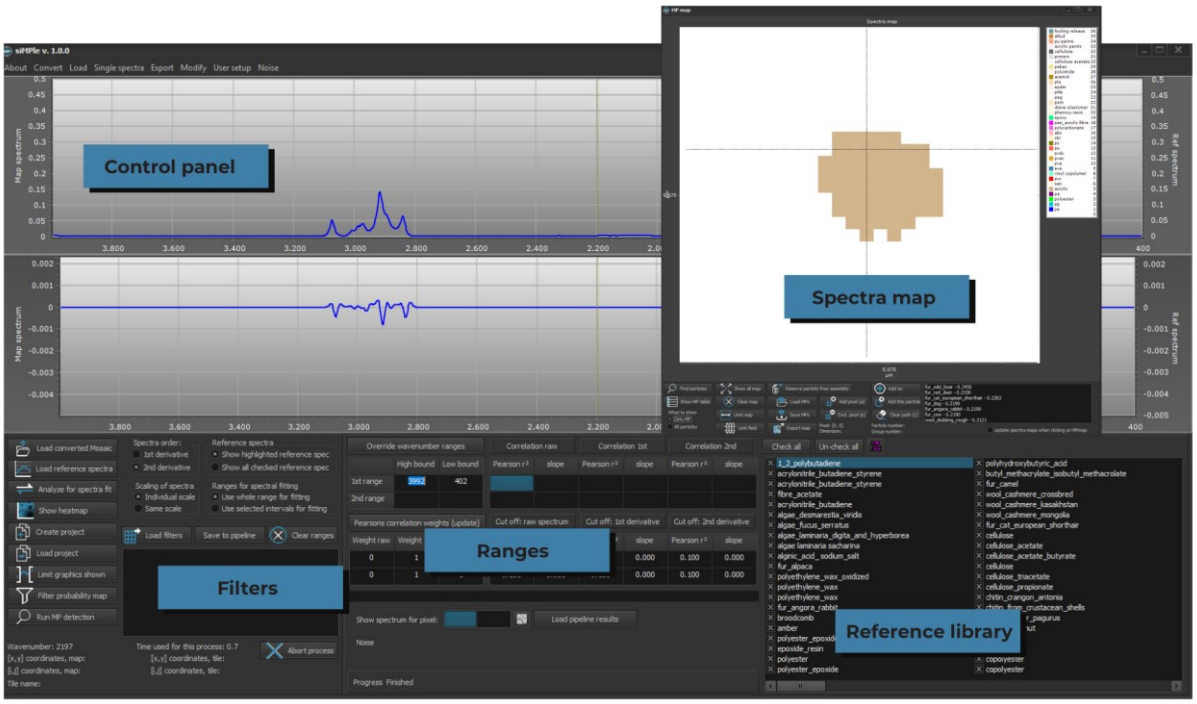
How to use siMPle?

This document is based on the current HowTos available on: http://simple-plastics.eu/howto.html

Overview of the interface:

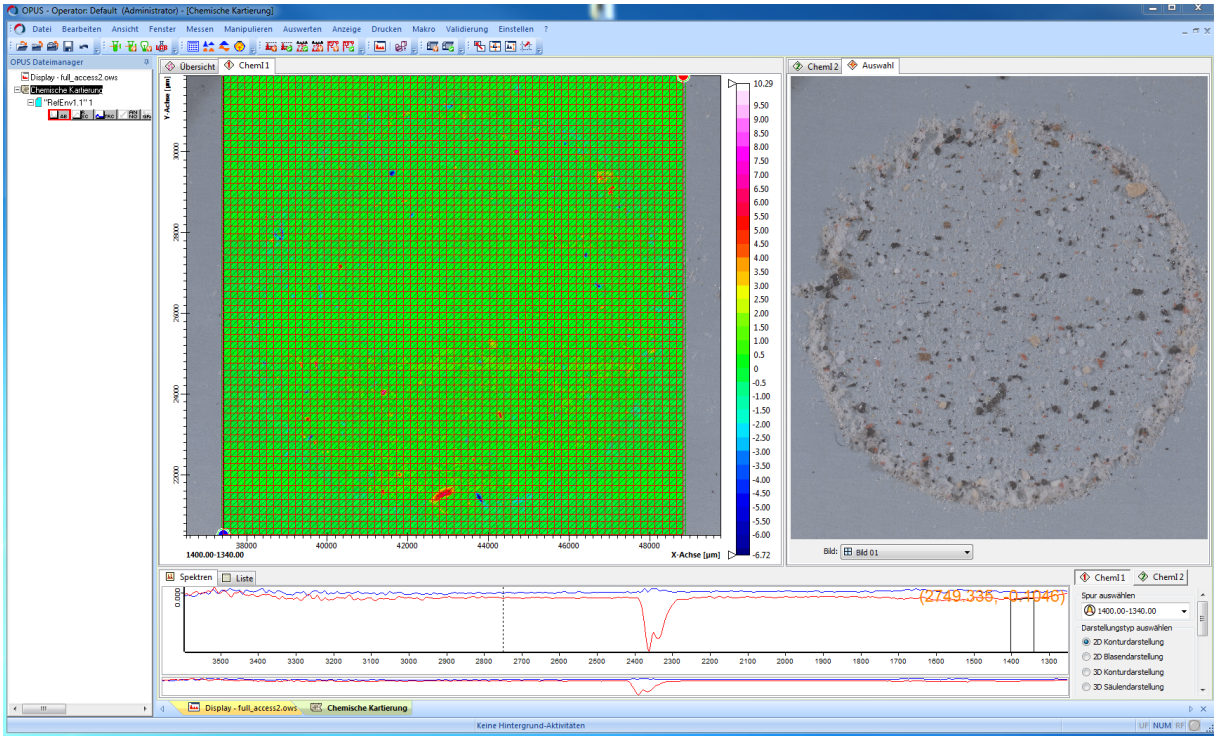


Converting Bruker and ThermoFisher Scientific Data into files suitable for siMPle.

