

FlowJo™ v11 Software, the future of data analysis

Mandy Chiang

Sr. product specialist

BD Biosciences

Mandy.chiang@bd.com



Power what matters to you.

Rationale

Update the code base to a modern language to:

- Improve performance
- Make 'enabling comparison' the primary design goal
- Build-in more tools for automation
- Build on a foundation compatible with cloud compute

Changes compared to version 10

- Login via BD[®] Access Portal
- Immersive application
- Themes, e.g. Dark mode
- Updated sample hierarchy
- New context: Metadata manager
 - Parameter sets
- Built-in QC
- Modern unmixing platform
 - Including adjustment matrices
- Graph gallery
 - Virtual concatenation
- Built-in clustering
- Auto-batched tables
- Charts
- Reports replaces Layout Editor
 - Page oriented
 - New batching options
 - Toggle-on batching
- Built-in support for imaging
- Preferences modeled after v10 advanced prefs.

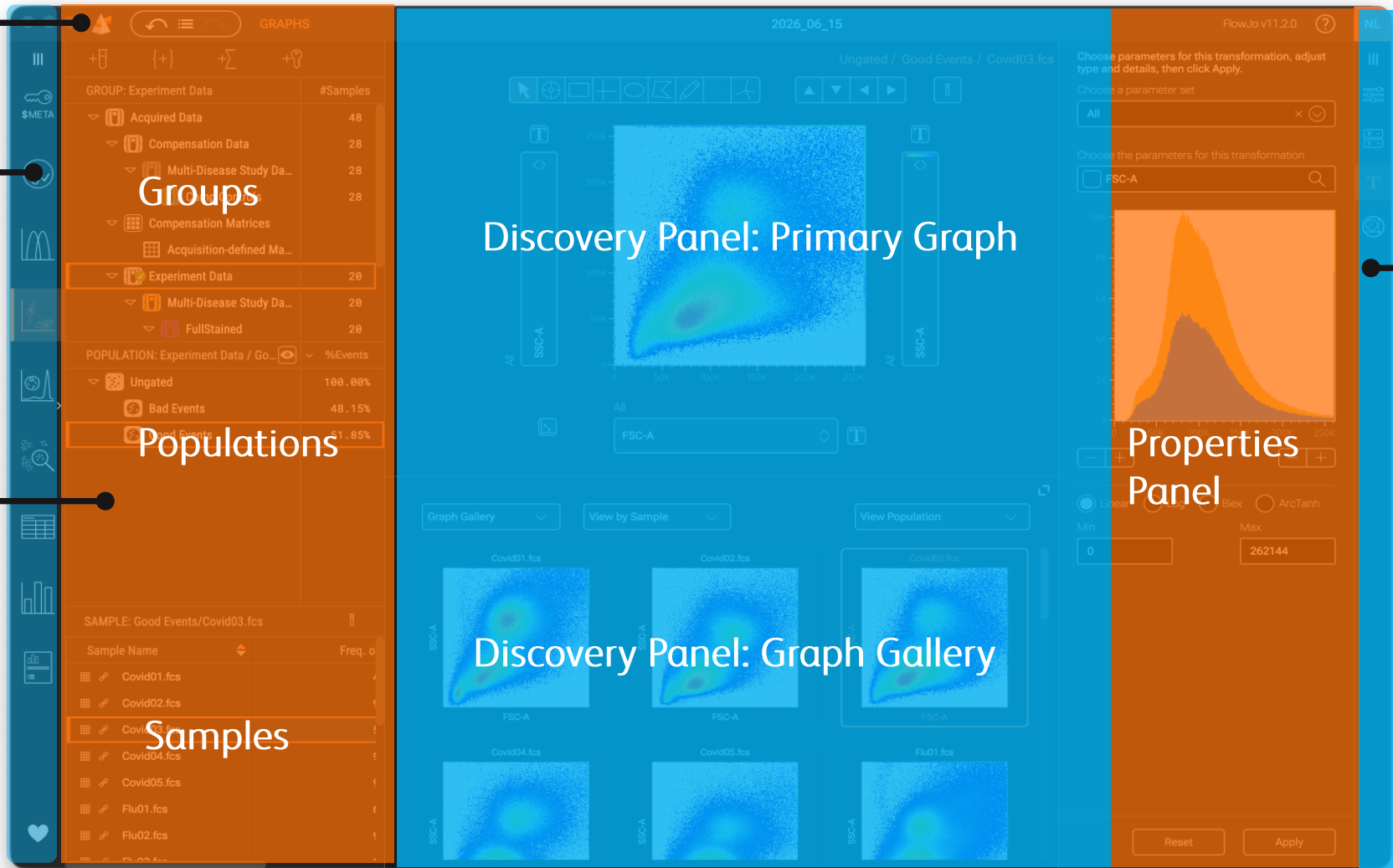
Immersive Application

Analysis toolbar

Navigation bar

*Each element is
a context*

Analysis Tree



Groups

Populations

Samples

Discovery Panel: Primary Graph

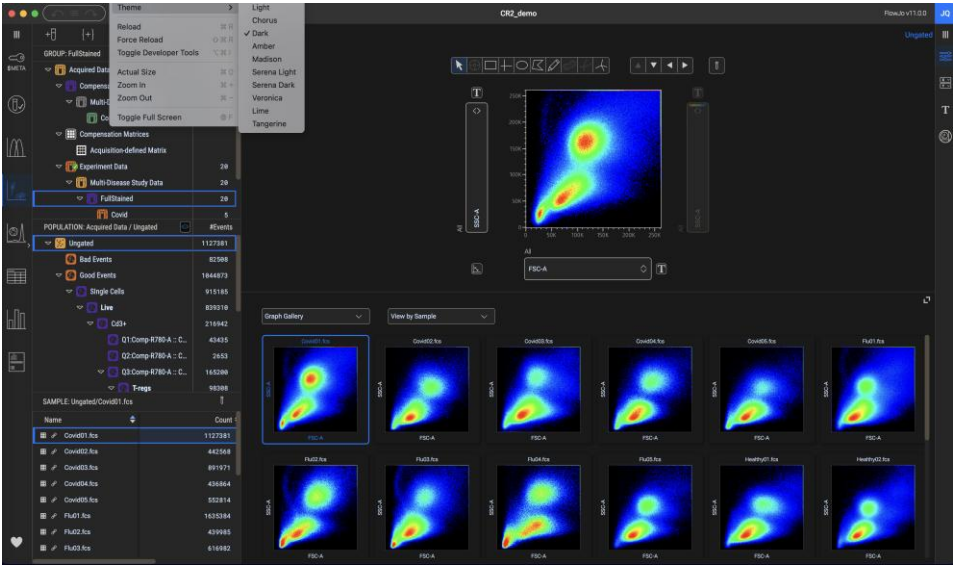
Discovery Panel: Graph Gallery

Properties Panel

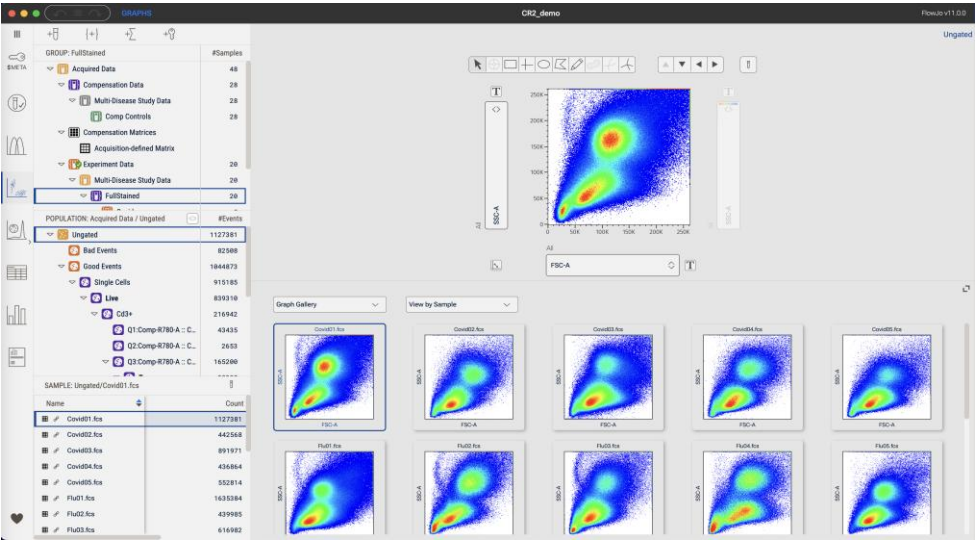
Properties navigation bar

Themes

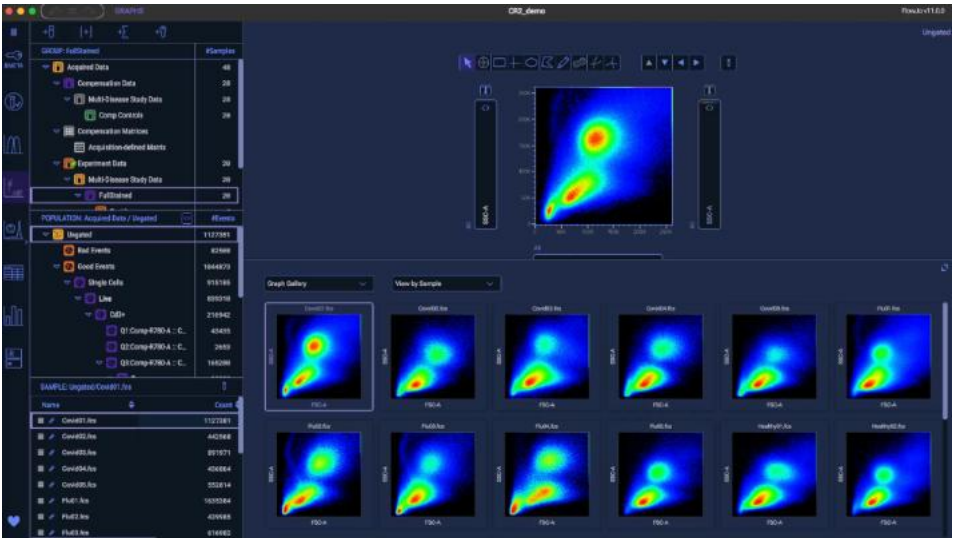
Dark



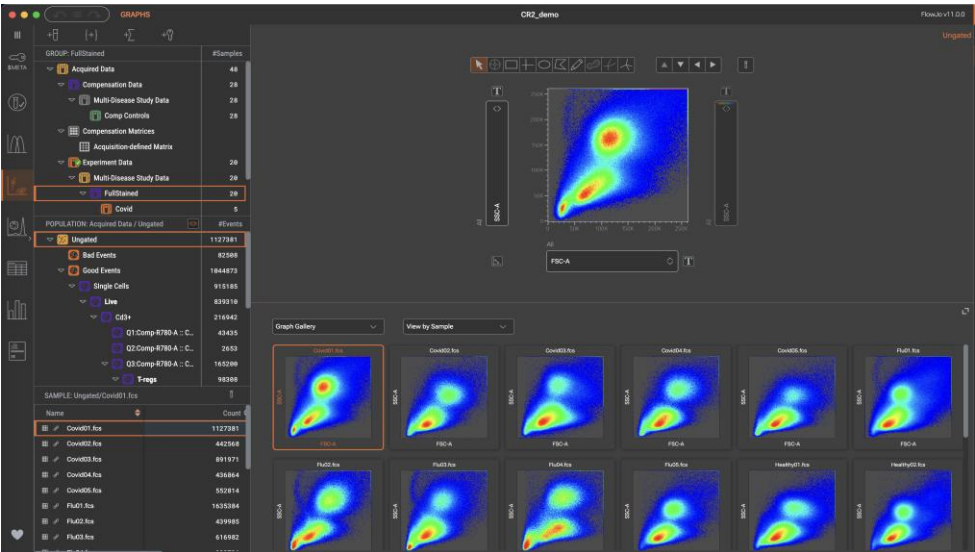
Light



Veronica



Ioannis



Updated Sample Hierarchy

Group Panel

- Selection filters populations and samples
