



● The Ease of Use workflow – A simple way to set up MALDI Imaging measurements

MALDI Mass Spectrometry Imaging (MSI) as a versatile and robust tool for analyzing different types of analytes in various samples requires a well-thought-out experiment setup.

Abstract

Here we present a straightforward workflow in flexImaging software, which facilitates and speeds up the measurement set-up in conjunction with dedicated consumables rapifleX Tissue typer. An important part of the new workflow also includes

improvement and monitoring of the performance level of the measurement setup.

Introduction

MALDI Imaging is a powerful label-free technique that allows users to simultaneously examine many different molecules in

their native location from one tissue section. This technique combines the molecular depth that comes with mass spectrometric detection with the ability to spatially view them on a cellular scale similar to microscopy, therefore giving the ability to investigate a sample's molecular fingerprint at both the overall and

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unique subregion level. One of the limitations of MALDI Imaging was the relative complexity for setting up experiments due to the disparate technical nature of sample preparation and measurement for microscopy and mass spectrometry. Bruker has developed solutions which simplify experiment set up to a matter of minutes.

With the development of the cutting-edge instrumentation, such as the rapifleX and timsTOF fleX, and the introduction of IntelliSlides™, MALDI Imaging experiments were greatly simplified. Users were not only instantly informed on sample handling, but it also became possible to automate instrument set up, such as alignment of the sample to the optics of the mass spectrometer. The latest version of flexImaging TissueTyper now contains the Ease of Use workflow which uses IntelliSlides to check instrument performance. Along with automatic sample scan loading and teaching, and new features in the simplified method selection, the Ease of Use workflow introduces automatic tissue recognition for measurement region assignment, and the ability to trigger instrument performance optimization tools directly from the flexImaging New Imaging

Run Wizard. In addition, this update offers the opportunity for online recalibration of tryptic peptide digest using the approach defined by Boskamp et al [1]. While this application note shows data acquired on a rapifleX TissueTyper using the respective acquisition software, the Ease of Use workflow is also available on the timsTOF fleX platform.

Taken together, use of IntelliSlides with this workflow now simplifies the use of MALDI Imaging for examining molecular markers and investigate biochemical changes at the touch of a button.

Imaging Workflow and Results

Instrumentation and Sample Preparation

All experiments were conducted on a rapifleX TissueTyper MALDI-TOF with flexControl 4.2 and flexImaging 6.0, using application-appropriate methods and parameters supplied from Bruker's global application specialists. For all measurements, adjacent regions were measured: one region using the automatic tools, and one region using manual set up. Data were analyzed using SciLS Lab 2020a.

Three different applications requiring different sample preparation methods were used to demonstrate the usability of the workflow. For all described samples, sections were mounted on IntelliSlides™ and scanned with the TissueScout prior to matrix coating. Matrix was sprayed on all samples using established protocols on the HTX™ Sprayer. Important to know for the Ease of Use workflow is that different regions of the IntelliSlides are used to automatically conduct specific steps that either require parts of the slide to be coated in a matrix layer or kept matrix-free. To do this, we employed the IntelliSlide sprayer mask, which made sure that the appropriate parts of the sample were prepared as required. Before measurement, 0.5 μL of an appropriate calibrant for the respective application was dotted on the top right region of the slide [Figure 1].

The New Imaging Run Wizard

The Ease of Use workflow New Imaging Run Wizard covers five steps:

- Ⓐ Sample Image: Use the drop-down menu to identify the sample position in the MTP Slide Adapter II and define if the sample is a Tissue Microarray (TMA). Completion of this step triggers barcode reading and slide teaching at once.
- Ⓑ Data Storage: Definition of the measurement name and the directory in which it is saved.
- Ⓒ Acquisition Settings: Definition of the measurement raster width and measurement method.
- Ⓓ Completing: Covering any extra time for completing teaching (if required).
- Ⓔ Performance Check (continuing on Ⓕ).

