

# High-throughput flow cytometry data and how to load, transform and visualise data and gate populations in Bioconductor (R)

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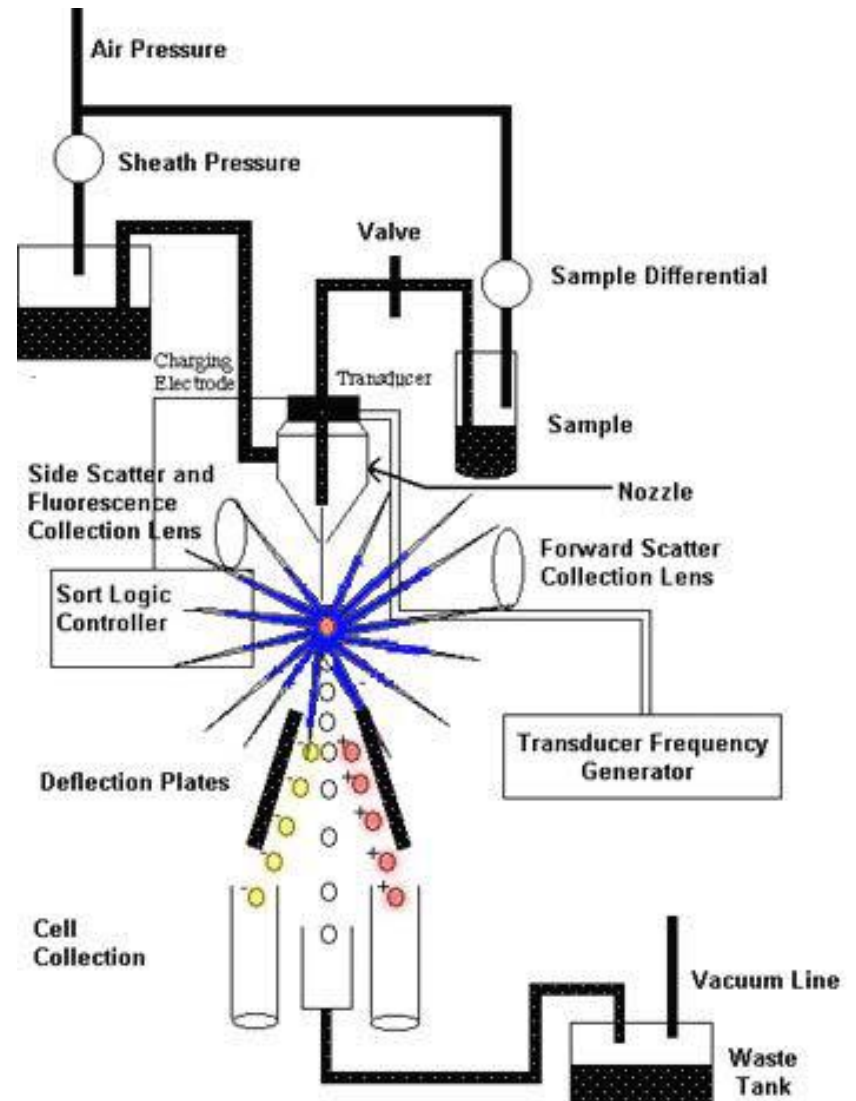
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# Flow Cytometry (FCM)

- **Flow Cytometry** is a technique for counting, examining and sorting microscopic particles suspended in a stream of fluid.



# Flow Cytometry

- Cells have been stained with monoclonal antibodies.
- Several Detectors are aimed at the point where the stream of fluid passes through a light beam and pick up the reflected/scattered light
- The detectors differ in what they measure:  
FSC (Forward Scatter) detector measures correlates with the cell volume and SSC (Side Scatter) measures correlate with the inner complexity of the particle

# High-throughput flow cytometry

- 21st Century technology
- large numbers of flow cytometric samples can be processed and analysed in a short period of time
- Challenge: high-dimensional complex data. Manual analysis time-consuming, subjective and error-prone  
→ Development of automatic gating methods

# Software for Analysis of Flow Cytometry Data

- Beckman Coulter Kaluza Software
- Cellular Symphony Flow Cytometry Software
- Millipore Flow Cytometry Software
- SPICE Data Mining & Visualization Software
- WEASEL Flow Cytometry Software
- ...

Today I'm only going to explain briefly how Flow Cytometry Data can be processed using

**Bioconductor**

# Bioconductor

- provides tools for the analysis and comprehension of high-throughput genomic data.
- uses the R statistical programming language, and is open source and open development

Installation of basic Bioconductor packages in R:

```
> source("http://bioconductor.org/biocLite.R")
```

```
> biocLite()
```

```
> biocLite("flowCore ")
```

```
> biocLite("curvHDR")
```

- install curvHDR package over the menu. This will also install the following packages: 'abind' 'akima' 'magic' 'locfit' 'ash' 'mvtnorm' 'feature' 'geometry' 'hdrcde' 'ks' 'misc3d' 'ptinpoly' 'rgl'

# Software packages in Bioconductor for flow cytometry data

- These packages use standard FCS files, including infrastructure, utilities, visualization and semi-automated gating methods for the analysis of flow cytometry data.
- [flowCore](#): Basic structures for flow cytometry data  
[flowViz](#): Visualization of flow cytometry  
[flowQ](#): Quality control for flow cytometry  
[flowStats](#): Statistical methods for the analysis of flow cytometry data  
[flowUtils](#): Utilities for flow cytometry  
[flowFP](#): Fingerprint generation of flow cytometry data, used to facilitate the application of machine learning and data mining tools for flow cytometry.  
[flowTrans](#): Profile maximum likelihood estimation of parameters for flow cytometry data transformations.  
[iFlow](#): Tool to explore and visualize flow cytometry



Algorithms for clustering flow cytometry data are found in these packages:

- [flowClust](#): Robust model-based clustering using a t-mixture model with Box-Cox transformation.
- [flowMeans](#): Identifies cell populations in Flow Cytometry data using non-parametric clustering and segmented-regression-based change point detection.
- [flowMerge](#): Merging of mixture components for model-based automated gating of flow cytometry data using the flowClust framework.
- [SamSPECTRAL](#): Given a matrix of coordinates as input, SamSPECTRAL first builds the communities to sample the data points.
- A typical workflow using the packages flowCore, flowViz, flowQ and flowStats is described in detail in [flowWorkflow.pdf](#). The data files used in the workflow can be downloaded from [here](#).

# Load the Data

Loading data is a complex step, that can take a long time. Flow cytometry experiments typically involve data from

- several patients
- several time points
- a number of antibody stain combinations

1. Understand the structure of the data

2. Read in the data. We decided to create for each patient a separate R workspace file (.Rdata)

# Format, and organising the data to read it into R

Time of this presentation is too short to give a detailed account so I will go only into some issues we encountered.

1. The available data is a time series. The number of days differ for each participant.
2. The days available differ for each study participant!
3. 10 different Antibody-combinations were used in our data.

# Transform the data, take out extremes

- Determine minima and maxima of each flow cytometry sample. Remove recordings that accumulate on the boundaries (usually upper boundaries)
- possibly transform samples to reduce their skewness  $x_{new} = \sinh^{-1}(x) = \log\left(x + \sqrt{x^2 + 1}\right)$

# Gating Flow Cytometry Data with curvHDR - Steps in the analysis process

**curvHDR** – package in R/Bioconductor for gating FCM data and displaying gates

Functions: **curvHDRfilter** and **plot**

The most important parameters of the function `curvHDRfilter` are the dataset `x`, `HDRlevel`, `growthFac` and `signifLevel`

- `x` a numerical vector or a matrix or data frame having 1-3 columns.
- `HDRlevel` number between 0 and 1 corresponding to the level of the highest density region within each high curvature region.
- `growthFac` growth factor parameter. High curvature regions are grown to have 'volume' `growthFac` times larger than the original region.
- `signifLevel` number between 0 and 1 corresponding to the significance level for curve region determination.

# Steps in the analysis process

## Defaults:

HDRlevel = 0.1

growthFac =  $5^{(d/2)}$  where  $d$  is the dimension of the input data;

signifLevel = 0.05

Start with trial values of signifLevel and growthFac and HDRlevel, set at defaults.

## Example:

```
xBiva <- cbind(c(rnorm(1000,-2),rnorm(1000,2)),  
              c(rnorm(1000,-2),rnorm(1000,2)))
```

```
gate2a <- curvHDRfilter(xBiva)
```

