

Package ‘flowcatchR’

April 8, 2025

Type Package

Title Tools to analyze in vivo microscopy imaging data focused on tracking flowing blood cells

Version 1.41.2

Date 2024-12-20

Description flowcatchR is a set of tools to analyze in vivo microscopy imaging data, focused on tracking flowing blood cells. It guides the steps from segmentation to calculation of features, filtering out particles not of interest, providing also a set of utilities to help checking the quality of the performed operations (e.g. how good the segmentation was). It allows investigating the issue of tracking flowing cells such as in blood vessels, to categorize the particles in flowing, rolling and adherent. This classification is applied in the study of phenomena such as hemostasis and study of thrombosis development. Moreover, flowcatchR presents an integrated workflow solution, based on the integration with a Shiny App and Jupyter notebooks, which is delivered alongside the package, and can enable fully reproducible bioimage analysis in the R environment.

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VignetteBuilder knitr

Suggests BiocStyle, knitr, rmarkdown

Depends R (>= 2.10), methods, EBImage

Imports colorRamps, abind, BiocParallel, graphics, stats, utils, plotly, shiny

SystemRequirements ImageMagick

LazyData true

URL <https://github.com/federicomarini/flowcatchR>,
<https://federicomarini.github.io/flowcatchR/>

BugReports <https://github.com/federicomarini/flowcatchR/issues>

biocViews Software, Visualization, CellBiology, Classification, Infrastructure, GUI, ShinyApps

RoxygenNote 7.3.2

Encoding UTF-8

Roxygen list(markdown = TRUE)

git_url <https://git.bioconductor.org/packages/flowcatchR>

git_branch devel

git_last_commit d498f26

git_last_commit_date 2024-12-20

Repository Bioconductor 3.21

Date/Publication 2025-04-08

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| | |
|--------------|---|
| add.contours | <i>Add object contours to a Frames object Creates a Frames object containing raw information, combined with the segmented images and the relative trajectory under analysis</i> |
|--------------|---|

Description

If a TrajectorySet is provided and mode is set to trajectories, returns a Frames with all trajectories included in the IDs vector painted accordingly. If the mode is set to particles, it will just plot the particles (all) on all frames. If no TrajectorySet is provided, it will be computed with default parameters. If no binary.frames is provided, it will be computed also with default parameters

Usage

```
add.contours(
  raw.frames,
  binary.frames = NULL,
  trajectoryset = NULL,
  trajIDs = NULL,
  mode = "particles",
  col = NULL,
  channel = NULL
)
```

Arguments

| | |
|---------------|--|
| raw.frames | A Frames object with raw images |
| binary.frames | A Frames object with preprocessed frames |
| trajectoryset | A TrajectorySet object |
| trajIDs | Numeric vector, the ID(s) of the trajectory. |
| mode | A character string, can assume the values particles or trajectories. Defaults to particles |
| col | A vector of color strings |
| channel | A character string, to select which channel to process |

Value

A new Frames object with contours of the objects added

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
## Not run:
paintedTrajectories <- add.contours(raw.frames = MesenteriumSubset,
                                   mode = "trajectories",channel="red")
paintedParticles <- add.contours(raw.frames = MesenteriumSubset,
                                 mode = "particles",channel="red")
inspect.Frames(paintedTrajectories)
inspect.Frames(paintedParticles)

## End(Not run)
```

| | |
|--------------|---|
| addParticles | <i>Combines the information from a raw Frames object and the corresponding preprocessed one</i> |
|--------------|---|

Description

All objects are painted with a unique colour - for sake of speed

Usage

```
addParticles(raw.frames, binary.frames, col = NULL)
```

Arguments

| | |
|---------------|---|
| raw.frames | A Frames object containing the raw images |
| binary.frames | A Frames object with the preprocessed versions of the images (e.g. segmented) |
| col | A color character string, to select which color will be used for drawing the contours of the particles. If not specified, it will default according to the objects provided |

Value

A Frames object, whose images are the combination of the raw images with the segmented objects drawn on them

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

axesInfo

Info on the dimensions of the FOV

Description

Auxiliary function to return the dimensions of the field of interest

Usage

```
axesInfo(frames)
```

Arguments

| | |
|--------|-----------------|
| frames | A Frames object |
|--------|-----------------|

Value

A list object, containing the extremes of the field of interest (x-y-z, where z is time)

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

candidate.platelets *A sample ParticleSet object*

Description

The sample ParticleSet object is constituted by the platelets identified from the MesenteriumSubset data

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

channel.Frames *Channel extraction for objects*

Description

channel

Usage

