Atomically Resolved Dynamics Group, University of Toronto

PDL-MS Manual

Developed by Steam Instruments and the Miller Atomically Resolved Dynamics Group, University of Toronto

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List of Acronyms

DOF: Distance of Flight

fs-IR: Femtosecond Infrared

MCP: Micro-Channel Plate

MS: Mass Spectrometer

ns-UV: Nanosecond Ultraviolet

PDL-MS: Pulsed-Deflection Lens Mass Spectrometry

PIRL: Picosecond Infared Laser

TOF: Time of Flight

U of T: University of Toronto

UT: University of Toronto

Preface

How to use this manual

Purpose of this manual:

This manual provides brief, step-by-step procedures for preparing and analyzing a sample. It is designed to help quickly learn how to use the PDL-MS system developed for the Miller group.

Audience:

This guide is intended for new group members to learn how to use the PDL-MS system and should always be viewed as a live document. The MS system will continue to be developed and used by the many students, post-doctoral fellows, and researchers in the Miller group for years to come.

Text conventions:

The guide uses the following conventions:

- Bold indicates user action. For example:
  Type 0, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis.
  For example: Before analyzing, *always* prepare a fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:
  Select **File** > **Open** > **Spot Set**.
  Right-click the sample row, then select **View Filter** > **View All Runs.**

User Attention Words

Two user attention words appear in the user documentation. Each word implies a particular level of observation or action as described below:

- Notes provide information that may be of interest or help but is not critical to the use of the product. For example:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

- Important provide information that is critical to the use of the product or the completion of a procedure. Importance can also emphasize the safe use of chemicals. For example:
  IMPORTANT! To verify your client connection to the database…

Safety Alert Words

IMPORTANT! Safety alert words also appear in the user documentation.

1. Before you begin

The following sections are modified from the *PDLS User's Manual* by Steam Instruments.

1.1 System operation modes

The PDL-MS system is a time of flight (TOF) mass spectrometer which uses a pulsed-laser ionizing source. The system was initially tested using a ns-UV laser which will be replaced with a picosecond infrared laser (PIRL) or a fs-IR laser for future studies at U of T. The PDL-MS has four operational modes which can be used to optimise mass spectra results. The modes are Static non-defection, static deflection, Pulsed deflection TOF, and Pulsed deflection DOF.

1.1.1 Static non-deflected mode

The PDL-MS functions as a conventional linear time-of-flight mass spectrometer in this mode. Extracted ions from the sample fly in a straight path, directed towards an ion detector located at the standard port (S-port in Figure 1). In this mode, no voltage is applied to the PDL. A diagram of the system configuration in this setup is shown in Figure 1.

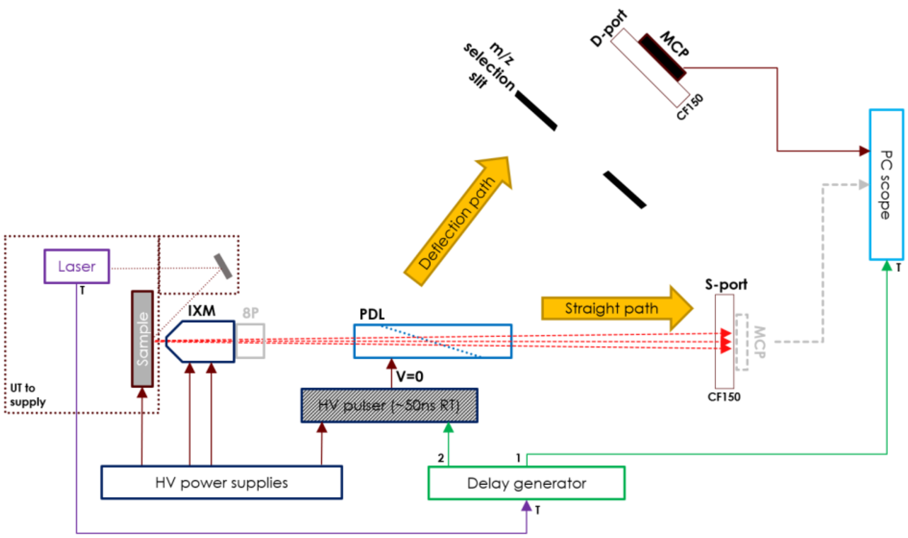


Figure 1: Diagram of static non-deflection mode operations of the PDL-MS.

