

# Package ‘infinityFlow’

April 9, 2025

**Title** Augmenting Massively Parallel Cytometry Experiments Using  
Multivariate Non-Linear Regressions

**Version** 1.17.0

**Description** Pipeline to analyze and merge data files produced by BioLegend's LEGEND-Screen or BD Human Cell Surface Marker Screening Panel (BD Lyoplates).

**Depends** R (>= 4.0.0), flowCore

**License** GPL-3

**Encoding** UTF-8

**LazyData** false

**Imports** stats, grDevices, utils, graphics, pbapply, matlab, png,  
raster, grid, uwot, gtools, Biobase, generics, parallel,  
methods, xgboost

**Suggests** knitr, rmarkdown, keras, tensorflow, glmnetUtils, e1071

**VignetteBuilder** knitr

**RoxygenNote** 7.3.0

**biocViews** Software, FlowCytometry, CellBasedAssays, SingleCell,  
Proteomics

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fitter_glmnet	<i>Wrapper to glmnet. Defined separately to avoid passing too many objects in parLapplyLB</i>
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### Description

Wrapper to glmnet. Defined separately to avoid passing too many objects in parLapplyLB

### Usage

```
fitter_glmnet(x = NULL, params = NULL)
```

### Arguments

x	passed from fit_regressions
params	passed from fit_regressions

### Value

A list with two elements: predictions and a fitted model

### Examples

```
fitter_glmnet()
```

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fitter_linear	<i>Wrapper to linear model training. Defined separately to avoid passing too many objects in parLapplyLB</i>
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**Description**

Wrapper to linear model training. Defined separately to avoid passing too many objects in parLapplyLB

**Usage**

```
fitter_linear(x = NULL, params = NULL)
```

**Arguments**

x	passed from fit_regressions
params	passed from fit_regressions

**Value**

A list with two elements: predictions and a fitted model

**Examples**

```
fitter_linear()
```

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fitter_nn	<i>Wrapper to Neural Network training. Defined separately to avoid passing too many objects in parLapplyLB</i>
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**Description**

Wrapper to Neural Network training. Defined separately to avoid passing too many objects in parLapplyLB

**Usage**

```
fitter_nn(x, params)
```

**Arguments**

x	passed from fit_regressions. Defines model architecture
params	passed from fit_regressions

**Value**

A list with two elements: predictions and a fitted model

**Examples**

```
fitter_xgboost()
```

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fitter_svm	<i>Wrapper to SVM training. Defined separately to avoid passing too many objects in parLapplyLB</i>
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**Description**

Wrapper to SVM training. Defined separately to avoid passing too many objects in parLapplyLB

**Usage**

```
fitter_svm(x = NULL, params = NULL)
```

**Arguments**

x	passed from fit_regressions
params	passed from fit_regressions

**Value**

A list with two elements: predictions and a fitted model

**Examples**

```
fitter_svm()
```

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fitter_xgboost	<i>Wrapper to XGBoost training. Defined separately to avoid passing too many objects in parLapplyLB</i>
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**Description**

Wrapper to XGBoost training. Defined separately to avoid passing too many objects in parLapplyLB

**Usage**

```
fitter_xgboost(x = NULL, params = NULL)
```

**Arguments**

x                    passed from fit\_regressions  
 params              passed from fit\_regressions

**Value**

A list with two elements: predictions and a fitted model

**Examples**

```
fitter_xgboost()
```

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infinity_flow	<i>Wrapper to the Infinity Flow pipeline</i>
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**Description**

Wrapper to the Infinity Flow pipeline

**Usage**

```
infinity_flow(
  path_to_fcs,
  path_to_output,
  path_to_intermediary_results = tempdir(),
  backbone_selection_file = NULL,
  annotation = NULL,
  isotype = NULL,
  input_events_downsampling = Inf,
  prediction_events_downsampling = 1000,
  cores = 1L,
  your_random_seed = 123,
  verbose = TRUE,
  extra_args_read_FCS = list(emptyValue = FALSE, truncate_max_range = FALSE,
    ignore.text.offset = TRUE),
  regression_functions = list(XGBoost = fitter_xgboost),
  extra_args_regression_params = list(list(nrounds = 500, eta = 0.05)),
  extra_args_UMAP = list(n_neighbors = 15L, min_dist = 0.2, metric = "euclidean", verbose
    = verbose, n_epochs = 1000L, n_threads = cores, n_sgd_threads = cores),
  extra_args_export = list(FCS_export = c("split", "concatenated", "none")[1], CSV_export
    = FALSE),
  extra_args_correct_background = list(FCS_export = c("split", "concatenated",
    "none")[1], CSV_export = FALSE),
  extra_args_plotting = list(chop_quantiles = 0.005),
  neural_networks_seed = NULL
)
```

