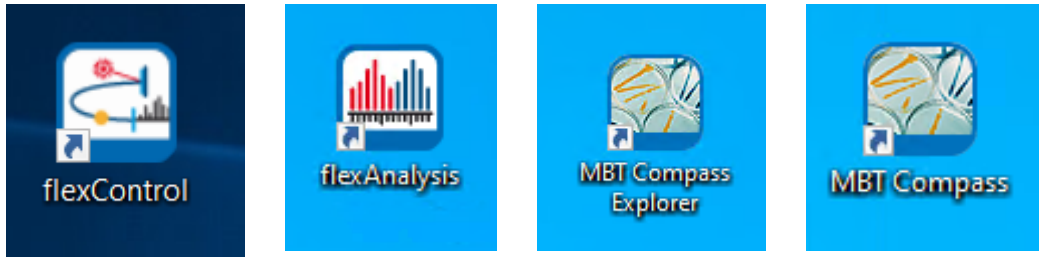
Creation of Library Entries (Main specta -MSPs)

- from raw spectrum to database entry -

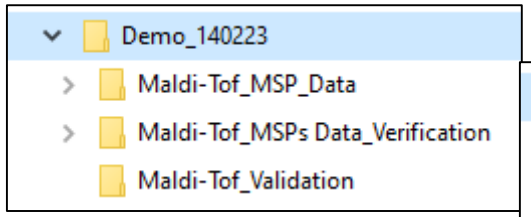
This document provides the basics for creating your own MSPs to expand your own MALDI-TOF database and is based on own experience. It does not claim to be complete and does not replace Bruker's application training courses. All image rights to the software systems shown belong to Bruker.

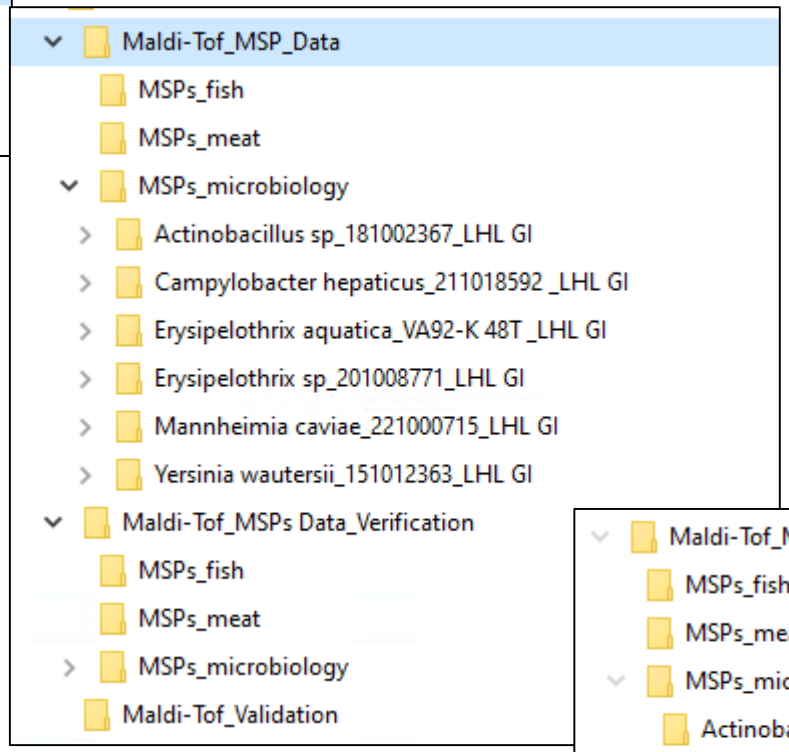
Agenda

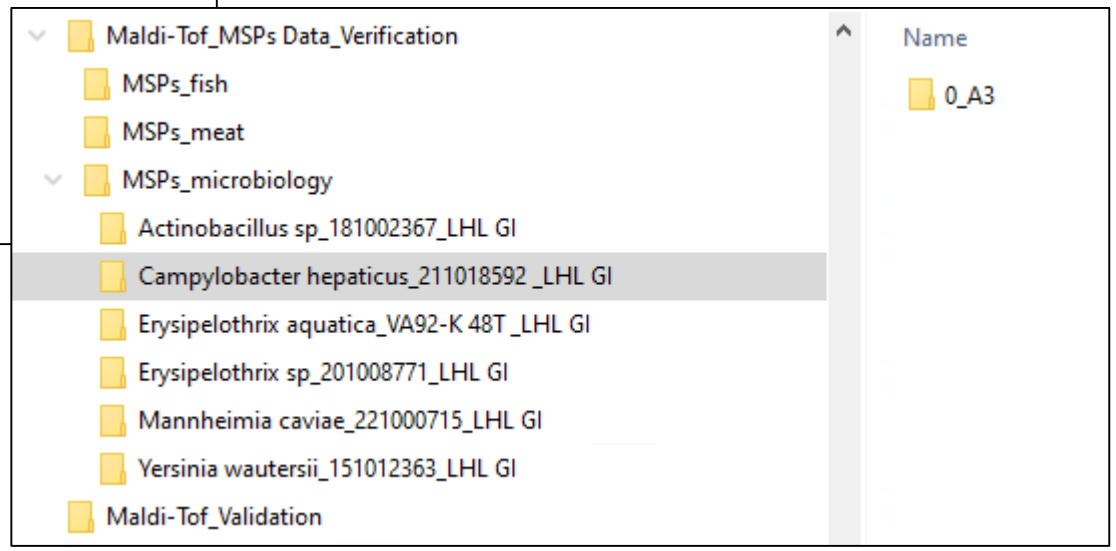


- data structure and nomenclature
- generation of raw spectra - flexControl
- raw spectra processing – flexAnalysis
- mass spectrum (MSP) creation – MBT Compass Explorer
- quality control – MBT Compass

Data structures







file directories for

- **raw data**
- **sorted/edited data**

➡ **always use copies of the raw file**

➡ **location? (MALDI PC, network storage …)**

Nomenclature (file-names)

data entries (MSPs)

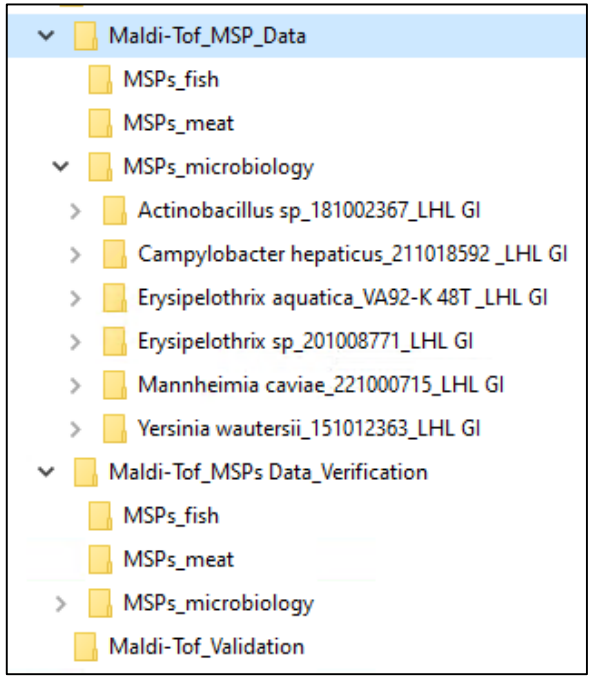
Bruker:

Genus Species Ssp(o.s.) ID Creator

e.g.: Staphylococcus aureus anaerobius AGES 001 CVUAS

Aerococcus sp-185-U1-2 185-U1-2 CVUAS

Campylobacter hepaticus_2110592_LHL GI



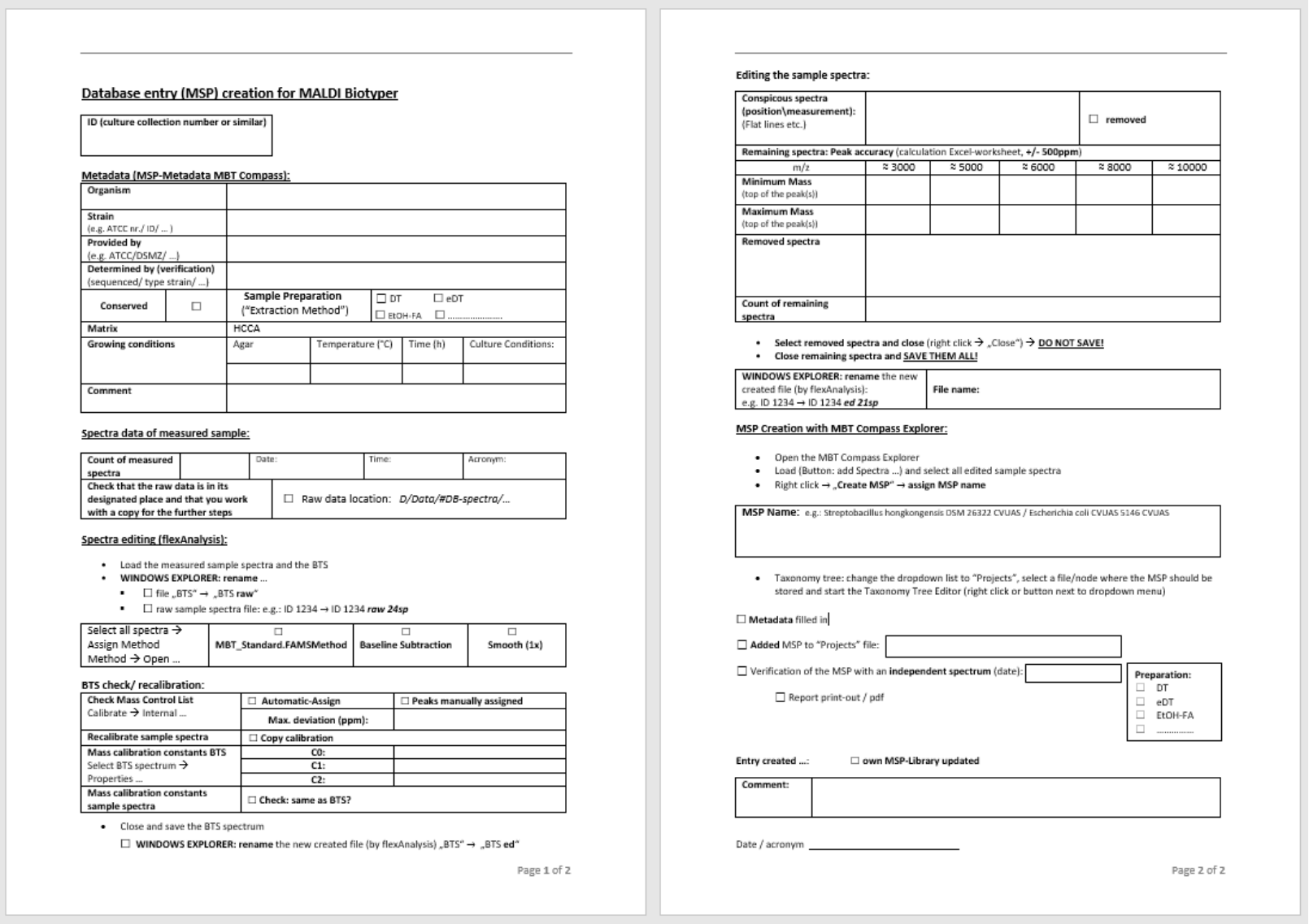
Attention:

- only use characters that are allowed by Windows for filenames
- no characters as [];:@ ….

  Problem: you can create MSPs with these characters but you cannot export these later

Fig: CVUA Stuttgart, 2022

Documentation



Database entry (MSP) creation for MALDI Biotyper

ID (culture collection number or similar)

Metadata (MSP-Metadata MBT Compass):

Organism					
Strain (e.g. ATCC nr./ ID/ …)					
Provided by (e.g. ATCC/DSMZ/ …)					
Determined by (verification) (sequenced/ type strain/ …)					
Conserved	☐	**Sample Preparation** ("Extraction Method")	☐ DT ☐ eDT ☐ EtOH-FA ☐ …….		
Matrix		HCCA			
Growing conditions		Agar	Temperature (°C)	Time (h)	Culture Conditions:
Comment					

Spectra data of measured sample:

Count of measured spectra		Date:	Time:	Acronym:
Check that the raw data is in its designated place and that you work with a copy for the further steps	☐ Raw data location: *D/Data/#DB-spectra/…*			

Spectra editing (flexAnalysis):

- Load the measured sample spectra and the BTS
- **WINDOWS EXPLORER: rename** …
  - ☐ file „BTS" → „BTS **raw**"
  - ☐ raw sample spectra file: e.g.: ID 1234 → ID 1234 ***raw 24sp***

Select all spectra → Assign Method Method → Open …	☐ **MBT_Standard.FAMSMethod**	☐ **Baseline Subtraction**	☐ **Smooth (1x)**

BTS check/ recalibration:

Check Mass Control List Calibrate → Internal …	☐ **Automatic-Assign**	☐ **Peaks manually assigned**
	Max. deviation (ppm):	
Recalibrate sample spectra	☐ **Copy calibration**	
Mass calibration constants BTS Select BTS spectrum → Properties …	**C0:**	
	C1:	
	C2:	
Mass calibration constants sample spectra	☐ **Check: same as BTS?**	

- Close and save the BTS spectrum
  - ☐ **WINDOWS EXPLORER: rename** the new created file (by flexAnalysis) „BTS" → „BTS **ed**"

Editing the sample spectra:

Conspicous spectra (position\measurement): (Flat lines etc.)				☐ **removed**	
Remaining spectra: Peak accuracy (calculation Excel-worksheet, **+/- 500ppm**)					
m/z	≈ 3000	≈ 5000	≈ 6000	≈ 8000	≈ 10000
Minimum Mass (top of the peak(s))					
Maximum Mass (top of the peak(s))					
Removed spectra					
Count of remaining spectra					

- **Select removed spectra and close** (right click → „Close") → **DO NOT SAVE!**
- **Close remaining spectra and SAVE THEM ALL!**

WINDOWS EXPLORER: rename the new created file (by flexAnalysis): e.g. ID 1234 → ID 1234 ***ed 21sp***	**File name:**

MSP Creation with MBT Compass Explorer:

- Open the MBT Compass Explorer
- Load (Button: add Spectra …) and select all edited sample spectra
- Right click → **„Create MSP" → assign MSP name**

MSP Name: e.g.: Streptobacillus hongkongensis DSM 26322 CVUAS / Escherichia coli CVUAS 5146 CVUAS

- Taxonomy tree: change the dropdown list to "Projects", select a file/node where the MSP should be stored and start the Taxonomy Tree Editor (right click or button next to dropdown menu)

☐ **Metadata** filled in

☐ **Added** MSP to "Projects" file:

☐ Verification of the MSP with an **independent spectrum** (date):

☐ Report print-out / pdf

Preparation:
☐ DT
☐ eDT
☐ EtOH-FA
☐ ……

Entry created …: ☐ **own MSP-Library updated**

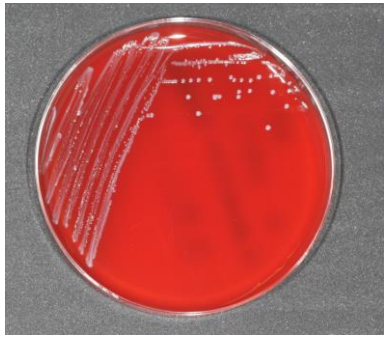
Comment:	

Date / acronym _____

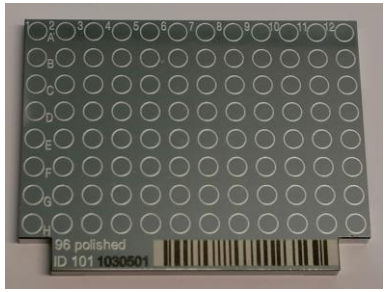
Generation of raw-spectra - sample preparation

- use fresh reagents
- prepare valid and well-characterized strains (e.g. 16s rRNA gene sequencing)
- preparation according to Bruker

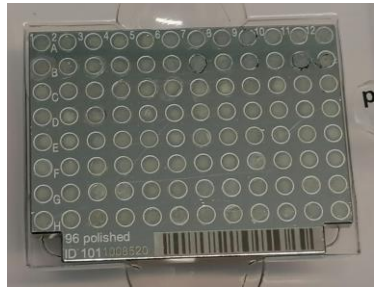
  https://www.bruker.com/en/services/training/microbiology-and-diagnostics/maldi-biotyper-training-movies.html
- sample preparation: extraction protocol
- make a test measurement of the extraction before use
- prepare spots on Target
- Prepare BTS



Fresh valid culture (max. 24 h)



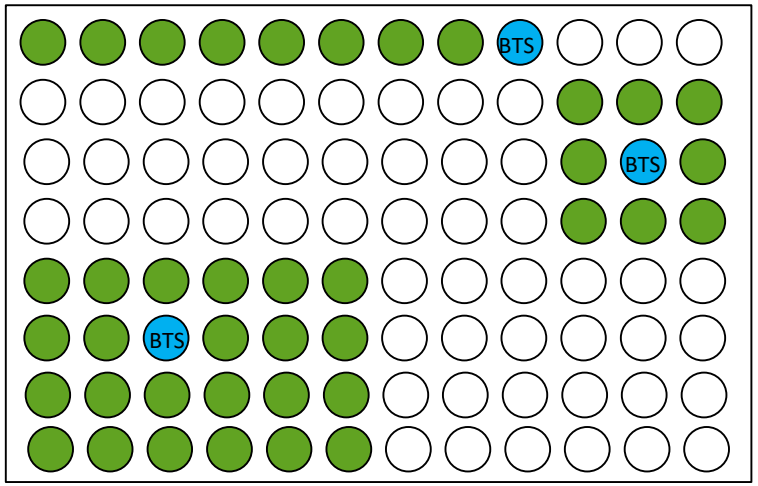
Target before preparation

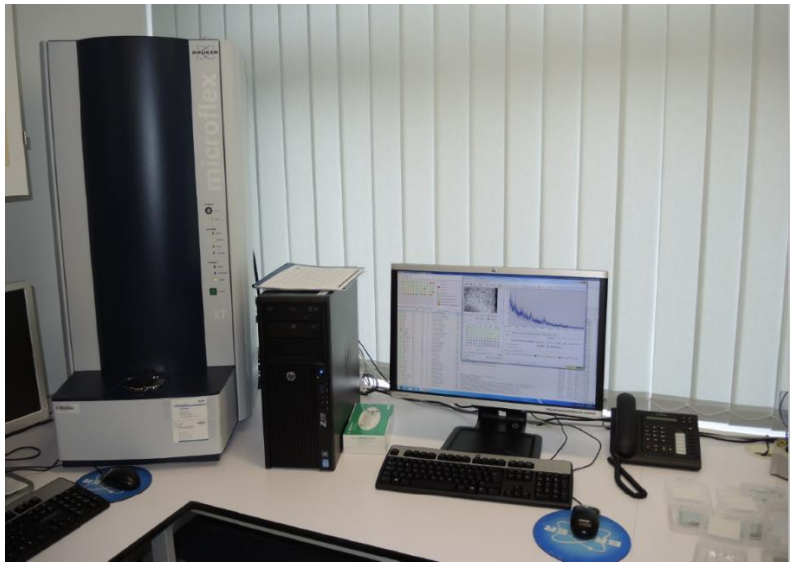


Target after preparation

Generation of raw-spectra - flexControl

- prepare 8 sample spots and measure each spot 3 times = 24 spectra
  alternative: 23 spots * 2 = 46 spectra
  8 spots * 4 = 32 spectra



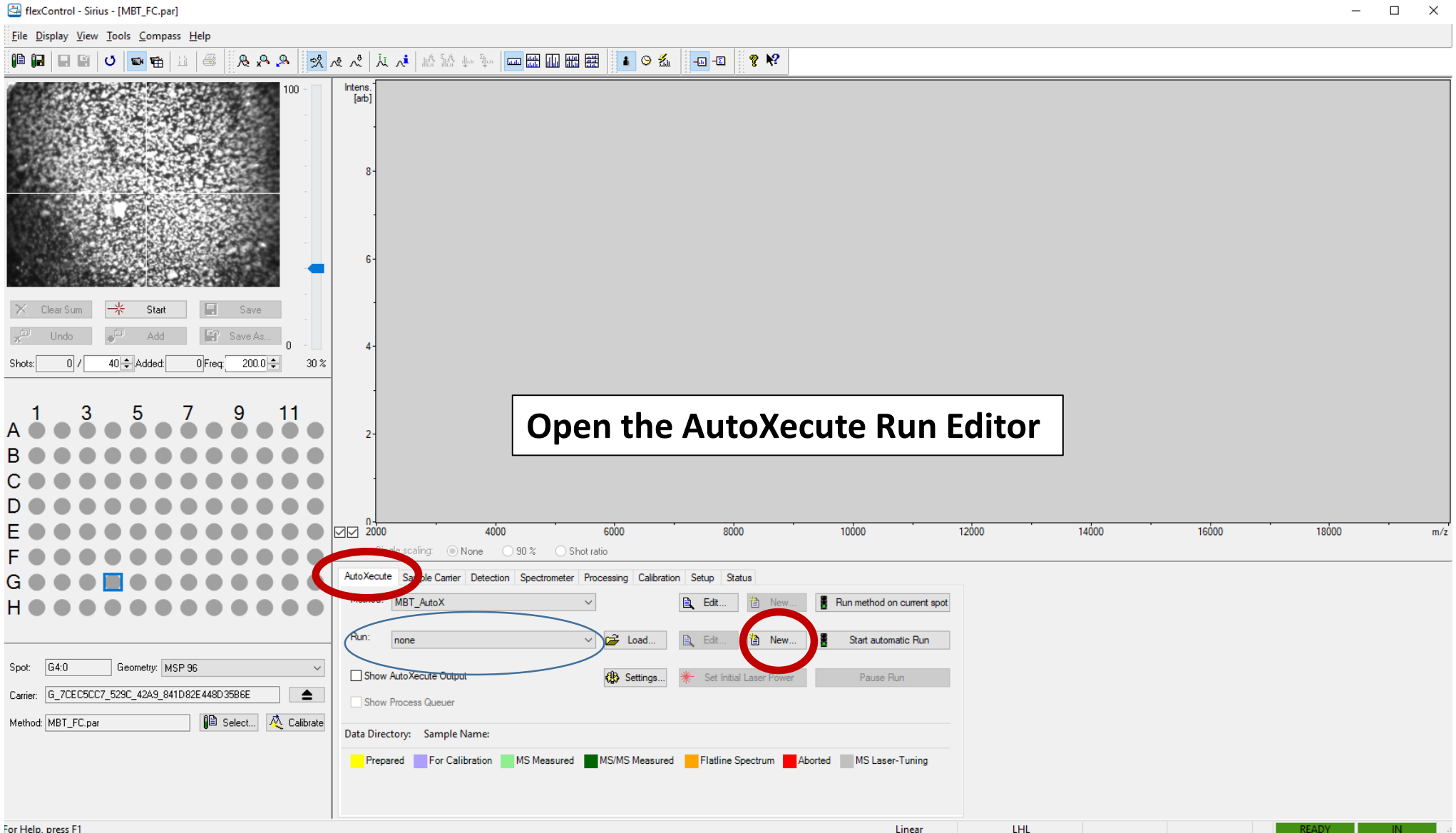


Biotyper LT-microflex, Bruker Daltonik

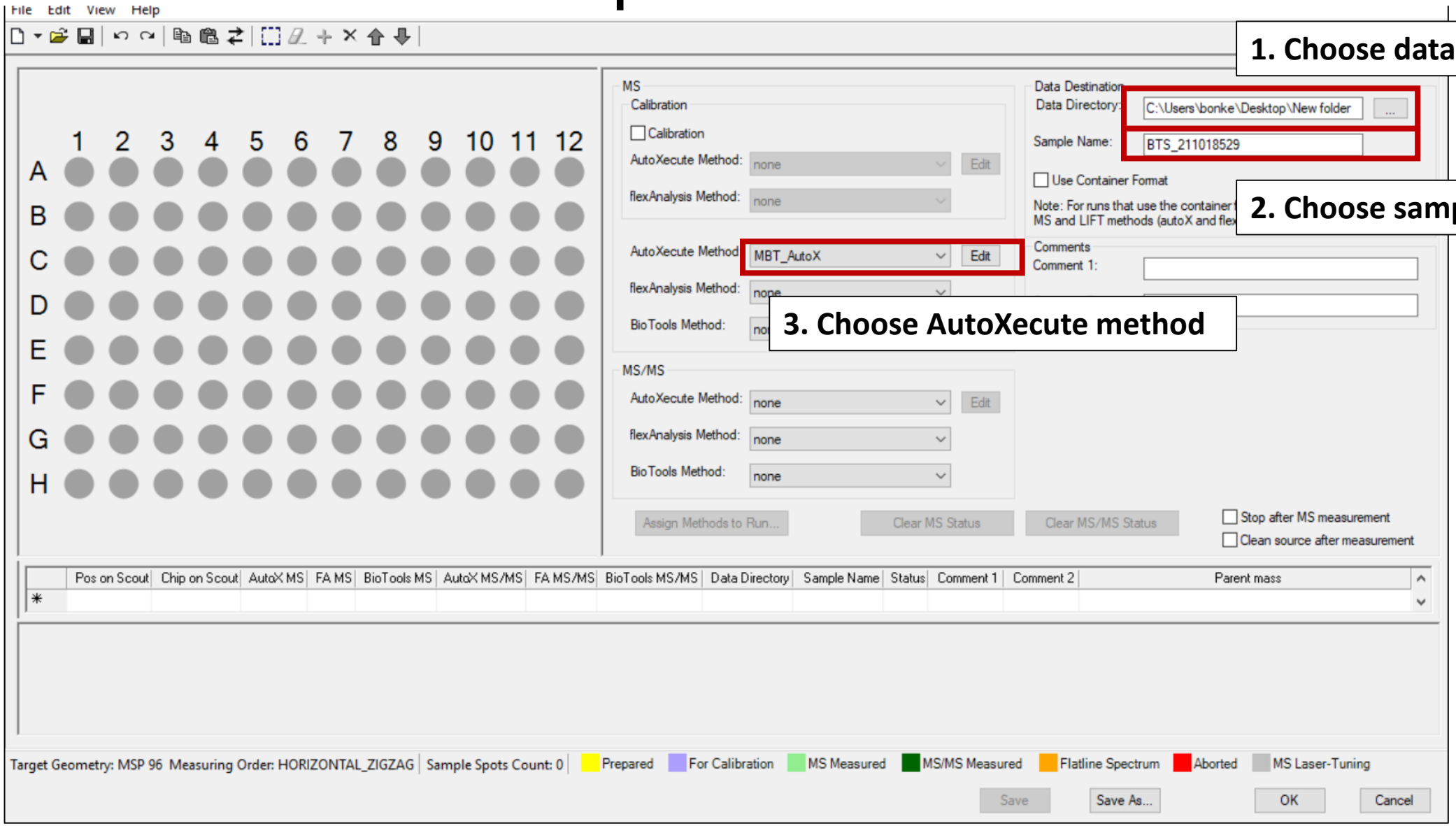
- one BTS preparation for calibration and quality control

