a comparison of dispense performance of manual pipetting versus automated pipetting for assay development

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introduction

Every new assay requires significant optimisation (e.g. enzyme and substrate concentrations, cell type and number, buffer constituents, pH, incubation timepoint) for robust performance and assured decision making. Hand pipettes are often utilised for this step, as they can handle a wide range of liquid viscosities that would be challenging for automated dispensers. However, with a manual approach the number of variables per assay is limited, due to the sheer difficulty and time it would take to dispense all desired reagent combinations. This leads to long and iterative assay development cycles.

Here we assess a positive displacement dispensing instrument that incorporates the versatility of a hand pipettor, but in an automated manner to reduce assay development times from weeks to just hours or days. The system uses up to ten independent channels to rapidly dispense liquids into high density plates, without any requirement for liquid classification. Each disposable tip has a low minimum dispense volume of 200nL and a low dead volume, which enables comprehensive miniaturised assay optimisation with precious reagents such as proteins or cells.

In this poster, we compare the performance in dispensing different liquids both by hand and by using the dragonfly® discovery automated pipettor. We also highlight the benefits in its utility in running complex design of experiments leading to faster assay development, in addition to obtaining more robust assays.

2. dispense technology

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 instruments 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.

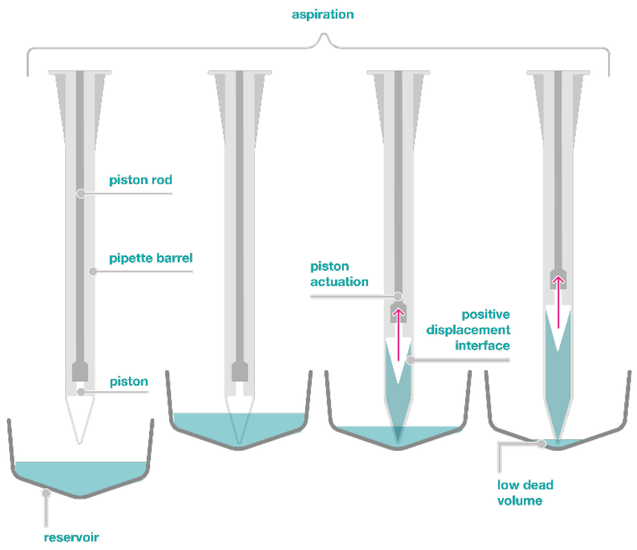


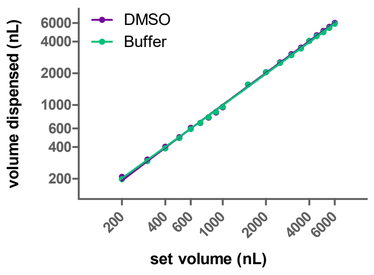
Fig 1. aspiration and dispensing with positive displacement tips

Each channel is fully independent of the others, yet they can all be operated simultaneously, giving rapid, but highly flexible dispensing. This enables complex combination gradients to be set up in high density (up to 1,536-well) microplates, as well as high speed bulk filling of common reagents.

Fig 2. dragonfly discovery

3. precision and accuracy

Tartrazine in either 100% DMSO or aqueous buffer (pH7) was dispensed in a range of volumes from 200 nL to 6 µL, and backfilled to a total well volume of 50 µL using the dragonfly discovery dispenser.



Material	Volume (nL)	CV (%)	inaccuracy (%)
Buffer	**200**	4.21	-1.16
Buffer	**500**	2.84	-2.91
Buffer	**1000**	1.59	-4.04
Buffer	**2000**	2.10	2.35
Buffer	**4000**	1.86	1.97
Buffer	**6000**	0.81	-1.45

Material	Volume (nL)	CV (%)	inaccuracy (%)
DMSO	**200**	2.78	3.98
DMSO	**1000**	1.51	-5.06
DMSO	**2000**	3.44	2.53
DMSO	**4000**	2.74	1.42
DMSO	**6000**	2.42	0.33

Fig 3. precision and accuracy of the dragonfly discovery dispenser

The automated dispenser shows excellent precision (<4% CV) and accuracy (<5% deviation) across the 200 nL – 6 µL volume range

4. fluorescein titration

Fluorescein in a variety of liquids was dispensed in a range of volumes from 500 nL to 20 µL, and backfilled with the same liquid to 20 µL (n=4). Liquid was dispensed using either a manual pipette or the dragonfly discovery dispenser (default settings).