```
channel.Frames(frames, mode)
```

Arguments

| | |
|--------|--|
| frames | A Frames object |
| mode | A character value specifying the target mode for conversion. |

Value

A Frames object with just the information on the selected channel

Examples

```
data("MesenteriumSubset")  
channel.Frames(MesenteriumSubset, "red")
```

| | |
|------------|--|
| computeMSD | <i>Calculates the Mean Squared Displacement for a trajectory</i> |
|------------|--|

Description

Calculates the Mean Squared Displacement for a trajectory

Usage

```
computeMSD(sx, sy, until = 4)
```

Arguments

| | |
|-------|---|
| sx | x axis positions along the trajectory |
| sy | y axis positions along the trajectory |
| until | how many points should be included in the Mean Squared Displacement curve |

Value

A numeric vector containing the values of the MSD

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

| | |
|-------------|---------------------------------------|
| crop.Frames | <i>Cut borders of a Frames object</i> |
|-------------|---------------------------------------|

Description

Performs cropping on the Frames object, selecting how many pixels should be cut on each side

Usage

```
crop.Frames(  
  frames,  
  cutLeft = 5,  
  cutRight = 5,  
  cutUp = 5,  
  cutDown = 5,  
  cutAll = 0,  
  testing = FALSE,  
  ...  
)
```

Arguments

| | |
|----------|---|
| frames | An input Frames object |
| cutLeft | Amount of pixels to be cut at the side |
| cutRight | Amount of pixels to be cut at the side |
| cutUp | Amount of pixels to be cut at the side |
| cutDown | Amount of pixels to be cut at the side |
| cutAll | Amount of pixels to be cut at all sides. Overrides the single side values |
| testing | Logical, whether to just test the cropping or to actually perform it. Default set to FALSE |
| ... | Arguments to be passed to <code>EImage::display()</code> (e.g. setting the method argument) |

Details

Cropping can be performed with careful choice of all cutting sides, or cropping a single value from all sides

Value

A Frames object, with cropped frames in the image slot

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
crop.Frames(MesenteriumSubset)
```

| | |
|---------------|--------------------------------|
| export.Frames | <i>Exports a Frames object</i> |
|---------------|--------------------------------|

Description

Writes the images contained in the image slot of the Frames object elements. The images can be exported as single frames, or as a .gif image that is composed by the single frames.

Usage

```
export.Frames(
  frames,
  dir = tempdir(),
  nameStub = "testExport",
  createGif = FALSE,
  removeAfterCreatingGif = TRUE
)
```


Arguments

| | |
|------------------------|---|
| frames | A Frames object |
| dir | The path of the folder where the image should be written |
| nameStub | The stub for the file name, that will be used as a prefix for the exported images |
| createGif | Logical, whether to create or not an animated .gif file |
| removeAfterCreatingGif | Logical, whether to remove the single exported .png images after creating the single .gif |

Value

Image files are written in the desired location

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
## Not run: export.Frames(MesenteriumSubset,nameStub="subset_export_",
                        createGif=TRUE,removeAfterCreatingGif=FALSE)
## End(Not run)
```

export.particles *Exports a ParticleSet object*

Description

Writes the particles contained in the particles data frame slot of the ParticleSet object elements. A track of the provenience of the particles is stored as a comment line above the header

Usage

```
export.particles(
  particleset,
  dir = tempdir(),
  nameStub = "testExport_particles"
)
```

Arguments

| | |
|-------------|--|
| particleset | A ParticleSet object |
| dir | The path of the folder where the particle sets should be written |
| nameStub | The stub for the file name, that will be used as a prefix for the exported particle sets |

Value

Particle sets files are written in the desired location

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
## Not run: export.particles(candidate.platelets)
```

```
extractKinematics.traj
```

Calculate a set of kinematics parameters from a single trajectory

Description

The computed set of parameters include `delta.x`, `delta.t` and `delta.v` (displacements and instantaneous velocity), `totalTime`, `totalDistance`, `distStartToEnd`, `curvilinearVelocity`, `straightLineVelocity` and `linearityForwardProgression`, Mean Squared Displacement, velocity autocorrelation, and more

Usage

```
extractKinematics.traj(
  trajectoryset,
  trajectoryID,
  acquisitionFrequency = 30,
  scala = 50
)
```

Arguments

| | |
|-----------------------------------|--|
| <code>trajectoryset</code> | A <code>TrajectorySet</code> object |
| <code>trajectoryID</code> | The ID of a single trajectory |
| <code>acquisitionFrequency</code> | The frame rate of acquisition for the images, in milliseconds |
| <code>scala</code> | The value of micro(?)meters to which each single pixel corresponds |

Value

A `KinematicsFeatures` object

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

| | |
|----------------|--|
| flowcatchR-pkg | <i>flowcatchR: analyzing time-lapse microscopy imaging, from detection to tracking</i> |
|----------------|--|

Description

A toolset to analyze in vivo microscopy imaging data focused on tracking flowing blood cells.

