

Ease of Use Workflow - rapifleX

Regarding Software
Compass 2.1 for rapifleX (FC 4.2.72 or higher), flexImaging 6.0

1. Introduction	1
2. What is the Ease of Use workflow?	1
3. Sample Preparation	2
4. Setting up the Ease of Use methods	3
5. Setting up experiments with the flexImaging <i>New Imaging Run Wizard</i> and the <i>Performance Check</i>	4
6. Laser preparation settings	7
7. Service Information	8

1. Introduction

This document outlines the combined flexControl and flexImaging workflow for setting up a MALDI Imaging experiment when using the Ease of Use workflow found in flexImaging 6.0, compatible with Compass 2.1 for rapiflex series.

These instructions will cover:

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

Please follow exactly the below steps to ensure optimal usage.

2. What is the Ease of Use workflow?

The Ease of Use Workflow is a streamlined process for setting up MALDI imaging experiments on the rapifleX 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 flexControl for instrument performance optimization tools directly from the flexImaging *New Imaging Run Wizard*.

These tools are:

- Detector Check,
- Target Height Profile generation,
- Laser Focus Adjustment, and
- Automatic Mass Calibration.

In addition, the option for *online peptide recalibration* for measurements of tryptic peptides is included.

Important: The Ease of Use workflow 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 Ease of Use workflow will *only* work with Bruker's IntelliSlides™. Please be aware of the following points:

1. Please create your optical pre-measurement scan before matrix coating. We recommend using the Bruker TissueScout for scanning samples. The entire IntelliSlide including all teach marks, barcode and Bruker logo must be scanned (see Figure 1).

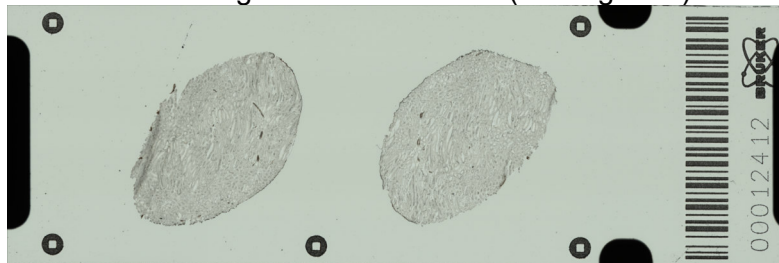


Figure 1. Example of IntelliSlide scan using TissueScout.

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

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

2. 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.

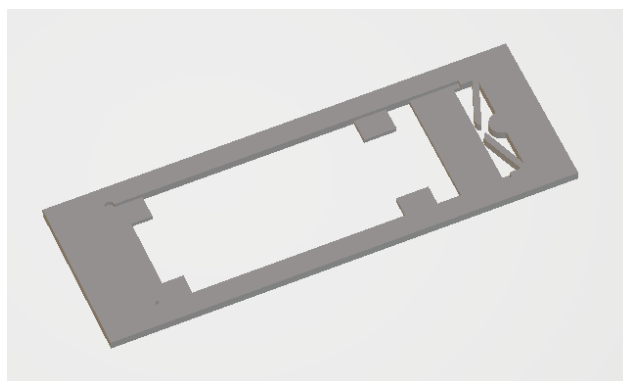


Figure 2. Example of modified IntelliSlide sprayer mask.

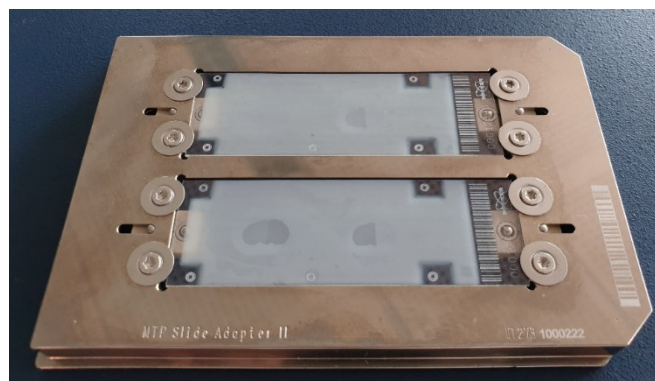


Figure 3. Example of IntelliSlides™ coated with matrix using the modified mask, showing barcode and teachmarks free of matrix.

