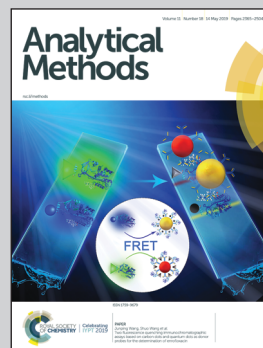


Showcasing research from Professor Francisco Fernandez-Lima's laboratory, Department of Chemistry, Biomolecular Science Institute, Florida International University, Miami, USA.

Workflow for fast lipid tissue screening using LESA-FT-ICR-MS

Recent studies have shown the potential of lipids biomarkers for disease screening and treatment. In this paper, we showcase a fast workflow for ambient, lipid screening from biological surfaces using LESA-FT-/CR MS/MS.

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## Workflow for fast lipid tissue screening using LESA-FT-ICR-MS†

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Lipid screening of biological substrates is an important step during biomarker detection and identification. In this work, a fast workflow is described capable of rapid screening for lipid components from biological tissues at ambient pressure based on liquid microjunction extraction in tandem with nano-electrospray ionization (nESI) with ultra-high resolution mass spectrometry, *i.e.*, liquid extraction surface analysis (LESA) coupled to Fourier-transform ion cyclotron resonance (tandem) mass spectrometry (LESA-FT-ICR-MS/MS). Lipid profiles are presented for thin tissue sections of mouse brain (MB) and liver (ML) samples, analyzed in both positive and negative mode by data-dependent acquisition (DDA) tandem FT-ICR-MS/MS. Candidate assignments were based on fragmentation patterns using mostly SimLipid software and accurate mass using mostly the LipidMaps database (average sub-ppm mass error). A typical, single point surface analysis (<1 mm spatial sampling resolution) lasted less than 15 minutes and resulted in the assignment of (unique and multiple) lipid identifications of ~190 (MB) and ~590 (ML) *m/z* values. Despite the biological complexity, this led to unique identifications of distinct lipid molecules (sub-ppm mass error) from 38 different lipid classes, corresponding to 10–30% of the lipid *m/z* identifications.

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## Introduction

Lipids are important components of living cells and frequently mediate biological processes.<sup>1</sup> Changes to a cell's environment are rapidly translated into changes in its lipid composition, making it an attractive target for biomarker discovery and disease screening and treatment.<sup>1–3</sup> Lipid analyses are typically performed using mass spectrometry (MS).<sup>1,3–8</sup>

Lipid MS analyses can be undertaken at several levels. For example, the sum composition of the fatty acids constituting a lipid can be obtained with an exact mass MS measurement (*e.g.* PC(40:6)); moreover, tandem MS (MS/MS) experiments, such as collision induced dissociation (CID), can further allow to elucidate the length and the number of double bonds of each of the fatty acid chains (*e.g.* PC(20:2\_20:4)). The double bond positions on the fatty acid chains can also be investigated through specific fragmentation techniques.<sup>9–14</sup> The coupling of different separation

techniques in front of the MS analysis, such as liquid chromatography (LC) or ion mobility (IM), can add an additional separation dimension to the MS lipid characterization.<sup>5,15–19</sup>

The challenges for global mass spectrometry analyses of lipids (lipidomics) are twofold. First, the sample preparation can bias the lipid composition by selecting only a partial lipid content of the sample.<sup>4,5</sup> Second, the mass spectrometry analysis must be capable of detecting both low and high abundance species, with a high resolving power and mass accuracy in order to resolve and confidently identify isobaric lipids. The latter challenge can be addressed by using instruments such as Fourier-transform ion cyclotron resonance mass spectrometers (FT-ICR MS). The choice of the sample preparation however depends on the ionization method used for the mass spectrometry analysis. For tissue analyses, imaging techniques such as secondary ion mass spectrometry (SIMS) or matrix assisted laser desorption ionization (MALDI) mass spectrometry are often used.<sup>20–28</sup> Ionization suppression and the matrix choice potentially bias the observed lipid composition.<sup>5,21,28,29</sup> When using electrospray ionization (ESI), the MS analyses are usually preceded by LC separations with long separation gradients (up to 2 hours) depending on the LC column, the LC solvent conditions, and the number of lipid classes.<sup>30</sup> LC–LC couplings have shown some advantages in lipid separations, with the tradeoff of increased analysis times.<sup>5,15</sup>

An alternative to lipid extraction (*e.g.*, LC-MS/MS) or surface mapping (*i.e.*, desorption electrospray ionization (DESI)<sup>31–34</sup> or MALDI), is liquid extraction surface analysis

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(LESA) in which a liquid microjunction between the surface and an extraction tip is created, followed by direct nano-electrospray infusion.<sup>35–37</sup> In a LESA experiment, the solvent (or solvent mixtures) of choice can direct the type of chemical class that is extracted (*e.g.*, lipids or proteins).<sup>38–48</sup> The choice of solvent composition in LESA can favor the extraction of certain lipid classes.<sup>46–49</sup> When compared to an LC-MS/MS or a MALDI-MS/MS experiment, LESA-MS/MS significantly decreases the sample preparation time and potential ionization suppression bias (*e.g.*, MALDI matrix). Moreover, LESA experiments lead to continuous sample infusions during several minutes, which allows performing MS/MS measurements for lipid identification, with the potential of adding spatial tissue profiling to the analyses.<sup>41</sup>

Here, we developed a fast, lipid screening workflow based on LESA-FT-ICR-MS and MS/MS for ambient analysis of thin tissue sections. Examples shown include mouse brain and mouse liver analyzed using data dependent acquisition (DDA) with ultra-high mass resolution and high mass accuracy in positive and negative ion mode. Candidate lipid assignments were performed using different databases, based on MS/MS fragmentation patterns, as well as on MS1 accurate mass measurements (<3 ppm mass accuracy database searches).

## Experimental

### Thin tissue sections

Liver and brain from wildtype mice (extraneous tissue from culled animals) were the gift of Prof. Steve Watson (University of Birmingham). Organs were frozen on dry ice prior to storage at  $-80^{\circ}\text{C}$ . Sections of murine liver tissue and brain tissue of area  $\sim 1.5\text{ cm}^2$  were obtained at a thickness of  $10\ \mu\text{m}$  using a CM1850 Cryostat (Leica Microsystems, Wetzlar, Germany) and thaw mounted onto glass slides.

### LESA-MS/MS analysis

Thin tissue section samples were loaded onto a universal LESA adapter plate and placed in the TriVersa Nanomate chip-based

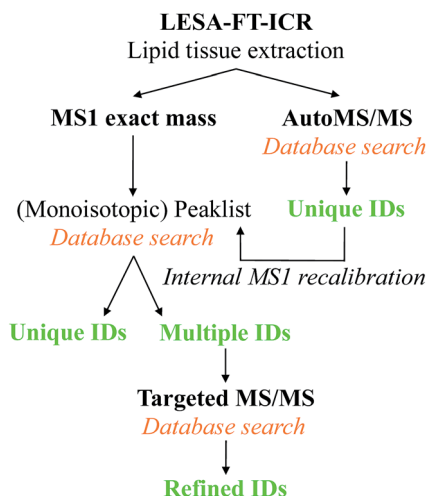


Fig. 1 LESA-FT-ICR workflow developed in this study.

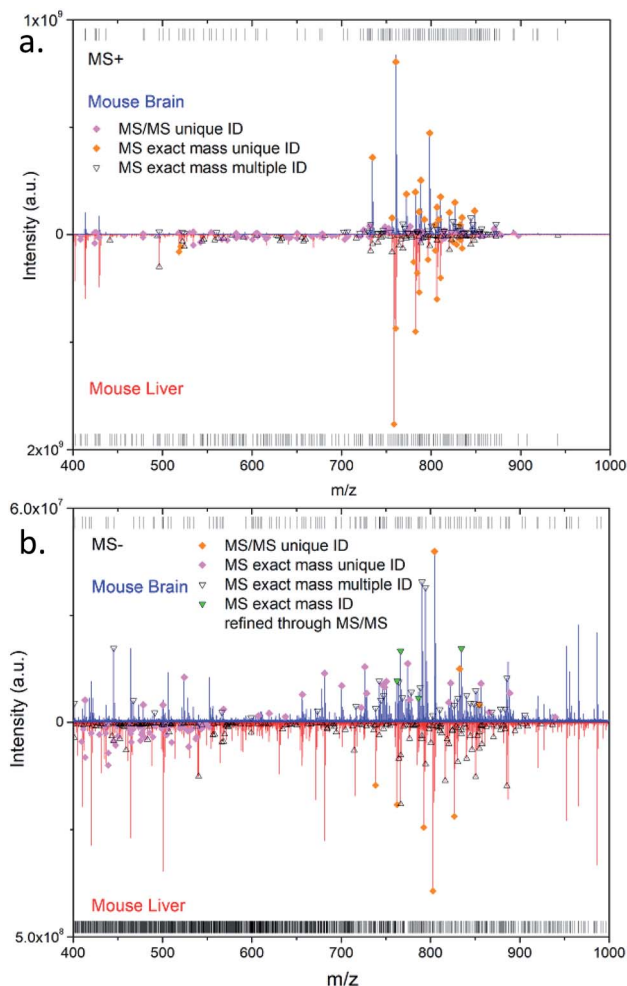


Fig. 2 Positive (a) and negative (b) ionization mode LESA-FT-ICR MS spectra of mouse brain (top, blue) and of mouse liver (bottom, red). The vertical lines on top of each spectrum represent the monoisotopic  $m/z$  peaks extracted for identification. The orange markers denote MS/MS identified peaks. The  $m/z$  peaks with unique and multiple lipid identifications are highlighted with pink and black markers. As proof-of-concept, the negative mode analysis of MB was subjected to targeted MS/MS experiments using MS1 accurate mass assignments (highlighted with green triangular markers).

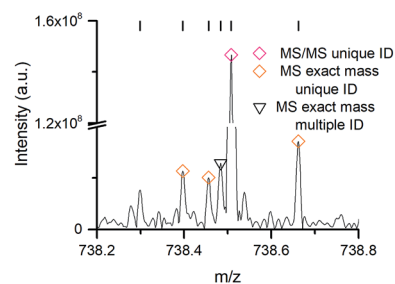


Fig. 3 Extract from the mouse liver mass spectrum in negative mode from  $m/z$  738.2 to 738.8. The black vertical lines represent the monoisotopic  $m/z$  peaks extracted for identification. The orange markers denote MS/MS identified peaks. The  $m/z$  peaks with unique and multiple lipid identifications are highlighted with pink and black markers.

**Table 1** Summary of the positive ionization mode LESA-FT-ICR-MS (MS1 and MS/MS) of a MB (left) and ML (right) sample. The molecular ion species, chemical composition, lipid class, theoretical mass, mass error, and identifiers are provided. HG denotes the head group and FA denotes fatty acids. MS1\* designates lipid identifications where odd-chained lipids were discarded as biologically unlikely