Details

flowcatchR is a set of tools to analyze in vivo microscopy imaging data, focused on tracking flowing blood cells. It guides the steps from segmentation to calculation of features, filtering out particles not of interest, providing also a set of utilities to help checking the quality of the performed operations (e.g. how good the segmentation was). The main novel contribution investigates the issue of tracking flowing cells such as in blood vessels, to categorize the particles in flowing, rolling and adherent. This classification is applied in the study of phenomena such as hemostasis and study of thrombosis development.

Author(s)

Federico Marini <marinif@uni-mainz.de>, Johanna Mazur <mazur@uni-mainz.de>, Harald Binder <binderh@uni-mainz.de>, 2015

Maintainer: Federico Marini <marinif@uni-mainz.de>

See Also

Useful links:

- <https://github.com/federicomarini/flowcatchR>
- <https://federicomarini.github.io/flowcatchR/>
- Report bugs at <https://github.com/federicomarini/flowcatchR/issues>

| | |
|--------|--|
| Frames | <i>Constructor for a Frames object</i> |
|--------|--|

Description

Constructor for a Frames object

Usage

Frames(x, channel)

Arguments

x A multi-dimensional Image object
channel A character vector, can be 'red', 'green', 'blue' or 'all' (if in color mode)

Value

The created Frames object.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
inputImg <- Image(MesenteriumSubset)
Frames(inputImg, "red")
```

Frames-class

Frames class

Description

S4 class for storing information on multiple images belonging to the same time-lapse experiment. It is designed as a subclass of the existing Image class from the EBImage package

Slots

channel A character vector, can be 'red', 'green', 'blue' or 'all' (if in color mode)

`initialize.LinkedParticleSet`

Initialize a ParticleSet object for subsequent linking/tracking

Description

Initialize a ParticleSet object for subsequent linking/tracking

Usage

```
initialize.LinkedParticleSet(particleset, linkrange = 1)
```

Arguments

| | |
|-------------|--|
| particleset | A ParticleSet object |
| linkrange | The number of frames to look for candidate particles potentially belonging to the same track |

Value

A ParticleSet object with slots dedicated for the tracking pre-filled

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

| | |
|----------------|---------------------------------------|
| inspect.Frames | <i>Explore the frames of a Frames</i> |
|----------------|---------------------------------------|

Description

The first frames of a Frames are displayed in the browser, and are interactively navigable.

Usage

```
inspect.Frames(
  frames,
  nframes = NULL,
  display.method = "browser",
  verbose = FALSE
)
```

Arguments

| | |
|----------------|---|
| frames | A Frames object |
| nframes | The number of frames to display (default value: NULL, all are displayed) |
| display.method | Method for displaying, can be either raster or browser. Defaults to browser, by opening a window in the browser |
| verbose | Logical, whether to provide additional output on the command line alongside with the images themselves |

Value

inspect.Frames returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
## Not run: inspect.Frames(MesenteriumSubset)
```

| | |
|------------|--|
| kinematics | <i>Calculate a set of kinematics parameter from a TrajectorySet object, or a single parameter, or from a single trajectory (all possible combinations)</i> |
|------------|--|

Description

The computed set of parameters include `delta.x`, `delta.t` and `delta.v` (displacements and instantaneous velocity), `totalTime`, `totalDistance`, `distStartToEnd`, `curvilinearVelocity`, `straightLineVelocity` and `linearityForwardProgression`, Mean Squared Displacement, velocity autocorrelation, and more. If a single trajectory is specified, the computation is performed for that trajectory alone. If a parameter is specified, only that parameter is reported, either for one or all trajectories

Usage

```
kinematics(
  trajectoryset,
  trajectoryIDs = NULL,
  acquisitionFrequency = 30,
  scala = 50,
  feature = NULL
)
```

Arguments

| | |
|-----------------------------------|--|
| <code>trajectoryset</code> | A TrajectorySet object |
| <code>trajectoryIDs</code> | The ID of a single trajectory |
| <code>acquisitionFrequency</code> | The frame rate of acquisition for the images, in milliseconds |
| <code>scala</code> | The value of micro(?)meters to which each single pixel corresponds |
| <code>feature</code> | Character string, the name of the feature to be computed |

