Travis Pies

# Overview

This readme should be followed in order during installation and will instruct users how to

* Install software required to run TraVis Pies
* Open the script of TraVis Pies in the installed software so TraVis Pies can be run
* Install required packages (software extensions) to run TraVis Pies on first use
* Run the script of TraVis Pies in the software to get the TraVis Pies interface
* Interact with the TraVis Pies interface to get the desired pie charts

# Installation guide

The install time using the guide below is estimated to be 30 minutes.

To run the provided code, the user needs to install the R base package and additional packages. The installation of package and running of the code is easier when using an integrated development environment (IDE). We recommend Rstudio, version 1.4.1103 of which was used in the writing of the R code. See <https://www.rstudio.com/products/rstudio/download/>

To install R, go to <https://cran.r-project.org/bin/>. Select your operating system and follow the instructions in that folder.

**For advanced users only**: if you wish to use the web version of this script where generated files have to be downloaded instead of saved locally, download and install Rtools from [Using Rtools4 on Windows (r-project.org)](https://cran.r-project.org/bin/windows/Rtools/rtools40.html). In this case, set the local\_version variable in the script you will load later to FALSE

# Instructions for opening the script

## Overview

If the software specified in section 1 of this document is installed, one still needs to save TraVis pies scripts, and open them in a suitable R studio project to run them. Finally, one needs to run the application.

## Saving the script files

Normally, the script is available from the attachment or as a GitHub download as shown below. Thes files can be saved (in the same directory) anywhere. Do not rename the folder Functions and modules nor any file inside it, or the script might not work anymore. The .Rproj file can be created again if not available or lost, see below.

Graphical user interface, text, application, chat or text message

Description automatically generated

Figure : folder with script, rpoj file and folder containing functions and modules

## Open script in R studio project

### Open already provided .Rproj file

In order to use the script, it has to be opened in an appropriate R studio project. If the R studio project (the .Rproj file) in the image above was available from the download source, you can simply open that one with Rstudio. If this file is not available or not working for some reason, follow the steps below to create a new one. If you managed to open the .Rproj file, skip to “Open the script”.

### Create new rstudio project (new .Rproj file)

In case no functioning .Rproj file is available, create a new one before trying to run the code by following these steps:

1. Open Rstudio
2. Click on File>New Project...
3. If you are prompted to save workspace data, you don’t have to but it does not matter.
4. Select “Existing directory” in the pop up
5. Click the browse button
6. Go into the folder where you saved the script (you should the Functions and modules folder like the figure below). Do not open the Functions and modules folder.
7. Click Open
8. Click Create Project
9. Now you can follow the steps in sections 2.3.1 and 2.3.2

Graphical user interface, text, application

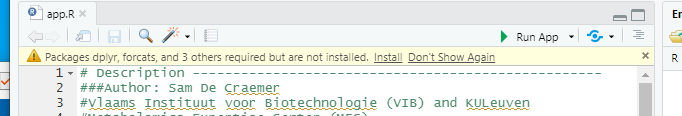
Description automatically generated

### Open the script

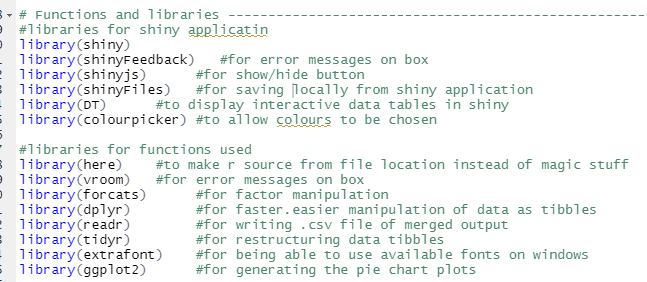
Once you have an r project loaded in Rstudio, open the script by clicking File>Open File. This should open a window showing the folder where you saved the .Rproj file, the TraVis Pies r script and the folder Functions and modules. If not, see below to create a new, correctly configured .Rproj file. Click the Travis pies.R (TraVis Pies v1.0.R in example above) file to load it.

# Installing required packages

One the script is opened for the first time on a computer, you will see a message like the one below. Click install to install these packages.



If the notice does not pop up, use the package installer under Tools>Install packages… and enter all the names that are between the () brackets of the library(XXX) commands near the top of the script. Note that the screenshot below might be outdated, best check your script itself.



# Using the TraVis pies interface

## Open and the script after opening

After opening the script, click the run app button (see below) to start the user interfaced application. Do not change the code in the script to avoid breaking the tool.



## Select input method and files

The user can choose to either create a new standardized TraVis pies input file from the 3 input files described in the previous section, or upload a previously created standardized TraVis pies input file to move on faster.

Creating a new file will allow the user to upload the three input files and specify which columns contain sample names, cohorts and normalization, and which compounds should be included in the analysis. The standardized TraVis pies input file created this way can be saved for uploading next time the app is used for this data, and will be used as input for this session.

