flowPlots: An R Package for Analyzing Gated ICS Flow Cytometry Data

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Abstract

The flowPlots package provides graphical displays with embedded statistical tests for gated ICS flow cytometry data, helper functions to prepare data for plotting, and a data class which stores "stacked" data and has methods for computing summary measures on stacked data, such as marginal, polyfunctional degree (pfd) data, and polyfunctional degree parts (pfdParts) data.

1 Introduction

The flowPlots package provides a boxplot function with embedded statistical tests to compare groups, data helper functions to prepare data for the boxplot, ternary plot, or bar plot, and a data storage class, *StackedData*, which has methods to create profile and summary data such as marginal, polyfunctional degree (pfd), and polyfunctional degree parts (pfdParts) data from "stacked" data. This document provides examples of the graphical displays which can be used to analyze gated ICS flow cytometry data. Most of these displays are based on summary data. If the summary data are already available, the displays can be made directly. If the summary data are not available, but the data is available in "stacked" format, methods from the *StackedData* class can be used to compute the summary data. Examples are given to illustrate the use of the *StackedData* class. If the summary data are not available and the data is available in a format different from "stacked", the user can take advantage of this package by converting the data to the "stacked" format. The examples are based on a small subset of the data described in Kollmann et al. (2009).

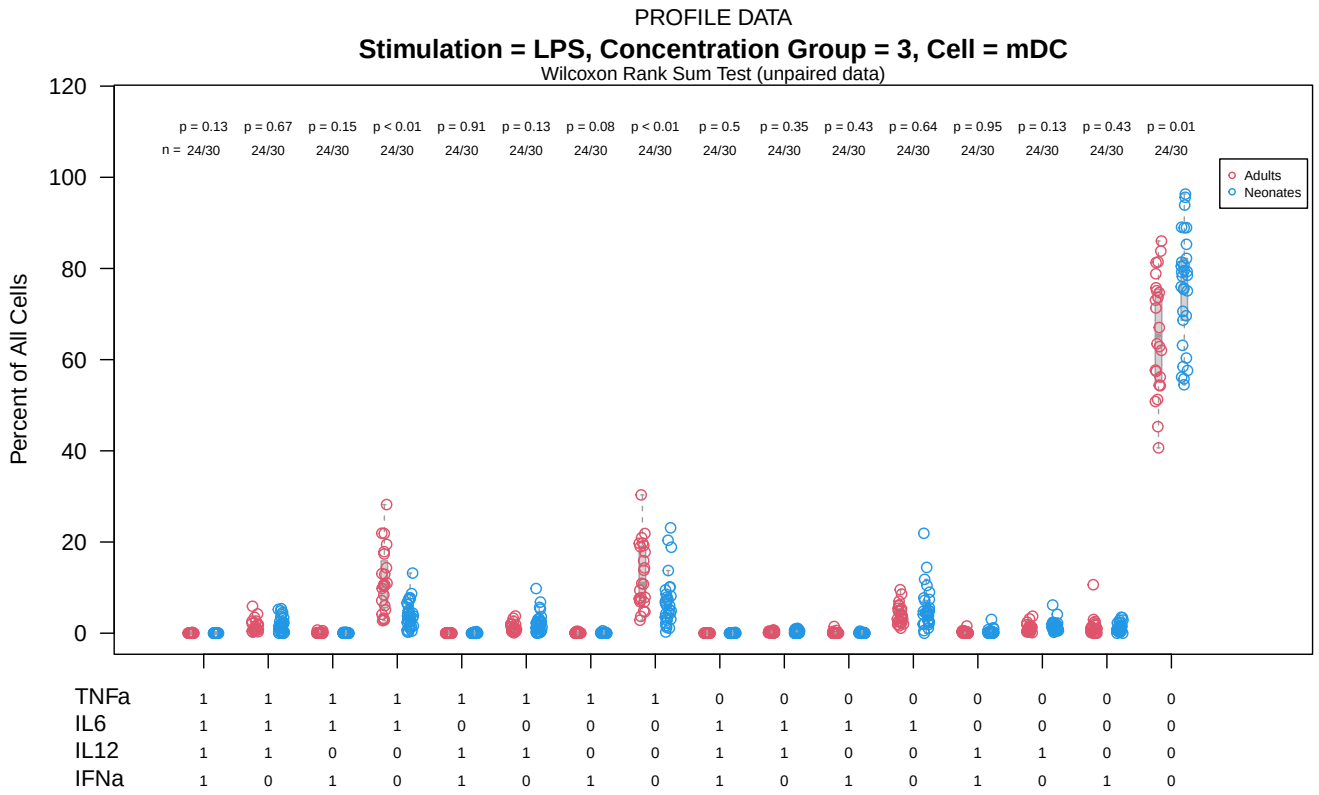
2 Plotting the data: boxplots, ternary plot, bar plot

The boxplot function and the data helper functions are used in the following examples. We load the profile data and the various summary data directly; i.e. we already have it available and do not need to compute it. For each example, we show the first few rows of the data frame being plotted.

