sptlabtech

Antha and dragonfly® discovery

Rapid, automated execution of a high-dimensional space-filling DoE for assay development

Synthace

Key findings

- **Antha's** automated data structuring provided a 94% time saving, accelerating the user's time to insight.
- Antha also provided a 75% time saving in execution planning. (Figure 7)
- **dragonfly® discovery** provided a 98% saving in liquid handling time compared with an average pipetting robot. (Figures 5 & 7)
- dragonfly® discovery further provided a 99.9% saving in tip usage compared with an average pipetting robot. (Figure 8)
- We demonstrate the ability to run thousands of runs of assay optimisation in a single day, opening up experimental possibilities that were previously intractable. (Figures 1, 2, 6 & 7)

Executive Summary

SPT Labtech and Synthace have joined forces to push the boundaries in physical execution of sophisticated Design of Experiment (DoE) campaigns. DoE investigations are extremely powerful experiments that enable the rapid optimisation of many inputs and parameters simultaneously. The ability to quickly and easily execute and analyse higher granularity, multifactorial characterisations of biological processes is particularly valuable to assay development groups, who are expected to work under time pressure with increasing modalities and more complexity without commensurate increases in resources.

Combining the speed and accuracy of SPT Labtech's dragonfly® discovery dispenser with the flexible planning and data aggregation capabilities of Synthace's Antha software, we were able to characterise a spectrophotometric enzymatic assay using a space-filling DoE, totalling 3,456 runs for two sets of triplicates of 384 runs and the corresponding controls. This DoE campaign was executed within one work day by a single user, providing a wealth of data for future assay optimisations.

The use of Antha reduced time spent on planning the 20,745 liquid handling steps and data aggregation from the microplate reader, by 75% and 94% respectively. The dragonfly® discovery reduced liquid handling time by 98% compared with an average pipetting robot, as well as eliminating 99.9% of the number of tips needed.

Antha, in combination with the dragonfly® discovery, provides an efficient and robust solution to reduce assay development cycles with automated DoE execution and data aggregation. This approach can also prevent future bottlenecks in assay transfer by enabling digitisation of assay protocols and use of the same liquid handler from assay development through to high-throughput screening.

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Introduction

Enabling sophisticated DoE for assay development

Pharmaceutical assay development groups work under constant time pressure to produce robust assays that can be transferred into high-throughput screening workflows. Facing ever more modalities and complexity without a commensurate increase in resources, many groups are adopting more powerful statistical approaches, such as Design of Experiments (DoE)[1], to shorten their assay development cycles.

DoE is a methodology often applied in process optimisation with the aim of gaining maximal insights in a time and cost-effective manner. Process conditions (factors) are simultaneously varied in a statistically optimised way to allow analysis of how different factors interact, which cannot be observed in one-factor-at-a-time (OFAT) experiments.[2] Conventional DoE campaigns executed by hand still require weeks of iterative cycles as the number of runs possible per cycle is limited by the need to minimise the complexity of manual calculation, liquid handling and data collection tasks to avoid human error.

In this case study, we combine the speed and accuracy of SPT Labtech's dragonfly® discovery dispenser with the flexible planning and data aggregation capabilities of Synthace's Antha software to unlock the possibility of physically executing a 6-factor space-filling DoE (Figure 1) to comprehensively characterise the design space for an enzymatic assay in a single rapid experiment.