Value

A KinematicsFeaturesSet object, or a KinematicsFeatures object, or an atomic value, or a list(eventually coerced to a vector)

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
platelets.trajectories <- trajectories(candidate.platelets)
# for all trajectories, all features
alltrajs.features <- kinematics(platelets.trajectories)
# for one trajectory, all features
traj11features <- kinematics(platelets.trajectories,trajectoryIDs = 11)
# for all trajectories, one feature
alltrajs.curvVel <- kinematics(platelets.trajectories,feature = "curvilinearVelocity")
```

KinematicsFeatures-class

KinematicsFeatures class

Description

S4 class for storing information on all kinematics features identified for a single trajectory

Slots

.Data A list storing the information for the kinematics features

KinematicsFeaturesSet-class

KinematicsFeaturesSet class

Description

S4 class for storing information on all kinematics features identified for all trajectories. Single KinematicsFeatures objects are the element of the main list

Slots

.Data A list storing the information for the sets of kinematics features

length.Frames *Compute the length of render frames in a Frames object*

Description

Compute the length of render frames in a Frames object

Usage

```
## S3 method for class 'Frames'  
length(x)
```

Arguments

x A Frames object

Value

An integer number

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")  
length(MesenteriumSubset)
```

link.particles *Links a ParticleSet object*

Description

Performs linking of the particles by tracking them through the frames

Usage

```
link.particles(  
  particleset,  
  L,  
  R = 2,  
  epsilon1 = 0.1,  
  epsilon2 = 2,  
  lambda1 = 1,
```



```

lambda2 = 1,
penaltyFunction = penaltyFunctionGenerator(),
verboseOutput = FALSE,
prog = FALSE,
include.intensity = TRUE,
include.area = FALSE
)

```

Arguments

| | |
|-------------------|---|
| particleset | A ParticleSet object |
| L | Maximum number of pixels an object can move in two consecutive frames |
| R | Linkrange, i.e. the number of consecutive frames to search for potential candidate links |
| epsilon1 | A numeric value, to be used in the formula. Jitter for allowing angular displacements |
| epsilon2 | A numeric value, to be used in the formula. Jitter for allowing spatial displacements |
| lambda1 | A numeric value. Multiplicative factor for the penalty function |
| lambda2 | A numeric value. Multiplicative factor applied to the angular displacement |
| penaltyFunction | A function structured in such a way to be applied as penalty function in the linking |
| verboseOutput | Logical, whether the output should report additional intermediate steps. For debugging use mainly |
| prog | Logical, whether the a progress bar should be shown during the tracking phase |
| include.intensity | Logical, whether to include also intensity change of the particles in the cost function calculation |
| include.area | Logical, whether to include also area change of the particles in the cost function calculation |

Value

A LinkedParticleSet object

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

References

I F Sbalzarini and P Koumoutsakos. "Feature point tracking and trajectory analysis for video imaging in cell biology." In: Journal of structural biology 151.2 (Aug. 2005), pp. 182-95. ISSN: 1047-8477. DOI: 10.1016/j.jsb.2005.06.002. URL: <http://www.ncbi.nlm.nih.gov/pubmed/16043363>

Examples

```
data("candidate.platelets")
tracked.platelets <- link.particles(candidate.platelets, L= 40)
```

LinkedParticleSet-class

LinkedParticleSet class

Description

S4 class for storing information of particles after they have been tracked. It inherits the slots from the ParticleSet class.

Slots

tracking A list storing all necessary information for the tracking algorithm to work, and for providing the information to the function to determine the trajectories

matchTrajToParticles *Match trajectories to related particles.*

Description

Match trajectories to the related particles in the TrajectorySet and ParticleSet objects. This function returns a new ParticleSet object that contains as additional column the trajectory ID that the particular particle was assigned to. Used also by other routines, such as [snap\(\)](#)

Usage

```
matchTrajToParticles(particleset, trajectoryset)
```

Arguments

particleset A ParticleSet object
trajectoryset A TrajectorySet object coupled to the particleset

Value

A ParticleSet object with an additional column with the trajectory IDs

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2015

Examples

```
data(candidate.platelets)
trajs <- trajectories(candidate.platelets)
matchTrajToParticles(candidate.platelets, trajs)
```

| | |
|-------------------|-------------------------------|
| MesenteriumSubset | <i>A sample Frames object</i> |
|-------------------|-------------------------------|

Description

The sample Frames object is constituted by a subset of a time-lapse intravital microscopy imaging dataset. Green channel marks leukocytes, red channel focuses on blood platelets. 20 frames are provided in this subset. Images are kindly provided by Sven Jaeckel (<Sven.Jaekkel@unimedizin-mainz.de>).

