Package: spatialFDA (via r-universe)

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Title A Tool for Spatial Multi-sample Comparisons

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Description spatialFDA is a package to calculate spatial statistics metrics. The package takes a SpatialExperiment object and calculates spatial statistics metrics using the package spatstat. Then it compares the resulting functions across samples/conditions using functional additive models as implemented in the package refund. Furthermore, it provides exploratory visualisations using functional principal component analysis, as well implemented in refund.

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`.dfToppp` *Convert SpatialExperiment object to ppp object*

Description

Convert SpatialExperiment object to ppp object

Usage

```
.dfToppp(df, marks = NULL, continuous = FALSE, window = NULL)
```

Arguments

df	A dataframe with the x and y coordinates from the corresponding SpatialExperiment and the ColData
marks	A vector of marks to be associated with the points, has to be either named 'cell_type' if you want to compare discrete celltypes or else continous gene expression measurements are assumed as marks.
continuous	A boolean indicating whether the marks are continuous defaults to FALSE
window	An observation window of the point pattern of class owin.

Value

A ppp object for use with spatstat functions

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
speSub <- subset(spe, , image_number == "138")
dfSub <- .speToDf(speSub)
pp <- .dfToppp(dfSub, marks = "cell_type")
```

.extractMetric *Compute a spatial metric on a SpatialExperiment object*

Description

A function that takes a SpatialExperiment object and computes a spatial statistics function as implemented in spatstat. The output is a spatstat object.

Usage

```
.extractMetric(
  df,
  selection,
  fun,
  marks = NULL,
  rSeq = NULL,
  by = NULL,
  continuous = FALSE,
  window = NULL,
  ...
)
```

Arguments

df	A dataframe with the x and y coordinates from the corresponding SpatialExperiment and the colData
selection	the mark(s) you want to compare
fun	the spatstat function to compute on the point pattern object
marks	the marks to consider e.g. cell types
rSeq	the range of r values to compute the function over
by	the spe colData variable(s) to add to the meta data
continuous	A boolean indicating whether the marks are continuous defaults to FALSE
window	a observation window for the point pattern of class owin.
...	Other parameters passed to spatstat.explore functions

Value

a spatstat metric object with the fov number, the number of points and the centroid of the image

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
speSub <- subset(spe, , image_number == "138")
dfSub <- .speToDf(speSub)
metricRes <- .extractMetric(dfSub, c("alpha", "Tc"),
    fun = "Gcross",
    marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
    by = c("patient_stage", "patient_id", "image_number")
)
```

.loadExample *load Example dataset from Damond et al. (2019)*

Description

load Example dataset from Damond et al. (2019)

Usage

```
.loadExample(full = FALSE)
```

Arguments

full	a boolean indicating whether to load the entire Damond et al. (2019) or only a subset

Value

A SpatialExperiment object as uploaded to `ExperimentHub()`

Examples

```
# retrieve the Damond et al. (2019) dataset
spe <- .loadExample()
```

`.speToDf` *Transform a SpatialExperiment into a dataframe*

Description

Transform a SpatialExperiment into a dataframe

Usage

```
.speToDf(spe)
```

Arguments

spe	A SpatialExperiment object subset to a single image

Value

A dataframe with the x and y coordinates from the corresponding SpatialExperiment and the colData

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
speSub <- subset(spe, , image_number == "138")
dfSub <- .speToDf(speSub)
```

`calcCrossMetricPerFov` *Calculate cross spatial metrics for all combinations per FOV*

Description

A function that takes a `SpatialExperiment` object as input and calculates a cross spatial metric as implemented by `spatstat` per field of view for all combinations provided by the user.

Usage