Fig: CVUAS, 2022

Generation of raw-spectra - flexControl



Generation of raw-spectra - flexControl



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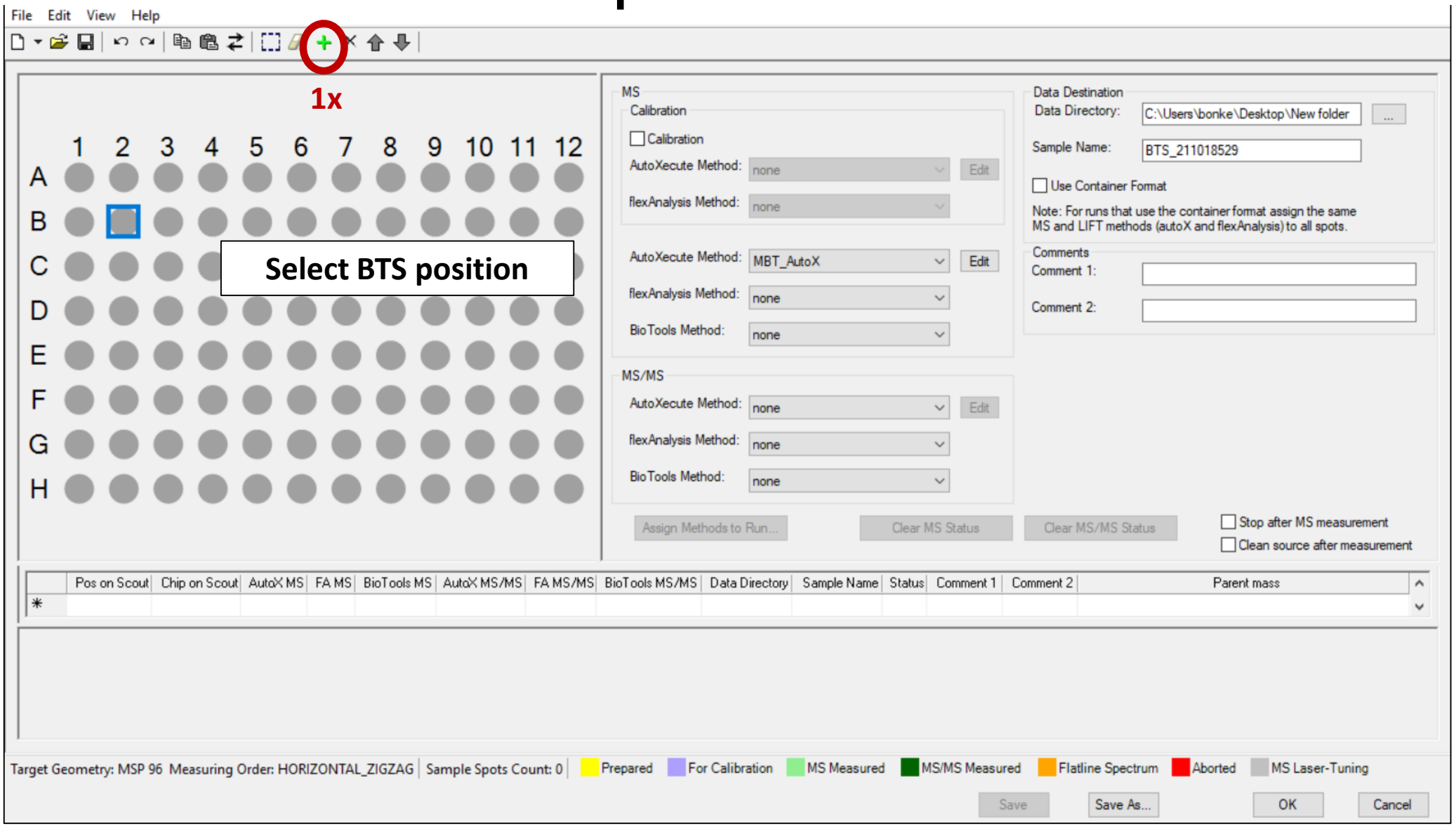

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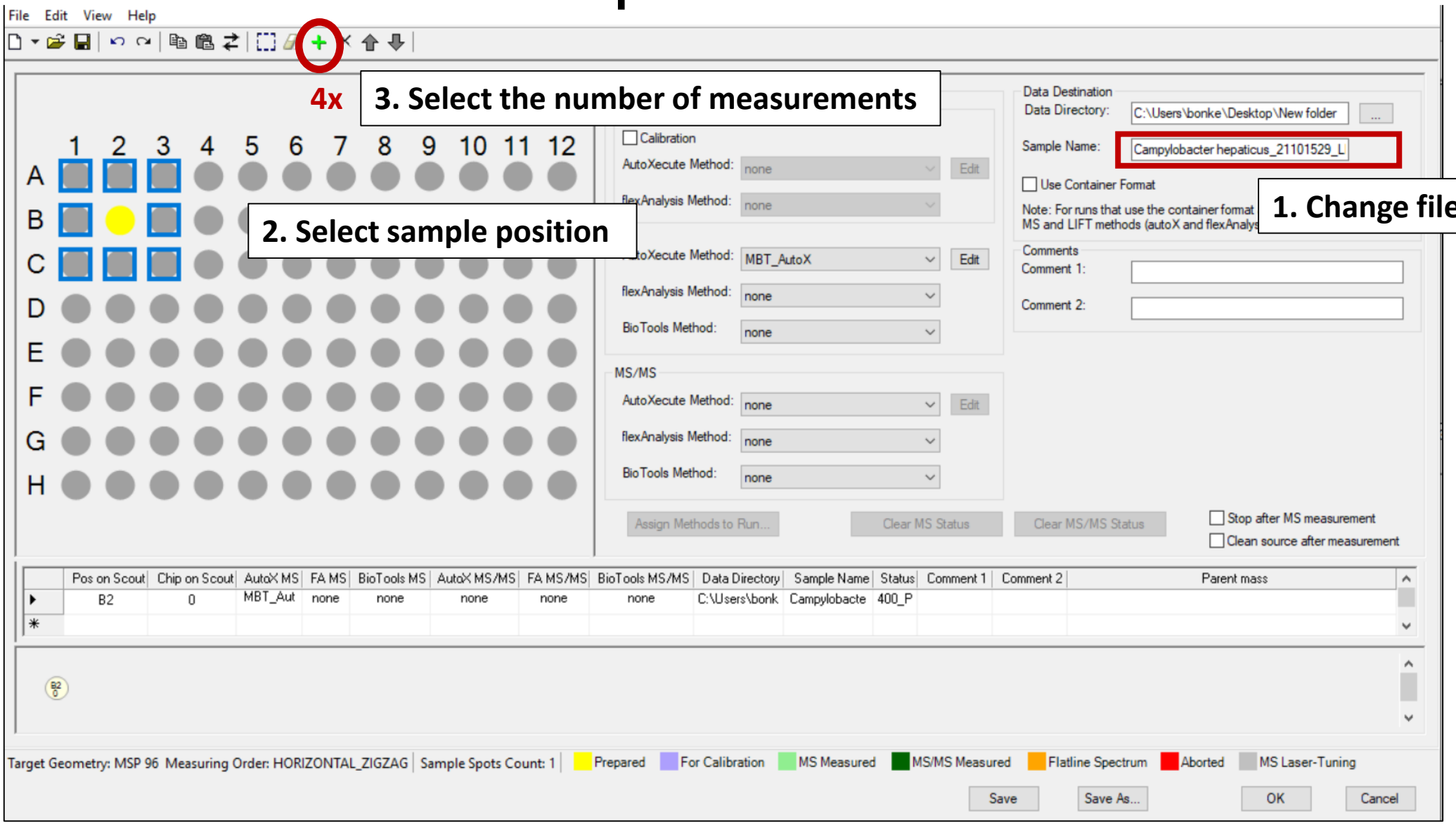

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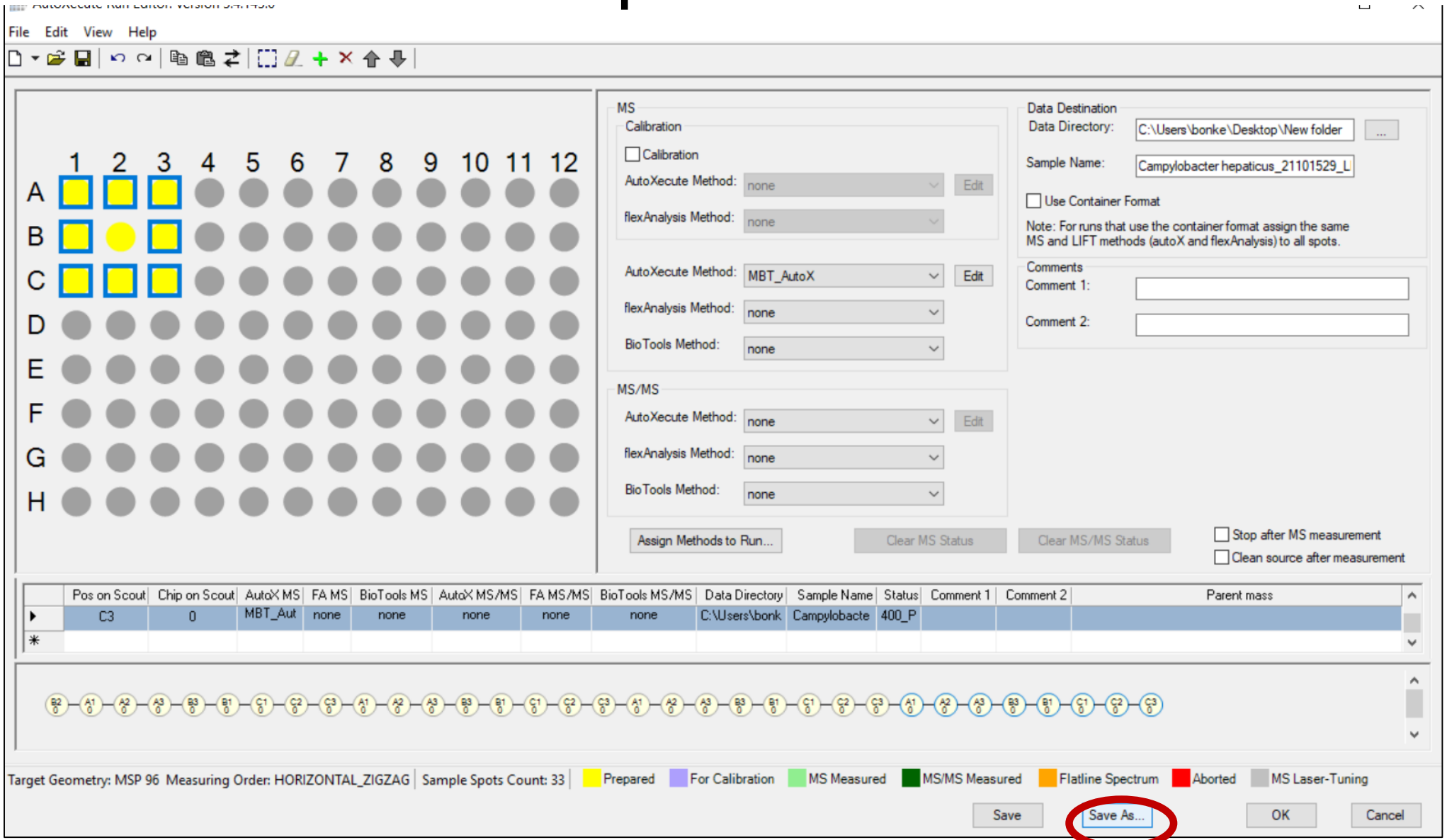
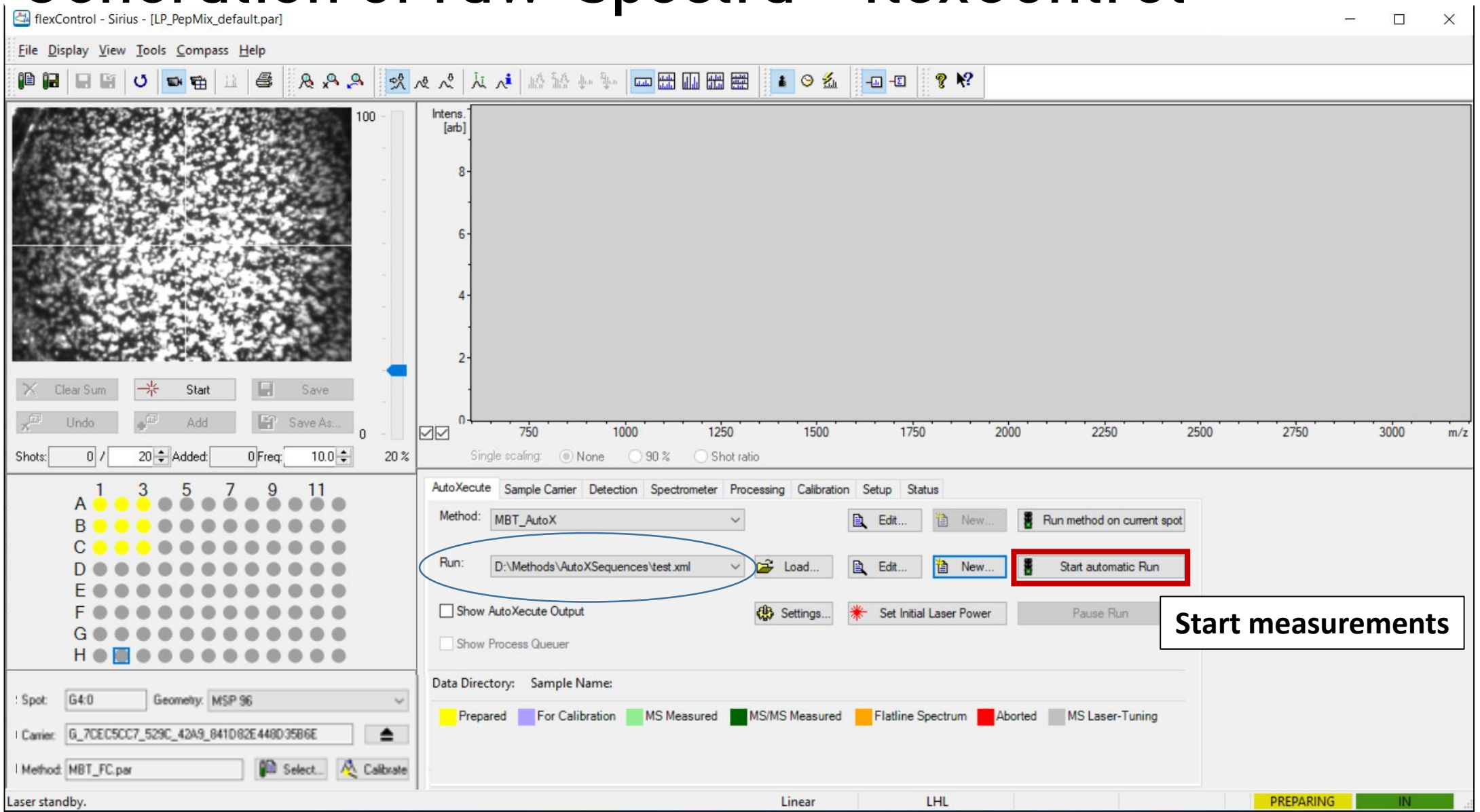
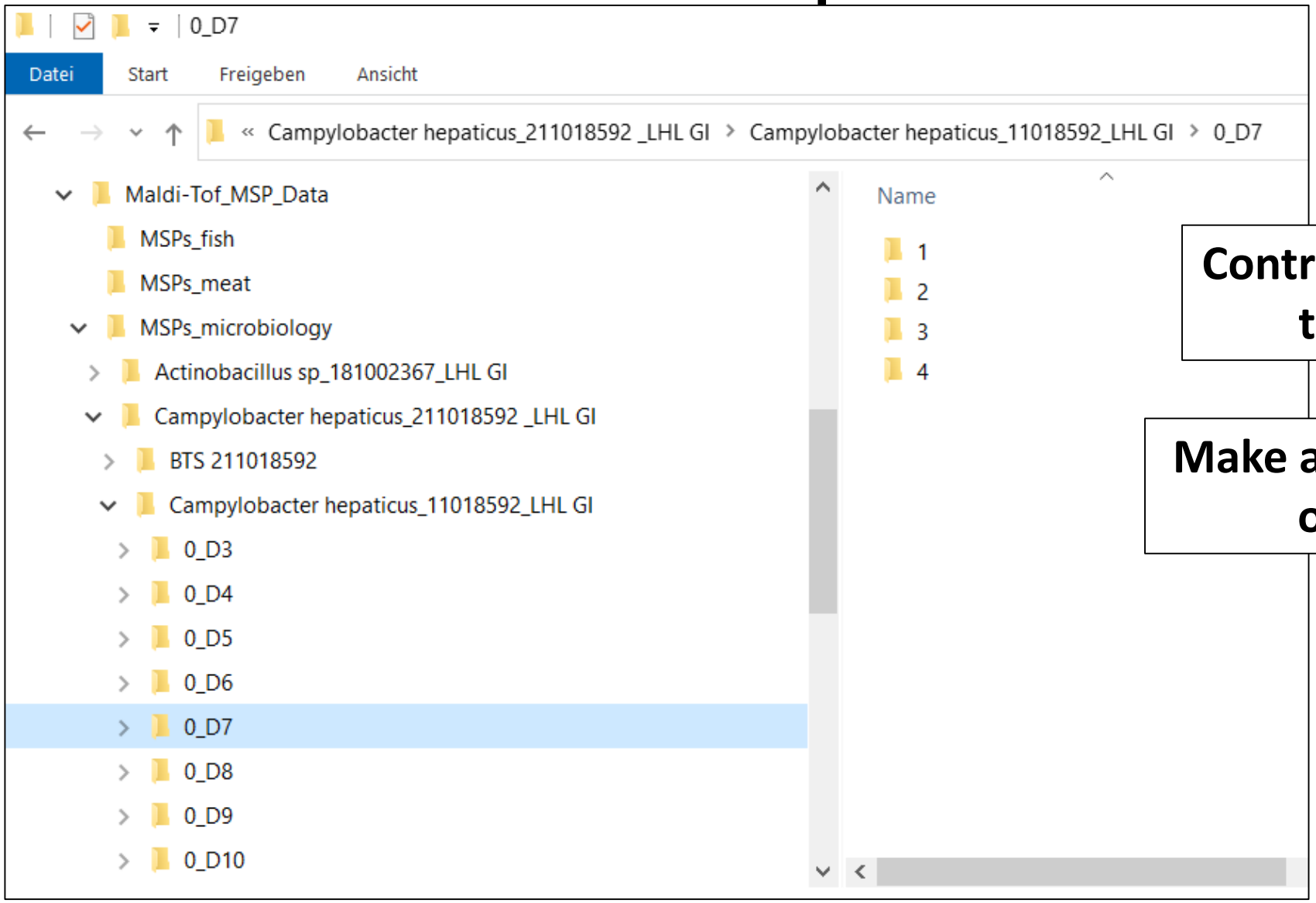


Generation of raw-spectra - flexControl



Start measurements

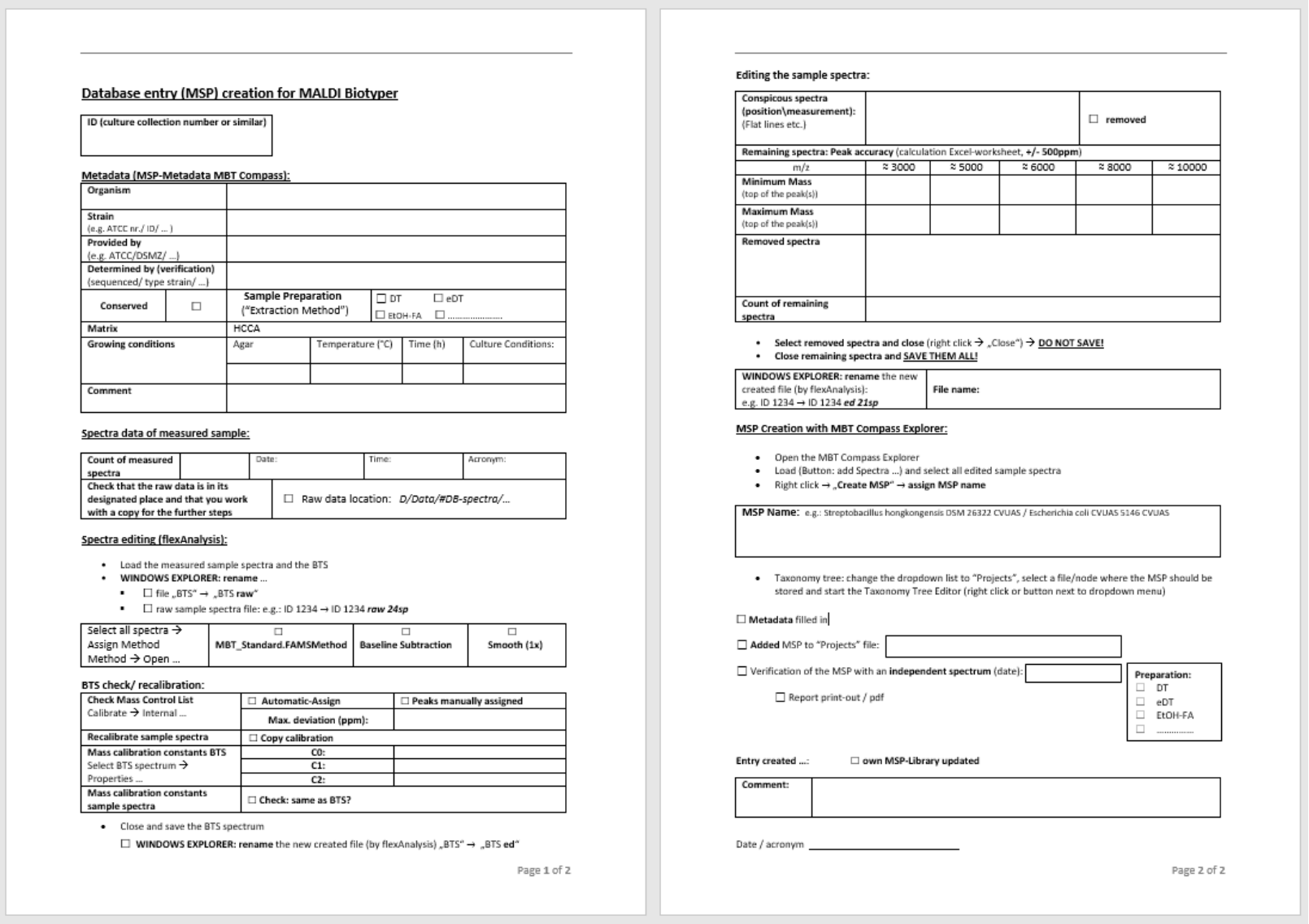
Generation of raw-spectra - flexControl



Control the measurement in the file directories.

Make a copy for the processing of the raw spectra!

