

How to work with “scripts”

➔ If you are familiar with Python , please directly move to section 5

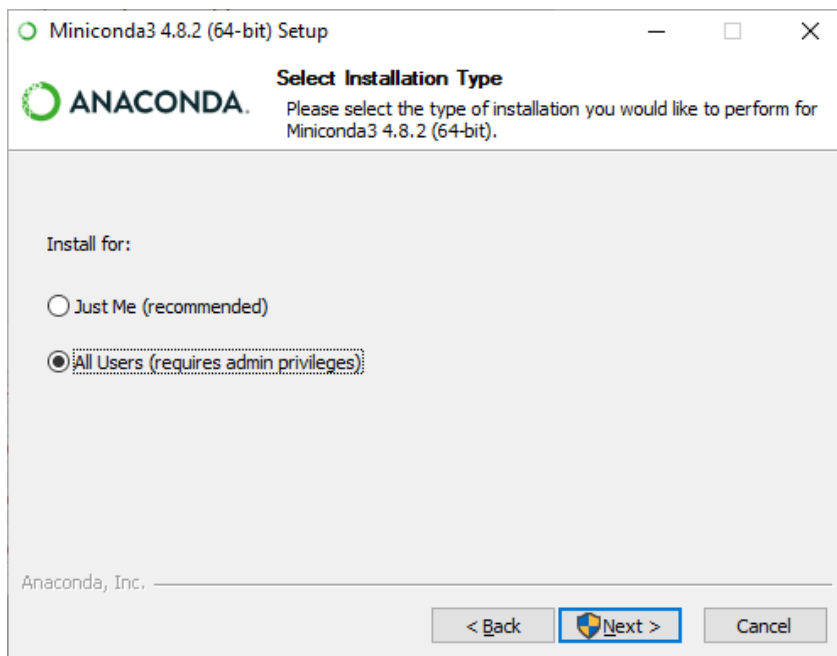
1. Install Miniconda (Python3.7, usually 64-bit)

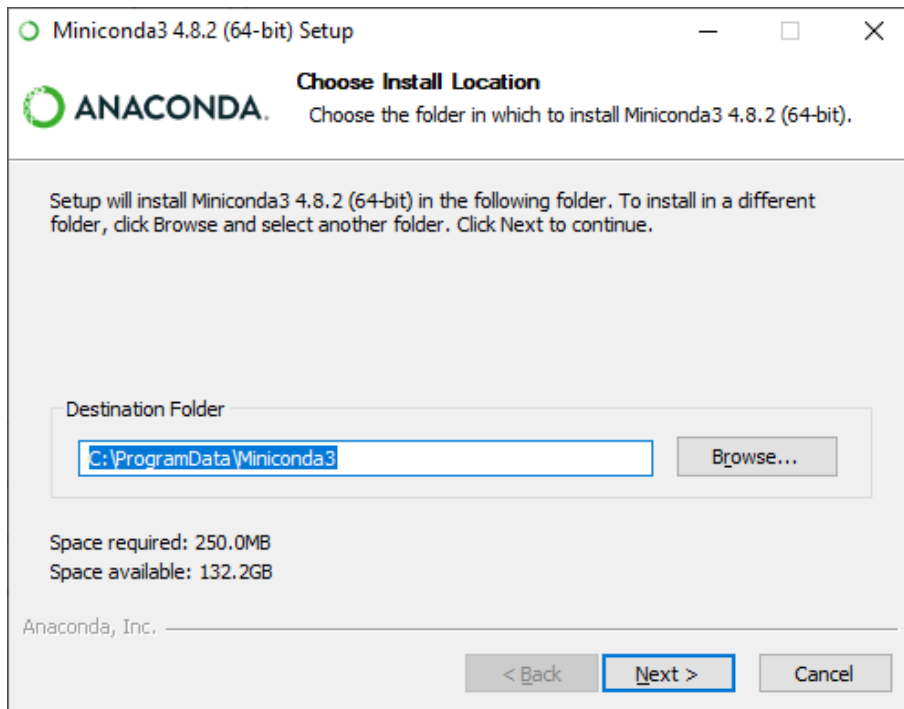
If you haven't done so, please install Miniconda first.

