TECH NOTE

Digital SPR STV Cartridge Sensor Performance

Overview

The reproducibility of several sensor metrics was assessed across four streptavidin (STV) cartridges from different lots. From this study, sensitivity, immobilization, and analyte response were all found to vary by <2% within and <5% across cartridges. A kinetics test was also performed, testing a single interaction across all sensors, showing variability of <6% for immobilization and Rmax and <20% for ka and kd across 72 replicates.

Introduction

Nicoya's Digital surface plasmon resonance™ (SPR) platform, is a benchtop SPR instrument with flexible throughput 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; Digital SPR 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's 16-Channel Streptavidin Cartridges.

Results & Discussion

Cartridge reproducibility

To assess sensitivity, three 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 immobilization of a biotinylated ligand 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 end of the analyte association phase.

The measured reproducibility in sensitivity, immobilization, and analyte binding is presented in Table 1, showing <2% variability within a cartridge and <5% variability across cartridges for all metrics.

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

The intra-assay variability was determined from 24 replicates on a single cartridge, while the inter-assay variability was determined from 96 replicates across four cartridges. In both cases, variability was calculated as %CV by dividing the standard deviation by the mean of all replicates.

Kinetics reproducibility

The reproducibility of kinetics determined on the streptavidin cartridges was evaluated with a biotin-oligo ligand and solution oligo analyte system. 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, displayed in Figure 3 and Table 2, showing <6% CV for the bt-ligand immobilization responses.

Kinetics data from each of the 72 interactions in a single cartridge is shown in Figure 4. These data were fit to a Langmuir 1:1 binding model analyzed in the Nicosystem software. As demonstrated in Table 2, k_a and k_d had variability of <20% across the cartridge, while R_{max} had variability of <6%.

Table 1: *Reproducibility of Digital SPR Streptavidin sensors*

Metric	Intra-cartridge mean	Intra-cartridge CV	Inter-cartridge mean	Inter-cartridge CV
Sensitivity	134 nm/RIU	1.5 %	134 nm/RIU	2.1 %
Immobilization	291 RU	1.9 %	297 RU	4.7 %
Analyte Response	1019 RU	1.9 %	1021 RU	2.7 %

