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## **Hybrid Valve Structure for High Throughput, Low Volume Liquid Handling Applications**

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## Abstract

A hybrid valve that integrates precision microfluidics for fluid routing, high-speed valving for fluid switching, and reagent-jetting devices for metering the fluid dispenses is described. The hybrid valve enables parallel switching between aspiration and dispense modes for multiple sample streams. This unique valve structure addresses many of the concerns with handling microscale volumes, including efficient use of samples, degradation of ink jet valves and speed of operation. A broad range of volumes can be manipulated with excellent reproducibility. The hybrid valve can be configured for a variety of applications. Pick-and-place aspiration and dispensing of unique reagents and rapid dispensing of a common reagent are possible. Together these features lead to higher-speed transfer of smaller volumes of reagent.

## Introduction

Advances in biomedical science, particularly in genomics and combinatorial synthesis, have greatly increased the potential number of reactions and analyses that must be performed by the biotechnology and pharmaceutical industries. Improved high-throughput analytical approaches, including lab-on-a-chip based analyses [Figeys, 2002], mass spectrometry-based characterizations [Ferguson and Smith, 2003] and protein crystallization techniques [Adams, 2003; Mueller, 2001], offer greater access to biological structure and function. Parallel implementation of microscale assays provides even greater advantages as demonstrated by the use of high-density microarray formats for gene expression profiling or for screening for protein-protein interactions [Ramsay, 1998; Cutler, 2003].

Implementation of these assays at small scales offers economies that are unmatched by conventional approaches. However, conventional liquid-handling devices often fail to interface to such devices or to deliver the required volumes. Therefore, a needed technology is a low volume liquid transfer technique that is robust and scalable for measurements of interest. With growing demand, the development of fluid handling devices adept at manipulating sub-microliter volumes of multiple reagents is needed.

Typically, conventional liquid handling instruments manipulate liquid volumes in the range of microliters to milliliters and use syringe pumps for fluid manipulations. These systems often employ a *pick-and-place* technique, in which a sample is transferred between a source and target location. For example, the technique can be used to transfer samples from a storage plate to a microassay plate, where the benefit of scale

reduction can be realized. Other liquid handling instruments dispense common reagents, such as reaction cocktails, wash solutions or diluents, to a sample plate. In either approach, parallel transfer of reagents is essential for high throughput. Likewise, reduced sample volumes and inter-sample spacings are required for higher degrees of miniaturization. When using syringe style pumps for transferring small volumes ( $< 1 \mu\text{l}$ ), contact between the dispensing tip and target site is required for fluid transfer. This is necessary for overcoming the capillary adhesion forces that would otherwise retain the sample with the dispensing tip. Unfortunately, the need for contact between the dispensing tip and target plate defines the arrangement of samples (when using multiple tips) and presents the opportunity for cross-contamination. Therefore, rearrangement of sample order, or reduction of the spacing between samples is not possible with touch-off spotting.

In other advancements, drop-on-demand ink jet technology has been adopted for accurately delivering volumes extending down to hundreds of picoliters [Cooley 2002; Calvert, 2001]. This technology is capable of high volumetric precision and employs either piezoelectric [Schober 1993] or solenoid actuation mechanisms [Hicks, 2001; Muller, 2001, Lemmo, 1997]. Piezoelectric devices dispense discrete droplets and therefore require multiple dispenses for delivering larger volumes. When volume metering is required, piezo-based approaches are slow compared to solenoid-actuated devices that allow for streaming of the sample. Both of these techniques offer noncontact dispensing as the sample is ejected from the tip.

Noncontact ink jet printing technology represents the basis for the next generation of automated liquid handlers. Currently available ink-jet-based systems are limited by their reliability, speed/throughput, and scalability [Bateman 1999]. These systems are often slow, cannot switch between liquid handling modes and are limited in the range of liquid volumes that can be manipulated. Here we describe a hybrid valve device for applications in high-throughput, low volume liquid handling. The valve integrates several functions into a single device and allows for rapid, parallel switching of multiple fluid channels. The hybrid valve structure offers the versatility needed to exploit the advantages afforded by ink jet delivery systems. The fluid channels are individually addressable and functions such as aspiration, dispensing and washing are rapidly carried out. Further, the device is modular and capable of being implemented in a variety of formats.

