TECH NOTE

nicoya

Characterization of Alto™ Sample Preparation Precision and Accuracy Enabled by Digital Microfluidics

Summary

Precise and accurate liquid handling is crucial to obtaining repeatable, high-quality data with any analytical instrument. This is especially true when preparing samples for surface plasmon resonance (SPR) experiments, since the sample concentration is the only known variable when solving for kinetic constants. Without accurate and precise liquid handling, error rapidly creeps into concentration calculations, and consequently into kinetic fits. From this study, we conclude that Alto is more precise than the majority of pipettes and liquid handlers, when operating at similar volumes. We also demonstrate that compared to those prepared with a mechanical pipette, Alto-made serial dilutions are more than twice as accurate after three dilutions.

Introduction

Alto is the world's first SPR instrument based on digital microfluidics (DMF). DMF technology is used to move nanoliter-sized droplets from various sample wells to sensors in a disposable cartridge, requiring as little as 2 µL of sample for a full kinetic analysis. Alto mixes reagents and automates analyte sample dilutions in the cartridge, saving the user the time and effort needed to prepare these on the bench.

In this technical note, we demonstrate the reproducibility of Alto's DMF fluid handling in preparing samples for SPR experiments. Two studies were run, which respectively examine the precision and accuracy of Alto's DMF operations compared to sample preparation using mechanical pipettes and automated liquid handler specifications.

Materials & Equipment

Precision Study

- Nicoya Alto 16-Channel Instrument (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-PEG-16)
- Basler Ace Aerial Scan Camera acA2440-35uc
- Imaging fluid

Accuracy Study

- Nicoya Alto 16-Channel Instrument (ALTO16)
- Alto 16-Channel Carboxyl Cartridge (KC-CBX-PEG-16)
- 32% (w/w), 16% (w/w), 8% (w/w), and 4% (w/w) in Milli-Q H2O-T glycerol solutions
- 16% (v/v), 8% (v/v), and 4% (v/v) in Milli-Q H2O-T glycerol solutions
- Milli-Q H2O-T (0.1% Tween20)
- Sartorius Tracta mechanical pipette, single channel 1000 µL
- Eppendorf mechanical pipette, single channel 200 µL
- Reichert Refractometer

Methods

Precision Study

Three Alto 16-channel cartridges were used to collect data for this study. Each cartridge was tested using the following steps:

1. Cartridge unpacked and placed into Alto.
2. Cartridge fluid was loaded into each cartridge.
3. Imaging fluid was loaded into the wells as follows:
   a. 5.5 µL in sample wells (S-wells)
   b. 70 µL in reagent wells (R-wells)

c. 180 µL in the buffer wells (BF-wells)

4. A custom electrowetting protocol containing image acquisition commands was used to dispense 5 droplets from each S-well, 92 from each R-well, and 160 from each BF-well.
5. The droplets were sequentially transported to a single location on the cartridge and imaged by a machine vision camera with macro lens, which was positioned above the cartridge.
6. Once all images were acquired, they were processed through Nicoya's droplet measurement algorithm to calculate their volumes (Figure 1).

Accuracy Study

For this study, various sample solutions were prepared, and were tested on three Alto 16-channel cartridges.

Sample Preparation:

1. Each of 32%, 16%, 8%, and 4% glycerol solutions (w/w) were prepared by weighing pure glycerol and diluting it in Milli-Q water and 10% Tween20 (T20) to achieve the target concentrations (these solutions will be referred to as "control solutions")
2. The 32% w/w glycerol solution was used to prepare 16%, 8%, and 4% glycerol solutions v/v using a pipette (these solutions will be referred to as "pipette solutions").
3. The refractive index (RI) of the w/w glycerol solutions was measured. The refractometer was first calibrated using Milli-Q water. Then, each solution was placed on the Sample Measurement Surface of the refractometer to get the RI reading. The sample well was cleaned with soap, Milli-Q water, and Kimwipe between each solution.

