Sample preparation free mass spectrometry using laser-assisted rapid evaporative ionisation mass spectrometry (LA-REIMS): applications to microbiology, metabolic biofluid phenotyping, and food authenticity

Simon J.S. Cameron (1,2)*, Alvaro Perdones-Montero (1), Lieven Van Meulebroek (3), Adam Burke (1), Kate Alexander-Hardiman (1), Daniel Simon (1,4), Richard Schaffer (4), Julia Balog (4), Tamas Karancsi (4), Tony Rickards (5), Monica Rebec (5), Sara Stead (6), Lynn Vanhaecke (2,3), Zoltán Takáts (1,2)*.

(1) Department of Metabolism, Digestion, and Reproduction, Imperial College London, London, SW7 2AZ, UK.

(2) Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, BT9 5DL, UK.

(3) Laboratory of Chemical Analysis, Department of Veterinary Public Health and Food Safety, Ghent University, Ghent, B-9820, Belgium.

(4) Waters Research Center, Budapest, 1031, Hungary.

(5) Department of Microbiology, Imperial College Healthcare NHS Trust, London, W6 8RD, UK.

(6) Waters Corporation, Wilmslow, SK9 4AX, UK.

* Joint Corresponding Authors:

Dr Simon J.S. Cameron: Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, BT9 5DL, UK. Telephone: 028 9097 6421. Email: s.cameron@qub.ac.uk

Professor Zoltán Takáts: Department of Metabolism, Digestion, and Reproduction, Imperial College London, South Kensington Campus, Sir Alexander Fleming Building, London, SW7 2AZ. Telephone: 020 7594 2760. Email: z.takats@imperial.ac.uk

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Abstract

Mass spectrometryhas established itself as a powerful tool in the chemical, biological, medical, environmental, and agricultural fields. However, experimental approaches and potential application areas have been limited by a traditional reliance on sample preparation, extraction, and chromatographic separation. Ambient ionisation mass spectrometry methods have addressed this challenge but are still somewhat restricted in requirements for sample manipulation to make it suitable for analysis. These limitations are particularly restrictive in view of the move towards high-throughput and automated analytical workflows. To address this, we present what we consider to be the first automated sample-preparation-free mass spectrometry platform utilising a carbon dioxide (CO_2) laser for sample thermal desorption linked to the rapid evaporative ionisation mass spectrometry (LA-REIMS) methodology. We show that the pulsatile operation of the CO_2 laser is the primary factor in achieving high signal-to-noise ratios. We further show that the LA-REIMS automated platform is suited to the analysis of three diverse biological materials within different application areas. Firstly, clinical microbiology isolates were classified to species level with an accuracy of 97.2%, being the highest reported in current literature. Secondly, faecal samples from a type 2 diabetes mellitus cohort were analysed with LA-REIMS, which allowed tentative identification of biomarkers which are potentially associated with disease pathogenesis and a disease classification accuracy of 94%. Finally, we showed the ability of the LA-REIMS system to detect instances of adulteration of cooking oil and determine the geographical area of production of three protected olive oil products with 100% classification accuracy.

Keywords: Mass spectrometry; ambient ionisation mass spectrometry; automation; microbiology; food authenticity; metabolic phenotyping;

Introduction

Mass spectrometry (MS) has a broad range of applications in, but not limited to, the biological chemistry,[1] medical,[2] environmental,[3] and agricultural fields.[4] As a result of instrument sensitivity and selectivity, they have become a powerful research tool for the chemical characterisation of biological samples.[5] However, most commonly used approaches require substantial analytical run times due to a requirement for complex sample preparation and extraction and typical combination with chromatographic separations.[6] This has arguably limited the wider adoption of mass spectrometry tools and techniques outside of research settings as the required user skill and time is prohibitive and does not integrate well with existing workflows; particularly those which are automated.[7] Although methods such as flow-injection,[8] matrix assisted laser desorption ionisation (MALDI) MS,[9] and liquid extraction surface analysis[10] have removed chromatographic separation to increase analytical throughput, these methods still require sample preparation and extraction prior to MS analysis. In addition to increasing the analytical run times, extraction and preparation processes may increase chemical alterations in a sample and deviates it from its *in situ* profile.[11] This may be important for studies involving disease biomarker discovery, food composition, and particularly applications with a microbial component.

