

# Package ‘cytomapper’

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**Version** 1.2.0

**Title** Visualization of highly multiplexed imaging data in R

**Description** Highly multiplexed imaging acquires the single-cell expression of selected proteins in a spatially-resolved fashion. These measurements can be visualised across multiple length-scales. First, pixel-level intensities represent the spatial distributions of feature expression with highest resolution. Second, after segmentation, expression values or cell-level metadata (e.g. cell-type information) can be visualised on segmented cell areas. This package contains functions for the visualisation of multiplexed read-outs and cell-level information obtained by multiplexed imaging technologies. The main functions of this package allow 1. the visualisation of pixel-level information across multiple channels, 2. the display of cell-level information (expression and/or metadata) on segmentation masks and 3. gating and visualisation of single cells.

**License** GPL (>= 2)

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**Imports** S4Vectors, RColorBrewer, viridis, utils, SummarizedExperiment, tools, graphics, raster, grDevices, stats, ggplot2, ggbeeswarm, svgPanZoom, svglite, shiny, shinydashboard, matrixStats

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CytoImageList-class *S4 class for list of images*

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### Description

This class facilitates the handling of multiple one- or multi-channel images. It inherits from [SimpleList](#) setting `elementType="Image"`. Therefore, each slot contains an either one- or multi-dimensional array in form of an [Image](#) object.

### Usage

```
CytoImageList(...)
```

### Arguments

... A list of images (or coercible to a list) or individual images

### Details

Similar to the [Image](#) class, the first two dimensions of each entry indicate the spatial dimension of the image. These can be different for each entry. The third dimension indicates the number of channels per Image. Each entry in the CytoImageList class object must contain the same number of channels. Here, each channel represents pixel values indicating measurement intensities or in case of segmentation masks the cells' ID. The CytoImageList class therefore only supports a Grayscale colormode (see [colormode](#)) representation of each individual image.

The class further contains an `elementMetadata` slot that stores image-level meta information. This slot should be accessed using the `mcols` accessor function.

### Value

A CytoImageList object

### Restrictions on entry names

The CytoImageList class only supports unique entry names to avoid duplicated images. Names of a CytoImageList object can be get and set via `names(x)`, where `x` is a CytoImageList object. Furthermore, only named or unnamed CytoImageList objects are allowed. Partially named objects causing empty or NA names return an error.

### Coercion

Coercion to and from list, [SimpleList](#) and [List](#):

`as.list(x)`, `as(x, "SimpleList")`, `as(x, "SimpleList")`: Coercion from a CytoImageList object `x`

`as(x, "CytoImageList")`: Coercion from a list, SimpleList or List object `x` to an CytoImageList object

### Looping

While `lapply` and `mapply` return regular list objects, `endoapply` and `mendoapply` return CytoImageList objects.

### Author(s)

Nils Eling (<nils.eling@dqbm.uzh.ch>)

### See Also

[Image](#), for further image analysis tools.

[SimpleList](#), for basics functions to handle SimpleList objects

`?loadImages`, for reading images into a CytoImageList object

`?"CytoImageList-naming"`, for setting and getting image and channel names

`?"CytoImageList-subsetting"`, for subsetting and accessor functions

### Examples

```
# Creation of CytoImageList
u <- matrix(rbinom(100, 10, 0.5), ncol=10, nrow=10)
v <- matrix(rbinom(100, 10, 0.5), ncol=10, nrow=10)
IL1 <- CytoImageList(image1 = Image(u), image2 = Image(v))

# Coercion
as.list(IL1)
as(IL1, "SimpleList")
as(list(image1 = Image(u), image2 = Image(v)), "CytoImageList")
```

---

CytoImageList-manipulation

*Manipulating CytoImageList objects*

---

### Description

Methods to change pixel values in CytoImageList objects. In the following sections, object is a [CytoImageList](#) object containing one or multiple channels.

### Value

A CytoImageList object containing the manipulated Images.

### Image scaling

In some cases, images need to be scaled by a constant (e.g.  $2^{16}-1 = 65535$ ) value to revert them back to the original pixel values after reading them in.

`scaleImages(object, value)` Scales all images in the [CytoImageList](#) object by value.

### Image normalization

Linear scaling of the intensity values of each [Image](#) contained in a [CytoImageList](#) object to a specific range. Images can either be scaled to the minimum/maximum value per channel or across all channels (default `separateChannels = TRUE`). Also, images can be scaled to the minimum/maximum value per image or across all images (default `separateImages = FALSE`). The latter allows the visual comparison of intensity values across images.

To clip the images before normalization, the `inputRange` can be set. The `inputRange` either takes `NULL` (default), a vector of length 2 specifying the clipping range for all channels or a list where each named entry contains a channel-specific clipping range.

`normalize(object, separateChannels = TRUE, separateImages = FALSE, ft = c(0, 1), inputRange = NULL)`:

**object:** A CytoImageList object

**separateChannels:** Logical if pixel values should be normalized per channel (default) or across all channels.

**separateImages:** Logical if pixel values should be normalized per image or across all images (default).

**ft:** Numeric vector of 2 values, target minimum and maximum intensity values after normalization (see [normalize](#)).

