# More Efficiency with Equivalent Data: MFI 5200 Comparison to DPA 4200

## Introduction

We all know that monitoring and characterizing visible and subvisible particles is a critical part of protein therapeutic drug development since these aggregates can compromise the efficacy and safety of a drug product. That's why Micro-Flow Imaging (MFI) is now the go-to application for analyzing 1– 300 µm particles![1] Because you can quantitate and characterize the size and morphology of a wide range of particle contaminants, MFI gives you the information you need to address regulatory guidelines.

Since the technology has been around for a while now, we thought you might want to know how the latest MFI system stacks up to its predecessor, the Dynamic Particle Analyzer (DPA). As a full-on DPA replacement, MFI systems cut the hands-on time for sample analysis without losing any of the DPA functionality and performance you've come to count on. So you get the same great data—only sooner, with the added bonus of freeing up way more FTE time.

## What Makes MFI Better?

MFI systems use the same optical system, camera, and flow cells as DPA systems do so you can keep the same methods you use today. We've just packed in some great enhancements that'll up your overall experience (**Figure 1**). Here are a few highlights:

- **Focus your flow cell with 5x less hands-on time.** The fully automated stage eliminates the need to manually perform the flow cell focus sweep (**Table 1**).
- **Increase your throughput.** When you add a Bot1 autosampler to a MFI system, you'll up your throughput by 5x (**Table 1**)[2].
- **Save time with the latest software features.** MFI can use MVSS 4.0 so you'll get automated protocol creation and instantaneous multi-sample analysis. That'll cut the hands-on time needed to acquire data for 80 samples down to just 1 hour[3].
- **Get the same high-quality data.** A comparison between one DPA 4200 and four MFI 5200 systems (**Figure 2**) shows the data across all five are equivalent.


**FIGURE 1.** MFI 5200 with the stirrer motor and pump integrated.

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| | DPA | MFI |
|---|---|---|
| Flow Cell Focus | 25 minutes | 5 minutes |
| Data Acquisition of 80 Samples | 12 hours (manual with MVSS 3.3) | 2.5 hours (Bot1 with MVSS 3.3) |
| Data Acquisition of 80 Samples | 12 hours (manual with MVSS 3.3) | 1 hour (Bot1 with MVSS 4.0) |

**TABLE 1.** Comparison of the hands-on time in front of the instrument required for MFI versus DPA.

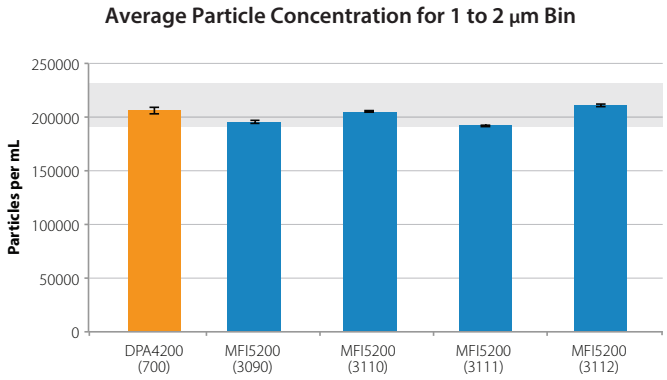



**FIGURE 2.** Concentration measured on the DPA 4200 and MFI 5200 using an internal standard that replicates protein aggregates. Error bars represent the standard deviation and the shaded region represents ±10% of the average particle concentration measured on the DPA 4200. Samples were run in triplicate on the MFI 5200 and 12 sample replicates were run on the DPA 4200.

While the comparison we'll discuss here shows the equivalency of the DPA 4200 and MFI 5200, enhancements made to the MFI 5100 mirror those on the 5200. So you'll get equivalent data between the DPA 4100 and MFI 5100 as well.

To demonstrate how the two systems compare when running samples that are more relevant and meaningful to you, we ran samples side-by-side. Read on to see how polystyrene COUNT-CAL standard beads and protein samples compare.

## Materials and Methods

### SETTING UP THE DPA 4200 AND MFI 5200

We set up a DPA 4200 connected to a Master Flex L/S Pump (Model 7550-50) and a Precision Stir Controller (ProteinSimple PN 4012-001-001) next to a MFI 5200 without the Bot1. Both instruments were run using MVSS 3.3.

