

Package ‘mxnorm’

September 8, 2025

Title Apply Normalization Methods to Multiplexed Images

Version 1.1.0

Description Implements methods to normalize multiplexed imaging data, including statistical metrics and visualizations to quantify technical variation in this data type. Reference for methods listed here: Harris, C., Wrobel, J., & Vandekar, S. (2022). mxnorm: An R Package to Normalize Multiplexed Imaging Data. Journal of Open Source Software, 7(71), 4180, <doi:10.21105/joss.04180>.

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 ``scikit-image``)))

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VignetteBuilder knitr

URL <https://github.com/ColemanRHarris/mxnorm>

BugReports <https://github.com/ColemanRHarris/mxnorm/issues>

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mx_dataset	<i>Generates mx_dataset</i>
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Description

Takes in data from data.frame of cell-level multiplexed data to create a mx_dataset S3 object.

Usage

```
mx_dataset(data, slide_id, image_id, marker_cols, metadata_cols = NULL)
```

Arguments

data	multiplexed data to normalize. Data assumed to be a data.frame with cell-level data.
slide_id	String slide identifier of input data. This must be a column in the data data.frame.
image_id	String image identifier of input data. This must be a column in the data data.frame.
marker_cols	vector of column name(s) in data corresponding to marker values.
metadata_cols	other identifiers of the input data (default=NULL). This must be a vector of column name(s) in the data data.frame.

Value

data.frame object in the mx_dataset format with attribute for input type

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
```

mx_normalize	<i>Normalizes multiplexed data</i>
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Description

Normalizes some given image input according to the method specified

Usage

```
mx_normalize(
  mx_data,
  transform = "None",
  method = "None",
  method_override = NULL,
  method_override_name = NULL,
  ...
)
```

Arguments

mx_data	mx_dataset object to normalize
transform	transformation to perform on the input data. Options include: c("None", "log10", "mean_divide", "log10_mean_divide")
method	normalization method to perform on the input data. Options include: c("None", "ComBat", "Registration")
method_override	optional user-defined function to perform own normalization method (default=NULL). If using a user-defined function, it must include a mx_data parameter.
method_override_name	optional name for method_override (default=NULL).
...	optional additional arguments for normalization functions

Value

Multiplexed data normalized according to the method specified, in the mx_dataset format. Normalized data will be included a new table with normalized values and attributes describing the transformation.

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
```

mx_sample	<i>Sample multiplexed dataset for mxnorm.</i>
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Description

A simulated multiplexed dataset containing: 4 slides, with 3 images each, and 3 markers, and 1 metadata column.

Usage

```
mx_sample
```

Format

A data frame with 3000 rows and 6 variables:

slide_id slide identifier

image_id image identifier

marker1_vals simulated marker 1 values

marker2_vals simulated marker 2 values

marker3_vals simulated marker 3 values

metadata1_vals simulated metadata 1 values ...

plot_mx_density	<i>Visualize marker density before/after normalization by marker and slide</i>
-----------------	--

Description

Visualize marker density before/after normalization by marker and slide

Usage

```
plot_mx_density(mx_data)
```

Arguments

mx_data	mx_dataset object that been used with run_otsu_discordance() to compute Otsu discordance scores (necessary for the density rug plot). Note that the table attribute must be set when running run_otsu_discordance().
---------	--

Value

ggplot2 object with density plot

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
## using `threshold_override` here in case users haven't installed `scikit-image`
mx_data = run_otsu_discordance(mx_data, table="normalized",
  threshold_override = function(thold_data){quantile(thold_data, 0.5)})
plot_mx_density(mx_data)
```

plot_mx_discordance	<i>Visualize Otsu discordance scores by marker and slide</i>
---------------------	--

Description

Visualize Otsu discordance scores by marker and slide

Usage

```
plot_mx_discordance(mx_data)
```

Arguments

mx_data	mx_dataset object that been used with run_otsu_discordance() to compute Otsu discordance scores. Note that the table attribute must be set when running run_otsu_discordance().
---------	---

Value

ggplot2 object with Otsu discordance scores plot

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
## using `threshold_override` here in case users haven't installed `scikit-image`
mx_data = run_otsu_discordance(mx_data, table="normalized",
  threshold_override = function(thold_data){quantile(thold_data, 0.5)})
plot_mx_discordance(mx_data)
```

plot_mx_proportions	<i>Visualize variance proportions by marker and table</i>
---------------------	---

Description

Visualize variance proportions by marker and table

Usage

```
plot_mx_proportions(mx_data)
```

Arguments

mx_data	mx_dataset object that been used with run_var_proportions() to run random effects modeling. Note that the table attribute must be set when running run_var_proportions().
---------	---

Value

ggplot2 object with proportions plot

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
mx_data = run_var_proportions(mx_data, table="both")
plot_mx_proportions(mx_data)
```

plot_mx_umap	<i>Visualize UMAP dimension reduction algorithm</i>
--------------	---

Description

Visualize UMAP dimension reduction algorithm

Usage

```
plot_mx_umap(mx_data, metadata_col = NULL)
```

Arguments

mx_data	mx_dataset object that been used with run_reduce_umap() to compute the UMAP dimensions for the dataset. Note that the table attribute must be set when running run_reduce_umap().
metadata_col	column denoted in the run_reduce_umap() to change the scale_color attribute of the ggplot (default=NULL)

Value

ggplot2 object with density plot

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
mx_data = run_reduce_umap(mx_data, table="normalized",
  c("marker1_vals", "marker2_vals", "marker3_vals"))
plot_mx_umap(mx_data)
```

```
print.summary.mx_dataset
```

