flexControl 3.4
User Manual

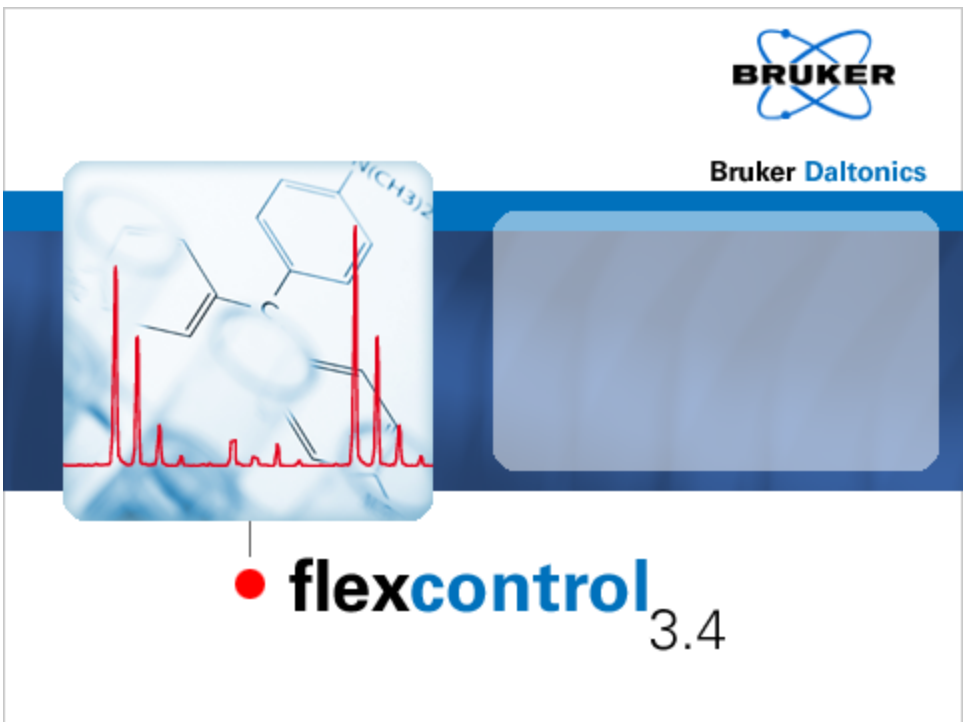

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Table of Contents

1 Introduction

flexControl is a program designed to configure and operate time-of-flight mass spectrometers of the Bruker flex series, such as the microflex, autoflex and ultraflex instruments. flexControl runs under Microsoft® Windows® XP® (SP3) or Windows 7 operating systems. The Bruker post processing software flexAnalysis 3.4 can reload recorded spectra from the disk for post processing.

Before installing flexControl 3.4 for the first time, Microsoft .NET Framework 2.0 must have been installed. For more information, refer to the installation instructions that are part of the Compass installation.

Figure 1-1 Dialog on the Compass installation CD

flexControl uses standard Microsoft® Windows® conventions to operate with windows, menus, dialog boxes, and the mouse. The operator should be familiar with the basic operation of the computer and with Microsoft® Windows® software. Some general

instructions are given here, but if additional support is necessary regarding the basic use of a computer, please review its accompanying documentation.

Depending on the mass spectrometer (autoflex, microflex, ultraflex TOF/TOF), flexControl offers features specific to the respective unit.

flexControl 3.4 (Compass 1.4 for flexSeries) is able to operate in combination with the Bruker Compass Security Pack. The Bruker Compass Security Pack and user management are described in a separate manual.

Main features of the Bruker Compass Security Pack are electronic signatures, audit trailing, and user management. Note that this is different to the user management of the operating system.

Bruker User Management is a tool that allows managing access to Bruker applications and assigning individual privileges to users. Operator IDs and passwords identify such privileges. The access of a user to features of Bruker applications depends on these privileges.

On saving data files, the operator can electronically sign them, if they have this specific privilege. Electronic signatures are created using the operator's ID and their password.

Audit trails are useful for tracing the operations performed at a specific time point.

2 Getting Started

2.1 Installation

Please refer to the separate installation instructions on the installation DVD, or contact Bruker via e-mail (maldi.sw.support@bdal.de) to install flexControl 3.4 on the instrument control computer.

2.2 Starting flexControl

In accordance with Windows conventions, flexControl can be started via the Windows **Start** menu. During installation of the Bruker Daltonics Applications package, a Bruker Daltonics folder was automatically created in the **Start** menu's **Programs** folder, which contains the respective applications.

A second way to launch flexControl is by double clicking the corresponding icon  on the Windows desktop.

During start up of the program (see Figure 3-1) the last loaded method automatically opens. If a dialog informing the user that the method has been saved with another flexControl version appears, click **OK** to save the method. flexControl can acquire five different types of spectra and therefore offers five different method types; each identified by a specific file extension:

- `<file name>.par` (for standard applications).
- `<file name>.lft` (for LIFT applications on a tandem mass spectrometer).
- `<file name>.psm` (for FAST applications).
- `<file name>.isd` (for applications with undigested proteins).
- `<file name>.srm` (for single reaction monitoring applications).

If a LIFT method is loaded, the **LIFT** page (see section 3.7.9) appears additionally and the **Calibration** page (see section 3.7.8) changes. If a FAST method is loaded, the **FAST** page is shown (see section 3.7.10). If a LIFT method is loaded on a non-LIFT instrument the following message appears:

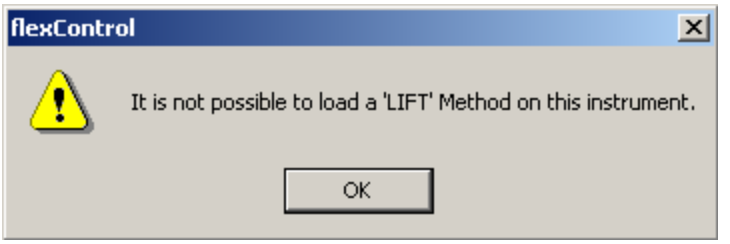


Figure 2-1 flexControl has not detected a tandem instrument

2.3 Closing flexControl

To close flexControl, click the application's **Close** button , select **File > Exit**, or press ALT + F4.

If the currently loaded method or instrument setting file (`*.isset`) have been changed but not saved, message dialogs appear asking the user to save the respective settings.

Select **No** or **Cancel** to stay in flexControl and save the files under different file names.

3 Graphical User Interface (GUI)

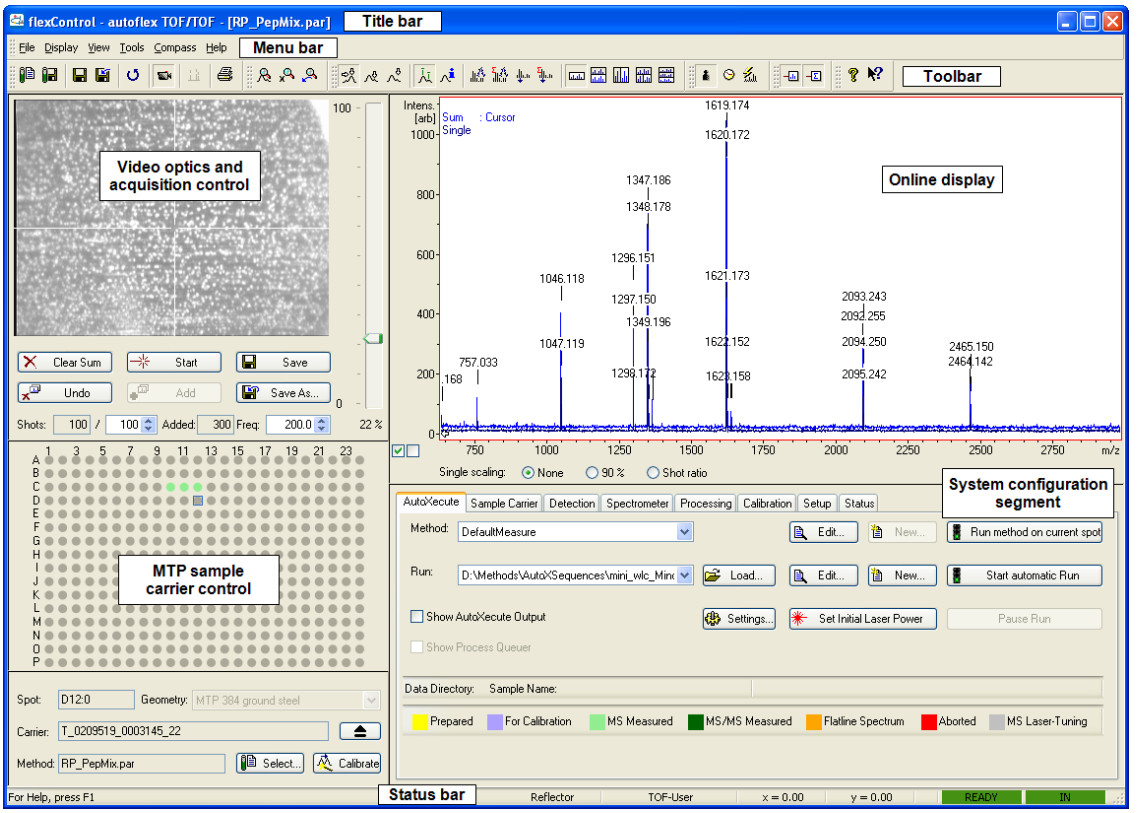


Figure 3-1 Graphical user interface of flexControl

The flexControl GUI shows the current entire status of the system. It is used to configure and control a mass spectrometer of the flex-series in order to acquire data.

The GUI (see Figure 3-1) is composed of the following items:

- Title bar (see section 3.1)
- Menu bar (see section 3.4)
- Toolbar (see section 3.2)
- Status bar (see section 3.4.3.2)
- Video optics and acquisition control (see section 3.5.1)

- MTP sample carrier control (see section 3.5.2)
- Online display (see section 3.6)
- System configuration segment (see section 3.7).

3.1 Title Bar

The title bar (see Figure 3-2) reflects the type of the mass spectrometer, for example, ultraflex and the currently loaded method. The left edge of the title bar contains the application's control menu button and the right edge contains the familiar Windows minimize, maximize, and close buttons.

Figure 3-2 Title bar in flexControl

3.2 Toolbar

Figure 3-3 Toolbar of flexControl

Commonly used menu commands can be accessed through toolbar buttons. These buttons are organized into five groups.

- **File** menu commands (see section 3.4.1).
- **Display** menu commands (see section 3.4.2).
- **View** menu commands (see section 3.4.3).
- **Tools** menu commands (see section 3.4.4).
- **Help** menu commands (see section 3.4.5).

The buttons available in the toolbar can be configured by the user (see section 3.4.3.1).

3.3 Status Bar

The status bar (see Figure 3-4) is located at the bottom of the GUI. It displays several data parameters, such as instrument mode, current user, coordinates of the mouse pointer, status of the instrument and target location.

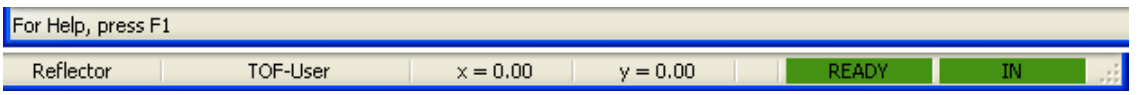


Figure 3-4 Status bar of flexControl

3.4 Menu Bar

The menu bar (see Figure 3-5) consists of six drop-down menus. Each menu contains groups of commands related to this menu. Many menu commands can also be accessed using toolbar buttons (see section 3.2) or short cuts.

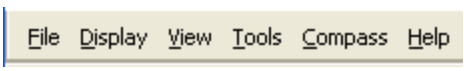


Figure 3-5 flexControl menu bar

3.4.1 File Menu

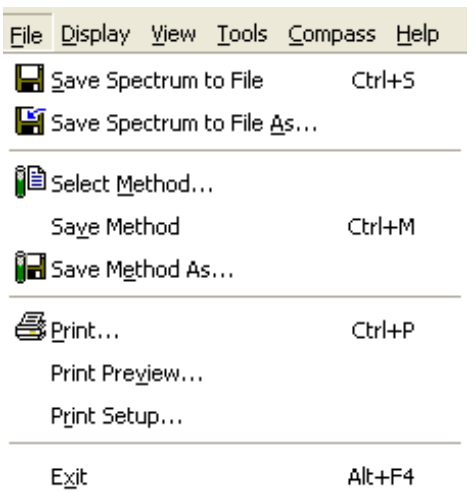


Figure 3-6 File menu commands

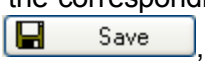
The **File** menu (see Figure 3-6) contains commands for handling methods, saving data, and printing reports.

Table 3-1 Feature buttons of the File menu with corresponding tasks

Toolbar button	Menu command	Shortcut	Description
	**Save Spectrum to File**	CTRL+S	Saves spectrum.
	**Save Spectrum to File As**		Saves spectrum under another name.
	**Select Method**		Opens a dialog box to select a method from the file system.
	Save Method	CTRL+M	Saves the method.
	**Save Method As**		Opens a dialog to save a method with a new name.
	**Print**	CTRL+P	Prints a report of the selected analyses.
	Print Preview		Displays print preview using the selected report layout.
	Print Setup		Displays the Windows dialog for printer setup.
	Exit	ALT+F4	Closes flexControl.

3.4.1.1 Save Spectrum to File

The current spectrum can be saved to disk by:

- selecting **File > Save Spectrum to File**,
- clicking the corresponding button in the toolbar  or the **Acquisition Control** section 

,
- or using the short cut (CTRL+S).

flexControl supports two spectrum data formats:

- the XMass format (see Figure 3-7) in which each spectrum is saved in a separate folder/file structure. This is the traditional format for spectra from flex series instruments.
- the new container format introduced with Compass 1.4.

flexControl handles names of saved spectra in such a manner that overwriting of spectra is automatically avoided. Every **Save** process performed in a directory generates a numerated subfolder with a new analysis tree.

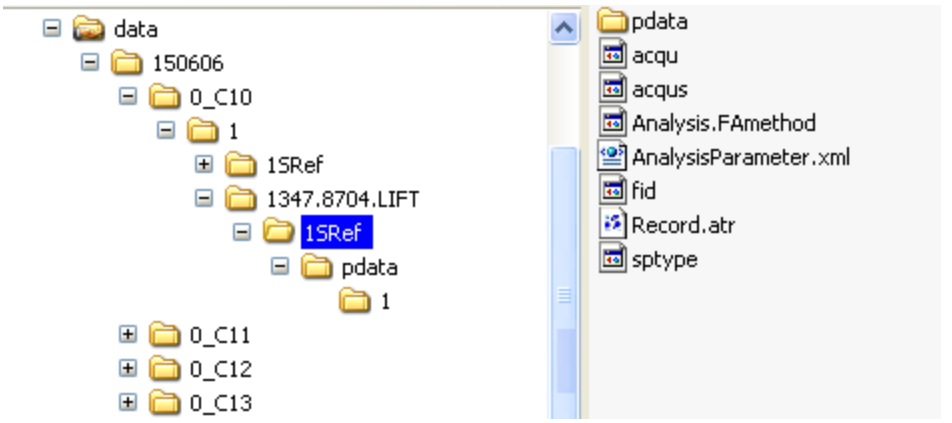


Figure 3-7 Example of the XMass file structure

Raw data are stored in a `fid` file. Processed data are stored in a `1r` file. Each analysis contains both files. If raw data are not yet processed both the `fid` and the `1r` files contain the same data.

In the container data format (see Figure 3-8), all data from an autoXecute run with the same sample name are saved in a single directory with a `.d` extension that contains only a few files. Bruker software never prompts for files within the directory, it only requests the `.d` directory.

The container format is only used for automatic measurements for LC-MALDI and MALDI imaging.

When saving a file for the first time, the file structure must be generated, and a dialog appears (see Figure 3-9). If the **Save** command has already been used in a flexControl session for this spectrum type, the file is automatically saved, since a valid path- and sample name exist.

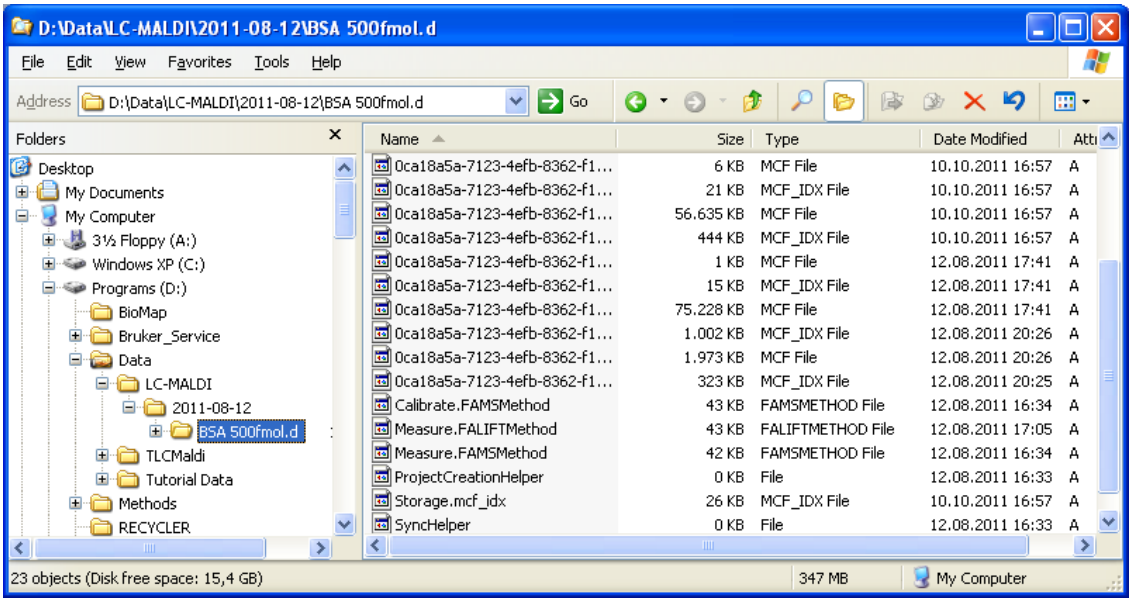


Figure 3-8 Example of the container data format

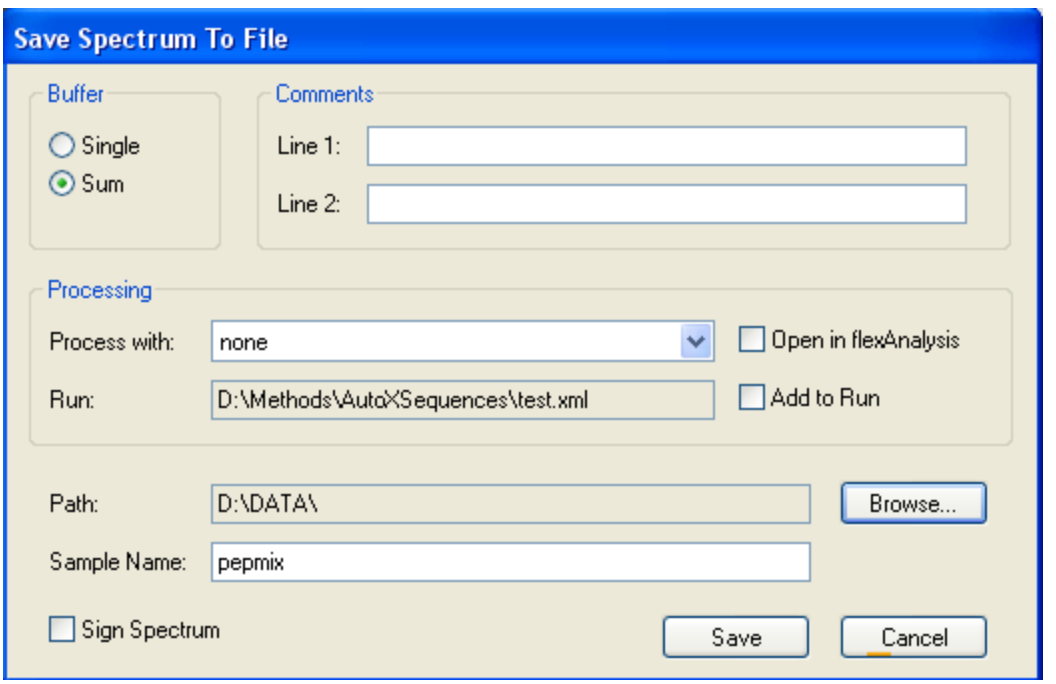


Figure 3-9 Save (As) dialog to specify data directory and possible post-processing

- **Single/Sum**: Select the desired option to save the single or the sum buffer.
- **Comments** : Comments are saved with the spectrum and appear in post-processing tools like flexAnalysis and BioTools.
- **Process with**: Specifies a method for post processing in flexAnalysis if it is installed on the same computer. When opening a spectrum the first time in flexAnalysis the assigned processing method will be executed.
- **Open in flexAnalysis**: If this check box is selected, the spectrum is automatically sent to flexAnalysis and any assigned method is executed. If no method is assigned, the spectrum opens in flexAnalysis with a defined default method (see *flexAnalysis User Manual*).
- **Run**: This feature should be used to save the information on a measured MS spot (path, sample name and comments) and use it for MS/MS measurement on this spot afterwards.
    - Select the **Add to Run** check box to store the MS information in the currently displayed AutoXecute Run. If no run is loaded, a new one, named yyyymmdd_hhmmss.xml, is automatically created in the folder `D:\Methods\AutoXSequences\ManualMeasurements`.
    - It is possible to transfer (different) comments and sample names for different spots in one run. During the subsequent LIFT measurement, the spot information is automatically loaded into the **Save (As)** dialog, enabling LIFT spectra to be saved alongside the corresponding MS spectrum.

►► To use this feature

1. In the **AutoXecute** tab, select **none** for **AutoXecute run**.
2. Acquire a MS spectrum, open the **Save As** dialog and select the check box **Add to Run**.
3. Change the settings (path, sample name,…) if necessary and save the spectrum.
4. Load a LIFT method and start the manual LIFT acquisition for any LIFT mass on the green spot where the MS spectrum has been acquired.
5. Open the **Save As** dialog where the correct spot information has been transferred and the LIFT spectrum can be saved directly.

This feature can also be used to perform manual MS measurement and/or select masses for LIFT manually and automatically perform subsequent LIFT measurements.

For details please refer to the `Quickstart_PrecursorSelection.pdf` available on the installation DVD.

- **Add to Run**: If a run is loaded and this check box is activated, the data directory, sample name information, comments and any selected flexAnalysis method are transferred from the run to the **Save As** dialog.
- **Path**: Select a data directory for the results. Use the Browse... button to change the path.
- **Sample**: Type a sample name.
- **Sign Spectrum**: If the Compass Security Pack is installed, spectra can be signed during the save process (if the user has the specific privilege). A valid operator and password are required.

Note If a spectrum is signed in flexControl, it is write-protected. No post processing results can be stored with flexAnalysis or any other programs.

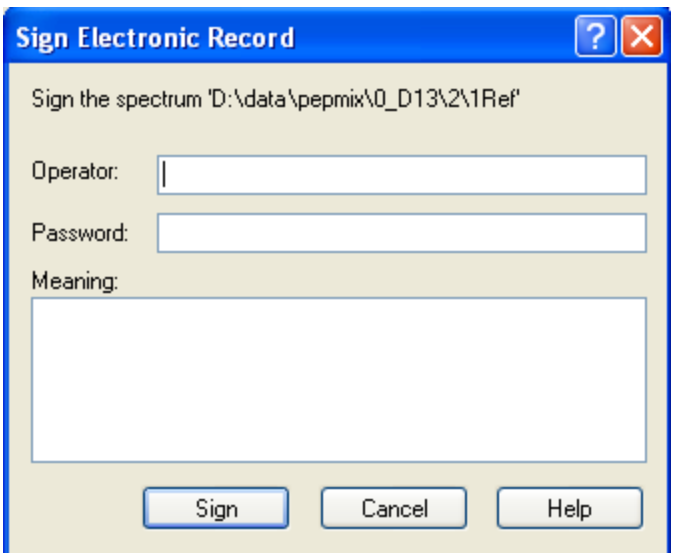
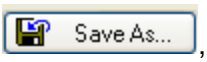


Figure 3-10 Signing a spectrum

3.4.1.2 Save Spectrum to File As

The current spectrum can be saved to disk under another name by:

- selecting **File > Save Spectrum to File As**,
- clicking the corresponding button in the toolbar  or the **Video Optics and Acquisition Control** section 

,

The dialog that appears every time **Save Spectrum to File As** is selected is the same as for the **Save Spectrum** command.

This command is unavailable in case of a FAST measurement, as saving is automatically done after switching to the next segment. Even at the end of a manual FAST measurement, the spectrum is automatically saved to the path and sample name selected on the FAST page (see section 3.7.10).

3.4.1.3 Select Method

The **Select Method** command and the corresponding icon  on the toolbar open a dialog for selecting one of the following flexControl methods:

- `*.par` for fingerprint spectra.
- `*.psm` for FAST measurements.
- `*.lft` for LIFT measurements on a tandem mass spectrometer.
- `*.isd` (Ion Source Decay) for measurements of fragments arising inside the ion source.
- `*.srm` (Single Reaction Monitoring) for measurements with only one fragment.

