APPLICATION NOTE

Customer Evaluation of the CyBi®-Drop 3D for Screening Applications

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Key words

CyBi®-Drop 3D, Non-contact Dispensing, Dead Volume, Precision and Accuracy, Dispensing to Cell-based Assays, Customer Evaluation for Screening Applications.

Introduction

Library screening applications require the rapid transfer of compounds and reagents into 96, 384, and 1536-well microplates. Traditionally, this has been accomplished with 96- or 384-tip automated pipettors. However, there are several advantages of utilizing a non-contact dispenser for reagents that are delivered to multiple columns of a screen, including elimination of disposable pipette tip costs, increased speed, and conservation of reagents. The CyBi®-Drop 3D is well-suited for this purpose since it has a volume range of 0.5 µL to 10 µL per stroke, can dispense accurately to 96, 384, and 1536-well plates, and can dispense to a pattern of columns across a plate. For these reasons, the CyBi®-Drop 3D was evaluated in the HTS Solutions Laboratory from Invitrogen for use in a screening environment.

Methods

Instrument Description

The CyBi®-Drop 3D offers fast and accurate non-contact dispensing of up to four reagents to microplates through 8-channel stainless steel or PEEK cannulae. Reagents are ejected from dispensing combs via a pair of ceramic pumps, where the volume per stroke can be adjusted without the need for pressurized reagent reservoirs. The CyBi®-Drop 3D a high-precision y- and z-drive unit, which allows for each comb to dispense by column to all wells of 96-, 384- and 1536-well plates. The system used in this study consisted of a standalone CyBi®-Drop 3D with four pumps and four 8-channel combs with stainless steel cannulae, a microplate transport system, one stacker with two towers (30-35 plate capacity) and a barcode reader (see Fig. 1).

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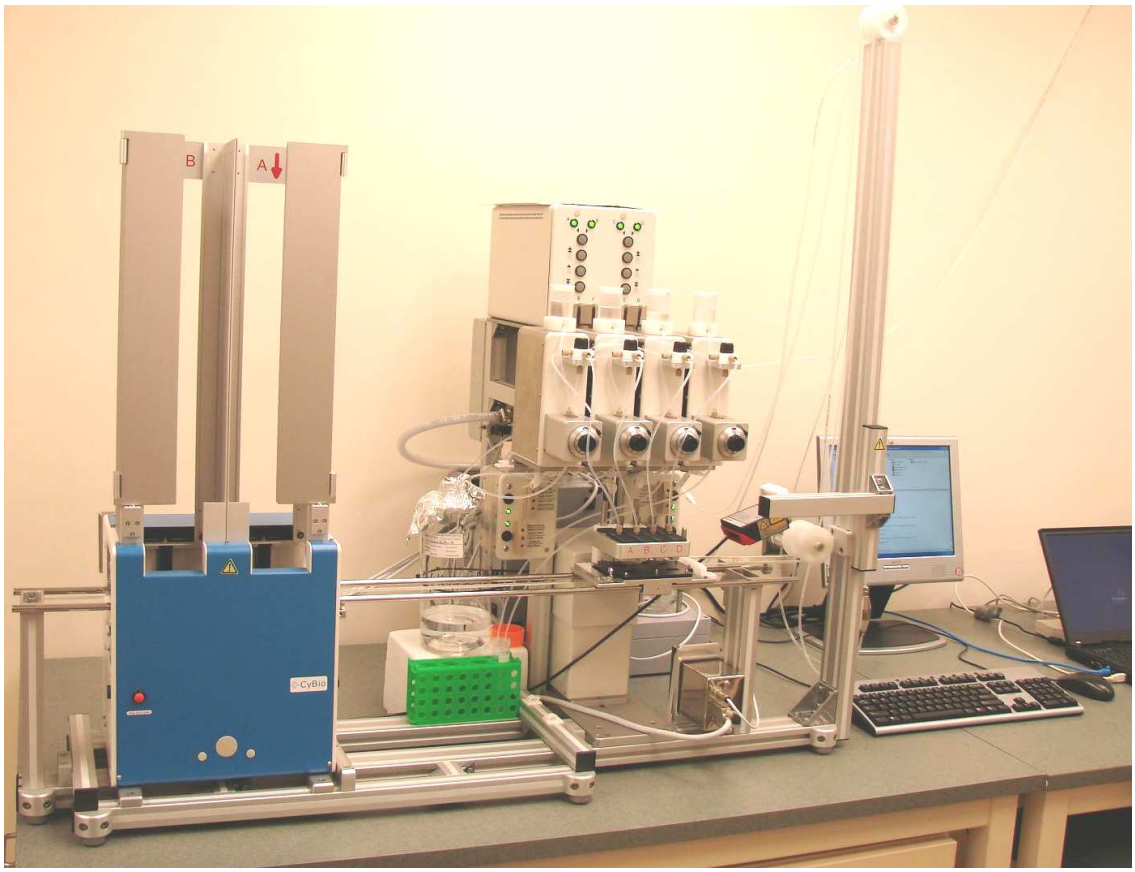

