Information and Examples for AP-MALDI Zoom Mode Using the Target Software (Version 7)

Revised: November 21, 2014

Introduction

A new feature, the AP-MALDI zoom mode, is introduced in Version 7 of the Target Software. The goal of the software is to:

1. Give the user more flexibility and options to run both the Target and MS Software together.
2. Create position information with the necessary details to create a map of ion signals.
3. Export the position information for the post processing of the data.

The description of the test procedure (in the Motion Modes section below) introduces the user to the capabilities of the AP-MALDI zoom mode.

Motion Modes: CSR and Pixel Map

The two different options that can be used for the AP-MALDI zoom mode include the constant speed raster motion (CSR) and pixel map, which can be seen in Figure 1. Both options have two different scan patterns that are available for use, which include the flyback and meandering modes displayed in Figure 2. The flyback mode includes line scans that move in the same direction (horizontally and vertically), while the meandering mode's line scans move in alternating directions so that the total scan time is minimal.

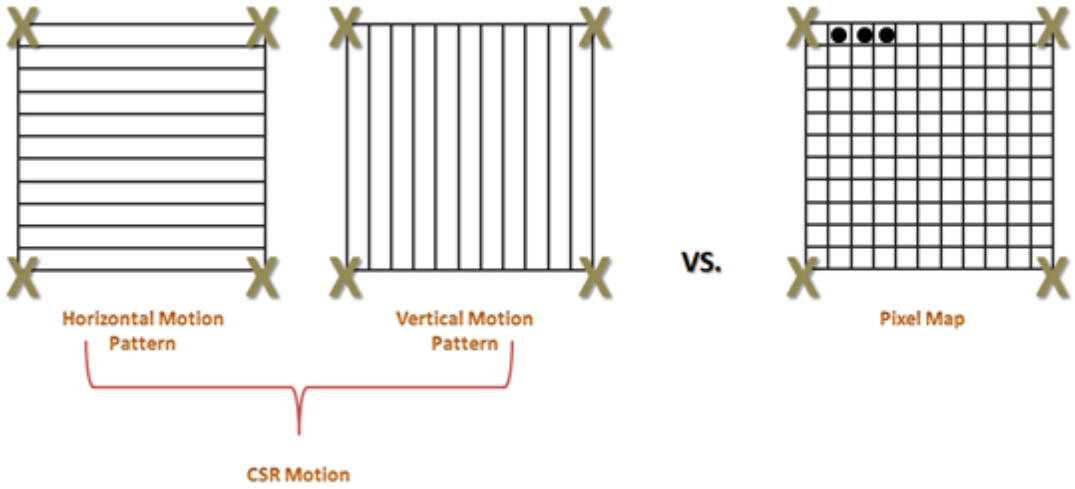

Figure 1 CSR Mode vs. Pixel Map Mode

Note: In the CSR mode, the laser fires constantly as the stage moves (except during transitions from one row/column) to the next. In thepixel map mode,tThe laser only fires within the pixel points.

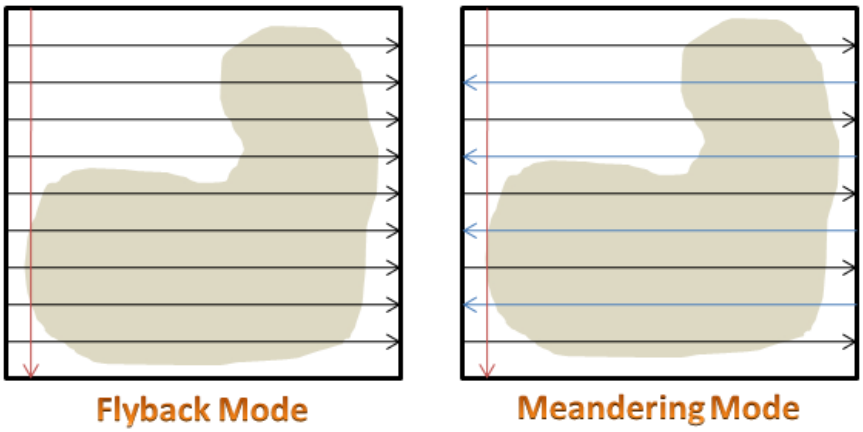

Figure 2 Flyback Mode vs. Meandering Mode

Based on the specific needs of the sample and available instrumentation, the user needs to decide which zoom mode should to be used. When making this decision, the user should consider the following preliminaries:

1. If the number of pixels are larger than the number of data files that the MS Software (e.g. XCalibur, Analyst, MassHunter) can handle, the CSR mode should be used. Each line will be a separate file.
2. The availability of the handshaking option (available in the Thermo Q Exactive Instrument Software) enables the use of one file for all the pixels. The use of such instruments creates a very efficient setup for the pixel map mode.
3. The scan time cannot be too long, as each pixel needs at least one mass spectrum per scan. If there is no mass spectrum available for a given pixel, the result of the scan will show an empty pixel (i.e. the number of scans is greater than the number of pixels). The amount of time the laser will fire To tune the pixel, the overall scan time for each MS scan needs to be known and should be minimized. Finding out how long the scan time is, depends on the MS software; e.g. for the XCalibur Program the overall scan time is displayed in two places:
    a. During the Acquisition - the scan is usually displayed as the ST in the LTQ tune window.
    b. Qual Browser- right click to the MS window and select the **View >Scan Header**(the user needs to look for the “Elapsed Scan Time”-see Figure 3).
4. If the sample area is more than 10 mm X 10mm, using the meandering mode is advised; the flyback mode can require a long transition time.

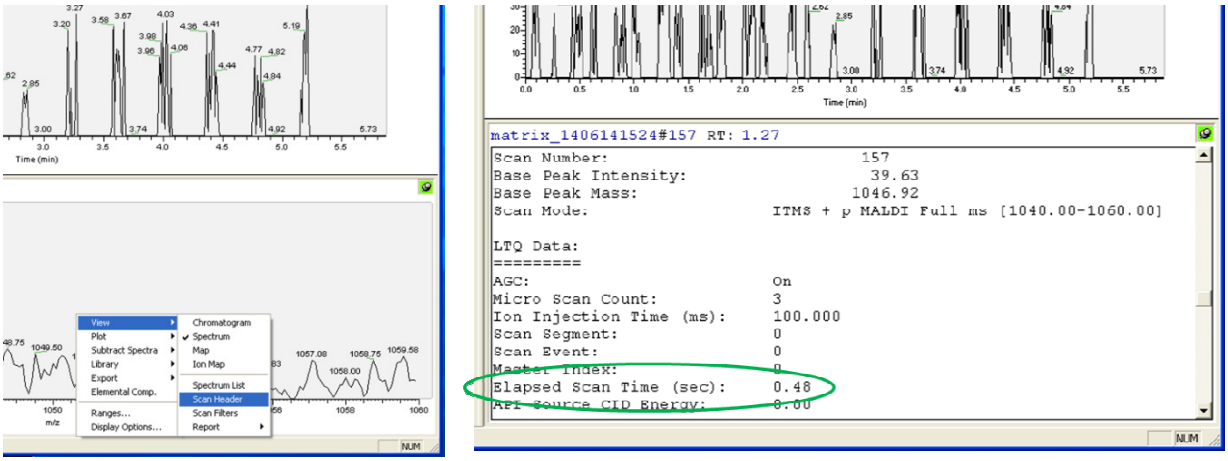

Figure 3 Viewing the Scan Time

Test Sample Preparations

1. On a cleaned AP/MALDI sample plate, spot a 0.5 µL of Angiotensin II solution (FW: 1046.18- or any other sample that is available – the suggested amount is 100 fmol/µl) near the top left hand corner of the plate. The solution must be pre-mixed with a matrix that consist of 1 mg/ml CHCA in 70%Acetonitrile and 0.1%TFA.
2. Spot the solution two more times so that the spots form a right triangle; more specifically, the spots will act as the endpoints (see Figure 1). Each spot should be approximately 1 mm (in distance) from the original point; try to avoid having the samples touch one another.

