

## Micro-flow Imaging (MFI) – ProteinSimple

4100HM, 4100LM/5100, 4200/5200

### INTRODUCTION

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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

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LINK requires the CSV data file exported from the MVSS software or PDF report. The JPG particle image files are optional.

The following raw curve data may be imported, in addition to all available instrument/analysis settings and parameters calculated by the instrument software:

- Particle Count Vs. Size/Shape
- Particle Conc. Vs. Size/Shape
- Particle Conc. Vs. Analysis Time

Particle count/concentration for each supported morphological parameter occurs at a pre-defined bin spacing and measurement range as per the table in the Users Manual located in the software help menu (section 4.27). 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 a given size range bin divided by the volume of particles in all bins, assuming particles are spherical in volume).
- 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.

New MFI feature permits removal of particles from the dataset that are visualized as bubbles, or particulate nestled within the air bubbles. To utilize this feature, in the analysis template dashboard, follow the below instructions

- Scroll to the thumbnail images and right click to display the context menu
- Select Image Explorer
- Click any images to select them for removal
- Click the button at the bottom of the screen called "Re-Import Measurements for Selected Particles". This will:
  - Determine all the constituent measurements in that thumbnail group, the source path for each, the import method used for each, and reimport referencing particle data that excludes those that have been tagged
  - Create a LNK\_Import\_Particles\_Excluded LINKdb field with a "Yes" "No"
  - Create a LNK\_Import\_Excluded\_Particle\_IDs LINKdb field to note the SampleName:AnalysisDate:Frame:ID
- After button selection, the re-import job has been added to the job
- Once the job is complete, all selected images will be removed from the dataset

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**
- Custom Particle Parameters: **None**
- Container Fill Volume: **None**
- Include Edge Particles: **No**
- Time Filter: **None**
- Stuck/Repeating Particle Removal: **OFF**
- Number of Thumbnails to Crop: **250**
- Ignore Thumbnails less than: **5 µm**
- Import Images in order of Largest-to-Smallest: **Yes**
- Dilution Factor: **1 (no dilution)**

