Development and deployment of the BioRAPTR 2.0 FRD Dispenser through collaboration, and necessity, and evaluation of dispense and liquid handling technologies.

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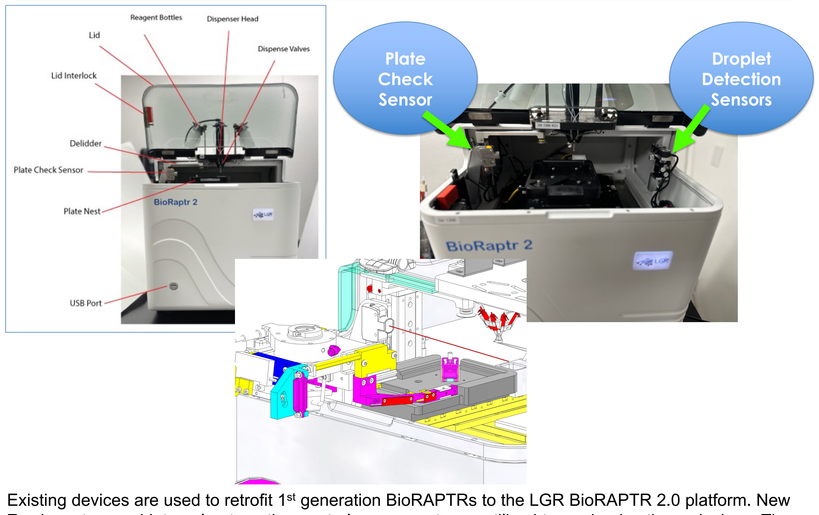
Introduction

The National Center For Advancing Translational Sciences (NCATS) has historically employed 1st generation BioRAPTR Flying Reagent Dispensers for offline assay validation/development and on our qHTS platforms. NCATS' fleet of 1st generation BioRAPTRs were at end-of-life due to unsupported hardware and software. The need to maintain continuity and retain the functionality of the 1st generation BioRAPTRs prompted NCATS to seek out a vendor to undertake significant revamp to these devices. Let's Go Robotics, Inc., in collaboration with NCATS, took on the task of retrofitting these 1st generation BioRAPTRs platforms with new internal electronics, controllers, software and GUI to create the BioRAPTR 2.0. Numerous software features were added beyond the 1st generation of BioRAPTRs. These include semi-auto volume scaling, multi-step dispense protocols, user access management, true column dispense, auto-calculated gradient dispenses, multiple liquid class calibrations stored on-board, backward compatibility with existing Excel based dispense definitions, simplified plate definition control widget, robot gripper compatible plate nest, droplet detection, among others. LGR has also added plate detection sensors and reporting to prevent crashes involving the dispenser tips, a common occurrence with the 1st generation BioRAPTRs. NCATS collaborated with LGR to perform in-lab testing of the software interface, mechanical performance and validation of dispense volumes across various liquid classes and plate types. Extensive testing and resulting feedback from benchwork completed at NCATS by staff engineers and scientists provided to LGR's engineers and software developer further refined the hardware and software.

Objective

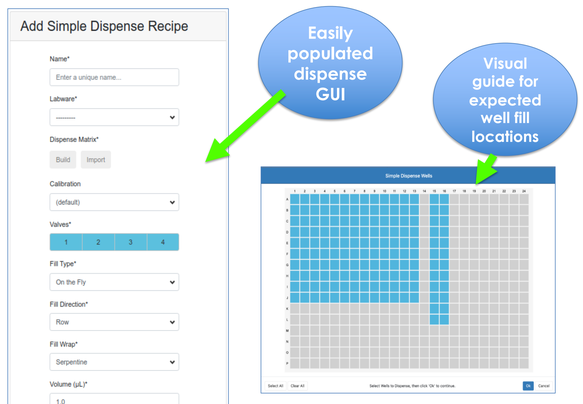
- **Evaluate hardware, software and dispense volume accuracy of Let's Go Robotics, Inc. BioRAPTR 2.0**

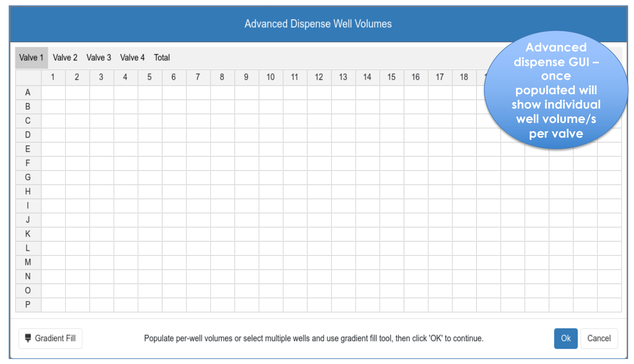
BioRAPTR 2.0 Overview

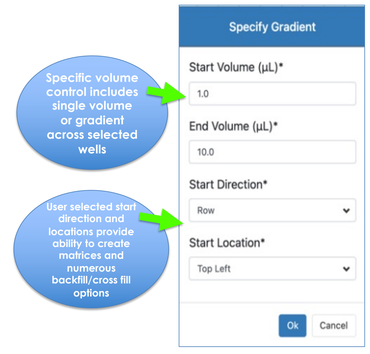


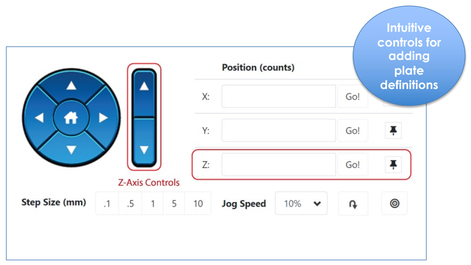
Existing devices are used to retrofit 1st generation BioRAPTRs to the LGR BioRAPTR 2.0 platform. New Z axis motors and internal automation control components are utilized to modernize these devices. The addition of plate detection and droplet detection sensors provides increased functionality and equipment protection. Also, alternative plate nests can be installed for the myriad robot grippers found on automation systems. LGR has removed the need for X Y stage end limit sensors, thus resolving issues related to valve leaks and/or other unintended liquid events. In the 1st generation BioRAPTR any liquid exposure to the X Y axis limit sensor would damage the stage, make it inoperable and result in costly repairs and device downtime.

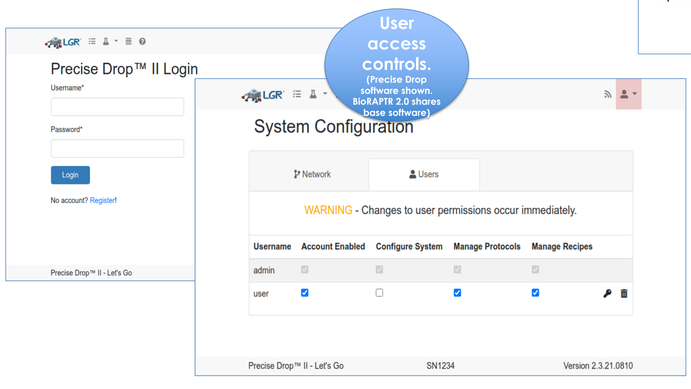
BioRAPTR 2.0 Software Overview

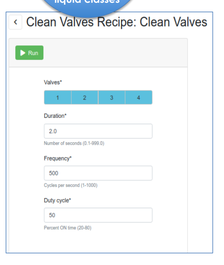










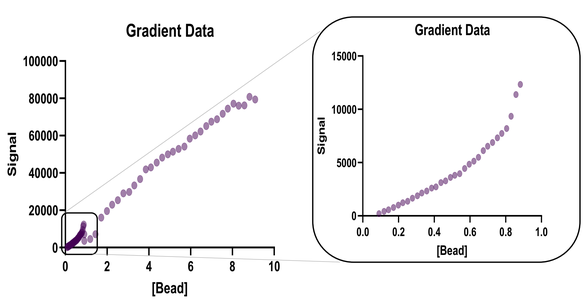


Examples of Gradient Dispense[1] and Comparative Dispense Accuracy[2]

0.1x to 10x Gradient Disp 0.1x to 10x Gradient Dispense – PerkinElmer TruHits Assay

B.) (Continued)

Back fill HEPES Buffer (uL)	Beads (uL)	total vol	[bead]	signal		
12	13 14	15 16				
0.3	3	3.3	9.090909	79382	0.909091	3553
0.33	2.97	3.3	8.818182	80769	0.909091	7125
0.39	2.91	3.3	8.575758	76228	0.881818	12331
0.47	2.83	3.3	8.30303	76057	0.857576	11381
0.56	2.74	3.3	8.054711	77121	0.830303	9348
0.64	2.65	3.29	7.781155	74499	0.805471	8189
0.73	2.56	3.29	7.537994	71706	0.778116	7733
0.81	2.48	3.29	7.264438	68761	0.753799	7315
0.9	2.39	3.29	6.990881	67488	0.726444	6878
0.99	2.3	3.29	6.74772	65094	0.699088	6536
1.07	2.22	3.29	6.474164	62188	0.674772	6118
1.16	2.13	3.29	6.219512	60116	0.647416	5491
1.24	2.04	3.28	5.957447	58349	0.621951	5130
1.33	1.96	3.29	5.701422	54131	0.595745	4845
1.41	1.87	3.28	5.426829	52877	0.570122	4446
1.5	1.78	3.28	5.152439	51414	0.542683	3952
1.59	1.69	3.28	4.908537	49932	0.515244	3800
1.67	1.61	3.28	4.634146	48203	0.490854	3610
1.76	1.52	3.28	4.373089	45524	0.463415	3287
1.84	1.43	3.27	4.097859	42959	0.437309	3116
1.93	1.34	3.27	3.853211	41914	0.409786	2717
2.01	1.26	3.27	3.577982	36746	0.385321	2603
2.1	1.17	3.27	3.302752	33269	0.357798	2337
2.19	1.08	3.27	3.058104	29792	0.330275	2147
2.27	1	3.27	2.782875	29013	0.30581	1881
2.36	0.91	3.27	2.515337	25403	0.278287	1653
2.44	0.82	3.26	2.239264	22971	0.251534	1368
2.53	0.73	3.26	1.993865	19570	0.223926	1235
2.61	0.65	3.26	1.717791	16055	0.199387	1007
2.7	0.56	3.26	1.441718	7144	0.171779	779
2.79	0.47	3.26	1.189024	4579	0.144172	570
2.89	0.39	3.28			0.118902	418
3	0.3	3.3			0.090909	209

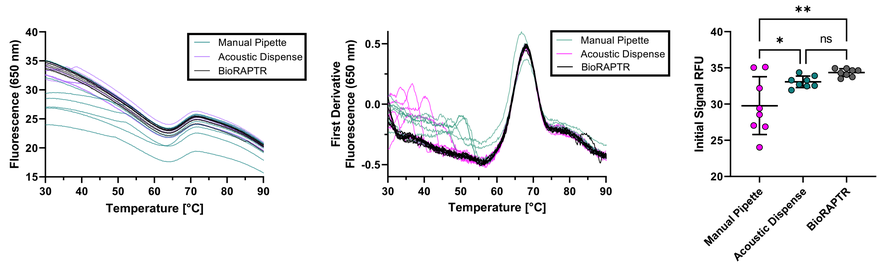


The gradient dispensing mode was used to try to assess if there was a cap in signal with respect to concentration of beads.

Two batches were dispensed; 0.1x to 1x and 1x to 10x concentrations.

Brooklyn Aragon[1]

Comparison of Dispense Methods for HIS-DSF



Testing dispense methods using an established HIS-DSF assay against in-house protease target. (Left) Raw thermogram traces for n = 8 replicates of each liquid handling method dispense of 1 µL protein-dye mixture into 1536-well Roche PCR plates and tested in a standard DSF melting experiment. (Middle) First derivative of the n=8 replicate samples from each dispense method. (Right) Scatter plot of the sample initial fluorescence signal from each dispense method. Lines represent the means of the n=8 replicates with error bars representing the standard deviation with significance values derived from one-way ANOVA with Tukey's multiple comparisons between groups (* = p<0.05, ** = p < 0.01).

Michael Ronzetti[1]

Conclusions

- **Retrofit of 1st generation BioRAPTRs to the LGR BioRAPTR 2.0 platform provides continuity of assay development/validations and qHTS assays performed at NCATS.**
- **Anecdotal user feedback of the software GUI has been extremely positive.**
- **Validation of accuracy of gradient dispenses (example[1]) shows consistent volumes dispensed per the onboard software calculations. This removes potential errors in user calculations when populating gradient dispenses.**
- **Validation of dispense volumes compared to manual and acoustic dispense methods (example[2]) show greater accuracy over manual dispenses and comparable dispenses using an acoustic dispenser vs. the BioRAPTR 2.0. This dispense was made to a 1536 well Roche Light Cycler Plate. The acoustic dispense took 36 +/- minutes vs 1.5 minutes with the BioRAPTR 2.0. 1st generation BioRAPTRs could not accomplish this dispense due to plate well targeting issues - droplet release timing, location within the well, plate materials and well design.**
- **Additionally, retrofit of existing 1st generation BioRAPTRs to the LGR BioRAPTR 2.0 platform provided significant cost savings over maintaining existing devices and/or acquiring, deploying and training staff to use new instrumentation.**