Package ‘RTCA’

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Type Package

Title Open-source toolkit to analyse data from xCELLigence System (RTCA)

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Description Import, analyze and visualize data from Roche(R) xCELLigence RTCA systems. The package imports real-time cell electrical impedance data into R. As an alternative to commercial software shipped along the system, the Bioconductor package RTCA provides several unique transformation (normalization) strategies and various visualization tools.

License LGPL-3

LazyLoad yes

Depends methods,stats,graphics,Biobase,RColorBrewer, gtools

Suggests xtable


biocViews CellBasedAssays, Infrastructure, Visualization, TimeCourse

NeedsCompilation no

R topics documented:

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alphaNames

Auxilliary functions for experiments with microtitre plates

Description

Functions to manipulate indices or names of microtitre plates

Usage

alphaNames(row = 8, column = 12, order=c("column","row"))
repairAlphaName(x)
alphaNames2Pos(x)
rowcol2pos(row = 1, column=1, plateFormat=c("96","384"))

Arguments

row integer, row index, 1,...,8 for 96-well plates
column integer, column index, 1,...,12 for 96-well plates
x character, Well alpha name, in the form of [A-Z][0-9][0-9], like 'A01'
order character, should the alpha names returned in a row-first or column-first order?
plateFormat integer, the microtitre format, either 96 or 384

Details

alphaNames returns so-called alpha well names in the form of [A-H][0-9][0-9] (i.e., A01, C03, D11, H12) for microtitre plates. The order of returned alphaNames is controlled by the option order, which can be set either as col or row
repairAlphaName attempts to fix incomplete alpha well names. Now it is mainly used to fix well names missing the leading 0 of numeric index, like A1.
alphaName2Pos returns the row and column number of the given alpha well name, in the form of two-column data frame with row and col as colnames.
rowcol2pos returns the row-wise position index of given row and column index.

Value

See details

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>
**Examples**

```r
wells <- alphaNames()

repairAlphaName("A1")

alphaNames2Pos(c("A01","B02","C03","H12"))

rowcol2pos(3,1)
```

---

**combineRTCA**

Combine a list of RTCA objects

**Description**

Combine a list of RTCA objects

**Usage**

```r
combineRTCA(list)
```

**Arguments**

- `list` A list of RTCA objects

**Details**

The current implementation requires all the objects have exactly the same time-points recorded (or at least of same length).

The combined RTCA object has an obligatory column in the `phenoData` 'Plate' (upper-case!), which matches the names of the RTCA list. When the `list` has no names, the 'Plate' field is filled with integer index starting from 1.

**Value**

A new RTCA object

**Note**

Special attention should be given to the cases where the `list` parameter partially has names. In this case all items without name will be assigned to a 'Plate' field of empty string ('"'). Therefore it is advised either to assign names to all items of the list, or leave them all off.

**Author(s)**

Jitao David Zhang <jitao_david.zhang@roche.com>
controlView

Examples

```r
## An artificial example
require(RTCA)

ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
xSub1 <- x[,1:3]
xSub2 <- x[,4:ncol(x)]
xComb <- combineRTCA(list(sub1=xSub1, sub2=xSub2))
identical(exprs(x), exprs(xComb))
pData(xComb)$Plate

## in case of nameless list
pData(combineRTCA(list(xSub1, xSub2)))$Plate

## partial names
pData(combineRTCA(list(a=xSub1, xSub2)))$Plate
```

controlView

### PLOT CONTROL WELLS IN RTCA DATA

Description

A convenience function to plot sample wells with control wells on an E-plate in RTCA system. To use the function the phenoData field of the RTCA object must contain a field named “GeneSymbol”.

