TDS
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Introduction to R
for flow cytometry data analysis
Day 2

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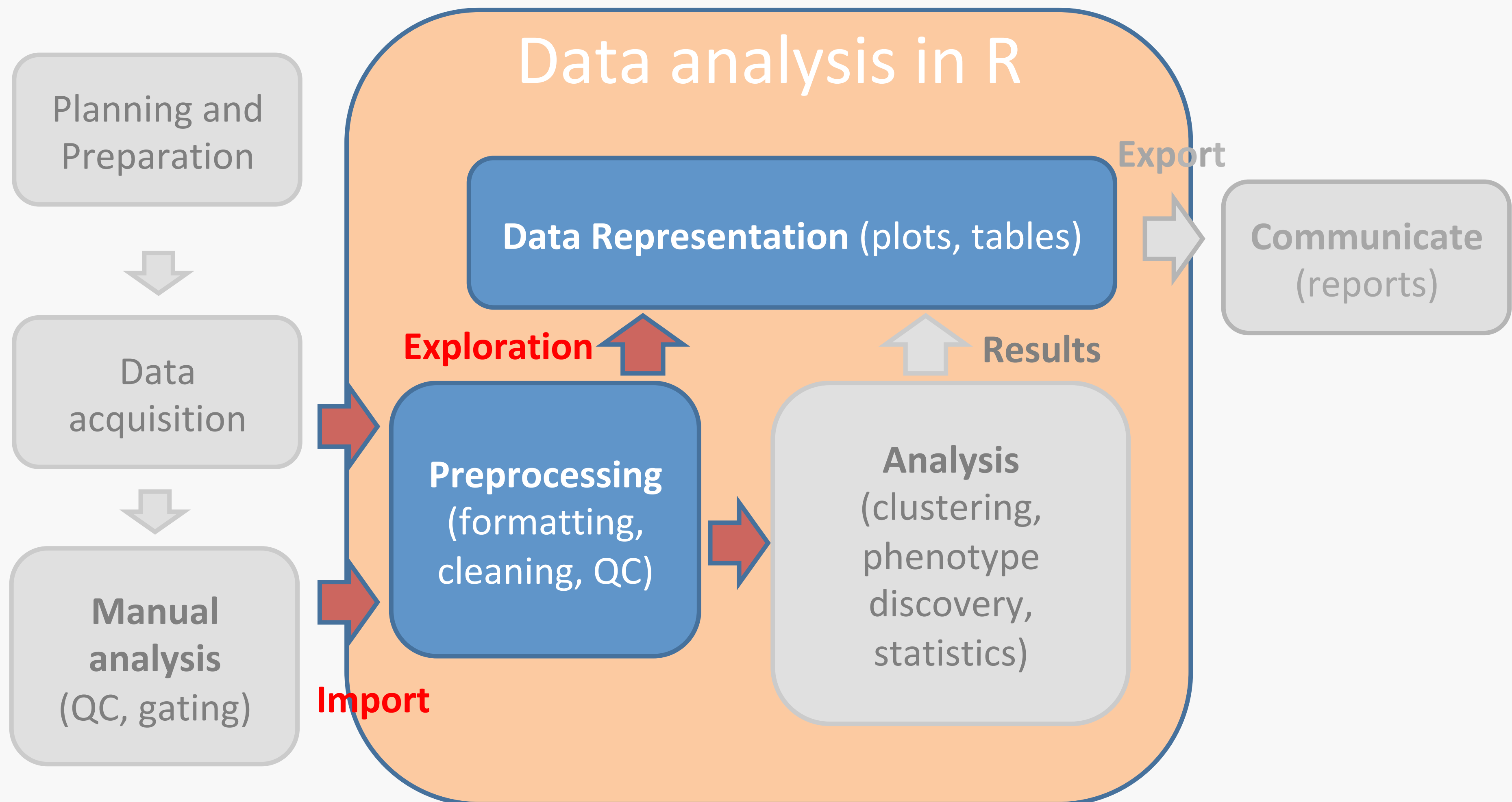
Translational Data Science – Facility

SIB Swiss Institute of Bioinformatics

Taking Advantage of R For Your Work



06

Starting to work with flow cytometry data

Workflow

frontiers in Immunology

METHODS
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How to Prepare Spectral Flow Cytometry Datasets for High Dimensional Data Analysis: A Practical Workflow

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Example of flow cytometry dataset

- Publicly available through the **FlowRepository database** at https://flowrepository.org/, using repository ID FR-FCM-Z4KT
- Data from **31-color spectral flow cytometry** on peripheral blood mononuclear cells (**PBMCs**) from healthy controls
- Data were acquired and unmixed using SpectroFlo® v2.2.0.3 software (Cytek Biosciences, Fremont, California, USA)
- **Resulting unmixed fcs files were analyzed using manual gating** in FlowJo v10.7 software (BD Biosciences, San Jose, California, USA)

Flow Cytometry Standard (FCS) files

- Data standard for the reading and writing of data from flow cytometry experiments
- File exported from the cytometer’s acquisition software
- Versions: FCS1.o (1984), FCS 2.0 (1990), FCS 3.0 (1997), FCS 3.1 (2010),
- File Format (main segments):
  - HEADER segment (ASCII text): version, …
  - TEXT segment (ASCII text): keywords and values which describe the data format and encoding
  - DATA segment (binary): contains the actual measurements
  - Others …

Data structure

- Array (matrix) with fluorescence and scatter channels represented in columns and individual «events» (cells…) forming the rows

