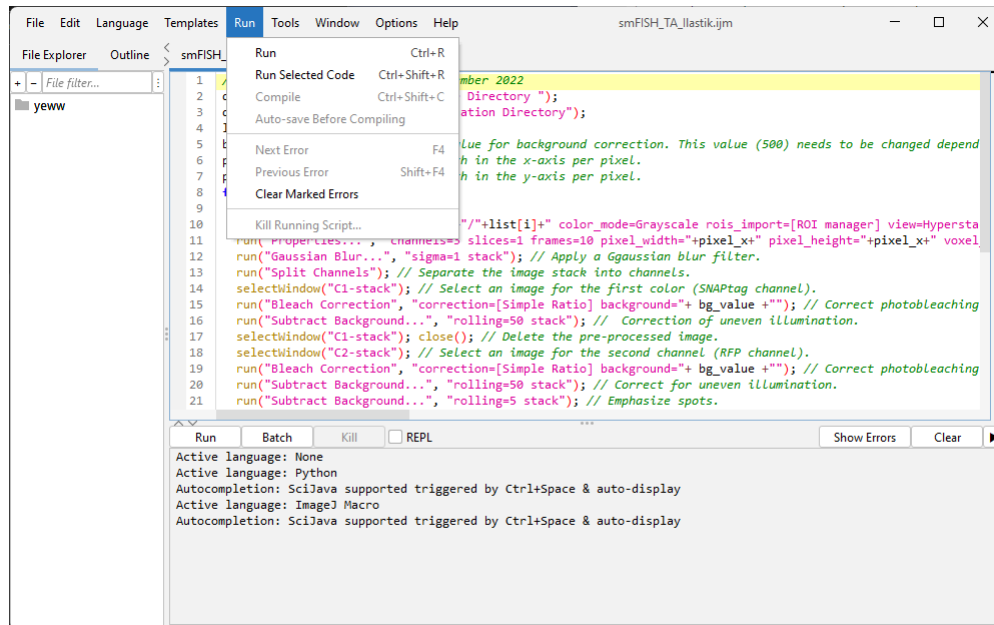


## 1 Getting the necessary software

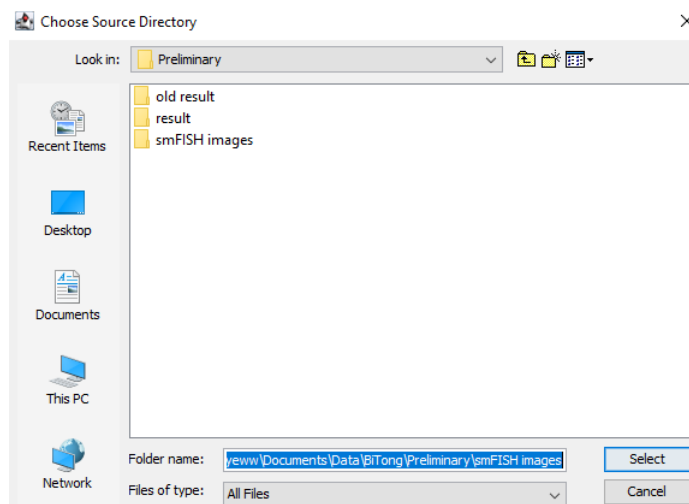
1. Download Fiji's distribution of the ImageJ from  
<https://imagej.net/software/fiji/downloads>.
2. Download MATLAB from  
[https://au.mathworks.com/login?uri=%2Fdownloads%2Fweb\\_downloads](https://au.mathworks.com/login?uri=%2Fdownloads%2Fweb_downloads).

## 2 Extracting the channels

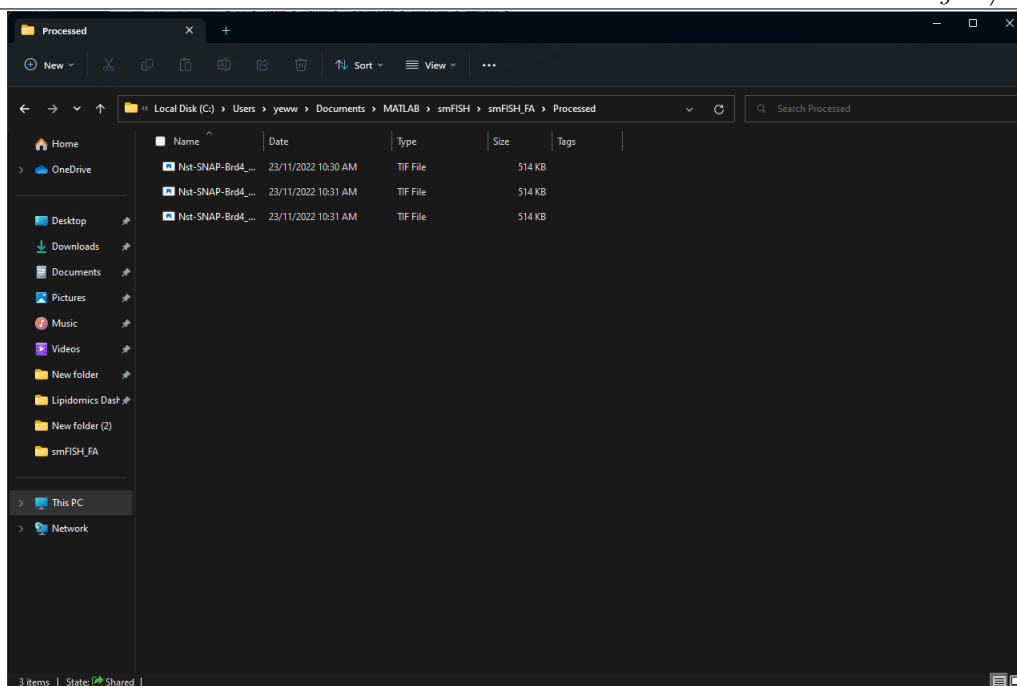
3. Start up Fiji.
4. Go File - Open.
5. Select the *smFISH-TA-Ilastik.ijm* file.
6. A new window like the one shown below should appear, go Run - Run.



7. Another new windows as follow will pop up, select the folder that contains the .nd2 files (make sure the folder only contains the nd2 files of interest and nothing else).



8. After that, another similar window will pop up. Select a folder to store the new images.
9. If everything is done correctly, your new folder should fill with images of different channels as follows.



10. You may now exit Fiji.

### 3 Identify the objects in each channel

11. Start up MATLAB by running the *smFISH-TA.m*. Define the parameters defined in line 3 - 7. Then click "Run" on the top bar.
12. A pop-up will appear and let you select the folder containing all the files from *Fiji*.
13. Select the folder and press "Select Folder".
14. Wait for MATLAB to finish running and a new "Solution.csv" file should appear in the folder.
15. The first column in that folder is the name of the file, follow up by whether the *RFP* and *mNG* is located within tolerance given in MATLAB, with 1 being yes and 0 being no. Finally, the third column is the minimum distance between the *SNAP* and *mNG*.