# 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.

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NeedsCompilation no

**Depends** R (>= 4.3.0), SingleCellExperiment, SpatialExperiment, cytomapper,

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

Suggests BiocStyle, knitr, rmarkdown, markdown, testthat

**biocViews** ExperimentData, ExperimentHub, SingleCellData, SpatialData, Homo\_sapiens\_Data, ImmunoOncologyData, TechnologyData, PackageTypeData, ReproducibleResearch, Tissue

VignetteBuilder knitr

URL https://github.com/BodenmillerGroup/imcdatasets

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

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Author Nicolas Damond [aut, cre] (<a href="https://orcid.org/0000-0003-3027-8989">https://orcid.org/0000-0002-4711-1176</a>),
Fischer Jana [ctb] (<a href="https://orcid.org/0000-0001-9047-8400">https://orcid.org/0000-0001-9047-8400</a>)

Maintainer Nicolas Damond <nicolas.damond@dqbm.uzh.ch>

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DamondPancreas2019-defunct

 $Defunct \ function \ - \ 'Damond Pancreas 2019' \ dataset$ 

## Description

These functions are defunct and no longer available.

#### **Details**

Defunct functions are: DamondPancreas2019\_sce, DamondPancreas2019\_masks, and DamondPancreas2019\_images. Please use the Damond\_2019\_Pancreas function instead.

DamondPancreas2019Data-defunct

Defunct function - 'DamondPancreas2019Data' dataset

#### **Description**

This function is defunct and no longer available.

#### **Details**

This function is defunct, please use the Damond\_2019\_Pancreas function instead.

Damond\_2019\_Pancreas Obtain the Damond\_2019\_Pancreas dataset

## **Description**

Obtain the Damond\_2019\_Pancreas dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry (IMC) of human pancreas sections from donors with type 1 diabetes.

#### Usage

```
Damond_2019_Pancreas(
  data_type = c("sce", "spe", "images", "masks"),
  full_dataset = FALSE,
  version = "latest",
  metadata = FALSE,
  on_disk = FALSE,
  h5FilesPath = NULL,
  force = FALSE
)
```

## Arguments

data_type	type of object to load, 'images' for multichannel images or 'masks' for cell segmentation masks. Single cell data are retrieved using either 'sce' for the SingleCellExperiment format or 'spe' for the SpatialExperiment format.
full_dataset	if FALSE (default), a subset corresponding to 100 images is returned. If TRUE, the full dataset (corresponding to 845 images) is returned. Due to memory space limitations, this option is only available for single cell data and masks, not for data_type = "images".
version	dataset version. By default, the latest version is returned.
metadata	if FALSE (default), the data object selected in data_type is returned. If TRUE, only the metadata associated to this object is returned.

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):

- images contains a hundred 38-channel images in the form of a CytoImageList class object.
- masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- sce contains the single cell data extracted from the multichannel 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.
- spe same single cell data as for sce, but in the SpatialExperiment format.

All data are downloaded from ExperimentHub and cached for local re-use.

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 and SpatialExperiment objects. Mapping at the image level can be performed with the image\_name or image\_number variables. Mapping between cell segmentation masks and single cell data is performed with the cell\_number variable, the values of which correspond to the intensity values of the 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. If full\_dataset = TRUE, the full dataset (845 images from 12 patients) is returned. This option is not available for multichannel images.

The assay slots of the SingleCellExperiment and SpatialExperiment objects contain three assays:

- counts contains raw mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.
- quant\_norm contains counts censored at the 99th percentile and scaled 0-1.

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

The cell-associated metadata are stored in the colData of the SingleCellExperiment and Spatial-Experiment objects. These metadata include cell types (in colData(sce)\$cell\_type) and broader cell categories, such as "immune" or "islet" cells (in colData(sce)\$cell\_category). In addition, for cells located inside pancreatic islets, the islet they belong to is indicated in colData(sce)\$islet\_parent.

For cells not located in islets, the "islet\_parent" value is set to 0 but the spatially closest islet can be identified with colData(sce)\$islet\_closest.

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

Neighborhood information, defined here as cells that are localized next to each other, is stored as a SelfHits object in the colPairs slot of the SingleCellExperiment and SpatialExperiment objects.

