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Document Abstract

Description of standardized sample for sample preparation for MALDI FTICR imaging

Table of Content

1. General Introduction

Standardized protocols are required to obtain reliable results at the optimum operating conditions. Specific case treated here is MS imaging. Unfortunately, ion mobility (IM) was planned to be delivered by Bruker at the beginning of the EU_FT-ICR_MS project is still not available as the company has finally chosen to not implement this equipment on the current generation of FT-ICR mass spectrometer. Consequently, this deliverable does not include a protocol for ion mobility (IM) on FT-ICR MS.

2. Analysis of bacterial strains

2.1 Application field

MALDI mass spectrometry imaging (MSI) allows the mapping and tentative identification of compounds based on their m/z ratio. In typical MSI, a spectrum is taken at incremental 2D coordinates (pixels) across a sample surface. Single pixel mass spectra show the resolving power of the mass analyser. [1]

2.2 Materials

2.2.1 Reagents

Chemicals

- MALDI matrix α-cyano-4-hydroxycinnamic acid (HCCA)

Solvents

- Acetonitrile (ACN)
- Water
- Trifluoroacteic acid (TFA)

2.3 Sample Preparation

(1) Spot five microliters of bacterial cell suspension on a semisolid agar-based root exudates mimicking medium.
(2) Incubate bacteria for 48 h at 25 °C.
(3) Cut the microbial colonies on agar and region of interest from the Petri dish.
(4) Transfer the samples to the target ITO plate, previously covered with double-sided conductive copper tape.
(5) Place the assembly in a vacuum desiccator at 70 mbar until dryness overnight.

2.4 Matrix deposition

(1) Mix HCCA matrix in 70:30 ACN:water v:v with 0.2% TFA to reach a final concentration of 5 mg/mL.
(2) Spray, in total, 10 layers of HCCA matrix onto the ITO slides. As spray instrument, e.g., SunCollect instrument (SunChrom, Friedrichsdorf, Germany) can be used.
(3) Spray the first layer at a flow rate of 10 µl/min. Increase the flow for each of the following layers by 10 µL/min until reaching 60 µL/min.

2.5 Measurement Procedure

2.5.1 LDI Parameters

Positive mode

laser power	70 %
laser shots	20
laser shots frequency	5 kHz
pixel step size	100 µm

FT-ICR Mass Spectrometry Parameters

mass-to-charge ratio (m/z) range	300-2000
mode	positive mode, broadband

*For a 9.4 T FT-ICR MS with para cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

See the ad hoc paper with a full description of parameters to tune to get optimum resolution and minimum mass shift in draft paper: ***FTICR Mass spectrometry imaging at extreme mass resolving power using a dynamically harmonized ICR cell with 1ω or 2ω detection.***

2.6 References

[1] A. McCann, S. Rappe, R. La Rocca, M. Tiquet, L. Quinton, G. Eppe, J. Far, E. de Pauw, C. Kune. Mass shift in mass spectrometry imaging: comprehensive analysis and practical corrective workflow, *Anal Bioanal Chem*. **2021**, *413*, 2831–2844.

3. MALD-FT-ICR MSI of mouse brain tissues slices

3.1 Application field

Observing the spatial distribution of ions is information of choice for pharmaceutical and toxicological fields. Matrix Assisted Laser Desorption/Ionization-Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry Imaging (MALDI-FT-ICR MSI) offers a label free localisation method of tissue slices with a high spectral resolution and mass accuracy measurements. Such performances allow the discerning of many isobaric ions often encountered during the analysis of complex biological samples.

3.2 Materials

3.2.1 Reagents

Powders

- Calibrant : Red Phosphorus (□97%, Merck)
- MALDI matrix : α-cyano-4-hydroxycinnamic acid (>97%, Merck)

Solvants

- milliQ water
- Acetonitrile (HPLC grade, Biosolve)
- TriFluoroacetic Acid (99%, Merck)
- Acetone (HPLC grade, Merck)
- Ethanol (70%, Biosolve)
- Methanol (technical grade, Biosolve)

3.3 Equipment

Instruments

- FT-ICR : solariX XR 9.4T with Paracell cell (Bruker Daltonics)
- Cryo-microtome : CryoStar NX70 (ThermoScientific)
- Automatic Sprayer : SunCollect (SunChrom)
- Analytical balance
- Vacuum desiccator

Software

- ftms control 2.2.0 (Bruker Daltonics GmbH)
- Bruker Compass Data Analysis 5.0 (Bruker Daltonics GmbH)
- FlexImaging 5.0 (Bruker Daltonics GmbH)
- SCiLS Lab 2016b or above (SCiLS)
- SunCollect Spray Control v2.6

Consumables

- ITO glass slides (Bruker Daltonics)
- Disposable spatulas (VWR)
- 2mL Eppendorf (Fisher)
- SEC 35e low profile blades (Fisher Scientific)
- Tissue-Tek NEG50 (Fisher Scientific)

Further equipment

- MTP slide adaptor

- Scanner
- Laboratory gloves
- Utrasonic bath
- 30mm specimen chuck
- Forceps
- Brushes

3.4 Sample Cutting Procedure

Cut process of a mouse brain tissue destined to mass spectrometry imaging.

3.4.1 Preparation

1) Wear gloves to avoid contamination of the sample and equipment.
2) Place the frozen tissue in the Cryostar NX70 enclosure to come up to the instrument temperature for 10 to 15 minutes.
3) Set the sample holder temperature to -20°C and the blade temperature to -12°C.
4) Place a pair of forceps inside the enclosure to cool down.
5) Clean an ITO glass slide with ethanol 70% then place in the Cryostar NX70 enclosure to come down to the instrument temperature.
6) Place with caution a SEC 35e low profile blade in the razor blade holder.
7) Put a Tissue-Tek NEG50 on a 30mm specimen chuck and place the chuck inside the Cryostar NX70 enclosure.
8) When the Tissue-Tek NEG50 starts to solidify, quickly dispose the sample on the Tissue-Tek NEG 50 with the cooled pair of forceps.
9) When the Tissue-TEK NEG50 has completely solidified place the specimen chuck on the cryostat sample holder.
10) If needed, correct the cutting incidence in the x and y axis using top and side knobs of the sample holder.
11) Set the sample cutting size to 12µm and bring the blade stage close to the sample using the left control know or the right crank.
12) If needed, trim the sample until a complete sample slice can be obtained or until the region of interest is reached. During the trimming process a bigger cutting size can be set to accelerate the process.
13) Perform a slice at 12µm by rotating the crank 360° while the anti-roll glass slide is down on the razor blade.
14) Lock the crank for safety and move the anti-roll glass slide to the side gently. Use a brush to gently remove the slice from the anti-roll glass slide if stick together.
15) Using brushes relocate the tissue slice on the sample plate for easier collection.
16) Warm up for two seconds a portion of an ITO glass slide by placing our thumb of the back of the slide (thumb on the non-coated side).
17) Press the heated up ITO coated side of the slide in contact with the sample to collect it. If a tissue sample is already on the ITO glass slide, avoid making a second contact of already collected samples with the sample plate.
18) Use your thumb again on the back of the ITO glass slide, while keeping it in the cryostat enclosure, to make the tissue slice completely adheres to the ITO glass slide.
19) Repeat step 13 to 18 until enough tissues slices have been collected.
20) Clean the cryostat by throwing unused slices in an adequate manner and clean the cryostat with ethanol 70%.

21) Keep ITO glass slides with sample at -80°C for storage or continue with the MALDI-Matrix Spray Procedure for mass spectrometry imaging acquisition.

3.5 MALDI-Matrix Spray Procedure

Spray process to cover an ITO glass slide with a coating of approximatively 5nm/mm² of α-cyano-4-hydroxycinnamic acid (HCCA).

3.5.1 Preparation

1) Wear gloves to avoid contamination of the sample and equipment.
2) Place ITO glass slides with samples in a desiccator for 10 minutes or until complete dryness.
3) Switch on the analytical balance and allow it to stabilize. Set the initial value to zero.
4) Weight an empty 2mL Eppendorf and set value to zero.
5) With a disposable spatula add around 8mg of HCCA in the Eppendorf.
6) Depending of the quantity of HCCA calculate the required amount of solvent to obtain a concentration of 5mg/mL.
7) Prepare excess amount of Acetonitrile / milliQ water / TriFluoroacetic Acid mixture in proportion 70 / 29.9 / 0.1.
8) Add calculated volume of the mixture to the Eppendorf containing the matrix.
9) Agitate the Eppendorf until the matrix is completely dissolved. To accelerate this process and ensure that no particles are left place the Eppendorf 5 minutes in an Ultrasonic.

3.5.2 MALDI-Matrix coating

1) Switch on the compressor linked to the SunCollect on position “II” and set the pressure to 2 bars.
2) Switch on the SunCollect and the connected computer.
3) Check the remaining amount of wash/waste solution and refill/dispose bottles if necessary.
4) Place the Eppendorf with MALDI matrix in the SunCollect.
5) Insert the tubing connected to inlet “F” inside the Eppendorf.
6) Place ITO glass slide in one of the SunCollect slide adapter or sample plate (

7) Figure 1).

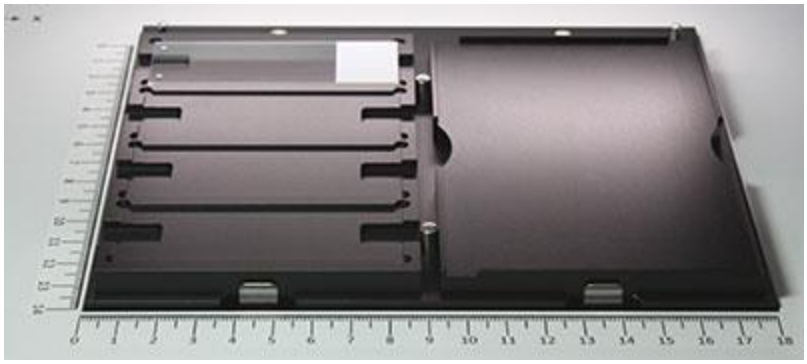

Figure 1 SunCollect slide adapter (right) and sample plate (left)

8) Open SunCollect Spray Control v2.6.
9) Select an existing spray method by clicking on “Load Method” or create a new method using the software “Method” menu. Used spray parameters are available in Table 1.

Table 1 Spray Method parameters corresponding to a coating around 5nM/mm².

z	(mm)	25
Line distance	(mm)	2
Speed while spraying	(mm/min)	600
Inlet Port		F(F)
# Layer		7
Drying time	(s)	0
Flow Rate		Variable
Flow Rate #1	(µL/min)	5
Flow Rate #2	(µL/min)	10
Flow Rate #3	(µL/min)	20
Flow Rate #4 to 7	(µL/min)	40

10) Select the region to spray on with “Load format 1” and a preconfigured existing format of the SunCollect slide adapter or by entering x1;y1 and x2;y2 coordinates while keeping a 0.5cm margin (Figure 2) in the “Format” menu.

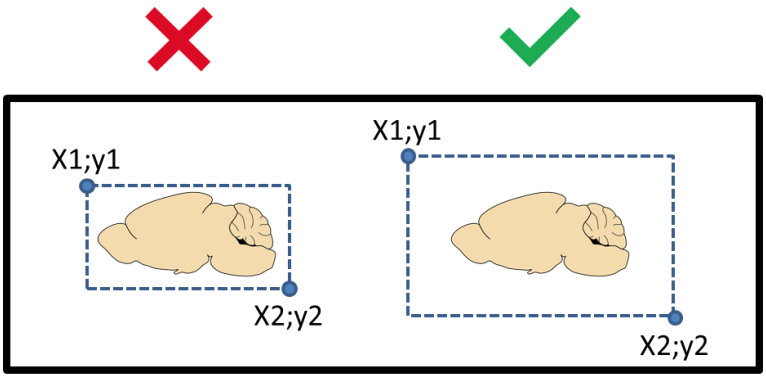

Figure 2 Schematic representation on how to properly select the spray area on the SunCollect

11) When the method and the spray area are selected press on “Start” to launch the spray process.
12) When the spray process is finished, unless another coating is planned is the coming minute, launch the “cleaning system” process and follows software instruction.
13) Clean the SunCollect with the wash solution or methanol technical grade.
14) Shut down the compressor, SunCollect and computer.
15) Place the coated ITO glass slide in an MTP slide adaptor II for MS acquisition or store at -20°C if immediate analysis is not required.

See the ad hoc paper describing parameters to get optimum resolution and minimum mass shift in draft paper: ***FTICR Mass spectrometry imaging at extreme mass resolving power using a dynamically harmonized ICR cell with 1ω or 2ω detection.***

4. co-Crystallisation methods for lipids samples

4.1 Application field

MALDI mass spectrometry imaging (MSI) allows the mapping and tentative identification of compounds based on their m/z ratio. In typical MSI, a spectrum is taken at incremental 2D coordinates (pixels) across a sample surface. Single pixel mass spectra show the resolving power of the mass analyser. [1]

4.2 Materials

4.2.1 Reagents

Chemicals

- MALDI matrix 2,5-Dihydroxybenzoic acid (DHB)

Solvents

- Chloroform
- Ethanol
- Ethyl acetate
- Formic acid
- Hexane
- Methanol

4.3 Sample Preparation

Method 1: heterogenous crystallization

(1) Dilute lipid sample if necessary for example in an ethanol-chloroform mixture (50:50 v:v).
(2) Dilute DHB matrix at 100 mg/mL in an ethyl acetate-hexane-formic acid mixture (98.9:1:0.1 v:v:v).
(3) Spot 1 µL of the lipid sample onto an ITO slide.
(4) Spot 1 µl of matrix solution onto the sample, which is mixed thoroughly with the dried droplet crystallization method.
(5) Dry the sample under air at room temperature.

Method 2: homogenous crystallization

(1) Dilute lipid sample if necessary for example in an ethanol-chloroform mixture (50:50 v:v).
(2) Spot 1 µl of the lipid sample on an ITO slide.
(3) Dilute DHB matrix at 100 mg/mL in an ethyl acetate-hexane-formic acid mixture (98.9:1:0.1 v:v:v).
(4) Spray 25 layers of matrix onto the sample with a flow rate of 5µl/min. As spray instrument, for example, a HTX TM-Sprayer (HTX Technologies, LLC, Chapel Hill, NC, USA) can be used.

Method 3: homogenous crystallization

(1) Dilute lipid sample if necessary for example in an ethanol-chloroform mixture (50:50 v:v).
(2) Spot 1 µl of the lipid sample on an ITO slide.
(3) Dissolve 50 mg of DHB matrix in 2 mL of methanol.
(4) Apply the matrix by sublimation using, for example, an HTX Sublimator (HTX technologies, Chapel Hill, NC, USA). Evaporate the solvent during Preheating at 65 °C. Sublimate the DHB matrix at 160 °C under vacuum for 160 s.

4.4 FTICR acquisition parameters

Sets of parameters used in the original and re-optimized method. The laser power was adjusted to get the lower power possible when the TIC signal was reaching 5×10^8 cps.

Parameters	(Unit)	solariX XR		scimaX 2XR 1 or 2ω	
		Original	Re-optimized	Original	Re-optimized
Laser focus[a]	%	98	80	93	85
Laser shots (#shots)		600	[2 ; 10]	400	6
Laser frequency	(Hz)	1000	#shots×10	1000	60
Sweep excitation power	(%)	22	[16 ; 18]	20	18
Front & back trap plate	(V)	1.5	1.35	3	3.06
Analyzer entrance	(V)	-10	-10	-10	-10
Side Kick	(V)	5	[6 ; 10]	0.2	3
Side Kick Offset	(V)	-1.0	-1.5	-1.5	-1.5
Time of flight	(ms)	1.2	1.2	1.0	0.7[b]

a small and medium laser focus for solariX XR and the scimaX 2XR, respectively

b time of flight set at 0,7ms for the 2ω acquisition for 16M data point only

4.5 References

[1] A. McCann, S. Rappe, R. La Rocca, M. Tiquet, L. Quinton, G. Eppe, J. Far, E. de Pauw, C. Kune. Mass shift in mass spectrometry imaging: comprehensive analysis and practical corrective workflow, *Anal Bioanal Chem*. **2021**, *413*, 2831–2844.

5. Workflow for brain tissues imaging

5.1 Application field

MALDI mass spectrometry imaging (MSI) allows the mapping and tentative identification of compounds based on their m/z ratio. In typical MSI, a spectrum is taken at incremental 2D coordinates (pixels) across a sample surface. Single pixel mass spectra show the resolving power of the mass analyser. [1]

5.2 Materials

5.2.1 Reagents

Chemicals

- MALDI matrix α-cyano-4-hydroxycinnamic acid (HCCA)

Solvents

- Acetonitrile (ACN)
- trifluoroacteic acid (TFA)
- Water

5.3 Sample Preparation

For brain tissue imaging, homogenate slices of the tissue are needed. These slices can be obtained by cryosectioning using, for example, a cryomicrotome (Cryostar NX70, Thermo Fischer Scientific, Waltham, MA, USA). [1]

(1) Set temperatures at -20 °C for the sample holder and -10 °C for the SEC 35e razor blade.
(2) Cut tissue slices at a thickness of 8-16 µm depending on brain tissue type.
(3) Mount the thawed slices onto indium-tin-oxide (ITO) coated slides.
(4) Dry the samples in a vacuum desiccator for 15 min or until visible dryness.

5.4 Matrix deposition

(1) Mix HCCA matrix in 70:30 ACN:water v:v with 0.2% TFA to reach a final concentration of 5 mg/mL.
(2) Spray, in total, 10 layers of HCCA matrix onto the ITO slides. As spray instrument, e.g., SunCollect instrument (SunChrom, Friedrichsdorf, Germany) can be used.
(3) Spray the first layer at a flow rate of 10 µl/min. Increase the flow for each of the following layers by 10 µL/min until reaching 60 µL/min.

5.5 Measurement Procedure

5.5.1 LDI Parameters

Positive mode

laser focus	minimum, small
laser power	70 %
laser shots	100-600
laser shots frequency	200-1000 Hz
pixel step size	60-100 µm

FT-ICR Mass Spectrometry Parameters

mass-to-charge ratio (m/z) range	300-2000
mode	positive mode, broadband
resolution	1000 k @ m/z 800 @ 8M
transient length	2-8 M

*For a 9.4 T FT-ICR MS with para cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

See the ad hoc drat paper describing parameters to get optimum resolution and minimum mass shift in draft paper: ***FTICR Mass spectrometry imaging at extreme mass resolving power using a dynamically harmonized ICR cell with 1ω or 2ω detection.***

5.6 References

[1] A. McCann, S. Rappe, R. La Rocca, M. Tiquet, L. Quinton, G. Eppe, J. Far, E. de Pauw, C. Kune. Mass shift in mass spectrometry imaging: comprehensive analysis and practical corrective workflow, *Anal Bioanal Chem*. **2021**, *413*, 2831–2844.