Usage

```r
controlView(rtca, genesymbol = c("Allstar", "COPB2", "GFP", "mock", "PLK1", "WEE1"), cols, ylim, smooth = FALSE, group = ..., ylab = "y axis label", xlab = "x axis label", drawsd = TRUE, normline = TRUE, ncol = 1, legendpos = "topleft", pData.column="GeneSymbol", ...)
```

Arguments

- `rtca` An object of RTCA. To use the function, the phenoData must contain a column which name is specified by the `pData.column` parameter.
- `genesymbol` character, gene symbols to be plotted.
- `cols` character, colors used by the provided gene symbols
- `ylim` y-axis lim
- `smooth` logical, whether the RTCA object should be smoothed before plotting
- `group` logical. If ‘group’ is set to TRUE, wells with the same GeneSymbol will be summarized and plotted. For instance, these could be biological replicates. Otherwise each well is plotted separately
- `ylab` y axis label
- `xlab` x axis label
- `drawsd` logical, should the error bar be drawn to represent standard deviation?
- `normline` logical, should the base-time indicated by a line? See `ratioTransform` for the concept of the base-time
- `ncol` integer, legend column number
- `legendpos` character, legend position
- `pData.column` The column which the genesymbol parameter will be matched with
- `...` other parameters passed to the `plot` function
derivativeTransform

Details

The function is often called to draw sample and control in one plot.

Value

NULL, the function is called for its side effect

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

See Also

RTCA

Examples

require(RTCA)
ofile <- system.file("extdata/testOutput.csv", package="RTCA")
pfile <- system.file("extdata/testOutputPhenoData.csv", package="RTCA")
pData <- read.csv(pfile, sep="\t", row.names="Well")
metaData <- data.frame(labelDescription=c(
  "Rack number",
  "siRNA catalogue number",
  "siRNA gene symbol",
  "siRNA EntrezGene ID",
  "siRNA targeting accession"
))
phData <- new("AnnotatedDataFrame", data=pData, varMetadata=metaData)
x <- parseRTCA(ofile, phenoData=phData)
controlView(x, genesymbol=c("mock","COPB2","PLK1"),ylim=c(0,2))
factor2numeric

Details

The first derivative of the cell impedance curve measured by RTCA. The derivative of the last time point is estimated by that of the next to last point.

Value

An RTCA object populated with derivative values

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

See Also

smoothTransform and interpolationTransform for smoothing and interpolating the RTCA data.

rgrTransform calculates relative growth rate, which calls derivativeTransform.

Examples

```r
require(RTCA)
ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
xDeriv <- derivativeTransform(x)
```

---

factor2numeric  FACTOR UTILITIES

Description

The functions implement easy interface to certain tasks of factor. See details for explanation

Usage

```r
factor2numeric(x)
relevels(x, refs)
```

Arguments

- `x` A vector of factor
- `refs` A vector of character, reference vector to give the order of levels

Details

relevels re-arrange the order of levels by the given character refs. Alternatively user could use factor(...,levels=refs) to achieve a similar effect, however the relevels enables also partial list. The missing levels in refs will be ordered to the last.

factor2numeric converts factor of numerics into their numeric form.
**Value**

A vector of factor

**Author(s)**

Jitao David Zhang <jitao_david.zhang@roche.com>

**Examples**

```r
## factor2numeric
numFac <- factor(c(3.5, 2.5, 2.5,3.5, 1))
numFac
levels(numFac)

factor2numeric(numFac)
levels(factor2numeric(numFac))

## relevels
relevels(numFac, c("3.5", "1", "2.5"))
relevels(numFac, c("3.5", "2.5"))
```

---

**interpolationTransform**

*TRANSFORM RTCA DATA WITH INTERPOLATION*

**Description**

Interpolate RTCA data

**Usage**

`interpolationTransform(object, interval=0.01, method=c("linear","constant","fmm","periodic","natural","monoH.FC"))`

**Arguments**

- `object` An RTCA object
- `...` other parameters, `interval` and `method` are implemented, see below
- `interval` numeric, the interval between interpolated points, set to 0.01 by default
- `method` character, specifying the method for interpolation, “linear” by default (for linear interpolation). Allowed options are: “linear” and “constant” for approx interpolation, and “fmm”, “periodic”, “natural” and “monoH.FC” for cubic spline interpolation

**Details**

Since most RTCA experiments record the experiments in the irregular time-series, sometimes however it is desired to have regular intervals. `interpolationTransform` interpolate between data points to estimate results of regular intervals.

