Package 'smoppix'

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Type Package

Title Analyze Single Molecule Spatial Omics Data Using the Probabilistic Index

Version 1.1.0

Description Test for univariate and bivariate spatial patterns in spatial omics data with single-molecule resolution. The tests implemented allow for analysis of nested designs and are automatically calibrated to different biological specimens. Tests for aggregation, colocalization, gradients and vicinity to cell edge or centroid are provided.

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Encoding UTF-8

Imports spatstat.geom(>= 3.2.0),spatstat.random,methods,BiocParallel,SummarizedExperiment,SpatialExperiment,scam,Rdpack,stats,utils,extra (>= 1.0.11),Matrix,spatstat.model,openxlsx

Suggests testthat,rmarkdown,knitr,DropletUtils,polyCub,RImageJROI,sp,ape,htmltools,funkycells,glmnet,doParallel

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Contents

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addCell *Add cell boundaries and event-wise cell identifiers to a hyperframe.*

Description

Add the list of the cells and their centroids in the hyperframe, check in which cell each event lies and add a cell marker.

Usage

```
addCell(
  hypFrame,
  owins,
  cellTypes = NULL,
  findOverlappingOwins = FALSE,
  warnOut = TRUE,
  coords = c("x", "y"),
  verbose = TRUE,
  addCellMarkers = TRUE,
  overwriteCells = FALSE,
  ...
)
```

Arguments

`hypFrame`	A hyperframe
`owins`	A list containing a list of owins per point pattern. The length of the list must match the length of the hyperframe, and the names must match. Also lists of geojson objects, coordinate matrices or rois are accepted, see details.
`cellTypes`	A dataframe of cell types and other cell-associated covariates. If supplied, it must contain a variable 'cell' that is matched with the names of the owins
`findOverlappingOwins`	a boolean, should windows be checked for overlap? Can be computationally intensive.
`warnOut`	a boolean, should warning be issued when points are not contained in window?
`coords`	The names of the coordinates, if the windows are given as sets of coordinates.
`verbose`	A boolean, should verbose output be printed?
`addCellMarkers`	A boolean, should cell identities be added? Set this to FALSE if cell identifiers are already present in the data, and you only want to add windows and centroids.
`overwriteCells`	A boolean, should cells already present in hyperframe be overwritten?
`...`	Further arguments passed onto convertToOwins

Details

First the different cells are checked for overlap per point pattern if 'findOverlappingOwins' is TRUE. If no overlap is found, each event is assigned the cell that it falls into. Events not belonging to any cell will trigger a warning and be assigned 'NA'. Cell types and other variables are added to the marks if applicable. This function employs multithreading through the BiocParallel package. If this leads to excessive memory usage and crashes, try serial processing by setting register(SerialParam()). Different formats of windows are allowed, if the corresponding packages are installed. A dataframe of coordinates or a list of spatstat.geom owins is always allowed, as the necessary packages are required by smoppix. A 'SpatialPolygonsDataFrame' object is allowed if the 'polycub' package is installed, and a list of 'ijroi' object or a single 'ijzip' object if the 'RImageJROI' package is installed.

Value

The hyperframe with cell labels added in the marks of the point patterns

Note

By default, overlap between windows is not checked. Events are assigned to the first window they fall in. If you are not sure of the quality of the segmentation, do check your input or set checkOverlap to TRUE, even when this make take time.

See Also

buildHyperFrame, convertToOwins

Examples

```
library(spatstat.random)
set.seed(54321)
n <- 1e3 # number of molecules
ng <- 25 # number of genes
nfov <- 3 # Number of fields of view
conditions <- 3
# sample xy-coordinates in [0, 1]
x <- runif(n)
y <- runif(n)
# assign each molecule to some gene-cell pair
gs <- paste0("gene", seq(ng))
gene <- sample(gs, n, TRUE)
fov <- sample(nfov, n, TRUE)
condition <- sample(conditions, n, TRUE)
# construct data.frame of molecule coordinates
df <- data.frame(gene, x, y, fov, "condition" = condition)
# A list of point patterns
listPPP <- tapply(seq(nrow(df)), df$fov, function(i) {
    ppp(x = df$x[i], y = df$y[i], marks = df[i, "gene", drop = FALSE])
}, simplify = FALSE)
# Regions of interest (roi): Diamond in the center plus four triangles
w1 <- owin(poly = list(x = c(0, .5, 1, .5), y = c(.5, 0, .5, 1)))
w2 <- owin(poly = list(x = c(0, 0, .5), y = c(.5, 0, 0)))
w3 <- owin(poly = list(x = c(0, 0, .5), y = c(1, 0.5, 1)))
w4 <- owin(poly = list(x = c(1, 1, .5), y = c(0.5, 1, 1)))
w5 <- owin(poly = list(x = c(1, 1, .5), y = c(0, 0.5, 0)))
hypFrame <- buildHyperFrame(df,
```

```
    coordVars = c("x", "y"),
    imageVars = c("condition", "fov")
)
nDesignFactors <- length(unique(hypFrame$image))
wList <- lapply(seq_len(nDesignFactors), function(x) {
    list("w1" = w1, "w2" = w2, "w3" = w3, "w4" = w4, "w5" = w5)
})
names(wList) <- rownames(hypFrame) # Matching names is necessary
hypFrame2 <- addCell(hypFrame, wList)
```

`addDesign` *Add design variables to hyperframe*

Description

Add design variables to hyperframe

Usage

```
addDesign(hypFrame, desMat, designVec)
```

Arguments

`hypFrame`	The hyperframe
`desMat`	The design matrix
`designVec`	The design vector

Value

The hyperframe with design variables added

`addNuclei` *Add nuclei to a hyperframe*

Description

Add the nuclei identifiers to a hyperframe already containing cells.

Usage

```
addNuclei(
  hypFrame,
  nucleiList,
  checkSubset = TRUE,
  verbose = TRUE,
  coords = c("x", "y"),
  overwriteNuclei = FALSE,
  ...
)
```

Arguments

hypFrame	A hyperframe
nucleiList	A list containing a list of owins per point pattern. The length of the list must match the length of the hyperframe, and the names must match. Also lists of geojson objects, coordinate matrices or rois are accepted, see addCell
checkSubset	A boolean, should be checked whether nuclei are encompassed by cells?
verbose	A boolean, should verbose output be printed?
coords	The names of the coordinates, if the nuclei are given as sets of coordinates.
overwriteNuclei	A boolean, should existing nuclei be replaced?
...	Further arguments passed onto convertToOwins

Details

The nuclei names must match the cell names already present, all other nuclei are dropped. A warning is issued when nuclei are not encompassed by their cell.