- Hierarchical
- Defaults: Compensation & Experimental

Population Panel

- All populations associated w/ selected group
- *See-all* mode shows all populations per sample

Sample Panel

- Shows samples in selected group
- Controls display

The screenshot displays a hierarchical tree view of sample data. The interface is divided into three main sections, each labeled with an orange bracket on the right:

- Groups:** This section shows a hierarchy starting with 'GROUP: Multi-Disease Study Data' (28 samples). It includes sub-groups like 'Acquired Data' (48), 'Compensation Data' (28), 'Multi-Disease Study Data' (28), 'Comp Controls' (28), 'Compensation Matrices' (Acquisition-defined Matrix), and 'Experiment Data' (20). The 'Multi-Disease Study Data' group is selected and highlighted with an orange border.
- Populations:** This section shows 'POPULATION: Acquired Data / Ungated' (1127381 events). It includes sub-populations like 'Ungated' (1127381), 'Bad Events' (82508), and 'Good Events' (1044873). The 'Ungated' population is selected and highlighted with an orange border.
- Samples:** This section shows 'SAMPLE: Ungated/Covid01.fcs'. It lists individual samples with their counts: 'Covid01.fcs' (1127381), 'Covid02.fcs' (442568), 'Covid03.fcs' (891971), 'Covid04.fcs' (436864), 'Covid05.fcs' (552814), 'Flu01.fcs' (1635384), 'Flu02.fcs' (439985), 'Flu03.fcs' (616982), 'Flu04.fcs' (333736), and 'Flu05.fcs' (591041). The 'Covid01.fcs' sample is selected and highlighted with an orange border.