Two classes of interpolations are supported by now: linear (using `approx`) and cubic spline (`spline`) interpolation. By default linear interpolation is used.
nearestTimeIndex

Value
An interpolated object of RTCA.

Author(s)
Jitao David Zhang <jitao_david.zhang@roche.com>

See Also
rgrTransform stands for relative growth rate transformation, ratioTransform for ratio normalization adopted by Roche commercial software. smoothTransform to smooth the RTCA readout.

Examples
require(RTCA)
ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
xInter <- interpolationTransform(x)

Description
Get index for the nearest time point to the given one. Called internally in many time-point related functions.

Usage
nearestTimeIndex(rtca, time)

Arguments
rtca An object of RTCA
time numeric, a time point

Details
The function finds the time point with minimum absolute difference to the given time and returns its index.

Value
An integer, the index of the nearest time point

Author(s)
Jitao David Zhang <jitao_david.zhang@roche.com>
parseRTCA

See Also
timepoints to return all time points of an RTCA object.

Examples

```r
require(RTCA)
ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)

x
xIndex <- nearestTimeIndex(x, 25)
timepoints(x)[xIndex]
```

**Description**

The function parses RTCA output file into RTCA object

**Usage**

```r
parseRTCA(file, dec = ".", phenoData, maskWell, ...)
```

**Arguments**

- `file` character, name of the RTCA output file
- `dec` decimal sign of the file
- `phenoData` phenoData
- `maskWell` character, either names or regular expression pattern(s) for well(s) to mask
- `...` other parameters passed to `read.table`

**Details**

A csv-like format file can be exported from the RTCA device, which can be fed into this function to set up an instance of RTCA object.

In the `/extdata` directory of the package, such a file is provided as an example. The first line contains the experiment ID, which is followed by a matrix of recorded data in the tabular form. The first and second column records the time-interval in the unit of hour and hour-minute-second format respectively. The rest columns then record the read-out (‘Cell-Index’, or ‘CI’) of the device, with each well a role.

`phenoData` allows user to annotate the wells. Its usage mimicks the ExpressionSet object in the Biobase package.

`maskWell` allows to mask wells in case, for example, they are known to be contaminated. The values can be either a vector of well names, or a regular expression pattern for wells to be masked. To learn regular expression patterns see `grep`.
Value

An object of RTCA-class

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

References

http://www.roche-applied-science.com/proddata/gpip/3_8_9_1_1_1.html

Examples

```r
require(RTCA)
ofile <- system.file("extdata/testOutput.csv", package="RTCA")
pfile <- system.file("extdata/testOutputPhenoData.csv", package="RTCA")

pData <- read.csv(pfile, sep="\t", row.names="Well")
metaData <- data.frame(labelDescription=c("Rack number", "siRNA catalogue number", "siRNA gene symbol", "siRNA EntrezGene ID", "siRNA targeting accession" ))

phData <- new("AnnotatedDataFrame", data=pData, varMetadata=metaData)
x <- parseRTCA(ofile, phenoData=phData)
print(x)

## mask wells, e.g. due to unusual values

x.skip <- parseRTCA(ofile, phenoData=phData, maskWell=c("D09"))
x.skip.multiWells <- parseRTCA(ofile, phenoData=phData, maskWell=c("A01", "B01", "C02"))

## skip the last row

x.skip.pattern <- parseRTCA(ofile, phenoData=phData, maskWell=c("H[0-9]{2}"))

## check the number of masked wells

noMasked <- function(x) sum(apply(x, 2, function(x) all(is.na(x)))))
noMasked(exprs(x))
noMasked(exprs(x.skip))
noMasked(exprs(x.skip.multiWells))
noMasked(exprs(x.skip.pattern))
```

Description

Plots a E-plate in RTCA assays in one plot to convey an overview of the plate
plateView

Usage

plateView(rtca, ylim, titles,...)

