www.lumetics.com

Flow Cytometry

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.

FlowJo™ Software is the leading platform for single-cell flow cytometry analysis that helps you interpret your data quickly and effectively with accessible features for immunophenotyping, cell cycle analysis, proliferation, kinetics studies, quantitative population comparison, high-dimensional analysis and more.

DETAILS

LINK requires an FCS, OMIQ CSV or FlowJo Workspace WSP output file to import Flow Cytometry measurements. Supplementary files and file formats will be available within the LINK analysis template as measurement attachments to view or download, including FCS, CSV, PPT/PPTX, PDF, and XML. LINK will also populate specific LNK fields to reflect the presence of the supplementary files as listed below:

- LNK_Import Attachment(s)
- LNK_Import CSV Attachment
- LNK_Import FCS Attachment
- LNK_Import PDF Attachment
- LNK_Import PPTX/PDF Attachment
- LNK_Import XML Attachment

LINK utilizes a specific combination of mandatory fields to generate a unique FCS/CSV/WSP measurement within the LINK software. These fields as are follows:

- Analysis Date
- Sample Name
- File Name
- Data Acquisition Date
- Parameter Name ($PnN)
- FCS File Name
- Plate Name
- Group Name

Data for Flow Cytometry can be imported in four different combinations, depending on the needs of the scientist. The import options are denoted in the Flow Cytometry Import Method. Once a selection in the import method is chosen, subsequent import method options are available. This will be described below in section 4.17.4. The file type import options are;

- All Flow Cytometry Files (FCS Files, FlowJo WSP files, and OMIQ CSV Files) – **Default Method** automatically applied at point of data import.
- Analysis Files – FlowJo WSP Files/OMIQ CSV files (FCS File data linked if available)
- FCS Files Only – no channel data imported
- FCS Files Only – with individual channel data

Import File Type – FCS Files

Within the Flow Cytometry import method, the option of FCS Files Only represents the import of only FCS data with either no channel data or channel data. LINK will continue to find any PPT/PPTX, PDF, and XML files present in the same folder, and import them as an attachment. All other file types (WSP and CSV) will be ignored as they are considered separate import types and are not valid under the FCS File Only import method options.

LINK will map certain fields from the Flow Cytometry files and populate LINKdb fields that are standard within the software. These fields are:

- Sample Name – derived from the FCS filename without the extension
- Analysis Date – derived from the values $Date + $Etim from the FCS file
- Operator – derived from the value $OP from the FCS file
- Channel and Measurement Type – derived from $PnN from the FCS file
- Group Name - derived from GROUPNAME from the FCS file
- Comment – derived from $COM from the FCS file

Helpful Notes:

- LINK will automatically populate a field called "FCS Metadata Only" (with respective yes or no). This provides the user with the ability to isolate measurements that include only FCS metadata and exclude channel data, if imported.
- When importing an FCS file, if there is an XML file in the same folder, LINK will try to match it to the FCS file based on the file name stored in the XML file under the 'data_filename' tag.
- If the FCS File contains information on the CST System Setup pass/fail criteria, the value of PASS or FAIL is mapped to a new field called "CST Setup Status (numeric)" with values of +1 or -1 respectively, so it can be visualized on a chart as per the example chart in example section #2.

FCS Files Only (no channel data) selection in the import method is for QA/QC application. LINK will import all "general" metadata in the FCS File, and not include individual channel data.

FCS Files Only (with channel data) selection in the import method will include the FCS channel data, which will be imported and broken out as individual measurement results. LINK also allows the option to select only specific channels (parameters) to import from the FCS file (next option within the import method explained further in section 4.17.4). The default setting is to import all channels found in the file. Each channel will represent a single measurement, plus one additional measurement with the general metadata on its own.

