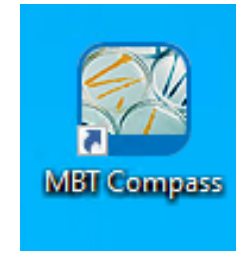
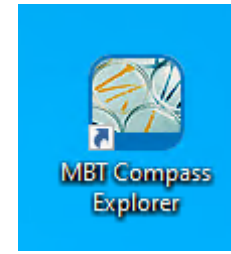
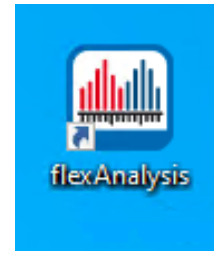
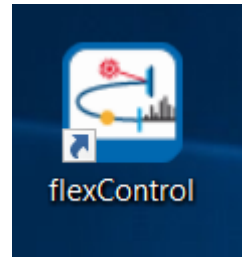


# Creation of Library Entries (Main spectra -MSPs)

*- from raw spectrum to database entry -*

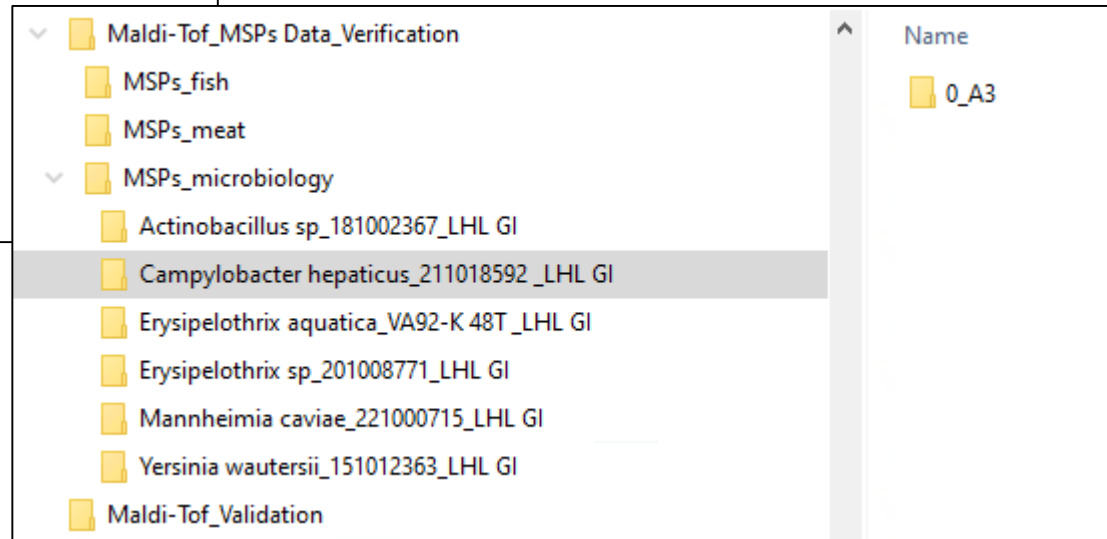
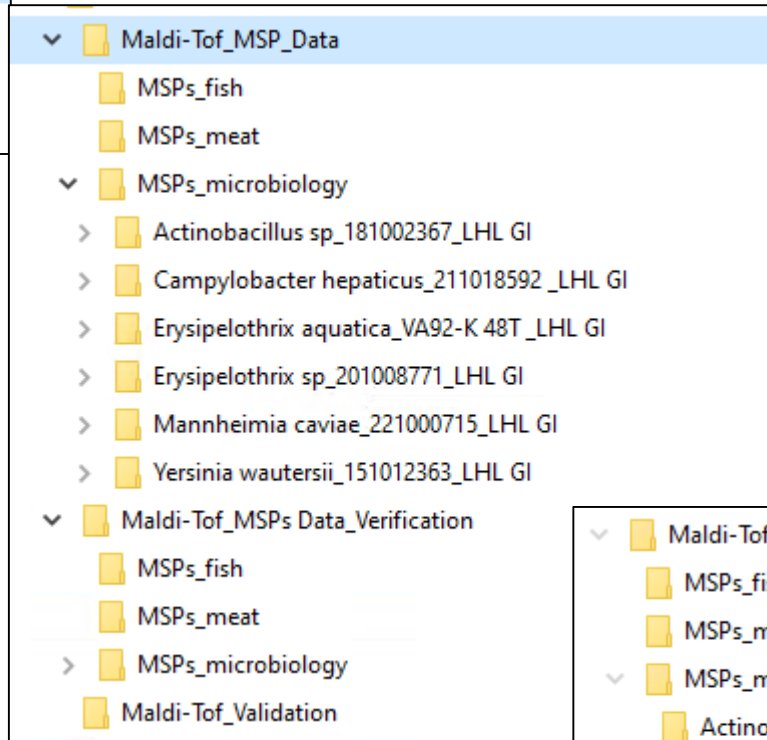
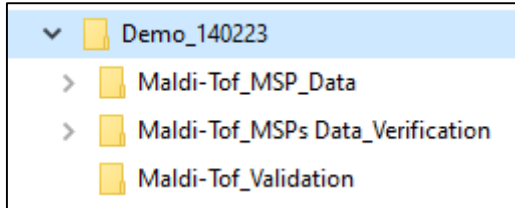
This document provides the basics for creating your own MSPs to expand your own MALDI-TOF database and is based on own experience. It does not claim to be complete and does not replace Bruker's application training courses. All image rights to the software systems shown belong to Bruker.

# 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 ...)

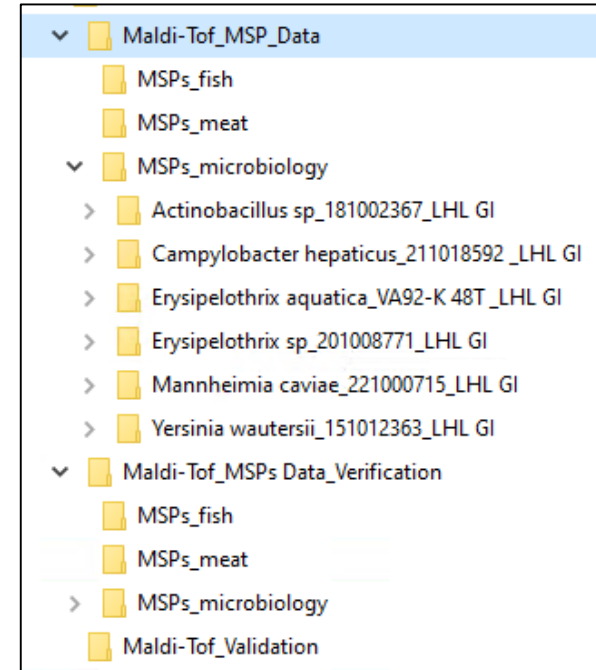
# Nomenclature (file-names)

## 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

# 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	<input type="checkbox"/>	Sample Preparation ("Extraction Method")	<input type="checkbox"/> DT <input type="checkbox"/> eDT <input type="checkbox"/> EtOH-FA <input type="checkbox"/> .....	
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		<input type="checkbox"/> 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 ...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	MBT_Standard.FAMSMETHOD	Baseline Subtraction	Smooth (1x)

### BTS check/ recalibration:

Check Mass Control List Calibrate → Internal ...	<input type="checkbox"/> Automatic-Assign	<input type="checkbox"/> Peaks manually assigned
	Max. deviation (ppm):	
Recalibrate sample spectra	<input type="checkbox"/> Copy calibration	
Mass calibration constants BTS Select BTS spectrum → Properties ...	C0:	
	C1:	
	C2:	
Mass calibration constants sample spectra	<input type="checkbox"/> 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:

Conspicuous spectra (position/measurement): (Flat lines etc.)	<input type="checkbox"/> 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.: Streptobadillus 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:
<input type="checkbox"/> DT
<input type="checkbox"/> eDT
<input type="checkbox"/> EtOH-FA
<input type="checkbox"/> .....

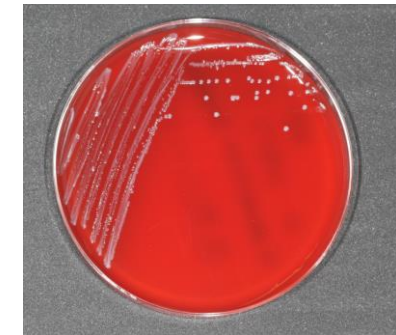
Entry created ...:  own MSP-Library updated

Comment:
----------

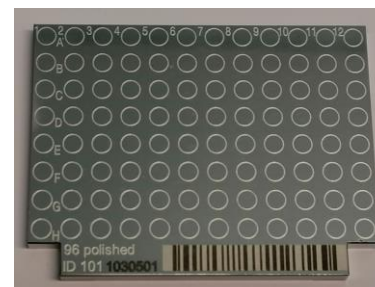
Date / acronym \_\_\_\_\_

# Generation of raw-spectra - sample preparation

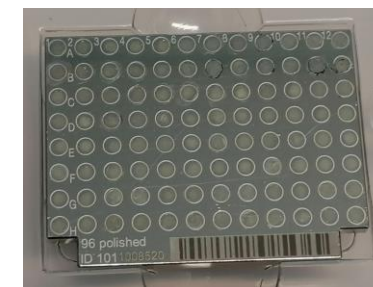
- use fresh reagents
- prepare valid and well-characterized strains (e.g. 16s rRNA gene sequencing)
- preparation according to Bruker  
<https://www.bruker.com/en/services/training/microbiology-and-diagnostics/maldi-biotyper-training-movies.html>
- sample preparation: extraction protocol
- make a test measurement of the extraction before use
- prepare spots on Target
- Prepare BTS



Fresh valid culture (max. 24 h)



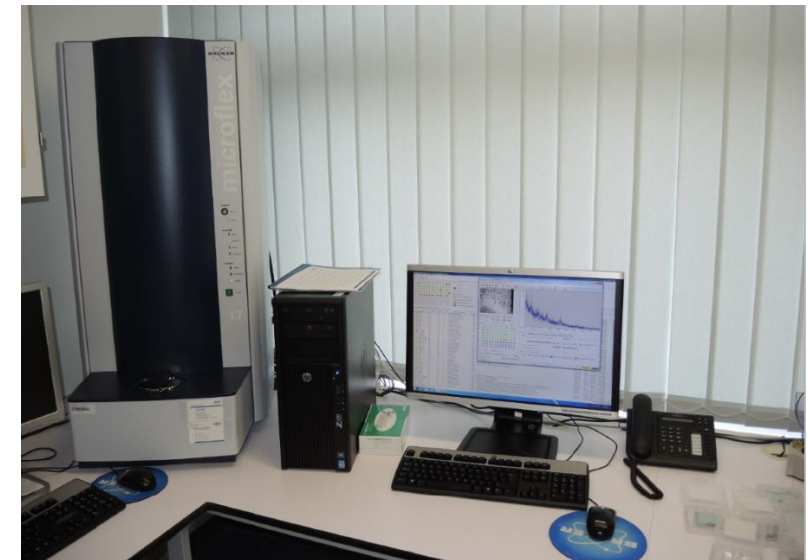
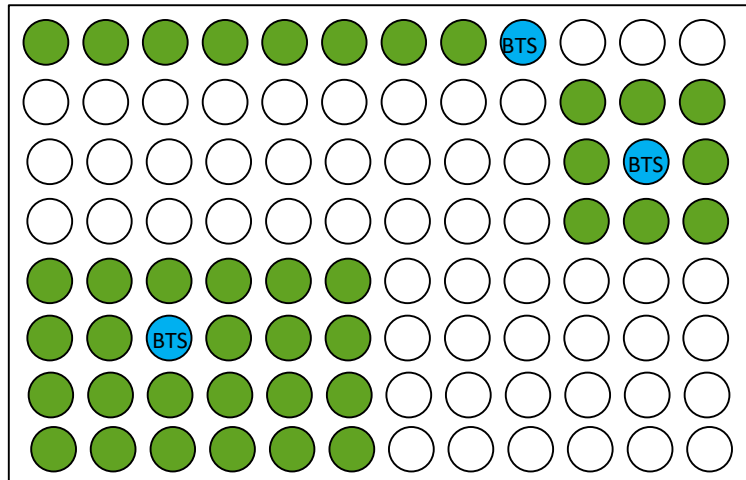
Target before preparation



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

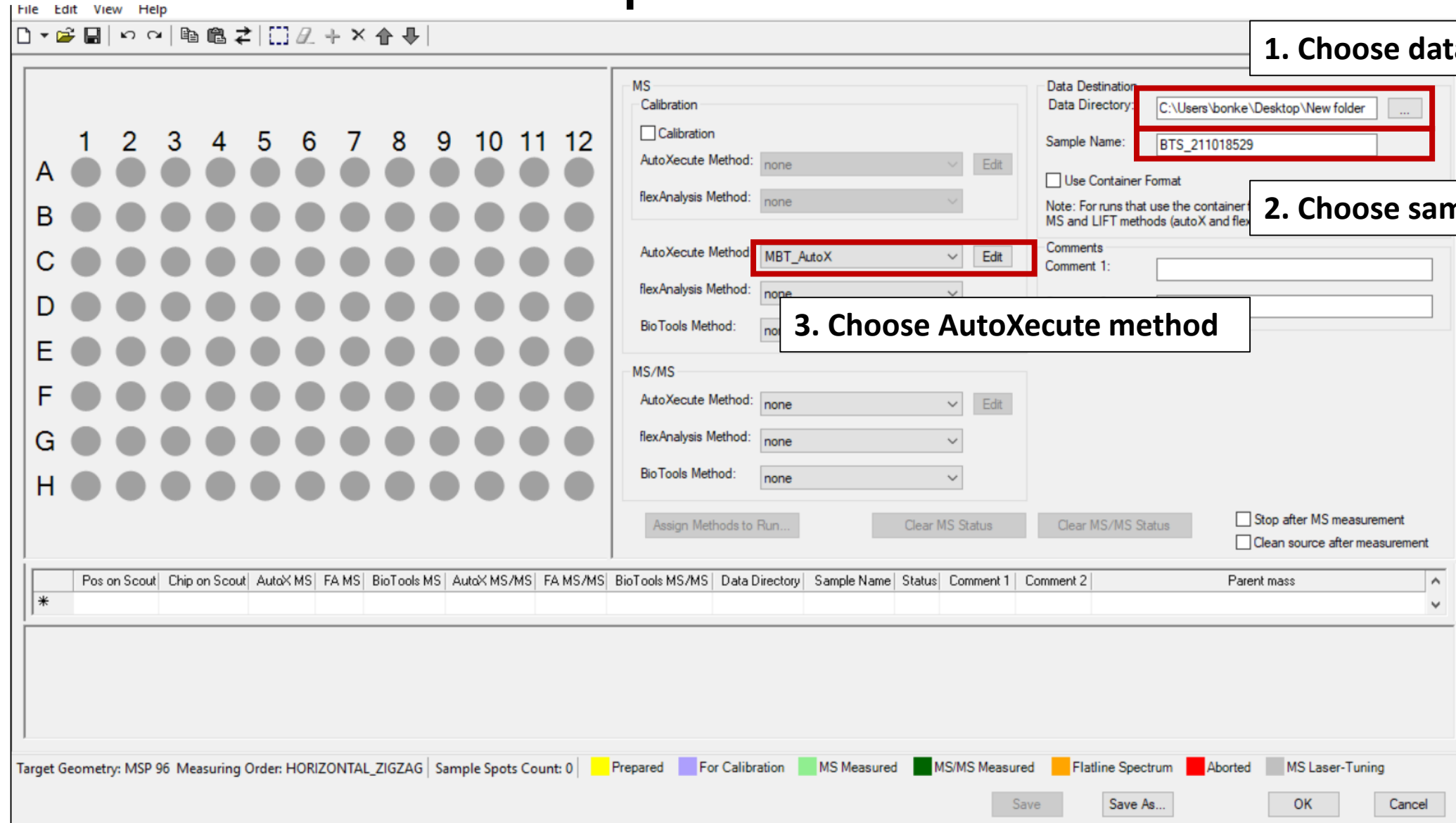
- one BTS preparation for calibration and quality control

# Generation of raw-spectra - flexControl

The screenshot displays the flexControl software interface. On the left, there is a grid of sample spots labeled A through H and 1 through 11. Below the grid, the 'Spot' is set to G4:0 and 'Geometry' to MSP 96. The 'Carrier' is G\_7CEC5CC7\_529C\_42A9\_841D82E448D35B6E and the 'Method' is MBT\_FC.par. The main area shows a mass spectrum plot with 'Intens. [arb]' on the y-axis (0 to 100) and 'm/z' on the x-axis (2000 to 18000). A text box in the center of the plot reads 'Open the AutoXecute Run Editor'. At the bottom, the 'AutoXecute Run Editor' window is open, showing a 'Run:' dropdown menu set to 'none' and a 'New...' button, both circled in red. The 'AutoXecute' tab is also circled in red. The status bar at the bottom shows 'READY' and 'IN'.



# Generation of raw-spectra - flexControl

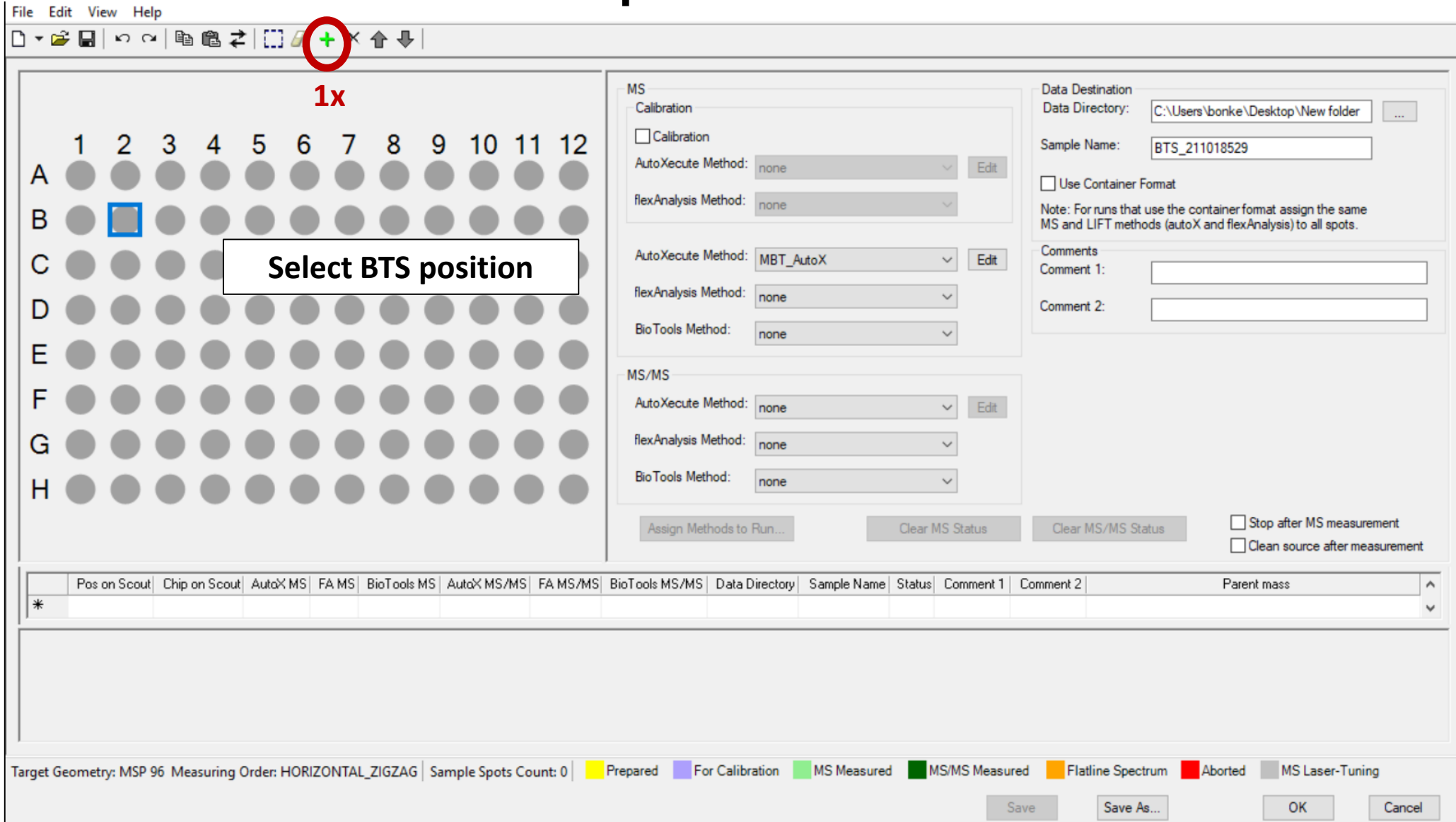


The screenshot shows the flexControl software interface. On the left is a 96-well plate layout (A-H, 1-12). On the right are configuration panels for MS and MS/MS. Three callout boxes highlight specific settings:

- 1. Choose data directory:** Points to the 'Data Directory' field in the MS panel, which contains 'C:\Users\bonke\Desktop\New folder'.
- 2. Choose sample name:** Points to the 'Sample Name' field in the MS panel, which contains 'BTS\_211018529'.
- 3. Choose AutoXecute method:** Points to the 'AutoXecute Method' dropdown in the MS panel, which is set to 'MBT\_AutoX'.

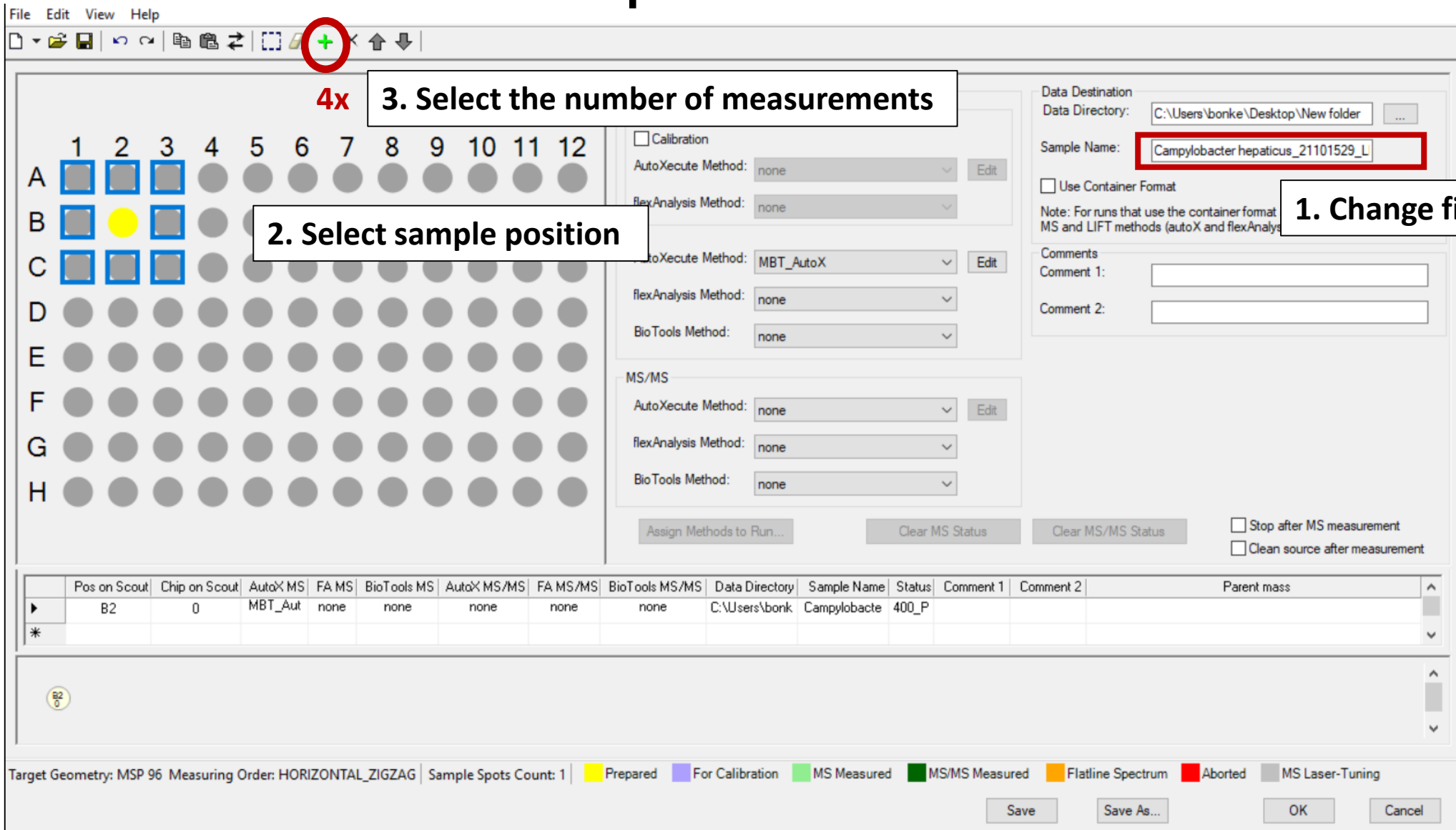
At the bottom, there is a table with columns: Pos on Scout, Chip on Scout, AutoX MS, FA MS, BioTools MS, AutoX MS/MS, FA MS/MS, BioTools MS/MS, Data Directory, Sample Name, Status, Comment 1, Comment 2, and Parent mass. The status bar at the very bottom shows 'Target Geometry: MSP 96 Measuring Order: HORIZONTAL\_ZIGZAG | Sample Spots Count: 0' and a legend for spot colors: Prepared (yellow), For Calibration (purple), MS Measured (green), MS/MS Measured (dark green), Flatline Spectrum (orange), Aborted (red), and MS Laser-Tuning (grey). Buttons for 'Save', 'Save As...', 'OK', and 'Cancel' are visible at the bottom right.

# Generation of raw-spectra - flexControl



The screenshot displays the flexControl software interface. On the left, a grid of spots is shown with columns numbered 1-12 and rows lettered A-H. A blue square highlights the spot at row B, column 2. A red circle with a plus sign is positioned above the grid, and a red '1x' label is placed above the highlighted spot. A white box with the text 'Select BTS position' is overlaid on the grid. The right side of the interface contains configuration panels for MS and MS/MS methods, including dropdown menus for 'AutoXecute Method', 'flexAnalysis Method', and 'BioTools Method'. The 'Data Destination' panel shows the 'Data Directory' set to 'C:\Users\bonke\Desktop\New folder' and the 'Sample Name' set to 'BTS\_211018529'. At the bottom, a status bar shows 'Target Geometry: MSP 96 Measuring Order: HORIZONTAL\_ZIGZAG Sample Spots Count: 0' and a legend for spot colors: Prepared (yellow), For Calibration (purple), MS Measured (green), MS/MS Measured (dark green), Flatline Spectrum (orange), Aborted (red), and MS Laser-Tuning (grey). Buttons for 'Save', 'Save As...', 'OK', and 'Cancel' are located at the bottom right.

# Generation of raw-spectra - flexControl



The screenshot shows the flexControl software interface. A red circle highlights the '+' icon in the toolbar. Three callout boxes provide instructions: '1. Change file name' points to the 'Sample Name' field containing 'Campylobacter hepaticus\_21101529\_L'; '2. Select sample position' points to the B2 well in the 96-well plate grid; '3. Select the number of measurements' points to the '4x' label above the grid. The interface includes a 96-well plate grid, method selection dropdowns (AutoXecute, flexAnalysis, BioTools), data destination settings, and a table of run parameters.

	Pos on Scout	Chip on Scout	AutoX MS	FA MS	BioTools MS	AutoX MS/MS	FA MS/MS	BioTools MS/MS	Data Directory	Sample Name	Status	Comment 1	Comment 2	Parent mass
▶	B2	0	MBT_Aut	none	none	none	none	none	C:\Users\bonk	Campylobacte	400_P			
*														

# Generation of raw-spectra - flexControl

File Edit View Help

**MS**

Calibration

Calibration

AutoXecute Method: none

flexAnalysis Method: none

AutoXecute Method: MBT\_AutoX

flexAnalysis Method: none

BioTools Method: none

**MS/MS**

AutoXecute Method: none

flexAnalysis Method: none

BioTools Method: none

Stop after MS measurement

Clean source after measurement

Data Destination

Data Directory: C:\Users\bonke\Desktop\New folder

Sample Name: Campylobacter hepaticus\_21101529\_L

Use Container Format

Note: For runs that use the container format assign the same MS and LIFT methods (autoX and flexAnalysis) to all spots.

Comments

Comment 1:

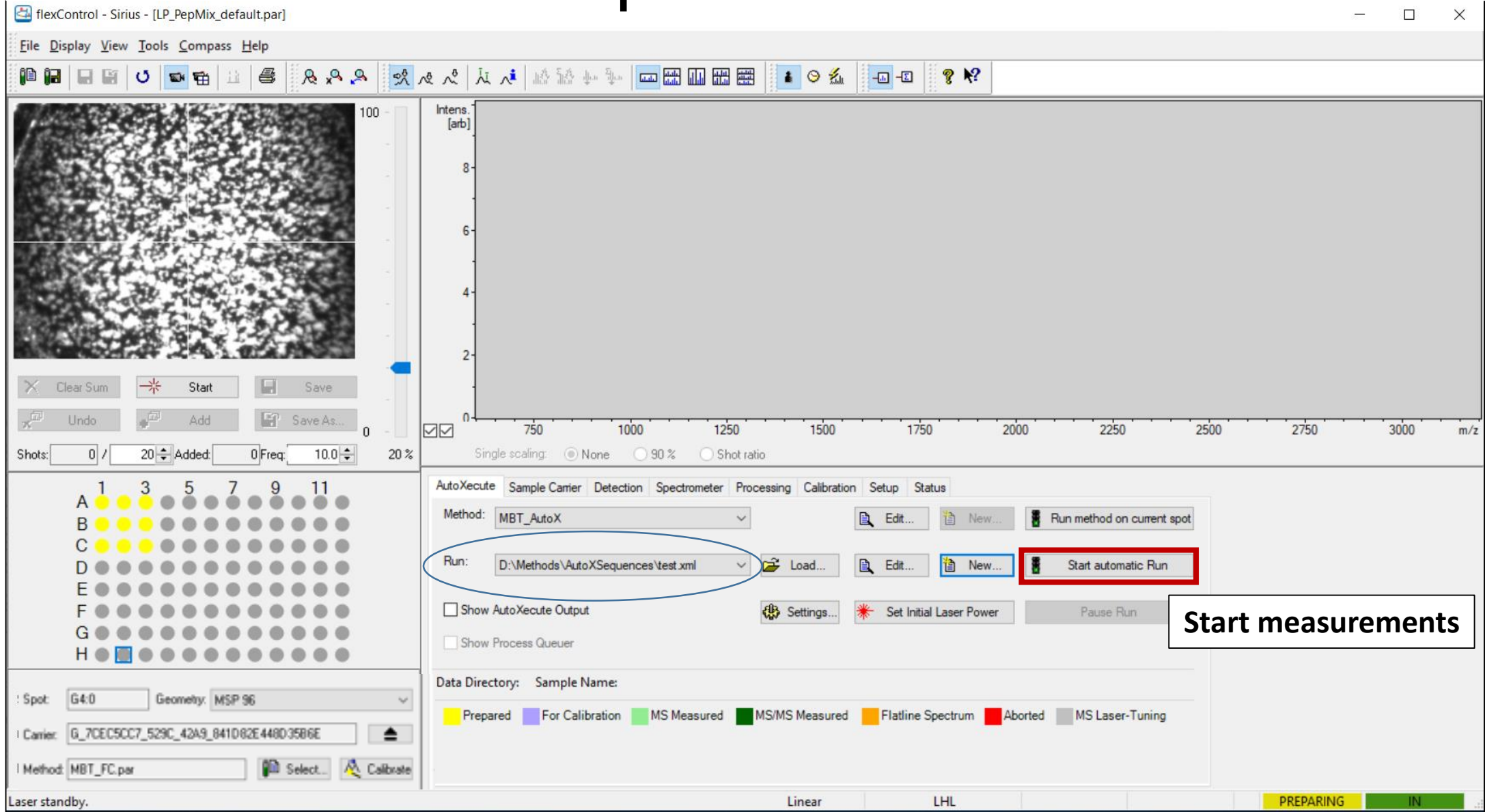
Comment 2:

	Pos on Scout	Chip on Scout	AutoX MS	FA MS	BioTools MS	AutoX MS/MS	FA MS/MS	BioTools MS/MS	Data Directory	Sample Name	Status	Comment 1	Comment 2	Parent mass
▶	C3	0	MBT_Aut	none	none	none	none	none	C:\Users\bonk	Campylobacte	400_P			
*														

Target Geometry: MSP 96 Measuring Order: HORIZONTAL\_ZIGZAG | Sample Spots Count: 33

■ Prepared 
 ■ For Calibration 
 ■ MS Measured 
 ■ MS/MS Measured 
 ■ Flatline Spectrum 
 ■ Aborted 
 ■ MS Laser-Tuning

# Generation of raw-spectra - flexControl



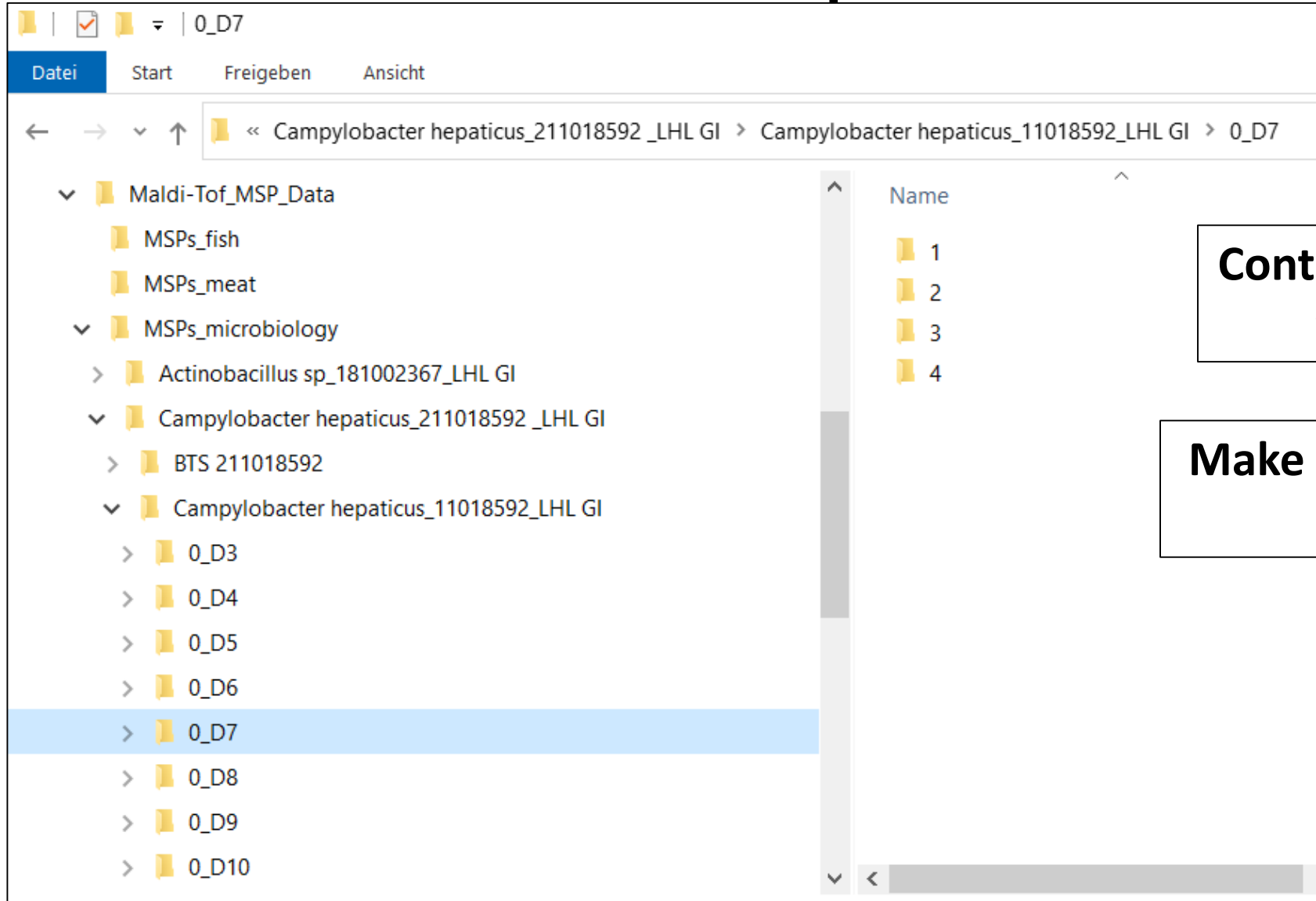
The screenshot shows the flexControl software interface for a mass spectrometer. The main window is titled "flexControl - Sirius - [LP\_PepMix\_default.par]". The interface includes a menu bar (File, Display, View, Tools, Compass, Help), a toolbar with various icons, and a large central plot area for intensity versus m/z. The plot area is currently empty, with the y-axis labeled "Intens. [arb]" ranging from 0 to 100 and the x-axis labeled "m/z" ranging from 750 to 3000. Below the plot area, there are several control panels:

- Control Panel 1:** Includes buttons for "Clear Sum", "Start", "Save", "Undo", "Add", and "Save As...". It also has input fields for "Shots: 0 / 20", "Added: 0", and "Freq: 10.0", along with a "20%" scale indicator.
- Control Panel 2:** A grid of buttons labeled A through H, with columns numbered 1, 3, 5, 7, 9, 11. Buttons A, B, and C are highlighted in yellow.
- Control Panel 3:** Includes fields for "Spot: G4:0", "Geometry: MSP 96", "Carrier: G\_70CEC5CC7\_529C\_42A9\_841D82E448D3586E", and "Method: HBT\_FC.par".
- Control Panel 4 (AutoXecute):** Contains tabs for "Sample Carrier", "Detection", "Spectrometer", "Processing", "Calibration", "Setup", and "Status". It features a "Method" dropdown set to "MBT\_AutoX", a "Run" dropdown set to "D:\Methods\AutoXSequences\test.xml", and a "Start automatic Run" button highlighted with a red box. Other buttons include "Edit...", "New...", "Load...", "Settings...", "Set Initial Laser Power", and "Pause Run".
- Control Panel 5 (Data Directory):** Includes a "Sample Name" field and a legend for measurement states: Prepared (yellow), For Calibration (purple), MS Measured (green), MS/MS Measured (dark green), Flatline Spectrum (orange), Aborted (red), and MS Laser-Tuning (grey).

At the bottom of the interface, there is a status bar with the text "Laser standby." and a progress indicator showing "PREPARING" and "IN".

**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

## 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	<input type="checkbox"/>	Sample Preparation ("Extraction Method")	<input type="checkbox"/> DT <input type="checkbox"/> eDT <input type="checkbox"/> EtOH-FA <input type="checkbox"/> .....
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		<input type="checkbox"/> 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	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Method → Open ...	MBT_Standard.FAMSMETHOD	Baseline Subtraction	Smooth (1x)

### BTS check/ recalibration:

Check Mass Control List Calibrate → Internal ...	<input type="checkbox"/> Automatic-Assign	<input type="checkbox"/> Peaks manually assigned
	Max. deviation (ppm):	
Recalibrate sample spectra	<input type="checkbox"/> Copy calibration	
Mass calibration constants BTS Select BTS spectrum → Properties ...	C0:	
	C1:	
	C2:	
Mass calibration constants sample spectra	<input type="checkbox"/> 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:

Conspicuous spectra (position/measurement): (Flat lines etc.)		<input type="checkbox"/> 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.: Streptobadillus 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:
<input type="checkbox"/> DT
<input type="checkbox"/> eDT
<input type="checkbox"/> EtOH-FA
<input type="checkbox"/> .....

Entry created ...:  own MSP-Library updated

Comment:	
----------	--

Date / acronym \_\_\_\_\_

# Raw spectra processing – flexAnalysis

**Spectrum Browser**

Root: U:\BONKE\AAA\_Tierarten\TGSH\_Reisek

**Browse...**

Filter Spectra

- MS
- LIFT
- LIFT (conv.)
- FAST
- FAST Seg.

From: 2020-05-27 0

To: 2020-05-27 0

Spectrum Properties

Selected spectra: 0  
Spectra: 4 / 4

**Open the raw spectra for processing**

**Spectrum Browser**

Root: C:\Users\bonke\Desktop\Demo\_140223\

**Browse...**

Filter Spectra

- MS
- LIFT
- LIFT (conv.)
- FAST
- FAST Seg.

From: 2023-02-10 21:05

To: 2023-02-10 21:05

**Apply**

Spectrum Properties

Selected spectra: 1  
Spectra: 33 / 33

**Open**

**Cancel**

**Select the BTS file and open.**

Select All Clear Selection  Load all selected spectra



# Raw spectra processing – flexAnalysis

flexAnalysis - unknown

File Edit Mass List Process Calibrate Annotation **Method** FAST View Report Tools Window Compass Help

Open...  
Save  
Save As...  
Run Script F2  
Edit Parameters... Alt+F2  
Edit Processing Parameters... Shift+F2  
Edit Script... Ctrl+F2  
Select Default...

Analysis List  
bts\_0\_C4\_1

Open flexAnalysis Method

Dieser PC > Lokaler Datenträger (C:) > Methods > flexAnalysisMethods

Name	Änderungsdatum	Typ	Größe
Calibrate_BSA_reISD_mono.FAMSMETHOD	07.07.2009 08:41	FAMSMETHOD-D...	35 KB
CalibratePeptideStandards.FAMSMETHOD	17.04.2009 14:57	FAMSMETHOD-D...	35 KB
CalibratePeptideStandards_SNAP2.FAMSMETHOD	18.11.2013 15:35	FAMSMETHOD-D...	45 KB
CalibratePeptideStatistic.FAMSMETHOD	17.04.2009 14:57	FAMSMETHOD-D...	35 KB
ExternalCalibration.FAMSMETHOD	17.04.2009 14:57	FAMSMETHOD-D...	34 KB
ExternalCalibration_SNAP2.FAMSMETHOD	18.11.2013 15:35	FAMSMETHOD-D...	44 KB
ExternalCalibrationPS.FAMSMETHOD	29.09.2011 15:17	FAMSMETHOD-D...	34 KB
MBT_Standard.FAMSMETHOD	18.05.2009 12:44	FAMSMETHOD-D...	28 KB
PAC_CalibratePeptideStandards.FAMSMETHOD	20.09.2010 15:29	FAMSMETHOD-D...	33 KB
PAC_ExternalCalibration.FAMSMETHOD	20.09.2010 15:29	FAMSMETHOD-D...	32 KB
PACII_CalibratePeptideStandards.FAMSMETHOD	20.09.2010 15:29	FAMSMETHOD-D...	34 KB
PACII_ExternalCalibration.FAMSMETHOD	20.09.2010 15:29	FAMSMETHOD-D...	33 KB
ParametersOnly.FAMSMETHOD	24.07.2009 07:08	FAMSMETHOD-D...	33 KB
ParametersOnly_linear.FAMSMETHOD	21.03.2011 11:04	FAMSMETHOD-D...	41 KB

Dateiname: MBT\_Standard.FAMSMETHOD MS Method (\*.FAMSMETHOD)

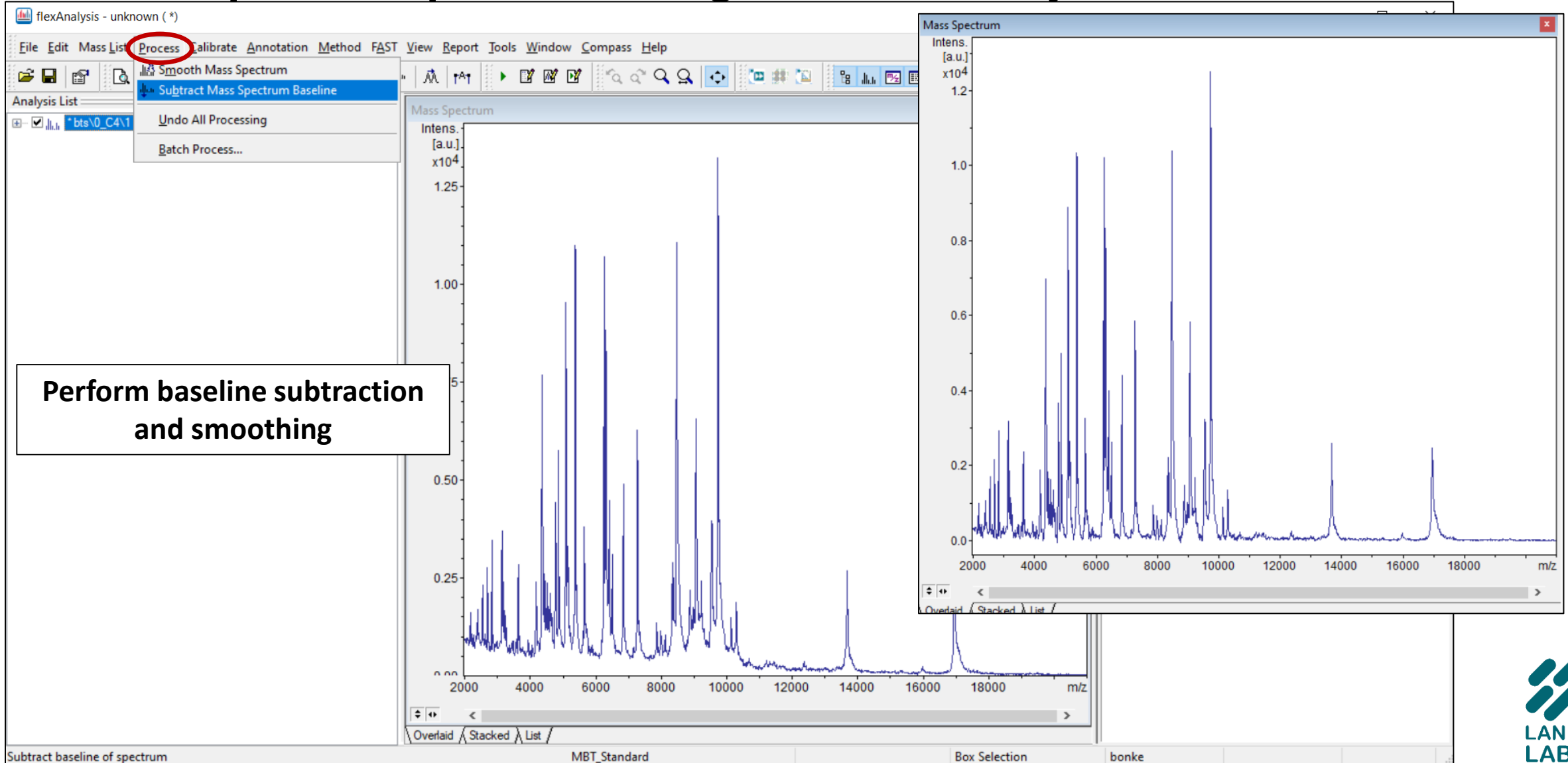
Öffnen Abbrechen

Select the BTS spectrum and open the method.

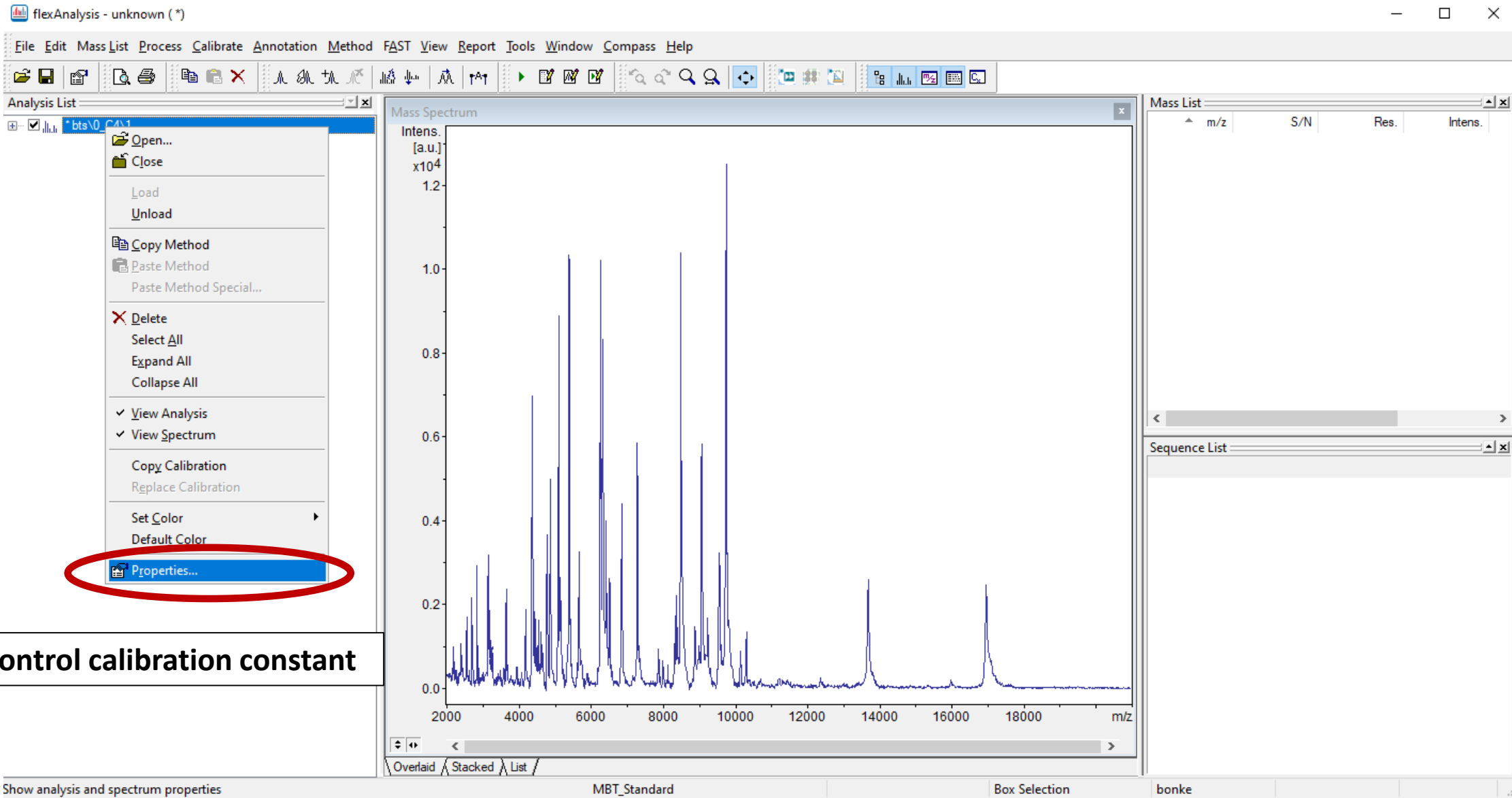
Mass Spectrum Plot: Y-axis (0.00 to 1.00), X-axis (2000 to 18000 m/z)

Open and attach a method      bts\_0\_C4\_1      Box Selection      bonke

# Raw spectra processing – flexAnalysis



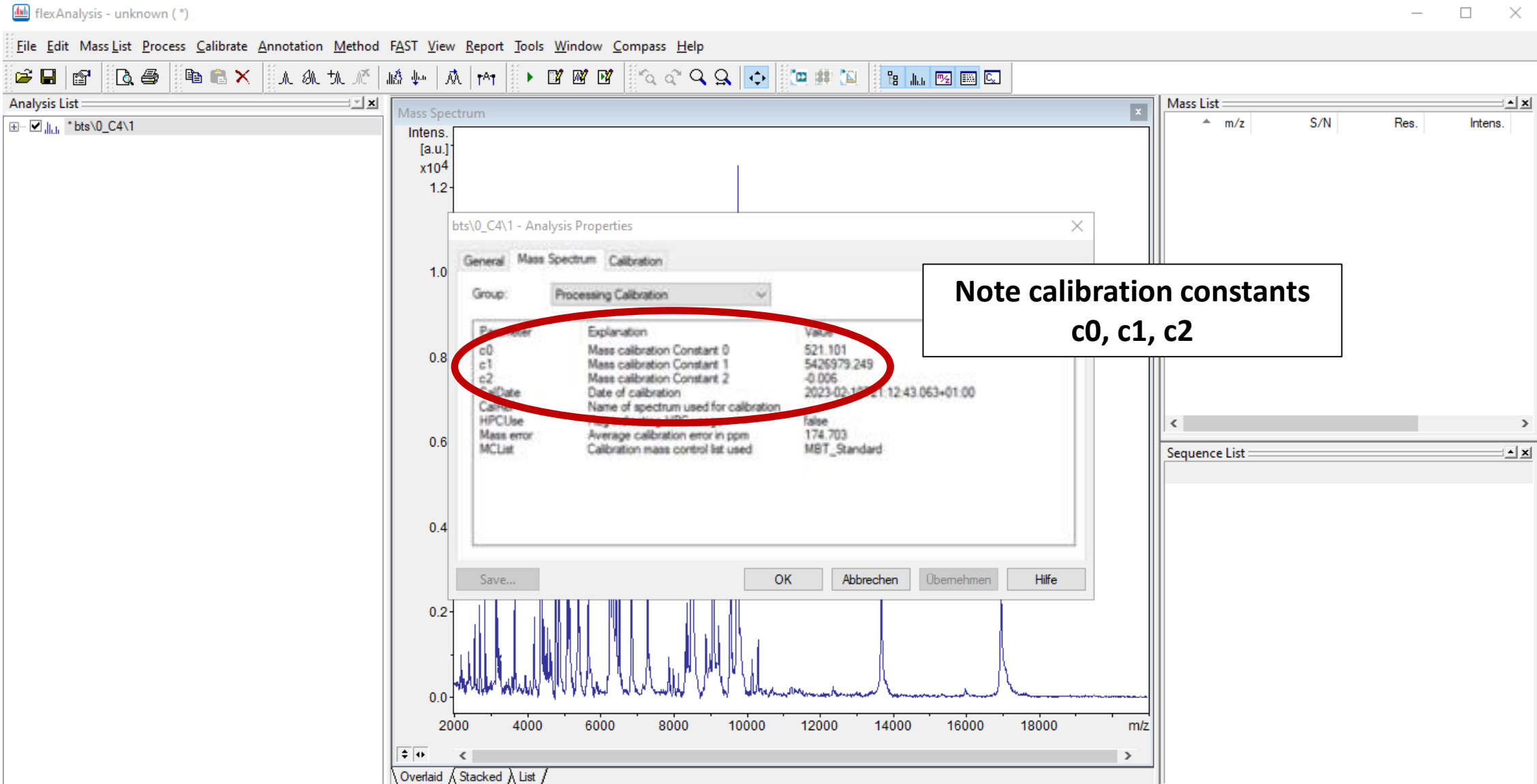
# Raw spectra processing – flexAnalysis



The screenshot shows the flexAnalysis software interface. The main window displays a mass spectrum plot with intensity (Intens. [a.u.] x10<sup>4</sup>) on the y-axis and m/z on the x-axis. The x-axis ranges from 2000 to 18000 m/z. The y-axis ranges from 0.0 to 1.2. The plot shows a complex pattern of peaks, with a prominent peak at approximately 10000 m/z. A context menu is open over the 'Analysis List' panel, with the 'Properties...' option highlighted by a red circle. The menu includes options such as 'Open...', 'Close', 'Load', 'Unload', 'Copy Method', 'Paste Method', 'Delete', 'View Analysis', 'View Spectrum', 'Copy Calibration', 'Set Color', and 'Properties...'. The 'Mass List' panel on the right shows a table with columns for m/z, S/N, Res., and Intens. The 'Sequence List' panel is also visible below the Mass List panel.

Control calibration constant

# Raw spectra processing – flexAnalysis



The screenshot shows the flexAnalysis software interface. The main window displays a mass spectrum plot with intensity on the y-axis (ranging from 0.0 to 1.2 x 10<sup>4</sup> a.u.) and m/z on the x-axis (ranging from 2000 to 18000). A dialog box titled "bts\0\_C4\1 - Analysis Properties" is open, showing the "Calibration" tab. The "Group" is set to "Processing Calibration". A red circle highlights the following parameters:

Parameter	Explanation	Value
c0	Mass calibration Constant 0	521.101
c1	Mass calibration Constant 1	5426979.249
c2	Mass calibration Constant 2	-0.006

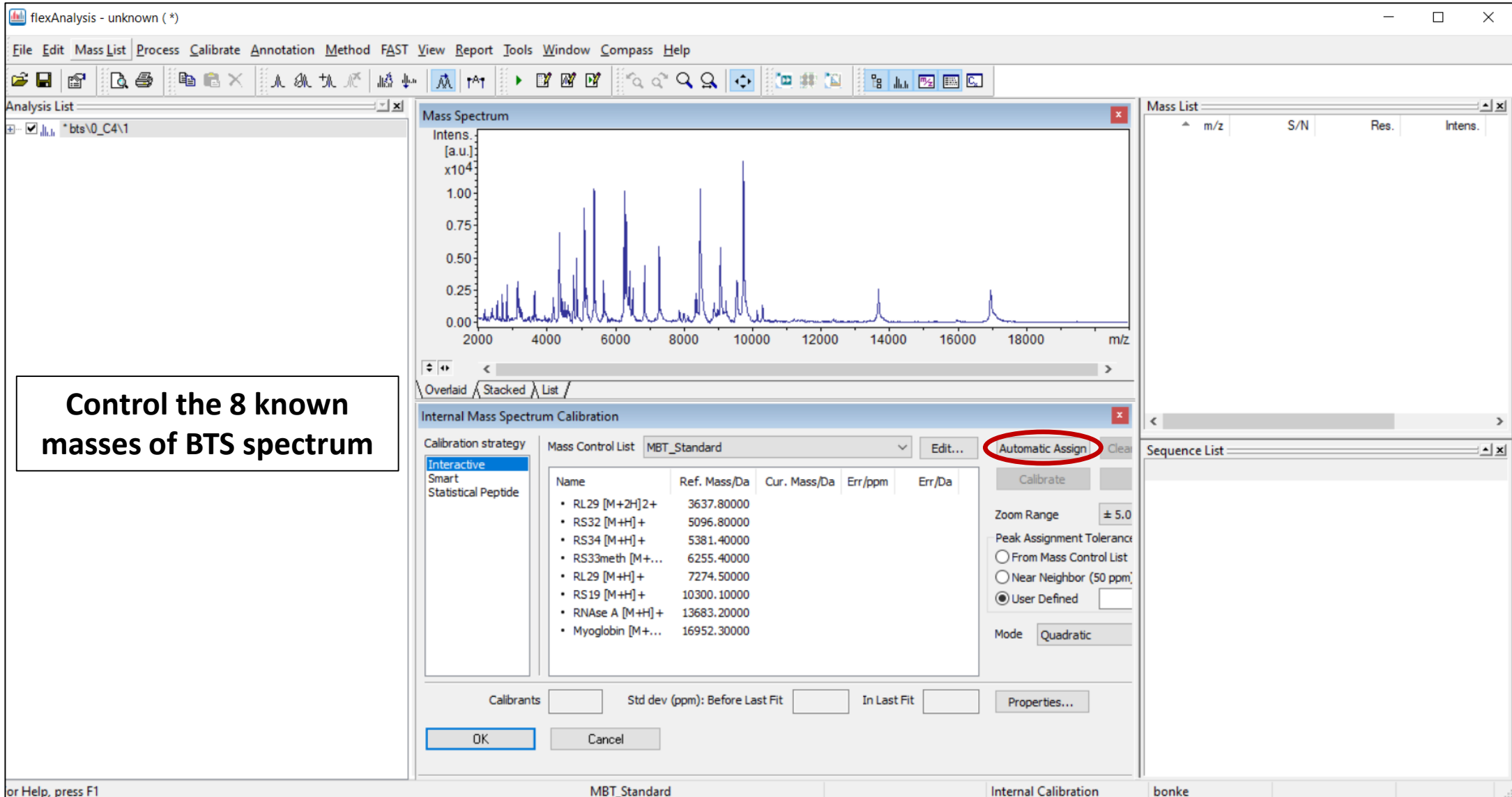
A callout box points to these constants with the text: "Note calibration constants c0, c1, c2". Other parameters in the dialog include CalDate (2023-02-10 21:12:43.063+01:00), Name of spectrum used for calibration (MRT\_Standard), HPCUse (false), Mass error (174.703), and MCList (MRT\_Standard).

# Raw spectra processing – flexAnalysis

The screenshot shows the flexAnalysis software interface. The main window displays a mass spectrum plot with intensity on the y-axis (ranging from 0.0 to 1.2 x 10<sup>4</sup> a.u.) and m/z on the x-axis (ranging from 2000 to 18000). The plot shows a complex pattern of peaks, with a prominent peak at approximately m/z 10000. The 'Calibrate' menu is open, showing options: 'Internal...', 'Copy Calibration', 'Replace Calibration', and 'Apply Raw Calibration'. The 'Internal...' option is circled in red. The 'Analysis List' on the left shows a file named 'bts\0\_C4\1'. The 'Mass List' on the right is empty. The 'Sequence List' at the bottom right is also empty. The status bar at the bottom indicates 'Calibrate mass spectrum internally', 'MBT\_Standard', 'Box Selection', and 'bonke'.

**Control the 8 known masses of BTS spectrum**

# Raw spectra processing – flexAnalysis



**Control the 8 known masses of BTS spectrum**

**Internal Mass Spectrum Calibration**

Calibration strategy: **Interactive**

Mass Control List: **MBT\_Standard**

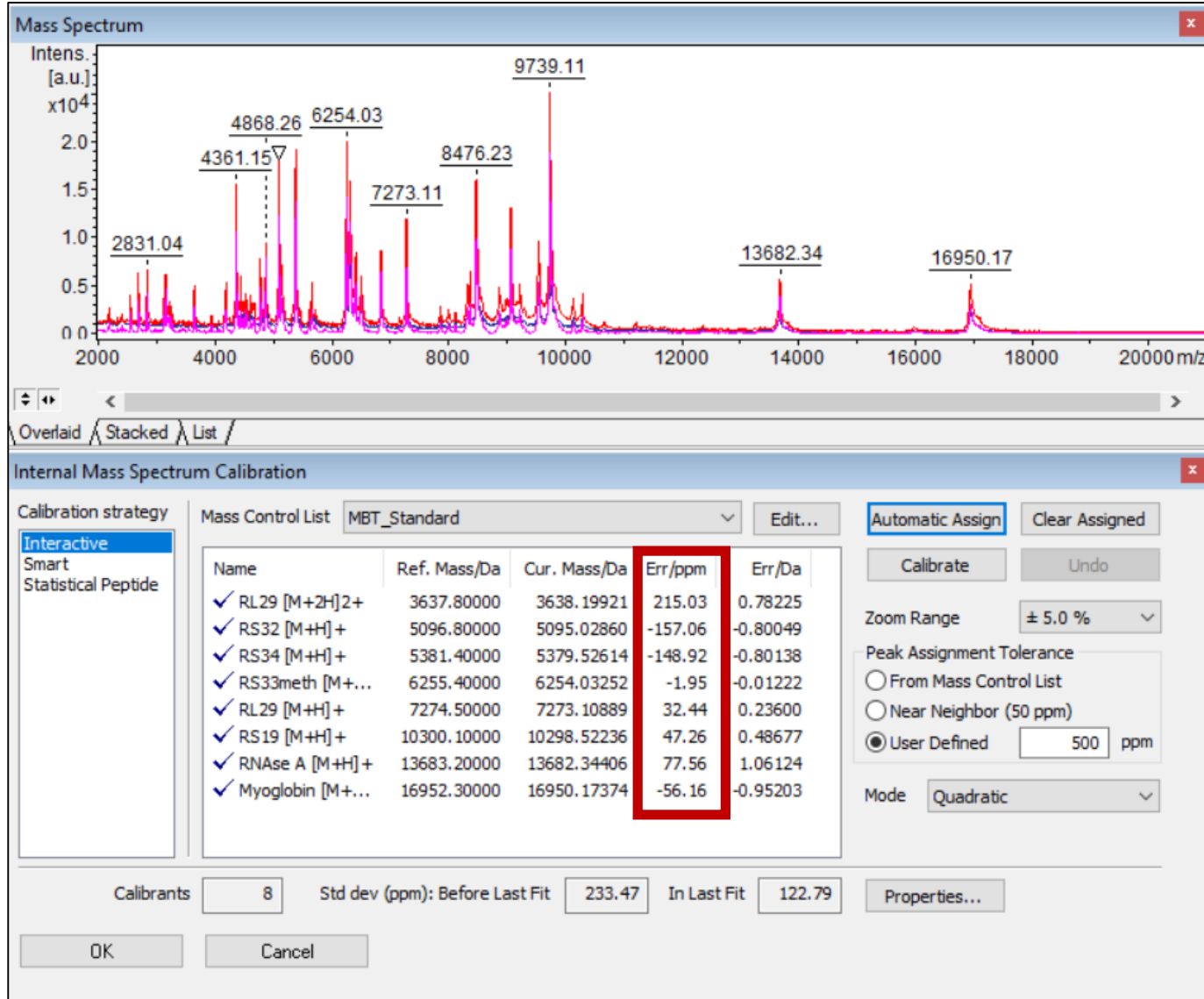
Name	Ref. Mass/Da	Cur. Mass/Da	Err/ppm	Err/Da
• RL29 [M+2H] <sup>2+</sup>	3637.80000			
• RS32 [M+H] <sup>+</sup>	5096.80000			
• RS34 [M+H] <sup>+</sup>	5381.40000			
• RS33meth [M+...]	6255.40000			
• RL29 [M+H] <sup>+</sup>	7274.50000			
• RS19 [M+H] <sup>+</sup>	10300.10000			
• RNase A [M+H] <sup>+</sup>	13683.20000			
• Myoglobin [M+...]	16952.30000			

Buttons: **Automatic Assign** (circled), Calibrate, Clear, Zoom Range: ± 5.0, Peak Assignment Tolerance:  From Mass Control List,  Near Neighbor (50 ppm),  User Defined, Mode: Quadratic

Calibrants:  Std dev (ppm): Before Last Fit  In Last Fit  Properties...

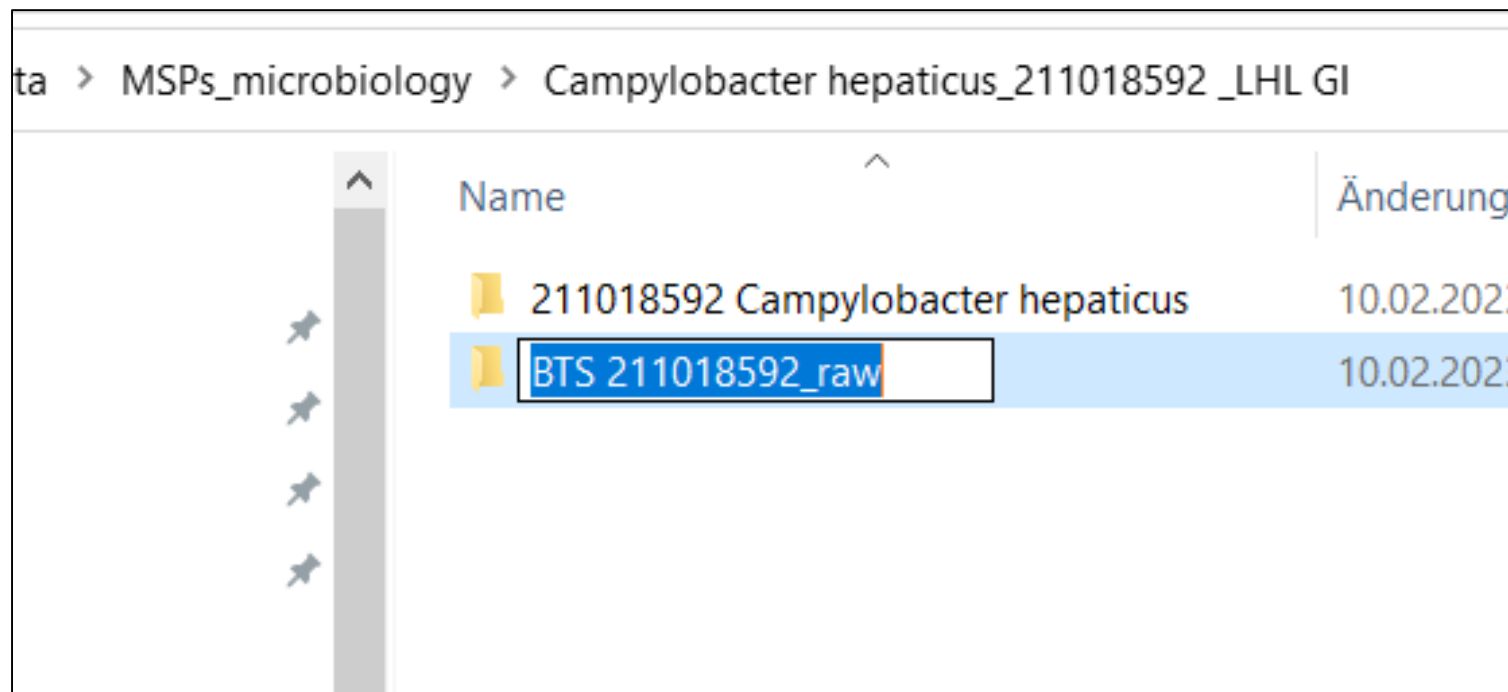
Buttons: OK, Cancel

# Raw spectra processing – flexAnalysis



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

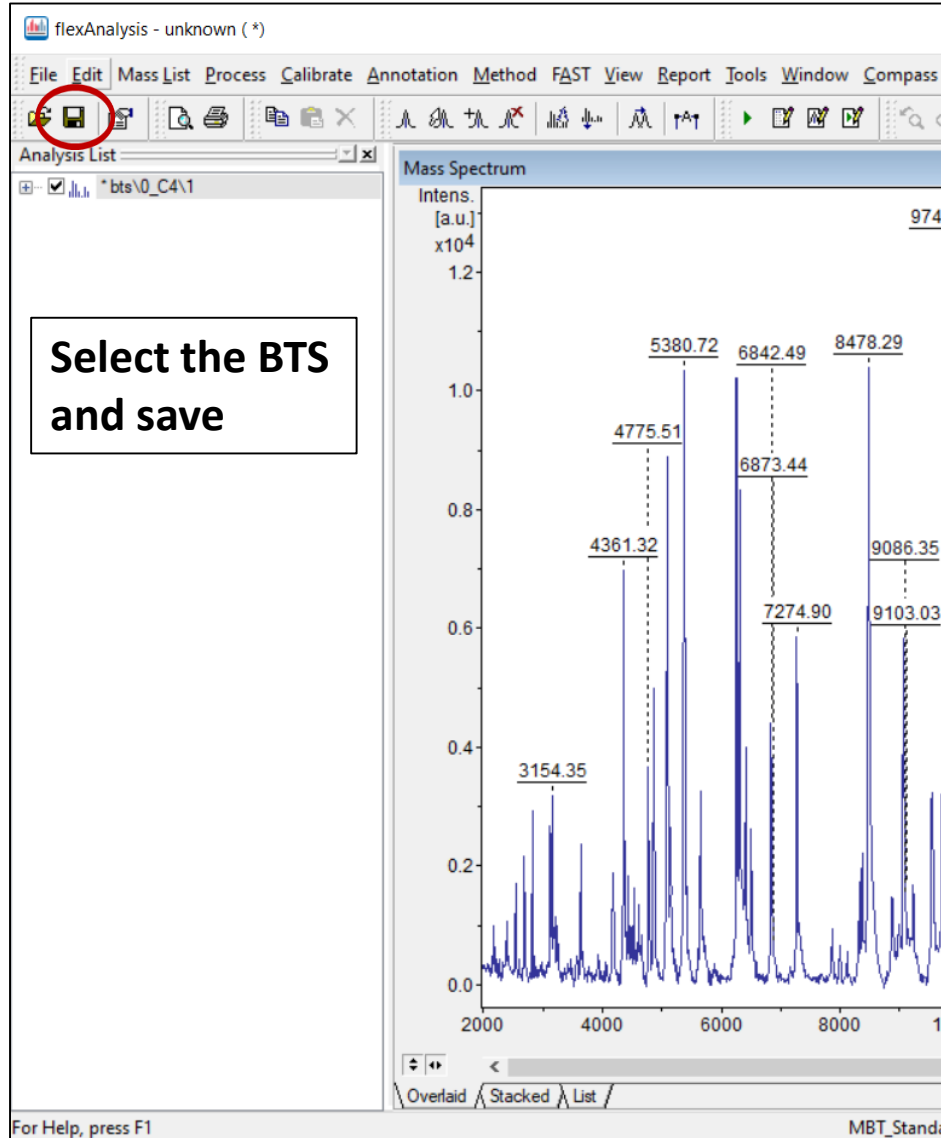
# Raw spectra processing – flexAnalysis



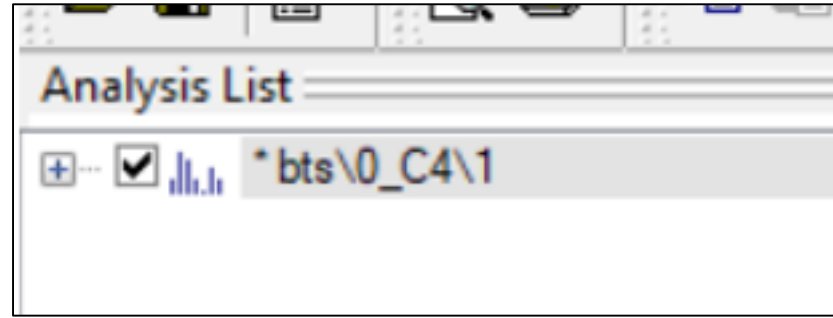
**Change the original  
BTS spectrum file  
name before saving!  
(e.g. add raw, ed, ...)**



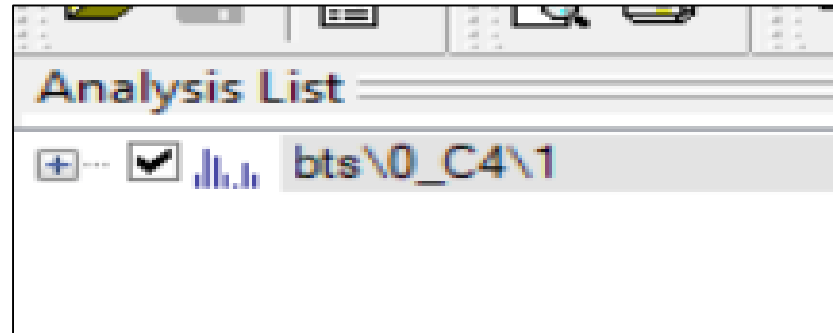
# Raw spectra processing – flexAnalysis



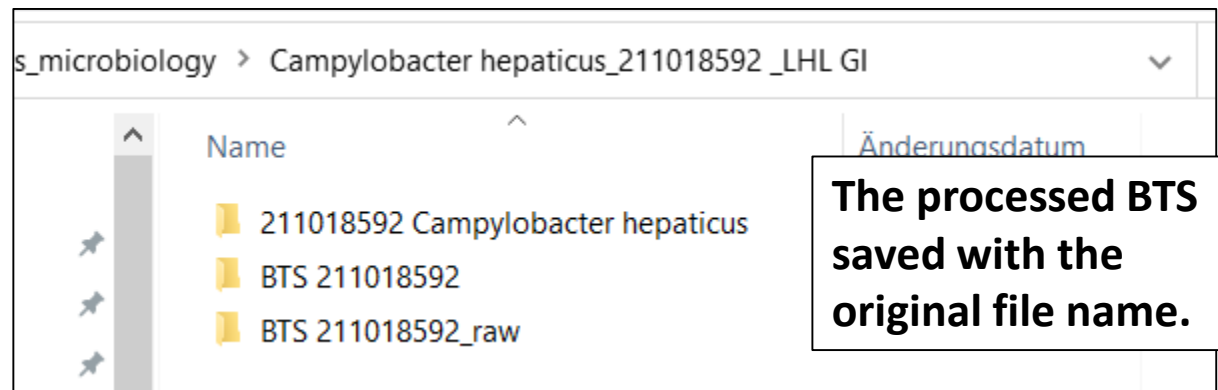
Select the BTS and save



BTS before saving

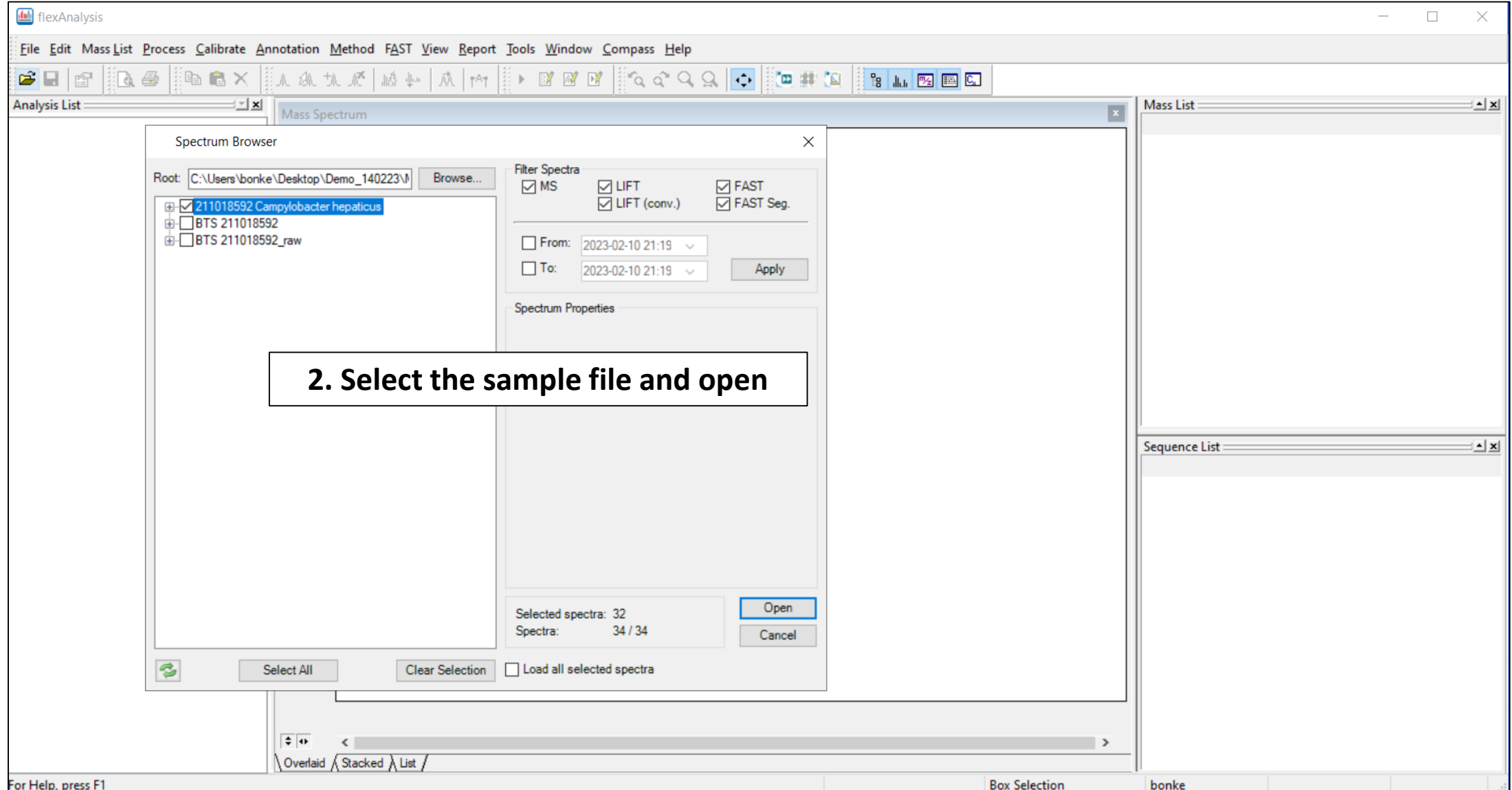


BTS after saving



The processed BTS saved with the original file name.

# Raw spectra processing – flexAnalysis



The screenshot shows the flexAnalysis software interface. The main window has a menu bar (File, Edit, Mass List, Process, Calibrate, Annotation, Method, FAST, View, Report, Tools, Window, Compass, Help) and a toolbar. The 'Spectrum Browser' dialog box is open, displaying a file tree with the following items:

- Root: C:\Users\bonke\Desktop\Demo\_140223\
- 211018592\_Campylobacter hepaticus (selected)
- BTS 211018592
- BTS 211018592\_raw

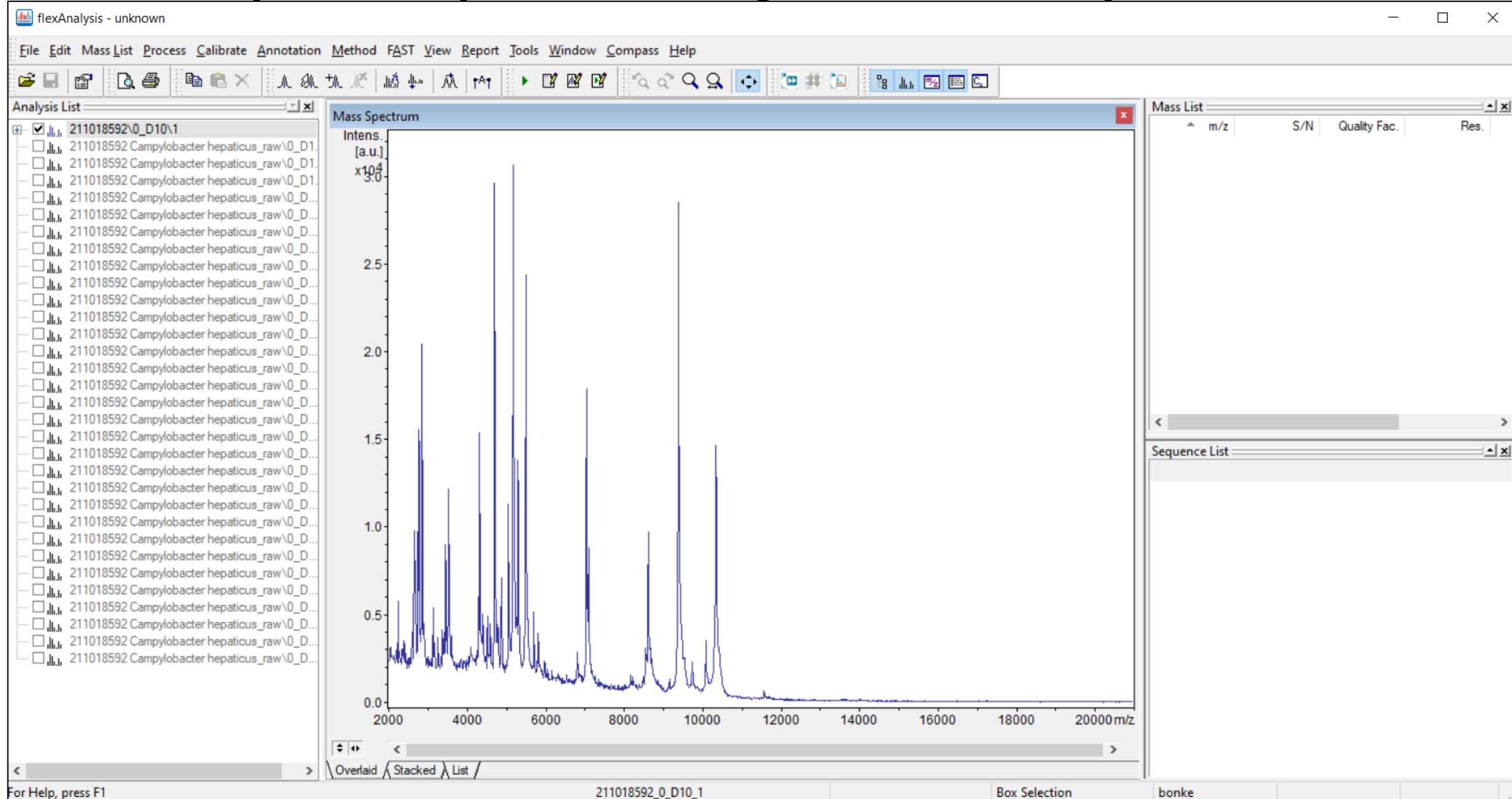
The 'Filter Spectra' section includes the following options:

- MS
- LIFT
- LIFT (conv.)
- FAST
- FAST Seg.

The 'From' and 'To' date pickers are both set to 2023-02-10 21:19. The 'Spectrum Properties' section is currently empty. At the bottom of the dialog, it shows 'Selected spectra: 32' and 'Spectra: 34 / 34'. There are 'Open' and 'Cancel' buttons. Below the dialog, there are 'Select All', 'Clear Selection', and 'Load all selected spectra' options.

**2. Select the sample file and open**

# Raw spectra processing – flexAnalysis



# Raw spectra processing – flexAnalysis

flexAnalysis

File Edit Mass List Process Calibrate Annotation **Method** FAST View Report Tools Window Comp

Open...  
Save  
Save As...  
Run Script F2  
Edit Parameters... Alt+F2  
Edit Processing Parameters... Shift+F2  
Edit Script... Ctrl+F2  
Select Default...

Analysis List

- 211018592\0\_D10\1
- 211018592 Campylobacter hepaticus\0\_D10\2
- 211018592 Campylobacter hepaticus\0\_D10\3
- 211018592 Campylobacter hepaticus\0\_D10\4
- 211018592 Campylobacter hepaticus\0\_D3\1
- 211018592 Campylobacter hepaticus\0\_D3\2
- 211018592 Campylobacter hepaticus\0\_D3\3
- 211018592 Campylobacter hepaticus\0\_D3\4
- 211018592 Campylobacter hepaticus\0\_D4\1
- 211018592 Campylobacter hepaticus\0\_D4\2
- 211018592 Campylobacter hepaticus\0\_D4\3
- 211018592 Campylobacter hepaticus\0\_D4\4
- 211018592 Campylobacter hepaticus\0\_D5\1
- 211018592 Campylobacter hepaticus\0\_D5\2
- 211018592 Campylobacter hepaticus\0\_D5\3
- 211018592 Campylobacter hepaticus\0\_D5\4
- 211018592 Campylobacter hepaticus\0\_D6\1
- 211018592 Campylobacter hepaticus\0\_D6\2
- 211018592 Campylobacter hepaticus\0\_D6\3
- 211018592 Campylobacter hepaticus\0\_D6\4
- 211018592 Campylobacter hepaticus\0\_D7\1
- 211018592 Campylobacter hepaticus\0\_D7\2
- 211018592 Campylobacter hepaticus\0\_D7\3
- 211018592 Campylobacter hepaticus\0\_D7\4
- 211018592 Campylobacter hepaticus\0\_D8\1
- 211018592 Campylobacter hepaticus\0\_D8\2
- 211018592 Campylobacter hepaticus\0\_D8\3
- 211018592 Campylobacter hepaticus\0\_D8\4
- 211018592 Campylobacter hepaticus\0\_D9\1
- 211018592 Campylobacter hepaticus\0\_D9\2
- 211018592 Campylobacter hepaticus\0\_D9\3
- 211018592 Campylobacter hepaticus\0\_D9\4

2.0  
1.5  
1.0  
0.5  
0.0

2000 4000 6000 8000

Overlaid Stacked List

Open and attach a method

Open flexAnalysis Method

Dieser PC > Lokaler Datenträger (C:) > Methods > flexAnalysisMethods

Organisieren Neuer Ordner

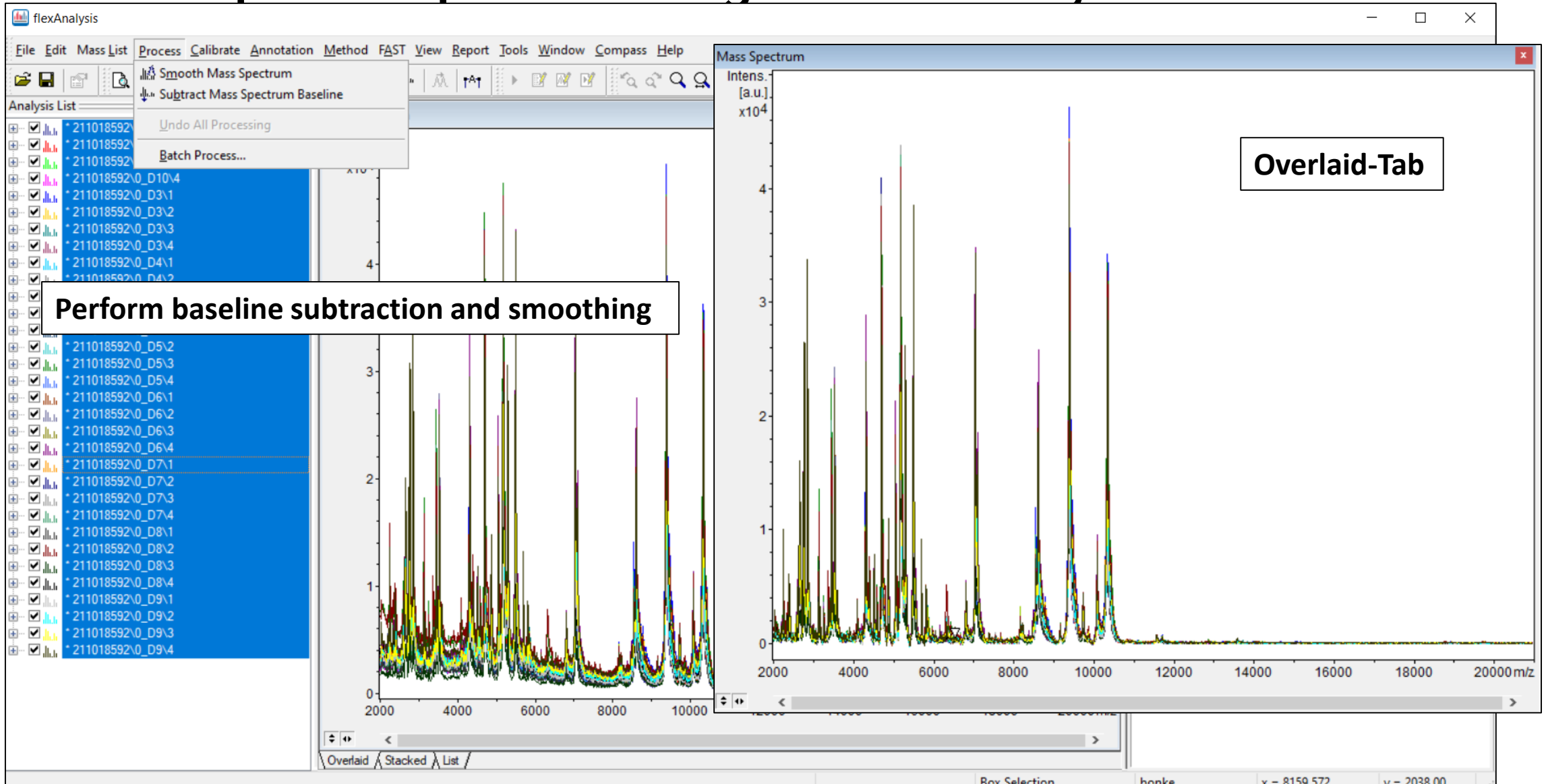
Name	Änderungsdatum	Typ	Größe
Calibrate_BSA_relSD_mono.FAMSMethod	07.07.2009 08:41	FAMSMETHOD-D...	35 KB
CalibratePeptideStandards.FAMSMethod	17.04.2009 14:57	FAMSMETHOD-D...	35 KB
CalibratePeptideStandards_SNAP2.FAMS...	18.11.2013 15:35	FAMSMETHOD-D...	45 KB
CalibratePeptideStatistic.FAMSMethod	17.04.2009 14:57	FAMSMETHOD-D...	35 KB
ExternalCalibration.FAMSMethod	17.04.2009 14:57	FAMSMETHOD-D...	34 KB
ExternalCalibration_SNAP2.FAMSMethod	18.11.2013 15:35	FAMSMETHOD-D...	44 KB
ExternalCalibrationPS.FAMSMethod	29.09.2011 15:17	FAMSMETHOD-D...	34 KB
MBT_External.FAMSMethod	07.07.2009 08:41	FAMSMETHOD-D...	32 KB
<b>MBT_Standard.FAMSMethod</b>	18.05.2009 12:44	FAMSMETHOD-D...	28 KB
PAC_CalibratePeptideStandards.FAMSMe...	20.09.2010 15:29	FAMSMETHOD-D...	33 KB
PAC_ExternalCalibration.FAMSMethod	20.09.2010 15:29	FAMSMETHOD-D...	32 KB
PACII_CalibratePeptideStandards.FASMe...	20.09.2010 15:29	FAMSMETHOD-D...	34 KB
PACII_ExternalCalibration.FAMSMethod	20.09.2010 15:29	FAMSMETHOD-D...	33 KB
ParametersOnly.FAMSMethod	24.07.2009 07:08	FAMSMETHOD-D...	33 KB
ParametersOnly_linear.FAMSMethod	21.03.2011 11:04	FAMSMETHOD-D...	41 KB

Dateiname: MBT\_Standard.FAMSMethod MS Method (\*.FAMSMethod)

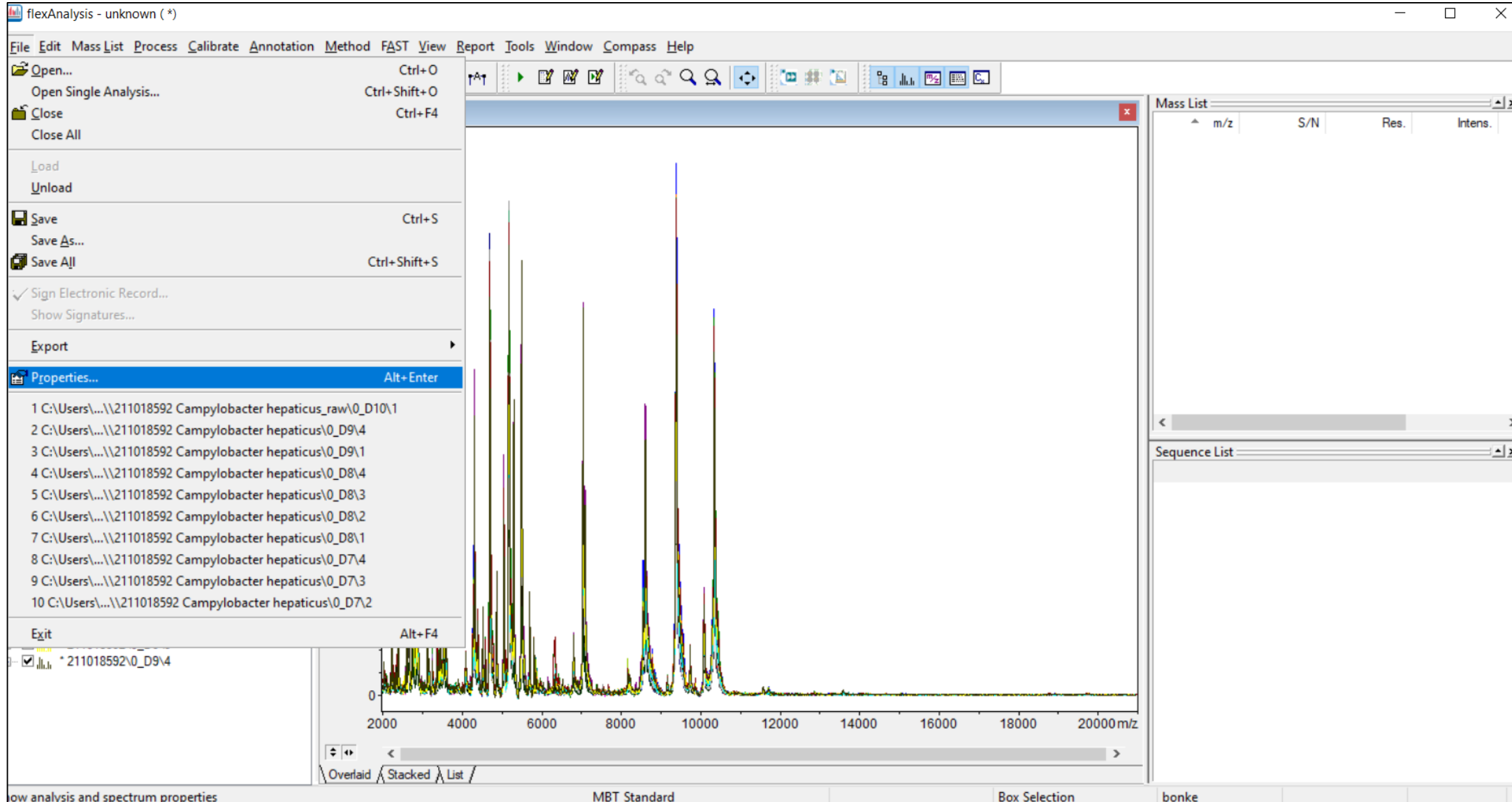
Öffnen Abbrechen

Select all spectra and open the method.

# Raw spectra processing – flexAnalysis

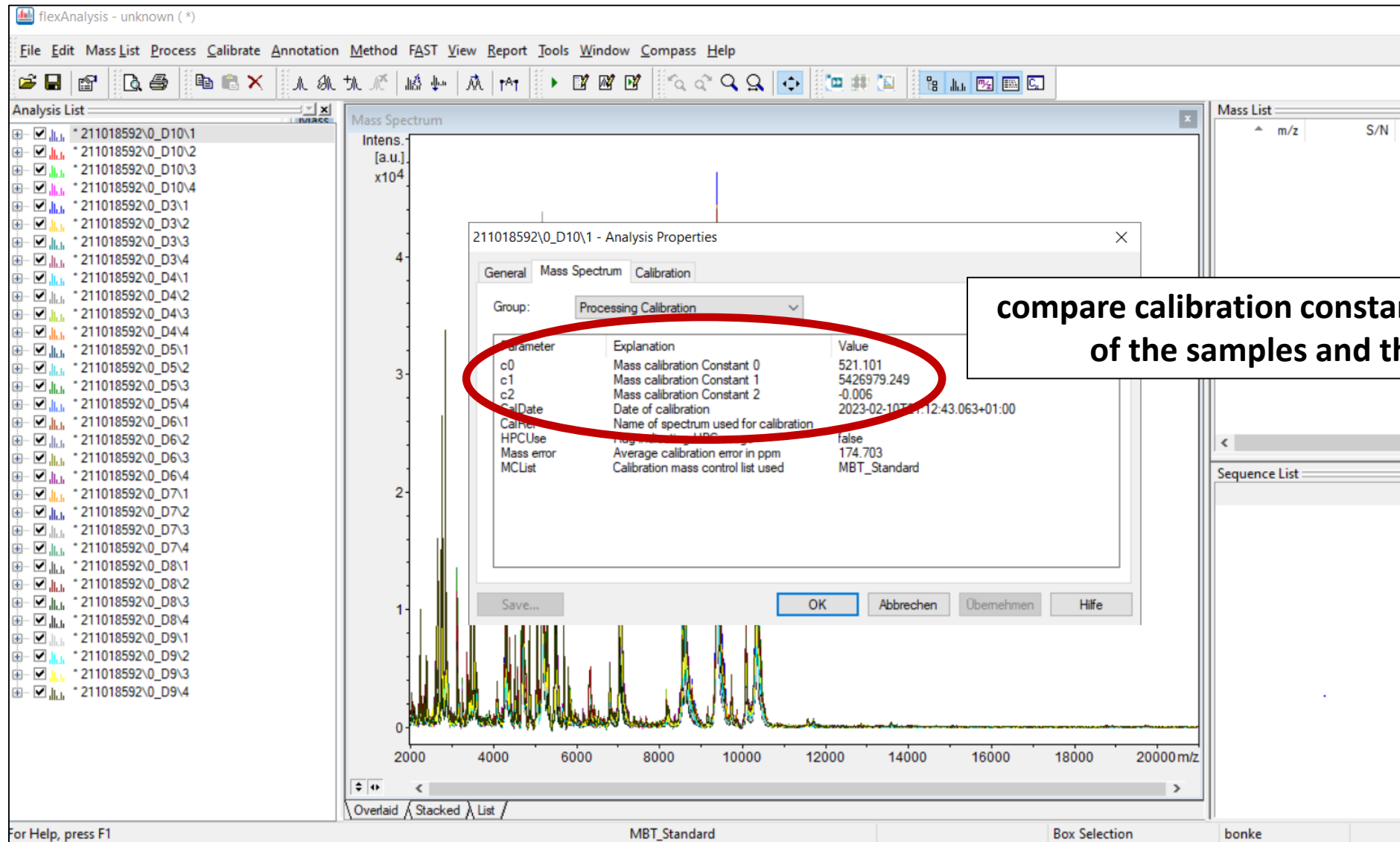


# Raw spectra processing – flexAnalysis



The screenshot displays the flexAnalysis software interface. The main window shows a mass spectrum plot with the x-axis labeled 'm/z' ranging from 2000 to 20000. The plot contains multiple overlapping spectra in various colors. A menu is open on the left side, listing various file operations such as 'Open...', 'Close', 'Save', and 'Export'. The 'Properties...' option is selected, showing a list of file paths for 'Campylobacter hepaticus' raw data files. The 'Mass List' panel on the right is empty, and the 'Sequence List' panel below it is also empty. The status bar at the bottom indicates 'low analysis and spectrum properties', 'MBT Standard', 'Box Selection', and 'bonke'.

# Raw spectra processing – flexAnalysis



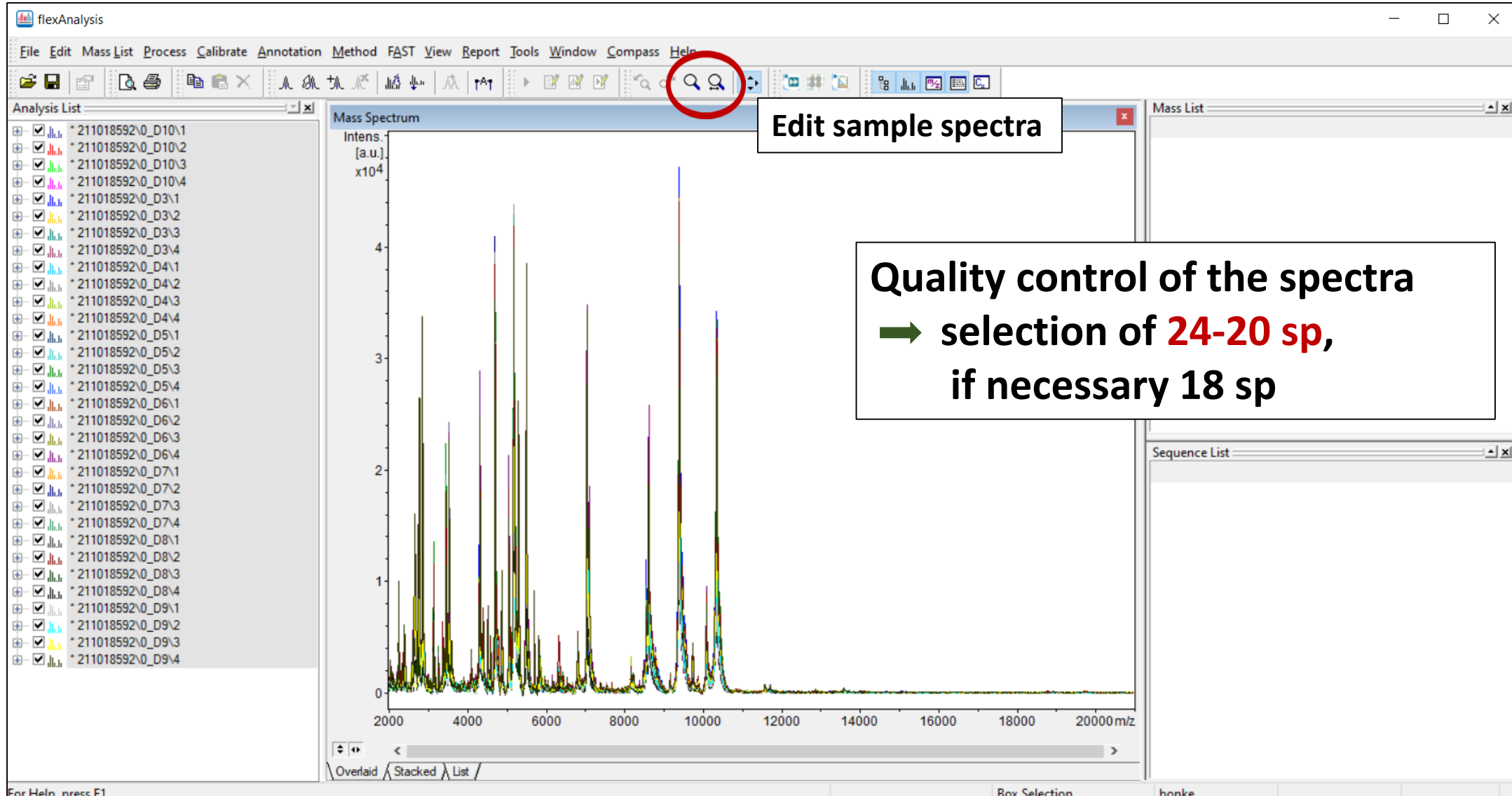
The screenshot displays the flexAnalysis software interface. On the left, an 'Analysis List' contains numerous sample files. The main window shows a 'Mass Spectrum' plot with intensity in arbitrary units (a.u.) on the y-axis (scaled by 10<sup>4</sup>) and m/z on the x-axis (ranging from 2000 to 20000). A dialog box titled '211018592\0\_D10\1 - Analysis Properties' is open, showing calibration parameters. A red circle highlights the 'Mass Spectrum' tab and the 'c0', 'c1', and 'c2' parameters in the table below.

Parameter	Explanation	Value
c0	Mass calibration Constant 0	521.101
c1	Mass calibration Constant 1	5426979.249
c2	Mass calibration Constant 2	-0.006
CalDate	Date of calibration	2023-02-10T11:12:43.063+01:00
CalFile	Name of spectrum used for calibration	
HPCUse	High Performance Computing	false
Mass error	Average calibration error in ppm	174.703
MCList	Calibration mass control list used	MBT_Standard

Buttons at the bottom of the dialog include 'Save...', 'OK', 'Abbrechen', 'Übernehmen', and 'Hilfe'.

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

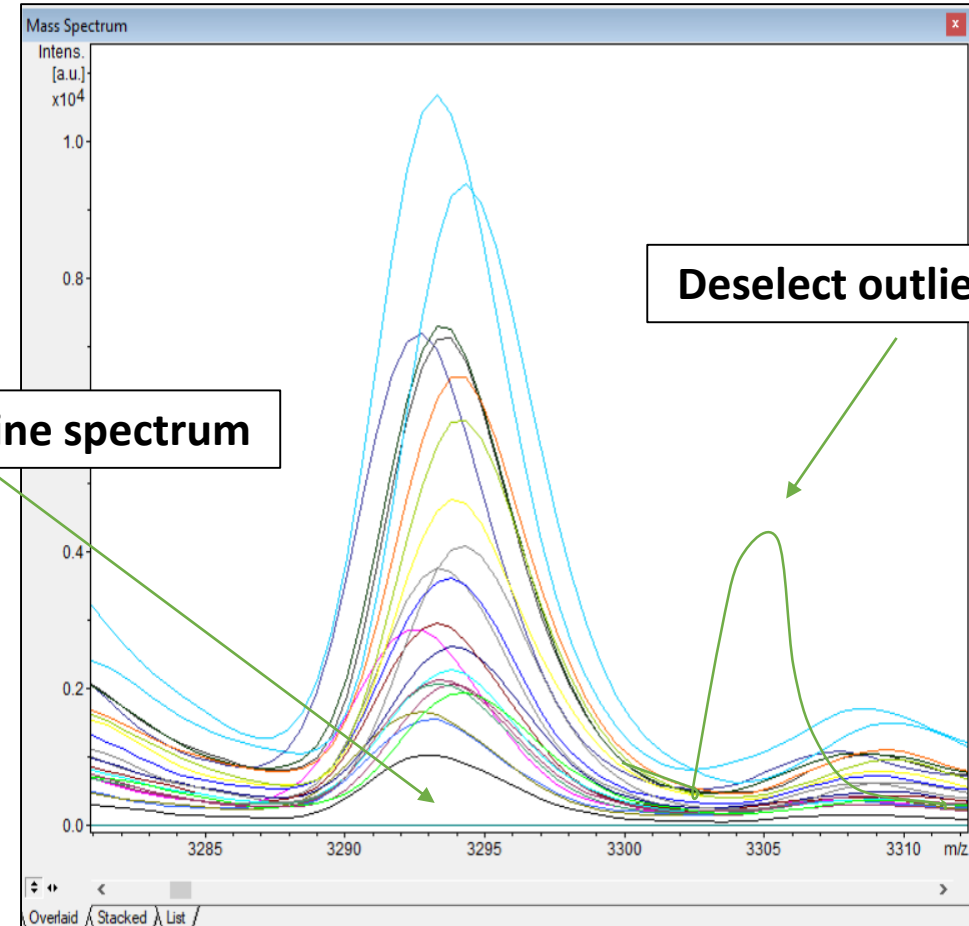
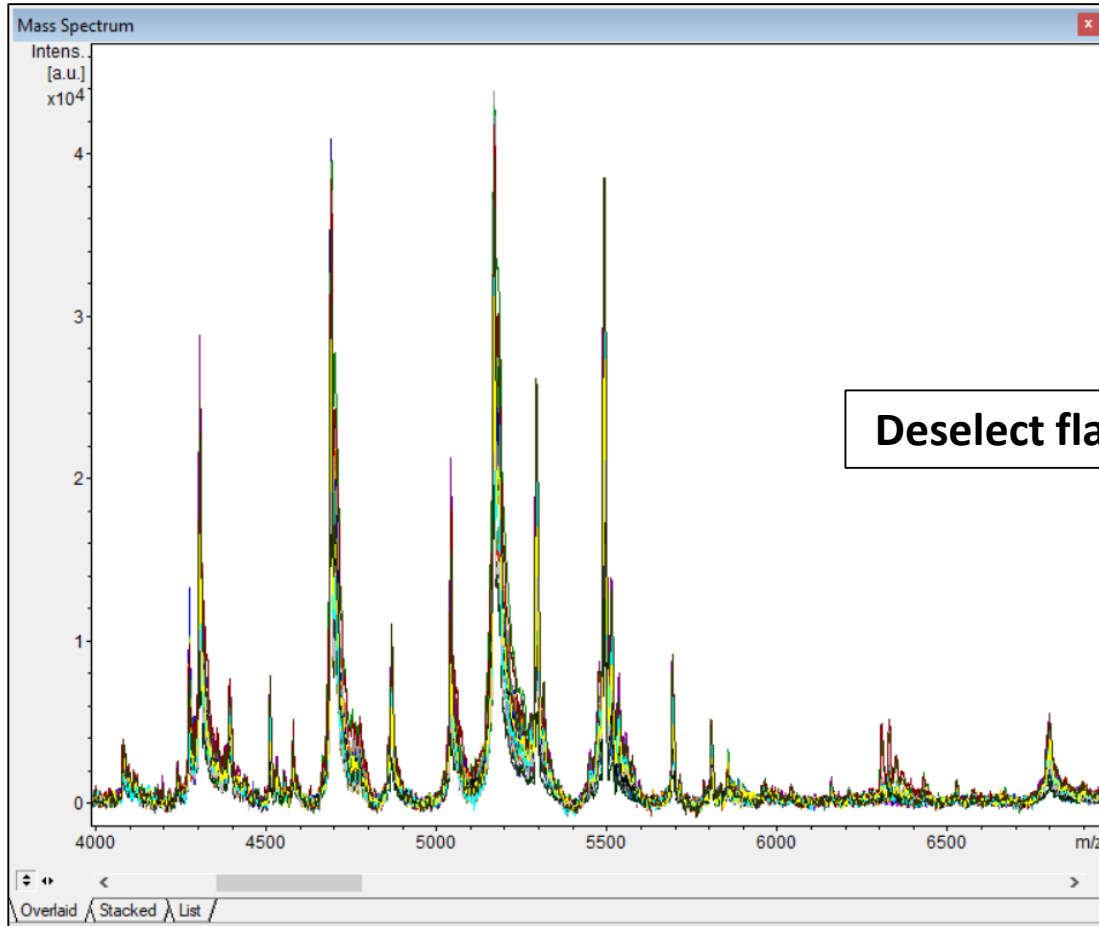
# Raw spectra processing – flexAnalysis



The screenshot displays the flexAnalysis software interface. On the left, the 'Analysis List' pane shows a list of 24 sample files, each with a small spectrum icon and a checkmark. The main window shows a 'Mass Spectrum' plot with 'Intens. [a.u.] x10<sup>4</sup>' on the y-axis and 'm/z' on the x-axis (ranging from 2000 to 20000). The plot shows multiple overlapping spectra in various colors. A red circle highlights the 'Edit sample spectra' icon in the toolbar. A text box points to this icon with the text 'Edit sample spectra'. Another text box on the right contains the text: 'Quality control of the spectra → selection of 24-20 sp, if necessary 18 sp'. The bottom status bar shows 'For Help: press F1', 'Box Selection', and 'honke'.



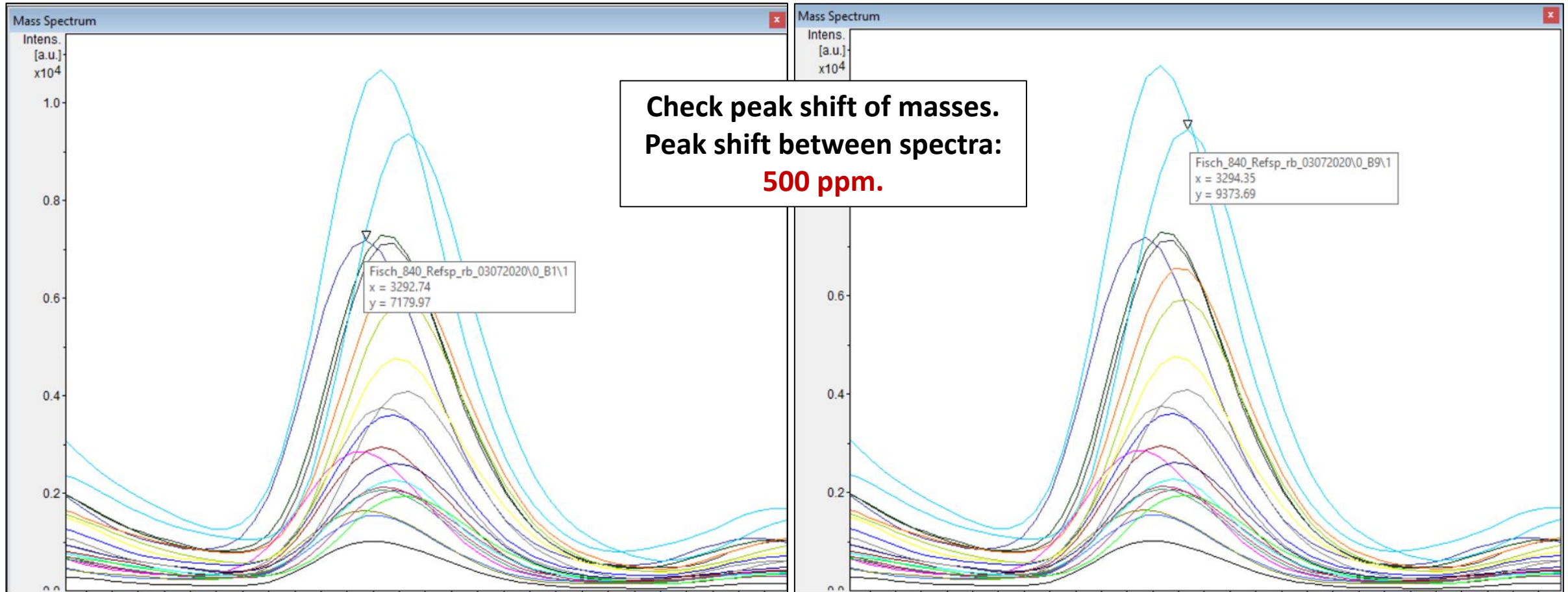
# Raw spectra processing – flexAnalysis



Deselect flat line spectrum

Deselect outlier peaks

# Raw spectra processing – flexAnalysis



# Raw spectrum processing – flexAnalysis

Name	Änderungsdatum
211018592 Campylobacter hepaticus_raw	02.2023 13:35
BTS 211018592	10.02.2023 21:16
BTS 211018592_raw	10.02.2023 21:05

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

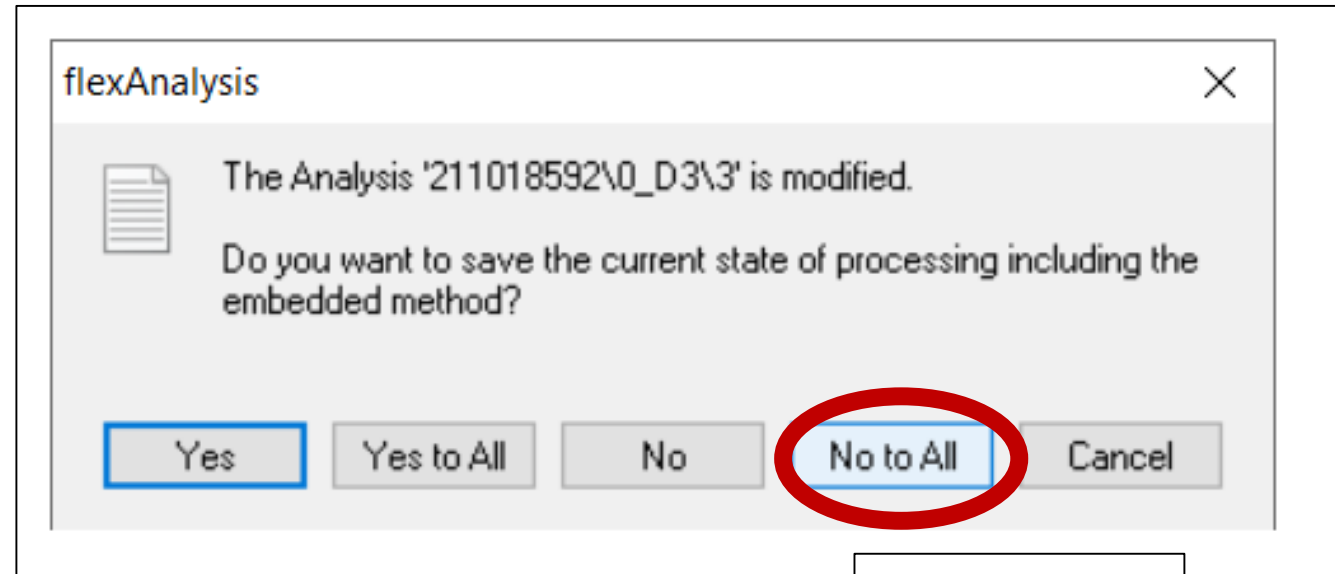
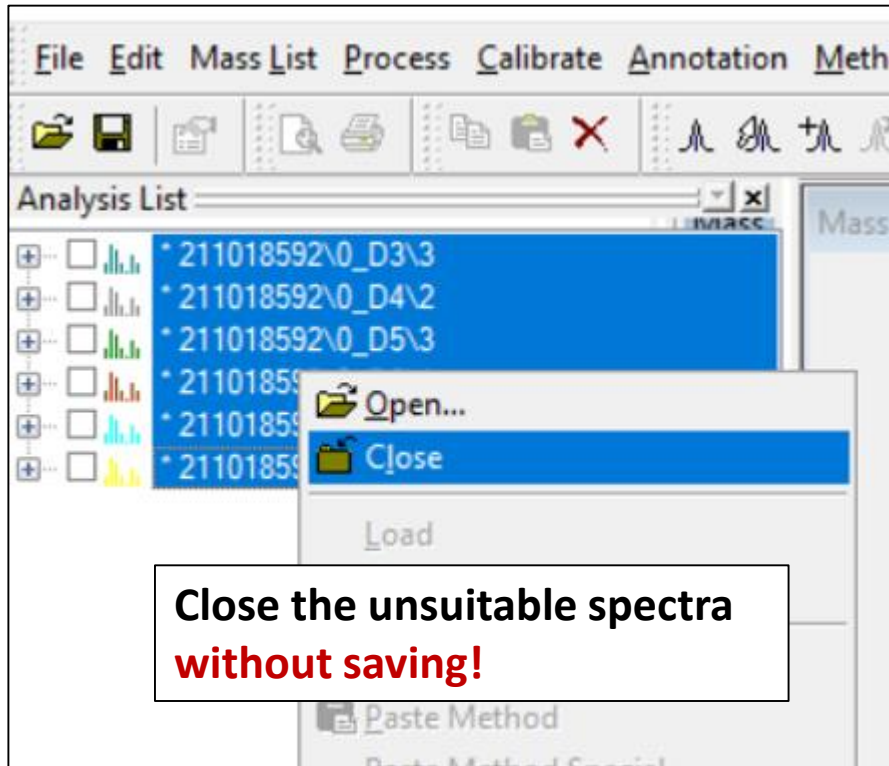
# Raw spectrum processing – flexAnalysis

The screenshot shows the flexAnalysis software interface. On the left is the 'Analysis List' with a list of 30 sample files, each with a checkbox and a small spectrum icon. The 'Save' icon in the top toolbar is circled in red. The main window displays a 'Mass Spectrum' plot with intensity on the y-axis (0 to 4) and m/z on the x-axis (2000 to 20000). A 'Mass List' window is open on the right. A text box is overlaid on the plot area.

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



Die verarbeiteten Probenspektren werden unter dem ursprünglichen Dateinamen gespeichert.

Name	Änderungsdatum
211018592 Campylobacter hepaticus	10.02.2023 21:31
211018592 Campylobacter hepaticus_raw	10.02.2023 13:35
BTS 211018592	10.02.2023 21:16
BTS 211018592_raw	10.02.2023 21:05

# 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	<input type="checkbox"/>	Sample Preparation ("Extraction Method")	<input type="checkbox"/> DT <input type="checkbox"/> eDT <input type="checkbox"/> EtOH-FA <input type="checkbox"/> .....
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		<input type="checkbox"/> 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	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Method → Open ...	MBT_Standard.FAMSMETHOD	Baseline Subtraction	Smooth (1x)

### BTS check/ recalibration:

Check Mass Control List Calibrate → Internal ...	<input type="checkbox"/> Automatic-Assign	<input type="checkbox"/> Peaks manually assigned
	Max. deviation (ppm):	
Recalibrate sample spectra	<input type="checkbox"/> Copy calibration	
Mass calibration constants BTS Select BTS spectrum → Properties ...	C0:	
	C1:	
	C2:	
Mass calibration constants sample spectra	<input type="checkbox"/> 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:

Conspicuous spectra (position/measurement): (Flat lines etc.)		<input type="checkbox"/> 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.: Streptobadillus 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:
<input type="checkbox"/> DT
<input type="checkbox"/> eDT
<input type="checkbox"/> EtOH-FA
<input type="checkbox"/> .....

Entry created ...:  own MSP-Library updated

Comment:	
----------	--

Date / acronym \_\_\_\_\_

# MSP creation – MBT Compass Explorer

The screenshot shows the 'Spectrum Browser' dialog box. The 'Root' field contains the path 'C:\Users\bonke\Desktop\Demo\_1402'. The 'Browse...' button is circled in red. The 'Filter Spectra' section has several checked options: MS, FAST, LIFT, FAST Segment, LIFT (converted), and Unknown. The 'From' and 'To' date fields are both set to '2023-02-10 22:37'. The 'Spectrum Properties' section is empty. At the bottom, the 'Selected spectra' count is 26 and the total 'Spectra' count is 26 / 26. The 'Open' button is circled in red. A text box with the text 'Open processed spectra' is overlaid on the dialog.

Root: C:\Users\bonke\Desktop\Demo\_1402

Filter Spectra

- MS
- FAST
- LIFT
- FAST Segment
- LIFT (converted)
- Unknown

From: 2023-02-10 22:37

To: 2023-02-10 22:37

Spectrum Properties

Selected spectra: 26

Spectra: 26 / 26

Open processed spectra

The screenshot shows the main window of MBT Compass Explorer. The 'Spectrum' tab is active, displaying a mass spectrum plot with intensity on the y-axis (0 to 40) and m/z (10<sup>3</sup>) on the x-axis (0 to 15). The plot shows several peaks, with the most prominent ones around m/z 5 and 10. The 'Identification' tab is also visible, showing a list of spectra files. The status bar at the bottom indicates 'Spectra loading running... Processed: 21/26 Remaining: 00:00:00'.

Spectrum [ C:\Users\bonke\Desktop\Demo\_140223\Maldi-Tof\_MSP\_Data\MSPs\_microbiology\Campylobacter hepaticu ]

Intensity [arb] (10<sup>3</sup>)

m/z (10<sup>3</sup>)

Spectra loading running... Processed: 21/26 Remaining: 00:00:00

# MSP creation – MBT Compass Explorer

The screenshot displays the MALDI Biotyper Compass Explorer interface. The 'Action' menu is open, showing 'MSP Creation' selected, with a sub-menu containing 'Create' and 'Create series'. A list of spectra is visible on the left, with several items highlighted in blue. A 'New MSP Name' dialog box is open in the foreground, showing the 'New Name' field containing 'Campylobacter hepaticus\_211018592\_LHL G'. A spectrum plot is visible in the background. The 'Bruker Taxonomy' panel on the right shows 'Unassigned MSPs (17/17)' circled in blue.

**1. Select all spectra**

**2. Create MSP**

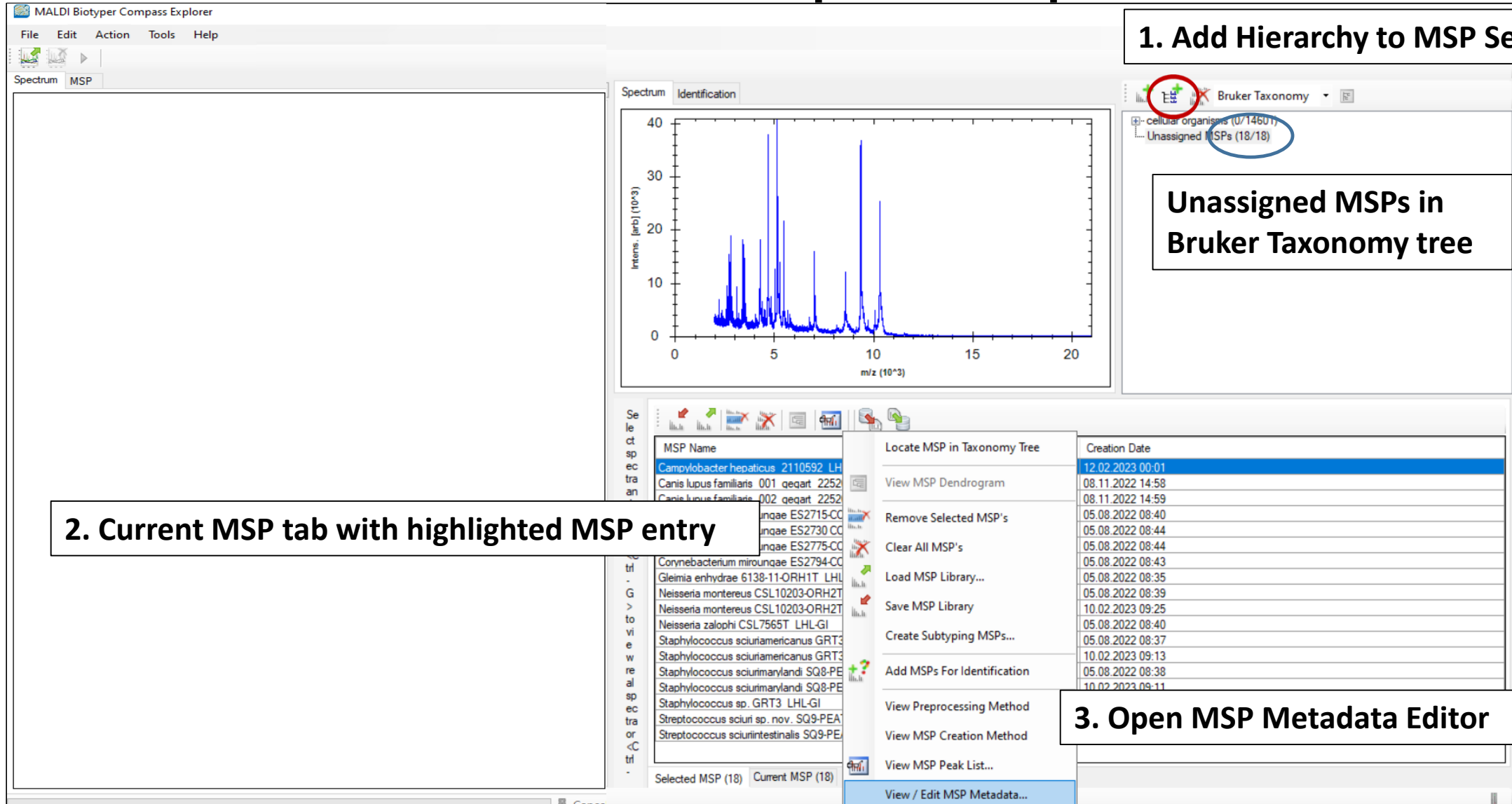
**3. Set MSP Name**

**Note: Name of MSP cannot be changed after creation!**

The created MSP appears under Unassigned MSPs.



# 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**

MSP Name	Creation Date
Campylobacter hepaticus_2110592_LH	12.02.2023 00:01
Canis lupus familiaris_001_0eqart_2252	08.11.2022 14:58
Canis lupus familiaris_002_0eqart_2252	08.11.2022 14:59
inqaee ES2715-CC	05.08.2022 08:40
inqaee ES2730-CC	05.08.2022 08:44
inqaee ES2775-CC	05.08.2022 08:44
Corynebacterium miroungaee ES2794-CC	05.08.2022 08:43
Gleimia enhydrae 6138-11-ORH1T_LHL	05.08.2022 08:35
Neisseria montereus CSL10203-ORH2T	05.08.2022 08:39
Neisseria montereus CSL10203-ORH2T	10.02.2023 09:25
Neisseria zalophi CSL7565T_LHL-GI	05.08.2022 08:40
Staphylococcus sciuriamericanus GRT3	05.08.2022 08:37
Staphylococcus sciuriamericanus GRT3	10.02.2023 09:13
Staphylococcus sciurimarylandi SQ8-PE	05.08.2022 08:38
Staphylococcus sciurimarylandi SQ8-PE	10.02.2023 09:11
Staphylococcus sp. GRT3_LHL-GI	
Streptococcus sciuri sp. nov. SQ9-PEA	
Streptococcus sciurintestinalis SQ9-PEA	

**3. Open MSP Metadata Editor**

# MSP creation – MBT Compass Explorer

MALDI Biotyper MSP Metadata Editor. Versi... [min] [max] [close]

Campylobacter hepaticus\_2110592\_LHL GI (32 Spectra)

Organism:

Strain:

Provided by:

Determined by:

Conserved

Extraction Method:

Matrix:

Growing Conditions:

Comment:

MALDI Biotyper MSP Metadata Editor. Versi... [min] [max] [close]

Campylobacter hepaticus 211018592\_LHL-GI (29 Spectra)

Organism:

Strain:

Provided by:

Determined by:

Conserved

Extraction Method:

Matrix:

Growing Conditions:

Comment:

Create MSP Metadata

# MSP creation – MBT Compass Explorer

## MSP organization

**1. Open Taxonomy Tree Editor**

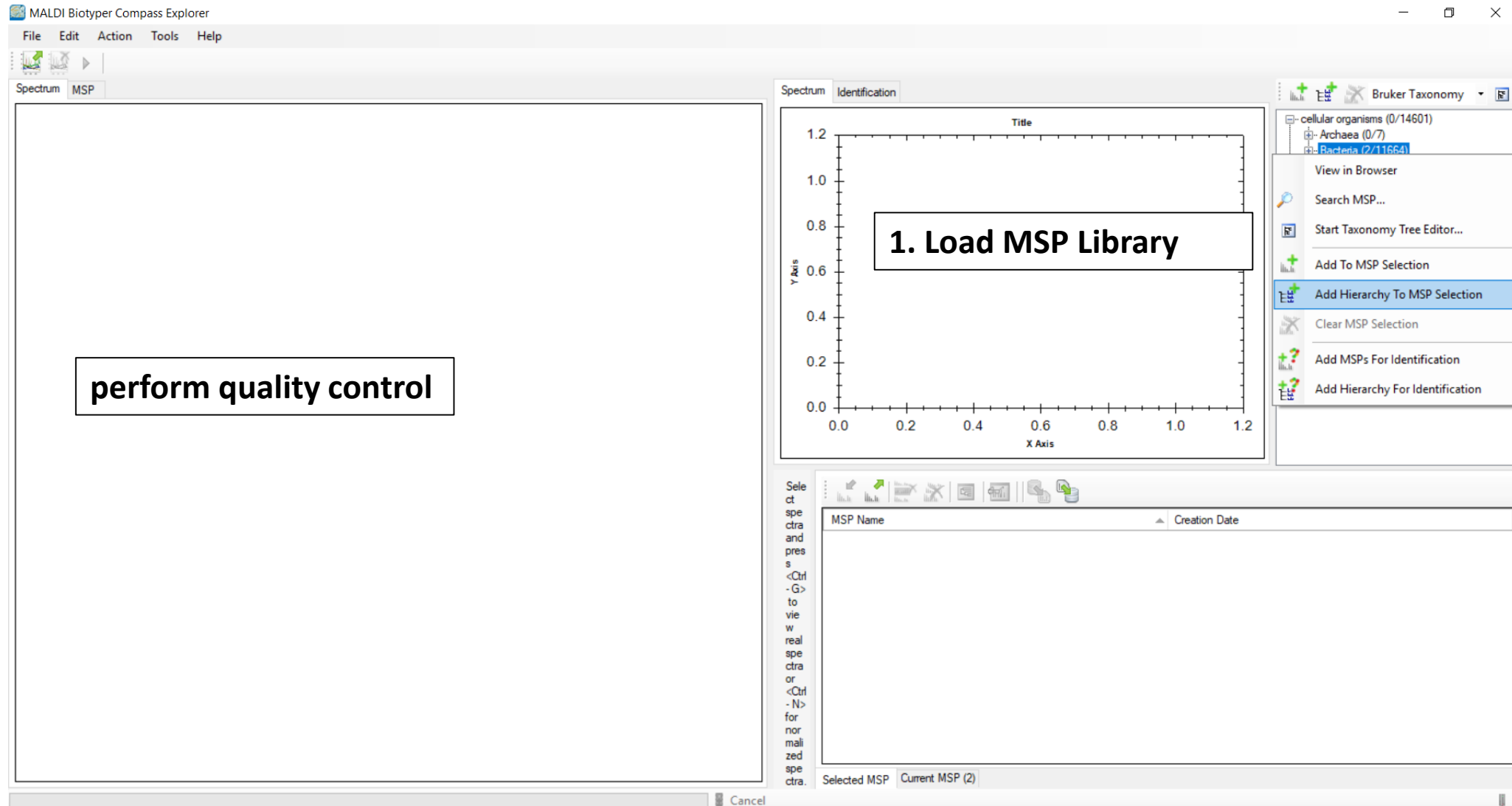
**2. Choose Project**

MSP Name	Creation Date	Peak I
<i>Campylobacter hepaticus</i> _2110185	2/10/2023 9:41:51	26
<i>Canis lupus familiaris</i> _001_gegart_	11/8/2022 2:58:52	20
<i>Canis lupus familiaris</i> _002_gegart_	11/8/2022 2:59:15	23
<i>Corynebacterium miroungae</i> _ES271	8/5/2022 8:40:51 AM	31
<i>Corynebacterium miroungae</i> _ES273	8/5/2022 8:44:59 AM	29
<i>Corynebacterium miroungae</i> _ES277	8/5/2022 8:44:11 AM	31
<i>Corynebacterium miroungae</i> _ES275	8/5/2022 8:43:35 AM	27
<i>Gleimia enhydrae</i> _6138-11-ORH1T_	8/5/2022 8:35:00 AM	25
<i>Neisseria montereus</i> _CSL10203-OF	8/5/2022 8:39:29 AM	27
<i>Neisseria montereus</i> _CSL10203-OF	2/10/2023 9:25:31	27
<i>Neisseria zolophi</i> _CSL7565T_LHL-G	8/5/2022 8:40:10 AM	28
<i>Staphylococcus sciuriamericanus</i> _G	8/5/2022 8:37:00 AM	30
<i>Staphylococcus sciuriamericanus</i> _G	2/10/2023 9:13:43	24
<i>Staphylococcus sciurimarylandi</i> _SQ	8/5/2022 8:38:47 AM	27
<i>Staphylococcus sciurimarylandi</i> _SQ	8/5/2022 8:38:47 AM	27

**3. Move created MSP to tree node**

MSP Name	Creation Date	Peak I
<i>Canis lupus familiaris</i> _001_gegart_	11/8/2022 2:58:52	20
<i>Canis lupus familiaris</i> _002_gegart_	11/8/2022 2:59:15	23
<i>Corynebacterium miroungae</i> _ES271	8/5/2022 8:40:51 AM	31
<i>Corynebacterium miroungae</i> _ES273	8/5/2022 8:44:59 AM	29
<i>Corynebacterium miroungae</i> _ES277	8/5/2022 8:44:11 AM	31
<i>Corynebacterium miroungae</i> _ES275	8/5/2022 8:43:35 AM	27
<i>Gleimia enhydrae</i> _6138-11-ORH1T_	8/5/2022 8:35:00 AM	25
<i>Neisseria montereus</i> _CSL10203-OF	2/10/2023 9:25:31	27
<i>Neisseria montereus</i> _CSL10203-OF	8/5/2022 8:39:29 AM	27
<i>Neisseria zolophi</i> _CSL7565T_LHL-G	8/5/2022 8:40:10 AM	28
<i>Staphylococcus sciuriamericanus</i> _G	8/5/2022 8:37:00 AM	30
<i>Staphylococcus sciuriamericanus</i> _G	2/10/2023 9:13:43	24
<i>Staphylococcus sciurimarylandi</i> _SQ	8/5/2022 8:38:47 AM	27
<i>Staphylococcus sciurimarylandi</i> _SQ	2/10/2023 9:11:56	27

# MSP creation – MBT Compass Explorer

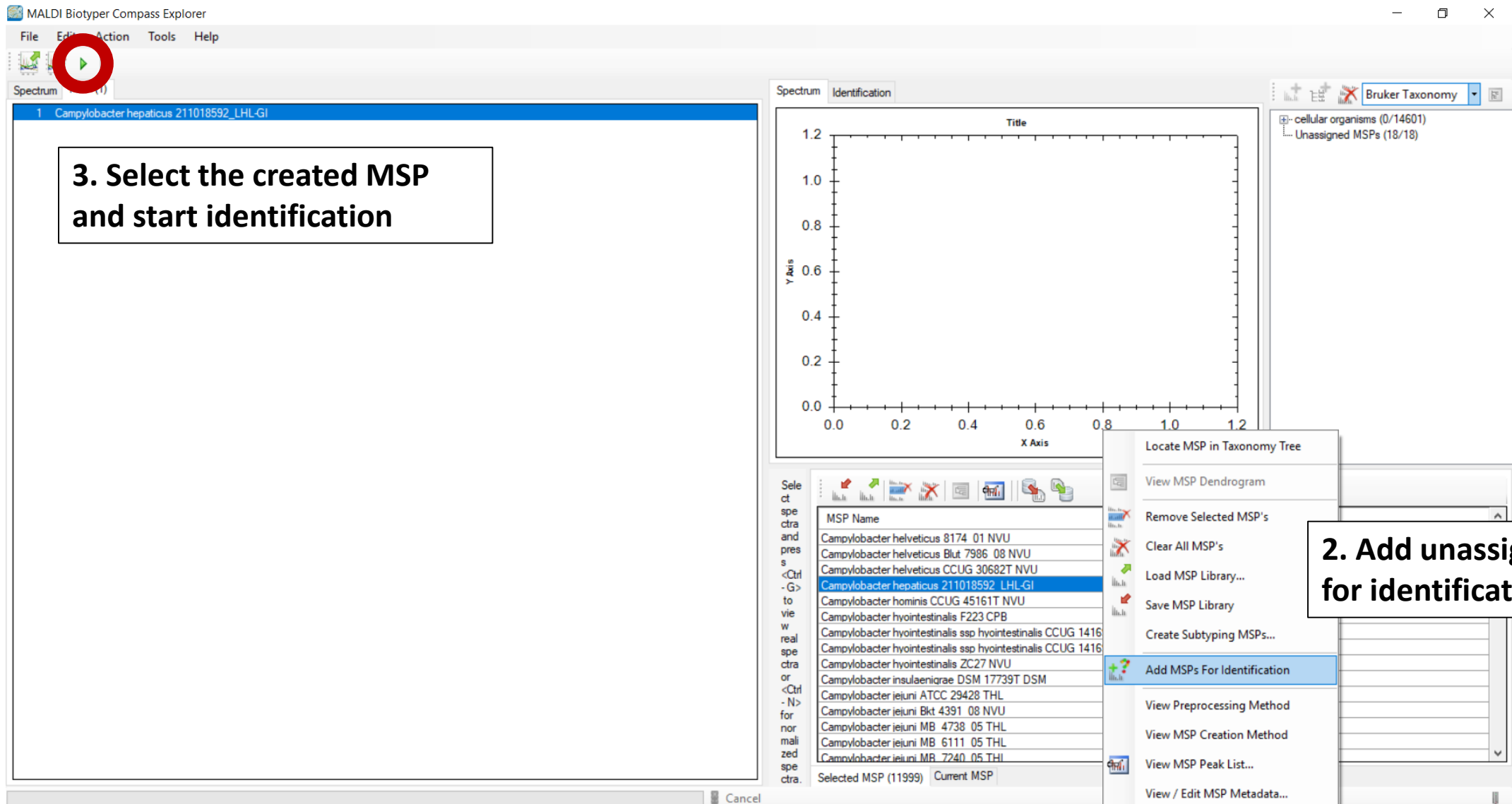


The screenshot shows the MALDI Biotyper Compass Explorer software interface. The main window is titled "MALDI Biotyper Compass Explorer" and has a menu bar with "File", "Edit", "Action", "Tools", and "Help". The interface is divided into several panels:

- Left Panel:** A large empty area with a text box that says "perform quality control".
- Top Panel:** A "Spectrum" tab is active, showing a plot with "Y Axis" and "X Axis" ranging from 0.0 to 1.2. A text box in the center of the plot says "1. Load MSP Library".
- Right Panel:** A "Bruker Taxonomy" tree is visible, showing a hierarchy of "cellular organisms (0/14601)", "Archaea (0/7)", and "Bacteria (2/11664)". A context menu is open over the "Bacteria" node, listing options such as "View in Browser", "Search MSP...", "Start Taxonomy Tree Editor...", "Add To MSP Selection", "Add Hierarchy To MSP Selection", "Clear MSP Selection", "Add MSPs For Identification", and "Add Hierarchy For Identification".
- Bottom Panel:** A table with columns "MSP Name" and "Creation Date". Below the table, it shows "Selected MSP" and "Current MSP (2)".

At the bottom of the window, there is a "Cancel" button.

# MSP creation – MBT Compass Explorer



The screenshot shows the MALDI Biotyper Compass Explorer software interface. The main window is titled "MALDI Biotyper Compass Explorer" and has a menu bar with "File", "Edit", "Action", "Tools", and "Help". A red circle highlights the "Action" menu. The "Spectrum" tab is active, showing a spectrum plot for "1 Campylobacter hepaticus 211018592\_LHL-GI". The "Identification" tab is also visible, showing a plot with "Y Axis" and "X Axis" ranging from 0.0 to 1.2. A "Bruker Taxonomy" panel on the right shows a tree structure with "cellular organisms (0/14601)" and "Unassigned MSPs (18/18)". A context menu is open over the MSP list, with "Add MSPs For Identification" highlighted. A text box on the left contains the instruction "3. Select the created MSP and start identification". A text box on the right contains the instruction "2. Add unassigned MSP for identification".

**3. Select the created MSP and start identification**

**2. Add unassigned MSP for identification**

MSP Name
Campylobacter helveticus 8174_01 NVU
Campylobacter helveticus Blut 7986_08 NVU
Campylobacter helveticus CCUG 30682T NVU
<b>Campylobacter hepaticus 211018592_LHL-GI</b>
Campylobacter hominis CCUG 45161T NVU
Campylobacter hyointestinalis F223 CPB
Campylobacter hyointestinalis ssp hyointestinalis CCUG 1416
Campylobacter hyointestinalis ssp hyointestinalis CCUG 1416
Campylobacter hyointestinalis ZC27 NVU
Campylobacter insulaenigræ DSM 17739T DSM
Campylobacter jejuni ATCC 29428 THL
Campylobacter jejuni Bkt 4391_08 NVU
Campylobacter jejuni MB 4738_05 THL
Campylobacter jejuni MB 6111_05 THL
Campylobacter jejuni MB 7240_05 THL

# MSP creation – MBT Compass Explorer

File Edit Action Tools Help

Spectrum MSP (1)

1 *Campylobacter hepaticus* 211018592\_LHL-GI

Spectrum Identification

rel. int.

*Campylobacter hepaticus* 211018592\_LHL-GI

m/z (10<sup>3</sup>)

Braker Taxonomy

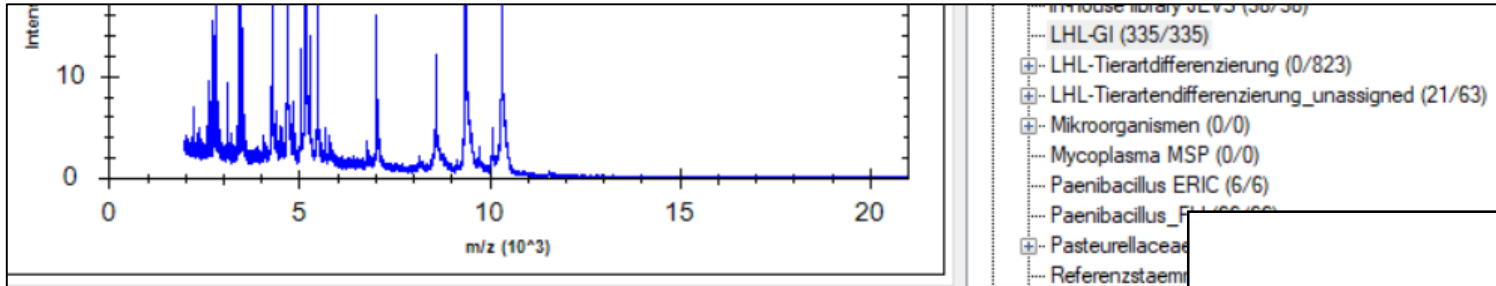
- cellular organisms (0/14601)
- Unassigned MSPs (18/18)

MID	Detected Species	Log(Score)
1	<i>Campylobacter hepaticus</i> 211018592_LHL-GI	3.000
2	<i>Campylobacter jejuni</i> MB 6111 05 THL	1.380
3	<i>Campylobacter jejuni</i> MB 7240 05 THL	1.300
4	<i>Pannonibacter phraamitetus</i> LMG 5414 HAM	1.270
5	<i>Campylobacter jejuni</i> ATCC 29428 THL	1.240
6	<i>Neisseria meningitidis</i> 24086406 MLD	1.200
7	<i>Campylobacter jejuni</i> MB 4738 05 THL	1.190
8	<i>Prevotella histicola</i> ENR 0213 ENR	1.180
9	<i>Clostridium polynesiense</i> DSM 27072T DSM	1.170
10	<i>Clostridioides difficile</i> MB 7869 05 THL	1.160

**MSP scores of detected species**

Selected MSP (11999) Current MSP MSP Scores (10)

# MSP creation – MBT Compass Explorer



Select spectra and press Ctrl-G to view real spectra or Ctrl-G to view

MSP Name	Creation Date
Campylobacter hepaticus 211018592 LHL-GI	18.02.2022 07:47
Campylobacter sp-W1 CVUAS 31484 CVUAS	01.03.2019 13:06
Caviibacter abscessus CCUG39713T	04.03.2016 13:43
Chelonobacter oris CVUAS 8268	21.02.2013 16:58
Chryseobacterium chaponense CVUAS 32831 CVUAS	19.04.2021 13:03
Chryseobacterium hominis CVUAS 5661 CVUAS	12.03.2015 14:04
Chryseobacterium manosquense 185-U1-6 CVUAS	04.05.2021 07:28
Chryseobacterium sp-I-7 CVUAS 11450.4 CVUAS	22.06.2018 09:08
Clostridium acidisoli DSM12555 LHL	22.02.2017 13:54
Clostridium akaqii DSM12554 LHL	22.02.2017 13:42
Clostridium alqidicamis DSM15099 LHL	22.02.2017 13:49
Clostridium alqidixylanolyticum DSM12273 LHL	22.02.2017 13:58
Clostridium alqorophilum DSM16153 LHL	22.02.2017 13:51
Clostridium botulinum E CVUAS 30006 CVUAS	29.06.2017 07:16
Clostridium bowmanii DSM14206 LHL	22.02.2017 14:03
Clostridium estertheticum ssp. estertheticum DSM8809 LHL	22.02.2017 14:10
Clostridium estertheticum ssp. laramiense DSM14864 LHL	22.02.2017 14:07
Clostridium fimetarium DSM9179 LHL	22.02.2017 14:13
Clostridium friqdicamis DSM12271 LHL	22.02.2017 14:19

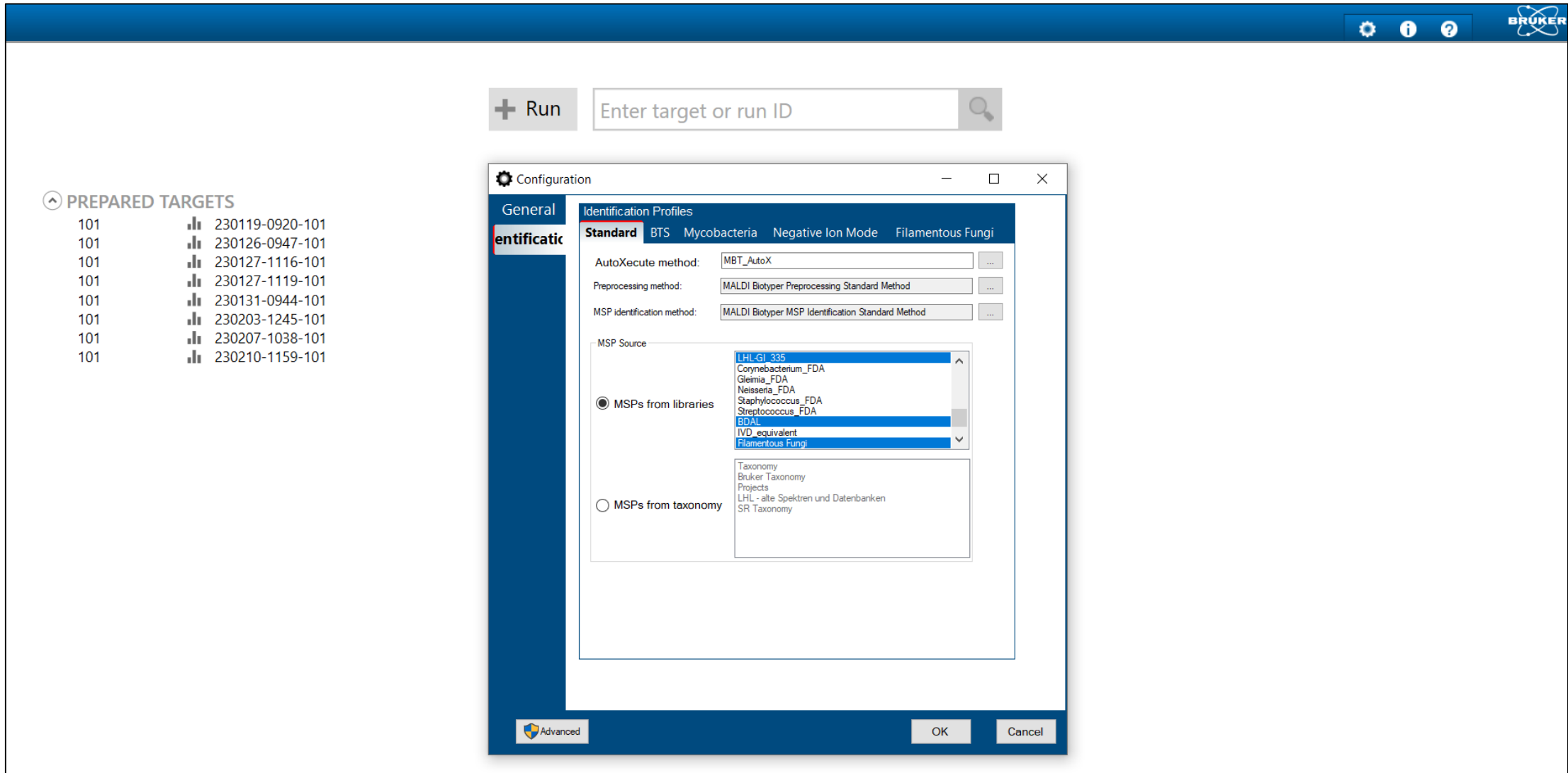
Selected MSP (335) Current MSP (335)



**Save MSP library!**

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

# Library selection- MBT Compass



The screenshot displays the MBT Compass software interface. At the top, there is a '+ Run' button and a search input field labeled 'Enter target or run ID'. On the left side, under 'PREPARED TARGETS', a list of 10 targets is shown, each with a bar chart icon and a numerical value of 101.

The main configuration window is titled 'Configuration' and has a 'General' tab selected. The 'Identification Profiles' section is active, with 'Standard' selected among other options like 'BTS', 'Mycobacteria', 'Negative Ion Mode', and 'Filamentous Fungi'. The configuration includes the following settings:

- AutoXecute method: MBT\_AutoX
- Preprocessing method: MALDI Biotyper Preprocessing Standard Method
- MSP identification method: MALDI Biotyper MSP Identification Standard Method

The 'MSP Source' section is divided into two radio button options:

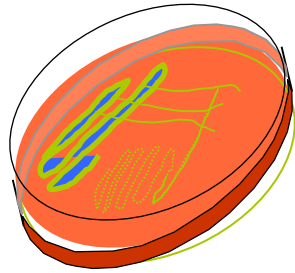
- MSPs from libraries** (selected): A list of library sources is shown, including LHL-GI\_335, Corynebacterium\_FDA, Gleimia\_FDA, Neisseria\_FDA, Staphylococcus\_FDA, Streptococcus\_FDA, EDAL, IVD\_equivalent, and Filamentous Fungi.
- MSPs from taxonomy** (unselected): A list of taxonomy sources is shown, including Taxonomy, Bruker Taxonomy, Projects, LHL - alte Spektren und Datenbanken, and SR Taxonomy.

At the bottom of the configuration window, there are 'Advanced', 'OK', and 'Cancel' buttons.



# Quality control – MBT Compass

fresh  
culture



Direct smear



**Bruker Daltonik MALDI Biotyper Classification Results**

**BRUKER**

**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_20	<i>Campylobacter hepaticus</i>	<u>2.61</u>	not reliable identification	<u>1.28</u>

➔ **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.

# 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	<input type="checkbox"/>	Sample Preparation ("Extraction Method")	<input type="checkbox"/> DT <input type="checkbox"/> eDT <input type="checkbox"/> EtOH-FA <input type="checkbox"/> .....
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		<input type="checkbox"/> 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	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Method → Open ...	MBT_Standard.FAMSMETHOD	Baseline Subtraction	Smooth (1x)

### BTS check/ recalibration:

Check Mass Control List Calibrate → Internal ...	<input type="checkbox"/> Automatic-Assign	<input type="checkbox"/> Peaks manually assigned
	Max. deviation (ppm):	
Recalibrate sample spectra	<input type="checkbox"/> Copy calibration	
Mass calibration constants BTS Select BTS spectrum → Properties ...	C0:	
	C1:	
	C2:	
Mass calibration constants sample spectra	<input type="checkbox"/> 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:

Conspicuous spectra (position/measurement): (Flat lines etc.)	<input type="checkbox"/> 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

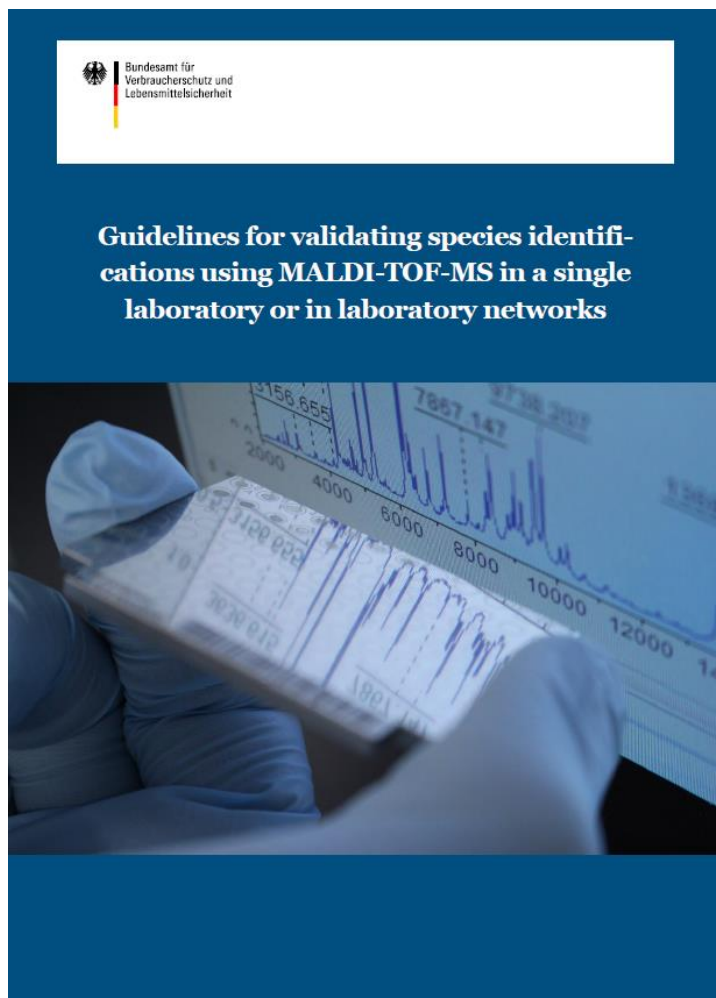
Preparation:
<input type="checkbox"/> DT
<input type="checkbox"/> eDT
<input type="checkbox"/> EtOH-FA
<input type="checkbox"/> .....

Entry created ...:  own MSP-Library updated

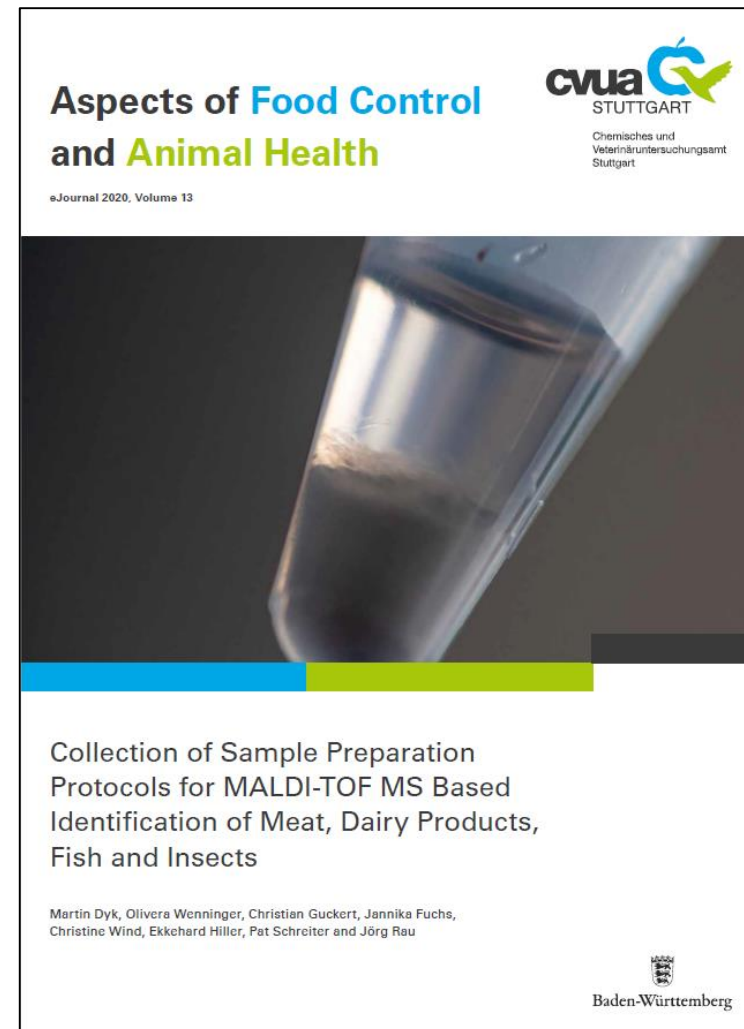
Comment:
----------

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://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>