## Package 'imcdatasets'

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- Title Collection of publicly available imaging mass cytometry (IMC) datasets
- **Description** The imcdatasets package provides access to publicly available IMC datasets. IMC is a technology that enables measurement of > 40 proteins from tissue sections. The generated images can be segmented to extract single cell data. Datasets typically consist of three elements: a SingleCellExperiment object containing single cell data, a CytoImageList object containing multichannel images and a CytoImageList object containing the cell masks that were used to extract the single cell data from the images.

License GPL-3

NeedsCompilation no

**Depends** R (>= 4.1), SingleCellExperiment, cytomapper,

Imports methods, utils, ExperimentHub, S4Vectors, DelayedArray, HDF5Array

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**biocViews** ExperimentHub, SingleCellData, TechnologyData, PackageTypeData, Tissue

VignetteBuilder knitr

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BugReports https://github.com/BodenmillerGroup/imcdatasets/issues

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DamondPancreas2019 'DamondPancreas2019' dataset

#### Description

This dataset consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry of human pancreas sections from donors with type 1 diabetes.

#### Usage

```
DamondPancreas2019_sce(metadata = FALSE)
DamondPancreas2019_images(metadata = FALSE)
DamondPancreas2019_masks(metadata = FALSE)
```

#### Arguments

metadata logical value indicating whether ExperimentHub metadata (describing the overall dataset) should be returned only, or if the whole dataset should be loaded. Default = FALSE, which loads the whole dataset.

#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Damond et al. (2019), consisting of three data objects:

- DamondPancreas2019\_images contains a hundred 38-channel images in the form of a CytoImageList class object.
- DamondPancreas2019\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- DamondPancreas2019\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment. This represents a total of 252,059 cells x 38 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/DamondPancreas2019\_sce, imcdatasets/DamondPancreas2019\_images, or imcdatasets/DamondPancreas2019\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageName or ImageNumber variables. Mapping

between cell segmentation masks and single cell data is performed with the CellNumber variable, the values of which correspond to the intensity values of the DamondPancreas2019\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset is a subset of the complete Damond et al. (2019) dataset comprising the data from three pancreas donors at different stages of type 1 diabetes (T1D). The three donors present clearly diverging characteristics in terms of cell type composition and cell-cell interactions, which makes this dataset ideal for benchmarking spatial and neighborhood analysis algorithms.

The assay slot of the SingleCellExperiment object contains two assays:

- counts contains mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object.

The cell-associated metadata are stored in the colData of the SingleCellExperiment object. These metadata include cell types (in colData(sce)\$CellType) and broader cell categories, such as "immune" or "islet" cells (in colData(sce)\$CellCat). In addition, for cells located inside pancreatic islets, the islet they belong to is indicated in colData(sce)\$ParentIslet. For cells not located in islets, the "ParentIslet" value is set to 0 but the spatially closest islet can be identified with colData(sce)\$ClosestIslet.

The donor-associated metadata are also stored in the colData of the SingleCellExperiment object. For instance, the donors' IDs can be retrieved with colData(sce)\$case and the donors' disease stage can be obtained with colData(sce)\$stage.

The three donors present the following characteristics:

- 6126 is a non-diabetic donor, with large islets containing many beta cells, severe infiltration of the exocrine pancreas with myeloid cells but limited infiltration of islets.
- 6414 is a donor with recent T1D onset (shortly after diagnosis) showing partial beta cell destruction and mild infiltration of islets with T cells.
- 6180 is a donor with long-duration T1D (11 years after diagnosis), showing near-total beta cell destruction and limited immune cell infiltration in both the islets and the pancreas.

File sizes:

- `DamondPancreas2019\_images`: size in memory = 7.4 Gb, size on disk = 1780 Mb.
- `DamondPancreas2019\_masks`: size in memory = 200.0 Mb, size on disk = 8.6 Mb.
- `DamondPancreas2019\_sce`: size in memory = 248.6 Mb, size on disk = 145 Mb.

Original source: Damond et al. (2019): https://doi.org/10.1016/j.cmet.2018.11.014

Original link to raw data, also containing the entire dataset: https://data.mendeley.com/datasets/cydmwsfztj/2

#### Value

Returns a SingleCellExperiment or CytoImageList object.

#### Author(s)

Nicolas Damond

#### Source

Publication Original dataset

#### References

Damond N et al. (2019). A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. *Cell Metab* 29(3), 755-768.

