Package ‘CRImage’
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Type    Package
Title   CRImage a package to classify cells and calculate tumour cellularity
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Description CRImage provides functionality to process and analyze images, in particular to classify cells in biological images. Furthermore, in the context of tumor images, it provides functionality to calculate tumour cellularity.

License Artistic-2.0
LazyLoad yes
Imports MASS, e1071, foreach, sgeostat
Depends EBImage, DNAcopy, aCGH


biocViews CellBiology, Classification

NeedsCompilation no
R topics documented:

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CRImage-package

CRImage is a package to analyze images and classify cells.

Description

CRImage allows classification of cells in biological images. It offers methods to segment cells or cell nuclei in biological images for example HE stained images. It offers methods to create a classifier and to classify cells in these images. Furthermore it allows the calculation of tumour cellularity for large microscope images.

CRImage makes use of the image processing package EBImage, which uses the 'ImageMagick' library for image I/O operations and the 'GTK' library to display images.

Details

Package: CRImage
Type: Package
Version: 1.0
Date: 2010-04-27
License: LGPL Version 2 or later
LazyLoad: yes
Package content

Image processing methods:

• calculateThreshold
• segmentImage

Classification:

• createTrainingSet
• createClassifier
• classifyCells

Tumour cellularity

• calculateCellularity
• processAperio

Author(s)

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Examples

eexample(segmentImage)
eexample(createClassifier)
eexample(classifyImage)
calculateCellularity  *Calculation of tumour cellularity*

**Description**

The function calculates the tumour cellularity of an image by counting tumour and non tumour cells.

**Usage**

```r
calculateCellularity(filename = "", image = NA, classifier = NULL, cancerIdentifier = NA, KS = FALSE, maxShape = NA, minShape = NA, failureregion = NA, colors = NA, threshold = "otsu", numwindows = 1, classifystructures = FALSE, pixelclassifier = NA, kstoexclude = NA, densitytoexclude = NA, numdensitywindows = 1)
```

**Arguments**

- `filename`: A path to an image file.
- `image`: If filename is undefined, an Image object.
- `classifier`: A SVM object, created with `createClassifier` or directly with the package `e1071`.
- `cancerIdentifier`: A string which describes, how the cancer class is named.
- `KS`: Apply kernel smoother?
- `maxShape`: Maximum size of cell nuclei.
- `minShape`: Minimum size of cell nuclei.
- `failureRegion`: Minimum size of failure regions.
- `colors`: Colors to paint the classes.
- `threshold`: Which threshold should be uses, "otsu" or "phansalkar".
- `classesToExclude`: Should a class be excluded from cellularity calculation?
- `numWindows`: Number of windows for the threshold.
- `classifyStructures`: Use hierarchical classification. If yes a pixel classifier has to be defined.
- `pixelClassifier`: A SVM to classify pixel based on their color values. Needed if hierarchical classification should be applied.
- `ksToExclude`: These classes are excluded from kernel smoothing.
- `densityToExclude`: This class is excluded from cellularity calculation.
- `numDensityWindows`: Number of windows for the density plot.
Details
The method calculates tumour cellularity of an image. The cells of the image are classified and the
cellularity is: numTumourCells/numPixel. Furthermore the number of cells of the different classes
are counted. A heatmap of cellularity is created. The image is divided in 16 subwindows and
cellularity is calculated for every subwindow. Green in the heatmaps indicates strong cellularity,
white low cellularity.

Value
A list containing
cellularity values
a vector, the n first values indicate the n numbers of cells in the n classes, the
n + 1th value indicates the tumour cellularity. The n + 2th value is the ratio of
tumour cells by all cells
cancerHeatmap Heatmap of cancer density

Author(s)
Henrik Failmezger, failmezger@mpipz.mpg.de

Examples

t = system.file("extdata", "trainingData.txt", package="CRImage")
#read training data
trainingData=read.table(t,header=TRUE)
#create classifier
classifier=createClassifier(trainingData)[[1]]
#calculation of cellularity
f = system.file("extdata", "exImg.jpg", package="CRImage")
exImg=readImage(f)
cellularity=calculateCellularity(classifier=classifier, filename=f, KS=TRUE, maxShape=800, minShape=40, failureRegion

-----------------------------------------------------------------
calculateMeanStdTarget
Calculates Mean and Standard deviation of an image
-----------------------------------------------------------------

Description
Mean and SD calculation

Usage

calculateMeanStdTarget(imgT)
The function applies Otsu thresholding on the image.