The three donors in the subset 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.

For information about other donors in the full dataset, please refer to the Damond et al. publication.

Dataset versions: a version argument can be passed to the function to specify which dataset version should be retrieved.

- `v0`: original version (Bioconductor <= 3.15).
- `v1`: consistent object formatting across datasets.

#### File sizes:

- 'images': size in memory = 7.4 Gb, size on disk = 1.7 Gb.
- `masks`: size in memory = 200 Mb, size on disk = 8.2 Mb.
- `sce`: size in memory = 353 Mb, size on disk = 204 Mb.
- `spe`: size in memory = 372 Mb, size on disk = 205 Mb.
- `sce\_full`: size in memory = 2.4 Gb, size on disk = 1.5 Gb.
- `spe\_full`: size in memory = 2.5 Gb, size on disk = 1.5 Gb.
- masks\_full: size in memory = 1.4 Gb, size on disk = 60 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 SpatialExperiment object with single cell data, a CytoImageList object containing multichannel images, or a CytoImageList object containing cell segmentation 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**

```
# Load single cell data
sce <- Damond_2019_Pancreas(data_type = "sce")
print(sce)

# Display metadata
Damond_2019_Pancreas(data_type = "sce", metadata = TRUE)

# Load masks on disk
library(HDF5Array)
masks <- Damond_2019_Pancreas(data_type = "masks", on_disk = TRUE,
h5FilesPath = getHDF5DumpDir())
print(head(masks))</pre>
```

HochSchulz\_2022\_Melanoma

Obtain the HochSchulz\_2022\_Melanoma dataset

#### **Description**

Obtain the HochSchulz\_2022\_Melanoma dataset, which is composed of two panels (rna and protein) that were acquired on consecutive sections. Each dataset (panel) is composed of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry (IMC) of a tissue microarray (TMA) with multiple cores of formalin-fixed paraffin-embedded (FFPE) tissue from 69 patients with metastatic melanoma.

#### Usage

```
HochSchulz_2022_Melanoma(
  data_type = c("sce", "spe", "images", "masks"),
  panel = "rna",
  full_dataset = FALSE,
  version = "latest",
  metadata = FALSE,
  on_disk = FALSE,
  h5FilesPath = NULL,
  force = FALSE
)
```

#### **Arguments**

data_type	type of object to load, 'images' for multichannel images or 'masks' for cell segmentation masks. Single cell data are retrieved using either 'sce' for the SingleCellExperiment format or 'spe' for the SpatialExperiment format.
panel	which panel should be returned? Can be set to "rna" (default) or "protein".
full_dataset	if FALSE (default), a subset corresponding to the 50 images containing the most B cells is returned. If TRUE, the full dataset (corresponding to 166 images) is returned. Due to memory space limitations, this option is only available for single cell data and masks, not for data_type = "images".
version	dataset version. By default, the latest version is returned.
metadata	if FALSE (default), the data object selected in data_type is returned. If TRUE, only the metadata associated to this object is returned.
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 Hoch, Schulz et al. (2022):

- images contains fifty 38-channel images in the form of a CytoImageList class object.
- masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- sce contains the single cell data extracted from the multichannel images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment object.
- spe same single cell data as for sce, but in the SpatialExperiment format.

All data are downloaded from ExperimentHub and cached for local re-use.

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 and SpatialExperiment objects. Mapping at the image level can be performed with the image\_name or image\_number variables. Mapping between cell segmentation masks and single cell data is performed with the cell\_number variable, the values of which correspond to the intensity values of the masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

The assay slots of the SingleCellExperiment and SpatialExperiment objects contain three assays:

- counts contains raw mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.
- scaled\_counts contains scaled counts.

• scaled\_exprs contains scaled asinh-transformed counts.

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

The cell-associated metadata are stored in the colData of the SingleCellExperiment and Spatial-Experiment objects. These metadata include various information about cells, milieu, samples, and patients. For instance, cell types can be retrieved with colData(sce)\$cell\_type and cell clusters with colData(sce)\$cell\_cluster.