**inputRange:** Numeric vector of 2 values, sets the absolute clipping range of the input intensity values (see [normalize](#)). Alternatively a names list where each entry corresponds to a channel-specific clipping range.

### Author(s)

Nils Eling (<[nils.eling@dqbm.uzh.ch](mailto:nils.eling@dqbm.uzh.ch)>)

### See Also

[normalize](#) for details on Image normalization

**Examples**

```

data(pancreasImages)

# Scale images to create segmentation masks
cur_files <- list.files(system.file("extdata", package = "cytomapper"),
                        pattern = "mask.tiff", full.names = TRUE)
x <- loadImages(cur_files)
# Error when running plotCells(x)
# Therefore scale to account for 16 bit encoding
x <- scaleImages(x, 2^16 - 1)
plotCells(x)

# Default normalization
x <- normalize(pancreasImages)
plotPixels(x, colour_by = c("H3", "CD99"))

# Setting the clipping range
x <- normalize(x, inputRange = c(0, 0.9))
plotPixels(x, colour_by = c("H3", "CD99"))

# Setting the clipping range per channel
x <- normalize(pancreasImages,
               inputRange = list(H3 = c(0, 70), CD99 = c(0, 100)))
plotPixels(x, colour_by = c("H3", "CD99"))

# Normalizing per image
x <- normalize(pancreasImages, separateImages = TRUE)
plotPixels(x, colour_by = c("H3", "CD99"))

```

---

CytoImageList-naming *Getting and setting the channel and image names*

---

**Description**

Methods to get and set the names of individual channels or the names of individual images.

**Setting and getting the channel names**

In the following code, `x` is a [CytoImageList](#) object containing one or multiple channels. The channel names can be replaced by `value`, which contains a character vector of the same length as the number of channels in the images.

`channelNames(x)` Returns the names of all channels stored in `x`

`channelNames(x) <- value` Replaces the channel names of `x` with `values`. For this, `value` needs to have the same length as the number of channels in `x`

**Setting and getting the image names**

Here, `x` is a [CytoImageList](#) object. The element names can be replaced by `value`, which contains a character vector of the same length as the number of images. In case of the `CytoImageList` object, elements are always images.

`names(x)` Returns the names of all images stored in `x`

`names(x) <- value` Replaces the image names of `x` with `value`. For this, `value` needs to have the same length as `x`

### Author(s)

Nils Eling (<nils.eling@dqbm.uzh.ch>)

### Examples

```
data("pancreasImages")

# Get channel and image names
channelNames(pancreasImages)
names(pancreasImages)

# Set channel and image names
channelNames(pancreasImages) <- paste0("marker", 1:5)
names(pancreasImages) <- paste0("image", 1:3)
```

---

CytoImageList-subsetting

*General subsetting methods for CytoImageList objects*

---

### Description

These getter and setter functions are used to extract, replace and merge entries in a [CytoImageList](#) object.

### Arguments

<code>x, y</code>	CytoImageList objects
<code>i</code>	integer, logical, character or vector of such indicating which element(s) to replace or extract
<code>value</code>	a CytoImageList or <a href="#">Image</a> object

### Value

A CytoImageList object

### Setting and getting images

Functions to extract and replace elements (= images) of a [CytoImageList](#) object. In the following code, `x` is a CytoImageList object. The parameter `i` indicates the element(s) of `x` that should be returned or replaced. Replacement is done by `value`, which takes a CytoImageList or Image object. If `length(i) > 0`, `value` has to be a CytoImageList object of length `length(i)`, otherwise `value` allows a CytoImageList object of length 1 or an Image object. If an Image object is provided, only the image entry in the CytoImageList object is replaced, not the corresponding elementMetadata entry.

`getImages(x, i)` Returns image(s) indicated by `i` of the CytoImageList object `x`

`setImages(x, i) <- value` Replaces the image(s) indicated by `i` of the CytoImageList object `x` with `value`. For this, `value` needs to have the same length as `i`

These setter and getter functions are the recommended way of extracting and replacing images in a `CytoImageList` object. Alternatively, the standard operations via ``[`, `[[`, `[<-`` and ``[[<-`` can be performed (see [?List](#) for `S4Vectors` subsetting functionality). However, these operations do not change element names during replacement calls. The `setImages()` function makes sure that element names are replaced if `value` is named or if `i` is a character or vector of characters.

### Getting and setting channels

Functions to extract and replace channels of a `CytoImageList` object. Here, `x` is a `CytoImageList` object. The parameter `i` indicates the channels of `x` that should be returned or replaced. Replacement is done by `value`, which takes a `CytoImageList` object. The `CytoImageList` object `value` needs to have the same length as `x`. Furthermore, the number of channels in `value` should be identical to `length(i)`.

`getChannels(x, i)` Returns channel(s) indicated by `i` of the `CytoImageList` object `x`

`setChannels(x, i) <- value` Replaces the channel(s) indicated by `i` of the `CytoImageList` object `x` with `value`. For this, `value` needs to have the same length as `i` and the same number of channels as `length(i)`.

The `setChannels()` setter function does not allow adding new channels to the `CytoImageList` object. For this operation, the `mergeChannels` function was implemented (see below).

