Developments in Quadruplexing Microplate Assays: From 1536 to 6444 Assays in a Standard Microplate

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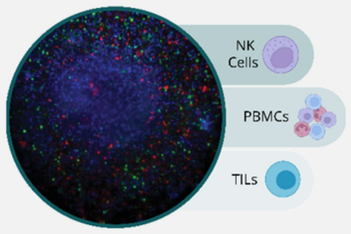
Overview

The ability to perform multiple assays within a single microtiter plate provides novel opportunities in high throughput screening, extending its utility in early-stage drug discovery. The technology development presented here aims to maximize the use of standard microtiter plates and HTS plate reader instrumentation through controlled, simultaneous dispensing and culturing of physically separated cell-lines that share a common microwell for drug dosing.

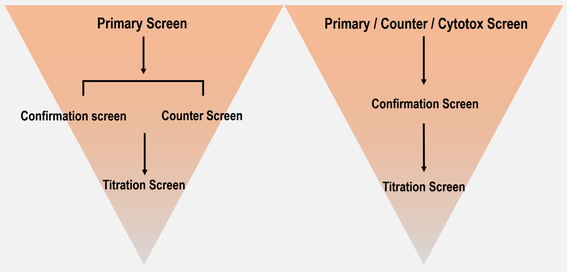
Currently the work presented here is proof of concept that integrates the development of a specialized dispenser for testing the quadruplexing approach. In this work, a BioRaptr FRD dispenser had been modified with a custom dispensing tip solution and alterations to the electronic wiring to enable 4-tip simultaneous dispensing. Initial testing has shown successful dispensing with a standard 1536-well microplate. Presented is the development of the quadruplexing dispenser along with the future goals aimed at evaluating its assay performance for early-stage drug screening.

Applications



Co-Culture Drug Screening

Co-culturing separate but adjacent cell-lines can enable the detection of cross-communications which is especially critical in cancer research. High content analysis of the wells can reveal the effect of the drug compound on each cell line in a single read.



Multiple Primary Drug Discovery

Assay development typically takes place in multiple stages, with hit compounds being tested against a variety of different cell lines. By testing multiple cell lines at once, the testing procedure is simplified. Dosing and readout variances are greatly reduced, and the overall analysis is improved.

Lion's Mane Mushroom

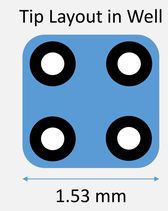
Natural Product (NP) Discovery

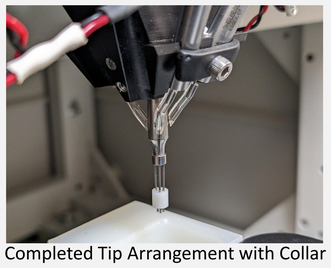
A critical bottleneck in natural product research is to ascertain the potential utility of any NP entity against different disease models. The ability to test each NP against four different disease models could significantly improve the speed and costs that arise with NP evaluation.

Development

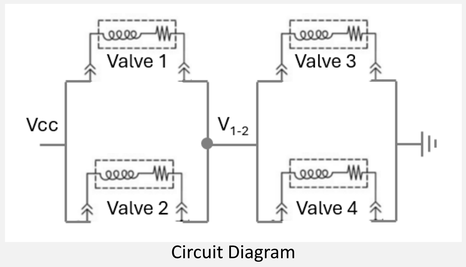
Creation of the Dispense Head

The FRD head design consists of 25-Gauge stainless steel syringe tubing attached at 0.3 mm spacing to maintain all tips fitting within a single 1.53 mm diameter well. A plastic collar was created to help prevent deflection in the tubing and ensure this spacing. A wiring harness distributes the input voltage of a single tip to all 4 dispense tips simultaneously, with the wiring setup so the overall resistance of the circuit is equivalent to a single tip circuit.

