RR Quant

Procedure overview and quick guide

Prerequisites

Software and plugins

- Fiji (https://fiji.sc/)
    - MorphoLibJ (“IJBP-Plugins” update site)
- RStudio (https://posit.co/download/rstudio-desktop/)
    - Libraries:
        - data.table
        - readr
        - stringr
        - tidyverse
        - shiny
        - plotly
        - bslib
        - sortable

Workflow scripts

- RRQuant_data-table.R
- RRQuant_app.R

Data

Installation

Fiji

RStudio

Find the installer fitting your system here: https://posit.co/download/rstudio-desktop/

To install the different packages needed to run the different scripts, you can run the following in the console of RStudio:

```
# Define the packages you want to install
packages <- c("data.table", "readr", "stringr", "tidyverse", "shiny", "plotly",
"bslib", "sortable")
# Find out which packages are already installed
installed_packages <- rownames(installed.packages())
# Determine which packages are not installed
packages_to_install <- setdiff(packages, installed_packages)
# Install the packages that are not yet installed
if (length(packages_to_install) > 0) {
  install.packages(packages_to_install)
} else {
  cat("All packages are already installed.\n")
}
```

The code is also available in GitHub “Install_R_packages”. The code checks if the required packages are installed and add the ones that are not. For more information on how to install packages, see https://cran.r-project.org/doc/manuals/r-release/R-admin.html#Default-packages .

The R scripts are available on GitHub to download. Once RStudio and the scripts are downloaded, double-click on the script to open it with RStudio. It is then ready to use.

