

### S1: Mass spectrometer and DESI ion source parameters

Exacte mass spectrometer (Thermo Fisher Scientific Inc., Bremen, Germany)

| Parameter             | Setting                             |
|-----------------------|-------------------------------------|
| Injection time        | 1000 ms                             |
| Micro-scans           | 1                                   |
| Mass Analyser         | Fourier transform mass spectrometer |
| Mass resolution       | 100,000                             |
| Mass range            | 150-1000                            |
| Capillary temperature | 250°C                               |
| Tube lens voltage     | -150V                               |
| Skimmer Voltage       | -40V                                |

Geometric and electro-solvent parameters for home built DESI ion source

| Parameter                                          | Setting                    |
|----------------------------------------------------|----------------------------|
| Distance from sprayer tip to sample surface        | 2mm                        |
| Distance from sprayer tip to MS inlet capillary    | 14mm                       |
| Collection angle of MS inlet capillary             | 10°                        |
| Distance of MS inlet capillary from sample surface | <<1mm                      |
| Solvent constitution                               | Methanol:water (90:10 v/v) |
| Flow rate of solvent                               | 1.5µl/min                  |
| Nebulising gas flow rate                           | 7 Bar                      |
| Spray Voltage                                      | 5 kV                       |

## S2: MSI data pre-processing

The MSI data was initially subjected to pre-processing which included peak detection, filtering of solvent/noise related peaks and variance stabilizing normalization with an in-house bio-informatics platform appointed on MATLAB (MathWorks). The molecular ion peaks within a  $m/z$  range smaller than the native accuracy of the mass spectrometer ( $<4\text{ppm}$  in this case) were assigned to the same molecular ion species uniformly for all pixels on a tissue section. The noise related  $m/z$  species were then found to be randomly located at  $<1\%$  of pixels based on the distribution of molecular ion features across pixels. Additionally,  $m/z$  species were deemed to be of solvent-related origin if their mean peak intensity within tissue object pixels was less than their mean background intensity. All identified noise and solvent molecular ion peaks were removed from subsequent analysis.

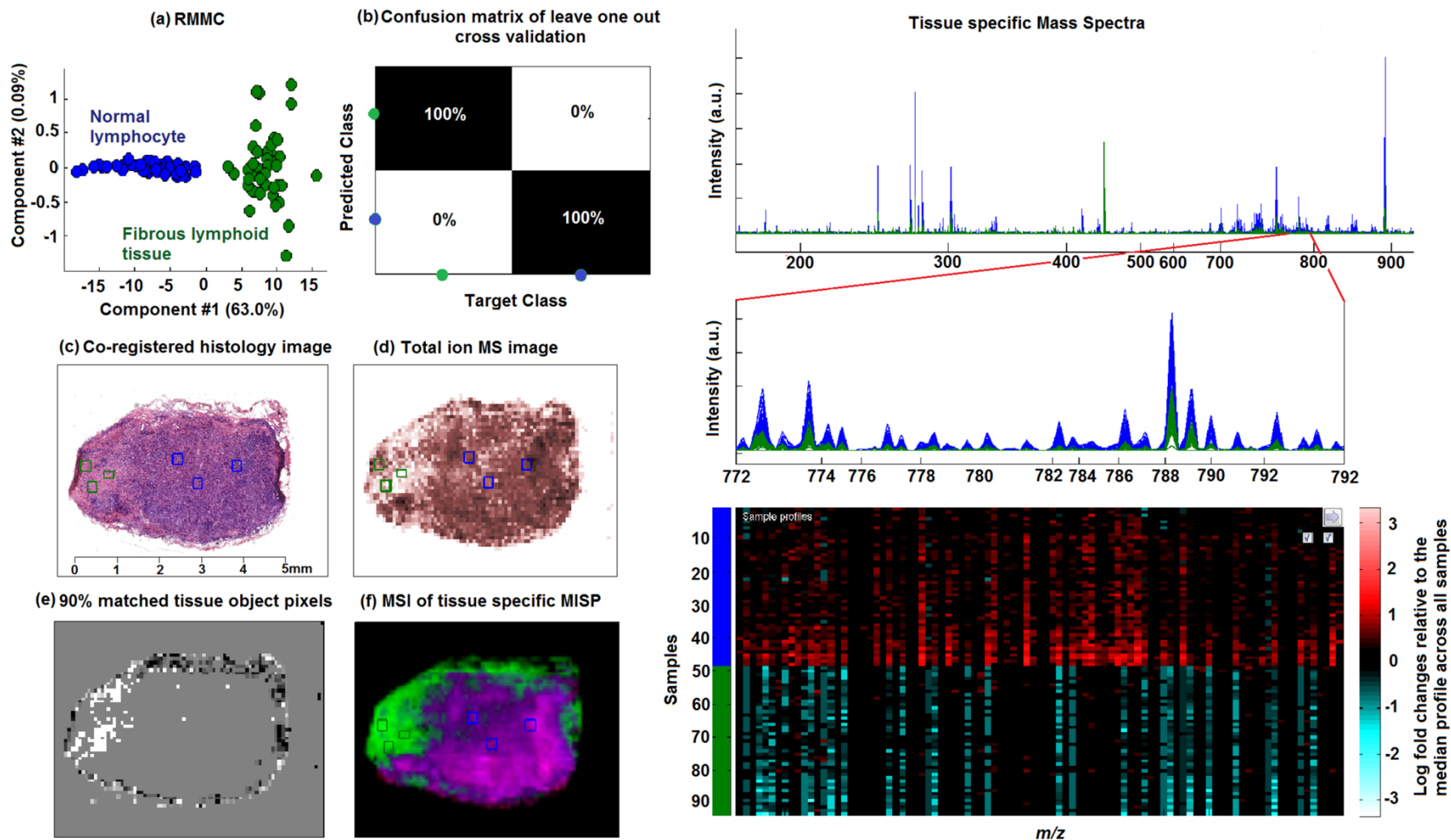
The combination of these processing steps resulted in a significant reduction of spectral data volume from  $>250,000$  to approximately  $1,000\text{-}5,000$   $m/z$  features, thus enabling more efficient data handling/mining and pixel-wise tissue classification. Log-based transformation was subsequently applied to stabilise the increased variance of peak intensity as a function of increased signal intensity ("heteroscedastic noise structure").<sup>1</sup>This ensured that the data structure is consistent with the downstream application of multivariate statistical tools.