Usage

\[ \text{calculateOtsu}(\text{allGreyValues}) \]

Arguments

\begin{itemize}
  \item \text{allGreyValues} Vector of grey values.
\end{itemize}

Details

The function calculates a value which separates the grey value histogram the best in foreground and background.
classifyCells

Value

the threshold

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

References


See Also

calculateThreshold localOtsuThreshold

Examples

f1 = system.file("extdata", "exImg2.jpg", package="CRImage")
print(f1)
img = readImage(f1)
print(img)
#convert to grayscale
imgG = EBImage::channel(img, 'grey')
#threshold value
t = calculateOtsu(as.vector(imgG))

classifyCells    A function to classify cells

Description

The function classifies cells and paints the different class types in the image.

Usage

classifyCells(classifier, filename="", image=NA, segmentedImage=NA, featuresObjects=NA, paint=TRUE, KS=FALSE, exclude=NA)

Arguments

classifier    A Support Vector Machine created by createClassifier or directly by the package e1071
filename      A path to an image file.
image         An 'Image' object or an array.
segmentedImage An 'Image' object or an array. The corresponding segmented image (created by segmentImage)
featuresObjects
  Cell feature file of the segmentedImage (created by segmentImage)
paint
  If true, the classified cells are painted with different colors in the image
KS
  Use Kernel Smoohter in classification?
cancerIdentifier
  A string which describes, how the cancer class is named.
maxShape
  Maximum size of cell nuclei
minShape
  Minimum size of cell nuclei
failureRegion
  minimum size of failure regions
colors
  Colors to paint the classes
classesToExclude
  Which class should be excluded?
threshold
  Which thresholding method should be used, "otsu" or "phansalkar"
numWindows
  Number of windows to use for thresholding.
structures
  If the image is already segmented, structures can be inserted to enable hierarchi-
  cal classification.
classifyStructures
  Use hierarchical classification. If yes a pixel classifier has to be defined.
pixelClassifier
  A SVM to classify pixel based on their color values. Needed if hierarchical
classification should be applied.
ksToExclude
  These classes are excluded from kernel smoothing.

Details
The kernels smoother improves the classification for cells which are likely to occur in clusters, like
tumour cells. The kernel smoothing method can only be applied for two classes. If there are more
classes only the normal svm without kernel smoothing is applied. Different classes are labeled with
different colors in the image.

Value
A list with

comp1  classes
comp2  Classes, painted in the image, if paint was true

Author(s)
Henrik Failmezger, failmezger@mpipz.mpg.de
Examples

t = system.file("extdata", "trainingData.txt", package="CRImage")
# read training data
trainingData = read.table(t, header=TRUE)
# create classifier
classifier = createClassifier(trainingData)[[1]]
# classify cells
f = system.file("extdata", "exImg.jpg", package="CRImage")
classesValues = classifyCells(classifier, filename=f, KS=TRUE, maxShape=800, minShape=40, failureRegion=2000)

colorCorrection

Description

The colors of one image are adapted to the colors of a target image.

Usage

colorCorrection(imgO, meanStdTarget, whiteMask = c())

Arguments

imgO The image who’s colors should be adapted
meanStdTarget Array with mean and standard deviation of the target image.
whiteMask Boolean mask of white pixel in the image. These pixels are excluded from color correction.

Details

Mean and standard deviation of the target image can be calculated using the function calculateMeanStdTarget.

Value

The image with adapted colors.