Figure 1: CyBi®-Drop 3D with four dispensing combs, one stacker and a barcode reader.

System Dead Volume

The purpose of this experiment was to determine the approximate dead volumes of the instrument, including priming volume, or the volume required for priming the system plus the residual volume required before air bubbles started to enter the pump; working volume, or sample volume available for dispensing; residual volume, or volume left in the lines that is recoverable but not available for dispensing; and total void volume, or priming volume and residual volume combined. The reagent tubing typically runs from the sample reservoir through a switch box, pump, and valve, and then to the 8 channel comb. For this experiment, the switch box and valve were bypassed.
A volume of 3 mL of water (sample volume) was added to a 5 mL conical sample reservoir tube, and the liquid lines were emptied by priming with air. A disposable trough was tared on an analytical balance and placed underneath the dispenser. The lines were then primed with sample until the lines were completely filled and no air bubbles were seen and a minimal volume of sample had been dispensed to ensure all combs were filled with sample and purged of air bubbles. The trough was re-weighed and the priming volume determined. The sample was then purged out of the system until just before the trailing air reached the pump. The trough was once again weighed and the working volume was determined. This process was repeated two more times to determine average values. Residual volume was calculated as sample volume (3 mL) – (priming volume + working volume). Finally, the void volume or priming volume + residual volume was calculated.

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Accuracy and Precision Testing

The purpose of this experiment was to test the accuracy and precision of the CyBi®-Drop 3D. In duplicate wells in Row B of four 384-well assay plates (Corning Cat. No. 3676), 1 µL to 12 µL 10 µM Fluorescein solution was manually combined with 14 µL to 3 µL 1X Kinase Buffer A (Invitrogen Cat. No. PV3189), respectively, for a total well volume of 15 µL. This standard curve will be used to assess the accuracy of the CyBi®-Drop 3D dispensing. Fluorescence signal intensity was determined using a Tecan Safire2 plate reader.

Assay plates 1 and 2. The calibration dial of the CyBi®-Drop 3D pump was set to 0.5 µL/rev and a program was written to dispense 2.5 µL (five revs) of 10 µM Fluorescein to the assay plate in rows A, C, E, G, I, K, M, and O. 1X Kinase Buffer A (12.5 µL) was manually added to all sample wells. Plates were incubated for 30 minutes prior to measuring fluorescence.

Assay plates 3 and 4. The calibration dial of the CyBi®-Drop 3D pump was set to 2.5 µL/rev, and a program was written to dispense 2.5 µL (one rev) of 10 µM Fluorescein to the assay plate in rows A, C, E, G, I, K, M, and O. 1X Kinase Buffer A (12.5 µL) was manually added to all sample wells. Plates were incubated for 30 minutes prior to measuring fluorescence.

