



BIOCONDUCTOR

flow.. packages

FICCS3 - March 2007

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flowCore version 0.3.16

- Last november
- ❑ data structure
- ❑ methods
- Release April

flowCore data structure

- Data structure
 - flowFrame
 - flowSet
 - filterSet
- Methods
 - compensation
 - transformation
 - Filter

Use cases

http://www.ficcs.org/software.html#Data_Files

- GvHD
- Compound screening
- Plate data
- Large datasets

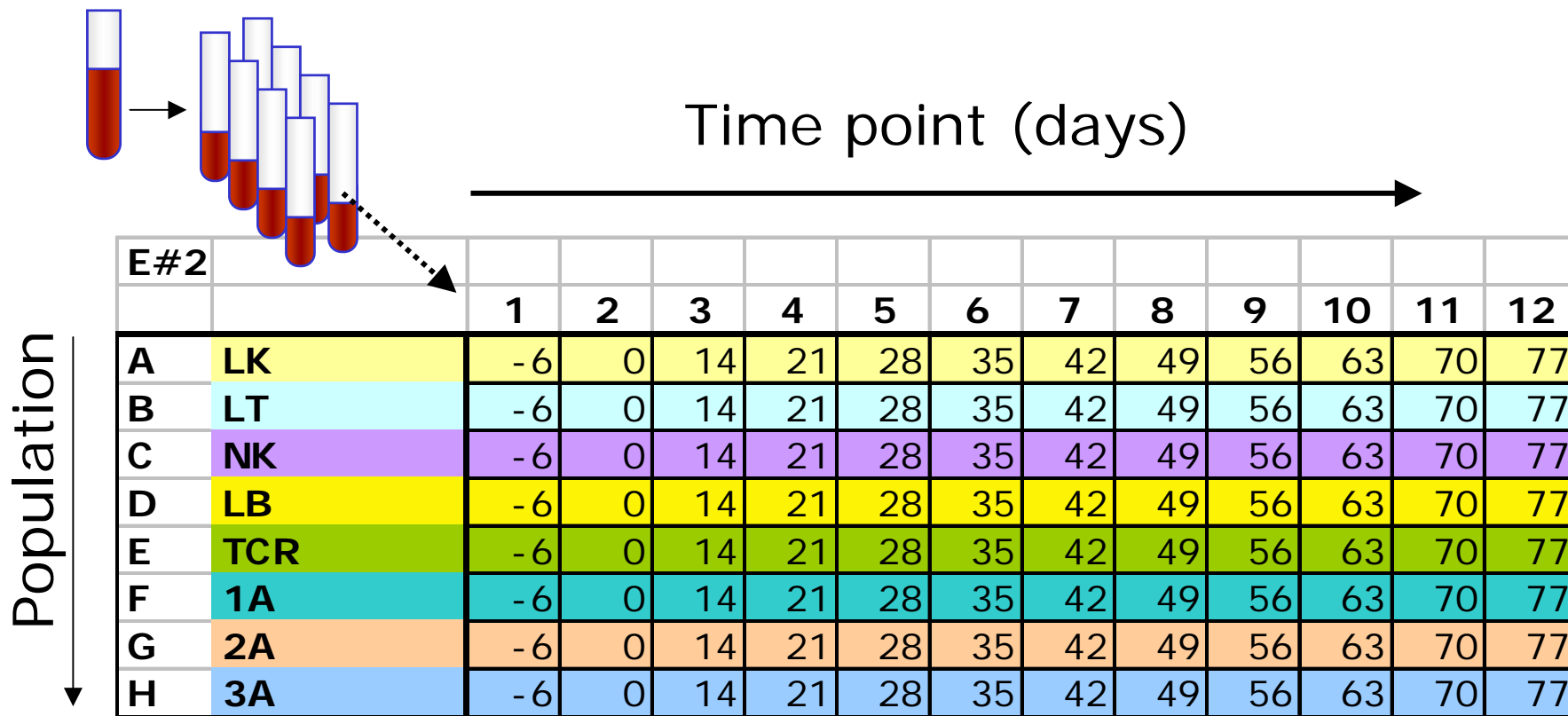
flowQ

Quality Control & Assessment

- Visualization
- Statistics
 - non paramateric KS
 - Grubb's test

N. Le Meur et al. (2007) Data Quality Assessment of Ungated Flow Cytometry Data in High Throughput Experiments.
Cytometry (In print)

