R-code for 'GCalignR. An R package for aligning Gas-Chromatography data'

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This document provides the code for the validation of alignments presented in our paper. Some parts presented here a computational demanding and run for several hours on a standard computer. Therefore, we provide these data files along with our manuscript.

Prerequisites

Most functions that are used in this analysis are part of our package **GCalignR**, while some more functions are provided in form of R scripts that are available along with this document in the subdirectory `R`. In order to run the code you need to have a subdirectory called `data` that contains the raw data.

- Installing the most recent version of **GCalignR**

```
## install devtools
if (!("devtools" %in% rownames(installed.packages()))) {
    install.packages("devtools")
} else if (packageVersion("devtools") < 1.6) {
    install.packages("devtools")
}
## install GCalignR
devtools::install_github("mastoffel/GCalignR")
## install plot3D
if (!"plot3D" %in% rownames(installed.packages())) {
    install.packages("plot3D")
}
```

Load packages, data and functions

```
## small function to test parameters in align_chromatograms
source("R/optimal_params.R")
## calculates errors by matching aligned data to a table of known substances
source("R/error_rate.R")
## custom function for simulations based on chromatograms
source("R/ChromaSimFunctions.R")
library(GCalignR)
library(ggplot2)
library(plot3D)
```

Explore the parameter space in `align_chromatograms`

There are two parameters of major importance in `GCalign`, namely `max_peak2mean` and `min_peak2peak`. While the first determines the finescale grouping of retention times the latter greatly influences the formation of substances by combining initially separated rows of similar retention times. Here, we evaluate the error rate as a function of the combination of these two parameters. The combinations are tested by iteratively running `aling_chromatograms` with the following combinations

```
max_diff_peak2mean = seq(0.01, 0.05, 0.01)
min_diff_peak2peak = seq(0.01, 0.2, 0.01)
## all combinations tested
expand.grid(peak2mean = max_diff_peak2mean, peak2peak = min_diff_peak2peak)
```

- Run alignments with all combinations of both parameters

```
## B. flavifrons
results_bfla <- optimal_params(data = "data/bfla.txt", rt_col_name = "RT", max_diff_peak2mean = seq(fro
    to = 0.05, by = 0.01), min_diff_peak2peak = seq(from = 0.01, to = 0.2, by = 0.01))
save(results_bfla, file = "data/results_bfla.RData")

## B. bimaculatus
results_bbim <- optimal_params(data = "data/bbim.txt", rt_col_name = "RT", max_diff_peak2mean = seq(fro
    to = 0.05, by = 0.01), min_diff_peak2peak = seq(from = 0.01, to = 0.2, by = 0.01))
save(results_bbim, file = "data/results_bbim.RData")

## B. ephippiatus
results_beph <- optimal_params(data = "data/beph.txt", rt_col_name = "RT", max_diff_peak2mean = seq(fro
    to = 0.05, by = 0.01), min_diff_peak2peak = seq(from = 0.01, to = 0.2, by = 0.01))
save(results_beph, file = "data/results_beph.RData")
```

```
## Load data
load("data/results_bbim.RData")
load("data/results_beph.RData")
load("data/results_bfla.RData")
```

- Estimate error rates Error rate calculations are executed with a custom function `error_rate` that uses a list of annotated substances as reference. See the functions code for details.

```
errors_bbim <- data.frame(p2p = results_bbim[[2]][["p2p"]], p2m = results_bbim[[2]][["p2m"]])
errors_bbim[["error"]] <- unlist(lapply(X = results_bbim[[1]], error_rate, "data/bbim_ms.txt"))

errors_beph <- data.frame(p2p = results_beph[[2]][["p2p"]], p2m = results_beph[[2]][["p2m"]])
errors_beph[["error"]] <- unlist(lapply(X = results_beph[[1]], error_rate, "data/beph_ms.txt"))

errors_bfla <- data.frame(p2p = results_bfla[[2]][["p2p"]], p2m = results_bfla[[2]][["p2m"]])
errors_bfla[["error"]] <- unlist(lapply(X = results_bfla[[1]], error_rate, "data/bfla_ms.txt"))
```

