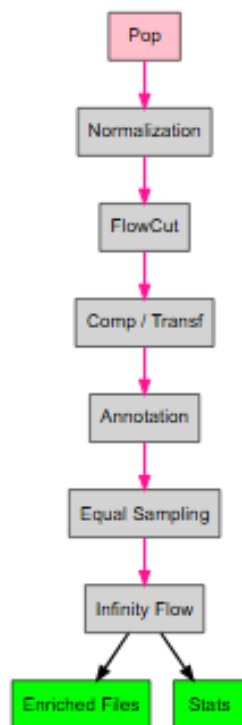


[illegible]

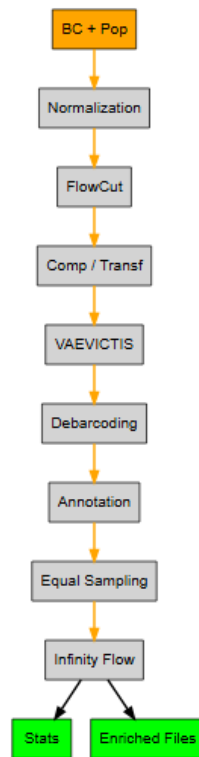
The aim of this application is to interface analysis tools in a workflow for the analysis of CMP (computational cytometry) data. This makes it possible to manage large quantities of files simultaneously.

3 workflows are possible in this app, depending on the project:

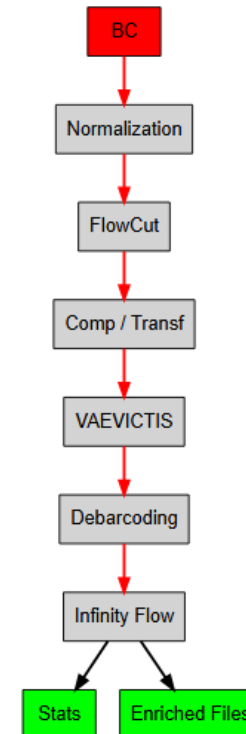
1- Analysis on pop without barcoding



2- Analysis on pop + barcoding



3- Analysis on one population with barcodes



10.71.1.6:1234

1 - To access to the app, go to your favorite browser and tap
10.71.1.6:1234
OR go to GoT (10,71,1,22)

2 – In WINSCP, create a folder for your experiment here:
/mnt/md0/CMP/input

And put your files in it (one folder per plate)

CIPHE CMP app: **DATA MANAGMENT**



Name of your
experiment

Select your
experiment
(Bc = barcode)

Indicate your
plate number

Choose the path
to your FCS files
for each plate

Load the FCS
files

UPLOADING ?

Experiment Name:

Leave blank for an automatic name

Upload backup analysis ...

Upload

Select :

- ☐ Pop
☐ Bc + Pop
☒ Bc

Upload Files

How many plates?

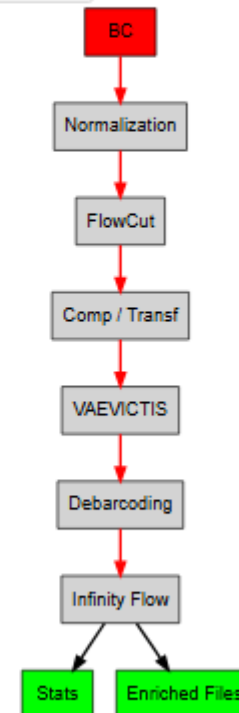
3

Select folder plate : 1

Select folder plate : 2

Select folder plate : 3

Load data



1- For each plate please click on the button

Upload Files

How many plates?

3

Select folder plate : 1

Select folder plate : 2

Select folder plate : 3

Load data

2- Click on path to your FCS for the selected plate

- ▼ CMP
 - folder backup
 - ▶ folder backupFCS
 - folder **input**
 - ▶ folder pyInfinityFlow
 - folder scaffold_models
 - ▶ folder scripts

3- Repeat these steps for the other plates

4- Click on Load data

Upload Files

How many plates?

3

Select folder plate : 1

Sele

Select folder plate : 3

Load data

plate_1 - 96 files

plate_2 - 96 files

plate_3 - 96 files

DATA MANAGEMENT

UPLOADING ⓘ

Experiment Name:

Experiment_20250425_171515

Upload backup analysis ...

UPLOADING ?

Experiment Name:












Experiment_20250425_171515

Upload backup analysis ...

RDS backup:

Click on **Upload backup analysis.**

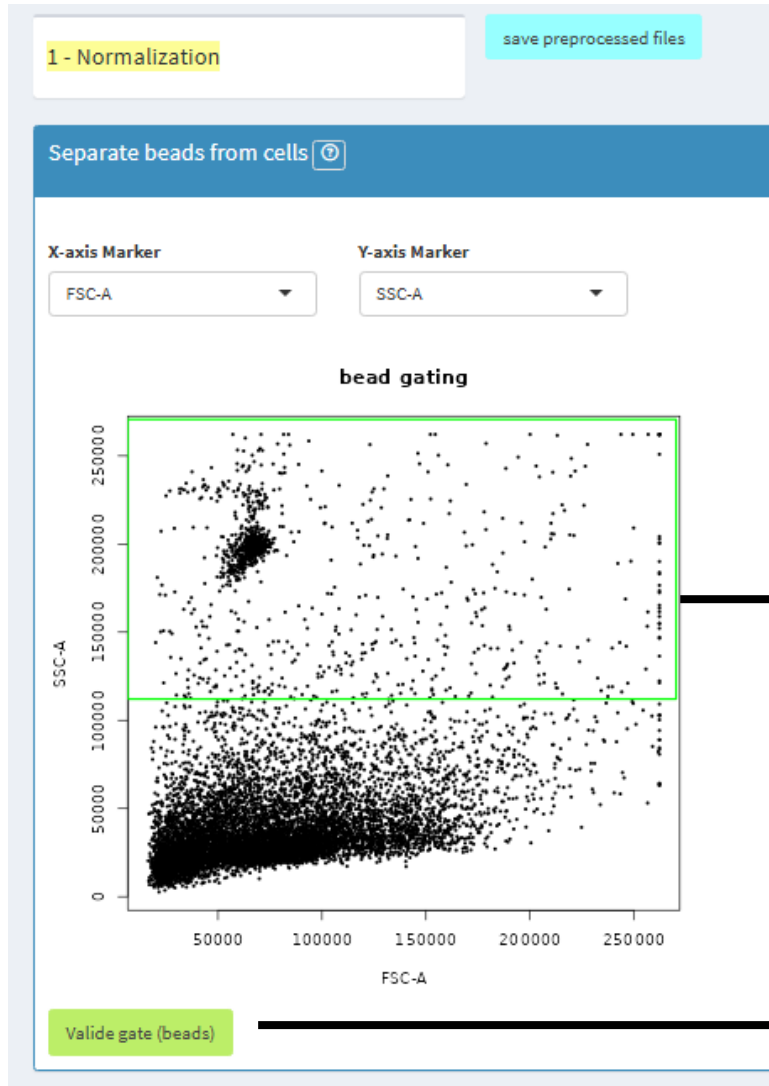
Select the RDS project file you previously saved.

	debarcoding_Experiment_20250411_142813.rds	78.3 MB	vendredi 11 avr. 2025, 14:30	vendredi 11 avr. 2025, 14:30
	debarcoding_Experiment_20250411_142813_1.rds	78.3 MB	vendredi 11 avr. 2025, 14:31	vendredi 11 avr. 2025, 14:31
	debarcoding_Experiment_20250411_153037.rds	78.3 MB	vendredi 11 avr. 2025, 15:32	vendredi 11 avr. 2025, 15:32
	debarcoding_Experiment_20250411_155620.rds	78.2 MB	vendredi 11 avr. 2025, 15:57	vendredi 11 avr. 2025, 15:56
	debarcoding_Experiment_20250411_160314.rds	78.6 MB	vendredi 11 avr. 2025, 16:04	vendredi 11 avr. 2025, 16:03
	debarcoding_Experiment_20250411_170313.rds	49.6 MB	vendredi 11 avr. 2025, 17:04	vendredi 11 avr. 2025, 17:04
	preprocessed_.rds	61.3 MB	jeudi 10 avr. 2025, 08:57	jeudi 10 avr. 2025, 08:56
	preprocessed_Experiment_20250409_183550.rds	67.3 MB	mercredi 9 avr. 2025, 18:36	mercredi 9 avr. 2025, 18:36
	preprocessed_Experiment_20250410_102038.rds	105.1 MB	jeudi 10 avr. 2025, 10:22	jeudi 10 avr. 2025, 10:22
	preprocessed_Experiment_20250424_150925.rds	237.0 MB	jeudi 24 avr. 2025, 15:14	jeudi 24 avr. 2025, 15:12
	preprocessed_Experiment_20250424_155836.rds	168.7 MB	jeudi 24 avr. 2025, 16:01	jeudi 24 avr. 2025, 16:00

Cancel

Select

CIPHE CMP app:
PREPROCESSING



With your mouse, select beads

Then, click on validate gate. This step will extract beads for the normalization and also remove all events in the selections from your files

