# Package 'beadarray'

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**Title** Quality assessment and low-level analysis for Illumina BeadArray data

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**Description** The package is able to read bead-level data (raw TIFFs and text files) output by BeadScan as well as bead-summary data from BeadStudio. Methods for quality assessment and low-level analysis are provided.

**Depends** R (>= 3.5.0), BiocGenerics (>= 0.3.2), Biobase (>= 2.17.8), hexbin

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**License** MIT + file LICENSE

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---

`beadarray-package` *The beadarray package: a tool for low-level analysis of Illumina BeadArrays*

---

## Description

The beadarray package: a tool for low-level analysis of Illumina BeadArrays

## Author(s)

Mark Dunning, Mike Smith, Jonathan Cairns, Matt Richie, Andy Lynch

addFeatureData *Add probe data*

## Description

Adds extra probe-specific data to an ExpressionSetIllumina object using an installed annotation package

## Usage

```
addFeatureData(data, toAdd = c("SYMBOL", "PROBEQUALITY",
"CODINGZONE", "PROBESEQUENCE"), annotation = NULL)
```

## Arguments

| | |
|---|---|
| data | An ExpressionSetIllumina object |
| toAdd | Either a pre-prepared data frame, or characters which refer to mappings within an annotation pacakge |
| annotation | Optional character identifying the annotation of the ExpressionSetIllumina object. e.g. Humanv3, Mousev2. |

## Details

The function will identify which package should be used by concatenating the character string *illumina* with the value of the annotation slot of the object, or the annotation argument passed in. If this package is not installed on the users computer, then the function will fail.

Assuming the package has been correctly loaded, the character vector toAdd is converted to the names of environments within the package. These environments are then queried with the featureNames of the input object. The result of each query is converted to a data frame and merged with the original feature data of the object.

Alternatively, rather than querying from an annotation pacakge, a pre-prepared data frame can be used.

## Value

An ExpressionSetIllumina object with modified featureData

## Examples

```
if(require(beadarrayExampleData)){

data(exampleSummaryData)

exampleSummaryData <- addFeatureData(exampleSummaryData)
head(fData(exampleSummaryData))
}
```

Annotation *Storage of annotation information*

## Description

An interface to set or retrieve information about the annotation of a beadLevelData or ExpressionSetIllumina object.

## Usage

```
suggestAnnotation(data,verbose=FALSE)
annotation(object,...)
```

## Arguments

| | |
|---|---|
| data | An object of class beadLevelData-class |
| . | |
| verbose | If TRUE, report overlaps with known platforms |
| object | Either a beadLevelData-class or ExpressionSetIllumina-class |
| ... | Extra arguments used by annotation |

## Details

A character string is used to specify the annotation with the currently supported values being; Humanv4, Humanv3, Humanv2, Humanv1, Mousev2, Mousev1, Mousev1p1 and Ratv1. This string is used within beadarray to retrieve control probe IDs within particular QC functions.

The suggestAnnotation function tries to determine a suitable value for a beadLevelData-class based on the probe IDs and compiled list of IDs from all expression platforms. This is based on the percentage of IDs on the array that overlap with IDs from known platforms. The platform with highest overlap is chosen.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

annotation(exampleBLData)

suggestAnnotation(exampleBLData,verbose=TRUE)

annotation(exampleBLData) <- "Humanv2"
```

```
data(exampleSummaryData)

annotation(exampleSummaryData)

annotation(exampleBLData) <- "Humanv1"

}
```

---

backgroundCorrectSingleSection
*Background correct an array-section*

---

## Description

Function to perform a basic bead-level background correction using a defined set of foreground and background intensities.

## Usage

```
backgroundCorrectSingleSection(BLData, array = 1, fg="Grn", bg="GrnB", newName = "Grn.bc")
```

## Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| array | the number of the array-section to be corrected |
| fg | the name under which the foreground intensities are stored |
| bg | the name under which the background intensities are stored |
| newName | Name to store the corrected intensities |

## Details

This function takes two attributes of a bead-level object and returns that bead-level object with an additional attribute consisting of the difference of the other two. We anticipate this being used as a simple background correction step, returning the difference between foreground and background intensities to be used as the specific intensity associated with a bead.

Functions to perform more advanced background correction steps can easily be constructed after consideration of this function.

## Value

beadLevelData object with modified beadData slot for the particular section

## Author(s)

Mark Dunning

## Examples

```
## Not run:

if(require(beadarrayExampleData)){

  data(exampleBLData)

  head(exampleBLData[[1]])

  for(i in 1:2){
   exampleBLData = backgroundCorrectSingleSection(exampleBLData, array=i)
  }


  head(exampleBLData[[1]])

  } else {

  stop("You will need the beadarrayExampleData package to run this example")
}


## End(Not run)
```

---

BASH *BASH - BeadArray Subversion of Harshlight*

---

## Description

BASH is an automatic detector of physical defects on an array. It is designed to detect three types of defect - COMPACT, DIFFUSE and EXTENDED.

## Usage

```
BASH(BLData, array, neighbours=NULL, transFun = logGreenChannelTransform,
    outlierFun = illuminaOutlierMethod, compn=3, wtsname=NULL, compact = TRUE,
    diffuse = TRUE, extended = TRUE, cinvasions = 10, dinvasions = 15,
    einvasions = 20, bgcorr = "median", maxiter = 10, compcutoff = 8,
    compdiscard = TRUE, diffcutoff = 10, diffsig = 0.0001, diffn = 3,
    difftwotail = FALSE, useLocs = TRUE, ...)
```

## Arguments

| | |
|---|---|
| BLData | BeadLevelList |
| array | integer specifying which section/array to plot. |
| neighbours | the user may specify the neighbours matrix, rather than have BASH calculate it. Time can be saved if using BASH and HULK, by calculating the neighbours matrix once and passing it to the two functions. |
| transFun | function to use to transform data prior to running BASH |
| outlierFun | the choice of outlier calling function to use. |
| compn | Numerical - when finding outliers in the compact analysis, how many MADs away from the median (for example) an intensity must be for it to be labelled an outlier. |
| wtsname | name under which bead weights are stored in the BLData object. It is only necessary to specify this if a) weights have already been set, and b) you wish BASH to observe them. |
| compact | Logical - Perform compact analysis? |
| diffuse | Logical - Perform diffuse analysis? |
| extended | Logical - Perform extended analysis? |
| cinvasions | Integer - number of invasions used whenever closing the image - see BASHCompact |
| dinvasions | Integer - number of invasions used in diffuse analysis, to find the kernel - see BASHDiffuse |
| einvasions | Integer - number of invasions used when filtering the error image - see BGFilter. |
| bgcorr | One of "none", "median", "medianMAD" - Used in diffuse analysis, this determines how we attempt to compensate for the background varying across an array. For example, on a SAM array this should be left at "median", or maybe even switched to "none", but if analysing a large beadchip then you might consider setting this to "medianMAD". (this code is passed to the method argument of BGFilter). Note that "none" may be the correct setting if HULK has already been applied. |
| maxiter | Integer - Used in compact analysis - the max number of iterations allowed. (Exceeding this results in a warning.) |
| compcutoff | Integer - the threshold used to determine whether a group of outliers is in a compact defect. In other words, if a group of at least this many connected outliers is found, then it is labelled as a compact defect. |
| compdiscard | Logical - should we discard compact defect beads before doing the diffuse analyis? |
| diffcutoff | Integer - this is the threshold used to determine the minimum size that clusters of diffuse defects must be. |
| diffsig | Probability - The significance level of the binomial test performed in the diffuse analysis. |
| diffn | Numerical - when finding outliers on the diffuse error image, how many MADs away from the median an intensity must be for it to be labelled an outlier. |

| | |
|---|---|
| difftwotail | Logical - If TRUE, then in the diffuse analysis, we consider the high outlier and low outlier images seperately. |
| useLocs | Logical - If TRUE then a .locs file corresponding to the array is sought and, if found, used to identify the neighbouring beads. If FALSE the neighbours are infered algorithmically. See generateNeighbours for more details. |
| ... | Logical - Perform compact analysis? |

## Details

The BASH pipeline function performs three types of defect analysis on an image.

The first, COMPACT DEFECTS, finds large clusters of outliers, as per BASHCompact. The outliers are found using findAllOutliers(). We then find which outliers are clustered together. This process is iterative - having found a compact defect, we remove it, and then see if any more defects are found.

The second, DIFFUSE DEFECTS, finds areas which are densely populated with outliers (which are not necessarily connected), as per BASHDiffuse. To make this type of defect more obvious, we first generate an ERROR IMAGE, and then find outliers based on this image. (The error image is calculated by using method = "median" and bgfilter = "medianMAD" in generateE, unless ebgcorr = FALSE in which case we use bgfilter = "median".) Now we consider a neighbourhood around each bead and count the number of outlier beads in this region. Using a binomial test we determine whether this is more that we would expect if the outliers were evenly spread over the entire array. If so, we mark it as a diffuse defect. (A clustering algorithm similar to the compact defect analysis is run to reduce false positives.)

After each of these two analyses, we "close" the image, filling in gaps.

The third, EXTENDED DEFECTS, returns a score estimating how much the background is changing across an array, as per BASHExtended. To estimate the background intensity, we generate an error image using the median filter (i.e. generateE with method = "median" and bgfilter = "median"). We divide the variance of this by the variance of an error image without using the median filter, to obtain our extended score.

It should be noted that to avoid repeated computation of distance, a "neighbours" matrix is used in the analysis. This matrix describes which beads are close to other beads. If a large number of beads are missing (for example, if beads with ProbeID = 0 were discarded) then this algorithm may be affected.

For more detailed descriptions of the algorithms, read the help files of the respective functions listed in "see also".

BASH is currently quite a slow, memory-intensive function. It will only run on a single array at a time, and for analysis of multiple arrays, we recommend parallelising the command. An example is shown using the base parallel package.

## Value

The output is a list with four attributes:

wts: A vector of weights for the matrix.

ext: A vector of extended scores (null if the extended analysis was disabled).

QC: A summary of the extended score and the number of beads masked.

call: The function you used to call BASH.

## Author(s)

Jonathan Cairns

## References

J. M. Cairns, M. J. Dunning, M. E. Ritchie, R. Russell, and A. G. Lynch (2008). BASH: a tool for managing BeadArray spatial artefacts. Bioinformatics 15; 24(24)

## See Also

BASHCompact, BASHDiffuse, BASHExtended, generateNeighbours, HULK

## Examples

```
## Not run:

if(require(beadarrayExampleData)){

 data(exampleBLData)
 output <- BASH(exampleBLData,array=1,useLocs=FALSE)
     exampleBLData <- setWeights(exampleBLData, output$wts, array=1) #apply BASH weights to exampleBLData

 ###BASH only accepts one array at a time, but it can be made to run in a parallel fashion
 library(parallel)

 output <- mclapply(c(1,2), function(x) BASH(exampleBLData, array=x, useLocs=FALSE))

 for(i in 1:2){
   exampleBLData <- setWeights(exampleBLData, output[[i]]$wts, array=i)
 }


 #diffuse test is stricter
 output <- BASH(exampleBLData, diffsig = 0.00001,array=1, useLocs=FALSE)

 #more outliers on the error image are used in the diffuse analysis
 output <- BASH(exampleBLData, diffn = 2,array=1, useLocs=FALSE)

 #only perform compact & diffuse analyses (we will only get weights)
 output <- BASH(exampleBLData, extended = FALSE,array=1, useLocs=FALSE)

 #attempt to correct for background.
 output <- BASH(exampleBLData, bgcorr = "median",array=1, useLocs=FALSE)
}

else{

  stop("You will need the beadarrayExampleData package to run this example")
}
```

```
## End(Not run)
```

---

BASHCompact *BASH - Compact Defect Analysis*

---

## Description

Creates a list of probes marked as being in compact defects.

## Usage

```
BASHCompact(inten, probeIDs, neighbours = NULL, wts=1, n=3, maxiter = 10,
        cutoff = 8,  cinvasions = 10, outlierFun=illuminaOutlierMethod, ...)
```

## Arguments

| | |
|---|---|
| inten | the (transformed) intensities associated with beads on an array. |
| probeIDs | the probe identities associated with those beads (i.e. indicating which beads are of which sort) |
| neighbours | A Neighbours matrix such as that generated by generateNeighbours. Compulsory - the function will no longer generate this for you. |
| wts | weights indicating any beads to be masked in calculations. |
| n | Specify a cut-off for outliers (e.g. as n median absolute deviations (MADs) from the median). The default value is 3 |
| maxiter | Integer - Maximum number of iterations. |
| cutoff | Integer - Size a cluster must be to be labelled a compact defect. |
| cinvasions | Integer - Number of invasions used when closing the image. |
| outlierFun | the choice of outlier calling function to use. |
| ... | Additional arguments to be passed to outmeth. |

## Details

BASHCompact finds "compact defects" on an array. A compact defect is defined as a large connected cluster of outliers.

This function first finds the outliers on an array. This is done via the user's choice of function (e.g. illuminaOutlierMethod or squeezedVarOutlierMethod.

Next, using the Neighbours matrix and a Flood Fill algorithm, it determines which beads are in large connected clusters of outliers (of size larger than cutoff). These beads are then temporarily removed and the process repeated with the remaining beads. The repetition continues until either no large clusters of outliers remain, or until we have repeated the process maxiter times (and in this case, a warning will be given). In this way, we obtain a list of defective probes.

Finally, we "close" the image, to fill in small gaps in the defect image. This consists of a "dilation" and an "erosion". In the dilation, we expand the defect image, by adding beads adjacent to defective beads into the defect image. This is repeated `cinvasions` times. In the erosion, we contract the defect image, by removing beads adjacent to non-defective beads from the defect image. (Erosion of the defect image is equivalent to a dilation of the non-defective image.)

## Value

A vector consisting of the BeadIDs of beads labelled as compact defects.

## Author(s)

Jonathan Cairns

## References

J. M. Cairns, M. J. Dunning, M. E. Ritchie, R. Russell, and A. G. Lynch (2008). BASH: a tool for managing BeadArray spatial artefacts. Bioinformatics 15; 24(24)

## See Also

BASH, generateNeighbours

## Examples

