# Package 'EBImage'

July 20, 2025

Version 4.50.0

Title Image processing and analysis toolbox for R

**Encoding** UTF-8

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**Depends** methods

**Imports** BiocGenerics (>= 0.7.1), graphics, grDevices, stats, abind, tiff, jpeg, png, locfit, fftwtools (>= 0.9-7), utils, htmltools, htmlwidgets, RCurl

Suggests BiocStyle, digest, knitr, rmarkdown, shiny

Description EBImage provides general purpose functionality for image processing and analysis. In the context of (high-throughput) microscopy-based cellular assays, EBImage offers tools to segment cells and extract quantitative cellular descriptors. This allows the automation of such tasks using the R programming language and facilitates the use of other tools in the R environment for signal processing, statistical modeling, machine learning and visualization with image data.

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LazyLoad true
biocViews Visualization
VignetteBuilder knitr

URL https://github.com/aoles/EBImage

BugReports https://github.com/aoles/EBImage/issues
git\_url https://git.bioconductor.org/packages/EBImage

git\_branch RELEASE\_3\_21
git\_last\_commit a01be15
git\_last\_commit\_date 2025-04-15

**Repository** Bioconductor 3.21 **Date/Publication** 2025-07-20

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abind

Combine Image Arrays

### **Description**

Methods for function abind from package abind useful for combining Image arrays.

#### Value

An Image object or an array, containing the combined data arrays of the input objects.

#### Usage

```
abind(...)
```

#### **Arguments**

... Arguments to abind

#### Methods

```
signature(... = "Image") This method is defined primarily for the sake of preserving the class of the combined Image objects. Unlike the original abind function, if dimnames for all combined objects are NULL it does not introduce a list of empty dimnames for each dimension.
```

```
signature(... = "ANY") Dispatches to the original abind function.
```

### Author(s)

```
Andrzej Oleś, <andrzej.oles@embl.de>, 2017
```

#### See Also

combine provides a more convenient interface to merging images into an image sequence. Use tile to lay out images next to each other in a regular grid.

```
f = system.file("images", "sample-color.png", package="EBImage")
x = readImage(f)

## combine images horizontally
y = abind(x, x, along=1)
display(y)

## stack images one on top of the other
z = abind(x, x, along=2)
display(z)
```

4 bwlabel

bwlabel

Binary segmentation

### **Description**

Labels connected (connected sets) objects in a binary image.

# Usage

```
bwlabel(x)
```

### **Arguments**

Χ

An Image object or an array. x is considered as a binary image, whose pixels of value 0 are considered as background ones and other pixels as foreground ones.

#### **Details**

All pixels for each connected set of foreground (non-zero) pixels in x are set to an unique increasing integer, starting from 1. Hence, max(x) gives the number of connected objects in x.

### Value

A Grayscale Image object or an array, containing the labelled version of x.

#### Author(s)

Gregoire Pau, 2009

### See Also

computeFeatures, propagate, watershed, paintObjects, colorLabels

```
## simple example
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
display(x, title='Binary')
y = bwlabel(x)
display(normalize(y), title='Segmented')

## read nuclei images
x = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
display(x)

## computes binary mask
y = thresh(x, 10, 10, 0.05)
y = opening(y, makeBrush(5, shape='disc'))
```

channel 5

```
display(y, title='Cell nuclei binary mask')
## bwlabel
z = bwlabel(y)
display(normalize(z), title='Cell nuclei')
nbnuclei = apply(z, 3, max)
cat('Number of nuclei=', paste(nbnuclei, collapse=','),'\n')
## paint nuclei in color
cols = c('black', sample(rainbow(max(z))))
zrainbow = Image(cols[1+z], dim=dim(z))
display(zrainbow, title='Cell nuclei (recolored)')
```

channel

Color and image color mode conversions

### **Description**

channel handles color space conversions between image modes. rgbImage combines Grayscale images into a Color one. toRGB is a wrapper function for convenient grayscale to RGB color space conversion; the call toRGB(x) returns the result of channel(x, 'rgb').

### Usage

```
channel(x, mode)
rgbImage(red, green, blue)
toRGB(x)
```

### **Arguments**

x An Image object or an array.

mode A character value specifying the target mode for conversion. See Details.

red, green, blue Image objects in Grayscale color mode or arrays of the same dimension. If

missing, a black image will be used.

#### **Details**

#### Conversion modes:

rgb Converts a Grayscale image or an array into a Color image, replicating RGB channels.

gray, grey Converts a Color image into a Grayscale image, using uniform 1/3 RGB weights.

luminance Luminance-preserving Color to Grayscale conversion using CIE 1931 luminance weights: 0.2126 \* R + 0.7152 \* G + 0.0722 \* B.

red, green, blue Extracts the red, green or blue channel from a Color image. Returns a Grayscale image.

asred, asgreen, asblue Converts a Grayscale image or an array into a Color image of the specified hue.

NOTE: channel changes the pixel intensities, unlike colorMode which just changes the way that EBImage renders an image.

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### Value

An Image object or an array.

### Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>
```

### See Also

colorMode

### **Examples**

```
x = readImage(system.file("images", "shapes.png", package="EBImage"))
display(x)
y = channel(x, 'asgreen')
display(y)

## rgbImage
x = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
y = readImage(system.file('images', 'cells.tif', package='EBImage'))
display(x, title='Cell nuclei')
display(y, title='Cell bodies')

cells = rgbImage(green=1.5*y, blue=x)
display(cells, title='Cells')
```

clahe

Contrast Limited Adaptive Histogram Equalization

### **Description**

Improve contrast locally by performing adaptive histogram equalization.

### Usage

```
clahe(x, nx = 8, ny = nx, bins = 256, limit = 2, keep.range = FALSE)
```

### **Arguments**

| X          | an Image object or an array.   |
|------------|--|
| nx         | integer, number of contextual regions in the X direction (min 2, max 256)  |
| ny         | integer, number of contextual regions in the Y direction (min 2, max 256)  |
| bins       | integer, number of greybins for histogram ("dynamic range"). Smaller values (eg. 128) speed up processing while still producing good quality output. |
| limit      | double, normalized clip limit (higher values give more contrast). A clip limit smaller than 0 results in standard (non-contrast limited) AHE.        |
| keep.range | logical, retain image minimum and maximum values rather then use the full available range  |

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#### **Details**

Adaptive histogram equalization (AHE) is a contrast enhancement technique which overcomes the limitations of standard histogram equalization. Unlike ordinary histogram equalization the adaptive method redistributes the lightness values of the image based on several histograms, each corresponding to a distinct section of the image. It is therefore useful for improving the local contrast and enhancing the definitions of edges in each region of an image. However, AHE has a tendency to overamplify noise in relatively homogeneous regions of an image. Contrast limited adaptive histogram equalization (CLAHE) prevents this by limiting the amplification.

The function is based on the implementation by Karel Zuiderveld [1]. This implementation assumes that the X- and Y image dimensions are an integer multiple of the X- and Y sizes of the contextual regions. The input image x should contain pixel values in the range from 0 to 1, inclusive; values lower than 0 or higher than 1 are clipped before applying the filter. Internal processing is performed in 16-bit precision. If the image contains multiple channels or frames, the filter is applied to each one of them separately.

#### Value

An Image object or an array, containing the filtered version of x.

#### Note

The interpolation step of the original implementation by Karel Zuiderveld [1] was modified to use double precision arithmetic in order to make the filter rotationally invariant for even-sized contextual regions, and the result is properly rounded rather than truncated towards 0 in order to avoid a systematic shift of pixel values.

#### Author(s)

```
Andrzej Oleś, <andrzej.oles@embl.de>, 2017
```

### References

[1] K. Zuiderveld: Contrast Limited Adaptive Histogram Equalization. In: P. Heckbert: Graphics Gems IV, Academic Press 1994

#### See Also

```
equalize
```

```
x = readImage(system.file("images", "sample-color.png", package="EBImage"))
y = clahe(x)
display(y)
```

8 colorLabels

colorLabels

Color Code Labels

### **Description**

Color codes the labels of object masks by a random permutation.

### Usage

```
colorLabels(x, normalize = TRUE)
```

### **Arguments**

x an Image object in Grayscale color mode or an array containing object masks.

Object masks are sets of pixels with the same unique integer value

normalize if TRUE normalizes the resulting color image

#### **Details**

Performs color coding of object masks, which are typically obtained using the bwlabel function. Each label from x is assigned an unique color. The colors are distributed among the labels using a random permutation. If normalize is set to TRUE the intensity values of the resulting image are mapped to the [0,1] range.

### Value

An Image object containing color coded objects of x.

### Author(s)

