

## Horizon/Aura – Halo Labs

### 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.

The Aura system provides quantitative subvisible particle analysis. The Aura system is powered by a highly sensitive Backgrounded Membrane Imaging (BMI) technology to provide high-throughput particle count, size, and ID formation.

### DETAILS

LINK requires the Histogram ParticlesPerML CSV and associated individual well Particle Data CSVs for measurements that have a particle count greater than 0. PNG or JPG particle images are imported if available and may be exported as thumbnail images from LINK in the same format as they were imported. LINK will map ‘Sample’ from the Aura csv file to ‘Sample Name’ within LINK. If ‘Sample’ is not in the csv, LINK will map ‘experiment’ or ‘File Name’ to ‘Sample Name’, depending on availability.

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

Particle count/concentration for each supported morphological parameter occurs at a pre-defined bin spacing and measurement range as per the table below. 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	Bin Spacing	Meas. Start	Meas. End
Area	100	0	20000000
AspectRatio	0.01	0	1
Circularity	0.01	0	1
Convexity	0.01	0	1
FeretDiameter	0.25	2	4000
IntensityMean	1	-255	255
MaxFeretDiameter	0.25	0	4000
MinFeretDiameter	0.25	0	4000
Particle Diameter (µm)	0.25	2	4000
Perimeter	0.25	2	4000
SIMIntensity	1	-255	255

Notes:

- 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.
- PDF reports are imported as series attachments, which are accessible and viewable within LINK.



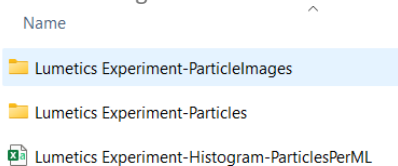
- LINK requires that the Particles Per ML CSV be unzipped as this file is a root CSV that ties all the images files to the Histogram CSV and LINK will not be able to search within a zipped folder.
- A wide range of Import Method options are available. These options are noted below.
  - Custom Particle 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.
  - PDF reports are imported as series attachments, which are accessible and viewable within LINK.
  - A wide range of Import Method options are available. These options are noted below.

The Horizon/Aura ParticlesPerML CSV data file example is as follows:

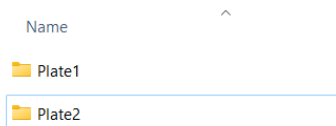
	A	B	C	D	E	F	G	H
1	Experiment	Lumetics Experiment						
2	plate	well	volume (uL)	Dilution Factor	Sample	2um - 3um	3um - 4um	4um - 5um
3	Plate1	A1	40	1	Sample A	148425	71675	32450
4	Plate1	A2	40	1	Sample B	234000	128400	64775
5	Plate1	A3	40	1	Sample C	335325	194000	95000
6	Plate1	A4	40	1	Sample D	293500	166250	83675
7	Plate1	A5	40	1	Sample E	161150	81050	41175

### Folder Structure Details

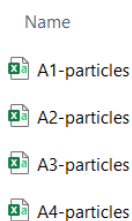
- LINK requires that the ParticlesPerML CSV be unzipped as this file is a root CSV that ties all image files and particle data to the Histogram CSV and LINK will not be able to search within a zipped folder.
- ParticlesPerML CSV contains three main columns to link to the particle data and particle image folders:
  - Experiment: This must match the corresponding “Particles” and “ParticleImages” folders. For example, if the experiment name is “Lumetics Experiment” than LINK will expect a “Lumetics Experiment-Particles” folder and a “Lumetics Experiment-ParticleImages” folder.



- Plate: The plate name will link to the corresponding subfolder names for both the “Particles” and “ParticleImages” folders.



- Well: The well name/number will link to the corresponding particle CSVs from the “Particles” and will link to the particle image subfolders within the “ParticleImages” folder
  - Particle CSVs have a naming convention of “well-particles”, therefore if the well is “A1” the CSV will be called “A1-particles”.



