Sample Preparation

Sample preparation for MALDI will have a **major** impact on your data quality. Considerations include:

1) Sample concentration
    a. Concentrations depend on sample type, look in the literature for specific sample types
    b. Optimal concentration ranges from picomolar to micromolar levels
    c. If you are not sure what the optimal concentration to use is set up a dilution series and test multiple concentrations
2) Sample solvent
    a. Solvent should be able to fully dissolve sample and not react with steel plate
3) Salt concentration in sample
    a. High salt concentrations can cause ion suppression
    b. Addition of specific salts can help ionization; Ag, Cu or alkali salts
4) Matrix selection
    a. Different matrix types are better for different samples
    b. Check sample and matrix guide at the end of this document for a starting point
    c. SuperDHB is a good general matrix that works with most sample types if you need a starting point
5) Matrix concentration
    a. Concentration depends on matrix selected but can range from 5 mg/mL up to 40 mg/mL
    b. Standard concentration of superDHB is 20 mg/mL in a 1:1 v:v mixture of acetonitrile:H_2O with 0.1% TFA
6) Matrix Solvent
    a. Solvent should dissolve matrix and be miscible with sample solvent
7) Ratio of matrix to sample
    a. Standard spotting involves a 1:1 v/v ratio of sample and matrix
8) Spotting method
    a. **Comixing/dried droplet**: mix equal volumes of matrix and sample solutions then spot 1 microliter of solution onto a ground steel plate
    b. **Sandwich**: spot 1 microL of matrix solution, dry, spot 1 microL of sample solution, dry, spot 1 microL of matrix, dry

Calibration concerns and setup

The MALDI should be calibrated every time a user loads a new method for best results.

Selection of calibrant is dependent on the mass range of your analytes, ideally expected masses will be within the calibrant range.

The most common calibrants are Peptides for a mass range from 700 m/z to 3500 m/z and Proteins for a mass range of 5500 m/z to 17000 m/z. These are normally spotted on the instrumentation center plates and are preselected in the appropriate instrument methods. Other standard samples can be used for calibration if needed.