Extension of print S3 method to print summary.mx_dataset objects

Description

Extension of print S3 method to print summary.mx_dataset objects

Usage

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

Arguments

x	summary.mx_dataset object to summarize
...	option for additional params given S3 logic

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
print(summary(mx_data))
```

run_otsu_discordance	<i>Calculate Otsu discordance scores using specified threshold for an mx_dataset object.</i>
----------------------	--

Description

The Otsu discordance analysis quantifies slide-to-slide agreement by summarizing the distance between slide-level Otsu thresholds and the global Otsu threshold for a given marker in a single metric.

Usage

```
run_otsu_discordance(
    mx_data,
    table,
    threshold_override = NULL,
    plot_out = FALSE,
    ...
)
```

Arguments

mx_data	mx_dataset object used to compute Otsu discordance scores
table	dataset in mx_data used to compute metrics. Options include: c("raw", "normalized", "both"), e.g. a y-axis parameter.
threshold_override	optional user-defined function or alternate thresholding algorithm adaptable from Python skimage module filters (Note: not all algorithms in filters adapted). Options include supplying a function or any of the following: c("isodata", "li", "mean", "otsu", "triangle", "yen"). More detail available here: https://scikit-image.org/docs/dev/api/skimage.filters.html . If using a user-defined function, it must include a thold_data parameter.
plot_out	boolean to generate Otsu discordance plots (default=FALSE)
...	optional additional arguments for Otsu discordance functions

Value

mx_dataset object with analysis results of Otsu discordance in otsu_data table

References

Otsu, N. (1979). A threshold selection method from gray-level histograms. IEEE transactions on systems, man, and cybernetics, 9(1), 62-66.

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
## using `threshold_override` here in case users haven't installed `scikit-image`
mx_data = run_otsu_discordance(mx_data, table="normalized",
  threshold_override = function(thold_data){quantile(thold_data, 0.5)})
```

run_reduce_umap	<i>Run UMAP dimension reduction algorithm on an mx_dataset object.</i>
-----------------	--

Description

Run UMAP dimension reduction algorithm on an mx_dataset object.

Usage

```
run_reduce_umap(
  mx_data,
  table,
  marker_list,
  downsample_pct = 1,
  metadata_cols = NULL
)
```

Arguments

mx_data	mx_dataset object used to compute UMAP dimensions
table	dataset in mx_data used to compute metrics. Options include: c("raw", "normalized", "both"), e.g. a y-axis parameter.
marker_list	list of markers in the mx_dataset object to use for UMAP algorithm
downsample_pct	double, optional percentage (0, 1] of sample rows to include when running UMAP algorithm. (default=1)
metadata_cols	other identifiers of the input data (default=NULL). This must be a vector of column name(s) in the mx_dataset object

Value

mx_dataset object with analysis results of UMAP dimension results in umap_data table

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",
  c("marker1_vals", "marker2_vals", "marker3_vals"),
  c("metadata1_vals"))
mx_data = mx_normalize(mx_data, transform="log10", method="None")
mx_data = run_reduce_umap(mx_data, table="normalized",
  c("marker1_vals", "marker2_vals", "marker3_vals"))
```

run_var_proportions	<i>Run random effects modeling on mx_dataset object to determine proportions of variance at the slide level</i>
---------------------	---

Description

Run random effects modeling on `mx_dataset` object to determine proportions of variance at the slide level

Usage

```
run_var_proportions(
  mx_data,
  table,
  metadata_cols = NULL,
  formula_override = NULL,
  save_models = FALSE,
  ...
)
```

Arguments

<code>mx_data</code>	<code>mx_dataset</code> object used to compute UMAP dimensions
<code>table</code>	dataset in <code>mx_data</code> used to compute metrics. Options include: <code>c("raw", "normalized", "both")</code> , e.g. a y-axis parameter.
<code>metadata_cols</code>	other identifiers of the input data to use in the modeling process (default=NULL). This must be a vector of column name(s) in the <code>mx_dataset</code> object
<code>formula_override</code>	String with user-defined formula to use for variance proportions modeling analysis (default=NULL). This will be the RHS of a formula with <code>marker~</code> as the LHS.
<code>save_models</code>	Boolean flag to save <code>lme4::lmer()</code> models in a list to the <code>mx_dataset</code> object
<code>...</code>	optional additional arguments for <code>lme4::lmer()</code> modeling

Value

`mx_dataset` object with modeling results in `var_data` table

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",  
  c("marker1_vals", "marker2_vals", "marker3_vals"),  
  c("metadata1_vals"))  
mx_data = mx_normalize(mx_data, transform="log10", method="None")  
mx_data = run_var_proportions(mx_data, table="both")
```

summary.mx_dataset	<i>Extension of summary S3 method to summarize mx_dataset objects</i>
--------------------	---

Description

Extension of summary S3 method to summarize mx_dataset objects

Usage

```
## S3 method for class 'mx_dataset'  
summary(object, ...)
```

Arguments

object	mx_dataset object to summarize
...	option for additional params given S3 logic

Value

summary.mx_dataset object

Examples

```
mx_data = mx_dataset(mxnorm::mx_sample, "slide_id", "image_id",  
  c("marker1_vals", "marker2_vals", "marker3_vals"),  
  c("metadata1_vals"))  
summary(mx_data)
```

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