# Imaging interspecies interactions in bacterial co-cultures using nanostructured DIUTHAME membranes in laser desorption/ionization mass spectrometry

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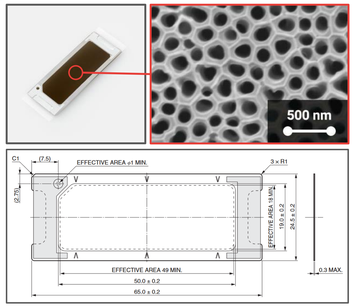
## Introduction: DIUTHAME




Courtesy of Hamamatsu Photonics K.K.

***Desorption/Ionization Using Through-Hole Alumina Membrane,*** *Hamamatsu Photonics K.K.*

- **DIUTHAME: Porous alumina ($Al_2O_3$) membrane**
  - Thickness = 5 µm
  - Pore size = 200 nm
  - Open aperture ratio = 50%
  - Coated with a 10-nm thick layer of platinum
- **Advantages:** no need for MALDI matrix, clean background in the low *m/z* region, higher reproducibility than DHB (*Hasan MM et al. (2021) RCM, 35(10), e9076*), dual-polarity capabilities, allows blotting preparation (*Enomoto H et al. (2020) Foods, 9(4), 408*), allows high lateral resolution imaging (*Müller MA et al. (2021) Metabolites, 11(9), 624*)
- **Applications:** imaging of fresh-frozen tissue sections, acetylcholinesterase reaction assays, characterization of polymer samples, imaging of metabolites in fruits by a blotting method, ...

## Methods: DIUTHAME vs MALDI-MSI

**Agar-based bacterial co-culture**

*Bacillus velezensis GA1*

*Pseudomonas sessilinigenes CMR12a*

**Imprinting with the DIUTHAME membrane**

- **Membrane:** A13331-5019-1B model for blotting (Hamamatsu Photonics K.K.)
- **Imprinting:** Membrane placed and kept on the agar-based bacterial co-culture during 5 min. No external pressure applied.

VS

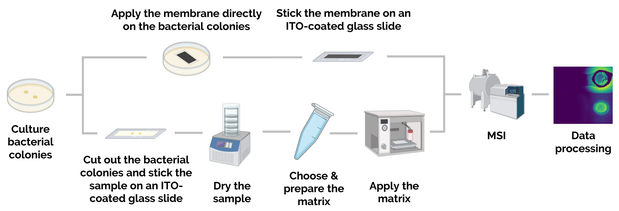
**MALDI sample preparation**

- **Sample drying:** 8 hours at 50°C
- **Matrix:** CHCA (5 mg/mL) in 70:30 ACN:$H_2O$ + 0.2% TFA
- **Spraying**: 10 layers with the SunCollect (*SunChrom*), flow rate gradient from 10 to 60 µL/min until the 6th layer, then constant 60 µL/min for the last 4 layers

**Instrumentation**

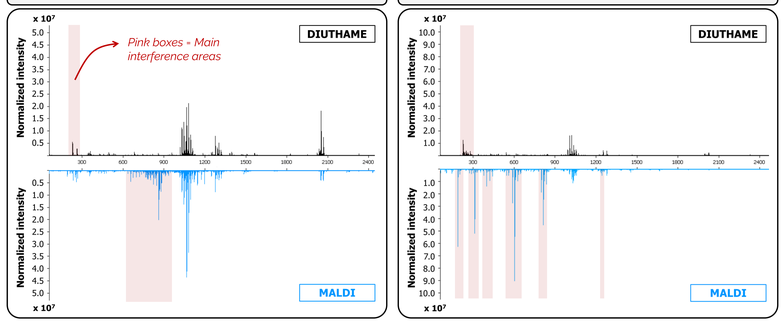
- **Instrument:** SolariX XR FT-ICR mass spectrometer (*Bruker*)
- **Ionization modes**: detection in the positive & negative ionization modes
- ***m/z* range**: From *m/z* 100 to *m/z* 2500
- **Data processing**: SCiLS Lab