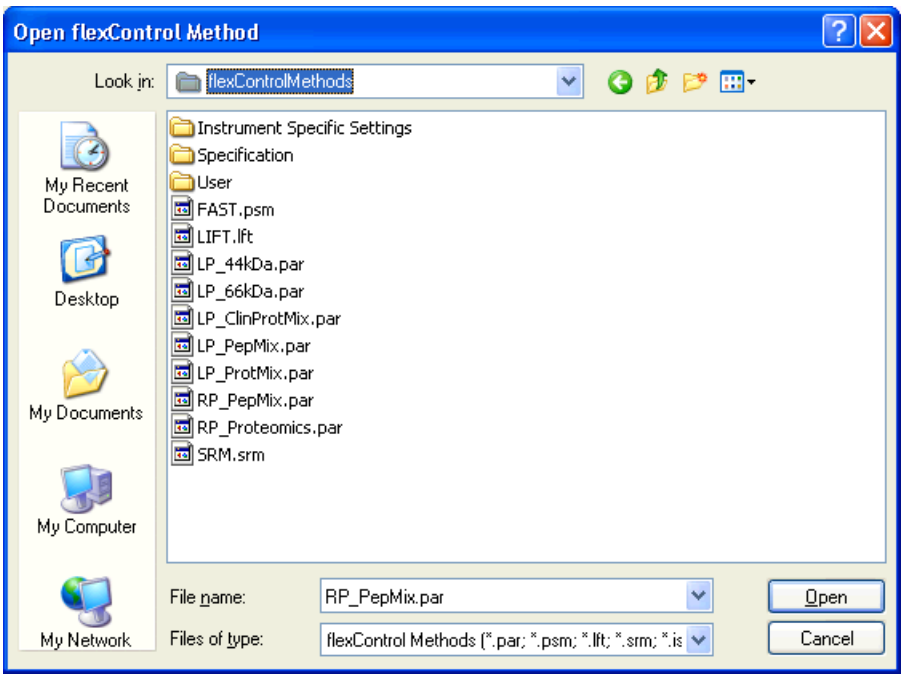


Figure 3-11 Open flexControl Method dialog

3.4.1.4 Save Method

The **Save Method** command saves the currently loaded (write-enabled) method.

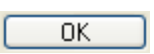
3.4.1.5 Save Method As

The **Save Method As** command, and the corresponding icon  of the toolbar open a dialog for saving the currently loaded method with a new name.

3.4.1.6 Print

A printer can be selected by:

- selecting **File > Print**
- clicking the corresponding icon  of the toolbar
- or using the short cut (CTRL+P)

Click  to start printing.

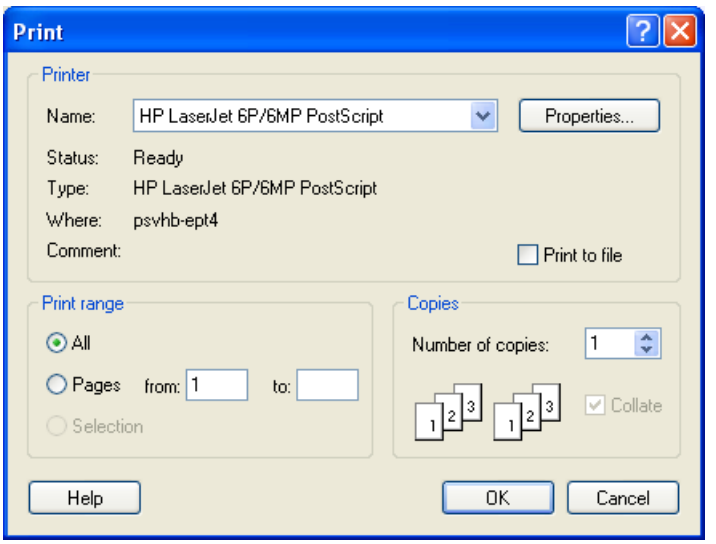


Figure 3-12 Print dialog

3.4.1.7 Print Preview

The **Print Preview** command is used to check the appearance of a spectrum before printing (see Figure 3-13). Click **Print** to start printing from this dialog.

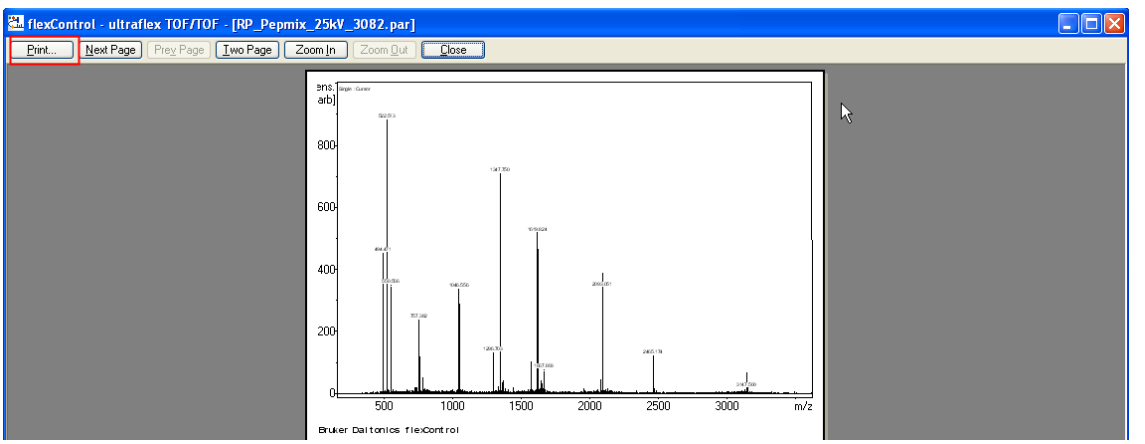


Figure 3-13 Print Preview dialog

3.4.1.8 Print Setup

The **Print Setup** command is used to adjust the default printer settings and opens the following dialog.

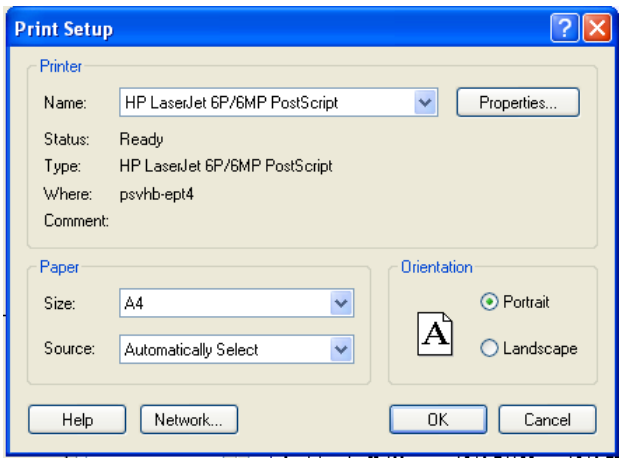


Figure 3-14 Print Setup dialog

3.4.1.9 Exit

The **Exit** command and the short cut ALT+F4 are used to close flexControl. Before closing a confirmation dialog appears (see Figure 3-15). Click **Yes** to close flexControl.

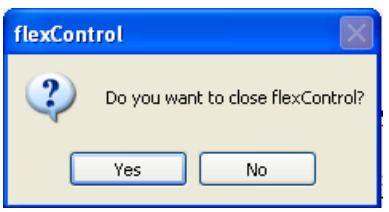


Figure 3-15 Close flexControl by applying “Yes”

If the currently loaded method or instrument setting file (`*.isset`) have been changed but not saved, message dialogs appear asking the user to save the respective method (see Figure 3-16) or instrument (see Figure 3-17) settings.

Select **Cancel** to stay in flexControl and save the files under different file names.

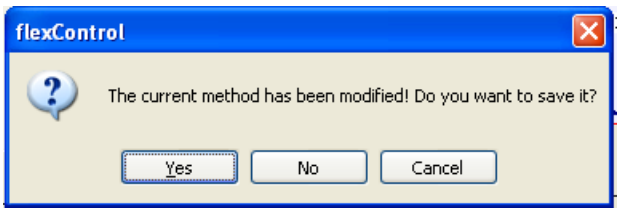


Figure 3-16 Save changed methods confirmation dialog

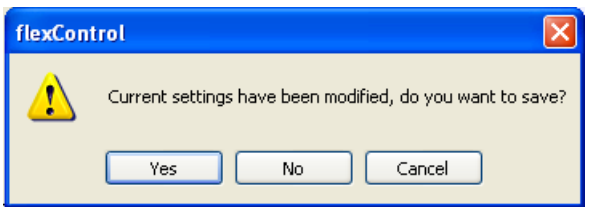


Figure 3-17 Save modified instrument settings confirmation dialog

3.4.2 Display Menu

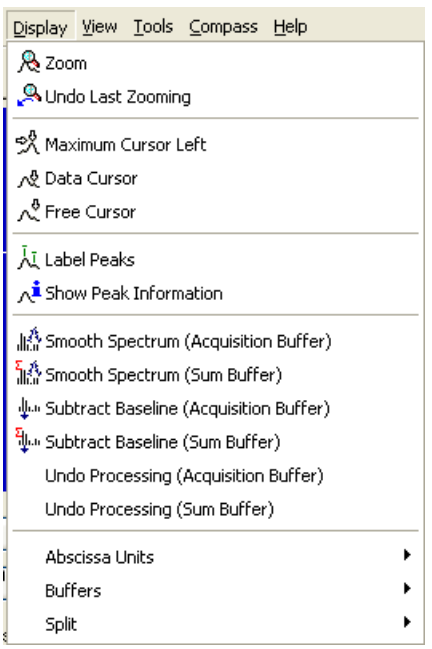


Figure 3-18 Display menu commands

The **Display** menu (see Figure 3-18) contains commands for manipulating spectra.

Table 3-2 Features of the Display menu

Toolbar button	Menu command	Description
	**Zoom**	Zooms the selected data range.
	**Undo Last Zooming**	Undoes the last zooming step.
	**Maximum Cursor Left**	Clicking to the left of a peak sets an arrow label at the top of the peak.
	**Data Cursor**	Sets an arrow label at the spectrum y-postion directly below the mouse pointer.
	**Free Cursor**	
	**Label Peaks**	
	**Show peak information**	
	**Smooth Spectrum (Acquisition Buffer)**	Smooths the spectrum in the active acquisition buffer.
	**Smooth Spectrum (Sum Buffer)**	Smooths the spectrum in the active sum buffer.
	**Subtract Baseline (Acquisition Buffer)**	Subtracts the baseline of the spectrum in the active acquisition buffer.
	**Subtract Baseline (Sum Buffer)**	Subtracts the baseline of the spectrum in the active sum buffer.
	Undo Processing (Acquisition Buffer)	Undoes smoothing and/or baseline subtraction of the acquisition buffer
	Undo Processing (Sum Buffer)	Undoes smoothing and/or baseline subtraction of the sum buffer

Toolbar button	Menu command	Description
	**Abscissa Units Mass**	Mainly used for standard operation. *m/z* scale represents a calibrated mass spectrum.
	**Abscissa Units Times**	Mainly used for debugging purposes. t [ns] denotes a raw time-of-flight-spectrum displayed in nanoseconds.
	**Abscissa Units Points**	Mainly used for debugging purposes. Shows how many data points are distributed over the peak shape.
	**Linear Detector Single Spectrum**	Displays a single shot, usually the last acquired sequence of shots.
	**Sum Spectrum**	Displays at least a single shot, usually the last acquired sequence of shots.
	**One Display**	One display is shown.
	**Two Displays**	Two displays, split vertically.
	**Two Displays**	Two displays, split horizontally.
	**Three Displays**	Three displays, split horizontally.
	**Three Displays**	Three displays, split vertically.

3.4.2.1 Zoom

The **Zoom** command and the corresponding icon  of the toolbar are used to enlarge a section within the Mass Spectrum window. The zoom function is active after the corresponding tool button is applied. Then the mouse pointer converts into the zoom pointer  (within the Mass Spectrum window).

To enlarge a mass range, move the zoom pointer to the initial position and press the left mouse button. Hold the button, move to the final position (in x and y direction), and release the mouse button. This action draws a rectangle inside the Mass Spectrum window. The content will be shown enlarged.

3.4.2.2 Undo Last Zooming

The **Undo Last Zooming** command and the corresponding icon  of the toolbar are used to reverse the last zooming step that was performed in the Mass Spectrum window via the **Zoom** command.

3.4.2.3 Maximum Cursor Left

The **Maximum Cursor Left** command and the corresponding icon  of the toolbar are used to create an additional cursor, which is always located left-hand to the mouse pointer. Left click of the mouse button fixes the cursor to that y-position (top of a peak) next to the mouse pointer, which is just higher.

3.4.2.4 Data Cursor

The **Data Cursor** command and the corresponding icon  of the toolbar are used to create an additional cursor that follows always the mouse pointer on the left side sliding over the peaks.

3.4.2.5 Free Cursor

The **Free Cursor** command and the corresponding icon  of the toolbar are used to create an additional cursor that accompanies the mouse pointer with an F. This mark might be fixed as desired by using the left mouse button.

3.4.2.6 Label Peaks

The **Label Peaks** command and the corresponding icon  of the toolbar are used to perform peak picking in the active buffer. The peak picking parameters can be set using the**Processing Parameter** dialog (see Figure 3-86).

3.4.2.7 Show Peak Information

The **Show Peak Information** command, or the corresponding icon  of the toolbar are used to open a window (see Figure 3-19), which contains the four (five) most important peak characteristics. This command is only available together with the activated **Maximum Cursor Left** button  .

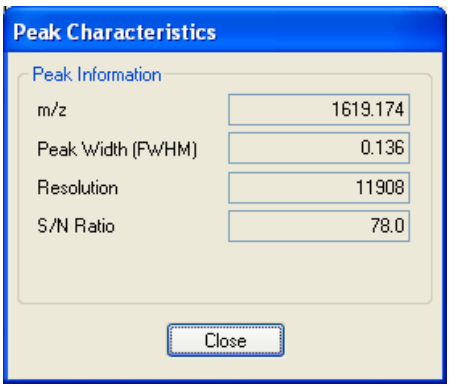


Figure 3-19 Example of peak characteristics

3.4.2.8 Smooth Spectrum (Acquisition/Sum Buffer)

The **Smooth Spectrum** (**Acquisition**/**Sum Buffer**) command and the corresponding icons / of the toolbar are used to smooth the spectrum in the respective buffer. The algorithm used for this can be adjusted via the Processing Parameter method (see section 3.7.5).

3.4.2.9 Subtract Baseline (Acquisition/Sum Buffer)

The **Subtract Baseline** (**Acquisition**/**Sum Buffer**) command, or the corresponding icon / of the toolbar are used to subtract the baseline of the spectrum in the respective buffer. The algorithm used for this can be adjusted via the Processing Parameter method (see section 3.7.5).

3.4.2.10 Undo Processing (Acquisition/Sum Buffer)

Undoes smoothing and/or baseline subtraction of the acquisition/sum buffer.

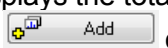
3.4.2.11 Abscissa Units

flexControl allows choosing between three scaling factors for the x-axis. The **Abscissa Units** commands and the respective icons of the toolbar are used to assign one of these to the abscissa.

Mass  :	Mainly used for standard operations. *m/z* scale is default.
Time  :	Mainly used for debugging purposes. t [ns] denotes a raw time-of flight-spectrum displayed in nanoseconds.
Points  :	Mainly used for debugging purposes. Shows how many data points are distributed over the peak shape.

3.4.2.12 Buffers

On the TOF-instruments of the flex series are two buffers available. One is used to store a single spectrum, the other one is used to add spectra.

- The **Single Spectrum** buffer  displays at least a single shot, usually the last acquired sequence of shots.
- The **Sum Spectrum** buffer  displays the total number of added spectra. The  button reverses the last  operation.

flexControl allows for scaling the single buffers with regard to the respective sum buffer. The available three scaling modes have no influence on the buffer contents. They are exclusively used for displaying spectra in the RTD (Real Time Display) (Mass Spectrum window).

flexControl performs only scaling when data are already stored on the sum buffer and the corresponding sum buffer is chosen. Toggling between the scale modes takes only effect, when an acquisition is running. Changing the mode before or after an acquisition has no influence on the display.

Below the RTD (see Figure 3-20) is an arrangement of three radio buttons to choose a suitable scaling mode.

- **None**: No scaling of the single buffer is performed.
- **90%**: The scaling of a single buffer is performed in a manner that its most intensive peak has 90% of the height of the most intensive peak of the sum buffer. This feature enables comparison of peaks in the single buffer and in the respective sum buffer on the basis of position, shape and size.

- **Shot ratio**: Using this mode, scaling of the single buffer is performed with regard to the total number of shots. After the shot sequence is finished the peak size of the single buffer corresponds to that of the sum buffer. In this mode the user can observe the increase of the peak intensity. Thus he can verify immediately the current peak intensity and its deviation from the average.

3.4.2.13 Split

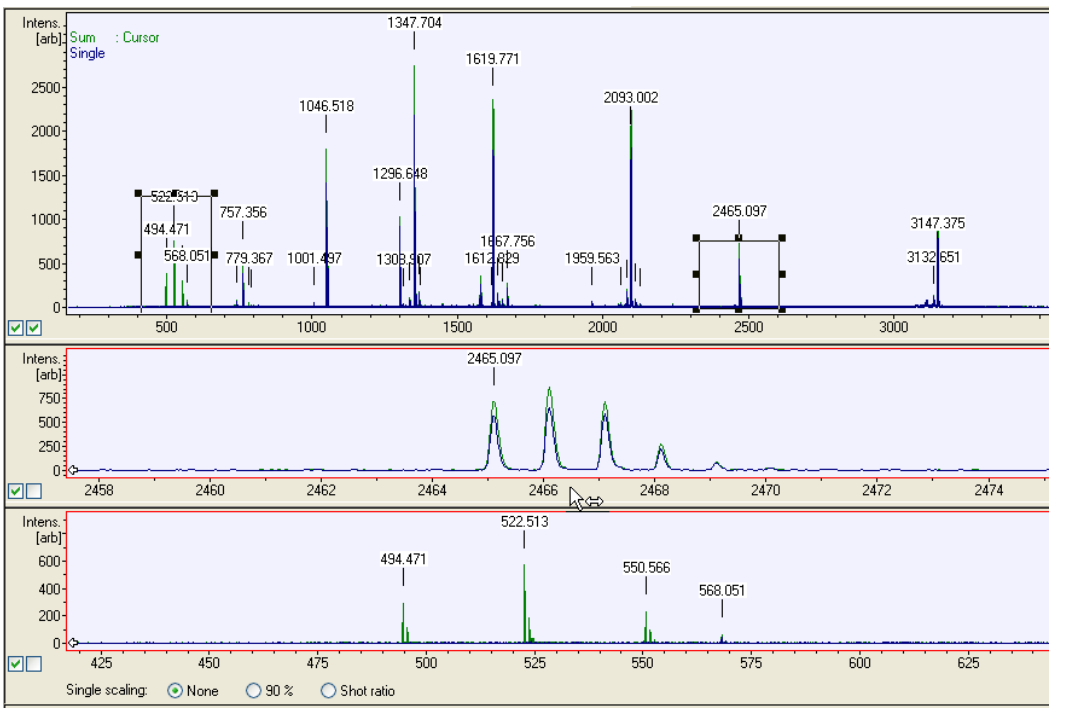


Figure 3-20 Mass Spectrum window displayed in one of the split modes

The **Split** command, or one of the corresponding icons on the toolbar are used to display more than one spectrum at a time (see Figure 3-20), in order to facilitate manipulations, e.g., to compare segments of a spectrum.

3.4.3 View Menu

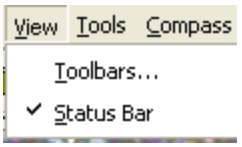


Figure 3-21 Contents of the View menu

The **View** menu (see Figure 3-21) is composed of commands to configure the GUI.

Table 3-3 Features of the View menu

Menu command	Description
Toolbars	Opens the dialog toolbars.
Status Bar	Show or hides the status bar.

3.4.3.1 Toolbars

The **Toolbars** command opens the **Toolbars** dialog box (see Figure 3-22) to customize the toolbar of the GUI. Select or clear the check box next to a toolbar to show or hide it in the flexControl GUI. The change is immediately shown in flexControl.

Click **New** to create a toolbar with a user-defined selection of buttons. The **Customize** dialog (see Figure 3-29) opens. Select the **Commands** tab to choose available buttons. Drag and drop the desired buttons into the toolbar.

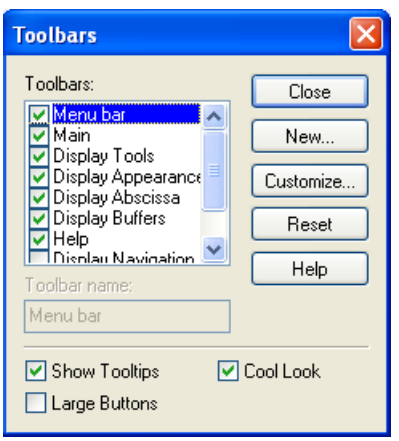


Figure 3-22 Toolbars dialog box

3.4.3.2 Status Bar

The **Status Bar** command is used to show or hide the status bar (see Figure 3-23), which is located at the bottom of the GUI. If the status bar is visible, this is indicated by a highlighted check mark left hand to the command.

The status bar contains the type of currently loaded method, the name of the current user, the coordinates of the mouse pointer and the status Information of the instrument.

Figure 3-23 Example of a status bar

3.4.4 Tools Menu

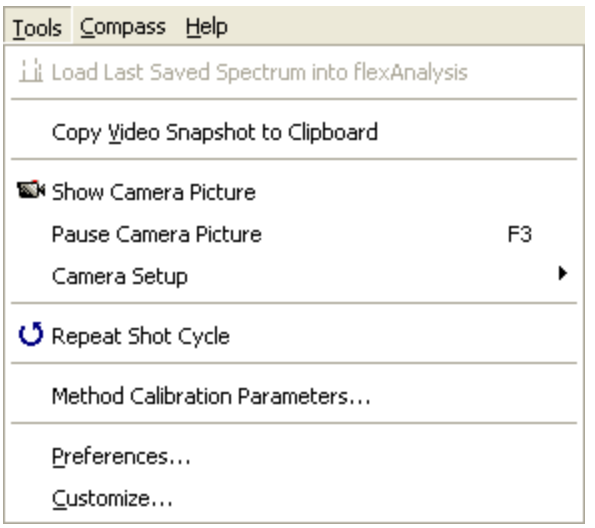


Figure 3-24 Contents of the Tools menu

The **Tools** menu (see Figure 3-24) is composed of various commands for different features, e.g., to re-align the video camera, choose a post processing application, etc.

Table 3-4 Feature buttons of the Tools menu with related functions

Feature button	ToolTip text	Menu command	Shortcut	Description
	Load last saved spectrum into post-processing application	**Load Last Spectrum into flexAnalysis**		Transfers the last saved spectrum directly into flexAnalysis.
		Copy Video Snapshot to Clipboard		Takes a snapshot of the video display and copies it to the clipboard
	Camera	**Show Camera Picture**		Toggles spot symbol and true video
		Pause Camera Picture	F3	Corresponds to pause on a video recorder.
	Camera teaching	**Teach Camera Control Features**		Opens a dialog for camera adjustments.
	Repeat shot cycle	**Repeat shot cycle**		Repeats the last shot sequence until termination.
		Method Calibration Parameters		Parameterizes parameters for the automatic MS calibration
		Preferences		Opens the **Preferences** dialog
		Customize		Customizes the toolbar.

3.4.4.1 Load Last Spectrum Into flexAnalysis

The **Load Last Spectrum Into flexAnalysis** command and the corresponding icon  of the toolbar are used to transfer the last saved spectrum directly into this post-processing software.

3.4.4.2 Copy Video Snapshot to Clipboard

The **Copy Video Snapshot to Clipboard** command takes a snapshot of the current image in the video display and copies it to the clipboard.

3.4.4.3 Show Camera Picture

The **Show Camera Picture** command and the corresponding icon  on the toolbar allow switching the camera video off and on.

3.4.4.4 Pause Camera Picture

The **Pause Camera Picture** command and the short cut (F3) are used to pause the video signal. In flexControl this is shown below the video (see Figure 3-25). During Webex sessions, pausing the video signal is recommended for faster data transfer.

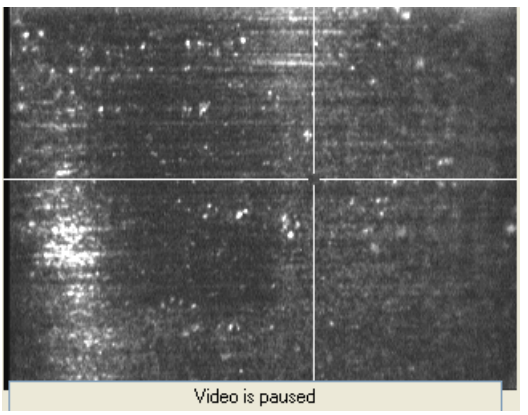


Figure 3-25 Paused video picture

3.4.4.5 Teach Camera Control Feature

The **Show Teach Camera Control Feature** command and the corresponding icon  on the toolbar are used to open a dialog for matching target size and position with the camera position (see Figure 3-26).

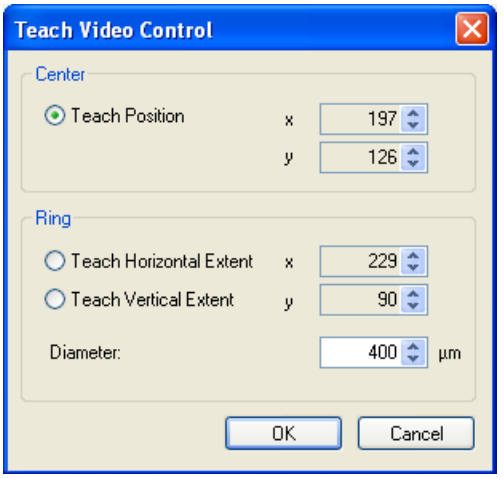


Figure 3-26 Parameters to match target size and camera position

The feature **Teach Video Control** (see Figure 3-26) is an alignment tool in order to match the screen coordinates and respective motor positions

Note The settings have been adjusted in the factory – they must not be changed by the user!