Beads Control Quality

STEP 1 : Remove doublets

Visualization group :

plate_1

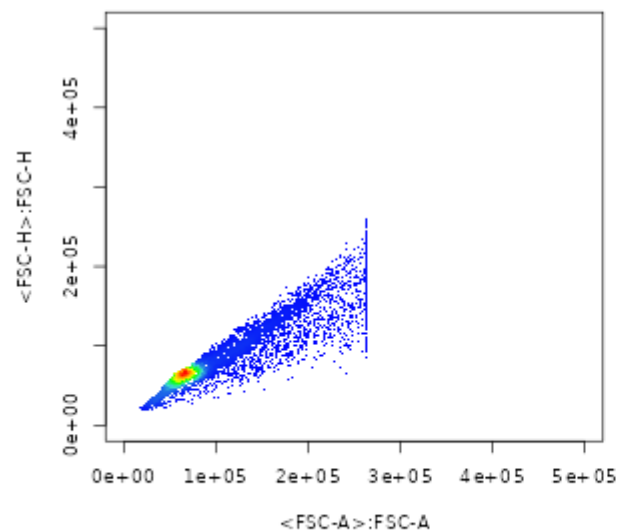
X-axis Marker

FSC-A

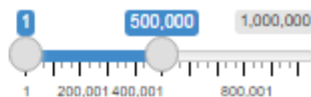
Y-axis Marker

FSC-H

Beads for Group: plate_1



X scale



Y scale



Remove doublets (All plates)

Bead gating step 1 :
Click to remove doublets
from your beads

1- Transform beads with $\log(500)$ (just for better visualization)

2- Choose marker with the better distinction between peaks

3- Indicate the number of bead peaks

4- Launch clusterint algorithm

5- Choose the bead peak that you want to use for the normalization

Select mode

Transform data (All plates)

X-axis Marker

G-PE-A

nb of Peaks

2

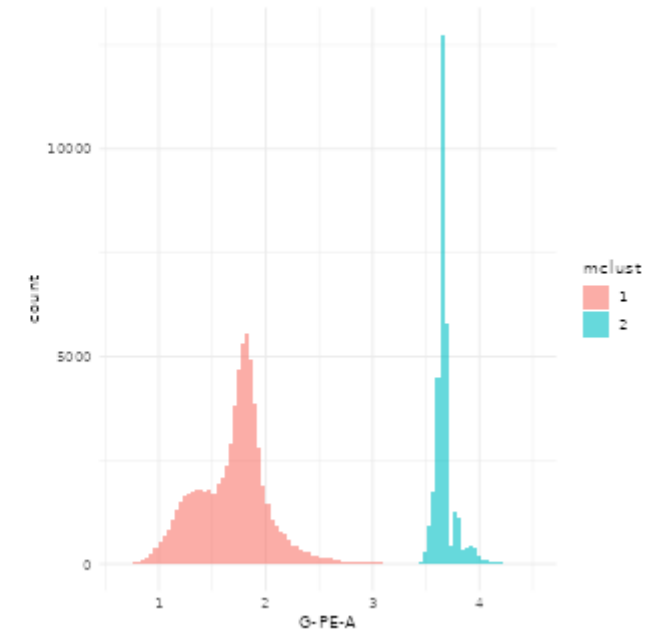
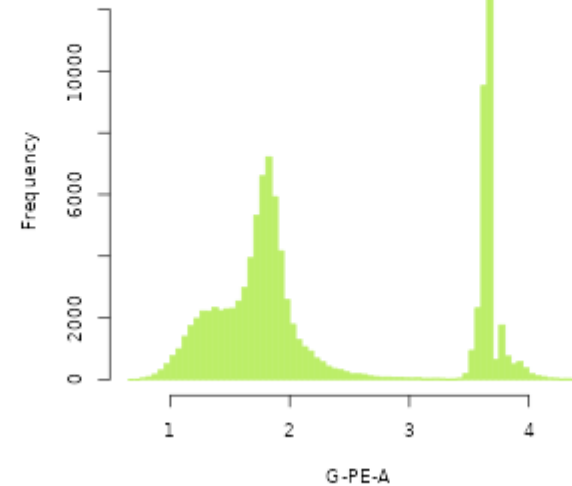
Launch mclust (All plates)

Select Peak

☐ 1

☒ 2

Beads before gating for : plate_1



(optionnal) STEP 3 : Re-gate

Select group

plate_1

X-axis Marker

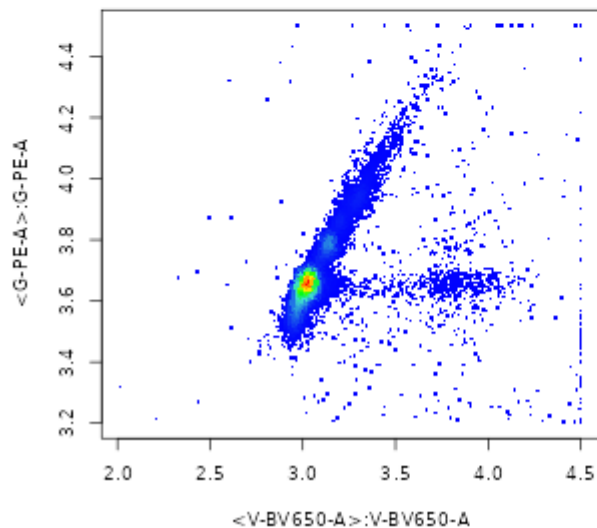
V-BV650-A

Y-axis Marker

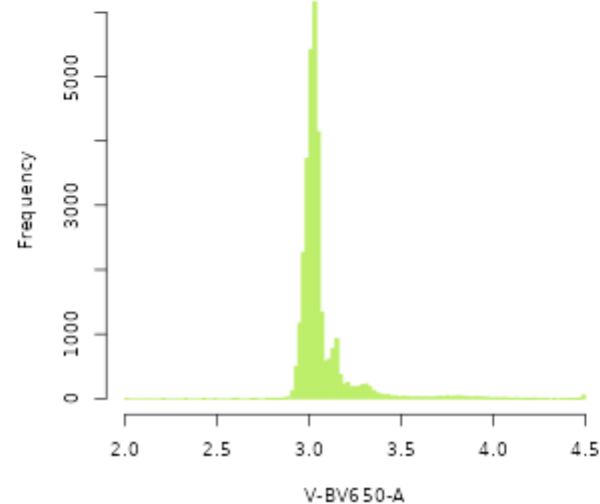
G-PE-A

Reset Gate

Beads peaks n° 2 for group : plate_1



Beads peaks n° 2 for group : plate_1



For each plate, perform manual gating to select only beads and not debris

And after

unTransform data (All plates)

Click on
untransform beads

Validate beads (All plates)

And finally, click on
validate beads

Normalization ⓘ

First : Select markers to normalize

Group
plate_2 ▼

Common markers with Ref

FSC-A	FSC-H	FSC-W	SSC-A	SSC-H
SSC-W	UV-BUV395-A	UV-BUV496-A		
UV-BUV737-A	UV-BUV805-A	V-BV421-A		
V-V500-A	V-BV610-A	V-BV650-A		
V-BV711-A	B-FITC-A	B-PE-Cy5-5-A		
G-PE-A	G-PE-TR-A	G-PE-Cy5-A		
G-PE-Cy7-A	R-APC-A	R-Alexa700-A		
R-APC-Cy7-A				

Normalize

→ Select markers to
normalize for each
Group

↓
Then, click on
Normalize

Harmonization table for :

plate_2

Show 10 entries

Search:

Factor

FSC-A	0.9856
FSC-H	0.9896
FSC-W	0.9971
SSC-A	0.9999
SSC-H	1.0016
SSC-W	0.9983
UV-BUV395-A	1.5335
UV-BUV496-A	0.5629
UV-BUV737-A	3.1914
UV-BUV805-A	4.2899

Showing 1 to 10 of 24 entries

Previous

1

2

3

Next

Enter ZIP file name (without extension):