Before running samples, we calibrated the pump and focused the same flow cell on each instrument during the initial flow cell installation, a process that is more streamlined on the 5200 thanks to the automated stage. This established the optimal focus position we used for all subsequent runs. Finally, we performed a size and concentration verification using 10 µm DUKE size standard beads and 5 µm COUNT-CAL™ Count Precision beads, respectively.

### ANALYZING SAMPLES USING THE SAME CONDITIONS ON BOTH INSTRUMENTS

Because it was important to avoid introducing sample order bias, we ran one sample on both instruments before moving on to the next. This meant running, for example, the 5 µm beads on one instrument, installing the flow cell onto the second instrument and then running the 5 µm beads on the second instrument before moving on to the 10 µm beads. Between each run, we flushed the flow cell with 0.2 µm filtered Milli-Q® water until the baseline particle count was ≤20 particles per mL to confirm we had eliminated any carry-over. This series was repeated until all the samples described in the next section were analyzed.

### RUNNING COUNT-CAL BEADS AND PROTEIN SAMPLES

#### Sample Types

We ran an assortment of COUNT-CAL Count Precision Standards on both systems to confirm similar behavior of the beads on both systems (**Table 2**).

| BEAD SIZE | PROTEINSIMPLE PN |
|---|---|
| 5 µm | 4004-003-002 |
| 10 µm | 4004-012-001 |
| 15 µm | 4004-013-001 |
| 25 µm | 4004-014-001 |
| 50 µm | 4004-015-001 |

**TABLE 2.** COUNT-CAL beads used to compare the DPA 4200 with the MFI 5200.

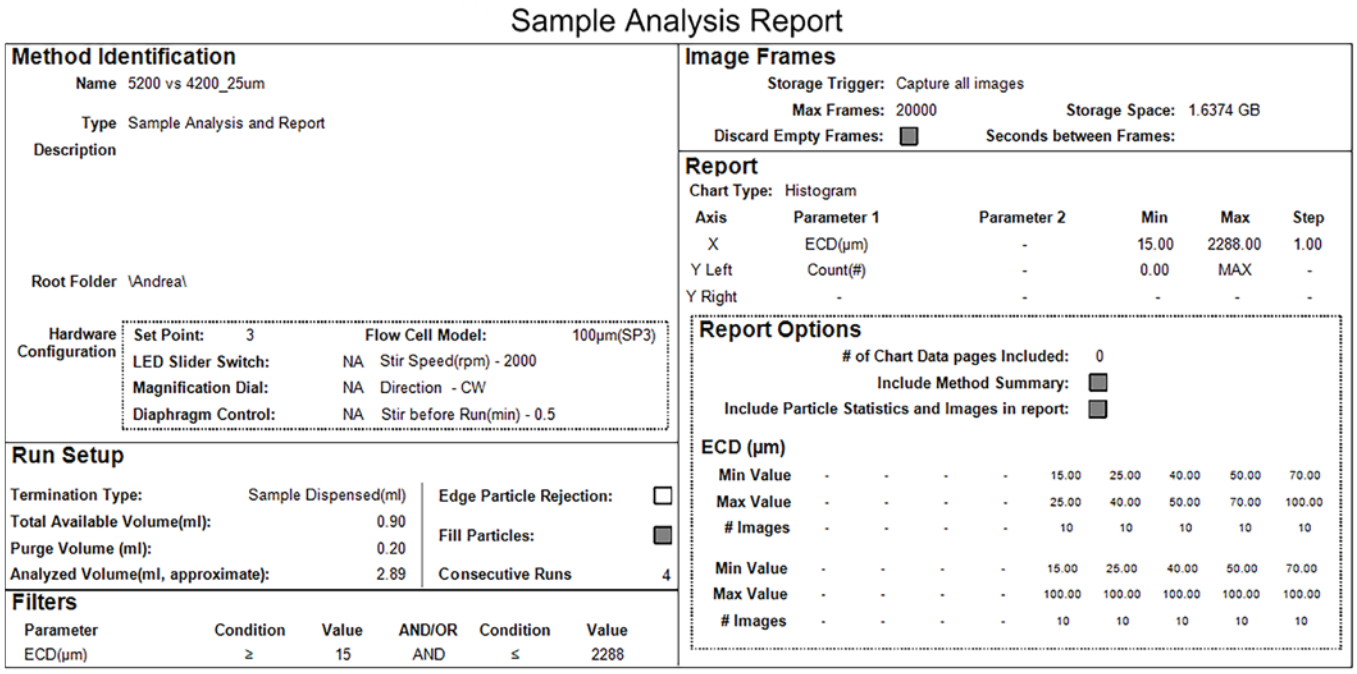
A variety of protein sample preparations were also run on both systems. We resuspended 1% protein, 5% protein, and 10% protein in 15 mL centrifuge tubes using 0.2 µm filtered Milli-Q water. Samples were stressed by heating at 60 °C with shaking at 500 rpm for 24 hours with continued agitation for another 24 hours at room temperature before storing at -20 °C. Samples were removed from the freezer to thaw the night before they were run.

