dragonfly® discovery: Assay development meets uHTS

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Introduction

Ultra-high-throughput screening (uHTS) demands homogeneous assay formats to leverage the density of 1536/3456 well plates to increase the throughput of large compound (> 1 million) library screens against disease targets. Such assay formats require automated reagent dispensing and rigorous assay development. The key liquid handlers in any uHTS system are non-contact reagent dispensers rather than tip-based multi-channel pipetting devices that are common in many lower throughput assay systems. Critical requirements for any uHTS reagent dispenser are accuracy, precision, and speed. Additionally, it is important to integrate the uHTS dispenser into assay development as early as possible. For most bulk dispensers, this integration typically occurs late in the process. The dragonfly discovery from SPT Labtech is a non-contact positive displacement dispenser designed to bridge dispensing needs from assay development through high-throughput screening. It provides ready access to 10 different reagents that can be dispensed in variable volumes as well as at high speeds. We evaluate the performance of this dispenser in high-throughput mode and examine its use in assay development for reagent concentration optimization in 1536-well plates and buffer component optimization by DOE.

1. dragonfly discovery has capabilities that span the needs of assay development and HTS

dragonfly discovery's innovative dispense technology and reliability not only simplify the process of assay development but also significantly aid the smooth transition into HTS. With its versatility and powerful software, dragonfly discovery provides the ideal platform for hit-to-lead and lead optimisation, thus enabling the same dispense technology to be used throughout the drug discovery process.

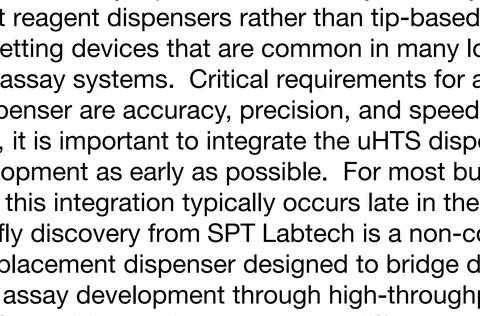


Fig 1. Non-contact dispensing from a disposable, positive displacement tip

In each of the channels (up to 10) there is a tight fitting piston that travels within a pipette barrel, when coupled to the instrument's piston rod, the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of the piston control how and when liquid is ejected from the tip.

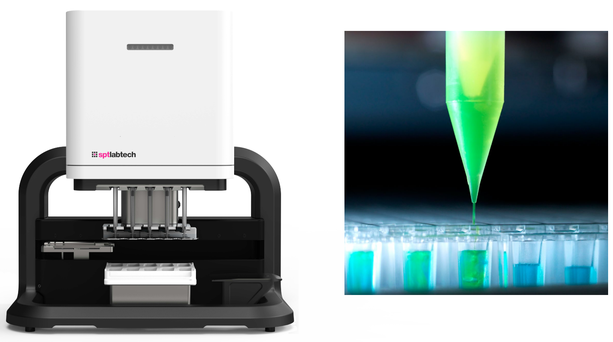


Fig 2. dragonfly discovery dispenser and tip

2. Robust dispenser performance serves as the foundation for successful HTS

Methods: To test the precision and accuracy of the dragonfly discovery (DFD), 80 µL of FluoSpheres® (Molecular Probes, cat.# F8805) were added to 80 mL of 1mM Tris Buffer (pH 7.5), mixed and placed in reagent trays of the DFD. The suspension was aspirated using 6 different tips of the DFD and dispensed into three 1536 well plates (Mako®, Brooks). The plates were centrifuged and read on a fluorescence plate reader (PheraStar® BMG Labtech, ex 360nm/em 450nm). The 1536 well plates were weighed before and after the dispense for gravimetric analysis.

