

# Analyzing flow cytometry data in Bioconductor

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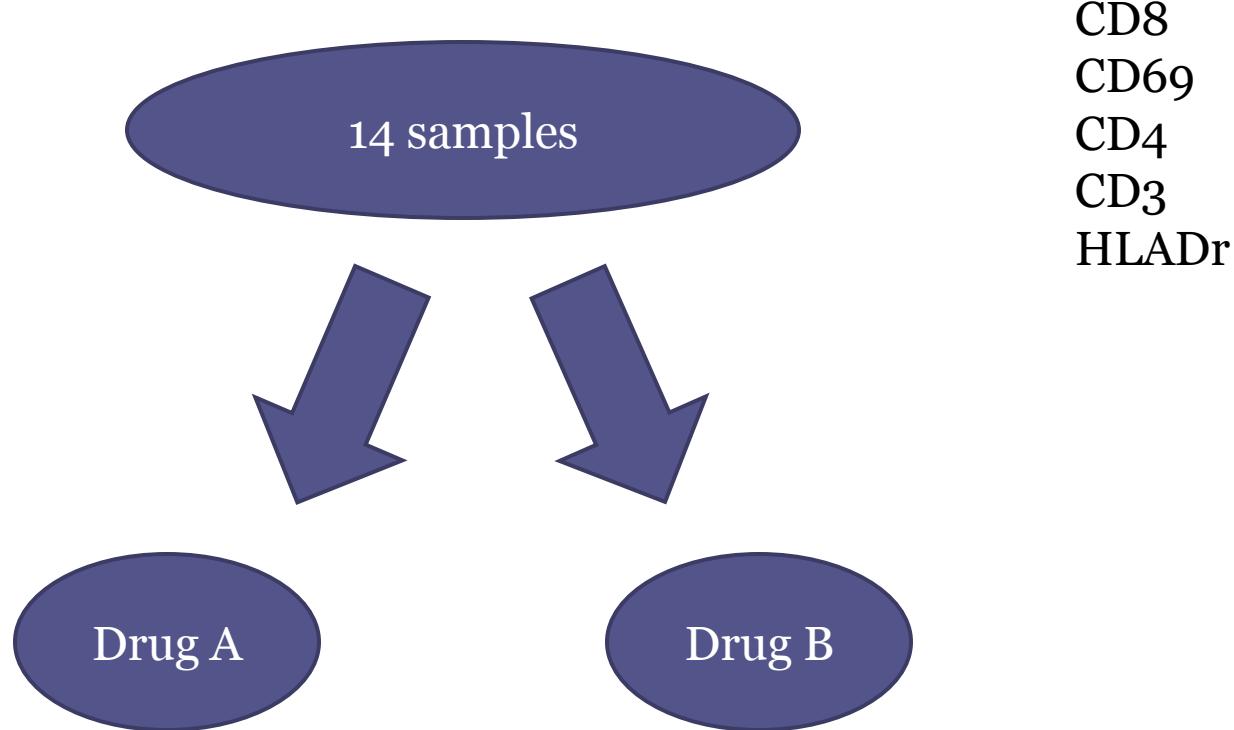
# Flow cytometry

- R – Bioconductor packages
  - Open source
    - flowCore
    - flowViz
    - flowQ
    - flowStats
    - flowUtils
    - flowClust
    - flowMerge
    - flowFP
    - flowFlowJo