Arguments

- rtca: An object of RTCA
- ylim: ylab lim
- titles: Titles of sub-figures representing each well. If missing, the function seeks whether a Well column is available in the pData of the RTCA object, and if so, its value will be used. If not, the sample names (by sampleNames function) will be used as titles.
- ...: Other parameters passed to the plot function. Currently options col, lty and lwd are supported. See details below.

Details

For now the function only supports the visualization of a 96-well E-plate.

The plate view plot draws lines indicating cell index (or its transformations) in a birdview. When ... are not specified, default color, line style and width are used. col, lty and lwd can be a vector, and if needed they will be expanded to have the same length as wells.

Value

NULL, the function is called for the side effect

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

See Also

RTCA for data structure, plot for the basic plot function.

Examples

```r
require(RTCA)

ofile <- system.file("extdata/testOutput.csv", package="RTCA")
rtca <- parseRTCA(ofile)

## Not run automatically, because of 'margin too large'
## plateView(rtca)
## plateView(rtca, lty=2)
## plateView(rtca, col=rep(1:8, each=12))

rtca.skip <- parseRTCA(ofile, maskWell="H[0-9]{2}")
## plateView(rtca.skip)
```
plotGridEffect  

PLOT GRID EFFECT OF RTCA

Description

Plot the mean and deviation of rows/columns of a RTCA E-plate, to provide hints of potential row/column effect of the plate

Usage

plotGridEffect(rtca, mode = c("column", "row"), xlab = "time point", ylab = "readout", legend = TRUE, col, ...)

Arguments

- rtca: An object of RTCA
- mode: character, either "column" or "row", to choose which effect to depict
- xlab: x-axis label
- ylab: y-axis label
- legend: logical, whether the legend should be added
- col: Color of the curves
- ... Further parameters passed to plot function

Details

The error bars depicts the standard deviations

Value

NULL, the function is called for its side effect

Author(s)

Jitao David Zhang

Examples

require(RTCA)

ofile <- system.file("extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
plotGridEffect(x)
ratioTransform  

**RATIO TRANSFORMATION OF RTCA DATA**

**Description**
Performs ratio transformation (normalisation) of RTCA data, as recommended by the producer Roche.

**Usage**
```
ratioTransform(object, time)
```

**Arguments**
- **object**: An object of `RTCA`
- **time**: numeric, the time point used to normalize the whole series of data

**Details**
The `xCelligence` software provided by Roche performs ratio transform implicitly by dividing the time-series impedance measurement by the value of a selected time point (so-called 'base-time'), for instance 5 hours after compound transfection, in each cell. The aim of this transformation was to scale (normalize) the data of different wells, since the normalized values of all wells are uniformly 1 at the base-time.

However, this method is vulnerable to arbitrary selection of the time point chosen to normalize. It may be helpful to try several base-time values before comparing normalized results.

See `derivativeTransform` and `rgrTransform` for other normalization (scaling) possibilities.

**Value**
An object of `RTCA`, populated with normalized value. The normalized values of all wells are uniformly 1 at the base-time.

**Author(s)**
Jitao David Zhang <jitao_david.zhang@roche.com>

**See Also**
- `smoothTransform` and `interpolationTransform` for smoothing and interpolating the RTCA data.
- `rgrTransform` calculates relative growth rate, `derivativeTransform` calculates derivative. The later two methods are not sensitive to the selection of base-time point.

**Examples**
```
require(RTCA)

ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
xNorm <- ratioTransform(x, 35)
```
rgrTransform

TRANSFORM RTCA DATA INTO RELATIVE GROWTH RATE

Description
Transform RTCA data into relative growth rate

Usage
rgrTransform(object, smooth)

Arguments
object An object of RTCA
smooth logical, should the object be smooth transformed after the rgrTransform? Set to TRUE by default

Details
TODO: relative growth rate

Value
An object of RTCA populated with relative growth rate instead of input data

Author(s)
Jitao David Zhang <jitao_david.zhang@roche.com>

References
TODO: reference

See Also
derivativeTransform for first derivative. ratioTransform for ratio normalization adopted by Roche commercial software. smoothTransform and interpolationTransform for other transformation possibilities.

