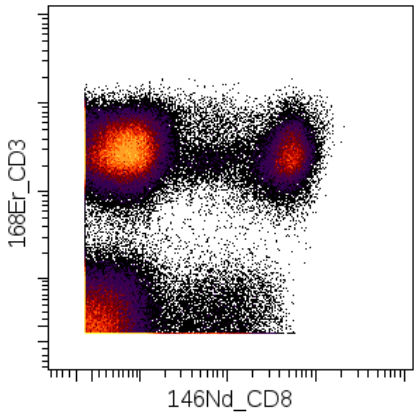
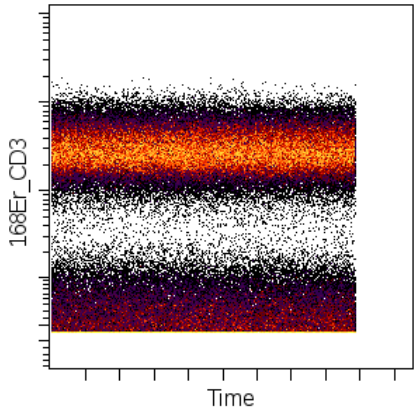
The Problem: sporadic samples with increased in background and consequent poor staining quality

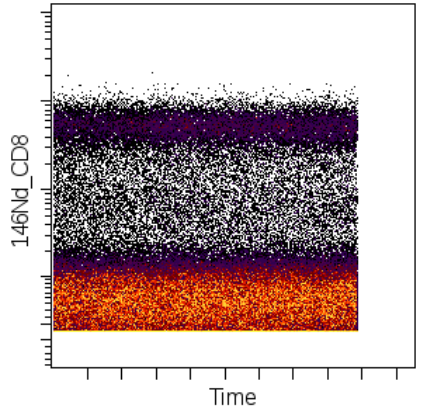
Example: Two aliquots of the same stained sample run sequentially on the same instrument (stored in CSM and washed and resuspended in H20 immediately prior to acquisition)

Same stained sample

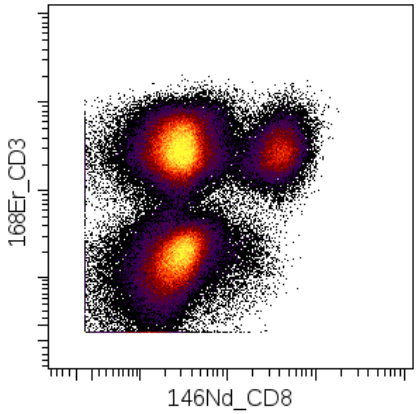
Aliquot 1

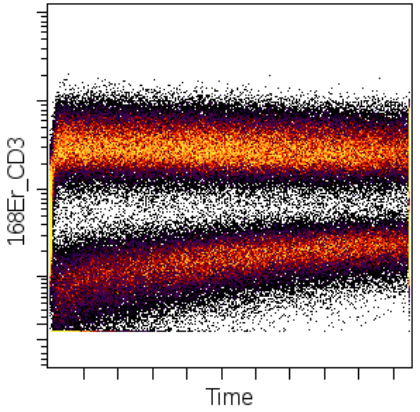


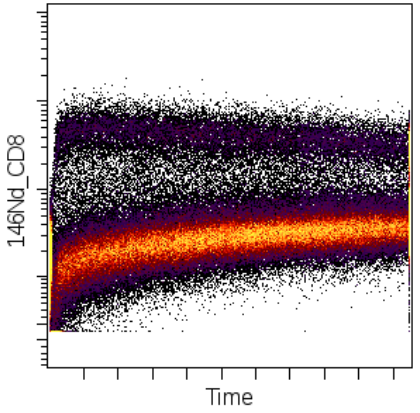




Aliquot 2







Both acquisitions came from aliquots the same sample, suggesting that this is not an issue of inadequate fixation

