Thermal Inkjet System Enabling Biomolecule Dispensing for Life Science Research

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Abstract

A system designed to dispense small-molecule compounds dissolved in dimethyl sulfoxide (DMSO) into ANSI-standard microtiter plate was developed and commercialized by Hewlett Packard (HP) in 2011. This commercial system, which included the HP D300 Digital Dispenser and the T8 Dispensehead Cassette, was reported at the Digital Fabrication 2011 Conference [1] and an early beta version of the system was reported at the Digital Fabrication 2006 Conference [2]. The D300, based on HP's Scalable Printing Technology (SPT), has proven to be a useful tool for conducting pharmaceutical research with compounds in DMSO [3,4,5]. The large dynamic range enabled by non-contact picoliter dispensing enables the rapid creation of dose-response experiments, including multi-level drug-drug interaction experiments.

In this contribution, we discuss key improvements to the system since commercial release, most importantly, the expansion of the capability of the system to enable the dispensing of various aqueous-based biomolecules, including proteins, DNA, lipids, and nanoparticles. We will discuss challenges in developing these new capabilities, especially around enabling DMSO and aqueous-based fluids to be dispensed accurately from a common dispensehead, and examples of the types of experiments that are now enabled. We will also discuss future directions to further expand the utility of the digital dispensing platform in pharmaceutical and life science research.

Introduction

Serial dilution is routinely used in pharmaceutical research to create varying concentrations of compounds for dose-response analyses, for secondary screening, and for various absorption, metabolism, toxicity, and compound-compound interaction studies. This dilution method is also used to create varying concentrations of biomolecules, such as proteins, DNA, or lipids for studies of interest, such as enzyme profiling experiments. The comparatively large minimum dispense volumes of existing technologies such as microliter-scale pipetting require a slow and expensive workflow including multiple serial dilution steps to span the concentration range of interest [6].

The solution developed by the Specialty Printing Systems division of Hewlett Packard, relies on the ability of SPT to reliably and rapidly dispense discrete picoliter volumes to create titrations without requiring time-consuming serial dilution steps. SPT directly titrates a solution by dispensing single drops to achieve low concentrations and hundreds or thousands of drops to reach high concentrations. These direct dilutions achieve an independently generated dose for each well without the typical constraints of serial dilution.

The original solution consisted of a cassette containing eight independent SPT-based dispenseheads topped with user-fillable cups, an instrument that operated the dispenseheads and moved standard 96 or 384-well wellplates below the dispenseheads, and software for designing and executing user-designed experiments[1]. In the time since the solution was introduced to the market in 2011, several key improvements have been released which have greatly expanded the utility of the original benchtop system. These include a refreshed version of the instrument, the addition of a new larger-cup cassette, an expansion of the enabled plate formats, and a modification of the original dispenseheads to enable aqueous-based biomolecules to be dispensed from the same system as DMSO-based compounds.

System Improvements

Refreshed instrument

The original D300 instrument has been refreshed as the D300e seen in Figure 1, with several improvements. First, the wellplate assembly now features no-force loading of plates by utilizing a stage-position based biasing mechanism. This design allows wellplate loading onto the instrument using standard robotic laboratory automation solutions. This, along with an instrument communication DLL, makes the benchtop instrument capable of full integration with laboratory automation workcells by enabling automated plate and cassette loading and unloading, as well as fluid loading.

Figure 1: *HP D300e Digital Dispenser*

Second, the wellplate stage mechanism has been modified to expand the range of wellplate depths enabled by the system. While the original D300 system only enabled plate heights from 7-21 mm, the D300e system now enables plate heights from 6-47 mm, enabling a new class of deep-wellplate-based experiments. Third, the footprint of the instrument has been reduced to enable easier integration with laboratory laminar flow hoods. Finally, the

instrument software has been improved to enable easier creation of and execution of complicated experiments. A screenshot of a four-plate protocol including plates with standard titrations, co-titrations, randomized layouts and multi-level co-titrations using both 384 and 1536 plates is shown in Figure 2.

