-target particles (t) derived from the **original sample** (Ot/Ont) will end up either in the **sorted** (St/Snt) or the **center stream catch or CSC** (Ct/Cnt), i.e., Ot=Ct+St & Ont=Cnt+Snt

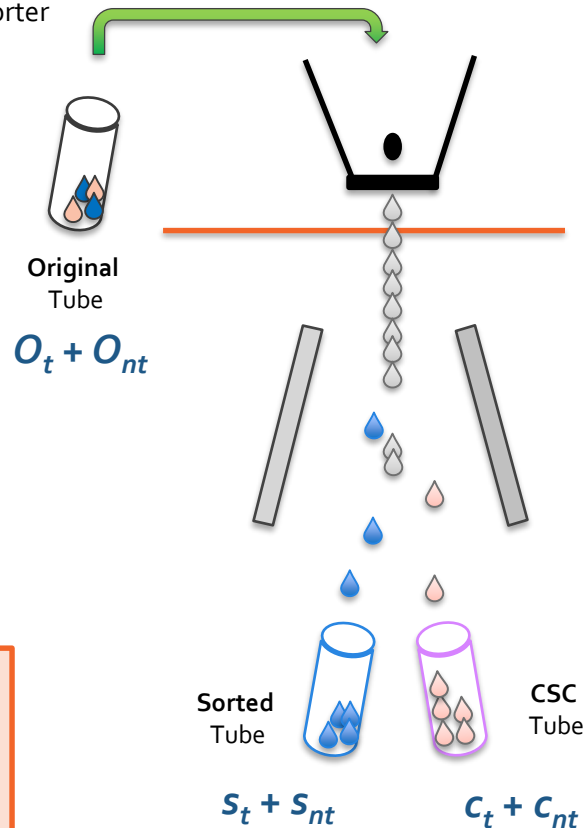$$Rmax = \frac{\frac{Cnt}{Ct} - \frac{Ont}{Ot}}{\frac{Cnt}{Ct} - \frac{Snt}{St}}$$

Assuming purity is close to 100% (or above 95%, which it typically is after measuring the drop-charge delay) we can simplify the above equation:

$$Rmax = 1 - \frac{Ont}{Ot} \times \frac{Ct}{Cnt}$$
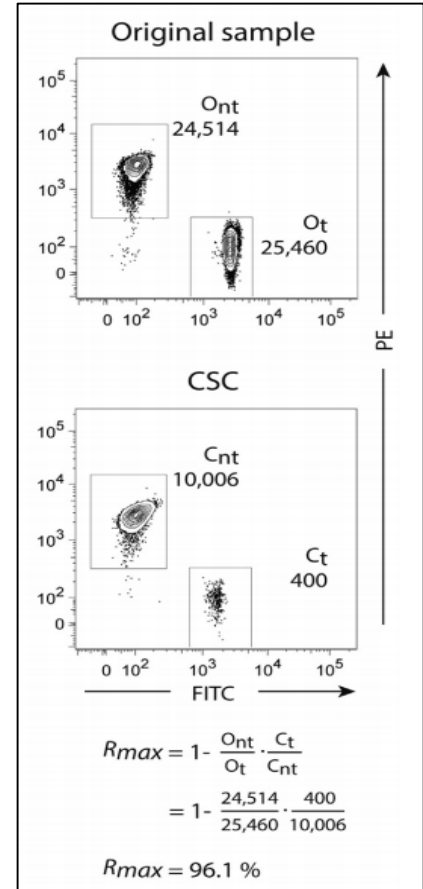
Rmax can also be used to:

- troubleshoot the instrument
- determine daily drop-charge delay
- evaluate & troubleshoot sample preparation

Rmax online protocol

Original Tube

$O_t + O_{nt}$

Sorted Tube

$S_t + S_{nt}$

CSC Tube

$C_t + C_{nt}$

Calculate Rmax

Original sample

O_{nt} 24,514

O_t 25,460

PE

CSC

C_{nt} 10,006

C_t 400

FITC

$$Rmax = 1 - \frac{O_{nt}}{O_t} \cdot \frac{C_t}{C_{nt}}$$

$$= 1 - \frac{24,514}{25,460} \cdot \frac{400}{10,006}$$

$$Rmax = 96.1\%$$

Riddell (2015) doi: 10.1016/j.ymeth.2015.02.017

Flow Cytometry Core Facility

https://fccf.mskcc.org

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