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<sup>1</sup>K. A. Veselkov, L. K. Vingara, P. Masson, S. L. Robinette, E. Want, J. V. Li, R. H. Barton, C. Boursier-Neyret, B. Walther, T. M. Ebbels, I. Pelczer, E. Holmes, J. C. Lindon, J. K. Nicholson, *Anal. Chem.*, 2011, 83(15), 5864-72.

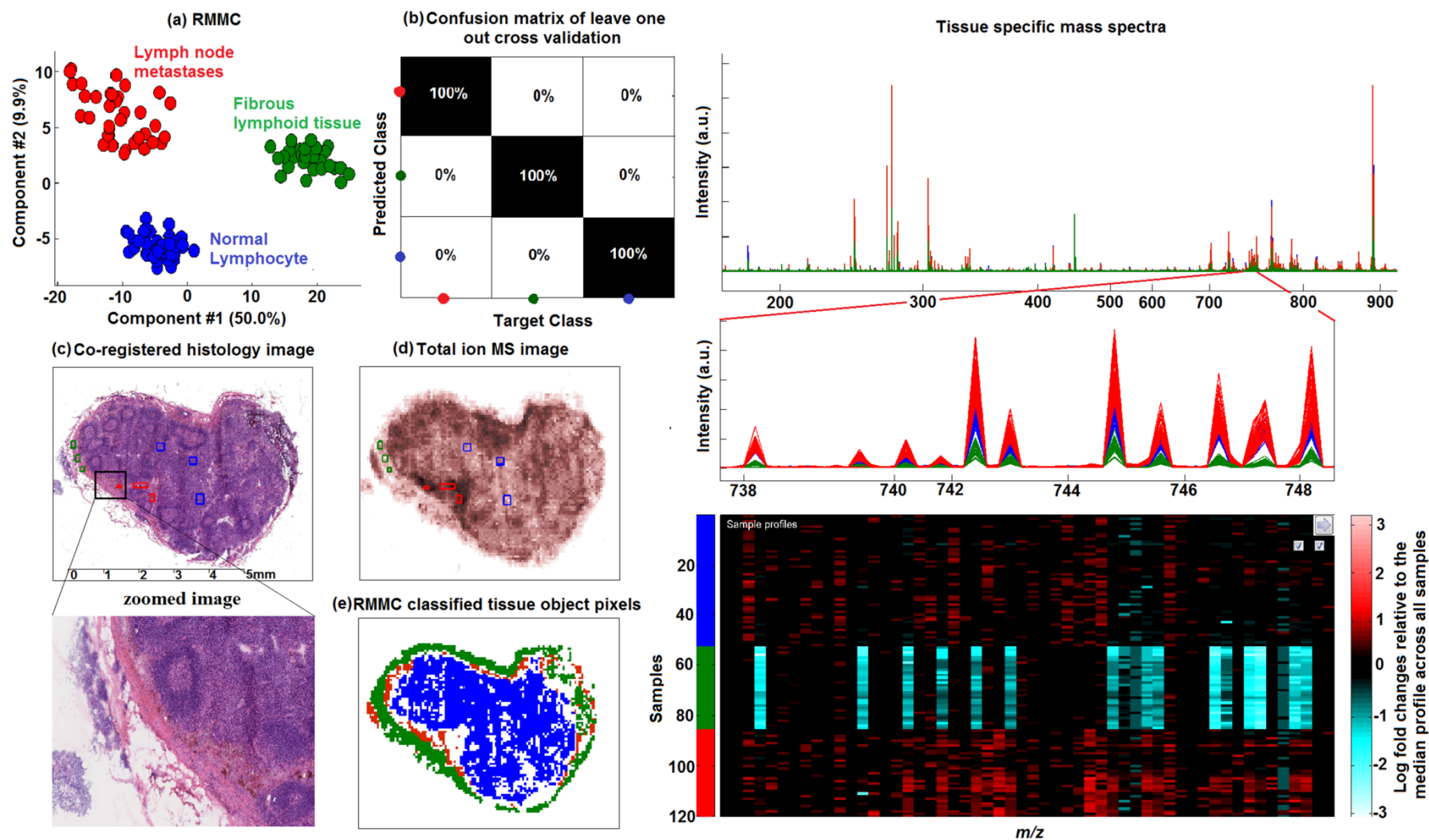
### S3: DESI-MSI data interrogation for a normal lymph node with no evidence of metastases

The blue and green regions highlighted in the histology image of the normal lymph node (Fig. c) represent normal lymphocyte and fibrous lymphocyte tissue, respectively. This colour coding theme is representative of these tissue specific regions throughout the different sections of the whole figure. Figure 1 RMMC, Recursive Maximum Margin Criterium; MS, Mass Spectrometry; MSI, Mass Spectrometry Image; MISP, Molecular Ion Spectral Pattern.

