REF 1859743
1887953

Quickstart SCiLS autopilot

Tutorial

Revision A (July 2021)

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Document History

Revision	Date	Changes	Responsible
A	04.06.2021	Document creation - Revision A	Janina Oetjen

Scope

This document is valid for the following instruments / software packages:

Instrument / Software package	***Serial / part Number***
timsTof FleX	*1859743*
SW Package Compass 2022 timsTOF Series	*1887953*

Contents

1 Introduction

This document outlines the combined timsControl and flexImaging workflow for setting up a MALDI Imaging experiment when using the SCiLS™ autopilot found in flexImaging 7.0.

These instructions will cover:

- An explanation of what this workflow encompasses
- Information regarding sample preparation
- Method settings in timsControl
- Experiment set up in flexImaging

Please follow exactly the below steps to ensure optimal usage.

2 What is the SCiLS™ autopilot?

The SCiLS™ autopilot is a streamlined process for setting up MALDI imaging experiments on the timsTOF fleX when using Bruker's IntelliSlides™. This workflow contains existing tools as automatic sample scan loading and teaching, and new features in the simplified method selection, 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**.

These tools are:

- Target Height Profile generation
- Laser Focus Adjustment
- Automatic Mass Calibration

Important: The SCiLS™ autopilot is currently available only for measuring one slide at a time and should not be used with the AutoXecute Batch Runner.

3 Sample preparation

The SCiLS™ autopilot will *only* work with Bruker's IntelliSlides™. Please be aware of the following points:

- Please create your optical pre-measurement scan before matrix coating. We recommend using the Bruker TissueScout for scanning samples. However, flatbed scanners such as the Epson Perfection V850 Pro can also be used. The entire IntelliSlide™ including all teach marks, barcode and Bruker logo must be scanned (see Figure 3-1).

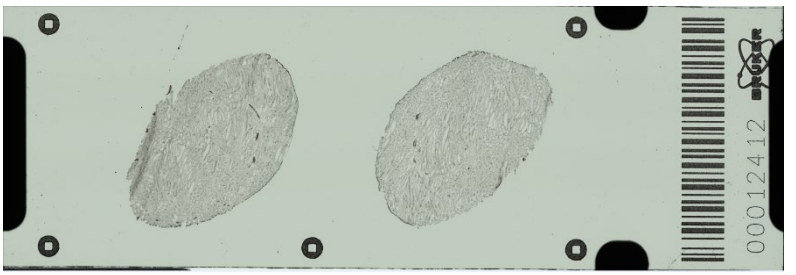

Figure 3-1 Example of IntelliSlide™ scan using TissueScout

After scanning, copy the scanned image into the Import folder located in your Methods directory (D:\Methods\images\Import) on your timsTOF fleX acquisition computer.

Important: As part of autoteaching, this file will be deleted from this folder and moved to D:\Methods\images\Imported.

- Please use the modified IntelliSlide™ sprayer mask when coating slides with matrix, as specific IntelliSlide™ regions must be kept matrix-free. Simply insert your slide/s into the mask/s before matrix coating. The following matrices were tested with the SCiLS autopilot™: Dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), 9-aminoacridine (9AA).