Results:

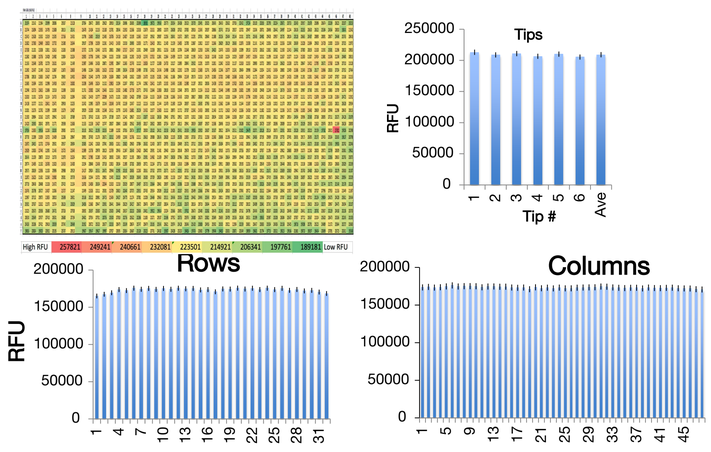


Fig 3. Dispense precision & accuracy: RFU by whole plate (top, left); DFD tip (top, right), row (bottom, left) and row (bottom, right)

- **Dispense time** for 4uL, 1536 plate fill (from 6 tips): 85 s
- **Precision** for 4 uL dispense of FluoSpheres® 2.51% CV
- **Accuracy** within 1% of requested volume.
- **Negligible tip-to-tip variation**

3. Assay development is often the bottleneck in HTS campaigns

Development of assays for optimal HTS performance requires: identification of key components/factors, optimization of component concentrations, and evaluation of assay logistics such as order of addition and incubation times. Manual assay development is often inefficient and frequently considers only a few factors at a time. To investigate the utility of the dragonfly discovery for assay development, we conducted preliminary studies that included optimization of buffer conditions using design of experiments (DOE) and simultaneous optimization of the concentrations of two assay components.

4. Buffer component optimization using design of experiments (DoE)

Assay: protease cleavage of peptide-AMC substrate with kinetic fluorescence read

White 384-well ProxiPlate
(PerkinElmer) Add 4 uL **Protease**
Add 4 uL **Peptide-AMC Substrate**
Read fluorescence kinetically for 30 min on SpectraMax M5 (Molecular Devices)(ex 340nm; em 615/665 nm)

Fig 4. Protease activity assay

Experiment 1: 5-Factor main effects screen

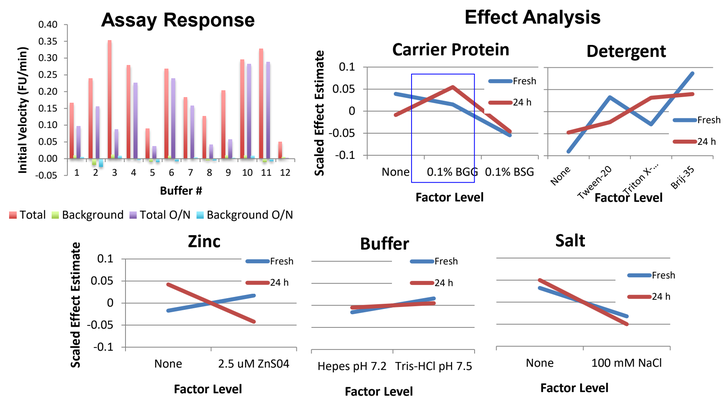
Purpose: to identify key buffer factors that maximize signal window and reagent stability

12 buffers (200 uL) were made from 10 stock solutions using the DFD. Enzyme and substrate were diluted into each buffer, combined in the assay plate and analyzed immediately and again after 24 h at 4°C (n=4 / condition).

