

Gut Baby

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Preliminary

```
library(tidyverse)
library(mixOmics)
walk(dir("../Rscript/"), pattern = ".R$", full.names = TRUE), source)
```

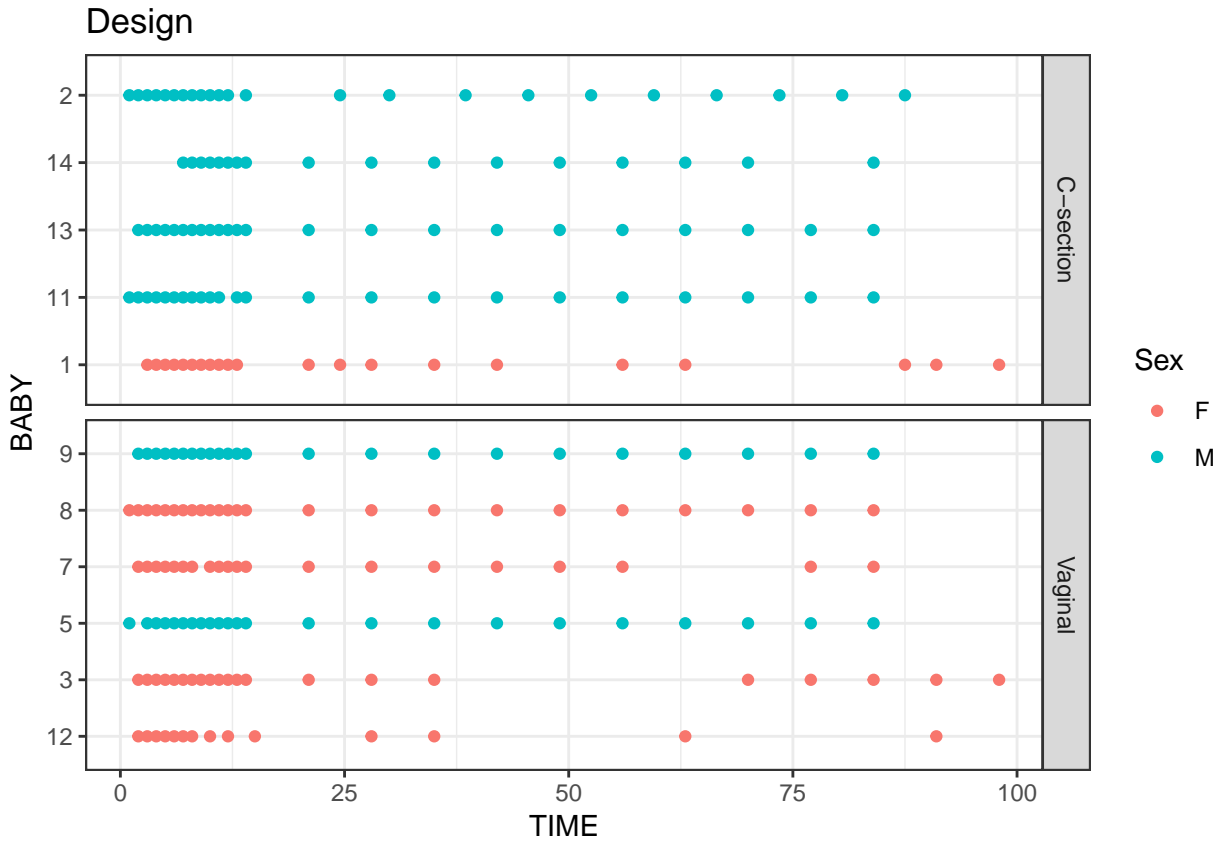
Data Description & Design

Original paper (Development of the Human Infant Intestinal Microbiota, Palmer et al. 2007) studied gastrointestinal microbiome of 14 babies during the first year of life. They collected an average of 26 stool samples from 14 healthy full-term human infants. They have also included vagina and milk microbiome composition from the mothers and stool samples from mothers and fathers.

For demonstration purposes and because babies' gut almost reach an "adult-like" composition, we have focused our attention on the first 100 days of life. We also excluded babies who recieved an antibiotic treatment during that period, because antibiotics can change drastically microbiome composition.

Our final design consists in an average of 21 timepoits for each of the 11 selected babies.

```
load("../Data/milk_data.RData")
ggplot(data= design %>% rename(Sex = Sexe), aes(x = TIME, y = BABY, color = Sex)) +
  geom_point() + facet_grid(Delivery~., scales = "free_y") + ggtitle("Design") +
  #scale_color_manual(values = color.mixo(1:2)) +
  theme_bw()
```



```
design %>% dplyr::select(BABY, TIME) %>% mutate(BABY = as.numeric(BABY)) %>%
  group_by(BABY) %>% summarise(n_timepoints = n()) %>% knitr::kable()
```

BABY	n_timepoints
1	21
2	23
3	21
5	23
7	20
8	24
9	23
11	23
12	14
13	23
14	17

Analysis

Pre-processing

We perform standard pre-processing steps :

- Low Count Removal
- Total Sum Scalling
- Centered Log Ratio Transformation

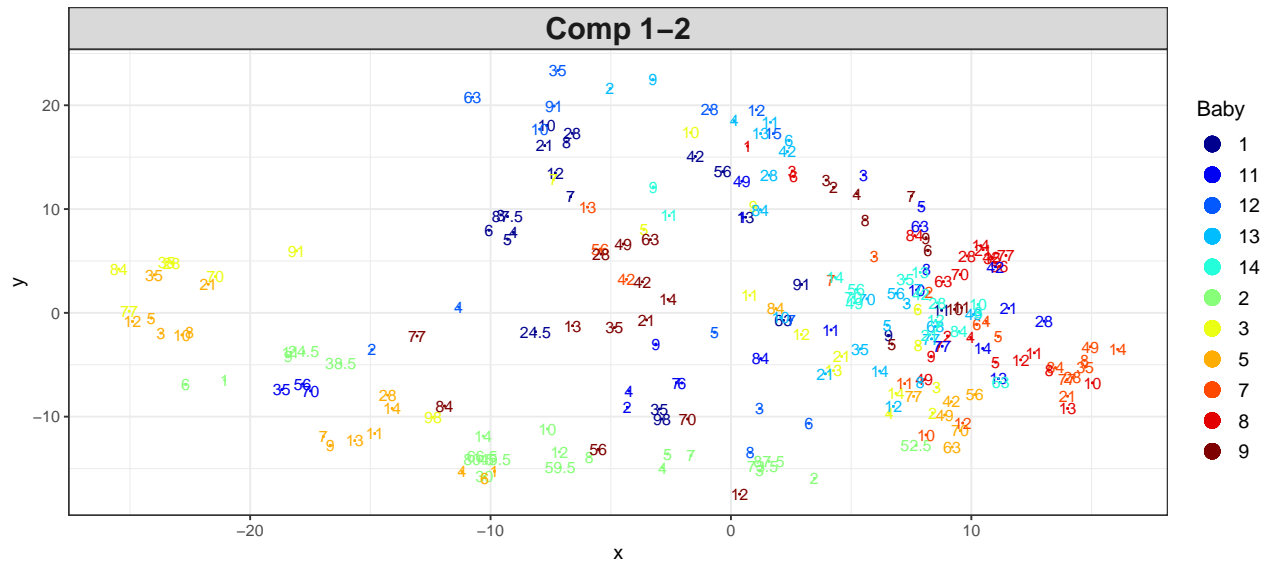
```

OTU_norm <- norm_OTU(OTU, AR = T)
# option AR(Abundance Relative) = data already in AR.
# need to add a "pouillème" in order to compute CLR.

#pca(OTU_norm, ncomp = 10) %>% plot
pca.res <- pca(OTU_norm, ncomp = 4)

plotIndiv(pca.res, group = design$BABY, ind.names = design$TIME,
  comp = c(1,2), legend.title = "Baby", legend = T, title = "Comp 1-2")

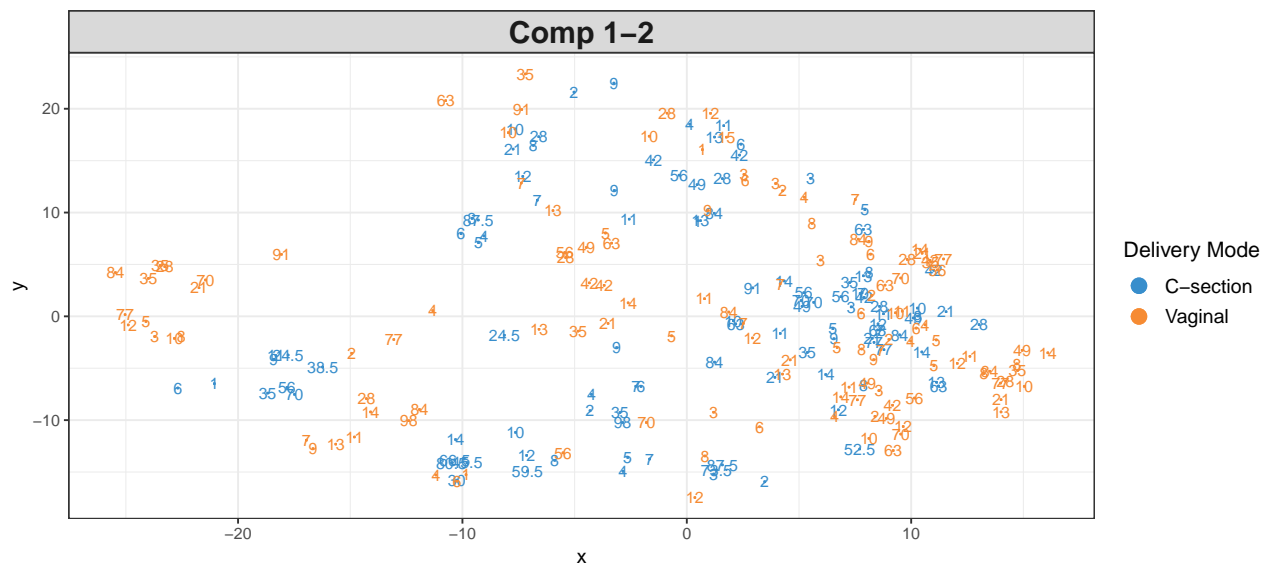
```



```

plotIndiv(pca.res, group = design$Delivery, ind.names = design$TIME,
  comp = c(1,2), legend.title = "Delivery Mode", legend = T, title = "Comp 1-2")

```

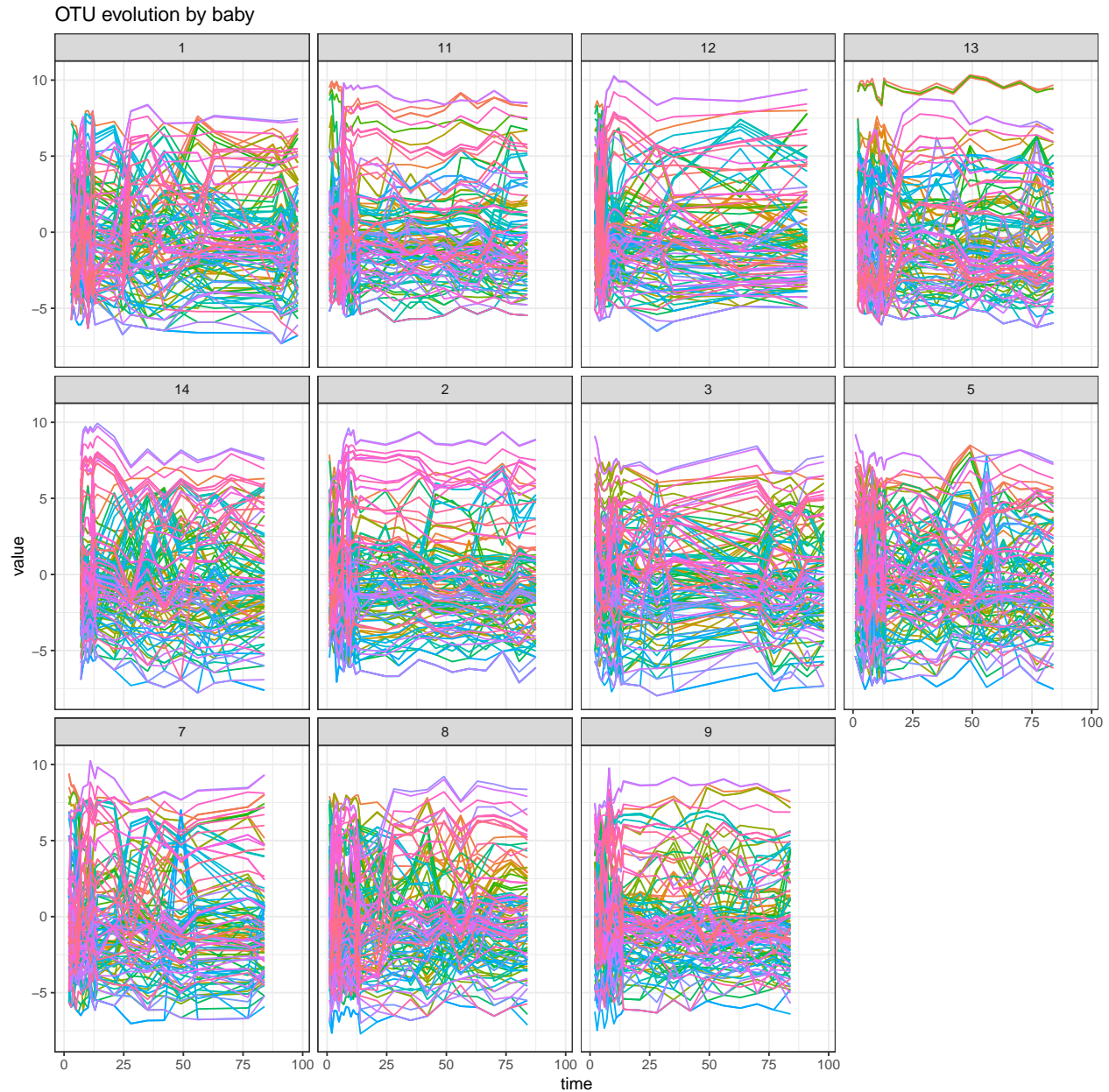


```

# per sample OTU evolution
OTU_norm %>% as.data.frame() %>% rownames_to_column("sample") %>%
  gather(OTU, value, -sample) %>%

