FlowCam – Fluid Imaging

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

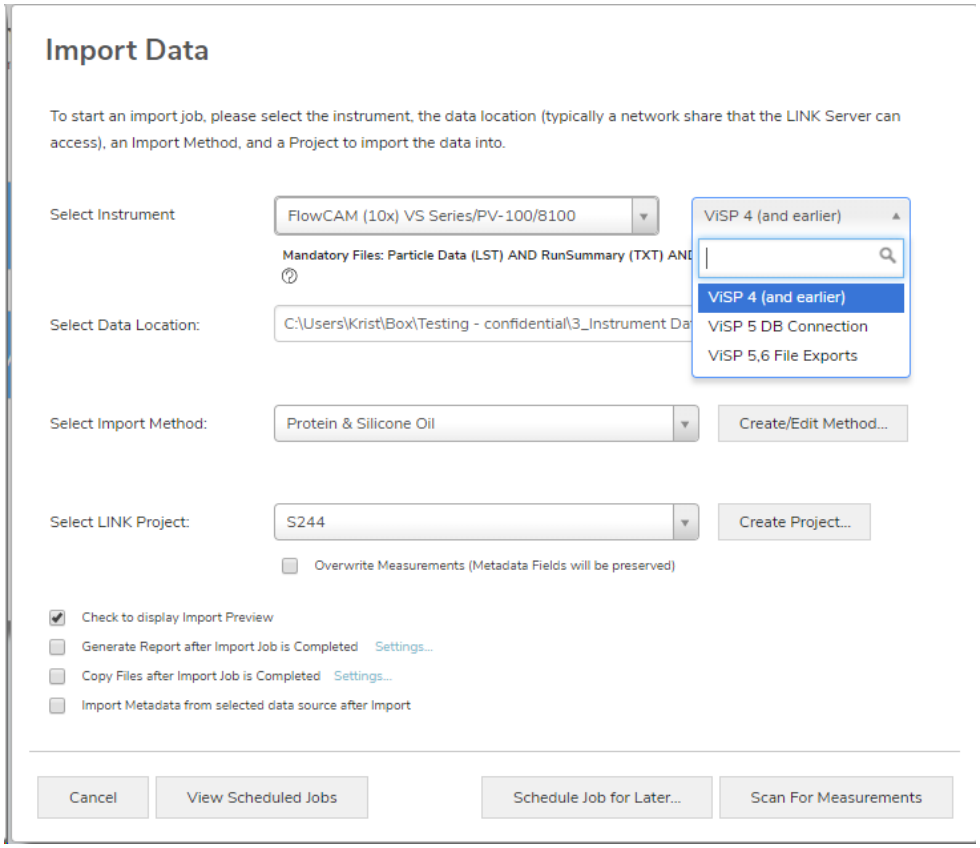
INTRODUCTION

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 to characterize the particles using a variety of measurements.

DETAILS

LINK may import FlowCam data from either Visual Spreadsheet version 4, 5 and 6. Particle images may optionally be imported as PNGs (ViSP 5 & 6) and TIFF (ViSP 4, ViSP 6). Once the FlowCam instrument and respective magnification is selected for import, another drop down will be populated where the user must select ViSP 4 (and earlier), ViSP 5 DB Connection, and ViSP 5,6 File Exports. Please refer to the below sections for specifics relating to each FlowCam version.



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

Parameter Name	FlowCam Nano (40X)			FlowCam 2X, 4X, 10X, 20X		
	Bin Spacing	Meas. Start	Meas. End	Bin Spacing	Meas. Start	Meas. End
abd_area	0.001	0	100	10	1	5000
abd_diameter	0.001	0	100	0.25	1	5000
Analysis Time	1	0	5000	1	0	5000
Area	10	1	5000	10	1	5000
AspectRatio	0.01	0	1	0.01	0	1
avg_blue	1	0	255	1	0	255
avg_green	1	0	255	1	0	255
avg_red	1	0	255	1	0	255
Circularity	0.01	0	1	0.01	0	1
circularity_hu	0.01	0	1	0.01	0	1
compactness	0.1	1	5000	0.1	1	5000
convex_perimeter	1	1	5000	1	1	5000
ECD	0.0001	0	100	0.0001	1	10000
edge_gradient	1	1	500	1	1	500
elongation	1	1	5000	1	1	5000
feret_max_angle	5	-85	90	5	-85	90
feret_min_angle	5	-85	90	5	-85	90
filled_area	10	1	500	10	1	500
Geodesic Aspect Ratio	0.01	0	1\N	0.01	0	1\N
Geodesic Length	0.1	0	1000\N	0.1	0	1000\N
Geodesic Thickness	0.1	0	1000\N	0.1	0	1000\N
intensity_calimage	1	0	255	1	0	255
IntensityMean	1	0	5000	1	0	5000
IntensitySTD	1	0	255	1	0	255
length	0.0001	0	100	0.0001	0	5000
MaxFeretDiameter	0.0001	0	100	0.0001	0	5000
perimeter	1	1	5000	1	1	5000
ppc	1	1	500	1	1	500
raw_convex_hull_area	10	1	500	10	1	500
raw_convex_perimeter	1	1	5000	1	1	5000
raw_feret_max	1	0	5000	1	1	5000
raw_feret_mean	1	0	5000	1	1	5000
raw_feret_min	1	0	5000	1	1	5000
raw_filled_area	10	1	500	10	1	500
raw_perimeter	1	1	5000	1	1	5000
roughness	0.01	1	5000	0.01	1	5000
sum_intensity	1000	1	5000	1000	1	5000
symmetry	0.01	0	1	0.01	0	1
timestamp	5	0	3600	5	0	3600
width	0.001	0	100	1	1	5000