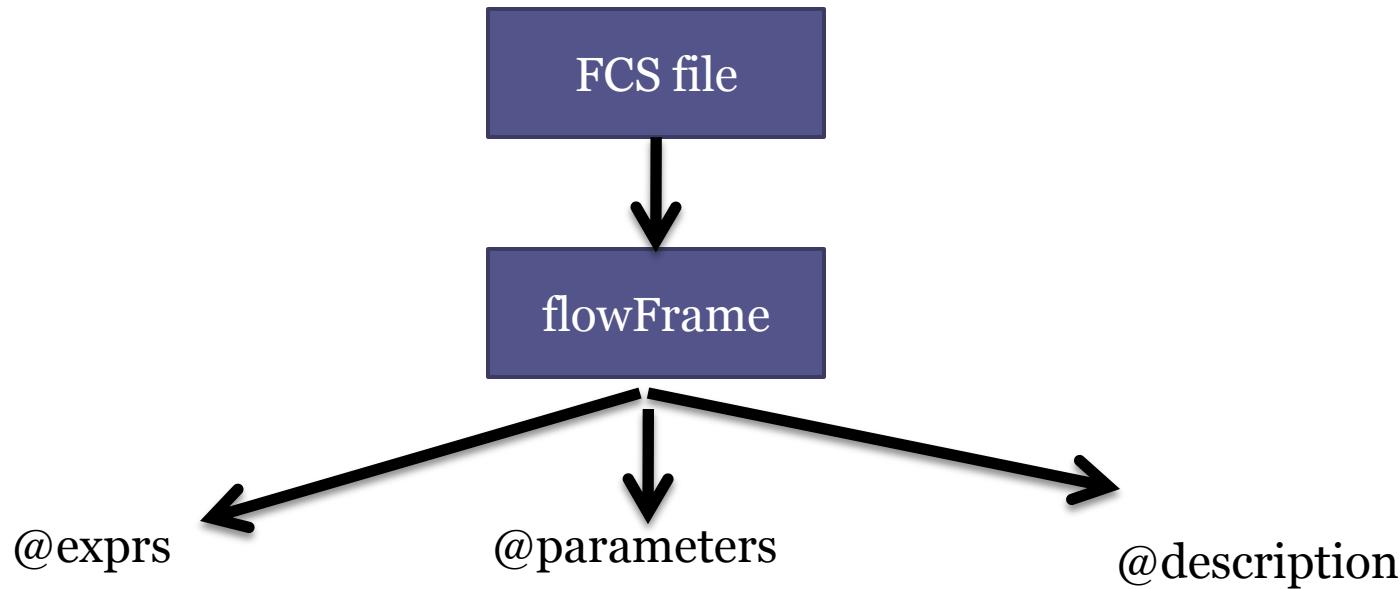
# Overview

- Walk through a simple flow data analysis
  - FCS files
  - Data structures in flowCore
  - Quality control reports
  - Transformation, sequential gating , visualization
  - Workflows

# FCS files



# Data structures - flowFrame



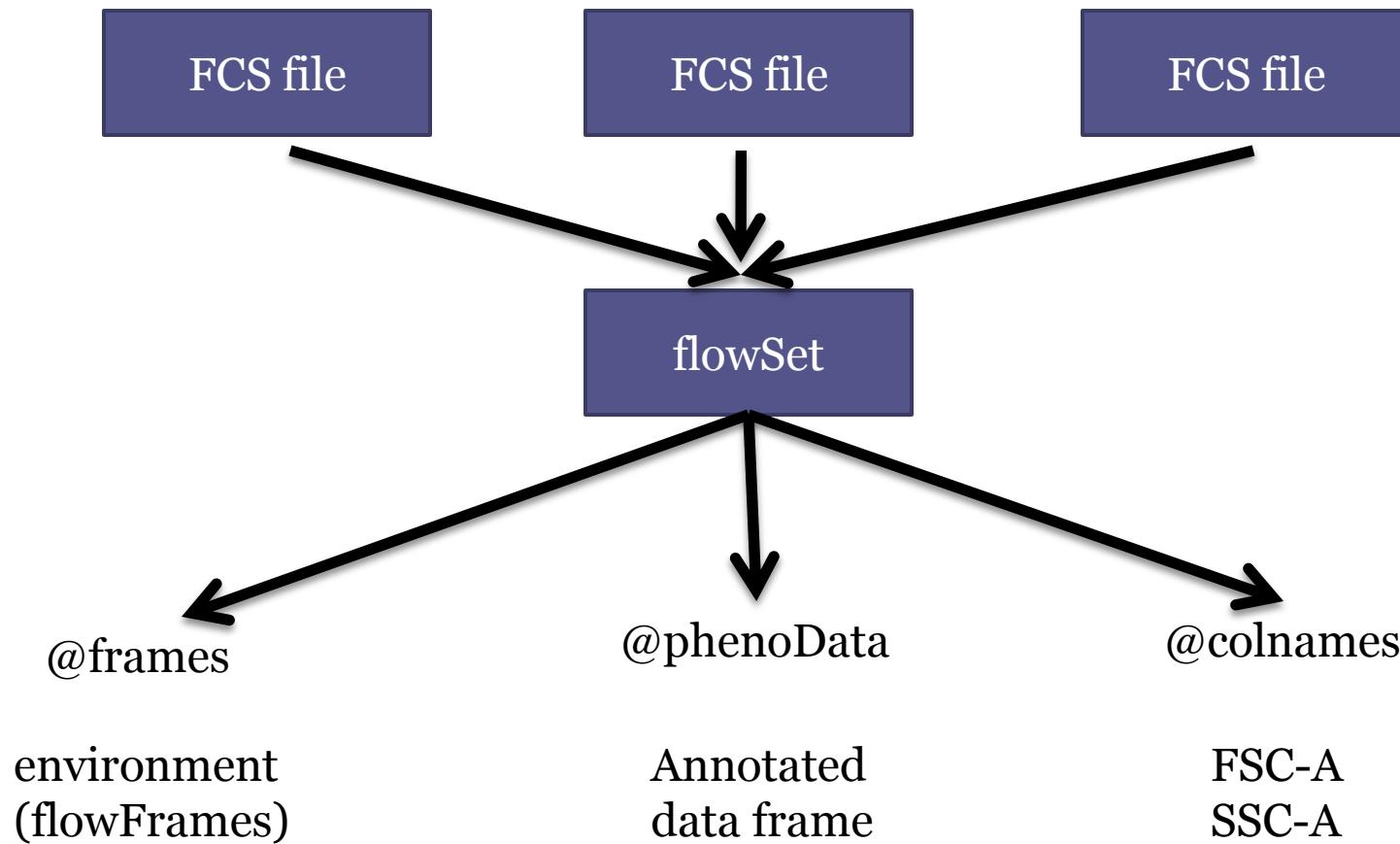
FSC	SSC	FITC-A	PE-A
600	444	4.5	5.4
500	333	3.4	4.5
700	450	4.3	3.3

