

## rapifleX - Ease of Use Workflow

### Required Software

Compass 2.1 for rapifleX (FlexControl 4.2.72 or higher),  
flexImaging 6.0

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### 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 in flexImaging 6.0, compatible with Compass 2.1 for rapifleX series.

These instructions will cover:

- An explanation of the workflow
- Sample preparation procedure
- Method settings in flexControl
- Experiment set-up in flexImaging

**Note:** Please follow the procedure carefully to produce optimal results.

### 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 such 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
- Automatic Mass Calibration

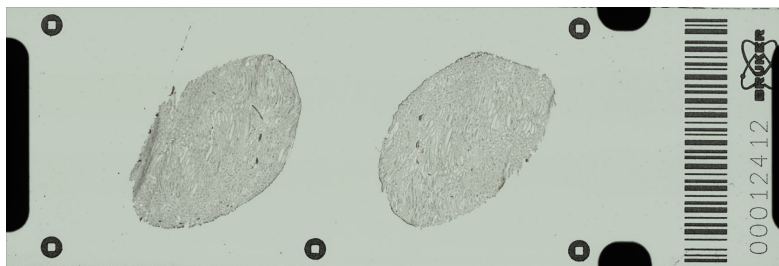
In addition, the option for **online peptide recalibration** for measurements of tryptic peptides is included.

**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™.

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

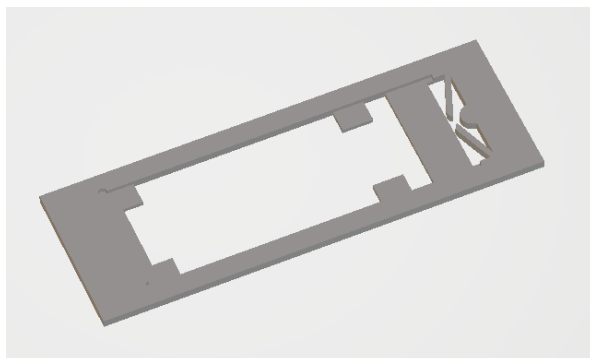


**Figure 1. Example of IntelliSlide scan using TissueScout.**

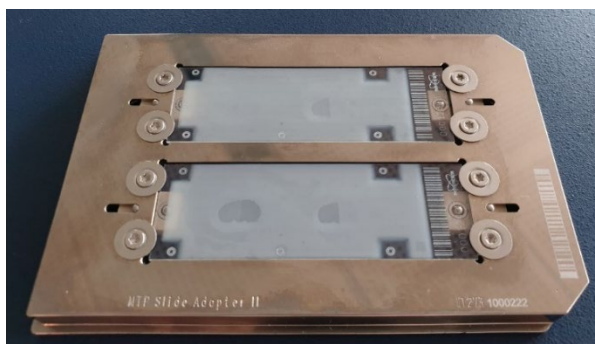
After scanning, copy the scanned image to D:\Methods\images\Import on your rapifleX acquisition computer.

**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 regions on the IntelliSlide must remain matrix-free. Simply insert your slide/s into the mask/s before matrix coating.