### 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 a 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.

### 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.

### 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.

### 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.



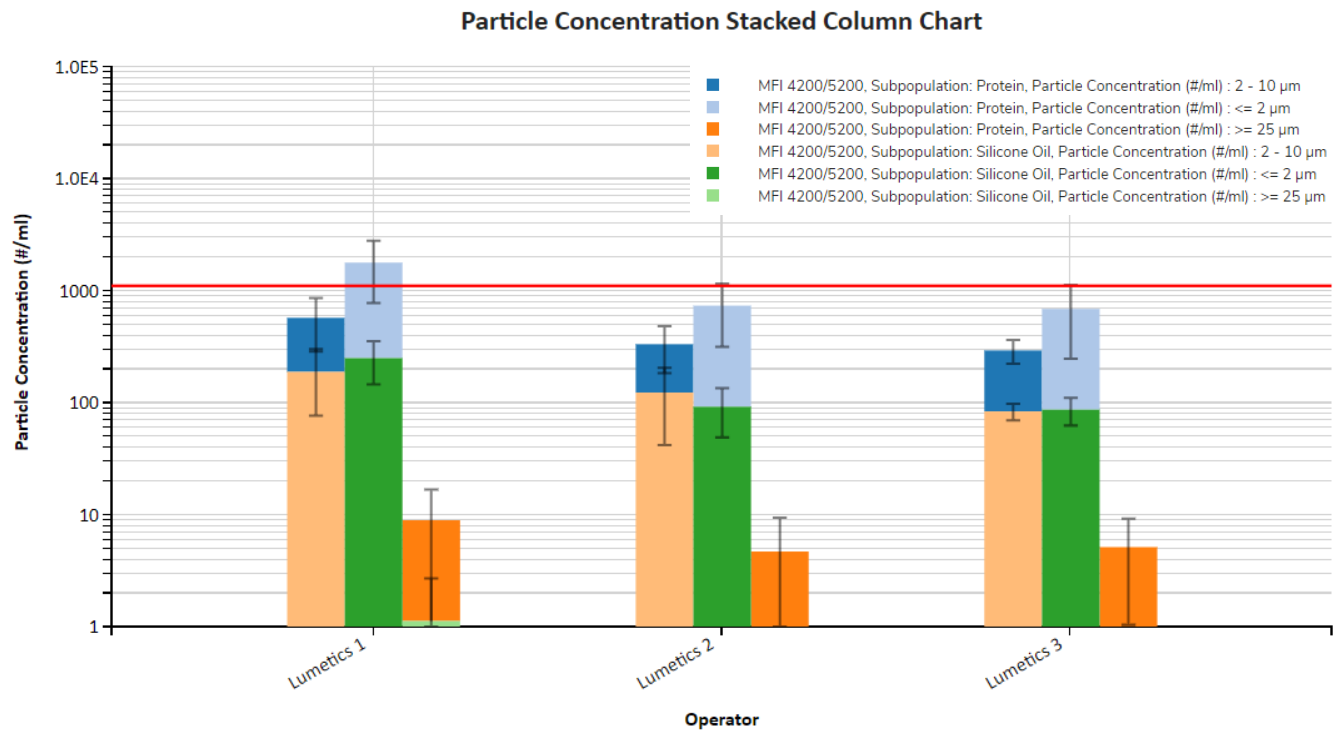
### Dilution Factor

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.