Design:

Factor	Carrier Protein	Detergent	Buffer	Salt	Zinc
# of Test Levels	3	4	2	2	2
1	0.1% BGG	None	Tris-HCl pH 7.5	100 mM NaCl	2.5 uM ZnSO4
2	None	Tween-20	Hepes pH 7.2	100 mM NaCl	None
3	None	Brij-35	Tris-HCl pH 7.5	100 mM NaCl	2.5 uM ZnSO4
4	0.1% BGG	Tween-20	Tris-HCl pH 7.5	None	None
5	0.1% BSG	None	Hepes pH 7.2	100 mM NaCl	None
6	None	Triton X-100	Tris-HCl pH 7.5	None	None
7	0.1% BGG	Brij-35	Hepes pH 7.2	100 mM NaCl	None
8	None	None	Hepes pH 7.2	None	2.5 uM ZnSO4
9	0.1% BSG	Tween-20	Hepes pH 7.2	None	2.5 uM ZnSO4
10	0.1% BSG	Brij-35	Tris-HCl pH 7.5	None	None
11	0.1% BGG	Triton X-100	Hepes pH 7.2	None	2.5 uM ZnSO4
12	0.1% BSG	Triton X-100	Tris-HCl pH 7.5	100 mM NaCl	2.5 uM ZnSO4

Results:



Carrier protein: BGG chosen for 24h reagent stability
Detergent: narrowed choice to Tween-20 or Brij-35
Buffer: no clear favorite
Salt: NaCl had a negative effect on assay performance
Zinc: enhances signal with fresh reagents, but leads to reagent instability

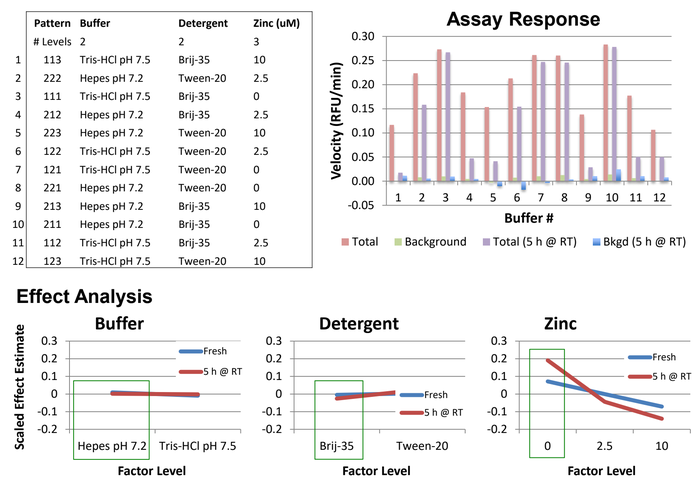
Experiment 2: 3-Factor Full Factorial

Purpose: to fine tune the three remaining factors in the presence of 0.1% BGG 12 buffers (200 uL) were made from 5 stock solutions using the DFD. Enzyme and substrate were diluted into each buffer and combined in assay plate. The assay was run immediately and after 5 h of reagent storage at room temperature (n=4 / condition).

Design:

Pattern	Buffer	Detergent	Zinc (uM)
# Levels	2	2	3
1	113 Tris-HCl pH 7.5	Brij-35	10
2	222 Hepes pH 7.2	Tween-20	2.5
3	111 Tris-HCl pH 7.5	Brij-35	0
4	212 Hepes pH 7.2	Brij-35	2.5
5	223 Hepes pH 7.2	Tween-20	10
6	122 Tris-HCl pH 7.5	Tween-20	2.5
7	121 Tris-HCl pH 7.5	Tween-20	0
8	221 Hepes pH 7.2	Tween-20	0
9	213 Hepes pH 7.2	Brij-35	10
10	211 Hepes pH 7.2	Brij-35	0
11	112 Tris-HCl pH 7.5	Brij-35	2.5
12	123 Tris-HCl pH 7.5	Tween-20	10

Results:

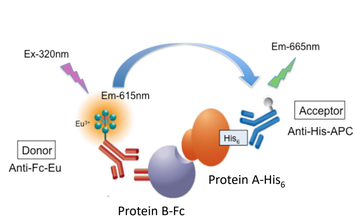


Buffer: Minimal difference; Hepes pH 7.2 was selected
Detergent: Minimal difference; Brij-35 was selected
Zinc: Most significant effect – negative; Zn was eliminated from the buffer

The use of DOE and the dragonfly discovery enabled fast, efficient optimization of assay buffer factors for signal magnitude and reagent stability.