Value

The hyperframe with nuclei added as entry

See Also

addCell, convertToOwins

Examples

```
library(spatstat.random)
set.seed(54321)
n <- 1e3 # number of molecules
ng <- 25 # number of genes
nfov <- 3 # Number of fields of view
conditions <- 3
# sample xy-coordinates in [0, 1]
x <- runif(n)
y <- runif(n)
# assign each molecule to some gene-cell pair
gs <- paste0("gene", seq(ng))
gene <- sample(gs, n, TRUE)
fov <- sample(nfov, n, TRUE)
condition <- sample(conditions, n, TRUE)
# construct data.frame of molecule coordinates
df <- data.frame(gene, x, y, fov, "condition" = condition)
# A list of point patterns
listPPP <- tapply(seq(nrow(df)), df$fov, function(i) {
    ppp(x = df$x[i], y = df$y[i], marks = df[i, "gene", drop = FALSE])
}, simplify = FALSE)
# Regions of interest (roi): Diamond in the center plus four triangles
w1 <- owin(poly = list(x = c(0, .5, 1, .5), y = c(.5, 0, .5, 1)))
w2 <- owin(poly = list(x = c(0, 0, .5), y = c(.5, 0, 0)))
w3 <- owin(poly = list(x = c(0, 0, .5), y = c(1, 0.5, 1)))
w4 <- owin(poly = list(x = c(1, 1, .5), y = c(0.5, 1, 1)))
w5 <- owin(poly = list(x = c(1, 1, .5), y = c(0, 0.5, 0)))
```

```
hypFrame <- buildHyperFrame(df,
    coordVars = c("x", "y"),
    imageVars = c("condition", "fov")
)
nDesignFactors <- length(unique(hypFrame$image))
wList <- lapply(seq_len(nDesignFactors), function(x) {
    list("w1" = w1, "w2" = w2, "w3" = w3, "w4" = w4, "w5" = w5)
})
names(wList) <- rownames(hypFrame) # Matching names is necessary
hypFrame2 <- addCell(hypFrame, wList)
# The nuclei
n1 <- owin(poly = list(x = c(0.2, .4, 0.8, .4), y = c(.4, .2, .4, .8)))
n2 <- owin(poly = list(x = c(0.1, 0.1, .4), y = c(.4, .1, .1)))
n3 <- owin(poly = list(x = c(0.1, 0.1, .4), y = c(1, .75, 1)))
n4 <- owin(poly = list(x = c(1, 1, .6), y = c(.7, .9, .9)))
n5 <- owin(poly = list(x = c(.95, .95, .7), y = c(.1, .4, .1)))
nList <- lapply(seq_len(nDesignFactors), function(x) {
    list("w1" = n1, "w2" = n2, "w3" = n3, "w4" = n4, "w5" = n5)
})
names(nList) <- rownames(hypFrame) # Matching names is necessary
hypFrame3 <- addNuclei(hypFrame2, nList)
```

`addTabObs` *Add tables with gene counts to the hyperframe, presort by gene and x-ccordinate and add design varibales*

Description

Add tables with gene counts to the hyperframe, presort by gene and x-ccordinate and add design varibales

Usage

```
addTabObs(hypFrame)
```

Arguments

`hypFrame`	The hyperframe

Value

The hyperframe with tabObs added

`buildDataFrame` *Extract a data frame for a certain gene and PI from a fitted object*

Description

Based on a fitted object, a dataframe with results for a certain feature and PI is built, e.g. in preparation for linear modelling.

Usage

```
buildDataFrame(
  obj,
  gene,
  pi = c("nn", "nnPair", "edge", "centroid", "nnCell", "nnPairCell"),
  piMat,
  moransI = FALSE,
  numNNs = 8,
  weightMats,
  pppDf
)
```

Arguments

`obj`	A results object. For distances to fixed objects, the result of a call to estPis; for nearest neighbour distances, the result of a call to addWeightFunction
`gene`	A character string indicating the desired gene or gene pair (genes separated by double hyphens)
`pi`	character string indicating the desired PI
`piMat`	A data frame. Will be constructed if not provided, for internal use.
`moransI`	A boolean, should Moran's I be calculated? in the linear mixed model
`numNNs`	An integer, the number of nearest neighbours in the weight matrix for the calculation of the Moran's I statistic
`weightMats`	List of weight matrices for Moran's I calculation.
`pppDf`	Dataframe of point pattern-wise variables. It is precalculated in fitLMMsSingle for speed, but will be newly constructed when not provided.

Value

A dataframe with estimated PIs and covariates

See Also

addWeightFunction, buildMoransIDataFrame

Examples

```
example(addWeightFunction, "smoppix")
dfUniNN <- buildDataFrame(yangObj, gene = "SmVND2", pi = "nn")
# Example analysis with linear mixed model
library(lmerTest)
mixedMod <- lmer(pi - 0.5 ~ day + (1 | root),
    weight = weight, data = dfUniNN,
    contrasts = list("day" = "contr.sum")
)
summary(mixedMod)
# Evidence for aggregation
```

---

buildFormula *Build a formula from different components*

Description

Build a formula from different components

Usage

```
buildFormula(Formula, fixedVars, randomVars, outcome = "pi - 0.5")
```

Arguments

`Formula`	A formula. If not supplied or equals NULL, will be overridden
`fixedVars, randomVars`	Character vectors with fixed and random variables
`outcome`	A character vector describing the outcome

Details

Random intercepts are assumed for the random effects, if more complicated designs are used, do supply your own formula.

Value

A formula

See Also

fitLMMs,formula

buildHyperFrame *Build a hyperframe containing all point patterns of an experiment.*

Description

Build a spatstat hyperframe with point patterns and metadata. Matrices, dataframe, lists and SpatialExperiment inputs are accepted.

Usage

```
buildHyperFrame(x, ...)

## S4 method for signature 'data.frame'
buildHyperFrame(
  x,
  coordVars,
  imageIdentifier = imageVars,
  imageVars,
  pointVars = setdiff(names(x), c(imageVars, imageIdentifier, coordVars, featureName)),
  featureName = "gene",
  ...
)

## S4 method for signature 'matrix'
buildHyperFrame(
  x,
  imageVars,
  imageIdentifier = imageVars,
  covariates,
  featureName = "gene",
  ...
)

## S4 method for signature 'list'
buildHyperFrame(
  x,
  coordVars = c("x", "y"),
  covariates = NULL,
  idVar = NULL,
  featureName = "gene",
  ...
)

## S4 method for signature 'SpatialExperiment'
buildHyperFrame(x, imageVars, pointVars, imageIdentifier = imageVars, ...)
```

Arguments

x	the input object, see methods('buildHyperFrame')
...	additional constructor arguments

`coordVars` Names of coordinates

`imageIdentifier` A character vector of variables whose unique combinations define the separate point patterns (images)

`imageVars` Covariates belonging to the point patterns

`pointVars` Names of event-wise covariates such as gene or cell for each single point

`featureName` The name of the feature identifier for the molecules.

