## Package 'RTCA'

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Type Package

```
Title Open-source toolkit to analyse data from xCELLigence System
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Description Import, analyze and visualize data from Roche(R) xCELLigence RTCA sys-
     tems. The package imports real-time cell electrical impedance data into R. As an alterna-
     tive to commercial software shipped along the system, the Bioconductor package RTCA pro-
     vides several unique transformation (normalization) strategies and various visualization tools.
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 ${\tt alphaNames}$ 

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                                 |

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## **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**

```
wells <- alphaNames()
repairAlphaName("A1")
alphaNames2Pos(c("A01","B02","C03","H12"))
rowcol2pos(3,1)</pre>
```

combineRTCA

Combine a list of RTCA objects

## **Description**

Combine a list of RTCA objects

## Usage

```
combineRTCA(list)
```

## **Arguments**

list

A list of RTCA objects

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## **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>

## **Examples**

```
## 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</pre>
```

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".

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## Usage

```
controlView(rtca, genesymbol = c("Allstar", "COPB2", "GFP", "mock", "PLK1", "WEE1"), cols, ylim, smooth
```

## **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 sum-

marized and plotted. For instance, these could be biological replicates. Other-

wise each well is plotted separatedly

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

#### **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** 

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## **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))</pre>
```

derivativeTransform

DERIVATIVE TRANSFORM OF RTCA OBJECT

## **Description**

Derivative transform of RTCA object, returning the change rate of cell impedance

## Usage

```
derivativeTransform(object)
```

## **Arguments**

object

An object of RTCA

## **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>

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## See Also

smoothTransform and interpolationTransform for smoothing and interpolating the RTCA data. rgrTransform calculates relative growth rate, which calls derivativeTransform.

#### **Examples**

```
require(RTCA)

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

xDeriv <- derivativeTransform(x)</pre>
```

factor2numeric

FACTOR UNITILITIES

## Description

The functions implement easy interface to certain tasks of factor. See datails for explaination

## Usage

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

## **Arguments**

x A vector of factor

refs A vector of character, reference vector to give the orderof 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**

```
## factor2numeric
numFac <- factor(c(3.5, 2.5, 2.5,3.5, 1))
numFac
levels(numFac)
factor2numeric(numFac)
class(factor2numeric(numFac))
## relevels
relevels(numFac, c("3.5", "1", "2.5"))
relevels(numFac, c("3.5", "2.5"))</pre>
```

interpolationTransform

#### TRANSFORM RTCA DATA WITH INTERPOLATION

## **Description**

Interpolate RTCA data

#### Usage

interpolationTransform(object, interval=0.01, method=c("linear","constant","fmm","periodic","natural

## **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 experiements 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.

## Value

An interpolated object of RTCA.

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## 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)</pre>
```

nearestTimeIndex

GET INDEX FOR NEAREST TIME

## 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>

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## See Also

timepoints to return all time points of an RTCA object.

#### **Examples**

```
require(RTCA)

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

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

parseRTCA

PARSE RTCA OUTPUT FILE

## **Description**

The function parses RTCA output file into RTCA object

## Usage

```
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.

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## 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**

```
require(RTCA)
ofile <- system.file("extdata/testOutput.csv", package="RTCA")</pre>
pfile <- system.file("extdata/testOutputPhenoData.csv", package="RTCA")</pre>
pData <- read.csv(pfile, sep="\t", row.names="Well")</pre>
metaData <- data.frame(labelDescription=c(</pre>
"Rack number",
"siRNA catalogue number",
"siRNA gene symbol",
"siRNA EntrezGene ID",
"siRNA targeting accession"
))
phData <- new("AnnotatedDataFrame", data=pData, varMetadata=metaData)</pre>
x <- parseRTCA(ofile, phenoData=phData)</pre>
print(x)
## mask wells, e.g. due to unusual values
x.skip <- parseRTCA(ofile, phenoData=phData, maskWell=c("D09"))</pre>
x.skip.multiWells <- parseRTCA(ofile, phenoData=phData, maskWell=c("A01", "B01",
"C02"))
## skip the last row
x.skip.pattern <- parseRTCA(ofile, phenoData=phData,</pre>
maskWell=c("H[0-9]{2}"))
## check the number of masked wells
noMasked \leftarrow 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))
```

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#### PLATE VIEW OF RTCA DATA

## Description

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

## 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 <i>Well</i> 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.

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## **Examples**

```
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)</pre>
```

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 funciton is called for its side effect

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#### Author(s)

Jitao David Zhang

## **Examples**

```
require(RTCA)

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

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>

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## 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 sensative 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)</pre>
```

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

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#### 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)</pre>
```

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 time line
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.

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## Methods

```
addAction signature(object = "RTCA", time = "numeric",action = "character"): add ac-
     tion 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"): up-
    date the action at the specified time, passed to the RTCAtimeline slot
plot signature(x = "RTCA", y): plot the RTCA running plot with matplot. y is interpretated 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

- 1 https://www.roche-applied-science.com/sis/xcelligence/index.jsp?id=xcect\_000000 introduces xCelligence system.
- 2 http://www.roche-applied-science.com/proddata/gpip/3\_8\_9\_1\_1\_1.html for brief introduction into RTCA

## Examples

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

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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</pre>

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 actiont track

timeUnit<- signature(object = "RTCAtimeline", value = "character"): assign the time unit
 used by the actiont track</pre>

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</pre>

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## Author(s)

Jitao David Zhang <jitao\_david.zhang@roche.com>

#### References

```
1 http://www.xcelligence.roche.com/introduces xCelligence system.
```

2 http://www.roche-applied-science.com/proddata/gpip/3\_8\_9\_1\_1.html for brief introduction into RTCA

#### See Also

**RTCA** 

## **Examples**

```
tl <- new("RTCAtimeline")
show(tl)</pre>
```

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>

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## **Examples**

```
require(RTCA)

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

subx <- sliceRTCA(x, 20, 50)</pre>
```

smoothTransform

SMOOTH TRANSFORM OF RTCA OBJECT

## **Description**

Smoothing the RTCA cell impedance measurement

## Usage

```
smoothTransform(object, ...)
```

## **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 visualiation purposes and in conjuction 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>

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## **Examples**

```
require(RTCA)

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

xSmooth <- smoothTransform(x)</pre>
```

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

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

## **Arguments**

file A Spectramax file

encoding File character encoding, by default "latin1"

## **Details**

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.

#### Value

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.

## Author(s)

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## See Also

cellHTS2 package documentation.

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## Examples

```
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</pre>
```

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