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

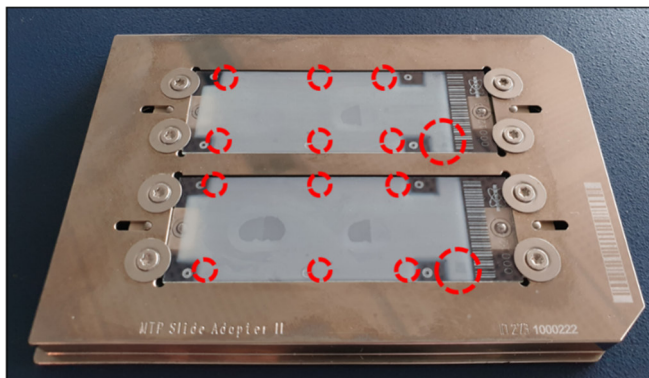


Figure 4. Areas that should be kept free of tissue sample.

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

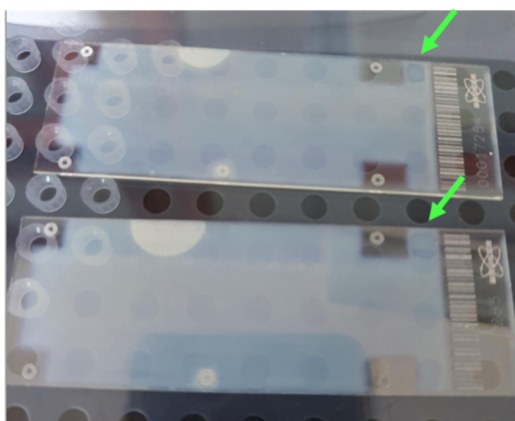


Figure 5. Area for MS calibration, with example of Peptide Calibration Standard II spread in the area.

4. Setting up the Ease of Use methods

The Ease of Use workflow is recommended only for the following applications:

- Protein (linear positive mode)
- Peptide (reflector positive mode)
- Tryptic peptide (reflector positive mode)
- Glycan (reflector positive mode)
- Lipid (reflector positive mode)
- Lipid (reflector negative mode)

Performance is not guaranteed if used for other applications.

For newly installed instruments, pre-set methods using recommended parameters from Bruker's application laboratory are preloaded onto your system. To set up an Ease of Use method from existing methods on your instruments, you can use the *CreateEaseOfUse Methods* utility, as outlined in the respective document (Quickstart_CreateEoUMethod_instructions.pdf).

Make sure that the following settings are confirmed for each of your methods in flexControl before starting any measurements:

- In the *Automation* tab
 - Recalibration for tryptic peptides should only be activated for the peptide digest method.
- In the *Calibration* tab
 - The appropriate mass control list for your calibrant has been selected.
 - The appropriate mode for calibration is loaded.
 - If you are unsure, *cubic enhanced* is the general recommendation, but requires a minimum of six (6) calibrant peaks. If you have less than six calibrant peaks picked in the spectrum, use quadratic.
- In the *Sample Carrier* tab, activate:
 - Use Target Profile
 - Use Focus Adjustment
 - Use IntelliSlides

Note: All 3 settings will be used automatically after the *Performance Check* has been executed as described in the following chapter.

5. Setting up experiments with the flexImaging *New Imaging Run Wizard* and the *Performance Check*

The flexImaging 6.0 *New Imaging Run Wizard* now offers three possible workflows:

- Ease of use workflow, covered in this document
- Automatic workflow, offered from flexImaging 5.1, not covered in this document
- Classic workflow, offered from flexImaging 5.0 and earlier, not covered in this document

Before starting flexImaging, make sure that flexControl 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 rapifleX instrument.

Important: MTP Slide Adapter II must be selected in the flexControl 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.

1. Open flexImaging and set up a new imaging run using the *New Imaging Run Wizard*. Select “Ease of use workflow”.

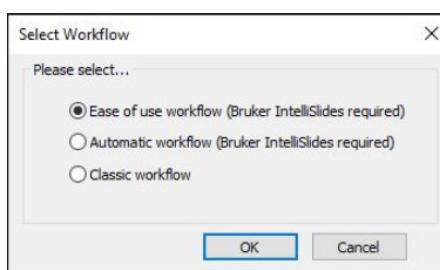


Figure 6. flexImaging Wizard – Select workflow

2. *New Imaging Run Wizard* window 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 barcode reading and 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.

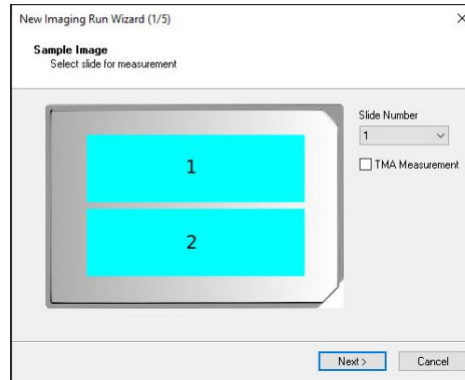


Figure 7. flexImaging Wizard – Sample Image

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

3. *New Imaging Run Wizard* window 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

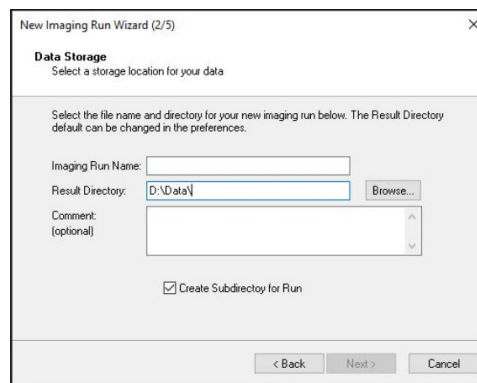


Figure 8. flexImaging Wizard – Data Storage

4. *New Imaging Run Wizard* window 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 EaseOfUse method folder (D:\Methods\flexControlMethods\EaseOfUse), with the recommended methods that have been created following section 4.

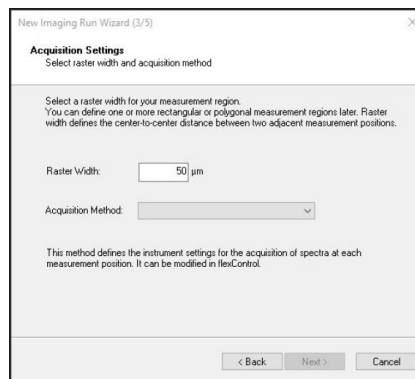


Figure 9. flexImaging Wizard – Acquisition Settings

5. *New Imaging Run Wizard* window 4/5 – Completing
 - If the automatic teaching is not yet complete, it is doing so now

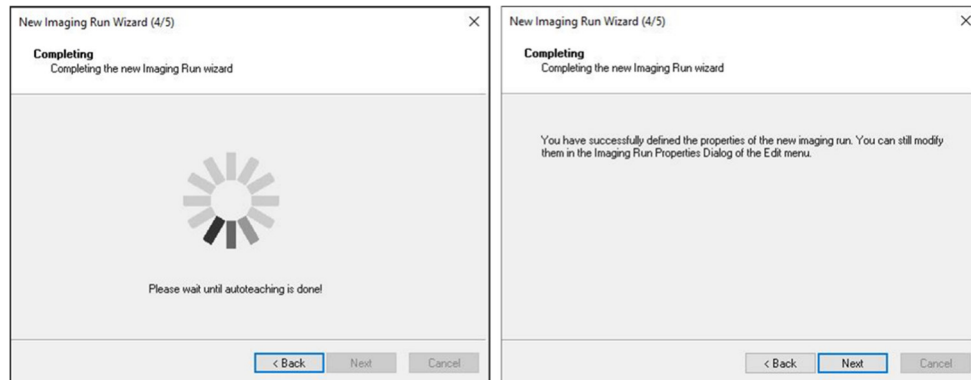


Figure 10. flexImaging Wizard – Completing

6. *New Imaging Run Wizard* window 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.

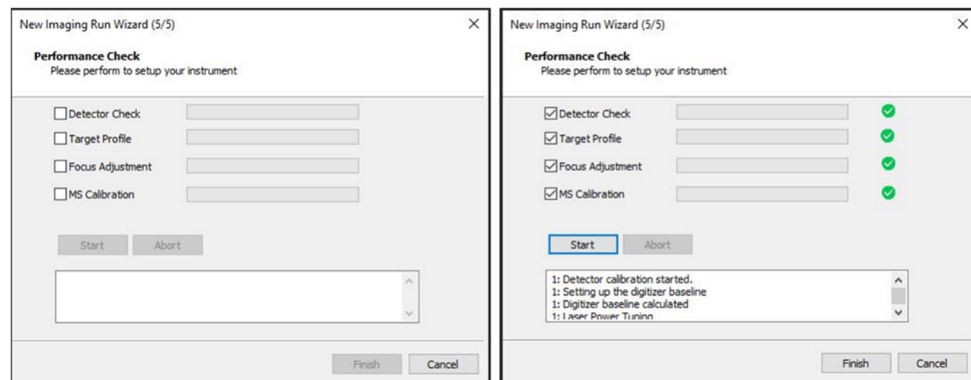


Figure 11. flexImaging Wizard – Performance Check showing initial settings and completed check

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

7. 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.

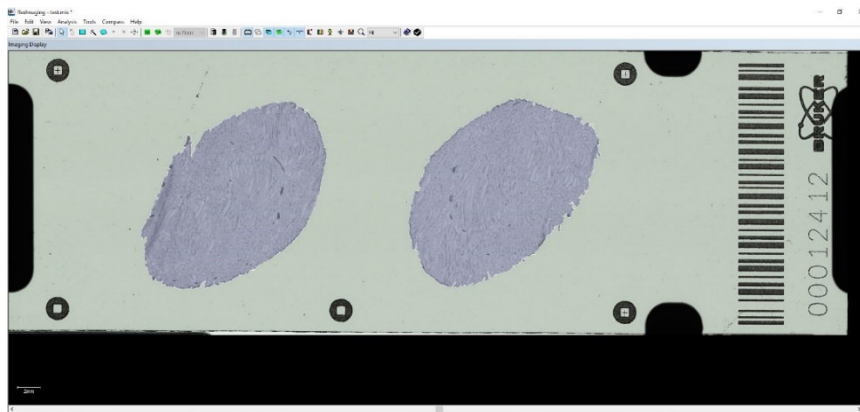


Figure 12. flexImaging Imaging Display showing automatically assigned measurement regions

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

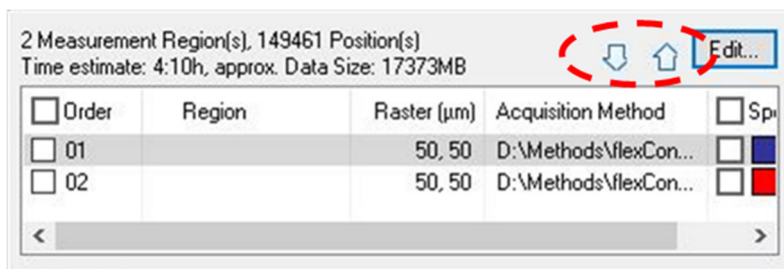


Figure 13. flexImaging Wizard – Regions window with arrows to re-order measurement regions circled in red dotted line

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 flexControl:

6. Laser preparation settings

1. In flexControl, 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 Fig 14).

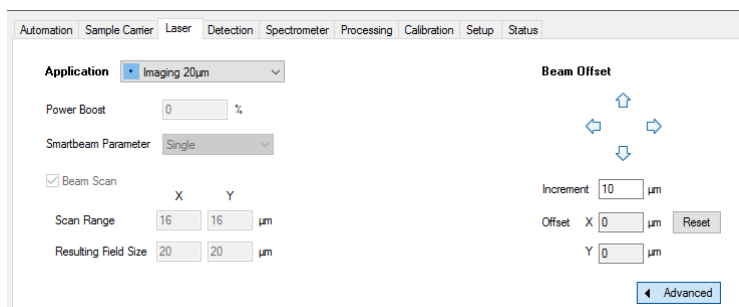


Figure 14. flexControl Laser Tab

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 (maldi.support@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 intensity of the spectra is on average in the range of $1.0\text{-}1.5 \times 10^4$.
4. For most experiments, the detector gain level output from the Detector Check is ideal. Default value here is gain 1x and after each Detector Check the current detector voltage will be automatically adapted to this gain.
*In some cases, the individual tissue/analyte character may require higher detector gain settings. It is possible to adapt the detector gain, for details please refer to MALDI.application.support@bruker.com.
Please be aware: a manually adjusted gain (e.g. 1.5x) leads to a detector boost. A following usage of the method will, after doing the automatic Detector Check again, not reset the gain. It is the detector voltage value for default gain 1x which is automatically adjusted, but the boost value remains.*
5. Confirm that the resulting field/pixel size for your method (in *Laser* tab) is appropriate for the raster width set in flexImaging. Please be aware that you will have overlapping pixels if the raster width is less than the resulting field size.
6. Save all new settings in your flexControl method.

Now the Ease of Use setup for your application in flexControl 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 flexControl 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
maldi.sw.support@bruker.com
November 2020, Version 1.5 (Alice Ly)