2.1 Boxplots to compare groups on profileData

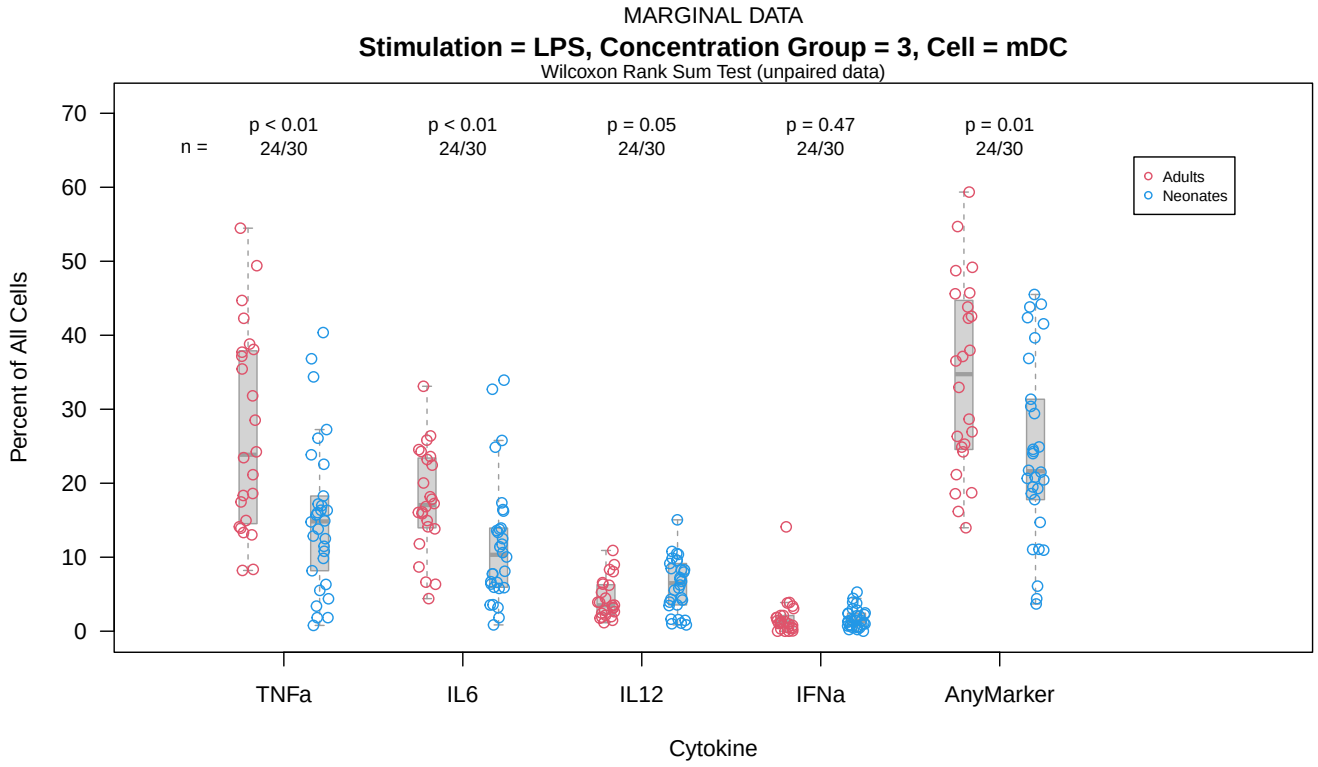
```
> data(profileDF)
> profileDataSubset = subset(profileDF, stim=="LPS" & concGroup==3 & cell=="mDC")
> profileDataSubset[1:3,]

  TNFa+IL6+IL12+IFNa+ TNFa+IL6+IL12+IFNa- TNFa+IL6+IL12-IFNa+
1                   0                0.43                   0
2                   0                0.32                   0
3                   0                0.44                   0
```

```
  TNFa+IL6+IL12-IFNa- TNFa+IL6-IL12+IFNa+ TNFa+IL6-IL12+IFNa-
1               21.86                   0                0.29
2                2.75                   0                0.50
3                3.06                   0                1.97
  TNFa+IL6-IL12-IFNa+ TNFa+IL6-IL12-IFNa- TNFa-IL6+IL12+IFNa+
1                   0               19.71                   0
2                   0                9.45                   0
3                   0                7.86                   0
  TNFa-IL6+IL12+IFNa- TNFa-IL6+IL12-IFNa+ TNFa-IL6+IL12-IFNa-
1                0.00                   0                 2.0
2                0.23                   0                 1.1
3                0.44                   0                 2.4
  TNFa-IL6-IL12+IFNa+ TNFa-IL6-IL12+IFNa- TNFa-IL6-IL12-IFNa+
1                0.14                0.29                1.00
2                0.32                1.05                0.46
3                0.00                2.40                0.00
  TNFa-IL6-IL12-IFNa- stim concGroup cell groups subjectId group
1               54.28  LPS         3  mDC      1     a2004 adult
2               83.82  LPS         3  mDC      1     a2005 adult
3               81.43  LPS         3  mDC      1     a2006 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(profileDataSubset ,"group", 1:16)
> # Make x-axis tick labels
> data(markerMatrix)
> theMarkers = colnames(markerMatrix)
> xTickLabels = cbind(theMarkers, t(markerMatrix))

> GroupListBoxplot(groupDataList, xlabel="",
+    ylabel="Percent of All Cells", boxOutliers=FALSE,
+    xAxisLabels=xTickLabels, xMtext="Marker Category",
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.6, pCEX=.6, legendColor=c(2,4), legendCEX=.6,
+    xAxisCEX=.7, xAtMtext=-1)
> mtext("PROFILE DATA", side=3, line=2)
```