Import File Type – Analysis Files

Within the Flow Cytometry import method, the option of Analysis Files includes importing FlowJo WSP Files and / or OMIQ CSV files. LINK will no longer import PPT/PPTX, PDF, and XML as attachments for this import method option. LINK will however import and attach FCS file(s) to the Analysis files under specific circumstances illustrated below:

- FlowJo WSP – the FCS file needs to be found either in the same folder as the WSP file or if the WSP is found in a subfolder called "attachments", LINK will look for the FCS files in the parent folder.
- OMIQ CSV – the FCS file needs to be found either in the same folder as the OMIQ CSV or located in subfolders generated by Auora when the OMIQ CSV is analyzed. The OMIQ CSV file needs to be located two folders up from the actual FCS files, with the first sub-folder being the plate name ($PLATENAME) in the CSV file, and the second folder being the Group Name (GROUPNAME) in the CSV file.

Once the matching FCS files are located, LINK will merge the FCS file metadata with the WSP or OMIQ CSV measurements. The following LINKdb fields will also be populated;

- FCS File – populated with the FCS file name
- Path to FCS File – populated with the path to the FCS file

For the OMIQ CSV, if there is already a field in the CSV file that is the same (case insensitive), LINK will not port it over. For example, if the CSV file had a field called "Cell line" and the FCS file had a field called "CELL LINE", LINK will ignore the FCS file field.

The FlowJo WSP data file example is as follows:

OMIQ CSV Specific Information

To import OMIQ CSV files, LINK has the following file criteria;

- The first row of the CSV file needs to start with "file"
- The header row needs to line up with the data
- No header values can be completely blank
- The header cannot have more than two pipe "|" symbols
- LINKdb field "File Name" is the name of the CSV file
- LINKdb field "Sample Name" is the CSV file name but with the extension stripped.

The OMIQ column headers are parsed into LINK based on Pipe (|). Therefore, LINK will detect and remove the pipe symbol if present in a column. Also, if a column header contains "count", LINK will place it in brackets as (count). An example is as follows: Ly6G+ NOT|count will be imported into LINK as Ly6G+ NOT (count).

The CSV data file example is as follows:

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	file	UR|FSC-A|median	UR|FSC-A|cv	UR|SSC-A|median	UR|SSC-A|cv	UR|FITC-A|median	UR|FITC-A|cv	UR|FITC-H|median	UR|FITC-H|cv	UR|BB630-A|median	UR|BB630-A|cv	UR|BB630-H|median	UR|BB630-H|cv
2	QC UltraRainbow_A3_UR_2022-12-05_021.fcs	89901	0.0287	89678	0.0531	95963	0.0309	104281	0.0313	94124	0.0368	102865	0.0381
3	QC UltraRainbow_A3_UR_2022-12-12_022.fcs	92256	0.0199	93385	0.0435	99913	0.0225	99199	0.0272	96520	0.028	96348	0.0333
4	QC UltraRainbow_A3_UR_2022-12-19_023.fcs	91445	0.023	93380	0.0463	99502	0.0256	99075	0.0269	95902	0.0297	95880	0.0309
5	QC UltraRainbow_A3_UR_2022-11-07_018.fcs	93308	0.0247	92634	0.0518	99465	0.0283	104874	0.0311	96710	0.0344	102198	0.0379
6	QC UltraRainbow_A3_UR_2022-11-21_019.fcs	92151	0.0309	93782	0.0569	97624	0.0343	100948	0.0387	94724	0.0412	98452	0.0456
7	QC UltraRainbow_A3_UR_2022-11-29_020.fcs	89956	0.0289	93702	0.0549	97546	0.0351	97884	0.0389	94041	0.0421	94829	0.0456
8													

Import File Type – All Flow Cytometry Files (Default)

Importing all Flow Cytometry files is the default import for the data. Therefore, all FCS Files, FlowJo WSP files and OMIQ CSV within the same folder or subsequent subfolders will be imported into LINK. Details from section 4.17.1 and 4.17.2 illustrated above apply to this import file method as well.

