Ultra-high resolution 2D-FTMS for truly data independent analysis of challenging systems

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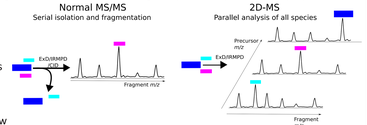
Introduction:

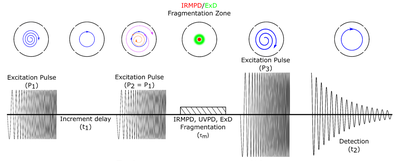
Two-dimensional mass spectrometry (2DMS) is a data-independent technique which can be used to analyse all species in a spectrum to provide accurate fragmentation patterns for each and every precursor ion observed.

2DMS uses modulation pulses within ion-trapping instruments (e.g. FT-ICR MS) to excite ions to different radii prior to fragmentation with a radius-dependent technique (e.g. IRMPD, UVPD, ExD). By repeatedly scanning a sample in this fashion and by incrementing the delay between excitation pulses a 2D map can be constructed revealing the fragments of each species in a parallel fashion. 2DMS has already been applied to a wide variety of samples including; small molecules, peptide digests, protein top down, and polymers.

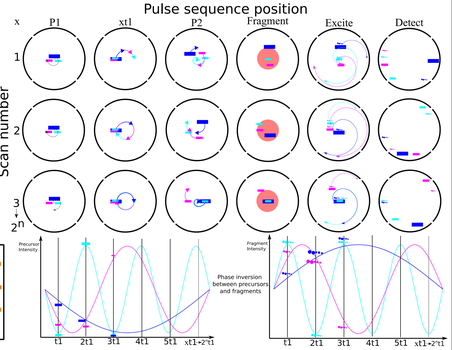


The layout of a general pulse-delay-pulse-fragmentation-excitation 2DMS experiment is shown below for several scans to illustrate the iterative creation of the vertical axis and correlation between precursors and fragments.

2D pulse sequence excites ions inside the ICR cell, then waits for a short amount of time (~micro seconds) to allow ions to orbit around the cell. This is then followed by another pulse which will excite or de-excite ions based on their position (phase), leading to a distribution of ions at different radii. A radius dependent MS/MS technique is then used to fragment the ions before a standard excite-detect sequence. A stack -plot of these spectra are then acquired with different delay times.



The 2D map produced (key shown below), shows 2 axis—the precursor m/z (y axis) and the fragment m/z (x axis). This map allows one to read the exact fragmentation pattern of each precursor by looking horizontally. The precursor spectrum is produced by reading the x=y diagonal line, diagonal lines offset from this show neutral losses from precursors.



A huge density of information can be mined from a single 2DMS plot.

Despite being a very powerful technique, this level of data acquisition generates large amounts of complex data and creates interesting analysis challenges. Herein the latest progress and solutions to many of those challenges are presented and discussed, the development of which has enabled the application of 2DMS to ever more complex and difficult samples.

Methods and processing:

All spectra were acquired on a 12 tesla Bruker SolariX FT-ICR mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an infinity cell.

IRMPD MS/MS and 2D MS spectra were acquired using a continuous wave CO2 laser (Synrad, US) using 50-70% laser power and 0.1-0.8 s pulse lengths. ECD MS/MS was achieved through the use of a hollow dispenser cathode, heated at 1.5 A, using 1.2 eV electrons, and a lens voltage of 20V. EID MS/MS was achieved using 16 eV electrons from the same setup. UVPD MS/MS was integrated with SolariX using the 213nm output of an Nd:YAG laser (Litron lasers, UK) and 3 crystals to move from the fundamental 1064nm, to 532nm, 355nm, and finally 213nm. Energies were measured to be 12mJ/pulse at laser and 6mJ/pulse into instrument. The UVPD laser fires at a repetition rate of 10Hz with a pulse width of ~7ns.