Plot parameter space using package *plot3D*

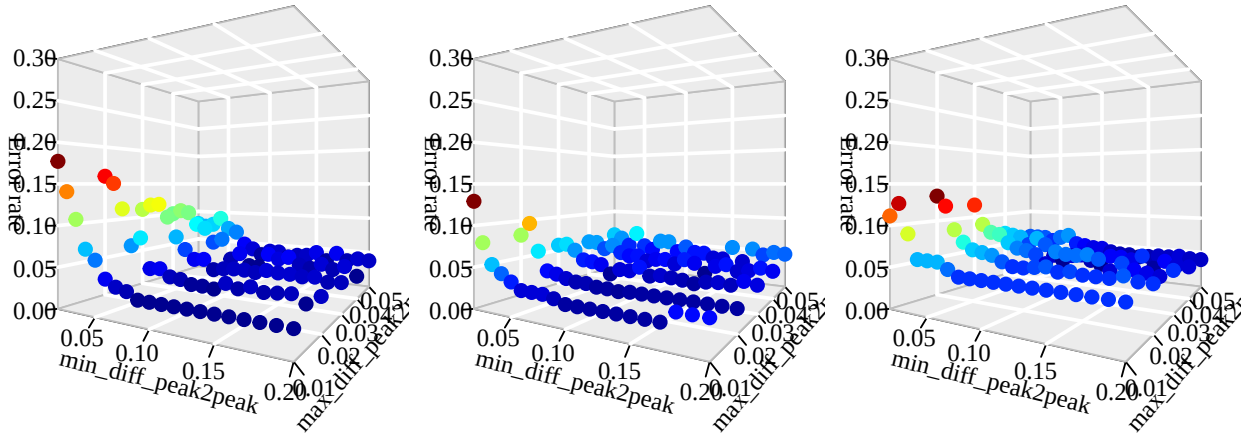
```
## Set up the margins
par(mfrow = c(1, 3), family = "serif", mai = c(0.1, 0.3, 0.5, 0.15))
## plotting
scatter3D(x = errors_bbim[["p2p"]], y = errors_bbim[["p2m"]], z = errors_bbim[["error"]],
    pch = 19, size = 2, theta = 30, phi = 0, ticktype = "detailed", main = "Bombus bimaculatus\nn = 717
    xlab = "min_diff_peak2peak", ylab = "max_diff_peak2mean", zlab = "Error rate",
    bty = "g", colkey = FALSE, cex = 1.5, cex.lab = 1.25, cex.axis = 1.25, cex.main = 2,
    zlim = c(0, 0.3))

with(errors_beph, scatter3D(x = p2p, y = p2m, z = error, pch = 19, size = 2, theta = 30,
    phi = 0, ticktype = "detailed", main = "Bombus ephippiatus\nn = 782", xlab = "min_diff_peak2peak",
    ylab = "max_diff_peak2mean", zlab = "Error rate", bty = "g", colkey = FALSE,
    cex = 1.5, cex.lab = 1.25, cex.axis = 1.25, cex.main = 2, zlim = c(0, 0.3)))
```

```
with(errors_bfla, scatter3D(x = p2p, y = p2m, z = error, pch = 19, size = 2, theta = 30,
    phi = 0, ticktype = "detailed", main = "Bombus flavifrons\nn = 457", xlab = "min_diff_peak2peak",
    ylab = "max_diff_peak2mean", zlab = "Error rate", bty = "g", colkey = FALSE,
    cex = 1.5, cex.lab = 1.25, cex.axis = 1.25, cex.main = 2, zlim = c(0, 0.3)))
```

Bombus bimaculatus
n = 717

Bombus ephippiatus
n = 782

Bombus flavifrons
n = 457



Simulate the effect of additional noise

The performance of GCalignR is clearly dependend on the quality of the raw data. To show the effect of noise (i.e. bad quality chromatograms) we used again the raw datasets of (Dellicour and Lecocq 2013) and added small errors to a varying proportion of peaks and repeated the error calculation as shown above. This time we choose default values for `min__diff_peak2peak`and `max_diff_peak2mean`that were shown to be robust.