Notes:

- 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).
- **Custom Particle Parameters** can be defined and be generated for each measurement during import (e.g., calculate particle mass assuming a density and volume) if it is first created/defined and then requested within an Import Method.

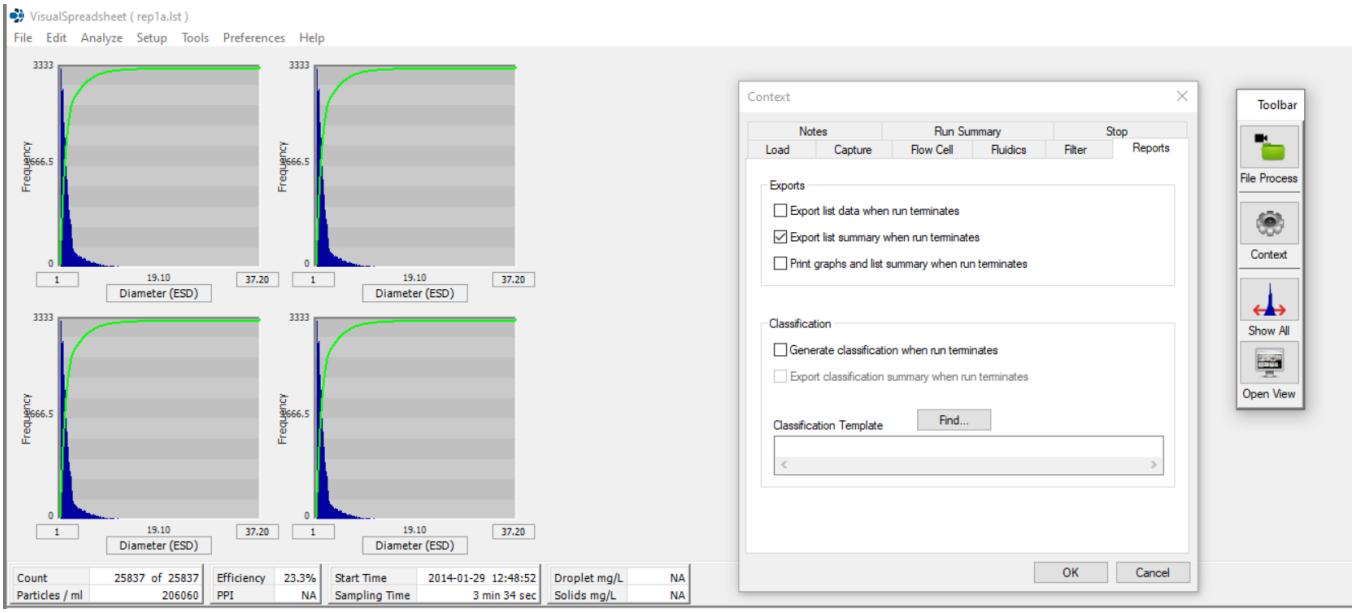
- A more precise volume analyzed is available for users of more recent versions of the Visual Spreadsheet version 4 software. Whether this has been applied on a given import shall be noted in a database field called: LNK_Sample_Analyzed_Calculated
- A wide range of Import Method options are available. These options are noted below.

Importing from Visual Spreadsheet v4 or earlier

LINK requires the CTX, LST, and TXT files for VisualSpreadsheet (ViSP) 4 and earlier. Particle images (TFF) files and Notes/Comments TXT are optional. If present, an optional CSV that will deliver improved precision on concentrations. The FlowCam system generates the required files automatically.

Note:

- Within LINK, the field 'circularity' is mapped from 'circle_fit' in the LST file.
- 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.