<>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Channel 1																							
B	1 µL		2 µL		3 µL		4 µL		5 µL		6 µL		7 µL		8 µL		9 µL		10 µL		11 µL		12 µL	
C	Channel 2																							
D																								
E	Channel 3																							
F																								
G	Channel 4																							
H																								
I	Channel 5																							
J																								
K	Channel 6																							
L																								
M	Channel 7																							
N																								
O	Channel 8																							
P																								

Figure 2: Plate map of the precision and accuracy test.

2.5 µL of 10 µM Fluorescein dispensed by the CyBi®-Drop 3D
1 to 12 µL 10 µM Fluorescein in 14 to 3 µL 1X Kinase Buffer A, 15 µL total volume
Blank

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Dispensing to Cell-based Assays

The purpose of this experiment was to evaluate if additional assay reagents could be added by the CyBi®-Drop 3D into wells of an assay plate containing 32 µL buffer and an adherent layer of HEK293 cells, without disrupting the cells (e.g. scattering the cells to the sides of the well). Several dispense speeds were evaluated microscopically: 4 µL (1 revolution) was dispensed at 300, 400, 500, and 600 rpm,
two revolutions of 2 µL were dispensed at 300 rpm.

Results

The residual volume and total void volume were calculated for the CyBi®-Drop 3D, and are shown in Table 1. The overall average priming volume for the CyBi®-Drop 3D system was 0.32 mL, average sample volume was 1.62 mL, average residual volume was 1.13 mL, and the average total void volume 1.39 mL.

Run	Priming Volume[1] (mL)	Working Volume[2] (mL)	Residual Volume[3] (mL)	Total Void Volume[4] (mL)
1	0.47	ND	ND	ND
2	0.29	1.45	1.26	1.55
3	0.21	1.79	1.00	1.21
Avg	**0.32 mL**	**1.62 mL**	**1.13 mL**	**1.39 mL**

Table 1: Dead volume determinations for the CyBi®-Drop 3D.

[1]Volume required to prime the lines with sample into the comb
[2]Volume of the total 3 mL sample available for dispensing
Working Volume = Sample Volume - Void Volume
[3]Volume left in the lines that is recoverable but not available for dispensing
Residual Volume = Sample Volume - (Priming Volume + Working Volume)
[4]Void Volume = Priming Volume + Residual Volume

Accuracy results are summarized in Table 2. For plates 1 and 2 (5 dispenses of 0.5 µL/stroke), an inaccuracy of 8.0% was observed for both plates. For assay plates 3 and 4 (1 dispense of 2.5 µL/stroke) inaccuracies of 2.8% and 0.8% were observed, respectively.

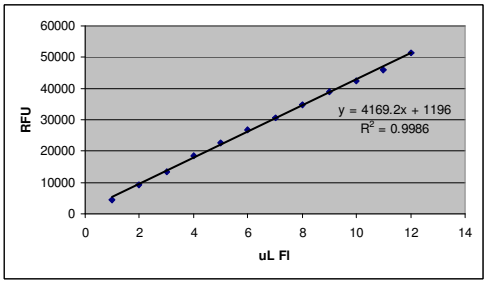
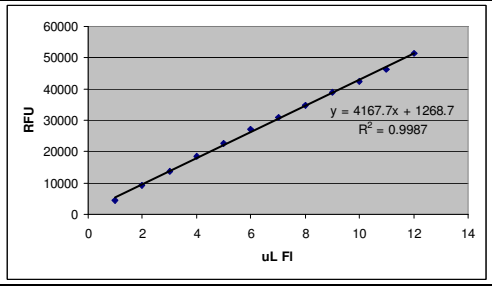
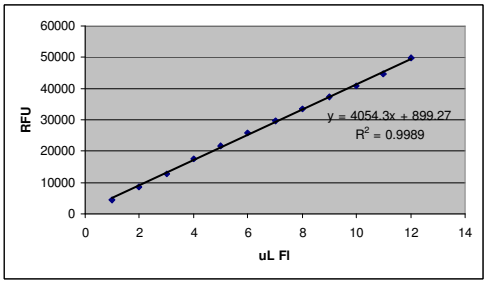
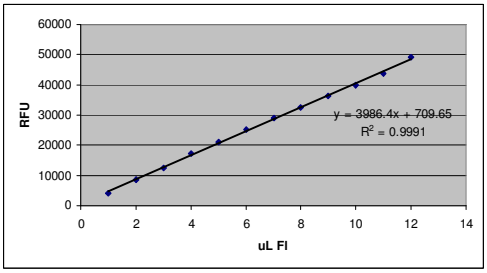
Plate	Standard Curve Graph	Average RFU	Dispensed Volume	Target Volume	Inaccuracy
1		10799	2.30 µL	2.5 µL	8.0%
2		10865	2.30 µL	2.5 µL	8.0%
3		11336	2.57 µL	2.5 µL	2.8%
4		10752	2.52 µL	2.5 µL	0.8%