Mouse brain MS+										Mouse liver MS+									
ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1	Identified fragments from AutoMS/MS or MS1	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1		
1	426.3568	[M + H] <sup>+</sup>	C <sub>25</sub> H <sub>48</sub> NO <sub>4</sub>	CAR	CAR (18:1)	426.3578	2.35	MS1	MS1	1	408.3074	[M + Na] <sup>+</sup>	C <sub>22</sub> H <sub>43</sub> NO <sub>4</sub> Na	CAR	CAR (15:0)	408.3084	-2.50	MS1	
2	604.5059	[M + K] <sup>+</sup>	C <sub>36</sub> H <sub>71</sub> NO <sub>3</sub> K	Cer	Cer (d36:1)	604.5065	1.06	MS1	MS1	2	428.3724	[M + H] <sup>+</sup>	C <sub>25</sub> H <sub>50</sub> NO <sub>4</sub>	CAR	CAR (18:0)	428.3734	-2.38	MS1	
3	836.6082	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>86</sub> NO <sub>13</sub>	LacCer	LacCer (d32:0)	836.6094	1.41	MS1	MS1	3	638.6075	[M + H] <sup>+</sup>	C <sub>40</sub> H <sub>80</sub> NO <sub>4</sub>	Cer	Cer (t40:1)	638.6082	-1.05	MS1*	
4	854.5578	[M + Na] <sup>+</sup>	C <sub>44</sub> H <sub>81</sub> NO <sub>13</sub> Na	LacCer	LacCer (d32:2)	854.56	2.57	MS1	MS1	4	666.6390	[M + H] <sup>+</sup>	C <sub>42</sub> H <sub>84</sub> NO <sub>4</sub>	Cer	Cer (t42:1)	666.6395	-0.80	MS1	
5	478.3282	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>24</sub> H <sub>49</sub> NO <sub>6</sub> P	LPC	LPC (16:0)	478.3292	2.17	MS1*	MS1*	5	589.4795	[M + Na] <sup>+</sup>	C <sub>35</sub> H <sub>66</sub> O <sub>5</sub> Na	DG	DG (32:1)	589.4802	-1.19	MS1	
5	518.3208	[M + Na] <sup>+</sup>	C <sub>24</sub> H <sub>50</sub> NO <sub>7</sub> PNa	LPC	LPC (16:0)	518.3217	1.77	MS1*	MS1*	6	617.5108	[M + Na] <sup>+</sup>	C <sub>37</sub> H <sub>70</sub> O <sub>5</sub> Na	DG	DG (34:1)	617.5115	-1.07	MS1	
5	534.2947	[M + K] <sup>+</sup>	C <sub>24</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (16:0)	534.2956	1.63	MS1*	MS1*	7	615.4952	[M + Na] <sup>+</sup>	C <sub>37</sub> H <sub>68</sub> O <sub>5</sub> Na	DG	DG (34:2)	615.4959	-1.19	MS1	
6	582.2949	[M + K] <sup>+</sup>	C <sub>28</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (20:4)	582.2956	1.27	MS1	MS1	8	613.4795	[M + Na] <sup>+</sup>	C <sub>37</sub> H <sub>66</sub> O <sub>5</sub> Na	DG	DG (34:3)	613.4802	-1.08	MS1	
7	606.2951	[M + K] <sup>+</sup>	C <sub>30</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (22:6)	606.2956	0.82	MS1	MS1	9	643.5266	[M + Na] <sup>+</sup>	C <sub>39</sub> H <sub>72</sub> O <sub>5</sub> Na	DG	DG (36:2)	643.5272	-0.95	MS1	
8	530.2868	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>26</sub> H <sub>45</sub> NO <sub>8</sub> P	LPS	LPS (20:3)	530.2877	1.62	MS1	MS1	10	641.5109	[M + Na] <sup>+</sup>	C <sub>39</sub> H <sub>70</sub> O <sub>5</sub> Na	DG	DG (36:3)	641.5115	-0.87	MS1	
9	554.2869	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>28</sub> H <sub>45</sub> NO <sub>8</sub> P	LPS	LPS (22:5)	554.2877	1.50	MS1	MS1	11	639.4952	[M + Na] <sup>+</sup>	C <sub>39</sub> H <sub>68</sub> O <sub>5</sub> Na	DG	DG (36:4)	639.4959	-1.05	MS1	
10	734.5693	[M + H] <sup>+</sup>	C <sub>40</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (32:0)	734.5694	0.11	HG (184.0725)	HG (184.0725)	12	897.5922	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>49</sub> H <sub>85</sub> O <sub>14</sub>	DGDG	DGDG (34:3)	897.5934	-1.35	MS1	
11	760.5851	[M + H] <sup>+</sup>	C <sub>42</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (34:1)	760.5851	0.00	HG (184.0725)	HG (184.0725)	13	572.4513	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>32</sub> H <sub>62</sub> NO <sub>7</sub>	HexCer	HexCer (d26:0)	572.4521	-1.48	MS1	
12	756.5514	[M + H] <sup>+</sup>	C <sub>42</sub> H <sub>79</sub> NO <sub>8</sub> P	PC	PC (34:3)	756.5538	3.16	HG (184.0724)	HG (184.0724)	14	586.4670	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>33</sub> H <sub>64</sub> NO <sub>7</sub>	HexCer	HexCer (d27:0)	586.4677	-1.28	MS1	
13	788.6166	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>87</sub> NO <sub>8</sub> P	PC	PC (36:1)	788.6164	-0.25	HG (184.0725)	HG (184.0725)	15	600.4826	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>34</sub> H <sub>66</sub> NO <sub>7</sub>	HexCer	HexCer (d28:0)	600.4834	-1.27	MS1	
14	786.6009	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (36:2)	786.6007	-0.26	HG (184.0725)	HG (184.0725)	16	409.2340	[M + H] <sup>+</sup>	C <sub>19</sub> H <sub>38</sub> O <sub>7</sub> P	LPA	LPA (16:1)	409.2350	-2.42	MS1	
15	782.5666	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (36:4)	782.5694	3.64	HG (184.0725)	HG (184.0725)	17	478.3282	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>24</sub> H <sub>49</sub> NO <sub>6</sub> P	LPC	LPC (16:0)	478.3292	-2.13	MS1*	
15	820.5258	[M + K] <sup>+</sup>	C <sub>44</sub> H <sub>80</sub> NO <sub>8</sub> PK	PC	PC (36:4)	820.5253	-0.58	M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> -H <sub>2</sub> O (637.4541)	M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> -H <sub>2</sub> O (637.4541)	17	534.2947	[M + K] <sup>+</sup>	C <sub>24</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (16:0)	534.2956	-1.65	MS1*	



Table 1 (Contd.)

