MALDI – TOF mass spectrometer

Shimadzu AXIMA CFR

CIC-MALDI 1.1  UMDS – This document
CIC-MALDI 1.2  MALDI – Basic Description & Generic Sample Preparation
CIC-MALDI 1.3  New User Tutorial & Basic Axima Operation, Quick Use Notes
CIC-MALDI 1.4  AXIMA – System Maintenance
CIC-MALDI 1.5  AXIMA – Calibration Performance Testing
MALDI – TOF mass spectrometer

1. INSTRUMENT DESCRIPTION and LOCATION

**Instrument**
Kratos Axima – CFR Maldi, Ser#60059/03

**Date of Manufacture**
9/2001

**Location**
Located in CIC Lab B87, which is second entrance on the left as you proceed from the elevator, through the security doors.

**Software**
Kompact v.2.4.1

**Technical Description for Publications**
All spectra were run on a KratosAXIMA CFR (Shimadzu Biotech, Kyoto Japan), Mass Spectrometer in positive ion mode and using linear mode.
2. DOCUMENTATION

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CIC-MALDI 1.4 AXIMA – System Maintenance
CIC-MALDI 1.5 AXIMA – Calibration Performance Testing

3. USER SUPPLIED MATERIALS

Common Matrices:

Source: Sigma Aldrich 2007, 1-800-325-3010,
Box 2060 Milwaukee WI 53209

10g C2020 ‘’CHCA’’ Alpha-Cyano-4-Hydroxy- Cinnamic Acid $29.00

\[\text{C2020 } \alpha\text{-Cyano-4-hydroxycinnamic acid}
\]
\[\text{Sigma } \geq98\% \text{ (TLC), powder}\]
1g  H5126  “HABA”  $9.00

[2-4\{-hydroxyphenylazo\} benzoic acid]

**H5126 2-(4-Hydroxyphenylazo)benzoic acid**

Sigma  ≥98% (TLC)

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

1g  “DMC CINNAMIC ACID”  $37.00

3,5-Dimethoxy-4-hydroxy-cinnamic Acid

**49508 Sinapic acid**

Fluka  puriss. p.a., matrix substance for MALDI-MS, ≥99.5% (HPLC)

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{C} \\
\text{O} & \quad \text{C} \\
\text{OCH}_3 & \quad \text{C}
\end{align*}
\]

Sample Plate:

Source: Justin Frey

Justin Frey

Field Sales Engineer, North Atlantic Territory

Shimadzu Scientific Instruments; [www.ssi.shimadzu.com](http://www.ssi.shimadzu.com)

Voice (800) 396-4943 Fax (781) 878-7212

Cell (617)821-6801 email:jwfrey@shimadzu.com
4. CIC MAINTENANCE MATERIALS

4.1 Instrument Materials

Source:
Justin Frey
Field Sales Engineer, North Atlantic Territory
Shimadzu Scientific Instruments; www.ssi.shimadzu.com
Voice (800) 396-4943 Fax (781) 878-7212
Cell (617)821-6801 email:jwfrey@shimadzu.com

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<tr>
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<td>$20.00</td>
</tr>
<tr>
<td>Door Seal</td>
<td>97486</td>
<td>$16.00</td>
</tr>
</tbody>
</table>

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Cell (617)821-6801 email:jwfrey@shimadzu.com

4.2 Vacuum materials
5. **CIC PERFORMANCE TESTING MATERIALS**

Calibration Kits

Source: Sigma Aldrich

**MSCAL4 Proteo Mass Kit** $248.00

This kit provides a set of standard Peptides with matrix and solvent solution. for calibrations in the region 523 – 3675 Daltons.