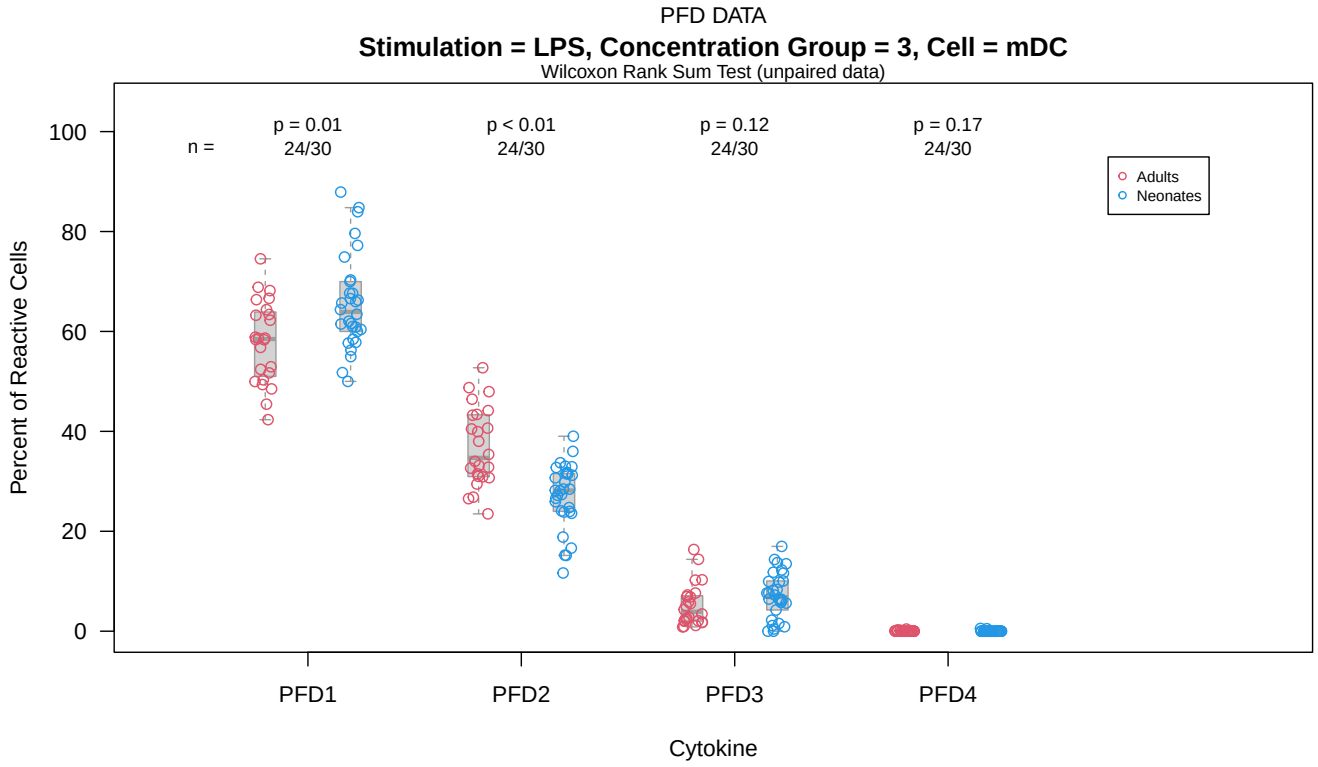
2.2 Boxplots to compare groups on marginalData

```
> data(marginalDF)
> marginalDataSubset = subset(marginalDF, stim=="LPS" & concGroup==3 & cell=="mDC")
> marginalDataSubset[1:3,]

   TNFa   IL6 IL12 IFNa AnyMarker stim concGroup cell groups subjectId group
1 42.29 24.29 1.15 1.14     45.72  LPS         3  mDC      1     a2004 adult
2 13.02  4.40 2.42 0.78     16.18  LPS         3  mDC      1     a2005 adult
3 13.33  6.34 5.25 0.00     18.57  LPS         3  mDC      1     a2006 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(marginalDataSubset ,"group", 1:5)

> GroupListBoxplot(groupDataList, xlabel="Cytokine",
+    ylabel="Percent of All Cells", boxOutliers=FALSE,
+    xAxisLabels=c("TNFa","IL6","IL12","IFNa","AnyMarker"),
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.8, pCEX=.8, legendColor=c(2,4), legendCEX=.7)
> mtext("MARGINAL DATA", side=3, line=2)
```



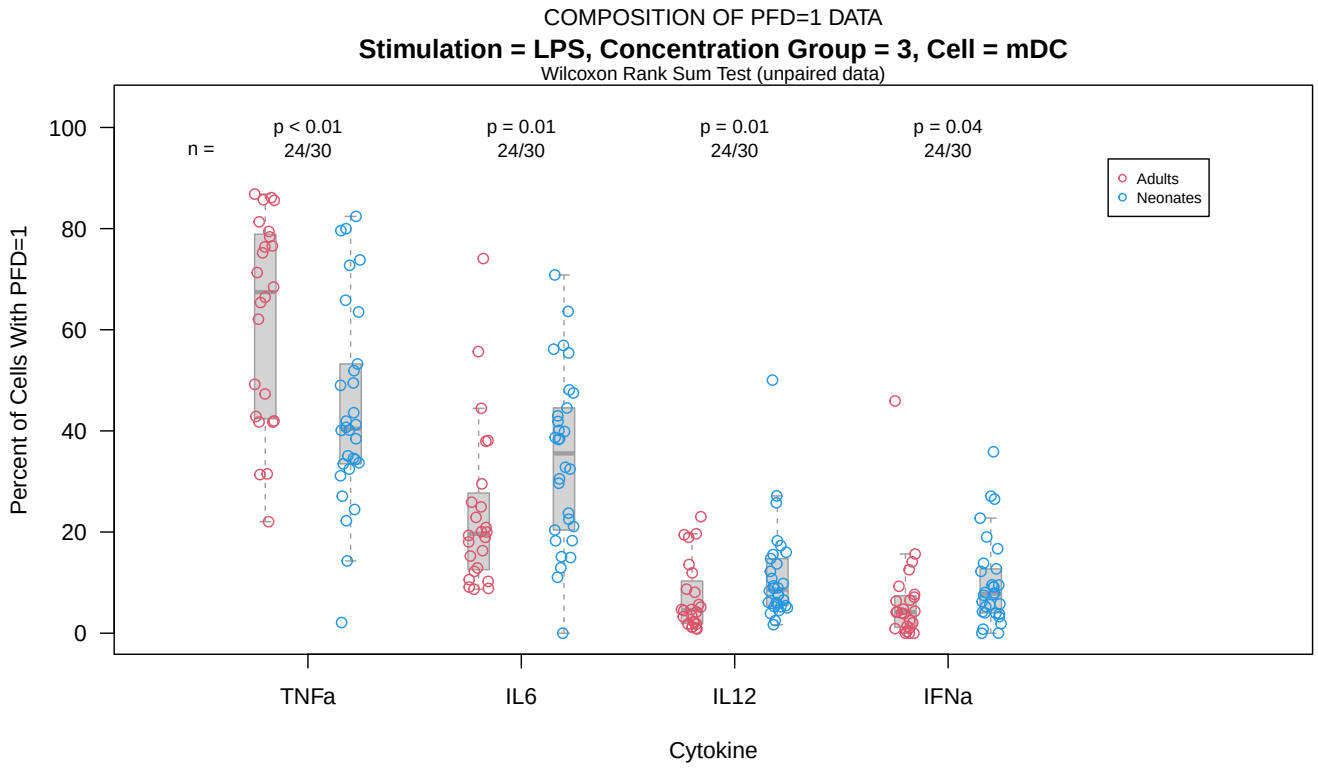
2.3 Boxplots to compare groups on pfdData

```
> data(pfdDF)
> pfdDataSubset = subset(pfdDF, stim=="LPS" & concGroup==3 & cell=="mDC")
> pfdDataSubset[1:3,]

      PFD1     PFD2      PFD3 PFD4 stim concGroup cell groups subjectId group
1 50.30621 48.75328 0.9405074    0  LPS         3  mDC      1     a2004 adult
2 74.53646 23.48578 1.9777503    0  LPS         3  mDC      1     a2005 adult
3 68.17447 29.45611 2.3694130    0  LPS         3  mDC      1     a2006 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(pfdDataSubset ,"group", 1:4)

> GroupListBoxplot(groupDataList, xlabel="Cytokine",
+    ylabel="Percent of Reactive Cells", boxOutliers=FALSE,
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.8, pCEX=.8, legendColor=c(2,4), legendCEX=.7)
> mtext("PFD DATA", side=3, line=2)
```