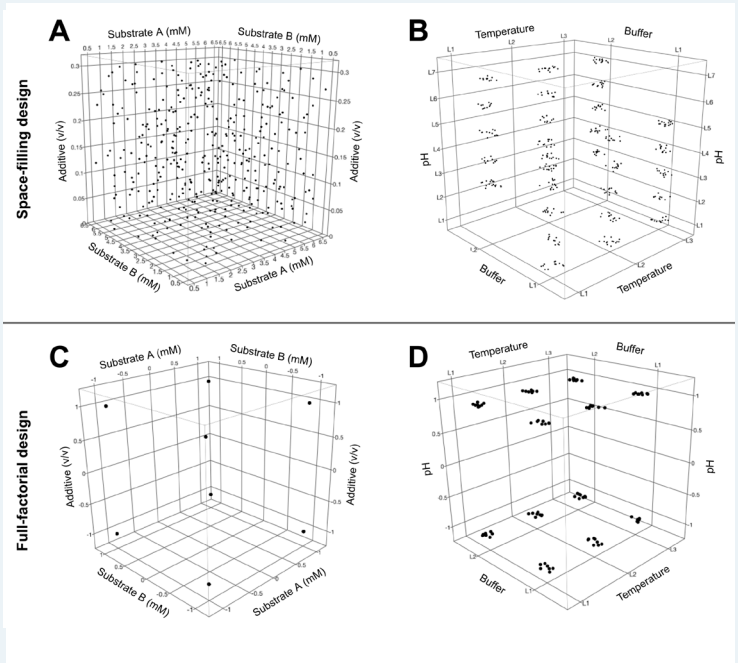

Figure 1. Combination of Antha and dragonfly® discovery enables physical execution of a space-filling DoE for characterisation of a spectrophotometric assay. *A 384-run space-filling design (A–B) was used in this case study to explore the 6 factors of interest for a spectrophotometric assay: concentrations of substrates A and B and an additive (A), as well as buffer type, buffer pH and temperature (B). To compare, a full factorial 2-level screening DoE would require 96 runs (C–D) but cover only a fraction of the design space. Traditionally, space-filling designs are only simulated in silico. Combining the Antha software with the dragonfly® discovery dispenser allows these designs to be executed physically.*

1. Fukuda, I. S., Fidelis Pinto, C. F., dos Santos Moreira, C., et al. (2018) Design of Experiments (DoE) applied to Pharmaceutical and Analytical Quality by Design (QbD) Brazilian Journal of Pharmaceutical Sciences. 54 (Special): e01006. In particular: Table VIII

2. Sadowski, M. S., Grant, C. and Fell ,T. S. (2016) Harnessing QbD, programming languages, and automation for reproducible biology. Trends in Biotechnology. 34: 3: 214-217

Introduction

Antha for automated DoE planning and data

To optimise the user experience with running their own automated DoE, we designed a streamlined process that could be generalised to all DoE campaigns, irrespective of complexity (Figure 2). Using Antha, we could rapidly and flexibly generate an automated liquid handling protocol from the input DoE design file to generate instructions to execute that campaign on the dragonfly® discovery (Figure 3) and reduce the need for physical dry-runs to validate the protocol.

The 6-factor space-filling DoE in this case study required 3,456 runs in total for two sets of triplicates of 384 runs and the corresponding blanks. This amounts to 20,745 liquid handling steps, 2,305 for each of nine 384-well plates. Antha makes this painless by taking care of all planning for the execution of the DoE, guiding the user on required reagents, volumes and labware with detailed schematics of how to set up the liquid dispenser and reagent reservoirs (Figure 4A). As the dragonfly® discovery only has 10 dispensing heads, Antha also calculates the optimal timing for the reservoirs to be swapped out after the first 10 liquids have been dispensed, when more liquids are required. The initial workflow set up and simulation took 30 minutes, providing a 75% time saving when compared to manually creating an equivalent instruction file in spreadsheet software (Figure 8).

The subsequent data aggregation is also made pain-free as Antha already knows the full set of factor conditions for each well from the protocol simulation (Figure 4B), and can therefore auto-structure raw time-course data from the microplate reader and apply a suite of pre-processing steps such as replicate grouping and blank correction to eliminate all manual data handling steps (Figure 2*). Within 30 minutes of exporting data from the plate ready, a single data table that can be easily mined was compiled by Antha, providing a 94% time saving when compared with manual data structuring (Figure 8).