```
calcCrossMetricPerFov(
  spe,
  selection,
  subsetby = NULL,
  fun,
  marks = NULL,
  rSeq = NULL,
  by = NULL,
  ncores = 1,
  continuous = FALSE,
  assay = "exprs",
  ...
)
```

Arguments

`spe`	a `SpatialExperiment` object
`selection`	the mark(s) you want to compare
`subsetby`	the spe `colData` variable to subset the data by
`fun`	the `spatstat` function to compute on the point pattern object
`marks`	the marks to consider e.g. cell types
`rSeq`	the range of r values to compute the function over
`by`	the spe `colData` variable(s) to add to the meta data
`ncores`	the number of cores to use for parallel processing, default = 1
`continuous`	A boolean indicating whether the marks are continuous defaults to FALSE
`assay`	the assay which is used if `continuous = TRUE`
`...`	Other parameters passed to spatstat.explore functions

Value

a dataframe of the `spatstat` metric objects with the radius r, the theoretical value of a Poisson process, the different border corrections the fov number, the number of points and the centroid of the image

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcCrossMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)
```

`calcMetricPerFov` *Calculate a spatial metric on a* `SpatialExperiment` *object per field of view*

Description

A function that takes a `SpatialExperiment` object as input and calculates a spatial metric as implemented by `spatstat` per field of view.

Usage

```
calcMetricPerFov(
  spe,
  selection,
  subsetby,
  fun,
  marks = NULL,
  rSeq = NULL,
  by = NULL,
  continuous = FALSE,
  assay = "exprs",
  ncores = 1,
  verbose = TRUE,
  ...
)
```

Arguments

`spe`	a `SpatialExperiment` object
`selection`	the mark(s) you want to compare. NOTE: This is directional. c(A,B) is not the same result as c(B,A).
`subsetby`	the spe `colData` variable to subset the data by. This variable has to be provided, even if there is only one sample.
`fun`	the `spatstat` function to compute on the point pattern object

marks	the marks to consider e.g. cell types
rSeq	the range of r values to compute the function over
by	the spe colData variable(s) to add to the meta data
continuous	A boolean indicating whether the marks are continuous defaults to FALSE
assay	the assay which is used if continuous = TRUE
ncores	the number of cores to use for parallel processing, default = 1
verbose	logical indicating whether to print all information or not
...	Other parameters passed to spatstat.explore functions

Value

a dataframe of the spatstat metric objects with the radius r, the theoretical value of a Poisson process, the different border corrections the fov number, the number of points and the centroid of the image

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)
```

---

coef.functionalGam *Coef for functionalGam object*

Description

Coef for functionalGam object

Usage

```
## S3 method for class 'functionalGam'
coef(object, ...)
```

Arguments

object	a fitted functionalGam-object
...	see coef.pffr() for options.

Value

coefficicents of the model, see `coef.pffr()`

Author(s)

Martin Emons, adapted from `coef.pffr()` by Fabian Scheipl

---

crossSpatialInference *Function for Cross Spatial Inference*

Description

This function is a wrappere function around `spatialInference`. It calculates `spatialInference` results either for all cell types in `marks` (if `selection == NULL`) or for a custom subset defined in `selection`.

Usage

```
crossSpatialInference(
  spe,
  selection = NULL,
  fun,
  marks = NULL,
  rSeq = NULL,
  correction,
  sample_id,
  image_id,
  condition,
  continuous = FALSE,
  assay = "exprs",
  transformation = NULL,
  eps = 0.001,
  delta = "minNnDist",
  family = stats::gaussian(link = "log"),
  verbose = TRUE,
  ncores = 1,
  ...
)
```

Arguments

`spe`	a `SpatialExperiment` object
`selection`	the mark(s) you want to compare. NOTE: This is directional. c(A,B) is not the same result as c(B,A).
`fun`	the `spatstat` function to compute on the point pattern object
`marks`	the marks to consider e.g. cell types

rSeq	the range of r values to compute the function over
correction	the edge correction to be applied
sample_id	the spe colData variable to mark the sample, if not NULL this will result in a mixed model estimation
image_id	the spe colData variable to mark the image
condition	the spe colData variable to mark the condition
continuous	A boolean indicating whether the marks are continuous defaults to FALSE
assay	the assay which is used if continuous = TRUE
transformation	the transformation to be applied as exponential e.g. 1/2 for sqrt
eps	some distributional families fail if the response is zero, therefore, zeros can be replaced with a very small value eps
delta	the delta value to remove from the beginning of the spatial statistics functions. Can be reasonable if e.g. cells are always spaced by 10 µm. If set to "minNnDist" it will take the mean of the minimum nearest neighbour distance across all images for this cell type pair.
family	the distributional family for the functional GAM
verbose	logical indicating whether to print all information or not
ncores	the number of cores to use for parallel processing, default = 1
...	Other parameters passed to spatstat.explore functions for parameters concerning the spatial function calculation and to refund::pffr for the functional additive mixed model inference

Value

a list of objects created by the function spatialInference with three objects: i) the dataframe with the spatial statistics results, ii) the designmatrix of the inference and iii) the fitted pffr object

Examples

