WHITEPAPER

Correcting SPR Signal Drift with Detrending on Alto™

Introduction

Signal drift is one of the most common signal artifacts seen in SPR data. Drift is particularly troublesome when measuring tight binding kinetics, as the signal change due to drift may be greater than that of the off-rate. This makes the k_d difficult to measure accurately. A typical method of overcoming signal drift is with a buffer blank subtraction, which involves subtracting a buffer baseline from the binding data. This method, known as "double referencing", therefore requires the length of the buffer baselines to match those of the binding curves. Adding these blanks to experiments is not advantageous because it extends the experiment time and increases the signal noise.

Detrending is another way of applying baseline drift correction, which is offered as part of Alto's analysis accommodations. To apply detrending, the slope of a set period of baseline data is extrapolated to measure and remove the signal drift from each binding curve in a dataset. Detrending only requires a baseline segment of 3 minutes to accurately calculate and remove drift from any binding curve. This is an effective alternative to double referencing due to the absence of any other artifacts in Alto, such as injection induced pressure changes, that are common in traditional SPR instruments.

Detrending

Buffer blanks are useful for subtracting both injection artifacts and drift from binding curves. However, unlike traditional SPR instruments that rely on injections, Alto uses digital microfluidics (DMF) to move solutions in the form of droplets. In the absence of injections, Alto has no injection artifacts to remove, making drift the only undesirable environmental effect to require correction.

Nicoya's detrending algorithm allows users to subtract

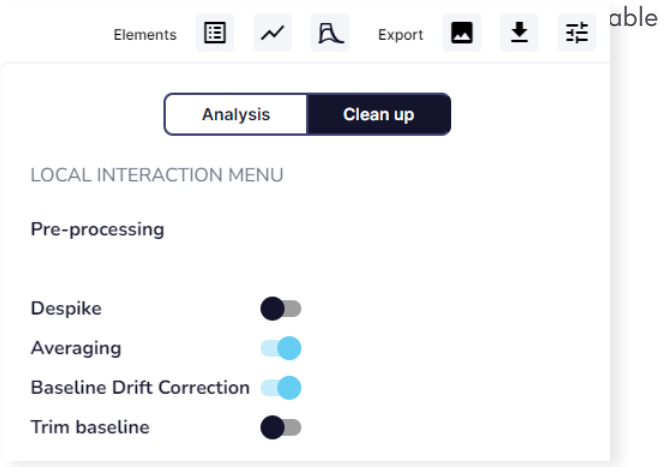


Figure 1: *Interaction tools showing the "Baseline Drift Correction" toggle.*

noise and/or length to their experiments. The algorithm works by calculating the slope of a 3-minute baseline segment, and extrapolating it over the duration of the subsequent binding curve. The extrapolated linear function is then subtracted from the binding curve, resulting in drift-free data.