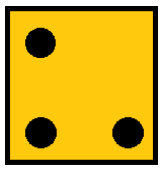

Figure 4 End Point Shapes

Creating the Test Data in the CSR Mode

1. Choose the zoom type in the **General** tab (see Figure 5):
    a. **Settings>Zoom Parameters>General**

    Zoom Type→CSR

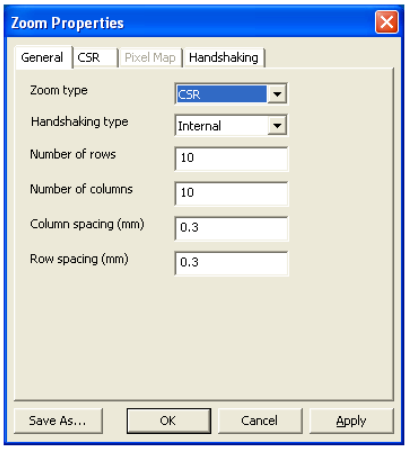

Figure 5 CSR –General Settings

2. When completed, press **Apply**.
3. Choose the **CSR** tab (see Figure 6):

Direction →Horizontal

Pattern →Flyback or Meandering

Velocity (mm/min) →20

Transition Time (sec) →10

a. The user must click the **Save As** button in order for the position file to be active; this file is known as the raster XML file.

b. When the file has been named, click the **Save** button.

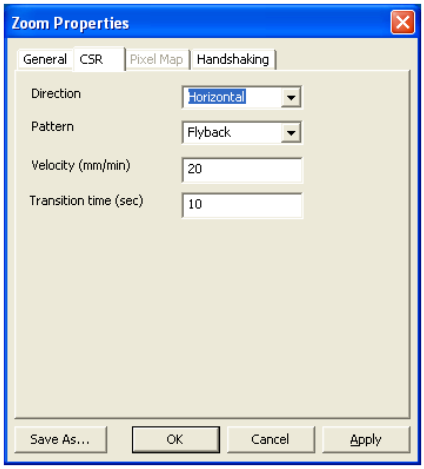

Figure 6 CSR Specific Settings

Creating the Test Data in the Pixel Map Mode:

1. Choose the zoom type in the **General** tab (see Figure 7):

   a. **Settings>Zoom Parameters> Genera*l***
   Zoom Type→Pixel Map
   Handshaking Type →Internal

   b. The remaining properties should be specified to the user's needs. In this case, the number of columns and rows were 10, while the spacing used was 0.3mm.

   c. The user must click the **Save As** button in order for the position file to be active; this file is known as the *raster XML file*.

2. When completed press the **Apply** button and then immediately following, press **OK**.
3. Choose the necessary properties for the data in the **Pixel Map** tab; the properties should be specified to the user's needs. Figure 8 shows a screenshot of the default settings:

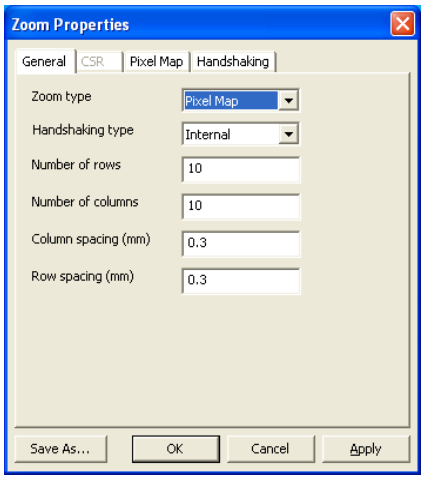

Figure 7 Pixel Map General Settings

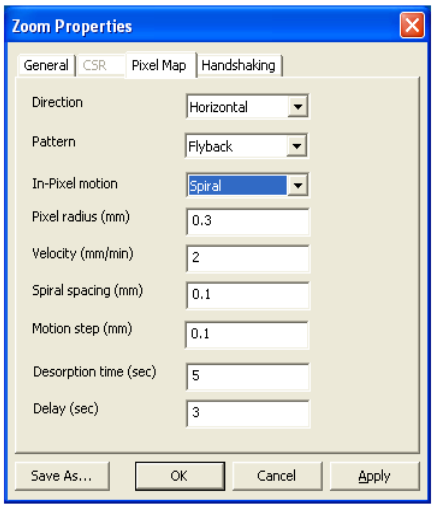

Figure 8 Pixel Map Properties

a. The in-pixel motion is typically enabled only when the “pixed area” is larger than the spot size.
b. The desorption time refers to the laser ‘on’ duration.
c. The delay time refers to the delay between the laser on.

Note: The handshaking action is needed only for the external synchronization.

