Protocol - Matrix application

An array of different tissue samples was prepared for MALDI-IMS to have a widely applicable protocol in hand.

- Prior to matrix application, cryo-sections were thaw-mounted onto ITO-coated glass slides (Bruker Daltonics) to provide a conductive surface.
- After drying, crosses were drawn onto areas around the tissue using a white edding® 752 pen. These markings are intended as teach marks for the MALDI imaging system. Furthermore, the markings serve the purpose of creating an alignment reference for MALDI-FISH correlation as the ink fluoresces when excited with laser.
- An matrix mix consisting of 7 $mg*mL^{-1}$ α-cyano-4- hydroxycinnamic acid (HCCA) in 70:30 acetonitrile /water with 0.2% Trifluoroacetic acid (TFA) was prepared.
- Using the automated spray coating system (SunChrom GmbH), 16 layers of matrix were distributed homogenously onto the glass slide containing the sample. This area was predefined with the "teaching" function incorporated into the SunCollect software module (SunChrom GmbH). The system uses a pump to maintain a constant pressure of 2 bars that produces a steady drizzle of matrix solution from a capillary nozzle. The z-distance of the capillary (25mm) and the pumping speed were adjusted via SunCollect. Samples were coated with $15\mu L*min^{-1}$, 20 µl/min, 20 µl/min and 20 µl/min twice resulting in 8 layers.