Calibration/Data Collection

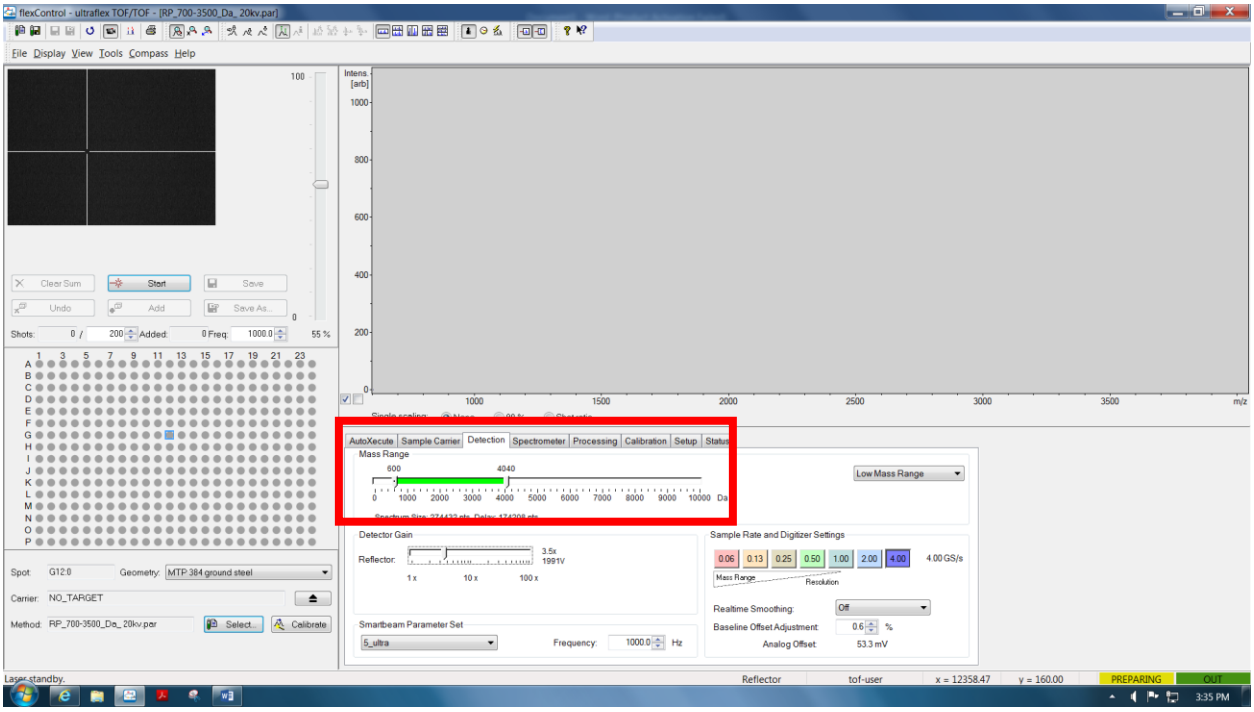
1) Open flexcontrol if it is closed

2) In bottom left corner of page click "Select" next to methods

3) A new popup screen will show up with all of the available methods
   a. RP = Reflectron positive mode
   b. RN = Reflectron negative mode
   c. LP = Linear positive mode
   d. LN = Linear negative mode
   e. The number range after the RP/RN/LP/LN describes the optimized mass range for that method

4) Select the method based on your expected sample mass and if you expect it to ionize as a positively charged ion or a negatively charged ion

5) Once the method is selected go to the **detection** tab



6) Select the desired mass range for your sample by dragging the arrows to the appropriate spots
   a. The mass range should cover both the expected mass of your compound and a large enough range to cover enough calibration points

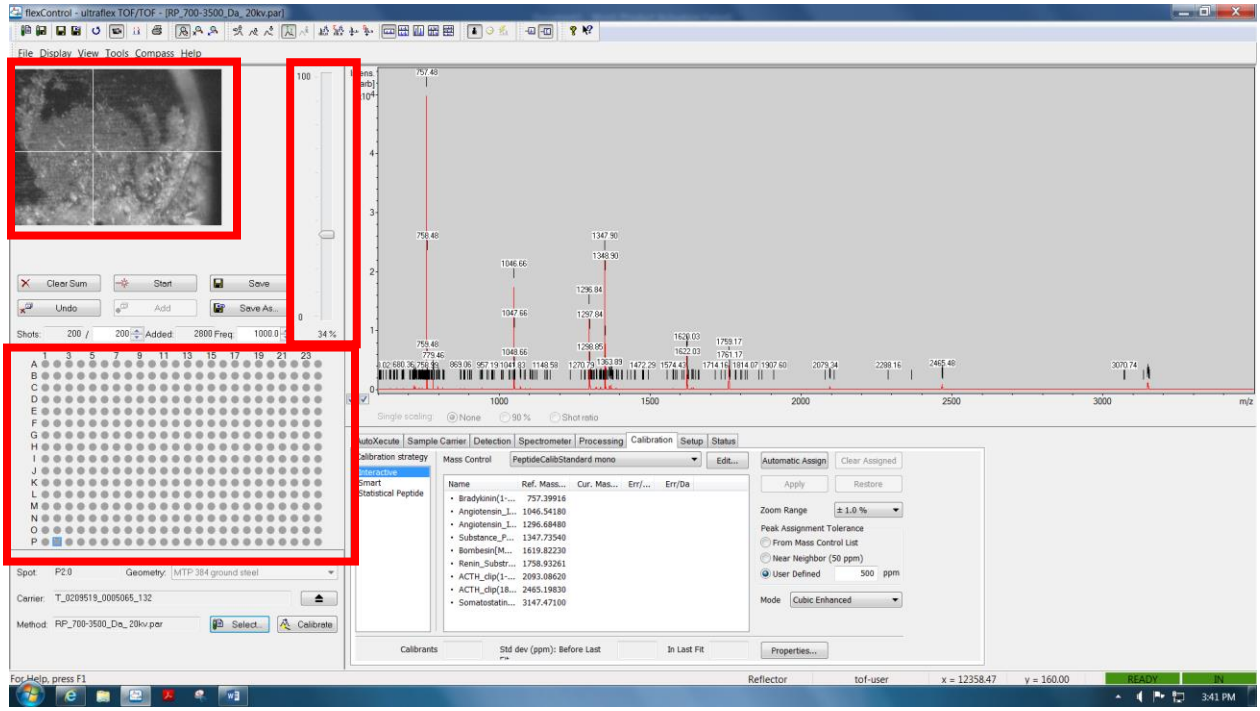
b. The number of calibration points needed varies based on what calibration method you are using and how accurate the measurement needs to be
    i. The cubic enhanced method requires 7 good calibration peaks
    ii. The quadratic method requires at least 3
    iii. The linear method requires at least 2.
c. To calibrate in reflectron mode use monoisotopic lists
d. To calibrate in linear mode use average mass lists

7) On the plate grid click on the spot where calibration standard is spotted to move the plate

8) Once the plate has stopped moving click on the IR camera screen to move the sample plate small distances and center the crystallized sample/matrix in the white crosshairs

