# This script wants fcs files as exported by the C6 Accuri Magic Flow Cytometer

# Get into the directory with the fcs files

require(ggplot2)
require(flowViz)
# this loads library(flowCore)

# Take all fcs and load them as a dataframe of exprs, store in a list
datz <- list()
for (fz in dir()[grep("fcs",dir())]) {
  datz[[fz]] <- data.frame(exprs(read.FCS(fz,transformation=F)))
}

# Exploratory plot to figure out gating
ggplot(data.frame(datz[[1]][1:1e4,]),aes(x=FL1.A,y=FSC.A)) +
  theme_bw() + geom_point(cex=0.01) +
  scale_x_log10(limits=c(1e1,1e7)) +
  scale_y_log10(limits=c(1e5,1e7)) +
  xlim(c(0,750000))+ylim(c(0,3e6)) +
  geom_abline(intercept=0,slope=15) # An exploratory gating line for
  # GFP pos/neg, can also use
  # something complex like
  # stat_function, or simple
  # like geom_vline

# Now that we know how to gate those suckas, we loop through and build
# a pretty dataframe ammenable to ggplot
# Change stuff to change your gates, what channels you're gating on etc
sdatz <- list()
for (fz in names(datz)) {
  sdatz[[fz]] <- cbind(datz[[fz]][,c("FSC.A","FL1.A")],
                       datz[[fz]]$FSC.A > 1e5 & datz[[fz]]$FL1.A > 1e5,
                       datz[[fz]]$FSC.A > 1e5 & datz[[fz]]$FL1.A < 1e7,
                       datz[[fz]]$FL1.A * 15 > datz[[fz]]$FSC.A
  names(sdatz[[fz]]) <- c("FSC","FL1","cell","gfp")
}
# And easier names than the names of the files from names(datz)
names(sdatz) <- c("blank","653","fy4","blank","mix1","mix2")

# So now you can explore your results
print(ggplot(sdatz[[fz]][sdatz[[fz]]$cell,],aes(x=FL1,y=FSC,col=gfp)) +
  theme_bw() + geom_point(cex=0.01,alpha=0.1) +
  scale_x_log10(limits=c(1e1,1e6)) +
  scale_y_log10(limits=c(1e5,5e6)) +
  ggtitle(paste0(fz," scatterplot")))

# Once you're happy, adjust this look to output a bunch of pngs
for (fz in names(sdatz)) {
  png(paste0(fz,"scatter",">.png"))
  print(ggplot(sdatz[[fz]][sdatz[[fz]]$cell,],aes(x=FL1,y=FSC,col=gfp)) +
        theme_bw() + geom_point(cex=0.01,alpha=0.1) +
        scale_x_log10(limits=c(1e1,1e6)) +
        scale_y_log10(limits=c(1e5,5e6)) +
        ggtitle(paste0(fz," scatterplot")))
  dev.off()
  # This is a density plot, if you like that kind of thing
  # png(paste0(fz,"hist",">,.png"))
  # print(ggplot(sdatz[[fz]][sdatz[[fz]]$cell,],aes(x=FL1,y=col="gfp")) +
  #        theme_bw() + geom_density(adjust=0.1) +
  #        scale_x_log10(limits=c(1e1,1e6)) +
  #        xlab("FL1-A signal") +
  #        ggtitle(paste0(fz," distributions of GFP signal per cell")))
  # dev.off()}

# How many GFP "pos" cells are detected as such?
frac653 <- sum(sdatz$`653`$gfp & sdatz$`653`$cell)/sum(sdatz$`653`$cell)
frac1 <- sum(sdatz$fy4$gfp & sdatz$fy4$cell)/sum(sdatz$fy4$cell)
fracmix1 <- sum(sdatz$mix1$gfp & sdatz$mix1$cell)/sum(sdatz$mix1$cell)
fracmix2 <- sum(sdatz$mix2$gfp & sdatz$mix2$cell)/sum(sdatz$mix2$cell)
fracmix3 <- sum(sdatz$mix3$gfp & sdatz$mix3$cell)/sum(sdatz$mix3$cell)

# And so forth