QuaSI – accompanying R import code

Usage instructions

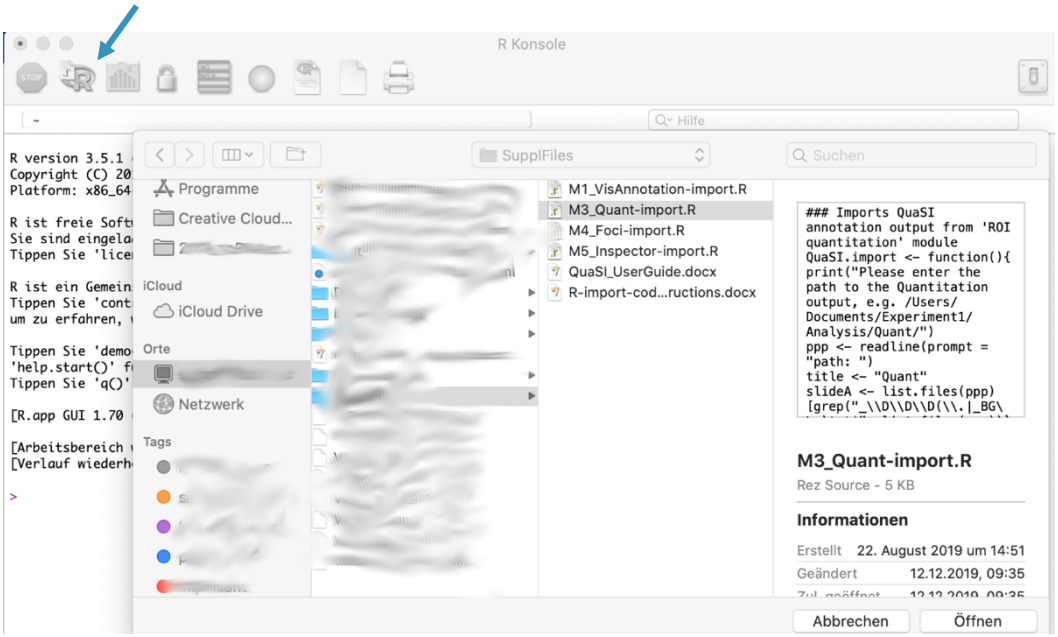
1. Download R und run source files

These instructions are targeted at users with no knowledge of R. No programming skills are needed to use the R import scripts. R can be downloaded free of charge from this website: https://www.r-project.org/.

1.1 For Mac users

Open the R Console.

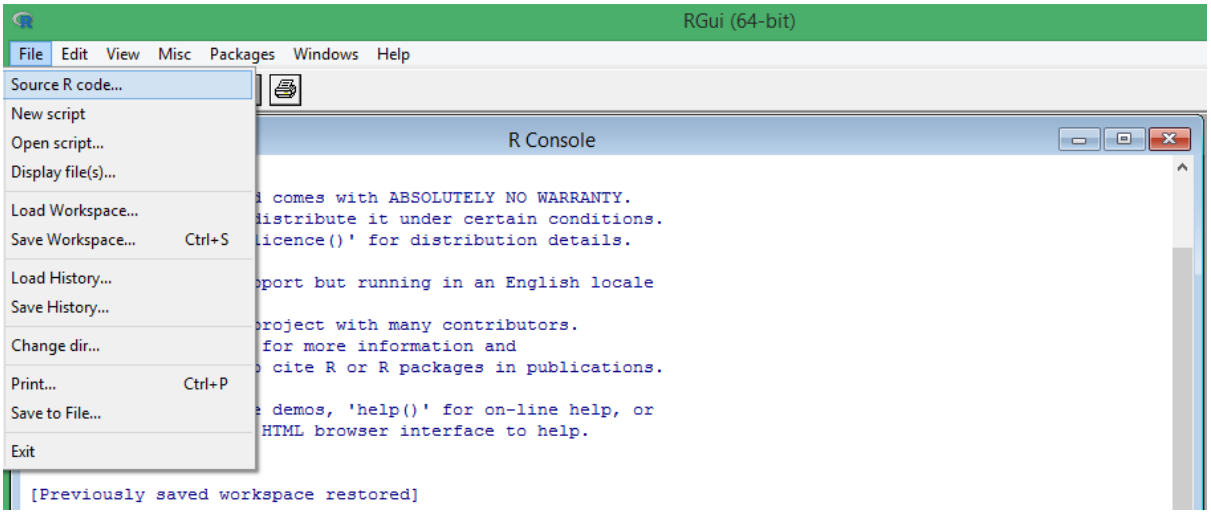
Click on the option to run a source file. Select the import script you want to run and click Open.