```

```
mutate(time = sample %>% str_split("_") %>% map_chr(~.x[2]) %>% as.numeric)%>%
mutate(baby = sample %>% str_split("_") %>% map_chr(~.x[1])) %>%
ggplot(aes(time, value, col=OTU)) + geom_line() + facet_wrap(~baby) + theme_bw() +
theme(legend.position = "none") + ggtitle("OTU evolution by baby")
```



Split

```
delivery_mode <- design %>% dplyr::select(BABY, Delivery) %>% unique
index.C <- rownames(OTU) %>% str_split("_") %>% map_chr(~.x[1]) %in%
(delivery_mode %>% filter(Delivery == "C-section") %>% pull(BABY))
OTU_norm.C <- OTU[index.C,] %>% norm_OTU(AR = T)
```

```
OTU_norm.V <- OTU[!index.C,] %>% norm_OTU(AR = T)
```

LMMS

C-section

```
time_lmms.C <- rownames(OTU_norm.C) %>% str_split("_") %>% map_chr(~.x[2]) %>% as.numeric
sample_id = rownames(OTU_norm.C)

# cubic p-spline
spline.MILK.C.cubicpspline = lmms::lmmSpline(data = OTU_norm.C, time = time_lmms.C,
                                              sampleID = sample_id,
                                              basis = 'cubic p-spline', keepModels = T,
                                              numCores = 2)

spline.MILK.C.pspline = lmms::lmmSpline(data = OTU_norm.C, time = time_lmms.C,
                                         sampleID = sample_id,
                                         basis = 'p-spline', keepModels = T,
                                         numCores = 2 )

spline.MILK.C.cubic = lmms::lmmSpline(data = OTU_norm.C, time = time_lmms.C,
                                       sampleID = sample_id,
                                       basis = 'cubic', keepModels = T,
                                       numCores = 2)

# summary
spline.MILK.C.cubicpspline@modelsUsed %>% table %>% as.data.frame() %>%
  set_names("ModelUsed", "Cubic P-spline") %>%
  left_join(spline.MILK.C.pspline@modelsUsed %>% table %>% as.data.frame() %>%
    set_names("ModelUsed", "P-spline")) %>%
  left_join(spline.MILK.C.cubic@modelsUsed %>% table %>% as.data.frame() %>%
    set_names("ModelUsed", "Cubic")) %>%
  knitr::kable()
```

	ModelUsed	Cubic P-spline	P-spline	Cubic
0		95	78	82
1		12	29	25

Vaginal

```
time_lmms.V <- rownames(OTU_norm.V) %>% str_split("_") %>% map_chr(~.x[2]) %>% as.numeric
sample_id = rownames(OTU_norm.V)

# cubic p-spline
spline.MILK.V.cubicpspline = lmms::lmmSpline(data = OTU_norm.V, time = time_lmms.V,
                                              sampleID = sample_id,
                                              basis = 'cubic p-spline', keepModels = T,
                                              numCores = 2)

spline.MILK.V.pspline = lmms::lmmSpline(data = OTU_norm.V, time = time_lmms.V,
```

```

        sampleID = sample_id,
        basis = 'p-spline', keepModels = T,
        numCores = 2 )

spline.MILK.V.cubic = lmms::lmmSpline(data = OTU_norm.V, time = time_lmms.V,
        sampleID = sample_id,
        basis = 'cubic', keepModels = T,
        numCores = 2)

# summary
spline.MILK.V.cubicpspline@modelsUsed %>% table %>% as.data.frame() %>%
  set_names("ModelUsed", "Cubic P-spline") %>%
  left_join(spline.MILK.V.pspline@modelsUsed %>% table %>% as.data.frame() %>%
    set_names("ModelUsed", "P-spline")) %>%
  left_join(spline.MILK.V.cubic@modelsUsed %>% table %>% as.data.frame() %>%
    set_names("ModelUsed", "Cubic")) %>%
knitr::kable()

```

ModelUsed	Cubic P-spline	P-spline	Cubic
0	107	95	98
1	10	22	19

More straight lines in every delivery mode. Less in pspline.

Filter

C-section

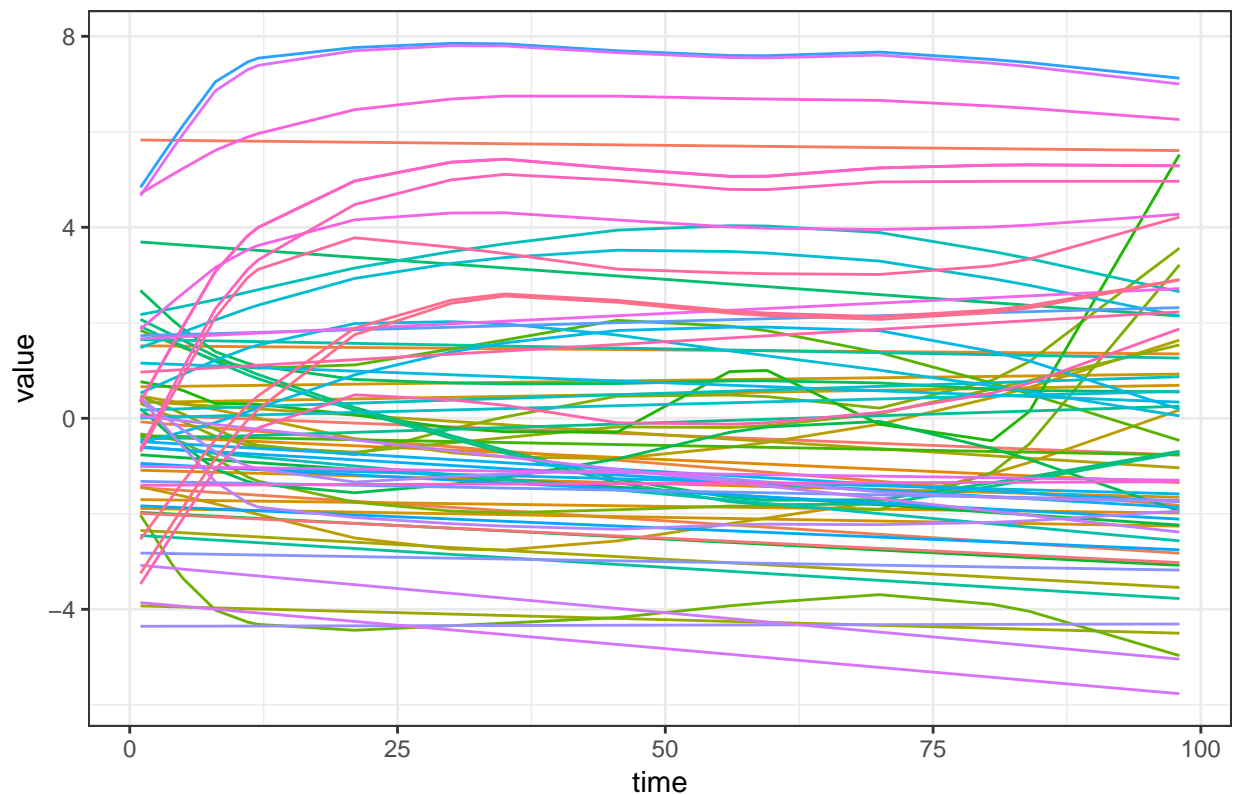
```

filter.spline.C.res <- wrapper.filter.splines(OTU_norm.C, spline.MILK.C.pspline)
index.filter.C <- (rownames(spline.MILK.C.pspline@predSpline) %in% filter.spline.C.res$to_keep) %>%
  which

spline.data.C <- spline.MILK.C.pspline@predSpline[index.filter.C,] %>% t %>% as.data.frame()
spline.data.C %>% rownames_to_column("time") %>%
  gather(Features, value, - time) %>% mutate(time =as.numeric(time)) %>%
  ggplot(aes(x=time, y = value, col = Features)) + geom_line() + theme_bw() +
  theme(legend.position = "none") + ggtitle("Modelled OTU evolution")

```

Modelled OTU evolution

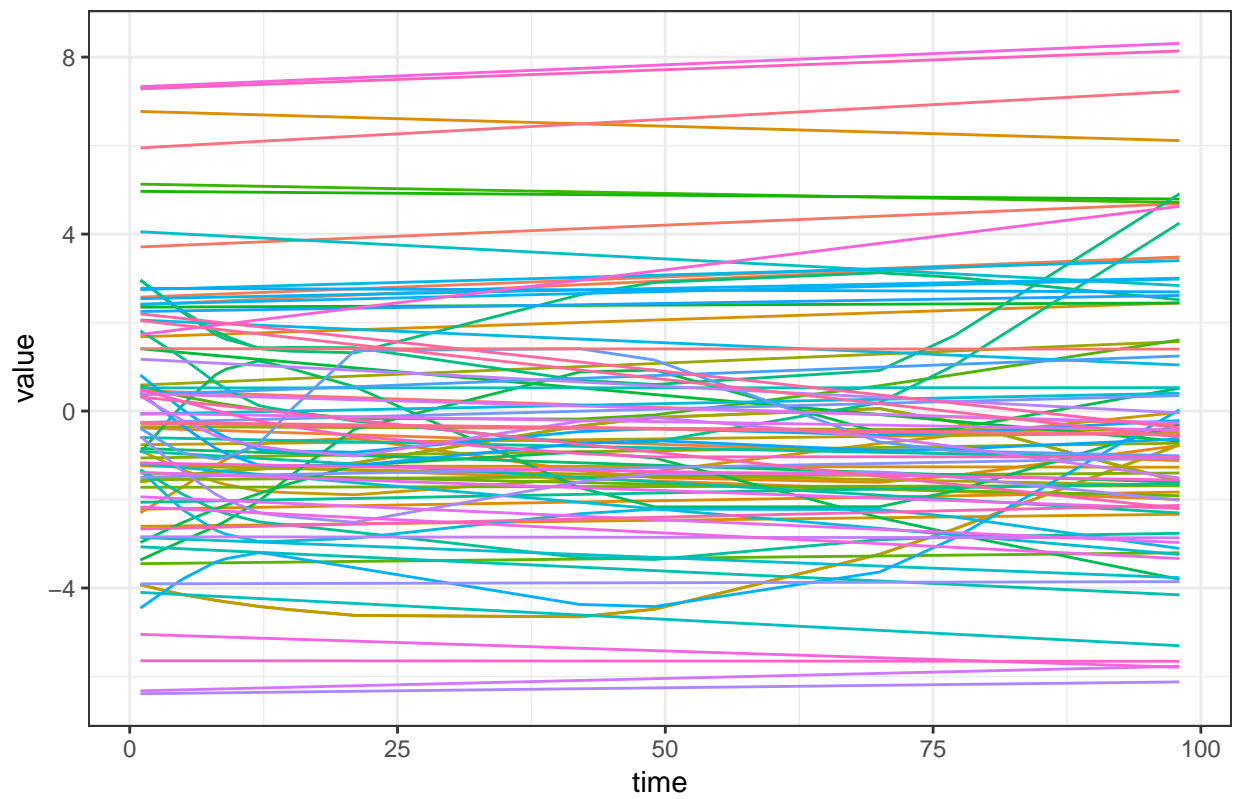


Vaginal

```
filter.spline.V.res <- wrapper.filter.splines(OTU_norm.V, spline.MILK.V.pspline)
index.filter.V <- (rownames(spline.MILK.V.pspline@predSpline) %in% filter.spline.V.res$to_keep) %>%
  which()

spline.data.V <- spline.MILK.V.pspline@predSpline[index.filter.V,] %>% t %>% as.data.frame()
spline.data.V %>% rownames_to_column("time") %>%
  gather(Features, value, - time) %>% mutate(time =as.numeric(time)) %>%
  ggplot(aes(x=time, y = value, col = Features)) + geom_line() + theme_bw() +
  theme(legend.position = "none") + ggtitle("Modelled OTU evolution")
```