Flow Cytometry Import Method

Import Method options can be defined and calculated with each measurement during import, if it is first created/defined and then requested within an Import Method. The Flow Cytometry Import Method is unique in that a user may select different file types to import. Based on the file type selection, other import options are available. The options are as follows:

- File types to Import – Default is All Flow Cytometry Files (FCS, WSP, CSV)
- Select Parameters for Import – Default is to import all parameters (only valid for FCS Files Only with channel data file type import)
- Exclude Sub-folders from Import – Default is none
- File Attachment – Default to save file path

- Automated Metadata Extraction

File Types to Import

Specify the types of Flow Cytometry files to import, from a combination of FCS files (with or without channel data as individual measurement results), or Analysis results (FlowJo / OMIQ), or both

- ● All Flow Cytometry Files (FCS Files, FlowJo WSP files, OMIQ CSV Files) - **Default**
- ○ Analysis Files (FlowJo WSP files / OMIQ CSV Files), FCS File data linked if available
- ○ FCS Files Only (no channels)
- ○ FCS Files Only (with individual channel data)

Cancel   Back Next

Select Parameters for Import: Optionally select specific parameters to import from the FCS file (with individual channel data). The default setting is to import all parameters found in the file. If the desired parameter(s) are missing, it means that it has not yet been imported into the LINK database and needs to be imported at least once to be selected.

Select Parameters for Import

Optionally select specific parameters to import from the FCS file. The default setting is to import all parameters found in the file.

Please note that if the desired parameter(s) are missing, it means that it has not yet been imported into the LINK database, and needs to be imported at least once to be selected.

- ● Import All Parameters
- ○ Select Specific Parameters to Import

Cancel   Back Next

Exclude Sub-Folders from Import: Type in the name of a folder where any FCS files found underneath this folder will be skipped for import. For example, the Aurora Flow Cytometer exports FCS data automatically by creating two sub-folders called "Raw" and "Unmixed" containing FCS files in each, where only one folder may be required for LINK, and (for example) typing the folder name "Raw" (without quotes) below will automatically not process any FCS files inside all folders with this name. If this feature is utilized, the following LINKdb Fields will be populated: LNK_Import_Exclude Folder and LNK_Import_File Types.

Exclude Sub-Folders from Import

Type in a name of a folder where any FCS files found underneath this folder will be skipped for import. For example, the Aurora Flow Cytometer exports FCS data automatically by creating two sub-folders called "Raw" and "Unmixed" containing FCS files in each, where only one folder may be required for LINK, and (for example) typing the folder name "Raw" (without quotes) below will automatically not process any FCS files inside all folders with this name.

Folder Name to Exclude: [   ] (default is blank)

Cancel   Back   Next

File Attachment: Select the checkbox below to import a copy of the file attached to the LINK database. De-selecting this checkbox will instead save a link to the attachment in the database, which keeps the size of the LINK database smaller, and is the preferred option if the attachment is in a location which will not change. Default is unchecked, which saves a path instead.

File Attachment

Select the checkbox below to import a copy of the file attachment to the LINK database. De-selecting this checkbox will instead save a link to the attachment in the database, which keeps the size of the LINK database smaller, and is the preferred option if the attachment is in a location which will not change.

☐ Save a copy of the attachment in the database (default is unchecked, which saves a path instead)

