Using the Artel PCS® to Calibrate the Beckman Coulter Biomek 3000

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ABSTRACT:

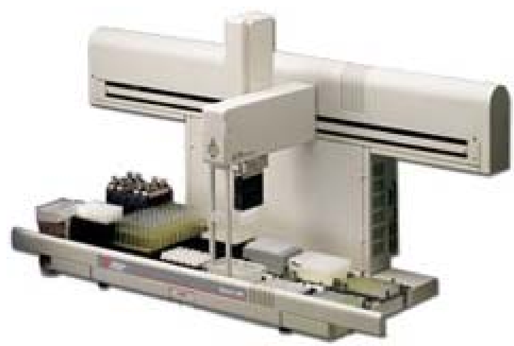
Pipetting tasks are the backbone of countless assay procedures and ensuring the accuracy and precision of dispensed volumes is paramount to the integrity of assay data. The purpose of this application note is to show how the Artel PCS® Pipette Calibration System can be used to test the performance of a Beckman Coulter Biomek® 3000 Laboratory Automation Workstation's pipetting tools.

INTRODUCTION:

Ensuring that pipetting devices (both manual and automated) are performing properly is often a regulatory requirement. Many of those labs that are not required to prove such documentation will proactively encourage that a quality assurance program exists and is followed within their labs as pipetting errors can lead to costly amounts of rework and damage reputations.

The Artel PCS is a universal system designed for measuring the volumes dispensed from a single channel pipetting device. It is commonly used to quickly and reliably generate accuracy and precision data of volumes dispensed from handheld pipettes. The PCS is not only used to verify the performance of the pipetting device, but has proven to be a powerful l tool for teaching proper pipetting technique. The system uses Ratiometric Photometry™ - a dual-dye, dual-wavelength absorbance method - to determine the volume of liquid dispensed in a single measurement. Measurement data generated using the PCS are traceable to the International System of Units (SI) enabling instruments and methods to be standardized regardless of location.

However, many automated systems are not compatible with a single-channel system like the PCS and for this reason the Artel MVS® Multichannel Verification System was developed. The MVS also employs Ratiometric Photometry, but in this case the 'sample cuvette' is a SBS-format 96 or 384 well microwell plate. This enables the system to accommodate any dispensing instrument that is capable of dispensing into a micro well plate.



The Biomek 3000 is an automated liquid handler that incorporates various pipetting tools for common pipetting tasks, including single- and multi-channel pipetting heads.

Many labs opt for a single channel instrument as they transition to the world of laboratory automation. Therefore the question of whether the PCS could be used to verify the performance of a Biomek 3000 equipped with a single channel head presented an interesting concept to investigate as there are many laboratories that already own a PCS that are on the cusp of making the transition to automate some of their work.

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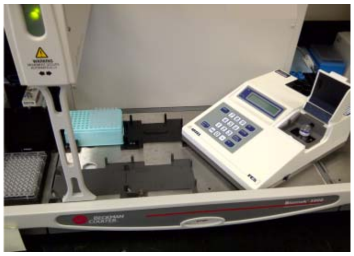
MATERIALS:

- Artel PCS
- PCS Standard Reagent Kit
- PCS Instrument Calibrator Kit
- Biomek 3000
- Biomek P200L Tool
- Biomek P250 Tips
- Biomek modular reservoir

PROCEDURE:



The PCS was placed directly onto the deck of the Biomek 3000 at an angle to allow optimal clearance for access by the robot. A 'labware' description of the PCS was created within the Biomek software. This accounted for the position of the vial in the PCS and the height of the vial (which was approximately 100 mm). The Biomek method was written so that a few pause steps were incorporated to allow the manual lifting and closing of the PCS lid as well as ensuring that the tip was centered over the PCS vial. The dispensing tip was then moved vertically down to approximately 30 mm below the top of the vial prior to delivering the Sample Solution. Other than the positioning parameters, the default water liquid class parameters were used as part of a single aspirate, single dispense protocol.

During the calibration of manual pipettes using the PCS, it is strongly advised that the sample is dispensed into the vial using a 'forward mode pipetting technique' and a tip-touch which ensures that the entire volume of sample is dispensed.

Due to the nature of the automated liquid handler, the tip-touch is not possible, and so a 'non-contact' dispense had to be utilized to deliver the target volume into the vial while the tip was approximately 15 mm above the liquid surface. Care had to be taken to ensure that the delivery of the target volume in "mid-air" was efficient and that the entire volume was dispensed from the tip.

One drawback of the approach described above is that it limits the usefulness of the optimization steps described herein to non-contact dispensing protocols. To demonstrate the applicability of this approach, three different optimization experiments were devised.

The first experiment used a P200L tool equipped with P250 tips to test a range of volumes using default dispensing parameters with a calibration scaling factor and offset of 1 and 0 respectively. Six different volumes spanning the volume capacity of the P200L tool were tested. Five measurements were taken at each volume. Once the dispensed volumes had been measured, the data were used to generate new calibration parameters to fine-tune the accuracy and precision. The scaling factor and offset, which can be defined by the user within the Biomek software, represent the slope and Y-intercept values, respectively, for the equation of a straight line. The target volumes (or points on the line) are the values for a linear standard curve. The curve is then used to calculate a new scaling factor and offset. The accuracy and precision of the volumes delivered by the Biomek 3000 were then retested.

