Continued development of high throughput MS within AstraZeneca's Discovery Sciences

Jonathan Wingfield[1], Adrià Escobet-Montalban[2]

Mechanistic and Structural Biology, Discovery Sciences, R&D, AstraZeneca, Cambridge, UK[1]
TTP plc, TTP Campus, Melbourn, Cambridge, UK[2]

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 to purchase our systems have a finite lifespan of support.

Here we will introduce an IR-MALDESI interface that can replace the acoustic mist ionization system. The IR-MALDESI prototype has been built with our collaborators at TTP.

Introduction

- Following the publications from Abbvie[1] where they describe a 1536 compatible laser based high throughput mass spectrometry loading system, AZ decided to collaborate with TTP to develop and test an IR-MALDESI interface that can replace AMI-MS.
- 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 solvent ions across the surface of the plate and the sample droplets merge with the cross spray and are sucked into the mass detector (Figure 1).
- Mechanically an IR-MALDESI interface should be relatively simple to build and would interface with our MS via a modified version of the existing AMI transfer optic.
- With limited knowledge of lasers and with no access to on site engineering we decided to work with a local engineering consultation company (TTP) to deliver a prototype.

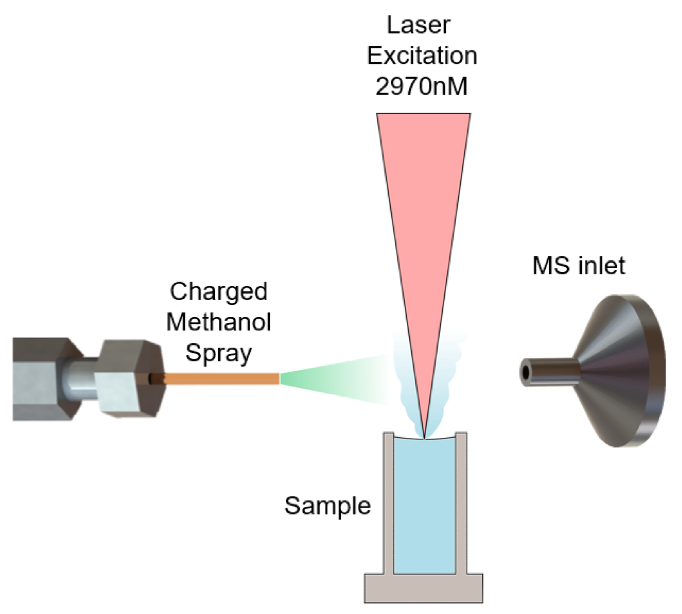


Figure 1. Schematic of IR-MALDESI.
The laser illumination of the sample generates a droplet spray which then merges with an ionized solvent stream generated by a secondary ionization electrospray (SESI) source. The merged droplets are drawn into the MS.

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 and 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.
- Significant thought was put into the design of the optical components of the system so as to minimize safety risks, reduce energy loss during light transmission and remove the requirement for lab staff make any adjustments to the light path.
- The estimated project timelines are shown in Figure 2.

