

## FlowCAM – Fluid Imaging

(10x) VS Series/PV-100/8100, (20x) VS Series/8100, (2x) VS Series, (4x) VS Series/PV-40/8100, Nano (40x)

### 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.

FlowCAM rapidly counts and images particles in a fluid stream in order to characterize the particles using a variety of measurements.

### DETAILS

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LINK requires the CTX, LST, and TXT files for VisualSpreadsheet (ViSP) 4 and earlier. LINK may directly connect to FlowCAM database and import data from ViSP 5. Particle images (TFF) files and Notes/Comments TXT are optional. The FlowCAM system generates the required files automatically.

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 or concentration vs. morphology

The following are advanced morphological parameters calculated upon import:

- Geodesic Aspect Ratio
- Geodesic Length
- Geodesic Thickness

New feature for FlowCAM permits removal of particles from the dataset that are visualized as bubbles, or particulate nestled within the air bubbles. The feature requires re-import of any FlowCAM imported prior to LINK software version 2.3.23.200925. 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 an LNK\_Import\_Particles\_Excluded LINKdb field with a "Yes" "No"
  - Create an 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

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 4.16). Default measurement ranges will dynamically widen based as required, based on the datasets imported to LINK.

- 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).

LINK allows direct database connection to ViSP 5 version 5.2.25. This connection is established when importing FlowCAM data, selectin ViSP 5 from the drop down and adding the FlowCAM database as a connection. The username and password for the FlowCAM database can be accessed from within the ViSP 5 software via the *File -> Show Read Only Login* menu option and requires a read-only license to be purchased from Fluid Imaging before using. For easy measurement search, the keyword field will query the 'name' & 'notes' fields in ViSP 5, and the date range field allows for custom date search ranges. Runs from ViSP 5 that have deleted particles or are flagged as "deleted" will not be imported.

The second requirement for successful ViSP 5 support involves activation of read only tables that LINK requires access to. The tables we expect to see from the ViSP software are:

- context\_history
- image\_frame
- particle
- particle\_image
- particle\_property
- run
- run\_summary

If none of these tables are visible, please follow the below instructions:

- Navigate t the ViSP 5 app to File -> "Update Read Only Tables"
- The software will prompt for the Password to the database
- Proceed to add the missing tables

### Import Data

To start an import job, please select the instrument, the data location (typically a network share that the LINK Server can access), an Import Method, and a Project to import the data into.

Select Instrument: FlowCAM (10x) VS Series/PV-100/8100 ViSP 5

Select Database Connection: Please Select a Data Source Add Connection...

Search Keywords: Optional (leave blank for all measurements)

Date Range: ▼

Select Import Method: Default Method Create/Edit Method...

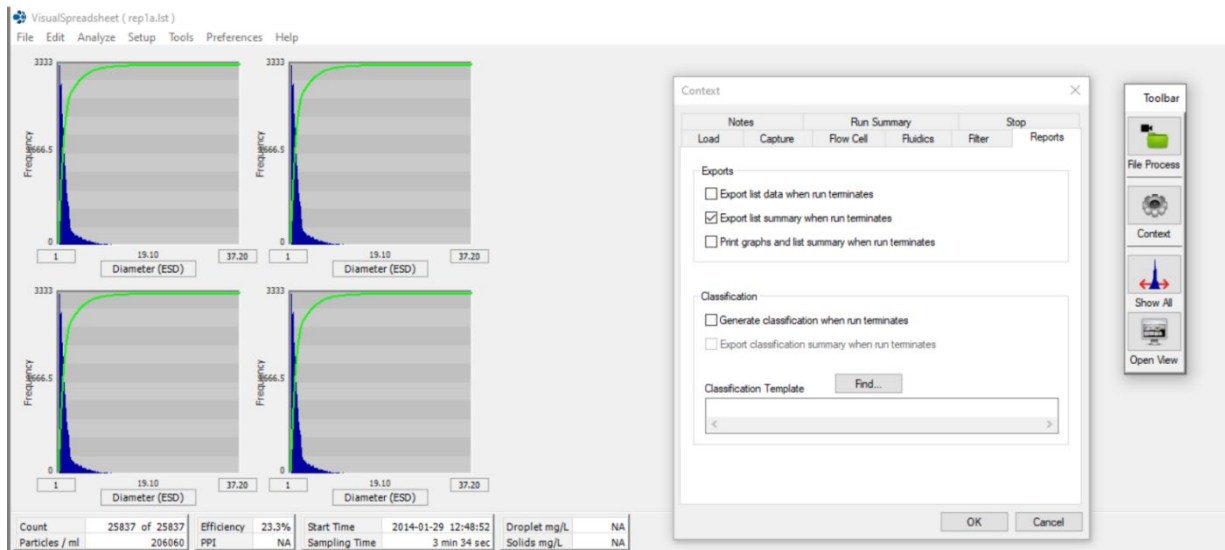
Select LINK Project: FlowCam Create Project...

