ABRF 2024
Annual Meeting

Preparing today's cores
for tomorrow's needs

Minneapolis, MN
April 21-24

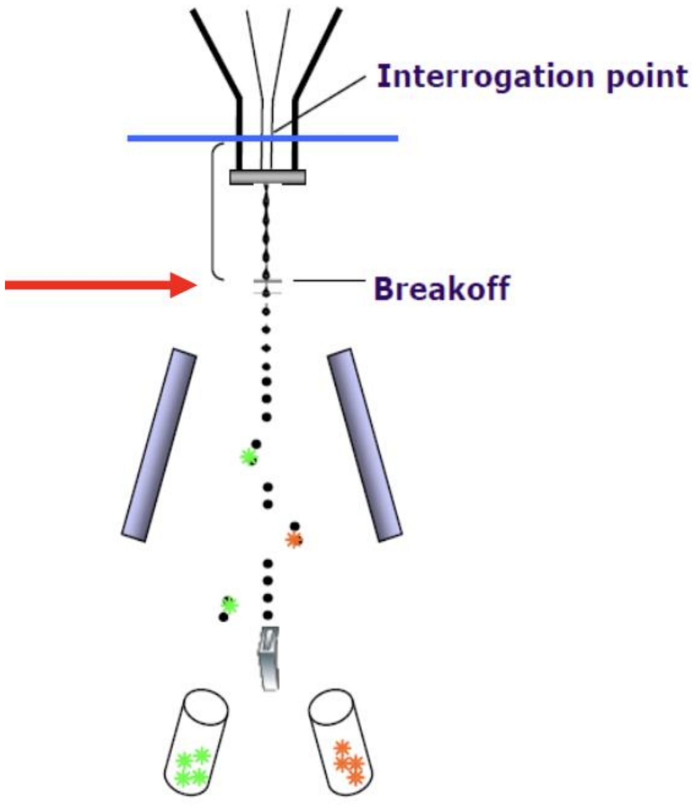
ABRF FCRG Drop Delay Study Year 2

ABRF Meeting – Monday April 22, 2024

Drop Delay

- Drop delay is the time needed by an event to travel from the interrogation point to the droplet breakoff point

