Condensed paragraph version

Background subtracted fluorescence values from the average of three line scans per each wing disc in each condition were transformed into R data frames using standard R packages [2-5]. Data frames consist of a column representing relative distance, in microns, from the start of the line scan that the fluorescence value was obtained at, this will be used as x. Then there is one column per wing disc per condition containing fluorescence values that will by used as y in all future calculation. The auc() function from the MESS [1] package was used to calculate auc values as auc(x, y, from = 0, type = ”linear”) using linear interpolation. We further optimized our usage of the auc() function by adjusting the subdivisions argument in positive increments of 1000 to auc(x, y, from = 0, type = ”linear”, subdivisions = 1000). We performed absolute and non-absolute calculations for each dataset by setting the auc() function argument “absolutearea” to TRUE and FALSE respectively. To separate the auc value of peak 1 from the auc value of peak 2, cut off values were manually determined and added into the auc() function. To get peak 1 auc values, the auc() function “from” argument was set to 0 and the “to” argument was assigned as the peak 1, auc(x, y, from = peak 1 cut off, type = ”linear”, subdivisions = 1000). To get peak 2 auc values, the “from” argument was assigned as the peak 1 cut off auc(x, y, from = peak 1 cut off, type = ”linear”, subdivisions = 1000), All auc values were combined into an array and exported as .csv files that were transferred to GraphPad PRISM for visualization and statistical analysis. All code, output, and input data are found in a Git repository at <https://github.com/jrb07/auc_bates.git>

Citations

1. Claus Thorn Ekstrøm (2022). MESS: Miscellaneous Esoteric Statistical Scripts. R package

version 0.5.9. <https://CRAN.R-project.org/package=MESS>

2. Hadley Wickham, Jim Hester and Jennifer Bryan (2022). readr: Read Rectangular Text Data.

R package version 2.1.2. <https://CRAN.R-project.org/package=readr>

3. H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

4. Hadley Wickham and Maximilian Girlich (2022). tidyr: Tidy Messy Data. R package version

1.2.0. <https://CRAN.R-project.org/package=tidyr>

5. Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2022). dplyr: A Grammar

of Data Manipulation. R package version 1.0.10. <https://CRAN.R-project.org/package=dplyr>

Expanded list version (supplemental?)

1. Install the required packages: {MESS} [**1**], {readr} [**2**], {ggplot2} [**3**], {tidyr} [**4**], and {dplyr} [**5**]. The user may either use the Packages tab or the install.packages function. The packages will remain installed in the environment using R. We use the if require template for convenience as this only installs the packages if the environment does not have them installed.
2. Load the installed packages by using the library(“package-name”) function.

Text

Description automatically generated

1. Format data for compilation. The format in our case is a column of distance in microns values followed by columns of fluorescence values with headers that will be used as identifiers.

Table, Excel

Description automatically generated

1. Convert the raw fluorescence values into R data frames.
   1. Load the main .csv file which contains all the fluorescence values in columns with a header that labels the columns with unique identifiers, these will be the row 1 values.
   2. Use the grepl function to create dataframes of each condition by using the identifiers.

Text, chat or text message

Description automatically generated

1. Use the MESS AUC function to calculate the AUC with a spline.

The MESS AUC function uses the splinefun function and an integration function to calculate an integral for the area under the curve of a spline that fits the curve that is a function of x and y.

* 1. The x in this function are the distances in microns along the line scan.
  2. The y in this function are the fluorescence values at those distances. In this case we use the brackets to grab a specific column and the the drop = TRUE argument to make our wide, or multiple columns, dataframe into a long, or multiple rows, dataframe.



* 1. Use the type = “spline” argument.
  2. Choose an appropriate number of subdivisions.
  3. Use absolutearea = TRUE if dealing with negative Y values.
  4. Use from = minimum and to = maximum to only calculate AUC values from a minimum x value to a maximum x value.



1. Create a ggplot2 multiplot that is ordered by decreasing AUC to visualize the resulting AUC data.

Text

Description automatically generated

The x = reorder(Identified, -AUC\_Values) is the argument that orders by decreasing AUC value.

1. Save the dotplot using the ggsave function.



1. Save the dataframe used to create the dotplot using the write.csv function.



An optional debug Boolean can be declared as TRUE to render and save x and y plots for each wing disc used by the auc functions.