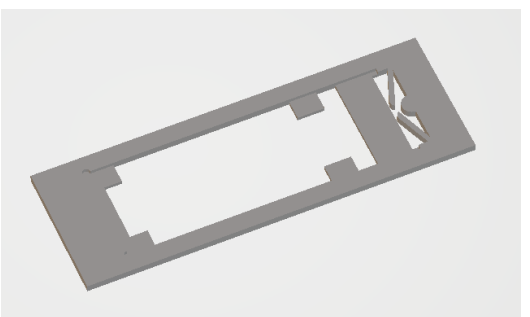

Figure 3-2 Example of modified IntelliSlide™ sprayer mask

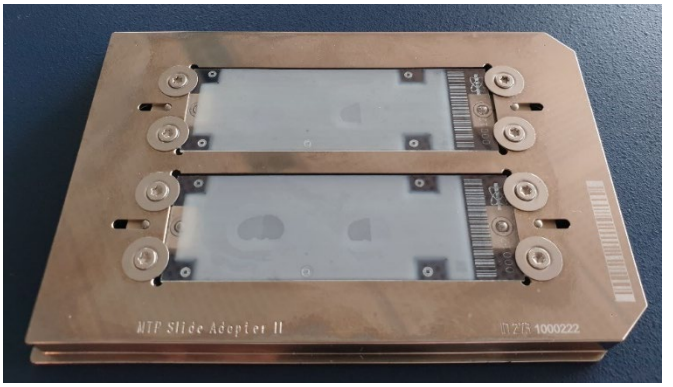

Figure 3-3 Example of IntelliSlides™ coated with matrix using the modified mask, showing barcode and teachmarks free of matrix

- The areas circled in red in Figure 3-4 are used for Target Height Profile Generation and Laser Focus Adjustment and should be kept free of tissue sample.

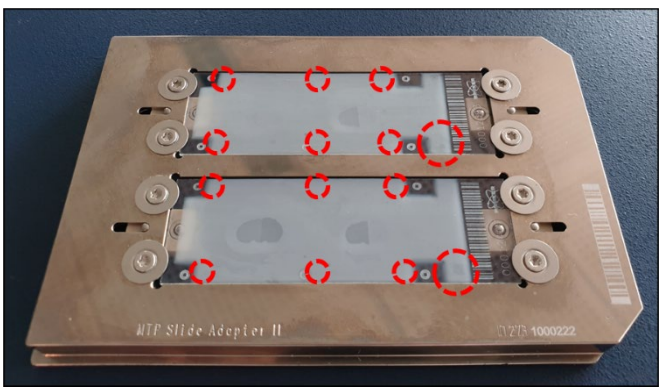

Figure 3-4 Areas that should be kept free of tissue sample

- The top right area of the slide is used for MS calibration (Figure 3-5). This area is approximately 5 mm x 5 mm in size. Please spot 0.5-1.0 µl of your application-appropriate calibrant in the position and spread over as much of the area as possible.

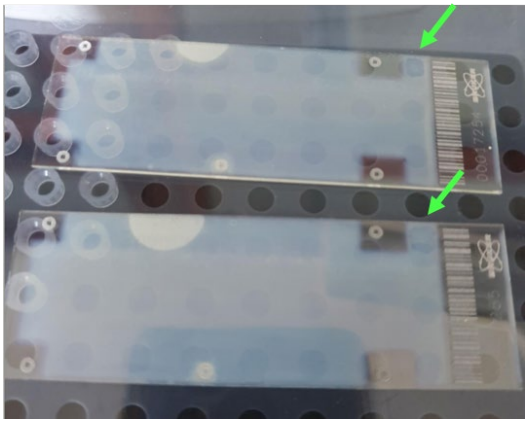

Figure 3-5 ***Area for MS calibration, with example of Peptide Calibration Standard II spread in the area.***

4 SCiLS™ autopilot application methods

The SCiLS™ autopilot on the timsTOF fleX is recommended only for the following applications:

- Peptide imaging (peptides tims off imaging 50 um pos.m)
- Lipid imaging (lipids tims off imaging 20 um pos.m)
- Small molecule imaging (small molecules tims off imaging 20 um pos.m)
- TIMS lipid imaging (lipids tims on imaging 20 um pos.m)
- TIMS small molecules imaging (small molecules tims on imaging 20 um pos.m)

Note: Negative mode methods can be generated by loading the respective positive method in timsControl, save it under a different name in D:\Methods\Default Application Methods\OTOF\timsTOF fleX\MALDI Imaging TIMS off (or …TIMS on), turn on negative mode, save the method and choose Method – Lock for changes.

The recommended methods can be downloaded from the Bruker homepage at https://www.bruker.com/protected/en/services/software-downloads/mass-spectrometry/methods-and-libraries.html

Performance is not guaranteed if used for other applications.

