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

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1. Automating complex DoE for faster assay development

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

In DoE, 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]. Manually executing conventional DoE campaigns still requires weeks of iterative cycles, as the number of runs possible per cycle is limited by the need to reduce calculation, liquid handling and data manipulation steps in order to minimise human error.

Here, the speed and accuracy of TTP Labtech's **dragonfly® discovery** dispenser is combined 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 an enzymatic assay in one rapid experiment.

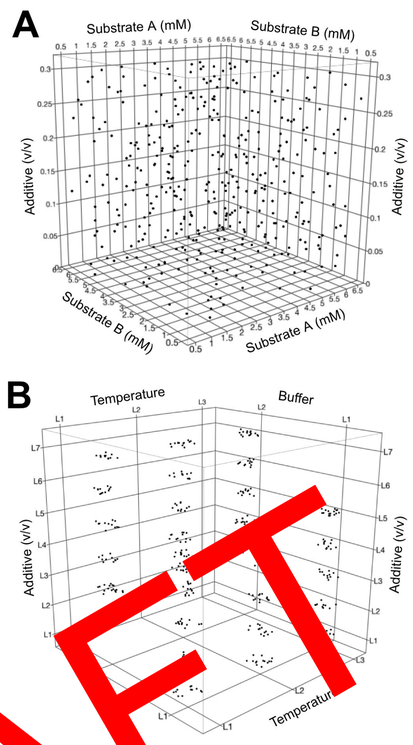

Figure 1. Space-filling design used to characterise an enzymatic assay. The 6 factors investigated were concentrations of substrates A and B and an additive (**A**), and buffer type, buffer pH and temperature (**B**).

2. Technologies designed to streamline assay transfer into HTS

dragonfly® discovery uses a unique non-contact, positive displacement mechanism to dispense a wide range of fluids from disposable syringes (Figure 2), and is compatible with a wide range of assay formats, including biochemical[3], cell-based[4] as well as genomics applications[5]. In an independent volumetric test using the Artel® MVS System, the dispense accuracy was found to be 0.2% CV (200 µL) and 1.9% CV (1 µL) across a 384-well plate. Its speed, versatility and accuracy allows teams to develop assays using the same dispenser and high-density plates (384 or 1,536-well) as would be used in subsequent high-throughput workflows.

Paired with Antha, the automated generation of a detailed, digital record of every liquid handling and data aggregation step (Figures 3-5) can significantly reduce risk of assays failing when transferring protocols into HTS. Taken together, this hardware/software combination represents a paradigm shift in automating DoE for assay development.

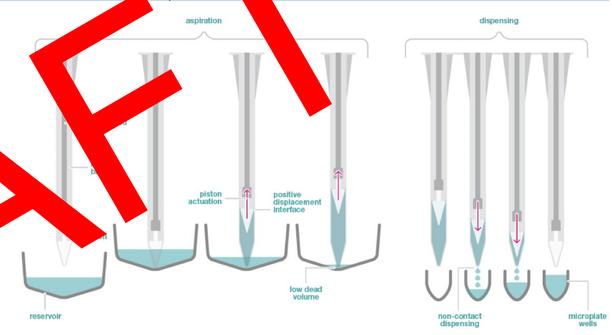

Figure 2. Each liquid channel comprises a tightly sealed plunger that travels vertically within a syringe body. When coupled to the instrument's piston rod, the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of each piston rod control how and when liquid is aspirated or dispensed. Each positive displacement syringe is independently controlled.

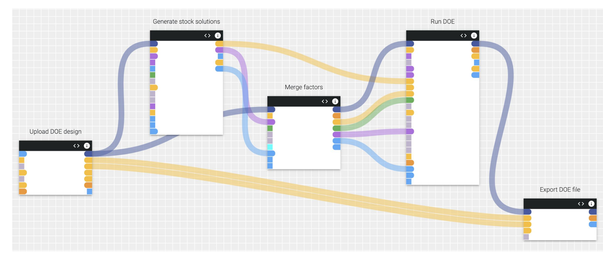

Figure 3. Simple, rapid and flexible DoE planning in Antha's Workflow Editor. The visual interface allows rapid prototyping of automation workflows using experiment modules that can be easily reordered and customised.

