Sublimation as a streamlined sample preparation approach for high-resolution multiomic imaging on the timsTOF fleX

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Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging enables spatially resolved molecular analysis with high sensitivity. Sample preparation, particularly matrix application, critically influences both sensitivity and lateral resolution. Spraying is widely used but involves trade-offs between resolution and sensitivity. Sublimation avoids solvents and produces smaller crystals, improving resolution, yet conventional sublimation devices demand expertise, effort, and often lack reproducibility. We present a novel automated sublimation and recrystallization device designed to deliver consistently superior resolution and sensitivity. Its performance was evaluated using three common MALDI matrices—DHAP, DHB, and CHCA—across multiple laboratories, operators, and sites.

Introduction

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Methods

To ensure reproducibility and ease of use, we systematically analyzed workflows and sources of variability in matrix deposition. Solutions were integrated into the design of a new automated sublimation device. Three late-stage prototypes were tested in three laboratories to assess consistency and effectiveness. DHAP, DHB, and CHCA were selected for comprehensive evaluation. Homogeneity and reproducibility of matrix coatings were assessed on plain conductive slides using pixel-intensity histograms and gravimetry. Tissue samples were analyzed by mass spectrometry imaging to evaluate sensitivity and lateral resolution.

Results

Key evaluation parameters included matrix layer thickness and homogeneity. All devices demonstrated reproducibility in both absolute deposition and uniformity of coatings.

Cross-site and cross-operator tests confirmed reproducibility, while also identifying variability sources such as human handling and ambient conditions. Failure modes were investigated, and mitigation strategies were implemented. Tissue imaging confirmed that the automated device consistently achieved high sensitivity and superior lateral resolution compared to conventional methods.

Conclusion

The automated sublimation and recrystallization device provides reproducible, standardized, and user-friendly matrix deposition for MALDI Imaging. By minimizing variability across sites and operators, it enables reliable parameter transfer and supports cohort-driven studies, including clinical research. This innovation addresses limitations of conventional sublimation approaches and establishes a robust foundation for routine high-resolution molecular imaging.Introduction