

FlowCam Light Obscuration– Fluid Imaging

INTRODUCTION

The Lumetecs 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 to characterize the particles using a variety of measurements.

DETAILS

The FlowCam instrument can support a Light Obscuration (LO) mode, and this data can be imported into the LINK software if exported from the Visual Spreadsheet 5,6+ software in CSV format. The Light Obscuration data can be exported in two ways:

- As data collected alongside an imaging run (mixed imaging + LO)
- Exported as an LO measurement on its own

An example of a summary CSV file containing both LO data and imaging data is shown below, where the LO data is contained in a section titled "LO Statistics"

```
===== LO Statistics =====
Name,LO Count,Count:>= 2 um ,Count: >=5 um ,Count: >=10 um ,Count: >=15 um ,Count: 2<=um<5,Count:
17032024-SAMPLE_A-14-42-23,572,572,35,2,0,521,46,9,0,1617.31,1617.31,154.43,25.27,0.00,1462.88,129
===== End LO Statistics =====
```

An example of a summary CSV file with LO data exported on its own (without imaging data) is shown below, where the data is contained in a section called "LO Data"

```
=====LO Module=====
Serial Number : 122222 Last Calibration : 2025-01-14 14:13:15
=====End LO Module=====

=====LO Data=====
Name,VS Count,LO Count,Count:>= 2 um ,Count: >=5 um ,Count: >=10 um ,Count: >=15 um ,Count: 2<=um<5,Count: 5<=
23012025-SAMPLE_A-15-04-05,314,5,4,0,0,0,4,0,0,0,922.44,3.15,2.05,0.00,0.00,0.00,3.15,0.00,0.00,0.00
=====End LO Data=====
```

To import only LO data, open the Import dialog and select the "FlowCam (LO)" instrument from the instrument drop-down list. This will search all summary CSV files and import LO data contained in both the mixed image/LO files, and LO data exported on its own as explained above.

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

Mandatory File: ViSP 5+ CSV summary export with LO data ?

For convenience, the LINK software will allow a user to select a FlowCam imaging instrument (e.g. FlowCam 10x) and have it optionally also found and import any LO measurements as its own measurements and instruments, saving having to perform two separate imports. This is shown below as the checkbox "Import LO data as separate instrument/measurements".

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

Mandatory Files: Particle Data (CSV) Export, AND Summary (CSV) Export ?

☒ Import LO data as separate instrument/measurements (if found)

When this is checked, the LINK software will look in summary CSV files containing mixed imaging and LO data, and in addition to importing the imaging measurements, it will also import any LO data found as a separate measurement and instrument (FlowCam LO). Note that it will not import LO data that is found in a summary CSV exported as only LO data alone (e.g. containing a LO Data section), these are ignored as they are not imaging exports.

If this checkbox is unchecked, then only imaging measurements will be imported and individual LO measurements will not be imported as a separate measurement.

In all cases for an imaging import regardless if the checkbox is checked or not, the LO data found in the "LO Summary" section is added as metadata LINKdb fields for the imaging measurements, so the imaging measurement will always have the LO data available as a LINKdb field (but not as raw data).

Available Metadata

The CSV files contain different information that is available as metadata, depending on if the LO data is exported on its own or exported as a mixed imaging/LO measurement. For the mixed imaging/LO files, the following fields in the summary CSV header are associated with the LO measurements as metadata LINKdb fields:

- Software (also mapped to the *Version* LINKdb field)
- SerialNo (also mapped to the *Serial Number* LINKdb field)
- Mode

After measurements have been imported, measurements imported as part of a mixed imaging/LO CSV file vs imported from an LO-only CSV file, can be determined by filtering on the LINKdb field called "LNK_LO_Measurement_From_Mixed_File" which will have a value of true or false for all FlowCam (LO) instrument measurements.

Analysis Date

For FlowCam mixed imaging/LO files, the Start Time field is used as the Analysis Date for the imaging measurements, but the FlowCam LO only summary CSV files do not contain this information. For LO measurements only, if the Sample Name of the LO measurement is in the format (e.g.) "MMddYYYY--HH-mm-ss"



with a leading day-month-year, and trailing 24-hour time value, then these values will be assigned to the LO measurement's Analysis Date LINKdb field. If the Sample Name format does not match this, then the Analysis Date will be set as NULL for the LO measurements found in a CSV exported as LO data only and will be set to the Start Time value for the mixed imaging/LO CSV export.

IMPORT METHOD

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**
- Machine Learning Particle Classification: **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 μ m**
- Import Images in order of Largest-to-Smallest: **Yes**
- Advanced Image Selection Criteria: **None**
- Automated Metadata Extraction: **None**
- 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.

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.

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.

