

Package ‘gateR’

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Title Flow/Mass Cytometry Gating via Spatial Kernel Density Estimation

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Description Estimates statistically significant marker combination values within which one immunologically distinctive group (i.e., disease case) is more associated than another group (i.e., healthy control), successively, using various combinations (i.e., “gates”) of markers to examine features of cells that may be different between groups. For a two-group comparison, the ‘gateR’ package uses the spatial relative risk function that is estimated using the ‘sparr’ package. Details about the ‘sparr’ package methods can be found in the tutorial: Davies et al. (2018) <doi:10.1002/sim.7577>. Details about kernel density estimation can be found in J. F. Bithell (1990) <doi:10.1002/sim.4780090616>. More information about relative risk functions using kernel density estimation can be found in J. F. Bithell (1991) <doi:10.1002/sim.4780101112>.

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gateR-package	<i>The gateR Package: Flow/Mass Cytometry Gating via Spatial Kernel Density Estimation</i>
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Description

Estimates statistically significant fluorescent marker combination values within which one immunologically distinctive group (i.e., disease case) is more associated than another group (i.e., healthy control), successively, using various combinations (i.e., "gates") of fluorescent markers to examine features of cells that may be different between groups.

Details

For a two-group comparison, the 'gateR' package uses the spatial relative risk function that is estimated using the sparr package. Details about the sparr package methods can be found in the tutorial: Davies et al. (2018) doi: [10.1002/sim.7577](https://doi.org/10.1002/sim.7577). Details about kernel density estimation can be found in J. F. Bithell (1990) doi: [10.1002/sim.4780090616](https://doi.org/10.1002/sim.4780090616). More information about relative risk functions using kernel density estimation can be found in J. F. Bithell (1991) doi: [10.1002/sim.4780101112](https://doi.org/10.1002/sim.4780101112).

This package provides a function to perform a gating strategy for flow cytometry data. The 'gateR' package also provides basic visualization for each gate.

Key content of the 'gateR' package include:

Gating Strategy

`gating` Extracts cells within statistically significant combinations of fluorescent markers, successively, for a set of markers. Statistically significant combinations are identified using two-tailed

p-values of a relative risk surface assuming asymptotic normality. This function is currently available for two-level comparisons of a single condition (e.g., case/control) or two conditions (e.g., case/control at time 1 and time 2). Provides functionality for basic visualization and multiple testing correction.

rrs Estimates a relative risk surface and computes the asymptotic p-value surface for a single gate with a single condition. Includes features for basic visualization. This function is used internally within the `gating` function to extract the points within the significant areas. This function can also be used as a standalone function.

lotrrs Estimates a ratio of relative risk surfaces and computes the asymptotic p-value surface for a single gate with two conditions. Includes features for basic visualization. This function is used internally within the `gating` function to extract the points within the significant areas. This function can also be used as a standalone function.

Flow Cytometry Data

randCyto A sample dataset containing information about flow cytometry data with two binary categorical variables. The data are a random subset of the 'extdata' data in the 'flowWorkspaceData' package found on Bioconductor <http://bioconductor.org/packages/release/data/experiment/html/flowWorkspaceData.html> and formatted for gateR input.

Dependencies

The 'gateR' package relies heavily upon `sparr`, `spatstat.geom`, and `raster`. For a two-level comparison, the spatial relative risk function uses the `risk` function. The calculation of a Bonferroni correction for multiple testing accounting for the spatial correlation of the estimated surface uses the `modified.ttest` function. Basic visualizations rely on the `image.plot` function.

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gating

Gating strategy for mass cytometry data using spatial relative risk functions

Description

Extracts cells within statistically significant combinations of fluorescent markers, successively, for a set of markers. Statistically significant combinations are identified using two-tailed p-values of a relative risk surface assuming asymptotic normality. This function is currently available for two-level comparisons of a single condition (e.g., case/control) or two conditions (e.g., case/control at time 1 and time 2). Provides functionality for basic visualization and multiple testing correction.

