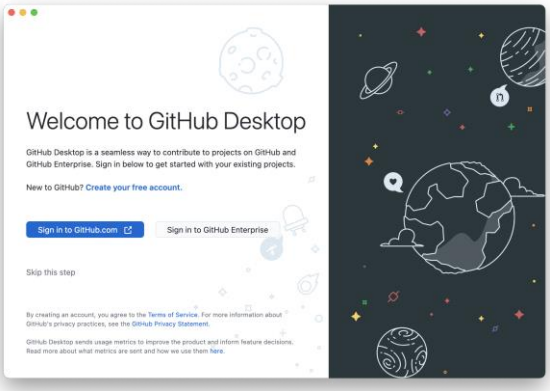
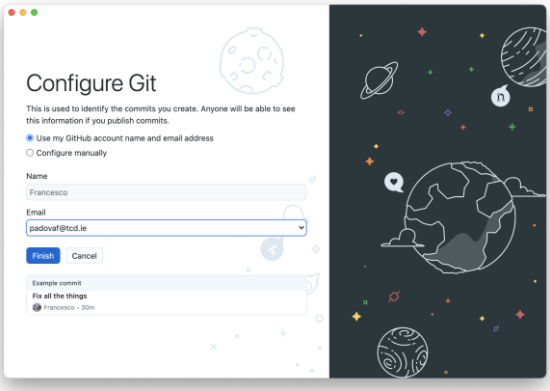
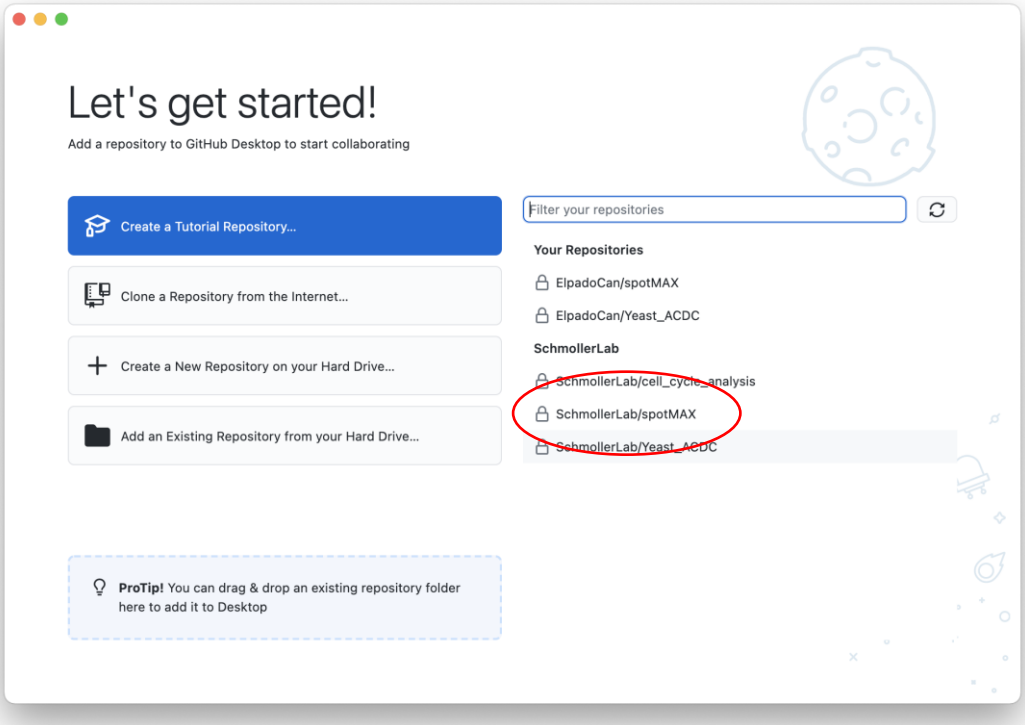
spotMAX Installation for MacOS:

1. Download Python 3.8.5 from here https://www.python.org/ftp/python/3.8.5/python-3.8.5-macosx10.9.pkg
2. Install downloaded Python with default settings
3. Download and install “GitHub Desktop” from here https://desktop.github.com/
4. Open “GitHub Desktop” and click on “Sign in to GitHub.com”. This will open a browser tab where you can Login to your GitHub account. **NOTE**: not all browsers are supported. Makes sure that you login onto a supported browser. This should not be a problem on MacOS with Safari or Chrome. I think the only one not being supported is Internet Explorer, but if your browser is not supported you will get a message. If the automatically opened browser is a non-supported one you have to change the default browser in your OS settings (in Windows type in the search “Default Apps” and change the default Web Browser) and the click again on “Sign in to GitHub.com” in “GitHub Desktop”. If the default browser was changed successfully now GitHub Desktop should open in the default browser.

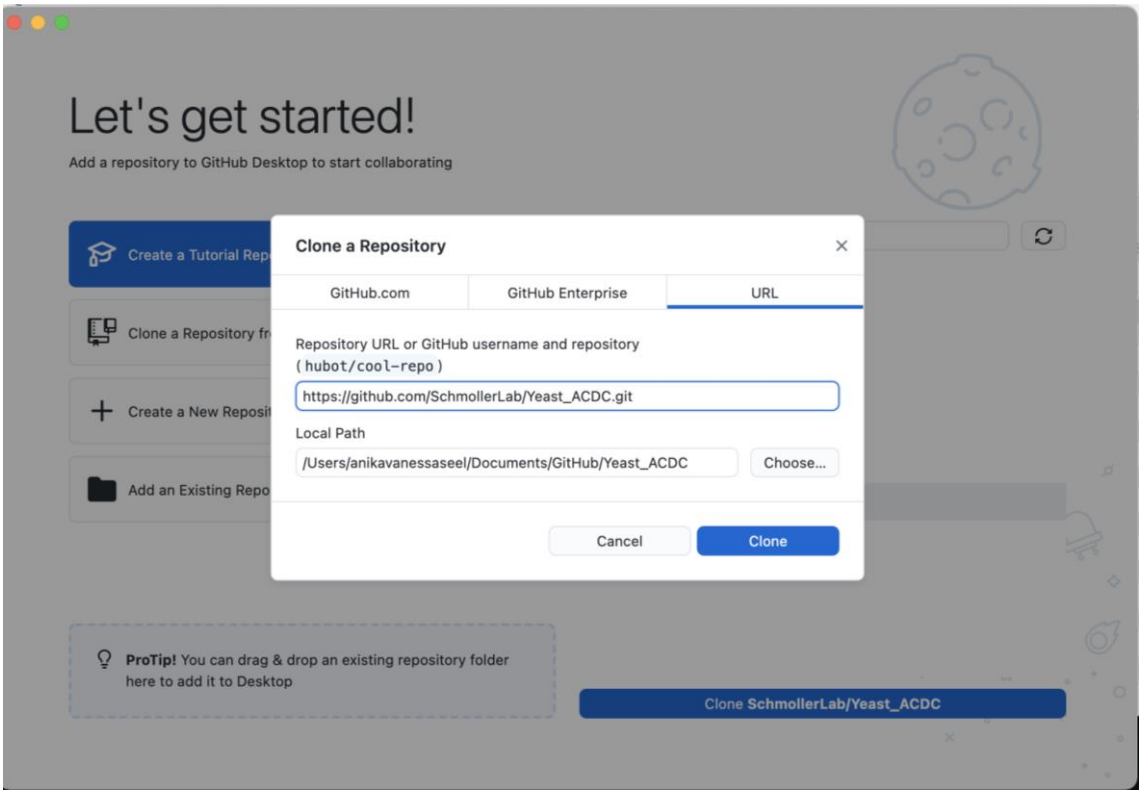




5. If the login was successful, “GitHub Desktop” should now look like this:



6. Click on “SchmollerLab/spotMAX” and then click on the button “**CLONE** SchmollerLab/spotMAX” (bottom-right)
7. In the “Local path” choose where you want to install spotMAX. The default one is fine.



8. Close GitHub desktop

9. Open **Terminal** (Click the Launchpad icon in the Dock, type Terminal in the search field, then click Terminal)

10. In the terminal navigate to the spotMAX folder with the `cd` command. In my case I have installed spotMAX (step 7) in "/Users/anikavanessaseel/Documents/GitHub/spotMAX" (in the screenshot it is Yeast_ACDC which is another software but it's the same with spotMAX. It is likely that you will also use Yeast_ACDC for segmentation anyway). When we open a Terminal in MacOS usually we are already in ""/Users/anikavanessaseel" so we only type `cd documents/github/spotmax` (capital letters do not matter, you can type all non-capital).

11. Now we are ready to install the environment for spotmax which are all the packages needed. Type the following commands one at a time:

```
python3 -m pip install --user --upgrade pip
python3 -m pip install --user virtualenv
```

12. If the terminal shows you the message

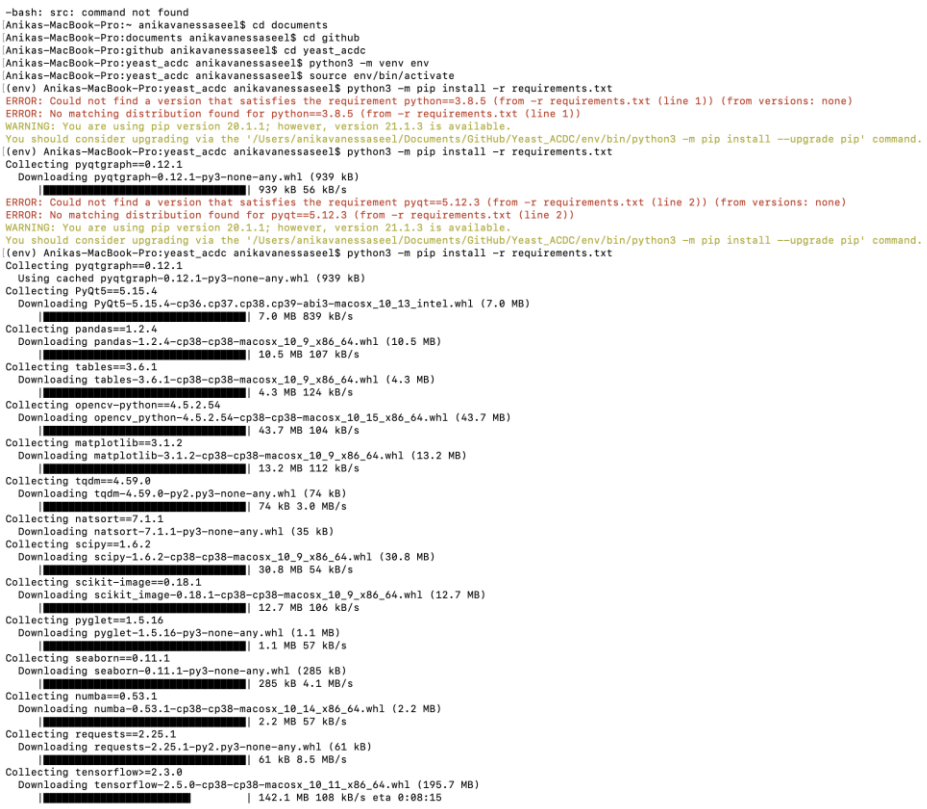
```
WARNING: The scripts pip, pip3 and pip 3.8 are installed in 'some-path' which is not in PATH
```

Then we have to add the path in the quotes to PATH. Copy that path and then type in the terminal `sudo nano /etc/paths` then paste the path at the end of the file and press "control+x" and then accept the saving with "y" and press "enter" to exit. See screenshot below

13. You should now be back in terminal (make sure you are in the spotmax folder as explained in step 10.) and we can finish installing spotMAX environment. Type the following commands one at a time:

```
python3 -m venv spotmax
source spotmax/bin/activate
python3 -m pip install -r requirements.txt
```

Now you will see several packages being installed. It will take several minutes (around 20 if I have to guess) depending on the speed of the internet connection. This is how my terminal looked like after the sequence of commands (it is installing the packages)



Preparing your data for spotMAX:

1. spotMAX automatically upload all the data it needs, but it needs it with a specific structure. To facilitate the creation of this structure we provide Fiji (ImageJ) scripts that automatically structure the data from any microscopy file (.czi, .nd2, .tif etc.). So first of all, if you don't have it, install Fiji from here https://imagej.net/software/fiji/
2. Extract the content of the downloaded Fiji folder and run "Fiji.app/ ImageJ-win64"
3. File → Open and select either "frames_to_tifs.ijm" or "snapshots_to_tifs.ijm" file from "/spotMAX/FijiMacros/" folder.
4. Before running the macro, you must edit the channels. At line 3 of modify the "channels" variable by writing a name for each channel in your file. They need to be in the exact same order they are in the original microscopy file. If you do not know the order, open the file in Fiji first and check the order of the channels there.
5. Run the script with the Run button. The script will ask you to select a folder containing the microscopy files (create a specific experiment folder for each different set of files). Select the folder (it MUST contain ONLY the microscopy files) and wait. You can see the progress in the Log Window. The script will silently open the microscopy files and save each Position (if the file contains multiple positions) into a separate folder. This is the structure that spotMAX requires for automatic loading of the relevant files.

Running spotMAX:

1. To run spotMAX open a Terminal window. Navigate to spotMAX folder as in step 10. And then type `source spotmax/bin/activate`
2. Navigate to src folder (spotMAX subfolder containing the scripts) by typing `cd src`
3. Now we can run spotMAX by typing `python3 main_v1.py`

Converting .h5 (single spots metrics) files to .csv

1. Open terminal and navigate to spotMAX folder as in step 10 (e.g., `cd documents/github/spotmax`). Then type `source spotmax/bin/activate`
2. Navigate to src/utils folder with command `cd src/utils`
3. Run script by typing `python3 h5_to_csv.py`
4. A file dialog window will pop-up. Select the .h5 file you want to convert
5. If successful, the terminal will print part of the table and another folder dialog window will pop-up. Select the FOLDER where you want to save the newly created .csv file. Done.