1.1.2 Static deflection mode

In "Static deflection mode", all ions are deflected along a secondary path toward a detector (D-port in Figure 2). In this mode, all ions travel down the "Deflection path" as shown in Figure 2. A constant voltage is applied to the PDL to deflect the ions that have travelled past the ion extraction module. The selection slits can be adjusted to prevent ions of high-angle trajectories from reaching the detector on the D-port, which may improve MRP at the expense of signal.

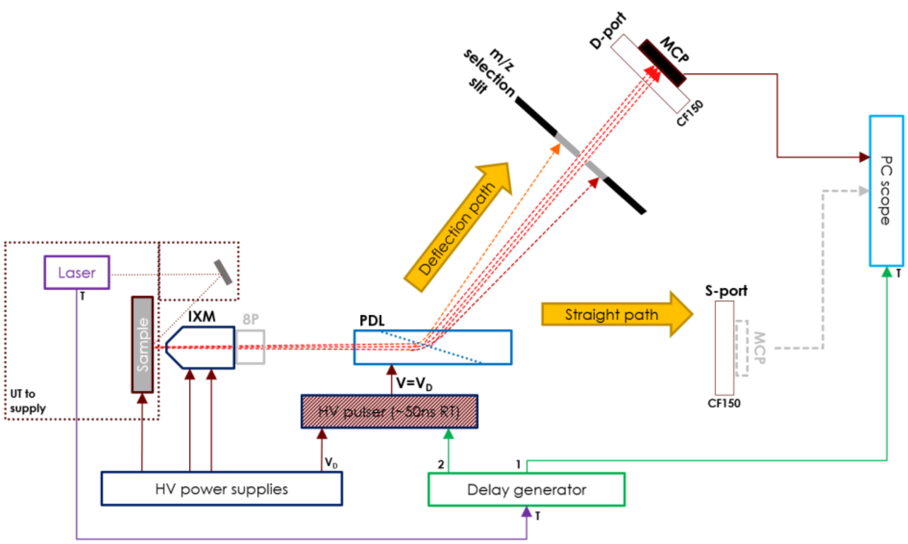


Figure 2: Diagram of static deflection mode operations of the PDL-MS.

1.1.3 Pulsed deflection TOF mode

In pulsed deflection TOF mode, the pulser and delay generator are used to generate a voltage pulse on the PDL. This will allow ions within a narrow mass range to be deflected towards the “deflected port” (D-port), while the other ions continue to the “straight port” (S-port). The delay and width of the voltage pulse can be modified to select the desired mass range that is to be directed towards the D-port. The width of the selection slits can be adjusted to potentially improve MRP.

This can be used to direct strong low-mass signals to the S-port, and rarer high-mass signals to the D-port where sensitivity can be boosted at the expense of dynamic range.

In the schematic below, each of ions #1 – 5 have increasingly higher m/z. For a short time after the laser pulse, the PDL voltage is zero, and ion #1 travels to the S-port. Soon after the PDL voltage is raised to V_D, ions #2 & #3 travel through the PDL and are deflected to the D-port. Ion #2 has a high-angle and is stopped by the slits, whereas ion #3 is detected. Ions #4 & #5 aren’t yet affected by the PDL voltage. If the PDL voltage is set to zero in time, then these ions will continue to the S-port. A diagram of the system configuration in this setup is shown in Figure 3.