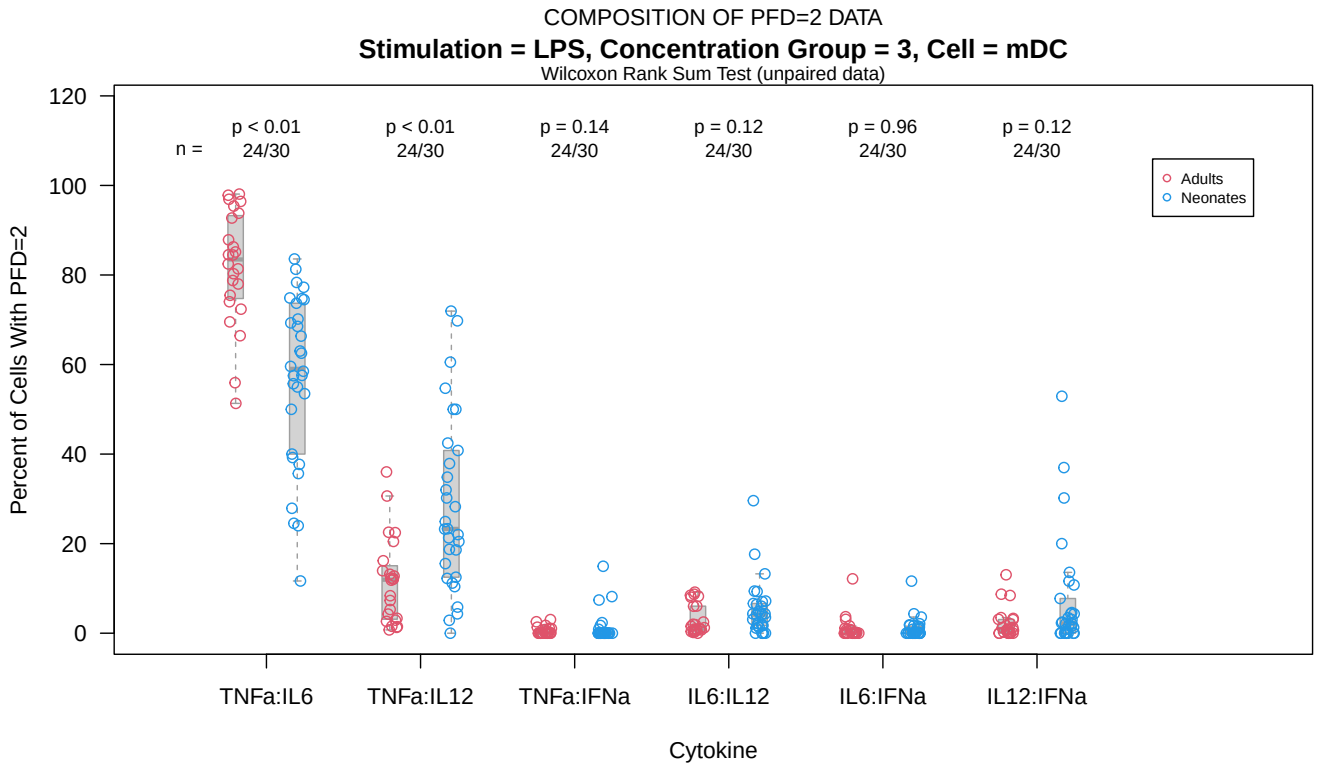


2.4 Boxplots to compare groups on pfdPartsData

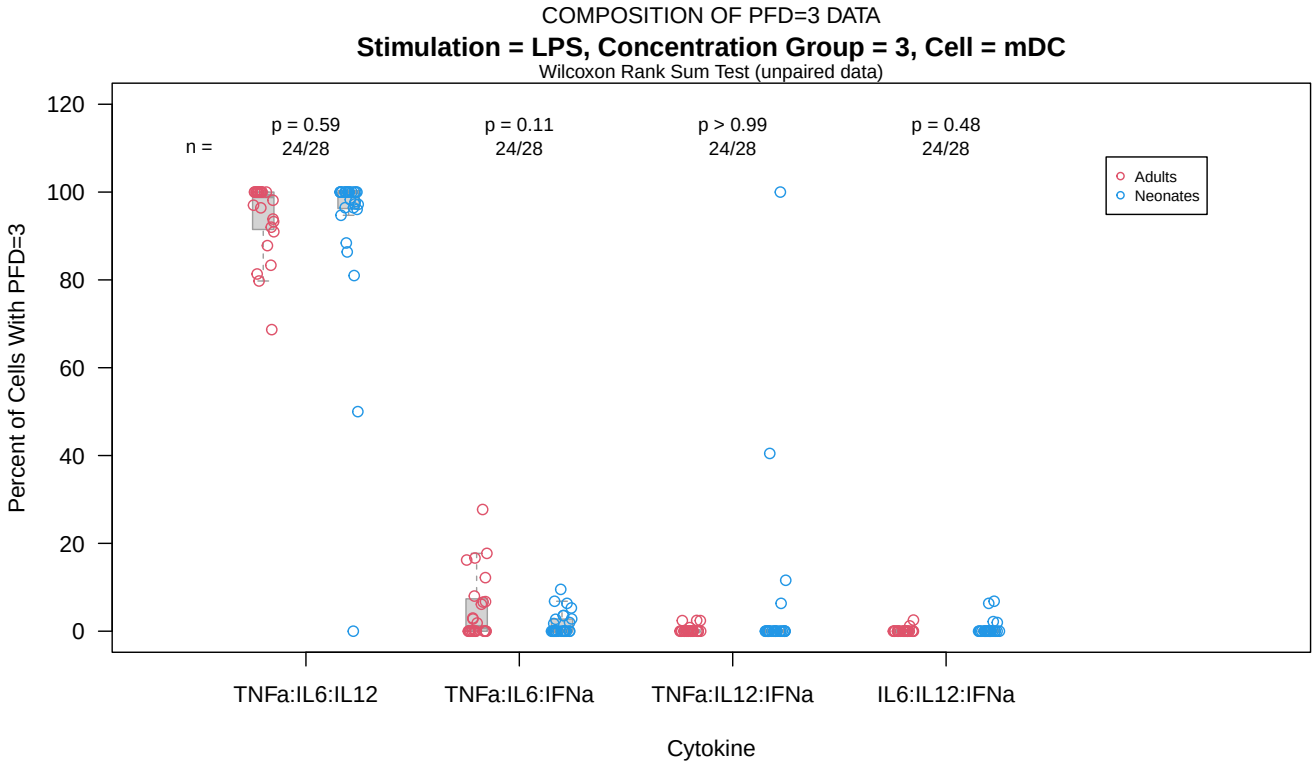
```
> data(pfdPartsList)
> # Look at the composition percentages for the case where PFD=1
> pfdEq1PartsDataSubset = subset(pfdPartsList[[1]], stim=="LPS" & concGroup==3 & cell=="mDC")
> pfdEq1PartsDataSubset[1:3,]

      TNFa       IL6      IL12     IFNa stim concGroup cell groups subjectId
1 85.69565  8.695652  1.260870 4.347826  LPS         3  mDC      1     a2004
2 78.35821  9.121061  8.706468 3.814262  LPS         3  mDC      1     a2005
3 62.08531 18.957346 18.957346 0.000000  LPS         3  mDC      1     a2006
  group
1 adult
2 adult
3 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(pfdEq1PartsDataSubset, "group", 1:4)

> GroupListBoxplot(groupDataList, xlabel="Cytokine",
+    ylabel="Percent of Cells With PFD=1", boxOutliers=FALSE,
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.8, pCEX=.8, legendColor=c(2,4), legendCEX=.7)
> mtext("COMPOSITION OF PFD=1 DATA", side=3, line=2)
```




```
> # Look at the composition percentages for the case where PFD=2
> pfdEq2PartsDataSubset = subset(pfdPartsList[[2]], stim=="LPS" & concGroup==3 & cell=="mDC")
> pfdEq2PartsDataSubset[1:3,]

  TNFa:IL6 TNFa:IL12 TNFa:IFNa IL6:IL12 IL6:IFNa IL12:IFNa stim concGroup cell
1 98.07088  1.301032         0 0.000000        0 0.6280843  LPS         3  mDC
2 72.36842 13.157895         0 6.052632        0 8.4210526  LPS         3  mDC
3 55.94150 36.014625         0 8.043876        0 0.0000000  LPS         3  mDC
  groups subjectId group