Examples
require(RTCA)

ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)
xRgr <- rgrTransform(x)
RTCA-class  

Class "RTCA"

Description

RTCA object

Objects from the Class

Objects can be created by calls of the form new("RTCA", assayData, phenoData, featureData, experimentData, annotation, exprs, ...). However, it is more common to be constructed by parseRTCA function by reading in RTCA output data directly.

Slots

expID: Object of class "character", experiment ID
timeline: Object of class "RTCAtimeline", recording action track along the timeline
assayData: Object of class "AssayData", assay data inherited from ExpressionSet-class
phenoData: Object of class "AnnotatedDataFrame", pheno data of the assay, annotating the wells
featureData: Object of class "AnnotatedDataFrame", feature data of the assay, preserved for time-line recording by the package
experimentData: Object of class "MIAME", idle
annotation: Object of class "character", idle
__classVersion__: Object of class "Versions", idle

Extends

Class ExpressionSet-class, directly. Class eSet-class, by class "ExpressionSet", distance 2. Class VersionedBiobase-class, by class "ExpressionSet", distance 3. Class Versioned-class, by class "ExpressionSet", distance 4.

Methods

addAction signature(object = "RTCA", time = "numeric", action = "character"): add action at the specified time, passed to the RTCAtimeline slot
getAction signature(object = "RTCA", time = "numeric"): get action at the specified time, passed to the RTCAtimeline slot
plotRTCA signature(x = "RTCA"): plot RTCA
rmAction signature(object = "RTCA", time = "numeric"): remove action at the specified time, passed to the RTCAtimeline slot
show signature(object = "RTCA"): print method
expID codesignature(object = "RTCA"): get Experiment ID
expID<- codesignature(object = "RTCA", value = "ANY"): set Experiment ID
time signature(x = "RTCA"): deprecated
timeline signature(object = "RTCA"): get the RTCAtimeline slot
timeline<- signature(object = "RTCA"): assign the RTCAtimeline slot
**timepoints** signature(object = "RTCA"): get the recording time points in a vector

**timepoints<-** signature(object = "RTCA"): assign the recording time points

**updateAction** signature(object = "RTCA", time = "numeric", action = "character"): update the action at the specified time, passed to the RTCA timeline slot

**plot** signature(x = "RTCA", y): plot the RTCA running plot with `matplot`. `y` is interpreted as the indices of the columns to be plotted, and will be expanded to all the columns in case it is missing.

**Author(s)**

Jitao David Zhang <jitao_david.zhang@roche.com>

**References**


2 [http://www.roche-applied-science.com/proddata/gpip/3_8_9_1_1_1.html](http://www.roche-applied-science.com/proddata/gpip/3_8_9_1_1_1.html) for brief introduction into RTCA

**Examples**

```r
new("RTCA", expID="testExp01")
```

---

**RTCAtimeline-class**

**Class** "RTCAtimeline"

**Description**

Time line of actions performed by the xCelligence device, supporting CRUD manipulations (create, read, update and delete).

**Objects from the Class**

Objects can be created by calls of the form `new("RTCAtimeline")`. However, it is more common to be called implicitly by creating an instance of RTCA object.

**Slots**

- **actionTrack**: Object of class ”data.frame", records action track in the form of two-column data.frame. The two columns must have the names ‘time’ and ‘action’.

- **timeUnit**: Object of class "character", recording the unit of time points stored in the actionTrack slot.