Neighborhood information, defined here as cells that are localized next to each other, is stored as a SelfHits object in the colPairs slot of the SingleCellExperiment and SpatialExperiment objects.

For more information, please refer to the Hoch, Schulz, et al. publication.

Dataset versions: a version argument can be passed to the function to specify which dataset version should be retrieved.

• `v1`: first published version

#### File sizes:

- `images\_rna`: size in memory = 13.9 Gb, size on disk = 954 Mb.
- `masks\_rna`: size in memory = 347 Mb, size on disk = 11 Mb.
- 'sce\_rna': size in memory = 774 Mb, size on disk = 401 Mb.
- `masks\_full\_rna`: size in memory = 1.1 Gb, size on disk = 30 Mb.
- `sce\_full\_rna`: size in memory = 2.0 Gb, size on disk = 1.1 Gb.
- `images\_protein`: size in memory = 16.8 Gb, size on disk = 1.2 Gb.
- `masks\_protein`: size in memory = 374 Mb, size on disk = 12 Mb.
- 'sce\_protein': size in memory = 856 Mb, size on disk = 531 Mb.
- `masks\_full\_protein`: size in memory = 1.2 Gb, size on disk = 35 Mb.
- `sce\_full\_protein`: size in memory = 2.2 Gb, size on disk = 1.4 Gb.

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: Hoch, Schulz et al. (2022): https://doi.org/10.1126/sciimmunol.abk1692

Original link to raw data: https://doi.org/10.5281/zenodo.5994136.

#### Value

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

## Author(s)

Nicolas Damond

#### References

Hoch, Schulz et al. (2022). Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the response to immunotherapy *Sci Immunol* 7(70):eabk1692.

## **Examples**

```
# Load single cell data
sce <- HochSchulz_2022_Melanoma(data_type = "sce")
print(sce)

# Display metadata
HochSchulz_2022_Melanoma(data_type = "sce", metadata = TRUE)

# Load masks on disk
library(HDF5Array)
masks <- HochSchulz_2022_Melanoma(data_type = "masks", on_disk = TRUE,
h5FilesPath = getHDF5DumpDir())
print(head(masks))</pre>
```

IMMUcan\_2022\_CancerExample

Obtain the IMMUcan\_2022\_CancerExample dataset

## **Description**

Obtain the IMMUcan\_2022\_CancerExample dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. Data were obtained by imaging mass cytometry (IMC) of sections of 4 patients with different tumor indications.

#### Usage

```
IMMUcan_2022_CancerExample(
  data_type = c("sce", "spe", "images", "masks"),
  version = "latest",
  metadata = FALSE,
  on_disk = FALSE,
  h5FilesPath = NULL,
  force = FALSE
)
```

#### Arguments

data\_type type of object to load, 'images' for multichannel images or 'masks' for cell segmentation masks. Single cell data are retrieved using either 'sce' for the SingleCellExperiment format or 'spe' for the SpatialExperiment format.

version dataset version. By default, the latest version is returned.

metadata	if FALSE (default), the data object selected in data_type is returned. If TRUE, only the metadata associated to this object is returned.
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

#### **Details**

This is an Imaging Mass Cytometry (IMC) dataset used in the IMC data analysis book

already exist on disk.

- images contains 14 multichannel images, each containing 50 channels, in the form of a CytoImageList class object.
- masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- sce contains the single cell data extracted from the multichannel images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment object. Single cell data can also be retrieved as a SpatialExperiment object. This represents a total of 46,825 cells x 40 channels.

All data are downloaded from ExperimentHub and cached for local re-use.

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 sample\_id or image\_name variables. Mapping between cell segmentation masks and single cell data is performed with the cell\_number variable, the values of which correspond to the intensity values of the masks object. For practical examples, please refer to the "Accessing IMC datasets" vignette.

This imaging mass cytometry dataset serves as an example to demonstrate downstream analysis tools including spatial data analysis. The data was generated as part of the Integrated iMMUnoprofiling of large adaptive CANcer patient cohorts (IMMUcan) project (immucan.eu) using the Hyperion imaging system.

