

# Package ‘CytoGLMM’

May 13, 2024

**Type** Package

**Title** Conditional Differential Analysis for Flow and Mass Cytometry Experiments

**Version** 1.13.0

**Description** The CytoGLMM R package implements two multiple regression strategies: A bootstrapped generalized linear model (GLM) and a generalized linear mixed model (GLMM). Most current data analysis tools compare expressions across many computationally discovered cell types. CytoGLMM focuses on just one cell type. Our narrower field of application allows us to define a more specific statistical model with easier to control statistical guarantees. As a result, CytoGLMM finds differential proteins in flow and mass cytometry data while reducing biases arising from marker correlations and safeguarding against false discoveries induced by patient heterogeneity.

**License** LGPL-3

**URL** <https://christofseiler.github.io/CytoGLMM>,  
<https://github.com/ChristofSeiler/CytoGLMM>

**BugReports** <https://github.com/ChristofSeiler/CytoGLMM/issues>

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|             |                                    |
|-------------|------------------------------------|
| cytoflexmix | <i>Logistic mixture regression</i> |
|-------------|------------------------------------|

---

## Description

Logistic mixture regression

**Usage**

```
cytoflexmix(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0,
  ks = seq_len(10),
  num_cores = 1
)
```

**Arguments**

|                                |  |
|--------------------------------|--|
| <code>df_samples_subset</code> | Data frame or tibble with proteins counts, cell condition, and group information |
| <code>protein_names</code>     | A vector of column names of protein to use in the analysis                       |
| <code>condition</code>         | The column name of the condition variable  |
| <code>group</code>             | The column name of the group variable  |
| <code>cell_n_min</code>        | Remove samples that are below this cell counts threshold                         |
| <code>cell_n_subsample</code>  | Subsample samples to have this maximum cell count                                |
| <code>ks</code>                | A vector of cluster sizes  |
| <code>num_cores</code>         | Number of computing cores  |

**Value**

A list of class `cytoglm` containing

|                                |  |
|--------------------------------|--|
| <code>flexmixfits</code>       | list of <code>flexmix</code> objects                     |
| <code>df_samples_subset</code> | possibly subsampled <code>df_samples_subset</code> table |
| <code>protein_names</code>     | input protein names                                      |
| <code>condition</code>         | input condition variable                                 |
| <code>group</code>             | input group names  |
| <code>cell_n_min</code>        | input <code>cell_n_min</code>                            |
| <code>cell_n_subsample</code>  | input <code>cell_n_subsample</code>                      |
| <code>ks</code>                | input <code>ks</code>                                    |
| <code>num_cores</code>         | input <code>num_cores</code>                             |

**Examples**

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 2)

mix_fit

```

cytoglm

*Fit GLM with bootstrap resampling***Description**

Fit GLM with bootstrap resampling

**Usage**

```

cytoglm(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  covariate_names = NULL,
  cell_n_min = Inf,
  cell_n_subsample = 0,
  num_boot = 100,
  num_cores = 1
)

```

**Arguments**

|                                |  |
|--------------------------------|--|
| <code>df_samples_subset</code> | Data frame or tibble with proteins counts, cell condition, and group information |
| <code>protein_names</code>     | A vector of column names of protein to use in the analysis                       |
| <code>condition</code>         | The column name of the condition variable  |
| <code>group</code>             | The column name of the group variable  |
| <code>covariate_names</code>   | The column names of covariates   |
| <code>cell_n_min</code>        | Remove samples that are below this cell counts threshold                         |
| <code>cell_n_subsample</code>  | Subsample samples to have this maximum cell count                                |
| <code>num_boot</code>          | Number of bootstrap samples  |
| <code>num_cores</code>         | Number of computing cores  |

**Value**

A list of class `cytoglm` containing

|                                |  |
|--------------------------------|--|
| <code>tb_coef</code>           | coefficient table  |
| <code>df_samples_subset</code> | possibly subsampled <code>df_samples_subset</code> table |
| <code>protein_names</code>     | input protein names                                      |
| <code>condition</code>         | input condition variable                                 |
| <code>group</code>             | input group names  |
| <code>covariate_names</code>   | input covariates   |
| <code>cell_n_min</code>        | input <code>cell_n_min</code>                            |
| <code>cell_n_subsample</code>  | input <code>cell_n_subsample</code>                      |
| <code>unpaired</code>          | true if unpaired samples were provided as input          |
| <code>num_boot</code>          | input <code>num_boot</code>                              |
| <code>num_cores</code>         | input <code>num_cores</code>                             |
| <code>formula_str</code>       | formula use in the regression model                      |

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glm_fit <- CytoGLMM::cytoglm(df,
                             protein_names = protein_names,
                             condition = "condition",
                             group = "donor",
                             num_boot = 10) # in practice >=1000
glm_fit
```