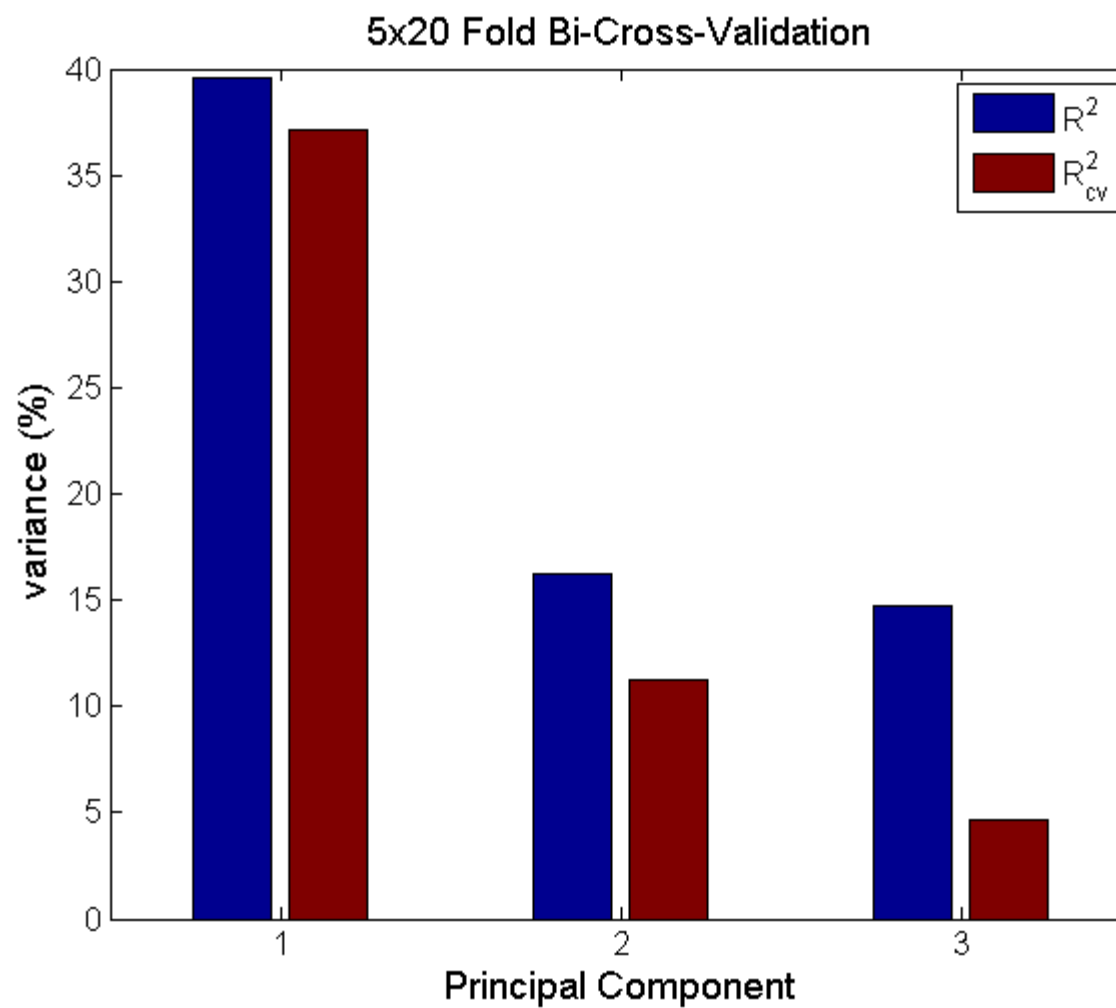


# S4: DESI-MSI data interrogation for a lymph node with evidence of micro-metastases

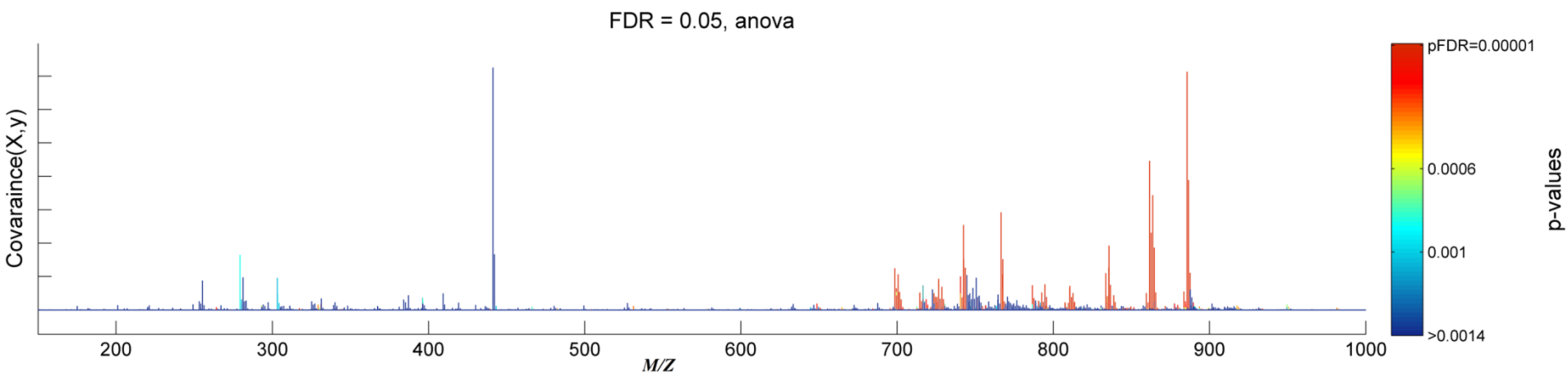
The blue, green and red regions highlighted in the histology image of the lymph node (Figure 3c), represent normal lymphocyte, fibrous lymphocyte tissue and lymph node micro-metastases, respectively. The colour coding theme is representative of these tissue specific regions throughout the different sections of the figure. The zoomed image shows the areas of micro-metastases highlighted by the brown staining of the AE1/AE1 anti-cytokeratin antibody. RMMC, Recursive Maximum Margin Criterium; MS, Mass Spectrometry; MSI, Mass Spectrometry Image; MISP, Molecular Ion Spectral Pattern.



