

# immunoClust - Automated Pipeline for Population Detection in Flow Cytometry

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## Contents

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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 assorts 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

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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
```

```
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 **
```

```
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 \*\***

Number of clusters: 16

Cluster	Proportion	Observations
1	0.091783	890
2	0.005886	57
3	0.008490	77
4	0.031803	313
5	0.033960	330
6	0.015891	157
7	0.008038	78
8	0.066041	643
9	0.048068	462
10	0.212217	1803
11	0.009426	91
12	0.002096	20
13	0.001494	15
14	0.003245	31
15	0.007413	72

16	0.454149	4643
Min.	0.001494	15
Max.	0.454149	4643

**\*\* Information Criteria \*\***

Log likelihood: -254122.5 -255397.4 -173577.5

BIC: -254122.5

ICL: -255397.4

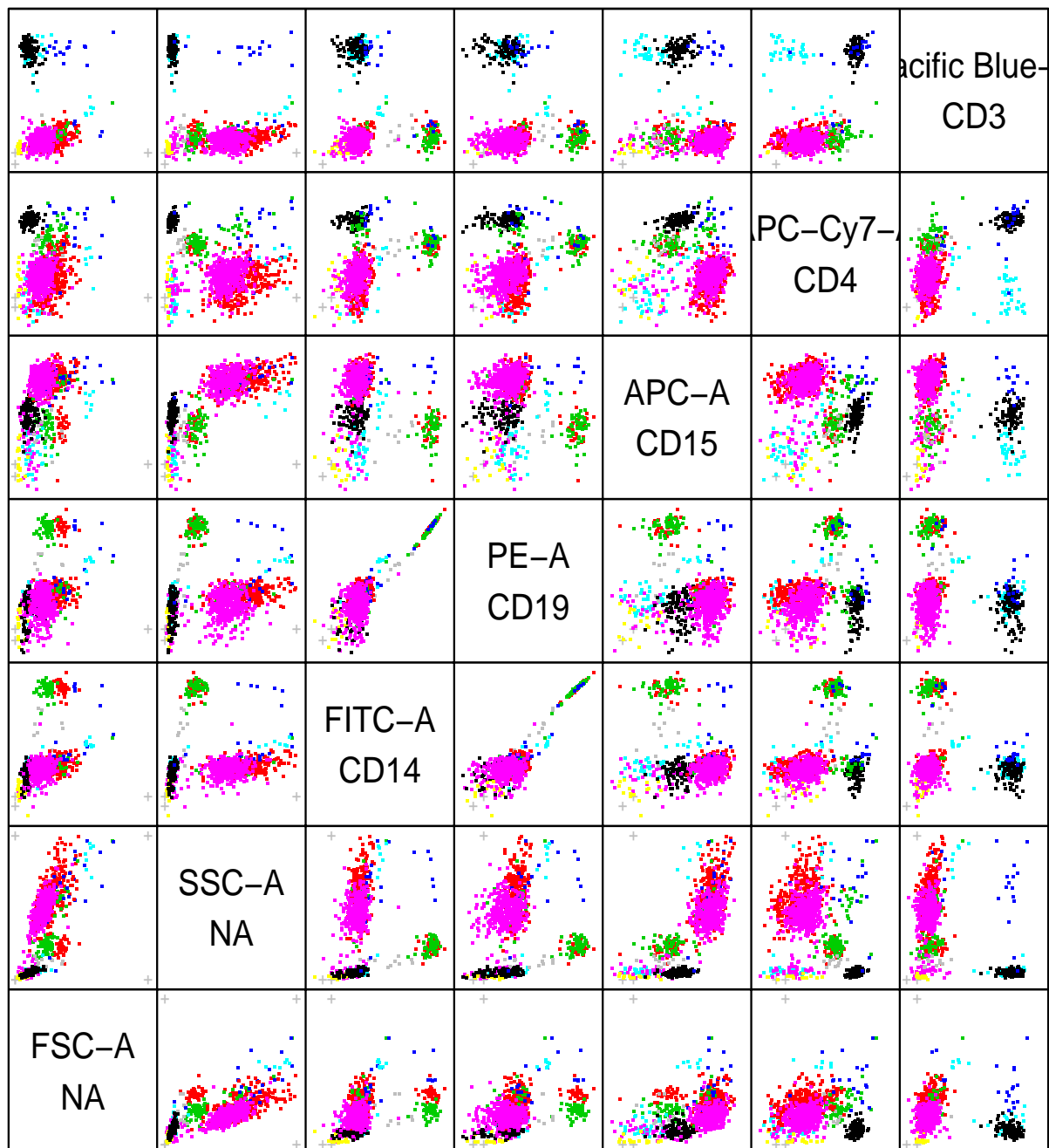
An ICL-bias of 0.3 is reasonable for fluorescence cytometry data based on our experiences, whereas the number of clusters increase dramatically when a bias below 0.2 is applied. A principal strategy for the ICL-bias in the whole pipeline is the use of a moderately small bias (0.2 - 0.3) for cell-clustering 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.