Loss of staining quality is due to progressive increase in background over time

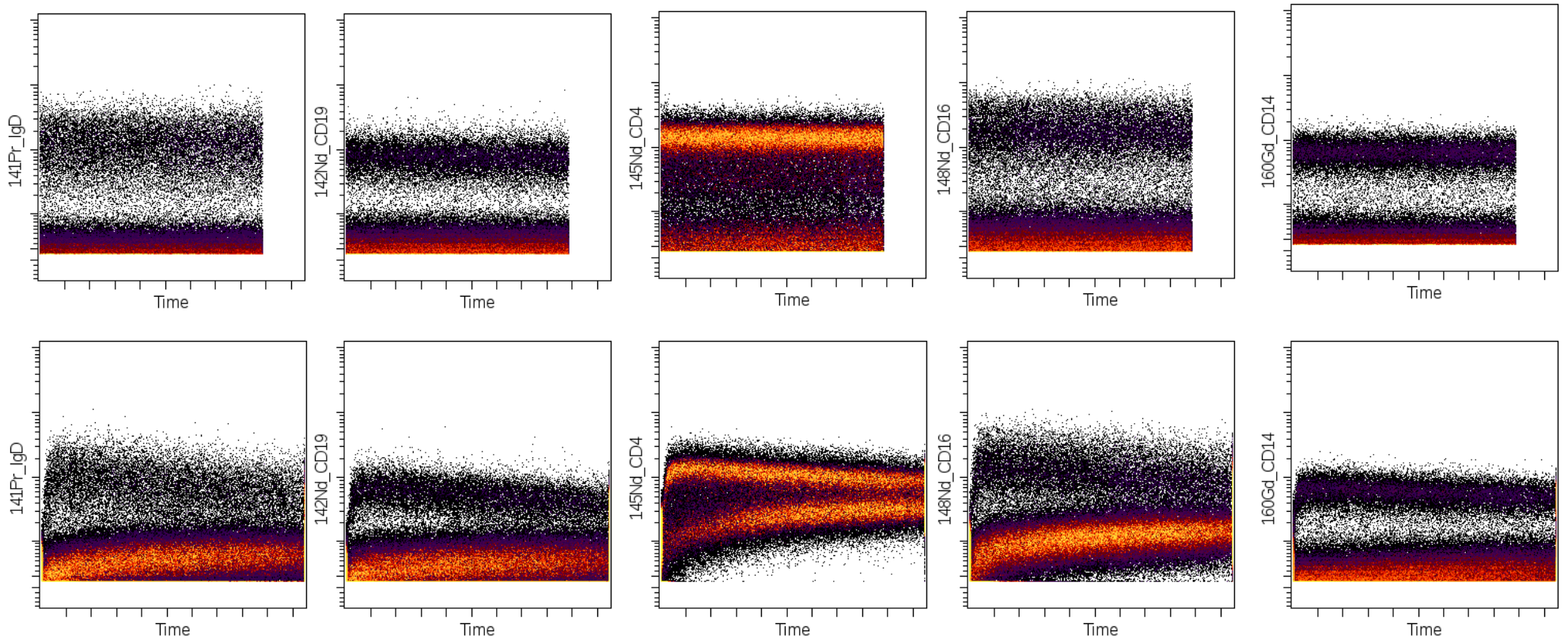
The Problem: increase in background and consequent loss of staining quality

Example: Two aliquots of the same stained sample run sequentially on the same instrument (stored in CSM and washed and resuspended in H20 immediately prior to acquisition)

Same stained sample

Aliquot 1

Aliquot 2



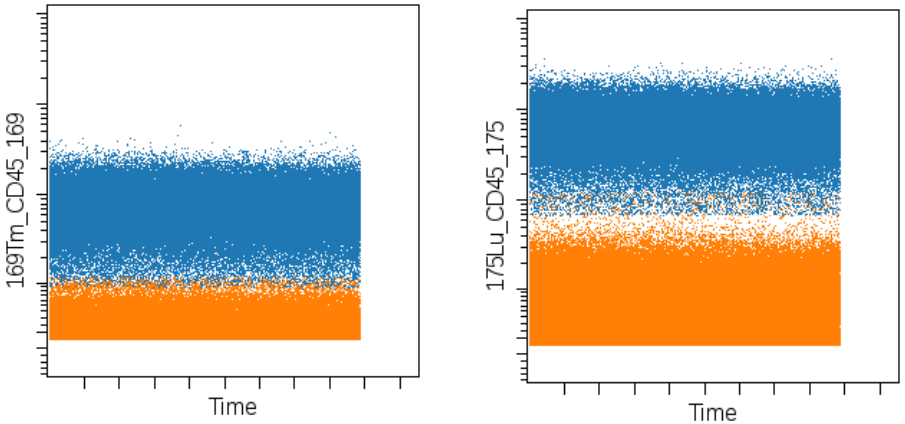
Multiple channels are affected, but the relative impact varies somewhat by channel. The Increase in background is also associated with a corresponding loss of positive signal (more noticeable with lower signal intensity markers due to the relative scale).

