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High-throughput bacterial profiling via MALDI spot analysis: Precise characterization of antibiotic-induced metabolic responses

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Introduction

Spot-based Matrix-Assisted Laser Desorption/Ionization mass spectrometry (MALDI-MS) is gaining traction in metabolomics for its **speed combined with minimal sample preparation and ease of automation across large sample sets**. This study presents a streamlined, fast, and selective **MALDI spot workflow** for metabolomics using the timsTOF fleX platform with Trapped Ion Mobility Spectrometry (TIMS)-enabled separation to generate **metabolic fingerprints** of complex bacterial profiles. We demonstrate the usage of this workflow for rapid and high-confidence discovery of **antibiotic-specific metabolic responses** including an efficient and intuitive data interpretation in MetaboScape 2026, covering efficient feature extraction, background removal, and statistical analysis.

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Technical Note 62 for more details

High-throughput metabolic fingerprinting by MALDI-TIMS-MS

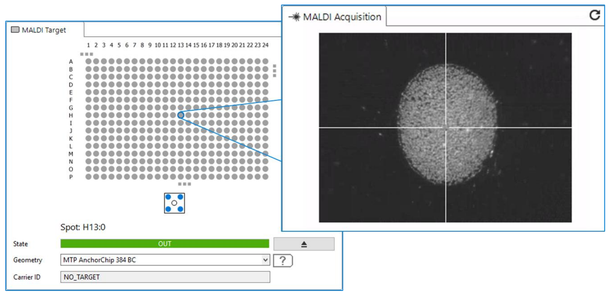

Figure 1: Scheme of the AnchorChip™ (800 µm) MALDI target plate used for bacterial profiling in timsControl acquisition software.
One spot is visualized more closely using the camera of the timsTOF fleX, showing the homogeneous distribution of the MALDI matrix α-cyano-4-hydroxycinnamic acid (HCCA).

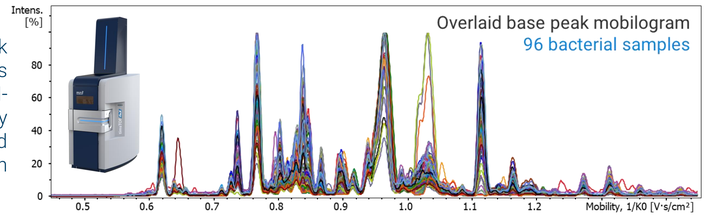

Figure 2: Overlaid base peak mobilograms of 96 bacterial samples (colored traces) analyzed by MALDI-TIMS-MS show reproducible mobility values, enabling straightforward metabolic fingerprint characterization in less than 10s per spot.

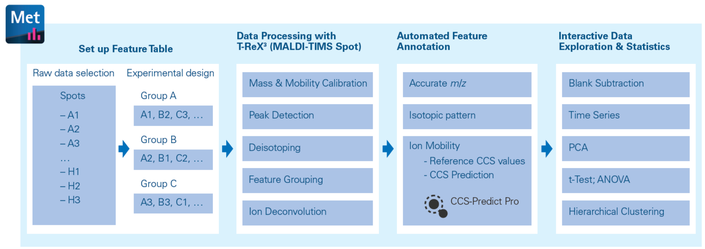

Figure 3: In MetaboScape® 2026, a new comprehensive workflow for MALDI Spot and MALDI-TIMS Spot analysis data was introduced providing a fully integrated pipeline from setting up feature tables to interactive data exploration and statistics.

Methods

Sample preparation *E. coli* cultures grown in Mueller-Hinton medium (n = 4 biological replicates) were exposed to various antibiotics, cultivated, and extracted in a 96-well format. Antibiotics included Chloramphenicol (CLO), Penicillin G (PENG), and Trimethoprim (TMP) at various minimum inhibitory concentrations (MIC).

MALDI spot analysis Diluted samples (1:10) were spotted on an AnchorChip™ MALDI target (800 µm) using the dried-droplet method with HCCA matrix solution (85% ACN, 0.1% TFA, 1 mM ammonium phosphate). MALDI-TIMS-MS measurements were acquired in Full-MS positive ionization mode on a timsTOF fleX with automated laser acquisition at 10 kHz.

Data analysis Data were processed in MetaboScape 2026 using the new "MALDI spot" workflow. MALDI spectra acquired from blank cultivation medium served as the background reference, and features with intensities ≤10 were excluded from statistics.

Competing interest statement: Authors Edward Rudt, Arndt Asperger, Sofie Weinkouff, Nikolas Kessler, Stefanie Wernisch, Heiko Neuweger, and Matthew R. Lewis are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sells analytical instruments including mass spectrometers and software. Bruker mass spectrometers and software were used in this study.