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

| | |
|-----------------|--|
| normalizeFrames | <i>Normalize the values of a Frames object</i> |
|-----------------|--|

Description

Applies a transformation to the Frames object in a way that the intensities throughout the acquisition are normalized overall in term of pixel values sums. It can be used to compensate for example a global change in the illumination values, e.g. due to changed acquisition conditions in experiments that span long timescales.

Usage

```
normalizeFrames(frames, normFun = "median")
```

Arguments

| | |
|---------|---|
| frames | A Frames object to normalize |
| normFun | The normalization function chosen. Can be one of mean or median |

Value

A Frames object with normalized pixel values.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data(MesenteriumSubset)
normalizeFrames(MesenteriumSubset, normFun="median")
```

particles *Extracts particles from the images of a Frames object.*

Description

Extracts particles from the images of a Frames object.

Usage

```
particles(
  raw.frames,
  binary.frames = NULL,
  channel = NULL,
  BPPARAM = bpparam()
)
```

Arguments

| | |
|---------------|--|
| raw.frames | A Frames object with the raw images (mandatory) |
| binary.frames | A Frames object with preprocessed images (optional, if not provided gets produced with standard default parameters) |
| channel | Character string. The channel to perform the operations on. Can be red, green or blue |
| BPPARAM | a MulticoreParam object, used to control the performances inside the BiocParallel call to process frames in parallel by taking advantage of the computing infrastructure available |

Value

A ParticleSet object, containing all detected particles for each frame

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2015

Examples

```
data("MesenteriumSubset")
```

| | |
|-------------------|--------------------------|
| ParticleSet-class | <i>ParticleSet class</i> |
|-------------------|--------------------------|

Description

S4 class for storing information on particles detected in distinct frames.

Slots

.Data A list storing the information for the particles

channel A character vector, can be 'red', 'green', or 'blue'. It refers to which channel the particles were detected

| | |
|--------------------------|------------------------------------|
| penaltyFunctionGenerator | <i>Generate a penalty function</i> |
|--------------------------|------------------------------------|

Description

A function to generate penalty functions to use while linking particles

Usage

```
penaltyFunctionGenerator(
  epsilon1 = 0.1,
  epsilon2 = 2,
  lambda1 = 1,
  lambda2 = 1
)
```

Arguments

| | |
|----------|---|
| epsilon1 | A numeric value, to be used in the formula. Jitter for allowing angular displacements |
| epsilon2 | A numeric value, to be used in the formula. Jitter for allowing spatial displacements |
| lambda1 | A numeric value. Multiplicative factor for the penalty function |
| lambda2 | A numeric value. Multiplicative factor applied to the angular displacement |

Value

A function object, to be used as penalty function

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
custom.function <- penaltyFunctionGenerator(epsilon1=0.1,epsilon2=6,lambda1=1.5,lambda2=0)
```

plot.TrajectorySet *3D representation of a TrajectorySet object*

Description

Provides a visual representation of a TrajectorySet object

Usage

```
## S3 method for class 'TrajectorySet'  
plot(x, frames, verbose = FALSE, ...)
```

Arguments

| | |
|---------|---|
| x | A TrajectorySet object |
| frames | A Frames object, used here to identify the limits of the region of interest |
| verbose | Logical, whether to provide additional output on the command line |
| ... | Arguments to be passed to methods |

Details

Based on the plotly library, the function extracts the region of interests from the dimensions of an image of the Frames object, and afterwards plots the x-y-time representation of the identified trajectories

Value

plot.TrajectorySet returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
data("candidate.platelets")
platelets.trajectories <- trajectories(candidate.platelets)
## Not run:
plot(platelets.trajectories, MesenteriumSubset)

## End(Not run)
```

plot2D.TrajectorySet *2D projection of a TrajectorySet object*

Description

Provides a bird's eye view of a TrajectorySet object on a bidimensional space

Usage