<https://docs.conda.io/en/latest/miniconda.html>

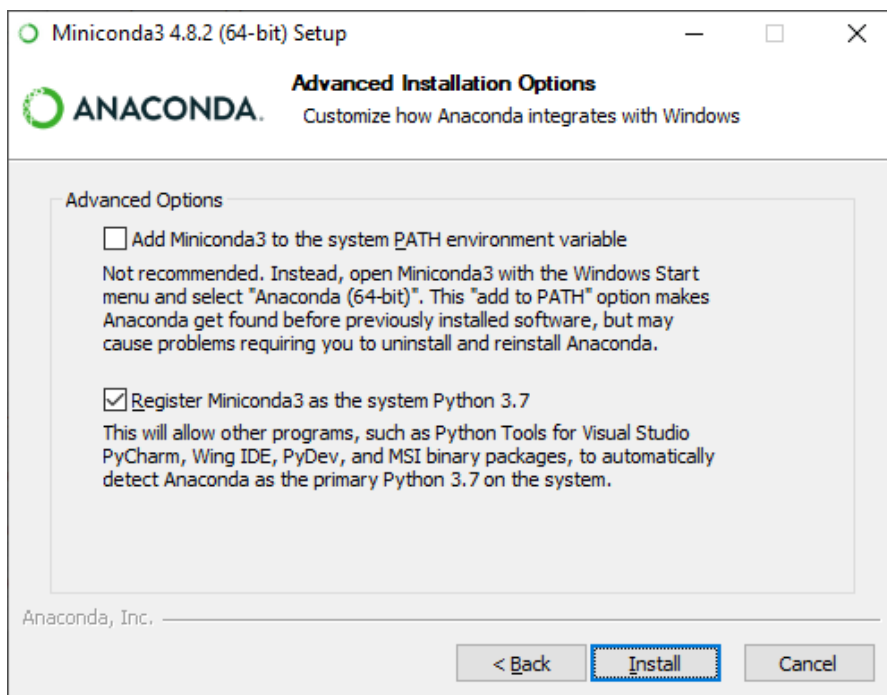
Python version	Name	Size	SHA256 hash
Python 3.7	Miniconda3 Windows 64-bit	51.6 MiB	1781955cd637d1d9d5a84958f6478649b79de973d1578541eb52857664b5856c
	Miniconda3 Windows 32-bit	52.2 MiB	ca74cb6eb0731db2b972c0fb512e29661a84c3f01ac6133121b4372eb1c41f46
Python 2.7	Miniconda2 Windows 64-bit	50.9 MiB	8647c54858f11842c37854edefff4d280c1fbdad8b88d9d34d76fda1638e64846
	Miniconda2 Windows 32-bit	48.7 MiB	8d186228d6a4618b5959df965d6d9bb659329a17e3d693e3274b28291a7c6f94

Install for all users:





Leave second option selected:



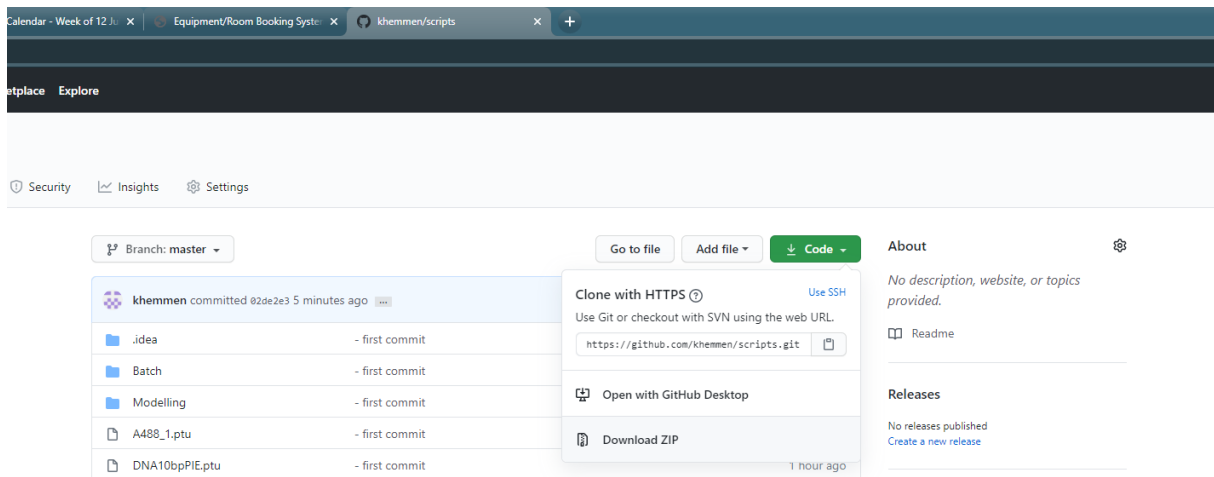
2. Getting "scripts" from Github

Go to the following website and download the newest version:

<https://github.com/khemmen/scripts>

You will need to create an account at Github to be able to download the files.

As this is a private repository, please send me your account name and I will send you an invite!



Best is to place the folder on "C:\Users\username"

3. Generating the "scripts" environment

<https://docs.conda.io/projects/conda/en/latest/user-guide/tasks/manage-environments.html>

Option: "Creating an environment from an environment.yml file"

In case you closed the command window, open it again by typing into your windows search bar "miniconda".

Navigate to your folder by typing into the console:

```
cd C:\Users\username\scripts
```

Generate the new environment named „scripts“:

```
conda env create -f environment.yml
```

```
Anaconda Prompt (Miniconda3) - conda env create -f environment.yml

(base) C:\Users\User>cd C:\Users\User\scripts

(base) C:\Users\User\scripts>conda env create -f environment.yml
Collecting package metadata (repodata.json): done
Solving environment: -
```

It will download quite some packages and dependencies.

When it is done, it will show the following:

```
Anaconda Prompt (Miniconda3)

ca-certificates-2020 125 KB ##### 100%
qt-5.9.7 72.5 MB ##### 100%
tbb-2020.0 161 KB ##### 100%
atomicwrites-1.4.0 11 KB ##### 100%
matplotlib-base-3.2. 5.4 MB ##### 100%
pickleshare-0.7.5 14 KB ##### 100%
pluggy-0.13.1 34 KB ##### 100%
prompt-toolkit-3.0.5 245 KB ##### 100%
git-2.23.0 10.5 MB ##### 100%
importlib-metadata-1 52 KB ##### 100%
icc_rt-2019.0.0 6.0 MB ##### 100%
setuptools-49.1.0 734 KB ##### 100%
python-utils-2.3.0 18 KB ##### 100%
fftw-3.3.8 926 KB ##### 100%
mkl-2020.1 99.3 MB ##### 100%
jedi-0.17.1 914 KB ##### 100%
Preparing transaction: done
Verifying transaction: done
Executing transaction: done
#
# To activate this environment, use
#
# $ conda activate scripts
#
# To deactivate an active environment, use
#
# $ conda deactivate
#
(base) C:\Users\User\scripts>
```

4. Starting “scripts” environment

Activate the environment by typing:

```
conda activate scripts
```

When you want to use “scripts” next time, you can directly start from section 4 using the environment activation.