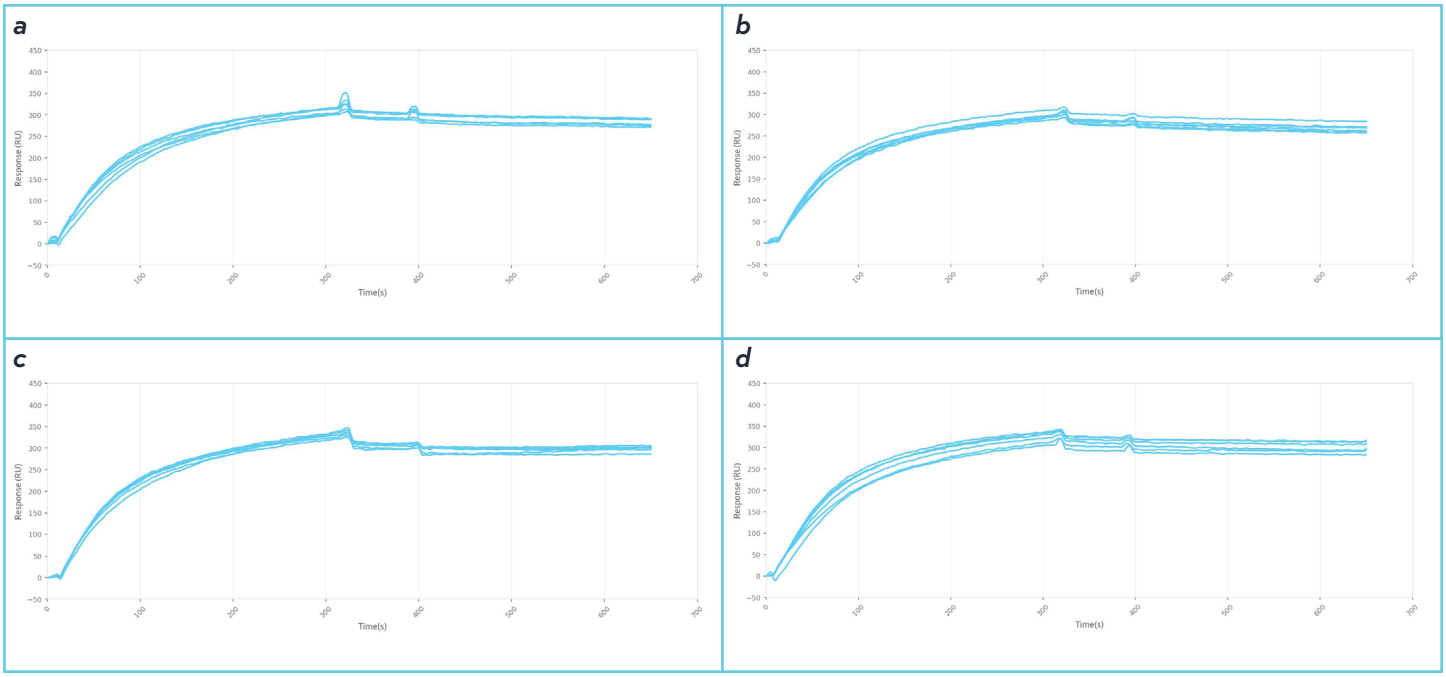

Figure 1: *Immobilization of 4 µg/mL of biotinylated Protein A on each of the four cartridges tested.*