```
spe <- .loadExample()
#make the condition a factor variable
colData(spe)[["patient_stage"]] <- factor(colData(spe)[["patient_stage"]])
#relevel to have non-diabetic as the reference category
colData(spe)[["patient_stage"]] <- relevel(colData(spe)[["patient_stage"]],
"Non-diabetic")

selection <- c("acinar", "ductal")
resLs <- crossSpatialInference(spe, selection, fun = "Gcross",
                     marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
                     correction = "rs", sample_id = "patient_id",
                     image_id = "image_number", condition = "patient_stage",
                     algorithm = "bam",
                     ncores = 1
                   )
```

`extractCrossInferenceData` *Reshaping the Result of a Cross Spatial Inference to a Dataframe*

Description

Reshaping the Result of a Cross Spatial Inference to a Dataframe

Usage

```
extractCrossInferenceData(
  resLs,
  QCMetric = "medianMinIntensity",
  QCThreshold = 0.1
)
```

Arguments

`resLs`	a list with four objects: i) the dataframe with the spatial statistics results transformed and filtered as used for fitting, ii) the raw spatial statistics results, iii) the designmatrix of the inference and iv) the fitted pffr object v) the residual standard error per condition defined as the residual sum of squares divided by the number of datapoints - sum of the estimated degrees of freedom for the model parameters as well as other QC metrics
`QCMetric`	the metric to relate the quality of the fit too.
`QCThreshold`	the threshold on the QC metric. Depends on the function used.

Value

a dataframe for plotting with ggplot2

Examples

```
spe <- .loadExample()
#make the condition a factor variable
colData(spe)[["patient_stage"]] <- factor(colData(spe)[["patient_stage"]])
#relevel to have non-diabetic as the reference category
colData(spe)[["patient_stage"]] <- relevel(colData(spe)[["patient_stage"]],
"Non-diabetic")

selection <- c("acinar", "ductal")
resLs <- crossSpatialInference(spe, selection,
                      fun = "Gcross", marks = "cell_type",
                      rSeq = seq(0, 50, length.out = 50), correction = "rs",
                      sample_id = "patient_id",
                      image_id = "image_number", condition = "patient_stage",
                      algorithm = "bam",
                      ncores = 1
```

```
                  )
df <- extractCrossInferenceData(resLs)
```

`functionalGam` *General additive model with functional response*

Description

A function that takes the output of a metric calculation as done by `calcMetricPerFov`. The data has to be prepared into the correct format for the functional analysis by the `prepData` function. The output is a `pffr` object as implemented by `refund`.

Usage

```
functionalGam(
  data,
  x,
  designmat,
  weights,
  formula,
  family = stats::gaussian(link = "log"),
  algorithm = "bam",
  bs.yindex = list(bs = "ps", k = 5, m = c(2, 1)),
  bs.int = list(bs = "ps", k = 20, m = c(2, 1)),
  sandwich = "cluster",
  discrete = TRUE,
  ...
)
```

Arguments

`data`	a dataframe with the following columns: Y = functional response; sample_id = sample ID; image_id = image ID;
`x`	the x-axis values of the functional response
`designmat`	a design matrix as defined by model.matrix()
`weights`	weights as the number of points per image. These weights are normalised by the mean of the weights in the fitting process
`formula`	the formula for the model. The colnames of the designmatrix have to correspond to the variables in the formula.
`family`	the distributional family as implemented in `family.mgcv`. For fast computation the default is set to `gaussian` with a log link. other interesting options can be `betar` and `scat` • for more information see `family.mgcv`.
`algorithm`	algorithm to fit the refund::pffr method. defaults to `bam`

bs.yindex	a list specifying the spline bases for the index. See refund::pffr for more details
bs.int	a list specfying the spline bases for the global function intercept. See refund::pffr for more details
sandwich	string indicating how and if to adjust for heterscedasticity of the residuals with a sandwich correction
discrete	option to discretise the function for faster computation. default is TRUE. See mgcv::bam for more information. When using gam, this option has to be FALSE
...	Other parameters passed to pffr

Value

a fitted pffr object which inherits from gam

Examples