### Sample Analysis

The methods used to run samples were created on the MFI 5200 and then imported into the DPA 4200 repository to ensure the same method for each sample was run on both instruments (**Figures 3**, **4**, and **Table 3**). Each sample was run in quadruplicate using a syringe barrel for sample introduction.

| BEAD SIZE | MORPHOLOGICAL FILTER | STIRRER? |
|---|---|---|
| 5 µm | ≥3 µm | No |
| 10 µm | ≥7.5 µm | No |
| 15 µm | ≥10 µm | Yes |
| 25 µm | ≥15 µm | Yes |
| 50 µm | ≥30 µm | Yes |

**TABLE 3.** Morphological filters applied to run the COUNT-CAL bead standards. The table also indicates if the stirrer was used to prevent samples from settling during the run.



Sample Analysis Report

**Method Identification**
Name 5200 vs 4200_25um
Type Sample Analysis and Report
Description
Root Folder \Andrea\
Hardware Configuration
Set Point: 3 Flow Cell Model: 100µm(SP3)
LED Slider Switch: NA Stir Speed(rpm) - 2000
Magnification Dial: NA Direction - CW
Diaphragm Control: NA Stir before Run(min) - 0.5

**Run Setup**
Termination Type: Sample Dispensed(ml)
Total Available Volume(ml): 0.90
Purge Volume (ml): 0.20
Analyzed Volume(ml, approximate): 2.89
Edge Particle Rejection:
Fill Particles:
Consecutive Runs 4

**Filters**

| Parameter | Condition | Value | AND/OR | Condition | Value |
|---|---|---|---|---|---|
| ECD(µm) | ≥ | 15 | AND | ≤ | 2288 |

**Image Frames**
Storage Trigger: Capture all images
Max Frames: 20000 Storage Space: 1.6374 GB
Discard Empty Frames: Seconds between Frames:

**Report**
Chart Type: Histogram

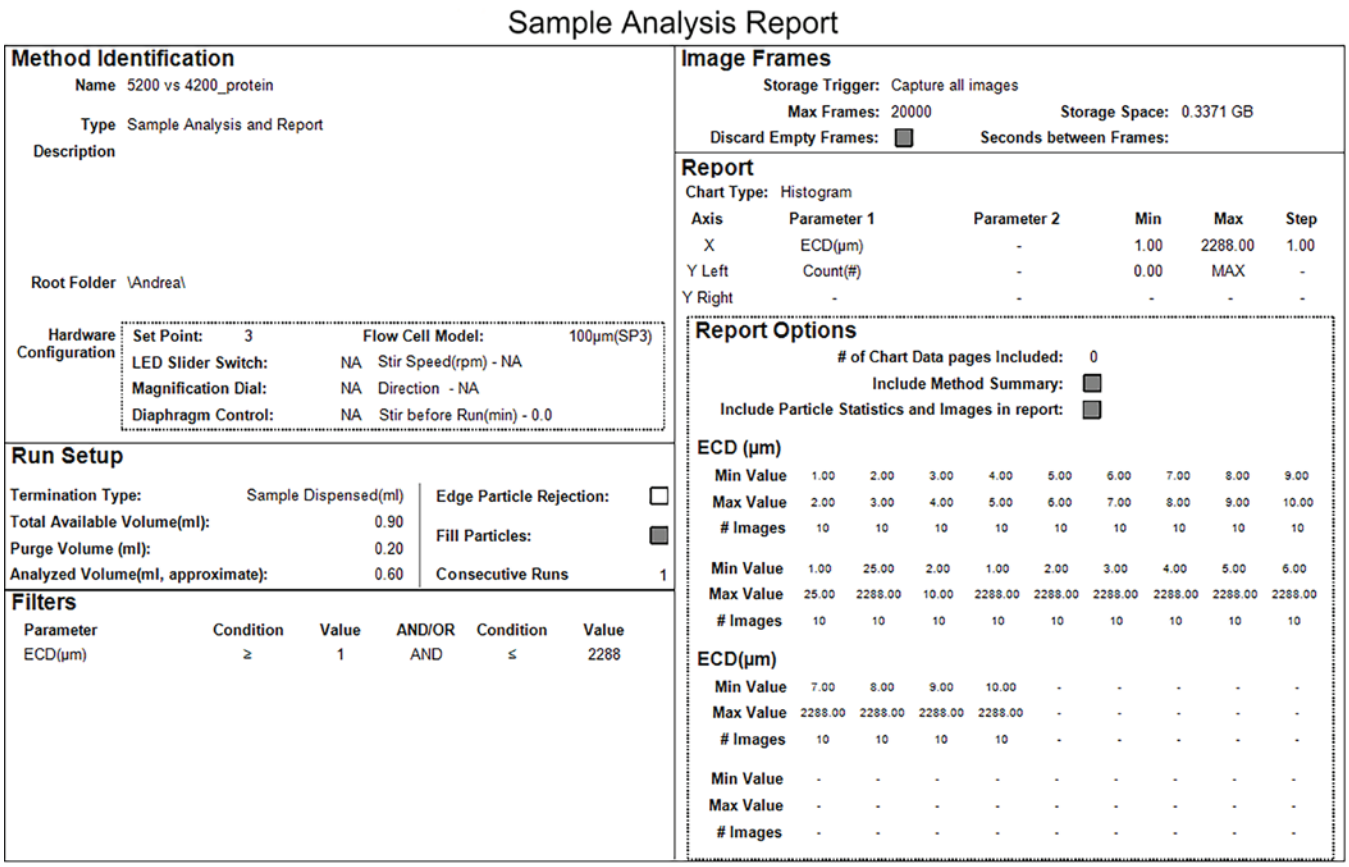
| Axis | Parameter 1 | Parameter 2 | Min | Max | Step |
|---|---|---|---|---|---|
| X | ECD(µm) | - | 15.00 | 2288.00 | 1.00 |
| Y Left | Count(#) | - | 0.00 | MAX | - |
| Y Right | - | - | - | - | - |

**Report Options**
# of Chart Data pages Included: 0
Include Method Summary:
Include Particle Statistics and Images in report:

**ECD (µm)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Min Value | - | - | - | - | 15.00 | 25.00 | 40.00 | 50.00 | 70.00 |
| Max Value | - | - | - | - | 25.00 | 40.00 | 50.00 | 70.00 | 100.00 |
| # Images | - | - | - | - | 10 | 10 | 10 | 10 | 10 |
| Min Value | - | - | - | - | 15.00 | 25.00 | 40.00 | 50.00 | 70.00 |
| Max Value | - | - | - | - | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| # Images | - | - | - | - | 10 | 10 | 10 | 10 | 10 |

**FIGURE 3.** Sample Analysis Report listing the method used to run COUNT-CAL beads on both systems.



Sample Analysis Report

**Method Identification**

Name 5200 vs 4200_protein

Type Sample Analysis and Report

Description

Root Folder \Andrea\

Hardware Configuration

| | | | |
|---|---|---|---|
| Set Point: | 3 | Flow Cell Model: | 100µm(SP3) |
| LED Slider Switch: | NA | Stir Speed(rpm) - NA | |
| Magnification Dial: | NA | Direction - NA | |
| Diaphragm Control: | NA | Stir before Run(min) - 0.0 | |

**Run Setup**

| | | | |
|---|---|---|---|
| Termination Type: | Sample Dispensed(ml) | Edge Particle Rejection: | ☐ |
| Total Available Volume(ml): | 0.90 | Fill Particles: | ■ |
| Purge Volume (ml): | 0.20 | | |
| Analyzed Volume(ml, approximate): | 0.60 | Consecutive Runs | 1 |

**Filters**

| Parameter | Condition | Value | AND/OR | Condition | Value |
|---|---|---|---|---|---|
| ECD(µm) | ≥ | 1 | AND | ≤ | 2288 |

