Effects of particle size on drop delay accuracy and subsequent recovery in cell sorting: a multi-institutional, multi-sorter study

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Introduction

Drop delay calibration is used by electrostatic cell sorters to calculate the time needed by an event of interest to travel from the interrogation point to the droplet that will be sorted. If the delay is measured incorrectly, the particle of interest will not be contained in the sorted drop, reducing the recovery of the target particles (1,2).

Early cell sorters required a time-consuming manual drop delay process. Now, sorters have fully automated drop delay calibration routines relying on standardized beads and may or may not provide an option to manually adjust drop delay if needed. Although automated drop delay streamlines sort set up, the ability to adjust the value to maximize recovery of a given sample allows sort operators the flexibility to handle a wider variety of sample types. This is especially advantageous on instruments that are limited in nozzle diameter options. Drop delay is influenced by a number of parameters including temperature, pressure, drop drive settings, fluidics design, and particle size (3). Of these, particle size is the most variable among sorts on a given instrument and increased size can subtly alter drop delay before causing noticeable deterioration of sort streams.

There has not been a comprehensive study of automated drop delay accuracy at different particle sizes across commercially available sorters. The Association of Biomolecular Resource Facilities (ABRF) Flow Cytometry Research Group (FCRG) has undertaken a study to measure the accuracy across a variety of sorters using 10- and 24-micron particles. The FCRG used beads to decrease the variability that could be introduced when working with cells. While beads are not cells, we hypothesized that if automated drop delay settings are not optimal for sorting beads, this effect would be exacerbated with cells. The initial study includes 11 institutions and 10 cell sorter models using 15 different automated configurations. For consistency, a 100-micron orifice was used on each configuration.

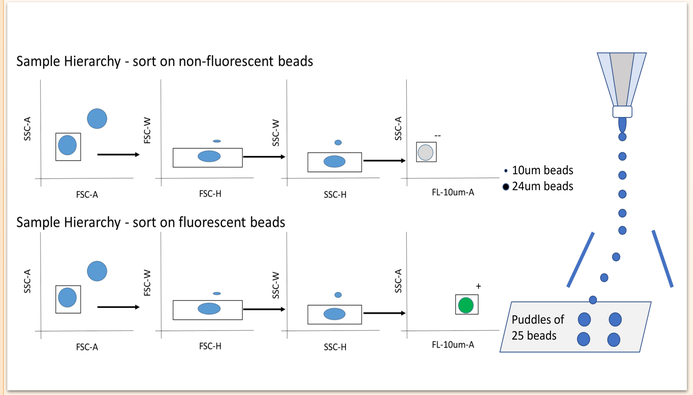
Objectives

- Develop a protocol to test automated drop delay accuracy for beads of various diameters that will work on any sorter without overriding safety interlocks
- Test the accuracy of the automated drop delay programs using 10- and 24-micron beads
- Determine sort recovery as it relates to the make, model, and sorter configuration

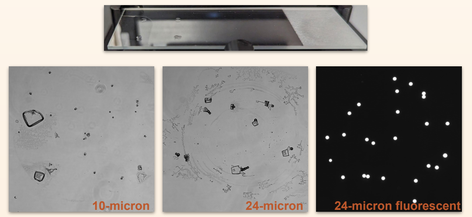
Materials

- 10 cell sorter models, using a 100-micron orifice for sorting
- 10- and 24-micron beads: ACURFP2.5-250-5, ACURFP20-100-1, PPX-100-10, PPX-200-10, Spherotech, Inc.

Method



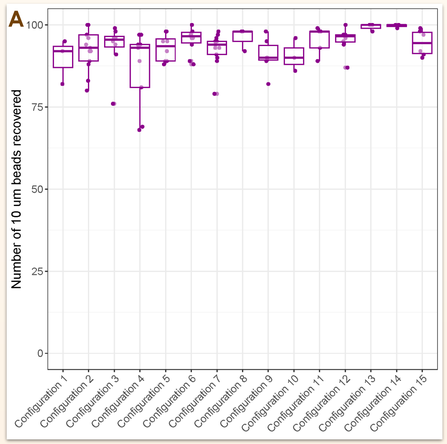
1. Drop delay was set using the automated drop delay value for each configuration with a 100-micron orifice.
2. 10- or 24-micron beads were sorted in four puddles of 25 beads each, using a one-drop envelope and set to maintain sort purity. (**Top figure**)
3. Beads were counted manually using a light or fluorescence microscope. (**Bottom figures**)



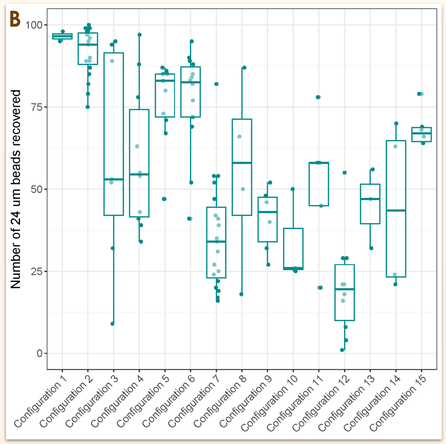
Results

Effects of automated drop delay calibration values on sorting of 10- and 24-micron beads

10-micron beads



24-micron beads



Fanning

(A) When evaluating differences in 10-micron and 24-micron sorting based on automated drop delay setup, there was minimal variability in 10-micron bead recovery across configurations.

(B) There was significant variability among 24-micron bead recovery across configurations.
- Configurations 1 and 2 show almost no change in the number of beads that were recovered using 10- or 24-micron beads
- All other configurations show a 17 to 84 percent decrease in the recovery of 24-micron beads compared to 10-micron

(C) Side stream fanning of varying degrees was observed on some models of cell sorters. This fanning was seen predominantly when sorting the 24-micron beads, although minor fanning was experienced while sorting the 10-micron beads with at least one model of sorter. **Left:** example of side stream fanning of 24-micron beads. **Right:** example of side stream fanning of 10-micron beads.

Conclusions

Accurate drop delay calculation is required to ensure proper sorting and recovery of target cells. Using automated drop delay settings to sort 10- and 24-micron beads across multiple commercially available sorters with a 100-micron orifice, the ABRF FCRG found the following in its initial drop delay study:

- 10-micron beads exhibited good recovery across all configurations
- 24-micron beads exhibited poor recovery across most configurations
- Automated drop delay settings were accurate for 10-micron but not 24-micron bead sorting across most sorters
- Fanning of 24-micron samples indicate that these size beads may be outside of the size tolerance of the 100-micron orifice
- From the data collected, some cell sorter manufacturers have taken into account the requirements for sorting larger particles using a 100-micron orifice

Future Directions

- Decrease large bead diameter from 24-micron to 20-micron to account for any size tolerance issues of the 100-micron orifice
- Test additional sorters for automated drop delay accuracy with assistance from the wider flow cytometry community
- Include calculated droplet volume as a variable in future testing, along with additional variables

Interested in helping with the Drop Delay study?

Volunteer!

References

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Acknowledgements

- Mehrnoosh Abshari, NIH/NIDCR
- Claudia Bispo, AbbVie Biotherapeutics
- Sara Bowen, Barrow Neurological Institute
- Thank you to the ABRF for funding of the FCRG drop delay study