# FociQ User Guide

November 14, 2018

# Contents

# 1 Installation

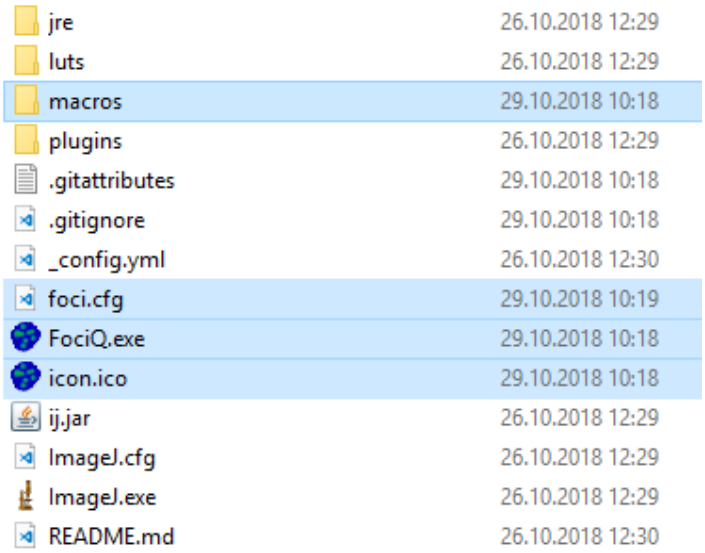
Before installing and using FociQ you will have to download ImageJ at: `https://imagej.nih.gov/ij/download.html` make sure to follow instructions on the ImageJ website. Additionally Java is required for FociQ to run `https://java.com/download/`. After installation you will have to choose your preferred installation method.

If you are on the Windows platform you may choose FociQ-Windows which the preferred way for users that are not familiar with executing Python programs from the Windows command line. Alternatively you can choose to use Python to directly launch the program. This method is valid for Windows, Linux, MacOS etc.

## 1.1 Windows Executable

Open and unzip the downloaded Windows repository. Select all files contained in the folder and paste them into your ImageJ path. Merge the macro folder - if prompted - into the existing one in your ImageJ path as shown in Figure 1. The program can be run by executing the FociQ.exe file.

Figure 1: Pasted program files in ImageJ directory.



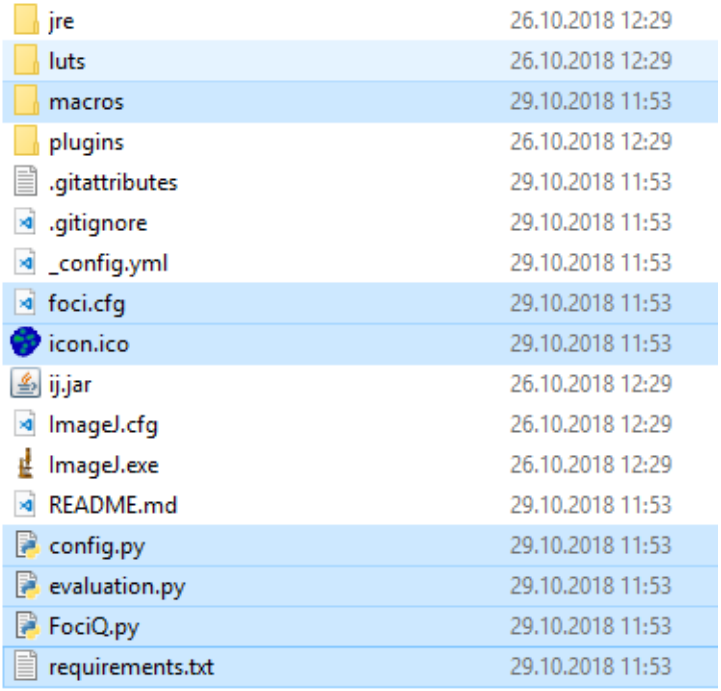
## 1.2 Python

To use the normal version of FociQ written in Python you will need Python 3.7 from `https://www.python.org/`. After installing Python you need to install required packages. You may do so by changing your directory to the downloaded FociQ folder and typing:

```
pip install -r requirements.txt
```

into your command line. Afterwards you will have to copy all contents of the extracted FociQ folder into your ImageJ folder as seen in Fig. 2.

Figure 2: Pasted program files in ImageJ directory.




the program may be executed by changing to your ImageJ folder in the command line and executing following line of code:

```
python FociQ.py
```

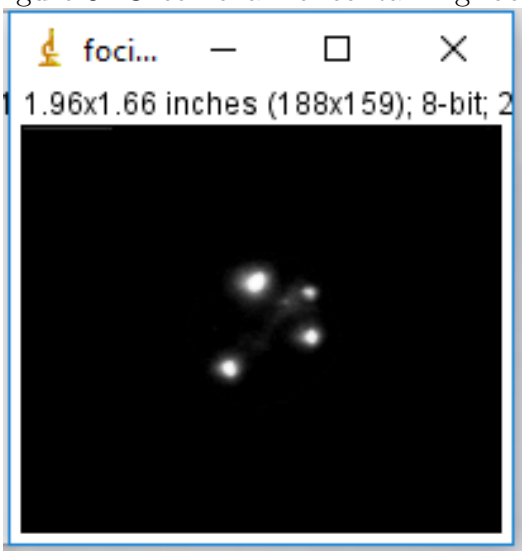
# 2 Preprocessing

Preprocessing is an important step when using this program. Before quantifying your foci you should always remove images that have artifacts which are not foci as these might be falsely interpreted by the program. Also image properties vary vastly between image sets making this step crucial.

Before preprocessing make sure that your images are in .tif or .jpg format and contain a green foci channel and a blue core marker.

Open up a test image of your set in ImageJ (File → Open), proceed by choosing Image → Color → Split Channels. You should now see three images in ImageJ these are the red, green an blue channels as grayscale. Select the green channel image containing your foci as in Fig. 3.

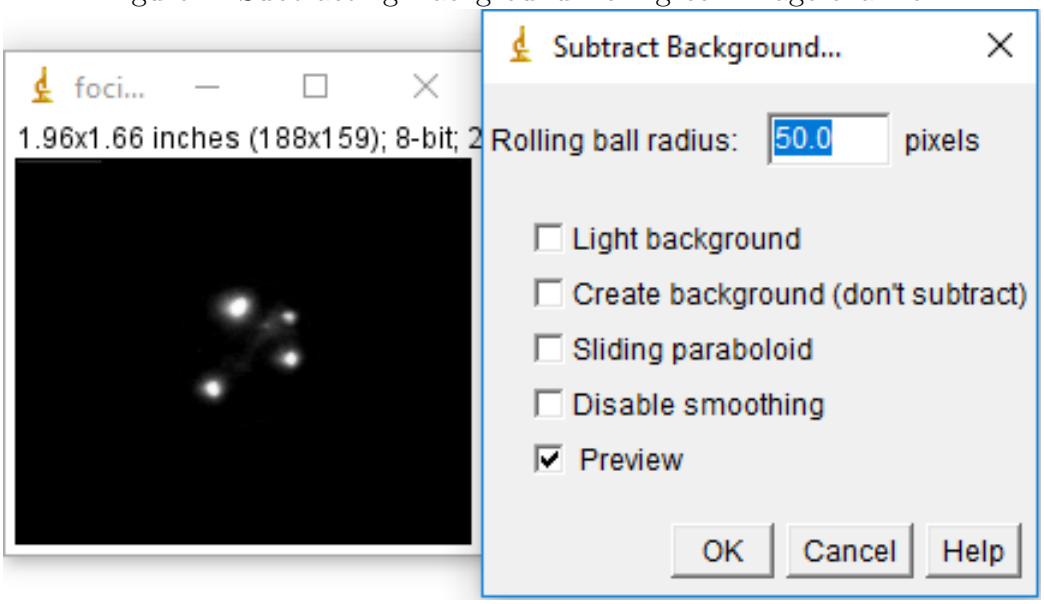
Figure 3: Green channel containing foci.



## 2.1 Subtract Background

The following step will subtract background intensity for better quantification. Start by choosing Process → Subtract Background. Check the "Preview" option and change the radius until you can clearly see your foci (Fig. 4). You might not need to change this setting if your images contain clear foci without too much background intensity. Don't forget your radius setting, you will need it later on.

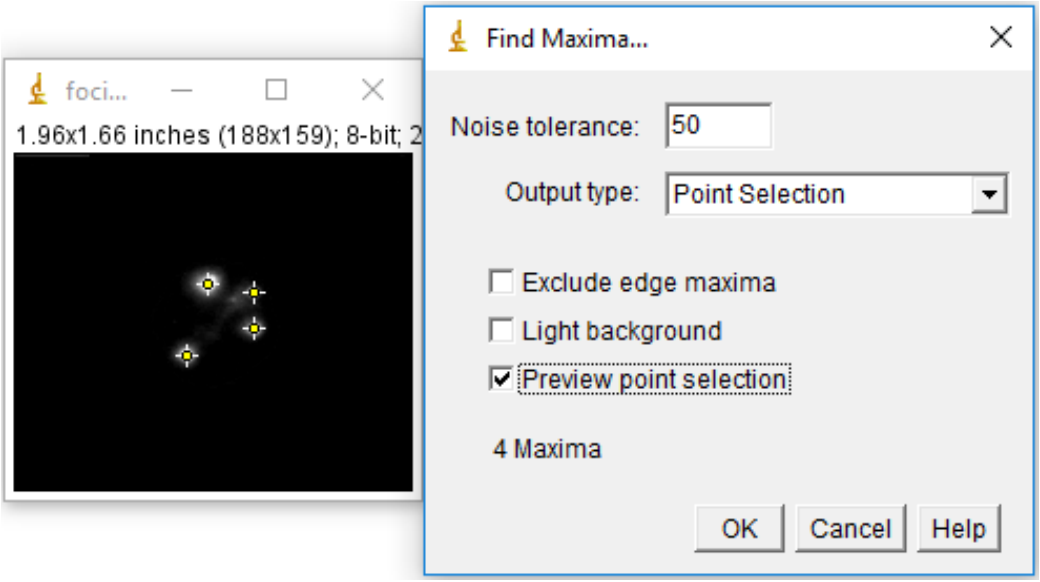
Figure 4: Subtracting Background from green image channel.



## 2.2 Noise Tolerance

The final step in preprocessing is to find out the perfect noise tolerance level for your image set. Click on Process → Find Maxima and select "Preview point selection". Now change the "Noise tolerance" until your foci are marked (Fig. 5).

Figure 5: Subtracting Background from green image channel.

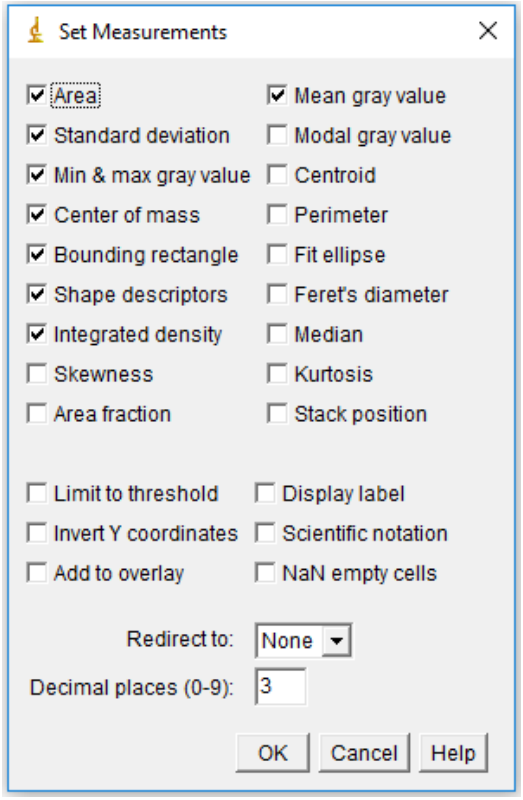



Make sure to remember the noise tolerance level, as it is a requirement for later steps.

# 3 Usage and processing

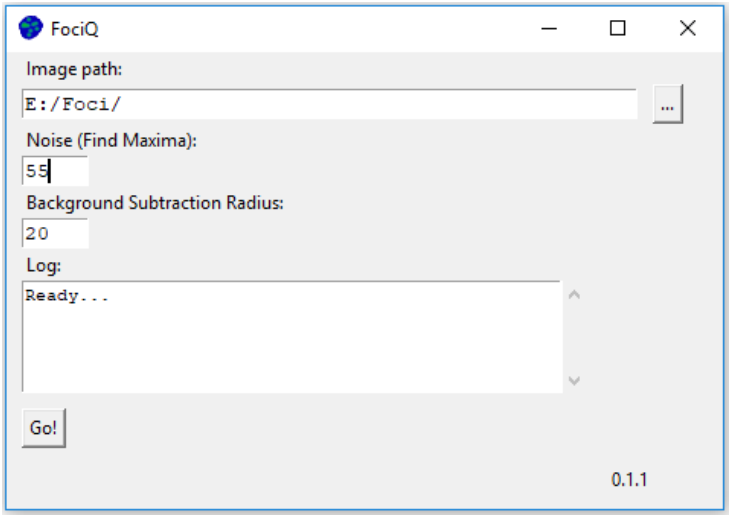
Prior to first time usage you may need to open up ImageJ and click on Analyze → Set Measurements and check "Integrated density" (Fig. 6). This option will be saved for future uses.

Figure 6: Set Measurements option window in ImageJ.



To process your images simply open FociQ (Fig. 7) and proceed by picking the path to your image set. Adjust the background subtraction radius and noise tolerance level to their according values from the preprocessing step. And continue by pressing "Go!". You should now see ImageJ opening and process your images. Do not close ImageJ as it will do so automatically after processing.

Figure 7: FociQ graphical user interface.

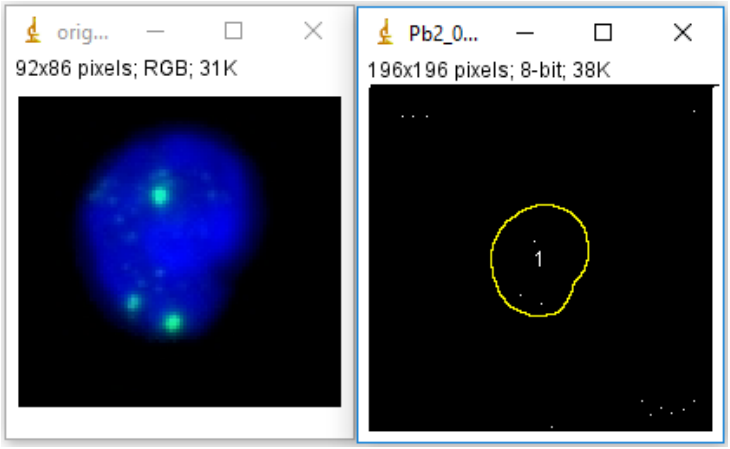



After processing the program will notify you that it is done in the log window. Check the "Results" folder in your image set path.

## 3.1 Results

You will see a .csv file containing foci and cells for each image. The .roi file contains all detected foci in the image and can be compared to the original image with ImageJ (Fig. 8).

Figure 8: ROI and original opened in ImageJ.




Output.xlsx (Fig. 9) will contain a summary of all processed files, their cells and foci.

Figure 9: Output file containing a summary of processed images.