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

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

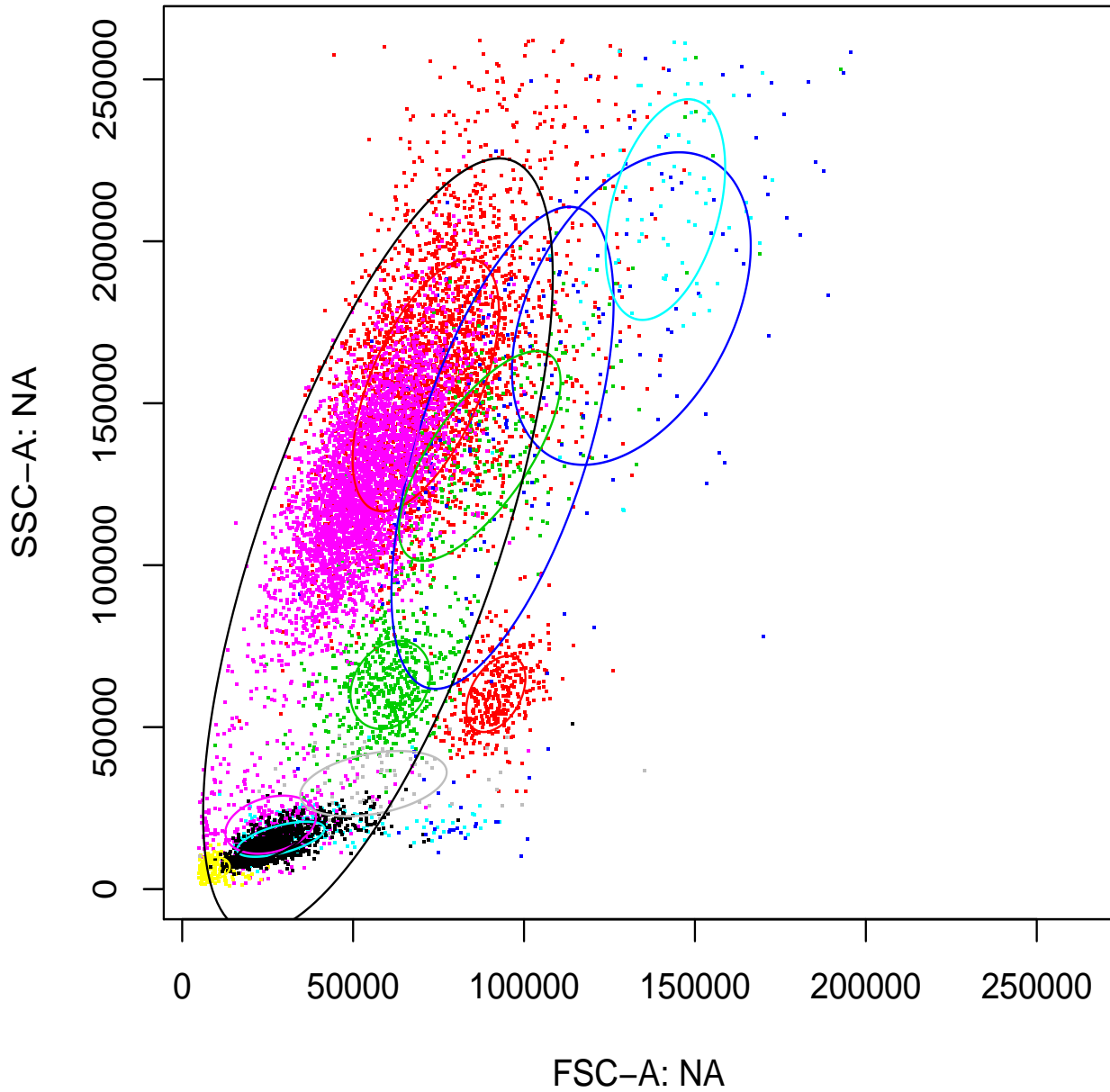
```
> splom(res.fcs, dat.transformed, N=1000)
```