```
# load the pancreas dataset
library("tidyr")
library("dplyr")
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
# calculate the Gcross metric for alpha and Tc cells
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross",
    marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
    c("patient_stage", "patient_id", "image_number"), ncores = 1
)
metricRes$ID <- paste0(
  metricRes$patient_stage, "|", metricRes$patient_id,
  "|", metricRes$image_number
)
dat <- prepData(metricRes, "r", "rs", sample_id = "patient_id",
    image_id = "image_number", condition = "patient_stage")

#' # drop rows with NA
dat <- dat |> drop_na()

# create a designmatrix
condition <- dat$patient_stage
# relevel the condition - can set explicit contrasts here
condition <- relevel(condition, "Non-diabetic")
designmat <- model.matrix(~condition)
# colnames don't work with the '-' sign
colnames(designmat) <- c(
    "(Intercept)", "conditionLong_duration",
    "conditionOnset"
)
# fit the model
mdl <- functionalGam(
    data = dat, x = metricRes$r |> unique(),
    designmat = designmat, weights = dat$npoints,
```

```
    formula = formula(Y ~ conditionLong_duration +
        conditionOnset + s(patient_id, bs = "re")),
        algorithm = "bam"
)
summary(mdl)
```

`functionalPCA` *Functional Principal Component Analysis*

Description

A function that takes as input the output of `calcMetricPerFov` which has to be converted into the correct format by `prepData`. The output is a list with the `fpca.face` output from refund.

Usage

```
functionalPCA(data, r, ...)
```

Arguments

`data`	a data object for functional data analysis containing at least the functional response Y.
`r`	the functional domain
`...`	Other parameters passed to `fpca.sc` functions

Value

a list with components of fpca.sc

Examples

```
# load the pancreas dataset
library("tidyr")
library("stringr")
library("dplyr")
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
# calculate the Gcross metric for alpha and Tc cells
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross",
    marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
    c("patient_stage", "patient_id", "image_number"), ncores = 1
)
metricRes$ID <- paste0(
  metricRes$patient_stage, "|", metricRes$patient_id,
  "|", metricRes$image_number
)
# prepare data for FDA
dat <- prepData(metricRes, "r", "rs")
```

```
# drop rows with NA
dat <- dat |> drop_na()
# create meta info of the IDs
splitData <- str_split(dat$ID, "x")
dat$condition <- factor(sapply(splitData, function(x) x[1]))
dat$patient_id <- factor(sapply(splitData, function(x) x[2]))
dat$image_id <- factor(sapply(splitData, function(x) x[3]))
# calculate fPCA
mdl <- functionalPCA(
    data = dat, r = metricRes$r |> unique()
)
```

`plotCrossFOV` *Creates a nXn plot of the cross metrics per sample*

Description

Helper function for `plotCrossMetricPerFov`. It applies `plotMetricPerFov` to all `n` marks defined in the variable `selection`. This gives an `nxn` plot of all marks.

Usage

```
plotCrossFOV(
  subFov,
  theo,
  correction,
  x,
  imageId,
  ID = NULL,
  ncol = NULL,
  nrow = NULL,
  legend.position = "none",
  ...
)
```

Arguments

`subFov`	a subset of the `dataframe` to the respective fov
`theo`	logical; if the theoretical line should be plotted
`correction`	the border correction to plot
`x`	the x-axis variable to plot
`imageId`	the ID of the image/fov
`ID`	the (optional) ID for plotting combinations
`ncol`	the number of columns for the facet wrap
`nrow`	the number of rows for the facet wrap

legend.position
: the position of the legend of the plot

... : Other parameters passed to ggplot2 functions

Value

a ggplot object

---

plotCrossHeatmap *Plotting the Result of a Cross Spatial Inference*

Description

Plotting the Result of a Cross Spatial Inference

Usage