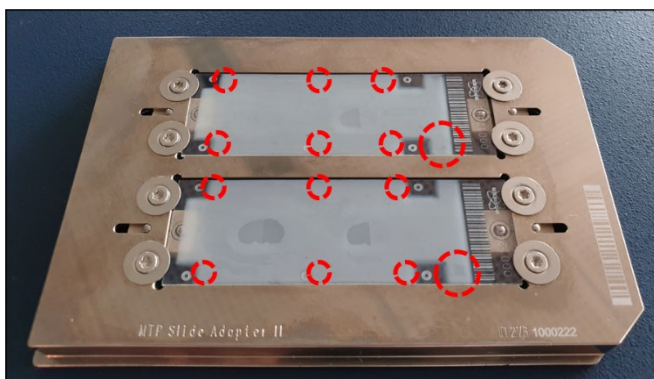


**Figure 2. Example of modified IntelliSlide sprayer mask.**



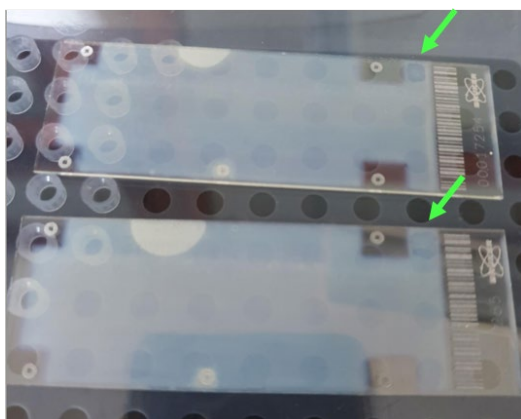
**Figure 3. Example of IntelliSlides™ coated with matrix using the modified mask, showing barcode and teachmarks free of matrix.**

3. 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.**

4. The top right area of the slide is used for MS calibration (Figure 5). This area is approximately 5 mm × 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 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 [Quickstart\\_CreateEoUMethod\\_instructions.pdf](#) provided on the installation medium.

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 digested 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 section 5.

## 5. flexImaging New Imaging Run Wizard and the Performance Check

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

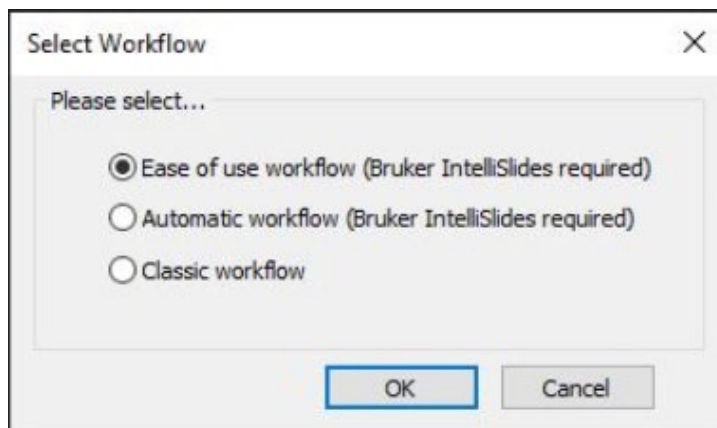
- **Ease of use workflow**, covered in this document
- **Automatic workflow**, offered from flexImaging 5.1, not covered in this document
- **Classic workflow**, offered from flexImaging 5.0 and earlier, not covered in this document

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 contain a preparation. When this is confirmed, please dock the MTP Slide Adapter into the rapifleX instrument.

**Important:** **MTP Slide Adapter II** must be selected in 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 (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 select the **TMA Measurement** checkbox in this window. Different algorithms are used to detect large tissue samples or the smaller cores found in TMAs. Selecting the **TMA Measurement** checkbox chooses the correct algorithm to detect and assign tissue samples in the scan as measurement regions.