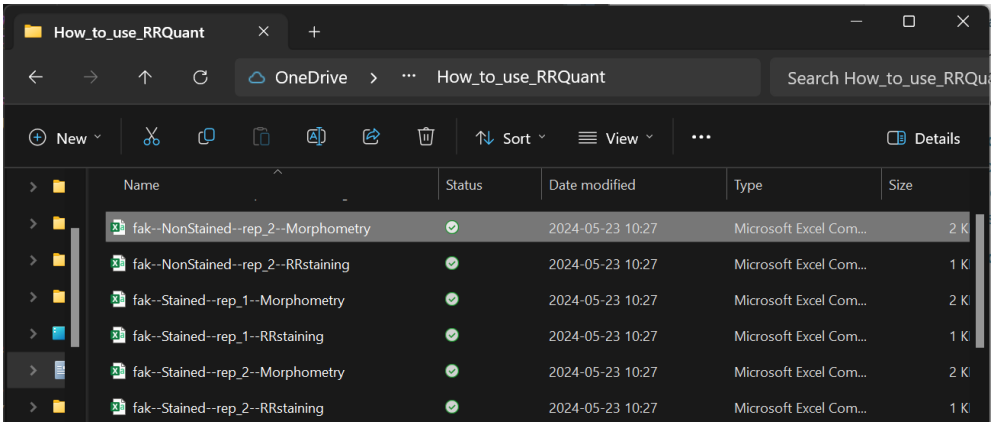
RR Quant Workflow

R script and shiny app

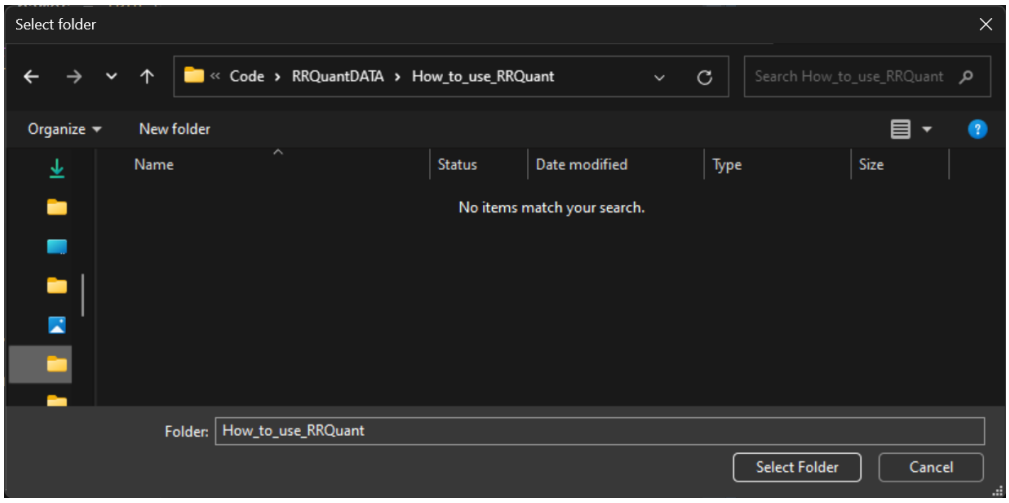
Make the data tables

Run the script RRQuant_data-table.R in RStudio. A pop-up window will open to choose the working folder. The working folder should be the one containing all the csv files issued from the macro.

Working folder:



Pop-up window:



Note 1: The working folder can also contain images or other file types, just make sure to not have csv files that should not be processed. The script will use ALL csv files to make the table and errors can occur if the csv does not correspond to what is expected (csv issued from the macro).

Note 2: when choosing the directory, several problems can happen:

- The popup window opens in the background: check your taskbar.
- The popup window does not open at all. Try to write choose.dir() in the console, it should return NA. In this case, write choose.file() in the console, when the window opens for choose.file(), do not select anything (click cancel). The function choose.dir() should now work as expected.

The script reorganizes the data to have one row = one sample and sort the data in any way we want to plot them later (per genotype, per replicate…). More information about the R script can be found directly in the code, as comments.

2 tables are saved as CSV files directly in the working folder:

- RRQuant-analysis_*Date*_*Time*: contains all data, with stained and non-stained samples.
- RRQuant-analysis-Stained_*Date*_*Time*: contains only data from stained samples. Filtering is applied based on the pixel count to make sure that no small dots from the segmentation: every object below 200 pixels is removed. This file is used to make plots in the next step.

Note 3: you can re-run the script several times in the same folder, it will not take in the "RRQuant-analysis files".

Explanation about the columns in the data table:

- **RRmean_absolute** is the RR mean measured by the macro.
- **RRmean_NS_genotype** is the mean of all non-stained samples RR mean per genotype (e.g.: mean of RR for all non-stained Col-0)
- **RRmean_NS_rep** is the mean of all non-stained samples RR mean per genotype and replicate (e.g.: mean of RR for all non-stained Col-0 for replicate 1)
- **RRmean_relative_NS_genotype** is the value of RRmean for each sample relative to the mean of the RR intensity for all the corresponding genotype non-stained samples.
- **RRmean_relative_NS_rep** is the value of RRmean for each sample relative to the mean of the RR intensity for all the corresponding genotype and replicate non-stained samples.

Example:

For the sample col-0-Stained-rep_2-2:

RRmean_NS_genotype	RRmean_NS_rep	RRmean_relative_NS_genotype	RRmean_relative_NS_rep
Mean of all non-stained col-0	Mean of all non-stained col-0 rep2	RRmean_absolute col-0-Stained-rep_2-2 / RRmean_NS_genotype col-0	RRmean_absolute col-0-Stained-rep_2-2 / RRmean_NS_rep col-0-rep_2