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

References

See Also

calculateMeanStandardTarget

Examples

# read the target image
f1 = system.file("extdata", "exImg2.jpg", package="CRImage")
targetImage = readImage(f1)
# read the image whose color values should be adapted
f2 = system.file("extdata", "exImg3.jpg", package="CRImage")
imgToConvert = readImage(f2)
# calculate mean and standard deviation of target color channels
mst = calculateMeanStdTarget(targetImage)
# create a white pixel mask
whitePixelMask = imgToConvert[,1] > 0.85 & imgToConvert[,2] > 0.85 & imgToConvert[,3] > 0.85
# adapt color channels of image
imgCorrected = colorCorrection(imgToConvert, mst, whitePixelMask)

convertHSVToRGB  Conversion from HSV color space to RGB color space

Description

The function converts images in the HSV colour space to the RGB colour space.

Usage

convertHSVToRGB(imgHSV)

Arguments

imgHSV  An 'Image' object or an array in the HSV colour space.

Details

Standard colour space conversion.

Value

An array in the RGB colour space.

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

See Also

convertRGBToHSV convertRGBToLAB convertLABToRGB
convertLABToRGB

Examples

f = system.file("extdata", "exImg.jpg", package="CRImage")
img = readImage(f)
#conversion to RGB color space
imgRGB = convertHSVtoRGB(img)

Description

Color space conversion.

Usage

convertLABToRGB(imgLAB)

Arguments

imgLAB LAB channel vectors.

Details

Color space conversion

Value

RGB channel vectors.

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

Examples

f = system.file("extdata", "exImg.jpg", package="CRImage")
img = readImage(f)
#conversion to HSV color space
imgRGB = convertLABToRGB(img)
convertRGBToHSV

Conversion from RGB color space to HSV color space

Description

The RGB Image is converted to an HSV image.

Usage

convertRGBToHSV(img)

Arguments

img The RGB image

Details

The entries of the array are Hue, Saturation and Value.

Value

The image in HSV color space.

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

See Also

convertHSVToRGB convertRGBToLAB convertLABToRGB

Examples

f = system.file("extdata", "exImg.jpg", package="CRImage")
img = readImage(f)
#conversion to HSV color space
imgHSV = convertRGBToHSV(img)
**convertRGBToLAB**  
*Converts RGB to LAB color space.*

**Description**  
Conversion of Color spaces.

**Usage**  
convertRGBToLAB(imgT)

**Arguments**  
- **imgT** The RGB image.

**Details**  
Color space conversion

**Value**  
The image in LAB color space.

**Author(s)**  
Henrik Failmezger, failmezger@cip.ifi.lmu.de

**Examples**  
f = system.file("extdata", "exImg.jpg", package="CRImage")
img = readImage(f)
# conversion to LAB color space
imgLAB = convertRGBToLAB(img)

---

**correctCopyNumber**  
*Allelic Copy Number correction for cellularity*

**Description**  
This function segments copy number and corrects log-ratios (LRR) and beta allele frequencies (BAF) values for cellularity.

**Usage**  
correctCopyNumber(arr="Sample1", chr=NULL, p=NULL, z=NULL, min.value=-5)
correctCopyNumber

Arguments

arr   Name of the array.
chr   Chromosome to run. If NULL, all chromosomes are run.
p     Percentage of tumoural cells.
z     Copy Number Data. Must be a dataframe with the following columns: Name (id of the probe), Chr (chromosome), Pos (position), LRR (log ratios) and BAF (beta allele frequencies).
min.value Value assigned to the probes that have 0 copies after correction.

Details

The data.frame z must contain only SNP probes, that is probes with both LRR and BAF values. It is recommended that all replicated probes are merged so the positions are unique. This function calls DNACopy to segment the LRR and then correct the segmented profiles for normal contamination according to the method described in the reference below (see for details).

Value

A list with 2 components:

y

a data.frame with as many rows as probes containing the following variables: Chrom (chromosome), Pos (position), Orig.LRR (LRR before correction) Orig.BAF (BAF before correction), Corr.LRR (LRR after cellularity correction) and Corr.BAF (BAF after correction)

seg

a data.frame with the segmented data. Contains the following columns: ID (name of the array), chrom (chromosome), loc.start (start of the region), loc.end (end of the region), num.mark (number of probes in the region), seg.mean (LRR of the region), BAF (BAF of the regions), num.BAF (number of SNP probes in the region), Sa (estimated absolute copy number for the first allele), Sb (estimated absolute copy number for the first allele), LRR.tum (corrected LRR for the region), BAF.tum (corrected BAF for the region).