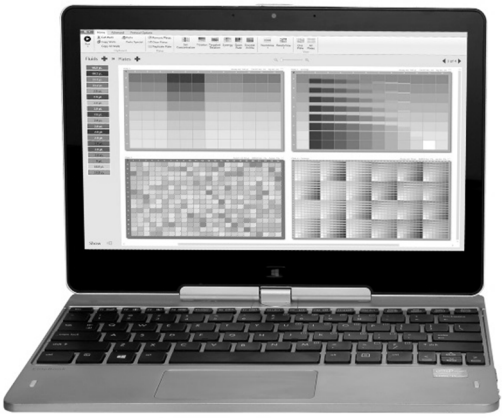

Figure 2: *Powerful software allows for easy creation of complicated plate layouts*

Large reservoir Cassette

The original T8 cassettes was released with a maximum total fill volume of 5 µL, which was later expanded to 10 µL. While this user-fillable cassette, seen in Figure 3, has an adequate volume for most titration-based experiments, including multi-level drug-drug interaction experiments, there were certain classes of experiments and dispensing operations that required the dispensing of larger total volumes, including DMSO normalization.

While the direct dispensing of compounds into assay plates is an extremely efficient way to create dose experiment, it results in a small but variable amount of DMSO in each assay well. Most assays are not sensitive to this DMSO variation, but certain experiments may require the DMSO content to be normalized via a second dispense operation. This normalization may require more than the 10 µL of fluid enabled by the T8.

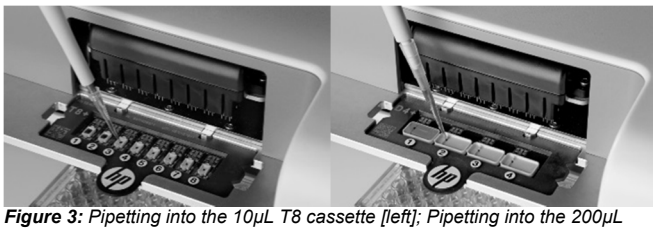

Figure 3: *Pipetting into the 10µL T8 cassette [left]; Pipetting into the 200µL D4 cassette [right]*

The D4 cassette was designed to enable DMSO normalization and other dispensing operations that require more than 10 µL of fluid. This cassette has four dispenseheads with large reservoirs which each are capable of dispensing up to 200 µL. Like on the T8, the user-loaded fluid travels through a hole in the bottom of the cup, then through a slot the center of the of the silicon die, and finally into the microfluidics where it is dispensed out of nozzles and into a wellplate.

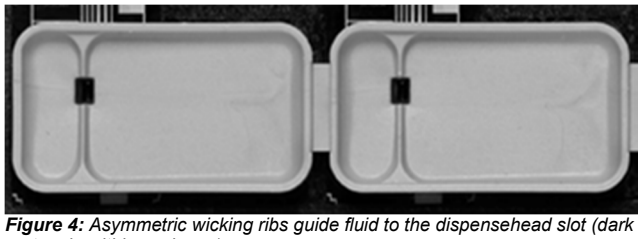

Figure 4: *Asymmetric wicking ribs guide fluid to the dispensehead slot (dark rectangle within each cup)*

To minimize the stranded fluid in these relatively-large fill cups, wicking rib features, seen in Figure 4, were designed into each cup. A variable radius molded into these ribs enable capillary forces to wick the fluid towards the dispensehead connection hole at the bottom of each cup.

Expanded Plate Formats

Though the original instrument only enabled plates with 96 or 384 wells, this has been expanded to now cover standard plates ranging between 12 wells and 1536 wells. The challenges for enabling either larger or smaller format plates are different.

With large-format wellplates, such as those with 12, 24, or 48 wells, one challenge is to ensure good mixing in a vessel that is much larger in diameter than the 900 µm tall swath of the dispensehead, seen in Figure 5. This is achieved by oscillating the wellplate under the dispensehead during and after the dispensing operation. Optimal oscillation amplitude, frequency, duration, and accelerations are dependent on wellplate size, and must account for both mixing efficacy as well as fluid containment (i.e. preventing 'sloshing' of fluid over the sides of the wells).

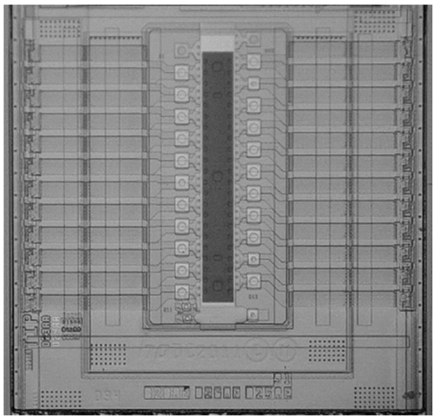

Figure 5: *T8+ Dispensehead with 22 functional nozzles on a 900 µm tall swath. Fluid slot is large dark rectangle in center.*

With small-format wellplates, such as 1536 wellplates, one challenge is to ensure good placement in a vessel that is only slightly larger in diameter than the 900 µm tall swath of the dispensehead. This is achieved by utilizing a subset of five of the available nozzles when dispensing into these plates to effectively shorten the swath. Because assay volumes and dispense volumes are smaller in 1536 plates, and because the dispensehead operates with DMSO using both 20pL and 13pL drops fired at 8 kHz, the dispense times are

quite fast even when using only a portion of the dispensehead. The consistency of dispense volumes, shown in Figure 6, is comparable between 1536 plates and the larger format plates.

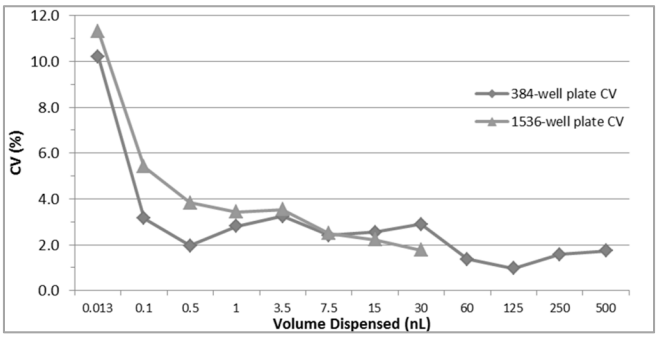

Figure 6: *Coefficient of Variation (CV) for 1536 plates relative to 384 plates.*

Aqueous Based Biomolecules

While the T8 and D4 supplies were originally designed for DMSO-based fluids, there was customer demand for bringing the same capabilities to aqueous-based biomolecules. Enabling this capability on the D300 platform required several changes, including a modification to the cassettes (now known as the T8+ and D4+ respectively) and posed several technical challenges.

First, because the drive bubble size in a Thermal Inkjet (TIJ) dispensehead is dependent on fluid properties, DMSO and water perform differently within the same dispensehead. Thus, the dispensehead was redesigned to enable either DMSO or water to be used. The dispensehead now has three different nozzles sizes distributed around the fluid slot, with the large nozzles used for both DMSO and water, the medium used only for DMSO, and the small used only for water, as shown in Figure 5. Using separate small nozzles for water and DMSO enabled optimization of performance and dynamic range, as the minimum dispense volume for DMSO stayed at 13 pL, while the water minimum dispense volume is 11 pL.

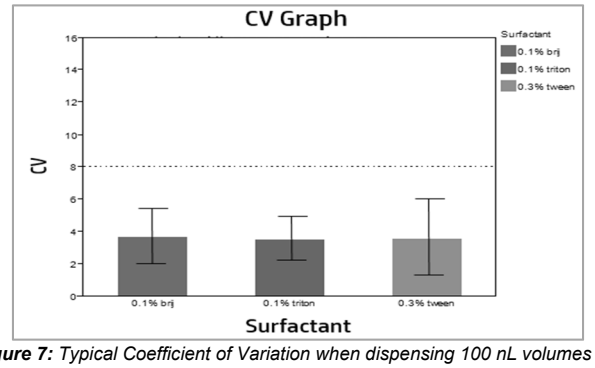

Figure 7: *Typical Coefficient of Variation when dispensing 100 nL volumes*

Second, a series of biocompatible surfactants needed to be characterized for the system to enable consistent priming and jetting. Specifically, Brij 35, Tween 20, and Triton X-100 were characterized with and without up to 20% glycerol to determine the optimum level for good performance, as shown in Figure 7. The amount of surfactant added is small (0.1% for Brij and Triton; 0.3% for Tween), and once even large volumes (100-500nL) are dispensed into typical assay plate volumes (50 µL), the total amount of surfactant in each well of the assay plate is <0.001%.

