# A Flexible, Multi-Purpose, Multi-Detector HTS Automated Platform

Diana K. Cohen[1], Jim Schools[2], and Paul Held, Ph. D.[3]

[1]Novartis Institute for Biomedical Research, Summit NJ 07901. [2]Hudson Control Group, Springfield NJ 07081. [3]Bio-Tek Instruments Inc. Winooski VT 05404

## ABSTRACT

Decentralized screening groups have increasing needs for improved productivity. However, the cost of "high-end" automation systems are prohibitive except for centralized groups. Thus, there is a need to develop flexible, low-cost systems for assay automation in decentralized screening lab environments.

A flexible, multi-purpose, multi-detector automated system allows for rapid reconfiguration to accommodate various screening technologies. Various combinations of washers, liquid handlers, detectors and other devices can be combined to build an automated system with high capacity. A cylindrical robotic arm allows such a system to be quickly reconfigured for different operations compared to large-scale dedicated linear track robot systems. This system allows the user to make rapid detector substitutions for maximum flexibility.

Flexibility is also required of the pipetting system used on such a platform. Pipetting capabilities, even at low volumes, of a new versatile automated 96/384 well microplate pipetting system is ideal. The small footprint allows for two pipettors to be used in this system providing numerous pipetting capabilities.

## INTRODUCTION

The Hudson Control Group PlateCrane robotic arm provides the capability to configure a lab automation workstation with multiple devices. The combination of liquid handlers and detectors described here can be combined to build an automated system with a high capacity for labware. The PlateCrane allows such a system to be quickly and easily configured and set up for operation to perform fluorescent, fluorescence polarization (FP), fluorescence resonance energy transfer (FRET), time-resolved fluorescent, absorbance, luminescent, and scintillation assays. This system is not limited to what is described here. Any number of other detectors, washers, etc. with readily available interfaces can be quickly configured into the system.

Because of the small volumes associated with the wells of plates, both 96- and 384-well, the ability to dispense small volumes accurately and precisely is necessary to perform most assays. Initially, the performance of the Bio-Tek Precision 2000 Automated Pipetting System was specified at relatively large volumes such as 100µl but its typical performance at much lower volumes is quite remarkable.

## MATERIALS AND METHODS

•Hudson Control Group PlateCrane Pick and Place System to supply microplates from stacks.
•Hudson Control Group Crane Software (based on Hudson Total Control for Windows) to control the PlateCrane, and to act as the supervisory control of all modules in the workstation.
•Bio-Tek Precision 2000 96/384-Well Automated Microplate Pipetting System for liquid pipetting and dispensing with Precision Power Software.
•PE Life Sciences Victor2 V Multilabel Counter with 1420 Workstation Software.
•PE Life Sciences MicroBeta Trilux Multidetector LSC with MicroBeta Windows Workstation Software.
•Molecular Devices Thermomax Absorbance Reader with Softmax Pro Software.
•Pentium Computer to run Crane software. The computer will require one serial port for each module in the workstation.

To determine the Precision 2000 microplate pipetting system's low volume pipetting capabilities, a concentrated dye solution was dispensed into plates using the Precision 2000 in all experiments. In some experiments, blue dye solution in TRIS buffer was dispensed into clean dry plates (either 96-well or 384-well) and colorless TRIS buffer diluent was added in order to obtain adequate absorbance measurements. The resulting absorbance was then read at 595nm using a microplate absorbance reader. In other experiments, a yellow dye solution was added to wells already containing the diluent. In these experiments, both the dye and the colorless buffer also contained 0.1% Tween 20 surfactant. After the addition of the aqueous solutions the absorbance of each well of the plate was determined at 450nm using a microplate absorbance reader. The data from both experiments was then exported to Microsoft Excel and the data reduction was performed.

Accuracy was determined using either a gravimetric method or the dilution of dye. Determinations using the gravimetric method were performed by weighing plates pre-filled with 100µl of PBS. Plates were weighed using an analytical balance. After dispensing the appropriate fluid to the plate using the Precision 2000, the plate was quickly re-weighed. The resultant weight change when divided by the number of plate wells returned an average per well dispense volume. When calculating the accuracy of dispense using the dye method, concentrated yellow dye was pipetted into pre-filled plates either manually or with the Precision 2000. After mixing on an orbital shaker for 60 seconds, the absorbance was read on a microplate spectrophotometer. The resultant absorbance values were compared to a pre-existing calibration curve and the dispense volume interpolated.

