

## Micro-flow Imaging (MFI) – ProteinSimple

4100HM, 4100LM/5100, 4200/5200

### INTRODUCTION

The Lumetics LINK™ software platform scans network locations for new measurement data files, copies data directly to a centralized database, and provides a powerful user interface for rapid multi-measurement multi-technique data aggregation, visualization, analysis, and reporting. LINK employs a client/server-based architecture where the LINK server hardware is provided by the end user and resides on the end user’s network. The LINK client is a portable web-based application that may be placed on any computer with network connectivity to the LINK server. For successful import, the LINK webserver requires read access to the folders where user data resides.

MFI is an image-based particle analysis that combines the direct imaging capabilities of digital microscopy with the precise control of microfluidics. Images of the sample are captured as it passes through the flow cell’s sensing zone. Every particle in every image is then analyzed to create a database of particle count, size, transparency, and morphology (or shape).

### DETAILS

LINK can import MFI from CSV data file exports from the MVSS software (with optional JPG particle images), or directly from a PDF report. If importing from the PDF report, only table data is available within LINK (vs. full particle size/morphology distributions, particle images, sub-population calculations etc....). Particle images may optionally be imported as JPGs, and therefore may be exported as thumbnail images from LINK as JPGs.

Particle count/concentration for each supported morphological parameter occurs at a pre-defined bin spacing and measurement range as per the table below. Default measurement ranges will dynamically widen based as required, based on the datasets imported to LINK.

- Raw particle size distribution data is imported at pre-defined bin widths (e.g. the bin width for ECD is 0.25um).
- Particle Volume % for the number of particles in each 0.25um bin is calculated automatically and imported to LINK. (Particle Volume % = volume of particles in each size range bin divided by the volume of particles in all bins, assuming particles are spherical in volume).

Parameter Name	MFI 4100HM & 4100LM/5100			MFI 4200/5200		
	Bin Spacing	Meas. Start	Meas. End	Bin Spacing	Meas. Start	Meas. End
Analysis Time (s)	1	0	3600	1	0	3600
Area	1	1	5000	1	1	5000
Aspect Ratio	0.01	0	1	0.01	0	1
AspectRatio_StdDev	-	-	-	0.01	0	1
Circularity	0.01	0	1	0.01	0	1
Circularity StdDev	-	-	-	0.01	0	1
ECD	0.25	2	2500	0.25	1	2500
IntensityMax	1	0	1023	1	0	1023
IntensityMean	1	0	1023	1	0	1023
IntensityMin	1	0	1023	1	0	1023
IntensitySTD	1	0	1023	1	0	1023
Max Feret Diameter (µm)	0.25	1	2500	0.25	1	2500
Perimeter	1	1	5000	1	1	5000

The MFI CSV data file example is as follows:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	Version	2-R2.5.0.14.1195																		
2	Sample_Name	Sample A																		
3	Run	1																		
4	Flow_Cell_Serial	2763																		
5	Flow_Cell_Model	100um(SP3)																		
6	Flow_Cell_Position	3268																		
7	System_Model	MFI5200																		
8	System_Serial	3130																		
9	Operator	Kristie																		
10	Comment																			
11	FileName	C:\Documents and Settings\MFI Admin																		
12	Start_Date	2013-02-17																		
13	Start_Time	13h44m37s																		
14	Stop_Date	2013-02-17																		
15	Stop_Time	13h47m34s																		
16	Sample_Analyzed(ml)	0.9																		
17	Total_Volume_Dispende	0.50013																		
18	Volume_Dispende_Befc	0.18																		
19	Set-Point	3																		
20	Resolution(um)	0.25																		
21	Images_Stored	949																		
22	Images_Result_Folder	\\imageFolder\																		
23	Image_Format	Jpg																		
24	Fill_Hollow	Disable																		
25	Threshold(%)	96																		
26	Threshold	811																		
27	Table_Name(ECD)	LINK																		
28	Table_Name(MFD)	LINK																		
29	#	Frame #	Particle #	Time Stamp(s)	ECD(um)	Area(pixel)s	Perimeter	Circularity	Intensity Mean	Intensity STD	Intensity Mir	Intensity Max	Max Feret	Diameter(um)	Aspect Ratio	X Left	X Right	Y Top	Y Bottom	Edge Particle
30		1 1	1	0	1.125	8	13.07	0.77	799.25	8	788	809		1.125	0.62	1136	1139	165	167	0
31		2 1	2	0	1.375	19	18.07	0.86	776.68	24.91	735	808		1.375	0.86	334	338	517	521	0
32		3 2	1	0.10938	2.125	27	20.83	0.88	734.3	49.48	641	808		2.875	0.74	930	934	556	561	0

