

flowWorkspace: A Package for Importing flowJo Workspaces into R

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1 Purpose

The purpose of this package is to provide functionality to import relatively simple *flowJo* workspaces into R. By this we mean, accessing the samples, groups, transformations, compensation matrices, gates, and population statistics in the *flowJo* workspace, and replicating these using (primarily) *flowCore* functionality.

2 Why Another flowJo Workspace Import Package?

There was a need to import *flowJo* workspaces into R for comparative gating. The *flowFlowJo* package did not meet our needs. Many groups have legacy data with associated flowJo XML workspace files in version 2.0 format that they would like to access using BioConductor's tools. Hopefully this package will fill that need.

3 Support

This package supports importing of **Version 2.0 XML workspaces only**. We cannot import **.jo** files directly. You will have to save them in XML workspace format, and ensure that that format is *workspace version 2.0*. The package has been tested and works with files generated using flowJo version 9.1 on Mac OS X. XML generated by older versions of *flowJo* on windows should work as well. We do not yet support *flowJo*'s **Chimera** XML schema, though that support will be provided in the future.

The package supports import of only a subset of the features present in a flowJo workspace. The package allows importing of sample and group names, gating hierarchy, compensation matrices, data transformation functions, a subset of gates, and population counts.

BooleanGates are now supported by flowWorkspace.

4 Data Structures

The following section walks through opening and importing a flowJo workspace.

4.1 Loading the library

Simply call:

```
> library(flowWorkspace)
```

Scalable Robust Estimators with High Breakdown Point (version 1.3-01)

The library depends on numerous other packages, including *graph*, *XML*, *Rgraphviz*, *flowCore*, *flowViz*, *RBGL*.

4.2 Opening a Workspace

We represent flowJo workspaces using `flowJoWorkspace` objects. We only need to know the path to, and filename of the flowJo workspace.

```
> d<-system.file("extdata",package="flowWorkspaceData");  
> wsfile<-list.files(d,pattern="A2004Analysis.xml",full=T)
```

In order to open this workspace we call:

```
> ws<-openWorkspace(wsfile)  
> summary(ws)
```

FlowJo Workspace Version 2.0

File location: /Library/Frameworks/R.framework/Versions/2.15/Resources/library/flowW

File name: A2004Analysis.xml

Workspace is open.

Groups in Workspace

	Name	Num.Samples
1	All Samples	2

We see that this is a version 2.0 workspace file. Its location and filename are printed. Additionally, you are notified that the workspace file is open. This refers to the fact that the XML document is internally represented using 'C' data structures from the *XML* package. After importing the file, the workspace must be explicitly closed using `closeWorkspace()` in order to free up that memory.

4.3 Parsing the Workspace

With the workspace file open, we have not yet imported the XML document. The next step parses the XML workspace and creates R data structures to represent some of the information therein. Specifically, by calling `parseWorkspace()` the user will be presented with a list of *groups* in the workspace file and need to choose one group to import. Why only one? Because of the way flowJo handles data transformation and compensation. Each group of samples is associated with a compensation matrix and specific data transformation. These are applied to all samples in the group. When a particular group of samples is imported, the package generates a *GatingHierarchy* for each sample, describing the set of gates applied to the data (note: polygons, rectangles, quadrants, and ovals and boolean gates are supported). The set of *GatingHierarchies* for the group of samples is stored in a *GatingSet* object. Calling `parseWorkspace()` is quite verbose, informing the user as each gate is created. The parsing can also be done non-interactively by specifying which group to import directly in the function call (either an index or a group name). An additional optional argument `execute=T/F` specifies whether you want to load, compensate, transform the data and compute statistics immediately after parsing the XML tree.

```
> G<-parseWorkspace(ws,name=1,path=ws@path,isNcdf=FALSE,cleanup=FALSE,keep.indices=TRUE)
> #Lots of output here suppressed for the vignette.
```

When `isNcdf` flag is set `TRUE`, the data is stored in `ncdf` format on disk.

```
> G
```

```
A GatingSet with 2 samples
1 .      FCS File:  a2004_01T2pb05i_A1_A01.fcs
      GatingHierarchy with 20 gates
2 .      FCS File:  a2004_01T2pb05i_A2_A02.fcs
      GatingHierarchy with 20 gates
```