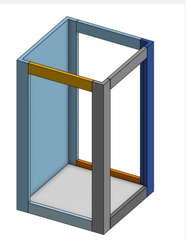




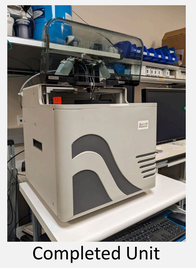
Completed Tip Arrangement with Collar



Circuit Diagram



Bottle Holder Design

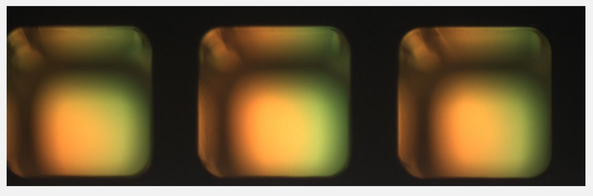


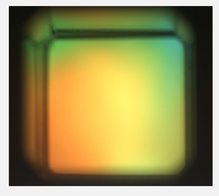
Completed Unit

Dye Testing

The head was tested with absorbance measurements to ensure even dispense volumes. A colored dye was added to each dispense tip one at a time, and absorbance measurements were taken to determine if each tip was dispensing an equal volume. Absorbance values measurements are shown in the table below. With the total volume confirmed, a different colored dye was dispensed from each tip into the same well. The initial results showed a promising localization of colors. However, diffusion caused the colors to mix over time and made analysis of location of each tip dispense difficult. It was at this point it was decided to test with dyed beads as their physical properties more closely match cells/organelles.

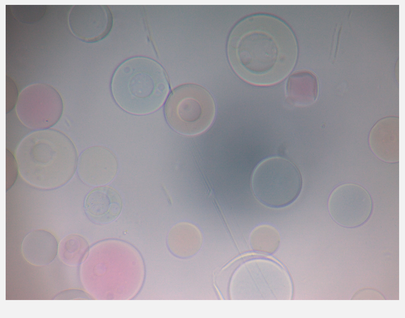
Absorbance Test								
Tip	OD Rep. 1	OD Rep. 2	OD Rep. 3	OD Rep. 4	OD Rep. 5	AVG	STDDEV	%CV
1	1.003	1.000	0.987	0.985	1.000	0.995	0.008	0.828
2	1.001	1.001	1.006	0.992	1.016	1.003	0.009	0.889
3	0.985	0.981	0.992	0.974	0.973	0.981	0.008	0.821
4	0.999	1.001	0.998	0.999	1.008	1.001	0.004	0.418

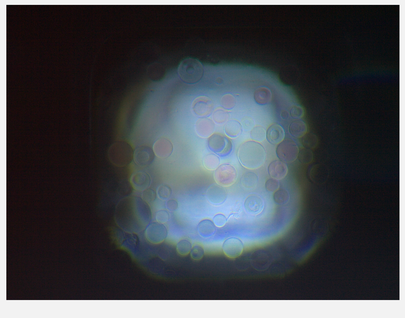


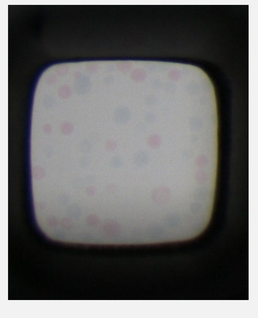


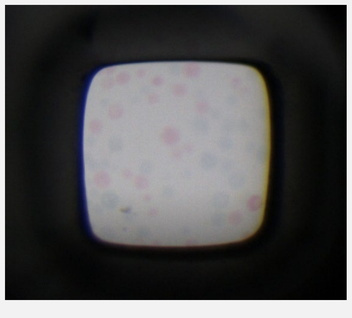
Dyed Sepharose Bead Testing

Sepharose beads do not experience the issue of diffusion in hydrogels like dyes, making them more ideal for testing the mixing behaviors of actual cells during a dispense. The Sepharose beads tested range in size from 60 uM to 200 uM, and each tip was given Sepharose beads dyed a different color. Initial testing shows that mixing of the beads is occurring upon addition to the plate, which is seen by the uniform distribution of the different bead colors across the well. The overall goal is to keep the different cell types localized. Currently we are in the process of testing hydrogels of varying viscosities to better control for the mixing seen. Another alternative we are testing is the reduction of pressure to reduce jet mixing once the gel impacts the well surface.









Conclusion

Development of the Quadruplex dispense head is still underway. The current goal is to identify a cellular medium with the appropriate viscosity to maintain cellular separation, while simultaneously being amenable to solenoid valve dispensing. Challenges remain in deconvolving results obtained from wells with multiple cell lines in localized positions, with high-content analysis being the most likely solution. Quadrant isolation of different cell lines may not be necessary with the possibility of AI-enhanced HCA image analysis. Future testing will involve cell-based assays to verify the biology is working as expected and that the technology is amenable to these different types of assays.