

# immunoClust - Automated Pipeline for Population Detection in Flow Cytometry

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## 1 Licensing

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Under the Artistic License, you are free to use and redistribute this software. However, we ask you to cite the following paper if you use this software for publication.

Sörensen, T., Baumgart, S., Durek, P., Grützkau, A. and Häupl, T.

immunoClust - an automated analysis pipeline for the identification of immunophenotypic signatures in high-dimensional cytometric datasets.

*Cytometry A* (accepted).

## 2 Overview

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*immunoClust* presents an automated analysis pipeline for uncompensated fluorescence and mass cytometry data and consists of two parts. First, cell events of each sample are grouped into individual clusters (cell-clustering). Subsequently, a classification algorithm assort these cell event clusters into populations comparable between different samples (meta-clustering). The clustering of cell events is designed for datasets with large event counts in high dimensions as a global unsupervised method, sensitive to identify rare cell types even when next to large populations. Both parts use model-based clustering with an iterative Expectation Maximization (EM) algorithm and the Integrated Classification Likelihood (ICL) to obtain the clusters.

The cell-clustering process fits a mixture model with  $t$ -distributions. Within the clustering process a optimisation of the *asinh*-transformation for the fluorescence parameters is included.

The meta-clustering fits a Gaussian mixture model for the meta-clusters, where adjusted Bhattacharyya-Coefficients give the probability measures between cell- and meta-clusters.

Several plotting routines are available visualising the results of the cell- and meta-clustering process. Additional helper-routines to extract population features are provided.

## 3 Getting started

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The installation on *immunoClust* is normally done within the Bioconductor.

The core functions of *immunoClust* are implemented in C/C++ for optimal utilization of system resources and depend on the GNU Scientific Library (GSL) and Basic Linear Subprogram (BLAS). When installing *immunoClust* form source using Rtools be aware to adjust the GSL library and include pathes in `src/Makevars.in` or `src/Makevars.win` (on Windows systems) repectively to the correct installation directory of the GSL-library on the system.

*immunoClust* relies on the *flowFrame* structure imported from the *flowCore*-package for accessing the measured cell events from a flow cytometer device.

## 4 Example Illustrating the immunoClust Pipeline

The functionality of the immunoClust pipeline is demonstrated on a dataset of blood cell samples of defined composition that were depleted of particular cell subsets by magnetic cell sorting. Whole blood leukocytes taken from three healthy individuals, which were experimentally modified by the depletion of one particular cell type per sample, including granulocytes (using CD15-MACS-beads), monocytes (using CD14-MACS-beads), T lymphocytes (CD3-MACS-beads), T helper lymphocytes (using CD4-MACS-beads) and B lymphocytes (using CD19-MACS-beads).

The example datasets contain reduced (10.000 cell-events) of the first Flow Cytometry (FC) sample in `dat.fcs` and the *immunoClust* cell-clustering results of all 5 reduced FC samples for the first donor in `dat.exp`. The full sized dataset is published and available under <http://flowrepository.org/id/FR-FCM-ZZWB>.

### 4.1 Cell Event Clustering

```
> library(immunoClust)
```

The cell-clustering is performed by the `cell.process` function for each FC sample separately. Its major input are the measured cell-events in a *flowFrame*-object imported from the *flowCore*-package.

```
> data(dat.fcs)
> dat.fcs

flowFrame object '2d36b4cf-da0f-4b8d-9a4c-fc7e4f5fccc8'
with 10000 cells and 7 observables:
      name desc  range  minRange maxRange
$P2      FSC-A <NA> 262144    0.00000  262143
$P5      SSC-A <NA> 262144 -111.00000  262143
$P8      FITC-A CD14 262144 -111.00000  262143
$P9       PE-A CD19 262144 -111.00000  262143
$P12     APC-A CD15 262144 -111.00000  262143
$P13    APC-Cy7-A CD4 262144 -111.00000  262143
$P14 Pacific Blue-A CD3 262144 -98.93999  262143
171 keywords are stored in the 'description' slot
```

In the `parameters` argument the parameters (named as observables in the *flowFrame*) used for cell-clustering are specified. When omitted all determined parameters are used.

```
> pars=c("FSC-A", "SSC-A", "FITC-A", "PE-A", "APC-A", "APC-Cy7-A", "Pacific Blue-A")
> res.fcs <- cell.process(dat.fcs, parameters=pars)
```