Mouse brain MS+										Mouse liver MS+									
ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1 identification	ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1 identification		
16	810.6014	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (38:4)	810.6007	-0.87	HG (184.0725)	18	520.3388	[M + H] <sup>+</sup>	C <sub>26</sub> H <sub>51</sub> NO <sub>7</sub> P	LPC	LPC (18:2)	520.3398	-1.80	M-C <sub>21</sub> H <sub>37</sub> O <sub>6</sub> P (104.1068), HG (184.0725), M-H <sub>2</sub> O (502.3254)		
16	848.5572	[M + K] <sup>+</sup>	C <sub>46</sub> H <sub>84</sub> NO <sub>8</sub> PK	PC	PC (38:4)	848.5566	-0.74	M-C <sub>23</sub> H <sub>43</sub> NO <sub>3</sub> P-H <sub>2</sub> O (665.4851)	18	542.3208	[M + Na] <sup>+</sup>	C <sub>26</sub> H <sub>50</sub> NO <sub>7</sub> PNa	LPC	LPC (18:2)	542.3217	-1.64	MS1		
17	808.5858	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (38:5)	808.5851	-0.85	HG (184.0725)	19	518.3208	[M + H] <sup>+</sup>	C <sub>26</sub> H <sub>49</sub> NO <sub>7</sub> P	LPC	LPC (18:3)	518.3241	-6.49	M-C <sub>21</sub> H <sub>35</sub> O <sub>6</sub> P (104.1068), HG (184.0725)		
18	806.5698	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (38:6)	806.5694	-0.46	HG (184.0725)	20	582.2948	[M + K] <sup>+</sup>	C <sub>28</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (20:4)	582.2956	-1.31	MS1		
19	804.5517	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>79</sub> NO <sub>8</sub> P	PC	PC (38:7)	804.5538	2.65	HG (184.0725)	20	566.3209	[M + Na] <sup>+</sup>	C <sub>28</sub> H <sub>50</sub> NO <sub>7</sub> PNa	LPC	LPC (20:4)	566.3217	-1.39	MS1		
20	834.6013	[M + H] <sup>+</sup>	C <sub>48</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (40:6)	834.6007	-0.67	HG (184.0725)	21	606.2949	[M + K] <sup>+</sup>	C <sub>30</sub> H <sub>50</sub> NO <sub>7</sub> PK	LPC	LPC (22:6)	606.2956	-1.09	MS1		
21	832.5833	[M + H] <sup>+</sup>	C <sub>48</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (40:7)	832.5851	2.20	HG (184.0725)	22	495.3073	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>24</sub> H <sub>48</sub> O <sub>8</sub> P	LPG	LPG (18:0)	495.3081	-1.72	MS1		
21	870.5417	[M + K] <sup>+</sup>	C <sub>48</sub> H <sub>82</sub> NO <sub>8</sub> PK	PC	PC (40:7)	870.541	-0.83	MS1*	23	689.2106	[M + K] <sup>+</sup>	C <sub>25</sub> H <sub>48</sub> O <sub>15</sub> P <sub>2</sub> K	LPIP	LPIP (16:1)	689.2100	0.93	MS1		
22	892.5261	[M + K] <sup>+</sup>	C <sub>50</sub> H <sub>80</sub> NO <sub>8</sub> PK	PC	PC (42:10)	892.5253	-0.85	MS1	24	839.3513	[M + K] <sup>+</sup>	C <sub>36</sub> H <sub>66</sub> O <sub>15</sub> P <sub>2</sub> K	LPIP	LPIP (27:3)	839.3509	0.44	MS1		
23	772.5254	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>75</sub> NO <sub>7</sub> P	PC	PC (O-37:9)	772.5276	2.75	HG (184.0725)	25	580.3601	[M + H] <sup>+</sup>	C <sub>28</sub> H <sub>55</sub> NO <sub>9</sub> P	LPS	LPS (22:1)	580.3609	-1.33	MS1		
24	798.5412	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>79</sub> NO <sub>7</sub> P	PC	PC (O-39:10)	798.5432	2.54	HG (184.0725)	26	429.2966	[M + Na] <sup>+</sup>	C <sub>25</sub> H <sub>42</sub> O <sub>4</sub> Na	MG	MG (22:4)	429.2975	-2.05	MS1		
25	826.5728	[M + H] <sup>+</sup>	C <sub>49</sub> H <sub>81</sub> NO <sub>7</sub> P	PC	PC (O-41:10)	826.5745	2.10	HG (184.0728)	27	457.3279	[M + Na] <sup>+</sup>	C <sub>27</sub> H <sub>46</sub> O <sub>4</sub> Na	MG	MG (24:4)	457.3288	-1.88	MS1		
26	650.4388	[M + H] <sup>+</sup>	C <sub>33</sub> H <sub>65</sub> NO <sub>6</sub> P	PE	PE (28:1 (OH))	650.4391	0.52	MS1*	28	593.3288	[M + Na] <sup>+</sup>	C <sub>30</sub> H <sub>50</sub> O <sub>10</sub> Na	MGDG	MGDG (21:3)	593.3296	-1.35	MS1		
27	678.4703	[M + H] <sup>+</sup>	C <sub>35</sub> H <sub>69</sub> NO <sub>6</sub> P	PE	PE (30:1 (OH))	678.4704	0.13	MS1*	29	665.4232	[M + Na] <sup>+</sup>	C <sub>35</sub> H <sub>62</sub> O <sub>10</sub> Na	MGDG	MGDG (26:2)	665.4235	-0.48	MS1		
28	792.5540	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>79</sub> NO <sub>8</sub> P	PE	PE (40:6)	792.5538	-0.29	M-C <sub>2</sub> H <sub>7</sub> NO <sub>3</sub> P-H <sub>2</sub> O (651.5296)	30	679.4389	[M + Na] <sup>+</sup>	C <sub>36</sub> H <sub>64</sub> O <sub>10</sub> Na	MGDG	MGDG (27:2)	679.4392	-0.40	MS1		

Table 1 (Contd.)

Mouse brain MS+										Mouse liver MS+									
Precursor ID	<i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1	Identified fragments from AutoMS/MS or MS1	Precursor ID	<i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1	Identified fragments from AutoMS/MS or MS1
29	828.4945	[M + K] <sup>+</sup>	C <sub>45</sub> H <sub>76</sub> NO <sub>8</sub> PK	PE	PE (40:7)	828.494	-0.60	MS1*	MS1	31	693.4546	[M + Na] <sup>+</sup>	C <sub>37</sub> H <sub>66</sub> O <sub>10</sub> Na	MGDG	MGDG (28:2)	693.4548	-0.26	MS1	MS1
30	724.5276	[M + H] <sup>+</sup>	C <sub>41</sub> H <sub>75</sub> NO <sub>7</sub> P	PE	PE (O-36:5) or PE (P-36:4)	724.5276	0.01	MS1	MS1	32	707.4703	[M + Na] <sup>+</sup>	C <sub>38</sub> H <sub>68</sub> O <sub>10</sub> Na	MGDG	MGDG (29:2)	707.4705	-0.30	MS1	MS1
31	752.5589	[M + H] <sup>+</sup>	C <sub>43</sub> H <sub>79</sub> NO <sub>7</sub> P	PE	PE (O-38:5) or PE (P-38:4)	752.5589	-0.04	MS1	MS1	33	721.4859	[M + Na] <sup>+</sup>	C <sub>39</sub> H <sub>70</sub> O <sub>10</sub> Na	MGDG	MGDG (30:2)	721.4861	-0.22	MS1	MS1
31	790.5150	[M + K] <sup>+</sup>	C <sub>43</sub> H <sub>78</sub> NO <sub>7</sub> PK	PE	PE (O-38:5) or PE (P-38:4)	790.5147	-0.38	MS1	MS1	34	878.5396	[M + H- H <sub>2</sub> O] <sup>+</sup>	C <sub>44</sub> H <sub>81</sub> NO <sub>14</sub> P	MIPC	MIPC (m32:2)	878.5389	0.75	MS1	MS1
32	750.5434	[M + H] <sup>+</sup>	C <sub>43</sub> H <sub>77</sub> NO <sub>7</sub> P	PE	PE (O-38:6) or PE (P-38:5)	750.5432	-0.28	MS1	MS1	35	730.5380	[M + H] <sup>+</sup>	C <sub>40</sub> H <sub>77</sub> NO <sub>8</sub> P	PC	PC (32:2)	730.5381	-0.14	MS1*	MS1*
33	780.5904	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>83</sub> NO <sub>7</sub> P	PE	PE (O-40:5) or PE (P-40:4)	780.5902	-0.31	MS1	MS1	36	760.5851	[M + H] <sup>+</sup>	C <sub>42</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (34:1)	760.5851	0.02	HG (184.0725), M-FA 18:1 (496.3364)	HG (184.0725), M-FA 18:1 (496.3364)
34	748.5275	[M + H] <sup>+</sup>	C <sub>43</sub> H <sub>75</sub> NO <sub>7</sub> P	PE	PE (P-38:6)	748.5276	0.11	MS1	MS1	37	758.5694	[M + H] <sup>+</sup>	C <sub>42</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (34:2)	758.5694	0.00	HG (184.0725)	HG (184.0725)
34	786.4836	[M + K] <sup>+</sup>	C <sub>43</sub> H <sub>74</sub> NO <sub>7</sub> PK	PE	PE (P-38:6)	786.4834	-0.29	MS1	MS1	38	786.6009	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (36:2)	786.6007	0.18	HG (184.0725)	HG (184.0725)
35	776.5591	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>79</sub> NO <sub>7</sub> P	PE	PE (P-40:6)	776.5589	-0.22	MS1	MS1	39	784.5853	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (36:3)	784.5851	0.21	HG (184.0725)	HG (184.0725)
35	814.5151	[M + K] <sup>+</sup>	C <sub>45</sub> H <sub>78</sub> NO <sub>7</sub> PK	PE	PE (P-40:6)	814.5147	-0.49	MS1	MS1	40	782.5697	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (36:4)	782.5694	0.33	HG (184.0725)	HG (184.0725)
36	876.5731	[M + K] <sup>+</sup>	C <sub>44</sub> H <sub>88</sub> NO <sub>11</sub> PK	PI-Cer	PI-Cer (d38:0)	876.5727	-0.48	MS1*	MS1*	41	780.5513	[M + H] <sup>+</sup>	C <sub>44</sub> H <sub>79</sub> NO <sub>8</sub> P	PC	PC (36:5)	780.5538	-3.18	HG (184.0725)	HG (184.0725)
37	822.5238	[M + Na] <sup>+</sup>	C <sub>43</sub> H <sub>78</sub> NO <sub>10</sub> PNa	PS	PS (37:3)	822.5256	2.25	MS1	MS1	42	810.6011	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (38:4)	810.6007	0.48	HG (184.0725), M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> P-H <sub>2</sub> O (627.5343)	HG (184.0725), M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> P-H <sub>2</sub> O (627.5343)
38	774.5234	[M + Na] <sup>+</sup>	C <sub>39</sub> H <sub>78</sub> NO <sub>10</sub> PNa	PS	PS (O-33:0) (OH)	774.5256	2.79	MS1	MS1	43	806.5698	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>81</sub> NO <sub>8</sub> P	PC	PC (38:6)	806.5694	0.49	HG (184.0725)	HG (184.0725)

