

Facilitating MALDI Imaging Sample Preparation: A High-Resolution, Reproducible, and User-Friendly Sublimation Device

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Introduction

MALDI Imaging enables laterally resolved molecular analysis with matrix application critically impacting sensitivity and resolution. While spraying is common, it involves trade-offs between these factors. Sublimation avoids solvents and yields finer crystals, improving lateral resolution. However, typical homemade laboratory setups require expertise, substantial effort, and often lack reproducibility.

Here, we developed an automated sublimation and recrystallization device to overcome the causes behind variability and consistently ensure superior lateral resolution and sensitivity. Testing with common MALDI matrices confirmed consistent performance across devices, operators, and sites.

Methods

Three identical sublimation devices were built and evaluated across three different laboratories:

Reproducibility & Homogeneity of Coating

- MALDI matrices: 5 mg of DHAP, DHB, CHCA
- Substrates: Plain IntelliSlides
- Set-up: 3 runs x 3 slides x 3 matrices x 3 sites
- Technique: Gravimetry & Pixel-intensity histograms of transmitted light scans

Sensitivity & Lateral resolution of MALDI Imaging

- MALDI matrices: DHAP, DHB, NEDC
- Substrates: Rat Testis, Brain, Kidney; Vero B4 cells
- Technique: MALDI Imaging (timsTOF fleX MALDI-2, partly with transmission prototype)
- Analytes: Lipids, Metabolites

Sublimator parameters:

	Heating (°C)	Cooling (°C)	Time (s)
DHAP	90	10	60
DHB	110	10	180
CHCA	160	10	300
NEDC	160	10	180
Recrystallization	50	18	10