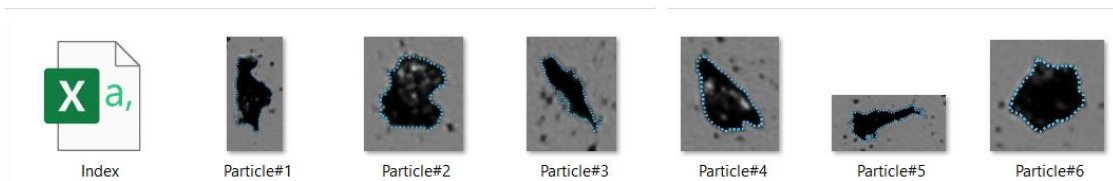
- Within the “ParticleImages” plate subfolder, are additional subfolders that have a naming convention of “well-particles”, therefore if the well is “A1” the subfolder will be called “A1-particles”.



Name

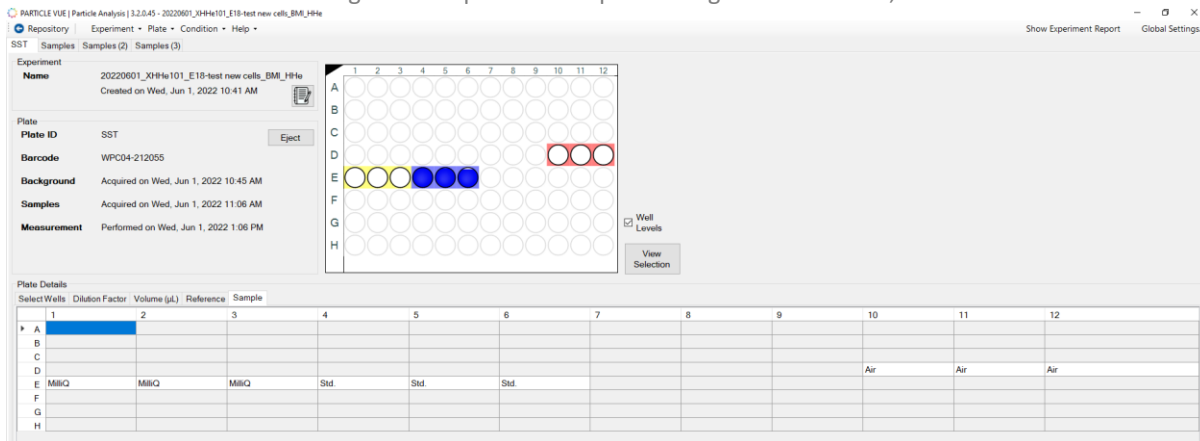
- A1-particles
- A2-particles
- A3-particles
- A4-particles

- Each particle image folder will contain the particle images themselves but also an “Index CSV”. This CSV will organize the particle number and particle Y data.

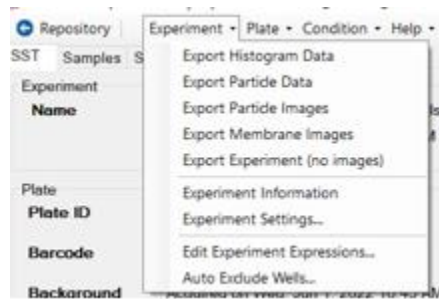


### Aura Software Export

Within the Aura software when creating a new experiment or performing measurements, the default screen is as follows:



In this example, the measurements have already been made. To export data, select “Experiment” and then select which data to export.



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. The following is the default MFI-specific import method settings:

- Default Sub-Populations: **None**
- Machine Learning Particle Classification: **None**
- Custom Particle Parameters: **None**
- Container Fill Volume: **None**
- Number of Thumbnails to Crop: **250**
- Ignore Thumbnails less than: **5 µm**
- Import Images in order of Largest-to-Smallest: **Yes**
- Advanced Image Selection: **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**