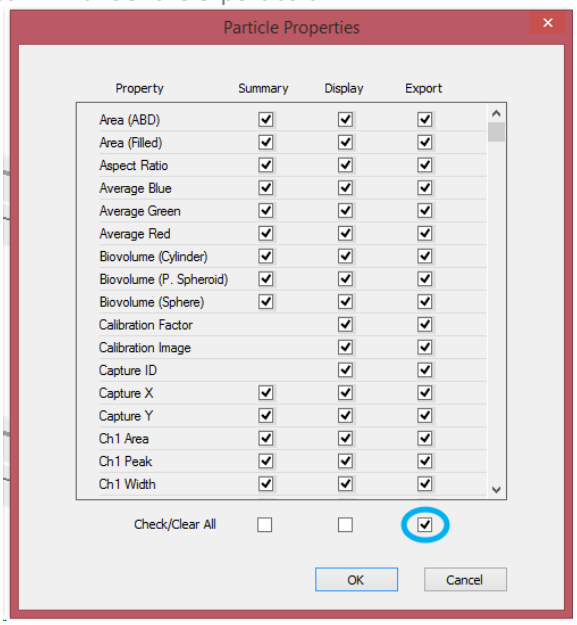
The FlowCam 10x lst data file example is as follows:

```
017
num-fields|50
id|int32
image_x|int32
image_y|int32
image_w|int32
image_h|int32
src_x|int32
src_y|int32
src_image|int32
cal_image|int32
ppc|int32
camera|int32
cal_const|double
fringe_size|double
raw_area|double
raw_feret_max|double
raw_feret_min|double
raw_feret_mean|double
raw_perimeter|double
raw_convex_perimeter|double
abd_area|double
abd_diameter|double
length|double
width|double
esd_diameter|double
perimeter|double
convex_perimeter|double
intensity|double
sigma_intensity|double
sum_intensity|double
compactness|double
elongation|double
roughness|double
edge_gradient|double
circle_fit|double
feret_max_angle|double
feret_min_angle|double
avg_red|double
avg_green|double
avg_blue|double
ch1_peak|double
ch1_width|double
ch1_area|double
ch2_peak|double
ch2_width|double
ch2_area|double
ch3_peak|double
ch3_width|double
ch3_area|double
timestamp|timestamp
collage_file|string
1|2|2|21|20|429|38|32|1|1|0|0.5515|4.5000|103.0000|14.0263|9.6603|12.3764|41.2132|
```

Importing from Visual Spreadsheet v5 CSV

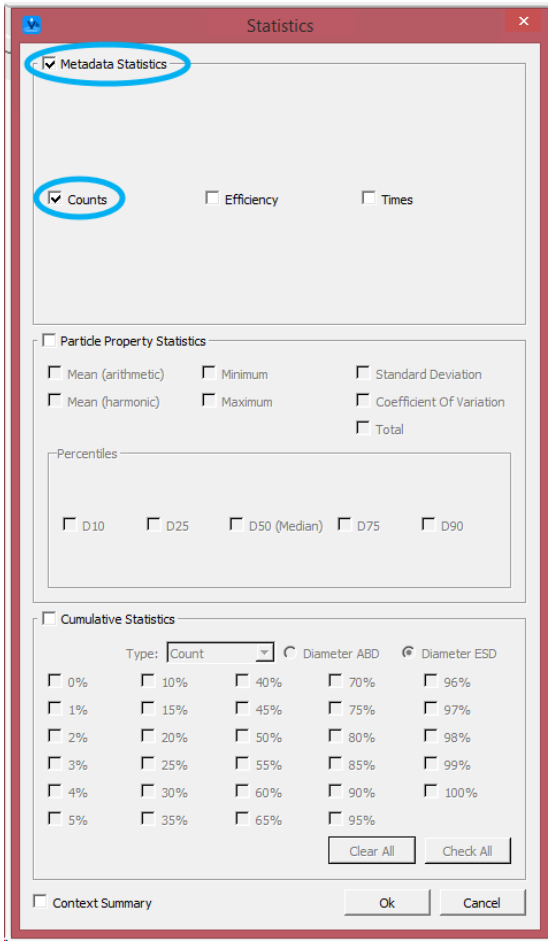
LINK allows the import from CSV's exported from the ViSP5 software. There are two required CSVs to export from the ViSP5 software for LINK import:

- Data CSV, which represents the particle raw data, where the first line must start with 'Name.'
    - To ensure all mandatory fields are exported, navigate to Preferences, select Particle Properties, and select "Check All" under the export column.