```
plot2D.TrajectorySet(
  trajectoryset,
  frames,
  trajIDs = NULL,
  addGrid = FALSE,
  verbose = FALSE,
  ...
)
```

Arguments

| | |
|---------------|---|
| trajectoryset | A TrajectorySet object |
| frames | A Frames object, used here to identify the limits of the region of interest |
| trajIDs | A vector containing the ids of the desired trajectories |
| addGrid | Logical, add an additional grid to the 2-dimensional plot (visual aid for back-tracking trajectory point locations) |
| verbose | Logical, whether to provide additional output on the command line |
| ... | Arguments to be passed to methods |

Details

This function extracts the region of interests from the dimensions of an image of the Frames object, and afterwards plots the x-y-time representation of the identified trajectories on a 2d plane. It is possible to subset the TrajectorySet object with the IDs of the desired trajectories

Value

plot2D.TrajectorySet returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
data("candidate.platelets")
platelets.trajectories <- trajectories(candidate.platelets)
plot2D.TrajectorySet(platelets.trajectories,MesenteriumSubset)
```

```
preprocess.Frames      Preprocessing function for Frames objects
```

Description

Frames objects are processed according to the chosen set of parameters. Many of them refer directly to existing EImage functions, please see the corresponding help for additional information

Usage

```
preprocess.Frames(
  frames,
  brush.size = 3,
  brush.shape = "disc",
  at.offset = 0.15,
  at.width = 10,
  at.height = 10,
  kern.size = 3,
  kern.shape = "disc",
  ws.tolerance = 1,
  ws.radius = 1,
  displayprocessing = FALSE,
  ...
)
```

Arguments

| | |
|-------------|--|
| frames | A Frames object |
| brush.size | Size in pixels of the brush to be used for initial smoothing (low-pass filtering) |
| brush.shape | Shape of the brush to be used for initial smoothing (low-pass filtering) |
| at.offset | Offset to be used in the adaptive thresholding step - see also EImage::thresh() . As an alternative thresholding method, see also EImage::otsu() in the EImage package. |
| at.width | Width of the window for the adaptive thresholding step - see also EImage::thresh() . As an alternative thresholding method, see also EImage::otsu() in the EImage package. |

| | |
|-------------------|---|
| at.wheight | Height of the window for the adaptive thresholding step - see also <code>EImage::thresh()</code> . As an alternative thresholding method, see also <code>EImage::otsu()</code> in the EImage package. |
| kern.size | Size in pixels of the kernel used for morphological operations - e.g., opening, which is an erosion followed by a dilation, and closing which is a dilation followed by an erosion - see also <code>EImage::opening()</code> , <code>EImage::closing()</code> |
| kern.shape | Shape of the kernel used for morphological operations |
| ws.tolerance | Tolerance allowed in performing the watershed-based segmentation (see also <code>EImage::watershed()</code>) |
| ws.radius | Radius for the watershed-based segmentation (see also <code>EImage::watershed()</code>) |
| displayprocessing | Logical, whether to display intermediate steps while performing preprocessing. Dismissed currently, it could increase runtime a lot |
| ... | Arguments to be passed to methods |

Value

A Frames object, whose frame images are the preprocessed versions of the input images

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
preprocess.Frames(channel.Frames(MesenteriumSubset,"red"))
```

read.Frames *Constructor for a Frames object*

Description

This function is used to create a Frames object from a vector of image files (or a folder specifying the directory containing them). The number of frames is also specified, as just a subset of the images can be used for this

Usage

```
read.Frames(image.files, nframes = NULL)
```

Arguments

| | |
|-------------|---|
| image.files | Vector of strings containing the locations where the (raw) images are to be found, or alternatively, the path to the folder |
| nframes | Number of frames that will constitute the Frames object |

Value

An object of the Frames class, which holds the info on a list of frames, specifying for each the following elements:

image The Image object containing the image itself
 location The complete path to the location of the original image

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
## see vignette
## Not run: fullData <- read.Frames(image.files = "/path/to/the/directory", nframes = 100)
```

read.particles *Constructor for a ParticleSet object*

Description

This function is used to create a ParticleSet object from a vector/list of tab separated text files, each of one containing one line for each particle in the related frame, alongside with its coordinates and if available, the computed features. The number of frames is also specified, as just a subset of the particle lists can be used for this.

