

# Package ‘IrisSpatialFeatures’

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**Type** Package

**Title** A package to extract spatial features based on multiplex IF images

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**Imports** Rcpp (>= 0.12.7), SpatialTools, gplots, spatstat, tiff, RColorBrewer, methods, grDevices, graphics, stats, utils, data.table, ggplot2, dplyr, magrittr, tibble

**Description** IrisSpatialFeatures reads the output of the PerkinElmer inForm software and calculates a variety of spatial statistics. In addition to simple counts, it can derive average nearest neighbors for each cell-type and interaction summary profiles for each celltype. These statistics are derived across images, both overall and regions of interest as defined by user defined masks.

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## R topics documented:

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as.data.frame,ImageSet-method

*Read inForm output and store it in an IrisSpatialFeatures ImageSet object.*

---

### Description

Read inForm output and store it in an IrisSpatialFeatures ImageSet object.

### Usage

```
## S4 method for signature 'ImageSet'
as.data.frame(x)
```

### Arguments

x	Iris ImageSet boject
...	Additional arguments

**Value**

A dataframe

**Examples**

```
dataset <- IrisSpatialFeatures_data
dataframe <- as.data.frame(dataset)
```

---

collapse_markers	<i>This function collapses two markers into one, and reruns the counting of cells. Mostly a convenience function for the Shiny interface so we start with a completely split set successively adding more markers</i>
------------------	---

---

**Description**

This function collapses two markers into one, and reruns the counting of cells. Mostly a convenience function for the Shiny interface so we start with a completely split set successively adding more markers

**Usage**

```
collapse_markers(image_set, marker1, marker2, combined)

## S4 method for signature 'ImageSet'
collapse_markers(image_set, marker1, marker2, combined)
```

**Arguments**

image_set	IrisSpatialFeatures ImageSet object.
marker1	Name of the first marker that should be collapsed.
marker2	Name of the second marker that should be collapsed.
combined	Name of the combined marker.

**Value**

IrisSpatialFeatures ImageSet object.

**Examples**

```
dataset <- IrisSpatialFeatures_data
ds <- collapse_markers(dataset, marker1="SOX10+ PDL1+", marker2="SOX10+ PDL1-", combined="SOX10+")
```

---

Coordinate-class	<i>Class to represent a single imaging coordinate</i>
------------------	---

---

### Description

Class to represent a single imaging coordinate

### Slots

ppp A spatstat ppp object that contains all coordinate information

raw Includes all raw data that is output from inForm

mask List of user defined masks that define regions of interest

coordinate\_name Name of the current coordinate

size\_in\_px Size of the image in pixel, accounting for mask size

### Examples

```
coord <- new("Coordinate")
```

---

extract_features	<i>Extract all spatial features</i>
------------------	-------------------------------------

---

### Description

Extract all spatial features

### Usage

```
extract_features(x, ...)
```

```
## S4 method for signature 'ImageSet'
```

```
extract_features(x, name = "", rm.na = FALSE)
```

### Arguments

x	IrisSpatialFeatures ImageSet object
...	Additional arguments
name	Prefix for all features, e.g. 'invasive_margin' (Default: '')
rm.na	Should features with NA values be removed (Default: FALSE)

### Value

dataframe of features

## Examples

```
#' #loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset,min_num_cells=2)
dataset <- extract_proximity(dataset,only_closest=TRUE,radii=25)
dataset <- extract_interactions(dataset)
extract_features(dataset)
```

---

extract\_interactions *Extract interactions between all cell-types*

---

## Description

Extract interactions between all cell-types

## Usage

```
extract_interactions(x, ...)
```

## S4 method for signature 'ImageSet'

```
extract_interactions(x)
```

## Arguments

x	IrisSpatialFeatures ImageSet object
...	Additional arguments

## Value

list of interactions

## Examples

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_interactions(dataset)
```

---

extract_mask	<i>Read inForm output from a single coordinate</i>
--------------	--

---

**Description**

Read inForm output from a single coordinate

**Usage**

```
extract_mask(filename)
```

**Arguments**

filename	Name of the .tif file that contains the mask.
----------	---

**Value**

Mask matrix

**Examples**

```
extract_mask(system.file("extdata",
                        "MEL2", "MEL2_080416_2_Invasive_Margin.tif",
                        package = "IrisSpatialFeatures"))
```

---

extract_nearest_neighbor	<i>Extract the distance to each nearest neighbor for each cell-type</i>
--------------------------	---

---

**Description**

Extract the distance to each nearest neighbor for each cell-type

**Usage**

```
extract_nearest_neighbor(x, ...)
```

```
## S4 method for signature 'ImageSet'
extract_nearest_neighbor(x, min_num_cells = 10)
```

**Arguments**

x	IrisSpatialFeatures ImageSet object
...	Additional arguments
min_num_cells	Minimum number of cell that a coordinate needs to have in order to calculate the statistics (Default: 10)

**Value**

distance to nearest neighbor for each

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
extract_nearest_neighbor(dataset)
```

---

extract_proximity	<i>Run a proximity analysis on all samples. There are two modes this function can be run. In the first mode it uses the major and minor axes for each cell as provided by inform. It then averages half of those two axes and adds an uncertainty margin, which are used to provide an estimate on whether two cells are touching. This mode can be used to approximate the interaction analysis. The second mode uses a user specified distance to count the cells within the proximity of a given cell-type. With increasing distances usually cells fall into the proximity of multiple cells of a given type so the function allows the restriction of only counting the cell only once.</i>
-------------------	--

---

**Description**

Run a proximity analysis on all samples. There are two modes this function can be run. In the first mode it uses the major and minor axes for each cell as provided by inform. It then averages half of those two axes and adds an uncertainty margin, which are used to provide an estimate on whether two cells are touching. This mode can be used to approximate the interaction analysis. The second mode uses a user specified distance to count the cells within the proximity of a given cell-type. With increasing distances usually cells fall into the proximity of multiple cells of a given type so the function allows the restriction of only counting the cell only once.

**Usage**

```
extract_proximity(x, ...)
```

```
## S4 method for signature 'ImageSet'
extract_proximity(x, radii = c("Entire.Cell.Major.Axis",
  "Entire.Cell.Minor.Axis"), uncertainty_margin = 1, only_closest = FALSE)
```

**Arguments**

x	IrisSpatialFeatures ImageSet object
...	Additional arguments
radii	The size of the radius in pixels or the default, which are the inForm output columns that indicated the minor and major axis of each cell.
uncertainty_margin	Only for the approximation of the interaction analysis, where it indicates how many pixels further should be search to find a touching cell (default: 1).
only_closest	For the proximity analysis a target cell can be in the vicinity of multiple source cells, so the counts are artificially inflated. E.g. a CD8 PD1+ T-cell is within <50 pixels of 30 HRS cells, this cell should only be counted for the closes HRS cell. (default: FALSE)

**Value**

The proximal events for each cell

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
extract_proximity(dataset)
```

---

extract_ROI	<i>Method that reduces the current dataset to a specific region of interest, discarding all cell coordinates outside of that region</i>
-------------	---

---

**Description**

Method that reduces the current dataset to a specific region of interest, discarding all cell coordinates outside of that region

**Usage**

```
extract_ROI(x, ...)
```

## S4 method for signature 'ImageSet'

```
extract_ROI(x, ROI = "invasive_margin")
```

**Arguments**

x	IrisSpatialFeatures ImageSet object
...	Additional arguments
ROI	Region of interest (default: 'invasive_margin')

**Value**

IrisSpatialFeatures ImageSet object

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
im_area <- extract_ROI(dataset,ROI='invasive_margin')
```



---

feature\_selection      *Function to extract all numeric features*

---

**Description**

Function to extract all numeric features

**Usage**

```
feature_selection(dat, lab)
```

**Arguments**

dat	A data matrix with features as rows and samples as columns
lab	Label annotation that contains 2 classes, which corresponds to the samples in the column

**Value**

t-test and wilcox test between the 2 classes

**Examples**

```
dat <- cbind(matrix(runif(400),ncol=10),matrix(runif(400)+0.2,ncol=10))
lab <- c(rep('classA',10),rep('classB',10))
rownames(dat) <- paste0('F',1:nrow(dat))
feature_selection(dat,lab)
```

---

get\_all\_interactions      *Get all interactions between all cell-types*

---

**Description**

Get all interactions between all cell-types

**Usage**

```
get_all_interactions(x, ...)
```

## S4 method for signature 'ImageSet'

```
get_all_interactions(x)
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object.
...	Additional arguments.

**Value**

For each cell-type return interactions

## Examples

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_interactions(dataset)
get_all_interactions(dataset)
```

---

```
get_all_nearest_neighbors
```

*Get the nearest neighbor for each cell-type*

---

## Description

Get the nearest neighbor for each cell-type

## Usage

```
get_all_nearest_neighbors(x, ...)
```

## S4 method for signature 'ImageSet'

```
get_all_nearest_neighbors(x)
```

## Arguments

x	An IrisSpatialFeatures ImageSet object
...	Additional arguments

## Value

Nearest neighbor for each cell-type

## Examples

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset)
get_all_nearest_neighbors(dataset)
```

---

get\_all\_proximities     *Get all proximity data for all cell-types in a sample*

---

### Description

Get all proximity data for all cell-types in a sample

### Usage

```
get_all_proximities(x, ...)
```

```
## S4 method for signature 'ImageSet'
```

```
get_all_proximities(x)
```

### Arguments

x                    An IrisSpatialFeatures ImageSet object  
 ...                  Additional arguments

### Value

all proximity data for cell-types

### Examples

```
#loading pre-read dataset
```

```
dataset <- IrisSpatialFeatures_data
```

```
get_all_proximities(dataset)
```

---

get\_counts\_per\_mm2     *Get all the counts on a per mm2 basis*

---

### Description

Get all the counts on a per mm2 basis

### Usage

```
get_counts_per_mm2(x, ...)
```

```
## S4 method for signature 'ImageSet'
```

```
get_counts_per_mm2(x, digits = 2, blank = FALSE)
```

### Arguments

x                    An IrisSpatialFeatures ImageSet object  
 ...                  Additional arguments  
 digits              Number of digits that are shown in the output (default: 2)  
 blank                (default: FALSE)

**Value**

counts per mm2 per sample, collapsing each coordinate and returning mean and standard error

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
get_counts_per_mm2(dataset)
```

---

```
get_counts_per_mm2_noncollapsed
```

*Get all the counts on a per mm2 basis non-collapsed*

---

**Description**

Get all the counts on a per mm2 basis non-collapsed

**Usage**

```
get_counts_per_mm2_noncollapsed(x, ...)
```

## S4 method for signature 'ImageSet'

```
get_counts_per_mm2_noncollapsed(x)
```

**Arguments**

x	IrisSpatialFeatures ImageSet object.
...	Additional arguments

**Value**

IrisSpatialFeatures ImageSet object.

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
get_counts_per_mm2_noncollapsed(dataset)
```

---

get\_count\_ratios      *Get ratio of counts between two markers*

---

**Description**

Get ratio of counts between two markers

**Usage**

```
get_count_ratios(x, ...)  
  
## S4 method for signature 'ImageSet'  
get_count_ratios(x, marker1, marker2, digits = 2)
```

**Arguments**

x	An IrisSpatialFeatures object
...	Additional arguments.
marker1	First cell-type.
marker2	Second cell-type.
digits	Number of digits that should be shown in the the results. (Default: 2)

**Value**

Count ratio between two markers

**Examples**

```
#loading pre-read dataset  
dataset <- IrisSpatialFeatures_data  
get_count_ratios(dataset, 'SOX10+ PDL1-', 'SOX10+ PDL1+')
```

---

get\_interactions      *Get interactions for a specific marker*

---

**Description**

Get interactions for a specific marker

**Usage**

```
get_interactions(x, ...)  
  
## S4 method for signature 'ImageSet'  
get_interactions(x, marker, normalize = TRUE)
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object
...	Additional arguments.
marker	Cell-type for which the interactions should be pulled
normalize	Flag to indicated whether to normalize each sample so all interactions sum up to 1 (Default: 1)

**Value**

interactions for a specific marker

**Examples**

```
#' #loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_interactions(dataset)
get_interactions(dataset, 'CD8+ PD1+')
```

---

get\_nearest\_neighbors *Get the nearest neighbor for a specified cell-type*

---

**Description**

Get the nearest neighbor for a specified cell-type

**Usage**

```
get_nearest_neighbors(x, ...)

## S4 method for signature 'ImageSet'
get_nearest_neighbors(x, marker)
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object
...	Additional arguments
marker	Cell type for which the nearest neighbor should be calculated

**Value**

nearest neighbors for the specified cell-type

**Examples**

```
#' #loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset, min_num_cells=2)
get_nearest_neighbors(dataset, "SOX10+ PDL1+")
```

---

get_proximities	<i>Get proximity data for a given cell-type</i>
-----------------	---

---

**Description**

Get proximity data for a given cell-type

**Usage**

```
get_proximities(x, ...)

