

Supplementary File 1

Identification of RNA Fragments Resulting from Enzymatic Degradation Using MALDI-TOF Mass Spectrometry

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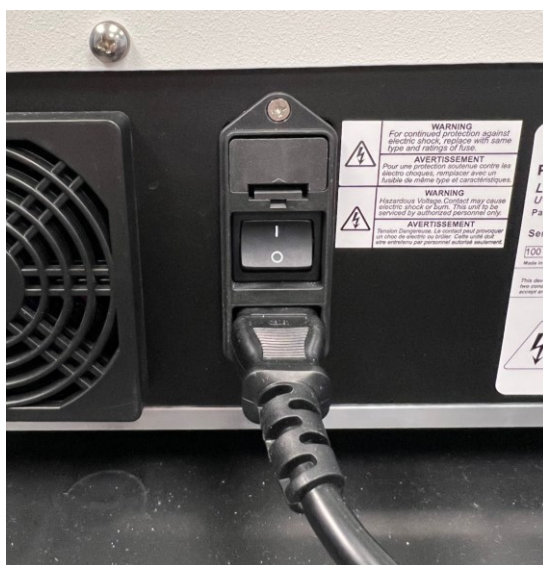
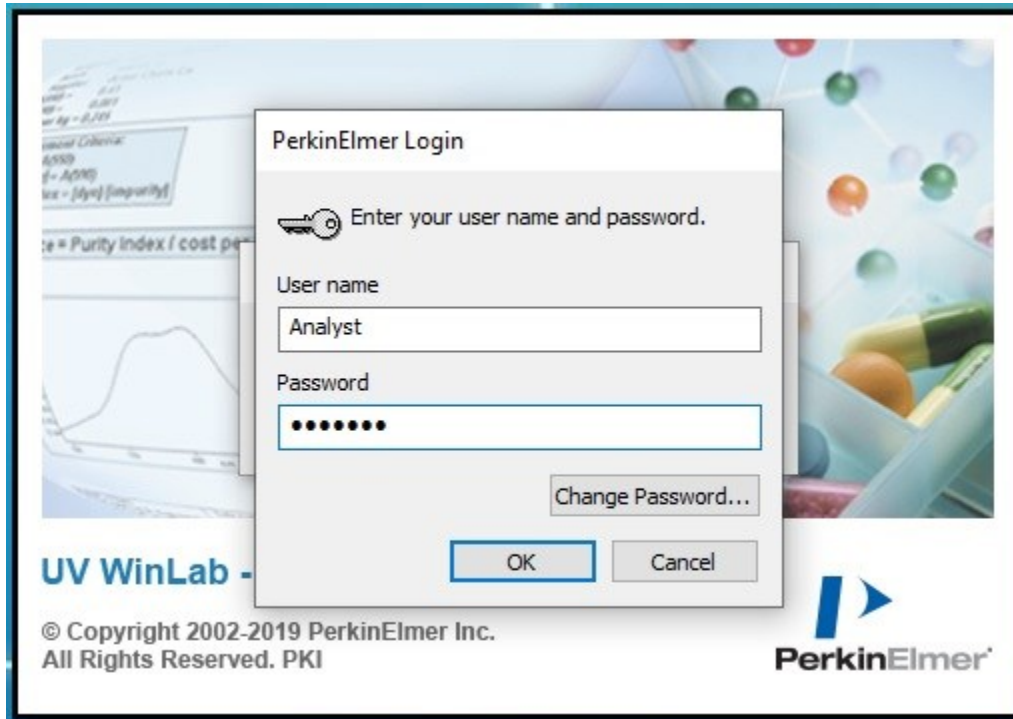
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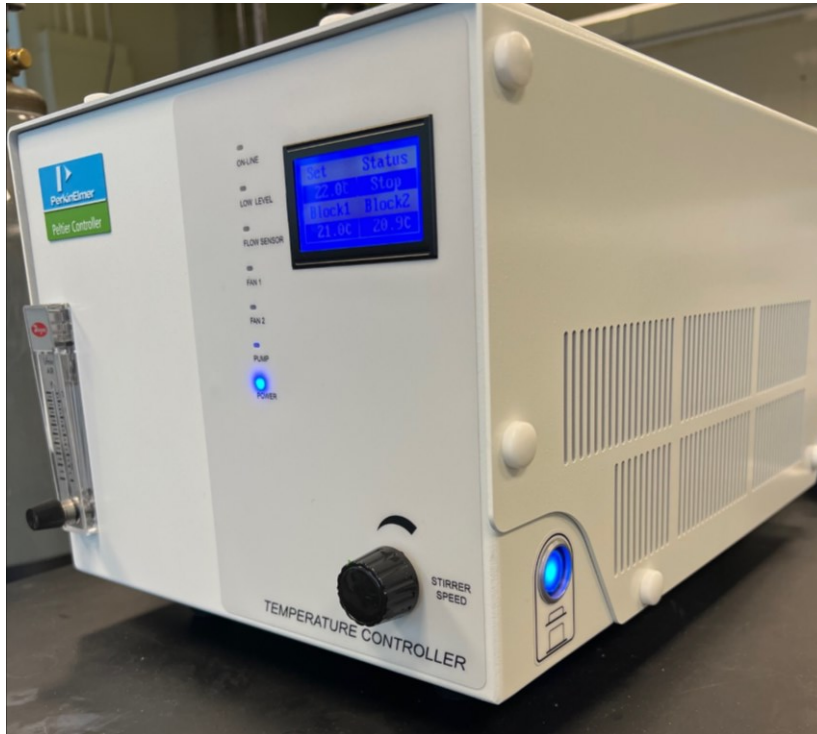
Page	Topic Description
S1–S7	UV-vis spectrophotometer usage (Steps 1.2.1 – 1.2.10)
S8–S23	Data acquisition (Steps 4.1.1 – 4.1.4.5)
S24–S30	Data processing (Steps 4.2.1 – 4.2.10.3)

Step 1.2: UV-vis spectrophotometer usage

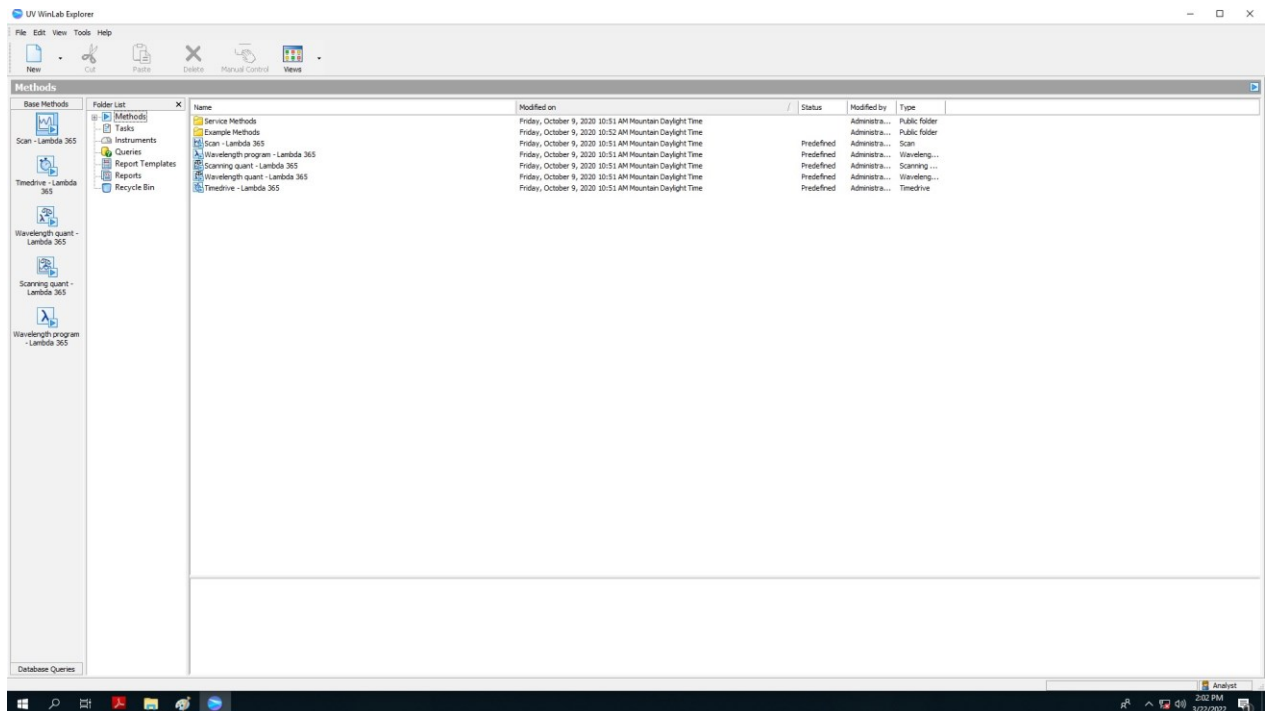
Step 1.2.1



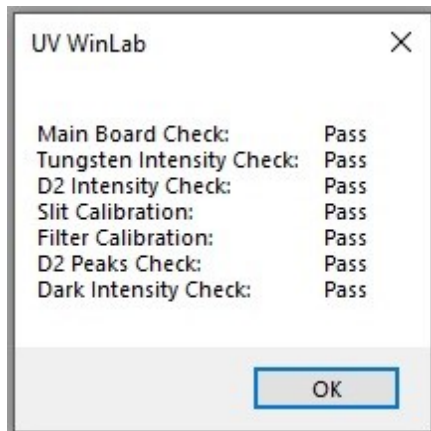
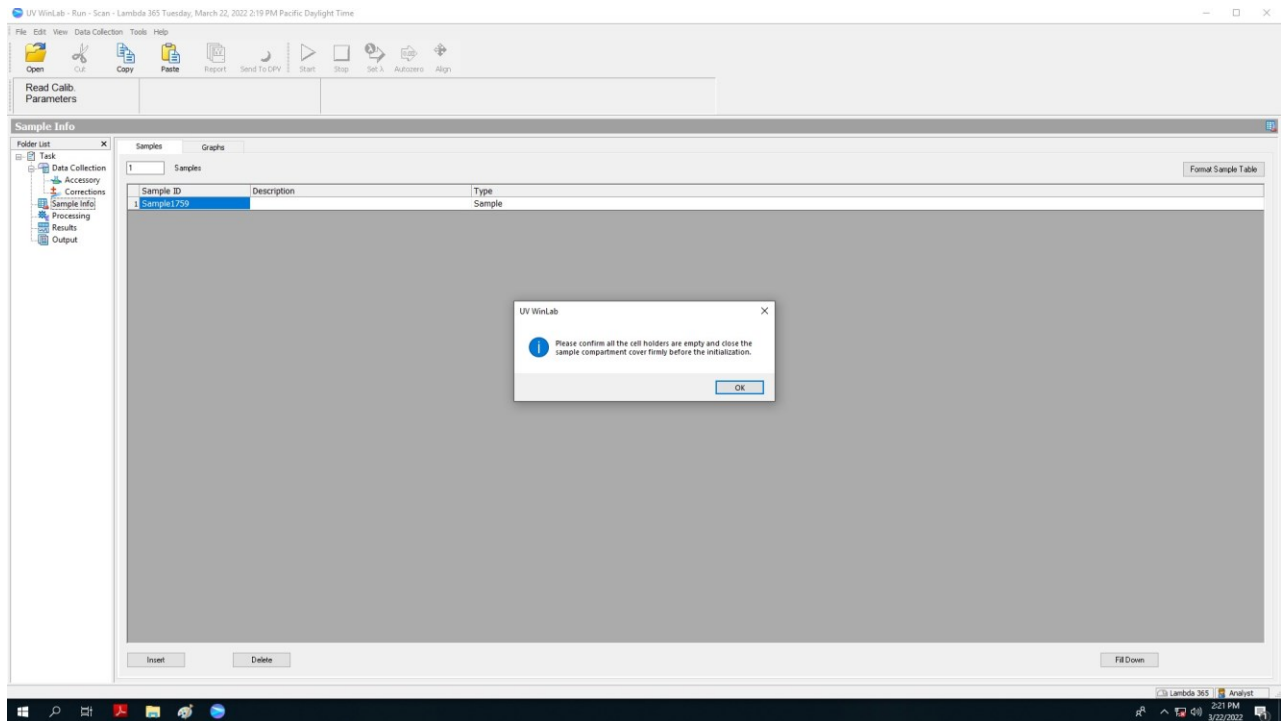
Step 1.2.2



Step 1.2.3



Step 1.2.3 (continued)



Step 1.2.4

The screenshot shows the 'Data Collection' software interface. On the left is a 'Folder List' with a tree structure: Task > Data Collection > Accessory, Corrections, Sample Info, Processing, Results, and Output. The 'Data Collection' folder is selected. The main panel is titled 'Data Collection' and contains several sections:

- Scan Settings:** Start (nm) is 350, End (nm) is 215, Ordinate mode is A, and Slit width (nm) is 1.
- Advanced Scan Settings:** Scan speed (nm/min) is 480, and Data interval (nm) is 1.
- Cycles:** Number of cycles is 1. There are two radio buttons: 'Cycle as fast as possible' (unselected) and 'Cycle time' (selected). The 'Cycle time' is set to 1 seconds.
- Lamps:** Both 'UV lamp on' and 'Visible lamp on' are checked. 'Lamp change at (nm)' is set to 400.

Step 1.2.5

The screenshot shows the 'Accessory' software interface. On the left is a 'Folder List' with a tree structure: Task > Data Collection > Accessory, Corrections, Sample Info, Processing, Results, and Output. The 'Accessory' folder is selected. The main panel is titled 'Accessory' and contains a 'General' section with the following settings:

- Default accessories:** 'Multiple Cell Peltier' is checked.

Step 1.2.5 (continued)

Multiple Cell Peltier

Folder List

- Task
 - Data Collection
 - Accessory
 - Multiple Cell Peltier**
 - Corrections
 - Sample Info
 - Processing
 - Results
 - Output

Cell Changer Accessory

Type: Auto

Description:

Autozero

Autozero every sample

Autozero at position Front Cell 2

Enabled Positions:

Setting

Temperature (°C): 25.0 Error Range (°C): 0.5

Monitor: Block

Temperature Display

Block Probe 1 Probe 2

Action

Cell: Front Cell 2 Goto

Peltier On Peltier Off Calibrate

Step 1.2.6

Sample Info

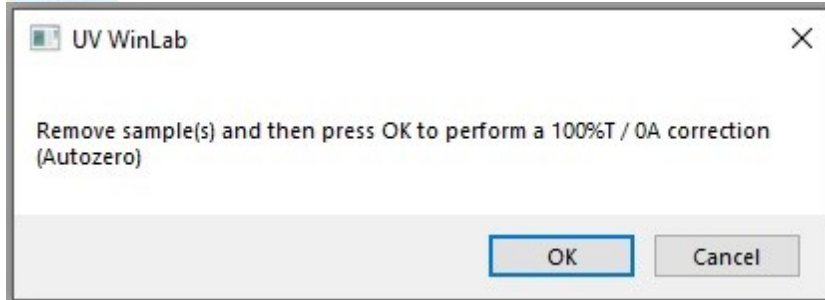
Folder List

- Task
 - Data Collection
 - Accessory
 - Corrections
 - Sample Info**
 - Processing
 - Results
 - Output

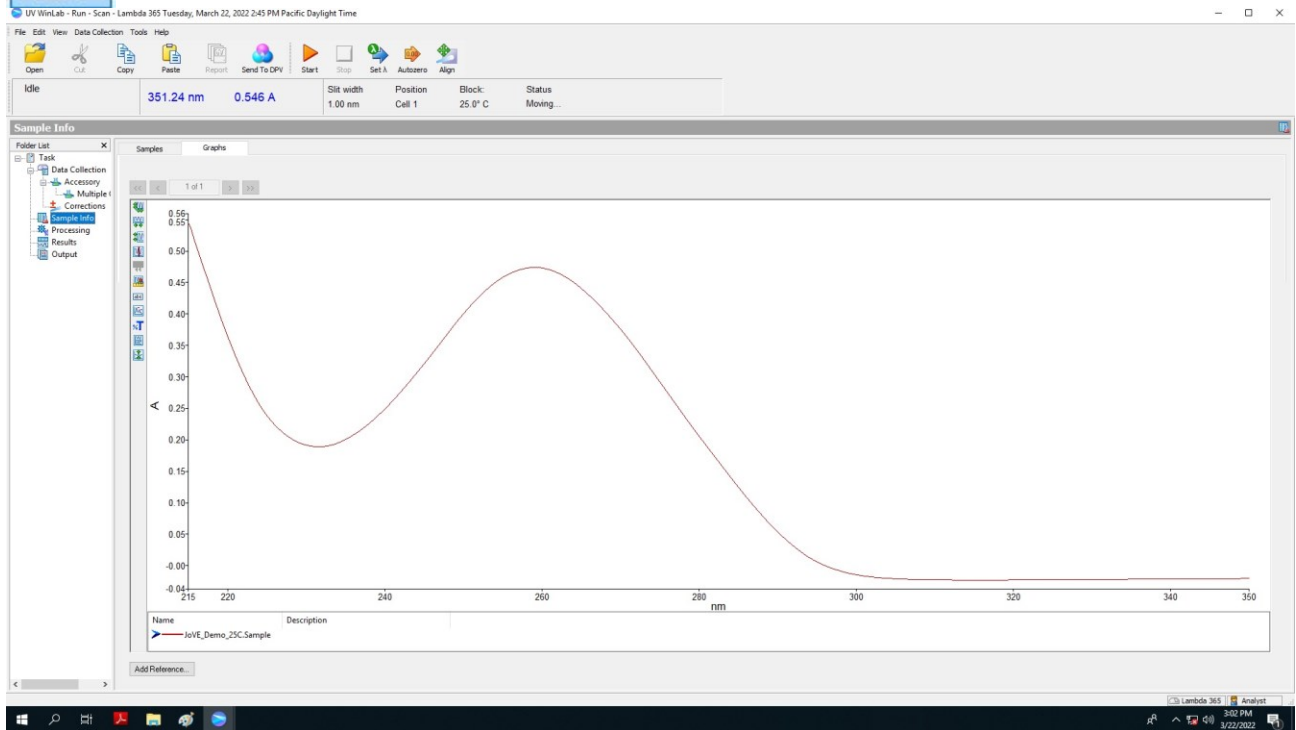
Samples 2 Samples

Sample ID	Description	Type	Cell Position	Carousel Position
1	JoVE_Demo_25C	Sample	1	Front
2	JoVE_Demo_90C	Sample	1	Front