### Merging images

Merging images is possible by merging two or more `CytoImageList` objects via:

`c(x, y)` Returns an composite `CytoImageList` object with elements of both `CytoImageList` objects `x` and `y`. More than two `CytoImageList` objects can be merged in that way.

### Merging channels

Merging channels is possible via:

`mergeChannels(x, y)`: Returns a `CytoImageList` in which the channels of the `CytoImageList` object `y` have been appended to the channels of the `CytoImageList` object `x`. Only channels of two `CytoImageList` objects can be merged in that way.

### Author(s)

Nils Eling (<nils.eling@dqbm.uzh.ch>)

### Examples

```
data("pancreasImages")

# Get images
getImages(pancreasImages, 1)
getImages(pancreasImages, "E34_imc")
getImages(pancreasImages, 1:2)
getImages(pancreasImages, c("E34_imc", "G01_imc"))
getImages(pancreasImages, grep1("E34_imc", names(pancreasImages)))

# Set images
setImages(pancreasImages, 1) <- pancreasImages[1]
setImages(pancreasImages, "J02_imc") <- pancreasImages[1]
```

```

setImages(pancreasImages, "J02_imc") <- NULL

# Get channels
getChannels(pancreasImages, 1)
getChannels(pancreasImages, "CD99")
getChannels(pancreasImages, c("CD99", "PIN"))

# Set channels
channel1 <- getChannels(pancreasImages, 1)
setChannels(pancreasImages, 1) <- channel1
channelPIN <- getChannels(pancreasImages, "PIN")
setChannels(pancreasImages, "CD8a") <- channelPIN
setChannels(pancreasImages, "CD8a") <- NULL

# Merge images
data("pancreasImages")
c(pancreasImages[c(1,3)], pancreasImages[2])

# Merge channels
channel12 <- getChannels(pancreasImages, c(1,2))
channel134 <- getChannels(pancreasImages, c(3,4))
mergeChannels(channel12, channel134)

```

---

cytomapperShiny

*Shiny application to visualise gated cells on images*


---

## Description

This shiny application allows users to gate cells based on their raw or transformed expression values and visualises gated cells on their corresponding images.

## Usage

```

cytomapperShiny(
  object,
  mask = NULL,
  image = NULL,
  cell_id = NULL,
  img_id = NULL,
  ...
)

```

## Arguments

object	a <a href="#">SingleCellExperiment</a> object.
mask	(optional) a <a href="#">CytoImageList</a> containing single-channel <a href="#">Image</a> objects.
image	(optional) a <a href="#">CytoImageList</a> object containing single or multi-channel <a href="#">Image</a> objects.
cell_id	character specifying the <code>colData(object)</code> entry, in which the integer cell IDs are stored. These IDs should match the integer pixel values in the segmentation mask object ( <code>mask</code> ).

img_id	character specifying the colData(object) and mcols(mask) and/or mcols(image) entry, in which the image IDs are stored.
...	parameters passed to the <code>plotCells</code> or <code>plotPixels</code> function.

**Value**

A Shiny app object for hierarchical gating of cells

**User inputs**

This function requires at least a `SingleCellExperiment` input object. Gating is performed on cell-specific marker counts stored in the assay slots of the object. These can either be raw counts (usually stored in the counts slot) or transformed/scaled counts stored in other assay slots. Gating can only be performed sample-wise; therefore, even if mask or image are not specified, img\_id needs to point to the colData entry storing unique sample IDs. Furthermore, the cell\_id entry is required to identify cells during hierarchical gating.

If mask is specified, marker expression values and selected cells will be visualised by using the `plotCells` function. To visualise pixel-level information with `plotPixels`, the user has to further provide multi-channel images stored in a `CytoImageList`.

**The user interface (UI)**

The UI's body is composed of two tabs (*Scatter Plots* and *Images*). The side bar contains the *General Control* and *Plots* panels. Both panels can be collapsed; however only one can be expanded again.. Using the control panel, the user can set the number of plots, which sample to display and which assay slot to use for plotting expression. The *Plots* panel can be used to select up to two markers per plot. A one-marker selection is displayed as violin and jittered points, two markers are shown as scatter plot. To define a subset of cells, the user can draw gates on the plots. When using multiple plots, the gate applied in a plot will define the subset of cells displayed in the next plot. The number of plots, the marker selection and the applied gates will be transferred when switching between samples. When switching markers or assay slots, the gates will be removed.

By switching to the Images tab, the user can inspect marker expression levels and the result of the gating. This requires either an entry to mask and (optionally) image. Depending on the input, the left image visualises the expression of the selected markers by using `plotCells` (if mask is supplied) or `plotPixels` (if image is supplied). Displayed markers can be changed using the dropdown menu. If images are provided, the user can change the contrast of the selected markers. The right image colors (masks) or outlines (images) gated cells. Both the left and the right image come with a zoom-in functionality.

The header section of the shiny allows to hide the side bar, to download selected cells (see below), or to display the sessionInfo output and the *Help* section (see below).