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.

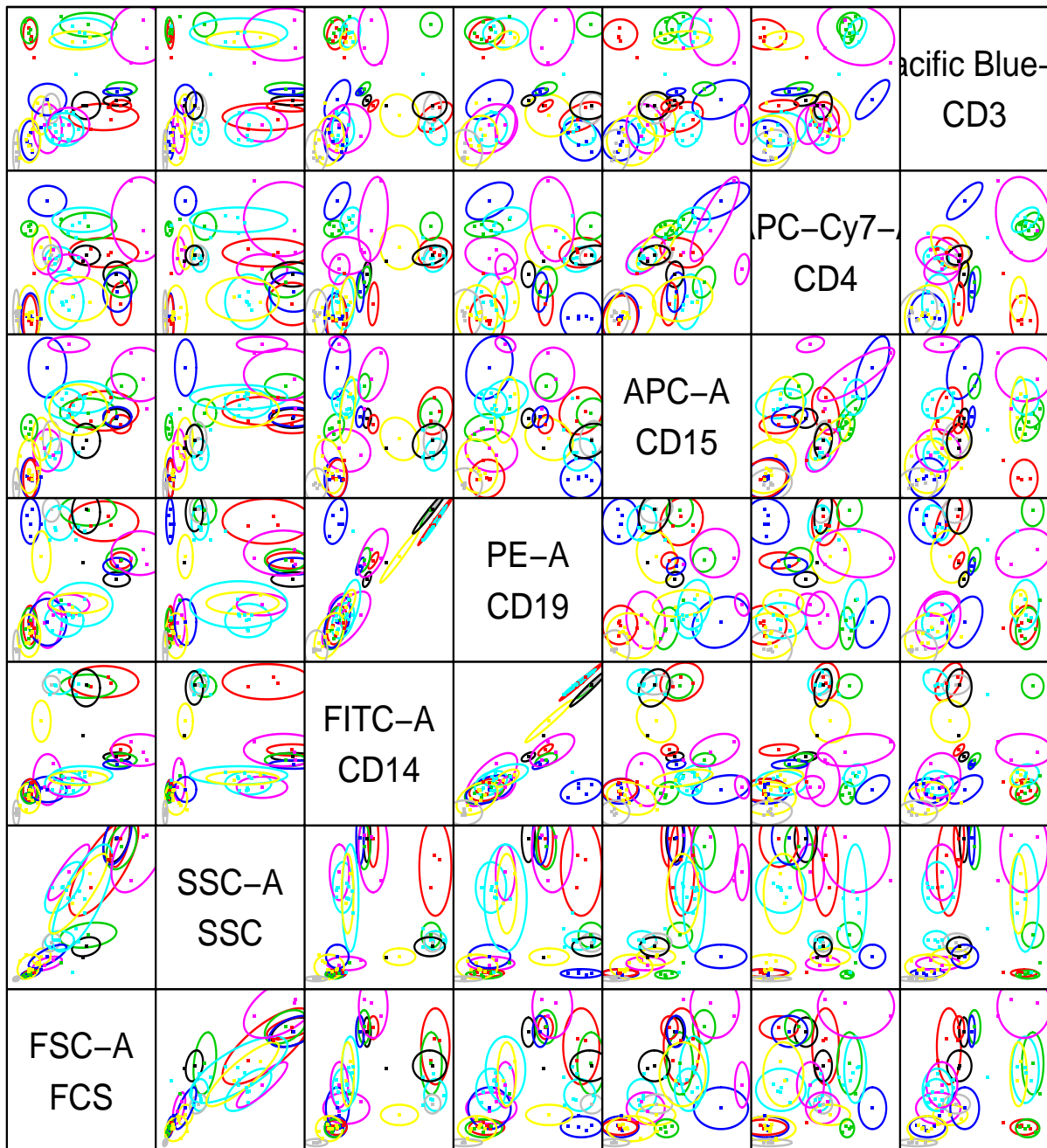
```
> data(dat.exp)
> meta<-meta.process(dat.exp, meta.bias=0.3, scatter.subset=c(1,2))
```