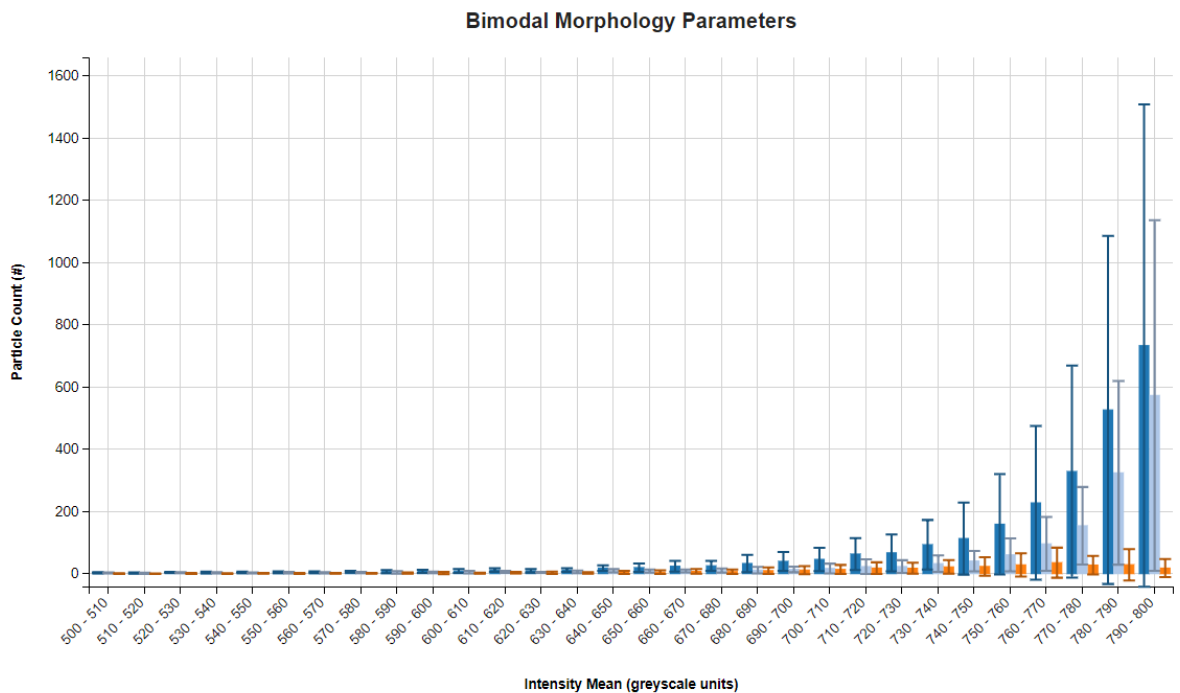
### EXAMPLES

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

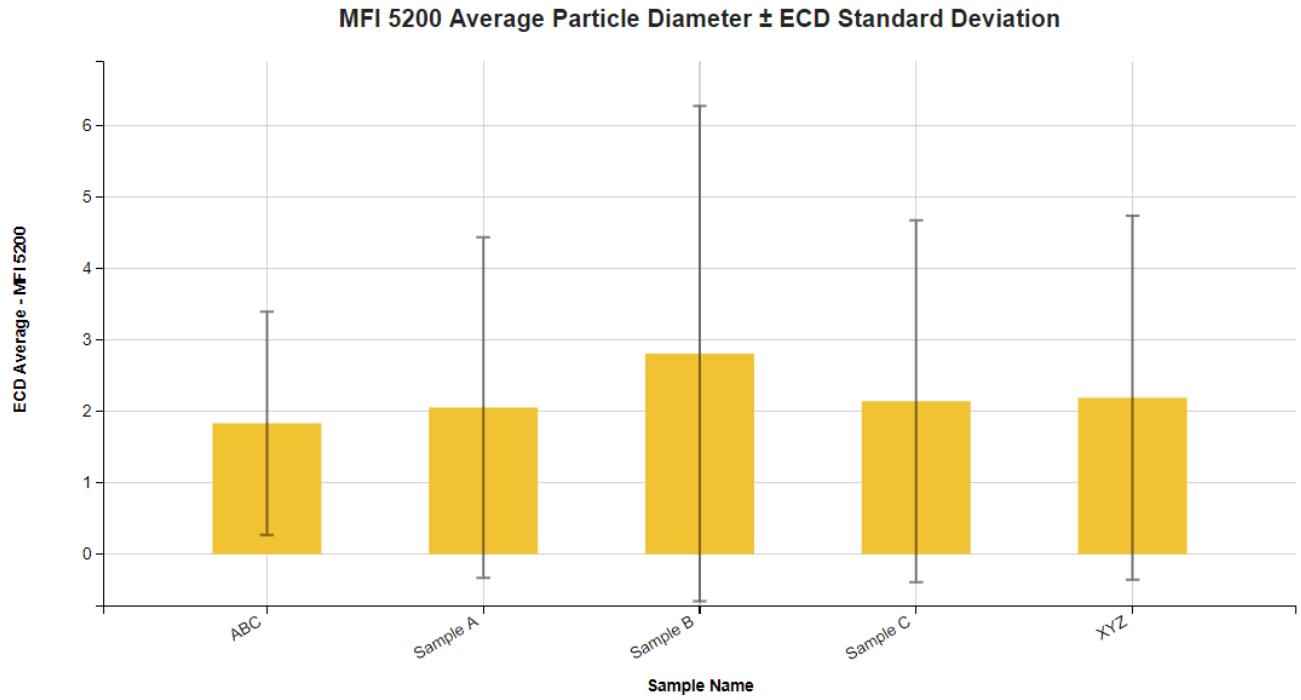
#### 1. Stacked column chart plotting raw data based on sub-populations and particle concentration ranges



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

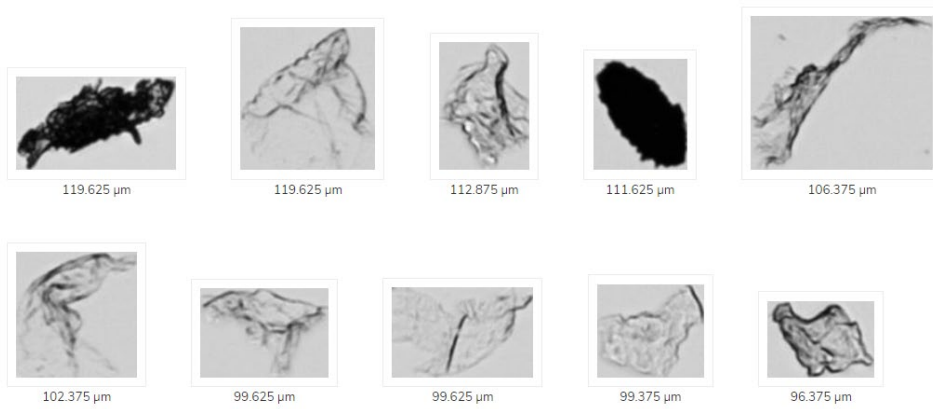


3. Column chart plotting binned instrument measurements with error bars for Average Particle Diameter vs. Sample Name

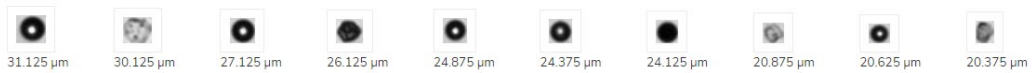


4. Particle Images organized based on sub-population

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	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

## DASHBOARD DOWNLOADS

Included below is a link to downloadable dashboards for MFI measurement files:

<http://lumetics.com/dashboards/MFI/MFI.zip>

## CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

**E-mail:** [support@lumetics.com](mailto:support@lumetics.com)

**Phone:** 1.613.614.874

**Website:** <http://lumetics.com/>