**S5: Bi-Cross Validation of PCA in Fig. 2**

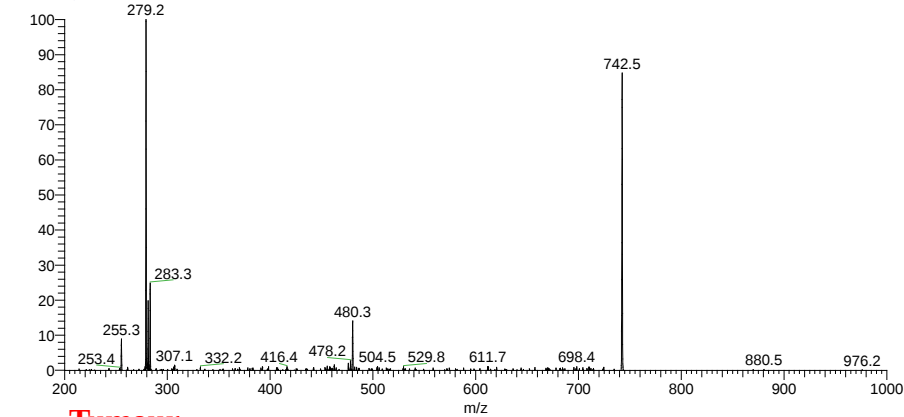


**S6: Discriminating features of comparative mass spectra of tissue specific regions ( Figure 2c, main text) as a function of ANOVA based analysis of identified peaks**



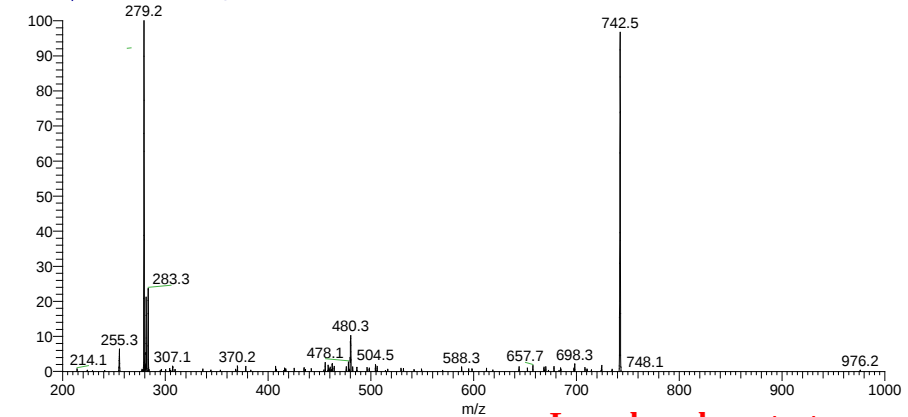
FDR, false discovery rate

MSMS\_742p54\_Tumor\_131031122422 #22-149 RT: 0.43-3.03 AV: 128 NL: 8.01E-1  
T: ITMS - p NSI Full ms2 742.54@cid25.0



Tumour

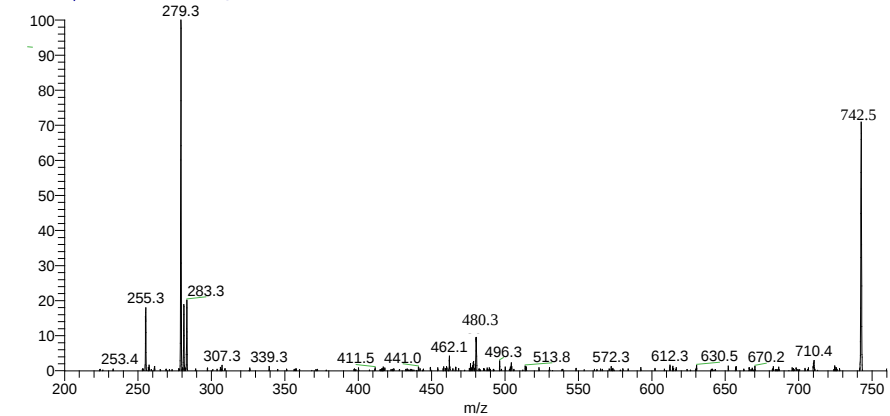
MSMS\_742p54\_LN3\_4\_131031122422 #25-75 RT: 0.49-1.52 AV: 51 NL: 9.30E-1  
T: ITMS - p NSI Full ms2 742.54@cid20.0