#### Examples

```
sce <- DamondPancreas2019_sce()
images <- DamondPancreas2019_images()
masks <- DamondPancreas2019_masks()</pre>
```

DamondPancreas2019Data

Obtain the damond-pancreas-2019 dataset

#### Description

Obtain the damond-pancreas-2019 dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry of human pancreas sections from donors with type 1 diabetes.

#### Usage

```
DamondPancreas2019Data(
   data_type = c("sce", "images", "masks"),
   on_disk = FALSE,
   h5FilesPath = NULL,
   force = FALSE
)
```

#### Arguments

data_type	type of data to load, should be 'sce' for single cell data, 'images' for multichan- nel images or 'masks' for cell segmentation masks.
on_disk	logical indicating if images in form of HDF5Array objects (as .h5 files) should be stored on disk rather than in memory. This setting is valid when downloading images and masks.
h5FilesPath	path to where the .h5 files for on disk representation are stored. This path needs to be defined when on_disk = TRUE. When files should only temporarily be stored on disk, please set h5FilesPath = getHDF5DumpDir()
force	logical indicating if images should be overwritten when files with the same name already exist on disk.

#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Damond et al. (2019), consisting of three data objects:

- DamondPancreas2019\_images contains a hundred 38-channel images in the form of a CytoImageList class object.
- DamondPancreas2019\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.

• DamondPancreas2019\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment. This represents a total of 252,059 cells x 38 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/DamondPancreas2019\_sce, imcdatasets/DamondPancreas2019\_images, or imcdatasets/DamondPancreas2019\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageName or ImageNumber variables. Mapping between cell segmentation masks and single cell data is performed with the CellNumber variable, the values of which correspond to the intensity values of the DamondPancreas2019\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset is a subset of the complete Damond et al. (2019) dataset comprising the data from three pancreas donors at different stages of type 1 diabetes (T1D). The three donors present clearly diverging characteristics in terms of cell type composition and cell-cell interactions, which makes this dataset ideal for benchmarking spatial and neighborhood analysis algorithms.

The assay slot of the SingleCellExperiment object contains two assays:

- counts contains mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object.

The cell-associated metadata are stored in the colData of the SingleCellExperiment object. These metadata include cell types (in colData(sce)\$CellType) and broader cell categories, such as "immune" or "islet" cells (in colData(sce)\$CellCat). In addition, for cells located inside pancreatic islets, the islet they belong to is indicated in colData(sce)\$ParentIslet. For cells not located in islets, the "ParentIslet" value is set to 0 but the spatially closest islet can be identified with colData(sce)\$ClosestIslet.

The donor-associated metadata are also stored in the colData of the SingleCellExperiment object. For instance, the donors' IDs can be retrieved with colData(sce)\$case and the donors' disease stage can be obtained with colData(sce)\$stage.

The three donors present the following characteristics:

- 6126 is a non-diabetic donor, with large islets containing many beta cells, severe infiltration of the exocrine pancreas with myeloid cells but limited infiltration of islets.
- 6414 is a donor with recent T1D onset (shortly after diagnosis) showing partial beta cell destruction and mild infiltration of islets with T cells.
- 6180 is a donor with long-duration T1D (11 years after diagnosis), showing near-total beta cell destruction and limited immune cell infiltration in both the islets and the pancreas.

File sizes:

- `DamondPancreas2019\_images`: size in memory = 7.4 Gb, size on disk = 1780 Mb.
- `DamondPancreas2019\_masks`: size in memory = 200.0 Mb, size on disk = 8.6 Mb.
- `DamondPancreas2019\_sce`: size in memory = 248.6 Mb, size on disk = 145 Mb.

When storing images on disk, these need to be first fully read into memory before writing them to disk. This means the process of downloading the data is slower than directly keeping them in memory. However, downstream analysis will lose its memory overhead when storing images on disk.

Original source: Damond et al. (2019): https://doi.org/10.1016/j.cmet.2018.11.014

Original link to raw data, also containing the entire dataset: https://data.mendeley.com/datasets/cydmwsfztj/2

#### Value

A SingleCellExperiment object with single cell data, a CytoImageList object containing multichannel images, or a CytoImageList object containing cell masks.

#### Author(s)

Nicolas Damond

#### References

Damond N et al. (2019). A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. *Cell Metab* 29(3), 755-768.