- Aligning the raw datasets with default settings

```
## we align that untreated datasets in order to extract input retention times
bbim_zero <- align_chromatograms(data = "data/bbim.txt", rt_col_name = "RT")
save(bbim_zero, file = "data/bbim_zero.RData")
beph_zero <- align_chromatograms(data = "data/beph.txt", rt_col_name = "RT")
save(beph_zero, file = "data/beph_zero.RData")
bfla_zero <- align_chromatograms(data = "data/bfla.txt", rt_col_name = "RT")
save(bfla_zero, file = "data/bfla_zero.RData")
```

```
load("data/bbim_zero.RData")
load("data/beph_zero.RData")
load("data/bfla_zero.RData")
```

- Convert to lists

```
bfla_chroma <- lapply(bfla_zero[["input_list"]], na.remove)  # remove NAs
bbim_chroma <- lapply(bbim_zero[["input_list"]], na.remove)  # remove NAs
beph_chroma <- lapply(beph_zero[["input_list"]], na.remove)  # remove NAs
```

Do the simulations

- Dataset *Bombus flavifrons*

```
bfla_out <- sim_linear_shift(bfla_chroma, rt_col_name = "RT", shifts = c(-0.03, 0.03))
bfla_shifted <- bfla_out[["Chromas"]]

p <- rep(seq(from = 0, to = 1, by = 0.05), each = 3)
bfla_data <- list()
names <- character()
for (i in 1:length(p)) {
    ## add errors
    temp <- lapply(bfla_shifted, add_peak_error, p = p[i], rt_col_name = "RT", conc_col_name = "Area",
        distr = c(-0.02, -0.01, 0.01, 0.02))
    ## extract peak list
    temp <- lapply(temp, FUN = function(x) x[["chroma"]])
    aligned <- align_chromatograms(temp, rt_col_name = "RT", max_linear_shift = 0.05)
    ## We need the 'true' retention times for referencing purposes
    aligned <- original_rt(org = bfla_chroma, aligned = aligned, rt_col_name = "RT")
    bfla_data <- append(bfla_data, list(aligned))
    names <- c(names, paste0("no_", as.character(i), "_noise_", as.character(p[i])))
}
names(bfla_data) <- names
bfla_simulations <- list(OptAlign = bfla_zero, SimAlign = bfla_data, noise = p)
save(x = bfla_simulations, file = paste0("data/", "bfla_simulations", ".RData"))
```

- Dataset *Bombus bimaculatus*

```
bbim_out <- sim_linear_shift(bbim_chroma, rt_col_name = "RT", shifts = c(-0.03, 0.03))
bbim_shifted <- bbim_out[["Chromas"]]  # linearly shifted sample

p <- rep(seq(from = 0, to = 1, by = 0.05), each = 3)
bbim_data <- list()
names <- character()
for (i in 1:length(p)) {
    ## add errors
```

```
    temp <- lapply(bbim_shifted, add_peak_error, p = p[i], rt_col_name = "RT", conc_col_name = "Area",
        distr = c(-0.02, -0.01, 0.01, 0.02))
    ## extract peak list
    temp <- lapply(temp, FUN = function(x) x[["chroma"]])
    aligned <- align_chromatograms(temp, rt_col_name = "RT", max_linear_shift = 0.05)
    ## We need the 'true' retention times for referencing purposes
    aligned <- original_rt(org = bbim_chroma, aligned = aligned, rt_col_name = "RT")
    bbim_data <- append(bbim_data, list(aligned))
    names <- c(names, paste0("no_", as.character(i), "_noise_", as.character(p[i])))
}
names(bbim_data) <- names
bbim_simulations <- list(OptAlign = bbim_zero, SimAlign = bbim_data, noise = p)
save(x = bbim_simulations, file = paste0("data/", "bbim_simulations", ".RData"))
```