The script will guide you through the import process. Type the requested information and hit Enter. For more details, see section 2.

1.2 For Windows users

Open the **RGui**. Select File>Source R code.

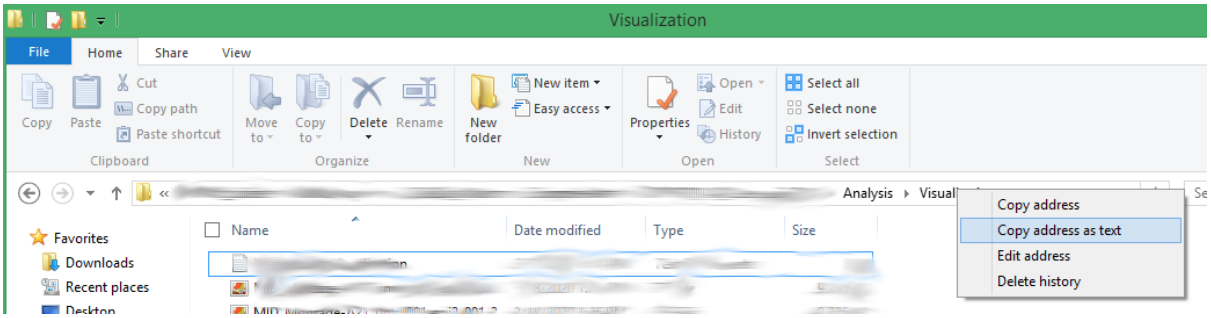


Select the import script you want to run and click Open. The script will guide you through the import process. Type the requested information and hit Enter. For more details, see section 2.

