A FULLY AUTOMATED DESI IMAGING PLATFORM- FROM SLIDE LOADING TO DATA PROCESSING.

3D TISSUE IMAGING OR LARGE SAMPLE COHORT STUDIES WITH MINIMAL USER INPUT

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INTRODUCTION

In order to maximise the potential of imaging mass spectrometry, integrated workflows and automation will be key requirements for the uptake of the technique by non-expert users. With advances in acquisition software, tissue object identification, data directed methodologies and robotics, a full 3D imaging data set from serial sections or a large cohort of patient samples can be analysed and processed with minimal interaction from the user.

Here we introduce a desorption electrospray ionization (DESI) mass spectrometry system which combines a commercially available source with an automated slide loader for high throughput mass spectrometry imaging. Figure 1 illustrates the components and workflow that are involved in this approach.

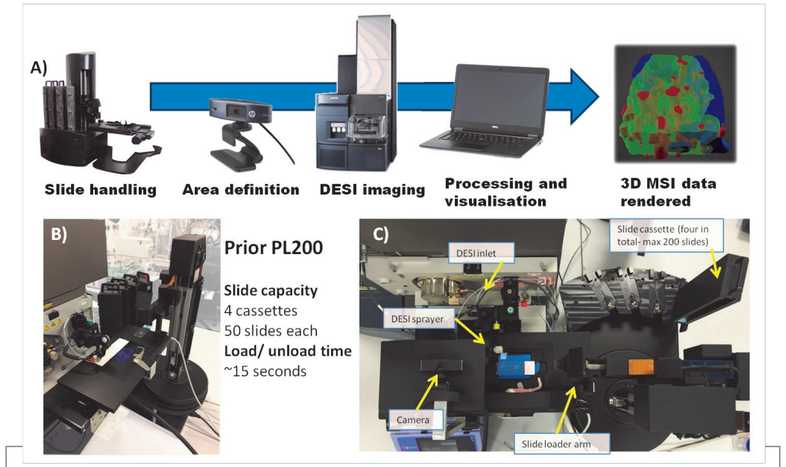

Figure 1. Setup for the automated DESI imaging platform . A) The components involved in the experiment. B) The Prior loader loading a slide to the DESI stage C) An aerial view of the system prior to operation.

METHODS

Hardware and mass spectrometry

All experiments were carried out on a Xevo G2-XS QToF (Waters, Wilmslow, UK) with a DESI 2D source from Prosolia (Indianapolis, US). The PL200 slide loader (Prior, Cambridge, UK) was obtained with a modified top plate for the DESI stage (fig 1b). No other hardware modifications were required. DESI analysis conditions were typical values as reported in the literature[1].

Acquisition and data processing software

The Prior software development kit was used to integrate the robotic slide loader into the MS workflow, C# and MATLAB applications were written to initiate and define the experimental parameters. HDI 1.4 (Waters) was used for data processing with an in-house MATLAB program used to reconstruct, correct and then visualise the data from the multiple experiments. In the case of the 3D data the results can be exported to 3rd party software such as Blender for enhanced rendering options.

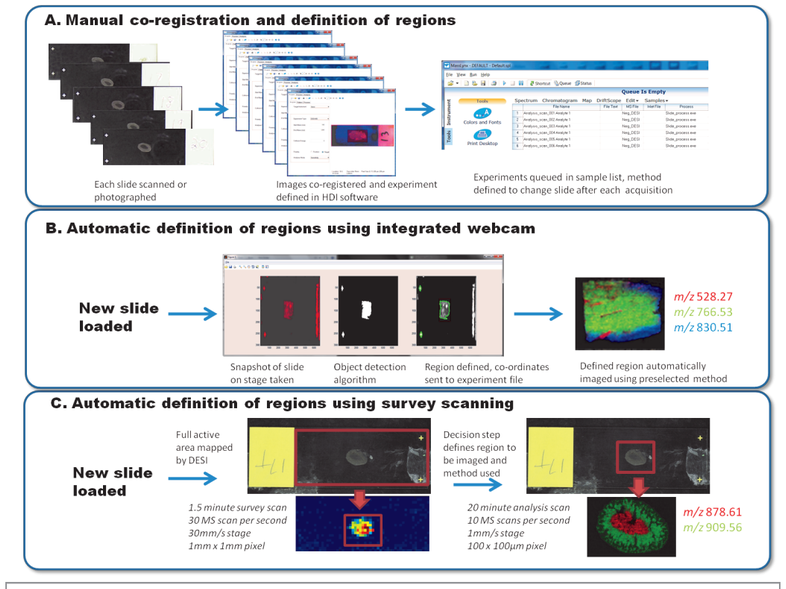

Figure 2: Available experimental modes for automated imaging experiments. A) All the regions are manually defined by the user B) Each slide is optically scanned for tissue sections prior to analysis C) A mass spec survey scan is used to define the region to image.

