

Supplemental Material

Characteristic lipid profiles of canine non-Hodgkin's lymphoma from surgical biopsy tissue sections and fine needle aspirate smears by desorption electrospray ionization – mass spectrometry

Alan K. Jarmusch,^{a,†} Kevin S. Kerian,^{a,†} Valentina Pirro,^a Tyler Peat,^b Craig A. Thompson,^b José A. Ramos-Vara,^b Michael O. Childress,^c and R. Graham Cooks^a

^a Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University, West Lafayette, IN 47907.

^b Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, IN 47907

^c Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN 47907

† Authors contributed equally to this work.

Methods

Metastatic Carcinoma Specimen

Surgical biopsy and FNA samples were also collected from a single pet dog, specimen 12, with metastatic carcinoma to a regional lymph node; this lymph node had been removed as part of standard surgical cancer staging at the Purdue University Veterinary Teaching Hospital (PUVTH). The primary tumor was not available for inclusion in this study. These samples were evaluated using DESI-MS, but the results excluded from subsequent statistical analysis.

Data Analysis

Multivariate exploration of chemical and spatial information

Raw data (.raw) was converted into .txt files and subsequently converted from .txt to .csv using in-house programs for datacube assembly in Matlab. PCA was performed on selected DESI-MS datacubes, each spatial pixel (and corresponding mass spectrum) considered independently. An interactive brushing procedure was performed, connecting object grouping in PCA space for their similarity in chemical information to their spatial location (*i.e.* pixels).²⁶ Objects are manually selected from the score plot and visualized in a 2D image - pixels associated with the selected region are color coded. This allowed heterogeneous samples to be manually investigated, correlating histopathology and chemical composition.

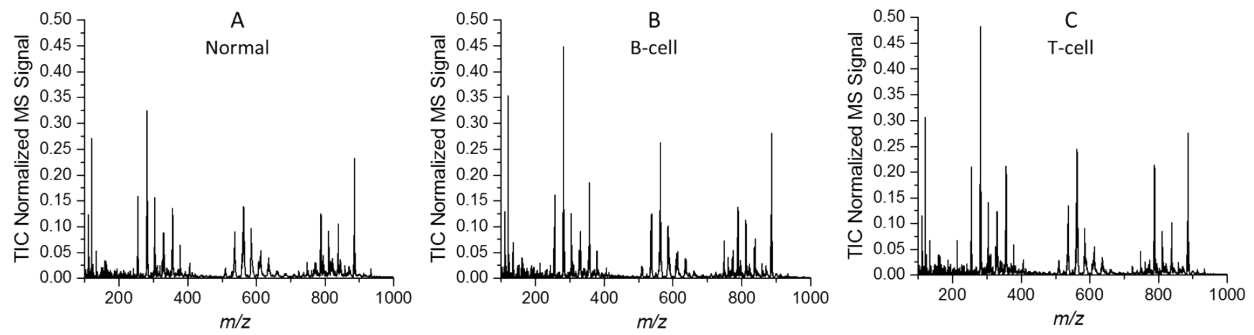
Supplemental Table 1. Tabulated specimen information

Specimen #	Histopathology Diagnosis (Formalin-fixed)	Sample Included in Statistics		Comments
		Surgical Biopsy	FNA	
1	T-zone lymphoma	x		
2	Diffuse large B-cell lymphoma	x	x	
3	Diffuse large B-cell lymphoma	x	x	
4	Peripheral T-cell lymphoma	x	x	
5	T-zone lymphoma	x	x	
6	B-cell lymphoma, tonsil	x	x	
7	Peripheral T-cell lymphoma	x		
8	Diffuse large B-cell lymphoma	x	x	5 FNA ROIs
9	Diffuse large B-cell lymphoma	x	x	
10	Diffuse large B-cell lymphoma	x	x	
11	Diffuse large B-cell lymphoma	x	x	
12	Metastatic carcinoma			Excluded
13	Diffuse large B-cell lymphoma	x	x	4 FNA ROIs
14	T-zone lymphoma	x		
15	Diffuse large B-cell lymphoma	x	x	
16	Diffuse large B-cell lymphoma	x	x	
17	Diffuse large B-cell lymphoma	x	x	
18	Diffuse large B-cell lymphoma	x	x	
19	Diffuse large B-cell lymphoma	x	x	4 FNA ROIs
20	Diffuse B-cell lymphoma	x		
21	Diffuse large B-cell lymphoma	x		
22	Diffuse large B-cell lymphoma	x	x	
23	Diffuse large B-cell lymphoma			Excluded (MS signal)
24	Diffuse large B-cell lymphoma	x		
25	Peripheral T-cell lymphoma	x	x	
26	Diffuse large B-cell lymphoma	x	x	
27	Peripheral T-cell lymphoma	x	x	
28	Diffuse large B-cell lymphoma	x		
29	Diffuse large B-cell lymphoma	x	x	
30	Diffuse large B-cell lymphoma	x	x	
31	Diffuse large B-cell lymphoma	x		
32	Normal	x		
33	Normal	x		
34	Normal	x		
35	Normal	x	x	2 FNA ROIs
36	Normal	x		
37	Normal	x		
38	Normal	x	x	
39	Normal	x		
40	Normal	x		
41	Normal	x		
42	Normal	x	x	