Overwrite Measurements (Metadata Fields will be preserved)

Cancel
View Scheduled Jobs
Schedule Job for Later...
Scan For Measurements

Note: LINK calculates particle concentration dynamically within LINK using the particle counts and the volume analyzed. To obtain the volume analyzed, the ml is stripped from the "Fluid Volume Imaged" field value and used the number for LINK calculations. If the run\_summary file reports both Count and Concentration values, these are used for Volume Analyzed and Sample Analyzed field calculations. If This method is used, the system LINKdb field 'LNK\_Volume\_Analyzed\_Calculated' will note a value of "yes" instead of "no". If using ViSP v4.x, for calculated concentrations to line up with Visual Spreadsheet, select the Context option from the Toolbar, select Reports, and select the "Export list summary when run terminates" option.





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 located in the software help menu (section 4.13). The following is the default FlowCAM-specific import method settings:

- Default Sub-Populations: **None**
- Custom Particle Parameters: **None**
- Container Fill Volume: **None**
- Stuck/Repeating Particle Removal: **Off**
- Number of Thumbnails to Crop: **250**
- Ignore Thumbnails less than: **5  $\mu\text{m}$**
- 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 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.

### 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.

### 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

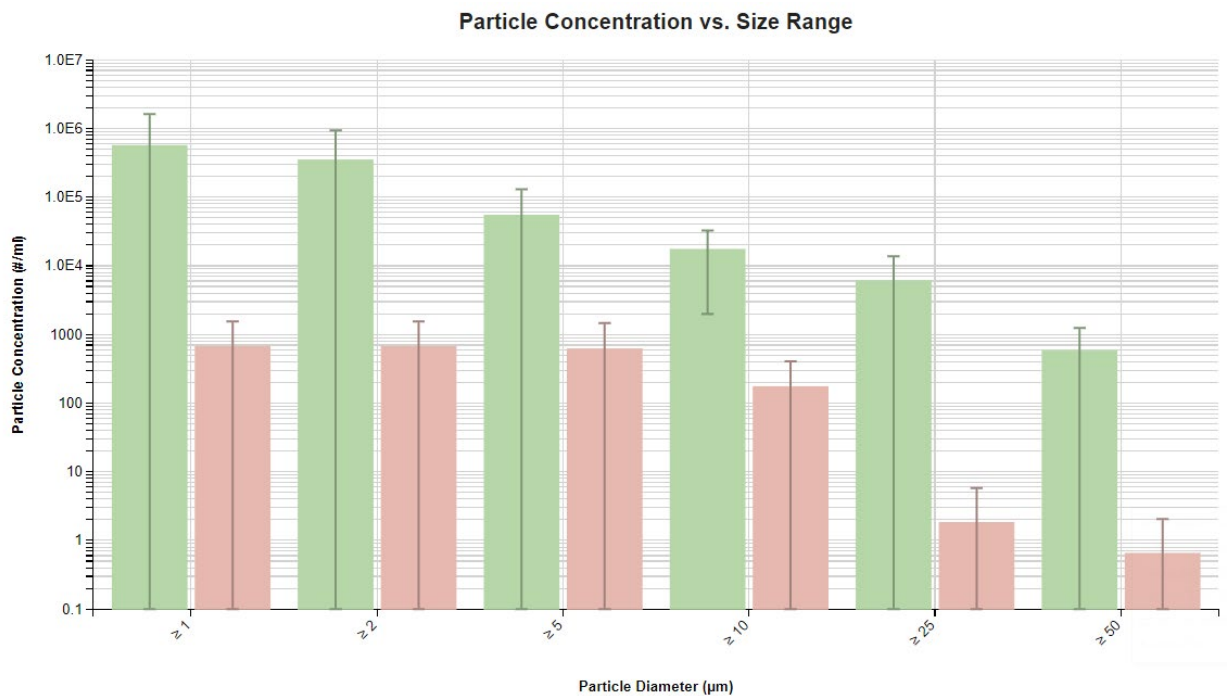
Specify a dilution factor for automated particle count/concentration data adjustment. This selection applies to the total particle population and all defined sub-populations. A LNK\_Dilution\_Factor may also be applied after import, utilizing the Add/Edit LINKdb Fields feature.

