Innovations in ambient mass spectrometry imaging

7.1. SUMMARY

Ambient mass spectrometry imaging is – even more so than MSI in general – an emerging, developing, and fragmented field, as we argue in **Chapter 2**. For some analytical challenges commercial MSI techniques are now available (such as DESI), while other challenges can only be addressed through academic prototypes or not at all. As an example, DESI has proven very useful for the analysis of highly soluble analytes present in the top sample layer. Biological tissues are therefore thinly sectioned prior to analysis and washed to remove undesired molecular classes. This somewhat moves away from the ambient ideal (minimal sample preparation) but can help to answer the research question. Laser and thermal desorption are less analyte specific and can penetrate deeper into the sample. This makes them less reliant on sample preparation but adds complexity and cost to the source design. Considerations will always need to be made to select an analysis technique suitable to answer the active research question, though progress will undoubtedly lead to more universal ambient mass spectrometry imaging methods in the future. In this thesis we described several technological and methodological advancements made towards this goal. We will now discuss and summarize these advancements in the grander scheme, working in order from desorption to detection.

Desorption / ionization

All desorption and ionization techniques in ambient mass spectrometry have their benefits and limitations. Laser based desorption is relatively a-selective and therefore useful for wider screening approaches. Solvent-based desorption can lead to high sensitivity because it can be naturally coupled to a highly efficient ionization technique (e.g. DESI or LESA). **Chapter 3** demonstrates the power and versatility of a platform that separates the desorption and ionization steps via the combination of ESI and APCI in a single infrared laser-ablation based MSI source.

LA-APCI works very well for a significant range of analytes, partly overlapping with LAESI but superior for less polar analytes. We have demonstrated the advantages of APCI for use in an MSI instrument over ESI, most notably the improvements in repeatability and reproducibility. As stated in **Chapter 3**, we feel this approach has great potential for agrochemical investigations, but also for non-biological imaging experiments where non-polar compounds are investigated such as synthetic polymer characterisation.

We have demonstrated that infrared laser desorption can be used to desorb non-covalent protein complexes from solutions in their native state. The analysis of intact non-covalent protein complexes has now, 25 years since its inception[329], reached the level of sensitivity and robustness that this "native-MS" can be used for analytical applications. **Chapter 4** extends this ground-breaking research by demonstrating that this can be coupled to laser sampling technologies. In fact, our

results suggest a significant part of the LAESI-generated ions might reach the mass spectrometer in a more native state than with the current state-of-the-art, nano-ESI. In addition, the method has proven robust and highly tolerant to salt contaminants – which is a significant limitation of nano-ESI. As the native-MS field advances we expect applications to move towards more routine, high-throughput screening of clinical samples for diagnosis - for which LAESI could be well suited.

Infrared lasers are a powerful tool for desorption of biological sample material. A separate desorption and ionization step is crucial for robust, broadly applicable ambient mass spectrometry imaging. Completely decoupling the ESI spray generation and the interaction with ablated sample material (i.e. adding a second ambient stage) in LAESI-MSI could add much in terms of repeatability and image quality.

Ion capture / transfer

The transfer of analyte ions from the ambient environment to the mass spectrometer vacuum is an important aspect in ambient mass spectrometry. In contrast to vacuum-based mass spectrometry, the ions need to be captured and separated from the excess of neutral molecules around them without losing ions. A very high ionization efficiency, as described in the previous paragraph, is only valuable if those ions manage to reach the detector. This principle holds for any ambient ionization technique, not just imaging techniques. ESI sources are optimized in distance, temperature, enclosures, angles and potentials for the best capture of ions possible. However, imaging sources must compromise in these respects due to the requirements of the sample (stage) and desorption setup. Ambient imaging sources are therefore less efficient in capturing ions than ambient non-imaging sources.

This is only true for the first stage of ion transfer, from ambient pressure to the first vacuum stage. The successive ion transfer stages are not impacted by the type of ion source, and they are commonly not considered as limiting factors in academic mass spectrometry imaging research. For **Chapter 4** however, these transfer stages were crucial to be able to measure the large protein complexes. Very heavy ions require quite different instrumental parameters in terms of quadrupole frequencies and timings, for instance. Typical instruments are designed for a much lower mass range and are therefore not sensitive enough for protein complexes. For this reason, we employed an ultra-high mass range (UHMR) Q-Exactive Orbitrap mass spectrometer in **Chapter 4**.

