Fitting a bivariate normal distribution to a 2D scatterplot

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1 Overview

Using FACS (fluorescence-activated cell sorter) one can measure certain properties of each individual cell in a population of cells. Examples for these properties:

- Forward light scatter (FSC): this measures a cell's size
- Sideward light scatter (SSC): this measures a cell's granularity
- Several fluorescence channels (typically 3 to 4) that measure the abundance of fluorophores, which may be bound to specific antibodies for surface or intracellular markers, or be encoded by a GFP-tagged transcript.

First, we load example data from a FACS analysis that was performed by Mamatha Sauermann at the German Cancer Research Center in Heidelberg.

```
> library(prada)
> sampdat <- readFCS(system.file("extdata", "fas Bcl2 plate323-04-04.A01",
+     package = "prada"))
> fdat <- exprs(sampdat)
```

The scatterplot of FSC vs SSC is often used for quality control. It is shown in Fig. 1.

```
> plot(fdat[, "FSC-H"], fdat[, "SSC-H"], pch = 20, col = "#303030",
+     xlab = "FSC", ylab = "SSC", main = "Scatter plot FSC vs SSC")
```

The cell population is often contaminated by cell debris or conjugates. These can be identified by their size: they are either much smaller or much larger than the main population, or they have an unusual degree of granularity. Segmentation is often performed manually by looking at the FSC-SCC scatterplot.

Here we describe an automated algorithm for this task.

2 Fitting

The package *prada* provides the functions `fitNorm2` and `plotNorm2`. We assume that the shape of the main population in the FSC vs SSC plot can be approximated by a normal distribution. The function `fitNorm2` fits a bivariate normal distribution into the data (by robust estimation

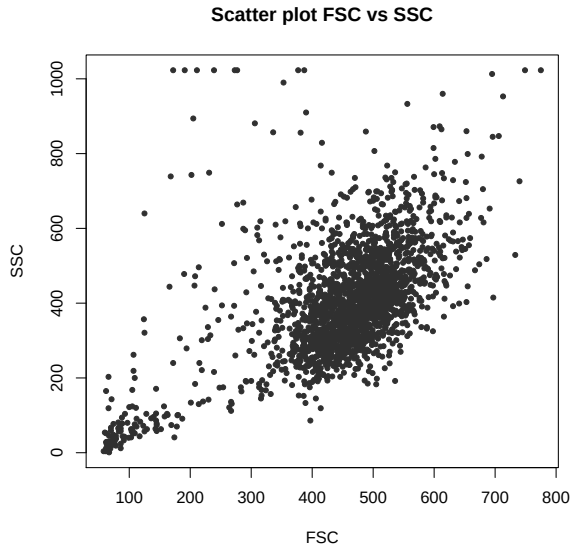

Figure 1: Scatter plot FACS data: FSC vs SSC.

of its covariance matrix). Contours of equal probability of a bivariate normal are ellipses. We select those cells as being part of the main population that lie within such an ellipse. Its size is controled by the parameter *scalefac*. The function returns a list.

```
> nfit <- fitNorm2(fdat[, "FSC-H"], fdat[, "SSC-H"], scalefac = 2)
```

We can plot this with the function `plotNorm2` (see Fig 2). It shows the ellipse, and the set of discarded points is marked by a red dot. Also the center of the normal distribution is marked by the red cross.

```
> plotNorm2(nfit, selection = TRUE, ellipse = TRUE)
```

```
Loading required package: geneplotter
Loading required package: annotate
```

```
> nfit3 <- fitNorm2(fdat[, "FSC-H"], fdat[, "SSC-H"], scalefac = 3)
> plotNorm2(nfit3, selection = TRUE, ellipse = TRUE)
```

To select the cells from within the ellipse, the list item `nfit$sel` is a logical vector with the same length as the number of data points.

```
> cleanfdat <- fdat[nfit$sel, ]
```

Fig. 3 shows again a scatter plot of the two fluorescense channels FL1 and FL4 this time using the 'clean' data set `cleanfdat`.

