

June, 19th

MALDI: A Nobel Prize Technique to celebrate 50 years of Mass Spectrometry



Various faces of MALDI – Application stories from all over Europe



Different locations – different stories



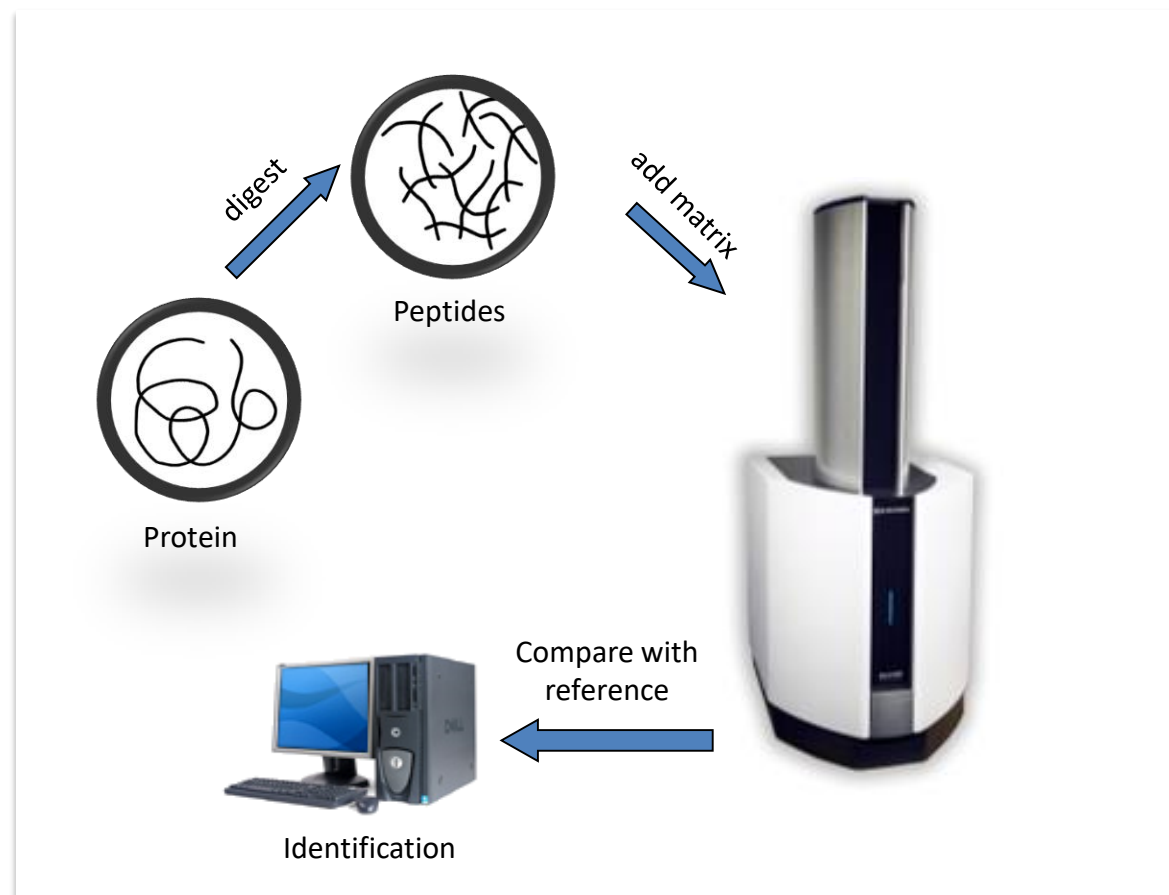
MALDI in Archeology

- After manufacturing the origin of fibres and bones is not visible anymore.
- Scientific interest which animals were kept / hunted



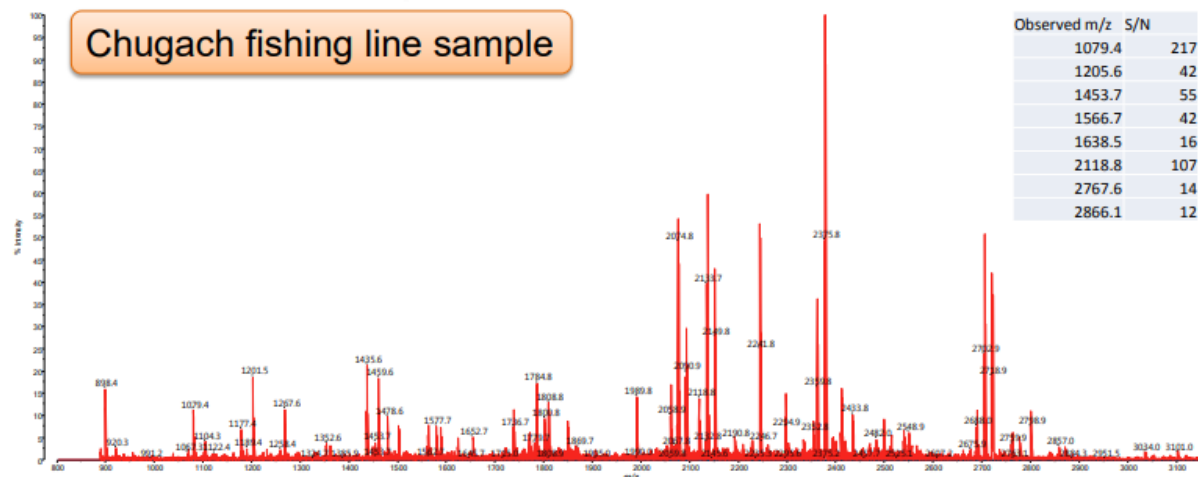
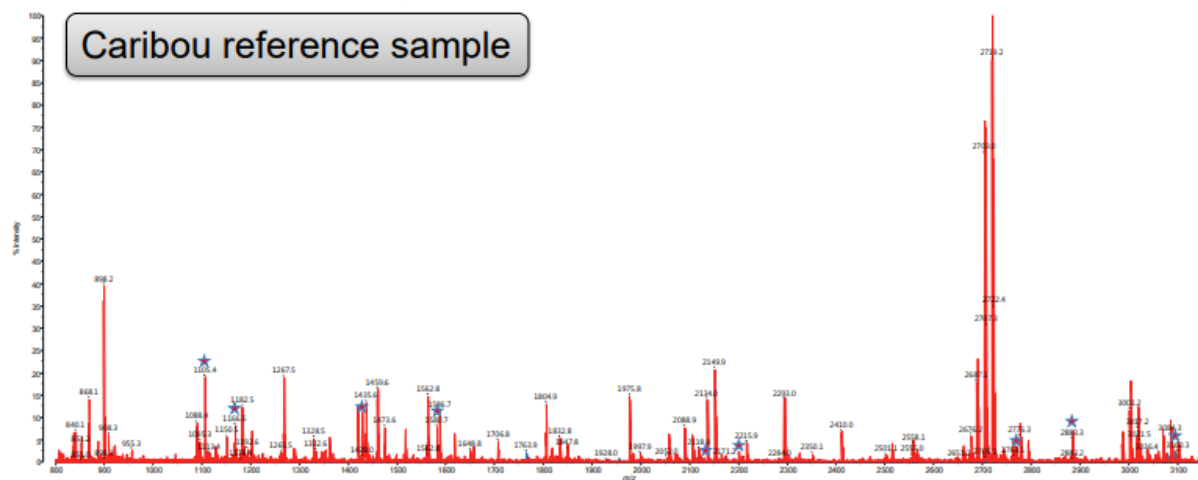
Workflow to identify proteins

- Extract proteins from sample.
- Digest Proteins into characteristic fragments.
 - Peptide Mass Fingerprint (PMF)
- Comparison with reference spectra / data base



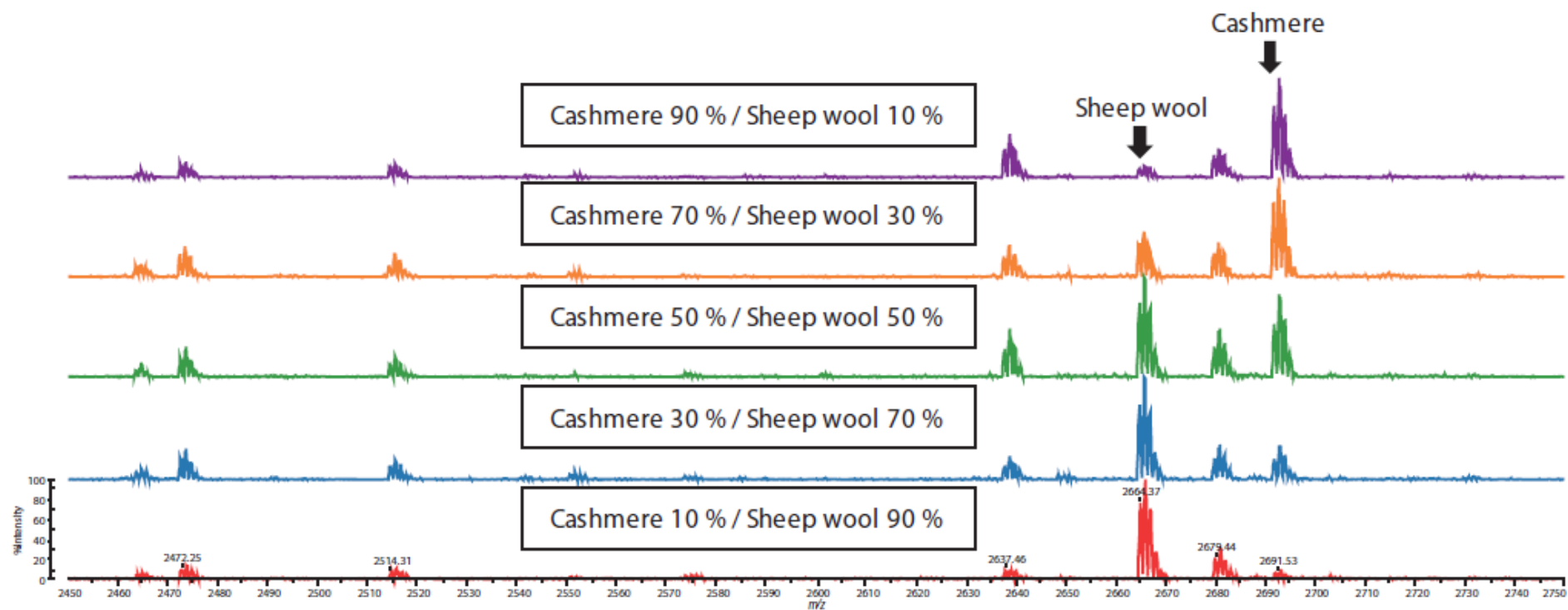
Example

- Mass spectra of reference and historical sample
- Characteristic peaks identified Caribou as origin of the fishing line fibre

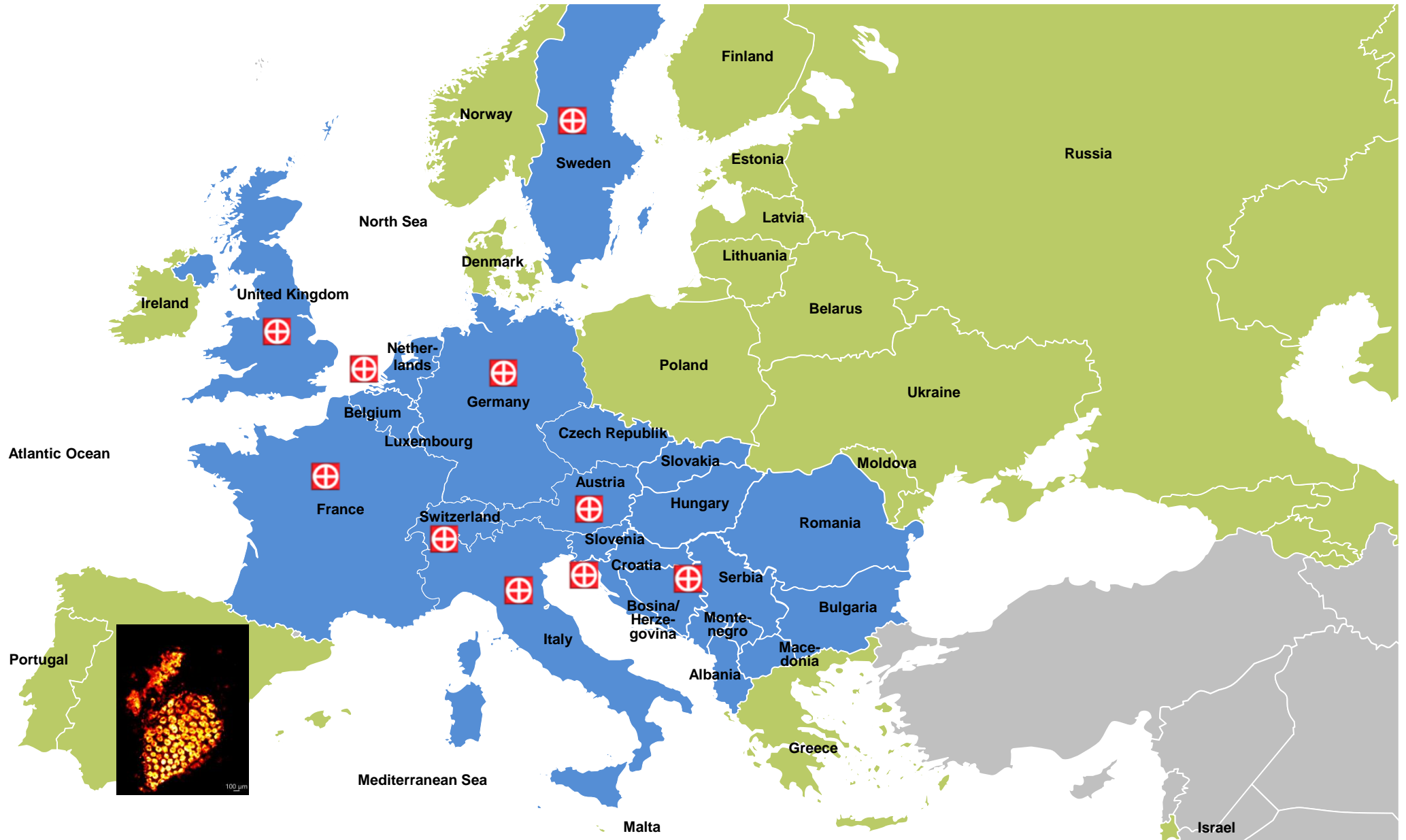


Similar approach

Quantification of Cashmere in fibre mixtures



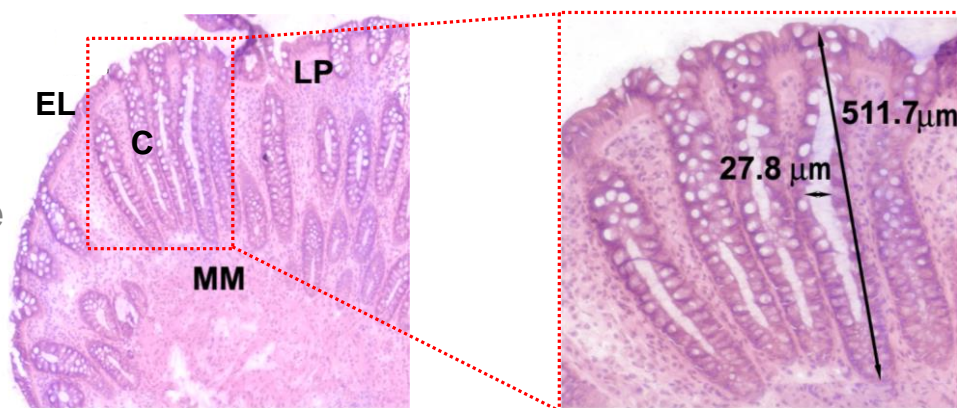
Different locations – different stories



MALDI imaging in clinical research

- Inflammatory bowel diseases (IBDs) are chronic and relapsing inflammatory conditions of unknown aetiology
- Subgroups: ulcerative colitis (UC) and Crohn's disease (CD)
- Current treatments are not universally effective and can have severe adverse effects
- Early detection and precise identification of the type of disease is important in order to apply the correct treatment