---

 cytoglmm

*Fit GLMM with method of moments*


---

**Description**

Fit GLMM with method of moments

**Usage**

```
cytoglmm(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  covariate_names = NULL,
  cell_n_min = Inf,
  cell_n_subsample = 0,
  num_cores = 1
)
```

**Arguments**

|                                |  |
|--------------------------------|--|
| <code>df_samples_subset</code> | Data frame or tibble with proteins counts, cell condition, and group information |
| <code>protein_names</code>     | A vector of column names of protein to use in the analysis                       |
| <code>condition</code>         | The column name of the condition variable  |
| <code>group</code>             | The column name of the group variable  |
| <code>covariate_names</code>   | The column names of covariates   |
| <code>cell_n_min</code>        | Remove samples that are below this cell counts threshold                         |
| <code>cell_n_subsample</code>  | Subsample samples to have this maximum cell count                                |
| <code>num_cores</code>         | Number of computing cores  |

**Value**

A list of class `cytoglm` containing

|                                |  |
|--------------------------------|--|
| <code>glmfit</code>            | <code>mbest</code> object                                |
| <code>df_samples_subset</code> | possibly subsampled <code>df_samples_subset</code> table |
| <code>protein_names</code>     | input protein names                                      |
| <code>condition</code>         | input condition variable                                 |
| <code>group</code>             | input group names  |
| <code>covariate_names</code>   | input covariates   |
| <code>cell_n_min</code>        | input <code>cell_n_min</code>                            |
| <code>cell_n_subsample</code>  | input <code>cell_n_subsample</code>                      |
| <code>num_cores</code>         | input <code>num_cores</code>                             |

**Examples**

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glmm_fit <- CytoGLMM::cytoglmm(df,
                               protein_names = protein_names,
                               condition = "condition",
                               group = "donor")

glmm_fit

```

---

|          |   |
|----------|---|
| cytgroup | <i>Group-specific fixed effects model</i> |
|----------|---|

---

**Description**

Group-specific fixed effects model

**Usage**

```

cytgroup(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0
)

```

**Arguments**

|                   |  |
|-------------------|--|
| df_samples_subset | Data frame or tibble with proteins counts, cell condition, and group information |
| protein_names     | A vector of column names of protein to use in the analysis                       |
| condition         | The column name of the condition variable  |
| group             | The column name of the group variable  |
| cell_n_min        | Remove samples that are below this cell counts threshold                         |
| cell_n_subsample  | Subsample samples to have this maximum cell count                                |

**Value**

A list of class `cytoglm` containing

|                   |  |
|-------------------|--|
| groupfit          | <code>glm</code> object                                  |
| df_samples_subset | possibly subsampled <code>df_samples_subset</code> table |

```

protein_names  input protein names
condition      input condition variable
group         input group names
cell_n_min    input cell_n_min
cell_n_subsample
              input cell_n_subsample

```

### Examples

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
group_fit <- CytoGLMM::cytogroup(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor")

group_fit

```

---

cytostab

*Evaluate parameter stability with respect to gating scheme*

---

### Description

Evaluate parameter stability with respect to gating scheme

### Usage

```

cytostab(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0
)

```

### Arguments

```

df_samples_subset      Data frame or tibble with proteins counts, cell condition, and group information
protein_names         A vector of column names of protein to use in the analysis
condition             The column name of the condition variable
group                 The column name of the group variable
cell_n_min           Remove samples that are below this cell counts threshold
cell_n_subsample     Subsample samples to have this maximum cell count

