

# Development of a Cross Platform IR-MALDESI Front End for High Throughput ToF Mass Spectrometry

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## Overview

AstraZeneca (AZ) has been a leader in the field of high throughput mass spectrometry since the development of Acoustic Mist Ionization Mass Spectrometry (AMI-MS). Despite successfully supporting more than 10 full HTS campaigns, the AMI-MS technology has some limitations.

Firstly, it is limited to 384 well format. A full 300 plate run takes around 20 hours to process but generates 21.5kg of plastic waste. Switching to 1536 well plate format would reduce the waste by around 90%.

Secondly, with no commercial AMI-MS systems available, our systems have a finite lifespan of support.

Here we describe an IR-MALDESI interface that is compatible with both Waters and Thermo MS platforms with the potential to replace the acoustic mist ionization system.

## Introduction

- Following the publications from Abbvie<sup>1</sup> where they describe a 1536 compatible laser based high throughput mass spectrometry loading system, AZ decided to collaborate with TTP to develop and test a “generic” IR-MALDESI interface that can connect with multiple MS detectors.
- IR-MALDESI uses a laser tuned to excite water molecules within a sample to generate a mist of droplets from the sample surface.
- A secondary ionization source sprays charged solvent into the sample plume, the merged droplets are drawn into the mass spectrometer (Figure 1).
- Mechanically an IR-MALDESI interface should be relatively simple to build and would interface with the MS via a simple heated capillary.

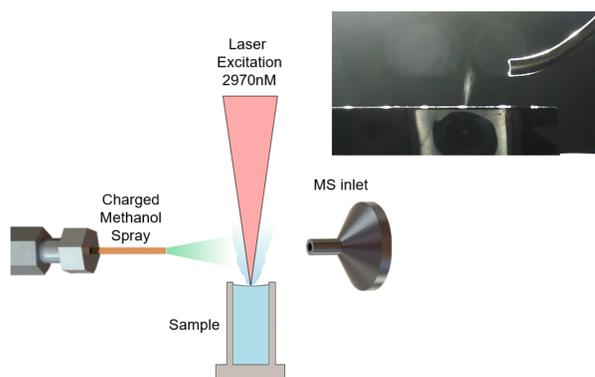


Figure 1. Schematic of IR-MALDESI. The laser illumination of the sample generates a droplet spray which then merges with an ionized solvent stream generated by a secondary electro-spray ionization (SESI) source. The merged droplets are drawn into the MS. Inset is an image of the sample plume generated by the laser.

## Project Planning and System Design

- With some literature precedent, we set a challenging delivery timeline for the generation of a breadboard instrument, 11 weeks.
- The laser source is a class 4 device with an excitation wavelength of 2970nm (JGMA inc, USA). This laser has the potential to cause serious injury, therefore, operator safety was paramount in the design concept.
- An optical fiber is used to pipe the light from the laser source to the sample. A series of collimators and lenses are used to maximize the energy transmission while minimizing the risks of laser strikes.
- With the additional safety features, this prototype has been assessed as a class 1 laser instrument.
- The breadboard system was built to time and connected to a Waters Xevo G2XS ToF instrument to demonstrate the fundamental ability to generate an ion beam.
- A second “demonstrator” instrument was designed to connect to either the Waters Xevo G2XS or a Thermo Exploris 480.
- In addition to incorporating some additional safety features the “demonstrator” has a mounting plate that enables reproducible connectivity to either MS system.
- Two different heated transfer capillaries are required since the configuration of the sample inlet and source block orientation are different on each detector.

## Ion Transfer Capillary

The ion transfer capillary is basically a heated stainless steel capillary which guides the sample plume (generated by the laser) and the charged methanol spray into the mass spectrometer using the vacuum generated behind the sample cone.

Since the ion blocks on the two mass spectrometers have different configurations, we designed two different transfer optics. For the Thermo instrument we use a straight heated letter box capillary while for the Waters MS we have a capillary with a 90° bend (Figure 2a).

The transfer capillary enters the IR-MALDESI enclosure via a port at the back of the instrument. To locate the IR-MALDESI interface with the Thermo instrument we use 2 mounting pins that would typically locate the electro-spray source (Figure 2b). We have modified the Waters source enclosure so that it also has 2 mounting pins (Figure 2c).

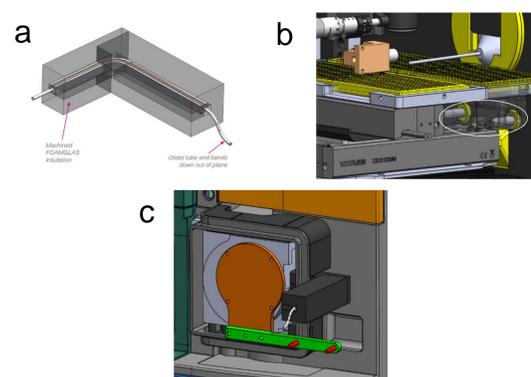


Figure 2. CAD representation of the transfer optics and mounting plates on the IR-MALDESI “demonstrator”. a) The transfer optic for the Waters Xevo G2XS with a 90° bend to enable the IR-MALDESI instrument to stand in front of the mass detector. b) Thermo transfer capillary reaching into the IR-MALDESI enclosure via the port (yellow) at the back of the device. The mounting pins are circled in white. c) A front view of the modified Waters source enclosure with the transfer optic in place and the mounting pins to locate the IR-MALDESI interface.