The Problem: increase in background and consequent loss of staining quality

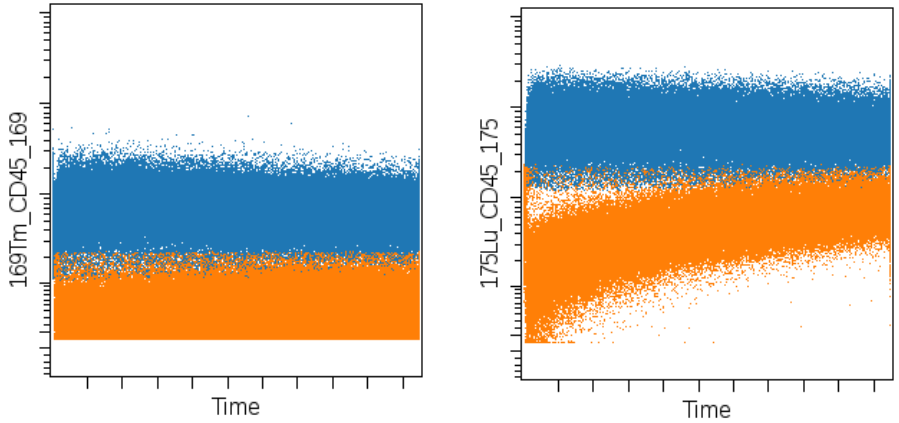
Example: Two aliquots of the same stained sample run sequentially on the same instrument (stored in CSM and washed and resuspended in H20 immediately prior to acquisition)

Same stained sample

Aliquot 1



Aliquot 2



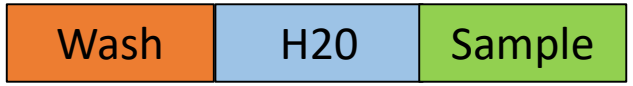
The impact is primarily due to the isotope, not the antigen (e.g., CD45 in two channel; 175Lu seems to be more affected than 169Tm)

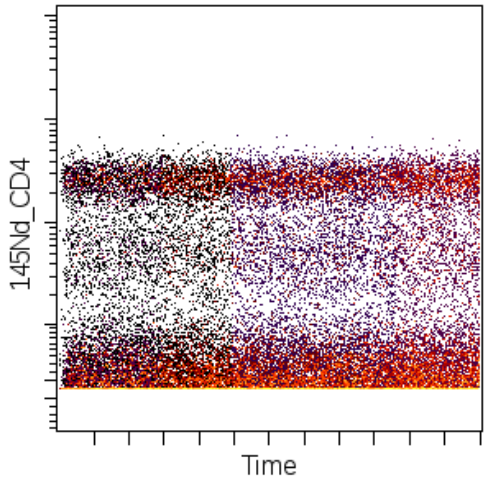
Overall isotope abundance in the sample (more cell positive for a given isotope and/or more isotopes on those cells, i.e., higher signal intensity) seem to be associated with a more noticeable increase in background

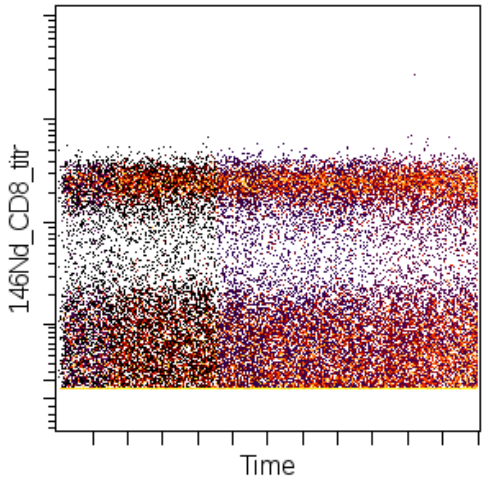
A Potential Cause

Two aliquots of the same stained sample run sequentially on the same instrument (stored in CSM and washed and resuspended in H20 immediately prior to acquisition)