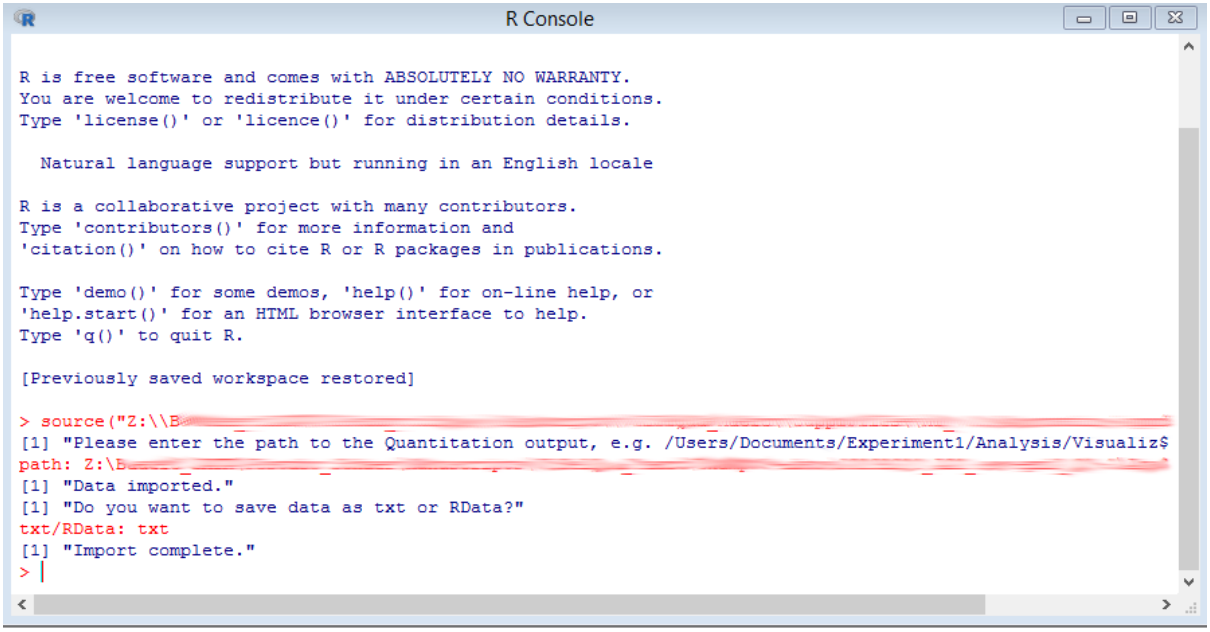
2. Import Script specifics

2.1 General

After starting the script, you are prompted to enter the path to the QuaSI output you want to import. Usually, this would be a subfolder of the "Analysis/" output folder generated by the QuaSI macro. The easiest way to enter the path is by copying from the path bar in the Explorer or Finder, for Windows or Mac users, respectively.



After specifying the path, the text files will be imported and combined into one table. This table can be saved as a txt-file that can be opened for example with Excel, or as an RData-file, if you want to proceed with your analyses in R. After successful import, the R Console looks like this:



The generated data file can be found in the folder where the data were imported from, following a naming convention of "Foci-import.txt", "Quant_import.RData" or similar.

2.2 Quantitation import

Import of the ROI quantitation can be much more time-consuming than for the other output types, dependent on the size of the dataset. Additionally, you can perform a background subtraction (for details, see section 3); you can indicate this in the R Console:

```
[1] "Data imported."
[1] "Do you want to subtract the background?"
y/n: y
[1] "Background subtracted."
[1] "Do you want to save data as txt or RData?"
txt/RData: RData
[1] "Import complete."
>
```

3. Output and further steps

Visualization output

The annotations performed with the Visualization module are combined into one data table with 4 columns, if saved as txt-file (see screenshot).

Annotation	type	user.input	image
Mitoses	Num	3	NANOG_2017-09-25_654503_MF001_Pos001_S001
MitoticPhase	Str	p,m,m	NANOG_2017-09-25_654503_MF001_Pos001_S001
Mitoses	Num	0	NANOG_2017-09-25_654503_MF001_Pos002_S001
MitoticPhase	Str	NA	NANOG_2017-09-25_654503_MF001_Pos002_S001
Mitoses	Num	0	NANOG_2017-09-25_654503_MF001_Pos003_S001
MitoticPhase	Str	NA	NANOG_2017-09-25_654503_MF001_Pos003_S001
Mitoses	Num	0	NANOG_2017-09-25_654503_MF001_Pos004_S001

If you choose to save your data as an RData-file, you will get a list with as many data frames as there are levels for the "Annotation" column. In this example, there will be 2 elements: "Mitoses" and "MitoticPhase". Each list element will contain a data frame with 2 columns: The user input (i.e. the number of mitoses or the assigned mitotic phases) and the name of the corresponding image.

```
> str(annot.list)
List of 2
 $ Mitoses     :'data.frame':   56 obs. of  2 variables:
  ..$ user.input: num [1:56] 3 0 0 0 0 0 0 0 0 0 ...
  ..$ image     : chr [1:56] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_20
17-09-25_654503_MF001_Pos003_S001" "NANOG_2017-09-25_654503_MF001_Pos004_S001" ...
 $ MitoticPhase:'data.frame':   56 obs. of  2 variables:
  ..$ user.input: chr [1:56] "p,m,m" NA NA NA ...
  ..$ image     : chr [1:56] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_20
17-09-25_654503_MF001_Pos003_S001" "NANOG_2017-09-25_654503_MF001_Pos004_S001" ...
```

Quantitation output

The txt-files from the Quant-folder will be combined for all images. In addition to the columns of the ImageJ-Results table, the output will contain several additional columns, indicating channel name, image name, projection type, and ROI number. If the user chooses to subtract the background, mean, median, and standard deviation of the entire image will also be included in the table as measures for the background level (indicated by the ".BG"-suffix). The median image intensity will be subtracted from the ROI intensities, indicated by the ".b"-suffix – this the background-subtracted ROI intensity as mean, median, or integrated density.

