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Design of an On-the-Fly Optical Detection System for Multi-tip Reagent Dispensing

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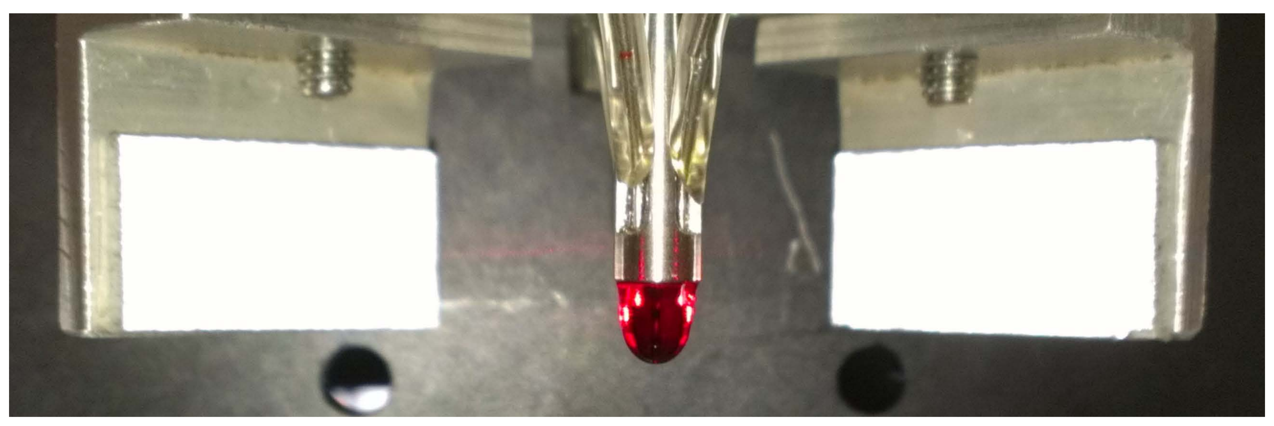
Overview

- One of the most common problems during high throughput screening (HTS) is errors in dispensing reagents into microplates.
- This is typically due to a failure of the dispense instrumentation itself (often a clogged tip) or incorrect calibration of the dispenser. These errors can cause plates to fail quality control. Rescreening compounds costs additional screening resources and time.
- We have developed a droplet measurement system for volumes as low as 50nL that can be used to monitor dispenses in real time providing immediate feedback in the event of any failure.
- Development of the droplet monitor required the selection of an appropriate sensor capable of rapidly detecting low liquid volumes, and the design of a mechanical fixture used to house the selected sensors for the simultaneous monitoring of multiple dispense tips.
- We have also developed software to read the output of all connected sensors for easy integration with existing commercially available dispensers.
- We implemented our solution on two Beckman BioRAPTR dispensers on one of our high throughput screening systems, and have had multiple instances where we could immediately respond to a detected dispensing error, allowing us run several thousand 1536 well plates without loss.
- We anticipate that our hardware and software combination could become a low-cost and straightforward tool to increase the robustness of high throughput screening.

Introduction



Problem Statement

After extended periods of use, the valves which control reagent dispenses may spontaneously fail, jeopardizing an entire assay and costing thousands of dollars.

- Significant attention has been given to the development of droplet based microfluidic devices due to their potential in high throughput applications.
- A fundamental challenge is to determine the quality of each dispense within a large scale biological screen.
- Ensuring the dispense of microfluidic droplets requires real-time detection and manipulation of droplets in multiple locations.

In this study, we present a cost effective sensor design that uses optics to measure droplet presence using commercially available and inexpensive laser optic sensors.

Methods

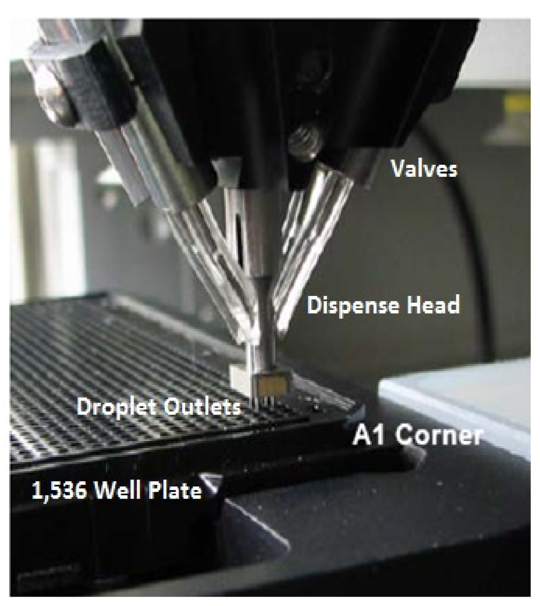
Design Objectives

Engineering Requirements:

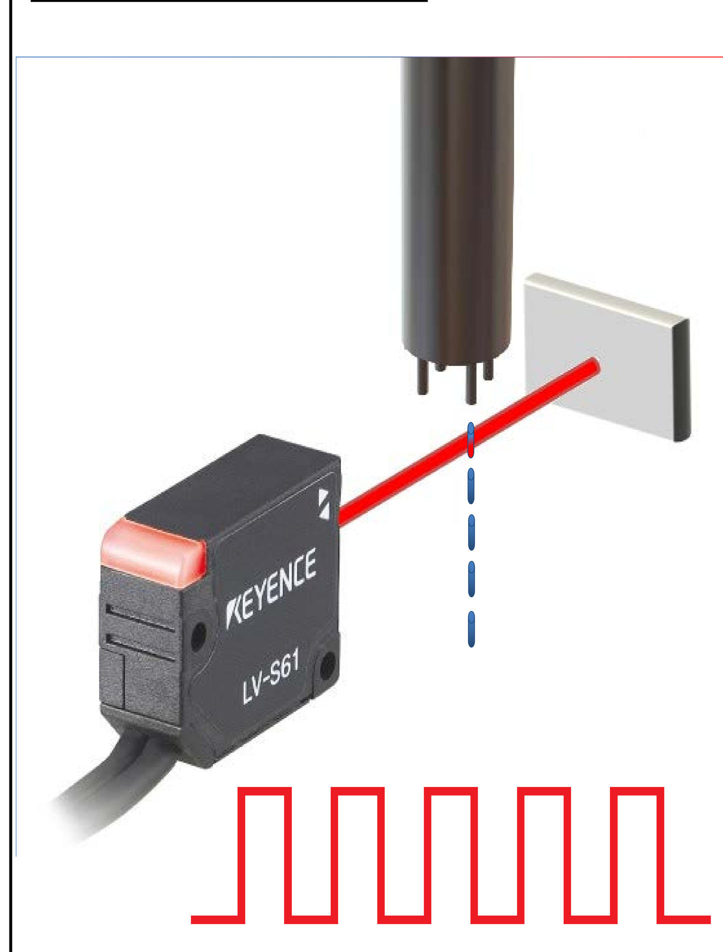
- Must be capable of 4 tip detection
- Able to detect droplets in varying volumes (50nL to 1mL)
- Must maintain a high accuracy (>98%)
- Acquire data rapidly to capture falling droplets (v = 9.6 m/s)
- Summarize data for Ethernet access
- Provide 1-3 mm well plate clearance
- Must detect clear fluid reliably
- Should avoid interference between channels
- Must maintain alignment
- Must fit in tight space requirements

Customer Requirements:

- Adaptable – attach to many dispense heads
- Flexible – ability to adjust programming
- Easy to install and maintain
- Minimum size on dispense head
- Potential to operate stand alone



Sensor Selection

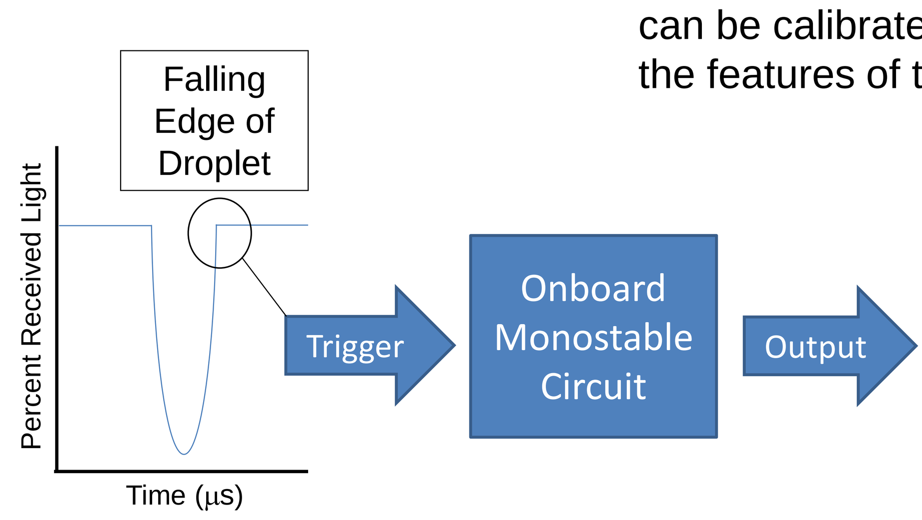


The retro-reflective optical sensor, LVS61 manufactured by Keyence was integrated into the system Operation: Light bounces off a reflector and returns to the source