## LAYOUT AND OPERATION

The PlateCrane can be installed on virtually any stable laboratory surface. Standard lab benchtops and tables provide sufficiently stable surfaces for reliable operation of the workstation. This provides the following advantages:

• Expensive laser-grade tables are not required to mount the robotic system
• The system can quickly and easily be moved to different locations and still maintain reliable operation
• The end user can reconfigure the system as desired without the need for a factory reinstallation

The system consists of a PlateCrane XL with two 90 degree bases with a total of 8 stacks. Each stack has a capacity of 30 standard 96- or 384-well microplates or 6 boxes of pipet tips. Leaving one stack empty for output, this brings the capacity of the system to 210 microplates or any combination of microplates and tip boxes necessary.

The workstation has flexible liquid handling capability via two Precision 2000 automated pipetting systems. The Precision 2000s are anchored to the PlateCrane base, allowing this assembly to be manually moved without the necessity of reteaching the pipettors' locations to the PlateCrane. As configured in Figure A, the PlateCrane can access 4 of the 6 available locations on one Precision 2000 and 2 of the available 6 locations on the other Precision 2000. The inaccessible Precision 2000 locations are utilized for fixed reagent reservoirs when using 8-channel pipetting of precious reagents versus manifold pipetting.

Three different detectors are available to accommodate a variety of assays. For each operation, the required detector is moved into position next to the PlateCrane, and manually positioned until the plate nest is properly accessed by the PlateCrane gripper. This system allows a detector change to be implemented in less than 15 minutes. The installation surface can me marked with outlines of the devices to make manual positioning even easier. Positional marking can also be outlined with high visibility tape or small brackets installed on the work surface.

The system is operated by Crane software based on Hudson's Total Control for Windows (TCW) software, which provides control of the PlateCrane and supervisory control of the Precision 2000s and each detector. Each detector has a software interface installed into the Crane software. Multiple methods controlling different devices can be stored in the Crane software. After physically positioning the desired detector into the workstation, the appropriate method is selected and run.