```
Bernd Fischer, Andrzej Oles, 2013-2014
```

### See Also

```
bwlabel, normalize
```

```
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
y = bwlabel(x)
z = colorLabels(y)
display(z, title='Colored segmentation')
```

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colormap

Map a Greyscale Image to Color

### **Description**

Maps a greyscale image to color using a color palette.

# Usage

```
colormap(x, palette = heat.colors(256L))
```

### **Arguments**

x an Image object of color mode Grayscale, or an array palette character vector containing the color palette

### **Details**

The colormap function first linearly maps the pixel intensity values of x to the integer range 1:length(palette). It then uses these values as indices to the provided color palette to create a color version of the original image.

The default palette contains 256 colors, which is the typical number of different shades in a 8bit grayscale image.

#### Value

An Image object of color mode Color, containing the color-mapped version of x.

### Author(s)

```
Andrzej Oleś, <andrzej.oles@embl.de>, 2016
```

```
x = readImage(system.file("images", "sample.png", package="EBImage"))

## posterize an image using the topo.colors palette
y = colormap(x, topo.colors(8))

display(y, method="raster")

## mimic MatLab's 'jet.colors' colormap
jet.colors = colorRampPalette(c("#00007F", "blue", "#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7
y = colormap(x, jet.colors(256))

display(y, method="raster")
```

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combine

Combine images

### **Description**

Merges images to create image sequences.

### Usage

```
combine(x, y, ...)
```

### **Arguments**

x An Image object, an array, or a list containing Image objects and arrays.

y An Image object or an array.

... Image objects or arrays.

### **Details**

The function combine uses abind to merge multi-dimensional arrays along the dimension depending on the color mode of x. If x is a Grayscale image or an array, image objects are combined along the third dimension, whereas when x is a Color image they are combined along the fourth dimension, leaving room on the third dimension for color channels.

#### Value

An Image object or an array.

### Author(s)

Gregoire Pau, Andrzej Oles, 2013

#### See Also

The method abind provides a more flexible interface which allows to specify the dimension along which to combine the images.

```
## combination of color images
img = readImage(system.file("images", "sample-color.png", package="EBImage"))[257:768,,]
x = combine(img, flip(img), flop(img))
display(x, all=TRUE)

## Blurred images
x = resize(img, 128, 128)
xt = list()
for (t in seq(0.1, 5, length.out=9)) xt=c(xt, list(gblur(x, s=t)))
```

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```
xt = combine(xt)
display(xt, title='Blurred images', all=TRUE)
```

computeFeatures Compute object features

### **Description**

Computes morphological and texture features from image objects.

#### Usage

```
compute Features (\texttt{x}, \texttt{ref}, \texttt{methods}. \texttt{noref=c("compute Features}. \texttt{moment"}, \texttt{"compute Features}. \texttt{shape"}),
 methods.ref=c("computeFeatures.basic", "computeFeatures.moment", "computeFeatures.haralick"),
 xname="x", refnames, properties=FALSE, expandRef=standardExpandRef, ...)
computeFeatures.basic(x, ref, properties=FALSE, basic.quantiles=c(0.01, 0.05, 0.5, 0.95, 0.99), xs, ..
computeFeatures.shape(x, properties=FALSE, xs, ...)
computeFeatures.moment(x, ref, properties=FALSE, xs, ...)
computeFeatures.haralick(x, ref , properties=FALSE, haralick.nbins=32, haralick.scales=c(1, 2), xs, ..
standardExpandRef(ref, refnames, filter = gblob())
```

# **Arguments** Х

ref

A matrix or a list of matrices, containing the intensity values of the reference objects. methods.noref A character vector containing the function names to be called to compute features without reference intensities. Default is computeFeatures.moment and computeFeatures.shape. methods.ref A character vector containing the function names to be called to compute features with reference intensities. Default is computeFeatures.basic, computeFeatures.moment and computeFeatures.haralick. A character string naming the object layer. Default is x. xname

An Image object or an array containing labelled objects. Labelled objects are

refnames A character vector naming the reference intensity layers. Default are the names of ref, if present. If not, reference intensity layers are named using lower-case letters.

pixel sets with the same unique integer value.

A logical. If FALSE, the default, the function returns the feature matrix. If TRUE, the function returns feature properties.

expandRef A function used to expand the reference images. Default is standardExpandRef.

See Details. basic.quantiles

properties

A numerical vector indicating the quantiles to compute.

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haralick.nbins An integer indicating the number of bins using to compute the Haralick matrix. See Details.

haralick.scales

A integer vector indicating the number of scales to use to compute the Haralick

features.

xs An optional temporary object created by computeFeatures used for perfor-

mance considerations.

filter The filter applied to reference images using filter2 in order to add granulom-

etry.

... Optional arguments passed to the feature computation functions.

#### **Details**

Features are named x.y.f, where x is the object layer, y the reference image layer and f the feature name. Examples include cell.dna.mean, indicating mean DNA intensity computed in the cell or nucleus.tubulin.cx, indicating the x center of mass of tubulin computed in the nucleus region.

The function computeFeatures computes sets of features. Features are organized in 4 sets, each computed by a different function. The function computeFeatures.basic computes spatial-independent statistics on pixel intensities:

• b.mean: mean intensity

· b.sd: standard deviation intensity

• b.mad: mad intensity

• b.q\*: quantile intensity

The function computeFeatures. shape computes features that quantify object shape:

- s.area: area size (in pixels)
- s.perimeter: perimeter (in pixels)
- s.radius.mean: mean radius (in pixels)
- s.radius.sd: standard deviation of the mean radius (in pixels)
- s.radius.max: max radius (in pixels)
- s.radius.min: min radius (in pixels)

The function computeFeatures.moment computes features related to object image moments, which can be computed with or without reference intensities:

- m.cx: center of mass x (in pixels)
- m.cy: center of mass y (in pixels)
- m.majoraxis: elliptical fit major axis (in pixels)
- m.eccentricity: elliptical eccentricity defined by sqrt(1-minoraxis^2/majoraxis^2). Circle eccentricity is 0 and straight line eccentricity is 1.
- m.theta: object angle (in radians)

The function computeFeatures.haralick computes features that quantify pixel texture. Features are named according to Haralick's original paper.

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### Value

If properties if FALSE (by default), computeFeatures returns a matrix of n cells times p features, where p depends of the options given to the function. Returns NULL if no object is present.

If properties if TRUE, computeFeatures returns a matrix of p features times 2 properties (translation and rotation invariance). Feature properties are useful to filter out features that may not be needed for specific tasks, e.g. cell position when doing cell classification.

### Author(s)

```
Gregoire Pau, <gregoire.pau@embl.de>, 2011
```

#### References

R. M. Haralick, K Shanmugam and Its'Hak Deinstein (1979). *Textural Features for Image Classification*. IEEE Transactions on Systems, Man and Cybernetics.

#### See Also

```
bwlabel, propagate
```

```
## load and segment nucleus
y = readImage(system.file("images", "nuclei.tif", package="EBImage"))[,,1]
x = thresh(y, 10, 10, 0.05)
x = opening(x, makeBrush(5, shape='disc'))
x = bwlabel(x)
display(y, title="Cell nuclei")
display(x, title="Segmented nuclei")
## compute shape features
fts = computeFeatures.shape(x)
## compute features
ft = computeFeatures(x, y, xname="nucleus")
cat("median features are:\n")
apply(ft, 2, median)
## compute feature properties
ftp = computeFeatures(x, y, properties=TRUE, xname="nucleus")
ftp
```

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|--|

### **Description**

Display images in an interactive JavaScript viewer or using R's built-in graphics capabilities.

#### Usage

```
display(x, method, ...)
## S3 method for class 'Image'
plot(x, ...)
```

### **Arguments**

x an Image object or an array.

method the way of displaying images. Defaults to "browser" when R is used interac-

tively, and to "raster" otherwise. The default behavior can be overridden by

setting options ("EBImage.display"). See Details.

... arguments to be passed to the specialized display functions; for details see the

sections on individual display methods.

#### Details

The default method used for displaying images depends on whether called from and interactive R session. If interactive() is TRUE images are displayed with the "browser" method, otherwise the "raster" method is used. This dynamic behavior can be overridden by setting options("EBImage.display") to either "browser" or "raster".

```
plot.Image S3 method is a wrapper for display(..., method="raster")
```

### "browser" method

The "browser" method runs an interactive JavaScript image viewer. A list of available features along with corresponding mouse and keyboard actions is shown by pressing 'h'. This method takes the following additional arguments.

embed logical(1), include images in the document as data URIs. Defaults to TRUE in non-interactive context (e.g. static R Markdown documents), otherwise to FALSE.

tempDir character(1), file path for storing any temporary image files. Defaults to tempfile("")

... arguments passed to createWidget, such as fixed width and height (in CSS units), elementId, or preRenderHook.

