

● The SCiLS™ autopilot – 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 set-up.

Abstract

Here we present a straightforward software add-on, which facilitates and speeds

up the measurement set-up in conjunction with dedicated consumables on the timsTOF fleX platform [1]. An important part of the new workflow

includes improvement and monitoring of the performance level of the measurement set-up.

Keywords:
MALDI Imaging,
IntelliSlides, flexImaging,
timsTOF fleX,
timsControl, TissueScout

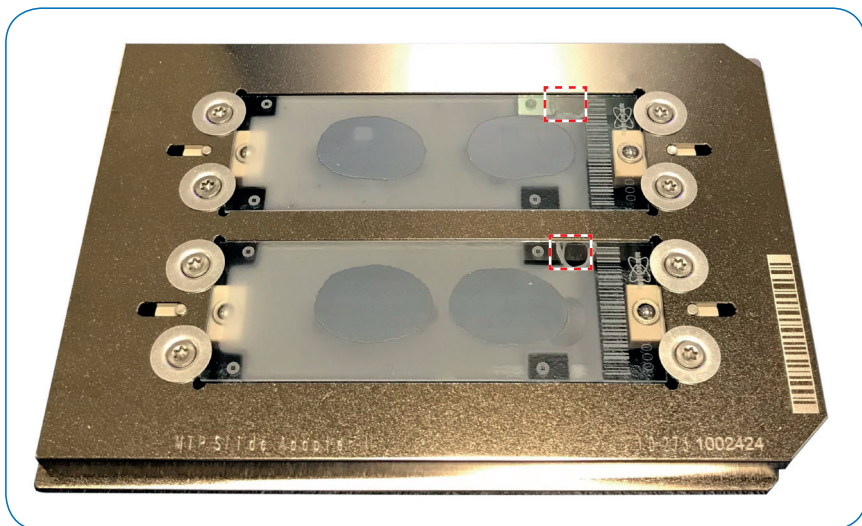


Figure 1. Example of samples on IntelliSlides prepared for the SCiLS autopilot. Samples prepared for measurement showing the barcode and teachmarks free of matrix coating. The red dotted squares indicate the location of the calibration standard.

Introduction

MALDI Imaging is a powerful label-free technique that allows users to simultaneously examine different molecules in their native location from one tissue section. This technique combines the molecular depth obtained by mass spectrometry with the ability to spatially view the analytes on a cellular scale similar to microscopy. Giving the ability to investigate a sample's molecular fingerprint at both the overall and unique subregion level. One of the limitations of MALDI Imaging is 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 and expedite the experimental set-up.

With the development of 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 versions of timsControl and flexImaging on the timsTOF fleX platform now contain the SCiLS autopilot which uses IntelliSlides to check instrument performance. Along with automatic sample scan loading and teaching, and new features in the simplified method selection, the SCiLS autopilot introduces automatic tissue recognition for measurement region assignment, and the ability to trigger timsControl for instrument performance optimization tools directly from the flexImaging New Imaging Run Wizard.

Use of IntelliSlides with the SCiLS autopilot simplifies and automates the use of MALDI Imaging for examining molecular markers and investigating biochemical changes.

Imaging workflow and Results

Instrumentation and Sample Preparation

All experiments were conducted on a timsTOF fleX with timsControl 3.0 and flexImaging 7.0. 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 2021b.

Fresh-frozen rat testis was cut at 10 μm thickness and sections were mounted on IntelliSlides and scanned with TissueScout prior to matrix coating. Matrix (15 mg/mL fleXmatrix for positive mode lipid imaging in 90% ACN/H₂O with 0.1% TFA) was sprayed on the sections using established protocols on the HTX TM-Sprayer. SCiLS autopilot uses different regions of the IntelliSlides to automatically conduct specific steps that either require parts of the slide to be coated in a matrix layer or kept matrix-free. We, therefore, employed the IntelliSlide sprayer mask, ensuring that the appropriate parts of the sample were prepared as required. Before measurement, 0.5 μL of red phosphorous was dotted on the top right region of the slide (Figure 1).