3.4.4.6 Repeat Shot Cycle

The **Repeat Shot Cycle** command, or the corresponding icon  of the toolbar are used to repeat the last shot sequence until the command or the button are applied a second time.

3.4.4.7 Method Calibration Parameters

This menu item opens the **Edit Auto Calibration Parameters** dialog (see Figure 3-27). It is used to adjust the parameters used for automatic re-calibration of the MS acquisition method (see section 3.7.7).

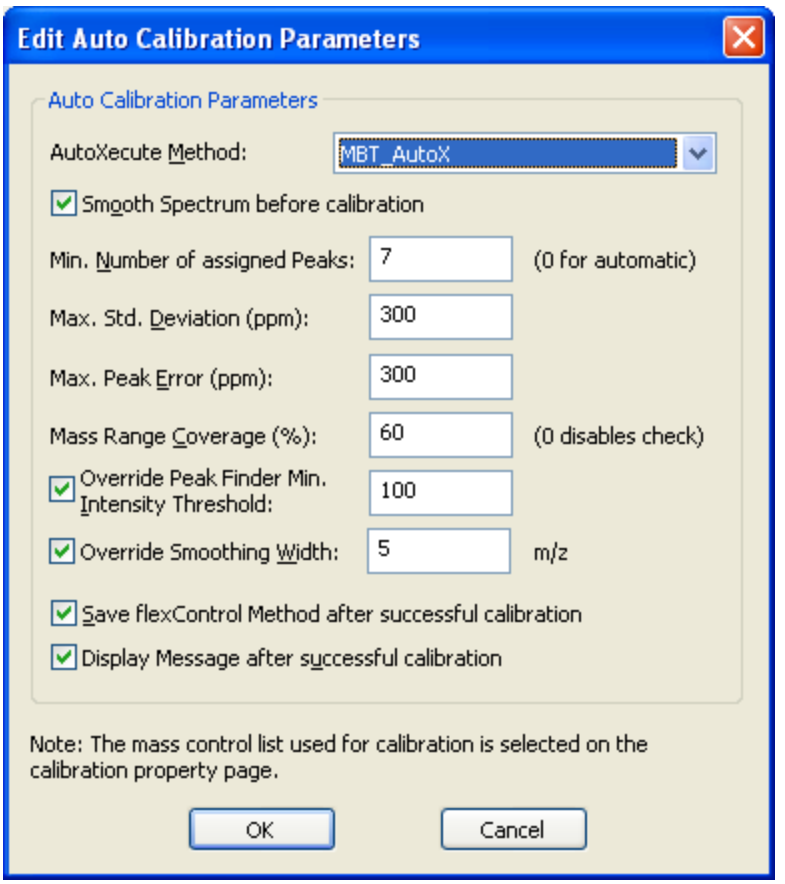


Figure 3-27 Edit Auto Calibration Parameters dialog (example settings)

The individual settings have the following effect:

AutoXecute Method

This selection specifies the AutoXecute method that is used for data acquisition during the automatic MS re-calibration.

Smooth Spectrum before calibration

If the entry is selected, the acquired spectrum is smoothed using the algorithm specified in the processing parameters before performing the peak finding and assignment algorithms.

Min. Number of assigned Peaks

Specifies the minimum number of peaks that be matched against the mass control list. If 0 is used, the minimum number of peaks depends on the calibration mode.

Max. Std. Deviation (ppm)

If the standard deviation of the new calculated calibration exceeds the value specified here, the new calibration is rejected.

Max. Peak Error (ppm)

If the error of an individual assigned peak exceeds the value specified here, the new calibration is rejected.

Mass Range Coverage (%)

This value specifies the minimum coverage for the assigned peaks in relation to the mass range. Example: The mass range of the acquisition method goes from 500 to 3500 Da. A coverage 60% then means that the first and last assigned peaks must have a minimum distance of 1800 Da (60% of 3000 Da).

Override Peak Finder Min. Intensity Threshold

If this element is selected, the value entered is used for the peak finder minimum intensity setting instead of the parameter specified in the processing parameters dialog.

Override Smoothing Width

If this element is selected, the value entered is used for the smoothing width setting instead of the parameter specified in the processing parameters dialog.

Save flexControl Method after successful calibration

If the entry is selected, the acquisition method is automatically saved after successfully completing the automatic MS re-calibration.

Display Message after successful calibration

If the entry is selected, a message will be displayed after successfully completing the automatic MS re-calibration.

The method calibration parameters are stored in the flexControl method so that different parameters can be used for automatic re-calibration e.g. on peptides and proteins.

3.4.4.8 Preferences

The **Preferences** command opens the **Preferences** dialog (see Figure 3-28). This dialog is used to alter the settings used for checking for updates.

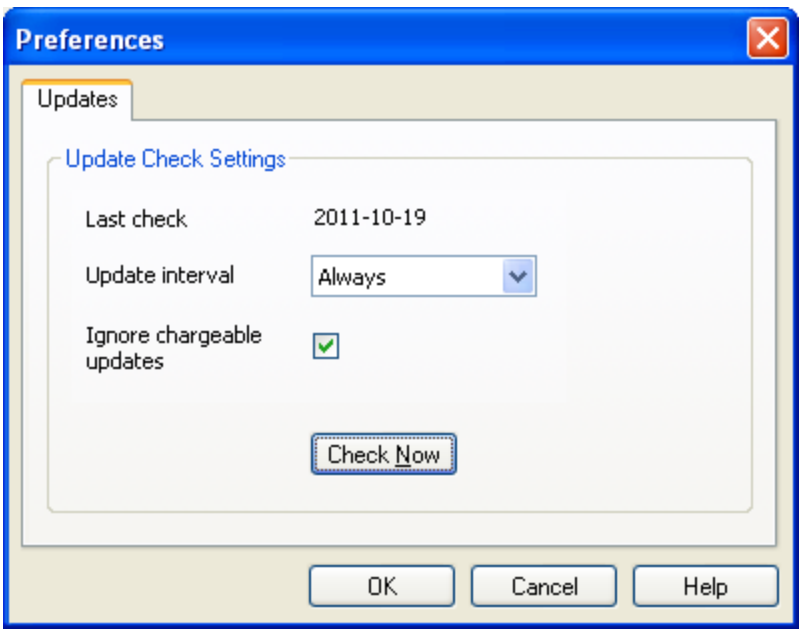


Figure 3-28 Preferences dialog

When flexControl is launched from the start menu or desktop icon, it checks if updates are available if the interval since the last check has expired. If an update is found, it informs the user.

The software contacts a Bruker server for the check. Refer to the release notes on implications such as firewall configuration.

Last Check

This field lists the data of the last update check.

Update interval

This element specifies the interval between automatic checks. Possible choices are **Never**, **Always** (on every start), **Daily**, **Weekly** and **Monthly**.

Ignore chargeable updates

If this element is selected, the software only checks for free updates and ignores future Compass releases.

Check Now

If this button is clicked, the software immediately performs the check for updates and displays the result.

3.4.4.9 Customize

The **Customize** command opens the **Customize** dialog (see Figure 3-29). It is used to vary the contents of the menu bar. Additionally the user can remove or add parts of the toolbar.

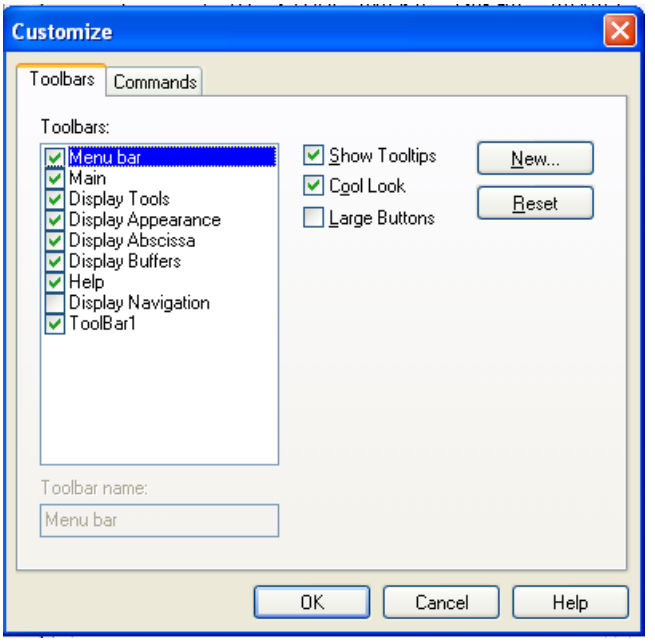


Figure 3-29 Customize > Toolbars dialog

3.4.5 Compass Menu

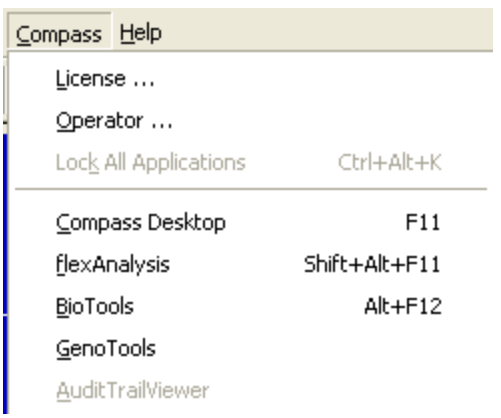


Figure 3-30 Content of the Compass menu

Table 3-5 Features of the Compass menu

Menu commands	Shortcut	Description
License		Enter the license number here.
Operator		Changes the operator.
Lock all Applications	CTRL+ALT+K	Locks all programs in case the Compass Security Pack is installed.
Compass Desktop	F11	Opens the Compass Desktop.
flexAnalysis	SHIFT+ALT+F11	Opens flexAnalysis.
BioTools	ALT+F12	Opens BioTools.
Genotools		Opens GenoTools.
Audit Trail Viewer		Opens the Audit Train Viewer in case the Compass Security Pack is installed.

3.4.5.1 License

The **License** command is used to open the **Bruker Daltonics License Viewer** dialog box (see Figure 3-31) for viewing or adding/removing licenses for Bruker products. The **LicenseViewer** (and **Manager**) look the same in nearly all programs. It is not necessary to enter a license for a certain Bruker program in the respective program; it can be entered via the **License Manager** of other Bruker programs.

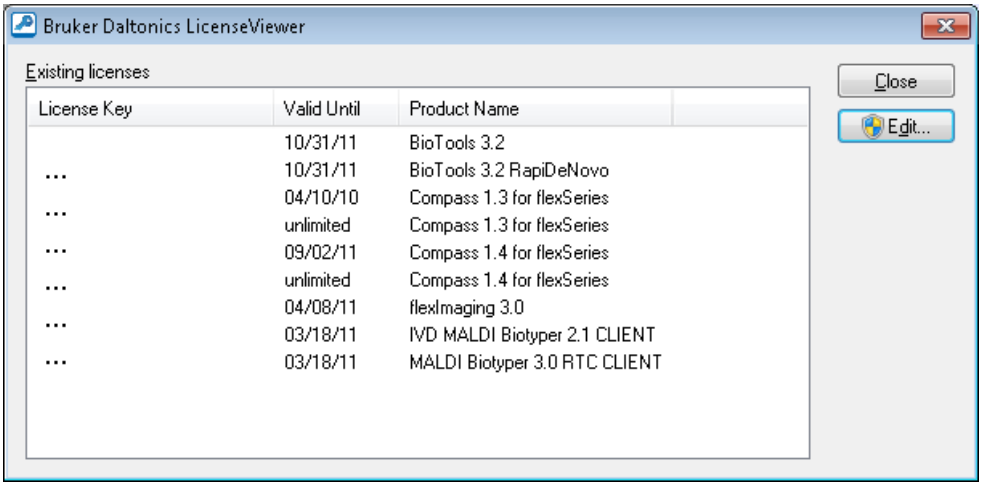


Figure 3-31 The Bruker Daltonics LicenseViewer

To enter a license click the **Edit** button. On some computers it might be necessary, even when the administrator is logged in, to explicitly select the administrator and enter the respective password. The **License Manager** will then open:

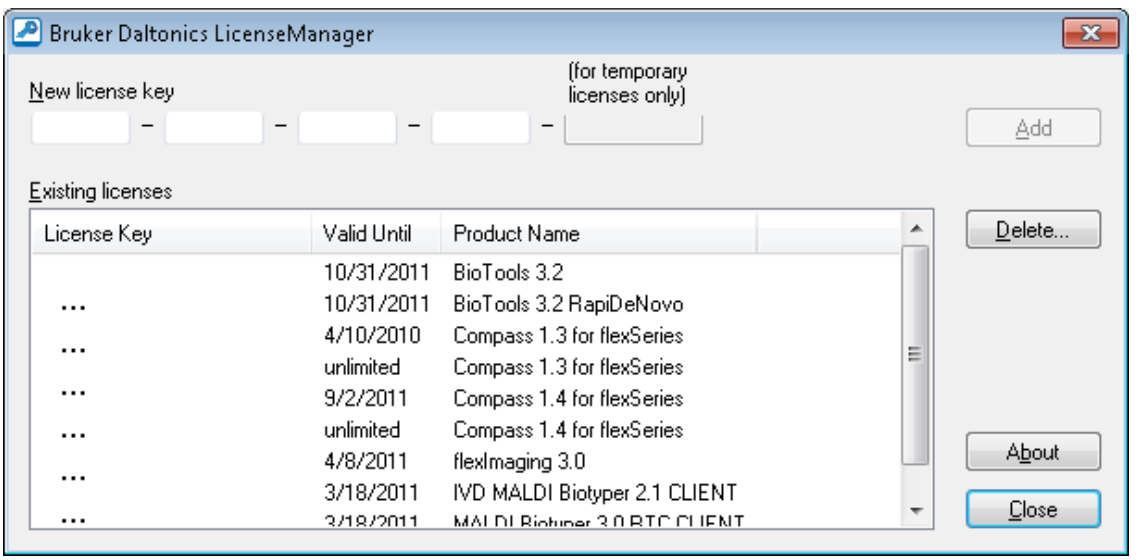


Figure 3-32 The Bruker Daltonics LicenceManager

3.4.5.2 Operator

The **Operator** command opens the flexControl log on dialog (see Figure 3-33) and is used to log on as new operator. The new operator name appears in the status bar at the bottom of the GUI. In case the Bruker Daltonics UserManagement is installed, an additional password is requested. If a user logs on with the **Operator** command the user automatically changes for all other Bruker programs that are currently opened and runnning with the UserManagement. In flexControl the **Select Method** dialog appears.

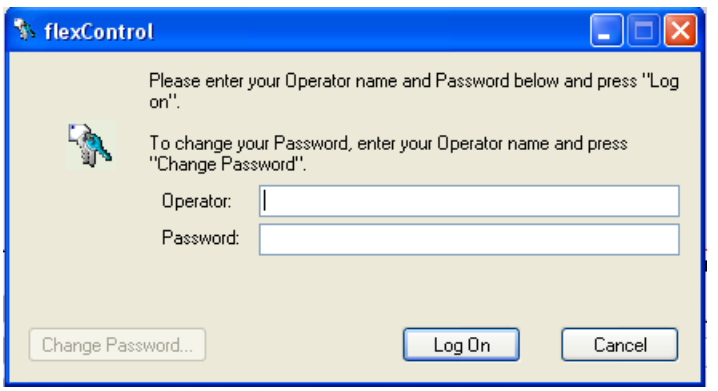


Figure 3-33 Log On operator dialog

If the Bruker UserManagement is not installed, the conventional **Operator log on** dialog appears (see Figure 3-34).

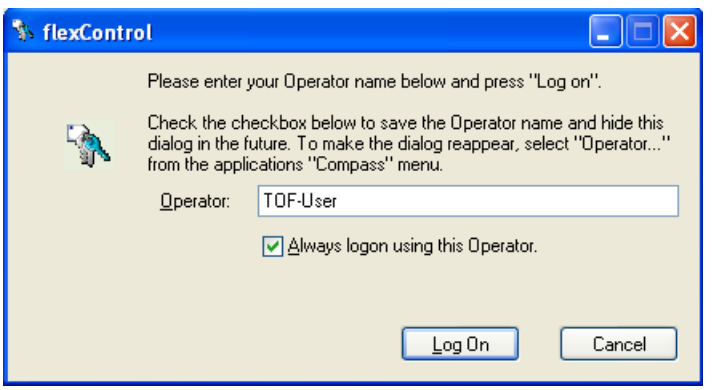


Figure 3-34 Conventional Log On operator dialog

3.4.5.3 Lock All Applications

The **Lock all Applications** command and the short cut (CTRL+ALT+K) lock all applications that are currently open depending on the lock-time that is defined in the UserManagement. Additionally the **Unlock** dialog opens. After a timeout, that is adjustable via the UserManagement, the software is locked automatically.

If one program is locked (manually or via timeout), all programs that run with the UserManagement are locked, since they all use the same UserManagement server. In this case it is also necessary to unlock only one program. A locked program can only be unlocked from the user who locked it, or from the UserManagement administrator.

Figure 3-35 Unlock applications dialog

3.4.5.4 Compass Desktop

The **Compass Desktop** command opens the so-called Bruker Compass. This GUI offers access to the installed Bruker programs categorized in **Acquisition**, **Processing**, **Data Interpretation**, and the corresponding manuals.

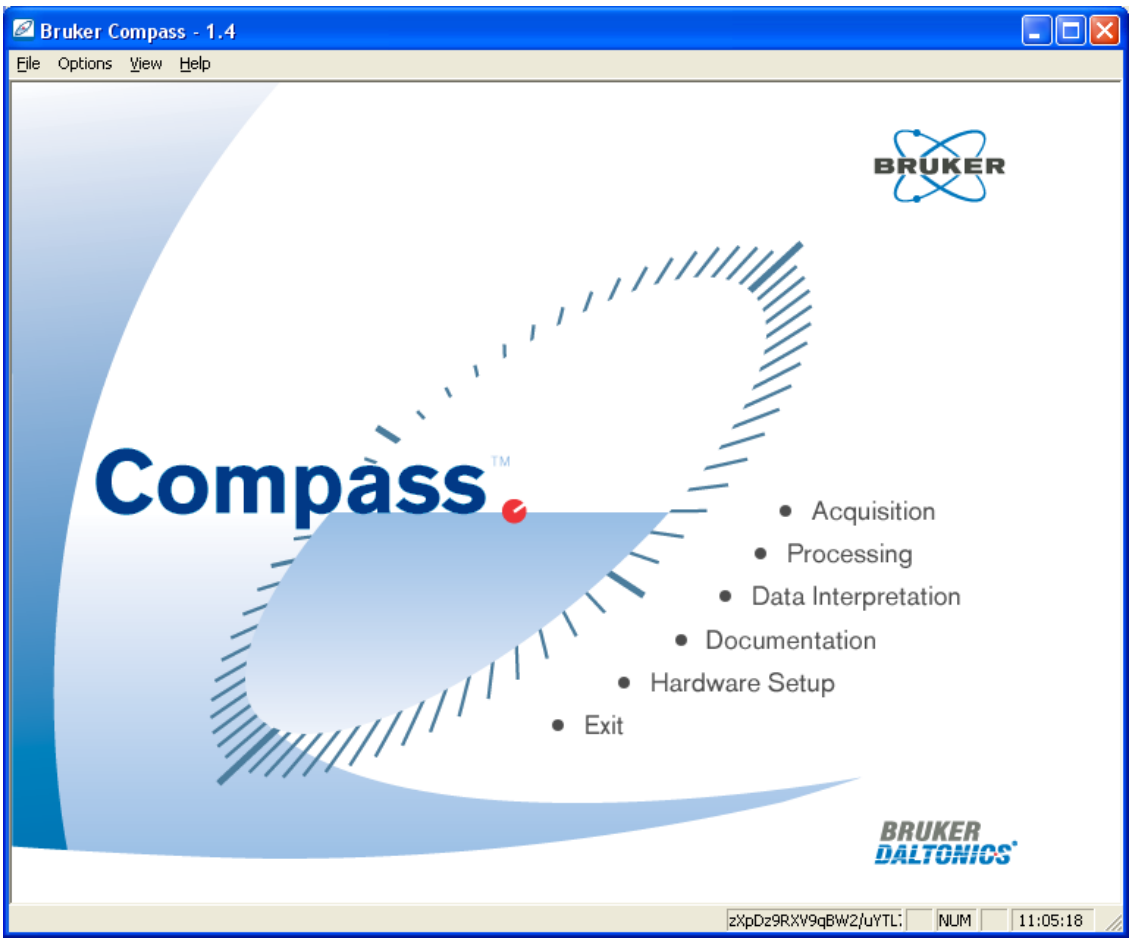


Figure 3-36 The Compass desktop

3.4.5.5 flexAnalysis

The **flexAnalysis** command is used to open flexAnalysis or bring it to front if it is already open. Data transfer is not achieved with this command, i.e. no spectrum is sent to flexAnalysis. To send a spectrum to flexAnalysis, use the button **Load last saved spectrum into flexAnalysis** (see section 3.4.4.1).

3.4.5.6 Audit Trail Viewer

The **Audit Trail Viewer** command opens the **Bruker Daltonics AuditTrailViewer** dialog. It is only available if the Bruker UserManagement is installed.

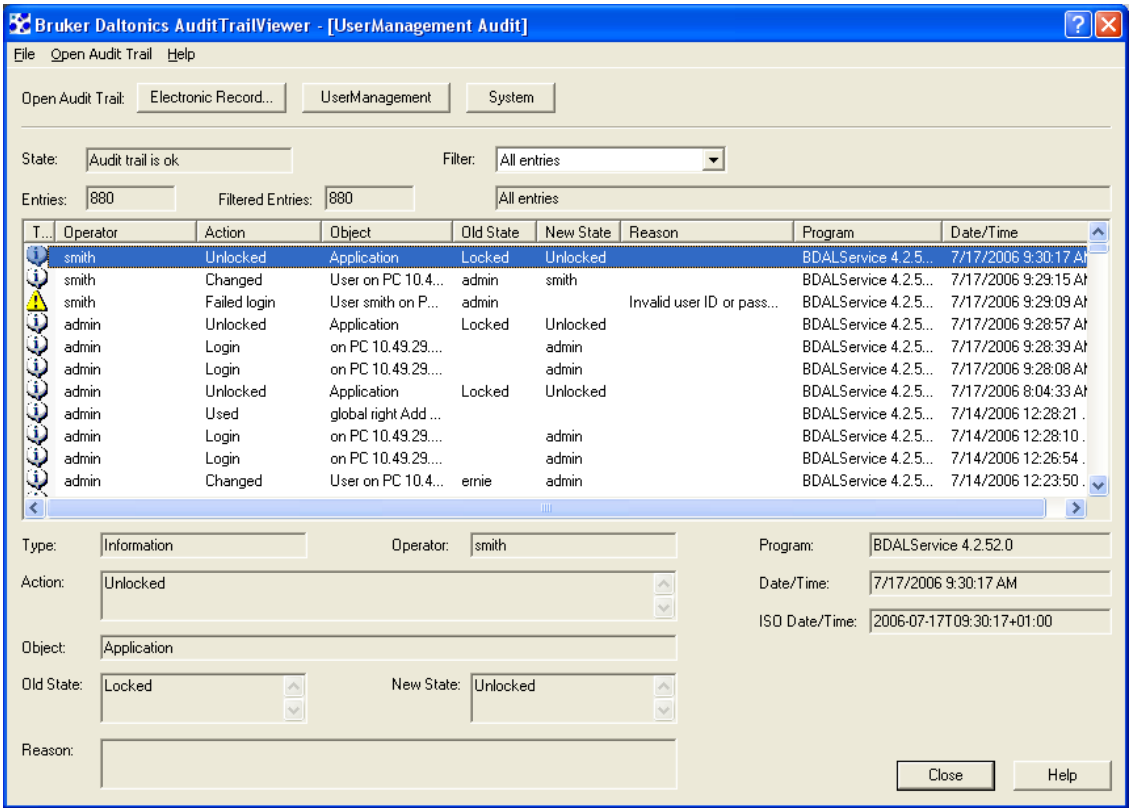


Figure 3-37 The Bruker Daltonics AuditTrailViewer

3.4.6 Help Menu

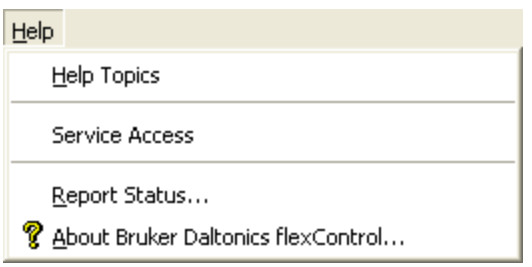


Figure 3-38 Contents of the Help menu

The **Help** menu (see Figure 3-38) is composed of commands referring to copyright and license information, and to electronic support.

3.4.6.1 Help Topics

The **Help Topics** command opens the online-help system and displays the help system's contents page.

3.4.6.2 Service Access

Some interface elements of flexControl are only available for authorized service personnel and read-only for other users. To gain access to these settings, this command opens the **Service Access** dialog (see Figure 3-39).

Figure 3-39 Service Access dialog

3.4.6.3 Report Status

The **Report Status** command launches the **Status Reporter** (see Figure 3-40) that is used to collect helpful information of the instrument and the computer. The **Status Reporter** also starts in case of an error in flexControl to help the user to collect necessary information for the support concerning the current system state.

