

Package ‘FISHalyseR’

April 12, 2022

Type Package

Title FISHalyseR a package for automated FISH quantification

Version 1.28.0

Date 2015-04-08

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Description FISHalyseR provides functionality to process and analyse
digital cell culture images, in particular to quantify FISH
probes within nuclei. Furthermore, it extract the spatial
location of each nucleus as well as each probe enabling spatial
co-localisation analysis.

VignetteBuilder knitr

License Artistic-2.0

Depends EBImage,abind

Suggests knitr

biocViews CellBiology

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

git_branch RELEASE_3_14

git_last_commit f2ce1a2

git_last_commit_date 2021-10-26

Date/Publication 2022-04-12

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| | |
|------------------|----------------|
| analyseParticles | <i>Analyse</i> |
|------------------|----------------|

Description

Cleans a given binary image according to area criteria specified by the user.

Usage

```
analyseParticles(Image,MaxSize,MinSize, isMask)
```

Arguments

| | |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Image | Binary image |
| MaxSize | Maximum area allowed for objects |
| MinSize | Minimum area allowed for objects |
| isMask | In case isMask=1, the function assumes that the binary images contains nuclei. Nuclei with an area smaller than MaxSize and greater than MinSize will be removed. If isMask=0, the function assumes that the binary images contains probes and subsequently probes with an area smaller than MinSize or larger than MaxSize are removed |

Value

Returns a labeled image

Author(s)

Karesh Arunakirinathan

Examples

```
f = system.file( "extdata", "SampleFISHgray.jpg", package="FISHalyseR")
img = readImage(f)

anaImg <- analyseParticles(img, 20000, 1000,0)
## anaImg contains now the cleaned-up image
```

calculateMaxEntropy *Max Entropy thresholding*

Description

The function converts a grayscale image to a binary image by computing a threshold using the Max Entropy method.

Usage

```
calculateMaxEntropy(Image)
```

Arguments

Image grayscale image

Details

Max Entropy thresholding can be used to detect the signals of probes in FISH cell culture images.

Value

The function returns the threshold value

Author(s)

Karesh Arunakirinathan

References

J.N KANPUR, P.K SHAOO, A.K.C WONG: A New Method for Gray-Level picture thresholding Using the Entropy of the Histogram. In COMPUTER VISION, GRAPHICS AND IMAGE PROCESSING,1985 p 273-285

See Also

calculateThreshold

Examples

```
f = system.file( "extdata", "SampleFISHgray.jpg", package="FISHalyseR")
img = readImage(f)

t = calculateMaxEntropy(img)

## Threshold grayscale image using the value computed by the Max Entropy method
img[img<t] <- 0
img[img>=t] <- 1
```

calculateThreshold *Compute threshold using Otsu's method*

Description

Computes the binary image of a grayscale image by using Otsu thresholding

Usage

```
calculateThreshold(Image)
```

Arguments

Image grayscale image

Details

The function computes a binary image using Otsu's method.

Value

calculateThreshold returns the threshold value

Author(s)

Karesh Arunakirinathan

References

Nobuyuki Otsu: A threshold selection method from grey level histograms. In: IEEE Transactions on Systems, Man, and Cybernetics. New York 9.1979, S.62-66. ISSN 1083-4419

See Also

calculateMaxEntropy

Examples

```
f = system.file( "extdata", "SampleFISHgray.jpg", package="FISHalyseR")
img = readImage(f)

t = calculateThreshold(img)

##Threshold image using the value computed via Otsu's method
img[img<t] <- 0
img[img>=t] <- 1
```

computeIlluminationCorrection
Multidimensional Illumination Correction

Description

Function to compute the multidimensional illumination correction (MDIC) using a stack of images

Usage

```
computeIlluminationCorrection(Images,pattern='*',AmountOfFiles=6)
```

Arguments

| | |
|---------------|---------------------------------------------------------------------|
| Images | Directory containing the images |
| pattern | Filenames have to match the pattern specified here |
| AmountOfFiles | Limit the amount of files used to compute the illumination gradient |

Value

```
computeIlluminationCorrection  
return the image containing the illumination background
```

Author(s)

Andreas Heindl

Examples

```
illuCorrection = dirname(system.file("extdata", "SampleFISHillu.jpg", package="FISHalyseR"))
```

processFISH *FISHalyseR - Automated fluorescence in situ hybridisation quantification in R*

Description

Function to automatically quantify FISH probes in cell-culture images.

Usage

```
processFISH(combinedImg, writedir, bgCorrMethod = list(1, 100), channelSignals = NULL,  
channelColours = NULL, sizeNucleus = c(5, 15000), sizeProbe = c(5, 100),  
gaussigma = 20, outputImageFormat = ".png")
```

Arguments

| | |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| combinedImg | Composite image of all available channels |
| writedir | Traget directory for output files |
| bgCorrMethod | Specifies the method used to correct for uneven background. Accepts only list types. Currently, four different methods are available: (1) Gaussian blurring, (2) Illumination correction image provided by the user, (3) multidimensional illumination correction (using a stack of images). In case no illumination correction should be applied, pass an empty list to the function |
| channelSignals | List of images containing the FISH probe |
| channelColours | List of colour vectors for each single channel |
| sizeNucleus | Minimum and maximum area (in pixel) of probes to be considered for further analysis |
| sizeProbe | Minimum and maximum area (in pixel) of probes to be considered for further analysis |
| gaussigma | Sigma of Gaussian used to blur the image |
| outputImageFormat | File format for the output image |

Value

processFISH does not return any value

Author(s)

Karesh Arunakirinathan, Andreas Heindl

See Also

computeIlluminationCorrection, analyseParticles

Examples

```
## Specify illumination correction image
illuCorrection = system.file( "extdata", "SampleFISHillu.jpg", package="FISHalyseR")

## Composite image containing available channels
combinedImage <- system.file( "extdata", "SampleFISH.jpg", package="FISHalyseR")

## Single FISH channels containing the probe signals
red_Og <- system.file( "extdata", "SampleFISH_R.jpg", package="FISHalyseR")
green_Gn <- system.file( "extdata", "SampleFISH_G.jpg", package="FISHalyseR")

## Output directory
writedir = paste(tempdir(),sep='')

## Use provided illumination correction image
bgCorrMethod = list(2,illuCorrection)
```

```
## Colour vector for three different probe channels (red, green and blue)
channelColours = list(R=c(255,0,0),G=c(0,255,0))

## Add probe channels to list
channelSignals = list(red_0g,green_Gn)

## Minimum and maximum area allowed for nuclei respectively probes
sizecell = c(1000,20000)
sizeprobe= c(5,20)

## Call processFISH with the specified parameters
processFISH(combinedImage,writedir,bgCorrMethod,channelSignals,
            channelColours,sizecell,sizeprobe)
```

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