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#### "raster" method

The "raster" method displays images as R raster graphics. The user coordinates of the plotting region are set to the image pixel coordinates with the origin (0, 0) in the upper left corner.

By default only the first frame of an image stack is shown; a different frame can also be specified. When all=TRUE the whole image stack is rendered and the frames are automatically positioned next to each other in a grid. The grid layout can be modified through nx and spacing and margin.

This method provides to following additional arguments to display.

interpolate a logical vector (or scalar) indicating whether to apply linear interpolation to the image when drawing.

frame a numeric indicating the frame number to display; only effective when all = FALSE.

- all logical, defaulting to FALSE. If set to TRUE, all frames of a stacked image are displayed arranged in a grid, otherwise (default) just a single frame specified in frame is displayed. The grid layout can be controlled by nx, spacing and margin.
- drawGrid a logical indicating whether to draw grid lines between individual frames. Defaults to TRUE unless spacing is non-zero. Line color, type and width can be specified through graphical parameters col, 1ty and 1wd, respectively; see par for details.
- nx integer. Specifies the number images in a row. Negative numbers are interpreted as the number of images in a column, e.g. use -1 to display a single row containing all the frames.
- spacing numeric. Specifies the separation between frames as a fraction of frame dimensions (positive numbers <1) or in pixels (numbers >=1). It can be either a single number or a vector of length 2, in which case its elements correspond to the horizontal and vertical spacing, respectively.
- margin numeric. Specifies the outer margins around the image, or a grid of images. Similarly as for spacing, different horizontal and vertical margins can be defined by providing a vector.
- ... graphical parameters passed to par

#### Author(s)

Andrzej Oles, <andrzej.oles@embl.de>, 2012-2017

#### See Also

```
display-shiny
```

```
## Display a single image
x = readImage(system.file("images", "sample-color.png", package="EBImage"))[257:768,,]
display(x)

## Display a thresholded sequence ...
y = readImage(system.file("images", "sample.png", package="EBImage"))[366:749, 58:441]
z = lapply(seq(from=0.5, to=5, length.out=6),
    function(s) gblur(y, s, boundary="replicate")
)
z = combine(z)
```

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display-shiny

Shiny Bindings for display

### **Description**

Output and render functions for using the interactive image viewer within Shiny applications and interactive R Markdown documents.

### Usage

```
displayOutput(outputId, width = "100%", height = "500px")
renderDisplay(expr, env = parent.frame(), quoted = FALSE)
```

### **Arguments**

| outputId      | output variable to read from   |
|---------------|--|
| width, height | Must be a valid CSS unit (like '100%', '400px', 'auto') or a number, which will be coerced to a string and have 'px' appended. |
| expr          | An expression that generates the image viewer (typicall through a call to ${\tt display}$ )                                    |
| env           | The environment in which to evaluate expr.   |
| quoted        | Is expr a quoted expression (with quote())? This is useful if you want to save an expression in a variable.                    |

### See Also

display

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```
# Only run this example in interactive R sessions
if (interactive()) {
 options(device.ask.default = FALSE)
 require("shiny")
 ui <- fluidPage(
    # Application title
    titlePanel("Image display"),
    # Sidebar with a select input for the image
    sidebarLayout(
      sidebarPanel(
     selectInput("image", "Sample image:", list.files(system.file("images", package="EBImage")))
     ),
      # Show a plot of the generated distribution
      mainPanel(
        tabsetPanel(
          tabPanel("Static raster", plotOutput("raster")),
          tabPanel("Interactive browser", displayOutput("widget"))
        )
     )
   )
 )
 server <- function(input, output) {</pre>
    img <- reactive({</pre>
     f = system.file("images", input$image, package="EBImage")
     readImage(f)
    })
   output$widget <- renderDisplay({</pre>
      display(img())
   })
    output$raster <- renderPlot({</pre>
      plot(img(), all=TRUE)
    })
 }
 # Run the application
 shinyApp(ui = ui, server = server)
}
```

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distmap

Distance map transform

### **Description**

Computes the distance map transform of a binary image. The distance map is a matrix which contains for each pixel the distance to its nearest background pixel.

### Usage

```
distmap(x, metric=c('euclidean', 'manhattan'))
```

### **Arguments**

| X      | An Image object or an array. x is considered as a binary image, whose pixels of value 0 are considered as background ones and other pixels as foreground ones. |
|--------|--|
| metric | A character indicating which metric to use, L1 distance (manhattan) or L2 distance (euclidean). Default is euclidean.  |

### **Details**

A fast algorithm of complexity O(M\*N\*log(max(M,N))), where (M,N) are the dimensions of x, is used to compute the distance map.

### Value

An Image object or an array, with pixels containing the distances to the nearest background points.

### Author(s)

```
Gregoire Pau, <gpau@ebi.ac.uk>, 2008
```

### References

M. N. Kolountzakis, K. N. Kutulakos. Fast Computation of the Euclidean Distance Map for Binary Images, Infor. Proc. Letters 43 (1992).

```
x = readImage(system.file("images", "shapes.png", package="EBImage"))
display(x)
dx = distmap(x)
display(dx/10, title='Distance map of x')
```

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| Draw a circle on an image. | rawCircle | rawCircle |
|----------------------------|-----------|-----------|
|----------------------------|-----------|-----------|

# Description

Draw a circle on an image.

# Usage

```
drawCircle(img, x, y, radius, col, fill=FALSE, z=1)
```

# Arguments

| img          | An Image object or an array.   |
|--------------|--|
| x, y, radius | numerics indicating the center and the radius of the circle.                               |
| col          | A numeric or a character string specifying the color of the circle.                        |
| fill         | A logical indicating whether the circle should be filled. Default is FALSE.                |
| Z            | A numeric indicating on which frame of the image the circle should be drawn. Default is 1. |

### Value

An Image object or an array, containing the transformed version of img.

### Author(s)

```
Gregoire Pau, 2010
```

```
## Simple white circle
x = matrix(0, nrow=300, ncol=300)
y = drawCircle(x, 100, 200, 47, col=1)
display(y)

## Simple filled yellow circle
x = channel(y, 'rgb')
y = drawCircle(x, 200, 140, 57, col='yellow', fill=TRUE)
display(y)
```

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**EBImage** 

Package overview

# Description

EBImage is an image processing and analysis package for R. Its primary goal is to enable automated analysis of large sets of images such as those obtained in high throughput automated microscopy.

EBImage relies on the Image object to store and process images but also works on multi-dimensional arrays.

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- haralickFeatures, haralickMatrix
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### **Authors**

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UK
```

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### **Examples**

```
example(readImage)
example(display)
example(rotate)
example(propagate)
```

EBImage-defunct

Defunct functions in package 'EBImage'

### **Description**

These functions are defunct and no longer available.

#### **Details**

The following functions are defunct and no longer available; use the replacement indicated below.

- animate: display
- blur: gblur
- drawtext: see package vignette for documentation on how to add text labels to images
- drawfont: see package vignette for documentation on how to add text labels to images
- getFeatures: computeFeatures
- getNumberOfFrames: numberOfFrames
- hullFeatures: computeFeatures.shape
- zernikeMoments: computeFeatures
- edgeProfile: computeFeatures
- edgeFeatures: computeFeatures.shape
- haralickFeatures: computeFeatures
- haralickMatrix: computeFeatures
- moments: computeFeatures.moment

equalize 23

```
• cmoments: computeFeatures.moment
```

• rmoments: computeFeatures.moment

• smoments: computeFeatures.moment

• dilateGreyScale: dilate

• erodeGreyScale: erode

openingGreyScale: openingclosingGreyScale: closing

whiteTopHatGreyScale: whiteTopHat

blackTopHatGreyScale: blackTopHat

• selfcomplementaryTopHatGreyScale: selfComplementaryTopHat

equalize

Histogram Equalization

### Description

Equalize the image histogram to a specified range and number of levels.

### Usage

```
equalize(x, range = c(0, 1), levels = 256)
```

#### **Arguments**

x an Image object or an array

range numeric vector of length 2, the output range of the equalized histogram

levels number of grayscale levels (Grayscale images) or intensity levels of each chan-

nel (Color images)

### **Details**

Histogram equalization is an adaptive image contrast adjustment method. It flattens the image histogram by performing linearization of the cumulative distribution function of pixel intensities.