Reproducibility & Homogeneity of Coating

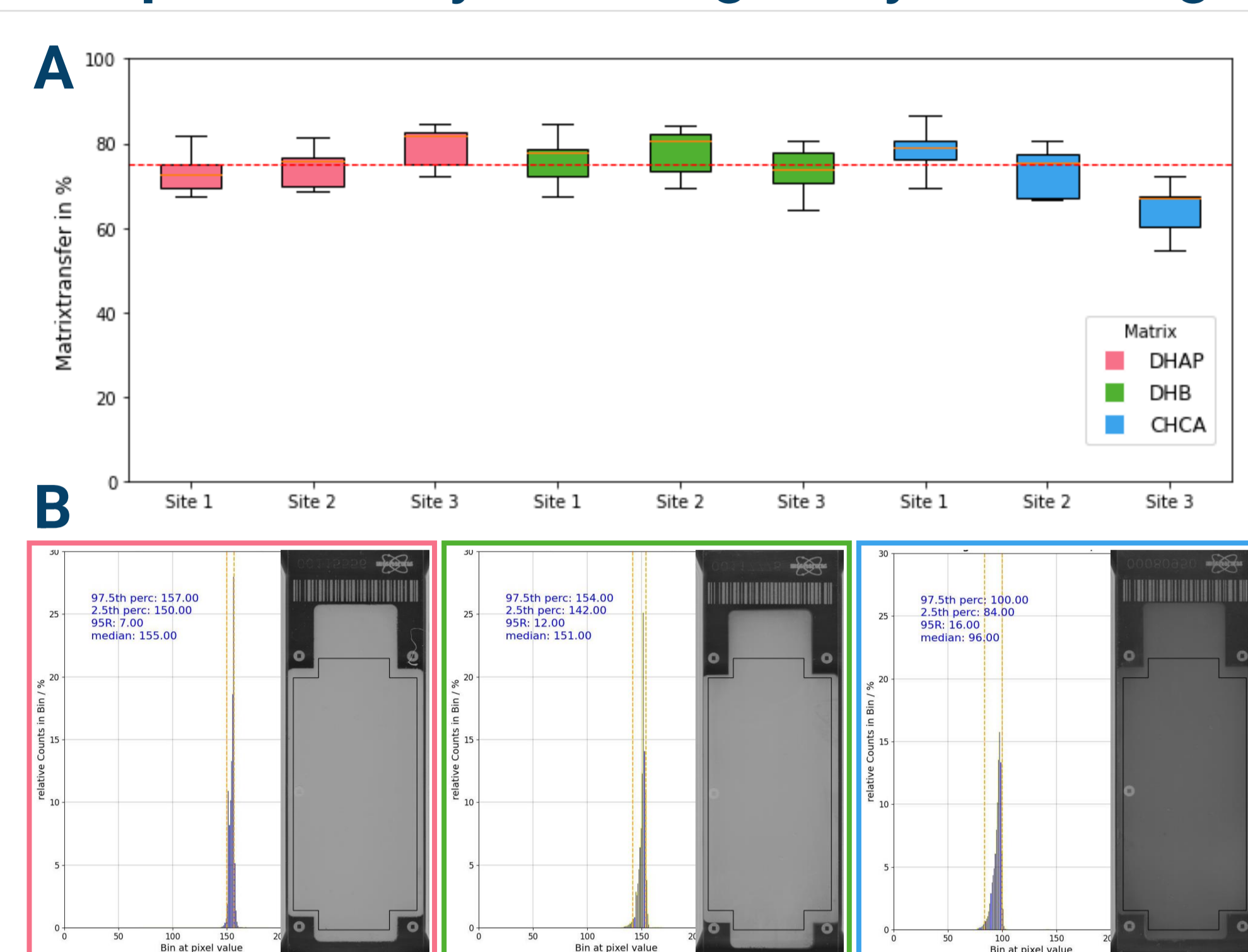


Fig. 1 (A) Matrix transfer measurements for three MALDI matrices at three different instruments and sites; (B) Homogeneous matrix distribution for three representative slides sublimated with DHAP (left), DHB (middle) and CHCA (right) prepared at site 2.

