

*BioRobotics***BIOMEK® FX: MICROLITER AND SUB-MICROLITER DRY-Well PIPETTING WITH THE BIOMEK FX 384-CHANNEL DISPOSABLE TIP HEAD**

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Introduction

The Biomek® FX, the newest liquid handler offered by Beckman Coulter, Inc., was introduced in 2000 with 96-channel pipetting. In 2001, the 384-Channel Disposable Tip Head and the Span-8 Pipettor were added to the system. The pressures of time and reagent cost reduction necessitate the miniaturization of assays to the higher-density formats like 384- and 1536-well. The new 384-Channel Disposable Tip Head extends the capabilities in applications like plate replication and high-throughput screening to include higher density formats such as 384-well and 1536-well assays. This multichannel dispense head has the capability to dispense into 384-well plates in a single step and 1536-well plates with the optional Positive Positioning ALP. What makes the Biomek FX Liquid Handling System unique is its versatility and expandability to meet the ever-changing needs in Drug Discovery. The Biomek FX offers complete solutions for a variety of specific applications including nucleic acid preparation, hit picking, and quantitation and normalization, as well as the aforementioned applications. Utilizing single- or dual-bridge systems configured with various pipetting heads and on-deck devices provides the user with both flexibility and throughput.

This Technical Information Bulletin provides details that characterize microliter and sub-microliter dry-spotting pipetting performance of the Biomek FX 384-Channel Disposable Tip Head utilizing DMSO to mimic plate replication and HTS applications. This multichannel head was designed to use AP384 P30 disposable tips that employ the industry standard of 4.5-mm center spacing within

the tip rack for use with 384-well and 1536-well microplates. A separate bulletin will cover wet-well pipetting with the 384-channel head and 1536-well pipetting.

Manufacturing Criteria

Beckman Coulter has established a set of specifications that each head must meet in a manufacturing test prior to shipment. For the Biomek FX 384-Channel Disposable Tip Head, the criteria are a dispense of 30 μL into a wet well at <2.00% CV, 5 μL at <3.00% CV, and 1 μL at <5.00% CV. The tests are performed as follows: Water is dispensed into three dry 384-well microplates so that total volume



Figure 1. The Biomek FX liquid handler equipped with a 384-Channel Disposable Tip Head and a Span-8 Pipettor.

in the well is 90 μL after the sample volume is dispensed. The sample volume (30, 5, or 1 μL) of Eosin Y dye solution being tested is dispensed into three 384-well microplates, mixed with the tips, and read on the microplate reader. The percent CV of each plate must fall below the current specification to qualify.

Testing Beyond the Specifications

It is understood that the Biomek[®] FX is used in the laboratory to pipette a wide variety of liquids at low volumes, far too many to include in the manufacturing test. Thus, the experiments described in this bulletin were carried out to characterize the performance of the systems under assay conditions used in the research laboratory.

The study conducted tested the DMSO dry-spotting capabilities at 1 μL , 0.5 μL , and 0.25 μL . This evaluation was performed on a dual-bridge Biomek FX equipped with the 384-Channel Disposable Tip Head and a Span-8 Pipettor. Biomek AP384 P30 tips were used in this study. Each volume was tested using three different 384-Channel Disposable Tip Heads.

Solutions and Techniques Used

A Biomek FX method (Figure 2) was created that dispenses Eosin Y, disodium salt (Sigma, cat. no. E-6003) solutions into 384-well flat-bottom microplates (Greiner cat. no. 781 101). Eosin Y dye stock solutions were made by adding varying quantities of Eosin Y to DMSO to obtain readings of approximately 1.0-1.5 absorbance at 510 nm for each volume pipetted. For each stock solution, the results of three plates from three instruments, nine plates total, were averaged at each volume.

A custom pipetting template (Figure 3) was created that employed a dispense of the sample volume and 5 μL of the blowout volume at a position that was 50% from the center of the well at 90 degrees. The head was moved to a position that was 50% from the center on the opposite side of the well, 270 degrees, to dispense the balance, 20 μL , of the blowout volume. While these blowout volumes may seem excessive, it was found that they were necessary to completely dispense the microliter and sub-microliter volumes accurately. Individual calibration numbers for Scaling Factor and Offset were used in the custom techniques to adjust the accuracy to meet