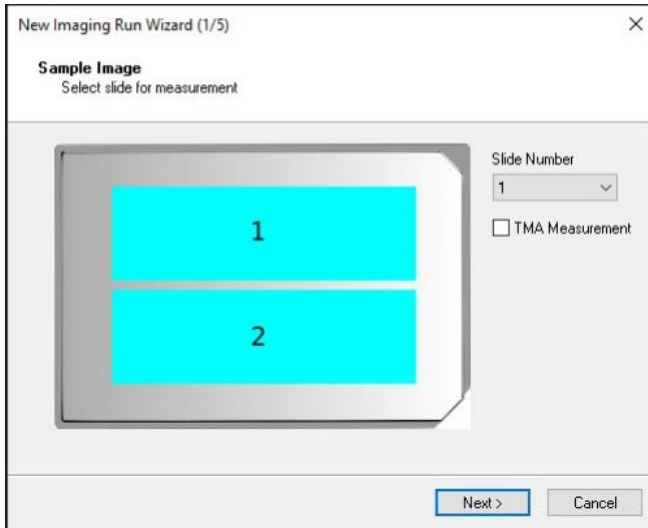


Figure 7. flexImaging Wizard – Sample Image

**Important:** From this point on, you must not undock the MTP Slide Adapter II from the instrument.

## 3. New Imaging Run Wizard (2/5) – Data Storage

- Type a name into the **Imaging Run Name** field.
- Use the **Browse** button to select the destination folder for results in the **Result Directory** field.
- Select the **Create Subdirectory for Run** checkbox.

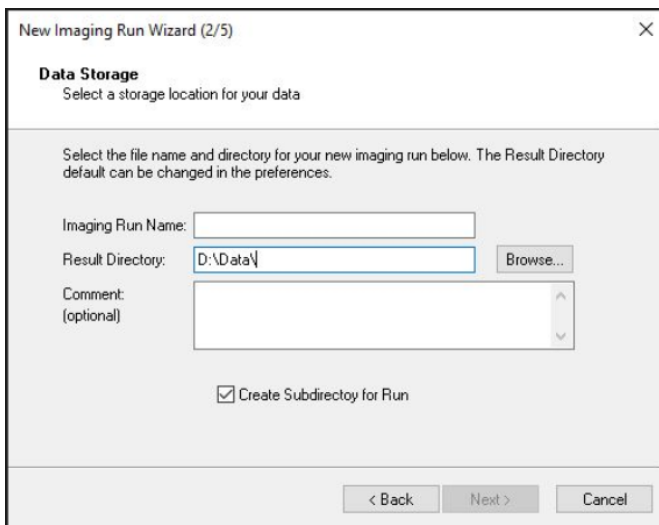


Figure 8. flexImaging Wizard – Data Storage

## 4. New Imaging Run Wizard (3/5) – Acquisition Settings

- Set an appropriate **Raster Width** for your acquisition method.
- Select your defined **Acquisition Method** from the drop-down menu. This menu only accesses `D:\Methods\flexControlMethods\EaseOfUse`, the EaseOfUse method folder, with the recommended methods that have been created following section 4.

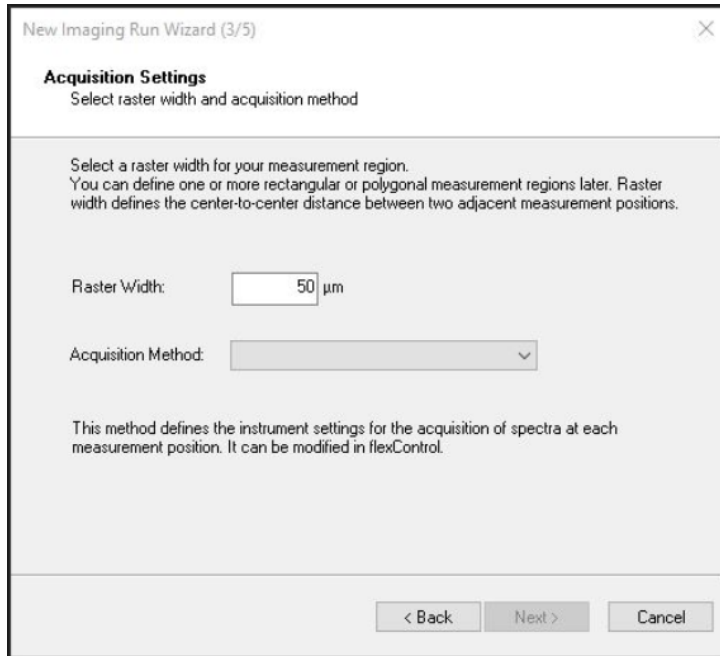


Figure 9. flexImaging Wizard – Acquisition Settings

## 5. New Imaging Run Wizard (4/5) – Completing

- This dialog displays either autoteaching progress or successful completion of the autoteaching.