```
## Not run:

if(require(beadarrayExampleData)){

data(exampleBLData)
o <- BASHCompact(getBeadData(exampleBLData,array=1,what="Grn"),
                 getBeadData(exampleBLData,array=1,what="ProbeIDs"))
##increased no of closure invasions
o <- BASHCompact(getBeadData(exampleBLData,array=1,what="Grn"),
                 getBeadData(exampleBLData,array=1,what="ProbeIDs"), cinvasions = 10)
##only larger defects will be found with this setting
o <- BASHCompact(getBeadData(exampleBLData,array=1,what="Grn"),
                    getBeadData(exampleBLData,array=1,what="ProbeIDs"), cutoff = 12)

}


## End(Not run)
```

BASHDiffuse *BASH - Diffuse Defect Analysis*

## Description

Creates a list of probes marked as being in diffuse defects.

## Usage

```
BASHDiffuse(inten, probeIDs, wts=NULL, neighbours = NULL, E = NULL, n = 3,
    compact = NULL, sig = 0.0001, invasions = 10, cutoff = 8, cinvasions = 10,
    twotail = FALSE, einvasions = 20, outlierFun = illuminaOutlierMethod, ...)
```

## Arguments

| | |
|---|---|
| inten | the (transformed) intensities associated with beads on an array. |
| probeIDs | the probe identities associated with those beads (i.e. indicating which beads are of which sort) |
| wts | weights indicating any beads to be masked in calculations. |
| neighbours | A Neighbours matrix such as that generated by generateNeighbours. Compulsory - the function will no longer generate this for you. |
| E | Numerical vector - The error image to use. Optional - if left blank, it will be computed, using generateE using bgfilter = "median". |
| n | Specify a cut-off for outliers (e.g. as n median absolute deviations (MADs) from the median). The default value is 3 |
| compact | Vector - Optional. BeadIDs of beads in compact defects to remove from the analysis. |
| sig | Numerical - Significance level of binomial test. |
| invasions | Integer - Number of invasions to use to find the kernel (see below). |
| cutoff | Integer - Size a cluster must be to be labelled a diffuse defect. |
| cinvasions | Integer - Number of invasions used when closing the image. |
| twotail | Logical - If TRUE, then we analyse positive and negative outliers separately, and then combine the diffuse defect images at the end. |
| einvasions | Integer - Number of invasions used when forming the error image E. |
| outlierFun | the choice of outlier calling function to use. |
| ... | Additional arguments to be passed to outmeth. |

## Details

BASHDiffuse finds "diffuse defects" on an array. A diffuse defect is defined as a region containing an unusually large number of (not necessarily connected) outliers.

Firstly, we consider the error image E, and find outlier beads on this image. Outliers for a particular bead type are determined using a 3 MAD cut-off from the median.

We now consider an area around each bead (known as the "kernel"). The kernel is found by an invasion process using the neighbours matrix - we choose the beads which can be reached from the central bead in cinvasions steps.

We count how many beads are in the kernel, and how many of these are marked as outliers. Using a binomial test, we work out if there are significantly more outliers in the kernel than would be expected if the outliers were equally distributed over the entire array. If so, then the central bead is marked as a diffuse defect.

Lastly, we run a clustering algorithm and a closing algorithm similar to those in BASHCompact.

## Value

A vector consisting of the BeadIDs of beads considered diffuse defects.

## Author(s)

Jonathan Cairns

## References

J. M. Cairns, M. J. Dunning, M. E. Ritchie, R. Russell, and A. G. Lynch (2008). BASH: a tool for managing BeadArray spatial artefacts. Bioinformatics 15; 24(24)

## See Also

BASH, generateNeighbours

## Examples

```
## Not run:


if(require(beadarrayExampleData)){

data(exampleBLData)
o <- BASHDiffuse(getBeadData(exampleBLData,array=1,what="Grn"), getBeadData(exampleBLData,array=1,what="ProbeID
o <- BASHDiffuse(getBeadData(exampleBLData,array=1,what="Grn"), getBeadData(exampleBLData,array=1,what="ProbeID
o <- BASHDiffuse(getBeadData(exampleBLData,array=1,what="Grn"), getBeadData(exampleBLData,array=1,what="ProbeID

}


## End(Not run)
```

BASHExtended *BASH - Extended Defect Analysis*

## Description

Returns a score, which assesses the extent to which the background is changing across the array/strip.

## Usage

```
BASHExtended(BLData, array, transFun = logGreenChannelTransform, neighbours = NULL, useLocs = TRUE, E =
```

## Arguments

| | |
|---|---|
| BLData | BeadLevelList |
| array | integer specifying which section/array to analyse |
| transFun | Function to use to transform data prior to running BASH. |
| neighbours | A Neighbours matrix. Optional - if left NULL, it will be computed, using default generateNeighbours settings. |
| useLocs | Logical value, specifiying whether the .locs file (if present) should be used to determine neighbours. |
| E | Numerical vector - The error image to use. Optional - if left blank, it will be computed, using generateE (with bgfilter = "none", i.e. no background filter applied). |
| E.BG | Numerical vector - The background error image to use. Optional - if left blank, it will be computed from E, using default BGFilter settings (i.e. method = "median"). |

## Details

BASHExtended assesses the change of background across an array.

The error image used should not be background filtered (as opposed to the error image used in BASHDiffuse). Here, E is the error image

## Value

Scalar (Extended defect score)

## Author(s)

Jonathan Cairns

## References

J. M. Cairns, M. J. Dunning, M. E. Ritchie, R. Russell, and A. G. Lynch (2008). BASH: a tool for managing BeadArray spatial artefacts. Bioinformatics 15; 24(24)

## See Also

BASH, generateNeighbours,

## Examples

```
## Not run:

if(require(beadarrayExampleData)){

 data(exampleBLData)
 extended <- BASHExtended(exampleBLData, 1)

}

## End(Not run)
```

---

# beadarrayUsersGuide *View beadarray User's Guide*

---

## Description

Finds the location of the beadarray User's Guide and opens it.

## Usage

```
beadarrayUsersGuide(view=TRUE, topic="beadlevel")
```

## Arguments

| | |
|---|---|
| view | logical, should the document be opened using the default PDF document reader? (default is TRUE) |
| topic | character string specifying topic ("beadlevel", "beadsummary" or "BASH") |

## Details

The function vignette("beadarray") will find the short beadarray vignette which describes how to obtain the more detailed user's guide on the analysis of raw "beadlevel" data, "beadsummary" data or how to use the "BASH" method for detecting spatial artefacts.

## Value

Character string giving the file location.

### Author(s)

Matt Ritchie

### Examples

```
beadarrayUsersGuide(view=FALSE)
beadarrayUsersGuide(view=FALSE, topic="beadsummary")
```

---

## beadIntensityPlots *Plotting the intensities of selected beads on a section*

---

### Description

The function will plot the intensities of selected beads on a specified array

### Usage

```
plotBeadIntensities(BLData, array = 1, BeadIDs, transFun = logGreenChannelTransform, cols = NULL, ...)
```

### Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| array | numeric specifying which array to plot the intensities from |
| BeadIDs | what ArrayAddress IDs to be plotted |
| transFun | function specifying what transformation to be applied to the beadLevelData prior to plotting |
| cols | a vector of colours to be used to plot each ID. If NULL the rainbow function is used to generate colours. |
| ... | other argument that may be passed along to plot. |

### Details

The function will take all data from the specified section, apply the transformation (the default is to do log2) and then find the subset of beads that have the specified ID. These IDs should match the numeric ArrayAddress IDs that are stored in the beadLevelData object.

### Value

Plot is produced on current graphical device.

### Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){


data(exampleBLData)

randIDs = sample(getBeadData(exampleBLData, array=1, what="ProbeID"),10)

plotBeadIntensities(exampleBLData, array=1, BeadIDs = randIDs)

}
```

---

# beadLevelData-class *Class "beadLevelData"*

---

## Description

A class for storing red and green channel foreground and background intensities from an Illumina experiment.

## Objects from the Class

Objects can be created by calls of the form `new("beadLevelData")`, but are usually created by `readIllumina`.

## Slots/List Components

Objects of this class contain the following slots

| | |
|---|---|
| `beadData`: | A list of arrays, indexed by array name. Each item in this list is itself a list, containing enviroments holdi |
| `sectionData`: | a list containing information. Each item in the list is a data frame containing one row for each section |
| `experimentData`: | a list containing the annotation of the platform, link to the sdf file and type of data (slide or Sentrix Array |
| `history`: | Character vector storing the operations performed on this object. |

## Methods

**show(beadLevelData)** Printing method for BeadLevelList

`sectionNames(object,arrays=NULL)` Returns the strip/array names from a

`numBeads(object,arrays=NULL)` Returns the number of beads on selected `arrays`

## Accessing data from the class

`getBeadData` retrieve data

`insertBeadData` Input or modify existing data

### Author(s)

Mark Dunning, Mike Smith

### See Also

readIllumina

### Examples

```
if(require(beadarrayExampleData)){


data(exampleBLData)

sectionNames(exampleBLData)

head(exampleBLData[[1]])

getBeadData(exampleBLData, array=1, what="Grn")[1:10]


}
```

---

## BeadLevelList-class *Class "BeadLevelList"*

---

### Description

A class for storing red and green channel foreground and background intensities from an Illumina experiment.

### Objects from the Class

Objects can be created by calls of the form `new("BeadLevelList")`, but are usually created by readIllumina.

### Slots/List Components

Objects of this class contain the following slots

| | |
|---|---|
| beadData: | an environment for storing the raw bead-level data. Each row correspond to a bead and columns the data. |
| phenoData: | an 'AnnotatedDataFrame' containing experimental information. |
| arrayInfo: | a list containing array information. |
| annotation: | character storing annotation package information. |

### Methods

`arrayNames(object,arrays=NULL)` Returns the strip/array names from a `BeadLevelList` object for selected `arrays`

`getArrayData(object,what="G",log=TRUE)` Retrieves the `what` intensities on the `log` scale from the `BeadLevelList`

### Author(s)

Mark Dunning and Matt Ritchie

---

## beadRegistrationData-class

*Class "beadRegistrationData"*

---

### Description

A class for storing information relating to the registration of the image.

### Slots/List Components

Objects of this class contain the following slots

| | |
|---|---|
| `layout`: | A list entry containing details of the structure of a BeadChip. By default entries for the number of secti |
| `registrationData`: | A list with length equal to the total number of segments registered. Each entry contains a vector of the |
| `coordinateData`: | A list with length equal to the total number of segments registered. Each entry contains the coordinates |
| `cornerData`: | A list with length equal to the total number of segments registered. Each entry contains a set of four co |
| `p95`: | A numeric vector storing the 95th percentile of bead intensities within each segment. |
| `imageLocations`: | Character vector storing the location of the tiff images of the array. These are obtained from the sectio |
| `metrics`: | A data.frame, where each line contains the appropriate entry from the Metrics.txt file. Currently not us |

### Methods

**boxplot(regScores, plotP95 = FALSE)**

### Author(s)

Mike Smith

### See Also

`checkRegistration`

boxplot-methods *Boxplots from summary data*

## Description

The standard boxplot function has been extended to work with the ExpressionSetIllumina class. Moreover, it generates graphics using the ggplot2 package and can incorporate user-defined factors into the plots.

## Details

Extra factors can be added to the plots provided they are present in either the phenoData or featureData or the object.

## Value

A ggplot object is produced and displayed on screen

## Author(s)

Mark Dunning

## Examples

```
    if(require(beadarrayExampleData)){

data(exampleSummaryData)

subset <- channel(exampleSummaryData, "G")[,1:8]

boxplot(subset)

boxplot(subset, what="nObservations")


###You can use columns from the featureData in the plots. Here we will use the control-type

head(fData(subset))

table(fData(subset)[,"Status"])

boxplot(subset, probeFactor = "Status")

###Similarly, we group samples according to colums in phenoData

pData(subset)


boxplot(subset, SampleGroup = "SampleFac")
```

```
##Both sample and probe factors can be combined into the same plot

boxplot(subset, SampleGroup = "SampleFac", probeFactor = "Status")

##Suppose we have found differentially expressed genes between experimental conditions and want to plot their respo

if(require(illuminaHumanv3.db)){

   ids <- unlist(mget("ALB", revmap(illuminaHumanv3SYMBOL)))
   subset2 <- subset[ids,]

   boxplot(subset2, SampleGroup = "SampleFac")
   boxplot(subset2, SampleGroup = "SampleFac", probeFactor = "IlluminaID")
}

   }
```

---

`calculateDetection` *Calculate detection scores*

---

## Description

Function to calculate detection scores for summarized data if they are not available.