## EXAMPLES

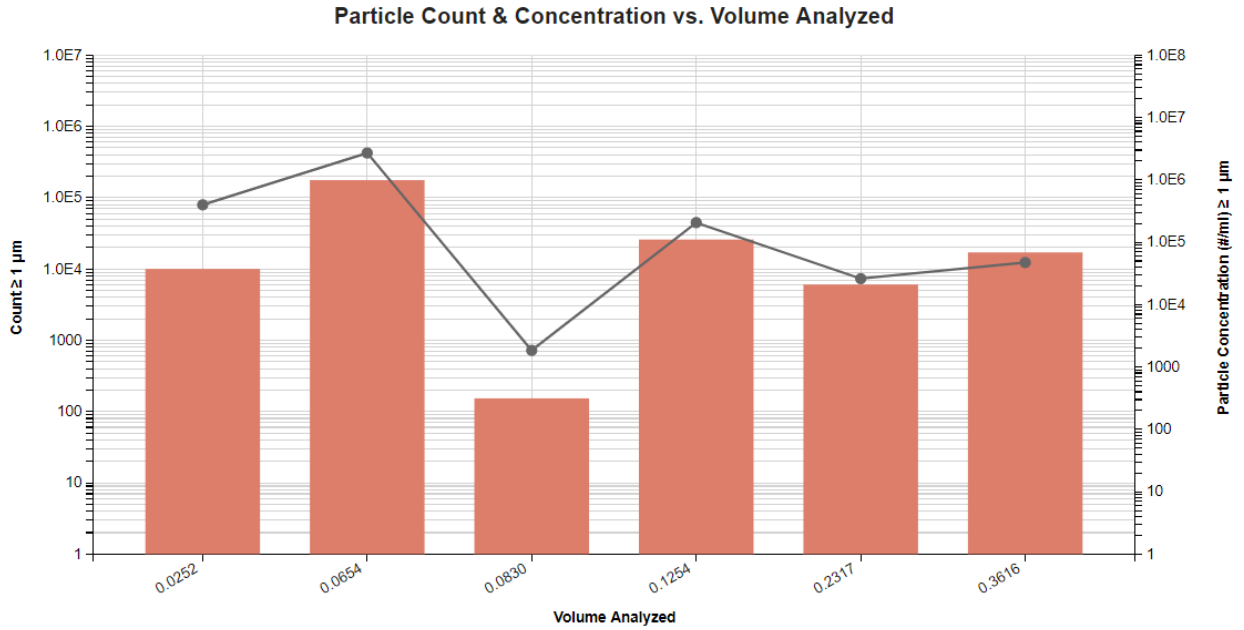
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Included below are example dashboards from FlowCAM measurement files:

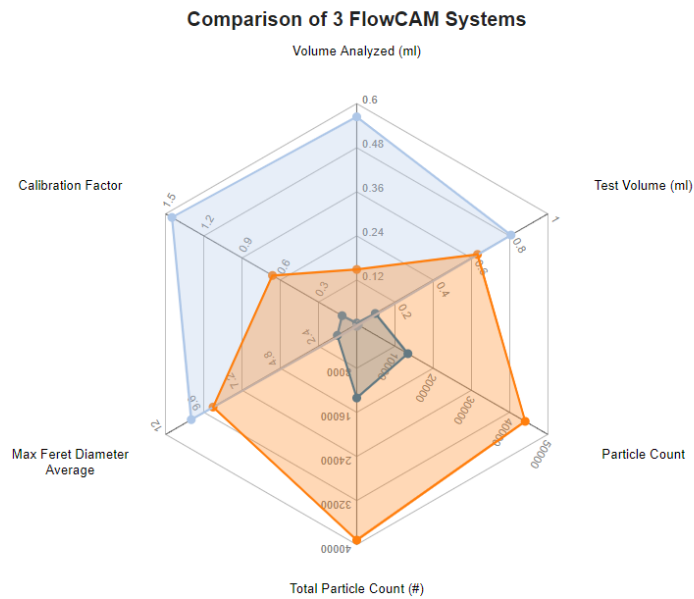
### 1. Histogram plotting binned raw data for Particle Concentration vs. Particle Diameter