Reproducibility of MALDI Imaging

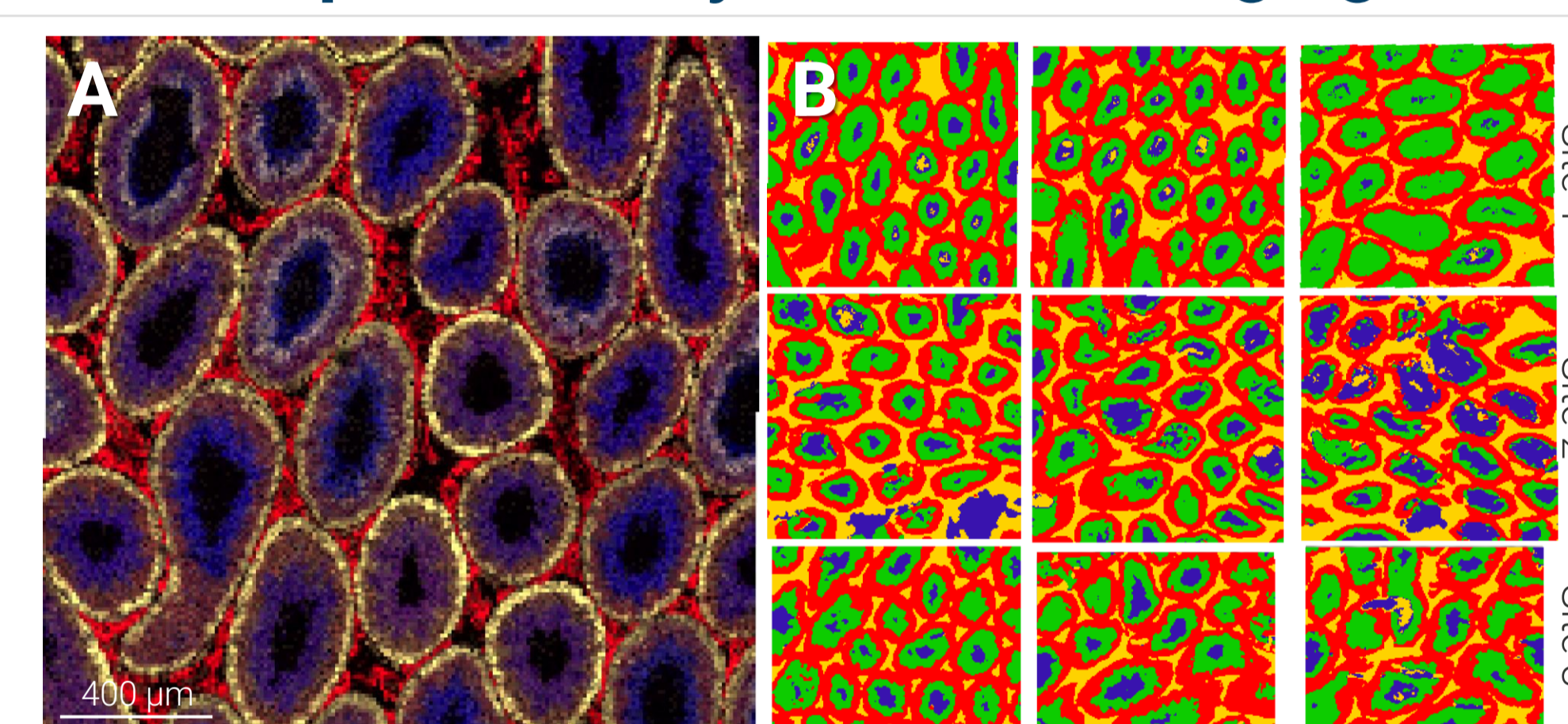


Fig. 2 MALDI Imaging data from rat testis tissue prepared with DHAP and measured at 10 μm pixel-size; (A) representative image result from site 1, (B) segmentation results from triplicates acquired at all 3 sites.

Fig. 4 MALDI Imaging data of (A) lipids in rat brain tissue (DHAP, 5 μm pixel-size), (B) lipids in single Vero B4 cells (DHAP, 5 μm pixel-size) and (C) metabolites in kidney (NEDC, 1.5 μm pixel-size) prepared with (D) the automated sublimation device.

Results

Reproducibility & Homogeneity of Coating:

Sublimation with the automated device yields a consistent matrix transfer efficiency of $75\% \pm 5\%$ (Fig. 1A). The coated slides show a high coating quality with the large-scale homogeneity expressed in narrow pixel-intensity histograms as seen for three representative slides in Fig 1B. Between MALDI matrices, fine differences in homogeneity of coating can be observed with DHAP (width: 8 ± 2) having an