EL: epithelial layer
LP: Lamina propria
MM: muscularis mucosae
C: crypt

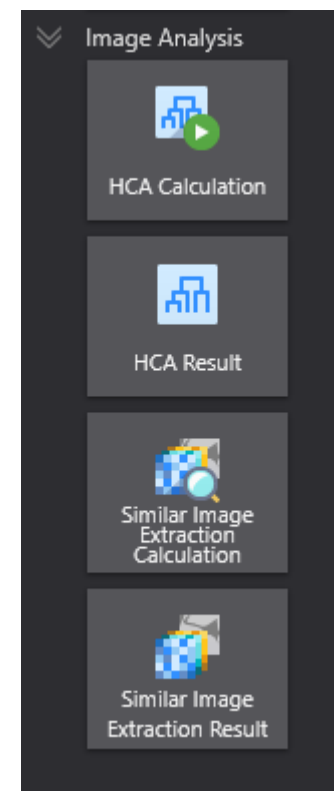
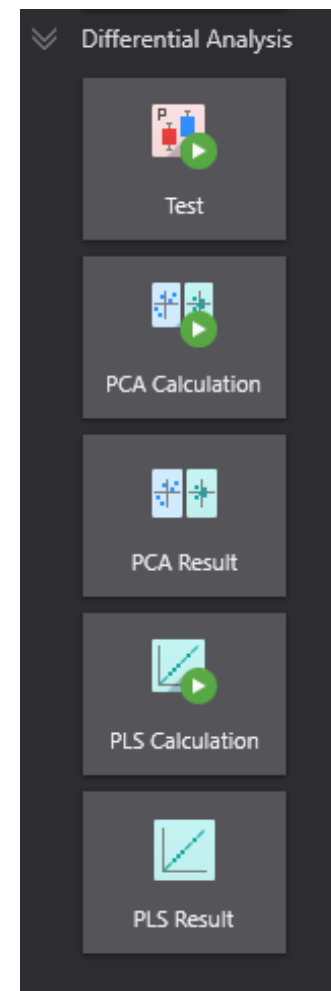
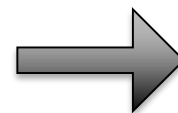
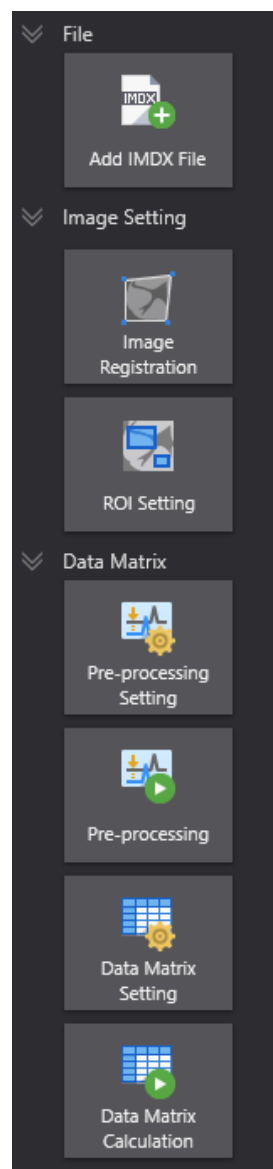
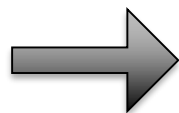
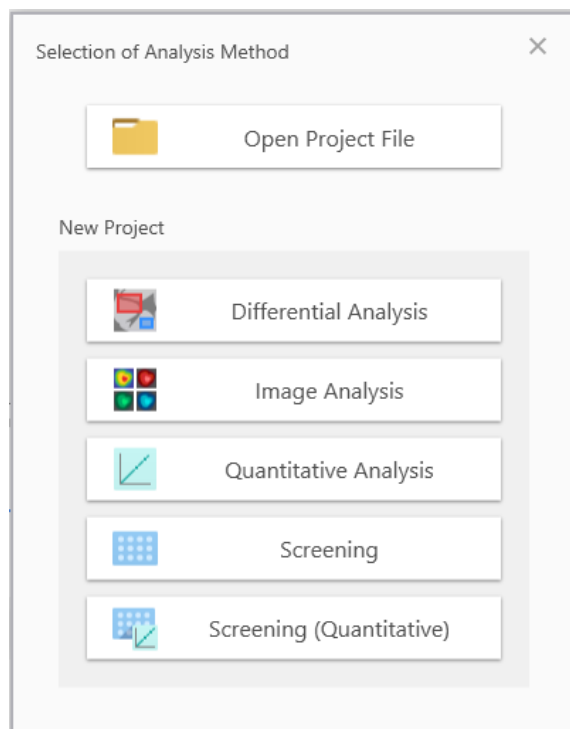


Method

- Human colon tissues from healthy and Crohn's disease
- Sublimation of DAN (1,5-diaminonaphthalene, DAN) for negative-ion detection
- MALDI-7090™ MALDI-TOF-TOF mass spectrometer (10 μm laser spot size)
- MALDI-MS/MS with HE-CID → a full structural elucidation of the fatty acid chains and positioning of the double bonds