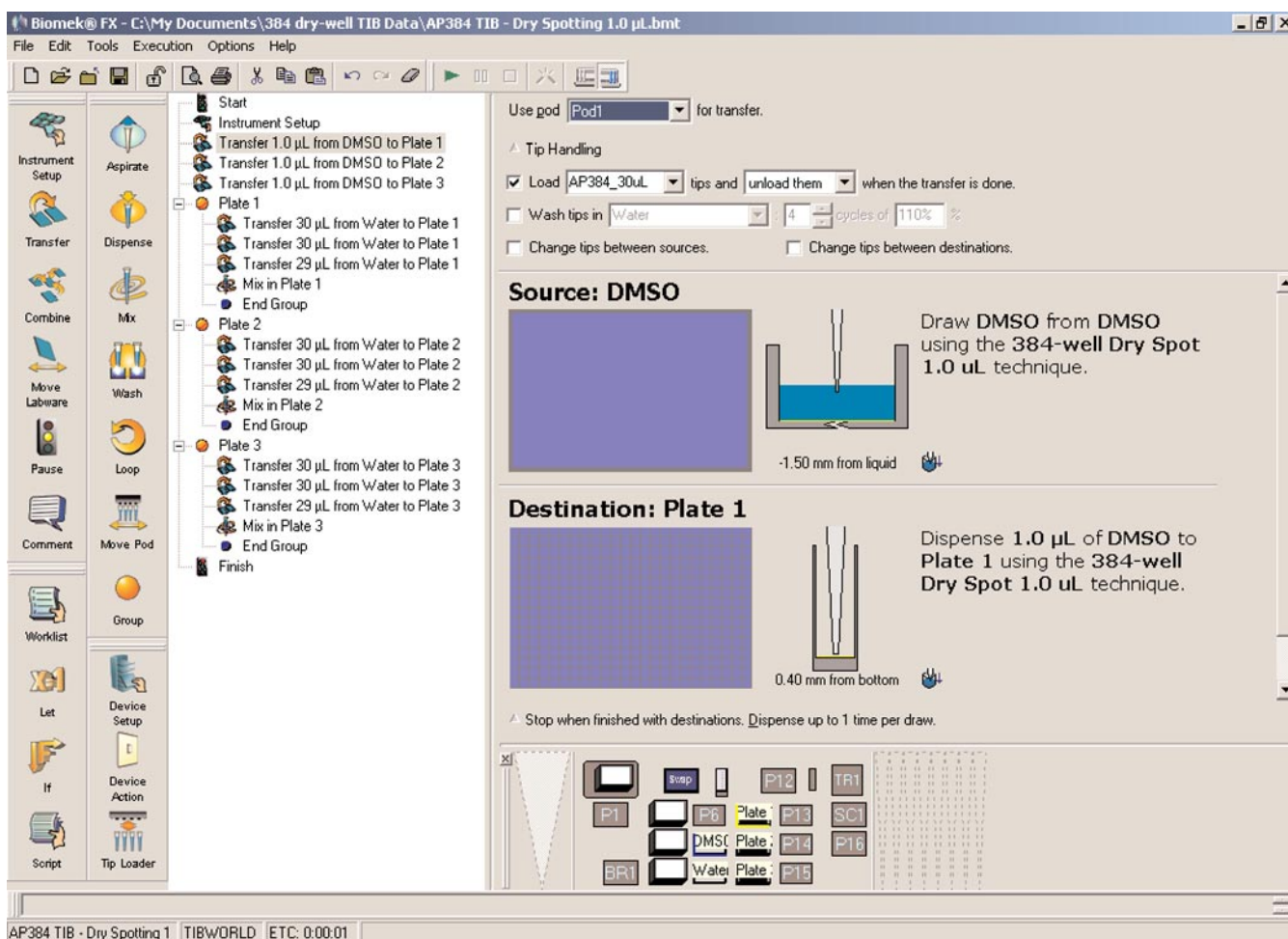


Figure 2. The Biomek FX method used for dry spotting with DMSO.