**Download of gated cells**

The user can download the selected cells in form of a `SingleCellExperiment` object. The output is a subset of the input `SingleCellExperiment` object. The downloaded object (in form of a .rds file) contains a new colData entry containing the label of the subset defined by the user (using the *Cell label* argument). The cell label is stored in colData(object)\$cytomapper\_CellLabel

The metadata slot of the `SingleCellExperiment` object will be converted to a list. It contains the original metadata (metadata(object)\$metadata), gating parameters (e.g. metadata(object)\$cytomapper\_gate\_1), sessionInfo() output (metadata(object)\$cytomapper\_SessionInfo), and the date (metadata(object)\$cytomapper\_date)

### Passing further parameters

To customise the visual output of cytomapperShiny, refer to [plotting-param](#). Further arguments can be passed to the [plotCells](#) and [plotPixels](#) function. As an example: To avoid interpolation of output images, set `interpolate = FALSE`. Passing the parameter `interpolate = FALSE` will make images less blurry, which might be useful while using the zoom-in functionality of cytomapperShiny.

### Getting further help

Please refer to the *Help* section found when clicking the '?' button in the upper right corner. The *Help* section contains a recommended workflow on how to use the app.

### Author(s)

Nils Eling (<nils.eling@dqbm.uzh.ch>  
Tobias Hoch (<tobias.hoch@dqbm.uzh.ch>)

### See Also

[plotCells](#) and [plotPixels](#) for the main plotting functions

### Examples

```
## Only run this example in interactive R sessions
if (interactive()) {
  # Load example data sets
  data("pancreasSCE")
  data("pancreasImages")
  data("pancreasMasks")

  # Use shiny with SCE object, images and masks
  cytomapperShiny(object = pancreasSCE, mask = pancreasMasks,
                  image = pancreasImages, cell_id = "CellNb",
                  img_id = "ImageNb")

  # Use shiny with SCE object and masks
  cytomapperShiny(object = pancreasSCE, mask = pancreasMasks,
                  cell_id = "CellNb", img_id = "ImageNb")

  # Use shiny with SCE object only
  cytomapperShiny(object = pancreasSCE,
                  cell_id = "CellNb", img_id = "ImageNb")
}
```

---

loadImages

*Read images into CytoImageList object*

---

### Description

Function to read in single- or multi-channel images from a specified path or file. The function returns a [CytoImageList](#) object containing one image per slot. Supported file extensions are: '.tiff', '.tif', '.png', '.jpeg', '.jpg'.

**Usage**

```
loadImages(x, pattern = NULL, ...)
```

**Arguments**

**x** The function takes a variety of possible character inputs:  
**A single file** Full path and file name of an individual image file.  
**A path** A path to where image files are located.  
**A vector of files** A character vector where each entry represents an individual file.

**pattern** Character inputs of the following form:  
**A single character** A pattern to search for in the specified path (regular expressions are supported).  
**A character vector** Unique entries are matched against file names in the specified path.

**...** arguments passed to the [readImage](#) function.

**Value**

A [CytoImageList](#) object

**Loading specific images**

This function loads images via the [readImage](#) function and stores them in a [CytoImageList](#) object. In the simplest case, `x` is an image file name. If `x` is a path, the `pattern` argument can be used to select image names with certain patterns. For convenience, `pattern` also takes a vector of characters (e.g. a `colData` entry in a [SingleCellExperiment](#) object) to select by unique image names. Furthermore, a vector of image names can be provided to read in multiple images.

**Author(s)**

Nils Eling (<nils.eling@dqbm.uzh.ch>),  
 Nicolas Damond (<nicolas.damond@dqbm.uzh.ch>)

**See Also**

[readImage](#), for reading in individual images.

**Examples**

```
# Providing a single file
single.image <- system.file("extdata/E34_mask.tiff", package = "cytomapper")
single.image <- loadImages(single.image)

# Providing a path and pattern
path.to.images <- system.file("extdata", package = "cytomapper")
image.list <- loadImages(path.to.images, pattern = "mask.tiff")

# Providing multiple patterns
data(pancreasSCE)
path.to.images <- system.file("extdata", package = "cytomapper")
image.list <- loadImages(path.to.images, pattern = pancreasSCE$MaskName)
```

```
# Providing multiple files
list.images <- list.files(system.file("extdata", package = "cytomapper"),
                          pattern = "_mask.tiff", full.names = TRUE)
image.list <- loadImages(list.images)
```

---

pancreasImages

*Example CytoImageList object of image files*

---

### Description

This [CytoImageList](#) object contains multi-channel stacks of three images acquired by imaging mass cytometry. Each channel represents the pixel-intensities of each of the 5 measured proteins. The data is part of a imaging mass cytometry study on the progression of Type 1 diabetes and contains pancreas cells.

### Usage

```
pancreasImages
```

### Format

A [CytoImageList](#) object containing 3 [Image](#) objects with 5 channels each. Channel names can be accessed via `?channelNames`.

### References

Damond, N. et al., A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry, *Cell Metabolism* 29:3, 2019

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pancreasMasks

*Example CytoImageList object of segmentation masks*

---

### Description

This [CytoImageList](#) object contains single-channel images representing the segmentation masks after preprocessing of imaging mass cytometry data. The data is part of a imaging mass cytometry study on the progression of Type 1 diabetes and contains pancreas cells.