# Example of output

## Output:

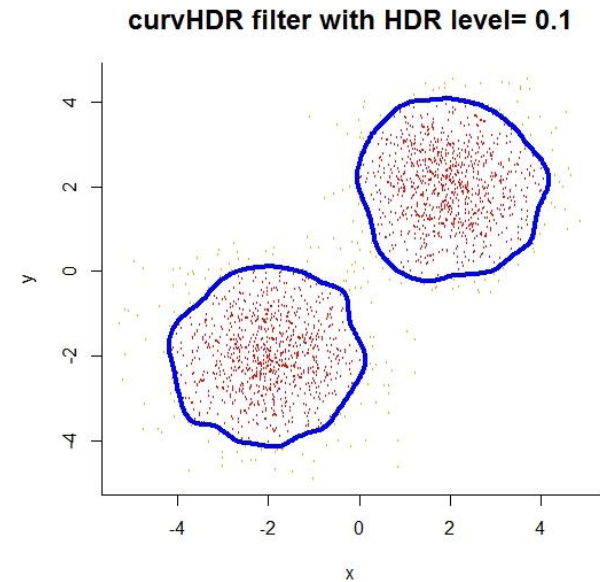
- data the input data (for use in plotting).
- insideFilter logical variable indicating the rows of the input data matrix corresponding to points inside the curvHDR filter.
- polys the curvHDR filter. Depending on the dimension  $d$  this is a list of intervals ( $d=1$ ), polygons ( $d=2$ ) or polyhedra ( $d=3$ ).
- HDRlevel highest density region level

`xBiva[gate2a$insideFilter,]` contains the data inside of the gate.

# Example of Output

We can visualise the output of curvHDRfilter with the plot command.

```
plot(gate2a)
```



In our actual dataset, we combined respectively 3 gates to obtain our final combined gates – a 2-dim gate on (FSC,SSC), a 1-dim gate Ab3, a 2-dim gate on (Ab1,Ab2). Our intention was to match the results of an already existing analysis (Brinkman 2007).

We selectively tested out a number of choices for the parameters HDRlevel, growthFac and signifLevel. Applying the same set of these 3 parameters globally for the entire dataset provided good results.



# How to summarise the output visually and interpret it

We want obtain for each patient and each patient day and each anti-body combination, a summary of the data that passed through the gate.

In curvHDR:

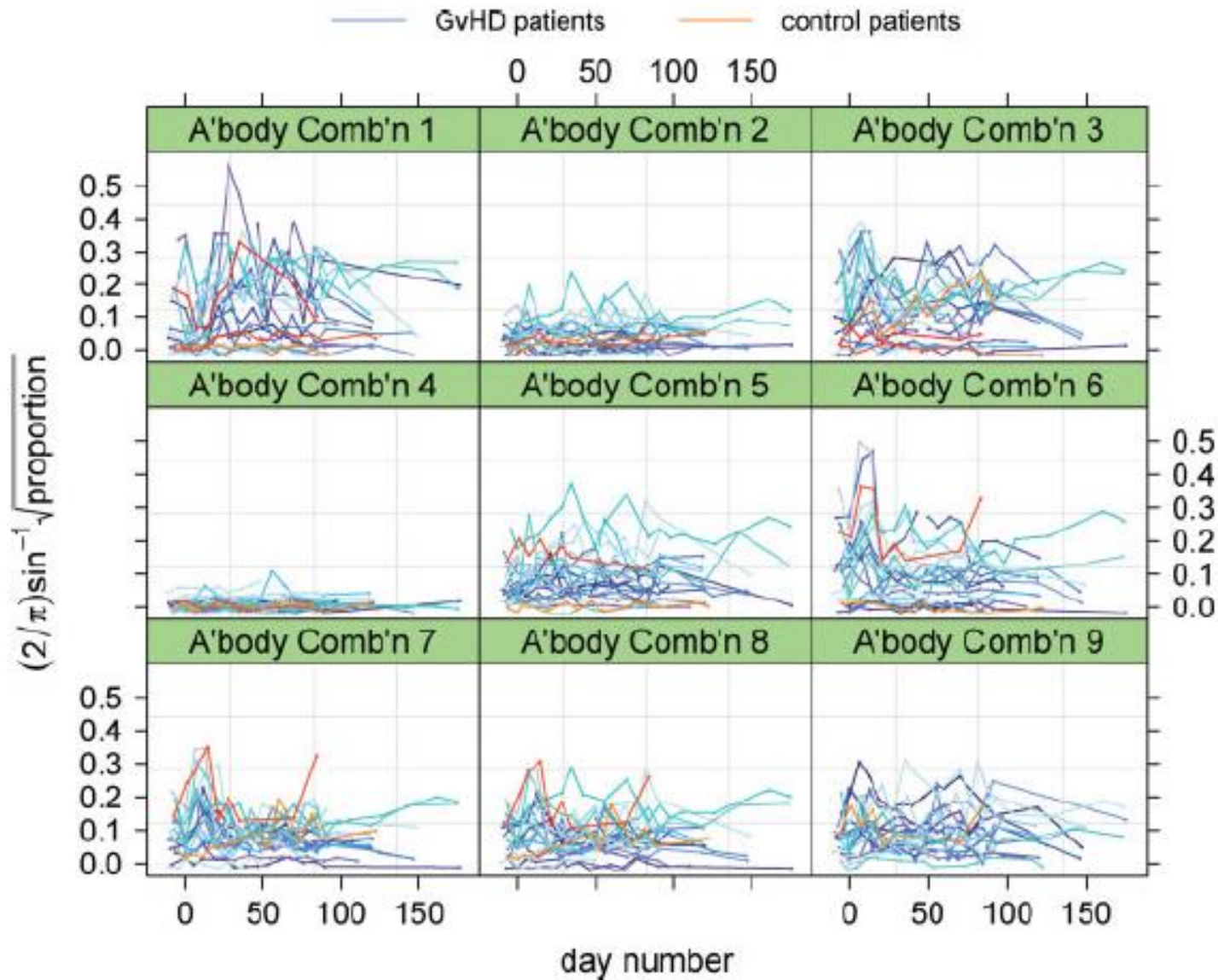
- get the proportion of gated cells for each patient, each day and each AB combination