Cancel   Back   Next

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.
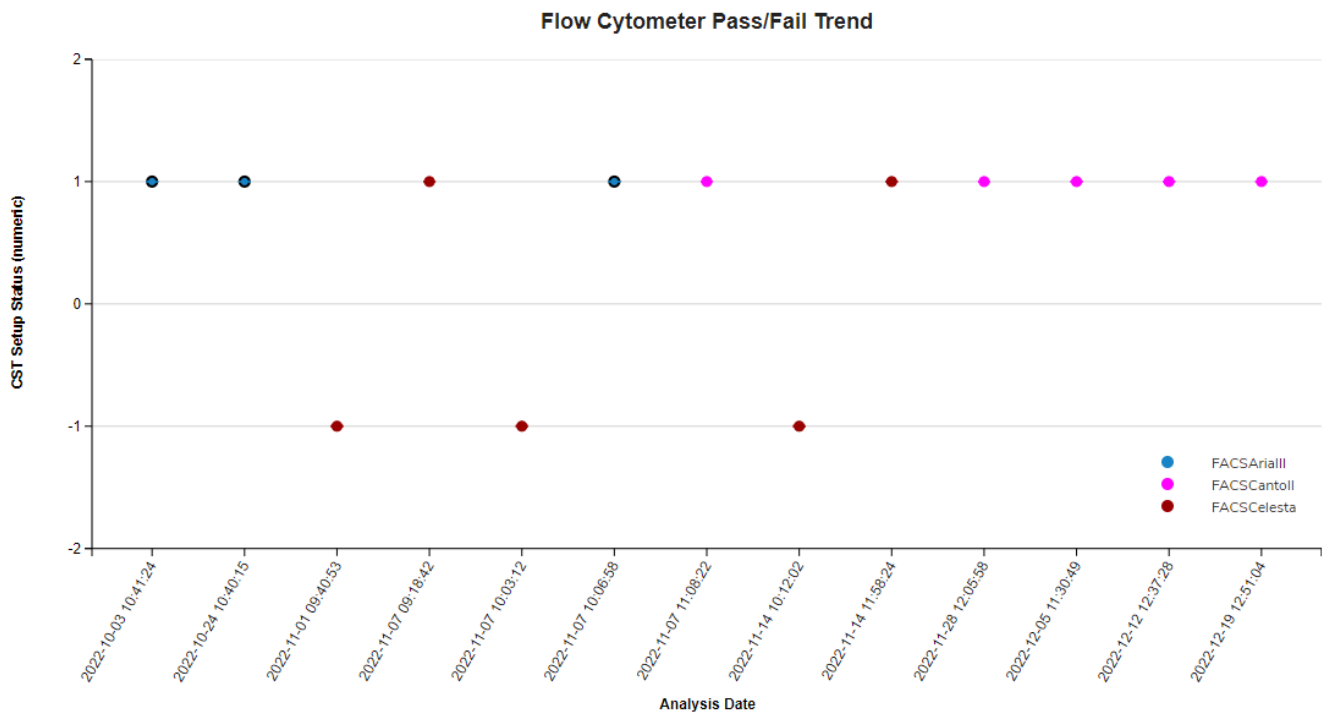
Included below are sample dashboards from Flow Cytometry measurement files:

1. Trend Chart plotting voltage vs. analysis date with confidence interval grid lines
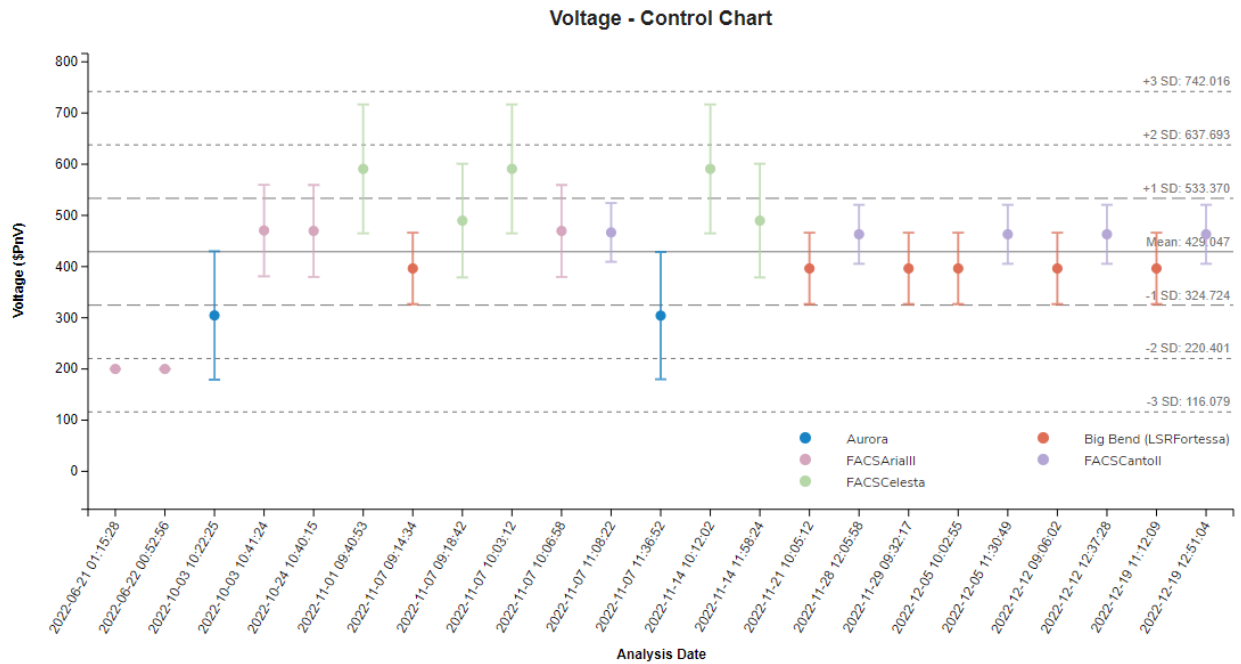
2. Trend Chart CST Setup Status (where a pass takes a value of 1, and a failure takes a value of -1) vs. Date on a per FCS file basis, broken out by Cytometer and Cytometer Setup Status.

