flowClean

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Introduction

This package contains the flowCore method for performing quality control on flow cytomery datasets. This method is described in [1].

```
> library(flowClean)
> library(flowViz)
> library(grid)
> library(gridExtra)
```

Data

Example data is a real FCS file in which we intentionally perturbed the fluorescent intensity (FI) of a subset of cells along the V705 channel ('<V705-A>').

```
> data(synPerturbed)
> synPerturbed
```

```
flowFrame object '9301d9e4-a160-477f-a5fb-ee7d785d5655'
with 76466 cells and 17 observables:
         name        desc     range  minRange  maxRange
$P1     FSC-A          NA    262144    0.0000    262144
$P2     FSC-H          NA    262144    0.0000    262144
$P3     SSC-A          NA    261589    0.0000    261589
$P4      Time          NA      2048    0.0000      2048
$P5  <B515-A>   CD27 FITC    260954  -26.8846    260954
...       ...         ...       ...       ...       ...
$P13 <V450-A> CD127 BV421    260964  -35.9838    260964
$P14 <V545-A>      Aq Blu    260949  -22.2072    260949
$P15 <V585-A>   CD8 QD585    261965 -111.0000    261965
$P16 <R660-A>  CD45RA APC    261023  -96.5092    261023
$P17 <V605-A>   CD4 BV605    261131 -111.0000    261131
212 keywords are stored in the 'description' slot
```

Quality Control

The full details are available in [1]. The motivating idea for this methodology is that populations in a flow experiment should be collected nearly uniformly with respect to time of collection. The primary actor in flowClean is the `clean`, which tests for deviations from uniformity of collection. Specifically, the collection time is discretized into l periods, each of which can be considered a N-part composition

$$D_{j=1..l} = [P_1, P_2, \ldots, P_N]$$

with each P_i the frequency of a population defined as +/- with respect to some threshold; the default is the median FI of a flow parameter. By default $l = 100$.

Each D_j then udnergoes the centered log ratio (CLR) transformation [2]:

$$CLR(D_j) = \left[ln\frac{P_1}{g(D_j)}; \ldots; ln\frac{P_N}{g(D_j)} \right]$$

where

$$g(D_j) = \sqrt[N]{P_1 P_2 ... P_N}$$

To avoid `-Inf` values, substitution of zeroes is performed using the 'modified Aitchison' of [3].

The L_p norm of the subset $CLR(D_j) > 0$, denoted $L_p = \|CLR(D_j)\|^+$, where $p = |CLR(D_j) > 0|$, is then calculated for each D_j and changepoint analysis is performed on the set of all $\|CLR(D_j)\|^+$. If there are no changes then the FCS is assumed to contain no errors. Otherwise, the means of the periods are compared relative to the mean of the longest period between changepoints and thresholded according to some k, which empirically works well with $k = 1.3$.

Actually calling `clean` requires only specifying a flowFrame, which markers are to be analyzed (generally without the 'scatter' parameters), the name to be given to the output (directory structure can be included) and the file extension:

```
> synPerturbed.c <- clean(synPerturbed, vectMarkers=c(5:16),
+                     filePrefixWithDir="sample_out", ext="fcs", diagnostic=TRUE)

[1] "flowClean has identified problems in synPerturbed.FCS with 24, 25, 26, 27, 28,

> synPerturbed.c

flowFrame object '9301d9e4-a160-477f-a5fb-ee7d785d5655'
with 76466 cells and 18 observables:
          name       desc     range  minRange  maxRange
$P1      FSC-A         NA    262144    0.0000    262144
$P2      FSC-H         NA    262144    0.0000    262144
$P3      SSC-A         NA    261589    0.0000    261589
$P4       Time         NA      2048    0.0000      2048
$P5   <B515-A>  CD27 FITC    260954  -26.8846    260954
```

```
...        ...         ...        ...        ...        ...
$P14  <V545-A>     Aq Blu    260949  -22.2072    260949
$P15  <V585-A>  CD8 QD585    261965 -111.0000    261965
$P16  <R660-A> CD45RA APC    261023  -96.5092    261023
$P17  <V605-A>  CD4 BV605    261131 -111.0000    261131
18   GoodVsBad  GoodVsBad    262144    0.0000    262143
222 keywords are stored in the 'description' slot
```