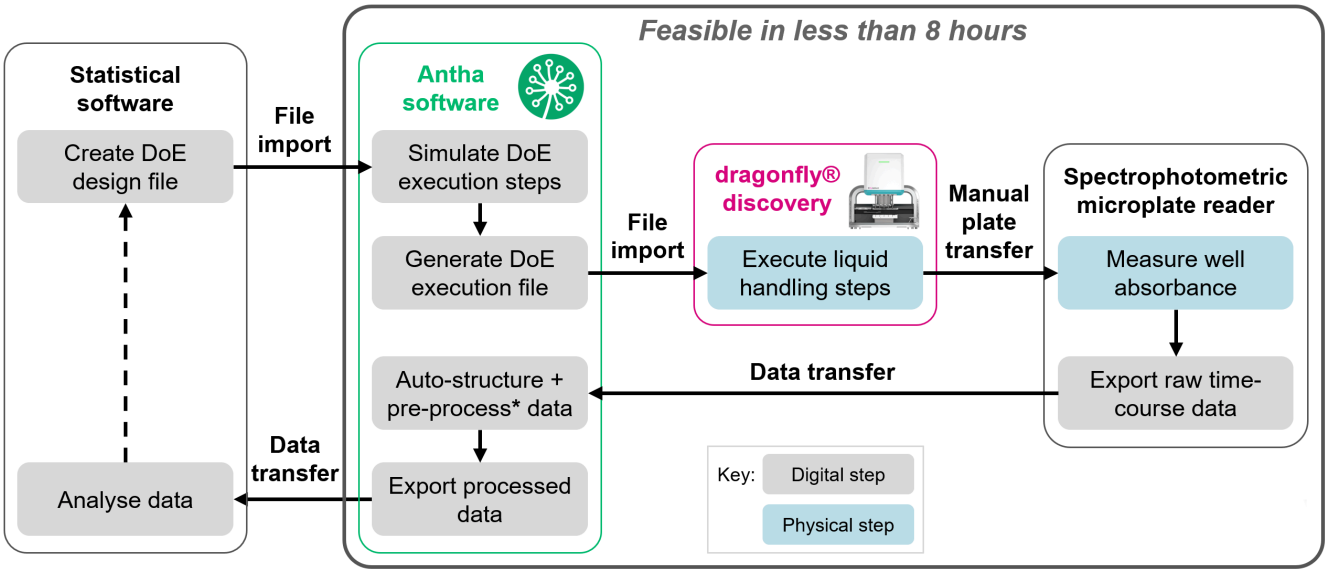

Figure 2. Generalised one-day process for automated DoE. *In order to reduce user time spent on low level calculations and data handling tasks, the Antha software transforms inputs from the statistical software (JMP) and microplate reader into liquid handling instruction files for the dragonfly® discovery and structured data sets for immediate analysis. *Antha pools the raw time-course data from the microplate reader and performs a suite of pre-processing steps to clean the data set, including replicate grouping, absorbance isolation by wavelength, blank correction, path length correction and basic statistics such as averages, standard deviation and %CV.*

Introduction

Antha for automated DoE planning and data

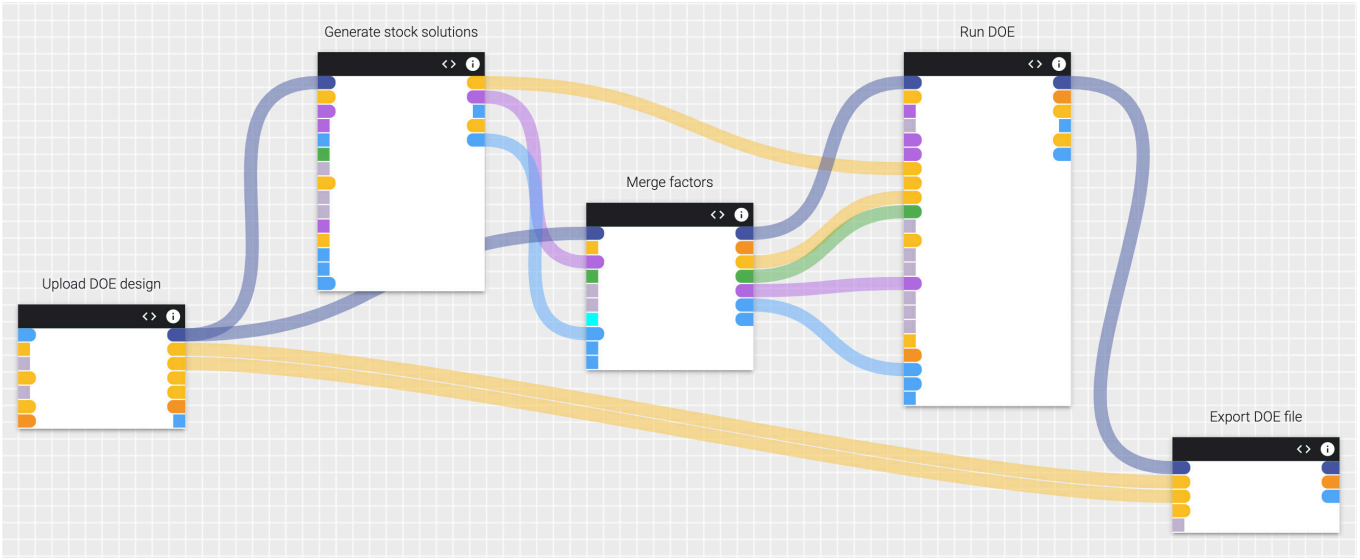

Figure 3. Simple, rapid and flexible DoE execution planning in Antha's Workflow Editor. *The visual interface of Antha's Workflow Editor allows users to rapidly prototype automated liquid handling workflows by providing modules that can be easily rearranged and customised. The workflow used for driving the execution of the space-filling DoE presented in this case study is shown here.*

Figure 4. Visual preview of 2,305 liquid handling steps onto a 384-well plate as simulated by Antha. *Antha's detailed preview page shows the in silico simulation of every single liquid handling step into a 384-well plate for the space-filling DoE in this case study (A). This allows the user to validate the experimental workflow prior to physical execution (see QR code for video). All low-level decisions are taken care of by Antha so that the user isn't required to manually determine plate maps and reservoir set-up before conducting a physical run in the lab, thereby reducing risk of human error or the need for repetitive dry-run physical testing in the lab before executing with valuable reagents. The two 10-well "plates" on the left side represent two sets of reagent reservoirs for the dragonfly® discovery. Antha prompts the user to change the reservoirs when necessary, allowing more than 10 liquids to be layered onto the experiment plate. In the last step of the simulation, the user can select any well to inspect all the details of the liquids added during the protocol (B).*

Scan QR code to watch preview

Synthace

Introduction

dragonfly® discovery for automated DoE execution

Two key strengths of SPT Labtech's dragonfly® discovery dispenser make it an ideal liquid handler for executing sophisticated DoE in assay development, such as the space-filling design selected for this case study.