Generation of raw-spectra - flexControl



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Conserved	☐	**Sample Preparation** ("Extraction Method")	☐ DT ☐ eDT ☐ EtOH-FA ☐ …….		
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Select all spectra → Assign Method Method → Open …	☐ **MBT_Standard.FAMSMethod**	☐ **Baseline Subtraction**	☐ **Smooth (1x)**

BTS check/ recalibration:

Check Mass Control List Calibrate → Internal …	☐ **Automatic-Assign**	☐ **Peaks manually assigned**
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Recalibrate sample spectra	☐ **Copy calibration**	
Mass calibration constants BTS Select BTS spectrum → Properties …	**C0:**	
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Page 1 of 2

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Preparation:
☐ DT
☐ eDT
☐ EtOH-FA
☐ ……

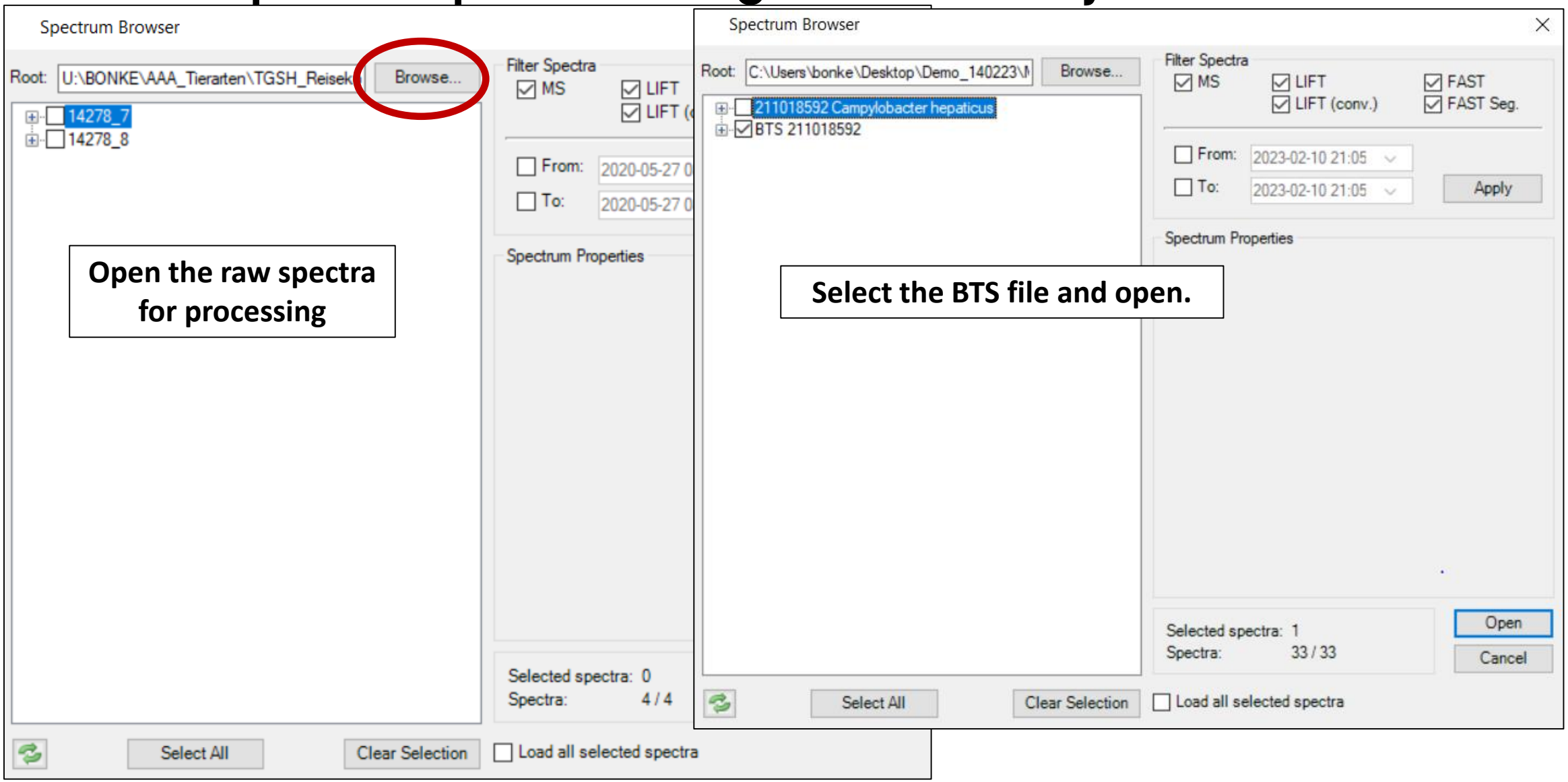
Entry created …: ☐ **own MSP-Library updated**

Comment:	

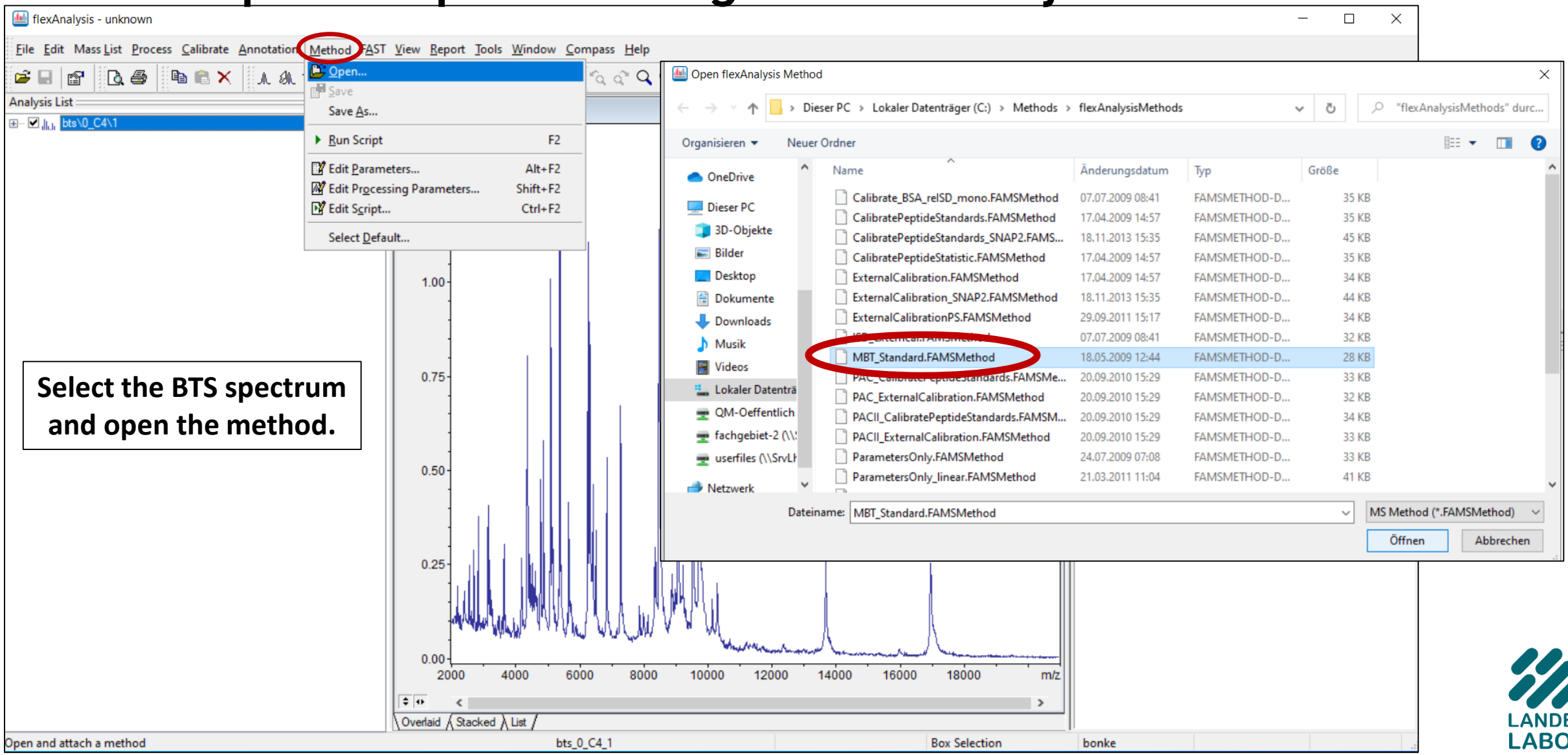
Date / acronym _____

Page 2 of 2

Raw spectra processing – flexAnalysis

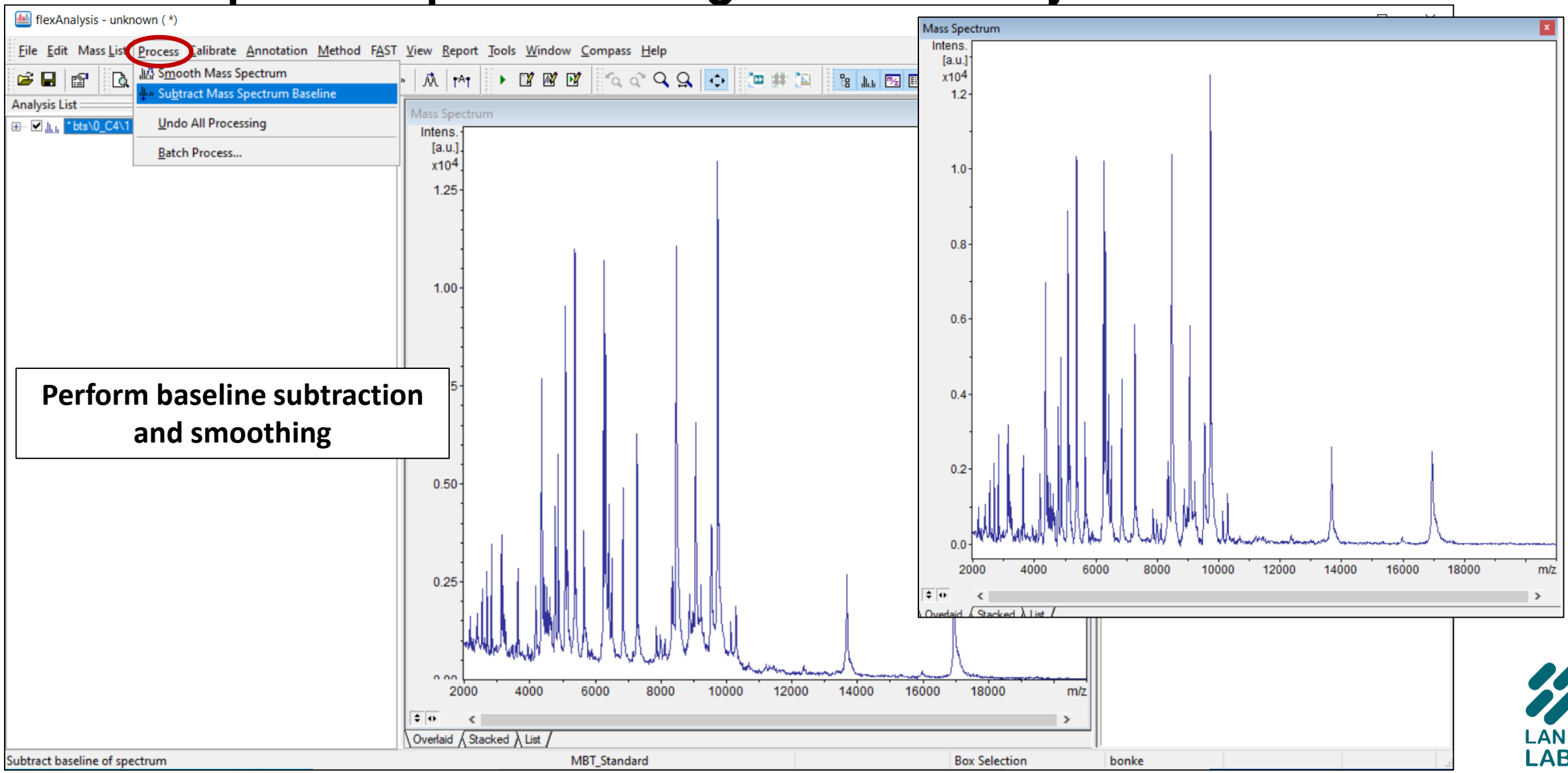


Raw spectra processing – flexAnalysis



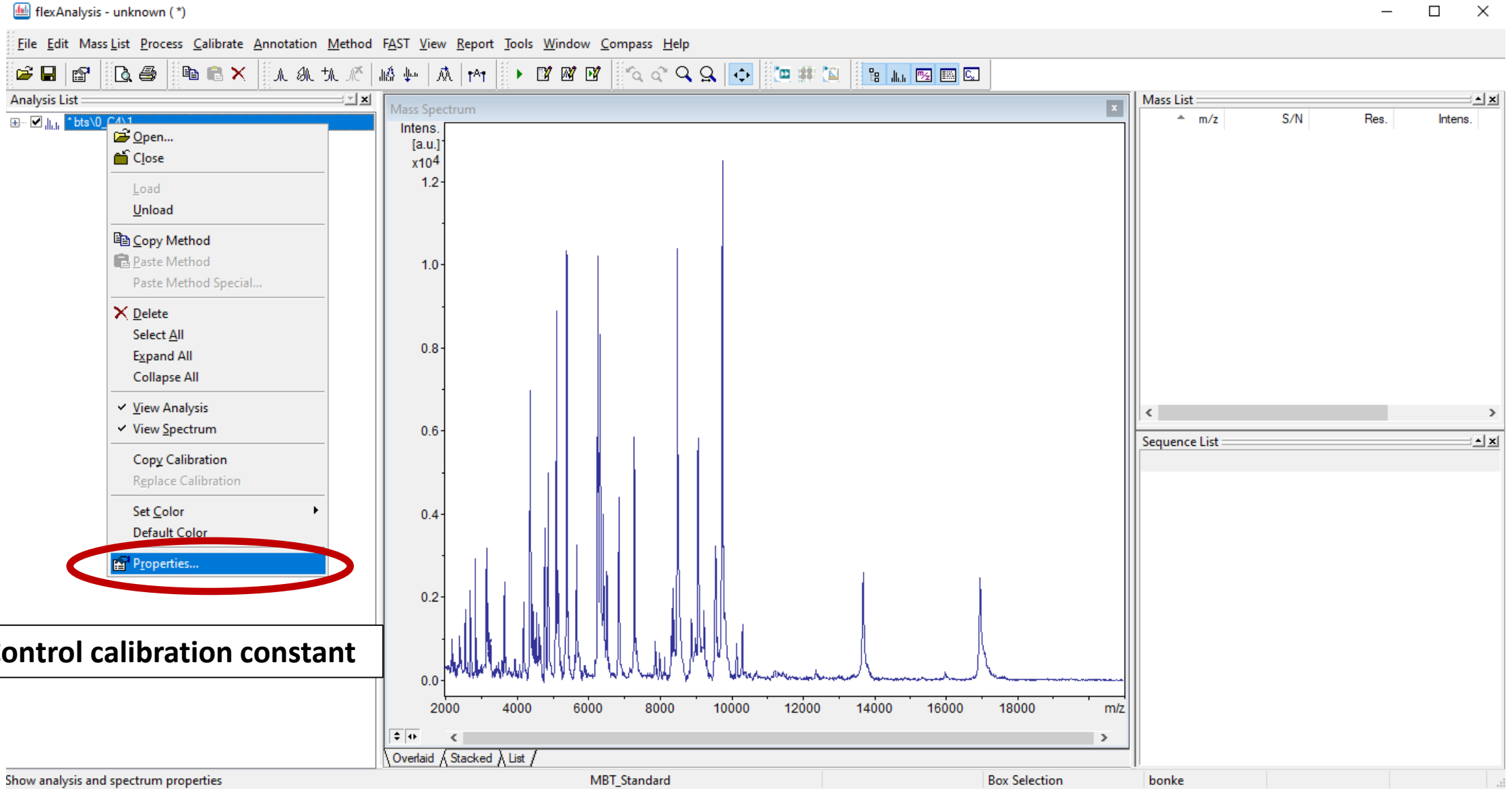
Select the BTS spectrum and open the method.

Raw spectra processing – flexAnalysis



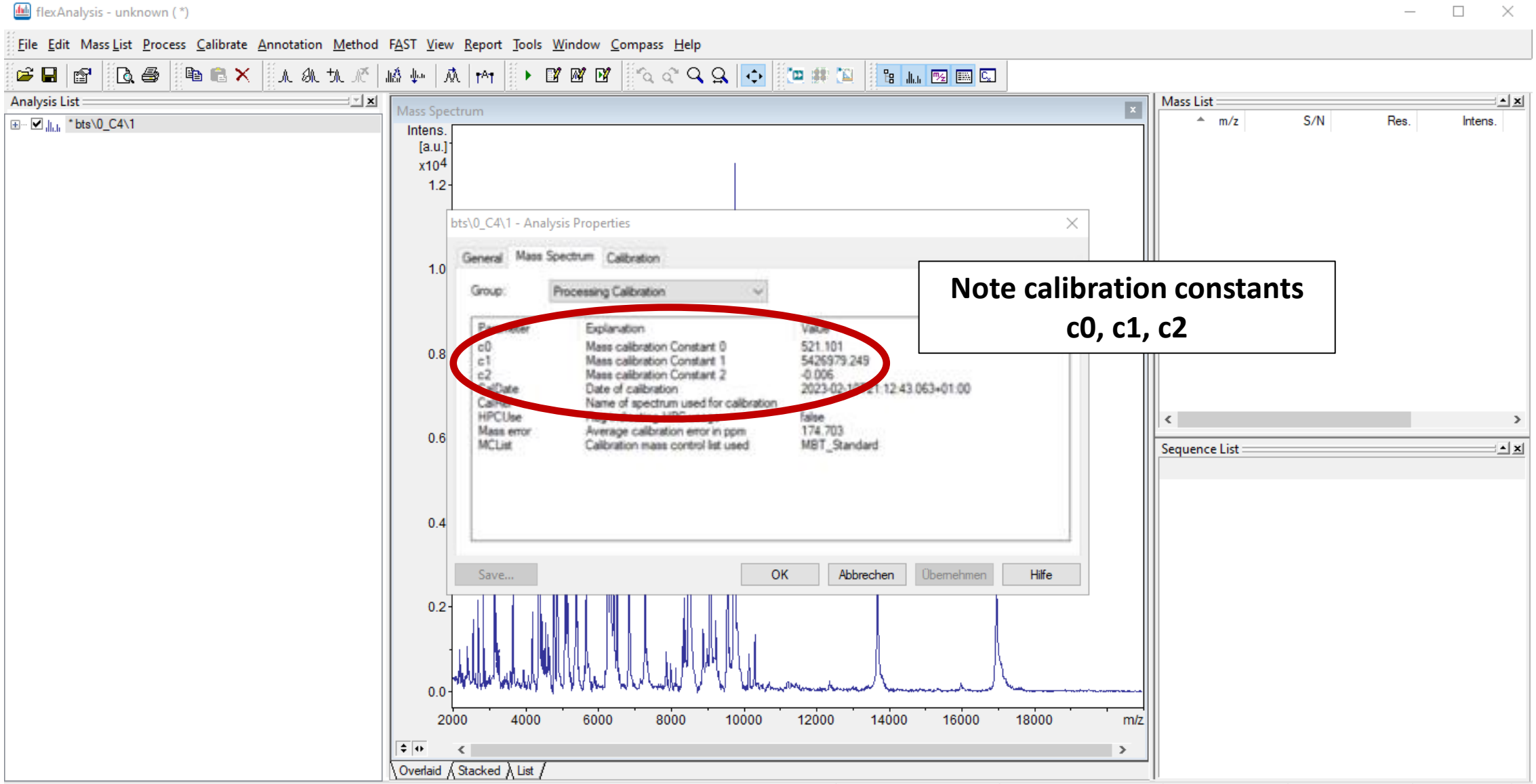
Perform baseline subtraction and smoothing

Raw spectra processing – flexAnalysis

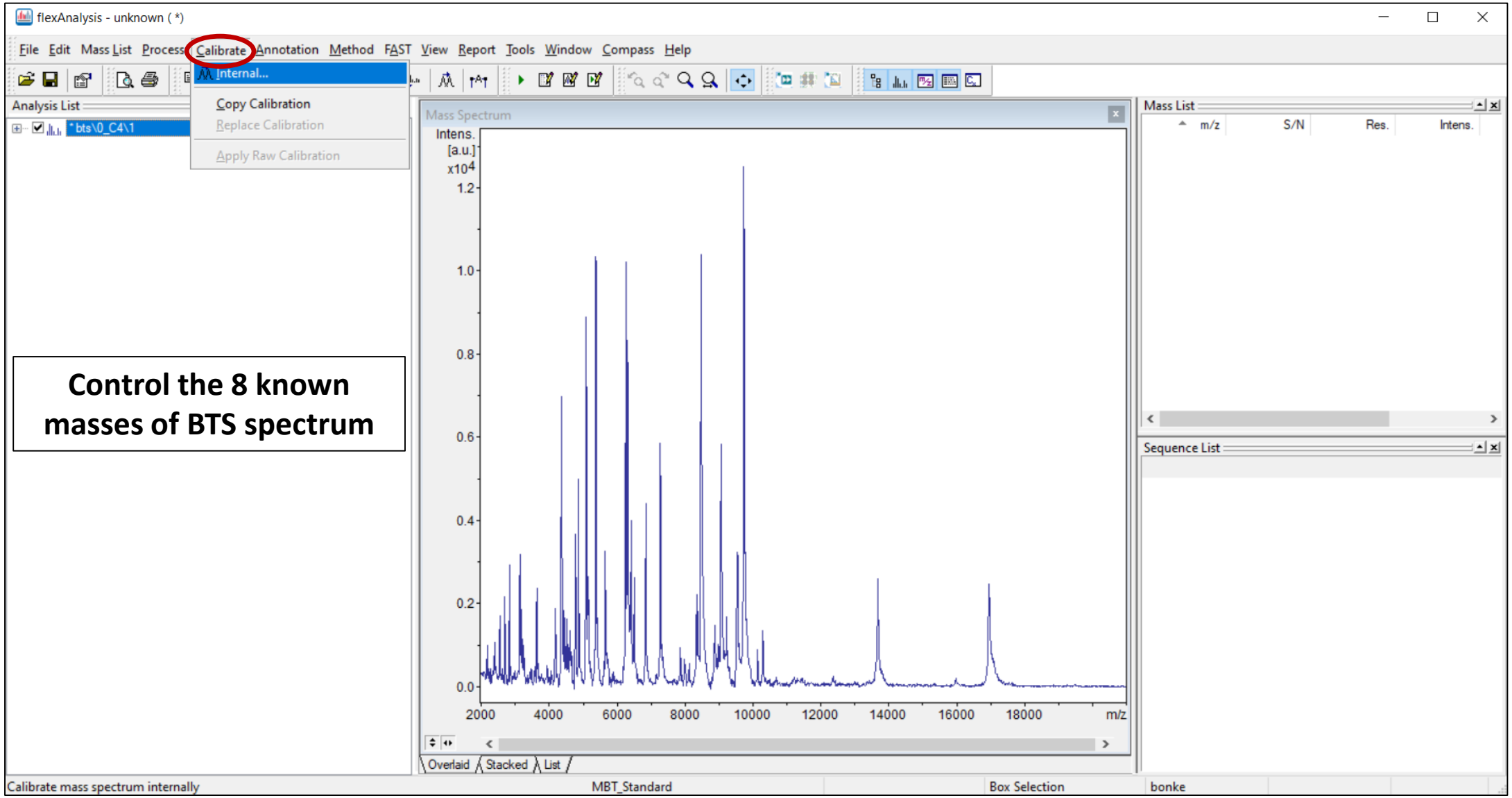


Control calibration constant

Raw spectra processing – flexAnalysis

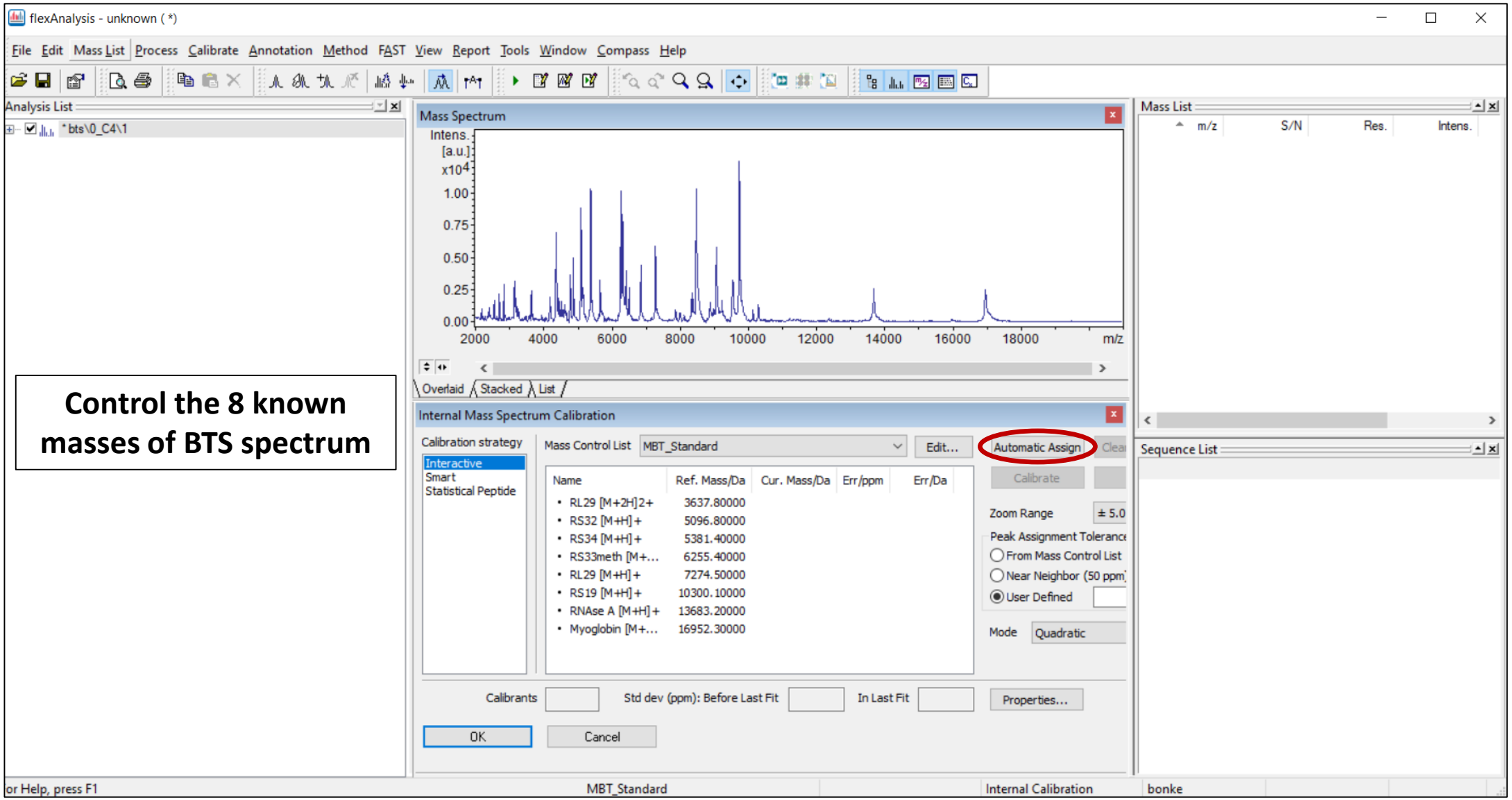


Raw spectra processing – flexAnalysis



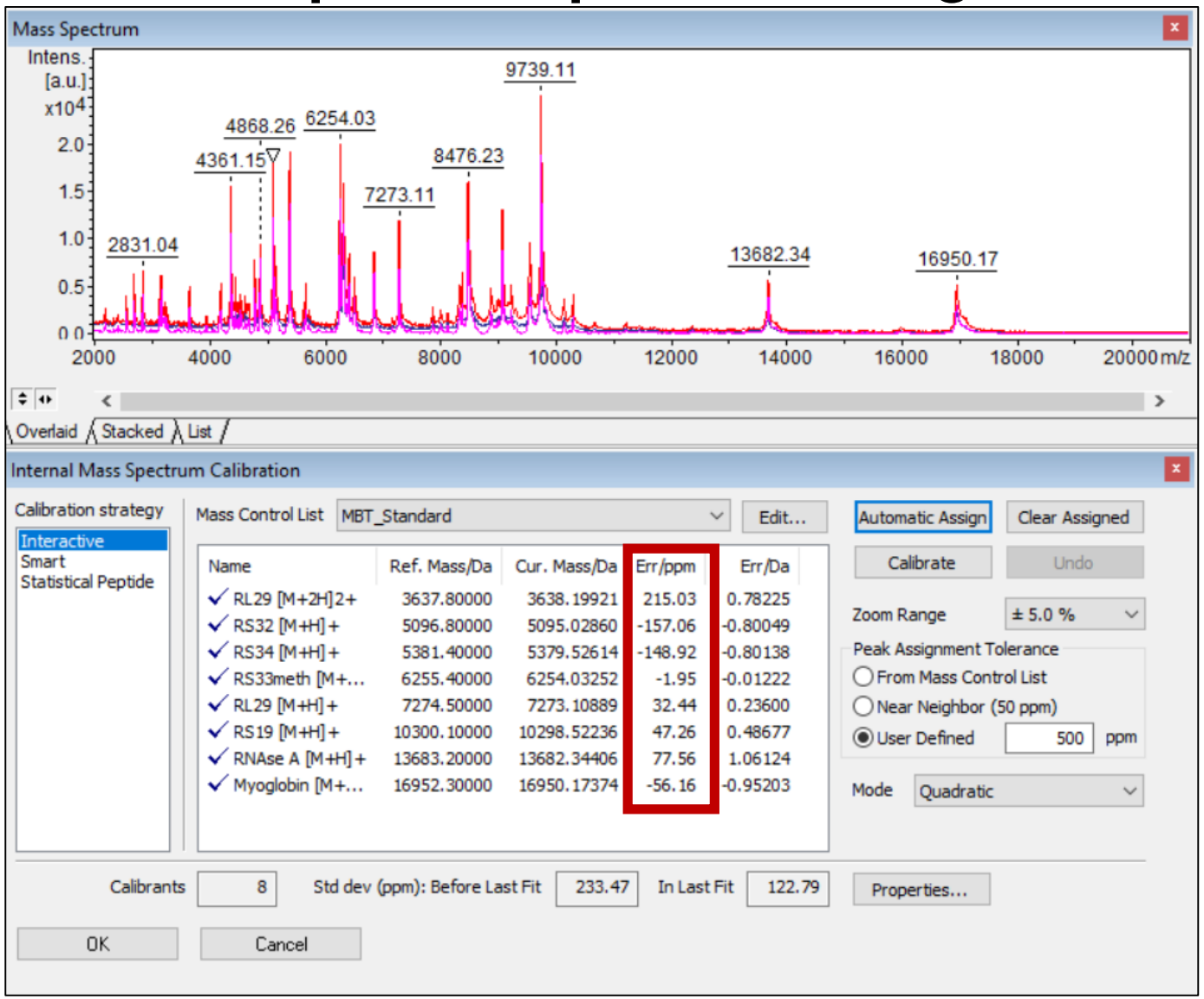
Control the 8 known masses of BTS spectrum