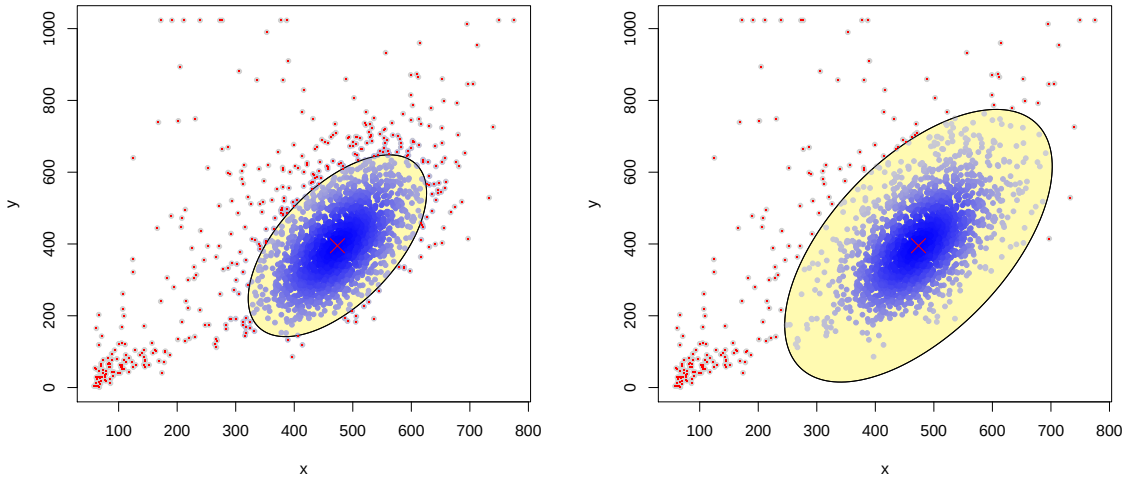

Figure 2: Selection of the main population, using two different values of the parameter `scalefac`.

```
> par(mfrow = c(1, 2))
> xlim <- range(fdat[, "FL1-H"])
> ylim <- range(fdat[, "FL4-H"])
> plot(fdat[, "FL1-H"], fdat[, "FL4-H"], pch = 20, col = "#303030",
+     xlab = "FL1", ylab = "FL4", main = "all data", xlim = xlim,
+     ylim = ylim)
> plot(cleanfdat[, "FL1-H"], cleanfdat[, "FL4-H"], pch = 20, col = "#303030",
+     xlab = "FL1", ylab = "FL4", main = "clean data only", xlim = xlim,
+     ylim = ylim)
```

3 Scatterplots

If you think that scatterplots with thousands of points are hard to read and annoying to view in a PDF viewer, have a look at the function `smoothScatter` (see Fig. 4):

```
> smoothScatter(fdat[, c("FSC-H", "SSC-H")], nrpoints = 50)
```

```
Loading required package: RColorBrewer
Loading required package: KernSmooth
KernSmooth 2.22 installed
Copyright M. P. Wand 1997
```

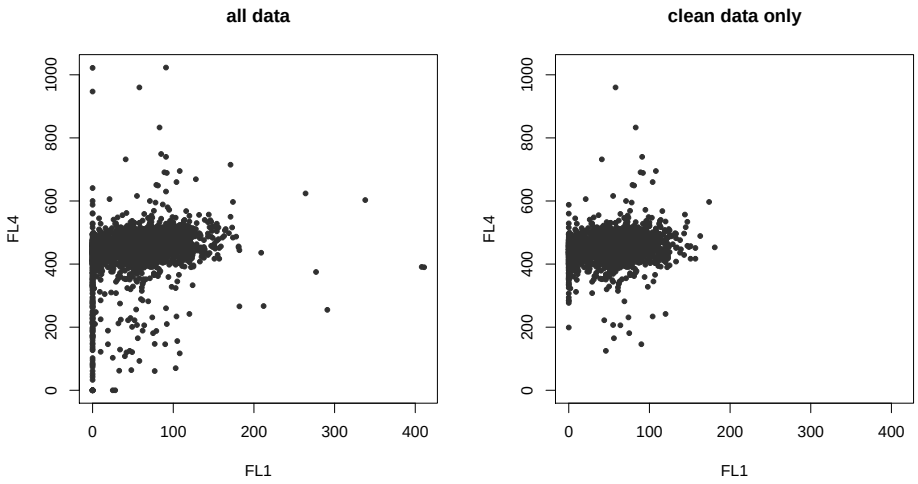

Figure 3: Scatter plots of FL1 vs FL4.