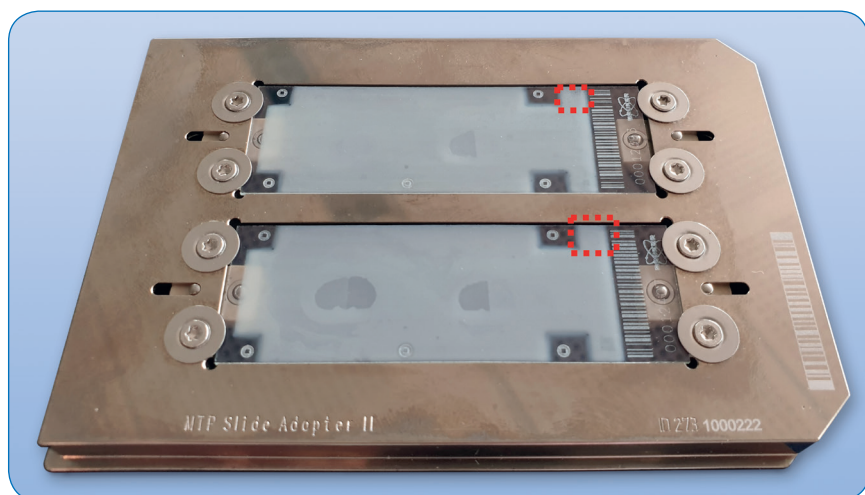


Figure 1: Example of samples on IntelliSlides prepared for Ease of Use workflow. Samples prepared for measurement showing the barcode and teachmarks free of matrix coating. The red dotted squares indicate the location of the calibration standard.

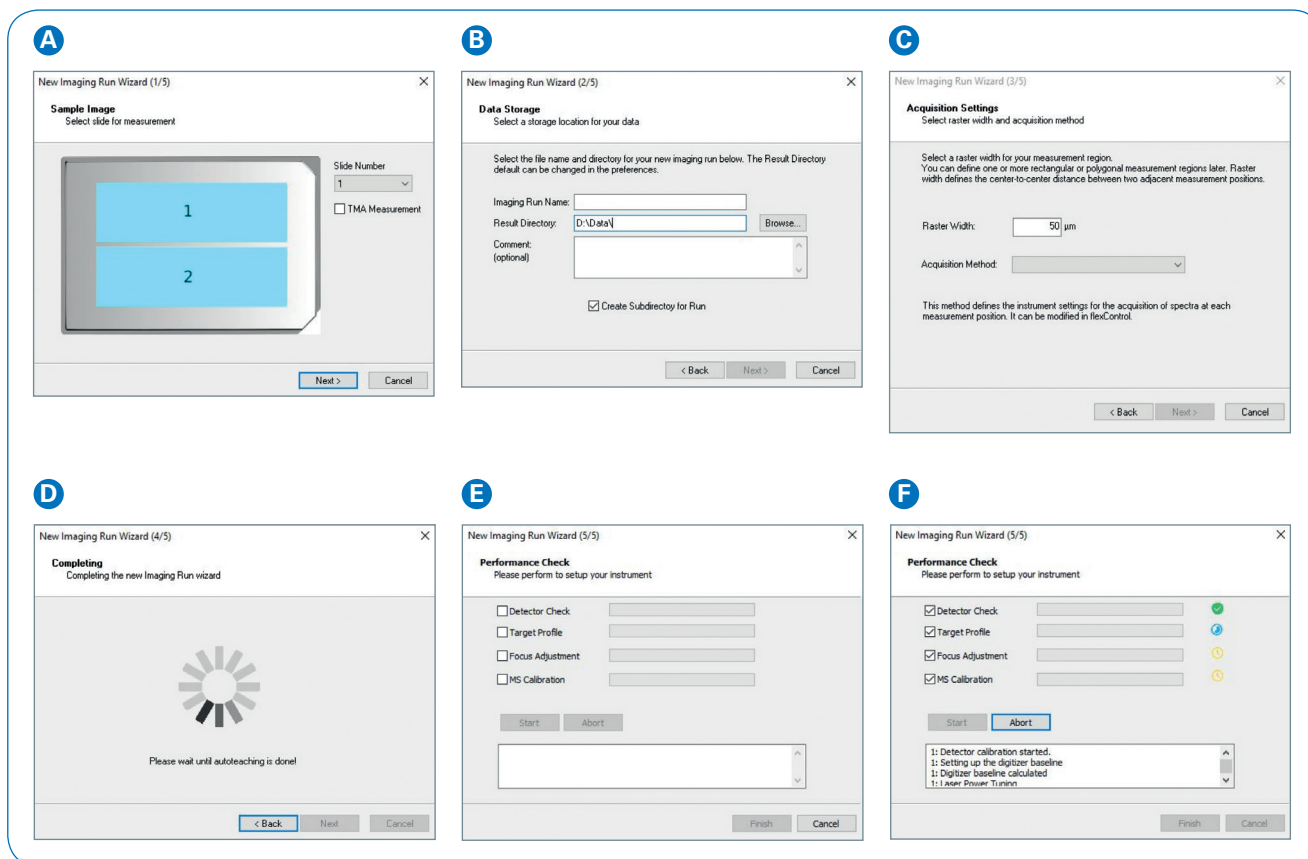


Figure 2: flexImaging New Imaging Run Wizard. The different Run Wizard windows guide through the Ease of Use workflow step-by-step as explained in text above (A-F).

