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I.DOT & DropDetection: A unique feature for quality control and dispensing protocol optimization.

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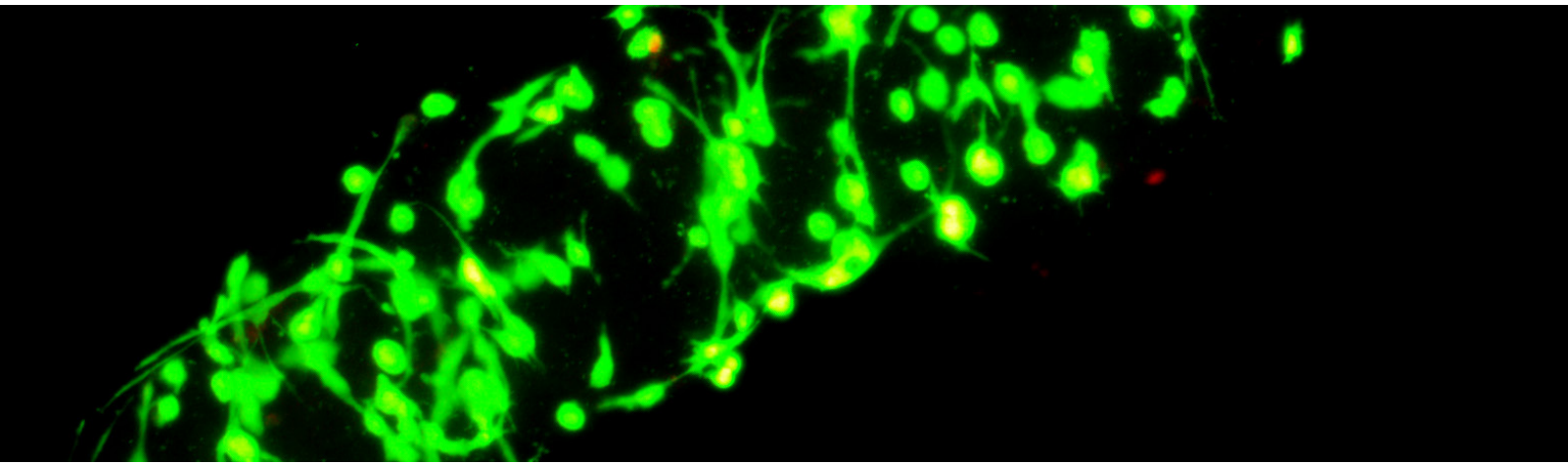

Introduction

Non-contact, low-volume dispensing depends on generating individual and discrete droplets. They typically range from pico- to microliters, and volumes this small can't be evaluated visually. Immediate Drop-On-Demand Technology (I.DOT) is a non-contact, low-volume dispensing platform. The general principle is based on a hole (less than 100 µm) in the bottom of a microtiter plate well, where surface tension and capillary forces retain the sample liquid in the cavity. Applying a pulse of air pressure on top of the well forms a precise droplet that can be released into or onto almost any target.

Each I.DOT is equipped with DropDetection to interface with the low-volume world.

DropDetection is a patented feature unique to the I.DOT. It enables detection and counting of every droplet released during a dispensing run. It acts as a simple yet powerful tool for quality control and protocol optimization.

DropDetection is mounted under the I.DOT One source tray (**Figure 1**). It's a circuit board that leverages 96 miniaturized light barriers to detect every droplet that each source plate position generates. DropDetection identifies changes in light intensity to detect droplets passing the light barrier. After dispensing is complete, DropDetection provides a text file and color-coded result. Green indicates successful dispensing and red indicates an error. If the software recognizes the error, it displays the potential reason as well.

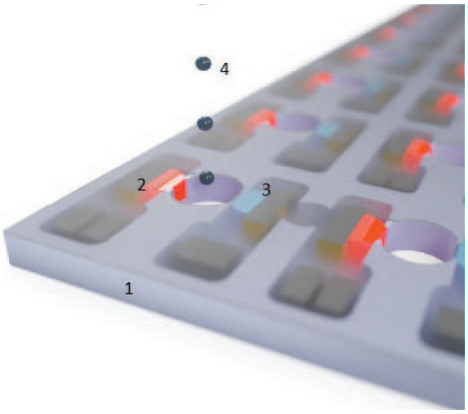

Figure 1. *Individual droplets dispensed through source tray of I.DOT One and DropDetection (**1**) circuit board underneath source tray of I.DOT One, LED (**2**), photodiode (**3**) and dispensed liquid (**4**).*

DropDetection output notes:

- Passed – everything went accordingly.
- Strange – unexplainable error.
- Unfilled/Clogged – the well wasn't filled to begin with, or got clogged.
- Became empty – the liquid in the well became empty; there was not enough for the completion of the assay.

We designed an experiment to validate DropDetection's performance.

Experiment design

We setup a dispensing protocol to demonstrate four scenarios (**Figure 2**). Four liquids were dispensed into a 384-well target plate.

"Water 1" (**yellow**) represented normal water.