- 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



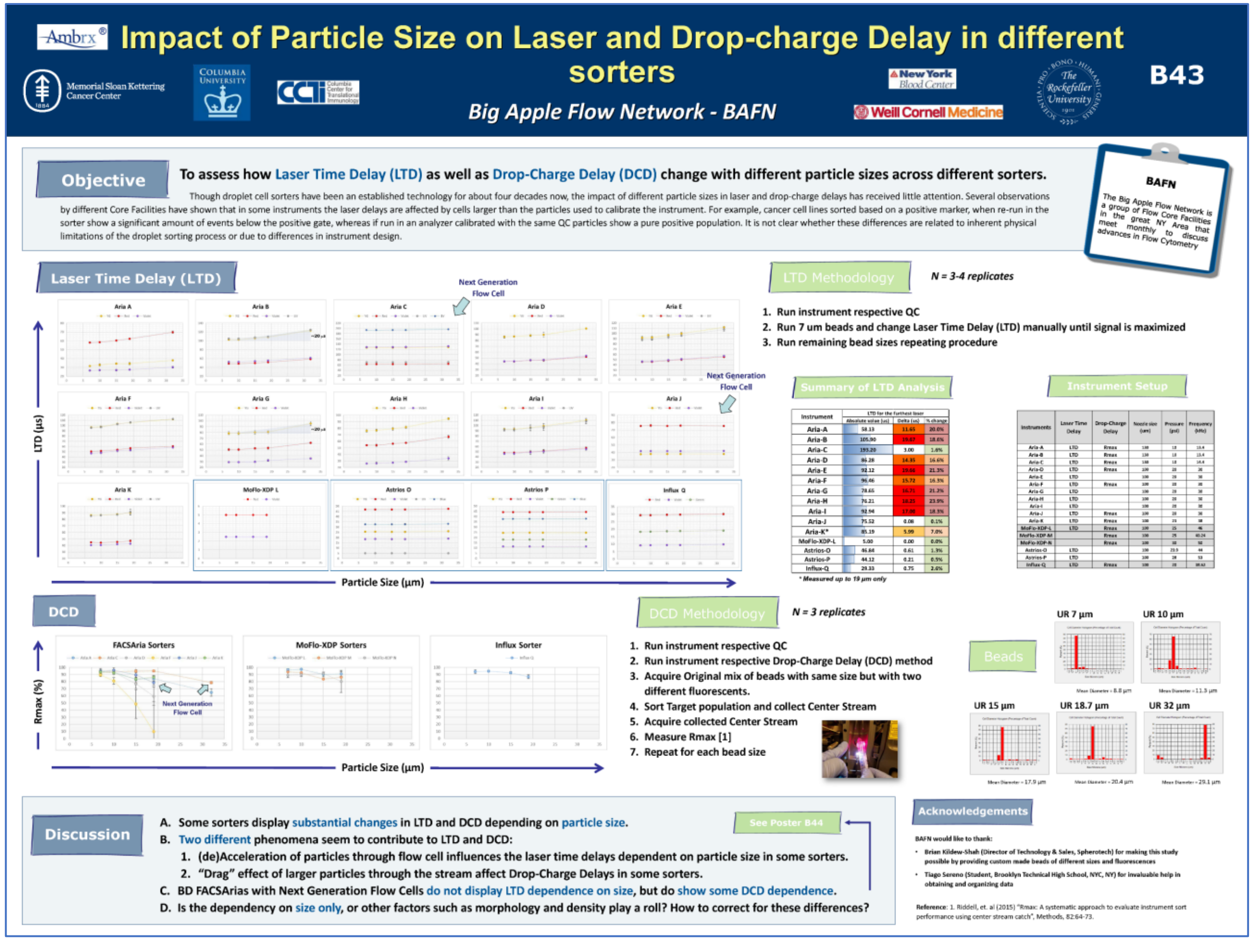
Evolution of Drop Delay

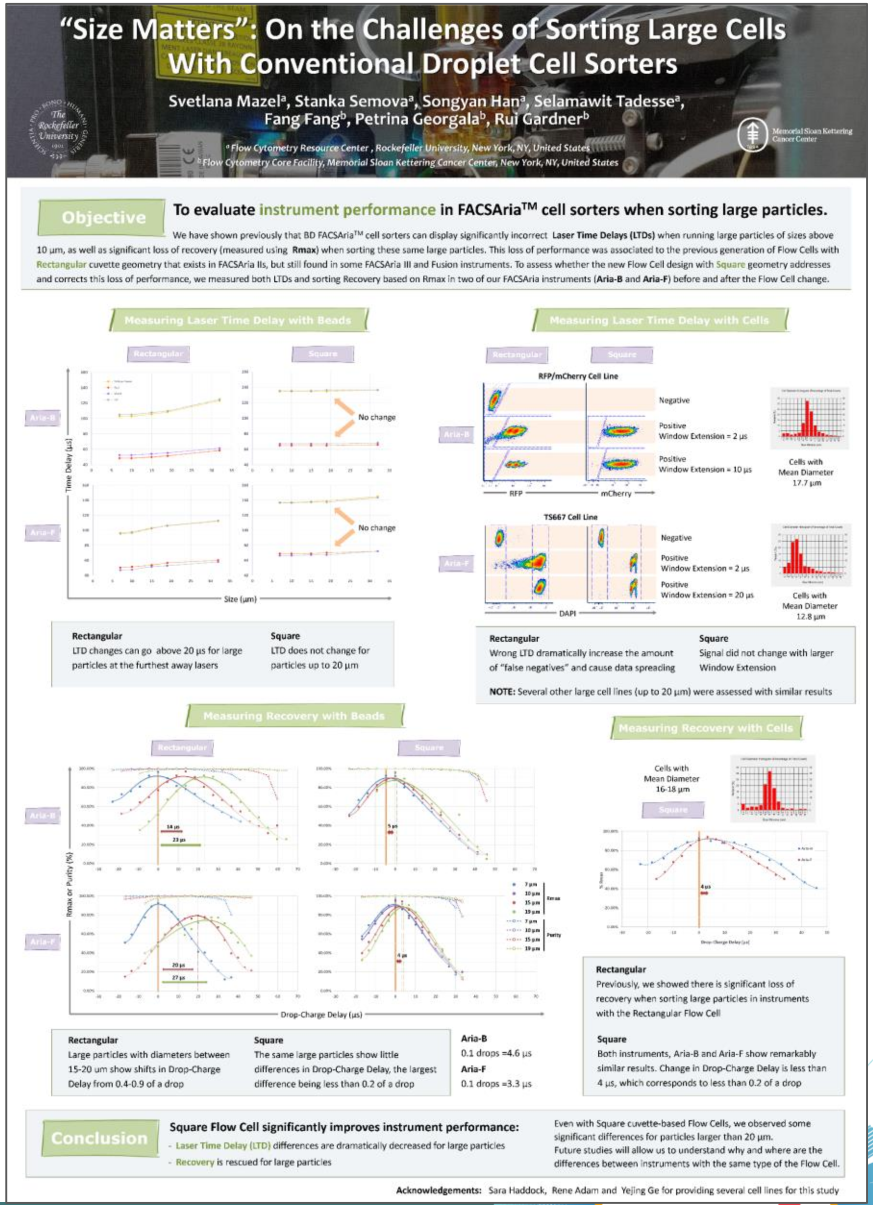
- Early cell sorters required a manual drop delay process = time consuming
- This evolved to an automated drop delay calibration:
  - Standardized beads
- Now - fully automated wizard driven instrument sort set up
  - Includes drop delay calibration
- New fully automated systems:
  - Streamline sort set up and drop delay calibration
  - **BUT** block the ability of the sort operator from adjusting the delay
- Why is changing drop delay necessary?
  - Allow sorting of a wider variety of sample types
  - Increases ability to sort larger cells on instruments with limited nozzle diameter options

What factors influence Drop Delay?

- Drop delay is influenced by several parameters including:
  - ✓ Temperature
  - ✓ Sheath Pressure
  - ✓ Drop drive settings
  - ✓ Fluidics design
  - ✓ Particle size
- Of these, particle size is the most variable among sorts on a given instrument
- Increased size can subtly alter drop delay before causing noticeable deterioration of sort streams

Previous drop delay testing





Rockefeller University, MSKCC, and the BAFN group

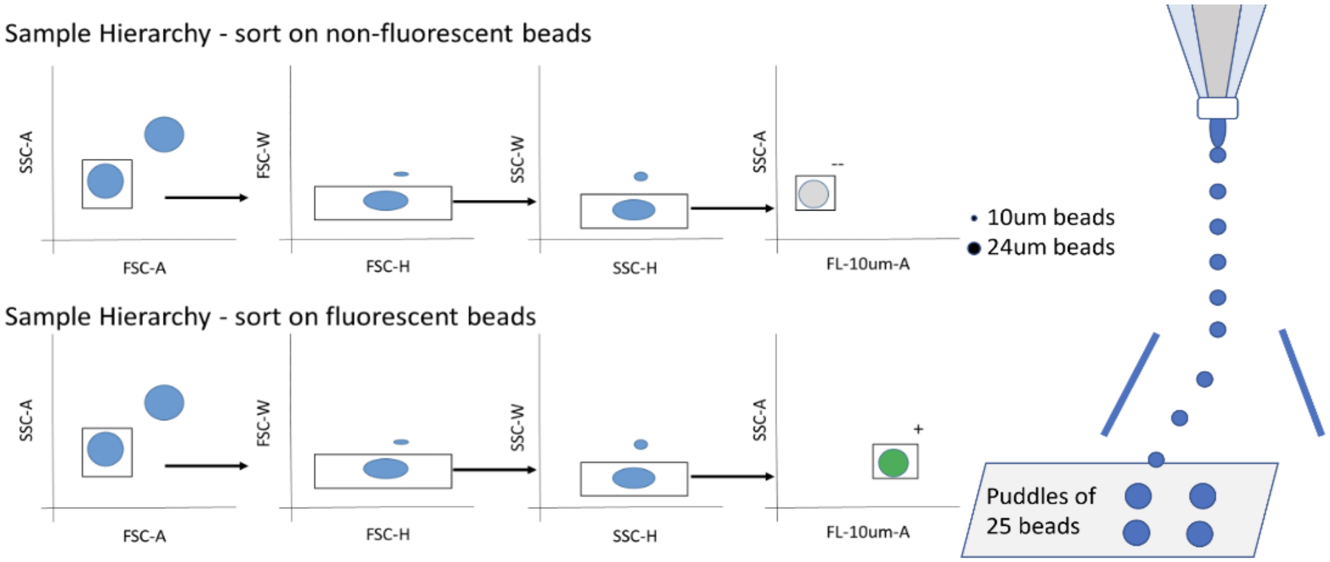
ABRF FCRG Drop Delay Study – Year 1 recap

- First Year: Measure automated drop delay accuracy with different particle sizes across commercially available sorters
  - ✓10- and 24-micron beads
  - ✓Beads decrease variability introduced when working with cells
  - ✓11 institutions and 10 cell sorter models, 15 different configurations
  - ✓For consistency, a 100-micron orifice was used for each configuration