#### Examples

```
sce <- DamondPancreas2019Data(data_type = "sce")
images <- DamondPancreas2019Data(data_type = "images")
masks <- DamondPancreas2019Data(data_type = "masks")</pre>
```

JacksonFischer2020 'JacksonFischer2020' dataset

#### Description

This dataset consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry of tumour tissue from patients with breast cancer.

#### Usage

```
JacksonFischer2020_sce(metadata = FALSE)
JacksonFischer2020_images(metadata = FALSE)
JacksonFischer2020_masks(metadata = FALSE)
```

#### Arguments

metadata

logical value indicating whether ExperimentHub metadata (describing the overall dataset) should be returned only, or if the whole dataset should be loaded. Default = FALSE, which loads the whole dataset.

#### JacksonFischer2020

#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Jackson, Fischer et al. (2020), consisting of three data objects:

- JacksonFischer2020\_images contains a hundred 42-channel images in the form of a CytoImageList class object.
- JacksonFischer2020\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- JacksonFischer2020\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment. This represents a total of 285,851 cells x 42 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/JacksonFischer2020\_sce, imcdatasets/JacksonFischer2020\_images, or imcdatasets/JacksonFischer2020\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageNb variable. Mapping between cell segmentation masks and single cell data is performed with the CellNb variable, the values of which correspond to the intensity values of the JacksonFischer2020\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset is a subset of the complete Jackson, Fischer et al. (2020) dataset comprising the data from tumour tissue from 100 patients with breast cancer (one image per patient).

The assay slot of the SingleCellExperiment object contains three assays:

- counts contains mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.
- quant\_norm contains quantile-normalized counts (0 to 1, 99th percentile).

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object.

The cell-associated metadata are stored in the colData of the SingleCellExperiment object. These metadata include clusters (in colData(sce)\$PhenoGraphBasel) and metaclusters (in colData(sce)\$metacluster), as well as spatial information (e.g., cell areas are stored in colData(sce)\$Area).

The patient-associated clinical data are also stored in the colData of the SingleCellExperiment object. For instance, the tumor grades can be retrieved with colData(sce)\$grade. File sizes:

- JacksonFischer2020\_images`: size in memory = 17.8 Gb, size on disk = 1996 Mb.
- JacksonFischer2020\_masks: size in memory = 433 Mb, size on disk = 10.2 Mb.
- JacksonFischer2020\_sce: size in memory = 517 Mb, size on disk = 272 Mb.

Original source: Jackson, Fischer et al. (2020): https://doi.org/10.1038/s41586-019-1876-x

Original link to raw data, also containing the entire dataset: https://doi.org/10.5281/zenodo.3518284

#### Value

Returns a SingleCellExperiment object with single cell data, a CytoImageList object containing multichannel images, or a CytoImageList object containing cell masks.

#### Author(s)

Jana Fischer

#### Source

Publication Original dataset

#### References

Jackson, Fischer et al. (2020). The single-cell pathology landscape of breast cancer. *Nature* 578(7796), 615-620.

#### Examples

```
sce <- JacksonFischer2020_sce()
images <- JacksonFischer2020_images()
masks <- JacksonFischer2020_masks()</pre>
```

JacksonFischer2020Data

Obtain the jackson-fischer-2020 dataset

#### Description

Obtain the jackson-fischer-2020 dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry of tumour tissue from patients with breast cancer.

#### Usage

```
JacksonFischer2020Data(
   data_type = c("sce", "images", "masks"),
   on_disk = FALSE,
   h5FilesPath = NULL,
   force = FALSE
)
```

#### Arguments

data_type	type of data to load, should be 'sce' for single cell data, 'images' for multichan- nel images or 'masks' for cell segmentation masks.
on_disk	logical indicating if images in form of HDF5Array objects (as .h5 files) should be stored on disk rather than in memory. This setting is valid when downloading images and masks.
h5FilesPath	path to where the .h5 files for on disk representation are stored. This path needs to be defined when on_disk = TRUE. When files should only temporarily be stored on disk, please set h5FilesPath = getHDF5DumpDir()
force	logical indicating if images should be overwritten when files with the same name already exist on disk.

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#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Jackson, Fischer et al. (2020), consisting of three data objects:

- JacksonFischer2020\_images contains a hundred 42-channel images in the form of a CytoImageList class object.
- JacksonFischer2020\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- JacksonFischer2020\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment. This represents a total of 285,851 cells x 42 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/JacksonFischer2020\_sce, imcdatasets/JacksonFischer2020\_images, or imcdatasets/JacksonFischer2020\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageNb variable. Mapping between cell segmentation masks and single cell data is performed with the CellNb variable, the values of which correspond to the intensity values of the JacksonFischer2020\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset is a subset of the complete Jackson, Fischer et al. (2020) dataset comprising the data from tumour tissue from 100 patients with breast cancer (one image per patient).