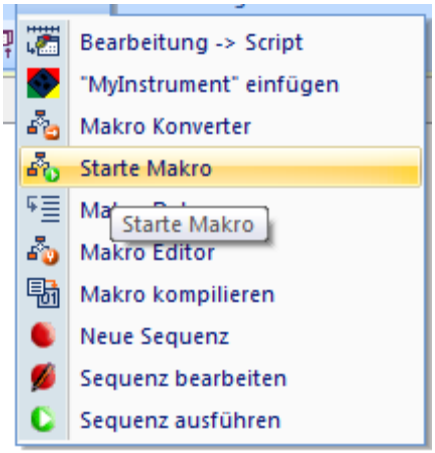
Bruker OPUS

Conversion of get OPUS Data to siMPle

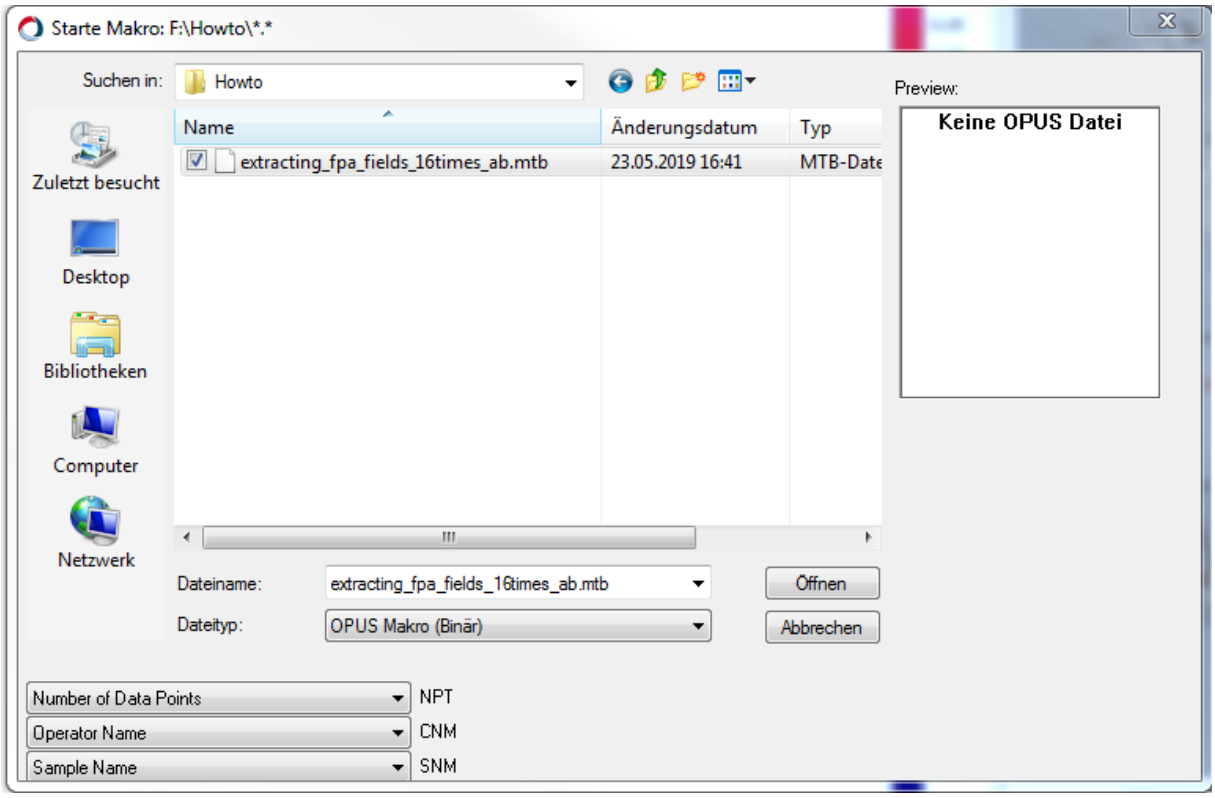
1. Load the respective file into OPUS: (This description is using the fullAccess.ows)



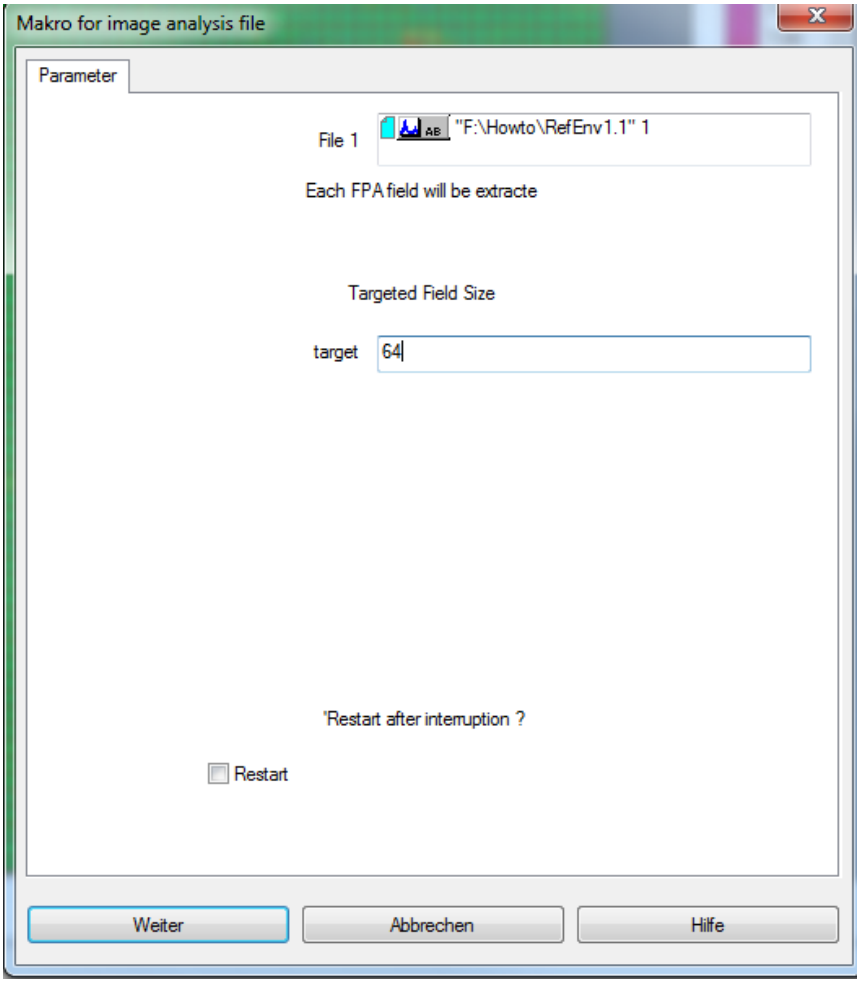
2. Go to Macro/Makro:



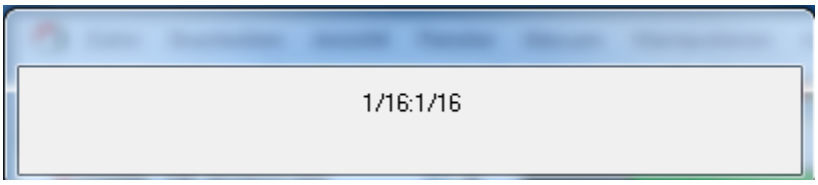
3. Choose the macro from the GoogleDrive (https://drive.google.com/open?id=1O3vtsb963KoGwsTTGvDZgo8KUqjGjNAD) (Choose as file type OPUS Macro (binary)) and open it.



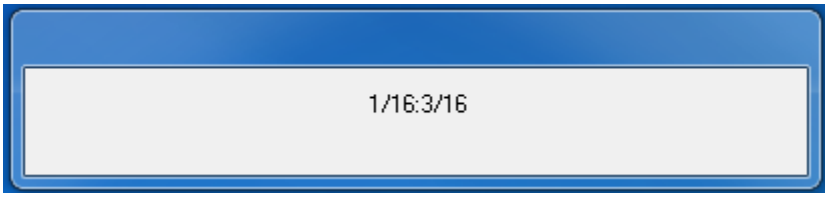
4. Choose your target field size (simple support 16,32,64,128 datapoints (will read in as 16 x 16, etc.) and click Weiter/Proceed. This case uses a value of 64.



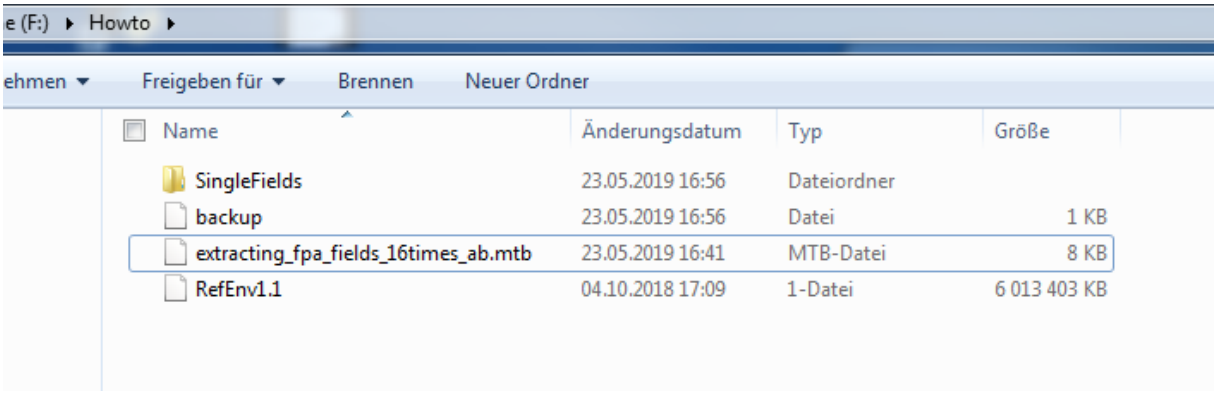
5. Data extraction starts, it is beneficial to minimize OPUS for performance:



6. These numbers should now increase:



7. Depending on the hard drive this process is rather slow or fast and one will find a new file and a folder generated:



The folder Single Fields contains the generated .dx files. Depending on the file size this process may be instable. If problems occur please inform the corresponding authors.