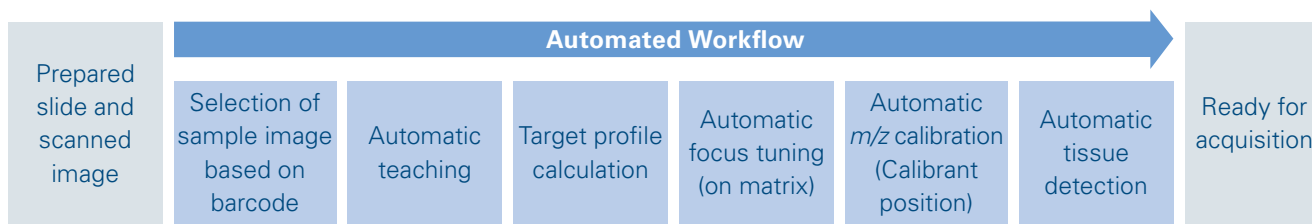


Figure 2. Figure 2. SCiLS autopilot workflow

The SCiLS Autopilot Workflow

Starting with a prepared slide and an image scanned before matrix application, the SCiLS autopilot performs the following steps (Figure 2):

1. The barcode of the slide is scanned in the instrument. Based on the barcode, the corresponding

sample image is automatically selected and used in the further processing steps.

2. Automatic teaching of the sample image using the reference marks on the IntelliSlides.

3. Calculation of the target profile to provide constant laser focus across the slide.

4. Laser focus tuning on a certain position on the slide that is covered with matrix. Steps 3 and 4 ensure optimum laser focus across the slide.

5. Automatic m/z calibration using a dedicated position on the slide using e.g. red phosphorus as calibrant. Other calibrants can be used by selecting the appropriate reference mass list in timsControl.

6. Finally, the measurement regions are automatically created based on the scanned image.

At the end of the workflow the MALDI Imaging experiment is fully prepared and ready for data acquisition.

The New Imaging Run Wizard

The SCiLS autopilot New Imaging Run Wizard consists of five steps (Figure 3):