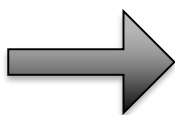
Data Analysis using IMAGEREVEAL MS



IMAGEREVEAL MS – PLS Analysis

▼ Differential Analysis

- Test
- PCA Calculation
- PCA Result
- PLS Calculation
- PLS Result

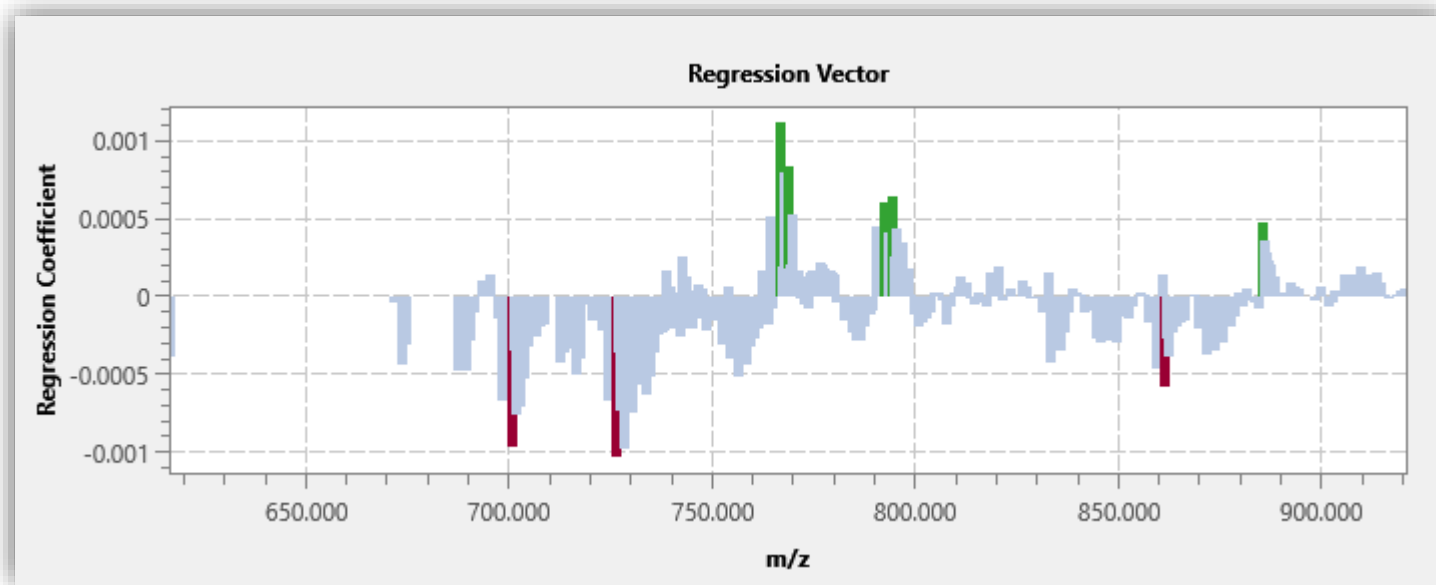


ROI List

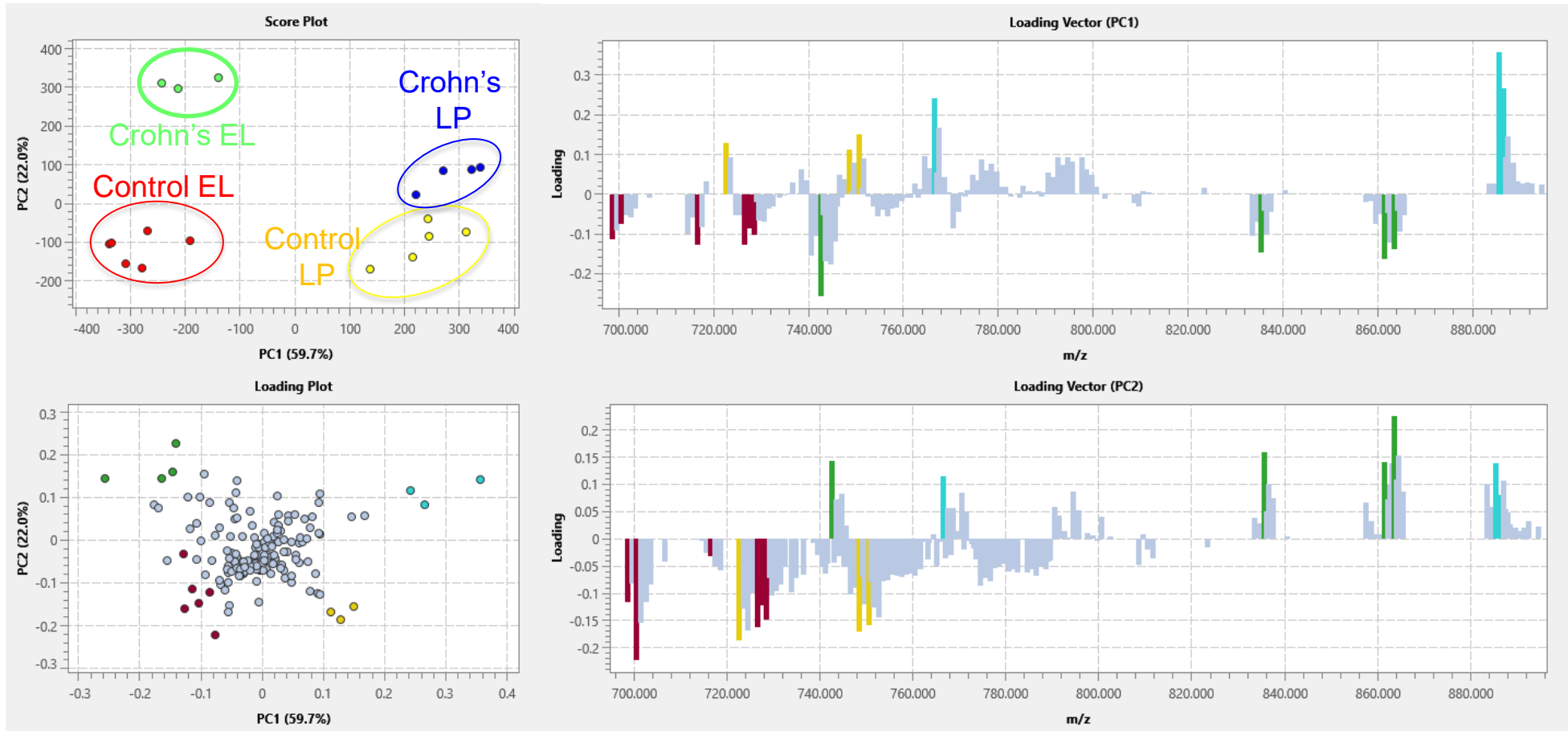
Import Export

| No. | File Name | ROI Name | Attribute | Y value |
|-----|----------------------------|----------|-----------|---------|
| 1 | IBD 018 1A (Control).im... | ROI001 | Control | 0.00000 |
| 2 | IBD 018 1B (Crohn's).imdx | ROI002 | Crohn | 1.00000 |
| 3 | IBD 019 1A (Control).im... | ROI003 | Control | 0.00000 |
| 4 | IBD 019 1B (Crohn's).imdx | ROI004 | Crohn | 1.00000 |
| 5 | IBD 020 1A (Control).im... | ROI005 | Control | 0.00000 |
| 6 | IBD 020 1B (Crohn's).imdx | ROI006 | Crohn | 1.00000 |