Please make sure you navigate to the scripts-folder before trying to run any command.

```
(base) C:\Users\User > conda activate scripts
(scripts) C:\Users\User > cd C:\Users\username\scripts
(scripts) C:\Users\username\scripts >
```

5. Running your first command

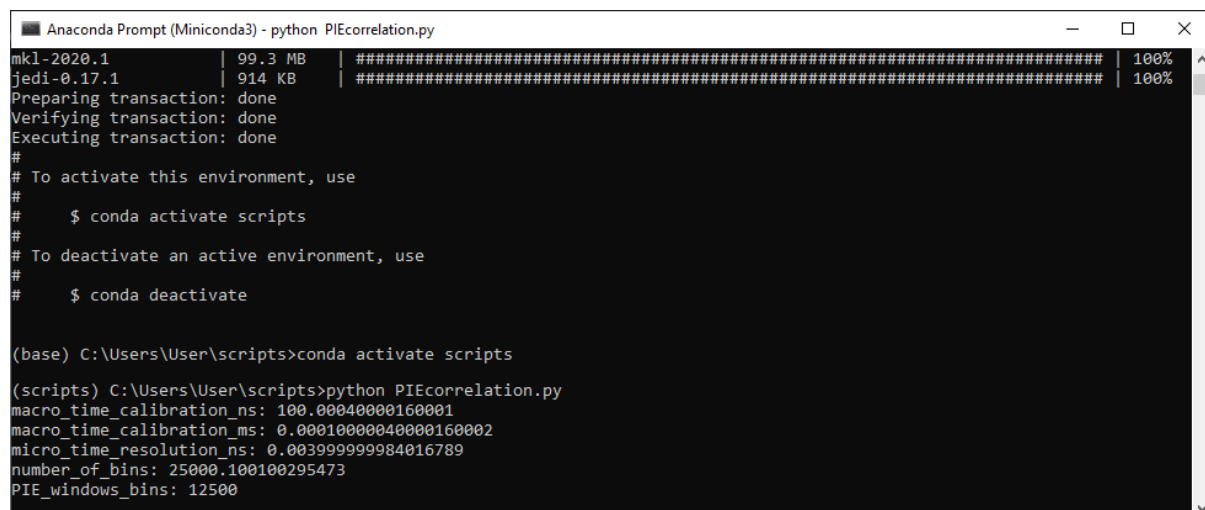
e.g. correlate a single data file, which had been obtained by a PIE measurement:

→ 2 channels from two colours each (“donor”, green, and “acceptor”, red), the two channels of a colour are arranged in two different polarizations “s” and “p”, horizontally and vertically to the excitation laser beam.

The example data is called: DNA10bpPIE.ptu

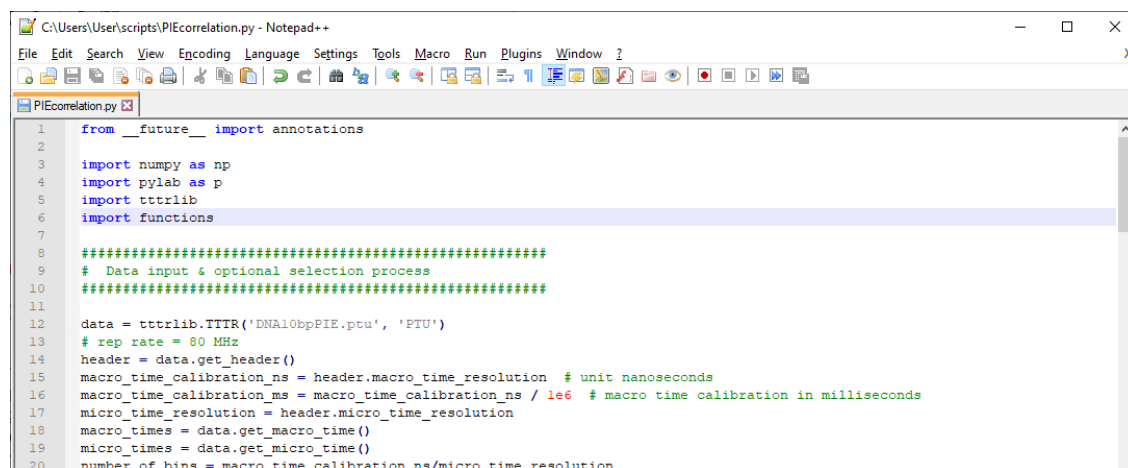
Start the correlation by typing “python PIEcorrelation.py”

```
(scripts) C:\Users\user\scripts > python PIEcorrelation.py
```