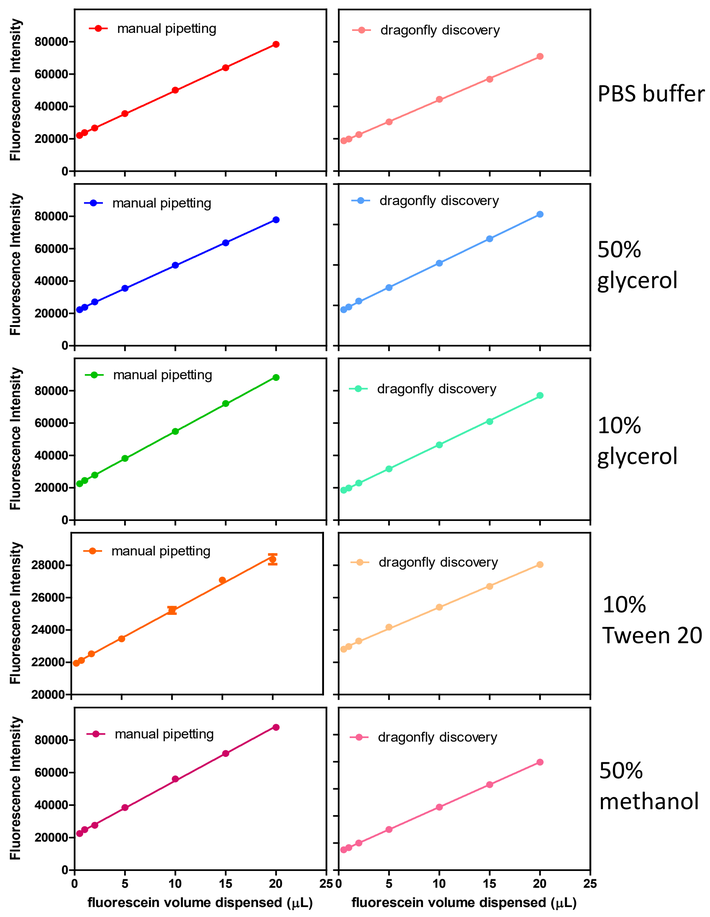


Fig 4. fluorescein titration: manual vs. automated dispensing

- very good linear correlation between volume of fluorescein dispensed and fluorescence intensity recorded for both manual and automated dispense
- Manual dispense of 10% Tween 20 required a tip change for each dispense and was more variable than the automated dispense
- overall, automated dispense times were much faster compared to hand pipetting

5. cell health assay

Human HeLa or Jurkat cells were seeded into 384-well plates (2000 cells/well in 50 µL cell culture medium) and incubated for 16 h at 37°C/ 5% CO_2. The cells were stained with 0.5 µM calcein-AM/ 1.5 µM propidium iodide, incubated for 45 minutes at RT and then analysed on the mirrorball fluorescence cytometer

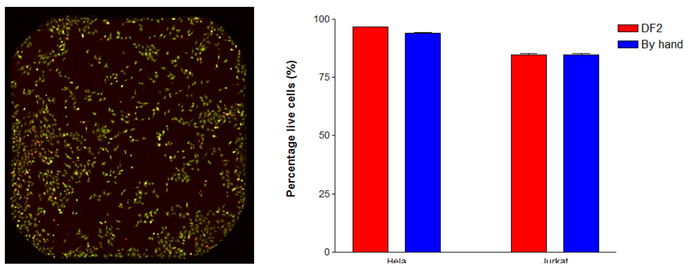


Fig 5. mirrorball whole well view of stained HeLa cells (left) and cell viability plots (right)

Results show excellent (>90%) cell viability for both cell lines, using either a manual or automated dispense approach.

6. assay development timescales

- A typical TR-FRET assay detection optimization experiment, with 5 donors and 1 acceptor with 6 points titration/ plate
- Dispense volume: 0.15 - 5µL of donor/acceptor, backfill with buffer