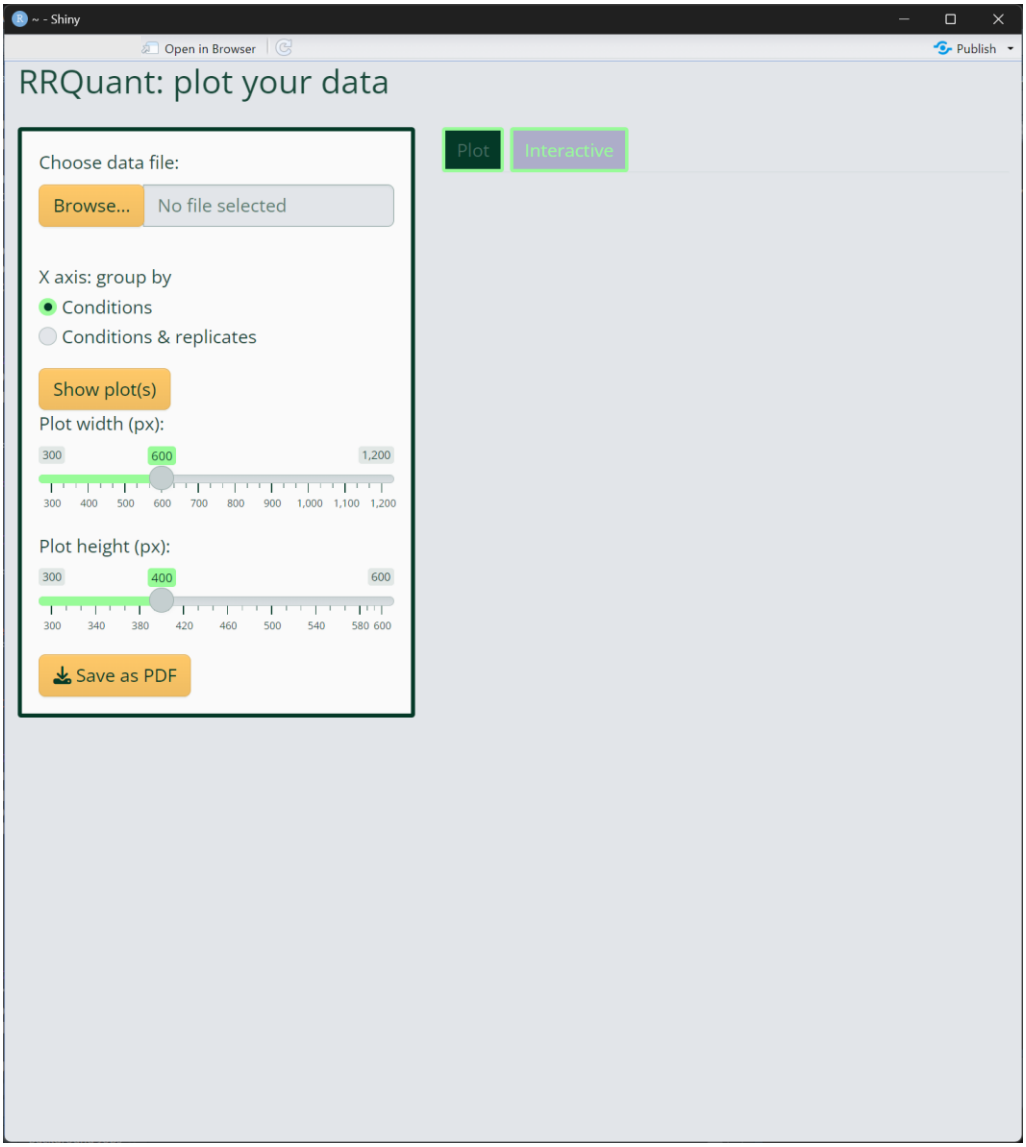
- **Length** corresponds to Geodesic Diameter:

  For particles with complex shapes, the geodesic diameter may be of interest. It corresponds of the largest geodesic distance between two points within a region, the geodesic distance being the length of the shortest path joining the two points while staying inside the region (Lantuéjoul & Beucher, 1981). (See MorpholibJ documentation)

Plot the data: RRQuant-app

Run the code for RRQuant_app.R.

RRQuant application:

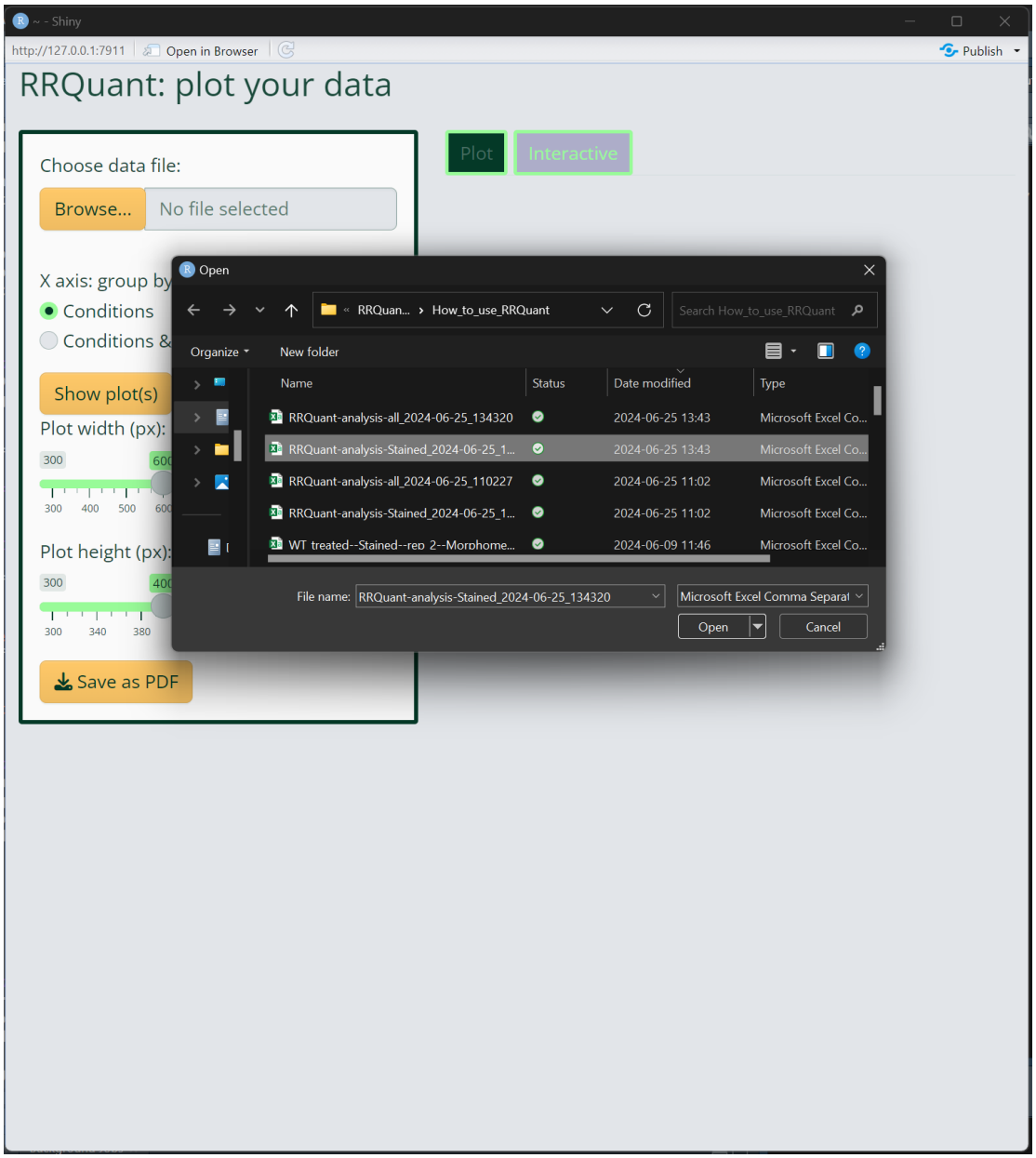


In the center, there are 2 tabs:

- Plot: basic plots to be downloaded, with size adjustable.
- Interactive: link the points in the graphs to the actual sample.

To select data to plot, choose a RRQuant-analysis-Stained csv file by clicking on the Browse button. It will open a window where you can choose the file of interest.

