

Use of a microlitre digital liquid handler for screening applications



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introduction

Assay optimisation entails the study of multiple parameters to maximise screening performance. As a result, the determination of the optimal conditions can be a costly, time consuming process requiring the set-up of multiple studies to analyse each individual parameter. Often, such studies are limited by manual pipetting when performed in microplates.

Digital dispensing offers researchers the most freedom for experimental design and sample placement with each microplate. Essentially, it allows the creation of any plate map through dispensation of any volume of liquid into any well. In doing so, a digital approach removes the burdens and technical constraints associated with manually handling such complexities, making it relatively simple to plan and execute the most desirable experimental design and not one predicated by manual or automated liquid handling.

The novel dispensing head technology in the dragonfly® digital liquid handler (TTP Labtech, UK) enables non-contact, positive displacement pipetting from disposable syringes directly into assay plates. The system can distribute fluids of a wide range of viscosities, ethanol to glycerol; with a minimum dispense volume of 0.5 µL with zero cross-contamination. Independent volume control and simultaneous digital dispensing from up to 10 pipetting heads provide total freedom of plate layout. Suitable applications range from formatting multi-dimensional reagent optimisation experiments to simple backfilling of each well with assay buffer.

Here, we demonstrate that a dragonfly liquid handler is a valuable compact low-cost addition to the discovery workbench, eliminating the tedium of complicated microplate set-up while maintaining flexibility.

1. dragonfly: digital liquid handler

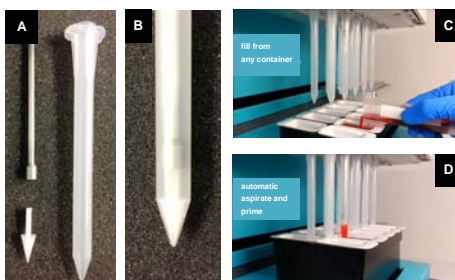


dragonfly provides automated non-contact, positive displacement dispensing enabling the rapid generation of microplates containing multiple reagents. With a minimum dispense volume of 0.5 µL and resolution of 0.1 µL, dragonfly offers digital microlitre dispensing for automation of a wide range of high throughput chemical and biological screening assays.

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2. pipetting technology



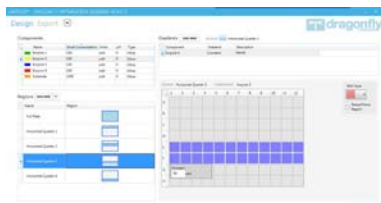
dragonfly is equipped with an array of either 5 or 10 pipetting heads. These utilise positive displacement pipettes providing reliable and accurate dispensing of a broad range of liquids without any classification (A & B). The use of disposable syringe and plunger guarantees no cross-contamination. Each pipette works independently to dispense any volume into any well for complete assay flexibility. Each liquid is aspirated from a dedicated easy-to-fill reservoir (C & D). Unused liquid at the end of a run can thus be recovered resulting in a dead volume of < 0.5 µL. The whole process is achieved without valves, air gaps or system fluid resulting in high reliability.

3. experimental design

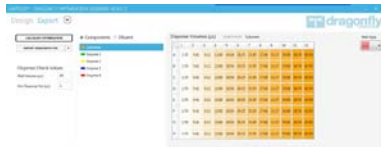
Assay optimisation requires the simultaneous study of multiple parameters to maximise assay performance. As a result the determination of the optimal conditions can be a costly and time consuming process requiring the set-up of multiple studies to analyse each individual parameter. Often, such studies are limited by manual pipetting. Such limitations are easily addressed with the dragonfly Designer software translating experimental design into dispensing worklists at the touch of a few buttons. The workflow for an enzyme optimisation is detailed below.



Step 1. Creating a 12-point substrate curve across the microplate is achieved by simply selecting the **Full Plate** region option and a **Horizontal** gradient. Entering the Start and End concentrations allows the Designer to calculate dispense volumes.



Step 2. A matrix of four enzyme samples (e.g. type, concentrations) can be formatted by selecting the appropriate **Horizontal Quarter** in the region option (in this example Enzyme 3 has been selected).



Step 3. In the Export view, the actual volumes that will be digitally dispensed into each well are displayed for all components in the assay. Colour coding each component and concentrations aid validation. Finally, the worklist is exported in CSV format for use by dragonfly control software.

4. example assay

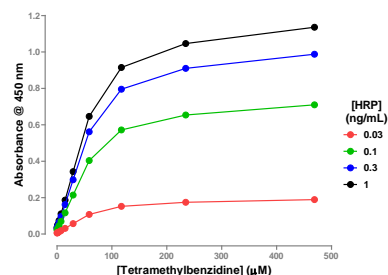
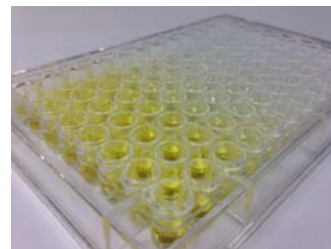
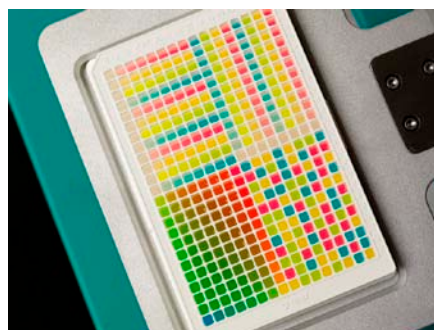


Figure. An assay comprising horseradish peroxidase (HRP) catalysis of tetramethylbenzidine (TMB) substrate was studied. A matrix of HRP and TMB concentrations was constructed using dragonfly and the assay started by addition of hydrogen peroxide using mosquito. The assay was stopped after a 45 minute incubation at room temperature by adding 1 M sulphuric acid to each well using dragonfly.

5. flexible digital dispensing



Assays performed in 96 and 384 microplates often utilise tens of microlitres of reagents that can be either beyond the volumetric range of a liquid handler or incredibly time-consuming operations. The dragonfly software optimises performance by controlling simultaneous dispensing from all heads while maintaining independent head control. This enables rapid set-up even the most complex tasks from formatting multi-dimensional reagent optimisation to backfilling.

conclusion

TTP Labtech's low cost assay optimiser, dragonfly, offers easy automated set-up of a wide range of concentration-dependent matrices and optimisation studies:

- reliable, accurate, non-contact dispensing and auto-aspiration is ensured by positive-displacement pipetting from easy to fill reservoirs
- zero cross-contamination is ensured and slow wash cycles eliminated using disposable pipettes
- dragonfly fills a 96-well plate in less than 5 minutes using up to 10 independent, modular liquid channels
- dispense an extensive viscosity range from alcohols through to glycerol without classification
- large volumetric range (0.5 µL – 4 mL).