## S4 method for signature 'ImageSet'
get_proximities(x, marker, normalize = TRUE)
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object.
...	Additional arguments.
marker	Cell type for which the proximity data should be extracted.
normalize	Flag indicating whether the populations should be normalized so that the sum of all is 1 (default: TRUE).

**Value**

proximities for a specific cell-type

---

ImageSet-class	<i>Class to represent an imaging dataset.</i>
----------------	---

---

**Description**

Class to represent an imaging dataset.

**Slots**

samples	A list of samples each containing multiple coordinates.
counts	A list of counts of different cell types for each coordinate in each sample.
nearest_neighbors	A list of mean and std of nearest neighbor distances for each samples.
interactions	A list of interaction information for each sample.
proximity	A list of mean and std of nearest neighbor distances for each sample.
microns_per_pixel	Scalar value that indicates the length of a pixel in micrometers.
markers	A vector of strings indicating all different cell types considered.
invasive_margin_in_px	The width of the invasive margin in pixels
readMasks	Flag indicating whether the "_Tumor.tif" and "_Invasive_Margin.tif" should be read (default: True)
ignore_scoring	Flag indicating whether the scoring file should be ignored (default: FALSE)

---

interaction\_maps      *Plot interaction maps for all samples*

---

### Description

Plot interaction maps for all samples

### Usage

```
interaction_maps(x, ...)

## S4 method for signature 'ImageSet'
interaction_maps(x, int_markers, int_marker_cols,
  silent_markers = c(), silent_col = c(), outline_transparency = 0.9,
  use_dapi = FALSE, outdir = "interaction_maps", useMask = NULL,
  format = ".png")
```

### Arguments

x	An IrisSpatialFeatures ImageSet object
...	Additional arguments.
int_markers	Cell-types that should be considered. If two cells from different cell-types interact they are filled in, if a cell is not interacting it is just outlined.
int_marker_cols	Colors for the cell-types
silent_markers	Cell-types that should only be outlined (Default: c())
silent_col	Colors for silent markers (Default: c())
outline_transparency	Dimming factor for the outlines cells(Default: 0.9)
use_dapi	Use the DAPI channel as a background (Default: FALSE)
outdir	Output directory (Default: './interaction_maps')
useMask	(Default: NULL)
format	Output format of the images. Can be '.png' or '.tiff' (Default: '.png')

### Value

plot of interactions for all samples

### Examples

```
## #loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_interactions(dataset)
get_interactions(dataset, 'CD8+ PD1+')
int_markers <- c('CD8+ PD1+', 'SOX10+ PDL1+')
int_marker_cols <- c('#dd1c77', '#99d8c9')
silent_markers <- c('CD8+ PD1-')
silent_col=c('yellow')
p <- interaction_maps(dataset, int_markers, int_marker_cols, silent_markers,
  silent_col)
```



---

IrisSpatialFeatures    *IrisSpatialFeatures: A package to extract spatial features based on multiplex IF images*

---

### Description

Iris reads the output of the PerkinElmer inForm software and calculates a variety of spatial statistics. In addition to simple counts, it can derive average nearest neighbors for each cell-type and interaction summary profiles for each celltype. These statistics are derived across images, both overall and regions of interest as defined by user defined masks.

### Author(s)

Daniel Gusenleitner <Daniel\_Gusenleitner@dfci.harvard.edu>

---

IrisSpatialFeatures\_data  
*Example IrisSpatialFeatures dataset*

---

### Description

A dataset that contains two small samples, with 5 imaging coordinates per samples

