TECH NOTE

nicoya

Nicoya Alto CBX Cartridge Sensor Performance

Summary

The reproducibility of several sensor metrics was assessed across 8 carboxyl (CMD) cartridges from different lots. From this study, sensitivity, immobilization, and analyte response were all found to vary by less than 5% both within and across cartridges.

A kinetics test was also performed, testing the same interaction across all sensors, showing a KD variability of only 10% across replicates.

Overview

Alto™, Nicoya's Digital surface plasmon resonance™ (SPR) platform, is a high-throughput benchtop SPR instrument that provides binding kinetics and affinity data for a wide variety of molecular interactions. Conventional SPR instruments use fluid handling methods that utilize pumps and valves for sample handling and delivery to the SPR sensors; Alto uses digital microfluidics (DMF).

DMF is a liquid handling technology capable of accurately controlling and manipulating discrete nanoliter droplets, providing flexibility for assay design and reducing sample volume requirements by up to 100X.

With Alto, the entirety of the fluidic control and sensing is done on single-use disposable cartridges. High accuracy between sensors in and across cartridges is important to achieve robust and repeatable results. This technical note presents the results of a study evaluating the reproducibility of Nicoya Alto® 16-Channel CMD-Carboxyl Cartridges.

Results

Cartridge reproducibility

The immobilization was calculated as the shift between the pre- and post-immobilization baselines. Analyte binding was calculated as the shift between the post-immobilization baseline and the steady-state binding response of the analyte. To assess sensitivity, 3 glycerol concentrations were exposed to each sensor. The responses for each refractive index change were plotted and fit linearly, and the slope was calculated to obtain the sensitivity values.

The measured reproducibility in sensitivity, immobilization, and analyte binding is presented in Table 1, showing less than 5% CV for all metrics.

Figures 1 and 2 present the immobilization and analyte responses, respectively, for all the cartridges tested in this study. These show similar responses with low errors across the different sensors and cartridges, demonstrating the excellent reproducibility of Alto sensor surfaces.

Kinetics reproducibility

The measured reproducibility in streptavidin and the immobilization of a biotinylated ligand (bt-ligand) are presented in Table 2, showing less than 6% CV for both metrics.

The immobilization of streptavidin was calculated as the shift between the pre- and post-immobilization baselines. Bt-ligand immobilization was calculated as the shift between the post-streptavidin immobilization baseline and the beginning of the baseline following the bt-ligand binding step.

Figures 3 and 4 present the streptavidin and bt-ligand immobilization responses, respectively. A standard direct kinetics protocol was used where even sensors are designated as active sensors and odd sensors are designated as reference sensors. The ligand was only immobilized on the active sensors.

Kinetics data from each of the 8 lanes in a single cartridge is shown in Figure 5. These data were fit to a Langmuir 1:1 binding model analyzed in the Nicosystem software. As demonstrated in Table 3, all kinetic parameters had a variability of 15% or less across the cartridge.

Conclusions

Consumable products are a significant potential source of variability in scientific research. This study showed that Alto's 16-Channel Carboxyl Cartridges offer consistent sensitivity, immobilization, and analyte response, both within cartridges and across different lots. These results also take into account differences in instruments and operators.

All metrics show variability below 5% both within a cartridge and across different lots. In a separate assay, KD was shown to vary by only 10% within a cartridge. These metrics show that Alto and its consumables offer consistent and reproducible data, allowing users to trust their results across different sensors and different lots of cartridges.

Appendix

Materials and Equipment

- Alto 16-Channel Instrument with Nicosystem Pro Software (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-CMD-16)
- Alto Carboxyl Surfacing Kit: Cleaning, normalization, activation solutions (ALTO-R-CBX-SURF)
- Running Buffer: PBS-T, pH 7.4 (ALTO-R-PBST)
- Immobilization Buffers: 10 mM Sodium Acetate, pH 5.0 (ALTO-R-IMB-5.0)
- Regeneration Solutions: 10 mM Glycine-HCl, pH 1.5 (ALTO-R-GLYHCL-1.5), 1 mM NaOH (ALTO-R-NaOH-1mM)
- Protein A Capture Kit (ALTO-R-PROA-KIT)
- Human Immunoglobulin G (HIgG), Sigma, Cat # I2511-10mg
- Streptavidin Kit (ALTO-R-STV-KIT)
- Ligand: bt-Oligo (ALTO-R-BT-OLIGO-L)
- Analyte: oligo (ALTO-R-OLIGO-A)

Methods

Cartridge reproducibility

The following test method was executed across eight Alto CMD-16 cartridges from different manufacturing lots on two different Alto instruments. All steps were completed automatically by Alto with no operator supervision.