Usage

```
gating(
  dat,
  vars,
  n_condition = c(1, 2),
  numerator = TRUE,
  bandw = NULL,
  alpha = 0.05,
  p_correct = "none",
  nbc = NULL,
  plot_gate = FALSE,
  save_gate = FALSE,
  name_gate = NULL,
  path_gate = NULL,
  rcols = c("#FF0000", "#CCCCCC", "#0000FF"),
  lower_lrr = NULL,
  upper_lrr = NULL,
  c1n = NULL,
  c2n = NULL,
  win = NULL,
  ...,
  doplot = lifecycle::deprecated(),
  verbose = lifecycle::deprecated()
)
```

Arguments

<code>dat</code>	Input data frame flow cytometry data with the following features (columns): 1) ID, 2) Condition A ID, 3) Condition B ID (optional), and a set of markers.
<code>vars</code>	A vector of characters with the name of features (columns) within <code>dat</code> to use as markers for each gate. See details below.
<code>n_condition</code>	A numeric value of either 1 or 2 designating if the gating is performed with one condition or two conditions.
<code>numerator</code>	Logical. If TRUE (the default), cells will be extracted within all statistically significant numerator (i.e., case) clusters. If FALSE, cells will be extracted within all statistically significant denominator (i.e., control) clusters.
<code>bandw</code>	Optional, numeric. Fixed bandwidth for the kernel density estimation. Default is based on the internal <code>[sparr]{OS}</code> function.
<code>alpha</code>	Numeric. The two-tailed alpha level for significance threshold (default is 0.05).
<code>p_correct</code>	Optional. Character string specifying whether to apply a correction for multiple comparisons including a False Discovery Rate <code>p_correct = "FDR"</code> , a spatially dependent Sidak correction <code>p_correct = "correlated Sidak"</code> , a spatially dependent Bonferroni correction <code>p_correct = "correlated Bonferroni"</code> , an independent Sidak correction <code>p_correct = "uncorrelated Sidak"</code> , an independent Bonferroni correction <code>p_correct = "uncorrelated Bonferroni"</code> , and a correction based on Random Field Theory using an equation by Adler and Hasofer <code>p_correct = "Adler and Hasofer"</code> or an equation by Friston et al. <code>p_correct</code>

	= "Friston". If <code>p_correct = "none"</code> (the default), then no correction is applied.
<code>nbc</code>	Optional. An integer for the number of bins when <code>p_correct = "correlated"</code> . Similar to <code>nbclass</code> argument in <code>modified.ttest</code> . The default is 30.
<code>plot_gate</code>	Logical. If TRUE, the output includes basic data visualizations.
<code>save_gate</code>	Logical. If TRUE, the output saves each visualization as a separate PNG file.
<code>name_gate</code>	Optional, character. The filename of the visualization(s). The default is "gate_k" where "k" is the gate number.
<code>path_gate</code>	Optional, character. The path of the visualization(s). The default is the current working directory.
<code>rcols</code>	Character string of length three (3) specifying the colors for: 1) group A (numerator), 2) neither, and 3) group B (denominator) designations. The defaults are <code>c("#FF0000", "#cccccc", "#0000FF")</code> or <code>c("red", "grey80", "blue")</code> .
<code>lower_lrr</code>	Optional, numeric. Lower cut-off value for the log relative risk value in the color key (typically a negative value). The default is no limit and the color key will include the minimum value of the log relative risk surface.
<code>upper_lrr</code>	Optional, numeric. Upper cut-off value for the log relative risk value in the color key (typically a positive value). The default is no limit and the color key will include the maximum value of the log relative risk surface.
<code>c1n</code>	Optional, character. The name of the level for the numerator of condition A. The default is null and the first level is treated as the numerator.
<code>c2n</code>	Optional, character. The name of the level for the numerator of condition B. The default is null and the first level is treated as the numerator.
<code>win</code>	Optional. Object of class <code>owin</code> for a custom two-dimensional window within which to estimate the surfaces. The default is NULL and calculates a convex hull around the data.
<code>...</code>	Arguments passed to <code>risk</code> to select resolution.
<code>doplot</code>	[Deprecated] <code>doplot</code> is no longer supported and has been renamed <code>plot_gate</code> .
<code>verbose</code>	[Deprecated] <code>verbose</code> is no longer supported; this function will not display verbose output from internal <code>risk</code> function.