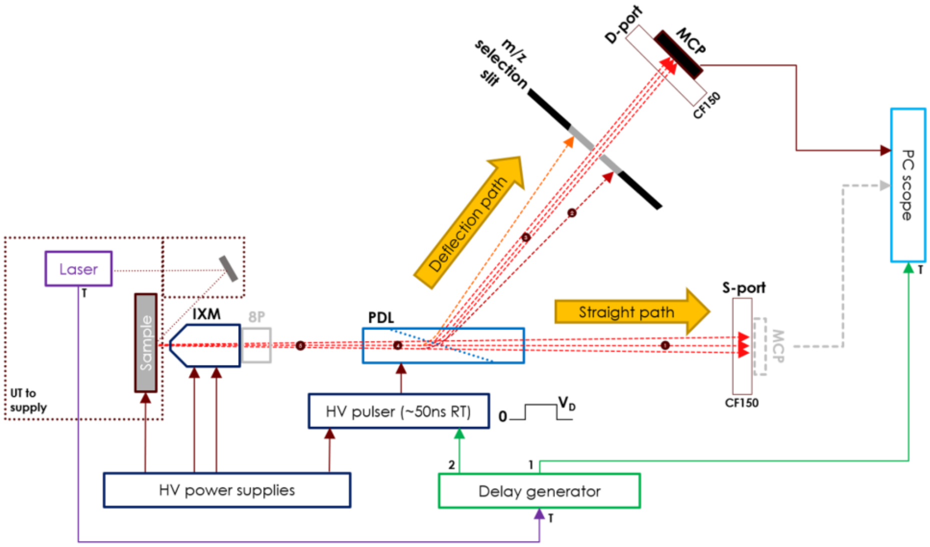


Figure 3: Diagram of Pulsed deflection mode operations of the PDL-MS.

1.1.4 Pulsed deflection DOF mode

This section is modified from the PDL-MS acceptance test presented by Tom Kelly from Steam Instruments Inc.

In pulsed deflection "Distance-of-Flight" (PD-DOF) mode, the pulser and delay generator are used to generate a voltage pulse on the PDL. This will allow ions within a narrow mass range to be deflected towards the "deflected port" (D-port), while the remaining ions continue to the "straight port" (S-port). The delay and width of the voltage pulse can be modified to select the mass range that is to be directed towards the D-port. The width of the selection slits can be adjusted to further isolate the mass range of interest. While the ions approach the PDL slit and pulsed voltages are in transition, a DOF spectrum is generated. With a narrow slit, the DOF resolution can exceed the TOF resolution and non-TOF detectors such as Faraday cups can be used. Alternately, the slits can be withdrawn, and a position sensitive (non-TOF) detector can be used. This mode permits measurements of MDa proteins when a Faraday cup is used on the D-port as the mass analyser. On the S-port with a MCP this allows for sub 100 KDa measurements. A diagram of the system configuration in this setup is shown in Figure 4.

Commented [KM1]: Maybe add a note that this is essentially the same as the previous mode but the analysis is what fundamentally changes

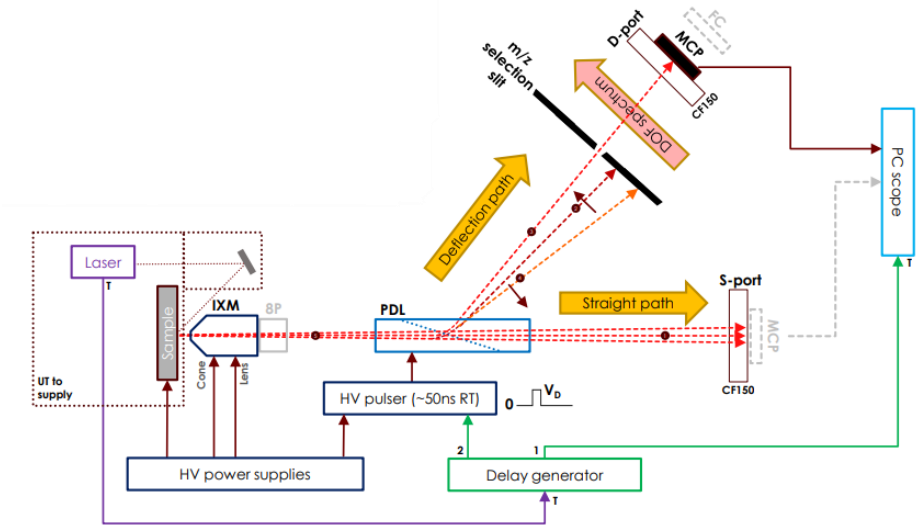


Figure 4: diagram of the pulsed distance of light mode of the PDL-MS.

1.2 System Diagram

The diagram below shows the systems configuration at Steam Instruments Inc. in July of 2022.