Metadata Manager

Three tools:

- Keywords
- Parameters
- Parameter Sets



KeywordsParametersParameter Sets

Select group in Analysis Tree to display parameters:
Multi-Disease Study Data

☒ Scatter ☐ Uncompensated

Fluorochrome Nam...	Stain Name (\$PnS)
SSC-W	
Comp-B515-A	TIM3
Comp-B610-A	BTLA
Comp-B660-A	CD27
Comp-B710-A	CD161
Comp-B780-A	CD38
Comp-G575-A	Lag3
Comp-G610-A	TCRgd
Comp-G660-A	CD137
Comp-G710-A	CD19
Comp-G780-A	CCR7
Comp-R660-A	Ki-67
Comp-R710-A	Granzyme B
Comp-R780-A	CD4
Comp-U395-A	CD8
Comp-U450-A	Dead
Comp-U500-A	CD3
Comp-U570-A	CD25
Comp-U660-A	HLA-DR
Comp-U730-A	ICOS
Comp-U780-A	CXCR6
Comp-V450-A	MR1 tet
Comp-V510-A	CD28
Comp-V570-A	CD45RA

Add SelectedAdd AllRemove SelectedRemove All

Build this Parameter Set:
Phenotyping

Fluorochrome Nam...	Stain Name (\$PnS)
Comp-G710-A	CD19
Comp-R780-A	CD4
Comp-U395-A	CD8
Comp-U500-A	CD3
Comp-U570-A	CD25

Create a parameter set to organize and quickly view fluorochromes or parameters of interest.

Phenotyping

enter a parameter set description

Built-in QC

QUALITY CONTROL

2026_05_12

FlowJo v11.2.0

Select parameters and adjust Quality Control settings. Press Apply to run tests.

☐ Select Parameter

No Results

☐ Signal Stability

Changepoints

Maximum: 5, Penalty: 500

Range: 1 to 10, Range: 1 to 1000

☐ Remove Outliers

☐ Flow Rate

MAD Range: 3.5

Range: 0.25 to 5

☐ Dynamic Range

Both Bounds

Please select some parameters.

Reset, Apply

GROUP: Experiment Data		#Samples
Acquired Data		0
Compensation Data		0
Compensation Matrices		0
Experiment Data		0

POPULATION: Acquired Data / Ungated

	%Events
Ungated	0.00%

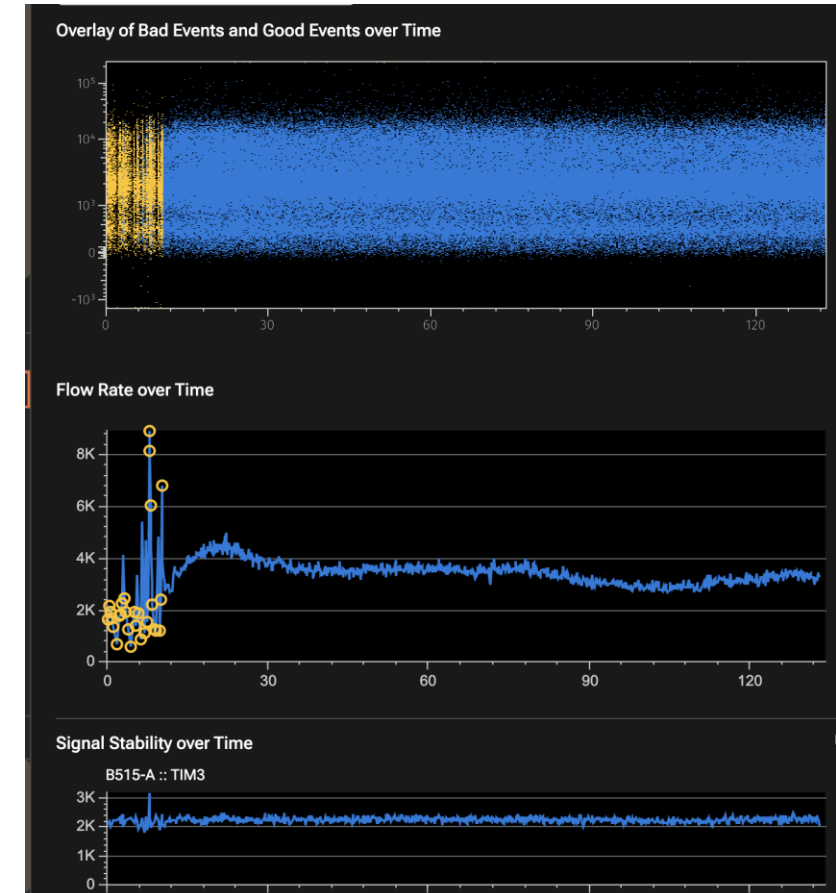
SAMPLE: Ungated

Sample Name	Freq. of Parent
-------------	-----------------

Built-in QC



Inspect fcs files
Remove anomalous events
Return high-quality events



POPULATION: Acquired Data / Un... ☐		#Events
▼ Ungated		442568
Bad Events		14007
Good Events		428561

Built-in QC

Extracts time bins with outliers in flow rate, signal stability, and margin events

Uses raw data

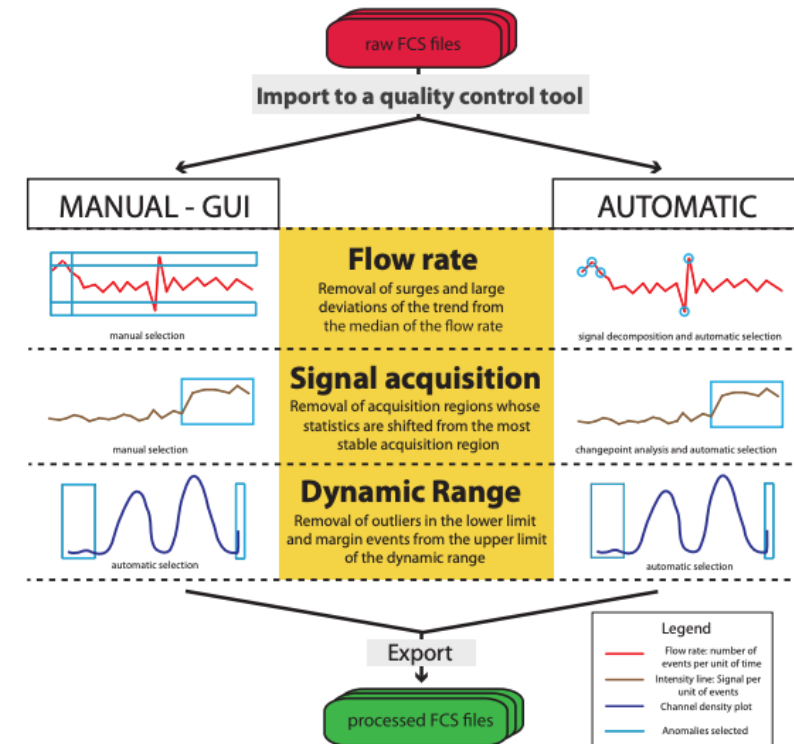
Flow rate check : MAD vs SD_{Mean}

Data and text mining

flowAI: automatic and interactive anomaly discerning tools for flow cytometry data