**MSCAL1-1KT Peptide and Protein Maldi MS Calibration kit** $269.00

This kit features a selection of prequalified standard peptides, protein matrix and solvent solutions. It is suitable for accurate calibration in the region 700- 66,000 Daltons.
**CIC-MALDI 1.2 MALDI Basic Description & Generic Sample Preparation**

**Introduction**

MALDI (Matrix Assisted Laser Desorption Ionization) is a mass spectroscopy (MS) technique whereby a sample consisting of an analyte in a matrix is irradiated and the molecular weight is determined using time of flight (TOF) MS. MALDI-TOF can analyze a wide range of masses, but is best used for determination of MW >1 kD. Typical applications are synthetic polymers, small peptides, proteins and biopolymers. The matrix absorbs energy from the laser and transfers this to the analyte, which is desorbed from the plate. The mass over charge ratio (m/z) of the analyte is then measured by the time between the laser pulses and detection by a linear detector (time of flight); 3ns pulses from a nitrogen laser with a wavelength at 337nm are the source of this ionization. This instrument uses a flight length of 120 cm and has a maximum resolution (R) of 4000 in linear mode, where:

\[ R = \frac{M}{\Delta M} \]

Where M is mass and \( \Delta M \) is the mass range at 1/2 peak height.

Samples are applied to a target on a plate, which is inserted in a slot at the front of the instrument. Samples must be dried before analysis – a dryer is situated next to the fume hood to help dry the plate. The following gives a description of how to prepare the matrix solutions and how to best prepare the samples that have the correct concentration and consistency.

A MALDI plate with 96 targets (circles). The plate is loaded with the notched edge in first.
Sample preparation guide

Solvent Systems (“Solvent”)
A standard solvent system suitable for analyzing most samples is 50:50 ACN:0.1% TFA in water (CAN = acetonitrile; TFA = trifluoroacetic acid). To enhance solubility of hydrophobic samples, this ratio can be increased to 70:30. However, as a general rule, any volatile solvent can be used, as long as the matrix and sample can co-crystallize after the solvent is evaporated from the target.

Stock Matrix Solution
In a separate tube, mix:
- 10 mg matrix material, and
- 1ml Solvent.

Vortex, and store in the dark. Discard after 1 week.

For a review of appropriate matrices, please refer to Sigma Aldrich’s catalog of available matrices, found at

http://www.sigmaaldrich.com/catalog/search/TablePage/9627857

Sample Solution
In a separate tube, dissolve samples for analysis to 10-100 µM of Solvent.
Sample application to MALDI targets

Method 1: Dried Droplet Method
This method is based on the original MALDI experiments and remains the most commonly used method in the Mass spectrometry community.

In a separate tube, vortex:
- 10 µl Stock Matrix Solution, and
- 1-10 µl Sample.

Apply 0.5-2 µl to the MALDI target well, and allow to dry in the sample dryer. Once the solvent has completely evaporated, the sample is ready for analysis.

Method 2: Overlayer (Two Layer) Method
While more complex, this method is believed to produce a more homogenous sample spot and to improve the resolution and mass accuracy, especially for peptides and proteins.

Solution A (matrix only)
In a separate tube, dissolve 10-50 mg of the appropriate matrix in methanol or acetone for fast evaporation.

Solution B
In a separate tube, vortex:
- 10 µl Stock Matrix Solution, and
- 1-10 µl Sample Solution.
Applying the sample:
To the MALDI target, apply 0.5 ul Solution A and dry to form a crystalline layer. Apply 0.5-2 ul Solution B on top of the crystalline layer and dry. Once the solvent has completely evaporated, the sample is ready for analysis.

NOTE:
The solvent system used in the second layer solution must not fully dissolve the first layer upon application.
CIC-MALDI 1.3  New User Tutorial & Basic Axima Operation

This is a description of basic operating techniques that will enable routine running of samples applied to MALDI targets. It presumes that samples have been prepared according to CIC-MALDI 1.2 It also presumes that the plate has been spotted and dried in the sample dryer. For more detailed description please refer to the Kompact users guide which is a yellow hardcover manual kept next to the instrument.

Opening the Kompact software

The Launchpad program (“Launchpad”) should open upon computer startup. If it has not automatically opened, it is found in the Start menu.