Graft vs. Host Disease (GVHD)



GvHD use case

```
> GvHD6 <- read.flowSet(filenames,  
  path=directory, alter.names=TRUE)  
> GvHD6  
A flowSet with 96 experiments.  
rowNames: a01, a02, ..., h12 (96 total)  
varLabels and varMetadata:  
  name: Name  
  
column names:  
FSC.H SSC.H FL1.H FL2.H FL3.H FL2.A FL4.H Time
```

phenoData

```
> phGvHD <- read.csv("patient6.csv"), sep="\t")
> var <- data.frame(labelDescription= c("Aliquot", "Visit"))
> rownames(var) <- colnames(phGvHD)
> annot <-new("AnnotatedDataFrame", data=phGvHD, varMetadata=var)
> GvHD6@phenoData <- annot
```

```
> GvHD6
```

A flowSet with 96 experiments.

rowNames: 1, 2, ..., 96 (96 total)

varLabels and varMetadata:

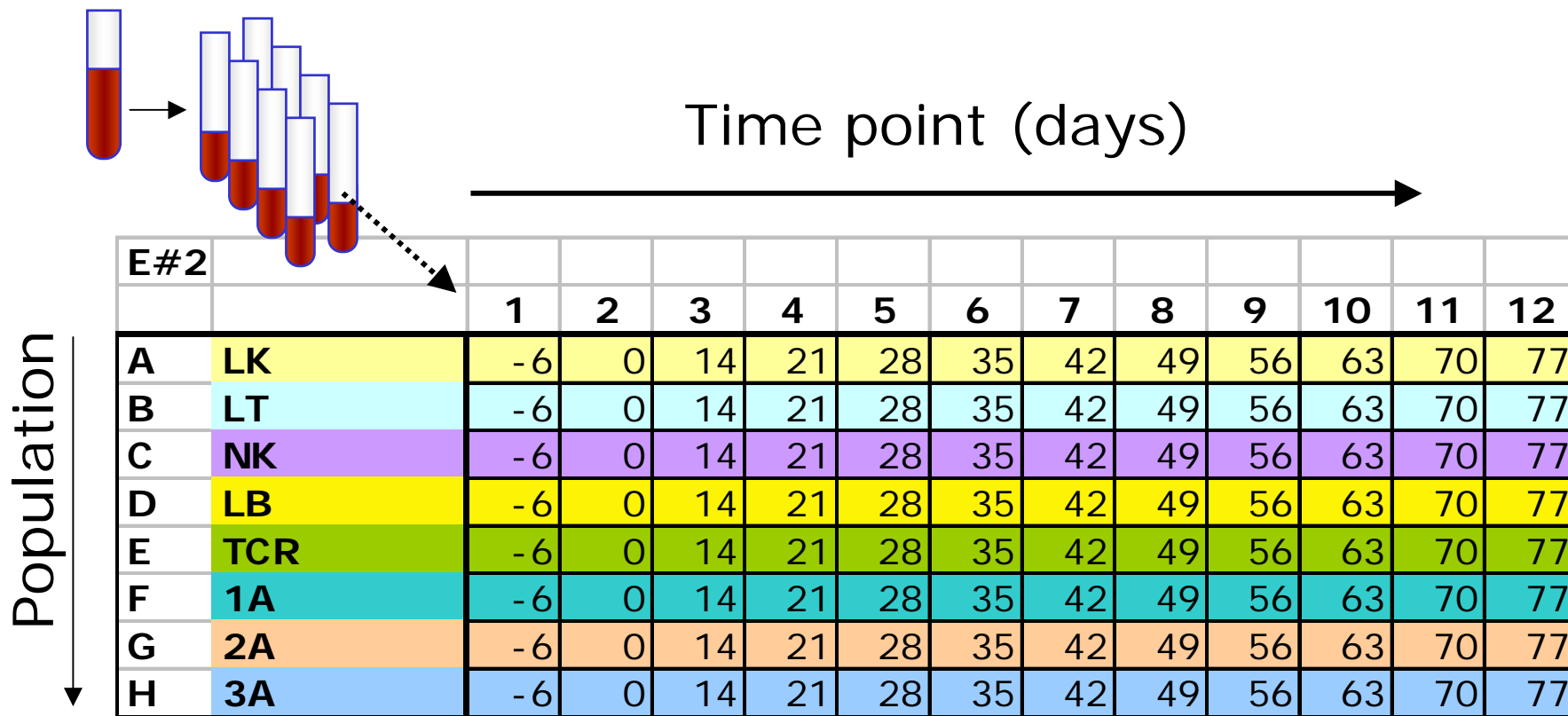
aliquot: Aliquot

timePoint: Visit

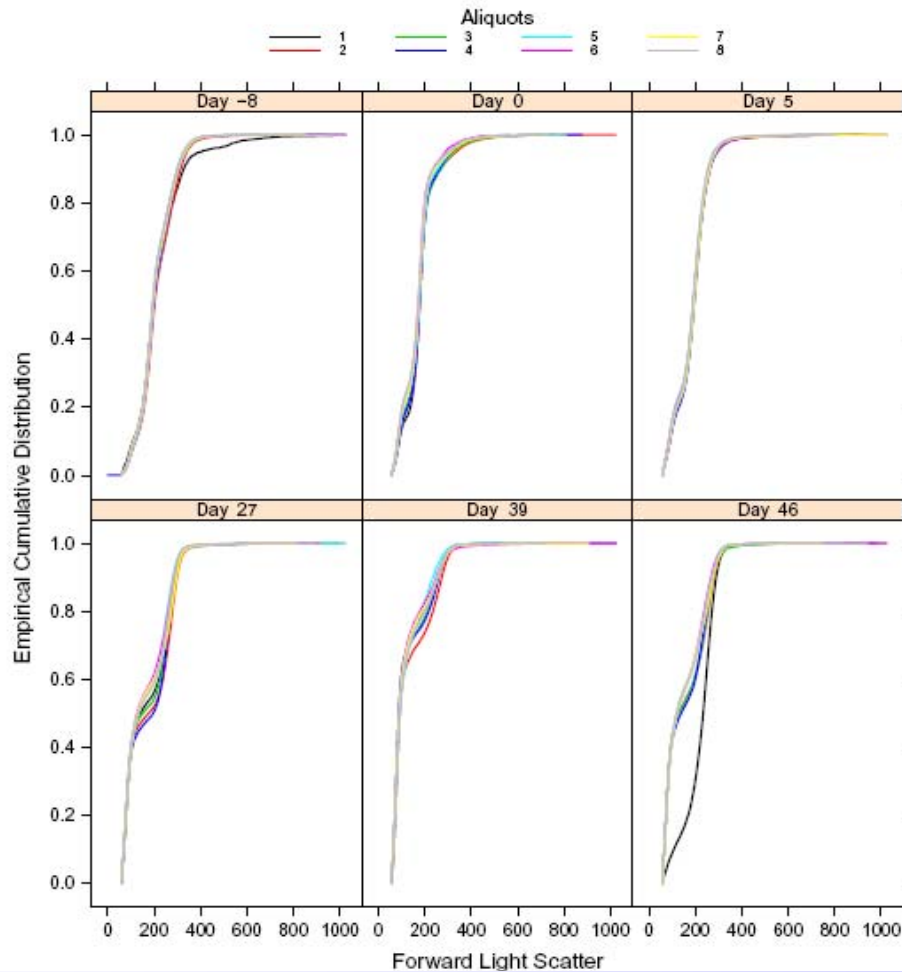
column names:

FSC.H SSC.H FL1.H FL2.H FL3.H FL2.A FL4.H Time

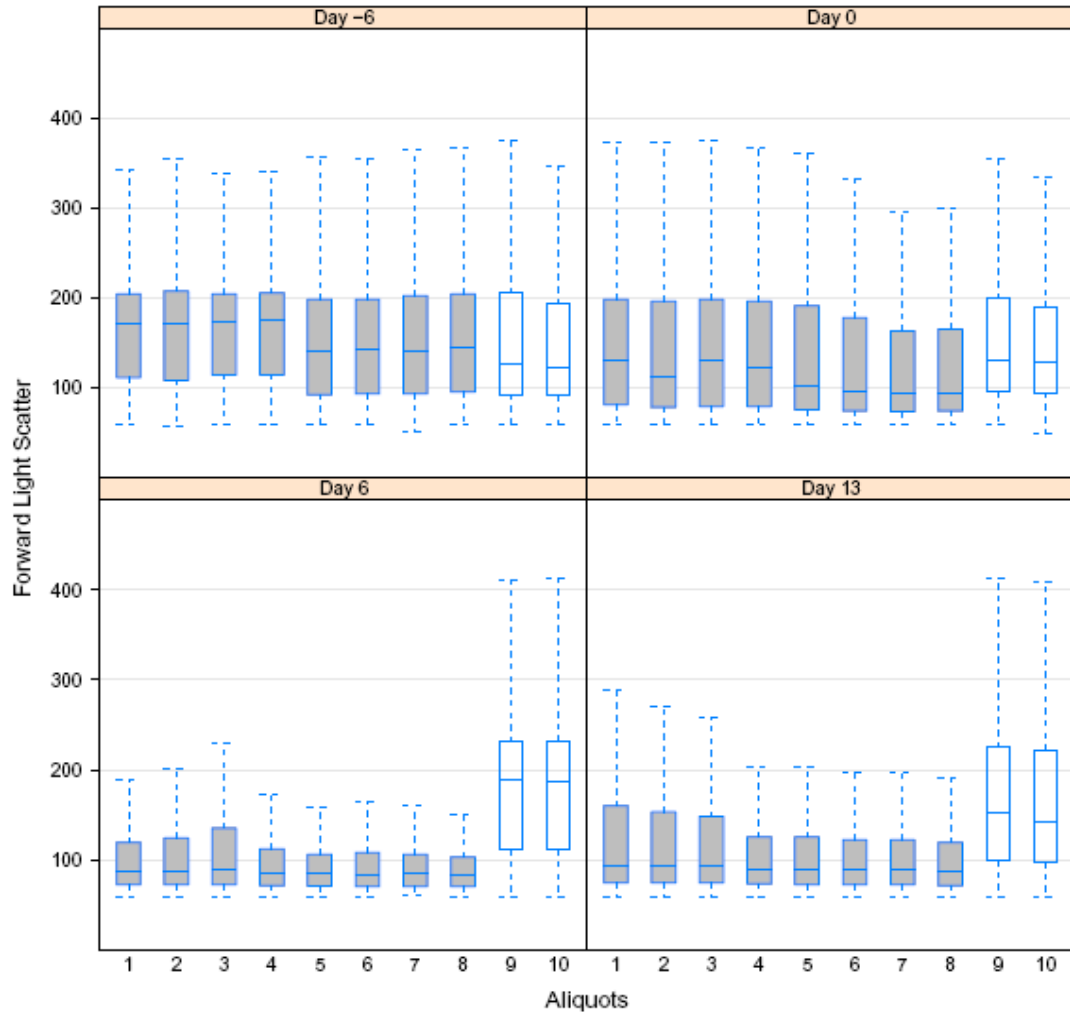
Graft vs. Host Disease (GVHD)