### 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)
```

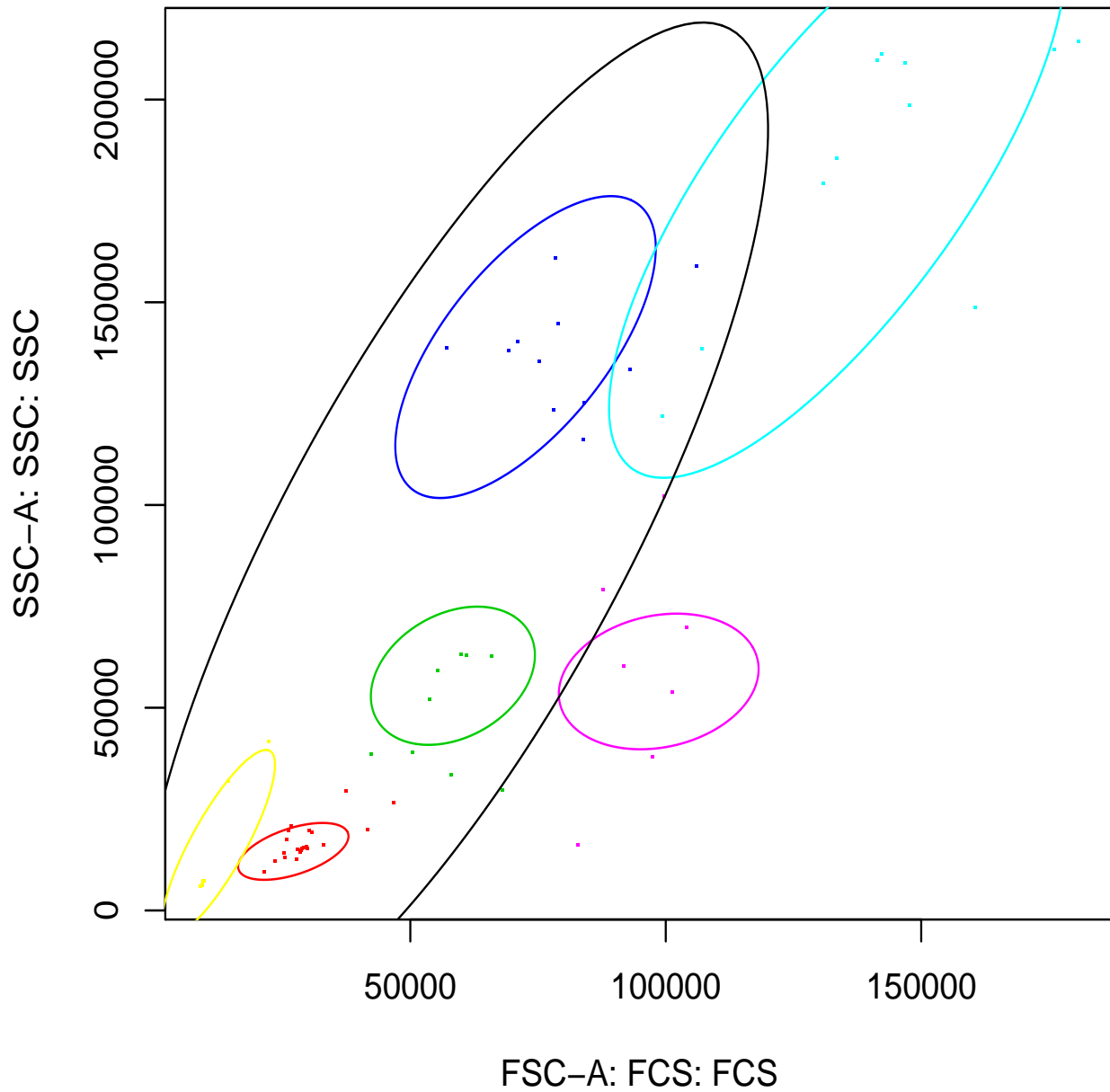


Scatter Plot Matrix

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]
```