The assay slot of the SingleCellExperiment object contains three assays:

- counts contains mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.
- quant\_norm contains quantile-normalized counts (0 to 1, 99th percentile).

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object.

The cell-associated metadata are stored in the colData of the SingleCellExperiment object. These metadata include clusters (in colData(sce)\$PhenoGraphBasel) and metaclusters (in colData(sce)\$metacluster), as well as spatial information (e.g., cell areas are stored in colData(sce)\$Area).

The patient-associated clinical data are also stored in the colData of the SingleCellExperiment object. For instance, the tumor grades can be retrieved with colData(sce)\$grade.

File sizes:

- `JacksonFischer2020\_images`: size in memory = 17.8 Gb, size on disk = 1996 Mb.
- JacksonFischer2020\_masks: size in memory = 433 Mb, size on disk = 10.2 Mb.
- JacksonFischer2020\_sce : size in memory = 517 Mb, size on disk = 272 Mb.

When storing images on disk, these need to be first fully read into memory before writing them to disk. This means the process of downloading the data is slower than directly keeping them in memory. However, downstream analysis will lose its memory overhead when storing images on disk.

Original source: Jackson, Fischer et al. (2020): https://doi.org/10.1038/s41586-019-1876-x

Original link to raw data, containing the entire dataset: https://doi.org/10.5281/zenodo.3518284

listDatasets

#### Value

A SingleCellExperiment object with single cell data, a CytoImageList object containing multichannel images, or a CytoImageList object containing cell masks.

#### Author(s)

Jana Fischer

#### References

Jackson, Fischer et al. (2020). The single-cell pathology landscape of breast cancer. *Nature* 578(7796), 615-620.

#### Examples

```
sce <- JacksonFischer2020Data(data_type = "sce")
images <- JacksonFischer2020Data(data_type = "images")
masks <- JacksonFischer2020Data(data_type = "masks")</pre>
```

listDatasets

Summary information for all available datasets in the **imcdatasets** package.

List all available datasets

#### Usage

Description

listDatasets()

#### Details

Each dataset contains single-cell data, multichannel images and cell segmentation masks.

#### Value

A DataFrame where each row corresponds to a dataset, containing the fields:

- Reference, a Markdown-formatted citation to scripts/ref.bib in the **imcdatasets** installation directory.
- Species, species of origin.
- Tissue, the tissue that was imaged.
- NumberOfCells, the total number of cells in the dataset.
- NumberOfImages, the total number of images in the dataset.
- NumberOfChannels, the number of channels per image.
- FunctionCall, the R function call required to construct the dataset.

#### Examples

listDatasets()

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ZanotelliSpheroids2020

'ZanotelliSpheroids2020' dataset

#### Description

This dataset consists consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data were obtained by imaging mass cytometry of sections of 3D spheroids generated from different cell lines.

#### Usage

```
ZanotelliSpheroids2020_sce(metadata = FALSE)
ZanotelliSpheroids2020_images(metadata = FALSE)
ZanotelliSpheroids2020_masks(metadata = FALSE)
```

#### Arguments

metadata	logical value indicating whether ExperimentHub metadata (describing the over-
	all dataset) should be returned only, or if the whole dataset should be loaded.
	Default = FALSE, which loads the whole dataset.

#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Zanotelli et al. (2020), consisting of three data objects:

- ZanotelliSpheroids2020\_images contains 517 multichannel images, each containing 51 channels, in the form of a CytoImageList class object.
- ZanotelliSpheroids2020\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- ZanotelliSpheroids2020\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCell-Experiment. This represents a total of 229,047 cells x 51 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/ZanotelliSpheroids2020\_sce, imcdatasets/ZanotelliSpheroids2020\_in or imcdatasets/ZanotelliSpheroids2020\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageName or ImageNumber variables. Mapping between cell segmentation masks and single cell data is performed with the CellNumber variable, the values of which correspond to the intensity values of the ZanotelliSpheroids2020\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset was obtained as following (the names of the experimental variables, located in the colData of the SingleCellExperiment object, are indicated in parentheses): *i*) Cells from four different cell lines (cellline) were seeded at three different densities (concentration, relative densities) and grown for either 72 or 96 hours (time\_point, duration in hours). In the appropriate experimental conditions (see the paper for details), the cells aggregate into 3D spheroids. *ii*) Cells were harvested and pooled into 60-well barcoding plates. *iii*) A pellet of each spheroid pool was

generated and cut into several 6 um-thick sections. iv) A subset of these sections (site\_id) were stained with an IMC panel and acquired as one or more acquisitions (acquisition\_id) containing multiple spheres each. v) Spheres in these acquisitions were identified by computer vision and cropped into individual images (ImageNumber).