In the last decade, the field of ambient ionisation MS has seen rapid expansion; driven by the ability to conduct analysis of a sample with little to no preparative steps and under normal atmosphere conditions.[12] However, methods such as desorption electrospray ionisation MS (DESI-MS),[13] direct analysis in real-time MS (DART-MS),[14] and paper-spray MS[15] typically require some manipulation of the sample to allow it to fit the instrumental set-up, driven by the limited distance available between sample and MS inlet. Rapid evaporative ionisation MS (REIMS) addressed this issue by allowing sample ionisation to take place up to 5 m away from the MS inlet and transporting ions in gas-phase through a tubing under the instrument's native vacuum.[16] This substantially increased versatility of the

experimental set-up and more easily fits the requirements of automated workflows.[17] REIMS works through the rapid heating of a sample to generate gas-phase ions. Initially, this was conducted through electrical diathermy with heating resulting from the sample's non-zero impedance of the radiofrequency electrical current. However, the use of an electrical current necessitates contact between the conductive probe and the sample, and for the sample to be electrically conductive.[18] This reduces analytical throughput as conductive probes require changing or cleaning in between samples and limits the analytical range as many sample types do not possess appropriate conductive properties.

Laser have seen use in MS for over 50 years,[19, 20] with direct sample analysis reported over 40 years ago, including the use of pulsed CO2 lasers with similar operating parameters, albeit restricted by the limited electronic controllers available at this time, to the CO2 laser system utilised in this study.[21] Lasers continue to see widespread use in MALDI-MS instruments today. Indeed, lasers paired with a secondary ionisation source, typically electrospray ionisation (ESI)[12] have seen use in ambient ionisation MS techniques such as laser ablation electrospray ionisation (LAESI) and matrix assisted laser desorption ESI (MALDESI). Nevertheless, these techniques maintained a requirement for sample preparation with analysis conducted within close proximity to the mass spectrometer. To counter this, optical fibres have previously been used to deliver the laser beam in order to increase distance between sample and mass spectrometer and maximise analytical flexibility.[22-25] The field of ambient ionisation MS has made substantial advances in the use of lasers to maximise analytical flexibility, but techniques have remained relatively low throughput. Here, we report on the first sample-preparation-free MS platform, utilising laser assisted REIMS (LA-REIMS) that allows high-throughput and automated mass spectrometry analysis of diverse sample types; all using the same analytical set-up in an ambient environment. Although various lasers, including ultraviolet and infrared, have been used with REIMS since 2010, applications have mainly remained limited to human and animal tissues, and biological fluids with high water content, and further, require the manual handling of samples.[25-27]

These limitations are associated either with the wavelength of the chosen lasers, such as the 210 to approximately 3000 nm range of the optical parametric oscillator (OPO) laser,[25] or a requirement for a fixed mirror focussing system that limits automation options.[28] Here, we have chosen a carbon dioxide laser operating at a 10.6 µM wavelength with a fibre optic beam delivery, and options for pulsed delivery of laser energy. This choice offers a flexible experimental approach which broadens the analytical sample range of the set-up. We have previously used this system in proof-of-concept studies in synthetic biology screeing[29] and cervical cancer screening[30] but here we show detailed construction and optimisation of the system for the first time and its wide analytical range. To this end, we show that the system provides highly-accurate species-level identification of clinically important bacteria and yeast species, metabolic profiling of human faecal samples to define biomarker signatures for type 2 diabetes mellitus, and detection of adulteration of cooking oils and the determination of geographical origin of protected olive oil types.

Experimental Section

Construction and Operation of Automated High-Throughput LA-REIMS Platform

To enable sample preparation-free mass spectrometry, we combined a 10.6 µM carbon dioxide laser (FELS-25A, OmniGuide, USA) with a modified liquid handling robot (Freedom Evo 75, TECAN, Switzerland), for which we previously reported on the adaptation of this robot for high-throughput electrical diathermy REIMS.[17, 31] A schematic of the automated high-throughput LA-REIMS system is shown in Figure 1a, with pictures of the implementation of the system given in Figure 1b, and of the analysis of an agar culture plate in Figure 1c. Further details on system construction, operation, and optimisation, acquisition of mass spectral data, instrument maintenance, data and statistical analysis, and tentative identification of important features from classification models are given in the supporting information accompanying this article. This includes reference to the bacterial isolates used in optimisation (Supplementary Table S1), and the combination of different operating parameters utilised (Supplementary Table S2).

Direct-from-Culture Speciation of Clinically Relevant Microorganisms

Clinical isolates were collected from routine diagnostic specimens at the Imperial College NHS Healthcare Trust Medical Microbiology Department at Charing Cross Hospital (London, UK). Isolates were identified using MALDI-ToF mass spectrometry as previously described.[17] Classification models were built on the randomised analysis of 15 isolates, collected from distinct clinical patient samples, from 25 clinically important microbial species and cultured as described in Supplementary Table S3. From each isolate culture plate, three different colonies were analysed. Random forest was used for the construction of taxonomic reference models and validated using leave-one-isolate-out cross-validation. In line with previous work, only negative ion detection mode acquisition was used for the construction of classification models.

139 **Direct-from-Sample Metabolic Fingerprinting of Diabetes Mellitus Type 2 (DMT2) Faeces**

140 Through Ghent University and Ghent University Hospital (Ghent, Belgium), faecal samples were
141 collected from patients diagnosed with DMT2 and healthy controls, being classified according to the
142 HbA1c level (60 mmol/mol as threshold). The main exclusion criteria were the presence of chronic
143 disorders (with exception of the typical co-morbidities of type 2 diabetes), acarbose or glucagon-like-
144 peptide 1 therapy, and recent antibiotic treatment. Ethical approval for the collection of material was
145 obtained from the UZ Ghent Ethical Committee (EC 2016/0673). Patient information is given in
146 Supplementary Table S4. It was verified that age, gender, and BMI were no dominant confounding
147 factors in previous work.[28] Faecal samples were freeze-dried and milled for long-term storage at -80°C.
148 Samples were transported to Imperial College London (London, UK) and reconstituted with HPLC
149 grade water in a 1:1 faecal powder to water (w/v) ratio into a 24 well tissue culture plate (Greiner Bio-
150 One, Austria), in a randomised order. For laser heating power optimisation, 100 mg was removed from
151 each sample and combined into one homogenate for subsequent use on separate plates. Different
152 combinations of laser power (1 W to 5 W in 0.5 W increments) and distance (1mm to 5 mm in 1mm
153 increments) were used to establish optimal analysis parameters in negative ion detection mode.

154

155 **Determination of Olive Oil Authenticity and Geographical Origin**

156 Commercially available cooking oils and extra virgin olive oils of geographically protected origins were
157 purchased from local retailers. For each oil sample, 10 µL droplets were placed onto a microscope
158 glass slide and analysed using optimised CO_2 laser operating parameters. Oils were analysed in a
159 randomised order with eight replicates of each oil used in the construction of statistical models.

160

161 **Safety considerations**

162 In this work, all microorganisms and samples were treated as potential Hazard Group 2 material. All
163 REIMS analyses were executed within a class 2 biological safety cabinet modified to contain the CO_2
164 laser, classified as a Class IVb hazard, which prevented any escape of uncontained laser beam. All

solvents, such as 2-propanol and methanol, were handled according to the material safety data sheet provided by their respective manufacturer.

167 **Results and Discussion**

168

169 After being initially developed as a tool for the real-time determination of cancer margins, utilising the
170 chemical contents of electro-surgical smoke,[16, 26] REIMS has seen wider applications in the areas of
171 clinical microbiology diagnostics,[17, 31-33] cell line analysis,[34-36] and food quality,[37-39] authenticity,[40] and
172 adulteration.[41] These applications, however, have relied on the use of electrical diathermy. This has
173 three drawbacks including limited analytical range, as a sample has to be electrically conductive,
174 sample throughput, as the heating surface has to be cleaned or replaced to prevent carry-over, and
175 potential analytical variability as each measurement is conducted by an individual, in a non-automated
176 fashion. Here we report on the successful integration of a high-throughput automated robotic
177 platform with a focussed CO_2 laser, Figure 1, that overcomes these three limitations of current REIMS
178 heating methods, allowing sample analysis in less than ten seconds, and thus increasing the analytical
179 throughput by three to five times that of electrical diathermy REIMS.

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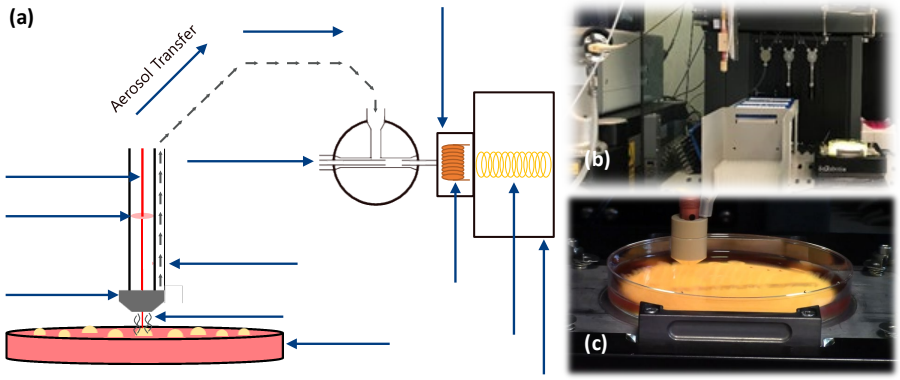

181

182 **Figure 1. Diagram and Implementation of Automated High-Throughput LA-REIMS Platform**

183 (a) A CO_2 laser passes through a flexible hollow core fibre guide system from an OmniGuide FELS-25A
184 and focussed in an modified Aesculight lens cell using two lenses into a spot size of ≈ 500 µM. The
185 resulting vapour is aspirated through a 3D printed PTFE capture head through PTFE tubing to a

stainless steel T-piece where the aerosol is mixed with 2-propanol solvent containing leucine enkephalin. The combined mixture enters the REIMS interface of the Xevo G2-XS QToF instrument and collides with a Kanthal ribbon heated (700°C) collision surface prior to entry of the ion guide of the mass spectrometer and subsequent time of flight mass analysis. Incorporation of the diagrammatic representation into a (b) TECAN Freedom Evo 75 platform is shown alongside analysis of a (c) bacterial culture directly from agar culture.

Optimisation of LA-REIMS Platform

We took a linear approach to achieve method optimisation, using 15 isolates of bacteria covering a range of species with different growth conditions, colony phenotype, and mass spectral features. Our overall aim was to maximise signal-to-noise ratios within two mass ranges which have previously been shown to contain fatty acids and metabolites (50 to 500 *m/z*) and complex lipids (600 to 1000 *m/z*).[32, 33] For laser heating power (Supporting Figure S1a), we identified a heating power of 2.0 W as optimum, although there were minimal differences across the heating powers. Although there appears to be a trend towards reduced signal-to-noise above 2.0 W, this was non-significant ($P > 0.05$) and suggests that the laser power is important for sample mobilisation and that this reached a point of saturation below the operating parameters of the CO_2 laser used. For both spectral regions, the pulsatile operation of the CO_2 laser showed significant changes in signal-to-noise ratios. The use of 'SuperPulse' pulsatile mode, which allows a high power delivery at the beginning of a pulse window, yielded a significant ($P < 0.05$) improvement in signal-to-noise ratios for spectral regions (Supporting Figure S1b). Additionally, introduction of multiple pulse windows within the analysis period, led to significant ($P < 0.01$) improvements in the signal-to-noise ratio of the complex lipid region, with 40 ms windows as the optimal parameter value. We hypothesise that this improvement is due to a reduced fragmentation of complex lipids within their gas-phase. As shown in Figure 1, the aspiration pathway of gas-phase ions within our system passes through the path of the focused laser beam, which may be an important contributor to fragmentation. The introduction of pulsatile windows may allow a clear

212 aspiration pathway for gas-phase ions and prevent further fragmentation during the periods the laser
213 is not firing. Interestingly, early applications of MALDI-MS to lipidomic experiments showed gas-phase
214 fragmentation which was moderated by matrix additives.[42]
215
216 LA-REIMS analysis was completed for eight seconds allowing for several combinations of coverage and
217 speeds to be investigated. Five laser head movements were tested for signal intensity (Supporting
218 Figure S1d), with two showing a significantly higher complex lipid signal-to-noise ratio ($P < 0.01$) which
219 share commonality with regards to the distance between movement lines. To minimise instrument
220 contamination, the maximum distance from sample that could be used before a significant reduction
221 in signal intensity was analysed (Supporting Figure S1e). For the low molecular weight region signal,
222 4.0 mm could be used before a significant reduction ($P < 0.001$) was seen but a limit of 2.0 mm was
223 observed for the complex lipid region signal ($P < 0.001$). To determine whether this resulted from
224 increased thermal distribution of the laser, we completed physical laser spot size measurements and
225 irradiance measurements from 1 to 5 mm distance in 1 mm increments (Supporting Figure S2). This
226 showed a spot size of approximately 1 mm from 2 to 5 mm distances with laser irradiance showing a
227 decline from 3 mm. As this decline was less than 10%, it suggests that the loss of signal intensity is a
228 combination of increased thermal distribution of the laser and reduced strength of aspiration of the
229 analyte containing vapour by the mass spectrometer. Based on these findings, a distance of 2.0 mm
230 was determined as optimal to maximise signal intensity and minimise potential debris build-up on the
231 vapour aspiration head.
232
233 **Direct-from-Culture Speciation of Clinically Relevant Microorganisms**
234 The use of electrical diathermy REIMS for the speciation of clinically relevant microorganisms has been
235 widely reported.[17, 31, 32] Here, a total of 25 bacterial and yeasts, Supplementary Table S3, were analysed
236 using the optimised laser operating parameters, whereby a species-level classification accuracy of
237 97.2% after leave-one-out cross-validation of random forest models was achieved (Figure 2a). This is

greater than all previously reported accuracies (96.3%[17]) using electrical diathermy REIMS and suggests that the utilisation of radiative heating addresses the issues of ionisation efficiency using monopolar probes whilst maintaining the analytical throughput of an automated system. As previously shown, using radiative heating as the evaporation modality in REIMS produces comparable spectra as for bipolar electrical diathermy REIMS.[43] Comparison of typical REIMS spectra of *E. coli* isolates (Figures 2b/c) further supported this and suggested that the even radiative heating distribution, offered by the use of a CO_2 laser, is similar to that offered by the bipolar electrical diathermy method and that the evenness of the thermal distribution is key in the ionisation efficiency of REIMS.

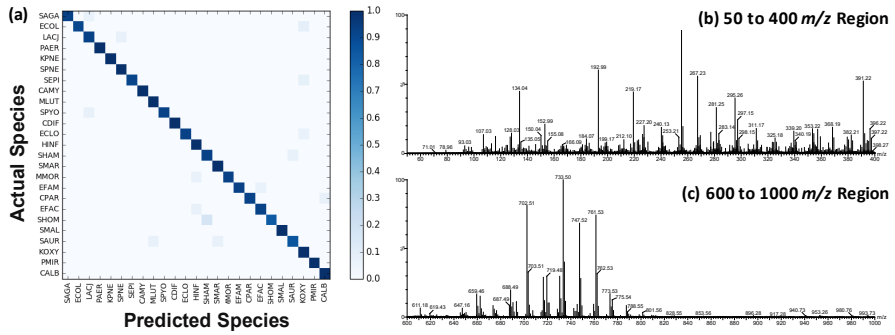

Figure 2. Microbial Speciation using LA-REIMS of 25 Clinically Significant Microorganisms

LA-REIMS was used for the speciation analysis of 15 isolates from 25 clinically significant microbial species. Resulting (a) confusion matrix of leave-one-out cross-validation or random forest speciation reference model is shown with an overall species-level accuracy of 97.2%. Representative spectra of an isolate of *E. coli* is shown for (a) the fatty acid and lower weight metabolite (50 to 400 *m/z*) region and (b) the complex lipid (600 to 1000 *m/z*) region.

One of the key benefits of REIMS to clinical microbiology laboratories is its suitability for high-throughput and automated analysis without sample preparation, which is currently not offered by

258 techniques such as MALDI-ToF-MS microbial speciation.[44] The move towards automation in clinical
259 microbiology is driven by a requirement for a reduced turn-around time, reduced costs associated
260 with speciation, and improved processes for quality control and assurance. The utilisation of LA-REIMS
261 for this purpose would streamline automation as no sample preparation, such as the addition of a
262 matrix to aid ionisation in MALDI-ToF-MS, is required.
263
264 **Metabolic Phenotyping of Faecal Samples from Diabetes Mellitus Type 2 (DMT2) Patients**
265 We have previously reported on the use of electrical diathermy REIMS for the analysis of faecal
266 material.[18] However, the conductivity of a sample can be affected by its water content and is highly
267 variable between donors; potentially preventing a universal power setting from being used. The use
268 of LA-REIMS potentially circumvents this as radiative sample heating is utilised. To establish the
269 operating parameters of LA-REIMS for faecal analysis, we reconstituted in water a mix of 50 mg of
270 freeze-dried faeces from each patient forming our DMT2 cohort and completed four analytical repeats
271 of parameter combinations. Parameter optimisation focused only on laser power and distance from
272 sample as laser pulsatile mode affects gas-phase ions and not the heating process. Supplementary
273 Figure S3 indicates that a laser power of 4.0W at 3 mm from the sample was optimal, as this
274 combination provided a plateau for signal intensity in both regions at the lowest laser power before a
275 reduction in intensity was observed from 4 mm distance or above.
276
277 We then applied these optimised parameters to the entire cohort of 39 DMT2 patients and 39 healthy
278 controls and conducted PLS-DA modelling and random forest classification modelling (Figure 3). In
279 both ion detection modes, separation was evident between DMT2 and healthy patient samples.
280 Stronger separation is shown in positive ion detection mode modelling which is further supported by
281 the accuracy of random forest models – 93.5% for positive and 87.1% for negative ion detection mode
282 models. Metabolic profiling of biofluids from DMT2 patients typically focuses on blood or urine due to
283 the systemic nature of the condition. However, as the growing importance of the gut microbiome is

realised in many non-communicable diseases, the study of the faecal metabolome is receiving increased attention[45]. Tentative identifications based on accurate mass determination of negative ion detection mode features (Supplementary Table S5) that are identified as important in random-

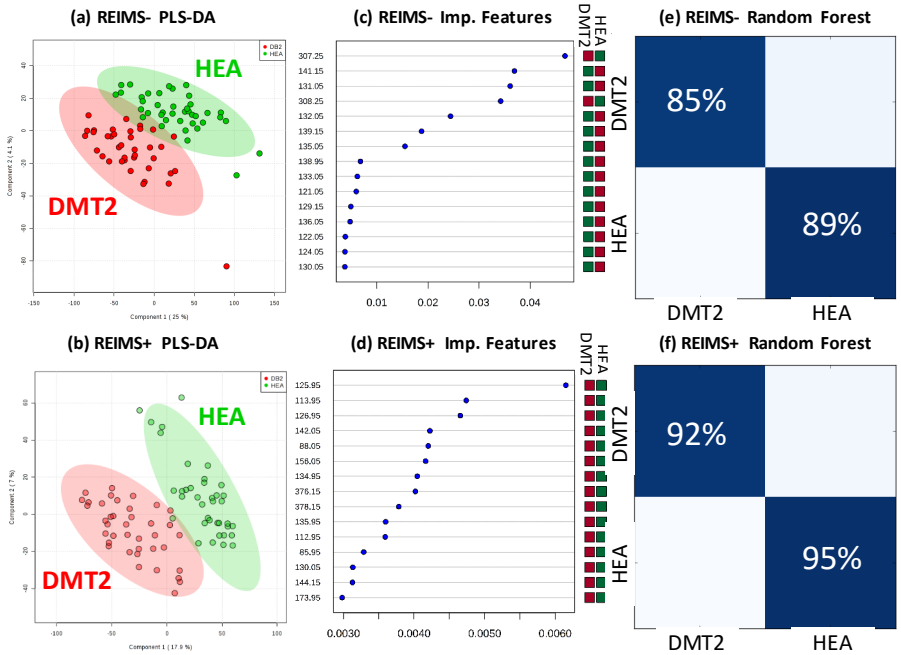

Figure 3. Multivariate Modelling and Random Forest Classification of DMT2 Faecal Samples

Supervised multivariate modelling using PLS-DA of (a) negative ion detection mode (Accuracy = 0.856, R2 = 0.961, Q2 = 0.609) and (b) positive ion detection mode (Accuracy = 0.987, R2 = 0.984, Q2 = 0.806). Healthy patient samples are shown in green and DMT2 in red. Features important in separation between DMT2 and healthy patient samples are given for (c) negative ion mode and (d) positive ion mode LA-REIMS analysis. Tentative identifications of important features is given in Supplementary Tables S5 and S6 respectively for each ion detection mode. Confusion matrices constructed from leave-one-out cross-validation random forest classification models are given for (e) negative ion mode (Accuracy = 87.1%) and (f) positive ion mode (Accuracy = 93.5%) LA-REIMS analysis.

297 forest model creation, showed several associations with dietary consumption (including aloesol 7-
298 glucoside and arecaidine), which may be a result of altered diet following DMT2 diagnosis.
299 Nevertheless, we also identified several metabolites that have previously been linked to diabetes
300 pathogenesis including 6-hydroxymelatonin[46] and nicotinamides,[47] and of particular interest are those
301 which may be of microbial origin including 5-aminopentanamide.[48] For positive mode ion detection,
302 more metabolite features closely related to diabetes pathogenesis and morbidity were identified; all
303 of which were higher in DMT2 patients (Supplementary Table S6). These included D-glucose which
304 may cause gastrointestinal issues,[49] biotripyrrin which may be responsible for pink urine syndrome in
305 diabetic patients,[50] and metabolites associated with homocysteine which is linked to major drivers for
306 morbidity and mortality.[51]
307
308 **Determination of Olive Oil Authenticity and Geographical Origin**
309 Olive oil is a high-value food product with considerable health-protective properties. As a result of the
310 different grades of olive oil, determined by production processes, it is considered one of the most
311 common targets for adulteration and food fraud.[52] To detect adulterated products, an analytical
312 technique is needed to compare samples against a validated database or reference model. These
313 techniques have traditionally been divided between vibrational spectroscopic techniques or
314 chromatographic mass spectrometry,[53] but typically require time consuming sample preparation and
315 analytical runs, making them both time and resource expensive. Whilst electrical diathermy REIMS is
316 unsuited to the analysis of olive oil, as it is insufficiently conductive, LA-REIMS overcomes this through
317 the utilisation of radiative heating using an appropriate wavelength that matches the absorbance
318 profile of the sample being analysed. To show that the analytical repertoire of the sample-preparation
319 extends beyond biological material into potential high-throughput screening of food products, we
320 analysed six commercially available cooking oils and three olive oils from origins of production
321 protected under European Union legislation.