Accurate, fast dispensing of widely varying volumes

SPT Labtech estimates that the dragonfly® discovery achieves ~5% CV for most liquid classes in 96, 384 and 1,536-well microplates, and an independent research group reported 2–3% CV for 2.5 and 5 µL aliquots. To validate these claims we carried out volumetric testing using the Artel® Multichannel Verification System and determined 0.2% CV (200 µL) and 2.9% CV (1 µL) across a 96-well plate. Satisfied with the device's accuracy, we also timed the dispensing of varying volumes of a single liquid into a 384-well plate, as would be executed in a DoE protocol: the dragonfly® discovery is up to 69% faster than an average pipetting robot (Figure 5). The time saving increases to over 95% when more liquids are involved as the dragonfly® discovery can dispense multiple liquids simultaneously while pipetting robots require tip changes to avoid contamination (Figure 8).

Suitability for high-throughput screening

The non-contact, positive displacement dispensing technology of the dragonfly® discovery was designed with assay robustness in mind for high-throughput screening (HTS) applications. The dragonfly® discovery allows teams to develop their assays using the same dispenser and high-density plates (384 or 1,536-well) as would be used in subsequent high-throughput workflows. When combined with the automated generation of a detailed, digital record of every liquid handling and data aggregation step in Antha, this can significantly reduce risk of assays failing when transferring protocols into HTS.

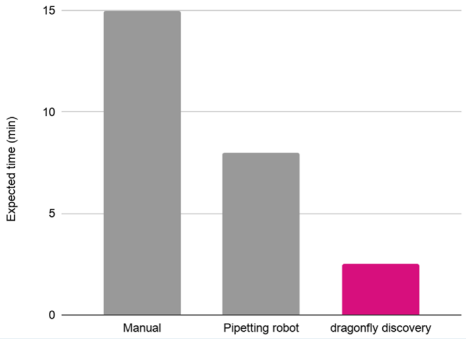

Figure 5. dragonfly® discovery provides up to a 69% time saving compared to a pipetting robot when filling a 384-well plate with a single liquid. *This graph shows the expected time taken to dispense >10µL of a single liquid with varying target volumes between each well into a 384-well plate without tip changes, when comparing the dragonfly® discovery against an average pipetting robot and manual operation by a trained biologist. Expected times are based on in-house comparisons and simulations performed by Antha for water.*

3. Scott, L., Craggs, P., Pemberton, M., et al. (2017) The application of Design of Experiment and the dragonfly® discovery to miniaturise, automate and accelerate assay optimization. Retrieved from https://www.sptlabtech.com/resources/posters/application-design-experiment-and-dragonfly-discov/
4. Gokhin, S., Chen, K., Cerruti, S. et al. (2017) Evaluation of a prototype dragonfly® dispenser for assay development. Retrieved from https://www.sptlabtech.com/resources/posters/evaluation-prototype-dragonfly-dispenser-assay-dev/
5. SPT Labtech. (2017) dragonfly® discovery: unifying drug discovery. Retrieved from https://www.sptlabtech.com/media/uploads/files/dragonfly_discovery_TTPLabtech_brochure_March17_web.pdf

Results

Full characterisation of a spectrophotometric assay

Having created and exported the design file for the space-filling DoE (Figure 1) from a standard statistical software (JMP), the campaign was carried out via Antha on the dragonfly® discovery, according to the one-day process described in Figure 2.

Physical execution of the DoE enabled the observation of a more comprehensive set of data points across the six factors of interest: the concentrations of substrates A and B and an additive, as well as buffer type, buffer pH and temperature. Figure 6 shows a subset of the resulting data set: the change in the assay response when the concentrations of substrate A and the additive, the buffer type and pH are varied.