The Performance check covers

- Detector Check
- Target Height Profile
- Laser Focus Adjustment
- Mass Spectrometer Method Calibration

These tools are conducted in the order that they are listed in the window, however you must conduct Target Height Profile and Laser Focus Adjustment for each run. The progress of each is displayed as well as the successful or unsuccessful completion of the tool/s. If a step cannot be completed, for example, if the Detector Check is at the end of its lifetime, users are clearly notified with a red x. It is possible to continue a measurement if a tool cannot be successfully completed, however this is not recommended.

The following shows results obtained using the Ease of Use workflow

versus measurements set up manually. For positive mode lipid measurements, 10 μm fresh frozen rat testis sections were coated in 15 mg/mL fleXmatrix for lipid imaging in 90% ACN/H₂O with 0.1% TFA. Figure 3 shows the distribution of three different lipids in the tissue; the intensity and distribution of these lipids show a comparable peak intensity and similar distribution. Spatial segmentation in SCiLS Lab demonstrated that the greatest difference in spectra in these measurements is based on anatomical features in the tissue, rather than how the data were acquired.

Protein measurements were conducted on 10 μm mouse brain sections that had been washed according to the Carnoy procedure [2], then coated with 10 mg/mL fleXmatrix for protein imaging in 50% ACN/H₂O with 0.1% TFA. Figure 4 shows the

distribution of m/z 11310, previously identified as histone H4 in a mouse brain section [3]. The signal is found in the hippocampus with similar intensity and distribution regardless of whether it was measured manually or with the Ease of Use workflow. Spatial segmentation of the sample indicates clustering of spectra based on brain histological regions rather than measurement.

Figure 5 shows tryptic peptide measurements conducted on FFPE mouse brain sections (3 μm), which were prepared according to Ly et al [4] and coated in 10 mg/mL fleXmatrix for peptide imaging. Different digest peaks of myelin basic protein were detected and localized to white matter regions, and segmentation again indicated that the greatest difference in spectra was found in brain regions and not measurement.