Make sure that the following settings in the **Calibration** tab are confirmed for each of your methods in timsControl before starting any measurements:

- The appropriate mass control list for your calibrant has been selected.
- The appropriate parameters for automatic calibration are set and the method is saved.
- If you are unsure, **enhanced quadratic** is the general recommendation but requires a minimum of five (5) calibrant peaks.

Note: All 3 settings will be used automatically during the **Performance Check** as described in the following chapter.

5 Setting up experiments

This chapter describes how to set up experiments with the flexImaging **New Imaging Run Wizard** and the Performance Check.

The flexImaging 7.0 **New Imaging Run Wizard** offers three possible workflows:

- SCiLS™ autopilot, covered in this document
- Automatic workflow, offered from flexImaging 5.1, not covered in this document
- Classic workflow, offered from flexImaging 5.1 and earlier, not covered in this document

Before starting flexImaging, make sure that timsControl is started, that your slide is correctly inserted into the MTP Slide Adapter II with the barcode towards the chamfered (right) corner, and that both positions in the Slide Adapter have a conductive slide inserted. It is not necessary for both slides to have a preparation. When this is confirmed, please dock your sample into the instrument.

Important: MTP Slide Adapter II must be selected in timsControl as the Geometry, either manually or from reading of the MTP Slide Adapter II barcode.

Important: Before starting, please make sure the scans are in folder D:\Methods\images\import.

5.1 Select workflow

Open flexImaging and set up a new imaging run using the **New Imaging Run Wizard**.

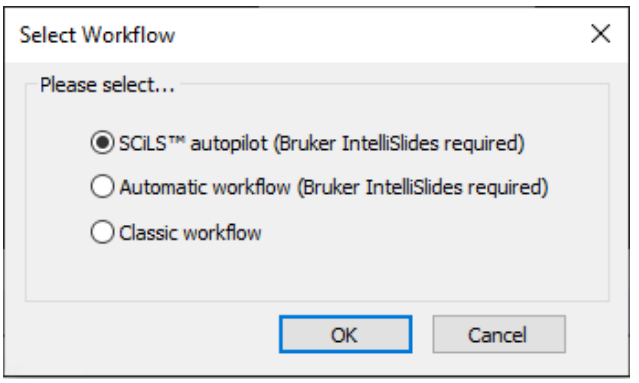

Figure 5-1 flexImaging Wizard – Select workflow

Select “SCiLS™ autopilot”.

5.2 Wizard (1/5) – Sample Image

Use the drop-down menu to select the position of your sample in the MTP Slide Adapter II. The system will begin reading the barcode from the IntelliSlide and start the automatic teaching.

When measuring a tissue microarray (TMA), please activate the checkbox in this window. Different algorithms are used to detect large tissue samples, or the smaller cores found in TMAs, check this box so that the correct algorithm is used to detect and assign tissue samples in the scan as measurement regions.

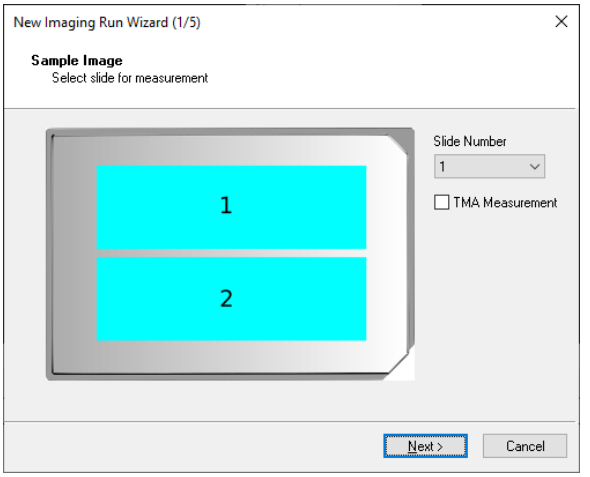

Figure 5-2 flexImaging Wizard – Sample Image

Important: From this point on, you must not undock your sample from your instrument.

