

# High Throughput Analysis of Flow Cytometry Data using *plateCore*

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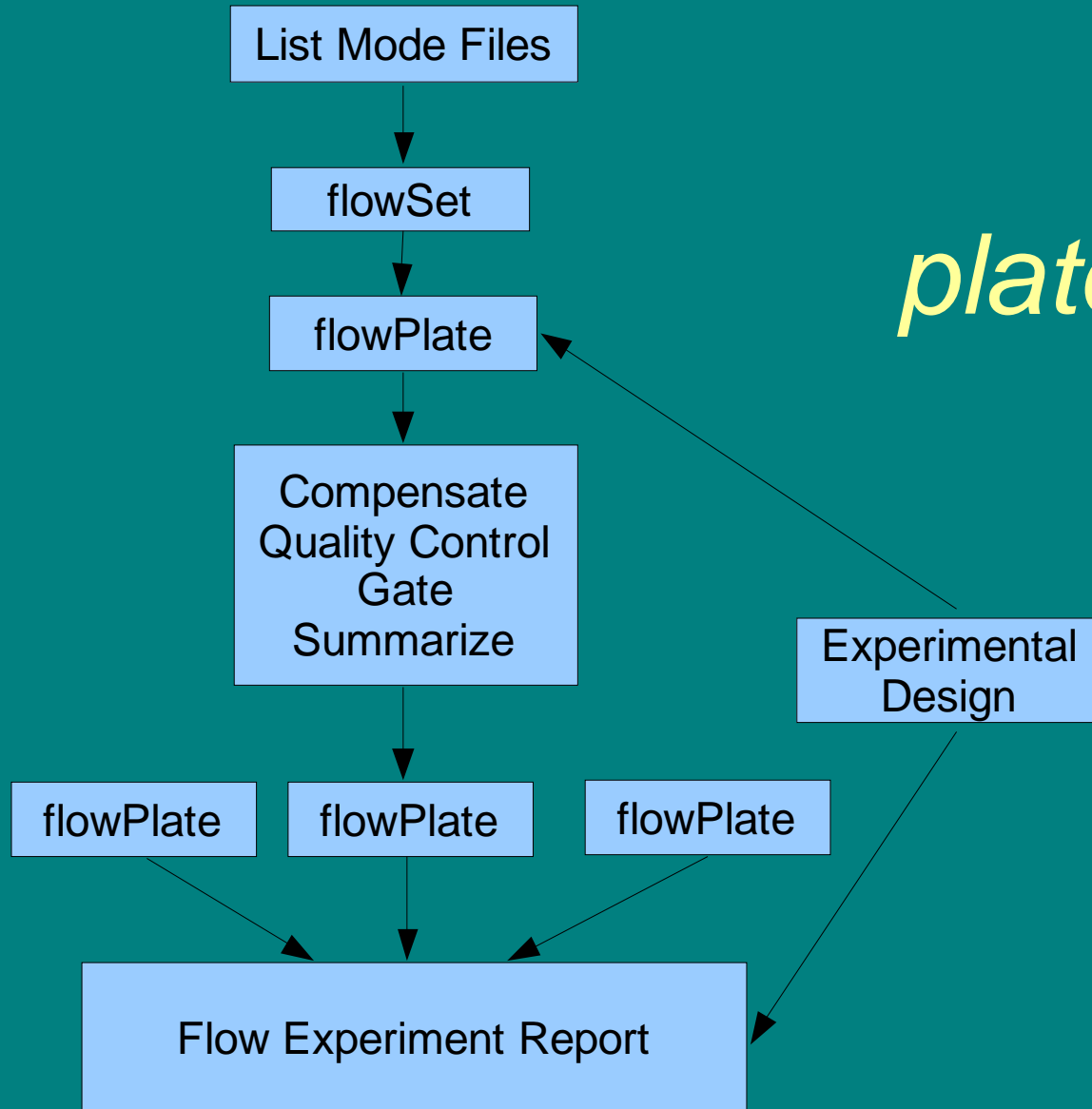
# Overview

- R for Flow Cytometry
- *plateCore*
- Getting Started
- Processing and Plotting
- Example Datasets
- Results
- Conclusions

# R for Flow Cytometry

- Bioconductor flow packages
  - *flowCore*
  - *flowViz*
  - *plateCore*
- Integrated with other bioinformatics tools
  - microarrays, proteomics, genomics, image analysis, etc.
- Graphing (*lattice*), reporting (Sweave), statistics

# *plateCore*



# *plateCore*

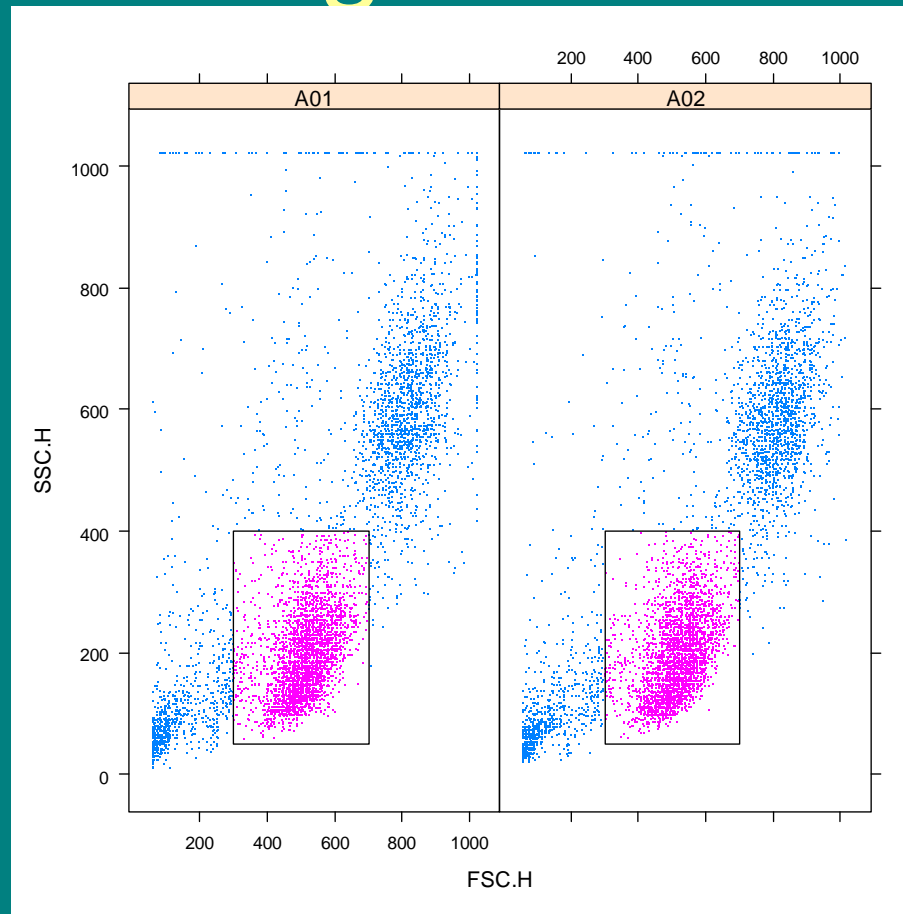
- Convenient interface to *flowCore*
  - *flowCore* data structures modeled on *AffyBatch* objects
  - Difficult to manage annotation (phenoData) for multiplex assays
- Screening
  - Correct for background fluorescence (1D gating)
  - Automatically determine threshold between positive and negative cells using negative controls
- Reporting
  - Summarizing information from multiple plates