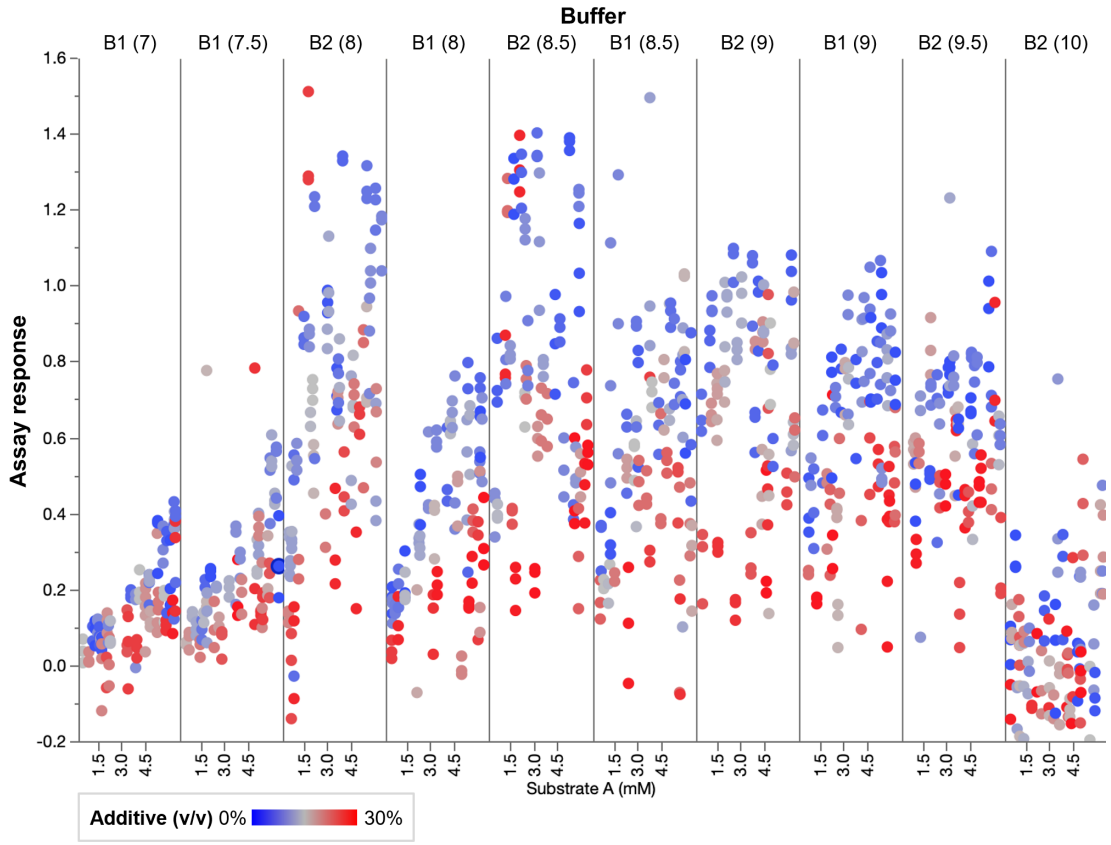

Figure 6. Full characterisation of a spectrophotometric enzymatic assay using a space-filling DoE achieved in a single day. *Physical execution of the space-filling DoE provided a more granular understanding of how the enzyme functions in this spectrophotometric assay under a wider range of conditions than would otherwise be possible when using a full-factorial design. The data shown here represents absorbance end-point measurements. The space-filling design execution allowed for more comprehensive data to be taken across varying ranges of substrate A concentration (mM), additive concentration (v/v), buffer type (B1 and B2) and pH (7–10). The additional granularity provides insights into how process limitations can be accommodated in future if necessary, e.g. a constraint to run the assay at pH 7 only.*

Results

Dramatic time and resource savings by combining Antha and dragonfly® discovery for DoE

A sophisticated DoE campaign was successfully carried out using a combination of Antha with the dragonfly® discovery according to the generalised one-day automated DoE process described in Figure 2. Totalling 3,456 runs for two sets of triplicates of 384 runs and the corresponding blanks, this campaign provided a wealth of data for future assay optimisations.

Physical execution of space-filling DoE campaigns of this sise (Figure 1) would not have been possible without this combination of state-of-the-art hardware and software. Antha and the dragonfly® discovery provided up to a 87% time saving for the entire process compared with using a pipetting robot or without using Antha (Figure 7):

- Antha reduced time spent on planning the 20,745 liquid handling steps for the dragonfly® discovery by 75%, compared with creating a custom spreadsheet.
- The dragonfly® discovery reduced liquid handling time by 98%, compared with an average pipetting robot.
- Antha reduced time spent on structuring and pre-processing the raw time-course data from the microplate reader by 94%, compared with manual data handling.
- The dragonfly® discovery furthermore allowed a 99.9% saving in tip usage (Figure 8).

To conclude, the dramatic time and resource savings provided by combining Antha and the dragonfly® discovery allow this one-day automated DoE process to be used as a routine assay optimisation approach in our in-house labs, irrespective of the demands of the DoE of interest. Users can now reallocate their time to focus on experimental design and analysing DoE data in their statistical software of choice in order to uncover vital scientific insights that can shorten assay development cycles. The experiment presented in this case study is a highly complex design with a great many input liquids at a huge number of levels and as such these results would transfer well to other assay DoEs.