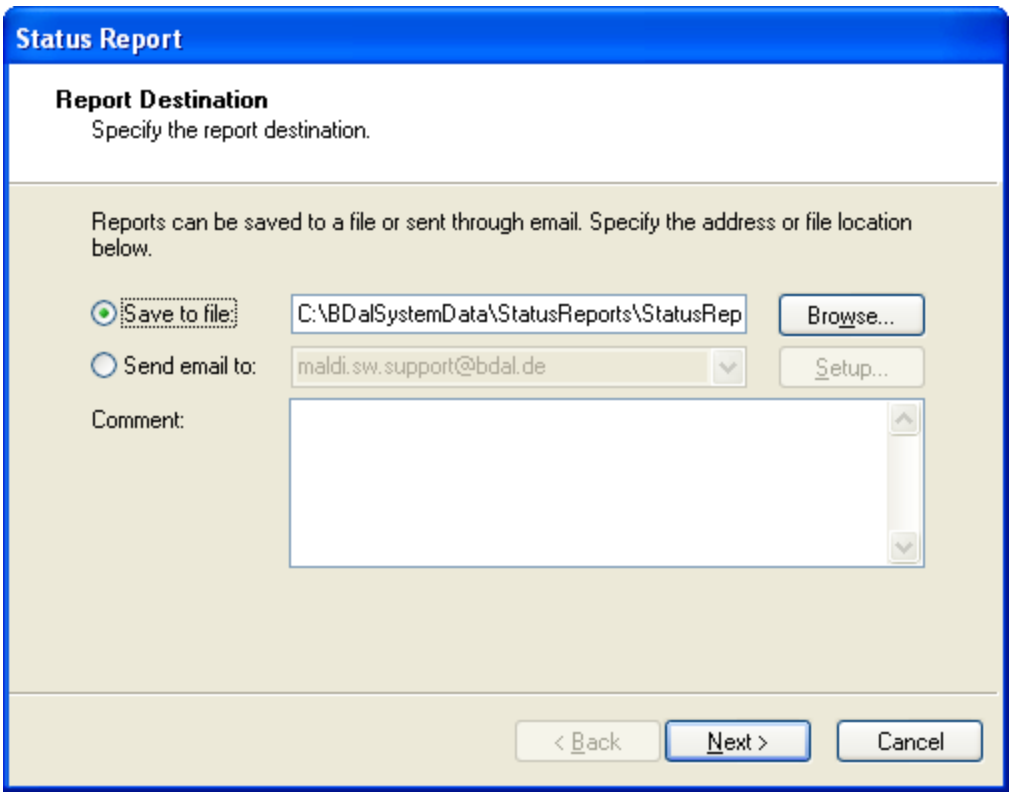


Figure 3-40 Status Reporter

3.4.6.4 About Bruker Daltonics flexControl

This command opens a window (see Figure 3-41) containing the software version, copyright information, the used mass spectrometer, and the support address.

Figure 3-41 The About Compass flexControl dialog box

3.5 Main Instrument Control

This part of the flexControl GUI comprises features of the video, data acquisition and method information as well as the sample carrier.

3.5.1 Video Optics and Acquisition Control

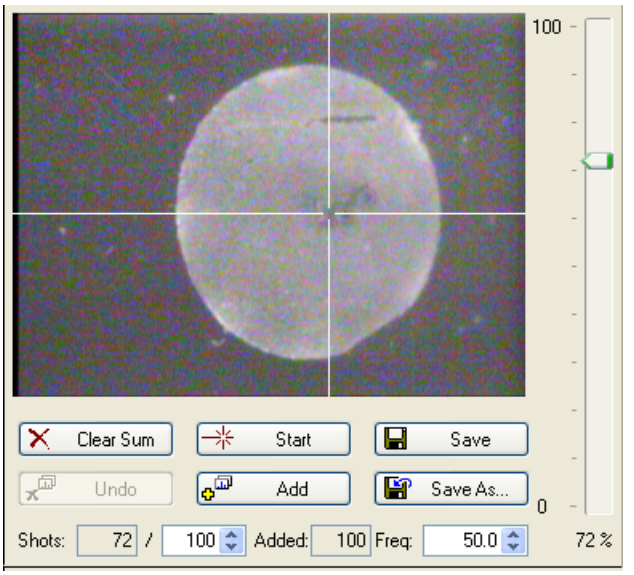
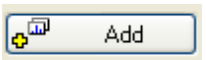
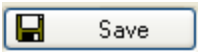
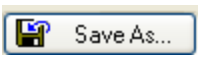
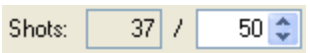
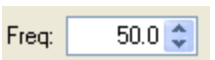


Figure 3-42 Video optics and acquisition control

The slider on the right of the video picture is used to adjust the laser intensity. This can be done with mouse clicks or with the mouse wheel since the focus is on the laser slider after a click in the video picture.

The six buttons located below the video are used for data acquisition and collection. Additional fields specify the laser settings.

Clear Sum	A click with the left mouse button (or ALT+C) will erase the contents of the linear and reflector sum buffers.
Undo	**Undo** (or ALT+U) deletes the last added shot sequence from the sum buffer.
Start	**Start** (or ALT+S) starts a manual acquisition. During a run it toggles to a Stop-button, which might be used to terminate the shot sequence immediately.
Stop	**Stop** (or ALT+T) stops a manual acquisition.

	**Add** (or ALT+A) transfers the current contents of the linear or reflection detector to the corresponding sum buffer. The data will be added to data previously acquired. This should be done only with good spectra in order to maximize the quality of the sum spectrum.
	**Save** (or CTRL+S) saves the current spectrum.
	Saves the current spectrum with a new name.
	Information on how many shots are currently acquired and how many are required.
	How many shots have been added to the sum buffer.
	Adjusts the laser trigger frequency. Higher frequencies result in faster data acquisition.

3.5.2 MTP Control and Method Selection

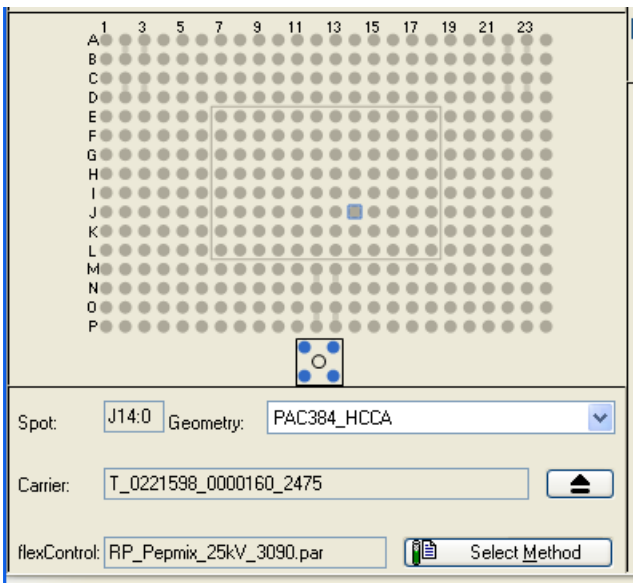


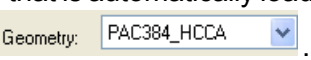
Figure 3-43 Target control features

Depending on the target that is currently used in the instrument the respective geometry is presented. If it is a target with chips (see Figure 3-43), the different available chips are visualized beneath the target () and can be chosen by clicking a circle. The currently active chip — chip 0 — (see Figure 3-43) is shown in blue. In the following example (see Figure 3-44) "chip 1" — i.e. the calibration spots — are selected.

The currently active sample spot is marked with a blue (system color dependent) square in the plate view. This information is additionally shown in the Spot information field. In the example 

denotes J14, chip 0, i.e. a sample spot. The other example, 

, identifies J14, chip 1, i.e. a calibration spot.

The geometry file that is automatically loaded for transponder targets is shown in the **Geometry** field 

. If a target geometry is detected during the docking process, the **Geometry** field becomes read-only. The only exceptions are PAC targets, due to the fact, that different (disposable) targets can be used on the same PAC frame (adapter).

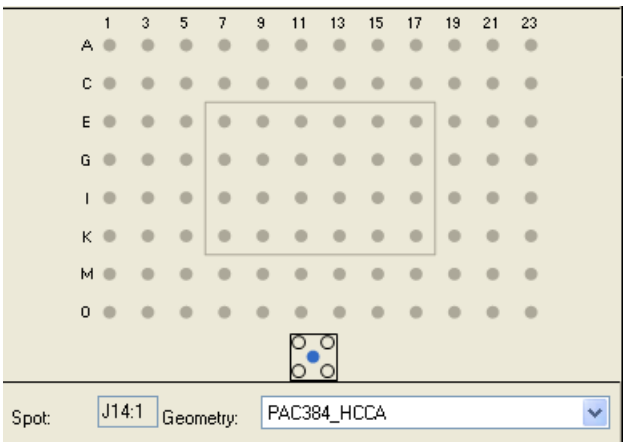


Figure 3-44 PAC target with selected calibration chip

During an AutoXecute run when flexControl acquires and saves data, the appearance of the plate view gets colored. This depends on the measurement status of the spots. Two main states are available:

- light green: MS measurement finished
- dark green: MS/MS measurement finished

Additionally five other states can be shown before and after the run.

- yellow: prepared MS and MS/MS positions
- mauve: prepared calibration spots
- red: unsuccessful acquisition
- orange: flatline spectrum saved
- gray: MS Laser-Tuning spot

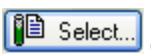
The light and dark green and red and orange states are saved when a run is finished.

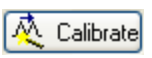
If a spot is green (light or dark) this denotes that a spectrum has been acquired and saved either automatically or manually with included path- and sample name information to a run (see section 3.4.1.1). In both cases the measurement status has also been saved in the AutoXecute run. This enables spectra to be sent to flexAnalysis by right-clicking the colored sample spot.

3.5.3 Method Selection Panel

Figure 3-45 Method selection panel

The method selection panel performs the following functions:

- It displays the name of the currently loaded acquisition method
- The **Select** button 

opens the method selection dialog (see section 3.4.1.3).
- The **Calibrate** button 

starts the automatic MS re-calibration procedure (see section 3.7.7).

3.6 Mass Spectrum Window

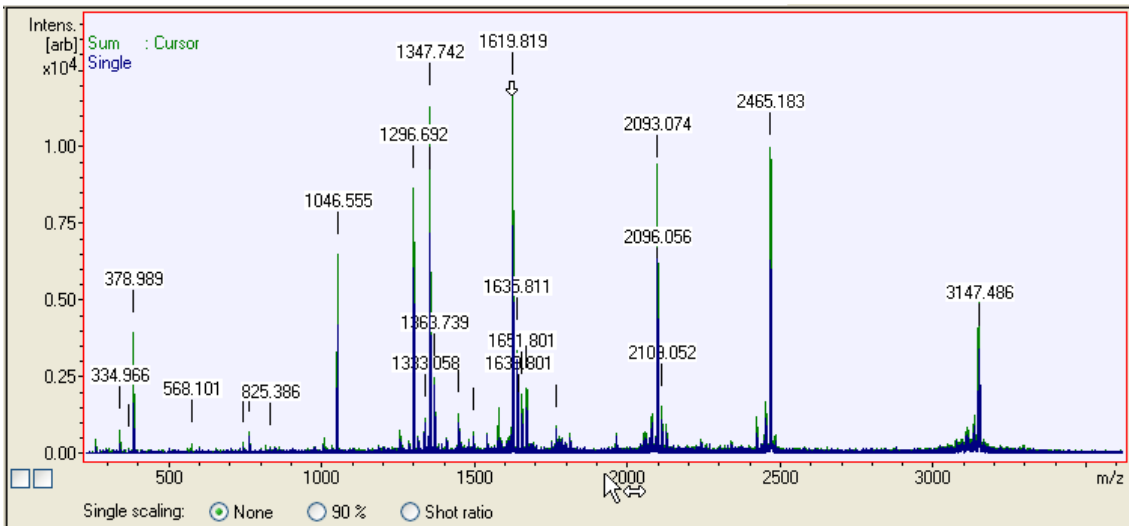


Figure 3-46 Example of a spectrum

The Mass Spectrum window (see Figure 3-46) is located top right on the GUI. It displays the currently acquired spectrum.

flexControl automatically normalizes the peak intensity scale. The x-axis may be scaled with one of the three available units (see section 3.4.2.11). Pre-selection of the scale can be performed on the menu bar by clicking the command **Display > Abscissa Units** (see section 3.4.2.11).

In the upper left corner the currently active acquisition buffer (Sum/Single) can be indicated. The setting is activated via the check box **Show legend** from the **Properties** dialog (see Figure 3-48).

The Mass Spectrum window can be divided in up to three windows (see Figure 3-20). This may be useful to simultaneously display a full spectrum and two different zoomed areas.

The two check boxes in the lower left corner are used for auto-scaling both the ordinate and the abscissa.

The three **Single scaling** options below the x-axis are used to select a scaling mode (see section 3.4.2.12).

For enhanced viewing of data, the color of one or more objects in the Mass Spectrum window may be changed (see section 3.6.2).

3.6.1 Scaling Pointers

To shift the currently displayed spectrum in the x- or in y-direction flexControl provides two additional pointers:

- Horizontal Scaling pointer:

This pointer appears when the mouse pointer hovers immediately below the x-axis of the Mass Spectrum window. It is used to shift the spectrum and the scale horizontally.

- Vertical Scaling pointer:

This pointer appears when the mouse pointer hovers immediately to the left of the y-axis of the Mass Spectrum window. It is used to shift the spectrum and the scale vertically.

3.6.2 Spectrum Manipulation

Right-clicking within the Mass Spectrum window opens a shortcut menu (see Figure 3-47). These features are used to customize the peak finder settings and the real-time display.

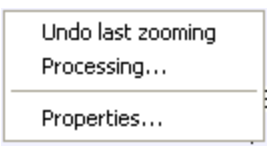


Figure 3-47 Content of the Mass Spectrum window shortcut menu

- Undo last zooming (see section 3.4.2.2):
- Processing: opens the **Processing Method Editor** (see section 3.7.5).
- Properties:

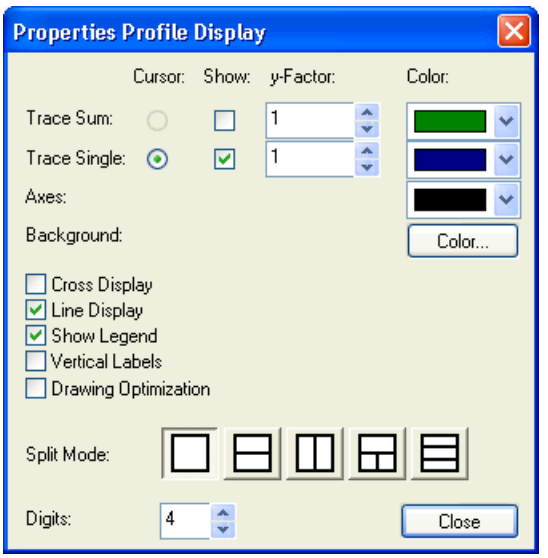


Figure 3-48 Features of the Properties dialog

The **Properties** dialog (see Figure 3-48) may be used to configure preferences for the Mass Spectrum window(s) (see section 3.4.2.13) and to display spectra in different ways.

- **Trace Single**/**Sum**: On selecting the respective check boxes spectra of the single and/or sum buffer are shown in the Spectrum Window. Clearing the boxes will fade out the spectra. The scale of the y-axis and the trace colors are to be adjusted in this dialog.
- **Axes**: A color can be specified for both the x- and y-axis.
- The button **Color** opens a dialog that is used to assign colors to the background of the Mass Spectrum window (see Figure 3-49).

Colors can be selected either from a set of basic colors or from a set of custom colors which the operator can define according to their own requirements.

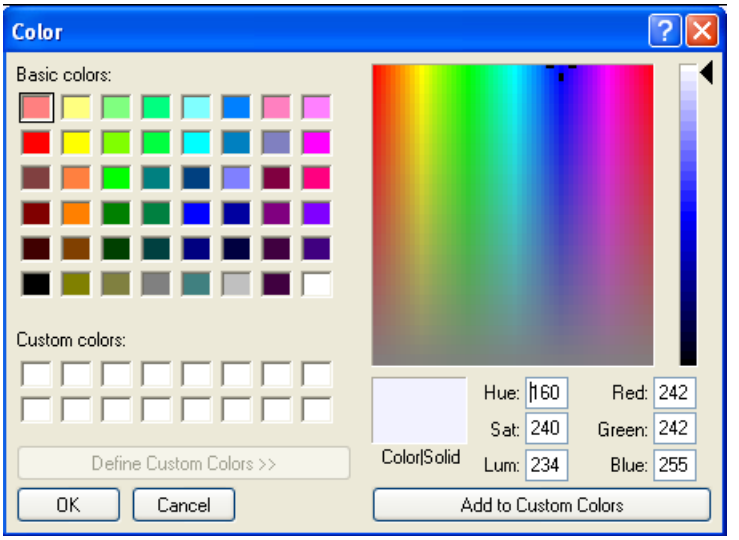


Figure 3-49 The color palette

- **Cross display**: When selected, the spectrum is shown as a series of crosses, each cross representing a data point.
- **Line display**: When selected, all data points of the spectrum are connected and shown as a line.
- **Show legend**: If selected, the RTD shows which acquisition buffer is currently in use (Sum/Single).
- **Vertical labels**: When selected, labels are displayed vertically.
- **Drawing Optimization**: Users can activate or deactivate the Local Maximum drawing optimization. By default this feature is activated and less noise is shown. This is only a display setting and does not affect the spectrum.
- **Split Mode**: Pre-selection of how much Mass Spectrum windows are displayed.
- **Digits**: Pre-selects the number of decimal places of the peak labels.

3.7 System Configuration Segment

Depending on the currently loaded method type flexControl provides respective pages or specific features in pages located in the System Configuration segment (see Figure 3-1):

- **AutoXecute** (see section 3.7.1),
- **Sample Carrier** (see section 3.7.1.8),
- **Spectrometer** (see section 3.7.4),
- **Detection** (see section 3.7.3),
- **Processing** (see section 3.7.5),
- **Setup** (see section 3.7.12),
- **Calibration** (see section 3.7.6),
- **LIFT** (see section 3.7.9), shown if a LIFT method is loaded
- **FAST** (see section 3.7.10), shown if a FAST method is loaded
- **SRM** (see section 3.7.11), shown if an SRM method is loaded
- **Status** (see section 3.7.13)

The **LIFT** page (see section 3.7.9) and the **LIFT Calibration** page (see section 3.7.8) appear if a flexControl LIFT method is loaded.

The **FAST** page (see section 3.7.10) is only available, if a FAST method is loaded.

The **SRM** page (see section 3.7.11) is only available, if a SRM method is loaded before.

Note New entries in the edit fields on all pages only take effect after pressing the ENTER key on the keyboard. The color of the new entry changes from blue to black.

3.7.1 AutoXecute

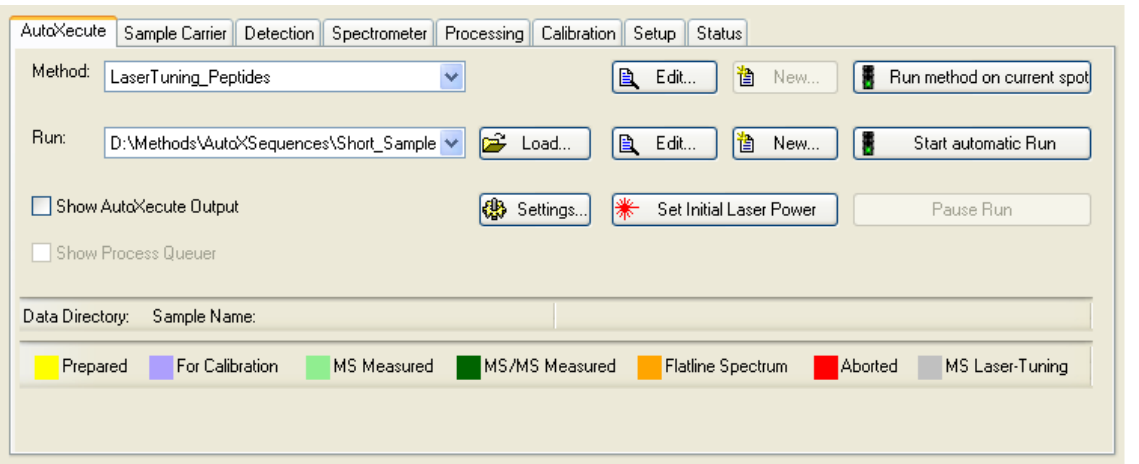


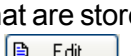
Figure 3-50 Features of the AutoXecute page

The **AutoXecute** page (see Figure 3-50) is used to create, edit and load AutoXecute methods and runs (sequences), to start, pause and stop AutoXecute runs and to configure some general settings concerning AutoXecute.

AutoXecute allows setup and execution of automatic runs utilizing data acquisition in flexControl, calibration, processing and annotation in flexAnalysis, and database search in BioTools or ProteinScape.

3.7.1.1 AutoXecute Methods

For automatic data acquisition in AutoXecute Bruker provides pre-installed write-protected methods. These method names start with `<Default>`. All pre-installed methods may be overwritten when a new version of the program is installed. Therefore it is strongly recommended to use the default methods only as templates to create own methods with user-defined names. These methods can be modified at any time and are not touched during de-installation. The tool for AutoXecute method development is the **AutoXecute Method Editor**.

The **Method** drop-down list on the **AutoXecute** page offers all available AutoXecute methods that are stored in the correct directory (typically `D:\Method\AutoXmethods`). The button 

opens the **AutoXecute Method Editor** with the selected method. It

consists of seven pages providing a variety of functions to customize automatic data acquisition. These functions are described in the following. To create a new AutoXecute method, select an existing one and save it with a new name.

AutoXecute Method Editor page	Description
General	General settings, such as method selection and comments
Laser	Adjustment of the laser (Fuzzy Control)
Evaluation	Customize the spectra evaluation
Accumulation	Customize accumulation (Fuzzy Control)
Movement	Specify the movement on the sample
Processing	Specify post processing settings
MS/MS	Setting parameters for an automatic MS/MS measurement

AutoXecute Method

The drop-down list on the top of the **AutoXecute Method Editor** shows the currently loaded AutoXecute method. The selection remains unchanged when switching between the pages.

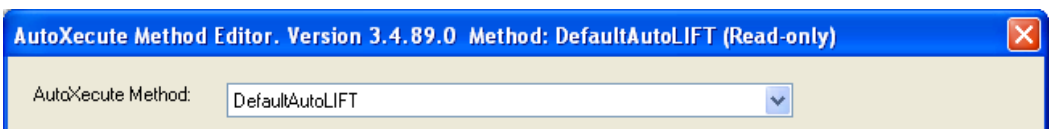


Figure 3-51 AutoXecute Method drop-down list

If another method should be loaded it can be selected from this list. If changes in the current method have not been saved a warning message appears asking the user to save the changes.

3.7.1.1.1 General page

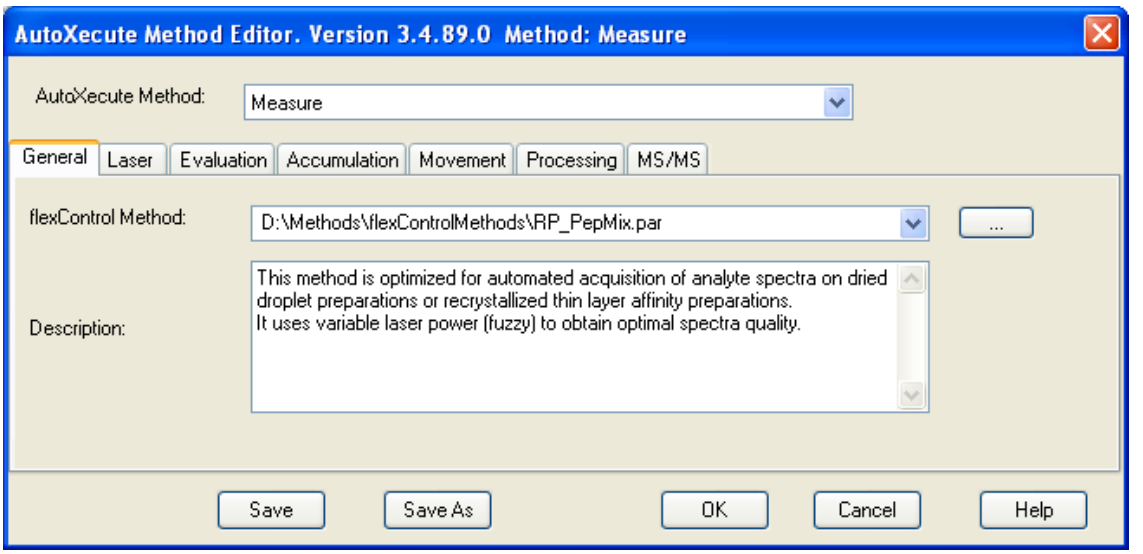


Figure 3-52 Content of the General page

The **General** page (see Figure 3-52) is used to perform general settings.

flexControl Method

Depending on the chosen flexControl method the AutoXecute method becomes a method for MS or MS/MS measurement. Some parameters will then be disabled depending on the selected use case. If a chosen flexControl method is no longer available on the hard drive, its name appears in red and a warning message appears when the AutoXecute method is saved.

For the MS use case it is possible to choose **None**, i.e. no flexControl method. In those cases the method currently loaded in flexControl is used for measurement. If it is not an MS method (but LIFT or FAST) a warning appears in the AutoXecute output and the measurement will not start.

Description

This field can be used for comments.