NormalizedFCS

Choose

rawFCS

X-axis Marker

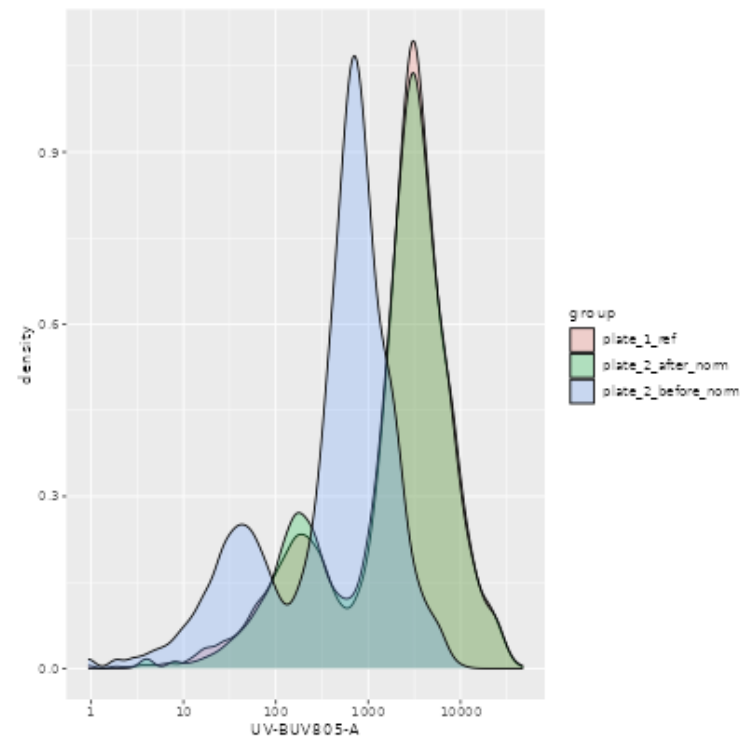
UV-BUV805-A

Group

plate_1_ref

plate_2_before_norm

plate_2_after_norm



Download normalized FCS

Download harmonized Table

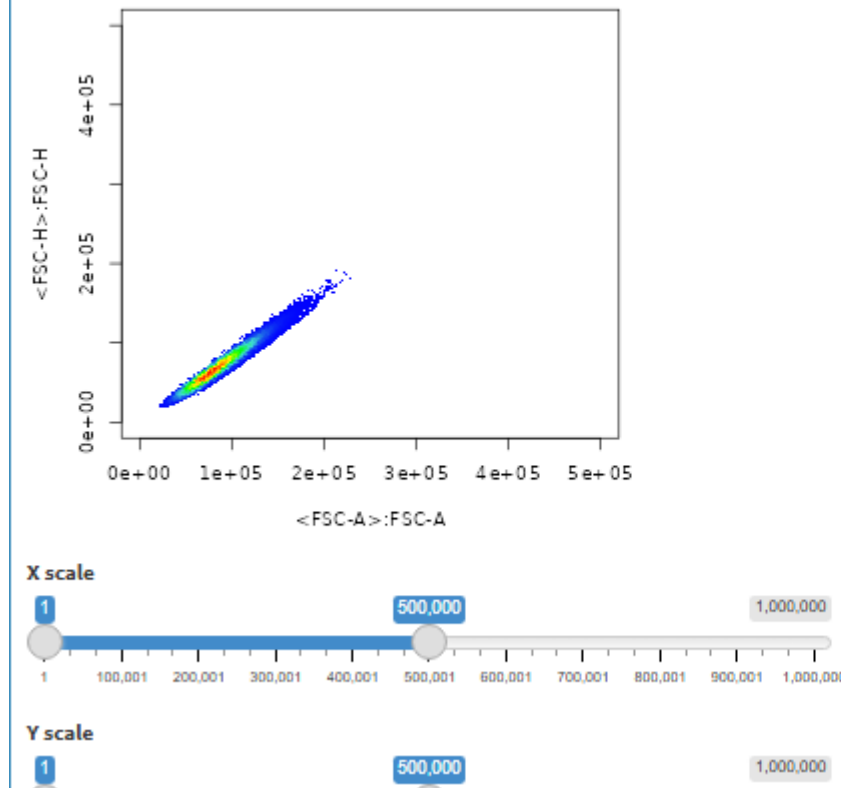
This section is designed to clean your FCS files by removing technical artifacts and unwanted events.

Remove margin events: These are extreme events that fall outside the detector range and may distort analysis.

Remove doublets: These are overlapping events (e.g., two cells passing together) that should be excluded from single-cell analysis.

Use FlowCut: FlowCut is an automated quality control algorithm that detects and removes abnormal regions in flow cytometry data over time. It uses statistical and signal drift detection to identify unstable acquisition periods, improving data consistency.

→ Configure FlowCut thresholds and select relevant markers before launching the cleaning process on all files.



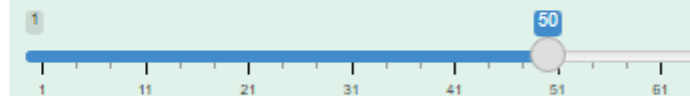
FlowQC

Preprocessing includes also:

- Remove margin events
- Remove doublets

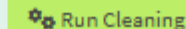
☒ Include flowCut step

Perc. max of cells removed (maxPercCut)



Markers flowcut :

FSC-A SSC-A UV-BUV395-A UV-BUV496-A UV-BUV737-A
 UV-BUV805-A V-BV421-A V-V500-A V-BV610-A V-BV711-A
 B-FITC-A V-BV650-A

 Run Cleaning

Results QC :

plate_1 plate_2

Show 10 entries

Search:

Perc.Removed

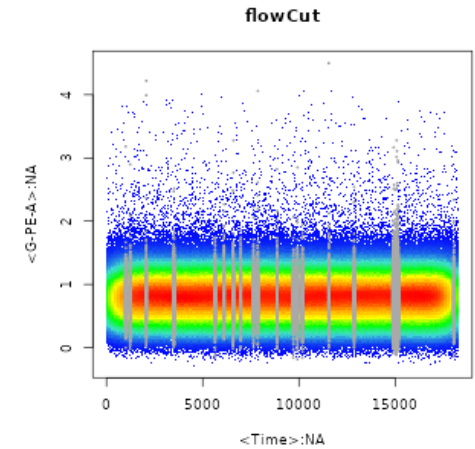
plate_2_Specimen_001_MIX+Billes-phoenix_003.fcs 2.8262

Showing 1 to 1 of 1 entries

Previous 1 Next

Is it monotonically increasing in time	[,1]
Largest continuous jump	"1"
Continuous - Pass	"0.828"
Mean of % of range of means divided by range of data	"F"
Mean of % - Pass	"0.153"
Max of % of range of means divided by range of data	"F"
Max of % - Pass	"0.871"
Has a low density section been removed	"F"
% of low density removed	"0"
How many segments have been removed	"41"
% of events removed from segments removed	"2.8262"
Worst channel	"G-PE-A"
% of events removed	"2.8262"
FileID	"2025_04_28_09_39_47"
Type of Gating	"AllCutMin"
Was the file run twice	"Yes"
Has the file passed	"F"

RemoveQC (Selected file) RemoveQC (Selected group) ValidateQc (All Files)



For each plate, you have a table with the file name, and the associated percentage of deleted cells. For more information on a particular file, click on the file in the table.

Click on Perc.Removed to display the files in ascending or descending order.

Results QC :

plate_1 plate_2

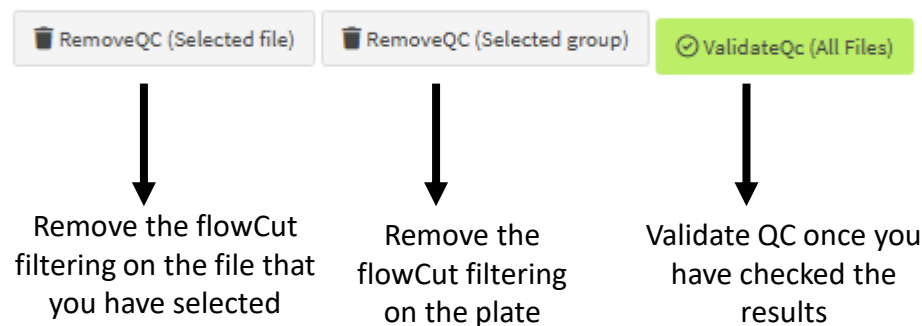
Show 10 entries Search:

Perc.Removed

plate_1_Specimen_044_D8_D08_044_CMCD8Tc.fcs	14.27
plate_1_Specimen_086_H2_H02_086_CMCD8Tc.fcs	13.92
plate_1_Specimen_010_A10_A10_010_CMCD8Tc.fcs	13.83
plate_1_Specimen_011_A11_A11_011_CMCD8Tc.fcs	13.68
plate_1_Specimen_062_F2_F02_062_CMCD8Tc.fcs	13.59
plate_1_Specimen_028_C4_C04_028_CMCD8Tc.fcs	13.55
plate_1_Specimen_002_A2_A02_002_CMCD8Tc.fcs	13.52
plate_1_Specimen_080_G8_G08_080_CMCD8Tc.fcs	13.45
plate_1_Specimen_094_H10_H10_094_CMCD8Tc.fcs	13.4
plate_1_Specimen_059_E11_E11_059_CMCD8Tc.fcs	13.32

Showing 51 to 60 of 96 entries Previous 1 ... 5 6 7 ... 10 Next

SAVE FLOWCUT FILTERING :



3 - Concatenate plates

Concatenate plates ⓘ

Choose plates to concatenate

plate_1 plate_2

Run concatenation

This section allows you to concatenate plates. The data parameters will be those present in the first plate you selected.

! Repeat this action as many times as you have plates to concatenate. !

3- Concatenate plates

Export deconcatenated FCS files

Select the plates you want to export. You can either export them as a ZIP file locally or save them on the server in /mnt/md0/CMP/backupFCS.

Plates to export:

☒ plate_1

Export to:

☐ Local ZIP

☒ Server Folder

Folder name (used only for server export):

Experiment_20250606_144942

Cancel

Start Export

After concatenation, export the plates containing the files (e.g. if you concatenated Plate 1 and Plate 2, export Plate 1).

Then refresh the application and re-import the files.