2. Column chart plotting Particle Count  $\geq 1\mu\text{m}$  & Concentration  $\geq 1\mu\text{m}$  vs. Volume



3. Radar chart comparing three FlowCAM systems (10x, 4x, nano)



4. Tabular Summary examples

Measurement Summary Table – Instrument Settings

Sample Name	File Name	Syringe Size	Serial Number	RunStartTime	RunEndTime	PumpFlowRate - AVG	Efficiency
Sample 6	Proteins_PPC=1.lst	1.00 ml	482	2013-06-14 15:50:58	2013-06-14 15:58:44	0.20	24.1%
Sample 6	Rep1.lst	1.00 ml	10021	2014-01-29 17:40:17	2014-01-29 17:43:34	0.05	63.0%
Sample 6	rep1a.lst	1.00 ml	10021	2014-01-29 17:48:52	2014-01-29 17:53:04	0.15	23.3%
Sample 6	Sample_1.lst		Unknown	2010-08-20 14:32:10	2010-08-20 14:33:52	0.00	
Sample 1	Silicone oil.lst	1.00 ml	NA	2019-02-13 09:14:54	2019-02-13 09:16:42	0.15	35.0%
Sample 6	testa.lst	1.00 ml	Unknown	2012-01-13 01:50:18	2012-01-13 01:55:42	0.15	29.5%

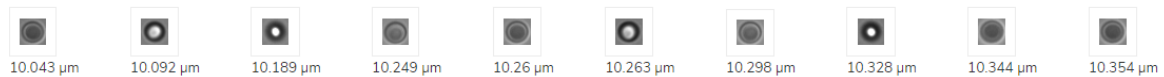


## Measurement Summary Table – Measurement Results

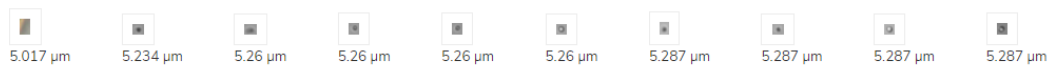
Sample Name	Volume Analyzed (ml)	≥1µm AVG	2-10µm - AVG	≥10µm AVG	≥25µm AVG	Total Particle Concentration (#/ml) - AVG	Total Particle Count (#) - AVG
Sample 1	Multiple (4 Values)	539,174	294,554	8,841	3,972	539174.50	36139.20
Sample 2	Multiple (10 Values)	565	403	161	1	564.60	298.90
Sample 3	Multiple (8 Values)	359	212	147	0	358.93	189.38
Sample 4	Multiple (6 Values)	100	66	34	1	100.39	51.00
Sample 5	Multiple (8 Values)	1,359	1,004	355	1	1358.60	716.00
Sample 6	Multiple (5 Values)	135,584	100,282	11,951	3,252	135583.66	11816.40

## 5. Particle Images organized by FlowCAM systems

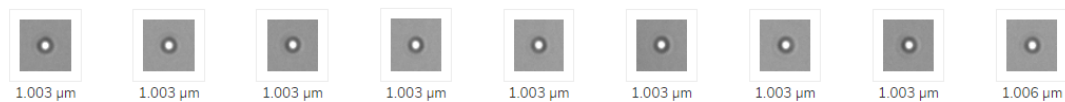
### FlowCAM (10x) VS Series/PV-100/8100, Total Population



### FlowCAM (4x) VS Series/PV-40/8100, Total Population



### FlowCAM Nano (40x), Total Population



## DASHBOARD DOWNLOADS

Included below is a link to downloadable dashboards for FlowCAM measurement files:  
<http://lumetics.com/dashboards/FlowCAM/FlowCAM.zip>

## CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

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