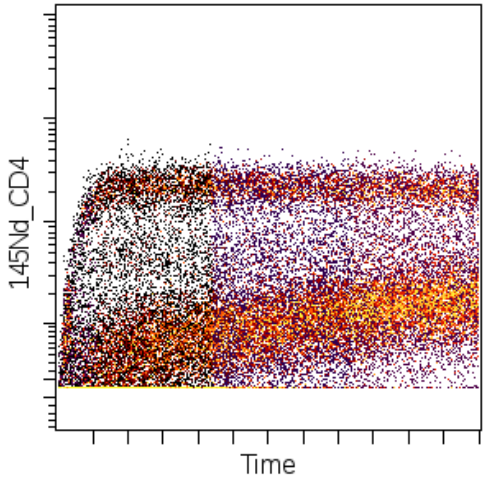
(Normal workflow)

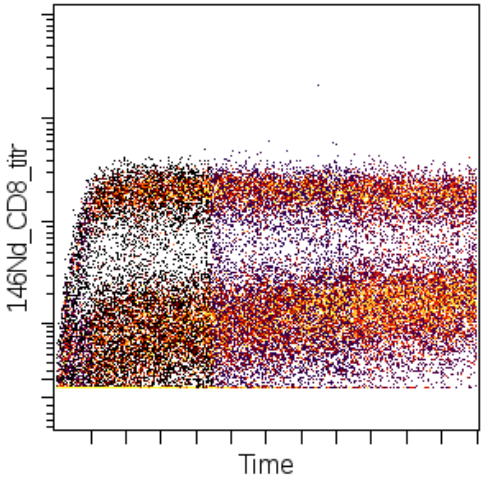






Test workflow)

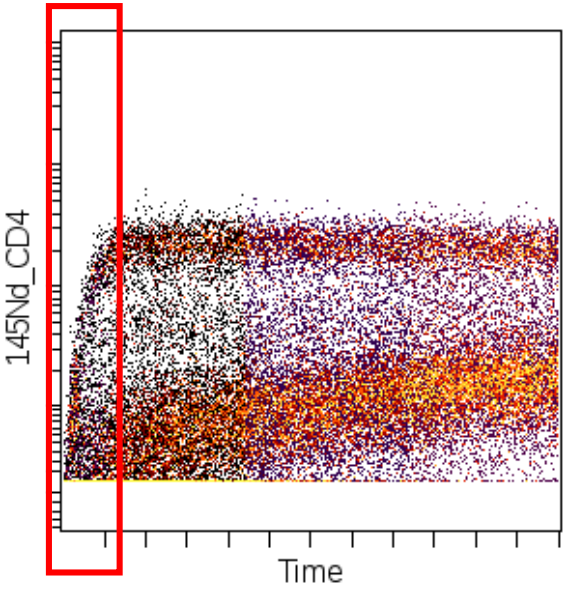




Skipping the water rinse recapitulates this data artifact, suggesting that it is due to exposure of the sample to residual acid from the Wash Solution

Some Additional Thoughts

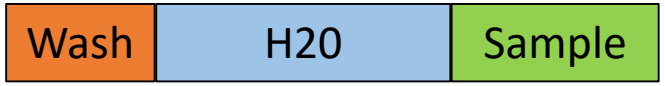
The Helios PSI is expected to be a one-way fluidics path, i.e., residual liquid in the lines should not be expected to back-drip and contaminate the sample. Why would the presence of residual wash buffer at the start of have a progressive impact over the entire duration of the acquisition?