Materials & Methods

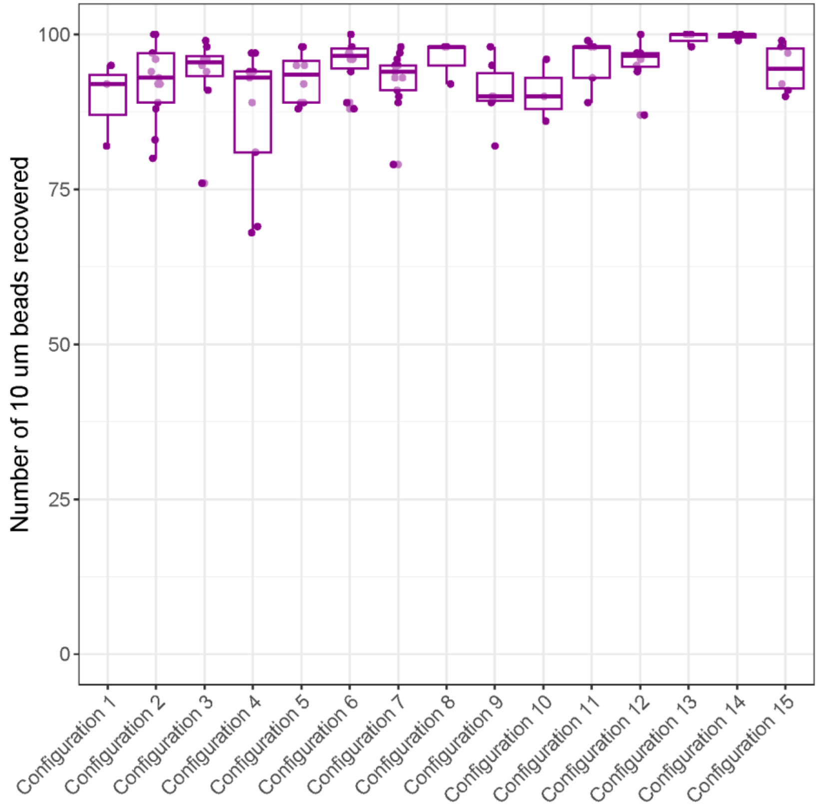
- 10 cell sorter models, using a 100-micron orifice for sorting
- 10- and 24-micron beads:
  - ACURFP2.5-250-5, ACURFP20-100-1, PPX-100-10, PPX-200-10, Spherotech, Inc.

- Drop delay was set using the automated drop delay for each configuration with a 100-micron orifice
- 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
- Sorting was repeated on two additional days for three total replicates

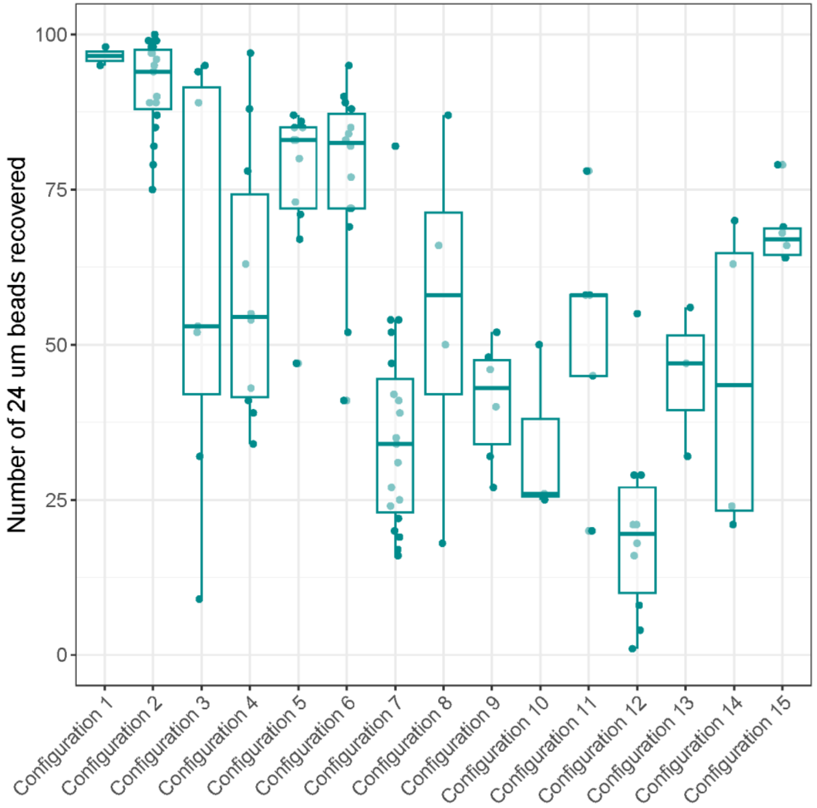


ABRF FCRG Drop Delay Study – Year 1 Results

10-micron beads



24-micron beads



ABRF FCRG Drop Delay Study – Year 2

Second year: increase robustness of study

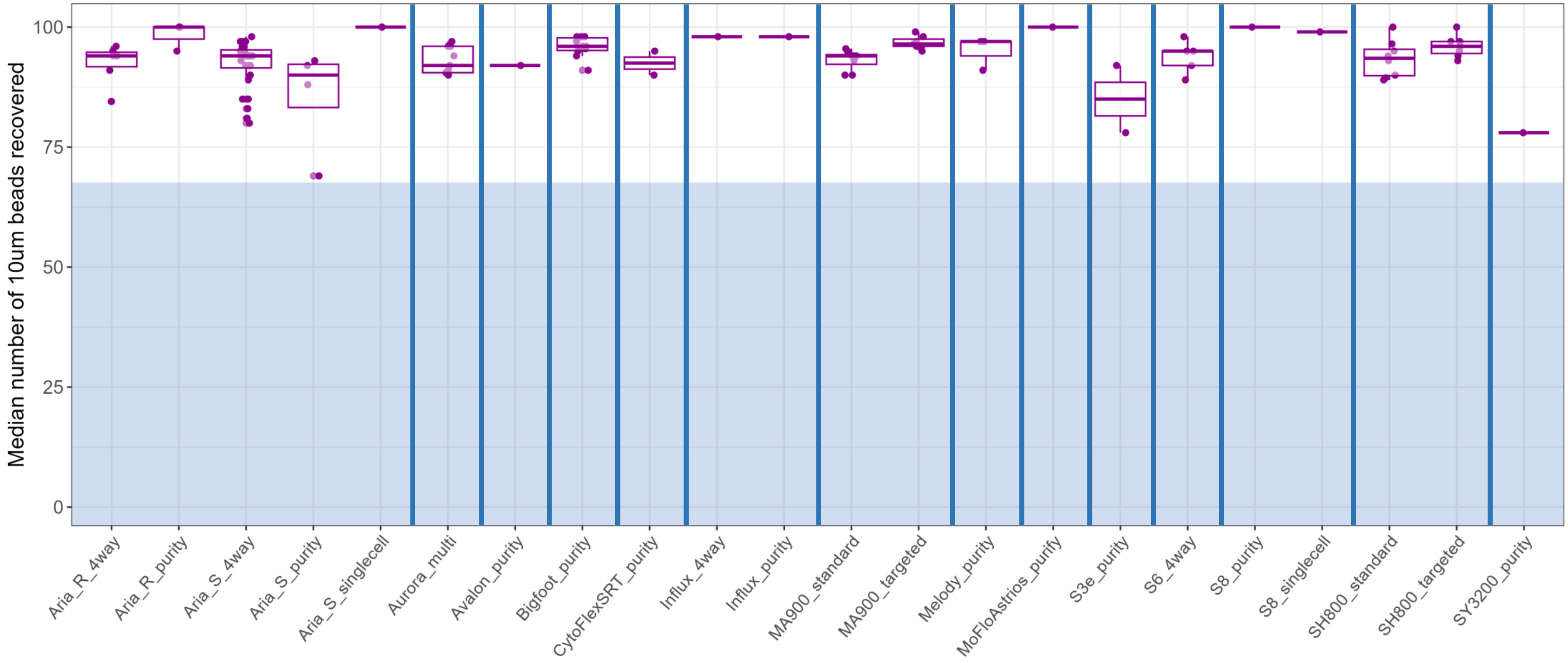
- Include more sorters
  - Sorters and configurations are named
- Similar materials and methods
  - Use fluorescent beads only - ACURFP2.5-250-5, ACURFP20-100-1, Spherotech Inc. – larger beads were closer to 25-micron diameter