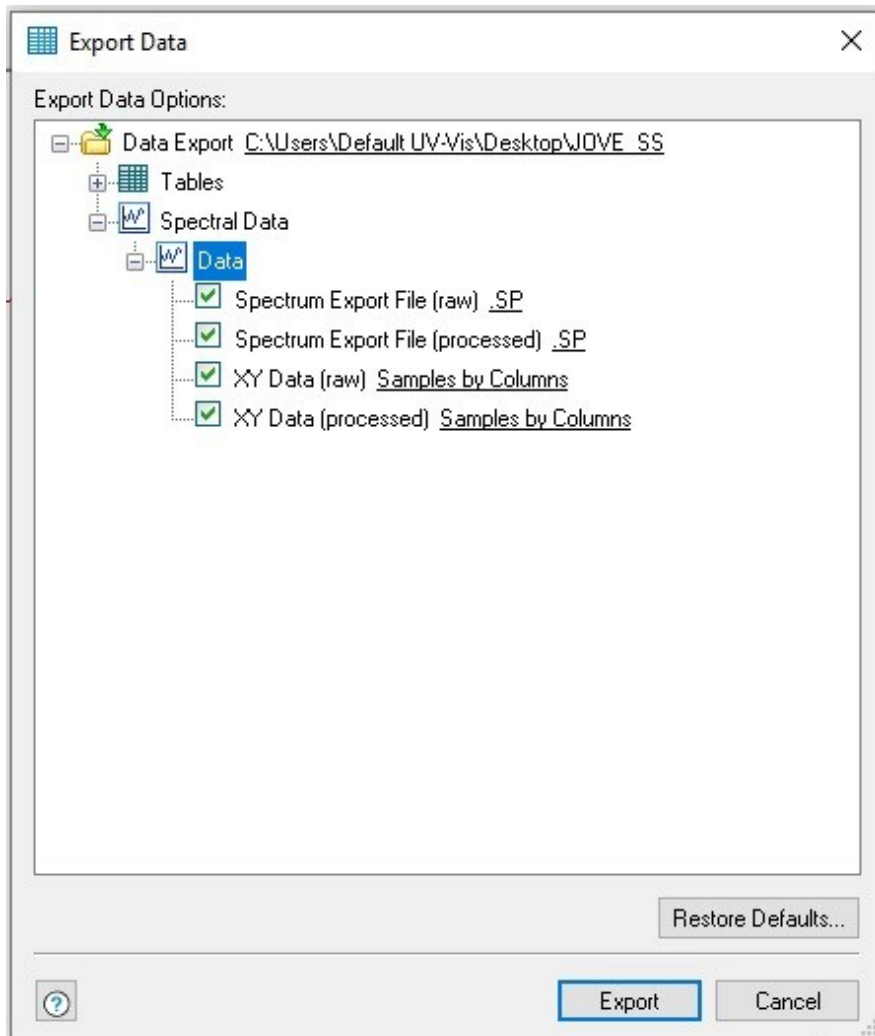
Step 1.2.7



Step 1.2.8

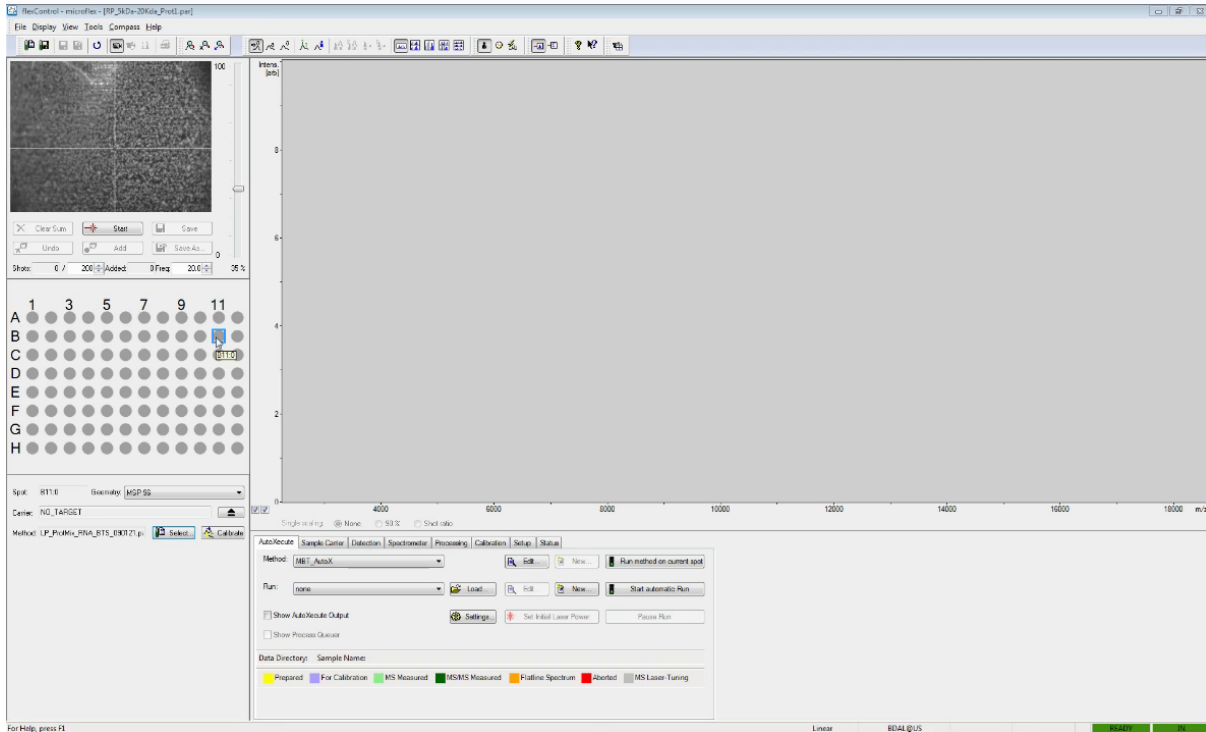
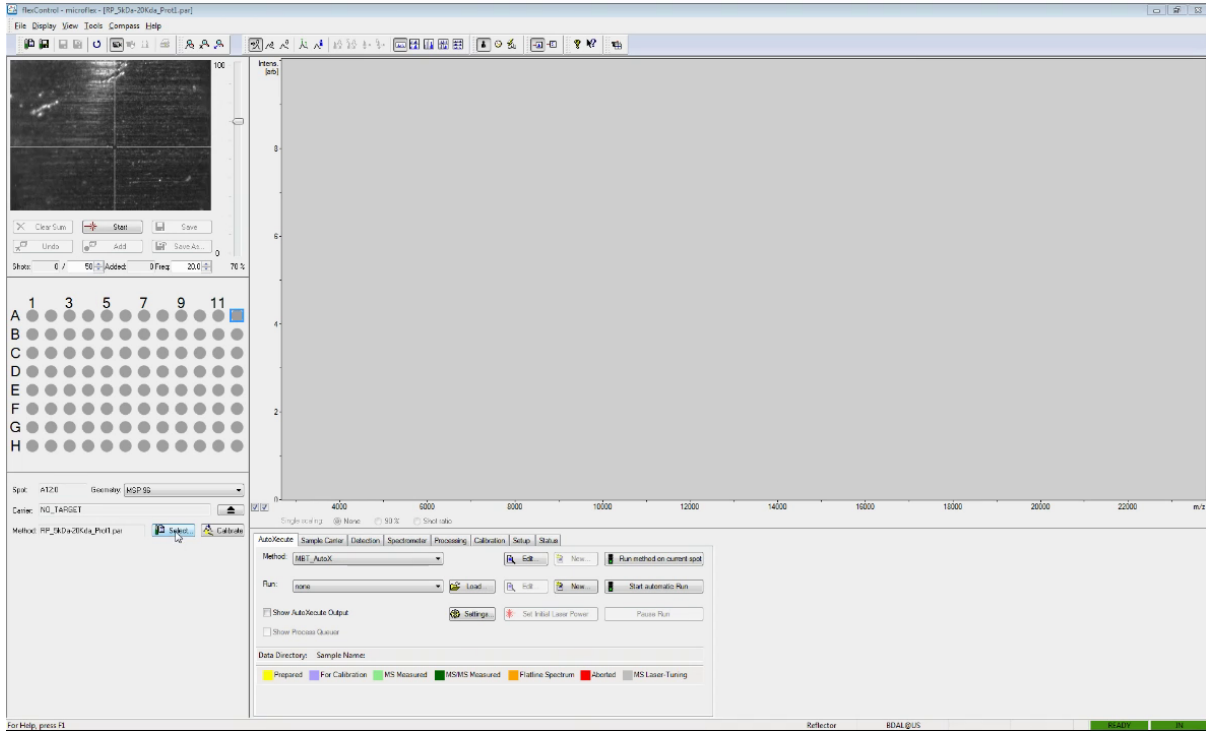


Step 1.2.10.



Step 4: Data acquisition and processing

Step 4.1.3.1



Step 4.1.3.1 (continued)

The screenshot shows the IlexControl software interface. The main window displays a mass spectrum plot with intensity on the y-axis (0 to 100) and m/z on the x-axis (4000 to 16000). A grid of sample positions (A-H, 1-11) is visible on the left. The 'Mass Control List' panel is open, showing a table of statistical peaks.

Name	Ref. Mass Da	Cur. Mass Da	Drift (ppm)	Err (Da)
• RL29 [M+2]Q+	3037.80000			
• E532 [M+H] ⁺	5096.80000			
• R524 [M+H] ⁺	5381.80000			
• R533[M+H] ⁺ ...	6285.80000			
• RL29 [M+H] ⁺	7274.30000			
• E510 [M+H] ⁺	8030.10000			
• R506 A [M+H] ⁺	13952.20000			
• Myoglobin [M+H] ⁺	16952.30000			

Below the table, there are options for 'Automatic Assign', 'Apply', 'Restore', and 'Zoom Range' (set to 5.5%). The 'Peak Assignment Tolerance' is set to 'User Defined' with a value of 1000 ppm. The 'Mode' is set to 'G4ic Enhanced'.

The screenshot shows the IlexControl software interface with the 'Teaching' panel open. The panel is divided into 'Teaching' and 'Automation' sections.

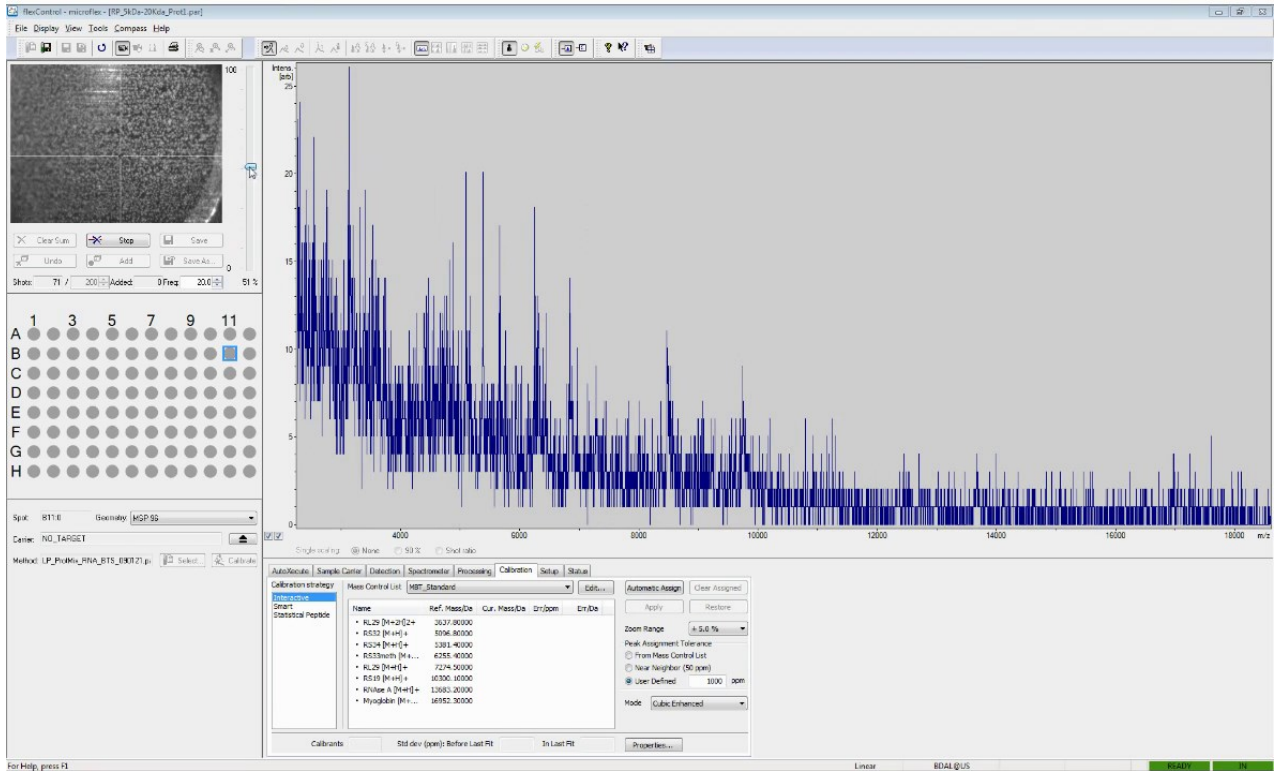
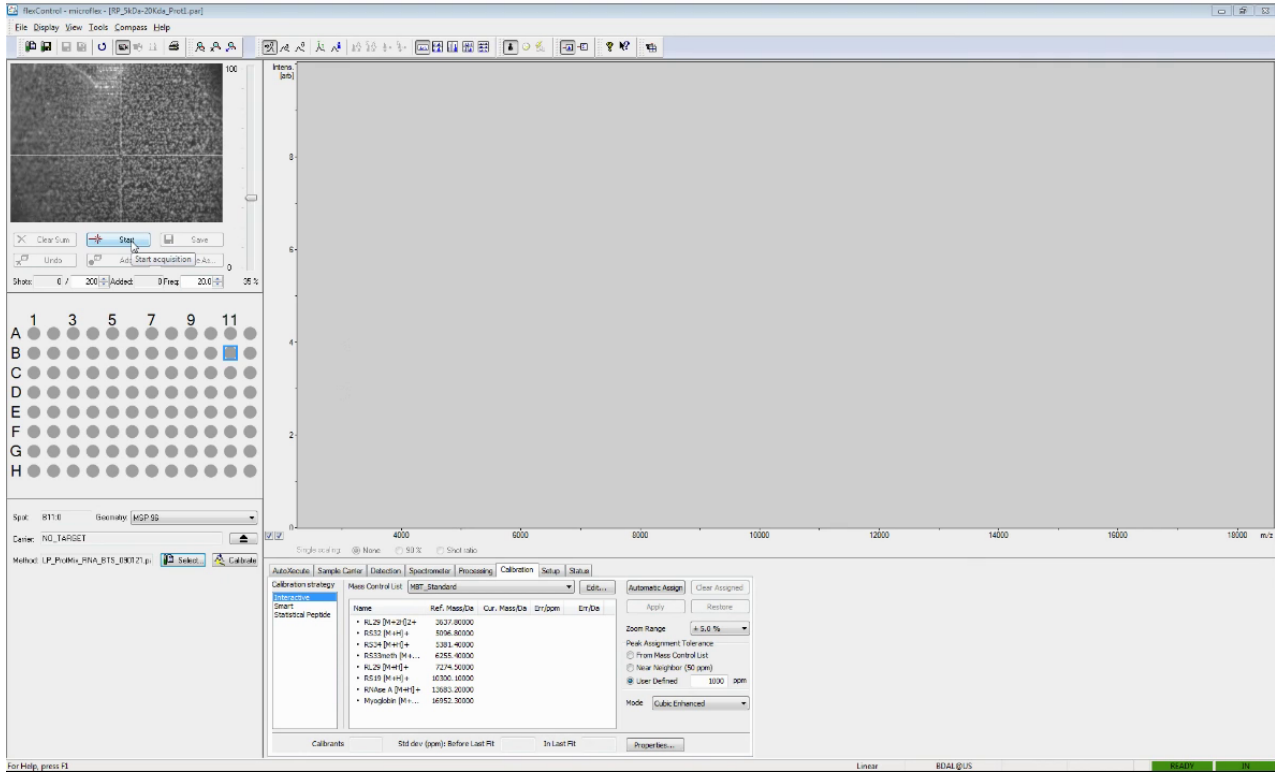
Teaching Section:

- File: []
- Mode: Off
- Partial sample: []
- Complete sample: []
- Shots at raster spot: 10
- Limit diameter: 2000 μm
- Buttons: Default Teaching, Auto Teaching, Teach, No random rastering, Advanced