Note

Includes an adaptation of aCGH mergeLevels function to fix a problem with ansari.test.

Author(s)

Oscar M. Rueda, rueda.om@gmail.com

References

**createBinaryImage**

**Thresholding**

Create a binary image from a grayscale image by thresholding.

**Usage**

```r
createBinaryImage(imgG, img=NULL, method="otsu", threshold=NULL, numWindows=1, whitePixelMask=c())
```

**Arguments**

- `img`: An Image object or an array.
- `imgG`: The grey valued Image object.
- `method`: Either "otsu" or "phansalkar".
- `threshold`: Fixed threshold.
- `numWindows`: Number of windows to use for threshold calculation.
- `whitePixelMask`: Boolean mask of white pixels, if they should be excluded from thresholding.

**Details**

The function returns the binary image resulting from the thresholding. If threshold is defined, all pixels smaller than this value will be fixed to 1 all other values will be set to 0. If threshold is undefined, the thresholding value is calculated automatically using 'otsu' or 'phansalkar' thresholding.

The function 'otsu' does Otsu thresholding on the grey level histograms of the image. The function 'phansalkar' does thresholding using the mean and standard deviation of a specified window. The thresholding is done on the RGB as well as the LAP color space and the results are ORed. The window size is dim(img)/numWindows. White pixel can be excluded from thresholding (e.g. if white is background) by defining a whitePixelMask.
createClassifier

Construction of a classifier

Description

Creates a classifier for a training set.

Usage

createClassifier(trainingData, cross = FALSE)

Arguments

trainingData  A table, created by segmentImage with manually added classes.
cross         Does 10-fold cross validation to test the classifiers performance.
Details

Topological features include the density of cells and the size of the surrounding cytoplasma of a cell. These features depend on the size of the image. If training image and the image to classify have different size, these features can fool the classification and should not be enabled.

Value

A List containing:

- classifier: The classifier
- performance: cross validation performance

Author(s)

Henrik Failmezger, failmezger@mpipz.mpg.de

See Also

'createTrainingSet', 'classifyCells'

Examples

```r
f = system.file("extdata", "trainingData.txt", package="CRImage")
# read training data
trainingData=read.table(f,header=TRUE)
# create classifier
classifier=createClassifier(trainingData)[[1]]
```

Description

The functions creates an interactive session in order to label cells with their classes. The labeled cells can be used as training set for the classifier. Note!! This is until now only tested for MacOsX.

Usage

```r
labelCells(img, segmentedImage, classes, classColours, nblocks = 3, labeledPoints = NULL, filename = NULL)
```
Arguments

- **img**: The image.
- **segmentedImage**: The segmented image.
- **classes**: The possible class labels.
- **classColours**: The colors for the class labels.
- **nblocks**: The image can be separated in several blocks, as zooming is not possible.
- **labeledPoints**: Labeled cells from a previous training session.
- **filename**: The table of labeled cells is saved at this location.
- **filenameImage**: The image with the labeled cells is saved at this location.
- **transformCoordinates**: deprecated

Details

Use the keys: 
- **a**: In order to add a label to a cell.
- **d**: In order to delete a label from a cell.
- **c**: To switch between classes.
- **q**: To quit the interactive session.
- **r**: To refresh the session (labeled cells will be shown after refreshing)

Value

A table with columns: 
- **index**: the index of the cell in the segmented image.
- **x**: x-coordinate of the cell
- **y**: y-coordinate of the cell
- **classCell**: Label of the cell
- **xLocal**: Local x coordinate in the subimage(block)
- **yLocal**: Local y coordinate in the subimage(block)
- **block**: Block number in which the cell arises.

