25 October, 2022 Issue 6

Research Flow Cytometry Core

Quarterly Newsletter

Information Provided by your CCHMC RFCC

Cells vs. Beads? Does it even matter?

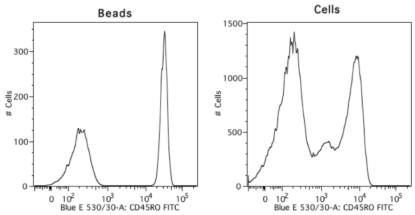
Single-Color Controls are a must but what particles should be used? Cells or beads? Accurate compensation is achieved if the positive signal for each fluorophore is as bright as, or brighter than the cells studied. Why? Because this is the part of the signal contributing the most to any spillover.

Cells as single color controls will:

- Give the most accurate compensation when an ample amount of bright and positive cells are recorded.
- Make compensation incorrect if they express the marker at a lower level than the population in the sample.
- Reflect true marker expression.
- Match the autofluorescence of the cells in the sample if the same cells are used.
- Use precious cells.

Beads as single color controls will:

- Be more reliable.
- Often put out a bright or brighter signal than cells and have a higher frequency of events which help avoid suboptimal compensation occurring with dim fluorescent markers.
- Stain uniformly and have a clear separation between positive and negative signals, which will aide in the reproducibility of the experiment.
- Need to be specific for viability dyes (for example ArC Beads from ThermoFisher…).



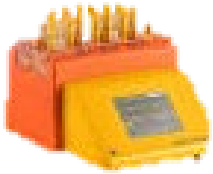
The good news is cells and beads can be used together to calculate a compensation matrix. For a new panel, consider bringing both single-color cells and beads for your experiment to determine which has the best outcome.

Did you know?

Are you interested in learning more about cell cycle analysis on FACSDiva? Have you attended a meeting presented by one of our flow cytometry staff members and want to get their slides? You can find those under our websites (Education and Training>Tutorials/Presentations on .org, Tutorials and Flow Cytometry Seminar Presentations on Centerlink).

New Equipments?

The RFCC now has a gentleMACS Octo Dissociator with Heaters available for sign out use. It can be used for tissue dissociation or homogenization with user-defined procedures or optimized ready-to-use gentleMACS programs.
Interested? Contact Mary Mullen.



Working with large particle/organoids? Did you hear about the VeloCyt?
Interested? Contact Andrew Rael from Bennubio or use this QR code to plan an onsite demo with your cells!

Looking into techniques to enrich your cells from debris? The LeviCell could be for you.
Interested? Contact Jason McKinney from LevitasBio to plan an onsite demo with your cells!

Core Updates

Fire Drills

Please pay attention to fire drills when in the Core area. The Core must be evacuated when there is a drill for R4, R5, and R6. Use the stairwell in the hall right outside the RFCC area. Go to floor R and exit to the Holmes lot. If confused, please refer to Alyssa or Sarah as they are the floor captains.

Reminder

When sorting on the Sony MA900 or SH800, please remain signed in when the sort is complete and everything is saved. The calibration settings will be lost when the software is not in use. You will not be charged for the time between you and the next user. If you feel you need to sign out, please inform a core staff member so they can sign in and keep the QC settings.

Staff

Congratulations to RFCC staff for their involvement in recent meetings.
Alyssa received a travel award and gave an oral presentation at GLIIFCA.
Celine hosted a roundtable at GLIIFCA.
Ken prepared a poster for CYTO & GLIIFCA, and hosted a breakout session at ORVCA.
Mary hosted a breakout session at ORVCA.
Sarah prepared a poster and hosted a roundtable at GLIIFCA, and hosted a breakout session at ORVCA.
Sherry and **Celine** helped coordinate the annual GLIIFCA and ORVCA meetings.
Sherry presented a poster at CYTO 2022.

Dates to Note

10/26: 1-2 PM: ORVCA meeting on Zoom by Canopy on Quantitative Single-cell Immune Profiling with ChipCytometry and CellScape.
11/16: 9-10 AM: High Parameter meeting in S9.130 to discuss consideration and pitfall when using high parameter flow cytometry. Interested to present your data to the group, contact Celine Silva Lages. Interested by a specific topic, fill out this form https://forms.gle/NGR6aZvEK6P6Gnhs7.
11/16: 1-2 PM: ORVCA meeting in S6.125 on Accelerating the next generation of immune medicine with cellular proteomics by IsoPlexis.
12/14: 1-2 PM: ORVCA meeting on updates in the RFCC.