

Plattform für explorative, zielgerichtete und orts aufgelöste Proteomik  
Platform for explorative, targeted and spatially resolved proteomics  
Core Facility (ProtCF)

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### a) Position

1. Before loading, place the slide onto the 384 wells plate within the marked area for scaling
2. Take a photo of slide on the plate
3. This is used to determine the coordinates for imaging

### b) Attachment to the MALDI plate

1. Transfer the slide onto the MALDI slide guide
2. Secure it with the two metal clamps and make sure they rest on the Matrix-free area
3. Tighten up the screws
4. Free the surface from any dust with a dust-removal spray
5. Place the MALDI slide guide in the loading chamber of the MALDI

### c) MALDI Software

1. Go into the 4000 Series Explorer Software
2. (This program is always open, don't close it!)
3. Load the plate by pressing "Load"  and select a plate ID. Make sure the ejection spot is empty if another MALDI plate is still in the measuring chamber
4. If the pumping cycle fails, press retry
5. When the loading process is finished, continue by choosing your settings

### d) Acquisition and processing settings

1. Click "Open acquisition mode" and select "Reflector-positive img"
2. Change settings according to your need. Things to be varied are: mass range (usually between 800 and 2500), focus mass, number of shots / subspectra per pixel and laser intensity
3. Click "Open processing mode" and select "Default calibration"
4. Turn on high voltage by pressing  and wait till everything is powered up.

### e) Update default calibration

1. Choose a tissue free spot on your slide using the photo you made earlier.

2. Navigate to the spot using a) the wells list and b) the controller for exact positioning .
3. Control the position with the live view camera feed
4. Press  to acquire a single MALDI spectrum
5. Check for calibrant mass peaks
6. Go to the calibration tab, switch to “Internal calibration”. Enter your calibrants and their mass peaks in the list below
7. Press “Run active processing method”
8. Control your calibrants peaks for corrected m/z 
9. Go into the “Reflector-positive img” → “Instrument” tab
10. Press “Open” in the Operating Mode field, a new tab opens
11. Click “Update default calibration”
12. Close the tab    , choose “Save” when asked
13. The default calibration is now updated. You can check by acquiring a new MALDI spectrum on a different spot on your slide. The calibrant mass peaks should now be accurate
14. Make sure to switch back to default calibration!

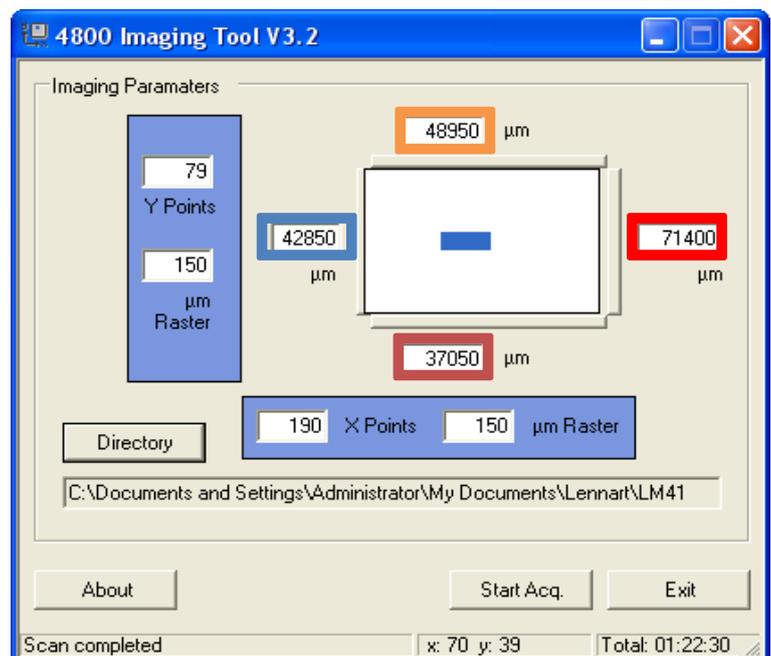
## Optional: Manual spectrum acquisition and saving

If you'd like to save single spectra for specific points of interests, follow the steps below:

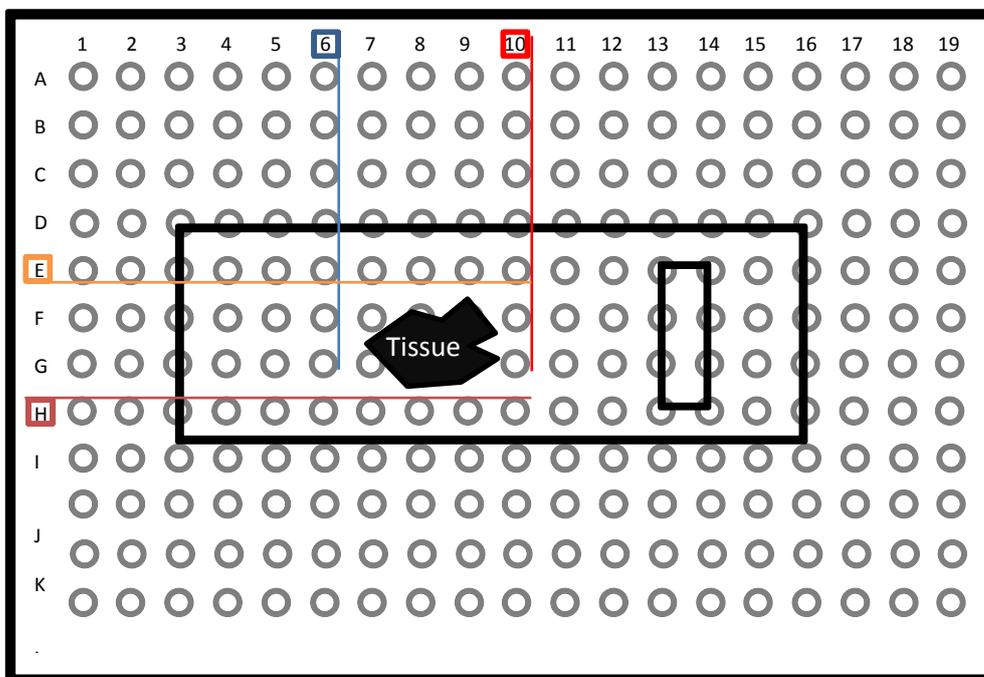
1. Navigate to point of interest using the methods described above
2. Acquire a MALDI spectrum by shooting once
3. Go into  and click “Save spectrum to file”
4. Choose a name and a location

## Defining area for imaging

1. Open the program “4800 Imaging tool”
2. Open the photo you made of your slide on the measuring plate
3. Look at the photo and determine the borders of the area you want to image by using the coordinates A-P and 1-24
4. Open / take the table that shows what coordinate corresponds to what distance from the MALDI plate border
5. You have to enter 4 parameters that represent the borders of the area you want to image



6. The parameters are determined like this example:  
The upper border of your tissue is around the lower end of the wells row **E**. *Always make sure you take some extra space into account (teach marks, digestion control spots, inaccurate placement of the slide on the measuring plate)*. Look up **E** in the table. The “s” column contains the parameter you are looking for (here: 57950). Repeat this step for the other remaining borders:  
Lower border: upper end of wells row **H** → column “t” (here: 46060)  
Left border: right end of wells row **6** → column “y” (here: 35450)  
Right border: right end of wells row **10** → column “x” (here: 53400)



7. Choose the correct file path where your data should be stored  
8. The files will be named after the plate ID you chose in the beginning. Make sure no other file with the same name is inside your desired folder, otherwise the data will be overwritten

## Reminder

1. Always make sure there is enough space left next to the tissue to ensure imaging of the whole tissue, digestion control spots and teach marks.
2. Be aware that a larger imaging area corresponds to more pixels what ends up in longer measuring times.
3. (500 shots per pixel → 2.9 seconds / pixel → 1241 pixels / hour ≈ 0.28 cm<sup>2</sup>)

## Info File

1. The template for the Info file is found on the desktop in the folder “MALDI imaging info file template)
2. Fill in the required information and take at least one screenshot with the acquisition settings visible
3. Save the file in the same folder as your imaging data

## Start imaging

1. Start imaging by pressing “Start Acq.” in the 4800 Imaging tool
2. You can monitor the imaging with the live camera view of the 4000 Series Explorer Software
3. A timer in the bottom right indicates the approximate duration of the imaging procedure

## Ejecting the plate

1. The imaging is finished when the MALDI isn't acquiring new spectra anymore
2. Make sure the eject pad is empty
3. Eject the plate by pressing “Eject” 