4-Compensation and transformation



Apply comp



Comp and Transfo

☒ Apply spillover matrix

(Option) Upload CSV transformation file

Upload CSV

No file selected

Transformation

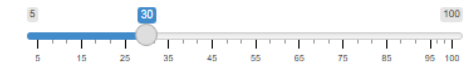
arsinh

Fluo	Arg
FSC-A	none
FSC-H	none
FSC-W	none
SSC-A	none
SSC-H	none
SSC-W	none
UV-BUV395-A	2000
UV-BUV496-A	500
UV-BUV737-A	none
UV-BUV805-A	none
V-BV421-A	none
V-V500-A	none
V-BV610-A	none
V-BV650-A	none
V-BV711-A	none
B-FITC-A	none
B-PE-Cy5-5-A	none
G-PE-A	none
G-PE-TR-A	none
G-PE-Cy5-A	none
G-PE-Cy7-A	none
R-APC-A	none
R-Alexa700-A	none
R-APC-Cy7-A	none
Time	none
vaevictis1	none
vaevictis2	none
barcodeId	none
Flag	none
fileId	none
FlaeApp	none

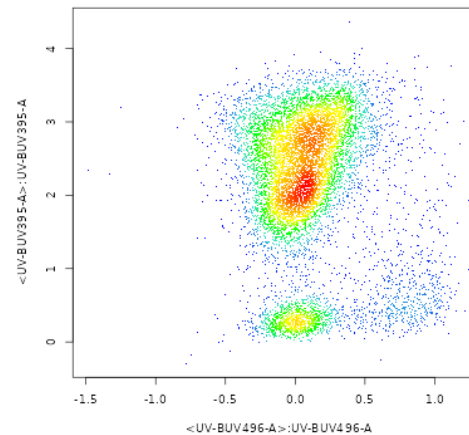
X-axis Marker
UV-BUV496-A

Y-axis Marker
UV-BUV395-A

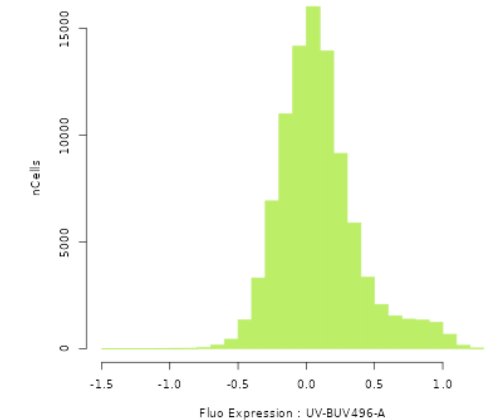
Number of Breaks:



plate_1



plate_1



Change cofactor



Export transformation table



Apply transform to visualize your data transformation



Transform

Export CSV

Validate transformation (Apply to all files)



FINAL : Click to validate the transformation table

CIPHE CMP app:
DEBARCODING

VAEVICTIS ⓘ

STEP 1: Run vaeictis

Select markers

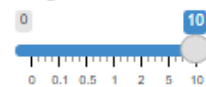
UV-BUV395-A V-BV650-A R-APC-A

N. of clusters

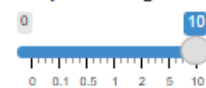
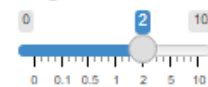
15

N.cells/clust

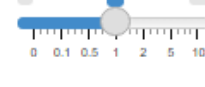
500

t-SNE regularization
weight

ivis pn loss weight

reconstruction error
weight

KL-divergence weight

UMAP regularization
weight☐ Use previously saved model☒ Save this model after training

Run

VAEVICTIS RUN:

- 1. Marker selection:** Choose the markers you want to use for clustering. These should be relevant for distinguishing cell populations.
- 2. Run on subset:** Click **Run** to apply VAEVICTIS on a subset of all your files (combined from all plates).

Run vaeictis on subset of each file and each plate

1- Run vaevictis

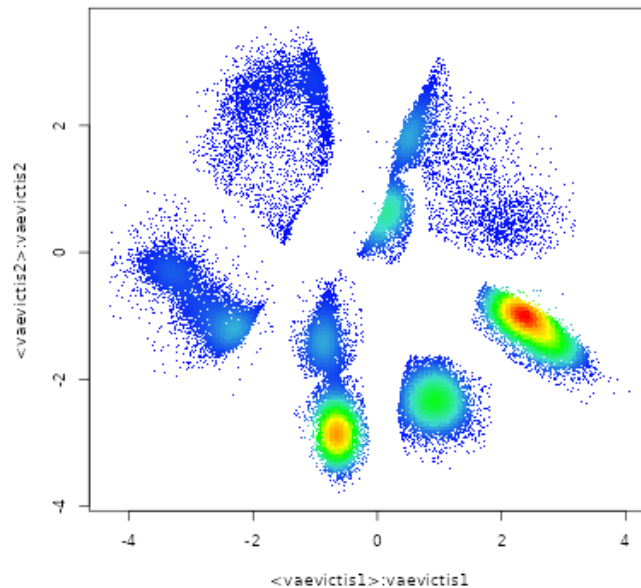


Choose Plate

Subset of all plates

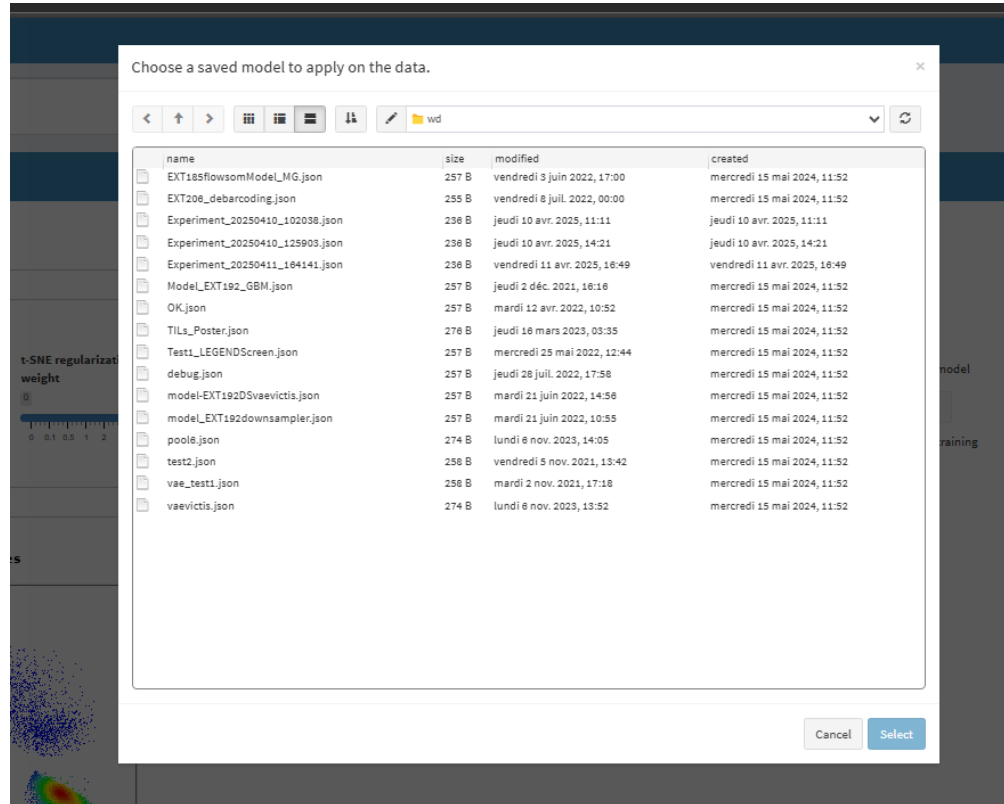
Apply (Run on all Files)

Subset of all plates



- 3. Visualize results:** Review the plots generated to assess cluster separation and quality.
- 4. Apply to all:** If satisfied with the preview, click **Apply (Run on all files)** to perform the full clustering process.

1- Run vaevictis



Load a saved model

Apply (Run on all Files)

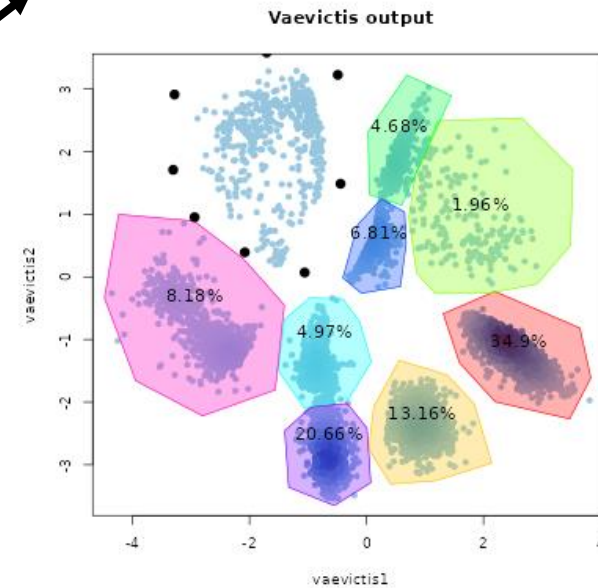
1- Run vaevictis

STEP 2: Attribute barcodes

X-axis Marker vaevictis1 Y-axis Marker vaevictis2

Apply Gate Reset Gates

Manual gating : just click on the plot to gate barcode, and then, click on apply Gate



gates

- Gate 1
- Gate 2
- Gate 3
- Gate 4
- Gate 5
- Gate 6
- Gate 7
- Gate 8

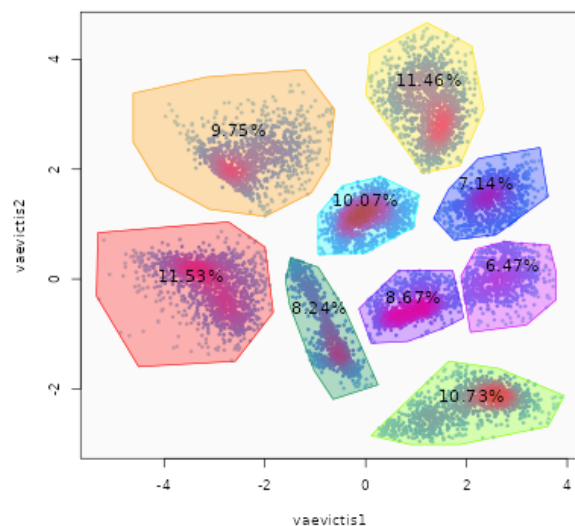
X-axis Marker
Y-axis Marker
X-axis Marker (gate)
Y-axis Marker (gate)
X-axis Marker (gate 2)
Y-axis Marker (gate 2)