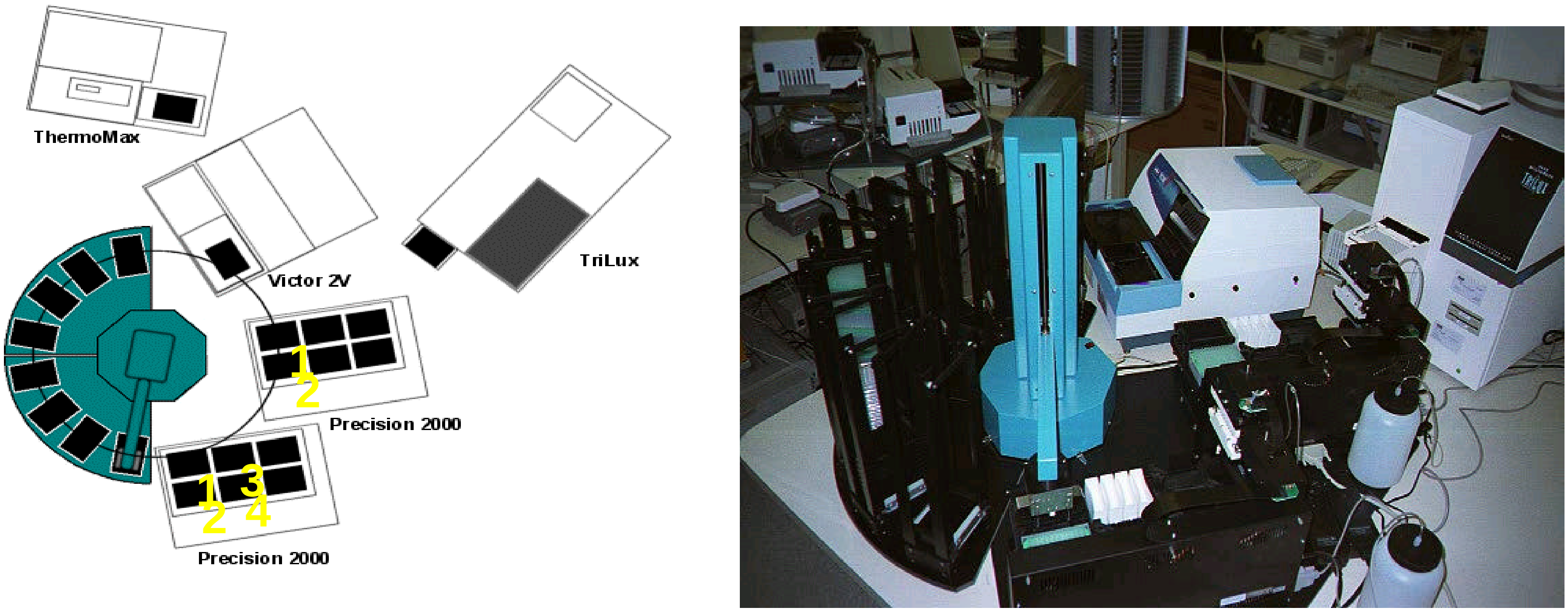


Figure A: Bench layout of PlateCrane XL, dual Precision 2000 pipetting systems, and three interchangeable detectors.

## RESULTS OF PRECISION 2000 LOW VOLUME PIPETTING

As demonstrated in Figure 1, when aqueous solutions were dispensed into dry plates, large well-to-well differences were observed with volumes less than 3µl. The coefficient of variance (%CV) for a plate with a 1µl dispense was found to be as great as 60%. However, the well-to-well differences quickly diminish with increasing dispense volume. Dispense volumes as small as 3µl have a %CV less than 4%. When small volumes of liquid are dispensed into 384-well plates, a similar pattern of well-to-well variation is observed (Figure 2). When dispensing into dry 384-well plates, very large differences between wells are observed at 1-2µl. However, dispense volume greater than 3µl demonstrate excellent results.

As demonstrated in Figure 3, the Precision 2000's rapid dispense mode option enables precise delivery of fluids as well. Using either a 96- or a 384-well plate, volumes as small as 10µl can be dispensed from the bulk reagent dispense manifold. When dispensing 10µl into a plate well, it appears that the 384-well plate offers better precision. However, at volumes of 20µl or greater, there appears to be little difference between the different plate matrices (Figure 3).

When small aqueous volumes are dispensed into partially filled plate wells, a marked improvement in the precision is observed (Figure 4.) Volumes as small as 1µl can be dispensed with a %CV less than 4%. With dispense volumes of 3µl per well or greater, the %CV is routinely less than 2%. The pattern of increased precision with dispense volumes of 3µl or greater is similar in nature as observed with the dispense into dry wells. However, the degree of improvement is lessened as results of the vastly improved results at 1-2µl dispense volumes (2-3.5% vs. 30-60 %CVs).

The accuracy of the Precision 2000 was tested using two different methods. Using a gravimetric method where a plate is weighed before and after a dispense cycle, the Precision 2000 generally dispenses slightly less than the indicated volume. As demonstrated in Table 1, there is a discrepancy between the expected volume and the calculated volume at all of the volumes tested. While the dispense at 2µl was approximately 25% less than expected, the degree of accuracy improved with a dispense at 20µl to being only 5% less than expected. Although the values were lower than expected, they were very consistent from plate to plate. This indicates that the discrepancy can be corrected to some extent by programming a slightly higher dispense volume.

As seen in Figure 6, the dispense volume is linear when one compares the resultant absorbance to the programmed dispense volume.

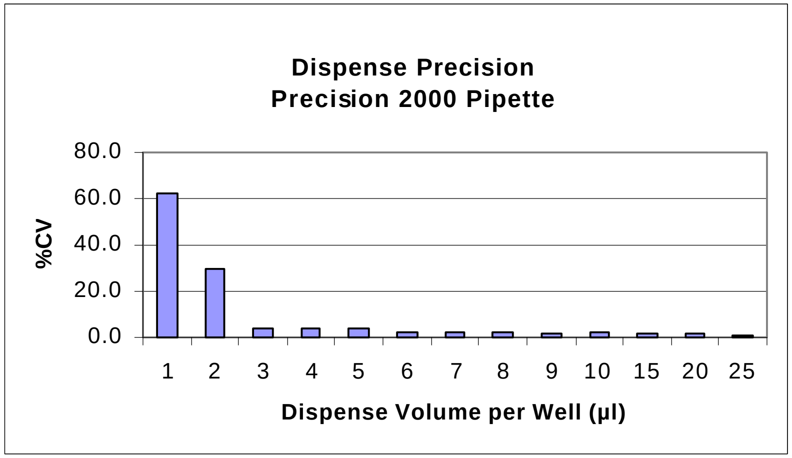


Figure 1. Dispense precision into dry 96-well plates using the Precision 2000 pipette at various dispense volumes. The indicated volume of TRIS buffer containing blue food coloring was dispensed to all the wells of a 96-well plate using the pipette. After dispensing the colored dye, 100µl of TRIS buffer was dispensed into each well using the dispense manifold at a speed setting of 5. The absorbance at 595nm for each well of every plate was determined using a microplate absorbance reader. Note that each data bar represents the %CV of an entire plate.