Table 2: Accuracy results of the CyBi®-Drop 3D testing for dispensing 5 strokes of 0.5 µL per well (plates 1 and 2) and one stroke of 2.5 µL per well (plates 3 and 4).

Results of precision testing are shown in Table 3. For assay plates 1 and 2 (5 dispenses of 0.5 µL per well), precision was 12.6% and 18.3% CV, respectively. For assay plates 3 and 4 (1 dispense of 2.5 µL per well), the observed precision was 3.3% and 3.8% CV, respectively.

	CyBi®-Drop 3D Instrument Settings				
Plate	Calibration Setting, µL/rev	Pump Revolutions	Total Dispense Vol., µL	Vol. Kinase Buffer, µL*	Plate %CV
1	9.02, 0.5	5	2.5	12.5	12.6
2	9.02, 0.5	5	2.5	12.5	18.3
3	8.27, 2.5	1	2.5	12.5	3.3
4	8.27, 2.5	1	2.5	12.5	3.8

Table 4. Precision results of the CyBi®-Drop 3D testing for dispensing 5 strokes of 0.5 µL per well (plates 1 and 2) and one stroke of 2.5 µL per well (plates 3 and 4).
*(*added manually)*

While dispensing of 4 µL assay reagent in one stroke into wells of an assay plate containing 32 µL buffer and an adherent layer of HEK293 cells the cell monolayer could not be preserved completely, independently from the used pump speed settings between 300 and 600 rpm. After this observation the dispense volume was reduced to 2 µL, and the 4 µL assay reagent were dispensed with two strokes per well at low dispense speed of 300 rpm. At these settings, the 4 µL assay reagent was added to the plate successfully with complete preservation of the cell monolayer.

Discussion

System residual volume of 1.13 mL was reasonable, and this sample volume was recoverable. For accuracy and precision testing, using 0.5 µL/rev setting to dispense 2.5 µL was much noisier and less accurate (12.6 and 18.3% CV, 8% inaccuracy) than using the 2.5 µL setting. Since 0.5 µL is the lowest setting for the CyBi®-Drop 3D pump, it is possible that dispenses using the corresponding calibrator dial setting were less accurate per stroke, and the error was additive. In addition, it is also possible for the 0.5 µL/rev dispensing precision and accuracy to be improved with a recalibration. However, the 0.5 µL/rev data was acceptable, except for pockets of lower RFU values, across the plate. For this experiment, it appeared advantageous to dispense 2.5 µL consistently using one rev of 2.5 µL/rev rather than five revs of 0.5 µL/rev. Accuracy and precision for dispensing using the 2.5 µL/rev setting were both acceptable (0.8 and 2.8% inaccuracy, 3.3 and 3.8% CV). According to the manufacturer, the calibration is stable once set. However, if the accuracy begins to slip over time, the instrument can quickly be recalibrated through the dials on the front-side of the pump(s).

Overall, the CyBi®-Drop 3D met or exceeded all validation requirements for the High Throughput Screening laboratory at Invitrogen. The low dead volume, high precision and high accuracy at 2.5 µL of the CyBi®-Drop 3D, and its ability to dispense in cell-based assays with complete preservation of the cell monolayer all contribute to the excellent suitability of the instrument for a high throughput screening environment.