The `summary` method for an *immunoClust*-object gives an overview of the clustering results.

```
> summary(res.fcs)

** Experiment Information **
Experiment name: immunoClust Experiment
Data Filename:   fcs/12443.fcs
```

## immunoClust

```
Parameters:  FSC-A SSC-A FITC-A PE-A APC-A APC-Cy7-A Pacific Blue-A
Description:  NA NA CD14 CD19 CD15 CD4 CD3

** Data Information **
Number of observations: 10000
Number of parameters:  7
Removed from above:    318 (3.18%)
Removed from below:    0 (0%)

** Transformation Information **
htrans-A:  0.000000 0.000000 0.010000 0.010000 0.010000 0.010000 0.010000
htrans-B:  0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000
htrans-decade:  -1

** Clustering Summary **
ICL bias: 0.30
Number of clusters: 13
Cluster   Proportion  Observations
    1      0.037370         367
    2      0.054586         523
    3      0.006003          58
    4      0.040367         391
    5      0.035039         341
    6      0.015410         149
    7      0.007836          79
    8      0.114686        1112
    9      0.271418        2537
   10      0.028004         280
   11      0.015558         149
   12      0.007284          70
   13      0.366438        3626

    Min.      0.006003          58
    Max.      0.366438        3626

** Information Criteria **
Log likelihood: -253652.8 -254974.9 -173649.5
BIC: -253652.8
ICL: -254974.9
```

With the `bias` argument of the `cell.process` function the number of clusters in the final model is controlled.

```
> res2 <- cell.process(dat.fcs, bias=0.25)
> summary(res2)

** Experiment Information **
Experiment name: immunoClust Experiment
Data Filename:  fcs/12443.fcs
Parameters:  FSC-A SSC-A FITC-A PE-A APC-A APC-Cy7-A Pacific Blue-A
Description:  NA NA CD14 CD19 CD15 CD4 CD3
```

```

** Data Information **
Number of observations: 10000
Number of parameters: 7
Removed from above: 318 (3.18%)
Removed from below: 0 (0%)

** Transformation Information **
htrans-A: 0.000000 0.000000 0.010000 0.010000 0.010000 0.010000 0.010000
htrans-B: 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000
htrans-decade: -1

** Clustering Summary **
ICL bias: 0.25
Number of clusters: 19

```

Cluster	Proportion	Observations
1	0.036971	367
2	0.054996	523
3	0.005985	58
4	0.008861	79
5	0.031426	311
6	0.035095	343
7	0.015409	149
8	0.007791	79
9	0.063226	632
10	0.048978	458
11	0.187527	1810
12	0.163055	1473
13	0.028275	291
14	0.009565	93
15	0.001858	17
16	0.001352	13
17	0.005394	49
18	0.007275	70
19	0.286961	2867
Min.	0.001352	13
Max.	0.286961	2867

```

** Information Criteria **
Log likelihood: -253740.7 -255874.5 -173560.5
BIC: -253740.7
ICL: -255874.5

```

An ICL-bias of 0.3 is reasonable for fluorescence cytometry data based on our experiences, whereas the number of clusters increase dramatically when a `bias` below 0.2 is applied. A principal strategy for the ICL-bias in the whole pipeline is the use of a moderately small `bias` (0.2 - 0.3) for cell-clustering and to optimise the `bias` on meta-clustering level to retrieve the common populations across all samples.