Finally, significant software changes were required to ensure that proper parameters are used to operate the cassettes depending on the protocol (experimental layout) that the customer has created and the fluids being used. These include accounting for dispensing parameters and other factors such fluid dispense order, destination plate swapping, and the time between filling each cup and dispensing. Furthermore, additional software tools, including layout wizards, were added to easily enable the generation of some particular aqueous-based experiments.

New Customer Application

Enzyme profiling is an example of the type of experiment that utilizes the combined capability of non-contact dispensing of DMSO-based compounds and aqueous-based biomolecules. In a typical experiment, an aqueous-based substrate is co-titrated against a DMSO-based inhibitor, with a constant enzyme concentration dispensed into each combination well in the experiment.

Figure 8: *Enzyme Wizard guides user through the generation of Enzyme Profiling experiments.*

An enzyme-profiling software wizard makes it easy for a user to easily design these experiments. As seen in Figure 8, the software walks the researcher through the process of selecting the compounds and substrates to use as well as the concentration ranges to target,

and the software automatically creates the complicated layout seen in Figure 9. The non-contact dispensing of both the compounds and enzymes into each well with the system enables the rapid and routine execution of enzyme profiling experiments.

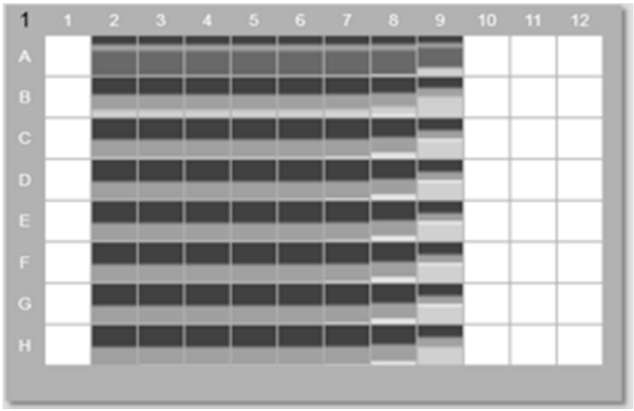

***Figure 9**: Plate layout for enzyme profiling experiment showing 3 different fluids dispensed in varying ratios into each well*

Co-titration experiments, such as enzyme profiling, have been difficult to design and execute with traditional pipetting processes, but are now practical to perform routinely and cost effectively with the T8+ and D4+ supplies. The ability to perform these experiments in 1536 plates with a D300e instrument integrated into a laboratory automation workcell further enhances the ability of researchers to scale up the execution of this class of experiments.

Future capabilities

An instrument that was introduced as a niche product for dispensing compounds in DMSO is gradually transitioning towards becoming a general purpose life science dispensing platform. The accretive capabilities of the system have come directly as a result of working with end users and potential users to understand workflow gaps and opportunities for non-contact picoliter dispensing. While not every investigation yields a new capability or product, a robust process exists for vetting and commercializing new capabilities in the life science space. End users show a great deal of continued interest in assay miniaturization in general, as well as in enhancing the platform to enable such capabilities as sample dispensing, cell dispensing, and qPCR.

Conclusion

The HP D300e Digital Dispenser brings the advantages of a non-contact digital dispensing technology to the creation of dose-response experiments. The small SPT dispenseheads built into the T8+ and D4+ cassettes are the key to making a cost-effective disposable titration solution. The introduction of new capabilities, such as the dispensing of aqueous-based biomolecules, have expanded the utility of the platform into a wider range of life science research. As the capabilities of the D300 platform continue to expand, the range of useful experiments will also grow.

References

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Author Biography

Jeff Nielsen is the Bioprinting Technology Strategist within the Specialty Printing System organization at Hewlett Packard. He has focused on thermal inkjet printhead technology and applications since joining the company in 1993. Jeff earned his Bachelors and Masters Degrees in Mechanical Engineering from the Massachusetts Institute of Technology in 1992 and 1993.