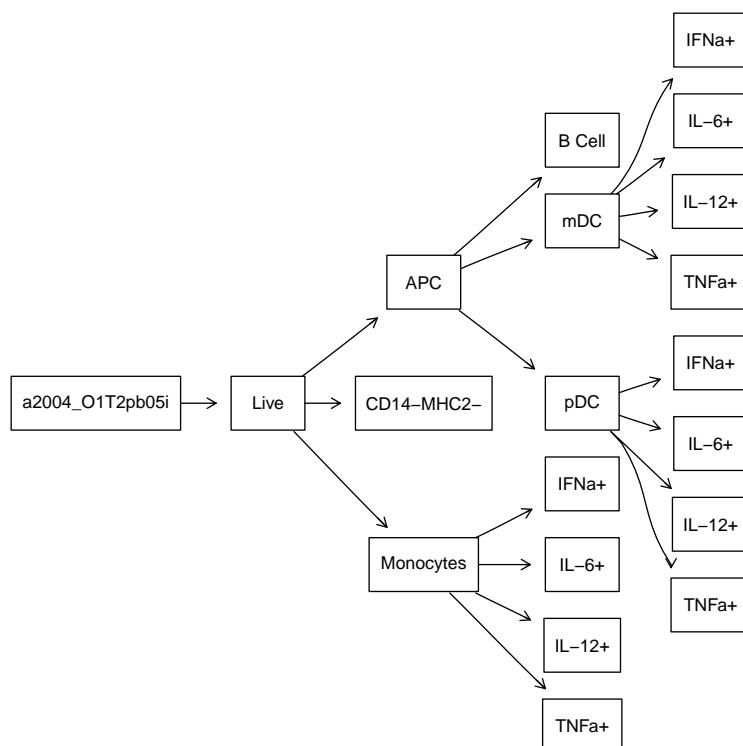
We have generated a *GatingSet* with 2 samples, each of which has 19 associated gates. Subsets of gating hierarchies can be accessed using the standard R subset syntax.

At this point we have parsed the workspace file and generate the gating hierarchy associated with each sample imported from the file. The data have been loaded, compensated, and transformed in the workspace, and gating has been executed. The resulting *GatingSet* contains a replicated analysis of the original flowJo workspace.

```
> G<-lapply(G,function(x)execute(x))
```

We can plot the gating hierarchy for a given sample:

```
> require(Rgraphviz)
> plot(G[[1]])
```



We can list the nodes (populations) in the gating hierarchy:

```
> getNodes(G[[1]])
```

[1]	"a2004_01T2pb05i"	"3.Live"	"4.APC"	"5.B Cell"
[5]	"6.mDC"	"7.IFNa+"	"8.IL-6+"	"9.IL-12+"
[9]	"10.TNFa+"	"11.pDC"	"12.IFNa+"	"13.IL-6+"
[13]	"14.IL-12+"	"15.TNFa+"	"16.CD14-MHC2-"	"17.Monocytes"
[17]	"18.IFNa+"	"19.IL-6+"	"20.IL-12+"	"21.TNFa+"

Note that the number preceding the period in the node names is just an identifier to help uniquely label populations in the gating hierarchy. It does not represent any information about population statistics. We can get a specific gate definition:

```
> getGate(G[[1]],getNodes(G[[1]])[3])
```

Polygonal gate '4.APC' with 14 vertices in dimensions <PerCP-CY5-5-A> and <PE-CY7-A>

We can extract the dimensions relating to a specific gate:

```
> getDimensions(G[[1]],getNodes(G[[1]])[3])
```

```
[1] "<PerCP-CY5-5-A>" "<PE-CY7-A>"
```

We can extract vertices of a gate:

```
> getBoundaries(G[[1]],getNodes(G[[1]])[3])
```

	<PerCP-CY5-5-A>	<PE-CY7-A>
[1,]	2349.993	2024.8746
[2,]	2163.383	1575.0085
[3,]	2240.899	992.3135
[4,]	2349.993	793.0647
[5,]	2585.516	696.7596
[6,]	3315.004	1138.4273
[7,]	3586.426	1354.9513
[8,]	3602.373	2040.1931
[9,]	3570.480	2256.4455
[10,]	3363.261	2318.7616
[11,]	3204.000	2240.8992
[12,]	3044.921	2209.8486
[13,]	2711.845	2070.8857
[14,]	2569.755	2055.5302