Usage

```
read.particles(particle.files, nframes = NULL)
```

Arguments

particle.files Vector of strings containing the locations where the particle coordinates are to be found, or alternatively, the path to the folder
 nframes Number of frames that will constitute the ParticleSet object

Value

An object of the ParticleSet class

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
## see vignette and export.particles
```

repmat *Function equivalent for MATLAB's repmat - Replicate and tile arrays*

Description

A more flexible and stylish alternative to replicate the behaviour of the repmat function of MATLAB

Usage

```
repmat(a, n, m)
```

Arguments

| | |
|---|----------------------------|
| a | The matrix to copy |
| n | The n value for the tiling |
| m | The m value for the tiling |

Value

Creates a large matrix consisting of an m-by-n tiling of copies of a.

Author(s)

Robin Hankin, 2001

References

<http://cran.r-project.org/doc/contrib/R-and-octave.txt>

rotate.Frames *Rotates all images in a Frames object*

Description

Rotation is performed exploiting the rotate function of the EBImage package. Could be automated if support for coordinate/pixel interaction is included

Usage

```
rotate.Frames(frames, angle, testing = FALSE)
```

Arguments

| | |
|---------|--|
| frames | A Frames object |
| angle | The rotation angle (clockwise) specified in degrees |
| testing | Logical, whether to just test the rotation or to actually perform it. Default set to FALSE |

Value

A Frames object containing the rotated frames

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
rotate.Frames(MesenteriumSubset, angle = 40)
```

| | |
|---------------|--|
| select.Frames | <i>Extracts subsets of frames from a Frames object</i> |
|---------------|--|

Description

An input Frames object is subject to subsetting. This function is useful e.g. when the trajectory of interest is presenting gaps (i.e. does not actually include a frame)

Usage

```
select.Frames(frames, framesToKeep = 1, ...)
```

Arguments

| | |
|--------------|--|
| frames | A Frames object |
| framesToKeep | A vector containing the indexes of the frames to keep in the selection |
| ... | Arguments to be passed to methods |

Value

A Frames object, composed by the subset of frames of the input Frames

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
select.Frames(MesenteriumSubset, framesToKeep = c(1:10, 14:20))
```

| | |
|------------------|---|
| select.particles | <i>Performs filtering on a ParticleSet object</i> |
|------------------|---|

Description

According to parameters of interests, such as size, eccentricity/shape, filters out the particles that do not satisfy the indicated requirements

Usage

```
select.particles(particleset, min.area = 1, max.area = 1000)
```

Arguments

| | |
|-------------|--|
| particleset | A ParticleSet object. A LinkedParticleSet object can also be provided as input, yet the returned object will be a ParticleSet object that needs to be linked again |
| min.area | Size in pixels of the minimum area needed to detect the object as a potential particle of interest |
| max.area | Size in pixels of the maximum area allowed to detect the object as a potential particle of interest |

Value

A ParticleSet object

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
selected.platelets <- select.particles(candidate.platelets, min.area = 5)
selected.platelets
```

| | |
|-----------|---|
| shinyFlow | <i>Shiny application for exploring the features and parameters provided by flowcatchR</i> |
|-----------|---|

Description

Launches a Shiny Web Application for interactive data exploration. Default data loaded are the frames from the `MesenteriumSubset` object, custom values can be inserted by typing the location of the data stored in a local folder. The Application is structured in a variety of tabs that mirror the steps in the usual workflow in time-lapse microscopy images. These can allow the user to interactively explore the parameters and their effect in the reactive framework provided by Shiny.

Usage

```
shinyFlow()
```

Value

The Shiny Application is launched in the web browser

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2015

Examples

```
## Not run: shinyFlow()
```

| | |
|--------------------|---|
| show,Frames-method | <i>Display conveniently a Frames object</i> |
|--------------------|---|

Description

Display conveniently a Frames object

Usage

```
## S4 method for signature 'Frames'  
show(object)
```

Arguments

object A Frames object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("MesenteriumSubset")
print(MesenteriumSubset)
```

show, KinematicsFeatures-method

Displaying conveniently a KinematicsFeatures object

Description

Displaying conveniently a KinematicsFeatures object

Usage

```
## S4 method for signature 'KinematicsFeatures'
show(object)
```

Arguments

object A KinematicsFeatures object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
platelets.trajectories <- trajectories(candidate.platelets)
traj11features <- kinematics(platelets.trajectories, trajectoryIDs = 11)
print(traj11features)
```

show,KinematicsFeaturesSet-method

Display conveniently a KinematicsFeatureSet object

Description

Display conveniently a KinematicsFeatureSet object

Usage

```
## S4 method for signature 'KinematicsFeaturesSet'  
show(object)
```

Arguments

object A KinematicsFeatureSet object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")  
platelets.trajectories <- trajectories(candidate.platelets)  
alltrajs.features <- kinematics(platelets.trajectories)  
print(alltrajs.features)
```

show,LinkedParticleSet-method

Display conveniently a LinkedParticleSet object

Description

Display conveniently a LinkedParticleSet object

Usage