Importing a MVSS PDF – this method does not provide raw data. Therefore, will not allow particle size distribution or particle images manipulation. Parameters are calculated based on full particle size distribution. The PDF import requires the first line of the document to be “Project Name”. Example file is as follows:

## Sample Analysis Report

**Sample Analysis Summary**

**Project Name:** Water - 1

User: General User

File: Water - 1\_PV 0.2 TV 0.5 AR filtered\_Run01\_0001.rpt

Method: PV 0.2 TV 0.5 AR filtered

Method: General method, >=1um particles. 0.20ml purge, 0.30ml sample. Particles filtered by aspect ratio < 0.85. Report size ranges of >= 1, >= 2, >= 10 and >= 25 um plus other ranges.

Description:

Comments:

Analysis Date/Time: 2016/07/15 11:05m

Duration: 2.94m

Import Method options can be defined and calculated each measurement during import if it is first created/defined and then requested within an Import Method. More information and instructions can be found in the Users Manual (section 4.27). The following is the default MFI-specific import method settings:

- Default Sub-Populations: **None**
- Number of Thumbnails to Crop: **250**
- Machine Learning Particle Classification: **None**
- Ignore Thumbnails less than: **5 µm**
- Custom Particle Parameters: **None**
- Import Images in order of Largest-to-Smallest: **Yes**
- Container Fill Volume: **None**
- Advanced Image Selection: **None**
- Include Edge Particles: **No**
- Automated Metadata Extraction: **None**
- Image Frame Trimming: **No**
- Dilution Factor: **1 (no dilution)**
- Time Filter: **None**
- Stuck/Repeating Particle Removal: **OFF**

### Subpopulations

In addition to the total particle population data set, sub-populations based morphological parameter filters may be generated at the point of measurement import. An unlimited number of sub-populations may be specified for each measurement. For each sub-population, the particle count/concentration vs. morphological parameter will be available, as well as representative particle thumbnail images. The sub-populations do not count towards the measurement limit per project. Additional Sub-Populations will affect import speeds.



When creating as sub-population, select a Morphological Parameter from the pre-defined list and specify the filter criteria. Select the + to add the filter term to the Live Expression View. The Live Expression View can be edited directly. Brackets, mathematical operators/values, logical ANDs or ORs are valid operators. Syntax must be exact, for successful application.

#### **Machine Learning Particle Classification**

A particle classification server can be selected to classify particle images into subpopulations (requires license to activate this functionality). The classification server will return the predicted name of the particle, as well as the confidence value of the prediction. The particle will be imported into the LINK database as a sub-population based on the name returned by the server.

#### **Custom Particle Parameters**

Select from the available user-defined Custom Particle Parameters, those that shall be calculated for each individual particle within each measurement. This selection applies to the total particle population and all defined sub-populations.

#### **Container Fill Volume**

Specify the Container Fill Volume, permitting LINK to calculate and report Particles per Container (in addition to Particle Count, and Particle Concentration). This selection applies to the total particle population and all defined sub-populations.

#### **Edge Particles**

Include or exclude Edge particles (particles intersecting the edge of the image frame). This selection applies to the total particle population and all defined sub-populations.

#### **Image Frame Trimming**

Permits specifying a subsection of the image frame that shall be used to determine particle count and related calculations.