1. 8, 16, and 32 % (w/w) glycerol droplets were oscillated on each sensor to assess refractive index sensitivity.
2. The sensor surfaces were conditioned with 10 mM HCl.
3. Carboxyl sensors were activated with 200 mM EDC/NHS for 600 s.
4. 5 µg/mL Protein A in pH 5.0 sodium acetate buffer was immobilized on the sensor surface with an interaction time of 600 s on 12 of the 16 sensors, leaving the remaining sensors as references.
5. All sensors were blocked with the 1 M ethanolamine for 300 s to quench any remaining active carboxyl groups.
6. All sensors were conditioned for 60 s with 10 mM, pH 1.5 Gly-HCl.
7. HIgG was diluted to 900 nM in the running buffer and exposed to the sensors for 320 s, followed by a 180 s rinse with the running buffer.
8. Regeneration was performed with 10 mM, pH 1.5 Gly-HCl for 60 s.

Kinetics reproducibility

The following steps were completed automatically by Alto with no operator supervision.

1. Carboxyl sensors were normalized with normalization solutions and primed with 10 mM HCl for 60 s.
2. Carboxyl sensors were activated with 200 mM EDC/NHS for 600 s.

3. The streptavidin from the Streptavidin Kit diluted in 10 mM Sodium Acetate, pH 5.0 was immobilized onto all sensors for 600 s.
4. All sensors were blocked with the 1 M ethanolamine for 300 s to quench any remaining active carboxyl groups.
5. 300 nM samples of bt-ligand in the running buffer (PBS-T) were introduced to each even-numbered active sensor for 300 s.
6. All sensors were conditioned for 60s with 1 mM NaOH.
7. Alto executed five automated analyte serial dilutions on the cartridge. Each sample was diluted from 600 nM stock, producing 2.47 nM, 7.41 nM, 22.2 nM, 66.6 nM, and 200 nM solutions in the running buffer.
8. The five analyte concentrations, from lowest to highest, are exposed to the sensor surface in tandem for 180 s, followed by dissociation in the running buffer for 600 s, and a 60 s regeneration step with 1 mM NaOH. This constitutes a round of single-cycle kinetics.

Metric	Intra-cartridge average	Intra-cartridge CV	Inter-cartridge average	Inter-cartridge CV
Sensitivity	144 nm/RIU	1.7%	146 nm/RIU	4.6%
Immobilization	3377 RU	3.2%	3492 RU	4.4%
Analyte Response	2682 RU	1.4%	2625 RU	4.4%

