Fast, Multi-sample Particle Analysis Using MVSS 4.0

Jessica Dermody[1], Senior Scientist, Scott Mack[1], Senior Scientist, Daniel Weinbuch[2], Scientist, Andrea Hawe[2], Chief Scientific Officer, Rick LeBold[1], MFI Software Manager, Angelica Olcott[1], MFI Product Manager

[1]ProteinSimple, San Jose, California; [2]Coriolis Pharma, Munich, Germany

Introduction

Micro-Flow Imaging™ (MFI™) has quickly become the go-to method for collecting the information regulatory agencies expect when it comes to characterizing sub-visible particles in biopharmaceuticals. MFI has always let you measure particle size, count and morphology. But now with our new MVSS 4.0 software, you can set up automated protocols and analyze multiple samples using sophisticated classification filters in a snap too!

Older techniques like light obscuration aren't able to detect particle morphology and image intensity. So they just can't distinguish silicone oil droplets from proteinaceous particles like MFI can — plus it's not unusual for them to miss translucent protein particles completely! MFI not only lets you detect the particles in your sample, it also lets you classify them too. In this application note, we will show how MVSS 4.0, together with the high-throughput Bot1 Autosampler on the MFI 5000, series lets you screen and analyze groups of samples to monitor the quality and safety of your biopharmaceutical product in no time.

A Quick Software Overview

You get two software products for particle analysis with every MFI system: MFI View System Software (MVSS) and MFI View Analysis Software (MVAS). MVSS controls the instrument and gives you a basic analysis format containing size, count and concentration data, while MVAS lets you classify particles based on images or a selection of custom parameters. And for those of you needing a bit more security, did we mention MVSS is 21 CFR Part 11-enabled?

Turbo-charged Workflow for Multi-sample Analysis

The power-packed, multi-sample particle characterization features in MVSS 4.0 simplify batch setup and analysis workflows so you can get straight to results. First you'll want to view samples acquired from MVSS in MVAS, as it lets you design and optimize particle classification filters based on image analysis of particle morphology and image intensity (**Figure 1**). Once that's done, MVSS 4.0 recreates the filter and applies it to multiple samples at once.

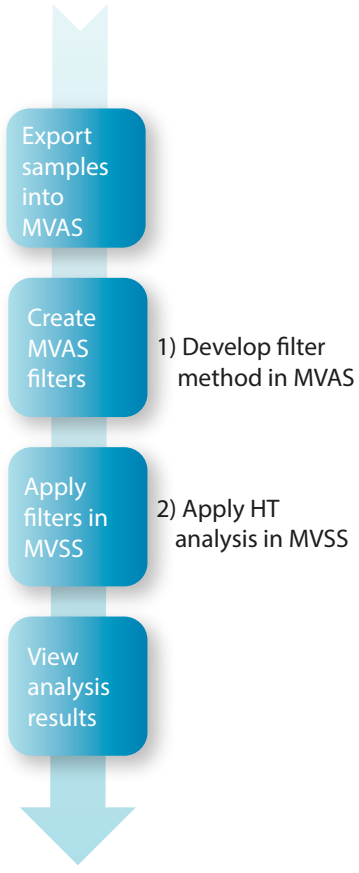

FIGURE 1. New multi-sample analysis workflow.

proteinsimple

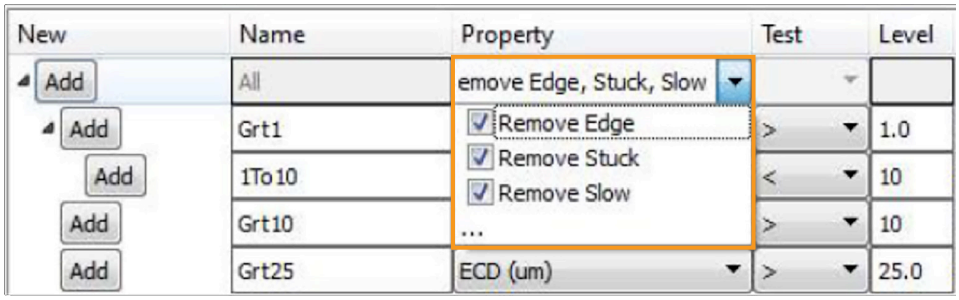

FIGURE 2. Figure 2. USP 788 filter created in MVSS 4.0 to remove edge, stuck and slow-moving particles. To eliminate sampling artifacts before filter analysis, we selected removal of **edge, stuck or slow-moving particles** in the **Property** menu for **All,** then applied the classification filter of interest to the remaining particles.