5.3 Wizard (2/5) – Data Storage

Name your Imaging Run and select the directory where you wish to save your measurement.

Create Subdirectory for Run should be checked.

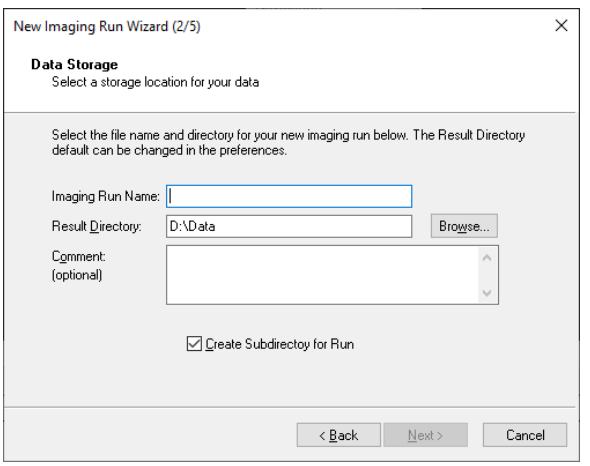

Figure 5-3 flexImaging Wizard – Data Storage

5.4 Wizard (3/5) – Acquisition Settings

Set an appropriate raster width for your acquisition method.

Select your defined method from the drop-down menu. This menu accesses only the SCiLS™ autopilot method folder (D:\Methods\Default Application Methods\OTOF\timsTOF fleX\MALDI Imaging TIMS off and D:\Methods\Default Application Methods\OTOF\timsTOF fleX\MALDI Imaging TIMS on), with the recommended measurement methods.

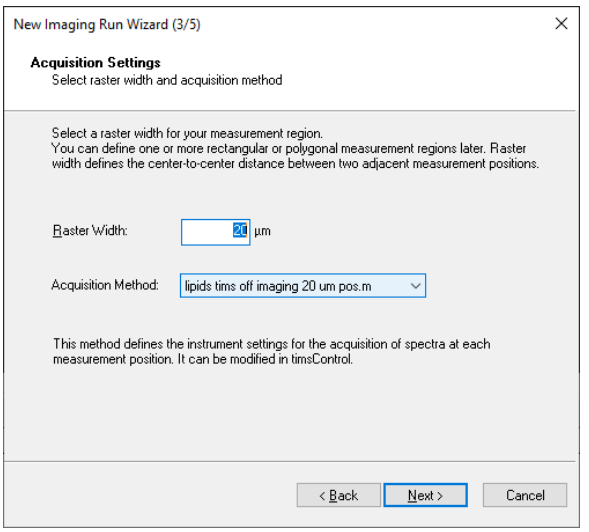

Figure 5-4 flexImaging Wizard – Acquisition Settings

5.5 Wizard (4/5) – Completing

If the automatic teaching is not yet complete, wait until it is done.

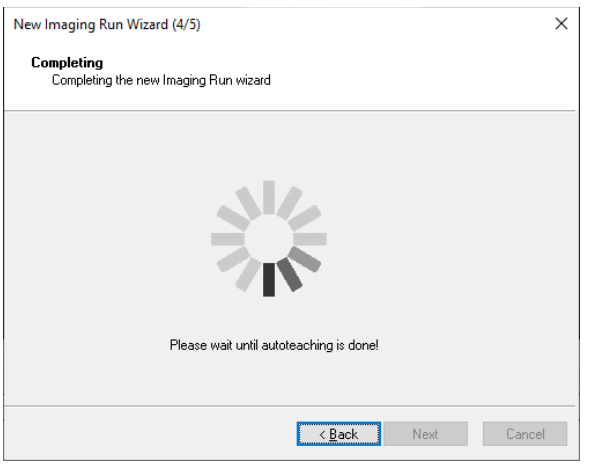

Figure 5-5 flexImaging Wizard – Completing

5.6 Wizard (5/5) – Performance Check

Users can select which tools to perform for their run, which are conducted in the order that they are listed in the window.