```
## S4 method for signature 'LinkedParticleSet'  
show(object)
```


Arguments

object A LinkedParticleSet object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
linked.platelets <- link.particles(candidate.platelets,L=26,R=3,epsilon1=0,
epsilon2=0,lambda1=1,lambda2=0,penaltyFunction=penaltyFunctionGenerator(),
include.area=FALSE)
print(linked.platelets)
```

show,ParticleSet-method

Display conveniently a ParticleSet object

Description

Display conveniently a ParticleSet object

Usage

```
## S4 method for signature 'ParticleSet'
show(object)
```

Arguments

object A ParticleSet object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
print(candidate.platelets)
```

show, TrajectorySet-method

Display conveniently a TrajectorySet object

Description

Display conveniently a TrajectorySet object

Usage

```
## S4 method for signature 'TrajectorySet'  
show(object)
```

Arguments

object A TrajectorySet object

Value

This returns an invisible NULL.

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")  
platelets.trajectories <- trajectories(candidate.platelets)  
print(platelets.trajectories)
```

snap

Snap the features of the closest particle identified

Description

This function combines all classes related to a single experiment in order to deliver a clickable feedback on one of the frames.

Usage

```

snap(
  raw.frames,
  binary.frames,
  particleset,
  trajectoryset,
  frameID = 1,
  infocol = "yellow",
  infoceX = 1,
  showVelocity = FALSE
)

```

Arguments

| | |
|----------------------------|--|
| <code>raw.frames</code> | A Frames object with the raw frames data |
| <code>binary.frames</code> | A Frames object with the preprocessed frames data |
| <code>particleset</code> | A ParticleSet object with the particles data |
| <code>trajectoryset</code> | A TrajectorySet object with the trajectories data |
| <code>frameID</code> | The ID of the frame to inspect |
| <code>infocol</code> | The color to use for plotting the contours and the information on the clicked particle |
| <code>infoceX</code> | The numeric character expansion value as in <code>cex</code> to be used for printing the text on the image |
| <code>showVelocity</code> | Logical, whether to display additional information on the instantaneous velocity of the particle |

Value

An image of the selected frame, rendered in R native graphics, and additionally a list with the coordinates as well as the trajectory ID of the particle closest to the clicked location

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2015

Examples

```

## Not run: data(MesenteriumSubset)
binary.frames <- preprocess.Frames(channel.Frames(MesenteriumSubset,"red"))
particleset <- particles(MesenteriumSubset,binary.frames,"red")
trajectoryset <- trajectories(particleset)
snap(MesenteriumSubset,binary.frames,particleset,trajectoryset,frameID=1)

## End(Not run)

```

toCartesianCoords *Converts polar coordinates to cartesian coordinates*

Description

Conversion from (radius,theta) to (x,y)

Usage

toCartesianCoords(Theta, Radius)

Arguments

| | |
|--------|---------------------------------------|
| Theta | The Theta angle |
| Radius | The radius value in polar coordinates |

Value

A list containing Theta and Radius, as in polar coordinates

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

toPolarCoords *Converts cartesian coordinates to polar coordinates*

Description

Conversion from (x,y) to (radius,theta)

Usage

toPolarCoords(x, y)

Arguments

| | |
|---|--------------|
| x | x coordinate |
| y | y coordinate |

Value

A list containing Theta and Radius, as in polar coordinates

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

| | |
|--------------|------------------------------|
| trajectories | <i>Generate trajectories</i> |
|--------------|------------------------------|

Description

Generates a TrajectorySet object from a (Linked)ParticleSystem

Usage

```
trajectories(particleset, verbose = FALSE, ...)
```

Arguments

| | |
|-------------|---|
| particleset | A (Linked)ParticleSystem object |
| verbose | Logical, currently not used - could be introduced for providing additional info on the trajectories |
| ... | Arguments to be passed to methods |

Value

A TrajectorySet object

Author(s)

Federico Marini, <marinif@uni-mainz.de>, 2014

Examples

```
data("candidate.platelets")
platelets.trajectories <- trajectories(candidate.platelets)
```

| | |
|---------------------|----------------------------|
| TrajectorySet-class | <i>TrajectorySet class</i> |
|---------------------|----------------------------|

Description

S4 class for storing information on the trajectories identified, including whether there were gaps, the number of points, and more

Slots

.Data A list storing the information for the particles
channel A character vector, can be 'red', 'green', or 'blue'. It refers to which channel the particles were detected

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