Channels

Events

	FSC-A	FSC-H	SSC-A	B515-A	R780-A	R710-A	R660-A	V800-A	V655-A
[1,]	27700.75	27291.75	177.52585	1984.485	625.0796	1232.1008	748.5101	1553.0295	1350.2565
[2,]	41264.25	39764.25	320.12296	3639.620	539.7032	1433.3112	1470.2659	2217.6750	2305.3516
[3,]	65054.75	57606.25	203.01607	2191.861	198.6541	726.9798	766.2198	802.2521	809.9579
[4,]	30584.00	31664.50	130.68690	1873.409	1304.0895	2528.7083	784.6980	1702.3671	1185.8608
[5,]	39505.75	39626.00	203.25166	2540.620	323.2625	857.1525	715.0004	1117.4775	1746.5798
[6,]	33171.50	34794.00	333.64246	2192.864	1408.8563	2573.5095	1604.2236	2128.1748	1727.5891
[7,]	63711.00	54475.50	1122.48340	3879.044	1730.8085	3573.5652	1691.8744	5106.0596	3578.0332
[8,]	40000.75	40213.50	236.54262	2545.858	1081.6753	2313.5962	1411.0983	2989.7524	1920.4047
[9,]	49286.00	49182.50	78.61845	1601.092	123.2834	493.6364	242.0255	633.3533	759.2227
[10,]	32209.75	33368.25	203.29897	2387.361	1056.0723	1769.4005	939.7758	1693.8635	1579.7000
[11,]	35937.25	36212.50	220.66580	2901.591	1218.1395	3202.3853	1059.7604	2443.0205	2253.0146
[12,]	32905.50	33897.50	233.98033	2726.240	1952.0721	3405.7139	2726.1091	2988.6882	2011.0159
[13,]	36028.50	35845.50	219.18674	3221.668	2542.3389	3895.0371	2283.0444	3331.8298	2479.6580
[14,]	38616.00	38775.00	218.46669	3218.305	582.6801	1022.7971	1255.5858	2150.4185	1993.2681
[15,]	45282.25	42223.25	1173.74487	6941.545	705.4651	1649.9570	1615.0811	4287.2036	3778.2302
[16,]	36246.25	36207.75	189.15569	3049.417	1736.7826	2823.7266	1031.0308	2824.6582	2053.6843
[17,]	29282.75	29884.00	209.64102	1836.197	612.2673	1149.7164	870.3303	1720.2170	1525.6914
[18,]	57757.25	54448.25	1999.17517	12972.877	4364.5908	11298.7070	6745.5039	20934.3457	17057.1934
[19,]	33301.00	33093.50	208.47151	2146.622	429.5022	855.5981	845.9418	1207.8969	1297.2683
[20,]	34478.25	35390.75	211.26921	3060.585	2016.3651	3442.5408	1348.4852	2673.9729	2259.8494
[21,]	29406.25	28219.50	231.55798	3008.380	997.8875	2319.5779	1514.2091	1757.2463	1675.9983
[22,]	49978.50	48517.75	537.04224	3122.343	981.1232	2252.1189	1861.3472	2518.4731	2230.5327
[23,]	39872.50	37620.75	198.75706	2719.222	1657.0939	2945.5713	1025.1293	2203.0527	1670.1367
[24,]	33395.00	35331.75	220.46056	2664.632	690.1926	1483.0898	1736.9537	1397.0316	1982.9124
[25,]	46976.00	47355.25	231.33037	2530.461	537.1376	1194.0681	1072.7083	1531.7494	1766.5841
[26,]	56663.75	51458.25	223.06416	3217.866	398.6222	1279.4880	1207.4561	1268.9905	1553.6884
[27,]	50818.75	48556.25	305.77182	3714.351	577.0732	1364.4095	1064.0983	1633.2513	2077.0466
[28,]	36225.25	36196.75	180.30524	2636.466	946.7570	2138.4143	1695.0502	1807.8429	2057.7292
[29,]	28509.25	30715.50	230.27397	1072.201	1867.2009	1643.1423	882.4811	1201.5806	688.1475
[30,]	37198.75	36200.50	237.67776	3046.719	1376.3452	2580.9287	1326.2197	2599.6101	2196.7258

flowCore R Package

- https://bioconductor.org/packages/release/bioc/html/flowCore.html
- Provides data structures and basic functions to deal with flow cytometry data in R
- Installation:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("flowCore")
```

- Vignette https://bioconductor.org/packages/release/bioc/vignettes/flowCore/inst/doc/HowTo-flowCore.pdf

Reading an FCS file into a *flowFrame*

- *A **flowFrame*** is the basic unit of manipulation
- Corresponds to a single FCS file

The function **read.FCS()** allows to read a single FCS file into R. Example:

```
> FCS_file <- read.FCS(
             filename = "course_datasets/FR_FCM_Z4KT/
T_cells_REU270_alive_T cells.fcs",
             transformation=FALSE,
            truncate_max_range = FALSE)
```

- Important arguments:
  - **filename** is the file path
  - **transformation** specifies the type of transformation to be applied. When set to FALSE, no transformation is applied.
  - **truncate_max_range**. Set to FALSE to avoid truncating the extreme positive value to the instrument measurement range.

What is a flowFrame object?

> help(flowFrame)

flowFrame-class {flowCore} R Documentation

'flowFrame': a class for storing observed quantitative properties for a population of cells from a FACS run

Description

This class represents the data contained in a FCS file or similar data structure. There are three parts of the data:

1. a numeric matrix of the raw measurement values with `rows=events` and `columns=parameters`
2. annotation for the parameters (e.g., the measurement channels, stains, dynamic range)
3. additional annotation provided through keywords in the FCS file

Details

Objects of class `flowFrame` can be used to hold arbitrary data of cell populations, acquired in flow-cytometry.

What is a flowFrame object ?

- In R, objects such as flowFrames are **collections of data (variables)** and **methods (functions).**
- They belong to a given **class** (a blueprint for that object)
- Member variables in R objects are called **slots**. There are three slots in a flowFrame: *exprs*, *parameters* and *description*

Slots

`exprs`

Object of class `matrix` containing the measured intensities. Rows correspond to cells, columns to the different measurement channels. The `colnames` attribute of the matrix is supposed to hold the names or identifiers for the channels. The `rownames` attribute would usually not be set.

`parameters`

An `AnnotatedDataFrame` containing information about each column of the `flowFrame`. This will generally be filled in by `read.FCS` or similar functions using data from the FCS keywords describing the parameters.

`description`

A list containing the meta data included in the FCS file.

Summarize a *flowFrame*