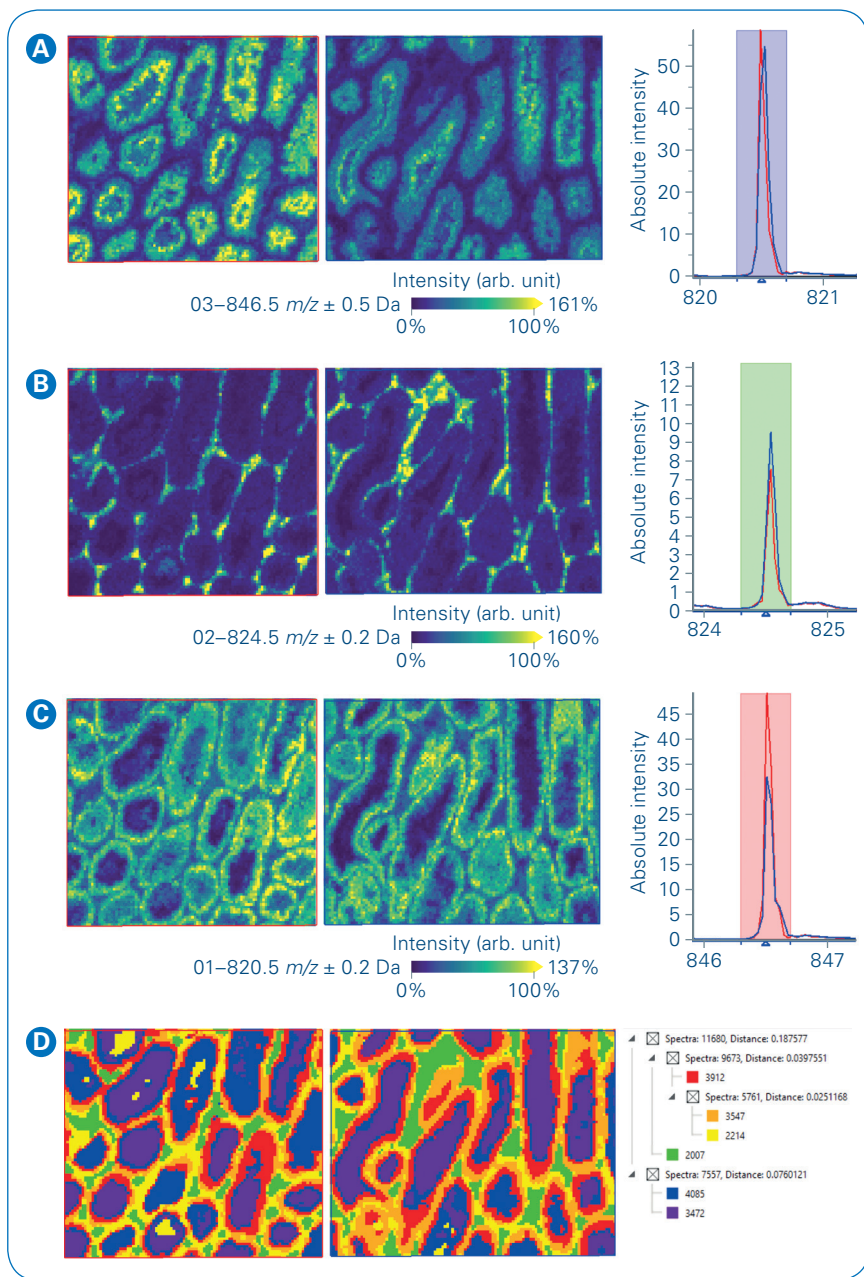


Figure 3: Measurement of lipids from rat testis. Shown here on the left pane is a comparison of the intensity distributions of 3 exemplary lipids on tissue (A) m/z 846.5; (B) m/z 824.5; (C) m/z 820.5, outer left – Ease of Use workflow; right – manual workflow. The right pane shows the resp. zoom into the overall mean spectrum in red (Ease of Use workflow) and in blue (manual workflow). (D) SCiLS Lab spatial segmentation results: left pane – segmentation map with Ease of Use workflow on the outer left and manual workflow on the right, right pane – segmentation tree.

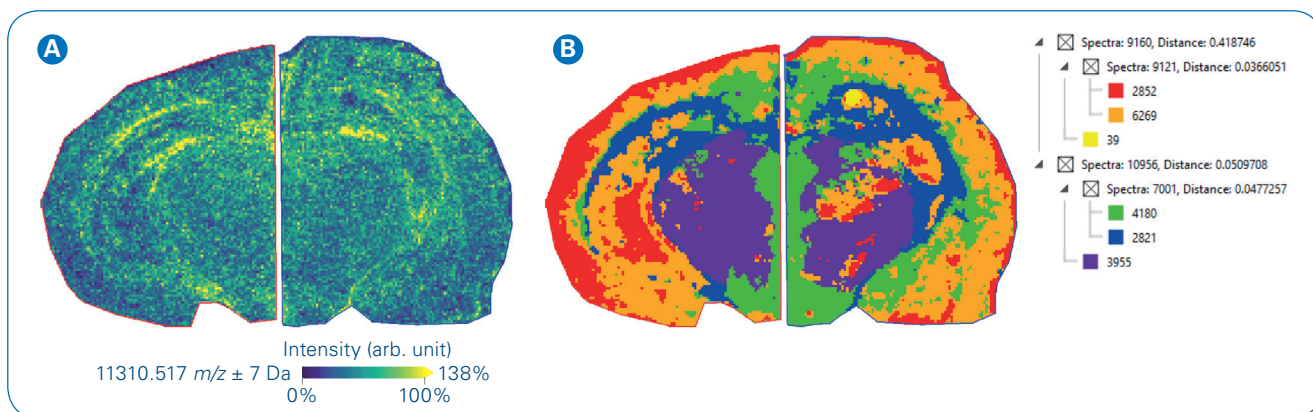


Figure 4: Measurement of proteins from mouse brain. Protein measurement showing (A) intensity distribution of m/z 11310 (histone H4) in typical hippocampus region on tissue and (B) SCiLS Lab spatial segmentation analysis with segmentation map and segmentation tree. The left side of the brain section was measured in Ease of Use mode, the right side with manual set-up.