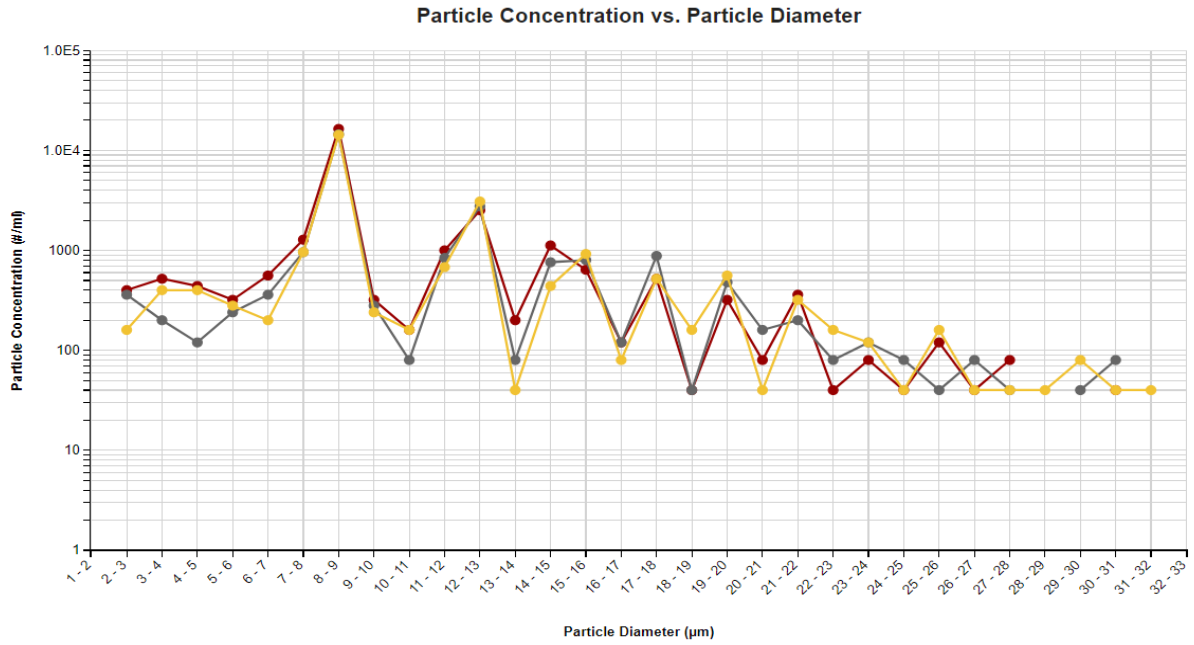
Specify a dilution factor for automated particle count/concentration data adjustment. This selection applies to the total particle population and all defined sub-populations. A LNK\_Dilution\_Factor may also be applied after import, utilizing the Add/Edit LINKdb Fields feature.