The progress of each is displayed as well as the successful or unsuccessful completion of the tool/s.

When the Performance Check is complete, press **Finish** to exit the Wizard.

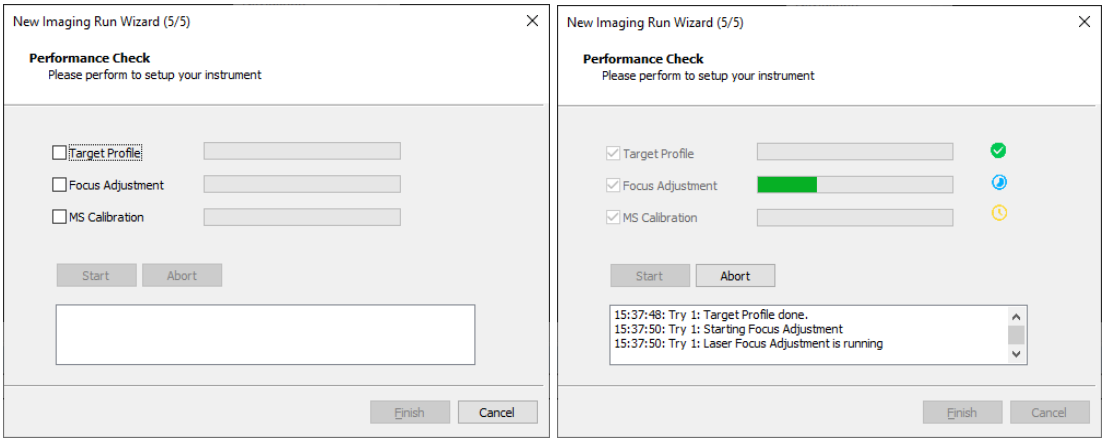

Figure 5-6 flexImaging Wizard – Performance Check showing initial settings and completed check

Important: As a minimum, you must conduct Target Profile and Focus Adjustment for each run.

5.7 Measurement regions

Following completion of the **New Imaging Run Wizard**, your measurement will open in flexImaging with the scan automatically loaded in the Imaging Display, and the tissue regions in the scan assigned as measurement regions.

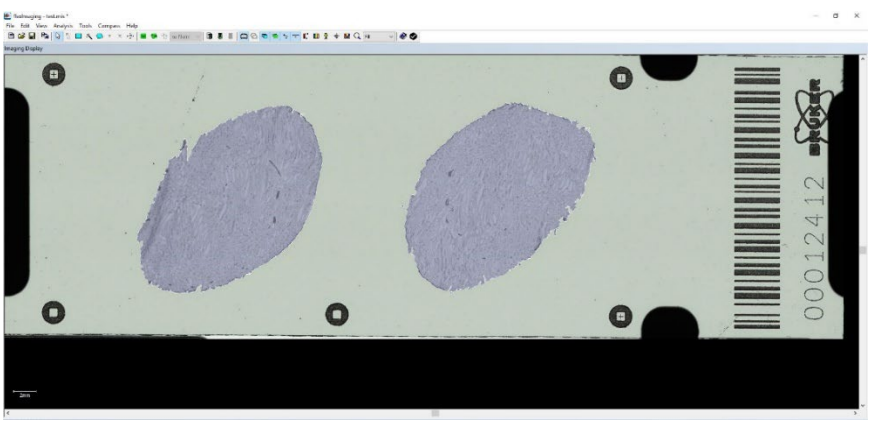

Figure 5-7 flexImaging Display showing automatically assigned measurement regions

It is possible to edit the region shape or delete regions by right clicking on the relevant region in the **Regions** dialog.

Users can also add more regions by using the rectangle, polygon, or magic wand tools.