Experimentation:

1. Cartridge was unpacked and placed in the Alto instrument.
2. Cartridge fluid was loaded into each cartridge.
3. Testing solutions were loaded into the wells as follows:
   a. 32%, 16%, 8%, and 4% glycerol control solutions (w/w) were added to four R-wells.
   b. 16%, 8%, and 4% glycerol pipette solutions (v/v) were added to three R-wells.
   c. 5 µL of 32% Glycerol (w/w) was added to the sample wells (S-wells) to make Alto dilutions.
   d. 180 µL H2O-T was added to the buffer wells (BF-wells)
4. A custom electrowetting protocol was used to automate serial dilutions of the 32% w/w glycerol solution in the Alto cartridge, to create 16%, 8% and 4% v/v glycerol samples.
5. The control, pipette, and DMF-prepared glycerol solutions were transported to, and measured with, each sensor in the Alto cartridge
6. Data was analyzed and compared using TraceDrawer and Microsoft Excel.

Results & Discussion

Precision Study

Over 2000 droplets were dispensed and measured in each cartridge. The average droplet volume and coefficient of variation (CV) were calculated for each individual well within each cartridge. Table 1 presents the average CV values obtained for each well type across the 3 cartridges tested, showing an average CV of 2% for S- and R-wells, and of 2.2% for BF-wells. Figure 2 shows representative droplet volume variability data for the different well types.

In Alto experiments, sample droplets from the S-wells in a lane are dispensed and mixed with buffer droplets dispensed from the BF-well in the same lane to create serial dilutions. Mixed droplets then oscillate on the functionalized sensor surface to generate binding curves. The precision and accuracy of dispenses is therefore of paramount importance in calculating reliable binding kinetics.

Most traditional SPR systems require manual sample preparation, meaning that serial analyte dilutions must be done with mechanical pipettes. As part of this study, three models of single and multi-channel pipettes were surveyed.

The manufacturer-reported error for each is presented in table 2, with test volumes selected as the ones closest to Alto's average droplet size of 0.675 µL. For single-channel pipettes, the random error varies between 1.6% and 6%, while the random error of 8-channel pipettes ranges between 4% and 7%. These errors further increase when adding different pipettes, users, and ambient conditions as variables, and propagate as serial dilutions are made.

The precision of three different liquid handler models were also investigated, and is reported in Table 3. All models surveyed reported a random error of 5% when pipetting a

volume of 1 µL under ideal conditions.

With its average dispense precision of 2%, Alto reduces the random error of dispenses more than twice compared to multi-channel pipettes and automated liquid handlers. Alto's dispense precision is also greater than all but one of the surveyed single-channel pipette models. Alto is capable of dispensing between 1 and 88 droplets at once, and can do so with more precision than most commercially available sample preparation options.

Accuracy Study

Localized surface plasmon resonance (LSPR) is a measurement technique that uses the refractive index (RI) properties of the sample, allowing for the sensitive detection of RI variations in interrogated samples. Glycerol, with its high RI, serves as a suitable substance for calibrating LSPR measurements and correlating signal shifts to glycerol concentrations. Figure 3 shows sample LSPR sensorgrams of glycerol solutions, illustrating how the LSPR sensors respond when presented with samples of varying RIs. Using this, it is possible to accurately determine the impact of different sample preparation methods on the signal shifts to see which is most accurate. In this study, w/w glycerol samples were used as controls to establish a reference standard. The LSPR signal shifts of these control solutions were then compared to solutions of the same expected concentration prepared using pipettes and Alto. By comparing the pipette and DMF-made samples to the controls, the accuracy of the different sample preparation methods was assessed.

The LSPR glycerol shift of the control samples, along with the refractometer measurements and glycerol concentrations, were used to develop sensor-specific equations that correlate the experimental glycerol concentrations with the LSPR signals. These equations were used to calculate the true concentration of pipette and Alto-prepared solutions for each glycerol concentration (Figure 4).

These calculations were performed across all sensors and cartridges tested. Table 4 presents the expected glycerol concentration for each dilution, along with the experimental concentrations obtained from the LSPR shifts. As shown in Figure 4, with both methods of sample preparation, error propagation grows exponentially, but at a much higher rate for pipette-prepared samples.