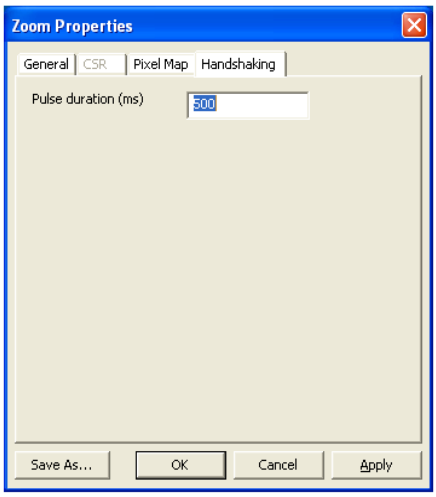

Figure 9 Pulse Duration

4. In the **Handshaking** tab, set the **Pulse Duration** to 500 (see Figure 9).
   a. Then, press the **Apply** and then **OK** button once all the zoom properties are set. **Pulse Duration (ms)** → 500.
   b. It is highly recommended to use the 500(ms) for the **Pulse Duration**.
5. Then press **Apply** and **OK**.

Acquiring the Data

1. In the Target program, this button  is used to enable the zoom mode. This  button, on the other hand, is used to scan the total area of the sample.
2. Now, the user needs to get the mass spectrometer ready.
3. The Target Software has a calculator that informs the user of the total acquisition time (found at the bottom of the CSR tab; the MS should scan for that period. Once finished, press **OK** and close the dialog box.
4. Set the scanning to a narrow mass range(see Figure 10):
   In the **Define Scanning** tab (on the mass spectrometer) set the beginning mass range from 1040 - 1060.

a. **Scan Mode> Define Scan**

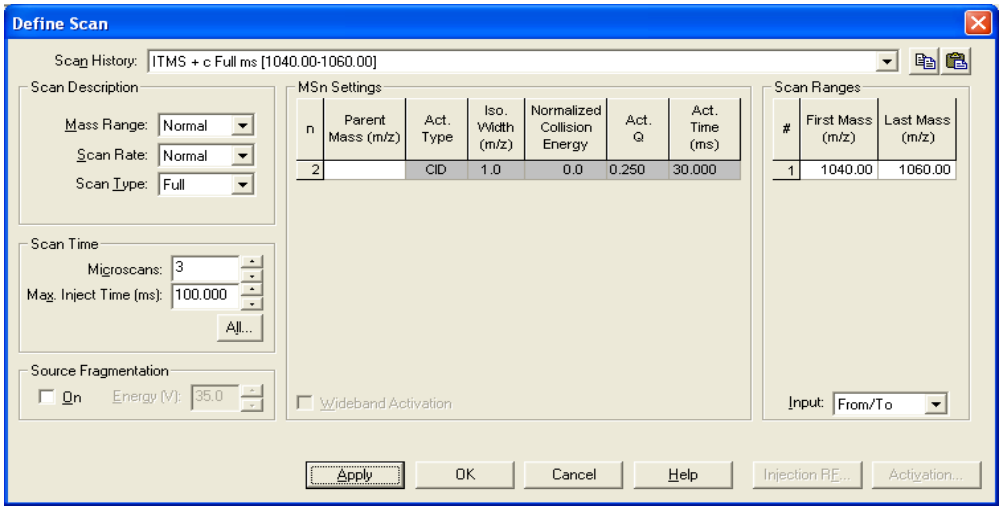

Figure 10 Define Scan as shown in XCalibur

5. Next, press **Apply** and immediately following that action, push the **OK** button before closing the window.
    a. The number of minutes for the MS data acquired is the same, or slightly more to the time calculated by the Target Software.
6. Turn on the laser.
7. Start the scan by pressing start 

on the Target Program, which activates the laser and sample motion scan. The filename needs to be the same as the user's raster XML file.
8. Next, acquire the data using the MS immediately following the start from the Target Software. (If MS software has the option, contact closure can be used to synchronize Target and MS Start) When acquiring the data, the user needs to make sure that only the single scans save and that no summation is used.

Processing the Test Data with Thermo Image Quest (Thermo .raw files only)
Thermo ImageQuest (version 1.1.0 or later) can create images directly from the .raw files and the raster XML file (see above) created under Target software.