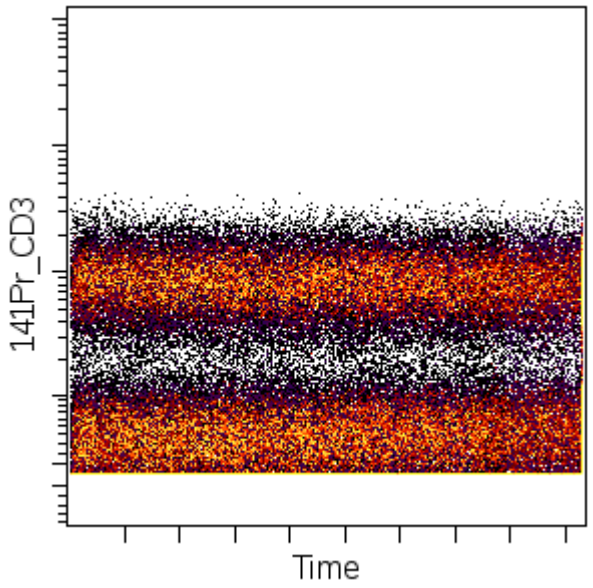


Note the loss of signal at the very start of the acquisition window. This could suggest that metals may be stripped from the cells that are exposed to residual acid at the very start of the acquisition. These metals may then stick to the tubing/nebulizer and then gradually fall off and bind non-specifically to cells over the course of the rest of the acquisition, thus increasing background. Isotopes that are more abundant in the sample or more sensitive to acid may be expected to contribute more significantly to background