In every case, the samples prepared with Alto have lesser error than those prepared with a pipette, with the lowest experimental pipette concentration having 29% error compared to the expected concentration, while the error of the equivalent Alto-prepared sample is only 12%. When these values are extrapolated to five total dilutions, which is the recommended number for kinetic analysis, the error of the pipette samples reaches 42%, while the equivalent extrapolation for Alto samples stays at 18%. This illustrates that serial dilutions prepared through DMF are significantly more accurate than those using a mechanical pipette, while also operating automatically, and at lower volumes.

Conclusion

Reliable and reproducible, sub-microliter sample dispenses are difficult to achieve, but essential for many analytical techniques. Mechanical pipettes are commonly used for SPR sample preparation, but when pipetting at these small volumes, random pipette error can surpass 6%, and climb even higher when accounting for user error, poorly calibrated pipettes, and environmental factors. Automated liquid handlers also suffer from this imprecision, in addition to being expensive and difficult to integrate with all analytical processes.

Alto's automated, and seamlessly integrated sample preparation saves scientists' time while removing both user and pipette error from dilution preparation, resulting in an average dispense error of only 2%, and a dilution accuracy that surpasses that of manually prepared samples. Alto also eliminates concentration errors that can arise due to evaporation from open well plates and dispersion during pressure driven pumping of samples through fluidic systems. Alto is therefore capable of creating, maintaining, and transporting precise and accurate sample concentrations, resulting in high-quality, reproducible data with minimal hands-on time.

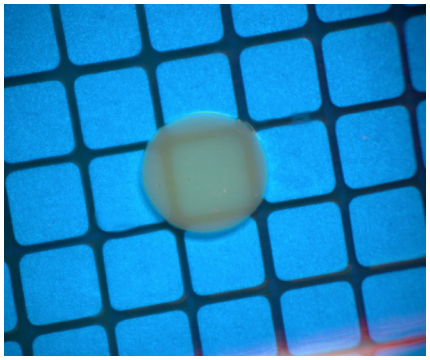

Figure 1: *Droplet image acquired with a machine vision camera after being processed through Nicoya's droplet measurement software.*