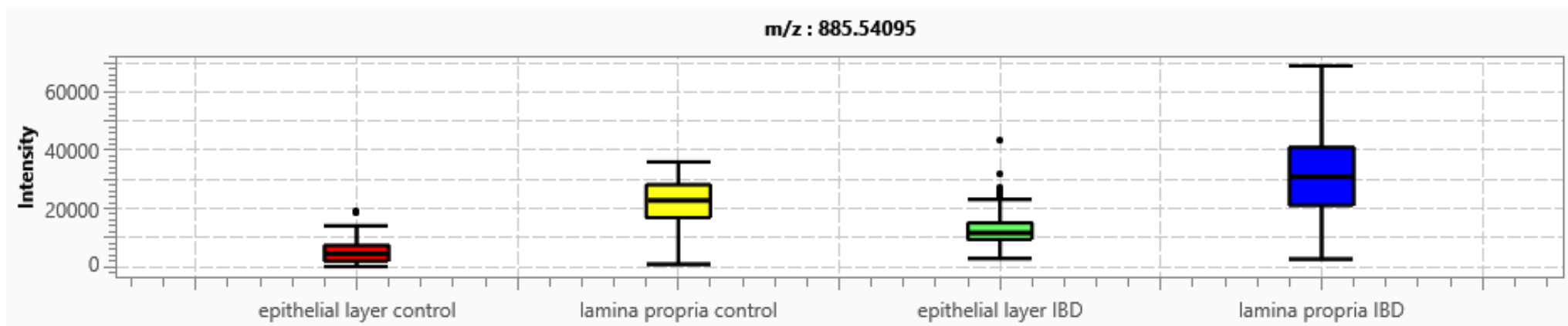
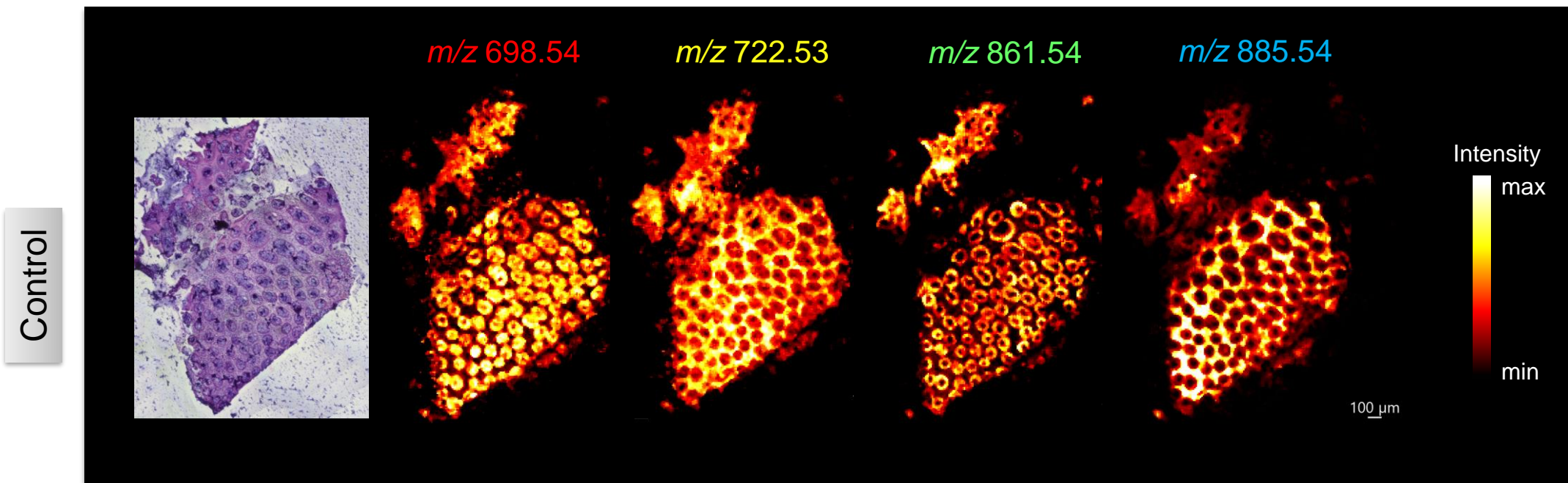
Execute Cancel