We can get the population proportion (relative to its parent) for a single population:

```
> getProp(G[[1]],getNodes(G[[1]])[3])
```

```
[1] 0.08402716
```

Or we can retrieve the population statistics for all populations in the sample:

```
> getPopStats(G[[1]])
```

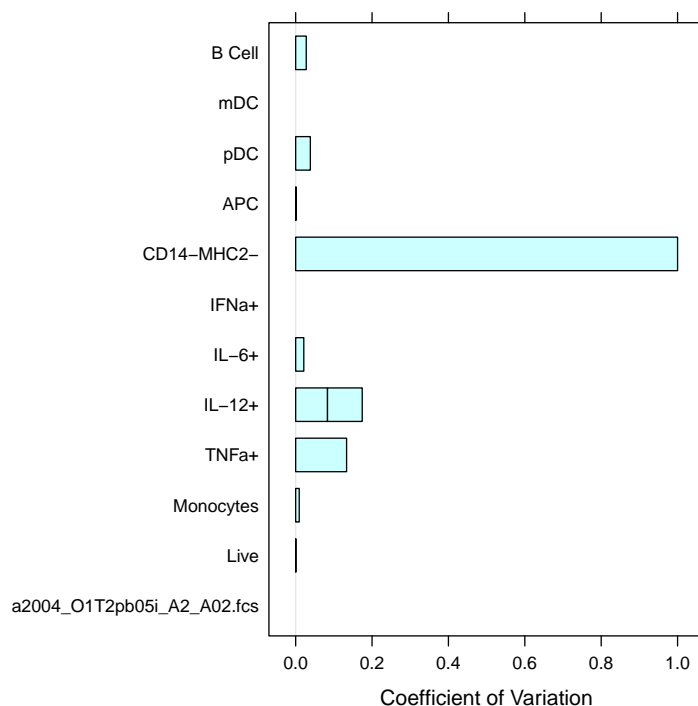
	flowCore.freq	flowJo.count	flowCore.count
a2004_01T2pb05i_A1_A01.fcs	1.000000000	61832	61832
/Live	0.800297581	49542	49484
/Live/Monocytes	0.058928138	2931	2916
/Live/Monocytes/TNFa+	0.250685871	754	731
/Live/Monocytes/IL-12+	0.047325103	146	138
/Live/Monocytes/IL-6+	0.237654321	694	693
/Live/Monocytes/IFNa+	0.003772291	13	11
/Live/CD14-MHC2-	0.543125051	26795	26876
/Live/APC	0.084027160	4141	4158
/Live/APC/pDC	0.104377104	446	434
/Live/APC/pDC/TNFa+	0.000000000	0	0
/Live/APC/pDC/IL-12+	0.571428571	250	248
/Live/APC/pDC/IL-6+	0.000000000	0	0
/Live/APC/pDC/IFNa+	0.002304147	1	1
/Live/APC/mDC	0.122174122	502	508
/Live/APC/mDC/TNFa+	0.141732283	71	72
/Live/APC/mDC/IL-12+	0.005905512	2	3
/Live/APC/mDC/IL-6+	0.043307087	22	22
/Live/APC/mDC/IFNa+	0.005905512	2	3
/Live/APC/B Cell	0.525493025	2271	2185

	parent.total	node
a2004_01T2pb05i_A1_A01.fcs	61832	a2004_01T2pb05i
/Live	61832	3.Live
/Live/Monocytes	49484	17.Monocytes
/Live/Monocytes/TNFa+	2916	21.TNFa+
/Live/Monocytes/IL-12+	2916	20.IL-12+
/Live/Monocytes/IL-6+	2916	19.IL-6+
/Live/Monocytes/IFNa+	2916	18. IFNa+
/Live/CD14-MHC2-	49484	16.CD14-MHC2-
/Live/APC	49484	4.APC
/Live/APC/pDC	4158	11.pDC