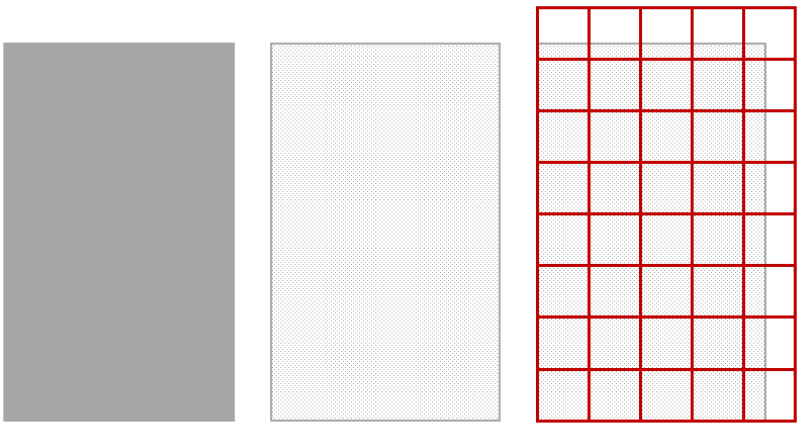
In this case the extraction of 1 million spectra in 256 single fields took 121 minutes. These values may change for different field sizes. Currently .dx files larger 128 datapoints are currently not supported by siMPle.

The files are ready to be converted via simple via BRUKER .DX INTO .SPE FILES by selecting all dx files.

THERMO µ-FTIR data

OMNIC PICTA

Preparation of map files that have been generated using the Thermo (single MCT) FTIR. Using Thermo software Picta to transform maps in a file type that can be read by the MPHunter (single jdx files).



Sample.map (original file)

Map splitted in individual spectra/files → >200.000 files

Grouping of jdx files in dx files (64x64 spectra), that is the size of an FPA detector used by Bruker

1. Open the .map file in Omnic Picta
2. Note the dimensions of the mapped area (in measuring points). As can be seen in above scheme the dx files might be larger than the original map. Rows will be 'filled up' by repeating the result of the last pixel, this needs to be corrected (see below *).
3. Create a .txt file with the bold text below. In Omnic, click ANALYZE (left bar) – SHOW MAP INFO, copy following information (bottom) in the .txt file and save this in a folder as "Map info.txt".

MAP DESCRIPTION

Tile name: MyTiles

First map location: -40.0, -5474.5

Last map location: 6880.0, 4845.5

Mapping stage X step size: 20.0 micrometers

Mapping stage Y step size: 20.0 micrometers

4. In Omnic, click ANALYZE – SPLIT MAP: Select path (created folder), choose jdx file and then OK. Splitting the data can take > 45 min (depending on the map size).

siMPle

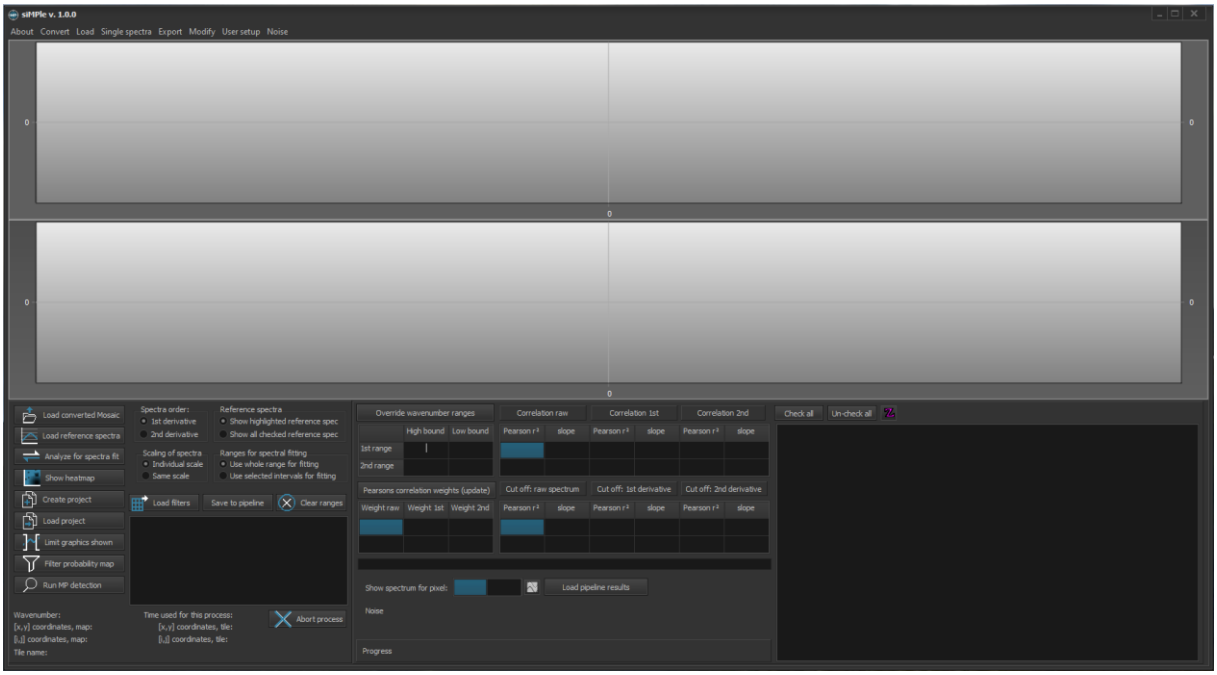
1. Click CONVERT – CONVERT NICOLET SPECTRA .JDX INTO .DX FILES: A window will open, then choose the created folder and click on the generated .txt file (map info). This will take a few minutes.

NOTE: the .jdx files must be in the same directory as the map info file, the names/ numbering done during the splitting cannot be changed!

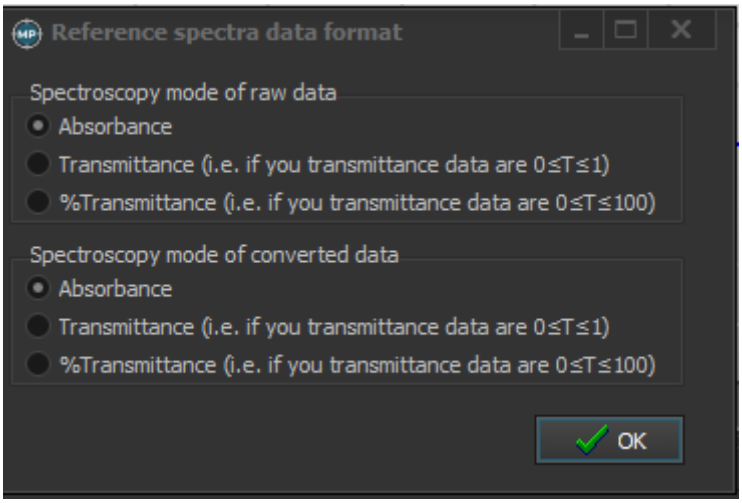
2. Click CONVERT – CONVERT BRUKER .DX INTO .SPE FILES by selecting all dx files (This will take also few minutes)
3. LOAD .SPE FILES