3. Tabular Summary examples:

Measurement Summary Table – Lab Overview

Experiment Name	$Op	Tube Name	$Cyt	Cytnum	CST Setup Date	CST Setup Status	$Par - AVG
EXP 1	Lumetics	ALL	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 1	Lumetics	UN	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 2	Lumetics	ALL	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 2	Lumetics	UN	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 3	Lumetics	ALL	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 3	Lumetics	UN	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 4	Lumetics	ALL	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12
EXP 4	Lumetics	UN	LSRII	H1122	2015-12-02T09:31:11-05:00	SUCCESS	12

Measurement Summary Table – Parameter vs. Metadata

Experiment Name	Analysis Date	$Cyt	$PnN	$PnV (avg)	$PnG (avg)	$PnB (avg)	&PnR (avg)	Laser1 Del (avg)	Laser2 Del (avg)	Laser3 Del (avg)	Laser4 Del (avg)
EXP 1	2023-07-18 15:31:02	LSRII	Alexa Fluor 488-A, APC-A, APC-Cy7-A, Comp-APC-Cy7-A, FSC-A, FSC-H, FSC-W, PE-A, PerCP-A, SSC-A, SSC-H, SSC-W, Time	382	0.93	32	262,144	14.38	34.14	44.34	22.36
EXP 2	2023-07-18 15:31:02	LSRII	Alexa Fluor 488-A, APC-A, APC-Cy7-A, Comp-APC-Cy7-A, FSC-A, FSC-H, FSC-W, PE-A, PerCP-A, SSC-A, SSC-H, SSC-W, Time	382	0.93	32	262,144	0.00	57.22	36.02	22.59
EXP 3	2023-07-18 15:31:02	LSRII	Alexa Fluor 488-A, APC-A, APC-Cy7-A, Comp-APC-Cy7-A, FSC-A, FSC-H, FSC-W, PE-A, PerCP-A, SSC-A, SSC-H, SSC-W, Time	377	0.94	32	262,144	0.00	56.00	32.88	21.58
EXP 4	2023-07-18 15:31:02	LSRII	Alexa Fluor 488-A, APC-A, APC-Cy7-A, FSC-A, FSC-H, FSC-W, PE-A, PerCP-A, SSC-A, SSC-H, SSC-W, Time	391	0.92	32	262,144	0.00	56.00	32.88	21.58

CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK^{TM} Support:

E-mail: support@lumetics.com
Phone: 1.613.417.1839
Website: http://lumetics.com/