`covariates` A matrix or dataframe of covariates

`idVar` An optional id variable present in covariates, that is matched with the names of covariates

`list` A list of matrices or of point patterns of class 'spatstat.geom::ppp'

Value

An object of class 'hyperframe' from the 'spatstat.geom' package

See Also

hyperframe

Examples

```
data(Yang)
hypYang <- buildHyperFrame(Yang,
    coordVars = c("x", "y"),
    imageVars = c("day", "root", "section")
)
```

---

buildMoransIDataFrame *Build a data frame with Moran's I as outcome variable*

Description

Build a data frame with Moran's I as outcome variable

Usage

```
buildMoransIDataFrame(pi, piMat, weightMats)
```

Arguments

`pi` character string indicating the desired PI

`piMat` A data frame. Will be constructed if not provided, for internal use.

`weightMats` List of weight matrices for Moran's I calculation.

Value

A modified data frame, containing the estimated Moran's I and its variance

See Also

Moran.I, buildMoransIWeightMat

buildMoransIWeightMat *Build a weight matrix based on nearest neighbourship for Moran's I calculations*

Description

Build a weight matrix based on nearest neighbourship for Moran's I calculations

Usage

```
buildMoransIWeightMat(coordMat, numNNs)
```

Arguments

coordMat	A matrix of centroid coordinates
numNNs	An integer, the number of nearest neighbours in the weight matrix for the calculation of the Moran's I statistic

Value

A sparse matrix of weights for Moran's I statistic, of equal value for the numNNs nearest neighbours and zero otherwise

Note

The choice of numNN nearest neighbours is far less memory-glutton than a weight decaying continuously with distance

See Also

buildMoransIDataFrame, Moran.I

calcIndividualPIs *Calculate individual PI entries of a single point pattern*

Description

Calculate individual PI entries of a single point pattern

Usage

```
calcIndividualPIs(
  p,
  tabObs,
  pis,
  pSubLeft,
  owins,
  centroids,
  null,
  features,
```

```
    ecdfAll,
    ecdfsCell,
    loopFun,
    minDiff,
    minObsNN
  )
```

Arguments

`p`	The point pattern
`tabObs`	A table of observed gene frequencies
`pis`	The PIs to be estimated or for which weighting functions is to be added
`pSubLeft`	The subsampled overall point pattern returned by subSampleP
`owins, centroids`	The list of windows corresponding to cells, and their centroids
`null`	A character vector, indicating how the null distribution is defined. See details.
`features`	A character vector, for which features should the probabilistic indices be calculated?
`ecdfAll, ecdfsCell`	Empirical cumulative distribution functions of all events and of cells within the cell, under the null
`loopFun`	The function to use to loop over the features. Defaults to bplapply except when looping over features within cells
`minDiff`	An integer, the minimum number of events from other genes needed for calculation of background distribution of distances. Matters mainly for within-cell calculations: cells with too few events are skipped.
`minObsNN`	An integer, the minimum number of events required for a gene to be analysed. See details.

Details

For the single-feature nearest neighbour distances, the PI is average over the point pattern

Value

A list containing PI entries per feature

See Also

estPis, calcNNPI

`calcNNPI` *Estimate the PI for the nearest neighbour distances, given a set of ranks, using the negative hypergeometric distribution*

Description

Estimate the PI for the nearest neighbour distances, given a set of ranks, using the negative hypergeometric distribution

Usage

```
calcNNPI(Ranks, n, m, ties, r = 1)
```

Arguments

`Ranks`	The (approximate) ranks, number of times observed distance is larger
`n`	the total number of observed distances minus the number of distances under consideration (the number of failures or black balls in the urn)
`m`	the number of observed distances (successes or white balls in the urn)
`ties`	The number of times the observed distance is equal to a null distance, of the same length as Ranks
`r`	The rank of distances considered, r=1 is nearest neighbour distance

Details

Ties are counted half to match the definition of the PI.

Value

A vector of evaluations of the negative hypergeometric distribution function

See Also

pnhyper, calcIndividualPIs

`calcWindowDistPI` *Estimate the PI for the distance to a fixed object of interest, such as a cell wall or centroid*

Description

Estimate the PI for the distance to a fixed object of interest, such as a cell wall or centroid

Usage

```
calcWindowDistPI(pSub, owins, centroids, ecdfAll, pi)
```

Arguments

`pSub`	The subset point pattern containing only a single gene
`owins, centroids`	The list of windows corresponding to cells, and their centroids
`ecdfAll`	the cumulative distribution function under the null
`pi`	The type of PI to calculate

Details

Analysis of the distance to the border was introduced by (Joyner et al. 2013) in the form of the B-function. The independent evaluations of the B-functions under the null hypothesis represented by *ecdfAll* per cell are here returned as realizations of the probabilistic index.

Value

A list of vectors of estimated probabilistic indeces per event

References

Joyner M, Ross C, Seier E (2013). "Distance to the border in spatial point patterns." *Spat. Stat.*, **6**, 24 - 40. ISSN 2211-6753, doi:10.1016/j.spasta.2013.05.002.

See Also

addCell, estPis

`centerNumeric` *Center numeric variables*

Description

Center numeric variables

Usage

```
centerNumeric(x)
```

Arguments

`x`	The dataframe whose numeric variables are being centered

Value

The adapted dataframe

checkFeatures *Check if features are present in hyperframe*

Description

Check if features are present in hyperframe

Usage

```
checkFeatures(hypFrame, features)
```

Arguments

`hypFrame`	A hyperframe
`features`	A character vector, for which features should the probabilistic indices be calculated?

Value

Throws error when features not found

checkPi *Check if the required PI's are present in the object*

Description

Check if the required PI's are present in the object

Usage

```
checkPi(x, pi)
```

Arguments

`x`	The result of the PI calculation, or a weighting function
`pi`	A character string indicating the desired PI

Value

Throws an error when the PIs are not found, otherwise returns invisible

constructDesignVars — *Check for or construct design matrix*

Description

Run checks on design variables, or construct them as vector them if missing

Usage

```
constructDesignVars(designVars, lowestLevelVar, allCell, resList)
```

Arguments

`designVars`	The initial design variables
`lowestLevelVar`	Variable indicating the lowest level of nesting
`allCell`	A boolean, are all PIs cell-related?
`resList`	The results list

Value

A vector of design variables

See Also

buildDataFrame

convertToOwins — *Convert windows to spatstat.geom owin format*

Description

Convert a list of windows in different possible formats to owins, for addition to a hyperframe.

Usage

```
convertToOwins(windows, namePPP, coords, ...)
```

Arguments

`windows`	The list of windows. See addCell for accepted formats.
`namePPP`	the name of the point pattern, will be added to the cell names
`coords`	The names of the coordinates, if the windows are given as sets of coordinates.
`...`	passed onto as.owin

Details

Order of traversion of polygons may differ between data types. Where applicable, different orders are tried before throwing an error.

Value

A list of owins

See Also

addCell, as.owin

---

crossdistWrapper — *A wrapper for C-functions calculating cross-distance matrix fast*

Description

A wrapper for C-functions calculating cross-distance matrix fast

Usage

```
crossdistWrapper(x, y)
```

Arguments

`x, y`	the matrices or point patterns between which to calculate the cross distances

Value

a matrix of cross distances

Eng — *Spatial transcriptomics data of mouse fibroblast cells*

Description

Single-molecule spatial transcriptomics seqFISH+ data containing measurements of 10,000 genes in NIH/3T3 mouse fibroblast cells by (Eng et al. 2019). Molecule locations, gene identity and design variables are included, a subset of eight most expressed genes is included in the package, and the dataset was subsampled to 100,000 observations for memory reasons. In addition, a list of regions of interest (rois) is given describing the cell boundaries.