1      1     a2004 adult
2      1     a2005 adult
3      1     a2006 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(pfdEq2PartsDataSubset, "group", 1:6)

> GroupListBoxplot(groupDataList, xlabel="Cytokine",
+    ylabel="Percent of Cells With PFD=2", boxOutliers=FALSE,
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.8, pCEX=.8, legendColor=c(2,4), legendCEX=.7)
> mtext("COMPOSITION OF PFD=2 DATA", side=3, line=2)
```

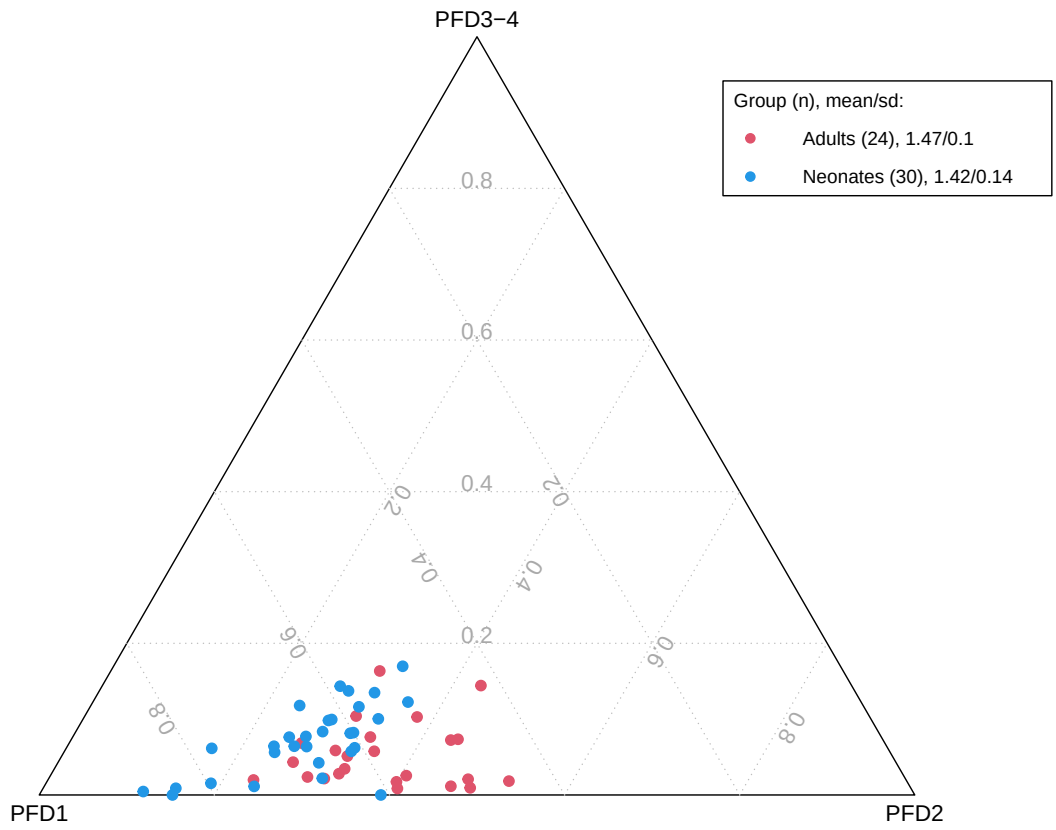



```
> # Look at the composition percentages for the case where PFD=3
> pfdEq3PartsDataSubset = subset(pfdPartsList[[3]], stim=="LPS" & concGroup==3 & cell=="mDC")
> pfdEq3PartsDataSubset[1:3,]

  TNFa:IL6:IL12 TNFa:IL6:IFNa TNFa:IL12:IFNa IL6:IL12:IFNa stim concGroup cell
1           100             0              0             0  LPS         3  mDC
2           100             0              0             0  LPS         3  mDC
3           100             0              0             0  LPS         3  mDC
  groups subjectId group
1      1     a2004 adult
2      1     a2005 adult
3      1     a2006 adult

> # Use data helper function, makeDataList()
> groupDataList = makeDataList(pfdEq3PartsDataSubset, "group", 1:4)

> GroupListBoxplot(groupDataList, xlabel="Cytokine",
+    ylabel="Percent of Cells With PFD=3", boxOutliers=FALSE,
+    mainTitle="Stimulation = LPS, Concentration Group = 3, Cell = mDC",
+    legendGroupNames=c("Adults","Neonates"), pointColor=c(2,4),
+    testTitleCEX=.8, nCEX=.8, pCEX=.8, legendColor=c(2,4), legendCEX=.7)
> mtext("COMPOSITION OF PFD=3 DATA", side=3, line=2)
```