How to use the AWI pipeline:

1. Load .spe files or convert (.dx, .dmd, .jdx or .fsm) files into .spe, files.

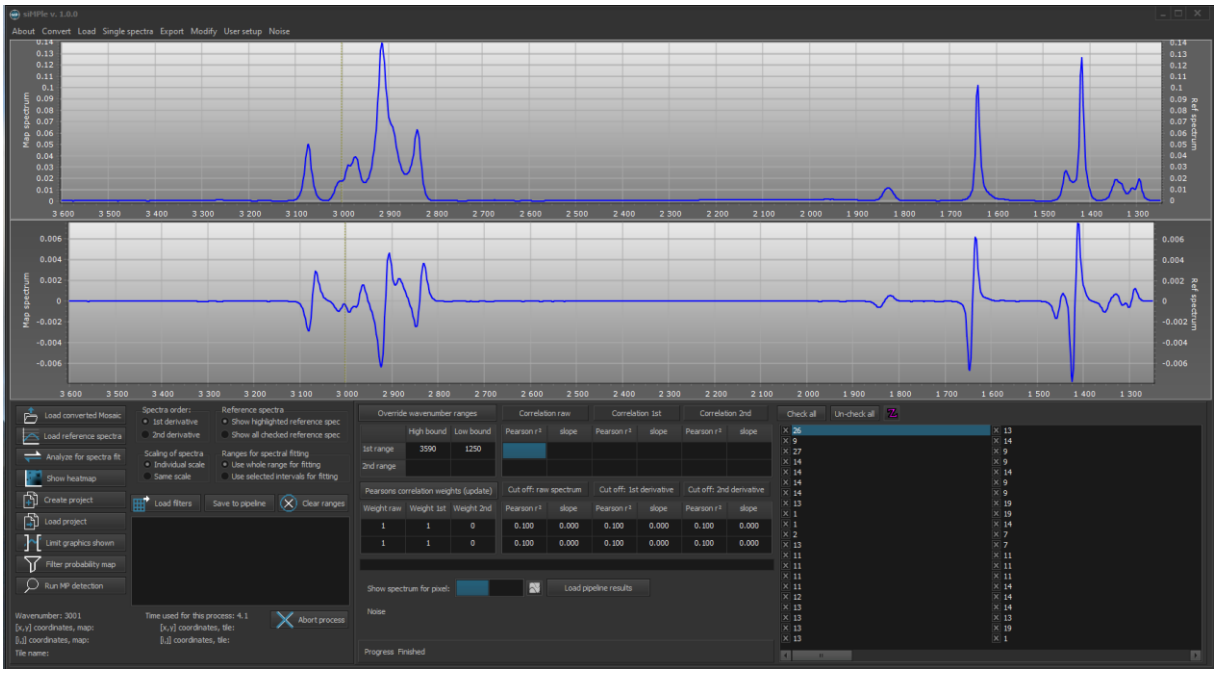


2. Load the file containing reference spectra, for AWI analysis choose: siMPle_database_Version1.0.txt

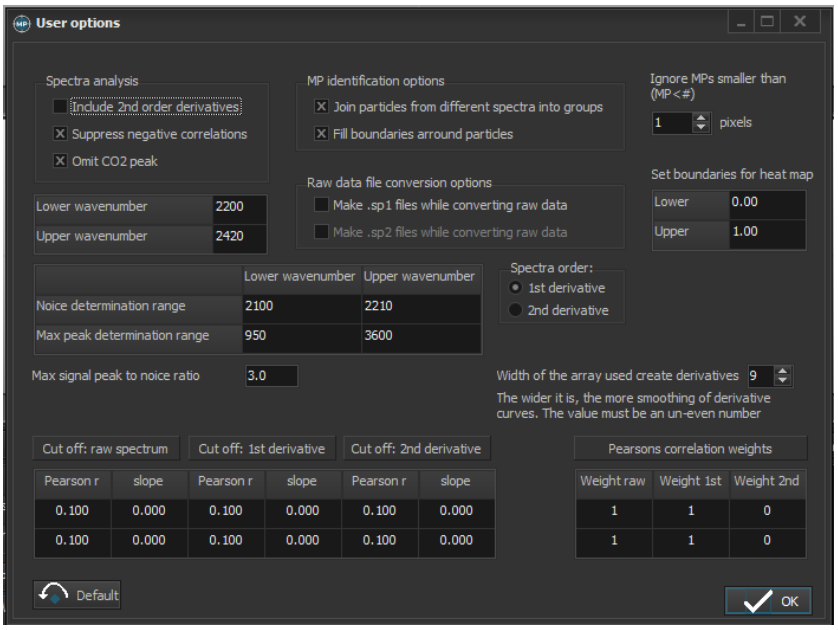


Please keep in mind that the reference spectra were measured in absorbance, press OK.

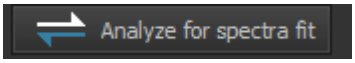
3. siMPle should now look like this:



4. Under User setup -> Options, values and options for the search can be checked and adjusted. Proceed by pressing OK.

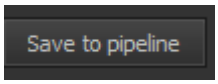


Proceed by clicking okay.

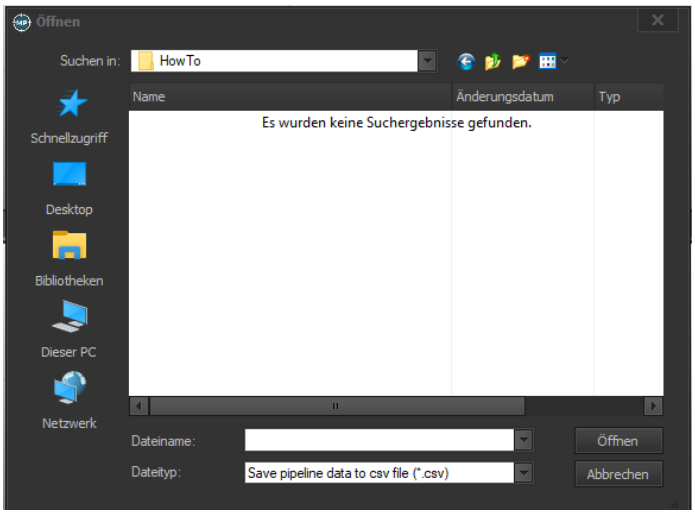
5. Start the calculation by pressing: 

The program will ask you to generate a project file (.ini), here called howto.ini, then click Save. The file will be saved automatically when the analysis is completed.

6. Wait until the calculations are finished.

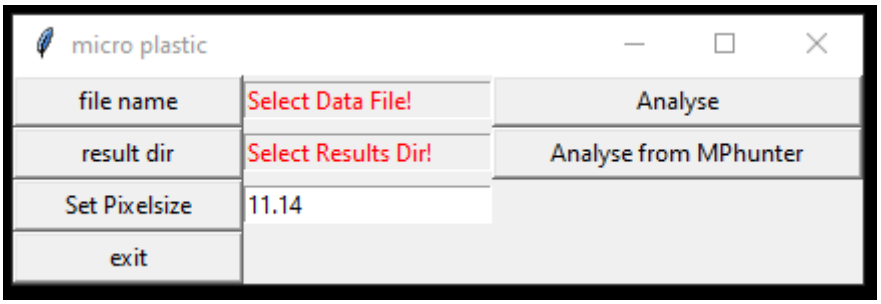
7. Afterwards, click on the button: 

8. Choose a file name (here howto.txt). Take care not to rename the database file (currently this is the default name generated by siMPle). Depending on the amount of identified MP this may take some time.