# Getting Started

Well.Id	Sample.Type	Ab.Name	Channel	Negative.Control
A01	Negative.Control	Isotype	FITC-A	A01
.....				
A10	Test	CD3	FITC-A	A01
A10	Test	CD4	PE-A	A02
A10	Test	CD8	APC-A	A03
H12	Unstained			

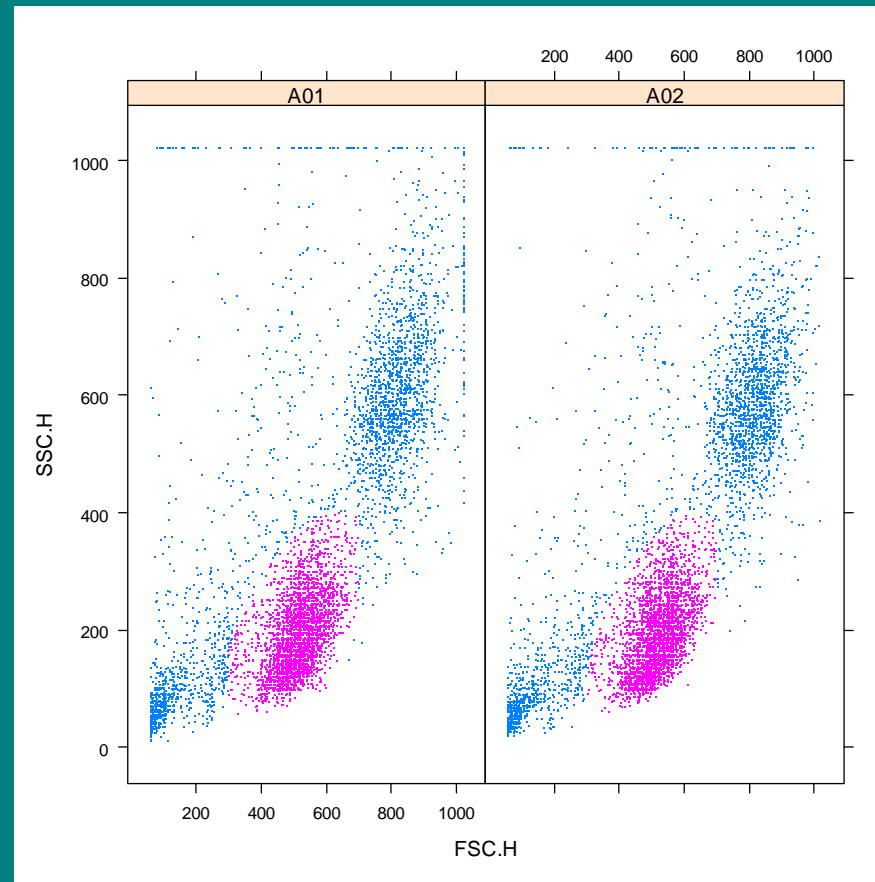
- Make a plate layout table
- Read in list mode files to a *flowSet* and create a *flowPlate*
  - > *pbmcFP* <- *flowPlate*(*pbmcFlowSet*, *wellAnnotation*,  
*plateName*="PBMC.001")

# Plotting and Gating



```
> rectGate <- rectangleGate("FSC.H" = c(300,700), "SSC.H"=c(50,400))  
> xyplot(SSC.H ~ FSC.H | as.factor(Well.Id), pbmcFP[1:2],  
  displayFilter=TRUE, filter =rectGate)
```