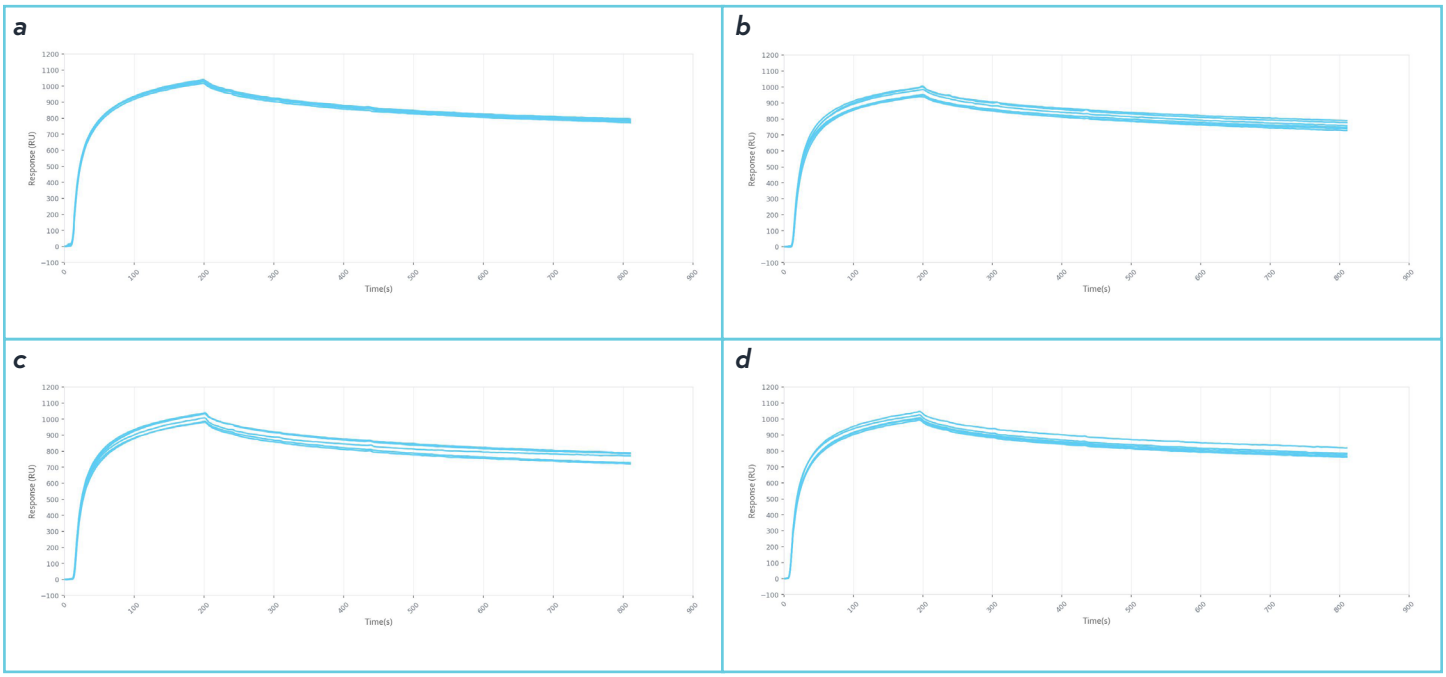

Figure 2: *900 nM HIgG binding to immobilized biotinylated Protein A on each of the four Alto 16-Channel Carboxyl Cartridges tested.*

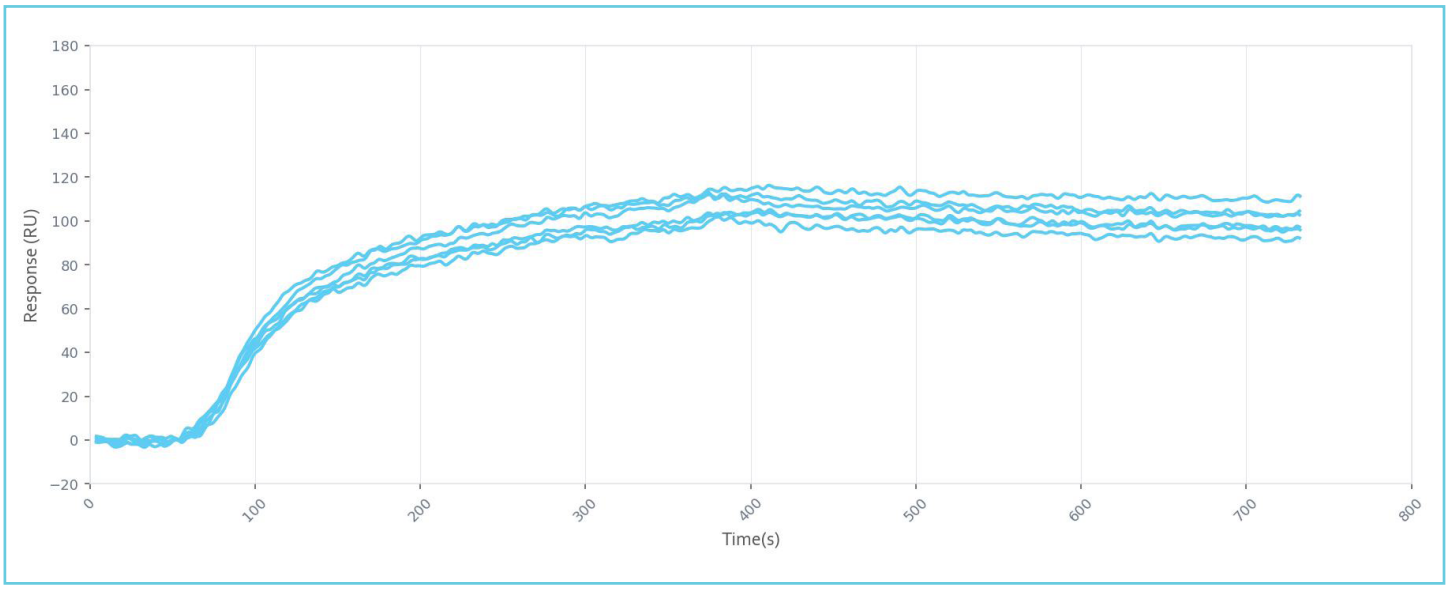

Figure 3: *300 nM bt-ligand non-reversibly binding to the streptavidin sensors (active channels only).*