#### **Time Filter**

Exclude all particles detected at the beginning or end of the analysis. This selection applies to the total particle population and all defined sub-populations.

#### **Stuck/Repeating Particles**

Ignore particles images which remain, or move slowly, in successive image frames. This selection applies to the total particle population and all defined sub-populations.

#### **Thumbnail Images**

Specify the maximum number of particle thumbnail images to be imported to the LINKdb for each measurement population. Apply a size threshold (the default is 5 microns). Choose whether particles should be first sorted from largest to smallest (based on ECD) or imported in their order of appearance during the analysis. These selections apply to the total particle population and all defined sub-populations. Advanced Image Selection Criteria: Specify the number of thumbnail images for various particle parameter ranges of interest. The total number of images may not exceed 5000.

#### **Automated Metadata Extraction**

Specify criteria for automated metadata extraction from measurement file fields (e.g. Comments, File Name, Import Path etc...) utilizing specified delimiters. Both metadata name and value may be extracted or only the value only.

#### **Dilution Factor**

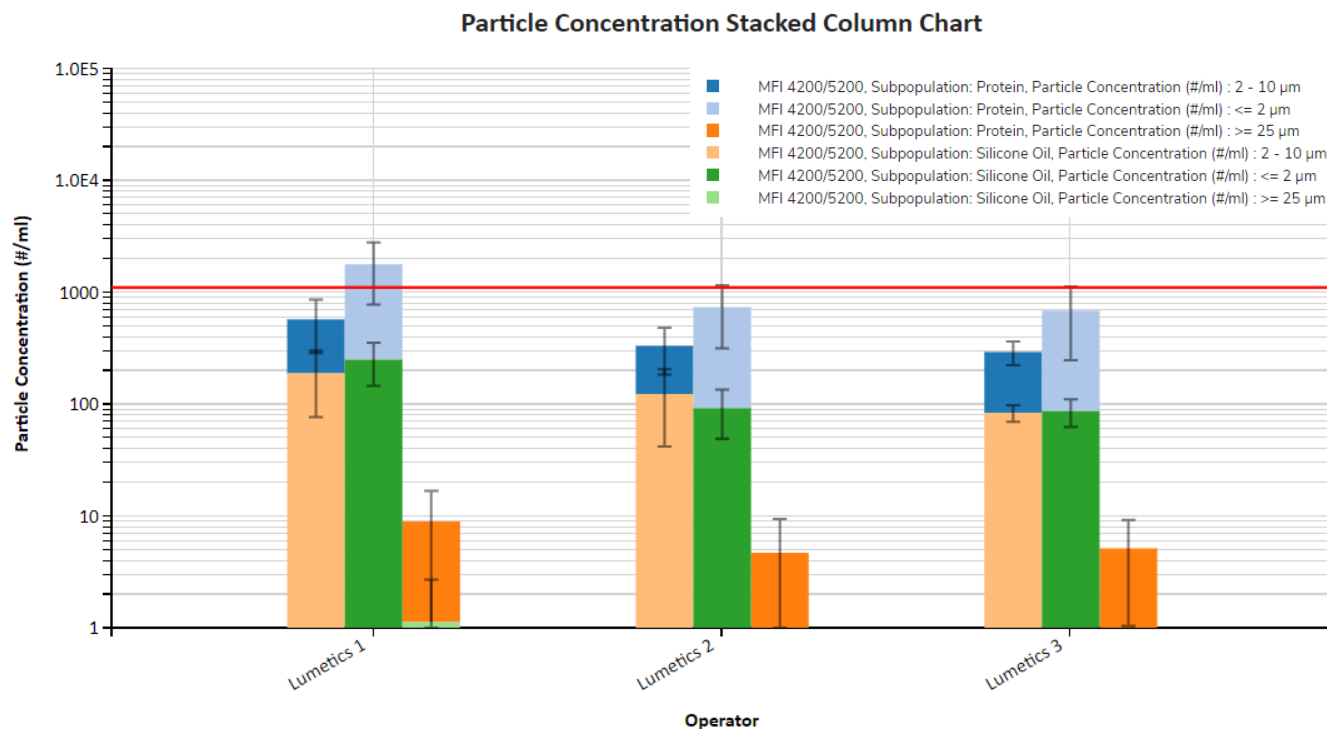
Specify a dilution factor for automated particle count/concentration data adjustment. This selection applies to the total particle population and all defined sub-populations. A LNK\_Dilution\_Factor may also be applied after import, utilizing the Add/Edit LINKdb Fields feature.