ABRF FCRG Drop Delay Study – Year 2 Results

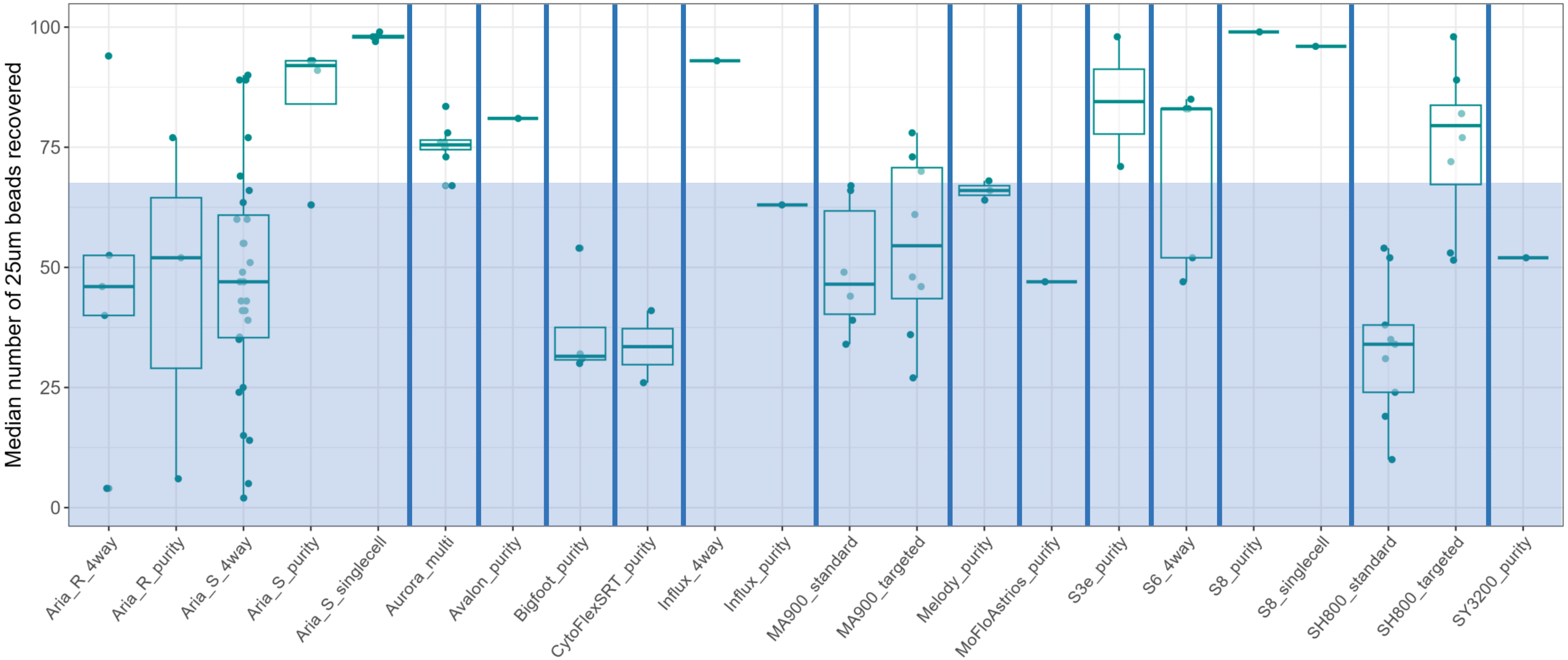
Before the big reveal

- Wanted to include at least 3 sorters for each model
  - In some cases, this was not possible
- To account for this, the FCRG is interested in conducting a continuation of the study for Year 3
  - Increase robustness of data for sorters of $n \leq 3$