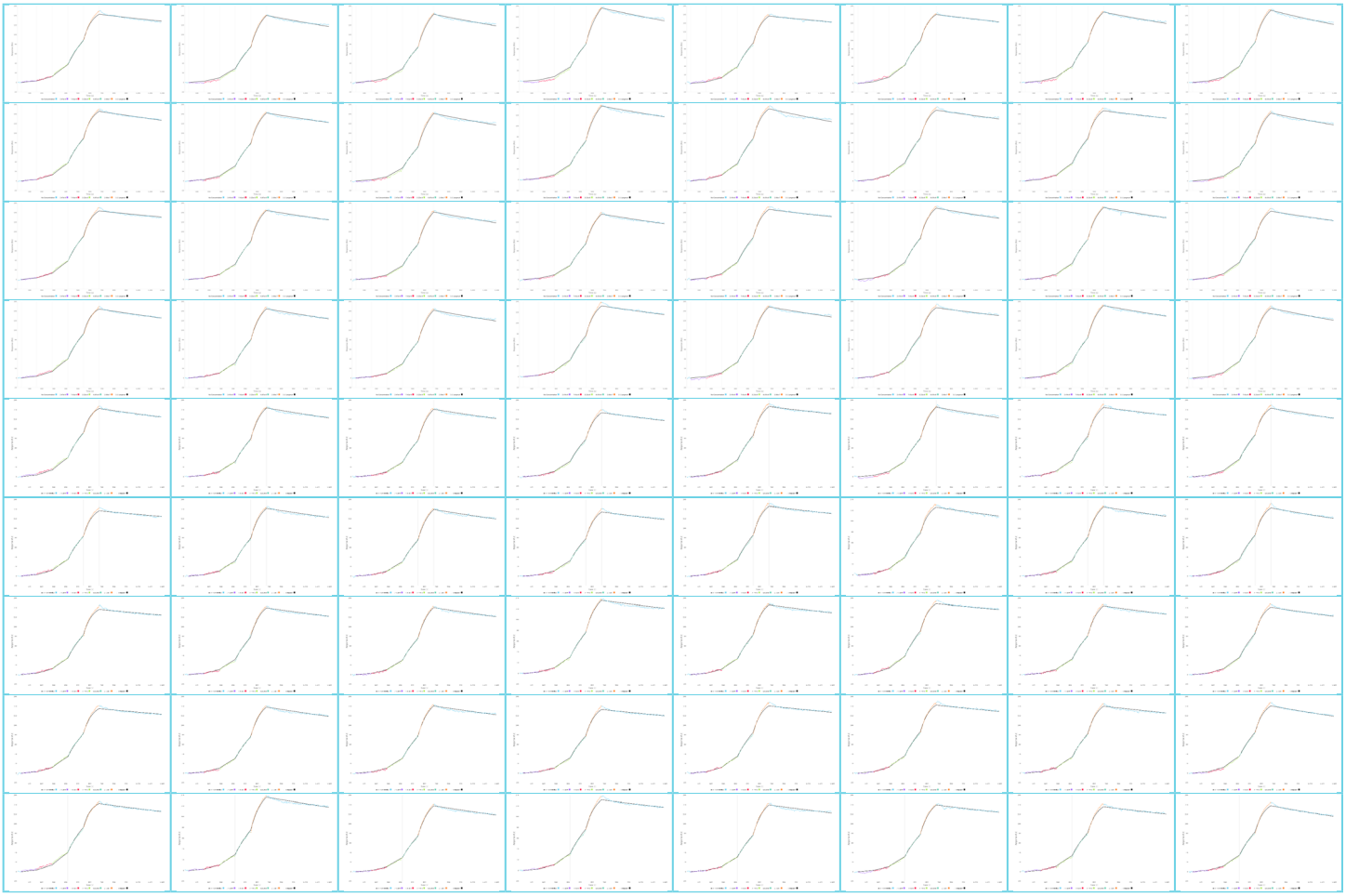

Figure 4: *Single-cycle kinetics of analyte binding to ligand in each lane of a single Alto cartridge. These sensorgrams show reference corrected data, where the paired reference sensor is subtracted from the active sensor to eliminate any background. The analyte was titrated using five concentrations 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.*

Table 2: Kinetics reproducibility of Digital SPR Streptavidin sensors.

Metric	Intra-cartrtidge mean	Intra-cartridge CV
Bt-Oligo immobilization	105 RU	5.9%
R_{max}	164 RU	5.3%
k_a	5.71 x 10^4 M^{-1} s^{-1}	13.9%
k_d	2.74 x 10^{-4} s^{-1}	19.9%

Conclusion

Consumable products are a potential source of variability in scientific research. This study shows Digital SPR Streptavidin Cartridges offer consistent sensitivity, immobilization, analyte response, and kinetics, both within cartridges and across different lots. These results also take into account differences in instruments and operators.

When measured across four, 16-channel cartridges, all metrics show variability <2% within and <5% across cartridges. In a separate kinetics assay, immobilization and R_{max} had variability of <6%, while kinetic parameters k_a and k_d were shown to vary by <20% within a cartridge. These metrics show that Digital SPR and its consumables offer consistent and reproducible data, allowing users to trust their results across different sensors and different lots of cartridges.

Materials

- Alto 16-Channel Instrument with Nicosystem Pro Software (DSPR16)
- Alto 16-Channel Streptavidin Cartridge (KC-STV-CMD-16)