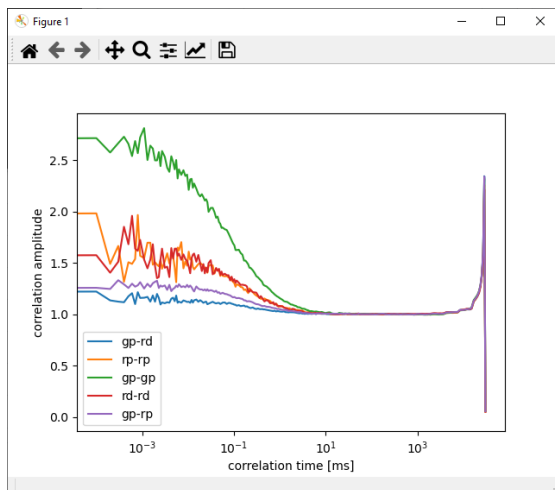
```
Anaconda Prompt (Miniconda3) - python PIEcorrelation.py
mk1-2020.1 | 99.3 MB | ##### | 100%
jedi-0.17.1 | 914 KB | ##### | 100%
Preparing transaction: done
Verifying transaction: done
Executing transaction: done
#
# To activate this environment, use
#
#     $ conda activate scripts
#
# To deactivate an active environment, use
#
#     $ conda deactivate
#
(base) C:\Users\User\scripts>conda activate scripts
(scripts) C:\Users\User\scripts>python PIEcorrelation.py
macro_time_calibration_ns: 100.00040000160001
macro_time_calibration_ms: 0.00010000040000160002
micro_time_resolution_ns: 0.003999999984016789
number_of_bins: 25000.100100295473
PIE_windows_bins: 12500
```

You might want to check the script in Notepad++ (or any editor) to check it and to modify your filename (line 12):



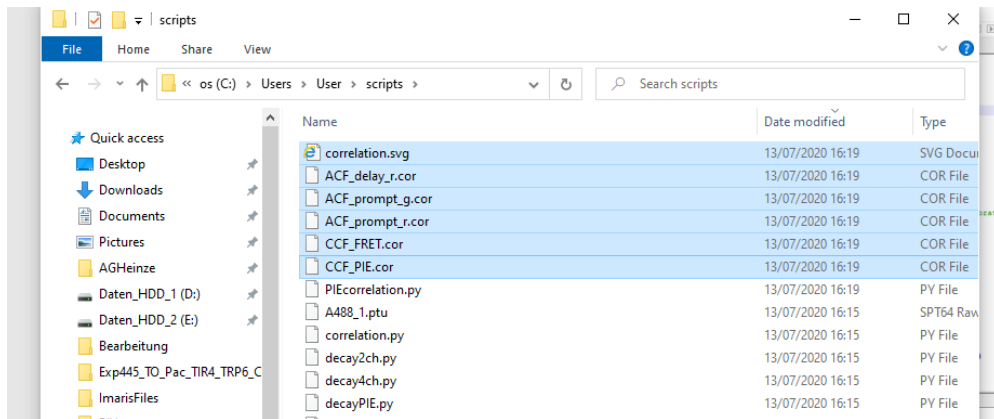
```
C:\Users\User\scripts\PIEcorrelation.py - Notepad++
File Edit Search View Encoding Language Settings Tools Macro Run Plugins Window ?
PIEcorrelation.py
1 from __future__ import annotations
2
3 import numpy as np
4 import pylab as p
5 import tttrlib
6 import functions
7
8 #####
9 # Data input & optional selection process
10 #####
11
12 data = tttrlib.TTTR('DNA10bpPIE.ptu', 'PTU')
13 # rep rate = 80 MHz
14 header = data.get_header()
15 macro_time_calibration_ns = header.macro_time_resolution # unit nanoseconds
16 macro_time_calibration_ms = macro_time_calibration_ns / 1e6 # macro time calibration in milliseconds
17 micro_time_resolution = header.micro_time_resolution
18 macro_times = data.get_macro_time()
19 micro_times = data.get_micro_time()
20 number of bins = macro time calibration ns/micro time resolution
```