Modelled OTU evolution

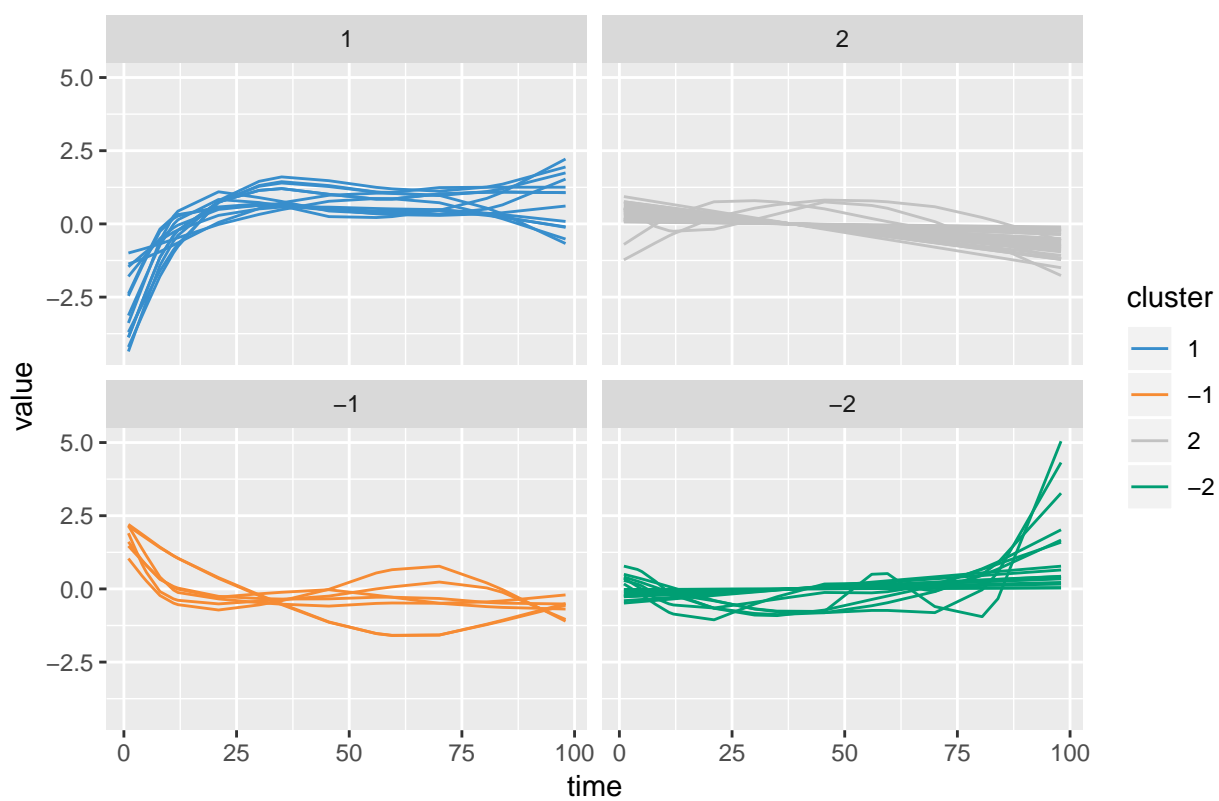


PCA Clustering

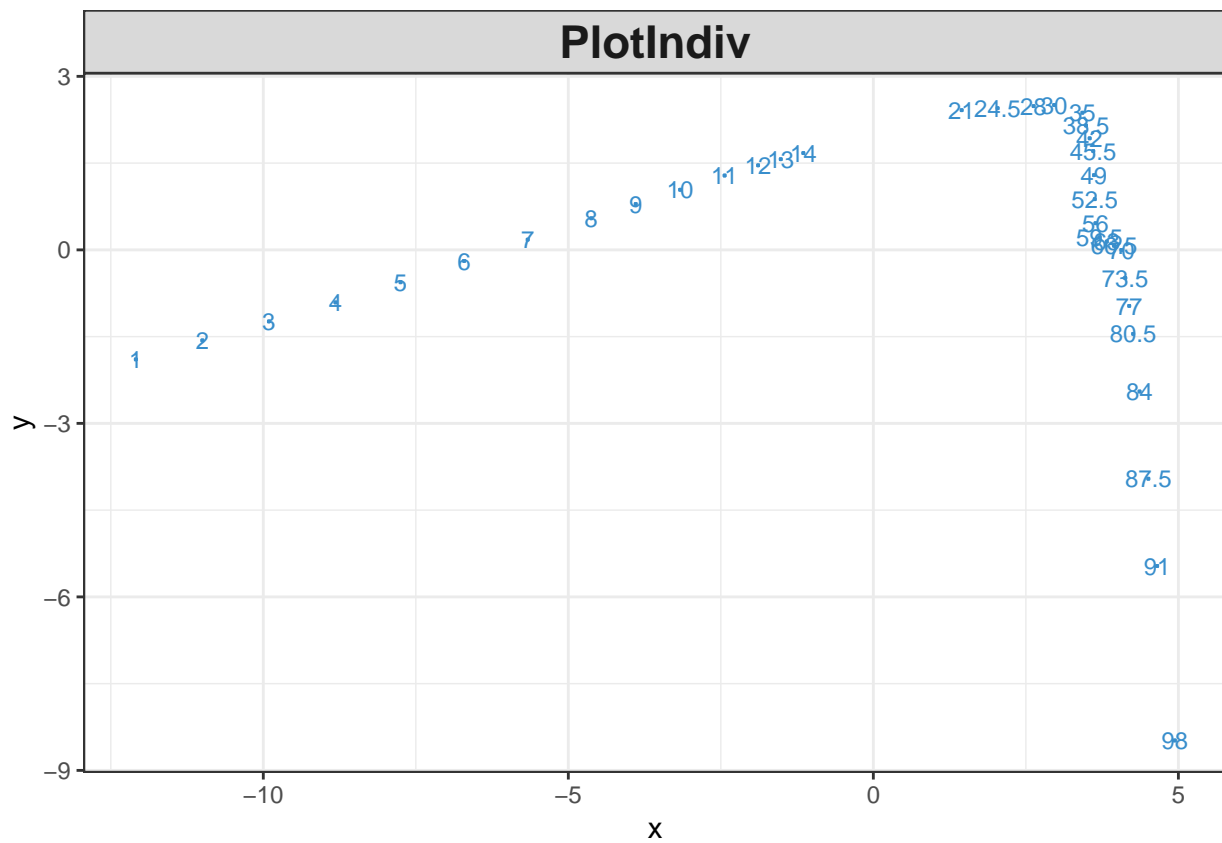
C-section

```
pca.res.C <- pca(spline.data.C, ncomp = 2, scale = F, center = T)
pca.plot(pca.res.C, title = "C-section PCA, scale = F")
```

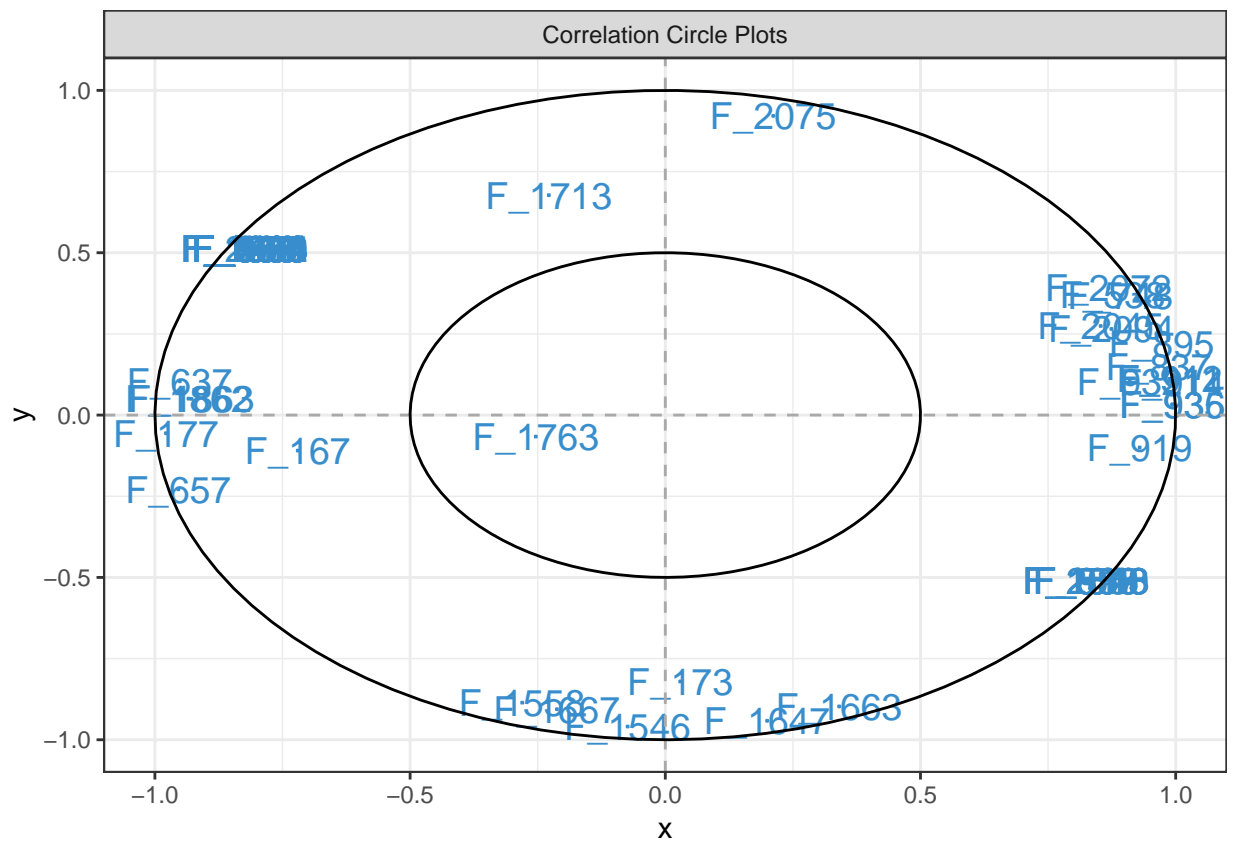

C-section PCA, scale = F



```
plotIndiv(pca.res.C)
```



```
plotVar(pca.res.C)
```

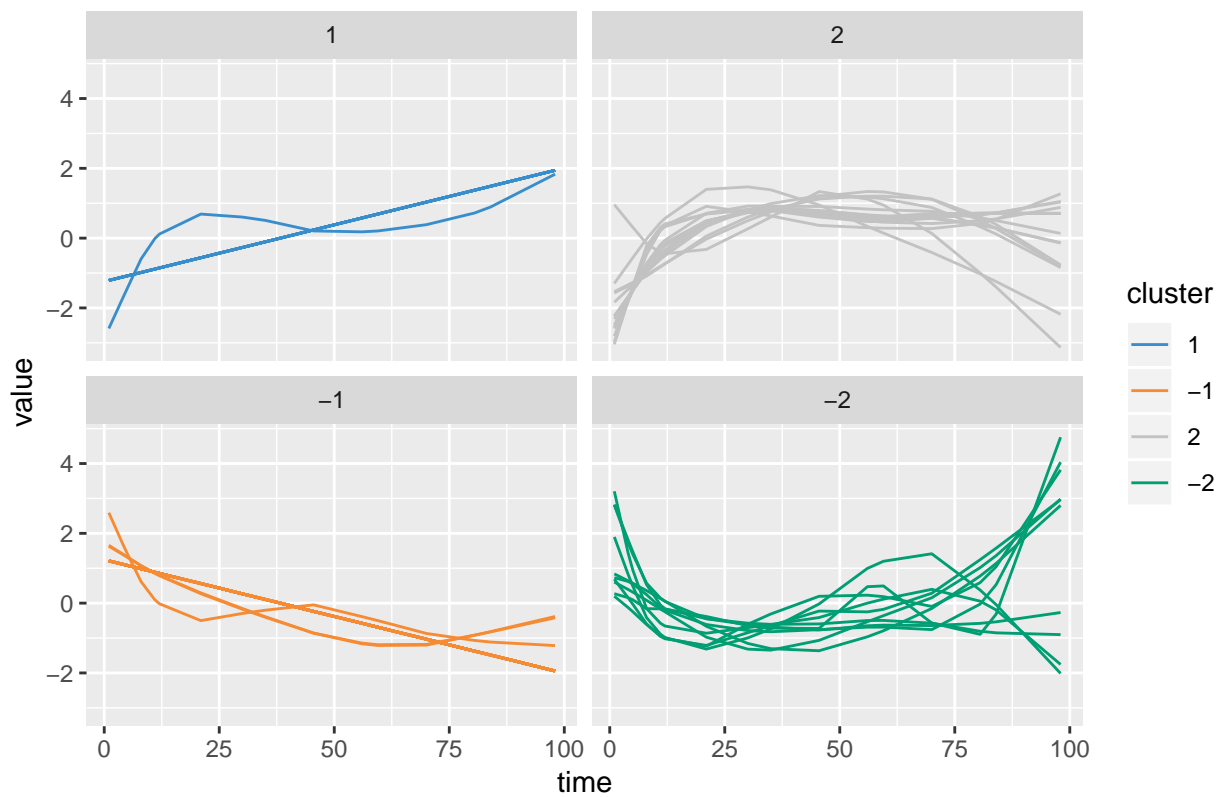


```
pca.res.C <- pca(spline.data.C, ncomp = 2, scale = T, center = T)
pca.get_cluster(pca.res.C) %>% pull(cluster) %>% table
```

```
## .
## -2 -1 1 2
## 10 35 11 15
```

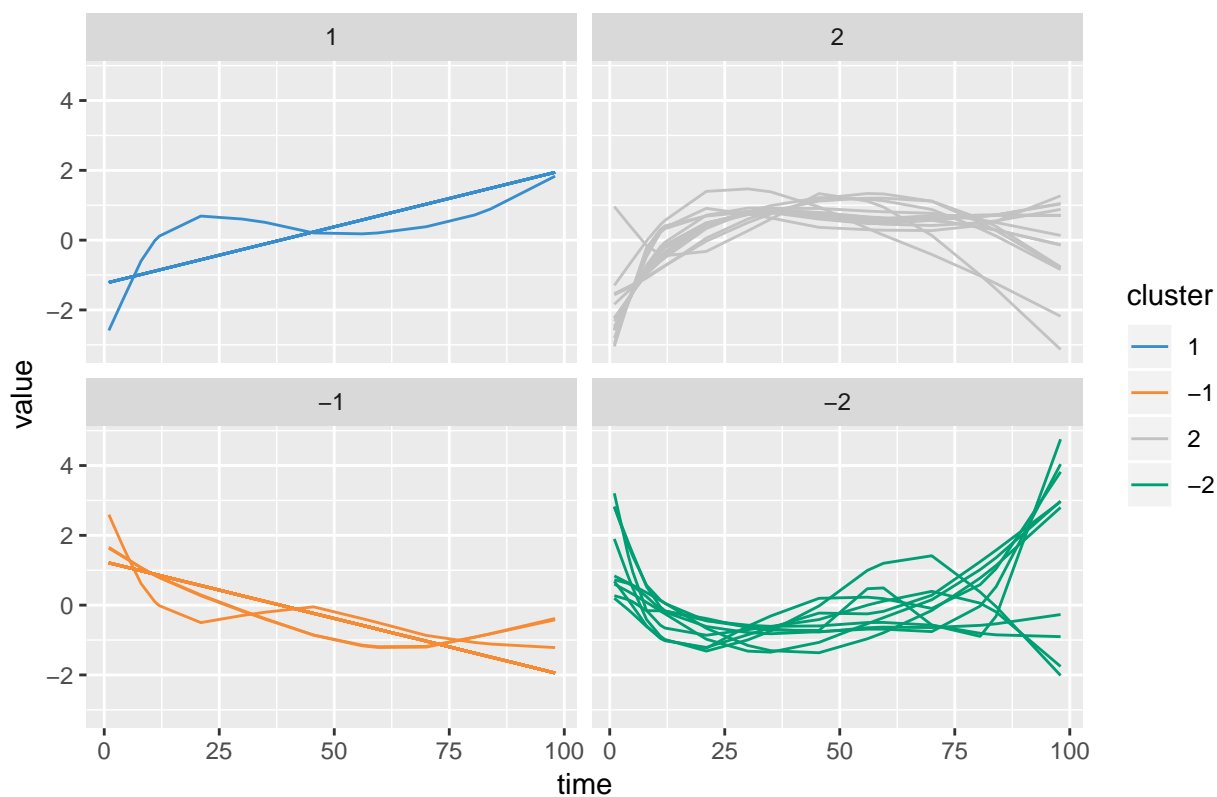
```
pca.plot(pca.res.C, title = "PCA, with lines, scale = T")
```