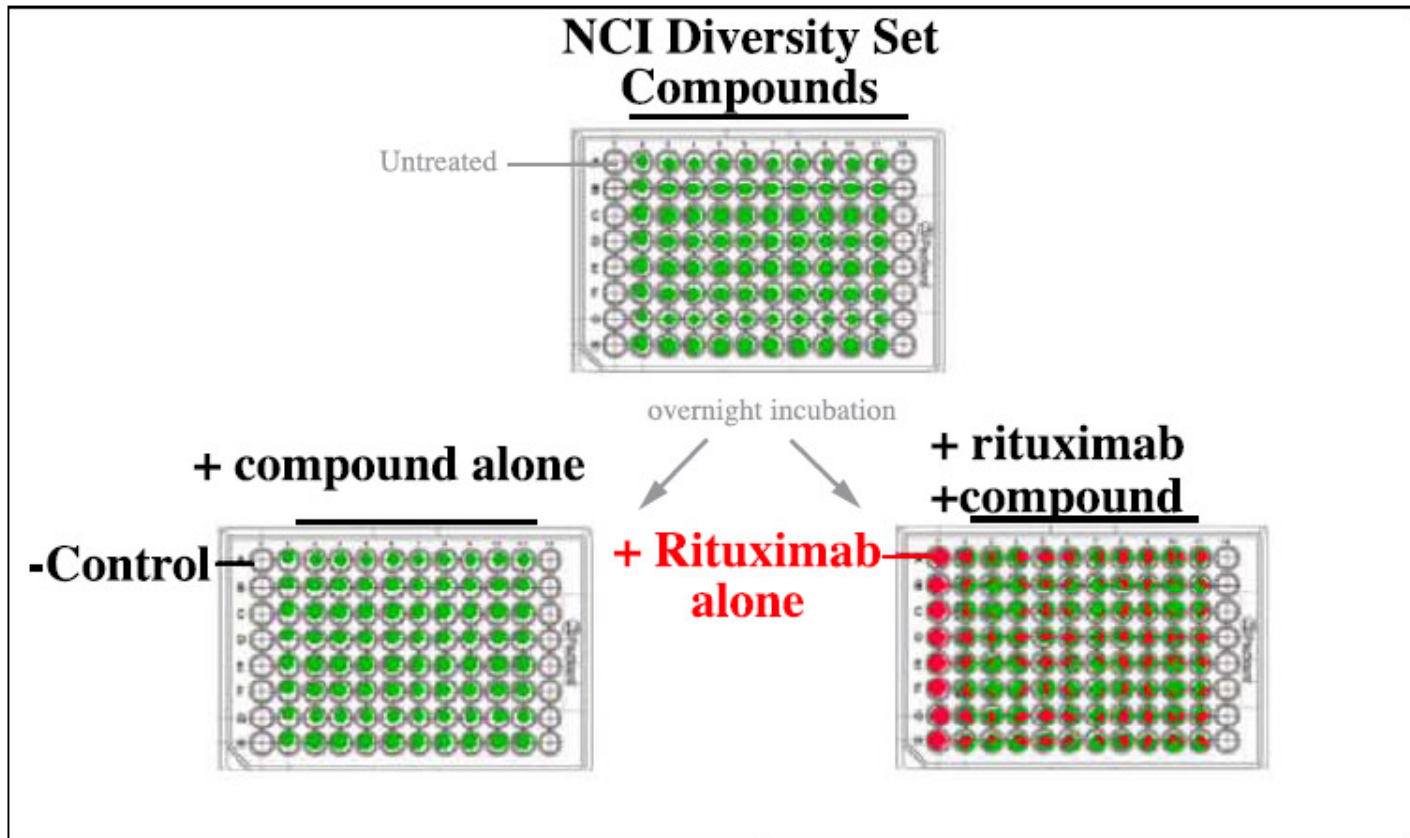
Quality Assessment ECDF plot



Quality Assessment boxplot

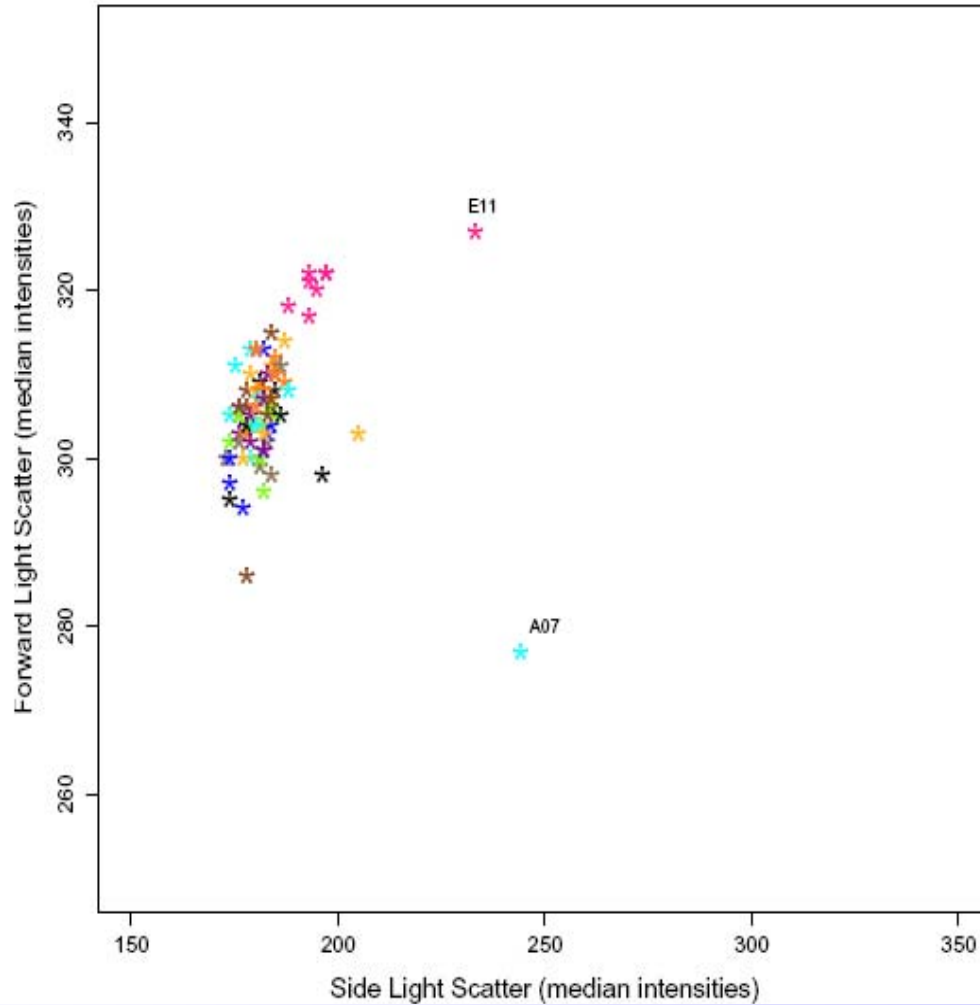


Rituximab Experiment

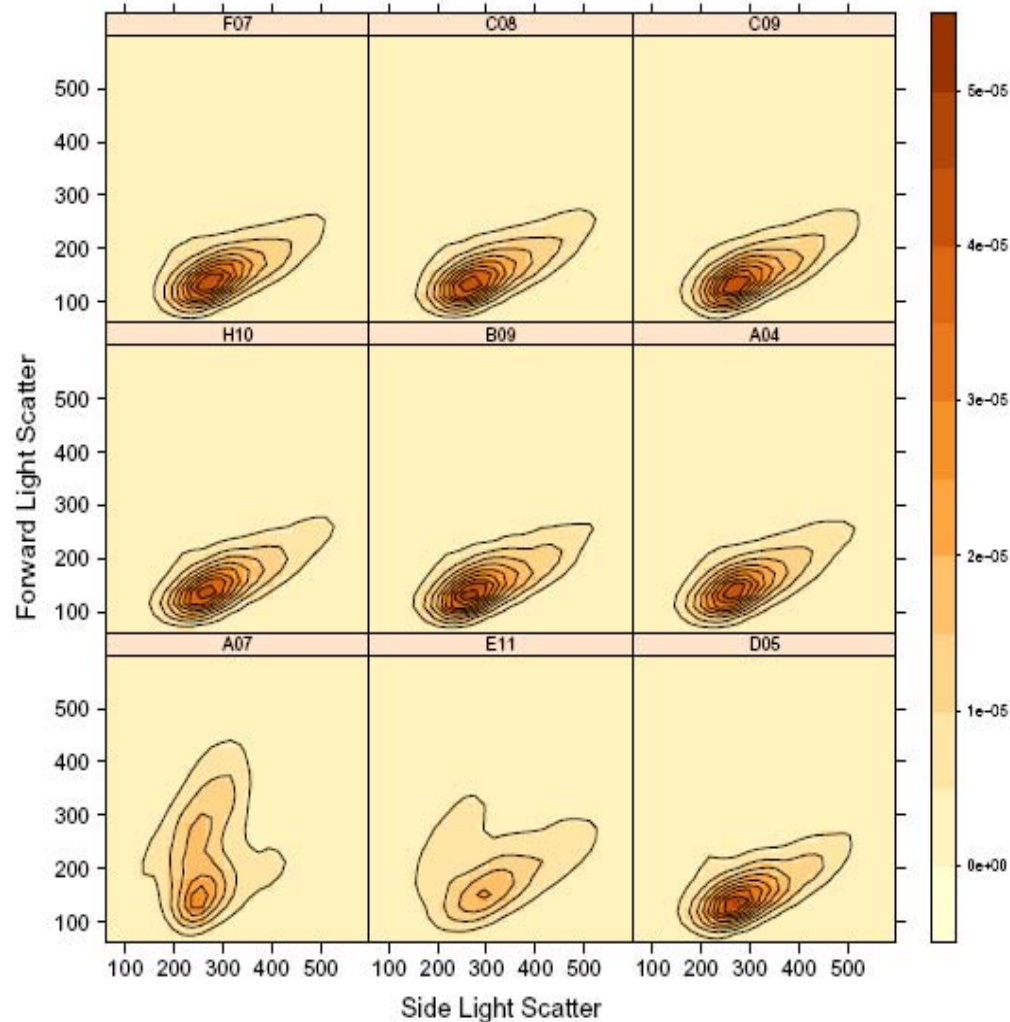


Journal of immunological Methods 292 (2004) 59-71

Quality Assessment



Quality Assessment contourplot



Next: flow packages

- ❑ flowQ
- ❑ flowUtils
- ❑ flowViz
- ❑ flowDB
- ❑ plateFlow
- ❑ ...

Analysis report

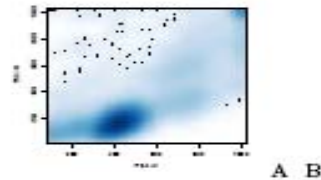


Figure 1: Example of smooth scatter plot.(A) FSC-H vs. SSC-H (B) FL2-H vs. FL3-H

```
> b08 - read.FCS(file.location[1])
> e07 - read.FCS(file.location[2])
> f06 - read.FCS(file.location[3])
```

We can plot the raw data for the FSC-H and SSC-H parameters.

```
[1] 10000

> basicplot(b08,plotParameters=c(1,2))
> basicplot(b08,plotParameters=c(3,4))
> basicplot(e07,plotParameters=c(1,2))
> basicplot(f06,plotParameters=c(1,2))
```

2.2 Transformation data

While analyzing flow cytometry data, various parameter transformations are performed to provide user-friendly visualization and/or to perform statistical analyses and interpret the data. Following the standard 8 transformations are pre-defined: linear, quadratic, natural logarithm, logarithm (with a specified base), hyperlog, bi-exponential, logicle, and split-scale.

As example we performed two different transformations on "FSC-H" channel of sample b08: a linear transformation and a log base 2 transformation.