Table 1: *Reproducibility of Alto 16-Channel Carboxyl Cartridge sensors*

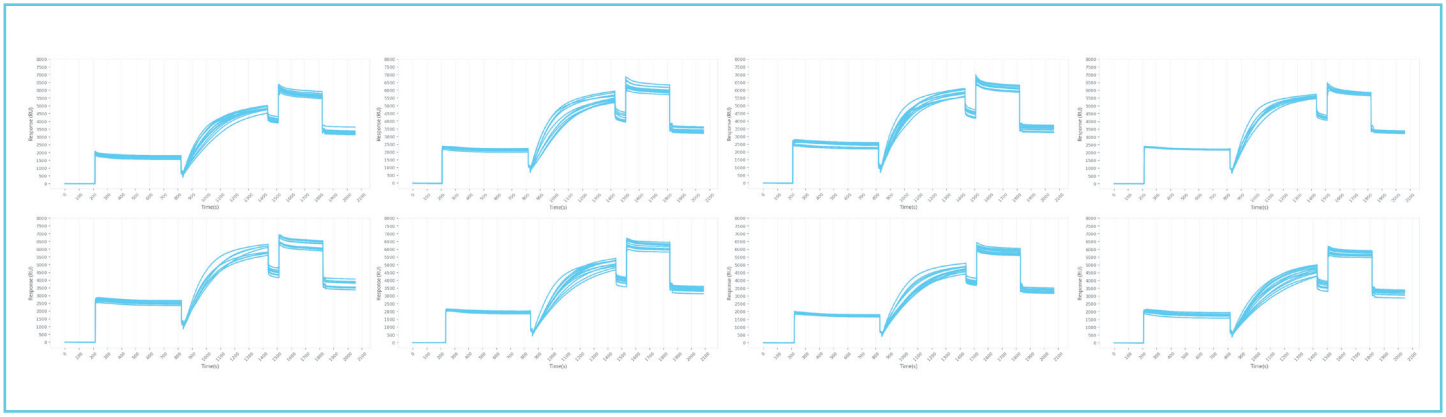

Figure 1: *Immobilization of 5 µg/mL of Protein A in 10 mM sodium acetate, pH 5.0 on each of the 8 cartridges tested. Sensors were first activated with 200 mM EDC/NHS from Nicoya's Carboxyl Surfacing Kit, followed by immobilization of Protein A and blocking of sensors with 1 M ethanolamine. Sensorgrams are zeroed before the EDC/NHS activation, and immobilization is measured after blocking with ethanolamine.*

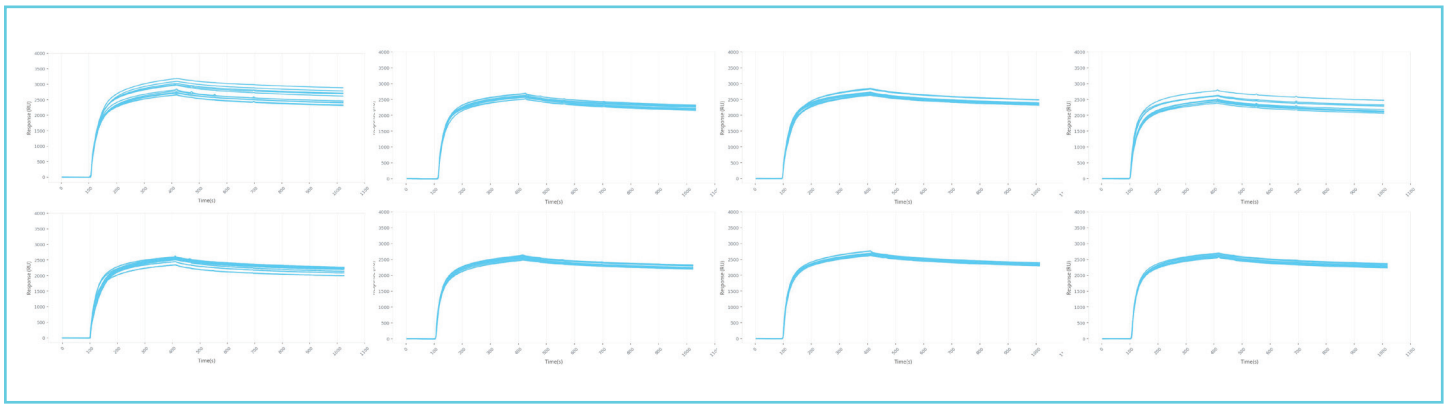

Figure 2: *900 nM HIgG binding to immobilized Protein A on each of the eight Alto 16-Channel Carboxyl Cartridges tested.*

Metric	Average	Intra-cartridge CV
Streptavidin Immobilization	4655 RU	2.4%
Bt-ligand Immobilization	139 RU	5.8%

Table 2: *Reproducibility of Alto CBX sensors in a direct kinetics protocol*

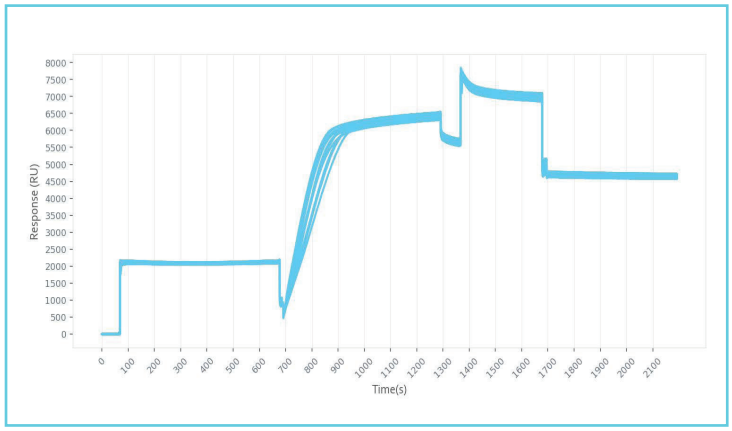

Figure 3: *Immobilization of 10 µg/mL of Streptavidin in 10 mM sodium acetate, pH 5.0 on 1 cartridge. Sensors were first activated with 200 mM EDC/NHS from Nicoya's Carboxyl Surfacing Kit, followed by immobilization of Streptavidin and blocking of sensors with 1 M ethanolamine. Sensorgrams are zeroed before the EDC/NHS activation, and immobilization is measured after blocking with ethanolamine.*