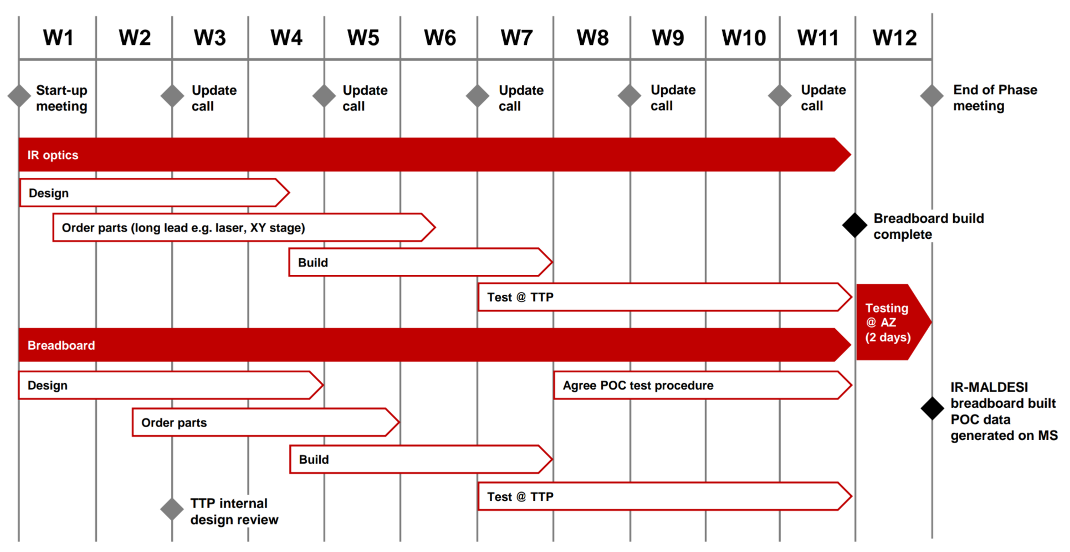


Figure 2. Initial timeline for project delivery.
This plan focuses on the development activities undertaken by TTP. In order to minimize the overall timelines AZ provided the MS infrastructure such as the SESI ionization components, heated transfer capillary and solvent delivery system.

Optics

For multiple reasons, we decided to use an optical fibre light guide to transmit the laser light onto the sample.

To maximize the laser energy reaching the sample a reflective collimator and compact Galilean beam expander were fitted. Figure 3 shows that a beam with a narrow numerical aperture (red) is difficult to focus into a small spot. A beam with a wide numerical aperture (green) can be more effectively focused brining more power to a smaller area.

To ensure that the laser has a reasonable focal depth a small lens was included in the optical module. This reduces the risk of laser power differences on the sample should the liquid height vary between samples.

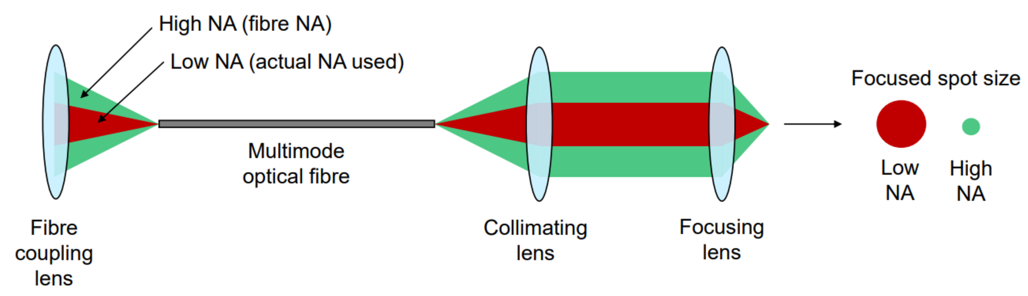


Figure 3. Schematic of the optical system.
The use of an optical fibre significantly reduces the safety risk associated with laser light as the beam is tightly controlled to the sample. Careful selection of the fibre, collimator and lenses reduces the energy loss between the source and the illumination event.

The final assembly of the optical module is shown in Figure 4a. The configuration was test fired using burn paper to visually check the beam characteristics (Figure 4b & c)

The beam appears to have a very reproducible circular shape with a high energy focus in the middle of the beam. At 8 pulses per burst (PPB) the paper is effectively burnt through.