FSC-A
FSC-A
R-APC-A
V-BV650-A
R-APC-A
UV-BUV395-A

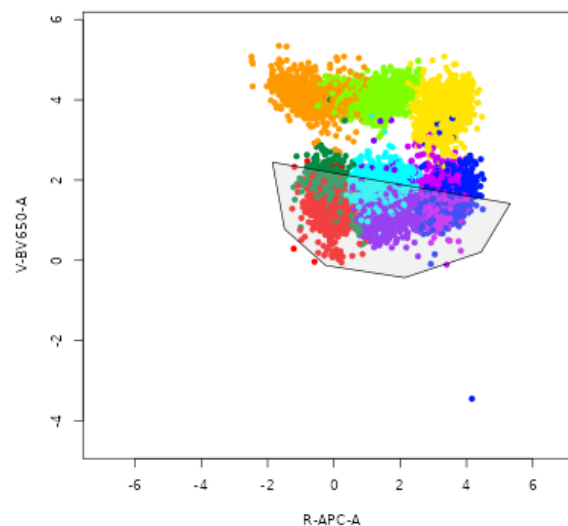
Apply Gate Reset Gates

Apply Gate Reset Gates

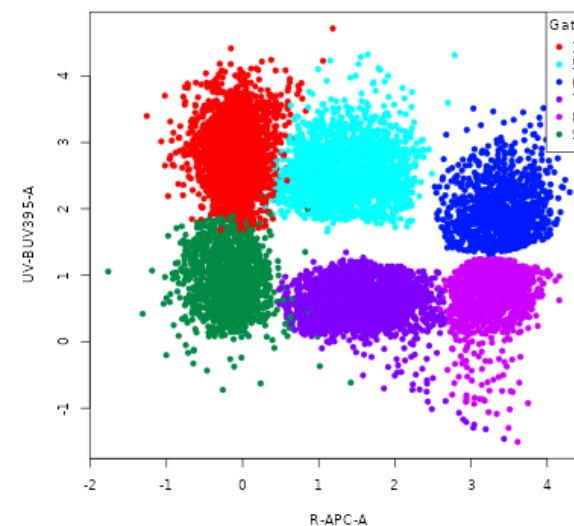
Vaevictis output



Gate Visualization



Gate 2 Visualization



gates

- Gate 1
- Gate 2
- Gate 3
- Gate 4
- Gate 5
- Gate 6
- Gate 7
- Gate 8
- Gate 9

Select

- ☒ Gate 1
- ☒ Gate 2
- ☒ Gate 3
- ☒ Gate 4
- ☒ Gate 5
- ☒ Gate 6
- ☒ Gate 7
- ☒ Gate 8
- ☒ Gate 9

STEP 3: Reattribute id to each gate (ONLY NUMERIC id)

Visualize each gate/barcode in a second plot. Select gate that you want to see in the plot

6	7	CC60
7	4	CC8
8	8	CC71

barcode_label

Export table

validate barcoding (All files)

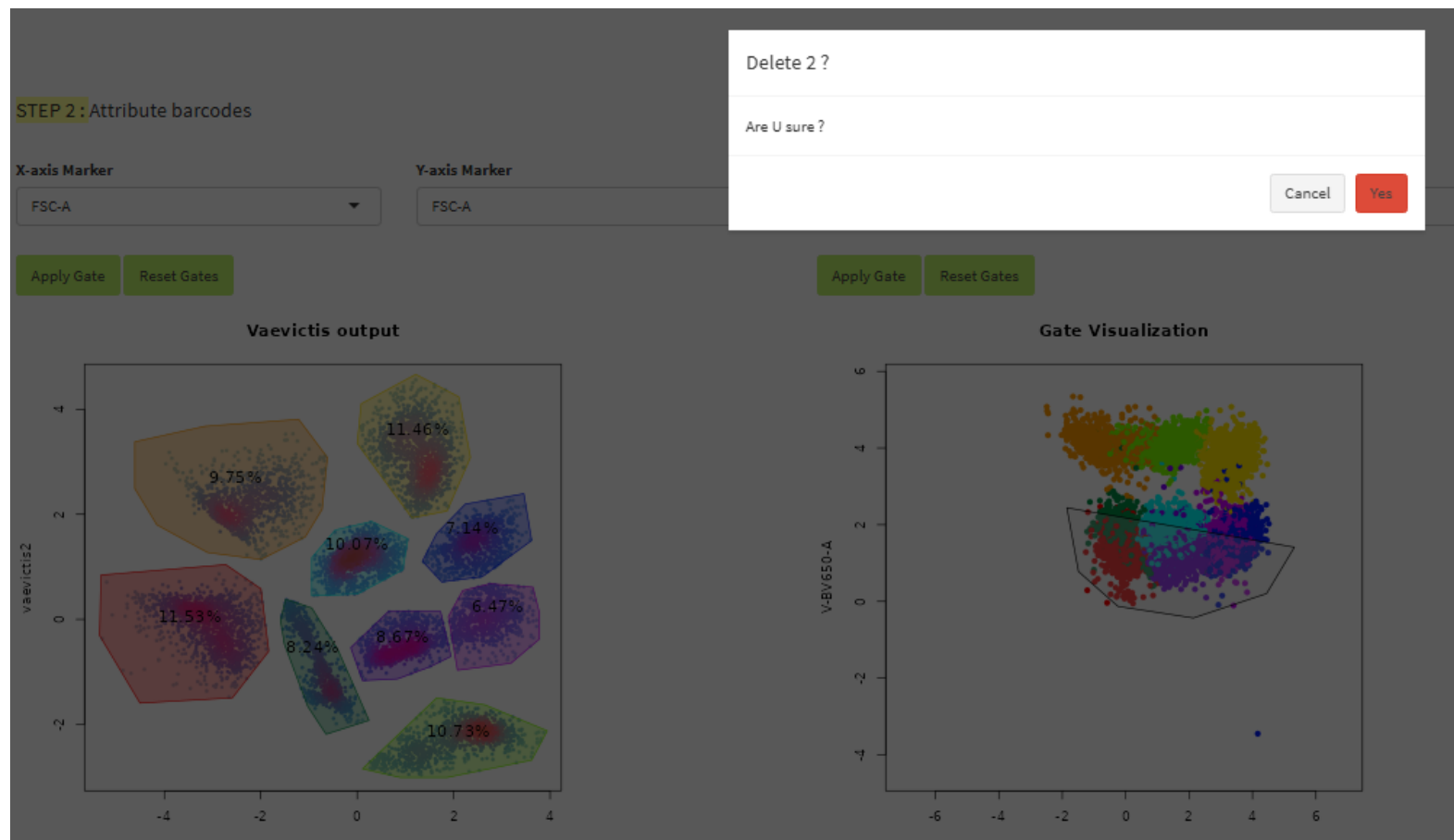
Information:

The column **barcode_id** refers to the NUMERICAL values that will be the FCS files to represent barcodes. FCS files can only store numerical values which is why this column is required.

The column **barcode_name** will not be stored in the FCS files but is a human-readable label to help match the **barcode_id** to a correct phenotype or condition. You can use any text here.

You can also export or import this table to preserve and reuse your mappings in future sessions.

To delete just one gate, click on the gate



STEP 3 : Reattribute id to each gate (ONLY NUMERIC id)

gate	barcode_id	barcode_label
1	1	B6J
2	3	CC80
3	9	CC16
4	6	CC10
5	5	CC12
6	7	CC60
7	4	CC8
8	8	CC71
9	2	CC3

Export table

validate barcoding (All files)

Information:

The column **barcode_id** refers to the NUMERICAL values that will be added to the FCS files to represent barcodes. FCS files can only store numeric values, which is why this column is required.

The column **barcode_name** will not be stored in the FCS files but serves as a human-readable label to help match the **barcode_id** to a corresponding phenotype or condition. You can use any text here.

You can also export or import this table to preserve and reuse your barcode mappings in future sessions.

Once the table is debarcoded, click on validate barcode so that it enriches all your files with barcodes (all cells outside the gates will be removed from the files)