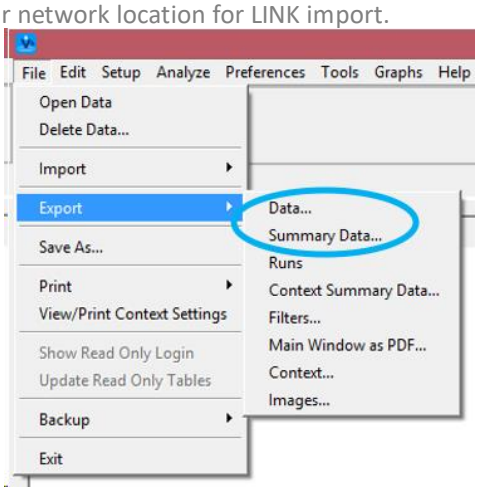
* Mandatory fields for import include: UUID and Timestamp

- Summary CSV, where the value in the Name column matches that of the Data CSV. LINK also requires “Metadata Statistics”. To include this statistic, navigate to Preferences, Statistics…, select “Metadata Statistics” and “Counts” under the statistics section. The other statistics are optional!



* “Run Summaries” must be in the first line and must complete with “Run Summaries End”. This should occur by default.

- To export the two data files, navigate to File, Export, and individually select “Data” and then “Summary Data” and save them to your network location for LINK import.



Notes:

- ViSP 5 allows users to group multiple runs together as a “Set”. The Raw Data CSV file will have the name of the “Set” as the “Name” column, but the Export Summary CSV will have the individual run(s) exported separately. If the value in the “Name” column doesn’t match the value(s) in an Export Summary CSV file, then the data will not be imported.
- Images may be imported but there is no information in the CSV file on where the image is located, therefore images cannot be cropped.
- Within LINK, ‘circularity’ is imported from CSV files or directly from the FlowCam database and ‘circle_fit’ will be ignored.
- If there is a difference between the particle count in the particle data csv and the total particle count in the run summary file, only a subset of the data will be imported.

The FlowCam 10x ViSP 5 CSV Export example required files:

1. Summary CSV

	A	B	C	D	E	F	G	H	I	J	K	L
1	Name	Area (ABD)	Area (Filled)	Aspect Ratio	Average Blue	Average Green	Average Red	Biovolume (Cylinder)	Biovolume (P. Spheroid)	Biovolume (Sphere)	Calibration Factor	Calibration Image
2	2020-09-14 10:01	541.79	612.89	0.85	131.25	131.34	130.87	12990.75	10624.79	9486.65	0.67	0
3	2020-09-14 10:01	610.87	682.36	0.97	111.02	110.95	110.39	14302.25	13222.38	11357.71	0.67	0
4	2020-09-14 10:01	622.16	693.71	1	102.15	101.69	100.38	14758.27	13743.72	11673.84	0.67	0
5	2020-09-14 10:01	622.16	693.71	1	84.37	83.15	81.32	14758.27	13743.72	11673.84	0.67	0
6	2020-09-14 10:01	1.12	1.12	0.16	128	128	128	4.35	0.66	0.89	0.67	0
7	2020-09-14 10:01	622.16	693.71	1	76.04	73.97	71.93	14758.27	13743.72	11673.84	0.67	0

2. Run (raw data) CSV

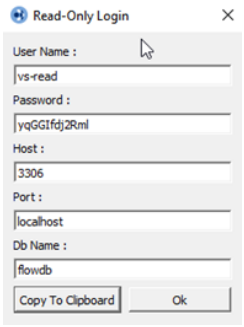
	A	B	C	D
1	========Run Summaries========			
2				
3	Name: 100 P11			
4				
5	Run:			
6	Mode	AutoImage		
7	Recalibrat	0		
8	Stop Reas	Particle Count		
9	Fluid Volu	0.0048 ml		
10	Efficiency	N/A		
11	Particle Cc	101		
12				
13	Images:			
14	Total	112		
15	Used	58		
16	Percentag	51.79%		
17	Particles P	0.9		
18	Frame Rat	310.25 fps		
19	Backgrour	127.93		
20	Backgrour	127.81		
21	Backgrour	128		
22				
23	Date/Time:			
24	Start Time	2020-09-14 09:55:10		
25	End Time	2020-09-14 09:55:11		
26	Sampling '	00:00:00		
27				
28	Environm	VisualSpreadsheet5 5.4.5		
29	Software	VisualSpreadsheet5 5.4.5		
30	Magnificat	10X		
31	Calibratio	0.668		
32	SerialNo	Unknown		
33	Number o	8		