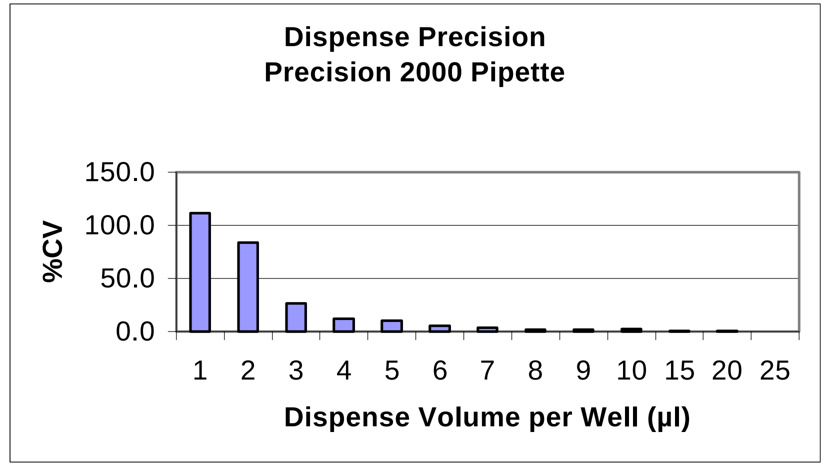


Figure 2. Dispense precision into dry 384-well plates using the Precision 2000 pipette at various dispense volumes. The indicated volume of TRIS buffer containing blue food coloring was dispensed to all the wells of a 384-well plate using the pipette. After dispensing the colored dye, 50µl of TRIS buffer was dispensed into each well using the dispense manifold at a speed setting of 5. The absorbance at 595nm for each well of every plate was determined using a microplate absorbance reader. Note that each data bar represents the %CV of an entire plate.

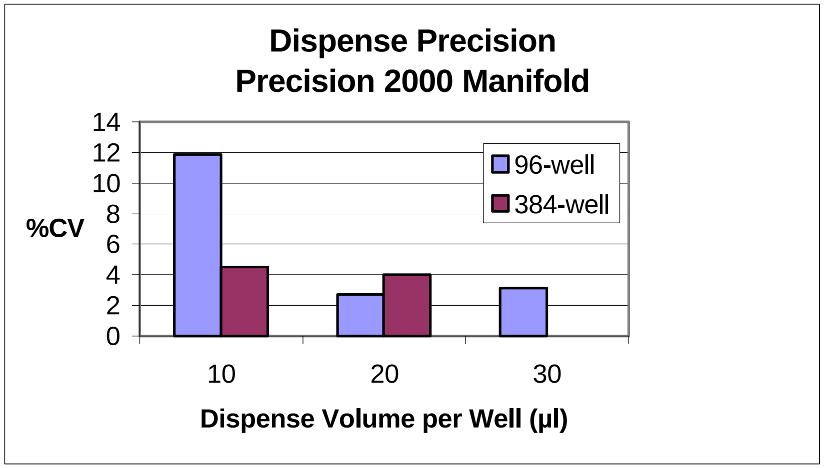


Figure 3. Dispense precision into dry 96- or 384-well plates using the Precision 2000 manifold at various dispense volumes. The indicated volume of TRIS buffer containing blue food coloring was dispensed to all the wells of either a 96- or a 384-well plate using the manifold. After dispensing the colored dye, the buffer bottle was changed to TRIS buffer only and either 100µl or 50µl of TRIS buffer was dispensed into each well of the 96-or 384-well plates respectively using the manifold at a speed setting of 5. The absorbance at 595nm for each well of every plate was determined using a microplate absorbance reader. Note that each data bar represents the %CV of an entire plate.