5. Simultaneous optimization of the concentrations of two binding partners

Assay: Protein-protein interaction in TR-FRET format



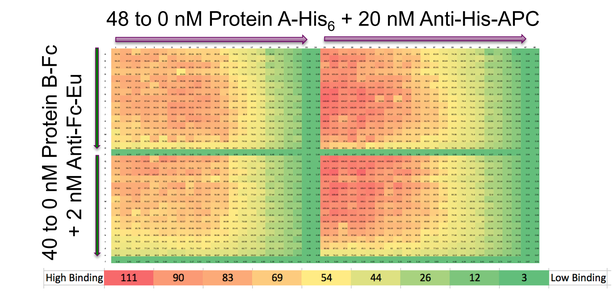
Black Aurora 1536-well plate
Add 2 uL **Protein A-His$_6$** + 20 nM Anti-His-APC
Add 2 uL **Protein B-Fc** or **buffer alone** + 2 nM Anti-Fc-Eu
2 h @ RT
Read TR-FRET on ViewLux (ex 340nm; em 615/665 nm)

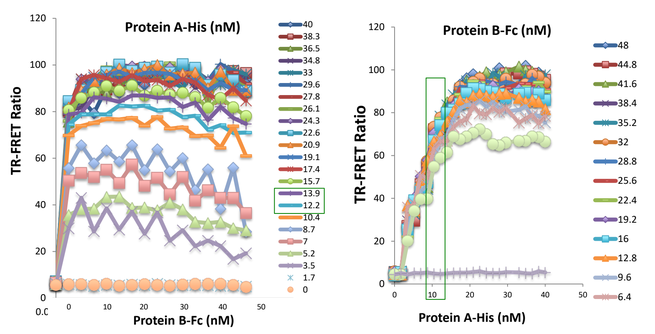
Reagents

A: 80 nM Protein A-His + 40 nM anti-His
B: 40 nM anti-His
C: 96 nM Protein B-Fc + 4 nM anti-Fc
D: 4 nM anti-Fc

Methods
Protein A-His was titrated across columns by diluting reagent A with reagent B.
Protein B-Fc was titrated across rows by diluting reagent C with reagent D.
All titrations were conducted directly in assay wells.
The plate was incubated for 2 h and read on a ViewLux® reader (PerkinElmer).

Results





Optimal protein B-Fc concentration is 2 nM
(below tested concentration range)

Optimal protein A-His concentration is 12 nM

- With the dragonfly discovery, reagent titrations can be carried out directly in the wells of a 1536-well assay plate to determine optimal concentrations.
- The significant real estate on such a plate facilitates optimization of multiple reagent concentrations simultaneously.
- The dragonfly designer software offers a user-friendly interface to create complex and wide range concentration gradients by taking care of calculating intermediate stocks where not all concentrations can be dispensed by a single reagent stock, as well as the calculation of the dispense volumes and the required backfill.

Conclusions

Our preliminary evaluation of the dragonfly discovery demonstrates:

- Positive displacement, non-contact dispensing gives excellent accuracy and precision in 1536-well plates
- Reagent titrations can be carried out directly in the wells of a 1536-well plate, thereby avoiding a potential miniaturization step later on.
- The significant real estate on 1536-well plates enables optimization of many more reagent combinations simultaneously than would be possible manually
- Experimental designs can readily be generated in the dragonfly discovery designer software (e.g. complex concentration gradients, calculation of requisite intermediate stocks concentrations and volumes) or uploaded as a .csv file

Key properties that make this system suitable for both HTS and assay development

- High speed
- Large dispense range (200 nL to > 1000 µL)
- Capacity for up to 10 reagents, with independent channel control
- Low dead volume
- High capacity reservoir

Next Steps

- Conduct additional studies for an in-depth assessment of assay development capabilities across multiple assay formats
- Evaluate system performance in high-capacity mode with auto-filling reservoirs