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Towards Integrating Synchrotron FTIR Microscopy and Mass Spectrometry at the Berkeley Synchrotron Infrared Structural Biology (BSISB) Program

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Synchrotron-radiation-based Fourier transform infrared (SR-FTIR or sFTIR) facilities worldwide are probing both live cells and tissues non-destructively under native-like conditions. From spectroscopic data such as those acquired through the Berkeley Synchrotron Infrared Structural Biology (BSISB) program [1–16], spatially and temporally resolved chemical information of molecular classes are obtained. However, these infrared data lack chemical specificity for precise and accurate species identification. High-resolution mass spectrometry (MS) directly contrasts with these IR data attributes in its ability to provide more specific identification of the full range of molecules at the cost of sample destruction [17–20]. Until recently, there were few mechanisms to bridge sFTIR spectral microscopy to spatially resolved mass spectrometry in a truly complementary manner [21, 22]. To address this challenge, the BSISB program recently developed spatially resolved ambient IR laser ablation mass spectrometry (AIRLAB-MS), which enabled the integration of this MS system into the currently sFTIR-capable infrared microscope to create an enduring bi-modal chemical imaging platform. An advantage of this integrated experimental set-up is that both sFTIR and AIRLAB-MS can share the same infrared microscope and sample stage; this design provides seamless data acquisition from the same sample site using the sFTIR imaging technique followed by AIRLAB-MS. Here, we describe our new set-up for integrating sFTIR into the newly developed AIRLAB-MS at ALS' BL 1.4.4—our solution for bridging and combining sFTIR capabilities with high-resolution mass spectrometry to further our abilities to study the chemical complexity of dynamic biological systems in a time-resolved manner.

an example being one which links between genetic and metabolic differences in functional metabolism [5, 8, 9, 27]. This has been demonstrated on at least 10 different biological systems, including four orders of magnitude in time, including dynamic processes such as stress-adaptive response, and protein phosphorylation. The chemical composition of biological samples can be determined with minimal modification/preparation is required. This improves our understanding of biochemical processes such as photosynthesis [7, 28, 29], photosynthesis [30, 31], cellular metabolism and adaptive response [6, 10, 32–35], and non-invasive monitoring of cardiac tissues induced by a high-glycemic-insulin challenge. Infrared data provide real-time information about the chemical changes that occur in living systems, infrared data lack the chemical specificity necessary for unambiguous molecular identification.

In contrast, high-resolution mass spectrometry provides chemical information with excellent specificity, albeit at the cost of destroying the target sample. Mass spectrometry specificity includes elemental composition of molecules, isotopic measurements and structural information gained from fragmentation products formed in tandem mass spectrometry. Mass spectrometry has the additional advantage that a wide range of compounds can be measured simultaneously [57]. The complementary and chemically informative nature of mass spectrometry has been historically combined with other analytical techniques that are performed upstream of mass spectrometry in the overall experimental workflow to provide multi-

Synchrotron-radiation-based Fourier transform infrared (SR-FTIR or sFTIR) facilities worldwide are probing both live cells and tissues non-destructively under native-like conditions. From spectroscopic data such as those acquired through the Berkeley Synchrotron Infrared Structural Biology (BSISB) program [[1–16](#)H. Yoo et al., *J. Phys. Chem. Lett.* 5, 947–952 (2014).

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M. Farre, Y. Pico, and D. Barcelo, *Journal of Chromatography A* 1328, 66–79 (2014).

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C. Junot et al., *Mass Spectrom Rev* 33(6), 471–500 (2014).]. Until recently, there were few mechanisms to bridge sFTIR spectral microscopy to spatially resolved mass spectrometry in a truly complementary manner [[21](#)V.W. Petit et al., *Analytical Chemistry* 82(9), 3963–3968 (2010).

[[Crossref](#)], [[PubMed](#)], [[Web of Science ®](#)], [[Google Scholar](#)], [22](#)F. Le Naour et al., *PLOS One* 4(10), e7408 (2009).

[[Crossref](#)], [[PubMed](#)], [[Web of Science ®](#)], [[Google Scholar](#)]]. To address this challenge, the BSISB program recently developed spatially resolved ambient IR laser ablation mass spectrometry (AIRLAB-MS), which enabled the integration of this MS system into the currently sFTIR-capable infrared microscope to create an enduring bi-modal chemical imaging platform. An advantage of this integrated experimental set-up is that both sFTIR and AIRLAB-MS can share the same infrared microscope and sample stage; this design provides seamless data acquisition from the same sample site using the sFTIR imaging technique followed by AIRLAB-MS. Here, we describe our new set-up for integrating sFTIR into the newly developed AIRLAB-MS at ALS' BL 1.4.4—our solution for bridging and combining sFTIR capabilities with high-resolution mass spectrometry to further our abilities to study the chemical complexity of dynamic biological systems in a time-resolved manner.

Overview

Fourier transform infrared (FTIR) spectromicroscopy is a non-destructive technique that provides a window into the changing chemistry of biological processes in living single cells that both structural [23B.C. Chen et al., *Science* 346(6208), 439–439 (2014). [\[Crossref\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)] and fluorescence [24M. Fernandez-Suarez and A.Y. Ting, *Nature Reviews Molecular Cell Biology* 9(12), 929–943 (2008). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)] imaging alone cannot achieve. At sFTIR facilities worldwide, the performance of FTIR spectromicroscopy is radically increased by switching from a thermal (globar) infrared source to a synchrotron infrared beam while coupling the beam to either a single-element MCT or a focal plane array (FPA) detector [9H.Y. Holman et al., *Analytical Chemistry* 82(21), 8757–65 (2010). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 25E. Stavitski et al., *Analytical Chemistry* 85(7), 3599–605 (2013). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 26M.J. Nasse et al., *Nat Methods* 8(5), 413–6 (2011). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. A prominent biological application of sFTIR at the Advanced Light Source involves measuring cellular and tissue response to perturbative experiments designed to uncover the temporal biochemical behavior—an example being one which links between genetic variations to differences in functional metabolism [5G. Birarda et al., *Biomedical Spectroscopy and Imaging* 2(3), 301–315 (2013). [\[PubMed\]](#), [\[Google Scholar\]](#), 8J. Baelum et al., *Environmental Microbiology* 14(9), 2405–16 (2012). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 9H.Y. Holman et al., *Analytical Chemistry* 82(21), 8757–65 (2010). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 27L. Chen et al., *Analytical Chemistry* 84(9), 4118–25 (2012). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. This sFTIR capability has been demonstrated on at least 10 different biological processes spanning four orders of magnitude in time, including muscle contraction, stress-adaptive response, and protein phosphorylation. Determining the chemical composition of biological samples under native conditions and with minimal modification/preparation is important to advancing our understanding of biochemical processes like cell differentiation [7L. Chen et al., *Analytical Chemistry* 84(9), 4118–4125 (2012). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 28K.P. Quinn et al., *Scientific Reports* 3, 3432 (2013). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 29B. Shrestha, J.M. Patt, and A. Vertes, *Analytical Chemistry* 83(8), 2947–2955 (2011). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)], photosynthesis [30G. Parsiegla et al., *Analytical Chemistry* 84(1), 34–38 (2012). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 31P. Nemes, A.A. Barton, and A. Vertes, *Analytical Chemistry* 81(16), 6668–6675 (2009). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)], cellular metabolism during stress-and-adaptive response [6O.U. Mason et al., *ISME Journal* 6(9), 1715–1727 (2012). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 10T.C. Hazen et al., *Science* 330(6001), 204–8 (2010). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#), 32–35T.C. Hazen et al., *Science* 330(6001), 204–208 (2010).

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In contrast, high-resolution mass spectrometry [21V.W. Petit et al., *Analytical Chemistry* 82(9), 3963–3968 (2010). [Crossref], [PubMed], [Web of Science ®], [Google Scholar], 29–31B. Shrestha, J.M. Patt, and A. Vertes, *Analytical Chemistry* 83(8), 2947–2955 (2011).

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Ambient infrared laser ablation (AIRLAB) for bridging sFTIR microscopy to mass spectrometry

An efficient approach to bridge sFTIR spectral microscopy with spatially resolved mass spectrometry is to have both methods share the same infrared microscope and reflective optics. The BSISB program began to test this concept recently by developing a new MS experimental set-up for spatially resolved ambient infrared laser ablation mass spectrometry (AIRLAB-MS) using an infrared microscope together with an infinity-corrected reflective objective and a continuous solvent flow probe [76] J.T. O'Brien, E.R. Williams, and H.Y.N. Holman, *Analytical Chemistry* 87(5), 2631–2638 (2015). [Crossref], [PubMed], [Web of Science®], [Google Scholar]. In this design, the flow probe provides the advantage of ablation into a non-contact surface sampling probe [73] O.S. Ovchinnikova, V. Kertesz, and G.J. Van Berkel, *Analytical Chemistry* 83(6), 1874–1878 (2011). [Crossref], [PubMed], [Web of Science®], [Google Scholar], [74] O.S. Ovchinnikova et al., *Analytical Chemistry* 85(21), 10211–10217 (2013). [Crossref], [PubMed], [Web of Science®], [Google Scholar], [77] O.S. Ovchinnikova, V. Kertesz, and G.J. Van Berkel, *Rapid Communications in Mass Spectrometry* 25(24), 3735–3740 (2011). [Crossref], [PubMed], [Web of Science®], [Google Scholar]. The output wavelength of the IR laser is tuned to 2.94 μm to take advantage of the water naturally present in biological samples as a matrix to absorb IR radiation. The IR laser pulse produces surface evaporation, phase explosion (explosive boiling) of water, and the secondary ejection of sample material into a plume of fine droplets [78] I. Apitz and A. Vogel, *Applied Physics A: Materials Science & Processing* 81(2), 329–338 (2005). [Crossref], [Web of Science®], [Google Scholar], [79] P. Nemes, H.H. Huang, and A. Vertes, *Physical Chemistry Chemical Physics* 14(7), 2501–2507 (2012). [Crossref], [PubMed], [Web of Science®], [Google Scholar]. The ejected sample material consists of mostly neutral droplets, particles, or molecules, which are known in other analytical systems to be ionized by intersection with an electrospray plume (ELDI, LAESI, MALDESI) [29] B. Shrestha, J.M. Patt, and A. Vertes, *Analytical Chemistry* 83(8), 2947–2955 (2011). [Crossref], [PubMed], [Web of Science®], [Google Scholar], [48] J.A. Stolee and A. Vertes, *Analytical Chemistry* 85(7), 3592–3598 (2013). [Crossref], [PubMed], [Web of Science®], [Google Scholar], [53] G. Robichaud et al., *Journal of the American Society for Mass Spectrometry* 24(1), 92–100 (2013). [Crossref], [PubMed], [Web of Science®], [Google Scholar], [67–72] I.X. Peng et al., *Analyst* 135(4), 767–772 (2010). J. Shiea et al., *Rapid Communications in Mass Spectrometry* 19(24), 3701–3704 (2005). M.Z. Huang et al., *Journal of Proteome Research* 5(5), 1107–1116 (2006). J.S. Sampson, A.M. Hawkrige, and D.C. Muddiman, *Journal of the American Society for Mass Spectrometry* 17(12), 1712–1716 (2006). J.S. Sampson, K.K. Murray, and D.C. Muddiman, *Journal of the American Society for Mass Spectrometry* 20(4), 667–673 (2009). P. Nemes and A. Vertes, *Analytical Chemistry* 79(21), 8098–8106 (2007).] or can be captured in solvent (LAST) [49–51] S.G. Park and K.K. Murray, *Rapid Communications in Mass Spectrometry* 27(15), 1673–1680 (2013). S.G. Park and K.K. Murray, *Journal of Mass Spectrometry* 47(10), 1322–1326 (2012). S.G. Park and K.K. Murray, *Journal of the American Society for Mass Spectrometry* 22(8), 1352–1362 (2011). , [73–75] O.S. Ovchinnikova, V. Kertesz, and G.J. Van Berkel, *Analytical Chemistry* 83(6), 1874–1878 (2011).

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M. Lorenz, O.S. Ovchinnikova, and G.J. Van Berkel, *Rapid Communications in Mass Spectrometry* 28(11), 1312–1320(2014).] for subsequent ionization by electrospray. The AIRLAB-MS instrumentation consists of four major components; an Opolette tunable infrared laser (Opotek, Carlsbad, CA), a Continuum XL infrared microscope (Thermo-Fisher, Waltham, MA) with a reflecting objective, a custom-built continuous flow probe to capture and deliver the ablated sample materials to electrospray ionization (ESI) emitter, and a home-built 7 T Fourier-transform ion cyclotron resonance mass spectrometer or, more recently, a Waters SYNAPT G1 time-of-flight mass spectrometer. A schematic diagram of the experimental set-up that includes the IR microscope, reflecting objective, continuous flow probe, and ESI emitter is shown in [Figure 1\(a\)](#).

Figure 1: (a) Schematic diagram (not to scale) of the AIRLAB-MS experimental set-up. The IR laser is focused through a 15× reflecting objective mounted on a Continuum XL infrared microscope. The ablation plume is captured by a hanging droplet at the tip of a stainless-steel capillary attached to a PEEK tee fitting (port A). Solvent is pumped with a syringe pump into port B. A fused silica capillary carries solvent and ablated material from the probe tip (enlarged to show solvent flow) out through port C and to the electrospray emitter. A stainless-steel union (attached to port D) is used to apply the electrospray voltage to the solution. Regulated N₂ gas enters the PEEK emitter tee fitting at port E and a fused silica capillary carries solvent and sample from the union, through the tee, and out port F, where ions are generated by pneumatically assisted electrospray ionization. (b-c) AIRLAB performance: Transfer efficiency (%) for the laser ablation from a 10 µL droplet of 85/15 glycerol/methanol containing 1 mM nicotine (c). Reproducibility as depicted by the ion abundance of protonated uridine by laser ablation from six nearby spots of a leaf from a tobacco seedling. Values of integrated areas for each peak are labeled (c). (d-e) Case studies: images of tobacco leaf samples from a John Williams plant and the areas selected for laser ablation indicated by circles. LE = leucine enkephalin (as internal standard). Representative mass spectra for the leaf tip and leaf base/stem are also shown (d). Average nicotine abundances measured for laser ablation of three 360 × 360 µm areas of plant tissue from each of the circled areas of John Williams versus mutant tobacco leaves (e). The average integrated nicotine abundance is indicated by the color of the circle. Expansion of the mutant tip shows ablation areas. From reference [76] J.T. O'Brien, E.R. Williams, and H.Y.N. Holman, *Analytical Chemistry* 87(5), 2631–2638 (2015).
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AIRLAB's material transfer efficiency (i.e., ablation and transfer of materials from a sample to the electrospray ionization emitter) was ~50%, which is surprisingly high. Here, the transfer efficiency was determined by comparing the protonated nicotine (a model demonstration compound) ion abundances from AIRLAB to that obtained from ESI of a standard nicotine solution. A 10 μL droplet was used in these experiments because the surface is flatter and results in higher reproducibility. Bursts of 10 laser shots were used to ablate the sample, and the protonated nicotine abundance as a function of time shows spikes for each set of 10 laser shots (Figure 1(b)). Protonated nicotine appeared ~90 s after the laser ablation, and the signal is observed for ~60–75 s. The area under each peak in the time-dependent nicotine signal was integrated, and the resulting integrated nicotine ion abundance values were scaled to account for the 10 s of each measurement cycle during which ions were not accumulated and measured by the mass spectrometer. These measured values were converted to mole equivalents using calibration data obtained under the same experimental conditions using nicotine standards in the same solvent (1:1 H₂O:MeOH; 1% acetic acid). The total volume of material ablated per laser pulse was obtained by measuring the number of laser pulses necessary to evaporate the droplet.

This transfer efficiency is significantly higher than values reported for similar techniques. High transfer efficiency of the sample to the mass spectrometer is important for the analysis of biological samples due to low chemical concentrations of many target molecular species within highly complex biological sample mixtures from living cells and tissue. The transfer efficiency of laser-ablated material from a surface to a solvent probe depends on the instrumental geometry. In experiments where backside laser ablation was used, the transfer efficiency for ablated angiotensin II in solution on a quartz slide to solution on a probe 1 mm away is reported to be 2%

[50] S.G. Park and K.K. Murray, *Journal of Mass Spectrometry* 47(10), 1322–

1326 (2012).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#). LAESI, in which the ablation plume expands into a flow of highly charged solvent droplets produced by electrospray, is reported to be “characterized by significant sample losses and low ionization efficiencies” [48J.A. Stolee and A. Vertes, *Analytical Chemistry* 85(7), 3592–3598 (2013).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. Vertes and co-workers reported that the transfer efficiency of LAESI was improved by the use of a capillary to confine the sample and to direct the radial expansion of the ablation plume, guiding more material directly into the electrospray flow [48J.A. Stolee and A. Vertes, *Analytical Chemistry* 85(7), 3592–3598 (2013).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. The AIRLAB reproducibility was evaluated with regard to the uridine ion abundance obtained by AIRLAB-MS from a live young (~3 weeks old) tobacco leaf from a tobacco plant John Williams (JW), our model demonstration biomaterials. Young leaves were selected to minimize variations in the leaf tissue due to vasculature (leaf veins) and trichomes (small hairs), both of which are more prevalent in mature leaves. Results show a standard deviation of <10% in the uridine signal obtained for six closely spaced areas (within 2 × 2 mm) ([Figure 1\(c\)](#)).

We also showed that spatial variations in chemical compositions could be obtained by AIRLAB-MS from different locations of live and mature leaves from JW and from TLA (a genetically modified JW with truncated light antenna (TLA)). Here, eight locations were selected (indicated by circles in [Figures 1\(d\) and 1\(e\)](#)). Four locations were within 1 cm of the tip of the leaf, three on the leaf mid-section, and one on the stem at the base of the leaf. These locations were selected to provide a variety of plant tissue types and ages. At each location, at least three 360 μm × 360 μm ablation areas using the same 4 μ 4 grid programmed for the reproducibility experiments were analyzed. The trichomes or leaf hairs were much more developed and prominent for these mature leaves compared to the young leaf used in the reproducibility measurements. There is a greater (20% on average) variability in ion abundances for these more mature leaves, likely caused by the unpredictable and variable effects of the leaf hairs on the sample transfer efficiencies. In [Figure 1\(d\)](#), two representative mass spectra from different ablation locations are also shown. Each mass spectrum consists of the averaged ion abundances from all spectra measured for the indicated area (tip or vein) where the S/N for protonated nicotine is greater than three. In total, approximately 50 ions, excluding isotope and background ions, were observed for laser ablation of the tip region and approximately 100 for the base/stem region. Of these ions, 28 were common to both regions. The greater number of ions for the base region may be due to the greater thickness of the stem, which provides more material for transfer and thus more signal for low concentration compounds. The most abundant ions in the mass spectra of the two ablation regions are at m/z 98.986, 120.968, 163.125, 203.044, and 219.025. Based on the comparison of the measured accurate masses to a tobacco plant metabolite database [65Y. Li, B. Shrestha, and A. Vertes, *Analytical Chemistry* 80(2), 407–420(2008).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)], these ions are assigned as protonated phosphoric acid, potassiated pyrimidine-ring, protonated nicotine, sodiated hexose (most likely glucose), and potassiated hexose, respectively. The abundant ion at m/z 158.025 is consistent with multiple sodiated metabolite isomers with elemental composition (C₅H₄N₄O), including hypoxanthine and 8-hydroxypurine.

Comparative analysis of the nicotine distribution for these two tobacco plant leaves indicates a higher nicotine level in the genetically modified plant ([Figure 1\(e\)](#)).

Initial integration of synchrotron FTIR microscopy with mass spectrometry

The integration of AIRLAB-MS with sFTIR spectral microscopy is in progress at BL 1.4.4 at the Advanced Light Source. Here, sFTIR and AIRLAB-MS share the same infrared microscope and sample stage through a set of computer-controlled switch mirrors. As depicted in [Figure 2](#), for the sFTIR microscopy mode ([Figure 2\(b\)](#), top panel), synchrotron infrared (SIR) photons from a bending magnet are collected, collimated, and transported to the commercial FTIR interferometer bench (Nicolet™ 6700 FTIR Spectrometer). After modulation by the interferometer, a commercial infrared microscope focuses the beam onto the sample with all-reflecting optics. The light, either transmitted or reflected from the sample, is collected by the same microscope optics and sent to an IR detector. For the AIRLAB-MS mode ([Figure 2\(b\)](#), bottom panel), the mirrors are switched to alter the optical path for laser ablation.

Figure 2: (a) Photo of the sFTIR-AIRLAB MS imaging platform at BL 1.4.4. On the left, the commercially available Waters SYNAPT QToF mass spectrometer with inset of the Venturi ESI source (also see 2d). In the center, the continuous solvent flow probe equipped with an automated mechanism to continuously monitor and feedback control of solvent droplet size (also see 2c). On the right, an instrumentation environment with switch mirrors for the interoperation of sFTIR vs AIRLAB-MS imaging (also see 2b). (b) A schematic of the interoperation sFTIR microscopy and AIRLAB-MS. The telescope is for laser beam shaping. (Insert) Top view of the telescope set-up for beam shaping. (c) A close-up view of our custom-built “Venturi” ESI. (d-i) On the left, a photo of the continuous solvent flow probe integrated with automated measurement and control of hanging droplet size for reproducible ion trapping. On the right, a close-up view of the probe tip and a hanging droplet. (d-ii) A typical time course of the hanging droplet size and the solvent flow rates before reaching equilibrium. Laser ablation starts when the droplet size reaches its steady state.

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The AIRLAB-MS instrumentation at the beamline is based upon our AIRLAB-MS prototype reported in [76J.T. O'Brien, E.R. Williams, and H.Y.N.Holman, *Analytical Chemistry* 87(5), 2631–2638 (2015).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. The current set-up at BL 1.4.4 consists of five components (Figures 2(a)–(d)): an Opolette tunable infrared laser (Opotek, Carlsbad, CA) for ablation, a telescope for beam shaping (Figure 2(b), insert), a shared Continuum XL infrared microscope (Thermo-Fisher, Waltham, MA) with a reflecting objective for sample viewing and site selection (Figure 2(b)), a home-built Venturi ESI source connected to the commercially available Waters SYNAPT QToF mass spectrometer (Figure 2(c)), and a computer-controlled non-contact continuous solvent flow probe (Figure 2(d)). As established in [76J.T. O'Brien, E.R. Williams, and H.Y.N.Holman, *Analytical Chemistry* 87(5), 2631–2638 (2015).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)], the IR Opolette laser system is tuned to generate 2.94 μm laser light for ablation of material from biological samples so that the integrity of our cellular biomolecular components are maintained. We can use the microscope equipped with reflective IR optics to focus the laser light to a certain spot size onto the sample secured on the x-y-z translational microscope stage. This spot size can be either increased by defocusing of the beam for ablating larger areas or decreased (i.e., an improved spatial resolution) by using an aperture to block part of the beam. A built-in camera controlled by our home-built software package enables us to view and define the area for laser ablation. The

horizontal continuous solvent flow probe is an assembly which consists of a notched outer capillary delivering solvent to the probe and a smaller inner capillary delivering solvent and the ablated sample materials into the electrospray source of the mass spectrometer ([Figure 2\(d\)](#)). Although the core of our “bridging” technology follows the prototype design with a non-contact sampling station and an improved version of the continuous solvent flow probe [[76](#)J.T. O'Brien, E.R. Williams, and H.Y.N. Holman, *Analytical Chemistry* 87(5), 2631–2638 (2015).

[[Crossref](#)], [[PubMed](#)], [[Web of Science](#)®], [[Google Scholar](#)]] based upon the original by Ovchinnikova et al. [[73](#)O.S. Ovchinnikova, V. Kertesz, and G.J. Van Berkel, *Analytical Chemistry* 83(6), 1874–1878 (2011). [[Crossref](#)], [[PubMed](#)], [[Web of Science](#)®], [[Google Scholar](#)], [77](#)O.S. Ovchinnikova, V. Kertesz, and G.J. Van Berkel, *Rapid Communications in Mass Spectrometry* 25(24), 3735–3740(2011).

[[Crossref](#)], [[PubMed](#)], [[Web of Science](#)®], [[Google Scholar](#)]], we included an additional droplet-size-measurement feedback component into the probe assembly in order to enhance the stability and reproducibility of the amount of materials trapped by the droplet. This modification to the continuous flow probe permits precise and automated laser ablation when the diameter of the hanging droplet reaches both our acceptable error range with respect to droplet diameter (± 0.2 mm) and the target droplet diameter (see [Figure 2\(d\)](#)).

In order to test the migration and integration of the prototype AIRLAB-MS with the sFTIR microscope, we performed our first application of AIRLAB-MS for metabolic profiling on yellow onion bulb epidermal cells at BL 1.4.4. Our previous sFTIR spectromicroscopy examination of the epidermis of a yellow onion scale revealed key IR absorption spectral features between 1800 and 900 cm^{-1} ([Figure 3\(a\)](#)), which are characteristic of such primary biochemical and macronutrient components as lipids, proteins, and carbohydrates in onions [[80](#)X. Lu et al., *J Agric Food Chem* 59(12), 6376–82 (2011). [[Crossref](#)], [[PubMed](#)], [[Web of Science](#)®], [[Google Scholar](#)]]. These features are well-described for the esters at ~ 1740 cm^{-1} , amide-stretching bands of protein at 1648 and 1542 cm^{-1} , and carbohydrate bands between 1200 and 900 cm^{-1} . As shown in [Figure 3\(a\)](#), the relative abundance of these classes of biomolecules varied spatially in accordance with the structure of interlocking cells. This makes onion epidermal cell layers an ideal model for evaluating the performance of our technology.

