

Manual for the use of flowEMMi

1. Set the path of the working directory containing the .fcs file(s) and the source file flowEMMi.cpp

```
> setwd("...")
```

2. Load all needed R libraries

```
> library("Rcpp")
```

```
> library("RcppEigen")
```

```
> library("mixtools")
```

```
> library("gtools")
```

```
> library("flowCore")
```

```
> library("ggplot2")
```

```
> library("flowViz")
```

```
> library("randomcoloR")
```

3. Read the .fcs file

```
> fr <- read.FCS("...",alter.names = TRUE,transformation = FALSE)
```

4. Source the .cpp file and make sure that the environmental variables are set correctly

```
> sourceCpp("flowEMMi.cpp")
```

5. At first, perform subsampling and determination of the most appropriate number of clusters. Select the channels used for clustering, the sample size and the range of the number of clusters. Start_cluster has to be ≥ 2 .

```
> flowEMMi_res<-flowEMMi(frame=fr, ch1="PMT.1", ch2="PMT.9", sample_size=50, prior=FALSE,  
separation = TRUE, max_inits = 10, use_log = FALSE, alpha = .7, img_format = "png",  
foreground_maxsd = 5000, start_cluster = 2, end_cluster = 20)
```

6. Save the outputs (distribution parameters) for the next run on the full data set

```
> pi_prior<-flowEMMi_res$pi
```

```
> mu_prior<-flowEMMi_res$mu
```

```
> sigma_prior<-flowEMMi_res$sigma
```

7. Run flowEMMi on the full data set using the prior distribution parameters. Adjust the maximum standard deviation used to separate cell clusters from background clusters, the alpha value to define the confidence interval and the range for the number of clusters.

```
> flowEMMi_res<-flowEMMi(frame=fr, ch1="PMT.1", ch2="PMT.9", sample_size=1, prior=TRUE, diff.ll  
= 1, pi_prior=pi_prior, mu_prior=mu_prior, sigma_prior=sigma_prior, separation = TRUE, use_log =  
FALSE, alpha = .7, img_format = "png", foreground_maxsd = 3000, start_cluster = 13, end_cluster =  
14)
```