Installing FIJI and ***QuoVadoPro***

1. Download and install FIJI:
   1. Download from: <https://imagej.net/Fiji/Downloads>
   2. Install Fiji from installer
2. Update Fiji:
   1. With the first run of FIJI, it will automatically ask for permission to update.
      1. Press “Yes, Update Now” and let the update happen
      2. After the update, the basics of FIJI should be ready
3. **Install StackReg plugin:**
   1. **This macro requires the stack reg plugin from EPFL for correcting registration defects in movies**
      1. To install this plugin do the following: HELP> UPDATE > MANAGE UPDATE SITES> click on BIG-EPFL>CLOSE
      2. Apply Changes
      3. If you don’t want to have this plugin or your movies don’t have any registration defects, contact Himanish (himanishbasu@gmail.com) for another version of the macro.
4. Run QuoVadoPro installer (*through Fiji*):
   1. Download the zipped folder. Extract everything in it into a separate folder anywhere on your computer (preferably on your desktop, so that you can access it easily. You can delete this folder after installation).
      1. In this folder, there should be the following subfolders : ***Example***, ***QuoVadoPro****,* the installer file named as ***installer\_* *QuoVadoPro \_.ijm,***and the ***instructions.docx*** file.
   2. From FIJI, go to Plugins>Macros>run
      1. Select the file named ***installer\_*** ***QuoVadoPro \_***
   3. Restart FIJI
   4. You should be able to see a new tab under Plugins>Macros called “***QuoVadoPro***”
5. In this case you are ready to run ***QuoVadoPro***!

Running ***QuoVadoPro***:

\*\* Note: For running ***QuoVadoPro***, preferably use single channel movies. Please refer to the example HeLa cell movie where I have split the GFP and TexasRed channels. Refer to the example (in the example folder) where I have the mitochondria movie is saved separately from the image of the GFP channel. This is not necessary but makes for an easier time using the macro

\*\* Note: Do not pre-open the images before running STEP 1 of the macro. As soon as you run Step1, it will prompt you to open an image. In this way the macro can read the metadata of the image.

1. **Step1\_preprocess\_Cells\_IntensitybasedSegmention**
   1. Select the movie and draw outline around the cell as asked by the macro
      1. if it is a dual channel movie, the macro will ask the user to select one channel, (choose the channel which has the moving particles that are to be traced)
   2. The processing should start at this point
   3. At some point during the processing, you will be prompted to enter the following:
      1. threshold level: Generally 9000 is good starting point, but feel free to play around such that the final image shows the correctly thresholded mitos. The value can be set to anywhere between 0-65535. This is dictated by SNR
      2. time smoothing value: Keep this to 1 unless you are getting a lot of blinking objects. Increase it to a minimum level such that you don’t miss movements
      3. minimum particle size: keep this to 1 unless you are getting a lot of noise even after tuning the threshold
   4. At the end of processing, the code will ask for fine tuning the polygon. Again at this step, you don’t need to exactly select the cell periphery, just make sure that all the mitos (or other organelles) are within the polygon. Also, it is a good practice to make sure that the polygon is away from the edges of the movie, as they are often mistakenly picked out by the thresholding code as having fluorescence.
   5. Step 1 is finished!
   6. You can use the final thresholded image for step 2
      1. This image is also saved in the same folder of original image, in case you need to open it again.
2. **Run step 2 of *QuoVadoPro*: Plugins>Macros> *QuoVadoPro* >Step2\_ variance\_Calculation**
   1. You can use the thresholded image generated at the end of step 1 to run step 2.
   2. If no images are open, an image selection window will open up as soon the step 2 is run.
      1. Select the **FOLDER with** the thresholded image
   3. Macro will open up the image and ask the user about the number of substacks you want to break the image into.
      1. Just using 1 sub-stack is good for most purposes.
      2. If you have a long movie with very densely packed organelles, think about increasing the number of sub-stacks.
   4. Step 2 is finished and the quantification is shown in a text window.
      1. The quantification is automatically saved in a text file as well in the same folder.
   5. The code will then bring up the variance image and ask you for a display range. At this point set a display range where the cells with a lot of moving mitochondria appear red. This is not going to be used for quantification but for saving the variance images as comparable RGB images for display. It is important that all the images are set to the same display range. The variance image will be stored in the same image folder
3. **Run step 3 of *QuoVadoPro*: Plugins>Macros> *QuoVadoPro* >Step3\_ collateDataFiles**
   1. When you run this step, it will simply ask you to select a folder with all the text files generated in step 2.
   2. Select the folder and the code will find all the text files in that folder and collate them into an excel sheet.
   3. You can name and save this excel sheet in the next dialogue box.