# Plotting and Gating

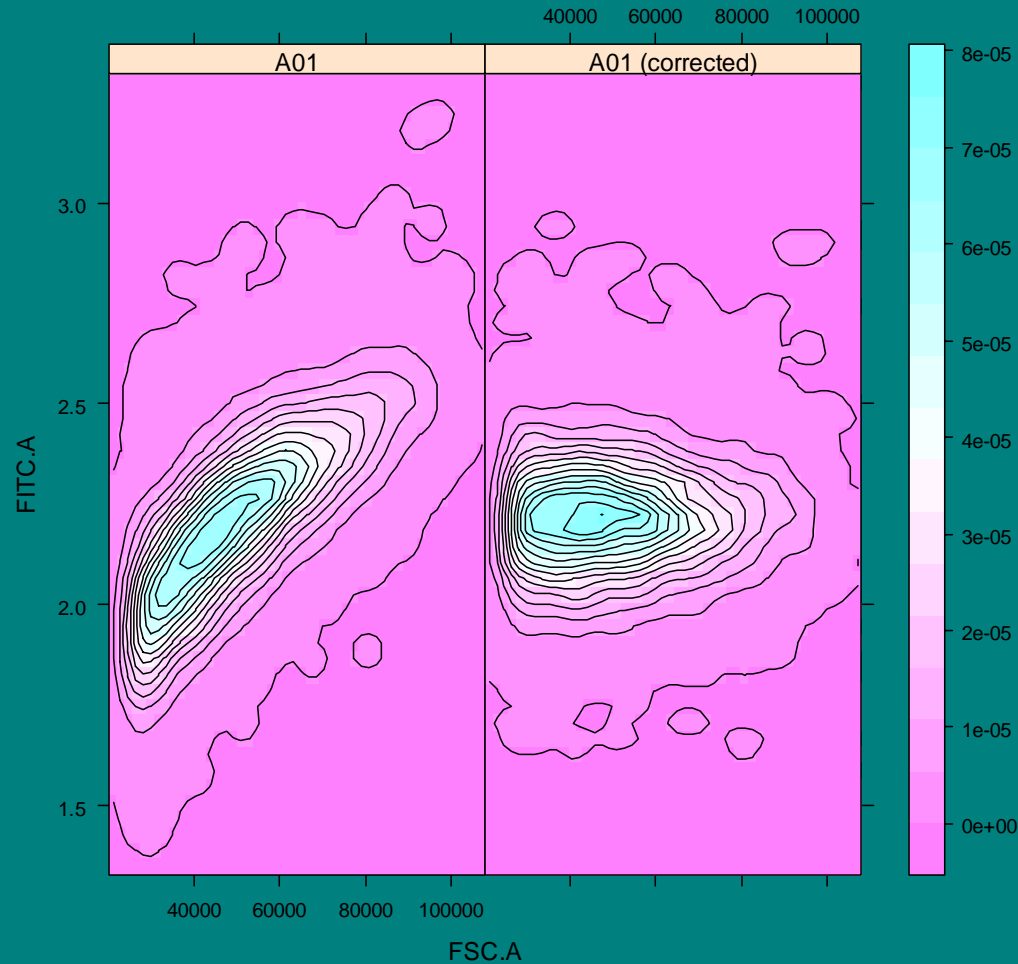


```
> normGate <- norm2Filter("SSC.H", "FSC.H", scale.factor=1.5)
> xyplot(SSC.H ~ FSC.H | as.factor(Well.Id), pbmcFP[1:2],
  displayFilter=TRUE, filter =rectGate & normGate)
```

# Data Processing

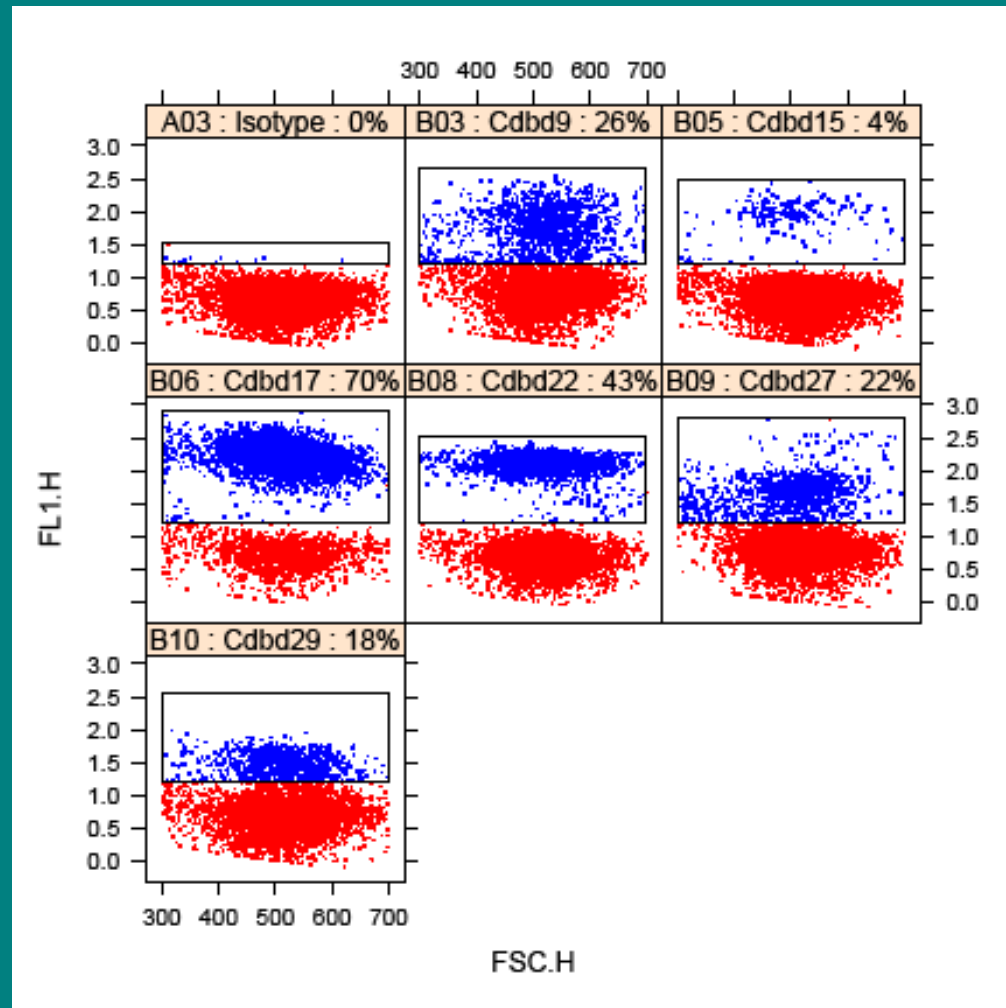
- Use the filters from xyplot
  - > Subset(pbmcFP, rectGate & normGate)
- Compensate based on wellAnnotation
  - > compensate(pbmcFP, comp.mat)
- Correct for auto fluorescence
  - > fixAutoFl(pbmcFP, fsc="FSC.H")