3.7.1.1.2 Laser Page

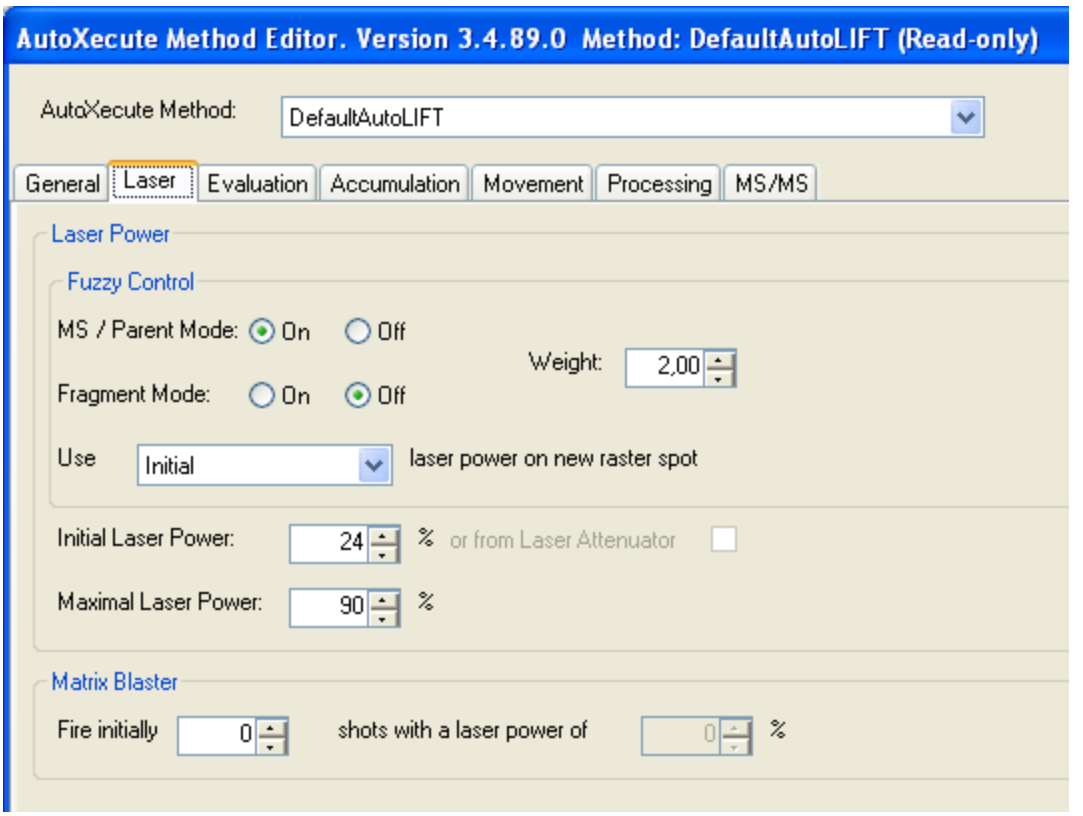


Figure 3-53 Content of the Laser page

The **Laser** page (see Figure 3-53) contains features to configure the laser power using the advantage of Fuzzy Control. All entries are global and valid during the entire acquisition.

Group box Laser Power

The module Fuzzy Control works like an automatic regulation circuit. It measures and processes two control arguments, such as peak resolution and peak intensity (S/N). As a result it delivers two output values, in this page to optimize the laser intensity, and accumulate spectra to the sum buffer, which are considered good enough.

In case of a MS/MS method, the Fuzzy Control can separately be switched on and off for MS/Parent and fragment measurement. The required parameter settings are accessible if a flexControl LIFT method has been selected.

Data recording of fragmented LIFT spectra can be controlled by the Fuzzy module analogue to the handling of MS data. Fuzzy logic for fragments handles fragmented spectra in the same manner as peptide fingerprint spectra.

In case of **Fragment Mode Off** the entered number of laser shots (edit field **Shots**) will be fired with the boosted laser power according to the **LIFT** page.

Weight is a parameter of the Fuzzy Control engine to scale the laser power. It is a configurable term of a couple of factors that are associated with the step width of the laser power changing gradually from the **Initial Laser Power** to the **Maximal Laser Power**. Variations are possible in minimum steps of 0.1 in the range between 0.5 and 2.0. The values in the pre-installed default methods are the recommended ones for the respective use cases.

Use…laser power on new raster spot

This feature is used to specify the laser power that is used on a new raster spot (measuring position on a sample spot (see Figure 3-59)) if the current raster position must be abandoned due to restrictions set in the AutoXecute method, that is, the required sum of spectra from this sample spot has not been reached. The four possible choices are:

1. **Initial**: Set the laser power to the initial value for this raster spot.

   This is either the value defined below (**Initial Laser Power**), or the one from the laser slider in flexControl, if the check box **from Laser Attenuator** is selected.

2. **First successful**: Set the laser power to the first successful acquisition that was done on the previous raster spot.

   If there was no successful acquisition the raster spot is left but the laser power will be increased further. For example, if you do not know the optimum laser power range, then type a low laser power and choose **First successful**. Then the laser power will be increased over several raster spots to the optimum.

3. **Last Successful**: Set the laser power to the last successful acquisition that was done on the previous raster spot.

4. **Locked**: As soon as a shot package has been acquired successfully, this laser power is locked and taken for all following shots until the number of required shots on the sample spot is reached; no evaluation of the shot packages takes place. A raster spot is left if the number of allowed shots per raster spot is reached.

5. **Last Successful**: Set the laser power to the last successful acquisition that was done on the previous raster spot.

6. **Locked**: As soon as a shot package has been acquired successfully, this laser power is locked and taken for all following shots until the number of required shots on the sample spot is reached; no evaluation of the shot packages takes place. A raster spot is left if the number of allowed shots per raster spot is reached.

The **Initial Laser Power** field determines the laser power (in %) that is used to start the acquisition on a sample spot.

The check box **from Laser Attenuator** can only be selected, if the **Laser Fuzzy Control** is switched off. In this case the laser power is taken from the laser slider in the flexControl GUI. The value for **Initial Laser Power** is then disabled.

Group box Matrix Blaster

If it is necessary to get rid of the first substance layer of a new sample spot, which shall not be measured, the Matrix Blaster function may be used by firing a number of initial laser shots with a specific intensity. Data obtained in this way is ignored. The default setting of 0 shots and 0 laser power means that this function is disabled.

Note This feature cannot be used together with random walk activated on the **Movement** page.

3.7.1.1.3 Evaluation page

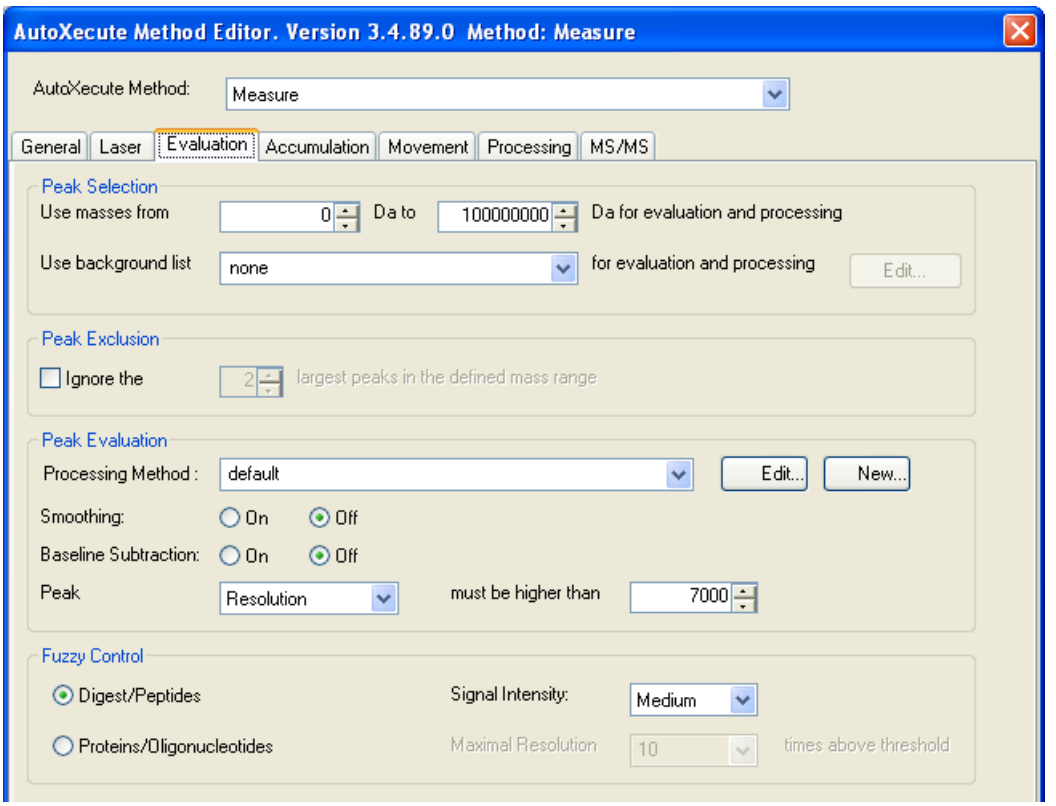


Figure 3-54 Content of the Evaluation page

The page **Evaluation** (see Figure 3-54) is used to configure the second output of the fuzzy engine for peak evaluation and processing.

Group box Peak selection

The row **Use masses from x Da to y Da for evaluation and processing** is used to define a mass range, which shall be evaluated during measurement. This has no influence on the acquisition mass range defined in the flexControl method.

If some specific peaks should not be evaluated during an AutoXecute run, you have to create a mass control list, where these masses are defined as background (see Figure 3-55). Since **Centroid** should be used as evaluation peak detection algorithm (every isotope is annotated) a fixed tolerance of ±2000 ppm is applied, so it is not necessary to enter all isotope masses in the list.

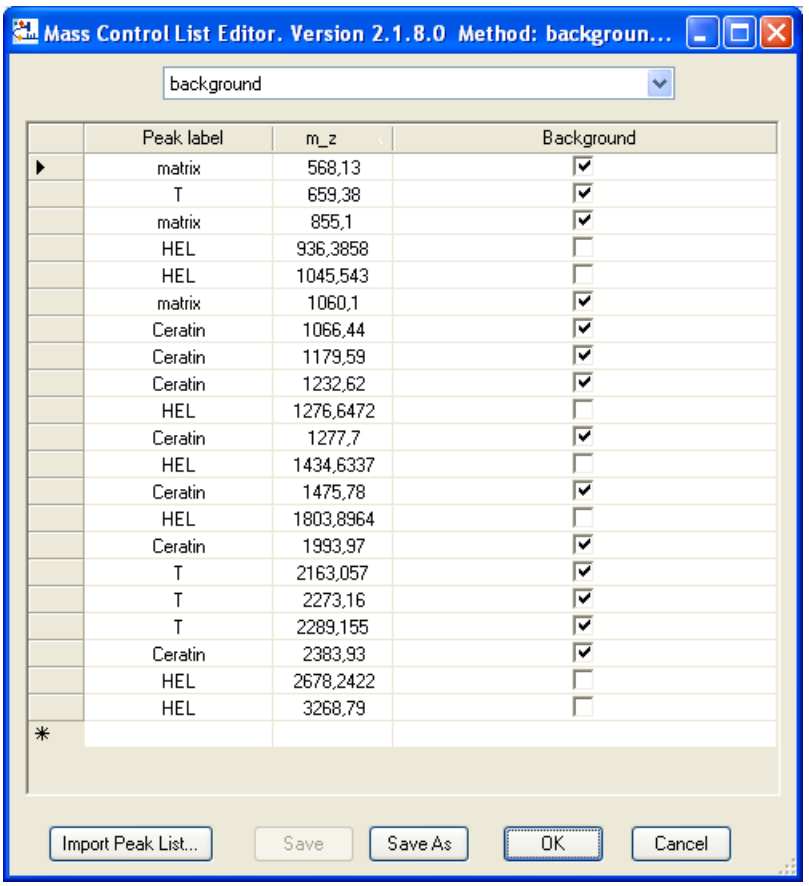


Figure 3-55 Mass Control List Editor for AutoXecute

The **background** feature can also be used in the MSMS mode to exclude special masses from LIFT measurement. Just define a MCL with masses marked as background and select it in the AutoXecute LIFT method. A fixed tolerance of ±2000 ppm is then also used and there will be no MSMS measurement of these masses.

The column **Peak label** contains the names of the substances.

The column **m_z** contains the theoretical values of the respective peaks.

The column **Background** contains check boxes to assign a mass as background.

When a Mass Control List is opened from the AutoXecute method editor only the **Background** column is shown, even if the list contains other masses that are marked as calibrants. Here, MCLs can only be used as background lists and not for calibration.

Group box Peak Exclusion

If the check box is activated, the entered number of highest peaks (from the defined mass range) is ignored during the evaluation. Again, a fixed tolerance of ±2000 ppm is used. If an additional background list is selected, at first the background peaks are excluded, then the x largest peaks. Detailed information is given in the AutoXecute output during the run.

Group box Peak Evaluation

The peak finder and related parameters used for evaluation during an AutoXecute run are offered via the Processing Methods known from flexControl and flexAnalysis. It is highly recommended to use **Centroid** as peak detection algorithm instead of **SNAP** because fast peak finding is required in AutoXecute runs. The signal/noise ratio of the highest peak in one shot package must exceed the signal/noise threshold in the Processing Method, otherwise the spectrum will be rejected. During the automatic run the Processing Method chosen in the AutoXecute method is transferred to flexControl (**Processing** page).

Smoothing may be applied to filter out spikes from a spectrum before the fuzzy module judges the spectrum.

If the parameter **Baseline Subtraction** is applied, the baseline is subtracted before the peak judgment operation is performed.

Note Smoothing and Baseline Subtraction are only applied for judging in AutoXecute. If a spectrum modified in this way is accepted, the original (unmodified) spectrum is added to the sum buffer (as known from manual data acquisition).

Entries in the last row concern the Peak Resolution or alternatively the Half Width of the most prominent peak of the current spectrum (shot package). It is recommended to use Half Width for LIFT measurements, since the Half Width of parent and fragments is comparable, in contrast to the respective Resolutions.

Detailed information on the currently evaluated peak that is given in the AutoXecute output, see `How to read AutoXecute log.pdf` in the `Manuals` folder of the installation DVD.

Group box Fuzzy Control

AutoXecute provides specific fuzzy engines for two substance classes. One is best suited to measuring peptides, the other one to measuring proteins.

Select **Digest/Peptides** for peptides in case of smaller molecules and high resolution. The **Signal Intensity** box contains criteria to specify a kind of threshold (Intensity per shot) peaks must exceed to be accepted. Four thresholds can be chosen: **High** (20), **Medium** (10), **Low** (5) and **Very Low** (2). From the AutoXecuteOutput the intensity per shot can be seen for the evaluated peak.

Example: the highest peak in a shot package that consists of 200 shots reaches an intensity value of 1200 (y-axis). So the intensity/shot ratio is 6. In an AutoXecute run this shot package would have been only accepted with the selected threshold **Low** (= 5) or **Very Low** (=2).

The fuzzy engine chosen by selecting **Proteins/Oligonucleotides** performs best with larger molecules and poor resolution. To avoid identifying noise as a peak a maximal resolution can be specified in the Maximal Resolution box. The chosen **Resolution** in the row **Peak** above is the reference.

3.7.1.1.4 Accumulation page

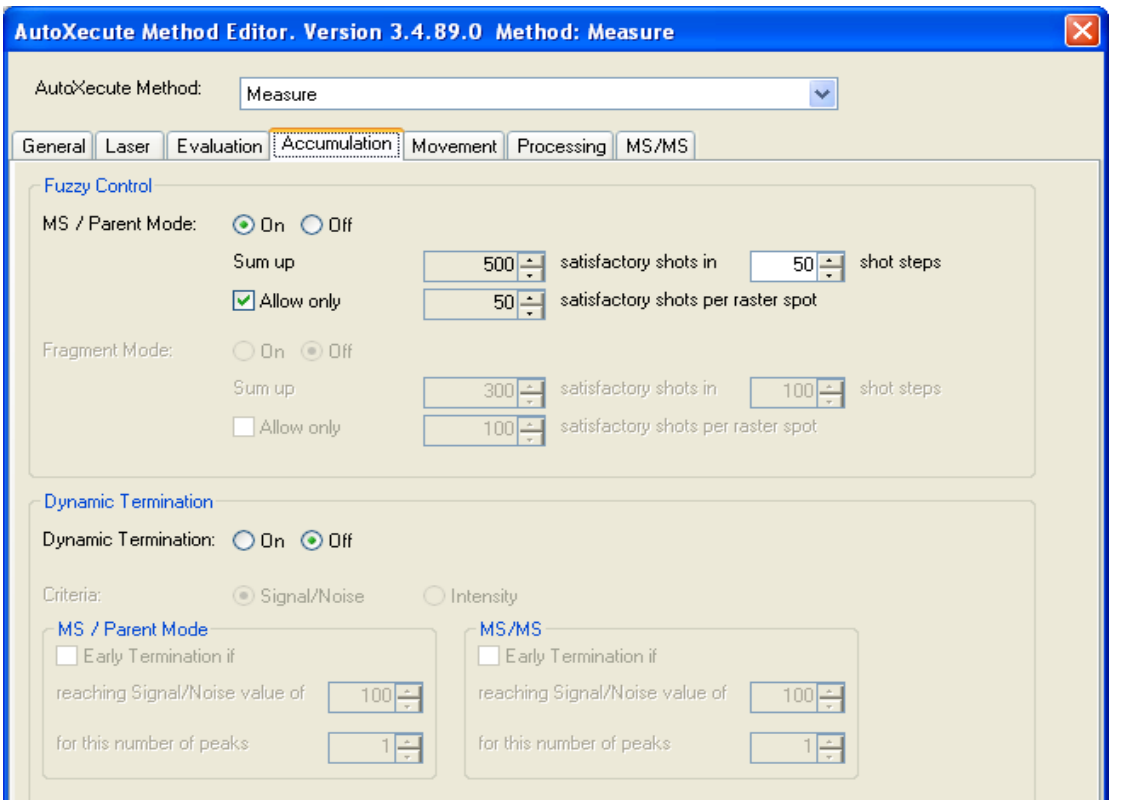


Figure 3-56 Content of the Accumulation page

The **Accumulation** page (see Figure 3-56) is used to determine by means of the fuzzy engine, whether spectra are added to the sum buffer.

When Fuzzy Control is switched off, any recorded spectrum is added, regardless of its quality. This is the same for MS/precursors and fragments in the MS/MS mode.

Note If both Fuzzy Controls (Laser & Accumulation) are switched off, a number of settings related to shot package evaluation are automatically disabled.

If the **Allow only** check box is selected, the raster spot is changed after the given number of satisfactory shots has been added.

Note The mass range for the evaluation of the LIFT fragment spectra starts at 200 Da and ends at “parent mass – 200 Da” (default value). It can be changed in the **AutoXecute Settings** dialog.

The **Dynamic Termination** (see Figure 3-57) feature offers the possibility to end the acquisition on a sample spot (if the spectrum is already good enough) before the required number of shots is reached: The already added shot packages in the sum buffer are evaluated after each new addition. If the defined criteria (**Signal/Noise** or **Intensity**) for the given number of peaks is reached the acquisition on this sample spot is complete. This feature works together with the common evaluation feature. So, a sample spot will be left as soon as one of the thresholds (**Evaluation** page or **Early Termination**) is reached. The feature can separately be used in the MS mode as well as during MS/MS measurement.

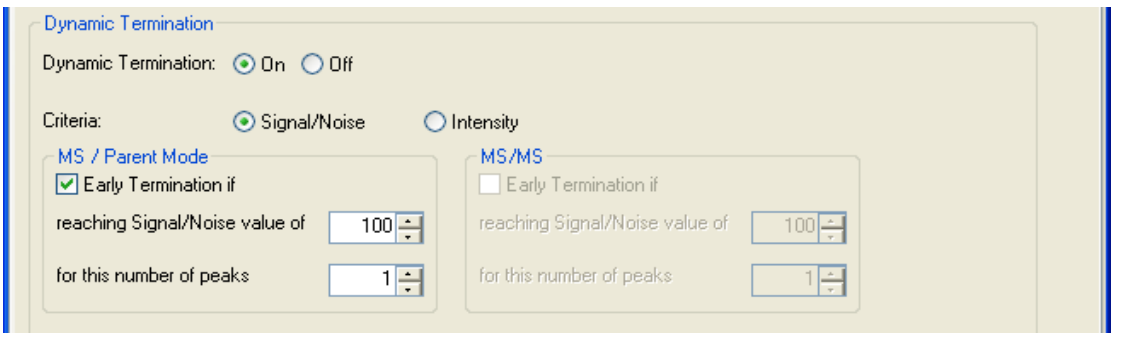


Figure 3-57 Activated Dynamic Termination feature

3.7.1.1.5 Movement page

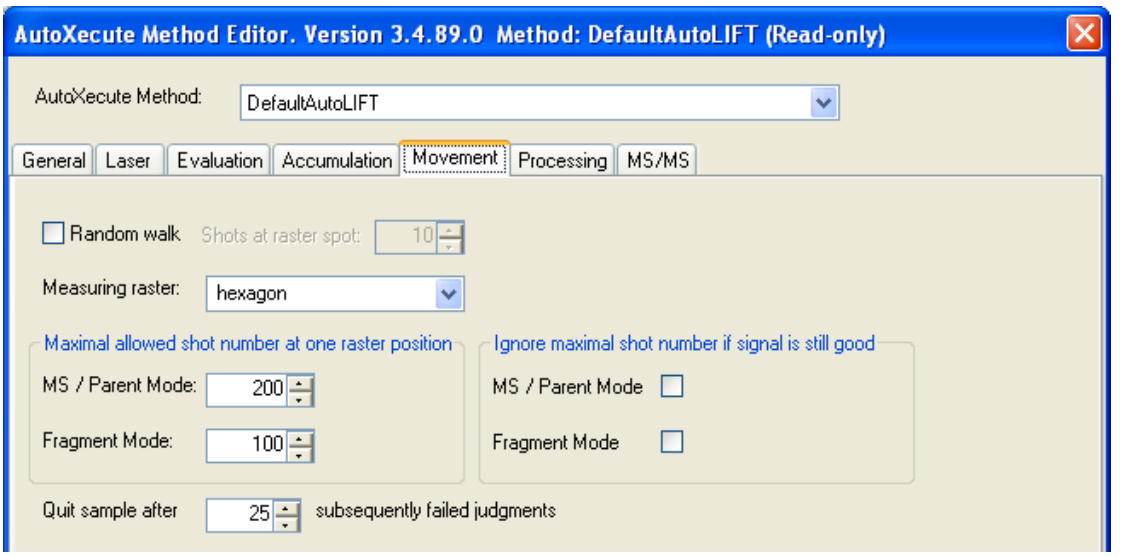


Figure 3-58 Content of the Movement page

The **Movement** page (see Figure 3-58) is used to specify whether the position on the sample spot (i.e. the raster spot) has to be changed, dependent on the sample quality. The different positions on a sample spot are named raster spots:

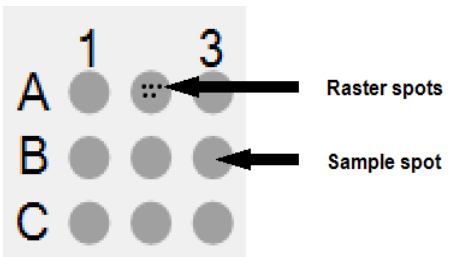


Figure 3-59 Sample spots and raster spots

It is possible to measure a sample spot with a pre-defined pattern (**Measuring raster**) or with the so called **Random walk**, where the laser fires on randomly chosen positions on the sample spot. All rastering features are de-activated in case of **Random walk**. To use this feature the number of shots per raster position has to be specified.

If a **Measuring raster** is activated and if all raster positions of one measuring raster have been used once, AutoXecute will restart with an additional offset at the first raster position.

The **Random walk** can be used for all preparations, if it is selected all raster-related settings are disabled. When random walk is used during autoXecute runs it is not possible to choose between **Partial sample** and **Complete sample**, as known from the manual measurement (see section 3.7.2), the movement is done with **Complete sample**. .

When the **Maximal allowed shot number at one raster position** is reached, the target moves to the next measure position (raster spot) on the same sample spot to continue with the acquisition.

However, if the check box **Ignore maximal shot number if signal is still good** is activated the measurement will be continued as long as the evaluation considers spectra as good enough.

The **Maximal allowed shot number** and **Ignore maximal shots** can both be separately used for precursors and fragments in an AutoXecute LIFT method.

The **Quit sample after** box is used to set a criterion to abort an acquisition on a sample spot. When the maximal number of subsequently failed judgments has been reached, the sample on the entire sample spot is considered unusable for data accumulation. The target moves to the next sample spot.

3.7.1.1.6 Processing Page

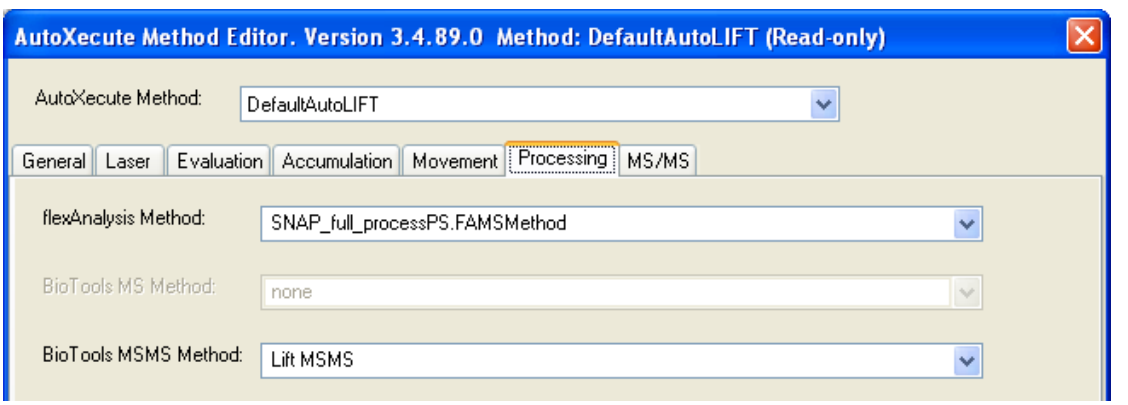


Figure 3-60 Content of the Processing page

The **Processing** page (see Figure 3-60) is used to specify different available methods for post processing.

Note It is recommended to specify the post processing methods in the AutoXecute Run editor and not here in the AutoXecute Method Editor. Nevertheless there are few use cases (e.g. flexImaging, WARP-LC), which require to specify the methods in the AutoXecute Method Editor. In case methods are specified in both the Run editor and in the referenced AutoXecute methods, the settings from the Run editor are taken.

The **flexAnalysis Method**, **BioTools MS Method**, and **BioTools MSMS Method** boxes allow choosing one of the offered methods for post processing. If a method is chosen the method name is entered as information in the parameters of the saved spectrum (FAMeth and BTMeth). During an automatic run a spectrum is only sent to flexAnalysis and BioTools, if a respective method is assigned (either here or via the RunEditor).

3.7.1.1.7 MS/MS Page

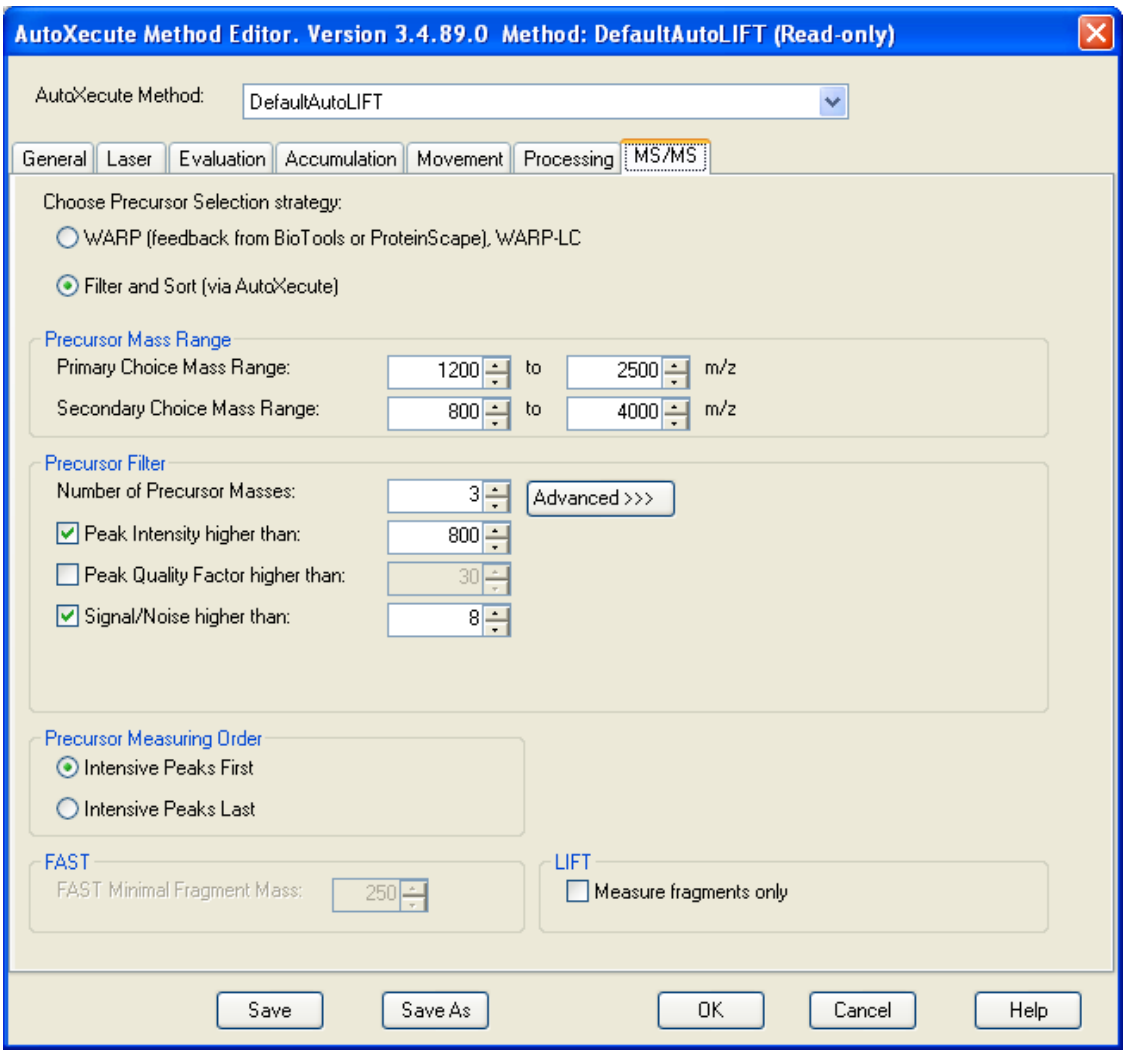


Figure 3-61 Content of the MS/MS page

If a flexControl LIFT or FAST method is loaded in the **General** page, the **MS/MS** page becomes activated. It is used to determine the LIFT and FAST measurements on the basis of the respective fingerprint information.

The main decision on this page is done with the **Precursor Selection strategy**. For automatic MS/MS measurements MS measurement must be done on a sample spot

before because it is essential to have MS fingerprint peak lists where special peaks are selected from. Two possibilities are offered to select these peaks from the MS peak list:

1. **WARP (feedback from BioTools or ProteinScape)**: BioTools (version 3.0 and higher) or ProteinScape mark peaks in the MS peak list and sort this peak list. AutoXecute then only reads this information and starts the MS/MS acquisition and evaluation without any further filtering. If WARP is chosen, the rest of the MS/MS page is not used and therefore de-activated (see section A.1). WARP-LC users have to use this precursor selection strategy.

2. **Filter and Sort (via AutoXecute)**: AutoXecute reads the MS fingerprint peak list and marks peaks for MS/MS, according to the filter criteria that are adjusted in the **Precursor Filter** group box. If this option is chosen, MS spectra can naturally also be sent to BioTools, but the Mascot result for these spectra does not affect the MS/MS measurement.

If you want to select the precursor masses from the MS peak list manually a different workflow has to be done. Please refer to the Quickstart document `Quickstart_PrecursorSelection.pdf` on the installation DVD.

Due to the process of a MS/MS- measurement there is a mass range with optimal fragmentation properties. This behavior is addressed with **Precursor Mass Range Selection**. Peaks in the **Primary Choice Mass Range** are preferred fragmentation targets. Only if there are too few peaks in the primary mass range after filtering, the peak list is gradually filled up from the **Secondary Choice Mass Range**. So the primary mass range is always included in the secondary mass range.

All parameters contained in the **Precursor Filters** box can be individually selected. They form a filter to ensure that only the best suitable precursors are taken for a measurement. The higher the values of the three parameters are set, the narrower is the bandwidth surrounding the precursor.

- Select the number of MS/MS measurements with **Number of Precursor Masses**. The selected masses will be listed in the AutoXecute output.
- With **Peak Intensity higher than** all peaks with lower intensity will be sorted out.
- With **Peak Quality** factor **higher than** all peaks with lower quality factor will be sorted out.
- With **Signal/Noise higher than** all peaks with lower S/N will be sorted out.

The information on the filtering steps during the run is shown in detail in the AutoXecute output. If you want to check the filter process you may load the MS spectrum manually in flexAnalysis and filter the peak list step by step on your own.

In the **Advanced** section (see Figure 3-62) a fourth filter, **Near Neighbor**, is offered with its belonging settings. It ensures that if peaks are in close proximity (ion selector window) of a possible precursor none of them is taken as precursor. The lower and upper limits define the mass window where no other peak must be detected.

There is an additional peak excluding filter available that can only be activated via the **Evaluation** tab of the autoXecute MSMS method: select a Mass Control List, where the masses that you want to ignore for MSMS precursor selection are marked as **Background** peaks.

A valid precursor must have at least the defined intensity (regarding his near neighbors) to be analyzed.

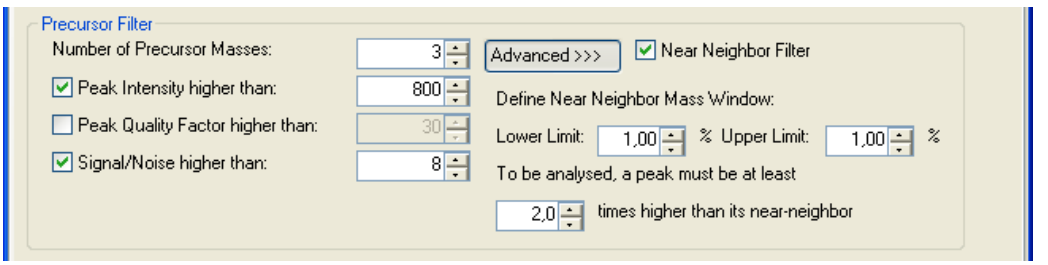


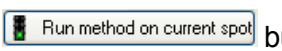
Figure 3-62 Precursor Filter - Advanced

Depending on the selected option in **Precursor Measuring Order** the MS/MS measurement begins with the intensive or the small peaks first.

In case of an AutoXecute FAST measurement the **minimal Fragment mass**, that determines the number of segments can be entered here and is transferred to flexControl during the run.

The LIFT option **Measure Fragments only** can be used e.g. for MALDI Imaging experiments. For more information, see the *FlexImaging User Manual*.

3.7.1.2 Run Method on Current Spot

To test the settings of an AutoXecute method the 

button can be used. Automatic data acquisition starts on the current spot with the currently selected AutoXecute method, but data is not saved automatically in the end. The flexControl

method loaded in the GUI has to fit to the AutoXecute method (both MS, or both LIFT). Otherwise a message appears in the AutoXecute output (see Figure 3-63) and the acquisition does not start.

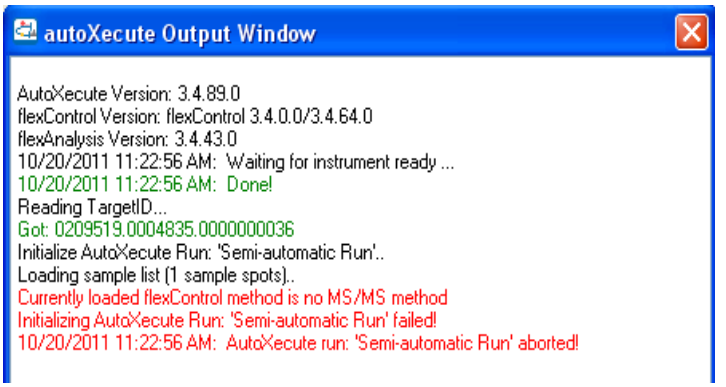


Figure 3-63 AutoXecuteoutput with error message

3.7.1.3 AutoXecuteRun

AutoXecute runs (sequences) are created with the **Run Editor** that is part of flexControl, but can also be used as stand alone program. An AutoXecute Run defines the workflow on every prepared target spot, i.e. what to measure (MS, MS/MS), where to save the data and which post processing is done.

After an AutoXecute run is finished the measurement status for each spot has automatically been transferred to the sequence. It can be seen in the sequence file, column **Status** (see Figure 3-66), as well as on the plate view (colored circles, (see Figure 3-73). A run that has been finished cannot be started again without resetting the status. If you try to start an already finished run, the AutoXecute output indicates that no measurement position is available by displaying the message `0 positions loaded` (see Figure 3-64). To run the sequence again the status for MS and/or MS/MS has to be reset in the **Run Editor** (see Figure 3-66).

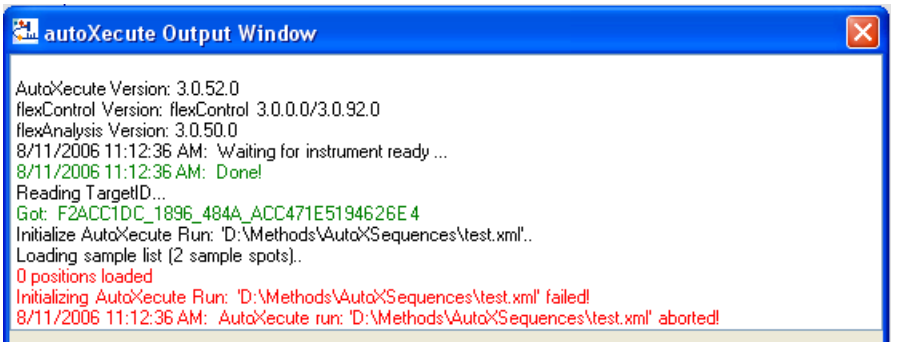
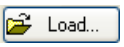
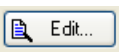


Figure 3-64 A finished run has been re-started without resetting the status

A list of the last used sequences is offered in the drop down box **Run** (see Figure 3-50). The number of sequences shown depends on the setting in the **AutoXecute Settings** dialog (see Figure 3-74). A selected sequence results automatically in visible colored circles on the Plate View. When you select **none** a plain plate view is shown.

The button 

is used to load sequences that are not offered in the drop down list. It is also possible to load old sequence files (`.txt`) that have been created with the Excel based Spreadsheet Generator. It is not possible to load a run that was created for a target geometry that does not match to the target that is currently in the instrument. A message appears (*The referenced geometry of the run does not fit to the geometry type of your target*). To load this run, insert the correct target first.

The button 

is used to open the **Run Editor** with the currently selected sequence file. If a `txt` file (spreadsheet generator generated) is selected it will be imported, the appearing message (see Figure 3-65) has to be confirmed. Methods, paths, sample names and comments will be transferred from the `txt` file to the Run Editor. If any method is not available it will be shown in red.

Figure 3-65 Import a spreadsheet generator created sequence

An AutoXecute run is started with the button 

. At the beginning the methods used in the run are selected. If a file is missing, the run stops and a message is given in the AutoXecute output.

During an AutoXecute run it is not possible to change any settings in either the AutoXecute methods or the sequence file. If you want to have a look to the method content, the run can be paused with the button Pause Run from the output window or the AutoXecute page. The **Method Editor** as well as the **Run Editor** can then be opened in the view mode where nothing can be changed. To continue click Resume Run.

A run can be stopped at any time and position. When it is restarted it continues on the spot where it has been stopped before (due to the **Status** information), no information is lost. If you want to restart a run from the beginning the **Status** has to be cleared and it is recommended to delete the already measured spectra from the hard drive.

3.7.1.4 The AutoXecute Run Editor

The button New... starts the **Run Editor** wizard (if activated) or opens the **Run Editor** immediately (like the Edit... button does). If you want to setup a run with calibration measurement as well as sample measurement you must use the wizard. The explanations in the wizard and in the help file and the *AutoXecute Run Editor Manual* are very detailed; therefore not much additional information is given here.

As denoted before, the status of a spot is shown in the sequence file. If the status is **finished** (light/dark green) but you want to measure the spot again, it is necessary to clear the status and save the sequence.

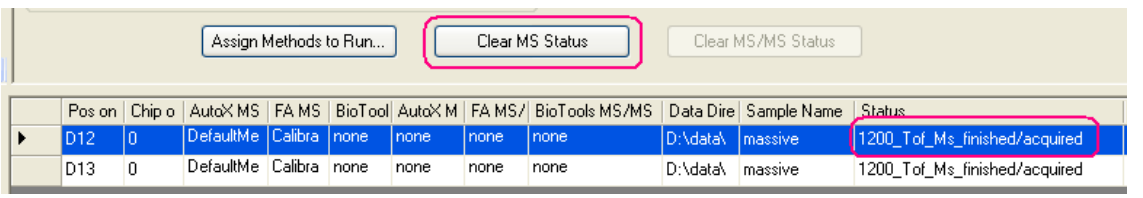


Figure 3-66 Status of a sample spot

In earlier flexControl versions where sequences had to be created with the spreadsheet generator some templates were offered, especially for the calibration use case. With the new **Run Editor** calibration templates are pre-installed for a lot of different target geometries. Additionally you can create your own calibration templates in the wizard. After you chose to work with a calibration template the **Calibration Mode** is automatically activated in the **Run Editor** wizard (see Figure 3-67) or the **Run Parameter** page (see Figure 3-68).

According to the loaded geometry the matching pre- defined or own user- defined calibration template is offered.

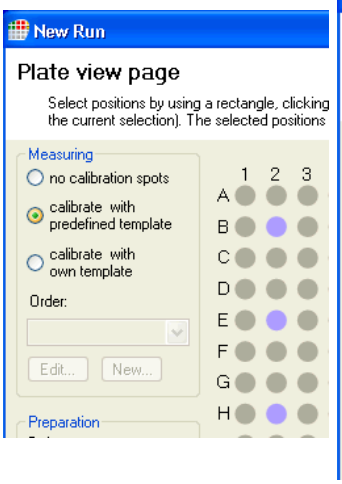
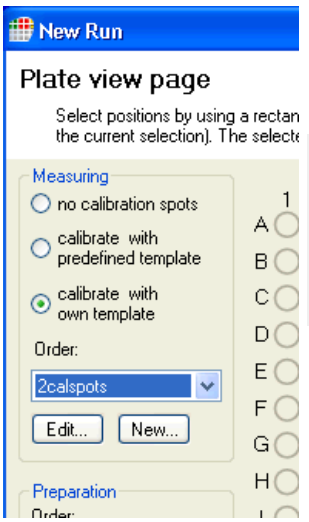


Figure 3-67 Activated calibration mode in the wizard



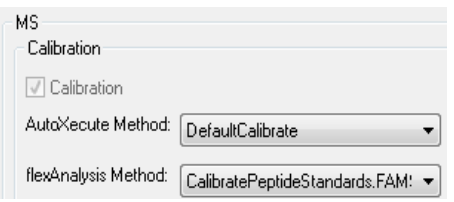


Figure 3-68 Activated calibration mode in the Run Editor GUI

3.7.1.5 Show AutoXecute Output

Selecting the **Show AutoXecute Output** check box opens the AutoXecute output window (see Figure 3-69) where detailed information concerning the current AutoXecute run is shown. In the beginning the complete version numbers of the programs are listed, afterwards follows the method check that assures that all selected methods from the run are available on the hard drive.

During the run a lot of information concerning laser power setting, peak evaluation etc is displayed. If errors occur they will be shown in red, successful evaluations are shown green colored. More information on how to interpret the fuzzy engine results (fuzzy input/output) are given in `How to read AutoXecute log.pdf` on the Compass for flex DVD.

A run can be paused and continued with the button Pause Run / Resume Run and aborted with Abort Run. Information on these actions are shown in the output.

The content of the AutoXecute Output Window is also saved on the hard drive in the file `C:\BDALSystemData\AutoXOutput.txt`.

Figure 3-69 AutoXecute output

3.7.1.6 Show Process Queuer

Selecting the **Show Process Queuer** check box opens the Process Queuer window (see Figure 3-70). The Process Queuer window shows information about spectra being processed in the background by flexAnalysis. This information contains the list of spectra being currently processed and information about the result of the processing. For details please refer to the *flexAnalysis User Manual*.

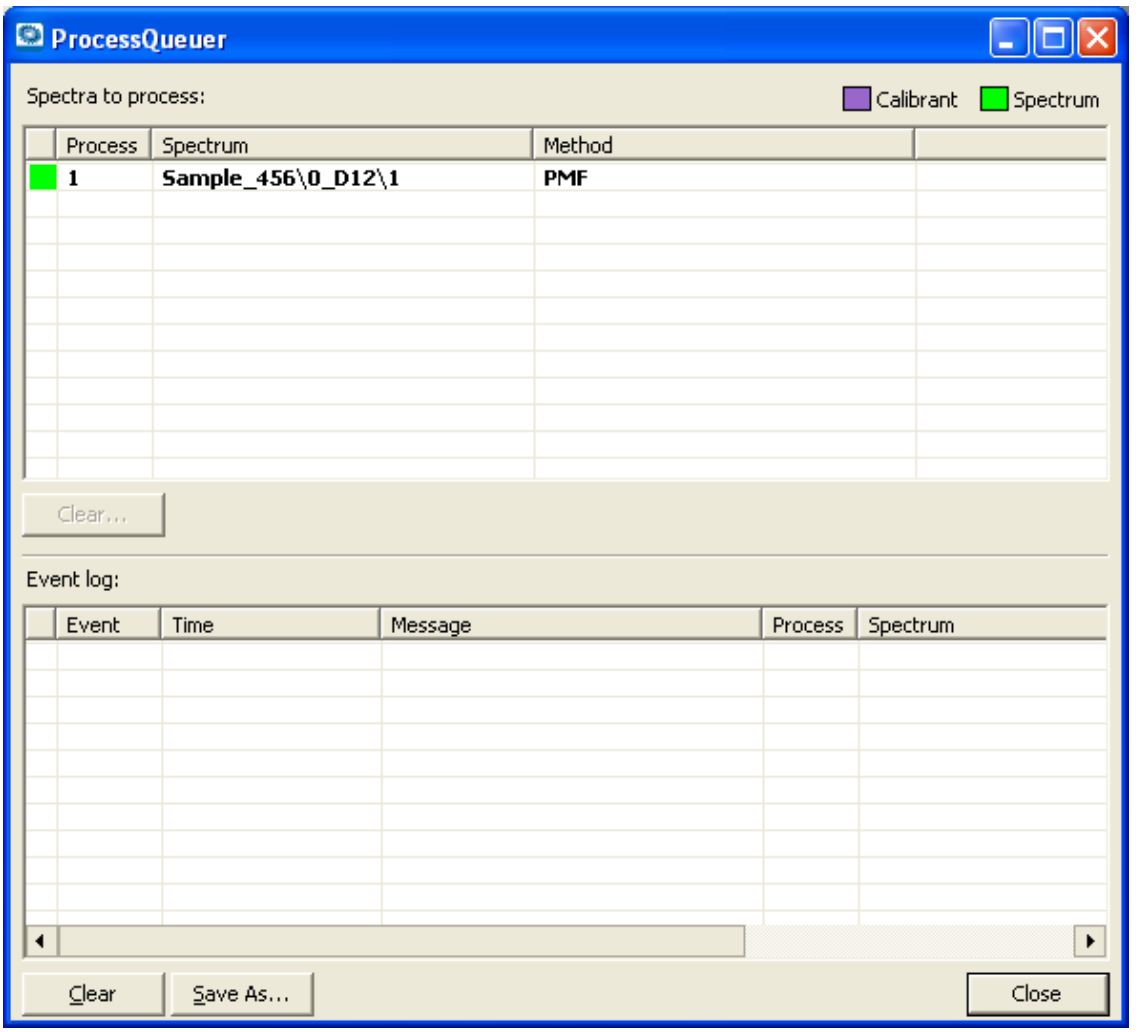


Figure 3-70 ProcessQueuer Window

3.7.1.7 Settings

The Settings... button opens the **AutoXecute Settings** dialog (see Figure 3-71) that helps to configure some general AutoXecute settings that do not need to be changed for every run.

Note After changes have been done in this dialog flexControl has to be re-started to be able to work with the new settings!

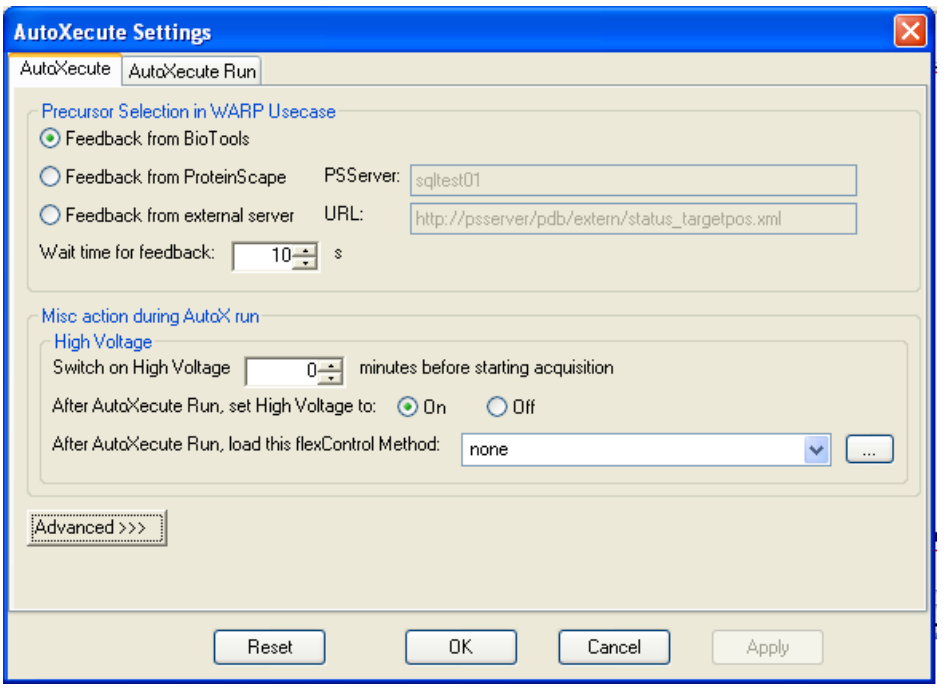


Figure 3-71 AutoXecute Settings – AutoX

Precursor selection in WARP use case

If the WARP use case is selected in the MS/MS AutoXecute method (section 3.7.1.1 and 3.7.1.1, page MS/MS), an external program like BioTools or ProteinScape selects the masses for later MS/MS measurement. In this dialog the respective **Feedback** option has to be chosen to determine to work with either of these programs.