- Carboxyl Surfacing Kit: Cleaning, normalization, activation solutions (DSPR-R-CBX-SURF)
- Running Buffer: PBS-T, pH 7.4 (DSPR-R-PBST)
- Regeneration Solutions: 10 mM Glycine-HCl, pH 1.5 (DSPR-R-GLYHCL-1.5), 1 mM NaOH (DSPR-R-NaOH-1mM)
- Ligand: Pierce™ Protein A, Biotinylated, ThermoFisher, Cat# 29989
- Analyte: Human Immunoglobulin G (HIgG), Sigma, Cat # I2511-10mg
- Ligand: bt-Oligo (DSPR-R-BT-OLIGO-L)
- Analyte: Oligo (DSPR-R-OLIGO-A)

Methods

Cartridge reproducibility

The following test method was executed across four Nicoya Streptavidin 16-channel cartridges from different manufacturing lots and using different instruments.

1. 8, 16, and 32 % (w/w) glycerol droplets were oscillated on each sensor to assess refractive index sensitivity.
2. 4 µg/mL biotinylated Protein A in running buffer was immobilized on the sensor surface for 300 s on 12 of the 16 sensors, the remaining sensors as references.
3. All sensors were conditioned for 60 s with 10 mM Gly-HCl, pH 1.5
4. 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 and 60 s regeneration with 10 mM Gly-HCl, pH 1.5.
5. Step 4 was repeated once for two rounds of binding.

Kinetics reproducibility

1. Sensors were normalized with normalization solutions for 60 s.
2. 300 nM samples of bt-Oligo in the running buffer (PBS-T) were introduced to each even-numbered active sensor for 300 s.
3. All sensors were conditioned for 60s with 1 mM NaOH.
4. Alto executed five automated Oligo 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.
5. The five analyte concentrations, from lowest to highest, were 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.
6. Steps 4 & 5 were repeated for nine rounds of binding.