Uploading a previously created file will perform some checks to make sure the format if the input is correct to avoid errors down the line, if this file was not manually modified no problems should be encountered. This input will then be used in the visualization module.

## Input cleaner module

Upload the input .csv files with the data of the experiment. See the example data provided with the article or with TraVis Pies.

* Metadata: Contains at least a column with sample names, and optionally a cohort column and a normalization column.
* Abundance data: Contains the column with sample names that should have the same name as the copy in the metadata. All other columns are abundances of the compounds measured.
* Fractional contribution data: Contains the column with sample names that should have the same name as the copy in the metadata. All other columns are the fractional contributions of the compounds measured, although compounds that are 100% unlabeled can be left out here (automatically happens in some software) and will be processed correctly. The fractional contributions can be formatted as fractions or as percentages.

Depending on whether the input data is consistent between the files, the user will get appropriate errors or warnings, or will be able to further finetune the input by selecting the sample column, and a cohort and normalisation column if present.

The user will then be able to select the compounds that should be taken along, by default all compounds in the input.

If all settings are as desired, click the merge inputdata button to create a standardized TraVis pies input table, which can optionally be saved as a .csv file for quicker input next time it is used. The user can then select a folder and name for the .csv file using the Save to any directory button, or (quicker) copy-pasting a folder path to output the .csv to the textbox, then click the “Save to specified directory” button. Click the “Continue with this data” button to proceed.

## Visualisation

This module shows the pie chart visualization for a compound of choice from the last dataset uploaded or created in the input module, combined with the current visualization settings. It allows users to adapt these settings, see the resulting pie chart plot for a compound of choice before the more time-consuming step of generating and downloading similar pie chart plots for all desired compounds. Below is a rundown of the settings that can be changed.

Once the settings are as desired, click the “Save plots with these settings” button in order to proceed to the output module where images can be generated for all desired compounds using these settings.

### Compound, cohort variable, cohort order and normalization

* Compound: select compound from the dataset should be shown as an example in the visualization app
* Cohort variable: select variable that should be used to group the samples in different cohorts
* Cohorts: Select the cohorts to be shown and their order. The first cohort will be used as a reference for statistical tests
* Use normalized abundances: if available and checked, uses normalized abundances instead of raw abundances for the pie radius and statistical tests.

### Chart layout options

* Maximum charts per row: The number of columns in the facet plot, equaling the maximal amount of pie charts that will be shown on one horizontal line in each figure. If the amount of cohorts exceeds this value, the next line will be used until all cohorts are plotted.
* Position FC label: Position of the label displaying the fractional contribution. If “center”, the FC is shown in the center of the graph. If “slice”, both the contribution of the labelled and unlabeled fractions are shown in their specific slices.
* Slice label distance from center: this is the minimal distance from pie center at which a label can be plotted if Position FC label is “slice”. 1 is the maximal radius a pie can have. Normally a label appears at half the radius of the pie, but if this is smaller than minLabDist it will be plotted at minLabDist distance instead.
* FC label decimals: the amount of decimals shown in the fractional contribution labels
* Add ‘%’ to FC label: if checked adds % to the fractional contribution label (eg. 13% instead of just 13)
* show\_P: if checked hows text relating to P values of statistical significant tests on pie charts
* Add legend: if checked include the labelling color legend for slice colors on the detailed figure. This is never plotted on the concise figure for pathways.
* Add compound name as title: if checked include the compound name on the detailed figure. This is never plotted on the concise figure for pathways.
* Pick colour (un)labeled fraction: select a colour to be used for the (un)labeled fraction slice of the pie
* Pick circle line colour: select a colour to be used for the concentric circles
* Set opacity: change the opacity of the pie slices to see the concentric circle lines more or less distinctly
* Circle linetypes: change the line type (none, solid, dotted…) of the concentric circles

### Chart font options

* Select font: specify the font to be used on the result figures. To get the option to use any but the default font in a local version of this application, a separate script must be run to allow R to use the fonts stored on the machine.
* Fontsize: change fontsize of specified text on the figure

## Output module

Accessed once the user clicked the button “Save plots with these settings”, this module has a few settings regarding which figures will be generated and the plot type

* The user can select whether they want to output one or both of the following plot types
* A detailed figure according to settings chosen
* A figure in more concise format without compound name and legend regardless of settings, more fit for overlaying on pathways.
* The user can select whether they want to output as .png or .tiff. They have similar quality but are suited for different purposes
* .png is more suited for screen and web display as the files are smaller
* .TIFF uses a colour scheme that is more compatible with conventional printing, however the files are very big
* The use can make any selection from the compounds in the input to output figures for, by default all

The user can then obtain the images for the desired compounds by selecting or copy-pasting a folder path to output the figures to, then clicking generate figures. A progress bar will pop up to show how many compounds still need to be processed, as this can take some time when 10’s of compounds are supplied