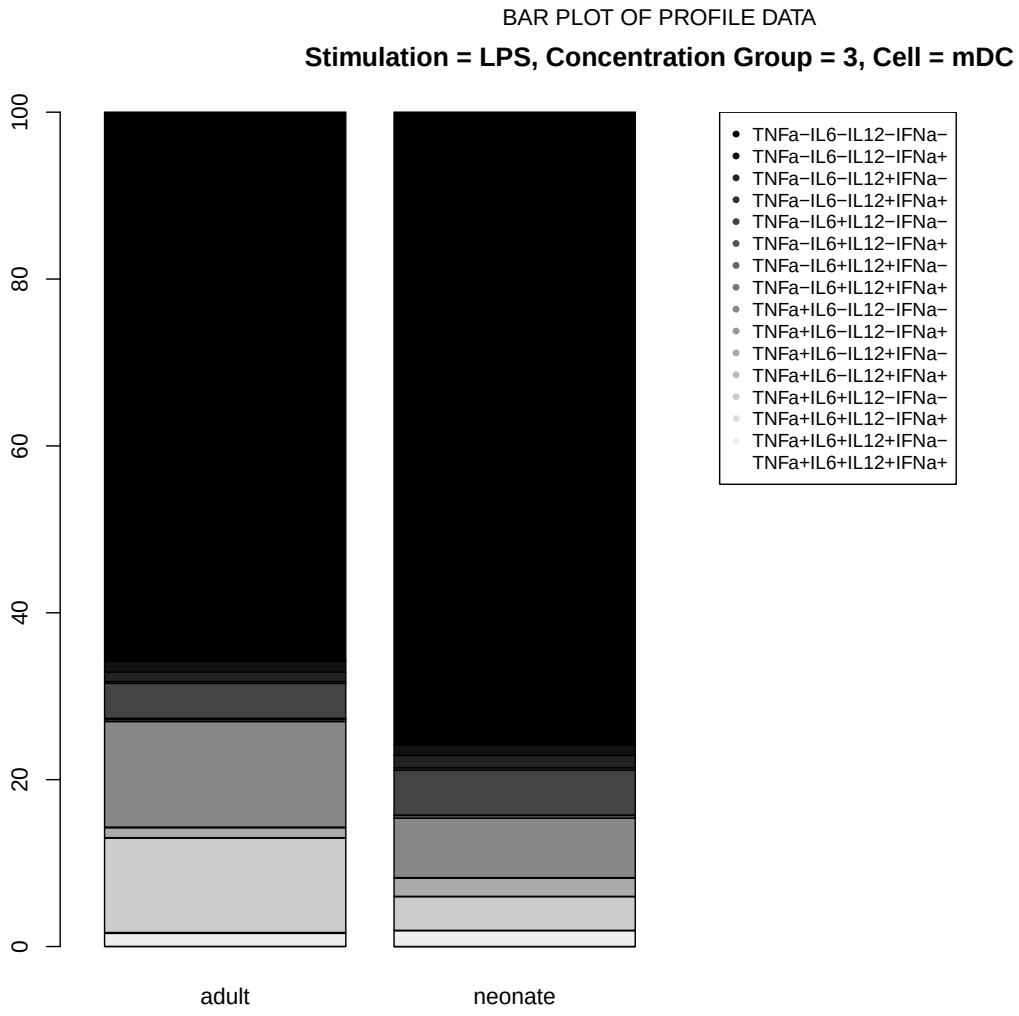
2.5 Ternary plots to compare groups on pfdData

```
> data(pfdDF)
> pfdDataSubset = subset(pfdDF, stim=="LPS" & concGroup==3 & cell=="mDC")
> # Use data helper function, makeTernaryData()
> ternaryData = makeTernaryData(pfdDataSubset, 1, 2, 3:4)
> colnames(ternaryData) = c("PFD1", "PFD2", "PFD3-4")
> adultPFDData = subset(pfdDataSubset, group=="adult", select=c(PFD1:PFD4))
> neoPFDData = subset(pfdDataSubset, group=="neonate", select=c(PFD1:PFD4))
> groupPFDDataList = list(adultPFDData, neoPFDData)
> pfdGroupStatsList = computePFDGroupStatsList(groupPFDDataList, pfdValues=1:4,
+    numDigitsMean=3, numDigitsSD=2)
> groupNames = c("Adults","Neonates")
> legendNames = legendPFDStatsGroupNames(pfdGroupStatsList,groupNames)

> # Load the package, vcd, for the ternaryplot() fcn
> library(vcd)
> ternaryplot(ternaryData, cex=.5, col=as.numeric(pfdDataSubset$group)*2,
+    main="TERNARY PLOT OF PFD DATA,
+ Stimulation = LPS, Concentration Group = 3, Cell = mDC")
> grid_legend(0.8, 0.7, pch=c(20,20), col=c(2,4), legendNames,
+    title = "Group (n), mean/sd:", gp=gpar(cex=.8))
```



2.6 Bar plots to compare groups on profileData

```
> data(profileDF)
> profileDataSubset = subset(profileDF, stim=="LPS" & concGroup==3 & cell=="mDC")
> profileColumns = 1:16
> # Use data helper function, makeBarplotData()
> barplotData = makeBarplotData(profileDataSubset, profileColumns, groupVariableName="group")
> barplotDataWithLegend = cbind(barplotData, NA, NA)
> barColors = gray(0:15/15)[16:1]

> barplot(barplotDataWithLegend, col=barColors,
+ main="Stimulation = LPS, Concentration Group = 3, Cell = mDC")
> legendNames = rownames(barplotData)
> legend(2.75,100,legend=legendNames[16:1],col=barColors[16:1],cex=.8,pch=20)
> mtext("BAR PLOT OF PROFILE DATA", side=3, line=3)
```