PCA, with lines, scale = T



```
# paper  
pca.plot(pca.res.C, title = "C-section PCA Clusters")
```

C-section PCA Clusters



```
pca.res.C <- pca(spline.data.C, ncomp = 2, scale = F, center = T)

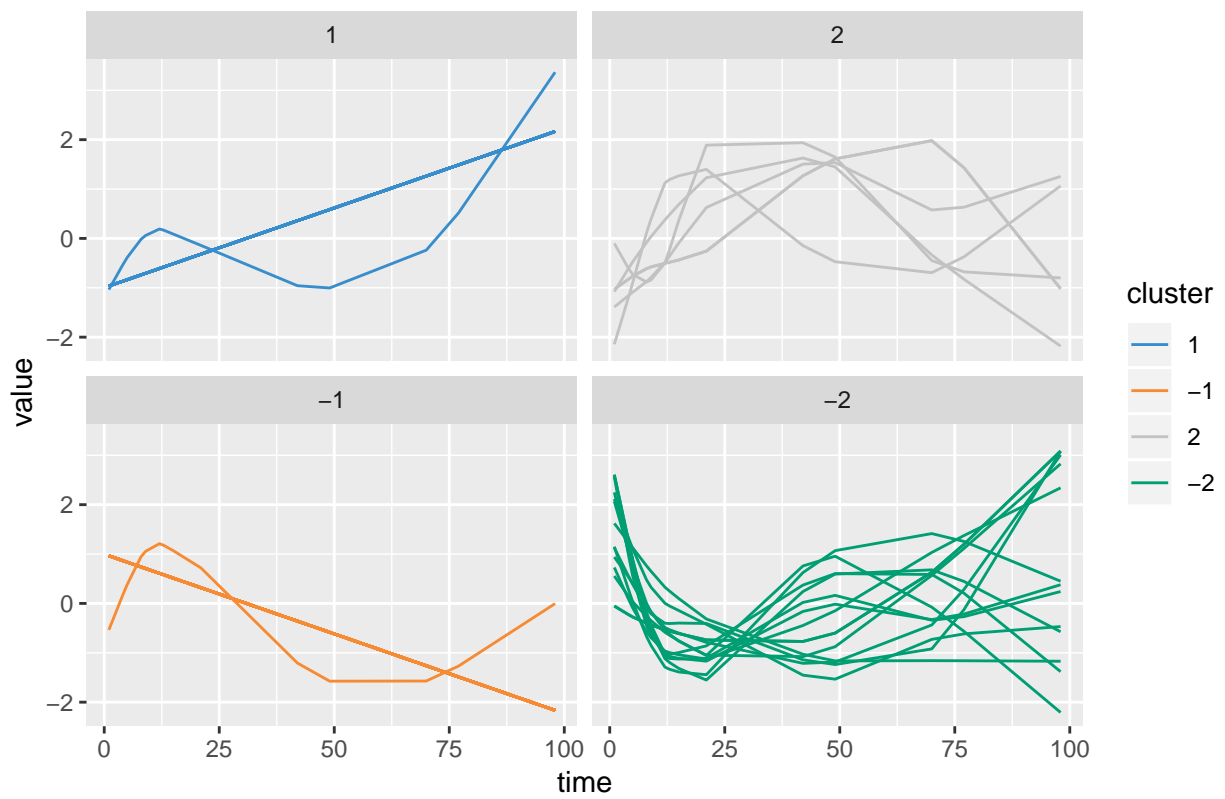
# silhouette coefficient for this clustering
wrapper.silhouette.pca(spline.data.C, ncomp = 2, scale = T, center=T)

## [1] 0.8426561
```

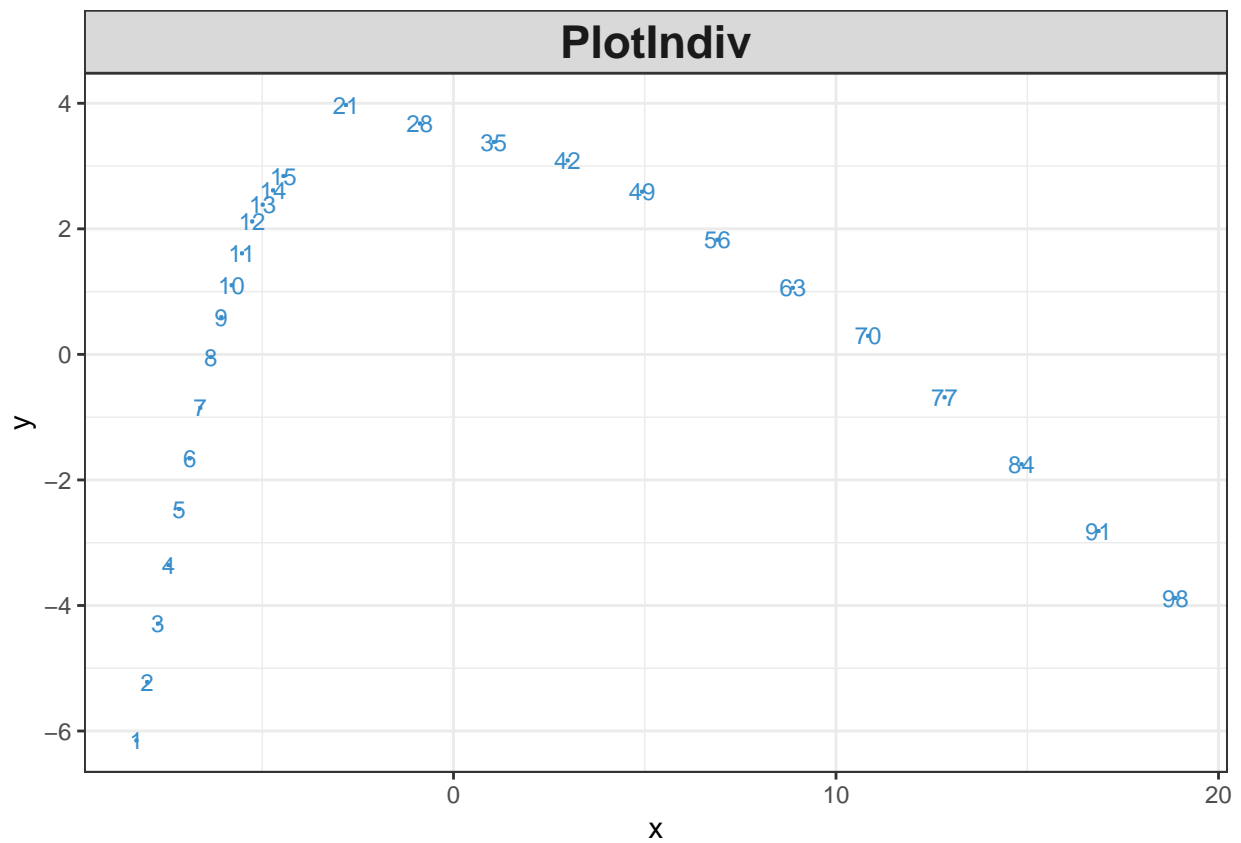
Vaginal

```
pca.res.V <- pca(spline.data.V, ncomp = 2, scale = T, center = T)
pca.plot(pca.res.V, title = "Vaginal PCA Clusters")
```

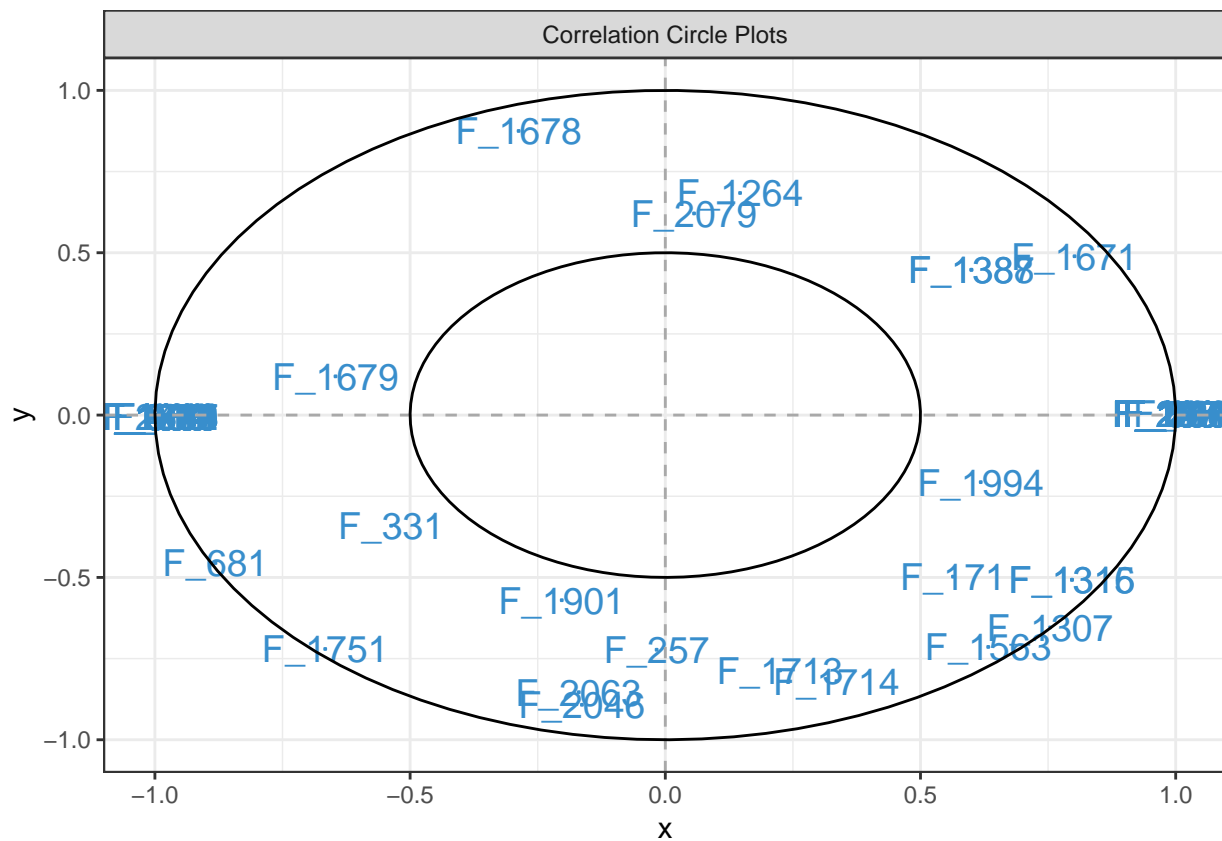
Vaginal PCA Clusters



```
plotIndiv(pca.res.V)
```



```
plotVar(pca.res.V)
```

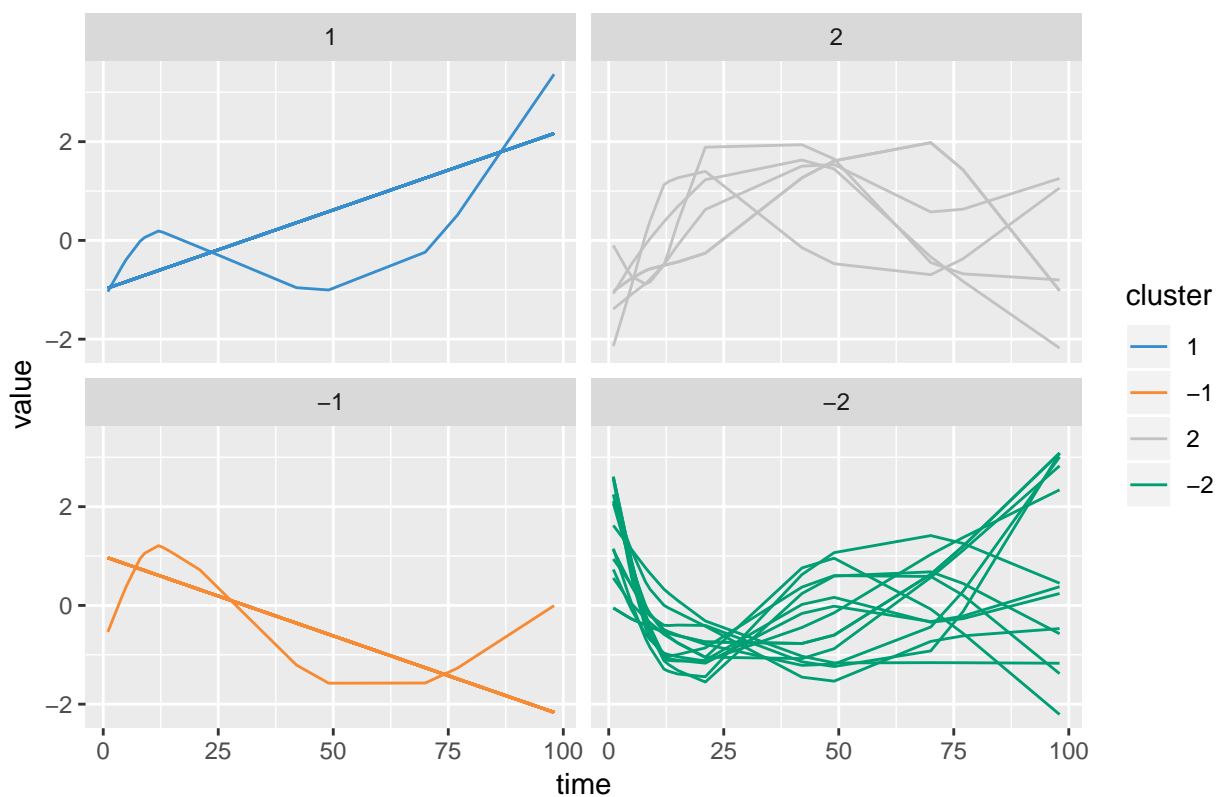


```
pca.res.V <- pca(spline.data.V, ncomp = 2, scale = T, center = T)
pca.get_cluster(pca.res.V) %>% pull(cluster) %>% table
```

```
## .
## -2 -1 1 2
## 14 38 32 6
```

```
pca.plot(pca.res.V, title = "PCA, with lines, scale = T")
```