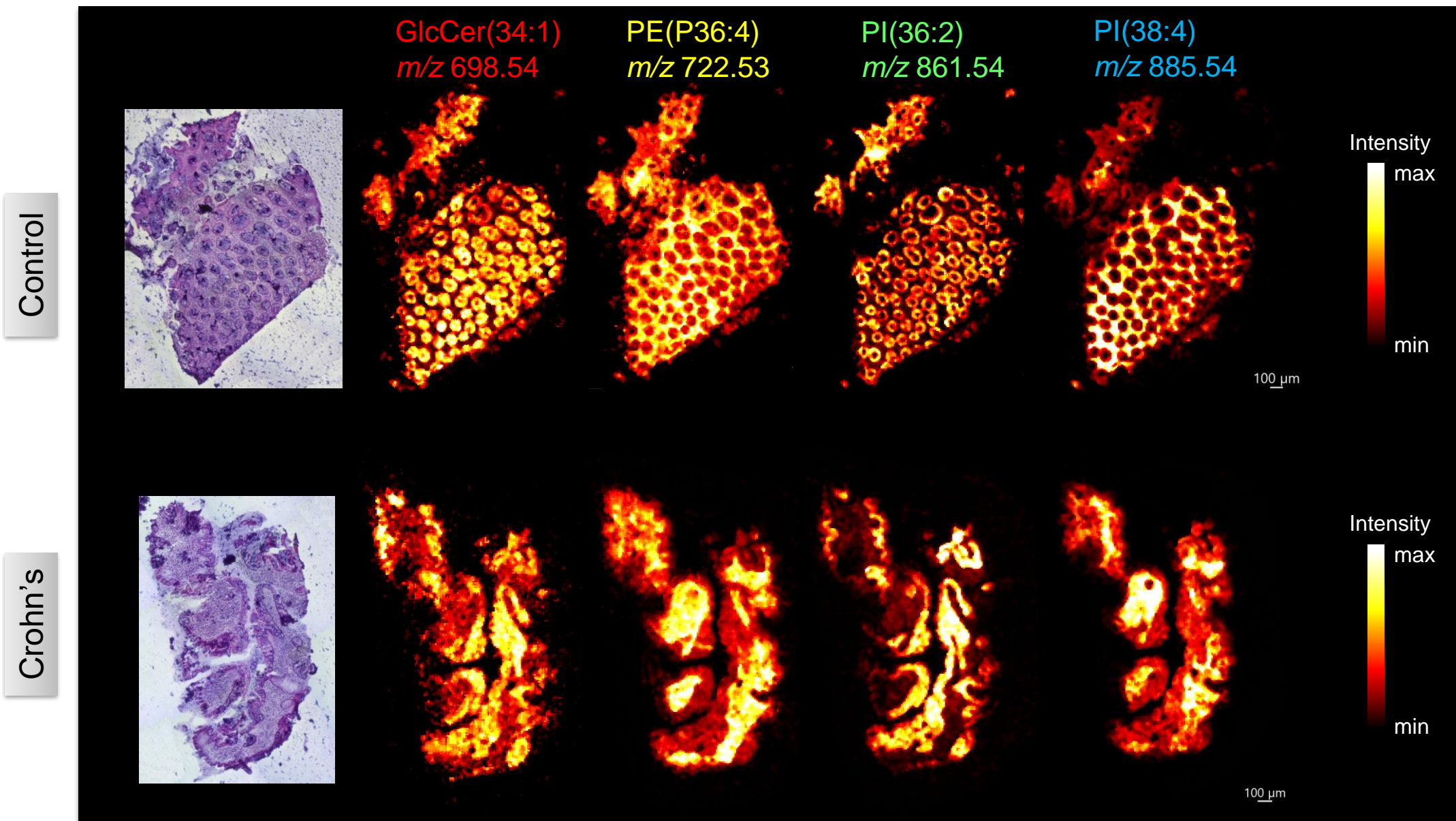
IMAGEREVEAL MS – PCA Analysis



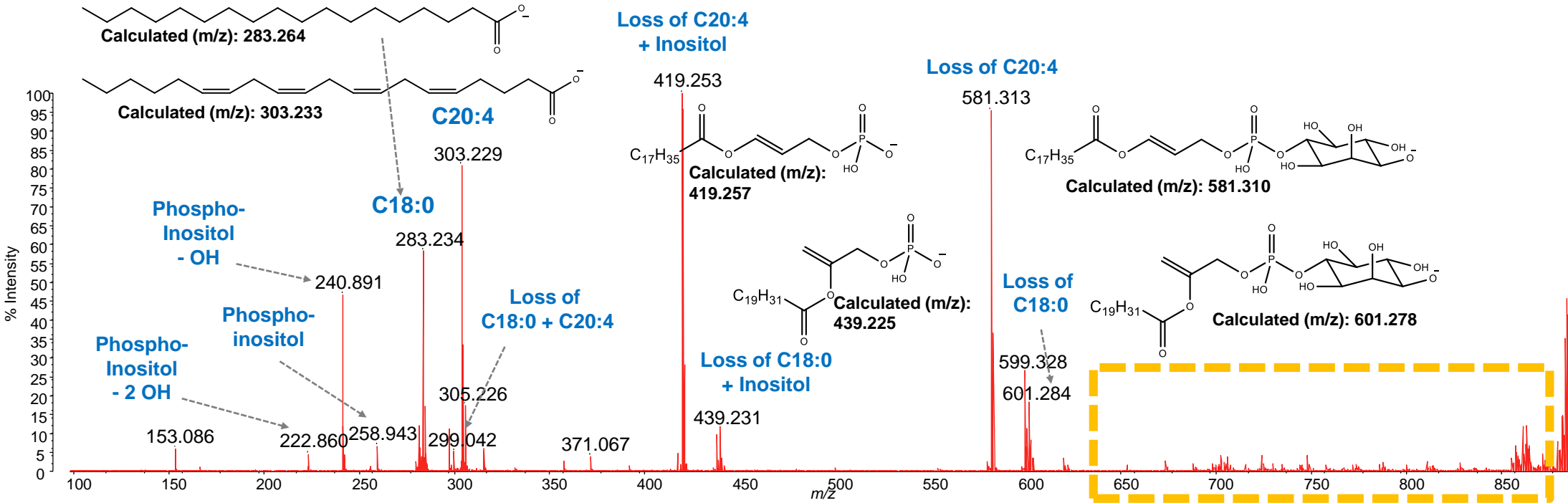
Results – Crohn’s vs Control



Results – Crohn's vs Control

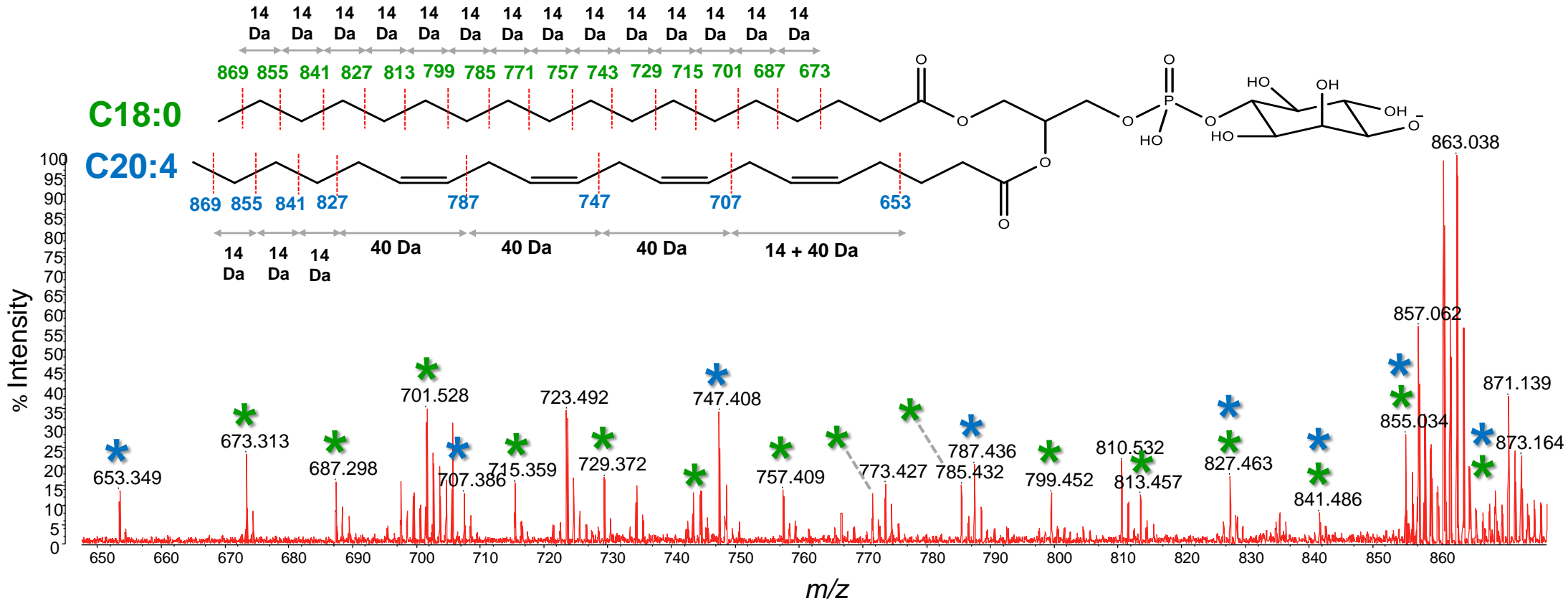


MALDI-MS/MS – m/z 885.5 (PI(38:4))



Identification of C18:0 and C20:4 chains

MALDI-MS/MS – m/z 885.5 (PI(38:4))

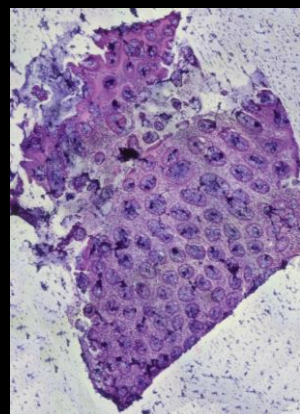


Identification of double bond positions

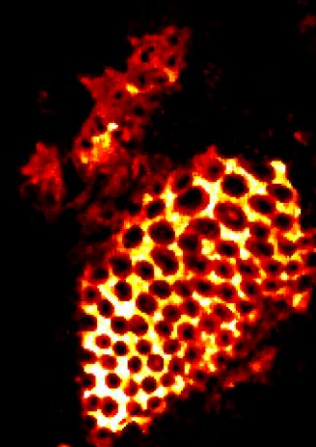
MALDI-MS/MS – m/z 885.5 (PI(38:4))

- Largest change of lipid expression related to Crohn's in lamina propria
- Infiltration of immune cells into lamina propria due to chronic inflammation
- PI(38:4) large reservoir of arachidonic acid
- Involved in signaling inflammation

Control



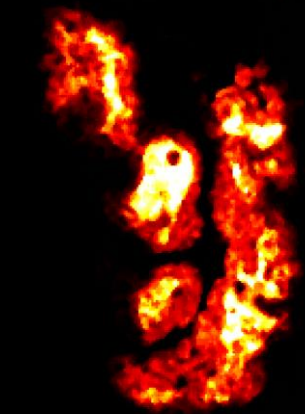
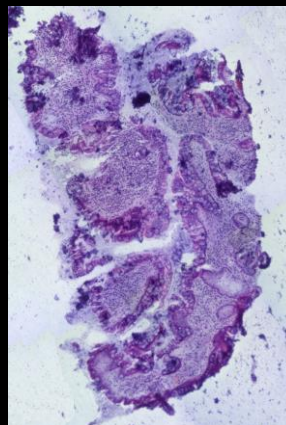
PI(38:4)
 m/z 885.54



Intensity
max
min

100 μ m

Crohn's

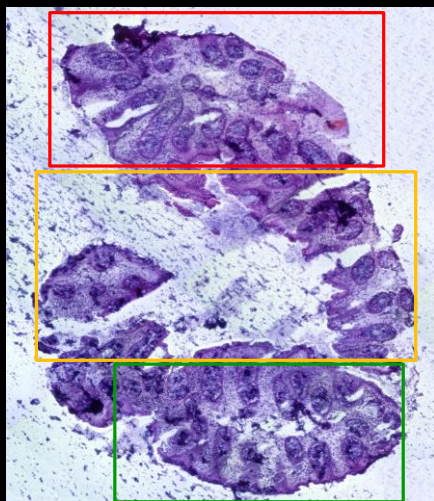


Intensity
max
min

100 μ m

MALDI-MS/MS

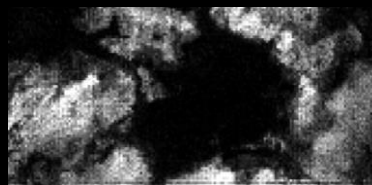
Control



Precursor:
m/z 742.539
PE(18:1/18:1)
PE(18:0/18:2)



Fragment:
m/z 281.244
C18:1



Precursor:
m/z 750.576
PE(P-18:0/20:4)