Author(s)

Henrik Failmezger

Examples

```r
## Should be DIRECTLY executable !! ----
##-- ==> Define data, use random,
##-- or do help(data=index) for the standard data sets.

## The function is currently defined as
```
plotCorrectedCN

Plot CN profiles corrected for cellularity

Description

This function takes the result of a call to correctCopyNumber and plots the results.

Usage

plotCorrectedCN(CN, chr=NULL)

Arguments

CN object result of a call to correctCopyNumber.

chr chromosome to plot.

Details

A panel with four plots is created. The top panel shows LRR (with DNAcopy segmentation overlaid) and BAF before correction and the bottom panel shows the plots after correction.

Value

No value is returned.

Author(s)

Oscar M. Rueda, rueda.om@gmail.com

References


Examples

LRR <- c(rnorm(100, 0, 1), rnorm(10, -2, 1), rnorm(3, 1),
          rnorm(100, 0, 1))

BAF <- c(rnorm(100, 0.5, 0.1), rnorm(5, 0.2, 0.01), rnorm(5, 0.8, 0.01), rnorm(10, 0.25, 0.1), rnorm(10, 0.75, 0.1),
         rnorm(100, 0.5, 0.1))

Pos <- sample(x=1:500, size=230, replace=TRUE)

Pos <- cumsum(Pos)

Chrom <- rep(1, length(LRR))

z <- data.frame(Name=1:length(LRR), Chrom=Chrom, Pos=Pos, LRR=LRR, BAF=BAF)

res <- correctCopyNumber(arr="Sample1", chr=1, p=0.75, z=z)

plotCorrectedCN(res, chr=1)
**processAperio**

*Cellularity Calculation of Aperio TX Scanner*

**Description**
Procession of Aperio TX Slides.

**Usage**

```
processAperio(classifier=classifier, inputFolder=inputFolder, outputFolder=outputFolder, identifier=identifier, numslides=numslides, cancerIdentifier=cancerIdentifier, classOther=classOther, maxShape=maxShape, minShape=minShape, failureRegion=failureRegion, slideToProcess=slideToProcess, KS=KS, colors=colors, classesToExclude=classesToExclude, threshold=threshold, numWindows=numWindows, colorCorrection=colorCorrection, classifyStructures=classifyStructures, ksToExclude=ksToExclude, pixelClassifier=pixelClassifier, densityToExclude=densityToExclude)
```

**Arguments**

- `classifier` The classifier.
- `inputFolder` The path to the image folder.
- `outputFolder` The path to the output folder.
- `identifier` The identifier of the files ("Ss" or "Da")
- `numSlides` The number of sections in the image.
- `cancerIdentifier` The identifier of the cancer class
- `classOther` deprecated
- `maxShape` Maximum size of cell nuclei
- `minShape` Minimum size of cell nuclei
- `failureRegion` minimum size of failure regions
- `slideToProcess` Set this parameter if only a certain slide should be processed
- `KS` Apply Kernel Smoother?
- `colors` Colors to paint the classes
- `classesToExclude` Which class should be excluded?
- `threshold` Which thresholding method should be used, "otsu" or "phansalkar" possible
- `numWindows` Number of windows to use for thresholding.
- `colorCorrection` deprecated
- `classifyStructures` Use hierarchical classification. If yes a pixel classifier has to be defined.
- `ksToExclude` These classes are excluded from kernel smoothing.
- `pixelClassifier` A SVM to classify pixel based on their color values. Needed if hierarchical classification should be applied.
- `densityToExclude` This class is excluded from cellularity calculation.
processAperio

numDensityWindows
Number of windows for the density plot.

resizeFactor
Specifies the size of the cell density image. If this variable is not defined, the size of the thumbnail is used for the cell density image, else the size is calculated by size(thumbnail) * resizeFactor. The thumbnail is the small overview image, created by the Aperio software.

plotCellTypeDensity
Plot the density of different cell types?

greyscaleImage
Color channel of the RGB image that should be used for thresholding

penClassifier
Classifier to exclude low quality images (will be part of next release)

referenceHist
Colour Histogram of a reference image that can be used to calculate the quality of the recent image. (will be part of next release)

fontSize
will be part of next release

Details
The function processes images of Aperio TX scanners. The images have to be saved in the CWS format.