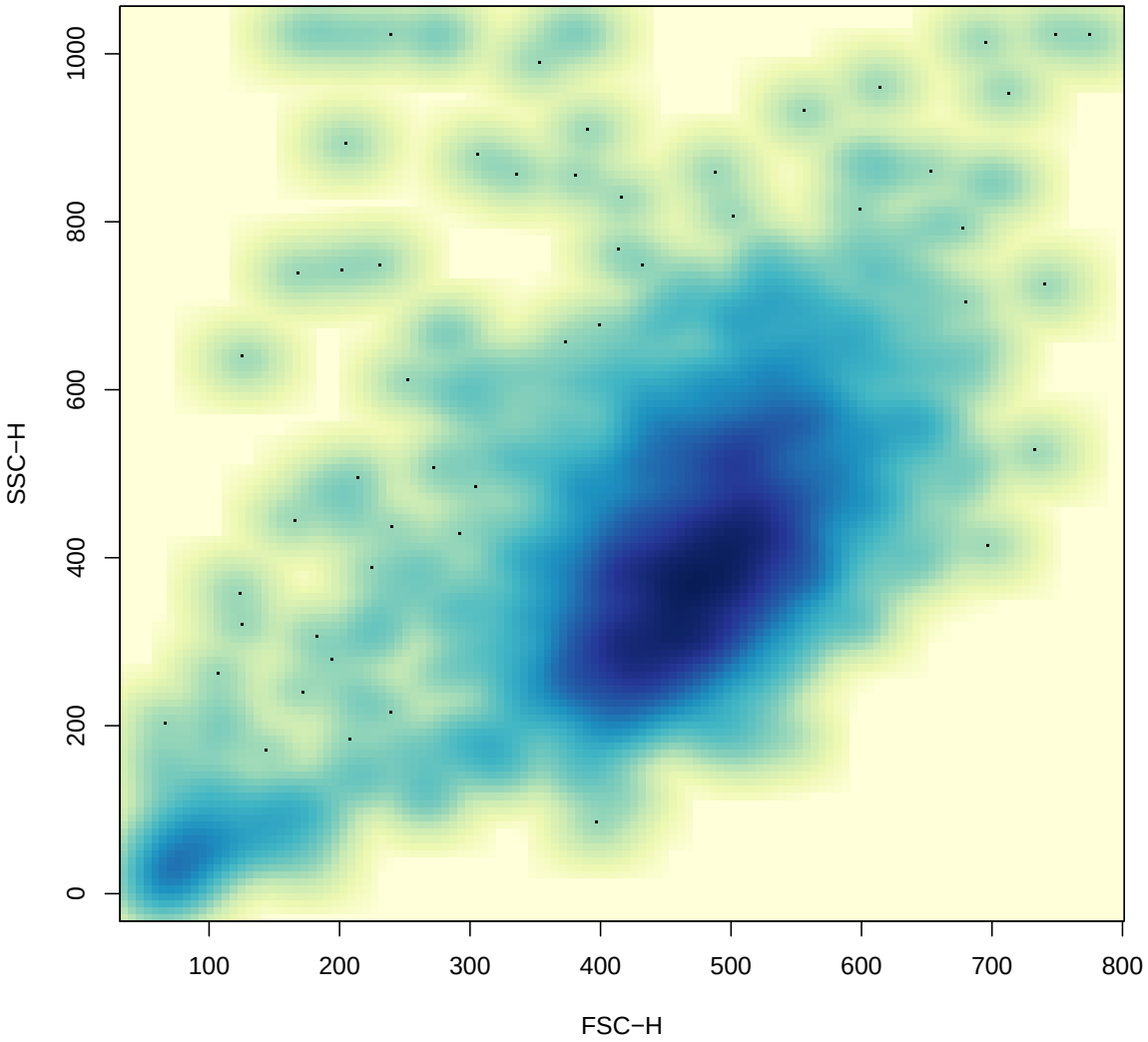

Figure 4: Smooth scatter plots.