X-axis Marker
FSC-A

Y-axis Marker
FSC-A

X-axis Marker (gate)
R-APC-A

Y-axis Marker (gate)
V-BV650-A

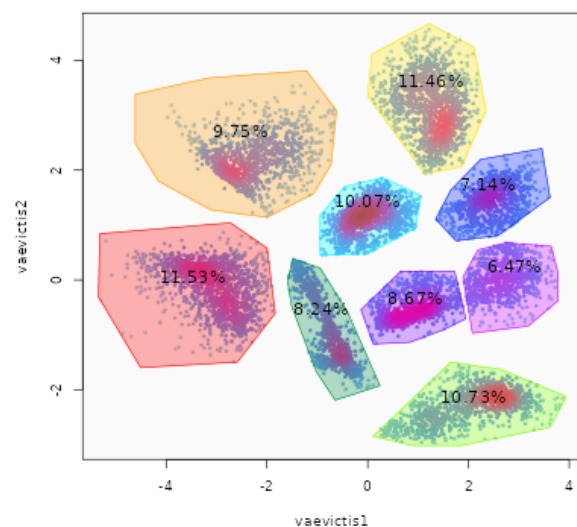
X-axis Marker (gate 2)
R-APC-A

Y-axis Marker (gate 2)
UV-BUV395-A

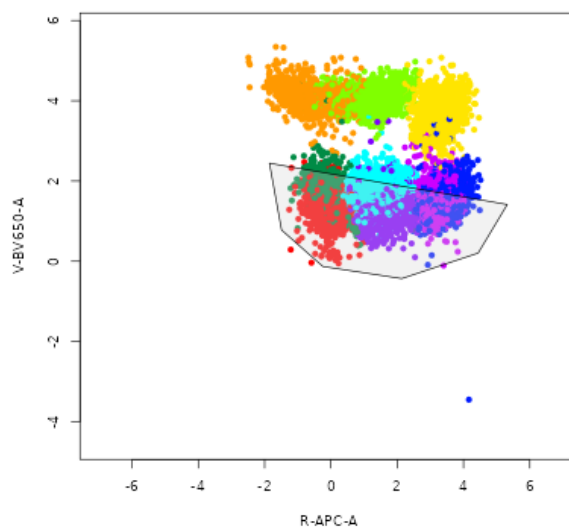
Apply Gate Reset Gates

Apply Gate Reset Gates

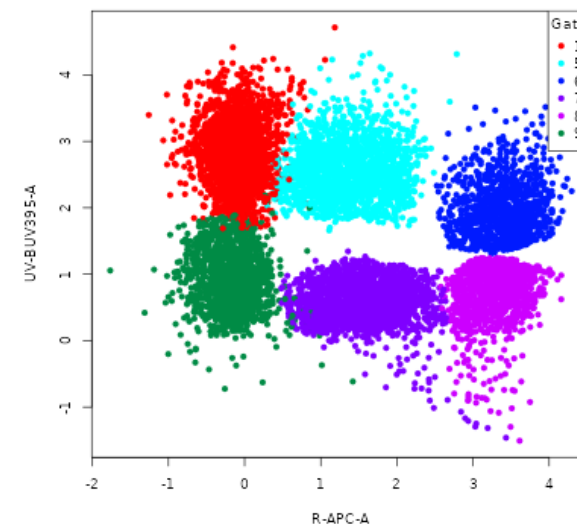
Vaevictis output



Gate Visualization



Gate 2 Visualization



Important information: Only the gates on the left-hand plot count; the gate on the middle plot is for visualization purposes only.

CIPHE CMP app:
ANNOTATION

This section allows you to annotate a subset of your dataset based on reference files. You can choose between two methods: Scyan or Scaffold. Both methods apply annotations to a subset of the data. Once the annotation is done, scroll down to the 'QC Annotation' section to inspect the results using UMAP and scatter plots. If you are satisfied with the results, click on 'Run on all files' to apply the annotation to all FCS files across your plates.

As with all parts, click on the help button for information on this part.

Help - Scyan Annotation

Scyan allows you to annotate all your FCS files using a reference knowledge table.

What is the knowledge table?

- It is a matrix where:
 - Each **column** corresponds to a marker (core panel).
 - Each **row** corresponds to a known cell population.
 - Each value should be between -1 and +1:
 - 1: the population does not express the marker
 - +1: the population does express the marker

How to provide it?

- Option 1:** Upload a CSV or Excel file. The first column must contain population names in the format: **1_TCD4** (a number, underscore, then the population name).
- Option 2:** Upload individual FCS files — each file must represent a single population. The file names must follow the same format: **1_CD4.fcs**.

Marker Matching: After the table is loaded, you will need to match the markers from your FCS files with those from the core panel used in the knowledge table.

Parameters:

- std (standard deviation):** Controls how strictly the model follows the knowledge table. A lower value enforces stricter adherence to the expected marker expressions, while a higher value allows more flexibility.
- lr (learning rate):** Determines how fast the model updates during training. A very low value may result in slow convergence; a very high value might make training unstable.

Default values generally work well, but you may adjust them depending on your data quality or model performance.

Once configured, Scyan will train a model using the table and annotate your ungated FCS files accordingly.

Close

Scyan Annotation : ?

Load or build scyan knowledge table

Upload knowledge Table (CSV or Excel) :

Upload... No file selected

OR upload landmark FCS ...

Match FCS markers with landmark markers

Markers in FCS

Markers in knowledge table

Annotation

Select column that contains annotation labels

std

lr

0,25

0,001

Run Scyan annotation

What is the knowledge table?

- It is a matrix where:
 - Each **row** corresponds to a known cell population.
 - Each value should be between **-1** and **+1**:
 - **-1**: the population does *not* express the marker
 - **+1**: the population *does* express the marker

How to provide it?

- **Option 1:** Upload a CSV or Excel file. The first column must contain population names in the format: **1_TCD4** (a number, underscore, then the population name).
- **Option 2:** Upload individual FCS files — each file must represent a single population. The file names must follow the same format: **1_CD4.fcs**.

Marker Matching: After the table is loaded, you will need to match the markers from your FCS files with those from the core panel used in the knowledge table.

⚙ Parameters:

•**std (standard deviation):** Controls how strictly the model follows the knowledge table. A lower value enforces stricter adherence to the expected marker expressions, while a higher value allows more flexibility.

•**lr (learning rate):** Determines how fast the model updates during training. A very low value may result in slow convergence; a very high value might make training unstable.

Default values generally work well, but you may adjust them depending on your data quality or model performance.

Once configured, Scyan will train a model using the table and annotate your ungated FCS files accordingly.

Match FCS markers with landmark markers

Markers in FCS

Markers in knowledge table

FSC.A SSC.A CD4 CD11b CD44 CD5.Ly6G IA.IE CD317 CD62L
CD8a.SiglecF F4.80 CD161 Ly6C CD25 CD11c CD19

Annotation

Select column that contains annotation labels

Populations ▼

std

0,25

lr

0,001

Run Scyan annotation

Step 1 – Clustering:

By default, the clustering algorithm used is **CLARA**.

Select the markers you want to use for clustering and click **Run CLARA clustering**.

Annotation Scaffold ⓘ

STEP 1 - Clustering

Markers used for clustering

FSC-A

SSC-H

SSC-W

UV-BUV395-A

UV-BUV496-A

UV-BUV737-A

UV-BUV805-A

V-BV421-A

V-V500-A

V-BV610-A

V-BV650-A

V-BV711-A

B-FITC-A

B-PE-Cy5-5-A

G-PE-A

G PE-TR-A

G-PE-Cy5-A

G-PE-Cy7-A

R-APC-A

R-Alexa700-A

R APC-Cy7-A

k_parameter

300

Run CLARA clustering

☐ My file(s) is(are) already clustered :

Step 1 – Clustering:

If your file is already clustered, check the box **“My files are already clustered”** and select the column that contains the cluster labels.

☒ My file(s) is(are) already clustered :

Choose name of column that contain clusters

fileId

G-PE-Cy5-A

G-PE-Cy7-A

R-APC-A

R-Alexa700-A

R APC-Cy7-A

Time

vaevictis1

Step 2 – Build or Load Scaffold Map:

- You can build a Scaffold reference map from annotated FCS files.
- Each FCS file must contain a single cell population, and the filename should follow the format: **1_TCD4.fcs** (ID number + underscore + population name).
- Alternatively, you can upload a previously built Scaffold map if you already have one.

STEP 2 - Build or load scaffold Map

Upload landmark FCS ...

OR upload scaffold map ...

Build Scaffold Map

This section allows you to visually assess the quality of your annotations using UMAP projection. A subset of all FCS files is used to generate a UMAP embedding. Annotations (from Scyan or Scaffold) are overlaid on the UMAP for inspection. If the clustering and annotation look correct, you can apply the annotation to all files by clicking the corresponding button. This step helps verify that the model-based annotations align with known population structures before full application.

Markers for UMAP

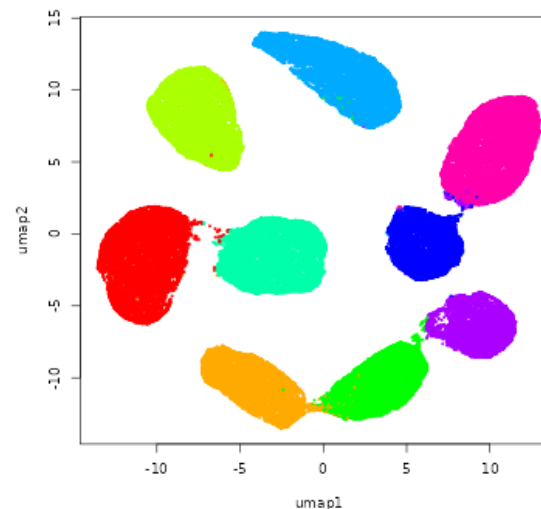
UV-BUV395-A V-BV650-A R-APC-A

Run UMAP

Color by :

barcodeID

Legend: barcodeID



This section allows you to visually assess the quality of your annotations using UMAP projection. A subset of all FCS files is used to generate a UMAP embedding. Annotations (from Scyan or Scaffold) are overlaid on the UMAP for inspection. If the clustering and annotation look correct, you can apply the annotation to all files by clicking the corresponding button. This step helps verify that the model-based annotations align with known population structures before full application.



If you are satisfied with the results, click on 'Run on all files' to apply the annotation to all FCS files across your plates.

Run Scyan on all files

Run Scaffold on all files

CIPHE CMP app:
pyInfinity Flow

Help – pyInfinityFlow

The **pyInfinityFlow** module allows you to predict unmeasured markers based on backbone markers using a deep learning model.

Step 1 – Data setup:

- For each plate, upload a TXT file containing plate information.
- This file must contain two tab-separated columns:
 - **Infinity_target**
 - **Infinity_isotype**

Step 2 – Marker selection:

- Select core panel markers as **backbone** markers (used for prediction).
- Designate the markers you want to impute as **exploratory**.

Step 3 – Run pyInfinityFlow:

- Select the number of cores to use, based on your machine's capabilities.
- Click **Run pyInfinityFlow** to start the prediction process.