Importing from Visual Spreadsheet v5 DB Connection

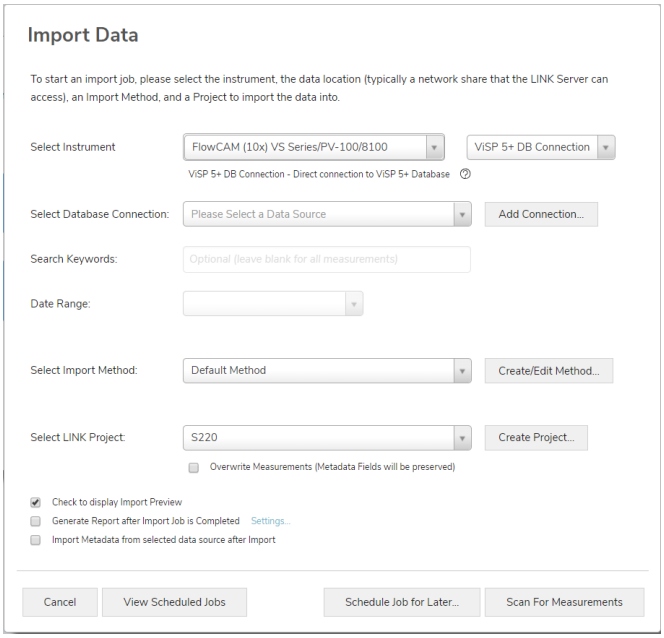
LINK provides a direct database connection to ViSP 5 software. To create and utilize a database connection, read only access to the ViSP5 database is required (typically provided by Fluid Imaging Technologies).

After acquiring and installing the read only license provided by Fluid Imaging Technologies, please follow these instructions to create a ViSP5 database connection:

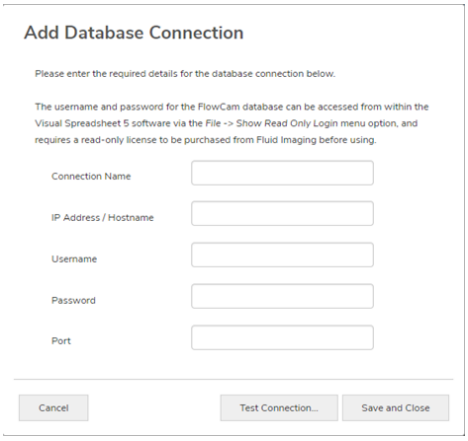
1. Within ViSP5 and select Update Read Only Tables from under the File menu
2. Within ViSP5, select Show Read Only Login from under the File menu. You should now have something that looks like this:



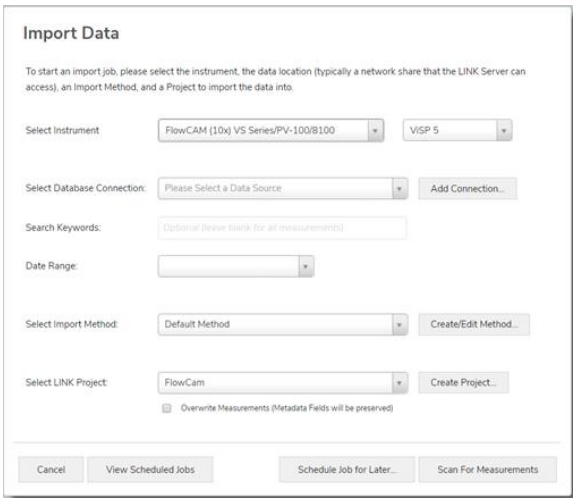
3. Within LINK, Launch an import for a FlowCam system. Select ViSP5 beside the instrument model. Then select the Add Connection button.



4. Assign a name to the database connection you are establishing (e.g., the specific ViSP5 system you are connecting to), and complete the rest of the fields based on the content of the read only login information in step 2. Test the connection, and if it reports success, select Save and Close. You are then ready to import data to LINK using this new database connection! Note: LINK splits the FlowCam instrument based on magnification (2x, 4x, 10x, 20x, 40x). When establishing a ViSP 5 database connection, a new connection needs to be made based on each magnification.

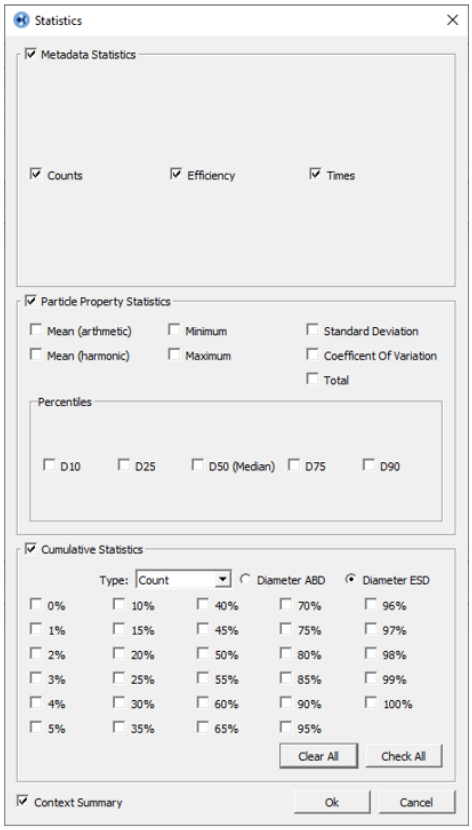


- Import Data using the newly established ViSP5 database connection. Keywords search will query the ‘name’ & ‘notes’ fields in ViSP 5 database, and the date range field allows for custom date search ranges. Please Note: Runs from ViSP 5 that have deleted particles or are flagged as “deleted” will not be returned from the search query.