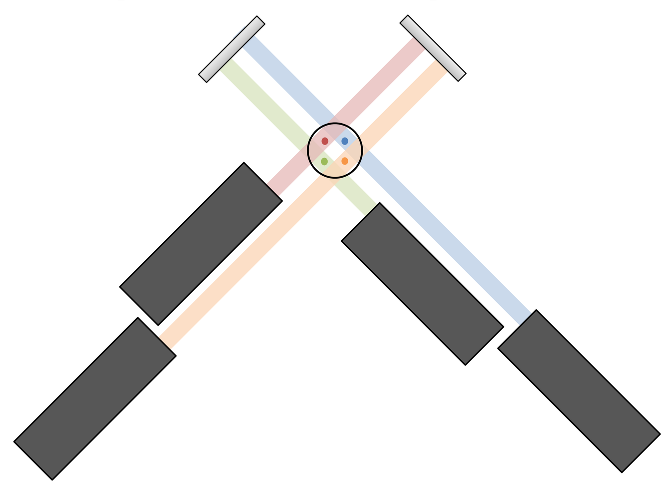
Sensor Features:

- Wave patterns prevent inference
- Alignment flexibility with 2.5mm beam width
- Return signal always decreases with a target
- No light anomaly issues
- No missed detection concerns
- Monostable Falling Edge Detection

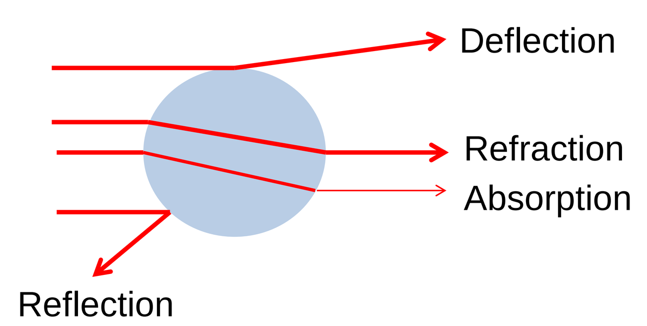
Optical sensing of microdroplets is based on the contrast of received light between the obstructed and unobstructed phases. A threshold can be calibrated by implementing the features of the NV-12 amplifier.



Design and Testing



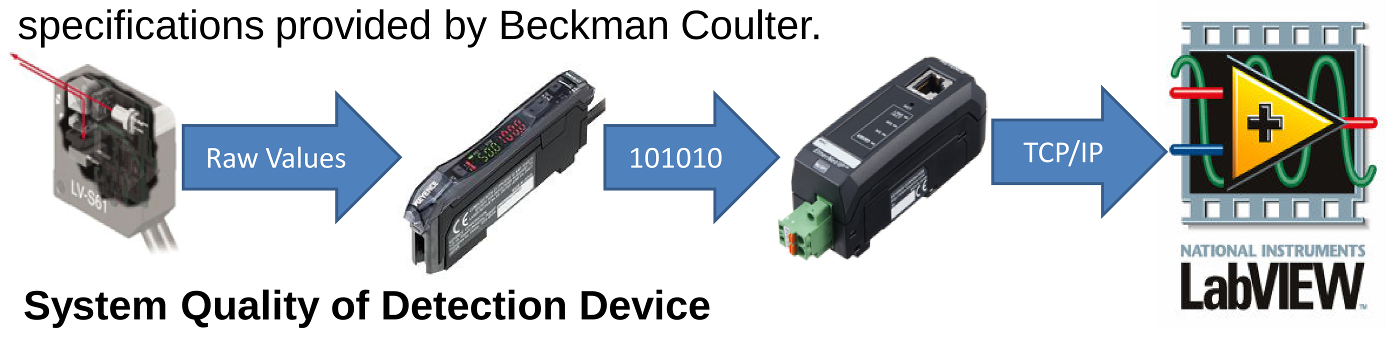
- A fundamental requirement to the final fixture size is to match the dispenser's lateral geometry.
- Because of the sensors large beam diameter and head size, it was necessary to develop a mounting bracket to prevent crosstalk between sensors.
- The fixture incorporates minute adjustment to properly align beams with their respective tips.