## Methods

### Hybrid Valve Construction

The hybrid valve architecture is the basis of a variety of derived platforms and OEM components, ranging from single channel to 96-channel versions. The hybrid valve is commercially available through Innovadyne Technologies, Inc. The valve is currently designed as a 12-channel device, but can also function with fewer active channels to arrive at configurations that perform as 4 or 8 channel systems. Additional designs are available for 24-, 48-, and 96-channel systems.

The hybrid valve integrates high-speed miniature solenoids with syringe drives to isolate the sample and reagent aspiration path from the dispense actuators.

A 4-layer diffusion-bonded ULTEM manifold, incorporated as an integral part of the parallel switching valve, permits the necessary access and exacting registration needed to combine multiple flow paths and components in a format that can deliver the high accuracy and precision required for low volume assays. The rotor and stator of the valve currently are made of TEFLON-based plastics and are highly inert.

Electronic control of the valve is accomplished using a microcontroller to control movements from one position to another, using RS 232 commands and/or Digital I/O. The valve has been integrated into both gantry and X-Y sub-stage systems using proprietary Innovadyne control boards. Firmware has been implemented which allows users to set parameters and actions to control the valves and associated electronics. A Graphical User Interface (GUI) has been developed for integrated end-user products. In its current product Innovadyne offers a high-speed, high-precision, 8-channel platform that includes both a stand-alone GUI and a remote automation control (ActiveX) for fully integrated robotic systems.

### Characterization of Dispensed Volumes

The hybrid valve was implemented on an 8-channel liquid handling system (Nanodrop, product of Innovadyne Technologies, Inc.). General characterization studies were performed using fluorescence and gravimetric measurements. Gravimetric measurements were employed in order to determine the relationship between pulse widths (solenoid valve open time) and dispensed volume for a given pressure, nozzle orifice size, solution viscosity, and system configuration. They were also used in order to verify dispensing accuracy of the systems studied. For this method, a number of dispenses (minimum of 10, but more for smaller volumes) is weighed using an analytical balance. The weight of these dispenses is divided by the total number of dispenses and corrected for solution density to obtain an average dispense volume. Calibration curves are generated from this data to provide pulse widths for all volumes in the calibration volume range.

Precision measurements were performed using the following fluorescence method. A solution of approximately 200  $\mu\text{M}$  fluorescein (Aldrich, F245-6) dissolved in tris buffered saline, pH=8.0 (Sigma, T-6664) was aspirated and dispensed. Unless otherwise stated, solutions are dispensed into black 384-well microtiter Cliniplates (ThermoLabsystems no. 95040020) and diluted with 50  $\mu\text{L}$  of the same tris buffer with the addition of 0.02% Tween 20 (Polyoxyethylenesorbitan monolaurate, Sigma, P-2287) using a Multidrop-384 (ThermoLabsystems). Fluorescence is read using a Fluoroskan Ascent reader (ThermoLabsystems) with an integration time of 100 ms.

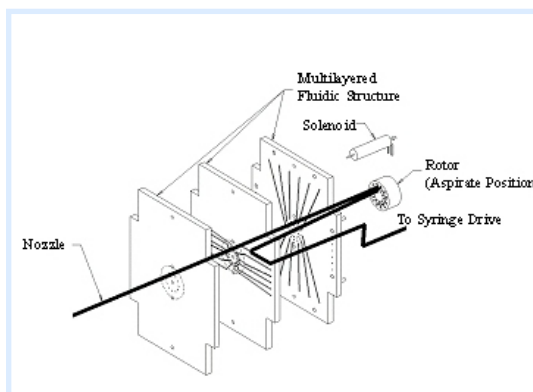
### Assay Miniaturization Example

For the luminescent assay miniaturization sample, the Nanodrop was used to dispense 250 nl of 100% DMSO, followed by 5  $\mu\text{L}$  each of two different proprietary solutions into a black polypropylene Matrical 384-well plate. The traditional sample assay used for comparison consisted of 1.25  $\mu\text{L}$  DMSO and 25  $\mu\text{L}$  each of the proprietary solutions dispensed using a Multidrop and CCS Platetrak. The results were read in a Perkin Elmer Trilux reader after a 60 minute incubation period (data courtesy of Dr. Timothy D. Dawes of the Chiron Corporation).