Details

This function performs a sequential gating strategy for mass cytometry data comparing two levels with one or two conditions. Gates are typically two-dimensional space comprised of two fluorescent markers. The two-level comparison allows for the estimation of a spatial relative risk function and the computation of p-value based on an assumption of asymptotic normality. Cells within statistically significant areas are extracted and used in the next gate. This function relies heavily upon the `risk` function. Basic visualization is available if `plot_gate = TRUE`.

The `vars` argument must be a vector with an even-numbered length where the odd-numbered elements are the markers used on the x-axis of a gate and the even-numbered elements are the markers used on the y-axis of a gate. For example, if `vars = c("V1", "V2", "V3", and "V4")` then the first gate is "V1" on the x-axis and "V2" on the y-axis and then the second gate is "V3" on the x-axis and "V4" on the y-axis. Markers can be repeated in successive gates.

The `n_condition` argument specifies if the gating strategy is performed for one condition or two conditions. If `n_condition = 1`, then the function performs a one condition gating strategy using the internal `rrs` function, which computes the statistically significant areas (clusters) of a relative risk surface at each gate and selects the cells within the clusters specified by the numerator argument. If `n_condition = 2`, then the function performs a two conditions gating strategy using the internal `lotrrs` function, which computes the statistically significant areas (clusters) of a ratio of relative risk surfaces at each gate and selects the cells within the clusters specified by the numerator argument. The condition variable(s) within `dat` must be of class 'factor' with two levels. The first level is considered the numerator (i.e., "case") value and the second level is considered the denominator (i.e., "control") value. The levels can also be specified using the `c1n` and `c2n` parameters. See the documentation for the internal `rrs` and `lotrrs` functions for more details.

The p-value surface of the ratio of relative risk surfaces is estimated assuming asymptotic normality of the ratio value at each gridded knot. The bandwidth is fixed across all layers.

Provides functionality for a correction for multiple testing. If `p_correct = "FDR"`, calculates a False Discovery Rate by Benjamini and Hochberg. If `p_correct = "uncorrelated Sidak"`, calculates an independent Sidak correction. If `p_correct = "uncorrelated Bonferroni"`, calculates an independent Bonferroni correction. If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, then the corrections take into account the into account the spatial correlation of the surface. (NOTE: If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, it may take a considerable amount of computation resources and time to calculate). If `p_correct = "Adler and Hasofer"` or if `p_correct = "Friston"`, then calculates a correction based on Random Field Theory. If `p_correct = "none"` (the default), then the function does not account for multiple testing and uses the uncorrected alpha level. See the internal `pval_correct` function documentation for more details.

Value

An object of class `list`. This is a named list with the following components:

- `obs` An object of class 'tibble' of the same features as `dat` that includes the information for the cells extracted with significant clusters in the final gate.
- `n` An object of class 'list' of the sample size of cells at each gate. The length is equal to the number of successful gates plus the final result.
- `gate` An object of class 'list' of 'rrs' objects from each gate. The length is equal to the number of successful gates.
- `note` An object of class 'character' of the gating diagnostic message.

The objects of class 'rrs' is similar to the output of the [risk](#) function with two additional components:

- `rr` An object of class 'im' with the relative risk surface.
- `f` An object of class 'im' with the spatial density of the numerator.
- `g` An object of class 'im' with the spatial density of the denominator.
- `P` An object of class 'im' with the asymptotic p-value surface.
- `lrr` An object of class 'im' with the log relative risk surface.
- `alpha` A numeric value for the alpha level used within the gate.

Examples

```
if (interactive()) {  
  ## Single condition, no multiple testing correction  
  test_gate <- gating(dat = randCyto,  
                      vars = c("arcsinh_CD4", "arcsinh_CD38",  
                              "arcsinh_CD8", "arcsinh_CD3"),  
                      n_condition = 1)  
}
```

lotrrs

A single gate for two conditions

Description

Estimates a ratio of relative risk surfaces and computes the asymptotic p-value surface for a single gate with two conditions. Includes features for basic visualization. This function is used internally within the [gating](#) function to extract the points within the significant areas. This function can also be used as a standalone function.