In this thesis we do not present instrumental developments on the ion capture or transfer. We have put significant efforts in the development of an interface for controlled ion capture in LAESI. An active aerodynamic and electrostatic ion funnel was built and tested for controlled, more gradual, and more efficient analyte particle-electrospray droplet interaction and capture in the mass spectrometer. Unfortunately, our ion funnel design did not improve the sensitivity of our LAESI setup and has

therefore not been published. The biggest challenge was to visualize the effect of many - often dependent - variables. Computational modelling of gas fluidics is a very powerful tool, and often used in the design of ion transfer systems. However, it is highly dependent on the accuracy of the input design. Small variations in clearances, fluid characteristics or air humidity (to name a few) can have a profound effect on the efficiency of the system. Which is something inherent to ambient MSI ion sources, unfortunately. The approach chosen has a lot of potential but requires more development efforts to mature. It is still a topic of interest and development.

Separation / detection

The analysis of complex samples with ionization across a broad range of analytes (i.e. the objective of this thesis research) automatically leads to highly complex mass spectra. This issue is further aggravated by the unavoidable background contamination experienced in any ambient ionization technique. In **Chapter 5** we describe the development of an electronic signal processing platform and software package for high mass resolution MSI to combat this issue. Using new advanced signal recording, processing, and post-processing techniques we are able to separate ion species closer in mass than ever before in an imaging experiment (see a picture of the physical setup in Figure 6.1). These results highlight the problem of isobaric ion species facing all one-dimensional separation techniques, such as MSI. Researchers can be more certain of the validity of their data during interpretation, if they would apply the method presented here. It comes with drawbacks, of course. Ultra-high resolution mass spectrometry imaging as shown in **Chapter 5** is too slow (and therefore costly) in terms of measurement and data processing time to become standard practise. However, the techniques we published are applicable to all Fourier transform mass spectrometry imaging experiments and will find their way into next generation equipment and software packages, as we discuss in **Chapter 6**.

High mass resolution alone will never be able to fully resolve the molecular complexity of biological samples. In imaging experiments orthogonal separation dimensions are difficult to implement, so MSI spectra are typically highly complex. Notable attempts to add separation dimensions are made with ion mobility spectrometry (IMS) and LESA, but thus far they fall short of the challenge in combination with MSI. Aside from isobaric compounds causing a complex spectrum, isomer analysis is a field that is largely untapped in MSI. A lot of biological information is hidden from sight by overlap with more abundant ion species. In the best case, this information remains hidden, in worst case it interferes with the interpretation of the more abundant ion species distribution. There is a clear need for an orthogonal separation dimension at the timescale of imaging experiments. IMS and infrared ion spectroscopy (IRIS) are exciting candidates for this position.

The constraints and outlook of ambient MSI

Ambient MSI is a fascinating, creative, and highly developing field of analytical chemistry. Where vacuum-based MSI has outgrown the academic "proof-of-principle stage" and its range of applications is growing, ambient MSI is not there yet. Ambient mass spectrometry imaging is mainly held back by the lacking repeatability and sensitivity of measurements. Real-world applications rarely concern the incidental detection of highly abundant analytes, and for those purposes usually a host of techniques can be used. To have an impact on the general society, ambient MSI techniques need to produce reliable results every time, which is in direct opposition to their ambient, minimal sample preparation, versatile nature. The sensitivity of ambient MSI methods is generally much lower for those same reasons. Both the sensitivity and the robustness of ambient MSI are primarily constrained by the efficiency of analyte capture towards the MS vacuum. Depending on the technique, this is more of an ionization or ion transfer challenge. A radical new design is needed for an interface that collects and guides neutral analytes towards the mass spectrometer, while promoting charge transfer regardless of the ionization principle. Control over the air flow between the sample and MS will be crucial in such a design.

Despite the hurdles left to take, ambient MSI is moving forward and has the potential to unlock molecular information in real-world situations that no other technique can. When combined with the advances made in the field of miniaturized mass spectrometers (outside of the scope of this thesis) the path forward seems bright. Star Trek tricorder-like devices - able to analyse any sample in real time by point-and-click – might still be the stuff of dreams for now, but who knows for how much longer? With the strides taken in recent decades - to which this thesis is proud to add - it is a matter of time before mass spectrometry leaves the analytical lab and moves into the hands of the general public.