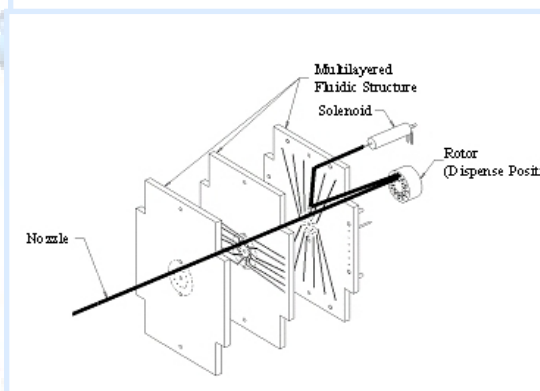
## Results and Discussion

The hybrid valve integrates several functions into a single device. A microfluidic manifold, shown in Figure 1, serves as a stator and allows fluidic connection between sets of components.

Figures 1A and 1B are expanded drawings of the microfluidic structure used for fluid switching and routing: In operation, a stepper motor turns the rotor through the actuator body. The position of the rotor, relative to the manifold (stator), determines the fluid pathway. The fluid pathways for the aspirate and dispense positions are shown in 1A and 1B. The multilayer manifold is used to connect the switching elements to the



**Figure 1A: Fluid Pathway for the Aspirate Position**



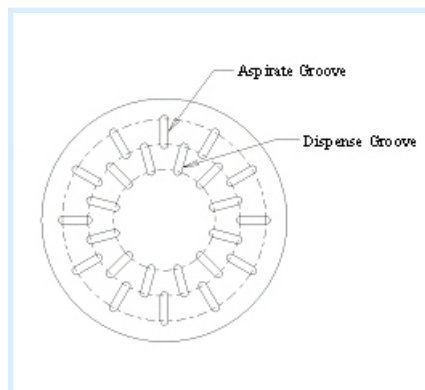
**Figure 1B: Fluid Pathway for the Dispense Position**

components to be switched. The manifold in 1A and 1B is represented by three layers as an exploded assembly. There is another layer, not shown, that converts the hole pattern from a circular arrangement to a linear arrangement. An expanded view of the rotor and grooves that allow the fluid connectivity is shown in 1C.

One set of components comprises high-speed solenoid ink jet valves. These ink jet valves, connected to

a common controlled-pressure source, maintain the flow of a hydraulic fluid such as deionized water and enable the controlled duration of a pressure pulse for ejecting samples. A second set of components is an array of fluidic tubes that are typically connected to either an array of syringe pumps or a selection valve (not shown). Use of a selection valve allows fluidic connection to either a syringe pump, for controlled aspiration or a purge source for washing of the fluidic lines.

In operation, a flat-faced switching valve, created between the rotor (Figure 1c) and stator face, accomplishes fluidic connectivity between a single set of components and an array of nozzles that are used for either aspirating or dispensing samples. The separation of the dispensing channels and positioning of the components are defined by the three-dimensional microfluidic structure that also serves as the valve stator. Finely machined grooves on the rotor



**Figure 1C: Expanded View of the Rotor and Grooves**

complete the fluidic paths and can be repositioned by a stepper motor. Therefore, the nozzles can be connected to either a syringe pump for aspiration or to the ink jets for dispensing. The actuator body applies pressure to the stator face to prevent leakage. These functions are diagrammed in Figure 1. Fluid switching is accomplished rapidly and in parallel for all fluidic channels thus reducing the number of parts and associated costs. Individual control over each channel's dispensed and aspirated volume is possible, allowing for calibration and customization of fluid-handling protocols.

In operation, the hybrid valve structure can be used to aspirate and dispense reagents directly from the array of nozzles. Keeping the dispensing tips proximal to the valve structure creates a compact design that is useful for filling or washing microassay plates. Alternatively, the nozzles can be connected with fluidic tubes for remote mounting of the hybrid valve. Tubing lengths up to two meters have been used with no loss in performance when dispensing volumes greater than 200 nl. This latter configuration is advantageous for gantry-style robotic liquid handlers as it reduces the weight of the robotically positioned arm.