### Usage

```
pancreasMasks
```

### Format

A [CytoImageList](#) object containing 3 [Image](#) objects with 1 channel each. These images are the result to segmentation and associated to the images stored in the [pancreasImages](#) object. Pixel values indicate the numeric cell identifier while a value of 0 represents the image background.

## References

Damond, N. et al., A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry, *Cell Metabolism* 29:3, 2019

---

pancreasSCE	<i>Example SingleCellExperiment object</i>
-------------	--

---

## Description

This `SingleCellExperiment` object contains the expression values of 5 proteins (rows) from 362 cells (columns) across 3 images. The data is part of a imaging mass cytometry study on the progression of Type 1 diabetes and therefore contains pancreas cells.

## Usage

```
pancreasSCE
```

## Format

A `SingleCellExperiment` object containing the raw and arcsinh-transformed mean pixel counts per cell as well as associated cell- and protein-specific metadata. Row names represent the names of the target proteins and column names represent the image name and cell id of each cell.

**colData** Cell-specific metadata where rownames represent the image name and cell id. It contains the

1. image number (`ImageNb`),
2. cell number/identifier (`CellNb`),
3. spatial position on the image (`Pos_X`, `Pos_Y`),
4. the associated image name (`ImageName`, see ?"pancreasImages"),
5. the associated mask name (`MaskName`, see ?"pancreasMasks"),
6. a arbitrary cell-type label (`CellType`)
7. a logical (`Pattern`) indicating exocrine cells

**rowData** Protein-specific metadata where rownames represent the names of the target proteins. It contains the

1. channel number (frame),
2. metal tag of the antibody (`MetalTag`)
3. Target (the expanded name of the targeted protein)
4. `clean_Target` (the abbreviated name of the targeted protein)

**assays** List of protein expression counts containing:

1. the raw expression counts (`counts`): mean pixel value per cell and protein
2. arcsinh-transformed raw expression counts using a co-factor of 1 (`exprs`)

## References

Damond, N. et al., A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry, *Cell Metabolism* 29:3, 2019

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plotCells

*Function to visualize cell-level information on segmentation masks*


---

### Description

This function takes a [SingleCellExperiment](#) and [CytoImageList](#) object containing segmentation masks to colour cells by marker expression or metadata.

### Usage

```
plotCells(
  mask,
  object = NULL,
  cell_id = NULL,
  img_id = NULL,
  colour_by = NULL,
  outline_by = NULL,
  exprs_values = "counts",
  colour = NULL,
  ...
)
```

### Arguments

mask	a <a href="#">CytoImageList</a> containing single-channel <a href="#">Image</a> objects (see section 'Segmentation mask object' below).
object	a <a href="#">SingleCellExperiment</a> object.
cell_id	character specifying the colData(object) entry, in which the integer cell IDs are stored. These IDs should match the integer pixel values in the segmentation mask object.
img_id	character specifying the colData(object) and mcols(mask) entry, in which the image IDs are stored (see section 'Linking the SingleCellExperiment and CytoImageList object' below)
colour_by	character or character vector specifying the features (rownames(object)) or metadata (colData(object) entry) used to colour individual cells. Cells can be coloured by single colData(object) entries or by up to six features.
outline_by	single character indicating the colData(object) entry by which to outline individual cells.
exprs_values	single character indicating which assay(object) entry to use when visualizing feature counts.
colour	a list with names matching the entries to colour_by and/or outline_by. When setting the colour for continous features, at least two colours need to be provided indicating the colours for minimum and maximum values. When colouring discrete vectors, a colour for each unique entry needs to be provided (see section 'Setting the colours' and examples)
...	Further arguments passed to <code>?plotting-param</code>

**Value**

a list if return\_images and/or return\_plot is TRUE (see [?"plotting-param"](#)).

- plot: a single plot object (display = "all") or a list of plot objects (display = "single")
- images: a [SimpleList](#) object containing three-colour [Image](#) objects.

**Segmentation mask object**

In the plotCells function, mask refers to a [CytoImageList](#) object that contains a single or multiple segmentation masks in form of individual [Image](#) objects. The function assumes that each object in the segmentation mask is a cell. The key features of such masks include:

- each Image object contains only one channel
- pixel values are integers indicating the cells' IDs or 0 (background)

**Linking SingleCellExperiment and CytoImageList objects**

To colour individual cells contained in the segmentation masks based on features and metadata stored in the SingleCellExperiment object, an img\_id and cell\_id entry needs to be provided. Image IDs are matched between the SingleCellExperiment and CytoImageList object via entries to the colData(object)[,img\_id] and the mcols(mask)[,img\_id] slots. Cell IDs are matched between the SingleCellExperiment and CytoImageList object via entries to colData(object)[,cell\_id] and the integer values of the segmentation masks.

**Setting the colours**

By default, features and metadata are coloured based on internally-set colours. To set new colours, a list object must be provided. The names of the object must correspond to the entries to colour\_by and/or outline\_by. When setting the colours for continuous expression values or continuous metadata entries, a vector of at least two colours need to be specified. These colours will be passed onto [colorRampPalette](#) for interpolation. Discrete metadata entries can be coloured by specifying a named vector in which each entry corresponds to a unique entry to the metadata vector.