2D acquisitions were acquired using a custom pulse sequence within SolariX control (Bruker), these have also been migrated to FTMS control (Bruker). A pulse-delay-pulse followed by MS/MS event was added into the excitation command section of the pulse program and the delay incrementally increased between scans for 2^n times. Usually 4096 or 8192 scans. 2D spectra were 2D-FFT'd, digitally demodulated, zero-filled, and sometimes denoised (using UrQrd), using the SPIKE software using a PC cluster.

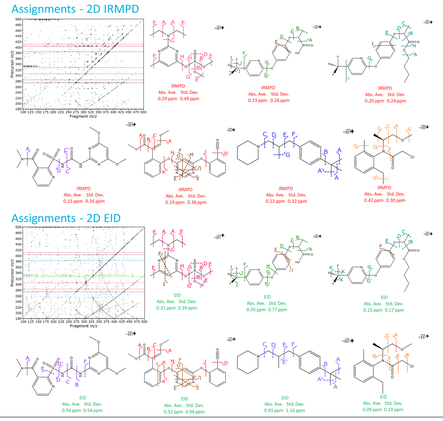
All 2D spectra were analysed, calibrated, peak picked and exported using the True-2D (T2D) software suite, coded in the O'Connor group.

Exported spectra were analysed in Bruker Data Analysis software package, tested versions include 4.0, 4.1, 4.2, 4.3, and 4.4.

Polymer, agrochemical, and peptide samples were ionised using nano-electrospray ionisation (nESI). Bio-oil samples were ionised using APPI with a 10.6eV krypton lamp (Bruker).

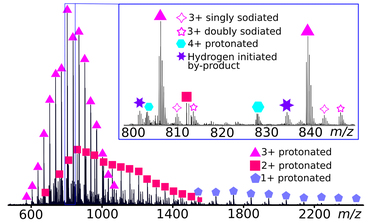
Sub-ppm to ppb accuracy

High resolution accurate mass (HRAM) measurements are particularly important for small molecule analysis to ensure confidence in assignments and discriminate species from outside matricies/contaminants. 2D-FT-ICR MS was applied to a mixture of agrochemicals using both infra-red multiphoton dissociation (IRMPD) and electron induced dissociation (EID) fragmentation methods to study the varying chemistries of the small molecules.

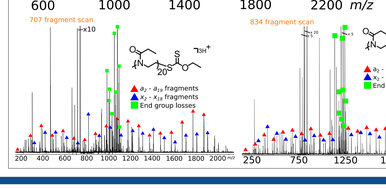


Calibration of 2D spectra

Internal recalibration of FT-ICR MS spectra reaches the sub-ppm assignment range, but only with careful recalibration. Using T2D software this is now possible for 2D-MS spectra. Spectra and tables below show the accuracy achievable for precursors on the auto-correlation line after quadratic recalibration within T2D, all peaks are assigned in the sub-ppm range. Interestingly and advantageously a single line in a 2D spectrum can be used to accurately recalibrate all other lines in the same spectrum, this has become particularly beneficial for proteomics analysis where a single fragmentation line (horizontal line) of a known or spiked peptide can be used to calibrate the entire data set and provide accurate assignments for hundreds to thousands of species.