PCA, with lines, scale = T



```
pca.res.V <- pca(spline.data.V, ncomp = 2, scale = F, center = T)

# silhouette coefficient for this clustering
wrapper.silhouette.pca(spline.data.V, ncomp = 2, scale = T, center=T)

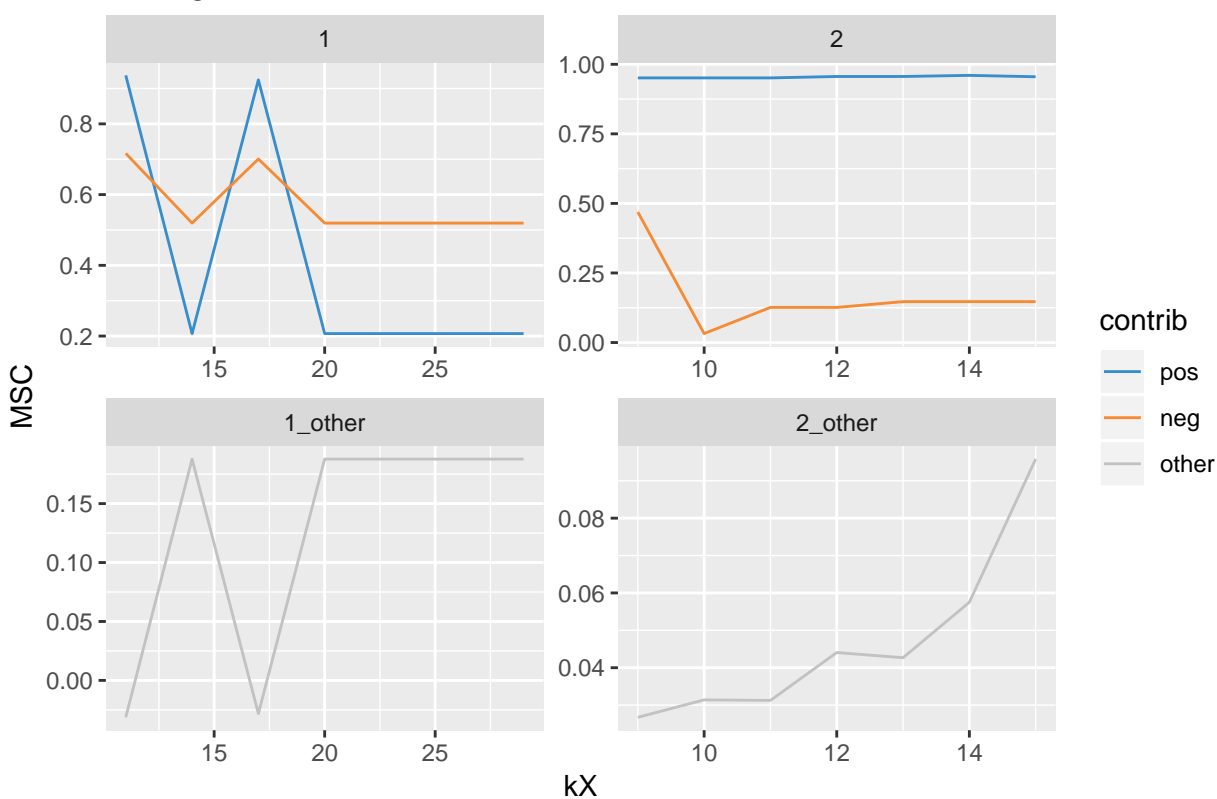
## [1] 0.8730084
```

sparse PCA Clustering

C-section

```
keepX = list(seq(11,29, 3), seq(9,15,1))
res.tune.spca.C <- tune.spca(X = spline.data.C, ncomp = 2, keepX = keepX)
tune.spca.choice.keepX(res.tune.spca.C, draw = T)
```

Tuning sPCA



```
## [1] 11 NA
```

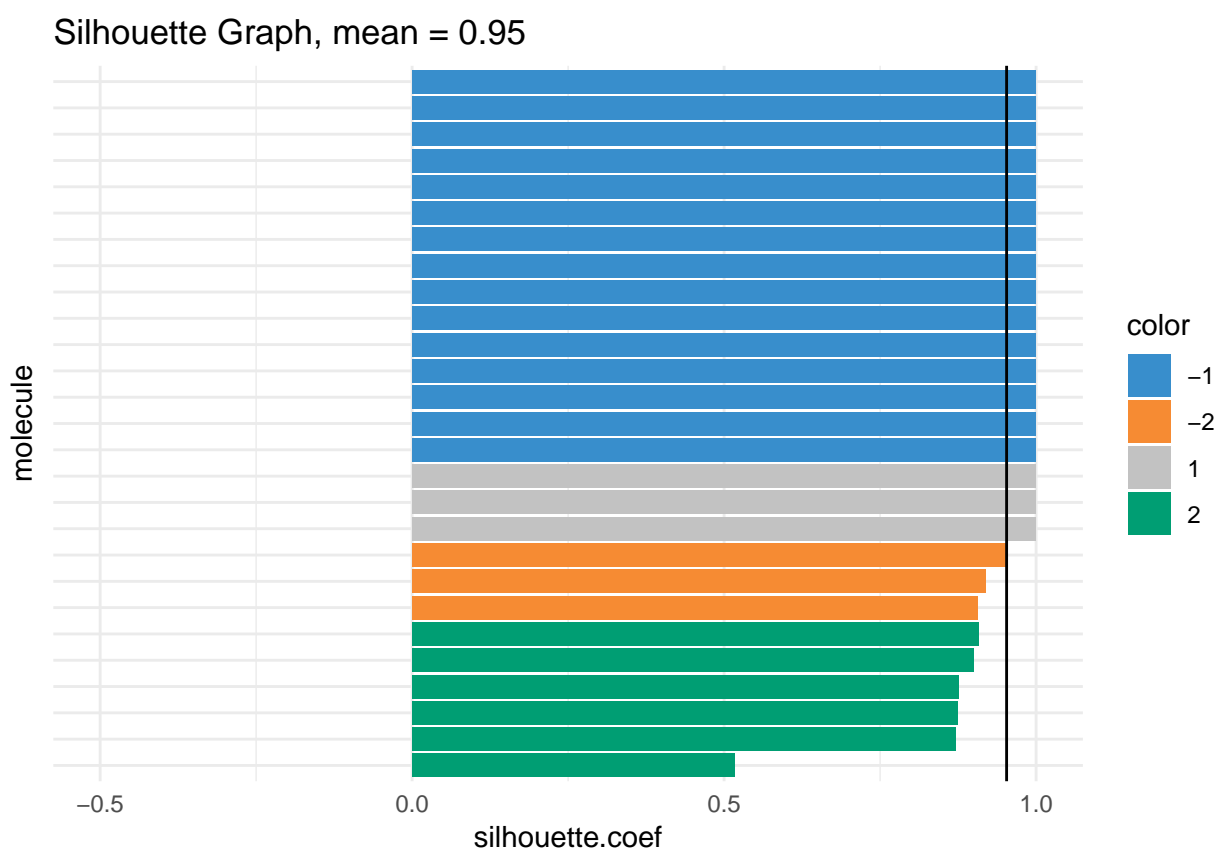
```
spca.res_f.C <- spca(spline.data.C, ncomp = 2, keepX = c(17,9))
pca.get_cluster(spca.res_f.C) %>% pull(cluster) %>% table
```

```
## .
```

```
## -2 -1 0 1 2
```

```
## 3 15 88 3 6
```

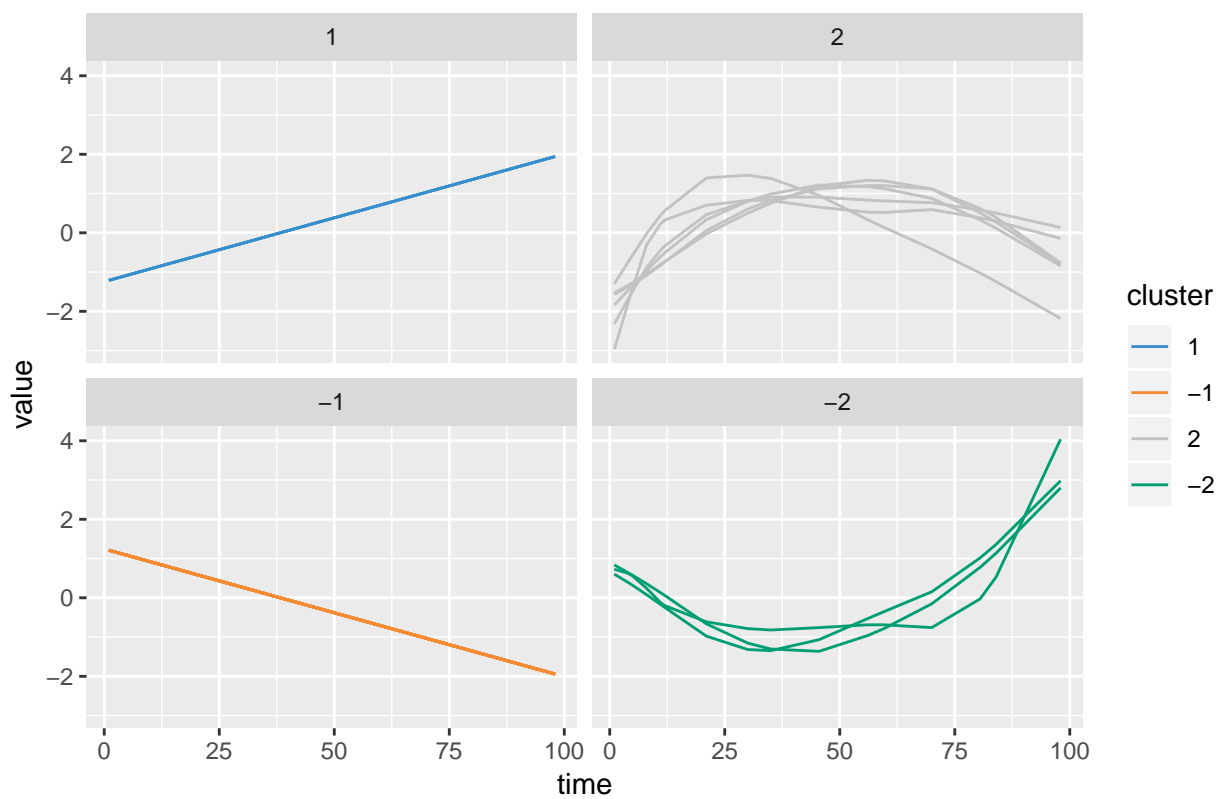
```
wrapper.silhouette.spca.paper(spline.data.C, keepX = c(17,9), ncomp = 2, scale = T, center=T, plot.t =
```



```
## [1] 0.9527507
```

```
spca.plot(spca.res_f.C, title = "C-section sparse PCA Clusters")
```

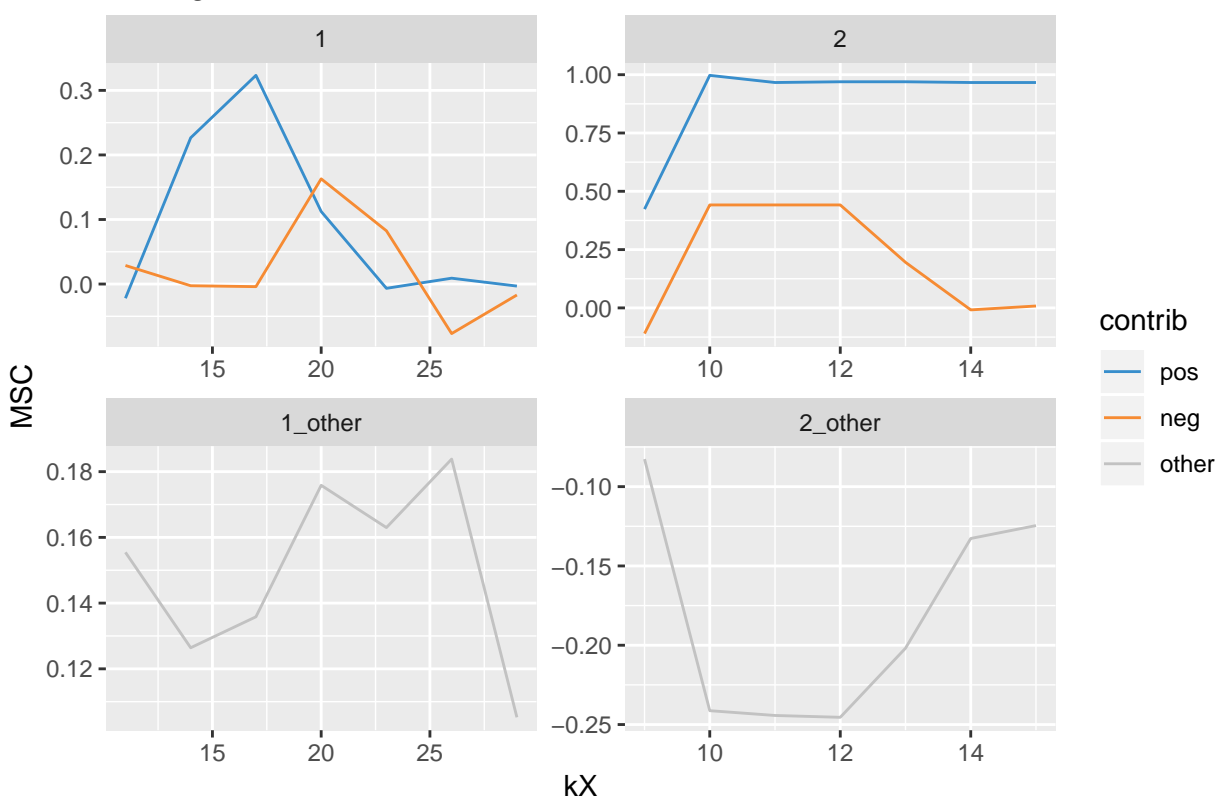
C-section sparse PCA Clusters



Vaginal

```
keepX = list(seq(11,29, 3), seq(9,15,1))
res.tune.spca.V <- tune.spca(X = spline.data.V, ncomp = 2, keepX = keepX)
tune.spca.choice.keepX(res.tune.spca.V, draw = T)
```

Tuning sPCA



```
## [1] 17 10
```

```
spca.res_f.V <- spca(spline.data.V, ncomp = 2, keepX = c(17,10))
pca.get_cluster(spca.res_f.V) %>% pull(cluster) %>% table
```