Sensitivity of MALDI Imaging

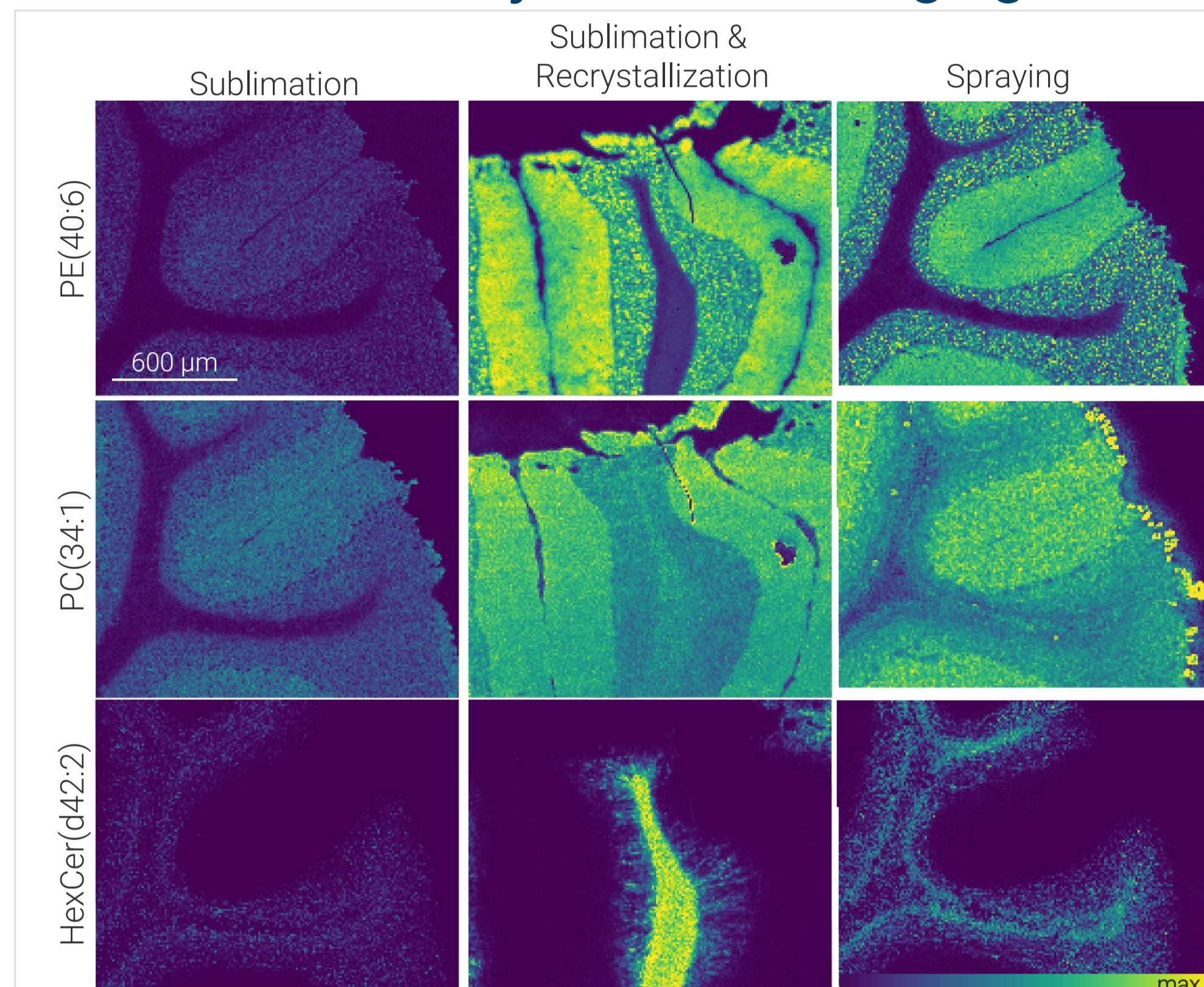
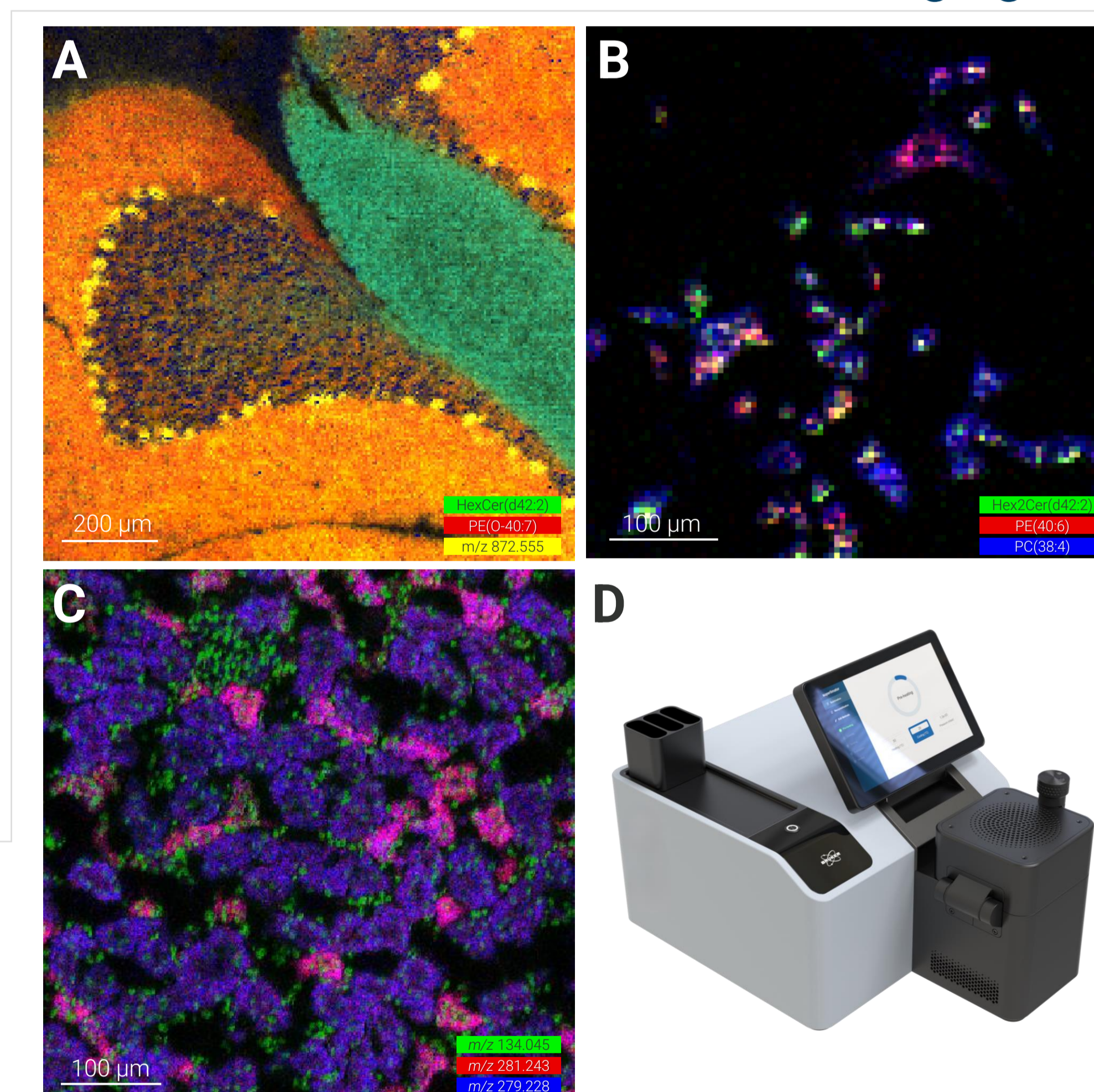