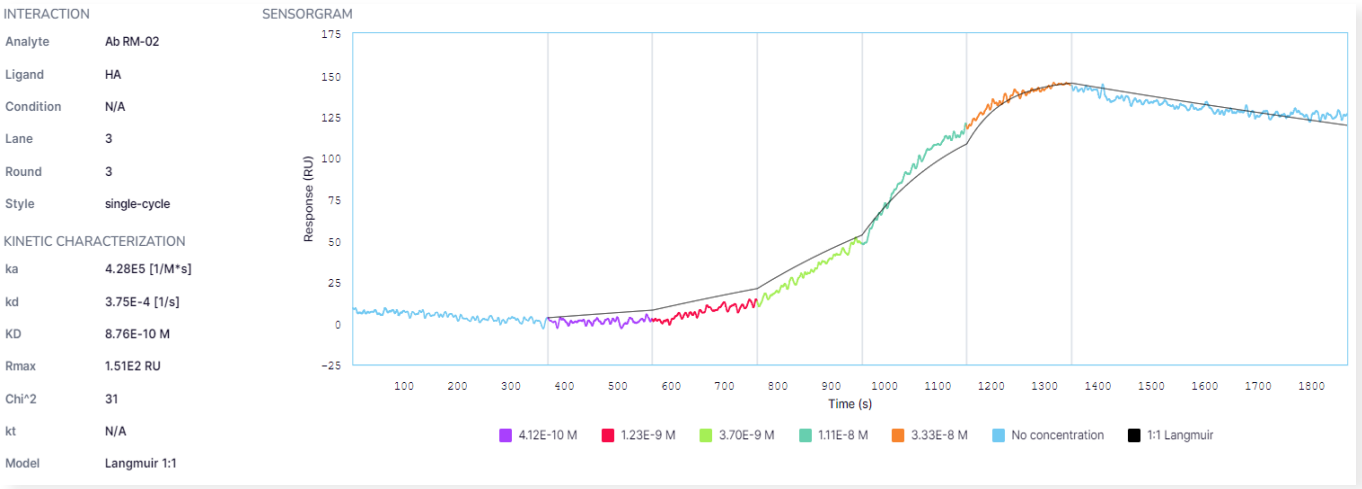
To use the detrending feature, users must ensure that the baseline droplet prior to their first analyte association drop is set to at least 180 s. Once the assay is run, and results are available, the user may open the interaction menu and toggle "Baseline Drift Correction" as shown in Figure 1. This will apply detrending to the entire interaction. Figure 2 shows an example of detrending being applied to a single-cycle kinetics binding curve.

Note: Before applying baseline drift correction, Alto ensures that the data are suitable for detrending. Drift correction will therefore only be applied to a binding curve if the baseline segment is found to be linear. Extending the baseline segment allows Alto to improve its estimation of the drift which leads to both more accurate drift removal.

Conclusion

Alto's use of DMF technology to deliver samples to its sensors makes it uniquely impervious to injection artifacts, thereby removing the need for the traditional buffer blank subtraction. Alto instead uses a detrending algorithm to correct for any baseline drift in its data. This allows users to accurately measure tight off-rates without the need for them to add lengthy buffer blanks to their experiments.

(A)



(B)

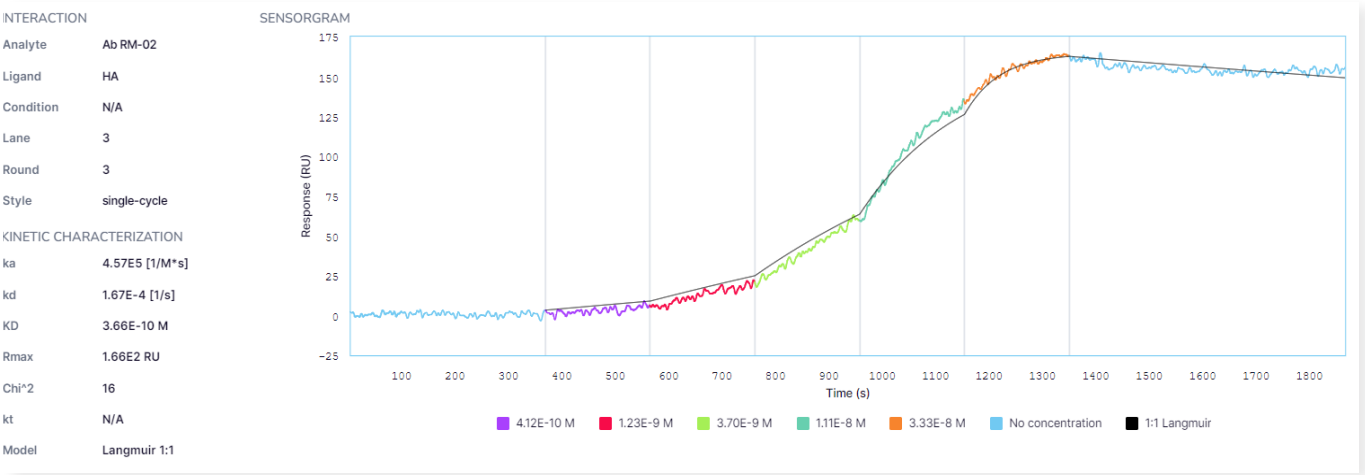


Figure 2: *Alto data before (A) and after (B) detrending.*