Modifications to a linear time-of-flight mass spectrometer for mass resolved microscopy with the PImMS camera

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- University of Oxford Physics - R. Nickerson
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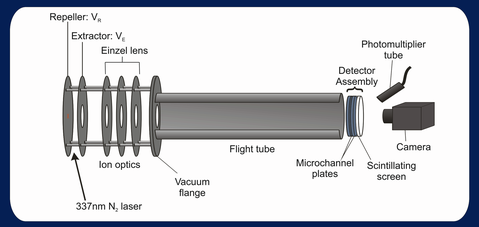
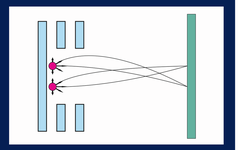
[1] S. Luxembourg, T. H. Mize, L. A. McDonnell, and R. M. A. Heeren, *Anal. Chem.* **76**, 5339 (2004)
[2] E. Halford, *et al.*, *Rapid Comms. Mass Spectrom.*, **in press**
[3] M. Brouard, *et al.*, *Rev. Sci. Instrum.* **83**, 114101, (2012)

1) Motivation



- Microscope-mode[1] imaging mass spectrometry (MSI) offers up large quantities of data that, with the correct equipment, can be recorded and analysed.



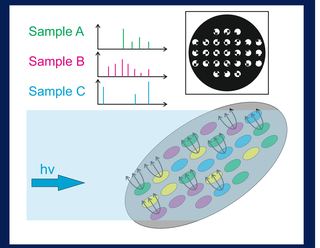
- With minor modifications[2], a linear time-of-flight mass spectrometry (ToF-MS) instrument can record MSI data in microscope-mode.

- The PImMS camera (Pixel Imaging Mass Spectrometry) is an event-triggered, time-stamping camera[3].

- This allows for data to be collected without the need to define acquisition frames.

- Every time an event is detected the camera records the (x,y,t) coordinate of that event.

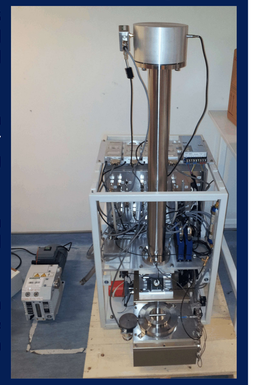
- This type of detector device allows for a much enhanced sample throughput as all of the spatial and mass information is obtained simultaneously.



- Micro-array samples could be analysed in parallel by a single machine using the spatial data to identify which particular sample well is being investigated.

2) Methods & Data Processing

- An Lt2 Plus linear MALDI ToF-MS instrument (SAI Ltd) was modified to record MSI data in microscope mode (pictured "stripped down" right).

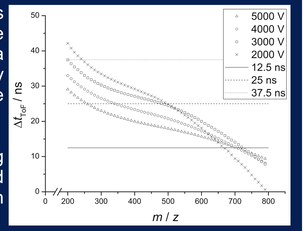


- The ToF detector was exchanged for a microchannel plate (MCP) and scintillator detector with a BC408 scintillator being used initially (decay time ~2.5ns).

- The laser optics were adjusted so that a large area of the sample was illuminated with each laser shot (400μm diameter).

- Finally, the voltages applied to the ion optics, and the pulsing regimes used were adapted to conserve the spatial information of the ablated ions.

- PImMS currently runs with a 12.5ns timing precision. Regardless of the ion optic resolution if features of a mass spectrum are seperated by less than 12.5ns they will not be resolvable.

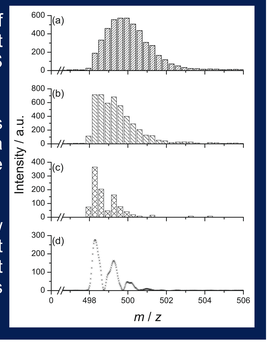


- By reducing the accelerating potentials ion ToFs are increased giving an increased seperation between neighbouring mass peaks.

- When an ion strikes an MCP a cloud of electrons is created. This single ion event will trigger multiple pixels on the PImMS camera.

