

# Theory and Practice in gene expression analysis

**Teacher:** Santiago Carmona

**Assistants:** Christian Halter, Shakiba Fadaei

**Dates:**

- 14.11.2023
- 15.11.2023
- 17.11.2023

**NOTE:** the exercises in this part will use R. Please [install R version 4.3.1](#) and [RStudio](#), if you have not installed it already. If you need help with the installation, we can support you.

**NOTE:** For experienced R users, as well as R newcomers, there are some heavily recommended tips & tricks at the end of this word file. Giving a whole tutorial on R and RStudio is out-of-scope for this course. However, for R newcomers we added some links to beginner guides.

## Overall goals

- **Using existing packages for single-cell omics analysis** which make your life easier and speed up analyses
- Creating a **reproducible** bioinformatics experiment
- **Cell type classification and annotation**
- **Combining datasets and correcting for batch effects**

## Table of Contents

<b>Goals.....</b>	<b>1</b>
<b>Introduction .....</b>	<b>2</b>
<b>Links to R + RStudio beginner guides .....</b>	<b>3</b>
<b>RStudio basic tips &amp; tricks .....</b>	<b>3</b>
Turn of .RData.....	3
Auto-save .....	4
<b>Exercise 1 .....</b>	<b>5</b>

## Introduction

Figure 1 shows an overview of the general single-cell RNA-seq (scRNA-seq) data analysis workflow. In this exercise, you will learn about **Seurat**, the most popular R package for scRNA-seq, and how you can use it for:

- Quality control (QC) & pre-processing
- Data exploration & visualization
- Downstream analysis (clustering, differential gene expression, etc.)

If you want to know more about Seurat, then there are great tutorials available on their website for a [basic introduction](#) as well as for more [advanced topics](#). Seurat can also be used for spatial transcriptomics and integrative multimodal analysis, e.g. combining scRNA-seq and scATAC-seq. If you prefer working in Python, there is the *Scanpy* package which reproduces many but not all functions of Seurat.

