High Precision Non-Contact Dispensing

innovadyne Technologies, Inc.

www.innovadyne.com

leading the way in high-precision dispensing

Introduction

From screening to crystallography, researchers seek to increase throughput while lowering the use of rare or hard-to-produce samples and reagents. Often the solution is found through miniaturization of assays, while other applications require delivery of small volumes to discrete target locations. In all cases instrument precision, accuracy, speed, reproducibility, reliability, and flexibility become critical success factors. Innovadyne's approach to liquid handling -- high precision non-contact dispensing, based on advances in solenoid valves and flow path technology, offers a robust and low-maintenance means of achieving assay volume reduction without compromising precision or accuracy. Precision is improved at all points in the dynamic range (from 100 nanoliters to 500 microliters).

Limitations of Current Approaches

Traditional approaches to low-volume liquid handling technologies range from classical liquid handlers, employing syringe-based dispensing, to piezo-electric dispensers. Some offer a fixed volume at the expense of accuracy and precision while others promote a variable volume range at the expense of delivery or dead volume. An additional class of dispense-only devices use a flow-through approach. In these systems, reagent flows through the entire fluid path propelled by a backpressure source such as a pressurized reservoir, syringe drive or perlistaltic pump. In all of these flow-through systems, the entire system must be primed with the reagent and most require a dispense actuator integral to the flow path.

The limiting factor of traditional liquid handling techniques is the fact that they rely on **low energy displacement**. To perform a reproducible and accurate dispense, the last task of any pipetting action relies on a **touch off**. Classical displacement techniques do not have enough energy to break the surface tension of the last droplet. So a dragging action -- touch off -- is employed, either against the solid surface of a vessel or a liquid surface. Consequently, this technique is variable -- it varies with liquid properties, temperature, humidity, surface adhesion, and other factors. At larger volumes the variation is small enough to have little impact on the end result. However, when the total volume pipetted is small, this variation has a significant negative impact on precision and the lower the volume, the larger is the contribution of the variation.

Advantages of Non-Contact Dispensing

Non-contact liquid handling relies on the combined use of rapidly actuated solenoid dispense valves, a controlled pressurized liquid source, and flow path control via hybrid valves. The speed and energy of the fluid displacement enables the surface tension of the liquid to break as it leaves the orifice, eliminating the need for a touch-off.

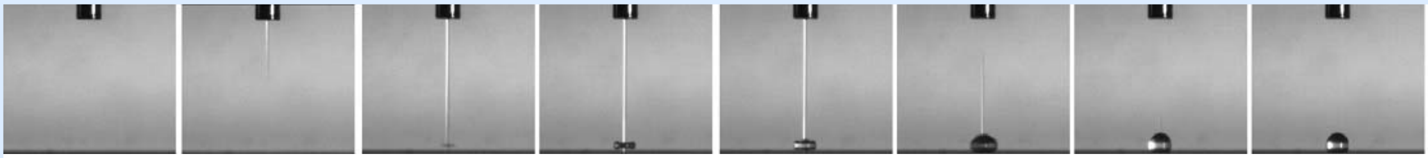

Figure 1: High Speed Photography of 100 nL Dispense (1000 frames/second)

The lack of a touch-off step in the dispense eliminates the variability issues associated with touching off. The following bar graph provides a typical example of the precision achieved with Innovadyne dispensing instruments at 100 nL:

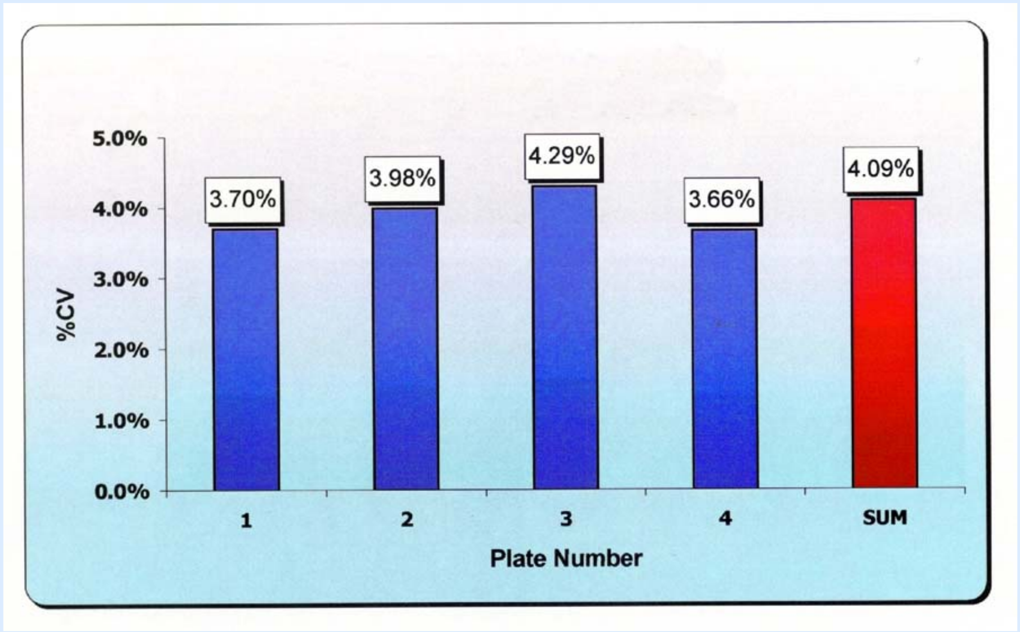

Figure 2: Overall %CV Across Four Plates (100 nL Dispense, 384-well Plate)

An important added benefit of non-contact dispensing is speed -- the non-contact technique allows the delivery nozzle to accurately deliver the droplet above the target well and rapidly move to the next well. With the non-contact technique dispensing becomes independent of the substrate, eliminating many of the reproducibility problems associated with motion control. Plate processing times fall dramatically. Using non-contact dispensing it is possible to deliver to all wells of a 96 well plate in approximately 5 seconds, 384 wells in approximately 7 seconds, and 1536 wells in approximately 14 seconds.

Isolation of Solenoid Valves from the Sample Path

The performance of solenoid-based delivery techniques relies on the speed of opening and closing of the valve. To be effective the actuation must be both rapid and reproducible. Many samples and reagents are either adsorbent (such as proteins) or particulate (samples marginally soluble in DMSO) and the introduction of these materials to the complex internal path of a solenoid valve can lead to deposits, obstructions, and wearing of the seal materials.

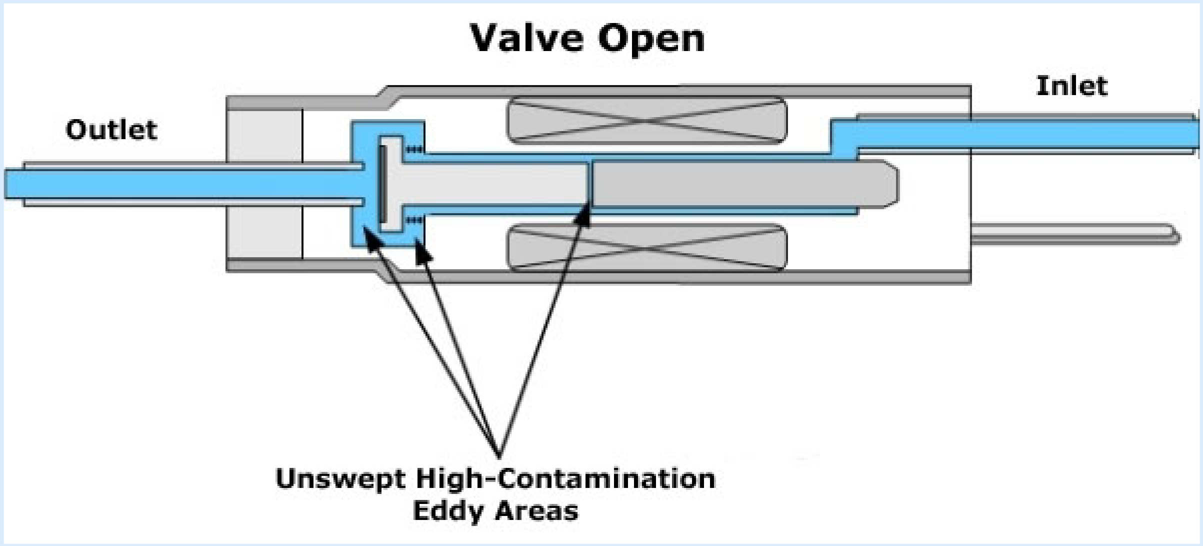

Figure 3: Problem Areas in Microsolenoid Valves (Valve Open)