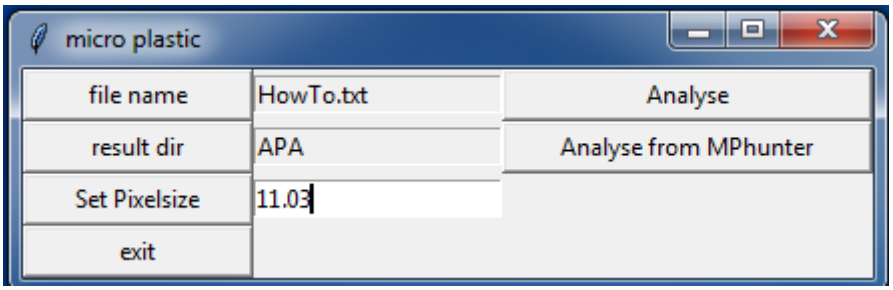


APA.exe or MPAPP.exe

1. Depending on the pipeline you want to use, open the APA.exe or MPAPP.exe (both filenames contain the current version number, e.g. APAv1.01.exe, which may differ due to updates).
2. After a short while the user interface shows up (here AAP):

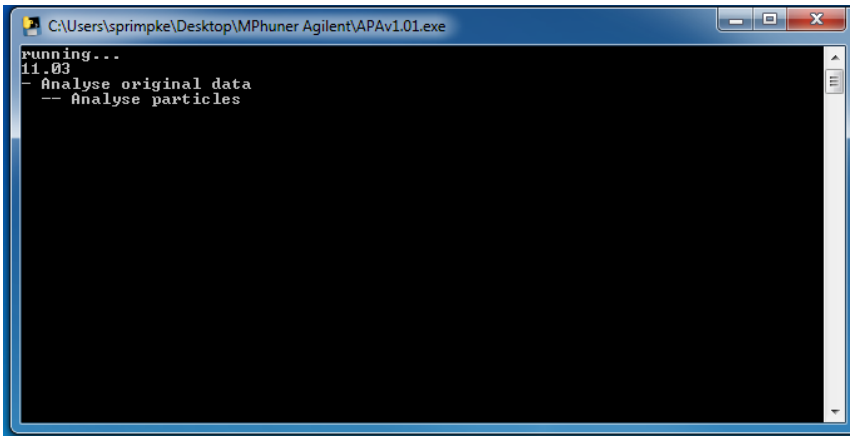


3. Set the pixel size to the value of your system, here 11.03, and press Set Pixelsize (otherwise 11.14 will be used as default).
4. By pressing “file name” you can choose the previously generated file (here HowTo.txt in the “MPhunter Agilent” folder).
5. Chose a result folder by pressing “result dir” (here called APA in the “MPhunter Agilent” folder). Important, this folder needs to be in the same folder as the executable, currently no sub folders are allowed (know bug, work in progress).

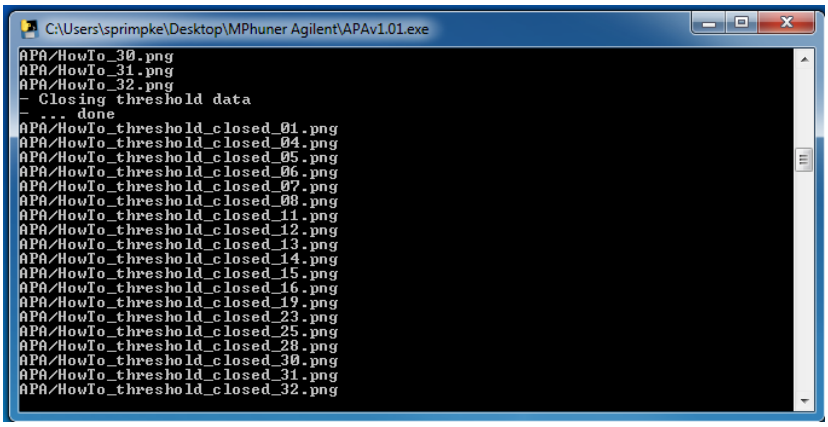


6. After folders and files are selected, and settings are set, press “Analyse from MPhunter” to start the analysis of generated data followed by an image analysis.

The start should look like this:



7. When the analysis is finished it will look like this:



8. Per samples various files are produced, all including data and images generated during image analysis. These are:

    a. A histogram for polymer type hits based on spectra.

    b. The combined (_closed_image.csv) and the individual (_closed_imageID.csv, ID = 01, etc.) image files.

    c. The number of particles found for each pixel area (_particle_histogramID.csv)

    d. All individual particle information (which number has which size) as _individualID.csv.

    e. A histogram for polymer type hits based on particle (_particles_perpolymer.csv)

    f. 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 for all polymers in one file):

	A	B	C	D	E	F	G	
1		s	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 labeled with s does not contain data and should be deleted before starting further calculations. Subsequent columns contain numbers per polymer cluster (# 1-32):

	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							

_forstatistics (all individual sizeclasses in one file):)

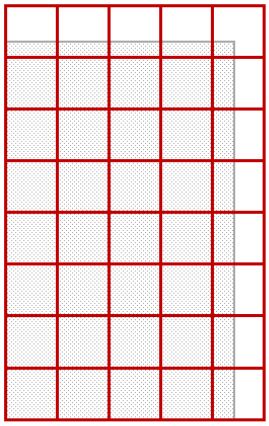
g. Greyscale image of the raw and final images.

Analysis is finished! Welcome to the data Jungle!

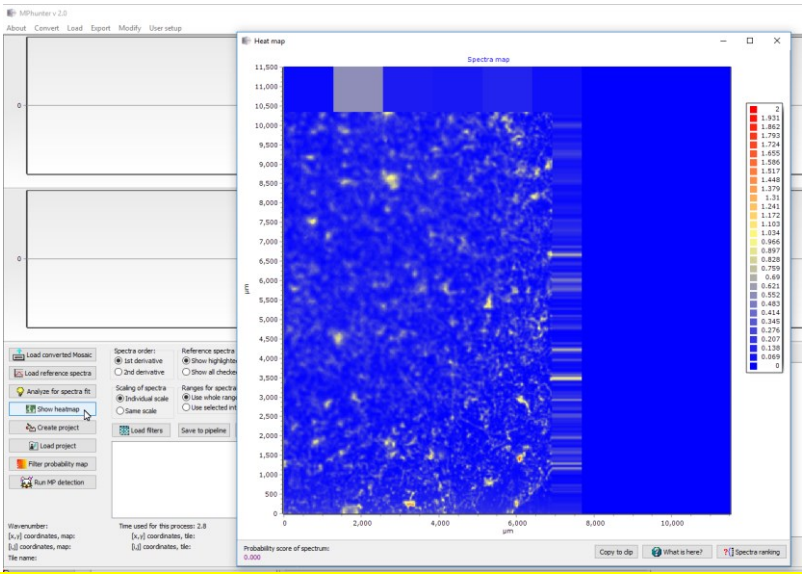
Note, using an FTIR system without an FPA detector, e.g. The Thermo system Nicolet iN10

simPLe

- Convert .jdx into .dx files, then convert .dx into .spe. While doing so MPHunter combines individual .jdx files into the shape of a 64x64 FPA format. At the right and upper edge, the last spectrum is repeated to fill up rows/ column. Do all steps with the MPHunter as indicated above. Before starting the APA/ MPAPP analysis remove these artefacts.



