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## **A Robust Nanolitre Dispensing System Applied to Cell-Based Assays**

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The ability to successfully handle sub-microlitre and nanolitre volumes has been of great importance in the practical operation of cell-based assays. It has been suggested that the dispensing of even picolitre volumes is achievable. It is important, however, to be able to distinguish between what is theoretically achievable and what is practical. Poor judgment, such as an overly optimistic assessment of the capabilities of a delivery system, can be a painful and potentially expensive mistake. This article reviews the evolution of this field that is fundamentally crucial to so many of today's assays.

The limiting factor of traditional liquid handling techniques is the fact that it is a "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, etc. 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 impact on precision and the lower the volume, the larger is the contribution of the variation.

## Solenoid Valve Systems

Successful low-volume pipetting was achieved, however, with solenoid valve systems. The concept of a controlled pressurised source and a rapidly actuated valve was simple and effective. The speed and energy of the fluid displacement produced non-contact dispensing. The surface tension of the liquid was broken as it left the orifice and immediately overcame the variability of the touch-off technique. An added benefit was the speed of dispensing the non-contact technique allowed the delivery nozzle to accurately deliver the droplet above the well and rapidly move to the next. In fact dispensing now became independent of the substrate, eliminating many of the reproducibility problems associated with motion control. Plate processing times fell dramatically for eight channel instruments. It was now possible to deliver to all wells of a 96 well plate in around 5 seconds, 384 wells in around 7 seconds, and 1536 wells in around 14 seconds.

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,

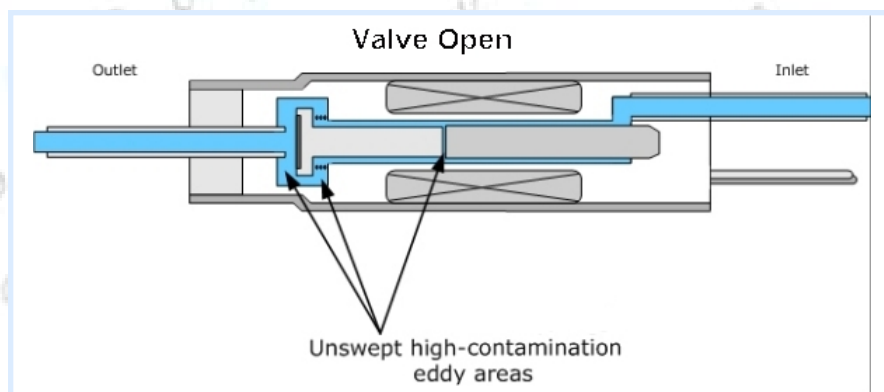
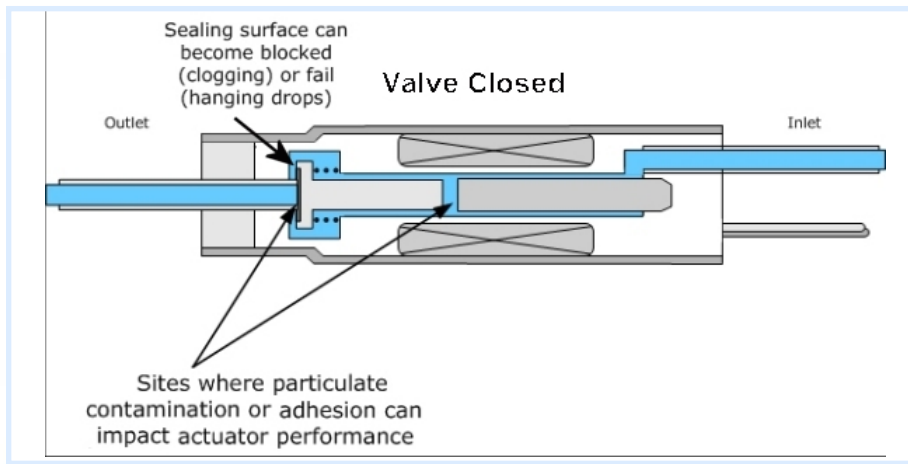


Figure 1A: Microsolenoïd Valves - Problem Areas (Valve Open)



**Figure 1B: Microsolenoïd Valves - Problem Areas (Valve Closed)**

obstructions, and wearing of the seal materials. This 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 (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.

At the outset it was recognised that microtitre plate technology helped to define the requirements of low-volume pipetting. The automated systems that have evolved to process plates better, faster and cheaper have also set new criteria for low volume instrumentation, namely—robustness. The incredibly high duty cycles of HTS and  $\mu$ HTS facilities, often greater than 250, 1536-well plates per day, will expose any fragility in a technique or instrument. The precision of solenoïd based systems has long been recognised but widespread acceptance has been tempered with concern regarding performance or maintenance issues.

### A More Robust System

This concern has been addressed by Innovadyne Technologies, Inc. with the introduction of the latest generation of non-contact pipetting systems. These are based on a technology developed under a collaborative research agreement with the Oak Ridge National Laboratory, the US Department of Energy research laboratory. The systems incorporate a proprietary hybrid valve ASAP architecture separating the sample aspiration path from the solenoïd dispense valves.

By isolating the critical measuring device, the solenoïd, from the sample flow path, the simple design instantly eliminates the risks present in earlier systems and takes a major step forward in terms of robustness. The solenoïd valves are now only exposed to deionised water at a constant pressure, allowing them to operate efficiently and effectively, as they were originally designed. Rather than lasting for only months, solenoïd 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.