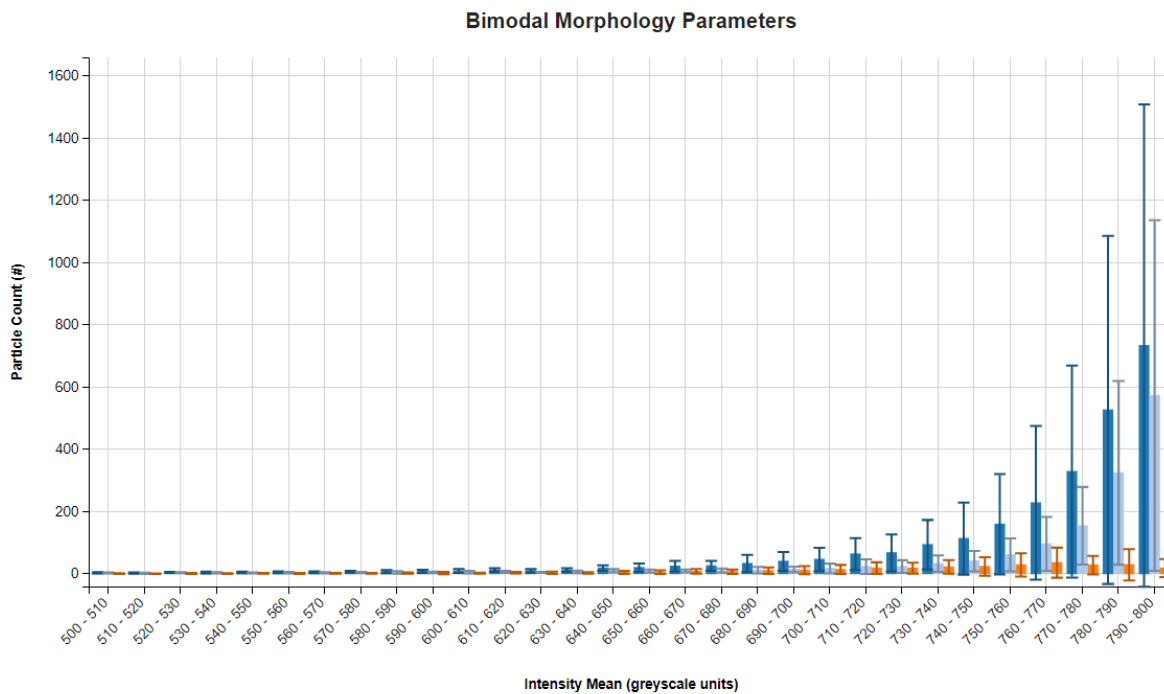
## EXAMPLES

Included below are example dashboards from MFI 4200/5200 measurement files:

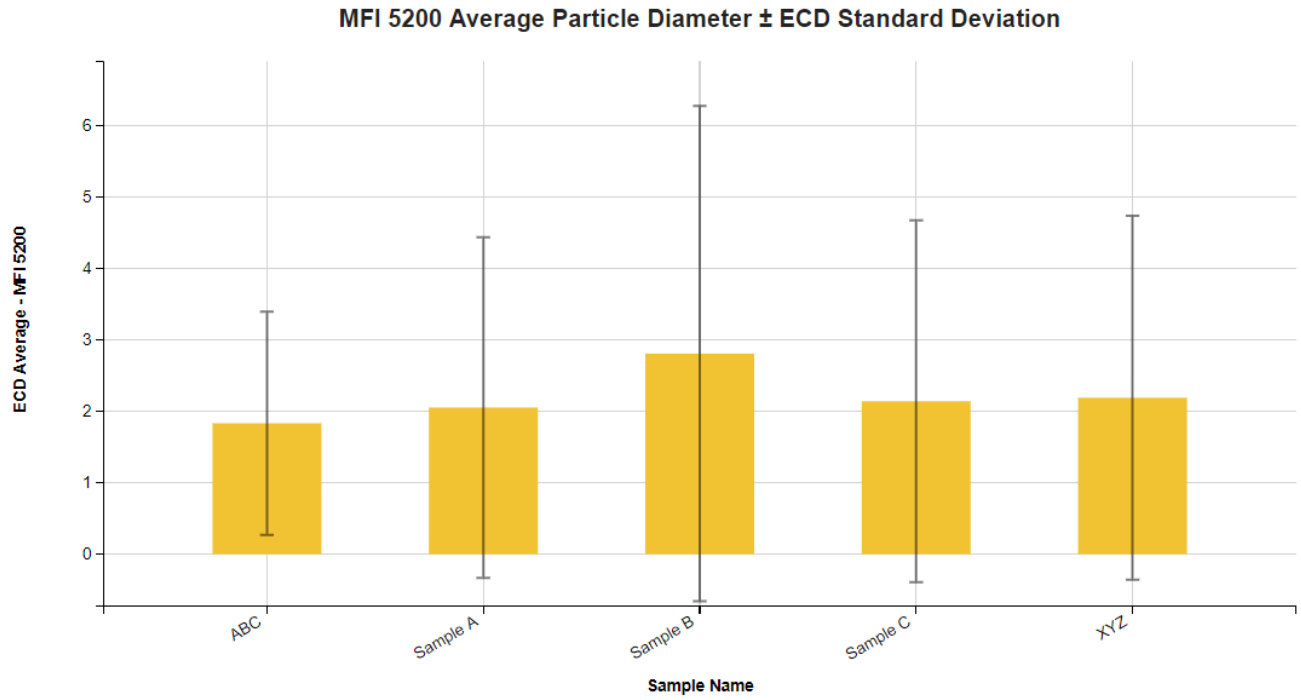
### 1. Stacked column chart plotting raw data based on sub-populations and particle concentration ranges



