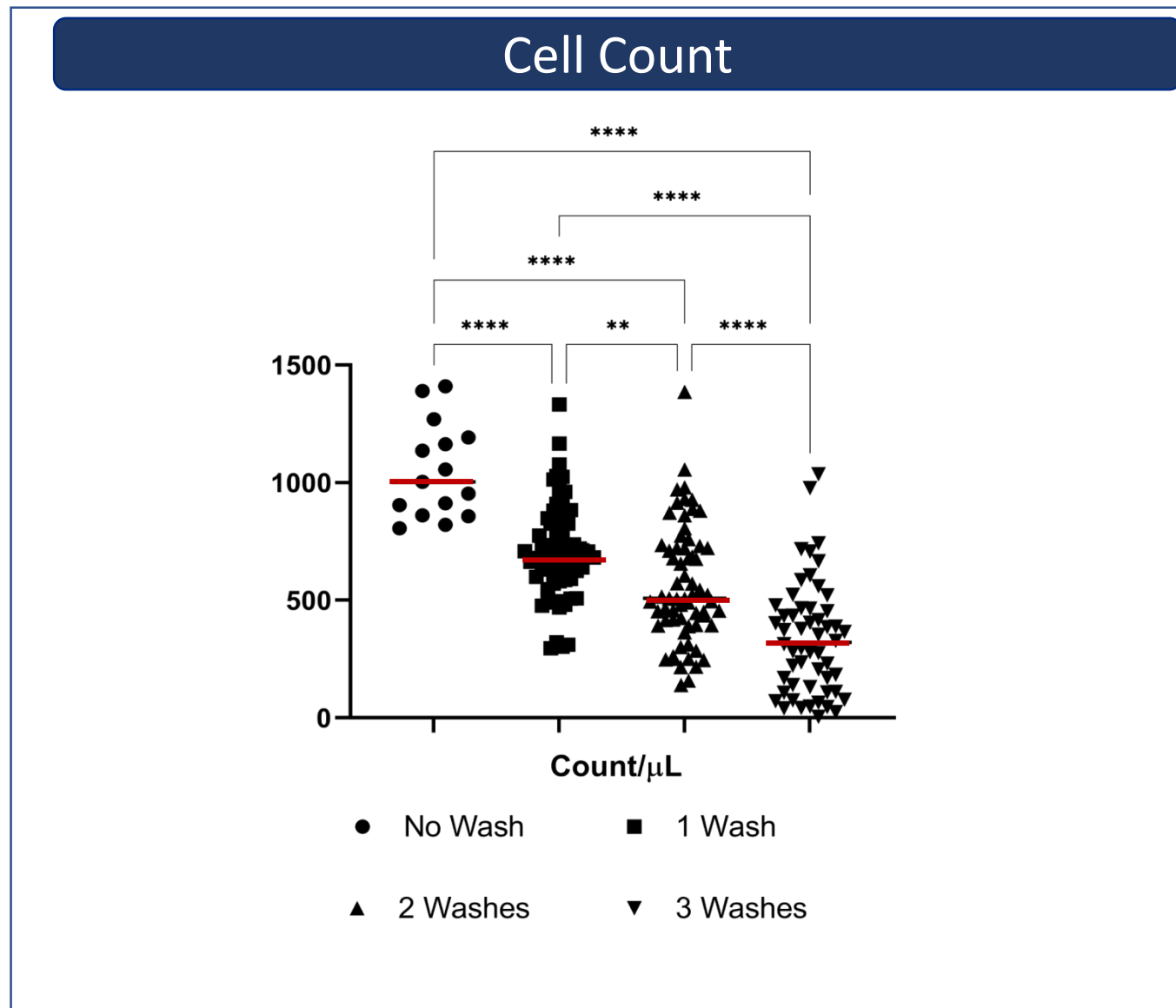
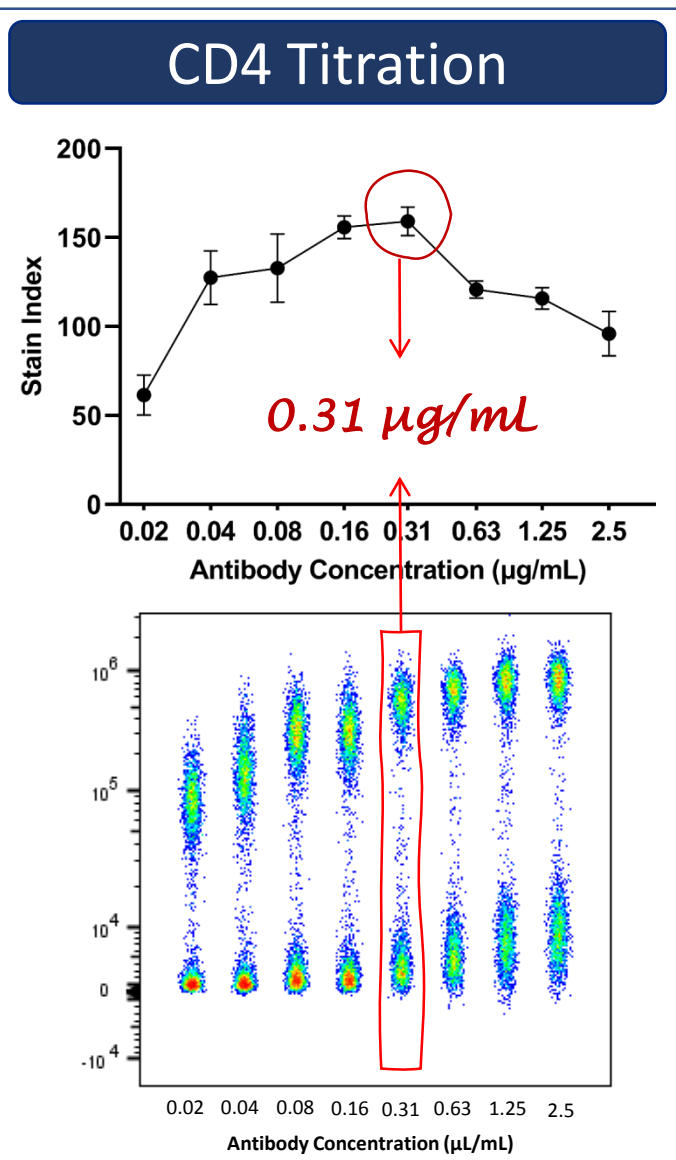
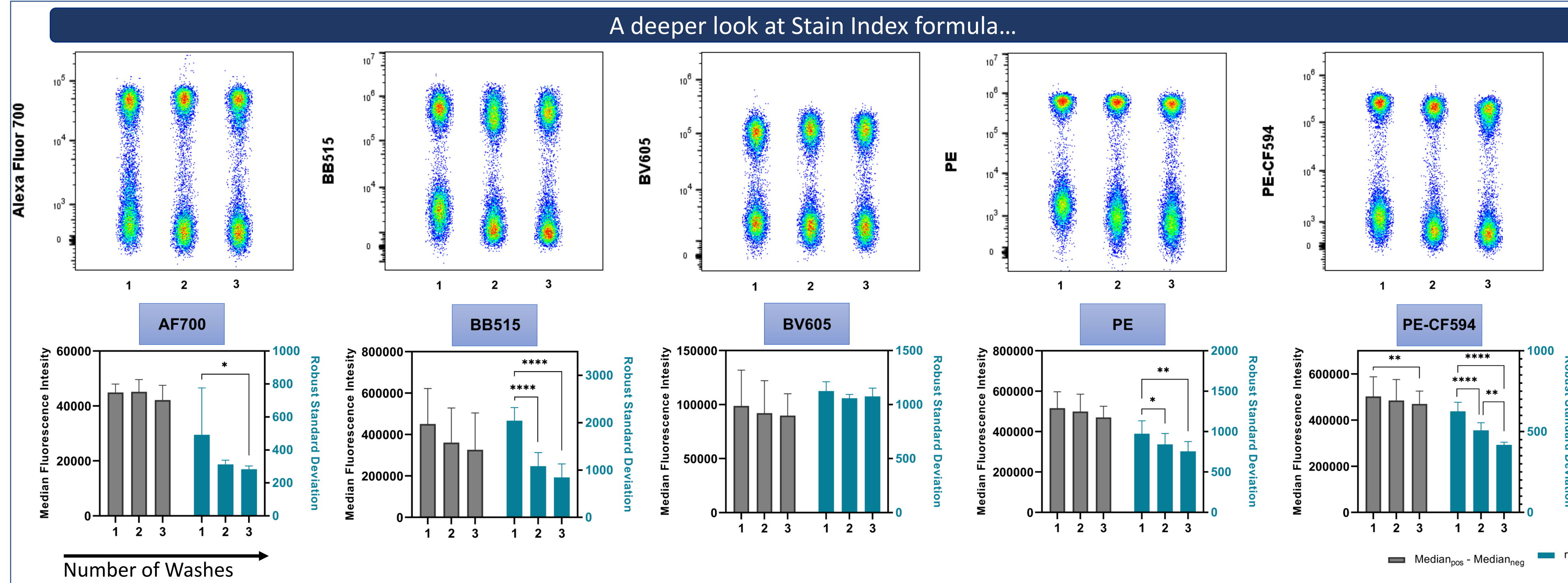
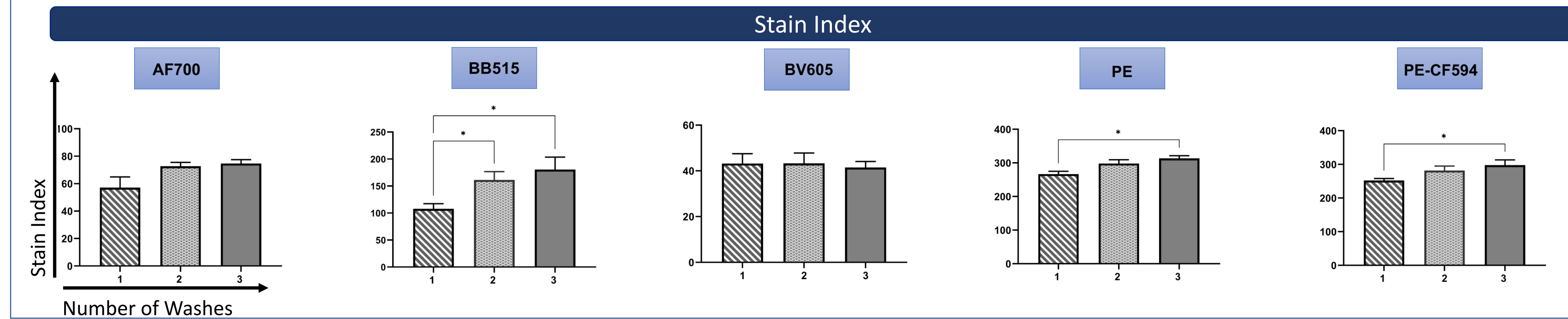


**Objective** To test whether increasing the number of washes improves resolution.

**Methods**

$$\text{Stain Index} = \frac{\text{median}_{\text{pos}} - \text{median}_{\text{neg}}}{2 \times rSD_{\text{neg}}}$$

- 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 titration was assessed, and the optimal concentration is 0.31 μg/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.

Including more washing steps in the staining protocol can improve resolution of populations and result in better quality data. This can be critical for low expressed antigens. However, impact of cell loss needs to be equated against any improvements in resolution and needs to be assessed for each antibody.

**Future Work**

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