Note: A more precise volume analyzed is available by following the below instructions in the visp5 software:

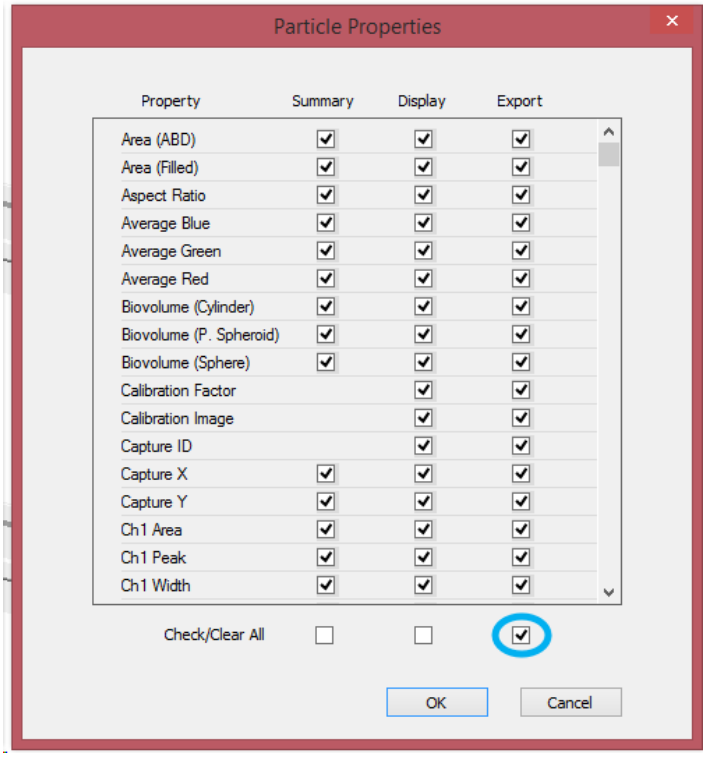
- Select preferences from the top menu bar, then select statistics
- Select the “Metadata Statistics” checkbox, then select ‘ok’
- This adds high efficiency particle counts and concentration to the run summary file. These values are used for Volume Analyzed and Sample Analyzed field LINK calculations.
- If this method is used, the system LINKdb field ‘LNK_Volume_Analyzed_Calculated’ will note a value of "yes" instead of "no".



Importing from Visual Spreadsheet v6 CSV

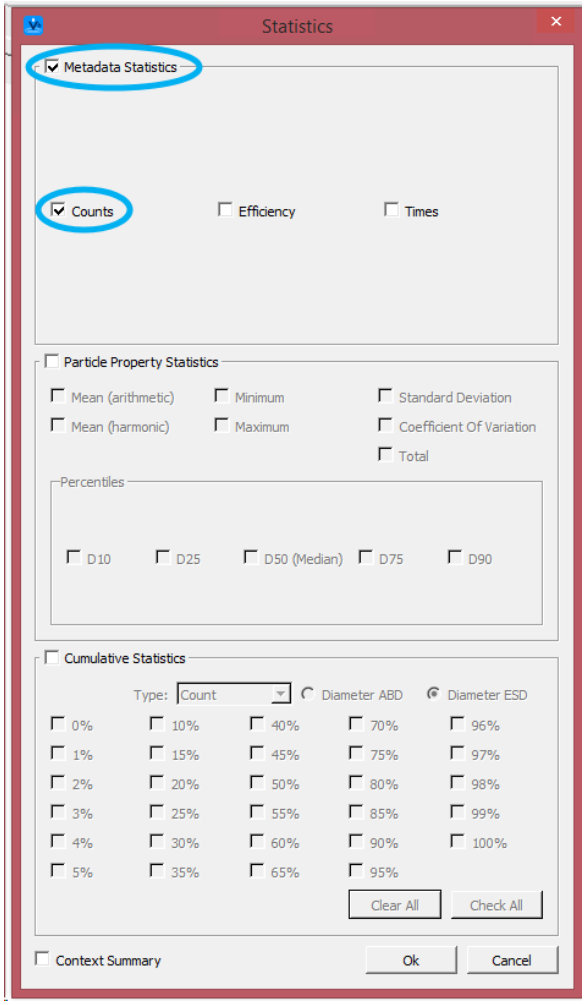
LINK allows the import from CSV's exported from the ViSP v6 software and optionally particle image files. Particle images may be PNG or TIFF files, however the TIFF will be converted to PNG upon LINK import. There are two required CSVs to export from the ViSP6 software for LINK import:

- Data CSV, which represents the particle raw data, where the first line must start with 'Name.'
    - To ensure all mandatory fields are exported, navigate to Preferences, select Particle Properties, and select "Check All" under the export column.