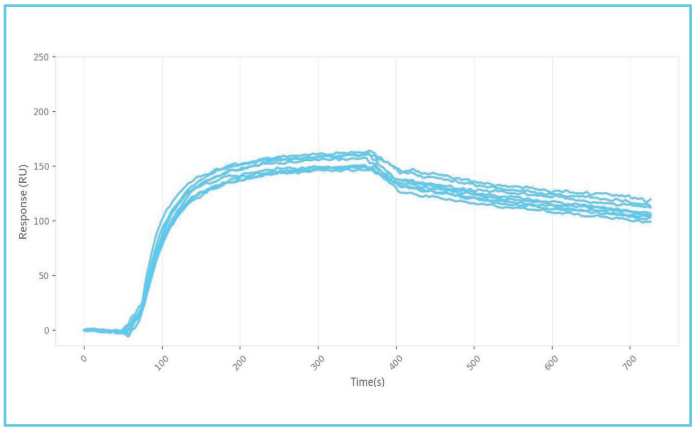

Figure 4: *300 nM bt-ligand non-reversibly binding to immobilized streptavidin on the 8 active sensors.*

Metric	Average	Intra-cartridge CV
R_{max} (RU)	229	7.8%
k_a (M^{-1} s^{-1})	4.60 x 10^4	15%
k_d (s^{-1})	3.77 x 10^{-4}	13%
K_D (nM)	8.25	10%

Table 3: *Kinetics reproducibility of Alto CBX sensors*

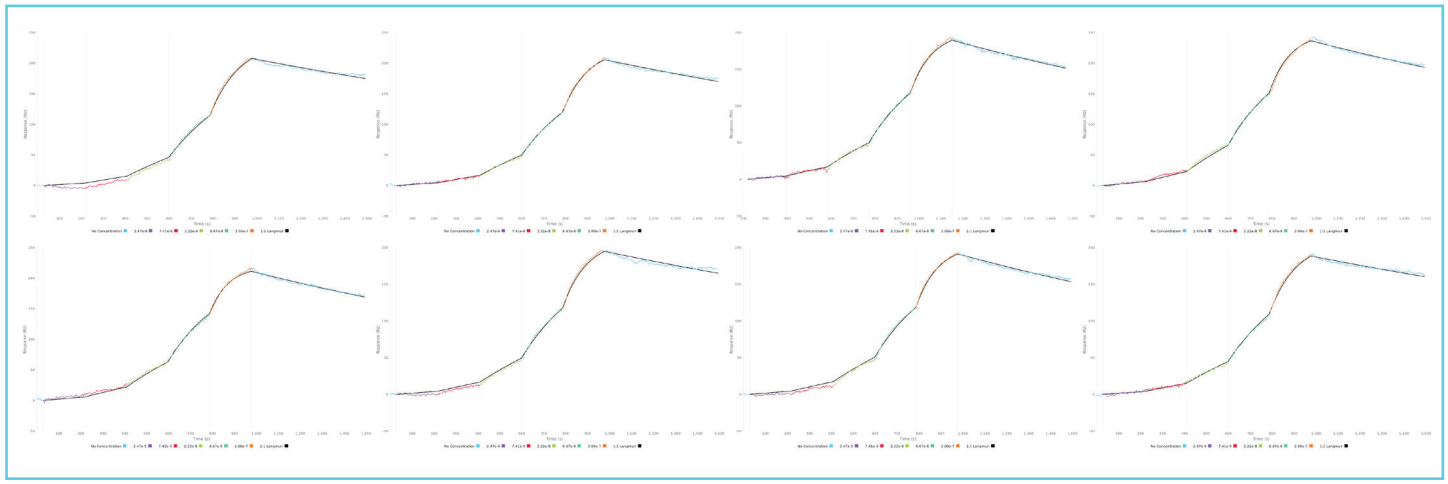

Figure 5: *Single-cycle kinetics of analyte binding to ligand in each lane of a single Alto cartridge. These sensorgrams show reference corrected data, where the paired reference sensor is subtracted from the active sensor to eliminate any background. The analyte was titrated using five concentrations in a 3x dilution series from 2.47 nM to 200 nM. Black curves represent the Langmuir 1:1 binding fit model generated by the Nicosystem software.*