The **Wait time** applies only for BioTools. It specifies how long AutoXecute waits for the modified fingerprint peak list. If after this time no peak list is available for the current sample

spot, AutoXecute continues with the next spot. Since common AutoXecute runs consist of more than a few spots, and first all MS measurements are done, BioTools or ProteinScape will normally have enough time to modify the MS peak lists. So, if AutoXecute starts with MS/MS, the MS information are available.

If ProteinScape has not been finished when AutoXecute starts with MS/MS the run stops immediately without any wait time.

Note In case BioTools or ProteinScape are not ready when AutoXecute wants to start the MS/MS measurement, the run ends without MS/MS. Afterwards, when the necessary modifications from BioTools or ProteinScape are available, i.e. peaks are marked, it is possible to simply re-start the run with measure MS/MS only.

Misc action during AutoXecute run

The **Misc action during AutoXecute run** group is used to define the time period the high voltage is switched on before starting the acquisition to put the instrument into a stable state. After a complete run the high voltage might be turned off by selecting the corresponding option.

Additionally an acquisition method may be loaded to prepare the mass spectrometer for the next run. If **none** is chosen for the flexControl method after a run, the last method loaded during the run remains in flexControl.

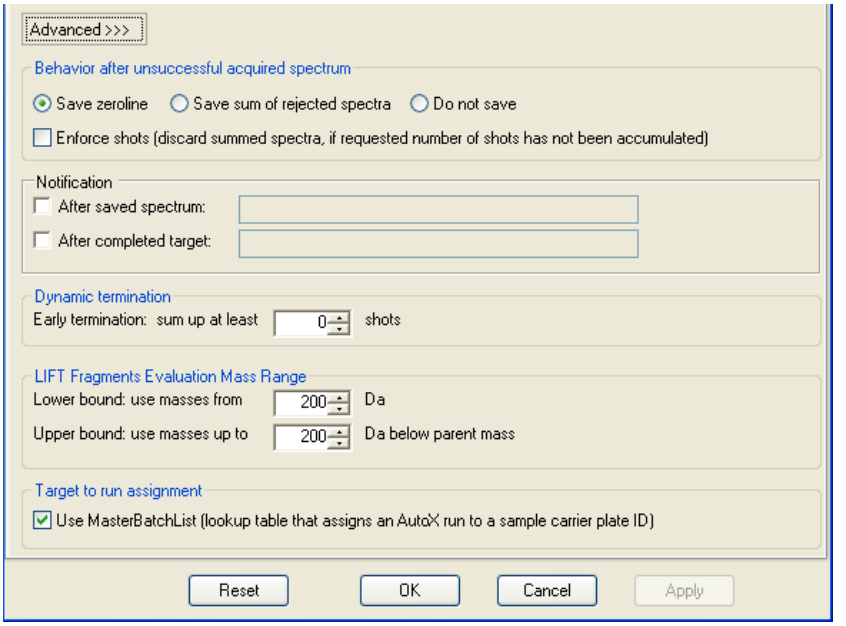


Figure 3-72 AutoXecute Settings – AutoXecute- Advanced

The **Advanced** section contains settings that should not be changed without exact knowledge (if questions occur, ask maldi.sw.support@bdal.de).
If changes have been done but you are not sure, the pre-defined settings can be restored with the button 

.

Behavior after unsuccessful acquired spectrum

Different save results are achieved, depending on the status combination of the check box **Enforce Shots** and the three **Save** options. The results of the combinations are displayed in the following two tables. The colors correspond to the colors shown on the plate view (see Figure 3-73) after the run has been finished.

1. **Enforce Shots** is selected (i.e. the requested number of successfully added shots has to be reached).

	Save zeroline	**Save sum of rejected**	**Do not save**
No shot package is summed	Zeroline	Zeroline	Nothing saved
Some shot packages are summed	Zeroline	Zeroline	Nothing saved

2. **Enforce Shots** is cleared, i.e. it is not necessary to have all requested shots in the end. Therefore you have to differentiate between ‘some shots have been added’ and ‘no shots have been added’:

	Save zeroline	**Save sum of rejected**	**Do not save**
No shot package is summed	Zeroline	Sum of rejected	Nothing saved
Some shot packages are summed	Sum of successfully added	Sum of successfully added	Sum of successfully added

The plate view may look as follows:

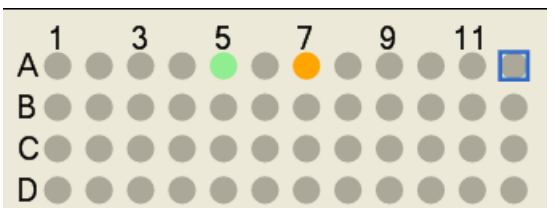


Figure 3-73 Plate view with colored results

Notification

After a spectrum has been saved or the measurement on a whole target is finished it is possible to get a notification on another server.

Dynamic Termination

This setting concerns the **Early Termination** feature from the **Accumulation** page in the **AutoXecute Method Editor** (Figure 3-57), where it is possible to finish the accumulation on a sample spot if a defined number of peaks have reached a defined **Signal/Noise** value. Here you can specify the number of shots that has to be added at least before the **Early Termination** feature is allowed to end the acquisition as soon as the other criteria are reached.

LIFT Fragments Evaluation Mass Range

During the automatic measurement of LIFT fragment spectra these values determine the respective mass range where peaks are used from for evaluation.

Target to run assignment

Activate this check box, if you want to use the MasterBatchList. Normally this is only used in conjunction with the Twister use.

History count

The **History count** determines the number of runs that is offered in the drop down box **AutoXecuteRun** (see Figure 3-50).

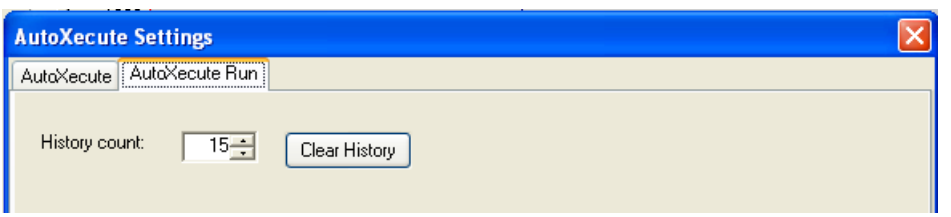


Figure 3-74 AutoXecute Settings – AutoXecuteRun

3.7.1.8 Set Initial Laser Power

If you don't know which laser power to use in the beginning of an AutoXecute run you can adjust the initial laser power before or during an AutoXecute run by pressing the button 

. Just follow the steps below:

1. Pause the run.
2. Adjust the laser power with the slide.
3. Resume the run.

The initial laser power, which is usually taken from the **Laser** page of the currently used AutoXecute method or from the automatic laser power tuning in the beginning of a run, is ignored. Additionally the maximum laser power value is adjusted accordingly. Both settings (new initial laser power and new maximum laser power) are only stored for this AutoXecute run. This means the used methods will not be changed. If you choose another run from the drop-down box, the initial and maximum laser power values are deleted, and the values from the AutoXecute methods are used again.

3.7.2 Sample Carrier

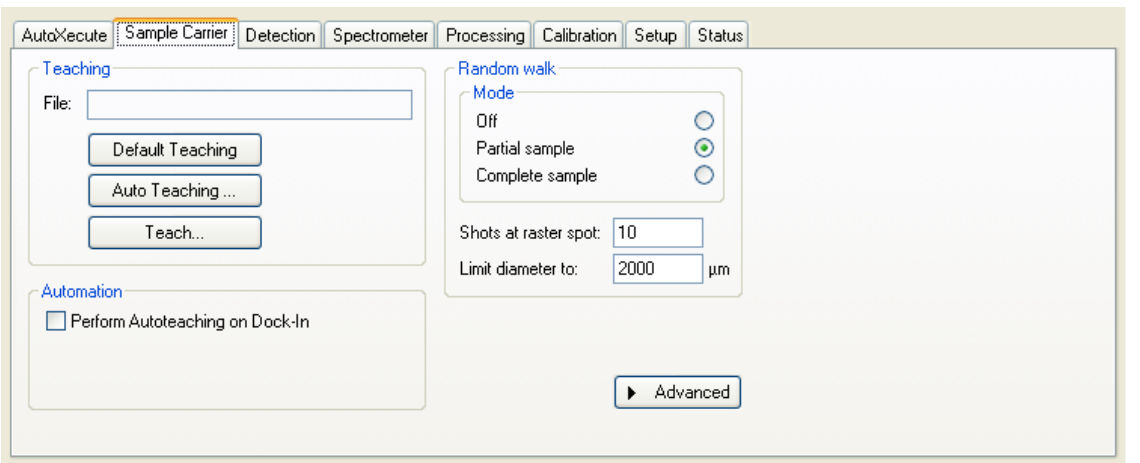


Figure 3-75 Features of the Sample Carrier page

The **Sample Carrier** page (see Figure 3-75) is used to configure target plate features. On the top the Teaching File is shown that belongs to the currently loaded target geometry (see Figure 3-43).

The Default Teaching button loads the default teaching file (named default.teach in the respective teachings directory for the current target plate, e.g. `D:\Methods\GeometryFiles\MTP AnchorChip 200-1536_teachings`) corresponding to the currently loaded target plate.

Auto teaching is an alignment procedure to automatically guide AnchorChip targets after docking into the best measure position. The button Auto Teaching ... starts the auto teaching process. If no auto teaching target is inserted a message appears and the process stops.

The button Teach... opens the **Teaching** dialog (see section 3.7.2.1).

The **Automation** group box contains elements that are used to configure workflow related actions. Depending on the options installed on the instrument, elements will be hidden, so that they cannot be set unintentionally.

Note If the **Perform Autoteaching on Dock-In** option is selected, the auto teaching process is performed whenever the target plate is changed, and if the target is identified as an AnchorChip type.

The button ▶ Advanced opens the group box **Manual Fine Control** (see Figure 3-76). Clicking the symbol in the center of the arrangement of arrows activates the keyboard cursors to move the tray. This option allows a sharp and exact move across a sample spot. A second way to alter a position on the spot is to enter corresponding values of the x-y-parameters entry fields on the right.

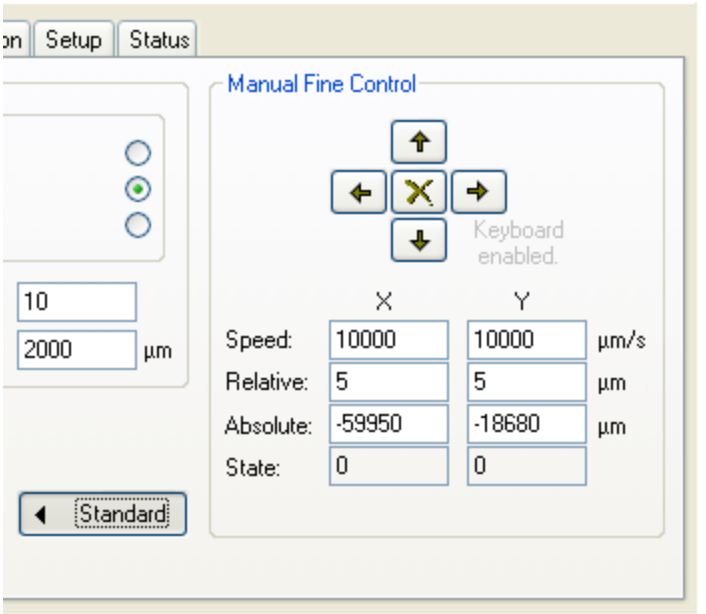


Figure 3-76 Manual control on teaching dialog

3.7.2.1 Teaching Dialog

Teaching is an alignment procedure to compensate possible mechanical tolerances of the sample loading facility.

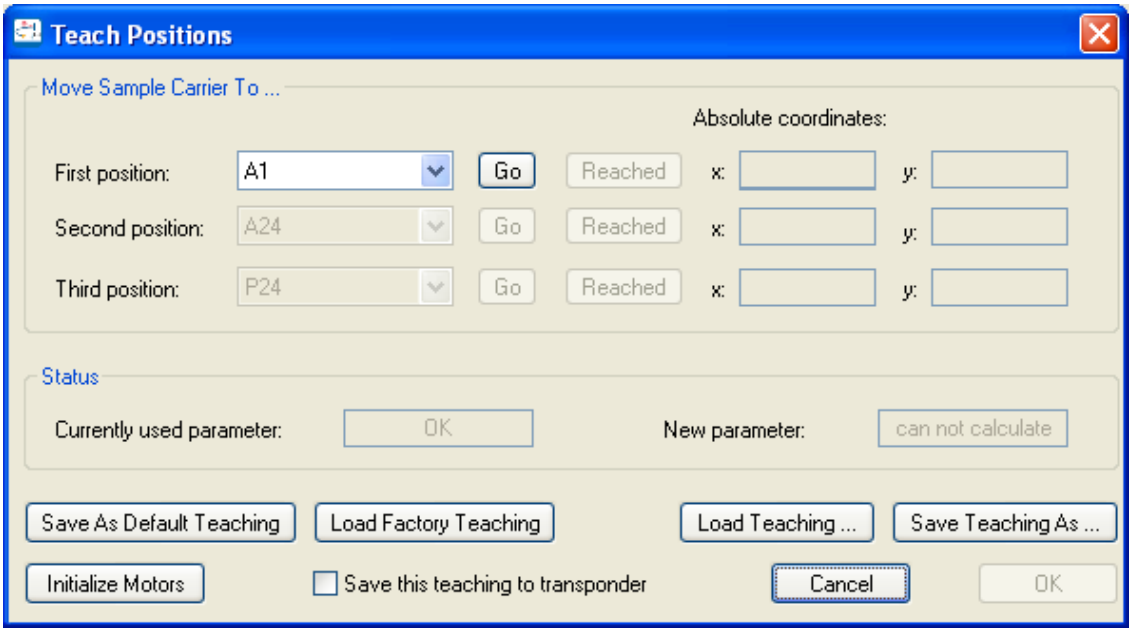


Figure 3-77 Features of the teaching dialog

The **Teach Positions** dialog (see Figure 3-77) appears for realignment. To perform a teaching select the **First Position** and click Go. When the target has been moved to this position, click Reached and continue with the **Second Position**. The new absolute coordinates will be calculated automatically.

When the group box **Status** acknowledges with **OK** quit with the OK button. The record of the new x-y-coordinates is finished and sent to the transponder in case of transponder targets, if the check box **Save this teaching to Transponder** is selected.

Use the button Initialize Motors before teaching to eliminate possible step losses. The task of the button is to re-define the zero point of the x-y-coordinates. The button Load Factory Teaching loads a file containing factory defaults of the target. The buttons Load Teaching ... and Save Teaching As ... are used to handle `<name>.teach` files, either to reload a stored file or to store a current file with a specific name. The Save As Default Teaching button saves the current teaching as default teaching, so that it is automatically loaded when a target plate with the same type is loaded for the next time.

3.7.2.2 Random Walk

The **Random walk** feature can either be used during manual measurement but also during automatic data acquisition (section 3.7.1, **Movement** page). It allows to measure on randomly selected raster positions.

Select **Mode Off** if you want to choose the measuring positions on your own.

If you select **Partial sample**, only a small area around the actual chosen raster position in the video is used for data acquisition.

Note In automatic mode **Partial sample** cannot be used – complete sample is used instead if the **Random walk** is activated.

With **Complete Sample** selected the laser fires on the whole sample spot.

Shots on a raster spot determine the number of shots that is fired on every randomly selected position.

The **Limit diameter to** field allows a temporary reduction of the sample spot diameter for manual measurements. The original sample spot diameter is restored e.g. on chip change or when a new target plate is inserted.

3.7.3 Detection

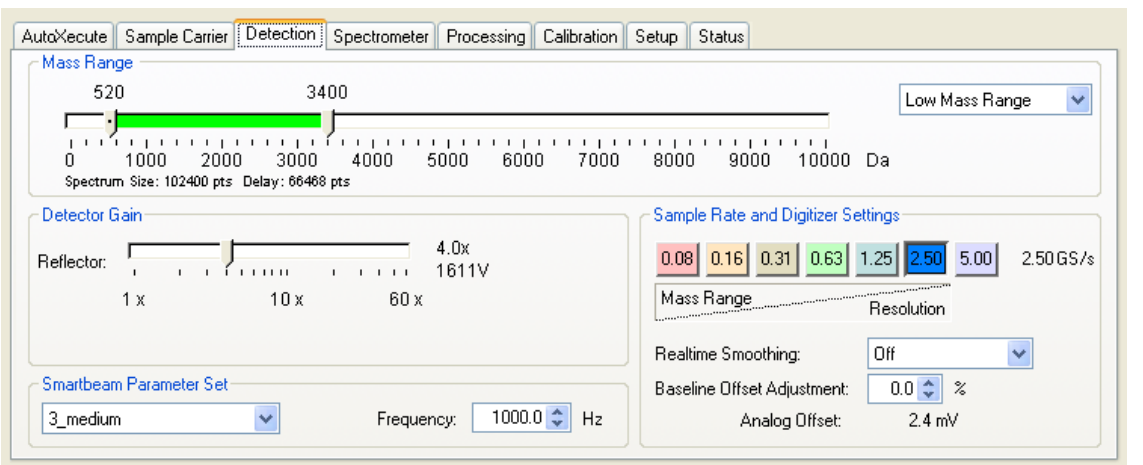


Figure 3-78 Features of the Detection page

The **Detection** page (see Figure 3-78) is used to re-adjust acquisition parameters, e.g., to configure the mass scale, or to vary the amplification factor of the detector.

The mass range is changed by moving the thumbs of the mass range slider.

The total mass range of 500,000 Da is divided into three sub ranges called **Low Mass Range**, **Medium Mass Range**, and **High Mass Range**. Anyone can be independently configured according to the application. This configuration comprises the definition of the start and end mass and the linear or logarithmic scaling of the range or sub ranges.

To configure a mass range select the **Define Range** item in the mass range drop list (see section 3.7.3.1).

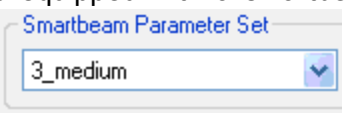
Figure 3-79 Mass Range drop-down list

Sometimes the S/N ratio of the acquired data requires an adaptation of the detector gain. The slider is used to adapt the amplification factor. The function of the slider corresponds to that of the edit field **Reflector Detector Gain** in the **FAST Method Editor** (see section 3.7.10.1). A change made in one of both features is simultaneously adopted by the other one.

The parameters **Resolution** and **Mass Range** of the group box **Sample Rate** correlate in a way that an increase of the **Sample Rate** causes an increase of the resolution and a decrease of the mass range (digitizer performance). To illustrate this, the invalid part of the mass range selector becomes red in color.

Realtime Smoothing reduces the bandwidth of the digitizer preamplifier. Depending on your digitizer type the **Detection** page contains a number of entries in the drop list (**on/off**, **off/low/medium/high**). If this entry is disabled, the instrument has no provision for real-time smoothing. This function is the same as that selectable by the **FAST Method Editor** (see section 3.7.10.1).

The **Baseline Offset Adjustment** box allows to fine tune the analog offset for the digitizer for the current flexControl method. The percentage is applied to half the digitizer sensitivity, the result analog offset is displayed in the field below. The percentage value is stored in the flexControl method.

If the instrument is equipped with a smartbeam laser, the smartbeam parameter set selection drop list 

allows to select a certain parameter set depending on the sample and preparation type to analyze. This setting is stored in the flexControl method.

The **Frequency** box duplicates the function of the corresponding element on the **Acquisition Control** (see section 3.5.1). It adjusts the laser trigger frequency. Higher frequencies result in faster data acquisition.

Certain flexSeries mass spectrometers are equipped with a High Mass detector instead of the standard MCP (Multi Channel Plate) detector . Operating in linear mode (linear method is loaded) with a High Mass instrument means that additional **High Mass** parameters appear on the **Detection** page (see Figure 3-80). High Mass detectors have an additional acceleration electrode, which operates best at a voltage of 8–10 kV. The task of this electrode is to accelerate secondary electrons.

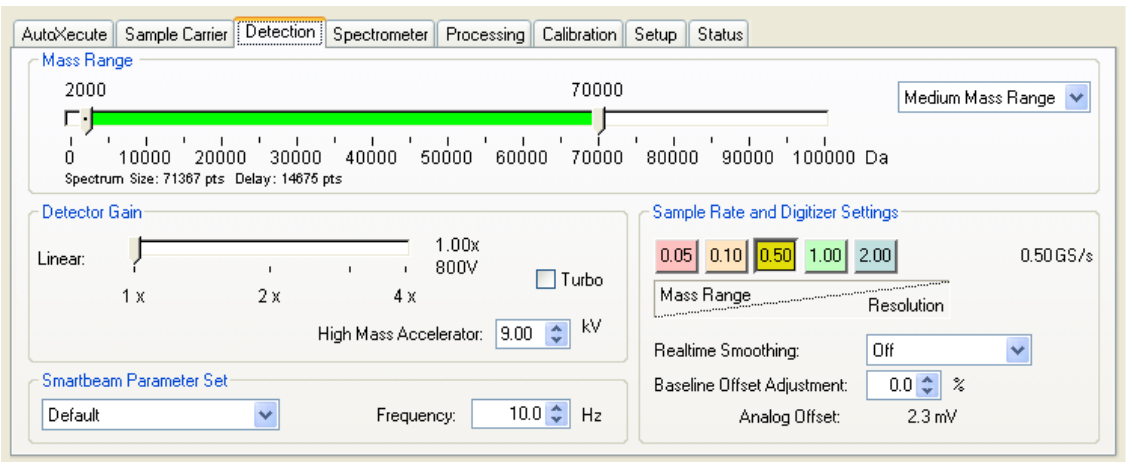


Figure 3-80 Features of the Detection page with installed Highmass detector

The check box **Turbo** available on certain instruments adapts the detector sensitivity for masses beyond 10 kDa (polymers). It is up to the operator to experiment with these settings for the best performance.

3.7.3.1 Mass Range Selector

The **Mass Range Selector** dialog is used to configure any of the three mass ranges. Concerning the layout all the boxes correspond to each other. They contain identical functions.

To customize the range corresponding values have to be entered into the **Lower Limit** and **Upper Limit** fields. The beginning or end of the **Medium Mass Range** can overlap the adjacent mass ranges. A mass range may be displayed as a whole, in linear mode, or logarithmic mode as well. A combination of both scales can be also performed at the user's discretion by entering a value into the field **Upper Limit of Linear scale**. The value in **Linear Display Portion** defines how both scales share the range.

Scale marking of a range can be performed by setting values, where ticks shall appear. Ticks may be assigned with a mass value to enhance viewing. The repetition rate of this assignment is associated with the factor in the entry field **Ticks with Text Repetition Factor** in the logarithmic portion.

To adopt all the new entries press the 

button.