To open the Kompact software (“Kompact”), click once on the Kompact icon in the Launchpad window to open the Kompact software (DON’T DOUBLE CLICK) XXXXX. Opening the Kompact software will open up the following windows:

1. The main Kompact software window,
2. The Acquisition window,
3. The Instrument Status window, and
4. The Camera Viewer.

Once these windows are open, you are ready to operate the software.
The main **Kompact window** is the window used to display and process spectra. It also includes the menus from which you can access the other windows necessary for operation of the MALDI.

The **Acquisition window** is used to set up experimental parameters and execute experiments. It is also the window used to control MALDI target selection for analysis. If the Acquisition window is not present it can be opened by the Kompact window in ‘Instrument > Acquisition’.

The **Instrument Status** window shows the working status of each of the main system components. The mode and status of the system is indicated in the top left hand corner. There are two vacuum pumps, labeled Turbo 1 and Turbo 2. There is also a pressure gauge (Gauge 1, shown in red), which tells the pressure within the apparatus. Under normal operation, all pumps should be green. *Operational pressure is $< 1.5e-5$ torr.*
The Camera Viewer shows the area in which the laser will fire on the sample plate. Clicking within the red circle will adjust the center of the viewfinder (and laser) to the point of click. The viewer can be manually opened by the Kompact window in ‘View > Camera’. If the image freezes, it is possible to refresh the camera by right-clicking, then selecting ‘Live’.

Inserting the Plate into the MALDI Chamber

**Before loading the plate:** The pressure in the apparatus **MUST BE LESS THAN 1.5e-5 torr**. If the pressure is too high, the vents in the chamber jam and prevent the door from opening. Pressure can be checked by looking at **Gauge 1** in the Instrument Status window.

**In the Acquisition window > Exp Tech tab:**

Select ‘Open Door’. At the prompt, select Yes. The vacuum pump labeled as Turbo 1 will run down, and pressure within the chamber will reach atmospheric pressure (both the pump and pressure are observable in the Instrument Status). The door will then open and expose the MALDI plate.

Make a note of the model number of the plate, located at the bottom center of the plate, as it may be needed later to load the correct parameters during analysis. Also note the locations of the targets with samples for analysis, denoted by letter and number (eg. A1). Load the plate into the chamber as shown, with the notched edge at front.
right corner. After the plate has been loaded, return to the Exp Tech tab and select ‘Close Door’.

**Note:** If the MALDI plate must be removed to load the samples in the targets, the door to the empty chamber must be closed by clicking the ‘Close Door’ button. Reopen the door when the loaded sample is ready for analysis, *but first check that the pressure in the chamber is* < 1.5e-5 *torr.*

After the door closes, the vacuum pumps will activate to decrease the pressure in the chamber to operating pressure (> 1.5e-5 torr). In the schematic in the Instrument Status window, the color of Turbo 1 will first be yellow as the pump accelerates, then green once the pump has reached full speed.

**Loading the Correct MALDI Plate Parameters**
There are various layouts of MALDI plates; in this guide, we show a standard 96-well plate. The correct plate (‘slide’) parameters should be loaded into the software to facilitate sample analysis.

In the Acquisition window > Slide tab:
Click ‘Load’ (circled in red in the figure) to open the explorer window. Select the file in which the last 6 digits/characters match the model number from the bottom of the plate. For example, the model number for the standard 96-well
plate is 1487TA; the last 6 digits/characters of the correct file is ‘1487ta.plt’. Click ‘Open’ to load the plate parameters into your current software session.