```



**Value**

A data frame

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
stab <- CytoGLMM::cytostab(df,
                           protein_names = protein_names,
                           condition = "condition",
                           group = "donor")

stab
```

---

cyto\_check

*Check if input to cytoxxx function have errors*

---

**Description**

Check if input to cytoxxx function have errors

**Usage**

```
cyto_check(cell_n_subsample, cell_n_min, protein_names)
```

**Arguments**

cell\_n\_subsample      Subsample samples to have this maximum cell count

cell\_n\_min            A vector of column names of protein to use in the analysis

protein\_names        A vector of column names of protein to use in the analysis

**Value**

NULL.

---

|               |  |
|---------------|--|
| generate_data | <i>Generate dataset for vignettes and simulation studies</i> |
|---------------|--|

---

**Description**

Generate dataset for vignettes and simulation studies

**Usage**

```
generate_data()
```

**Value**

[tibble](#) data frame

**Examples**

```
set.seed(23)
df <- generate_data()
str(df)
df
```

---

|             |   |
|-------------|---|
| glmm_moment | <i>Generalized linear mixed model with maximum likelihood</i> |
|-------------|---|

---

**Description**

Generalized linear mixed model with maximum likelihood

**Usage**

```
glmm_moment(
  df_samples,
  protein_names,
  response,
  group = "donor",
  covariate_names = NULL,
  num_cores = 1
)
```

**Arguments**

|                 |  |
|-----------------|--|
| df_samples      | Data frame or tibble with proteins counts, cell condition, and group information |
| protein_names   | A vector of column names of protein to use in the analysis                       |
| response        | The column name of the condition variable  |
| group           | The column name of the group variable  |
| covariate_names | The column names of covariates   |
| num_cores       | Number of computing cores  |

**Value**

`mbest` object

---

|             |  |
|-------------|--|
| is_unpaired | <i>Check if samples match or paired on condition</i> |
|-------------|--|

---

**Description**

Check if samples match or paired on condition

**Usage**

```
is_unpaired(df_samples_subset, condition, group)
```

**Arguments**

|                   |  |
|-------------------|--|
| df_samples_subset | Data frame or tibble with proteins counts, cell condition, and group information |
| condition         | The column name of the condition variable  |
| group             | The column name of the group variable  |

**Value**

A boolean

---

```
plot.cytoflexmix      Plot all components of mixture regression
```

---

**Description**

Plot all components of mixture regression

**Usage**

```
## S3 method for class 'cytoflexmix'
plot(x, k = NULL, separate = FALSE, ...)
```

**Arguments**

|          |   |
|----------|---|
| x        | A cytoflexmix class                                 |
| k        | Number of clusters                                  |
| separate | create two separate <a href="#">ggplot2</a> objects |
| ...      | Other parameters                                    |

**Value**

[ggplot2](#) object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 2)

plot(mix_fit)
```

---

```
plot.cytoglm      Plot bootstrapped coefficients
```

---

**Description**

Plot bootstrapped coefficients

**Usage**

```
## S3 method for class 'cytoglm'
plot(x, order = FALSE, separate = FALSE, ...)
```

**Arguments**

|          |   |
|----------|---|
| x        | A cytoglm class   |
| order    | Order the markers according to the mangintute of the coefficients |
| separate | create two separate <code>ggplot2</code> objects                  |
| ...      | Other parameters  |

**Value**

`ggplot2` object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glm_fit <- CytoGLMM::cytoglm(df,
                             protein_names = protein_names,
                             condition = "condition",
                             group = "donor",
                             num_boot = 10) # in practice >=1000
plot(glm_fit)
```

---

plot.cytoglm *Plot fixed coefficients of random effects model*

---

**Description**

Plot fixed coefficients of random effects model

**Usage**

```
## S3 method for class 'cytoglm'
plot(x, order = FALSE, separate = FALSE, ...)
```

**Arguments**

|          |   |
|----------|---|
| x        | A cytoglm class   |
| order    | Order the markers according to the mangintute of the coefficients |
| separate | create two separate <code>ggplot2</code> objects                  |
| ...      | Other parameters  |

**Value**

`ggplot2` object



---

|            |   |
|------------|---|
| plot_coeff | <i>Helper function to plot regression coefficient</i> |
|------------|---|

---

**Description**

Helper function to plot regression coefficient

**Usage**