- Dataset *Bombus ephippiatus*

```
beph_out <- sim_linear_shift(beph_chroma, rt_col_name = "RT", shifts = c(-0.03, 0.03))
beph_shifted <- beph_out[["Chromas"]]  # linearly shifted sample

p <- rep(seq(from = 0, to = 1, by = 0.05), each = 3)
beph_data <- list()
names <- character()
for (i in 1:length(p)) {
    ## add errors
    temp <- lapply(beph_shifted, add_peak_error, p = p[i], rt_col_name = "RT", conc_col_name = "Area",
        distr = c(-0.02, -0.01, 0.01, 0.02))
    ## extract peak list
    temp <- lapply(temp, FUN = function(x) x[["chroma"]])
    aligned <- align_chromatograms(temp, rt_col_name = "RT", max_linear_shift = 0.05)
    ## We need the 'true' retention times for referencing purposes
    aligned <- original_rt(org = beph_chroma, aligned = aligned, rt_col_name = "RT")
    beph_data <- append(beph_data, list(aligned))
    names <- c(names, paste0("no_", as.character(i), "_noise_", as.character(p[i])))
}
names(beph_data) <- names
beph_simulations <- list(OptAlign = beph_zero, SimAlign = beph_data, noise = p)
save(x = beph_simulations, file = paste0("data/", "beph_simulations", ".RData"))
```