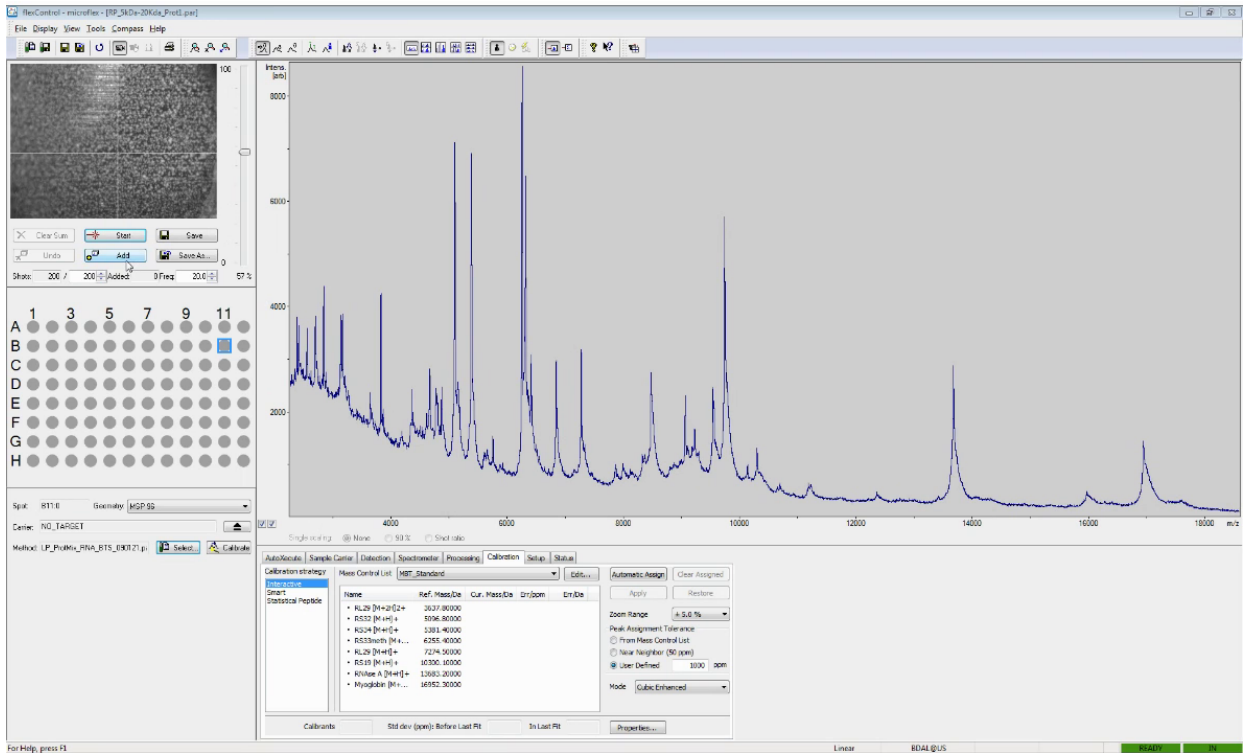
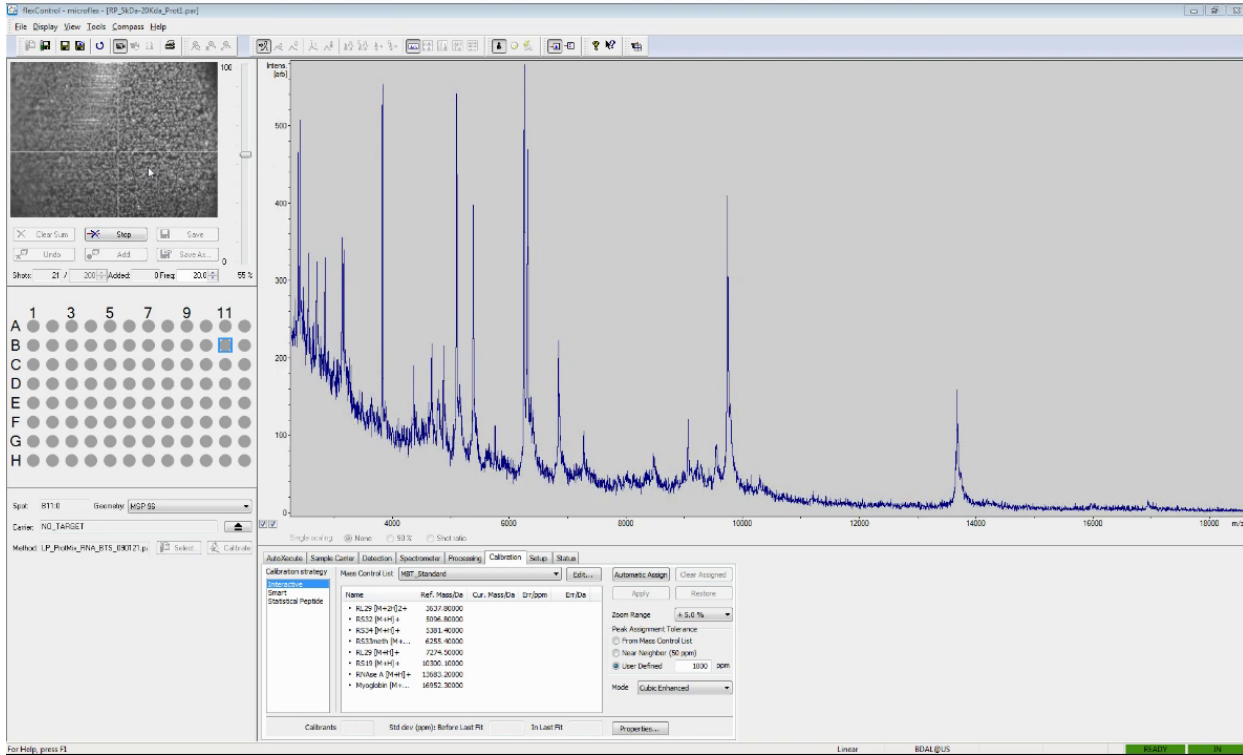
Automation Section:

- Automation: Perform AutoTeaching on Deck-in

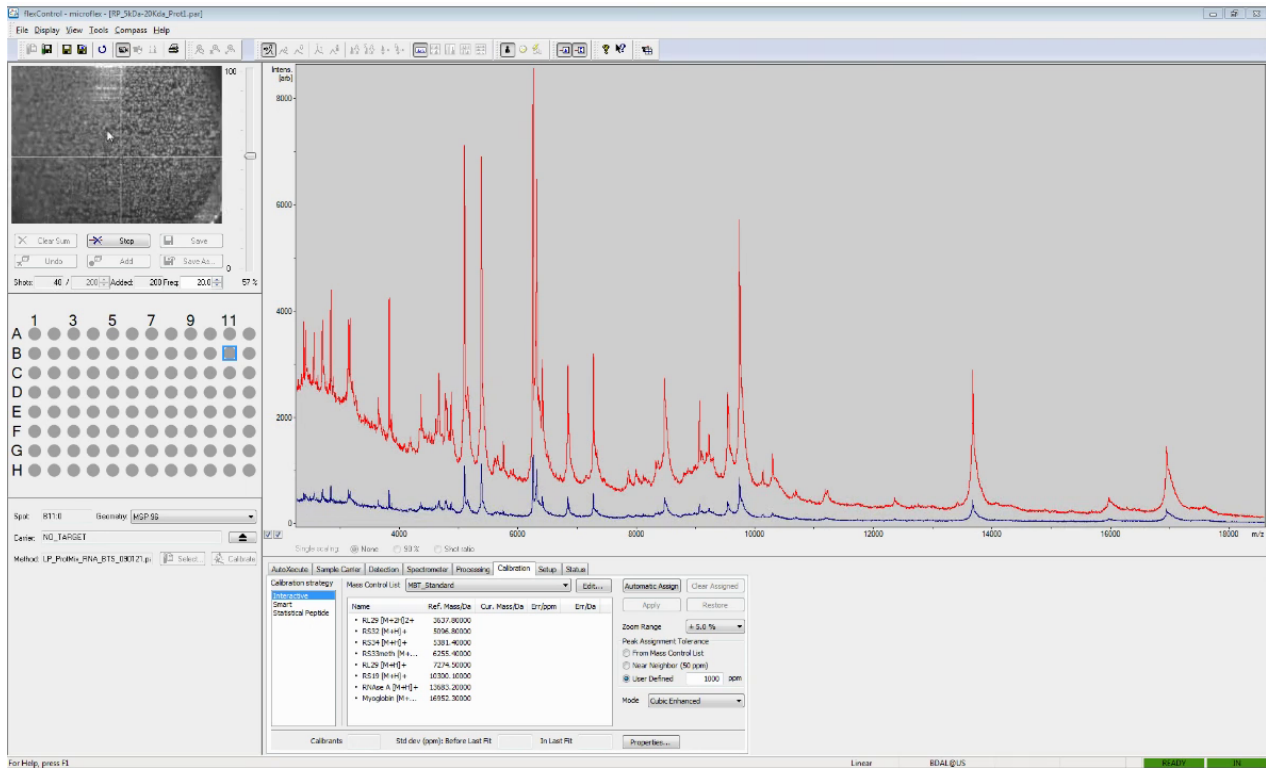
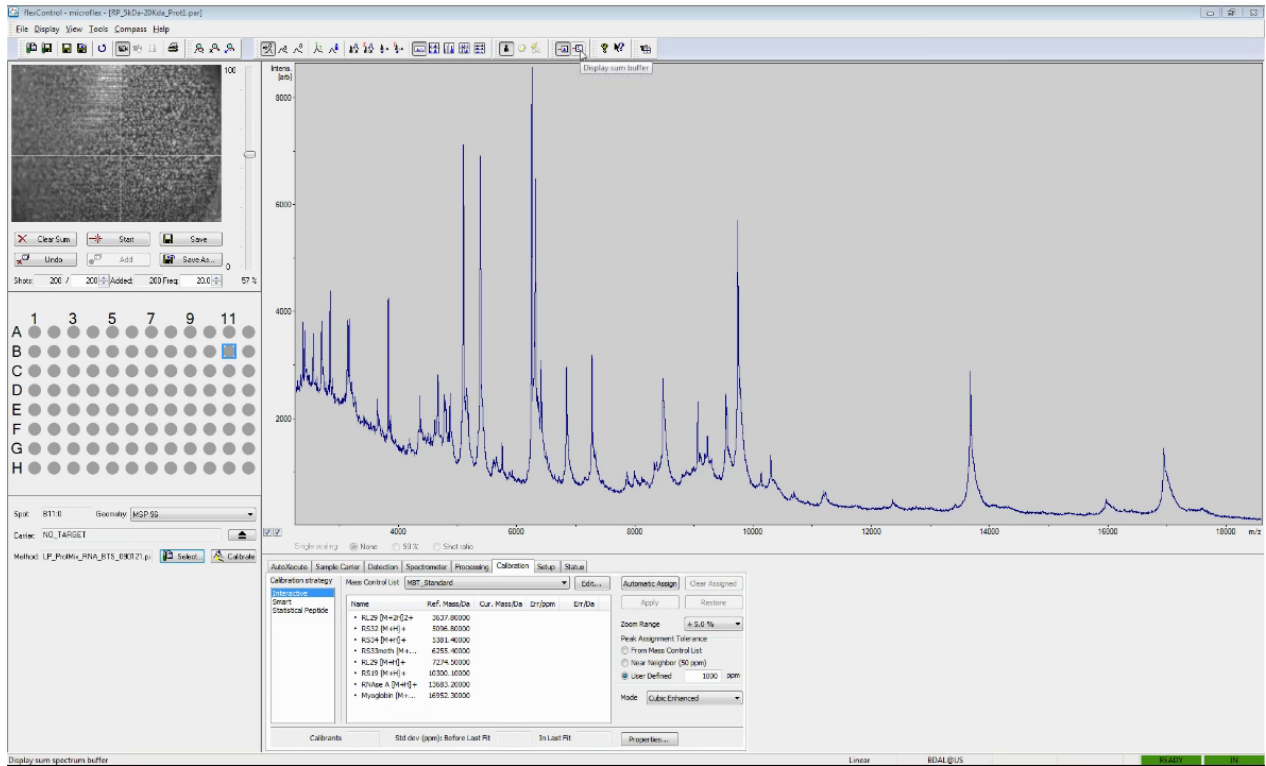
Step 4.1.3.2



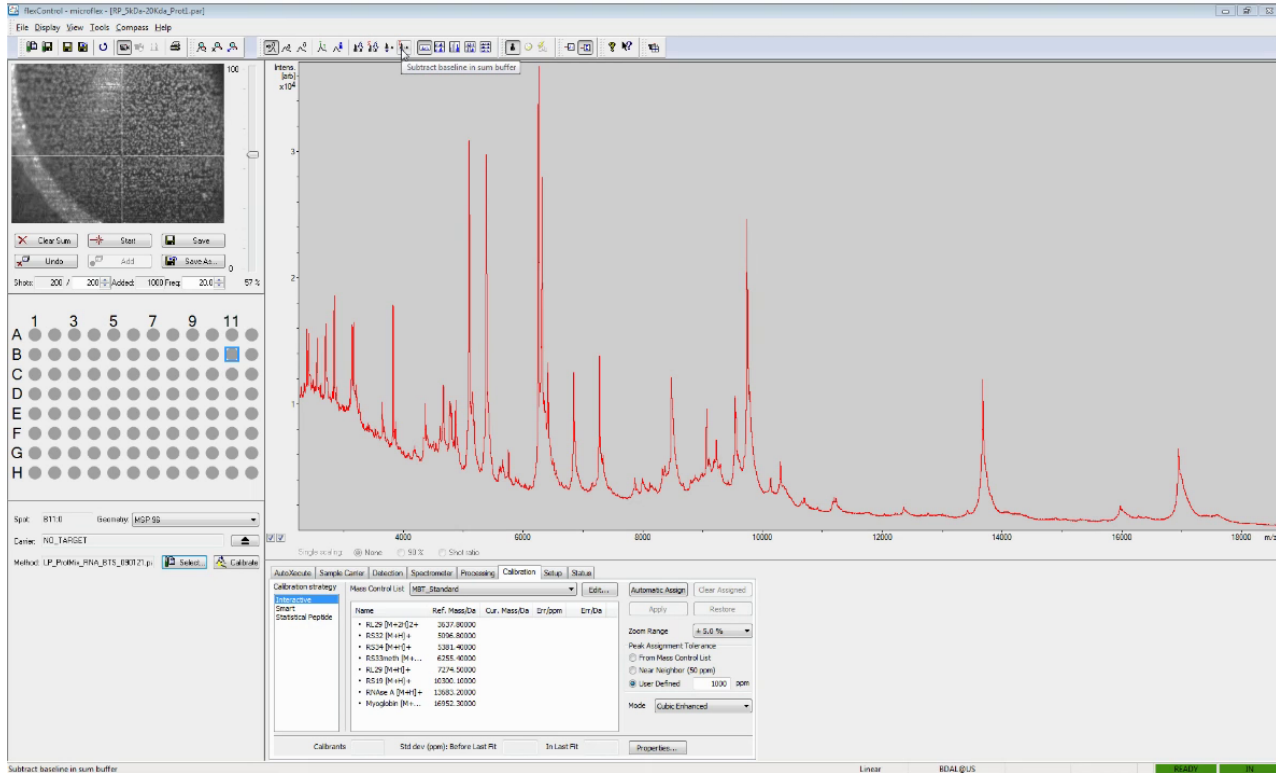
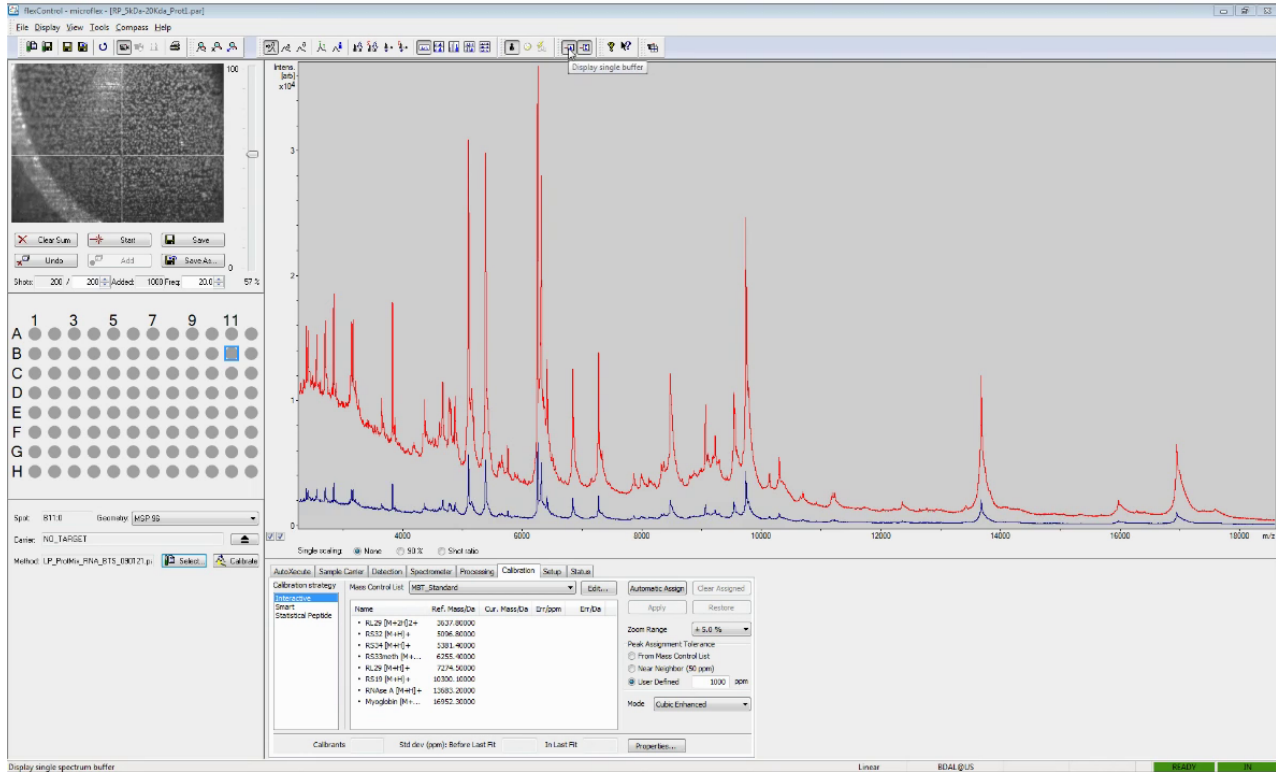
Step 4.1.3.2 (continued)