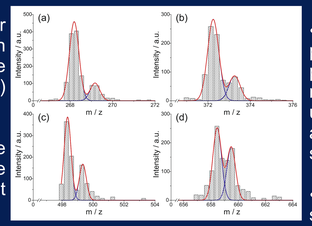
- By analysing these clusters of pixels one can reduce each cluster down to a single coordinate: the centroid of the group.

- The successive plots (a), (b), & (c) show raw PImMS data undergoing different levels of the processing algorithm to best reproduce the data in plot (d) which was acquired with a photo-multiplier tube.



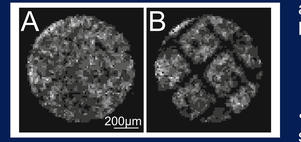
3) Results

- The plot on the right shows four different dyes which were acquired with the modified Lt2 Plus. The species are (a) Auramine O, (b) Crystal Violet, (c) Exalite 384, & (d) Exalite 404.



- Data were acquired under the same conditions, and all four species were resolved down to the isotopic limit (Δm<1).

- The two images on the right were acquired simultaneously. The represent an homogenous layer of Auramine O (A), and a grid pattern of Crystal Violet (B). The images were found to have a resolution of ~20um.



- The experimental data acquired can then be compared to data that would be expected from simulations using the ion trajectory simulation software SIMION 8.0.

- The results from SIMION simulations do not take detector response times into consideration so a convolution for these is necessary.

- The scintillating screens used were composed of BC408 and E404. Both have a decay time of ~2.5ns.

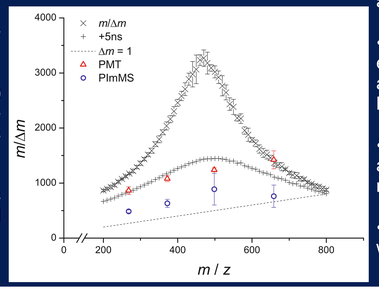
- This has to be convoluted with a response time for a photomultiplier tube. This is needed to acquire a spectrum and has a response of ~2ns.

- The final convolution is found to be ~5ns and can then be compared to the resolution obtained with the PMT.

- This plot shows data for the four dye species shown above.

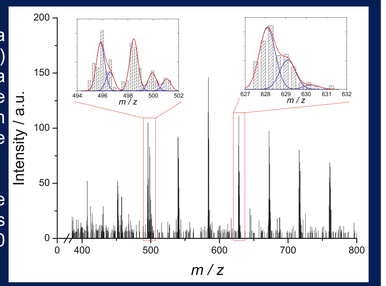
- The mass resolutions (m/Δm) obtained experimentally are compared to those resolutions obtained by simulation.

- The experimental data is shown for both the PMT and PImMS.



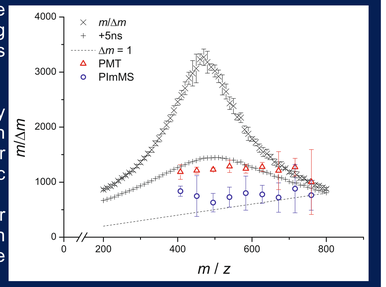
- Data was also obtained using a polyethylene glycol (PEG) polymer. This sample gives a range of mass peaks which can be used to measure the resolution across the useable range of the spectrometer.

- Closer inspection of the spectrum shows that the peaks across the range 400 < m/z < 800 have an isotopic resolution.



- The data shown above can be summarised in this plot showing the achievable mass resolutions across the range 400 < m/z < 800.

- The PImMS data is reliably above the isotopic limit although the error bars extend at the higher end of the range as the ion optic resolution drops off.
- The PMT data exhibits a higher resolution that than acquired with PImMS and fits closely to the simulated values.



- The processing algorithm explained earlier can also be extended to interpolate data points.

- This opens up the possibility of increasing the effective timing precision of the camera although at some point the inherent camera resolution will limit this.

- By increasing the timing precision of the data set a higher quality spectrum can be seen better reflecting the spectrum obtained with the PMT.

- This method also reduces the amount of data wasted in the processing steps.