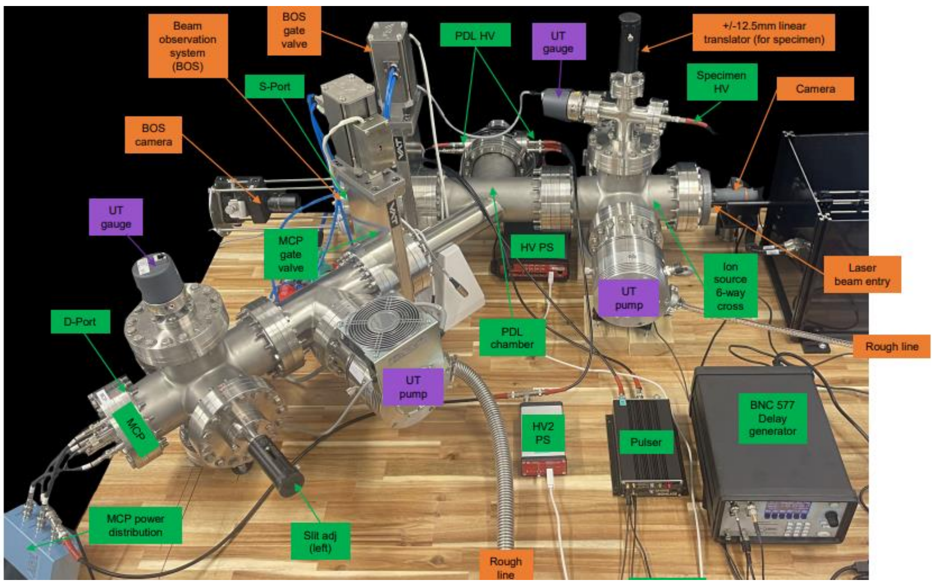


Figure 5: Ladled system image from July of 2022, provided by steam instruments in the PDL-MS acceptance test presented by Tom Kelly. The green boxes are purchased with the MS, the purple boxes are provided by U of T and the orange boxes are not provided with the MS and will be replaced at U of T.

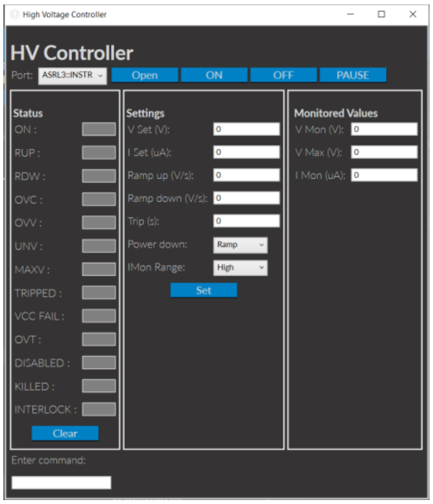
2. System Operations Manual

The following section is modified from the *PDLS User's Manual* written by Steam Instruments.

2.1 Conditioning the Jordan TOF MCP

Steam's High Voltage controller software can be used to control the voltage applied to the MCPs. Other software can be used such as LabView or serial communication (see Caen DT54xx user manual).

1) *Make sure* the Caen DT54xx unit is connected to computer via USB.
2) **Open** HV Controller software.



3) **Open** port to HV power supply. Function will be successful if unit model is displayed to user. (It may be helpful to check device manager first and determine which COM port is associated to DT54xx)
4) If this is the first time operating the high voltage unit, *ensure the hardware <u>V Max</u> is set to 2200 V*. This is done by rotating the screw on the front panel of the unit.

Fig. 10: HVmax trimmer

5) *Ensure no flags are red* in status (verify that hardware switch is enabled). If any of the flags are red, **mitigate the issue** and **select <u>Clear</u>**.

Bit 0 → ON	1 : ON 0 : OFF
Bit 1 → RUP	1 : Channel Ramping UP
Bit 2 → RDW	1 : Channel Ramping DOWN
Bit 3 → OVC	1 : Over current
Bit 4 → OVV	1 : Over voltage
Bit 5 → UNV	1 : Under voltage
Bit 6 → MAXV	1 : VOUT in MAXV protection
Bit 7 → TRIP	1 : Current generator
Bit 8 → OVP	1 : Over temperature
Bit 9	reserved
Bit 10 → DIS	1 : Ch disabled
Bit 11 → KILL	1 : Ch in KILL
Bit 12 → ILK	1 : Ch in INTERLOCK
Bit 13 → NOCAL	1 : Calibration Error
Bit 14, 15 → N.C.	reserved

6) **Start** PicoScope and enable Channel A (channel that is connected to the MCP out).