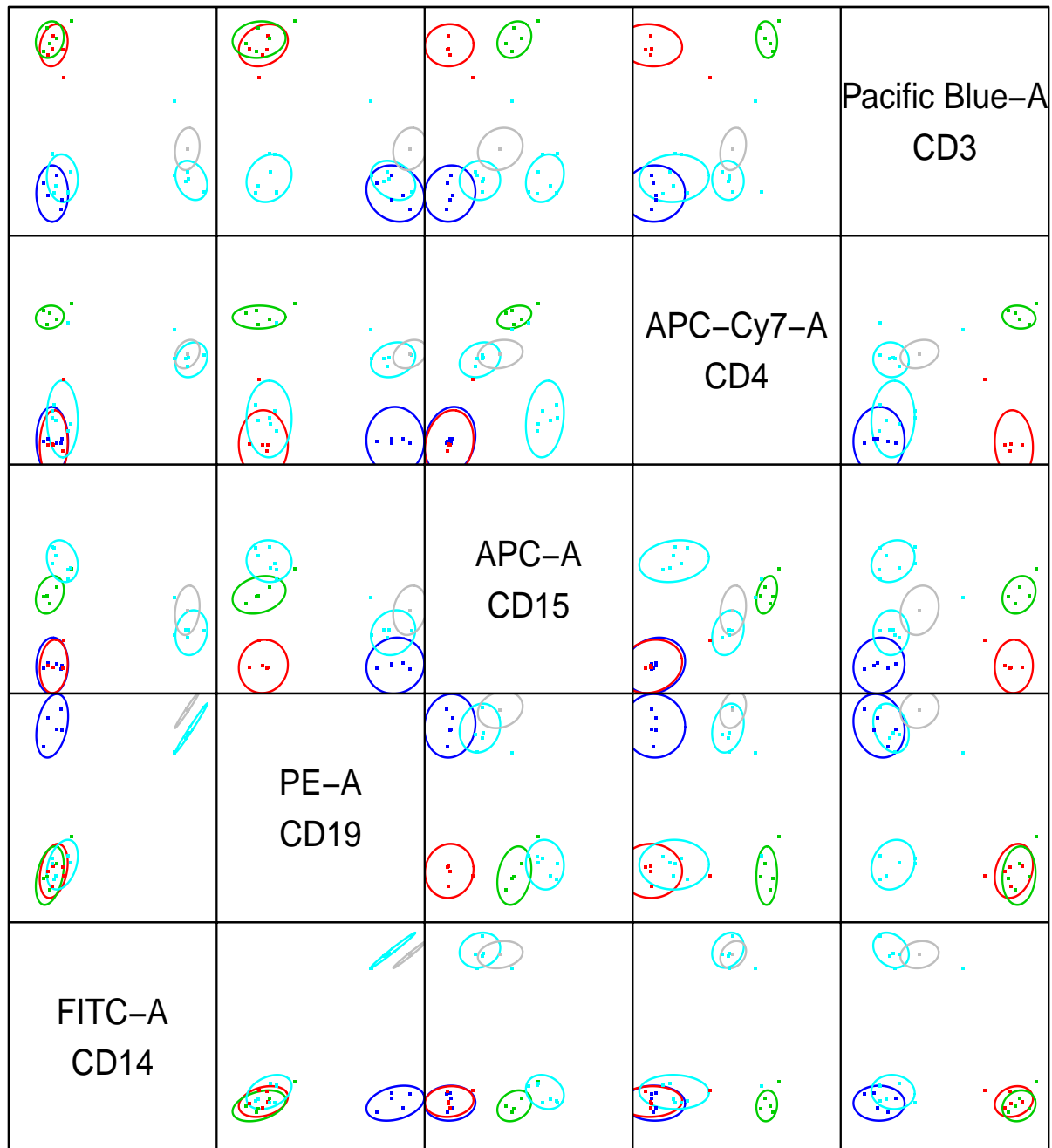
```
12543 12546 12549 12552 12555
```

1.1.1.1.P1_CD3neg_CD19neg_CD4neg.14.yellow	344	695	780	527	400
1.1.1.2.P1_CD3neg_CD19neg_CD4pos.5.magenta	71	145	0	0	0
1.1.2.P1_CD3neg_CD19pos.3.blue	0	926	452	331	325
1.2.1.P1_CD3pos_CD4neg.9.red	389	1079	574	433	46
1.2.2.P1_CD3pos_CD4pos.10.green3	1107	3425	1585	0	0
2.1.P2_CD14neg.11.blue	0	220	0	0	0
2.2.1.P2_CD14pos_CD3neg.4.cyan	898	0	0	761	950
2.2.2.P2_CD14pos_CD3pos.7.gray	0	1447	0	0	0
2.3.P2_CD14dim.6.yellow	0	173	0	0	0
3.1.1.P3_CD3neg_CD15neg.12.cyan	6459	0	5717	7280	7417
3.1.2.P3_CD3neg_CD15pos.13.magenta	0	24	0	0	0
3.2.1.P3_CD3pos_CD4neg.22.yellow	0	0	40	0	0
3.2.2.P3_CD3pos_CD4pos.20.cyan	143	8	199	0	0
4.1.1.1.1.P4_CD14neg_CD3neg_CD15neg_CD19neg.16.black	70	0	0	0	0
4.1.1.1.2.P4_CD14neg_CD3neg_CD15neg_CD19pos.17.red	0	0	0	95	0
4.1.1.1.2.P4_CD14neg_CD3neg_CD15neg_CD19pos.19.blue	0	0	132	0	0
4.1.1.2.P4_CD14neg_CD3neg_CD15pos.18.green3	0	77	0	0	0
4.1.2.P4_CD14neg_CD3pos.21.magenta	0	103	10	0	0
4.1.2.1.P4_CD14neg_CD3pos.21.magenta_CD15pos.21.magenta	0	103	10	0	0
4.2.P4_CD14pos.1.red	50	0	0	62	94
5.1.P5_CD3neg.8.black	0	923	0	0	0
5.2.P5_CD3pos.2.green3	0	102	0	0	0
6.P6.15.gray	151	495	247	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

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

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

- R version 3.3.1 (2016-06-21), x86\_64-apple-darwin13.4.0
- Locale: C/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, utils
- Other packages: flowCore 1.40.0, immunoClust 1.6.0, lattice 0.20-34
- Loaded via a namespace (and not attached): Biobase 2.34.0, BiocGenerics 0.20.0, BiocStyle 2.2.0, DEoptimR 1.0-6, Rcpp 0.12.7, cluster 2.0.5, corpcor 1.6.8, graph 1.52.0, matrixStats 0.51.0, mvtnorm 1.0-5, parallel 3.3.1, pcaPP 1.9-61, robustbase 0.92-6, rrcov 1.4-3, stats4 3.3.1, tools 3.3.1