Lymph node metastases

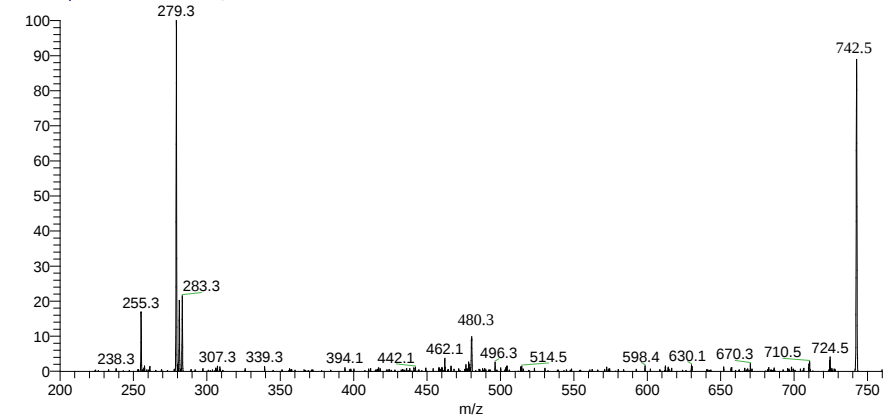
| m/z ratio | Ion Description                                        |
|-----------|--------------------------------------------------------|
| 742.54    | Precursor ion [M-H]-                                   |
| 480.31    | Loss of sn1 acyl chain as ketene (RCH=C=O) from [M-H]- |
| 279.23    | sn1 RCOO- ion                                          |
| 283.26    | sn2 RCOO- ion                                          |

MSMS\_742p54\_n1\_131031122422 #28-50 RT: 0.53-0.97 AV: 23 NL: 6.28  
T: ITMS - p NSI Full ms2 742.53@cid20.0



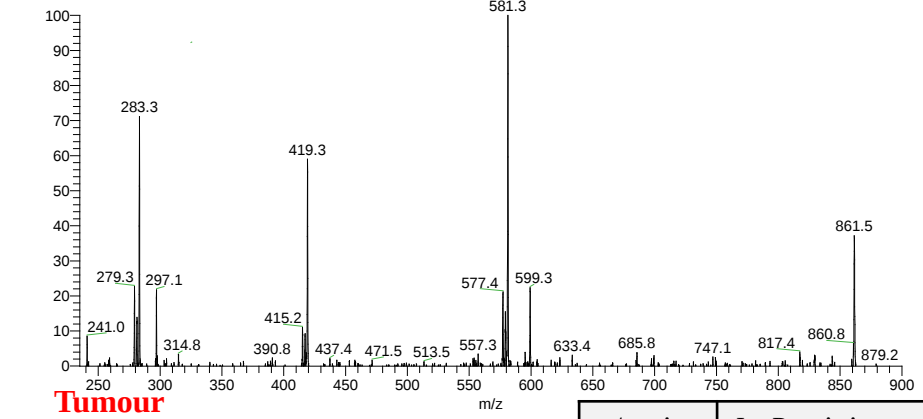
Normal lymphocytes

MSMS\_742p54\_fl\_131031122422 #29-66 RT: 0.55-1.28 AV: 38 NL: 4.10  
T: ITMS - p NSI Full ms2 742.53@cid20.0



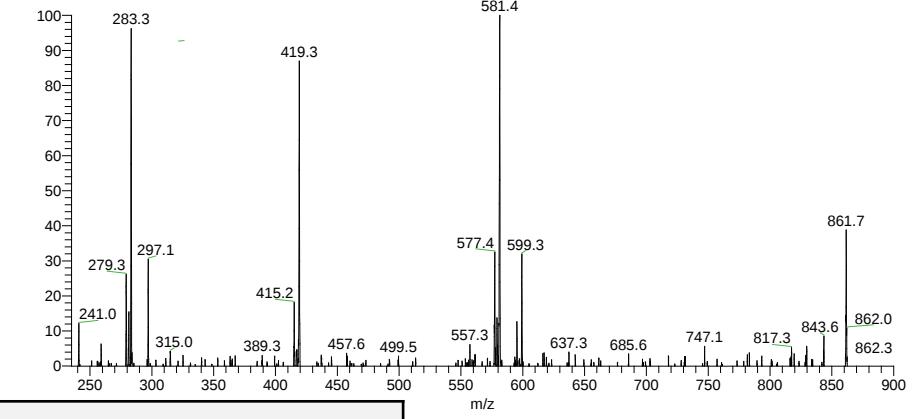
Fibrous lymphoid tissue

MSMS\_861p55\_131031122422\_#49-119 RT: 0.96-2.36 AV: 71 NL: 1.28  
T: ITMS - p NSI Full ms2 861.55@cid20.0