# Autofluorescence Correction



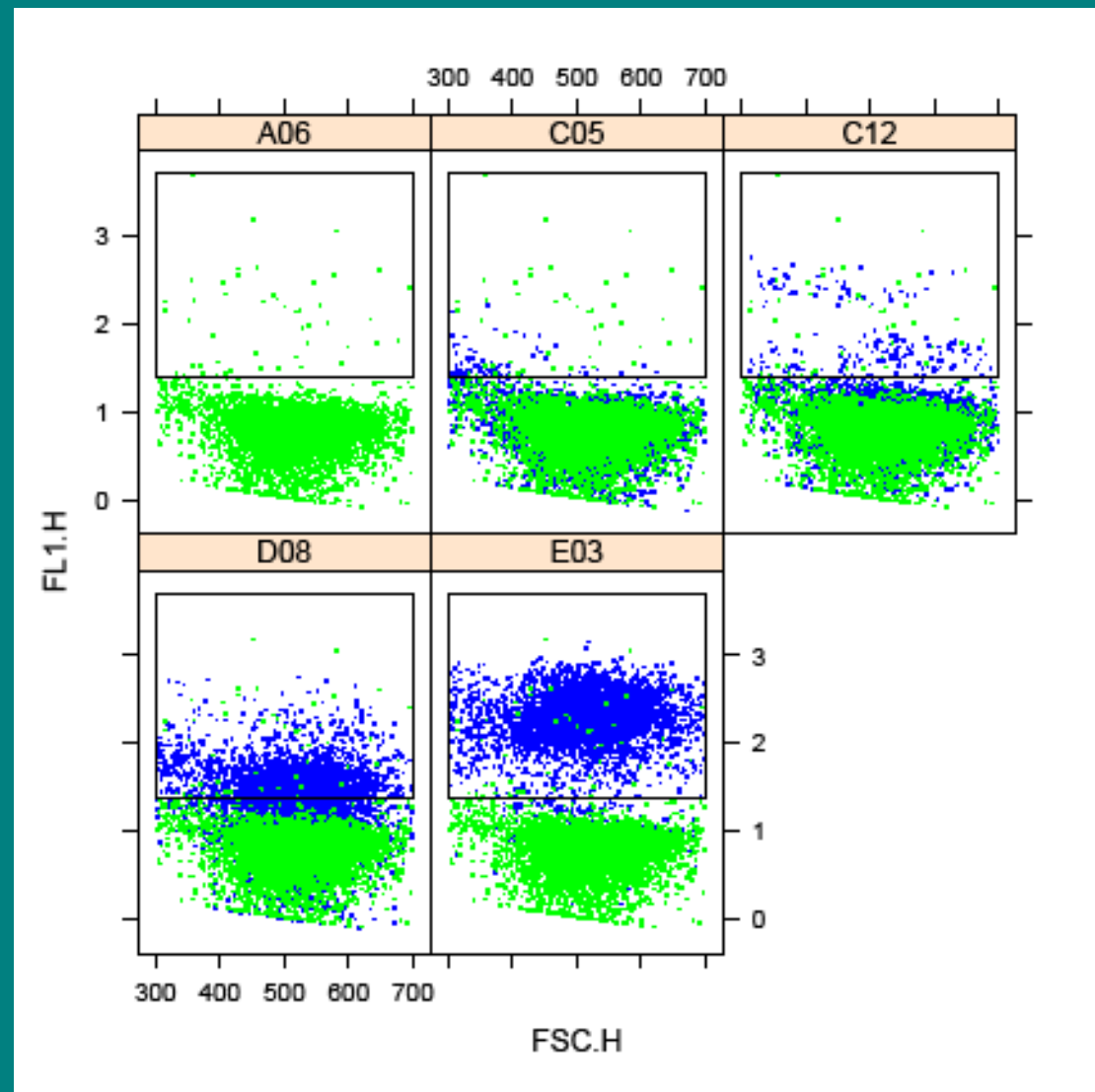
```
> fixAutoFI(flowPlate, fsc="FSC.A")
```

# Control Gates



```
> setControlGates(pbmcFP, gateType = "Negative.Control", numMads=5)  
> applyControlGates(pbmcFP)
```

# Overlay Plots



```
> xyplot(FL1.H ~ FSC.H | as.factor(Well.Id), transform("FL1.H"=log10)  
%on% pbmcFP[samples], displayFilter=TRUE,  
filter = "Negative.Control", filterResults="Negative.Control)
```

# Public Datasets

- Peripheral Blood Mononucleocyte Cells (PMBC)
  - Expression phenotype for 189 antibodies
  - Two Donors (2 or 3 replicate 96-well plates)
- Normal Donor Study
  - Immune response in 40 normal donors to tumor and infectious agents, multiple time points

# PBMC Lymphocytes

- What surface proteins are expressed?
- Donor Variation?
- 3 antibodies per well
- 6 different fluorochromes



# PBMC Analysis Setup

- Well Annotation File

<u>Well.Id</u>	<u>Sample.Type</u>	<u>Ab.Name</u>	<u>Channel</u>	<u>Negative.Control</u>
A01	Negative.Control	Isotype	FL1-H	A01

.....

- Morphology Gate Lymphocytes

- > *Subset(platePBMC, rectangleGate("FSC.H"=c(300,700),"SSC.H"=c(50,400)) & norm2Filter("SSC.H", "FSC.H", scale.factor=1.5))*

