

# Package ‘mxnorm’

July 23, 2025

**Title** Apply Normalization Methods to Multiplexed Images

**Version** 1.0.3

**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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**Config/testthat/edition** 3

**Config/reticulate** list( packages = list( list(package =  
 ``scikit-image" ) ) )

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**VignetteBuilder** knitr

**NeedsCompilation** no

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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> |
|--------------|------------------------------------|

---

## 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).<br>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> |
|-----------|-----------------------------------------------|

---

### 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: <a href="https://scikit-image.org/docs/dev/api/skimage.filters.html">https://scikit-image.org/docs/dev/api/skimage.filters.html</a> . 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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