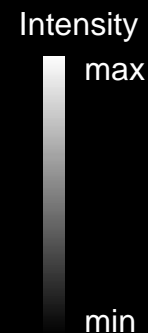
Fragment:
m/z 303.205
C20:4



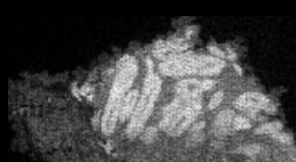
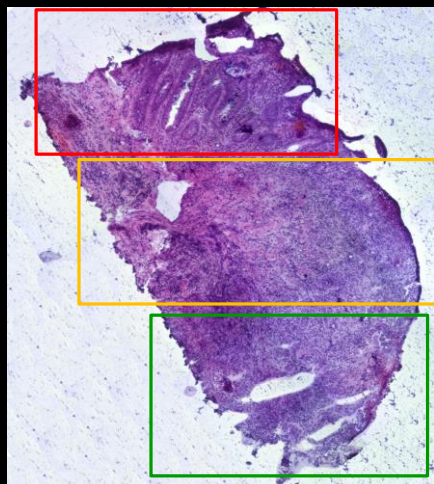
Precursor:
m/z 885.550
PI(18:0/20:4)



Fragment:
m/z 283.234
C18:0



Crohn's



Precursor:
m/z 742.539
PE(18:1/18:1)
PE(18:0/18:2)



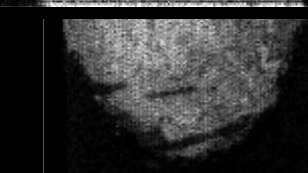
Fragment:
m/z 279.241
C18:1



Precursor:
m/z 750.576
PE(P-18:0/20:4)



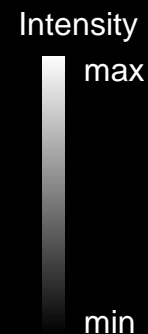
Fragment:
m/z 303.309
C20:4



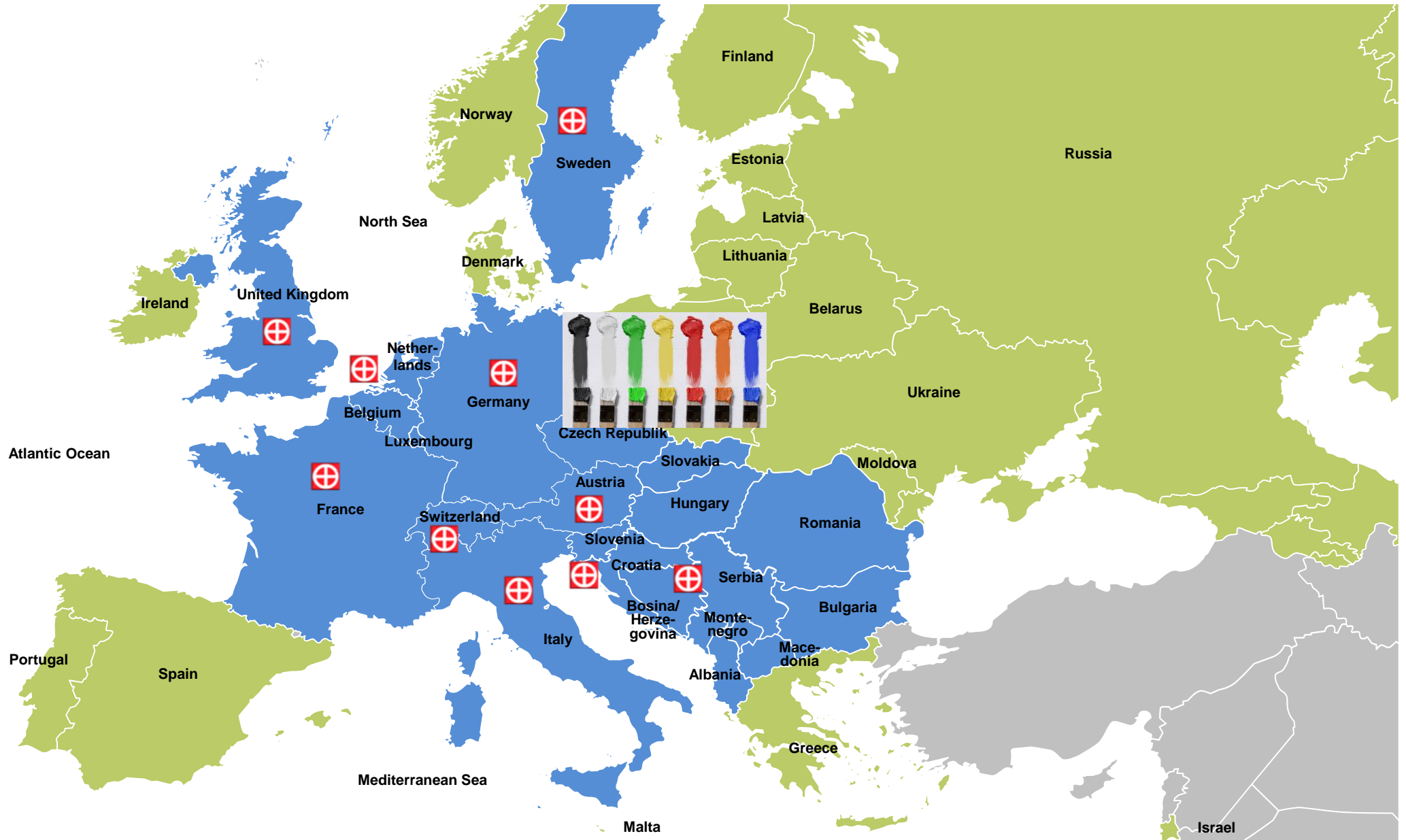
Precursor:
m/z 885.550
PI(18:0/20:4)



Fragment:
m/z 283.254
C18:0



Different locations – different stories



Polymers in paints

- Different polymers are used in paints
- Interpretation of homopolymer ions:
 - A_nEC
 - A repeating unit
 - n number of repeating units
 - E combined end group
 - C Adduct of Cation donator
- Distribution statistics (some examples):
 - M_w weight average molecular weight
 - PD polydispersity

Polymerix Software

Polymerix
File Edit View Help

Find Homopolymer Series

| Enabled | Excess Mass | Monoisotopic m/z 1 | Monoisotopic m/z 2 | Alpha End Group | Repeat | Omega End Group | Charge State | Adduct | Adduct Charge | Loss | Low Mass | High Mass |
|-------------------------------------|-------------|--------------------|--------------------|-----------------|--------|-----------------|--------------|--------|---------------|------|----------|-----------|
| <input checked="" type="checkbox"/> | | | | | | | | | 1 | | 1.0 | 100000.0 |

Source

Current Series

Residuals

Select repeat for mass difference 44.02110, from m/z 865.68108 to m/z 909.70218

| # | Repeat Formula | DBE | Charge | m/z | Error mDa | Error mDa | Error ppm |
|---|----------------|-----|--------|----------|------------|-----------|-----------|
| 1 | C2H4O | 0.0 | 1 | 44.02621 | 5.120 | -5.120 | -116.302 |

Monoisotopic m/z values are 865.68108 and 909.70218, repeat m/z is 44.02110, tolerance is 0.1 Da, electron mode is normal, restricted element ratios, DBE range is from -0.5 to 10.0, charge count is 1, maximum result count is 25, H count is from 0 to 20, O count is from 0 to 10, N count is from 0 to 3, Si count is from 0 to 1, C count is from 0 to 20.