Sample Analysis in MVSS 4.0 vs. MVSS 3.3

To give you an idea of the time you'll save with MVSS 4.0, we ran mixed sets of either 40 or 90 samples containing buffer, calibration standard or protein on an MFI 5200 with a Bot1 Autosampler — first with MVSS 4.0 and then with MVSS 3.3.

We created a USP 788 filter to categorize particles by size from 1 to 10 microns, greater than 10 microns, and greater than 25 microns. Since MVSS 4.0 now has the added bonus of being able to apply a unique proprietary filter to remove stuck and slow-moving particles, we used it to eliminate any sampling artifacts up front too (**Figure 2**). Once created, we applied the classification filter to compliant project files from MVSS or non-compliant CSV files from MVAS.

Set Up Automated Protocols in No Time

The new Repeat operation in MVSS 4.0 dramatically cuts the setup time to create a Bot1 batch protocol (**Figure 3**), so you can easily create protocols in just a few minutes! The Repeat operation duplicates the steps listed within it for a specified group of samples. Just fill in the steps within the Repeat operation, then click and drag any samples from the plate map to the start of the Repeat operation that you want it to apply to — that's it!

As you can see in **Table 1**, we only needed 5 minutes in MVSS 4.0 to set up a 40-sample protocol compared to 60 minutes in MVSS 3.3. And for a 90-sample protocol using two repeat steps, setup only took 10 minutes compared to the 2.5 hours it would have taken without the Repeat operation.

	MVSS 3.3	MVSS 4.0
Bot1 Protocol Setup for 40 Samples	60 minutes	5 minutes
Bot1 Protocol Setup for 90 Samples	2.5 hours	10 minutes

TABLE 1. The Repeat operation dramatically cuts setup time for automated Bot1 batch protocols.