RESULTS

Acquisition times

In order to justify the requirement for an automated sample loading system, the acquisition times of an imaging experiment should be on the scale of minutes not hours. Recent advances in DESI methodologies have greatly increased the data quality achievable both from a spatial resolution and signal intensity standpoint. This has allowed for imaging experiments at sub 10 minute time scales and with no overhead time considerations due to sample preparation and vacuum chamber loading times, as seen with MALDI-MS analysis, this is the actual time per sample.

Figure 3 shows a seven minute mouse brain acquisition at 150 x 150µm pixel size, which including sample removal from freezer, warming to room temperature, experiment definition and data processing is still in the time frame of ten minutes.

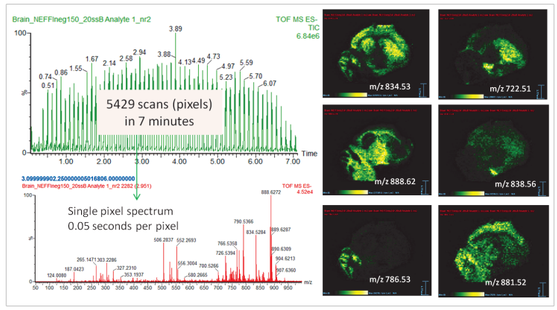

Figure 3: A DESI-MS imaging experiment of a sagittal section of mouse brain in negative ion mode. Total MS time was 7 minutes, total time from storage to data viewing 15 minutes. Pixel size is 150 x 150µm.

Automation

Being able to analyse samples at these timeframes justifies the development of an automated system. With the Prior slide loader, up to 200 slides can be loaded into four cassettes with a load time of approximately 20 seconds for a slide. Currently, the slides cannot be kept cold or in an inert environment which limits the length of experiment conducted to date. A MATLAB executable is run from the MassLynx sample list at the start of the whole run (loading slide 1) and then after each experiment. This executable unloads/ loads the slides, carries out the tissue region definition by one of three methods (figure 2) before the DESI analysis is started.

Data Processing

Figure 4 demonstrates the workflow for the processing of a three dimensional mass spectrometry imaging experiment. By automating this process a large bottleneck in the carrying out of multiple section experiments is removed. Firstly a combined peak list is generated from all raw files, this is then used to extract the relevant peak areas from all the data together. As they all share a common peak list these can now be combined and processed, typically by non-negative matrix factorisation[2] so that the distinct chemical regions within the tissue can be visualised. These can then be viewed within the software or exported as *.obj files to be rendered in more suitable software[3]. These workflows lend themselves to in-line processing and automation as shown in figure 7.

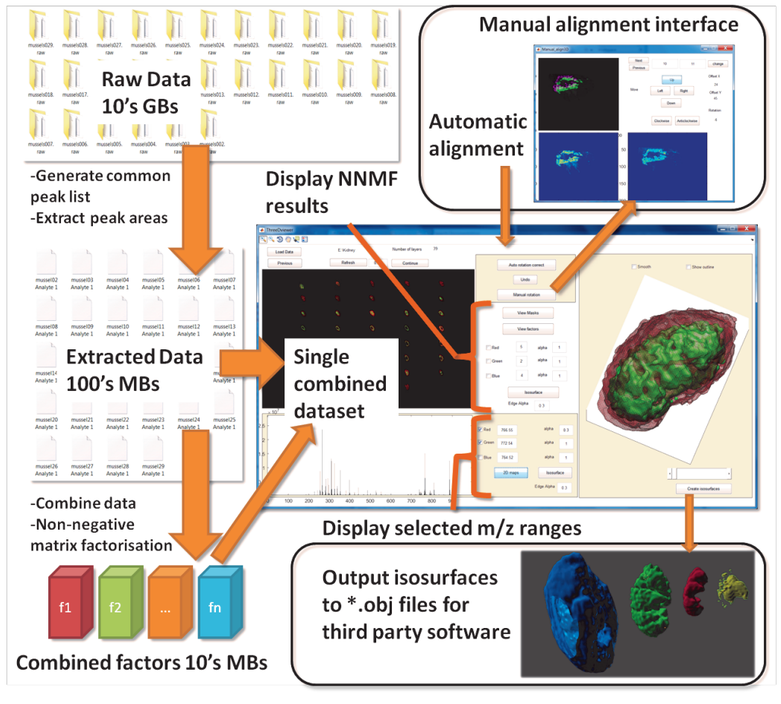

Figure 4. Data processing workflow that incorporates the data extraction component of HDI 1.4 and ThreeDViewer software. The later allows multiple image datasets to be combined and subjected to multivariate analysis as a single collection of pixels. These MVA output or selected ion intensity maps can then be plotted in 2D or if relevant as a 3D isosurfaces.