**Arguments**

- `path_to_fcs` Path to the input directory where input FCS files are stored (one file per well). Will look for FCS files recursively in that directory.
- `path_to_output` Path to the output directory where final results will be stored
- `path_to_intermediary_results` Path to results to store temporary data. If left blank, will default to a temporary directory. It may be useful to store the intermediary results to further explore the data, tweak the pipeline or to resume computations.
- `backbone_selection_file` If that argument is missing and R is run interactively, the user will be prompted to state whether each channel in the FCS file should be considered backbone measurement, exploratory measurement or ignored. Otherwise, the user should run [select\\_backbone\\_and\\_exploratory\\_markers](#) in an interactive R session, save its output using `write.csv(row.names=FALSE)` and set this `backbone_selection_file` parameter to the path of the saved output.
- `annotation` Named character vector. Elements should be the targets of the exploratory antibodies, names should be the name of the FCS file where that exploratory antibody was measured.
- `isotype` Named character vector. Elements should be the isotype used in each of the well and that (e.g. IgG2). The corresponding isotype should be present in *annotation* (e.g. Isotype\_IgG2, with this capitalization exactly). Autofluorescence measurements should be listed here as "Blank"
- `input_events_downsampling` How many event should be kept per input FCS file. Default to no downsampling. In any case, half of the events will be used to train regression models and half to test the performance. Predictions will be made only on events from the test set, and downsampled according to `prediction_events_downsampling`.
- `prediction_events_downsampling` How many event should be kept per input FCS file to output prediction for. Default to 1000.
- `cores` Number of cores to use for parallel computing. Defaults to 1 (no parallel computing)
- `your_random_seed` Deprecated: was used to set a seed for computationally reproducible results but is not allowed by Bioconductor. Please set a random seed yourself using `set.seed(somenumber)` if you desire computationally-reproducible results.
- `verbose` Whether to print information about progress
- `extra_args_read_FCS` list of named arguments to pass to `flowCore::read.FCS`. Defaults to `list(emptyValue=FALSE,truncate_max)`, which in our experience avoided issues with data loading.
- `regression_functions` named list of `fitter_*` functions (see `ls("package:infinityFlow")` for the complete list). The names should be desired names for the different models. Each object of the list will correspond to a machine learning model to train. Defaults to `list(XGBoost = fitter_xgboost)`.

- extra\_args\_regression\_params**  
list of lists the same length as the regression\_functions argument. Each element should be a named list, that will be passed as named arguments to the corresponding fitter\_function. Defaults to list(list(nrounds = 500, eta = 0.05)).
- extra\_args\_UMAP**  
list of named arguments to pass to uwot:umap. Defaults to list(n\_neighbors=15L,min\_dist=0.2,metric="euclidean").
- extra\_args\_export**  
Whether raw imputed data should be exported. Possible values are list(FCS\_export = "split") to export one FCS file per input well, list(FCS\_export = "concatenated") to export a single concatenated FCS file containing all the dataset, list(FCS\_export = "csv") for a single CSV file containing all the dataset. You can export multiple modalities by using for instance extra\_args\_export = list(FCS\_export = c("split", "concatenated", "csv"))
- extra\_args\_correct\_background**  
Whether background-corrected imputed data should be exported. Possible values are list(FCS\_export = "split") to export one FCS file per input well, list(FCS\_export = "concatenated") to export a single concatenated FCS file containing all the dataset, list(FCS\_export = "csv") for a single CSV file containing all the dataset. You can export multiple modalities by using for instance extra\_args\_export = list(FCS\_export = c("split", "concatenated", "csv"))
- extra\_args\_plotting**  
list of named arguments to pass to plot\_results. Defaults to list(chop\_quantiles=0.005) which removes the top 0.05% and bottom 0.05% of the scale for each marker when mapping color palettes to intensities.
- neural\_networks\_seed**  
Seed for computationally reproducible results when using neural networks (in addition to the other sources of stochasticity - sampling - that are made reproducible by the your\_random\_seed argument.

**Value**

Raw and background-corrected imputed expression data for every Infinity antibody

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select\_backbone\_and\_exploratory\_markers

*For each parameter in the FCS files, interactively prompts whether it is part of the Backbone, the Infinity (exploratory) markers or should be ignored.*

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**Description**

This function will load the first of the input FCS files and extract the measured parameters as well as their labels. For each of these, it will ask the user whether it is part of the backbone measurements (which will be used as a predictor variable in regressions models), Infinity (exploratory) measurements (usually PE-conjugated or APC-conjugated, used as dependent/target variable in regressions) or discarded (e.g. for parameter such as Time, Sample IDs, Event number IDs, ...).

**Usage**

```
select_backbone_and_exploratory_markers(files)
```

**Arguments**

files            character vector of paths to FCS files

**Value**

A data.frame

**Examples**

```
data(steady_state_lung)
dir <- tempdir()
fcs_tmp <- file.path(dir, "tmp.fcs")
library(flowCore)
write.FCS(steady_state_lung[[1]], file <- fcs_tmp)
if(interactive()){
  select_backbone_and_exploratory_markers(fcs_tmp)
}
```

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steady\_state\_lung        *Subset of a massively parallel cytometry experiment of mouse lung single cells*

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**Description**

Subset of a massively parallel cytometry experiment of mouse lung single cells

**Usage**

```
data(steady_state_lung)
```

**Format**

a flowSet containing 10 flowFrames (thus corresponding to 10 FCS files)

**Source**

<https://flowrepository.org/id/FR-FCM-Z2LP>

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steady\_state\_lung\_annotation

*Target and isotypes annotation for the data object infinityFlow::steady\_state\_lung*

---

**Description**

Target and isotypes annotation for the data object infinityFlow::steady\_state\_lung

**Usage**

data(steady\_state\_lung\_annotation)

**Format**

a data.frame specifying the Infinity antibody targets and isotypes for each flowFrame of the steady\_state\_lung flowSet

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steady\_state\_lung\_backbone\_specification

*Backbone and Infinity antibodies specification for the data object infinityFlow::steady\_state\_lung*

---

**Description**

Backbone and Infinity antibodies specification for the data object infinityFlow::steady\_state\_lung

**Usage**

data(steady\_state\_lung\_backbone\_specification)

**Format**

a data.frame specifying the Infinity antibody targets and isotypes for each flowFrame of the steady\_state\_lung flowSet

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