Usage

```
data(Eng)
```

Format

1. **Eng** A data frame with variables

   x,y Molecule coordinates

   gene Character vector with gene identities

   experiment,fov Design variables

2. **EngRois** A list of lists of regions of interest (ROIs): the cell boundaries

Source

doi:10.1038/s415860191049y

References

Eng CL, Lawson M, Zhu Q, Dries R, Koulena N, Takei Y, Yun J, Cronin C, Karp C, Yuan G, Cai L (2019). "Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+." *Nature*, **568**(7751), 235 - 239. ISSN 1476-4687, doi:10.1038/s415860191049y.

`estGradients` *Estimate gradients over multiple point patterns, and test for significance*

Description

estGradients() estimate gradients on all single-molecule point patterns of a hyperframe. estGradientsSingle() is the workhorse function for a single point pattern. getPvaluesGradient() extracts the p-values of the fits.

Usage

```
estGradients(
  hypFrame,
  gradients = c("overall", if (!is.null(hypFrame$owins)) "cell"),
  fixedEffects = NULL,
  randomEffects = NULL,
  verbose = FALSE,
  features = getFeatures(hypFrame),
  silent = TRUE,
  loopFun = "bplapply",
  ...
)

estGradientsSingle(
  hypFrame,
  gradients,
  fixedForm,
  randomForm,
  fixedFormSimple,
  effects = NULL,
  ...
)

getPvaluesGradient(res, gradient, method = "BH")
```

Arguments

`hypFrame`	A hyperframe
`gradients`	The gradients types to be estimated: "overall" or within cell ("cell")

`fixedEffects`, `randomEffects`
: Character vectors of fixed and random effects present in the hyperframe, modifying the baseline intensity. See details.

`verbose` A boolean, whether to report on progress of the fitting process.

`features` A character vector, for which features should the gradients indices be calculated?

`silent` A boolean, should error messages from spatstat.model::mppm be printed?

`loopFun` The function to use to loop over the features.

`...` Passed onto fitGradient

`fixedForm`, `randomForm`, `fixedFormSimple`
: Formulae for fixed effects, random effects and fixed effects without slopes respectively

`effects` Character vector of fixed and random effects

`res` The fitted gradients

`gradient` The gradient to be extracted, a character vector equal to "overall" or "cell".

`method` Method of multiplicity correction, see p.adjust. Defaults to Benjamini-Hochberg.

Details

The test for existence of a gradient revolves around interaction terms between x and y coordinates and image identifiers. If this interactions are significant, this implies existence of gradients in the different point patterns, albeit with different directions. Yet be aware that a gradient that is significant for a computer may look very different from the human perspective; many spatial patterns can be captured by a gradient to some extent. Baseline intensity corrections for every image or cell are included by default. The fixed and random effects modify the baseline intensity of the point pattern, not the gradient! Random effects can lead to problems with fitting and are dissuaded.

Value

For estGradients(), a list with the estimated gradients

For estGradientsSingle(), a list containing

`overall` Overall gradients

`cell` Gradients within the cell

For getPvaluesGradient(), a vector of p-values

Note

Fitting Poisson point processes is computation-intensive.

See Also

fitGradient

Examples

```
# Overall Gradients
data(Yang)
hypYang <- buildHyperFrame(Yang,
    coordVars = c("x", "y"),
    imageVars = c("day", "root", "section")
```

```
)
yangGrads <- estGradients(hypYang[seq_len(2), ],
    features = getFeatures(hypYang)[1],
    fixedEffects = "day", randomEffects = "root")
# Gradients within cell
data(Eng)
hypEng <- buildHyperFrame(Eng[Eng$fov %in% c(1,2),],
    coordVars = c("x", "y"),
    imageVars = c("fov", "experiment")
) #Subset for speed
hypEng <- addCell(hypEng, EngRois[rownames(hypEng)], verbose = FALSE)
# Limit number of cells and genes for computational reasons
engGrads <- estGradients(hypEng[seq_len(2),],
    features = feat <- getFeatures(hypEng)[1])
pVals <- getPvaluesGradient(engGrads, "cell")
```

`estPis` *Estimate probabilistic indices for single-molecule localization patterns, and add the variance weighting function.*

Description

Estimate different probabilistic indices for localization on all point patterns of a hyperframe, and integrate the results in the same hyperframe. estPisSingle() is the workhorse function for a single point pattern.

addWeightFunction() adds a weighting function based on the data to the object by modeling variance as a non-increasing spline as a function of the number of events.

Usage

```
estPis(
  hypFrame,
  pis = c("nn", "nnPair", "edge", "centroid", "nnCell", "nnPairCell"),
  verbose = TRUE,
  null = c("background", "CSR"),
  nPointsAll = switch(null, background = 20000, CSR = 1000),
  nPointsAllWithinCell = switch(null, background = 2000, CSR = 500),
  nPointsAllWin = 1000,
  minDiff = 20,
  minObsNN = 1L,
  features = getFeatures(hypFrame),
  ...
)

estPisSingle(
  p,
  pis,
  null,
  tabObs,
  owins = NULL,
  centroids = NULL,
  window = p$window,
```

```
  loopFun = "bplapply",
  features,
  nPointsAll,
  nPointsAllWithinCell,
  nPointsAllWin,
  minDiff,
  minObsNN
)

addWeightFunction(
  resList,
  pis = resList$pis,
  designVars,
  lowestLevelVar,
  maxObs = 1e+05,
  maxFeatures = 1000,
  minNumVar = 3,
  ...
)
```

Arguments

`hypFrame`	A hyperframe
`pis`	The PIs to be estimated or for which weighting functions is to be added
`verbose`	A boolean, whether to report on progress of the fitting process.
`null`	A character vector, indicating how the null distribution is defined. See details.
`nPointsAll`, `nPointsAllWithinCell`	How many points to subsample or simulate to calculate the overall nearest neighbour distance distribution under the null hypothesis. The second argument (nPointsAllWithinCell) applies to within cell calculations, where a lower number usually suffises.
`nPointsAllWin`	How many points to subsample or simulate to calculate distance to cell edge or centroid distribution
`minDiff`	An integer, the minimum number of events from other genes needed for calculation of background distribution of distances. Matters mainly for within-cell calculations: cells with too few events are skipped.
`minObsNN`	An integer, the minimum number of events required for a gene to be analysed. See details.
`features`	A character vector, for which features should the probabilistic indices be calculated?
`...`	Additional arguments passed on to the scam function, fitting the spline
`p`	The point pattern
`tabObs`	A table of observed gene frequencies
`owins`, `centroids`	The list of windows corresponding to cells, and their centroids
`window`	An window of class owin, in which events can occur
`loopFun`	The function to use to loop over the features. Defaults to bplapply except when looping over features within cells

`resList`	A results list, from a call to estPis().
`designVars`	A character vector containing all design factors (both fixed and random), that are also present as variables in hypFrame.
`lowestLevelVar`	The design variable at the lowest level of nesting, often separating technical replicates. The conditional variance is calculated within the groups of PIs defined by this variable.
`maxObs, maxFeatures`	The maximum number of observations respectively features for fitting the weighting function. See details.
`minNumVar`	The minimum number of observations needed to calculate a variance. Groups with fewer replicates are ignored.