Value
Four folders are created in the output folder.

Files
Cellularity values and cell numbers are saved in the file

classifiedImage
Subimages with labeled tumour and non tumour cells

tumourDensity
Cancer heatmaps for every subimage

cellCoordinates
Coordinates and cell class for every cell in the subimage

resizeFactor
Size of the cellularity density image, calculated by size(thumbnail) * resizeFactor. Whereas the thumbnail is the small overview image produced by Aperio.

Author(s)
Henrik Failmezger, failmezger@mpipz.mpg.de

Examples
#t = system.file("extdata", "trainingData.txt", package="CRImage")
#read training data
#trainingData=read.table(t,header=TRUE)
#create classifier
#classifier=createClassifier(trainingData,topo=FALSE)[[1]]
#classify aperio
#f = system.file("extdata", package="CRImage")
#f=file.path(f,"8905")
#dir.create("AperioOutput")
#takes long time!
SauvolaThreshold

Do Sauvola thresholding

Description

Thresholding method using mean and standard deviation.

Usage

SauvolaThreshold(allGreyValues)

Arguments

allGreyValues Vector of gray values.

Details

A threshold for the gray values is returned

Value

The threshold.

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

References


See Also

createBinaryImage
**Examples**

```r
f1 = system.file("extdata", "exImg2.jpg", package="CRImage")
print(f1)
img = readImage(f1)
print(img)
# convert to grayscale
imgG = EBImage::channel(img, 'grey')
# threshold value
t = SauvolaThreshold(as.vector(imgG))
```

---

**segmentImage**  
*Segmentation of an image*

**Description**

The function segments cells or cell nuclei in the image.

**Usage**

```r
segmentImage(filename="", image=NA, maxShape=NA, minShape=NA, failureRegion=NA, threshold="otsu", numWindows=NA, colorCorrection=FALSE, classifyStructures=FALSE, pixelClassifier=NULL, greyscaleImage=0, penclassifier=NULL, referenceHist=NULL)
```

**Arguments**

- **filename**  
  A path to an image
- **image**  
  An 'image' object, if no filename is specified.
- **maxShape**  
  Maximum size of cell nuclei
- **minShape**  
  Minimum size of cell nuclei
- **failureRegion**  
  Minimum size of failure regions
- **threshold**  
  Thresholding method, "otsu" or "phansalkar"
- **numWindows**  
  Number of windows to use for thresholding.
- **colorCorrection**  
  Deprecated
- **classifyStructures**  
  Segment structures in the image, if yes a pixel classifier has to be defined
- **pixelClassifier**  
  A SVM which classifies RGB color values in foreground and background.
- **greyscaleImage**  
  Channel of the RGB image, to use for thresholding, if 0 use a joined greyscale image.
- **penClassifier**  
  Classifier to exclude low quality images (will be part of next release)
- **referenceHist**  
  Color histogram of a reference image, that can be used to estimate the quality of the recent image (will be part of next release)
Details

The image is converted to greyscale and thresholded. Clutter is deleted using morphological operations. Clustered objects are separated using watershed algorithm. Segmented Cell nuclei, which exceed the maximum size are thresholded and segmented again. Cell nuclei which fall below the minimum size are deleted. Dark regions which exceed the parameter failureRegion are considered as artefacts and deleted. If the parameters are not defined, the operations will not be executed. Features are generated for every segmented object.

Value

A list is returned containing

- image The original image
- segmented image The segmented image

Author(s)

Henrik Failmezger, failmezger@cip.ifi.lmu.de

References


Examples

```r
#segment image
#f = system.file('extdata', 'exImg.jpg', package='CRImage')
#segmentationValues=segmentImage(f, maxShape=800, minShape=40, failureRegion=2000, threshold="otsu", numWindows=4)
#image=segmentationValues[[1]]
#segmentedImage=segmentationValues[[2]]
#imageFeatures=segmentationValues[[3]]
```
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