This approach can also prevent future bottlenecks in assay transfer. Antha automatically enables digitisation of assay protocols, reducing human error in knowledge transfer. Moreover, the dragonfly® discovery allows use of the same liquid handling technology from assay development through to high-throughput screening (HTS), reducing the need to miniaturise or re-optimise assays when proceeding into HTS campaigns.

Results

Dramatic time and resource savings by combining Antha and dragonfly® discovery for DoE

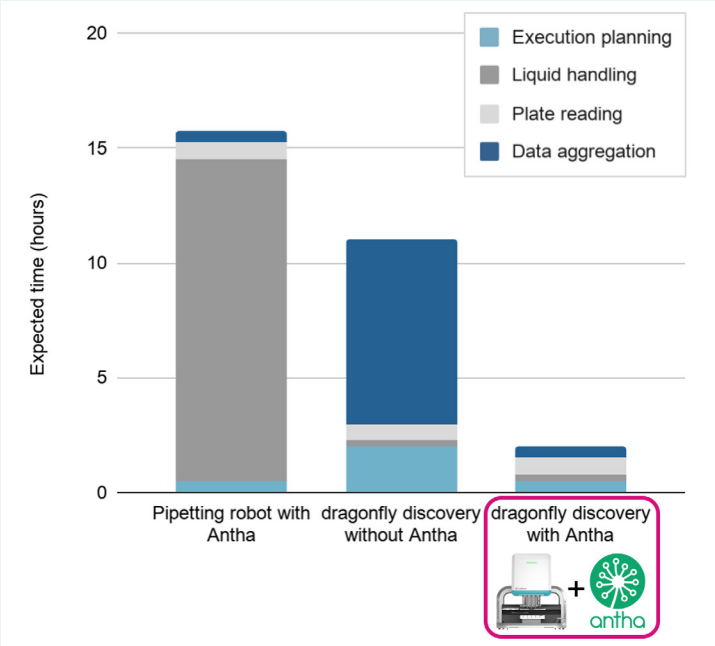

Figure 7. Greater than 90% time savings made when using Antha and dragonfly® discovery for planning, execution and data aggregation for a space-filling DoE. *Comparison of time expected to perform a single replicate of a 384-run space-filling design of a spectrophotometric assay showed that Antha provides a 90% time saving in execution planning and data aggregation, while the dragonfly® discovery provides a 98% time saving in liquid handling time compared with an average pipetting robot. Expected times are based on our in-house experience and liquid handling simulations in Antha. We found that a pipetting robot was not suitable for this application due to significant evaporation effects over 14 hours of liquid handling.*