Details

The null distribution used to calculate the PIs can be either 'background' or 'null'. For 'background', the observed distributions of all genes is used. Alternatively, for null = 'CSR', Monte-Carlo simulation under complete spatial randomness is performed within the given window to find the null distribution of the distance under study.

The 'nn' prefix indicates that nearest neighbour distances are being used, either univariately or bivariately. The suffix 'Pair' indicates that bivariate probabilistic indices, testing for co- and antilocalization are being used. 'edge' and 'centroid' calculate the distance to the edge respectively the centroid of the windows added using the addCell function. The suffix 'Cell' indicates that nearest neighbour distances are being calculated within cells only.

It can be useful to set the minObsNN higher than the default of 5 for calculations within cells when the number of events is low, not to waste computation time on gene (pairs) with very variable PI estimates.

Provide either 'designVars' or 'lowestLevelVar'. The 'designVars' are usually the same as the regressors in the linear model. In case 'lowestLevelVar' is provided, the design variables are set to all imageVars in the hypFrame object except lowestLevelVar. When the PI is calculated on the cell level ("nnCell" or "nnPairCell"), the cell is always the lowest nesting level, and inputs to 'designVars' or 'lowestLevelVar' will be ignored for these PIs. The registered parallel backend will be used for fitting the trends of the different PIs. For computational and memory reasons, for large datasets the trend fitting is restricted to a random subset of the data through the maxObs and maxFeatures parameters.

Value

For estPis(), the hyperframe with the estimated PIs present in it

For estPisSingle(), a list of data frames with estimated PIs per gene and/or gene pair:

`pointDists`	PIs for pointwise distances overall
`windowDists`	PIs for distances to cell wall or centroid
`withinCellDists`	PIs for pointwise distances within cell

For addWeightFunction(), the input object 'resList' with a slot 'Wfs' added containing the weighting functions.

See Also

buildDataFrame, estPis

Examples

```
data(Yang)
hypYang <- buildHyperFrame(Yang,
    coordVars = c("x", "y"),
    imageVars = c("day", "root", "section")
)
yangPims <- estPis(hypYang[c(seq_len(4), seq(27, 29)), ], pis = "nn",
    nPointsAll = 4e2)
# Univariate nearest neighbour distances
yangObj <- addWeightFunction(yangPims, designVars = c("day", "root"))
# Add the weight functions
yangObj2 <- addWeightFunction(yangPims, lowestLevelVar = "section",
pi = "nn")
# Alternative formulation with 'lowestLevelVar'
```

`evalWeightFunction` *Evaluate a variance weighting function*

Description

Evaluate the variance weighting function to return unnormalized weights

Usage

```
evalWeightFunction(wf, newdata)
```

Arguments

`wf`	The weighting function
`newdata`	A data frame with new data

Value

A vector of weights, so the inverse of predicted variances, unnormalized

See Also

predict.scam, addWeightFunction

Examples

```
data(Yang)
hypYang <- buildHyperFrame(Yang, coordVars = c("x", "y"),
    imageVars = c("day", "root", "section")
)
yangPims <- estPis(hypYang, pis = "nn", features = getFeatures(hypYang)[12:19], nPointsAll = 1e3)
# First Build the weighting function
yangObj <- addWeightFunction(yangPims, designVars = c("day", "root"))
evalWeightFunction(yangObj$Wfs$nn, newdata = data.frame("NP" = 2))
```

`extractResults` *Extract results from a list of fitted LMMs. For internal use mainly.*

Description

Extract results from a list of fitted LMMs. For internal use mainly.

Usage

```
extractResults(
  models,
  hypFrame,
  subSet = "piMod",
  fixedVars = NULL,
  method = "BH"
)
```

Arguments

`models`	The models
`hypFrame`	The original hyperframe
`subSet`	The name of the subset to be extracted, either PI or Moran's I
`fixedVars`	The fixed effects for which the effect is to be reported
`method`	Multiplicity correction method passed onto p.adjust

Value

A list of matrices, all containing estimate, standard error, p-value and ajdusted p-value

See Also

fitLMMs, p.adjust

`findEcdfsCell` *Construct empirical cumulative distribution functions (ecdfs) for distances within the cell*

Description

The distance distribution under the null hypothesis of complete spatial randomness (CSR) within the cell is the same for all genes. This function precalculates this distribution using Monte-Carlo simulation under CSR, and summarizes it in an ecdf object

Usage

```
findEcdfsCell(p, owins, nPointsAllWin, centroids, null, pis, loopFun)
```

Arguments

`p`	The point pattern
`owins, centroids`	The list of windows corresponding to cells, and their centroids
`nPointsAllWin`	How many points to subsample or simulate to calculate distance to cell edge or centroid distribution
`null`	A character vector, indicating how the null distribution is defined. See details of estPis.
`pis`	The PIs to be estimated or for which weighting functions is to be added
`loopFun`	The function to use to loop over the features. Defaults to bplapply except when looping over features within cells

Value

The list of ecdf functions

See Also

ecdf

---

findOverlap *Find overlap between list of windows*

Description

The function seeks overlap between the list of windows supplied, and throws an error when found or returns the id's when found.

Usage

```
findOverlap(owins, centroids = NULL, returnIds = FALSE, numCentroids = 30)
```

Arguments

`owins`	the list of windows
`centroids`	The centroids of the windows
`returnIds`	A boolean, should the indices of the overlap be returned? If FALSE an error is thrown at the first overlap
`numCentroids`	An integer, the number of cells with closest centroids to consider looking for overlap

Value

Throws an error when overlap found, otherwise returns invisible. When returnIds=TRUE, the indices of overlapping windows are returned.

Examples

```
library(spatstat.geom)
owins <- replicate(10, owin(
    xrange = runif(1) + c(0, 0.2),
    yrange = runif(1) + c(0, 0.1)
), simplify = FALSE)
idOverlap <- findOverlap(owins, returnIds = TRUE)
```

`fitGradient` *Test for presence of gradient in a hyperframe of point patterns*

Description

A Poisson process is fitted to the data assuming exponential relationship wit intensity of the interaction between x and y variables and image identifier. This is compared to a model without this interaction to test for the significance of the gradient.