- Correct for autofluorescence

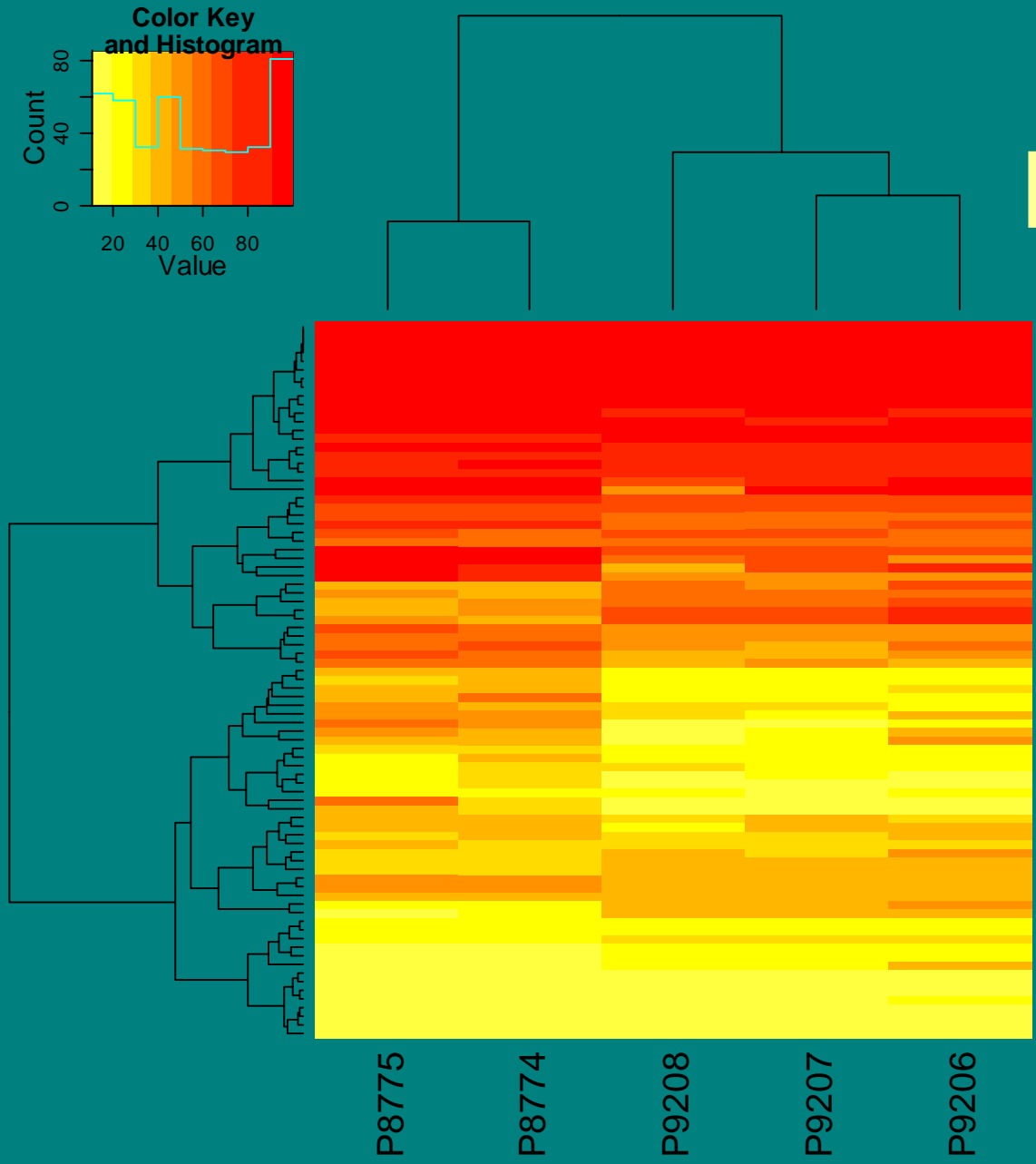
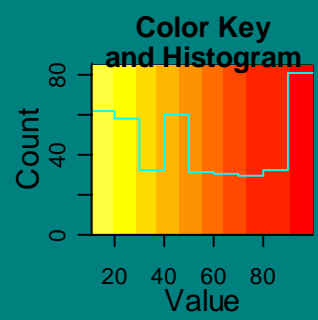
- > *fixAutoFl(platePBMC, fsc="FSC.H")*

- Create and apply isotype gates

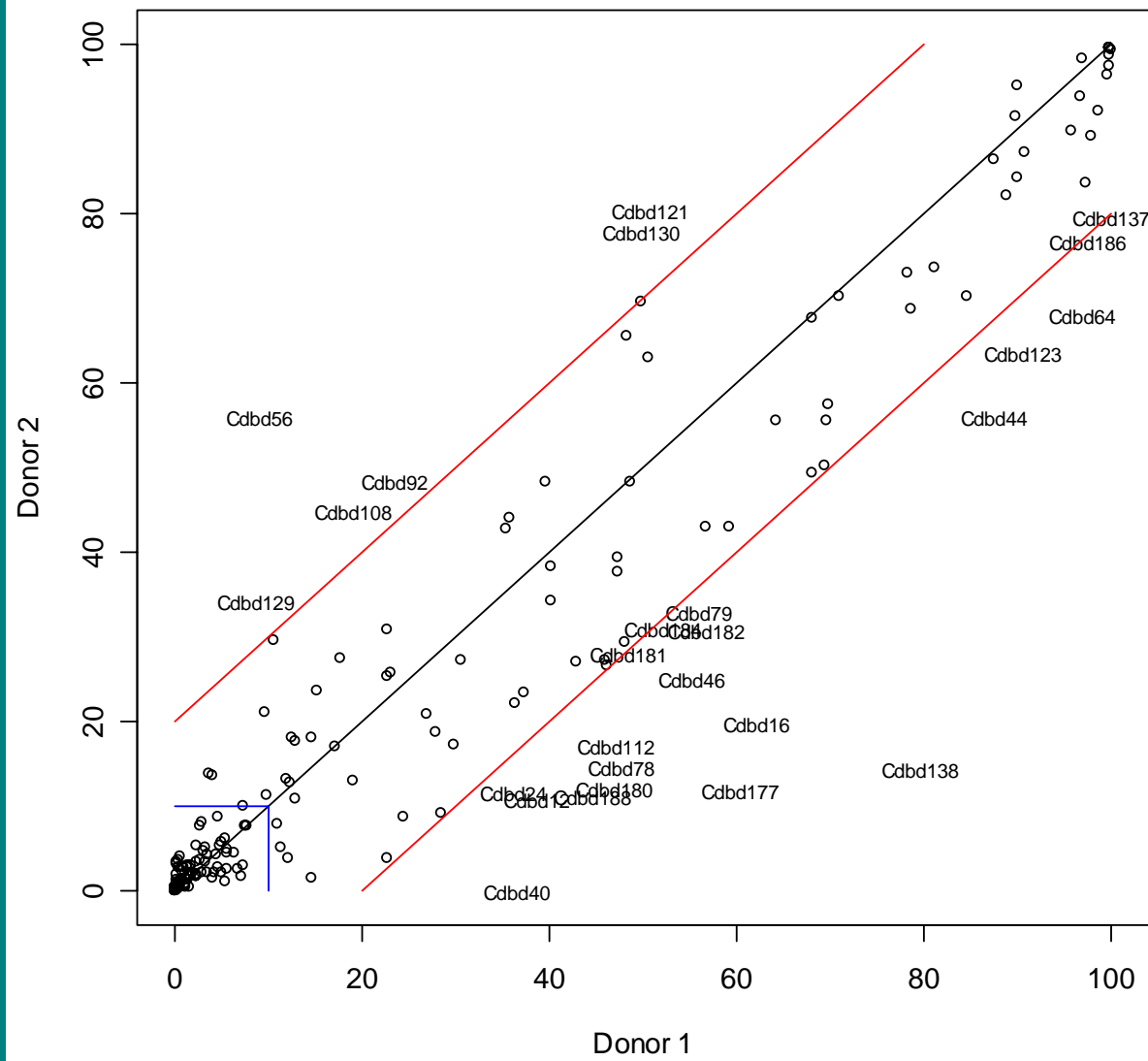
- > *setControlGates(platePBMC, gateType="Negative.Control")*