Features of Mechanical Fixture:

- Provides access to each tip
- Easily adjusted with set screws
- 2 mm clearance between head and plate
- Aluminum body

In order to record the falling edges of the signal, the rate of data acquisition should be at least two times higher than the frequency of interest. The frequency of the signal was calculated from technical specifications provided by Beckman Coulter.



System Quality of Detection Device

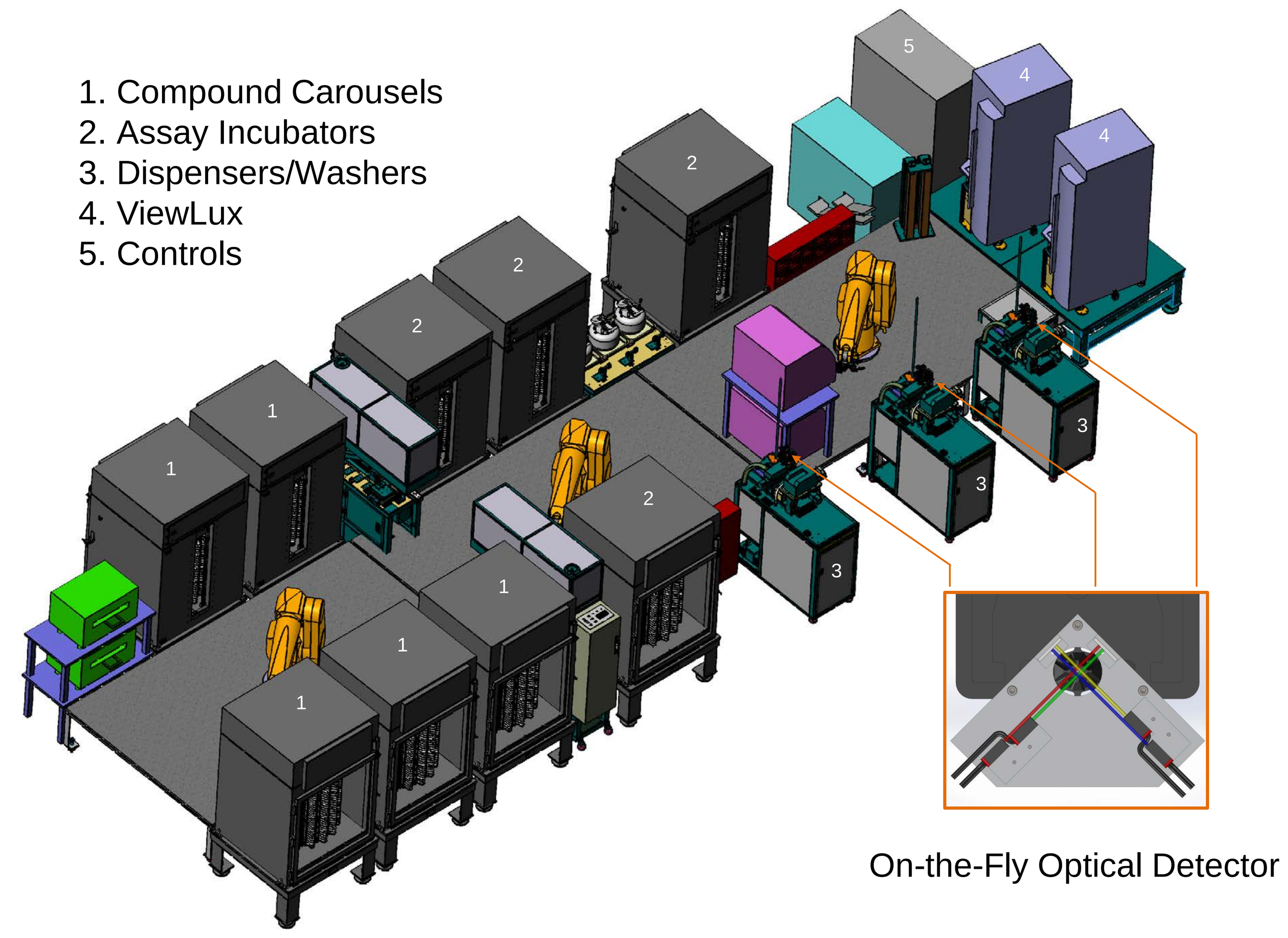
The uniformity amongst droplets in a series can be guaranteed by selecting parameters such as pressure, frequency and volume. The method of dispensing on a BioRAPTR FRD ensures a calculated Coefficient of Variation of ~4% amongst all volumes. This series of tests consisted of 1,000,000 dispenses for 4 liquid volumes (0.5 µL, 2.5 µL, 4 µL, 6 µL) for each solvent.