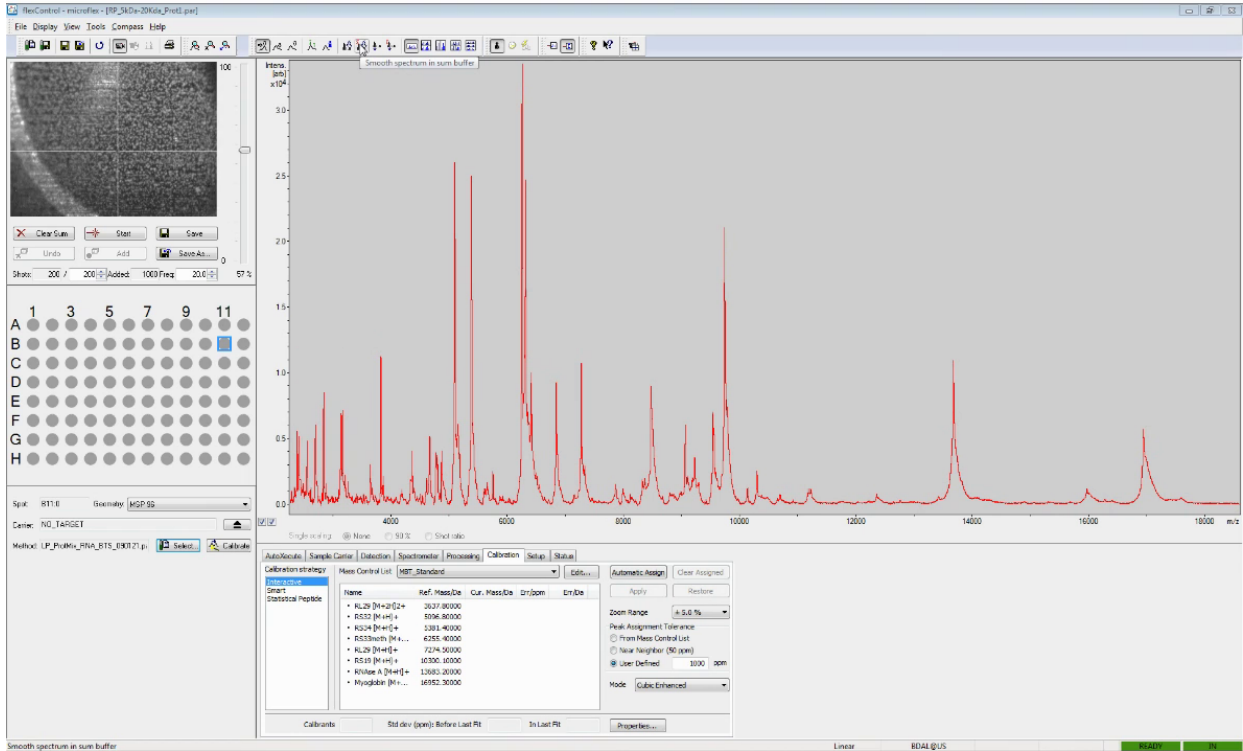
Step 4.1.3.2 (continued)



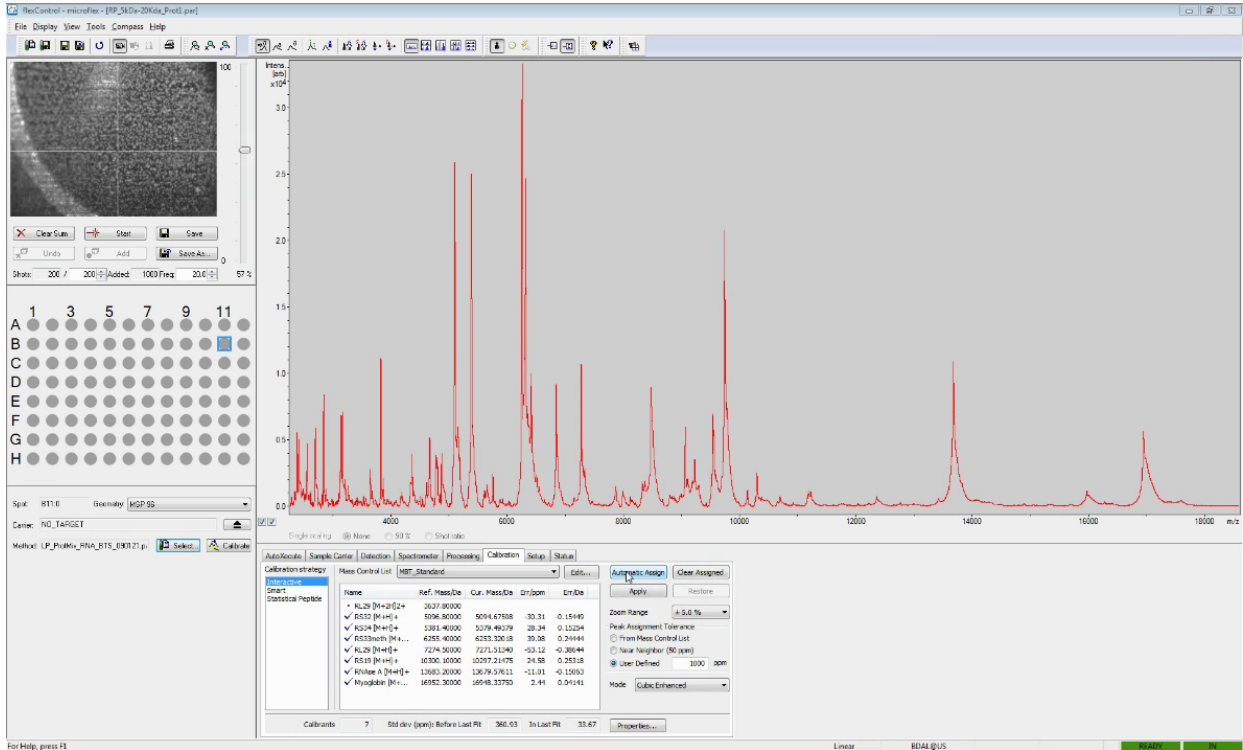
Step 4.1.3.2 (continued)



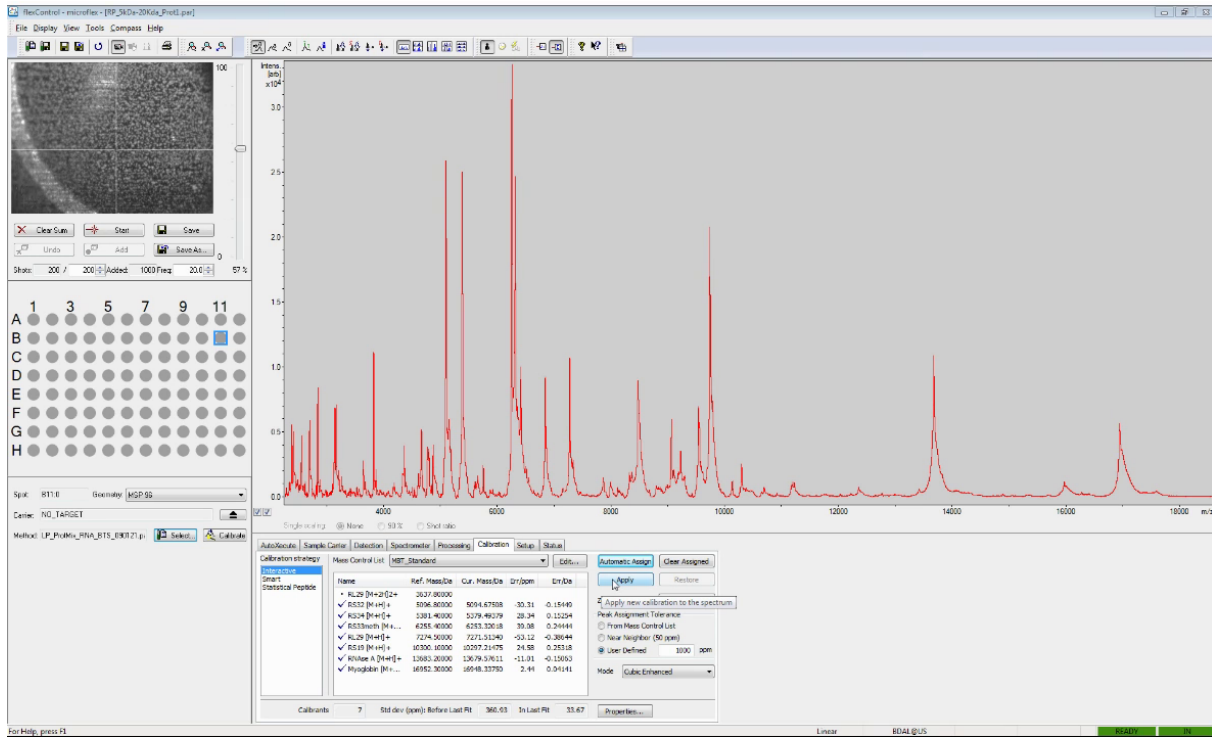
Step 4.1.3.2 (continued)



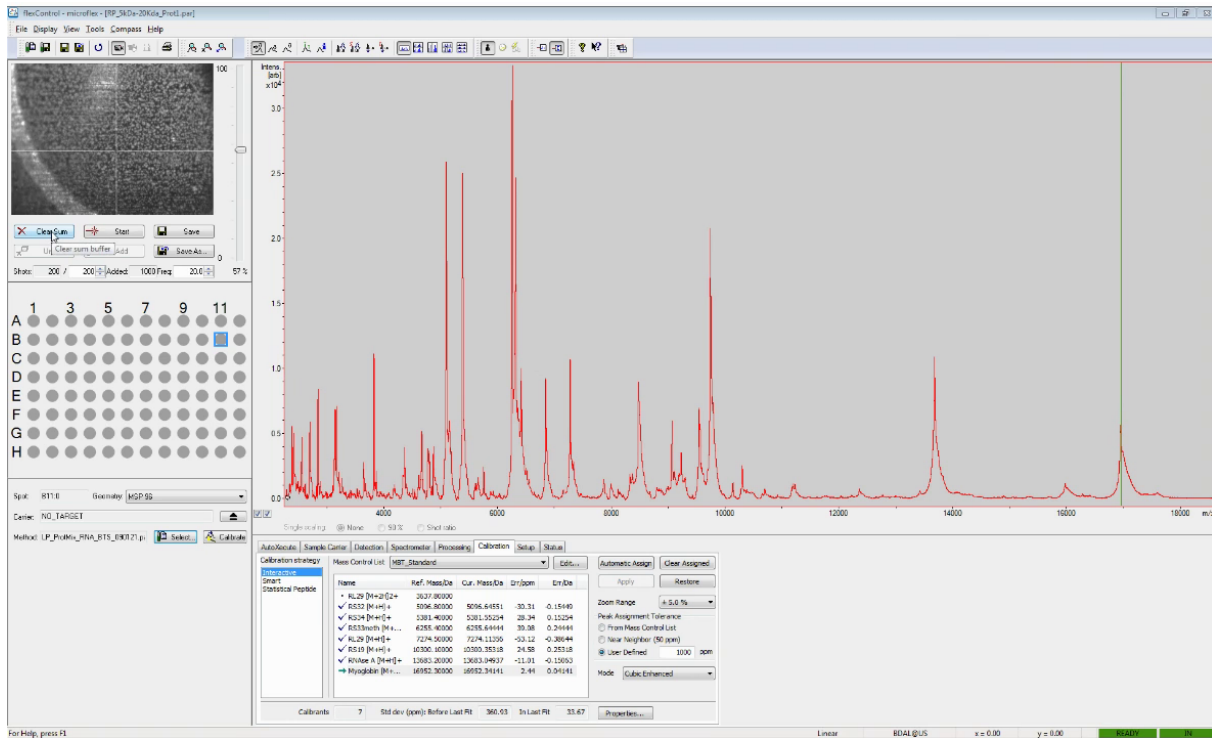
Step 4.1.3.3.



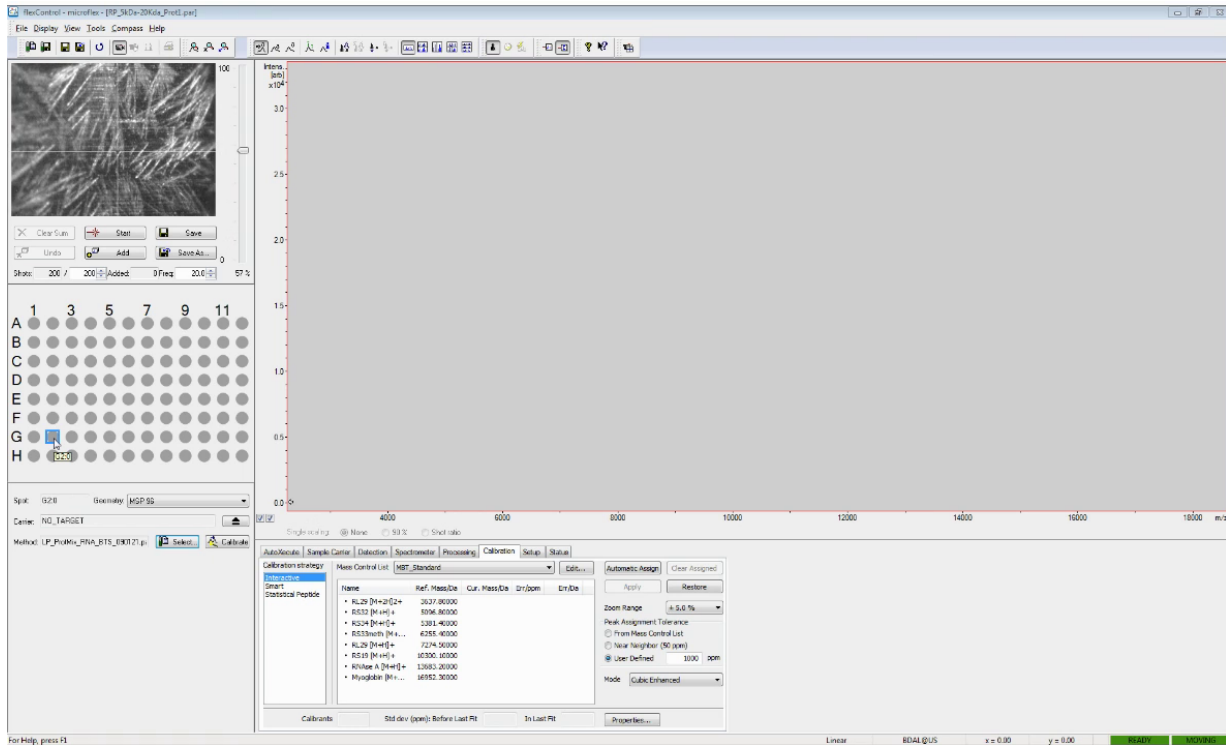
Step 4.1.3.3 (continued)