- **startTime**: Object of class "POSIXct", the absolute time when the measurement started (at the time point ‘0’)
Methods

- **addAction** signature(object = "RTCAtimeline", time = "numeric", action = "character"): add action at the specified time
- **actionTrack** signature(object = "RTCAtimeline"): get the action track in the form of data.frame
- **actionTrack<-** signature(object = "RTCAtimeline", value = "data.frame"): assign the action track
- **getAction** signature(object = "RTCAtimeline", time = "numeric"): get action at the specified time
- **orderAction** signature(object = "RTCAtimeline"): order the action track by the time
- **reset** signature(object = "RTCAtimeline"): undo all editing of the object and reset it to the initial state
- **rmAction** signature(object = "RTCAtimeline", time = "numeric"): remove the action at the specified time
- **timeUnit** signature(object = "RTCAtimeline"): return the time unit used by the action track
- **timeUnit<-** signature(object = "RTCAtimeline", value = "character"): assign the time unit used by the action track
- **start** signature(object = "RTCAtimeline"): return the starting POSIXct time of the experiment
- **timeUnit<-** signature(object = "RTCAtimeline", value = "character"): assign the starting POSIXct time of the experiment

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

References

2. [http://www.roche-applied-science.com/proddata/gpip/3_8_9_1_1_1.html](http://www.roche-applied-science.com/proddata/gpip/3_8_9_1_1_1.html) for brief introduction into RTCA

See Also

RTCA

Examples

tl <- new("RTCAtimeline")
show(tl)
sliceRTCA

SLICE RTCA OBJECT WITH TIME

Description
Subset (slice) RTCA object with starting- and ending-time

Usage
sliceRTCA(x, start, end)

Arguments
- x: An object of RTCA
- start: numeric, start time
- end: numeric, end time

Details
In case the exact starting- or ending-time is not matched, the nearest time point will be used to subset.

Value
An object of RTCA

Author(s)
Jitao David Zhang <jitao_david.zhang@roche.com>

Examples
```r
require(RTCA)
ofile <- system.file("/extdata/testOutput.csv", package="RTCA")x <- parseRTCA(ofile)subx <- sliceRTCA(x, 20, 50)
```

smoothTransform

SMOOTH TRANSFORM OF RTCA OBJECT

Description
Smoothing the RTCA cell impedance measurement

Usage
smoothTransform(object, ...)
spectramaxImport

Arguments

object  An object of RTCA
...  Parameters passed to smooth.spline

Details

smoothTransform smooths the RTCA cell impedance measurement by calling the function smooth.spline. This feature can be useful for visualization purposes and in conjunction with other transformations.

Value

An RTCA object populated with smoothed values

Note

ratioTransform performs ratio transformation recommended by the machine provider. interpolationTransform for interpolating the RTCA data. derivativeTransform returns cell impedance change rates and rgrTransform calculates relative growth rate.

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

Examples

```r
require(RTCA)

ofile <- system.file("/extdata/testOutput.csv", package="RTCA")
x <- parseRTCA(ofile)

xSmooth <- smoothTransform(x)
```

spectramaxImport  Import output files from Spectramax spectrophotometer

Description

Import output files from Spectramax spectrophotometer (plate reader) into the list format compatible with the cellHTS2 package.

Usage

```r
spectramaxImport(file, encoding="latin1")
```

Arguments

file  A Spectramax file
encoding  File character encoding, by default “latin1”
The function imports output files from Spectramax plate reader, with which single-channel cell-based assays could be performed. Such assay includes WST-1 viability assay, which can be used to validate RTCA assay results.

A list of two items: one data frame (no name) and one character vector (txt). The data frame contains following columns:

- **well**: Well indices ([A-Z][0-9][0-9] format) on the microtitre plate
- **val**: Value of each well

The character vector `txt` contains a copy of the file contents.

Jitao David Zhang <jitao_david.zhang@roche.com>

See Also
cellHTS2 package documentation.

```r
wstFiles <- dir(system.file("extdata", package="RTCA"),
    pattern="^WST.*csv$", full.names=TRUE)
spectramaxImport(wstFiles[1])

## NOT RUN
## spectramaxImport also supports multiple files, in which case the
## result is a list of individual lists
spectramaxImport(wstFiles)
## END NOT RUN
```
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