Other relevant cell metadata include:

- condition\_name: experimental conditions in the format: "Cell line name"\_c"seeding density"\_tp"time
  point".
- Center\_X/Y: object centroid position in image.
- Area: area of the cell (um<sup>2</sup>).
- dist.rim: estimated distance to spheroid border.
- dist.sphere: distance to spheroid section border.
- dist.other: distance to the closest of the other spheroid sections in the same image (if there is any).
- dist.bg: distance to background pixels.
- counts\_neighb: contains arsinh-transformed counts, with cofactor 1.
- exprs\_neighb: contains arsinh-transformed counts, with cofactor 1.

For a full description of the other experimental variables, please refer to the publication (https://doi.org/10.15252/msb.202 and to the original dataset repository (https://doi.org/10.5281/zenodo.4271910).

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object. The channels with names starting with "BC\_" are the channels used for barcoding. Post-transcriptional modification of the protein targets are indicated in brackets.

The assay slot of the SingleCellExperiment object contains four assays:

- counts: mean ion counts per cell.
- exprs: arsinh-transformed counts per cell, with cofactor 1.
- counts\_neighb: mean ion counts of the neighboring cells.
- exprs\_neighb: arsinh-transformed counts (cofactor 1) of the neighboring cells.

The metadata slot of the SingleCellExperiment object contains a graph of cell neighbors, generated with the igraph::graph\_from\_data\_frame function. File sizes:

- `ZanotelliSpheroids2020\_images`: size in memory = 21.2 Gb, size on disk = 881 Mb.
- `ZanotelliSpheroids2020\_masks`: size in memory = 426 Mb, size on disk = 11.6 Mb.
- `ZanotelliSpheroids2020\_sce`: size in memory = 584 Mb, size on disk = 340 Mb.

Original source: Zanotelli et al. (2020): https://doi.org/10.15252/msb.20209798

Original link to raw data, also containing the entire dataset: https://doi.org/10.5281/zenodo.4271910

#### Value

Returns a SingleCellExperiment or CytoImageList object.

#### Author(s)

Nicolas Damond

#### Source

Publication Original dataset

#### References

Zanotelli VRT et al. (2020). A quantitative analysis of the interplay of environment, neighborhood, and cell state in 3D spheroids *Mol Syst Biol* 16(12), e9798.

#### Examples

```
sce <- ZanotelliSpheroids2020_sce()
images <- ZanotelliSpheroids2020_images()
masks <- ZanotelliSpheroids2020_masks()</pre>
```

ZanotelliSpheroids2020Data

Obtain the zanotelli-spheroids-2020 dataset

#### Description

Obtain the zanotelli-spheroids-2020 dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data were obtained by imaging mass cytometry of sections of 3D spheroids generated from different cell lines.

#### Usage

```
ZanotelliSpheroids2020Data(
   data_type = c("sce", "images", "masks"),
   on_disk = FALSE,
   h5FilesPath = NULL,
   force = FALSE
)
```

#### Arguments

data_type	type of data to load, should be sce for single cell data, images for multichannel images or masks for cell segmentation masks.
on_disk	logical indicating if images in form of HDF5Array objects (as .h5 files) should be stored on disk rather than in memory. This setting is valid when downloading images and masks.
h5FilesPath	path to where the .h5 files for on disk representation are stored. This path needs to be defined when on_disk = TRUE. When files should only temporarily be stored on disk, please set h5FilesPath = getHDF5DumpDir()
force	logical indicating if images should be overwritten when files with the same name already exist on disk.

#### Details

This is an Imaging Mass Cytometry (IMC) dataset from Zanotelli et al. (2020), consisting of three data objects:

- ZanotelliSpheroids2020\_images contains 517 multichannel images, each containing 51 channels, in the form of a CytoImageList class object.
- ZanotelliSpheroids2020\_masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- ZanotelliSpheroids2020\_sce contains the single cell data extracted from the images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCell-Experiment. This represents a total of 229,047 cells x 51 channels.