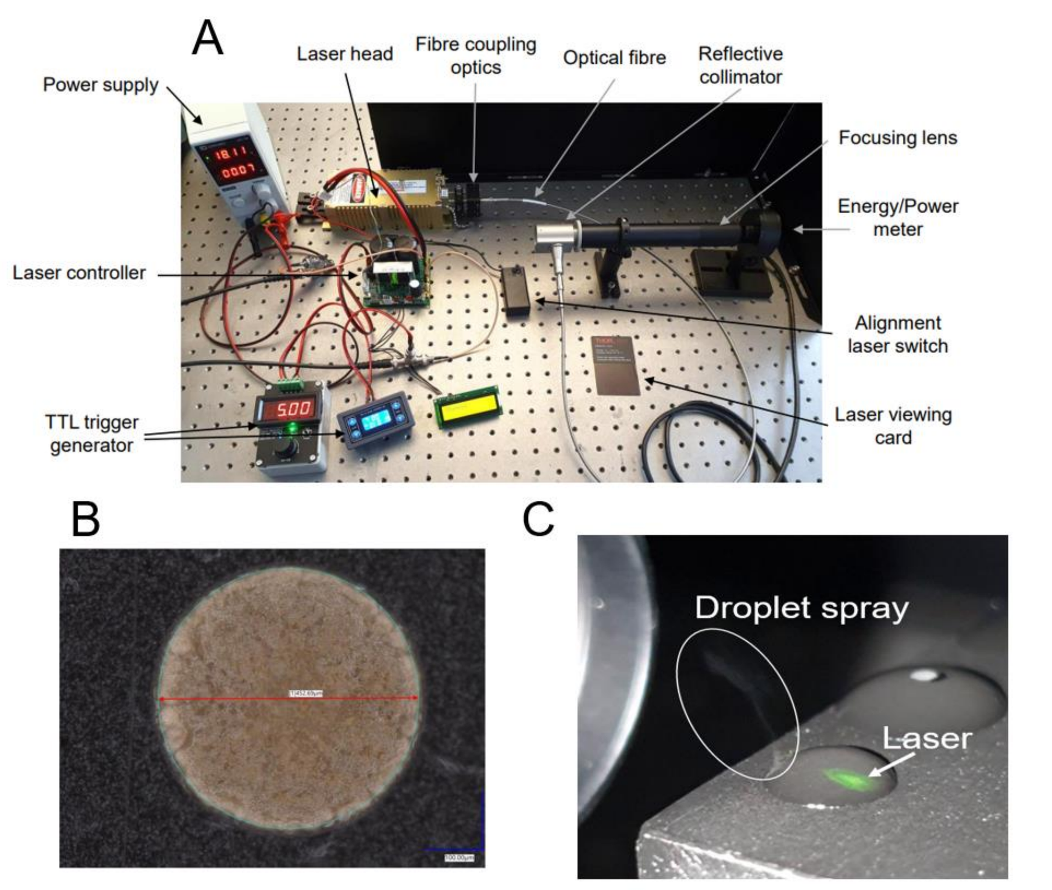
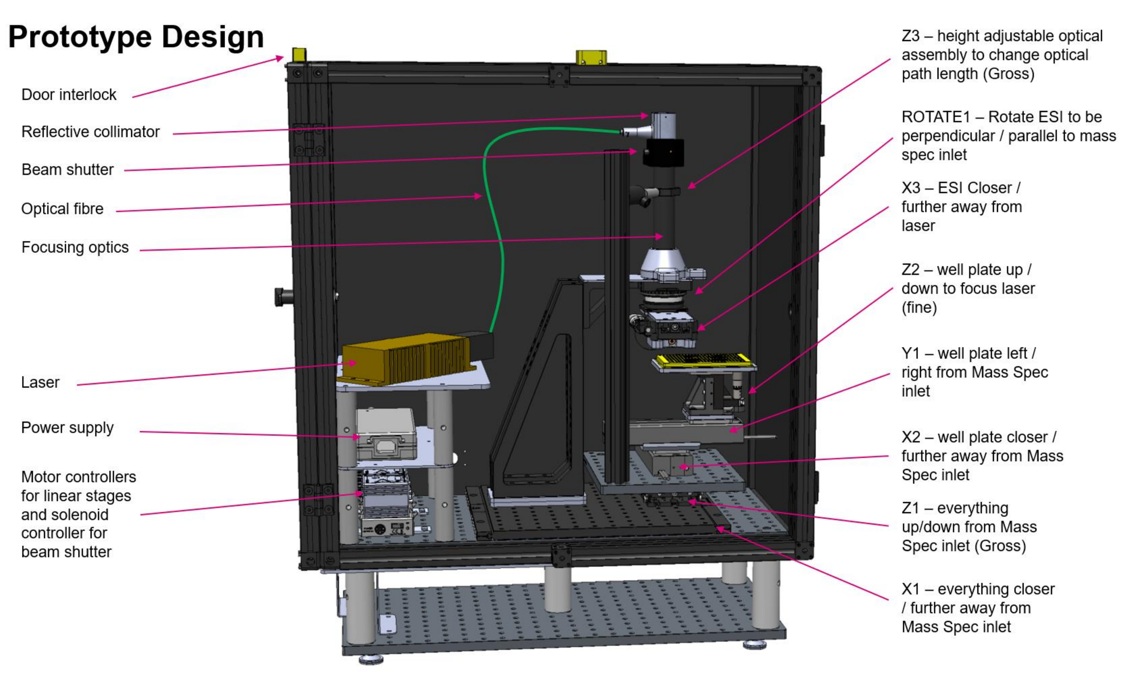