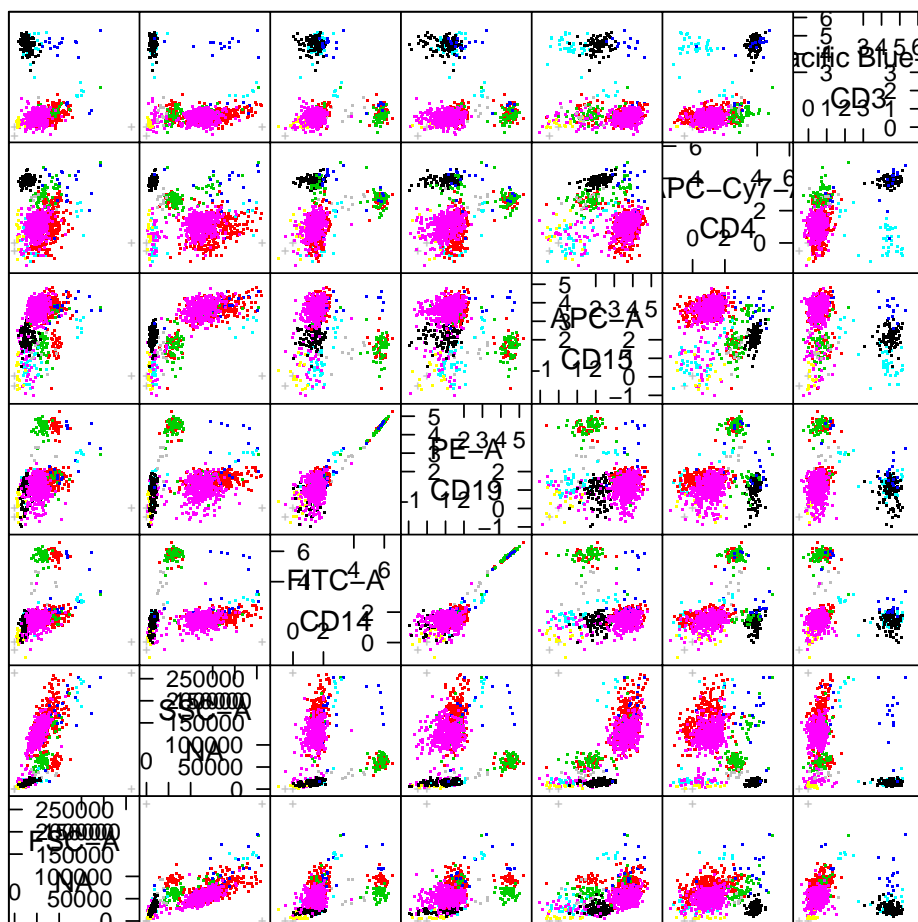
For plotting the clustering results on cell event level, the optimised *asinh*-transformation has to be applied to the raw FC data first.

## immunoClust

```
> dat.transformed <- trans.ApplyToData(res.fcs, dat.fcs)
```

A scatter plot matrix of all used parameters for clustering is obtained by the `splom` method.

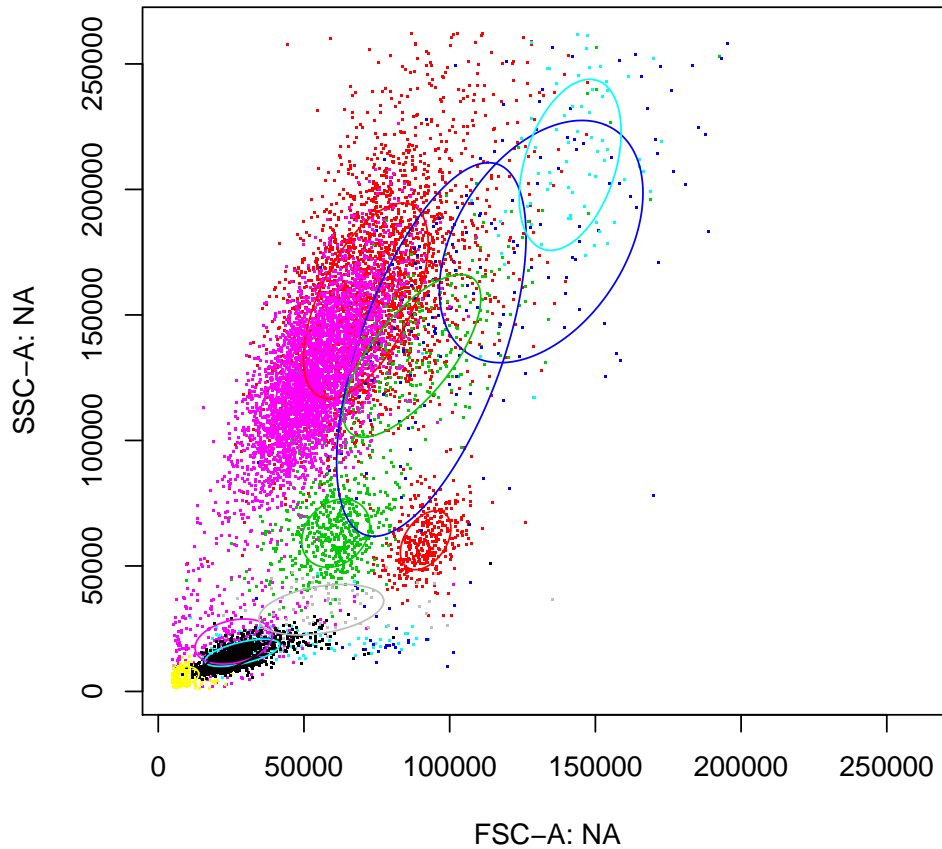
```
> splom(res.fcs, dat.transformed, N=1000)
```