**Subsetting the CytoImageList object**

The CytoImageList object can be subsetted before calling the plotCells function. In that case, only the selected images are displayed.

**Subsetting the SingleCellExperiment object**

The SingleCellExperiment object can be subsetted before calling the plotCells function. In that case, only cells contained in the SingleCellExperiment object are coloured/outlined.

**Colour scaling**

When colouring features using the plotCells function, colours are scaled between the minimum and maximum per feature across the full assay contained in the SingleCellExperiment object. When subsetting images, cell-level expression is not scaled across the subsetted images but the whole SingleCellExperiment object. To avoid this, the SingleCellExperiment object can be subsetted to only contain the cells that should be displayed before plotting.

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**See Also**

For further plotting parameters see ?"[plotting-param](#)"

**Examples**

```

data(pancreasMasks)
data(pancreasSCE)

# Visualize the masks
plotCells(pancreasMasks)

# Colour the masks based on averaged expression
plotCells(pancreasMasks, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = c("CD99", "CDH"))

# Colour the masks based on metadata
plotCells(pancreasMasks, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = "CellType")

# Outline the masks based on metadata
plotCells(pancreasMasks, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = "CD99",
          outline_by = "CellType")

# Colour the masks based on arcsinh-transformed expression
plotCells(pancreasMasks, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = "CD99",
          exprs_values = "exprs")

# Subset the images
cur_images <- getImages(pancreasMasks, 1:2)
plotCells(cur_images, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = "CD99")

# Set colour
plotCells(pancreasMasks, object = pancreasSCE, img_id = "ImageNb",
          cell_id = "CellNb", colour_by = "CD99", outline_by = "CellType",
          colour = list(CD99 = c("black", "red"),
                        CellType = c(celltype_A = "blue",
                                    celltype_B = "green",
                                    celltype_C = "red")))

```

## Description

This function takes a [CytoImageList](#) object to colour pixels by marker expression. Additionally, a [SingleCellExperiment](#) object and [CytoImageList](#) object containing segmentation masks can be provided to outline cells based on metadata.

## Usage

```
plotPixels(
  image,
  object = NULL,
  mask = NULL,
  cell_id = NULL,
  img_id = NULL,
  colour_by = NULL,
  outline_by = NULL,
  bcg = NULL,
  colour = NULL,
  ...
)
```

## Arguments

image	a <a href="#">CytoImageList</a> object containing single or multi-channel <a href="#">Image</a> objects (see details below).
object	an optional <a href="#">SingleCellExperiment</a> object.
mask	an optional <a href="#">CytoImageList</a> object containing segmentation masks in form of single-channel <a href="#">Image</a> objects (see details below)
cell_id	character specifying the <code>colData(object)</code> entry, in which the integer cell IDs are stored. These IDs should match the integer pixel values in the segmentation mask object.
img_id	character specifying the <code>colData(object)</code> , <code>mcols(image)</code> and <code>mcols(mask)</code> entry, in which the image IDs are stored (see section 'Linking the <a href="#">SingleCellExperiment</a> and <a href="#">CytoImageList</a> objects' below)
colour_by	character or character vector specifying the features (contained in <code>channelNames(image)</code> ) used to colour individual cells. Pixels can be coloured by up to six features.
outline_by	single character indicating the <code>colData(object)</code> entry by which to outline individual cells
bcg	a list with names matching the entries to <code>colour_by</code> . Each entry contains a numeric vector of three entries: <ol style="list-style-type: none"> <li>1. brightness value added to the specified channel</li> <li>2. contrast value multiplied with the specified channel</li> <li>3. gamma value (channel is exponentiated by this value)</li> </ol> Default is <code>c(0,1,1)</code> .
colour	a list with names matching the entries to <code>colour_by</code> and/or <code>outline_by</code> . When setting the colour for continuous features, at least two colours need to be provided indicating the colours for minimum and maximum values. When outlining by discrete values, a colour for each unique entry needs to be provided (see section 'Setting the colours' and examples)
...	Further arguments passed to <code>?"plotting-param"</code>

**Value**

a list if return\_images and/or return\_plot is TRUE (see [?"plotting-param"](#)).

- plot: a single plot object (display = "all") or a list of plot objects (display = "single")
- images: a [SimpleList](#) object containing three-colour [Image](#) objects.

**Multi-channel image and segmentation mask objects**

In the plotPixels function, image refers to a [CytoImageList](#) object that contains a single or multiple single- or multi-channel [Image](#) objects. Up to six channels can be overlaid to generate a composite image. When outlining cells, a [SingleCellExperiment](#) object and [CytoImageList](#) object containing segmentation masks must be provided. The function assumes that each object in the segmentation mask is a cell. The key features of such segmentation masks include:

- each Image object contains only one channel
- pixel values are integers indicating the cells' IDs

**Linking SingleCellExperiment and CytoImageList objects**

To outline individual cells contained in the segmentation masks based on metadata stored in the SingleCellExperiment object, an img\_id and cell\_id entry needs to be provided. Image IDs are matched between the SingleCellExperiment and CytoImageList objects via entries to the colData(object)[,img\_id], mcols(image)[,img\_id] and the mcols(image)[,img\_id] slots. Cell IDs are matched between the SingleCellExperiment and CytoImageList object via entries to colData(object)[,cell\_id] and the integer values of the segmentation masks.