Gianni Monaco^{1,2,*}, Hao Chen¹, Michael Poidinger¹, Jinmiao Chen¹, João Pedro de Magalhães² and Anis Larbi^{1,*}

¹Singapore Immunology Network (SiGN), Agency for Science Technology and Research (A*STAR), Singapore 138648, Singapore and ²Integrative Genomics of Ageing Group, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK



Built-in QC



Experiment Data

POPULATIONS: Acquired Data / Ung...		#Events
Ungated		315610
Bad Events		7391
Good Events		308219

2025_05_05

FlowJo v11.0.0

JQ

QUALITY CONTROL

GROUP: Experiment Data

#Samples

Acquired Data48

Compensation Data28

Multi-Disease Study Data28

Comp Controls28

Compensation Matrices

Acquisition-defined Matrix

Experiment Data20

Multi-Disease Study Data20

POPULATION: Acquired Data / Ungated

#Events

Ungated1127381

SAMPLE: Ungated/Covid01.fcs

Name

Count

Covid01.fcs1127381

Covid02.fcs442568

Covid03.fcs891971

Covid04.fcs436864

Covid05.fcs552814

Flu01.fcs1635384

Add samples then choose parameters and adjust settings to apply quality control to Experiment Data.

Select parameters and adjust Quality Control settings. Press Apply to run tests.