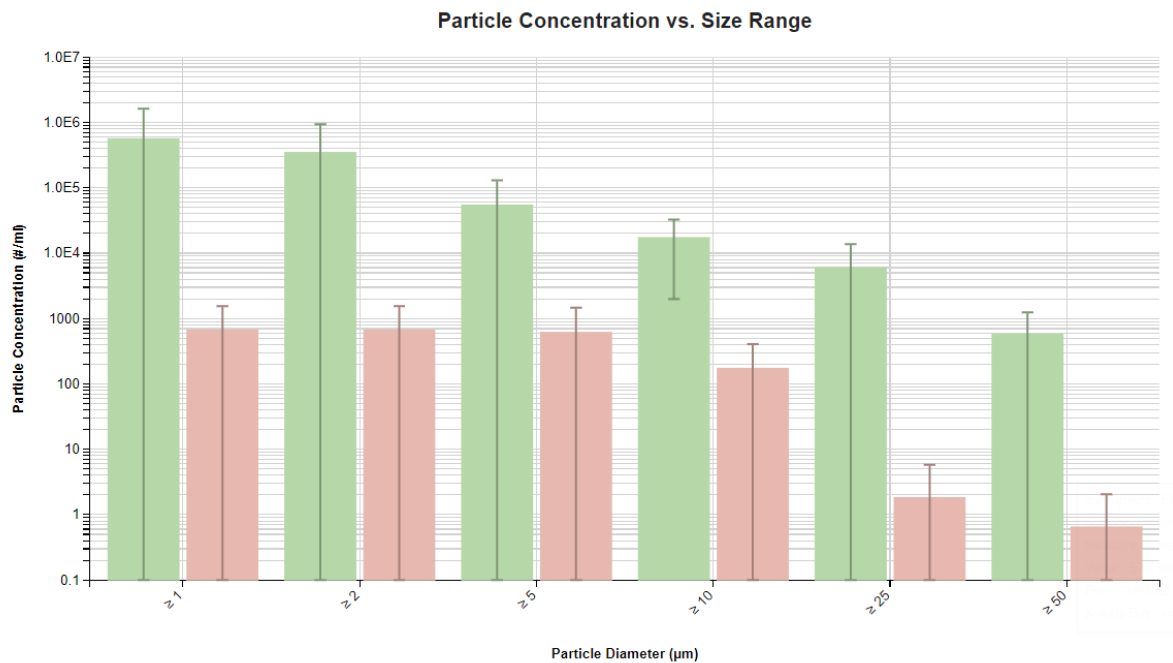
Dilution Factor

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.

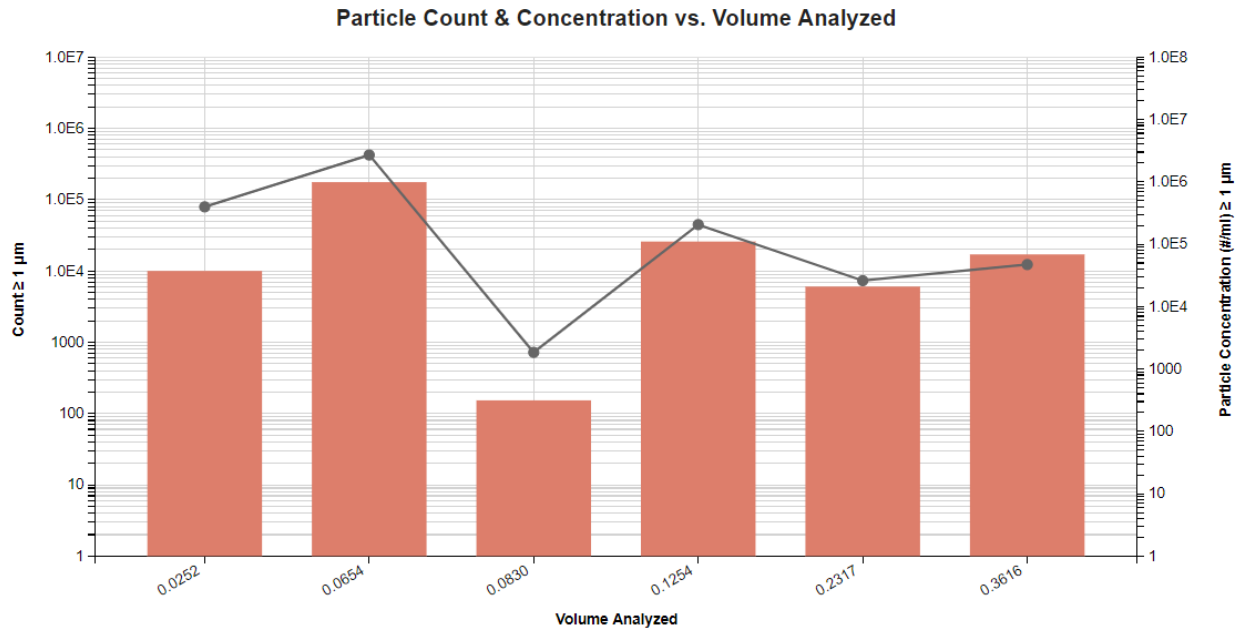
EXAMPLES

Included below are example dashboards from 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. Tabular Summary examples

Measurement Summary Table – Instrument Settings

Sample Name	File Name	Syringe Size	Serial Number	RunStartTime	RunEndTime	PumpFlowRate - AVG	Efficiency
Sample 6	Proteins_PPC1.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	Silicocone 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

4. 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



CONTACT LUMETICS

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