**Image Frames**

Storage Trigger: Capture all images

Max Frames: 20000 Storage Space: 0.3371 GB

Discard Empty Frames: ☐ Seconds between Frames:

**Report**

Chart Type: Histogram

| Axis | Parameter 1 | Parameter 2 | Min | Max | Step |
|---|---|---|---|---|---|
| X | ECD(µm) | - | 1.00 | 2288.00 | 1.00 |
| Y Left | Count(#) | - | 0.00 | MAX | - |
| Y Right | - | - | - | - | - |

**Report Options**

# of Chart Data pages Included: 0

Include Method Summary: ■

Include Particle Statistics and Images in report: ■

**ECD (µm)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Min Value | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 | 7.00 | 8.00 | 9.00 |
| Max Value | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 | 7.00 | 8.00 | 9.00 | 10.00 |
| # Images | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Min Value | 1.00 | 25.00 | 2.00 | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | 6.00 |
| Max Value | 25.00 | 2288.00 | 10.00 | 2288.00 | 2288.00 | 2288.00 | 2288.00 | 2288.00 | 2288.00 |
| # Images | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**ECD(µm)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Min Value | 7.00 | 8.00 | 9.00 | 10.00 | - | - | - | - | - |
| Max Value | 2288.00 | 2288.00 | 2288.00 | 2288.00 | - | - | - | - | - |
| # Images | 10 | 10 | 10 | 10 | - | - | - | - | - |
| Min Value | - | - | - | - | - | - | - | - | - |
| Max Value | - | - | - | - | - | - | - | - | - |
| # Images | - | - | - | - | - | - | - | - | - |

**FIGURE 4.** Sample Analysis Report listing the method used to run protein samples on both systems.

### ANALYZING THE DATA

The projects were analyzed using MVAS 1.4 with an initial filter to remove stuck particles. This proprietary filter ensures that artifacts occurring from particles stuck to the flow cell aren't included in the particle analysis. When applicable, we analyzed the data with the same morphological filters used when running the samples. Particle counts per mL from the four replicate runs were averaged and the standard deviation and %CV were calculated.

## Results

### POLYSTYRENE BEAD DATA

Let's look at the bead data first. As expected, our results confirmed equivalent and consistent performance between the MFI 5200 and the DPA 4200 (**Figure 5**). All concentrations measured fell within ±10% of the average of all the bead concentrations obtained on the DPA 4200 and easily fell between the expected particle counts of 2700-3000 per mL (**Table 4**). All CVs were ≤5.1%, and more importantly, all intra-instrument CVs were ≤4.1%. This clearly demonstrates that both systems generate equivalent results (**Table 4**).

| BEAD SIZE | DPA 4200 | | MFI 5200 | | INTRA-INSTRUMENT (DPA 4200 + MFI 5200) | |
|---|---|---|---|---|---|---|
| | Average Concentration | %CV | Average Concentration | %CV | Average Concentration | %CV |
| 5 µm | 3002.12 | 2.5% | 2923.04 | 3.9% | 2947.15 | 3.5% |
| 10 µm | 2720.81 | 1.8% | 2764.34 | 2.0% | 2756.19 | 1.7% |
| 15 µm | 2814.49 | 2.1% | 2743.40 | 2.1% | 2769.07 | 2.4% |
| 25 µm | 2634.94 | 2.8% | 2803.86 | 2.3% | 2820.84 | 2.9% |
| 30 µm | 2783.74 | 5.1% | 2830.61 | 2.8% | 2778.39 | 4.1% |

**TABLE 4.** Average concentration measured for COUNT-CAL beads on the DPA 4200 and MFI 5200 with %CV. All CVs were ≤5.1% showing accurate concentrations across a broad range of bead sizes.