channel	image	type	roi	Mean.BG	Median.BG	Stddev.BG	Mean.b	Median.b	IntDen.b
DAPI	A21_untr_001_epi2_001	SEP	1	1373.473	1086	1779.155	13269.508	13921	2077166.64
DAPI	A21_untr_001_epi2_001	SEP	2	1373.473	1086	1779.155	11673.925	11002	1863117.67
Nanog	A21_untr_001_epi2_001	SEP	1	9431.104	9402	658.987	2091.831	2170	327447.00
Nanog	A21_untr_001_epi2_001	SEP	2	9431.104	9402	658.987	1739.054	1721	277542.76
DAPI	A21_untr_001_epi2_002	SEP	1	2292.673	1134	4363.178	16529.072	16269	2182882.99
DAPI	A21_untr_001_epi2_002	SEP	2	2292.673	1134	4363.178	20717.520	19822	12705253.73
Nanog	A21_untr_001_epi2_002	SEP	1	9795.809	9567	1310.563	3166.647	3327	418199.86
Nanog	A21_untr_001_epi2_002	SEP	2	9795.809	9567	1310.563	5408.132	5480	3316601.15

If you opt not to subtract the background, ROI quantitations and background quantitations will be saved as separate txt-files (or data frames if you export as RData-file).

Foci output

The foci import script will aggregate all Maxima-txt-files and add an additional column with the associated image name.

ROI inspection

The ROI inspection import script will combine all files for a particular annotation type (general (also referred to as “manual”), ROI, or foci annotations) into one txt-file. If you choose to save as an RData-file, an object called insp.list is saved which contains up to three entries: ‘manual’, ‘ROIs’, and/or ‘Foci’. Within these list entries, the annotations are split by the user-defined categories. In the example given below, there are four different types of ROI annotations – ‘apoptotic’, ‘cellNo’, ‘damaged’, ‘mitotic’. Each of these is a separate data frame within insp.list$ROIs containing three columns: image name, ROI number, and user input.

```
> str(insp.list)
List of 2
 $ manual:List of 1
  ..$ Note:'data.frame':        56 obs. of  2 variables:
  .. ..$ user.input: chr [1:56] NA NA NA NA ...
  .. ..$ image     : chr [1:56] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001_
Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos003_S001" "NANOG_2017-09-25_654503_MF001_Pos004_S001" ...
 $ ROIs  :List of 4
  ..$ apoptotic:'data.frame':   169 obs. of  3 variables:
  .. ..$ ROI..     : int [1:169] 1 1 2 1 2 3 1 2 1 2 ...
  .. ..$ user.input: num [1:169] 0 0 0 0 0 0 0 0 0 0 ...
  .. ..$ image     : chr [1:169] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001
_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos003_S001" ...
  ..$ cellNo   :'data.frame':   169 obs. of  3 variables:
  .. ..$ ROI..     : int [1:169] 1 1 2 1 2 3 1 2 1 2 ...
  .. ..$ user.input: num [1:169] 15 6 1 1 1 5 1 8 2 1 ...
  .. ..$ image     : chr [1:169] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001
_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos003_S001" ...
  ..$ damaged  :'data.frame':   169 obs. of  3 variables:
  .. ..$ ROI..     : int [1:169] 1 1 2 1 2 3 1 2 1 2 ...
  .. ..$ user.input: num [1:169] 0 0 0 0 0 0 0 0 0 0 ...
  .. ..$ image     : chr [1:169] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001
_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos003_S001" ...
  ..$ mitotic  :'data.frame':   169 obs. of  3 variables:
  .. ..$ ROI..     : int [1:169] 1 1 2 1 2 3 1 2 1 2 ...
  .. ..$ user.input: num [1:169] 2 0 0 0 0 0 0 0 0 0 ...
  .. ..$ image     : chr [1:169] "NANOG_2017-09-25_654503_MF001_Pos001_S001" "NANOG_2017-09-25_654503_MF001
_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos002_S001" "NANOG_2017-09-25_654503_MF001_Pos003_S001" ...
```

For data export as txt-file, annotation subcategories are kept in one file, similar to the Visualization output data.