The result is an FCS file identical to the input file with a new parameter, 'GoodVsBad', in which 'Good' cells all are given $FI < 10000$ and 'Bad' cells are given $FI \geq 10000$, which allows for easy programmatic gating out of 'Bad' cells from multiple FCS files. This parameter can also be used in plots as any other flow parameter as well.

```
> lgcl <- estimateLogicle(synPerturbed.c, unname(parameters(synPerturbed.c)$name[5
> synPerturbed.cl <- transform(synPerturbed.c, lgcl)
> p1 <- xyplot(`<V705-A>` ~ `Time`, data=synPerturbed.cl,
+              abs=TRUE, smooth=FALSE, alpha=0.5, xlim=c(0, 100))
> p2 <- xyplot(`GoodVsBad` ~ `Time`, data=synPerturbed.cl,
+              abs=TRUE, smooth=FALSE, alpha=0.5, xlim=c(0, 100), ylim=c(0, 20000))
> rg <- rectangleGate(filterId="gvb", list("GoodVsBad"=c(0, 9999)))
> idx <- filter(synPerturbed.cl, rg)
> synPerturbed.clean <- Subset(synPerturbed.cl, idx)
> p3 <- xyplot(`<V705-A>` ~ `Time`, data=synPerturbed.clean,
+              abs=TRUE, smooth=FALSE, alpha=0.5, xlim=c(0, 100))
> grid.arrange(p1, p2, p3, ncol=3)
```

SessionInfo

- R version 4.5.0 beta (2025-04-02 r88102), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_GB`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Time zone: `America/New_York`
- TZcode source: `system (glibc)`
- Running under: `Ubuntu 24.04.2 LTS`
- Matrix products: default
- BLAS: `/home/biocbuild/bbs-3.22-bioc/R/lib/libRblas.so`

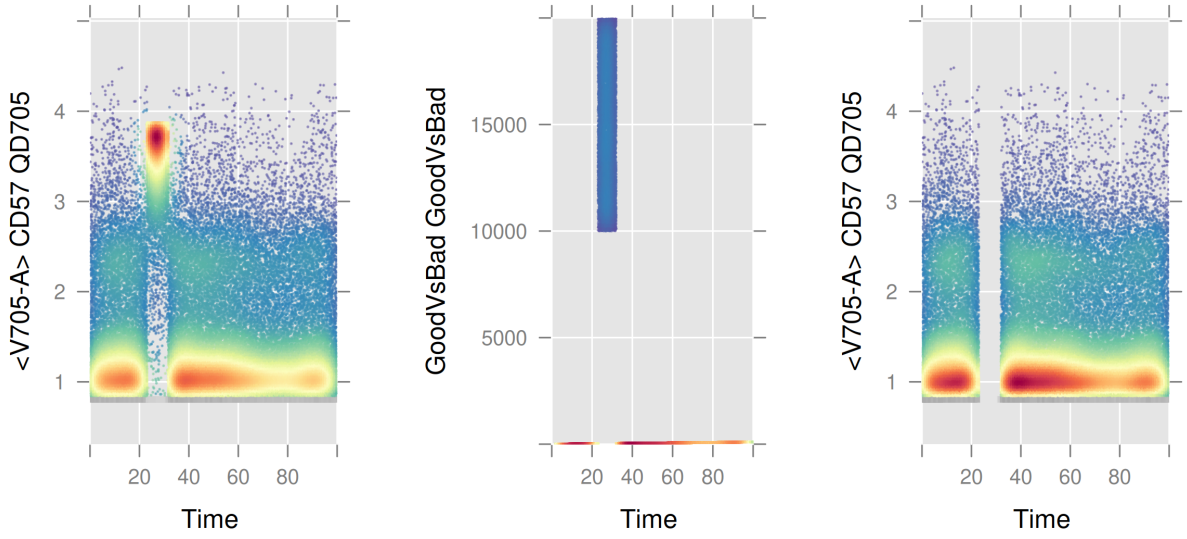

Figure 1: Left) FCS before flowClean. Center) New 'GoodVsBad' parameter. Right) FCS after flowClean and filtering.

- LAPACK: `/usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.12.0`
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, utils
- Other packages: flowClean 1.47.0, flowCore 2.21.0, flowViz 1.73.0, gridExtra 2.3, lattice 0.22-7
- Loaded via a namespace (and not attached): Biobase 2.69.0, BiocGenerics 0.55.0, IDPmisc 1.1.21, KernSmooth 2.23-26, MASS 7.3-65, RColorBrewer 1.1-3, RProtoBufLib 2.21.0, Rcpp 1.0.14, S4Vectors 0.47.0, bit 4.6.0, changepoint 2.3, cli 3.6.4, compiler 4.5.0, cytolib 2.21.0, deldir 2.0-4, generics 0.1.3, glue 1.8.0, gtable 0.3.6, hexbin 1.28.5, interp 1.1-6, jpeg 0.1-11, latticeExtra 0.6-30, lifecycle 1.0.4, matrixStats 1.5.0, png 0.1-8, rlang 1.1.6, sfsmisc 1.1-20, stats4 4.5.0, tools 4.5.0, zoo 1.8-14

References

[1] Fletez-Brant C, Spidlen J, Brinkman R, Roederer M, Chattopadhyay P. Quailty Control of flow cytometry data through compositional data analysis. In preparation.

[2] Aitchison J. A concise guide to compositional data analysis. Compositional Data Analysis Workshop; Girona, Italy.

[3] Fry J, Fry T, McLaren K. Compositional data analysis and zeros in micro data. CoPS/IMPACT Working Paper Number G-120.