```
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title: "QA FCM data in R"
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output:
  html_notebook:
    toc: yes
  pdf_document:
    highlights: tango
    keep_tex: yes
    number_sections: yes
    toc: yes
---

# QA FCM data in R


```{r setup, include=FALSE}
require("knitr")
knitr::opts_chunk$set(echo = FALSE)
opts_knit$set(root.dir = "./") #~/gits/project/")
```


Make sure R knows which directory our data will be read from

```{r}
getwd()
setwd('./data')
```

```{r}
rm(list=ls())
graphics.off() # close all graphics, for efficiency
```

Ensure flowCore library is loaded:

```{r}
library(flowCore)
```

To see a list of files in the 'fullFCS' folder, use dir(), it returns a vector of file names

```{r}
files <- dir('./data/fullFCS/')
files
```

To select the first file in the directory, subset the vector on its first index:

```{r}
firstFile <- paste('./data/fullFCS/', files[1], sep = "")
firstFile
f <- read.FCS(firstFile)
f
```

##

Quality Assurance

Load helper package and one support function from Radina

```{r}
library(flowQ)
source("./code/supportCode/qaProcess.GenericNumber.R")
```


Define directory where QA results will be saved:

```{r}
save.dir <- "./data/QA/"
```

This removes the current contents of the QA folder. Do this for space efficiency and to ensure correct results: (You may get an error the first tim

```{r}
system(paste('rm -r ', save.dir, "*", collapse="", sep=""))
```

First, check the raw cell counts (use 'fs')

See ?qaProcess.cellnumber for explanation

```{r}
load('./data/fs.RData') # Make sure we get the raw counts -- these should all be 20,000 for us!
qa.raw.count <- qaProcess.cellnumber(fs, outdir=save.dir, cFactor=Inf)
```

Next, examine non-debris cell counts from 'clean.fs'
```

```{r}
qa.nonDebris.count <- qaProcess.cellnumber(set = clean.fs, outdir=save.dir)
```

Use Radina's "pretty" non-debris count and set threshold at 10000 cells:

```{r}
numbers <- as.vector(fsApply(clean.fs, nrow))
frameIDs <- as.vector(sampleNames(fs))
qa.nonDebris.pretty <- qaProcess.GenericNumber(numbers=numbers, frameIDs=frameIDs, outdir=save.dir, cutoff=10000, name="Cell count")
```

flowQ provides an easy html report function:

```{r}
url <- writeQAReport(fs, list(qa.raw.count, qa.nonDebris.count, qa.nonDebris.pretty), outdir=save.dir)
browseURL(url)
```

You can do quality checks without relying on flowQ.

Can you say anything about the sample quality based on the following plot?

```{r}
graphics.off() # closes all current plots, good for computer speed
plot(density(exprs(trans.fs[[1]])[, "FSC-A"]), xlim=c(35000, 125000), ylim = c(0,0.00005), lwd=2, main = "FSC Density", sub="", xlab="FSC-A")
for (i in 2:10){
  lines(density(exprs(trans.fs[[i]])[, "FSC-A"]), col=i, lwd=2)
}
```

Save the transformed flowSet object for tomorrow!

```{r}
save(trans.fs, file='/home/rguru/Documents/Workshop/data/trans.fs.RData')
```

```
######################################################################
# Radina has provided a set of functions to process typical data sets:
#graphics.off()
#source("../code/supportCode/flowPrep.R")
#source("../code/supportCode/support_functions.R")
#fs.prep <- flowPrep(fs, apply.comp=FALSE, plot.preproc=TRUE, plot.for.lympho=TRUE)
```