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Application Note

Automation of Routine Microplate Pipetting and Dispensing

Quantitative assays of all sorts are common to many applications in basic science and clinical research. Most of these assays require the movement, either addition or subtraction, of liquid reagents. Historically these assays were performed in test tubes. However, regardless of the assay method employed, laboratories requiring high throughput have often adapted the described protocol to a 96-well, and more recently, a 384-well format. Despite the adaptation, processing large numbers of samples generally represents a large investment in manpower, with automation primarily taking place at the endpoint of reading the plate. The standardized matrix and spacing of the wells in microplates, 8 x 12 and 16 x 24, for 96- and 384-well microplates, respectively, has allowed the use of manual multichannel pipettes to pick up and dispense liquids when these microplates are employed. While these devices have certainly made pipetting fluids into these arrays much easier, they still require a high degree of manual manipulation. With today's throughput requirements, this still represents a very tedious and time-consuming task. Towards that end, BioTek Instruments (Winooski, VT) has developed the Precision 2000™ Automated Pipetting System to eliminate the need for manual pipetting into these plate formats. Although the performance of the Precision 2000 Automated Pipetting System has only been specified at volumes near 100 µl, its typical performance at much lower volumes is quite remarkable. In this monograph we will describe the dispense accuracy, linearity, and precision of the Precision 2000 at low volumes.

Figure 1. Precision 2000 Automated Pipetter with Rapid Dispense Manifold and Aerosol Hood

The Precision 2000 has a completely configurable six-station platform to hold the required pipette tips, reagent troughs, and microplates (96- and 384-well) for fluid transfer (Figure 1). The platform is removable, allowing for multi-user friendliness, easy cleaning, and setup of the instrument. The 8-channel pipette arm moves up and down as well as side to side, while the platform moves front to back to provide complete access to all locations on the work platform and complete configurability. The pipette arm uses a proprietary technology to reliably pick up and seal any standard tip with individual, free-floating barrels that compensate for tips out of position. An optional rapid dispense 8-channel manifold, which uses a precise bi-directional syringe pump to accurately and rapidly dispense fluids from a large unpressurized reservoir, is also available.

The Precision 2000 has a built in microprocessor that controls all movements. The flexible onboard software, which provides complete programming for the most complex fluid transfers, can store up to 80 programmed assays. For more complete automation robotics, interfaces can be developed using ActiveX® software commands. The Precision 2000's small size, with a 15 x 21-inch footprint and a height of 16 inches, allows it to be used almost anywhere including most biological safety cabinets or chemical fume hoods. Also available is an optional aerosol cover for use on the laboratory bench.

In all experiments, a concentrated dye solution was dispensed into microplates using the Precision 2000. In some experiments, blue dye solution was dispensed into clean dry plates (either 96- or 384-well) and colorless TRIS buffer diluent was added. The resulting absorbance was then read at 595 nm using a microplate absorbance reader. In other experiments, QC check solution #1 (BioTek Instruments), which contains FD&C Yellow #5 dye, 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 450 nm using an ELx800™ microplate absorbance reader (BioTek Instruments). The data from both experiments was then exported to Microsoft® Excel and the data reduction was performed.

Dispense 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 100 µl of phosphate buffered saline (PBS) using a Sartorius A 120S 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 with the Precision 2000. After mixing on an orbital shaker for 60 seconds, the absorbance was read on a PowerWaveX™ microplate spectrophotometer (BioTek Instruments). The resultant absorbance values were compared to a pre-existing calibration curve and the dispense volume interpolated.

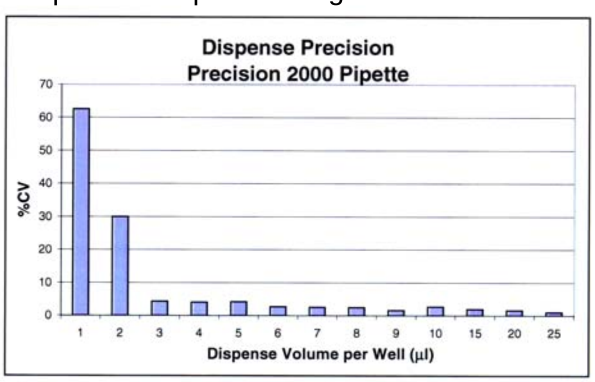

Figure 2. Dispense precision into dry 96-well plates using the Precision 2000 pipette at various dispense volumes.

When aqueous solutions were dispensed into dry plates, large well-to-well differences were observed with volumes less than 3 µl (Figure 2). The coefficient of variance (%CV) for a plate with a 1 µl dispense was found to be as great as 60%. However, the well-to-well differences quickly diminished with increasing dispense volume. Dispense volumes as small as 3 µl had a %CV less than 4%. When small volumes of liquid were dispensed into 384-well plates, a similar pattern of well-to-well variation was observed (Figure 3), with very large differences between wells are observed at 1-2µl. However, dispense volumes greater than 3µl demonstrate excellent results. When small aqueous volumes are dispensed into partially filled plate wells, a marked improvement in the precision was observed (Figure 4.) Volumes as small as 1 µl were dispensed with a coefficient of variance less than 4%. With dispense volumes of 3 µl per well or greater, the %CV was routinely less than 2%. The pattern of increased precision with dispense volumes of 3 µl or greater was similar to that observed when dispensing into dry wells. However, the degree of improvement was lessened due to the vastly improved results at 1-2 µl dispense volumes (2-3.5% vs. 30-60 %CVs).

