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# **guide template Documentation**

***Release 0.1.0***

**Open Microscopy Environment**

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This section shows how to install and use the R Gateway to analyze data. Jupyter notebooks are available to demonstrate some of the functionalities available.



## CONTENTS

### 1.1 Installing rOMERO-gateway

rOMERO-gateway will use rJava to communicate with an OMERO server.

#### 1.1.1 Install in a Conda environment

In this section, we show how to install rOMERO-gateway and additional packages for image analysis (see `environment.yml` and `postBuild`) in a [Conda](#) environment.

- Install [Miniconda](#) if necessary.
- If you do not have a local copy of the [omero-guide-r repository](#), first clone the repository:

```
$ git clone https://github.com/ome/omero-guide-r.git
```

- Go into the directory:

```
$ cd omero-guide-r
```

- Create a programming environment using Conda:

```
$ conda create -n rgateway
```

- Install dependencies and some packages used in the notebooks using an installation file:

```
$ conda env update -n rgateway --file binder/environment.yml
```

- Run a bash script to install rOMERO-gateway and the Java dependencies:

```
$ bash binder/postBuild
```

- Activate the environment:

```
$ conda activate rgateway
```

### 1.1.2 Install in RStudio

See *Installing and using romero.gateway package in RStudio*

#### Installing and using romero.gateway package in RStudio

##### Linux

This example uses Debian 10. On other distributions the commands and packages might be called slightly differently.

As *root* user install the system dependencies: `apt install r-base openjdk-11-jdk libssl-dev libcurl4-openssl-dev`

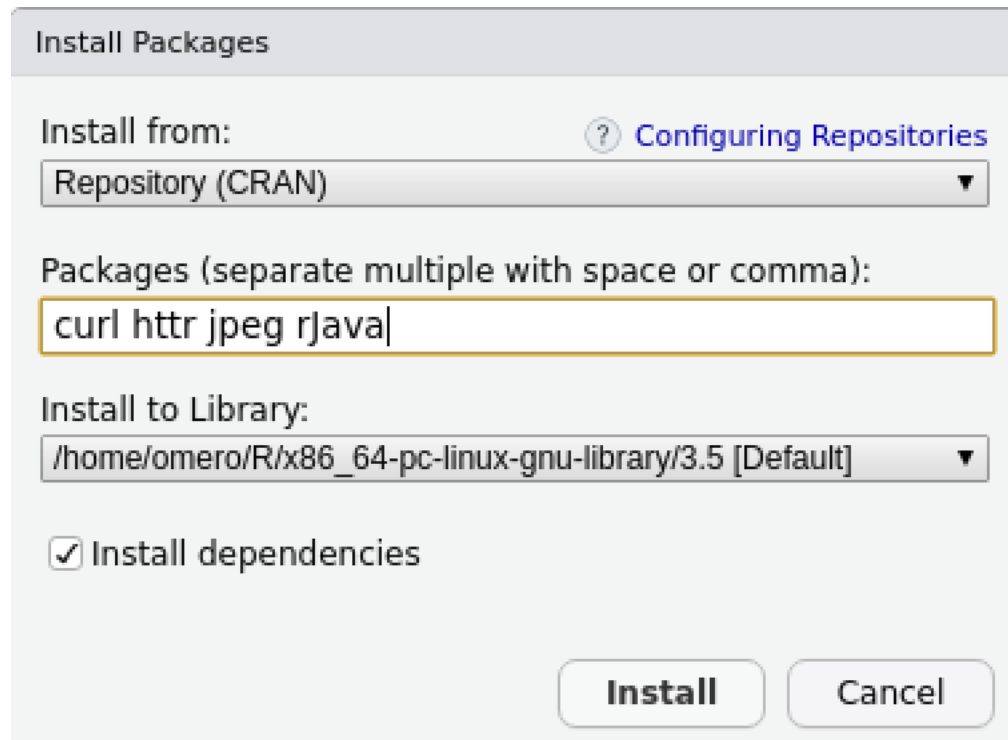
Then you need to find out where exactly the JDK was installed: `ls -l /etc/alternatives/ | grep java`

Lookout for 'openjdk' to find the location.

Configure Java for R (still using *root* user):

```
export JAVA_HOME=/usr/lib/jvm/java-11-openjdk-amd64 # Whatever your path to openjdk is
R CMD javareconf
```

As normal user install and start up RStudio. In RStudio install the dependencies from CRAN needed for the romero.gateway package:

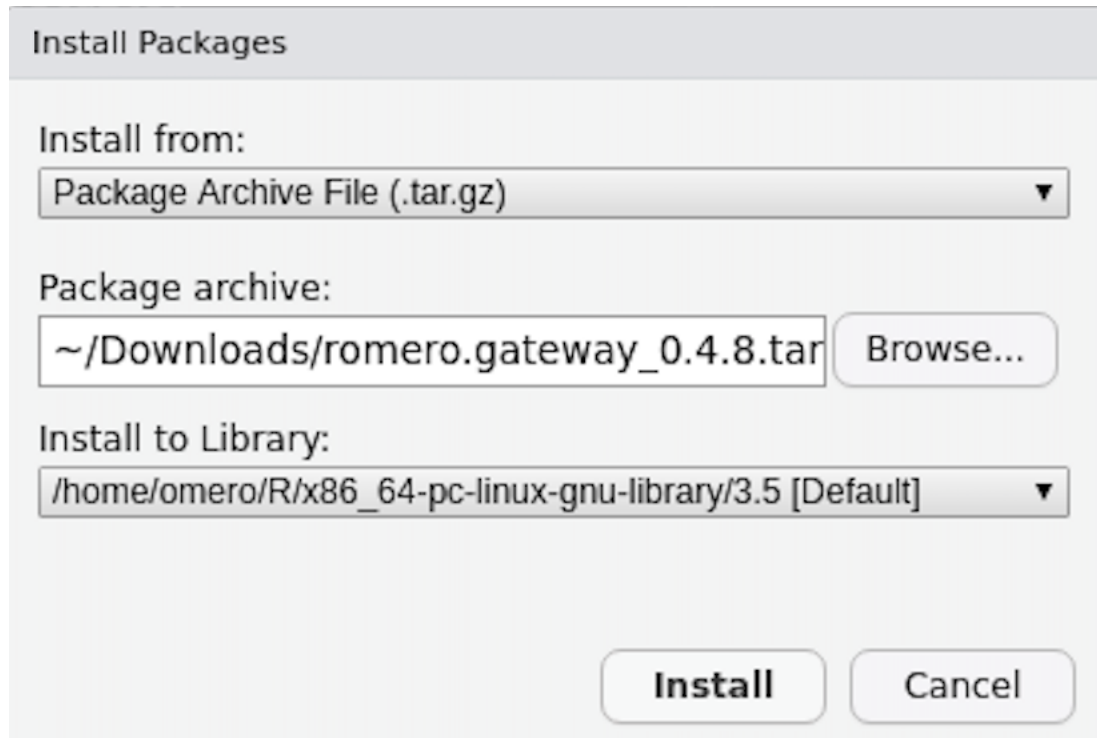


The screenshot shows the 'Install Packages' dialog in RStudio. The 'Install from:' dropdown is set to 'Repository (CRAN)'. The 'Packages (separate multiple with space or comma):' text box contains 'curl httr jpeg rJava'. The 'Install to Library:' dropdown is set to '/home/omero/R/x86\_64-pc-linux-gnu-library/3.5 [Default]'. The 'Install dependencies' checkbox is checked. 'Install' and 'Cancel' buttons are at the bottom right.

Alternative: Run the following in the console: `install.packages(c("jpeg", "curl", "httr", "rJava"))`

Download the latest romero.gateway release tar.gz from [Github](#). Install it in RStudio:





Install Packages

Install from:  
Package Archive File (.tar.gz) ▼

Package archive:  
~/Downloads/romero.gateway\_0.4.8.tar Browse...

Install to Library:  
/home/omero/R/x86\_64-pc-linux-gnu-library/3.5 [Default] ▼

Install Cancel

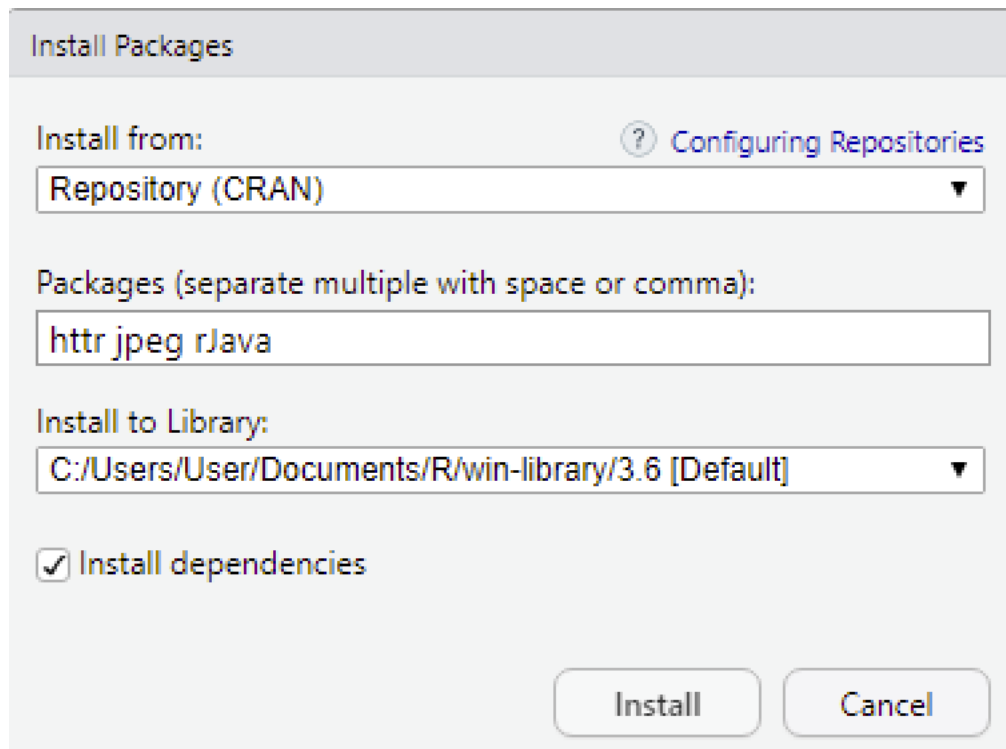
Alternative: Run the following in the console: `install.packages("~/Downloads/romero.gateway_0.4.8.tar.gz", repos = NULL, type = "source")`

## Windows

This example uses Windows 10, other Windows version should be similar.

Install a JDK, for example [AdoptOpenJDK](#). Make sure to tick the checkbox to set the *JAVA\_HOME* and *PATH* environment variables!

Install the dependencies `httr`, `jpeg` and `rJava` from CRAN:



The 'Install Packages' dialog box in RStudio. It has a title bar 'Install Packages'. Below it, 'Install from:' is a dropdown menu set to 'Repository (CRAN)', with a help icon and 'Configuring Repositories' link. 'Packages (separate multiple with space or comma):' is a text box containing 'httr jpeg rJava'. 'Install to Library:' is a dropdown menu set to 'C:/Users/User/Documents/R/win-library/3.6 [Default]'. There is a checked checkbox for 'Install dependencies'. At the bottom are 'Install' and 'Cancel' buttons.

Install Packages

Install from: [? Configuring Repositories](#)  
Repository (CRAN) ▼

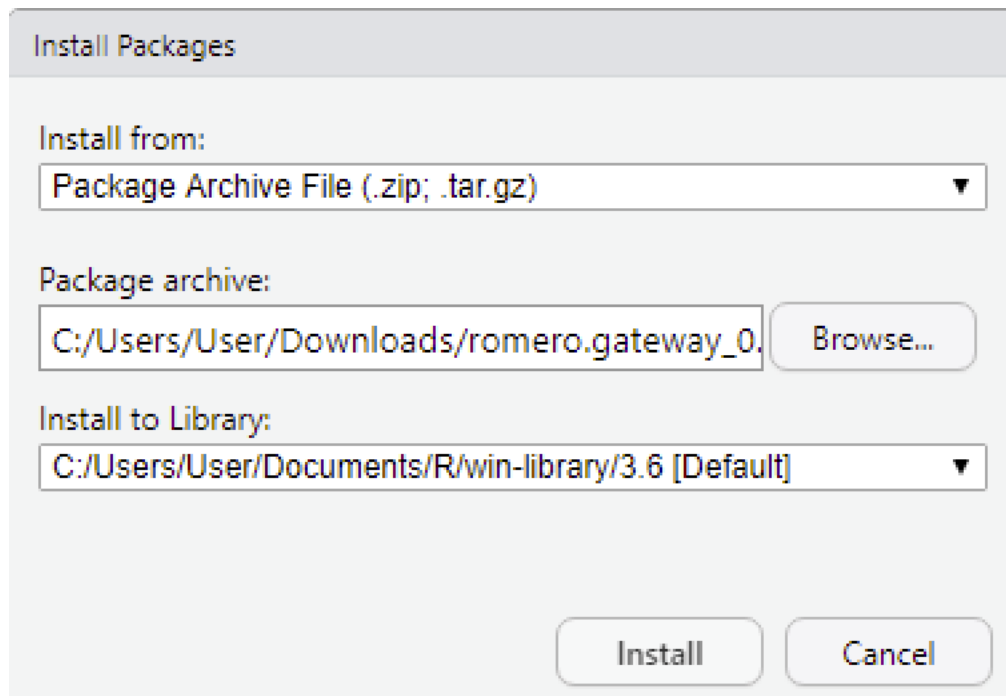
Packages (separate multiple with space or comma):  
httr jpeg rJava

Install to Library:  
C:/Users/User/Documents/R/win-library/3.6 [Default] ▼

☒ Install dependencies

Install Cancel

Download the latest `romero.gateway` release zip from [Github](#) and install it:



The 'Install Packages' dialog box in RStudio, configured for installing from a package archive. It has a title bar 'Install Packages'. Below it, 'Install from:' is a dropdown menu set to 'Package Archive File (.zip; .tar.gz)'. 'Package archive:' is a text box containing 'C:/Users/User/Downloads/romero.gateway\_0.' with a 'Browse...' button next to it. 'Install to Library:' is a dropdown menu set to 'C:/Users/User/Documents/R/win-library/3.6 [Default]'. At the bottom are 'Install' and 'Cancel' buttons.

Install Packages

Install from:  
Package Archive File (.zip; .tar.gz) ▼

Package archive:  
C:/Users/User/Downloads/romero.gateway\_0. Browse...

Install to Library:  
C:/Users/User/Documents/R/win-library/3.6 [Default] ▼

Install Cancel

## 1.2 Analyse data using R (statistical package)

R is a free software environment for statistical computing and graphics. See <https://www.r-project.org/> for more information.

### 1.2.1 Description

Here we demonstrate how to connect to OMERO using R, load images and other objects from OMERO and perform image analysis followed up by statistical analysis in R. We use R as part of a Jupyter notebook. In the notebook we are going to use the `idr0021` data. These are super resolution microscopy images showing certain proteins around the centrioles. With these images the authors of the article [Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material](#) showed that the area around the centrioles has a specific structure. By measuring the shape and diameter of these proteins and comparing them to each other they could show that each protein builds a specific part of this structure, see [Figure 1](#). Instead of manually measuring each centriole ring like the authors did, we are going to try to do this in an automated way in order to show that there is a significant difference between these proteins and create a plot similar to the one seen on [Figure 1](#).

We will show:

- How to connect to OMERO using R.
- How to load images from OMERO to R.
- How to perform segmentation of objects in an image in R.
- How to calculate properties of the segmented objects (e.g. diameter) in R.
- How to save the properties of the objects as an R dataframe.
- How to create and save ellipse representations of the ROI for each segmented object onto the original image in OMERO.
- How to save the properties of the objects as attachments in OMERO.
- How to load images in a whole Dataset or Project from OMERO and perform the steps above on all images.
- How to perform statistical analysis on the generated data in R and save results back to OMERO.

### 1.2.2 Setup

In order to be able to connect to OMERO from within R we need the `rOMERO-gateway` and `rJava` packages.

Note: Everything we are going to do there can be done in Rstudio as well, if the `rOMERO-gateway` package and its dependencies are available on your system.

See [Installing rOMERO-gateway](#).

### 1.2.3 Resources

We will use data from the Image Data Resource (IDR).

- IDR data `idr0021`.
- Notebook `idr0021_Segmentation.ipynb`.

For convenience, the IDR data have been imported into the training OMERO.server. This is only because we cannot save results back to IDR which is a read-only OMERO.server.

### 1.2.4 Step-by-step

All the steps are described in detail inside the Jupyter notebook. In you are running locally or in mybinder, go to:

- In the *notebooks* folder, select the notebook *idr0021\_Segmentation.ipynb*.

Note: We are going to show the image segmentation and feature calculation on a small subset of the *idr0021* data. The full analysis of the *idr0021* project has already been done and the result saved as ‘Summary from R’ table. We are going to load this table in order to do the statistical analysis at the end of the notebook.

Here, we give a digest of the steps inside the notebook: The notebook steps will:

1. Fetch the images from OMERO using rOMERO-gateway.
2. Perform image segmentation to identify the protein rings around the centrioles using EBImage in R.
3. Calculate the properties (features) of the identified objects (shape, diameter, etc.) using EBImage.
4. Store these features in R as an R dataframe.
5. The segmentation and feature calculation is a rather costly process. You don’t want to do this all over again. Therefore we save the identified objects (ROIs, region of interests) and the properties back to OMERO using rOMERO-gateway.
6. Perform statistical analysis on that data in R.
7. At the end of the notebook a plot is created which is similar to the one shown in [Figure 1](#) of the article. One dataset in particular has extreme outliers. OMERO.parade <https://github.com/ome/omero-parade> can be used to find the images that cause the outliers.
8. Note: OMERO.parade uses the *Summary from R* table that is attached to the project in order to provide enhanced filtering and plotting features.

## CONTRIBUTE

Changes to the documentation or the materials should be made directly in the [omero-guide-r repository](#).