**Annotated data frame**

- Stain
- Channel
- Range

List containing meta data information

# Data structures -flowSet



# R session 1

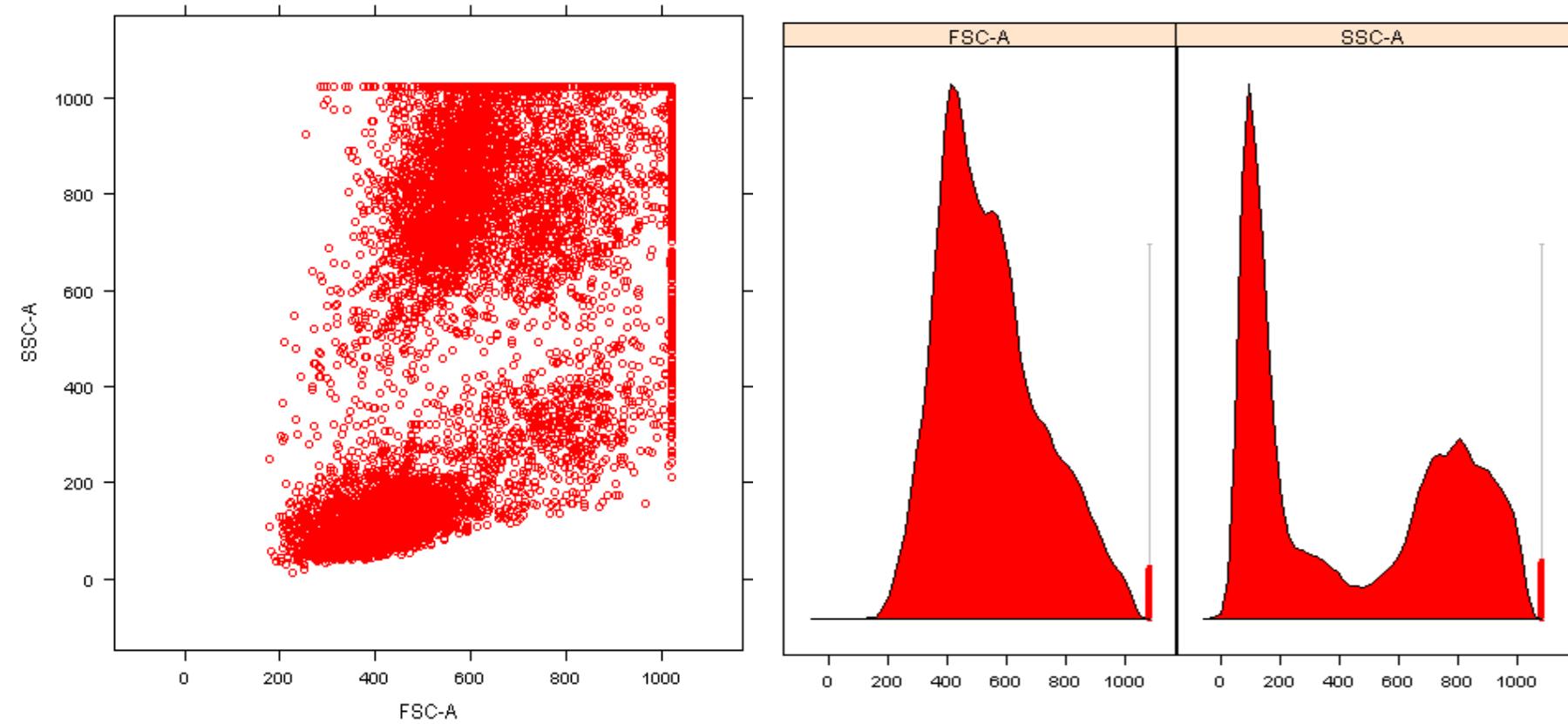
- Create a flowSet
- Transform Data using asinh transformation
  - `transform()`
    - FITC-A, PE-A, FL3-A, PE-CY7-A , APC-A
  - `transformList`
- Visualize data

# Quality Assessment - flowQ

- Boundary events
- Cell number
- Time Line
- ECDF plots
- Density plots
- KL distance plots

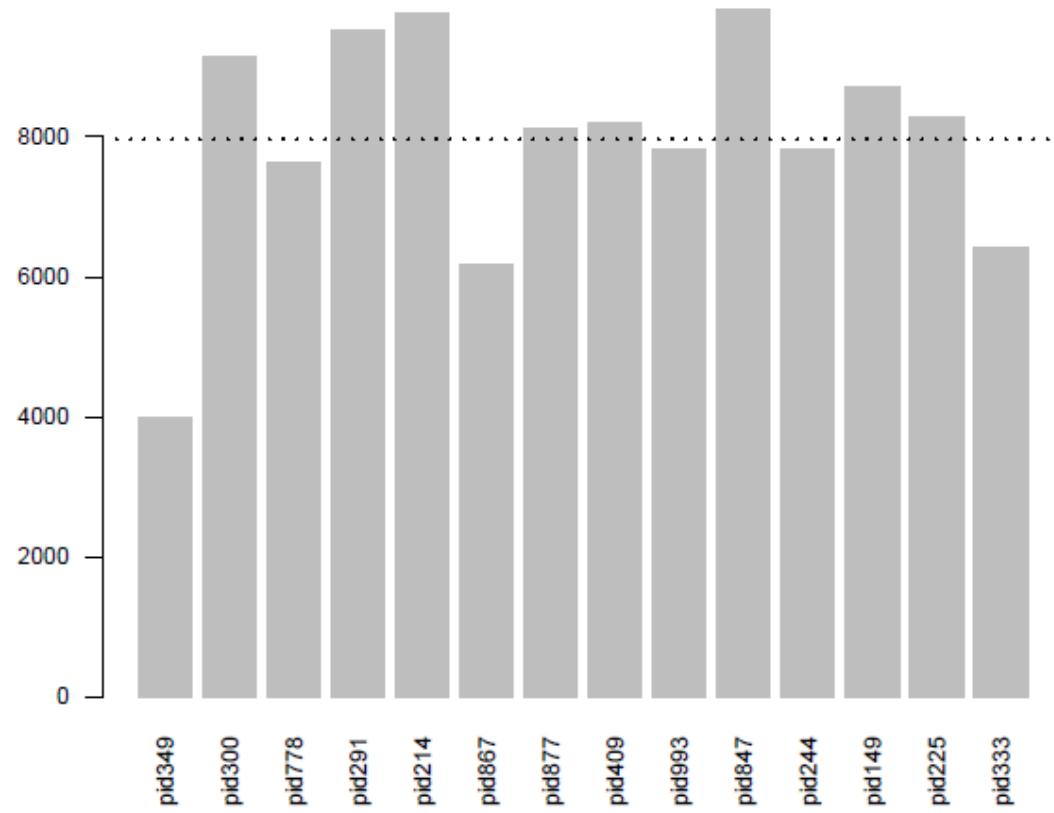
# Boundary events

- qaProcess.marginevents()



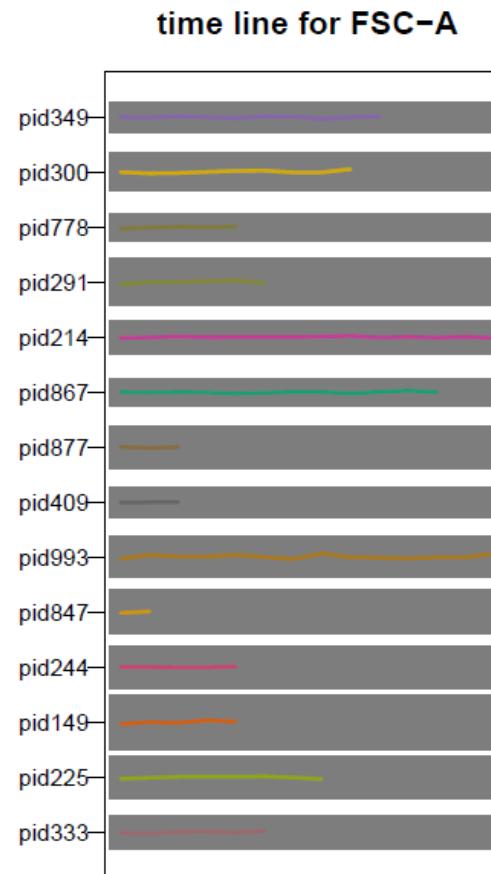
# Cell number

- qaProcess.cellnumber()



# Time line

- qaProcess.timeline()



# R session 2

- Generate quality reports

# Goals

Compare amongst two treatment protocols -  
Drug A & B

- T helper cells that exhibit HLADr activation marker
- T cytotoxic cells that exhibit HLADr activation marker

# Sequential gating strategy

