Package 'FISHalyseR'

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Type Package

Title FISHalyseR a package for automated FISH quantification

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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

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R topics documented:

`analyseParticles` *Analyse*

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 consided 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_Og,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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