## 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:

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>alphaNames</td>
<td>2</td>
</tr>
<tr>
<td>combineRTCA</td>
<td>3</td>
</tr>
<tr>
<td>controlView</td>
<td>4</td>
</tr>
<tr>
<td>derivativeTransform</td>
<td>5</td>
</tr>
<tr>
<td>factor2numeric</td>
<td>6</td>
</tr>
<tr>
<td>interpolationTransform</td>
<td>7</td>
</tr>
<tr>
<td>nearestTimeIndex</td>
<td>8</td>
</tr>
<tr>
<td>parseRTCA</td>
<td>9</td>
</tr>
<tr>
<td>plateView</td>
<td>10</td>
</tr>
<tr>
<td>plotGridEffect</td>
<td>12</td>
</tr>
<tr>
<td>ratioTransform</td>
<td>13</td>
</tr>
</tbody>
</table>
**alphaNames**

**rgrTransform** ................................................................. 14
**RTCA-class** ........................................................................ 15
**RTCAtimeline-class** ............................................................ 16
**sliceRTCA** ........................................................................... 18
**smoothTransform** ................................................................. 18
**spectramaxImport** ................................................................ 19

---

**Index**

| alphaNames | Auxilliary functions for experiments with microtitre plates |

---

**Description**

Functions to manipulate indices or names of microtitre plates

**Usage**

```r
doi:alphaNames(row = 8, column = 12, order=c("column","row"))
doi:repairAlphaName(x)
doi:alphaName2Pos(x)
doi: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 = ... = "Time interval (hour)", 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.
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

```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)

controlView(x, genesymbol=c("mock","COPB2","PLK1"), ylim=c(0,2))
```

### derivativeTransform

**DERIVATIVE TRANSFORM OF RTCA OBJECT**

#### Description

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

#### Usage

```r
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>

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)
class(factor2numeric(numFac))

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

**Description**

Interpolate RTCA data

**Usage**

```r
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

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>

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

```
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**

```r
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 depict the standard deviations.

**Value**

`NULL`, the function is called for its side effect.

**Author(s)**

Jitao David Zhang

**Examples**

```r
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)
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.

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 RTCAtimeline 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

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

2 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**

```r
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**

```r
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)
```

---

**Description**

Import output files from Spectramax spectrophotometer 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”
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.

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Examples

```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
```
Index

*Topic IO
  parseRTCA, 9

*Topic classes
  RTCA-class, 15
  RTCAtimeline-class, 16

*Topic file
  parseRTCA, 9

*Topic hplot
  controlView, 4
  plateView, 10
  plotGridEffect, 12

*Topic misc
  factor2numeric, 6

*Topic models
  rgrTransform, 14

*Topic ts
  interpolationTransform, 7
  actionTrack (RTCAtimeline-class), 16
  actionTrack, RTCAtimeline-method (RTCAtimeline-class), 16
  actionTrack<- (RTCAtimeline-class), 16
  addAction (RTCAtimeline-class), 15
  alphaNames, 2
  alphaNames2Pos (alphaNames), 2
  approx, 7
  combineRTCA, 3
  controlView, 4
  derivativeTransform, 5, 13, 14, 19
  derivativeTransform, RTCA-method (RTCA-class), 15
  expID (RTCA-class), 15
  expID, RTCA-method (RTCA-class), 15
  expID<- (RTCA-class), 15
  expID<-, RTCA-method (RTCA-class), 15
  factor2numeric, 6, 6
  getAction (RTCAtimeline-class), 16
  getAction, RTCA, numeric-method (RTCA-class), 15
  getAction, RTCAtimeline, numeric-method (RTCAtimeline-class), 16
  grep, 9
  interpolationTransform, 6, 7, 13, 14, 19
  interpolationTransform, RTCA-method (RTCA-class), 15
  matplot, 16
  nearestTimeIndex, 8
  orderAction (RTCAtimeline-class), 16
  orderAction, RTCAtimeline-method (RTCAtimeline-class), 16
  parseRTCA, 9, 15
  plateView, 10
  plot, 4, 11, 12
  plot, RTCA-method (RTCA-class), 15
  plotGridEffect, 12
  plotRTCA, RTCA-method (RTCA-class), 15
  ratioTransform, 4, 8, 13, 14, 19
  ratioTransform, RTCA-method (RTCA-class), 15
  read.table, 9
  relevels, 6
  relevels (factor2numeric), 6
  repairAlphaName (alphaNames), 2
  reset (RTCAtimeline-class), 16
  reset, RTCAtimeline-method (RTCAtimeline-class), 16
  rgrTransform, 6, 8, 13, 14, 19
  rgrTransform, RTCA-method (RTCA-class), 15
  rmAction (RTCAtimeline-class), 16
  rmAction, RTCA, numeric-method (RTCA-class), 15
Index

rmAction, RTCAtimeline, numeric-method (RTCAtimeline-class), 16
rowcol2pos (alphaNames), 2
RTCA, 4–6, 8, 9, 11–14, 16–19
RTCA-class, 15
RTCAtimeline, RTCA-method (RTCA-class), 15
RTCAtimeline-class, 16
RTCAtimeline<-, RTCA-method (RTCA-class), 15
show, RTCA-method (RTCA-class), 15
sliceRTCA, 18
smooth.spline, 19
smoothTransform, 6, 8, 13, 14, 18
smoothTransform, RTCA, ANY-method (RTCA-class), 15
smoothTransform, RTCA, missing-method (RTCA-class), 15
spectramaxImport, 19
spline, 7
startTime (RTCAtimeline-class), 16
startTime, RTCAtimeline-method (RTCAtimeline-class), 16
startTime<-, (RTCAtimeline-class), 16
startTime<-, RTCA, POSIXct-method (RTCAtimeline-class), 16

time, RTCA-method (RTCA-class), 15
timeline (RTCA-class), 15
timeline, RTCA-method (RTCA-class), 15
timeline<-, (RTCAtimeline-class), 15
timeline<-, RTCA-method (RTCA-class), 15
timepoints, 9
timepoints (RTCA-class), 15
timepoints, RTCA-method (RTCA-class), 15
timepoints<-, (RTCAtimeline-class), 15
timepoints<-, RTCA-method (RTCA-class), 15
timeUnit (RTCAtimeline-class), 16
timeUnit, RTCAtimeline-method (RTCAtimeline-class), 16
timeUnit<-, (RTCAtimeline-class), 16
timeUnit<-, RTCA, character-method (RTCAtimeline-class), 16

updateAction (RTCAtimeline-class), 16
updateAction, RTCA, numeric, character-method (RTCA-class), 15
updateAction, RTCAtimeline, numeric, character-method (RTCAtimeline-class), 16