In order to ensure ImageQuest matching the raw file with raster xml file, the user needs to ensure identical file names of the raw file and the raster xml file. e.g. if the raw file name is 'mydata1234.raw' , then the raster xml file name should be 'mydata1234.xml'. When

ImageQuest asks for 'MALDIPos not found' prompt, the user just should click 'no' and your image should appear instantly.

Note: when saving raster xml, make sure to click **Apply** before saving.

Processing the Test Data with demo software (any format files that can be converted to mzML)

Note: The steps outlined here are for demonstration purposes only. They involve freely available software that is not supported by MassTech. There may be alternative ways of doing similar tasks by other commercially available software.

There are few major steps needed to create the images from the gathered MS data:

- Converting the raw data file to the mzML format.
- Converting the mzML formatted data file to an imzML data file with the help of position information.
- Create digital images from the imzML data file.

1. The native data from the mass spectrometer in order to create a universal mzML; this allows for the data file to be properly formatted. Use the Proteowizard Software, which is available at the following website:

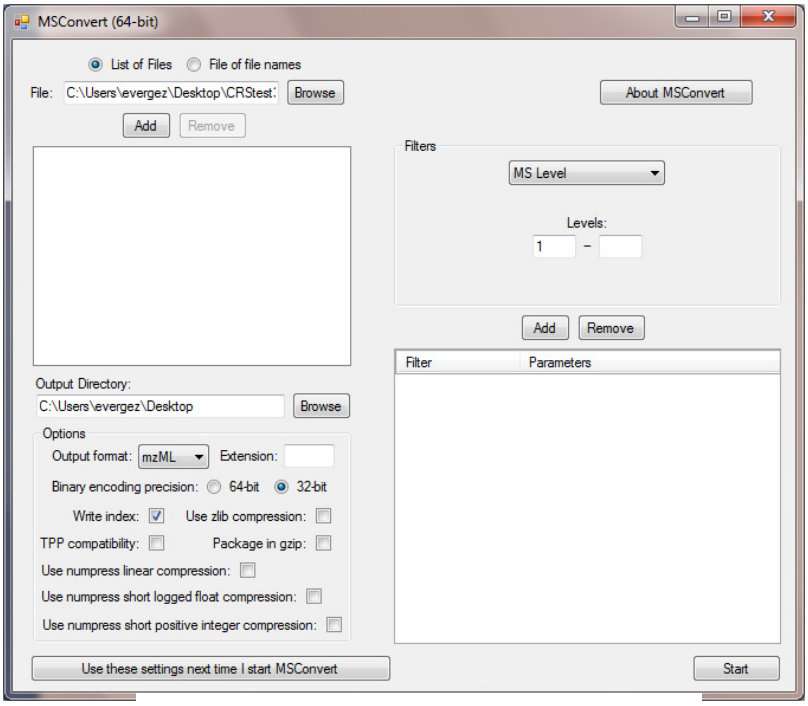

Figure 11 MS Convert Settings

proteowizard.sourceforge.net. The MS Component will be used, which can be found directly on the website's download page..

a. Download the MS Convert (if not installed on the user's computer).
b. Launch the MS Convert and load the *Xcalibur RAW file* into the browser.
c. The user should use the settings shown in Figure 11 to convert the data file..

2. If successful, the user should have an mzML data file that should be somewhat larger than the original MS data file (e.g. Thermo, AB Sciex, Agilent). If unsuccessful, the user will get either an error message or a file with no data in it. Check the settings if there is an issue. In some cases, the data files may be too big (especially Windows XP created files; they cannot be larger than 2 GB) or corrupt.
3. Next, find the Raster XML file created by the Target Software and the mzML file; make a copy of the files and place them in a folder that is easily accessible.
4. Open the MT imzML Converter- the demo software provided by MassTech. Populate the dialog box with the information as shown in Figure 12.
    a. When completed, press the  button to set the figures. Then press the "Convert" button.