Tumour

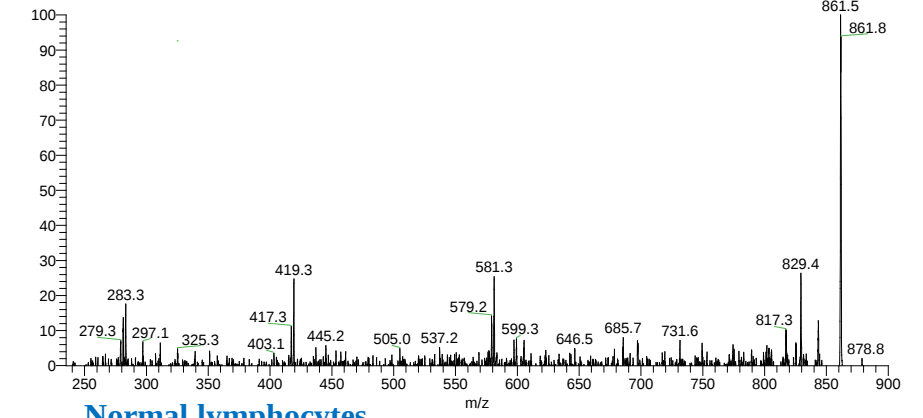
MSMS\_861p55\_LN3\_4\_131031122422\_#91-139 RT: 1.80-2.76 AV: 49 NL: 8.61E-1  
T: ITMS - p NSI Full ms2 861.55@cid20.0



Lymph node metastases

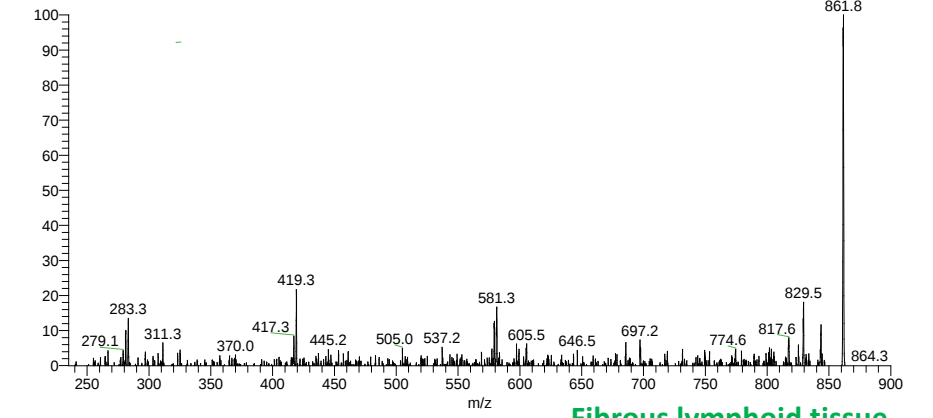
| m/z ratio | Ion Description                                          |
|-----------|----------------------------------------------------------|
| 861.55    | Precursor ion [M-H]-                                     |
| 599.32    | Loss of sn1 acyl chain as ketene (RCH=C=O) from [M-H]-   |
| 581.31    | Neutral loss of sn1 RCOOH group from [M-H]-              |
| 577.28    | Neutral loss of sn2 RCOOH group from [M-H]-              |
| 419.26    | Neutral loss of sn1 RCOOH group and inositol from [M-H]- |
| 415.23    | Neutral loss of sn2 RCOOH group and inositol from [M-H]- |
| 297.04    | Glycerophosphoinositol -2H2O                             |
| 283.26    | sn2 RCOO- ion                                            |
| 279.23    | sn1 RCOO- ion                                            |

MSMS\_861p55\_n1\_131113110640\_#78-205 RT: 1.56-4.13 AV: 128 NL: 3.29E-1  
T: ITMS - p NSI Full ms2 861.55@cid20.0



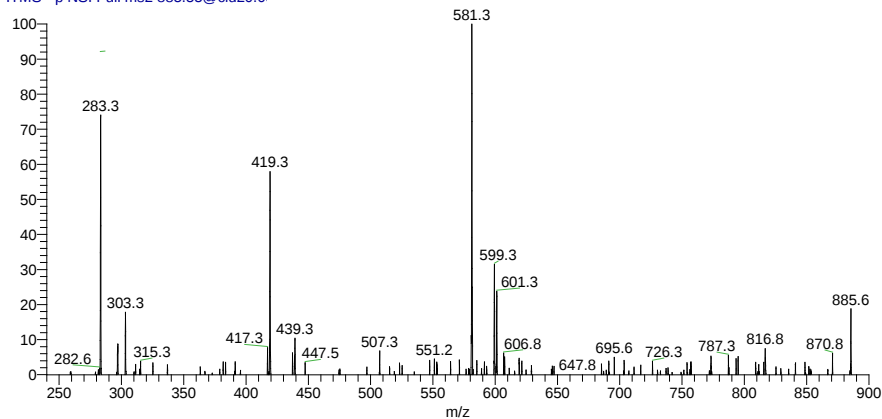
Normal lymphocytes

MSMS\_861p55\_f1\_131113110640\_#69-171 RT: 1.38-3.44 AV: 103 NL: 4.01E-1  
T: ITMS - p NSI Full ms2 861.55@cid20.0



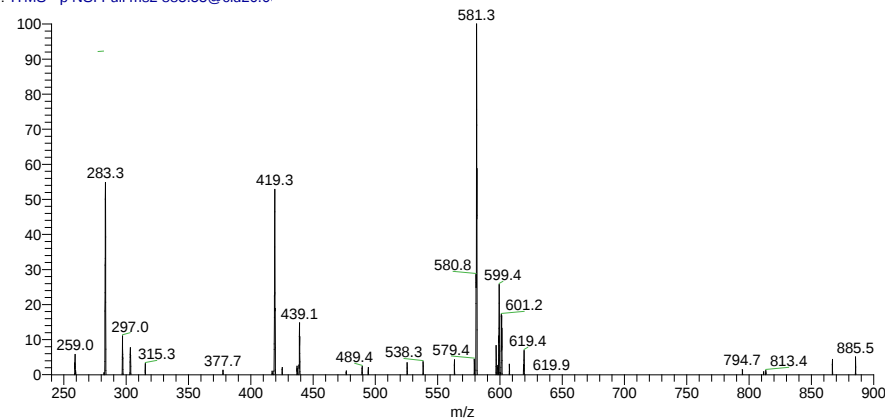
Fibrous lymphoid tissue

MSMS885\_131024155248 #16-47 RT: 0.27-2.02 AV: 32 NL: 1.99E-1  
T: ITMS - p NSI Full ms2 885.55@cid20.0