DISCUSSION

With the ability to automatically load samples onto an imaging mass spectrometry platform (fig 5), along with analysis times in the 10s of minutes time scale, the potential impact of imaging mass spectrometry can be greatly increased. For example, in figure 6 three different tissues are shown as three dimensional chemical maps, with the total acquisition time not exceeding 12 hours in any of the examples. With the automated data pipelines also presented here what was once an experiment that could take weeks can be done in a single day.

Other than the imaging of serial sections for 3D MS imaging, having an automated aspect to process could simplify the collection of data from a range of study samples or large patient sample cohorts. The issue of tissue stability over these timeframes is something that needs to be addressed and is currently being investigated.

As more applications are assessed with this approach then further developments in acquisition modes and data analysis will follow.

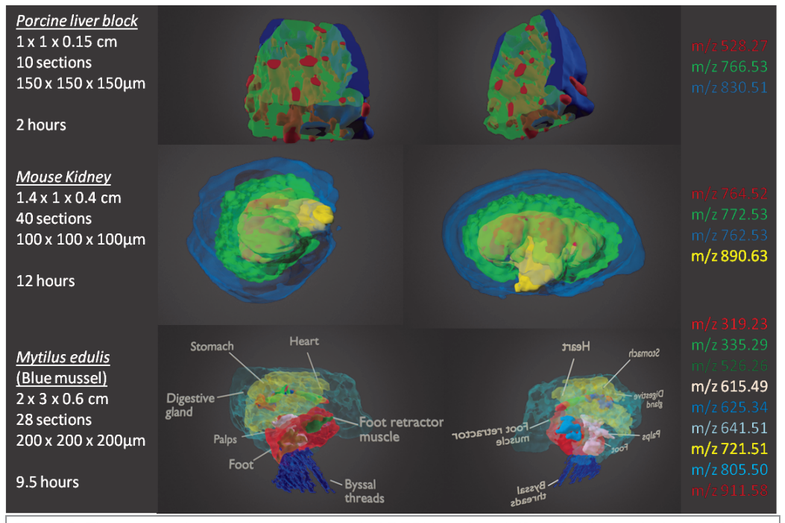

Figure 6. Three examples of using the automated DESI imaging platform for the analysis of serial sections through the same tissue to create three dimensional chemical maps. The total acquisition time of the data is included with the experimental parameters. All analysis done in negative ion mode with a mass range of m/z 50-1200.

Data pipeline management software such as Symphony (Waters, Wilmslow) should allow flexible experimental workflows to be tailored to the project that is being undertaken, figure 7 illustrates one potential application where large numbers of sample slides have MS imaging data co-registered with H&E for pathologists to draw regions of interest for database compiling.

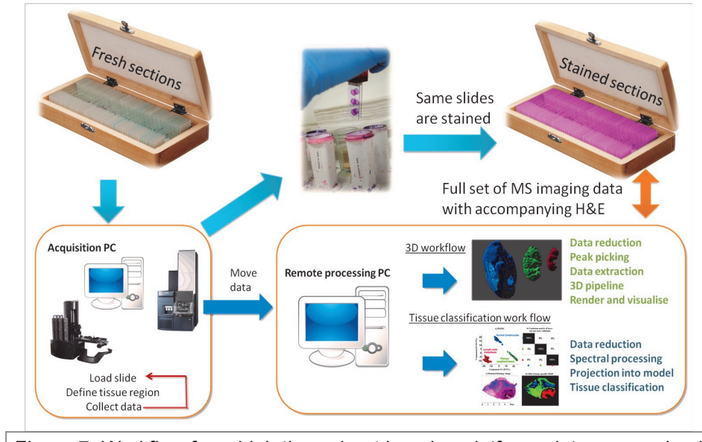

Figure 7: Workflow for a high throughput imaging platform, data processing is done on a separate processing station, workflow is selected depending upon experiment.

CONCLUSION

- **Fully automated system for mass spectrometry imaging**
- **DESI-MS platform coupled to 200 slide capacity loader**
- **Automatic tissue region definition done optically or by MS scan**
- **Can be applied to serial sections of a tissue for 3D analysis**
- **Alternatively, can be used for large number of samples for disease studies or tissue type fingerprinting**

References

1. Abbassi-Ghadi, N. Et al. *Anal. Methods* **2015**, 7 (1), 71–80.
2. Lee, D.D; Seung, H.S. *Nature* **1999**, 401, 788-901
3. Blender (Amsterdam, Netherlands) www.blender.org

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