Table 1 (Contd.)

Mouse brain MS+										Mouse liver MS+									
ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1 identification	ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i>	ppm	Identified fragments from AutoMS/MS or MS1 identification		
39	424.2813	[M + H] <sup>+</sup>	C <sub>20</sub> H <sub>43</sub> NO <sub>6</sub> P	S1P	S1P (t20:1)	424.2822	2.07	MS1*	44	804.5515	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>79</sub> NO <sub>8</sub> P	PC	PC (38:7)	804.5538	-2.84	HG (184.0725)		
40	731.6060	[M + H] <sup>+</sup>	C <sub>41</sub> H <sub>84</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d36:1)	731.6061	0.14	MS1*	45	834.6013	[M + H] <sup>+</sup>	C <sub>48</sub> H <sub>85</sub> NO <sub>8</sub> P	PC	PC (40:6)	834.6007	0.66	HG (184.0725), M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> P-H <sub>2</sub> O (651.5293)		
41	753.5882	[M + Na] <sup>+</sup>	C <sub>41</sub> H <sub>83</sub> N <sub>2</sub> O <sub>6</sub> PNa	SM	SM (d36:1)	753.5881	-0.17	MS1*	46	832.5857	[M + H] <sup>+</sup>	C <sub>48</sub> H <sub>83</sub> NO <sub>8</sub> P	PC	PC (40:7)	832.5851	0.72	HG (184.0725)		
42	813.6846	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>94</sub> N <sub>2</sub> O <sub>6</sub> P	PE	SM (d42:2)	813.6844	-0.23	MS1*	46	870.5418	[M + K] <sup>+</sup>	C <sub>48</sub> H <sub>82</sub> NO <sub>8</sub> PK	PC	PC (40:7)	870.5410	0.88	MS1*		
									47	828.5518	[M + H] <sup>+</sup>	C <sub>48</sub> H <sub>79</sub> NO <sub>8</sub> P	PC	PC (40:9)	828.5538	-2.37	HG (184.0724), M-C <sub>5</sub> H <sub>13</sub> NO <sub>3</sub> P-H <sub>2</sub> O (546.4805)		
									48	796.5255	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>75</sub> NO <sub>7</sub> P	PC	PC (O-39:11)	796.5276	-2.60	HG (184.0725)		
									49	824.5571	[M + H] <sup>+</sup>	C <sub>49</sub> H <sub>79</sub> NO <sub>7</sub> P	PC	PC (O-41:11)	824.5589	-2.20	HG (184.0725)		
									50	650.4386	[M + H] <sup>+</sup>	C <sub>33</sub> H <sub>65</sub> NO <sub>9</sub> P	PE	PE (28:1)	650.4391	-0.75	MS1*		
									50	672.4207	[M + Na] <sup>+</sup>	C <sub>33</sub> H <sub>64</sub> NO <sub>9</sub> PNa	PE	PE (28:1)	672.4211	-0.64	MS1*		
									51	676.4544	[M + H] <sup>+</sup>	C <sub>35</sub> H <sub>67</sub> NO <sub>9</sub> P	PE	PE (30:2)	676.4548	-0.55	MS1*		
									52	790.5391	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>77</sub> NO <sub>8</sub> P	PE	PE (OH))	790.5381	1.21	MS1*		
									53	789.4676	[M + Na] <sup>+</sup>	C <sub>42</sub> H <sub>71</sub> O <sub>10</sub> PNa	PG	PG (40:7)	789.4677	-0.13	MS1*		
									54	860.5057	[M + K] <sup>+</sup>	C <sub>42</sub> H <sub>80</sub> NO <sub>12</sub> PK	PI-Cer	PI-Cer (36:6)	860.5050	0.84	MS1*		
									55	858.5861	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>85</sub> NO <sub>11</sub> P	PS	PS (40:3)	858.5855	0.72	MS1		
									56	854.5549	[M + H] <sup>+</sup>	C <sub>46</sub> H <sub>81</sub> NO <sub>11</sub> P	PS	PS (OH))	854.5542	0.77	MS1		

Table 1 (Contd.)

Mouse liver MS <sup>+</sup>															
ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i> ppm	Identified fragments from AutoMS/MS or MS1 identification	ID	Precursor <i>m/z</i>	Species	Chemical composition	Main class	Short name	Theo. <i>m/z</i> ppm	Identified fragments from AutoMS/MS or MS1 identification
57	718.4624	[M + Na] <sup>+</sup>	C <sub>35</sub> H <sub>70</sub> NO <sub>10</sub> PNa	PS	PS (O-29:0 (OH))	-0.82	MS1	57	718.4630	[M + Na] <sup>+</sup>	C <sub>35</sub> H <sub>70</sub> NO <sub>10</sub> PNa	PS	PS (O-29:0 (OH))	-0.82	MS1
58	424.2813	[M + H] <sup>+</sup>	C <sub>20</sub> H <sub>43</sub> NO <sub>6</sub> P	S1P	S1P (t20:1)	-2.05	MS1*	58	424.2813	[M + H] <sup>+</sup>	C <sub>20</sub> H <sub>43</sub> NO <sub>6</sub> P	S1P	S1P (t20:1)	-2.05	MS1*
59	703.5745	[M + H] <sup>+</sup>	C <sub>39</sub> H <sub>80</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d34:1)	-0.38	MS1*	59	703.5745	[M + H] <sup>+</sup>	C <sub>39</sub> H <sub>80</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d34:1)	-0.38	MS1*
60	787.6690	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>92</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d40:1)	0.32	MS1*	60	787.6690	[M + H] <sup>+</sup>	C <sub>45</sub> H <sub>92</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d40:1)	0.32	MS1*
61	815.7005	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>96</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d42:1)	0.56	MS1*	61	815.7005	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>96</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d42:1)	0.56	MS1*
62	813.6848	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>94</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d42:2)	0.50	MS1*	62	813.6848	[M + H] <sup>+</sup>	C <sub>47</sub> H <sub>94</sub> N <sub>2</sub> O <sub>6</sub> P	SM	SM (d42:2)	0.50	MS1*

electrospray device (Advion, Ithaca, NY) coupled to a 7T Solarix XR FT-ICR MS (Bruker Daltonics, Germany). The solvent was EtOH/H<sub>2</sub>O/HCOOH 80/19.9/0.1 (v/v/v). It allowed to extract similar lipid classes as with other solvent mixtures.<sup>46–49</sup> A total of 6 μL were aspirated from the solvent well. The robotic arm relocated to a position above the tissue and descended to a height 0.2 mm above the surface of the sample. A total of 3 μL of the solution was dispensed onto the sample surface to form a liquid microjunction. The liquid microjunction was maintained for 5 seconds; then 3.5 μL were reaspirated into the pipet tip. This liquid dispensing and reaspiration was repeated twice before MS injection.

The FT-ICR MS instrument was operated in both negative and positive ionization mode and data were collected for 15 minutes. Data dependent acquisition of MS/MS spectra was performed using the AutoMS/MS function and spectra were recorded with 500 kW. CID was utilized as a fragmentation tool (typically 15–35 eV), with nominal mass quadrupole isolation prior to injection into the CID cell. Spectra were externally calibrated using a Tuning Mix solution (Agilent, SC)<sup>50</sup> and internally calibrated using single point correction with identified lipids. For example, the internal recalibration was performed using PC(34:1) (*m/z* = 760.5851) for MB and using PC(34:2) (*m/z* = 758.5694) for ML in positive mode and PC(34:1) (*m/z* = 804.5760) for MB and using PC(34:2) (*m/z* = 802.5604) for ML in negative mode. Data was analyzed using Data Analysis 5.2 (Bruker Daltonics, Germany) and SimLipid software (Premier Biosoft, US). Assignments were manually curated using Alex123 (ref. 51) and the LIPID MAPS Lipidomics Gateway.<sup>52,53</sup> MS1 exact mass identifications were performed using the LIPID MAPS Lipidomics Gateway<sup>52,53</sup> with a ± 3 ppm mass error search criterion. During lipid candidate assignments, protonated species (with and without the loss of H<sub>2</sub>O according to the lipid class), sodium and potassium cation adduct species were considered for positive mode; deprotonated species, chloride and formate anion adduct species were considered for negative mode analysis. Lipids with odd sum compositions were discarded as biologically unlikely when multiple identification possibilities were found, but the assignments were kept if they constituted unique identifications within the ± 3 ppm mass error search. MS1 exact mass measurements were recorded with 4 MW and 2 MW for positive and negative modes, respectively. The mass resolution was around 170 000 at *m/z* 760.5851 and 758.5694 for positive mode MB and ML, respectively and around 60 000 at *m/z* 804.5760 and 802.5604 for negative mode MB and ML, respectively. For data completeness, targeted MS/MS after preliminary MS1 lipid assignment was performed on species where only little interfering *m/z* peaks were found in the spectra, using an Impact Q-ToF instrument (Bruker Daltonics Inc., Billerica, MA).

## Results and discussion

The fast lipid screening workflow is based on LESA of thin tissue sections (without any other surface treatment) followed by ultra-high-resolution MS/MS analysis (see Fig. 1). The full-scan MS1 analyses and DDA MS/MS take advantage of the ultra-high mass resolution and high mass accuracy of the FT-



ICR mass spectrometers. For example, during DDA using CID as a fragmentation method, typical neutral losses and lipid headgroups were utilized during candidate assignment with high mass accuracy. While not the focus of this paper, it should be noted that other MS/MS fragmentation techniques (*e.g.*, EID, OzID, CTD)<sup>9–14</sup> may be easily implemented and provide better and/or complementary structural information during lipid candidate assignment. In the proposed workflow, an initial search provides candidate lipids from the DDA dataset. Following DDA interpretation, the MS1 spectrum is processed (*i.e.*, internal single point correction) and a list of monoisotopic  $m/z$  signals is created. This list is used to search among lipid databases using mass accuracy as a criterion. In many cases, the accurate mass database search will return multiple lipid hits, which will require secondary analysis (*e.g.*, targeted MS/MS experiments). While not currently implemented, online processing of the MS1 scan using accurate mass lipid database searches can be performed to retrofit the DDA acquisition target list; this procedure can be easily implemented during static nESI since no major changes in the spray occur during 15 minutes, and each MS/MS acquisition requires typically 10–20 seconds.

The LESA-FT-ICR-MS (MS1 and MS/MS) analysis of two biological substrates (*i.e.*, mouse brain, MB, and mouse liver, ML) resulted in the unique identification (within a  $\pm 3$  ppm database search for MS1) of distinct lipids from 38 different lipid classes in the 400–1000  $m/z$  range. The unsupervised analysis resulted in the identification of  $\sim 190$  (MB) and  $\sim 590$  (ML) monoisotopic  $m/z$  peaks as lipids. Despite the biological complexity, 10–30% of these lipid identifications yielded unique lipid assignments (in contrast to multiple lipid assignments to one  $m/z$  peak; see Fig. 2). The comparison of the MB and ML MS1 profiles (either positive or negative ionization mode) shows abundant lipid signal in the 700–900  $m/z$  range. Overall, a larger number of monoisotopic  $m/z$  peaks were observed and picked in the ML sample when compared to the MB sample (*e.g.*, 226(MS+)/2215(MS–) for ML and 157(MS+)/174(MS–) for MB (see ESI Fig. SI1† for an extract of the negative mode spectra)). Fig. 3 highlights the importance of performing these analyses using ultra-high-resolution mass spectrometers such as FT-ICR. Between  $m/z$

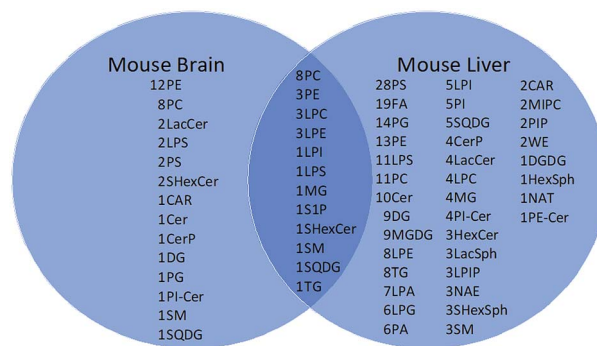


Fig. 5 Diagram of the lipid compositions (lipid classes) of healthy mouse brain and mouse liver samples identified from LESA-FT-ICR-MS (MS/MS and MS1) measurements, including both positive and negative ionization modes. The circle overlap represents the number of distinct lipids from the different lipid classes which were found in both tissues.

738.2 and 738.8, 5 out of the 6  $m/z$  values were correlated to lipid identifications.

In positive mode, the most intense  $m/z$  peaks with unique lipid identifications correspond to phosphatidylcholines (PC) in the MB and ML samples, with minor lipid signals corresponding to PE, LPC, DG, MGDG, SM, PS, CAR, Cer, HexCer, LPS, LacCer, LPIP, MG, PI-Cer, S1P, DGDG, LPA, MIPC and PG (see Table 1 and Fig. 4a and c; all abbreviations are described in the ESI†). For the case of PC, the AutoMS/MS identification (without fatty acid chain or double bond identification) relied mostly on the detection of the headgroup and the neutral loss of a fatty acid chain; other lipids assignments were mostly based on MS1 accurate mass. Tables SI1 and SI2 in the ESI† summarize all MS1  $m/z$  signals with multiple lipid identifications within the  $\pm 3$  ppm database search. It should be noted that multiple adducts were observed for the most abundant lipids, increasing the confidence during their identifications. All uniquely-identified lipids yielded sub-ppm average  $m/z$  deviations (*e.g.*, 0.70 ppm for MB and  $-0.85$  ppm for ML).

In negative mode, the lipid classes with the most unique identifications correspond to phosphatidylethanolamines (PE), LPE, PC and SHexCer in MB, and to phosphatidylserines (PS), fatty acyls (FA), glycerophosphoglycerols (PG) and LPS in ML

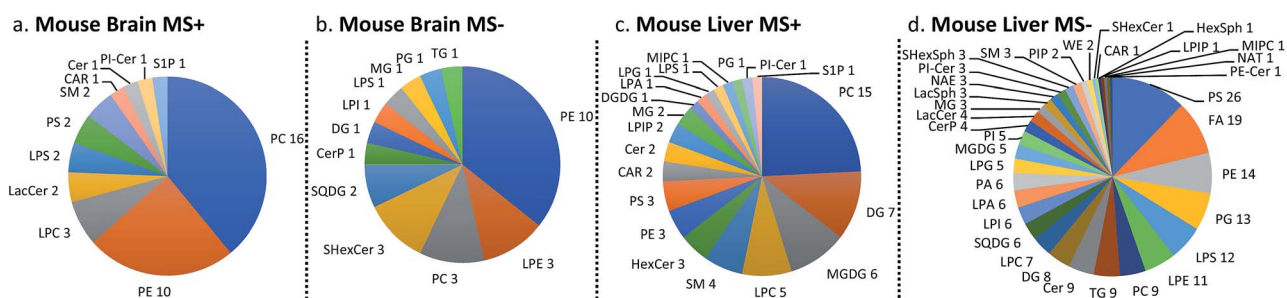


Fig. 4 Representations of the identified lipid classes in MB and ML (from both MS/MS and MS1), weighted by the number of unique and distinct lipid identifications for each class. (a and c) represent positive ionization for MB and ML, respectively, and (b and d) represent negative ionization for both samples.

**Table 2** Negative ion mode targeted MS/MS after preliminary MS1 accurate mass database search from the MB. The different lipid identification possibilities are shown, with the MS/MS fragment ion interpretations which refine the  $m/z$  identification

Mouse Brain MS- with multiple MS1 identifications								
Precursor $m/z$	Species	Chemical composition	Main class	Short name	Theo. $m/z$	ppm	Identified fragments from MS/MS	MS/MS refined identification
762.5079	[M - H] <sup>-</sup>	C <sub>43</sub> H <sub>73</sub> NO <sub>8</sub> P	PE	PE(38:6)	762.5079	0.05	FA 16:0 (+COO) (255.2306), FA 22:6 (-CO) (283.2423), FA 22:6 (+COO) (327.2305), M-FA 22:6 (-H) (452.2765)	PE(22:6_16:0)
766.5393	[M - H] <sup>-</sup>	C <sub>43</sub> H <sub>73</sub> NO <sub>8</sub> P	PE	PE(P-38:6(OH))	762.5079	0.05		
	[M - H] <sup>-</sup>	C <sub>43</sub> H <sub>77</sub> NO <sub>8</sub> P	PE	PE(38:4)	766.5392	0.14	FA 18:0 (+COO) (283.2615), FA 20:4 (+COO) (303.2312), M-FA 20:4 (+COO) (480.3029)	PE(20:4_18:0)
786.5276	[M - H] <sup>-</sup>	C <sub>43</sub> H <sub>77</sub> NO <sub>8</sub> P	PE	PE(O-38:5(OH)) or PE(P-38:4(OH))	766.5392	0.14		
	[M + HCOO] <sup>-</sup>	C <sub>42</sub> H <sub>77</sub> NO <sub>10</sub> P	PC	PC(33:3)	786.5291	-1.91		
	[M + HCOO] <sup>-</sup>	C <sub>42</sub> H <sub>77</sub> NO <sub>10</sub> P	PE	PE(36:3)	786.5291	-1.91		
	[M + HCOO] <sup>-</sup>	C <sub>42</sub> H <sub>77</sub> NO <sub>10</sub> P	PE	PE(O-36:4(OH)) or PE(P-36:3(OH))	786.5291	-1.91		
834.52904	[M - H] <sup>-</sup>	C <sub>42</sub> H <sub>77</sub> NO <sub>10</sub> P	PS	PS(36:2)	786.5291	-1.91	FA 18:1 (+COO) (281.2468), M-FA 18:1 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (417.2372), M-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (699.4904)	PS(18:1_18:1)
	[M - H] <sup>-</sup>	C <sub>42</sub> H <sub>77</sub> NO <sub>10</sub> P	PS	PS(O-36:3(OH)) or PS(P-36:2(OH))	786.5291	-1.91	FA 18:1 (+COO) (281.2468), M-FA 18:1 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (417.2372), M-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (699.4904)	PS(O-18:1_18:1(OH)) or PS(18:0_18:1(OH))
834.52904	[M + HCOO] <sup>-</sup>	C <sub>46</sub> H <sub>77</sub> NO <sub>10</sub> P	PC	PC(37:7)	834.5291	-0.07		
	[M + HCOO] <sup>-</sup>	C <sub>46</sub> H <sub>77</sub> NO <sub>10</sub> P	PE	PE(40:7)	834.5291	-0.07		
	[M - H] <sup>-</sup>	C <sub>46</sub> H <sub>77</sub> NO <sub>10</sub> P	PS	PS(40:6)	834.5291	-0.07	FA 22:6 (-CO) or FA 18:0 (+O) (283.2643), FA 22:6 (+O) (327.2323), FA 22:6 (+OH) (419.2576), M-FA 20:1 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> or FA 22:6 (+O) (303.2339), M-FA 22:6 (-H)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (437.2675), M-FA 18:0 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> or FA 20:5-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (463.2265), M-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (747.4984)	PS(22:6_18:0) or PS(20:1_20:5)
	[M - H] <sup>-</sup>	C <sub>46</sub> H <sub>77</sub> NO <sub>10</sub> P	PS	PS(P-40:6(OH))	834.5291	-0.07	FA 18:1 (+O) (281.2489), FA 22:6 (-CO) or FA 18:0 (+O) (283.2643), FA 20:4 (+O) or FA 23:3 (-CO) (303.2339), FA 22:6 (+O) or FA 25:5 (-CO) (327.2323), FA 23:4 (-H)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> or FA 22:6 (+OH) (419.2576), M-FA 20:1 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> or FA 22:6 (-H)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (437.2675), M-FA 18:0 (+HO)-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> or FA 20:5-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (463.2265), M-C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub> (747.4984)	PS(P-23:4_17:2(OH)) or PS(23:3_17:3(OH)) or PS(P-22:6_18:0(OH)) or PS(P-20:4_20:2(OH)) or PS(P-20:1_20:5(OH)) or PS(P-18:1_22:5(OH))

(see Fig. 4b and d, Tables SI3 and SI4†). Most of the AutoMS/MS assignments were based on the observation of the fatty acid losses and fragments; other lipids assignments were mostly based on MS1 accurate mass (see ESI Tables SI3 and SI4†). Tables SI5 and SI6 in the ESI† summarize all MS1  $m/z$  signals with multiple lipid identifications within the  $\pm 3$  ppm database search. For example, 64 out of the 174 picked  $m/z$  values for MB and 437 out of the 2215 values for ML were identified with unique or multiple matches. Further dataset descriptions can be found in Fig. SI2† for both MB and ML in positive and negative ionization. All uniquely-identified lipids yielded sub-ppm average  $m/z$  deviations (e.g., 0.27 ppm for MB and 0.02 ppm for ML).

An estimate of the specificity of the LESA-FT-ICR-MS workflow as a function of the biological surface was obtained from the comparison of the unique lipid assignments in the MB and ML (see Fig. 5, including both positive and negative mode MS1 and MS/MS identifications). 25 lipids were found common to the MB and ML, with the most abundant being 8 PC, 3 PE, 3 LPE, and 3 LPC. One lipid from the LPI, LPS, MG, S1P, SHexCer, SM, SQDG, and TG classes were found to be common. In the case of MB, 20 different lipid classes were identified (ranked according to the number of identified lipids: PC, PE, LPS, LPE, LPC, SHexCer, PS, SQDG, LacCer, SM, PG, Cer, DG, TG, LPI, CerP, PI-Cer, MG, CAR, and S1P). The most abundant lipid class for MB was PC (16 lipids), followed by PE (15 lipids) (see Table SI7†). In the case of ML, 38 different lipid classes were identified (ranked according to the number of identified lipids: PS, FA, PC, PE, PG, LPS, LPE, Cer, DG, TG, MGDG, LPC, LPA, SQDG, LPI, LPG, PA, MG, PI, LacCer, SM, CerP, PI-Cer, HexCer, LacSph, LPIP, NAE, SHexSph, CAR, MIPC, PIP, WE, SHexCer, S1P, DGDG, HexSph, NAT, and PE-Cer). The most abundant lipid class for ML was PS (28 lipids), followed by FA and PC (19 lipids each), and PE (16 lipids) (see Table SI5†).

An example of the use of targeted MS/MS following the MS1 accurate mass search is shown for the case of MB in negative ion mode (see Table 2). The added fragment ion information enables the exclusion of accurate mass identifications as well as to increase the structural information. For example, the identification of PE (22:6\_16:0) ( $m/z = 762.5079$ ) and PE (20:4\_18:0) ( $m/z = 766.5393$ ) is illustrated in Table 2.

## Conclusions

A fast and high-throughput analysis workflow for lipid screening in biological tissues at ambient conditions without the need for pre-separations or sample treatment is shown. The LESA-FT-ICR MS(/MS) analysis of mouse brain and liver sections resulted in the identification of 38 lipid classes in a single analysis (<15 min), with lipid markers specific to each tissue. The combination of accurate mass and AutoMS/MS resulted in the identification of unique and common lipid molecules from the biological tissues, with average sub-ppm mass accuracy. The workflow was presented using CID as a proof of concept, but other fragmentation techniques providing further structural lipid information are equally

suitable. The most abundant lipid species are typically observed and identified in several adduct forms (e.g., protonated, sodiated and potassiated), thus increasing the confidence in the molecular assignment. In the examples shown,  $\sim 190$  (MB) and  $\sim 590$  (ML)  $m/z$  values were identified by unique or multiple lipid assignments in positive and negative mode, with 10–30% of these identifications being unique and distinct lipids assignments. In addition to MS analysis, further integration of post-ionization ion mobility separation can provide additional structural information.<sup>15–19</sup>

## Conflicts of interest

There are no conflicts to declare.

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