The Impact of Washes on staining for Flow Cytometry
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Objective
To test whether increasing the number of washes improves resolution.

Methods
- Single stained lyophilized lymphocytes for anti-human CD4 (clone SK3) antibody (5 fluorochromes) were acquired in Cytek™ Aurora after passing vendor specified QC before running.
- CD4 antibody titeration was assessed, and the optimal concentration is 0.31µg/mL.
- The impact of 1, 2 and 3 washes were assessed by evaluating Stain Index.
- Count/µL was done by using the volumetric count by Cytek™ Aurora.
- Centrifugation settings were not optimized (1000 g for 3.5 minutes).
- One-way ANOVA followed by Tukey’s multiple comparisons test was performed using GraphPad Prism.

Discussion
- Spread of the negative population decreases with the number of washes leading to increased Stain Index.
- Reduced spread of the negative with more washing steps can result in improved population resolution for proteins expressed at low levels. However, this may also depend on fluorochrome choice.
- Cell loss will occur when more washes are included.

Future Work
- Evaluate the impact of antibody concentration, fluorochrome chemistry and wash number.

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<th>Antibody</th>
<th>CD4 Titration</th>
<th>Cell Count</th>
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Stain Index

\[
\text{Stain Index} = \frac{\text{median}_{\text{pos}} - \text{median}_{\text{neg}}}{2 \times \text{SD}_{\text{neg}}}
\]

CD4 Titration

0.31 µg/mL

Future Work
- Evaluate the impact of antibody concentration, fluorochrome chemistry and wash number.