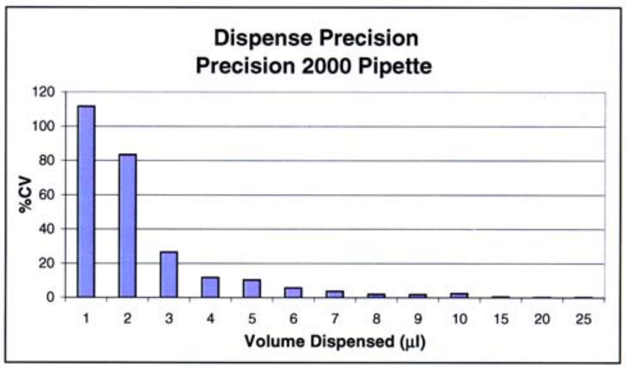

Figure 3. Dispense precision into dry 384-well plates using the Precision 2000 pipette at various dispense volumes.

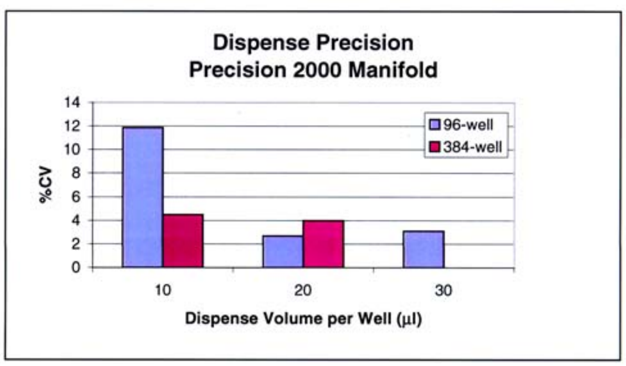

Figure 4. Dispense precision into dry 96- or 384-well plates using the Precision 2000 rapid dispense manifold at various dispense volumes.

The Precision 2000's Rapid Dispense Manifold option enables precise delivery of fluids as well. This bulk reagent dispense manifold has the advantage of being very rapid and is quite useful with dispense volumes as low as 10 µl per well into both 96- and 384-well plates. There is a caveat: being that the manifold requires much greater amounts of fluid than the pipette, 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 20 ml of fluid, and best results have been observed when the system is primed with 25 ml 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. When dispensing 10 l into a plate well, it appears that the 384-well plate offers better precision. However, at volumes of 20 l or greater, there appears to be little difference between the different plate matrices (Figure 5).

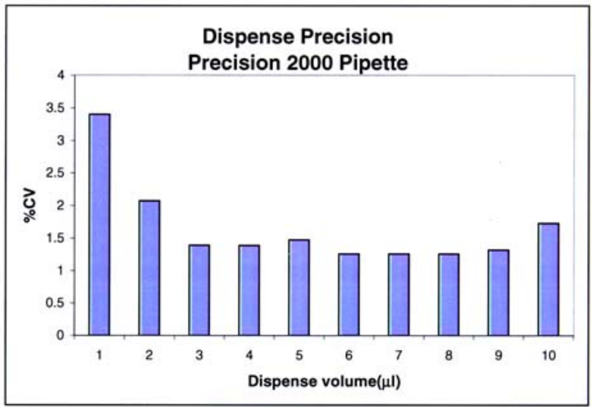

Figure 5. Dispense precision into partially filled 96-well plates using the Precision 2000 pipette at various dispense volumes.

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 2 l was approximately 25% less than expected, the degree of accuracy improved with a dispense at 20 l 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. These data indicate 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.

Table 1. Dispense accuracy into 96-well plates.

Dispense Accuracy Determined by Gravimetric Measurements

Expected Volume (l)	Calculated Volume (l)#	%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

Note that these data represent the mean and standard deviation of six determinations

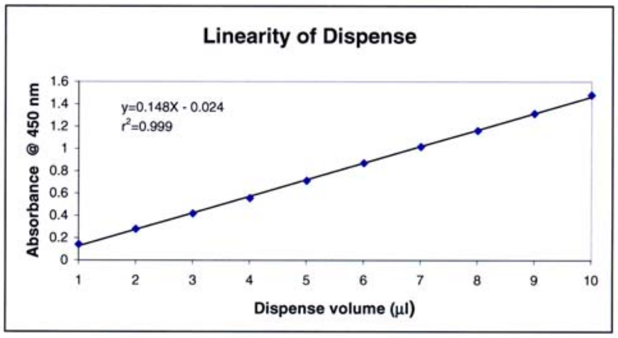

Figure 6. Linearity of dispense using the Precision 2000 pipette.

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, because gaseous materials are prone to compression, accuracy at large dispense volumes (e.g., 100 l) is accomplished in part, by filling the pipette tip with fluid and replacing the compressable air. This often precludes the ability to be accurate at distinctly smaller volumes. Because air is also prone to expansion and contraction because 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 of liquid. This will provide some reduction in the air (gas) compression, and, 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 amounts 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 because of surface tension.

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

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