Relevant entries to the colData slot are as follows:

- sample\_id image name.
- cell\_number cell identifier.
- width\_px width of the image.
- height\_px height of the image.
- patient\_id patient identifier.
- ROI region of interest identifier.
- indication cancer type.
- cell\_labels labels of manually labelled cells.

- cell\_type cell type as defined by classification.
- spatial\_community identifiers of each spatial tumor or non-tumor community
- cn\_celltypes cellular neighborhoods as defined by clustering cells based on the frequency of neighboring cell types.
- cn\_expression cellular neighborhoods as defined by clustering cells based on the mean expression of neighboring cells
- lisa\_clusters cellular neighborhoods as detected by the lisaClust package.
- spatial\_context spatial contexts defined in cn\_celltype.
- spatial\_context\_filtered filtered spatial context identifiers.
- patch\_id identifier of the spatial tumor patch.
- cell\_x spatial x coordinate.
- cell\_y spatial y coordinate.

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

The assay slot of the SingleCellExperiment object contains two assays:

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

The colPair slot of the SingleCellExperiment object contains the following spatial object graphs:

- neighborhood steinbock generated graph.
- knn\_interaction\_graph 20-nearest neighbor graph.
- expansion\_interaction\_graph expansion graph using a threshold of 20.
- delaunay\_interaction\_graph interaction graph constructed by delaunay triangulation.
- knn\_spatialcontext\_graph 40-nearest neighbor graph.

## File sizes:

- `images`: size in memory = 1.5 Gb, size on disk = 786 Mb.
- `masks`: size in memory = 19 Mb, size on disk = 1.2 Mb.
- `sce`: size in memory = 182 Mb, size on disk = 82 Mb.
- `spe`: size in memory = 183 Mb, size on disk = 81 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.

## Value

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

#### Author(s)

Nils Eling

## **Examples**

```
# Load single cell data
sce <- IMMUcan_2022_CancerExample(data_type = "sce")
print(sce)

# Display metadata
IMMUcan_2022_CancerExample(data_type = "sce", metadata = TRUE)

# Load masks on disk
library(HDF5Array)
masks <- IMMUcan_2022_CancerExample(data_type = "masks", on_disk = TRUE,
h5FilesPath = getHDF5DumpDir())
print(head(masks))</pre>
```

JacksonFischer2020-defunct

Defunct function - 'JacksonFischer2020' dataset

## **Description**

These functions are defunct and no longer available.

#### **Details**

Defunct functions are: JacksonFischer2020\_sce, JacksonFischer2020\_masks, and JacksonFischer2020\_images. Please use the JacksonFischer\_2020\_BreastCancer function instead.

```
JacksonFischer2020Data-defunct
```

Defunct function - 'JacksonFischer2020Data' dataset

## Description

This function is defunct and no longer available.

#### **Details**

This function is defunct, please use the JacksonFischer\_2020\_BreastCancer function instead.

```
JacksonFischer_2020_BreastCancer
```

Obtain the JacksonFischer\_2020\_BreastCancer dataset

## Description

Obtain the JacksonFischer\_2020\_BreastCancer dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data was obtained by imaging mass cytometry (IMC) of tumour tissue from patients with breast cancer.

## Usage

```
JacksonFischer_2020_BreastCancer(
  data_type = c("sce", "spe", "images", "masks"),
  full_dataset = FALSE,
  cohort = "Basel",
  version = "latest",
  metadata = FALSE,
  on_disk = FALSE,
  h5FilesPath = NULL,
  force = FALSE
)
```

## **Arguments**

data_type	type of object to load, 'images' for multichannel images or 'masks' for cell segmentation masks. Single cell data are retrieved using either 'sce' for the SingleCellExperiment format or 'spe' for the SpatialExperiment format.
full_dataset	if FALSE (default), a subset corresponding to 100 images is returned. If TRUE, the full dataset is returned, including both "Basel" and "Zurich" cohorts. Due to memory space limitations, this option is only available for single cell data and masks, not for data_type = "images".
cohort	which patient cohort should be returned? Can be set to "Basel" (default) or "Zurich". Ignored if full_dataset is set to TRUE.
version	dataset version. By default, the latest version is returned.
metadata	if FALSE (default), the data object selected in data_type is returned. If TRUE, only the metadata associated to this object is returned.
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 Jackson, Fischer et al. (2020):

- images contains a hundred 42-channel images in the form of a CytoImageList class object.
- masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- sce contains the single cell data extracted from the multichannel 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.
- spe same single cell data as for sce, but in the SpatialExperiment format.