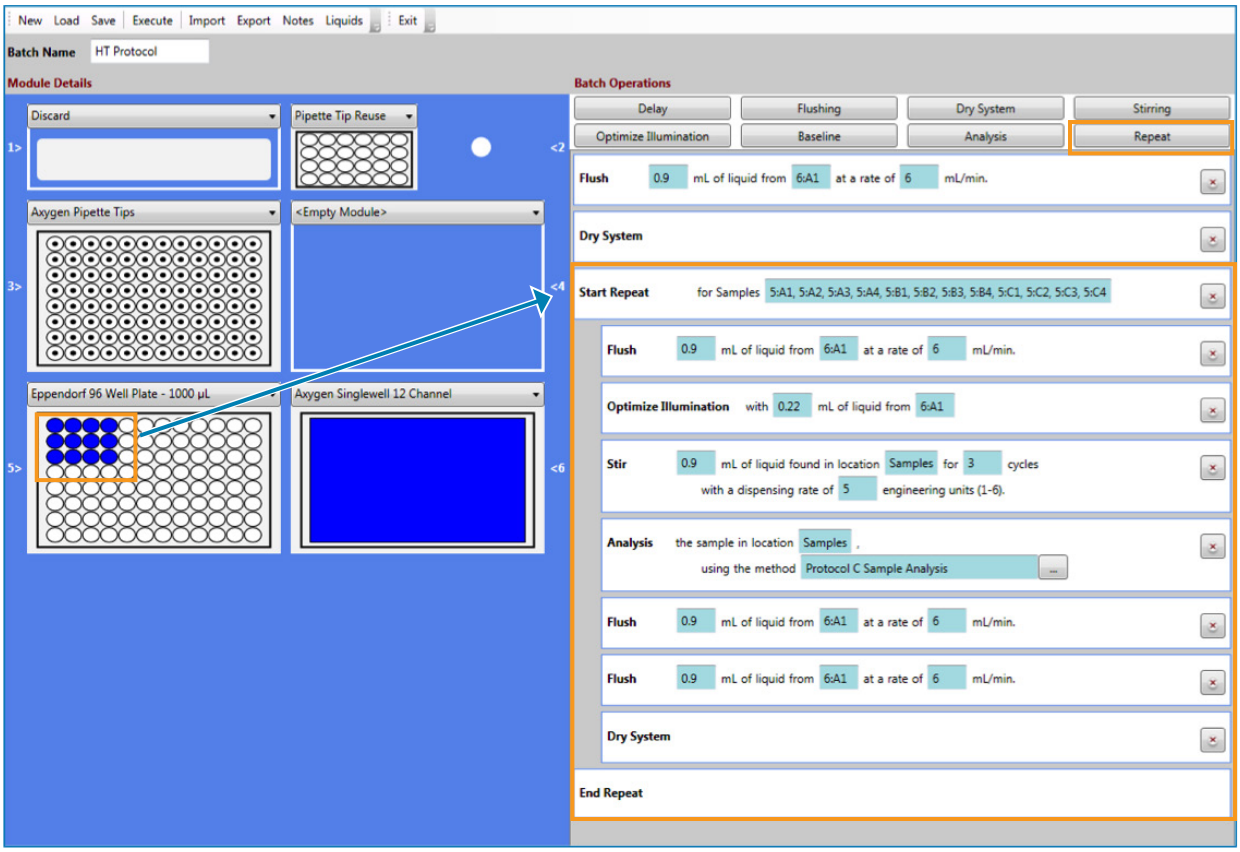

FIGURE 3. Repeat operation created within the automated Bot1 batch protocol.

Multi-Sample Analysis at Light Speed

To determine how fast the new MVSS 4.0 multi-sample analysis workflow is compared to MVSS 3.3, we evaluated analysis times using a broad mix of both MVSS project files and non-compliant CSV files.

When we imported 90 MVSS project files into the MVSS 4.0 multi-sample analysis window, all aspects of our USP-788 filter were applied instantly (**Figure 4**). MVSS 4.0 was also able to analyze both compliant (MVSS projects under 21 CFR Part 11) and non-compliant data (imported CSV files).

The time savings for multi-sample analysis with MVSS 4.0 was truly amazing! With the MVSS 3.3 particle analysis workflow, you could only analyze one sample at a time with MVAS (non-21 CFR Part 11-enabled) to get filters and classification of particle morphology. The new MVSS 4.0 workflow reduced analysis time by at least 3.5X for 40- or 90-sample runs. And, we only needed 5 minutes to create a simple filter in MVSS 4.0, which then instantaneously updated with the results of the classification once sample runs were selected.

Filter Creation Packed with Flexibility *and* Power

In collaboration with Coriolis Pharma, we also looked at the ability to create simple and complex classification filters. Results using multi-sample analysis hands-down solidified that MVSS 4.0 has the flexibility and power to duplicate more complex filters for silicone oil and protein-like particles like the S-factor filter[1,2], not to mention analysis formats routinely used by Coriolis Pharma.