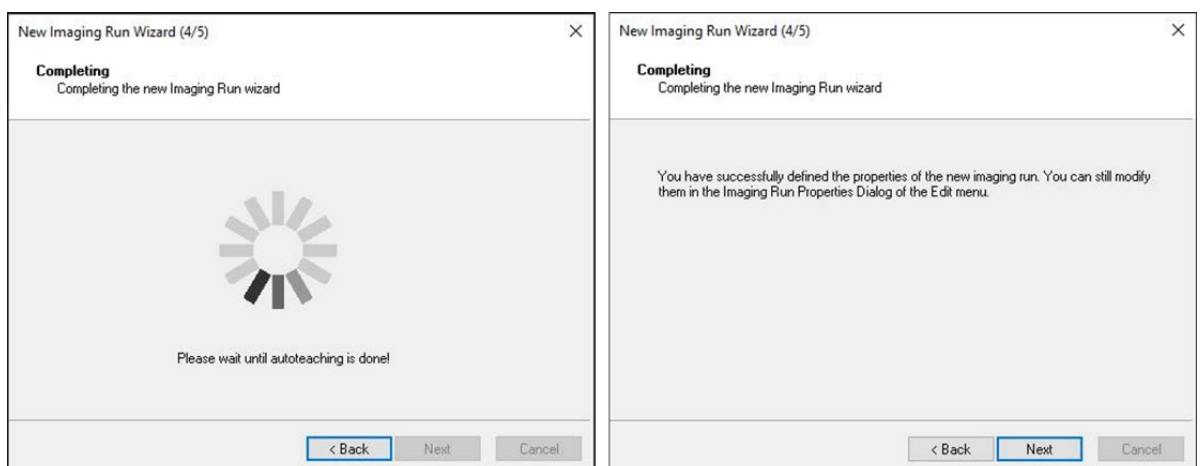
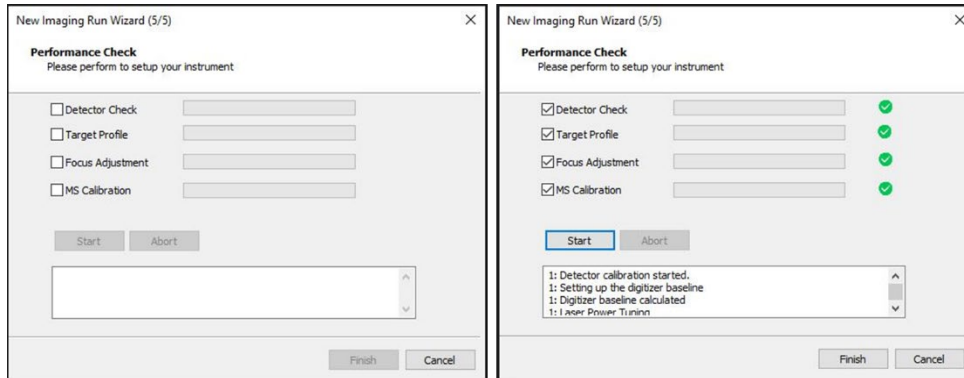


Figure 10. flexImaging Wizard – Completing



## 6. New Imaging Run Wizard (5/5) – Performance Check

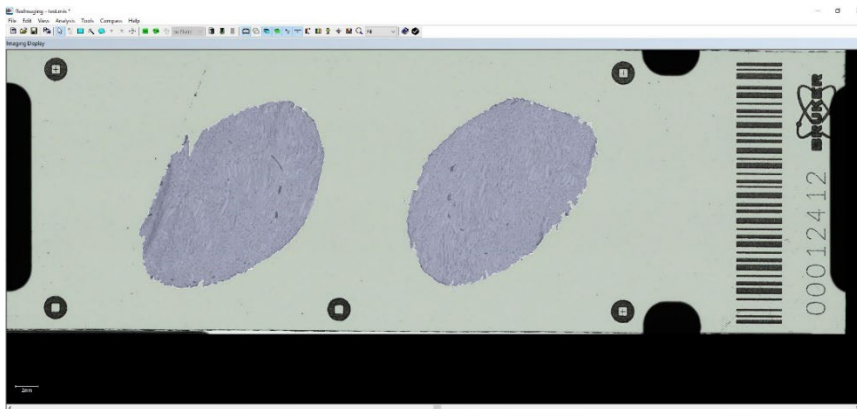
- Users can select which tools to perform for their run, which are conducted in the order that they are listed in the window.
- Progress is displayed and the successful or unsuccessful completion of the tool(s).
- When the Performance Check is complete, click **Finish** to exit the Wizard.



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

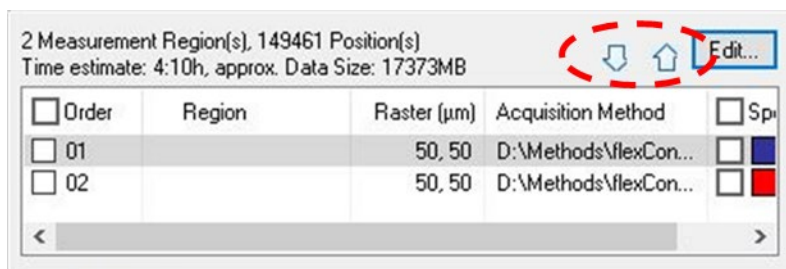
**Important:** As a minimum, you must conduct **Target Profile** and **Focus Adjustment** for each run.

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 showing arrows used to re-order measurement regions**

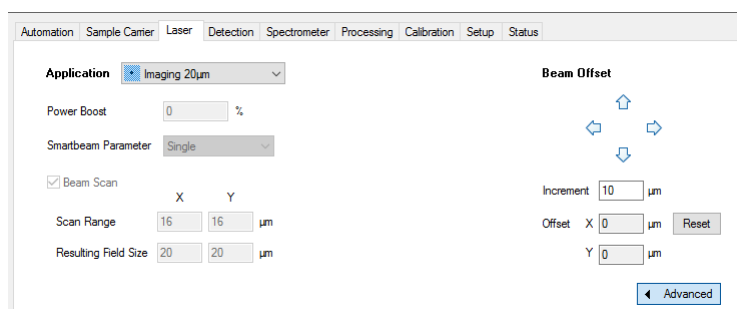
**Note:** Remember to save your flexImaging sequence after any editing

The Imaging sequence is now ready for use. Select **File > Save Imaging Run** to save the Imaging sequence.

## 5. Laser preparation settings

As a final step, the laser (power and beam offset) must be adjusted in flexControl.

1. In flexControl, go to a section of the tissue and conduct several test shots. Click **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 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 conducting a test shot again.
- Repeat as often as necessary until the laser within the crosshairs

**Important:** This offset correction is limited to 50 µm to avoid possible misalignments. After the docking process these values are set to zero again. If the maximal allowed optimization is not sufficient, please contact Bruker hardware support ([maldi.support@bruker.com](mailto:maldi.support@bruker.com)).



3. Conduct a sample shot 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.

**Note:** 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](mailto:MALDI.application.support@bruker.com).

A manually adjusted gain (e.g. 1.5x) leads to a detector boost. A following usage of the method will not reset the gain after performing the automatic Detector Check again. The detector voltage value for default gain 1x is automatically adjusted, but the boost value remains unchanged.

5. Confirm that the resulting field/pixel size for your method in the **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.

Ease of Use setup for your application in flexControl and fleximaging is now complete. Switch to flexImaging and start the measurement. Select **Automatic Eject** to remove the sample 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 (see **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 including the filename of the upload.

For further queries, please contact  
[maldi.sw.support@bruker.com](mailto:maldi.sw.support@bruker.com)

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