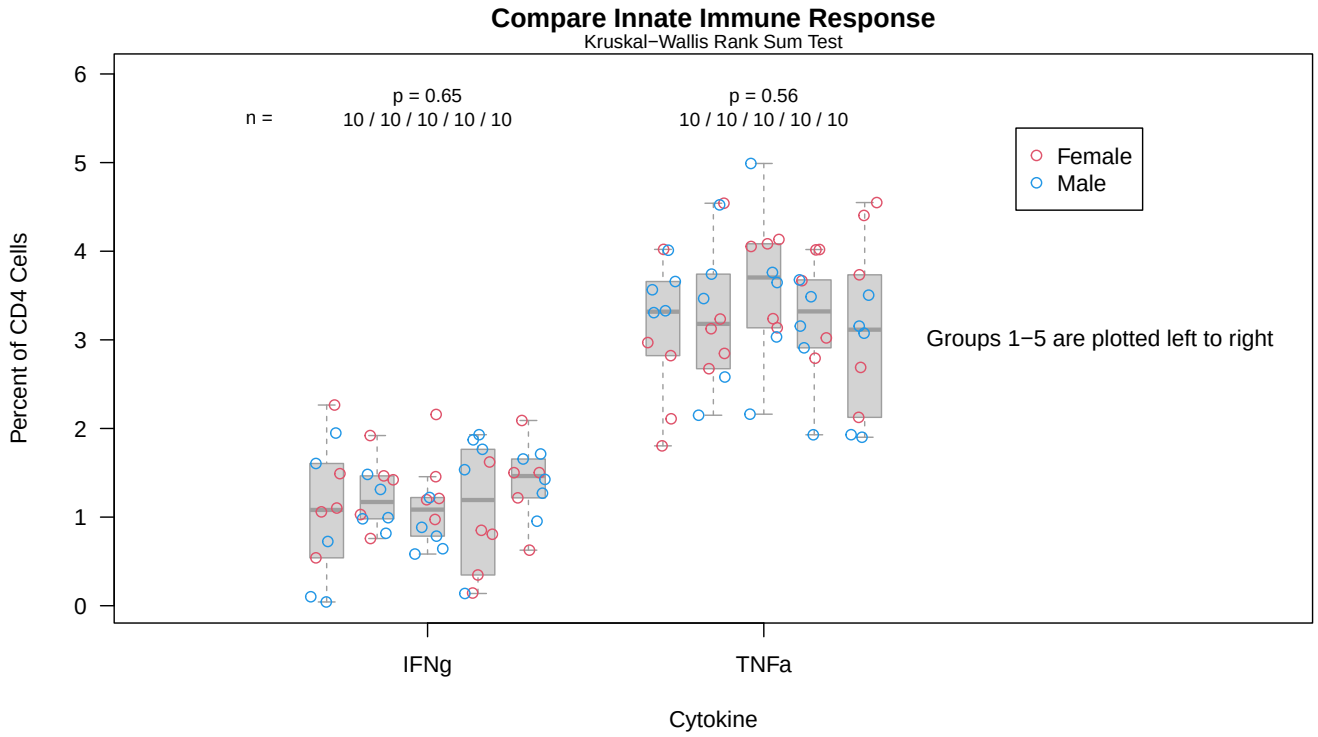


3 A Boxplot Example to Illustrate Multiple Groups and Point Colors

As many as 11 groups can be compared using the `GroupListBoxplot` function. Data points overlaid on the boxplots can be a single color, a color for each group (by specifying pointColor as a vector), varying colors for categories on the x-axis and for groups (by specifying pointColor as a matrix, where rows represent groups and cols represent x-cats), or a color can be specifically assigned for each point on the plot (by setting pointColor equal to a list of matrices, where each matrix represents a group, rows represent subjects and cols represent x-cats). In the following made-up example, we compare five groups and use point coloring to identify gender.

```
> # Create Example data list
> group1 = as.data.frame(cbind(rnorm(10,1.2,.6),rnorm(10,3.2,.8)))
> group2 = as.data.frame(cbind(rnorm(10,1.2,.6),rnorm(10,3.2,.8)))
> group3 = as.data.frame(cbind(rnorm(10,1.2,.6),rnorm(10,3.2,.8)))
> group4 = as.data.frame(cbind(rnorm(10,1.2,.6),rnorm(10,3.2,.8)))
> group5 = as.data.frame(cbind(rnorm(10,1.2,.6),rnorm(10,3.2,.8)))
> dataList = list(group1,group2,group3,group4,group5)
```

```
> # Create pointColor list
> colorGroup1 = cbind(c(rep(2,5),rep(4,5)),c(rep(2,5),rep(4,5)))
> colorGroup2 = cbind(c(rep(2,5),rep(4,5)),c(rep(2,5),rep(4,5)))
> colorGroup3 = cbind(c(rep(2,5),rep(4,5)),c(rep(2,5),rep(4,5)))
> colorGroup4 = cbind(c(rep(2,5),rep(4,5)),c(rep(2,5),rep(4,5)))
> colorGroup5 = cbind(c(rep(2,5),rep(4,5)),c(rep(2,5),rep(4,5)))
> pointColorList = list(colorGroup1,colorGroup2,colorGroup3,colorGroup4, colorGroup5)

> GroupListBoxplot(dataList, xlabel="Cytokine", ylabel="Percent of CD4 Cells",
+ xAxisLabels=c("IFNg","TNFa"), mainTitle="Compare Innate Immune Response",
+ legendGroupNames=c("Female","Male"), legendColors=c(2,4), boxOutliers=FALSE,
+ pointColor=pointColorList, testTitleCEX=.8, nCEX=.8, pCEX=.8)
> text(3,3,"Groups 1-5 are plotted left to right")
```