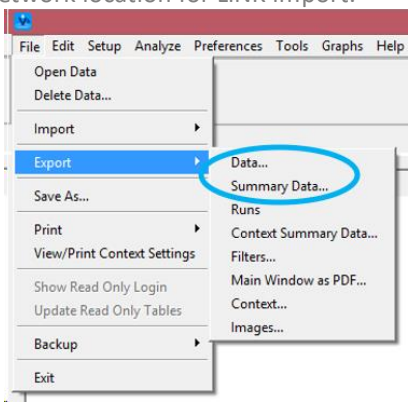
* Mandatory fields for import include: UUID and Timestamp

    - Summary CSV, where the value in the Name column matches that of the Data CSV. LINK also requires "Metadata Statistics". To include this statistic, navigate to Preferences, Statistics…, select "Metadata Statistics" and "Counts" under the statistics section. The other statistics are optional!



* "Run Summaries" must be in the first line and must complete with "Run Summaries End". This should occur by default.

- To export the two data files, navigate to File, Export, and individually select "Data" and then "Summary Data" and save them to your network location for LINK import.



The FlowCam 10x ViSP 6 CSV Export example required files:

1. Summary CSV

	A	B	C	D
1	========Run Summaries========			
2				
3	Name: 17052023-GN004681_001_12_3-18-01-34			
4				
5	Run:			
6	Mode	AutoImage Light Obscuration		
7	Priming Method	machine prime		
8	Flow Rate	0.200 mL/min		
9	Recalibrations	0		
10	Stop Reason	Sample Volume Processed		
11	Sample Volume Aspirated	0.3636 mL		
12	Sample Volume Processed	0.3598 mL		
13	Fluid Volume Imaged	0.1937 mL		
14	Efficiency	53.82%		
15	Particle Count	13206		
16				
17	Images:			
18	Total	3233		
19	Used	3179		
20	Percentage Used	98.33%		
21	Frame Rate	29.96 fps		
22	Background Intensity Mean	172.45		
23	Background Intensity Min	171.8		
24	Background Intensity Max	173.16		
25				
26	Date/Time:			
27	Start Time	2023-05-17 18:01:35		
28	End Time	2023-05-17 18:03:38		
29	Sampling Time	00:01:47		
30				
31	Environment	VisualSpreadsheet6 6.0.2.167		
32	Software	VisualSpreadsheet6 6.0.2.167		
33	Magnification	10X		
34	Calibration Factor	0.6944		
35	SerialNo	10866		
36	Number of Processors	8		
37	Pump	C80 Syringe		
38	Syringe Size	2.50 mL		
39				
40	========Run Summaries End========			

2. Data/Run (raw data) CSV

	A	B	C	D	E	F	G	H	I	J	K	L
1	Name	Area (ABD)	Area (Filled)	Aspect Ratio	Average Blue	Average Green	Average Red	Biovolume (Cylinder)	Biovolume (P. Spheroid)	Biovolume (Sphere)	Calibration Factor	Calibration Image
2	2020-09-14 10:01	541.79	612.89	0.85	131.25	131.34	130.87	12990.75	10624.79	9486.65	0.67	0
3	2020-09-14 10:01	610.87	682.36	0.97	111.02	110.95	110.39	14302.25	13222.38	11357.71	0.67	0
4	2020-09-14 10:01	622.16	693.71	1	102.15	101.69	100.38	14758.27	13743.72	11673.84	0.67	0
5	2020-09-14 10:01	622.16	693.71	1	84.37	83.15	81.32	14758.27	13743.72	11673.84	0.67	0
6	2020-09-14 10:01	1.12	1.12	0.16	128	128	128	4.35	0.66	0.89	0.67	0
7	2020-09-14 10:01	622.16	693.71	1	76.04	73.97	71.93	14758.27	13743.72	11673.84	0.67	0

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 µm**
- 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 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.

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.