Usage

```
lotrrs(  
  dat,  
  bandw = NULL,  
  alpha = 0.05,  
  p_correct = "none",  
  nbc = NULL,  
  plot_gate = FALSE,  
  save_gate = FALSE,  
  name_gate = NULL,  
  path_gate = NULL,  
  rcols = c("#FF0000", "#CCCCCC", "#0000FF"),  
  lower_lrr = NULL,  
  upper_lrr = NULL,  
  c1n = NULL,  
  c2n = NULL,  
  win = NULL,  
  ...,  
  doplot = lifecycle::deprecated(),  
  verbose = lifecycle::deprecated()  
)
```

Arguments

dat Input data frame flow cytometry data with five (5) features (columns): 1) ID, 2) Condition A ID, 3) Condition B ID, 4) Marker A as x-coordinate, 5) Marker B as y-coordinate.

bandw	Optional, numeric. Fixed bandwidth for the kernel density estimation. Default is based on the internal <code>[sparr]{OS}</code> function.
alpha	Numeric. The two-tailed alpha level for significance threshold (default is 0.05).
p_correct	Optional. Character string specifying whether to apply a correction for multiple comparisons including a False Discovery Rate <code>p_correct = "FDR"</code> , a spatially dependent Sidak correction <code>p_correct = "correlated Sidak"</code> , a spatially dependent Bonferroni correction <code>p_correct = "correlated Bonferroni"</code> , an independent Sidak correction <code>p_correct = "uncorrelated Sidak"</code> , an independent Bonferroni correction <code>p_correct = "uncorrelated Bonferroni"</code> , and a correction based on Random Field Theory using an equation by Adler and Hasofer <code>p_correct = "Adler and Hasofer"</code> or an equation by Friston et al. <code>p_correct = "Friston"</code> . If <code>p_correct = "none"</code> (the default), then no correction is applied.
nbc	Optional. An integer for the number of bins when <code>p_correct = "correlated"</code> . Similar to <code>nbclass</code> argument in <code>modified.ttest</code> . The default is 30.
plot_gate	Logical. If TRUE, the output includes basic data visualization.
save_gate	Logical. If TRUE, the output saves the visualization as a separate PNG file.
name_gate	Optional, character. The filename of the visualization. The default is "gate".
path_gate	Optional, character. The path of the visualization. The default is the current working directory.
rcols	Character string of length three (3) specifying the colors for: 1) group A (numerator), 2) neither, and 3) group B (denominator) designations. The defaults are <code>c("#FF0000", "#cccccc", "#0000FF")</code> or <code>c("red", "grey80", "blue")</code> .
lower_lrr	Optional, numeric. Lower cut-off value for the log relative risk value in the color key (typically a negative value). The default is no limit and the color key will include the minimum value of the log relative risk surface.
upper_lrr	Optional, numeric. Upper cut-off value for the log relative risk value in the color key (typically a positive value). The default is no limit and the color key will include the maximum value of the log relative risk surface.
c1n	Optional, character. The name of the level for the numerator of condition A. The default is null and the first level is treated as the numerator.
c2n	Optional, character. The name of the level for the numerator of condition B. The default is null and the first level is treated as the numerator.
win	Optional. Object of class <code>owin</code> for a custom two-dimensional window within which to estimate the surfaces. The default is NULL and calculates a convex hull around the data.
...	Arguments passed to <code>risk</code> to select resolution.
doplot	[Deprecated] <code>doplot</code> is no longer supported and has been renamed <code>plot_gate</code> .
verbose	[Deprecated] <code>verbose</code> is no longer supported; this function will not display verbose output from internal <code>risk</code> function.

Details

This function estimates a ratio of relative risk surfaces and computes the asymptotic p-value surface for a single gate with two conditions using three successive `risk` functions. A relative risk surface is estimated for Condition A at each level of Condition B and then a ratio of the two relative risk surfaces is computed.

$$RR_{ConditionB1} = \frac{ConditionA2ofB1}{ConditionA1ofB1}$$

$$RR_{ConditionB2} = \frac{ConditionA2ofB2}{ConditionA1ofB2}$$

$$\ln(rRR) = \ln\left(\frac{RR_{ConditionB2}}{RR_{ConditionB1}}\right)$$

The p-value surface of the ratio of relative risk surfaces is estimated assuming asymptotic normality of the ratio value at each gridded knot. The bandwidth is fixed across all layers. Basic visualization is available if `plot_gate = TRUE`.