```
plotCrossHeatmap(
  resLs,
  adj.pvalue = "BH",
  coefficientsToPlot = NULL,
  QCThreshold = 1e-05,
  QCMetric = "medianMinIntensity",
  ...
)
```

Arguments

resLs
: a list with four objects: i) the dataframe with the spatial statistics results transformed and filtered as used for fitting, ii) the raw spatial statistics results, iii) the designmatrix of the inference and iv) the fitted pffr object v) the residual standard error per condition defined as the residual sum of squares divided by the number of datapoints - sum of the estimated degrees of freedom for the model parameters as well as other QC metrics

adj.pvalue
: a pvalue adjustment method as passed to stats::p.adjust defaults to Benjamini-Hochberg correction of the false discovery rate.

coefficientsToPlot
: list of which coefficients to plot in the heatmap defaults to NULL in which case all coefficients are plotted

QCThreshold
: the threshold on the Quality control metric. Depends on the function used.

QCMetric
: the metric to relate the quality of the fit too.

... : other parameters passed to ggplot2 functions

Value

a ggplot2 object

Examples

```
spe <- .loadExample()
#make the condition a factor variable
colData(spe)[["patient_stage"]] <- factor(colData(spe)[["patient_stage"]])
#relevel to have non-diabetic as the reference category
colData(spe)[["patient_stage"]] <- relevel(colData(spe)[["patient_stage"]],
"Non-diabetic")

selection <- c("acinar", "ductal")
resLs <- crossSpatialInference(spe, selection,
                      fun = "Gcross", marks = "cell_type",
                      rSeq = seq(0, 50, length.out = 50), correction = "rs",
                      sample_id = "patient_id",
                      image_id = "image_number", condition = "patient_stage",
                      algorithm = "bam",
                      ncores = 1
                    )
p <- plotCrossHeatmap(resLs, adj.pvalue = "BH")
```

`plotCrossMetricPerFov` *Plot a cross type spatial metric per field of view*

Description

This function plots the cross function between two marks output from `calcMetricPerFov`. It wraps around helper function and applies this function to all samples.

Usage

```
plotCrossMetricPerFov(
  metricDf,
  theo = NULL,
  correction = NULL,
  x = NULL,
  imageId = NULL,
  ID = NULL,
  nrow = NULL,
  ncol = NULL,
  legend.position = "none",
  ...
)
```

Arguments

`metricDf`	the metric dataframe as calculated by `calcMetricPerFov`
`theo`	logical; if the theoretical line should be plotted
`correction`	the border correction to plot

x	the x-axis variable to plot
imageId	the ID of the image/fov
ID	the (optional) ID for plotting combinations
nrow	the number of rows for the facet wrap
ncol	the number of columns for the facet wrap
legend.position	the position of the legend of the plot
...	Other parameters passed to ggplot2 functions

Value

a ggplot object

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcCrossMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)

metricRes$ID <- paste0(
 metricRes$patient_stage, "|", metricRes$patient_id
)

metricRes <- subset(metricRes, image_number %in% c(138, 139, 140))
p <- plotCrossMetricPerFov(metricRes,
    theo = TRUE, correction = "rs",
    x = "r", imageId = "image_number", ID = "ID"
)
print(p)
```

---

plotFbPlot *Functional boxplot of spatstat curves*

Description

This function creates a functional boxplot of the spatial statistics curves. It creates one functional boxplot per aggregation category, e.g. condition.

Usage

```
plotFbPlot(metricDf, x, y, aggregateBy)
```

Arguments

metricDf	the metric dataframe as calculated by calcMetricPerFov
x	the name of the x-axis of the spatial metric
y	the name of the y-axis of the spatial metric
aggregateBy	the criterion by which to aggregate the curves into a functional boxplot. Can be e.g. the condition of the different samples.