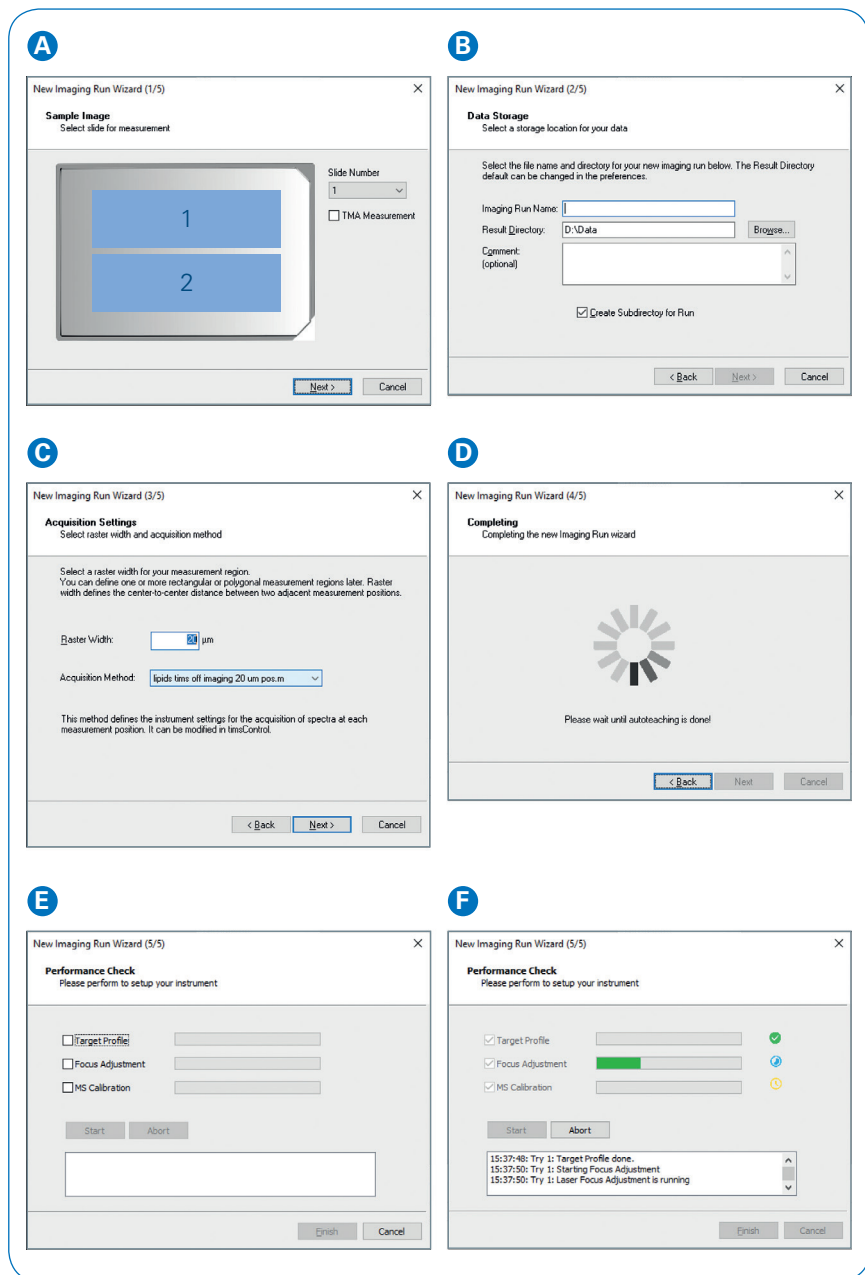


Figure 3. flexImaging New Imaging Run Wizard. The different Run Wizard windows guide through the SCiLS autopilot step-by-step as explained in text above (A)-(F).

A 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.

B Data Storage: Definition of the measurement name and the directory in which it is saved.

C Acquisition Settings: Definition of the measurement raster width and measurement method.

D Completing: covering any extra time for completing teaching (if required); and

E Performance Check: Selection of the desired performance check points.

F Completion of the Performance Check and indication of its status.

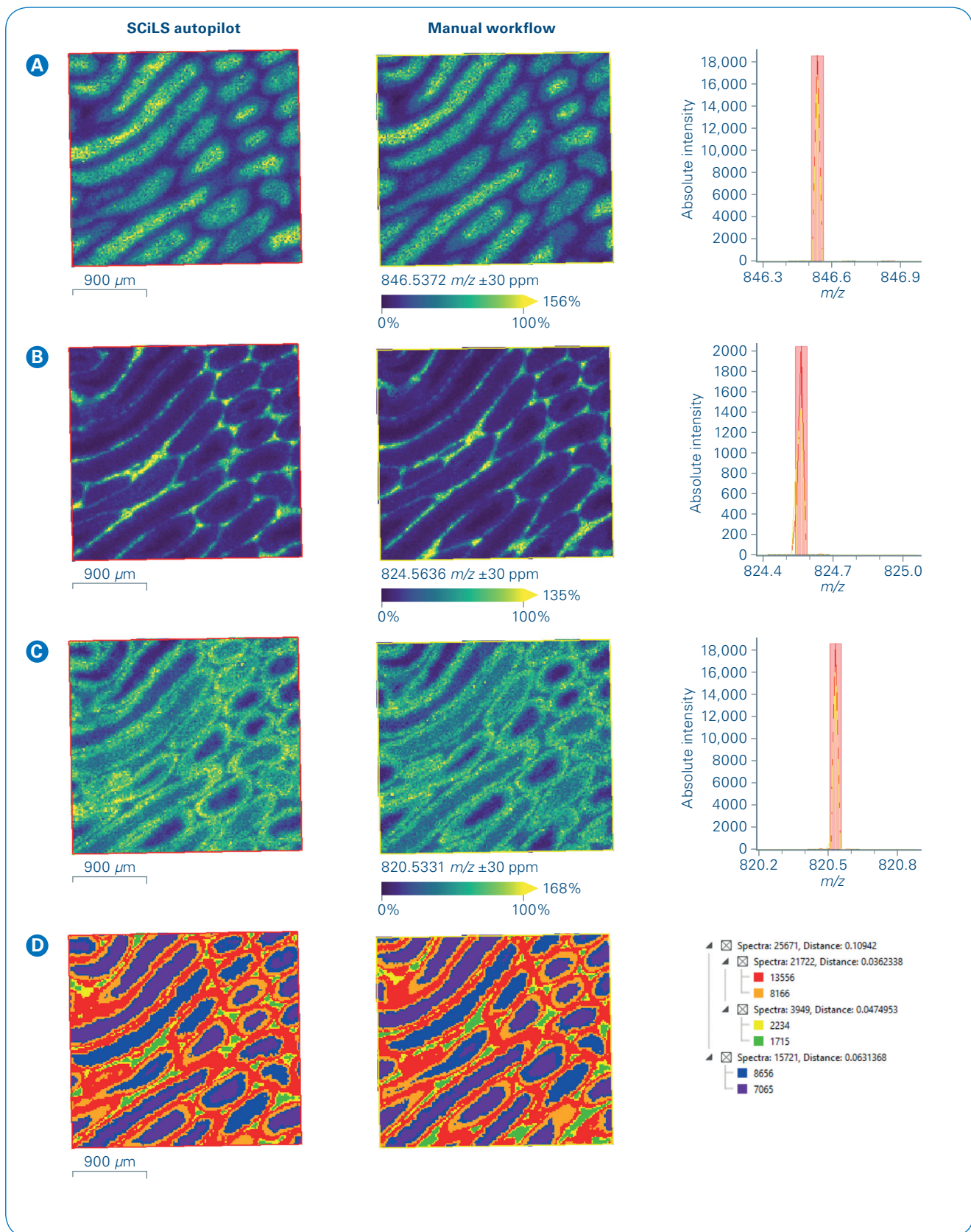


Figure 4. Measurement of lipids from rat testis. Left pane is a comparison of the intensity distributions of 3 exemplary lipids on tissue ((A) m/z 846.537; (B) m/z 824.564; (C) m/z 820.533), outer left obtained with SCiLS autopilot; right panel – manual workflow without normalization. The right pane shows the respective zoom into the overall mean spectrum in red (SCiLS autopilot) and in yellow (manual workflow). (D) SCiLS Lab spatial segmentation results: left pane - segmentation map with SCiLS autopilot on the outer left and manual workflow on the right, right pane - segmentation tree.

The Performance check covers

- Target Height Profile,
- Laser Focus Adjustment, and
- Mass Spectrometer m/z Calibration.

These steps are conducted in the order as they are listed in the window. Target Profile and Focus Adjustment are recommended to be conducted 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, users are 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 SCiLS autopilot versus manual set-up measurements.

Figure 4 shows the distribution of three different lipids in the tissue without normalization; the intensity and distribution of these lipids show 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.

A great advantage of measurement setup with the SCiLS autopilot is the ease of use. Users are guided through the measurement setup process. Different check points are performed automatically by selecting the respective bullet points in the New Imaging Run Wizard. This enables stable and reproducible measurements to be routinely obtained regardless of users' expertise.

The IntelliSlides enable precise co-registration for exact laser positioning and easy referencing due to the inscribed barcode. Target Height Profile determination and Laser Focus Adjustment are important parameters to ensure that the laser rasters in-focus over the tissue and generates high-quality images. In addition, automatic m/z calibration facilitates the calibration process for accurate mass measurements.

Conclusion

- With SCiLS autopilot, 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 such as lipid, metabolite or peptide imaging and can also be conducted on the rapifleX.



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References

- [1] Spraggins JM, Djambazova KV, Rivera ES, Migas LG, Neumann EK, Fuetterer A, Suetering J, Goedecke N, Ly A, Van de Plas R, Caprioli RM (2019). *High performance Molecular Imaging with MALDI Trapped Ion Mobility Time-of-Flight (timsTOF)*, Mass Spectrometry. Anal Chem. **91**(22):14552-14560.

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