After the correlation is done, a new window containing the data plot is opened:



CLOSE this window to be able to proceed!

Additionally, the data is saved to your input folder:



To process a different data set change the filename in line 12 of the scripts.

As it is unpractical to copy all your data to your “C:\Users\User\scripts” folder, you can replace the filename by a data path:

```

PIEcorrelation.py
1  from __future__ import annotations
2
3  import numpy as np
4  import pylab as p
5  import tttrlib
6  import functions
7
8  #####
9  # ..Data input & optional selection process
10 #####
11
12 data = tttrlib.TTTR(r'\\HC1008\Data\FCS\2020\DNA10bpPIE.ptu', 'PTU')
13 #.rep.rate = 80.MHz
14 header = data.get_header()

```

6. Batch correlation of whole folders

If you have many data files, which you all would to process the same way, you can use the scripts in the “batch” folder. These are made for batch processing.

Navigate to the “batch folder” in the console:

```
(scripts) C:\Users\user\scripts > cd C:\Users\user\scripts\batch
(scripts) C:\Users\user\scripts\batch
```

This scripts consist of two parts:

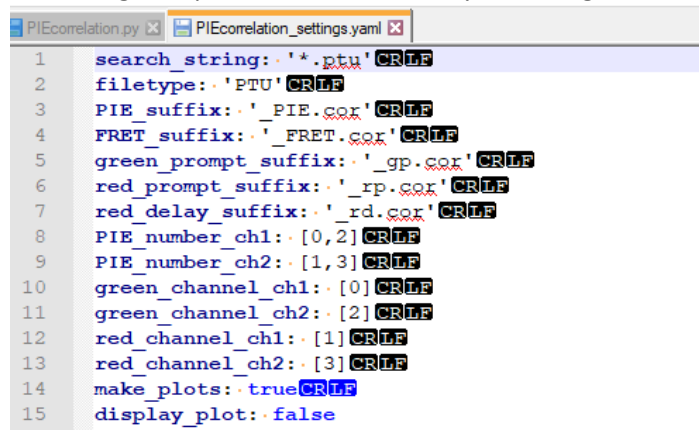
- your python script, which does the job and
- your “settings” file, which is a so-called YAML-file. This can also be edited in Notepad.

For correlating a folder of PIE data sets open the “PIEcorrelation_settings.yaml” file in Notepad and adjust your settings as required:

i.e.

- make sure the channel definitions are correct
- adjust the naming suffix for autosaving