Acceptor 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0
B	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0	0
C	0.25	0.25	0.25	0.25	0.25	0.25	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0	0
D	0.13	0.13	0.13	0.13	0.13	0.13	0	0	0.13	0.13	0.13	0.13	0.13	0.13	0	0	0.13	0.13	0.13	0.13	0.13	0.13	0	0
E	0.06	0.06	0.06	0.06	0.06	0.06	0	0	0.06	0.06	0.06	0.06	0.06	0.06	0	0	0.06	0.06	0.06	0.06	0.06	0.06	0	0
F	0.03	0.03	0.03	0.03	0.03	0.03	0	0	0.03	0.03	0.03	0.03	0.03	0.03	0	0	0.03	0.03	0.03	0.03	0.03	0.03	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
J	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0	0	0	0	0	0
K	0.25	0.25	0.25	0.25	0.25	0.25	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0	0	0	0	0	0	0	0	0	0
L	0.13	0.13	0.13	0.13	0.13	0.13	0	0	0.13	0.13	0.13	0.13	0.13	0.13	0	0	0	0	0	0	0	0	0	0
M	0.06	0.06	0.06	0.06	0.06	0.06	0	0	0.06	0.06	0.06	0.06	0.06	0.06	0	0	0	0	0	0	0	0	0	0
N	0.03	0.03	0.03	0.03	0.03	0.03	0	0	0.03	0.03	0.03	0.03	0.03	0.03	0	0	0	0	0	0	0	0	0	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Acceptor2	Row	Donor 1					Donor 2					Donor3				
	nM	6.00	3.00	1.50	0.75	0.38	6.00	3.00	1.50	0.75	0.38	6.00	3.00	1.50	0.75	0.38
A	104	3.4	3.2	3.5	3.0	2.5	3.3	3.3	3.4	2.8	3.1	3.4	3.9	3.7	3.2	3.4
B	52	2.5	2.5	2.7	2.1	2.0	2.6	2.3	2.3	2.1	2.3	2.5	3.0	2.7	2.5	2.7
C	26	1.9	1.9	2.1	1.7	1.5	1.9	1.9	1.9	1.7	2.0	1.9	2.1	2.0	1.9	1.8
D	13	1.6	1.5	1.5	1.4	1.4	1.7	1.6	1.6	1.4	1.4	1.5	1.8	1.8	1.1	1.4
E	6.5	1.2	1.2	1.2	1.2	1.0	1.4	1.3	1.3	1.0	1.1	1.3	1.3	1.3	1.3	1.2
F	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
G																
	nM	6.00	3.00	1.50	0.75	0.38	6.00	3.00	1.50	0.75	0.38					
I	104	4.2	4.3	4.2	3.9	3.7	2.2	2.1	2.3	2.2	2.0					
J	52	2.7	2.9	2.9	2.7	2.4	1.9	1.7	1.9	1.8	1.6					
K	26	2.2	2.4	2.2	2.1	2.1	1.6	1.4	1.5	1.5	1.4					
L	13	1.7	1.8	1.6	1.5	1.6	1.4	1.3	1.3	1.3	1.2					
M	6.5	1.4	1.4	1.3	1.4	1.1	1.1	1.1	1.2	1.1	1.1					
N	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0					
O																
		Donor 4					Donor 5									

Fig 6. Dispense of a detection reagent: .CSV dispense file (left) and results (right)

- Automated dispensing time/plate: < 5 mins
- manual dispense time 1.5 – 2 hrs
- titration of signal matrix is as expected

conclusions

Compared to manual pipetting, this automated dispenser offers to the following benefits

- **robust dispense** –excellent accuracy and precision over a large dynamic range, with very low tip dead volumes (100 nL)
- **versatility of a handheld pipette** – a wide range of liquids & viscosities can be dispensed in an automated fashion, with no requirement for liquid classification.
- **gentle on cells** – instrument dimensions compatible with setup inside a biosafety cabinet for sterility. Rapid dispense of cells, with no loss of viability or drop in cell number
- **increased assay development efficiency** – complex matrix plates are dispensed in a matter of minutes, not hours. Potential to decrease assay development cycles from weeks to days.
- **effortless assay transition to HTS** – assay development directly in 384- or 1,536-well plates, using the same dispensing technology as in HTS to further reduce assay development timelines

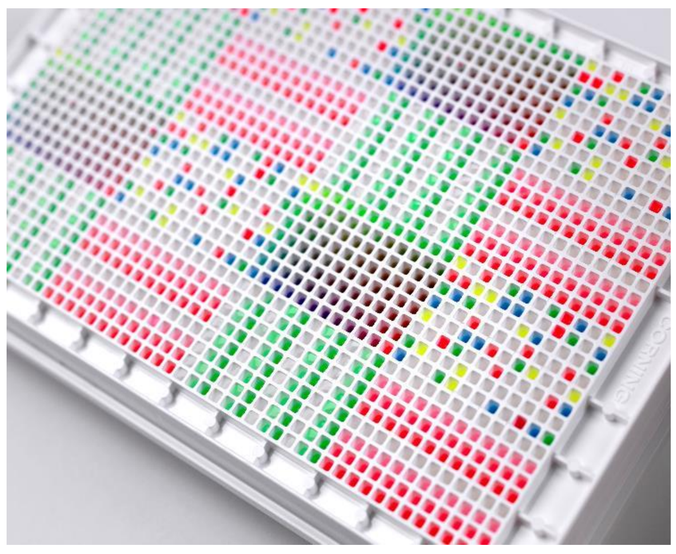


Fig 7. assay development in a 1,536-well plate using dragonfly discovery

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