3. Combining Antha software and dragonfly® discovery dispenser to execute 3,456-run DoE in single work day

We designed a streamlined process that could be generalised to automate any DoE campaign, irrespective of complexity (Figure 4). Using Antha's visual interface, we could rapidly and flexibly generate an automated liquid handling protocol from the input DoE design file and instructions to execute that campaign on the dragonfly® discovery (Figure 3), reducing the need for physical dry-runs to validate the protocol.

The 6-factor space-filling DoE presented here required 3,456 runs 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 operator on required reagents, volumes and labware with detailed schematics of how to set up the instruments (Figure 5A).

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 5B), and can therefore auto-structure raw 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 4*).

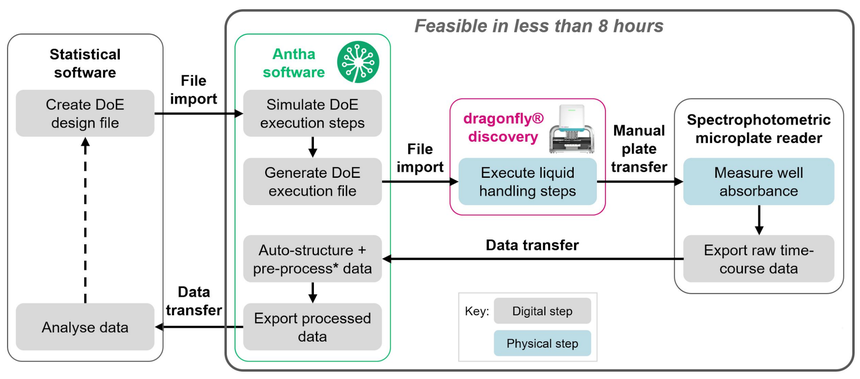

Figure 4 (above). Generalised one-day process for automated DoE. Antha transforms inputs from statistical software (JMP) into liquid handling instruction files for the dragonfly® discovery. *Antha then pools raw time-course data from the microplate reader and performs a suite of pre-processing steps to clean the data set for immediate analysis, including replicate grouping, absorbance isolation by wavelength, blank correction, path length correction and basic statistics such as averages, standard deviation and CV.

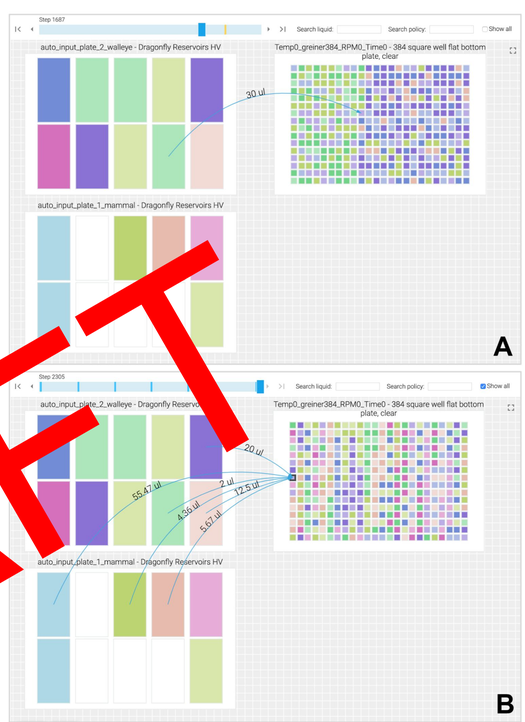

Figure 4 (right). Visual preview of 2,305 liquid handling steps onto a 384-well plate as simulated by Antha. Antha's detailed preview page shows the simulation of every single liquid handling step into a 384-well plate for the space-filling DoE in this case study (**A**). This allows the operator to validate the experimental workflow prior to physical execution. The two 10-well "plates" on the left side represent the set 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 DoE 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**).

4. Full characterisation of an enzymatic assay with DoE

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.

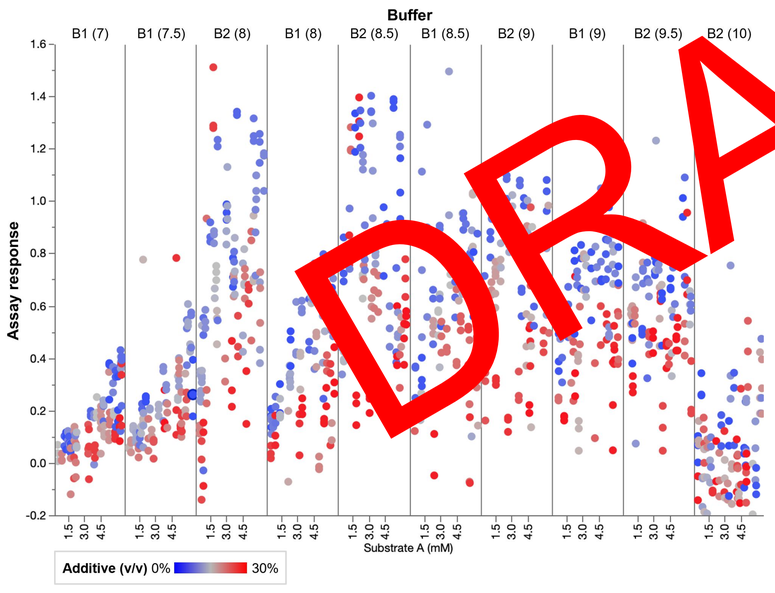

Figure 6. Full characterisation of a spectrophotometric enzymatic assay using a space-filling DoE achieved in a single day. Physical execution of the space-filling DoE provided a more granular understanding of how the enzyme functions in this spectrophotometric assay under a wider range of conditions 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.

References

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
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.ttplabtech.com/resources/posters/application-design-experiment-and-dragonfly-discov/
4. TTP 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.ttplabtech.com/media/uploads/files/dfd_cell_health_apoptosis_TTPL0060_app_note.pdf
5. Darmanis, S. (2019) tabula muris, a single cell atlas of the adult mouse. Webinar. Available from https://www.ttplabtech.com/resources/webinars/webinar-tabula-muris-a-single-cell-atlas/

5. Dramatic savings using Antha with dragonfly® discovery

Antha and the **dragonfly® discovery** provided up to a **87% time saving** for the entire process compared with using an average pipetting robot, or without Antha (Figure 7):

- **75% time saved** with Antha in liquid handling planning compared with creating a custom spreadsheet;
- **98% time saved** with dragonfly® discovery in liquid handling time by compared with a pipetting robot;
- **94% time saved** with Antha in data structuring and pre-processing compared with manual handling;
- dragonfly® discovery furthermore provided a **99.9% saving in tip usage** (Figure 8).

To conclude, the dramatic time and resource savings provided by Antha and dragonfly® discovery allow this one-day automated DoE process to be used routinely in assay optimisation, **irrespective of the complexity of the DoE**, allowing scientists to reallocate their time to focus on uncovering scientific insights.

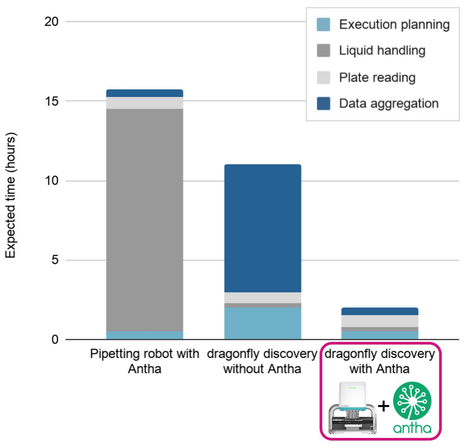

Figure 7. Up to 87% time saving made overall when using Antha and dragonfly® discovery together for planning, execution and data aggregation for a space-filling DoE. Expected times are based on our in-house experience and simulations in Antha.

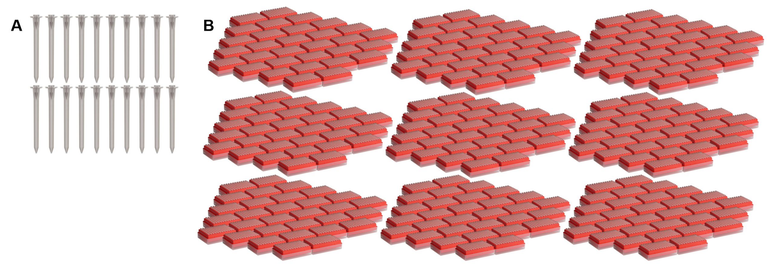

Figure 8. dragonfly® discovery provided a 99.9% saving in tip usage. To execute this space-filling DoE, 20 tips for the dragonfly® discovery were used (**A**), comprising 6 tips for the active component that were not reused between replicates and 14 reused tips for the other liquid components. Simulations 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**).