## Usage

```
calculateDetection(BSData, status=fData(BSData)$Status, negativeLabel="negative")
```

## Arguments

| | |
|---|---|
| `BSData` | An ExpressionSetIllumina object |
| `status` | character vector giving probe types |
| `negativeLabel` | character giving identifer for negative controls |

## Details

Detection scores are a measure of whether the probe is showing any specific expression. This function implements Illumina's method for calculating the detection scores for all bead types on a given array. Within an array, Illumina discard negative control bead-types whose summary values are more than three MADs from the median for the negative controls. Illumina then rank the summarized intensity for each other bead-type against the summarized values for the remaining negative control bead-types and calculate a detection p-value 1-R/N, where R is the relative rank of the bead intensity when compared to the $N$ remaining negative controls. Thus, if a particular bead has higher intensity than all the negative controls it will be assigned a value of 0. This calculation is repeated for all arrays.

The function expects the negative controls to be indicated by the `Status` column in the `featureData` slot of the `ExpressionSetIllumina` object. If this is not present the user can supply a status vector with the same length as the number of rows in the ExpressionSetIllumina object.

## Value

Matrix of detection scores with the same dimensions as the exprs matrix of BSData. This matrix can be stored in a ExpressionSetIllumina object using the `Detection` function

## Author(s)

Mark Dunning and Andy Lynch

## Examples

```
if(require(beadarrayExampleData)){

data(exampleSummaryData)
##By default, the status column of featureData is used

exampleSummaryData.log2 <- channel(exampleSummaryData ,"G")

det <- calculateDetection(exampleSummaryData.log2)

Detection(exampleSummaryData.log2) <- det

##Example of specifying own status vector

exampleSummaryData.log2 <- addFeatureData(exampleSummaryData.log2)

pq <- fData(exampleSummaryData.log2)$PROBEQUALITY

det2 <- calculateDetection(exampleSummaryData.log2, status="pq", negativeLabel="No match")
}
```

---

# `calculateOutlierStats` *Outlier distribution stats*

---

## Description

Function that determines the outlier beads on an array and how they are distributed among the segments

## Usage

```
calculateOutlierStats(BLData, array = array, transFun = logGreenChannelTransform, outlierFun = illumin
```

## Arguments

| | |
|---|---|
| `BLData` | a `beadLevelData-class` object |
| `array` | the number of the array of interest |
| `transFun` | how the section data is to be transformed prior to calculating outliers |

| | |
|---|---|
| outlierFun | a function for calculating outliers |
| n | an indicator of how extreme an observation must be (e.g. how many MADs from the median), to be passed to the function that will identify outliers |
| useLocs | use locs and sdf information (if available) to determine section layout |
| nSegments | manually set how many segments the section is divided into |
| ... | Additional arguments to be passed to outmeth. |

## Details

A section of an expression BeadChip (e.g. the Humanv3 or HumanHT-12) is made up of 9 physcially-separate segments. A useful QA check is to see how the outliers are distributed among these segments. Outliers are beads that have outlying intensities according to some rule that the user can specify. The default (as used by Illumina) is to exclude beads that are more than 3 median absolute deviations from the median. Once outliers are determined, the coordinates for these outliers are binned into segments by assuming that the segments are evenly spaced across the section surface.

Note that sections from Sentrix Array Matrix do not have segments, so the results may not be informative

## Value

vector with the percentage of beads found in each segment that were determined to be outliers

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

##Artificial example, there are no segments on this type of BeadArray

calculateOutlierStats(exampleBLData, array=1, nSegments=10, useLocs=FALSE)

calculateOutlierStats(exampleBLData, array=2, nSegments=10, useLocs=FALSE)


}
```

checkRegistration *Perform check for misregistered array segments.*

## Description

Occasionally the registration of an array can go wrong, with the bead centres found in the wrong place in an image. The effective result of this is a scrambling of the bead IDs.

Note that the function requires that the sdf file and locs file are present, and has particular expectations towards their file names and locations.

## Usage

```
checkRegistration(BLData, array = 1)
```

## Arguments

| | |
|---|---|
| BLData | An object of class beadLevelData-class. |
| array | Integer specifying the index of the arrays to be checked. Can be a vector to process multiple arrays e.g. 1:12. |

## Details

In order to check for mis-registration we can examine the within bead-type variance across the array. This function computes this statistic twice for each array segment (since each segment is registered independently), once using the given bead IDs and once using a randomly assigned set of IDs. The former is then subtracted from the later. In cases where the registration has worked successfully we expect the majority of these values to be greater than zero, which for misregistered arrays the differences should be centred about zero.

## Value

Returns an object of class beadRegistrationData.

## Author(s)

Mike Smith

## References

Smith ML, Dunning MJ, Tavare S, Lynch AG. Identification and correction of previously unreported spatial phenomena using raw Illumina BeadArray data. BMC Bioinformatics (2010) 11:208

combine *Combine two objects.*

## Description

Combine two seperate objects into a single object.

## Usage

```
## S4 method for signature 'beadLevelData,beadLevelData'
combine(x, y)

## S4 method for signature 'ExpressionSetIllumina,ExpressionSetIllumina'
combine(x,y)
```

## Arguments

| | |
|---|---|
| x | An object of class beadLevelData or ExpressionSetIllumina. |
| y | An object of the same class as x. |

## Details

The combine function allows two objects of the same class that have been created seperately to be combined into one.

## Value

Returns an object of the same class as the two inputs.

## Author(s)

Mark Dunning, Mike Smith

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

sectionNames(exampleBLData)

data2 <- combine(exampleBLData, exampleBLData)

sectionNames(data2)

}
```

controlProbeDetection *Percentage of beads detected*

## Description

Function to calculate the percentage of beads matching a defined set of control types that are detected as having intensity above background level on an array-section.

## Usage

```
controlProbeDetection(BLData, transFun = logGreenChannelTransform, array = 1, controlProfile = NULL, ta
```

## Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| transFun | transformation to be applied to data |
| array | a numeric index of the array section |
| controlProfile | optional data frame defining ArrayAddressIDs belonging to each control type |
| tagsToDetect | vector of character strings defined which control types to interrogate |
| negativeTag | character string defining which control type to use as background |
| detThresh | numeric value for threshold for detection |

## Details

Details of the controls on the array-section can be inferred from the annotation of the beadLevelData object or supplied as a data frame. The first column of the data frame should contain ArrayAddressIDs, with the control type of the each ID in the second column. The strings supplied in the tagsToDetect and negativeTag parameters should be present in this column.

The ArrayAddressIDs that correspond to the specified tags are matching to the ArrayAddressIDs for the chosen array and intensities for all beads are extracted. The function implements Illumina's method for calculating the detection scores for all bead types on a given array. Within an array, Illumina discard negative control bead-types whose summary values are more than three MADs from the median for the negative controls. Illumina then rank the summarized intensity for each other bead-type against the summarized values for the remaining negative control bead-types and calculate a detection p-value 1-R/N, where R is the relative rank of the bead intensity when compared to the $N$ remaining negative controls. Thus, if a particular bead has higher intensity than all the negative controls it will be assigned a value of 0. This calculation is repeated for all arrays.

The percentage reported is the percentage of beads of each control type that are detected at the defined threshold.

## Author(s)

Mark Dunning

## See Also

beadStatusVector,calculateDetection

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

for(i in 1:2){

print(controlProbeDetection(exampleBLData, array = i, tagsToDetect=c("housekeeping", "biotin"), negativeTag="neg

}

}
```

---

convertBeadLevelList *Convert a BeadLevelList object into a beadLevelData object*

---

## Description

As of beadarray version 2.0 the BeadLevelList class has been deprecated and replaced by the beadLevelData class. Whilst these are superficially similar, the way the data are stored is quite different, meaning most functionality within the package is no longer compatible with the original BeadLevelList class.

This function converts any object that is of the old BeadLevelList class into a beadLevelData object.

## Usage

```
convertBeadLevelList(BeadLevelList)
```

## Arguments

| | |
|---|---|
| BeadLevelList | An object of class BeadLevelList |

## Value

Returns an object of class beadLevelData.

## Author(s)

Mike Smith

## See Also

beadLevelData-class

createTargetsFile *A function to generate a targets file given a directory of Illumina bead-level files*

## Description

This function, when pointed to a directory containing Illumina bead-level files (e.g. txt, idat, locs, tif) will return a simple targets file of the sort expected by beadarray. Note that a user created targets file is likely to be of greater value.

## Usage

```
createTargetsFile(dir = NULL, nochannels = 1, channel1 = "Grn", channel2 = "Red", txtsuff = "txt", imgsu
```

## Arguments

| | |
|---|---|
| dir | dir: The directory containing the Illumina bead-level files. By default, will search the working directory. |
| nochannels | nochannels: Does the directory contain 1 or 2 channel arrays? Setting this argument to be null will result in the function making its best guess. |
| channel1 | channel1: The string indicating that files are associated with the first channel (usually Grn). |
| channel2 | channel2: The string indicating that files are associated with the second channel (usually Red). |
| txtsuff | txtsuff: The suffix of files containing the bead-level intensities (usually txt, but occasionally csv). |
| imgsuff | imgsuff: The suffix of files containing the images. |
| locssuff | locssuff: The suffix of files containing the precise bead locations (usually locs). |
| xmlsuff | xmlsuff: The suffix of files containing the meta-data (usually xml). |
| verbose | verbose: Determines whether or not the function reports on its progress as it goes along. |
| special | special: Files with names containing special words (such as fiducial) are ignored. |
| ColourConfusionStop | ColourConfusionStop: This determines the behaviour of the function if there is a discrepancy between the number of channels specified, and the number apparently present. |
| metricsflag | codemetricsflag: This gives the key word that can be used to identify metrics files. |
| metsep | metsep: This gives the cell separator used in the metrics file. |
| metricsection | metricsection: This gives the column heading used in the metrics file to indicate array section names. |
| metricchip | metricchip: This gives the column heading used in the metrics file to indicate the chip name. |

## Details

This function bases its resultant targets file on the files with suffix txtsuff.

## Value

This returns a dataframe containing

## Author(s)

Andy Lynch

## See Also

readIlluminaData()

## Examples

```
#createTargetsFile(verbose=T)
```

---

`deprecatedFunctions` *Deprecated Functions*

---

## Description

Functions that have been renamed in the latest version of beadarray, but kept in for backwards compatibility.

## Author(s)

Mark Dunning

---

`dim` *Retrieve the dimensions of an object*

---

## Description

Retrieve the dimension of an object.

## Usage

```
## S4 method for signature 'beadLevelData'
dim(x)

## S4 method for signature 'ExpressionSetIllumina'
dim(x)
```

### Arguments

| | |
|---|---|
| x | An object of class beadLevelData or ExpressionSetIllumina |

### Author(s)

Mark Dunning

---

## expressionQCPipeline *Flexible bead-level QC pipeline*

---

### Description

Function to produce various QC plots and HTML summary pages for bead-level data.

### Usage

```
expressionQCPipeline(BLData, transFun = logGreenChannelTransform, qcDir = "QC", plotType = ".jpeg", hor
```

### Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| transFun | what transformation function to apply |
| qcDir | a directory to write output to |
| plotType | desired file extension for plots (jpeg or png) |
| horizontal | if TRUE imageplots and outlier plots are produced with longest edge on x axis |
| controlProfile | a data frame defining all control types. not required if annotation information is stored in the bead-level object |
| overWrite | if FALSE any plots that exist in the directory will not be recreated |
| nSegments | how many segments each section is divided into |
| outlierFun | a function to removed outliers |
| tagsToDetect | which control types to used in the detection metrics |
| zlim | the range of the imageplots |
| boxplotFun | what transformation function to be used in boxplots |
| imageplotFun | what transformation function to be used for imageplots |
| positiveControlTags | character strings defining which positive controls to plot |
| hybridisationTags | additional control types to be plotted |
| negativeTag | character string to identify which control type in the control profile corresponds to negative controls |

## Details

This function is a convient way of automatically generating QC plots for each section within a beadLevelData object. The following plots are produced for each section. i) scatter plots of all bead observation of the positive controls. See poscontPlot. ii) Further scatter plots of other controls of interest using poscontPlot. iii) imageplot (imageplot) of section data after applying transformation function iv) plot of outlier locations using specified outlier function. A HTML page displaying all the plots is produced.

After plots have been produced for each section, makeQCTable is run to make a table of mean and standard deviations for the defined control types, followed by the results of calculateOutlierStats and controlProbeDetection for each section and written to a HTML page in the requested directory.

The function should be able to run automatically for expression data that has its annotation stored using setAnnotation or using readIllumina. Otherwise the controlProfile data frame can be used to define the control types on the array and their associated ArrayAddressIDs. Similarly, the function assumes single-channel data but a transformation function can be passed.

## Author(s)

Mark Dunning

## See Also

poscontPlot imageplot outlierplot controlProbeDetection

## Examples

```
if(require(beadarrayExampleData)){

## Not run:

data(exampleBLData)

expressionQCPipeline(exampleBLData, horizontal=T)