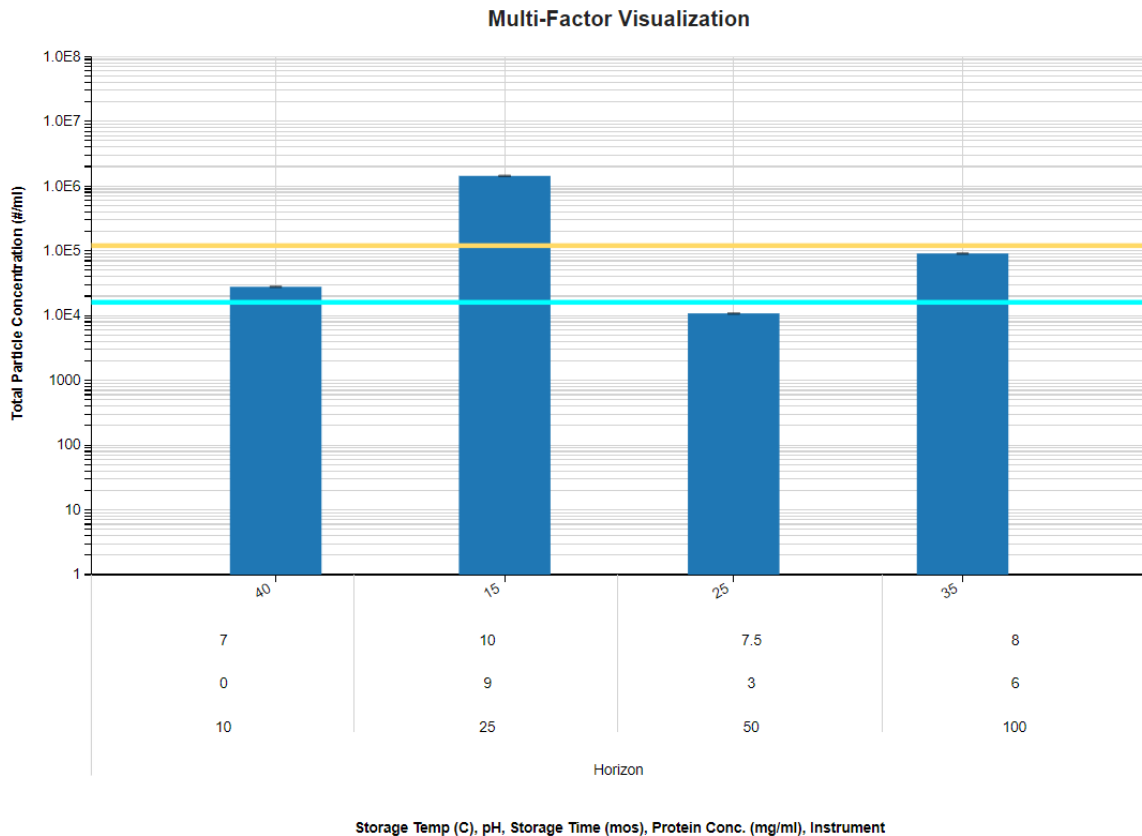
## EXAMPLES

Included below are sample dashboards from HORIZON measurement files:

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

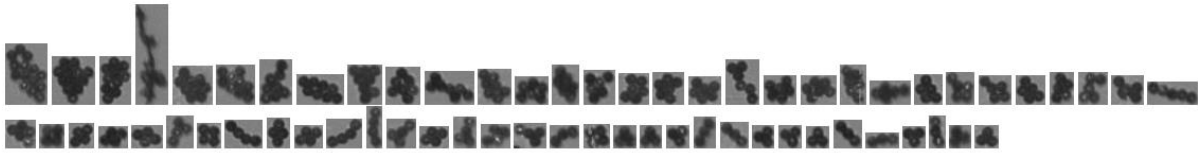


### 2. Column chart plotting multiple metadata parameters including; storage temp, pH, storage time, protein concentration vs. Total concentration.

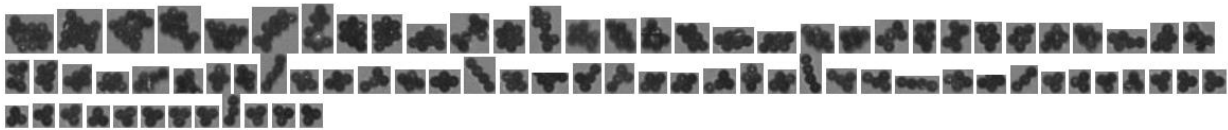


### 3. Particle Images organized by chart metadata parameters and ECD $\geq 15$

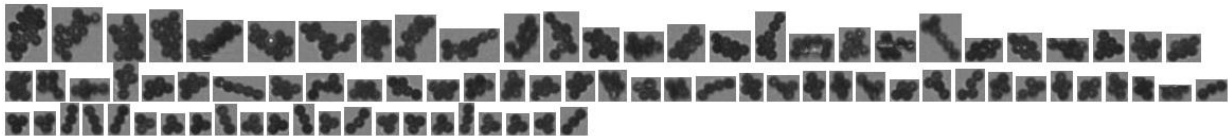
0, 10, 40, 7, ECD  $\geq 15$



3, 50, 25, 7.5, ECD  $\geq 15$



6, 100, 35, 8, ECD  $\geq 15$



### 4. Tabular Summary examples

Measurement Summary Table – Instrument Settings

LINK Record ID #	Sample Name	Dilution Factor - AVG	Volume Analyzed (ml) - AVG	Well Row - AVG	Well Col - AVG	Well
1	Sample A	1	0.03	1	1	A1
2	Sample B	1	0.03	1	2	A2
3	Sample C	1	0.03	1	3	A3
4	Lumetics 4	100	0.03	6	12	F12

Measurement Summary Table – Measurement Settings

LINK Record ID #	Sample Name	Particle Count (#) (Aspect Ratio $\geq 1$ ) - AVG	Particle Conc. (#/ml) (Circularity $\geq .01$ ) - AVG	Particle Conc. (#/ml) (Max Feret Diam. ( $\mu\text{m}$ ) $\geq .01$ ) - AVG	Particle Conc. (#/ml) (Area ( $\mu\text{m}^2$ ) $\geq .01$ ) - AVG	Volume Analyzed (ml) - AVG	Total Particle Count (#) - AVG	Total Particle Concentration (#/ml) - AVG
1	Sample A	201	27800	27800	7360	0.03	695	27800
2	Sample B	166	24800	24800	7640	0.03	620	10800
3	Sample C	155	24760	24760	7480	0.03	619	90760
4	Lumetics 4	2852	1432960	1432960	100120	0.03	35824	1432960

### CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

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