## The Black Box

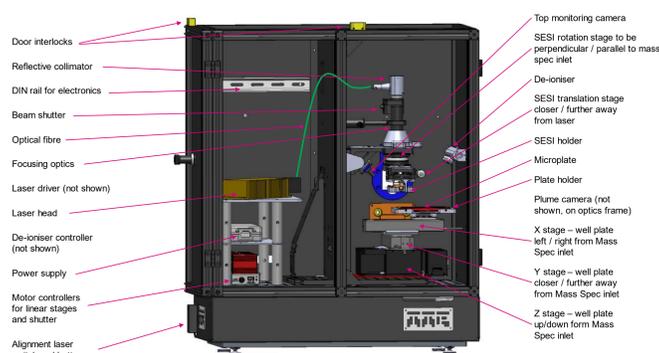


Figure 3 above shows a schematic of the internal workings of the IR-MALDESI prototype. The plate handler with the adjustment in the X, Y and Z plane is mounted on the deck plate, the electronics to control the laser and the plate position were mounted under the main assembly. All the moving parts were enclosed in a large black box with safety interlocks.

The SESI spray was mounted in a 3D printed holder above the plate stage and was connected to an external Agilent 1100 binary pump delivering between 0.5-1µL/min of 90% methanol: 9% water: 1% formic solvent.

Initially we are using a simple T piece fitted with a fused silica capillary which generates the charged spray, but we have the option to replace this with a commercial DESI XS spray head from Waters (Figure 4).

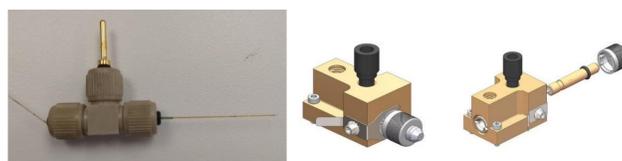


Figure 4. SESI spray options for IR-MALDESI “demonstrator”. Left – standard fused silica capillary emitter option, right – CAD drawing of commercial DESI XS spray head from Waters.

High voltage (~3kV) was supplied into the solvent stream with voltage controlled directly from the MS.

## First MS Data

The first test was to take a small molecule standard, warfarin and ensure that we could generate a signal in both positive and negative ion mode on both mass spectrometers. Typical data is shown in Figure 5.

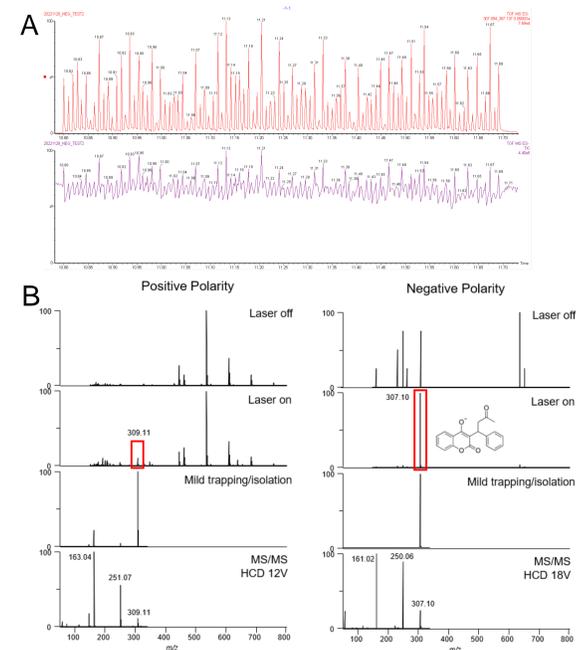


Figure 5A. Total ion current and extracted spectra from Waters Xevo G2XS for samples of warfarin. The data matches that generated by AMI-MS, though the samples were taken from a 1536 well source rather than 384. B – Initial data from Thermo Exploris 480 using the same IR-MALDESI source. The parent ion for warfarin is only present when the laser is firing, we confirmed the peak by carrying out MS/MS fragmentation.

The IR-MALDESI interface requires some optimization for the Thermo Exploris 480, we are currently working to improve the sensitivity and understand the range of analytes that we can detect.

Longer term our aim is to understand the relative merits and differences of using IR-MALDESI with the different MS systems from the two companies.

We plan to operate the system as an open access platform.

## Conclusions

- Initially we built a breadboard system to validate an IR-MALDESI source with the Waters Xevo G2XS ToF.
- Subsequently we improved the breadboard to build a “demonstrator” prototype with the ability to connect to either a Waters Xevo G2XS or Thermo Exploris 480 mass spectrometer.
- The “demonstrator” has been independently assessed as a class 1 laser instrument.
- Once we have established the basic robustness and usability of the system, we plan to make the platform open access for the external science community.
- If you are interested in access to this technology please contact [jonathan.wingfield@astrazeneca.com](mailto:jonathan.wingfield@astrazeneca.com)
- Scan the barcodes to see video footage of the system in action



## References

- Pu, F. *et al.* High-Throughput Label-Free Biochemical Assays Using Infrared Matrix-Assisted Desorption Electro-spray Ionization Mass Spectrometry. *Anal. Chem.* **93**, 6792-6800 (2021). <https://doi.org/10.1021/acs.analchem.1c00737>

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