### Usage

IrisSpatialFeatures\_data

### Format

IrisSpatialFeatures dataset

---

neighbor\_ray\_plot    *Plot nearest neighbor ray plots for each samples*

---

### Description

Plot nearest neighbor ray plots for each samples

### Usage

```
neighbor_ray_plot(x, ...)

## S4 method for signature 'ImageSet'
neighbor_ray_plot(x, from_type, to_type,
  from_col = "#EE7600", to_col = "#028482", format = ".pdf",
  plot_dir = "./", lineColor = "#666666", use_pixel = FALSE, height = 7,
  width = 10)
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object
...	Additional arguments.
from_type	Cell type from which the rays are drawn
to_type	Cell type to which the rays are drawn
from_col	Color for the 'from' cell-type (Default: '#EE7600')
to_col	Color for the 'to' cell-type (Default: '#028482')
format	Format of the output file, can be '.pdf' or '.png' (Default: '.pdf')
plot_dir	Directory in which the images are written (Default: './')
lineColor	Color of the rays (Default: '#666666')
use_pixel	use pixels instead of micrometer for distance measurements (default: FALSE)
height	Height of the pdf. (Default: 7)
width	Width of the pdf. (Default: 10)

**Value**

nearest neighbor ray plots

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset)
get_nearest_neighbors(dataset, "SOX10+ PDL1+")
plot_dir <- file.path('./ray_plots')
if (!file.exists(plot_dir)){
  dir.create(file.path(plot_dir))
}
```

---

overview\_plot

*Plot all coordinates in a given dataset*

---

**Description**

Plot all coordinates in a given dataset

**Usage**

```
overview_plot(x, ...)
```

```
## S4 method for signature 'ImageSet'
overview_plot(x, outdir = "./", palette = NULL,
  type = "pdf", width = 10, height = 7)
```

**Arguments**

x	Sample ImageSet object of the IrisSpatialFeatures package.
...	Additional arguments
outdir	Output directory (default: './')
palette	Color palette used for the different cell-types. (default: NULL)
type	File format for the plots. Can be either 'pdf' or 'png'. (default: 'pdf')
width	Width of the plot in inches for pdf and pixels for png. (default: 10)
height	Height of the plot in inches for pdf and pixels for png. (default: 7)

**Value**

plot of all coordinates

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
overview_plot(dataset)
```

---

plot,ImageSet-method *Plot all frames of an IrisSpatialFeatures ImageSet object.*

---

**Description**

Plot all frames of an IrisSpatialFeatures ImageSet object.

**Usage**

```
## S4 method for signature 'ImageSet'
plot(x)
```

**Arguments**

x	Iris ImageSet object
---	----------------------

**Value**

A plot

**Examples**

```
dataset <- IrisSpatialFeatures_data
plot(dataset)
```

---

plot\_interactions      *Interaction summary plot for all cell-types and all samples in a dataset*

---

### Description

Interaction summary plot for all cell-types and all samples in a dataset

### Usage

```
plot_interactions(x, ...)

## S4 method for signature 'ImageSet'
plot_interactions(x, label, ordering = NULL,
  normalize = TRUE, palette = NULL, celltype_order = NULL,
  xlim_fix = 13, topbar_cols = "darkgrey")
```

### Arguments

x	IrisSpatialFeatures ImageSet object to be plotted
...	Additional arguments
label	The cell type the interaction profile should be plotted for
ordering	Ordering of the samples (default: NULL)
normalize	Normalize the interactions with a given cell-type, so they sum up to 1 (default: TRUE)
palette	Color palette for all the cell-types (default: Spectral scheme from RColorbrewer)
celltype_order	Order in which the cell-types are displayed. (default: Alphabetically)
xlim_fix	Whitespace on the right side so the legend can be displayed clearly. (default: 13)
topbar_cols	Color of the barplots that are shown on top. (default: 'darkgrey')

### Value

plot of all cell-types and samples interactions

### Examples

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_interactions(dataset)
plot_interactions(dataset, 'SOX10+ PDL1+', xlim_fix=3)
```

---

`plot_nearest_neighbor` *Plot average nearest neighbor barplots for two cell types. This measurement is not symmetric, so if 'from' and 'to' are switched it will result in different results. For the 'to' parameter this function allows a cell-type without '+' or '-' in the end. Indicating that the distances from the first cell-type should be calculated against both '+/-' and a paired t-test should be calculated. For example we want to calculate the average distance between SOX10 PDL1+ melanoma cells against both CD8 PD1+ and CD8 PD1- cells, the 'CD8 PD1' would be specified as 'to' parameter, 2 distances would be calculated for each sample and a two-sided paired t-test calculated to test for significant differences.*

---

### Description

Plot average nearest neighbor barplots for two cell types. This measurement is not symmetric, so if 'from' and 'to' are switched it will result in different results. For the 'to' parameter this function allows a cell-type without '+' or '-' in the end. Indicating that the distances from the first cell-type should be calculated against both '+/-' and a paired t-test should be calculated. For example we want to calculate the average distance between SOX10 PDL1+ melanoma cells against both CD8 PD1+ and CD8 PD1- cells, the 'CD8 PD1' would be specified as 'to' parameter, 2 distances would be calculated for each sample and a two-sided paired t-test calculated to test for significant differences.