All data are downloaded from ExperimentHub and cached for local re-use.

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 and Spatial-Experiment objects. Mapping at the image level can be performed with the image\_name variable. Mapping between cell segmentation masks and single cell data is performed with the cell\_number variable, the values of which correspond to the intensity values of the 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). By default, data from the "Basel" cohort are returned. By setting cohort = "Zurich", data from the "Zurich" cohort, corresponding to images and associated data from 72 patients, are returned. For details about the patient cohorts, refer to the publication. If full\_dataset = TRUE, the full dataset is returned (including both "Basel" and "Zurich" patient cohorts). This option is not available for multichannel images.

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 and SpatialExperiment objects.

The cell-associated metadata are stored in the colData of the SingleCellExperiment and SpatialExperiment objects. These metadata include clusters (in colData(sce)\$cell\_cluster\_phenograph) and metaclusters (in colData(sce)\$cell\_metacluster), as well as spatial information (e.g., cell areas are stored in colData(sce)\$cell\_area).

The clinical data are also stored in the colData of the SingleCellExperiment and SpatialExperiment objects. For instance, the tumor grades can be retrieved with colData(sce)\$tumor\_grade.

Dataset versions: a version argument can be passed to the function to specify which dataset version should be retrieved.

- `v0`: original version (Bioconductor <= 3.15).
- `v1`: consistent object formatting across datasets.
- `v2`: added full datasets and Zurich cohort.

#### File sizes:

- 'images\_basel': size in memory = 19 Gb, size on disk = 2.0 Gb.
- `masks\_basel`: size in memory = 433 Mb, size on disk = 10 Mb.
- `sce\_basel`: size in memory = 513 Mb, size on disk = 270 Mb.
- 'images\_zurich': size in memory = 6.0 Gb, size on disk = 724 Mb.
- `masks\_zurich`: size in memory = 137 Mb, size on disk = 3.4 Mb.
- `sce\_zurich`: size in memory = 188 Mb, size on disk = 105 Mb.
- `masks\_full`: size in memory = 2.1 Gb, size on disk = 10 Mb.
- `sce\_full`: size in memory = 2.2 Gb, size on disk = 1.2 Gb.

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

#### Value

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

#### Author(s)

Nicolas Damond

#### References

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

#### **Examples**

```
# Load single cell data
sce <- JacksonFischer_2020_BreastCancer(data_type = "sce")
print(sce)

# Display metadata
JacksonFischer_2020_BreastCancer(data_type = "sce", metadata = TRUE)

# Load masks on disk
library(HDF5Array)
masks <- JacksonFischer_2020_BreastCancer(data_type = "masks", on_disk = TRUE, h5FilesPath = getHDF5DumpDir())
print(head(masks))</pre>
```

listDatasets

List all available datasets

## **Description**

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

## Usage

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:

- FunctionCall, the R function call required to construct the dataset.
- 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.
- Reference, a Markdown-formatted citation to scripts/ref.bib in the **imcdatasets** installation directory.

## **Examples**

listDatasets()

ZanotelliSpheroids2020-defunct

Defunct function - 'ZanotelliSpheroids2020' dataset

## **Description**

These functions are defunct and no longer available.

#### **Details**

Defunct functions are: ZanotelliSpheroids2020\_sce, ZanotelliSpheroids2020\_masks, and ZanotelliSpheroids2020\_images. Please use the Zanotelli\_2020\_Spheroids function instead.

ZanotelliSpheroids2020Data-defunct

Defunct function - 'ZanotelliSpheroids2020Data' dataset

## **Description**

This function is defunct and no longer available.

## **Details**

This function is defunct, please use the Zanotelli\_2020\_Spheroids function instead.