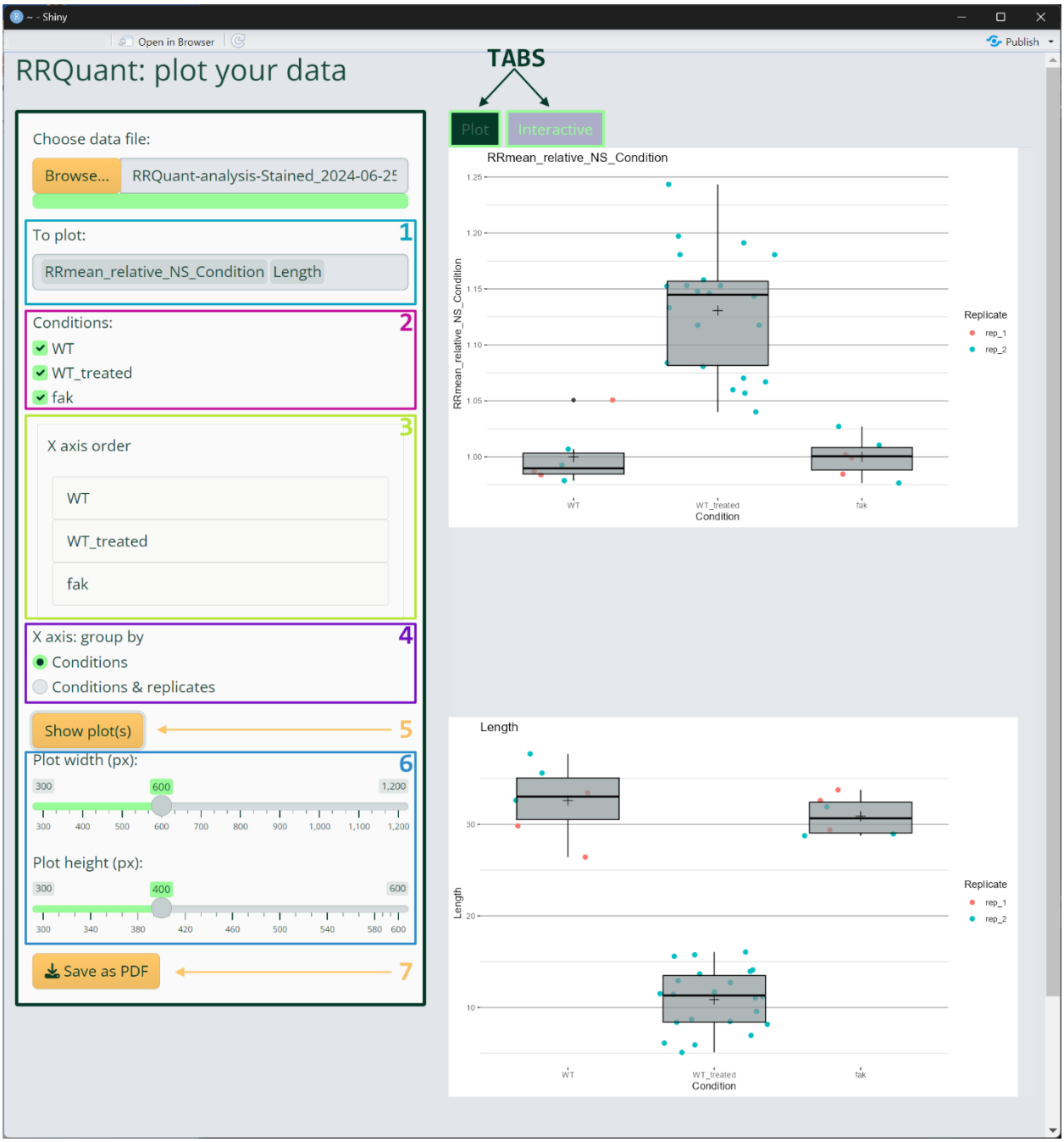
How to choose the file:



Once the file is uploaded, other functions of the app appear:

1- **To plot**: choose what you want to plot, several choices are available as Y axis. Click on the grey bar and the choices will appear. You may choose several at the same time.
2- **Conditions**: choose the genotypes or conditions that you want to plot by ticking/unticking the squares.
3- **X axis order**: choose the order of the conditions to display if you do not want alphabetical order by dragging and dropping the names of the conditions in the list.
4- **X axis: group by**: the data can be plotted by condition (with all replicates for a condition) or separate the different replicates done for one condition to compare them.
5- **Show plot(s) button**: once all the settings have been chosen, click on this button to make the plots. This allows the app to update only when the user is ready and happy with their choices instead of updating live which can take some time if there is a lot of data.
6- **Plot width** and **Plot height**: chose dimensions of the plots
7- **Save as PDF button**: click to save all the plots displayed in the app as a PDF file. By default, the name is RRplot-*Date*_*Time*. The PDF files are then editable with vectorial software (such as Inkscape).

App description with Plot tab activated:



To easily trace back the image corresponding to a data point, the second tab called “Interactive” shows additional information directly on the graph. When passing the mouse on top of the dots of the graphs, information appears: the sample name and the value corresponding to the Y axis.

App with Interactive tab activated: