Diagnosing Channel-to-Channel Dispense Variation in Multi-Channel Liquid Handling Systems

Using the AVENTIX Scarlett™ 01 Volume Analysis System

Abstract

Systematic channel-to-channel variation is an inherent characteristic of many multi-channel liquid handling systems. While these differences are often small, they can produce observable banding ("striping") across microplates and contribute to variability in downstream analytical workflows. Rapid identification of these systematic dispensing patterns can assist laboratories in troubleshooting assay performance, evaluating liquid handler condition, and verifying corrective actions following maintenance or calibration.

This application note demonstrates the ability of the AVENTIX Scarlett™ 01 Volume Analysis System to directly visualize and quantify channel-dependent dispense variation across an entire 384-well microplate. Using an eight-channel bulk reagent dispenser as an example, Scarlett identified distinct dispense-channel offsets corresponding to the individual dispensing channels while simultaneously quantifying the magnitude of the observed variation. Although this study utilized a Thermo Scientific Multidrop Combi dispenser, the analytical approach is broadly applicable to any multi-channel liquid handling platform.

Introduction

Multi-channel liquid handling systems are widely used throughout pharmaceutical, biotechnology, and diagnostic laboratories for reagent addition, media dispensing, plate washing, and assay preparation. These systems are designed to deliver highly uniform dispense volumes across an entire microplate; however, because each dispense channel is an independent fluidic system, small systematic differences between channels are a consequence of normal manufacturing and calibration tolerances. Additional variation may arise from fluidic characteristics, component wear, routine maintenance, and normal instrument aging.

Because individual dispense channels repeatedly service the same rows or columns of a microplate, systematic channel-to-channel differences may produce characteristic horizontal or vertical banding ("striping") patterns across a microplate. When similar spatial patterns are observed in downstream assay data, it can be difficult to determine whether the underlying cause originates from the liquid handling process, the assay itself, or other experimental variables.

Scarlett 01 provides rapid, non-contact measurement of liquid volume across an entire microplate. The resulting full-plate dataset allows

systematic dispensing patterns to be visualized and quantified, providing insight into channel-to-channel variation that may contribute to downstream assay variability.

This application note demonstrates Scarlett's ability to identify and quantify systematic dispense-channel variation using an eight-channel bulk reagent dispenser and illustrates how full-plate volume measurements can be used to visualize characteristic dispensing patterns.

Materials and Methods

Dispensing Platform

- Thermo Scientific Multidrop Combi
- Eight-channel dispensing cassette

Test Conditions

- Liquid: Deionized water
- Nominal dispense volume: 20 µL
- Microplate: Greiner Bio-One 781101, 384-well flat-bottom microplate
- Measurement system: Scarlett 01 Volume Analysis System

The Multidrop Combi was equipped with an eight-channel dispense cassette. During dispensing of a 384-well microplate, each dispense channel dispensed into two adjacent rows of the plate (Tip A → Rows A–B, Tip B → Rows C–D, ..., Tip H → Rows O–P), resulting in 48 dispense measurements per channel.

Dispense volume was measured for every well using Scarlett 01. To quantify systematic dispense-channel variation, wells were grouped according to dispense channel. For each channel, the mean dispense volume, channel offset relative to the plate mean, standard deviation, coefficient of variation, and 95% confidence interval were calculated. Channel offsets were calculated relative to the plate mean to isolate systematic dispense-channel differences independent of the nominal dispense volume.

Results

Figure 1 shows the measured dispense volumes across the entire microplate. Distinct horizontal banding corresponding to the eight dispense channels is readily apparent, demonstrating systematic differences in average dispense

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Tip A	A	22.36	20.15	21.92	21.45	20.63	21.64	21.54	21.99	21.94	21.97	21.68	21.78	21.97	21.96	21.90	21.76	21.81	21.40	21.45	21.75	21.26	21.19	20.99	21.90
	B	20.92	22.39	21.63	21.59	21.58	21.84	21.83	22.04	21.86	22.19	21.91	21.96	21.87	21.86	22.11	21.87	21.84	21.89	21.84	21.77	21.82	21.93	22.26	21.30
Tip B	C	22.19	22.64	22.55	22.60	22.64	22.67	22.73	22.72	22.68	22.68	22.79	22.71	22.37	22.67	22.75	22.71	22.42	22.76	22.49	22.49	22.47	22.44	22.84	22.90
	D	21.79	22.29	22.38	22.57	22.52	22.48	22.65	22.57	22.73	22.75	22.88	22.61	22.62	22.89	22.88	22.77	22.84	22.82	22.83	22.61	22.59	22.46	22.53	21.84
Tip C	E	22.34	22.56	22.86	22.71	22.69	22.83	22.67	22.79	22.77	22.75	22.81	22.75	22.68	22.71	22.73	22.68	22.73	22.70	22.63	22.60	22.75	22.30	22.67	22.10
	F	22.67	22.17	22.36	22.33	22.59	22.48	22.57	22.51	22.54	22.78	22.71	22.60	22.64	22.79	22.78	22.71	22.75	22.75	22.86	22.73	22.68	22.56	22.67	22.38
Tip D	G	22.45	22.40	22.52	22.47	22.53	22.50	22.51	22.37	22.35	22.55	22.51	22.42	22.40	22.39	22.39	22.00	22.27	22.40	22.26	22.39	22.50	22.23	22.29	21.82
	H	22.12	22.44	22.35	22.53	22.40	22.39	22.45	22.38	22.44	22.56	22.52	22.50	22.59	22.65	22.52	22.61	22.55	22.56	22.62	22.58	22.40	22.56	22.32	22.65
Tip E	I	22.68	22.78	22.73	22.71	22.73	22.64	22.69	22.59	22.60	22.67	22.60	22.63	22.57	22.55	22.56	22.58	22.46	22.57	22.40	22.47	22.65	22.56	22.46	22.08
	J	22.33	22.33	22.46	22.51	22.52	22.39	22.52	22.54	22.62	22.65	22.62	22.64	22.75	22.74	21.31	22.62	22.65	22.73	22.45	22.54	22.74	22.64	22.78	22.47
Tip F	K	22.90	22.88	22.83	22.88	22.92	22.30	22.80	22.80	22.77	22.82	22.76	22.76	22.75	22.78	22.66	22.70	22.70	22.64	22.64	22.77	22.62	22.79	22.68	22.58
	L	22.65	22.61	22.66	22.59	22.71	22.78	22.42	22.69	22.76	22.89	22.79	22.86	22.94	22.93	22.98	22.87	22.94	22.73	23.07	22.79	22.95	23.08	22.82	23.05
Tip G	M	23.38	23.14	23.07	23.11	23.18	22.94	23.22	22.88	23.03	23.22	22.96	23.10	23.01	22.96	23.00	23.03	22.64	23.12	22.91	22.97	23.01	23.01	22.79	22.94
	N	22.90	22.74	22.93	22.85	22.88	22.78	22.98	22.16	22.67	23.21	22.92	23.02	23.11	23.01	22.51	22.53	23.19	23.07	22.95	23.16	23.13	23.19	23.01	23.19
Tip H	O	22.31	22.10	22.04	21.90	21.61	21.39	22.09	22.09	21.86	21.49	20.91	21.89	22.02	21.26	21.44	21.41	21.48	21.88	21.41	21.81	21.88	21.86	21.80	21.93
	P	22.08	22.05	21.65	21.63	22.11	21.73	21.93	22.15	22.25	22.16	21.35	21.83	22.40	21.50	21.96	22.16	22.63	21.91	22.27	22.13	22.10	22.14	22.02	22.43

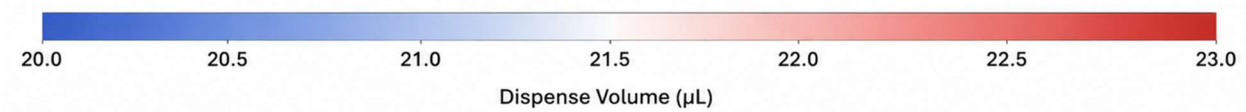

Figure 1. *Full-plate dispense volume measurements obtained with Scarlett 01 from a 384-well microplate following dispensing with an eight-channel bulk reagent dispenser. Distinct horizontal banding corresponding to the eight dispense channels is readily visible, with each channel producing a characteristic dispense volume across the two rows it services.*

To quantify these observations, dispense volumes were grouped according to dispense channel. Figure 2 summarizes the measured channel offsets relative to the plate mean.