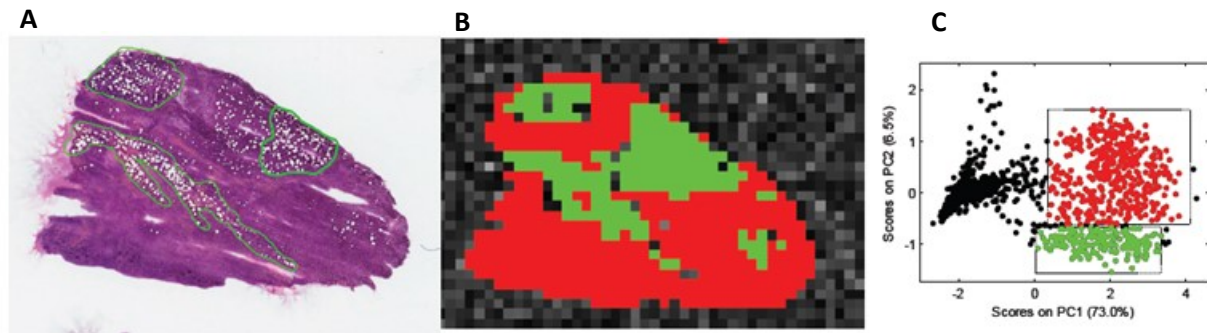
43	Normal	x	
44	Normal	x	
45	Normal	x	
46	Normal	x	
47	Normal	x	
48	Normal	x	x
49	Normal	x	x
50	Normal	x	
51	Normal	x	x
52	Normal	x	
53	Normal	x	

Supplemental Table 2. Tabulated negative ion mode DESI high resolution mass spectrometry data for predominate ions observed in the negative mode with identifications

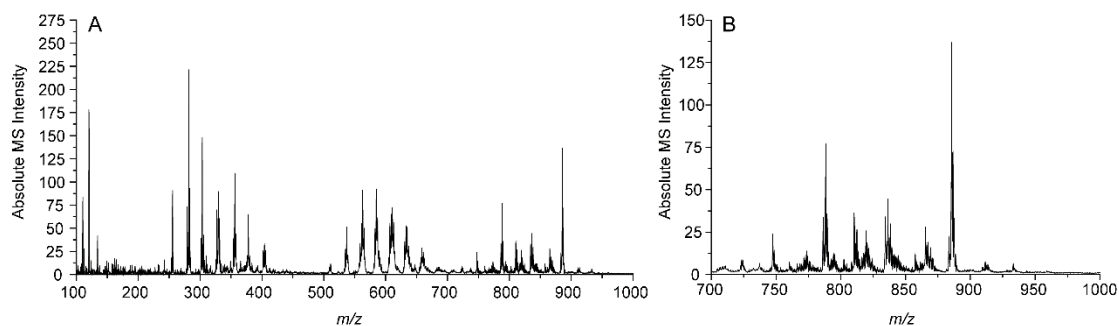
Name	Ion formula	Theoretical mass	Normal		B-cell lymphoma		T-cell lymphoma	
			Measured mass	Mass error (ppm)	Measured mass	Mass error (ppm)	Measured mass	Mass error (ppm)
Palmitic acid	[C ₁₆ H ₃₁ O ₂ -H] ⁻	255.2324	255.2324	0.0	255.2323	-0.4	255.2327	1.2
Oleic acid	[C ₁₈ H ₃₂ O ₂ -H] ⁻	281.248	281.2481	0.4	281.248	0.0	281.2483	1.1
Stearic acid	[C ₁₈ H ₃₅ O ₂ -H] ⁻	283.2637	283.2637	0.0	283.2636	-0.4	283.264	1.1
Arachidonic acid	[C ₂₀ H ₃₁ O ₂ -H] ⁻	303.2324	303.2323	-0.3	303.2323	-0.3	303.2327	1.0
PG(34:1)	[C ₄₀ H ₇₆ O ₁₀ P-H] ⁻	747.5176	747.5173	-0.4	747.5171	-0.7	747.5184	1.1
PS(34:1)	[C ₄₀ H ₇₅ NO ₁₀ P-H] ⁻	760.5128	760.5128	0.0	760.5122	-0.8	760.5136	1.1
PE(38:4)	[C ₄₃ H ₇₇ NO ₈ P-H] ⁻	766.5386	766.5386	0.0	766.5383	-0.4	766.5393	0.9
PG(36:2)	[C ₄₂ H ₇₈ O ₁₀ P-H] ⁻	773.5332	773.5334	0.3	773.533	-0.3	773.5342	1.3
PS(36:1)	[C ₄₂ H ₇₉ NO ₁₀ P-H] ⁻	788.5441	788.5442	0.1	788.5439	-0.3	788.5450	1.1
PC(34:1)+Cl ⁻	[C ₄₂ H ₈₂ NO ₈ P+Cl] ⁻	794.5466	794.5465	-0.1	794.5464	-0.3	794.5471	0.6
PS(38:4)	[C ₄₄ H ₇₇ NO ₁₀ P-H] ⁻	810.5284	810.5285	0.1	810.5282	-0.2	810.5293	1.1
PS(40:4)	[C ₄₆ H ₈₁ NO ₁₀ P-H] ⁻	838.5598	838.5597	-0.1	838.56	0.2	838.5607	1.1
PI(38:4)	[C ₄₇ H ₈₂ O ₁₃ P-H] ⁻	885.5493	885.5491	-0.2	885.5488	-0.6	885.5500	0.8



