The study of derivatization prior mass spectrometry imaging (MSI) analysis – charge tagging based on the cholesterol and betaine aldehyde

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Supplementary materials:

- ## Polystyrene lid – sample preparation

The way of dealing with different ways of sample preparation on a single glass during the optimization of the MSI tissue measurements:

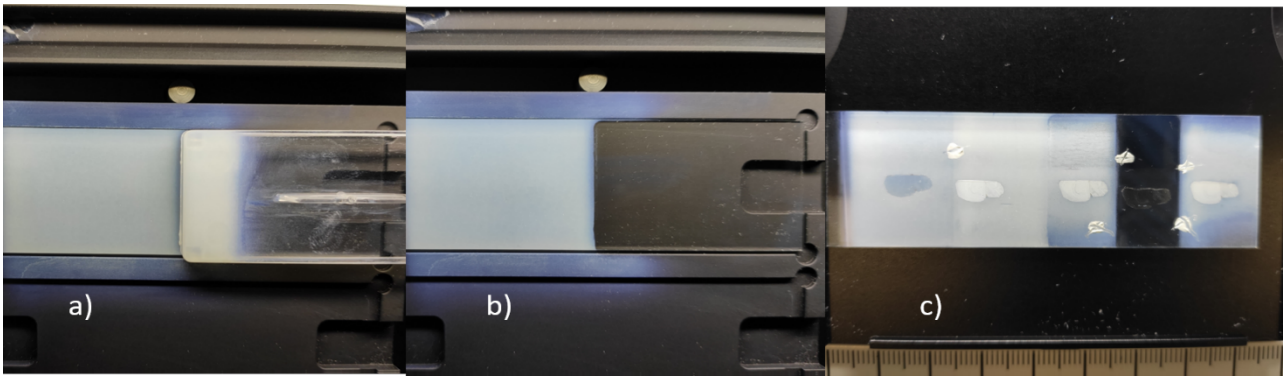

Figure S1. (a) ITO glass slide partially covered with polystyrene lid during matrix deposition (semi-transparent lid visible on the right); (b) sharp edges of matrix obtaining after lid removal; (c) regions of ITO glass slide with different matrix and derivatizing strategy.

- ## Nissl staining

Histological Nissl staining of the tissue sections after MALDI measurements were proceed as follows:

1. 5 minutes washes in 96% ethanol,
2. 5 minutes washes in 96% ethanol,
3. Drying for 5 min in desiccator,
4. Immersion in the water for 1 minute,
5. Cresyl violet stain* - 15 minutes
6. Dipping in water to remove cresyl violet solution (Sigma-Aldrich)
7. 30 second 50% ethanol
8. 30 second 95% ethanol
9. 30 second Histochoice Clearing Agent (Sigma-Aldrich)
10. Mounting tissue section with DPX mountant for histology (Sigma-Aldrich)
11. Obtaining a picture of tissue section

*Cresyl violet stain: 1.25g cresyl violet acetate and 0.75 ml glacial acetic acid to 250 ml warm H_20, cool and filter

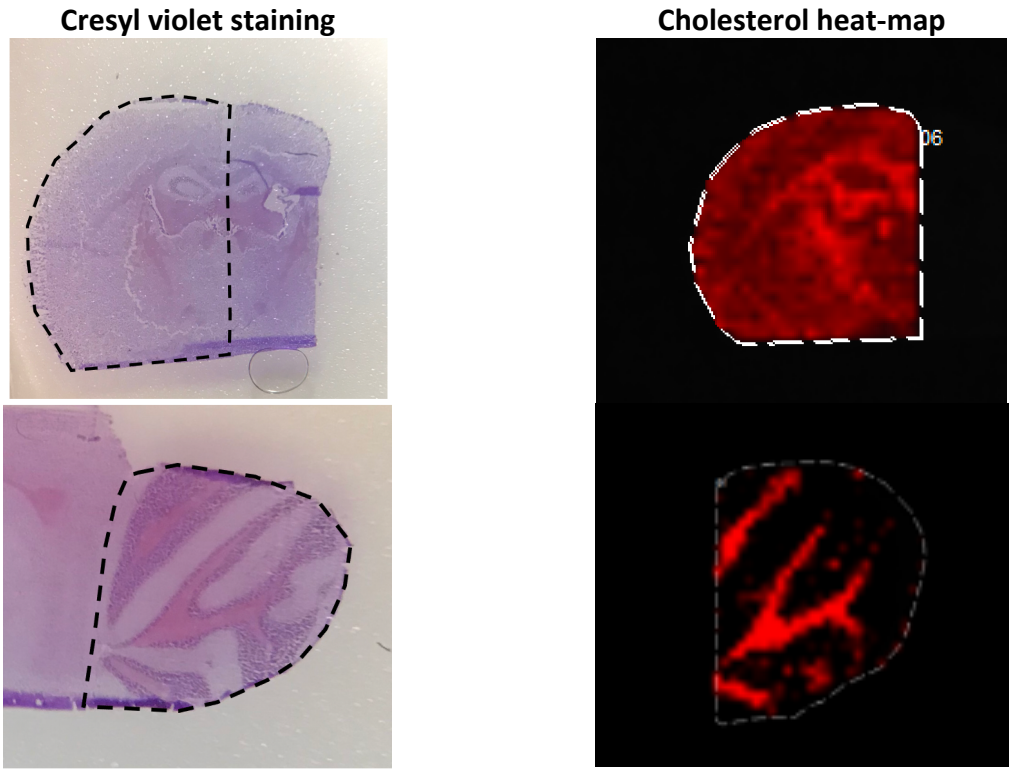

Figure S2. Exemplary tissue sections stained with cresyl violet, with the heat-map of cholesterol distribution.

- **The number of betaine aldehyde layers – cholesterol derivatization**

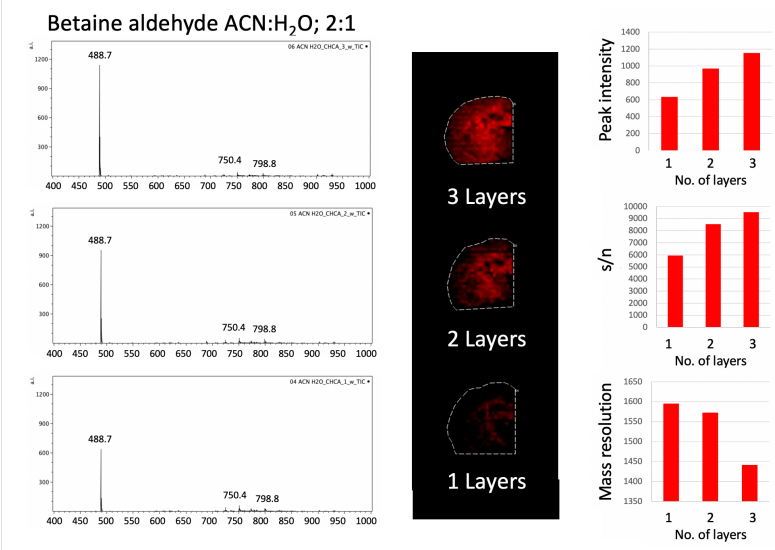

Figure S3. A slight saturation of the signal's response with the increasing number of layers could be observed when we consider the signal intensity from derivatized cholesterol from the whole tissue area.