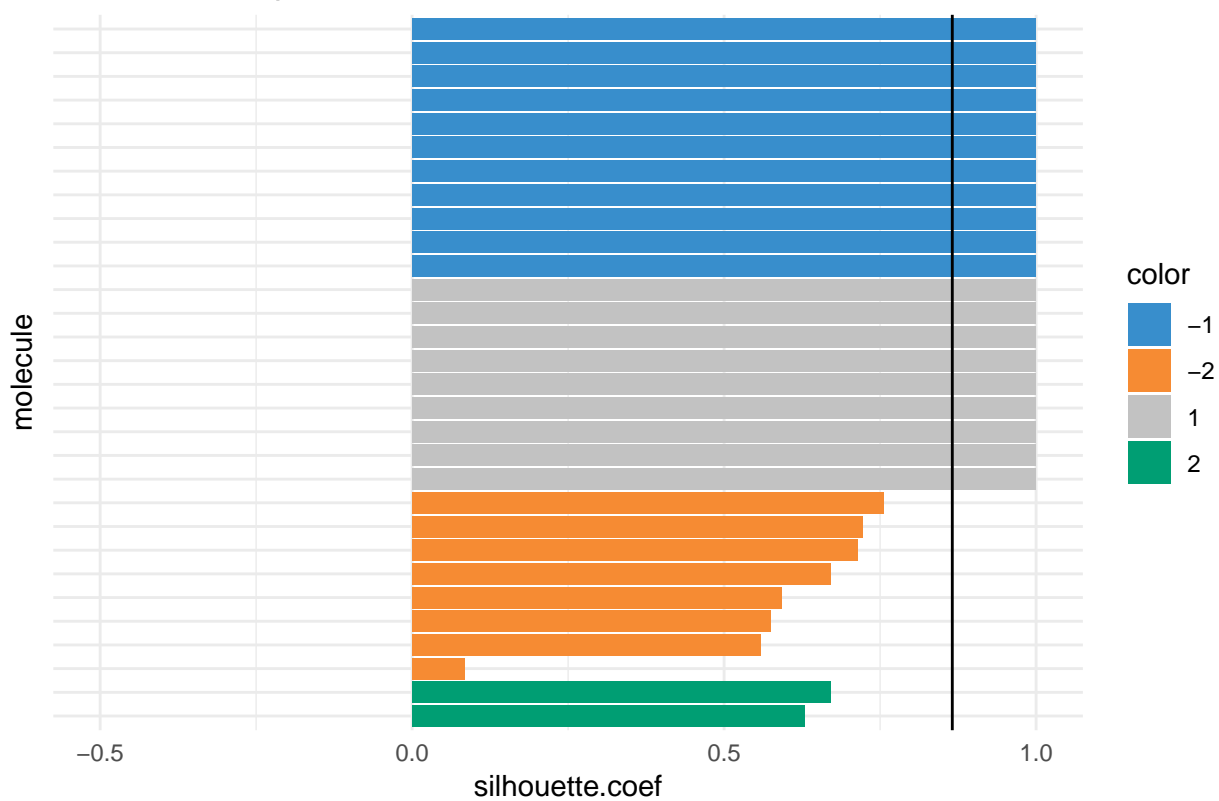
```
## .
```

```
## -2 -1 0 1 2
```

```
## 8 11 120 9 2
```

```
wrapper.silhouette.spca.paper(spline.data.V, keepX = c(17,10), ncomp = 2, scale = T, center=T, plot.t = F)
```

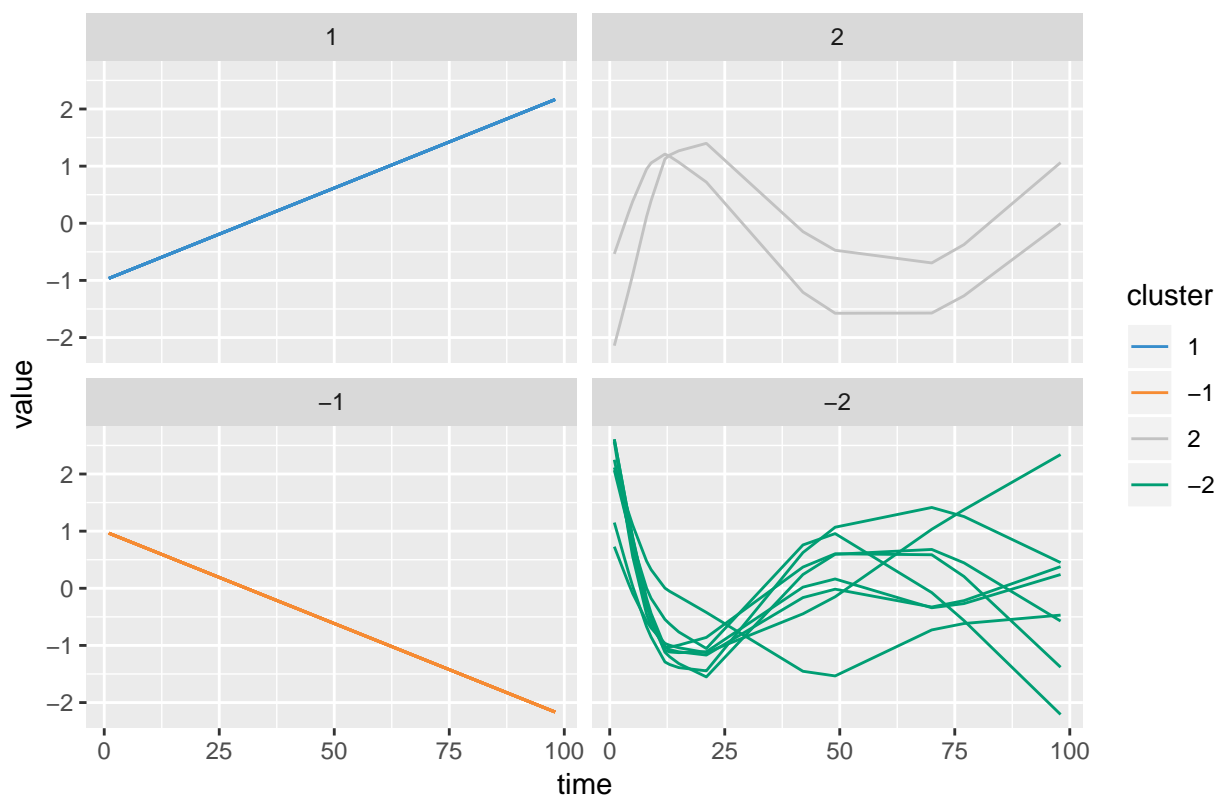
Silhouette Graph, mean = 0.87



```
## [1] 0.8656932
```

```
scca.plot(scca.res_f.V, title = "Vaginal sparse PCA Clusters")
```

Vaginal sparse PCA Clusters



Results

Silhouette coefficient :

	PCA	PCA w/o lines	sPCA	sPCA w/o lines
C-section	0.84	0.69	0.95	0.72
Vaginal	0.87	0.44	0.86	0.58

nb of molecules :

	0 (after filter)	1
C-section	42	29
Vaginal	68	22

Comparison with fPCA

C-section

```
library(fdapace)
```

```

data <- as.matrix(spline.data.C)

# prepare fclust input
FPCA_input <- MakeFPCAInputs(IDs = colnames(data) %>% rep(each=dim(data)[1]),
                             tVec = rep(rownames(data) %>% as.numeric(),dim(data)[2]),
                             yVec = data)

set.seed(123)
fclust.res <- FClust(FPCA_input$Ly, FPCA_input$Lt,
                    optsFPCA = list(userBwCov= 2, FVEthreshold = 0.90),
                    k = 4, cmethod = "EMCluster")

tmp <- bind_cols(as.data.frame(colnames(data)),
                 as.data.frame(as.character(fclust.res$cluster))) %>%
  set_names(c("molecule", "cluster"))

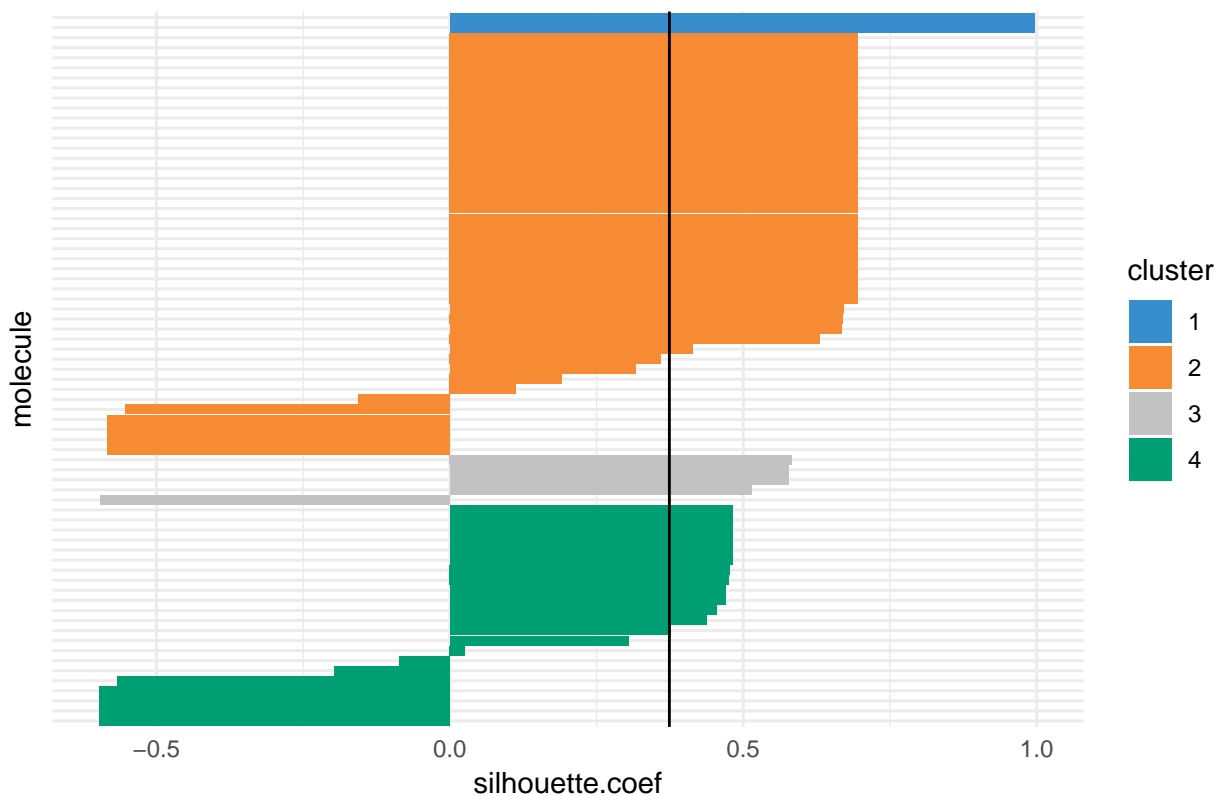
DF <- Spearman_distance(data)
B <- Add_Cluster_metadata(DF, tmp)
SC.fpca.1 <- Silhouette_coef_df(B)
mean(SC.fpca.1$silhouette.coef)

## [1] 0.3740866

#plot_silhouette_order_color(SC.fpca.1)
plot_fig.paper2(SC.fpca.1)

```

Silhouette Graph, mean = 0.37



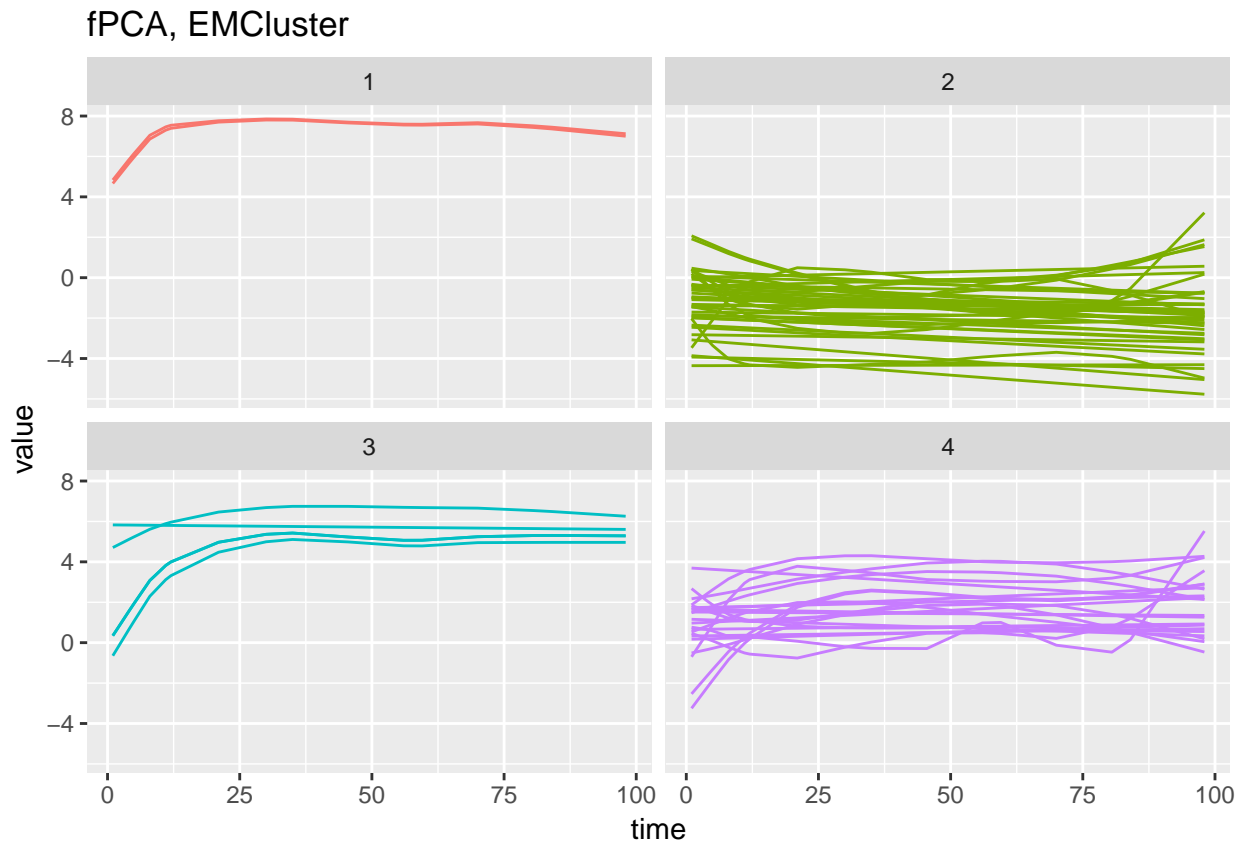
```

## plot clusters
data %>% as.data.frame() %>% rownames_to_column("time") %>%