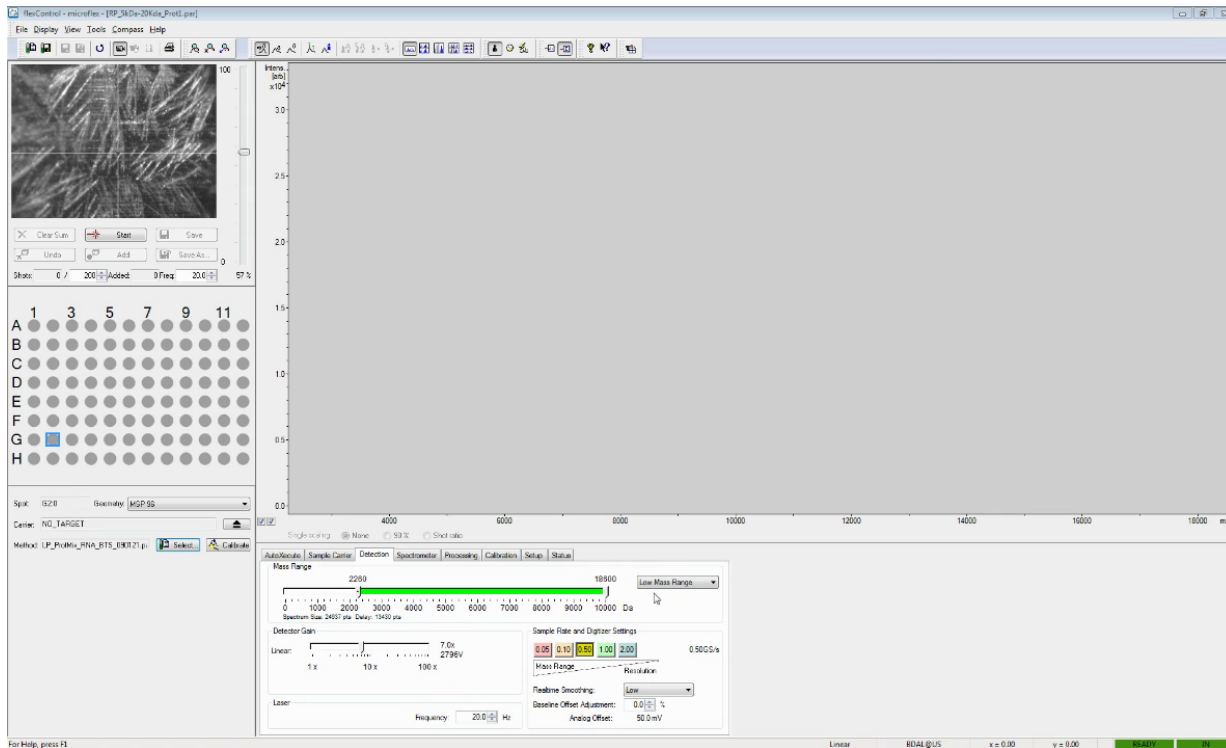
Step 4.1.3.4



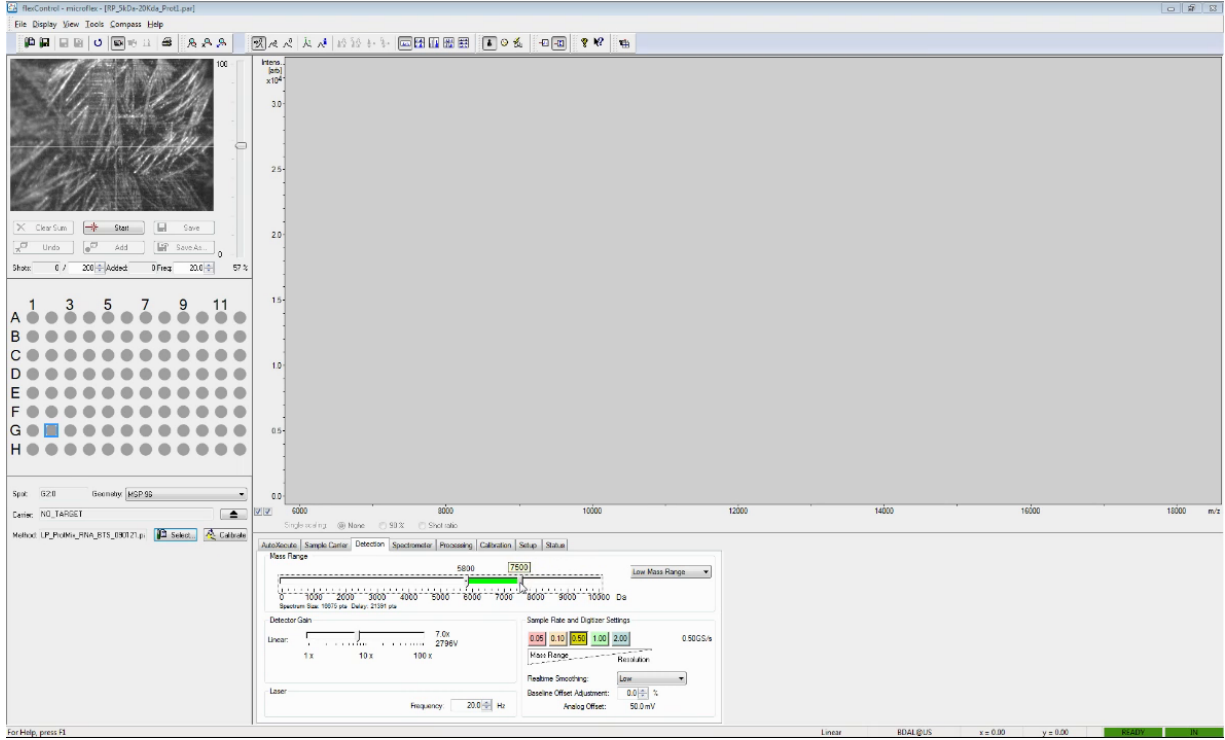
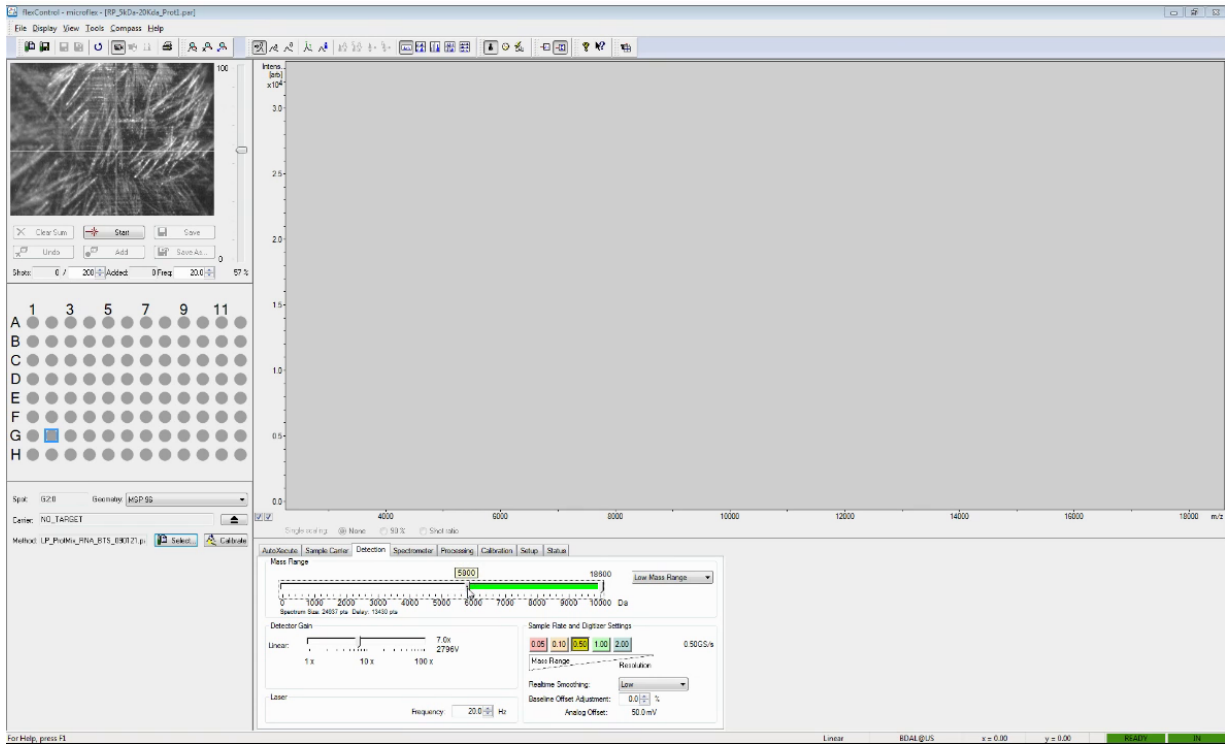
Step 4.1.3.4 (continued)



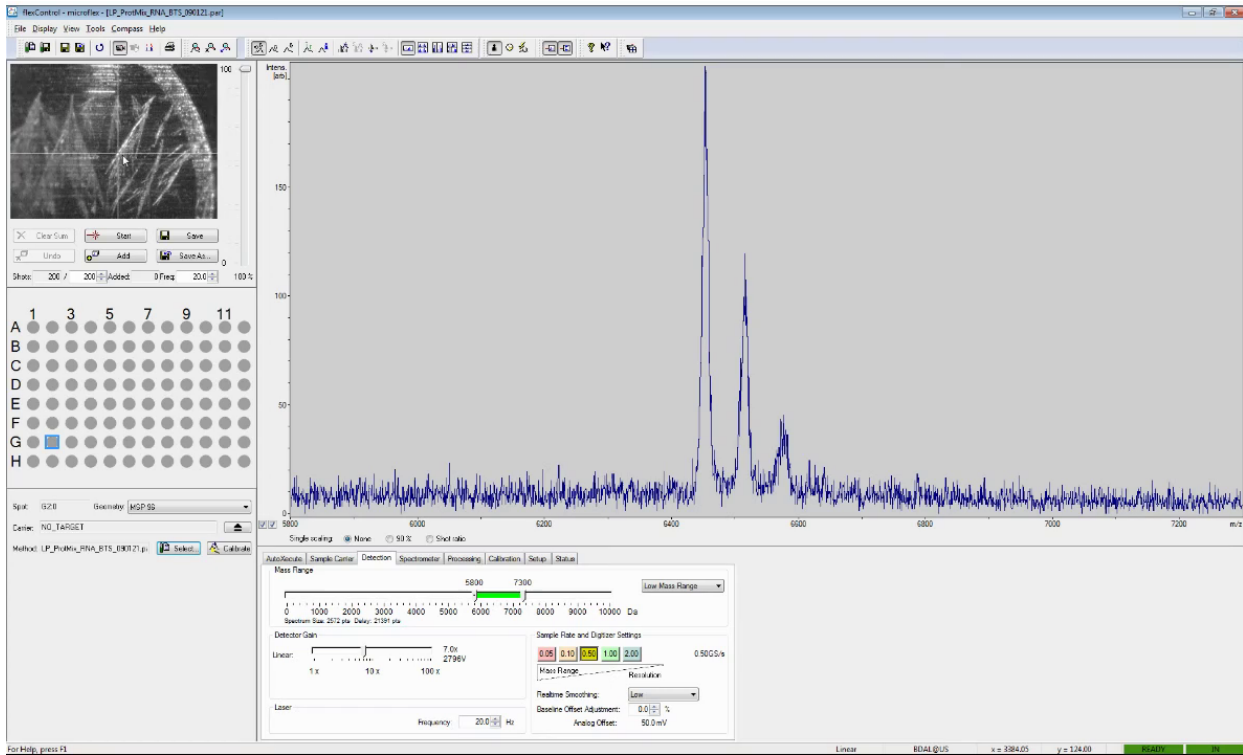
Step 4.1.3.5.



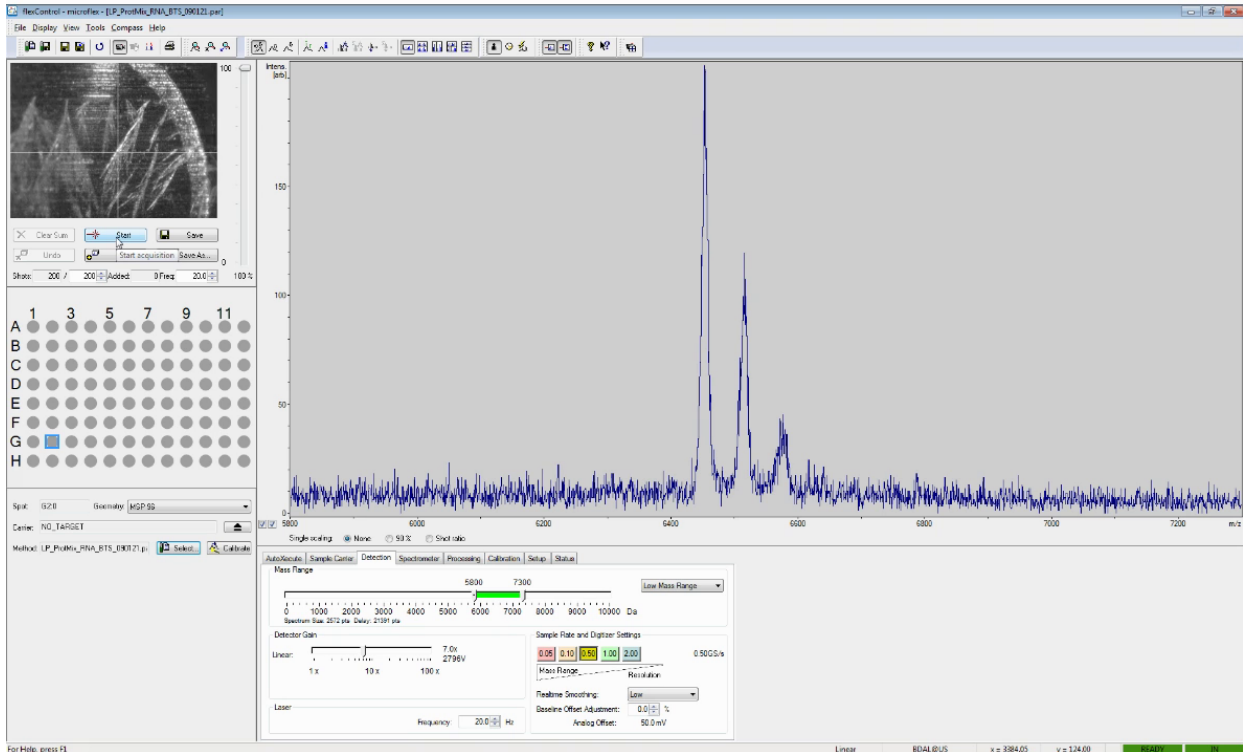
Step 4.1.3.5. (continued)



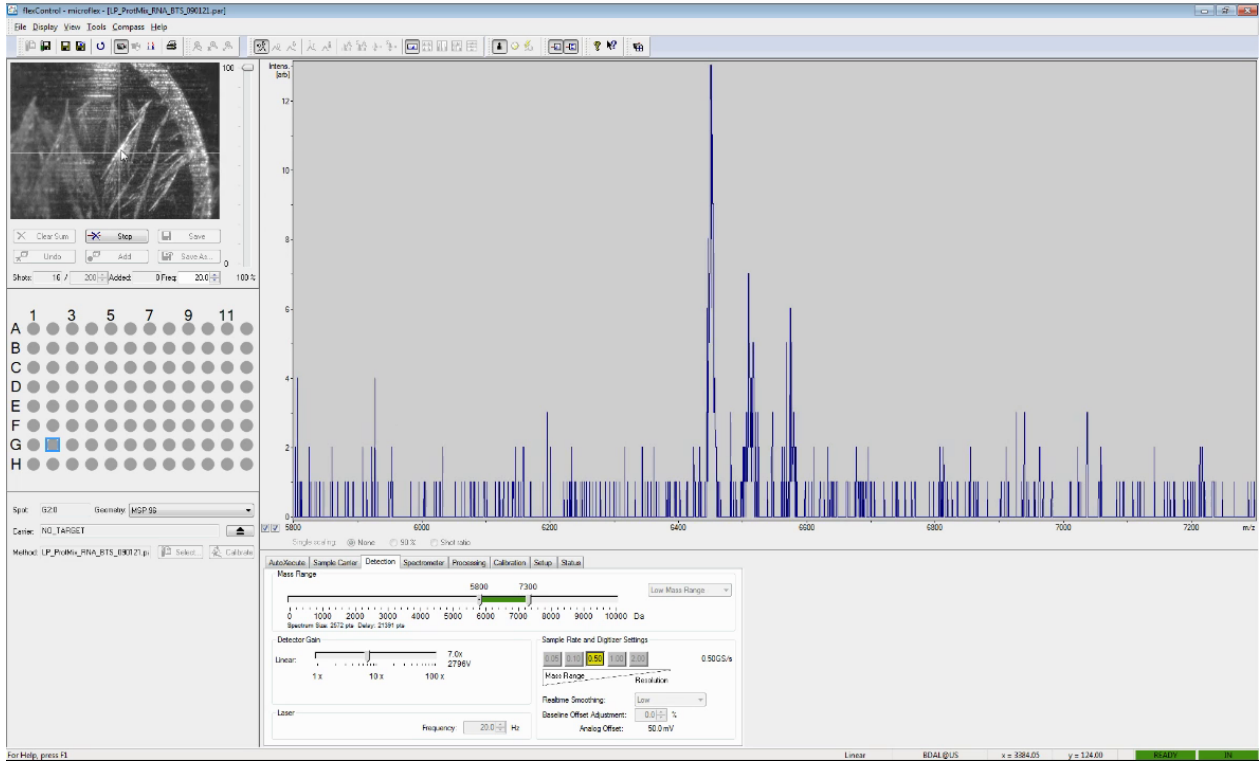
Steps 4.1.3.6 – 4.1.3.7.