/Live/APC/pDC/TNFa+	434	15. TNFa+
/Live/APC/pDC/IL-12+	434	14. IL-12+
/Live/APC/pDC/IL-6+	434	13. IL-6+
/Live/APC/pDC/IFNa+	434	12. IFNa+
/Live/APC/mDC	4158	6. mDC
/Live/APC/mDC/TNFa+	508	10. TNFa+
/Live/APC/mDC/IL-12+	508	9. IL-12+
/Live/APC/mDC/IL-6+	508	8. IL-6+
/Live/APC/mDC/IFNa+	508	7. IFNa+
/Live/APC/B Cell	4158	5. B Cell

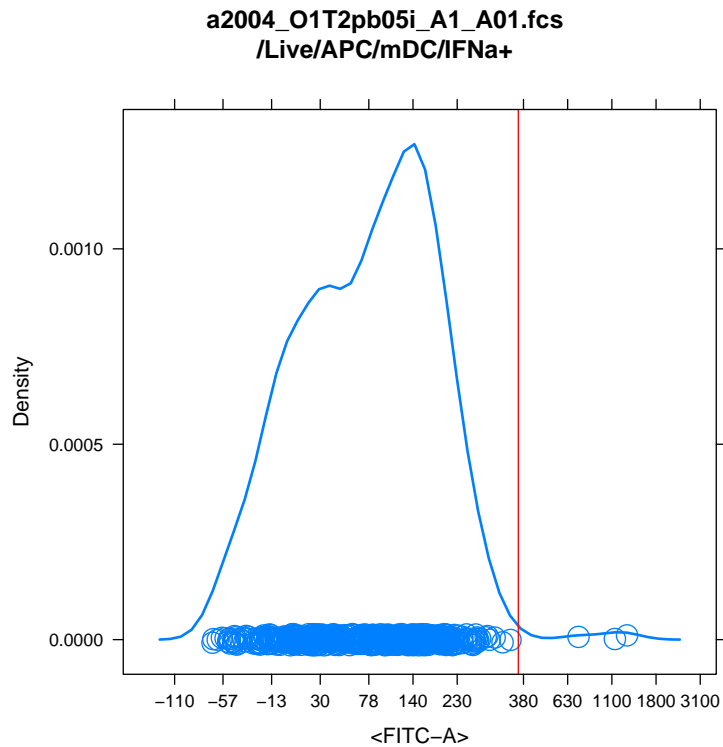
We can plot the coefficients of variation between the counts derived using flowJo and flowCore for each population:

```
> print(plotPopCV(G[[2]]))
```



We can plot individual gates: note the scale of the transformed axes.

```
> print(plotGate(G[[1]],getNodes(G[[1]])[6],lwd=2,cex=2))
```



If we have metadata associated with the experiment, it can be attached to the `GatingSet`.

```
> d<-data.frame(sample=factor(c("sample 1", "sample 2")),treatment=factor(c("sample",
> G@metadata<-new("AnnotatedDataFrame",data=d)
> pData(G);
```

```
      sample treatment
1 sample 1      sample
2 sample 2      control
```

We can retrieve the subset of data associated with a node:

```
> getData(G[[1]],getNode(G[[1]])[3]);
```

flowFrame object '1be493f5-51dd-4359-b2ed-524cd104eb5f'
with 4158 cells and 23 observables:

	name	desc	range	minRange	maxRange
\$P1	FSC-A	<NA>	262254.000	-111.00000	262143.000
\$P2	FSC-H	<NA>	262143.000	0.00000	262143.000


```

$P3          FSC-W <NA> 262143.000    0.00000 262143.000
$P4          SSC-A <NA> 262254.000 -111.00000 262143.000
$P5          SSC-H <NA> 262143.000    0.00000 262143.000
$P6          SSC-W <NA> 262143.000    0.00000 262143.000
$P7    <Am Cyan-A> CD123   3661.959  435.34379   4097.303
$P8          Am Cyan-H CD123 262143.000    0.00000 262143.000
$P9    <Pacific Blue-A> IL-12   3927.974  169.60860   4097.582
$P10   Pacific Blue-H IL-12 262143.000    0.00000 262143.000
$P11          <APC-A> CD11c   4405.818 -308.01302   4097.805
$P12          APC-H CD11c 262143.000    0.00000 262143.000
$P13    <APC-CY7-A> IL-6   3714.446  382.93207   4097.378
$P14          APC-CY7-H IL-6 262143.000    0.00000 262143.000
$P15    <Alexa 700-A> TNFa   3712.753  384.62271   4097.376
$P16          Alexa 700-H TNFa 262143.000    0.00000 262143.000
$P17          <FITC-A> IFNa   4180.519 -82.81306   4097.706
$P18          FITC-H IFNa 262143.000    0.00000 262143.000
$P19    <PerCP-CY5-5-A> MHCII   4942.398 -844.59317   4097.805
$P20   PerCP-CY5-5-H MHCII 262143.000    0.00000 262143.000
$P21          <PE-CY7-A> CD14   4942.398 -844.59317   4097.805
$P22          PE-CY7-H CD14 262143.000    0.00000 262143.000
$P23          Time <NA>   9918.400   89.00000  10007.400
322 keywords are stored in the 'description' slot

```

Or we can retrieve the indices specifying if an event is included inside or outside a gate using:

```
> getIndices(G[[1]],getNodes(G[[1]])[3])
```

The indices returned are relative to the parent population (member of parent AND member of current gate), so they reflect the true hierarchical gating structure.

If we wish to do compensation or transformation manually, we can retrieve all the compensation matrices from the workspace:

```
> C<-getCompensationMatrices(ws);
> C
```

```
$`A2004-A2005_06i`
```

	Am Cyan-A	Pacific Blue-A	APC-A	APC-CY7-A	Alexa 700-A
Am Cyan-A	1.00000	0.04800	0.000000	0.0000	0.00000
Pacific Blue-A	0.38600	1.00000	0.000529	0.0000	0.00000

APC-A	0.00642	0.00235	1.000000	0.0611	0.19800
APC-CY7-A	0.03270	0.02460	0.084000	1.0000	0.02870
Alexa 700-A	0.07030	0.05800	0.016200	0.3990	1.00000
FITC-A	0.74500	0.02090	0.001870	0.0000	0.00000
PerCP-CY5-5-A	0.00368	0.00178	0.015300	0.0269	0.07690
PE-CY7-A	0.01330	0.00948	0.000951	0.1380	0.00182
	FITC-A	PerCP-CY5-5-A	PE-CY7-A		
Am Cyan-A	0.028500	0.00104	0.00000		
Pacific Blue-A	0.000546	0.00000	0.00000		
APC-A	-0.000611	0.00776	0.00076		
APC-CY7-A	0.002690	0.00304	0.01010		
Alexa 700-A	0.001530	0.10800	0.00679		
FITC-A	1.000000	0.04180	0.00281		
PerCP-CY5-5-A	0.000000	1.00000	0.07030		
PE-CY7-A	0.002340	0.03360	1.00000		

Or we can retrieve transformations:

```
> T<-getTransformations(ws)
> names(T)

[1] "InputParameterTransform_Gain1_Offset1"
[2] "A2004-A2005_06i"
[3] "InputParameterTransform_Gain1_Offset1262144"

> names(T[[1]])

[1] "InputParameterTransform_Gain1_Offset1"
[2] "InputParameterTransform_Gain1_Offset1262144"

> T[[1]][[1]]

function (x, deriv = 0)
{
  deriv <- as.integer(deriv)
  if (deriv < 0 || deriv > 3)
    stop("'deriv' must be between 0 and 3")
  if (deriv > 0) {
    z0 <- double(z$n)
    z[c("y", "b", "c")] <- switch(deriv, list(y = z$b, b = 2 *
      z$c, c = 3 * z$d), list(y = 2 * z$c, b = 6 * z$d,
```

```

        c = z0), list(y = 6 * z$d, b = z0, c = z0))
    z[["d"]] <- z0
  }
  res <- .C(C_spline_eval, z$method, as.integer(length(x)),
    x = as.double(x), y = double(length(x)), z$n, z$x, z$y,
    z$b, z$c, z$d, PACKAGE = "stats")$y
  if (deriv > 0 && z$method == 2 && any(ind <- x <= z$x[1L]))
    res[ind] <- ifelse(deriv == 1, z$y[1L], 0)
  res
}
<bytecode: 0x5b5b868>
<environment: 0x4ce9014>

```

`getTransformations` returns a list, each element of which corresponds to a transformation applied to a group of samples. The transformation is presented as a list of functions to be applied to different dimensions of the data. Above, the transformation is applied to all samples of the group and for each sample in the group, the appropriate dimension is transformed using a channel-specific function from the list.