Figure 3: (a) sFTIR images (~ 550 $\mu\text{m} \times 520$ μm ; $\Delta = 10$ μm) showing the distribution of lipids, proteins, and carbohydrates in a sheet of yellow onion bulb epidermal cells. (Left) A typical sFTIR spectrum. The band assignment was according to the onion FTIR database [[80](#)X. Lu et al., *J Agric Food Chem* 59(12), 6376–82 (2011). [[Crossref](#)], [[PubMed](#)], [[Web of Science](#)®], [[Google Scholar](#)]]. (Right) Distribution heat map of the fatty acid carbonyl vibration mode at ~ 1740 cm^{-1} , protein amide II vibration modes at ~ 1540 cm^{-1} , flavonoid C–OH vibration mode at 1102 cm^{-1} , condensed cellulose C–O plus C–C plus C=O vibration mode at 1245 cm^{-1} , and carbohydrate C–C and C–O vibration modes between 1060 and 1000 cm^{-1} . (Insert) Microscope view of the onion sample before and after sFTIR imaging showing little visible changes. Scale bars: 100 μm . (b) AIRLAB MS results. Microscope view of the onion sample before and after 50 shots. (Left) Typical mass spectrum of onion (from 10 laser shots). The mass assignment was on the basis of the comparison of the measured accurate masses to an

onion metabolite database [81 B. Shrestha and A. Vertes, *Analytical Chemistry* 81(20), 8265–8271(2009).[\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)]. The mass at $m/z = 556.3$ corresponds to the enkephalin used as our internal standard. (Right) Spatial variations in the relative abundance of selected target metabolites in seven different locations are indicated with respect to time and are measured by the AIRLAB-MS at BL 1.4.4.

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For our first ablation experiment at BL 1.4.4, the AIRLAB mass spectra were acquired every 16 s and stored individually. The reported ion abundances were relative to the abundance of protonated leucine enkephalin, which was added to the pumped solution

at a concentration of 0.75 μM as an internal standard. As shown in [Figure 3\(b\)](#) (insert), there was a remarkable difference between the ablated and non-ablated onion cells. The ablation mark A indicates a large ($\sim 50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$) “depression” after ablation by the laser (10 shots). A typical mass spectrum shows the presence of ions representing small metabolites and along with lipids at higher masses. Some of the prominent ions include species at m/z 132.626, 175.143, 219.054, 325.155, 381.121, and 594.260. Comparing these accurately measured masses to an onion metabolite database [81B. Shrestha and A. Vertes, *Analytical Chemistry* 81(20), 8265–8271(2009). [\[Crossref\]](#), [\[PubMed\]](#), [\[Web of Science ®\]](#), [\[Google Scholar\]](#)], these ions are assigned as 5-oxoproline, arginine, monosaccharide, glucosan, disaccharide, and heptasaccharide. To evaluate the spatial distribution of these metabolites, we programmed the AIRLAB-MS system to ablate another area of $50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$ by moving the samples through a grid, each area separated by $\sim 100\text{ }\mu\text{m}$. The location-to-location variations in relative abundance of these identified metabolites are shown in [Figure 3\(b\)](#) (right). The heptasaccharide and glucosan ion counts varied spatially by 15–30%, and arginine by more than 50%.

Conclusion

In migrating our AIRLAB set-up to BL 1.4.4, we have shown that our AIRLAB-MS system is well-suited for integration with an IR microscope for sFTIR studies of biological systems. We built upon our previous AIRLAB set-up prototype by adding several modifications for system automation and a user-friendly interface. First, we introduced a set of computer-controlled switching mirrors to easily switch between the sFTIR and AIRLAB-MS imaging modes. Second, we automated the continuous solvent flow probe control for increased reproducibility and sample capture efficiency with respect to laser-activated plume generation and the optimal sample-capturing droplet size. We have demonstrated the capability of sFTIR and AIRLAB, separately, as two complementary chemical imaging tools under ambient conditions. We are continuing to optimize our sFTIR AIRLAB-MS integrated set-up through equipment parameter settings, additional plume confinement hardware and supporting software, as well as biological system-specific alterations to our parameters as we expand our target systems of study. With all of these variables in mind, we aim to further increase our sample capture efficiency, which subsequently increases our instrument sensitivity to ultimately effect the range of chemical species and concentrations that we can detect through our integration of sFTIR microscopy and high-resolution mass spectrometry.

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