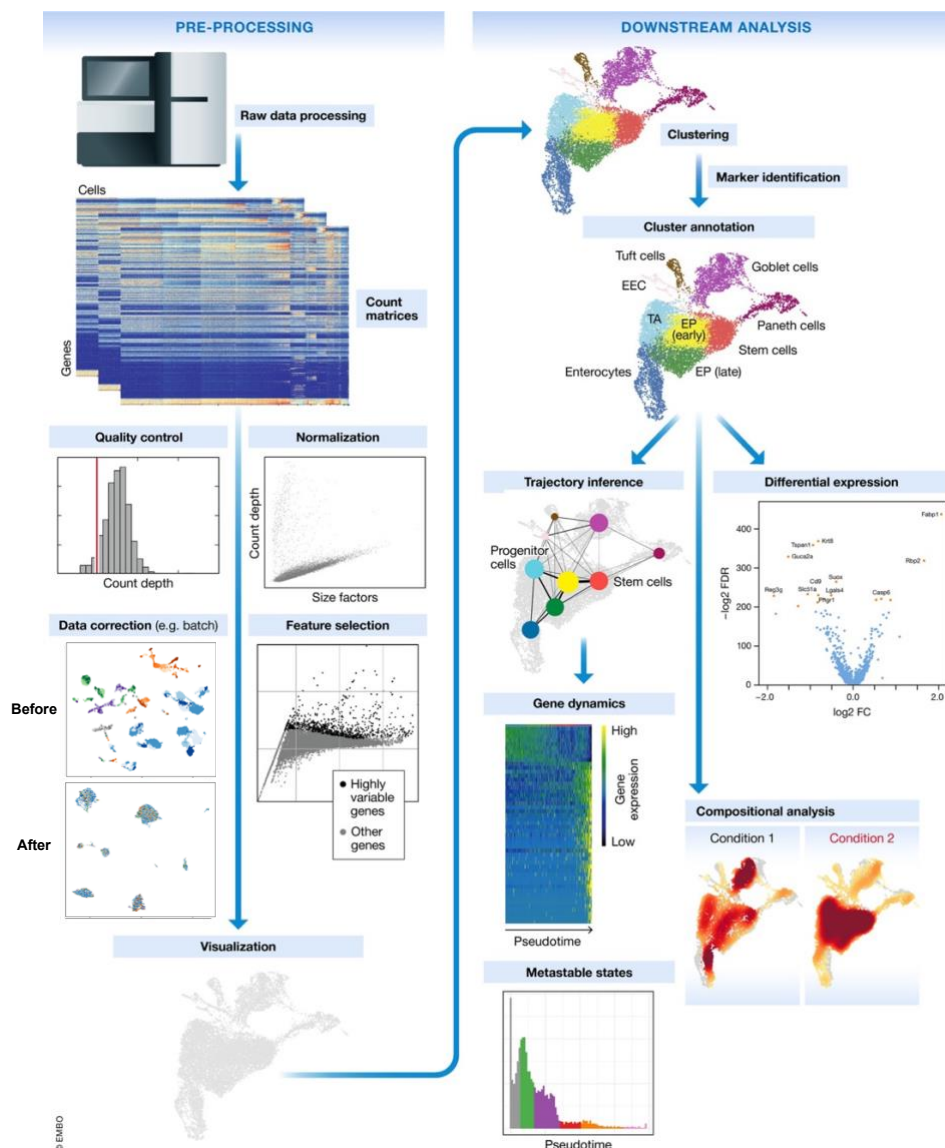


Figure 1: Overview of a standard scRNA-seq processing workflow. Modified from: [Luecken MD, Theis FJ. Current best practices in single-cell RNA-seq analysis: a tutorial. Mol Syst Biol. 2019 Jun 19;15\(6\):e8746.](#)

## Links to R + RStudio beginner guides

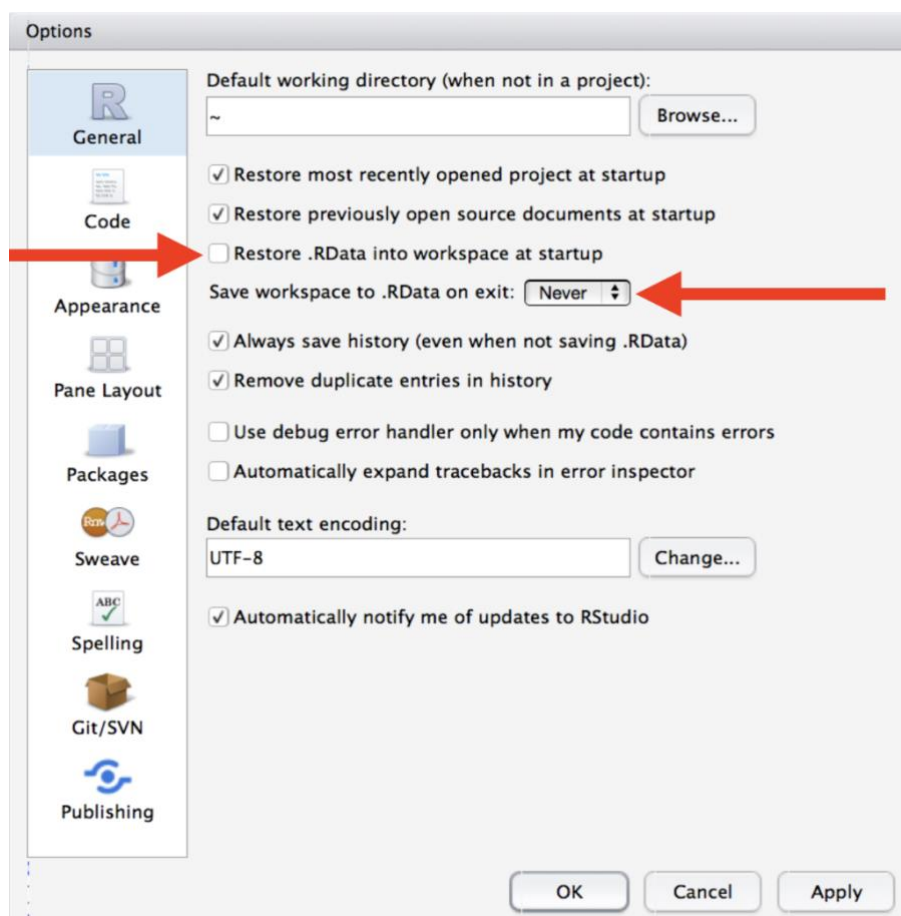
<https://www.datacamp.com/tutorial/r-studio-tutorial>  
<https://moderndive.netlify.app/1-getting-started.html>  
<https://education.rstudio.com/learn/beginner/>  
<https://r4ds.had.co.nz/index.html>

