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NEDC matrix application for metabolite imaging using MALDI-MSI

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Protocol status: Working

We use this protocol and it's working

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Abstract

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has emerged as a analytical technique more than 30 years ago. Originally developed as a method to analyze large biomolecules like proteins or peptides, MALDI-MS has over the years found a new application in the field of biological tissue imaging (MALDI-MSI).

MALDI-MS has been utilized as an imaging tool for biological tissues, giving insight into the spatial distribution of molecules within complex tissue samples. Metabolomics research, which focuses on the study of metabolites present in a biological system, can benefit from this technology.

Small molecules such as metabolites or lipids can be analyzed under the aspect of spatial distribution across tissue sections. This enables the investigation of metabolic pathways, biomarkers or the correlation of metabolic changes with specific tissue regions, uncovering potential links between metabolite variations and physiological or pathological conditions.

Despite its potential, untargeted metabolomics using MALDI-MSI still faces several limitations. One major challenge is the diversity of matrices and protocols which are used to enhance ionization efficiency and the lack of information on metabolome coverage within different types of methods. One matrix of interest is NEDC (N-(1-naphthyl)ethylenediamine dihydrochloride), which has gained recognition for its efficiency to visualize a broad range of metabolites.

This protocol provides a guide on how to prepare fresh frozen tissue samples for MALDI-MSI of metabolites using the NEDC matrix. It covers cryosectioning and preparation of tissue samples and provides a method for NEDC matrix application using the Sunchrom Sprayer.

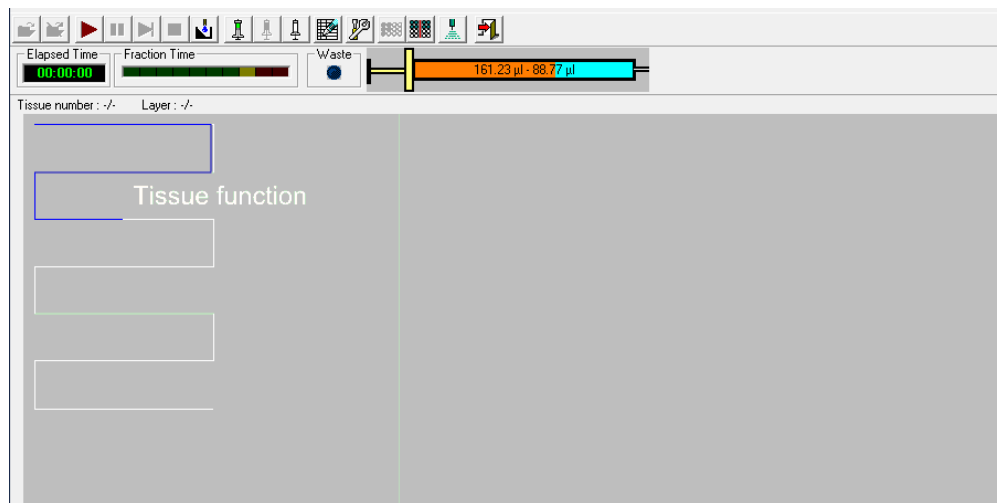
Sample preparation and cryosectioning

- 1 Remove fresh frozen tissue sample stored at -80°C and place in cryostat at -20°C (kidney)/ -17°C (liver)/ -22°C (brain)
- 2 Mount the sample on chuck by pipetting small droplets of water and placing the sample on top until the water is frozen
- 3 Make sections of $12\text{ }\mu\text{m}$ thickness
- 4 Collect the sections on an ITO slide (for MALDI-MSI) or a glass slide (for H&E staining) and thaw mount the tissue onto the slide by warming the slide with the back of your hand
- 5 Perform serial sectioning and collect the samples alternately on ITO and glass slides to allow for localization and orientation of each ion image generated in MALDI-MSI to its histological counterpart
- 6 Place the slides in a slide holder and store at -80°C

Matrix application

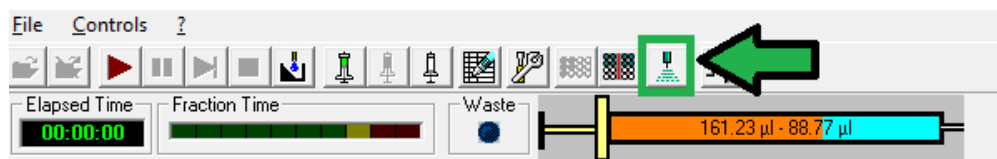
- 7 Dry the sample in a vacuum desiccator at -0.1 MPa until dry (20-25 min)
- 8 Prepare N-(1-naphthyl)ethylenediamine hydrochloride (NEDC) at a concentration of 7 mg/mL in $\text{MeOH}:\text{ACN}:\text{H}_2\text{O}$ (70:25:5)
- 9 Turn on sprayer, pump (35 psi) and the connected computer
- 10 Take $250\text{ }\mu\text{L}$ syringe, fill it with MeOH and rinse the lines of the sprayer manually
- 11 Open the Suncollect software and enter the sprayer settings and coordinates of your tissue (based on the size of your sample)