7) **Set trigger** to Channel A at a threshold of approximately -50 mV and Trigger mode Auto.
8) **Set**:
   a) V Set to 1650 V
   b) I Set to 315 uA
   c) Ramp up to 5 V/s
9) **Press ON**. User will be prompted to confirm chamber vacuum is at appropriate level as prompted by HV controller.
10) As VMon approaches VSet, **watch the oscilloscope**. If there are many continuous spikes, pause voltage rise and wait for them to disappear. Then **set** VSet to desired voltage again.
11) Once VMon reaches VSet, MCPs are ready to be used. The voltage can be modified by changing VSet and selecting the Set button.

2.2 Setting the high voltages on the ion optics

1. Make sure *Caen DT55xx high voltage supply is connected via USB* to laptop and power is on.
2. **Open** GECO software and **connect** to high voltage supply DT55xx through COM port (you may need to verify in device manager which COM port is associated to the device).
3. **Set** the ion optics voltages to desired values The connections are as follows:
   a. HV1-Ch0 to Cone 1
   b. HV1-Ch1 to Lens 1
   c. HV1-Ch2 to PDL 1
   d. HV1-Ch3 to Sample
4. **Select power on** for each channel.

Recommended voltages for different operation modes (below)

Operation Mode	HV1-Ch0 (V)	HV1-Ch1 (V)	HV1-Ch2 (V)	HV1-Ch3 (V)
Static non-deflection	700	850	0	1800
Static deflection	700	850	1340	1800
Pulsed deflection	700	850	4000	1800

2.3 Setting up PicoScope and the oscilloscope

Initial settings for PicoScope to trigger from the laser OptoSync pulse should be as follows. These settings should be saved as the user start-up settings and can be loaded through **File > Start-up Setting > Load User Default Settings**.

Trigger settings:

- Source: AUX (connected to BNC Channel A)
- Mode: Repeat
- Level: 1 V (assuming trigger source is emitting 5V TTL and signal is terminated to 50 Ohms at oscilloscope)
- Trigger on rising edge
- Pre-trigger: 0%

Cable connections to the oscilloscope are as follows:

- PicoScope Channel A to MCP
- PicoScope AUX to BNC Channel A
- (Optional) PicoScope Channel C to Behlke pulser trigger out. This will decrease Sampling rate to 2.5 GS/s

2.4 Setting up the oscilloscope and BNC for data acquisition

1. **Turn** the BNC (pulse generator) on. The unit can be controlled by the buttons on the front panel or using TeraTerm (see BNC577 user manual for SCPI commands if controlling through TeraTerm). There are two values to adjust: the pulse delay and pulse width of Channel A. The BNC is set to trigger externally from OptoSync on the laser.
2. Start the BNC by **pressing Run**
3. **Turn** pulser switch on.
4. For static deflection, set the BNC delay to zero and the pulse width to over 80 us.
5. To enable the oscilloscope settings required for acquisition, in PicoScope go to **File > Start-up Settings > Load User Default Settings**. This will set appropriate trigger settings and voltage ranges for spectrum acquisition.
6. For maximum sampling rate on Channel A, **connect** the BNC Channel A to EXT on the oscilloscope. **Disable** all other channels and set the trigger source in PicoScope to EXT. The voltage resolution can also be modified to higher than 8 bits, this will however decrease sampling rate.

2.5 Using the BNC remotely through TeraTerm

The BNC can be used remotely using the terminal program TeraTerm installed on the Laptop. Current settings have been saved to connect automatically to the BNC using COM8. If the BNC is unplugged or it's COM port has changed, these settings will need to be changed.

TeraTerm is command based, each command is sent after a carriage return. If the response is 'ok' the command was successful. Otherwise, and error code will be sent.