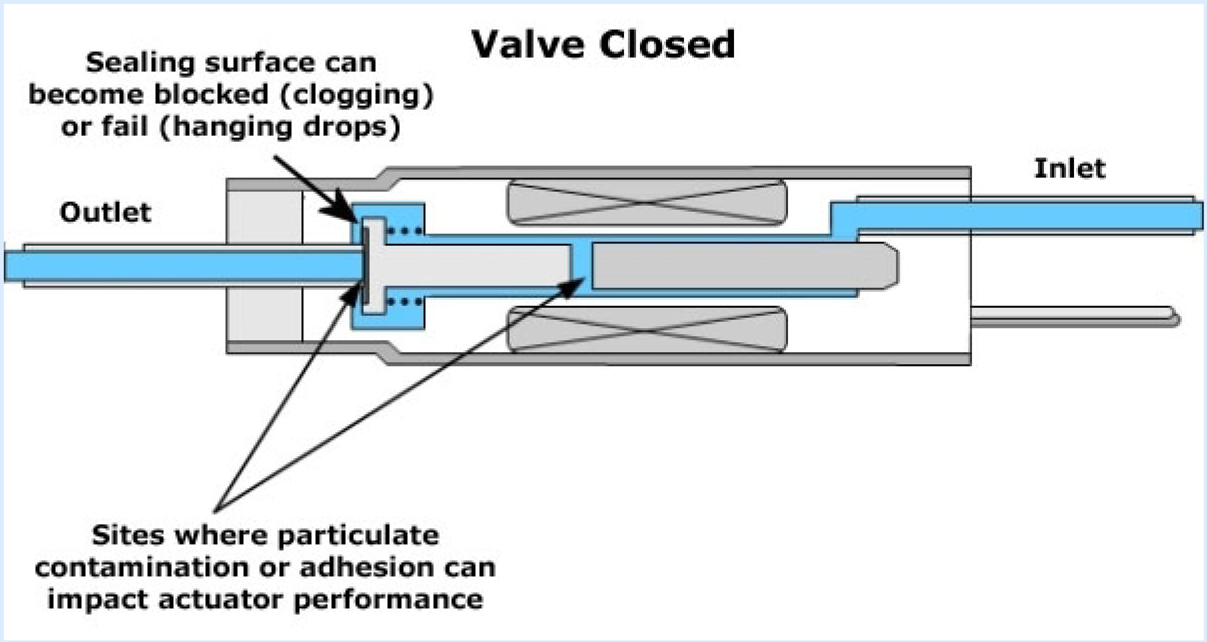

Figure 4: Problem Areas in Microsolenoid Valves (Valve Closed)

Valve deterioration from deposits and wear is not only costly, requiring valve replacement, but can also be difficult to diagnose because the effect can be a gradual deterioration of performance or intermittent failure (that is, no dispense). It can be very confusing to try to sort out the variation in the day-to-day reproducibility of the overall method when one is unsure whether the hardware or the actual chemistry is to blame.

The incredibly high duty cycles of HTS and μHTS facilities, often 250 or more 1536-well plates per day, will expose any fragility in a technique or instrument. The precision of solenoid-based systems has long been recognized but widespread acceptance has been tempered with concern regarding performance and maintenance issues. This concern has been addressed by Innovadyne with the introduction of a proprietary hybrid valve architecture separating the sample aspiration path from the solenoid dispense valves, based on a technology developed under a collaborative research agreement with the Oak Ridge National Laboratory (the US Department of Energy research laboratory).

An Innovadyne dispensing instrument utilizes two separate flow paths -- a syringe path and a pressure path -- to aspirate and dispense reagent. The **syringe path** is used to aspirate an air gap and reagent. The syringe path is the flow path between the syringes and the tips, shown below:

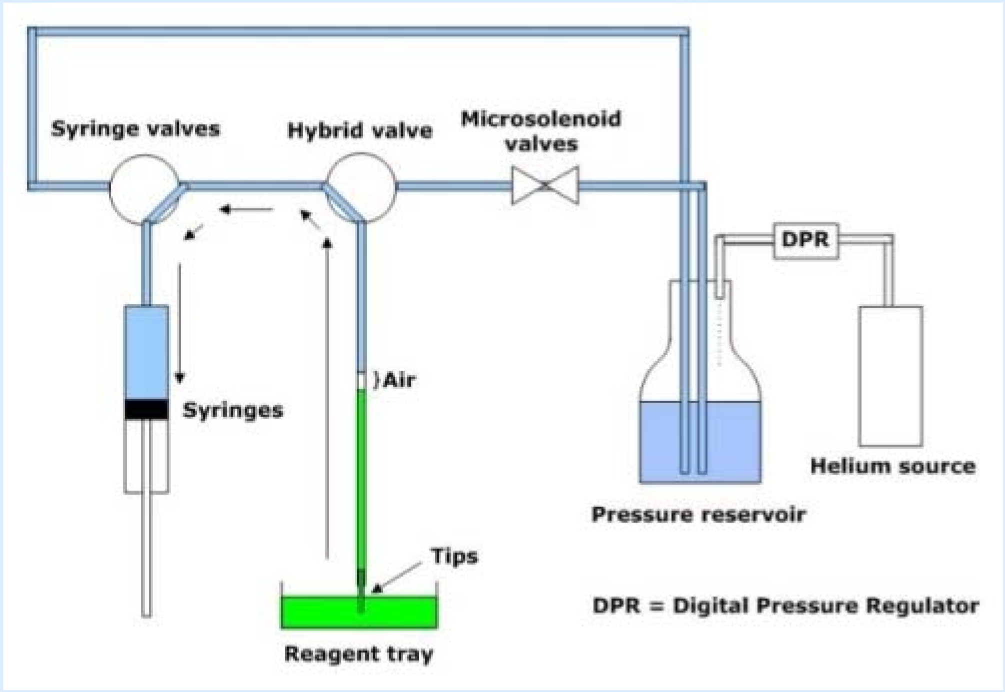

Figure 5: Reagent Aspiration, using the Syringe Path

To aspirate an air gap, the tips are exposed to the atmosphere (not descended into the reagent tray), allowing air to flow into the tips. The valves are switched into position, allowing flow from the tips to the syringes. The syringes are pulled down, creating a vacuum, and system fluid flows from above the reagent tray to the syringes. Air is aspirated through the tips and system fluid flows toward the syringes. Next, to aspirate reagent, the tips are descended into the reagent tray, then the syringes are pulled down further. Reagent flows from the reagent tray to the syringes, separated from system fluid by the air gap.

The **pressure path**, using a pressure reservoir filled with de-ionized system liquid held at system pressure, is used to dispense reagent. The pressure path is the flow path from the pressure reservoir, via the microsolenoid valves, to the tips, as shown:

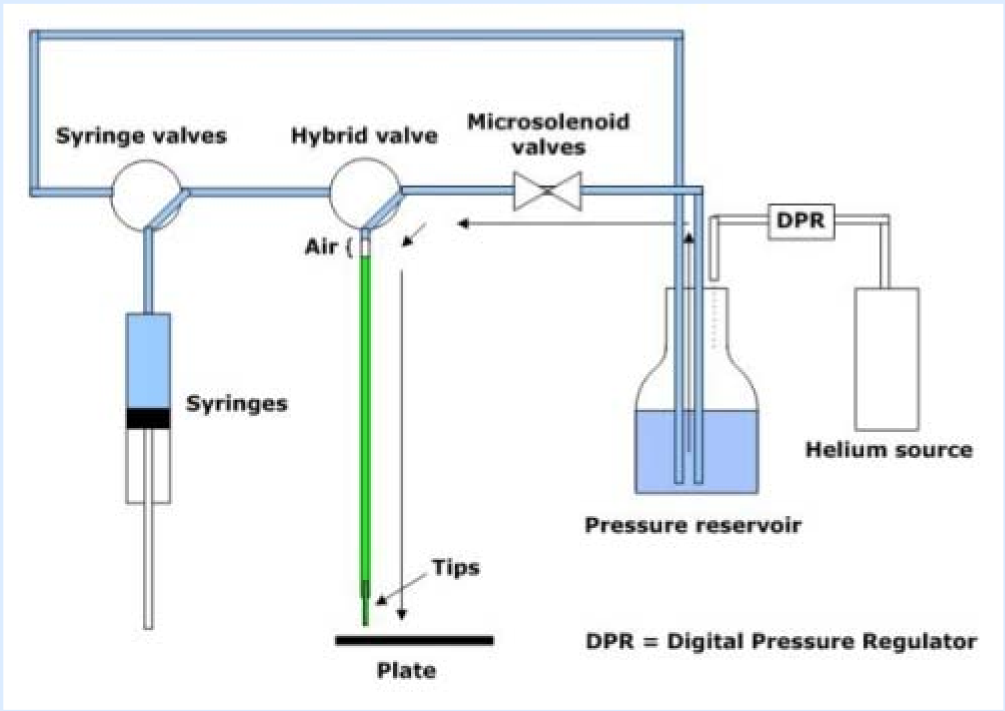

Figure 6: Reagent Dispense, using the Pressure Path

The pressure reservoir contains system liquid, maintained by a digital pressure regulator (DPR) at a specified system pressure. To dispense reagent, the hybrid valve switches the flow from the syringe path to the pressure path, and then the microsolenoid valves are opened and closed the requisite number of times to permit the desired volume of system liquid to flow from the pressure reservoir toward the tips. Note that the reagent does not itself flow through the microsolenoid valves; system liquid does. The motion of the system liquid towards the tips displaces the desired volume of reagent out through the tip, and reagent is dispensed into the plate.

By isolating the critical flow-regulating device, the solenoid, from the sample flow path, this simple design eliminates the risks present in earlier systems and takes a major step forward in terms of robustness. The solenoid valves are now only exposed to de-ionized water at a constant pressure, allowing them to operate efficiently and effectively, as they were originally designed.

Rather than lasting for only months, solenoid valves can now be expected to perform for years. In addition to improving robustness, the system design ensures that the aspirated sample never contacts moving parts and only encounters a very simple flow path. The benefits for difficult samples are clear. A wide range of reagent viscosities together with beads, cells, and complex mixtures have been pipetted successfully.

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