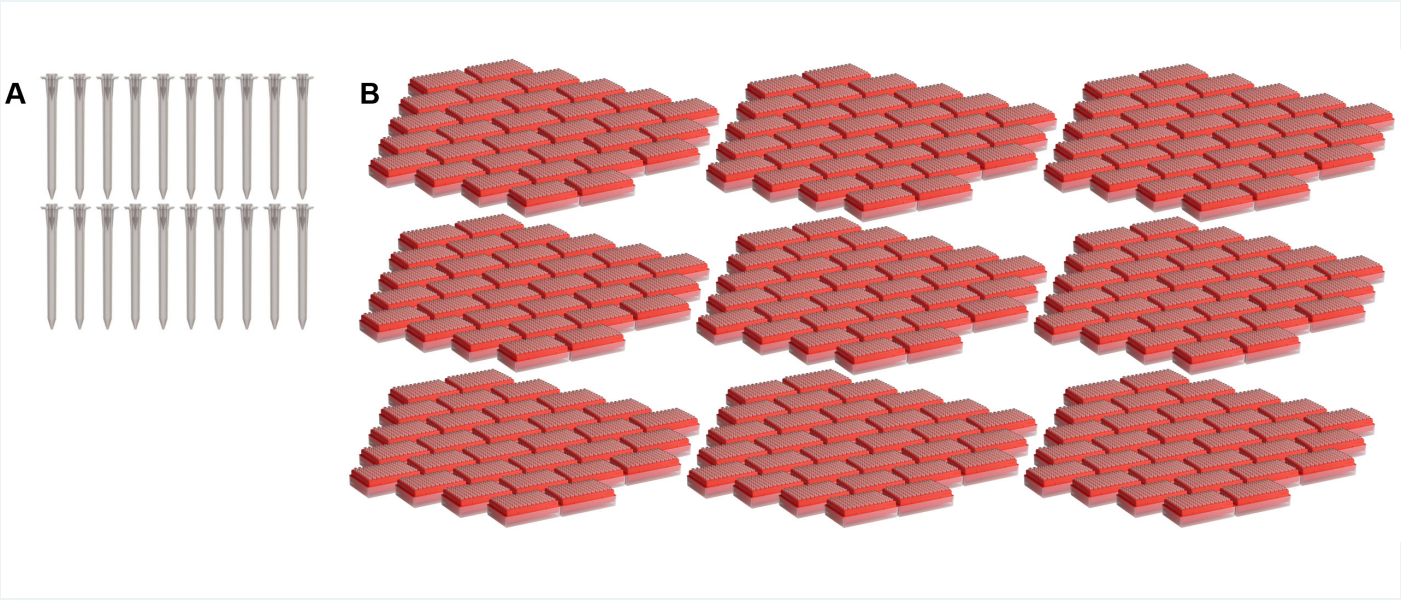

Figure 8. dragonfly® discovery provided a 99.9% saving in tip usage. *To execute the 3,456 runs required for this space-filling DoE, 20 tips for the dragonfly® discovery were used (A), comprising 6 tips for the active component that were not reused between replicates and 14 reused tips for the other liquid components. Simulations using an optimised planner of the same experimental setup against in Antha showed that 25,371 tips from 279 boxes would have been required by a pipetting robot (B).*

dragonfly® discovery for assay development and Design of Experiments (DoE)

Assay development and validation can be a slow and iterative process, often requiring multiple rounds of optimisation before meeting high-throughput screening requirements. Automation of assay development, and Design of Experiments (DoE) in particular, have been hampered by a lack of suitable instrumentation that can (i) easily handle a wide range of liquid factors, (ii) provide the requisite dispense performance and (iii) integrate smoothly with statistical DoE programs. Combining the speed and accuracy of SPT Labtech's dragonfly® discovery dispenser with the flexible planning and data aggregation capabilities of Synthace's Antha software provides an ideal platform for efficient and robust automated DoE execution and data aggregation.

Instrument hardware and dispense mechanism

The dragonfly® discovery instrument comprises up to 10 independently controlled dispensing heads inside the top cover (Figure I). It uses a unique non-contact, positive displacement mechanism to dispense a wide range of fluids from disposable syringes (Figure II) into a plate placed on an *x,y,z* stage. With 10 syringes the instrument has the flexibility to dispense up to 10 liquid factors per run, or more with manual syringe changes. Dispenses from multiple syringes occur across different regions of the plate simultaneously to ensure rapid plate fill times, but can be offset or delayed to control the order and timing of reagent additions, if required.