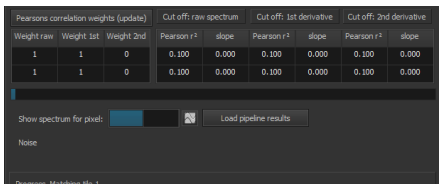
Grouping of jdx files in dx files (64x64 spectra), that is the size of an FPA detector used by Bruker

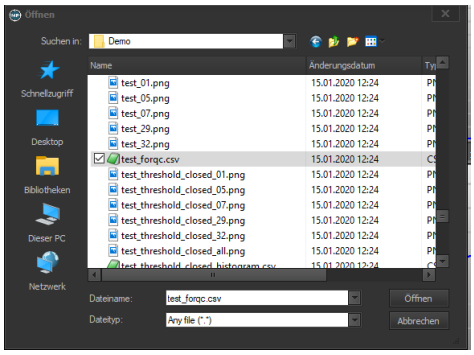


- This could be done using the software Origin, for this:
- Open – File – Import Wizard
- Choose file (howto.forqc.csv) – ‘Add file’ - ‘Ok’
- Mark column 3 and 4, right click ‘set as Z’

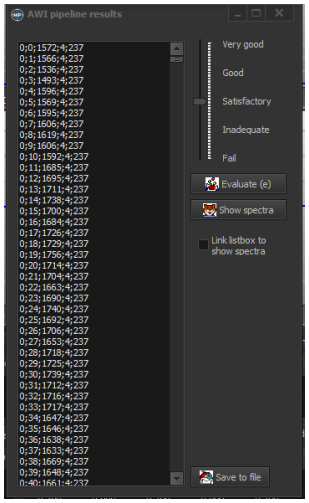
QA/QC

1. Load the file labeled “_for_qc” via the Load pipeline results function from Image Analysis:

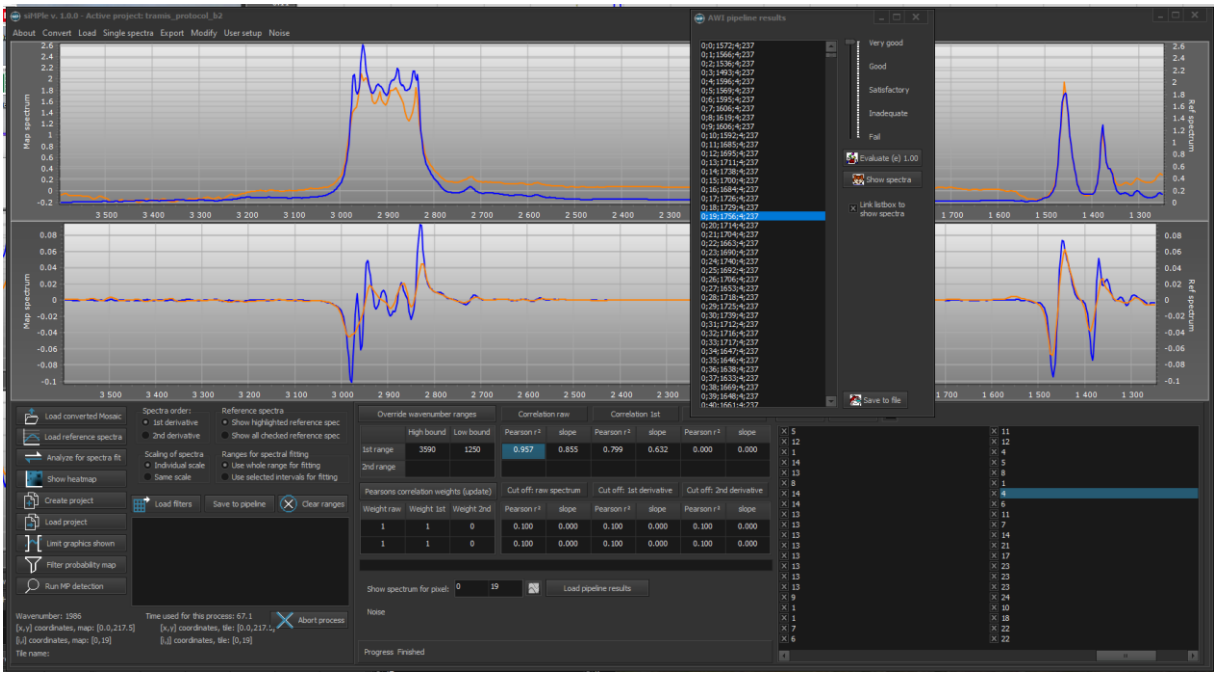




2. Activate “Link listbox to show spectra”:



3. Start QA/QC as described in the main document.

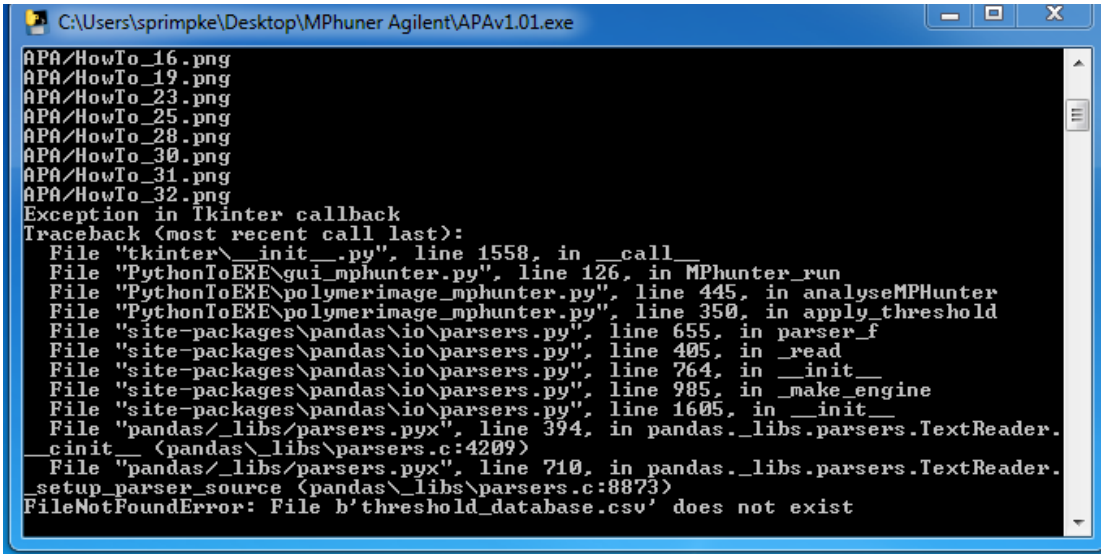


4. Click on “Save to file” so reassess the data later or for further evaluation.

Troubleshooting:

Error Messages (Traceback):

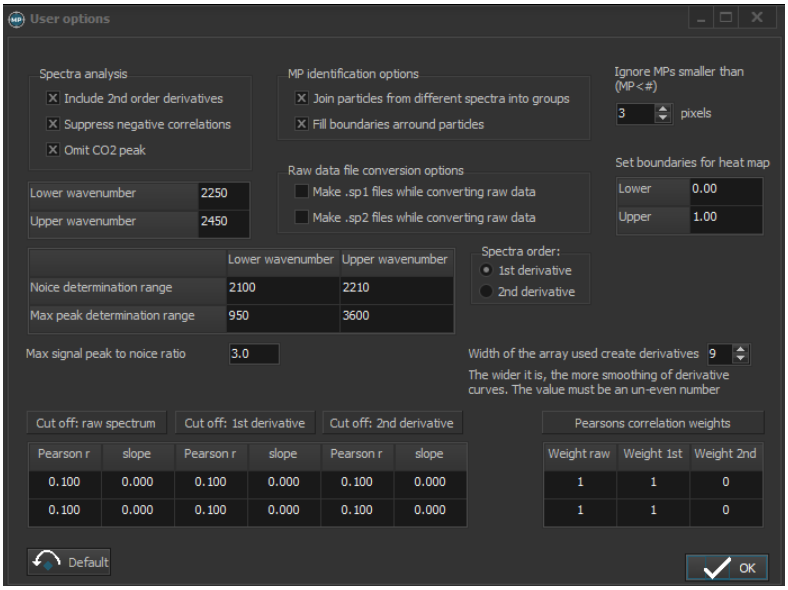
a. Missing the threshold.csv file in the folder with the executable for APA/MPAPP:



Please copy the provide threshold_database.csv into the folder.