```



```
gather(molecule, value, -time) %>%
left_join(tmp) %>% # add cluster metadata %>%
mutate(time = as.numeric(time)) %>%
ggplot(aes(x=time, y=value, group=molecule, color = as.factor(cluster))) +
geom_line() + facet_wrap(~as.factor(cluster)) + theme(legend.position="none") +
ggtitle("fPCA, EMCluster")
```



```
# idem with
set.seed(123)
fclust.res.2 <- FClust(FPCA_input$Ly, FPCA_input$Lt,
                      optsFPCA = list(userBwCov= 2, FVEthreshold = 0.90),
                      k = 4, cmethod = "kCFC")

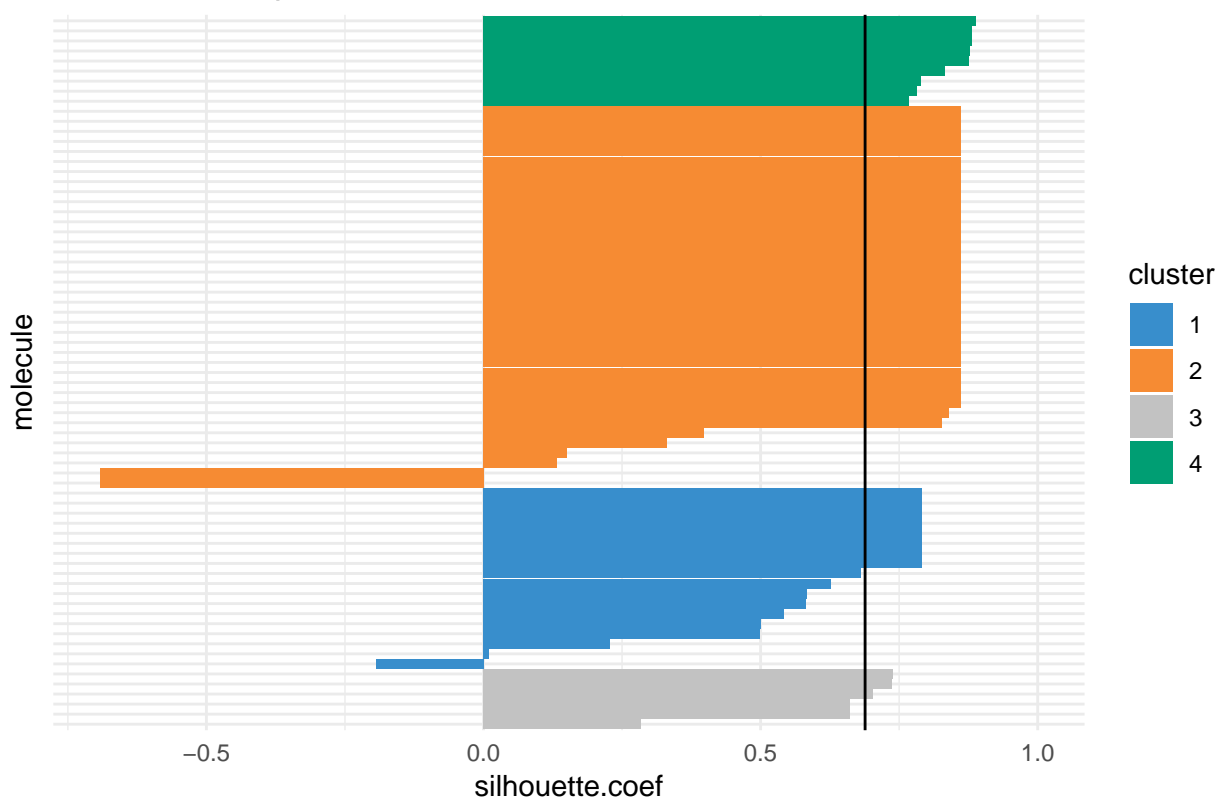
tmp <- bind_cols(as.data.frame(colnames(data)),
                 as.data.frame(as.character(fclust.res.2$cluster))) %>%
  set_names(c("molecule", "cluster"))

B <- Add_Cluster_metadata(DF, tmp)
SC.fpca.2 <- Silhouette_coef_df(B)
mean(SC.fpca.2$silhouette.coef)

## [1] 0.6885726

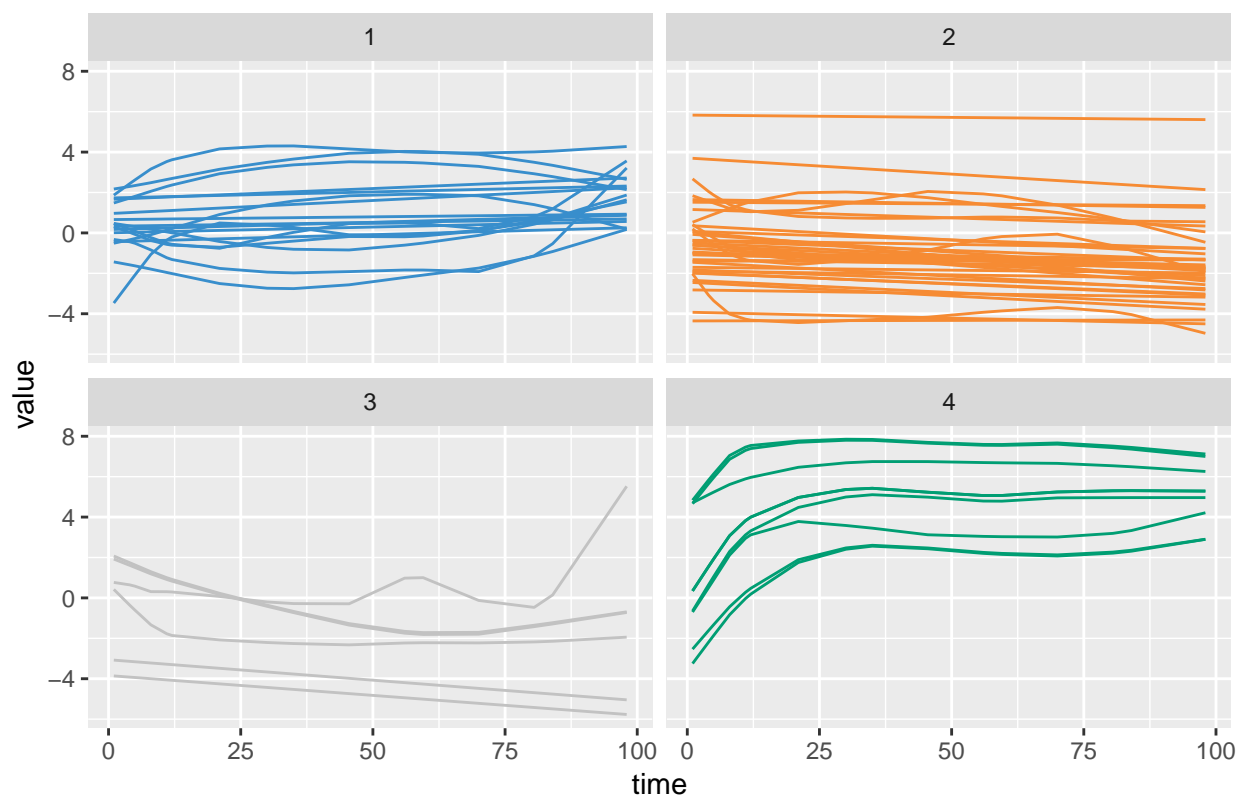
#plot_silhouette_order_color(SC.fpca.2)
plot_fig.paper2(SC.fpca.2)
```

Silhouette Graph, mean = 0.69



```
## plot clusters
data %>% as.data.frame() %>% rownames_to_column("time") %>%
  gather(molecule, value, -time) %>%
  left_join(tmp) %>% # add cluster metadata %>%
  mutate(time = as.numeric(time)) %>%
  ggplot(aes(x=time, y=value, group=molecule, color = as.factor(cluster))) +
  geom_line() + facet_wrap(~as.factor(cluster)) + theme(legend.position="none") +
  scale_color_manual(values=color.mixo(1:4)) + ggtitle("fPCA, kCFC")
```

fPCA, kCFC



Vaginal

```
data <- as.matrix(spline.data.V)

# prepare fclust input
FPCA_input <- MakeFPCAInputs(IDs = colnames(data) %>% rep(each=dim(data)[1]),
                             tVec = rep(rownames(data) %>% as.numeric(), dim(data)[2]),
                             yVec = data)

set.seed(123)
fclust.res <- FClust(FPCA_input$Ly, FPCA_input$Lt,
                    optnsFPCA = list(userBwCov= 2, FVEthreshold = 0.90),
                    k = 4, cmethod = "EMCluster")

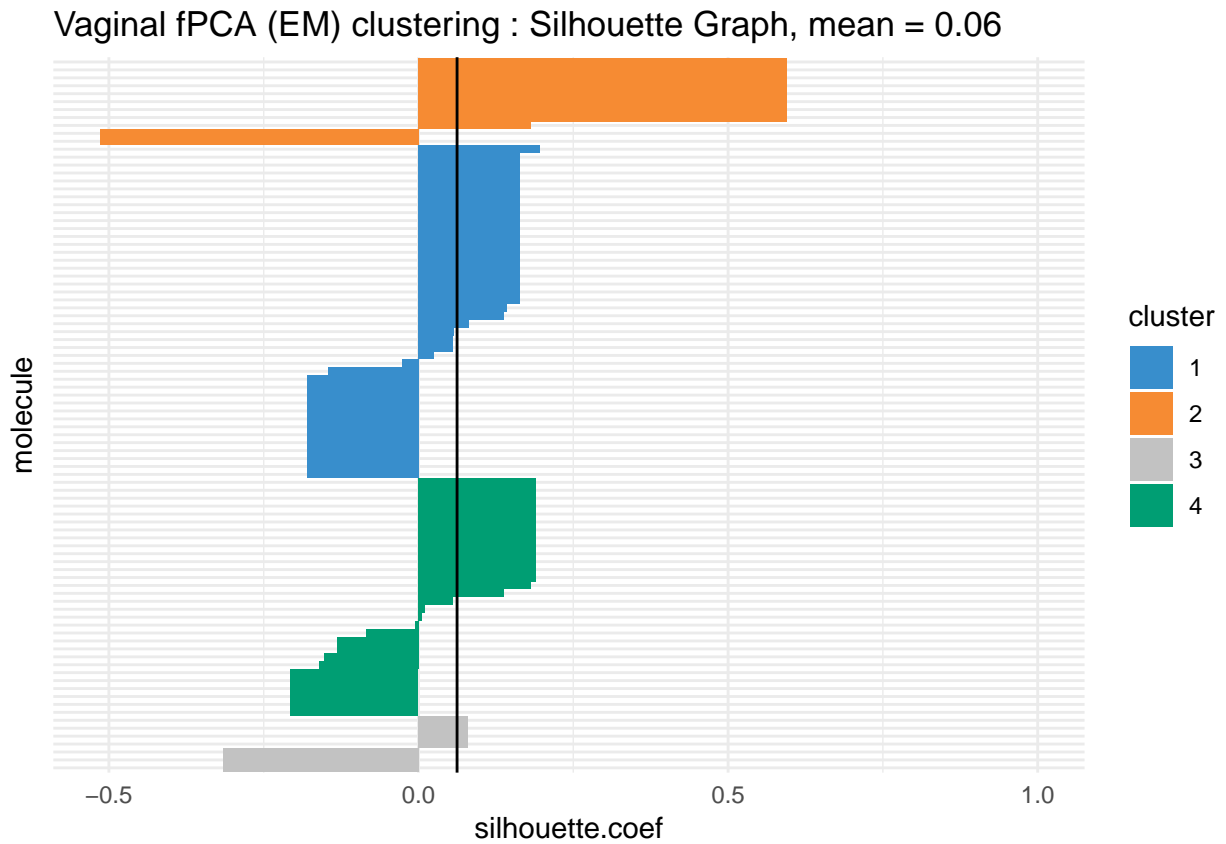
tmp <- bind_cols(as.data.frame(colnames(data)),
                 as.data.frame(as.character(fclust.res$cluster))) %>%
  set_names(c("molecule", "cluster"))

DF <- Spearman_distance(data)
B <- Add_Cluster_metadata(DF, tmp)
SC.fpca.1 <- Silhouette_coef_df(B)
mean(SC.fpca.1$silhouette.coef)

## [1] 0.06230024

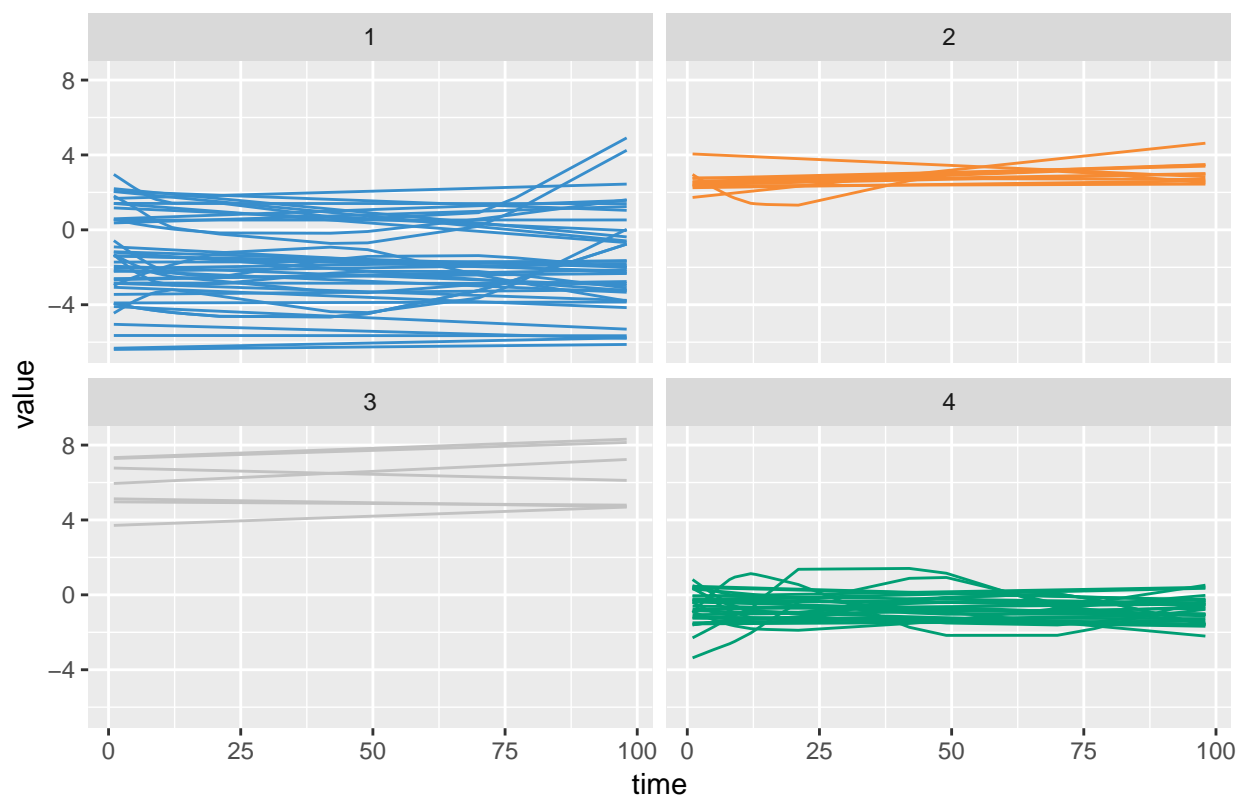
#plot_silhouette_order_color(SC.fpca.1)
```

```
title = "Vaginal fPCA (EM) clustering : "  
plot_fig.paper2(SC.fpca.1, title)
```



```
## plot clusters  
data %>% as.data.frame() %>% rownames_to_column("time") %>%  
  gather(molecule, value, -time) %>%  
  left_join(tmp) %>% # add cluster metadata %>%  
  mutate(time = as.numeric(time)) %>%  
  ggplot(aes(x=time, y=value, group=molecule, color = as.factor(cluster))) +  
  geom_line() + facet_wrap(~as.factor(cluster)) + theme(legend.position="none") +  
  ggtitle("Vaginal fPCA (EM) Clusters") +  
  scale_color_manual(values = color.mixo(1:4))
```