Value

a list of base R plots

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)
# create a unique ID for the data preparation
metricRes$ID <- paste0(
  metricRes$patient_stage, "|", metricRes$patient_id,
  "|", metricRes$image_number
)

plotFbPlot(metricRes, 'r', 'rs', 'patient_stage')
```

---

plotFpca *Plot a biplot from an fPCA analysis*

Description

A function that takes the output from the functionalPCA function and returns a ggplot object of the first two dimensions of the PCA as biplot.

Usage

```
plotFpca(data, res, colourby = NULL, labelby = NULL)
```

Arguments

data	a data object for functional data analysis containing at least the functional response Y.
res	the output from the fPCA calculation
colourby	the variable by which to colour the PCA plot by
labelby	the variable by which to label the PCA plot by

Value

a list with components of fpca.face

Examples

```
# load the pancreas dataset
library("tidyr")
library("stringr")
library("dplyr")
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
# calculate the Gcross metric for alpha and beta cells
metricRes <- calcMetricPerFov(spe, c("alpha", "beta"),
    subsetby = "image_number", fun = "Gcross",
    marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
    c("patient_stage", "patient_id", "image_number"), ncores = 1
)
metricRes$ID <- paste0(
  metricRes$patient_stage, "|", metricRes$patient_id,
  "|", metricRes$image_number
)

# prepare data for FDA
dat <- prepData(metricRes, "r", "rs")

# drop rows with NA
dat <- dat |> drop_na()
# create meta info of the IDs
splitData <- str_split(dat$ID, "|")
dat$condition <- factor(sapply(splitData, function(x) x[1]))
dat$patient_id <- factor(sapply(splitData, function(x) x[2]))
dat$image_id <- factor(sapply(splitData, function(x) x[3]))
# calculate fPCA
mdl <- functionalPCA(
    data = dat, r = metricRes$r |> unique()
)
p <- plotFpca(
    data = dat, res = mdl, colourby = "condition",
    labelby = "patient_id"
)
print(p)
```

plotMdl *Plot a pffr model object*

Description

A function that takes a pffr object as calculated in `functionalGam` and plots the functional coefficients. The functions are centered such that their expected value is zero. Therefore, the scalar intercept has to be added to the output with the argument `shift` in order to plot the coefficients in their original range.

Usage

```
plotMdl(mdl, predictor, shift = NULL)
```

Arguments

`mdl`	a `pffr` model object
`predictor`	predictor to plot
`shift`	the value by which to shift the centered functional intercept. this will most often be the constant intercept

Value

ggplot object of the functional estimate

Examples

```
library("tidyr")
library("stringr")
library("dplyr")
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)
# create a unique ID for each row
metricRes$ID <- paste0(
    metricRes$patient_stage, "x", metricRes$patient_id,
    "x", metricRes$image_number
)

dat <- prepData(metricRes, "r", "rs", sample_id = "patient_id",
    image_id = "image_number", condition = "patient_stage")
```

```
#' # drop rows with NA
dat <- dat |> drop_na()

# create a designmatrix
condition <- dat$patient_stage
# relevel the condition - can set explicit contrasts here
condition <- relevel(condition, "Non-diabetic")
designmat <- model.matrix(~condition)
# colnames don't work with the '-' sign
colnames(designmat) <- c(
    "(Intercept)", "conditionLong_duration",
    "conditionOnset"
)
# fit the model
mdl <- functionalGam(
    data = dat, x = metricRes$r |> unique(),
    designmat = designmat, weights = dat$npoints,
    formula = formula(Y ~ conditionLong_duration +
        conditionOnset + s(patient_id, bs = "re")),
        algorithm = "bam"
)
summary(mdl, re.test = FALSE)
plotLs <- lapply(colnames(designmat), plotMdl,
    mdl = mdl,
    shift = mdl$coefficients[["(Intercept)"]]
)
```

---

plotMetricPerFov *Plot a spatial metric per field of view*

Description

A function that plots the output of the function `calcMetricPerFov`. The plot contains one curve per FOV and makes subplots by samples.

Usage