Adjusting experimental parameters for sample analysis

In the Acquisition window > Exp Tech tab:

- **Tuning mode**: Describes the path the ionized sample will take to detector. The instrument only has a linear detector. Select ‘**Linear**’.
- **Mass Range**: Describes the range of masses the detector will analyze. Default range is 1 to 10^6 daltons. The range can be focused on the sample to be analyzed, but in general does not need to be adjusted.
- **Mode**: Describes the status of the laser within the chamber. Default is ‘**Standby**’, with the laser off. The laser will not operate unless the pressure in the chamber is < 1.5e-5 torr (chamber pressure can be viewed in the Instrument Status window). To operate the laser, select ‘**Operate**’. When the laser is in operation, the ‘Ionizing’ light on the instrument will turn orange.

  **Note**: After analysis is complete, return the Instrument to Standby mode by selecting ‘**Standby**’.

![Acquisition window > Exp Tech tab. Gives access to the Tuning mode, laser operation (‘Mode’), Mass Range for detection, and Door controls.](image)
Data collection

In the Acquisition window > Firing tab:

- **Selecting a MALDI target:** If the correct plate parameters have been loaded, 2 yellow schematics showing the locations of MALDI targets will appear on the right side of the window. There are several ways to align targets for analysis:
  1. Right-click anywhere in the yellow schematic and select ‘Goto Location’. Enter the target address (eg., A1) and select ‘OK’. The laser will align with the target, which is viewable in the Camera Viewer.
  2. In the large schematic, right-click on the desired MALDI target and select ‘Focus Well’. The laser will align with the target, which is viewable in the Camera Viewer.
  3. Within the small schematic, click and drag to the location for analysis, using the Camera Viewer as a visual guide.
  4. Underneath the plate schematics, there is a cluster of 5 buttons: ![buttons]. Click these buttons to shift the laser in the direction of the arrow, using the Camera Viewer as a visual guide.

- **Power:** Laser power can be adjusted to optimize the signal. It can be adjusted from 0-200. **Default = 85**

- **Profiles:** Describes the number of areas the laser will cover. Increasing the number of profiles ensures an accurate calculation of average mass. **Good default = 100**

- **Shots:** Describes the number of times the laser will fire per profile. **Good default = 2**
• **Ion Gate**: Refers to hardware that the instrument does not have. Should always be ‘Off’.

• **Raster Scan**: Rastering moves the laser in a pattern over the MALDI target thereby gathering a more uniform sample and eliminating any need to locate a “sweet spot” for a more accurate signal. Underneath the smaller plate schematic, this button appears: 📢. **Click once to enable rastering**, click again to disable.

• **Mass Range**: This optimizes the detector to collect masses within a given range, but does not limit data collection (as opposed to the Mass Range in the Exp Tech tab). Under the buttons for the Ion Gate, there is a text window next to the phrase ‘Optimise for:’. **Enter the theoretical mass value** of the sample in the text box.

**Note**: Parameters for an experiment can be saved by selecting ‘File > Parameters > Save’ in the Acquisition window. They can then be reloaded in the future.

When all these parameters have been set, the instrument is ready to fire the laser and collect data. Click the 🔥 button to fire the laser. As the ions reach the detector, peaks will begin to appear in the main Kompact window (refer to “Processing Data” in this guide for more information). If data collection needs to be paused or stopped during operation, use the ‘Suspend’ or ‘Abort’ buttons, respectively.
**Processing Data After Collection**

Displaying Processed Data: the Spectrum Contents window

Trace data can be displayed as real-time Profiles, Averaged profiles, smoothed Processed data (fit to a Gaussian or Savitsky-Golay distribution), or just the processed Peaks above a certain threshold. In the Kompact window, select ‘View > Display Contents…’ to open the Spectrum Contents window. To select which traces to view, click on the ‘Profile’, ‘Average’, ‘Process’, or ‘Peaks’ buttons. Multiple traces can be simultaneously viewed by selecting...
multiple buttons. Select ‘Stack’ or ‘Overlay’ to adjust how data is displayed. For more information on adjusting the spectrum contents, please refer to the yellow Shimadzu manual.

