

# flowBin: a Package for Combining Multitube Flow Cytometry Data

Kieran O'Neill'

April 15, 2025

koneill@bccrc.ca

## Contents

<b>1</b>	<b>Licensing</b>	<b>2</b>
<b>2</b>	<b>Introduction</b>	<b>2</b>
<b>3</b>	<b>An example of constructing a flowExprSet de novo</b>	<b>2</b>
<b>4</b>	<b>Looking a little more closely at the example data</b>	<b>3</b>
<b>5</b>	<b>Quantile normalisation</b>	<b>5</b>
<b>6</b>	<b>Running flowBin</b>	<b>6</b>

# 1 Licensing

Under the Artistic License, you are free to use and redistribute this software.

# 2 Introduction

flowBin is a package for combining multitube flow cytometry data together using common population markers included in each tube.

# 3 An example of constructing a flowExprSet de novo

Although for the main example we will use a pre-constructed flowSample, it is useful to understand how one may be constructed de novo (using the example data from flowFP):

```
> library(flowBin)
> library(flowFP)
> data(fs1)
> show(fs1)
```

A flowSet with 7 experiments.

column names(7): FS Lin SS Log ... FL4 Log FL5 Log

Let's take a look at the parameters stored in this flowSet:

```
> fsApply(fs1, function(x){x@parameters@data[, 'desc']})
```

	\$P1S	\$P2S	\$P3S	\$P4S	\$P5S
FI05_000942_001.LMD	"FS Lin"	"SS Log"	"IgG1-FITC"	"IgG1-PE"	"CD45-ECd"
FI05_000942_002.LMD	"FS Lin"	"SS Log"	"Kappa-FITC"	"Lambda-PE"	"CD45-ECd"
FI05_000942_003.LMD	"FS Lin"	"SS Log"	"CD7-FITC"	"CD4-PE"	"CD45-ECd"
FI05_000942_004.LMD	"FS Lin"	"SS Log"	"CD15-FITC"	"CD13-PE"	"CD45-ECd"
FI05_000942_005.LMD	"FS Lin"	"SS Log"	"CD14-FITC"	"CD11c-PE"	"CD45-ECd"
FI05_000942_006.LMD	"FS Lin"	"SS Log"	"HLA-DR-FITC"	"CD117-PE"	"CD45-ECd"
FI05_000942_007.LMD	"FS Lin"	"SS Log"	"CD5-FITC"	"CD19-PE"	"CD45-ECd"
	\$P6S	\$P7S			
FI05_000942_001.LMD	"IgG1-PC5"	"IgG1-PC7"			
FI05_000942_002.LMD	"CD19-PC5"	"CD20-PC7"			
FI05_000942_003.LMD	"CD8-PC5"	"CD2-PC7"			
FI05_000942_004.LMD	"CD16-PC5"	"CD56-PC7"			
FI05_000942_005.LMD	"CD64-PC5"	"CD33-PC7"			
FI05_000942_006.LMD	"CD34-PC5"	"CD38-PC7"			
FI05_000942_007.LMD	"CD3-PC5"	"CD10-PC7"			

P1, P2 and P5 are our common parameters, while 3,4,6,7 are the tube-specific measurement parameters. Also, tube 1 appears to contain isotype controls (IgG1). So, to make a flowSample:

```
> aml.sample <- new('FlowSample',
+
+                                     name='Example flowSample',
+                                     tube.set=as.list(fs1@frames),
+                                     control.tubes=c(1),
+                                     bin.pars=c(1,2,5),
+                                     measure.pars=list(c(3,4,6,7)))
> show(aml.sample)
```

A FlowSample containing 7 tubes

```
Control tube(s): 1
Parameters to bin: 1 2 5
Parameters to measure: 3 4 6 7

>
```

## 4 Looking a little more closely at the example data

For our example, we will use a leukemia diagnostic panel for one patient, down-sampled for inclusion in the package. This example data set was taken from FlowRepository data set FR-FCM-ZZYA, which contains full data for 359 patients, and is a good data set to try out flowBin on. (It is also most likely the same data set which the flowFP example was taken from).

Let's plot two of the population (binning) markers in tube 1:

```
> data(aml.sample)
> tube1.frame <- tube.set(aml.sample)[[1]]
> show(tube1.frame)
```

flowFrame object '0409.FCS'  
with 2000 cells and 7 observables:

	name	desc	range	minRange	maxRange
\$P1	FS Lin	FS Lin	1024	0.000000000	1023
\$P2	SS Log	SS Log	1024	0.000400391	4
\$P3	FL1 Log	IgG1-FITC	1024	0.000400391	4
\$P4	FL2 Log	IgG1-PE	1024	0.000400391	4
\$P5	FL3 Log	CD45-ECD	1024	0.000400391	4
\$P6	FL4 Log	IgG1-PC5	1024	0.000400391	4
\$P7	FL5 Log	IgG1-PC7	1024	0.000400391	4

180 keywords are stored in the 'description' slot

```
> plot(exprs(tube1.frame)[,c(5,2)], pch=16, cex=0.6, xlim=c(0,4), ylim=c(0,4))
```



Let's look at two of the measurement markers in tube 1, which is a negative control tube:

```
> plot(exprs(tube1.frame)[,c(3,6)], pch=16, cex=0.6, xlim=c(0,4), ylim=c(0,4))
```