```
plotMetricPerFov(
  metricDf,
  theo = FALSE,
  correction = NULL,
  x = NULL,
  imageId = NULL,
  ID = NULL,
  nrow = NULL,
  ncol = NULL,
  legend.position = "none",
  ...
)
```

Arguments

metricDf	the metric dataframe as calculated by calcMetricPerFov
theo	logical; if the theoretical line should be plotted
correction	the border correction to plot
x	the x-axis variable to plot
imageId	the ID of the image/fov
ID	the (optional) ID for plotting combinations
nrow	the number of rows for the facet wrap
ncol	the number of columns for the facet wrap
legend.position	the position of the legend of the plot
...	Other parameters passed to ggplot2 functions

Value

a ggplot object

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)
# ceate a unique plotting ID
metricRes$ID <- paste0(
 metricRes$patient_stage, "|", metricRes$patient_id
)

p <- plotMetricPerFov(metricRes,
    correction = "rs", x = "r",
    imageId = "image_number", ID = "ID"
)
print(p)
```

prepData *Prepare data from calcMetricRes to be in the right format for FDA*

Description

Prepare data from calcMetricRes to be in the right format for FDA

Usage

```
prepData(metricRes, x, y, sample_id = NULL, image_id = NULL, condition = NULL)
```

Arguments

metricRes	a dataframe as calculated by calcMetricRes - requires the columns ID (unique identifier of each row)
x	the name of the x-axis of the spatial metric
y	the name of the y-axis of the spatial metric
sample_id	the spe colData variable to mark the sample
image_id	the spe colData variable to mark the image
condition	the spe colData variable to mark the condition

Value

returns a list with three entries, the unique ID, the functional response Y and the weights

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
metricRes <- calcMetricPerFov(spe, c("alpha", "Tc"),
    subsetby = "image_number", fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), by = c(
        "patient_stage", "patient_id",
        "image_number"
    ),
    ncores = 1
)

# create a unique ID for each row
metricRes$ID <- paste0(
    metricRes$patient_stage, "|", metricRes$patient_id,
    "|", metricRes$image_number
)
dat <- prepData(metricRes, "r", "rs", sample_id = "patient_id",
image_id = "image_number", condition = "patient_stage")
```

print.fpca *print the fPCA results*

Description

this is a function that prints a summary of the fPCA result of class fpca

Usage

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

Arguments

x	the result of function functionalPCA
...	other parameters passed to base generic function print

Value

a formatted overview of the fPCA result

Examples

```
# load the pancreas dataset
library("tidyr")
library("stringr")
library("dplyr")
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
# calculate the Gcross metric for alpha and beta cells
metricRes <- calcMetricPerFov(spe, c("alpha", "beta"),
    subsetby = "image_number", fun = "Gcross",
    marks = "cell_type", rSeq = seq(0, 50, length.out = 50),
    c("patient_stage", "patient_id", "image_number"), ncores = 1
)
metricRes$ID <- paste0(
  metricRes$patient_stage, "|", metricRes$patient_id,
  "|", metricRes$image_number
)
# prepare data for FDA
dat <- prepData(metricRes, "r", "rs")

# drop rows with NA
dat <- dat |> drop_na()

# create meta info of the IDs
splitData <- strsplit(dat$ID, "|", fixed = TRUE)
dat$condition <- factor(sapply(splitData, function(x) x[1]))
dat$patient_id <- factor(sapply(splitData, function(x) x[2]))
```

```
dat$image_id <- factor(sapply(splitData, function(x) x[3]))
# calculate fPCA
mdl <- functionalPCA(
    data = dat, r = metricRes$r |> unique()
)
mdl
```

`rMaxHeuristic` *Heuristic for the choice of rMax*

Description

Heuristic for the choice of rMax

Usage

```
rMaxHeuristic(spe, subsetby, marks)
```

Arguments

`spe`	a `SpatialExperiment` object
`subsetby`	the spe `colData` variable to subset the data by. This variable has to be provided, even if there is only one sample.
`marks`	the marks to consider e.g. cell types

Value

a ggplot histogram of the bounding radius of all the

Examples

```
# retrieve example data from Damond et al. (2019)
spe <- .loadExample()
p <- rMaxHeuristic(spe,
subsetby = "image_number", marks = "cell_type"
)
```

spatialInference *Statistical Inference on Spatial Statistics Functions*

Description

A function to perform spatial statistical inference on spatial omics data. This function works so far only on functions of radius "r".

Usage