Scatter Plot Matrix

For a scatter plot of 2 particular parameters the `plot` method can be used, where parameters of interest are specified in the `subset` argument.

```
> plot(res.fcs, data=dat.transformed, subset=c(1,2))
```



## 4.2 Meta Clustering

For meta-clustering the cell-clustering results of all FC samples obtained by the `cell.process` function are collected in a vector of *immunoClust*-objects and processed by the `meta.process` function.

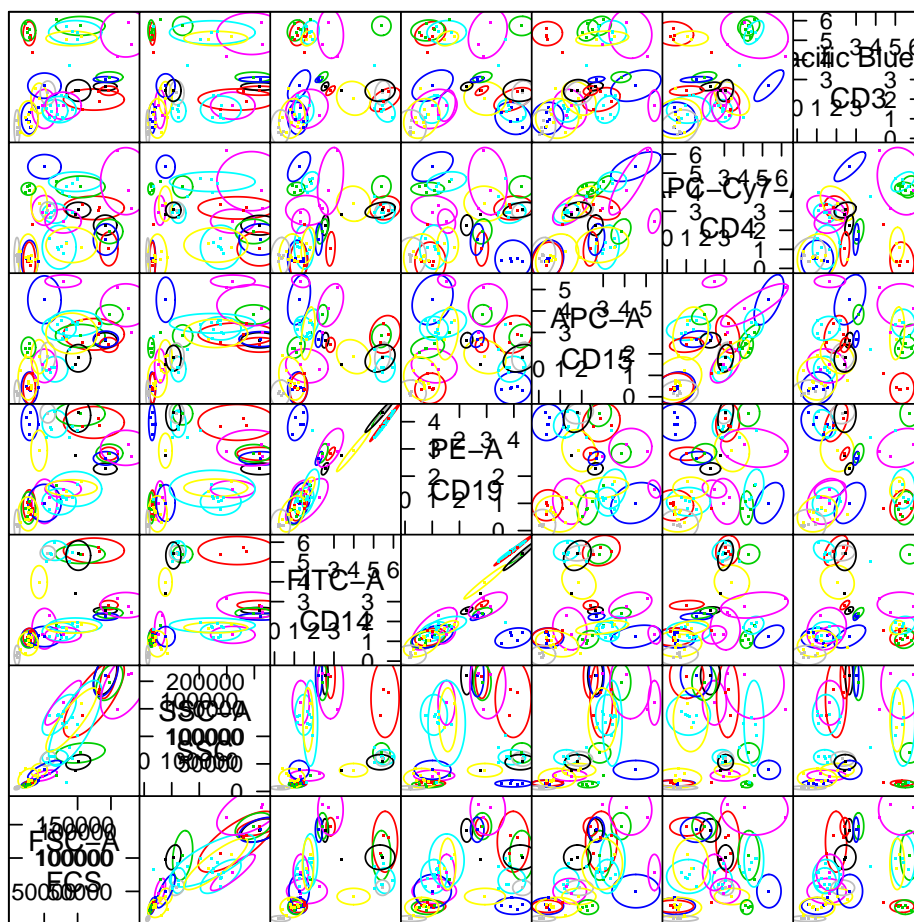
```
> data(dat.exp)
> meta<-meta.process(dat.exp, meta.bias=0.3, scatter.subset=c(1,2))
```

Scatter Gating

The obtained `list`-object contains the meta-clustering result in `$res.clusters`, and the used cell-clusters information in `$dat.clusters`. Additionally a meta-clustering using only the scatter parameter is performed within the `meta.process` function with results in `$res.scatter` and `$dat.scatter`. In a preliminary state of development an automated hierarchical gating on the meta-cluster is performed with results in `$gating`.