B515-A :: TIM3 + 27 more

V510-A :: CD28

V570-A :: CD45RA

V610-A :: PD1

V655-A :: CD69

V710-A :: OX40

V750-A :: CD103

V780-A :: CD127

Signal Stability

Maximum5

Penalty500

Range: 1 to 10

Range: 1 to 1000

Remove Outliers

Flow Rate

MAD Range3.5

Range: 0.25 to 5

Dynamic Range

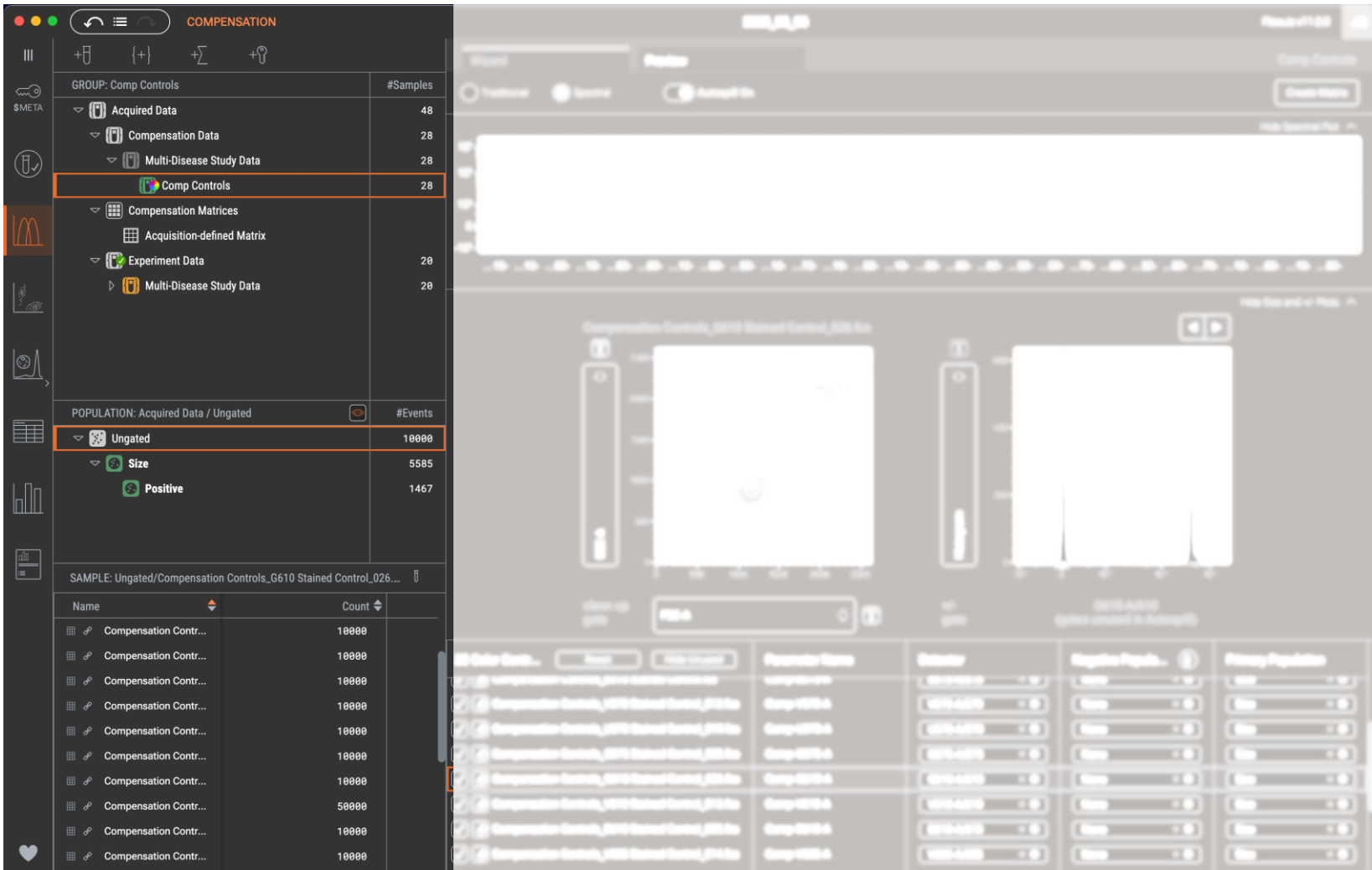
Both Bounds

Reset

Apply

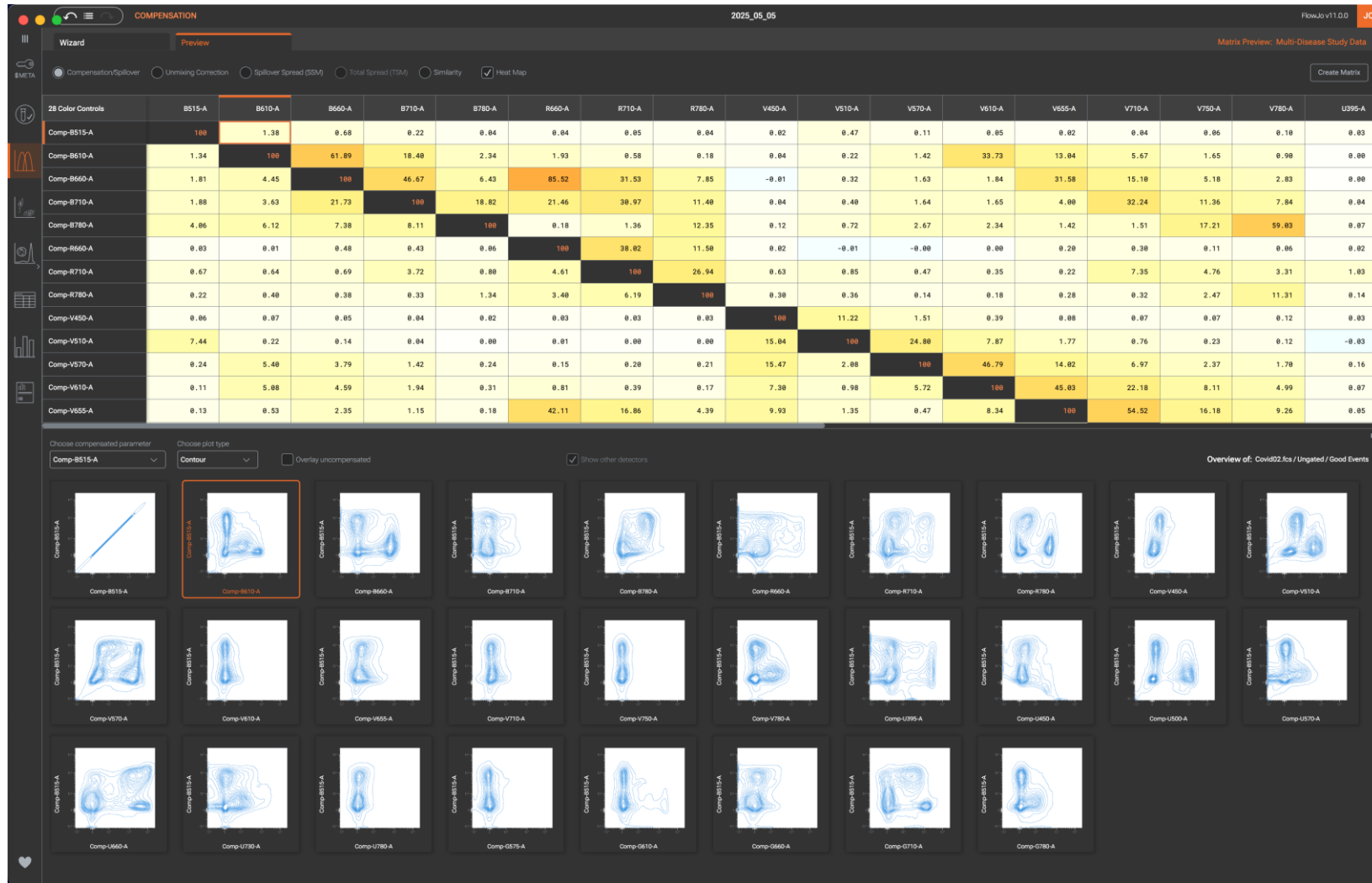
Modern Unmixing Platform

- Defaults to Spectral & AutoSpill



Modern Unmixing Platform

- Defaults to Spectral & AutoSpill
- View
 - SSM / TSM
 - CSM



Graph gallery

Selections controlled by hierarchy

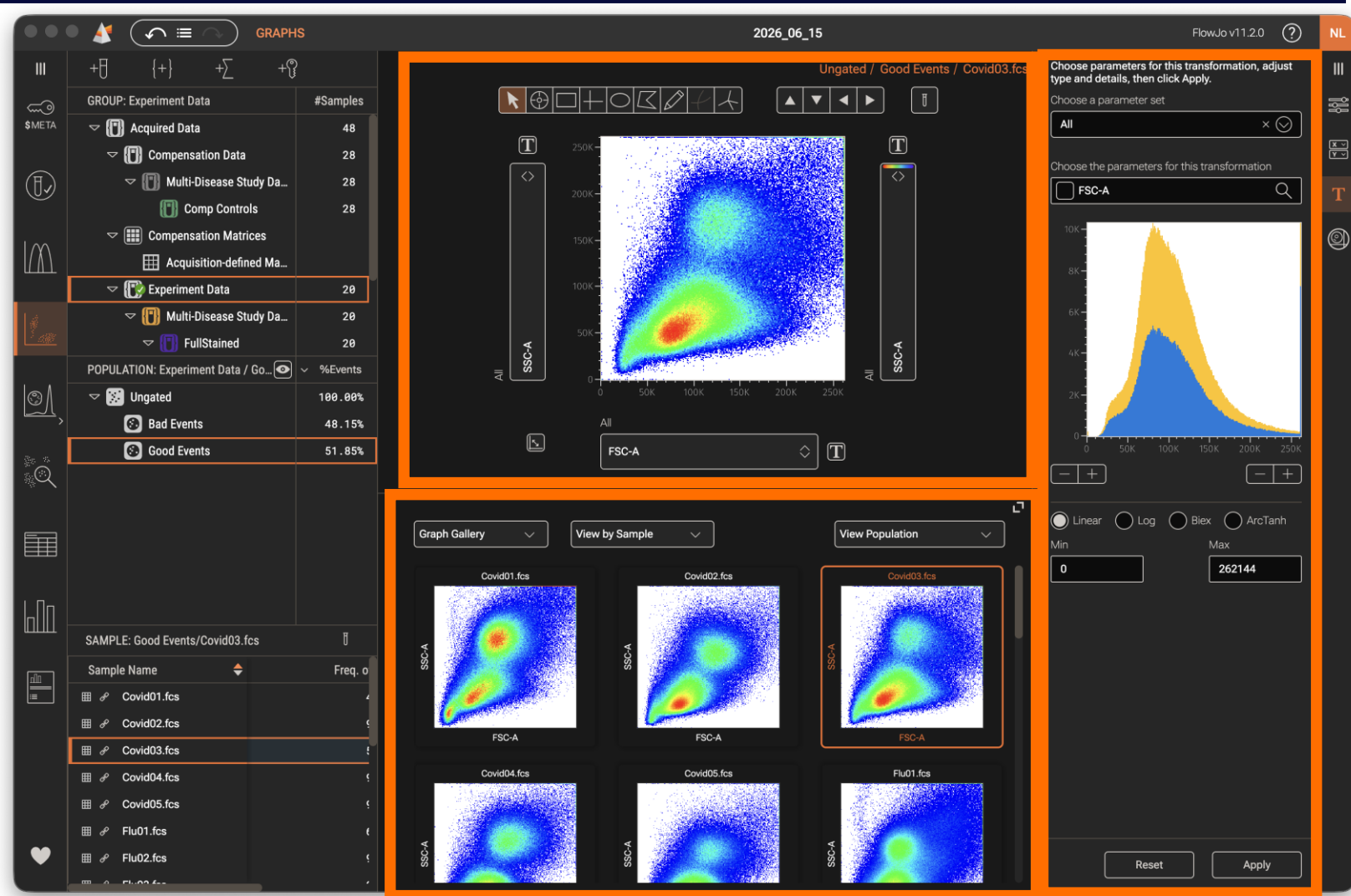
Primary graph

- Focus sample(s)