Provides functionality for a correction for multiple testing. If `p_correct = "FDR"`, calculates a False Discovery Rate by Benjamini and Hochberg. If `p_correct = "uncorrelated Sidak"`, calculates an independent Sidak correction. If `p_correct = "uncorrelated Bonferroni"`, calculates an independent Bonferroni correction. If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, then the corrections take into account the into account the spatial correlation of the surface. (NOTE: If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, it may take a considerable amount of computation resources and time to calculate). If `p_correct = "Adler and Hasofer"` or if `p_correct = "Friston"`, then calculates a correction based on Random Field Theory. If `p_correct = "none"` (the default), then the function does not account for multiple testing and uses the uncorrected alpha level. See the internal `pval_correct` function documentation for more details.

The two condition variables (Condition A and Condition B) within `dat` must be of class 'factor' with two levels. The first level in each variable is considered the numerator (i.e., "case") value and the second level is considered the denominator (i.e., "control") value. The levels can also be specified using the `c1n` and `c2n` parameters.

Value

An object of class 'list' where each element is a object of class 'rrs' created by the `risk` function with two additional components:

- `rr` An object of class 'im' with the relative risk surface.
- `f` An object of class 'im' with the spatial density of the numerator.
- `g` An object of class 'im' with the spatial density of the denominator.
- `P` An object of class 'im' with the asymptotic p-value surface.
- `lrr` An object of class 'im' with the log relative risk surface.
- `alpha` A numeric value for the alpha level used within the gate.

Examples

```
test_lotrrs <- lotrrs(dat = randCyto)
```

`randCyto`*Subset of the 'extdata' data in the 'flowWorkspaceData' package*

Description

A sample dataset containing information about flow cytometry data with two binary conditions and four markers. The data are a random subset of the 'extdata' data in the 'flowWorkspaceData' package found on Bioconductor <http://bioconductor.org/packages/release/data/experiment/html/flowWorkspaceData.html> and formatted for 'gateR' input. The selected markers are arcsinh transformed.

Usage

```
randCyto
```

Format

A data frame with 11763 rows and 7 variables:

id cell ID number

g1 binary condition #1

g2 binary condition #2

arcsinh_CD4 arcsinh-transformed CD4

arcsinh_CD38 arcsinh-transformed CD38

arcsinh_CD8 arcsinh-transformed CD8

arcsinh_CD3 arcsinh-transformed CD3

Source

<https://github.com/Waller-SUSAN/gateR/blob/master/README.md>

Examples

```
head(randCyto)
```

rrs

*A single gate for a single condition***Description**

Estimates a relative risk surface and computes the asymptotic p-value surface for a single gate with a single condition. Includes features for basic visualization. This function is used internally within the `gating` function to extract the points within the significant areas. This function can also be used as a standalone function.

Usage

```
rrs(
  dat,
  bandwidth = NULL,
  alpha = 0.05,
  p_correct = "none",
  nbc = NULL,
  plot_gate = FALSE,
  save_gate = FALSE,
  name_gate = NULL,
  path_gate = NULL,
  rcols = c("#FF0000", "#CCCCCC", "#0000FF"),
  lower_lrr = NULL,
  upper_lrr = NULL,
  c1n = NULL,
  win = NULL,
  ...,
  doplot = lifecycle::deprecated(),
  verbose = lifecycle::deprecated()
)
```

Arguments

<code>dat</code>	Input data frame flow cytometry data with four (4) features (columns): 1) ID, 2) Condition A ID, 3) Marker A as x-coordinate, 4) Marker B as y-coordinate.
<code>bandw</code>	Optional, numeric. Fixed bandwidth for the kernel density estimation. Default is based on the internal <code>[sparr]{OS}</code> function.
<code>alpha</code>	Numeric. The two-tailed alpha level for significance threshold (default is 0.05).
<code>p_correct</code>	Optional. Character string specifying whether to apply a correction for multiple comparisons including a False Discovery Rate <code>p_correct = "FDR"</code> , a spatially dependent Sidak correction <code>p_correct = "correlated Sidak"</code> , a spatially dependent Bonferroni correction <code>p_correct = "correlated Bonferroni"</code> , an independent Sidak correction <code>p_correct = "uncorrelated Sidak"</code> , an independent Bonferroni correction <code>p_correct = "uncorrelated Bonferroni"</code> , and a