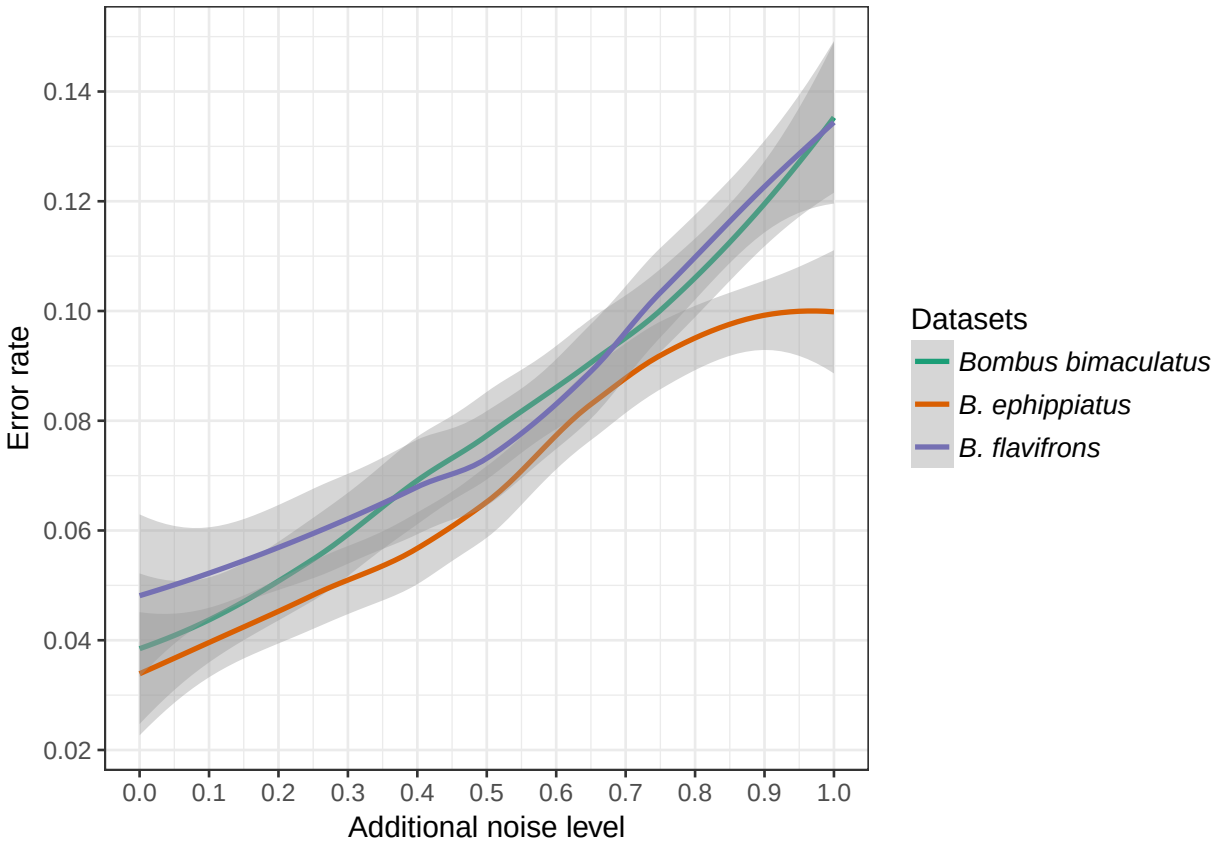
Estimate errors

```
load("data/bfla_simulations.RData")
load("data/beph_simulations.RData")
load("data/bbim_simulations.RData")
## set up data frames
bfla <- data.frame(data.frame(noise = bfla_simulations[["noise"]]))
bbim <- data.frame(data.frame(noise = bbim_simulations[["noise"]]))
beph <- data.frame(data.frame(noise = beph_simulations[["noise"]]))
## calculate errors
bfla[["error"]] <- unlist(lapply(X = bfla_simulations[["SimAlign"]], error_rate,
    Reference = "data/bfla_ms.txt", rt_col_name = "RT", linshift = FALSE))
bbim[["error"]] <- unlist(lapply(X = bbim_simulations[["SimAlign"]], error_rate,
    Reference = "data/bbim_ms.txt", rt_col_name = "RT", linshift = FALSE))
```

```
beph[["error"]] <- unlist(lapply(X = beph_simulations[["SimAlign"]], error_rate,
    Reference = "data/beph_ms.txt", rt_col_name = "RT", linshift = FALSE))
## Combine data into one data frame
df <- rbind(bbim, bfla, beph)
df[["id"]] <- rep(c("bbim", "bfla", "beph"), each = nrow(df)/3)
save(df, file = "data/df.RData")
```

Plotting

```
load("data/df.RData")
ggplot2::ggplot(data = df, aes(x = noise, y = error, group = id, col = id)) + geom_smooth(level = 0.95)
    theme_bw(base_size = 12) + theme(aspect.ratio = 1) + xlab("Additional noise level") +
    ylab("Error rate") + scale_x_continuous(breaks = seq(0, 1, 0.1)) + scale_y_continuous(breaks = seq(0
    0.3, 0.02)) + scale_colour_manual(values = c("#1B9E77", "#D95F02", "#7570B3"),
    name = "Datasets", breaks = c("bbim", "beph", "bfla"), labels = c("Bombus bimaculatus",
        "B. ephippiatus", "B. flavifrons"), guide = guide_legend(label.theme = element_text(face = "ital
        angle = 0, size = 11)))
> `geom_smooth()` using method = 'loess'
```



The plot reveals the potential of high noise levels to increase the error rate. Errors typically resemble the fact that a few retention times associated to a certain substance were not combined with the majortiy of retention times of the same substance because the initially formed groups have been too far a part (i.e. distance greater than 'min_diff_peak2peak). An inspection of heatmaps and aligned data tables will help in these situations to identify such cases.

References

Dellicour, Simon, and Thomas Lecocq. 2013. "GCALIGNER 1.0: An Alignment Program to Compute a Multiple Sample Comparison Data Matrix from Large Eco-Chemical Datasets Obtained by Gc." *Journal of Separation Science* 36 (19): 3206–9. doi:10.1002/jssc.201300388.