Individual channels of Color images and frames of image stacks are equalized separately.

### Value

An Image object or an array, containing the transformed version of x.

### Author(s)

```
Andrzej Oles, <andrzej.oles@embl.de>, 2014
```

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### See Also

clahe

### **Examples**

```
x = readImage(system.file('images', 'cells.tif', package='EBImage'))
hist(x)
y = equalize(x)
hist(y)
display(y, title='Equalized Grayscale Image')

x = readImage(system.file('images', 'sample-color.png', package='EBImage'))
hist(x)
y = equalize(x)
hist(y)
display(y, title='Equalized Grayscale Image')
```

fillHull

Fill holes in objects

### **Description**

Fill holes in objects.

### Usage

```
fillHull(x)
```

### **Arguments**

Х

An Image object or an array.

### **Details**

fillHull fills holes in the objects defined in x, where objects are sets of pixels with the same unique integer value.

# Value

An Image object or an array, containing the transformed version of x.

### Author(s)

```
Gregoire Pau, Oleg Sklyar; 2007
```

### See Also

bwlabel

filter2

### **Examples**

```
x = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
display(x)

y = thresh(x, 10, 10, 0.05)
display(y, title='Cell nuclei')

y = fillHull(y)
display(y, title='Cell nuclei without holes')
```

filter2

2D Convolution Filter

## Description

Filters an image using the fast 2D FFT convolution product.

### Usage

```
filter2(x, filter, boundary = c("circular", "replicate"))
```

### **Arguments**

x An Image object or an array.

filter An Image object or an array, with odd spatial dimensions. Must contain only

one frame.

boundary Behaviour at image borders. The default is to wrap the image around borders.

For other modes see details.

### **Details**

Linear filtering is useful to perform low-pass filtering (to blur images, remove noise...) and high-pass filtering (to detect edges, sharpen images). The function makeBrush is useful to generate filters.

The default "circular" behaviour at boundaries is to wrap the image around borders. In the "replicate" mode pixels outside the bounds of the image are assumed to equal the nearest border pixel value. Numeric values of boundary yield linear convolution by padding the image with the given value(s).

If x contains multiple frames, the filter is applied separately to each frame.

#### Value

An Image object or an array, containing the filtered version of x.

### Author(s)

Andrzej Oleś, Gregoire Pau

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### See Also

```
makeBrush, convolve, fft, blur
```

### **Examples**

```
x = readImage(system.file("images", "sample-color.png", package="EBImage"))
display(x, title='Sample')
## Low-pass disc-shaped filter
f = makeBrush(21, shape='disc', step=FALSE)
display(f, title='Disc filter')
f = f/sum(f)
y = filter2(x, f)
display(y, title='Filtered image')
## Low-pass filter with linear padded boundary
y = filter2(x, f, boundary=c(0, .5, 1))
display(y, title='Filtered image with linear padded boundary')
## High-pass Laplacian filter
la = matrix(1, nc=3, nr=3)
la[2,2] = -8
y = filter2(x, la)
display(y, title='Filtered image')
## High-pass Laplacian filter with replicated boundary
y = filter2(x, la, boundary='replicate')
display(y, title='Filtered image with replicated boundary')
```

floodFill

Region filling

### **Description**

Fill regions in images.

#### **Usage**

```
floodFill(x, pts, col, tolerance=0)
```

### **Arguments**

Χ

An Image object or an array.

pts

Coordinates of the start filling points given as either of the following: a vector of the form c(x1, y1, x2, y2, ...), a list of points, a matrix or data frame where rows represent points and columns are the x and y coordinates. For image stacks different points for each frame can be specified by providing them in a list of length matching the number of 'render' frames.

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col Fill color. This argument should be a numeric for Grayscale images and an R

color for Color images. Values are recycled such that their length matches the number of points in pts. Can be a list of length matching the number of 'render'

frames similarly as pts.

tolerance Color tolerance used during the fill.

#### **Details**

Flood fill is performed using the fast scan line algorithm. Filling starts at pts and grows in connected areas where the absolute difference of the pixels intensities (or colors) remains below tolerance.

#### Value

An Image object or an array, containing the transformed version of x.

### Author(s)

Gregoire Pau, Oleg Sklyar; 2007

```
x = readImage(system.file("images", "shapes.png", package="EBImage"))
## fill a shape with 50% shade of gray
y = floodFill(x, c(67, 146), 0.5)
display(y)
## fill with color
y = toRGB(y)
y = floodFill(y, c(48, 78), 'orange')
display(y)
## fill multiple shapes with different colors
y = y[110:512,1:130,]
points = rbind(c(50, 50), c(100, 50), c(150, 50))
colors = c("red", "green", "blue")
y = floodFill(y, points, colors)
display(y)
## area fill
x = readImage(system.file("images", "sample.png", package="EBImage"))
y = floodFill(x, rbind(c(200, 400), c(200, 325)), 1, tolerance=0.1)
display(y)
## application to image stacks
f = system.file("images", "nuclei.tif", package="EBImage")
x = readImage(f)[1:250,1:250,]
x = opening(thresh(x, 12, 12), makeBrush(5, shape='disc'))
xy = lapply(getFrames(bwlabel(x)), function(x) computeFeatures.moment(x)[,1:2])
y = floodFill(toRGB(x), xy, c("red", "green", "blue"))
display(y)
```

28 gblur

gblur

Low-pass Gaussian filter

### **Description**

Filters an image with a low-pass Gaussian filter.

### Usage

```
gblur(x, sigma, radius = 2 * ceiling(3 * sigma) + 1, ...)
```

# Arguments

| X      | An Image object or an array.  |
|--------|---|
| sigma  | A numeric denoting the standard deviation of the Gaussian filter used for blurring. |
| radius | The radius of the filter in pixels. Default is 2*ceiling(3*sigma)+1).               |
|        | Arguments passed to filter2.  |

### **Details**

The Gaussian filter is created with the function makeBrush.

### Value

An Image object or an array, containing the filtered version of x.

### Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2005-2007
```

### See Also

```
filter2, makeBrush
```

```
x = readImage(system.file("images", "sample.png", package="EBImage"))
display(x)

y = gblur(x, sigma=8)
display(y, title='gblur(x, sigma=8)')
```

29 Image

Image class Image

### **Description**

EBImage uses the Image class to store and process images. Images are stored as multi-dimensional arrays containing the pixel intensities. Image extends the base class array and uses the colormode slot to store how the color information of the multi-dimensional data is handled.

The colormode slot can be either Grayscale or Color. In either mode, the first two dimensions of the underlying array are understood to be the spatial dimensions of the image. In the Grayscale mode the remaining dimensions contain other image frames. In the Color mode, the third dimension contains color channels of the image, while higher dimensions contain image frames. The number of channels is not limited and can be any number >= 1; these can be, for instance, the red, green, blue and, possibly, alpha channel. Note that grayscale images containing an alpha channel are stored with colormode=Color.

All methods from the EBImage package work either with Image objects or multi-dimensional arrays. In the latter case, the color mode is assumed to be Grayscale.

### Usage

```
Image(data, dim, colormode)
as.Image(x)
is.Image(x)
## S3 method for class 'Image'
as.array(x, ...)
## S3 method for class 'Image'
as.raster(x, max = 1, i = 1L, ...)
colorMode(y)
colorMode(y) <- value
imageData(y)
imageData(y) <- value</pre>
getFrame(y, i, type = c('total', 'render'))
getFrames(y, i, type = c('total', 'render'))
numberOfFrames(y, type = c('total', 'render'))
```

#### **Arguments**

data A vector or array containing the pixel intensities of an image. If missing, the

default 1x1 zero-filled array is used.

dim A vector containing the final dimensions of an Image object. If missing, equals

to dim(data).

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| colormode | A numeric or a character string containing the color mode which can be either Grayscale or Color. If missing, equals to Grayscale.  |
|-----------|---|
| x         | An R object.  |
| У         | An Image object or an array.  |
| max       | Number giving the maximum of the color values range.  |
| i         | $Number(s) \ of \ frame(s). \ A \ single \ number \ in \ case \ of \ getFrame, \ or \ a \ vector \ of \ frame \ numbers \ for \ getFrames. \ If \ missing \ all \ frames \ are \ returned.$ |
| value     | For colorMode, a numeric or a character string containing the color mode which can be either Grayscale or Color. For imageData, an Image object or an array.                                |
| type      | A character string containing total or render. Default is total.  |
|           | further arguments passed to or from other methods.  |

#### **Details**

Depending on type, numberOfFrames returns the total number of frames contained in the object y or the number of rendered frames. The total number of frames is independent of the color mode and equals to the product of all the dimensions except the two first ones. The number of rendered frames is equal to the total number of frames in the Grayscale color mode, or to the product of all the dimensions except the three first ones in the Color color mode.

getFrame returns the i-th frame contained in the image y. If type is total, the function is unaware of the color mode and returns an xy-plane. For type=render, the function returns the i-th image as shown by the display function.

### Value

Image and as. Image return a new Image object.

is. Image returns TRUE if x is an Image object and FALSE otherwise.

as.raster coerces an Image object to its raster representation. For stacked images the i-th frame is returned (by default the first one).

colorMode returns the color mode of y and colorMode<- changes the color mode of y.

imageData returns the array contained in an Image object.

#### Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2005-2007
```