Vaginal fPCA (EM) Clusters



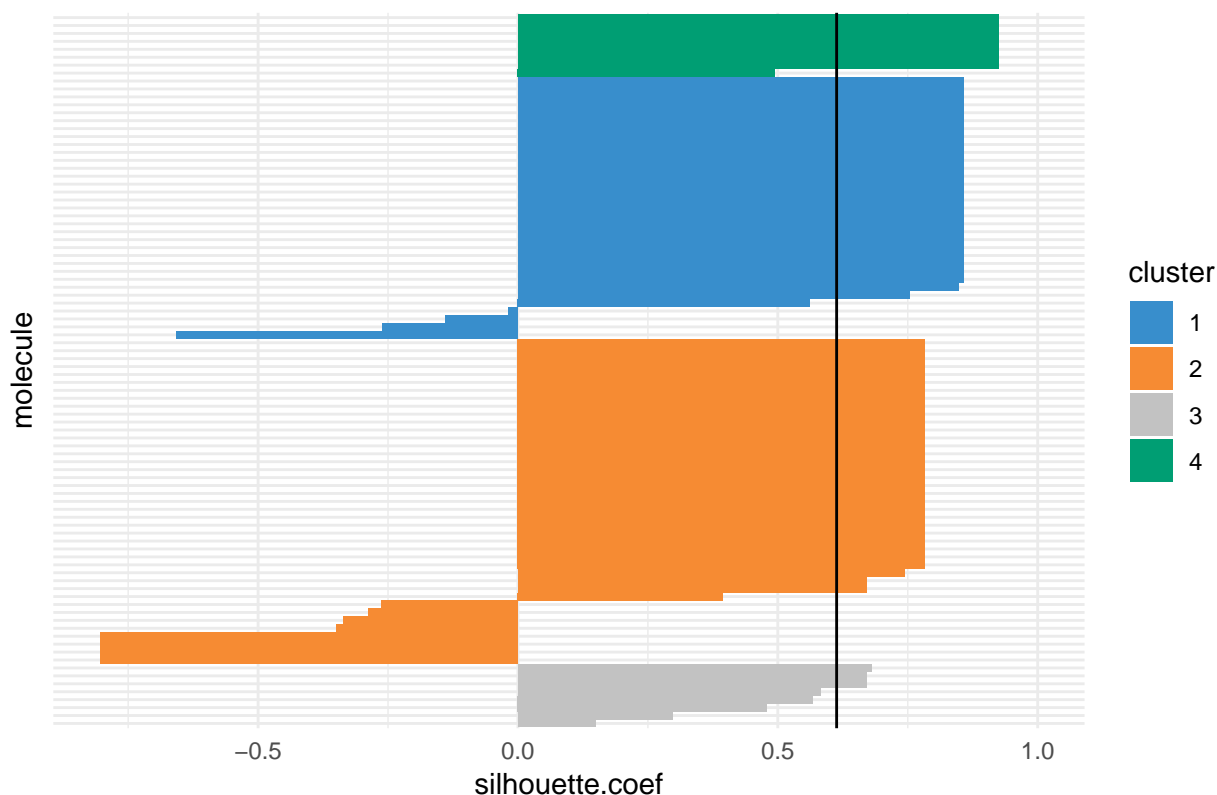
```
# idem with
set.seed(123)
fclust.res.2 <- FClust(FPCA_input$Ly, FPCA_input$Lt,
                      optnsFPCA = list(userBwCov= 2, FVEthreshold = 0.90),
                      k = 4, cmethod = "kCFC")
tmp <- bind_cols(as.data.frame(colnames(data)),
                 as.data.frame(as.character(fclust.res.2$cluster))) %>%
  set_names(c("molecule", "cluster"))

B <- Add_Cluster_metadata(Df, tmp)
SC.fpca.2 <- Silhouette_coef_df(B)
mean(SC.fpca.2$silhouette.coef)
```

```
## [1] 0.6131915
```

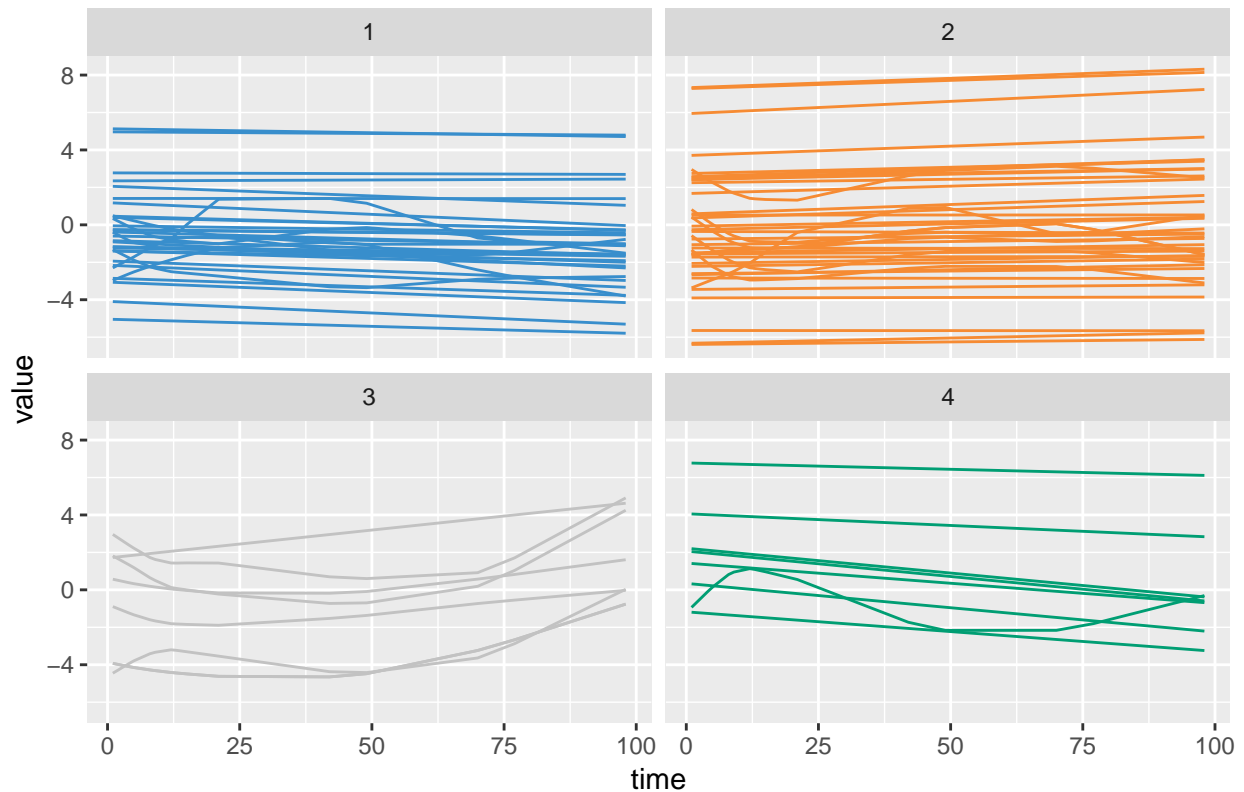
```
#plot_silhouette_order_color(SC.fpca.2)
title = "Vaginal fPCA (k-CFC) clustering : "
plot_fig.paper2(SC.fpca.2, title)
```

Vaginal fPCA (k-CFC) clustering : Silhouette Graph, mean = 0.61



```
## plot clusters
data %>% as.data.frame() %>% rownames_to_column("time") %>%
  gather(molecule, value, -time) %>%
  left_join(tmp) %>% # add cluster metadata %>%
  mutate(time = as.numeric(time)) %>%
  ggplot(aes(x=time, y=value, group=molecule, color = as.factor(cluster))) +
  geom_line() + facet_wrap(~as.factor(cluster)) + theme(legend.position="none") +
  scale_color_manual(values=color.mixo(1:4)) + ggtitle("Vaginal fPCA (k-CFC) Clusters")
```

Vaginal fPCA (k-CFC) Clusters



```
sessionInfo()
```

```
## R version 3.5.2 (2018-12-20)
## Platform: x86_64-redhat-linux-gnu (64-bit)
## Running under: Fedora 29 (Workstation Edition)
##
## Matrix products: default
## BLAS/LAPACK: /usr/lib64/R/lib/libRblas.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel stats      graphics  grDevices utils      datasets  methods
## [8] base
##
## other attached packages:
##  [1] fdapace_0.4.0      bindrcpp_0.2.2      pspline_1.0-18
##  [4] geiger_2.0.6       ape_5.2             usethis_1.4.0
##  [7] devtools_2.0.1     ciValid_0.6-6       edci_1.1-3
## [10] mclust_5.4.2       cluster_2.0.7-1     dynOmics_1.2
## [13] lmeSplines_1.1-10 nlme_3.1-137        knitr_1.20
```

```

## [16] reshape2_1.4.3      lmms_1.3.3           lmtest_0.9-36
## [19] zoo_1.8-4           tseries_0.10-46      mixOmics_6.6.0
## [22] lattice_0.20-38     MASS_7.3-51.1        forcats_0.3.0
## [25] stringr_1.3.1       dplyr_0.7.8          purrr_0.2.5
## [28] readr_1.3.0         tidyr_0.8.2          tibble_1.4.2
## [31] ggplot2_3.1.0       tidyverse_1.2.1
##
## loaded via a namespace (and not attached):
## [1] colorspace_1.3-2     class_7.3-14         rprojroot_1.3-2
## [4] htmlTable_1.12       corpcor_1.6.9        base64enc_0.1-3
## [7] fs_1.2.6             rstudioapi_0.8       remotes_2.0.2
## [10] RSpectra_0.13-1     mvtnorm_1.0-8        lubridate_1.7.4
## [13] xml2_1.2.0           splines_3.5.2        pkgload_1.0.2
## [16] Formula_1.2-3        jsonlite_1.6         broom_0.5.1
## [19] compiler_3.5.2       httr_1.4.0           backports_1.1.2
## [22] assertthat_0.2.0     Matrix_1.2-15        lazyeval_0.2.1
## [25] cli_1.0.1            acepack_1.4.1        htmltools_0.3.6
## [28] prettyunits_1.0.2    tools_3.5.2          igraph_1.2.2
## [31] coda_0.19-2          gtable_0.2.0         glue_1.3.0
## [34] Rcpp_1.0.0           cellranger_1.1.0     gdata_2.18.0
## [37] ps_1.2.1             rvest_0.3.2          gtools_3.8.1
## [40] scales_1.0.0         subplex_1.5-4        hms_0.4.2
## [43] RColorBrewer_1.1-2  yaml_2.2.0           quantmod_0.4-13
## [46] curl_3.2             memoise_1.1.0        gridExtra_2.3
## [49] rpart_4.1-13         latticeExtra_0.6-28  stringi_1.2.3
## [52] desc_1.2.0           checkmate_1.8.5      TTR_0.23-4
## [55] caTools_1.17.1.1     pkgbuild_1.0.2       rlang_0.3.0.1
## [58] pkgconfig_2.0.2      matrixStats_0.54.0   bitops_1.0-6
## [61] pracma_2.2.2         evaluate_0.12        bindr_0.1.1
## [64] htmlwidgets_1.3      tidyselect_0.2.5     processx_3.2.1
## [67] deSolve_1.21         plyr_1.8.4           magrittr_1.5
## [70] R6_2.3.0             Hmisc_4.1-1          gplots_3.0.1
## [73] generics_0.0.2       foreign_0.8-71        pillar_1.3.0
## [76] haven_2.0.0          withr_2.1.2          xts_0.11-2
## [79] nnet_7.3-12          survival_2.43-3      modelr_0.1.2
## [82] crayon_1.3.4         rARPACK_0.11-0       KernSmooth_2.23-15
## [85] ellipse_0.4.1        rmarkdown_1.10.14    grid_3.5.2
## [88] readxl_1.1.0         data.table_1.11.8    callr_3.1.0
## [91] htmldeps_0.1.1       digest_0.6.18        numDeriv_2016.8-1
## [94] munsell_0.5.0        sessioninfo_1.1.1    quadprog_1.5-5

```