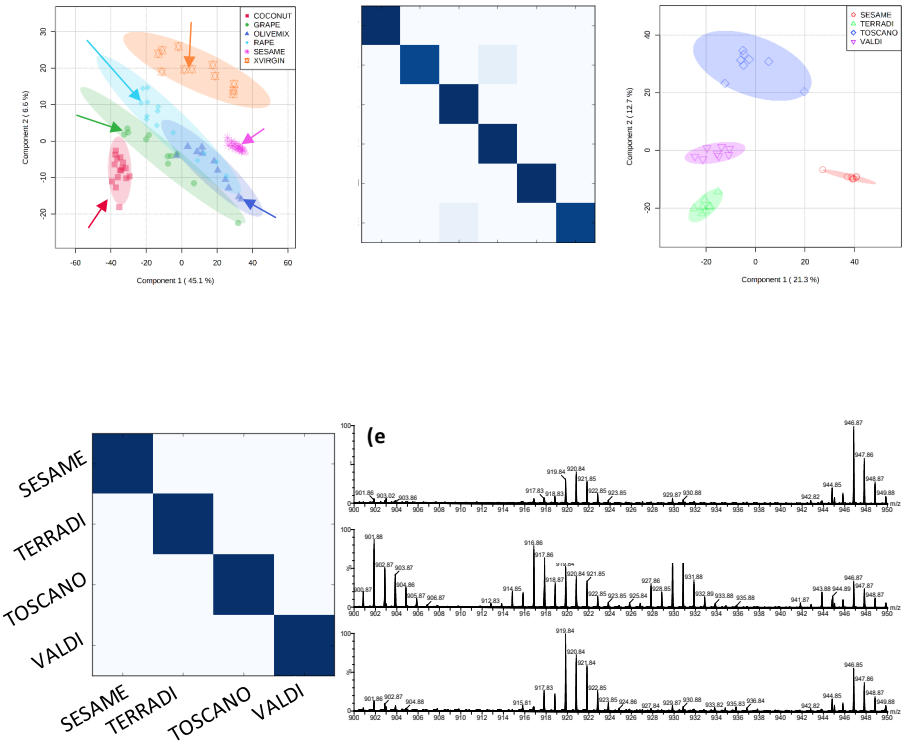

Figure 4. Multivariate Modelling and Random Forest Classification of Food Oil Type and Origin

Negative ion detection mode LA-REIMS analysis was used for the determination of oil type through supervised multivariate analysis through (a) PLS-DA modelling (accuracy = 0.869, R2 = 0.993, Q2 = 0.952), and (b) random forest analysis with leave-one-out cross-validation (classification accuracy = 97.5%). The same analytical method was used for the determination of the protected geographical origin of three Italian olive oil region (with sesame oil used as outlier anchor) through (c) PLS-DA modelling (accuracy = 1.000, R2 = 1.000, Q2 = 0.924) and (d) random forest leave-one-out cross-validation (classification accuracy = 100.0%). Representative mass spectra of the (e-g) 900 to 950 *m/z* region of three protected geographical origin Italian olive oils show clear spectral differences.

Tentative identifications of important differentiating features for PLS-DA are given in Supplementary Tables S7 and S8 for oil type and olive oil geographical origin respectively.

Both multivariate supervised PLS-DA modelling and random forest machine learning algorithms were applied for oil type analysis data (Figure 4a/b). Clear and significant separation between coconut, extra virgin olive oil, and sesame oil was observed, whilst overlapping of technical repeats was observed for rapeseed, grape, and an olive oil mixture type. This may be because of a rapeseed or grape oil being used as the non-olive component of the oil mixture. The high accuracy of 97.5%, as achieved with random forest classification, suggests that LA-REIMS could be used as a high-throughput and sample-preparation free method for food product monitoring in a context of adulteration – either through substitution or mixtures of low-value oils to dilute high-value oils such as extra virgin olive oil. Of issue in food authenticity is the monitoring of products from legally protected production methods or locations. Here, for extra virgin olive oils we analysed three oils from three geographically protected areas of production in Italy; Terra di Bari, Toscano, and Val di Mazara oils. Using the same multivariate modelling (Figure 4c/d), significant separation between the three oil types and 100% classification accuracy using random forest modelling was achieved. Based on tentative identification of important features in statistical models, the driving differential lipid classes are phosphatidylglycerols and phosphatidic acids. Although triacylglycerols are the main constituents of olive oil – around 98% - they are not usually shown to provide sufficient discriminatory power to detect instances of olive oil adulteration and fraud.[54] Polar lipids, as we identified in this study, have shown promise as molecular markers of product purity.[55] We hypothesise that our detected biomarkers are related to the climate and altitude of olive cultivation used for the production of the three geographically protected oils: Terra di Bari (190 m), Toscano (up to 500 m), and Val di Mazara (up to 200 m). Glycerophospholipid composition in plants has been shown to be affected by growth conditions, including temperature,[56] which is likely reflected in cultivation climate and altitude, being the discriminating factors found upon LA-REIMS analysis.

358 **Conclusions**

359

360 Mass spectrometry has proven itself as a powerful tool in chemical and biological research and with
361 significant impacts in clinical diagnostics. Application developments beyond these areas, however, are
362 limited by the complexity of sample preparation and the time typically required for chromatographic
363 separation prior to ion generation and mass spectrometry analysis – which can create a substantial
364 bottleneck in workflows. Although advances in direct infusion MS methods and ambient ionisation
365 mass spectrometry methods have reduced analysis turn-around times, the necessary sample
366 extraction and/or preparation has limited their overall throughput and application potential. Here, we
367 have shown that sample-preparation-free MS can be achieved by modifying the heating modality of
368 REIMS from electrical diathermy to radiative heating using a CO_2 laser and combining it with an
369 automation platform. By moving away from electrical diathermy, we have eliminated the requirement
370 for a sample to be conductive in order to be suitable for analysis and improved the throughput by
371 removing the requirement for cleaning or changing of the analysis probe between samples. We have
372 shown that this LA-REIMS platform can analyse a diverse range of sample types, including
373 microorganisms, human faeces, and cooking oils. As these samples show a diverse range of properties,
374 we therefore believe that the LA-REIMS system is capable of the analysis of all biological and organic
375 material with no sample preparation nor extraction. This will increase the throughput of systems
376 employing LA-REIMS to a 'tipping point' where a value proposition can be achieved to allow mass
377 spectrometry analysis to expand into novel application areas. Although we have shown that LA-REIMS
378 has sufficient analytical power to delivery meaningful insights into three different biological systems,
379 in some cases, such as where there is a requirement to resolve structural isomers, there will remain a
380 need for traditional profiling techniques, such as LC-MS. Here, we propose that LA-REIMS can act as a
381 screening tool to reduce the number of samples that require alternative profiling techniques and act
382 as the mechanism by which bottlenecks in analytical workflows can be removed. The pursuit of
383 sample-preparation-free mass spectrometry further offers the potential to move towards

miniaturisation of mass spectrometry analysers. This has seen limited progress in recent years; arguably because miniaturisation exhibits continued reliance on sample preparation and extraction and chromatographic separation to address the reduced analytical resolution of miniaturised MS systems. As we have shown that sample-preparation-free mass spectrometry can provide both decision-based classifications and biological insights, combining this capability with miniaturised instruments will greatly enhance the application areas for MS.

Supporting Information

Detailed information regarding bacterial and fungal species analysed in this study, and on additional data analyses completed as referenced within this text is given within the supporting information accompanying this manuscript.

Author Contributions

Study was planned by SJSC and ZT. Experiments were conducted by SJSC, LVM, AB, KAH, TR, and SS. Data was analysed and interpreted by SJSC and APM. Technical assistance was provided by DS, RS, JB, TK, MR, and LV. The manuscript was written by SJSC and ZT with input from all authors. All authors have given approval to the final version of the manuscript.

Conflict of Interest Statement

This work was funded and technically supported by the Waters Corporation and funded by the Biotechnology and Biological Sciences Research Council under grant BB/L020858/1 and European Research Council under contract number 617896. ZT provides remunerated consultancy to the Waters Corporation. The work detailed in this manuscript does not promote any available commercial product from Waters Corporation.

Acknowledgments

The authors would like to thank Imperial College Healthcare Trust for access to samples and to the participants/patients who agreed to donate samples which were utilised in this work. This work was funded and technically supported by the Waters Corporation and funded by the Biotechnology and Biological Sciences Research Council under grant BB/L020858/1 and European Research Council under contract number 617896. This article is independent research funded by the NIHR BRC, and the views expressed in this publication are those of the authors and not necessarily those of the NHS, NIHR, or the Department of Health and Social Care.

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Sample preparation free mass spectrometry using laser-assisted rapid evaporative ionisation mass spectrometry (LA-REIMS): applications to microbiology, metabolic biofluid phenotyping, and food authenticity

Simon J.S. Cameron, Alvaro Perdones-Montero, Lieven Van Meulebroek, Adam Burke, Kate Alexander-Hardiman, Daniel Simon, Richard Schaffer, Julia Balog, Tamas Karancsi, Tony Rickards, Monica Rebec, Sara Stead, Lynn Vanhaecke, Zoltán Takáts.

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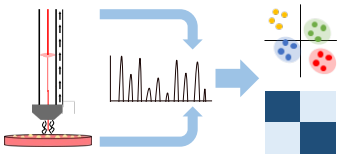

Optimisation and application of LA-REIMS, requiring no sample preparation, for rapid and direct MS-based metabolomics.