```
> FCS_file
```

```
flowFrame object 'T_cells_REU270_alive_T cells.fcs'
with 315735 cells and 39 observables:
                          name        desc     range  minRange  maxRange
$P1                      FSC-A          NA   4194304         0   4194303
$P2                      FSC-H          NA   4194304         0   4194303
$P3                      SSC-A          NA   4194304         0   4194303
$P4                    SSC-B-A          NA   4194304         0   4194303
$P5                    SSC-B-H          NA   4194304         0   4194303
...                        ...         ...       ...       ...       ...
$P35 FJComp-PerCP-eFluor ..          CD127    100000      -111     99999
$P36 FJComp-Spark Blue 55..            CD3    100000         0     99999
$P37      FJComp-Zombie UV-A Zombie UV    100000      -111     99999
$P38     FJComp-eFluor 660-A      CTLA-4    100000      -111     99999
$P39                      Time          NA       166         0       165
278 keywords are stored in the 'description' slot
```

```
> summary(FCS_file)
```

```
            FSC-A     FSC-H     SSC-A   SSC-B-A   SSC-B-H     SSC-H FJComp-AF-A         Time
Min.     248252.4  200055.0  104527.1  54734.91   43496.0   71728.0  -85312.109      0.00000
1st Qu.  694239.2  544927.0  371822.5 216510.48  163184.0  290444.0  -11530.027     39.13176
Median   794500.6  628644.0  453873.5 263345.84  195338.0  348003.0   -7938.739 ... 81.05503
Mean     809134.7  639853.2  452449.1 263406.04  195808.8  347746.4   -8392.375     81.00381
3rd Qu.  908499.2  722281.5  527944.0 307300.50  226672.5  402455.0   -4571.343    121.98465
Max.    1608623.4 1358182.0  946566.2 608151.94  449530.0  791039.0   37643.547    162.51257
```

Access data elements in a *flowFrame*

- To access data: use the @ operator or a method (function)
- Matrix of expression values (as a matrix)

```
> FCS_file@exprs
```

or

```
> exprs(FCS_file)
```

```
        FSC-A  FSC-H    SSC-A   SSC-B-A SSC-B-H  SSC-H FJComp-AF-A FJComp-APC-A
[1,] 708579.4 593958 331966.4 195681.8  161726 273584  -12322.742   -4990.1958
[2,] 587231.9 489906 323881.8 209247.5  165442 265458  -10672.745   -5642.0508
[3,] 828618.7 662813 487978.5 289251.3  215334 379895   -1366.873   -3940.6289
[4,] 733458.1 606898 447868.5 242895.0  188230 357695   -2092.956    -998.5401
[5,] 576551.5 461784 428876.1 238000.4  175819 326038   -6251.983   -5225.1035
[6,] 762848.1 606807 583804.5 344976.0  251346 444231  -10864.361   -4390.9263
```

```
> colnames(FCS_file)
```

```
 [1] "FSC-A"                    "FSC-H"                      "SSC-A"
 [4] "SSC-B-A"                  "SSC-B-H"                    "SSC-H"
 [7] "FJComp-AF-A"              "FJComp-APC-A"               "FJComp-APC-Fire 750-A"
[10] "FJComp-APC-Fire 810-A"    "FJComp-APC-R700-A"          "FJComp-BB515-A"
[13] "FJComp-BB700-A"           "FJComp-BUV395-A"            "FJComp-BUV496-A"
[16] "FJComp-BUV563-A"          "FJComp-BUV615-A"            "FJComp-BUV661-A"
[19] "FJComp-BUV737-A"          "FJComp-BUV805-A"            "FJComp-BV421-A"
[22] "FJComp-BV480-A"           "FJComp-BV510-A"             "FJComp-BV570-A"
[25] "FJComp-BV605-A"           "FJComp-BV650-A"             "FJComp-BV711-A"
[28] "FJComp-BV750-A"           "FJComp-BV785-A"             "FJComp-PE-A"
[31] "FJComp-PE-Cy5-A"          "FJComp-PE-Cy7-A"            "FJComp-PE-Dazzle594-A"
[34] "FJComp-PerCP-A"           "FJComp-PerCP-eFluor 710-A"  "FJComp-Spark Blue 550-A"
[37] "FJComp-Zombie UV-A"       "FJComp-eFluor 660-A"        "Time"
```

Access data elements in a *flowFrame*

- Metadata (panel)

```
> pData(FCS_file@parameters)
```
or
```
> pData(parameters(FCS_file))
```

	name	desc	range	minRange	maxRange
$P1	FSC-A	NA	4194304	0	4194303
$P2	FSC-H	NA	4194304	0	4194303
...	...	...	...	...	...
$P35	FJComp-PerCP-eFluor ..	CD127	100000	-111	99999
$P36	FJComp-Spark Blue 55..	CD3	100000	0	99999
$P37	FJComp-Zombie UV-A	Zombie UV	100000	-111	99999
$P38	FJComp-eFluor 660-A	CTLA-4	100000	-111	99999
$P39	Time	NA	166	0	165

How to replace the channel names by the antigen names in the expression matrix

- Copy the metadata to a data frame

```
> panel <- pData(FCS_file@parameters)
```

- Copy the names to a new column

```
> pData(FCS_file@parameters)$channel <- panel$name
```

- Replace the names by the antigens

```
> colnames(FCS_file)[!is.na(panel$desc)] <- panel$desc[!
  is.na(panel$desc)]
```

