Australian and New Zealand
SOCIETY FOR IMMUNOLOGY INC.

2021 IgV Masterclass Session #1

Getting started with cytometry data analysis using Spectre and R

Givanna Putri: g.putri@unsw.edu.au

Felix Marsh-Wakefield: felix.marsh-wakefield@sydney.edu.au

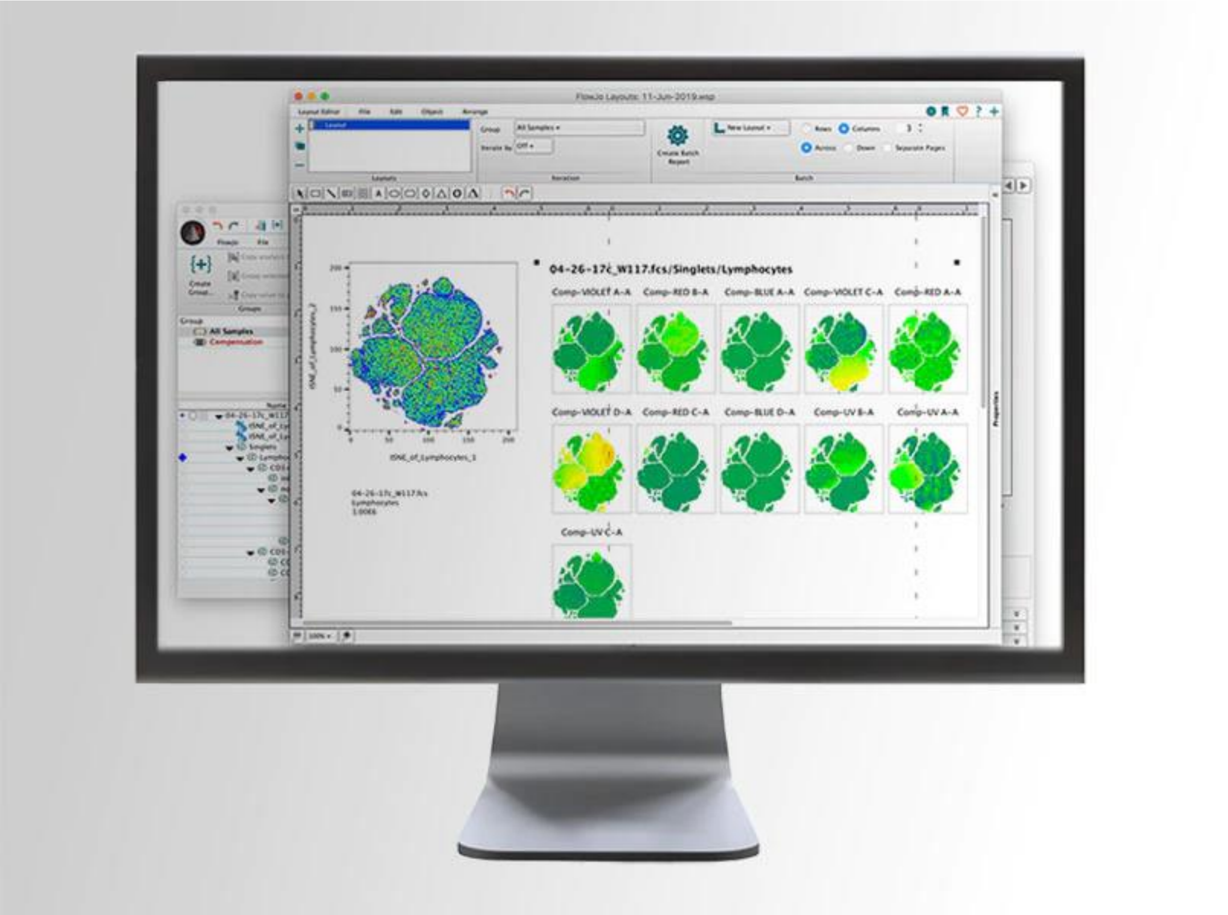
Outline

- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

Outline

- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

Traditional data analysis



https://www.bdbiosciences.com/en-au/products/software/flowjo-v10-software

Analysing data using programming/code

Image by: https://gowithcode.com/top-programming-languages

Analysing data using programming/code

Image by: https://gowithcode.com/top-programming-languages

=

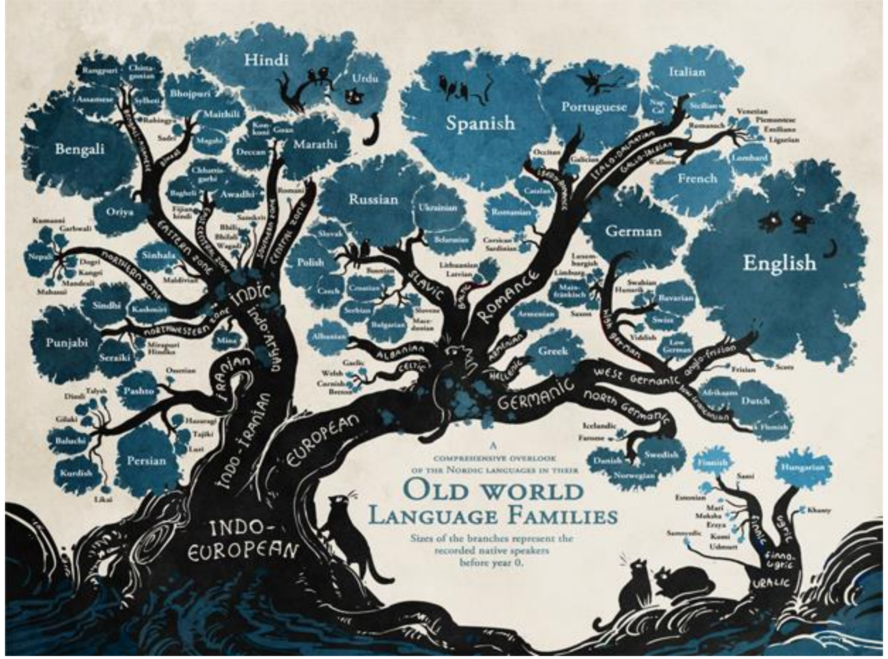


Image by: https://www.linguisticsociety.org/content/how-many-languages-are-there-world

Analysing data using programming/code

Image by: https://gowithcode.com/top-programming-languages

=

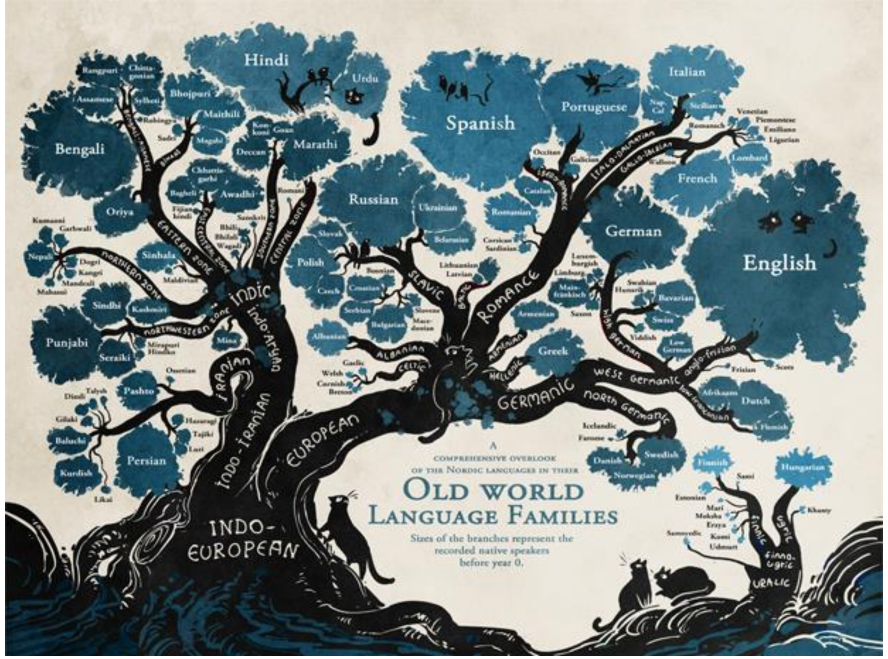

A comprehensive overlook of the Nordic languages in their
Old World Language Families
Sizes of the branches represent the recorded native speakers before year 0.
Indo-European
Indo-Iranian
European
Germanic
Romance
Slavic
Hindi
Spanish
English
Russian
German
Bengali

Image by: https://www.linguisticsociety.org/content/how-many-languages-are-there-world

RStudio for writing and running R code

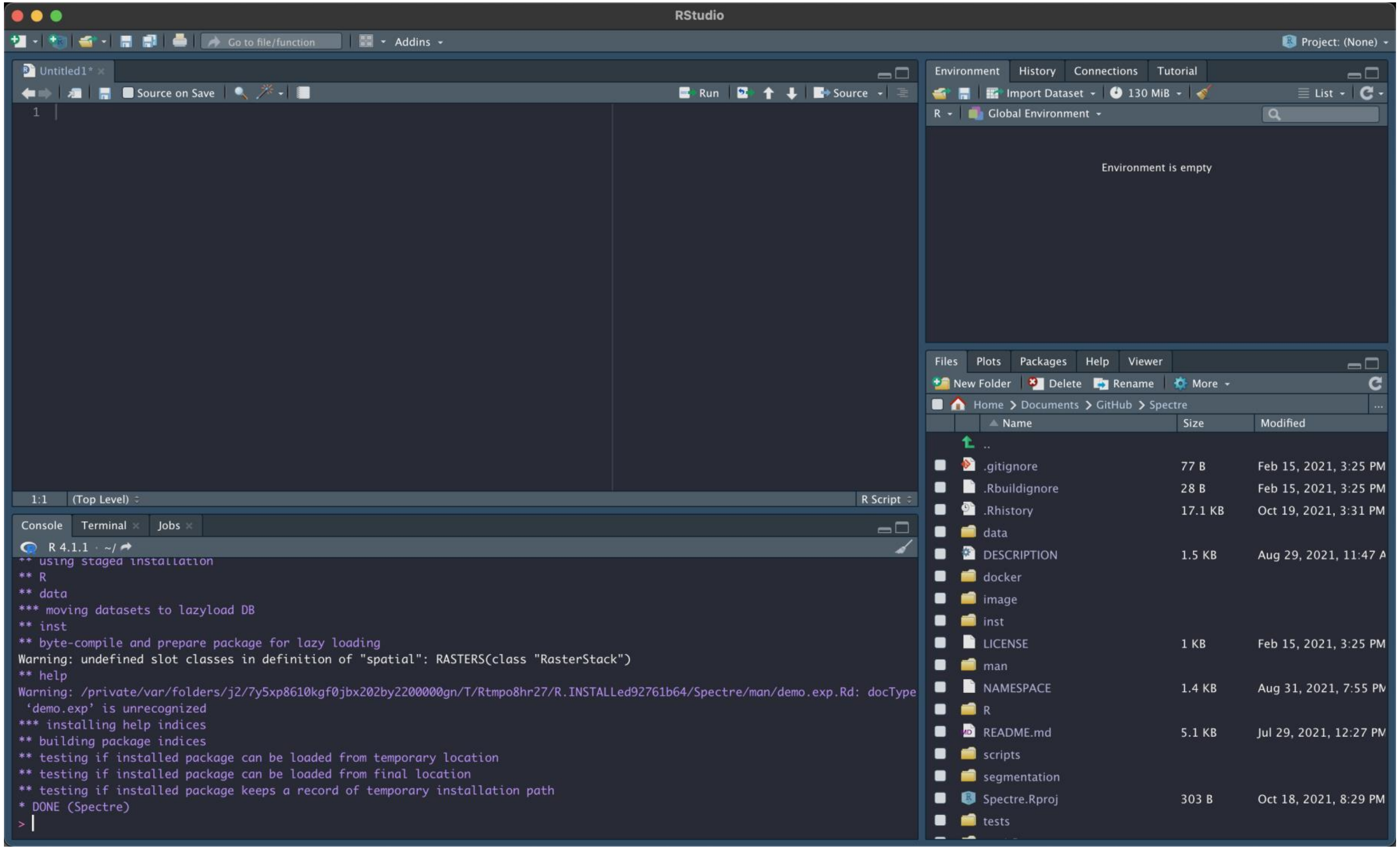
Using text editor and command line

- You can write R code using any text editor software then run it using command line
- Can be tedious and complicated

RStudio for writing and running R code

Using text editor and command line	**Using Rstudio**
• You can write R code using any text editor software then run it using command line • Can be complicated to set up and use	• Or using desktop application like Rstudio • Freely available for both Mac and Windows • Equipped with text editor and functionality to run R code

Rstudio layout



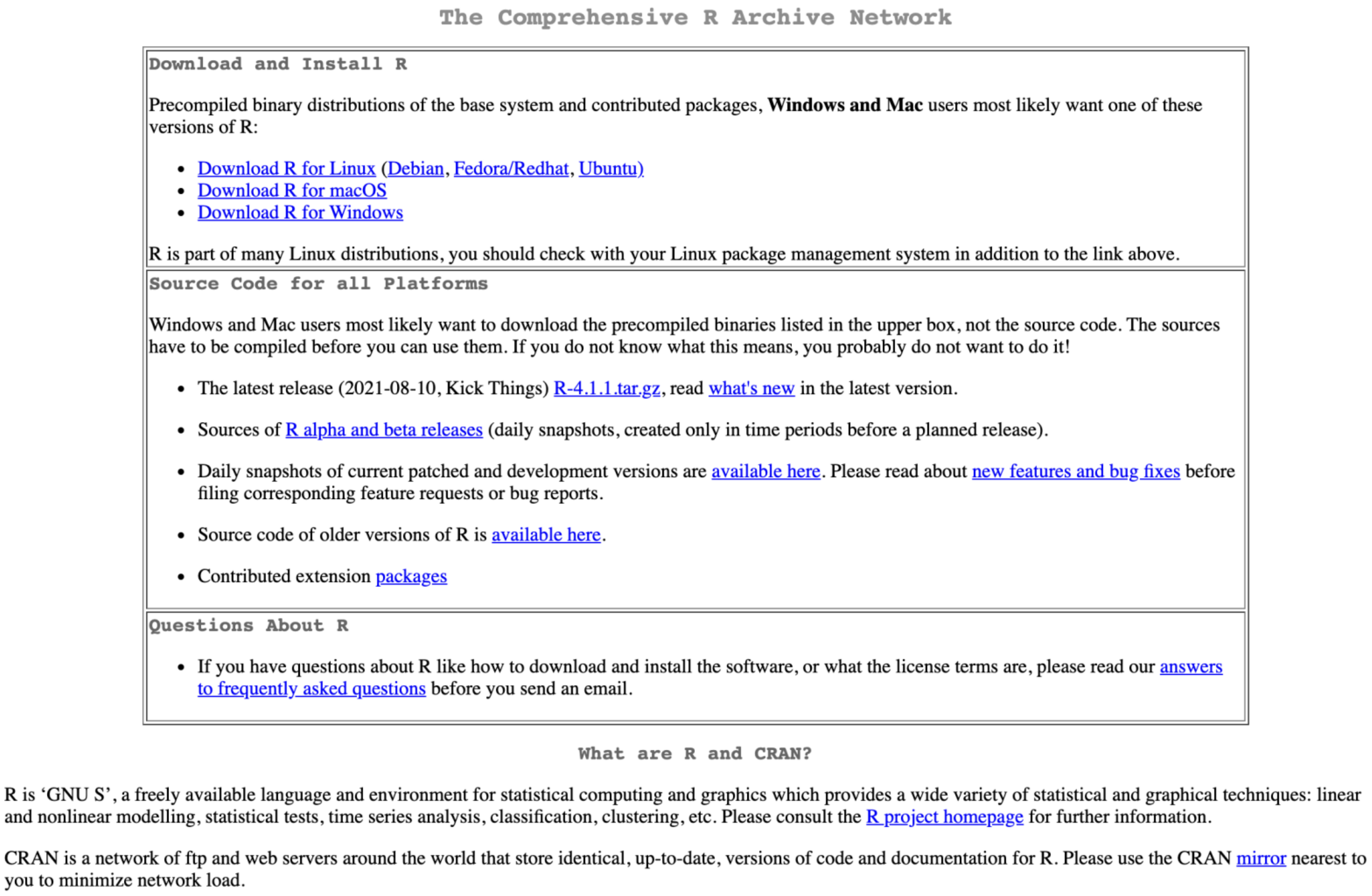
```
** using staged installation
** R
** data
*** moving datasets to lazyload DB
** inst
** byte-compile and prepare package for lazy loading
Warning: undefined slot classes in definition of "spatial": RASTERS(class "RasterStack")
** help
Warning: /private/var/folders/j2/7y5xp8610kgf0jbx202by2200000gn/T/Rtmpo8hr27/R.INSTALLed92761b64/Spectre/man/demo.exp.Rd: docType
 'demo.exp' is unrecognized
*** installing help indices
** building package indices
** testing if installed package can be loaded from temporary location
** testing if installed package can be loaded from final location
** testing if installed package keeps a record of temporary installation path
* DONE (Spectre)
> 
```

Name	Size	Modified
..		
.gitignore	77 B	Feb 15, 2021, 3:25 PM
.Rbuildignore	28 B	Feb 15, 2021, 3:25 PM
.Rhistory	17.1 KB	Oct 19, 2021, 3:31 PM
data		
DESCRIPTION	1.5 KB	Aug 29, 2021, 11:47 A
docker		
image		
inst		
LICENSE	1 KB	Feb 15, 2021, 3:25 PM
man		
NAMESPACE	1.4 KB	Aug 31, 2021, 7:55 PM
R		
README.md	5.1 KB	Jul 29, 2021, 12:27 PM
scripts		
segmentation		
Spectre.Rproj	303 B	Oct 18, 2021, 8:29 PM
tests		

Outline

- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

How to install R



The Comprehensive R Archive Network

Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- Download R for Linux (Debian, Fedora/Redhat, Ubuntu)
- Download R for macOS
- Download R for Windows

R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.

Source Code for all Platforms

Windows and Mac users most likely want to download the precompiled binaries listed in the upper box, not the source code. The sources have to be compiled before you can use them. If you do not know what this means, you probably do not want to do it!

- The latest release (2021-08-10, Kick Things) R-4.1.1.tar.gz, read what's new in the latest version.
- Sources of R alpha and beta releases (daily snapshots, created only in time periods before a planned release).
- Daily snapshots of current patched and development versions are available here. Please read about new features and bug fixes before filing corresponding feature requests or bug reports.
- Source code of older versions of R is available here.
- Contributed extension packages

Questions About R

- If you have questions about R like how to download and install the software, or what the license terms are, please read our answers to frequently asked questions before you send an email.

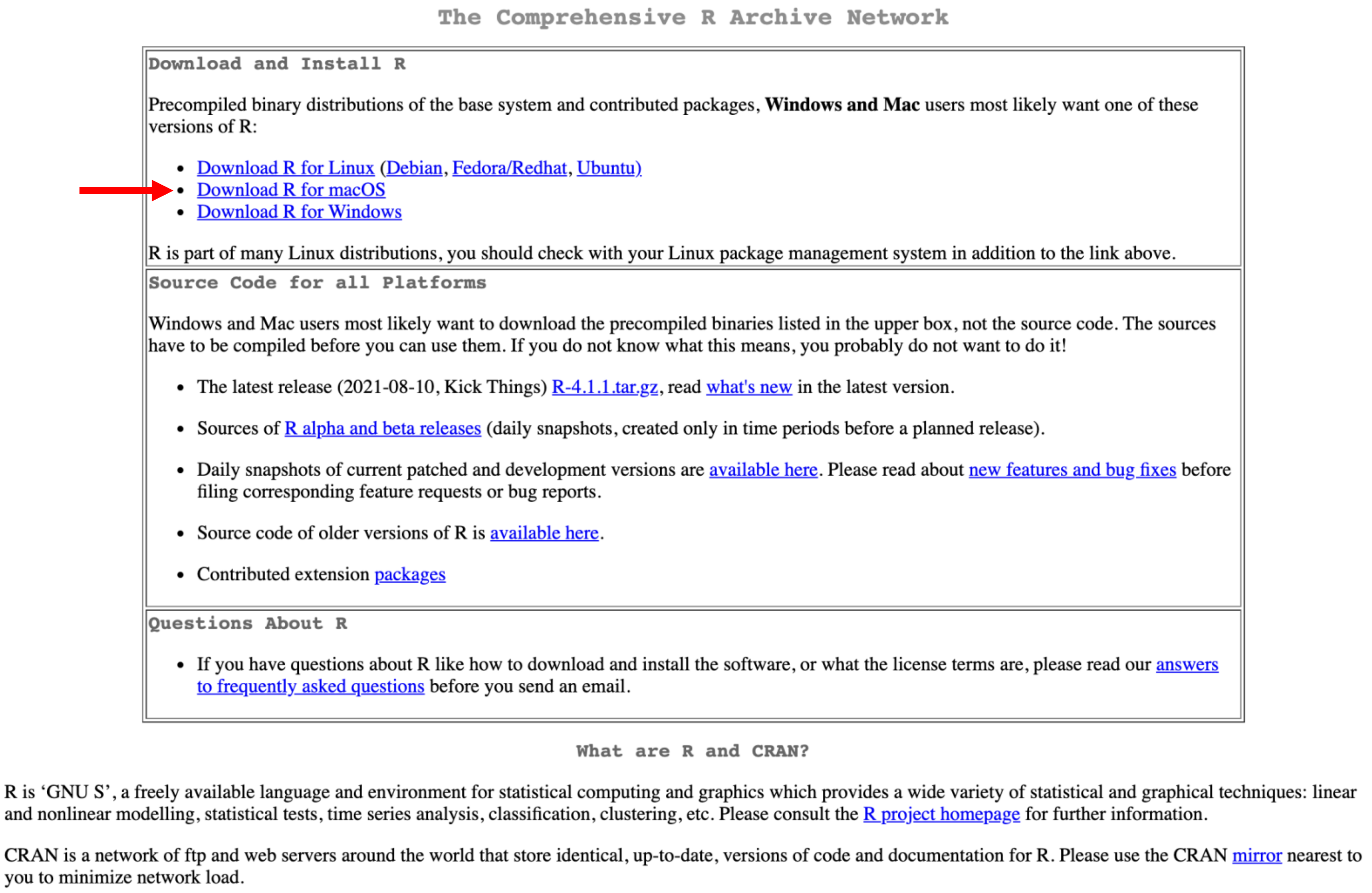
What are R and CRAN?