Selecting Regions of the Spectrum
It is possible to **zoom in** on displayed data in the Kompact window by:

- **Left clicking** near the lower limit and drag the mouse to the upper limit. The window will zoom in on the defined region.
- **Manually entering** the region you wish to display in the text box near the top of the Kompact window.

To **zoom out**, click on the button.

Data Selection Cursors
When working within the Kompact spectrum window, it is possible to manually select peaks and define regions using **data selection cursors**. To create a cursor, click on the **middle mouse button** within the spectrum; this will create a cross ( ) on your data spectrum. Adjust the mass/charge location and the threshold level of the cursor by clicking the middle mouse button and dragging horizontally and vertically, respectively. To define a region, create two cursors. Cursors can be cleared from data by clicking the button on the far right side of the Kompact window. The buttons found in various setup and processing windows are used to import manually cursor-selected mass peaks and regions.

Adding a Title to a Spectrum
In the Kompact window, select ‘**File > Comments**…’ to open the **Comments window**. Enter a **Title** to name your spectrum. In the bottom

The **Comments window** can be used to add comments to spectra.
left corner of the Comments window, select ‘Apply to: Current dataset’. Click ‘OK’ to close.

**Adjusting the Peak Threshold: the Peak Processing window**

Manually select a threshold using data selection cursors. In the Kompact window, select ‘Processing > Peak Processing...’ to open the Peak Processing window. The bottom half of the ‘Peak Cleanup’ tab is labeled ‘Peaks’. Click on the ↑↓ button to import the manually set threshold level from the cursors in the Kompact window. Click ‘Close’ to apply the new threshold levels.

**Data Calibration**

Data must be set to a named (saved) calibration file. Prepare a **standard calibration sample** with various molecules with **monodisperse** weights (avoid polymers if possible). Your standard molecule(s) does not need to be similar in structure to your experimental sample, but the molecular weight(s) should be within ±1kD of your sample. Run a MALDI experiment with your calibration samples, then refer to the yellow Shimadzu manual to create and save a named calibration file. For a list of commonly used molecules for calibration, please refer to the **Sigma Aldrich ProteoMass™** line.
Loading Named Calibrations
In the Kompact window, select ‘Processing > Calibration…’ to open the Calibration window. On the right side of the window, in the drop down list labeled ‘Load:’, select ‘named calibration’ and click the ‘List’ button to display a list of saved calibration files. In the drop down menu, select ‘Selected dataset’, then click to highlight the named calibration file you wish to use. Closing the window will load the file into the Calibration window. Click the ‘Load’ button to load the calibration file. The data you have collected should now be adjusted for your calibration.

Exporting Data in ACSII Format
Data can be exported in ACSII format for import into spreadsheet software such as Microsoft Excel. Begin in the Kompact window by zooming into the desired data range. Open the ASCII Export window by selecting ‘File > Export > ASCII’. The following parameters may be useful, but are readily adjusted:

- **File format**: PC
- **Columns**: 2
- **Delimiter**: comma
- **Decimal Places**: 2
- **Export**: Headings, Peaks (or whichever processed data you wish)
- **Format**: Mass, Intensity
- **Report intensities as mV**: Yes

Click ‘Save as…’ to open the Save As window. Browse to your desired location, enter a File name, and click ‘Save’. The ASCII file has now been created as a .txt file.
Manually close the Export ASCII window. You can now import your data into your personal spreadsheet software.
**Procedure to clean an AXIMA sample target**

1. Wipe target with methanol using a Kimwipe to remove all visible sample.
2. Soak the target in acetone in a glass container and put into a sonication bath for 10 – 15 minutes.
3. Replace the acetone with methanol and sonicate for 10-15 minutes.
4. Replace the methanol with 1% of formic acid in water and sonicate for 10-15 minutes.
5. Replace the 1% of formic acid with water and sonicate for 20 min.
6. Remove the target from water.
7. Flood target with methanol and drain off.
8. Air-dry thoroughly in the drying box for 30 minutes prior to use.
9. Note: Step 4 can be skipped if the target is not very dirty.