A similar procedure was followed for Experiment 2, during which the instrument was optimized for dispensing volumes of 10 µL, 5 µL and 2 µL.

During Experiment 3, a similar procedure was again followed, but this time the instrument was only optimized to deliver 10 µL. To perform this optimization a target volume of 10 µL was delivered using the standard scaling factor and offset values. Once this had been completed the accuracy could be tuned in by adjusting the offset accordingly.

The need for such limited optimization occurs when there is a critical transfer step within an automated method and the goal is to have a very accurate volume delivery for a specific target volume.

RESULTS:

The results of the first experiment measured six volumes in the range of 200 µL to 5 µL and show that optimization of the scaling factor and offset improved the pipetting performance in the range from 200 µL to 25 µL, where the relative inaccuracy is improved to less than 3% and the imprecision improved to less than 1.5% CV, as shown in Table 1 and Figure 1. The data presented in Table 1 and Figure 1 also demonstrate that the calibration loses effectiveness below a volume of 25 µL.

Often times, a different calibration value is employed for lower volume ranges to improve the pipetting performance. Therefore, a second experiment was performed at the lower end of the volume range for the P200L tool using P250 tips. The same default technique was used with scaling factor 1 and offset of 0 to calculate a new standard curve for this specific volume range.

For this test, three different volumes were tested with at least five measurements per volume.

New calibration values were generated and the performance at the target volumes was retested. The data are shown in Table 2 and Figure 2, and it should be noted that although the accuracy was significantly improved, imprecision also increased. It should also be noted that it is not standard practice to pipette such small volumes with such a large tip, which may explain the increased imprecision.

In order to further improve the low volume accuracy and precision of the Biomek instrument a final test was conducted to optimize the system to deliver 10 µL. Data using the default technique, scaling factor and offset were collected for a target volume of 10 µL and used to calculate the offset needed for that specific volume.

The optimized instrument was then set to dispense 10 µL. It delivered volumes with a mean of 9.975 µL and imprecision of 0.35% CV which is highly accurate considering the volume is at the lower limit for the tip size selected (P200L tool with a P250 tip), see Table 3.

CONCLUSION:

The data presented herein show that a PCS incorporated onto the bed of a Biomek 3000 automated liquid handler can be used to assess the accuracy and precision of volumes dispensed by the robot.

Furthermore, this data can then be used to generate a calibration curve that can significantly improve pipetting performance.

The data also serve to tell a cautionary tale about trusting calibration curves over a full range of a pipetting tool's range. While a calibration curve may be sufficient to produce accurate and precise dispenses at one specific range of volumes, that calibration curve may not hold for another part of the range and it is advisable to develop calibration curves for the specific ranges being used.

It is important to note that the optimization process described herein will only be valid for non-contact dispenses and should not be used with other dispensing methods such as a wet or dry-dispense with a tip touch at the bottom of a microwell.

Test Volume (µL)	Mean (µL)	Relative Inaccuracy (%)	Imprecision (%CV)	Calibration Parameters
200	191.9	-4.06	0.3	Scaling Factor and Offset = 1 and 0
100	94.61	-5.39	0.55	Scaling Factor and Offset = 1 and 0
50	45.53	-8.95	1.14	Scaling Factor and Offset = 1 and 0
25	22.3	-10.79	1.81	Scaling Factor and Offset = 1 and 0
15	12.92	-13.88	2.75	Scaling Factor and Offset = 1 and 0
5	3.89	-22.2	2.43	Scaling Factor and Offset = 1 and 0
200	200.4	0.21	0.18	Scaling Factor and Offset = 1.032 and 2.213
100	99.03	-0.97	0.6	Scaling Factor and Offset = 1.032 and 2.213
50	48.64	-2.71	0.58	Scaling Factor and Offset = 1.032 and 2.213
25	25	0	1.43	Scaling Factor and Offset = 1.032 and 2.213
15	15.49	3.28	2.62	Scaling Factor and Offset = 1.032 and 2.213
5	5.969	19.37	4.82	Scaling Factor and Offset = 1.032 and 2.213

Table 1. Pipetting performance data for volumes delivered by the Biomek 3000 using a P200L tool with P250 tips.