### 2. Histogram plotting raw binned data and split by sub-populations for Intensity Mean vs. Particle Count

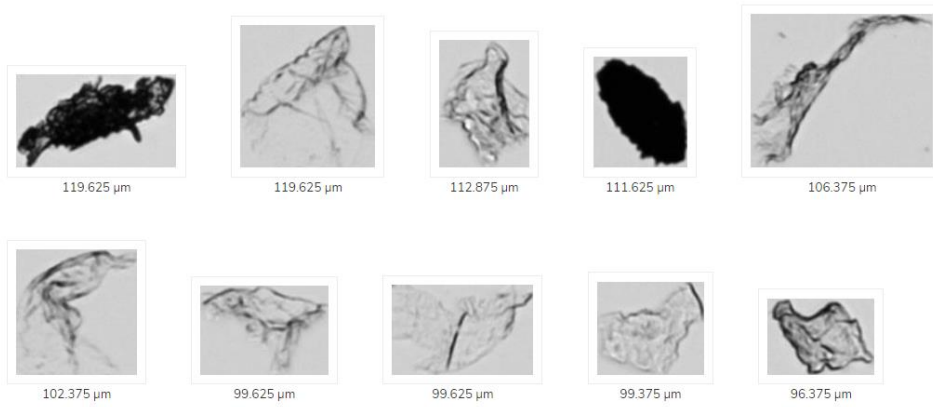


3. Column chart plotting binned instrument measurements with error bars for Average Particle Diameter vs. Sample Name

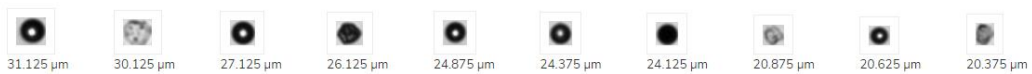


4. Particle Images organized based on sub-population

MFI 4200/5200, Protein



MFI 4200/5200, Silicon Oil



## 5. Tabular Summary examples

### Measurement Summary Table – Instrument Settings

LINK Record ID #	Sub-Population	Sample Name	Analysis Date	Stop Time	Start Time	Sample Analyzed (ml) - AVG	Purge Volume - AVG	Flow Cell Position - AVG	Flow Cell Position - AVG	Edge Particle Count - AVG
384	Multiple (3 Values)	Sample A	2013-02-17 13:...	13h47m34s	13h44m37s	0.90	0.18	3268	3268	25
385	Multiple (3 Values)	Sample A	2013-07-17 12:...	12h39m45s	12h36m49s	0.90	0.18	3268	3268	86
386	Multiple (3 Values)	Sample B	2013-02-17 13:...	13h16m27s	13h13m30s	0.50	0.18	3268	3268	16
387	Multiple (3 Values)	Sample C	2013-01-17 13:...	13h22m15s	13h19m18s	0.75	0.18	3268	3268	15
388	Multiple (3 Values)	Sample B	2013-01-17 10:...	10h42m52s	10h39m56s	0.75	0.18	3268	3268	22
389	Multiple (3 Values)	Sample C	2013-02-17 11:...	11h45m12s	11h42m15s	0.90	0.18	3268	3268	23
390	Multiple (3 Values)	XYZ	2013-02-17 13:...	13h47m34s	13h44m37s	0.90	0.18	3268	3268	25
391	Multiple (3 Values)	XYZ	2013-07-17 12:...	12h39m45s	12h36m49s	0.90	0.18	3268	3268	86
392	Multiple (3 Values)	XYZ	2013-02-17 13:...	13h16m27s	13h13m30s	0.50	0.18	3268	3268	16
393	Multiple (3 Values)	XYZ	2013-01-17 13:...	13h22m15s	13h19m18s	0.75	0.18	3268	3268	15
394	Multiple (3 Values)	ABC	2013-10-17 12:...	12h54m07s	12h51m10s	0.75	0.18	3268	3268	92
395	Multiple (3 Values)	ABC	2013-04-17 13:...	13h35m30s	13h32m33s	0.50	0.18	3268	3268	43

### Measurement Summary Table – Measurement Results

LINK Record ID #	Sub-Population	Count (Max Feret Diam. (µm) >1) - AVG	Particle Conc. (#/ml) (Circularity <1) - AVG	Particle Conc. (#/ml) (Aspect Ratio <1) - AVG	Volume (%) (Particle Diam. (µm) >1) - AVG	Total Particle Count (#) - AVG	Total Particle Concentration (#/ml) - AVG
384	Multiple (3 Values)	734.33	1477.04	1435.93	99.23	2088	2320.00
385	Multiple (3 Values)	1983.67	3656.67	3567.41	99.78	5118	5686.67
386	Multiple (3 Values)	521.33	1634.67	1598.67	99.76	1274	2548.00
387	Multiple (3 Values)	299.33	651.11	629.78	99.61	769	1025.33
388	Multiple (3 Values)	360.00	696.00	672.89	99.90	824	1098.67
389	Multiple (3 Values)	744.67	1381.85	1352.22	99.72	1929	2143.33
390	Multiple (3 Values)	572.33	1193.33	1154.45	98.86	2088	2320.00
391	Multiple (3 Values)	1633.67	3039.63	2961.11	99.76	5118	5686.67
392	Multiple (3 Values)	432.67	1367.33	1336.00	99.66	1274	2548.00
393	Multiple (3 Values)	241.00	533.33	514.22	99.31	769	1025.33
394	Multiple (3 Values)	2226.33	6697.78	6380.89	94.77	9878	13170.67
395	Multiple (3 Values)	704.67	2252.00	2189.33	99.59	2208	4416.00

## CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

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