**Tumour**

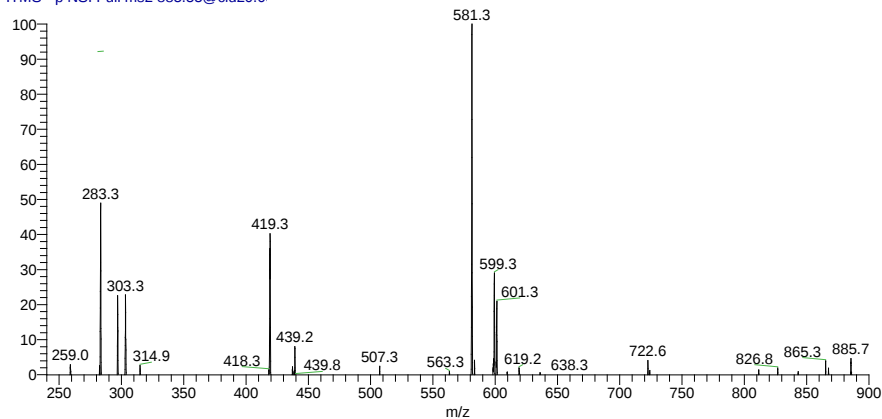
LN3\_8nl\_885\_131024155248 #22-30 RT: 1.20-1.65 AV: 9 NL: 5.61E-1  
T: ITMS - p NSI Full ms2 885.55@cid20.0



**Lymph node metastases**

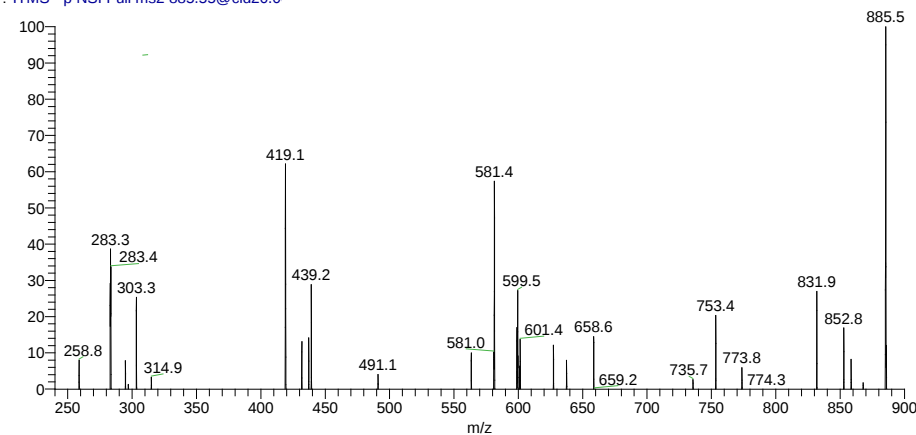
| m/z ratio | Ion Description                                          |
|-----------|----------------------------------------------------------|
| 885.55    | Precursor ion [M-H]-                                     |
| 599.32    | Loss of sn2 acyl chain as ketene (RCH=C=O) from [M-H]-   |
| 581.31    | Neutral loss of sn2 RCOOH group from [M-H]-              |
| 419.26    | Neutral loss of sn2 RCOOH group and inositol from [M-H]- |
| 303.23    | sn2 RCOO- ion                                            |
| 283.26    | sn1 RCOO- ion                                            |

LN3\_8nl\_885\_131024155248 #7-16 RT: 0.34-0.86 AV: 10 NL: 7.20E-1  
T: ITMS - p NSI Full ms2 885.55@cid20.0



**Normal lymphocytes**

LN3\_8fl\_885\_131024155248 #27-39 RT: 0.50-0.77 AV: 13 NL: 2.11E-1  
T: ITMS - p NSI Full ms2 885.55@cid20.0



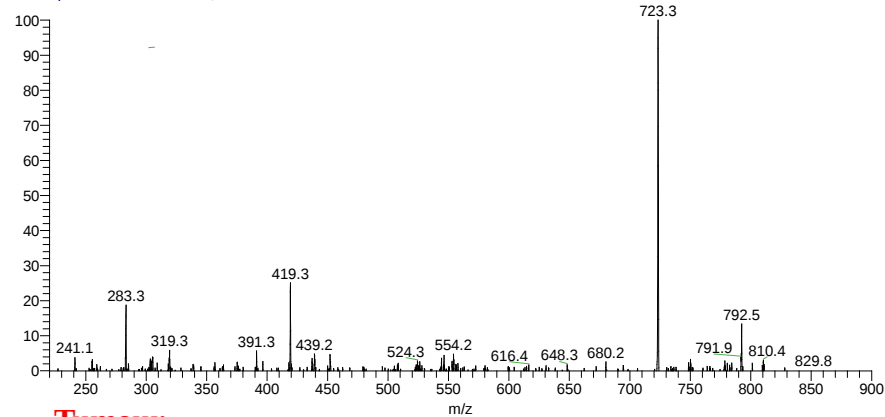
**Fibrous lymphoid tissue**

**Fig. S7d** Direct tissue DESI-MS/MS Spectra of  $m/z$  810.53 [Phosphatidylserine (20:4/18:0)-H] from tissue specific regions