## RStudio basic tips & tricks

### Turn of .RData

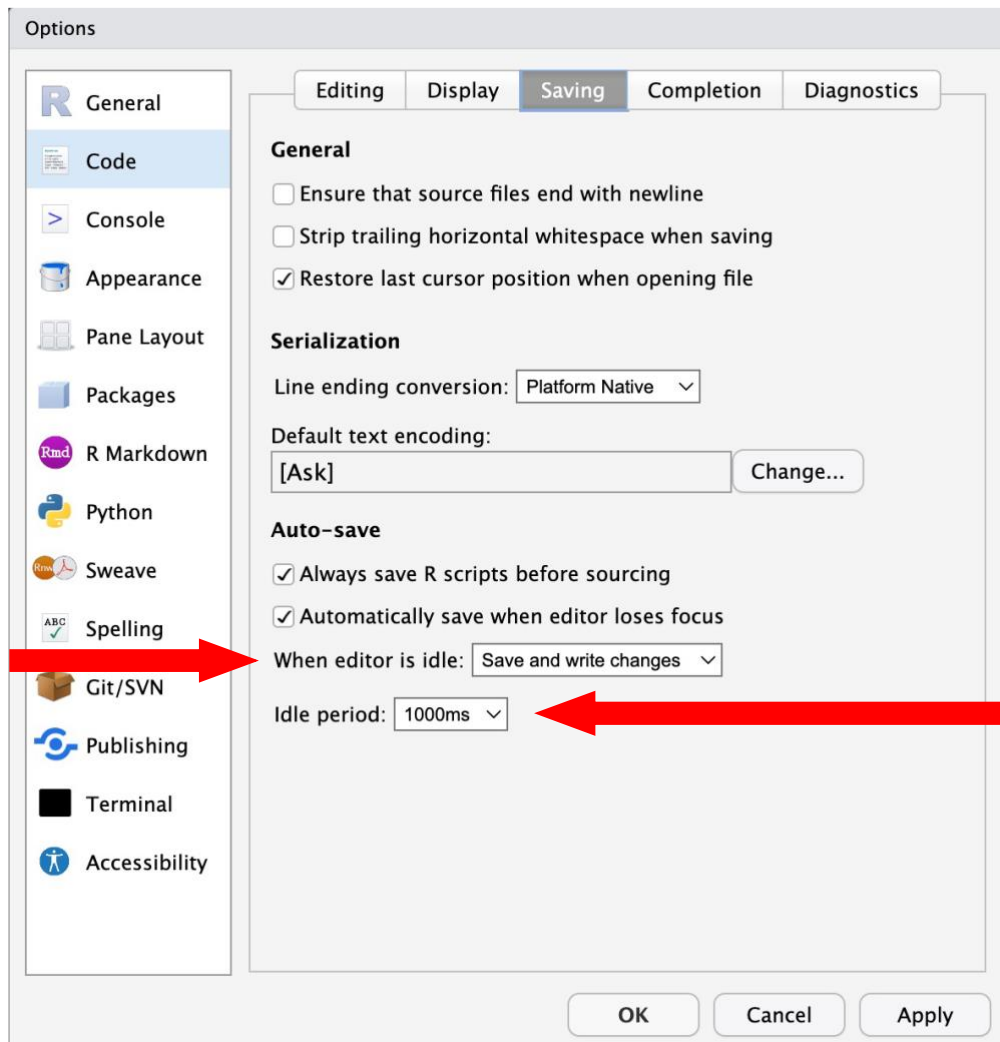
By default, RStudio has an undisable functionality: it creates an .RData file, where it stores the workspace, such as variables you created during your work on an R script or project. When you close and re-open RStudio it will load this workspace from the .RData. However, **it can be very misleading to re-load some variable, rather than re-creating it from your script**, for example if you manually assigned a variable or it was created before you made some change to the source code and did not re-run the code to create the newly updated variable. To turn this behaviour off, go to:

*Tools -> Global options ... ->* and set the settings like in the figure below:



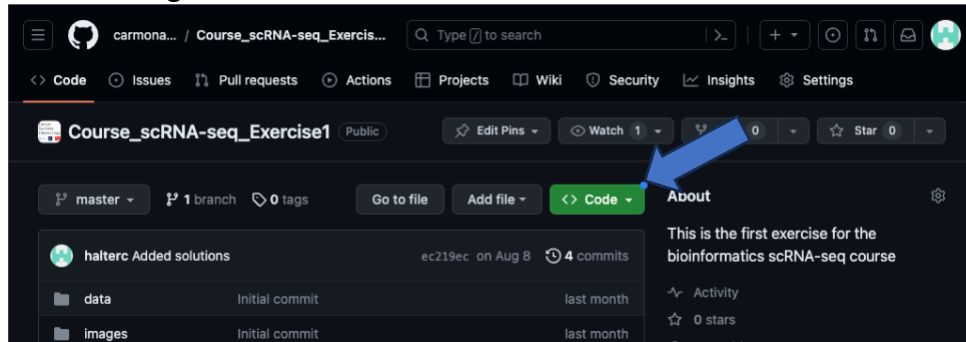
## Auto-save

Another very useful function to prevent data loss in case R hangs itself up (e.g. due to out-of-memory or some other freezing error) is located here:

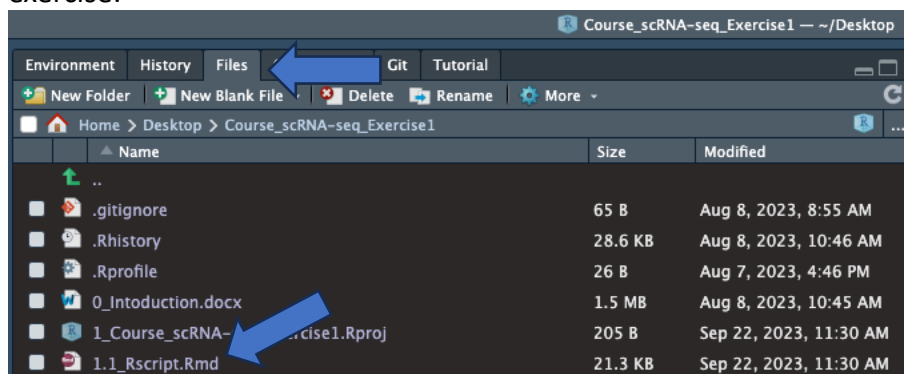


## Exercise 1

- Clone the GitHub repository for exercise 1 to your computer
  - Open this link:  
[https://github.com/carmonalab/Course\\_scRNA-seq\\_Exercise1](https://github.com/carmonalab/Course_scRNA-seq_Exercise1)
  - Click on the green “Code” button:



- Select *Download ZIP*
- Open the downloaded ZIP file containing the exercise repo and extract it anywhere you like
- Open the folder and open the file “1\_Course\_scRNA-seq\_Exercise.Rproj”
- If there is no script file open in RStudio yet, navigate to “Files” and open the R markdown file “1.1\_Exercise1\_Rscript.Rmd” containing the script for this exercise:



- The R markdown file is intended to be viewed in the “Visual” mode within RStudio, so make sure to select it in the top-left corner:

