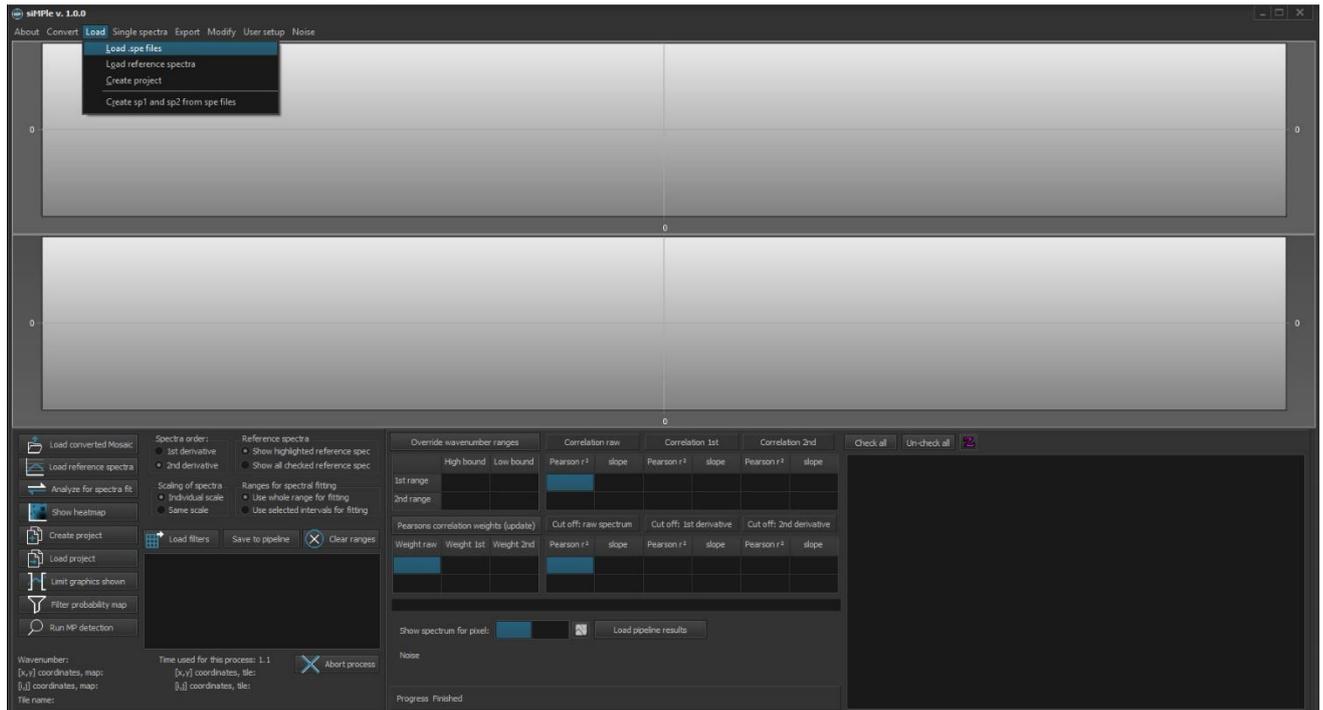




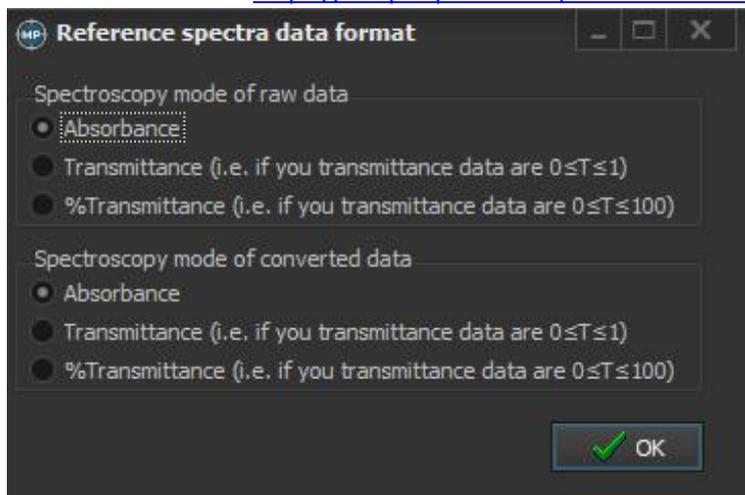
## How to use APP with siMPle

### 1. Load or convert the files (.spe, or .dmd, .dx, etc.)



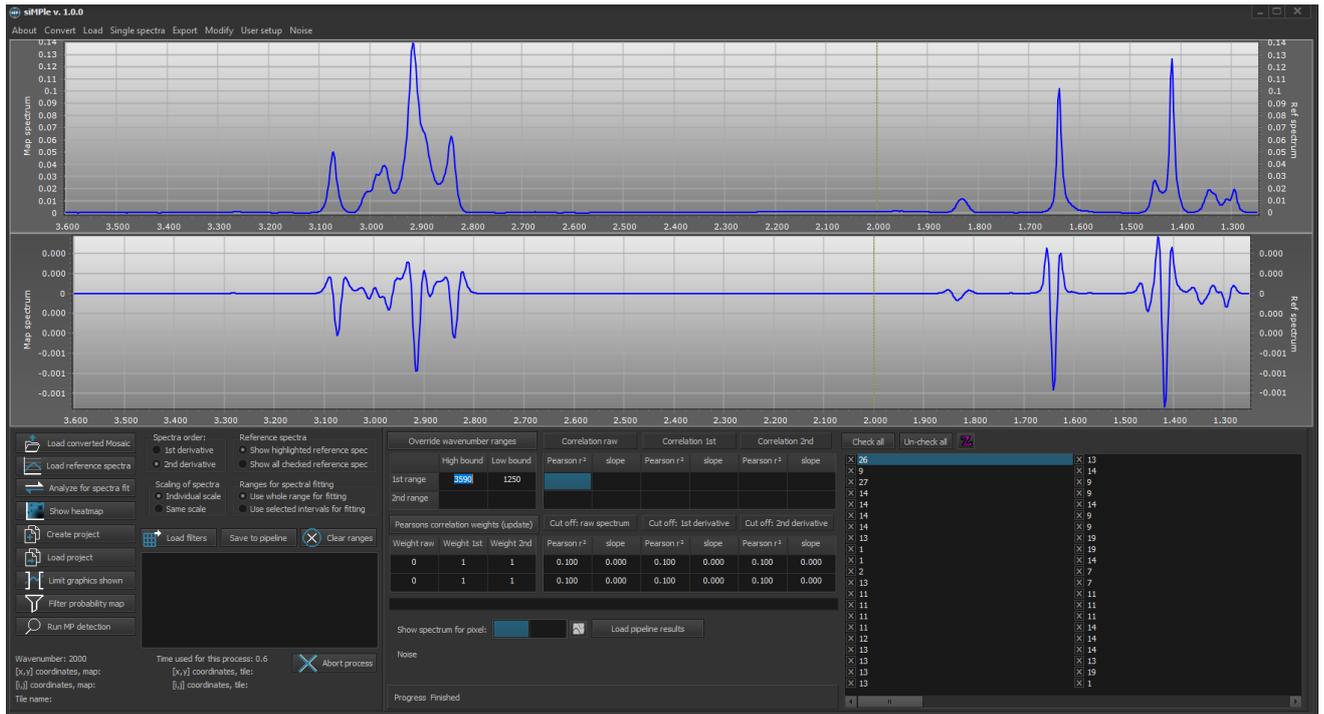
### 2. Load the reference file, for AWI analysis: siMPle\_automated\_analysis\_library\_VersionX.txt

It is available here: <https://simple-plastics.eu/download.html>



Please keep in mind that the reference data was measured in absorbance and press okay.

### 3. It should now look like this:



### 4. Please check user setup -> options and adjust to these values and options:

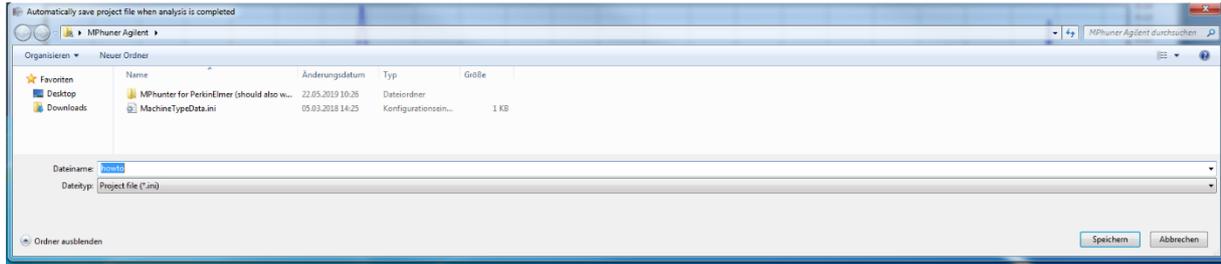
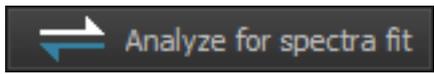
The 'User options' dialog box is shown with the following settings:

- Spectra analysis:**
  - Include 2nd order derivatives
  - Suppress negative correlations
  - Omit CO2 peak
- MP identification options:**
  - Join particles from different spectra into groups
  - Fill boundaries around particles
- Ignore MPs smaller than (MP < #):** 3 pixels
- Raw data file conversion options:**
  - Make .sp1 files while converting raw data
  - Make .sp2 files while converting raw data
- Set boundaries for heat map:**
  - Lower: 0.00
  - Upper: 1.00
- Wavenumber ranges:**
  - Lower wavenumber: 2200
  - Upper wavenumber: 2420
- Noise determination range:** 2100 (Lower), 2210 (Upper)
- Max peak determination range:** 950 (Lower), 3600 (Upper)
- Spectra order:**
  - 1st derivative
  - 2nd derivative
- Max signal peak to noise ratio:** 3.0
- Width of the array used create derivatives:** 9
- The wider it is, the more smoothing of derivative curves. The value must be an un-even number**
- Cut off:**
  - raw spectrum: Pearson r (0.100), slope (0.000)
  - 1st derivative: Pearson r (0.100), slope (0.000)
  - 2nd derivative: Pearson r (0.100), slope (0.000)
- Pearsons correlation weights:**
  - Weight raw: 1
  - Weight 1st: 1
  - Weight 2nd: 0

Buttons for 'Default', 'OK', and 'Cancel' are visible at the bottom.

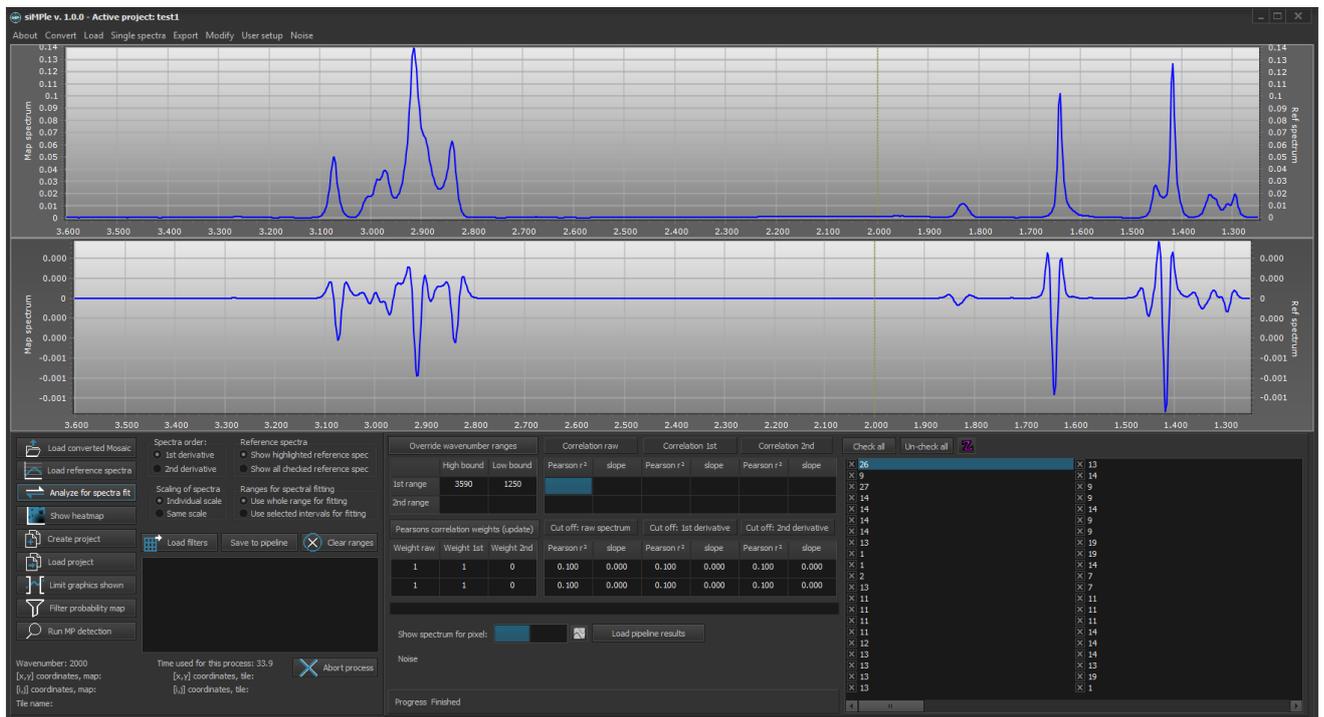
Proceed by clicking okay.

5. Start the calculation via pressing:

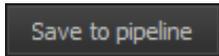


And generate a project file, here "How to" and click save.

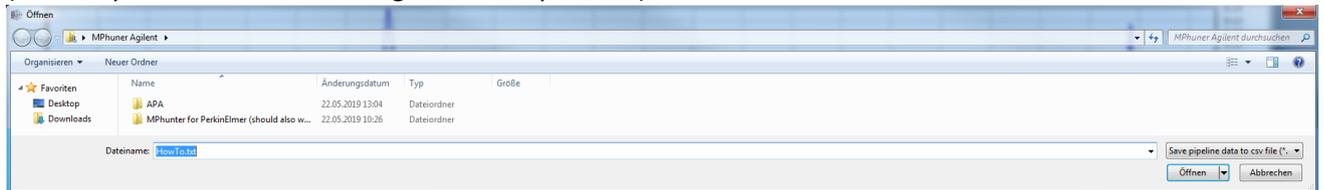
6. Wait until the calculations are finished.



7. Afterwards, click on the button:



8. Chose a file name and take care not to rename the database file (currently it is the default name generated by siMPle):

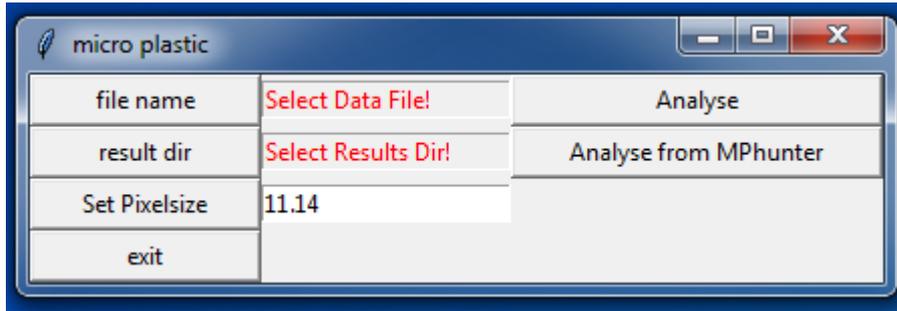


Saving the file may take a while.

9. Open the **APA.exe** or **MPAPP.exe** (both filenames contain the current version number as eg. APAv1.01.exe, which may differ due to updates) depending on the pipeline you want to use.

<https://drive.google.com/open?id=1O3vtsb963KoGwsTTGvDZgo8KUqjGjNAD>

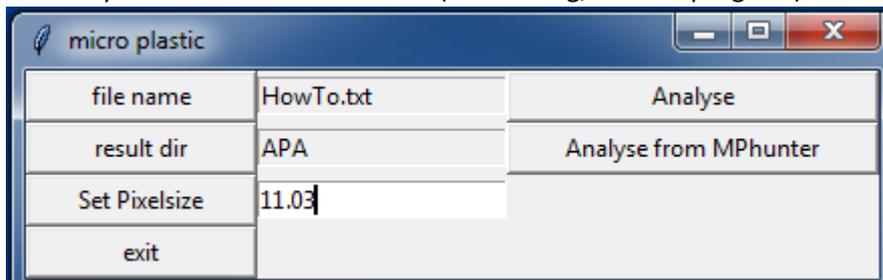
10. After a short while the user interface shows up:



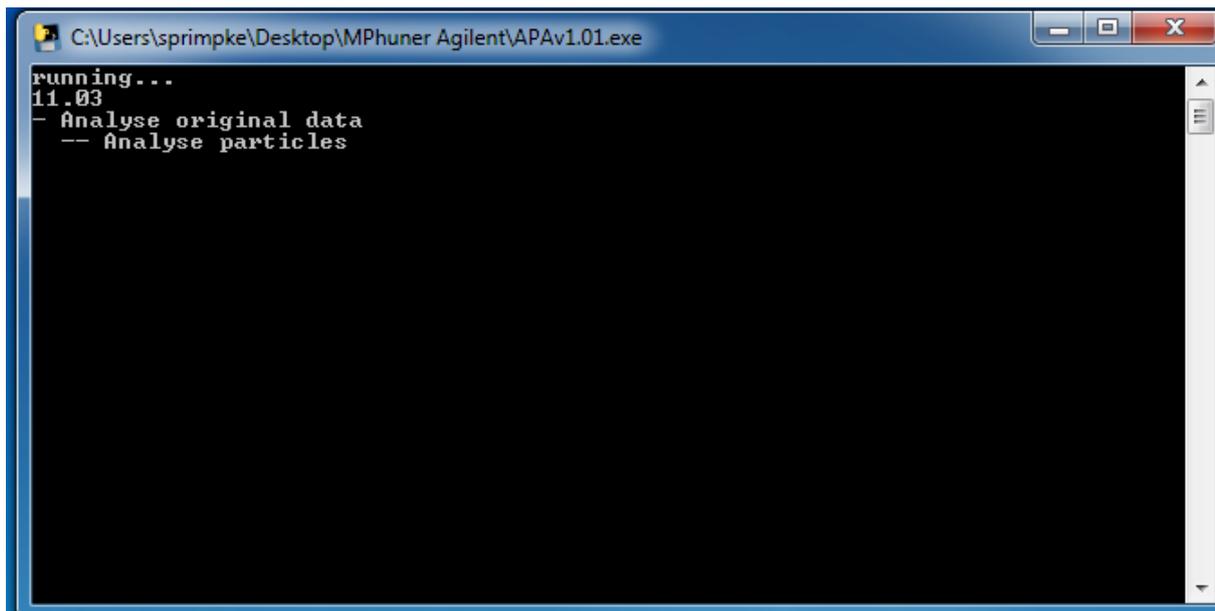
11. Please set the pixelsize to the value of your system, here 11.03, and press Set Pixelsize (otherwise 11.14 will be used as default).

12. By pressing "file name" you can chose the generated file (here HowTo.txt in the "MPhunter Agilent" folder).

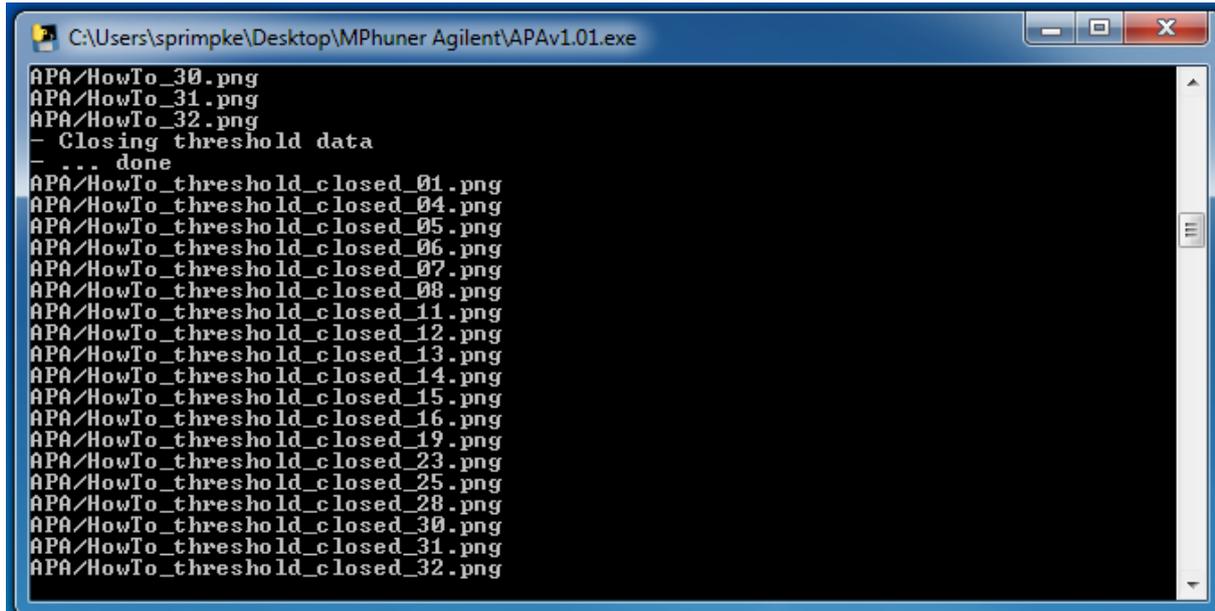
13. Choose a result folder by pressing "result dir" (here APA in the "MPhunter Agilent" folder). This folder needs to be in the same folder as the executable and currently no subfolders are allowed (known bug, work in progress).



14. After all folders, files and settings are set, please press Analyse from MPhunter to start the analysis of the generated data followed by Image Analysis. The start should look like this:



15. Finished analysis:



16. The generated data contains various different files including all data and images generated during image analysis:

- A histogram for polymer type hits based on spectra.
- The combined (\_closed\_image.csv) and the individual (\_closed\_imageID.csv, ID = 01, etc.) image files.
- The number of particles found for each pixel area (\_particle\_histogramID.csb)
- All individual particle information (which number has which size) as \_individualID.csv.
- A histogram for polymer type hits based on particle (\_particles\_perpolymer.csv)
- The size classes used in various publication:
  - \_sizeclassesID.csv for individual particles types,
  - \_bio for natural materials,
  - \_polymer for plastic materials,
  - \_other for all other materials,
  - \_bulk (bio, polymer and other combined),
  - \_forcalculations (all individual sizeclasses in one file):

	A	B	C	D	E	F	G
1		s	s	s	s	s	s
2	single	0	4	0	0	22	0
3	25	0	0	0	0	30	1
4	50	0	1	0	0	14	3
5	75	0	1	0	0	8	2
6	100	0	0	0	0	4	1
7	125	0	0	0	0	2	0
8	150	0	0	0	0	0	0
9	175	0	0	0	0	0	0
10	200	0	0	0	0	0	0
11	225	0	0	0	0	0	0
12	250	0	0	0	0	0	0
13	275	0	0	0	0	0	0
14	300	0	0	0	0	0	0
15	325	0	0	0	0	0	0
16	350	0	0	0	0	0	0
17	375	0	0	0	0	0	0
18	400	0	0	0	0	0	0
19	425	0	0	0	0	0	0
20	450	0	0	0	0	0	0
21	475	0	0	0	0	0	0
22	500	0	0	0	0	0	0
23	large	0	0	0	0	0	0
24							

Data reads as followed (the first column label with “s” is not containing data and needs has be skipped for

	A	B	C	D	E	F	G
1	s		1	2	3	4	5
2	single	0	4	0	0	22	0
3	25	0	0	0	0	30	1
4	50	0	1	0	0	14	3
5	75	0	1	0	0	8	2
6	100	0	0	0	0	4	1
7	125	0	0	0	0	2	0
8	150	0	0	0	0	0	0
9	175	0	0	0	0	0	0
10	200	0	0	0	0	0	0
11	225	0	0	0	0	0	0
12	250	0	0	0	0	0	0
13	275	0	0	0	0	0	0
14	300	0	0	0	0	0	0
15	325	0	0	0	0	0	0
16	350	0	0	0	0	0	0
17	375	0	0	0	0	0	0
18	400	0	0	0	0	0	0
19	425	0	0	0	0	0	0
20	450	0	0	0	0	0	0
21	475	0	0	0	0	0	0
22	500	0	0	0	0	0	0
23	large	0	0	0	0	0	0
24							

calculations):

\_forstatistics (all individual sizeclasses in one file):)

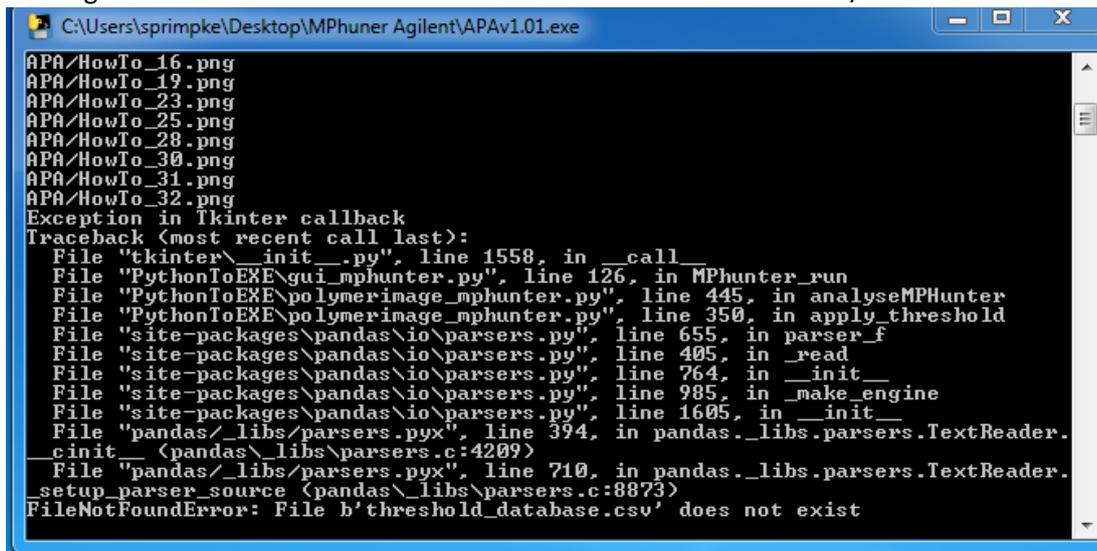
g. Greyscale image of the raw and final images.

## 17. Welcome to the data Jungle! Analysis finished!

## 18. Troubleshooting:

### a. Error Messages (Traceback):

- i. Missing the threshold.csv file in the folder with the executable for APA/MPAPP:



```
C:\Users\sprimpke\Desktop\MPHunter Agilent\APAv1.01.exe
APA/HowTo_16.png
APA/HowTo_19.png
APA/HowTo_23.png
APA/HowTo_25.png
APA/HowTo_28.png
APA/HowTo_30.png
APA/HowTo_31.png
APA/HowTo_32.png
Exception in Tkinter callback
Traceback (most recent call last):
  File "tkinter\__init__.py", line 1558, in __call__
  File "PythonToEXE\gui_mphunter.py", line 126, in MPHunter_run
  File "PythonToEXE\polymerimage_mphunter.py", line 445, in analyseMPHunter
  File "PythonToEXE\polymerimage_mphunter.py", line 350, in apply_threshold
  File "site-packages\pandas\io\parsers.py", line 655, in parser_f
  File "site-packages\pandas\io\parsers.py", line 405, in _read
  File "site-packages\pandas\io\parsers.py", line 764, in __init__
  File "site-packages\pandas\io\parsers.py", line 985, in _make_engine
  File "site-packages\pandas\io\parsers.py", line 1605, in __init__
  File "pandas\_libs\parsers.pyx", line 394, in pandas._libs.parsers.TextReader.
  cinit_ (pandas\_libs\parsers.c:4209)
  File "pandas\_libs\parsers.pyx", line 710, in pandas._libs.parsers.TextReader.
  _setup_parser_source (pandas\_libs\parsers.c:8873)
FileNotFoundError: File b'threshold_database.csv' does not exist
```

Please copy the provide threshold\_database.csv into the folder.