All data are downloaded from ExperimentHub and cached for local re-use. Specific resources can be retrieved by searching for imcdatasets/ZanotelliSpheroids2020\_sce, imcdatasets/ZanotelliSpheroids2020\_in or imcdatasets/ZanotelliSpheroids2020\_masks.

Mapping between the three data objects is performed via variables located in their metadata columns: mcols() for the CytoImageList objects and ColData() for the SingleCellExperiment object. Mapping at the image level can be performed with the ImageName or ImageNumber variables. Mapping between cell segmentation masks and single cell data is performed with the CellNumber variable, the values of which correspond to the intensity values of the ZanotelliSpheroids2020\_masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This dataset was obtained as following (the names of the experimental variables, located in the colData of the SingleCellExperiment object, are indicated in parentheses): *i*) Cells from four different cell lines (cellline) were seeded at three different densities (concentration, relative densities) and grown for either 72 or 96 hours (time\_point, duration in hours). In the appropriate experimental conditions (see the paper for details), the cells aggregate into 3D spheroids. *ii*) Cells were harvested and pooled into 60-well barcoding plates. *iii*) A pellet of each spheroid pool was generated and cut into several 6 um-thick sections. *iv*) A subset of these sections (site\_id) were stained with an IMC panel and acquired as one or more acquisitions (acquisition\_id) containing multiple spheres each. *v*) Spheres in these acquisitions were identified by computer vision and cropped into individual images (ImageNumber).

Other relevant cell metadata include:

- condition\_name: experimental conditions in the format: "Cell line name"\_c"seeding density"\_tp"time point".
- Center\_X/Y: object centroid position in image.
- Area: area of the cell (um<sup>2</sup>).
- dist.rim: estimated distance to spheroid border.
- dist.sphere: distance to spheroid section border.
- dist.other: distance to the closest of the other spheroid sections in the same image (if there is any).
- dist.bg: distance to background pixels.
- counts\_neighb: contains arsinh-transformed counts, with cofactor 1.
- exprs\_neighb: contains arsinh-transformed counts, with cofactor 1.

For a full description of the other experimental variables, please refer to the publication (https://doi.org/10.15252/msb.202 and to the original dataset repository (https://doi.org/10.5281/zenodo.4271910).

The marker-associated metadata, including antibody information and metal tags are stored in the rowData of the SingleCellExperiment object. The channels with names starting with "BC\_" are the

channels used for barcoding. Post-transcriptional modification of the protein targets are indicated in brackets.

The assay slot of the SingleCellExperiment object contains four assays:

- counts: mean ion counts per cell.
- exprs: arsinh-transformed counts per cell, with cofactor 1.
- counts\_neighb: mean ion counts of the neighboring cells.
- exprs\_neighb: arsinh-transformed counts (cofactor 1) of the neighboring cells.

The metadata slot of the SingleCellExperiment object contains a graph of cell neighbors, generated with the igraph::graph\_from\_data\_frame function.

File sizes:

- ZanotelliSpheroids2020\_images: size in memory = 21.2 Gb, size on disk = 881 Mb.
- `ZanotelliSpheroids2020\_masks`: size in memory = 426 Mb, size on disk = 11.6 Mb.
- ZanotelliSpheroids2020\_sce: size in memory = 584 Mb, size on disk = 340 Mb.

When storing images on disk, these need to be first fully read into memory before writing them to disk. This means the process of downloading the data is slower than directly keeping them in memory. However, downstream analysis will lose its memory overhead when storing images on disk.

Original source: Zanotelli et al. (2020): https://doi.org/10.15252/msb.20209798

Original link to raw data, also containing the entire dataset: https://doi.org/10.5281/zenodo.4271910

#### Value

A SingleCellExperiment object with single cell data, a CytoImageList object containing multichannel images, or a CytoImageList object containing cell segmentation masks.

#### Author(s)

Nicolas Damond

#### References

Zanotelli VRT et al. (2020). A quantitative analysis of the interplay of environment, neighborhood, and cell state in 3D spheroids *Mol Syst Biol* 16(12), e9798.

#### Examples

```
sce <- ZanotelliSpheroids2020Data(data_type = "sce")
images <- ZanotelliSpheroids2020Data(data_type = "images")
masks <- ZanotelliSpheroids2020Data(data_type = "masks")</pre>
```

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