Sublimation/Rehydration of SA or CHCA for imaging proteins/peptides

Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process should be done with fume hood ventilation.

Equipment and Materials:

Equipment

Petri dish (plastic, Fisher, 08-757-100D, 100mm x 15mm); stainless steel plate (AB, 4347680); heat conductive copper tape (Electron Microscopy Sciences, 77801);Tape (Fisher, 35901R); Oven set at 85°C.

Chemicals

Acetic acid, methanol; MilliQ water; Carnoy's fluid (6 ethanol, 3 chloroform, 1 acetic acid)

Procedure

Tissue preparation prior to sublimation.

1. Following tissue sectioning and placement on plate or slide, it may be necessary to wash tissue.
2. Washing conditions for detection of proteins: 70% ethanol (30s), 100% ethanol (30s), Carnoy's fluid (2min), 100% ethanol (30s), H_2O (30s), 100% ethanol (30s). The section is sublimated with SA to obtain a coating at optimized 0.25mg/cm^2. (MSRC-R-025)
3. For sections to detect peptides, the section is not washed and is sublimated with CHCA at 0.2 mg/cm^2.

Rehydration of sublimated matrices (Figure 1).

1. The slide with sublimated matrix is attached to a stainless-steel plate (used as a heat sink).
2. The plate is attached to the underside of the top part of the Petri dish using a heat conductive copper tape.

3. This top part of Petri dish is placed into a pre-heated oven at 85°C for 2 min.
4. A piece of filter paper is placed in the bottom part of the Petri dish, for SA:1 mL of MilliQ water and 100 µL of acetic acid or methanol pipetted onto the paper to subsequently create a vapor for the recrystallization process; for peptide analysis using CHCA: 200 µL MilliQ water, 50 µL TFA are pipetted onto the paper.
5. The two parts of Petri dish are reassembled to form a hydration chamber, sealed using a tape (Fisher, 35901R) and left in the oven for 3.5min.
6. The slide is taken out from the petri dish and allow to dry.

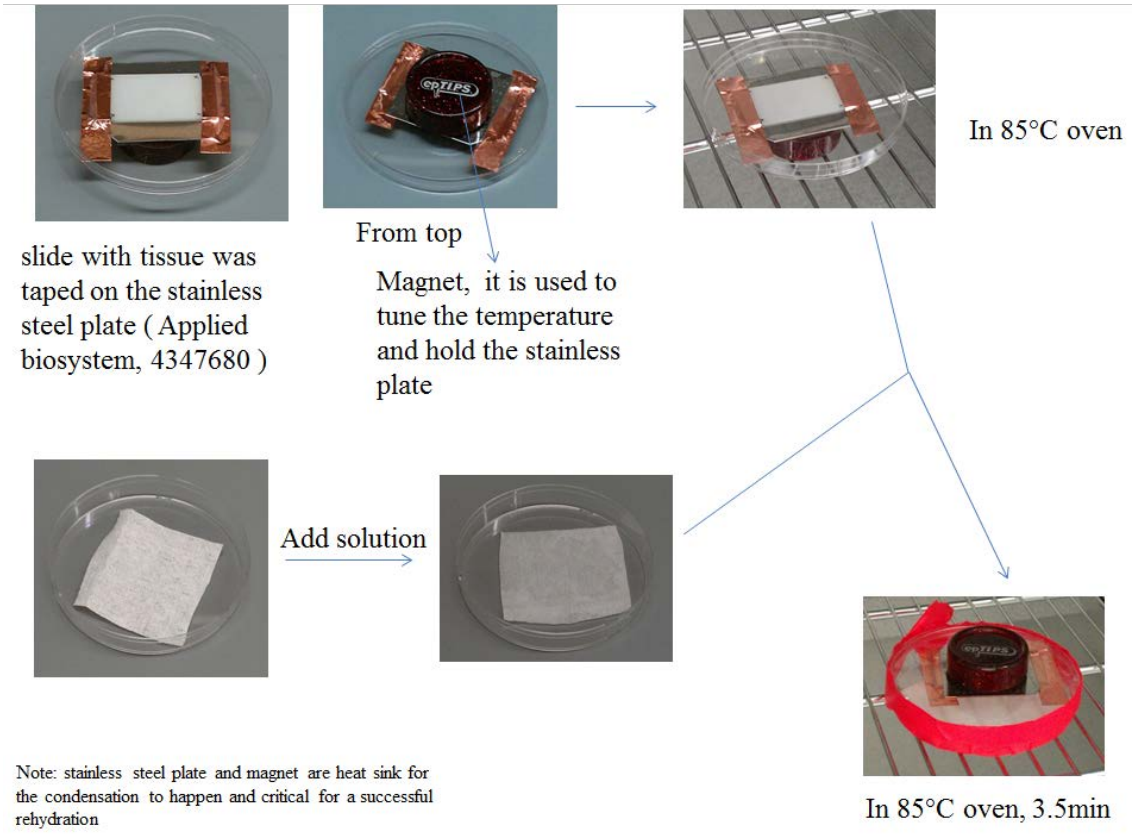

Figure 1 Construction of rehydration/recrystallization chamber with a Petri dish, magnet, stainless steel plate and tape.

Expected outcome:

The sign of a successful rehydration is determined by a quick color change at the moment the Petri dish is opened. This phenomenon is caused by the evaporation of solvent condensed on the surface of matrix.

Reference:

1. Matrix Sublimation/Recrystallization for Imaging Proteins by Mass Spectrometry at High Spatial Resolution, Junhai Yang and Richard M. Caprioli *Analytical Chemistry* **2011** *83* (14), 5728-5734
2. High spatial resolution imaging mass spectrometry and classical histology on a single tissue section, F. Deutskens, J. Yang, R. M. Caprioli. *Journal of Mass Spectrometry* 2011, 46, 568-571.