Raw spectra processing – flexAnalysis



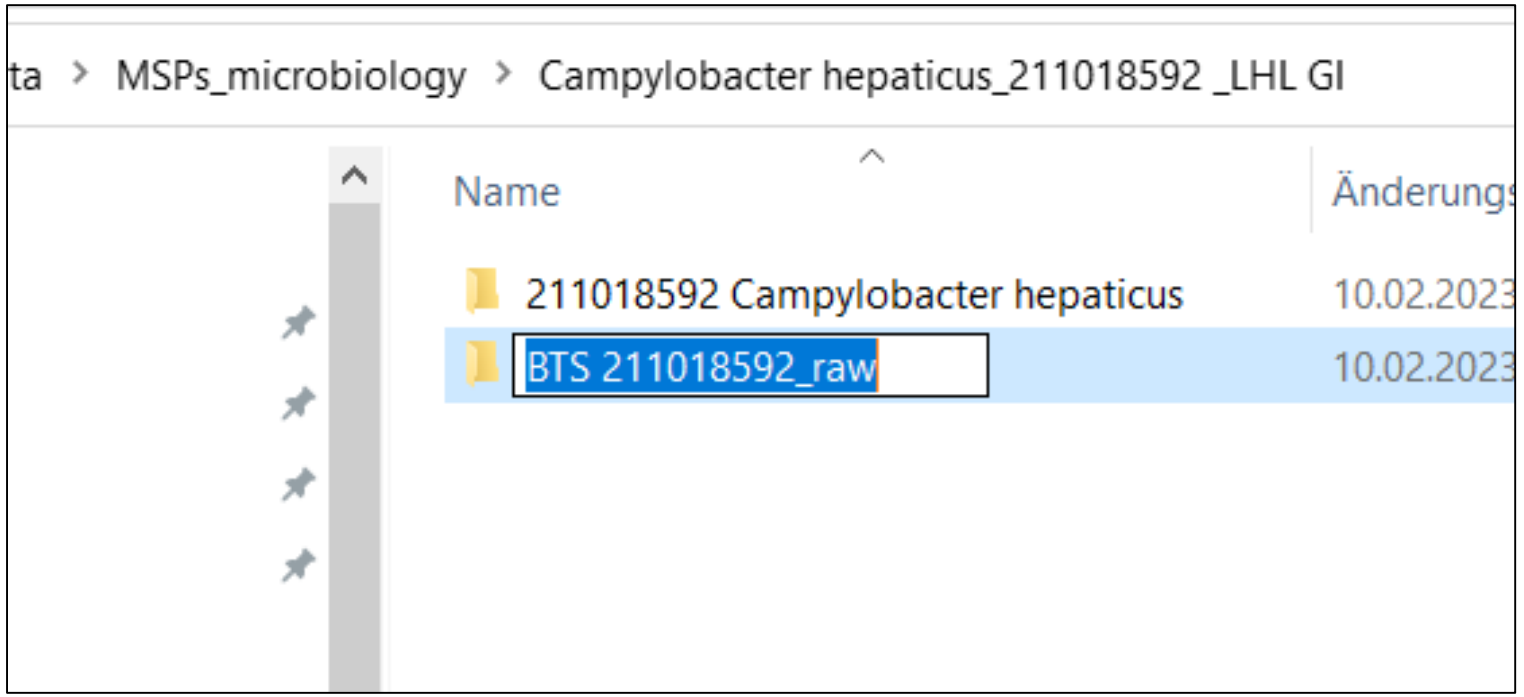
Control the 8 known masses of BTS spectrum

Raw spectra processing – flexAnalysis



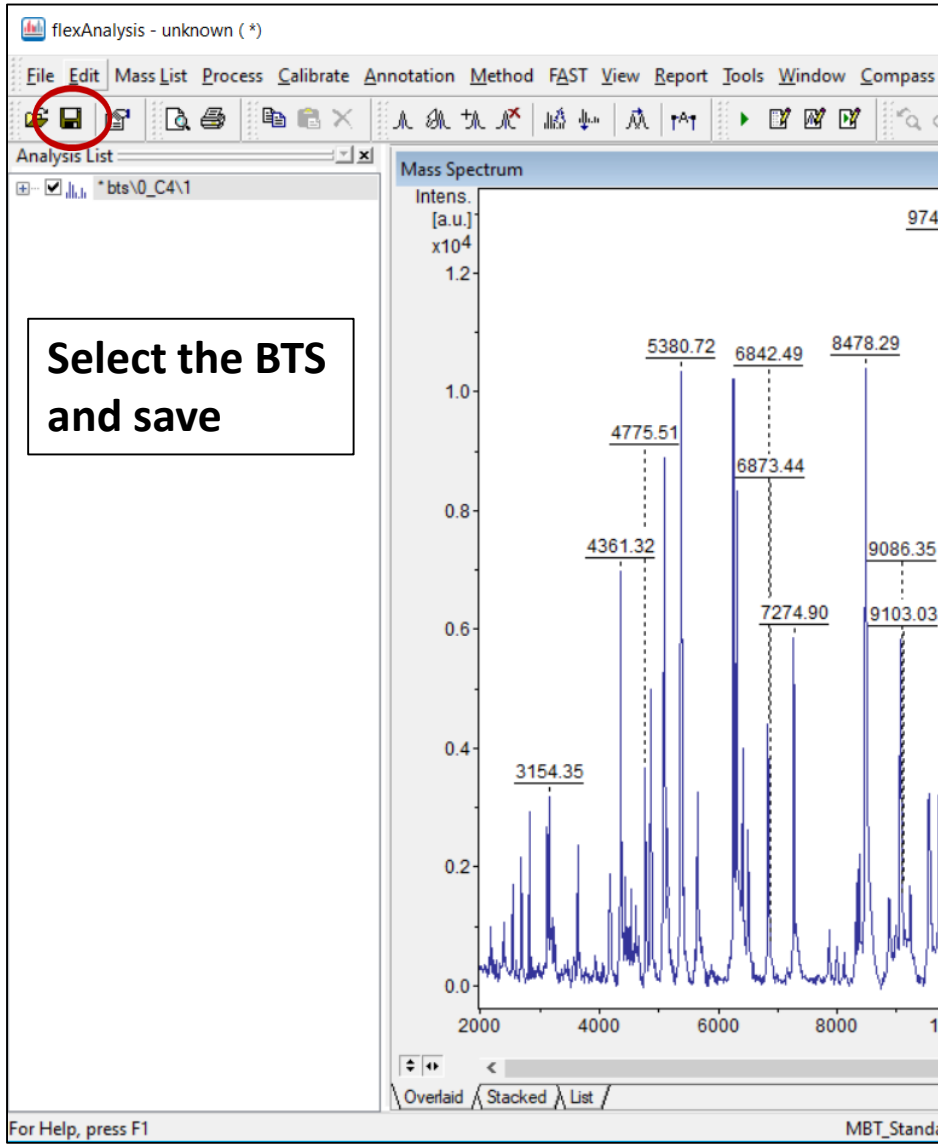
The masses of the measured BTS spectrum must be within the tolerance range of +/- 300 ppm.

Raw spectra processing – flexAnalysis

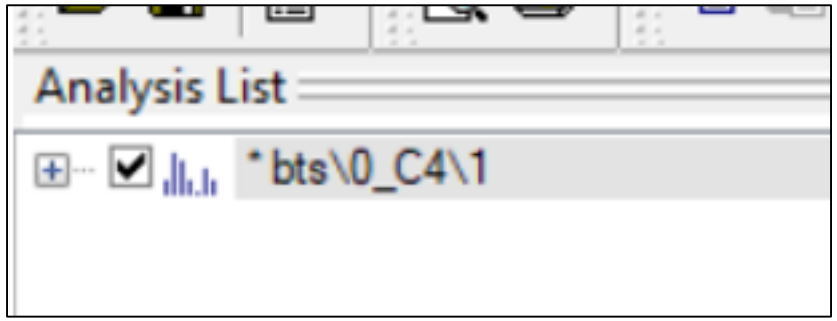


Change the original BTS spectrum file name before saving! (e.g. add raw, ed, ...)

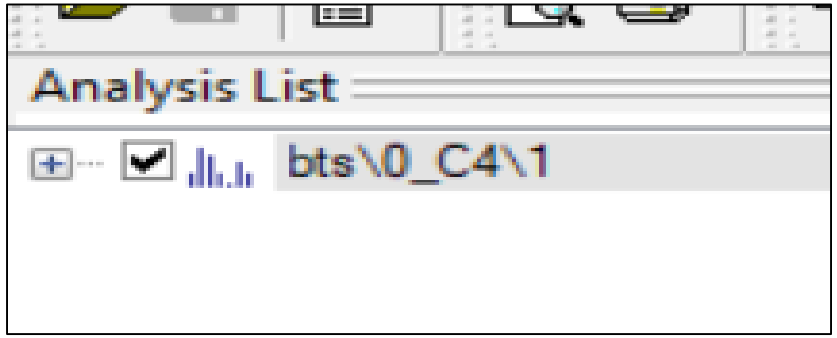
Raw spectra processing – flexAnalysis



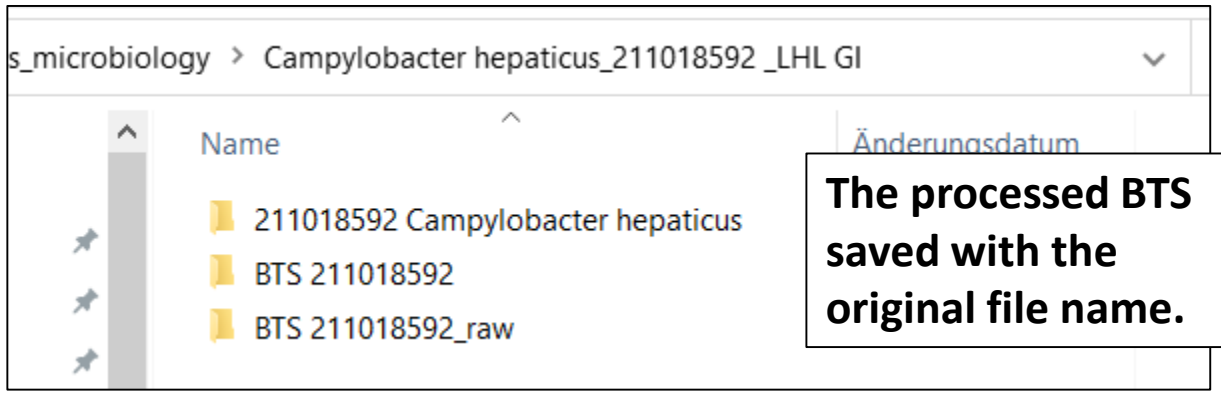
Select the BTS and save



BTS before saving

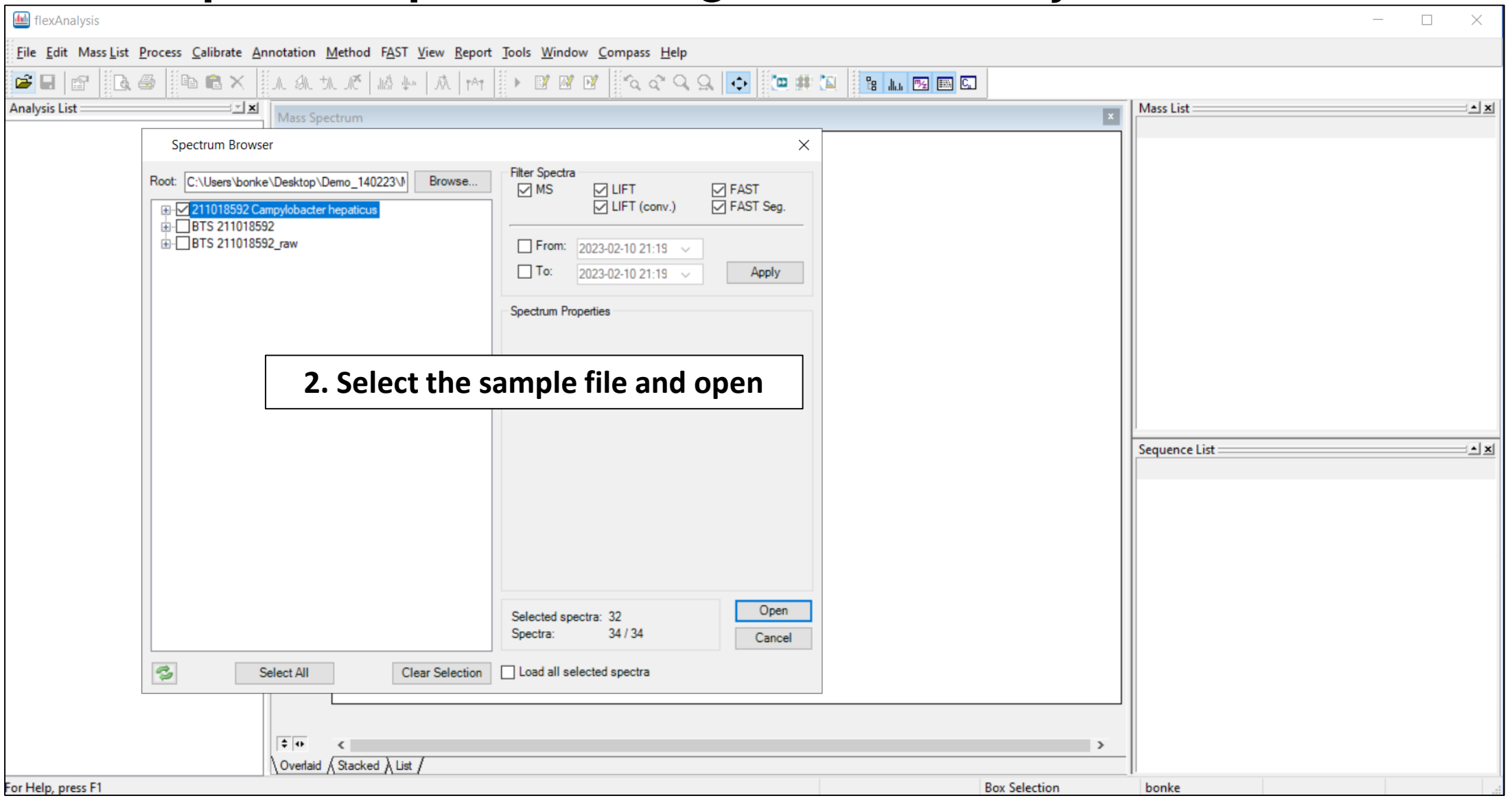


BTS after saving

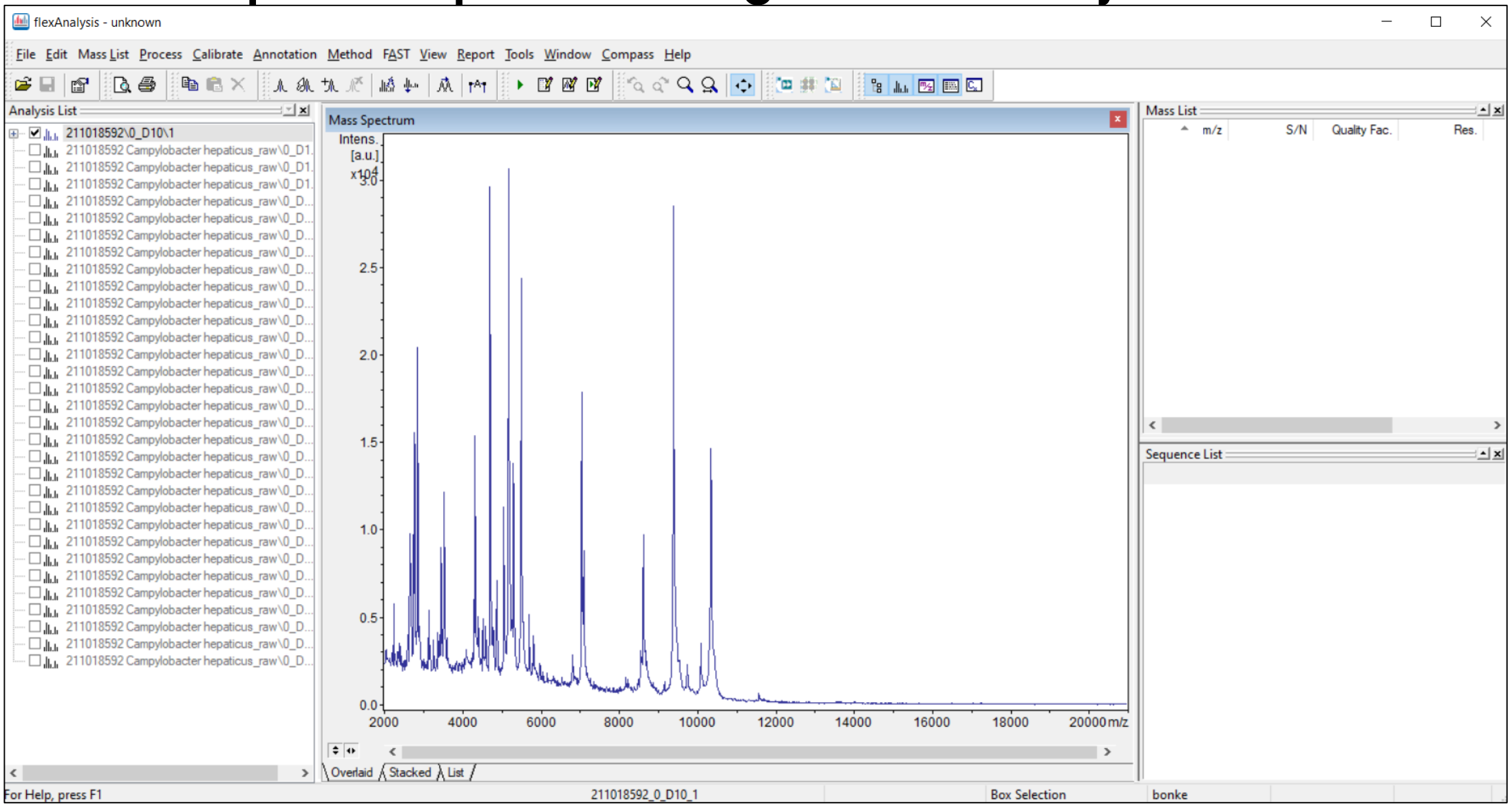


The processed BTS saved with the original file name.

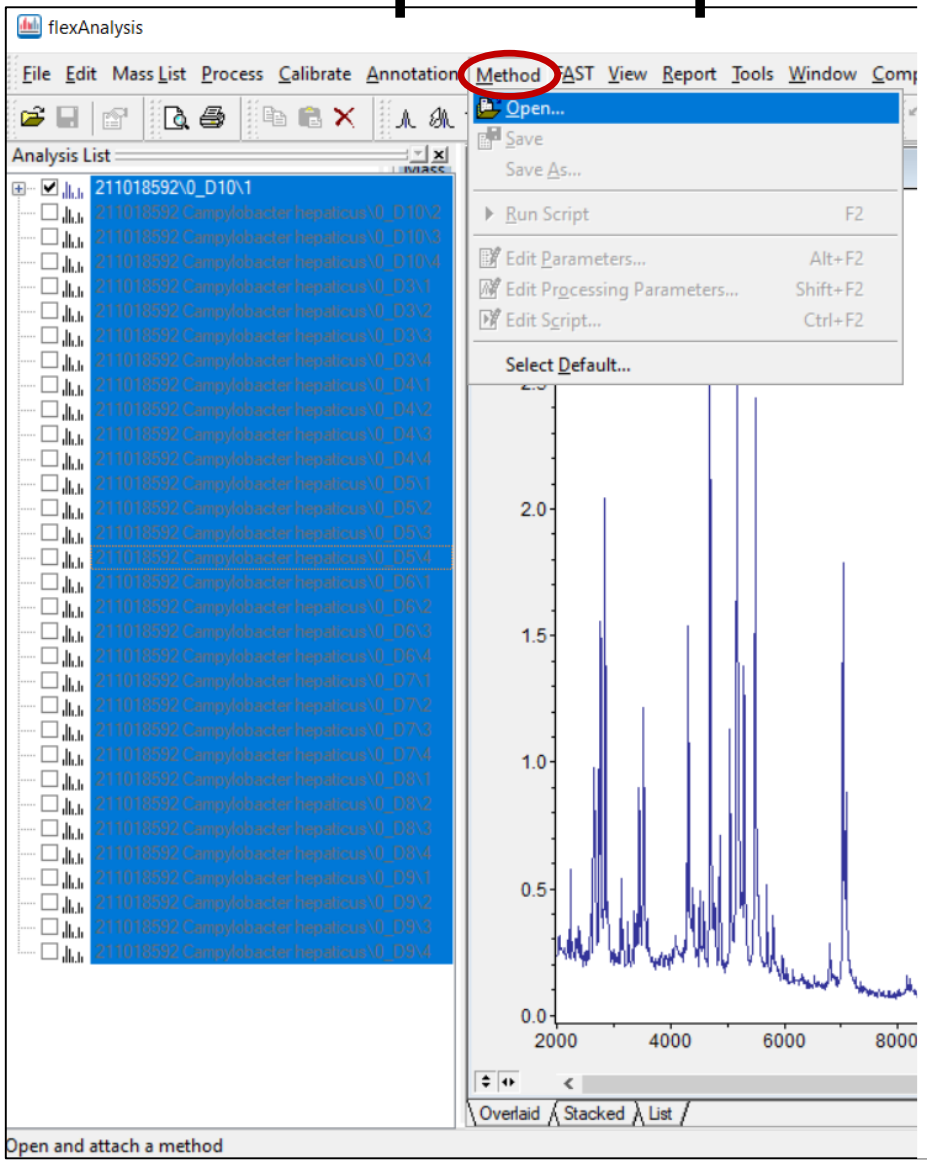
Raw spectra processing – flexAnalysis

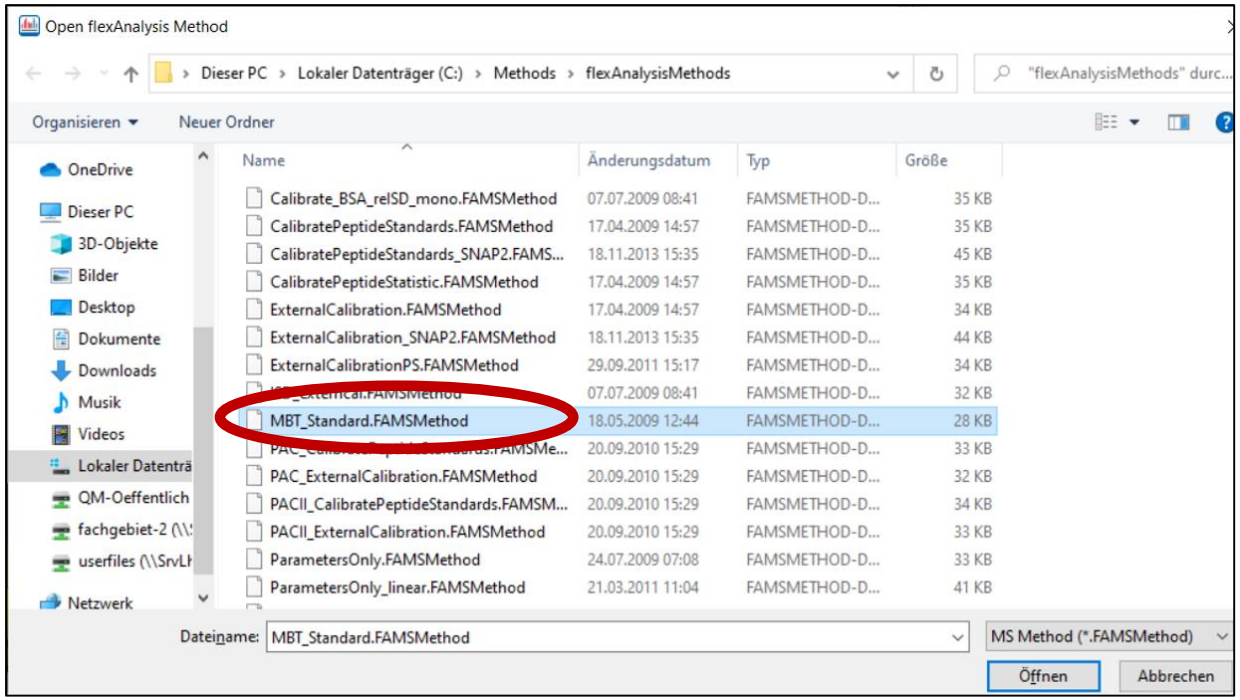


Raw spectra processing – flexAnalysis



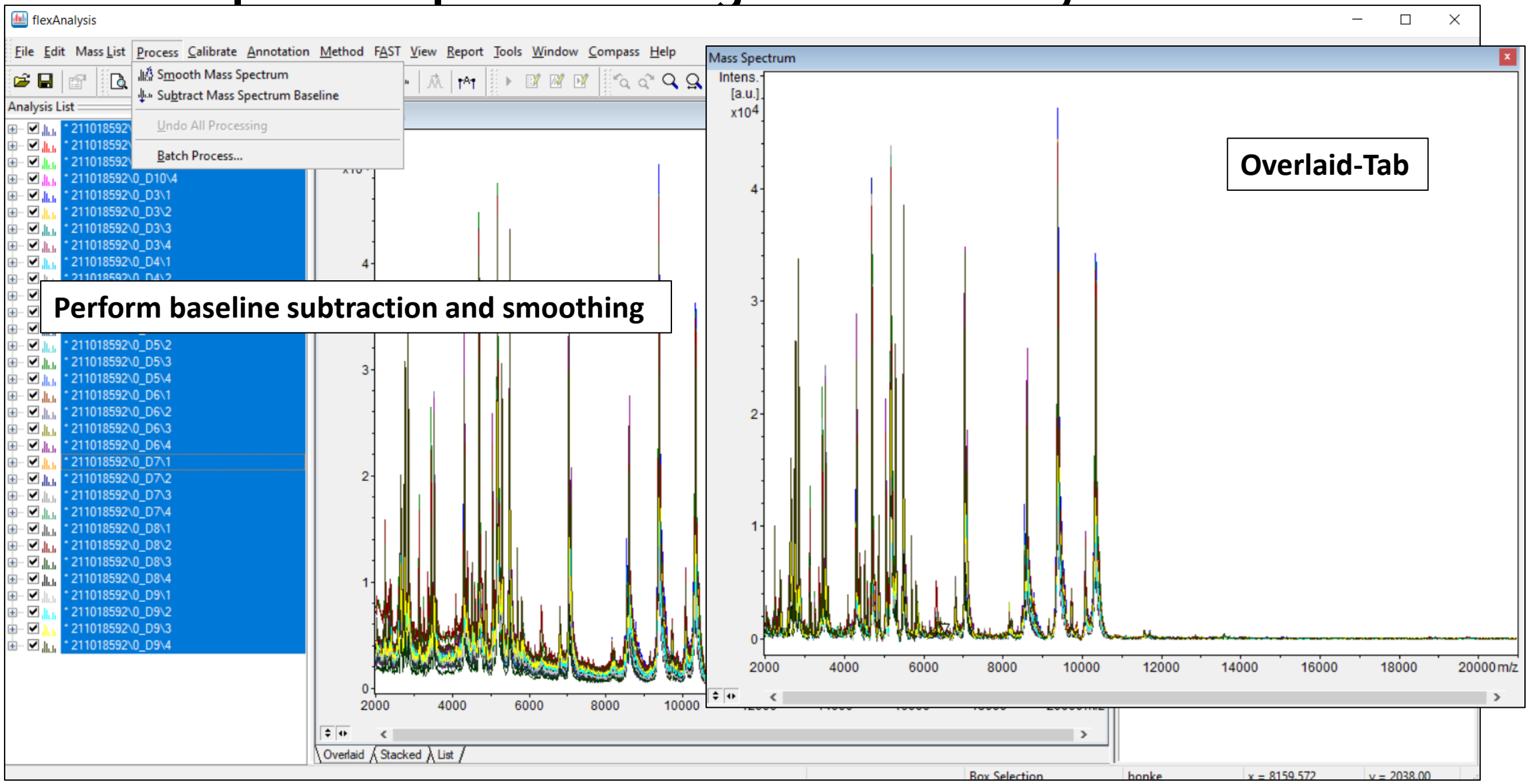
Raw spectra processing – flexAnalysis



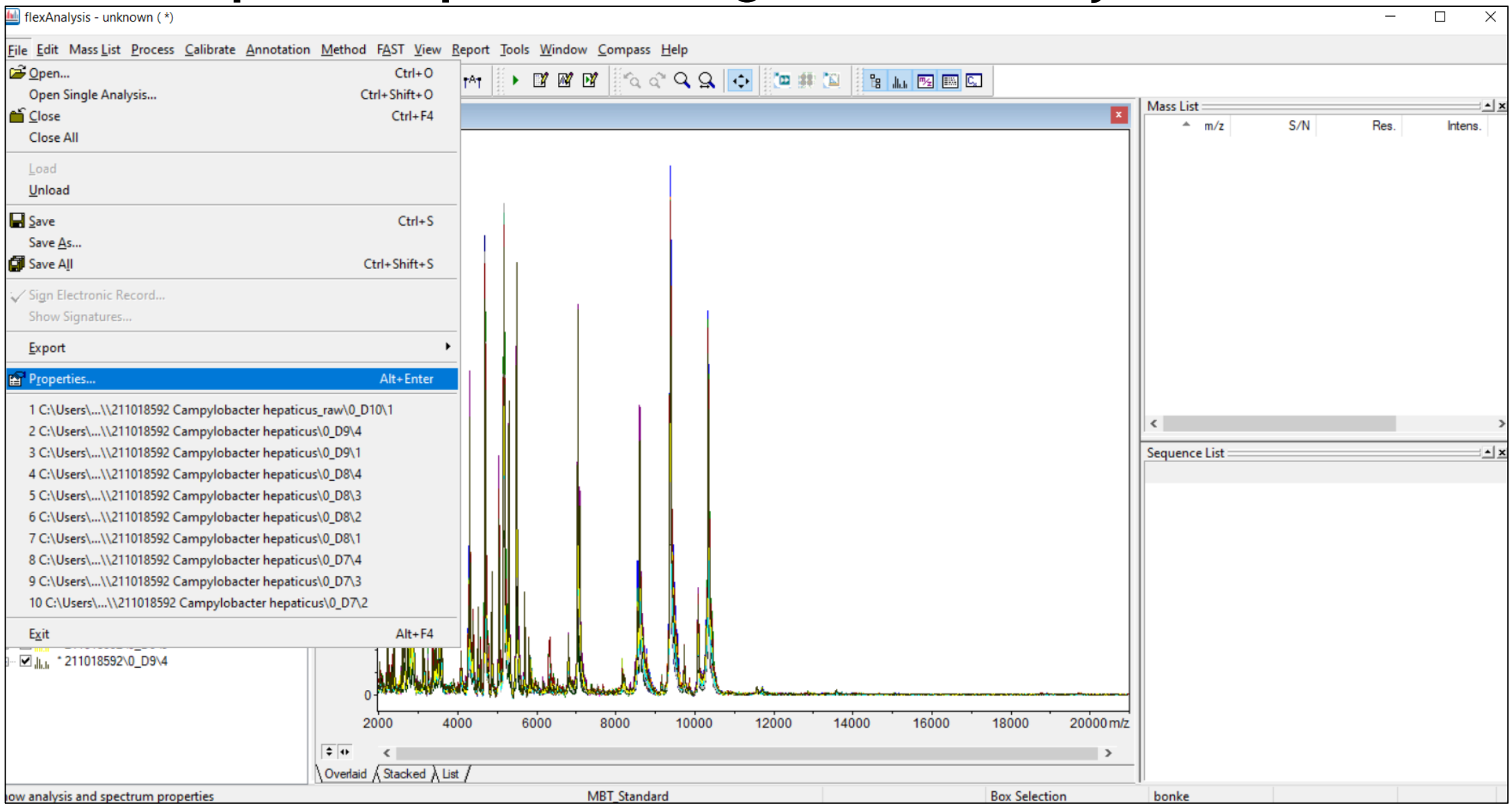


Select all spectra and open the method.

Raw spectra processing – flexAnalysis



Raw spectra processing – flexAnalysis



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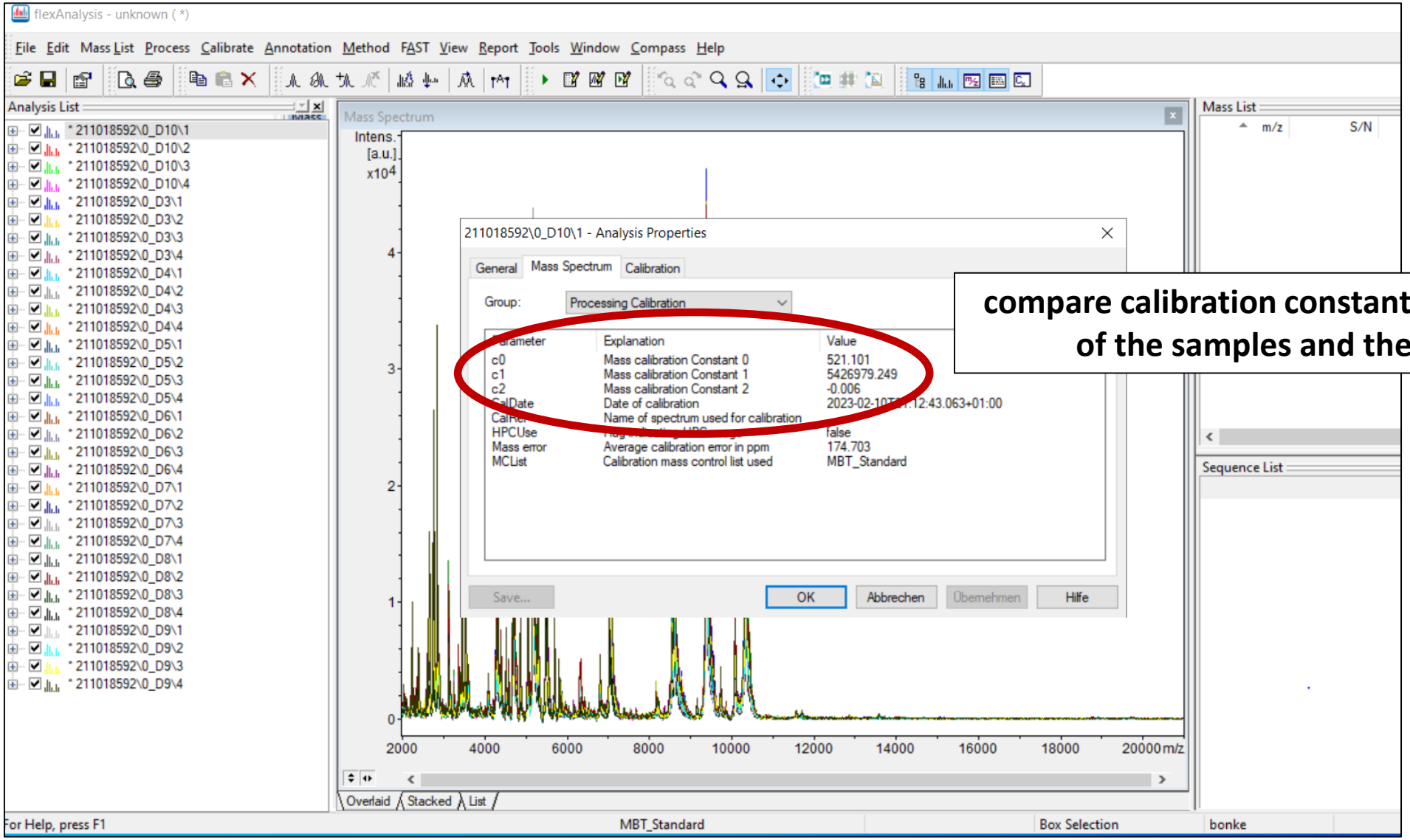
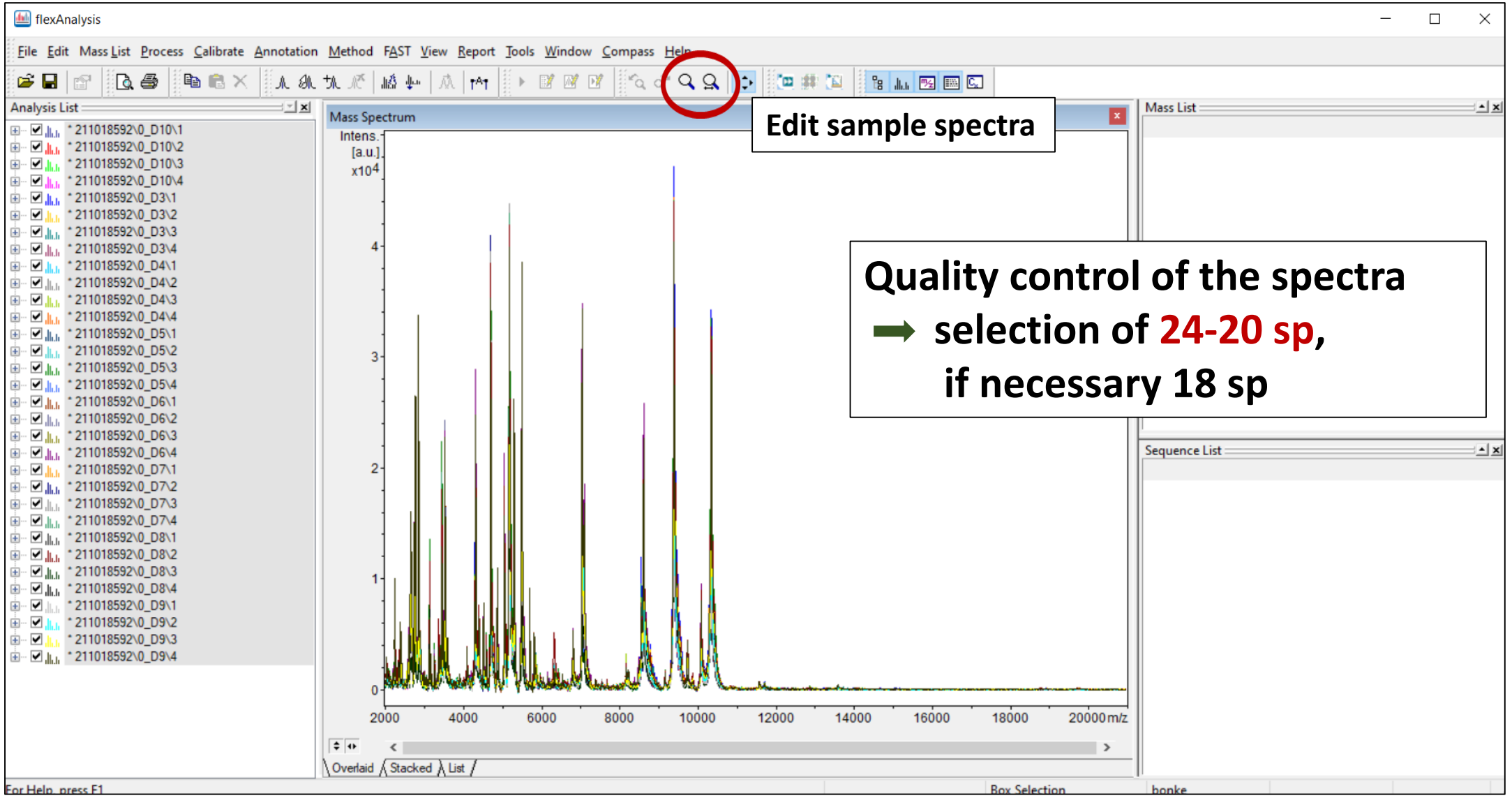
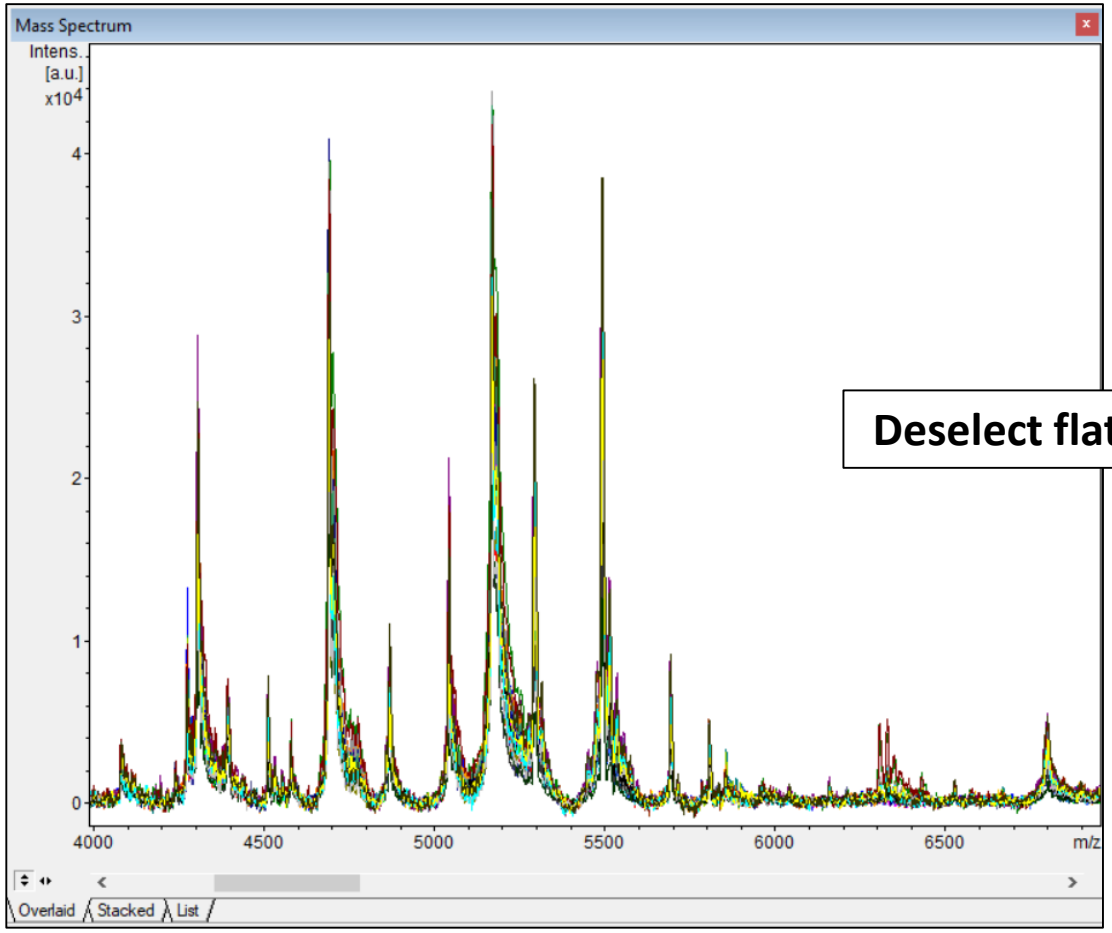


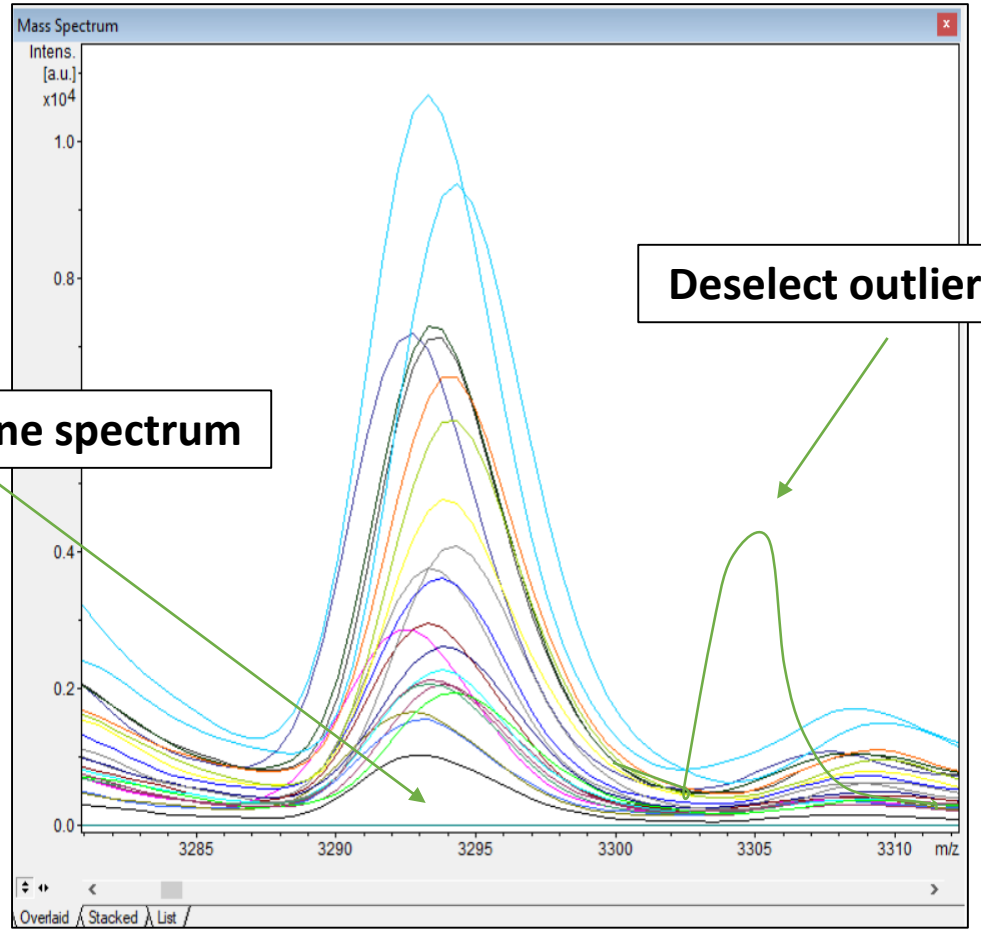
compare calibration constants c0, c1, c2 of the samples and the BTS

Raw spectra processing – flexAnalysis



Raw spectra processing – flexAnalysis

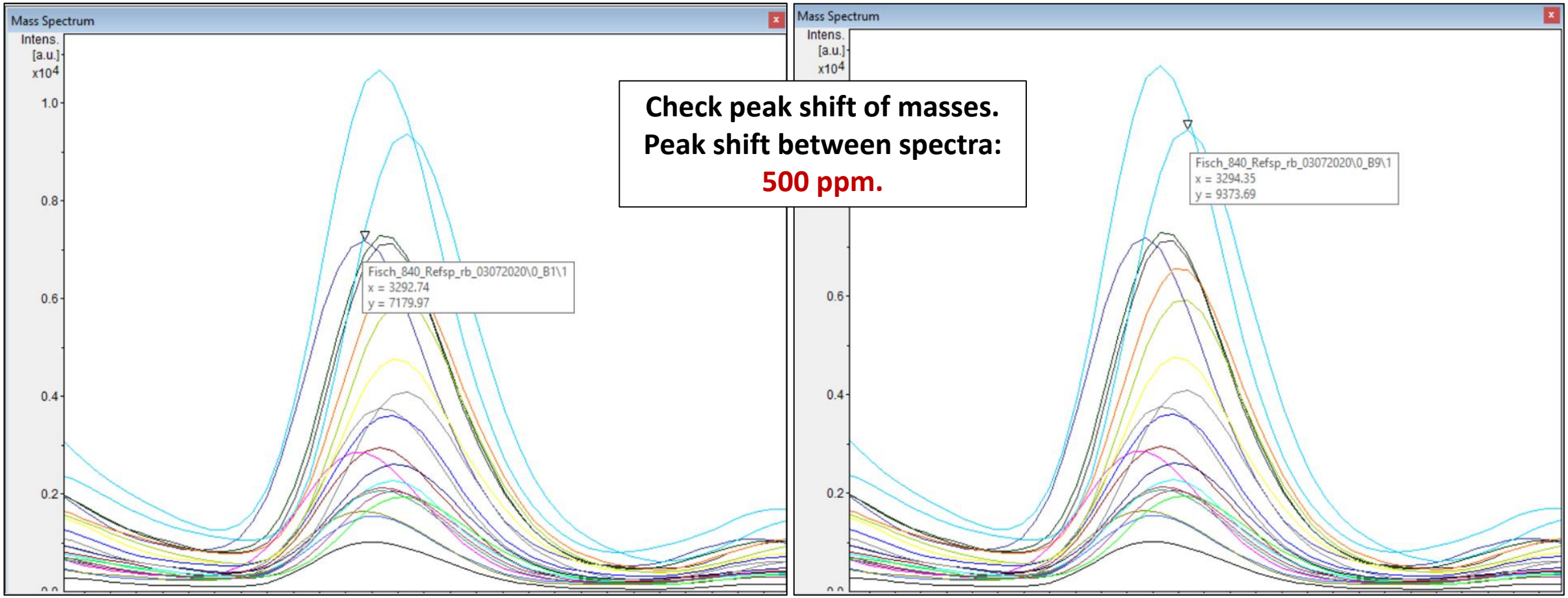




Deselect flat line spectrum

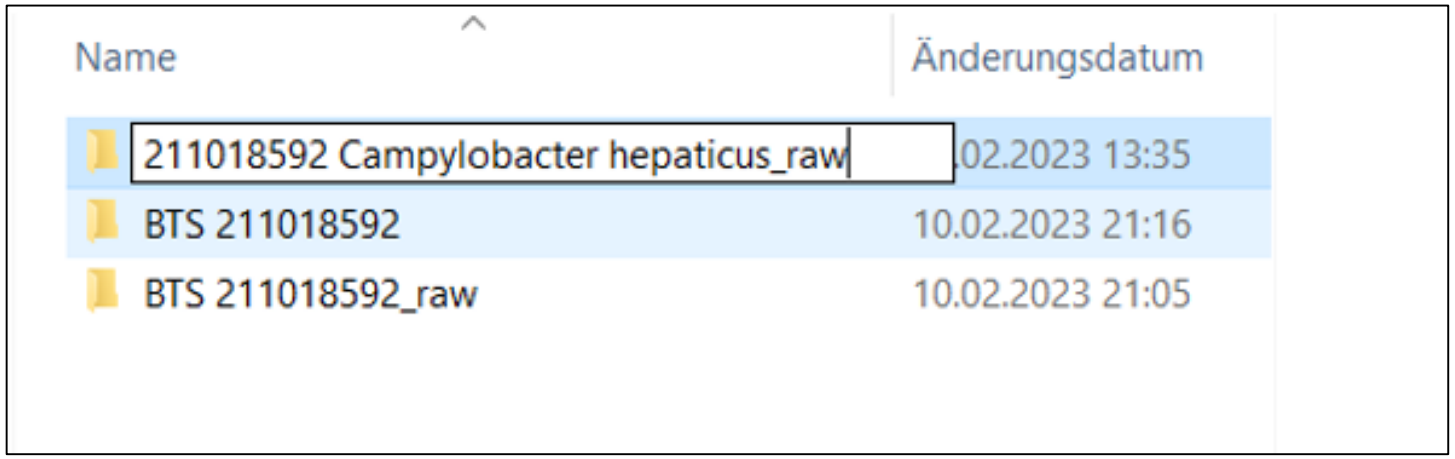
Deselect outlier peaks

Raw spectra processing – flexAnalysis



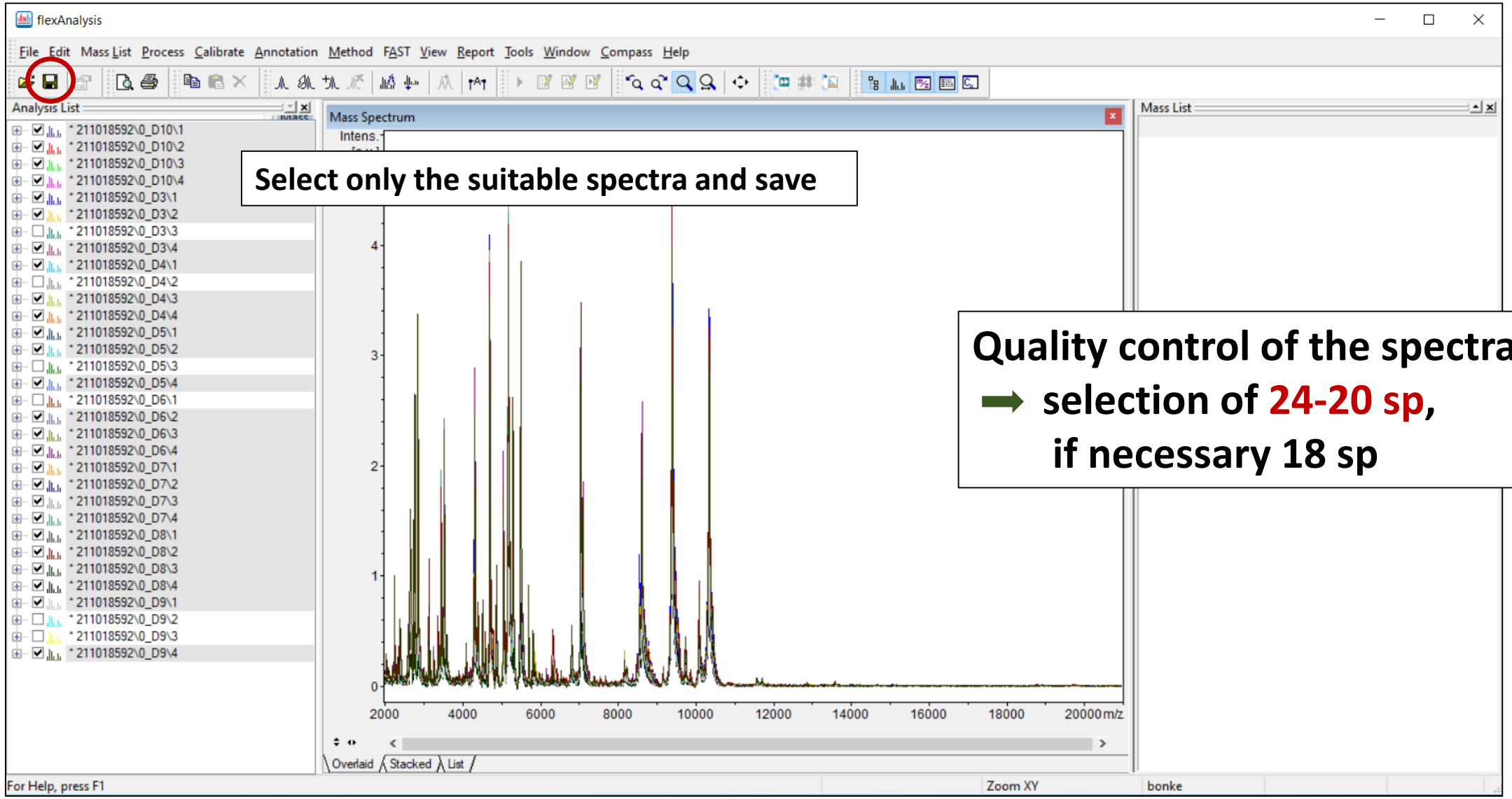
Check peak shift of masses.
Peak shift between spectra:
500 ppm.

Raw spectrum processing – flexAnalysis



Change the original sample spectra file name before saving!
(e.g. add raw, ed, ...)

Raw spectrum processing – flexAnalysis

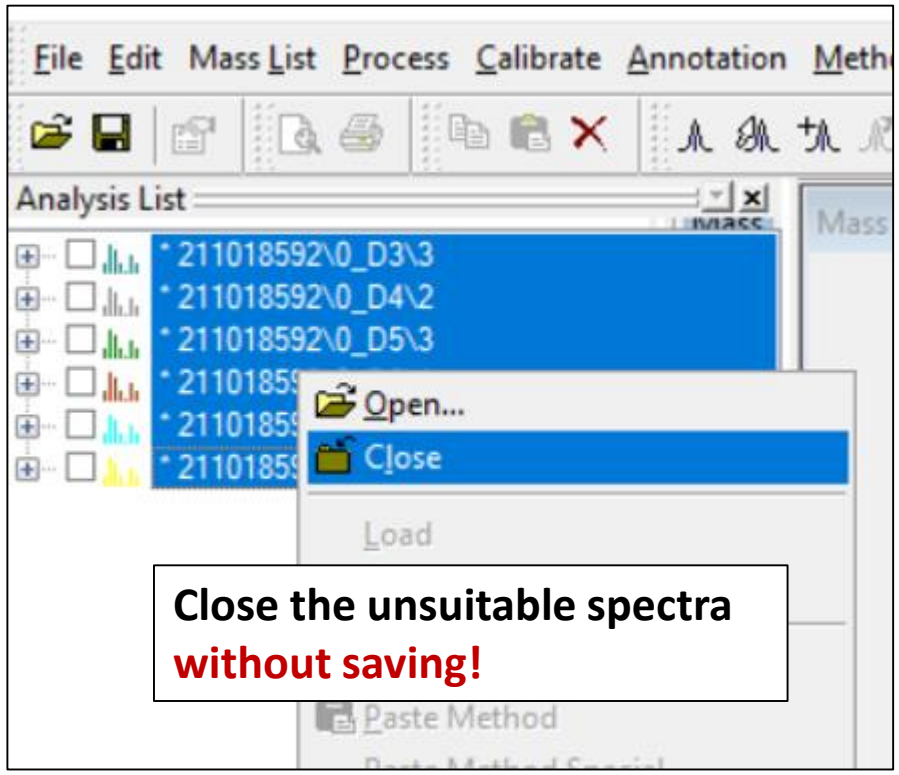


Select only the suitable spectra and save

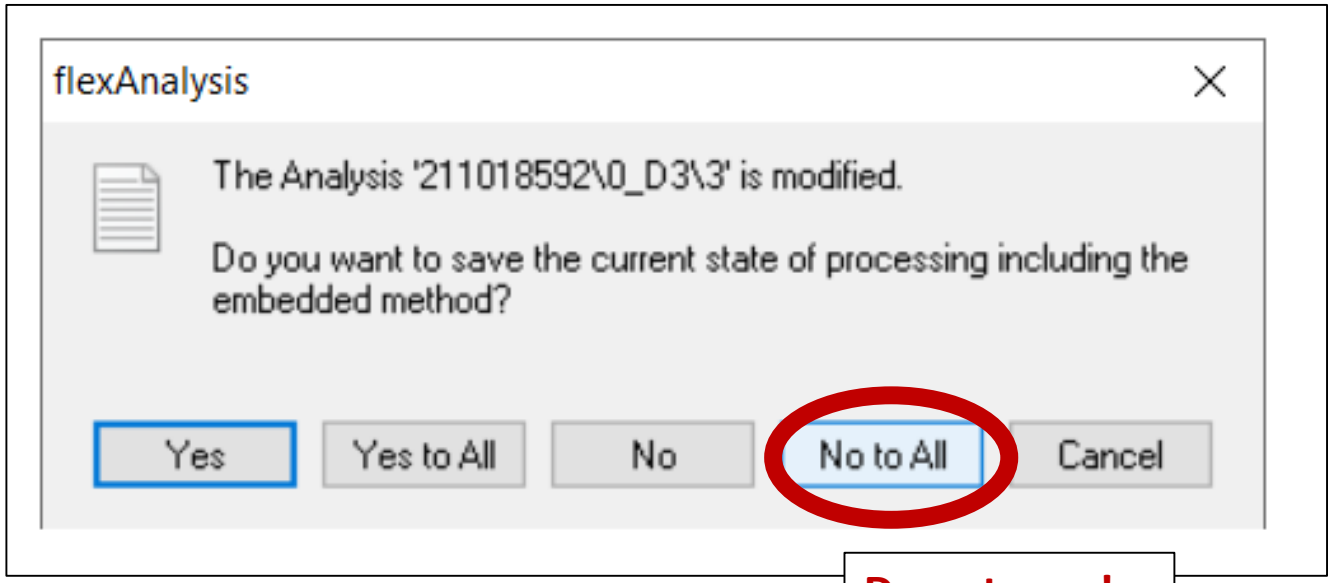
Quality control of the spectra

➡ **selection of 24-20 sp,**
if necessary 18 sp

Raw spectrum processing – flexAnalysis

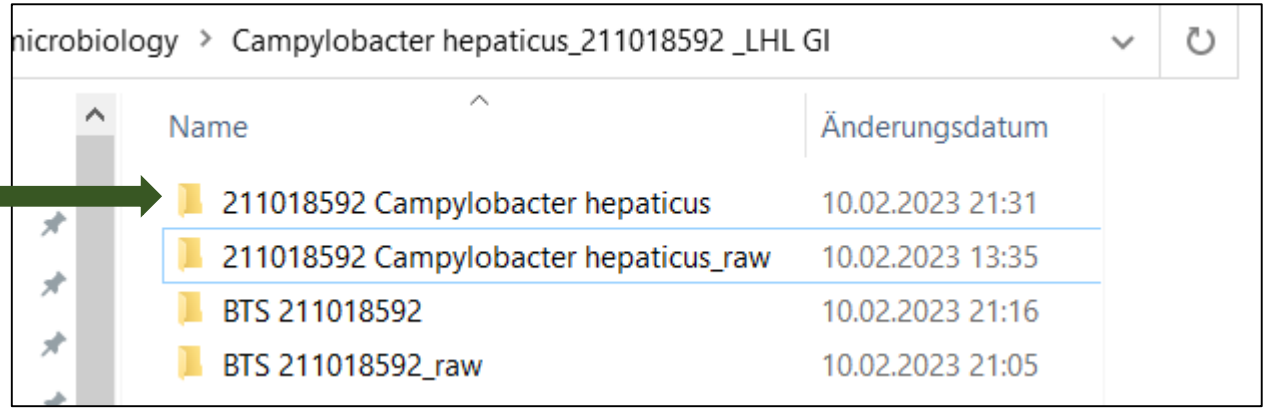


Close the unsuitable spectra without saving!

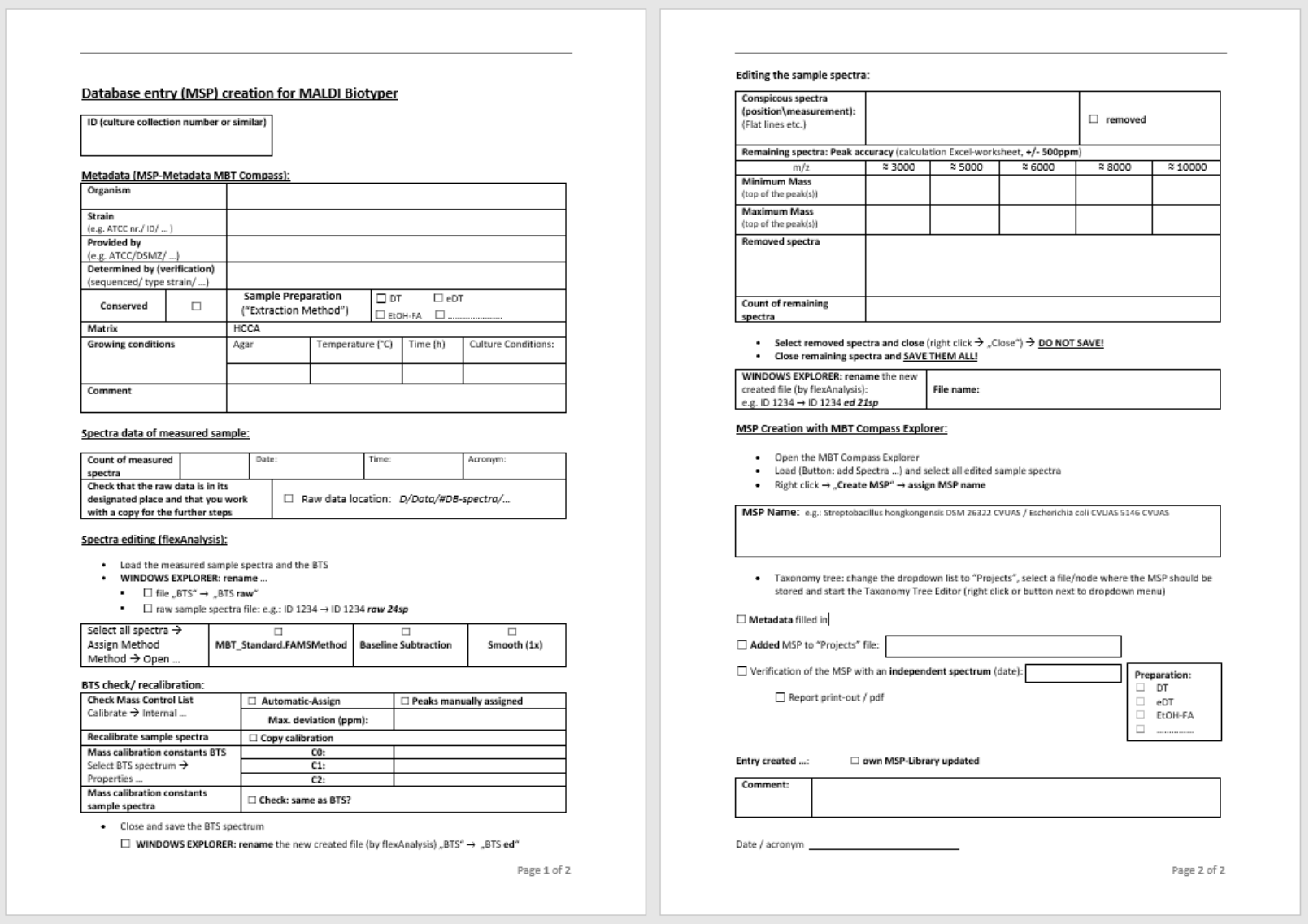


Do not save!

Die verarbeiteten Probenspektren werden unter dem ursprünglichen Dateinamen gespeichert.



Raw spectrum processing – flexAnalysis



Database entry (MSP) creation for MALDI Biotyper

ID (culture collection number or similar)

Metadata (MSP-Metadata MBT Compass):

Organism					
Strain (e.g. ATCC nr./ ID/ …)					
Provided by (e.g. ATCC/DSMZ/ …)					
Determined by (verification) (sequenced/ type strain/ …)					
Conserved	☐	**Sample Preparation** ("Extraction Method")	☐ DT ☐ eDT ☐ EtOH-FA ☐ …….		
Matrix		HCCA			
Growing conditions		Agar	Temperature (°C)	Time (h)	Culture Conditions:
Comment					

Spectra data of measured sample:

Count of measured spectra		Date:	Time:	Acronym:
Check that the raw data is in its designated place and that you work with a copy for the further steps	☐ Raw data location: *D/Data/#DB-spectra/…*			

Spectra editing (flexAnalysis):

- Load the measured sample spectra and the BTS
- **WINDOWS EXPLORER: rename** …
  - ☐ file „BTS" → „BTS **raw**"
  - ☐ raw sample spectra file: e.g.: ID 1234 → ID 1234 ***raw 24sp***

Select all spectra → Assign Method Method → Open …	☐ **MBT_Standard.FAMSMethod**	☐ **Baseline Subtraction**	☐ **Smooth (1x)**

BTS check/ recalibration:

Check Mass Control List Calibrate → Internal …	☐ **Automatic-Assign**	☐ **Peaks manually assigned**
	Max. deviation (ppm):	
Recalibrate sample spectra	☐ **Copy calibration**	
Mass calibration constants BTS Select BTS spectrum → Properties …	**C0:**	
	C1:	
	C2:	
Mass calibration constants sample spectra	☐ **Check: same as BTS?**	

- Close and save the BTS spectrum
  - ☐ **WINDOWS EXPLORER: rename** the new created file (by flexAnalysis) „BTS" → „BTS **ed**"

Editing the sample spectra:

Conspicous spectra (position\measurement): (Flat lines etc.)				☐ **removed**	
Remaining spectra: Peak accuracy (calculation Excel-worksheet, **+/- 500ppm**)					
m/z	≈ 3000	≈ 5000	≈ 6000	≈ 8000	≈ 10000
Minimum Mass (top of the peak(s))					
Maximum Mass (top of the peak(s))					
Removed spectra					
Count of remaining spectra					

- **Select removed spectra and close** (right click → „Close") → **DO NOT SAVE!**
- **Close remaining spectra and SAVE THEM ALL!**

WINDOWS EXPLORER: rename the new created file (by flexAnalysis): e.g. ID 1234 → ID 1234 ***ed 21sp***	**File name:**

MSP Creation with MBT Compass Explorer:

- Open the MBT Compass Explorer
- Load (Button: add Spectra …) and select all edited sample spectra
- Right click → **„Create MSP" → assign MSP name**

MSP Name: e.g.: Streptobacillus hongkongensis DSM 26322 CVUAS / Escherichia coli CVUAS 5146 CVUAS

- Taxonomy tree: change the dropdown list to "Projects", select a file/node where the MSP should be stored and start the Taxonomy Tree Editor (right click or button next to dropdown menu)

☐ **Metadata** filled in

☐ **Added** MSP to "Projects" file:

☐ Verification of the MSP with an **independent spectrum** (date):

☐ Report print-out / pdf

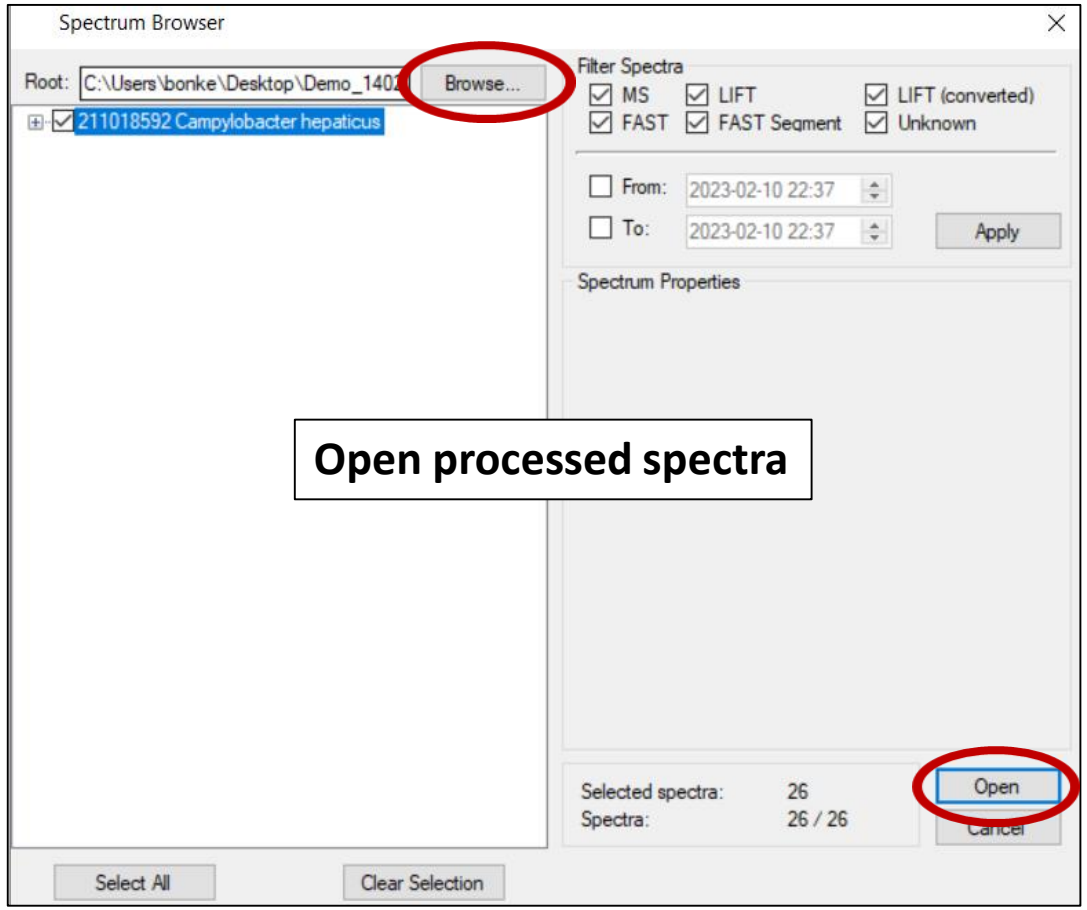
Preparation:
☐ DT
☐ eDT
☐ EtOH-FA
☐ ……

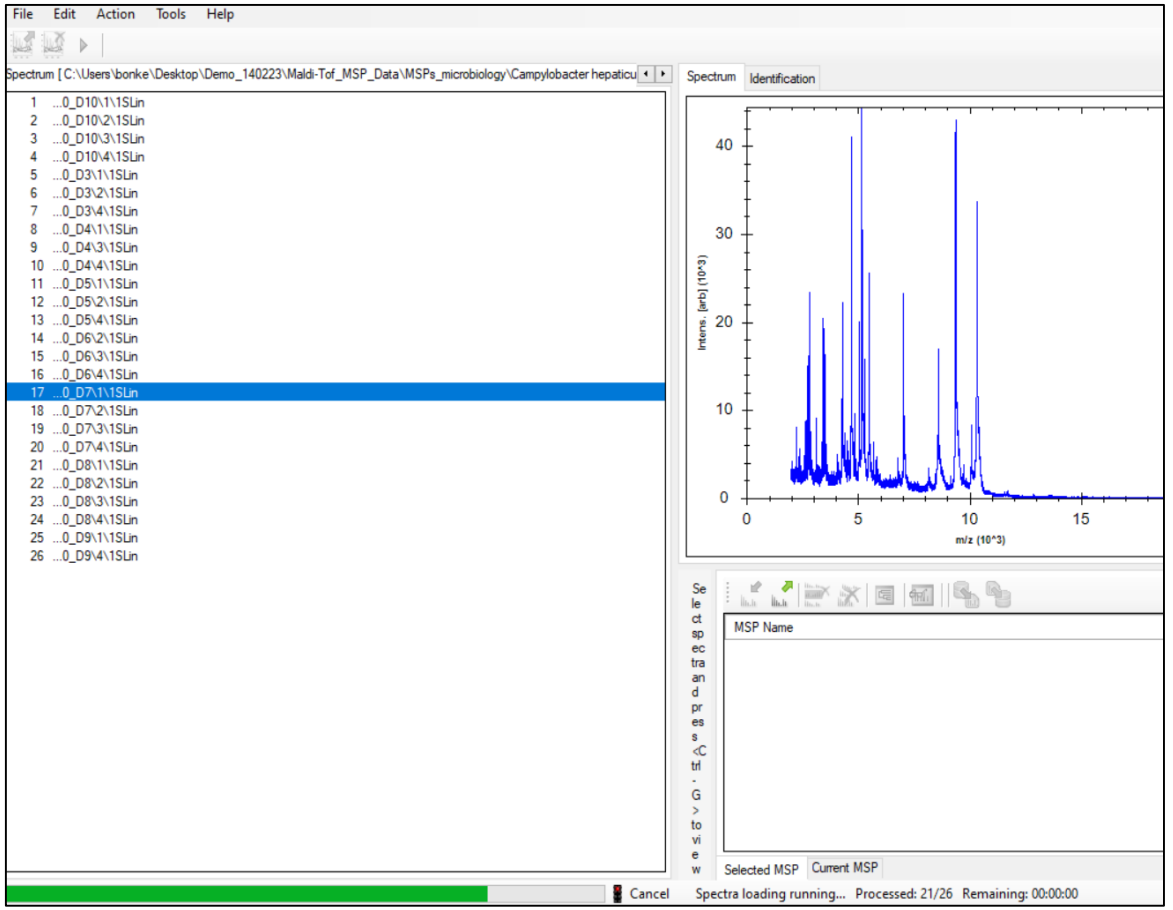
Entry created …: ☐ **own MSP-Library updated**

Comment:	

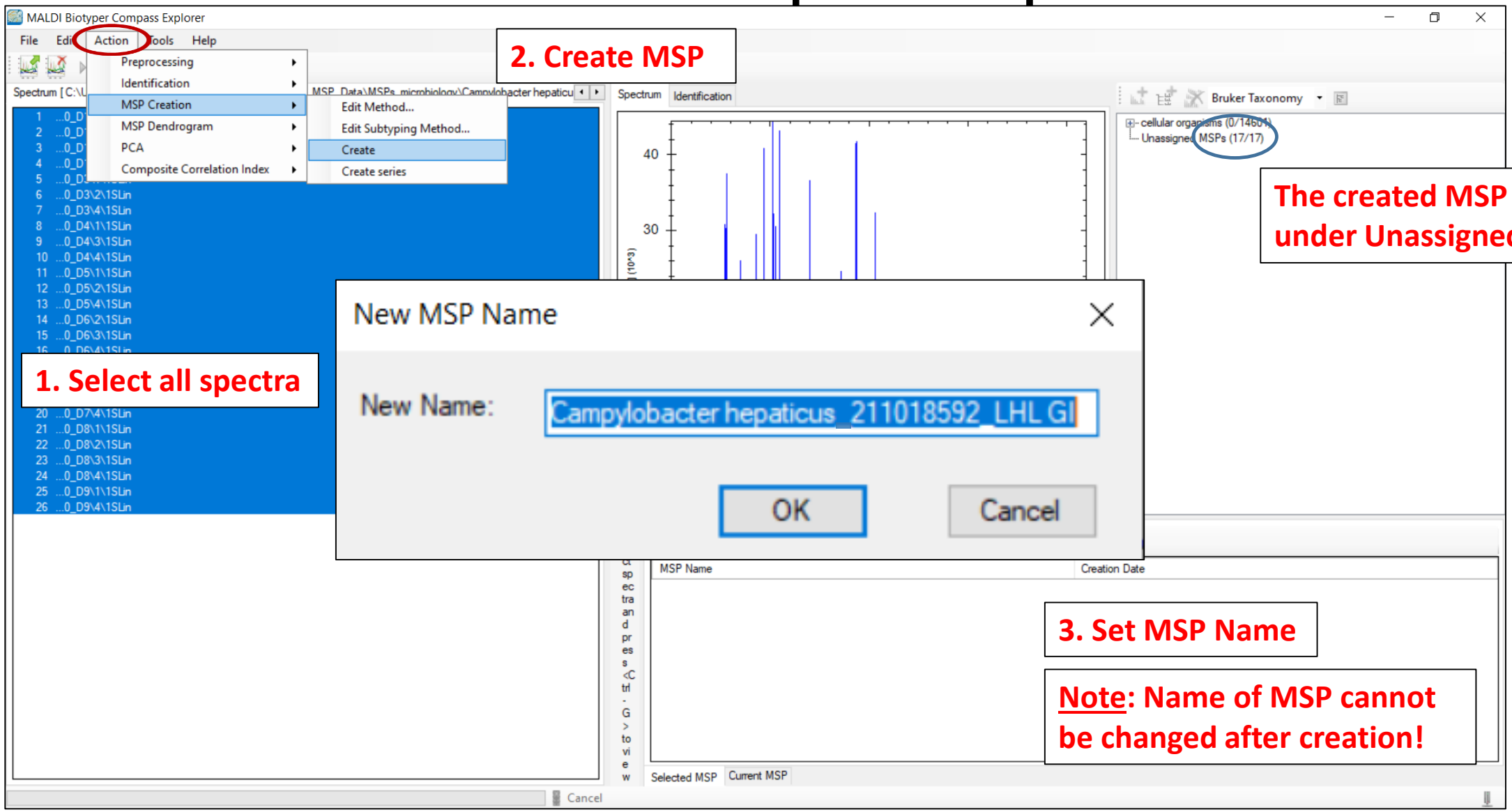
Date / acronym _____

MSP creation – MBT Compass Explorer

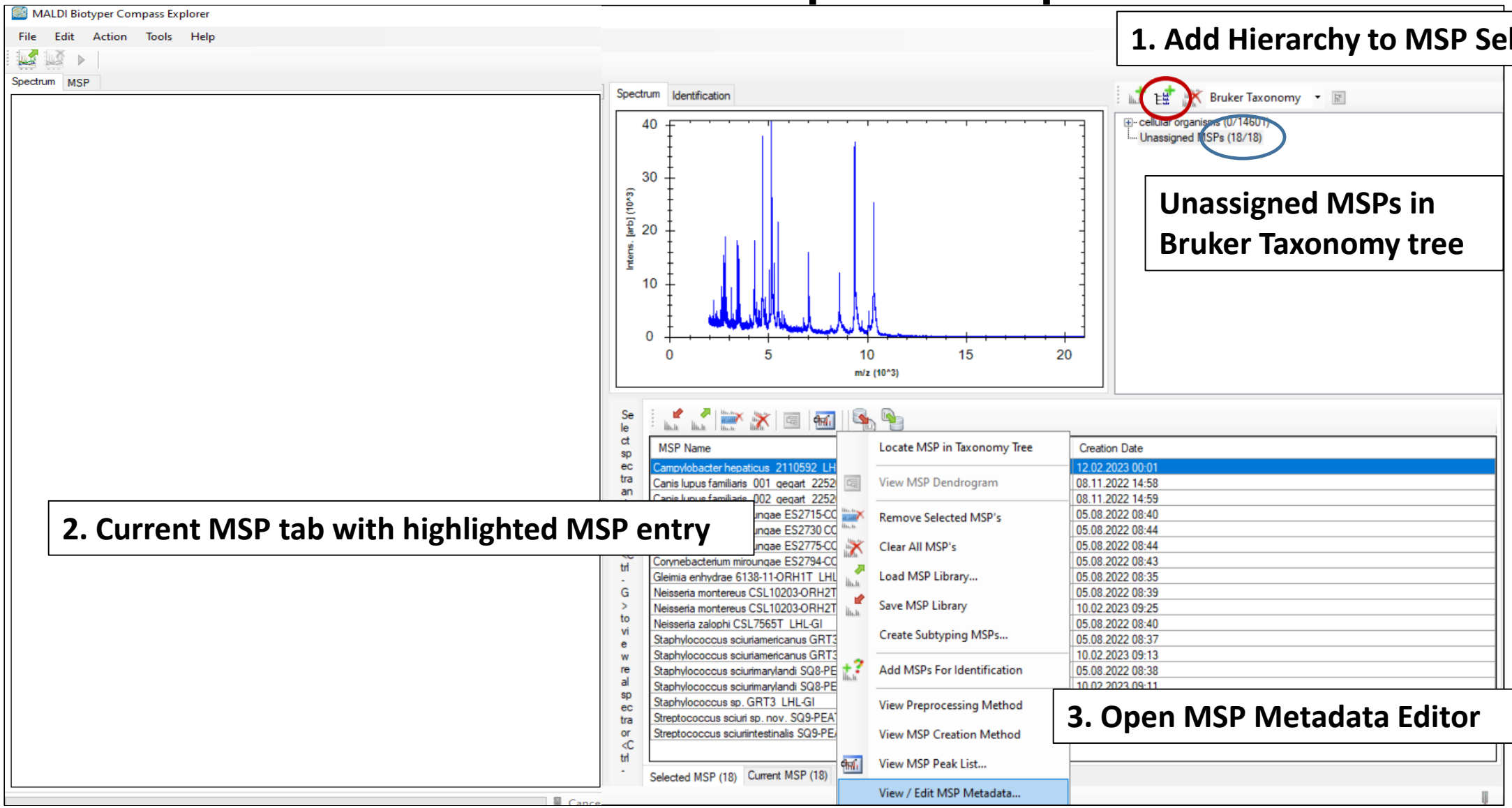




MSP creation – MBT Compass Explorer



MSP creation – MBT Compass Explorer



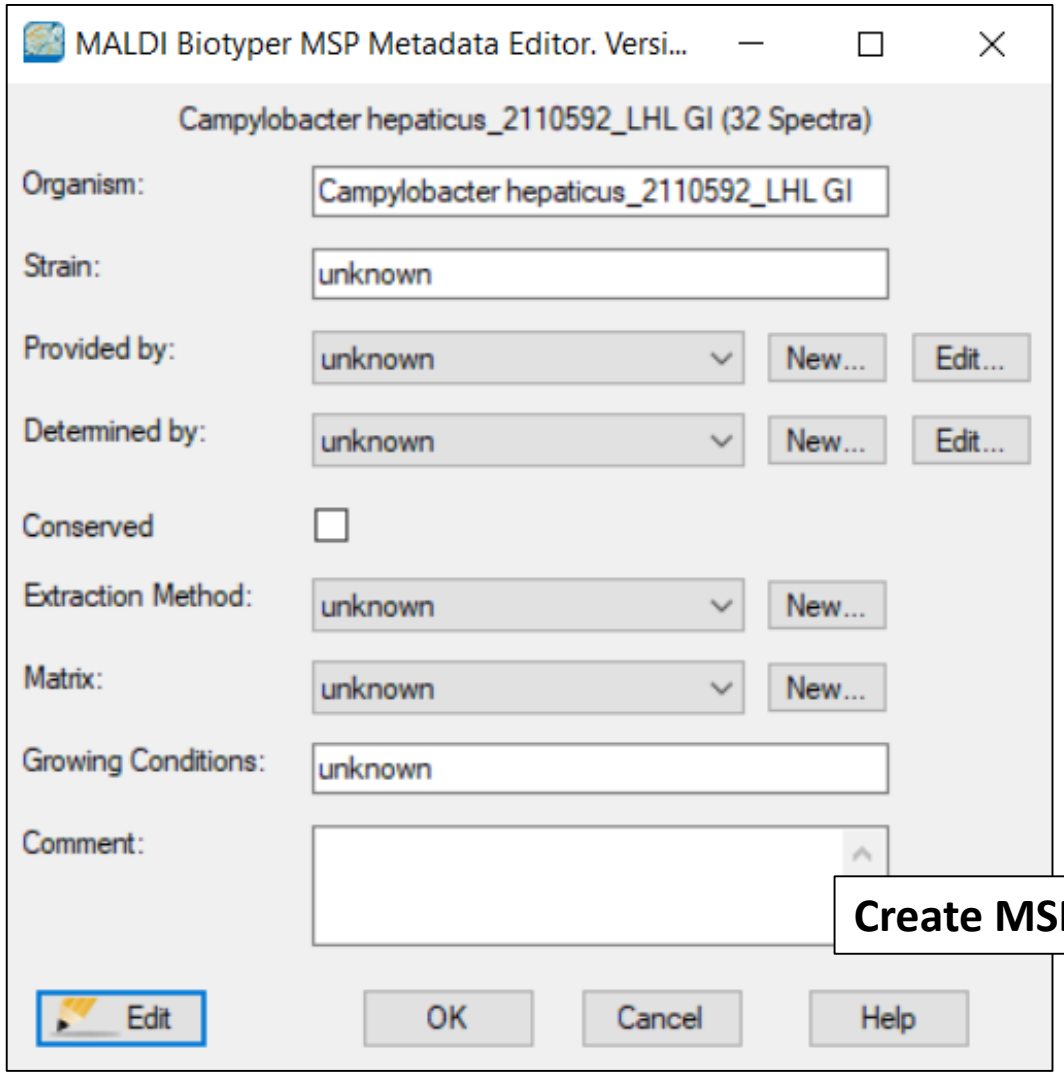
1. Add Hierarchy to MSP Selection

Unassigned MSPs in Bruker Taxonomy tree

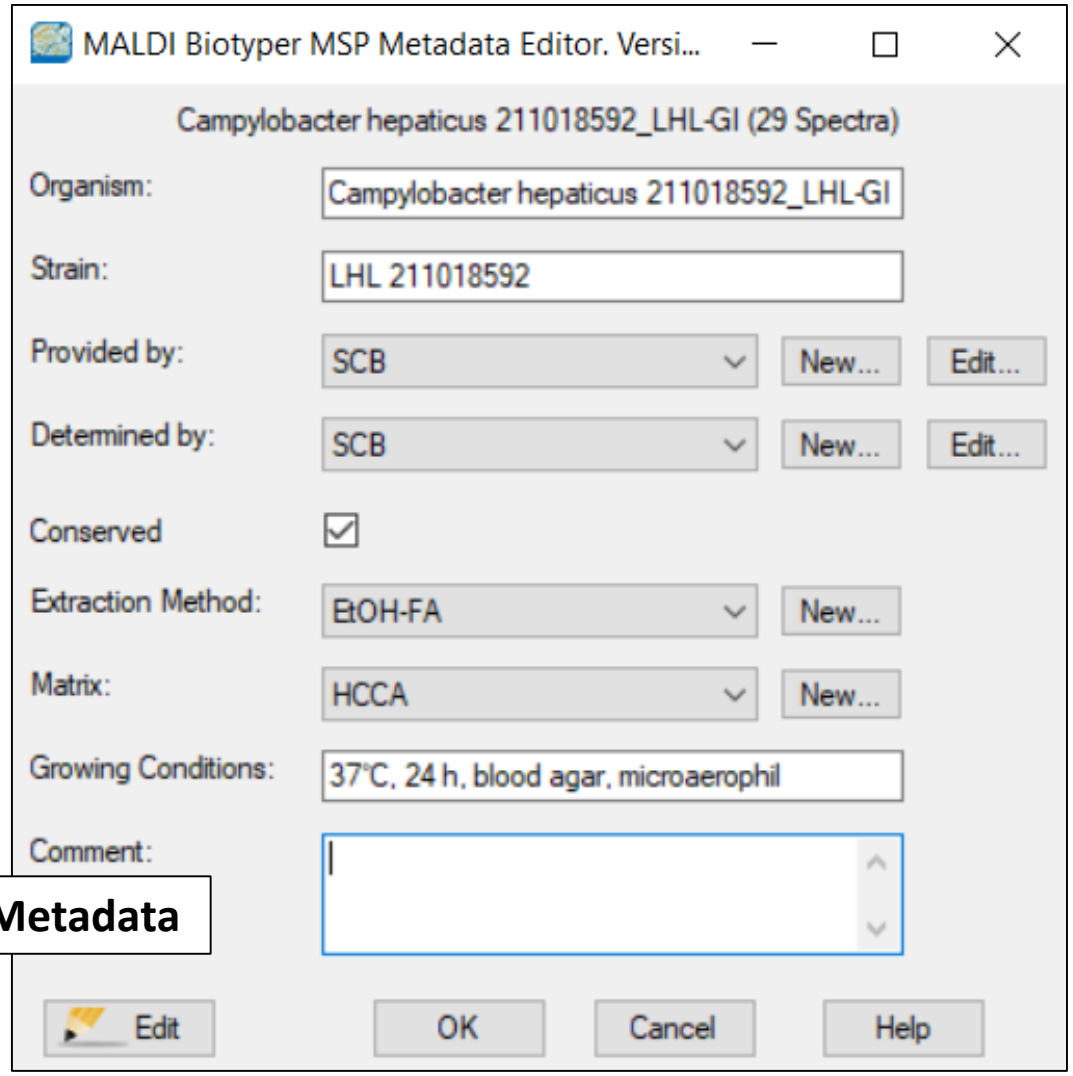
2. Current MSP tab with highlighted MSP entry

3. Open MSP Metadata Editor

MSP creation – MBT Compass Explorer

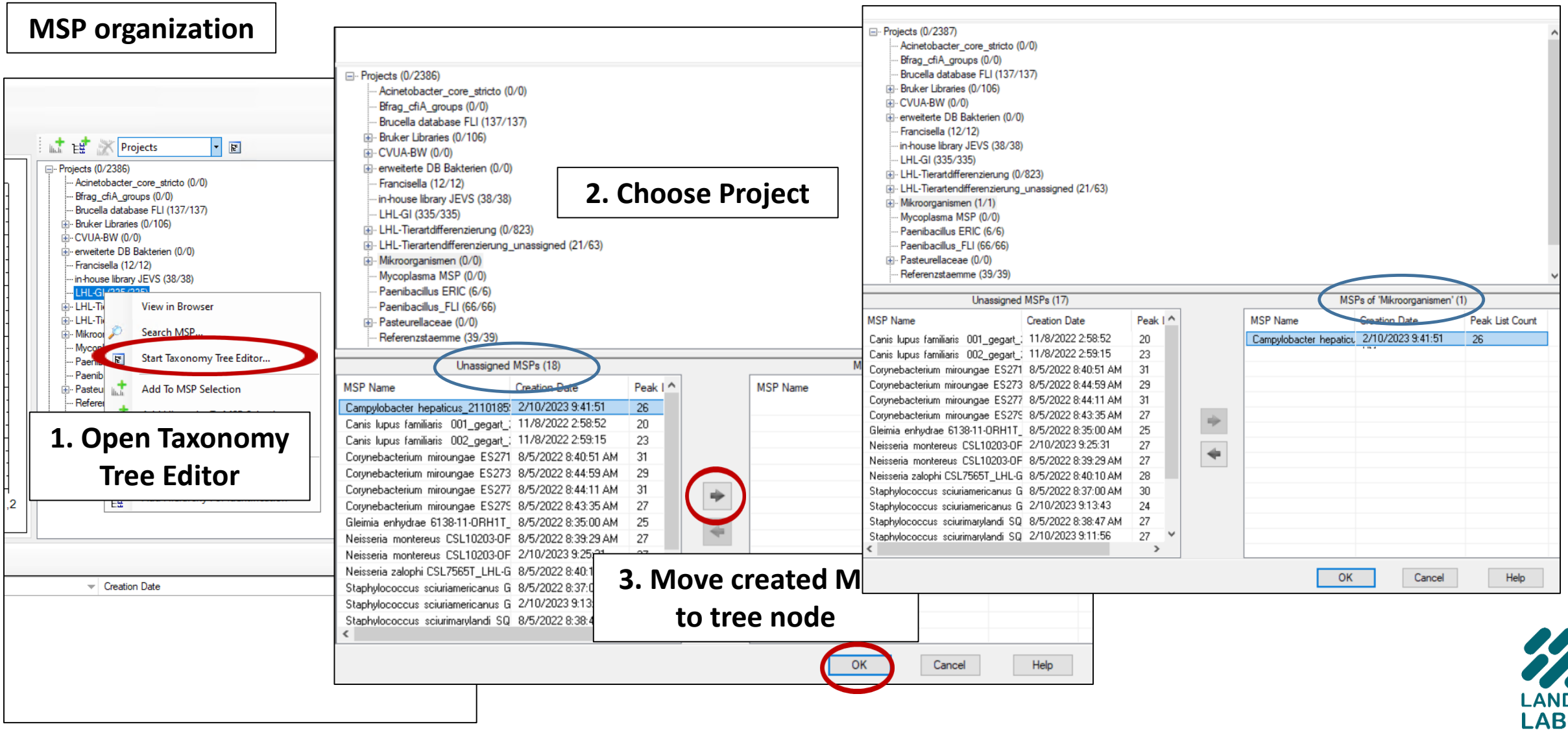


Create MSP Metadata

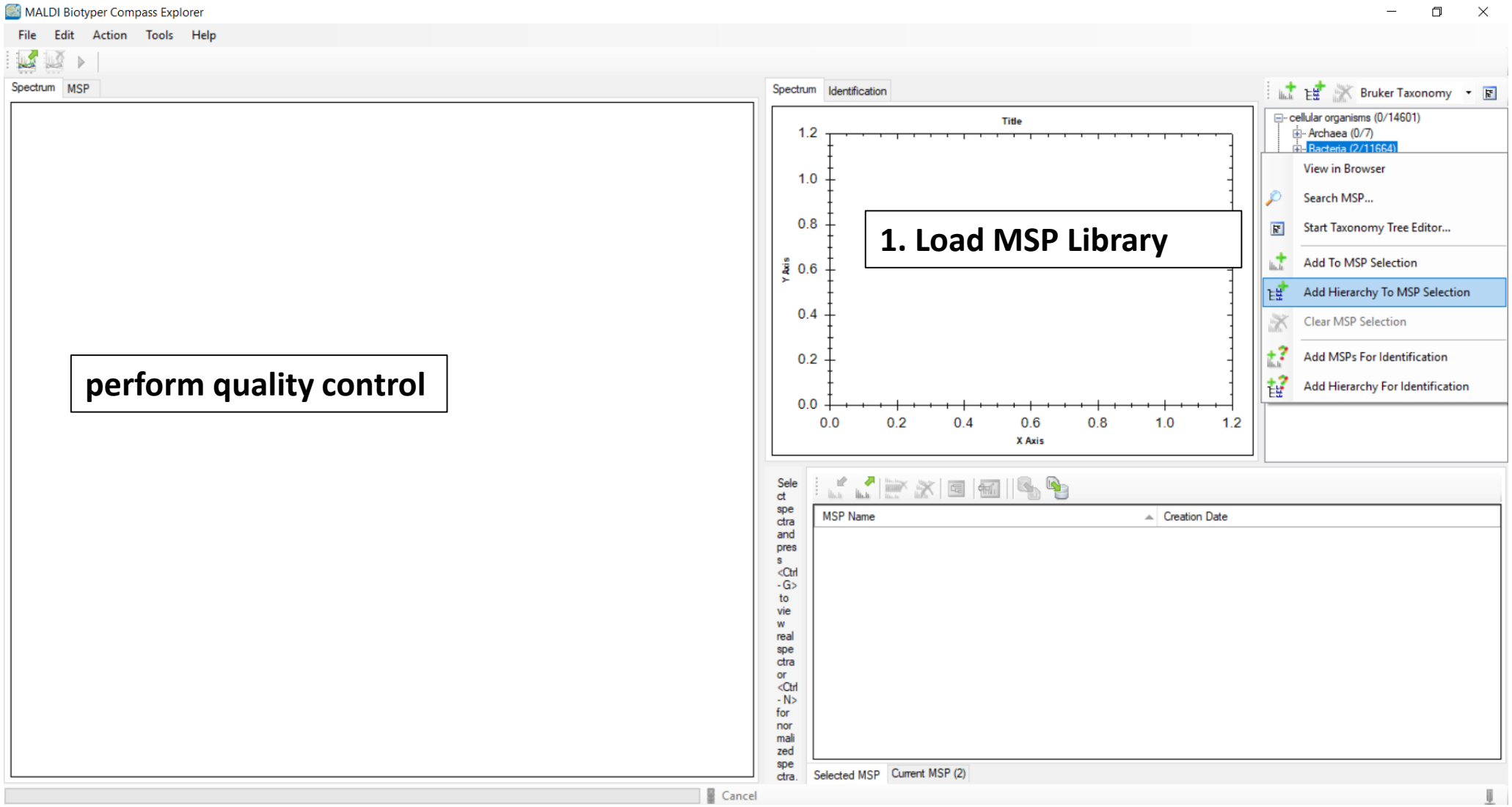


MSP creation – MBT Compass Explorer

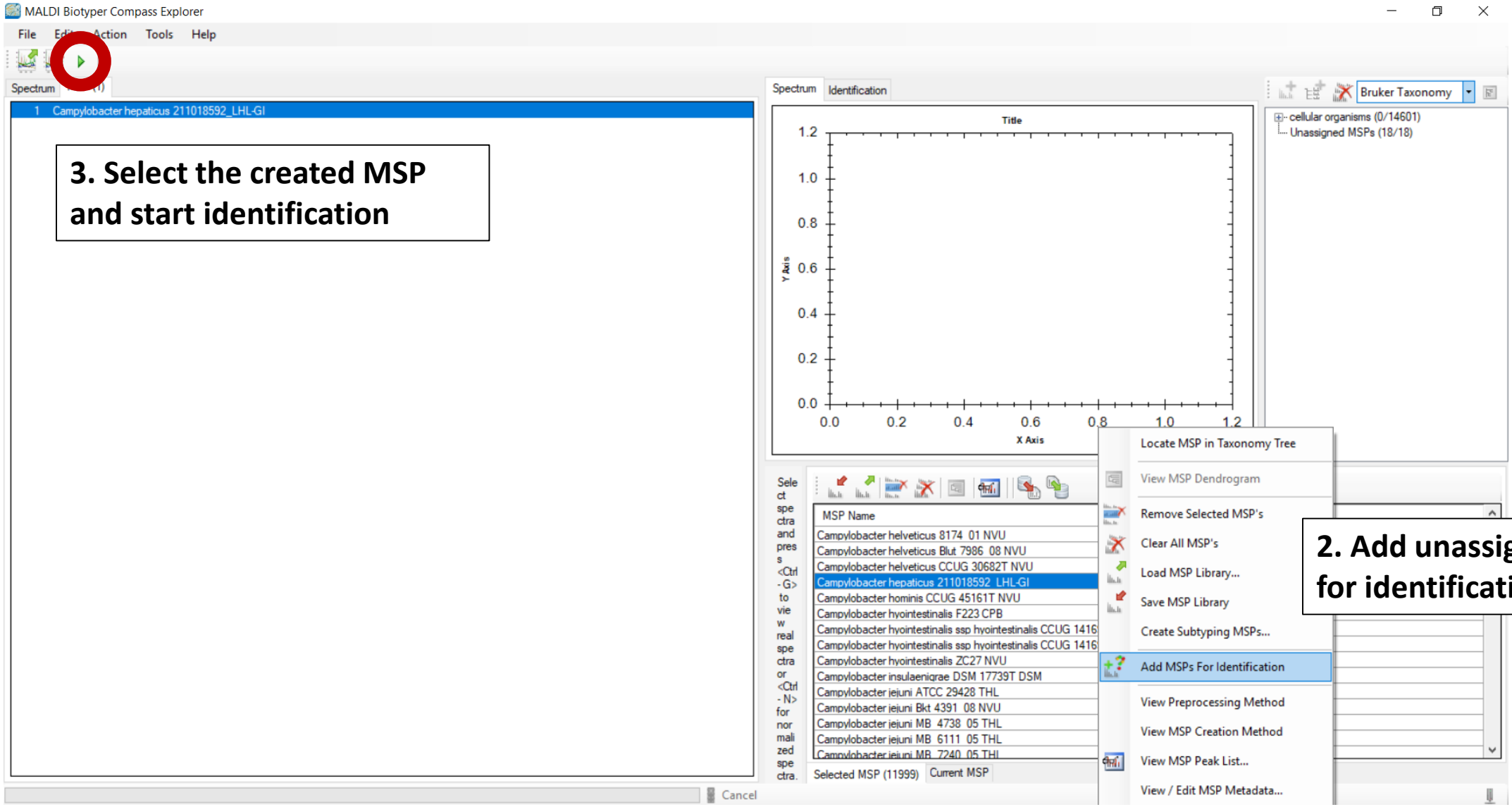
MSP organization



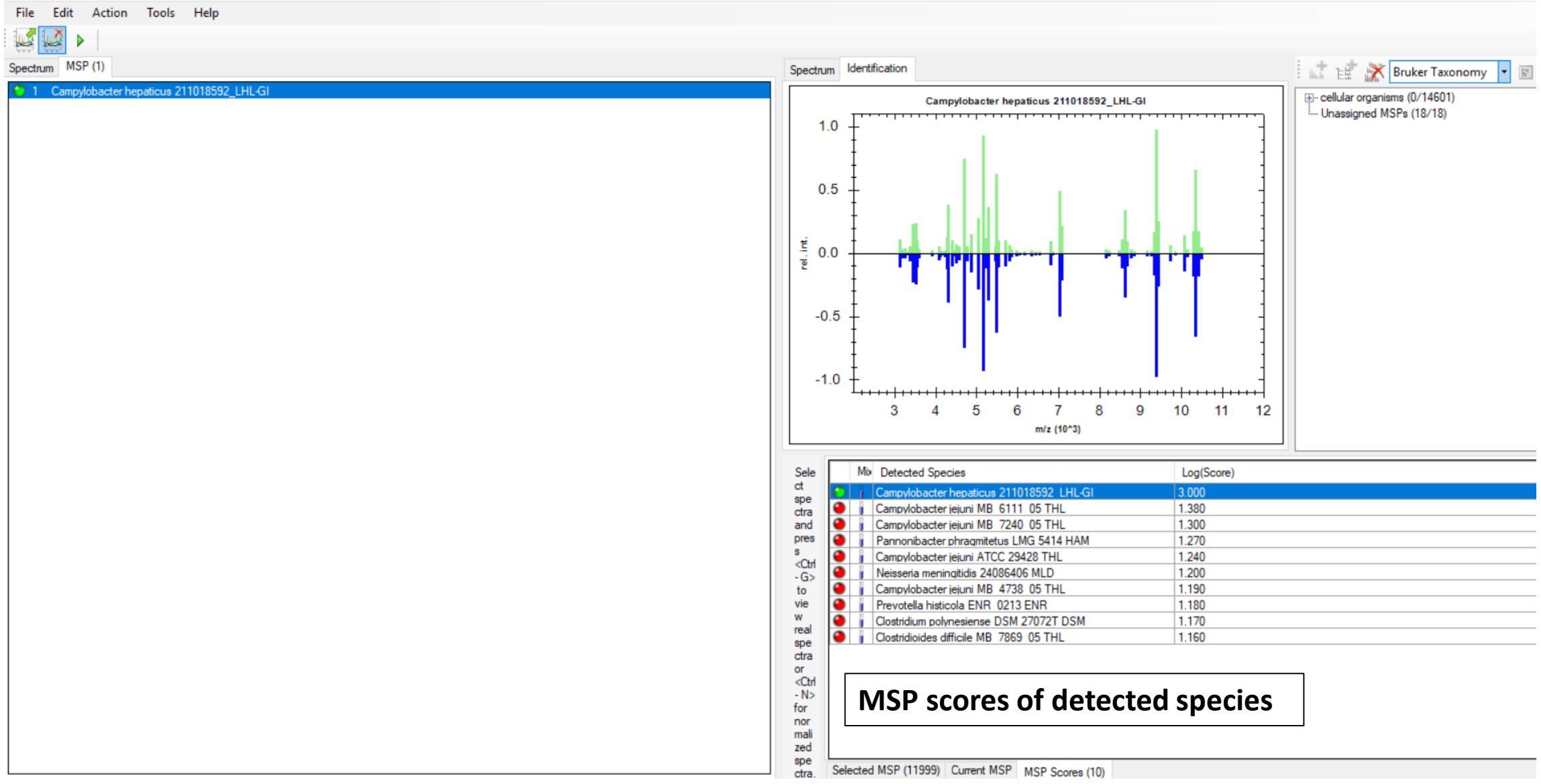
MSP creation – MBT Compass Explorer



MSP creation – MBT Compass Explorer

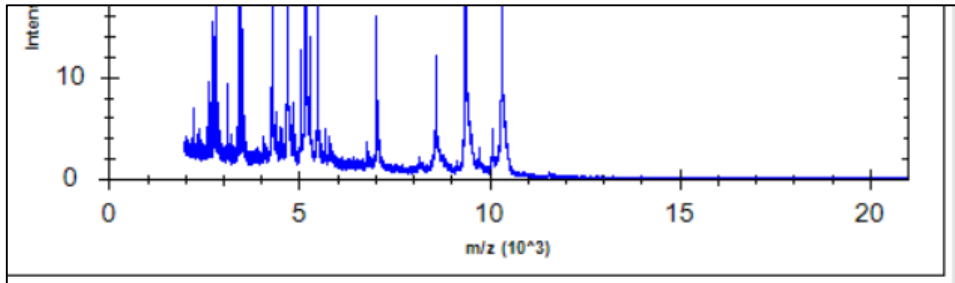


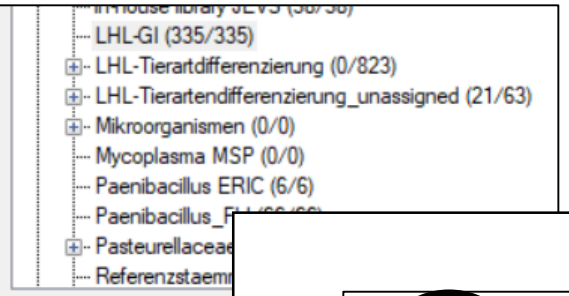
MSP creation – MBT Compass Explorer

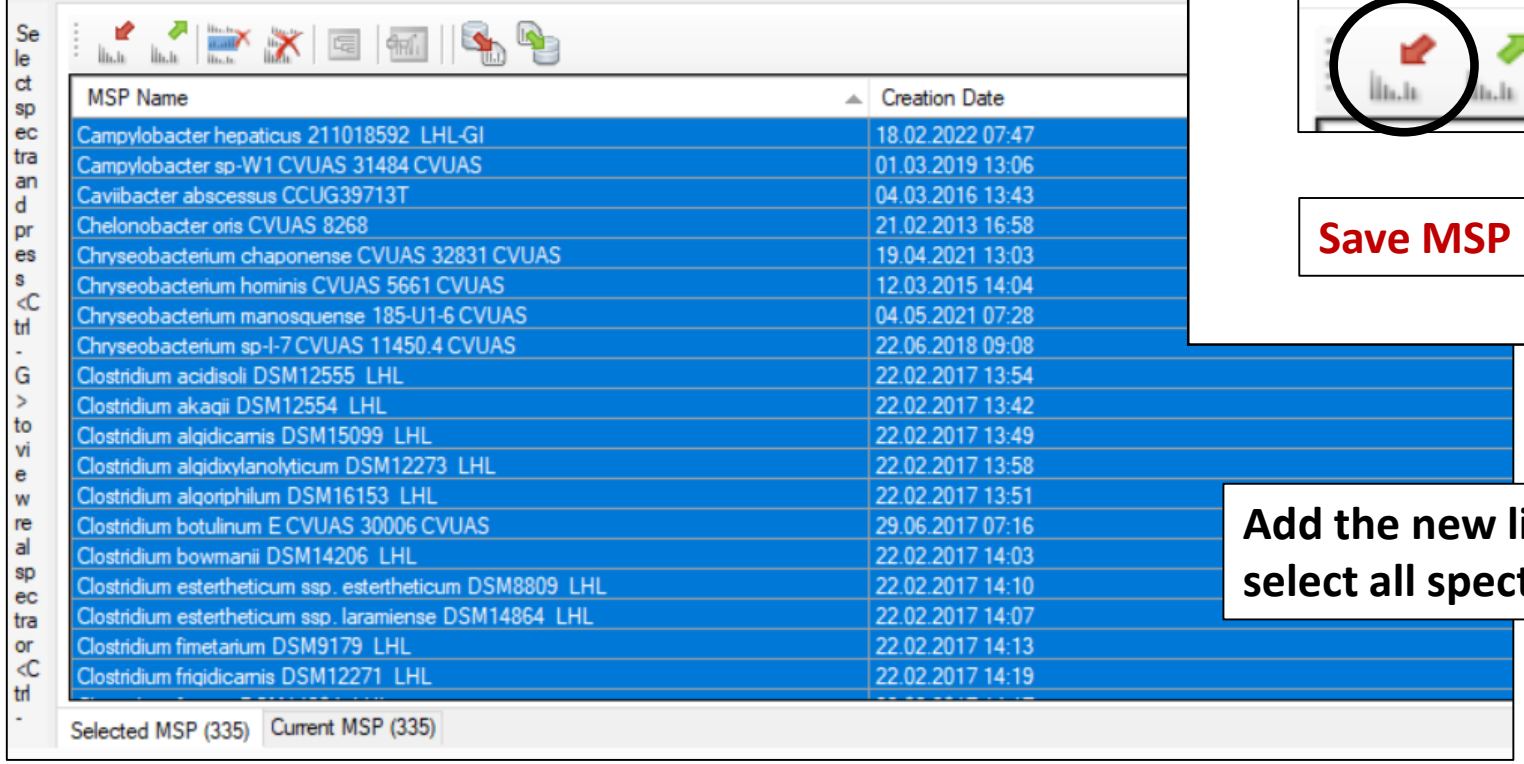


MSP scores of detected species

MSP creation – MBT Compass Explorer



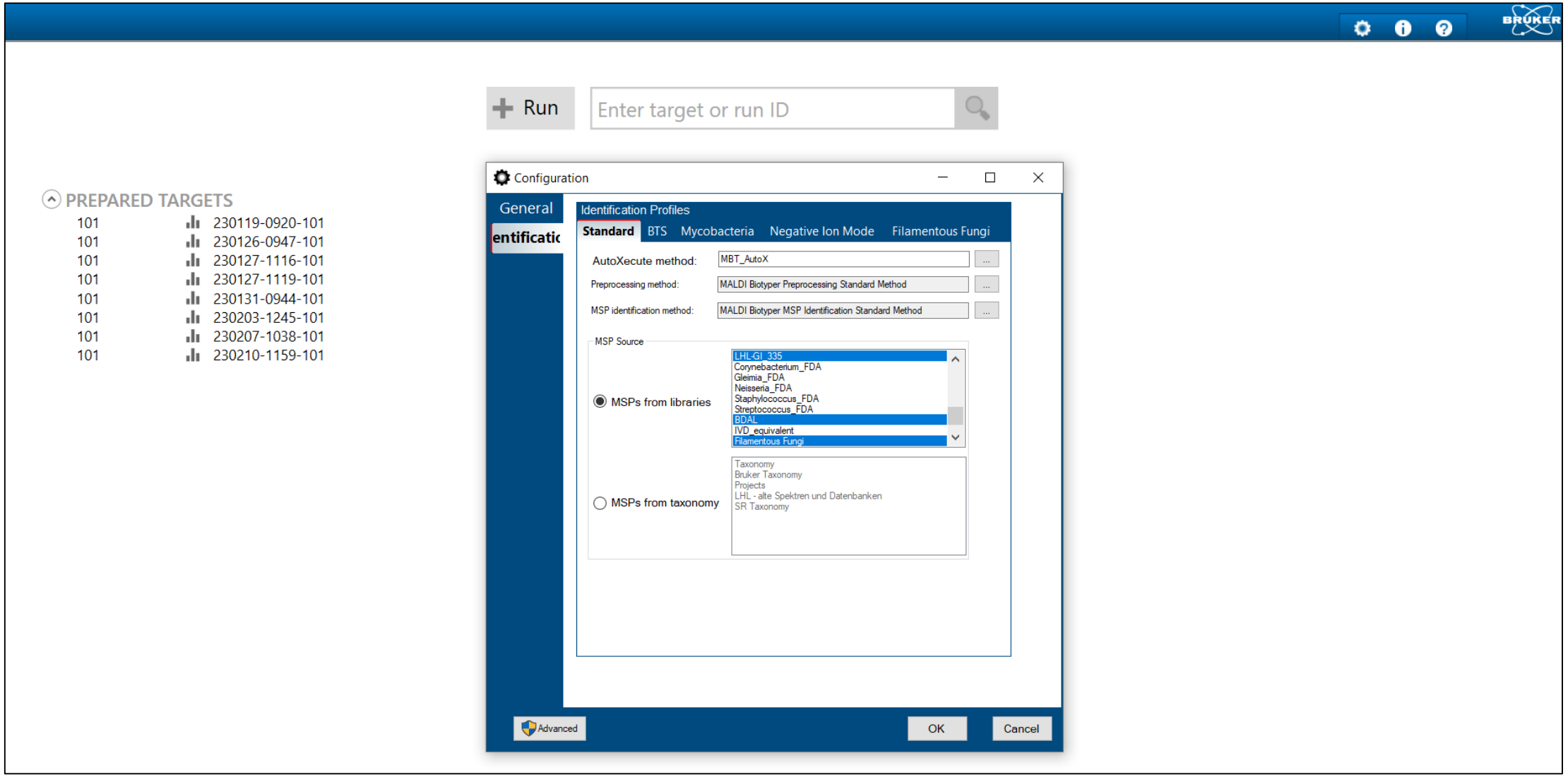




Save MSP library!

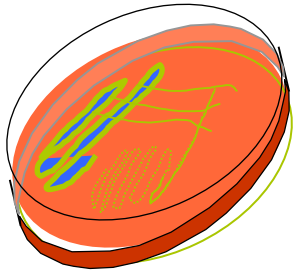
Add the new library to the table view, select all spectra and save.

Library selection– MBT Compass



Quality control – MBT Compass

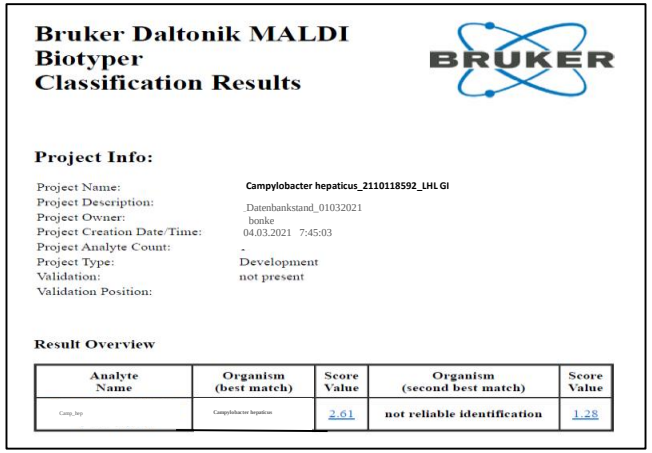
fresh culture



Direct smear

➡ Additional quality control

- freshly prepared subculture of the same strain for an additional quality control step.
- prepare a direct transfer and measure against the new library.
- strain should be identified within the 10 best matches.



Bruker Daltonik MALDI Biotyper Classification Results

Project Info:

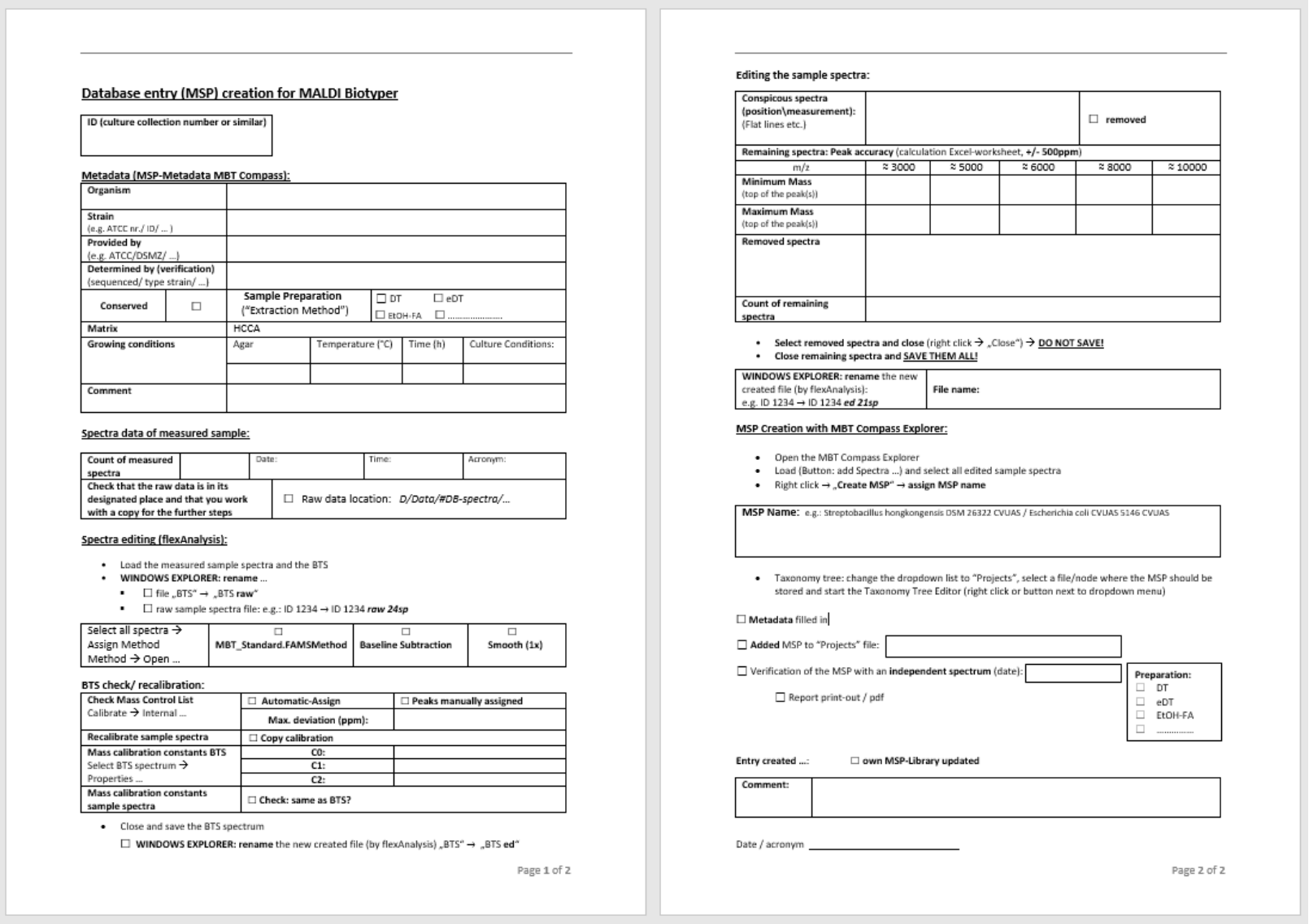
Project Name:	Campylobacter hepaticus_2110118592_LHL GI
Project Description:	_Datenbankstand_01032021
Project Owner:	bonke
Project Creation Date/Time:	04.03.2021 7:45:03
Project Analyte Count:	-
Project Type:	Development
Validation:	not present
Validation Position:	

Result Overview

Analyte Name	Organism (best match)	Score Value	Organism (second best match)	Score Value
Camp_hep	Campylobacter hepaticus	2.61	not reliable identification	1.28

Fig: CVUA Stuttgart, 2022

Documentation



Database entry (MSP) creation for MALDI Biotyper

ID (culture collection number or similar)

Metadata (MSP-Metadata MBT Compass):

Organism					
Strain (e.g. ATCC nr./ ID/ …)					
Provided by (e.g. ATCC/DSMZ/ …)					
Determined by (verification) (sequenced/ type strain/ …)					
Conserved	☐	**Sample Preparation** ("Extraction Method")	☐ DT ☐ eDT ☐ EtOH-FA ☐ …….		
Matrix		HCCA			
Growing conditions		Agar	Temperature (°C)	Time (h)	Culture Conditions:
Comment					

Spectra data of measured sample:

Count of measured spectra		Date:	Time:	Acronym:
Check that the raw data is in its designated place and that you work with a copy for the further steps	☐ Raw data location: *D/Data/#DB-spectra/…*			

Spectra editing (flexAnalysis):

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  - ☐ file „BTS" → „BTS **raw**"
  - ☐ raw sample spectra file: e.g.: ID 1234 → ID 1234 ***raw 24sp***

Select all spectra → Assign Method Method → Open …	☐ **MBT_Standard.FAMSMethod**	☐ **Baseline Subtraction**	☐ **Smooth (1x)**

BTS check/ recalibration:

Check Mass Control List Calibrate → Internal …	☐ **Automatic-Assign**	☐ **Peaks manually assigned**
	Max. deviation (ppm):	
Recalibrate sample spectra	☐ **Copy calibration**	
Mass calibration constants BTS Select BTS spectrum → Properties …	**C0:**	
	C1:	
	C2:	
Mass calibration constants sample spectra	☐ **Check: same as BTS?**	

- Close and save the BTS spectrum
  - ☐ **WINDOWS EXPLORER: rename** the new created file (by flexAnalysis) „BTS" → „BTS **ed**"

Editing the sample spectra:

Conspicous spectra (position\measurement): (Flat lines etc.)				☐ **removed**	
Remaining spectra: Peak accuracy (calculation Excel-worksheet, **+/- 500ppm**)					
m/z	≈ 3000	≈ 5000	≈ 6000	≈ 8000	≈ 10000
Minimum Mass (top of the peak(s))					
Maximum Mass (top of the peak(s))					
Removed spectra					
Count of remaining spectra					

- **Select removed spectra and close** (right click → „Close") → **DO NOT SAVE!**
- **Close remaining spectra and SAVE THEM ALL!**

WINDOWS EXPLORER: rename the new created file (by flexAnalysis): e.g. ID 1234 → ID 1234 ***ed 21sp***	**File name:**

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☐ **Metadata** filled in

☐ **Added** MSP to "Projects" file:

☐ Verification of the MSP with an **independent spectrum** (date):

☐ Report print-out / pdf

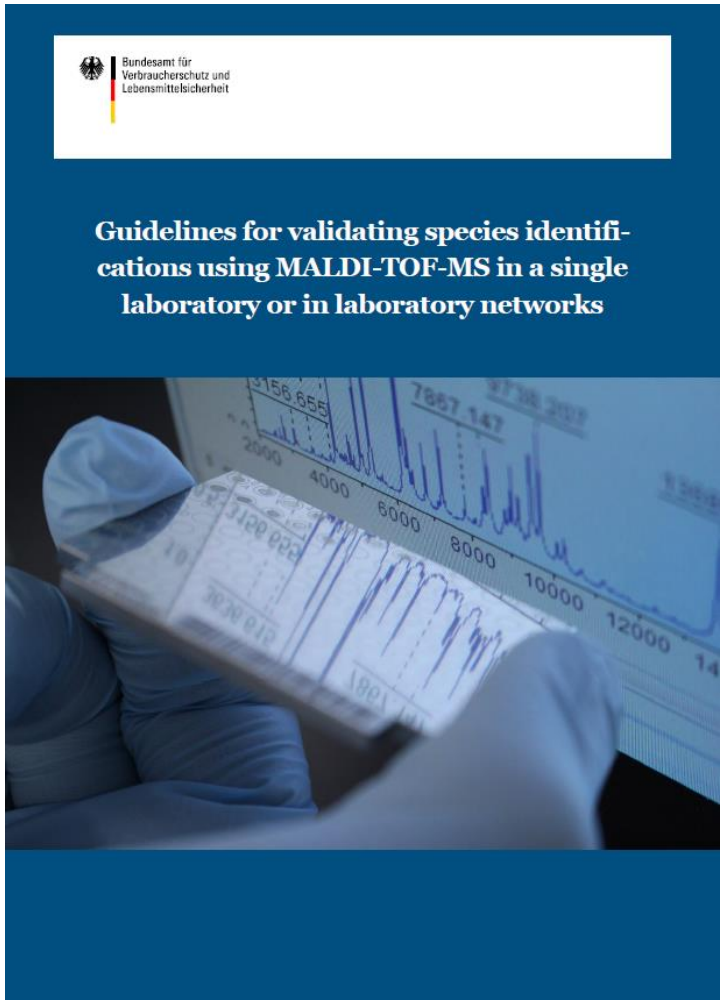
Preparation:
☐ DT
☐ eDT
☐ EtOH-FA
☐ ……

Entry created …: ☐ **own MSP-Library updated**

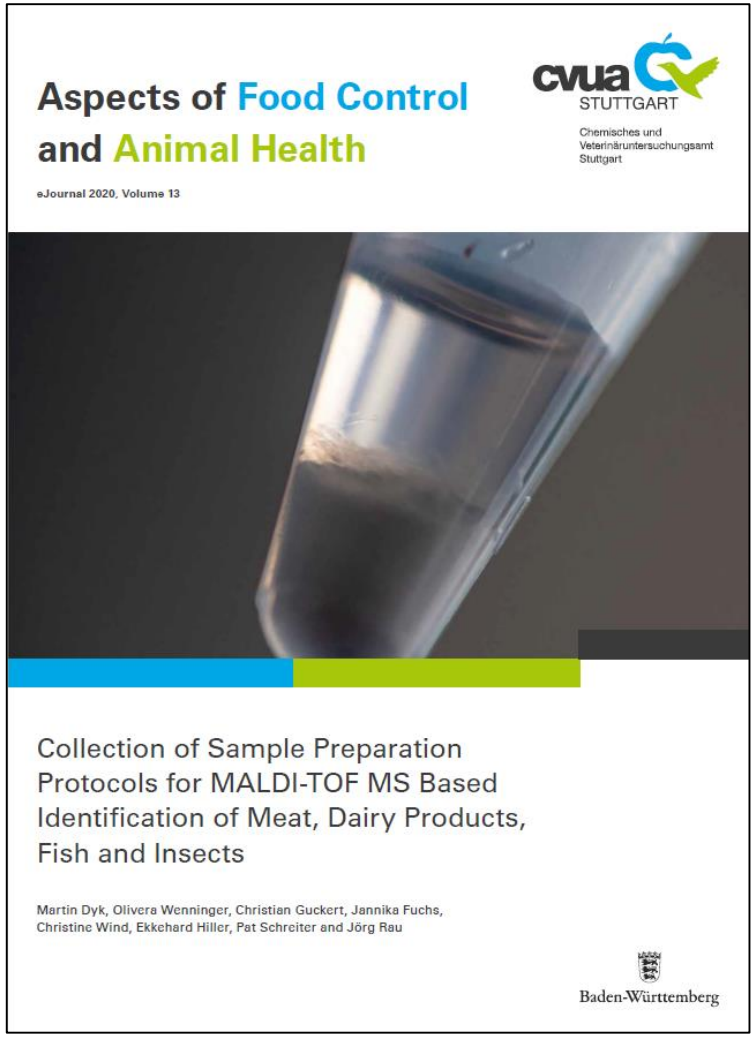
Comment:	

Date / acronym _____

Validation and other applications



https://www.bvl.bund.de/SharedDocs/Downloads/07_Untersuchungen/Guidelines_for_validating_species_identifications_using_MALDI-TOF-MS.pdf?__blob=publicationFile&v=4



https://ejournal.cvuas.de/issue202013.asp