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Technical note

Automated imaging MS: Toward high throughput imaging mass spectrometry

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ABSTRACT

The term molecular histology has been used to convey the potential of imaging mass spectrometry to describe tissue by its constituent peptides and proteins, and to link this with established histological features. The low throughput of imaging mass spectrometry has been one of the factors inhibiting a full investigation of the clinical potential of molecular histology. Here we report the development of an automated set-up, consisting of a controlled environment sample storage chamber, a sample loading robot, and a MALDI-TOF/TOF mass spectrometer, all controlled by a single user interface. The automated set-up is demonstrated to have the positional stability and experimental reproducibility necessary for its clinical application.

MALDI imaging MS is a rapidly developing technique that now allows the simultaneous analysis of the distributions of a large number of peptides and proteins directly from a tissue section or tissue array [1–3]. The technique uses the masses of the peptides and proteins to distinguish between different species and thus does not require any form of labeling. These profiles can be used to obtain biomolecular signatures associated with specific histological features, to distinguish different regions of tissue and to differentiate and classify tissues [3–5]. Previous studies have established effective sample preparation techniques [4,6–8], how to integrate the results with histology [9,10] and magnetic resonance imaging [11], and how to analyze the rich biomolecular datasets [4,12–14].

One of the aims of peptide and protein imaging MS is to develop into a multiplex peptide/protein screening analogue of histopathological analysis, termed molecular histology. Though the gold standard and an integral tool for diagnosis, prognosis, and predicting response to therapy, histopathological techniques are often insufficient for evaluating individual patients [15]. The potential strength of molecular histology is that its classifications are based on profiles incorporating multiple peptides and proteins, a feat beyond immunohistochemical analyses and in accordance with the view that a single biomarker is mostly insufficient to annotate the complexity of real biological systems [16].

To date the slow analysis speed of imaging MS of peptides and proteins has limited its clinical application. A small tissue micro array (TMA) [4] imaged with 20 μm spatial resolution would generate ≈280 k mass spectra, take 7 days continuous measurement time and consist of ≈50 Gb of data (113 needle biopsies, each analyzed using a 1×1 mm array of pixels spaced 20 μm apart, 2 s analysis time per pixel, and 200 kb per spectrum). The recently announced high speed MALDI-TOF/TOF mass spectrometers, the AB Sciex TOF/TOF 5800 from Applied Biosystems and the UltrafleXtreme from Bruker Daltonics, will provide the robustness and increased throughput to

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enable such analyses to be performed in approximately 1.5 days if the samples can be automatically delivered to the mass spectrometer and the experiment automatically initiated. Automated imaging MS will enable the increased throughput to be exploited for larger TMA's or arrays of larger tissues, for example the analysis of sequential tissue sections for 3D imaging MS [11,17]. This automation also increases the throughput of existing infrastructure by increasing the effective measurement time of existing instruments. Here we report the development of an automated imaging MS system for increasing the throughput of imaging MS experiments.

Fig. 1 shows a schematic of a MALDI imaging MS experiment and demonstrates how we have co-opted the existing software to provide high throughput capabilities while maintaining the full capabilities of the commercial mass spectrometer. First an optical image of the prepared tissue section is obtained using a flatbed scanner. The sample is then placed inside the mass spectrometer and aligned with the optical image using fiducial markers visible in both the optical image and the mass spectrometer's sample visualization system. The imaging MS experiment can then be set-up by manually selecting the tissue section(s), selecting the desired spatial resolution and finally defining the mass analysis and data analysis methods. The commercial software allows this entire experiment including alignment, pixel positions, mass analysis method, and data analysis method to be saved as an autoexecutable file. The automated sample handling system is based upon linking these autoexecute files to the unique transponders on the sample holders and then using an external piece of software to load the appropriate autoexecute file when each sample holder is loaded into the mass spectrometer (Fig. 1d).

Fig. 2a shows a photograph of the automated sample handling system. It consists of a Cytomat 2 DR (ThermoFisher) sample repository that can accommodate up to 52 sample holders in a humidity and oxygen controlled environment as well as move selected samples to an external pick-up location. A CRS F3 robot (ThermoFisher) transfers the sample between the sample repository and the mass spectrometer, an UltraFlex III (Bruker Daltonics). The three components of this high throughput system, sample repository, transfer robot and mass spectrometer, have been unified in an easy-to-use interface precisely because it co-opts the existing instrument software

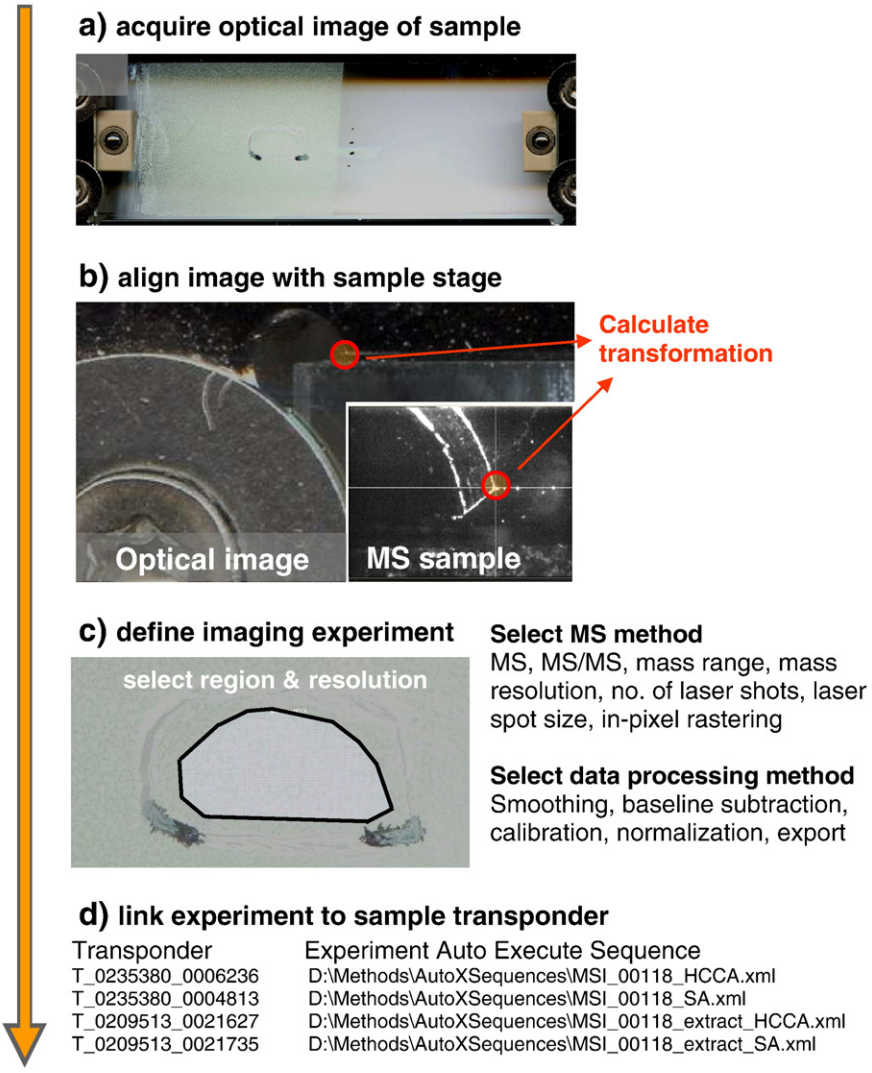

Fig. 1 – Workflow of an imaging mass spectrometry experiment and the linking of the autoexecute files to sample holder transponders, the basis of this automated system.