### See Also

```
readImage, writeImage, display
```

```
s1 = exp(12i*pi*seq(-1, 1, length.out=300)^2)
y = Image(outer(Im(s1), Re(s1)))
display(normalize(y))
```

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```
x = Image(rnorm(300*300*3),dim=c(300,300,3), colormode='Color')
display(x)

w = matrix(seq(0, 1, length.out=300), nc=300, nr=300)
m = abind::abind(w, t(w), along=3)
z = Image(m, colormode='Color')
display(normalize(z))

y = Image(matrix(c('red', 'violet', '#ff51a5', 'yellow'), nrow=10, ncol=10))
display(y, interpolate=FALSE)

## colorMode example
x = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
x = x[,,1:3]
display(x, title='Cell nuclei')
colorMode(x) = Color
display(x, title='Cell nuclei in RGB')
```

io

Image I/O

### **Description**

Read images from files and URLs, and write images to files.

### Usage

```
readImage(files, type, all = TRUE, names = sub("\\.[^.]*$", "", basename(files)), ...)
writeImage(x, files, type, quality = 100, bits.per.sample, compression = "none", ...)
```

### **Arguments**

| files          | a character vector of file names or URLs.   |
|----------------|---|
| type           | image type (optional). Supported values are: jpeg, png, and tiff. If missing, file format is automatically determined by file name extension.                             |
| all            | logical: when the file contains more than one image should all frames be read, or only the first one?   |
| names          | a character vector used for frame names. Should have the same length as files.  |
| X              | an Image object or an array.  |
| bits.per.sampl | e   |
|                | a numeric scalar specifying the number of bits per sample (only for tiff files). Supported values are 8 and 16.   |
| compression    | the desired compression algorithm (only for tiff files). For a list of supported values consult the documentation of the writeTIFF function from the <b>tiff</b> package. |
| quality        | a numeric ranging from 1 to 100 (default) controlling the quality of the JPEG output.   |
| • • •          | arguments passed to the corresponding functions from the <b>jpeg</b> , <b>png</b> , and <b>tiff</b> packages.   |

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#### **Details**

readImage loads all images from the files vector and returns them stacked into a single Image object containing an array of doubles ranging from 0 (black) to 1 (white). All images need to be of the same type and have the same dimensions and color mode. If type is missing, the appropriate file format is determined from file name extension. Color mode is determined automatically based on the number of channels. When the function fails to read an image it skips to the next element of the files vector issuing a warning message. Non-local files can be read directly from a valid URL.

writeImage writes images into files specified by files, were the number of files needs to be equal 1 or the number of frames. Given an image containing multiple frames and a single file name either the whole stack is written into a single TIFF file, or each frame is saved to an individual JPEG/PNG file (for files = "image.\*" frames are saved into image-X.\* files, where X equals the frame number less one; for an image containing n frames this results in file names numbered from 0 to n-1).

When writing JPEG files the compression quality can be specified using quality. Valid values range from 100 (highest quality) to 1 (lowest quality). For TIFF files additional information about the desired number of bits per sample (bits.per.sample) and the compression algorithm (compression) can be provided. For a complete list of supported values please consult the documentation of the **tiff** package.

#### Value

readImage returns a new Image object.
writeImage returns an invisible vector of file names.

### Note

Image formats have a limited dynamic range (e.g. JPEG: 8 bit, TIFF: 16 bit) and writeImage may cause some loss of accuracy. In specific, writing 16 bit image data to formats other than TIFF will strip the 8 LSB. When writing TIFF files a dynamic range check is performed and an appropriate value of bits.per.sample is set automatically.

#### Author(s)

```
Andrzej Oles, <andrzej.oles@embl.de>, 2012
```

### See Also

Image, display, readJPEG/writeJPEG, readPNG/writePNG, readTIFF/writeTIFF

```
## Read and display an image
f = system.file("images", "sample-color.png", package="EBImage")
x = readImage(f)
display(x)

## Read and display a multi-frame TIFF
y = readImage(system.file("images", "nuclei.tif", package="EBImage"))
display(y)
```

localCurvature 33

```
## Read an image directly from a remote location by specifying its URL
try({
   im = readImage("http://www-huber.embl.de/EBImage/ExampleImages/berlin.tif")
   display(im, title = "Berlin Impressions")
})

## Convert a PNG file into JPEG
tempfile = tempfile("", , ".jpeg")
writeImage(x, tempfile, quality = 85)
cat("Converted '", f, "' into '", tempfile, "'.\n", sep="")

## Save a frame sequence
files = writeImage(y, tempfile("", , ".jpeg"), quality = 85)
cat("Files created: ", files, sep="\n")
```

localCurvature

Local Curvature

# Description

Computes signed curvature along a line.

### Usage

```
localCurvature(x, h, maxk)
```

### Arguments

| Χ    | A data frame or matrix of dimensions N x 2 containing the coordinates of the        |
|------|---|
|      | line, where N is the number of points. The points should be ordered according to    |
|      | their position on the line. The columns should contain the x and y coordinates.     |
|      | The curvature calculation is unaffected by any permutation of the columns. Di-      |
|      | rectly accepts a list element from ocontour.  |
| h    | Specifies the length of the smoothing window. See $locfit::lp\ for\ more\ details.$ |
| maxk | See locfit::locfit.raw for details.   |

### **Details**

localCurvature fits a local non-parametric smoothing line (polynomial of degree 2) at each point along the line segment, and computes the curvature locally using numerical derivatives.

### Value

Returns a list containing the contour coordinates x, the signed curvature at each point curvature and the arc length of the contour length.

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#### Author(s)

Joseph Barry, Wolfgang Huber, 2013

#### See Also

ocontour

#### **Examples**

```
## curvature goes as the inverse of the radius of a circle
range=seq(3.5, 33.5, by=2)
plotRange=seq(0.5,33.5,length.out=100)
circleRes=array(dim=length(range))
names(circleRes)=range
for (i in seq_along(1:length(range))) {
 y=as.Image(makeBrush('disc', size=2*range[i]))
 y=ocontour(y)[[1]]
 circleRes[i]=abs(mean(localCurvature(x=y,h=range[i])$curvature, na.rm=TRUE))
plot(range, circleRes, ylim=c(0,max(circleRes, na.rm=TRUE)), xlab='Circle Radius', ylab='Curvature', type='p', x
points(plotRange, 1/plotRange, type='l')
## Calculate curvature
x = readImage(system.file("images", "shapes.png", package="EBImage"))[25:74, 60:109]
x = resize(x, 200)
y = gblur(x, 3) > .3
display(y)
contours = ocontour(bwlabel(y))
c = localCurvature(x=contours[[1]], h=11)
i = c$curvature >= 0
pos = neg = array(0, dim(x))
pos[c$contour[i,]+1] = c$curvature[i]
neg[c$contour[!i,]+1] = -c$curvature[!i]
display(10*(rgbImage(pos, , neg)), title = "Image curvature")
```

medianFilter

2D constant time median filtering

#### **Description**

Process an image using Perreault's modern constant-time median filtering algorithm [1, 2].

# Usage

```
medianFilter(x, size, cacheSize=512)
```

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### **Arguments**

x an Image object or an array. size integer, median filter radius.

cacheSize integer, the L2 cache size of the system CPU in kB.

### **Details**

Median filtering is useful as a smoothing technique, e.g. in the removal of speckling noise.

For a filter of radius size, the median kernel is a 2\*size+1 times 2\*size+1 square.

The input image x should contain pixel values in the range from 0 to 1, inclusive; values lower than 0 or higher than 1 are clipped before applying the filter. Internal processing is performed using 16-bit precision. The behavior at image boundaries is such as the source image has been padded with pixels whose values equal the nearest border pixel value.

If the image contains multiple channels or frames, the filter is applied to each one of them separately.

#### Value

An Image object or an array, containing the filtered version of x.

#### Author(s)