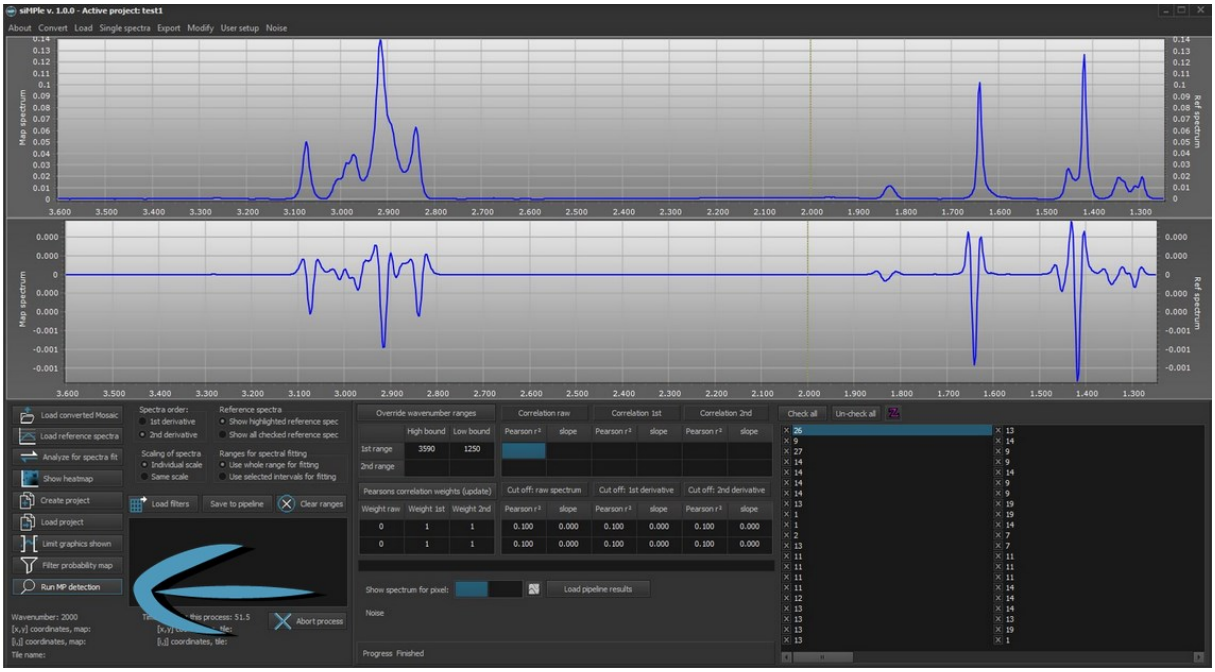
The AAU pipeline:

The second pipeline uses the following user setting prior to spectral fit (see above for the procedure).

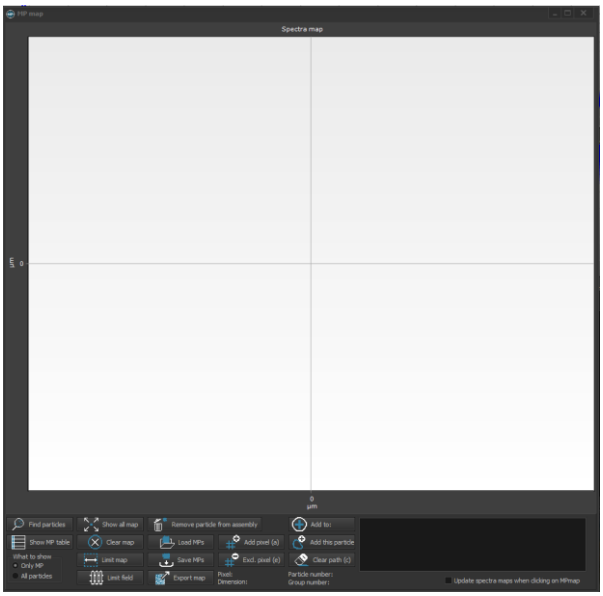


1. After spectral fit click on run MP detection

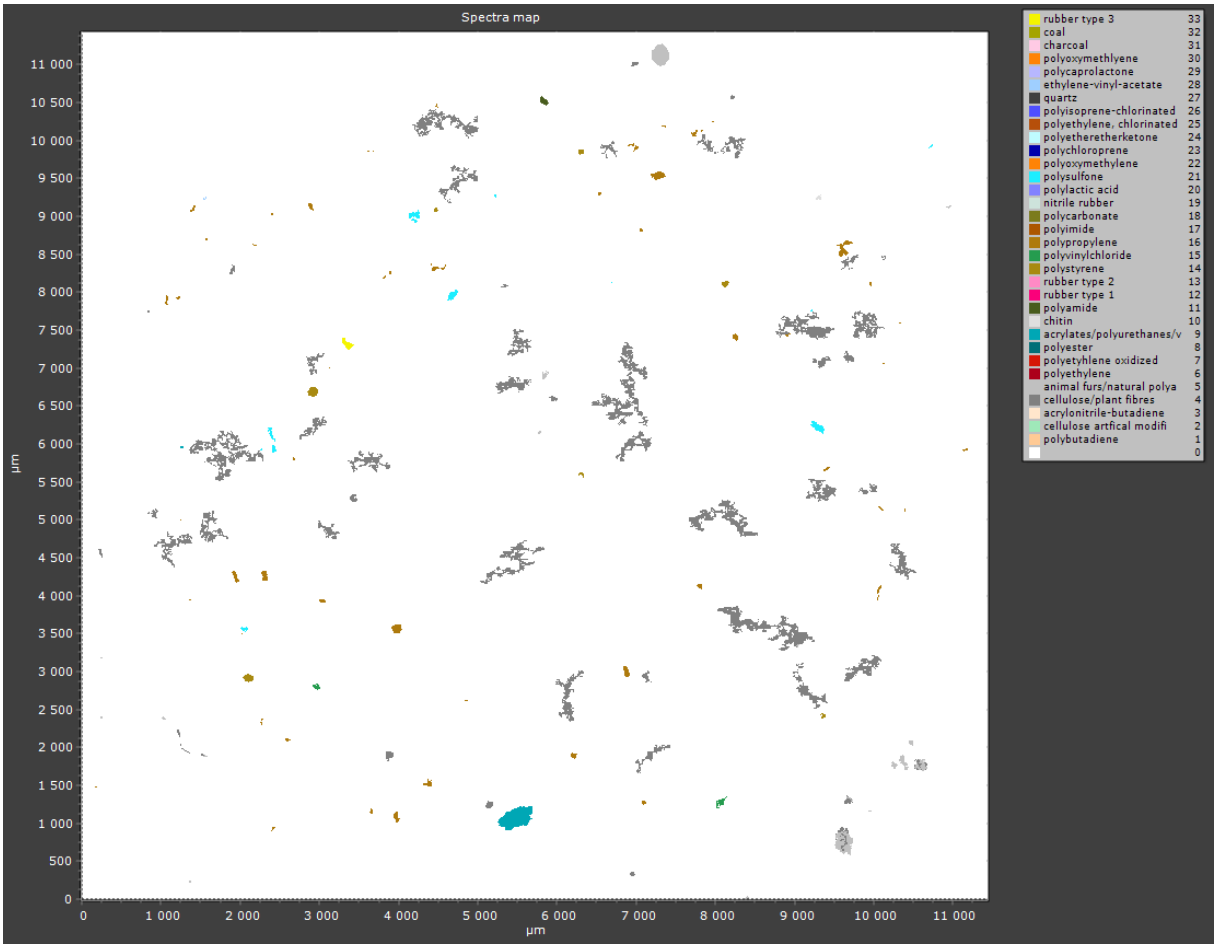
You can setup the probability thresholds before that: "Modify -> Modify loaded parameters for reference spectra"