We can see that they are well in the lower end of the expression range. By contrast, here are the same channels in a measurement tube, with specific antibodies conjugated to them:

```

> tube7.frame <- tube.set(aml.sample)[[7]]
> show(tube7.frame)

flowFrame object '0415.FCS'
with 2000 cells and 7 observables:
      name      desc      range      minRange      maxRange
$P1 FS Lin    FS Lin      1024 0.000000000      1023
$P2 SS Log    SS Log      1024 0.000400391         4
$P3 FL1 Log   CD5-FITC     1024 0.000400391         4
$P4 FL2 Log   CD19-PE      1024 0.000400391         4
$P5 FL3 Log   CD45-ECD     1024 0.000400391         4
$P6 FL4 Log   CD3-PC5      1024 0.000400391         4
$P7 FL5 Log   CD10-PC7     1024 0.000400391         4
180 keywords are stored in the 'description' slot

> plot(exprs(tube7.frame)[,c(3,6)], pch=16, cex=0.6, xlim=c(0,4), ylim=c(0,4))

```



Notice how most of the cells are still in the negative region, but there is a clear (but small) positive population.

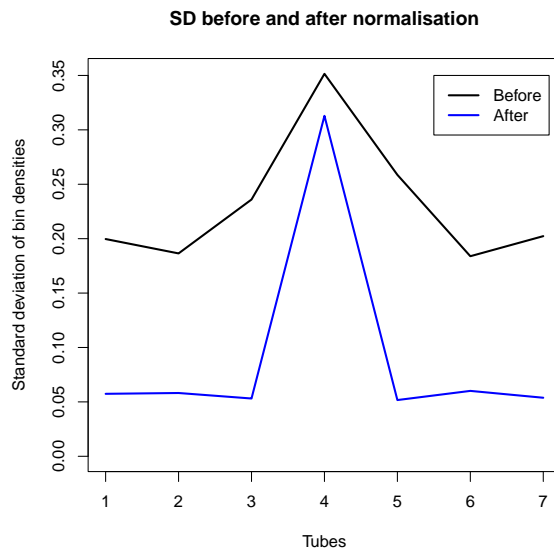
## 5 Quantile normalisation

While the negative controls can allow us to account for minor technical variation across tubes in the measurement markers, we do not have that luxury for the binning markers. However, since each tube is an aliquot from a common biological sample, stained with the same antibodies, we expect that they should all have the same underlying distribution. So, `flowBin` provides functionality to quantile normalise the binning markers across tubes.

```
> normed.sample <- quantileNormalise(aml.sample)
```

There is also a function to perform a quick check on the performance of the normalisation, using the quality control functionality of flowFP to measure the average standard deviation in probability bin densities across tubes. Here we plot the before and after densities (lower is better).

```
> qnorm.check <- checkQNorm(aml.sample, normed.sample, do.plot=F)
> plot(qnorm.check$sd.before, type='l', lwd=2,
+      ylim=c(0, max(qnorm.check$sd.before)),
+      xlab='Tubes',
+      ylab='Standard deviation of bin densities',
+      main='SD before and after normalisation')
> lines(qnorm.check$sd.after, lwd=2, col='blue')
> legend(x=5.5, y=0.35, legend=c('Before', 'After'), lwd=2, col=c('black', 'blue'))
```



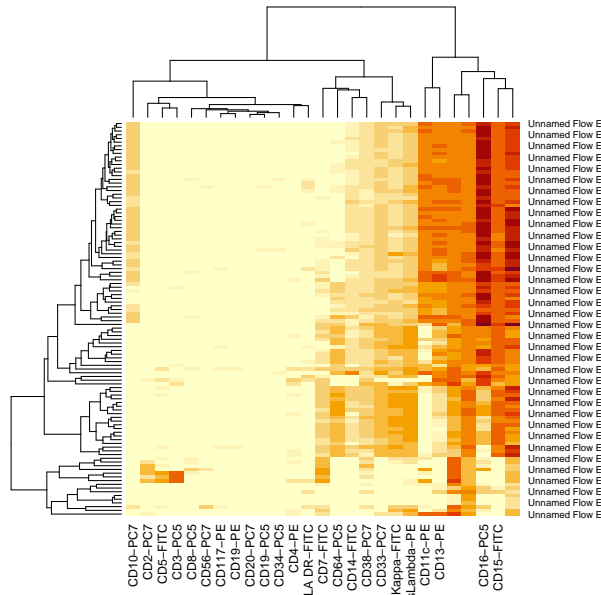
Quantile normalisation definitely seems to have improved things, although tube 4 might be worth examining for QC purposes. For this example, we'll run with it, though.

## 6 Running flowBin

```
> tube.combined <- flowBin(tube.list=aml.sample@tube.set,
+ bin.pars=aml.sample@bin.pars,
+ control.tubes=aml.sample@control.tubes,
+ expr.method='medianFIDist',
+ scale.expr=T)
```

We use `scale.expr` to scale the results to the interval  $[0, 1]$  by dividing by their range as specified in the `flowFrame`. For our example, this puts the FSC channel on the same scale as the others, facilitating plotting (and other downstream uses):

```
> heatmap(tube.combined, scale='none')
```



We can try another method of determining bin expression, `propPos`, which sets cutoffs at the 98th percentile of the negative control, and counts what proportion of events fall above the cutoff.

```
> tube.propPos <- flowBin(tube.list=aml.sample@tube.set,
+ bin.pars=aml.sample@bin.pars,
+ control.tubes=aml.sample@control.tubes,
+ expr.method='propPos',
+ scale.expr=T)

> heatmap(tube.propPos, scale='none')
```