Zanotelli\_2020\_Spheroids

Obtain the Zanotelli\_2020\_Spheroids dataset

## Description

Obtain the Zanotelli\_2020\_Spheroids dataset, which consists of three data objects: single cell data, multichannel images and cell segmentation masks. The data were obtained by imaging mass cytometry (IMC) of sections of 3D spheroids generated from different cell lines.

## Usage

```
Zanotelli_2020_Spheroids(
  data_type = c("sce", "spe", "images", "masks"),
  version = "latest",
  metadata = FALSE,
  on_disk = FALSE,
  h5FilesPath = NULL,
  force = FALSE
)
```

#### **Arguments**

data_type	type of object to load, 'images' for multichannel images or 'masks' for cell segmentation masks. Single cell data are retrieved using either 'sce' for the SingleCellExperiment format or 'spe' for the SpatialExperiment format.
version	dataset version. By default, the latest version is returned.
metadata	if FALSE (default), the data object selected in data_type is returned. If TRUE, only the metadata associated to this object is returned.
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:

- images contains 517 multichannel images, each containing 51 channels, in the form of a CytoImageList class object.
- masks contains the cell segmentation masks associated with the images, in the form of a CytoImageList class object.
- sce contains the single cell data extracted from the multichannel images using the cell segmentation masks, as well as the associated metadata, in the form of a SingleCellExperiment. This represents a total of 229,047 cells x 51 channels.
- spe same single cell data as for sce, but in the SpatialExperiment format.

All data are downloaded from ExperimentHub and cached for local re-use.

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 and SpatialExperiment objects. Mapping at the image level can be performed with the image\_name or image\_number variables. Mapping between cell segmentation masks and single cell data is performed with the cell\_number variable, the values of which correspond to the intensity values of the 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 and SpatialExperiment objects, are indicated in parentheses): *i*) Cells from four different cell lines (cell\_line) were seeded at three different densities (treatment\_concentration, relative densities) and grown for either 72 or 96 hours (treatment\_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 (image\_number).

Other relevant cell metadata include:

- treatment\_name: experimental conditions in the format: "Cell line name"\_c"seeding density"\_tp"time point".
- cell\_x/cell\_y: cell centroid position in the image.
- cell\_area: area of the cell (um^2).
- distance\_rim: estimated distance to spheroid border.
- distance\_sphere: distance to spheroid section border.
- distance\_other\_sphere: distance to the closest of the other spheroid sections in the same image (if there is any).

distance\_background: distance to background pixels.

For a full description of the other experimental variables, please refer to the publication (https://doi.org/10.15252/msb.202097 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 and SpatialExperiment objects. 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 slots of the SingleCellExperiment and SpatialExperiment objects contain three assays:

- counts contains raw mean ion counts per cell.
- exprs contains arsinh-transformed counts, with cofactor 1.
- quant\_norm contains counts censored at the 99th percentile and scaled 0-1.

In addition, the altExp slot of the SingleCellExperiment object contains another SingleCellExperiment object where the counts matrix represents raw mean ion counts for cells neighboring the current cell.

Neighborhood information, defined here as cells that are localized next to each other, is stored as a SelfHits object in the colPairs slot of the SingleCellExperiment and SpatialExperiment objects. Cells in the SelfHits object are represented by unique integers that map to the cell\_number\_absolute column of colData(sce).

Dataset versions: a version argument can be passed to the function to specify which dataset version should be retrieved.

- `v0`: original version (Bioconductor <= 3.15).
- `v1`: consistent object formatting across datasets.

## File sizes:

- `images`: size in memory = 21.2 Gb, size on disk = 860 Mb.
- masks: size in memory = 426 Mb, size on disk = 12 Mb.
- `sce`: size in memory = 564 Mb, size on disk = 319 Mb.
- `spe`: size in memory = 596 Mb, size on disk = 320 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 SpatialExperiment 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**

```
# Load single cell data
sce <- Zanotelli_2020_Spheroids(data_type = "sce")
print(sce)

# Display metadata
Zanotelli_2020_Spheroids(data_type = "sce", metadata = TRUE)

# Load masks on disk
library(HDF5Array)
masks <- Zanotelli_2020_Spheroids(data_type = "masks", on_disk = TRUE,
h5FilesPath = getHDF5DumpDir())
print(head(masks))</pre>
```

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