```
Joseph Barry, <joseph.barry@embl.de>, 2012
Andrzej Oleś, <andrzej.oles@embl.de>, 2016
```

### References

```
[1] S. Perreault and P. Hebert, "Median Filtering in Constant Time", IEEE Trans Image Process 16(9), 2389-2394, 2007
```

```
[2] http://nomis80.org/ctmf.html
```

#### See Also

gblur

```
x = readImage(system.file("images", "nuclei.tif", package="EBImage"))
display(x, title='Nuclei')
y = medianFilter(x, 5)
display(y, title='Filtered nuclei')
```

36 morphology

| morphology Perform morphological operations on images |
|---|
|---|

### **Description**

dilate(x, kern)
erode(x, kern)

Functions to perform morphological operations on binary and grayscale images.

# Usage

```
opening(x, kern)
closing(x, kern)
whiteTopHat(x, kern)
blackTopHat(x, kern)
selfComplementaryTopHat(x, kern)
makeBrush(size, shape=c('box', 'disc', 'diamond', 'Gaussian', 'line'), step=TRUE, sigma=0.3, angle=45)
```

### **Arguments**

| X     | An Image object or an array.   |
|-------|--|
| kern  | An Image object or an array, containing the structuring element. kern is considered as a binary image, with pixels of value 0 being the background and pixels with values other than 0 being the foreground. |
| size  | A numeric containing the size of the brush in pixels. This should be an odd number; even numbers are rounded to the next odd one, i.e., size = 4 has the same effect as size = 5. Default is 5               |
| shape | A character vector indicating the shape of the brush. Can be box, disc, diamond, Gaussian or line. Default is box.   |
| step  | a logical indicating if the brush is binary. Default is TRUE. This argument is relevant only for the disc and diamond shapes.  |
| sigma | An optional numeric containing the standard deviation of the Gaussian shape. Default is $0.3$ .  |
| angle | An optional numeric containing the angle at which the line should be drawn. The angle is one between the top of the image and the line.  |

### **Details**

dilate applies the mask kern by positioning its center over every pixel of the image x, the output value of the pixel is the maximum value of x covered by the mask. In case of binary images this is equivalent of putting the mask over every background pixel, and setting it to foreground if any of the pixels covered by the mask is from the foreground.

erode applies the mask kern by positioning its center over every pixel of the image x, the output value of the pixel is the minimum value of x covered by the mask. In case of binary images this is

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equivalent of putting the mask over every foreground pixel, and setting it to background if any of the pixels covered by the mask is from the background.

opening is an erosion followed by a dilation and closing is a dilation followed by an erosion.

whiteTopHat returns the difference between the original image x and its opening by the structuring element kern.

blackTopHat subtracts the original image x from its closing by the structuring element kern.

selfComplementaryTopHat is the sum of the whiteTopHat and the blackTopHat, simplified the difference between the closing and the opening of the image.

makeBrush generates brushes of various sizes and shapes that can be used as structuring elements.

**Processing Pixels at Image Borders (Padding Behavior):** Morphological functions position the center of the structuring element over each pixel in the input image. For pixels close to the edge of an image, parts of the neighborhood defined by the structuring element may extend past the border of the image. In such a case, a value is assigned to these undefined pixels, as if the image was padded with additional rows and columns. The value of these padding pixels varies for dilation and erosion operations. For dilation, pixels beyond the image border are assigned the minimum value afforded by the data type, which in case of binary images is equivalent of setting them to background. For erosion, pixels beyond the image border are assigned the maximum value afforded by the data type, which in case of binary images is equivalent of setting them to foreground.

#### Value

dilate, erode, opening, white Top Hat, black Top Hat and self Complementary Top Hat return the transformed Image object or array x, after the corresponding morphological operation.

makeBrush generates a 2D matrix containing the desired brush.

#### Note

Morphological operations are implemented using the efficient Urbach-Wilkinson algorithm [1]. Its required computing time is independent of both the image content and the number of gray levels used.

#### Author(s)

```
Ilia Kats <<ili>lia-kats@gmx.net>> (2012), Andrzej Oles <<andrzej.oles@embl.de>> (2015)
```

#### References

[1] E. R. Urbach and M.H.F. Wilkinson, "Efficient 2-D grayscale morphological transformations with arbitrary flat structuring elements", IEEE Trans Image Process 17(1), 1-8, 2008

```
x = readImage(system.file("images", "shapes.png", package="EBImage"))
kern = makeBrush(5, shape='diamond')
```

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```
display(x)
display(kern, title='Structuring element')
display(erode(x, kern), title='Erosion of x')
display(dilate(x, kern), title='Dilatation of x')

## makeBrush
display(makeBrush(99, shape='diamond'))
display(makeBrush(99, shape='disc', step=FALSE))
display(2000*makeBrush(99, shape='Gaussian', sigma=10))
```

normalize

Intensity values linear scaling

#### **Description**

Linearly scale the intensity values of an image to a specified range.

# Usage

```
## S4 method for signature 'Image'
normalize(object, separate=TRUE, ft=c(0,1), inputRange)
## S4 method for signature 'array'
normalize(object, separate=TRUE, ft=c(0,1), inputRange)
```

#### **Arguments**

object an Image object or an array

separate if TRUE, normalizes each frame separately

ft a numeric vector of 2 values, target minimum and maximum intensity values

after normalization

inputRange a numeric vector of 2 values, sets the range of the input intensity values; values

exceeding this range are clipped

#### **Details**

normalize performs linear interpolation of the intensity values of an image to the specified range ft. If inputRange is not set the whole dynamic range of the image is used as input. By specifying inputRange the input intensity range of the image can be limited to [min, max]. Values exceeding this range are clipped, i.e. intensities lower/higher than min/max are set to min/max.

#### Value

An Image object or an array, containing the transformed version of object.

# Author(s)

Oleg Sklyar, <osklyar@ebi.ac.uk>, 2006-2007 Andrzej Oles, <andrzej.oles@embl.de>, 2013

ocontour 39

#### **Examples**

```
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
y = bwlabel(x)
display(x, title='Original')

print(range(y))
y = normalize(y)
print(range(y))
display(y, title='Segmented')
```

ocontour

Oriented contours

## **Description**

Computes the oriented contour of objects.

#### Usage

```
ocontour(x)
```

# Arguments

Χ

An Image object or an array, containing objects. Only integer values are considered. Pixels of value 0 constitute the background. Each object is a set of pixels with the same unique integer value. Objects are assumed connected.

#### Value

A list of matrices, containing the coordinates of object oriented contours.

#### Author(s)

```
Gregoire Pau, <gpau@ebi.ac.uk>, 2008
```

```
x = readImage(system.file("images", "shapes.png", package="EBImage"))
x = x[1:120,50:120]
display(x)
oc = ocontour(x)
plot(oc[[1]], type='1')
points(oc[[1]], col=2)
```

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otsu

Calculate Otsu's threshold

# **Description**

Returns a threshold value based on Otsu's method, which can be then used to reduce the grayscale image to a binary image.

# Usage

```
otsu(x, range = c(0, 1), levels = 256)
```

#### **Arguments**

x A Grayscale Image object or an array.

range Numeric vector of length 2 specifying the histogram range used for thresholding.

levels Number of grayscale levels.

#### **Details**

Otsu's thresholding method [1] is useful to automatically perform clustering-based image thresholding. The algorithm assumes that the distribution of image pixel intensities follows a bi-modal histogram, and separates those pixels into two classes (e.g. foreground and background). The optimal threshold value is determined by minimizing the combined intra-class variance.

The threshold value is calculated for each image frame separately resulting in a output vector of length equal to the total number of frames in the image.

The default number of levels corresponds to the number of gray levels of an 8bit image. It is recommended to adjust this value according to the bit depth of the processed data, i.e. set levels to  $2^16 = 65536$  when working with 16bit images.

#### Value

A vector of length equal to the total number of frames in x. Each vector element contains the Otsu's threshold value calculated for the corresponding image frame.

# Author(s)

Philip A. Marais <philipmarais@gmail.com>, Andrzej Oles <andrzej.oles@embl.de>, 2014

#### References

[1] Nobuyuki Otsu, "A threshold selection method from gray-level histograms". IEEE Trans. Sys., Man., Cyber. 9 (1): 62-66. doi:10.1109/TSMC.1979.4310076 (1979)

#### See Also

thresh

paintObjects 41

## **Examples**

```
x = readImage(system.file("images", "sample.png", package="EBImage"))
display(x)

## threshold using Otsu's method
y = x > otsu(x)
display(y)
```

paintObjects

Mark objects in images

## **Description**

Higlight objects in images by outlining and/or painting them.