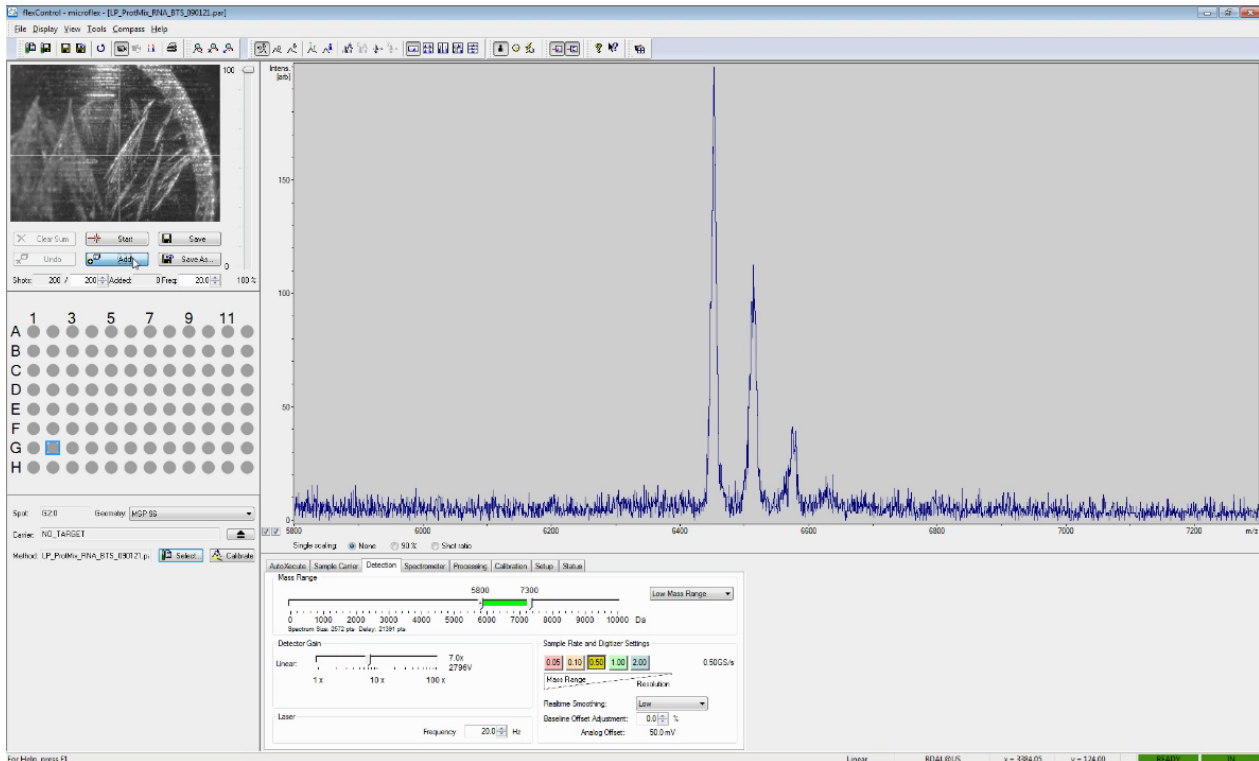
Step 4.1.4.1



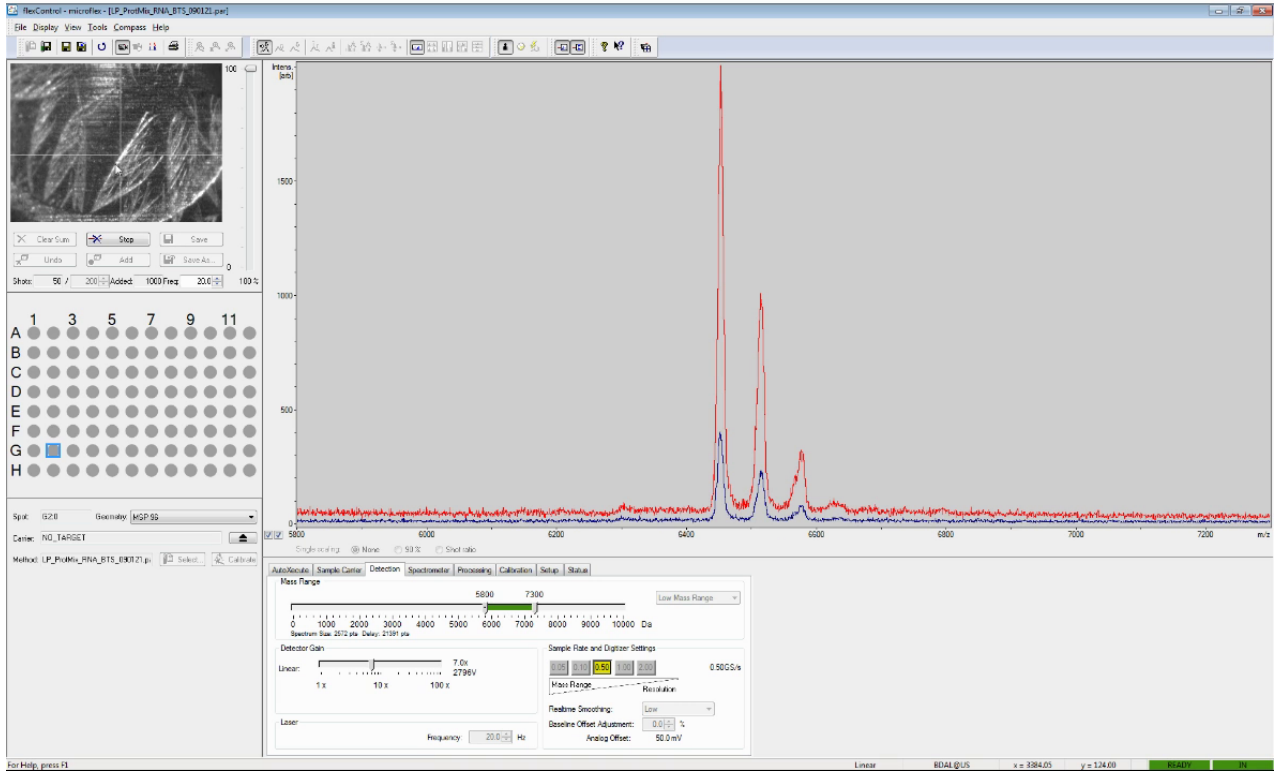
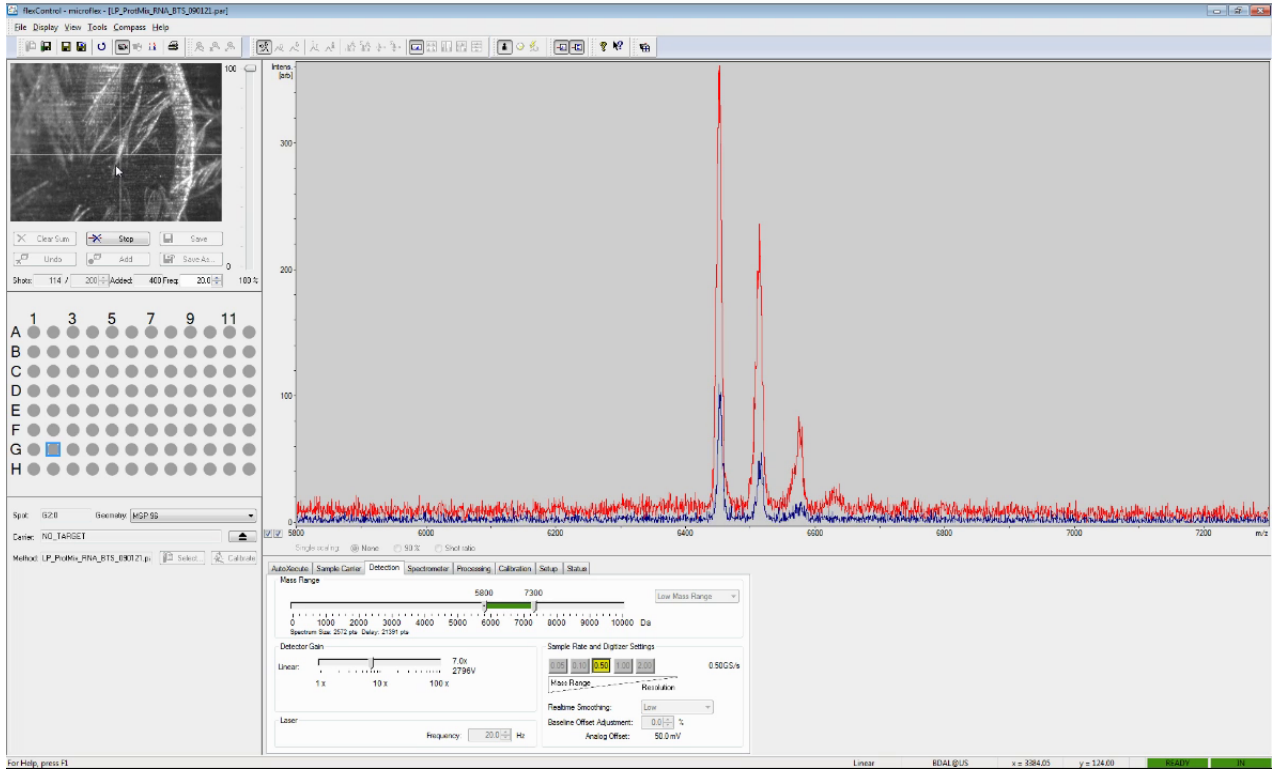
Step 4.1.4.1 (continued)



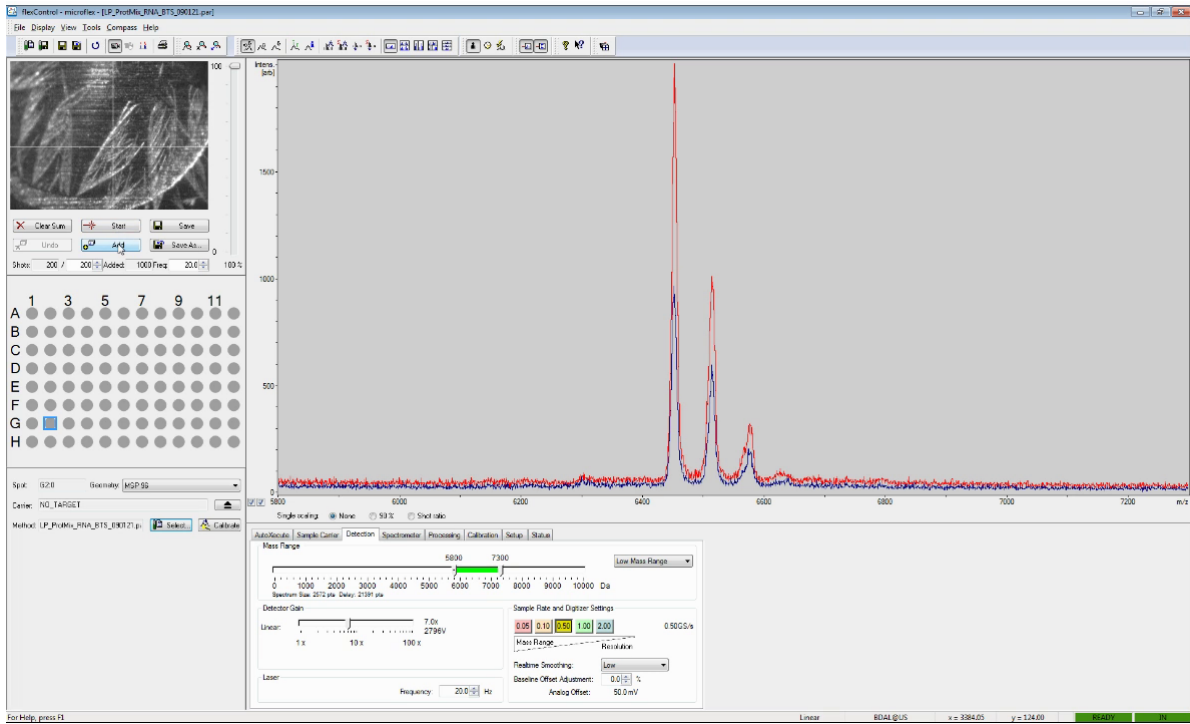
Step 4.1.4.2.



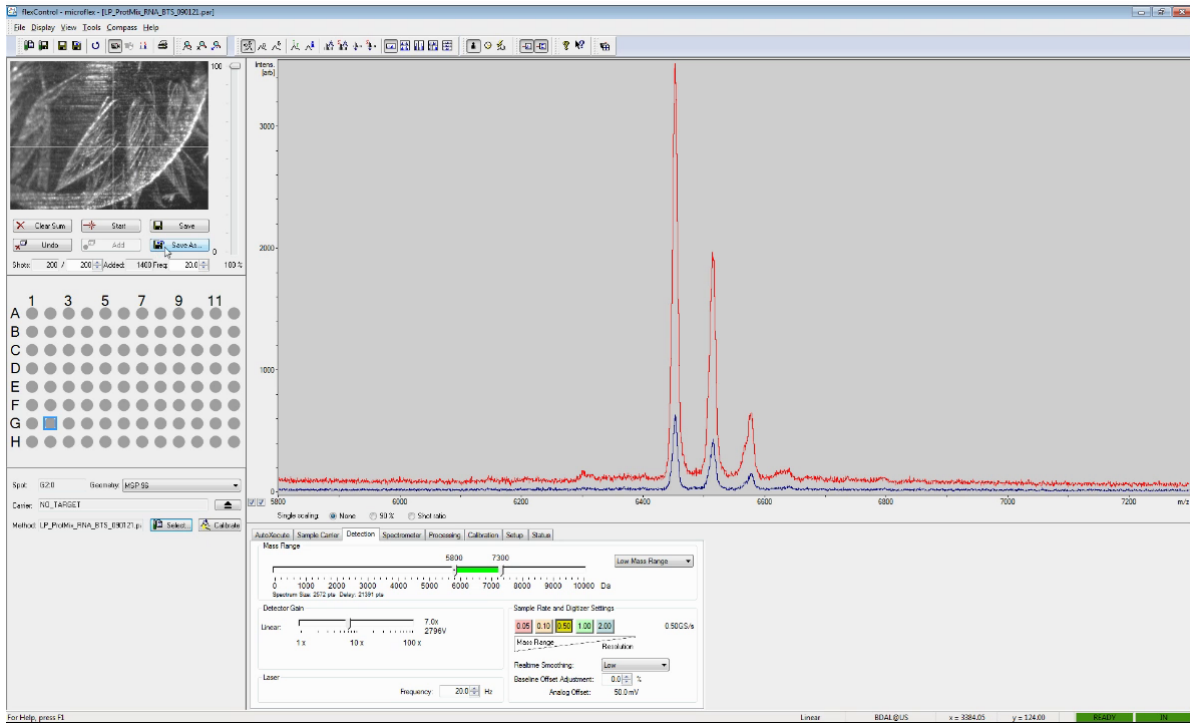
Step 4.1.4.3



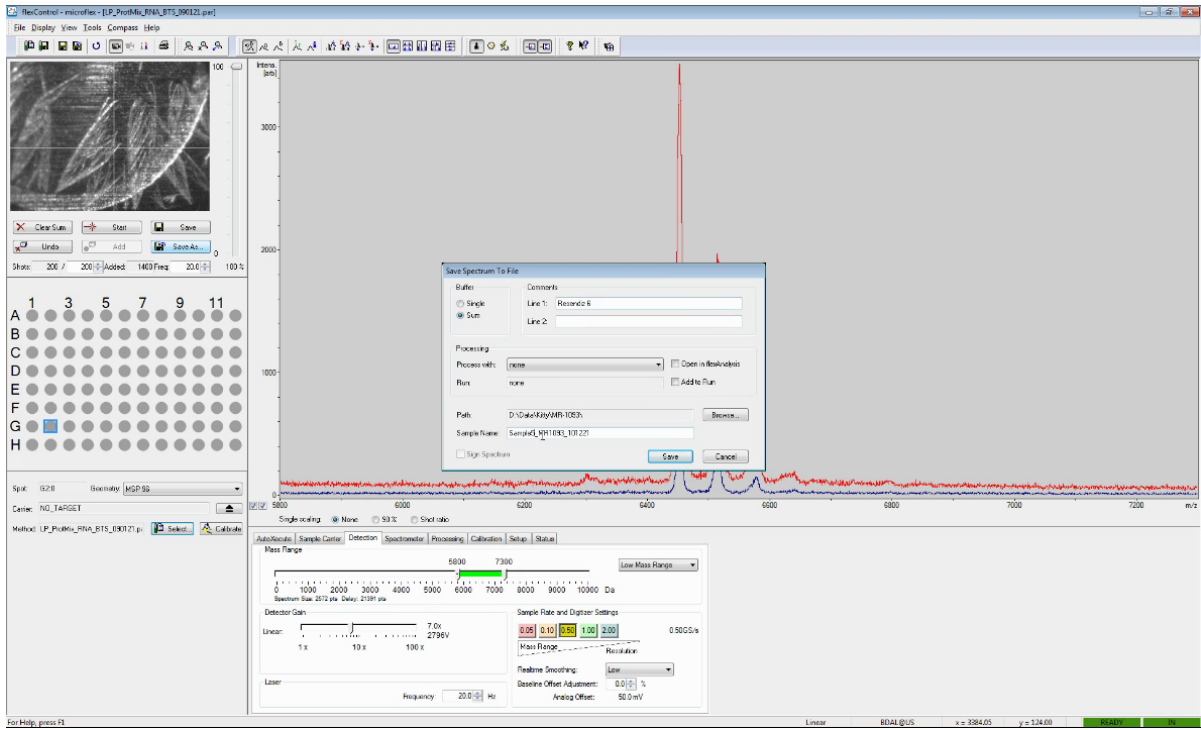
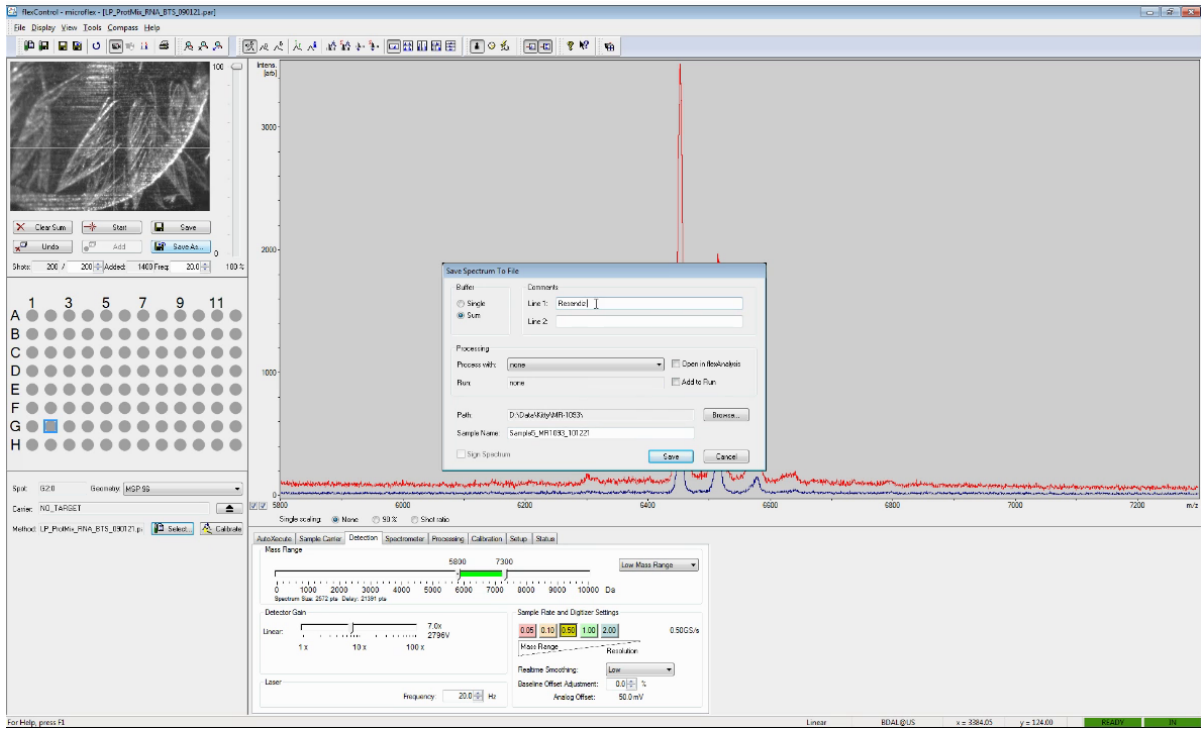
Step 4.1.4.3 (continued)