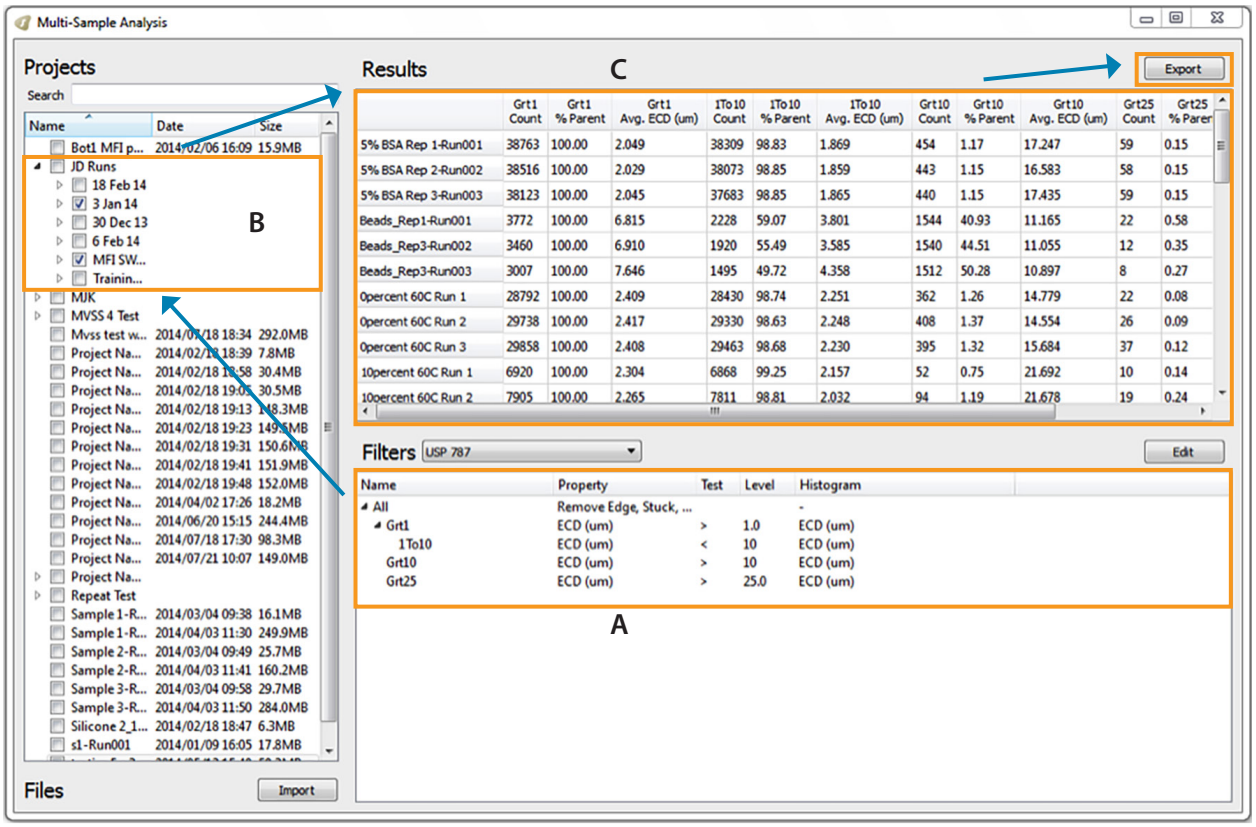

FIGURE 4. A USP 788 filter applied to 90 samples updates data instantly. With multi-sample analysis you can easily import many samples, apply a variety of particle classification filters for classification, and export all of your results right away.

MVSS 4.0 easily created a sophisticated filter with a variety of different morphologic and image intensity-based parameters (**Figure 5**). The filters designed to separate silicone oil and protein populations were non-linear as described by Weinbuch et al.[2] at Coriolis Pharma, so a custom threshold for each parameter was created (**Figure 6**). The custom threshold lets you define size-dependent cutoff values for each morphologic or image parameter, so now you can truly customize classification for challenging particle populations like silicone oil and protein.

Same Great System Performance

To verify system specifications were on par, we ran an IQ/OQ protocol on both MVSS 4.0 and 3.3. Using 5 and 10 micron polystyrene bead calibration standards on the same instrument, we evaluated both software versions for any difference in sizing and concentration accuracy. As expected, our results were well within the manufacturing specifications for the MFI system. Variability was only 2.8%, well below the ±10% specification. On top of that, our statistical analysis showed no significant differences between the two software versions, with a P-value of 0.159 (**Table 2**).

GROUPS	SAMPLES (N)	SUM	AVERAGE	VARIANCE
MVSS 4.0	3	9,671	3,224	5,230
MVSS 3.3	3	9,400	3,133	2,958

SOURCE OF VARIATION	SS	DF	MS	P-VALUE
Between groups	12,208.57	1	12,208.57	0.159
Within groups	16,378.68	4	4,094.67	
Total	2857.25	5		

TABLE 2. ANOVA statistical analysis of variance between the mean IQ/OQ values for MVSS versions 4.0 and 3.3. The resulting P-value of 0.159 showed no significant differences between the mean results for either software version. Abbreviations: SS=Sum of Squares, df=degrees of freedom, MS=Mean of Square.