4 The *StackedData* Class

"Stacked" data refers to data originating from an ICS Flow Cytometry experiment, after gating has been applied. The marker categories (for example, cytokine combinations) are "stacked" in the table of data. An example subset:

```
> # load an .rda file of stacked data
> data(adultsNeonates)
> adultsNeonates[1:16,]
```

```
      id group stim concGroup cell percentAll count totalCount percentReactive
1  a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
```

```
2  a2004 adult  LPS         3  mDC       0.43     3        700        0.940625
3  a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
4  a2004 adult  LPS         3  mDC      21.86   153        700       47.818750
5  a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
6  a2004 adult  LPS         3  mDC       0.29     2        700        0.634375
7  a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
8  a2004 adult  LPS         3  mDC      19.71   138        700       43.115625
9  a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
10 a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
11 a2004 adult  LPS         3  mDC       0.00     0        700        0.000000
12 a2004 adult  LPS         3  mDC       2.00    14        700        4.375000
13 a2004 adult  LPS         3  mDC       0.14     1        700        0.306250
14 a2004 adult  LPS         3  mDC       0.29     2        700        0.634375
15 a2004 adult  LPS         3  mDC       1.00     7        700        2.187500
16 a2004 adult  LPS         3  mDC      54.28   380        700              NA
              cytCombo
1  TNFa+IL6+IL12+IFNa+
2  TNFa+IL6+IL12+IFNa-
3  TNFa+IL6+IL12-IFNa+
4  TNFa+IL6+IL12-IFNa-
5  TNFa+IL6-IL12+IFNa+
6  TNFa+IL6-IL12+IFNa-
7  TNFa+IL6-IL12-IFNa+
8  TNFa+IL6-IL12-IFNa-
9  TNFa-IL6+IL12+IFNa+
10 TNFa-IL6+IL12+IFNa-
11 TNFa-IL6+IL12-IFNa+
12 TNFa-IL6+IL12-IFNa-
13 TNFa-IL6-IL12+IFNa+
14 TNFa-IL6-IL12+IFNa-
15 TNFa-IL6-IL12-IFNa+
16 TNFa-IL6-IL12-IFNa-
```

The *StackedData* class has 6 slots for data:

1. stackedData, a `data.frame`
2. profileData, a `data.frame`
3. marginalData, a `data.frame`.
4. pfdData, a `data.frame`.
5. pfdPartsData, a `list` of `data.frame`'s.
6. markers, a `matrix`.

The stackedData often is provided by the lab as a table in CSV format.

The markers data is a matrix of 0's and 1's in which each row of the matrix represents one of the possible marker combinations in the "stacked" data and each column represents a marker. The stackedData data frame

should be sorted within each subset of interest (for example, subjectID, cellType, stimulation, and concentration) so that the marker combinations match the order of the rows in the markers matrix before using the *StackedData* class methods to compute summary data.

The *StackedData* class provides methods to compute the matrix of markers, and the profileData, marginalData, pfdData, and pfdPartsData data frames.

4.1 Marker Data: markers

The marker categories (eg. cytokine combinations) should be stacked in this order within each subset of interest in the stackedData.

```
> data(markerMatrix)
> print(markerMatrix)
```

```
      TNFa IL6 IL12 IFNa
 [1,]    1   1    1    1
 [2,]    1   1    1    0
 [3,]    1   1    0    1
 [4,]    1   1    0    0
 [5,]    1   0    1    1
 [6,]    1   0    1    0
 [7,]    1   0    0    1
 [8,]    1   0    0    0
 [9,]    0   1    1    1
[10,]    0   1    1    0
[11,]    0   1    0    1
[12,]    0   1    0    0
[13,]    0   0    1    1
[14,]    0   0    1    0
[15,]    0   0    0    1
[16,]    0   0    0    0
```

4.2 The Other Data Slots: stackedData, profileData, marginalData, pfdData, pfdPartsData

Examples of the data held in these slots have been given elsewhere in this document: stackedData (Section 4), profileData (Section 2.1), marginalData (Section 2.2), pfdData (Section 2.3), and pfdPartsData (Section 2.4).

5 Using the *StackedData* Class

In this section we create a StackedData object and use the class methods to compute profile and summary data.

5.1 Create a `stackedDataObject`

```
> # create the StackedData object based on the adultsNeonates data
> stackedDataObject = new("StackedData", stackedData = adultsNeonates)
```

5.2 Create the markers

The stackedData can either include or exclude the "all-negative" combination of markers (eg. the TNFa-IL6-IL12-IFNa- row). If that row is included, set the `computeMarkers` parameter `includeAllNegativeRow` to `TRUE`, else set it to `FALSE`. If you create this matrix on your own, create a matrix which matches the data.

```
> markerNames = c("TNFa","IL6","IL12","IFNa")
> markers = computeMarkers(markerNames, includeAllNegativeRow=TRUE)
> markers(stackedDataObject) = markers
```

5.3 Sort the stackedData

The stackedData should be sorted within subsets of interest (such as: subjectID, cellType, stimulation, and concentration) so that the data within each subset matches the marker combinations specified by the marker matrix. Usually the lab provides data sorted in this way. If not, sort it before applying the *StackedData* class methods.

5.4 Create the profileData

```
> # The byVarNames specify the subsets in the data
> byVarNames = c("stim", "concGroup", "cell")
> profileData = computeProfileData(stackedDataObject, byVarNames, "id", "percentAll", "group")
> profileData(stackedDataObject) = profileData
```

5.5 Create the marginalData

```
> # The byVarNames specify the subsets in the data
> byVarNames = c("stim", "concGroup", "cell")
> marginalData = computeMarginalData(stackedDataObject, byVarNames, "id", "percentAll", "group")
> marginalData(stackedDataObject) = marginalData
```

5.6 Create the pfdData

```
> # The byVarNames specify the subsets in the data
> byVarNames = c("stim", "concGroup", "cell")
> pfdData = computePFDData(stackedDataObject, byVarNames, "id", "percentAll", "group")
> pfdData(stackedDataObject) = pfdData
```

5.7 Create the pfdPartsData

```
> # The byVarNames specify the subsets in the data
> byVarNames = c("stim", "concGroup", "cell")
> pfdPartsData = computePFDPartsData(stackedDataObject, byVarNames, "id", "percentAll", "group")
> pfdPartsData(stackedDataObject) = pfdPartsData
```

6 Acknowledgments

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References

TR Kollmann, J Crabtree, A Rein-Weston, D Blimkie, F Thomai, X Wang, J Furlong, E Fortuno III, A Hajjar, N Hawkins, S Self, and C Wilson. The neonatal innate immune system is not less responsive than the adult but responds differently. *J Immunology*, 183:7150–7160, 2009.