## End(Not run)

}
```

---

# ExpressionSetIllumina-class

*Class "ExpressionSetIllumina"*

---

## Description

Container for high-throughput assays and experimental metadata. ExpressionSetIllumina class is derived from eSet, and requires matrices exprs, se.exprs, nObservations, Detection as assay data members. The slots featureData, phenoData are accessed in the usual manner using fData and pData functions.

For ExpressionSetIllumina objects created from bead-level data (using the summarize function), a QC slot is used to contain any quality control data that was present in the beadLevelData object. This is a change from previous versions of beadarray, where the intensities of the control probes themselves were stored in this slot. From version 2.0.0 onwards, control probes are stored in the assayData slot with the regular probes and the featureData slot has a reference for which rows correspond to controls.

The ExpressionSetIllumina class is able to accomodate different channels when created from bead-level data. The channelNames function may be used to find out what channels are present in the object. The channel function can be used to select a particular channel, returning an ExpressionSetIllumina object.

## Author(s)

Mark Dunning

---

# generateNeighbours *Generate matrix of neighbouring beads*

---

## Description

Generates a neighbours matrix from either a .locs file or the X and Y coordinates in a beadLevelData object.

## Usage

```
generateNeighbours(BLData, array = 1, useLocs = TRUE, window = 30, margin = 10, thresh = 2.2)
```

## Arguments

| | |
|---|---|
| BLData | An object of class beadLevelData-class |
| array | integer specifying which section/array to process |
| useLocs | logical value, specifiying whether the .locs file (if present) should be used to determine neighbours. |
| window | numeric value, specifying window size (see below) |
| margin | numeric value, specifying size of window margin (see below) |
| thresh | numeric value, which determines how large links are removed. (see below) |

## Details

generateNeighbours determines, for each bead on the array, which beads are next to it. It assumes that the beads are in a hexagonal lattice.

If the `.locs` file is present and `useLocs = TRUE` then the ordering of the `.locs` file is used to infere the grid layout. This is far quicker than the alternative and is thus recommended, but can only be used on BeadChip platforms. If the data is from a Sentrix Array `useLocs` is automatically set to `FALSE` and the following algorithm is applied instead.

The algorithm used first links each bead to its 6 closest neighbours. It then removes the longest link if its squared length is more than `thresh` multiplied by the squared length of the next longest link. A similar process is applied to the 2nd and 3rd longest links.

Finally, any one way links are removed (i.e. a link between two beads is only preserved if each bead considers the other to be its neighbour).

To ease computation, the algorithm only computes neighbours of beads in a square window of side length `2*(window)` which travels across the array. Beads in a margin around the square, of width `(margin)`, are also considered as possible neighbours.

## Value

A matrix with 6 columns. Each row corresponds to a bead in the passed `beadLevelData-class` and the six entries are the indices of the 6 neighbouring beads. Values of NA indicate that the neighbouring bead appears to be missing, either due to failing Illumina's decoding or being at the edge of the array.

## Author(s)

Jonathan Cairns, Mike Smith

## References

Lynch AG, Smith ML, Dunning MJ, Cairns JM, Barbosa-Morais NL, Tavare S. beadarray, BASH and HULK - tools to increase the value of Illumina BeadArray experiments. In A. Gusnato, K.V. Mardia, & C.J. Fallaize (eds), Statistical Tools for Challenges in Bioinformatics. 2009 pp. 33-37. Leeds, Leeds University Press.

## See Also

`HULK`, `BASH`

## Examples

```
## Not run:
if(require(beadarrayExampleData)){

data(exampleBLData);
neighbours <- generateNeighbours(exampleBLData, array = 1, useLocs = FALSE);
}
```

```
## End(Not run)
```

---

getBeadData *Get raw data from a beadLevelData object*

---

## Description

Retrieves the raw bead data from a beadLevelData object for a given section/array.

## Usage

```
getBeadData(BLData, what="Grn", array=1)
```

## Arguments

| | |
|---|---|
| BLData | BeadLevelList |
| what | character string specifying the values to retrieve (e.g. "ProbeID", "Grn" etc.). |
| array | integer specifying the section/array to use |

## Value

A vector containing the specified bead data for the particular array.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

summary(getBeadData(exampleBLData))

}
```

HULK *HULK - Bead Array Normalization by NEighbourhood Residuals*

## Description

Normalizes an probe intensities by calculating a weighted average residual based on the residuals of the surrounding probes.

## Usage

```
HULK(BLData, array = 1, neighbours = NULL, invasions = 20, useLocs = TRUE, weightName = "wts", transFun =
```

## Arguments

| | |
|---|---|
| BLData | An object of class beadLevelData-class |
| array | integer specifying which section/array to process |
| neighbours | A Neighbours matrix. Optional - if left NULL, it will be computed. |
| invasions | Integer - Number of invasions used when identifying neighbouring beads. |
| useLocs | If information from an associated .locs file is to be used. If available using a .locs file can improve both the speed and accuracy of determining the network of neighbouring beads. |
| weightName | Column name where bead weights are to be taken from. |
| transFun | Transformation function. |
| outlierFun | Name or definition for the function to be used to calculated outliers. |

## Details

HULK is a method of intensity normalization based upon the BASH framework. Firstly For each bead a local neighbourhood of beads is determined, using the same process as the other BASH functions.

For each bead a weighted average residual is calculated. The average residual is calculated as the sum of the residuals for each bead in the neighbourhood, divided by 1 plus the number of invasions it took to reach that bead. This calculation is made by a call to HULKResids.

The average residuals are then subtracted from each bead and a vector of the resulting corrected intensities object is returned. These corrected intensities can be saved in the original beadLevelData object using insertBeadData

## Value

A vector of corrected intensities.

## Author(s)

Mike Smith

## References

Lynch AG, Smith ML, Dunning MJ, Cairns JM, Barbosa-Morais NL, Tavare S. beadarray, BASH and HULK - tools to increase the value of Illumina BeadArray experiments. In A. Gusnato, K.V. Mardia, & C.J. Fallaize (eds), Statistical Tools for Challenges in Bioinformatics. 2009 pp. 33-37. Leeds, Leeds University Press.

## See Also

BASH, insertBeadData, logGreenChannelTransform, squeezedVarOutlierMethod, illuminaOutlierMethod

## Examples

```
## Not run:

    if(require(beadarrayExampleData)){


 data(exampleBLData)
 o <- HULK(exampleBLData, 1)

}


## End(Not run)
```

---

# identifyControlBeads *Classify each bead according to its control status*

---

## Description

Using the control annotation specified for the array, the function will classify each bead as belonging to a control group, or as being a regular probe.

## Usage

```
identifyControlBeads(BLData, array = 1, controlProfile = NULL)
```

## Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| array | the numeric id of the array section |
| controlProfile | an optional control profile data frame |

## Details

The function requires either that a control profile data frame is specified (This associates probe IDs with their control status - see the example), or that the annotation of the `beadLevelData` object be set to an array that the package recognises (see `getAnnotation`). Note that some positive control bead-types may also be functioning 'regular' probes.

## Value

a vector of character strings giving the status of each bead

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

statVec = identifyControlBeads(exampleBLData)

table(statVec)


}
```

---

# illuminaChannel-class *Class "illuminaChannel"*

---

## Description

A class to define how illumina bead-level data are summarized

## Details

From beadarray version 2.0 onwards, users are allowed more flexibility in how to create summarized data from bead-level data. The `illuminaChannel` is a means of allowing this flexibility by defininining how summarization will be performed on each array section in the bead-level data object. The three keys steps applied to each section are; 1) use a transform function to get the quantities to be summarized (one value per bead). The most common use-case would be to extract the Green channel intensities and possibly perform a log2 transformation. 2) remove any outliers from this list of values 3) split the values according to ArrayAddressIDs and apply the definied `exprFun` and `varFun` to the quantities belonging to each ArrayAddress.

### Slots/List Components

Objects of this class contain the following slots

| | |
|---|---|
| transFun: | function to transform the data from each array-section. |
| outlierFun: | A function for identifying outliers from a list of bead intensiites and associated ArrayAddressIDs . |
| exprFun: | A function for producing a single summary of expression level from a vector of bead-type intensities. e.g. mea |
| varFun: | A function for producing a single summary of variability from a vector of bead-type intensities. e.g. sd |
| name: | Character vector that defines a name for the channel |

### Author(s)

Mark Dunning

### See Also

summarize

### Examples

```
greenChannel

redChannel = new("illuminaChannel", redChannelTransform, illuminaOutlierMethod, mean, sd, "R")

logRatio = new("illuminaChannel", logRatioTransform, illuminaOutlierMethod, mean, sd, "M")
```

---

## illuminaOutlierMethod *Identifier outliers on an array section*

---

### Description

Implementation of the illumina method for excluding outliers using a fixed number of MADs (median absolute deviations) cutoff for each bead type

### Usage

```
illuminaOutlierMethod(inten, probeList, wts=1, n = 3)
```

### Arguments

| | |
|---|---|
| inten | a list of intensities |
| probeList | the IDs corresponding to each intensity value |
| wts | Weights associated with beads, indicating those recommended for removal by, for example, BASH |
| n | number of MADs cutoff used |

## Details

This function is called within the summarisation routine of beadarray to exclude outliers from an array-section prior to summary. The intensities are not assumed to be on any particular scale and can result from any user-defined transformation function.

Beads with weight zero do not contribute to the outlier calling.

## Value

the positions in the original vector that were determined to be outliers

## Author(s)

Mark Dunning

## See Also

squeezedVarOutlierMethod

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

oList = illuminaOutlierMethod(logGreenChannelTransform(exampleBLData, 1), getBeadData(exampleBLData, array=1, wh

}
```

---

# imageplot *imageplot for beadLevelData object*

---

## Description

Generates an image plot for data from a beadLevelData object.

## Usage

```
imageplot(BLData, array = 1, transFun = logGreenChannelTransform, squareSize = NULL, useLocs = TRUE, hor
```

## Arguments

| | |
|---|---|
| BLData | beadLevelData |
| array | integer specifying what section to plot |
| transFun | Function that defines how values from the BLData object are to be transformed prior to plotting. |

| | |
|---|---|
| squareSize | Numeric specifying how many pixels in the original image make up each square in the imageplot. If NULL, the function will guess a suitable value from the data. |
| useLocs | If TRUE the function will read the locs file associated with the section in order to include the physical properties of the section in the plot |
| horizontal | If TRUE the image will be plotted so that the longest edge of the section is on the x axis. |
| low | colour to use for lowest intensity |
| high | colour to use for highest intensity |
| ncolors | The number of colour graduations between high and low |
| zlim | numerical vector of length 2 giving the extreme values of 'z' to associate with colours 'low' and 'high'. |
| legend | logical, if TRUE, zlim and range of data is added to plot. |
| ... | other arguments to plot |

## Details

Produces a standard imageplot for the specified section. The default, transformation logGreenChannelTransform, takes the log2 of the green channel. For two channel data, the red channel or log ratio can be plotted by logRedChannelTransform or logRatioTransform functions can be used. The user can also specify their own functions.

The default plotting orientation is such that the longest edge of the section is along the x axis. If horizontal = FALSE, the longest edge will by on the y axis and should match how the corresponding TIFF image from the BeadScan directory is orientated.

If locs = TRUE and locs file were made available to readIllumina, the segments that the section is comprised of will be visible (For expression BeadChips, each section is made of nine physically separate segments). The squareSize parameter will also be set appropriately.

As a result of both having identical function names this function can conflict with the imageplot method in 'limma'. If both packages are loaded, the function from whichever package was loaded last takes precedence. If the 'beadarray' imageplot() function is masking that from 'limma', one can directly call the 'limma' method using the command "limma::imageplot()". Alternatively, one can detach the 'beadarray' package using "detach(package:beadarray)". Similar techniques can be used if 'limma' is masking the 'beadarray' method.

## Value

A ggplot object which is printed to screen by default

## Author(s)

Mike Smith and Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

##By default the first array is plotted, here we plot the 2nd which should give a more interesting example
imageplot(exampleBLData, array=2)

}


## Not run:

ip <- imageplot(exampleBLData, array=2, low="lightgreen", high="darkgreen", horizontal=FALSE)

ggsave(ip, filename="myimageplot.png")

ip2 <- imageplot(exampleBLData, array=2, low="lightgreen", high="darkgreen", horizontal=TRUE)

ggsave(ip2, filename="myimageplot2.png")



## End(Not run)
```

---

# imageProcessing *Image processing functions*

---

## Description

Functions for obtaining bead intensity values from raw tiff images. The three commands with the illumina prefix attempt to emulate the image processing implemented by Illumina. The medianBackground function implements a more robust background calculation recommended by Smith et al.

## Usage

```
illuminaForeground(pixelMatrix, beadCoords)
illuminaBackground(pixelMatrix, beadCoords)
illuminaSharpen(pixelMatrix)

medianBackground(pixelMatrix, beadCoords)
```

## Arguments

| | |
|---|---|
| pixelMatrix | A matrix storing the individual pixel values of an image. Intended to be created by readTIFF, although any matrix can be passed as input. |
| beadCoords | Two column matrix with each row containing a pair of coordinates representing a bead centre. |

## Value

illuminaForeground, illuminaBackground and medianBackground return a vector of intensity values, with one entry for every row in the beadCoords argument. Any pairs of coordinates that fall outside the dimensions of the image return NA.

illuminaSharpen returns a matrix with the same dimensions as the pixelMatrix argument.

## Author(s)

Mike Smith

## References

Smith ML, Dunning MJ, Tavare S, Lynch AG. Identification and correction of previously unreported spatial phenomena using raw Illumina BeadArray data. *BMC Bioinformatics* (2010) 11:208

---

`insertBeadData` *Add, modify or remove data in a beadLevelData object*

---

## Description

Add, modify or remove data in a `beadLevelData` object.

## Usage

```
insertBeadData(BLData, array = 1, what, data)
removeBeadData(BLData, array = 1, what)
```

## Arguments

| | |
|---|---|
| `BLData` | An object of class `beadLevelData-class`. |
| `array` | Positive integer specifying what section should be modified. |
| `what` | Name of the data that is being modified. If 'what' doesn't exist then a new entry is created using the name specified in this argument. |
| `data` | A numeric vector to be stored, the same length as the number of beads in the section specified by the array argument. |

## Details

These functions allow the `beadData` slot of the `beadLevelData-class` object to be modified for a given array.

## Value

Returns an object of class `beadLevelData-class`.

### Author(s)

Mike Smith

### Examples

```
     if(require(beadarrayExampleData)){

data(exampleBLData)
logIntensity <- log2(getBeadData(exampleBLData, what = "Grn", array = 1))

## This will add a new entry called "LogGrn" to BLData
exampleBLData <- insertBeadData(exampleBLData, array = 1, what = "LogGrn", data = logIntensity)
head(exampleBLData[[1]])

## Supplying an existing entry to "what" will overwrite the current data
  exampleBLData <- insertBeadData(exampleBLData, array = 1, what = "Grn", data = logIntensity)
head(exampleBLData[[1]])

   }
```

---

## insertSectionData *Modify the sectionData slot*

---

### Description

A function to modify the sectionData slot of a beadLevelData object. Data can be be added if it is a data frame with a number of rows equal to the number of sections in the beadLevelData object.

### Usage

```
insertSectionData(BLData, what, data)
```

### Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| what | a character string specifiying a name for the new data |
| data | a data frame containing the data we wish to add |

### Details

This function allows users to modifiy the per\-section information that is included in the sectionData slot. Typical usage would be to store quality control data that has been computed.

### Value

a modified beadLevelData object with the new data attached to sectionData

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

qct = makeQCTable(exampleBLData)

exampleBLData = insertSectionData(exampleBLData, what="ProbeQC", data = qct)

exampleBLData@sectionData

}
```

---

# limmaDE *Differential expression using limma*

---

## Description

Function to perform a standard limma analysis using a single command.