The order in which regions are measured can be changed by selecting the region you wish to move using the arrows in the **Regions** dialog**.**

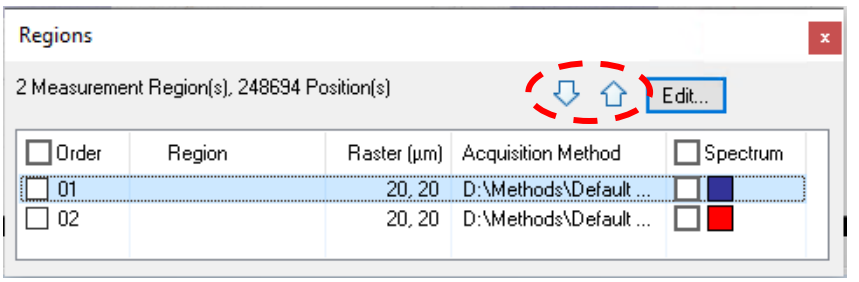

Figure 5-8 flexImaging Wizard – Regions dialog with arrows to re-order measurement regions circled in red dotted line

Note: Remember to save your flexImaging sequence after any editing

The Imaging sequence is now ready for use, save it, please. As final step, the laser (power and beam offset) must be adjusted in timsControl.

6 Laser preparation settings

1. In timsControl, go to a section of the tissue and conduct several test shots by pressing **Start**, and confirm that you have spectra.
2. Make sure that the laser shot is in the cross hairs of the camera view. If necessary, realign the laser spot using the **Beam Offset** settings hidden in the **Advanced** area of the **Laser** tab (see Figure 6-1).

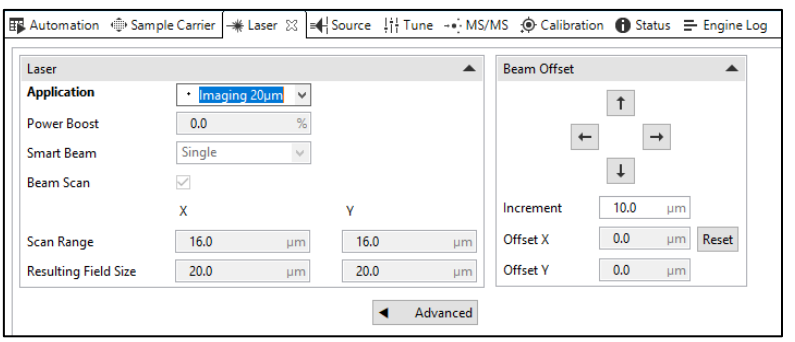

Figure 6-1 timsControl Laser Tab

To realign the laser spot:

- Move to a matrix-coated, off-tissue location and conduct a test shot to check the laser reflection (white flash) position relative to the crosshairs. It is helpful to burn a hole into the matrix.
- If the burn mark is not centered in the crosshairs, adjust the beam offset with the arrow buttons.
- Move to another off-tissue position.
- Check the new laser position by firing the laser again by shooting again.
- Repeat as often as necessary to get the laser within the crosshairs.

Important: This offset correction is limited to 50 µm to avoid possible misalignments. After a docking process these values are set to zero again. If the maximal allowed optimization is not enough, please contact the Bruker hardware support (service.bdal.de@bruker.com).

3. Shoot on your sample to check the spectrum signal intensity. Adjust the laser slider percentage power (higher or lower) to increase or decrease the spectrum intensity. Repeat the shoot/adjust steps multiple times until the base peak intensity is on average in the range of 1.0-1.5 x10e4.
4. Confirm that the resulting field/pixel size for your method (in **Laser** tab) is appropriate for the raster width set in flexImaging.
5. Save all new settings in your timsControl method.

Now the SCiLS™ autopilot setup for your application in timsControl and flexImaging is done. Switch to flexImaging and start the measurement. Select **Automatic Eject** so that the sample is removed from the instrument when the measurement is finished.

7 Service Information

It is helpful to have some information ready when contacting us:

Run the StatusReporter utility from the application in question (**Help menu** in timsControl and in flexImaging) and upload (i.e. copy & paste via Windows Explorer) the generated report to our ftp-server (**ftp://ftp.bdal.de/uploads/**). Afterwards, please send us an email with the name of the uploaded file.

For questions, please contact
service.bdal.de@bruker.com