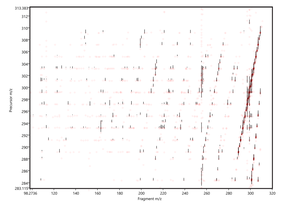
Species	Observed m/z	Intensity	Exact m/z	Error/
$M_{14}H^{3+}$	541.683208	5.26E+08	541.683232	-0.044
$M_{14}H^{3+}$	574.706036	2.64E+08	574.706036	-0.001
$M_{14}H^{3+}$	607.728726	8.33E+09	607.728841	-0.189
$M_{14}H^{3+}$	640.751560	1.69E+10	640.751646	-0.134
$M_{14}H^{3+}$	673.774451	3.38E+10	673.774456	0.001
$M_{14}H^{3+}$	706.797352	4.67E+10	706.797255	0.138
$M_{14}H^{3+}$	739.820060	6.36E+10	739.820060	0.001
$M_{14}H^{3+}$	772.842900	6.52E+10	772.842864	0.046
$M_{14}H^{3+}$	805.865789	7.4E+10	805.865669	0.149
$M_{14}H^{3+}$	838.888201	6.53E+10	838.888473	-0.325
$M_{14}H^{3+}$	871.911087	6.37E+10	871.911278	-0.219
$M_{14}H^{3+}$	904.933737	4.87E+10	904.934083	-0.382
$M_{14}H^{3+}$	937.956756	3.45E+10	937.956887	-0.140
$M_{14}H^{3+}$	970.979408	2.73E+10	970.979692	-0.292
$M_{14}H^{3+}$	1004.002499	1.63E+10	1004.002497	0.002
$M_{14}H^{3+}$	1037.024801	8.69E+09	1037.025301	-0.482
$M_{14}H^{3+}$	1070.047356	4.44E+09	1070.048106	-0.701
$M_{14}H^{3+}$	1103.070365	2.19E+09	1103.070910	-0.494
$M_{14}H^{3+}$	1136.093201	1.04E+09	1136.093715	-0.452
$M_{14}H^{3+}$	1169.116371	5.57E+08	1169.116520	-0.127
			abs. average	0.22 ppm
			std. dev.	0.23 ppm



Precursor assignments of a polymer distribution observed in the spectrum opposite. Species used for calibration are marked in red. Mx indicated the number of monomer units within the polymeric species

Ultra-high resolution DIA

2D-FT-ICR MS analysis benefits from FT-ICR MS's inherent ultra-high resolution in the x (fragment) dimension with ease using standard parameters. However the vertical (precursor/y) axis is also based on the ion frequencies and so benefits from high magnetics fields and scales with increasing number of scan lines and Nyquist frequency (frequency of the lowest m/z ion). By adjusting these parameters even modest data sets such as 4096 x 1M data point transients can produce very high precursor resolution and enable analysis of particularly complex samples such as petroleum.

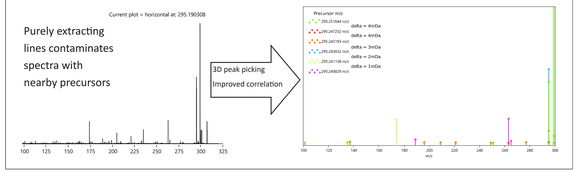


Zoom in of bio-oil sample ionised by APPI and fragmented using 2DMS. Small sections can be quad isolated to improve intensity and adjust Nyquist. ~500 precursors isolated.

After 3D peak picking (below), a precursor resolution of 2 mDa was achieved, allowing species-specific analysis of complex mixture data without individual isolation or optimisation.

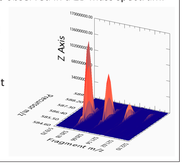
One-click peak picking and export for the whole 2D enables throughput and output to 3rd party interpretation software on a per-precursor basis.

8166 peaks were 3D picked using T2D for the entire spectrum. Peaks labelled in spectrum opposite.



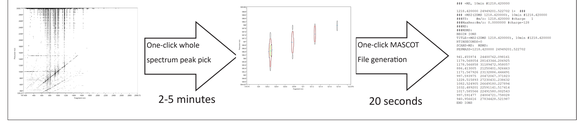
Peak picking in 3D

Peak picking to ppb accuracy in standard MS or MS/MS data can be challenging depending on spectral complexity, peak shapes, artefacts (Gibbs oscillations) etc., unfortunately this challenge is increased much further when moving into 2 dimensions and attempting to assign the centre of the 3 dimensional peaks observed in a 2D mass spectrum. T2D uses a newly developed peak picking algorithm to first assign all peak features in the spectrum, by runing an analysis on the several hundred thousand peaks to correlate their 2D position relative to each other. Since FT-ICR MS peaks are a predictable peak width (in data points), the boundary conditions for correlation can be easily set to correlate all x picked peaks within a y area without overlap or double assignment. This is then combined with a y axis evaluation which considers all of the peak features involved to increase the accuracy of y assignment 5-10 fold over purely parabolic fitting which has a hindered accuracy due to the limitation of a given fraction of bin size.