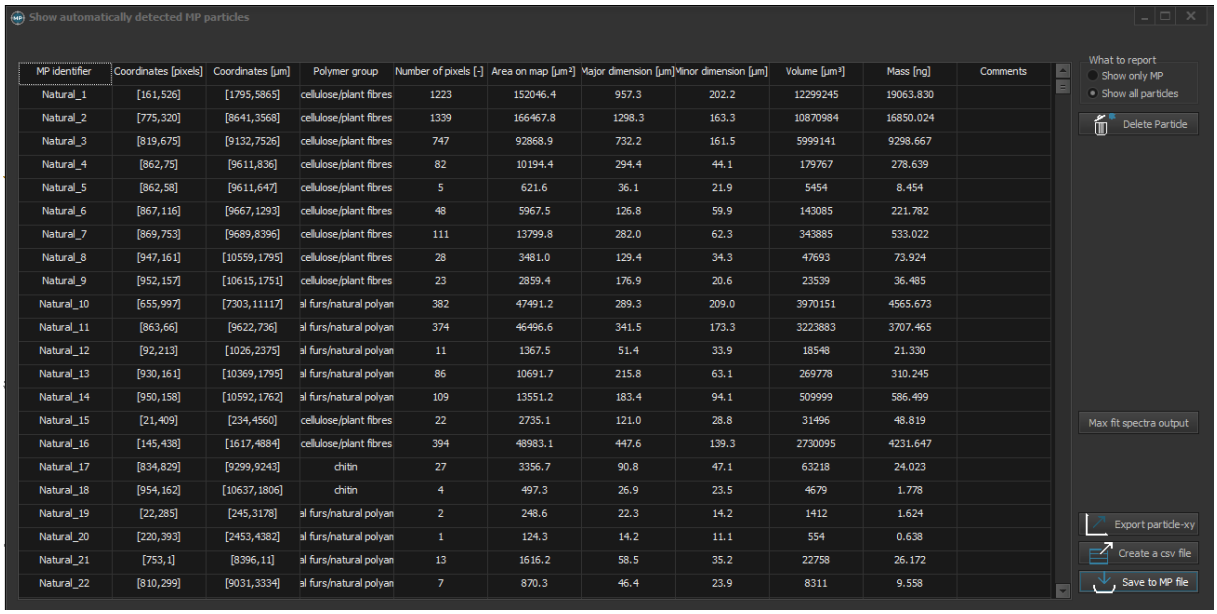
2. Click on Run MP detection and afterwards Find particles in the following window:



3. This results in a false color image (here RefEnv1):

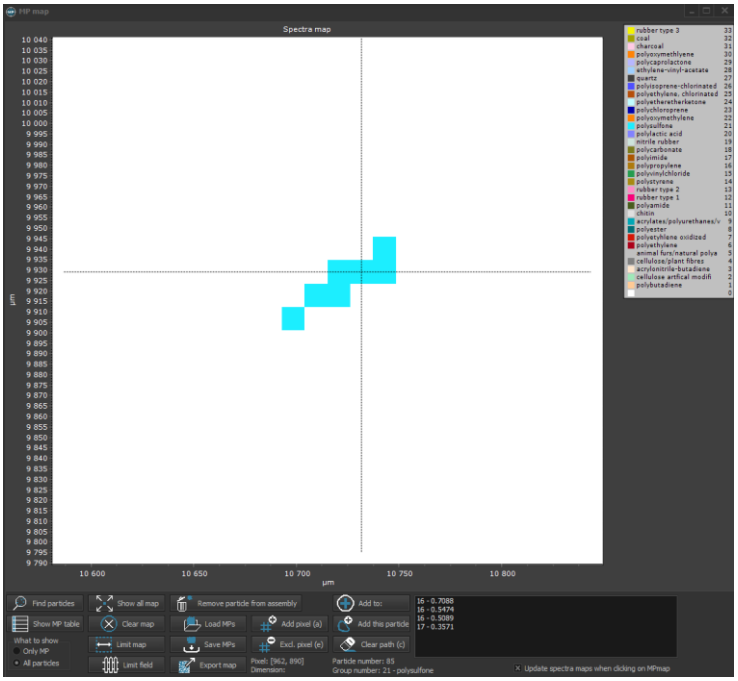


4. All particles are listed via Show MP table containing all results which can be exported to .csv files for future analysis.



MP identifier	Coordinates [pixels]	Coordinates [µm]	Polymer group	Number of pixels [-]	Area on map [µm²]	Major dimension [µm]	Minor dimension [µm]	Volume [µm³]	Mass [ng]	Comments
Natural_1	[161,526]	[1795,5865]	cellulose/plant fibres	1223	152046.4	957.3	202.2	12299245	19063.830	
Natural_2	[775,320]	[8641,3568]	cellulose/plant fibres	1339	166467.8	1298.3	163.3	10870984	16850.024	
Natural_3	[819,675]	[9132,7526]	cellulose/plant fibres	747	92868.9	732.2	161.5	5999141	9298.667	
Natural_4	[862,75]	[9611,836]	cellulose/plant fibres	82	10194.4	294.4	44.1	179767	278.639	
Natural_5	[862,58]	[9611,647]	cellulose/plant fibres	5	621.6	36.1	21.9	5454	8.454	
Natural_6	[867,116]	[9667,1293]	cellulose/plant fibres	48	5967.5	126.8	59.9	143085	221.782	
Natural_7	[869,753]	[9689,8396]	cellulose/plant fibres	111	13799.8	282.0	62.3	343885	533.022	
Natural_8	[947,161]	[10559,1795]	cellulose/plant fibres	28	3481.0	129.4	34.3	47693	73.924	
Natural_9	[952,157]	[10615,1751]	cellulose/plant fibres	23	2859.4	176.9	20.6	23539	36.485	
Natural_10	[655,997]	[7303,11117]	al furs/natural polyan	382	47491.2	289.3	209.0	3970151	4565.673	
Natural_11	[863,66]	[9622,736]	al furs/natural polyan	374	46496.6	341.5	173.3	3223883	3707.465	
Natural_12	[92,213]	[1026,2375]	al furs/natural polyan	11	1367.5	51.4	33.9	18548	21.330	
Natural_13	[930,161]	[10369,1795]	al furs/natural polyan	86	10691.7	215.8	63.1	269778	310.245	
Natural_14	[950,158]	[10592,1762]	al furs/natural polyan	109	13551.2	183.4	94.1	509999	586.499	
Natural_15	[21,409]	[234,4560]	cellulose/plant fibres	22	2735.1	121.0	28.8	31496	48.819	
Natural_16	[145,438]	[1617,4884]	cellulose/plant fibres	394	48983.1	447.6	139.3	2730095	4231.647	
Natural_17	[834,829]	[9299,9243]	chitin	27	3356.7	90.8	47.1	63218	24.023	
Natural_18	[954,162]	[10637,1806]	chitin	4	497.3	26.9	23.5	4679	1.778	
Natural_19	[22,285]	[245,3178]	al furs/natural polyan	2	248.6	22.3	14.2	1412	1.624	
Natural_20	[220,393]	[2453,4382]	al furs/natural polyan	1	124.3	14.2	11.1	554	0.638	
Natural_21	[753,1]	[8396,11]	al furs/natural polyan	13	1616.2	58.5	35.2	22758	26.172	
Natural_22	[810,299]	[9031,3334]	al furs/natural polyan	7	870.3	46.4	23.9	8311	9.558	

5. The particles can either be selected by clicking on the entry or on the MP map for QA/QC (Activate “Update spectra maps when clicking on MP map”)



Spectrum of the selected pixel of the identified particle.