```
plot_coeff(
  tb,
  title_str,
  title_str_right,
  xlab_str,
  redline = 0,
  order = FALSE,
  separate = FALSE
)
```

**Arguments**

|                 |  |
|-----------------|--|
| tb              | A data frame   |
| title_str       | Title string for summary plot                                    |
| title_str_right | Title for bootstrap sample plot                                  |
| xlab_str        | Label on x-axis  |
| redline         | Point on x-axis to draw the red line                             |
| order           | Order the markers according to the magnitude of the coefficients |
| separate        | Plot both summary and bootstrap samples                          |

**Value**

[ggplot2](#) object or list of two objects if separate is true

---

|              |  |
|--------------|--|
| plot_heatmap | <i>Heatmap of median marker expression</i> |
|--------------|--|

---

**Description**

Heatmap of median marker expression

**Usage**

```
plot_heatmap(
  df_samples,
  sample_info_names,
  protein_names,
  arrange_by_1,
  arrange_by_2 = "",
  cluster_cols = FALSE,
  fun = median
)
```

**Arguments**

|                   |  |
|-------------------|--|
| df_samples        | Data frame or tibble with proteins counts, cell condition, and group information                     |
| sample_info_names | Column names that contain information about the cell, e.g. donor, condition, file name, or cell type |
| protein_names     | A vector of column names of protein to use in the analysis   |
| arrange_by_1      | Column name  |
| arrange_by_2      | Column name  |
| cluster_cols      | Apply hierarchical cluster to columns  |
| fun               | Summary statistics of marker expression  |

**Value**

phemap object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_heatmap(df,
  protein_names = protein_names,
  sample_info_names = c("donor", "condition"),
  arrange_by_1 = "condition")
```

---

plot\_lda

*LDA on marker expression*

---

**Description**

LDA on marker expression



**Usage**

```
plot_lda(
  df_samples,
  protein_names,
  group,
  cor_scaling_factor = 1,
  arrow_color = "black",
  marker_color = "black",
  marker_size = 5
)
```

**Arguments**

|                    |  |
|--------------------|--|
| df_samples         | Data frame or tibble with proteins counts, cell condition, and group information |
| protein_names      | A vector of column names of protein to use in the analysis                       |
| group              | The column name of the group variable  |
| cor_scaling_factor | Scaling factor of circle of correlations   |
| arrow_color        | Color of correlation circle  |
| marker_color       | Colors of marker names   |
| marker_size        | Size of markerr names  |

**Value**

`ggplot2` object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
df$condition <- rep(c("A", "B", "C", "D"), each = length(df$condition)/4)
CytoGLMM::plot_lda(df,
  protein_names = protein_names,
  group = "condition",
  cor_scaling_factor = 2)
```

---

plot\_mds

*MDS on median marker expression*


---

**Description**

MDS on median marker expression

**Usage**

```
plot_mds(
  df_samples,
  protein_names,
  sample_info_names,
  color,
  sample_label = ""
)
```

**Arguments**

|                   |  |
|-------------------|--|
| df_samples        | Data frame or tibble with proteins counts, cell condition, and group information                     |
| protein_names     | A vector of column names of protein to use in the analysis   |
| sample_info_names | Column names that contain information about the cell, e.g. donor, condition, file name, or cell type |
| color             | Column name  |
| sample_label      | Column name  |

**Value**

cowplot object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_mds(df,
  protein_names = protein_names,
  sample_info_names = c("donor", "condition"),
  color = "condition")
```

---

plot\_model\_selection *Plot model selection to choose number optimal number of clusters*

---

**Description**

Plot model selection to choose number optimal number of clusters

**Usage**

```
plot_model_selection(fit, k = NULL)
```

**Arguments**

fit                    A cytoflexmix class  
 k                     Number of clusters

**Value**

cowplot object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 1:2)
plot_model_selection(mix_fit)
```

---

 plot\_prcomp

---

*Plot PCA of subsampled data using ggplot*


---

**Description**

Plot PCA of subsampled data using ggplot

**Usage**