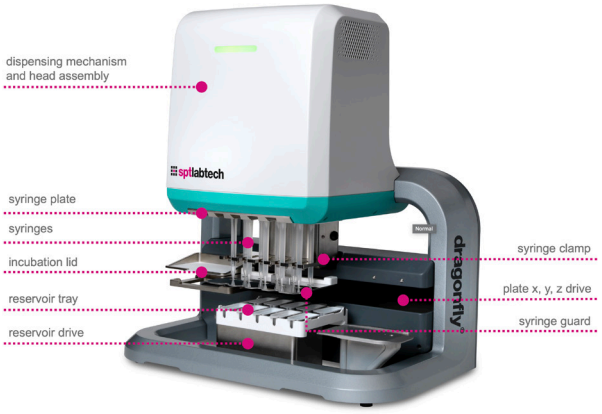

Figure I. *The dragonfly® discovery dispenser.*

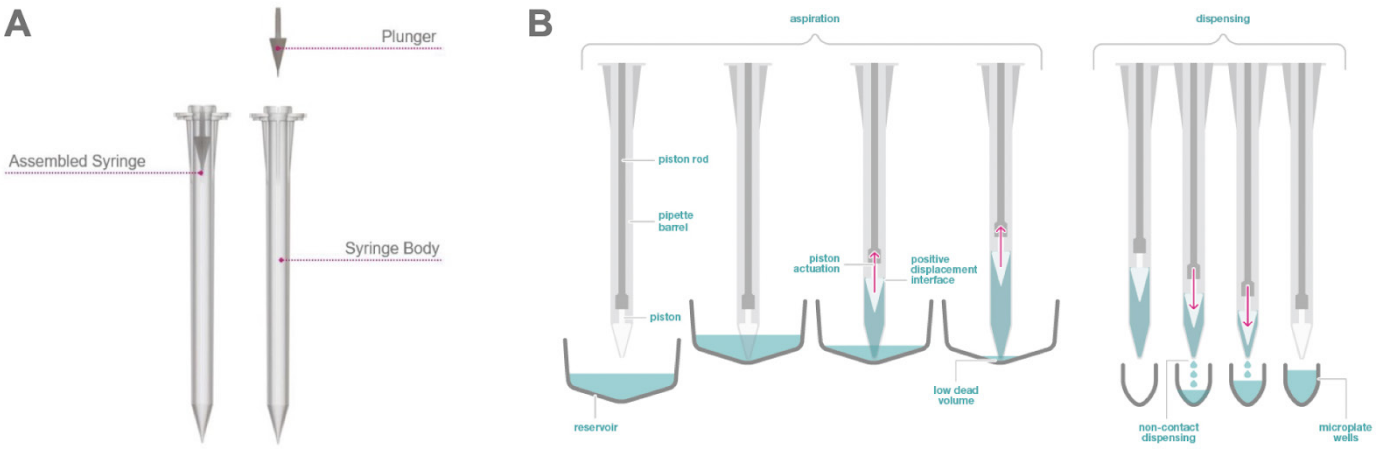

Figure II. *A) Each liquid channel comprises a tightly sealed plunger that travels vertically within a syringe body. B) When coupled to the instrument's piston rod, the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of each piston rod control how and when liquid is aspirated or dispensed. Each positive displacement syringe is independently controlled.*

Each syringe aspirates liquid from a disposable, low dead volume reservoir ensuring no reagent carryover. The dispense range of each syringe is 200 nL – 4 mL, with a dispense resolution of 12.5 nL. Positive displacement dispensing is liquid agnostic, meaning that liquid viscosities ranging from 70% EtOH to 70% glycerol can be dispensed using the instrument's factory pre-sets. The instrument is compatible with a wide range of 96-1536 well SBS microplates, so it is possible to develop assays directly in the plate type and reaction scale that will be used for subsequent high throughput screening.

Once an assay has been optimised and validated, it is possible to utilise dragonfly® discovery as an HTS reagent dispenser. By splitting a single reagent across 6 dispense heads, plate fill speeds as fast as 30 seconds per 384-well microplate or 1.5 minutes per 1536-well microplate can be achieved. In addition, plate stacker and autofeed reservoir modules provide the automation and throughput required for screening campaigns. Each syringe can perform hundreds of aspiration and non-contact dispense cycles to minimise tip use and maximise walk away time during operation. A validation screen comprising 3200 compounds demonstrated excellent reproducibility between independent runs, confirming the robustness of the dragonfly discovery dispense mechanism and its suitability for HTS.

Compatible assay formats

dragonfly® discovery is compatible with a wide range of assay formats, including biochemical assays[6], cell based assays[7] as well as genomics applications[8]. The broad dynamic dispense range is ideally suited to setting up complex reagent gradients for assay development (Figure III) or executing space filling DoE designs. The applicability of dragonfly® discovery to DoE execution has been demonstrated previously[9], however, the partnership with Synthace's Antha platform dramatically reduces the amount of time spent planning the experiment and structuring the data generated. Taken together, this represents a paradigm shift in automating DoE approaches for assay development.

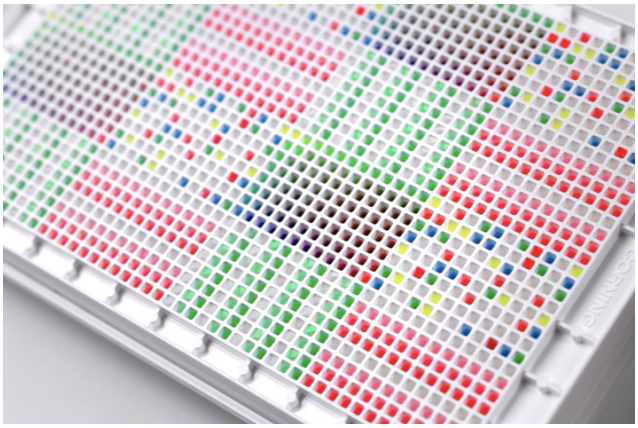

Figure III. *1536-well microplate showing representative reagents gradients and technical replicates dispensed with dragonfly discovery.*

6. Scott, L., Craggs, P., Pemberton, M., et al. (2017) The application of Design of Experiment and the dragonfly® discovery to miniaturise, automate and accelerate assay optimization. Retrieved from https://www.sptlabtech.com/resources/posters/application-design-experiment-and-dragonfly-discov/

7. SPT Labtech. (2018) Cells dispensed by dragonfly® discovery show normal proliferation, health and apoptotic responses in a range of cell types. dragonfly® discovery application note. Retrieved from https://www.sptlabtech.com/media/uploads/files/dfd_cell_health_apoptosis_TTPL0060_app_note.pdf

8. Darmanis, S. (2019) tabula muris, a single cell atlas of the adult mouse. Webinar. Available from https://www.sptlabtech.com/resources/webinars/webinar-tabula-muris-a-single-cell-atlas/

9. Dawes, T., Schenker, B., Jenkins, J. and Beresini, M. H. (2018, February) dragonfly discovery: Assay development meets uHTS. Poster session presented at the Society for Laboratory Automation and Screening annual conference, San Diego, CA.

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