```
> head(exprs(FCS_file)[,10:15])
```

	CD27	LAG-3	CD25	CD49b	CD8	CD4
[1,]	34010.4844	-726.74323	2337.454	622.7443	-1674.8558	54145.7031
[2,]	26705.5781	-447.67514	3196.554	1380.7151	-1855.1270	64054.7617
[3,]	846.9209	246.76016	1000.591	581.6843	-977.6837	-219.4745
[4,]	1110.7271	-507.84625	1215.742	1224.3079	1438.7726	-2148.8167
[5,]	3685.3149	-1773.50989	4398.276	818.2174	-1662.8772	88996.8984
[6,]	505.4961	-21.17858	2401.317	-358.6945	451.0627	-2950.0181

Reading a list of FCS files into a *flowSet*

- *A* ***flowSet*** is a collection of *flowFrame*
- Convenient way to apply methods to all *flowFrame* simultaneously

The function **read.flowSet()** allows to read several FCS files in a given directory.

Example:

```
> fcs_data <- read.flowSet(path="course_datasets/FR_FCM_Z4KT/",
                           pattern="*.fcs",
                            transformation = FALSE,
                            truncate_max_range = FALSE)
```

- Important arguments:
  - **path** is the path to the directory containing the FCS files
  - **pattern** sets which files to read (* is a wildcard replacing the file names)

You can coerce a list of *flowFrames* into a *FlowSet*, but is less convenient

Slots in a *flowSet*

> help(flowSet)

flowSet-class {flowCore} R Documentation

'flowSet': a class for storing flow cytometry raw data from quantitative cell-based assays

Description

This class is a container for a set of `flowFrame` objects

Slots

`frames`

An `environment` containing one or more `flowFrame` objects.

`phenoData`

An `AnnotatedDataFrame` containing the phenotypic data for the whole data set. Each row corresponds to one of the `flowFrame`s in the `frames` slot. The `sampleNames` of `phenoData` (see below) must match the names of the `flowFrame` in the `frames` environment.

List sample names

```
> sampleNames(fcs_data)
```

```
 [1] "T_cells_REU267_alive_T_cells.fcs"          "T_cells_REU268_alive_T_cells.fcs"
 [3] "T_cells_REU269_alive_T_cells.fcs"          "T_cells_REU270_alive_T_cells.fcs"
 [5] "T_cells_REU271_12_july_alive_T_cells.fcs"  "T_cells_REU271_13_april_alive_T_cells.fcs"
 [7] "T_cells_REU271_14_april_alive_T_cells.fcs" "T_cells_REU271_7_apr_alive_T_cells.fcs"
 [9] "T_cells_REU271_9_april_alive_T_cells.fcs"  "T_cells_REU271_alive_T_cells.fcs"
[11] "T_cells_REU272_12_july_alive_T_cells.fcs"  "T_cells_REU272_13_april_alive_T_cells.fcs"
[13] "T_cells_REU272_14_april_alive_T_cells.fcs" "T_cells_REU272_7_apr_alive_T_cells.fcs"
[15] "T_cells_REU272_9_april_alive_T_cells.fcs"  "T_cells_REU272_alive_T_cells.fcs"
```

We can change the sample names:

```
> sampleNames(fcs_data) <- c("REU267","REU268","REU269","REU270",
                             "REU271_12_july","REU271_13_april",
                              "REU271_14_april","REU271_7_apr",
                              "REU271_9_april","REU271","REU272_12_july",
                              "REU272_13_april","REU272_14_april",
                              "REU272_7_apr","REU272_9_apri","REU272")
```

Phenotypic data

- Extract / replace the data frame (or columns thereof) containing actual phenotypic information from the phenoData slot

```
> pData(fcs_data)
```

```
                                                         name
REU267                          T_cells_REU267_alive_T cells.fcs
REU268                          T_cells_REU268_alive_T cells.fcs
REU269                          T_cells_REU269_alive_T cells.fcs
REU270                          T_cells_REU270_alive_T cells.fcs
REU271_12_july    T_cells_REU271_12_july_alive_T cells.fcs
REU271_13_april T_cells_REU271_13_april_alive_T cells.fcs
REU271_14_april T_cells_REU271_14_april_alive_T cells.fcs
REU271_7_apr          T_cells_REU271_7_apr_alive_T cells.fcs
REU271_9_april    T_cells_REU271_9_april_alive_T cells.fcs
REU271                          T_cells_REU271_alive_T cells.fcs
REU272_12_july    T_cells_REU272_12_july_alive_T cells.fcs
REU272_13_april T_cells_REU272_13_april_alive_T cells.fcs
REU272_14_april T_cells_REU272_14_april_alive_T cells.fcs
REU272_7_apr          T_cells_REU272_7_apr_alive_T cells.fcs
REU272_9_apri     T_cells_REU272_9_april_alive_T cells.fcs
REU272                          T_cells_REU272_alive_T cells.fcs
```

Add a new column to the phenotypic data

```
> pData(fcs_data)$gender <- c(rep("male",8), rep("female",8))
> pData(fcs_data) # or fcs_data@phenoData@data
```

```
                                                 name gender
REU267                    T_cells_REU267_alive_T cells.fcs   male
REU268                    T_cells_REU268_alive_T cells.fcs   male
REU269                    T_cells_REU269_alive_T cells.fcs   male
REU270                    T_cells_REU270_alive_T cells.fcs   male
REU271_12_july    T_cells_REU271_12_july_alive_T cells.fcs   male
REU271_13_april T_cells_REU271_13_april_alive_T cells.fcs   male
REU271_14_april T_cells_REU271_14_april_alive_T cells.fcs   male
REU271_7_apr        T_cells_REU271_7_apr_alive_T cells.fcs   male
REU271_9_april    T_cells_REU271_9_april_alive_T cells.fcs female
REU271                    T_cells_REU271_alive_T cells.fcs female
REU272_12_july    T_cells_REU272_12_july_alive_T cells.fcs female
REU272_13_april T_cells_REU272_13_april_alive_T cells.fcs female
REU272_14_april T_cells_REU272_14_april_alive_T cells.fcs female
REU272_7_apr        T_cells_REU272_7_apr_alive_T cells.fcs female
REU272_9_apri     T_cells_REU272_9_april_alive_T cells.fcs female
REU272                    T_cells_REU272_alive_T cells.fcs female
```

Manipulating a *FlowSet*

- Extract a *flowFrame* from a flowSet object using the [[operator

```
> fcs_data[[1]]
```

```
flowFrame object 'T_cells_REU267_alive_T_cells.fcs'
with 265857 cells and 39 observables:
               name      desc     range  minRange  maxRange
$P1           FSC-A        NA   4194304         0   4194303
$P2           FSC-H        NA   4194304         0   4194303
$P3           SSC-A        NA   4194304         0   4194303
$P4         SSC-B-A        NA   4194304         0   4194303
$P5         SSC-B-H        NA   4194304         0   4194303
```

- Create a new *flowSet* object by subsetting with the [operator

```
> fcs_data[1:5]
```

```
A flowSet with 5 experiments.

column names(39): FSC-A FSC-H ... FJComp-eFluor 660-A Time
```

Manipulating a *FlowSet*

- Subset a *flowSet* based on a condition

```
> fcs_data_males <- fcs_data[pData(fcs_data)$gender=="male"]
> fcs_data_females <- subset(fcs_data, pData(fcs_data)$gender"female”)
```

- Split the *flowSet* based on a condition

```
> fcs_data_split <- split(fcs_data, pData(fcs_data)$gender)
> names(fcs_data_split)
[1] "female" "male"
```

Manipulating a *FlowSet*

- Combine *flowSets* (or *flowSets* and *flowFrames*)

```
> fcs_data_combined <- rbind2(fcs_data_split$female,fcs_data_split$male)
> pData(fcs_data_combined)
```

```
                                                      name gender  split
REU271_9_april   T_cells_REU271_9_april_alive_T cells.fcs female female
REU271                   T_cells_REU271_alive_T cells.fcs female female
REU272_12_july   T_cells_REU272_12_july_alive_T cells.fcs female female
REU272_13_april T_cells_REU272_13_april_alive_T cells.fcs female female
REU272_14_april T_cells_REU272_14_april_alive_T cells.fcs female female
REU272_7_apr       T_cells_REU272_7_apr_alive_T cells.fcs female female
REU272_9_apri    T_cells_REU272_9_april_alive_T cells.fcs female female
REU272                   T_cells_REU272_alive_T cells.fcs female female
REU267                   T_cells_REU267_alive_T cells.fcs   male   male
REU268                   T_cells_REU268_alive_T cells.fcs   male   male
REU269                   T_cells_REU269_alive_T cells.fcs   male   male
REU270                   T_cells_REU270_alive_T cells.fcs   male   male
REU271_12_july   T_cells_REU271_12_july_alive_T cells.fcs   male   male
REU271_13_april T_cells_REU271_13_april_alive_T cells.fcs   male   male
REU271_14_april T_cells_REU271_14_april_alive_T cells.fcs   male   male
REU271_7_apr       T_cells_REU271_7_apr_alive_T cells.fcs   male   male
```

Vizualizing Cytometry Data with *ggcyto* R Package

- https://www.bioconductor.org/packages/release/bioc/html/ggcyto.html
- Interface to the ggplot2 graphics system
- Installation:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("ggcyto")
```

- Vignettes

https://www.bioconductor.org/packages/release/bioc/vignettes/ggcyto/inst/doc/Top_features_of_ggcyto.html

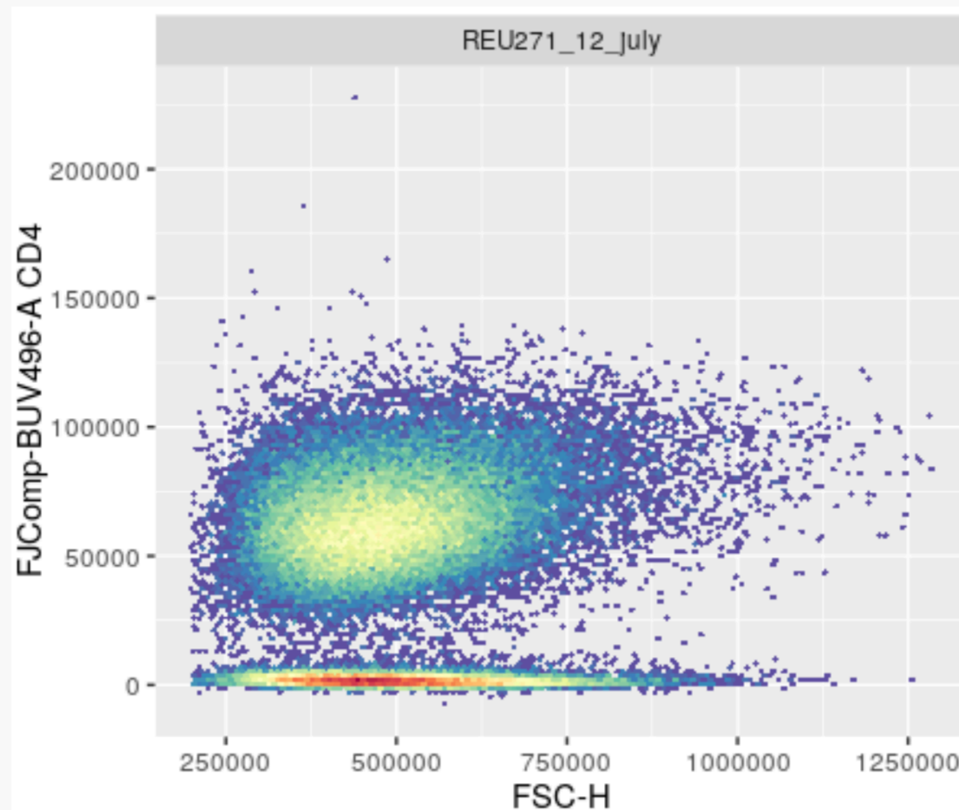
https://www.bioconductor.org/packages/release/bioc/vignettes/ggcyto/inst/doc/ggcyto.flowSet.html

Vizualizing a single *flowFrame*

The function **autoplot()** can be used to create a **bivariate density plot**.

Example:

```
> autoplot(object = fcs_data[[5]], x=”FSC-H", y="FJComp-BUV496-A",
  bins = 2^7)
```

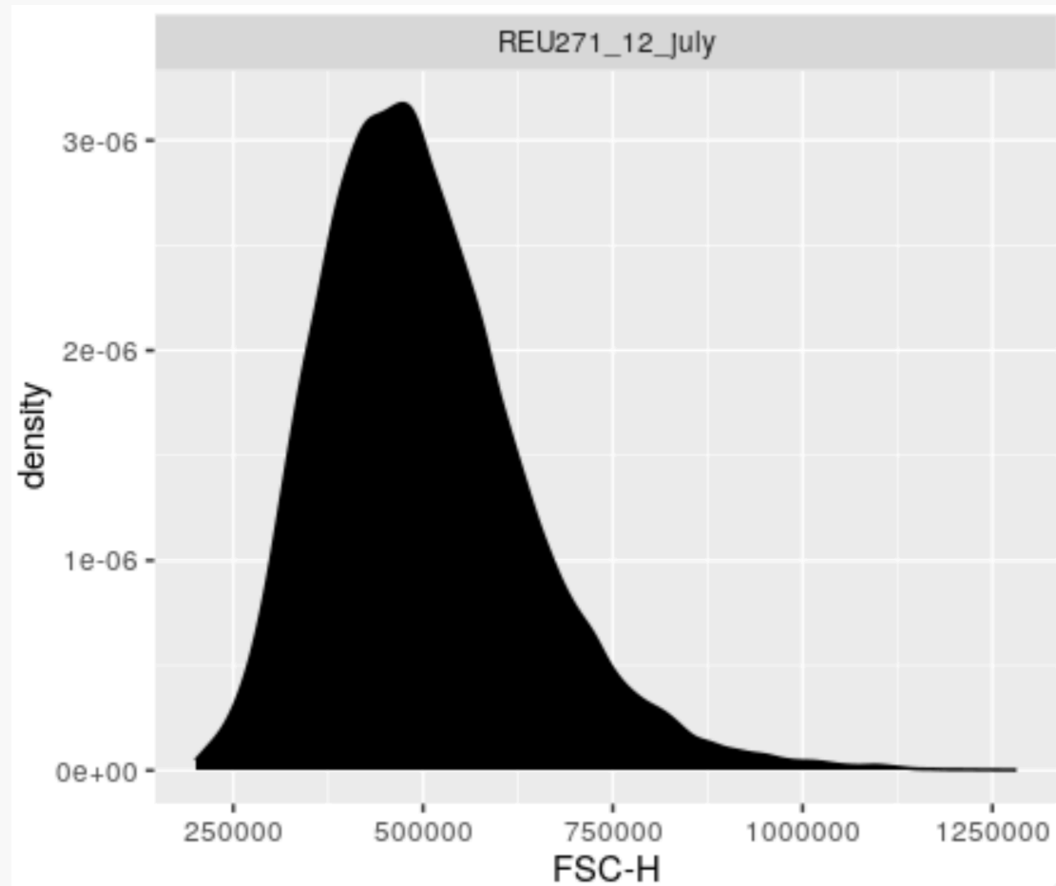


- **bins** sets the granularity of the plot. The higher the number of bins, the finer the granularity

Vizualizing a single *flowFrame*

Similarly, to get a **univariate densityplot**:

```
> autoplot(object = fcs_data[[5]], x="FSC-H")
```



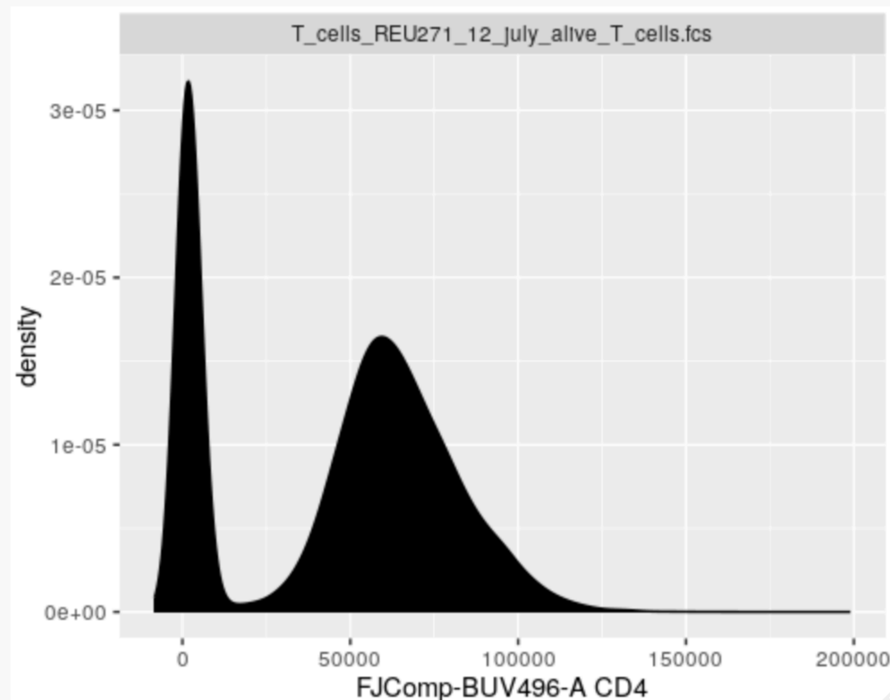
In-line transformation

Use a different scale for the data

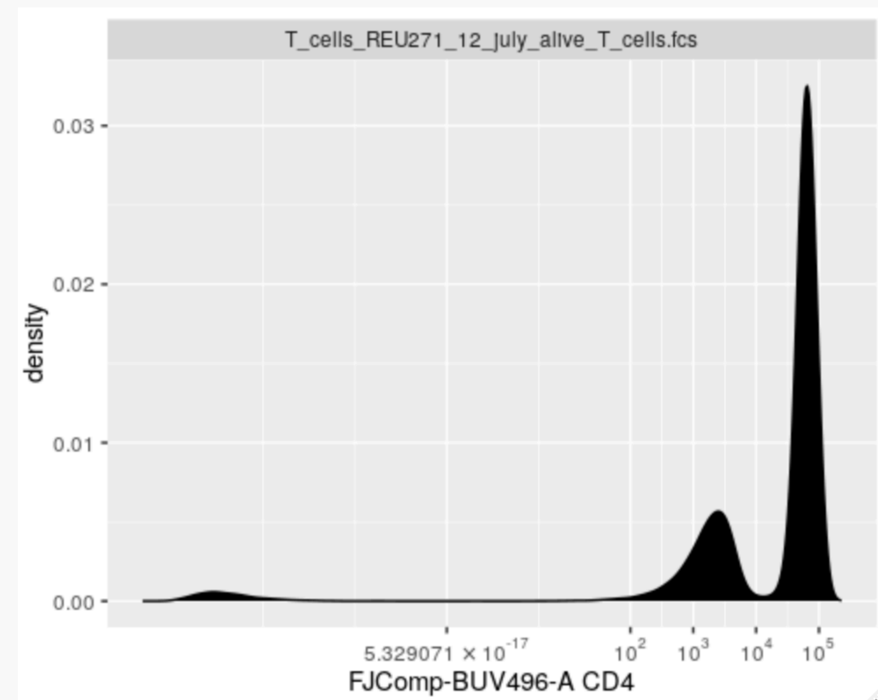
```
> autoplot(fcs_data[[5]],
           x="FJComp-BUV496-A")
```

```
> autoplot(fcs_data[[5]],
  x="FJComp-BUV496-A") +
  scale_x_flowjo_fasinh()
```

Original scale (raw intensity measurements)



flowJo inverse hyperbolic sine

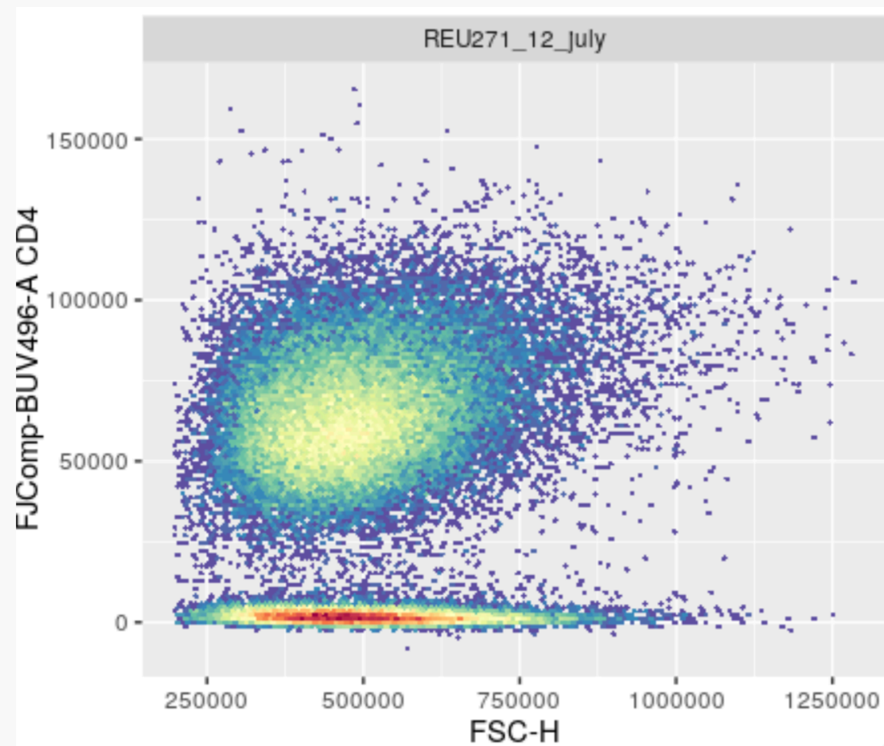


In-line transformation

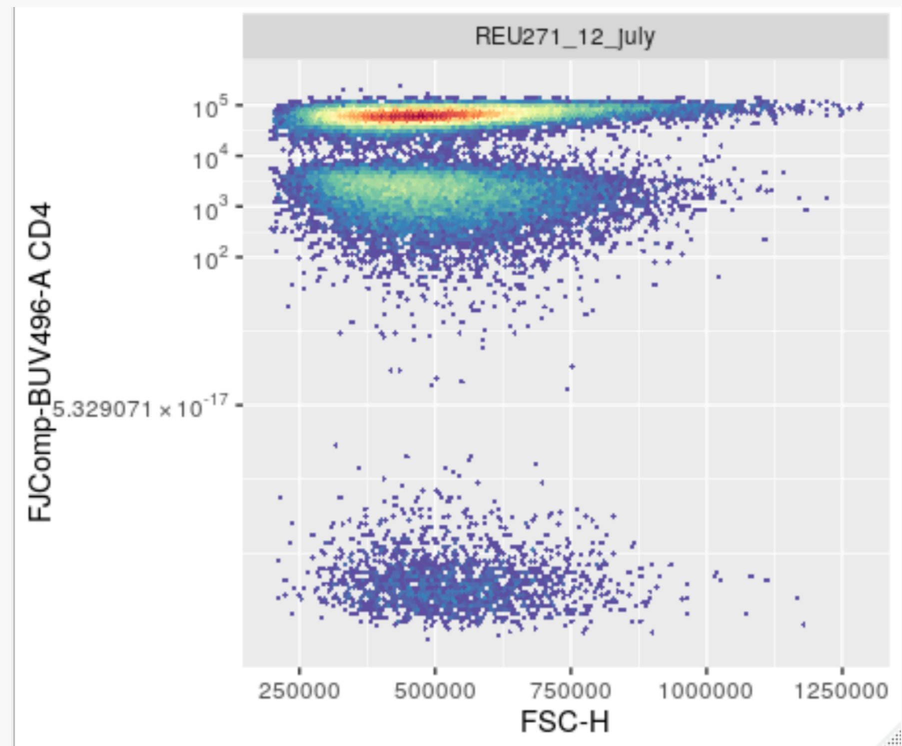
Example in a **bivariate density plot**.

```
> autoplot(object = fcs_data[[5]], x="FSC-H", y="FJComp-BUV496-A",
  bins = 2^7) + scale_y_flowjo_fasinh()
```