## Usage

```
limmaDE(summaryData, SampleGroup, DesignMatrix = NULL, makeWts = TRUE, ...)
```

## Arguments

| | |
|---|---|
| summaryData | ExpressionSetIllumina object |
| SampleGroup | Name of column in phenoData that will be used to construct sample groups for analysis |
| DesignMatrix | Optional design matrix |
| makeWts | if TRUE weights will be calculated for each array |
| ... | Other arguments that lmFit can accept |

## Details

The function automates the steps used in a typical limma analysis. Firstly, the lmFit is used to fit a linear model from the specified column in phenoData. Array weights can be calculated (arrayWeights) and used in the fit. A contrast matrix of all possible contrasts is created (makeContrasts) and fitted (makeContrasts). The empirical Bayes shrinkage of variances is then applied (eBayes). The design matrix, contrast matrix and array weights can all be extracted for the resulting object.

## Value

a limmaResults object

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){
  data(exampleSummaryData)
  rawdata <- channel(exampleSummaryData, "G")
  normdata <- normaliseIllumina(rawdata)
  limmaResults <- limmaDE(normdata, SampleGroup = "SampleFac")
  limmaResults
  DesignMatrix(limmaResults)
}
```

---

# limmaResults-class *Class "limmaResults"*

---

## Description

Container for results from a limma differential expression analysis

## Author(s)

Mark Dunning

---

# makeGEOSubmissionFiles *Create files for a Gene Expression Omnibus submission*

---

## Description

Following the guidelines at http://www.ncbi.nlm.nih.gov/geo/info/geo_illu.html, we provide functionality to create the three types of file required for a GEO submission of Illumina expression data. These are 1) sample metadata 2) Normalised intensities 3) Raw intensities.

## Usage

```
makeGEOSubmissionFiles(normData, rawData, forceDetection = TRUE, basename = "GEO", softTemplate = NULL)
createGEOMeta(normData,basename)
createGEOMatrix(normData,forceDetection=TRUE,basename="GEO",softTemplate=NULL,normalised=T)
```

## Arguments

| | |
|---|---|
| normData | A normalised ExpressionSetIllumina object |
| rawData | A 'raw' non-normalised ExpressionSetIllumina object |
| forceDetection | TRUE forces the calculation of detection scores (if they are not present). |
| basename | A prefix that all the output files will be given |
| softTemplate | A SOFT file downloaded from GEO that we wish to annotate against. e.g. GPL10558.annot for Humanv4 |
| normalised | if TRUE the suffix ProcessedMatrix will be used to write the intensities to. Otherwise the Raw suffix is used. |

## Details

Each file is written as a tab-delimited file in the current working directory. Note that the metadata contains the correct number of columns for the number of samples in the data, but the contents should be entered / curated manually prior to submission.\

Meta file: A template file that can be used to record all the relevant metadata for the experiment, plus the information required by GEO. The SAMPLES section of the file contains the per-sample metadata and has columns generate from the required GEO data an the phenoData stored with the input data\

Processed Matrix - GEO seems to want normalised intensities and detection scores for each probe on each array. Detection scores can be generated if they are not present in the data. The probes that are reported in this file are decided using the annotation slot of the object and querying the appropriate annotation package. Otherwise, a SOFT file downloaded from GEO can be used. \ Raw Matrix - As above, but non-normalised intensities are used

## Author(s)

Mark Dunning

## References

http://www.ncbi.nlm.nih.gov/geo/info/geo_illu.html

## Examples

```
## Not run:
if(require(beadarrayExampleData)){
data(exampleSummaryData)
rawdata <- channel(exampleSummaryData, "G")
normdata <- normaliseIllumina(rawdata)
makeGEOSubmissionFiles(normdata, rawdata)


##To annotate against a SOFT file that can be downloaded from GEO
##e.g. ftp://ftp.ncbi.nlm.nih.gov/geo/platforms/GPL10nnn/GPL10558/annot/GPL10558.annot.gz

makeGEOSubmissionFiles(normdata, rawdata,softTemplate="GPL10558.annot")
}
```

```
## End(Not run)
```

---

`makeQCProfile` *Retrieve control beads*

---

## Description

Function to retrieve the bead IDs for a particular annotation platform

## Usage

```
makeControlProfile(annoName, excludeERCC = TRUE)
```

## Arguments

| | |
|---|---|
| `annoName` | An abberviated name for the annotation packaget. e.g. Humanv3, MouseV2 |
| `excludeERCC` | Ignore any controls with REPORTERGROUPNAME beginning with ERCC. These are a large collection of spike controls on the Humanv4 chip and can make some control tables too big if they are included. |

## Details

The function will use the relevant annotation package (if it is installed) and return all IDs that are present in the REPORTERGROUPNAME mapping. Note that this mapping is only available in versions 1.12.0 and greater.

## Value

A matrix with one row per control bead-type giving the bead-level ID (ArrayAddressID) and control type.

## Author(s)

Mark Dunning

## Examples

```
makeControlProfile("Humanv3")
```

makeQCTable *Tabulate QC scores*

## Description

Function to make a table of quality control scores for every section in a beadLevelData object. Either the annotation of the data needs to be specified, or a control profile data frame that lists ArrayAddress IDs and control types. The supplied summary functions are applied to each control type on each section.

## Usage

```
makeQCTable(BLData, transFun = logGreenChannelTransform, controlProfile = NULL, summaryFns = list(Mean
```

## Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| transFun | a function to be applied to the beadLevelData object prior to tabulation |
| controlProfile | an optional data frame that specifies ID and type of controls to be used to generate the table |
| summaryFns | list of functions to apply to each control type on every section |
| channelSuffix | optional character string to append to the column names of the resulting table |

## Details

For each section in turn, the function groups together IDs of the same control type (e.g. housekeeping), and uses an lapply with the specified summary functions. A transformation function is applied to BLData prior to the summary, with the default being to take the log2 of the green channel.

If the annotation of the beadLevelData has been set by readIllumina or setAnnotation then the controlProfile data frame is calculated automatically and the controlProfile argument may be omitted.

## Value

A matrix with one row per section and one column for each combination of control type and summary function.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)


qct = makeQCTable(exampleBLData)

qct

}
```

---

`medianNormalise` *Median normalise data in a matrix*

---

## Description

Normalises expression intensities so that the intensities or log-ratios have equal median values across a series of arrays (columns).

## Usage

```
medianNormalise(exprs, log=TRUE)
```

## Arguments

| | |
|---|---|
| `exprs` | a matrix of expression values |
| `log` | if TRUE then do a log2 transformation prior to normalising |

## Details

Normalisation is intended to remove from the expression measures any systematic trends which arise from the microarray technology rather than from differences between the probes or between the target RNA samples hybridized to the arrays.

For median normalisation, the intensity for each gene is adjusted by subtracting the median of all genes on the array and then adding the median across all arrays. The effect is that each array then has the same median value.

## Value

Produces a matrix of normalised intensity values (on the log2 scale by default) with the same dimensions as `exprs`.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleSummaryData)

exampleSummaryData.log2 <- channel(exampleSummaryData,"G")

exampleSummaryData.med = assayDataElementReplace(exampleSummaryData.log2, "exprs", medianNormalise(exprs(example

}
```

---

`metaTemplate` *GEO required fields*

---

## Description

Template containing latest required fields for GEO submission

## Usage

```
data(metaTemplate)
```

## Examples

```
data(metaTemplate)
```

---

`metrics-methods` *Accessing metrics information in bead-level objects*

---

## Description

The `metrics`, `p95` and `snr` methods conveniently access metrics information that is stored in a beadLevelData object. These data are generated by the Illumina scanning software and can be an early indicator of array quality. These include the 95th (P95) and 5th (P05) quantiles of all pixel intensities on the image. A signal-to-noise ratio (SNR) can be calculated as the ratio of these two quantities. These metrics can be viewed in real-time as the arrays themselves are being scanned. By tracking these metrics over time, one can potentially halt problematic experiments before they even reach the analysis stage. Illumina recommend that the SNR ratio should be above 10, so these arrays are acceptable. However, the P95 and P05 values will fluctuate over time and are dependant upon the scanner setup. Including SNR values for arrays other than those currently being analysed will give a better indication of whether any outlier arrays exist.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

    data(exampleBLData)

    metrics(exampleBLData)

    p95(exampleBLData, "Grn")

    snr(exampleBLData, "Grn")

}
```

---

`noOutlierMethod` *returns no outliers on an array section*

---

## Description

The `summarize` function demands that an outlier function is called. This function allows one to satisfy this requirement and still not remove any outliers.

## Usage

```
noOutlierMethod(inten, probeList, wts=1,n=3)
```

## Arguments

| | |
|---|---|
| `inten` | a list of intensities but the intensities are not in fact used |
| `probeList` | the IDs corresponding to each intensity value (not used) |
| `wts` | Weights associated with beads. Again, these are not actually used. |
| `n` | another parameter that is not, in fact, used. |

## Details

This function returns integer(0).

## Value

integer(0)

## Author(s)

Andy Lynch

## See Also

`squeezedVarOutlierMethod`

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

oList = noOutlierMethod(logGreenChannelTransform(exampleBLData, 1), getBeadData(exampleBLData, array=1, what="Pr

}
```

---

normaliseIllumina *Normalise Illumina expression data*

---

## Description

Normalises expression intensities from an ExpressionSetIllumina object so that the intensities are comparable between arrays.

## Usage

```
normaliseIllumina(BSData, method="quantile", transform="none", T=NULL, status=fData(BSData)$Status,ne
```

## Arguments

| | |
|---|---|
| BSData | an ExpressionSetIllumina object |
| method | character string specifying normalisation method (options are "quantile", "qspline", "vsn", "rankInvariant", "median" and "none". |
| transform | character string specifying transformation to apply to the data prior to normalisation (options are "none", "log2", neqc, rsn and "vst" |
| T | A target distribution vector used when method="rankInvariant" normalisation. If NULL, the mean is used. |
| status | character vector giving probe types (used in neqc normalisation only) |
| negctrl | character vector giving negative control probes (used in neqc normalisation only) |
| regular | character vector giving regular probes (used in neqc normalisation only) |
| ... | further arguments to be passed to lumiT or neqc |

## Details

Normalisation is intended to remove from the expression measures any systematic trends which arise from the microarray technology rather than from differences between the probes or between the target RNA samples hybridized to the arrays.

In this function, the transform specified by the user is applied prior to the chosen normalisation procedure.

When transform="vst" the variance-stabilising transformation from the 'lumi' package is applied to the data. Refer to the lumiT documentation for further particulars. Note that the Detection P

values are only passed on when they are available (i.e. not NA). The rsn option calls code directly from lumi.

For further particulars on the different normalisation methods options refer to the individual help pages (?normalize.quantiles for "quantile", ?normalize.qspline for "qspline", ?rankInvariantNormalise for "rankInvariant", ?medianNormalise for "median" and ?vsn2 for "vsn".

For median normalisation, the intensity for each gene is adjusted by subtracting the median of all genes on the array and then adding the median across all arrays. The effect is that each array then has the same median value.

Note: If your BSData object contains data already on the log-scale, be careful that you choose an appropriate transform to avoid transforming it twice. The same applies for the "vst" transformation and "vsn" normalisation methods which require the expression data stored in BSData to be on the original (un-logged) scale. When method="vsn", transform must be set to "none", since this method transforms and normalises the data as part of the model.

The neqc normalisation is described in Shi et al (2010) and documented in the limma package. Note that the output from this method has control probes removed.

## Value

An 'ExpressionSetIllumina' object which conatains the transformed and normalised expression values for each array.

## Author(s)

Matt Ritchie and Mark Dunning

## References

Shi. W, Oshlack. A, Smyth, GK, (2010) Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips. Nucleic Acids Research

Lin, S.M., Du, P., Kibbe, W.A., (2008) 'Model-based Variance-stabilizing Transformation for Illumina Microarray Data', Nucleic Acids Res. 36, e11

## Examples