ABRF FCRG Drop Delay Study – Year 2 results – 10-micron beads

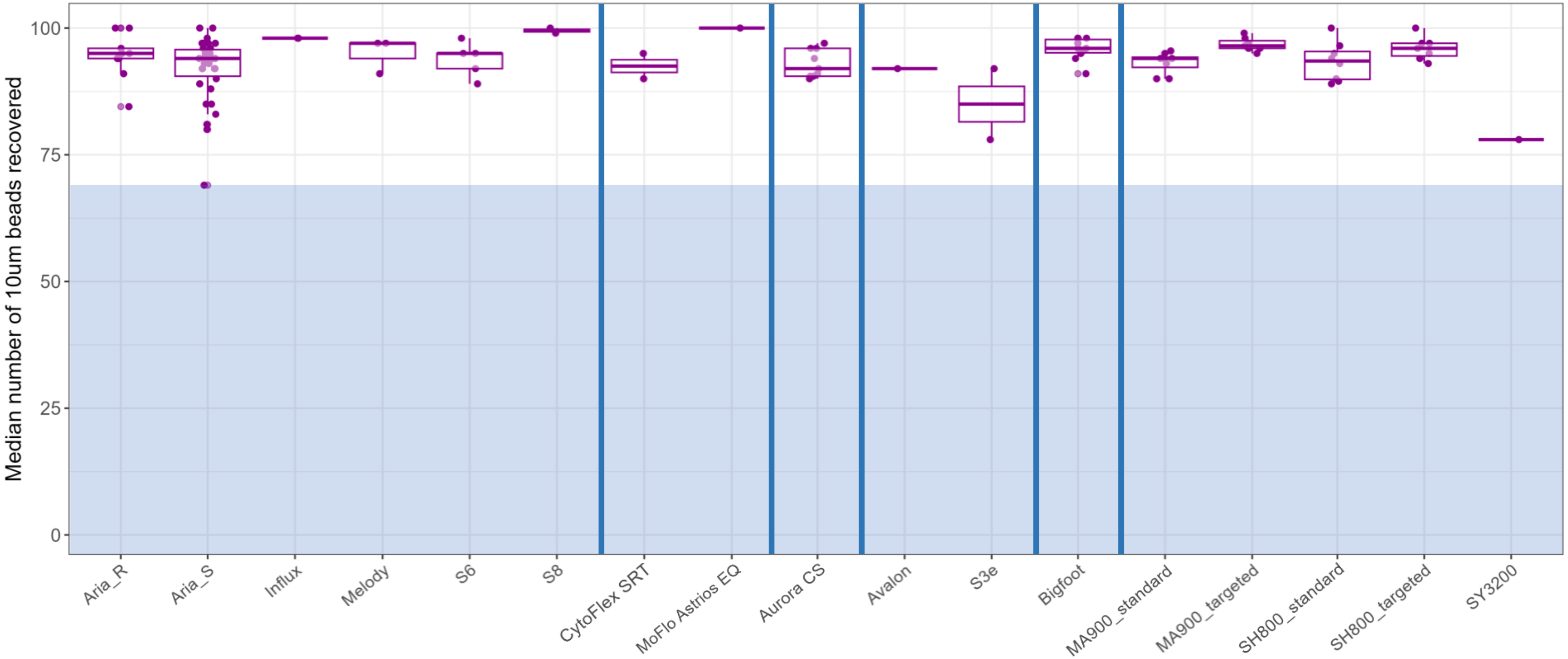


ABRF FCRG Drop Delay Study – Year 2 results – 25-micron beads



Data consolidated by instrument

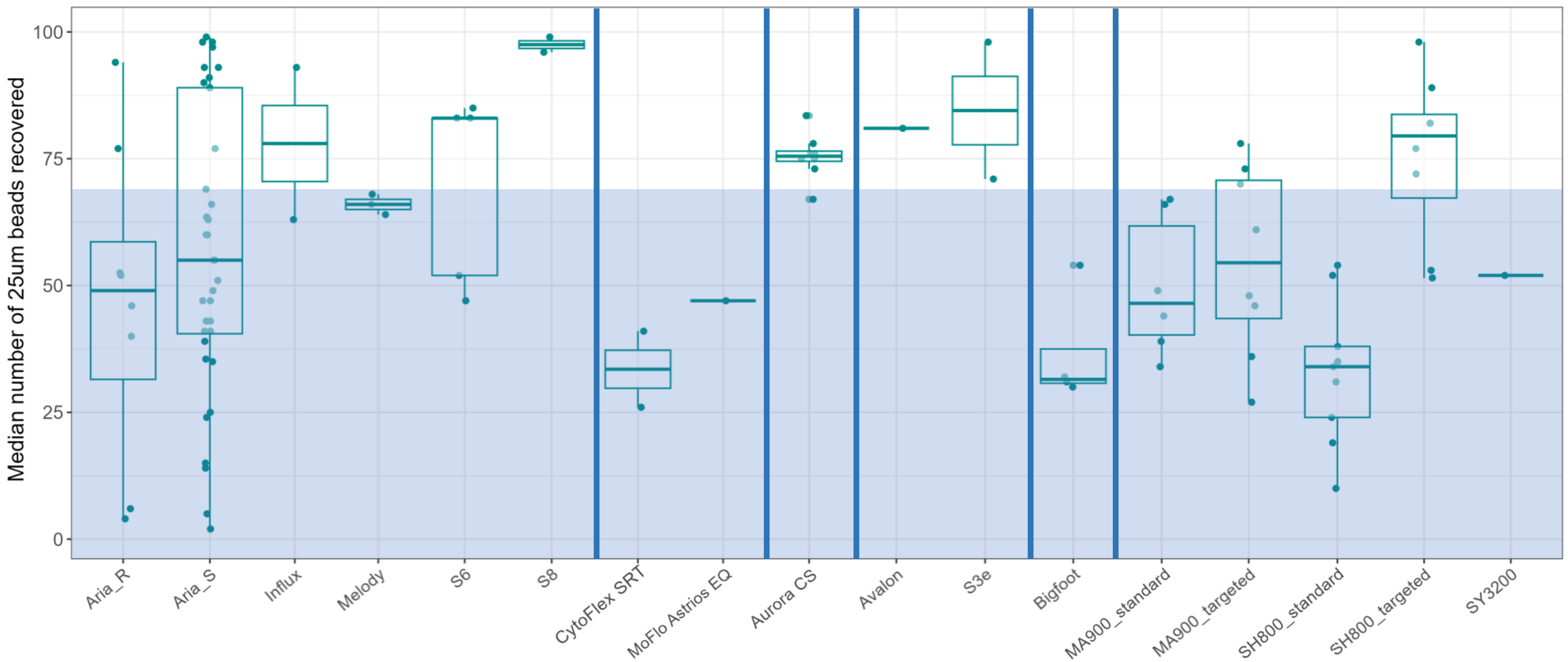
Consolidated 10-micron data



Number of each instrument:

- Aria-R - 10
- Aria-S - 27
- Influx - 2
- Melody - 2
- S6 - 3
- S8 - 1
- CytoFLEX SRT - 2
- MoFlo Astrios EQ - 1
- Aurora CS - 7
- Avalon - 1
- S3e - 1
- Bigfoot - 4
- MA900-S - 7
- MA900-T - 7
- SH800-S - 7
- SH800-T - 7
- SY3200 - 1

Consolidated 25-micron data



Number of each instrument:

- Aria-R - 10
- Aria-S - 27
- Influx - 2
- Melody - 2
- S6 - 3
- S8 - 1
- CytoFLEX SRT - 2
- MoFlo Astrios EQ - 1
- Aurora CS - 7
- Avalon - 1
- S3e - 1
- Bigfoot - 4
- MA900-S - 7
- MA900-T - 7
- SH800-S - 7
- SH800-T - 7
- SY3200 - 1

Conclusions – Year 2

- Like Year 1, 10-micron beads exhibited good recovery and 25-micron exhibited poor recovery across instruments
  - More variation in 25-micron data in year 2; number of instruments increased
- Automated drop delay settings were accurate for 10-micron but not 25-micron bead sorting across most sorters
- Some variability may be due to a 1-drop vs. 2-drop sort envelope
  - Study participants were instructed to use a 1-drop envelope, but this did not always occur
- Need additional data for sorters where the sorter number was $n \leq 3$

Future Directions – Year 3

- Test additional sorters where n ≤ 3
- On sorters where changing drop delay is possible, test whether incremental changes improve recovery of 25-micron particles
  - This has been shown previously for Aria instruments by the BAFN group: https://cdn.fccf.aws.mskcc.org/CYTO_2017_B43_impact_of_size_on_LTD_and_DCD_12_049764b2fa.jpg
- Test sort precisions

Acknowledgments

Amsterdam UMC - Microscopy and Cytometry Core Facility
Sara Garcia-Garcia
Tanja Konijn