The following are useful commands to send to the BNC for adjusting PDLS parameters:

1. Start the instrument,
   :INST:STATE 1
2. Stop the instrument,
   :INST:STATE 0
3. Set the pulse delay to 10 ns
   :PULS2:DEL 1e-8
4. Set the pulse width to 1 us
   :PULS2:WIDT 1e-6

2.6 Cable connections

A diagram of the system cable connections for the setup at Steam Instruments in Madison WI is shown in Figure 6.

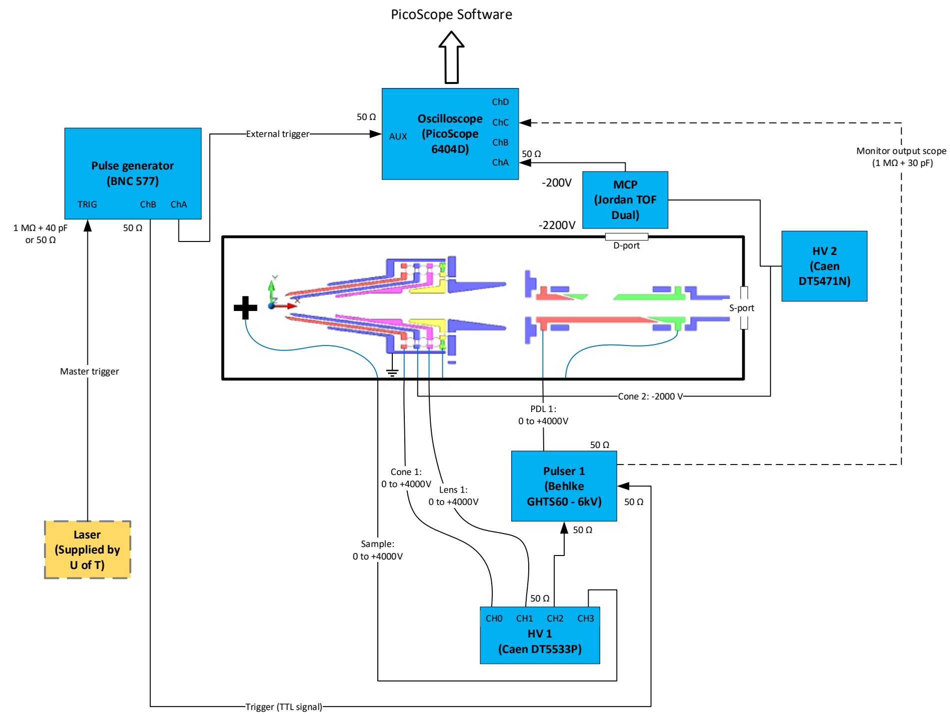
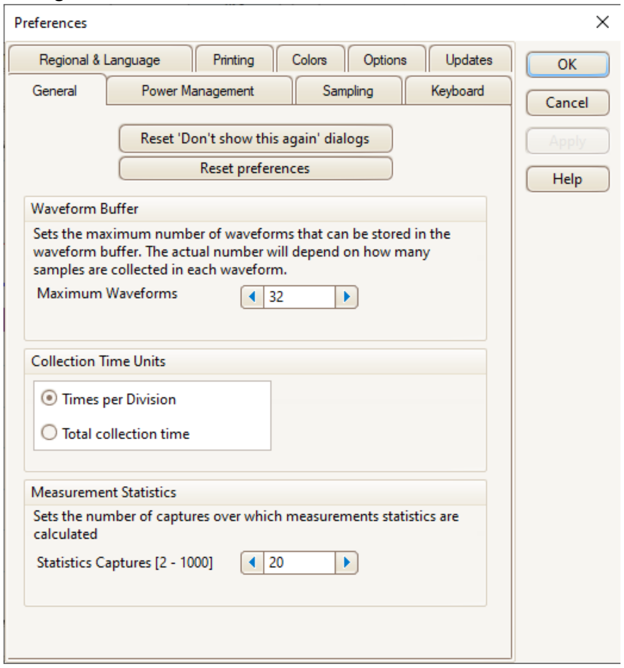


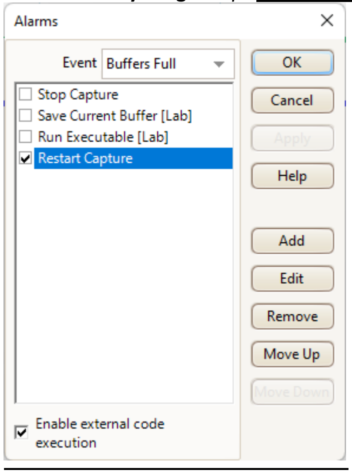
Figure 6: Cable wiring diagram for the setup at steam instruments.