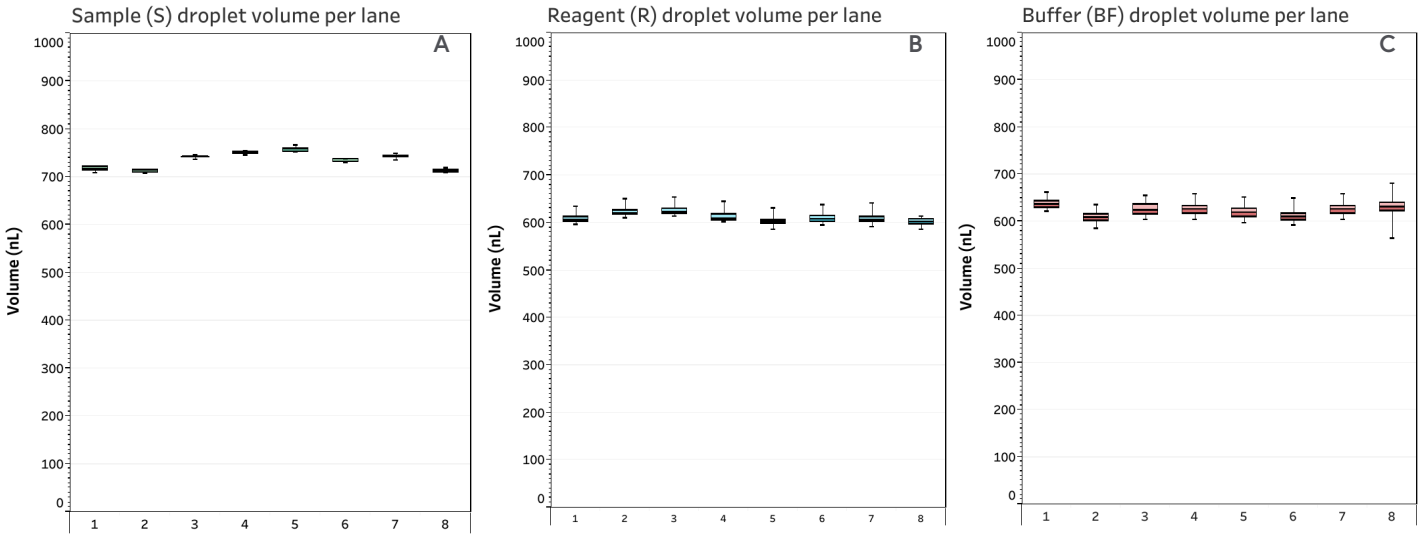

Figure 2: Sample droplet volume measurements and variability for A) S-wells B) R-wells and C) BF-wells in one row in an Alto cartridge.

Table 1: CV of droplets dispensed from the different well types across 3 Alto cartridges.

	S-Well Volume CV (%)	R-Well Volume CV (%)	BF-Well Volume CV (%)
Cartridge 1	3.2	1.6	2.4
Cartridge 2	2.0	3.1	2.3
Cartridge 3	0.8	1.3	1.9
Average	2.0	2.0	2.2

Table 2: Manufacturer-reported error for three single-channel and three 8-channel mechanical pipette models showing the reported test volume closest to Alto's average droplet size of 0.675 µL.

	Channels	Volume Range (µL)	Test Volume (µL)	Systematic Error (%)	Random Error (%)
Pipette 1	1	0.1 - 3.0	0.3	10.0	6.0
Pipette 2	1	0.5 - 10.0	0.5	8.0	2.6
Pipette 3	1	0.2 - 2.0	1.0	3.0	1.6
Pipette 4	8	0.5 - 10.0	1.0	5.5	4.0
Pipette 5	8	0.5 - 10.0	0.5	10.0	4.0
Pipette 6	8	1.0 - 10.0	1.0	12.0	7.0

Table 3: Manufacturer reported error for three different automated liquid handlers showing the reported test volume closest to Alto's average droplet size of 0.675 µL.

	Tip Size (µL)	Test Volume (µL)	Accuracy (%)	Precision (%)
Model 1	10.0	1.0	5.0	5.0
Model 2	10.0	1.0	5.0	5.0
Model 3	10.0	1.0	15.0	5.0