## Usage

```
paintObjects(x, tgt, opac=c(1, 1), col=c('red', NA), thick=FALSE, closed=FALSE)
```

## **Arguments**

| X      | An Image object in Grayscale color mode or an array containing object masks. Object masks are sets of pixels with the same unique integer value.                          |
|--------|---|
| tgt    | An Image object or an array, containing the intensity values of the objects.  |
| opac   | A numeric vector of two opacity values for drawing object boundaries and object bodies. Opacity ranges from 0 to 1, with 0 being fully transparent and 1 fully opaque.    |
| col    | A character vector of two R colors for drawing object boundaries and object bodies. By default, object boundaries are painted in red while object bodies are not painted. |
| thick  | A logical indicating whether to use thick boundary contours. Default is FALSE.  |
| closed | A logical indicating whether object contours should be closed along image edges or remain open.   |

#### Value

An Image object or an array, containing the painted version of tgt.

# Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2006-2007 Andrzej Oles, <andrzej.oles@embl.de>, 2015
```

#### See Also

bwlabel, watershed, computeFeatures, colorLabels

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#### **Examples**

```
nuc = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
cel = readImage(system.file('images', 'cells.tif', package='EBImage'))
img = rgbImage(green=cel, blue=nuc)
display(img, title='Cells')
## segment nuclei
nmask = thresh(nuc, 10, 10, 0.05)
nmask = opening(nmask, makeBrush(5, shape='disc'))
nmask = fillHull(nmask)
nmask = bwlabel(nmask)
display(normalize(nmask), title='Cell nuclei mask')
## segment cells, using propagate and nuclei as 'seeds'
ctmask = opening(cel>0.1, makeBrush(5, shape='disc'))
cmask = propagate(cel, nmask, ctmask)
display(normalize(cmask), title='Cell mask')
## using paintObjects to highlight objects
res = paintObjects(cmask, img, col='#ff00ff')
res = paintObjects(nmask, res, col='#ffff00')
display(res, title='Segmented cells')
```

propagate

Voronoi-based segmentation on image manifolds

#### **Description**

Find boundaries between adjacent regions in an image, where seeds have been already identified in the individual regions to be segmented. The method finds the Voronoi region of each seed on a manifold with a metric controlled by local image properties. The method is motivated by the problem of finding the borders of cells in microscopy images, given a labelling of the nuclei in the images.

Algorithm and implementation are from Jones et al. [1].

# Usage

```
propagate(x, seeds, mask=NULL, lambda=1e-4)
```

# **Arguments**

mask

| X     | An Image object or an array, containing the image to segment.                                 |
|-------|---|
| seeds | An Image object or an array, containing the seeding objects of the already identified ragions |
|       | tified regions.   |

An optional Image object or an array, containing the binary image mask of the

regions that can be segmented. If missing, the whole image is segmented.

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lambda

A numeric value. The regularization parameter used in the metric, determining the trade-off between the Euclidean distance in the image plane and the contribution of the gradient of x. See details.

#### **Details**

The method operates by computing a discretized approximation of the Voronoi regions for given seed points on a Riemann manifold with a metric controlled by local image features.

Under this metric, the infinitesimal distance d between points v and v+dv is defined by:

```
d^2 = ((t(dv)*g)^2 + lambda*t(dv)*dv)/(lambda + 1)
```

, where g is the gradient of image x at point v.

lambda controls the weight of the Euclidean distance term. When lambda tends to infinity, d tends to the Euclidean distance. When lambda tends to 0, d tends to the intensity gradient of the image.

The gradient is computed on a neighborhood of 3x3 pixels.

Segmentation of the Voronoi regions in the vicinity of flat areas (having a null gradient) with small values of lambda can suffer from artifacts coming from the metric approximation.

#### Value

An Image object or an array, containing the labelled objects.

#### License

The implementation is based on CellProfiler C++ source code [2, 3]. An LGPL license was granted by Thouis Jones to use this part of CellProfiler's code for the propagate function.

#### Author(s)

The original CellProfiler code is from Anne Carpenter <carpenter@wi.mit.edu>, Thouis Jones <thouis@csail.mit.edu>, In Han Kang <inthek@mit.edu>. Responsible for this implementation: Greg Pau.

## References

- [1] T. Jones, A. Carpenter and P. Golland, "Voronoi-Based Segmentation of Cells on Image Manifolds", CVBIA05 (535-543), 2005
- [2] A. Carpenter, T.R. Jones, M.R. Lamprecht, C. Clarke, I.H. Kang, O. Friman, D. Guertin, J.H. Chang, R.A. Lindquist, J. Moffat, P. Golland and D.M. Sabatini, "CellProfiler: image analysis software for identifying and quantifying cell phenotypes", Genome Biology 2006, 7:R100
- [3] CellProfiler: http://www.cellprofiler.org

#### See Also

bwlabel, watershed

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#### **Examples**

```
## a paraboloid mountain in a plane
n = 400
x = (n/4)^2 - matrix(
(rep(1:n, times=n) - n/2)^2 + (rep(1:n, each=n) - n/2)^2,
nrow=n, ncol=n)
x = normalize(x)
## 4 seeds
seeds = array(0, dim=c(n,n))
seeds[51:55, 301:305] = 1
seeds[301:305, 101:105] = 2
seeds[201:205, 141:145] = 3
seeds[331:335, 351:355] = 4
lambda = 10^seq(-8, -1, by=1)
segmented = Image(dim=c(dim(x), length(lambda)))
for(i in seq_along(lambda)) {
  prop = propagate(x, seeds, lambda=lambda[i])
  prop = prop/max(prop)
  segmented[,,i] = prop
}
display(x, title='Image')
display(seeds/max(seeds), title='Seeds')
display(segmented, title="Voronoi regions", all=TRUE)
```

resize

Spatial linear transformations

# Description

The following functions perform all spatial linear transforms: reflection, rotation, translation, resizing, and general affine transform.

#### Usage

```
flip(x)
flop(x)
rotate(x, angle, filter = "bilinear", output.dim, output.origin, ...)
translate(x, v, filter = "none", ...)
resize(x, w, h, output.dim = c(w, h), output.origin = c(0, 0), antialias = FALSE, ...)
affine(x, m, filter = c("bilinear", "none"), output.dim, bg.col = "black", antialias = TRUE)
```

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#### **Arguments**

| X             | An Image object or an array.   |
|---------------|--|
| angle         | A numeric specifying the image rotation angle in degrees.  |
| V             | A vector of 2 numbers denoting the translation vector in pixels.   |
| w, h          | Width and height of the resized image. One of these arguments can be missing to enable proportional resizing.  |
| filter        | A character string indicating the interpolating sampling filter. Valid values are 'none' or 'bilinear'. See Details.   |
| output.dim    | A vector of 2 numbers indicating the dimension of the output image. For affine and translate the default is $\dim(x)$ , for resize it equals $c(w, h)$ , and for rotate it defaults to the bounding box size of the rotated image. |
| output.origin | A vector of 2 numbers indicating the output coordinates of the origin in pixels.   |
| m             | A 3x2 matrix describing the affine transformation. See Details.  |
| bg.col        | Color used to fill the background pixels, defaults to "black". In the case of multi-frame images the value is recycled, and individual background for each frame can be specified by providing a vector.                           |
| antialias     | If TRUE, perform bilinear sampling at image edges using bg.col.  |
| • • •         | Arguments to be passed to affine, such as filter, output.dim, $\log.$ col or antialias.  |
|               |  |

#### **Details**

flip mirrors x around the image horizontal axis (vertical reflection).

flop mirrors x around the image vertical axis (horizontal reflection).

rotate rotates the image clockwise by the given angle around the origin specified in output.origin. If no output.origin is provided, the result will be centered in a recalculated bounding box unless output.dim is provided.

resize scales the image x to the desired dimensions. The transformation origin can be specified in output.origin. For example, zooming about the output.origin can be achieved by setting output.dim to a value different from c(w, h).

affine returns the affine transformation of x, where pixels coordinates, denoted by the matrix px, are transformed to cbind(px, 1)%\*%m.

All spatial transformations except flip and flop are based on the general affine transformation. Spatial interpolation can be either none, also called nearest neighbor, where the resulting pixel value equals to the closest pixel value, or bilinear, where the new pixel value is computed by bilinear approximation of the 4 neighboring pixels. The bilinear filter gives smoother results.

#### Value

An Image object or an array, containing the transformed version of x.

#### Author(s)

Gregoire Pau, 2012

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#### See Also

transpose

#### **Examples**