Baylor College of Medicine - Cytometry and Cell Sorting Core
Amanda White
Madhavi Chintalapati
Claude Chew
Courtney Harvey
Mason Perry
Paul Porter

Columbia University - Columbia Stem Cell Initiative
Michael Kissner

Cornell Institute of Biotechnology - Flow Cytometry Facility
Amanda Ferguson
Lydia Tesfa

Emory University - Emory + Pediatric's/Winship Flow Cytometry Core
Aaron Rae
Prasanthi Chappa

Houston Methodist Research Institute
David Haviland
Ellie Jardinella

Johns Hopkins University - Integrated Imaging Center
Hanhvy Bui
Erin Pryce
Ian Dobbie

Massachusetts General Hospital
Daire Daly

Mayo Clinic - Cytometry and Cell Imaging Lab (CCIL)
Laura Lewis-Tuffin
Margaret Ushman

Michigan State University - MSU Flow Cytometry Core
Daniel Vocelle

Rice University - Shared Equipment Authority (SEA)
Harshavardhan Deshmukh

University of Auckland - Auckland Cytometry
Thaize Chometon
Tanvi Damani
Sandy Chen

University of Iowa - University of Iowa Flow Cytometry Facility
Heath Vignes
Mike Shey
Tom Kaufman

University of Nebraska Medical Center - Flow Cytometry Research Facility
Craig L. Semerad

University of Utah - Flow Cytometry Core Facility
James Marvin
Eduardo Salustiano Jesus dos Santos, Ph.D.

University of Wisconsin - Madison - UWCCC Flow Cytometry Laboratory
Kathryn Fox
Dagna Sheerar

West Virginia University - WVU Flow Cytometry & Single Cell Core Facility
Kathleen Brundage
Raven Forshee

ABRF FCRG
(includes current and former study participants)

- **Mehrnoosh Abshari**, NIH/NIDCR
- **Roxann Ashworth** (Executive Board liaison), Johns Hopkins University, School of Medicine
- **Claudia Bispo**, University of California San Francisco
- **Sara Bowen**, Dignity Health St. Joseph's Hospital and Medical Center (*former*)
- **Ching-Yuan (Steve) Chen**, Columbia University Irving Medical Center
- **Xiaoxuan Fan**, University of Maryland School of Medicine
- **Kevin Ferro**, Stowers Institute for Medical Research (*former*) – **statistical analysis year 1**
- **Claire Fraser**, Barrow Neurological Institute – **organized data and ran statistical analysis year 2**
- **Christiane Hassel**, Indiana University – **study co-author**
- **Celine Lages**, Cincinnati Children's Hospital Medical Center
- **Pam Moody**, Cold Spring Harbor Laboratory – **study co-author**
- **Steven Polter**, University of Rochester Medical Center
- **Kenneth Quayle**, Cincinnati Children's Hospital Medical Center – **organized data year 2**
- **Kathy Schaefer**, HHMI Janelia Research Campus (*former*)
- **Rachael Sheridan** (Co-Chair), Van Andel Research Institute Flow Cytometry Core
- **Jane Srivastava** (Chair), Gladstone Institutes
- **John Tigges**, Beth Israel Deaconess Medical Center (*former*)
- **Eric Wieder**, University of Miami Miller School of Medicine

Thank you to the ABRF for funding the study!

Do you have these instruments?

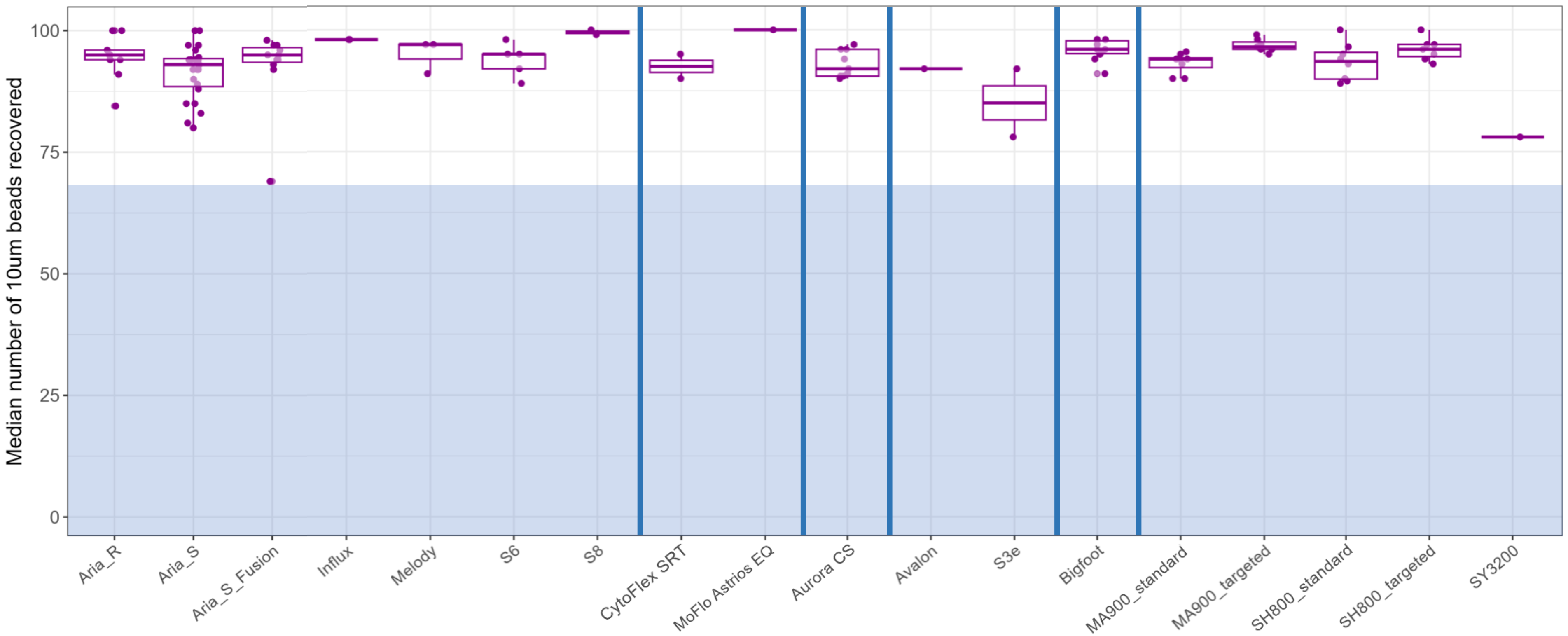
- Beckman Coulter – CytoFLEX SRT & MoFlo Astrios EQ
- BD Biosciences – Influx, FACSMelody & FACSDiscover S8
- Bio-Rad - S3e
- Cytek Biosciences – Aurora CS
- ThermoFisher – Bigfoot

Join Year 3 of the ABRF DD Study! →

Thank you!