**Setting the colours**

By default, features and metadata are coloured based on internally-set colours. To set new colours, a list object must be provided. The names of the object must correspond to the entries to colour\_by and/or outline\_by. When setting the colours for continuous expression values or continuous metadata entries, a vector of at least two colours need to be specified. These colours will be passed onto [colorRampPalette](#) for interpolation. Cells can be outlined by discrete metadata entries when specifying a named vector in which each entry corresponds to a unique entry to the metadata vector.

**Subsetting the CytoImageList objects**

The CytoImageList object(s) can be subsetted before calling the plotPixels function. In that case, only the selected images are displayed.

**Subsetting the SingleCellExperiment object**

The SingleCellExperiment object can be subsetted before calling the plotPixels function. In that case, only cells contained in the SingleCellExperiment object are outlined.

**Colour scaling**

When plotting pixel intensities, colours are scaled to the minimum and maximum per channel across all images that are being displayed. Therefore, when subsetting images, displayed intensities might change. However, the colour legend will display the correct numeric minimum and maximum pixel intensity across all displayed images per channel.

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**See Also**

For further plotting parameters see [?"plotting-param"](#). For instructions on how to normalize images see [normalize](#).

**Examples**

```
data(pancreasMasks)
data(pancreasImages)
data(pancreasSCE)

# Visualize the images - by default the first channel
plotPixels(pancreasImages)

# Colour the channels
plotPixels(pancreasImages, colour_by = c("CD99", "CDH"))

# Outline the cells based on metadata
plotPixels(pancreasImages, object = pancreasSCE, mask = pancreasMasks,
           img_id = "ImageNb", cell_id = "CellNb",
           colour_by = c("CD99", "CDH"), outline_by = "CellType")

# Enhance individual channels
plotPixels(pancreasImages, colour_by = c("CD99", "CDH"),
           bcg = list(CD99 = c(0, 2, 1)))

# Subset the images
cur_images <- getImages(pancreasImages, 1:2)
plotPixels(cur_images, colour_by = c("CD99", "CDH"))

# Set colour
plotPixels(pancreasImages, colour_by = c("CD99", "CDH"),
           colour = list(CD99 = c("black", "green"),
                        CDH = c("black", "blue")))
```

---

plotting-param

*Further plotting parameters for the cytomapper package*

---

**Description**

The [plotCells](#) and [plotPixels](#) functions share a number of parameter that can be set to change the visual representation of plotted images.

**Arguments**

`missing_colour` a single character specifying a valid colour. Cells that are not contained in the `SingleCellExperiment` object will be coloured based on `missing_colour`. In the [plotPixels](#) function, `missing_colour` defines the outline of the cells if `outline_by` is not set.

background_colour	(only <a href="#">plotCells</a> ) a single character specifying a valid colour that is set as the background of the image.
scale_bar	<p>a list specifying features of the scale bar. One or multiple of the following entries are supported:</p> <ul style="list-style-type: none"> <li>• length: numeric length in pixels (default 20% of the largest image width).</li> <li>• label: single character specifying the scale bar label.</li> <li>• cex: numeric value indicating the size of the scale bar label.</li> <li>• lwidth: numeric value indicating the line width of the scale bar in pixels. By default, the line width is adjusted relative to the maximum height of the images.</li> <li>• colour: single character specifying the colour of scale bar and label (default "white").</li> <li>• position: position of scale bar. Supported values: "topleft", "topright", "bottomleft", "bottomright" (default "bottomright").</li> <li>• margin: vector of two numeric entries specifying the x and y margin between image boundary and the scale bar (default c(10,10)).</li> <li>• frame: either "all" to display scale bar on all images or a single number specifying the image for which the scale bar should be displayed (default "all").</li> </ul> <p>Plotting of the scale bar is suppressed if set to NULL.</p>
image_title	<p>a list specifying features of the image titles. One or multiple of the following entries are supported:</p> <ul style="list-style-type: none"> <li>• text: character vector of image titles. Same length as the <code>CytoImageList</code> object.</li> <li>• position: single character specifying the position of the title. Supported entries: "top", "bottom", "topleft", "bottomleft", "topright", "bottomright" (default "top").</li> <li>• colour: single character specifying the colour of image title (default "white").</li> <li>• margin: vector of two numeric entries specifying the x and y margin between image boundary and the image title (default c(10,10)).</li> <li>• font: numeric entry specifying the font of the image title (default 1, see <a href="#">par</a> for details)</li> <li>• cex: numeric value indicating the size of the image title.</li> </ul> <p>Plotting of the image title is suppressed if set to NULL.</p>
save_plot	<p>a list specifying how to save the plot. One or multiple of the following entries are supported:</p> <ul style="list-style-type: none"> <li>• filename: single character specifying a valid file name. The file extension specifies the format in which the file is saved. Supported formats are: jpeg, tiff and png. If <code>display = "single"</code>, each image will be written in an individual file. The file names obtain a "_x" ending where x indicates the position of the image in the <code>CytoImageList</code> object or "legend".</li> <li>• scale: by default the height and width of the saved image is defined by the maximum image size times the number of rows and number of columns. This resolution is often not sufficient to clearly display the text. The scale parameter can be set to increase the resolution of the image while keeping the text size constant (default 1 but can be increased for optimal results).</li> </ul>
return_plot	logical indicating whether to return the plot (see <a href="#">recordPlot</a> for more infos).