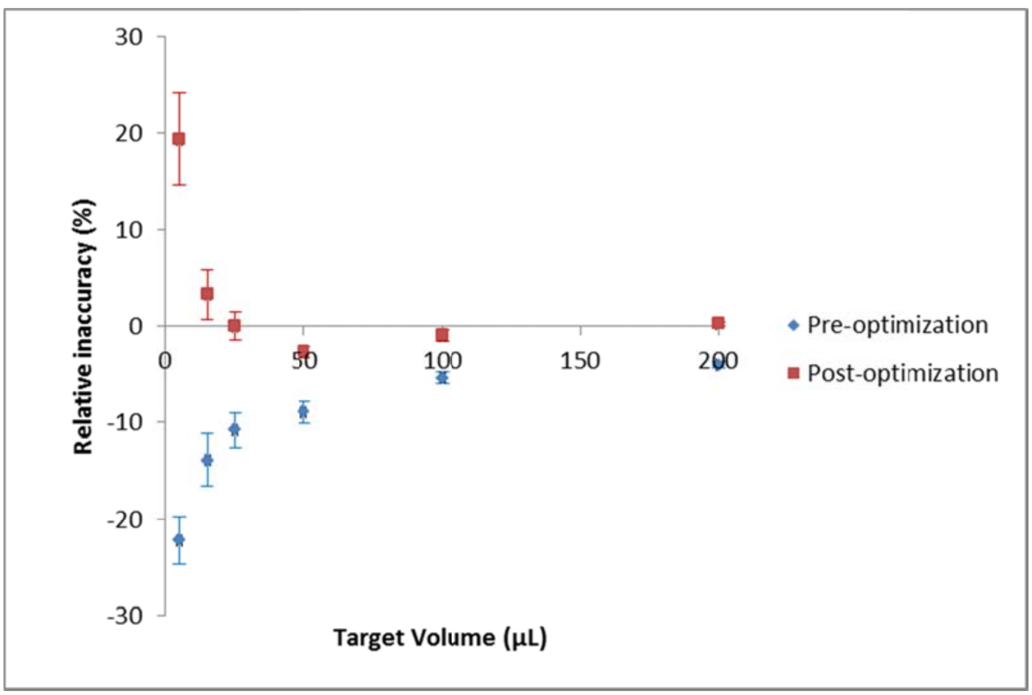


Figure 1. Pipetting performance data for volumes delivered by the Biomek 3000 using a P200L tool with P250 tips.

Test Volume (µL)	Mean (µL)	Relative Inaccuracy (%)	Imprecision (%CV)	Calibration Parameters
10	8.795	-12.05	0.75	Scaling Factor and Offset = 1 and 0
5	3.74	-25.2	6.72	Scaling Factor and Offset = 1 and 0
2	1.596	-20.19	10.33	Scaling Factor and Offset = 1 and 0
10	9.837	-1.63	2.9	Scaling Factor and Offset = 1.092 and 0.545
5	4.862	-2.75	8.63	Scaling Factor and Offset = 1.092 and 0.545
2	1.868	-6.61	15.95	Scaling Factor and Offset = 1.092 and 0.545

Table 2. Pipetting performance data for 2, 5, and 10 µL delivered by the Biomek 3000 using a P200L tool with P250 tips.

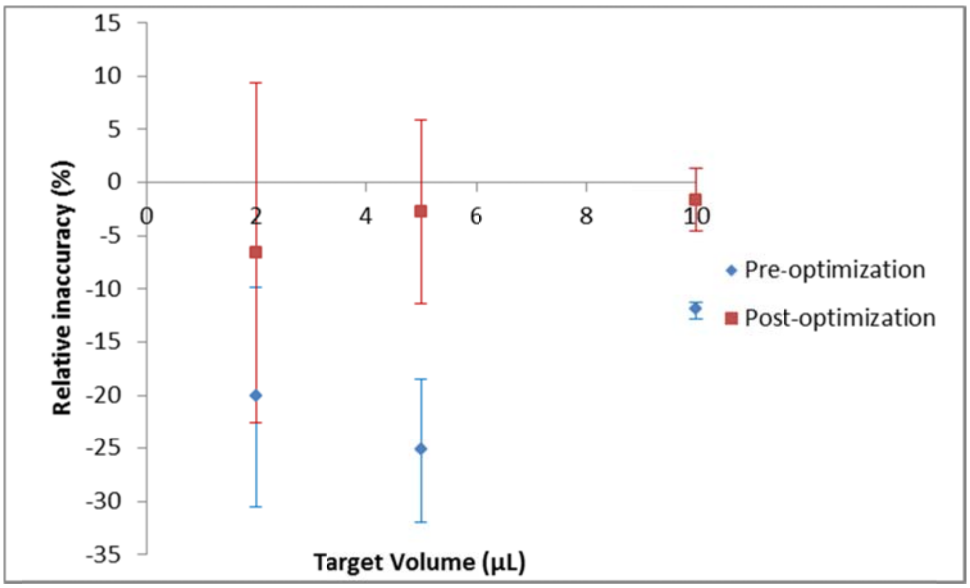


Figure 2. Pipetting performance data for 2, 5, and 10 uL delivered by the Biomek 3000 using a P200L tool with P250 tips.

Test Volume (µL)	Mean (µL)	Relative Inaccuracy (%)	Imprecision (%CV)	Calibration Parameters
10	9.975	-0.25	0.21	Scaling Factor and Offset = 1 and 1.2

Table 3 Pipetting performance data for a series of 10 µL dispenses delivered by the Biomek 3000 using a P200L tool with P250 tips.