Exporting data and proteomics

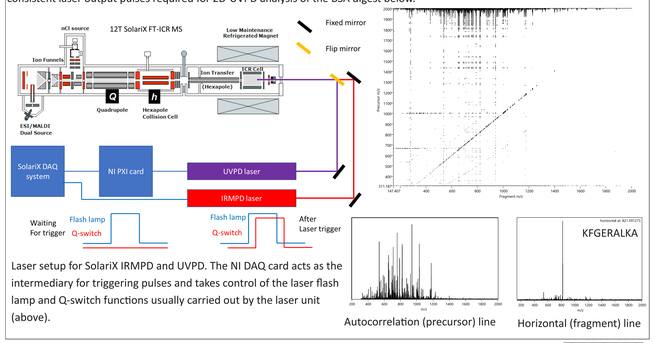
2D MS spectra can be peak picked inside T2D to export vast lists of x-y-z co-ordinates for each peak in the spectrum. For a proteomic standard such as BSA digest this was ~40,000 peaks—consisting of each precursor and each associated set of fragments (including all isotopes of each). This list is then easily grouped by y axis value (precursor m/z) to create a list of precursor specific ions consisting of the precursor itself and all fragment ions which modulated with said precursor. These lists can then be viewed independently (offline program) and/or be exported to MASCOT proteomics file format for database searching. Raw x-y-z co-ordinates can also be directly exported for other applications/downstream analysis.



UVPD

Implementation of 213nm UVPD was carried out in the UHV region of the FT-ICR MS instrument after trapping ions in the ICR cell.

The Nd:YAG laser provided a flash lamp pulse at a repetition rate of 10Hz, requiring a Q-switch delay 230 micro seconds afterwards. The SolariX scan rate can be tuned from ~1Hz to 0.25Hz depending on application, but is a serial instrument and is not based on absolute timing, thus the 2 systems can never be synchronised for division of repetition rate firing sequence. Instead a National Instruments PXI DAQ card was used as an interface between the SolariX acquisition system and the UVPD laser creating a flash lamp pulse every 0.1s to maintain the flash lamp and keep the laser in a state ready to output a consistent pulse. Upon receiving a trigger from the SolariX for laser dissociation, the request would be stored until the next pulse was scheduled, at which point a flash lamp pulse and a secondary pulse (230 micro seconds later) was generated to trigger the Q-switch at the correct timing and produce consistent laser output pulses required for 2D-UVPD analysis of the BSA digest below.



Laser setup for SolariX IRMPD and UVPD. The NI DAQ card acts as the intermediary for triggering pulses and takes control of the laser flash lamp and Q-switch functions usually carried out by the laser unit (above).

T2D is available to all:

T2D is packaged as a single (~47MB) .exe program and is easily distributed to anyone interested in pushing the limits of 2D-FT-ICR MS.

T2D is written in LabView (National Instruments coding language) and is compatible with all versions of Windows. The program can either be run purely from the .exe (NI driver required, free download), or can be installed from an installer (driver included).

Please contact me on C.Wootton.1@warwick.ac.uk if you would like a free copy of T2D.

T2D is written as a low-resource requirement program, the handling of the large 2D-FT-ICR files is all done in binary format from the file on the hard drive to increase speed of computing operations over xml style formats previously used and avoids excessive use of RAM. All the data shown was analysed on a 8GB RAM tablet PC. The program is configured for default SolariX acquisition assumptions (notes included in program), happy to discuss easy ways to input files for non-standard setups.

Acknowledgements:

Thank you to all of the co-authors for providing their latest and greatest data analysed using T2D and its various functions. The authors would also like to thank the other members of the O'Connor group for their research into 2DMS, especially Maartje van Agthoven for her assistance in setting up 2DMS on our SolariX FT-ICR MS.

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