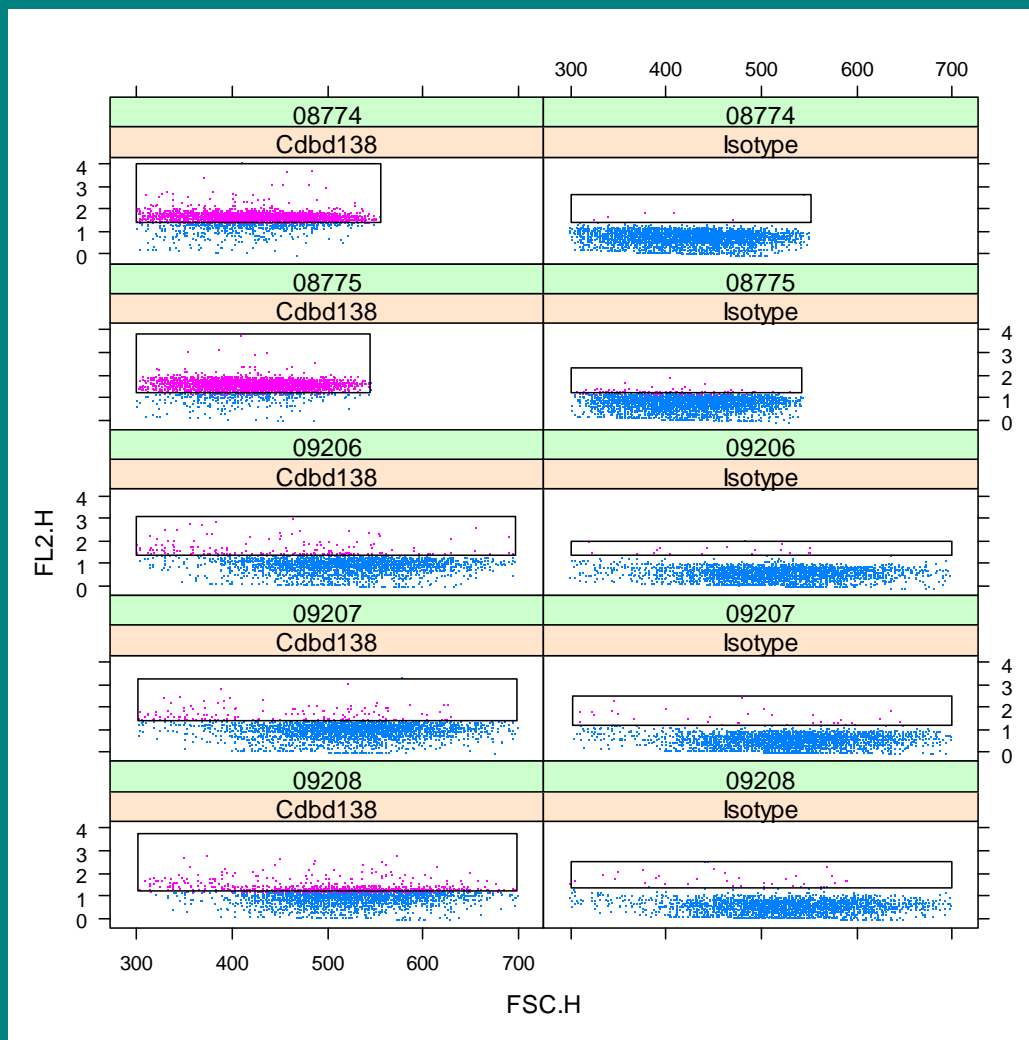
- > *applyControlGates(platePBMC)*

# PBMC Results



# PBMC Lymphocytes





```
> xyplot(FL2.H ~ FSC.H | as.factor(FL2.H) * as.factor(plateName),
  transform("FL2.H"=log10) %on% virtPlate,
  displayFilter=TRUE, filter="Negative.Control")
```

# Normal Donor Experiment

- Characterize T-cell responses in healthy individuals
- Negative control for breast cancer study
- Tumor Associated Antigen (HER-2/neu, CEA, and MAGE-3) endogenous T-cell cytokine responses
- Infectious Agents (CMV, Flu, and HIV)
- Do we see a response to tumor or viral stimuli?

# Normal Donor Stimulation Plate

blank	CD28+49d 1 ug/ml ea	pp65 mix 3.4 ug/ml	IE mix 3.4 ug/ml	A2 CMV pep 1 ug/ml	B7 CMV pep 1 ug/ml	flu HA+M1 3.4 ug/ml	HIV gag mix 3.4 ug/ml	CEA mix 3.4 ug/ml	MAGE-3 mix 3.4 ug/ml	her2/neu ICD mix 3.4 ug/ml	CEF
BFA	CMV lysate 1 ug/ml +CD28/49d	pp65 mix 1.7 ug/ml	IE mix 1.7 ug/ml	A2 CMV pep 0.2 ug/ml	B7 CMV pep 0.2 ug/ml	flu HA+M1 1.7 ug/ml	HIV gag mix 1.7 ug/ml	CEA mix 1.7 ug/ml	MAGE-3 mix 1.7 ug/ml	her2/neu ICD mix 1.7 ug/ml	CEF
DMSO cntl	BFA	pp65 mix 0.8 ug/ml	IE mix 0.8 ug/ml	A2 CMV pep 0.04 ug/ml	B7 CMV pep 0.04 ug/ml	flu HA+M1 0.8 ug/ml	HIV gag mix 0.8 ug/ml	CEA mix 0.8 ug/ml	MAGE-3 mix 0.8 ug/ml	her2/neu ICD mix 0.8 ug/ml	PMA 10 ng+ ion 1 ug/ml
DMSO cntl	CD28+49d 1 ug/ml ea	pp65 mix 0.4 ug/ml	IE mix 0.4 ug/ml	A2 CMV pep 0.008 ug/ml	B7 CMV pep 0.008 ug/ml	flu HA+M1 0.4 ug/ml	HIV gag mix 0.4 ug/ml	CEA mix 0.4 ug/ml	MAGE-3 mix 0.4 ug/ml	her2/neu ICD mix 0.4 ug/ml	SEB 1 ug/ml
blank	CD28+49d 1 ug/ml ea	pp65 mix 3.4 ug/ml	IE mix 3.4 ug/ml	A2 CMV pep 1 ug/ml	B7 CMV pep 1 ug/ml	flu HA+M1 3.4 ug/ml	HIV gag mix 3.4 ug/ml	CEA mix 3.4 ug/ml	MAGE-3 mix 3.4 ug/ml	her2/neu ICD mix 3.4 ug/ml	CEF
BFA	CMV lysate 1 ug/ml +CD28/49d	pp65 mix 1.7 ug/ml	IE mix 1.7 ug/ml	A2 CMV pep 0.2 ug/ml	B7 CMV pep 0.2 ug/ml	flu HA+M1 1.7 ug/ml	HIV gag mix 1.7 ug/ml	CEA mix 1.7 ug/ml	MAGE-3 mix 1.7 ug/ml	her2/neu ICD mix 1.7 ug/ml	CEF
DMSO cntl	BFA	pp65 mix 0.8 ug/ml	IE mix 0.8 ug/ml	A2 CMV pep 0.04 ug/ml	B7 CMV pep 0.04 ug/ml	flu HA+M1 0.8 ug/ml	HIV gag mix 0.8 ug/ml	CEA mix 0.8 ug/ml	MAGE-3 mix 0.8 ug/ml	her2/neu ICD mix 0.8 ug/ml	PMA 10 ng+ ion 1 ug/ml
DMSO cntl	CD28+49d 1 ug/ml ea	pp65 mix 0.4 ug/ml	IE mix 0.4 ug/ml	A2 CMV pep 0.008 ug/ml	B7 CMV pep 0.008 ug/ml	flu HA+M1 0.4 ug/ml	HIV gag mix 0.4 ug/ml	CEA mix 0.4 ug/ml	MAGE-3 mix 0.4 ug/ml	her2/neu ICD mix 0.4 ug/ml	SEB 1 ug/ml