**Sample preparation with DIUTHAME**
***turn-around times in minutes***



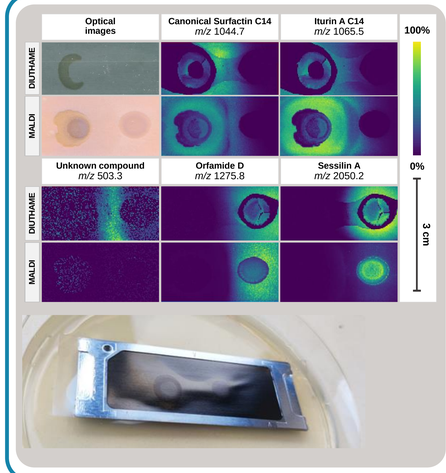

**Sample preparation for MALDI**
***turn-around times in hours***

## Advantages of the DIUTHAME method




- **Rapid and easy blotting sample preparation** (see *Methods*).
  - The blotting procedure only **takes a few minutes** and **does not require advanced skills** of the operator.
- **Analytes directly transferred from the sample to the DIUTHAME membrane, without sampling the agar medium**
  - **Avoiding the degradation** of labile compounds and the **deformation** of the sample caused by the drying step required in the MALDI procedure;
  - **Preventing the ion suppression** caused by agar.
- **The DIUTHAME membrane acts as the assisting material, and offers clean chemical background compared to MALDI**
  - **Few interference** in the low *m/z* region**, avoiding ion suppression** → suitable for the analysis of **small molecules.**
- **Detection of lipopeptides in both ionization modes**
  - Mainly as $[M + Na]^+$ ions in the **positive** ion mode and $[M - H]^-$ ions in the **negative** ion mode.
- **Reduced mass shift between pixels with the DIUTHAME membrane**
  - Due to lower signal intensities in DIUTHAME than MALDI-MSI → **less space charge effect;**
  - The imprinting allows **minimizing the effects of the sample topology** on the mass accuracy;
  - **No MALDI matrix** whose uneven application can induce mass shifts.

## Limitations of the DIUTHAME method




- **« Biased » visualization of the metabolite distributions**
  - **"Dark" areas** with little to no signal appear on the ion images, where the sample has **not been properly in contact** with the membrane.
- **Selectivity and sensitivity issues with DIUTHAME**
  - With a blotting sample preparation, some analytes may be **preferentially imprinted** on the membrane and others not at all.
  - **Signal intensities are lower** when using DIUTHAME than MALDI-MS, leading to a **lower sensitivity** of DIUTHAME.
- **Membrane fragility**
  - Adjusting the irradiating laser power to compensate for the low signal intensity is often not possible in DIUTHAME. Indeed, if the laser power is too high, it may **damage** or even **break the membrane**.

## Conclusion & Perspectives

***Imaging metabolites in agar-based bacterial co-cultures with minimal sample preparation using a DIUTHAME membrane in SALDI-MSI***

**PROS**

- ☑ *Rapid & easy sample preparation*
- ☑ *Suitable for the analysis of small molecules with limited interference*
- ☑ *Effective in both ionization modes*

**CONS**

- ☒ *Imprinting failure ⇨ biased ion images*
- ☒ *Low signal intensity*
- ☒ *Potential preferential blotting ⇨ selectivity issues*
- ☒ *Membrane damage (tear)*

**PERSPECTIVES**

- *Optimization of the blotting step to avoid artifacts*
- *Optimization of the MSI parameters to gain signal intensity without damaging the membrane*
- *Modification of the membrane chemical composition (→ selectivity)*
- *Testing the blotting method on other samples*


- Müller, W. H., et al. (**2022**). Surface-assisted laser desorption/ionization mass spectrometry imaging: A review. *Mass Spectrometry Reviews, 41(3)*, 373-420.
- Müller, W.H., et al. (**2022**) Imaging Metabolites in Agar-Based Bacterial Co-Cultures with Minimal Sample Preparation using a DIUTHAME Membrane in Surface-Assisted Laser Desorption/Ionization Mass Spectrometry. *ChemistrySelect, 7(18)*, e202200734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