Usage

```
fitGradient(
  hypFrame,
  fixedForm,
  randomForm,
  fixedFormSimple,
  returnModel = FALSE,
  silent,
  ...
)
```

Arguments

`hypFrame`	the hyperframe
`fixedForm`, `randomForm`, `fixedFormSimple`	Formulae for fixed effects, random effects and fixed effects without slopes respectively
`returnModel`	A boolean, should the entire model be returned? Otherwise the p-value and coefficient vector are returned
`silent`	A boolean, should error messages from spatstat.model::mppm be printed?
`...`	passed onto mppm

Value

A list contraining

`pVal`	The p-value for existence of gradients
`coef`	The model coefficients

or a mppm model when returnModel is true

See Also

estGradients

fitLMMs *Fit linear (mixed) models for all probabilistic indices (PIs) and all genes*

Description

The PI is used as outcome variable in a linear (mixed) model, with design variables as regressors. Separate models are fitted for every combination of gene and PI. fitLMMsSingle() is the workhorse function for a single point pattern,

getResults() extracts effect size estimates, standard errors and adjusted p-values for a certain parameter from a linear model.

Usage

```
fitLMMs(
  obj,
  pis = obj$pis,
  fixedVars = NULL,
  randomVars = NULL,
  verbose = TRUE,
  returnModels = FALSE,
  Formula = NULL,
  randomNested = TRUE,
  features = getFeatures(obj),
  moranFormula = NULL,
  addMoransI = FALSE,
  numNNs = 10,
  ...
)

fitLMMsSingle(
  obj,
  pi,
  fixedVars,
  randomVars,
  verbose,
  returnModels,
  Formula,
  randomNested,
  features,
  addMoransI,
  weightMats,
  moranFormula
)

getResults(obj, pi, parameter, moransI = FALSE)
```

Arguments

obj	The result object

`pis`	Optional, the pis required. Defaults to all pis in the object
`fixedVars`	Names of fixed effects
`randomVars`	Names of random variables
`verbose`	A boolean, should the formula be printed?
`returnModels`	a boolean: should the full models be returned? Otherwise only summary statistics are returned
`Formula`	A formula; if not supplied it will be constructed from the fixed and random variables
`randomNested`	A boolean, indicating if random effects are nested within point patterns. See details.
`features`	The features for which to fit linear mixed models. Defaults to all features in the object
`moranFormula`	Formula for Moran's I model fitting
`addMoransI`	A boolean, include Moran's I of the cell-wise PIs in the calculation
`numNNs`	An integer, the number of nearest neighbours in the weight matrix for the calculation of the Moran's I statistic
`...`	Passed onto fitLMMsSingle
`pi`	The desired PI
`weightMats`	A list of weight matrices for Moran's I
`parameter`	The desired parameter
`moransI`	A boolean, should results for the Moran's I be returned?

Details

Genes or gene pairs with insufficient observations will be silently omitted. When randomVars is provided as a vector, independent random intercepts are fitted for them by default. Providing them separated by '\' or ':' as in the lmer formulas is also allowed to reflect nesting structure, but the safest is to construct the formula yourself and pass it onto fitLMMs.

It is by default assumed that random effects are nested within the point patterns. This means for instance that cells with the same name but from different point patterns are assigned to different random effects. Set 'randomNested' to FALSE to override this behaviour.

The Moran's I statistic is used to test whether cell-wise PIs ("nnCell", "nnCellPair", "edge" and "centroid") are spatially autocorrelated across the images. The numeric value of the PI is assigned to the centroid location, and then Moran's I is calculated with a fixed number of numNNs nearest neighbours with equal weights.

Value

For fitLMMs(), a list of fitted objects

For fitLMMsSingle(), a list of test results, if requested also the linear models are returned

For getResults(), the matrix with results, with p-values in ascending order

`Estimate`	The estimated PI
`se`	The corresponding standard error
`pVal`	The p-value
`pAdj`	The Benjamini-Hochberg adjusted p-value

See Also

buildMoransIDataFrame, buildDataFrame

Examples

```
example(addWeightFunction, "smoppix")
lmmModels <- fitLMMs(yangObj, fixedVars = "day", randomVars = "root")
res <- getResults(lmmModels, "nn", "Intercept") #Extract the results
head(res)
```

---

fitPiModel *Fit a linear model for an individual gene and PI combination*

Description

Fit a linear model for an individual gene and PI combination

Usage

```
fitPiModel(Formula, dff, contrasts, Control, MM, Weight = NULL)
```

Arguments

Formula	A formula; if not supplied it will be constructed from the fixed and random variables
dff	The dataframe
contrasts	The contrasts to be used, see model.matrix
Control	Control parameters
MM	A boolean, should a mixed model be tried
Weight	A weight variable

Value

A fitted model

See Also

fitLMMsSingle

`getCoordsMat` *Extract coordinates from a point pattern or data frame*

Description

Extract coordinates from a point pattern or data frame

Usage

```
getCoordsMat(x)
```

Arguments

`x`	the point pattern, dataframe or matrix

Value

the matrix of coordinates

`getDesignVars` *Return all design variables, both at the level of the point pattern and the level of the event*

Description

Return all design variables, both at the level of the point pattern and the level of the event

Extract variables from point patterns

Extract variables from events (the marks)

Usage

```
getDesignVars(x)

getPPPvars(
  x,
  exclude = c("tabObs", "centroids", "owins", "ppp", "pimRes", "image", "inSeveralCells",
    "nuclei")
)

getEventVars(x, exclude = c("x", "y", "z"))
```

Arguments

`x`	The results list, output from estPis
`exclude`	variables to exclude

Details

getDesignVars() returns all design variables, getPPPvars returns design variables related to the different images and getEventVars returns design variables related to the individual events

Value

A vector of design variables

A vector of variables

A vector of variables

`getElement` *Extract en element from a matrix or vector*

Description

Extract en element from a matrix or vector

Usage

```
getElement(x, e)
```

Arguments

x	the matrix or vector
e	The column or element name

Value

The desired element

`getFeatures` *Extract all unique features from an object*

Description

Extract all unique features from an object

Usage

```
getFeatures(x)
```

Arguments

x	A hyperframe or a results list containing a hyperframe

Value

A vector of features

Examples

```
data(Yang)
hypYang <- buildHyperFrame(Yang,
    coordVars = c("x", "y"),
    imageVars = c("day", "root", "section")
)
head(getFeatures(hypYang))
```

getGp *Helper function to get gene pair from a vector or list*

Description

When provided with argument "geneA–geneB", looks for this gene pair as well as for "geneB–geneA" in the provided object.

Usage

```
getGp(x, gp, drop = TRUE, Collapse = "--")
```

Arguments

x	The object in which to look
gp	A character string describing the gene pair
drop	A boolean, should matrix attributes be dropped in [] subsetting
Collapse	The character separating the gene pair

Value

The element sought

Examples

```
mat <- t(cbind(
    "gene1--gene2" = c(1, 2),
    "gene1--gene3" = c(2, 3)
))
getGp(mat, "gene3--gene1")
```

getHypFrame *Extract the hyperframe*

Description

Extract the hyperframe

Usage

```
getHypFrame(x)
```

Arguments

x	The hyperframe, or list containing one

Value

the hyperframe

loadBalanceBplapply *Parallel processing with BiocParallel with load balancing*

Description

The vector to iterate over (iterator) is split into as many parts as there are cores available, such that each core gets an equal load and overhead is minimized. The registered backend is then used by default to multithread using bplapply.