Step 4 – Statistics:

- This part allows you to compute statistics either on:
 - The original measured values, or
 - The predicted values from pyInfinityFlow.
- First, define the positivity threshold for each ISO file to interpret marker expression

pyInfinityFlow : 

STEP 1 : Please upload Infinity Markers

Upload for plate: 1 (TXT format)

Browse...

No file selected

Upload for plate: 2 (TXT format)

Browse...

No file selected

Upload for plate: 3 (TXT format)

Browse...

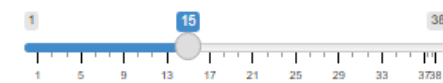
No file selected

STEP 2 : Please select backbone and exploratory markers :

	name	desc	type
\$P1	FSC-A	FSC-A	backbone
\$P2	FSC-H	FSC-H	backbone
\$P3	FSC-W	FSC-W	backbone
\$P4	SSC-A	SSC-A	backbone
\$P5	SSC-H	SSC-H	backbone
\$P6	SSC-W	SSC-W	backbone
\$P7	UV-BUV395-A	UV-BUV395-A	discard
\$P8	UV-BUV496-A	UV-BUV496-A	backbone
\$P9	UV-BUV737-A	UV-BUV737-A	backbone
\$P10	UV-BUV805-A	UV-BUV805-A	backbone
\$P11	V-BV421-A	V-BV421-A	backbone
\$P12	V-V500-A	V-V500-A	backbone
\$P13	V-BV610-A	V-BV610-A	backbone
\$P14	V-BV650-A	V-BV650-A	discard
\$P15	V-BV711-A	V-BV711-A	backbone
\$P16	B-FITC-A	B-FITC-A	backbone
\$P17	B-PE-Cy5-5-A	B-PE-Cy5-5-A	backbone
\$P18	G-PE-A	G-PE-A	exploratory
\$P19	G-PE-TR-A	G-PE-TR-A	backbone
\$P20	G-PE-Cy5-A	G-PE-Cy5-A	backbone
\$P21	G-PE-Cy7-A	G-PE-Cy7-A	backbone
\$P22	R-APC-A	R-APC-A	discard
\$P23	R-Alexa700-A	R-Alexa700-A	backbone
\$P24	R-APC-Cy7-A	R-APC-Cy7-A	backbone
\$P25	Time	Time	discard
\$P26	vaevictis1	vaevictis1	discard
\$P27	vaevictis2	vaevictis2	discard
\$P28	barcodeId	barcodeId	discard
\$P29	Flag	Flag	discard
\$P30	fileId	fileId	discard
\$P31	FlaeApp	FlaeApp	discard

STEP 3 : Run pyInfinityFlow

n cores



☐ Transform with pyInfinityFlow method (est.logicle)

Run (py) InfinityFlow

Step 1 – Data setup:

- For each plate, upload a TXT file containing plate information.
- This file must contain two tab-separated columns:
 - Infinity_target
 - Infinity_isotype

STEP 1 : Please upload Infinity Markers

Upload for plate: 1 (TXT format)

Browse...

No file selected

Upload for plate: 2 (TXT format)

Browse...

No file selected

Upload for plate: 3 (TXT format)

Browse...

No file selected

```
Infinity_target Infinity_isotype
"Rat IgG1,k" "Rat IgG1,k"
CD369 (Dectin-1/CLEC7A) "Rat IgG1,k"
PIR-A/B "Rat IgG1,k"
CD22 "Rat IgG1,k"
CD324 (E-Cadherin) "Rat IgG1,k"
CD172a (SIRPa) "Rat IgG1,k"
CD319 "Rat IgG1,k"
"Rat IgG2a,k" "Rat IgG2a,k"
MAIR-V "Rat IgG2a,k"
CD146 "Rat IgG2a,k"
PD-1H (VISTA) "Rat IgG2a,k"
CD8a "Rat IgG2a,k"
" CD275 (B7-H2, B7-RP1, ICOS Ligand)" "Rat IgG2a,k"
Ly-6A/E (Sca-1) "Rat IgG2a,k"
CD40 "Rat IgG2a,k"
CD45R/B220 "Rat IgG2a,k"
CD197 (CCR7) "Rat IgG2a,k"
CD47 "Rat IgG2a,k"
CD98 (4F2) "Rat IgG2a,k"
CD14 "Rat IgG2a,k"
CD107a (LAMP-1) "Rat IgG2a,k"
CD18 "Rat IgG2a,k"
Ly-6G "Rat IgG2a,k"
CD21/CD35 (CR2/CR1) "Rat IgG2a,k"
Mac-2 (Galectin-3) "Rat IgG2a,k"
CD199 (CCR9) "Rat IgG2a,k"
Ly-51 "Rat IgG2a,k"
IgD "Rat IgG2a,k"
Tim-4 "Rat IgG2a,k"
CD71 "Rat IgG2a,k"
```

STEP 2 : Please select backbone and exploratory markers :

	name	desc	type
SP1	FSC-A	FSC-A	backbone ▼
SP2	FSC-H	FSC-H	backbone ▼
SP3	FSC-W	FSC-W	backbone ▼
SP4	SSC-A	SSC-A	backbone ▼
SP5	SSC-H	SSC-H	backbone ▼
SP6	SSC-W	SSC-W	backbone ▼
SP7	UV-BUV395-A	UV-BUV395-A	discard ▼
SP8	UV-BUV496-A	UV-BUV496-A	backbone ▼
SP9	UV-BUV737-A	UV-BUV737-A	backbone ▼
SP10	UV-BUV805-A	UV-BUV805-A	backbone ▼
SP11	V-BV421-A	V-BV421-A	backbone ▼
SP12	V-V500-A	V-V500-A	backbone ▼
SP13	V-BV610-A	V-BV610-A	backbone ▼
SP14	V-BV650-A	V-BV650-A	discard ▼
SP15	V-BV711-A	V-BV711-A	backbone ▼
SP16	B-FITC-A	B-FITC-A	backbone ▼
SP17	B-PE-Cy5-5-A	B-PE-Cy5-5-A	backbone ▼
SP18	G-PE-A	G-PE-A	exploratory ▼
SP19	G PE-TR-A	G PE-TR-A	backbone ▼
SP20	G-PE-Cy5-A	G-PE-Cy5-A	backbone ▼
SP21	G-PE-Cy7-A	G-PE-Cy7-A	backbone ▼
SP22	R-APC-A	R-APC-A	discard ▼
SP23	R-Alexa700-A	R-Alexa700-A	backbone ▼
SP24	R APC-Cy7-A	R APC-Cy7-A	backbone ▼
SP25	Time	Time	discard ▼
SP26	vaevictis1	vaevictis1	discard ▼
SP27	vaevictis2	vaevictis2	discard ▼
SP28	barcodeId	barcodeId	discard ▼
SP29	Flag	Flag	discard ▼
SP30	fileId	fileId	discard ▼
SP31	FlaeApp	FlaeApp	discard ▼

Step 2 – Marker selection:

- Select core panel markers as **backbone** markers (used for prediction).
- Designate the markers you want to impute as **exploratory**.

Please indicate the number of cores and check box if you want to transform your data with estimate logicle. If you have already transform your data in section 2-Preprocessing, please do not check box

n cores

1 15 38

1 5 9 13 17 21 25 29 33 3738

☐ Transform with pyInfinity flow method (est.logicle)

Run (py) InfinityFlow

Step 3 – Run pyInfinityFlow:

- Select the number of cores to use, based on your machine's capabilities.
- Click **Run pyInfinityFlow** to start the prediction process.

Step 4 – Statistics:

- This part allows you to compute statistics either on:
 - The original measured values, or
 - The predicted values from pyInfinityFlow.

First, define the positivity threshold for each ISO file to interpret marker expression.



Calculate and export stats for PE measured or estimated by pyInfinityFlow

Stats :

Missing values is when there are 0 positive cells (above ISO threshold) :

Select :

PE mesured

Show 10 entries

Search:

Marker	threshold.pos	barcodeId	popID	count.pos	mean.pos
PIR-A/B	1000	12	8		
PIR-A/B	1000	16	8	1	2408.09
PIR-A/B	1000	3	8		
PIR-A/B	1000	60	8	1	1724.11
PIR-A/B	1000	71	8		
PIR-A/B	1000	8	8		
PIR-A/B	1000	80	8	1	1052.92
CD22	1000	6	8	5	2946.68
CD22	1000	10	8	7	1441.96
CD22	1000	12	8	16	1511.31

Showing 21 to 30 of 859 entries

Previous

1

2

3

4

5

...