The list of samples in a workspace can be accessed by:

```
> getSamples(ws);
```

	sampleID	name	count	compID	pop.counts
1	1	a2004_01T2pb05i	61832	1	19
2	2	a2004_01T2pb05i	45363	1	19

And the groups can be accessed by:

```
> getSampleGroups(ws)
```

	groupName	groupID	sampleID
1	All Samples	0	1
2	All Samples	0	2

The `compID` column tells you which compensation matrix to apply to a group of files, and similarly, based on the name of the compensation matrix, which transformations to apply.

4.4 Converting to flowCore Objects

You may want to convert the imported workspace into `flowCore` objects, such as workflows. We provide this functionality via the `flowWorkspace2flowCore` function.

`flowWorkspace2flowCore` extracts the compensation matrices, transformation functions and all the gates from GatingHierarchies generated by `flowWorkspace` package and converts them to the respective views and actionItems of workflows defined by `flowCore` package. It takes a `gatingHierarchy`, `flowJoWorkspace` or `GatingSet` as the input, and returns one or multiple workflows as the result, depending on whether the gating hierarchies for each sample (including gate coordinates) are identical.

```
> wfs<-flowWorkspace2flowCore(G,path=ws@path);  
> wfs
```

```
[[1]]
```

```
A flow cytometry workflow called 'default'
```

```
The following data views are provided:
```

```
Basic view 'base view'
```

```
on a flowSet
```

```
not associated to a particular action item
```

```
View 'CompensationView'
```

```
on a flowSet linked to
```

```
compensation action item 'action_defaultCompensation'
```

```
View 'a2004_01T2pb05i'
```

```
on a flowSet linked to
```

```
transform action item 'action_defaultTransformation'
```

```
View '3.Live+'
```

```
on a flowSet linked to
```

```
gate action item 'action_3.Live'
```

```
View '4.APC+'
```

```
on a flowSet linked to
```

```
gate action item 'action_4.APC'
```

```
View '5.B Cell+'
```

on a flowSet linked to
gate action item 'action_5.B Cell'

View '6.mDC+'
on a flowSet linked to
gate action item 'action_6.mDC'

View '7.IFNα++'
on a flowSet linked to
gate action item 'action_7.IFNα+'

View '8.IL-6++'
on a flowSet linked to
gate action item 'action_8.IL-6+'

View '9.IL-12++'
on a flowSet linked to
gate action item 'action_9.IL-12+'

View '10.TNFα++'
on a flowSet linked to
gate action item 'action_10.TNFα+'

View '11.pDC+'
on a flowSet linked to
gate action item 'action_11.pDC'

View '12.IFNα++'
on a flowSet linked to
gate action item 'action_12.IFNα+'

View '13.IL-6++'
on a flowSet linked to
gate action item 'action_13.IL-6+'

View '14.IL-12++'
on a flowSet linked to
gate action item 'action_14.IL-12+'

View '15.TNFα++'

```

        on a flowSet linked to
        gate action item 'action_15.TNFa+'

View '16.CD14-MHC2-+'
  on a flowSet linked to
  gate action item 'action_16.CD14-MHC2-'

View '17.Monocytes+'
  on a flowSet linked to
  gate action item 'action_17.Monocytes'

View '18.IFNα++'
  on a flowSet linked to
  gate action item 'action_18.IFNα+'

View '19.IL-6++'
  on a flowSet linked to
  gate action item 'action_19.IL-6+'

View '20.IL-12++'
  on a flowSet linked to
  gate action item 'action_20.IL-12+'

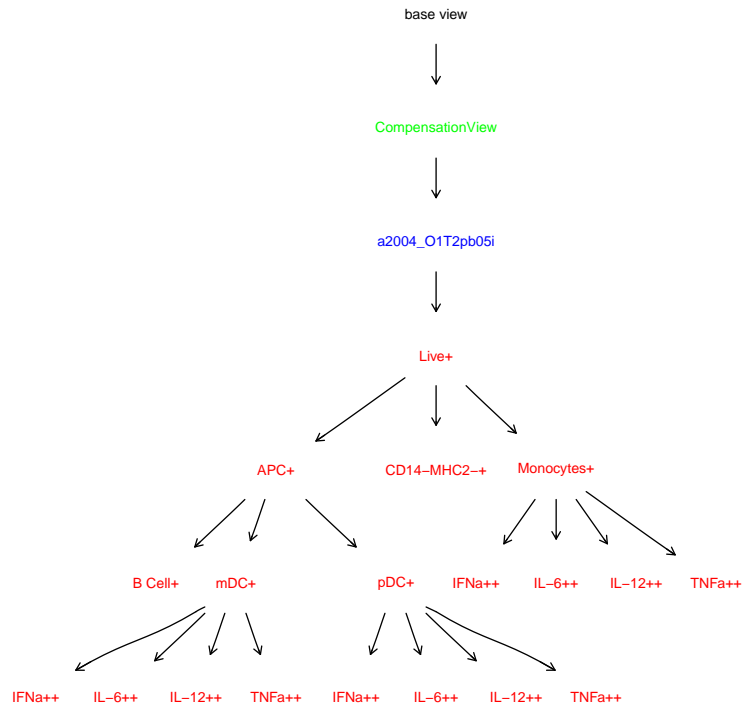
View '21.TNFα++'
  on a flowSet linked to
  gate action item 'action_21.TNFα+'

>

plotWf plots the workflow tree

> plotWf(wfs[[1]])

```



Finally, when we are finished with the workspace, we close it:

```
> closeWorkspace(ws);
> ws
```

FlowJo Workspace Version 2.0

File location: /Library/Frameworks/R.framework/Versions/2.15/Resources/library/flowW

File name: A2004Analysis.xml

Workspace is closed.

4.5 Exporting to FlowJo OSX 9.2

The `exportAsFlowJoXML` function can be used to export a `flowCore::workFlow` as an XML workspace for FlowJo 9.2 OSX. If `flowWorkspace` has been used to import an existing FlowJo workspace, `flowWorkspace2flowCore` can be used to obtain a `workFlow` for exporting. Currently this function can export one `workFlow` at a time.

4.6 Parallel Support

Parsing and gating can be time-consuming. This latest version (>1.0.0) of `flowWorkspace` supports parallelization via `multicore`, `snowfall`, and `Rmpi`. If `multicore` is loaded, or a `snowfall` cluster is initialized, `flowWorkspace` will use `snowfall` or `multicore` (in that order of preference) to parse the workspace. Parallel gating of the workspace can be performed by loading `Rmpi` and running `parseWorkspace()`. This corresponds to the `execute()` step of the `parseWorkspace` function. `Rmpi` is needed to handle concurrent reads/writes to the `ncdfFlowSet` file. Parallel gating / parsing will work with `netCDF`-backed data or if data is stored in RAM.

4.7 Deprecated Functionality

The following behaviour is no longer supported and has been replaced by more extensive `netCDF` support via the `ncdfFlow` package. If you have particularly large data files (millions of events), then you won't want to keep the data around, nor the indices for gate membership. Instead, pass the options `cleanup=TRUE`, `keep.indices=FALSE` to the `execute()` function, and the data will be scrubbed after computing population statistics. With future improvements making use of the `netCDF` framework, and bitvector representations of population memberships; this will improve memory usage in high-throughput unsupervised analysis settings.

5 Troubleshooting

If this package is throwing errors when parsing your workspace, and you are certain your workspace is version 2.0, contact the package author. If you can send your workspace by email, we can test, debug, and fix the package so that it works for you. Our goal is to provide a tool that works, and that people find useful.

6 Future Improvements

We are working on support for `flowJo` XML workspaces exported from the Windows version of `flowJo`. Efforts are underway to integrate `GatingSet` and `GatingHierarchy` objects more closely with the rest of the `flow` infrastructure.