Cryosectionning recipe

Mélanie Lagarrigue & Régis Lavigne

PREFACE

This book is primarily intended for beginners in mass spectrometry imaging (MSI) who have never experienced this discipline before.

This book is produced for the purpose of providing didactic recipes for the preparation of cryosections before MSI experiments.

We describe with as much detail as possible what we consider to be a good way to prepare your cryosections.

We also try to present you with some tips and possible alternatives that can be useful during this sample preparation step.

However, we do not claim to have exhaustively listed all existing sample preparation techniques as well as all the matrices available for the analysis of different types of molecules.

Freezing of Tissue

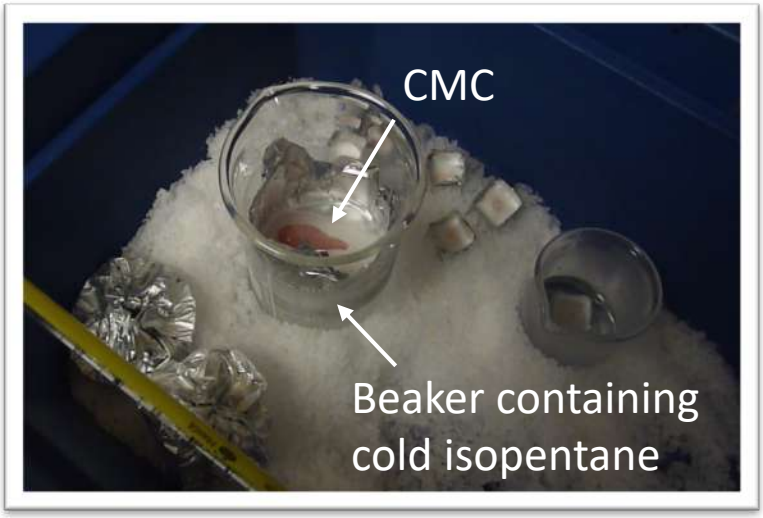
Cryosections prepared from fresh frozen tissue are best suited for MSI and the quality of tissue samples is crucial for obtaining the best results.

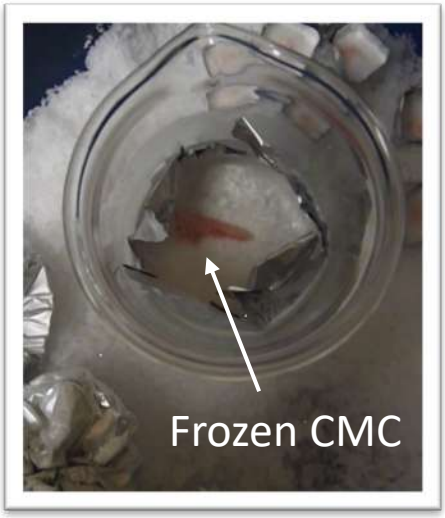
Tissue must be frozen immediately after harvesting with no additional treatment such as perfusion or fixation. If possible, blood and connective tissues should be removed to some extent.

Snap freezing in liquid nitrogen is the most commonly used technique. We recommend to loosely wrap the organ in aluminum foil and let the tissue float on liquid nitrogen for progressive freezing.

If the tissue is damaged during freezing in liquid nitrogen, alternatively you can use isopentane pre-cooled at -80°C and placed on dry ice.

Embedding of Tissue





Solid tissues such as brain, liver, kidney etc. can generally be cryosectioned without any embedding. However, embedding may be required to preserve the morphology of delicate tissues during freezing. Embedding media composed of 1% or 2% carboxymethyl cellulose (CMC) are compatible with MSI experiments. In contrast, embedding media such as optimal cutting temperature (OCT) polymer must be avoided. Indeed, polymers contained in OCT are highly detectable by mass spectrometry and can thus lead to ion suppression effects.

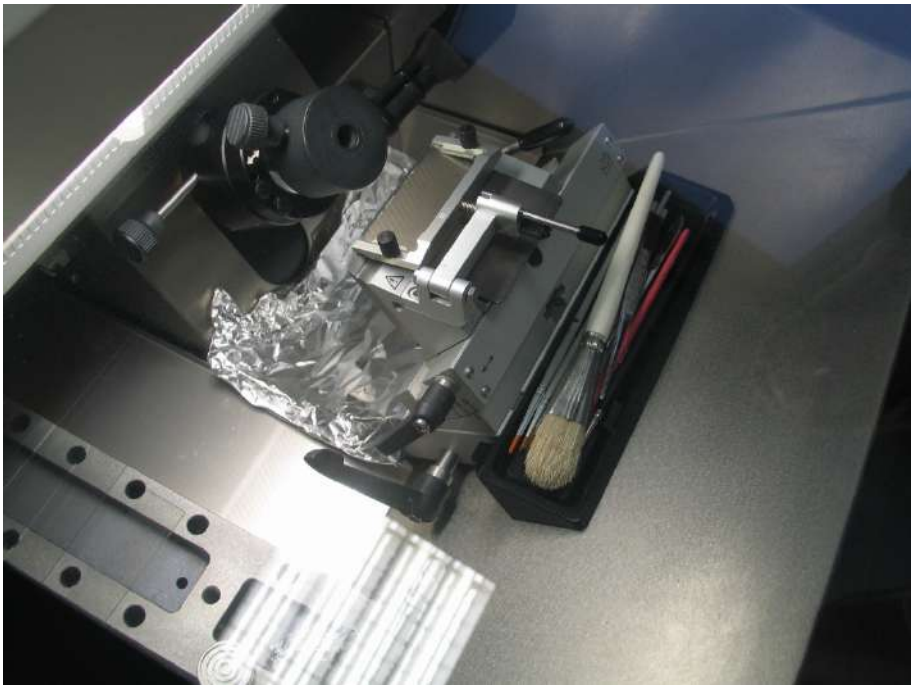
After freezing, tissues can be stored at –80°C for 1 or 2 years.

Preparation of the Cryostat

Prepare the cryostat by adjusting the chamber temperature at -20 °C and the specimen temperature between -10 °C and -20°C. The specimen temperature required for optimal cutting depend on each tissue. Select the desired tissue thickness (typically between 10 and 20 µm).

We recommend to transport the tissue on dry ice between the -80°C freezer and the cryostat. The tissue must be stored at chamber temperature (-20°C) for approximately 45 minutes before the start of cryosectioning.

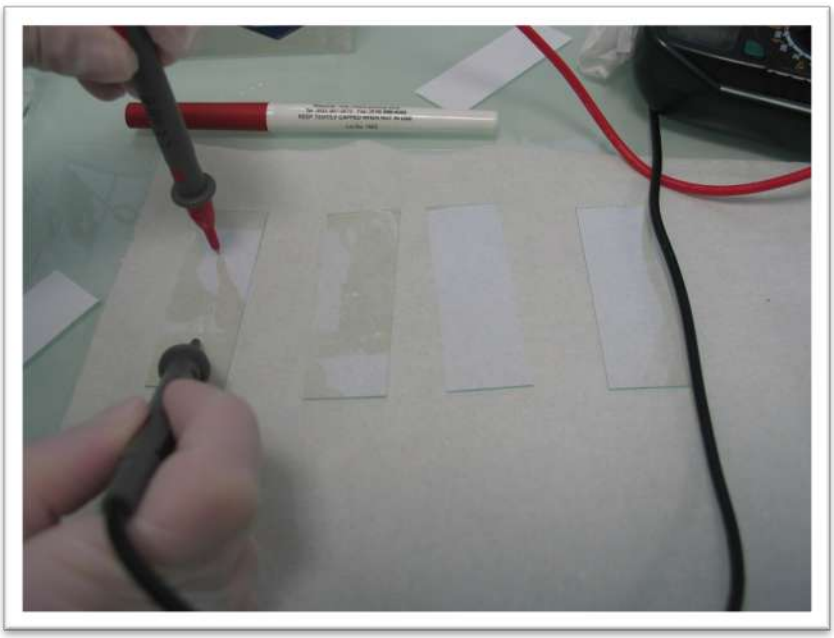
Preparation of the Cryostat



Place brushes, forceps and the tissue holder inside the cryostat and wait until they reach the cryostat temperature.

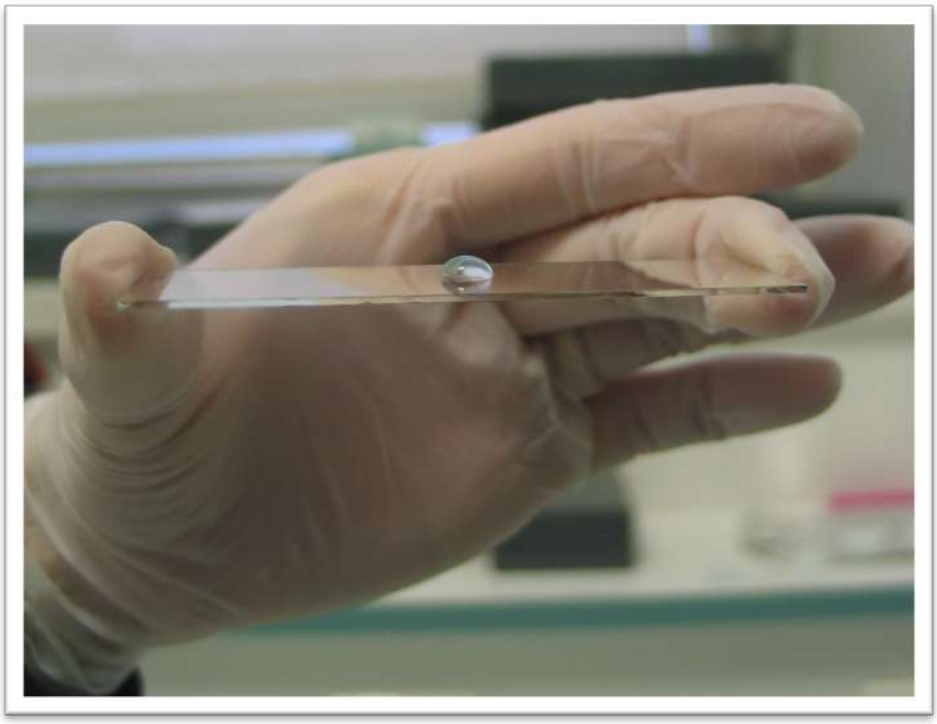
TRICK : To keep your cryostat clean, you can protect the chamber with Kim wipes or aluminium foil. Section debris can thus be very easily eliminated at the end of sectioning.

Preparation of Conductive Glass Slides

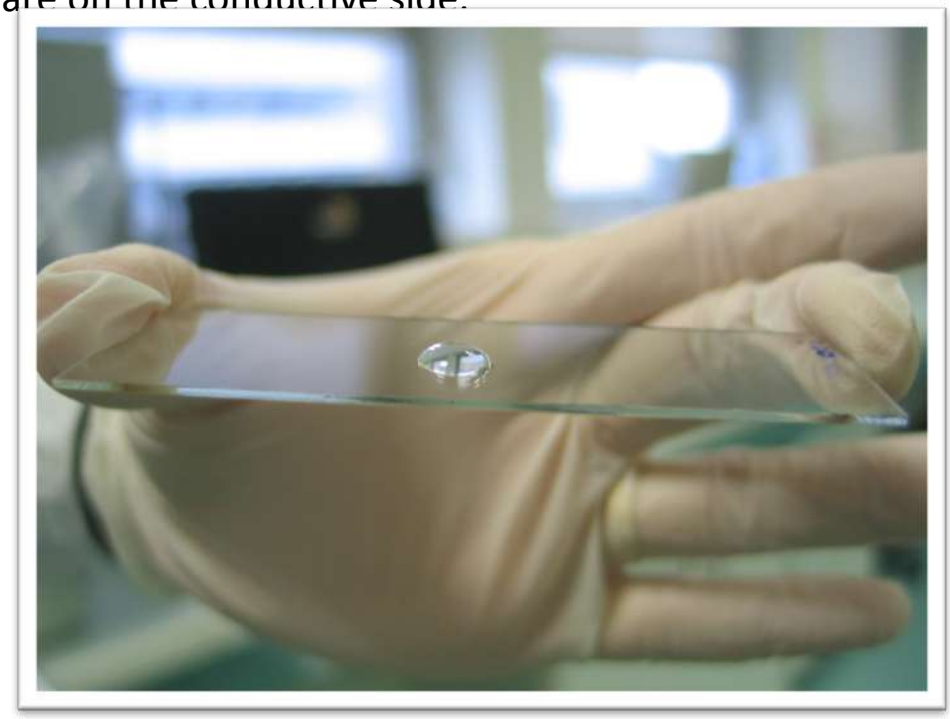


Tissue sections can be deposited onto MALDI steel targets or any type of conductive supports. In most cases, tissue sections are deposited onto conductive glass slides coated on one side with indium tin oxide (ITO). In the ITO slides package taken as an example, the conductive side is indicated by an arrow. The conductive side of ITO slides can be checked using a multimeter. Wash ITO slides with ethanol before use to remove dust or debris.

Preparation of Conductive Glass Slides

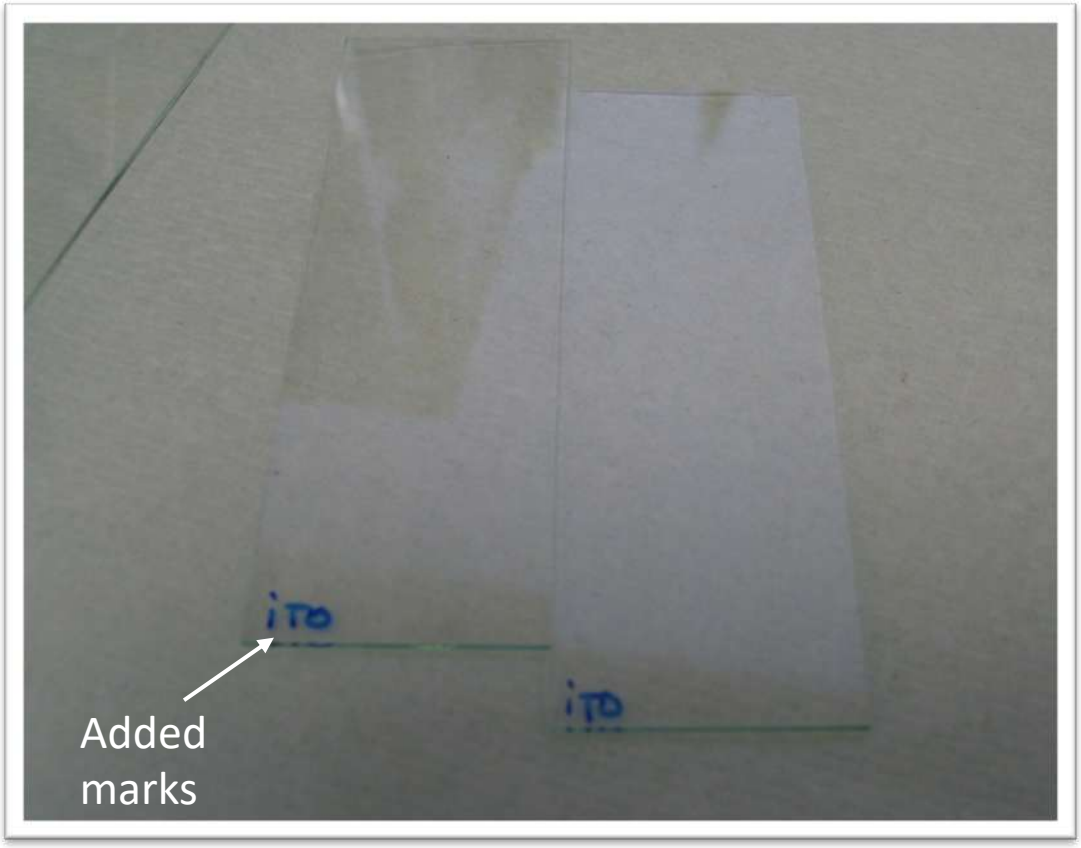


TRICK : As the conductive side is hydrophobic, you can also test the conductivity of the slide by depositing a drop of water (≈50 µL) on one of the two surfaces. If the drop of water is perfectly rounded and has no resistance to flow over the surface, you are on the conductive side.



TRICK : On the contrary, if the drop of water spreads out and seems to be more resistant to flow, you are on the non-conductive side.

Preparation of Glass slides for MALDI Imaging



TRICK : Prior to deposition of tissue sections on the ITO slide it is necessary to apply a characteristic and non-symetric mark on the conductive side.

Preparation of Histological Slides

Few histological stains are compatible with subsequent MSI. Histological staining is generally performed on a consecutive section collected on a standard glass slide (ie non-conductive glass slide). It is then possible to superimpose a microscopic image of the stained section with the MALDI image. Wash histological slides with ethanol before use.

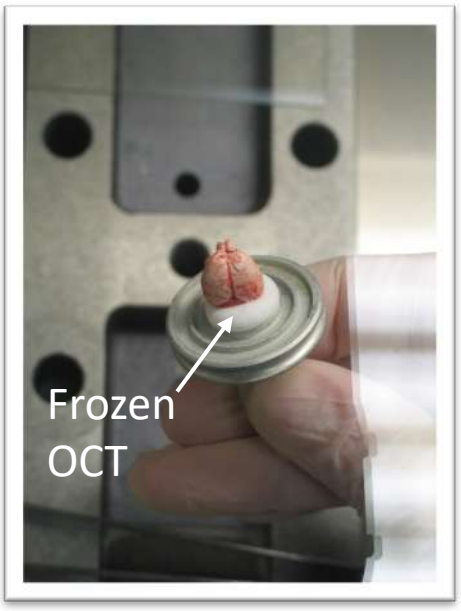
TRICK : If the ITO slide analyzed by MSI has been washed with alcohol solutions before matrix deposition (typically performed for MSI of proteins), you can remove the matrix by dipping the slide in ethanol for few seconds and then stain it.

Tissue Mounting

Place the conductive ITO glass slides (and histological slides, if needed) into the chamber of the cryostat.
Take your sample out of the aluminium foil.

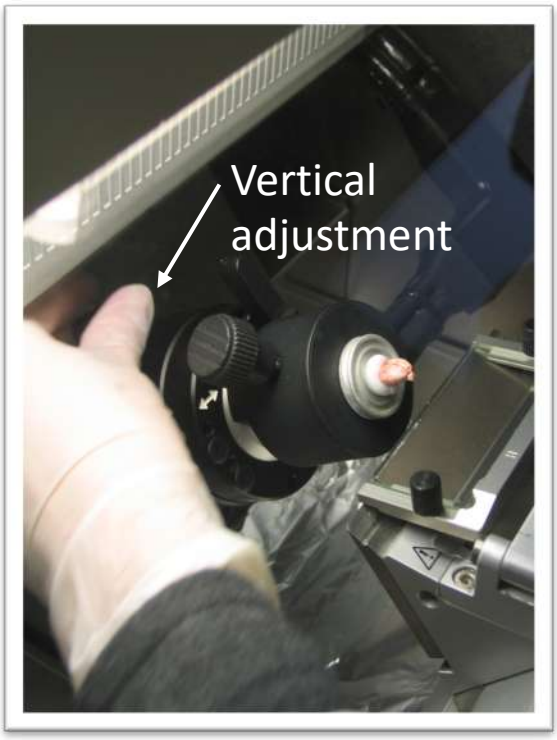
Tissue Mounting

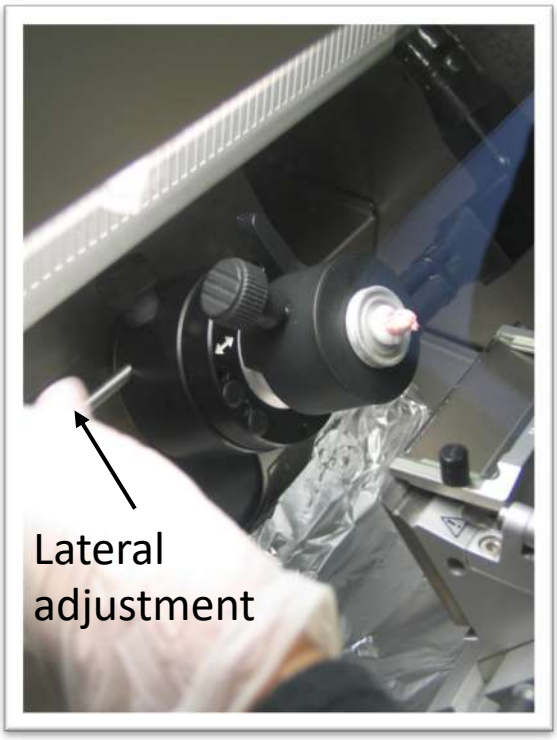
Deposit a drop of OCT on the tissue holder and place it in the chamber of the cryostat. Avoid any contact between OCT and regions of the tissue from which sections will be collected.



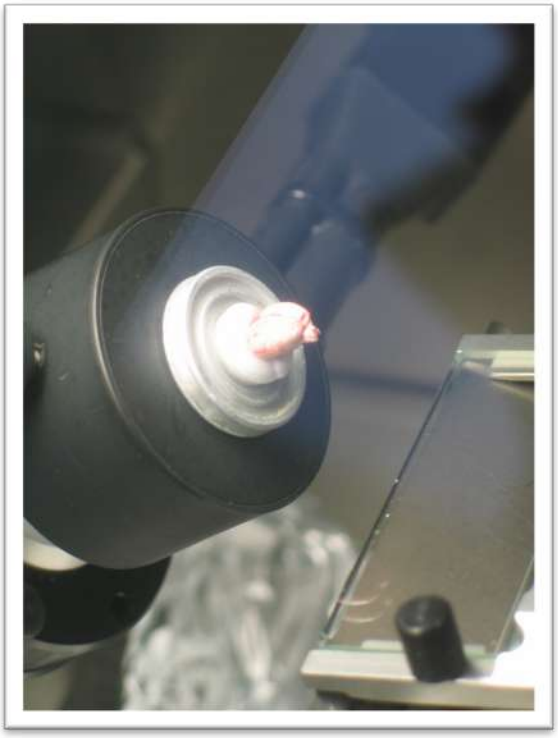
Promptly place the organ on the tissue holder and adjust its orientation before solidification of OCT. After a few minutes, OCT is totally frozen (and turns white) : the tissue is ready for sectioning.

Adjustment of Tissue Orientation

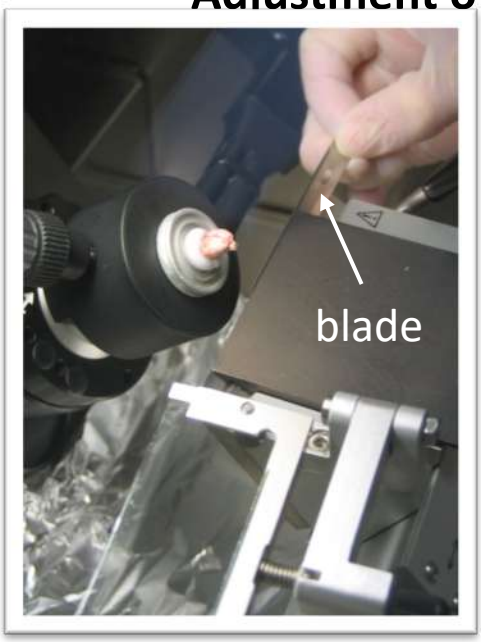




Place the tissue holder on the specimen head. Depending on the cryostat model, you can adjust the orientation of the specimen head, as illustrated below.



Installation of the Blade and Adiustment of the Anti-roll Guide



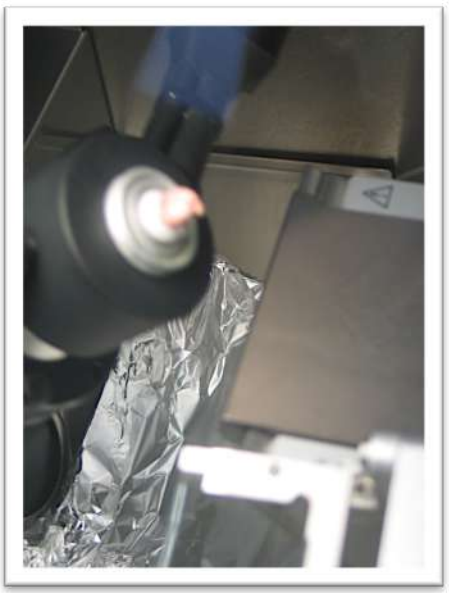
Wipe the cryostat blade with ethanol to remove contaminants. Insert the blade in the knife holder and lock the blade in place (right picture).

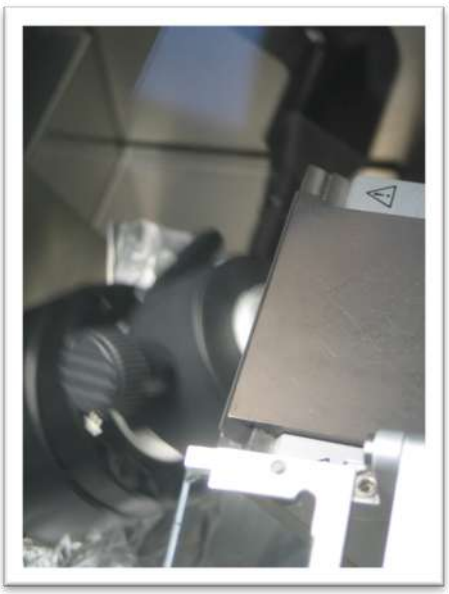
Place the anti-roll guide onto the blade. Carefully check the fine alignment of the anti-roll guide relative to knife edge.

TRICK : A thin bright line appears on the knife edge when the adjustment is correct.

Cryosectioning

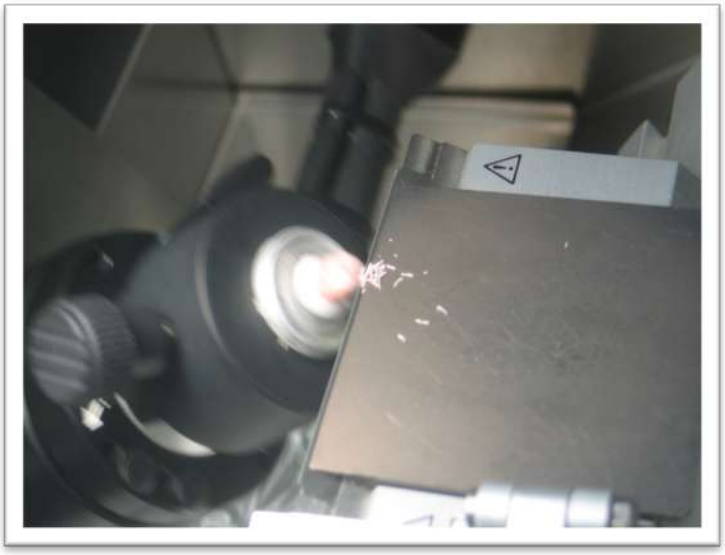
We recommend to wait for 15 minutes to allow temperature equilibration before starting cryo-sectionning.





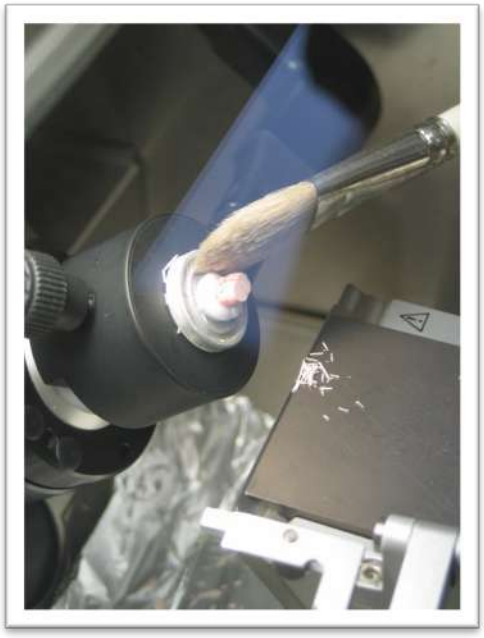
Move the anti-roll guide away from the knife. Move forward the specimen head using the four coarse feed keys (or any other command at your disposal depending on the instrument used) to bring the specimen close to the knife.

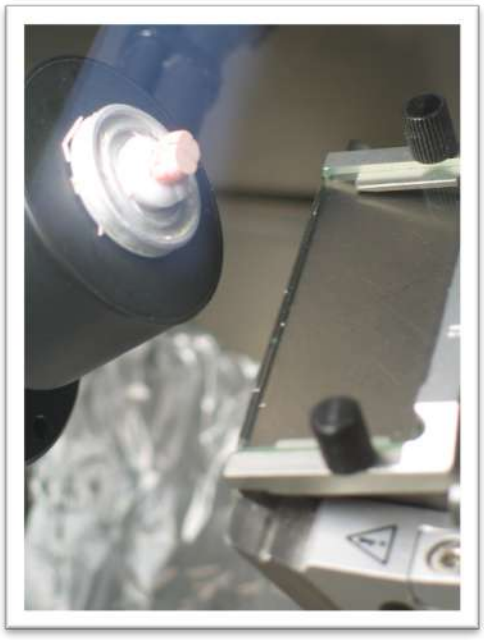
Cryosectioning

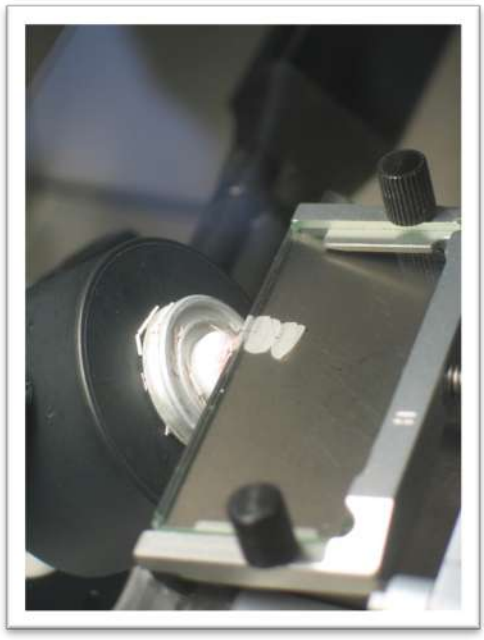


Rotate the handwheel to trim specimen down to the desired sectioning plane. You can use a higher section thickness (30-50 µm) to go faster (use the trim option if available).

Cryosectioning

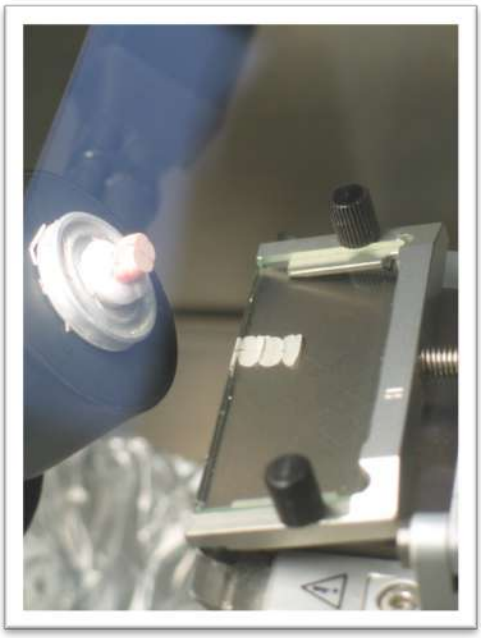


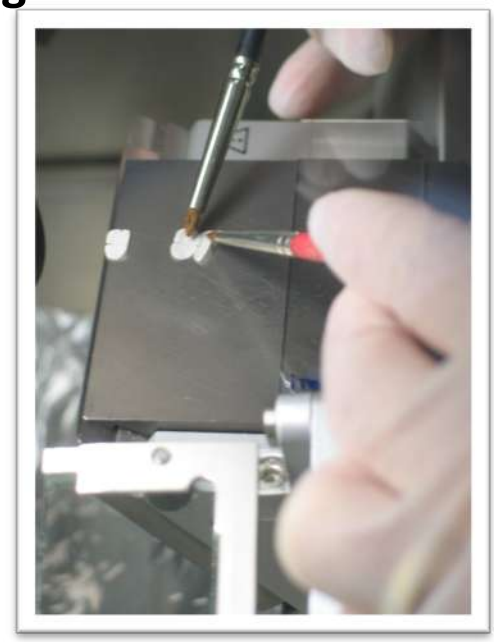




Once the region of interest is reached, gently remove all section debris using a large brush. Select the desired section thickness (10-20 µm). Place the anti-roll guide on the knife and discard the first 2 to 3 sections before collecting the sections.

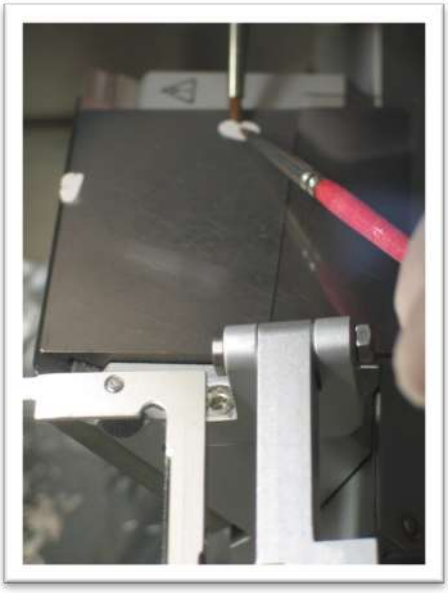
Section Mounting on ITO slides

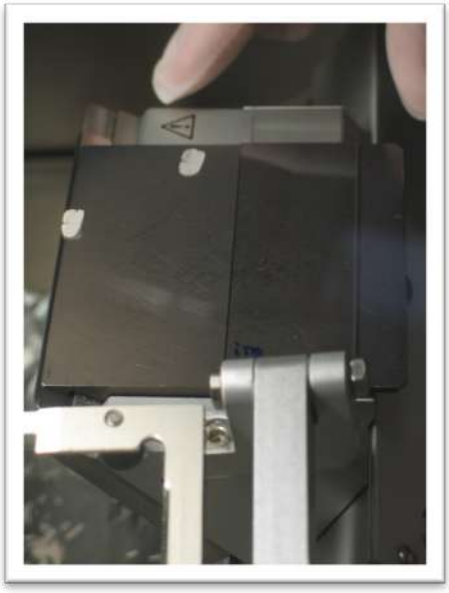


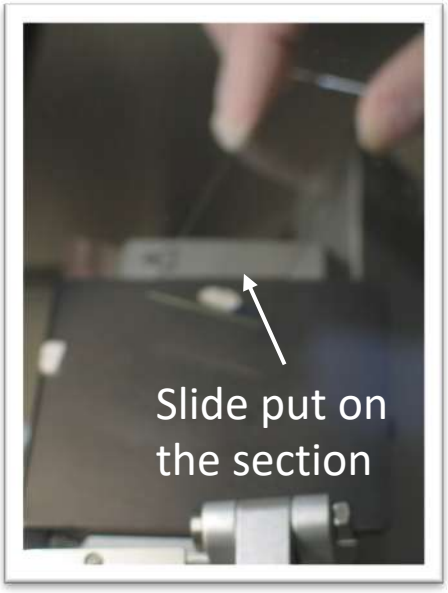


You are now ready to transfer a section onto the conductive side. There are two methods to do that.

Method 1 : Isolate a section with the brush, put the conductive side of the slide onto the section and flip the conductive slide.



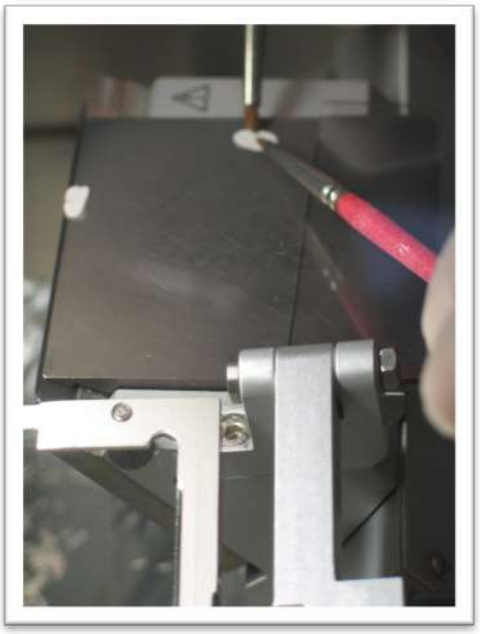


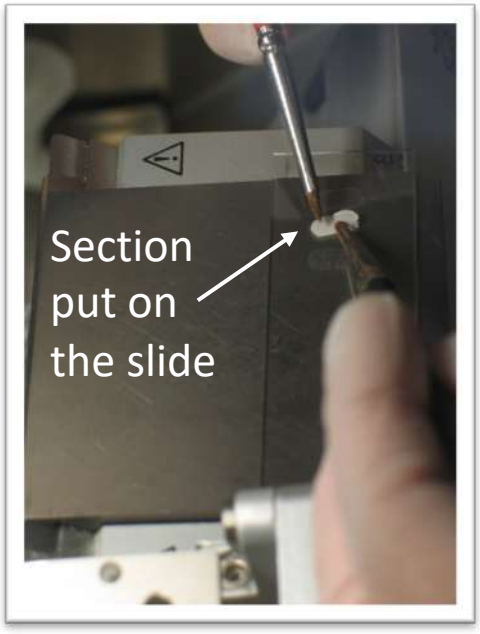


TRICK : If you deposit another section on the same conductive slide with this method, avoid any contact of the first section with the cold metal plate to prevent damage of the first section.

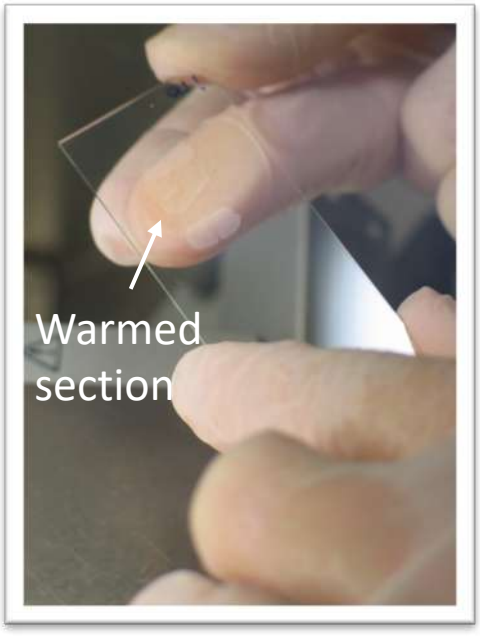
TRICK : Transferring of the section onto a warm slide is commonly used in histology but does not work very well with ITO slides (from our experience).

Section Mounting on ITO slides





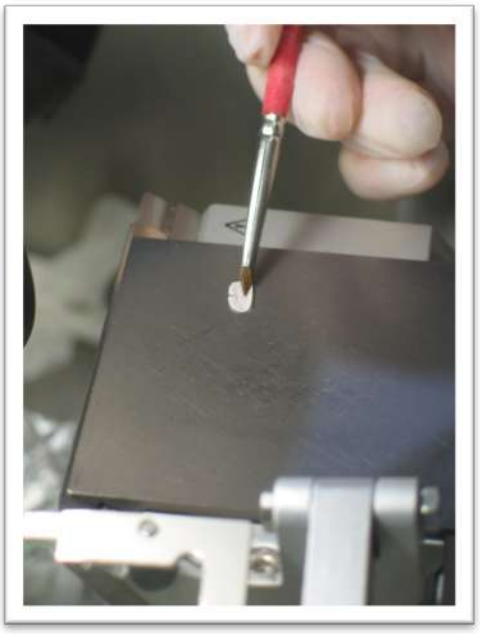
Method 2 : Grab a section with a brush and put it on the conductive side of the slide. This technique can be used with a steel target.

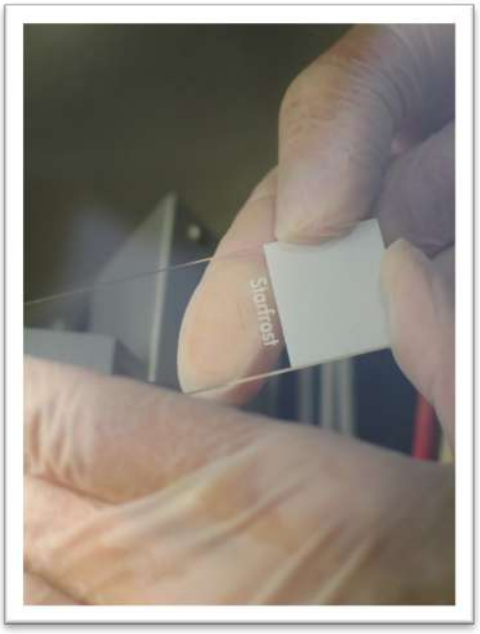


With both methods, inside the cryostat, place your finger on the non-conductive side back to the section to warm it. Wait until the section appears to be fully dry. You can put the slide back to the chamber and prepare another section.

TRICK : If you do not wait enough, the section can frost when you put the slide back to the chamber. This lead to very poor signals in MSI.

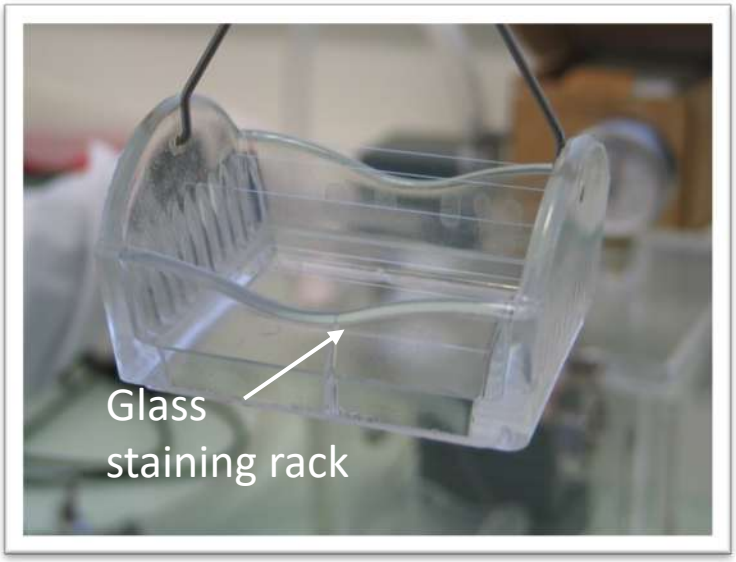
Section Mounting on Histological Slides





If histological staining is needed for annotation of the section, repeat the operation with the adjacent section on a standard glass slide (non-conductive slide).

Section Drying



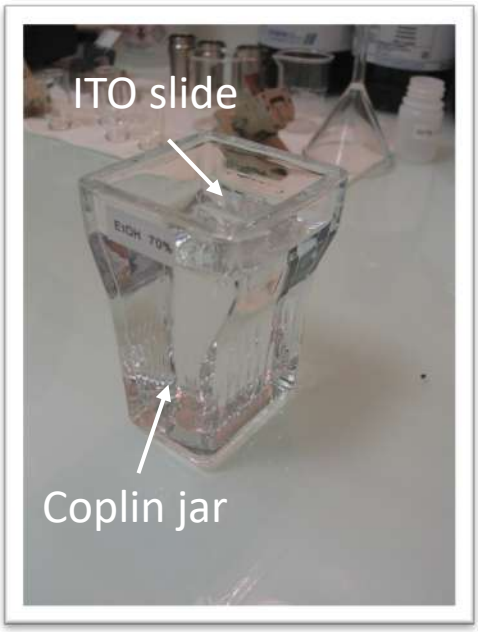
Once all desired sections have been collected, take the slides out of the cryostat.

TRICK : Warm the slides few seconds with your hand just before taking the slides out of the cryostat to avoid condensation.

Desiccate the slides vertically for 5 to 15 minutes. Glass staining rack is ideal for desiccation and transport of the slides.

TRICK : Depending on the tissue, up to 45 minutes of desiccation can be required.

Washing before MALDI Imaging of Proteins



If MSI of proteins is planned, the section must be washed after desiccation to remove interfering compounds (salts, lipids…). Plenty of washing procedures are described in the literature, we just present here a basic procedure that can be used at first intention.

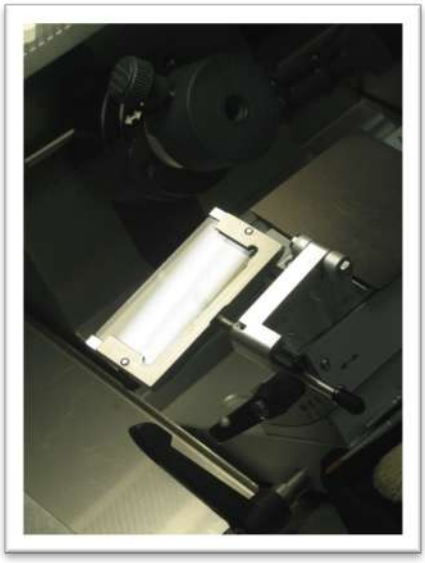
Prepare two Coplin jars (or Petri dishes) with 70% ethanol and one with 100% ethanol. Dip the slide twice in 70% ethanol for 60–120 seconds and once for 30–120 seconds in 100% ethanol.

After washing, the slide must be desiccated for another 5-15 minutes.

TRICK : If you are interested in small peptides (e.g. neuropeptides), decrease the washing times. If you are interested in drugs or lipids, you can skip this washing step.

Cryostat Cleaning



Don’t forget to clean your cryostat !

Remove the blade from the knife holder : this step is mandatory to clean the chamber and all other parts of the cryostat in safe conditions.

Remove all section debris and remove the specimen holder.

Desinfect all parts : knife holder, anti-roll glass, specimen holder arm and chamber with 70% ethanol.

Storage

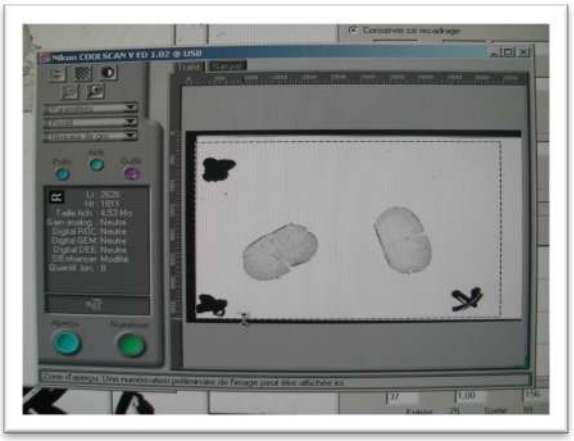
Short term storage : Desiccated sections can be stored under vacuum for 1 week.

Long-term storage : Slides can be stored at -80°C after desiccation. When you take the slides out of the freezer, place them in the desiccator for 5 minutes. The slide can then be washed if needed and treated as other slides.

Slide Scanning

The slides must be scanned before matrix deposition. Teach marks are required for the correlation of the optical image with the sample in the instrument, it is therefore necessary to have teach marks that are visible both on the optical image and underneath the matrix layer in the camera optic. The easiest way is to spot at least three correction fluid (e.g. Tipp-Ex®) marks around the sample before taking the optical image.

Slide Scanning



To acquire an optical image, any device that is capable of taking a picture can be used. Specific considerations apply to different possible devices (such as microscopes, gel-scanners, office scanners or digital cameras).
It will usually be necessary to adjust the brightness and contrast settings for the acquisition of the optical image (e.g. in the scanning application) or to adjust these settings after the acquisition.

Your slide is ready for matrix deposition !

Examples of Matrices

HCCA — α- Cyano- 4- hydroxycinnamic acid. HCCA enables highly sensitive MALDI- TOF-MS measurement of peptides and proteins from 0.7 to 20 kDa.

2,5-DHB —2,5-Dihydroxybenzoic acid. 2,5-DHB can be used for MALDI-TOF-MS analysis of a wide variety of peptides, proteins, polymers and carbohydrates, including phosphopeptides and glycoproteins.

2,5-DHAP — 2,5-Dihydroxyactetophenone. 2,5-DHAP is a MALDI matrix used for preparations of proteins with a mass of 8–100 kDa. 2,5-DHAP prevents ISD fragmentation and is recommended for proteomic profiling studies and for the analysis of glycoproteins.

SA — Sinapinic acid (trans-3,5-dimethoxy-4-hydroxycinnamic acid). SA is a good choice for analysis of larger proteins (10–150 kDa) and some polar polymers. It is also suitable for generation of ISD spectra of intact proteins. Small peptides (<3 kDa) may not produce strong signals with SA, and in such cases we recommend using HCCA as a MALDI matrix

SDHB — 90:10 mixture of 2,5-DHB and 2-Hydroxy-5-methoxybenzoic acid. We recommend using SDHB MALDI matrix instead of 2,5-DHB for MALDI-TOF- MS analysis of very large proteins and glycoproteins. SDHB is also suitable for the generation of ISD spectra of intact proteins.

3-HPA — 3-Hydroxypicolinic acid. 3-HPA has proved useful as a MALDI matrix material for the analysis of mixed oligonucleotide samples (DNA/RNA) between 1 and 30 kDa.

1,5-DAN — 1,5-Diaminonaphthalene. 1,5-DAN effectively promotes reduction of disulfide bonds in the gas phase. This greatly facilitates analysis of proteins and peptides containing disulfide linkages in top-down sequencing of intact proteins (ISD; T3).

9-AA — 9 Aminoacridine. 9-AA can improve detection and characterization of a wide range of small molecules (low molecular weight compounds having acidic protons, such as phenols, carboxylic acids, sulfonates, amines and alcohols) and lipids in negative polarity.

Dithranol — 1,8,9-Anthracenetriol. Dithranol can improve detection and characterization of a wide range of small molecules and lipids in positive polarity.

DCTB — trans – 2 - [3 - (4 – tert - Butylphenyl) – 2 – methyl – 2 - propenylidene] malononitrile. DCTB can be used for the detection of organic compounds in negative mode

NMR — Norhamane. NRM can improve detection and characterization of a wide range of lipids in both positive and negative polarities, and can be used as matrix for analysis of cyclodextrins and for sulfated oligosaccharides in combination with DHB as co-matrix.