return_images	logical indicating whether to return the coloured images in form of a <a href="#">SimpleList</a> object. Each entry to this list is a three-colour <a href="#">Image</a> object. However, the image title and scale bar are not retained.
legend	<p>a list specifying features of the legend. One or multiple of the following entries are supported:</p> <ul style="list-style-type: none"> <li>• <code>colour_by.title.font</code>: numeric entry specifying the font of the legend title for features specified by <code>colour_by</code>.</li> <li>• <code>colour_by.title.cex</code>: numeric entry specifying the size of the legend title for features specified by <code>colour_by</code>.</li> <li>• <code>colour_by.labels.cex</code>: numeric entry specifying the size of the legend labels for features specified by <code>colour_by</code>.</li> <li>• <code>colour_by.legend.cex</code>: (only discrete features) numeric entry specifying the size of the legend for features specified by <code>colour_by</code>.</li> <li>• <code>outline_by.title.font</code>: numeric entry specifying the font of the legend title for features specified by <code>outline_by</code>.</li> <li>• <code>outline_by.title.cex</code>: numeric entry specifying the size of the legend title for features specified by <code>outline_by</code>.</li> <li>• <code>outline_by.labels.cex</code>: numeric entry specifying the size of the legend labels for features specified by <code>outline_by</code>.</li> <li>• <code>outline_by.legend.cex</code>: (only discrete features) numeric entry specifying the size of the legend for features specified by <code>outline_by</code>.</li> <li>• <code>margin</code>: numeric value indicating the margin (in pixels) between the legends and the outer boundary (default 2)</li> </ul> <p>Plotting of the legend is suppressed if set to NULL.</p>
margin	numeric value indicating the gap (in pixels) between individual images (default 0).
display	one of two possible values: "all" or "single". When set to "all", all images are displayed at once in a grid-like fashion. When set to "single", individual images are plotted in single graphics devices. The second option is useful when saving individual images in pdf format or when displaying in Rmarkdown files.
scale	logical indicating whether to scale each feature individually to its minimum/maximum across the <code>SingleCellExperiment</code> object (see <a href="#">plotCells</a> ) or across all displayed images (see <a href="#">plotCells</a> ). If set to FALSE each value is displayed relative to the maximum of all selected features.
interpolate	a logical indicating whether to apply linear interpolation to the image when drawing (see <a href="#">rasterImage</a> ) (default TRUE).

**Value**

a list if `return_images` and/or `return_plot` is TRUE.

- `plot`: a single plot object (`display = "all"`) or a list of plot objects (`display = "single"`)
- `images`: a [SimpleList](#) object containing three-colour [Image](#) objects.

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**See Also**

[plotCells](#) and [plotPixels](#) for the main plotting functions

**Examples**

```

data("pancreasImages")
data("pancreasMasks")
data("pancreasSCE")

# Setting missing colour
plotCells(pancreasMasks, missing_colour = "blue")

# Setting background colour
plotCells(pancreasMasks, background_colour = "blue")

# Setting the scale bar
plotCells(pancreasMasks, scale_bar = list(length = 10,
                                          cex = 2,
                                          lwidth = 10,
                                          colour = "red",
                                          position = "bottomleft",
                                          margin = c(5,5),
                                          frame = 3))

# Setting the image title
plotCells(pancreasMasks,
          image_title = list(text = c("image1", "image2", "image3"),
                             position = "topleft",
                             colour = "blue",
                             margin = c(0,5),
                             font = 2,
                             cex = 2))

# Return plot
cur_out <- plotPixels(pancreasImages, return_plot = TRUE)
cur_out$plot

# Return images
cur_out <- plotPixels(pancreasImages, return_images = TRUE)
cur_out$images

# Setting the legend
plotCells(pancreasMasks, object = pancreasSCE,
          img_id = "ImageNb", cell_id = "CellNb",
          colour_by = c("CD99", "CDH"),
          outline_by = "CellType",
          legend = list(colour_by.title.font = 0.5,
                        colour_by.title.cex = 0.5,
                        colour_by.labels.cex = 0.5,
                        outline_by.legend.cex = 0.5,
                        margin = 0))

# Setting the margin between images
plotPixels(pancreasImages, margin = 3)

# Displaying individual images

```

```
plotPixels(pancreasImages, display = "single")

# Suppress scaling
plotPixels(pancreasImages, colour_by = c("CD99", "PIN"),
           scale = TRUE)
plotPixels(pancreasImages, colour_by = c("CD99", "PIN"),
           scale = FALSE)

# Suppress interpolation
plotPixels(pancreasImages, colour_by = c("CD99", "PIN"),
           interpolate = TRUE)
plotPixels(pancreasImages, colour_by = c("CD99", "PIN"),
           interpolate = FALSE)
```

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