```
spatialInference(
  spe,
  selection,
  fun,
  marks = NULL,
  rSeq = NULL,
  correction,
  sample_id,
  image_id,
  condition,
  continuous = FALSE,
  assay = "exprs",
  transformation = NULL,
  weights = "total",
  eps = 0.001,
  delta = "minNnDist",
  family = stats::gaussian(link = "log"),
  verbose = TRUE,
  upperDeltaProb = NULL,
  weightTransform = TRUE,
  AR1 = FALSE,
  sandwich = "cluster",
  ncores = 1,
  algorithm = "bam",
  ...
)
```

Arguments

spe	a SpatialExperiment object
selection	the mark(s) you want to compare. NOTE: This is directional. c(A,B) is not the same result as c(B,A).
fun	the spatstat function to compute on the point pattern object
marks	the marks to consider e.g. cell types
rSeq	the range of r values to compute the function over

correction	the edge correction to be applied
sample_id	the spe colData variable to mark the sample, if not NULL this will result in a mixed model estimation
image_id	the spe colData variable to mark the image
condition	the spe colData variable to mark the condition
continuous	A boolean indicating whether the marks are continuous defaults to FALSE
assay	the assay which is used if continuous = TRUE
transformation	the transformation to be applied as exponential e.g. 1/2 for sqrt or Fisher's variance-stabilising transformation if "Fisher"
weights	the weighting to be applied to the functional GAM. Either NULL (equal weights), total (npoints of total pattern), min (npoints of the smaller subpattern) or max (npoints of the larger subpattern) or a user defined value of same length as the number of curves to be estimated
eps	some distributional families fail if the response is zero, therefore, zeros can be replaced with a very small value eps
delta	the delta value to remove from the beginning of the spatial statistics functions. Can be reasonable if e.g. cells are always spaced by 10 µm. If set to "minNnDist" it will take the mean of the minimum nearest neighbour distance across all images for this cell type pair.
family	the distributional family for the functional GAM
verbose	logical indicating whether to print all information or not
upperDeltaProb	the quantile to filter out the constant 1 part for Gest and Gcross. If NULL no upper filtering is applied.
weightTransform	logical indicating whether the weights (number of points) should be sqrt transformed
AR1	logical indicating whether to calculate the autocorrelation of the residuals along the domain and account for this in a second fitting step
sandwich	string indicating how and if to adjust for heterscedasticity of the residuals with a sandwich correction
ncores	the number of cores to use for parallel processing, default = 1
algorithm	algorithm to fit the refund::pffr method. defaults to bam
...	Other parameters passed to spatstat.explore functions for parameters concerning the spatial function calculation and to refund::pffr for the functional additive mixed model inference

Value

a list with four objects: i) the dataframe with the spatial statistics results transformed and filtered as used for fitting, ii) the raw spatial statistics results, iii) the designmatrix of the inference and iv) the fitted pffr object v) the residual standard error per condition defined as the residual sum of squares divided by the number of datapoints - sum of the estimated degrees of freedom for the model parameters as well as other QC metrics

Examples

```
spe <- .loadExample()
#make the condition a factor variable
colData(spe)[["patient_stage"]] <- factor(colData(spe)[["patient_stage"]])
#relevel to have non-diabetic as the reference category
colData(spe)[["patient_stage"]] <- relevel(colData(spe)[["patient_stage"]],
"Non-diabetic")
res <- spatialInference(spe, c("alpha", "Tc"),
    fun = "Gcross", marks = "cell_type",
    rSeq = seq(0, 50, length.out = 50), correction = "rs",
    sample_id = "patient_id",
    image_id = "image_number", condition = "patient_stage",
    ncores = 1,
    algorithm = "bam"
)
```

---

summary.functionalGam *Summary for functionalGam object*

Description

Summary for functionalGam object

Usage

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

Arguments

object	a fitted functionalGam-object
...	see summary.gam() for options.

Value

A list with summary information, see summary.gam()

Author(s)

Martin Emons, adapted from summary.pffr() by Fabian Scheipl

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