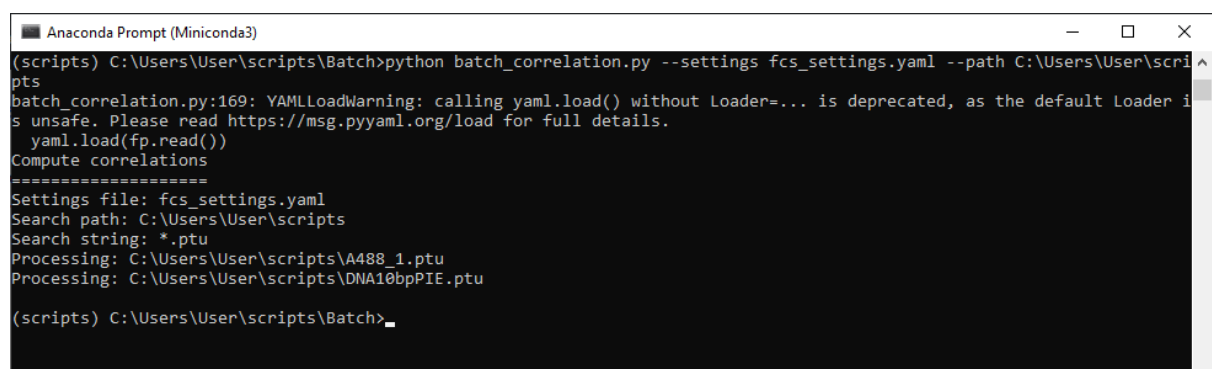
Don’t forget to press save after made your changes!



```
1 search_string: '*.ptu'
2 filetype: 'PTU'
3 PIE_suffix: '_PIE.cor'
4 FRET_suffix: '_FRET.cor'
5 green_prompt_suffix: '_gp.cor'
6 red_prompt_suffix: '_rp.cor'
7 red_delay_suffix: '_rd.cor'
8 PIE_number_ch1: [0,2]
9 PIE_number_ch2: [1,3]
10 green_channel_ch1: [0]
11 green_channel_ch2: [2]
12 red_channel_ch1: [1]
13 red_channel_ch2: [3]
14 make_plots: true
15 display_plot: false
```

Now switch back to the console and type:

```
(scripts) C:\Users\user\scripts\batch > python batch_correlation.py --settings
PIEcorrelation_settings.yaml --path "\\HC1008\Data\FCS\2020"
```



```
Anaconda Prompt (Miniconda3)
(scripts) C:\Users\User\scripts\Batch>python batch_correlation.py --settings fcs_settings.yaml --path C:\Users\User\scri
pts
batch_correlation.py:169: YAMLLoadWarning: calling yaml.load() without Loader=... is deprecated, as the default Loader i
s unsafe. Please read https://msg.pyyaml.org/load for full details.
  yaml.load(fp.read())
Compute correlations
=====
Settings file: fcs_settings.yaml
Search path: C:\Users\User\scripts
Search string: *.ptu
Processing: C:\Users\User\scripts\A488_1.ptu
Processing: C:\Users\User\scripts\DNA10bpPIE.ptu
(scripts) C:\Users\User\scripts\Batch>
```

This scripts needs two input parameter:

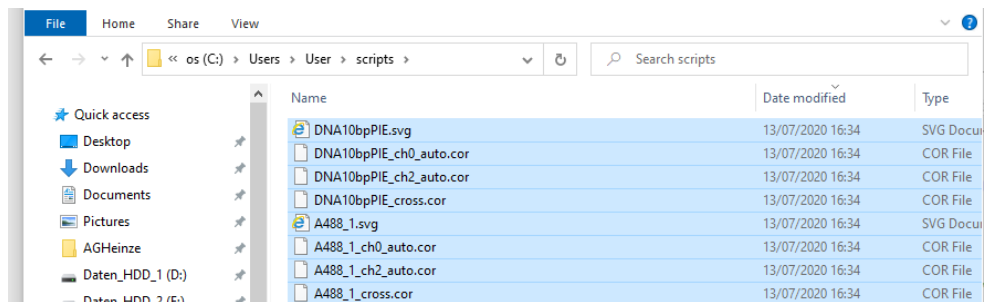
--settings: your modified settings file

--path: the path to your data, please enclose the path in “ “

CAUTION!!!

- **No dots (.sptw!),**
- **no special characters (μ m!),**
- **to change drive add path in “E:\path-to-data”**

Here, all folders within your specified path will be search recursive for the key phrase “.ptu” and all ptu-files will be correlated (ch0 and ch2), the auto- and crosscorrelation and a figure for visual inspection is saved:



In case you want to correlate e.g. only your cell data, you can change the search phrase to ‘cell*.ptu’, if your data is saved as cell1.ptu, cell2.ptu etc. Calibration measurements named e.g. A488.ptu will not be correlated.