Error Simulation of BioRAPTR Dispenser

In order to test this system's ability to detect dispensing errors, multiple trials were conducted to observe the sensor's response to a specific failure. In this test, particular errors were induced to match common errors of HTS automation.

- Insufficient amount reagent in reservoir
- Valve failure due to corrosion (H_2SO_4)
- High viscosity accumulation

NCATS HTS Platform



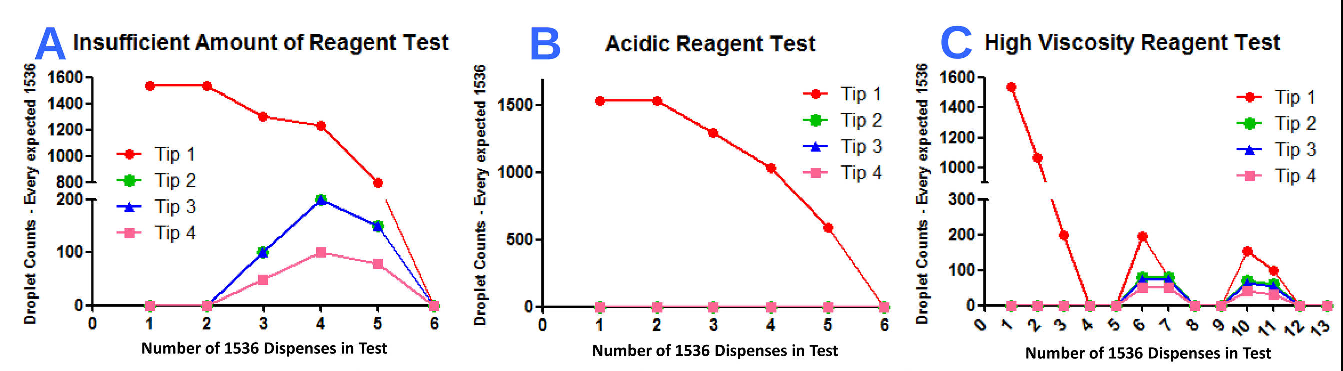
Results

Microfluidic Dispenses of Different Solvents After Optimization

Solvent	Volume	Count of Droplets	
		Expected	Observed
Water	0.5 µL	100000	100000
	2.5 µL	100000	100000
	4 µL	100000	100000
	6 µL	100000	100000
Methanol	0.5 µL	100000	100000
	2.5 µL	100000	100000
	4 µL	100000	100000
	6 µL	100000	100000
Dimethyl sulfoxide	0.5 µL	100000	100000
	2.5 µL	100000	100000
	4 µL	100000	100000
	6 µL	100000	100000

- We selected three commonly used solvents with different viscosity and density.
- This series of experiments consisted of 4 liquid volumes (0.5 µL, 2.5 µL, 4 µL, 6 µL) for each solvent.
- In order to determine the error of the optical system, the observed quantity of droplets counted by the optical system was compared to the expected number of droplets.

Error Simulation of BioRAPTR Dispenser



In all Error Simulation tests, only Tip 1 was used for the 1 µL dispenses. A – Indicates insufficient reagent in reservoir and cross-contamination with other tips; B – Indicates valve failure because of acidic reagent; C – Indicates accumulation of reagent on the dispense head and cross-contamination

Conclusion

- Designed and developed a device that enables users to detect the presence and absence of a free flying droplet in the microliter volume range.
- Although the signal does not allow for a quantitative droplet volume evaluation, it is highly sensitive to errors commonly found in HTS.
- Provides high sensitivity to errors, such as clogging and broken hardware, and an instantaneous read out, thus it can be utilized for online process control of non-contact dispensing systems.
- Can provide a reliable tool for lab technicians to easily diagnose and fix the error.

Acknowledgements

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The work is dedicated to Michael Balcom, whose unexpected death prevented him from seeing the final results of his contributions.