\*Image taken from normal donor dataset description.doc on ficcs.org

# Normal Donor Staining Plate

IFNg FITC / CD69 PE / CD4 PerCP-Cy5.5 / CD3 APC			
IFNg FITC / CD69 PE / CD4 PerCP-Cy5.5 / CD3 APC			
IFNg FITC / CD69 PE / CD4 PerCP-Cy5.5 / CD3 APC			
IFNg FITC / CD69 PE / CD4 PerCP-Cy5.5 / CD3 APC			
TNFa FITC / IL-2 PE / CD4 PerCP-Cy5.5 / CD3 APC			
TNFa FITC / IL-2 PE / CD4 PerCP-Cy5.5 / CD3 APC			
TNFa FITC / IL-2 PE / CD4 PerCP-Cy5.5 / CD3 APC			
TNFa FITC / IL-2 PE / CD4 PerCP-Cy5.5 / CD3 APC			

\*Image taken from normal donor dataset description.doc on ficcs.org

# Normal Donor Analysis Setup

- Well Annotation File

<u>Well.Id</u>	<u>Sample.Type</u>	<u>Ab.Name</u>	<u>Channel</u>	<u>Negative.Control</u>	<u>Treatment</u>	<u>Concentration</u>
A04	Test	IFNg	FL1.H	A01	IE	3.4

.....

- Morphology Gate Lymphocytes

```
> Subset(plateNorm,  
  rectangleGate("FSC.H"=c(200,700),"SSC.H"=c(35,400)) &  
  norm2Filter("SSC.H","FSC.H",scale.factor=1.75))
```

- Correct for autofluorescence

```
> fixAutoFl(plateNorm, fsc="FSC.H",  
  chanCols=c("FL1.H","FL2.H","FL3.H","FL4.H"), unstain=unstain)
```

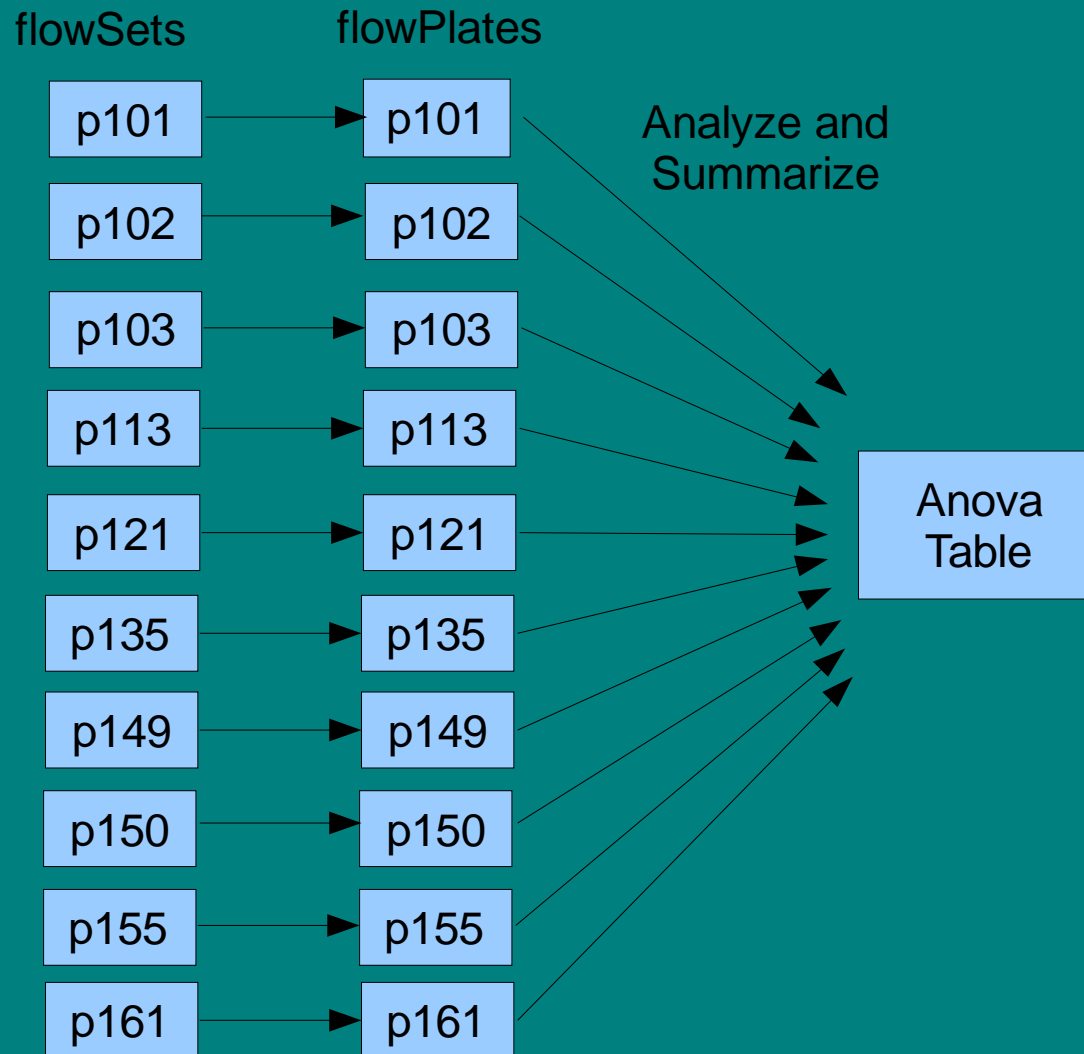
- Create and apply isotype gates

```
> setControlGates(plateNorm, gateType="Negative.Control", numMads=6)
```