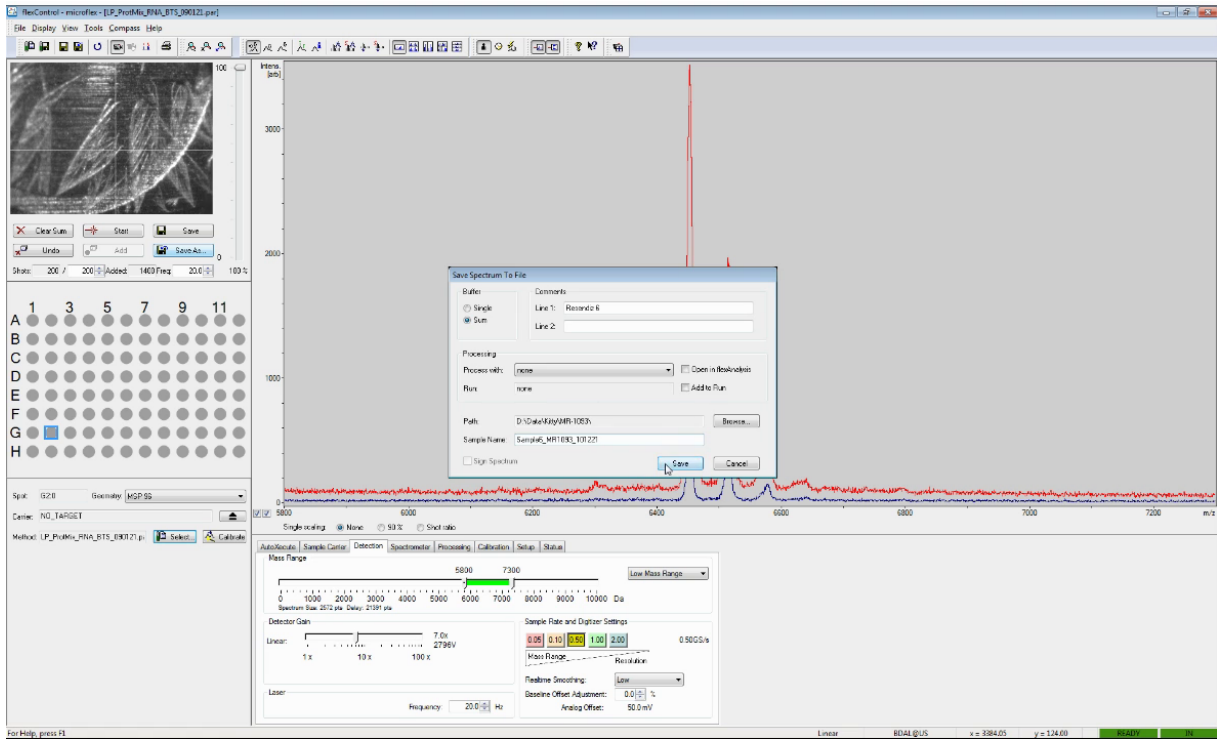
Step 4.1.4.5.



Step 4.1.4.5. (continued)

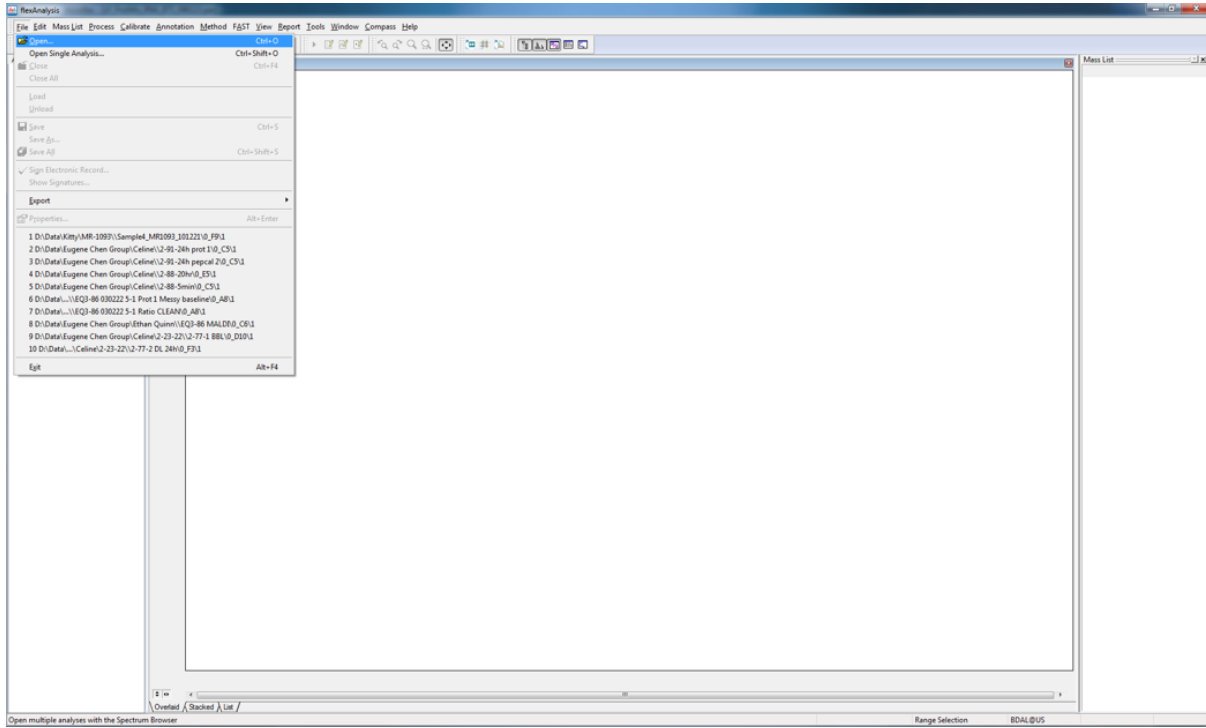


Step 4.1.4.5. (continued)

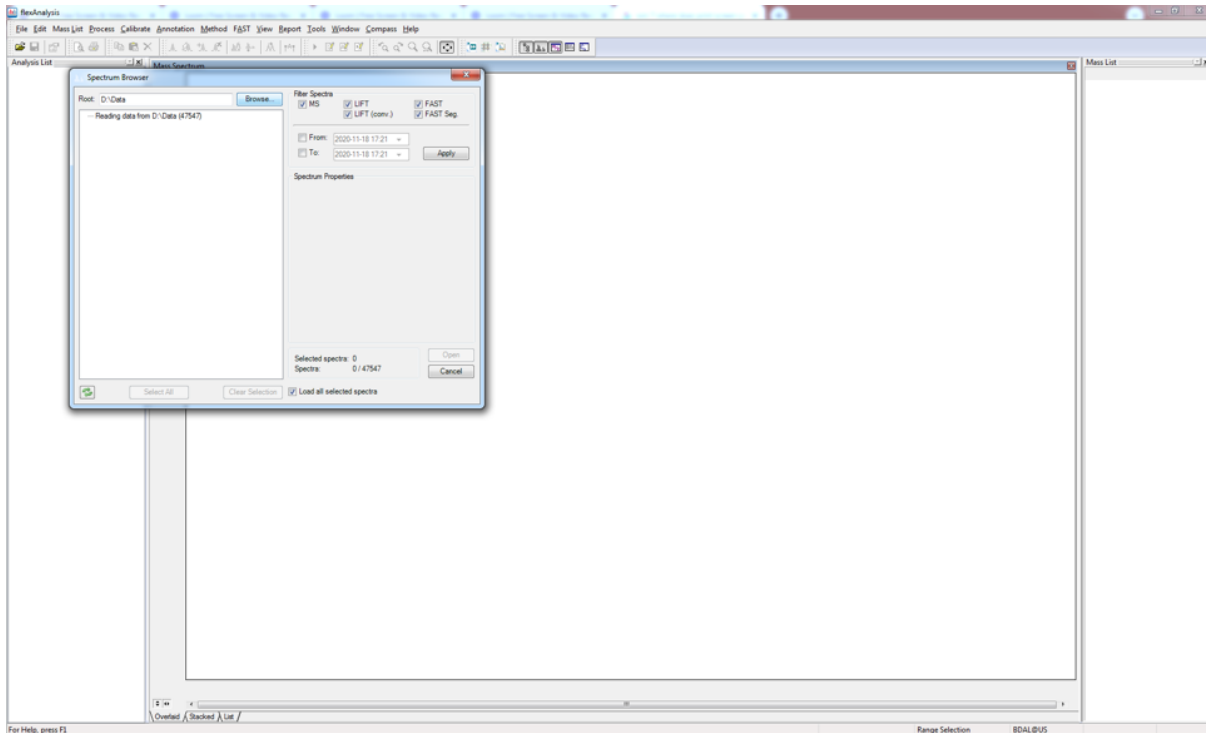


Step 4.2: Data processing

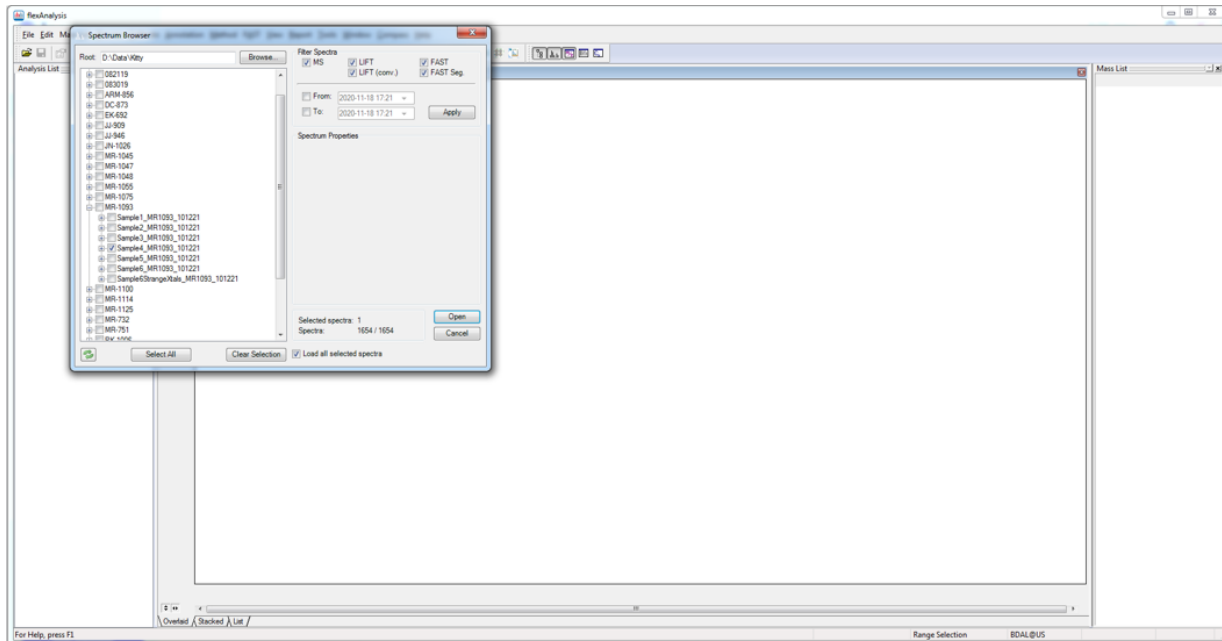
Step 4.2.1.



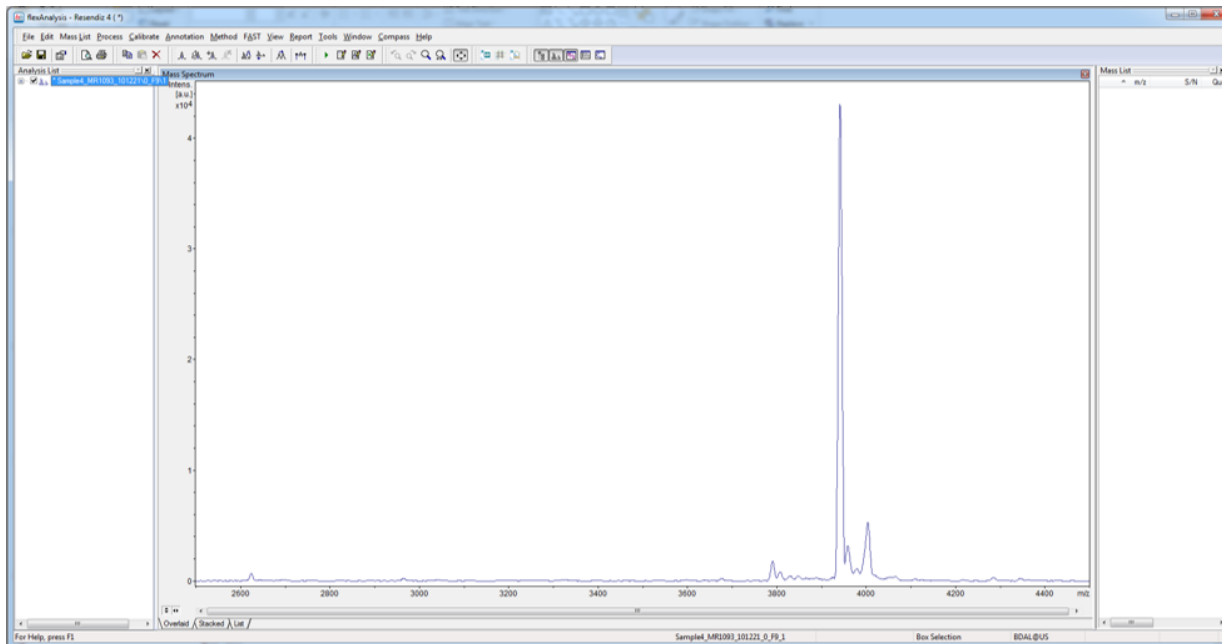
Step 4.2.2.



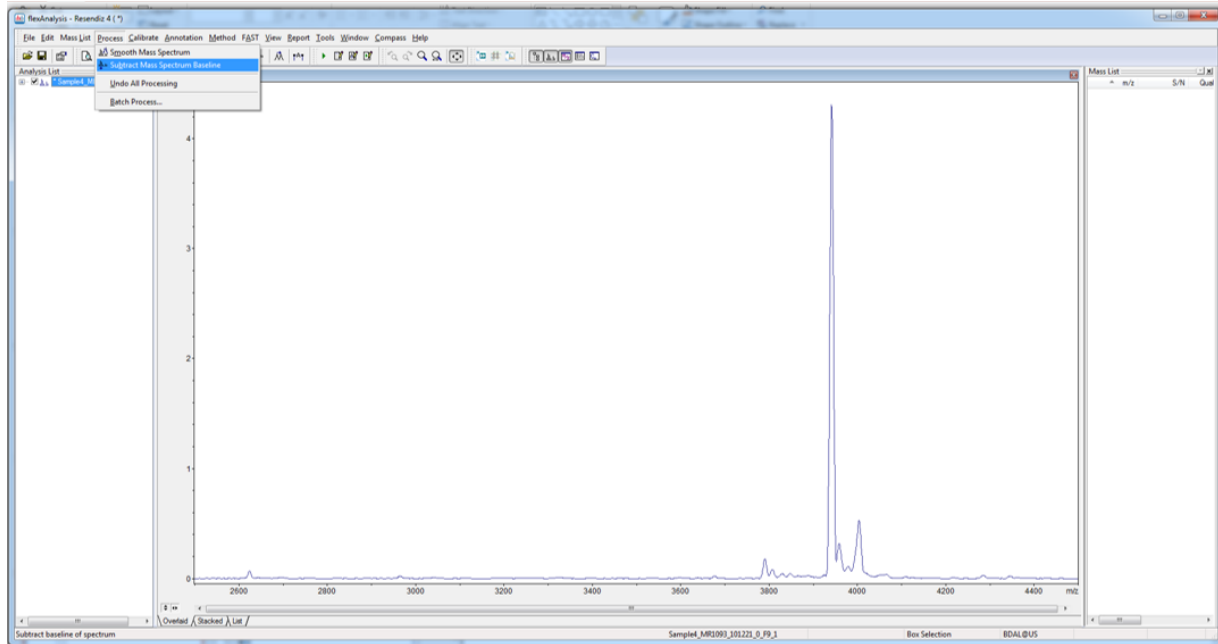
Step 4.2.2. (continued)



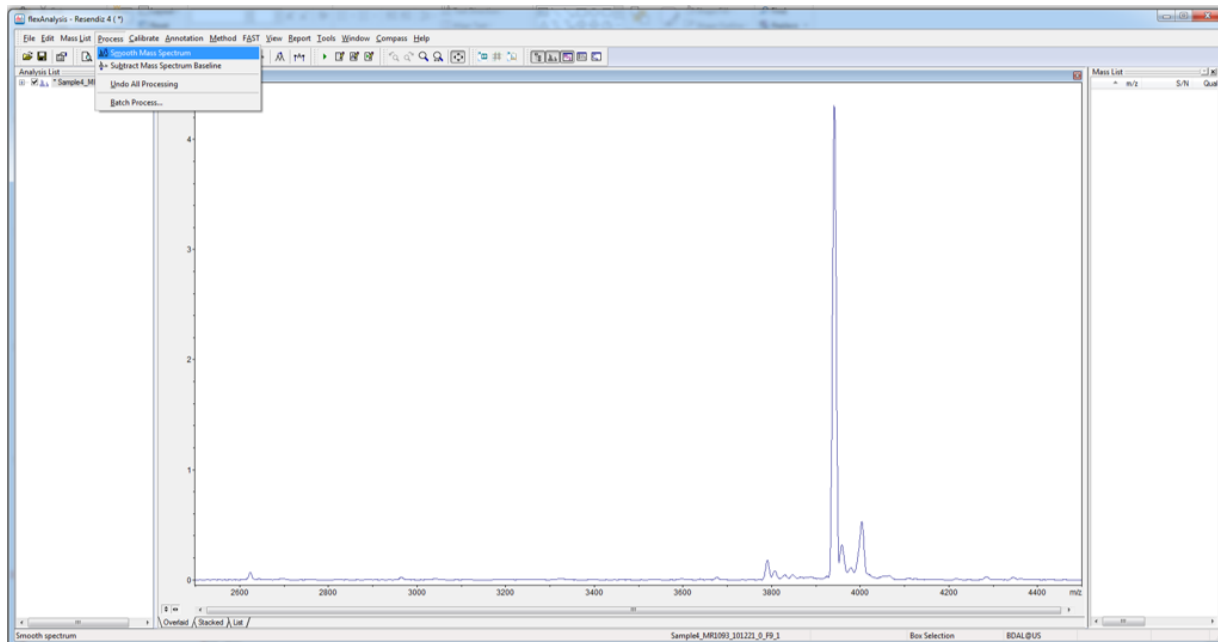
Step 4.2.3.



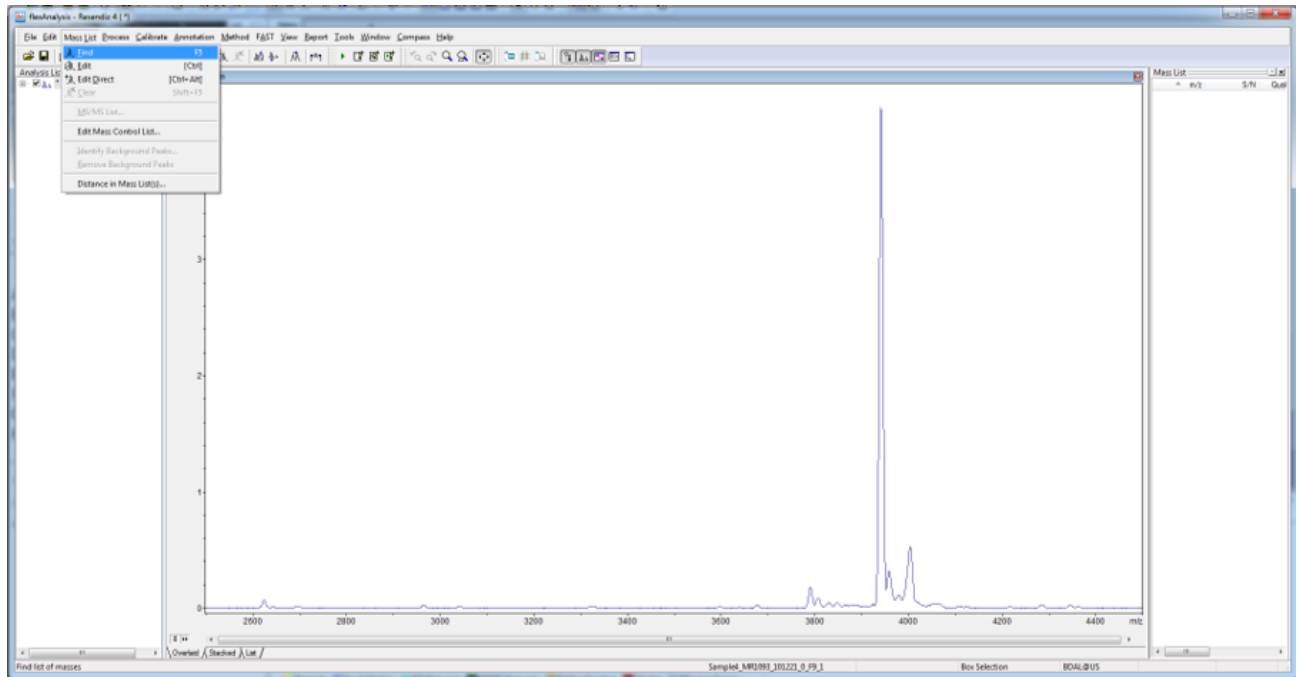
Step 4.2.3. (continued)



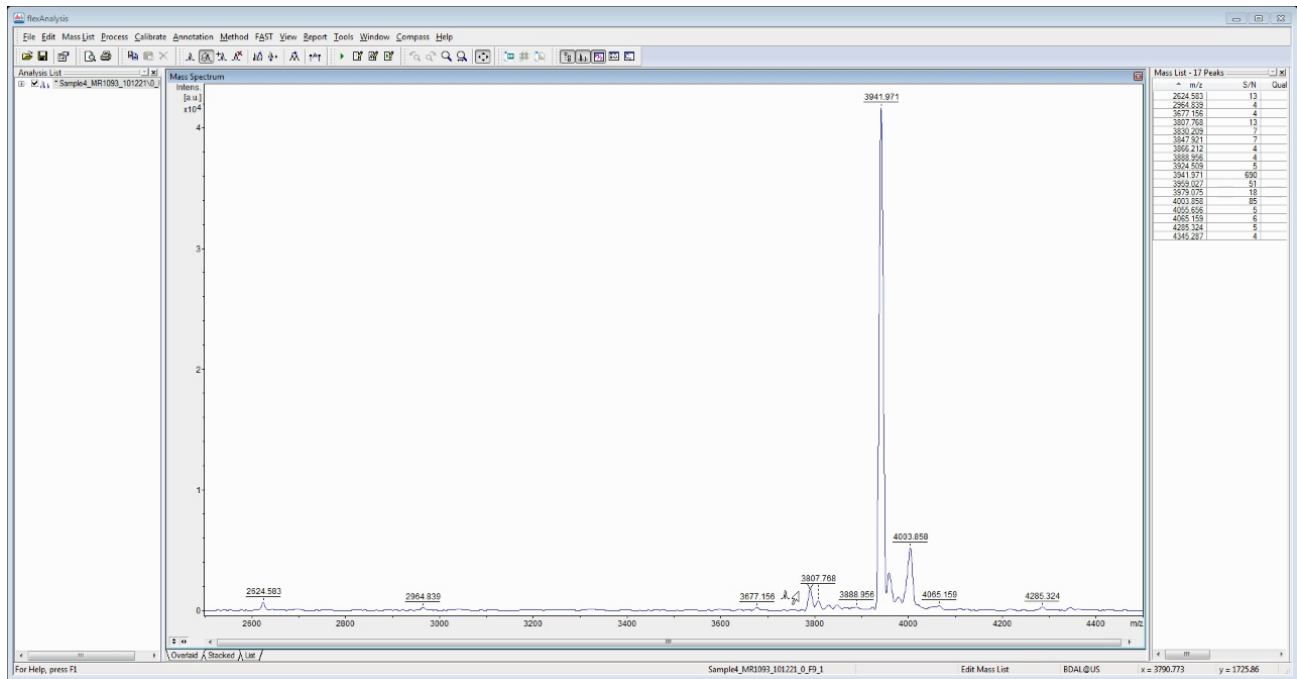
Step 4.2.4



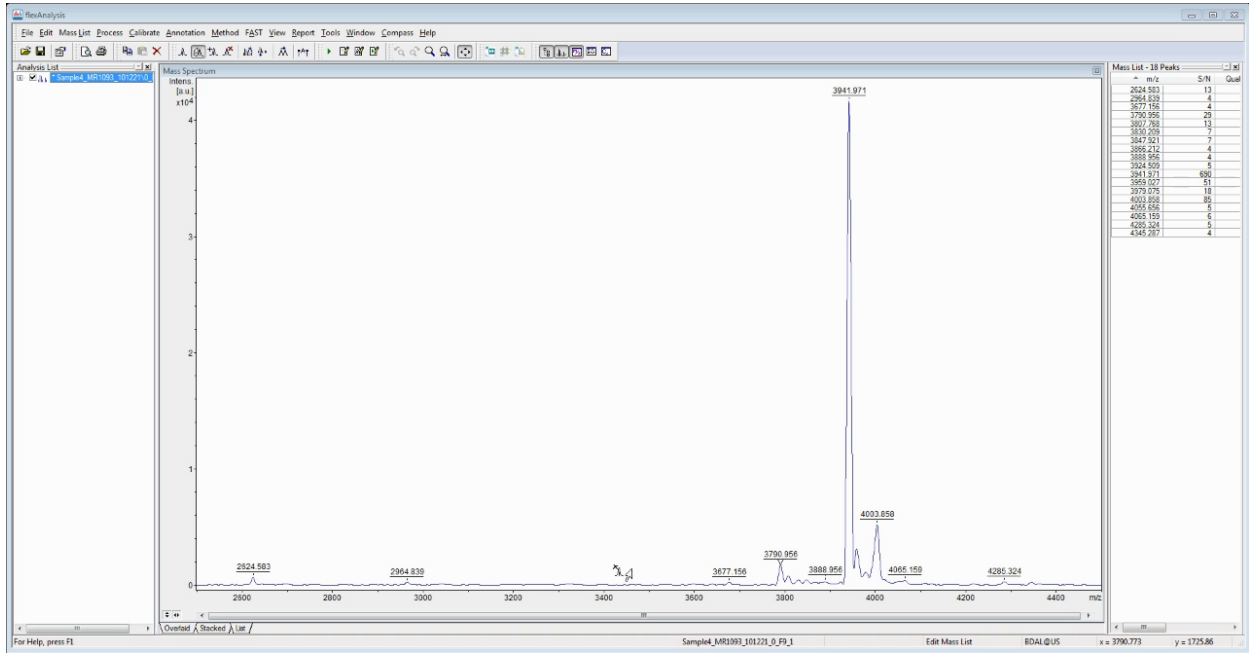
Step 4.2.5.



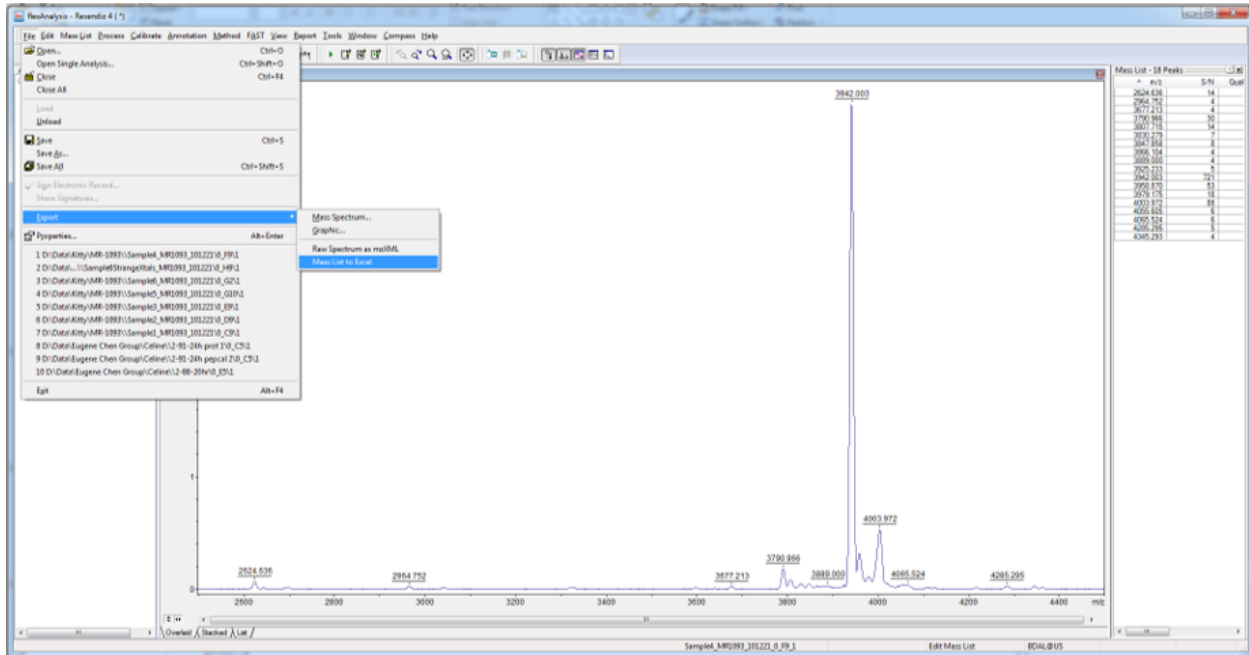
Step 4.2.7.



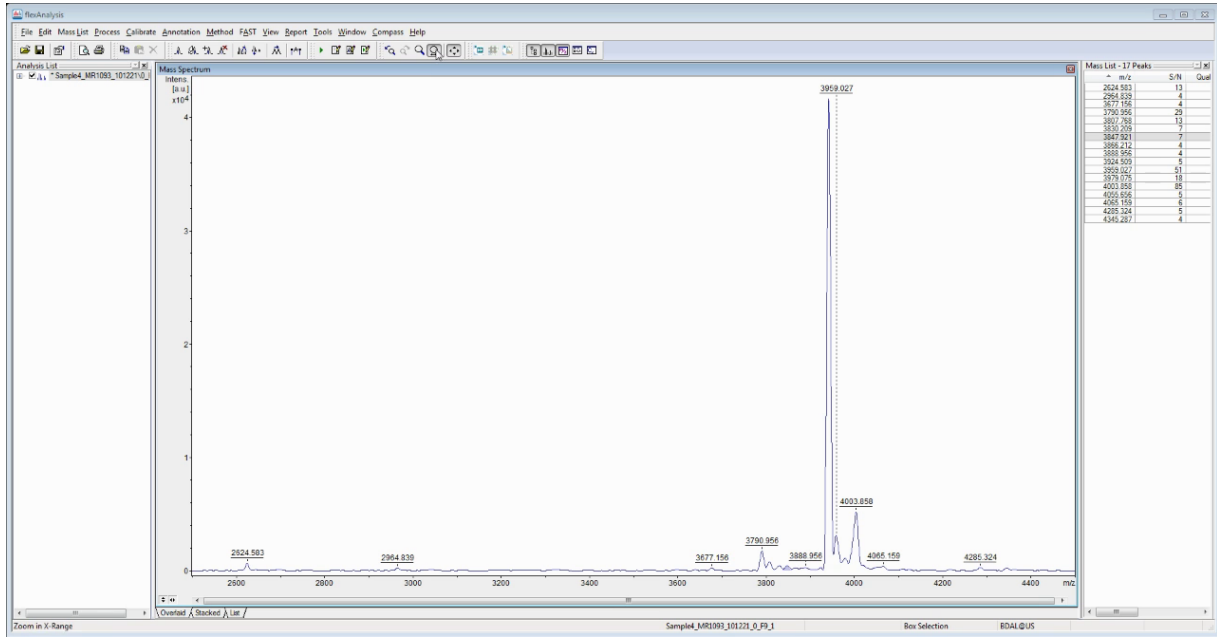
Step 4.2.7. (continued)



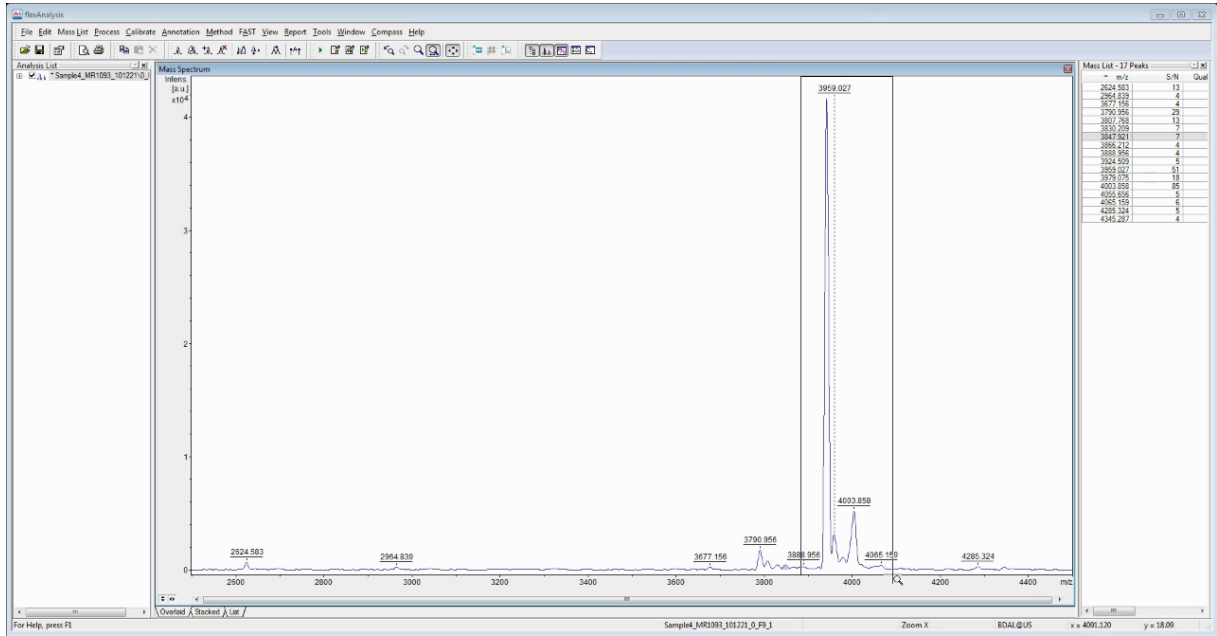
Step 4.2.8.



Step 4.2.10.1



Step 4.2.10.2



Step 4.2.10.3