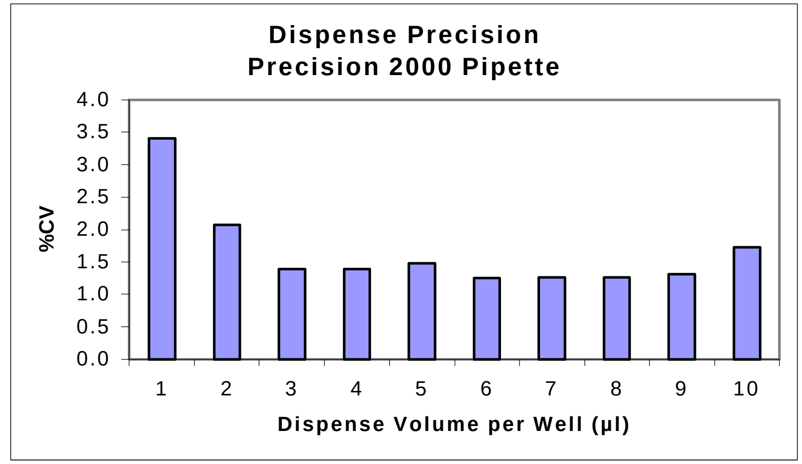


Figure 4. Dispense precision into partially filled 96-well plates using the Precision 2000 pipette at various dispense volumes. Into each well of a plate, 100µl of diluent was dispensed using the pipette. Next, the indicated volume of diluent containing yellow dye was dispensed to all the wells of a 96-well plate using the pipette. The absorbance at 450nm for each well of every plate was determined using a microplate absorbance reader. Note that each data bar represents the %CV of an entire plate.

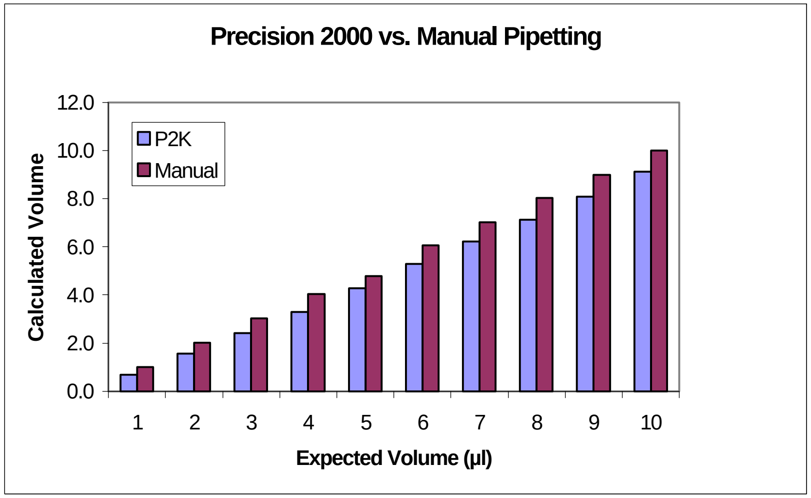


Figure 5. Comparison of dispense accuracy between the Precision 2000 and manual pipetting. The indicated volumes of yellow dye solution were pipetted into plate wells partially filled with 100µl of diluent. Absorbance values were then interpolated against a previously prepared standard curve. The manual data represents the mean of eight determinations, while the Precision 2000 data represents the mean of 96 wells.

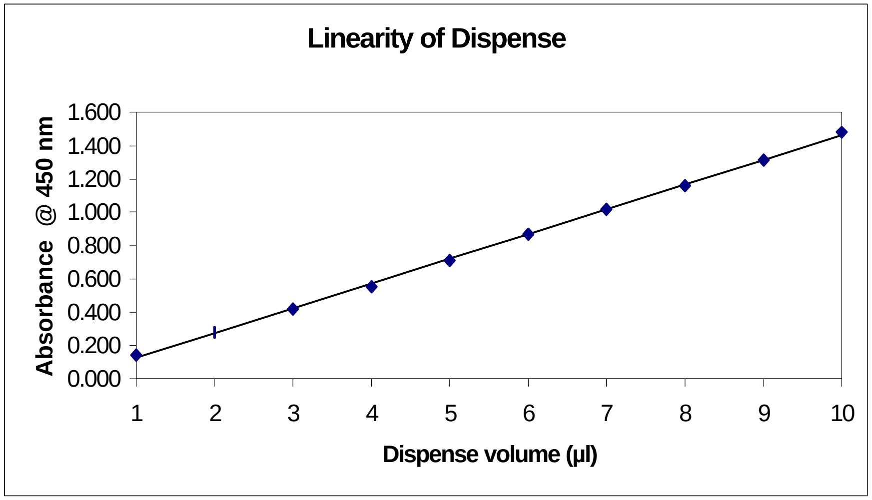


Figure 6. Linearity of dispense using the Precision 2000 pipette. Using the pipette, 100µl of diluent was pipetted into each well of a plate. After the addition of diluent, the indicated volumes of yellow dye was dispensed into each well of the plate again using the pipette. In order to mix the dye, the plate was placed on an orbital shaker for 60 seconds, after which the absorbance at 450nm was determined using a microplate absorbance reader. Data points at each volume were plotted (diamonds) along with the linear regression (line).