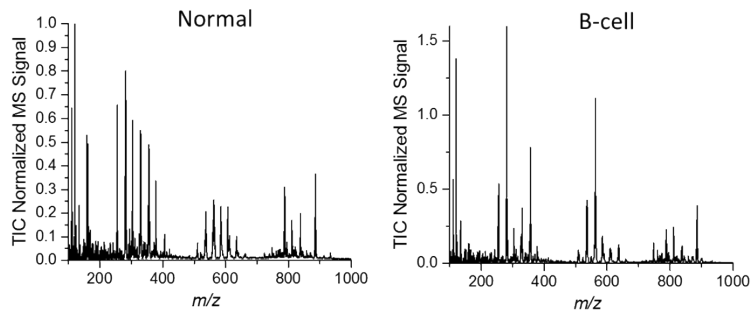
Supplemental Figure 1. TIC normalized average full scan (m/z 100-1000) negative mode DESI mass spectrum for **(A)** normal, $n=22$; **(B)** B-cell lymphoma, $n=22$; and **(C)** T-cell lymphoma, $n=7$.



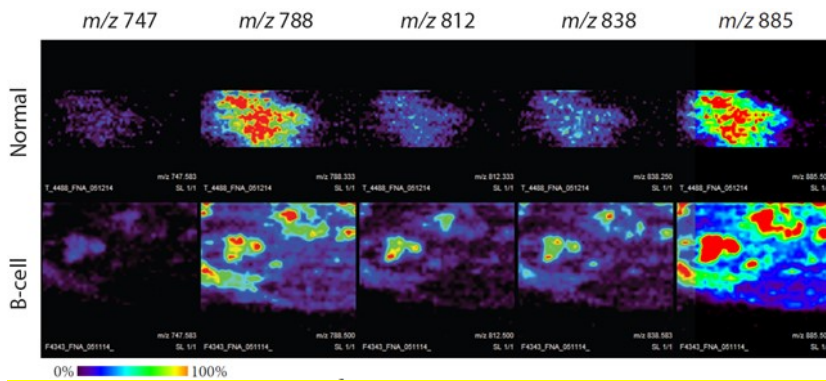
Supplemental Figure 2. **(A)** Sample from specimen 31, H&E stain with annotated non-neoplastic regions in green. **(B)** DESI-MS image, colors indicating chemical similarity. **(C)** PCA score plot of pixels with subgrouping selected.



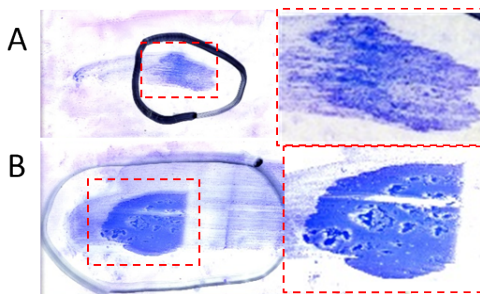
Supplemental Figure 3. **(A)** Metastatic carcinoma to lymph node, specimen 12, negative mode DESI-MS. **(B)** Zoomed region m/z 700-1000 containing GPLs.



Supplemental Figure 6. TIC normalized average full scan (m/z 100-1000) FNA mass spectra for normal samples ($n=7$) and tumor samples ($n=22$).



Supplemental Figure 7. DESI ion images of normal (top) and B-cell lymphoma (bottom) FNA smears, the latter contains areas of higher intensity corresponding to amount of material present.



Supplemental Figure 8. Wright's stained FNA smears displaying **(A)** an even distribution of material and **(B)** uneven distribution of material with corresponding zoomed insets.