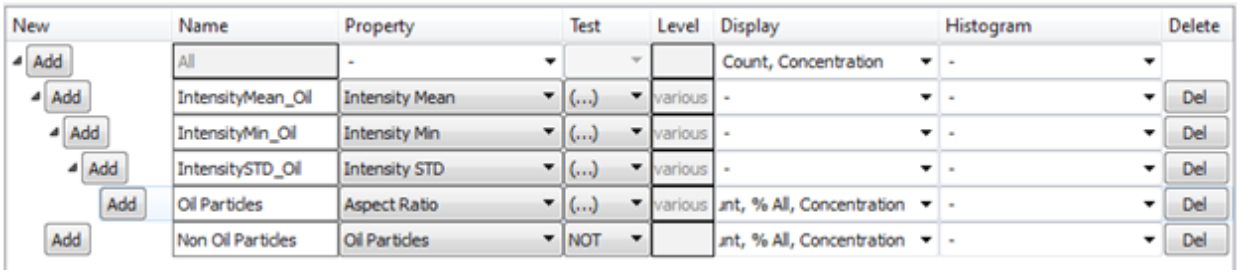

FIGURE 5. Custom filter created in MVSS 4.0 with several different morphologic and image intensity parameters. Results can be viewed immediately once you've applied the filter to imported projects from MVSS or non-compliant CSV files.

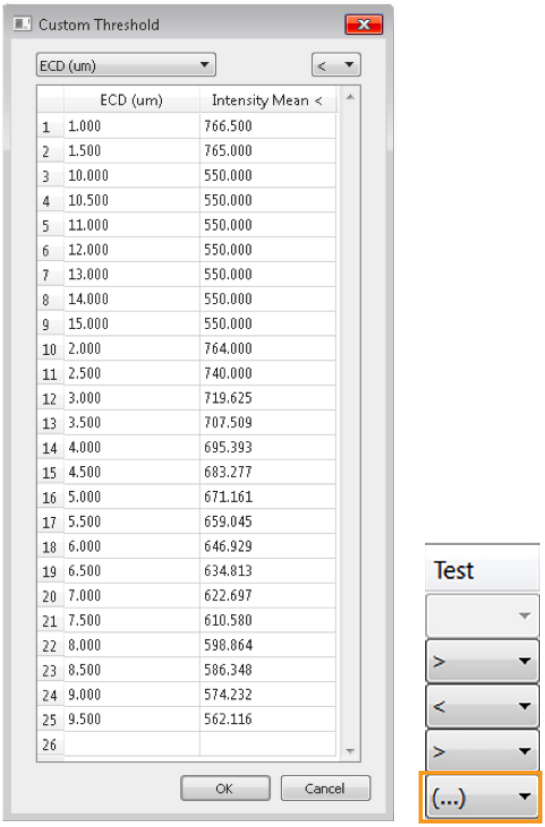

FIGURE 6. The custom threshold setting creates more defined filter cutoffs for classification. Simply pair the values for a size parameter (ECD) with a morphologic or image intensity parameter. You can access a custom threshold by selecting the **Custom** option (see highlighted symbol) from the **Test** menu of the parameter.

Conclusion

MFI is a must-have solution for sensitive detection of sub-visible particles for size, count and morphology — letting you accurately assess the quality of your biopharmaceutical products. And now with the new batch setup and multi-sample analysis features in MVSS 4.0 software, it's just gotten light years faster. Our study showed that with the new Repeat operation workflow in MVSS 4.0, we could cut the time needed to set up a Bot1 batch protocol for up to 90 samples down from 2.5 hours to just 5 minutes! And once the filter and samples

were selected, the new multi-sample analysis workflow instantly updated results in real time, not to mention we could easily export sample results as CSV files.

MVSS 4.0 ups your particle analysis game to a whole new throughput level. Now you can compare the results of a custom filter across multiple samples at once with full 21 CFR Part 11 security, and build powerful, highly sophisticated filters with ease.

References

1. Discrimination between silicone oil droplets and protein aggregates in biopharmaceuticals: a novel multiparametric image filter for sub-visible particles in microflow imaging analysis, R Strehl, V Rombach-Riegraf, M Diez, K Egodage, M Bluemel, M Jeschke and A Koulov, *Pharmaceutical Research*, 2012; 29(2):594–602.

2. Micro–flow imaging and resonant mass measurement (Archimedes) — complementary methods to quantitatively differentiate protein particles and silicone oil droplets, D Weinbuch, S Zölls, M Wiggenhorn, W Friess, G Winter, W Jiskoot and A Hawe, *Journal of Pharmaceutical Sciences*, 2013; 102(7):2152–2165.

proteinsimple

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

99-4005-00 / Rev A