2.7 For averaging a fixed amount of spectra

1. In **Tools > Preferences**, change the Maximum Waveforms to the number of pulses that the average should be taken over.



2. In **Tools > Alarms**, from the dropdown menu select Buffers Full. In the list of commands, **unselect everything** *except* *Restart Capture*. ***Make sure the Buffers Full checkbox is checked*.**



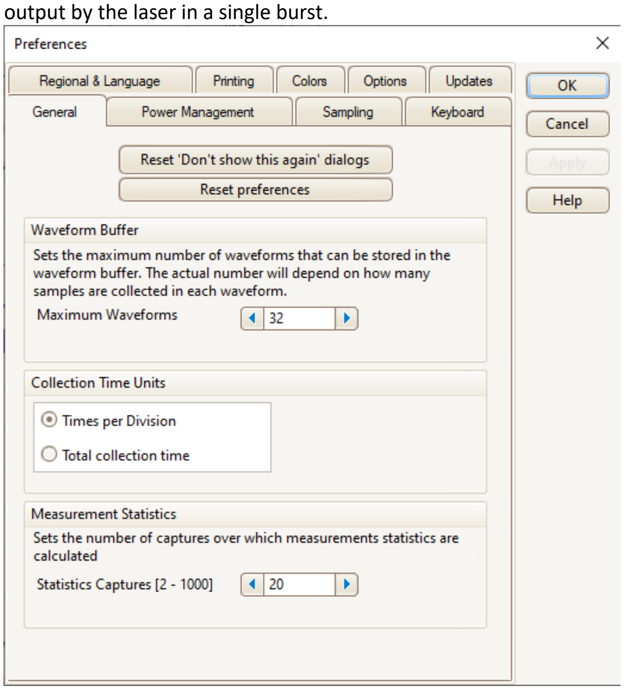
3. **Press** Ok and **start** capturing.

2.8 For Burst mode and full data processing (alpha)

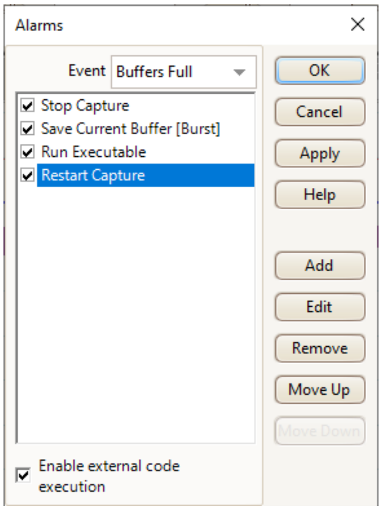
The program will acquire a set amount of waveforms, average them and save the average. A python script will then open the most recent file in the save directory, convert the average to a sum and output a file containing m/z vs counts. The waveforms are all saved to %userprofile%\Documents\waveforms\burst\. Do not change this directory as the location of the waveforms must be hard coded into the .bat script run by PicoScope. You can change the name of the saved files but ensure that the string "%time%" appears in the name and that the file is saved as a .csv. Ensure that the parameters are correct in the CurrentConfig.xlsx file on the desktop.

To conduct burst mode experiments and output m/z vs. Intensity curves, do the following:

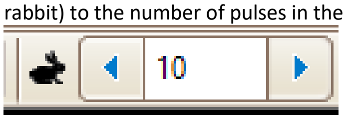
1. Make sure an instance of PicoScope is running and connected to the oscilloscope. In PicoScope, make sure Channel A and average(A) are active.
2. In **Tools > Preferences**, **change** the <u>Maximum Waveforms</u> to the number of pulses that will be output by the laser in a single burst.



3. In **Tools > Alarms,** from the dropdown menu select Buffers Full.



4. **Press** OK.
5. *Ensure trigger mode is Rapid* and **change** number of acquisitions (textbox next to image of a rabbit) to the number of pulses in the laser burst mode.