.....

```
> applyControlGates(gvhd150lymph)
```

# Normal Donor Analysis

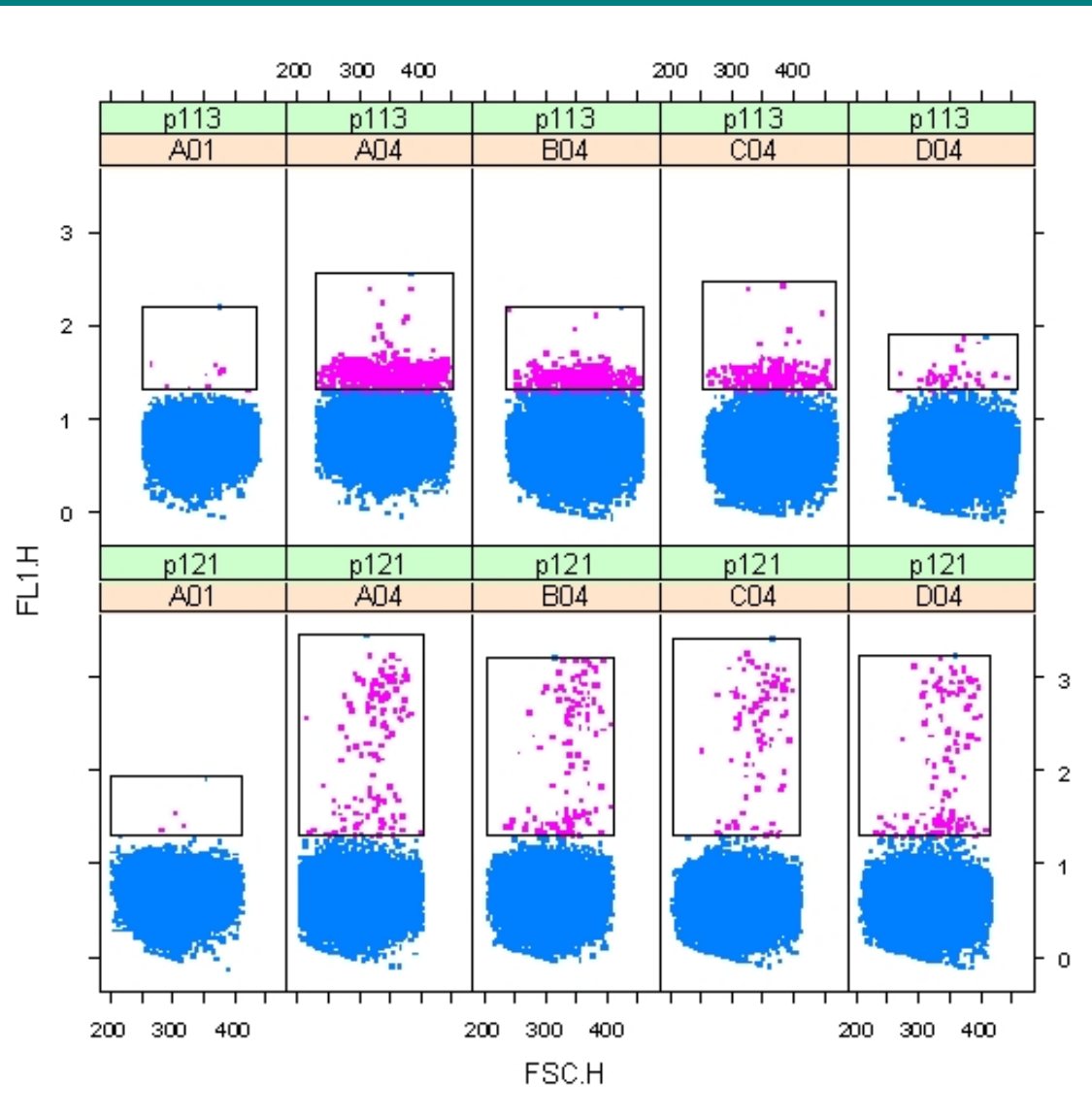


# Normal Donor Results

```
> summary(aov(IFNg ~ Patient + pp65 + IE + FLU.HA.M1 +  
HIV.gag.mix + CEA + MAGE.3 + her2.neu.ICD,  
data=normalDonorAOV))
```

	Df	SumSq	MeanSq	F value	Pr(>F)
Patient	8	1.469	0.184	5.3728	1.011e-06 ***
pp65	4	0.065	0.016	0.4740	0.7548
IE	4	1.276	0.319	9.3329	1.737e-07 ***
FLU.HA.M1	4	0.017	0.004	0.1221	0.9746
HIV.gag.mix	4	0.007	0.002	0.0487	0.9956
CEA	4	0.004	0.001	0.0292	0.9984
MAGE.3	4	0.004	0.001	0.0313	0.9981
her2.neu.ICD	4	0.031	0.008	0.2249	0.9246
Residuals	2555	87.313	0.034		

# Normal Donor Results



# Conclusions

- *plateCore* works well for screening a large number of antibodies on adherent and non-adherent cells
  - Not suitable for interactive gating
- Easily adapted to normal donor study
  - Response to CMV pp65
- Reporting capabilities
  - Sweave (R and LaTeX)

# Future Work

- Positive Controls
- Interact with FlowJo
  - Read/write FlowJo workspaces
- Graphically adjust control gates
  - One-dimension
- Release

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