```
if(require(beadarrayExampleData)){

data(exampleSummaryData)
exampleSummaryData.norm = normaliseIllumina(channel(exampleSummaryData, "G"), method="quantile", transform="none

exampleSummaryData.rsn = normaliseIllumina(channel(exampleSummaryData, "G.ul"),method="rsn", transform="none")

}
```

numBeads *Gets the number of beads from a beadLevelData object*

## Description

Retrieves the number of beads on selected sections from a beadLevelData object.

## Usage

```
numBeads(object, arrays=NULL)
```

## Arguments

| | |
|---|---|
| object | beadLevelData |
| arrays | either NULL to return the bead numbers for all arrays, or a scalar or vector of integers specifying a subset of strips/arrays |

## Details

numBeads retrieves the number of beads on arrays from the arrayInfo slot.

## Value

A vector containing the number of beads on individual array sections.

## Author(s)

Matt Ritchie

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)
numBeads(exampleBLData)
numBeads(exampleBLData, arrays=2)

}
```

`outlierplot` *Plot outlier locations*

## Description

Function to plot where the outliers are located on a given array

## Usage

```
outlierplot(BLData, array = 1, transFun = logGreenChannelTransform, outlierFun = illuminaOutlierMethod
```

## Arguments

| | |
|---|---|
| `BLData` | a `beadLevelData` object |
| `array` | the number of the array to plot |
| `transFun` | a function defining how to transform the data prior to calculating outliers |
| `outlierFun` | function that will identify outliers |
| `n` | an indicator of how extreme an observation must be (e.g. how many MADs from the median), to be passed to the function that will identify outliers |
| `wtsname` | column name of BLData object containing weights to feed to the outlier function |
| `horizontal` | if TRUE the longest edge of the array section will be on the x axis |
| `nSegments` | How many segments the section is divided into. If this argument is left as the default value (NULL) the code will attempt to extract this information from the relevant .sdf file. If it can't be found then the segments will not be indicated on the final plot. |
| `lowOutlierCol` | what colour to plot outliers below the median |
| `highOutlierCol` | what colour to plot outliers above the median |
| `outlierPch` | plotting character for the outliers |
| `main` | an optional title for the plot |
| `...` | additional arguments |

## Details

The function calls the specified outlier function to determine the outliers on the array and then plots their location. Points are coloured according the intensity of the bead is above or below the median for that bead-type.

## Value

plot produced on current graphical device

## Author(s)

Mark Dunning and Mike Smith

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)
outlierplot(exampleBLData, array=1, horizontal = FALSE)


}
```

---

`platformSigs` *Annotation definitions*

---

## Description

A list of bead-level IDs for each Illumina expression array platform, as obtained by the lumi mapping packages. These are used to suggest the annotation of a possibly unknown bead-level dataset.

## Usage

```
data(platformSigs)
```

---

`plotBeadLocations` *Plot bead locations*

---

## Description

Can plot where specified beads, or bead types were located on the array surface

## Usage

```
plotBeadLocations(BLData, ProbeIDs = NULL, BeadIDs = NULL, array = 1, SAM = FALSE, xCol = "GrnX", yCol =
```

## Arguments

| | |
|---|---|
| `BLData` | a `beadLevelData` object |
| `ProbeIDs` | a list of ArrayAddress IDs to plot |
| `BeadIDs` | a list of beads (rows in the beadLevelData object) to plot |
| `array` | the number of the section to plot |
| `SAM` | if TRUE the array is treated as a Sentrix Array Matrix (hexagonal) |
| `xCol` | column name for the x coordinates |
| `yCol` | column name for the x coordinates |
| `xlab` | optional label for the x axis |
| `ylab` | optional label for the y axis |
| `horizontal` | if TRUE the longest edge of the array surface will be plotted on the x axis |
| `main` | an optional title for the plot |
| `...` | any arguments to be passed to plots |

## Value

plot to current graphical device

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

##Plot location of first 100 beads as they are listed in beadLevelData object

plotBeadLocations(exampleBLData, BeadIDs = 1:100, array=1, horizontal = FALSE)



}
```

---

# plotChipLayout *Function to Plot the Layout of an Illumina BeadChip*

---

## Description

Using the values obtained from a sentrix descriptor object generated by simpleXMLparse, a plot is generated showing samples and sections.

## Usage

```
plotChipLayout(SD, subsC = NULL, sampC = NULL, sectC = NULL, desectC = "red", markC = "white", main = NUL
```

## Arguments

| | |
|---|---|
| SD | A sentrix descriptor object such as that generated by simpleXMLparse from an Illumina sdf file |
| subsC | The colour for the array substrate. Will be extracted from the sentrix descriptor object if null. |
| sampC | The colour for the samples. Will be extracted from the sentrix descriptor object if null. |
| sectC | The colour for the sections. Will be extracted from the sentrix descriptor object if null. |
| desectC | The colour with which to outline the decode sections |
| markC | The colour for plotting decoding and analytical markers |

| | |
|---|---|
| main | A title to give the figure (e.g. chip name) |
| samplab | labels for the samples. Will be extracted from the sentrix descriptor object if null. |
| sectlab | labels for the sections (if appropriate). Will be extracted from the sentrix descriptor object if null. |

## Value

returns a plot to the current device (recommend this is tall and thin)

## Author(s)

Andy Lynch

## Examples

```
## SD<-simpleXMLparse(mysdf)
## plotChipLayout(SD)
```

---

# plotMA-methods *Function to construct the classic MA plots from a dataset*

---

## Description

Note that a ggplot2 object is returned, which can be modified programatically.

The default operation is to construct a reference array be averaging all probes across the whole datasets, and then making an MA plot by comparing each array to this reference. A log2 transformation is performed, but this can be turned-off by setting `do.log=FALSE`.

Alternatively, a SampleGroup may be specified. This is a character string that should match a column in the phenoData for the object. Then, the resulting MA plot will contain all pairwise comparisons between samples belonging to the same group. A list of ggplot objects is returned, each item in the list being a different group.

## Details

Densities are computed using the 'hexbin' package

## Value

A list of ggplot2 objects is returned.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){
library(ggplot2)
data(exampleSummaryData)
rawdata <- channel(exampleSummaryData, "G")
 mas <- plotMA(rawdata,do.log=FALSE)
mas
##Smoothed lines can be added
mas + geom_smooth(col="red")
##Added lines on the y axis
mas + geom_hline(yintercept=c(-1.5,1.5),col="red")
##Changing the color scale
mas + scale_fill_gradient2(low="yellow",mid="orange",high="red")

mas <- plotMA(rawdata,do.log=FALSE,SampleGroup="SampleFac")

## Not run:
mas[[1]]
mas[[2]]

## End(Not run)

}
```

---

`plotMAXY` *Scatter plots and MA-plots for all specified arrays*

---

## Description

Produces smoothed scatter plots of M versus A and X versus Y for all pairwise comparisons from a set of arrays.

## Usage

```
plotMAXY(exprs, arrays, log = TRUE, genesToLabel=NULL,
         labels=colnames(exprs)[arrays],labelCol="red",
         labelpch=16,foldLine=2,sampleSize=NULL,...)
```

## Arguments

| | |
|---|---|
| `exprs` | a matrix of expression values |
| `arrays` | integer vector giving the indices of the arrays (columns of `exprs`) to plot |
| `log` | if TRUE then all values will be log2-transformed before plotting |
| `genesToLabel` | vector of genes to highlight on the plot. These must match the rownames of `exprs`. |
| `labels` | vector of array names to display on the plot |

| | |
|---|---|
| labelCol | plotting colours for highlighted genes |
| labelpch | plotting characters for highlighted genes |
| foldLine | a numeric value defining where to draw horizontal fold change lines on the plot |
| sampleSize | The number of genes to plot. Default is NULL, which plots every gene |
| ... | other graphical parameters to be passed |

## Details

This graphical tool shows differences that exist between two arrays and can be used to highlight biases between arrays as well as highlighting genes which are differentially expressed. For each bead type, we calculate the average (log2) intensity and difference in intensity (log2-ratio) for each pair of arrays.

In the lower-left section of the plot we see XY plots of the intensities for all pairwise comparisons between the arrays and in the upper right we have pairwise MA plots. Going down the first column we observe XY plots of array 1 against array 2 and array 1 against array 3 etc. Similarly, in the upper-right corner we can observe pairwise MA plots.

## Author(s)

Mark Dunning

## Examples

```
##This function is to be replaced. Please see plotMA
```

---

# plotTIFF *Produce plots of the Illumina tiff images*

---

## Description

Produces a plot of an Illumina tiff image, which can be useful for observing spatial artifacts on an array and checking the alignment of spot centres features in the image.

## Usage

```
plotTIFF(tiff, xrange = c(0, ncol(tiff)-1), yrange = c(0, nrow(tiff)-1),
high = "cyan", low = "black", mid = NULL, ncolours = 100,
log = TRUE, values = FALSE, textCol = "black", ...)
```

## Arguments

| | |
|---|---|
| tiff | Intended to the the result of readTIFF, but in reality can be any matrix |
| xrange | Range of X coordinates to plot. |
| yrange | Range of Y coordinates to plot. |
| high | Colour to plot the brightest pixels in the image. |
| low | Colour to plot the dimmest pixels. |
| mid | If specified the colour gradient will go from low to mid to high. If not specified then the gradient simply goes from low to high. |
| ncolours | Specify how many steps there should be in the gradient between the high and low colours |
| log | If TRUE the pixel values are logged before the colour gradient is created. |
| values | When set to TRUE each pixel in the image has it's value displayed over it. This should only be used when displaying a very small number of pixels as the text very quickly covers the entire image. |
| textCol | If values is TRUE this argument specifies the colour of the text. |
| ... | Other graphical parameters specified in par |

## Details

This can be very slow, especially when the Cairo graphics library is being used. When using the Cairo library, if one is plotting a large tiff with 10s of millions of pixels, the plotting time increases from around 20 seconds to 5 minutes on an Intel Xeon E5420.

If running on a Linux system it is recommended to use:

```
x11(type = "Xlib")
```

before running plotTIFF(), on order to force the quicker plotting mechanism.

Of course it is debatable whether it is useful to plot all of those pixels, given that there are far more than can be displayed on a normal screen, and future revisions of the code may address this.

## Value

A plot is produced on the current graphical device.

## Author(s)

Mike Smith

poscontPlot *Plot the positive controls*

## Description

Function for retrieving and plotting the biotin and housekeeping controls for an expression array. We know these controls should show high signal and are therefore useful for QA purposes. The housekeeping control targets a bead-type believed to be universally expressed whereas the biotin control targets the biotin used for staining.

## Usage

```
poscontPlot(BLData, array = 1, transFun = logGreenChannelTransform, positiveControlTags = c("housekeep
```

## Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| array | The section to be plotted |
| transFun | What transformation function to be applied prior to plotting |
| positiveControlTags | What identifiers to be used as positive controls |
| colList | vector of colours to be used to each positive control |
| controlProfile | an optional data frame with columns defining the ArrayAddress IDs and control\-type for all controls on the platform. |
| ... | other arguments to plot |

## Details

Function for plotting the observed intensites for all replicates of the specified control probes on a given array\-section. The identity of the control probes can be specified by passing a ControlProfile data frame, with the first column being a vector of ArrayAddress IDs and the second column being a corresponding set of characters tags. The beads to be plotted are found by matching the positiveControlTags argument to these character tags. Users with expression data can have the ControlProfile data frame defining automatically within the function, provided the annotation of the beadLevelData object has been defined by readIllumina or setAnnotation.

## Value

Plot to current graphical device

## Author(s)

Mark Dunning

## References

www.illumina.com/downloads/GX_QualityControl_TechNote.pdf

## Examples

```
###Load the example beadLevelData and associated controlProfile

if(require(beadarrayExampleData)){


data(exampleBLData)

poscontPlot(exampleBLData, array=1)

poscontPlot(exampleBLData, array=2)

}
```

---

processSwathData *Prepare iScan data for use with beadarray*

---

## Description

Data from Illumina's newer iScan system come in a different format to the previous BeadScan data. This function is intended to transform the data into a format compatible with beadarray.

## Usage

```
processSwathData(inputDir = NULL, outputDir = NULL, twoColour=NULL, textstring="_perBeadFile.txt", seg
```

## Arguments

| | |
|---|---|
| inputDir | Character string specifying the directory containing the data to be processed. If left NULL this defaults to the current working directory. |
| outputDir | Similar to the above, specifying the directory where the output should be written. If left NULL this defaults to the current working directory. |
| twoColour | Boolean value specifying whether the data is one or two channel. If left NULL the function will attempt to determing the number of channels be examing the files present in the directory. |
| textstring | String specifying the suffix to identify the text file containing original bead-level data. |
| segmentHeight | Each array section is made up of several segments of beads, arranged in an hexagonal pattern. This value specifies the number of rows in a segment, and can be found in the accompanying .sdf file (<SizeGridX>). In the future we will attempt to automate this. |
| segmentWidth | Similar to the above argument, this specifies the number of columns in an array segment (<SizeGridY>). |
| fullOutput | Boolean value specifying the type of output. More details are given below. |
| newTextString | Suffix for the two new "per swath" bead-level text file. |
| verbose | Boolean value that, if TRUE, directs the function to print progress to the screen. |

## Details

Data from the iScan system comes with two images of each array section (along with two .locs files), which are labelled Swath1 and Swath2. These two images are of the two halves of the array section, with an overlapping region in the middle. However, there is only one bead-level text file, with no indication as to which of the two images each entry comes from. Given this, simply reading the bead-level text file will result in any function that uses bead locations performing undesirably.

This function works to try and deconvolute the bead-level data and create two files, one per swath, which can then be read independently into beadarray.

The exact content of the output files depends upon the `fullOutput` argument. If the default value of `FALSE` is selected the function compares the coordinates in the "perBeadFile.txt" file with those found in the two .locs files, in order to determine which swath each bead is from. From this two new bead-level text files (with names containing "Swath1" and "Swath2" are created, containing all the beads from the orginal file. If the .locs files are not present this process will fail and the assigning of beads to swaths cannot be performed.

If the `fullOutput` argument is set to `TRUE` both text files contain all the beads that can be identified in their respective images. Assuming that both the .locs and .tif files are present, we can use the bead-centre coordinates stored in the .locs files to calculate intensity values for beads in both images, even if no intensity is recorded Illumina's text file. Any bead that is found in the overlapping region, and thus appearing in both images, will have two intensities calculated. The files that are created contain the same data as regular bead-level text files, but with an additional column entitled "Weights". Beads that appear in both images are assigned a weight of 0.5 (since the are two intensity values for them), whilst all other beads are given a weigth of 1. If the TIFF images aren't available the intensities cannot be calculate, so the output will default to the same as if `fullOutput = FALSE`.

## Value

This function is called for its side effects, which is to produce two text files containing the beads match to the approriate swath. No value it returned by the function.

## Author(s)

Mike Smith and Andy Lynch

---

`quickSummary` *Create summary values for specified IDs*

---

## Description

A utility function for quickly creating summary values for particular IDs (e.g. control IDs) on a given section.

## Usage

```
quickSummary(BLData, array = 1, transFun = logGreenChannelTransform, reporterIDs = NULL, reporterTags =
```

### Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| array | Which section to summarize |
| transFun | a transformation to be applied prior to summarization |
| reporterIDs | vector specifying the set of IDs to be summarized |
| reporterTags | a vector that divides the supplied IDs into categories |
| reporterFun | a function used to summarize each category |

### Details

The function can be used to calculate summarized values for particular control types on a section. The IDs for all controls are supplied in the reporterIDs argument along with which control type they belong to in the reporterTags argument. A summarized value for each control type is then calculated with the specified function (default is mean).

### Author(s)

Mark Dunning

### Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

quickSummary(exampleBLData, array=1)
quickSummary(exampleBLData, array=2)

}
```

---

## readBeadSummaryData *Read BeadStudio gene expression output*

---

### Description

Function to read the output of Illumina's BeadStudio software into beadarray

### Usage

```
readBeadSummaryData(dataFile, qcFile=NULL, sampleSheet=NULL,
                    sep="\t", skip=8, ProbeID="ProbeID",
                   columns = list(exprs = "AVG_Signal", se.exprs="BEAD_STDERR",
                     nObservations = "Avg_NBEADS", Detection="Detection Pval"),
                    qc.sep="\t", qc.skip=8, controlID="ProbeID",
                  qc.columns = list(exprs="AVG_Signal", se.exprs="BEAD_STDERR",
```

```
nObservations="Avg_NBEADS", Detection="Detection Pval"),
 illuminaAnnotation=NULL, dec=".", quote="", annoCols = c("TargetID", "PROBE_ID","SYMBOL"))
```

## Arguments

| | |
|---|---|
| dataFile | character string specifying the name of the file containing the BeadStudio output for each probe on each array in an experiment (required). Ideally this should be the 'SampleProbeProfile' from BeadStudio. |
| qcFile | character string giving the name of the file containing the control probe intensities (optional). This file should be either the 'ControlProbeProfile' or 'ControlGeneProfile' from BeadStudio. |
| sampleSheet | character string used to specify the file containing sample infomation (optional) |
| sep | field separator character for the dataFile ("\t" for tab delimited or "," for comma separated) |
| skip | number of header lines to skip at the top of dataFile. Default value is 8. |
| ProbeID | character string of the column in dataFile that contains identifiers that can be used to uniquely identify each probe |
| columns | list defining the column headings in dataFile which correspond to the matrices stored in the assayData slot of the final ExpressionSetIllumina object |
| qc.sep | field separator character for qcFile |
| qc.skip | number of header lines to skip at the top of qcFile |
| controlID | character string specifying the column in qcFile that contains the identifiers that uniquely identify each control probe |
| qc.columns | list defining the column headings in qcFile which correspond to the matrices stored in the QCInfo slot of the final ExpressionSetIllumina object |
| illuminaAnnotation | character string specifying the name of the annotation package (only available for certain expression arrays at present) |
| dec | the character used in the dataFile and qcFile for decimal points |
| quote | the set of quoting characters (disabled by default) |
| annoCols | additional columns containing annotation to be read from the file |

## Details

This function can be used to read gene expression data exported from versions 1,2 and 3 of the Illumina BeadStudio application. The format of the BeadStudio output will depend on the version number. For example, the file may be comma or tab separated of have header information at the top of the file. The parameters sep and skip can be used to adapt the function as required (i.e. skip=7 is appropriate for data from earlier version of BeadStudio, and skip=0 is required if header information hasn't been exported.

The format of the BeadStudio file is assumed to have one row for each probe sequence in the experiment and a set number of columns for each array. The columns which are exported for each array are chosen by the user when running BeadStudio. At a minimum, columns for average

intensity standard error, the number of beads and detection scores should be exported, along with a column which contains a unique identifier for each bead type (usually named "ProbeID").

It is assumed that the average bead intensities for each array appear in columns with headings of the form 'AVG_Signal-ARRAY1', 'AVG_Signal-ARRAY2',...,'AVG_Signal-ARRAYN' for the N arrays found in the file. All other column headings are matched in the same way using the character strings specified in the `columns` argument.

NOTE: With version 2 of BeadStudio it is possible to export annotation and sequence information along with the intensities. We _don't_ recommend exporting this information, as special characters found in the annotation columns can cause problems when reading in the data. This annotation information can be retrieved later on from other Bioconductor packages.

The default object created by readBeadSummaryData is an `ExpressionSetIllumina` object.

If the control intensities have been exported from BeadStudio ('ControlProbeProfile') this may be read into beadarray as well. The `qc.skip`, `qc.sep` and `qc.columns` parameters can be used to adjust for the contents of the file. If the 'ControlGeneProfile' is exported, you will need to set `controlID="TargetID"`.

Sample sheet information can also be used. This is a file format used by Illumina to specify which sample has been hybridised to each array in the experiment.

Note that if the probe identifiers are non-unique, the duplicated rows are removed. This may occur if the 'SampleGeneProfile' is exported from BeadStudio and/or `ProbeID="TargetID"` is specified (the "ProbeID" column has a unique identifier in the 'SampleProbeProfile', whereas the "TargetID" may not, as multiple beads can target the same transcript).

## Value

An `ExpressionSetIllumina` object.

## Author(s)

Mark Dunning and Mike Smith

## Examples

```
##Read the example data from
##http://www.switchtoi.com/datasets/asuragenmadqc/AsuragenMAQC_BeadStudioOutput.zip
##To follow this example, download the zip file


## Not run:
dataFile = "AsuragenMAQC-probe-raw.txt"

qcFile = "AsuragenMAQC-controls.txt"

BSData = readBeadSummaryData(dataFile=dataFile, qcFile=qcFile, controlID="ProbeID",skip=0,qc.skip=0, qc.columns=


## End(Not run)
```

readIdatFiles *Read BeadScan gene expression output*

## Description

Function to read IDAT files into beadarray.

## Usage

```
readIdatFiles(idatFiles = NULL)
```

## Arguments

| | |
|---|---|
| idatFiles | A vector of file paths to the idat files that are to be read. |

## Details

This function allows IDAT files, produced during the BeadArray scanning, to be read directly into beadarray. This removes the requirement to use Illumina's GenomeStudio software to convert such files into an ASCII format before they can be processed.

The expection is that IDAT files from expression BeadArrays will be read using this function. However, this is not currently checked for explicitly and it is possible that files from other platforms may be read successfully.

If files from a two-colour array are to be read, they should all be listed in the idatFiles argument. The function will determine two-colour data is present and act accordingly. If unequal numbers of green and red files are supplied, or the names of the array sections do not match, the function will fail.

IDAT files often contain probes that are used as internal controls and are not annotated by Illumina. Such probes are not included in the output of this function.

## Value

An ExpressionSetIllumina object.

## Author(s)

Mike Smith

`readIllumina` *Read bead-level Illumina data*

## Description

Reads bead-level ouput by Illumina's BeadScan software.

## Usage

```
readIllumina(dir = ".", useImages = FALSE, illuminaAnnotation = NULL, sectionNames = NULL, metricsFile
```

## Arguments

| | |
|---|---|
| `dir` | Directory from which to read the data, the default being the current working directory. If this is set to NULL, a sample sheet can be used (see below) |
| `useImages` | Boolean value specifying if bead intensities should be read directly from the source text files or calculated using the bead centre coordinates and image files. More details on the intensity calculation used are given below. |
| `illuminaAnnotation` | Character string specifiying the Illumina platform on which the data were generated. This is optional and will help to automate some analyses on expression data. Currently the choices for this argument are Humanv4, Humanv3, Humanv2, Humanv1, Mousev2, Mousev1, Mousev1p1, Ratv1. |
| `sectionNames` | Optional vector of character strings corresponding to section names to be read in. Typically these are the 10 digit numeric ID of Illumina chip followed by an underscore and capital letter |
| `metricsFile` | Optional name of a metrics file to be read in from the directory. Defaults to Metrics.txt. See details below for more information. |
| `forceIScan` | Only necessary for data from iScan machines. When TRUE, this argument forces beadarray to read data from files with the "perBeadFile.txt" extension, rather than seeking a file per swath. Use this if you are receiving the error "iScan data detected. This must first be processed with the function processSwathData()", but don't have access to .locs and .tif files for the array. |
| `dec` | Decimal point character. This should be a character string containing just one single-byte character. |
| `sampleSheet` | A comma-separated file that can be used to define the sections that make up the experiment to be analysed. See readSampleSheet for the specifications of this file |
| `...` | Other arguments to pass when reading bead-level text files. |

## Details

The bead-level data can be generated by any Illumina assay (expression, genotyping, methylation) and via BeadChips or Sentrix Array Matrix. However, some operations within the package are optimised for expression data. For optimal performance, BeadScan needs to be modified to output coordinates for each bead and to include outliers. See http://www.compbio.group.cam.ac.uk/Resources/illumina/index.html for details.

If present, the function will automatically read the following files from the directory

.txt Text file that lists the ID, coordinates and intensity for every decoded bead on an array section. The intensities have been subjected to a local background correction. If `useImages = FALSE` these intensities will be used as a starting point for analysis

.sdf Illumina\'s Sample Description File for the entire chip or SAM. This is used within beadarray to determine the physical properties of a section.

.locs Locations of all beads on the array (i.e. including all those that could not be decoded)

Metrics.txt Illumina\'s metrics that are produced at the time of scanning. Identification of the Metrics file is defined by the `metricsFile` argument. Internally, this argument is passed to grep. The default will thus find other items such as "Metrics.txt2" or "OtherMetrics.txt". To avoid these being read you can use a more specific regex such as "^Metrics.txt$".

Separate functionality exists to read and manipulate TIFF images that may be found in the same directory. See `readTIFF`.

If `useImage` is set to `TRUE`, intensities are extracted from the TIFF images using the bead-centre coordinates provided in the text file. Foreground values are calculated from a sharpened version of the image as described in Kuhn et al. Background values are calculated as the median of the 5 lowest pixels in a 17x17 pixel square around the bead-centre, detailed in Smith et al.

## Value

Returns an object of class `beadLevelData-class`

## Author(s)

Mike Smith, Mark Dunning, Andy Lynch

## References

Kuhn K, Baker SC, Chudin E, Lieu MH, Oeser S, Bennett H, Rigault P, Barker D, McDaniel TK, Chee MS. A novel, high-performance random array platform for quantitative gene expression profiling. Genome Research. 2004;14(11):2347-2356

Smith ML, Dunning MJ, Tavare S, Lynch AG. Identification and correction of previously unreported spatial phenomena using raw Illumina BeadArray data. BMC Bioinformatics. 2010;11:208

---

# readLocsFile *Read ".locs" file.*

---

## Description

Reads the binary Illumina bead location files and returns a matrix of the coordinate pairs for every bead on the array.

## Usage

```
readLocsFile(fileName)
```

### Arguments

| | |
|---|---|
| fileName | A string containing the name of the ".locs" file to be read. |

### Details

The locs file contains bead centre locations for every bead on the array, unlike the bead level text files, with contain just the beads that were decoded. Reading these can be useful if one wants to verify that the image registration was successful, or is interested in the locations of the undecoded beads.

The locs file itself is in a binary format, with each of the bead locations stored as a pair of doubles. The first 2 bytes contain header information, with the 3rd byte containing the number of probes on the array. The location information begins with the 4th byte.

### Value

Returns a two column matrix of bead coordinates, one row per bead.