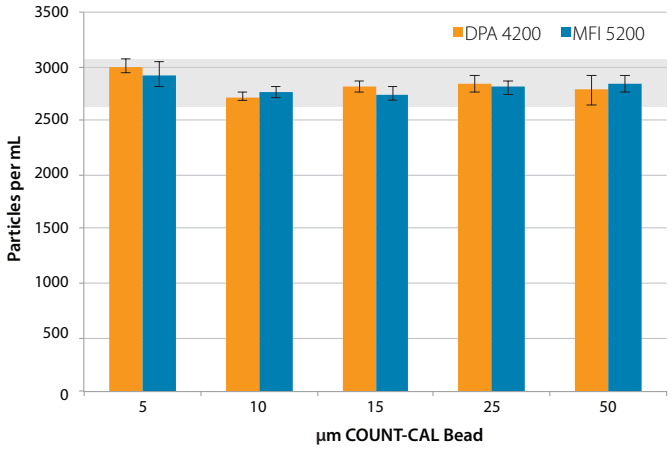



**FIGURE 5.** Comparison of the average concentration measured on the DPA 4200 and MFI 5200 when running COUNT-CAL beads. Results were consistently within the equivalent range for both instruments. Error bars represent the standard deviation and the shaded region represents ±10% of the average particle concentration for all the samples measured on the DPA 4200. All samples were run in quadruplicate.

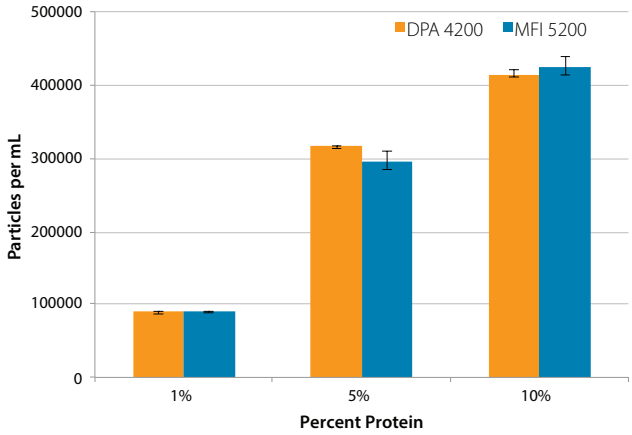



**FIGURE 6.** Concentration results for protein samples measured on the DPA 4200 and MFI 5200. Error bars represent the standard deviation. All samples were run in quadruplicate.

### PROTEIN SAMPLE DATA

Now let's look at how the protein samples compared. All of the protein samples behaved as expected and confirmed equivalent performance between the MFI 5200 and the DPA 4200 (**Figure 6**). Concentrations for 1%, 5%, and 10% protein resulted in an increase in protein particles detected by both instruments. Once again, the data was extremely reproducible as all CVs between the four replicates were ≤4.2%, and intra-instrument CVs for all three protein samples were ≤4.6% (**Table 5**).

| % PROTEIN | DPA 4200 | | MFI 5200 | | INTRA-INSTRUMENT (DPA 4200 + MFI 5200) | |
|---|---|---|---|---|---|---|
| | Average Concentration | %CV | Average Concentration | %CV | Average Concentration | %CV |
| 1% | 89279.49 | 3.2% | 90633.62 | 1.9% | 90512.33 | 2.7% |
| 5% | 316275.82 | 0.9% | 295631.33 | 4.2% | 305717.17 | 4.7% |
| 10% | 416318.48 | 1.0% | 425898.13 | 3.0% | 419966.72 | 2.8% |

**TABLE 5.** Average concentration measured for protein samples on the DPA 4200 and MFI 5200 with %CV. Instrument equivalency is demonstrated by the intra-instrument CV of ≤4.7%.

## Conclusion

The MFI 5200 can clearly give you equivalent data to the DPA 4200 as the intra-instrument CVs of ≤4.7% for COUNT-CAL bead standards and protein samples show. So you'll have the same high quality performance you've come to expect from DPA with MFI too. But on top of that, the MFI 5000 series simplifies system setup, frees up FTE time, increases throughput when you add a Bot1, and streamlines protocol set-up and analysis with multi-sample processing. When you combine that with all the other enhancements we've added into our MFI systems, you'll be getting way more done in no time!

## References

1. Micro-flow Imaging: flow microscopy applied to subvisible particulate analysis in protein formations, D Sharma, D King, P Oma, and C Merchant, *AAPS Journal* 2010; 12 (3).
2. Comparability study of manual and automated particle characterization with MFI, D Kristensen, T Meiborg-Sloth, S Mack, A Olcott, and E Gentalen, *ProteinSimple Application Note*.
3. Rapid and high throughput particle analysis on the MFI 5000, J Dermody, S Mack, D Weinbuch, A Hawe, R LeBold, A Olcott, *ProteinSimple Application Note*.

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Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
info@proteinsimple.com
proteinsimple.com



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