Figure 4A. Assembled optical components to bench test. This assembly have to fit inside the box for future use but was tested externally for ease of use.
4B. Burn tests show the beam pattern when focused. With optimum lens configuration the beam diameter is a reproducible 345µm (B). Testing the laser with a water droplet on a surface we can see the spray being generated (C).

The Black Box



The 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 syringe pump delivering between 0.5-1µL/min of 90% methanol: 9% water: 1% formic solvent.

High voltage (3kV) was supplied into the solvent stream directly from the MS

A modified (shortened) AMI-MS heated transfer capillary connected to the MS source block was fed into the back of the IR-MALDESI unit and aligned with the SESI spray above the sample plate. The temperature of the transfer optic was initially set to 300 Celsius.

The alignment of the SESI spray with the MS inlet was checked by turning on the solvent pump and applying the high voltage to generate a methanol ion beam.

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.

Wells of a 1536 well plate were filled with 10µM warfarin solution and the plate loaded into the plate handler.

The laser was set to 2 ppb, 40msec on and 500msec off for the initial test and then fired, the total ion current and the extracted mass peak associated with the 307Da mass were collected and are shown in Figure 5A.

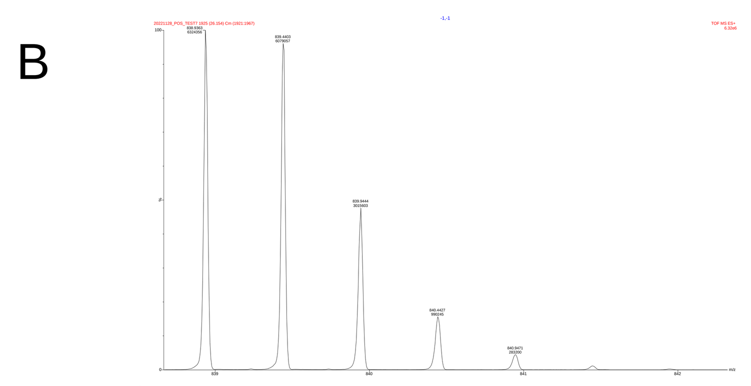


Figure 5A. Total ion current and extracted spectra from samples of warfarin. The data matches that generated by AMI-MS, though the samples were taken from a 1536 well source rather than 384. B – extracted spectra from a sample of peptide, the 0.5Da spacing between the peaks confirms that this is double charged species.

As a final test, wells were loaded with a 10uM solution of peptide and fired into the MS. As shown in Figure 5B, the extracted spectra from these samplings contained the double charge species of the peptide. Interestingly the single charge species was present but at very low abundance while the triple charge was not found. This was different from the patterns seen with AMI-MS

Conclusions

- Construction of the prototype instrument ran to the 11 week target timeline and came in on budget
- The instrument was connected to a Waters Xevo G2XS once the AMI interface had been disconnected. Conversion time was around 30 minutes
- Small molecule and peptide data was collected from 1536 well plates using an illumination time of 40msec
- Sample flow rate was estimated at 10nL per second, 20 times less than AMI-MS though the data quality was similar
- We will continue to develop IR-MALDESI as a platform to take over from AMI-MS

References

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

Acknowledgements
Andreas Dannhorn (AZ) provided the SESI parts and knowledge to help set up a stable ion beam. Wayne Bowen (TTP) provided excellent project management, Ethan Nott (TTP) and Matthew Sadler (TTP) mechanical engineering support.

AstraZeneca

Presented at SLAS Europe 2023.