11.1



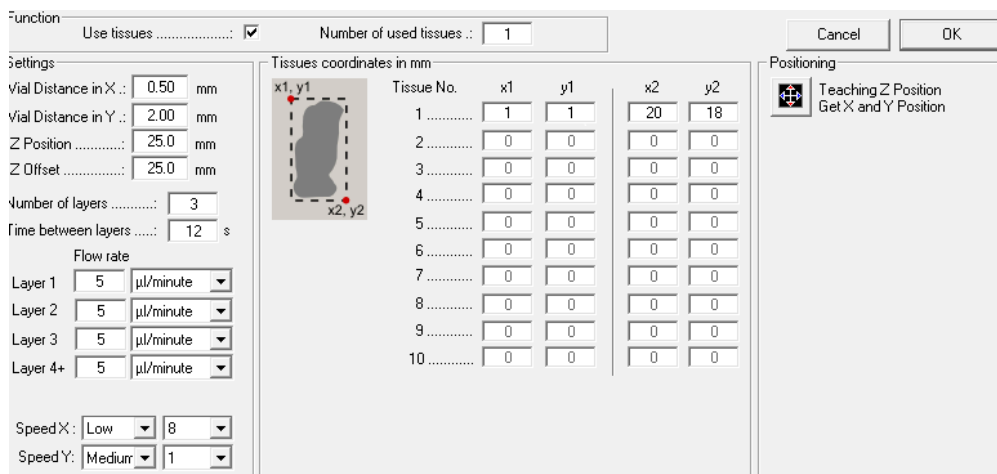
Screenshot of the SunCollect software

11.2



Screenshot of the commands in the SunCollect software

11.3



Screenshot of the software parameters and tissue coordinates in the suncollect software

11.4 Sprayer setting:

A	B
Vial Distance in X	0.50 mm
Vial distance in Y	2.00 mm
Z Position	35.00 mm
Z Offset	25.00 mm
Speed X	Low, 8
Speed Y	Medium 1

Method parameters for the SunChrom sprayer

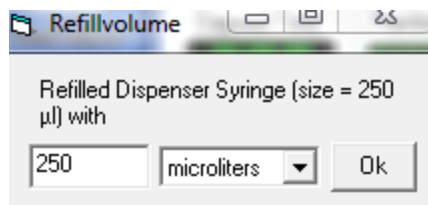
- 12 Fill the syringe with the prepared NEDC (solution (overfill), remove airbubbles and connect the syringe to the dispenser
- 13 Manually press the syringe until you have removed remaining airbubbles in the lines (the spray will stop shortly if there's an airbubble)
- 14 Place your tissue
- 15 In the suncollect software select your syringe size and set it to filled

15.1



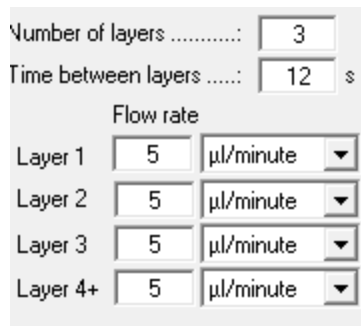
Screenshot of the commands in the SunCollect Software

15.2



Screenshot of the refill syringe command

- 16 Prepare your sprayer method. For this protocol you will need four separate methods (Layer 1-3, Layer 4-6, Layer 7-9 and Layer 10-21).



Screenshot of the flow rate method for layer 1-3. In total 4 methods are needed

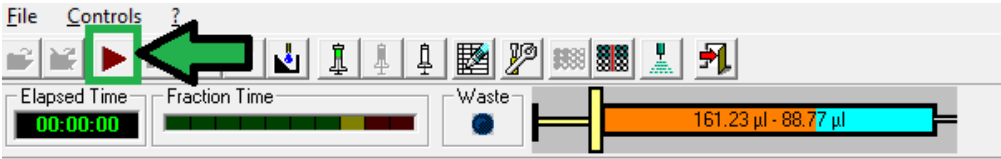
16.1 Sprayer method

	A	B
	Layer 1-3	5 µL/mL
	Layer 4-6	10 µL/mL
	Layer 7-9	15 µL/mL
	Layer 10-21	20 µL/mL

Flowrate for the different layers of the NEDC method

- 17 Start the spraying method. While spraying, watch the syringe and refill inbetween the layers if empty. While refilling, also set the syringe status to filled again in the software

17.1



Screenshot of the commands in the Suncollect Software

18 After spraying, remove the tissue

19 Clean you sample holder and fill the syringe again with MeOH to manually clean the lines

Consumables

20

A	B
Cryostat	Cm 1860, Leica, Nußloch, Germany
Blades	DB80 LX Low Profile Microtome Blades, Leica, Nußloch, Germany
ITO-slides	Part No. CG-90IN-S115, Delta Technologies, Loveland, USA
Glass slides	Micro Slides 2948-75X25, Corning Incorporated, NY, USA
N-(1-naphtyl)ethylenediamine hydrochloride	CAS: 1465-25-4, PCode: 1003246811, Merck, St Louis, USA
Sprayer	SunChrom Micro Fraction Collector MALDI Spotter Suncollect
Sprayer software	SunCollect Version 1.7.43



Used consumables and instruments

Protocol references

Wang, G., Heijs, B., Kostidis, S. *et al.* Analyzing cell-type-specific dynamics of metabolism in kidney repair. *Nat Metab* **4**, 1109–1118 (2022). <https://doi.org/10.1038/s42255-022-00615-8>

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