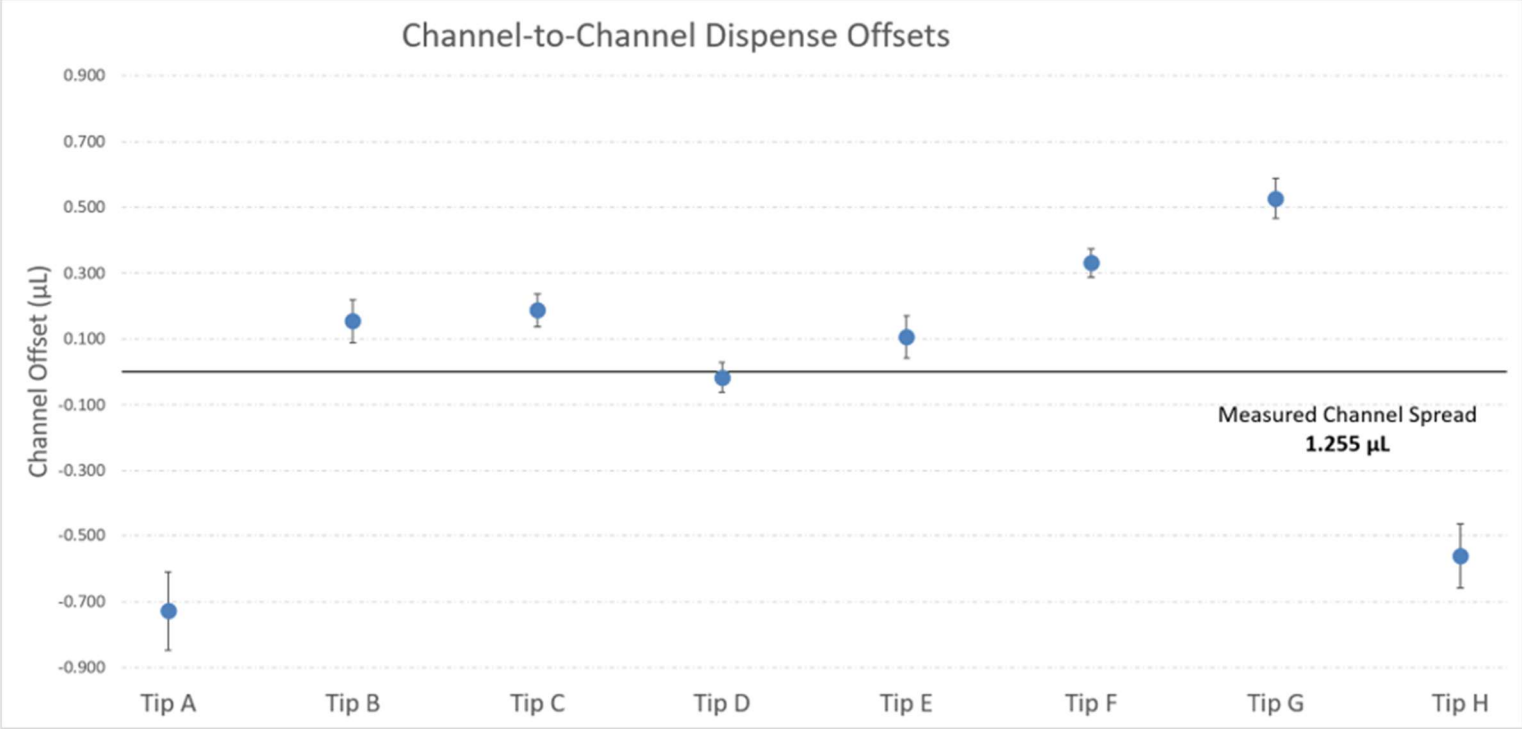

Figure 2. *Mean dispense volume offset for each dispense channel relative to the plate mean. Error bars represent the 95% confidence interval of the channel mean (n = 48 wells per channel), illustrating the magnitude and reproducibility of systematic channel-to-channel variation.*