Usage

```
loadBalanceBplapply(iterator, func, loopFun = "bplapply")
```

Arguments

iterator	The vector to iterate over
func	The function to apply to each element
loopFun	The looping function, can also be 'lapply' for serial processing

Value

A list with the same length as iterator

makeDesignVar *Make design variable by combining different design variables*

Description

Make design variable by combining different design variables

Usage

```
makeDesignVar(x, designVars, sep = "_")
```

Arguments

x	the design matrix
designVars	the design variables to be combined
sep	The string to separate the components

Value

a vector of design levels

makePairs — *An aux function to build gene pairs*

Description

An aux function to build gene pairs

Usage

```
makePairs(genes)
```

Arguments

genes	The genes to be combined

Value

A character vector of gene pairs

Examples

```
genes <- paste0("gene", seq_len(4))
makePairs(genes)
```

moransI — *Calculate the Moran's I test statistic for spatial autocorrelation*

Description

The Moran's I test statistic and its variance are calculated

Usage

```
moransI(x, W)
```

Arguments

x	A vector of outcomes
W	The matrix of weights, with dimensions equal to the lenght of x

Details

The implementation is inspired on the one from ape::Moran.I, but more bare-bones for a sparse weight matrix with certain properties as prepared by buildMoransIWeightMat, making it faster and using less memory.

Value

A vector of length 2: the Moran's I statistic and its variance

Note

Calculations are only correct for weight matrices as prepared by buildMoransIWeightMat!

See Also

Moran.I

`named.contr.sum` *A version of contr.sum that retains names, a bit controversial but also clearer*

Description

A version of contr.sum that retains names, a bit controversial but also clearer

Usage

```
named.contr.sum(x, ...)
```

Arguments

`x, ...`	passed on to contr.sum

Value

The matrix of contrasts

Note

After https://stackoverflow.com/questions/24515892/r-how-to-contrast-code-factors-and-retain-meaningful-labels-in-output-summary

---

`nestRandom` *Nest random effects within fixed variables, in case the names are the same*

Description

Nest random effects within fixed variables, in case the names are the same

Usage

```
nestRandom(df, randomVars, fixedVars)
```

Arguments

`df`	The dataframe
`randomVars`	The random variables
`fixedVars`	The fixed variables

Value

The dataframe with adapted randomVars

`plotCells` *Plot the n cells with highest abundance of a feature*

Description

After testing for within-cell patterns, it may be useful to look at the cells with the most events for certain genes. These are plotted here, but the spatial location of the cells in the point pattern is lost! The choice and ranking of cells is one of decreasing gene (pair) expression.

Usage

```
plotCells(
  obj,
  features = getFeatures(obj)[seq_len(3)],
  nCells = 100,
  Cex = 1.5,
  borderColVar = NULL,
  borderCols = rev(palette()),
  Mar = c(0.5, 0.1, 0.75, 0.1),
  warnPosition = TRUE,
  summaryFun = "min",
  plotNuclei = !is.null(getHypFrame(obj)$nuclei),
  nucCol = "lightblue",
  ...
)
```

Arguments

`obj`	A hyperframe, or an object containing one
`features`	The features to be plotted, a character vector
`nCells`	An integer, the number of cells to be plotted
`Cex`	The point expansion factor
`borderColVar`	The variable to colour borders of the cell
`borderCols`	Colour palette for the borders
`Mar`	the margins
`warnPosition`	A boolean, should a warning be printed on the image that cells are not in their original location?
`summaryFun`	A function to summarize the gene-cell table in case multiple genes are plotted, to determine which cells are plotted. Choose "min" for cells with the highest minimum, or "sum" for highest total expression of the combination of genes
`plotNuclei`	A boolean, should nuclei be added?
`nucCol`	A character string, the colour in which the nucleus' boundary is plotted
`...`	Additional arguments, currently ignored

Value

Plots cells with highest expression to the plotting window, returns invisible

Examples

```
example(addCell, "smoppix")
plotCells(hypFrame2, "gene1")
plotCells(hypFrame2, "gene1", borderColVar = "condition", nCells = 10)
```

---

plotExplore *Plot a hyperframe with chosen features highlighted*

Description

All points of the hyperframe are plotted in grey, with a subset of features highlighted in colour. A selection of point patterns is plotted that fit in the window, by default the first six. This function is meant for exploratory purposes as well as for visual confirmation of findings.

Usage

```
plotExplore(
  hypFrame,
  features = getFeatures(hypFrame)[seq_len(6)],
  ppps,
  numPps,
  maxPlot = 1e+05,
  Cex = 1,
  plotWindows = !is.null(hypFrame$owins),
  plotPoints = TRUE,
  plotNuclei = !is.null(hypFrame$nuclei),
  piEsts = NULL,
  Xlim = NULL,
  Ylim = NULL,
  Cex.main = 1.1,
  Mar = c(0.5, 0.1, 0.9, 0.1),
  titleVar = NULL,
  piColourCell = NULL,
  palCols = c("blue", "yellow"),
  nucCol = "lightblue",
  border = NULL,
  CexLegend = 1.4,
  CexLegendMain = 1.7,
  Nrow
)
```

Arguments

hypFrame	The hyperframe
features	A small number of features to be highlighted. Defaults to the first 5.
ppps	The rownames or indices of the point patterns to be plotted. Defaults to maximum 99.
numPps	The number of point patterns with highest expression to be shown. Ignored is pps is given.
maxPlot	The maximum number of events plotted per point pattern

`Cex, Cex.main`	Point and title expansion factors, repsectively
`plotWindows`	A boolean, should windows be plotted too?
`plotPoints`	A boolean, should the molecules be plotted as points?
`plotNuclei`	A boolean, should the nuclei be plotted?
`piEsts`	Set of PI estimates, returned by estPis
`Xlim, Ylim`	plotting limits
`Mar`	the margins
`titleVar`	Image variable to be added to the title
`piColourCell`	PI by which to colour the cell
`palCols`	Two extremes of the colour palette for colouring the cells
`nucCol`	The colour for the nucleus window
`border`	Passed on to plot.owin, and further to graphics::polygon
`CexLegend, CexLegendMain`	Expansion factor for the legend and its title respectively
`Nrow`	Number of rows of the facet plot. Will be calculated if missing.

Details

When cell-specific PIs are calculated ("nnCell’, "nnCellPair", "edge", "centroid"), the cells can be coloured by them to investigate their spatial distribution, for instance those discovered through Moran’s I statistic. The colour palette is taken from the output of palette(), so set that one to change the colour scheme.

Value

Plots a facet of point patterns to output

Note

palCols sets the pseudo-continuous scale to colour cells.