9) To shoot the sample click on the **Start** button and wait for the laser to fire, this will be visible on the IR camera screen

10) Laser power can be controlled by the sliding bar next to the IR camera video



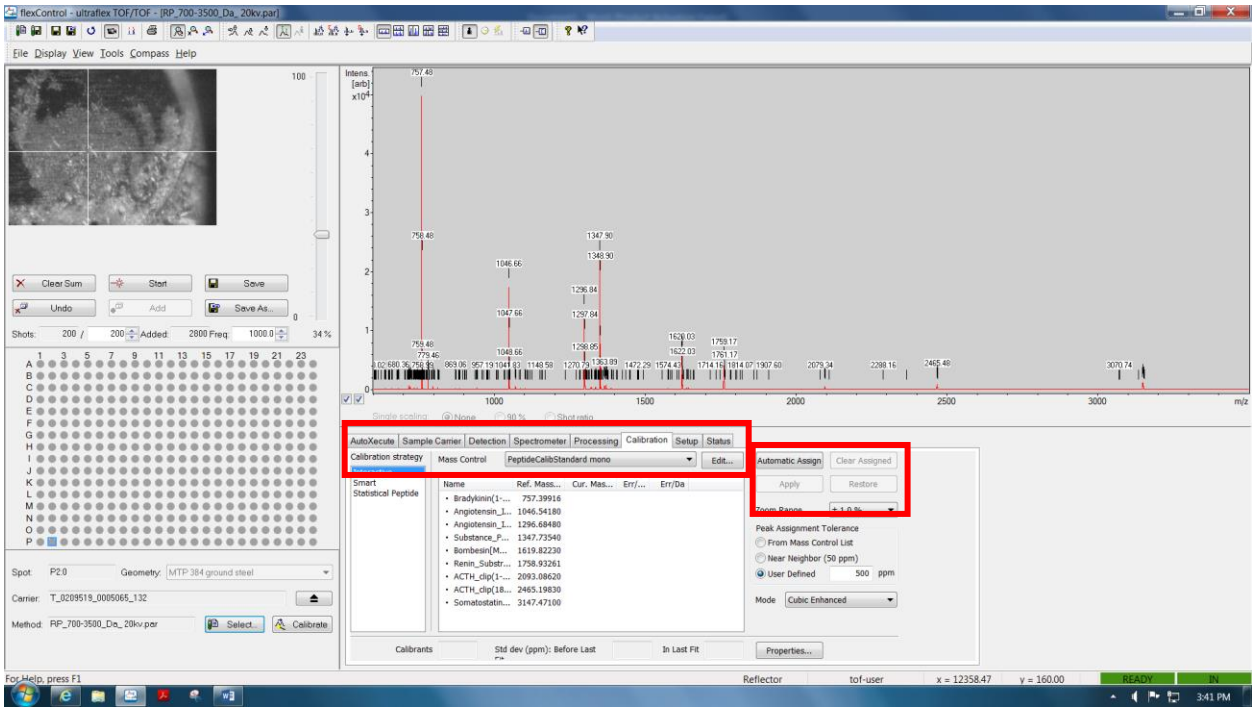
11) On the spectra screen a signal will appear as a blue line
a. If there are no distinct peaks try another area of the sample before slowly increasing the laser power
b. Do not increase laser power over 90%
c. If the sample is in the crosshairs and is ionizing well a spectra with a mostly flat baseline and distinct peaks will appear

12) To improve signal quality click **Add**, button under the laser **Start** button, then move to another location and collect another spectra before clicking **Add** again

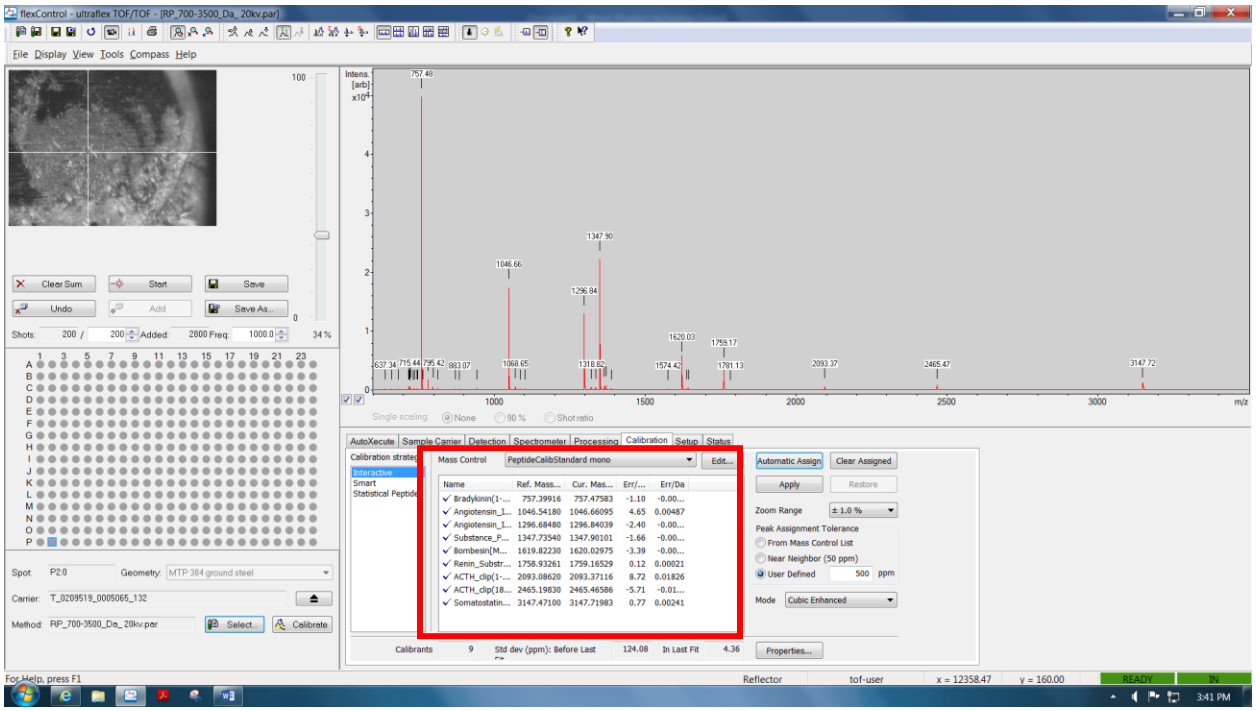
a. Repeat the shoot and add process until you have good signal to noise, with calibrants this will only take 2-3 shots while research samples may require more
b. Not all shots have to be added, if the collected spectra does not have a good signal you can just move to a different spot

13) When calibrating click on the **Calibration** tab and then click on **Automatic Assign**

a. Each method has a default **Mass Control** list that will be selected
b. If you need a different calibrant click on the drop down menu and select the calibrant being used

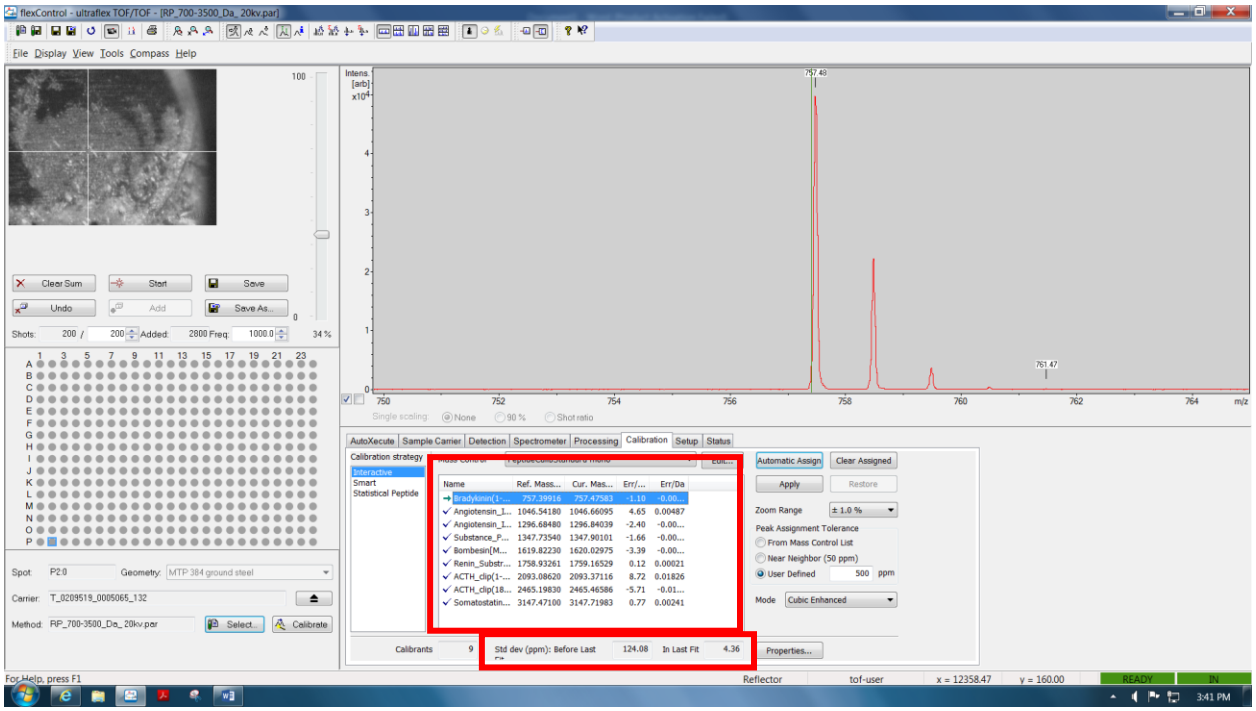


14) After clicking **Automatic Assign** check marks will appear next to all calibrants that the program found



15) Click on the first calibrant in the list to see the assigned peak

16) On the spectra click to the bottom left of the first isotopic peak to select the peak



17) Repeat 14 and 15 for each calibrant

18) Once each calibrant has been checked click on **Apply** under **Automatic Assign** to apply the calibration

19) Check the ppm value by **In Last Fit**

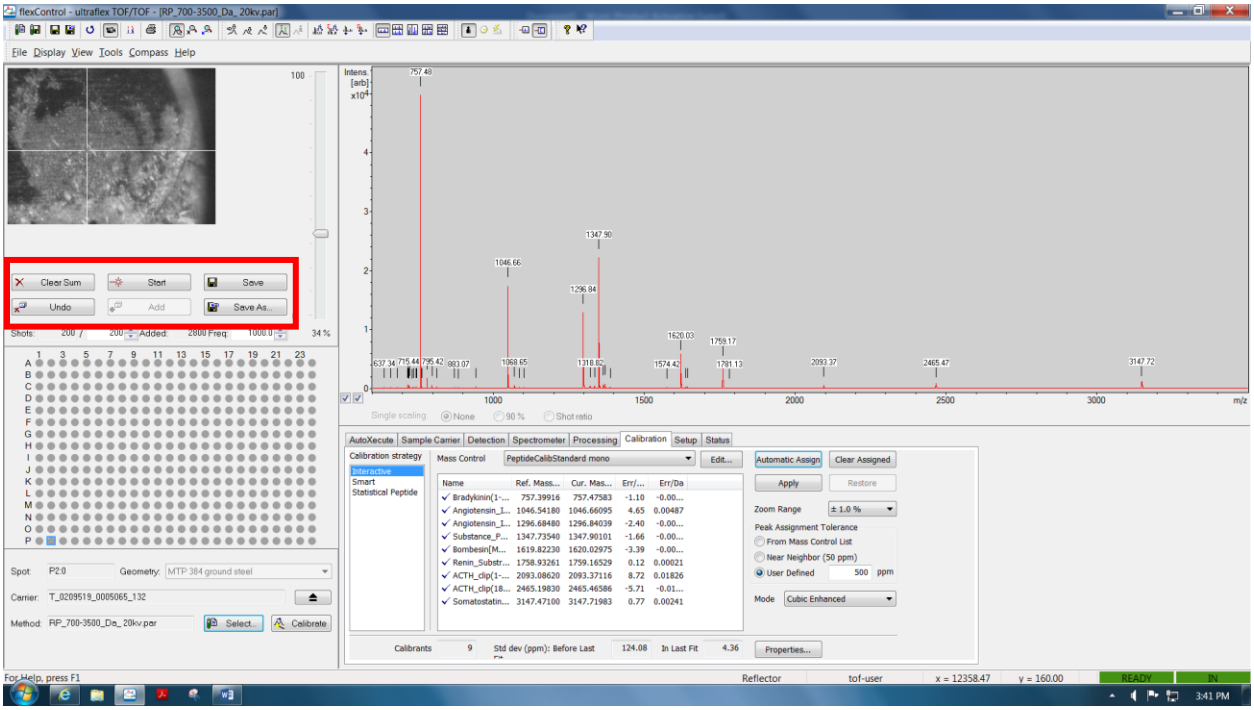
a. For reflectron mode this value should be below 10 ppm
b. For linear mode this value should be below 50 ppm

20) After calibration move plate to where your sample is spotted then collect data as outlined in steps **8** through **12**

21) After collected data save spectra by clicking **Save As**

a. After clicking on **Save As** a pop up will appear
b. Select the folder that you want to save your data in
c. Change the sample name to something you will recognize
d. Click **Save**

22) To move on to another sample click **Clear Sum** to clear the sum spectra before collecting data on the next sample



Matrix Guide to Sample Preparation