Fifty µL were added to the source well. No error was expected.

"Water 2" (**green**) also represented water. Here, an insufficient amount (less than 14.4 µL as calculated by the software) to run the assay was added to the source well. The error "became empty" was expected.

"Water 7" (**blue**) represents water mixed with a foaming agent to mimic unreliable dispensing behavior. The error "strange" was expected.

"Water 5" (**purple**) is not filled. The error "unfilled/clogged" was expected.

After setting up and filling the source wells with liquids, the I.DOT was ready for dispensing. To dispense a total volume of 150 nL per target well, 3 droplets of 50 nL droplet volume are generated.

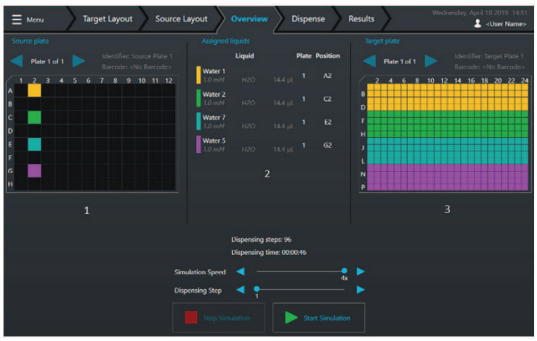

Figure 2. *Example setup of a dispensing protocol. (**1**) source plate layout, (**2**) liquids used in the setup (with indication of minimum volume to complete the dispensing task), (**3**) target plate layout.*

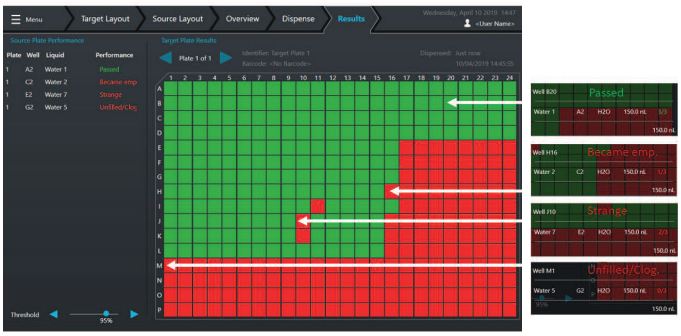

Figure 3. *Color-coded DropDetection results.*

Results

The results demonstrate the I.DOT's dispensing accuracy in each scenario (**Figure 3**).

"Water 1" was dispensed successfully. The software indicates this with the performance note "passed."

Three droplets of 50 nL were expected and dispensed in well B20.

"Water 2", as expected, began dispensing successfully. Due to insufficient volume in the dispensing well, the well ran empty. The software indicates this with the performance note "became empty." Three droplets of 50 nL were expected in well H16, but only 1 droplet was dispensed.

For "Water 7", the software shows the performance note "strange," and the color scheme notifies the user of a dispensing error. Three droplets of 50 nL were expected in well J10, but only 2 were dispensed, possibly due to air bubbles produced by the foaming agent.

For "Water 5", the software indicates that the source well was "unfilled" or "clogged" from the beginning. Three 50 nL droplets were expected and none were released. Each dispensing run generates a DropDetection report file (**Figure 4**). The report log displays the dispensed liquid, positions of source and target wells and the detected droplets of each individual liquid-transfer step. These report files can be used to evaluate dispensing performance, and act as immediate quality control.

DateTime: 2019-04-10 14:46:45
SoftwareVersion: 1.3.20180912

Source Plate ID: Source Plate 1 (PurePlate
Target Plate ID: Target Plate 1 (MWP 384)

Liquid	Source	Target	Drop	Miss	TargetVolume
Water 1	A2	A1	3	0	150 nL
Water 2	C2	E1	3	0	150 nL
Water 7	E2	I1	3	0	150 nL
Water 5	G2	M1	0	3	150 nL
Water 1	A2	B1	3	0	150 nL
Water 2	C2	F1	3	0	150 nL
Water 7	E2	J1	3	0	150 nL
Water 5	G2	N1	0	3	150 nL
Water 1	A2	C1	3	0	150 nL
Water 2	C2	G1	3	0	150 nL
Water 7	E2	K1	3	0	150 nL
Water 5	G2	O1	0	3	150 nL
Water 1	A2	D1	3	0	150 nL
Water 2	C2	H1	3	0	150 nL
Water 7	E2	L1	3	0	150 nL
Water 5	G2	P1	0	3	150 nL
Water 1	A2	A2	3	0	150 nL
Water 2	C2	E2	3	0	150 nL
Water 7	E2	I2	3	0	150 nL

Figure 4. *DropDetection report file*

Conclusion

DropDetection reliably and robustly detected the expected outcome.

This example was performed with water. However, the DropDetection works with every dispensable liquid — including DMSO, glycerol-containing solutions and more. Contact Dispendix for more information on dispensable liquids.

DropDetection has great potential to control dispensing quality. It's a noninvasive recording technology that accurately identifies dispensing faults to optimize dispensing protocols.

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