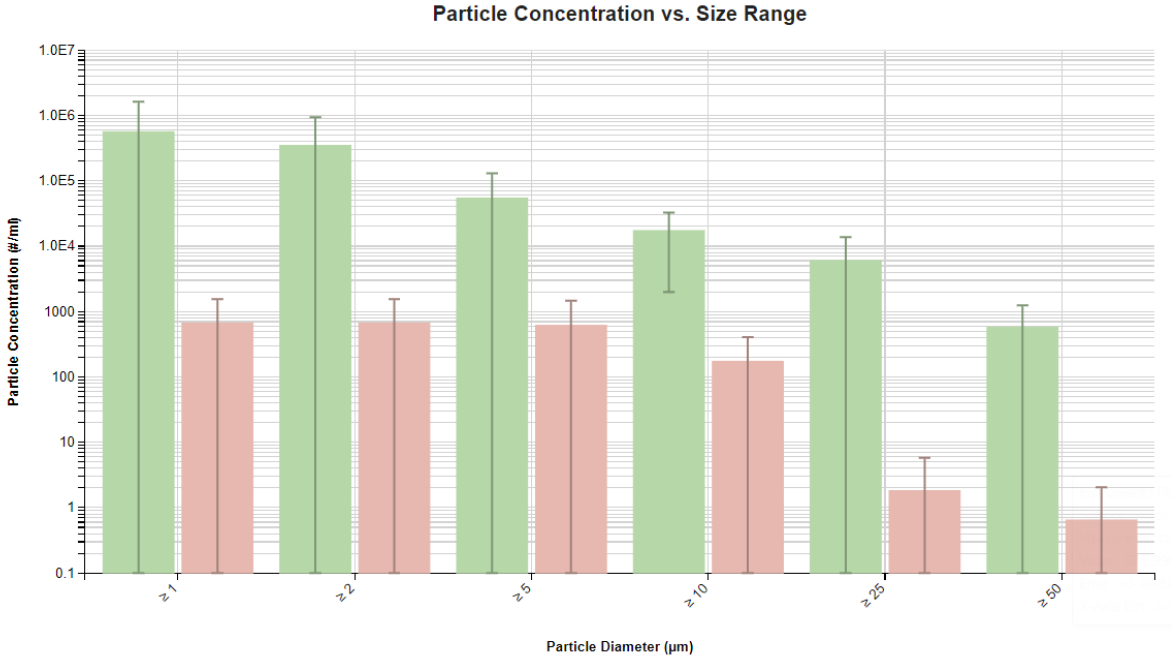
Automated Metadata Extraction
Specify criteria for automated metadata extraction from measurement file fields (e.g. Comments, File Name, Import Path etc…) utilizing specified delimiters. Both metadata name and value may be extracted or only the value only.

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.

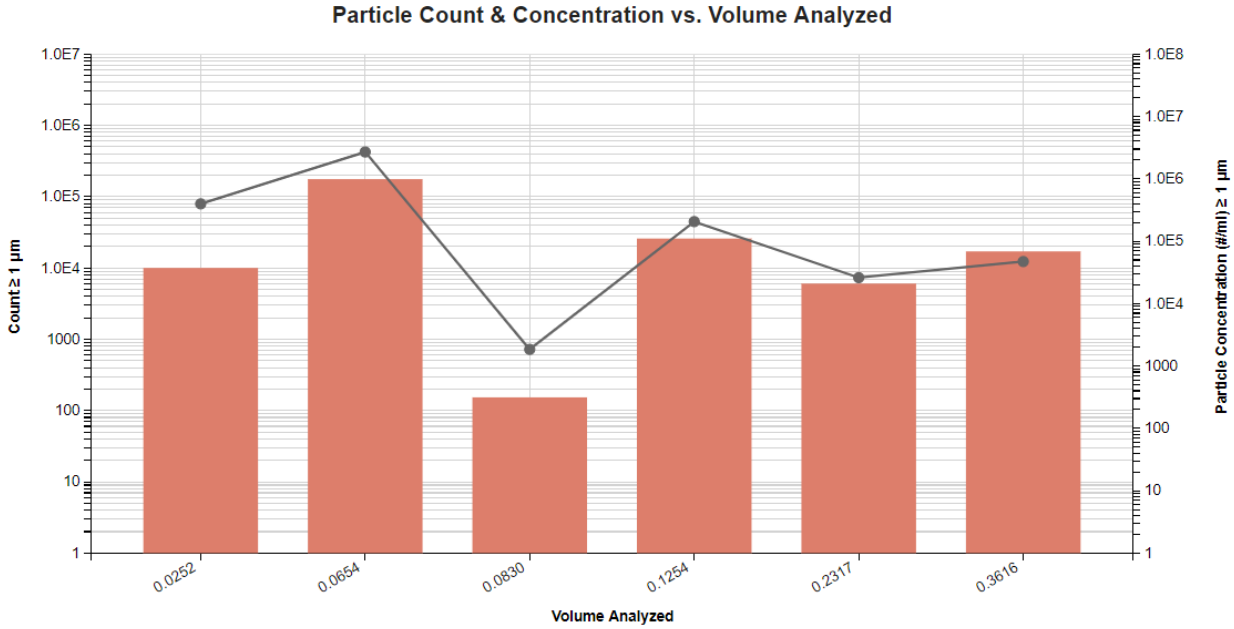
EXAMPLES

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 >=1µm & Concentration >=1 µm 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_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	Siliocone 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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