86

Next

Export stats

A	B	C	D	E	F	G	H	I	J	K	L	M
Marker	threshold.pc	barcodeId	popID	count.pos	mean.pos	median.pos	Perc.PE.pos	standard.dev	p5.pos	p25.pos	p75.pos	p95.pos
Rat IgG1,k	1000	6	8									
Rat IgG1,k	1000	10	8									
Rat IgG1,k	1000	12	8									
Rat IgG1,k	1000	16	8									
Rat IgG1,k	1000	3	8									
Rat IgG1,k	1000	60	8									
Rat IgG1,k	1000	71	8									
Rat IgG1,k	1000	8	8									
Rat IgG1,k	1000	80	8									
CD369 (Dect	1000	6	8									
CD369 (Dect	1000	10	8									
CD369 (Dect	1000	12	8	1	1403.23	1403.23	0.17		1403.23	1403.23	1403.23	1403.23
CD369 (Dect	1000	16	8	6	1432.96	1109.66	0.37	747.42	1019.83	1035.07	1345.05	2547.13
CD369 (Dect	1000	3	8									
CD369 (Dect	1000	60	8	1	1042.67	1042.67	1.05		1042.67	1042.67	1042.67	1042.67
CD369 (Dect	1000	71	8									
CD369 (Dect	1000	8	8									
CD369 (Dect	1000	80	8									
PIR-A/B	1000	6	8									
PIR-A/B	1000	10	8	1	1040.97	1040.97	0.35		1040.97	1040.97	1040.97	1040.97
PIR-A/B	1000	12	8									
PIR-A/B	1000	16	8	1	2408.09	2408.09	0.06		2408.09	2408.09	2408.09	2408.09
PIR-A/B	1000	3	8									
PIR-A/B	1000	60	8	1	1724.11	1724.11	0.97		1724.11	1724.11	1724.11	1724.11
PIR-A/B	1000	71	8									
PIR-A/B	1000	8	8									
PIR-A/B	1000	80	8	1	1052.92	1052.92	0.25		1052.92	1052.92	1052.92	1052.92
CD22	1000	6	8	5	2946.68	1621.85	0.64	2598.71	1267.62	1494.66	2972.04	6541.6
CD22	1000	10	8	7	1441.96	1279.13	3.4	342.15	1083.47	1214.35	1720.41	1888.55
CD22	1000	12	8	16	1511.31	1555.48	2.94	271.98	1156.55	1295.44	1718.41	1877.28
CD22	1000	16	8	44	1856.13	1719.18	3.05	646.07	1081.14	1441.28	2120.77	3125.29
CD22	1000	3	8									
CD22	1000	60	8	7	1710.64	1489.15	8.43	567.49	1189.23	1342.25	1932.27	2575.18
CD22	1000	71	8									
CD22	1000	8	8									
CD22	1000	80	8	25	2092.75	2033.98	7.16	744.68	1196.61	1562.55	2344.83	3424.34
CD324 (E-Cac	1000	6	8	3	2438.53	1309.52	0.33	2167.61	1092.57	1189	3123.56	4574.79
CD324 (E-Cac	1000	10	8	2	1503.85	1503.85	0.76	497.11	1187.49	1328.09	1679.61	1820.21

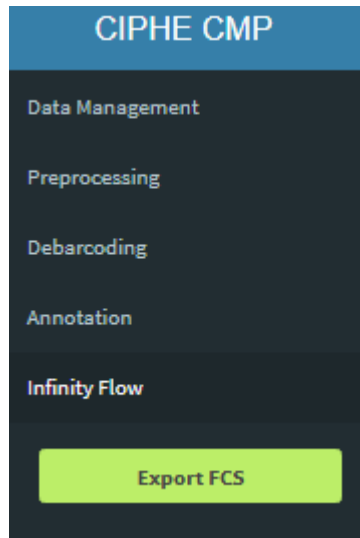
PE mesured

PE estimated (XGBoost)

+

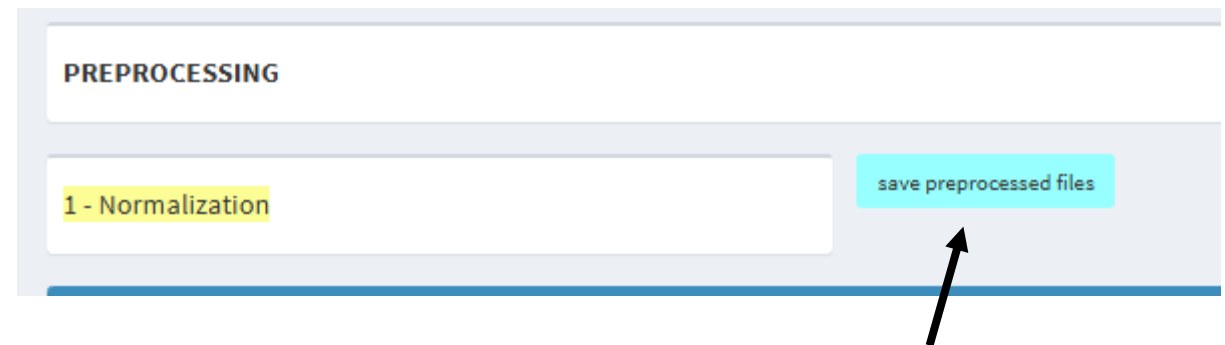
CIPHE CMP app: Backups

1 – Save FCS files



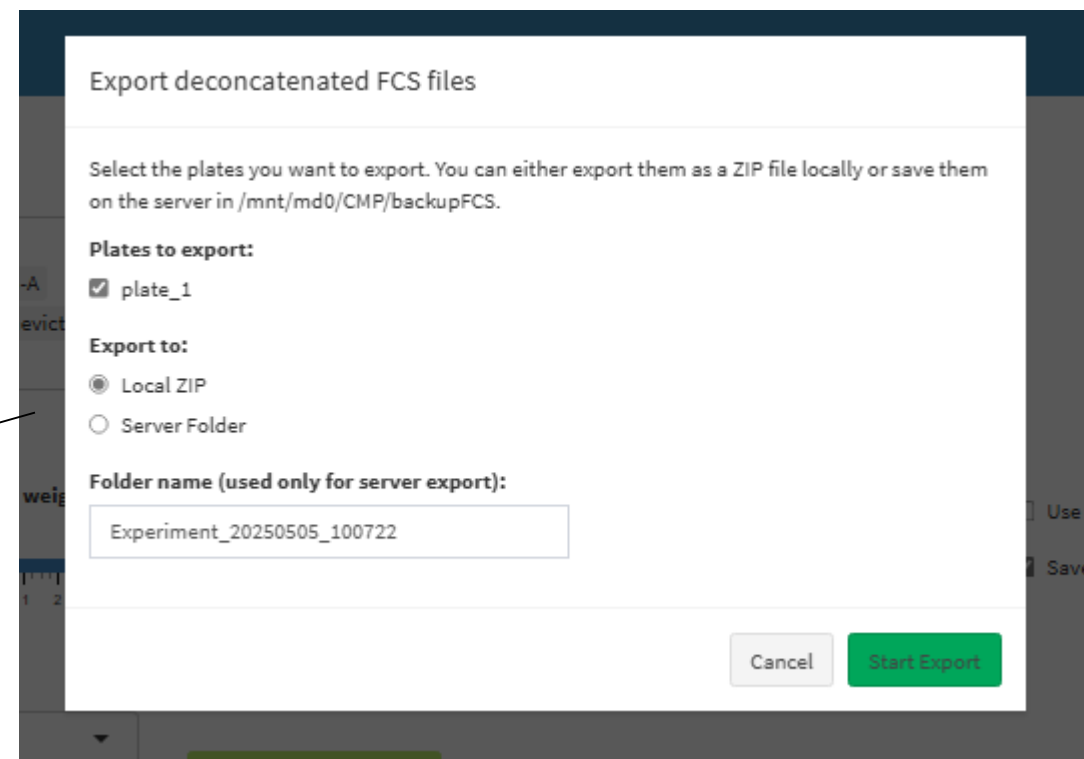
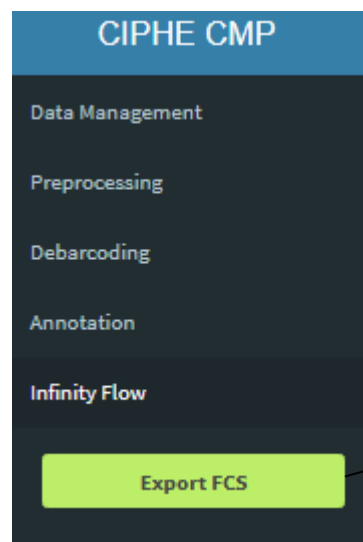
Useful for saving only the FCS files of one or more plates for further analysis in another tool.

2 – Save project



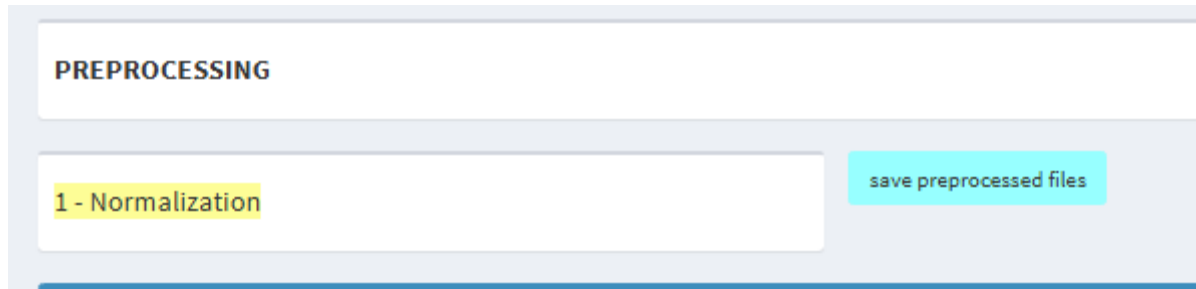
Saves the entire project and everything you've done in the application, so you can continue the analysis later.

1 – Save FCS files



Useful for saving only the FCS files of one or more plates for further analysis in another tool.

2 – Save project

A screenshot of the Ciphe application's preprocessing section. The interface has a light blue header with the word "PREPROCESSING" in blue. Below this, there is a white input field containing the text "1 - Normalization". To the right of the input field is a light blue button with the text "save preprocessed files".

PREPROCESSING

1 - Normalization

save preprocessed files

Saves the entire project and everything you've done in the application, so you can continue the analysis later.

You'll find save buttons for each section (I recommend saving after each minimum section).