

Deep learning software instructions for the Article:

"Continuous biomarker monitoring with single molecule resolution by
measuring free particle motion"

1. Python download

Download Python. Instructions can be find on this website:

<https://www.python.org/downloads/>

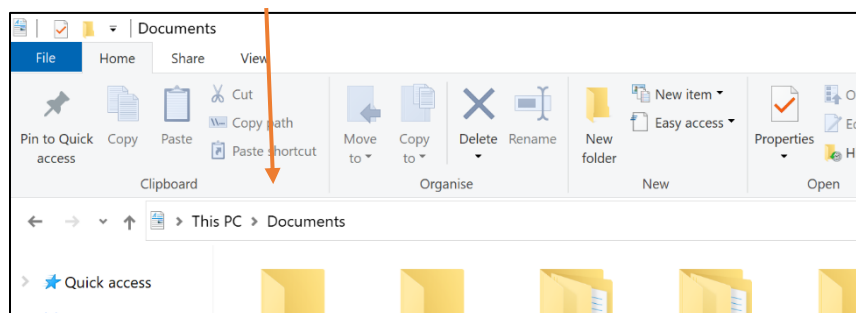
Make sure you have a version between 3.5-3.8.

2. PIP download

PIP, a package installer for python, is required. Check if PIP is already installed via the command prompt window.

A command prompt window can be opened by going to the explorer, typing cmd and pressing enter (see red arrow below). A black window should pop up. Then type 'pip --version'. If it says that pip is not installed, instructions can be found on this website: <https://pip.pypa.io/en/stable/installing/>

How to open command
prompt window:



Check version/installation of pip:

```
C:\Windows\System32\cmd.exe
Microsoft Windows [Version 10.0.18363.1198]
(c) 2019 Microsoft Corporation. All rights reserved.

:\Users\evaho\OneDrive\Documenten\Graduate project>python --version
Python 3.6.1

:\Users\evaho\OneDrive\Documenten\Graduate project>pip --version
pip 20.1.1 from c:\users\evaho\python36\lib\site-packages\pip (python 3.6)

:\Users\evaho\OneDrive\Documenten\Graduate project>
```

3. Installation of Jupyter notebook (environment to work with python)

With pip it is possible to install Jupyter notebook by typing 'pip install jupyter'.

```
C:\Windows\System32\cmd.exe

Microsoft Windows [Version 10.0.18362.959]
(c) 2019 Microsoft Corporation. All rights reserved.

C:\Users\evaho\Python36>pip install jupyter
```

4. Open jupyter notebook

After that open the jupyter notebook by typing 'jupyter notebook' in the command prompt window.

```
C:\Windows\System32\cmd.exe

Microsoft Windows [Version 10.0.18362.959]
(c) 2019 Microsoft Corporation. All rights reserved.

C:\Users\evaho\Python36>jupyter notebook
```

5. Start new python file in jupyter notebook:

By clicking 'new', a new python file to work in can be started.



6. Download extra libraries:

Several python libraries are required: numpy, matplotlib and pandas. These can be installed by typing 'pip install numpy', etc.

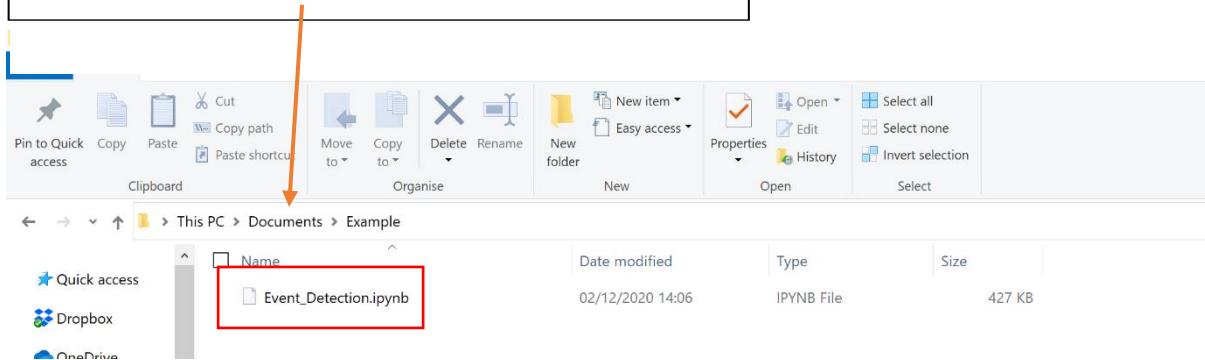
Any other missing libraries can be installed in the same way.

7. Installation of keras & tensorflow (deep learning environments in python)

Install keras & Tensorflow. Type in the command prompt window: *'pip install keras'* and *'pip install tensorflow'*.

To open an already written python file: go to the folder in the explorer, where the file is saved. Open the command prompt window from there, type jupyter notebook. Open the .ipynb file in the jupyter notebook browser.

Type cmd here and press enter. Type 'jupyter notebook' to open jupyter in the browser and select an .ipynb file.



8. Section 'Data import and Model selection'

Place the .ipynb (analysis script) and .h5 (model) files in the same folder as the measurement data (the particle trajectories are saved as .txt files).

Input the correct pixel size (in $\mu\text{m}/\text{pixel}$) and measurement framerate (in Hz).

Specify the Deep Learning models (.h5 files) to be used:

01 distinguishes unbound from bound states, 12 distinguishes single bound from multivalent bound states. Choose the correct model for the particle size (1 μm or 3 μm in the filename).

Specify the measurement windows to be used, which are in the model filenames after 'meas'.

```
PixelSize=0.345; #Leica M1/M3 with grasshopper3 = 0.345 um/pixel; M2 = 0,588; correct for c-mount x1
FrameRate=60 # Fill in the framerate of the measurement. (not the framerate of the deep Learning model)

# Model to differentiate between unbound/bound.
model01 = keras.models.load_model('DLmodel01_3um_meas120_norm4e-7_FR30.h5')
# Model to differentiate between single/double bound.
model12 = keras.models.load_model('DLmodel12_3um_meas180_norm4e-7_FR30.h5')
measurementwindow = 120
measurementwindow2 = 180
```

9. Run the analysis

Start analyzing the particle trajectory files in the folder by selecting 'run all' in the drop-down menu. The results will be output in the notebook, and analysis files will be saved in separate folders corresponding to each measurement.