A scatter plot matrix of the meta-clustering is again obtained by the `splom` method.

```
> splom(meta$res.clusters, meta$dat.clusters$M, ellipse=TRUE)
```



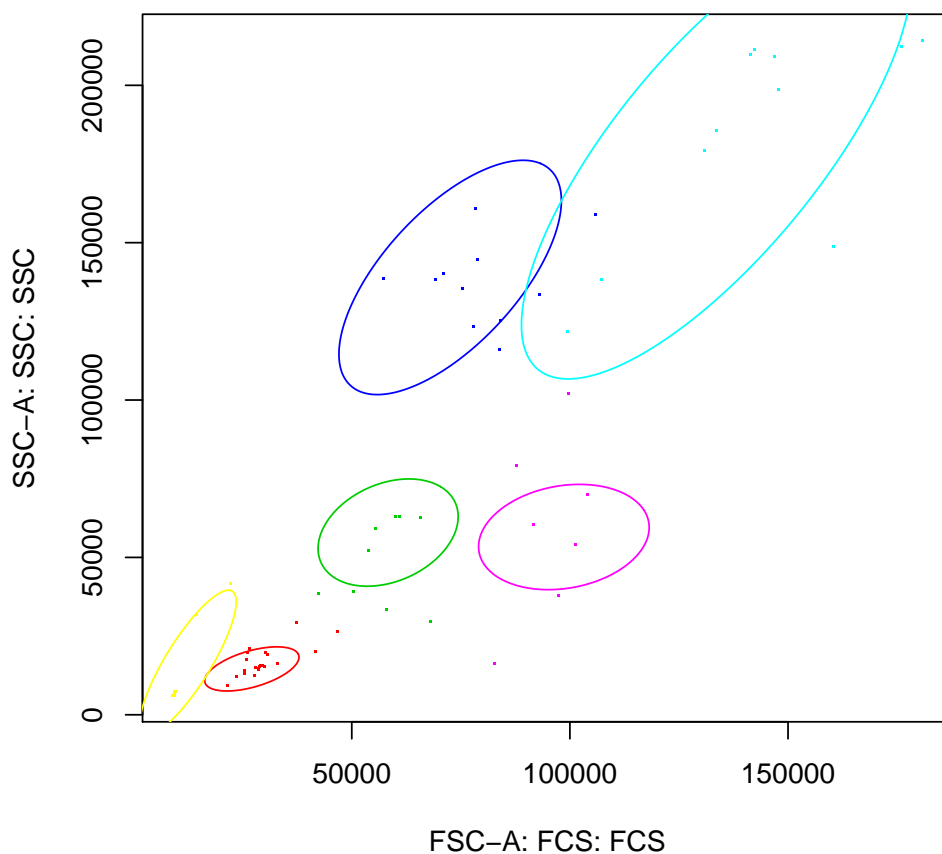
Scatter Plot Matrix

In these scatter plots each cell-cluster is marked by a point of its centre. With the `ellipse=TRUE` argument the meta-clusters are outlined by ellipses of the 90% quantile.

A scatter plot of the scatter parameter distribution of the cell-clusters is obtained by the `plot` method using `$res.scatter`.

```
> plot(meta$res.scatter, data=meta$dat.scatter$M)
```





The scatter distribution is divided into 6 major regions P1 - P6 with default colors red, green, blue, cyan, magenta and yellow.

The event numbers of each meta-cluster and each sample are extracted in a numeric matrix by the `meta.numEvents` function.

```
> tbl <- meta.numEvents(meta, out.all=FALSE)
> tbl[,1:5]
```

