Automatic spraying with TM sprayer for imaging proteins with 2,5-DHA

Equipment and Materials:

Equipment

- Petri dish (glass, Fisher, 100mm x 15mm),
- TM sprayer from HTX
- Indium oxide coated glass slide(ITO)
- Metal target

Chemicals

- Acetic acid (AcOH), trifluoroacetic acid (TFA), acetonitrile
- 2,5-dihydroxyacetophenone (DHA), recrystallized twice from commercial source
- MilliQ water
- Carnoy's fluid: 6 ethanol, 3 chloroform, 1 acetic acid

Procedure

1) Tissue preparation prior to spraying.
   The tissue, sectioned and placed on the target plate (ITO slides, metal target, etc.), is dried under ambient conditions for 10 minutes and stored in a slides mailer box (Electron microscope science, 71548-01) sealed with parafilm at -80°C. When ready for analysis, the slides box was placed into a vacuum desiccator under room temperature for 30 minutes to allow the slides to reach room temperature while preventing water condensation on the sections which could cause delocalization of analytes.
2) Washing conditions for detection of proteins: 70% ethanol (30s), 100% ethanol (30s), Carnoy's fluid (2min), 100% ethanol (30s), 40% ethanol (30s), 100% ethanol (30s).
3) Matrix solutions:
   make solution of DHA as: 90 mg DHA, 7mL ACN, 3mL H_2O, 100 µL TFA, 50 µL ammonium hydroxide

4) Spraying with TM sprayer:

   8 passes, 0.075mL/min, 45°C, 1.5 mm track, 1050 mm/min, HPLC solvent of 70% ACN.

Note:

1. The flow rate is critical for spraying DHA: too slow a rate will result a very wet surface and could cause delocalization of analytes and too fast a rate will result in poor extraction of proteins.
2. The acid in the matrix solution is to promote dissolution of analytes of interest. This can be changed to just TFA, or just AcOH. Small amount of ammonium hydroxide is sometimes used to improve sensitivity.
3. The coating from this spraying procedure is about 0.2-0.3mg/cm^2.
4. The washing protocol is extremely critical to obtain high quality spectra.