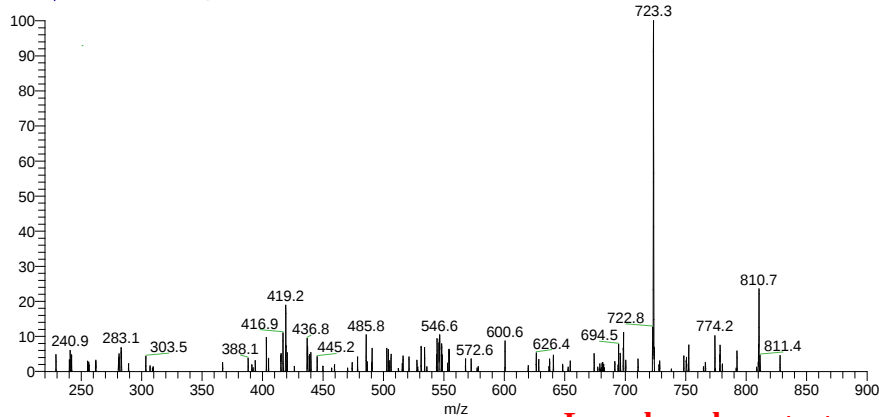
MSMS810\_1D\_oldtumor\_131024155248 #9-40 RT: 0.42-2.06 AV: 32 NL: 3.19E-1  
T: ITMS - p NSI Full ms2 810.53@cid20.0

PS - 20:4/18:0

LN3\_4\_810\_second\_131024155248 #18-64 RT: 0.96-3.57 AV: 47 NL: 9.64E-2  
T: ITMS - p NSI Full ms2 810.55@cid20.0



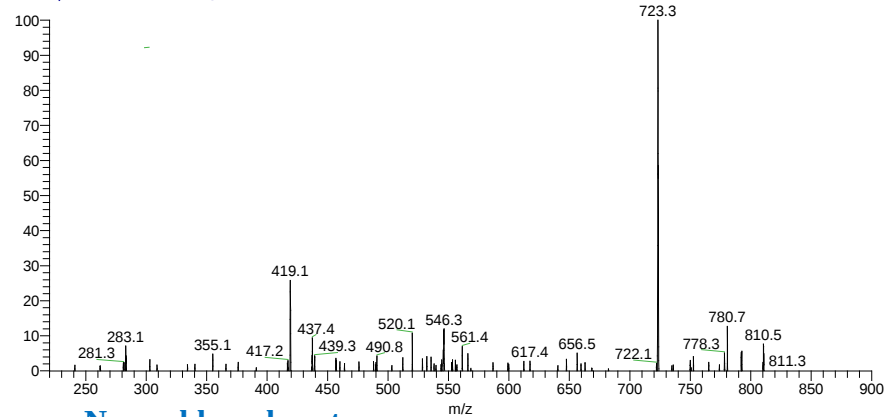
**Tumour**



**Lymph node metastases**

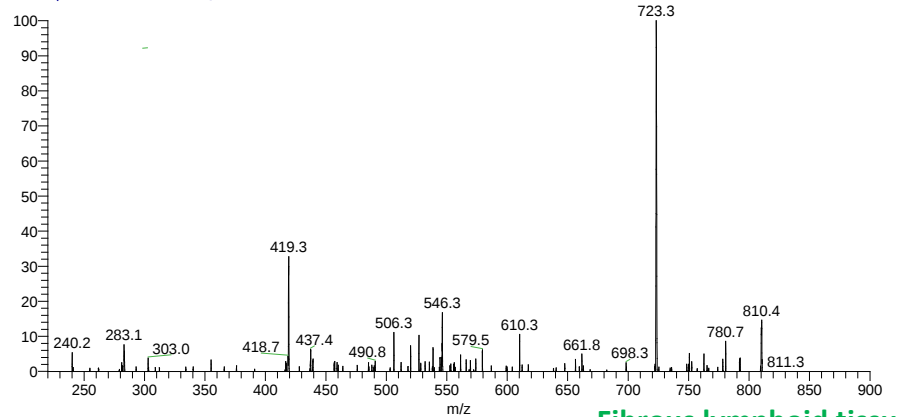
| m/z ratio | Ion Description                                        |
|-----------|--------------------------------------------------------|
| 810.53    | Precursor ion [M-H]-                                   |
| 723.50    | Loss of serine from precursor ion                      |
| 419.26    | Neutral loss of sn1 RCOOH group and serine from [M-H]- |
| 283.26    | sn2 RCOO- ion                                          |

LN3\_8nl\_810\_131024155248 #62-147 RT: 2.42-5.57 AV: 86 NL: 8.77E-2  
T: ITMS - p NSI Full ms2 810.55@cid20.0



**Normal lymphocytes**

LN3\_8fl\_810\_1310241552482 #1-146 RT: 0.17-5.53 AV: 143 NL: 7.81E-2  
T: ITMS - p NSI Full ms2 810.55@cid20.0



**Fibrous lymphoid tissue**