Original scale



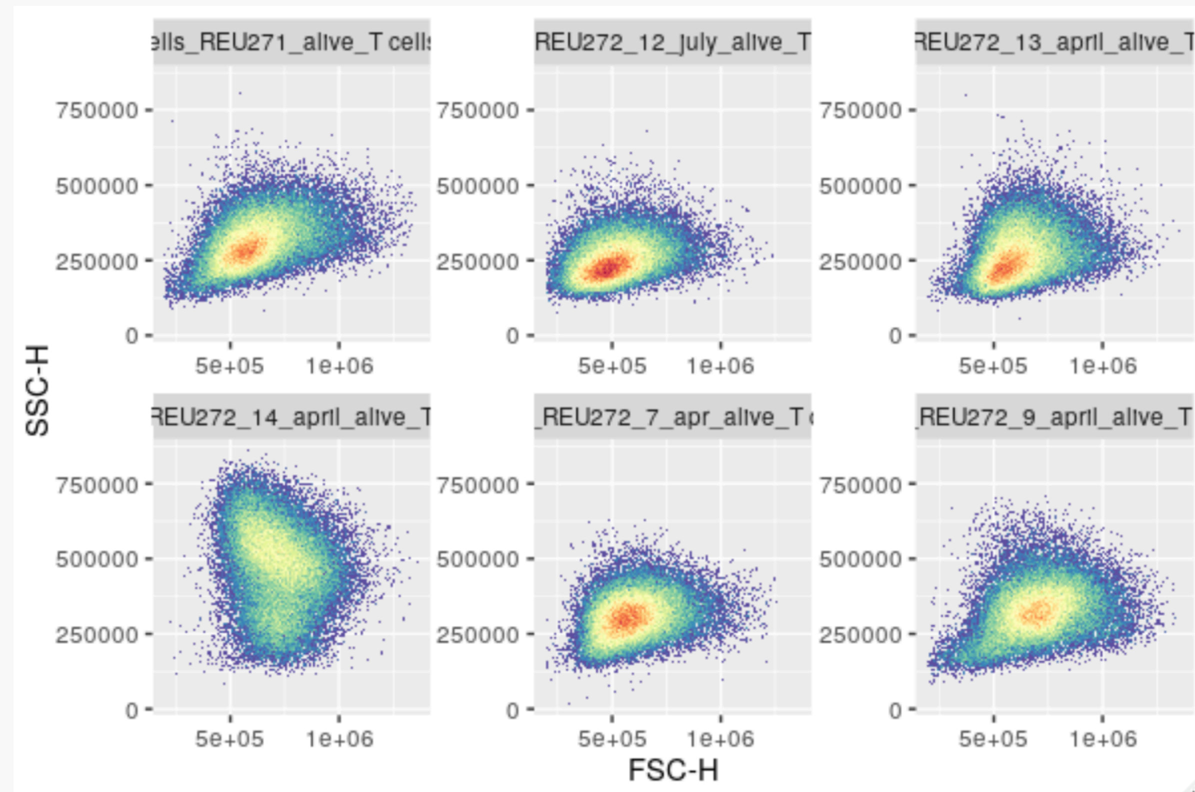
flowJo inverse hyperbolic sine



Vizualizing a *flowSet*

The syntax is basically the same for *flowSet* objects, with the output now being a grid of plots corresponding to each *flowFrame*. Example:

```
> autoplot(object = fcs_data[10:15], x="FSC-H", y="SSC-H", bins = 2^7)
```



Let’s practice – 9

In this exercise we will use a 36-color spectral flow cytometry dataset from a study performed in the context of Covid-19 research. Only a subset from 4 healthy donors will be used. For each healthy donor, there are three time points, as indicated in FCS file names. Data was downloaded through the Flow Repository database (FR-FCM-Z3WR) at https://flowrepository.org/id/FR-FCM-Z3WR. FCS files were pre-gated on live CD3+CD19-T cells in FlowJo.

Create a new script in which you will

1) Import the FCS files (course_datasets/FR_FCM_Z3WR/). Do not transform or truncate the values
2) Create a data frame with the list of channels and corresponding antigens, and plot it . **Hint:** get the antigens from the parameters of one of the flowFrame in the set
3) Convert the channel names in the expression matrices to the corresponding antigen names (where applicable)
4) Add a new column to the phenotypic data with the time point of the sample. Plot the phenotypic data
5) Create a bivariate density plot showing «FSC-H» against «HLA-DR» for all samples from day 0. Apply a flowJo inverse hyperbolic sine scale to the y axis («HLA-DR»)

In a nutshell

- **FCS files** include the cell measurements and metadata
- FCS files can be imported into the R environment with the **flowCore package**.
- flowCore provides data structures, such as ***flowFrames*** and ***flowSets***, and basic functions to deal with flow cytometry data.
- The **ggcyto package** implements methods for visualization of *flowFrame* and *flowSet* objects, including an interface to the ggplot2 graphics system

More to explore…

- **R manuals:** http://cran.r-project.org/manuals.html
- **Posit support (resources for learning and using R):** https://support.posit.co/hc/en-us/articles/200552336-Getting-Help-with-R
- **Datacamp free tutorials:** https://www.datacamp.com/courses/free-introduction-to-r
- **Stackoverflow** documentation, resources and user forum: http://stackoverflow.com/tags/r/info
- **Rseek -** search engine on numerous online R resources: http://www.rseek.org

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Thank you for your attention!

https://agora-cancer.ch/scientific-platforms/translational-data-science-facility/

Any questions? Contact us !

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