example: `length(xBiva[gate2a$insideFilter,1])`

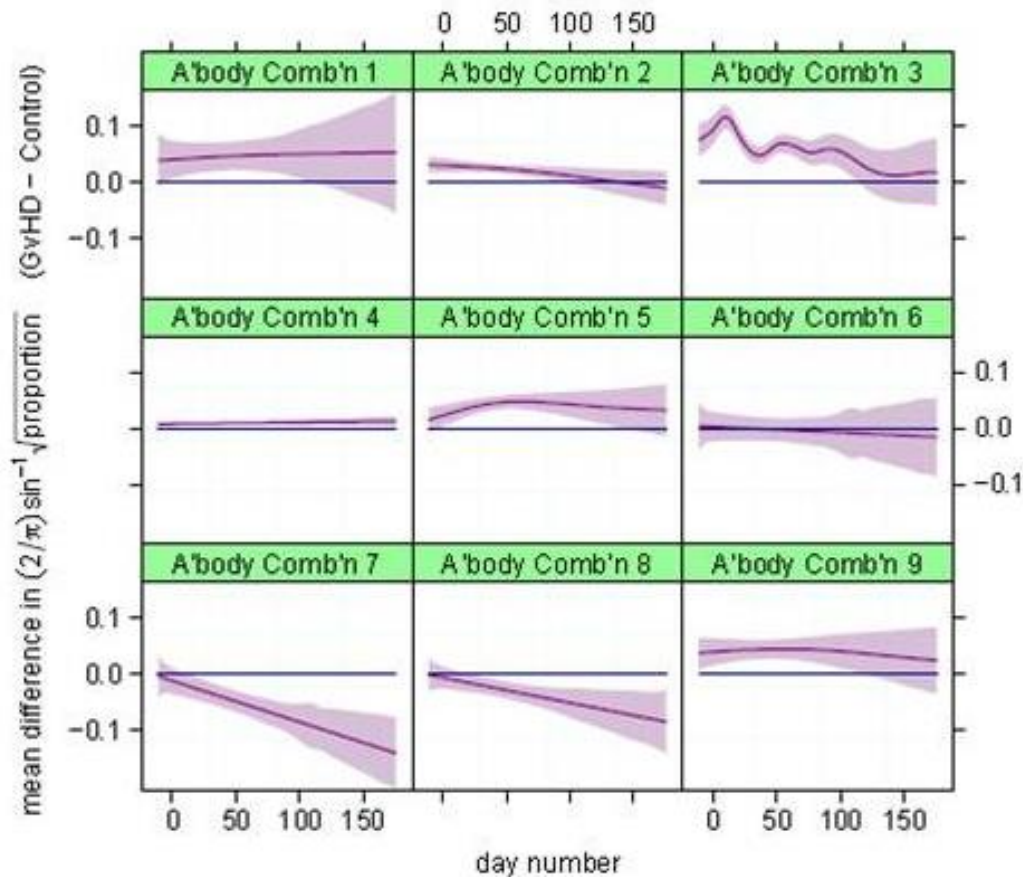
- Apply variance stabilising transformation

$y_{new} = \left(\frac{2}{\pi}\right) \sin^{-1}(\sqrt{y})$  on the proportion data

# Summary using Lattice graphics - ggplot



# Findings – Signatures for Graft-versus-Host Disease



- Estimated contrast curves (cellular signatures) arising from fitting the longitudinal data.
- The shading around each curve corresponds to approximate point-wise 95% confidence intervals.

# References

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