Results

The MALDI-TIMS-MS workflow enabled rapid acquisition of metabolic fingerprints from **96 *E. coli* samples treated with different antibiotics at various concentrations**, achieving **acquisition times of less than 10 seconds per spot**.

Software-guided MALDI background removal in MetaboScape® 2026 (Fig 3), based on features present in the cultivation medium, improved specificity and enabled confident detection of **sample-related and treatment-specific metabolomic responses**.

Furthermore, the MALDI-TIMS-MS results demonstrate **high reproducibility**, a critically important requirement for reliably detecting metabolic changes following antibiotic treatment (Fig 2 and Fig 4).

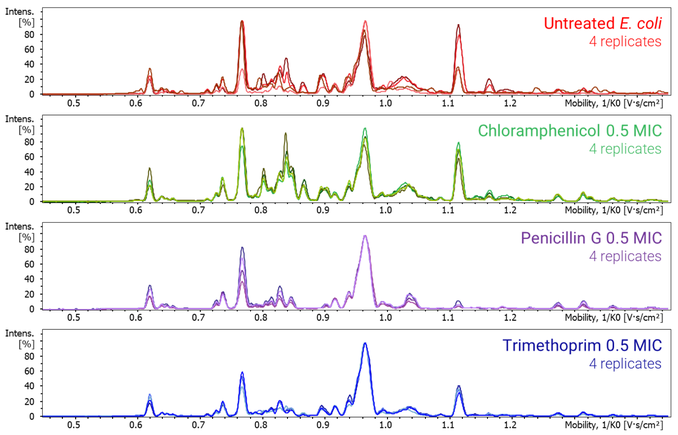

Figure 4: Overlaid base peak mobilograms of biological replicates (n=4) for untreated (red), Chloramphenicol (green), Penicillin G (violet) and Trimethoprim-treated (blue) *E. coli* samples analyzed by MALDI-TIMS-MS demonstrate high reproducibility, a cornerstone of high-quality metabolomics.

The speed, combined with high reproducibility, confirms the suitability of MALDI-TIMS-MS for high-throughput metabolomics and large-scale screening. The incorporation of TIMS separation significantly improved spectral quality and peak capacity, enabling **high-confidence discovery of antibiotic-specific metabolomic responses** (Fig 5).

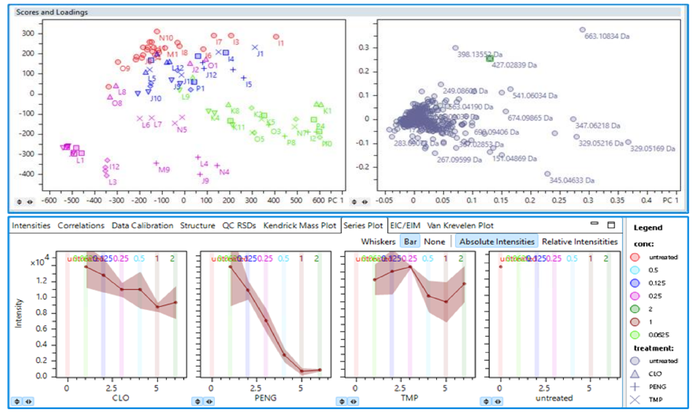

Figure 5: PCA (top) shows distinct clusters of PENG (violet) and CLO (light green) treated cultures, while TMP-treated samples (blue) clustered closely with controls (red), confirming antibiotic-specific metabolic responses.
Series plots (bottom) in MetaboScape 2026 highlight metabolic response trends across antibiotic concentration gradients, confirming specificity of MALDI-TIMS-MS spot profiling data.

Principal Component Analysis (PCA) revealed distinct clustering of samples treated with CLO and PENG, **reflecting strong metabolic perturbations** linked to these antibiotics. In contrast, TMP-treated samples clustered closely with controls, indicating less pronounced metabolic disturbance in the TMP group (consistent with *E. coli*´s known resistance to this treatment).

Series plots confirmed **concentration-dependent trends** for antibiotic specific features, demonstrating **high confidence and specificity** of the developed MALDI spot analysis workflow (Fig 5).

Conclusion

- High-throughput, TIMS-enhanced MALDI spot analysis for efficient and confident bacterial profiling
- Complemented by software workflow for intuitive interpretation of antibiotic-induced metabolic responses
- Basis for detailed structural identification of statistically relevant features

TIMS-enhanced MALDI Spot Analysis

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