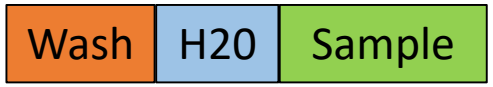
Some Additional Thoughts

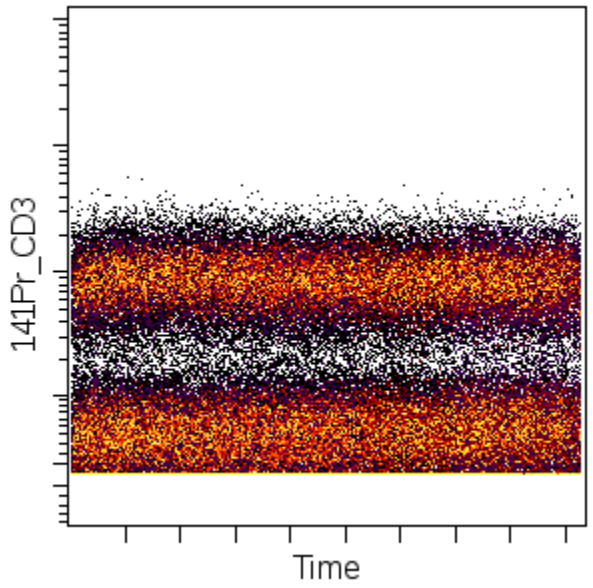
3 mins

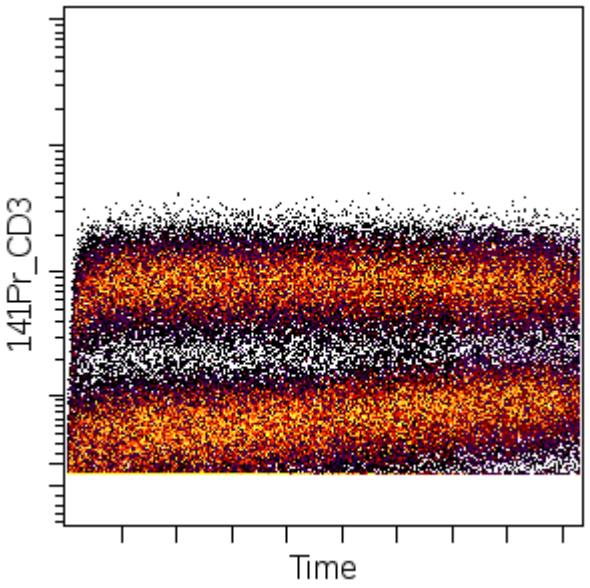




30 sec

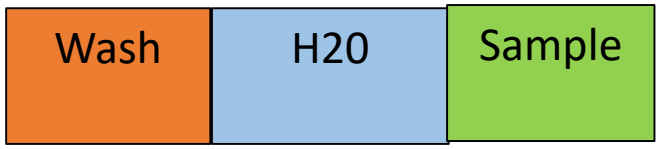


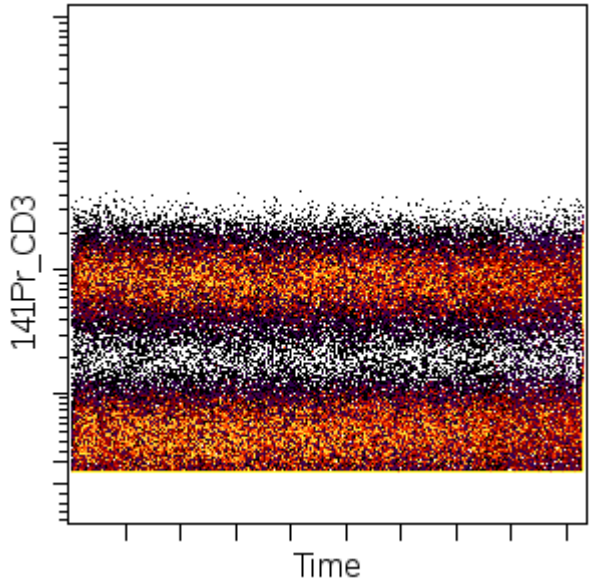


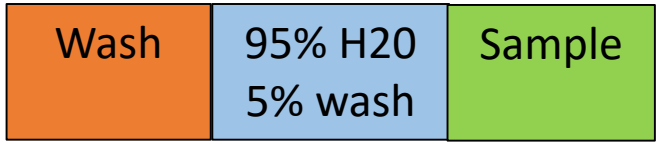


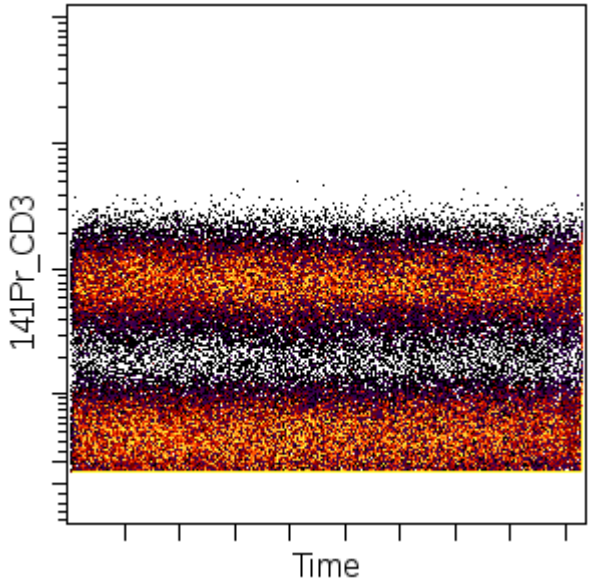
Even a minimal 30s rinse with H_20 is enough to mitigate the effect of the residual acid from the wash buffer

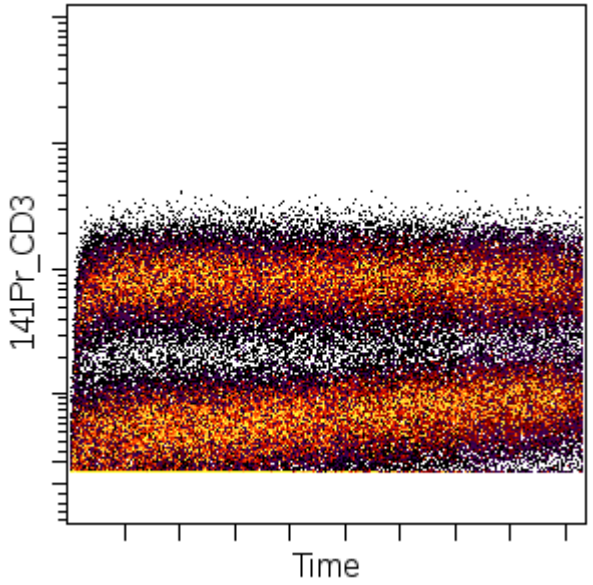
Some Additional Thoughts











Even rinsing with H_20 contaminated with residual wash solution is sufficient to prevent the effect caused by direct exposure to the wash buffer. This suggests that this data artifact results when a sample that has been exposed to fairly high concentrations of residual acid. Unclear why this would occur during normal instrument operation, assuming that normal cleaning procedures (i.e., always follow Wash buffer with a water rinse) are being followed.