```
x <- readImage(system.file("images", "sample.png", package="EBImage"))
display(x)

display( flip(x) )
display( flop(x) )
display( resize(x, 128) )
display( rotate(x, 30) )
display( translate(x, c(120, -20)) )

m <- matrix(c(0.6, 0.2, 0, -0.2, 0.3, 300), nrow=3)
display( affine(x, m) )</pre>
```

rmObjects

Object removal and re-indexation

## **Description**

The rm0bjects functions deletes objects from an image by setting their pixel intensity values to 0. reenumerate re-enumerates all objects in an image from 0 (background) to the actual number of objects.

## Usage

```
rmObjects(x, index, reenumerate = TRUE)
reenumerate(x)
```

## **Arguments**

x An Image object in Grayscale color mode or an array containing object masks.

Object masks are sets of pixels with the same unique integer value.

index A numeric vector (or a list of vectors if x contains multiple frames) containing

the indexes of objects to remove in the frame.

reenumerate Logical, should all the objects in the image be re-indexed afterwards (default).

#### Value

An Image object or an array, containing the new objects.

#### Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2006-2007
```

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#### See Also

bwlabel, watershed

# **Examples**

```
## make objects
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
y = bwlabel(x)
## number of objects found
max(y)
display(normalize(y), title='Objects')
## remove every second letter
objects = list(
 seq.int(from = 2, to = max(y), by = 2),
  seq.int(from = 1, to = max(y), by = 2)
z = rmObjects(combine(y, y), objects)
display(normalize(z), title='Object removal')
## the number of objects left in each image
apply(z, 3, max)
## perform object removal without re-enumerating
z = rmObjects(y, objects, reenumerate = FALSE)
## labels of objects left
unique(as.vector(z))[-1L]
## re-index objects
z = reenumerate(z)
unique(as.vector(z))[-1L]
```

stackObjects

Places detected objects into an image stack

## **Description**

Places detected objects into an image stack.

# Usage

```
stackObjects(x, ref, combine=TRUE, bg.col='black', ext)
```

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## **Arguments**

| X       | An Image object or an array containing object masks. Object masks are sets of pixels with the same unique integer value.   |
|---------|--|
| ref     | An Image object or an array, containing the intensity values of the objects.   |
| combine | If x contains multiple images, specifies if the resulting list of image stacks with individual objects should be combined using combine into a single image stack. |
| bg.col  | Background pixel color.  |
| ext     | A numeric controlling the size of the output image. If missing, ext is estimated from data. See details.   |

#### **Details**

stackObjects creates a set of n images of size (2\*ext+1, 2\*ext+1), where n is the number of objects in x, and places each object of x in this set.

If not specified, ext is estimated using the 98% quantile of m.majoraxis/2, where m.majoraxis is the semi-major axis descriptor extracted from computeFeatures.moment, taken over all the objects of the image x.

#### Value

An Image object containing the stacked objects contained in x. If x contains multiple images and if combine is TRUE, stackObjects returns a list of Image objects.

# Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2006-2007
```

#### See Also

```
combine, tile, computeFeatures.moment
```

```
## simple example
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
y = bwlabel(x)
display(normalize(y), title='Objects')
z = stackObjects(y, normalize(y))
display(z, title='Stacked objects')

## load images
nuc = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
cel = readImage(system.file('images', 'cells.tif', package='EBImage'))
img = rgbImage(green=cel, blue=nuc)
display(img, title='Cells')

## segment nuclei
nmask = thresh(nuc, 10, 10, 0.05)
```

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```
nmask = opening(nmask, makeBrush(5, shape='disc'))
nmask = fillHull(bwlabel(nmask))

## segment cells, using propagate and nuclei as 'seeds'
ctmask = opening(cel>0.1, makeBrush(5, shape='disc'))
cmask = propagate(cel, nmask, ctmask)

## using paintObjects to highlight objects
res = paintObjects(cmask, img, col='#ff00ff')
res = paintObjects(nmask, res, col='#fff00')
display(res, title='Segmented cells')

## stacked cells
st = stackObjects(cmask, img)
display(st, title='Stacked objects')
```

thresh

Adaptive thresholding

#### **Description**

Thresholds an image using a moving rectangular window.

# Usage

```
thresh(x, w=5, h=5, offset=0.01)
```

# **Arguments**

x An Image object or an array.

w, h Half width and height of the moving rectangular window.

offset Thresholding offset from the averaged value.

# **Details**

This function returns the binary image resulting from the comparison between an image and its filtered version with a rectangular window. It is equivalent of doing { f = matrix(1, nc=2\*w+1, nr=2\*h+1); f = f/sum(f);  $x > (filter2(x, f, boundary="replicate") + offset) } but faster.$  The function filter2 provides hence more flexibility than thresh.

#### Value

An Image object or an array, containing the transformed version of x.

#### Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2005-2007
```

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## See Also

filter2

#### **Examples**

```
x = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
display(x)
y = thresh(x, 10, 10, 0.05)
display(y)
```

tile

Tiling/untiling images

# **Description**

Given a sequence of frames, tile generates a single image with frames tiled. untile is the inverse function and divides an image into a sequence of images.

## Usage

```
tile(x, nx=10, lwd=1, fg.col="#E4AF2B", bg.col="gray")
untile(x, nim, lwd=1)
```

# **Arguments**

| X      | An Image object, an array or a list of these objects.                       |
|--------|---|
| nx     | The number of tiled images in a row.  |
| lwd    | The width of the grid lines between tiled images, can be 0.                 |
| fg.col | The color of the grid lines.  |
| bg.col | The color of the background for extra tiles.                                |
| nim    | A numeric vector of 2 elements for the number of images in both directions. |

## **Details**

After object segmentation, tile is a useful addition to stackObjects to have an overview of the segmented objects.

#### Value

An Image object or an array, containing the tiled/untiled version of x.

# Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2006-2007
```

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#### See Also

```
stackObjects
```

#### **Examples**

```
## make a set of blurred images
img = readImage(system.file("images", "sample-color.png", package="EBImage"))[257:768,,]
x = resize(img, 128, 128)
xt = list()
for (t in seq(0.1, 5, length.out=9)) xt=c(xt, list(gblur(x, s=t)))
xt = combine(xt)
display(xt, title='Blurred images')

## tile
xt = tile(xt, 3)
display(xt, title='Tiles')

## untile
xu = untile(img, c(3, 3))
display(xu, title='Blocks')
```

transpose

Image Transposition

#### **Description**

Transposes an image by swapping its spatial dimensions.

# Usage

```
transpose(x)
```

#### **Arguments**

Х

an Image object or an array.

# **Details**

The transposition of an image is performed by swapping the X and Y indices of its array representation.

#### Value

A transformed version of x with its first two dimensions transposed.

#### Note

The function is implemented using an efficient cash-oblivious algorithm which is typically faster than R's aperm and t functions.

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#### Author(s)

```
Andrzej Oles, <andrzej.oles@embl.de>, 2012-2017
```

#### See Also

```
flip, flop, rotate
```

## **Examples**

```
x = readImage(system.file("images", "sample-color.png", package="EBImage"))
y = transpose(x)

display(x, title='Original')
display(y, title='Transposed')

## performing the transposition of an image twice should result in the original image
z = transpose(y)
identical(x, z)
```

watershed

Watershed transformation and watershed based object detection

# Description

Watershed transformation and watershed based object detection.

# Usage

```
watershed(x, tolerance=1, ext=1)
```

#### **Arguments**

x An Image object or an array.

tolerance The minimum height of the object in the units of image intensity between its

highest point (seed) and the point where it contacts another object (checked for every contact pixel). If the height is smaller than the tolerance, the object will be combined with one of its neighbors, which is the highest. Tolerance should be chosen according to the range of x. Default value is 1, which is a reasonable

value if x comes from distmap.

ext Radius of the neighborhood in pixels for the detection of neighboring objects.

Higher value smoothes out small objects.

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## **Details**

The algorithm identifies and separates objects that stand out of the background (zero). It inverts the image and uses water to fill the resulting valleys (pixels with high intensity in the source image) until another object or background is met. The deepest valleys become indexed first, starting from 1.

The function bwlabel is a simpler, faster alternative to segment connected objects from binary images.

#### Value

An Grayscale Image object or an array, containing the labelled version of x.

## Author(s)

```
Oleg Sklyar, <osklyar@ebi.ac.uk>, 2007
```

#### See Also

```
bwlabel, propagate
```

```
x = readImage(system.file('images', 'shapes.png', package='EBImage'))
x = x[110:512,1:130]
display(x, title='Binary')
y = distmap(x)
display(normalize(y), title='Distance map')
w = watershed(y)
display(normalize(w), title='Watershed')
```

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