6. **Press** enter and capturing on PicoScope will begin. Now you can set a number of bursts on the laser using LWin and start acquisitions from LWin.

3. Method of signal optimization

The resulting mass spectra can be improved by varying several different parameters, starting from laser ionization to voltage optimization.

3.1 Sample Ionization

The sample ionization determines the ions which will enter the system. Because a laser is used for ionization, the critical value to consider is fluence. If the fluence is below threshold for ablation, then no ionization will occur. Because no ionization occurs, there will be no mass spectra produced. At threshold fluence, the system will have ionization and a minimal amount of plasma. This is optimal for mass spectra production and will lead to the best signal. Above threshold fluence, plasmas start to form and the mass spectra will have many peaks. This is unadventurous as the peaks formed will not be highly fragmented, and harder to analyse. As a result, to get the *best results to* ***set the laser system at or just above threshold ablation fluence***.

3.2 MCPs Voltage

The voltage applied to the MCPs determines the mass spectra amplitude. The higher the voltage on the MCP the larger the amplitude of the output signal. To optimise the mass spectra, this voltage should be set to the maximum amount which does not cause saturation of the MCP's mass spectra signal. This will be visible on the MS if a peak is defined as infinite. Note that this does not represent damage to the MCP. If a peak is defined as infinite on the MS, it may be wise to lower the Voltage on the MCP. The MCPs voltage *should not exceed 2000 V*.

3.3 Ion Optics Settings

To optimise the signal output at the MCP, the ions of interest from the source need to reach the MCP. This is achieved by adjusting the Ion optics settings so that the ions of interest from the source reach the MCP. Doing so changes the mass calibration of the Mass spectra, requiring a new mass calibration. Mass calibration can be easily achieved because of the linear relationship between time of flight and mass to charge ratio as shown in equation 1:

$$tof = a\sqrt{\frac{m}{z}} \tag{1}$$

Where a is the calibration constant, tof is the time of flight, and $\frac{m}{z}$ is the mass to charge ratio. For most ions produced from laser ionization, it is reasonable to assume single or double ionization. As a result, it is relatively easy to determine the calibration constant if two peaks can be identified based on the mass of the ion, assuming single ionization.

The suggested ion optics setting given in section 2 are based on observations of silicon ablation using the ns-UV laser and have been found to work for identifying carbon.

3.4 m/z selection slit

The m/z selection slit if useful for determining which ions reach the MCP. The slit works be creating a two-dimensional window though which the ions can pass. By leaving the slit wide open, more ions can reach the MCP. By Narrowing the slit, only ions travelling through the centre of the channel will reach the MCP. Narrowing the slit increases the selectivity of the mass spectrometer, and decreases the number of ions reaching the MCP, lowering the signal strength. Overall, the effect of changing the m/z selection slit size was the least significant factor in improving the quality of the mass spectra formed.

4. Standard operating procedures

The following are the standard operating procedures for using the PDL-MS at Steam Instruments Inc.

4.1 System Startup

First, one must ensure that all the electrical connections are properly made and all the corresponding software open on the designated computer. Also ensure that all the high voltage is turned off.

1. Introduce the sample into the sample chamber of the PDL-MS. Ensure that the leak valve of the vacuum chamber is closed.
2. Turn on roughing pumps.
3. Once the vacuum in the sample chambre reaches 10 mbar, turn on the turbo. With the current apparatus, it takes 4 to 5 hours for the sample chamber to reach the desired operational vacuum level.
4. Open valve gates.
5. Switch on the voltages. Ensure all the proper values are inputted into the corresponding software.
6. Turn on the laser.

4.2 System shutdown

1. Turn off laser.
2. Switch off all the voltages from the software control. Once they have completely ramped down, switch off the high voltage control box. To ensure extra safety, one may decide to unplug the high voltage cable from the sample chamber.
3. Close the valve gates.
4. Turn off the turbo.
5. Once the turbo is completely stopped, turn off the roughing pumps.
6. Slightly open the leak valve. A hiss should be heard.

After completion of these steps, the system has been safely shut down and a new sample may be introduced.