Dispense Accuracy Determined by Gravimetric Measurements

| Expected Volume (μl) | Calculated Volume (μl)[#] | %CV |
|---|---|---|
| 2 | 1.479±0.0009 | 0.609 |
| 5 | 4.324±0.017 | 0.398 |
| 10 | 9.144±0.062 | 0.683 |
| 20 | 18.870±0.014 | 0.075 |

Table 1. Dispense accuracy into 96-well plates. Plates were pre-filled with 100μl of liquid and weighed. After the Precision 2000 had dispensed the indicated volume of fluid into each well of the 96-well plate, the plate was then re-weighed. The volume of each well was calculated by dividing the change in weight from dispensing liquid by the total number of wells.

**DISCUSSION**

This data indicates that the Precision 2000 can be used to dispense small aqueous volumes precisely and accurately. While dispense accuracy deviates slightly from expected values, it does so in a consistent fashion. This allows the user to change the programmed aspiration and dispense volumes and compensate for the discrepancy.

Several factors influence the ability to dispense small volumes with the Precision 2000. The positive displacement syringes depend on the displacement of air to push the fluid out of the barrel of the tip. Unfortunately, gaseous materials are prone to compression and accuracy at large volumes (e.g. 100µl). This often precludes the ability to be accurate at distinctly smaller volumes. Because air is also prone to expansion and contraction as a result of temperature changes, having samples and reagents equilibrated to ambient temperature will result in more accurate and precise dispensing as well. For more accurate and precise dispenses, it is advisable to include an initial pre-pickup of a small volume. This will provide some compensation for air gas compression, as well as provide a humid environment inside the pipette tip.

Dispensing into a liquid generally is more accurate and precise than dispensing into an empty well. This phenomenon is most likely due to small amount of the fluids wicking onto the outside of the pipette tip and not being dispensed into the well. When dispensing into a pre-existing liquid, that fluid is more likely to disperse into the fluid than onto the tip as a result of surface tension.

The manifold used in Precision 2000's rapid dispense mode option is also quite useful for low volume dispenses. This bulk reagent dispense manifold has the advantage of being very rapid and is quite useful when dispensing down to as low as 10µl per well. The caveat being that the manifold requires much greater amounts of fluid, as the bi-directional pump and tubing need to be completely filled with fluid prior to any being dispensed. The pump and tubing combine to hold about 20ml of fluid and best results have been observed when the system is primed with 25ml of fluid. The manifold also has the limitation of having only one reservoir, whereas the pipette head can pick up out of any number of reagent vessels and does so with disposable tips. Aside from this, the manifold allows for a quick dispense to a full 96-well plate in less than 20 seconds and a full 384-well plate in less than 50 seconds.

Up to six plates can be filled at one time on Precision 2000's customizable work surface. Easily removable furniture allows for various setup options. With a PlateCrane robotic arm, the number of processed plates is virtually unlimited. As demonstrated, the Precision 2000 is an accurate and precise liquid handling system capable of automating most routine pipetting, dispensing, and diluting applications.

## CONCLUSIONS

- The capability of this system illustrates how a single PlateCrane can provide a laboratory automation solution that is not only very simple to implement, but also a very cost-effective approach. In a case where there are a variety of processes to be accomplished, but each is not constantly required, it is much more cost-effective to build such a re-configurable system versus multiple dedicated systems that require more hardware and valuable benchspace.

- This system will allow for any number of fluorescent, fluorescence polarization (FP), fluorescence resonance energy transfer (FRET), time-resolved fluorescent, absorbance, luminescent, and scintillation assays. Assays can be performed in 96- or 384-well plates and assay volumes can be as low as 25ul in 96 well plates and 10ul in 384 well plates.

- The Precision 2000 automated microplate pipetting system is an accurate and precise liquid handling system capable of automating most routine pipetting, dispensing, and diluting applications. Ideal for a flexible and versatile automated system with its small footprint, it has proven to be very capable of low volume pipetting (1µl) to accommodate today's low assay volumes.

- The flexibility and mobility of this system also allows for any instrument or pipettor to be accessed manually for assay development allowing for direct transfer of any assay into HTS format with little assay re-optimization.

## REFERENCES


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