Extra slides

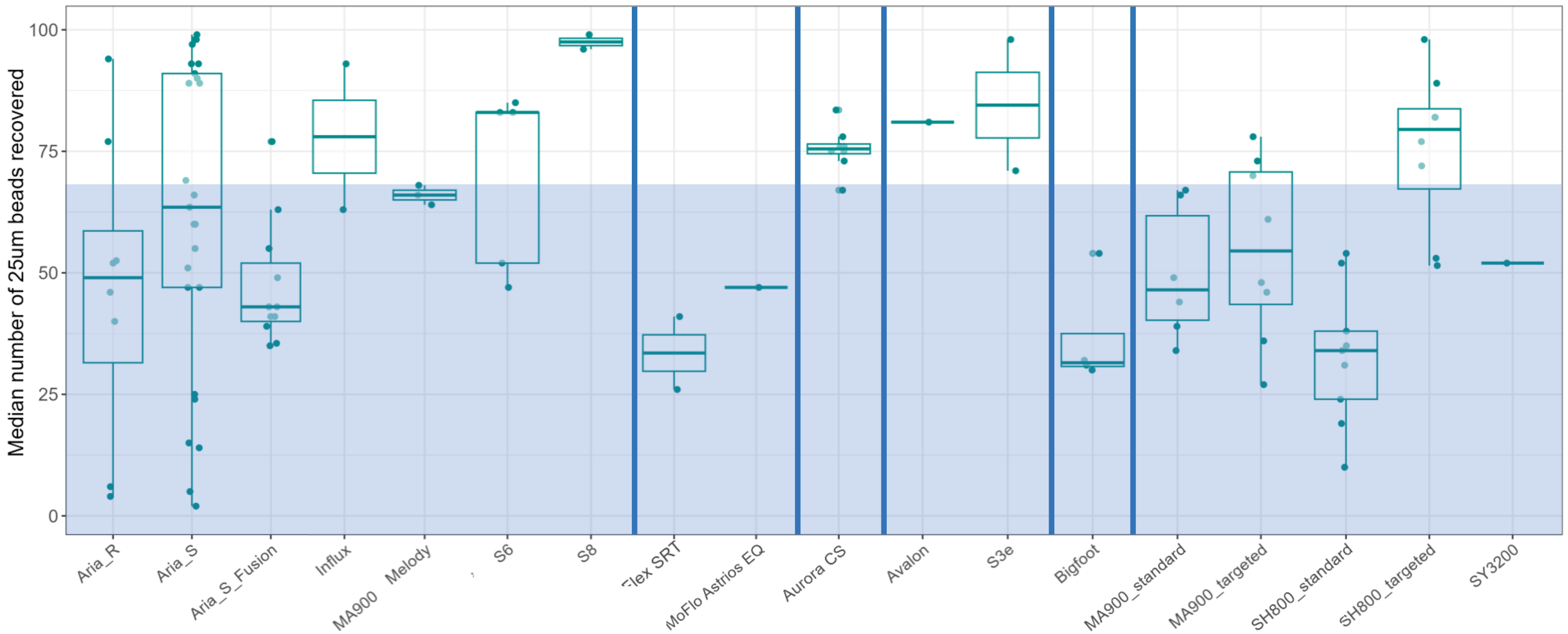
Consolidated 10-micron data – Fusion separate



Number of each instrument:

- Aria-R - 10
- Aria-S - 19
- Aria Fusion - 8
- Influx - 2
- Melody - 2
- S6 - 3
- S8 - 1
- CytoFLEX SRT - 2
- MoFlo Astrios EQ - 1
- Aurora CS - 7
- Avalon - 1
- S3e - 1
- Bigfoot - 4
- MA900-S - 7
- MA900-T - 7
- SH800-S - 7
- SH800-T - 7
- SY3200 - 1

Consolidated 25-micron data – Fusion separate

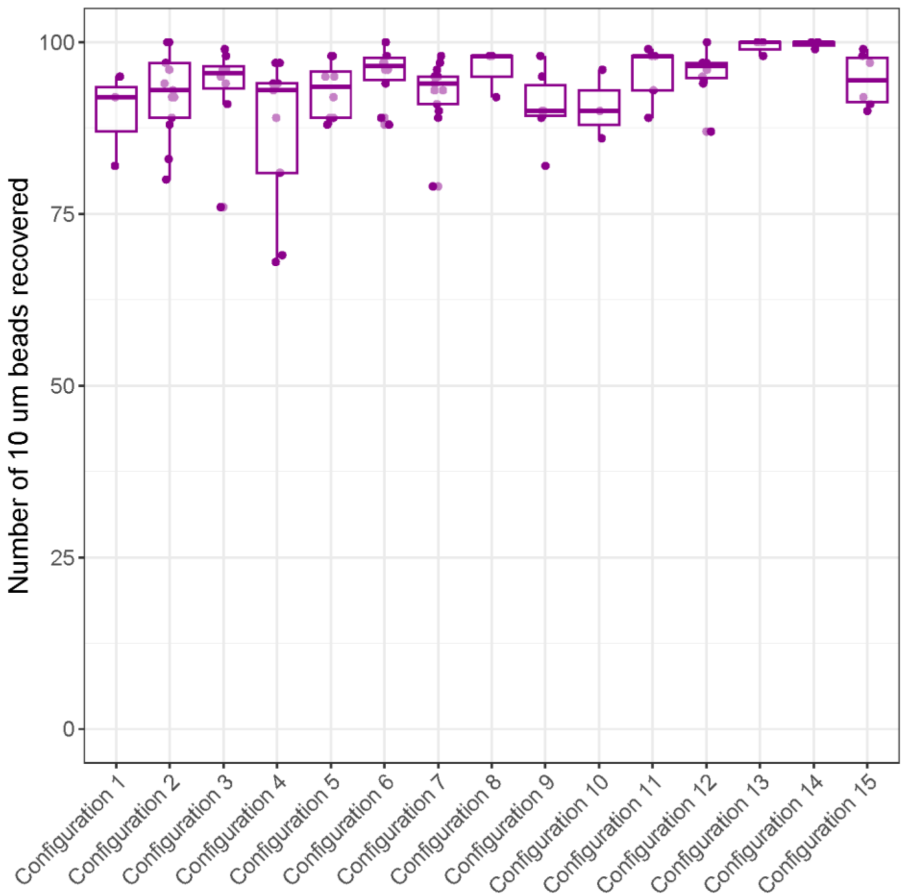


Number of each instrument:

- Aria-R - 10
- Aria-S - 19
- Aria Fusion - 8
- Influx - 2
- Melody - 2
- S6 - 3
- S8 - 1
- CytoFLEX SRT - 2
- MoFlo Astrios EQ - 1
- Aurora CS - 7
- Avalon - 1
- S3e - 1
- Bigfoot - 4
- MA900-S - 7
- MA900-T - 7
- SH800-S - 7
- SH800-T - 7
- SY3200 - 1