```
plot_prcomp(
  df_samples,
  protein_names,
  color_var = "treatment",
  subsample_size = 10000,
  repel = TRUE
)
```

**Arguments**

df\_samples            Data frame or tibble with proteins counts, cell condition, and group information  
 protein\_names        A vector of column names of protein to use in the analysis  
 color\_var            A column name  
 subsample\_size      Subsample per color\_var variable  
 repel                Repel labels

**Value**

cowplot object

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_prcomp(df,
                      protein_names = protein_names,
                      color_var = "condition")
```

---

```
print.cytoglm
```

```
Extract and print bootstrap GLM fit
```

---

**Description**

Extract and print bootstrap GLM fit

**Usage**

```
## S3 method for class 'cytoglm'
print(x, ...)
```

**Arguments**

```
x          A cytoglm class
...        Other parameters
```

**Value**

NULL.

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glm_fit <- CytoGLMM::cytoglm(df,
                             protein_names = protein_names,
                             condition = "condition",
                             group = "donor",
                             num_boot = 10) # in practice >=1000

print(glm_fit)
```

---

|                |                                  |
|----------------|----------------------------------|
| print.cytoglmm | <i>Extact and print GLMM fit</i> |
|----------------|----------------------------------|

---

**Description**

Extact and print GLMM fit

**Usage**

```
## S3 method for class 'cytoglmm'  
print(x, ...)
```

**Arguments**

|     |                  |
|-----|------------------|
| x   | A cytoglmm class |
| ... | Other parameters |

**Value**

NULL.

**Examples**

```
set.seed(23)  
df <- generate_data()  
protein_names <- names(df)[3:12]  
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))  
glmm_fit <- CytoGLMM::cytoglmm(df,  
                               protein_names = protein_names,  
                               condition = "condition",  
                               group = "donor")  
  
print(glmm_fit)
```

---

|                |  |
|----------------|--|
| remove_samples | <i>Remove samples based on low cell counts</i> |
|----------------|--|

---

**Description**

Remove samples based on low cell counts

**Usage**

```
remove_samples(df_samples_subset, condition, group, unpaired, cell_n_min)
```

**Arguments**

|                   |  |
|-------------------|--|
| df_samples_subset | Data frame or tibble with proteins counts, cell condition, and group information |
| condition         | The column name of the condition variable  |
| group             | The column name of the group variable  |
| unpaired          | true if unpaired samples were provided as input                                  |
| cell_n_min        | Remove samples that are below this cell counts threshold                         |

**Value**

NULL.

---

|                 |  |
|-----------------|--|
| summary.cytoglm | <i>Extract and calculate p-values of bootstrap GLM fit</i> |
|-----------------|--|

---

**Description**

Extract and calculate p-values of bootstrap GLM fit

**Usage**

```
## S3 method for class 'cytoglm'
summary(object, method = "BH", ...)
```

**Arguments**

|        |                                       |
|--------|---------------------------------------|
| object | A cytoglm class                       |
| method | Multiple comparison adjustment method |
| ...    | Other parameters                      |

**Value**

[tibble](#) data frame

**Examples**

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glm_fit <- CytoGLMM::cytoglm(df,
                             protein_names = protein_names,
                             condition = "condition",
                             group = "donor",
                             num_boot = 10) # in practice >=1000

summary(glm_fit)
```

---

|                  |   |
|------------------|---|
| summary.cytoglmm | <i>Extract and calculate p-values of GLMM fit</i> |
|------------------|---|

---

### Description

Extract and calculate p-values of GLMM fit

### Usage

```
## S3 method for class 'cytoglmm'  
summary(object, method = "BH", ...)
```

### Arguments

|        |                                       |
|--------|---------------------------------------|
| object | A cytoglmm class                      |
| method | Multiple comparison adjustment method |
| ...    | Other parameters                      |

### Value

[tibble](#) data frame

### Examples

```
set.seed(23)  
df = generate_data()  
protein_names = names(df)[3:12]  
df = dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))  
glmm_fit = CytoGLMM::cytoglmm(df,  
                               protein_names = protein_names,  
                               condition = "condition",  
                               group = "donor")  
  
summary(glmm_fit)
```

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