Graph gallery

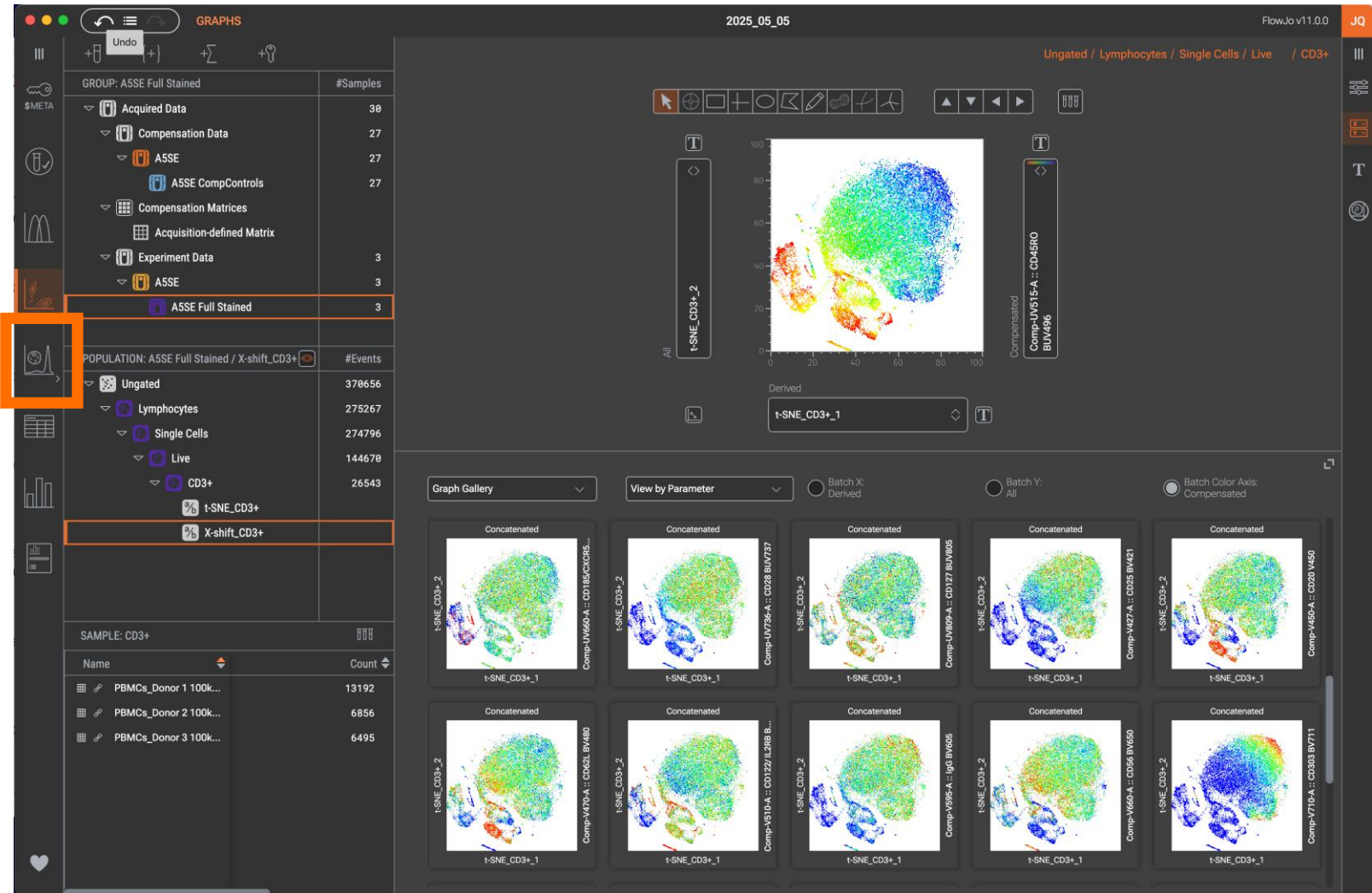
- Other samples
- Other parameters
- Ancestry
- Image gallery

Properties panel



Built-in HD workflow

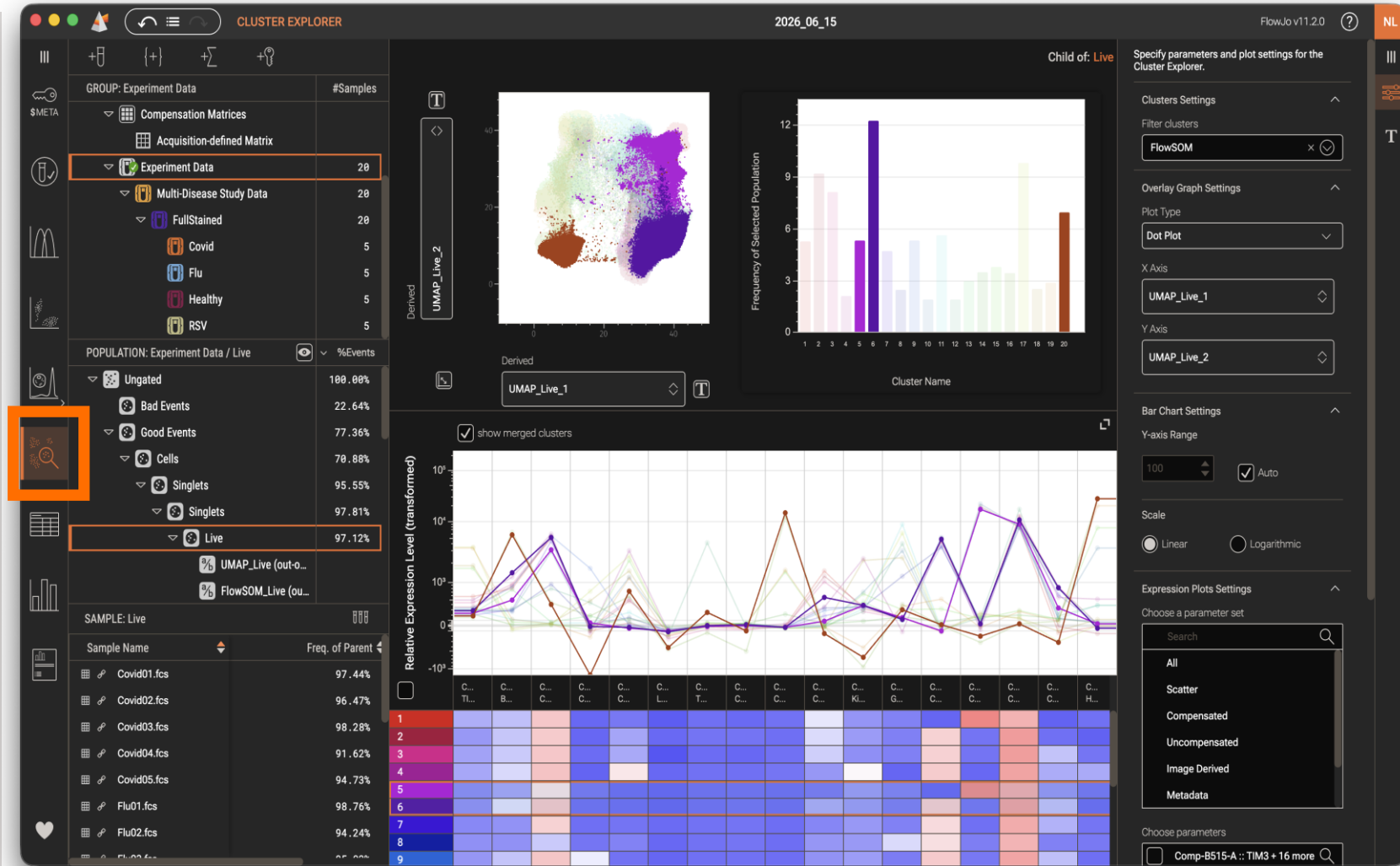
- Virtual Concatenation
- Dimensionality Reduction
 - tSNE
 - UMAP
- Clustering
 - X-shift
 - FlowSOM
- With built-in embedding