### Author(s)

Mike Smith

---

## readSampleSheet *Read a Sample sheet for a BeadArray experiment*

---

### Description

Read a comma-separated sample sheet into beadarray, which can then be used to annotate the data that beadarray reads in and add relevant metadata to the analysis.

### Usage

```
readSampleSheet(sheet = "sampleSheet.csv")
```

### Arguments

| | |
|---|---|
| sheet | A file name for the sample sheet |

### Details

The sample sheet should describe all sections are expected to be part of the experiment, along with any relevant metadata. We assume the GenomeStudio sample sheet specification, with [Data] and [Header] sections. The Data section is where the sample metadata are defined. One row is required for each section, and the columns Sentrix_ID, Sentrix_Position, Sample_Name, Sample_Group must be present. The Sentrix_ID and Sentrix_Position columns give the name of the chip (usually a 10 digit number) and slot (a letter A to L) where a given sample was hybridised to. The Sample_Group and Sample_Name columns can take any valid R name. If there are muliple images per section, for example for Mouse WG-6 data, which have bead-level data suffixed by A_1, A_2, B_1, B_2 etc, only one entry is required in the sample sheet for sections A and B. beadarray will duplicate the metadata as neccessary.

## Value

a list comprised of a tabular sample table and any header information that is present.

## Author(s)

Mark Dunning

---

`readTIFF` *Read the Illumina tiff images*

---

## Description

Reads Illumina tiff images and produces a matrix of pixel values.

## Usage

```
readTIFF(fileName, path = NULL, verbose = FALSE, xlim = NULL, ylim = NULL)
```

## Arguments

| | |
|---|---|
| `fileName` | String specifying the name of the tiff image to be read. |
| `path` | String specifying the path to the desired image. The default value of NULL means the current working directory will be used. |
| `verbose` | If TRUE then details from the header of the tiff are printed as it is read. These include things like the byte order, the number of pixels in the image, the number of tags in the header etc. Defaults to FALSE as this is generally not of interest. |
| `xlim` | Used to specify a subsection of the image to read in. Takes a two entry vector e.g c(m,n) specifying that only pixels with x-coordinates between m and n (inclusive) should be read in. |
| `ylim` | Same a xlim, but for the y-cordinates. |

## Details

This function has been specifically written to read the grayscale tiff images which are produced by the Illumina scanners. It is not generic enough to read all tiff files, although this functionality may be added in the future.

Given that the raw images can be quite large, functionality has also been included to read tiffs that have been compressed as either .bz2 or .gz files. Identification is performed based on the file extension and it is assumed that each tif is compressed individually. Support for zip files may be added in the future.

## Value

Returns a matrix with the same dimensions as the pixels in the tiff file to be read in.

## Author(s)

Mike Smith

`sectionNames` *Gets the section names from a beadLevelData Object*

## Description

Retrieves the section names from a `beadLevelData` object.

## Usage

```
sectionNames(object, arrays=NULL)
```

## Arguments

| | |
|---|---|
| `object` | Object of class `beadLevelData` |
| `arrays` | integer (scalar or vector) specifying the sections/arrays to retrieve the names of. When `NULL` the names of all sections/arrays are returned. |

## Details

`sectionNames` retrieves the name of the sections from the sectionInfo slot.

## Value

A character vector containing the names of the individual sections.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)
sectionNames(exampleBLData)


}
```

## setWeights *Set weights from BASH*

### Description

Function for committing the weights calculated by BASH into the beadLevelData object

### Usage

```
setWeights(BLData, wts, array, combine = FALSE, wtName = "wts")
```

### Arguments

| | |
|---|---|
| BLData | a beadLevelData object |
| wts | the wts component of the BASH output |
| array | a vector of arrays that we want to set the weights for |
| combine | if TRUE combine the weights with existing weights (if they exist) |
| wtName | name of column to assign weights to |

### Value

Modified beadLevelData object

### Author(s)

Mark Dunning

## show-method *Display object summary*

### Description

Prints a summary of an objects contents.

### Usage

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

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

### Arguments

| | |
|---|---|
| object | An object of class beadLevelData or ExpressionSetIllumina |

### Details

show is commonly invoked by simply entering the name of an object. Calling it on the classes defined in beadarray will print a summary of the object contents, with the actual output dependent on the class of the object.

Output for the beadLevelData class is broken down into three sections: experiment information, data that relate to each array section and data for individual beads. The full information relating to the first two groups will be display, with only a short summary of the per-bead information shown (currently 5 beads from the first section).

The ExpressionSetIllumina class is based up the eSet class and the output from show is closely related, with a short summary of the contents of available slots.

### Author(s)

Mark Dunning

---

## showArrayMask *Show Array Mask*

---

### Description

Function to display beads masked by BASH. The masked beads are assumed to have a weight of 0 in the specified weights column.

### Usage

```
showArrayMask(BLData,array = 0, override = FALSE, wtsName = "wts", transFun = logGreenChannelTransform,
```

### Arguments

| | |
|---|---|
| BLData | A BeadLevelList object. |
| override | Logical. Plotting a large mask can cause slowdown problems. By default, if more than 200 000 beads are masked, the current mask will not be plotted. You can force the mask to be plotted by setting this argument to TRUE, however beware as this may cause slower systems to freeze. |
| wtsName | name under which the bead weights are stored |
| array | numeric index of the array to plot |
| transFun | function to transform intensities prior to calculating outliers |
| outlierFun | function to remove outliers |
| horizontal | if TRUE the resulting image is plotting with the longest edge along the x axis |

## Details

showArrayMask plots the beads on an array that have been assigned a weight of 0 by BASH in red, and beads determined to be outliers in black.

## Value

None returned

## Author(s)

Jonathan Cairns and Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

  data(exampleBLData)

  showArrayMask(exampleBLData,2)

}
```

---

# squeezedVarOutlierMethod

*Identifier outliers on an array section*

---

## Description

An outlier calling method that shrinks the observed variance for a bead-type towards the predicted variance based on all bead-types on the array-section.

## Usage

```
squeezedVarOutlierMethod(inten, probeList, wts=1, n = 3, predictNlim=14)
```

## Arguments

| | |
|---|---|
| inten | a list of intensities |
| probeList | the IDs corresponding to each intensity value |
| wts | Weights associated with beads, indicating those recommended for removal by, for example, BASH |
| n | number of SDs cutoff used |
| predictNlim | how many beads of a bead-type must be present for that bead-type to contribute to prediction of variances? |

## Details

This function is called within the summarize routine of beadarray to exclude outlying beads from an array-section prior to summary. The intensities are not assumed to be on any particular scale and can result from any user-defined transformation function, however a log-transformation is recommended.

Bead-types that have predictNlim numbers are used to locally regress bead-type precision against bead-type mean, as well as the squared residual error of bead-type precision against bead-type mean. These are then used as prior values for the distribution of precision to feed into a standard Bayesian calcuation to obtain an estimate of the posterior variance.

Beads with weight zero do not contribute to the outlier calling.

## Value

the positions in the original vector that were determined to be outliers

## Author(s)

Andy Lynch

## See Also

illuminaOutlierMethod

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

oList = squeezedVarOutlierMethod(
        logGreenChannelTransform(exampleBLData, 1),
        getBeadData(exampleBLData, array=1, what="ProbeID")
    )

}
```

---

summarize *Create a summarized object*

---

## Description

Function to summarize the data in a beadLevelData object into a form more ameanable for downstream analysis (with the same number of observations for each bead type).

## Usage

```
summarize(BLData, channelList = list(greenChannel),
probeIDs=NULL, useSampleFac = FALSE, sampleFac= NULL,
weightNames = "wts", removeUnMappedProbes = TRUE)
```

## Arguments

| | |
|---|---|
| BLData | An object of class beadLevelData |
| channelList | List of objects of class illuminaChannel that defines the summarisation to be performed. The default is to do a log2 transformation and report the mean and standard deviation of each bead type after outlier removal. |
| probeIDs | Vector of ArrayAddressIDs to be included in the summarized object. The default is to summarize all probes. |
| useSampleFac | if TRUE sections belonging to the same biological sample will be combined. The default is to summarize each section separately. |
| sampleFac | optional character vector giving which a sample identifer for each section |
| weightNames | name of column in the beadLevelData to take extract weights |
| removeUnMappedProbes | if TRUE and annotation information is stored in the beadLevelData object, any ArrayAddressIDs that cannot be mapped to ILMN IDs will be removed. |

## Details

From beadarray version 2.0 onwards, users are allowed more flexibility in how to create summarized data from bead-level data. The illuminaChannel is a means of allowing this flexibility by defininining how summarization will be performed on each array section in the bead-level data object. The three keys steps applied to each section are; 1) use a transform function to get the quantities to be summarized (one value per bead). The most common use-case would be to extract the Green channel intensities and possibly perform a log2 transformation. 2) remove any outliers from this list of values 3) split the values according to ArrayAddressIDs and apply the definied exprFun and varFun to the quantities belonging to each ArrayAddress.

Some Illumina chips have multiple sections for the same biological sample; for example the HumanWG-6 chip or the Infinium genotyping chips. For such cases it may be more convenient to produce a summarized object where each column in the output is a different biological sample. This is especially important for genotpying chips where different SNPs are interrogated on the different sections, making a section-based summary problematic.

If the useSampleFac argument is set to TRUE, beadarray will try and combine sections belonging to the same sample. If the location of the sdf file for the chip is sucessfully stored in the experimentData slot of the beadLevelData object, the sdf will be interrogated to determine how samples were allocated to the chip. Otherwise the user can specifiy a sample factor that is the same length as the number of sections. If the sample factor is not supplied, or cannot be determined, then beadarray will summarize each section separately.

During the course of the summary, ArrayAddressIDs present in the beadLevelData object will be converted to Illumina IDs (prefix ILMN) if the annotation of the object was set by readIllumina or setAnnotation. The rownames of the resulting ExpressionSetIllumina will be set to these new IDs, and the featureData slot will contain the original and new IDs. Any control probes present

in the beadLevelData object will retain their original ArrayAddressID and the Status vector in featureData will report if each probe is a control or regular probe. Some ArrayAddressIDs present in the beadLevelData object may be neither regular probes will ILMN IDs, or control probes. These are internal controls used by Illumina and can be stopped from appearing in the summarized object by choosing the removeUnmappedProbes = TRUE option.

The user can specify a vector of ArrayAddressIDs to be summarized using the probeIDs argument. Otherwise, a unique set of IDs is derived from all the array sections in the beadLevelData object.

## Value

Returns an object of class ExpressionSetIllumina

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

 data(exampleBLData)

 bsd = summarize(exampleBLData)


 myMean = function(x) mean(x,na.rm=TRUE)
 mySd = function(x) sd(x,na.rm=TRUE)

 greenChannel = new("illuminaChannel",
         logGreenChannelTransform,
         illuminaOutlierMethod,
         myMean, mySd, "G"
    )

 bsd = summarize(exampleBLData, channelList = list(greenChannel))

 bsd


}
```

---

transformFunctions *Functions for transforming the data store in a* beadLevelData *object for easier visualisation or summarisation.*

---

## Description

Functions for transforming the data store in a beadLevelData object for easier visualisation or summarisation.

## Usage

```
logGreenChannelTransform(BLData, array)
logRedChannelTransform(BLData, array)
logRatioTransform(BLData, array)
```

## Arguments

| | |
|---|---|
| BLData | beadLevelData object |
| array | numeric specifying the section to be transformed |

## Details

beadarray aims to support the whole range of data that can be generated by the Illumina BeadArray technology and allows users to build upon the functionality in the package to make pipeline to automtically process their own data and develop new methodologies. Therefore we have made the quality assessment and summarisation tools general enough to take any kind of values that can be derived from the beadLevelData object. This is achieved by the definition of transformation functions that can be used throughout the package whenever a function is operating on data on a per-section basis.

The default transformation is to take the data from the Green channel (column Grn in the beadLevelData object) and perform a log2 transformation and is the default to functions such as `boxplot` or `imageplot`.

Users with two channel data (e.g. data from methylation and SNP assays) can use the `logRedChannelTransform` function which instead extracts the red channel on the log2 scale or `logRatioTransform`.

## Value

Transformation functions return a numeric vector with the same length as the number of beads for the particular section.

## Author(s)

Mark Dunning

## Examples

```
if(require(beadarrayExampleData)){

data(exampleBLData)

head(exampleBLData[[1]])

log2(getBeadData(exampleBLData, array=1,what="Grn")[1:10])

logGreenChannelTransform(exampleBLData, array=1)[1:10]

}
```

`weightsOutlierMethod` *returns all beads with weight=0.*

## Description

This function identifies those beads that have been set to having a weight of 0. Primarily intended to allow deprecation of the function `showArrayMask` by the more flexible outlierplot.

## Usage

```
weightsOutlierMethod(inten, probeList, wts,n=3)
```

## Arguments

| | |
|---|---|
| `inten` | a list of intensities but the intensities are not in fact used |
| `probeList` | the IDs corresponding to each intensity value (not used) |
| `wts` | Weights associated with beads. |
| `n` | another parameter that is not, in fact, used. |

## Details

This function the locations at which wts is equal to zero.

## Value

the positions in the original vector that had weight zero

## Author(s)

Andy Lynch

## See Also

`squeezedVarOutlierMethod`

## Examples

```
## Not run:

if(require(beadarrayExampleData)){
data(exampleBLData)
outlierplot(exampleBLData, array=1, outlierFun = weightsOutlierMethod,horizontal = FALSE)
}


## End(Not run)
```

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