### Usage

```
plot_nearest_neighbor(x, ...)

## S4 method for signature 'ImageSet'
plot_nearest_neighbor(x, from, to, ttest = TRUE,
  transposed = FALSE, remove_NAs = FALSE, use_pixel = FALSE)
```

### Arguments

<code>x</code>	IrisSpatialFeatures ImageSet object.
<code>...</code>	Additional arguments.
<code>from</code>	Cell-type from which the nearest neighbor is calculated.
<code>to</code>	Cell-type to which the nearest neighbor is calculated.
<code>ttest</code>	Flag indicating whether a paired t-test should be calculated. (default: TRUE)
<code>transposed</code>	Switches 'from' and 'to' cell-type. This way the (default: FALSE)
<code>remove_NAs</code>	dont plot samples with less than min cells
<code>use_pixel</code>	show the distances in pixels or micrometers (default: FALSE)

### Value

plot average nearest neighbor barplots for two cell types

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset)
p <- plot_nearest_neighbor(dataset,'CD8+ PD1+', 'SOX10+ PDL1')
```

---

plot\_proximities      *Plot proximity analysis data*

---

**Description**

Plot proximity analysis data

**Usage**

```
plot_proximities(x, ...)

## S4 method for signature 'ImageSet'
plot_proximities(x, label, ordering = NULL,
  normalize = TRUE, palette = NULL, celltype_order = NULL,
  xlim_fix = 13, topbar_cols = "darkgrey")
```

**Arguments**

x	An IrisSpatialFeatures ImageSet object
...	Additional parameters.
label	Cell-type for which the proximit profile is plotted
ordering	Ordering of the samples (Default: NULL)
normalize	Flag, should the populations of different cell-types sum up to one in each sample? (Default: TRUE)
palette	Color palette, by default it uses Spectral from RColorbrewer (Default:NULL)
celltype_order	Ordering of the cell-type. (Default: NULL)
xlim_fix	Space on the right side to show the legend (Default: 13)
topbar_cols	Color of the barplots on top (Default: 'darkgrey')

**Value**

plot proximity analysis

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_proximity(dataset,only_closest=TRUE,radii=25)
p <- plot_proximities(dataset,"SOX10+ PDL1-",xlim_fix=3)
```

---

raw_data-class	<i>Class to represent the raw data that is output by inForm</i>
----------------	---

---

**Description**

Class to represent the raw data that is output by inForm

**Slots**

data Raw coordinate data sheet  
summary Inform summary (optional)  
score Score table that indicates intensity cutoff for certain markers  
mem\_seg\_map Membrane map as output by inForm  
nuc\_seg\_map Nuclear map as output by inForm (optional)  
component\_tiff component tiff as output by inForm (optional)

---

rayplot_single_coordinate	<i>Plot nearest neighbor ray plots for a single coordinate</i>
---------------------------	--

---

**Description**

Plot nearest neighbor ray plots for a single coordinate

**Usage**

```
rayplot_single_coordinate(x, from_type, to_type, samp_name = "",
  from_col = "#EE7600", to_col = "#028482", lineColor = "#666666",
  use_pixel = FALSE, microns_per_pixel = 0.496)

## S4 method for signature 'Coordinate'
rayplot_single_coordinate(x, from_type, to_type,
  samp_name = "", from_col = "#EE7600", to_col = "#028482",
  lineColor = "#666666", use_pixel = FALSE, microns_per_pixel = 0.496)
```

**Arguments**

x	An Coordinate object
from_type	Cell type from which the rays are drawn
to_type	Cell type to which the rays are drawn
samp_name	Name of the sample
from_col	Color for the 'from' cell-type (Default: '#EE7600')
to_col	Color for the 'to' cell-type (Default: '#028482')
lineColor	Color for the line (Default: '#666666')
use_pixel	Express units as pixels (Default: FALSE)
microns_per_pixel	Conversion (Default: 0.496)

**Value**

a plot

**Examples**

```
#loading pre-read dataset
dataset <- IrisSpatialFeatures_data
dataset <- extract_nearest_neighbor(dataset)
rayplot_single_coordinate(x = dataset@samples[[1]]@coordinates[[1]],
                          samp_name = dataset@samples[[1]]@sample_name,
                          from_type = "SOX10+ PDL1+",
                          to_type = "CD8+ PD1+")
```

---

read_raw	<i>Read inForm output and store it in an IrisSpatialFeatures ImageSet object.</i>
----------	---

---

**Description**

Read inForm output and store it in an IrisSpatialFeatures ImageSet object.