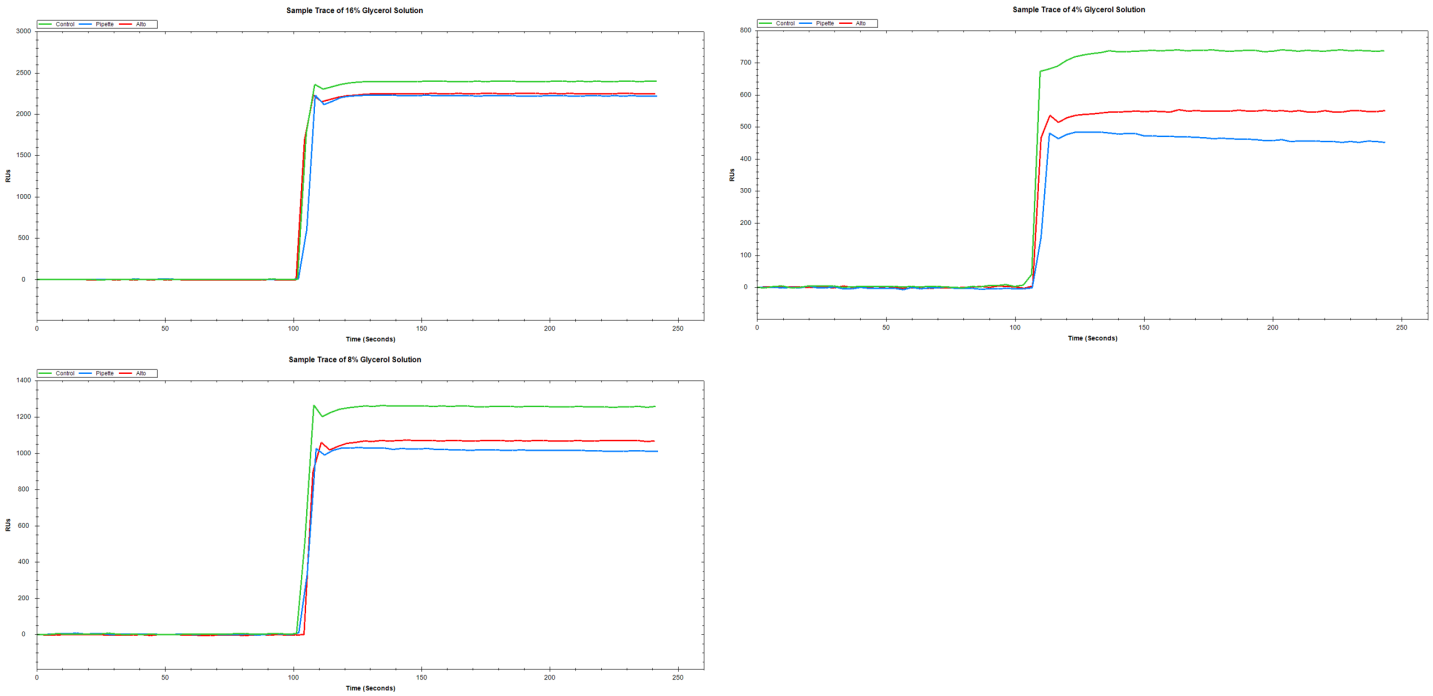

Figure 3: *Sample sensorgrams of the control, pipette and Alto-prepared dilutions for each glycerol concentrations (4%, 8%, and 16%).*

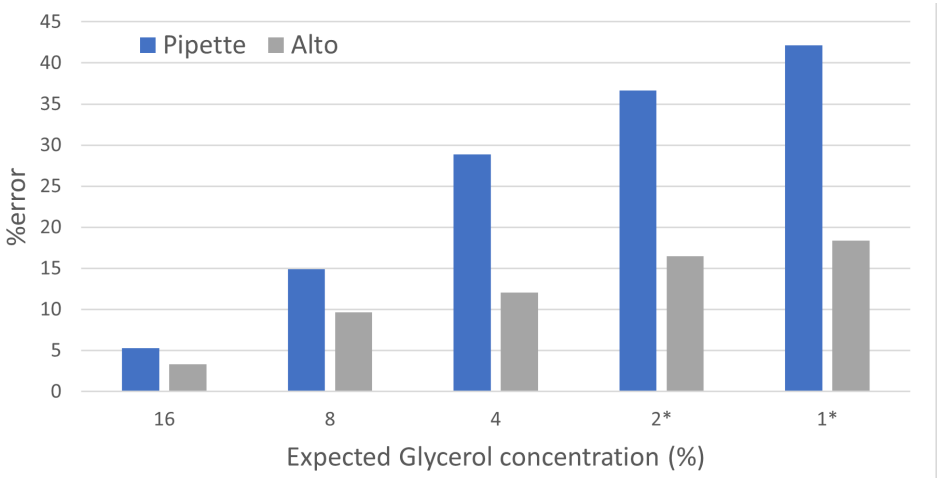

Figure 4: *Calculated error for glycerol samples prepared with Alto and with a mechanical pipette.*

Table 4: Calculated concentrations of pipette and Alto-prepared glycerol solutions compared to the expected concentration.

Expected Conc.(%)	Pipette-Prepared Conc. (%)	SD	%Error	Alto-Prepared Conc. (%)	SD	%Error
1*	N/A	N/A	42.16	N/A	N/A	18.4
2*	N/A	N/A	16.66	N/A	N/A	16.48
4	2.85	0.24	28.87	3.52	0.24	12.06
8	6.81	0.27	14.93	7.23	0.32	9.66
16	15.15	0.25	5.29	15.47	0.37	3.33

**Extrapolated data*