A significant operational advantage of the valve configuration is the removal of the solenoid valves from the sample flow path. This reduces degradation of the seals within the solenoid valve that can be caused by aggressive solvents. Furthermore, it significantly simplifies cleaning and decontamination of the reagent flow paths, as all moving parts reside in hydraulic fluid flow paths. Solvents including dimethylsulfoxide, acetonitrile and methanol have been used successfully. Additionally, various buffer, salt solutions and detergents, including a wide range of weight percents of sodium dodecyl sulfate, Brij, and Tween have been used effectively. The modular design of the hybrid valve can also allow for the switching of components. For example, alternate dispensing actuators, such as piezoelectric pumps, could replace the nozzles. Likewise alternative valves or pumps required for washing procedures or for use with other solvents could be used in place of the syringes.

As with other applications of solenoid jets, a desired dispense volume can be obtained by controlling the pressure or valve opening time [Hicks 2001]. The valve opening time is the easiest to modulate, with changes on the order of 10  $\mu$ s allowing for nanoliter scale resolution in volume. A relationship between valve opening time and the dispensed volume can be seen in Figure 2.

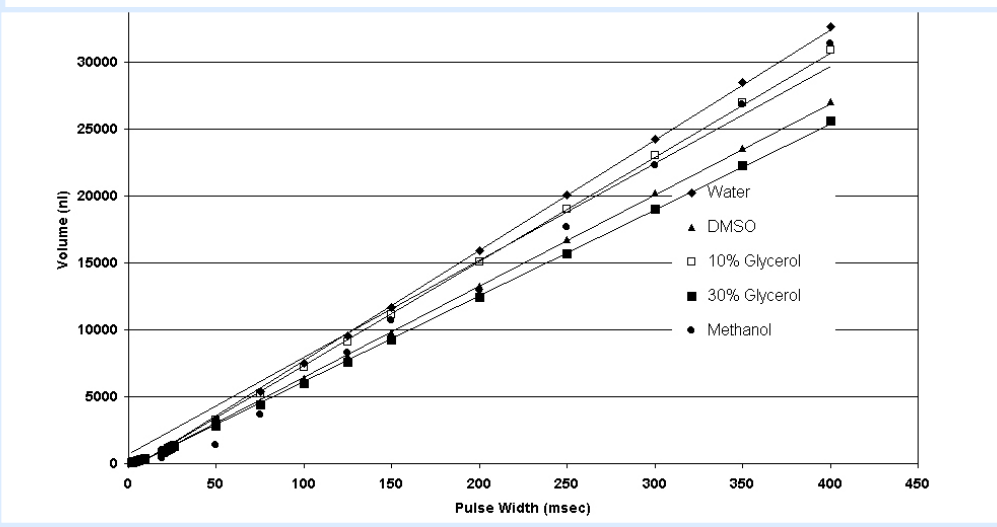
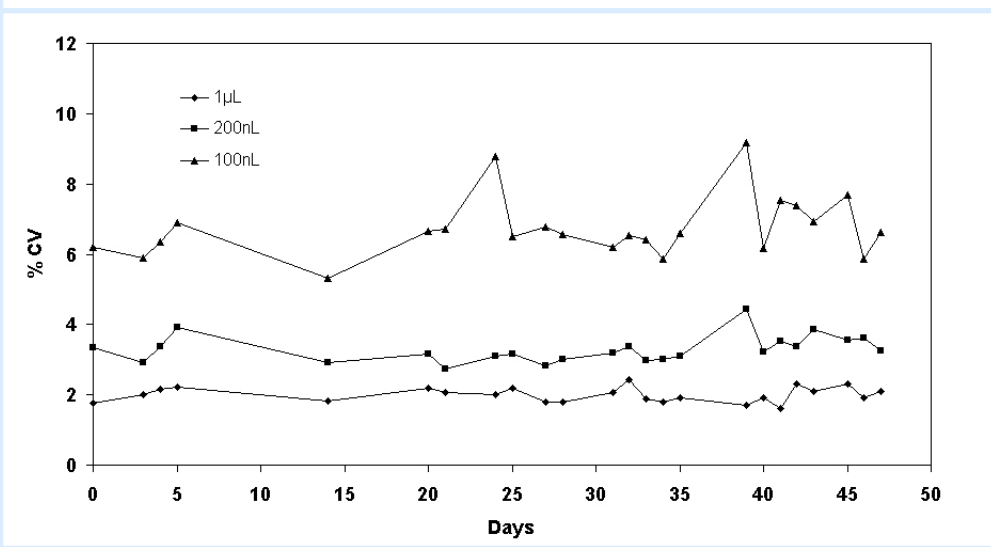