Fig. 3 MALDI Imaging data from rat brain tissue measured at 10 μm pixel-size and prepared with DHB by sublimation, sublimation & recrystallization, and spraying.

Lateral Resolution of MALDI Imaging



amorphous structure and CHCA (width: 22 ± 10) forming small and dense micro-crystals and being more sensitive to inhomogeneities.

Reproducibility of MALDI Imaging: Rat testis tissue prepared independently at three sites using different tissues, sublimators, users, and mass spectrometers yielded consistently high-quality and comparable MALDI Imaging data (Fig. 2A). k-Means clustering revealed groupings based on tissue structure rather than site- or instrument-specific differences (Fig. 2B), underscoring the reproducibility of the sublimation

method and its negligible impact on cross-laboratory comparability.

Sensitivity of MALDI Imaging: Rat brain tissue prepared with DHB using three methods—sublimation, sublimation & recrystallization, and spraying—shows superior MALDI Imaging performance when sublimation is combined with recrystallization (Fig. 3). While spraying often yields high signal intensities for certain analytes, it frequently leads to delocalization, particularly for outer membrane lipids such as PC's. For instance, PC(34:1) can leak up to 100 μm beyond the tissue border. Sublimation alone produces relatively low signal intensities compared to spraying, but adding a recrystallization step not only surpasses spraying in terms of signal intensity but also preserves the spatial localization of all lipid species. Notably, some analytes like HexCer are scarcely detectable with spraying but are prominently revealed when using the sublimation–recrystallization approach.

Lateral resolution of MALDI Imaging: Extending sublimation protocols to various tissue types and analytes at $\leq 5\ \mu\text{m}$ pixel sizes highlights the broad applicability of sublimation with optional in-instrument recrystallization (Fig. 4). Tissue architecture is resolvable at the single-cell level using standard MALDI Imaging, while subcellular features such as nuclei can be visualized with prototype transmission-mode systems. Overall, sublimation enables artifact-free sample preparation for high-resolution spatial imaging with accurate analyte localization, offering clear advantages in data quality and interpretability.

Conclusion

The automated sublimation device:

- ensures uniform, reproducible coating for three common MALDI matrices
- demonstrates consistent MALDI imaging results across sites, instruments, and tissue types
- enables artifact-free sample preparation for MALDI imaging at $\leq 5\ \mu\text{m}$ pixel size

Imaging MS: Instrumentation

COI Disclosure: T.B., C.H., W.E., J.H. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.