RESULTS – year 1

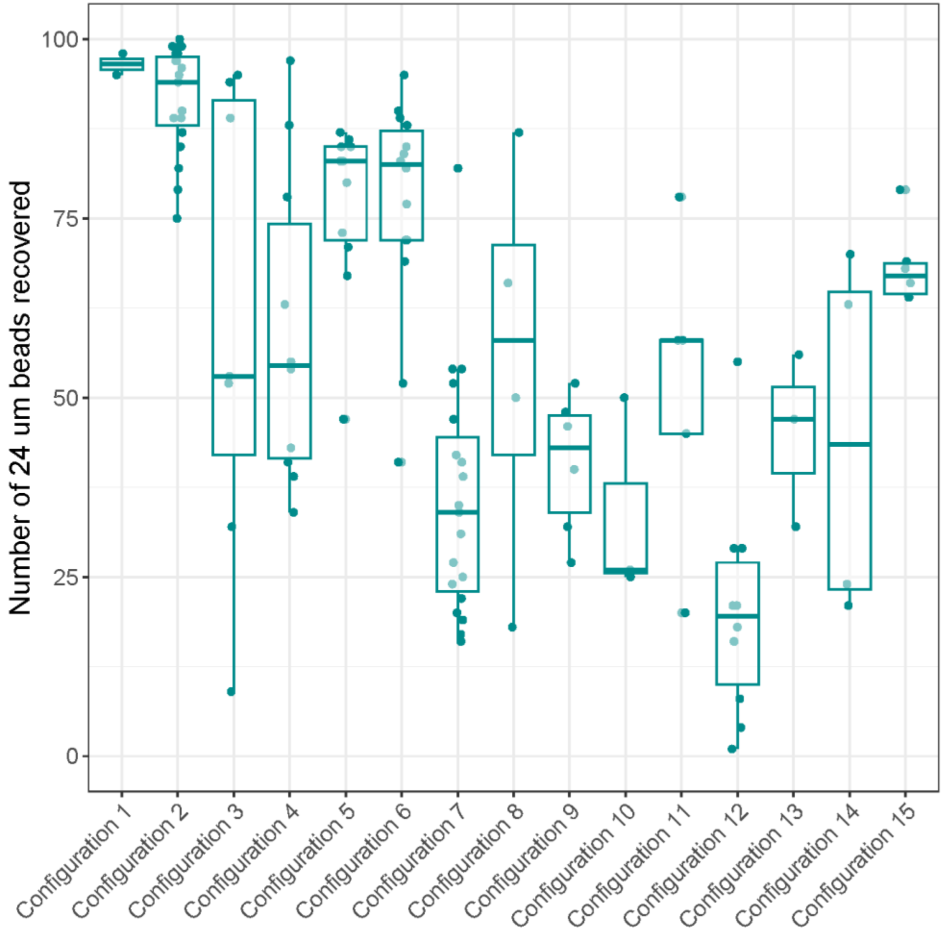
There was minimal variability in 10-micron bead recovery across all configurations using the Automated Drop Delay Setting.

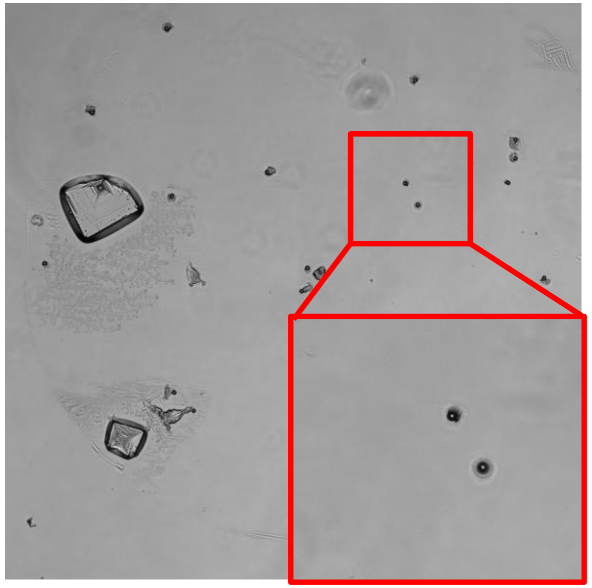


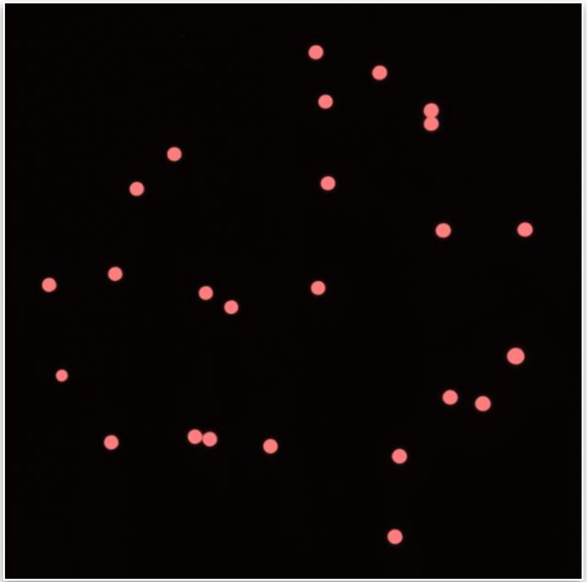
There was significant variability among 24-micron bead recovery across most configurations.

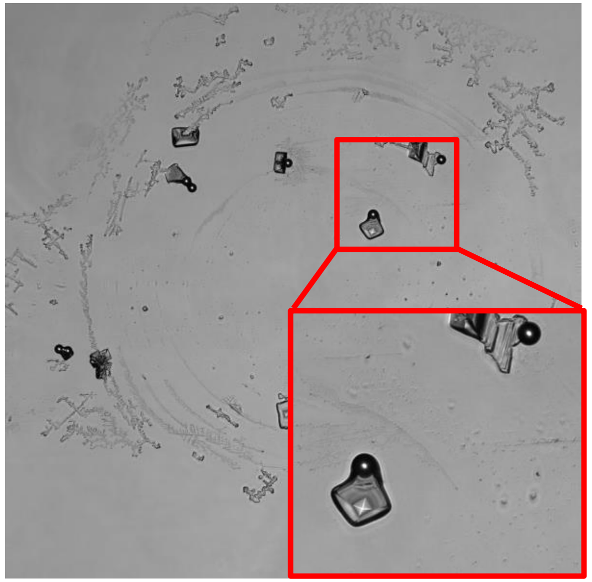
Configurations 1 and 2 show almost no change in the number of beads that were recovered using 10- or 24-micron beads

All other configurations show a 17 to 84 percent decrease in the recovery of 24-micron beads compared to 10-micron









Beads were sorted on to a slide and counted manually using a fluorescence or light microscope.

Side stream fanning of varying degree was observed on some models of cell sorter. 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.