Figure 2: Calibration Curves for Solutions of Various Viscosities

Solutions shown in the figure range from 0.58 cP to 2.4 cP and were determined at 12 psi (8.3 x 10<sup>4</sup> Pa) with a 125 µm orifice nozzle.

For this graph, the dispensed volumes were quantified by gravimetric measurements. This allows for a calibration that relates the valve opening time and the dispensed volume. Changes in pressure and solvent viscosity alter the slope of this relationship and can be easily accounted for. A wide range of viscous solutions such as 30% glycerol and 25% PEG 8000 have been calibrated and dispensed.

The coefficient of variation (CV, CV = standard deviation/average size) of the dispensed volume for a single valve depends on the volume dispensed. For larger volumes (~1 µl), the CV is on the order of 2%, while volumes on the order of 200 nl yield a CV of 3.5%. For smaller volumes, in the range of 100 nl, the CV increases slightly to about 6%. This precision can be optimized by varying the applied system pressure. Further, dispensing performance is maintained over months of constant use (Figure 3).



**Figure 3: Day-to-day Reliability of the Nanodrop at 100 nl, 200 nl, and 1 µL**

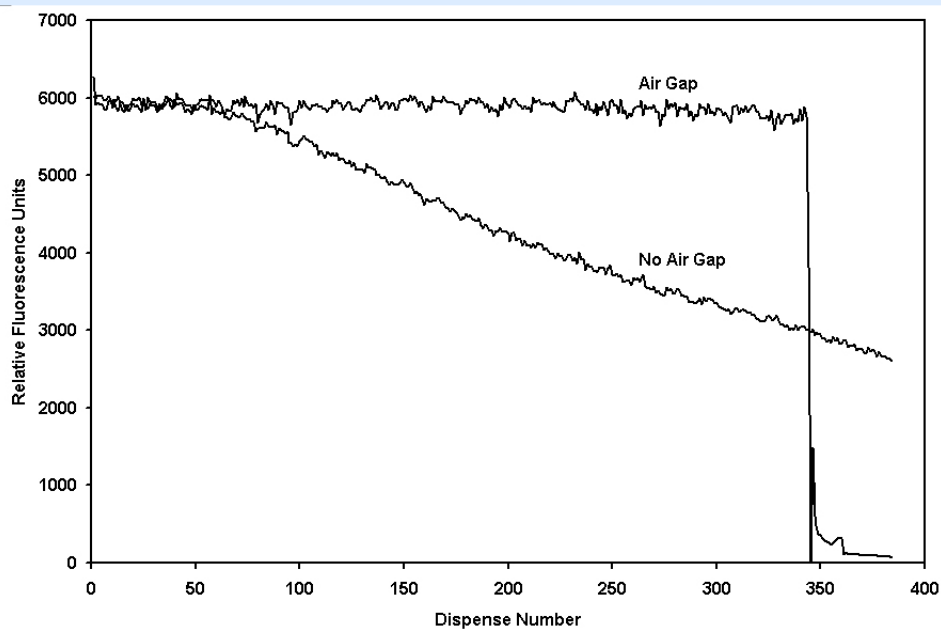
In the figure, coefficients of variation are measured over all eight tips with no individual calibration of solenoid valves. Three plates per day were averaged at 1 µL and 5 plates were averaged at 100 and 200 nl.

These CV values were collected using a nozzle diameter of 125 µm. The use of smaller diameter nozzles can improve the precision of low volume dispenses but at the expense of possible clogging and slower aspiration times. For example, the CV of a 200 nl dispense is increased to 5% when using a 300 µm nozzle. Volumes from nanoliters to microliters can be easily dispensed with barely perceptible changes in dispense time.

Variability in the dispensing volume between different tips is a concern with multi-tip dispensing. The use of different flow paths and ink jet valves can affect the dispensed volume for a given set of conditions (valve opening time, pressure, solution used). Although the performance of an individual tip is highly reproducible the variation in dispensed volume among different tips can increase the overall plate CVs by about 50%. Individual calibration of each valve can improve precision of the dispensed volume and reduce the variability between dispensing tips. Using this technique, the tip-to-tip variability can be

largely eliminated.

A concern related to low volume dispensing is low volume aspiration and efficient use of a sample. Using the hybrid valve structure, aspiration is typically accomplished using a syringe pump. Volume increments on the order of 50 nl can be routinely aspirated. However, the hydraulic fluid that is used to translate the pressure pulse to effect ejection can also cause mixing of the sample with the hydraulic fluid. This mixing can lead to incomplete use of the aspirated sample due to dilution. An example of this effect is shown in Figure 4.



**Figure 4: Reduction of Reagent Dilution by Using Airgaps to Separate the System Fluid from the Aspirated Reagent**

Shown is the relative fluorescence per well over many dispenses when using an airgap compared to a liquid-liquid interface. The aspirate volume is 100  $\mu\text{L}$  and the dispense volume is 300 nl.

More efficient use of the sample can be obtained by placing a micro air gap between the sample and hydraulic fluid (also shown in Fig. 4). This gap prevents diffusive mixing and enables more efficient use of the aspirated sample. The use of a micro air gap has no deleterious effect on aspiration or dispensing volumes greater than 200 nl. The CVs associated with dispense volumes less than 200 nl increased slightly (up to  $\sim 8\%$ ).

Liquid dispensing instruments built with the hybrid valve structure are limited in their dispense rates by the practical limits of the motion-control platforms on which they are integrated and the volumes to be dispensed. Actual firing rates of the micro-solenoids can be as fast as 1200 Hz, but practical limitations for dispensing liquids limits the dispense rate to a more modest nominal 20Hz or less. Other factors include the supply pressure and nozzle orifice size. As the volume to be dispensed increases, the open time on the valve becomes a significant driver for overall cycle time. For example, when operating at 8 psi ( $5.5 \times 10^4$  Pa) with 125  $\mu\text{m}$  nozzles and 1.6 m of tubing, a 100 nl stream can be dispensed in about 2.9 ms while a 1  $\mu\text{l}$  stream is dispensed in 28 ms. Innovadyne's platforms, dispensing in a non-stop mode, typically fill a 384-well plate (48 dispenses/tip, 8-tip system) with 1  $\mu\text{l}$  in about 6 s. The above example shows the disparity between the actual dispensing capabilities ( $\sim 2000$

s-1) and practical dispense speeds (8 s-1). In comparison, conventional syringe-based dispense systems require washing after each reagent addition, to avoid contamination, with a typical cycle time of approximately 16 minutes/plate including the necessary wash times.

The cost benefit of assay miniaturization using this technology was shown in a typical luminescence assay. The assay, which when using traditional technology used 50  $\mu\text{L}$  of total reagent volume, was reduced to 10  $\mu\text{L}$  of total reagent volume resulting in a 5-fold reduction in reagent from the traditional assay. In addition to the cost savings, the whole plate CVs decreased from 10% to 8%, the signal to noise ratio increased from 100 to 183, and the Z-factor (a measure of data quality) increased from 0.68 to 0.77 (data courtesy of Dr. Timothy D. Dawes of the Chiron Corporation).

## Conclusion

The use of the hybrid valve enables the ability to switch modes from aspirate to dispense rapidly and in parallel for all channels. Accomplishing this with a single valve reduces instrumentation costs while providing faster and more reproducible performance. The modular design of the hybrid valve allows for simple changes of components for meeting the needs of different applications. The hybrid valve also allows the remote mounting of the solenoid ink jet valves. This feature prevents contamination of the ink jet with sample, leading to significantly longer valve lifetimes and reduced chances of cross-contamination. Most significantly, the hybrid valve extends sample dispensing down to the nanoliter level with individual control of each dispense tip. Delivery volumes can be as low as 50 nanoliters, with a coefficient of variation of less than 5%. This low-volume dispensing is possible without contact with the target surface, allowing for repeated dispenses without the worry of cross-contamination. Such performance is necessary for matching the research requirements in drug discovery and genomics applications.

## Acknowledgements

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