	n	Mean (µL)	Channel Offset (µL)	Standard Deviation (µL)	%CV	95% Confidence Interval (µL)
Tip A	48	21.72	-0.73	0.42	1.92%	0.12
Tip B	48	22.60	0.15	0.23	1.03%	0.07
Tip C	48	22.63	0.19	0.17	0.77%	0.05
Tip D	48	22.43	-0.02	0.16	0.70%	0.04
Tip E	48	22.55	0.11	0.23	1.01%	0.06
Tip F	48	22.78	0.33	0.15	0.67%	0.04
Tip G	48	22.97	0.53	0.22	0.94%	0.06
Tip H	48	21.88	-0.56	0.34	1.56%	0.10

Table 1. *Summary statistics for each dispense channel, including mean dispense volume, channel offset relative to the overall plate mean, standard deviation, coefficient of variation (%CV), and 95% confidence interval (n = 48 wells per channel).*

Across the eight dispense channels, Scarlett measured a peak-to-peak channel spread of approximately 1.26 µL, corresponding to approximately 5.6% of the overall plate mean dispense volume. The narrow 95% confidence intervals indicate that these differences represent systematic dispense-channel bias rather than random well-to-well variability.

Discussion

The objective of this study was not to evaluate the performance of a specific liquid handling instrument, but rather to demonstrate Scarlett's ability to identify, visualize, and quantify systematic dispense-channel variation when present.

Although a Thermo Multidrop Combi was used for this evaluation, the analytical approach is broadly applicable to any multi-channel liquid handling system in which individual dispense channels repeatedly dispense into defined regions of a microplate. Similar channel-dependent patterns may be observed with reagent dispensers, peristaltic pumps, pipetting heads, and other multi-channel liquid handling technologies.

The presence of measurable channel-to-channel differences does not necessarily indicate poor liquid handler performance. Because each dispense channel represents an independent fluidic system, small systematic differences are expected within normal manufacturing, calibration, and operating tolerances. Characterizing these differences provides insight into dispensing performance and can support

instrument characterization, preventive maintenance, troubleshooting, process optimization, and long-term performance monitoring.

Perhaps more importantly, direct measurement of dispense volume within the actual assay plate enables liquid handling artifacts to be evaluated within the context of the experiment itself. When channel-dependent striping is observed in downstream assay results, corresponding dispense-volume measurements can help determine whether the observed pattern originates from the liquid handling process or from the underlying assay. Where appropriate, measured dispense-volume differences may also support normalization of downstream assay data to reduce systematic channel-dependent bias.

Unlike methods that characterize liquid handling performance using surrogate liquids (e.g., dye solutions), direct measurement of the actual experimental samples within the assay plate provides information that cannot otherwise be obtained. Because the measurement is performed directly on the experimental samples that generate the assay results, the measured dispense-volume distribution directly reflects the dispensing conditions experienced by the assay. Consequently, channel-dependent liquid handling artifacts can be distinguished from assay-related effects, providing a basis for more informed interpretation of experimental results and, where appropriate, normalization of systematic channel-dependent bias.

Conclusion

Scarlett 01 directly measured, visualized, and quantified systematic dispense-channel variation across an entire 384-well microplate, clearly identifying characteristic channel-dependent dispensing patterns through full-plate visualization and quantitative statistical analysis.

By measuring dispense volume directly within the experimental assay plate, Scarlett provides

information that cannot be obtained using surrogate measurement approaches alone. This capability extends liquid volume measurement beyond traditional liquid handler qualification by enabling laboratories to distinguish dispensing artifacts from assay-related effects, improve interpretation of downstream experimental results, and, where appropriate, support normalization of systematic channel-dependent bias.

AVENTIX AB
Gemenskapens Gata 9
Mölndal, 431 53
Sweden

+1 (617)-553 7534
hello@aventix.io