	correction based on Random Field Theory using an equation by Adler and Hasofer <code>p_correct = "Adler and Hasofer"</code> or an equation by Friston et al. <code>p_correct = "Friston"</code> . If <code>p_correct = "none"</code> (the default), then no correction is applied.
<code>nbc</code>	Optional. An integer for the number of bins when <code>p_correct = "correlated"</code> . Similar to <code>nbclass</code> argument in <code>modified.ttest</code> . The default is 30.
<code>plot_gate</code>	Logical. If TRUE, the output includes basic data visualization.
<code>save_gate</code>	Logical. If TRUE, the output saves the visualization as a separate PNG file.
<code>name_gate</code>	Optional, character. The filename of the visualization. The default is "gate".
<code>path_gate</code>	Optional, character. The path of the visualization. The default is the current working directory.
<code>rcols</code>	Character string of length three (3) specifying the colors for: 1) group A (numerator), 2) neither, and 3) group B (denominator) designations. The defaults are <code>c("#FF0000", "#cccccc", "#0000FF")</code> or <code>c("red", "grey80", "blue")</code> .
<code>lower_lrr</code>	Optional, numeric. Lower cut-off value for the log relative risk value in the color key (typically a negative value). The default is no limit and the color key will include the minimum value of the log relative risk surface.
<code>upper_lrr</code>	Optional, numeric. Upper cut-off value for the log relative risk value in the color key (typically a positive value). The default is no limit and the color key will include the maximum value of the log relative risk surface.
<code>c1n</code>	Optional, character. The name of the level for the numerator of condition A. The default is null and the first level is treated as the numerator.
<code>win</code>	Optional. Object of class <code>owin</code> for a custom two-dimensional window within which to estimate the surfaces. The default is NULL and calculates a convex hull around the data.
<code>...</code>	Arguments passed to <code>risk</code> to select resolution.
<code>doplot</code>	[Deprecated] <code>doplot</code> is no longer supported and has been renamed <code>plot_gate</code> .
<code>verbose</code>	[Deprecated] <code>verbose</code> is no longer supported; this function will not display verbose output from internal <code>risk</code> function.

Details

This function estimates a relative risk surface and computes the asymptotic p-value surface for a single gate and single condition using the `risk` function. Bandwidth is fixed across both layers (numerator and denominator spatial densities). Basic visualization is available if `plot_gate = TRUE`.

Provides functionality for a correction for multiple testing. If `p_correct = "FDR"`, calculates a False Discovery Rate by Benjamini and Hochberg. If `p_correct = "uncorrelated Sidak"`, calculates an independent Sidak correction. If `p_correct = "uncorrelated Bonferroni"`, calculates an independent Bonferroni correction. If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, then the corrections take into account the into account the spatial correlation of the surface. (NOTE: If `p_correct = "correlated Sidak"` or if `p_correct = "correlated Bonferroni"`, it may take a considerable amount of computation resources and time to calculate). If `p_correct = "Adler and Hasofer"` or if `p_correct = "Friston"`, then calculates

a correction based on Random Field Theory. If `p_correct = "none"` (the default), then the function does not account for multiple testing and uses the uncorrected alpha level. See the internal `pval_correct` function documentation for more details.

The condition variable (Condition A) within `dat` must be of class 'factor' with two levels. The first level is considered the numerator (i.e., "case") value and the second level is considered the denominator (i.e., "control") value. The level can also be specified using the `c1n` parameter.

Value

An object of class 'list' where each element is a object of class 'rrs' created by the [risk](#) function with two additional components:

- `rr` An object of class 'im' with the relative risk surface.
- `f` An object of class 'im' with the spatial density of the numerator.
- `g` An object of class 'im' with the spatial density of the denominator.
- `P` An object of class 'im' with the asymptotic p-value surface.
- `lrr` An object of class 'im' with the log relative risk surface.
- `alpha` A numeric value for the alpha level used within the gate.

Examples

```
test_rrs <- rrs(dat = randCyto)
```

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