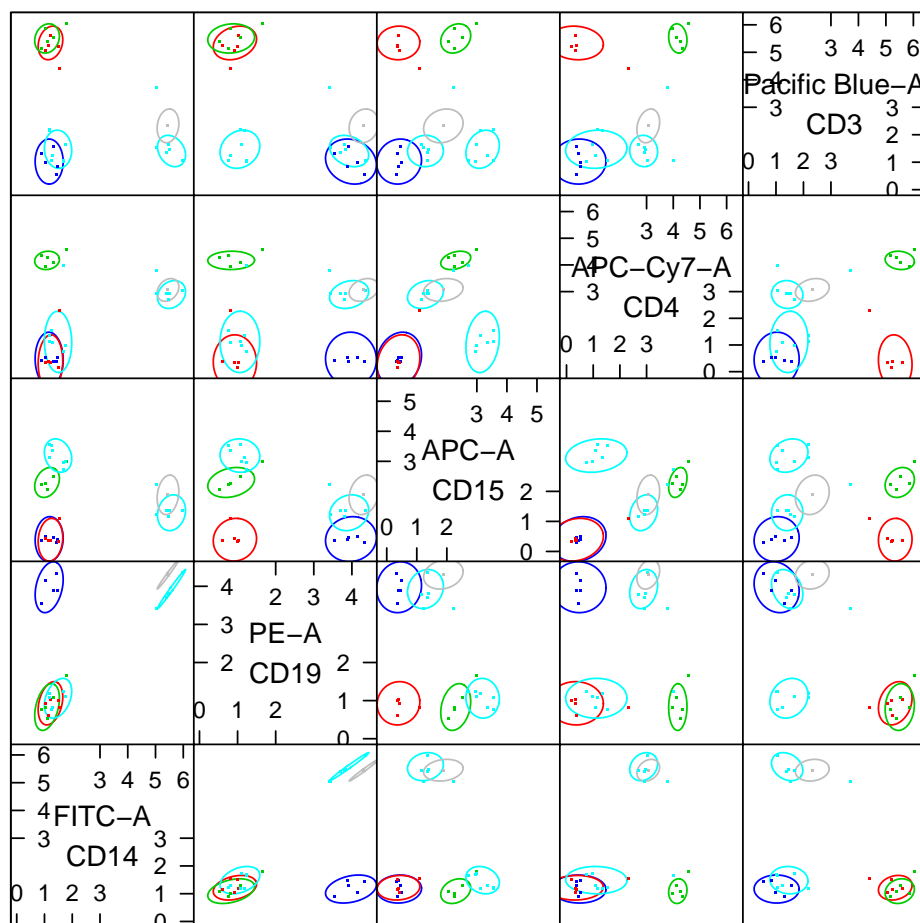
	12543	12546	12549
1.1.1.1.all_P1_CD3neg_CD19neg_CD4neg.14.yellow	344	695	780
1.1.1.2.all_P1_CD3neg_CD19neg_CD4pos.5.magenta	71	145	0
1.1.2.all_P1_CD3neg_CD19pos.3.blue	0	926	452
1.2.1.all_P1_CD3pos_CD4neg.9.red	389	1079	574
1.2.2.all_P1_CD3pos_CD4pos.10.green3	1107	3425	1585
2.1.all_P2_CD14neg.11.blue	0	220	0
2.2.1.all_P2_CD14pos_CD3neg.4.cyan	898	0	0
2.2.2.all_P2_CD14pos_CD3pos.7.gray	0	1447	0
2.3.all_P2_CD14dim.6.yellow	0	173	0
3.1.1.all_P3_CD3neg_CD15neg.12.cyan	6459	0	5717
3.1.2.all_P3_CD3neg_CD15pos.13.magenta	0	24	0

3.2.1.all_P3_CD3pos_CD4neg.22.yellow	0	0	40
3.2.2.all_P3_CD3pos_CD4pos.20.cyan	143	8	199
4.1.1.1.1.all_P4_CD14neg_CD3neg_CD15neg_CD19neg.16.black	70	0	0
4.1.1.1.2.all_P4_CD14neg_CD3neg_CD15neg_CD19pos.17.red	0	0	0
4.1.1.1.2.all_P4_CD14neg_CD3neg_CD15neg_CD19pos.19.blue	0	0	132
4.1.1.2.all_P4_CD14neg_CD3neg_CD15pos.18.green3	0	77	0
4.1.2.all_P4_CD14neg_CD3pos.21.magenta	0	103	10
4.1.2.1.all_P4_CD14neg_CD3pos.21.magenta_CD15pos.21.magenta	0	103	10
4.2.all_P4_CD14pos.1.red	50	0	0
5.1.all_P5_CD3neg.8.black	0	923	0
5.2.all_P5_CD3pos.2.green3	0	102	0
6.all_P6.15.gray	151	495	247
	12552	12555	
1.1.1.1.all_P1_CD3neg_CD19neg_CD4neg.14.yellow	527	400	
1.1.1.2.all_P1_CD3neg_CD19neg_CD4pos.5.magenta	0	0	
1.1.2.all_P1_CD3neg_CD19pos.3.blue	331	325	
1.2.1.all_P1_CD3pos_CD4neg.9.red	433	46	
1.2.2.all_P1_CD3pos_CD4pos.10.green3	0	0	
2.1.all_P2_CD14neg.11.blue	0	0	
2.2.1.all_P2_CD14pos_CD3neg.4.cyan	761	950	
2.2.2.all_P2_CD14pos_CD3pos.7.gray	0	0	
2.3.all_P2_CD14dim.6.yellow	0	0	
3.1.1.all_P3_CD3neg_CD15neg.12.cyan	7280	7417	
3.1.2.all_P3_CD3neg_CD15pos.13.magenta	0	0	
3.2.1.all_P3_CD3pos_CD4neg.22.yellow	0	0	
3.2.2.all_P3_CD3pos_CD4pos.20.cyan	0	0	
4.1.1.1.1.all_P4_CD14neg_CD3neg_CD15neg_CD19neg.16.black	0	0	
4.1.1.1.2.all_P4_CD14neg_CD3neg_CD15neg_CD19pos.17.red	95	0	
4.1.1.1.2.all_P4_CD14neg_CD3neg_CD15neg_CD19pos.19.blue	0	0	
4.1.1.2.all_P4_CD14neg_CD3neg_CD15pos.18.green3	0	0	
4.1.2.all_P4_CD14neg_CD3pos.21.magenta	0	0	
4.1.2.1.all_P4_CD14neg_CD3pos.21.magenta_CD15pos.21.magenta	0	0	
4.2.all_P4_CD14pos.1.red	62	94	
5.1.all_P5_CD3neg.8.black	0	0	
5.2.all_P5_CD3pos.2.green3	0	0	
6.all_P6.15.gray	247	278	

Each row denotes a meta-cluster and each column a data sample used for meta-clustering. The row names give the automated generated gating name, the meta-cluster index and the default color used in the plot routines for each meta-cluster. With an argument `out.all=TRUE` additionally the event numbers in each gating hierarchy level are extracted. In the last columns additionally the meta-cluster centre values in each parameter are given, which helps to identify the meta-clusters. Further export functions retrieve relative cell event frequencies and sample meta-cluster centre values in a particular parameter.

Picking the meta-clusters of the five commonly found population, with respect to the technical depletion the scatter plot matrix reduces to

```
> splom(meta$res.clusters, meta$dat.clusters$M,
+ include=c(3,9,10,4,7,12), subset=3:7, ellipse=TRUE)
```



Scatter Plot Matrix

The whole analysis is performed on uncompensated FC data, thus the high CD19 values on the CD14-population is explained by spillover of FITC into PE. The variation of the CD3 expression in the CD14-population of sample 12546 is caused artificially due to depletion of the granulocytes, which constitute about 60% - 75% of the cells in the other samples.

## 5 Session Info

The documentation and example output was compiled and obtained on the system:

```
> toLatex(sessionInfo())
```

- R version 3.5.1 Patched (2018-07-12 r74967), x86\_64-apple-darwin15.6.0
- Locale: C/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Running under: OS X El Capitan 10.11.6
- Matrix products: default

- BLAS:  
/Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
- LAPACK:  
/Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, utils
- Other packages: flowCore 1.48.0, immunoClust 1.14.1, lattice 0.20-38
- Loaded via a namespace (and not attached): Biobase 2.42.0, BiocGenerics 0.28.0, BiocManager 1.30.4, BiocStyle 2.10.0, DEoptimR 1.0-8, MASS 7.3-51.1, Rcpp 1.0.0, backports 1.1.2, cluster 2.0.7-1, compiler 3.5.1, corpcor 1.6.9, digest 0.6.18, evaluate 0.12, graph 1.60.0, htmltools 0.3.6, knitr 1.20, matrixStats 0.54.0, mvtnorm 1.0-8, parallel 3.5.1, pcaPP 1.9-73, rmarkdown 1.10, robustbase 0.93-3, rprojroot 1.3-2, rrcov 1.4-7, stats4 3.5.1, tools 3.5.1, yaml 2.2.0