Cluster Explorer

Phenotyping

- Easily phenotype populations from clustering algorithms
- Filter populations of interest
- Back-gate clusters



Auto Batched Tables

Easier to use

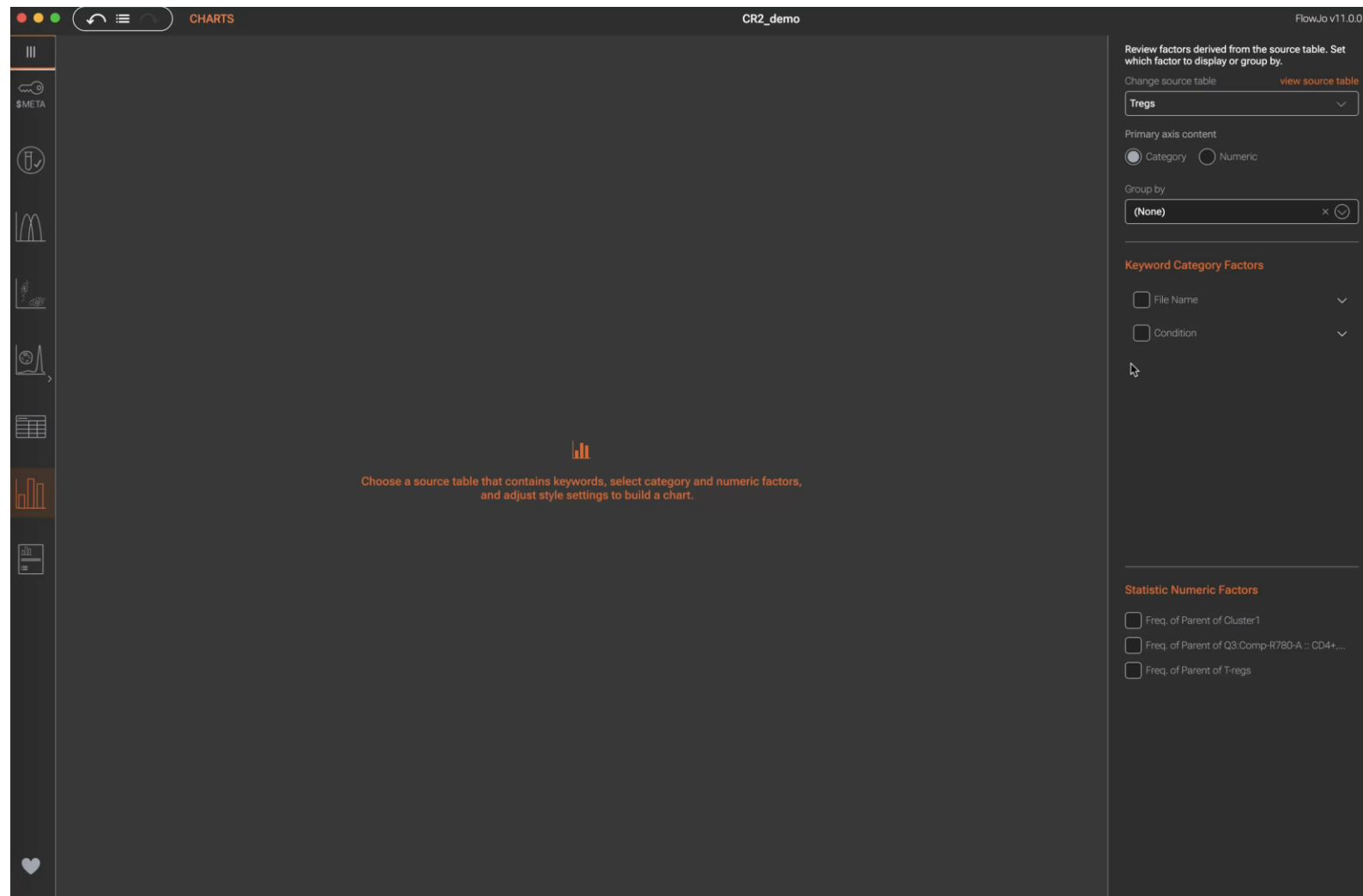
- Drag samples or groups for rows
- Drag populations for stats
- Multiplex stats from properties panel

The screenshot displays the FlowJo v11.0.0 interface. On the left, the 'TABLES' panel shows a hierarchical tree of data. The 'GROUP: FullStained' section is expanded, showing 'Acquired Data' (48), 'Compensation Data' (28), 'Multi-Disease Study Data' (28), 'Comp Controls' (28), 'Compensation Matrices', 'Acquisition-defined Matrix', 'Experiment Data' (20), and 'Multi-Disease Study Data' (20). The 'FullStained' group is selected, showing 'Covid' (5) and 'Tregs' (98308). The 'POPULATION: FullStained / Tregs' section is expanded, showing 'Q3.Comp-R780-A :: CD4+ Co...' (165200) and 'Tregs' (98308). The 'SAMPLE: Tregs/Covid01.fcs' section is expanded, showing a list of samples with their counts: Covid01.fcs (98308), Covid02.fcs (61711), Covid03.fcs (104609), Covid04.fcs (29122), Covid05.fcs (39257), Flu01.fcs (140516), Flu02.fcs (14206), Flu03.fcs (70298), and Flu04.fcs (15008). The 'Table' editor on the right shows a table with columns for 'Sample Name', 'MEAN', 'MEDIAN', 'MAX', and 'SD'. A message box in the center of the table says: 'Drag samples or groups to add rows to the table. Add populations, statistics and keywords to the table to form columns.' The right sidebar contains a 'Choose a population, statistics and parameters to create new table columns.' section, a 'Statistics will be applied to this population' section with a 'Tregs' dropdown, a 'Select one or more statistics, then select parameters' section with a list of statistics (Median, Mean, Mode, Geometric Mean, Robust CV, Robust SD, CV, SD, Median Absolute Deviation, Percentile), a 'Choose population for Frequency of' section with an 'Ungated' dropdown, a 'Select parameters for parametric statistics' section with a search bar and a list of parameters (FSC-A, SSC-A, FSC-H, SSC-H, FSC-W, SSC-W, Comp-B515-A :: TIM3, Comp-B610-A :: BTLA, Comp-B660-A :: CD27, Comp-B710-A :: CD161), and a '0 statistic columns will be added to the table.' section with an 'Add Statistic' button.