R is 'GNU S', a freely available language and environment for statistical computing and graphics which provides a wide variety of statistical and graphical techniques: linear and nonlinear modelling, statistical tests, time series analysis, classification, clustering, etc. Please consult the R project homepage for further information.

CRAN is a network of ftp and web servers around the world that store identical, up-to-date, versions of code and documentation for R. Please use the CRAN mirror nearest to you to minimize network load.

https://cran.csiro.au/

How to install R



The Comprehensive R Archive Network

Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- Download R for Linux (Debian, Fedora/Redhat, Ubuntu)
- Download R for macOS
- Download R for Windows

R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.

Source Code for all Platforms

Windows and Mac users most likely want to download the precompiled binaries listed in the upper box, not the source code. The sources have to be compiled before you can use them. If you do not know what this means, you probably do not want to do it!

- The latest release (2021-08-10, Kick Things) R-4.1.1.tar.gz, read what's new in the latest version.
- Sources of R alpha and beta releases (daily snapshots, created only in time periods before a planned release).
- Daily snapshots of current patched and development versions are available here. Please read about new features and bug fixes before filing corresponding feature requests or bug reports.
- Source code of older versions of R is available here.
- Contributed extension packages

Questions About R

- If you have questions about R like how to download and install the software, or what the license terms are, please read our answers to frequently asked questions before you send an email.

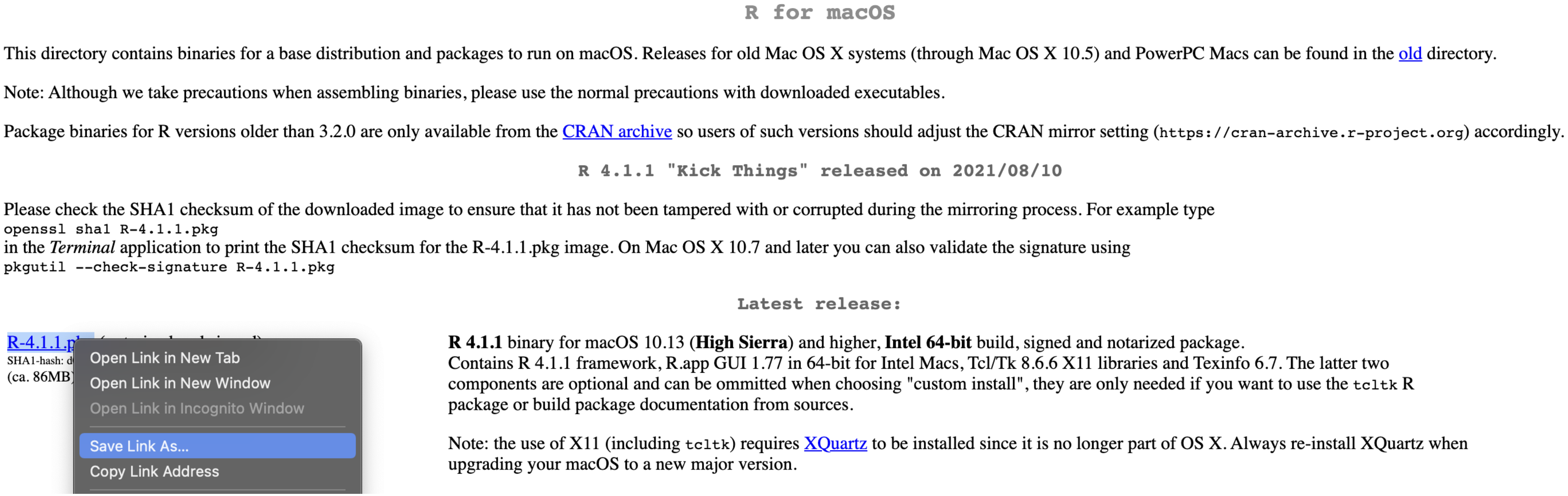
What are R and CRAN?

R is ‘GNU S’, a freely available language and environment for statistical computing and graphics which provides a wide variety of statistical and graphical techniques: linear and nonlinear modelling, statistical tests, time series analysis, classification, clustering, etc. Please consult the R project homepage for further information.

CRAN is a network of ftp and web servers around the world that store identical, up-to-date, versions of code and documentation for R. Please use the CRAN mirror nearest to you to minimize network load.

https://cran.csiro.au/

How to install R (Mac)



R for macOS

This directory contains binaries for a base distribution and packages to run on macOS. Releases for old Mac OS X systems (through Mac OS X 10.5) and PowerPC Macs can be found in the old directory.

Note: Although we take precautions when assembling binaries, please use the normal precautions with downloaded executables.

Package binaries for R versions older than 3.2.0 are only available from the CRAN archive so users of such versions should adjust the CRAN mirror setting (`https://cran-archive.r-project.org`) accordingly.

R 4.1.1 "Kick Things" released on 2021/08/10

Please check the SHA1 checksum of the downloaded image to ensure that it has not been tampered with or corrupted during the mirroring process. For example type

```
openssl sha1 R-4.1.1.pkg
```

in the *Terminal* application to print the SHA1 checksum for the R-4.1.1.pkg image. On Mac OS X 10.7 and later you can also validate the signature using

```
pkgutil --check-signature R-4.1.1.pkg
```

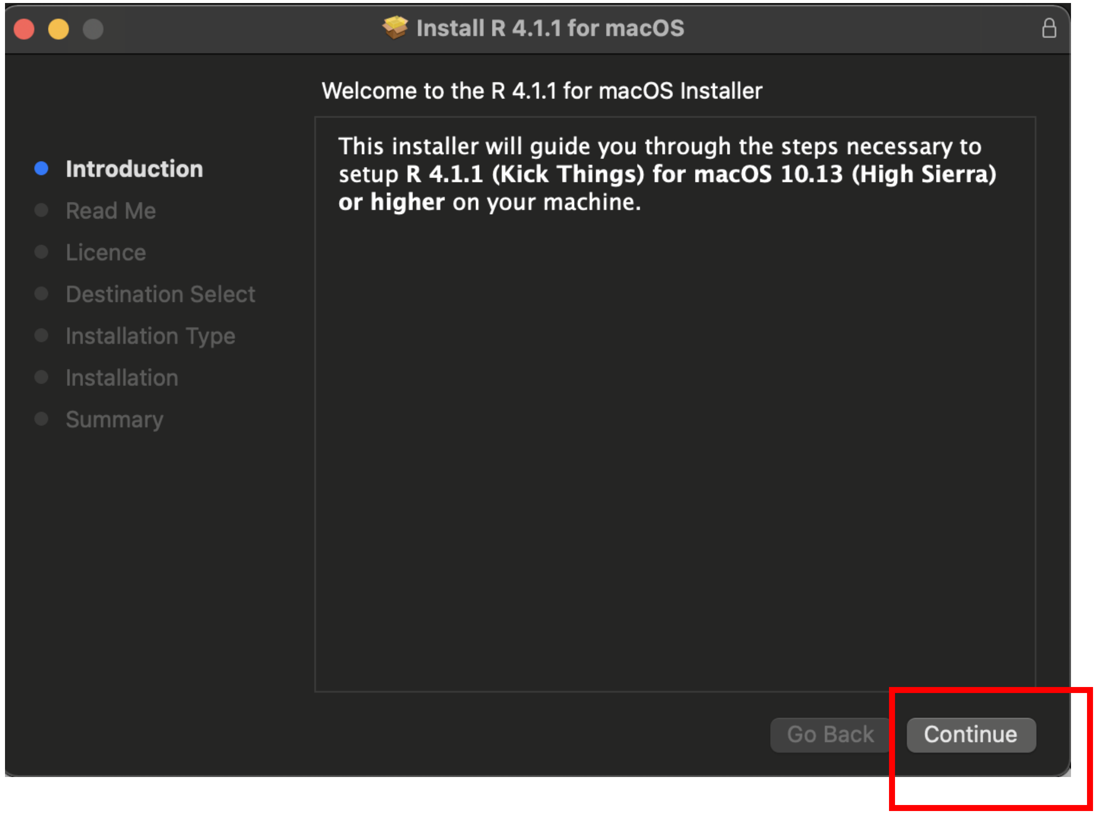
Latest release:

R-4.1.1.pk
SHA1-hash: d
(ca. 86MB)

R 4.1.1 binary for macOS 10.13 (**High Sierra**) and higher, **Intel 64-bit** build, signed and notarized package.
Contains R 4.1.1 framework, R.app GUI 1.77 in 64-bit for Intel Macs, Tcl/Tk 8.6.6 X11 libraries and Texinfo 6.7. The latter two components are optional and can be ommitted when choosing "custom install", they are only needed if you want to use the `tcltk` R package or build package documentation from sources.

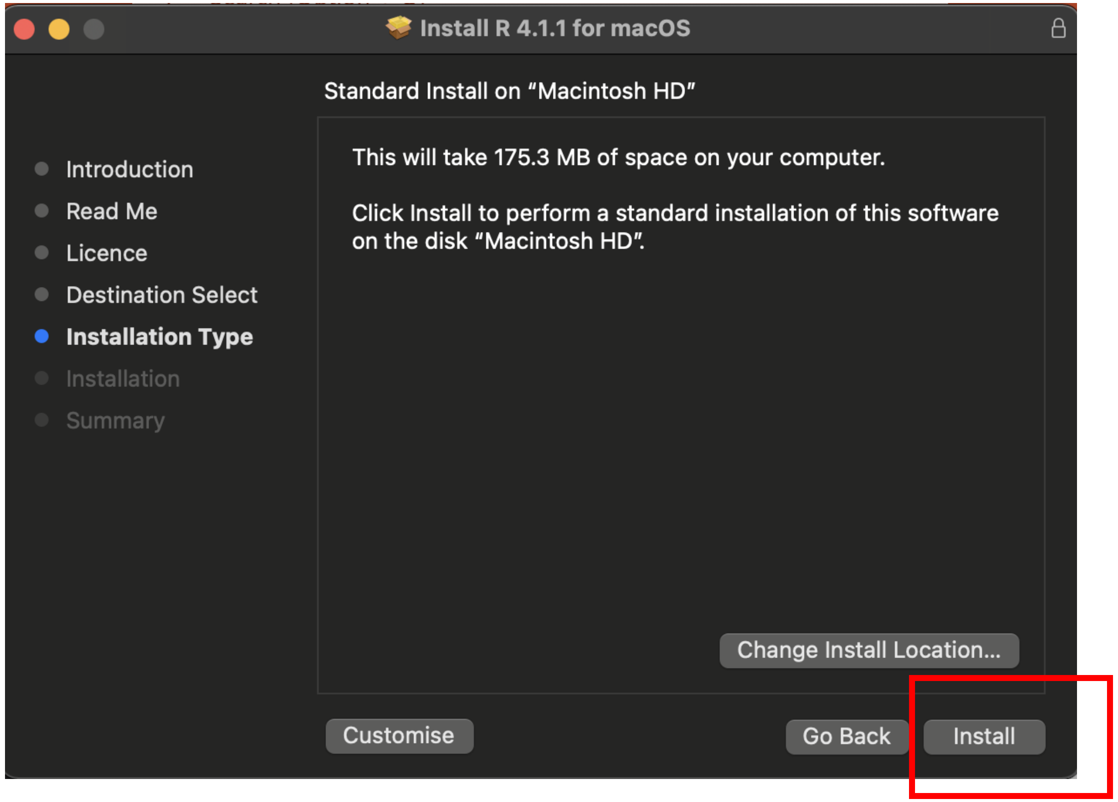
Note: the use of X11 (including `tcltk`) requires XQuartz to be installed since it is no longer part of OS X. Always re-install XQuartz when upgrading your macOS to a new major version.

How to install R (Mac)



Welcome to the R 4.1.1 for macOS Installer

This installer will guide you through the steps necessary to setup **R 4.1.1 (Kick Things) for macOS 10.13 (High Sierra) or higher** on your machine.

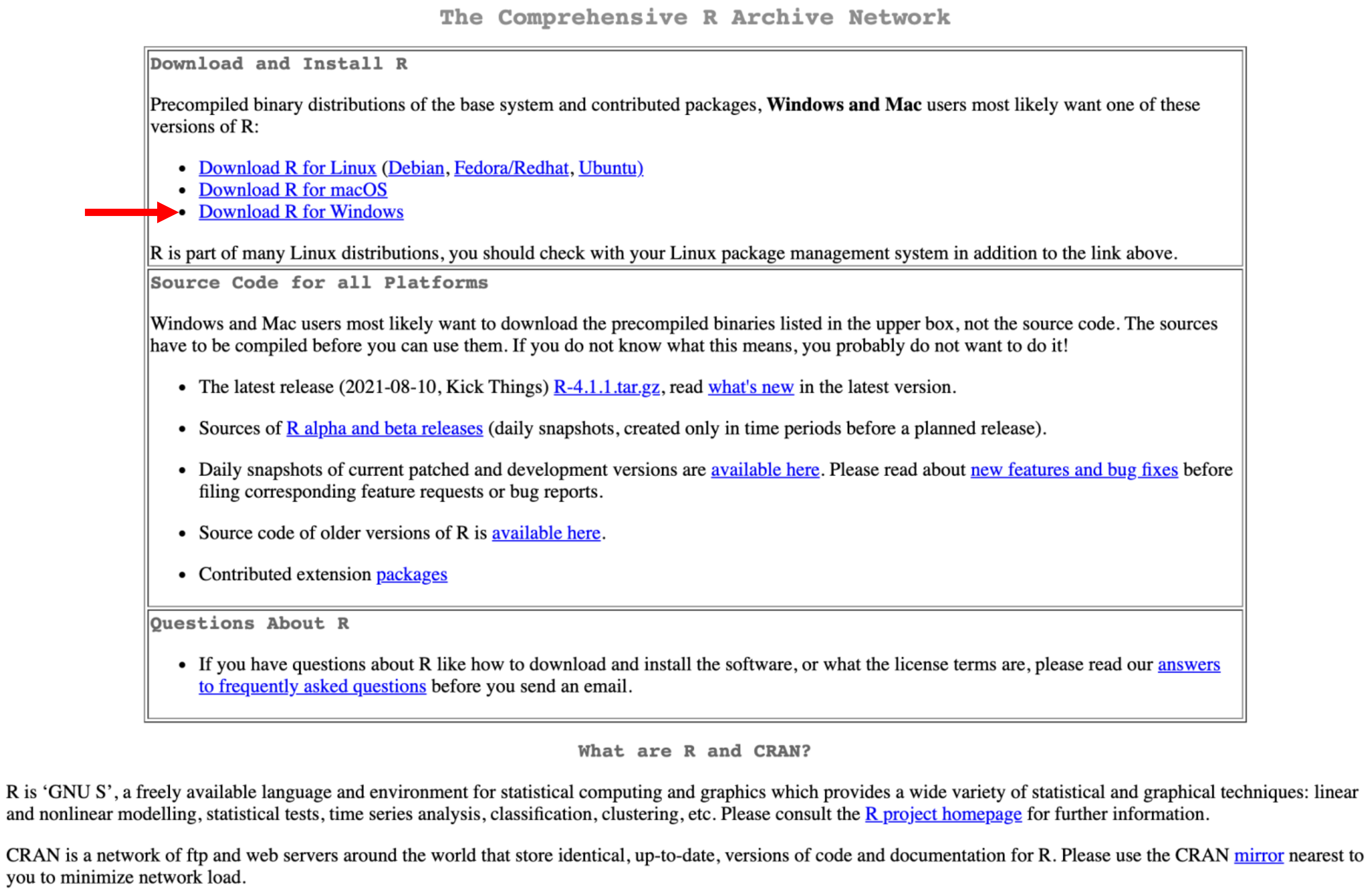


Standard Install on “Macintosh HD”

This will take 175.3 MB of space on your computer.

Click Install to perform a standard installation of this software on the disk “Macintosh HD”.

How to install R



The Comprehensive R Archive Network

Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- Download R for Linux (Debian, Fedora/Redhat, Ubuntu)
- Download R for macOS
- Download R for Windows

R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.

Source Code for all Platforms

Windows and Mac users most likely want to download the precompiled binaries listed in the upper box, not the source code. The sources have to be compiled before you can use them. If you do not know what this means, you probably do not want to do it!

- The latest release (2021-08-10, Kick Things) R-4.1.1.tar.gz, read what's new in the latest version.
- Sources of R alpha and beta releases (daily snapshots, created only in time periods before a planned release).
- Daily snapshots of current patched and development versions are available here. Please read about new features and bug fixes before filing corresponding feature requests or bug reports.
- Source code of older versions of R is available here.
- Contributed extension packages

Questions About R

- If you have questions about R like how to download and install the software, or what the license terms are, please read our answers to frequently asked questions before you send an email.

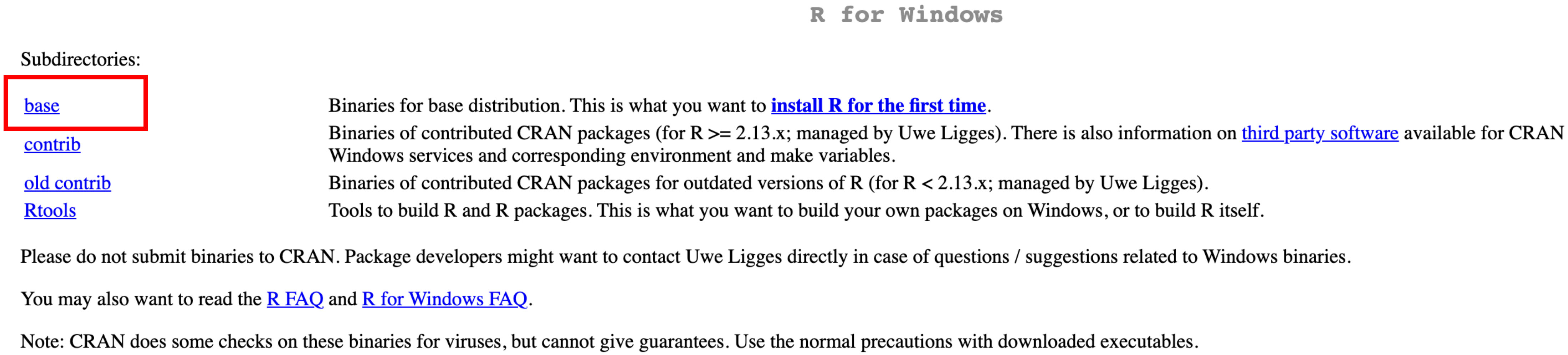
What are R and CRAN?

R is ‘GNU S’, a freely available language and environment for statistical computing and graphics which provides a wide variety of statistical and graphical techniques: linear and nonlinear modelling, statistical tests, time series analysis, classification, clustering, etc. Please consult the R project homepage for further information.

CRAN is a network of ftp and web servers around the world that store identical, up-to-date, versions of code and documentation for R. Please use the CRAN mirror nearest to you to minimize network load.

https://cran.csiro.au/

How to install R (windows)



R for Windows

Subdirectories:

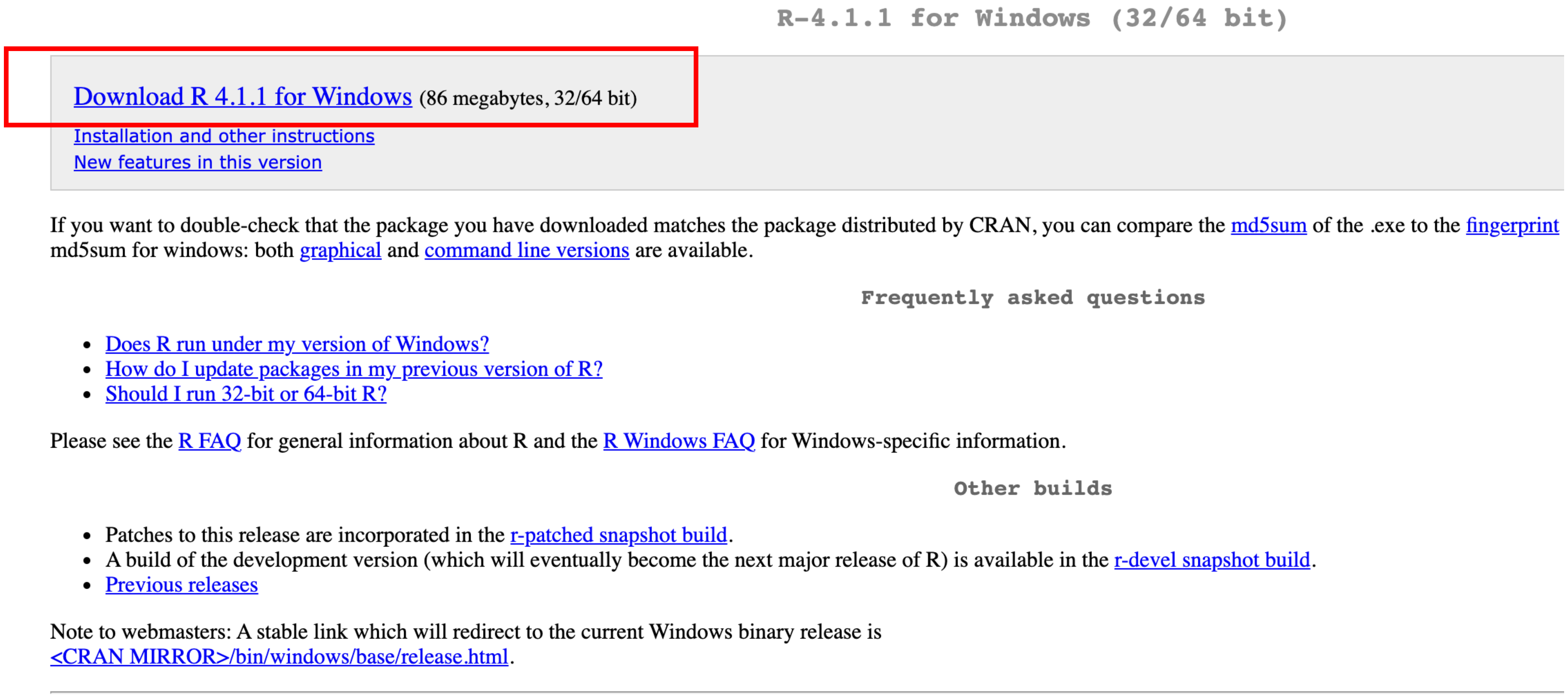
base	Binaries for base distribution. This is what you want to **install R for the first time**.
contrib	Binaries of contributed CRAN packages (for R >= 2.13.x; managed by Uwe Ligges). There is also information on third party software available for CRAN Windows services and corresponding environment and make variables.
old contrib	Binaries of contributed CRAN packages for outdated versions of R (for R < 2.13.x; managed by Uwe Ligges).
Rtools	Tools to build R and R packages. This is what you want to build your own packages on Windows, or to build R itself.

Please do not submit binaries to CRAN. Package developers might want to contact Uwe Ligges directly in case of questions / suggestions related to Windows binaries.

You may also want to read the R FAQ and R for Windows FAQ.

Note: CRAN does some checks on these binaries for viruses, but cannot give guarantees. Use the normal precautions with downloaded executables.

How to install R (windows)



R-4.1.1 for Windows (32/64 bit)

Download R 4.1.1 for Windows (86 megabytes, 32/64 bit)

Installation and other instructions

New features in this version

If you want to double-check that the package you have downloaded matches the package distributed by CRAN, you can compare the md5sum of the .exe to the fingerprint md5sum for windows: both graphical and command line versions are available.

Frequently asked questions

- Does R run under my version of Windows?
- How do I update packages in my previous version of R?
- Should I run 32-bit or 64-bit R?

Please see the R FAQ for general information about R and the R Windows FAQ for Windows-specific information.

Other builds

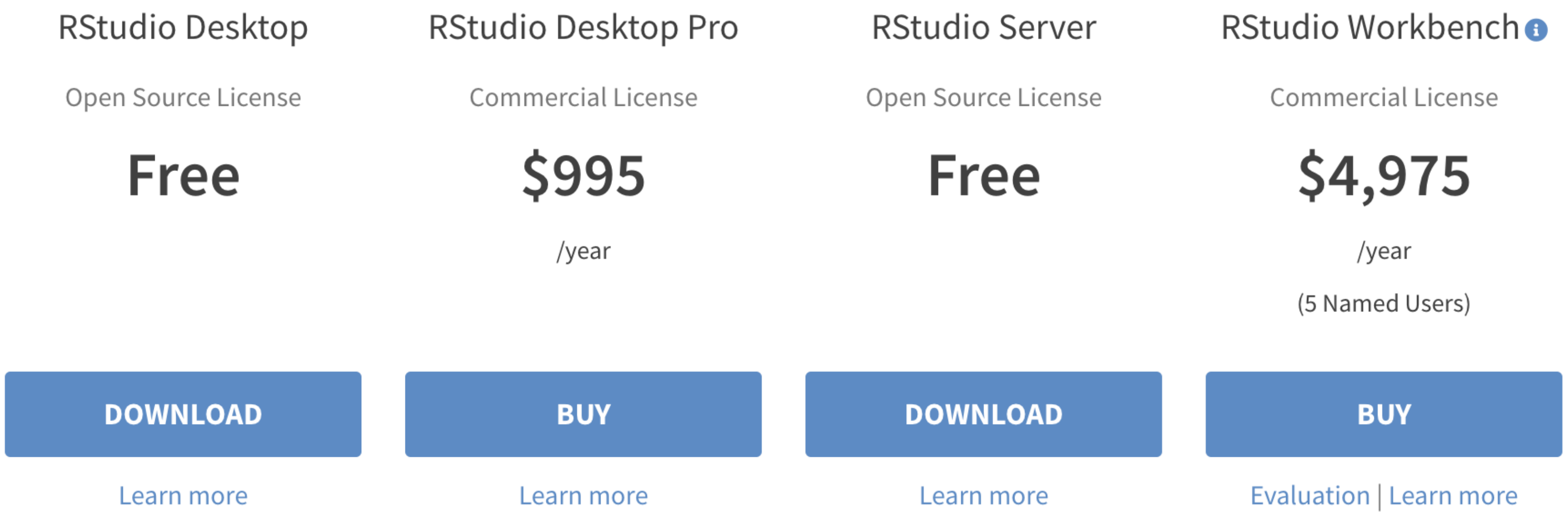
- Patches to this release are incorporated in the r-patched snapshot build.
- A build of the development version (which will eventually become the next major release of R) is available in the r-devel snapshot build.
- Previous releases

Note to webmasters: A stable link which will redirect to the current Windows binary release is
<CRAN MIRROR>/bin/windows/base/release.html.

Hands on practical #1

Install R on your computer

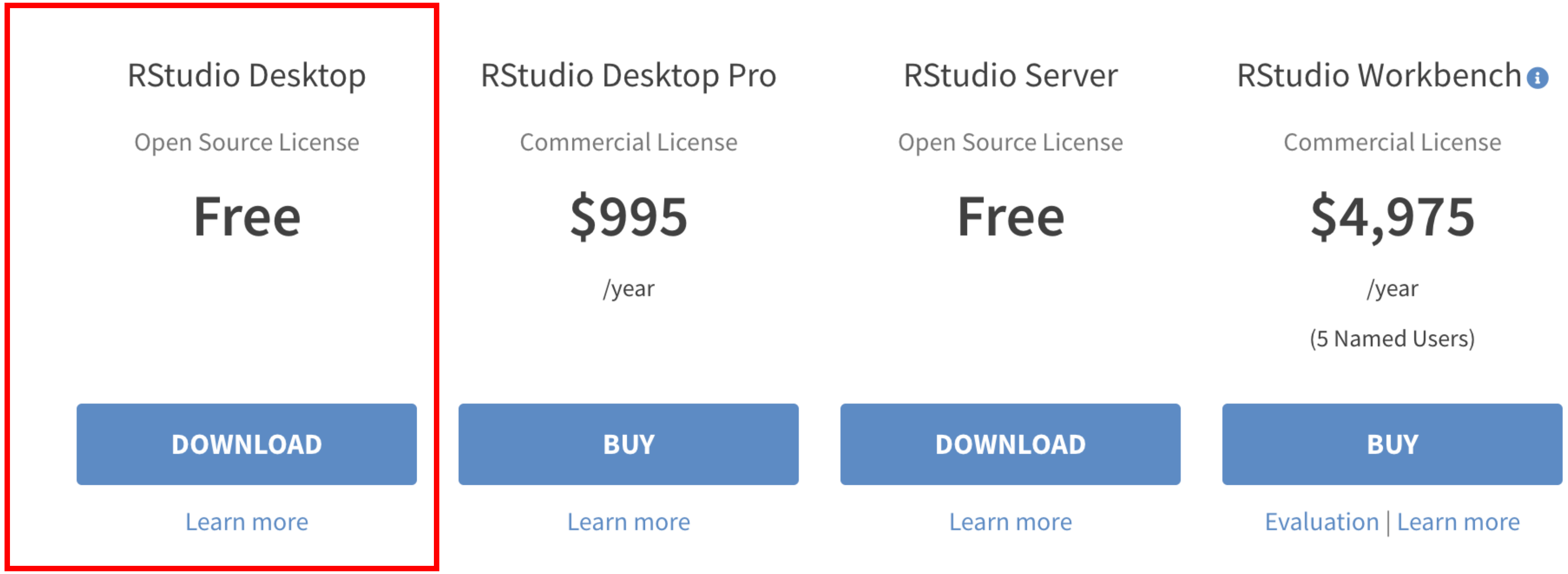
How to install Rstudio



RStudio Desktop	RStudio Desktop Pro	RStudio Server	RStudio Workbench
Open Source License	Commercial License	Open Source License	Commercial License
Free	$995	Free	$4,975
	/year		/year
			(5 Named Users)
DOWNLOAD	BUY	DOWNLOAD	BUY
Learn more	Learn more	Learn more	Evaluation | Learn more

https://www.rstudio.com/products/rstudio/download/

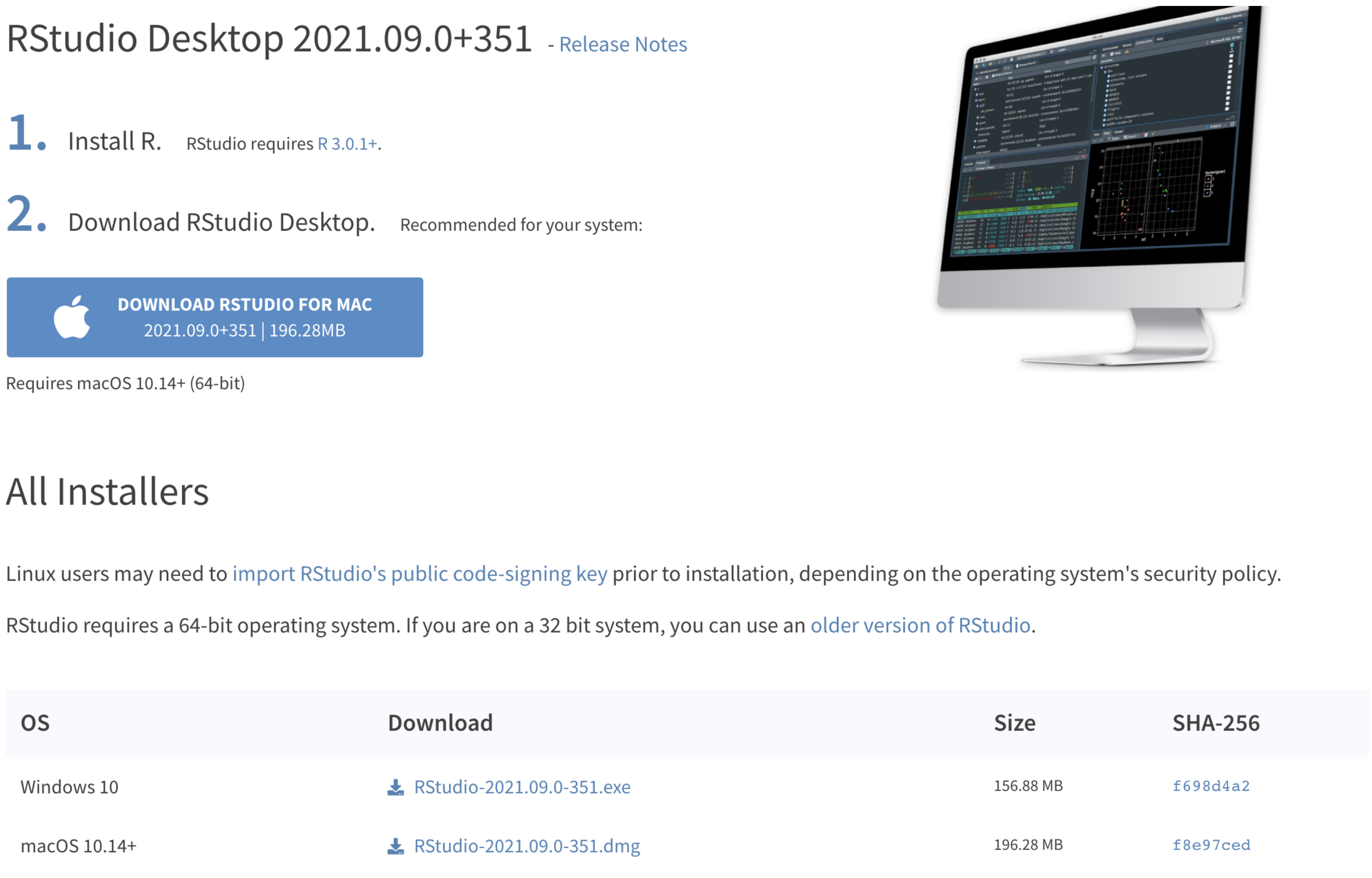
How to install Rstudio



RStudio Desktop	RStudio Desktop Pro	RStudio Server	RStudio Workbench
Open Source License	Commercial License	Open Source License	Commercial License
Free	$995	Free	$4,975
	/year		/year
			(5 Named Users)
DOWNLOAD	BUY	DOWNLOAD	BUY
Learn more	Learn more	Learn more	Evaluation | Learn more

https://www.rstudio.com/products/rstudio/download/

How to install Rstudio



RStudio Desktop 2021.09.0+351 - Release Notes

1. Install R. RStudio requires R 3.0.1+.

2. Download RStudio Desktop. Recommended for your system:

Requires macOS 10.14+ (64-bit)

All Installers

Linux users may need to import RStudio's public code-signing key prior to installation, depending on the operating system's security policy.

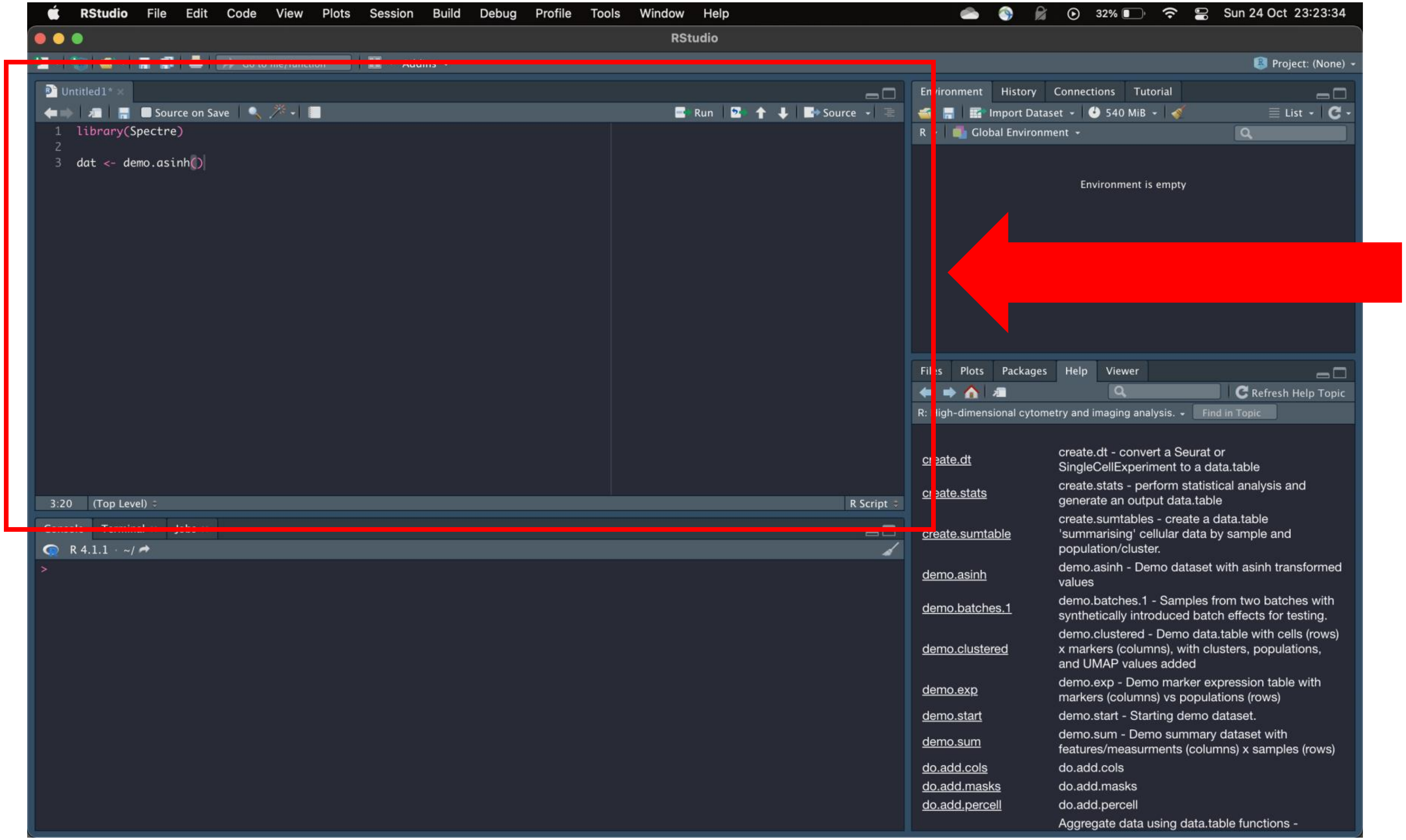
RStudio requires a 64-bit operating system. If you are on a 32 bit system, you can use an older version of RStudio.

OS	Download	Size	SHA-256
Windows 10	RStudio-2021.09.0-351.exe	156.88 MB	f698d4a2
macOS 10.14+	RStudio-2021.09.0-351.dmg	196.28 MB	f8e97ced

Hands on practical #2

Install Rstudio on your computer

Rstudio layout – text editor panel



```
library(Spectre)

dat <- demo.asinh()
```

3:20 (Top Level) R Script

Environment is empty

create.dt create.dt - convert a Seurat or SingleCellExperiment to a data.table

create.stats create.stats - perform statistical analysis and generate an output data.table

create.sumtable create.sumtables - create a data.table 'summarising' cellular data by sample and population/cluster.

demo.asinh demo.asinh - Demo dataset with asinh transformed values

demo.batches.1 demo.batches.1 - Samples from two batches with synthetically introduced batch effects for testing.

demo.clustered demo.clustered - Demo data.table with cells (rows) x markers (columns), with clusters, populations, and UMAP values added

demo.exp demo.exp - Demo marker expression table with markers (columns) vs populations (rows)

demo.start demo.start - Starting demo dataset.

demo.sum demo.sum - Demo summary dataset with features/measurments (columns) x samples (rows)

do.add.cols do.add.cols

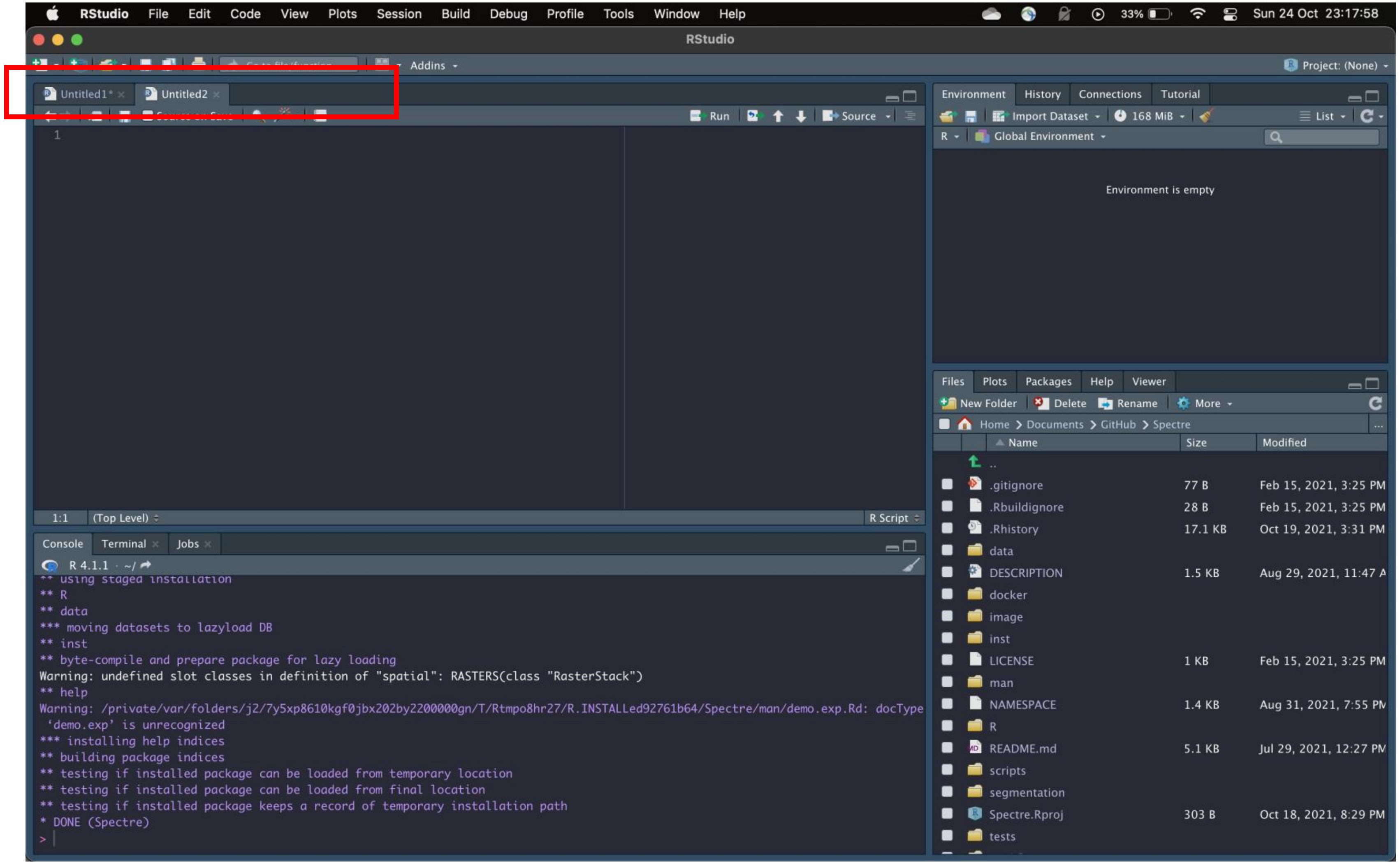
do.add.masks do.add.masks

do.add.percell do.add.percell

Aggregate data using data.table functions -

Text editor panel

Rstudio layout – R scripts

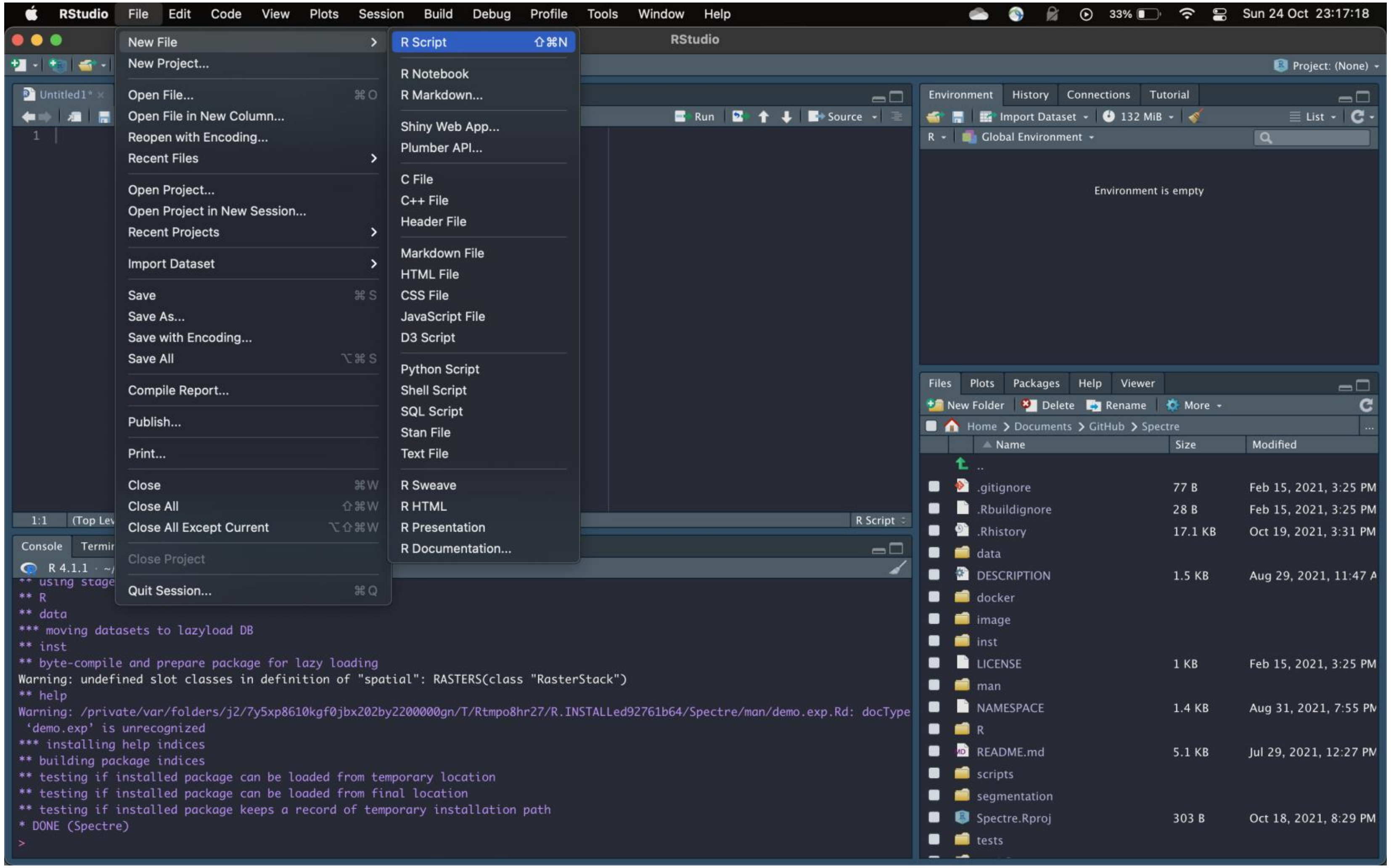


Environment is empty

```
** using staged installation
** R
** data
*** moving datasets to lazyload DB
** inst
** byte-compile and prepare package for lazy loading
Warning: undefined slot classes in definition of "spatial": RASTERS(class "RasterStack")
** help
Warning: /private/var/folders/j2/7y5xp8610kgf0jbx202by2200000gn/T/Rtmpo8hr27/R.INSTALLed92761b64/Spectre/man/demo.exp.Rd: docType
 'demo.exp' is unrecognized
*** installing help indices
** building package indices
** testing if installed package can be loaded from temporary location
** testing if installed package can be loaded from final location
** testing if installed package keeps a record of temporary installation path
* DONE (Spectre)
>
```

Name	Size	Modified
..		
.gitignore	77 B	Feb 15, 2021, 3:25 PM
.Rbuildignore	28 B	Feb 15, 2021, 3:25 PM
.Rhistory	17.1 KB	Oct 19, 2021, 3:31 PM
data		
DESCRIPTION	1.5 KB	Aug 29, 2021, 11:47 A
docker		
image		
inst		
LICENSE	1 KB	Feb 15, 2021, 3:25 PM
man		
NAMESPACE	1.4 KB	Aug 31, 2021, 7:55 PM
R		
README.md	5.1 KB	Jul 29, 2021, 12:27 PM
scripts		
segmentation		
Spectre.Rproj	303 B	Oct 18, 2021, 8:29 PM
tests		

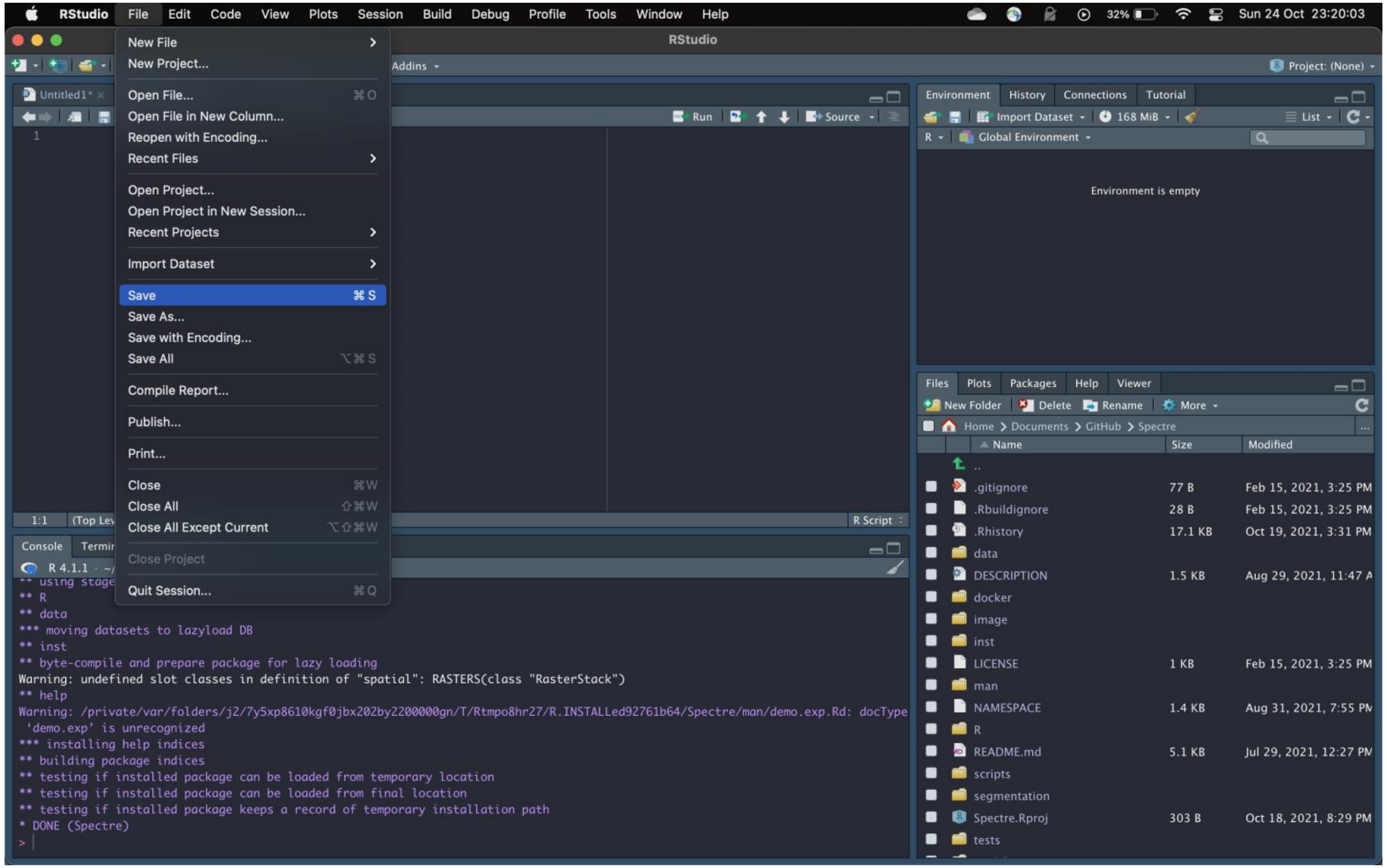
Rstudio layout – create new R script



```
** using stage
** R
** data
*** moving datasets to lazyload DB
** inst
** byte-compile and prepare package for lazy loading
Warning: undefined slot classes in definition of "spatial": RASTERS(class "RasterStack")
** help
Warning: /private/var/folders/j2/7y5xp8610kgf0jbx202by2200000gn/T/Rtmpo8hr27/R.INSTALLed92761b64/Spectre/man/demo.exp.Rd: docType
 'demo.exp' is unrecognized
*** installing help indices
** building package indices
** testing if installed package can be loaded from temporary location
** testing if installed package can be loaded from final location
** testing if installed package keeps a record of temporary installation path
* DONE (Spectre)
>
```

Name	Size	Modified
..		
.gitignore	77 B	Feb 15, 2021, 3:25 PM
.Rbuildignore	28 B	Feb 15, 2021, 3:25 PM
.Rhistory	17.1 KB	Oct 19, 2021, 3:31 PM
data		
DESCRIPTION	1.5 KB	Aug 29, 2021, 11:47 A
docker		
image		
inst		
LICENSE	1 KB	Feb 15, 2021, 3:25 PM
man		
NAMESPACE	1.4 KB	Aug 31, 2021, 7:55 PM
R		
README.md	5.1 KB	Jul 29, 2021, 12:27 PM
scripts		
segmentation		
Spectre.Rproj	303 B	Oct 18, 2021, 8:29 PM
tests		

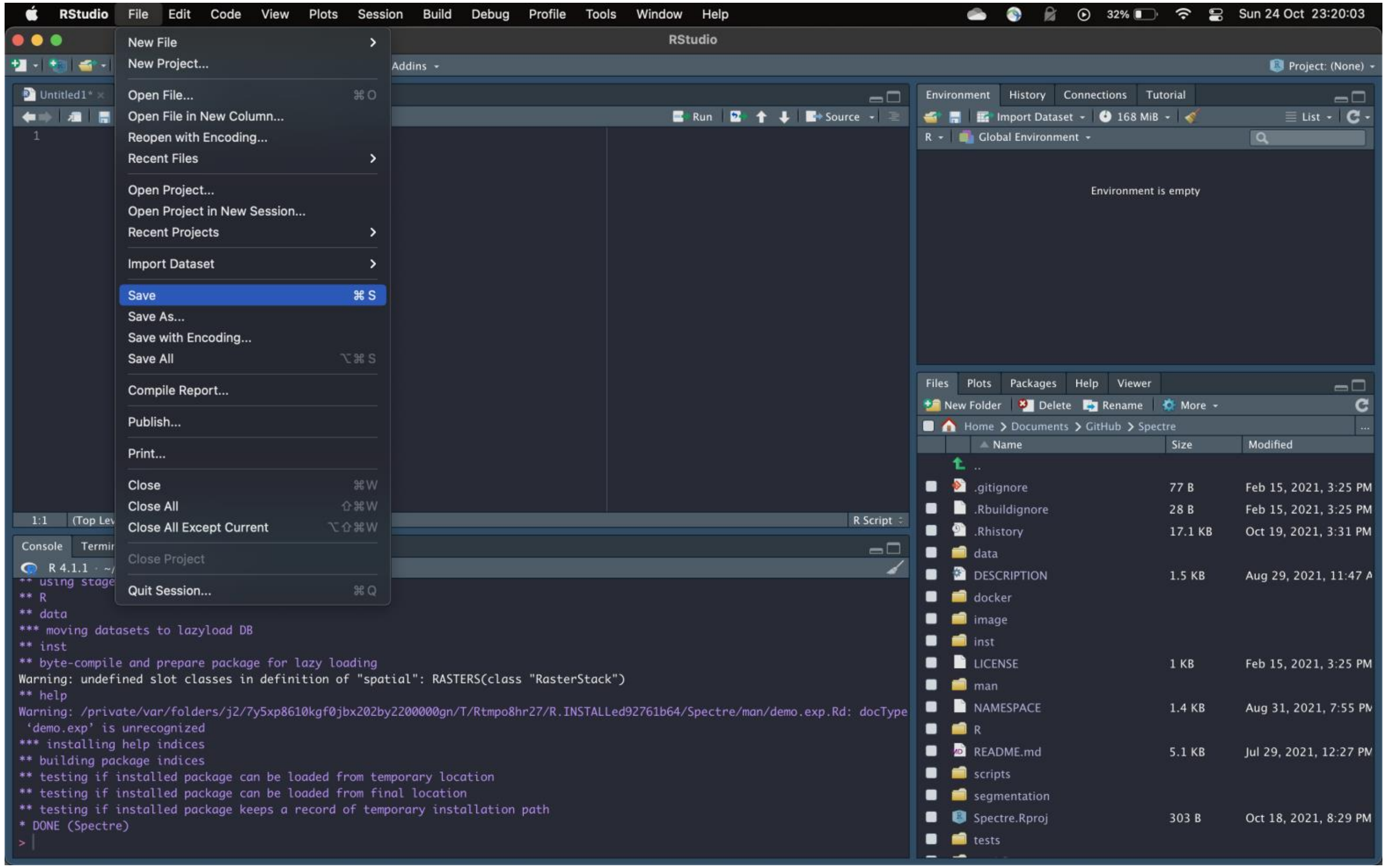
Rstudio layout – save R script



```
** using stage
** R
** data
*** moving datasets to lazyload DB
** inst
** byte-compile and prepare package for lazy loading
Warning: undefined slot classes in definition of "spatial": RASTERS(class "RasterStack")
** help
Warning: /private/var/folders/j2/7y5xp8610kgf0jbx202by2200000gn/T/Rtmpo8hr27/R.INSTALLed92761b64/Spectre/man/demo.exp.Rd: docType
 'demo.exp' is unrecognized
*** installing help indices
** building package indices
** testing if installed package can be loaded from temporary location
** testing if installed package can be loaded from final location
** testing if installed package keeps a record of temporary installation path
* DONE (Spectre)
>
```

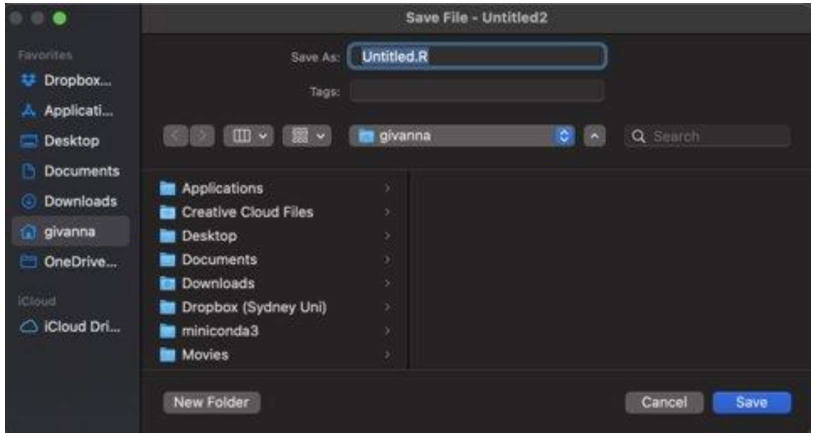
Name	Size	Modified
..		
.gitignore	77 B	Feb 15, 2021, 3:25 PM
.Rbuildignore	28 B	Feb 15, 2021, 3:25 PM
.Rhistory	17.1 KB	Oct 19, 2021, 3:31 PM
data		
DESCRIPTION	1.5 KB	Aug 29, 2021, 11:47 A
docker		
image		
inst		
LICENSE	1 KB	Feb 15, 2021, 3:25 PM
man		
NAMESPACE	1.4 KB	Aug 31, 2021, 7:55 PM
R		
README.md	5.1 KB	Jul 29, 2021, 12:27 PM
scripts		
segmentation		
Spectre.Rproj	303 B	Oct 18, 2021, 8:29 PM
tests		

Rstudio layout – save R script



```
** using stage
** R
** data
*** moving datasets to lazyload DB
** inst
** byte-compile and prepare package for lazy loading
Warning: undefined slot classes in definition of "spatial": RASTERS(class "RasterStack")
** help
Warning: /private/var/folders/j2/7y5xp8610kgf0jbx202by2200000gn/T/Rtmpo8hr27/R.INSTALLed92761b64/Spectre/man/demo.exp.Rd: docType
 ‘demo.exp’ is unrecognized
*** installing help indices
** building package indices
** testing if installed package can be loaded from temporary location
** testing if installed package can be loaded from final location
** testing if installed package keeps a record of temporary installation path
* DONE (Spectre)
>
```

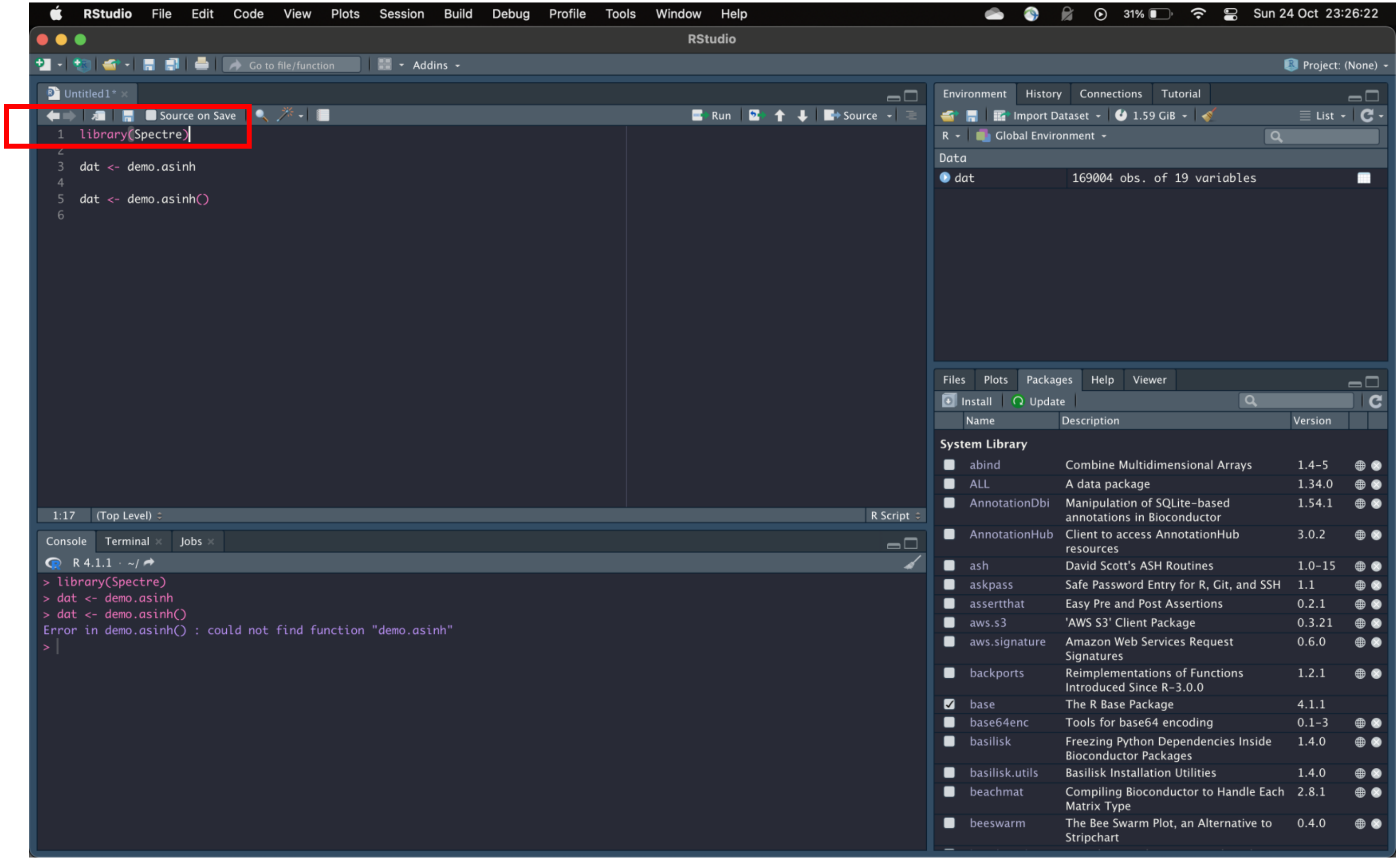
Name	Size	Modified
..		
.gitignore	77 B	Feb 15, 2021, 3:25 PM
.Rbuildignore	28 B	Feb 15, 2021, 3:25 PM
.Rhistory	17.1 KB	Oct 19, 2021, 3:31 PM
data		
DESCRIPTION	1.5 KB	Aug 29, 2021, 11:47 A
docker		
image		
inst		
LICENSE	1 KB	Feb 15, 2021, 3:25 PM
man		
NAMESPACE	1.4 KB	Aug 31, 2021, 7:55 PM
R		
README.md	5.1 KB	Jul 29, 2021, 12:27 PM
scripts		
segmentation		
Spectre.Rproj	303 B	Oct 18, 2021, 8:29 PM
tests		



Save As: Untitled.R

Tags:

Rstudio – running R code



```r
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()

```

```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
> 
```

Data

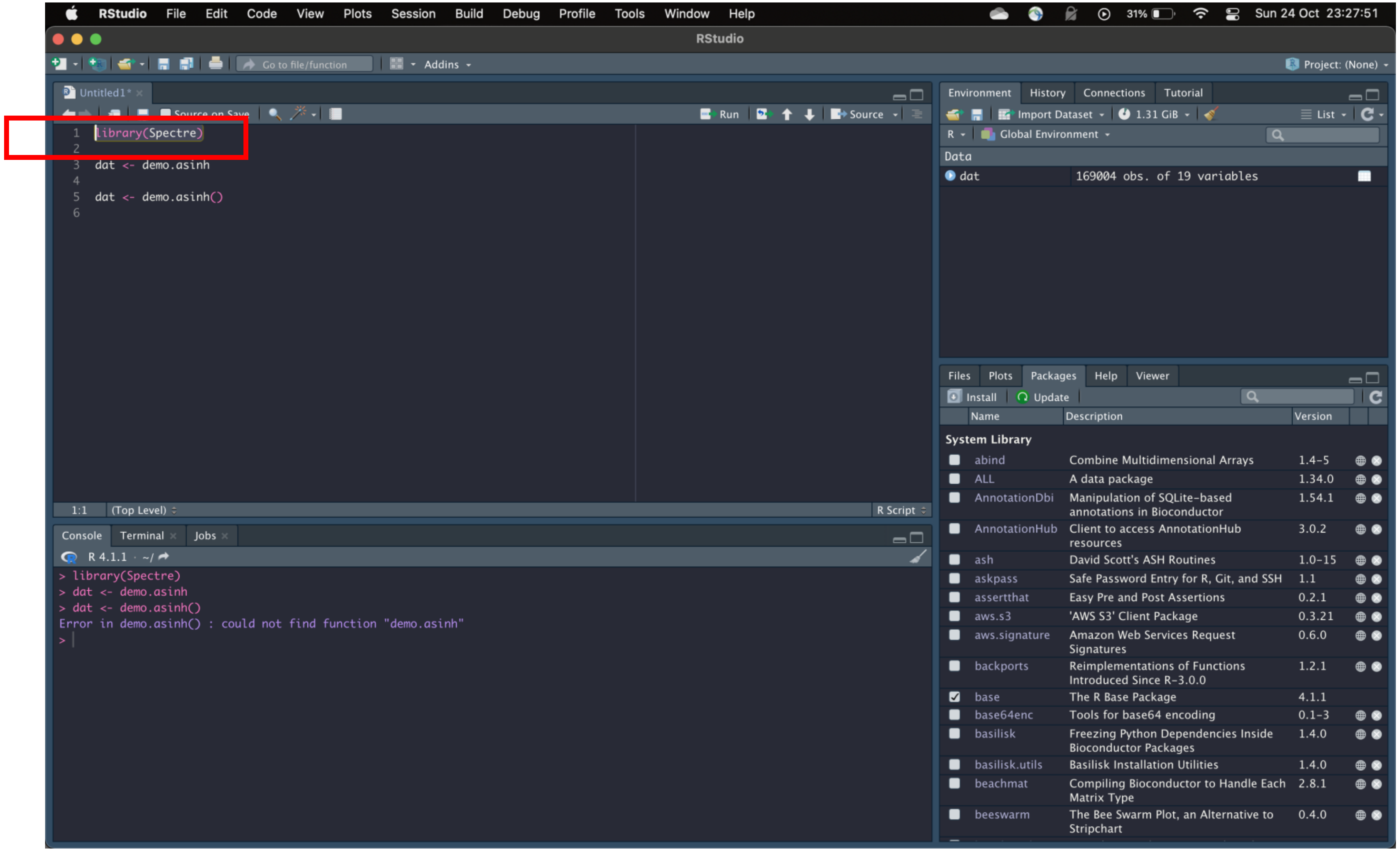
dat 169004 obs. of 19 variables

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Run a line of code:

1. Indicate which line to run by either:
   a) Put your cursor on the line

Rstudio – running R code



```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()
```

```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
>
```

Data

```
dat                 169004 obs. of 19 variables
```

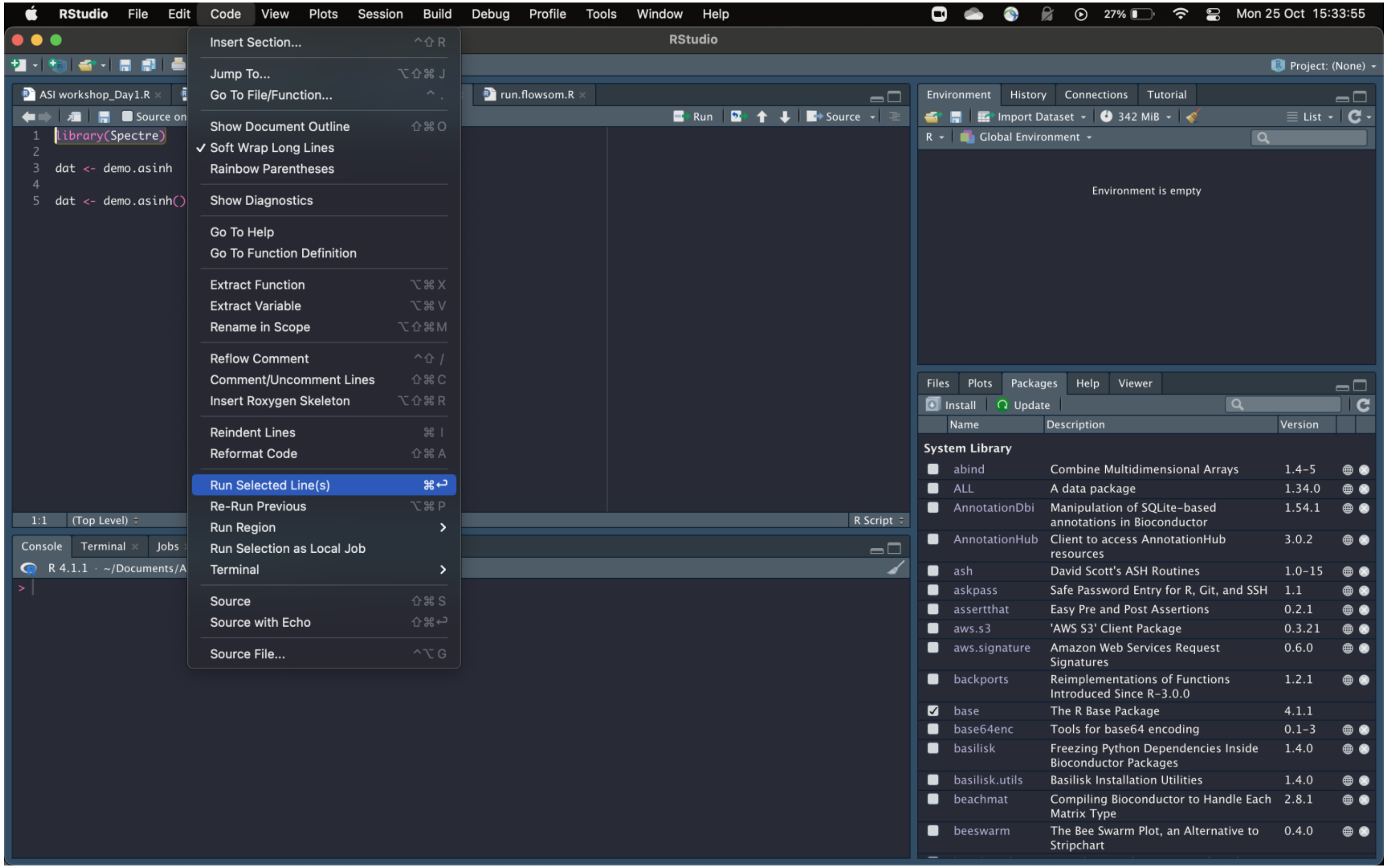
System Library

	Name	Description	Version
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Run a line of code:

1. Indicate which line to run by either:
   a) Put your cursor on the line
   b) Highlight the whole line

Rstudio – running R code



```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()
```

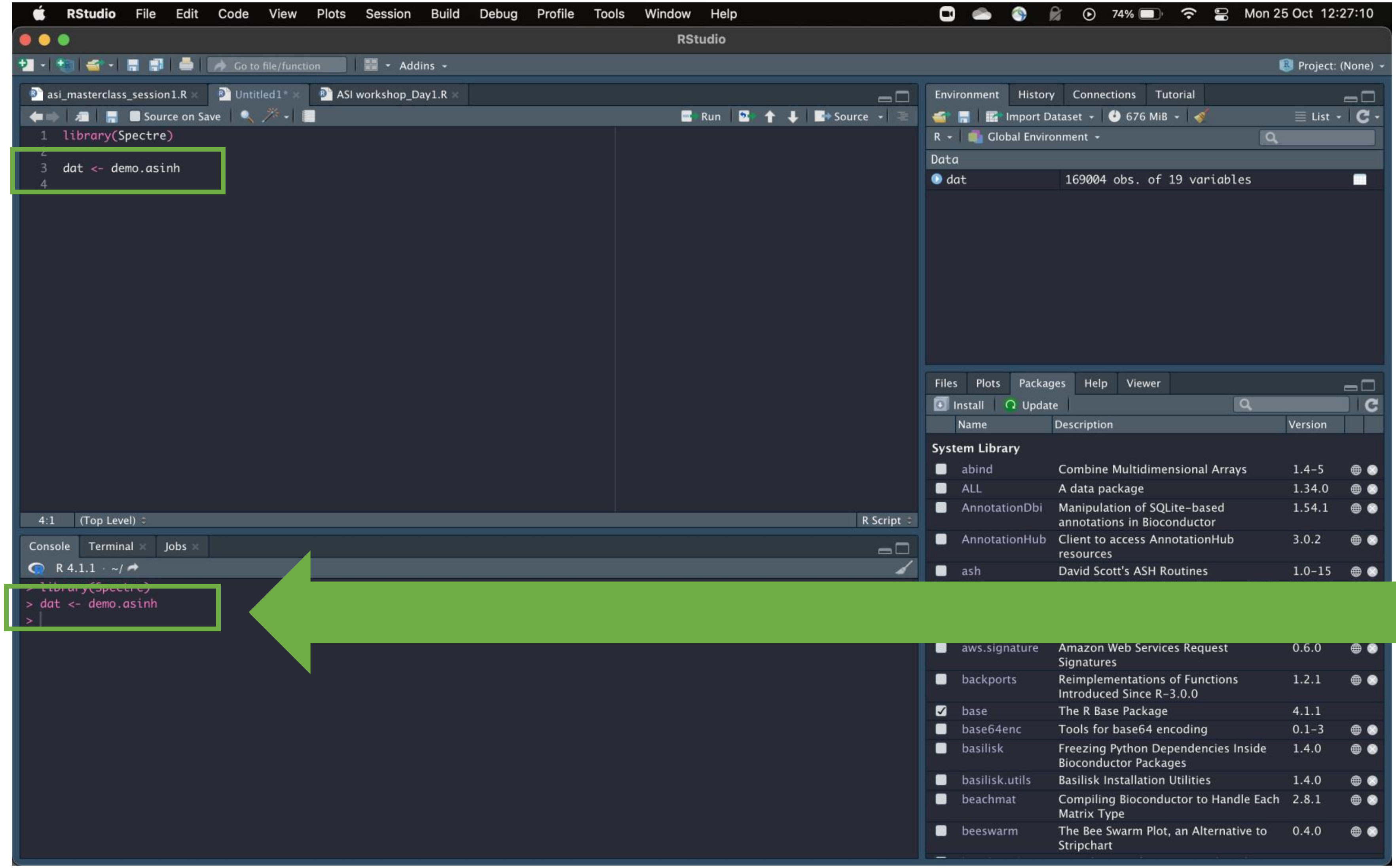
System Library

	Name	Description	Version
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Run a line of code:

1. Indicate which line to run by either:
   a) Put your cursor on the line
   b) Highlight the whole line
2. Either:
   a) Mac: cmd + enter
   b) Windows: ctrl + enter
   c) Code > Run Selected Line(s)

Rstudio layout – console panel



```
library(Spectre)

dat <- demo.asinh

```

```
> library(Spectre)
> dat <- demo.asinh
> 
```

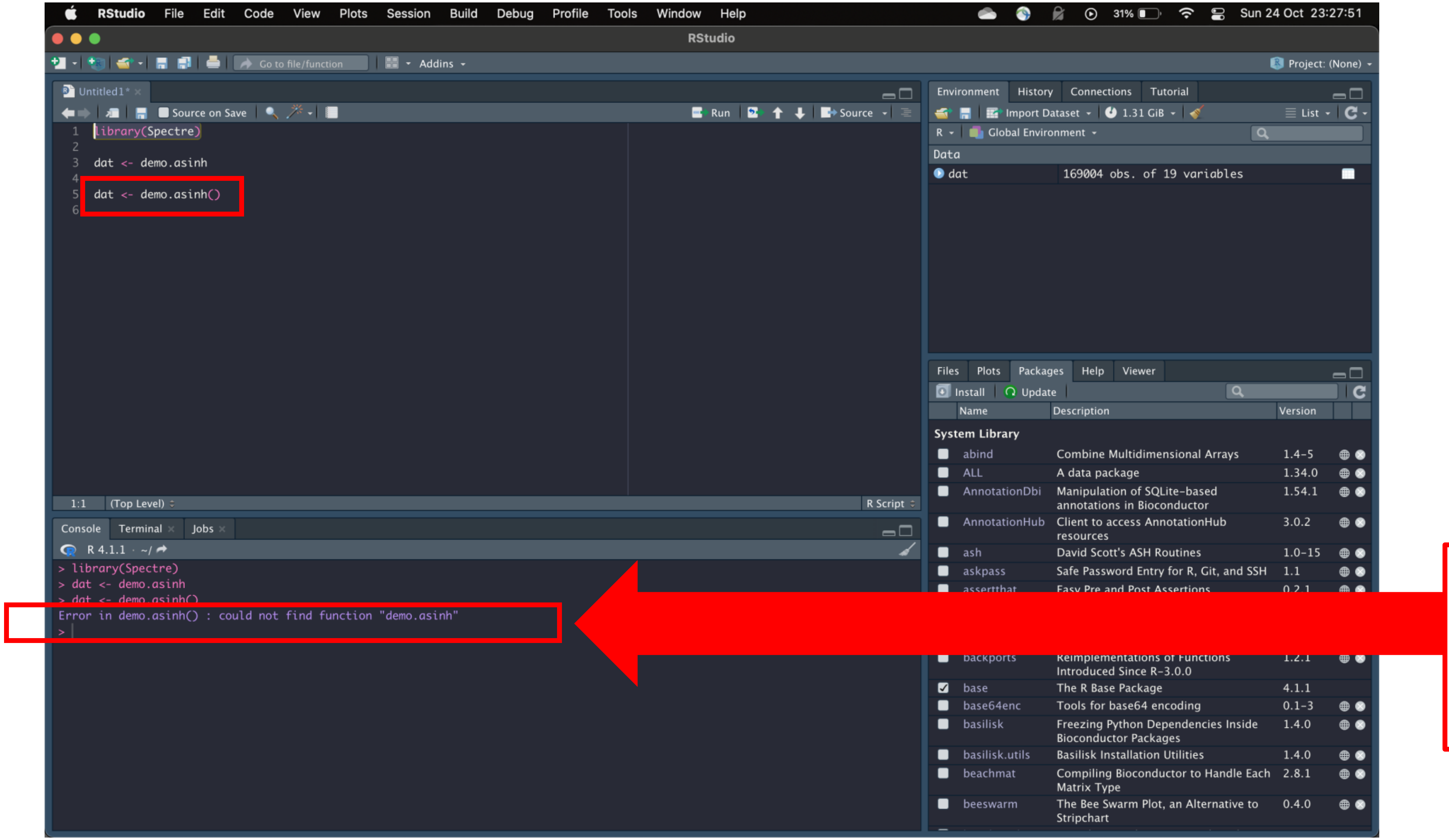
Data

dat	169004 obs. of 19 variables

	Name	Description	Version
System Library			
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

If all is well, it will just show you the chunk of code you just run

Rstudio layout – console panel



```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()

```

```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
> 
```

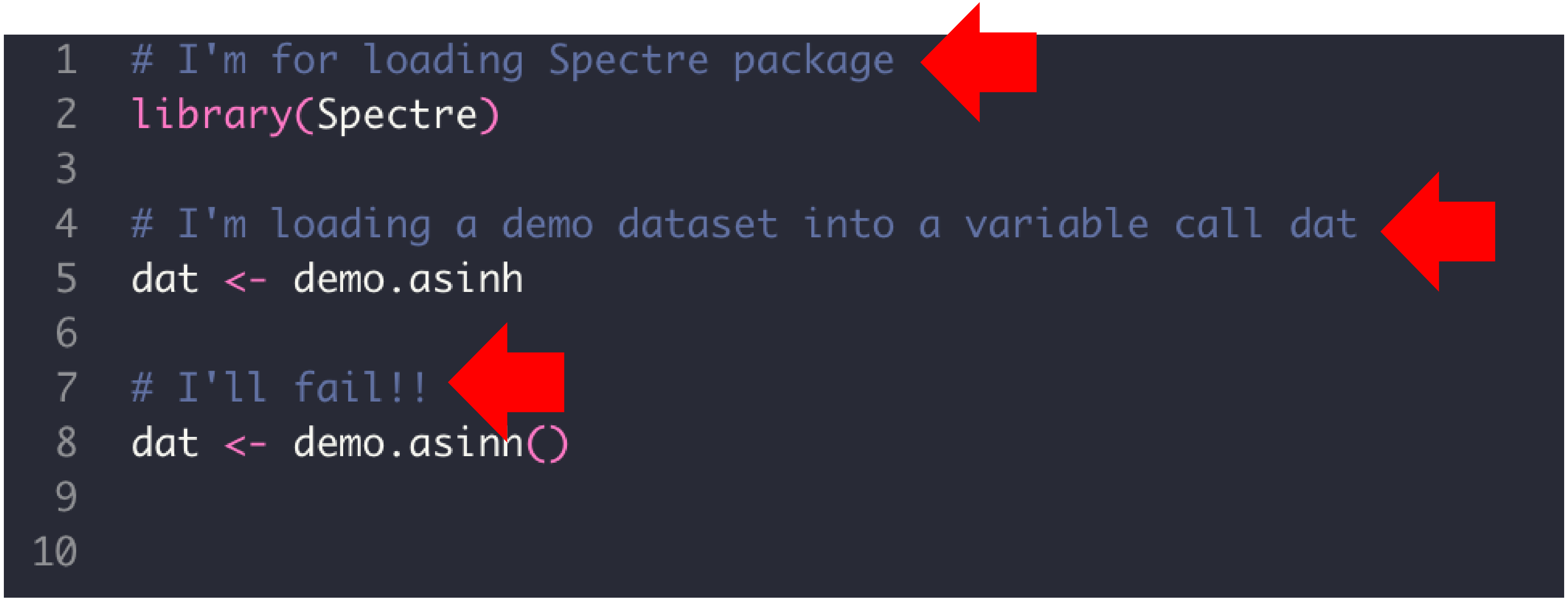
Data

dat 169004 obs. of 19 variables

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Otherwise, it will tell you something is wrong (error) and the reason behind it

Rstudio – comment within R script

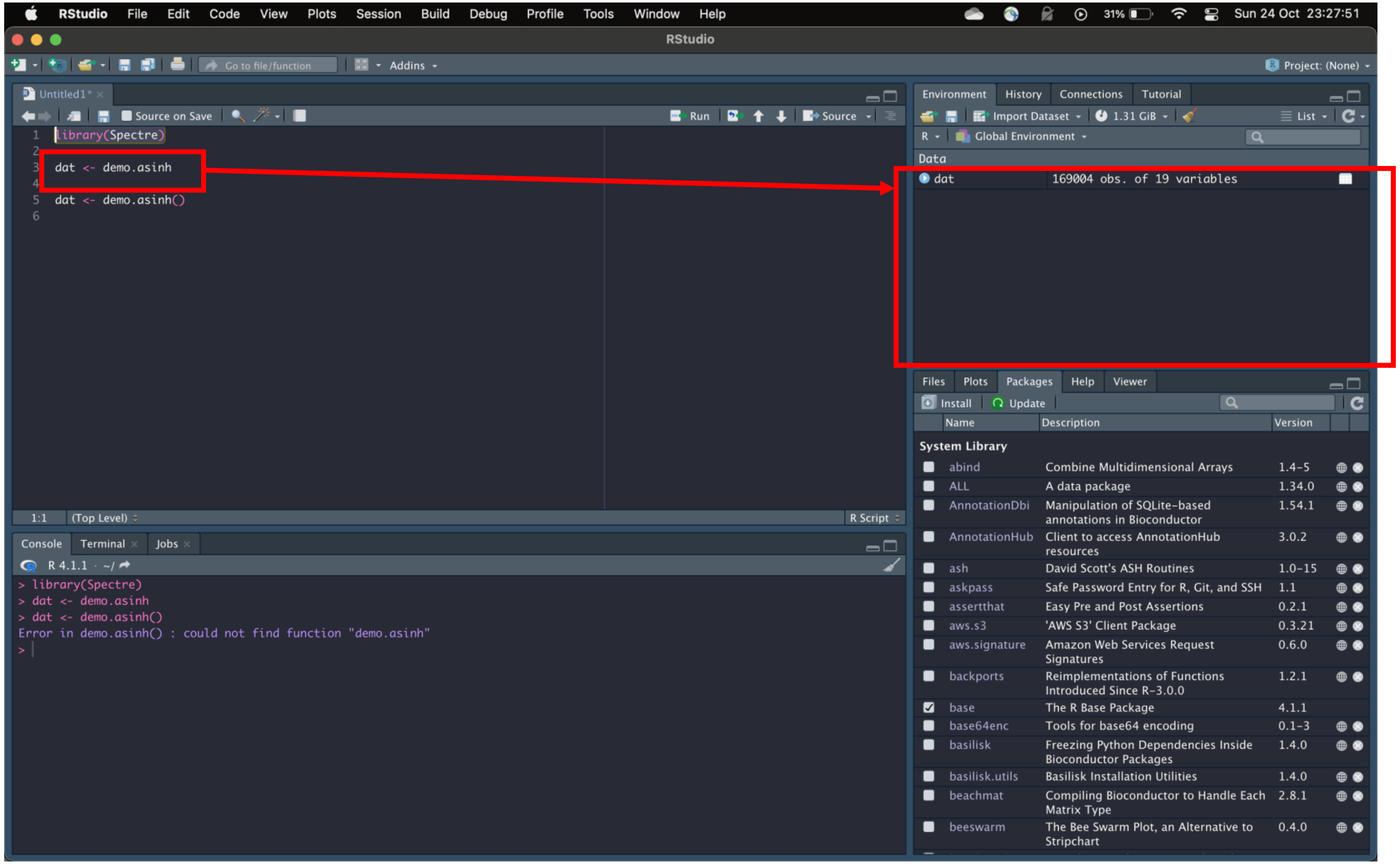


```r
# I'm for loading Spectre package
library(Spectre)

# I'm loading a demo dataset into a variable call dat
dat <- demo.asinh

# I'll fail!!
dat <- demo.asinh()

```

Comment in R script:

- Non-executable code.
- Useful to document what a line of code meant to do as a note or reminder to yourself or collaborators.
- Begin with a hash symbol (#).

Rstudio layout – environment panel



```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()
```

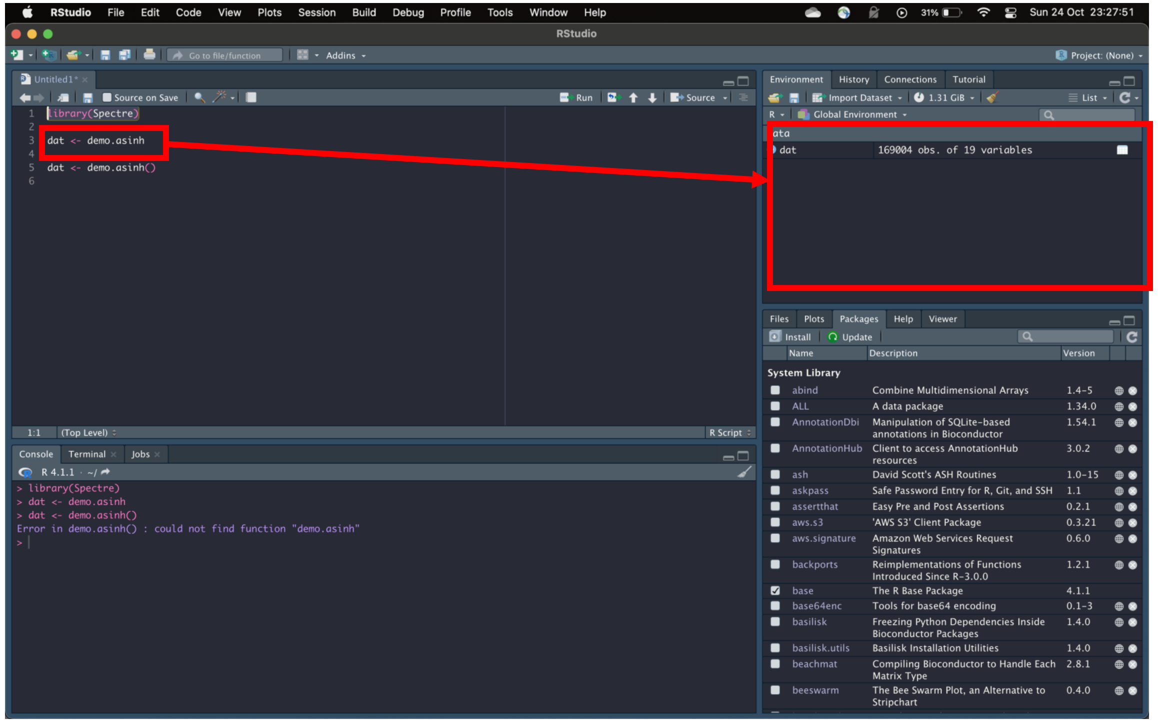
```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
>
```

Data

dat 169004 obs. of 19 variables

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Rstudio layout – what is a variable?



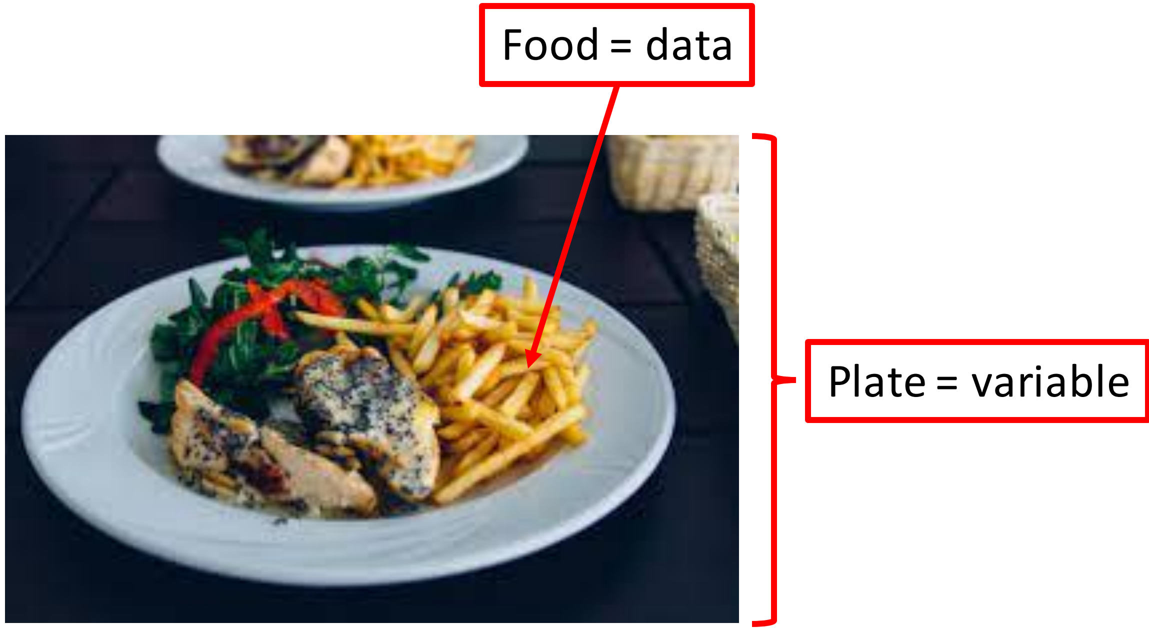
```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()

```

```
ata
dat        169004 obs. of 19 variables
```

```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
> 
```

System Library

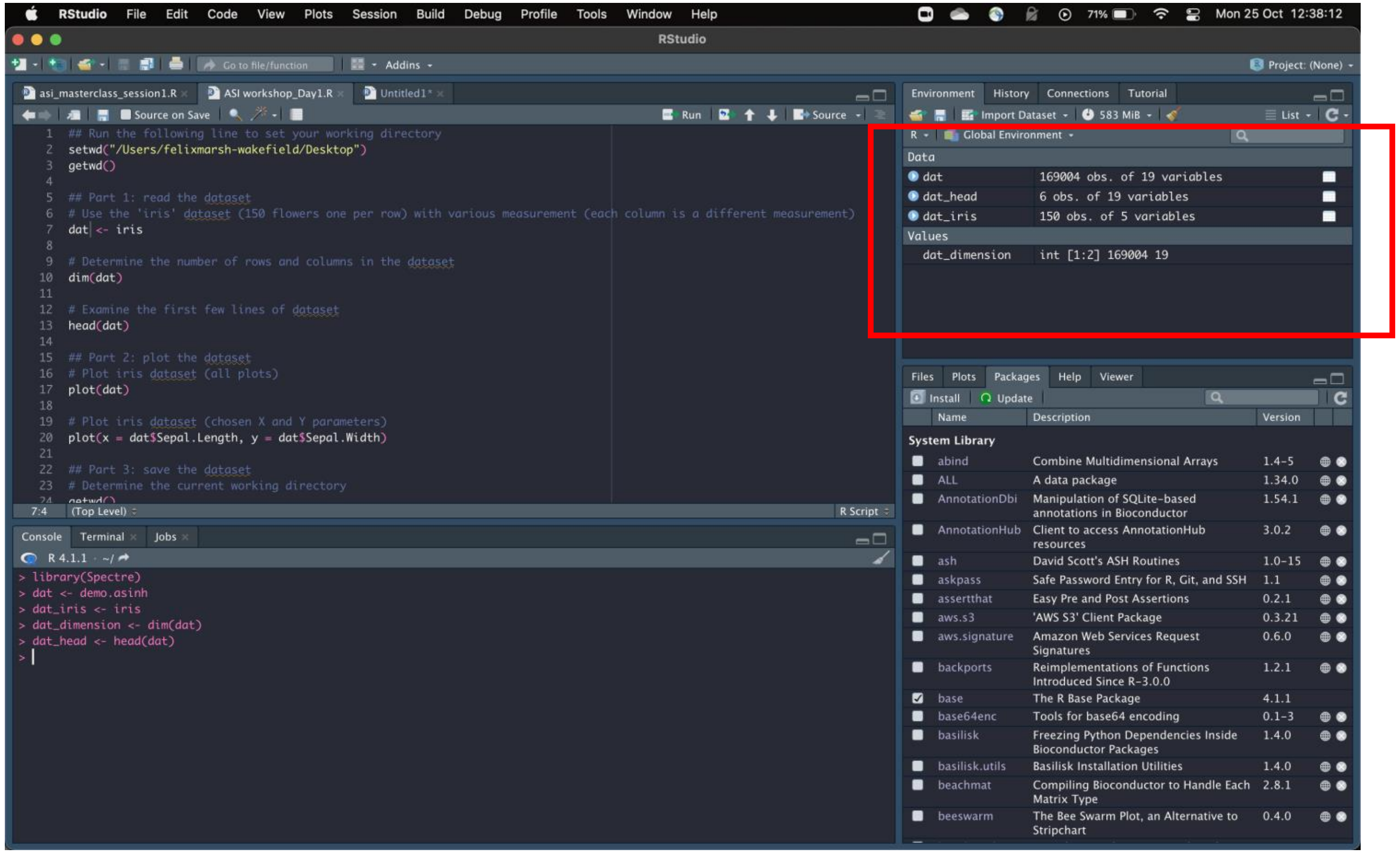
	Name	Description	Version
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0



Food = data

Plate = variable

Rstudio layout – what is a variable?



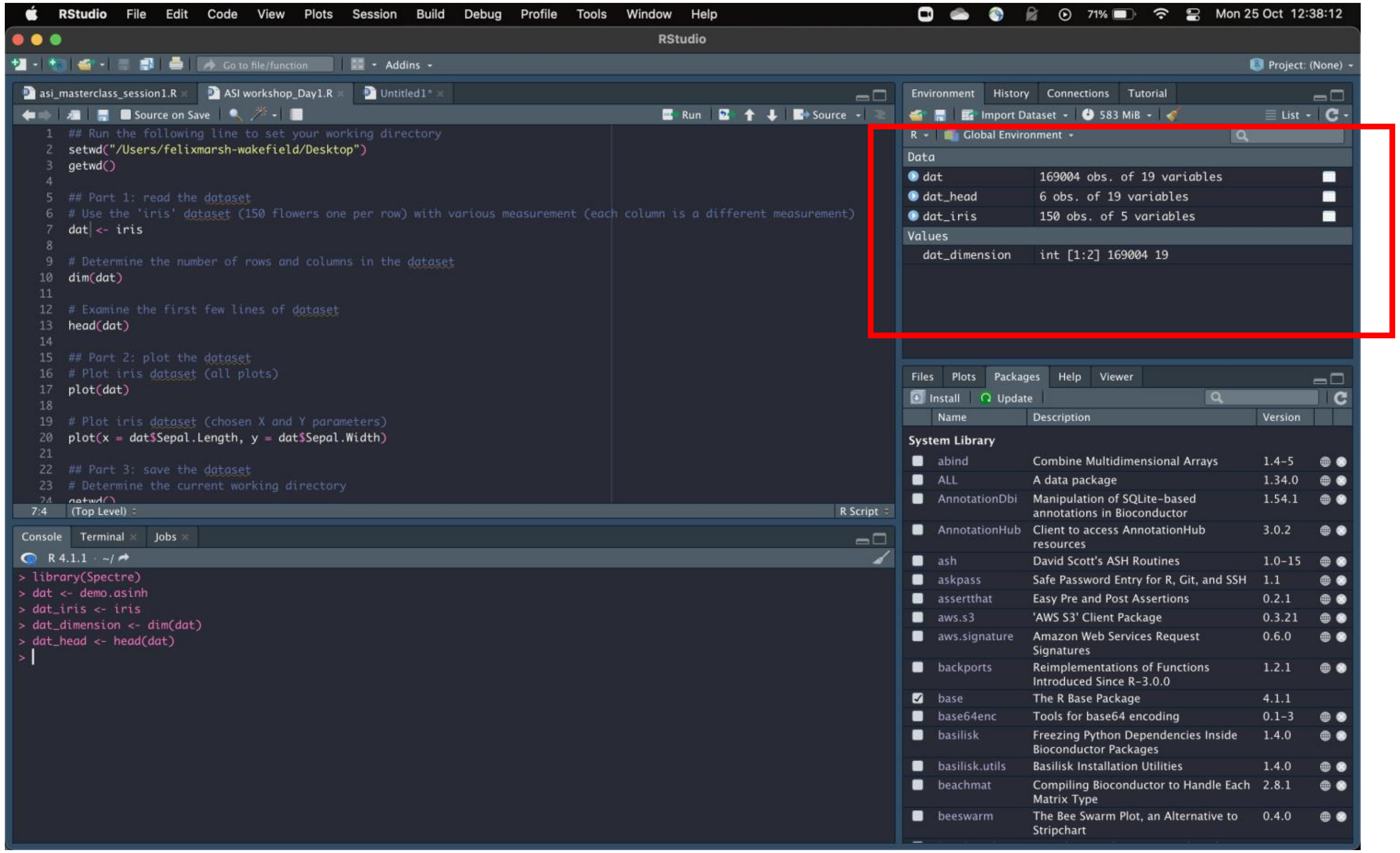
```r
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
> library(Spectre)
> dat <- demo.asinh
> dat_iris <- iris
> dat_dimension <- dim(dat)
> dat_head <- head(dat)
>
```

```
Data
dat             169004 obs. of 19 variables
dat_head        6 obs. of 19 variables
dat_iris        150 obs. of 5 variables
Values
dat_dimension   int [1:2] 169004 19
```

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Rstudio layout – different variable type



```r
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
R 4.1.1 · ~/
> library(Spectre)
> dat <- demo.asinh
> dat_iris <- iris
> dat_dimension <- dim(dat)
> dat_head <- head(dat)
> |
```

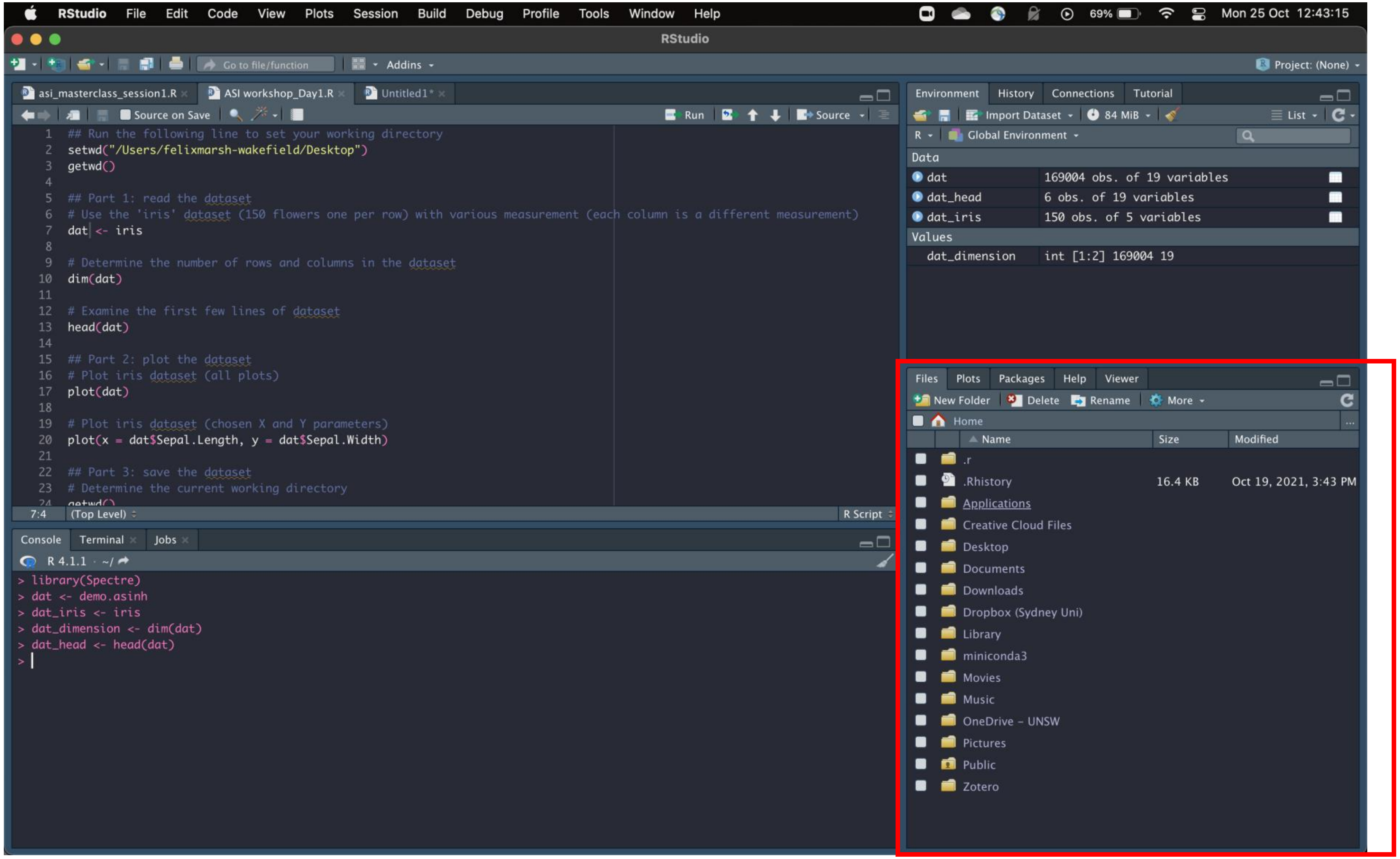
```
Data
dat            169004 obs. of 19 variables
dat_head       6 obs. of 19 variables
dat_iris       150 obs. of 5 variables
Values
dat_dimension  int [1:2] 169004 19
```

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Variable can be of various "type":

- Character: letters, words, sentences
- Numeric
- Logical: TRUE or FALSE
- Data structure:
  - Vector: a collection of the above
  - List
  - Data frame/data.table

Rstudio layout – file manager



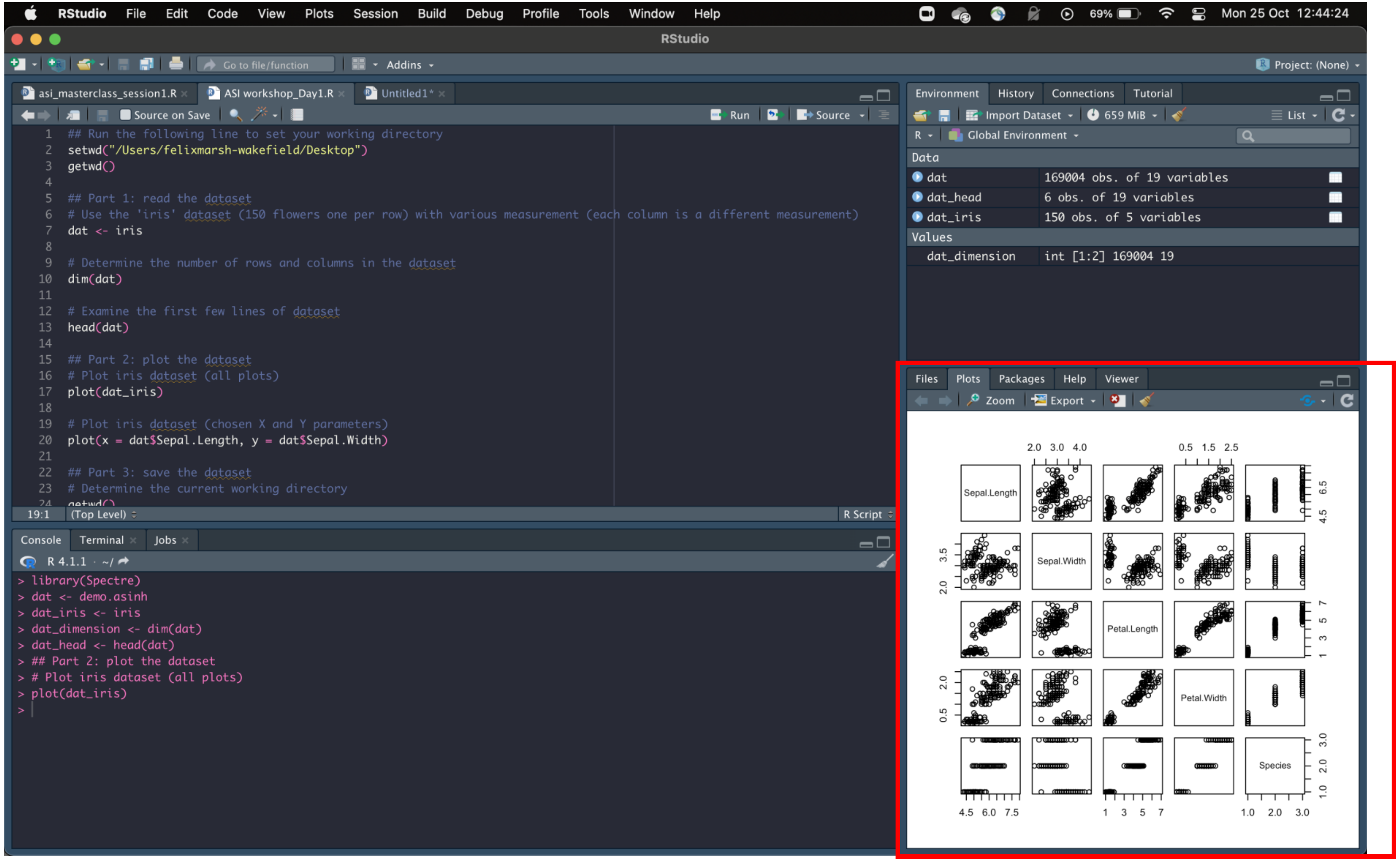
```
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
> library(Spectre)
> dat <- demo.asinh
> dat_iris <- iris
> dat_dimension <- dim(dat)
> dat_head <- head(dat)
>
```

Data	
dat	169004 obs. of 19 variables
dat_head	6 obs. of 19 variables
dat_iris	150 obs. of 5 variables
Values	
dat_dimension	int [1:2] 169004 19

Name	Size	Modified
.r		
.Rhistory	16.4 KB	Oct 19, 2021, 3:43 PM
Applications		
Creative Cloud Files		
Desktop		
Documents		
Downloads		
Dropbox (Sydney Uni)		
Library		
miniconda3		
Movies		
Music		
OneDrive – UNSW		
Pictures		
Public		
Zotero		

Rstudio layout – view plots



```
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat_iris)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
> library(Spectre)
> dat <- demo.asinh
> dat_iris <- iris
> dat_dimension <- dim(dat)
> dat_head <- head(dat)
> ## Part 2: plot the dataset
> # Plot iris dataset (all plots)
> plot(dat_iris)
>
```

```
Data
dat              169004 obs. of 19 variables
dat_head         6 obs. of 19 variables
dat_iris         150 obs. of 5 variables
Values
dat_dimension    int [1:2] 169004 19
```

Outline

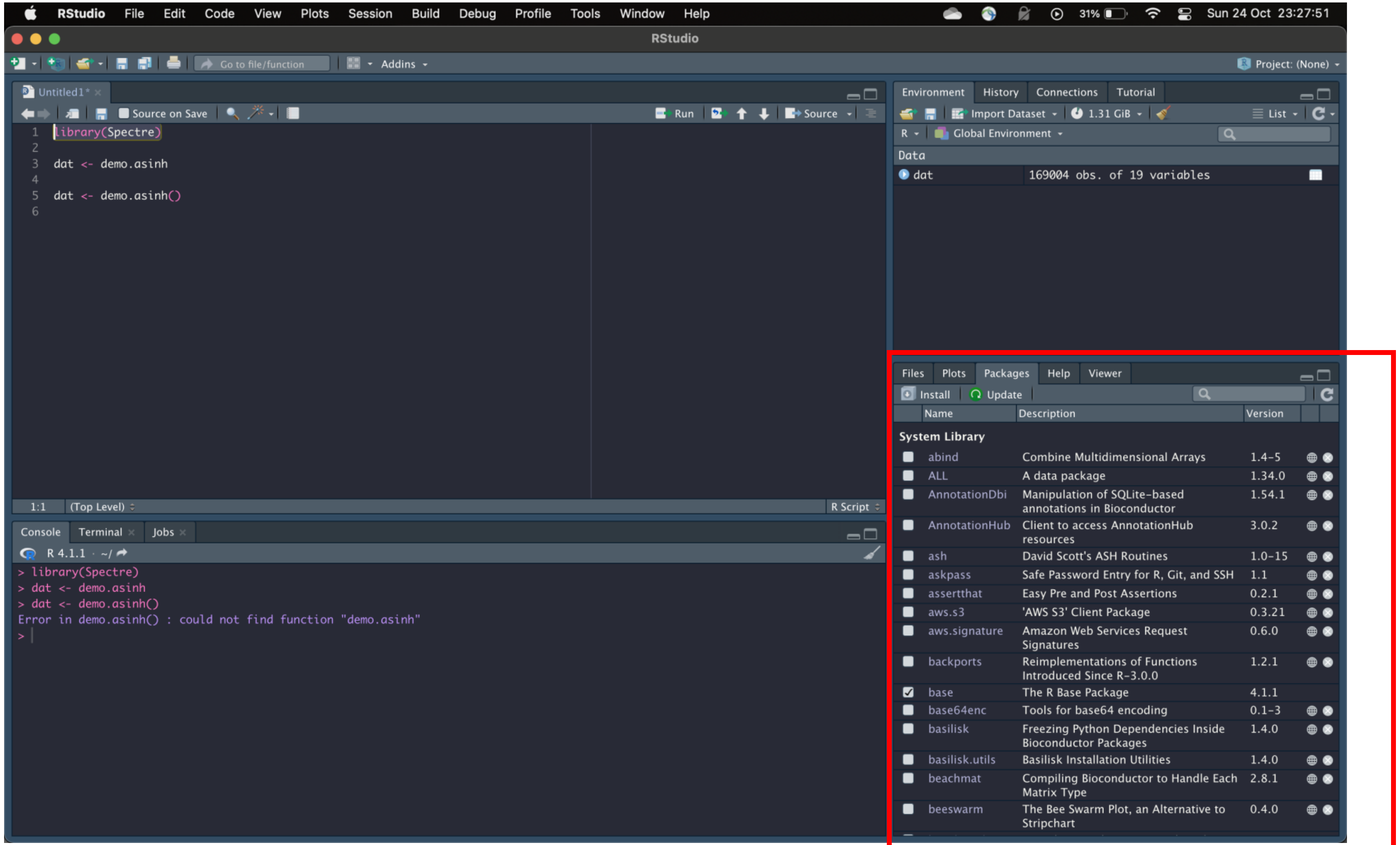
- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

What is a package

- Contain reusable R codes – functions
- Each function do something
- Package = recipe book
- A function = a recipe in the recipe book

SPECTRE

Rstudio layout – what are packages?



```
library(Spectre)

dat <- demo.asinh

dat <- demo.asinh()

```

```
> library(Spectre)
> dat <- demo.asinh
> dat <- demo.asinh()
Error in demo.asinh() : could not find function "demo.asinh"
>
```

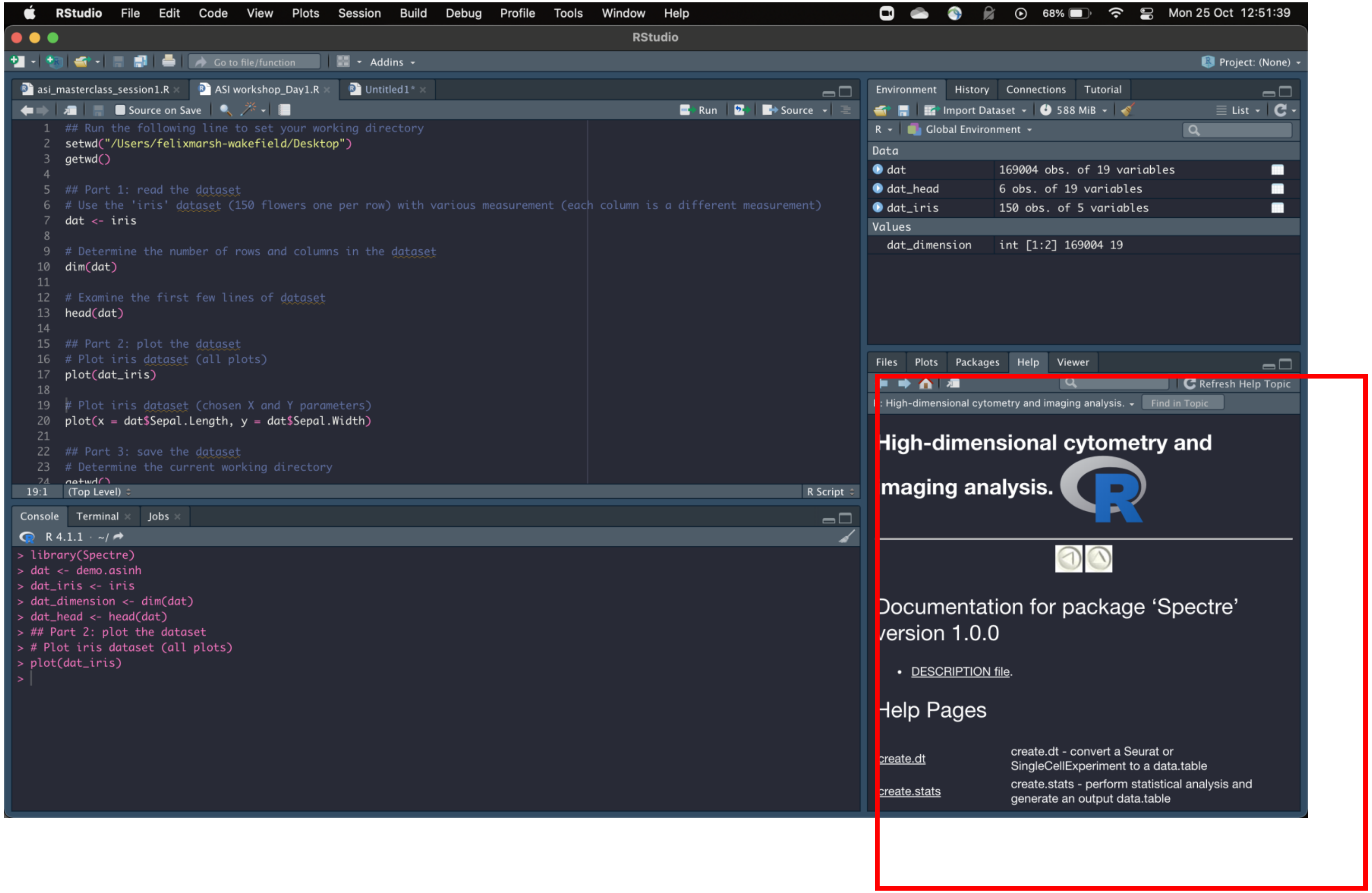
Data

dat	169004 obs. of 19 variables

System Library

	Name	Description	Version
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1
□	aws.s3	'AWS S3' Client Package	0.3.21
□	aws.signature	Amazon Web Services Request Signatures	0.6.0
□	backports	Reimplementations of Functions Introduced Since R-3.0.0	1.2.1
☑	base	The R Base Package	4.1.1
□	base64enc	Tools for base64 encoding	0.1-3
□	basilisk	Freezing Python Dependencies Inside Bioconductor Packages	1.4.0
□	basilisk.utils	Basilisk Installation Utilities	1.4.0
□	beachmat	Compiling Bioconductor to Handle Each Matrix Type	2.8.1
□	beeswarm	The Bee Swarm Plot, an Alternative to Stripchart	0.4.0

Rstudio layout – what are packages?



```
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat_iris)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
> library(Spectre)
> dat <- demo.asinh
> dat_iris <- iris
> dat_dimension <- dim(dat)
> dat_head <- head(dat)
> ## Part 2: plot the dataset
> # Plot iris dataset (all plots)
> plot(dat_iris)
>
```

Data	
dat	169004 obs. of 19 variables
dat_head	6 obs. of 19 variables
dat_iris	150 obs. of 5 variables
Values	
dat_dimension	int [1:2] 169004 19

High-dimensional cytometry and maging analysis.

Documentation for package 'Spectre' version 1.0.0

- DESCRIPTION file.

Help Pages

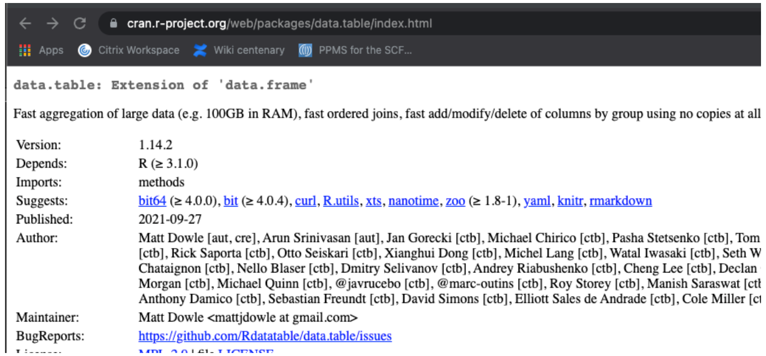
create.dt	create.dt - convert a Seurat or SingleCellExperiment to a data.table
create.stats	create.stats - perform statistical analysis and generate an output data.table

Package?

- Contain reusable R codes – functions
- Each function do something
- Package = recipe book
- A function = a recipe in the recipe book

Installing packages

- Packages can be installed from different CRAN or Bioconductor or GitHub
- How do you know whether to install a package from?
    - Google the package name and it should tell you.
    - Or the manuscript for the package should tell you.



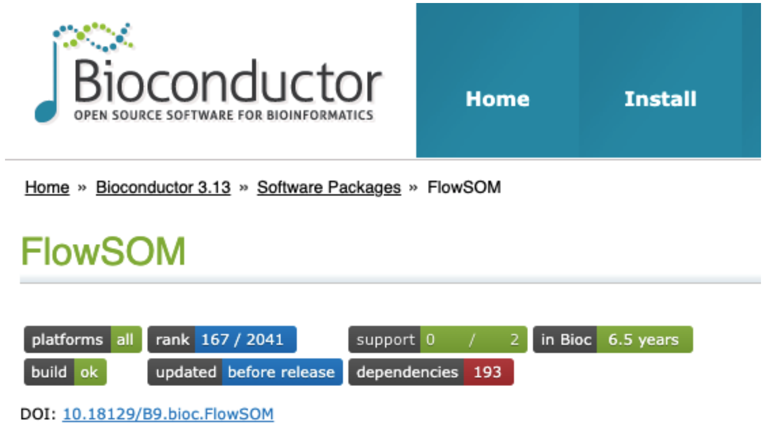
cran.r-project.org/web/packages/data.table/index.html

`data.table: Extension of 'data.frame'`

Fast aggregation of large data (e.g. 100GB in RAM), fast ordered joins, fast add/modify/delete of columns by group using no copies at all

Version:	1.14.2
Depends:	R (≥ 3.1.0)
Imports:	methods
Suggests:	bit64 (≥ 4.0.0), bit (≥ 4.0.4), curl, R.utils, xts, nanotime, zoo (≥ 1.8-1), yaml, knitr, rmarkdown
Published:	2021-09-27
Author:	Matt Dowle [aut, cre], Arun Srinivasan [aut], Jan Gorecki [ctb], Michael Chirico [ctb], Pasha Stetsenko [ctb], Tom [ctb], Rick Saporta [ctb], Otto Seiskari [ctb], Xianghui Dong [ctb], Michel Lang [ctb], Watal Iwasaki [ctb], Seth W Chataignon [ctb], Nello Blaser [ctb], Dmitry Selivanov [ctb], Andrey Riabushenko [ctb], Cheng Lee [ctb], Declan Morgan [ctb], Michael Quinn [ctb], @javrucebo [ctb], @marc-outins [ctb], Roy Storey [ctb], Manish Saraswat [ctl Anthony Damico [ctb], Sebastian Freundt [ctb], David Simons [ctb], Elliott Sales de Andrade [ctb], Cole Miller [ct
Maintainer:	Matt Dowle <mattjdowle at gmail.com>
BugReports:	https://github.com/Rdatatable/data.table/issues

CRAN

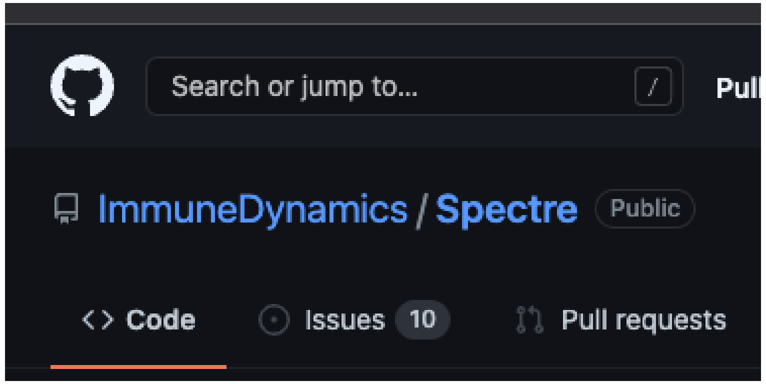


Home » Bioconductor 3.13 » Software Packages » FlowSOM

FlowSOM

DOI: 10.18129/B9.bioc.FlowSOM

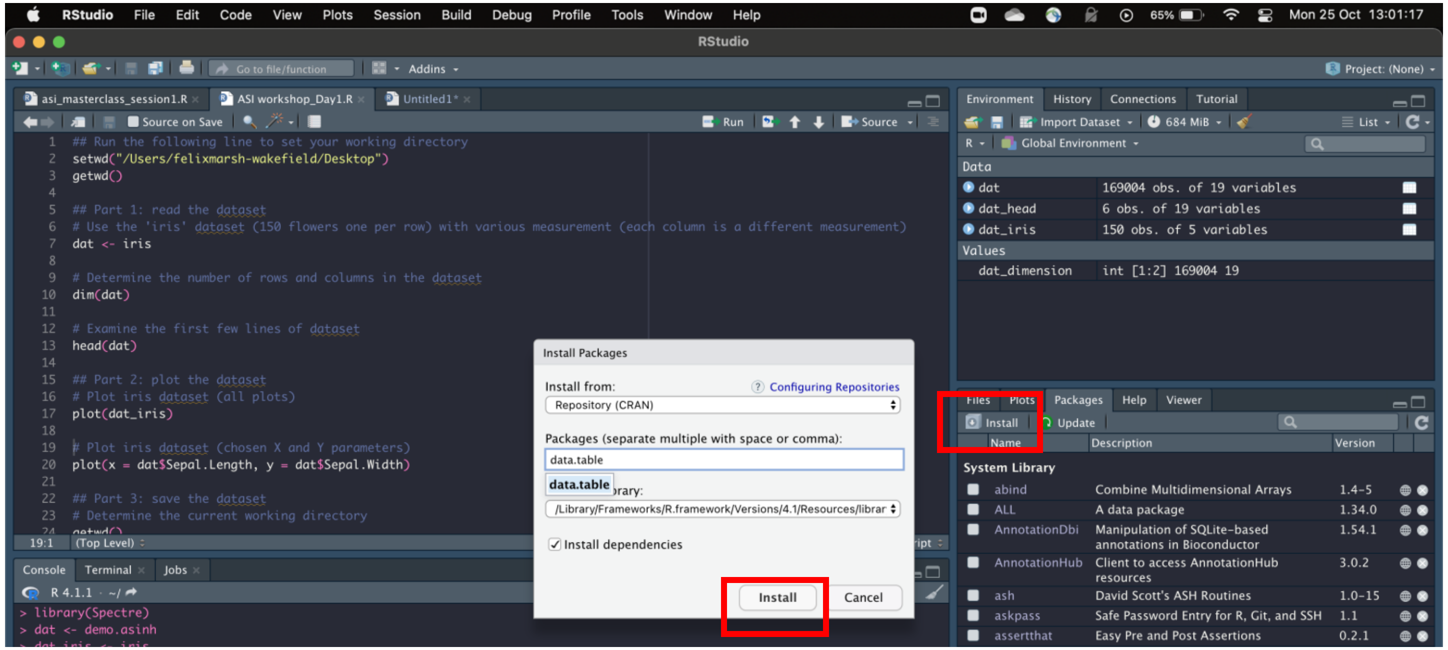
Bioconductor



GitHub

Installing packages

- Installing from CRAN:
  - install.packages(“data.table”)
    - Data.table is the name of the package
  - Or by using the packages tab on bottom right panel of Rstudio



```r
## Run the following line to set your working directory
setwd("/Users/felixmarsh-wakefield/Desktop")
getwd()

## Part 1: read the dataset
# Use the 'iris' dataset (150 flowers one per row) with various measurement (each column is a different measurement)
dat <- iris

# Determine the number of rows and columns in the dataset
dim(dat)

# Examine the first few lines of dataset
head(dat)

## Part 2: plot the dataset
# Plot iris dataset (all plots)
plot(dat_iris)

# Plot iris dataset (chosen X and Y parameters)
plot(x = dat$Sepal.Length, y = dat$Sepal.Width)

## Part 3: save the dataset
# Determine the current working directory
getwd()
```

```
> library(Spectre)
> dat <- demo.asinh
```

Install Packages

Install from: Repository (CRAN)

Configuring Repositories

Packages (separate multiple with space or comma): data.table

data.table

/Library/Frameworks/R.framework/Versions/4.1/Resources/librar

☑ Install dependencies

Install   Cancel

Data	
dat	169004 obs. of 19 variables
dat_head	6 obs. of 19 variables
dat_iris	150 obs. of 5 variables
Values	
dat_dimension	int [1:2] 169004 19

	Name	Description	Version
	System Library		
□	abind	Combine Multidimensional Arrays	1.4-5
□	ALL	A data package	1.34.0
□	AnnotationDbi	Manipulation of SQLite-based annotations in Bioconductor	1.54.1
□	AnnotationHub	Client to access AnnotationHub resources	3.0.2
□	ash	David Scott's ASH Routines	1.0-15
□	askpass	Safe Password Entry for R, Git, and SSH	1.1
□	assertthat	Easy Pre and Post Assertions	0.2.1

Installing packages

- Installing from Bioconductor:
  - Install the “BiocManager” package first:
    - install.packages(“BiocManager”)
  - Then use the install function within BiocManager to install the desired package:
    - BiocManager::install(”FlowSOM”)

Installing packages

- Installing from GitHub:
  - Install the “remotes” package first:
    - install.packages(“remotes”)
  - Then use the install function within the remotes package to install the desired package:
    - remotes::install_github(“immunedynamics/Spectre”)

Repository containing the package

Hands on practical #3

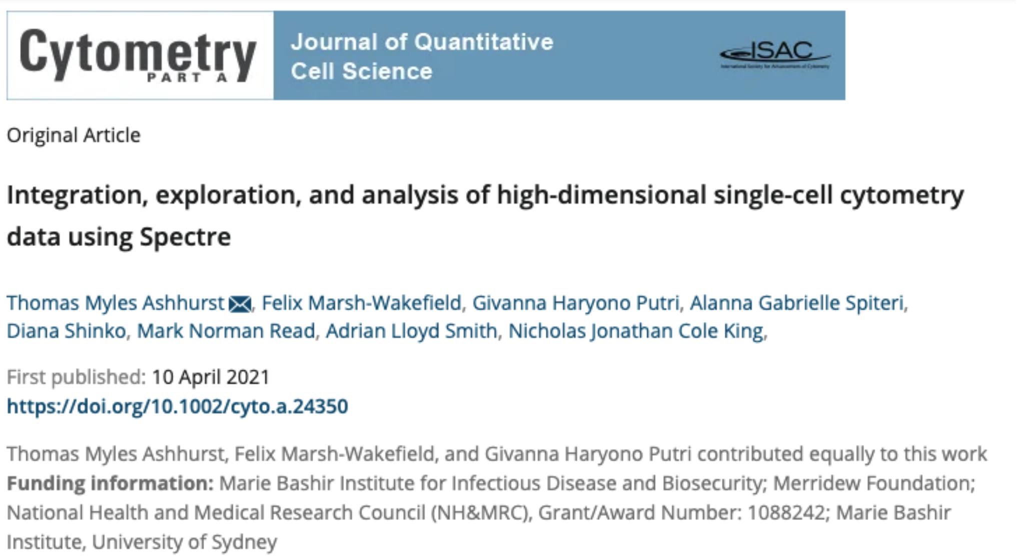
Installing packages using CRAN, Bioconductor, Github

Outline

- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

What is Spectre?

- R package containing various computational tools for exploring and analysing single-cell cytometry data.
- DOI: https://doi.org/10.1002/cyto.a.24350



Cytometry PART A | Journal of Quantitative Cell Science | ISAC

Original Article

Integration, exploration, and analysis of high-dimensional single-cell cytometry data using Spectre

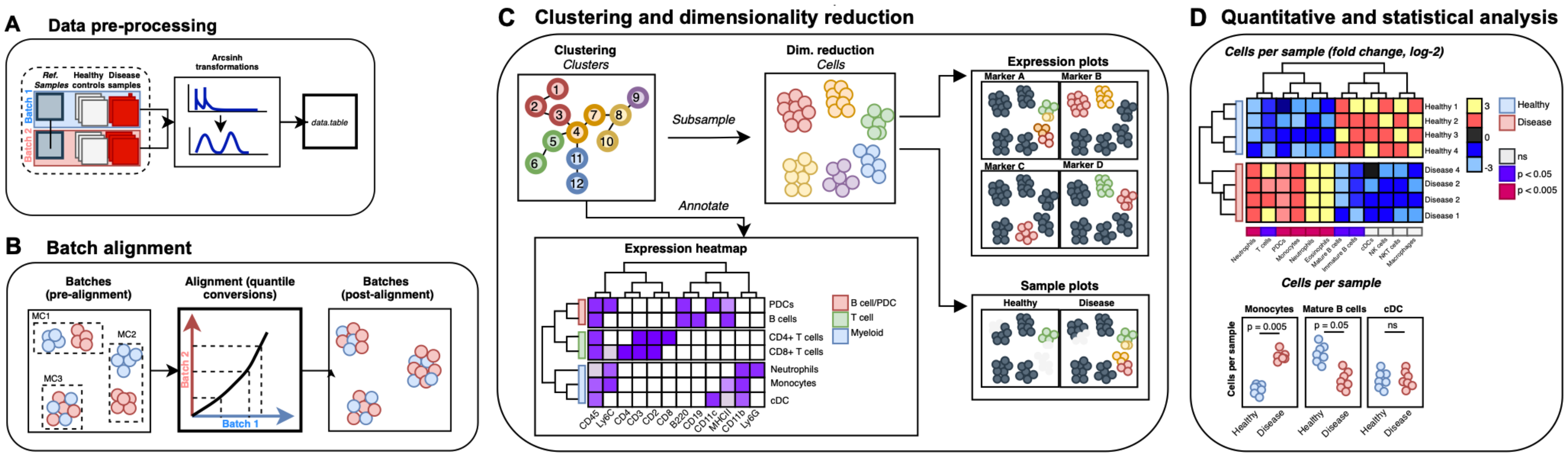
Thomas Myles Ashhurst, Felix Marsh-Wakefield, Givanna Haryono Putri, Alanna Gabrielle Spiteri, Diana Shinko, Mark Norman Read, Adrian Lloyd Smith, Nicholas Jonathan Cole King,

First published: 10 April 2021
https://doi.org/10.1002/cyto.a.24350

Thomas Myles Ashhurst, Felix Marsh-Wakefield, and Givanna Haryono Putri contributed equally to this work
Funding information: Marie Bashir Institute for Infectious Disease and Biosecurity; Merridew Foundation; National Health and Medical Research Council (NH&MRC), Grant/Award Number: 1088242; Marie Bashir Institute, University of Sydney

SPECTRE

What is Spectre?



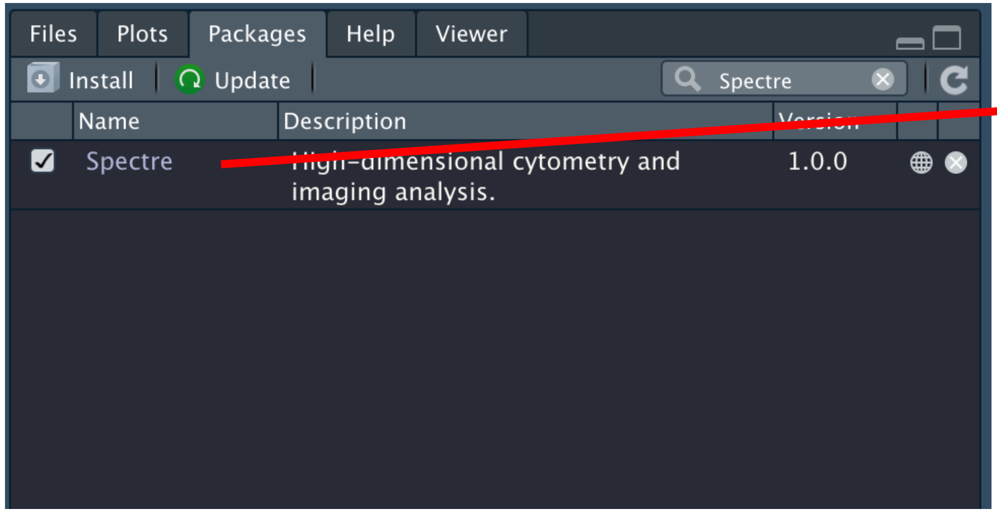
Outline

- Introduction to R and Rstudio
- Installing R and Rstudio on your computer
- What are packages and how to install them
- Introduction to the Spectre package
- Basic data manipulation using R and Spectre

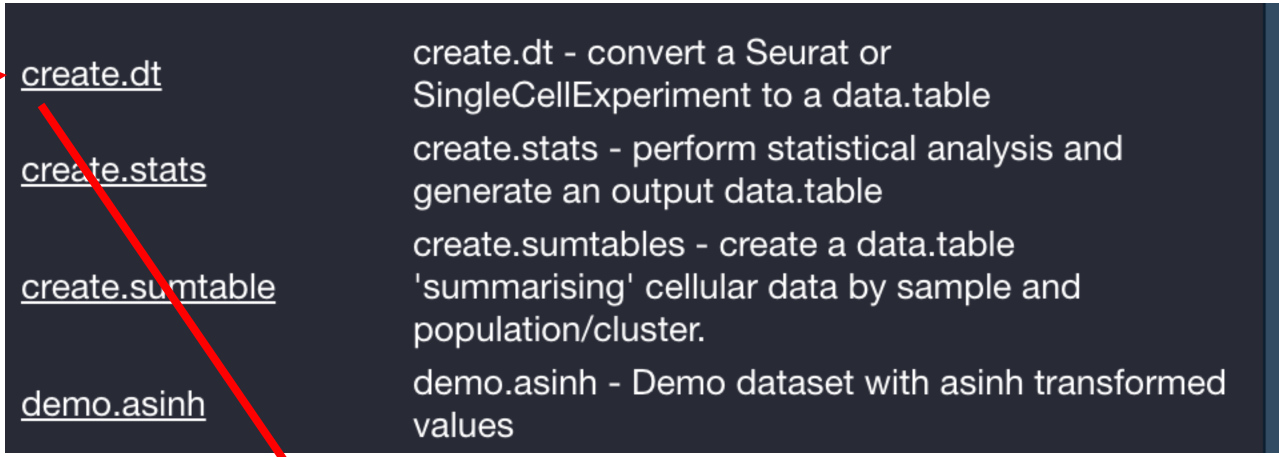
Anatomy of functions

- Each package contains various functions.
- Each function do something – akin to a recipe within a book.
- Each function has name and *sometimes* parameter
    - Parameter govern how the function behave
- A function *may* return data which you can store in a variable.

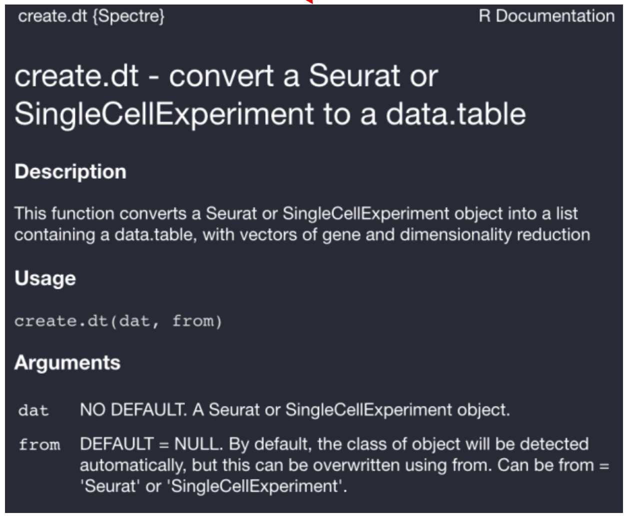
Get list of functions in a package



	Name	Description	Version
☑	Spectre	High-dimensional cytometry and imaging analysis.	1.0.0



create.dt	create.dt - convert a Seurat or SingleCellExperiment to a data.table
create.stats	create.stats - perform statistical analysis and generate an output data.table
create.sumtable	create.sumtables - create a data.table 'summarising' cellular data by sample and population/cluster.
demo.asinh	demo.asinh - Demo dataset with asinh transformed values



create.dt {Spectre} R Documentation

create.dt - convert a Seurat or SingleCellExperiment to a data.table

Description

This function converts a Seurat or SingleCellExperiment object into a list containing a data.table, with vectors of gene and dimensionality reduction

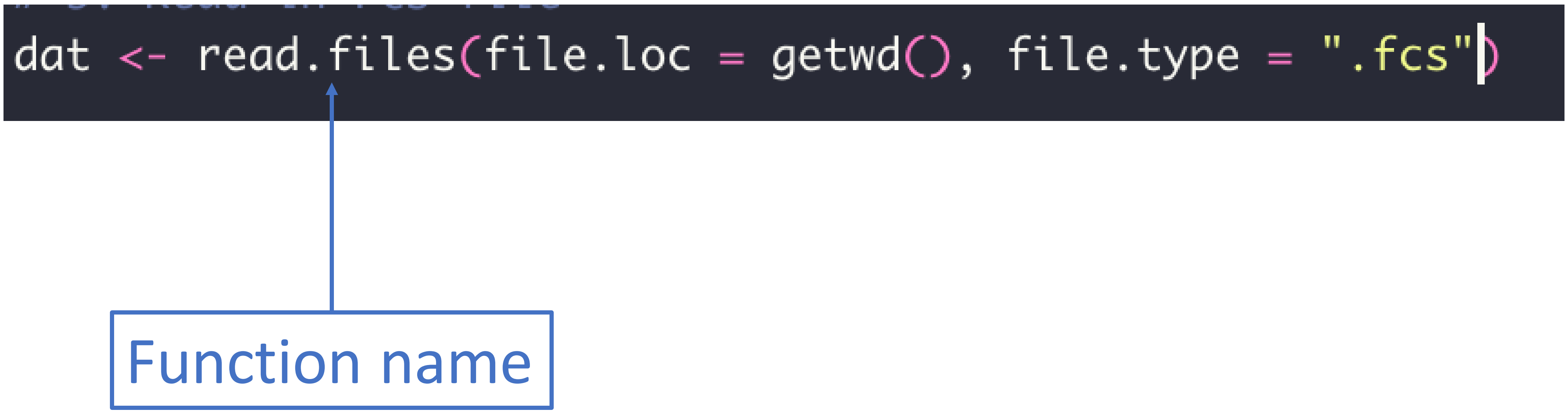
Usage

```
create.dt(dat, from)
```

Arguments

`dat`	NO DEFAULT. A Seurat or SingleCellExperiment object.
`from`	DEFAULT = NULL. By default, the class of object will be detected automatically, but this can be overwritten using from. Can be from = 'Seurat' or 'SingleCellExperiment'.

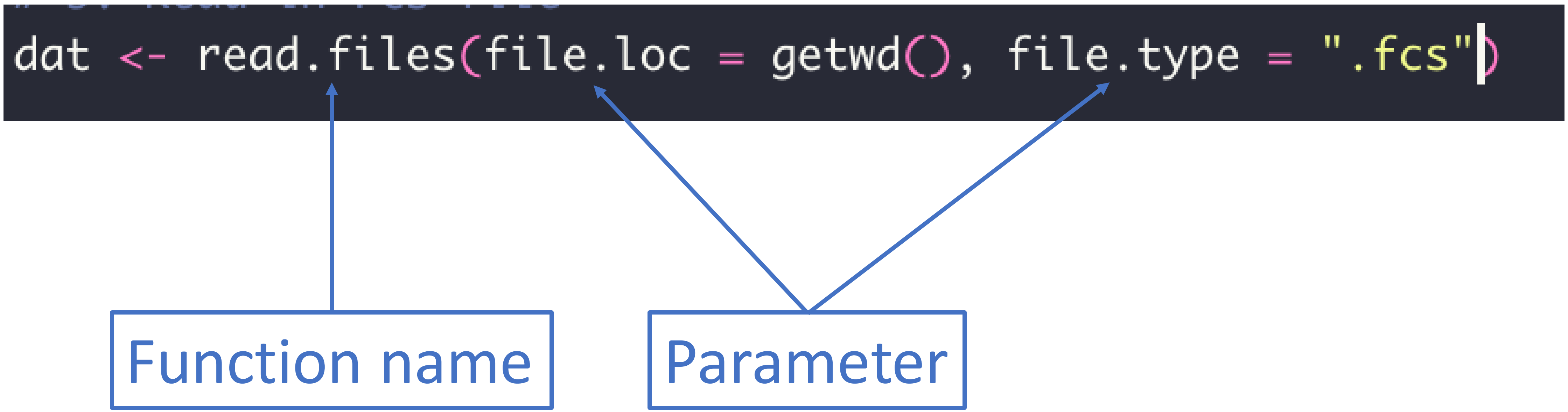
Anatomy of functions



```
dat <- read.files(file.loc = getwd(), file.type = ".fcs")
```

Function name

Anatomy of functions

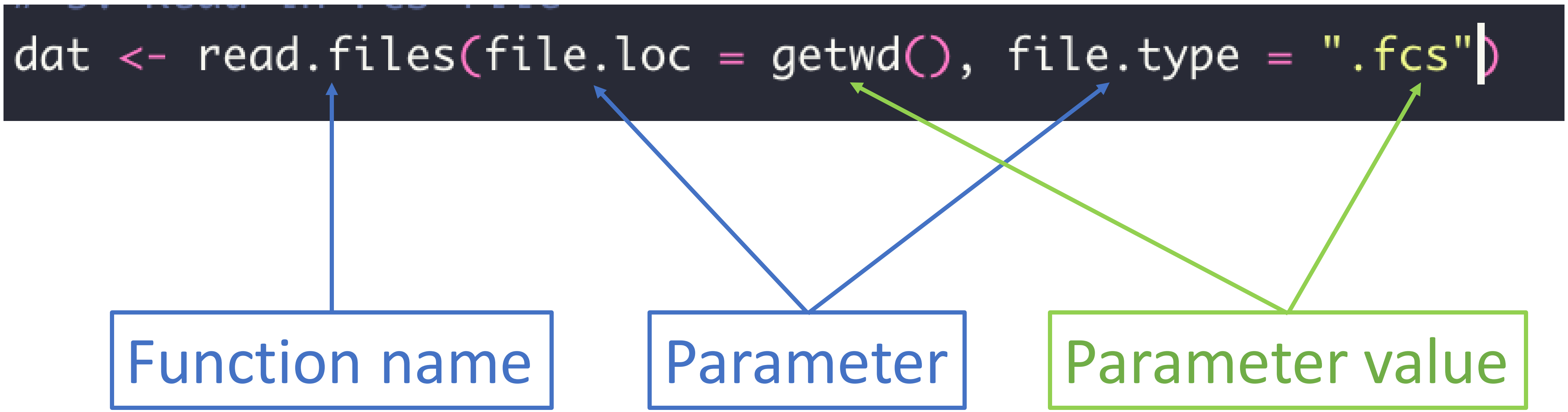


```r
dat <- read.files(file.loc = getwd(), file.type = ".fcs")
```

Function name

Parameter

Anatomy of functions



```
dat <- read.files(file.loc = getwd(), file.type = ".fcs")
```

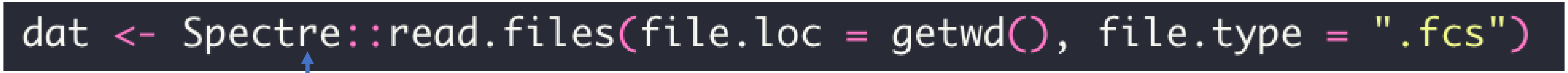
Function name

Parameter

Parameter value

Anatomy of functions

Different packages can share same function name even though they work differently!



```r
dat <- Spectre::read.files(file.loc = getwd(), file.type = ".fcs")
```

Prepend the function name with the package name

Basic data manipulation using R and Spectre

1. Setting working directory
2. Loading Spectre package
3. Read in FCS or CSV files into variable
4. Investigate the data
5. Save the result as FCS or CSV file

Hands on practical #4

Basic data manipulation using R and Spectre

What you have learnt so far?

- Alternative to proprietary software like FlowJo to analyse your data.
- You learnt how to install R and Rstudio in your own computer.
- There are many freely available (but please cite) packages online you can download and use from CRAN, Bioconductor, and GitHub.
- Spectre is an R package for exploring and analysing single-cell cytometry data (as well as imaging and scRNAseq).
- You can write and run R code to perform basic data manipulation.
- https://immunedynamics.io/masterclass2021/workshop-mainpage/session1