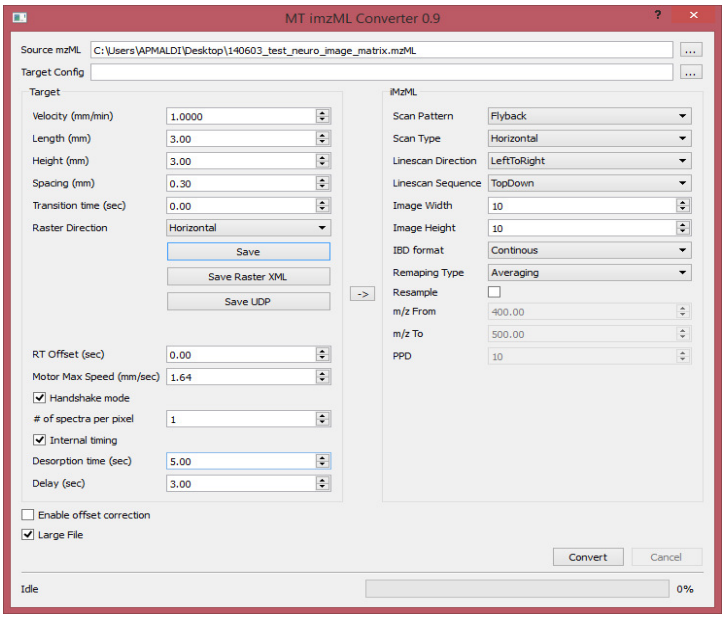

Figure 12 The MT imzML Converter 0.9

5. If successful, the user will have an imzML data file that is somewhat smaller than the original mzML data file.
6. Next, the user will be able to open the imzML data file with the appropriate software. Here the Datacube Explorer by FOM Institute-AMOLF. **http://www.amolf.nl/download/datacubeexplorer/** is shown.
7. When the file is open, the default settings will point to the lowest m/z available in the user's MS data file. Also, the user needs verify that there is a decent number of ion counts appearing at the MS display; MassTech recommends 1000 ion counts to be present. (depending on the instrument)

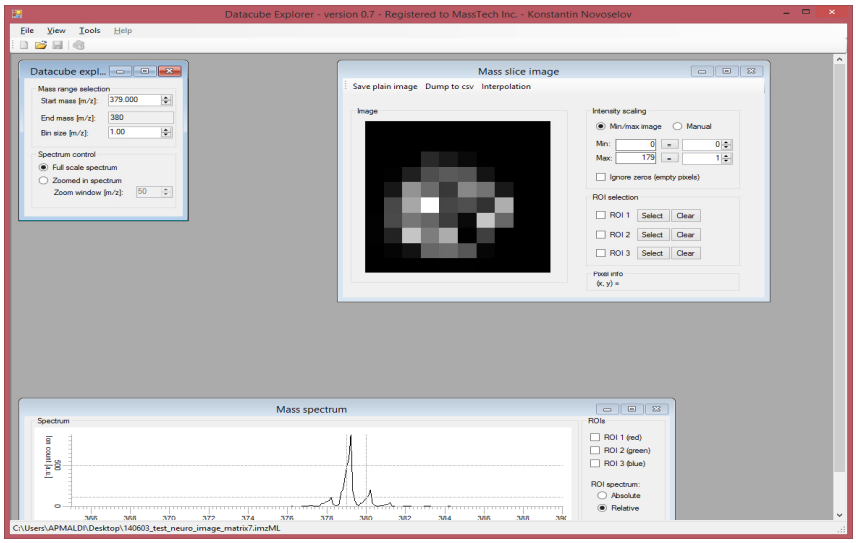

Figure 13 Datacube Explorer – Version 0.7

8. By changing the minimum/maximum value and selecting the appropriate m/z interval, the user's test data should look like in Figure 13.
9. Now the zoom mode is ready use. Please contact MassTech Inc. if there are any questions or concerns.

Notes:

- If the user needs an area larger than 30x30 mm, please inform MassTech; the limit may be stretched based on the sample plate geometry.

- Verify that the cross hair is located where the laser beam hits the sample.

- MassTech recommends the scanning narrow mass for specific applications: for example, if the user is interested in m/z 750, the scan range should only be 740-760; define scanning is based on the sample. MassTech used 1040 – 1060 because the analyte was at 1046; the user's analyte may be different.

- Due to the nature of the CSR motion, once scanning on a row starts, the process is unable to be stopped. Therefore, if the user needs to stop a run, he/she should click on "Stop" button and wait until the current row is finished.

- The Laser energy is dependent upon the sample; if the laser is too high then the sample erodes while on the target plate.

- Please do not hesitate to call or e-mail for more information:

Phone: (443)539-1758. Please ask to be directed to the MassTech Technical Team.

Email: msms@apmaldi.com