**Usage**

```
read_raw(path, label_fix = list(), format = "Vectra", dir_filter = "",
         read_nuc_seg_map = FALSE, MicronsPerPixel = 0.496,
         invasive_margin_in_px = 100, readMasks = TRUE, ignore_scoring = FALSE,
         read_only_relevant_markers = TRUE)
```

```
## S4 method for signature 'character'
read_raw(path, label_fix = list(), format = "Vectra",
         dir_filter = "", read_nuc_seg_map = FALSE, MicronsPerPixel = 0.496,
         invasive_margin_in_px = 100, readMasks = TRUE, ignore_scoring = FALSE,
         read_only_relevant_markers = TRUE)
```

**Arguments**

path	Directory that contains the raw files
label_fix	List of length 2 character vector that is used to fix filenames.
format	Output format: Currently only "Vectra" and "Mantra" are supported.
dir_filter	Filter to select only certain directory names.
read_nuc_seg_map	Flag indicating whether the nuclear map should be read.
MicronsPerPixel	Length of one pixel. Default: 0.496, corresponding to a 20x Mantra/Vectra images
invasive_margin_in_px	The width of the invasive margin in pixels
readMasks	Flag indicating whether the "_Tumor.tif" and "_Invasive_Margin.tif" should be read (default: True)



`ignore_scoring` Flag indicating whether the scoring file should be ignored (default: False)  
`read_only_relevant_markers` Flag that indicates whether all read inform output should be kept or only the relevant markers

**Value**

IrisSpatialFeatures ImageSet object.

**Examples**

```
raw_data <- read_raw(path=system.file("extdata", package = "IrisSpatialFeatures"),
  format='Mantra')
```

---

Sample-class	<i>An S3 class to represent a single imaging sample with multiple coordinates.</i>
--------------	--

---

**Description**

An S3 class to represent a single imaging sample with multiple coordinates.

**Slots**

`coordinates` A list of coordinate objects that contain all of the raw and coordinate data.  
`sample_name` Name of the contained sample.

---

<code>threshold_dataset</code>	<i>This function reads the manually determined thresholds of certain markers (e.g. PD1, PD-L1) and splits selected celltypes into marker+ and marker- celltypes.</i>
--------------------------------	--

---

**Description**

This function reads the manually determined thresholds of certain markers (e.g. PD1, PD-L1) and splits selected celltypes into marker+ and marker- celltypes.

**Usage**

```
threshold_dataset(image_set, marker, marker_name, base = NULL,
  pheno_name = "Phenotype", remove_blanks = TRUE)
```

```
## S4 method for signature 'ImageSet'
threshold_dataset(image_set, marker, marker_name,
  base = NULL, pheno_name = "Phenotype", remove_blanks = TRUE)
```

**Arguments**

<code>image_set</code>	IrisSpatialFeatures ImageSet object.
<code>marker</code>	Name of the marker used in the score file.
<code>marker_name</code>	corresponding name, which should be appended at the selected cell types.
<code>base</code>	Vector of cell types for which the marker should be used.
<code>pheno_name</code>	Name of the phenotype column to be used. (Default from inForm is "Phenotype")
<code>remove_blanks</code>	Flag that indicates whether or not not called cells are to be removed. (Default: TRUE)

**Value**

IrisSpatialFeatures ImageSet object.

**Examples**

```
dataset <- read_raw(path=system.file("extdata", package = "IrisSpatialFeatures"),
                   format='Mantra')
dataset <- threshold_dataset(dataset,
                             marker='PD-Ligand-1 (Opal 690)',
                             marker_name='PDL1',
                             base=c('SOX10+'))
dataset <- threshold_dataset(dataset,
                             marker='PD-1 (Opal 540)',
                             marker_name='PD1',
                             base=c('CD8+', 'OTHER'))
```

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