Examples

```
example(buildHyperFrame, "smoppix")
plotExplore(hypYang)
plotExplore(hypYang, titleVar = "day")
plotExplore(hypYang, features = c("SmRBRb", "SmTMO5b", "SmWER--SmAHK4f"))
```

`plotTopResults` *Plot the most significant findings for a certain PI*

Description

Extract the most significant features for a certain PI and direction of effect, and plot them using an appropriate function: either plotExplore or plotCells

Usage

```
plotTopResults(
  hypFrame,
  results,
  pi,
  effect = "Intercept",
  what = if (pi %in% c("nn", "nnCell")) {
     "aggregated"
 } else if (pi %in%
    c("nnPair", "nnPairCell")) {
     "colocalized"
 } else if (pi %in% c("edge",
    "centroid")) {
     "close"
 },
  sigLevel = 0.05,
  numFeats = 2,
  piThreshold = switch(effect, Intercept = 0.5, 0),
  effectParameter = NULL,
  ...
)
```

Arguments

`hypFrame`	The hyperframe with the data
`results`	The results frame
`pi`	A character string, specifying the probabilistic index
`effect`	The name of the effect
`what`	Which features should be detected? Can be abbreviated, see details.
`sigLevel`	The significance level
`numFeats`	The number of features to plot
`piThreshold`	The threshold for PI, a minimum effect size
`effectParameter`	A character string, indicating which parameter to look for when effect is provided
`...`	passed onto plotting functions plotCells or plotExplore

Details

The "what" argument indicates if features far from or close to cell wall or centroid should be shown for pi "edge" or "centroid", aggregated or regular features for "nn" and "nnCell" and colocalized or antilocalized features for "nnPair" and "nnPairCell". Partial matching is allowed. Defaults to small probabilistic indices: proximity, aggregation and colocalization. For fixed effects, provide the name of the parameter, in combination with what. For instance, what = "regular", effect = "Var1" and effectParameter = "level1" will return features more regular at level1 of the variable than at baseline.

Value

A plot from plotCells or plotExplore, throws an error when no features meet the criteria

See Also

plotCells,plotExplore,fitLMMs

Examples

```
example(fitLMMs, "smoppix")
plotTopResults(hypYang, lmmModels, "nn")
#For the sake of illustration, set high significance level, as example dataset is small
plotTopResults(hypYang, lmmModels, "nn",
    effect = "day", what = "reg",
    effectParameter = "day0", sigLevel = 1-1e-10)
```

---

plotWf — *Plot the variance weighting function*

Description

The observation weights are plotted as a function of number of events. For a univariate PI, this is a line plot, for a bivariate PI this is a scatterplot of majority gene as a function of minority gene, with the weight represented as a colour scale. The minority respectively majority gene are the genes in the gene pair with least and most events

Usage

```
plotWf(obj, pi = obj$pis[1])
```

Arguments

obj	The result of a call to addWeightFunction
pi	The PI for which to plot the weighting function

Value

For univariate PI, returns a line plot; for bivariate PI a ggplot object

Examples

```
example(addWeightFunction, "smoppix")
plotWf(yangObj, "nn")
```

smoppix	*smoppix: Analyze Single Molecule Spatial Omics Data Using the Probabilistic Index*

Description

Test for univariate and bivariate spatial patterns in spatial omics data with single-molecule resolution. The tests implemented allow for analysis of nested designs and are automatically calibrated to different biological specimens. Tests for aggregation, colocalization, gradients and vicinity to cell edge or centroid are provided.

Author(s)

Maintainer: Stijn Hawinkel `<stijn.hawinkel@psb.ugent.be>` (ORCID)

See Also

Useful links:

- `https://github.com/sthawinke/smoppix`
- Report bugs at `https://github.com/sthawinke/smoppix/issues`

splitWindow	*Split a number of plots into rows and columns*

Description

Split a number of plots into rows and columns

Usage

```
splitWindow(x)
```

Arguments

x	The number of plots

Value

A vector of length 2 with required number of rows and columns

subSampleP *Subsample a point pattern when it is too large*

Description

Subsample a point pattern when it is too large

Usage

```
subSampleP(p, nSims, returnId = FALSE)
```

Arguments

p	The point pattern
nSims	The maximum size
returnId	A boolean, should the id of the sampled elements be returned?

Value

A point pattern, subsampled if necessary

sund *Helper function to spit gene pairs*

Description

Helper function to spit gene pairs

Usage

```
sund(x, sep = "--")
```

Arguments

x	character string
sep	The character used to split

Value

The split string

Examples

```
GenePair <- "gene1--gene2"
sund(GenePair)
```

`writeToXlsx` *Write effect sizes and p-values results to excel worksheet*

Description

The results of the linear models are written to an excel spreasdsheet with different tabs for every sign (PI smaller than or larger than 0.5) of every PI, sorted by increasing p-value.

Usage

```
writeToXlsx(obj, file, overwrite = FALSE, digits = 3, sigLevel = 0.05)
```

Arguments

`obj`	The results of linear model fitting
`file`	The file to write the results to
`overwrite`	A boolean, should the file be overwritten if it exists already?
`digits`	An integer, the number of significant digits to retain for the PI, raw and adjusted p-values
`sigLevel`	The significance level threshold to use for the adjusted p-values, only features exceeding the threshold are written to the file. Set this parameter to 1 to write all features

Details

If no feature exceeds the significance threshold for a certain pi and parameter combination, an empty tab is created. For fixed effects, a single tab is written for PI differences of any sign. The "baseline" tabs indicate the overall patterns, the other tabs are named after the fixed effects and indicate departure from this baseline depending on this fixed effect

Value

Returns invisible with a message when writing operation successful, otherwise throws an error.

See Also

createWorkbook,writeData, addWorksheet, saveWorkbook

Examples

```
example(fitLMMs, "smoppix")
writeToXlsx(lmmModels, "tmpFile.xlsx")
file.remove("tmpFile.xlsx")
```

Yang *Spatial transcriptomics data of Selaginella moellendorffii roots*

Description

Single-molecule spatial transcriptomics smFISH data of Selaginella moellendorffii roots of a replicated experiment by (Yang et al. 2023). Molecule locations, gene identity and design variables are included. Only a subset of the data, consisting of roots 1-3 and sections 1-5 is included in the package for computational and memory reasons. The data are in table format to illustrate conversion to hyperframe using buildHyperFrame.

Usage

```
data(Yang)
```

Format

A data matrix

x,y Molecule coordinates

gene Character vector with gene identities

root,section,day Design variables

Source

doi:10.1016/j.cub.2023.08.030

References

Yang X, Poelmans W, Grones C, Lakehal A, Pevernagie J, Bel MV, Njo M, Xu L, Nelissen H, Rybel BD, Motte H, Beeckman T (2023). "Spatial transcriptomics of a lycophyte root sheds light on root evolution." *Curr. Biol.*, **33**(19), 4069 - 4084. ISSN 0960-9822, doi:10.1016/j.cub.2023.08.030.

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