A linear transformation is like $\text{parameter} = a * \text{parameter} + b$

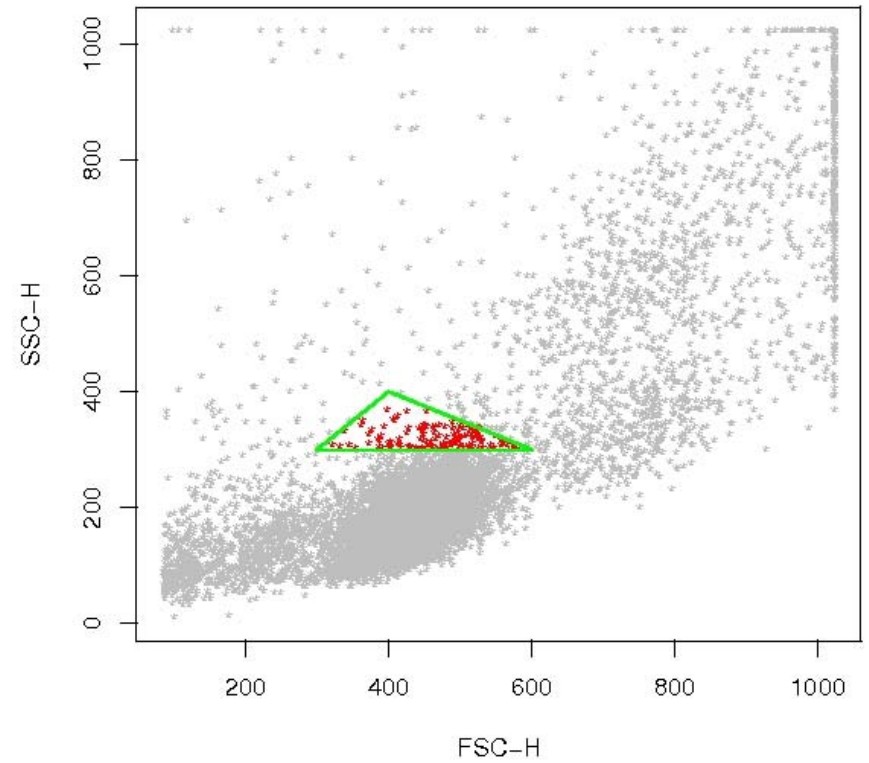
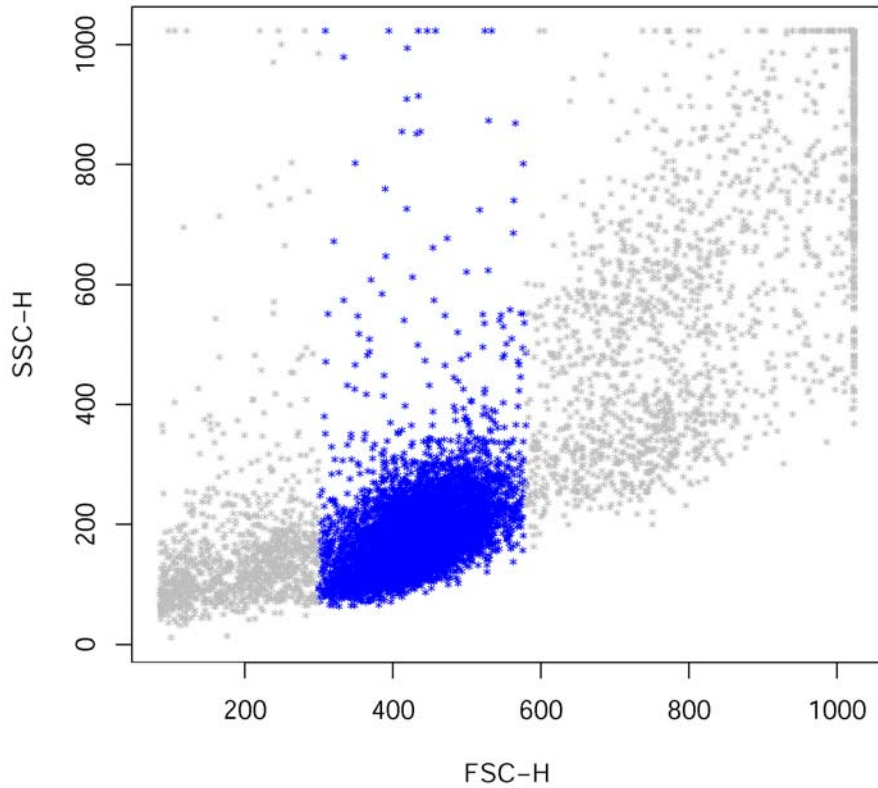
```
>linear <- new("linearTransformation",transformationId="Linear-transformation",
+             parameters="FSC-H", a=2, b=0)
>data08Transform <- applyTransformation(linear,b08)
```

A log transformation is of type: $\text{parameter} = \log(\text{parameter}, \text{logbase}) * r/d$

```
>logTransform <- new("logTransformation",transformationId="Log-Transformation",
+                   parameters="FSC-H", r=1, d=1,logbase=2)
>data08LogTransform <- applyTransformation(logTransform,b08)
```

```
> b08@exprs[1:5]
```

Other plots



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