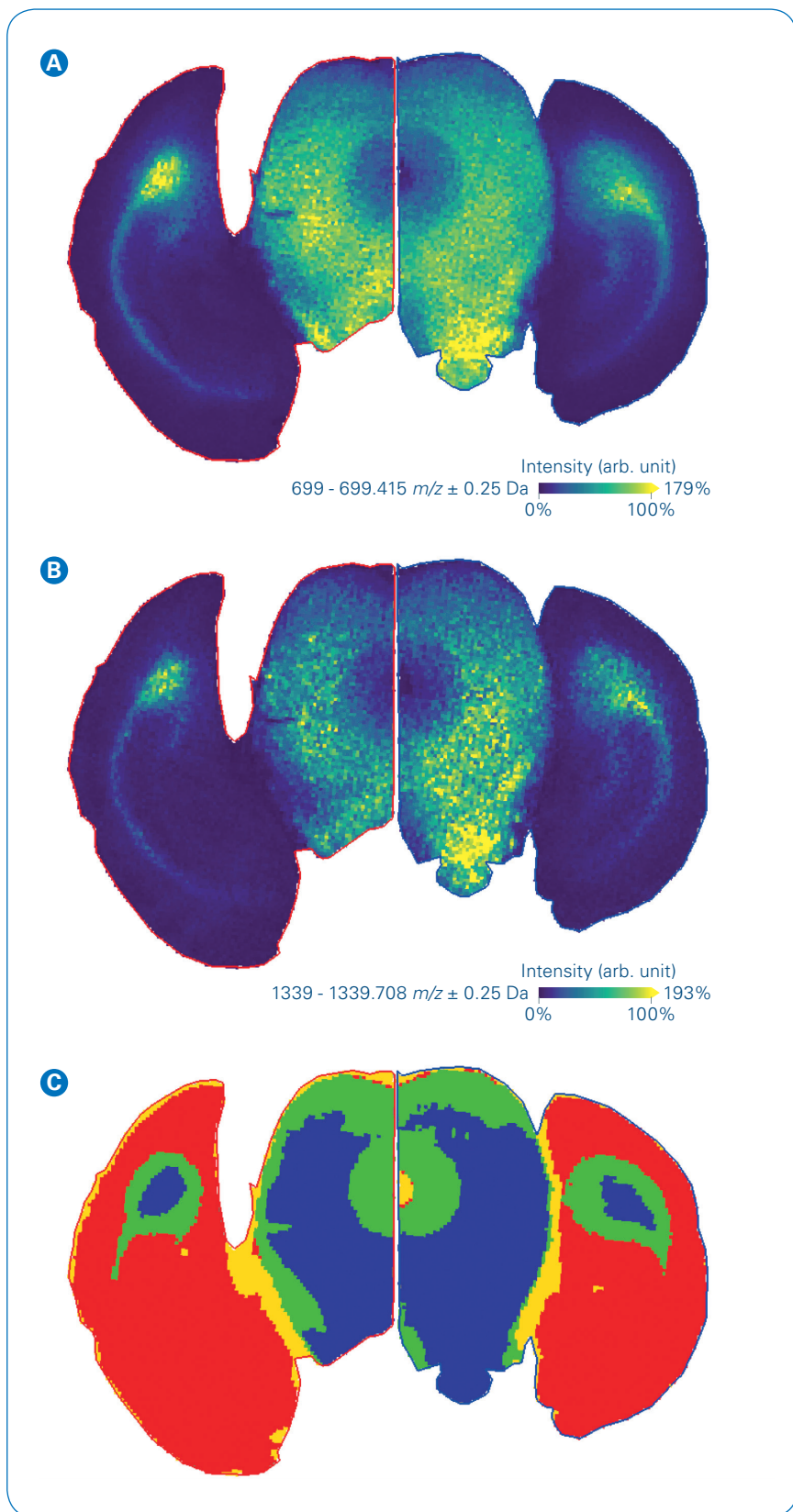


Figure 5: Measurement of tryptic peptides from FFPE mouse brain section. Peptide measurement revealing intensity distributions for two tryptic peptides of myelin basic protein (**A**) m/z 699.4; (**B**) m/z 1339.7). **C** shows SCiLS Lab spatial segmentation map. The left side of the brain section was measured in Ease of Use mode, the right side with manual.

Conclusion

- Using the Ease of Use workflow, the time to set up a MALDI Imaging experiment including measurement region assignment is approximately 10 minutes.
- The automatic performance check allows users of all levels of experience to quickly confirm instrument operation or identify errors before starting any measurements, thus saving time and samples.
- This workflow is available for different MALDI Imaging applications.



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