Find Series Settings

Repeat Tolerance: 100 (mDa)
End Group Tolerance: 200 (mDa)
Maximum Result Count: 25

End Group Mass: Minimum 0, Maximum 300
Charge: Minimum 1, Maximum 1
DBE: Minimum -0.5, Maximum 10

Electron Mode: Normal
 Element Ratio Restrictions
 Positive Charge Mixed adducts

Spectrum Match Tolerance:
 Constant: 0.1 +/- Da
 Proportional: 100 +/- ppm
 TOF: 0.5 +/- Da, at m/z 1000

Repeat Unit Elements Table

| + | Enable | Atomic Symbol | Min. Count | Max. Count |
|---|-------------------------------------|---------------|------------|------------|
| - | <input checked="" type="checkbox"/> | H | 0 | 20 |
| - | <input checked="" type="checkbox"/> | D | 0 | 10 |
| - | <input type="checkbox"/> | N | 0 | 3 |
| - | <input type="checkbox"/> | Si | 0 | 1 |
| - | <input checked="" type="checkbox"/> | C | 0 | 20 |

End Group Elements Table

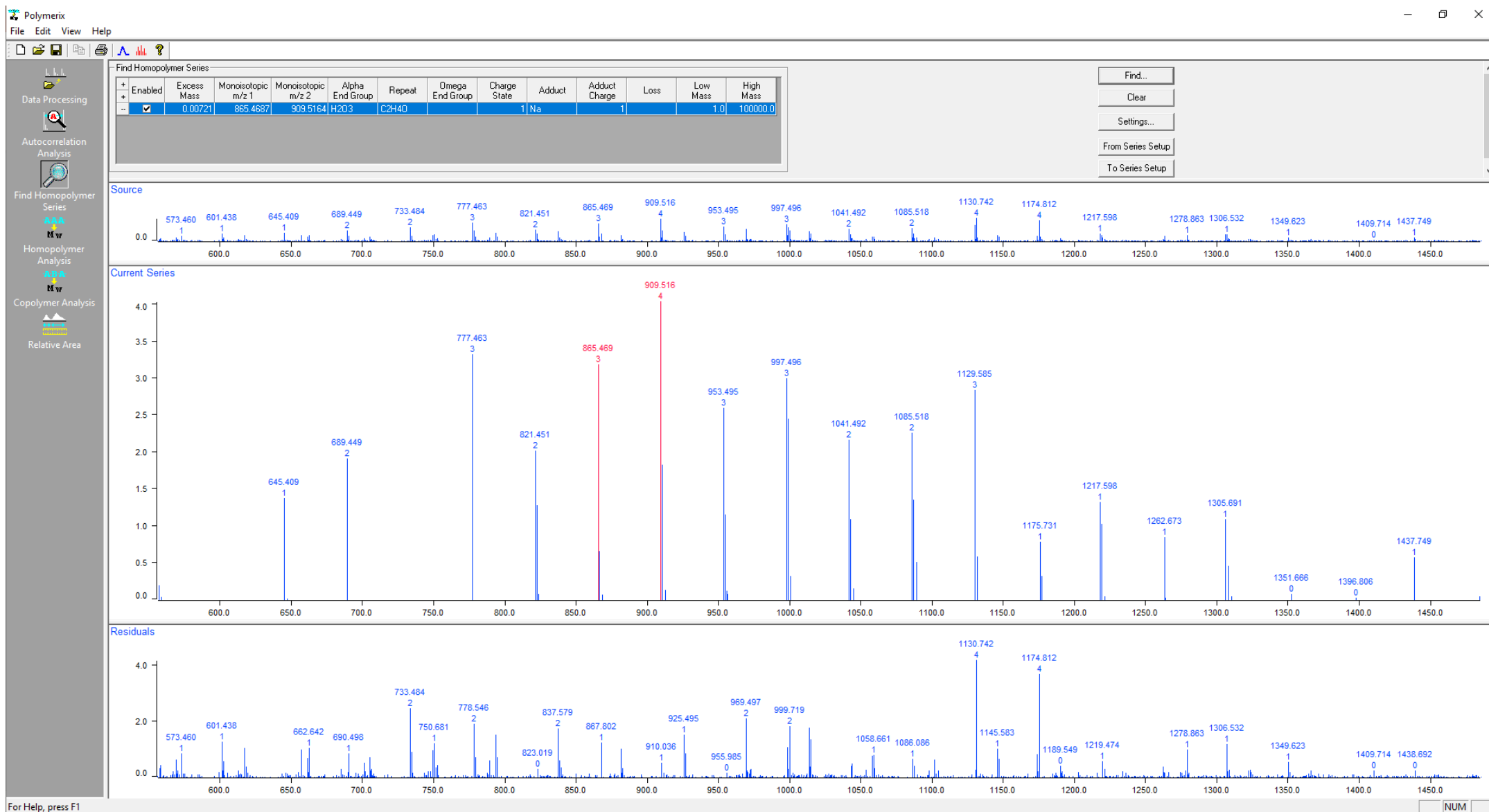
| + | Enable | Atomic Symbol | Min. Count | Max. Count |
|---|-------------------------------------|---------------|------------|------------|
| - | <input checked="" type="checkbox"/> | C | 0 | 20 |
| - | <input checked="" type="checkbox"/> | H | 0 | 40 |
| - | <input checked="" type="checkbox"/> | O | 0 | 20 |
| - | <input type="checkbox"/> | N | 0 | 6 |
| - | <input type="checkbox"/> | F | 0 | 3 |

Adducts

| + | Enable | Adduct | Loss |
|---|-------------------------------------|----------|------|
| - | <input type="checkbox"/> | H | |
| - | <input checked="" type="checkbox"/> | Na | |
| - | <input type="checkbox"/> | K | |
| - | <input type="checkbox"/> | | H |
| - | <input type="checkbox"/> | Ag | |
| - | <input type="checkbox"/> | C2F3NaO | |
| - | <input type="checkbox"/> | C2F3Na2I | |
| - | <input type="checkbox"/> | Fe | |

For Help, press F1

Polymerix Software



Polymerix Software

Polymerix
File Edit View Help

Match Tolerance
 Constant 0.1 +/- Da Use clusters
 Proportional 100 +/- ppm Positive charge
 TDF 0.5 +/- Da, at m/z 1000

Combine Within Series With Same Repeat
 Charge states
 Adducts and losses
 End groups

Processing Series Setup Mass Adjust

| Homopolymer Results Summary | | | | | | | | | | | | | | | | |
|-----------------------------|---------|---------|----------|-------|--------|--------|--------|----------------|------------------|-----------------|--------|-----------------|--------------|--------|------|---------------------------------|
| Series Label | Mn | Mw | Mz | PD | DPn | DPw | DPz | Percent Series | Percent Spectrum | Alpha End Group | Repeat | Omega End Group | Charge State | Adduct | Loss | Series Formula |
| Total/Average | 949.808 | 979.971 | 1009.674 | 1.032 | 20.338 | 21.023 | 21.698 | 100.00 | 48.64 | | C2H4O | | | 1 Na | | |
| S1 | 953.709 | 983.722 | 1012.974 | 1.031 | 20.527 | 21.208 | 21.873 | 72.48 | 35.26 | H2O3 | C2H4O | | 1 Na | | | H2O3 [C2H4O] _n + Na |
| S2 | 939.532 | 970.093 | 1000.980 | 1.033 | 19.841 | 20.535 | 21.237 | 27.52 | 13.39 | C4H2O | C2H4O | | 1 Na | | | C4H2O [C2H4O] _n + Na |

Homopolymer Assignments

Assignments Filtered Assignments Filtered Residuals Details Mass Ranges Distribution Plots

For Help, press F1

More options of Polymer analysis

- **Copolymer analysis:**
 - A_nB_mEC
 - Two different repeating units
 - For each signal the software calculates a polymer formula
- **Biopolymers**
- **Polymers in Fabrics / Leathers**



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Thank you...

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Matthew E. Openshaw

The British Museum, London, UK
Chris Mussell
Amber Lincoln

... for your attention!

Thank you!

Questions?

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