Charts

Built from tables

- Keyword oriented
- Extremely flexible
- Comparative stats



Reports Replaces Layout Editor

- Drag samples in to create rows
- Drag in populations to create columns
- Multiplex by using properties panel

The screenshot displays the FlowJo v11.0.0 Reports interface. On the left, a hierarchical tree shows the data structure: GROUP: FullStained (48 samples), POPULATION: FullStained / T-regs (98308 events), and SAMPLE: T-regs/Covid01.fcs (98308 events). The tree is expanded to show sub-populations like Q1, Q2, Q3, and T-regs. The main panel shows a t-SNE plot of the data. On the right, the properties panel allows users to choose iterators, discriminators, and batch intervals. The interface is titled 'CR2_demo' and 'FlowJo v11.0.0'.

GROUP: FullStained #Samples

- Acquired Data 48
 - Compensation Data 28
 - Multi-Disease Study Data 28
 - Comp Controls 28
 - Compensation Matrices 28
 - Acquisition-defined Matrix 28
 - Experiment Data 28
 - Multi-Disease Study Data 28
 - FullStained 28
 - Covid 5

POPULATION: FullStained / T-regs #Events

 - Q1:Comp-R780-A :: CD4+,Co... 43435
 - Q2:Comp-R780-A :: CD4+,Co... 2653
 - Q3:Comp-R780-A :: CD4+,Co... 165200
 - T-regs 98308
 - t-SNE_T-regs
 - X-shift_T-regs
 - X-shift_Tregs_1
 - Cluster1 12201
 - Cluster2 25192
 - Cluster3 10261

SAMPLE: T-regs/Covid01.fcs

Name	Count
Covid01.fcs	98308
Covid02.fcs	61711
Covid03.fcs	104609
Covid04.fcs	29122
Covid05.fcs	39257
Flu01.fcs	140516
Flu02.fcs	14206
Flu03.fcs	70298
Flu04.fcs	15008

CR2_demo

FlowJo v11.0.0

Choose iterators, discriminators and page tiling for reports. Batch by group or population.

☐ Group ☐ Population

Experiment Data

Apply Analysis Tree Selection

Choose an iterator

Sample

Iterate by: Sample

Ascending ☐ Descending ☐

Choose a discriminator

Off

Discriminate by: none

Set batch interval

1

Range: 1 to 10000

Samples per page should not exceed interval.

Rows 1 Columns 1

Range: 1 to 10000 Range: 1 to 10000

Across ☐ Down ☐

Each page in an un-batched report is a "tile".

Select the number of rows and columns to specify how many tiles appear on a batched report page.

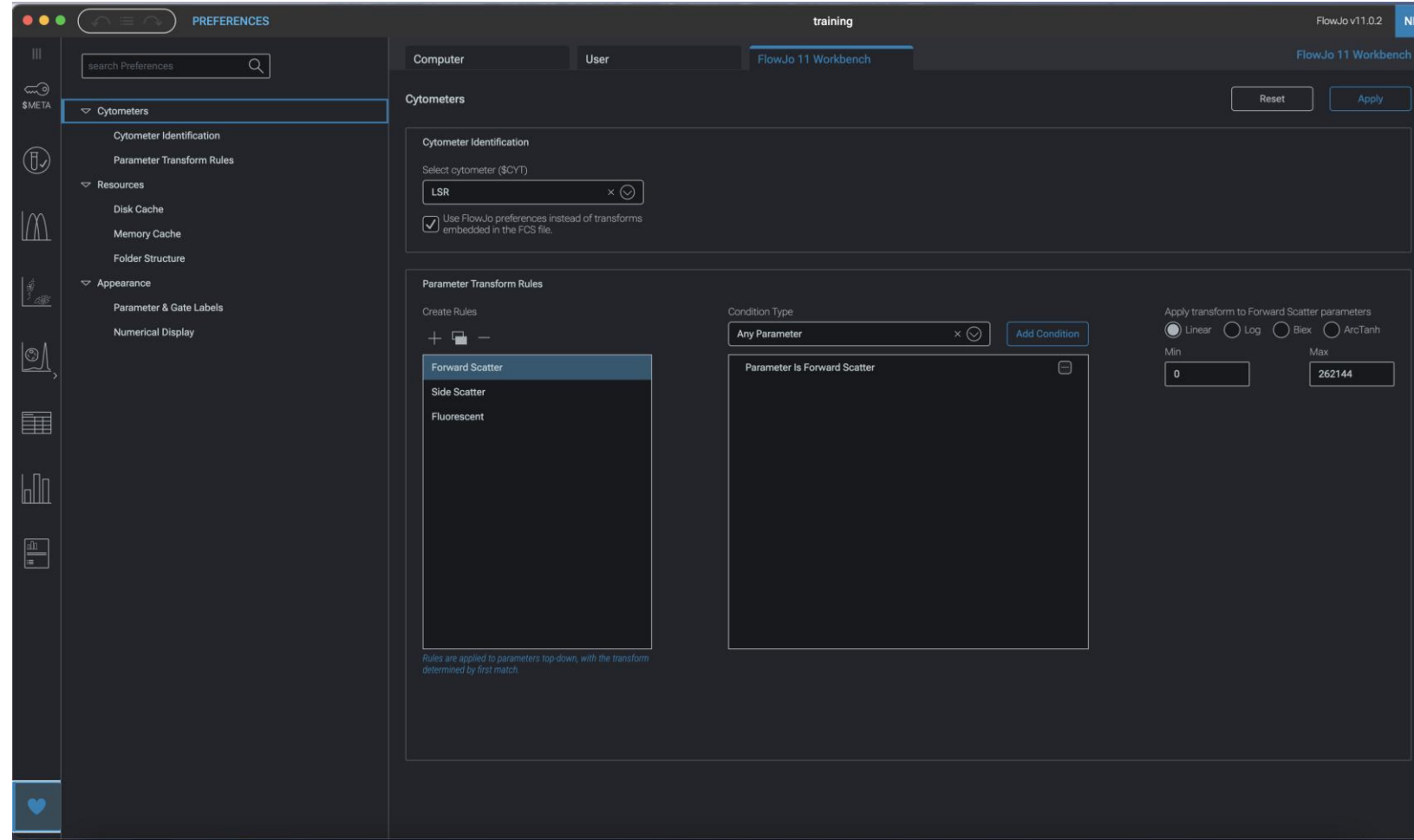
Select Across or Down for tile placement on a page.

Shorten batches by placing multiple tiles per page.

Page 1 of 1 + Page - Page

Preferences

- Tiered levels
 - Computer
 - User
 - Workbench
- Currently limited to Cytometer prefs
 - Following format of v10 advanced prefs



Development beyond 11.2

Long term

- Cloud compute as an option
- Solve plug-in handling
- Pipelines
- Match CellView Lens plugin functionalities
- Additional platforms
 - Cell Cycle, kinetics, proliferation

Demo time



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