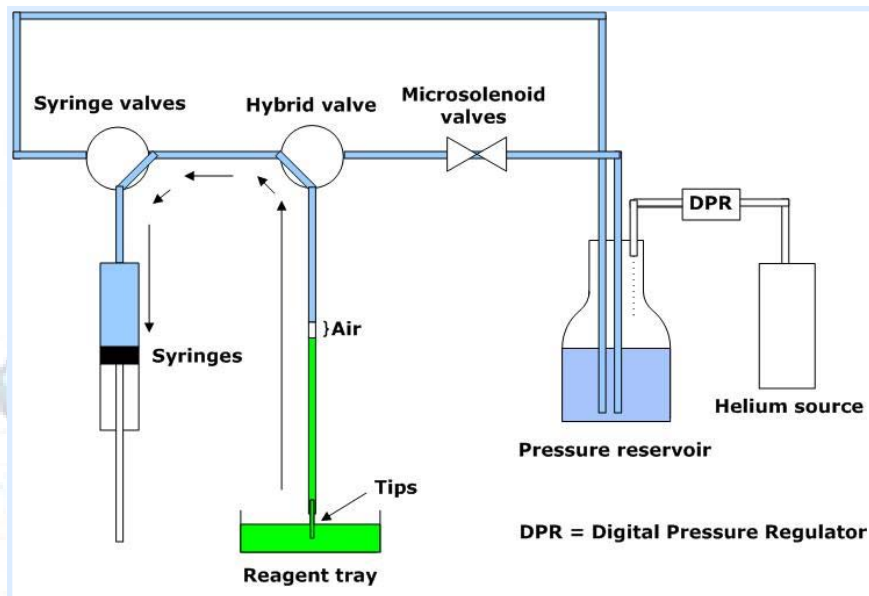


Figure 2A: Schematic of ASAP Technology, Aspirate Step

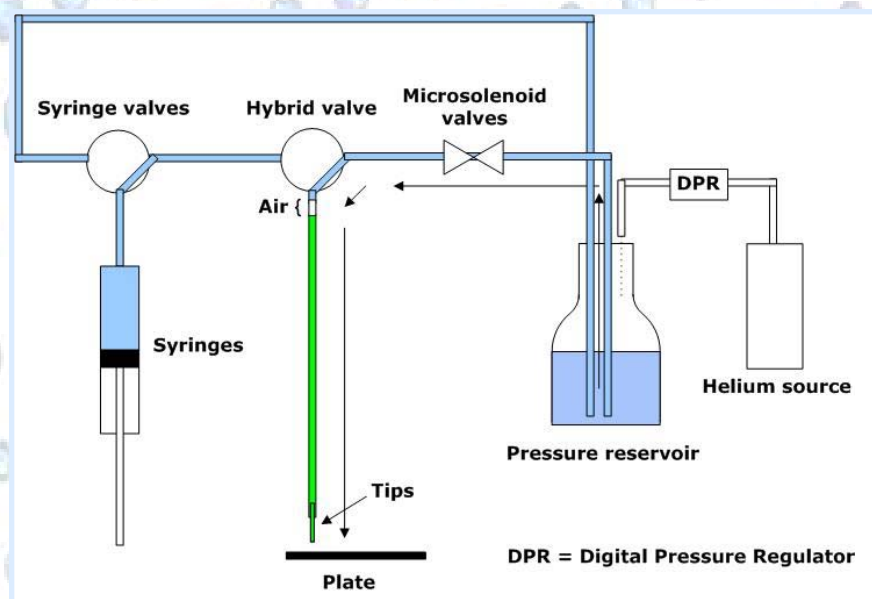


Figure 2A: Schematic of ASAP Technology, Dispense Step

## Cell Based Assay Applications

A good example of the application of this new design has been in the field of cell-based assays. The Nanodrop has been used to dispense many different cell-types but can also be used for measuring cell viability. A key factor in any successful liquid handler in this field, is that the fluidics must be gentle enough not to significantly damage or even kill dispensed cells. Most work to date has been performed using the standard Nanodrop tips. These tips have a tightly controlled "clean" aperture (sapphire insert) that measures 125 $\mu\text{m}$  in diameter. Using relatively low and consistent backpressures of a few psi, the shearing forces on the cells are minimised. Several cell lines recognised as being especially fragile have been successfully dispensed with good viability. Further investigation into the impact of even wider aperture tips is ongoing. Tables 1 and 2 summarise the utility of the Nanodrop for scaling cell viability assays to low volume 384-well plates. The volume dispensed is tabulate in the first column and reagent volumes are scaled from the standard 100 microlitre total volume per well of a 96-well kit.

### Steady-Glo Luciferase Assay System

Dispense volume/(total volume)	%Cv (whole plate)	Mean relative luminescence units (rlu)	Background rlu mean
2.5 $\mu$ L/(5 $\mu$ L)	4.8%	4501	189
1.5 $\mu$ L/(3 $\mu$ L)	3.8%	2674	316
1.0 $\mu$ L/(2 $\mu$ L)	3.4%	2190	285
1.0 $\mu$ L/(2 $\mu$ L)	3.1%	1924	332

Table 1: Steady-Glo Luciferase Assay System using the Nanodrop

### CellTiter-Glo Luminescent Cell Visibility Assay

Dispense volume/(total volume)	%Cv (whole plate)	Mean relative luminescence units (rlu)	Background rlu mean
5.0 $\mu$ L/(10 $\mu$ L)	6.7%	2950	729
2.5 $\mu$ L/(5 $\mu$ L)	7.0%	1991	600
2.0 $\mu$ L/(4 $\mu$ L)	7.5%	1798	671
1.5 $\mu$ L/(3 $\mu$ L)	7.6%	1602	748

Table 2: CellTiter-Glo Luminescent Cell Visibility Assay using the Nanodrop

Note: Assay systems available from ProMega Corporation.