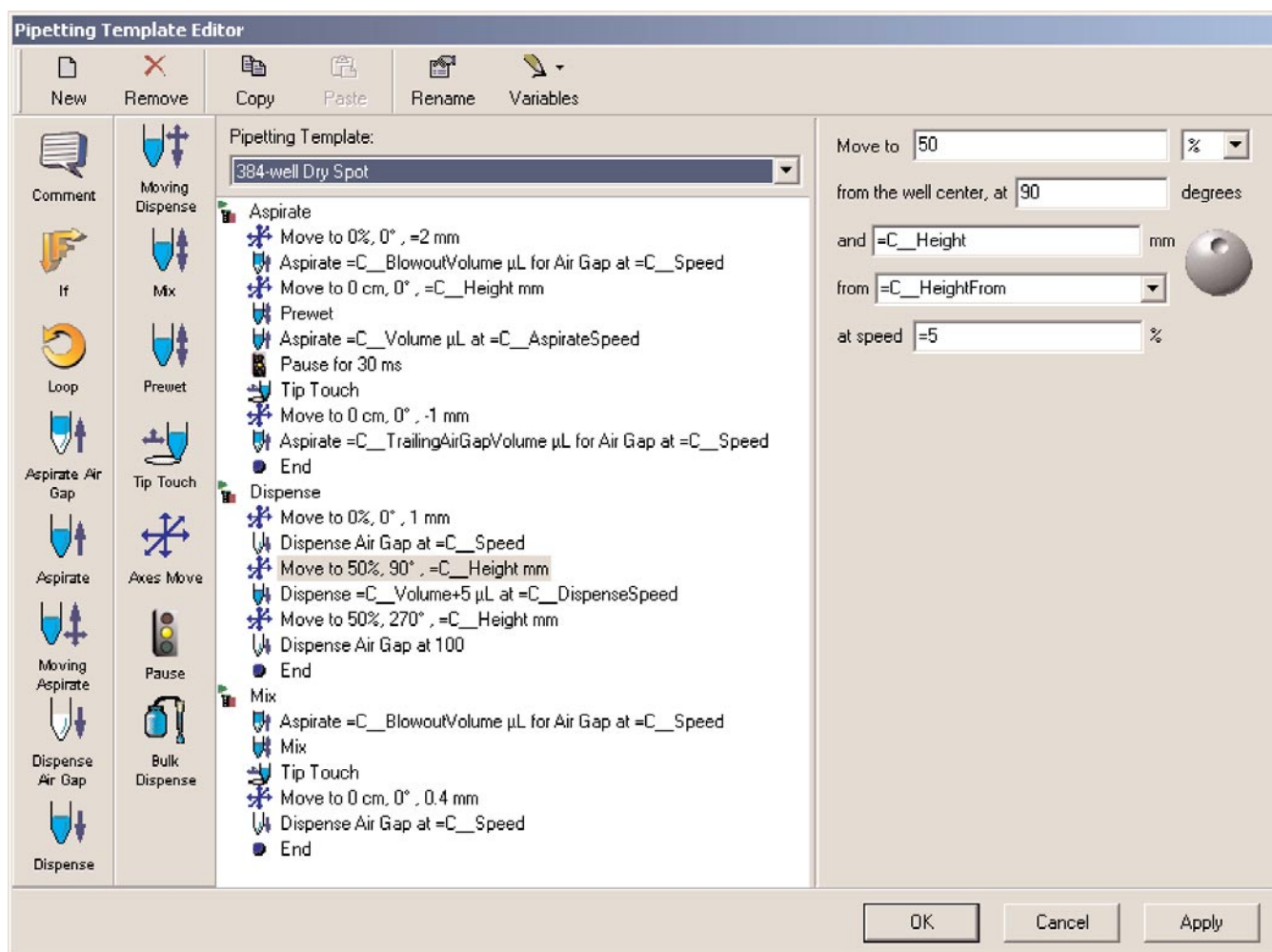


Figure 3. Custom pipetting template created for dry spotting with DMSO.

a standard of less than $\pm 10\%$ difference from the desired dispense volumes. The calibration numbers were determined and accuracy requirements were achieved by the methods described in a separate Technical Information Bulletin, “Improving Accuracy by Use of Technique Calibration”⁽¹⁾.

Following the sample dispense into the dry plate, the total volume of each well in the microplates was normalized to 90 μL with distilled water. For example, the 1.0 μL assay was performed by dispensing 1.0 μL of DMSO with Eosin Y to each of three empty 384-well flat-bottom microplates. Distilled water, 89 μL , was added with the 384-Channel Disposable Tip Head to each plate, and the contents of the well were mixed. The plates then were transported manually to the SAGIAN™ Plate Shaker for a two-minute shake, then taken to the Molecular Devices SPECTRAMax® PLUS³⁸⁴ plate reader for an immediate read at 510 nm and 650 nm (to account for background).

The Biomek® FX method incorporated the following parameters in the technique for DMSO dry spotting:

- Speed within the well: 100% (set in the custom Pipetting Template to 5%)
- Aspiration Height: -1.5 mm from Liquid
- Dispense Height: 0.4 mm from Bottom
- Aspiration
 - Trailing Airgap: 0 μL
 - Delay: 5 ms
 - Speed: 5%
- Blowout
 - Volume: 25 μL
 - Delay: 10 ms
- Dispense
 - Delay: 0 ms
 - Speed: 100%
- Do not follow liquid level when aspirating or dispensing liquid
- No Tiptouch on aspiration or dispense
- No Prewet (except for 0.25 μL dispenses)
- No Mix prior to aspirating liquid
- Do not aspirate a trailing airgap after leaving liquid
- Blowout all leading airgaps
- No mix after dispensing liquid

The Results of This Study

A total of three experiments were run on each 384-Channel Disposable Tip Head, one at each volume. Three individual 384-Channel Disposable Tip Heads were used in this study. The values reported in Table 1 represent the average % C.V. for nine plates, three plates dispensed with each multichannel head at each volume.

Table 1: Results of DMSO Microliter and Sub-Microliter Dry-Spotting Experiments

Volume	%CV
1.0 µL	1.71%
0.5 µL	3.18%
0.25 µL	5.48%

Conclusion

This bulletin shows the characteristic pipetting performance of the Biomek® FX 384-Channel Disposable Tip Head performing a typical laboratory application. End-user results will likely vary when using reagents and labware other than those described. However, the Biomek FX software is rich in pipetting control features that should enable most laboratories to obtain similar results for their respective application.

References

1. Improving Accuracy by Use of Technique Calibration. *Technical Information Bulletin T-1915A*, Beckman Coulter, Inc. (2001)

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