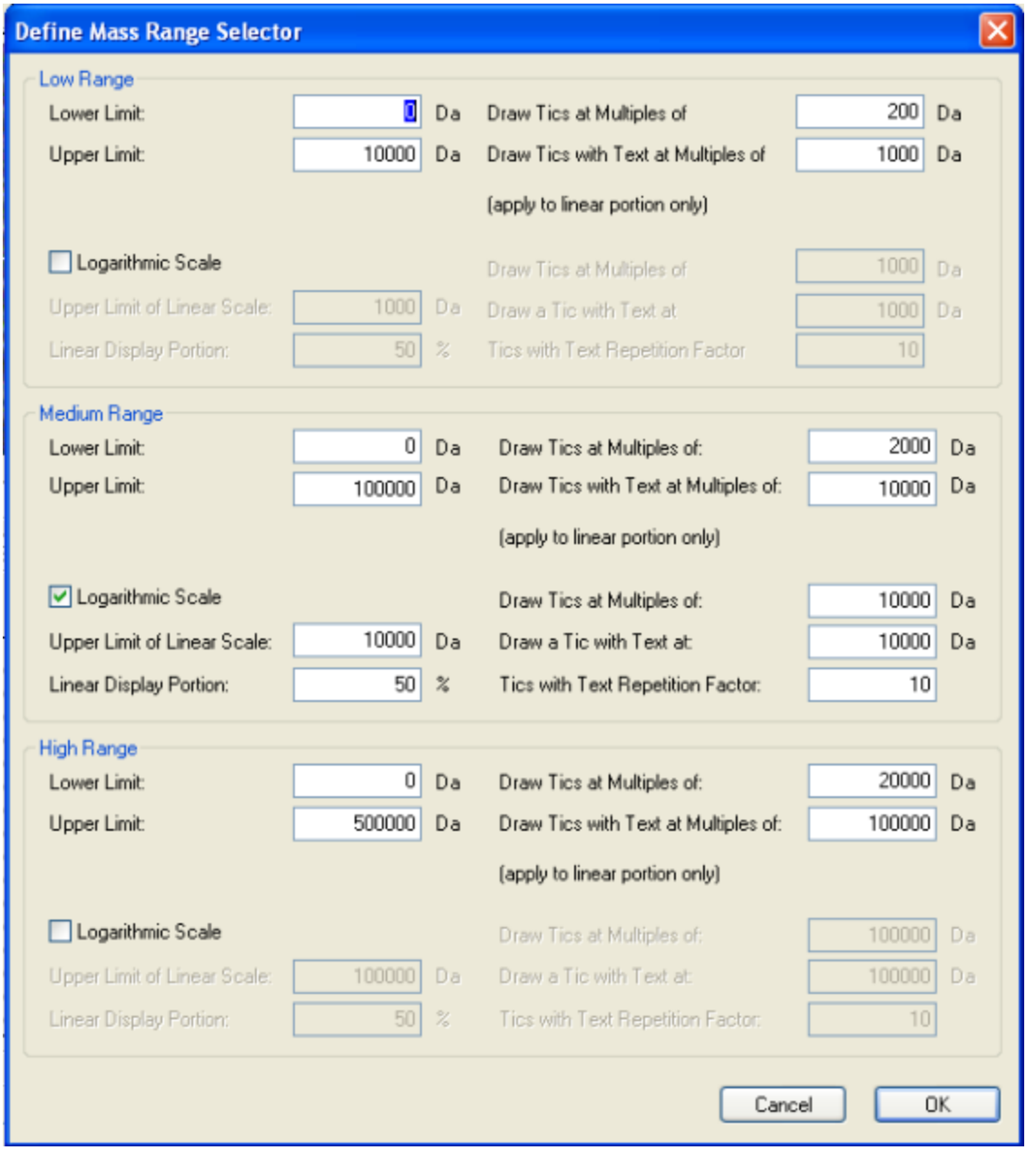


Figure 3-81 Configuring the mass ranges

3.7.4 Spectrometer

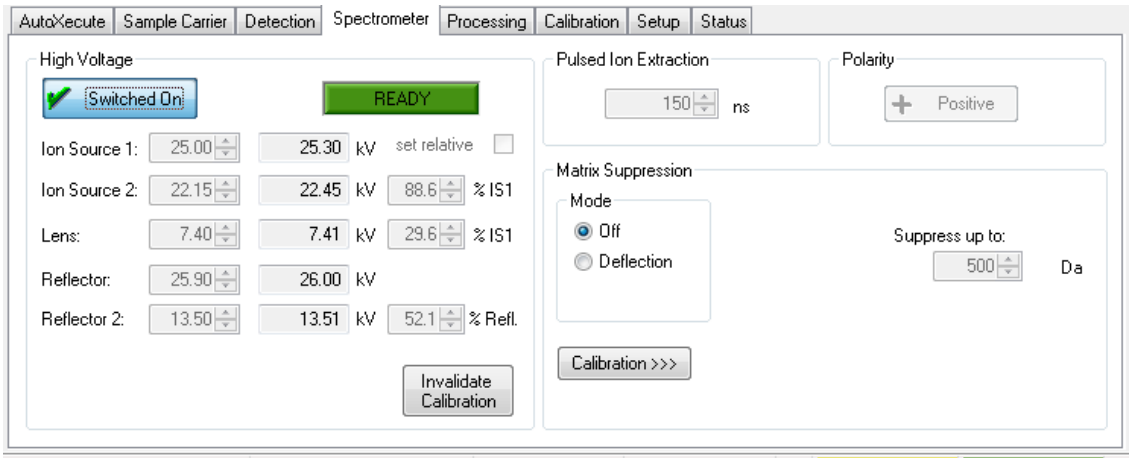


Figure 3-82 Features of the Spectrometer page (reflector method)

The **Spectrometer** page (see Figure 3-82) is used to configure the ion optics. According to the loaded method this page offers specific features.

The toggle key 

| 

is used to switch on or off following components, depending on the loaded method:

- Ion Source 1,
- Ion Source 2,
- Lens,
- Reflector,
- Reflector 2,
- LIFT,
- LIFT 2.

The status field 

| 

right beside the 

| 

button is the corresponding feedback, whether the HV-supply is ready or not. In case of a

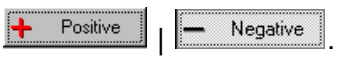
bad vacuum, or when the instrument is vented this field toggles to 

(high voltage is definitely off) independent of the switch position.

Below the toggle buttons are edit fields to adjust voltages for the target plate (ion source 1), the electrode P2 (ion source 2), and the lens arrangement. The unavailable fields reflect the actual measured values for diagnostics. They may deviate and fluctuate, as threshold values are not very accurate.

Selecting the box **set relative** activates the two **%IS1** boxes . The upper box is used to adjust the IS2 value relative to IS1. The lower box is used to set the lens voltage relative to IS1. These two adjustments may be varied to get good results in resolution and sensitivity.

The value for **Pulsed Ion Extraction** is the delay measured in ns between the laser shot and the moment when the electrode P2 is pulsed down to let the ions pass.

The current polarity is shown by one of the unavailable fields 

. You can change the polarity in the program flexConfigurator under **flexControl Misc**.

Matrix Suppression is a feature useful for suppressing matrix components of a compound and avoiding saturation effects of the detector. If the option **Off** inside the group box **Mode** is not applied either the button **Deflection** or the button **Gating** (available in the linear mode) becomes active.

The matrix deflection process is performed inside the ion source by coupling a pulse to the related deflection plate. The pulse length corresponds to the mass entered into the field **Suppress up to**. The mass entered here specifies the upper mass boundary, below which mass ions are suppressed.

The gating process occurs inside the linear detector by applying fractions of the supply voltage for few µs to the first channel plate of the detector. A couple of ms later the voltage returns back to its nominal value.

flexControl detects instruments that do not have an ion source with deflection electrodes, such as the microflex. The program automatically uses for deflection either the PCIS or PLMS (tandem spectrometers) electrode.

Setting a second reflector voltage (Reflector 2) is required using an ultraflex TOF/TOF spectrometer.

If the check box **set relative** is activated the second reflector voltage can be adjusted in the box **%Refl.** as a percentage of the first reflector voltage. In reflector mode, only the **Deflection** option is available for matrix suppression.

All methods that are used for data acquisition should have a so called valid calibration, i.e. the access to all the entry fields is deactivated. If the user wants to change voltage settings, they must apply the Invalidate Calibration button. Then the message below appears.

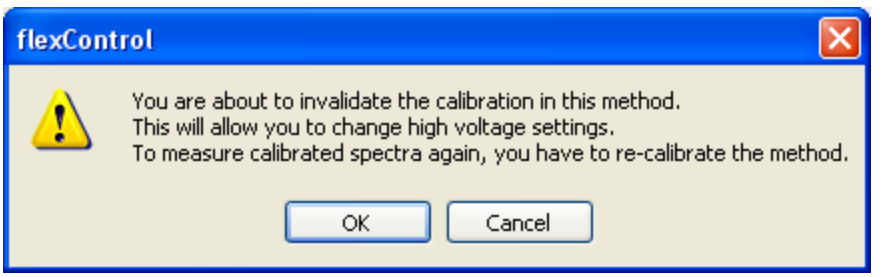


Figure 3-83 Warning

Clicking **OK** releases the edit fields for new voltage adjustments. This action requires a new calibration of the method, that is accepted by clicking the **Apply** button (see section 3.7.6).

In case of a tandem mass spectrometer and a loaded LIFT method the content of the **Spectrometer** page changes (see Figure 3-84). Compared to the previous example (see Figure 3-82) the voltages for the additional two LIFT-electrodes are available.

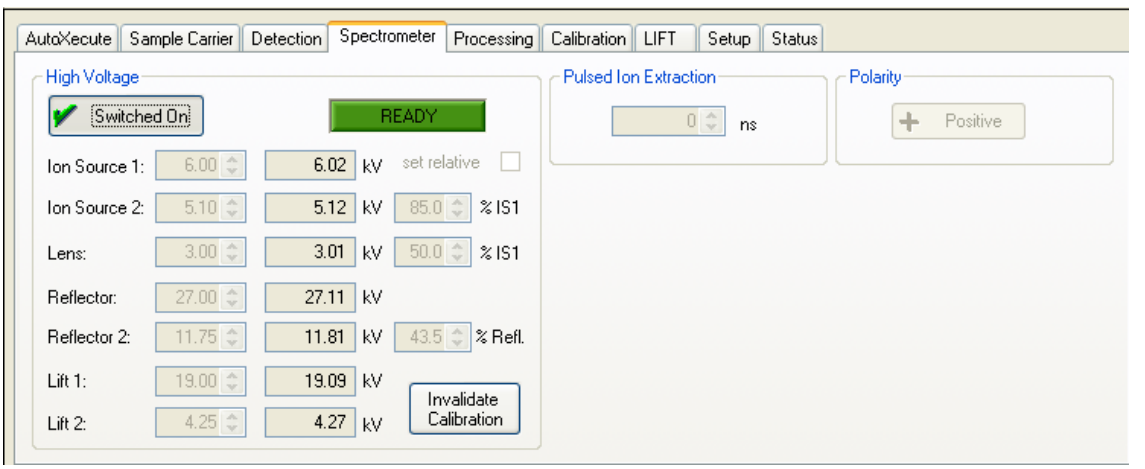


Figure 3-84 Features of the Spectrometer page in case of a LIFT method

3.7.5 Processing

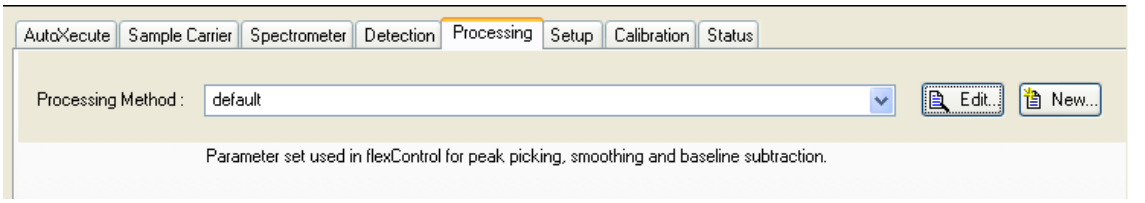


Figure 3-85 The Processing page

The **Processing** page (see Figure 3-85) offers access to the processing parameter methods that contain the currently active peak picking and processing settings. Processing methods (`*.prp`) are used, created and changed from flexControl, flexAnalysis and AutoXecute. If a processing method is edited in one of these programs the changes are immediately available in the other programs.

A Processing method is part of the flexControl method in a way that the flexControl method contains a reference to a Processing method. If a new Processing method is selected, this is detected as change of the FC method that has to be saved then. If a parameter in the current Processing method is changed only the Processing method has to be saved, the FC method does not notice this change.

The processing method that is loaded with a flexControl method defines the peak picking algorithm that is used for manual calibration and annotation in the display, as well as the algorithms and parameters that are used for manual baseline subtraction and smoothing.

When methods created and used with a flexControl version 2.x are loaded the first time in flexControl 3.4, a processing method is automatically created and the old processing parameters are transferred into a processing method. The new parameters get the name of the flexControl method. For more information see the `Quickstart_ProcessingParameter.pdf` on the Compass for flex DVD.

Existing processing methods can be edited and new processing methods can be created on the basis of the pre-installed default method. With the buttons 

the **Processing Method Editor** (Figure 3-86) opens. The section **Mass List > Find** covers the settings for the peak picking algorithm. Smoothing and baseline subtraction can be adjusted in the **Processing** section. The section **Mass List > Edit** is not relevant for flexControl and AutoXecute, but only for flexAnalysis.

Note The processing settings used in flexControl during the data acquisition are not saved with the spectrum and are not automatically available in flexAnalysis afterwards. This parameter set is only used in flexControl. The assignment of special processing parameters to a spectrum has to be done via the flexAnalysis method like this:

1. Create a flexAnalysis method with a reference to your special processing parameter method (see the *flexAnalysis User Manual*).
2. Acquire a spectrum in flexControl and open the **Save As** dialog (3.4.1).
3. Select the special flexAnalysis method **Process with** and save the spectrum.
4. Send the spectrum to flexAnalysis. The assigned method with the respective processing parameters will be applied automatically.

For data acquisition in flexControl and evaluation during automatic runs the recommended peak finder is **Centroid**. Using **SNAP** will only extend the processing time but not improve the spectra quality. **SNAP** should be used later on in flexAnalysis for creating peak lists in the peptide mass range.

Three different smoothing algorithms are offered. Which one is used depends on the application and on the user's experience.

The baseline subtraction algorithm **TopHat** should be used for all spectra except for protein spectra. For this use case **Median** is recommended.

For detailed information on the peak picking, smoothing and baseline subtraction algorithms, see the *flexAnalysis User Manual*.

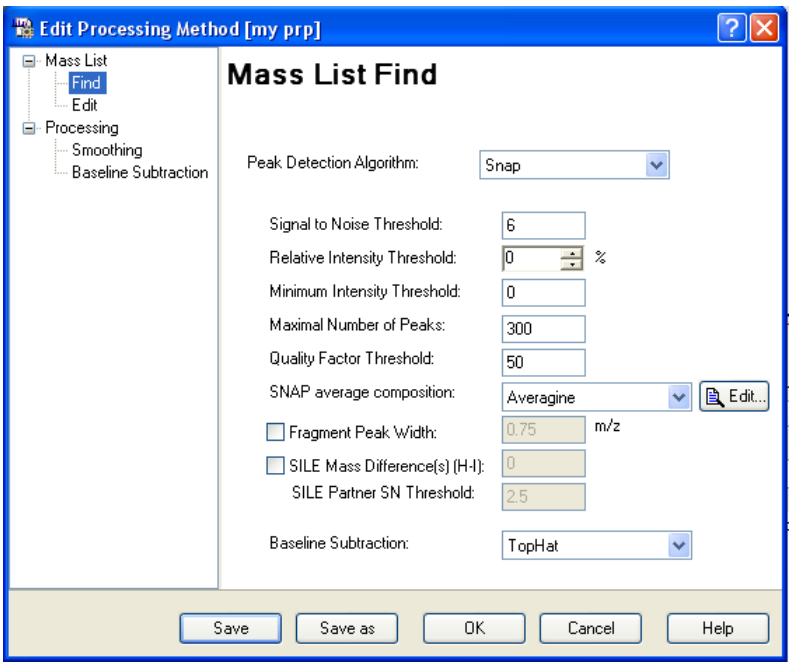


Figure 3-86 Processing Parameter Editor

3.7.6 Calibration (non-LIFT Method)

The **Calibration** dialog (see Figure 3-87) appears in case of a non-LIFT method. The **Calibration** page is used to recalibrate the method. During the calibration, particular masses of the acquired mass spectrum are assigned to reference masses of the loaded calibration list (depending on the calibration strategy).

flexControl offers three calibration strategies:

- **Interactive calibration**, done automatically or manually, using calibration lists (MCLs).
- **Smart**, that is an easy-to-use calibration procedure for optimum results. The program reappraises the statistical frequency of occurrence of calibrants.
- **Statistical calibration** for peptide spectra.

Interactive Calibration

In the **Interactive** mode the operator can choose between automatic (Automatic Assign) or manual (click in the list) assignment of reference mass peaks.

Depending on the mass range (see **Mass Range** slider on the **Detection** tab) **Automatic Assign** uses different peakfinders to create the peak list that is used for the calibration:

a. **SNAP** is used automatically in the mass range up to 6000 Da

b. in the higher mass range the peak finder currently selected in the active processing parameters is used (mostly **Centroid**).

If the masses are assigned manually, the peak detection algorithm that is currently chosen in the **Processing Parameter Editor** is used.

To assign the masses manually to the calibrants, click in the list and afterwards in the spectrum left to the corresponding peak (it is not necessary to hit exactly the top of the peak, just click left to it, the correct peak will be found). Masses are automatically transferred to the calibration list. You may use the scroll wheel of your mouse for further manual assignments.

The calibrant list shows names and masses of known substances, the corresponding masses found in the currently active spectrum, and the error in ppm and in Dalton. The error is re-calculated with each new assignment. The error in Dalton is calculated with $(current\ mass - reference\ mass)$, the error in ppm is calculated with $\frac{(current\ mass - reference\ mass)}{reference\ mass} \times 10^6$.

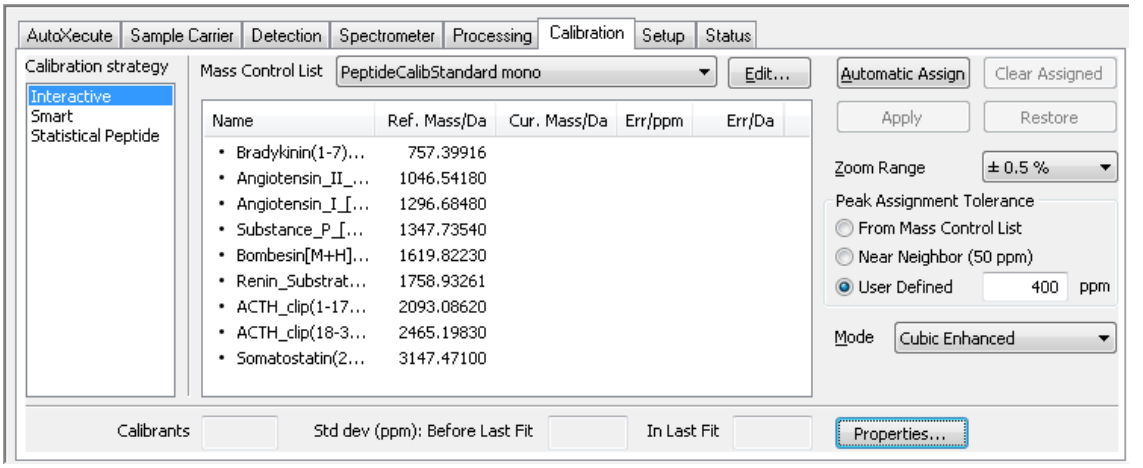


Figure 3-87 Features of the Calibration page

Mass Control List selection: This drop-down menu contains all mass control lists (MCLs) where at least one mass is marked as calibrant. Only the masses marked as calibrants of the selected list are displayed in the box below, the others are invisible in the calibration context.

Button Edit... : Applying this button opens the **Mass Control List Editor** (see Figure 3-88). Calibration lists can be changed and created from here, as well as from AutoXecute from flexAnalysis. Pre-installed lists are write protected but can be used as templates for own user created lists. Whole peak lists (`peak list.xml`) can be imported to use the masses as calibrants.

For more detailed information on mass control lists see `Quickstart_MassControlLists.pdf` on the Compass DVD.

Note If the **Mass Control List Editor** is opened from flexControl it shows only the possibility to mark masses as calibrants (column **Calibrant**). The column **Background** is not offered here, because flexControl only works with calibration lists. If your MCL of interest contains masses that are used as calibrants and as background peaks (activated via AutoXecute or in flexAnalysis), both check marks are active, but the **Background** check mark is invisible (compare section 3.7.1.1, **Evaluation** tab).

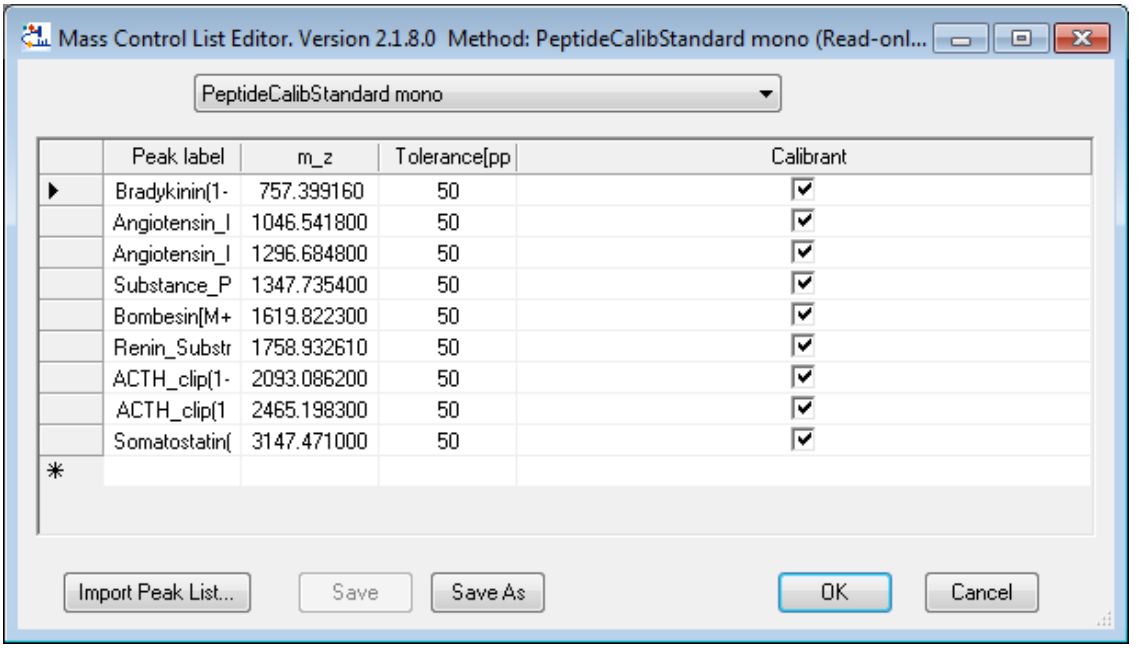


Figure 3-88 Mass Control List Editor

Automatic Assign: Peaks from the spectrum are automatically assigned to the reference masses; a new fit result and errors are calculated but not yet applied to the spectrum. The peak finder algorithm used for automatic calculation is **SNAP** for a mass range < 6000 Da. This value can be changed in the **flex Configuration** dialog (see Figure 3-89). For a mass range > 6000 Da the peak finder algorithm from the processing parameters is used.

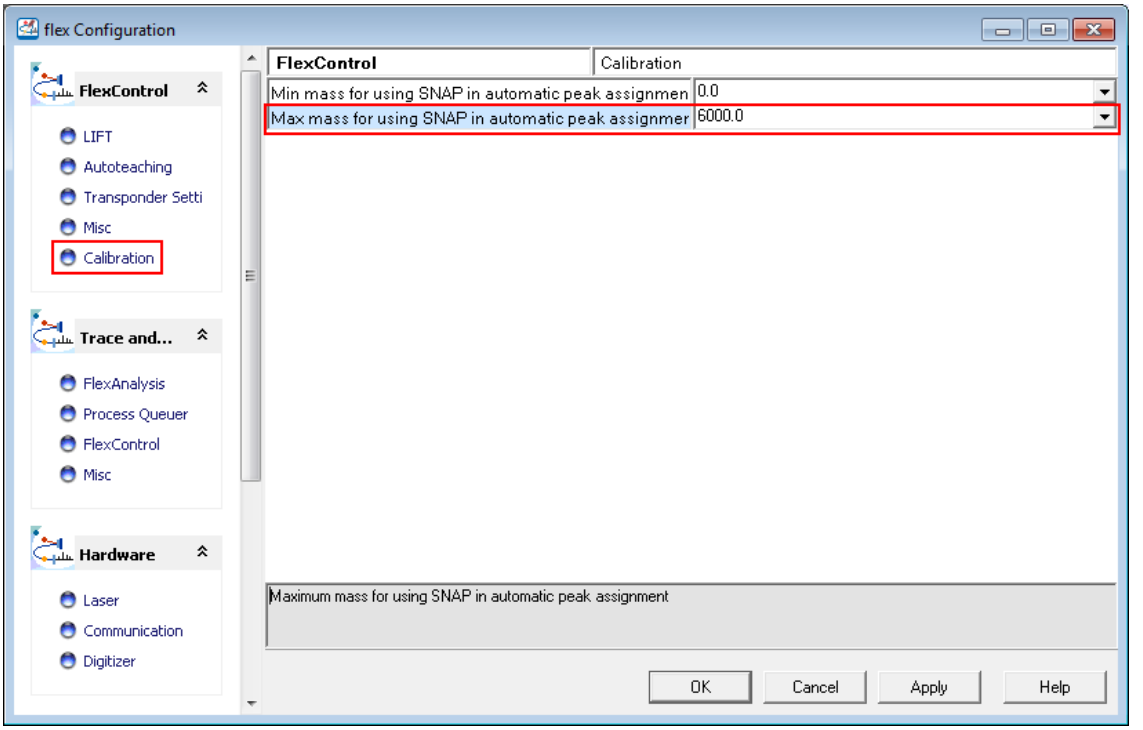


Figure 3-89 flex Configuration dialog

Apply : With a click on this button the new fit result, calculated with the button **Automatic Assign** or manual assignment is applied to the spectrum (the spectrum is moved in the display).

Restore : After the new fit result has been applied with **Calibrate** this can be canceled with the **Undo** button. The original calibration, currently saved in the spectrum, is applied again and peaks are re-annotated.

Clear Assigned : This button deletes the assignments from the calibration list; it does not affect any existing calibration.

Zoom Range off : For manual assignment of masses to reference masses it is necessary to click in the spectrum to the left of the peak of interest. Therefore it is helpful to zoom automatically the respective mass range corresponding to the selected reference mass. It is possible to choose between some relative and absolute zoom ranges.

Note You can use the mouse wheel to select the next reference mass (or to go back to a previous one). This simplifies the manual assignment.

Peak Assignment Tolerance: The tolerance defines a mass interval that is used for the calibrant assignment. If the difference between a reference mass and a peak in the peak list is smaller than the tolerance, the peak is assigned to the calibrant. If more than one peak of the peak list fits this criterion the peak with the smallest mass difference to the calibrant is assigned to the calibrant. Therefore be careful with accepting automatically created calibration results without having checked them!

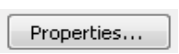
Mode: This drop down box offers the three calibration modes; **linear** (c0, c1, c2=0), **quadratic**, and **cubic enhanced**, as well as the correction mode **linear correction**.

Cubic enhanced is a proprietary Bruker calibration algorithm. In contrast to the classical quadratic approach, it provides three special and additional degrees of freedom for the calibration least square fit. These additional fit coefficients allow matching the instrument function down to sub ppm accuracy.

It should be used whenever the required number of supporting points is available because it will deliver superior accuracy. The **Cubic Enhanced** algorithm should replace the previous **High precision calibration** (HPC) which was used for high accuracy before. In contrast to the HPC, the **cubic enhanced** algorithm needs fewer coefficients but provides better accuracy and better extrapolation at the same time.

Typical use cases for highest precision use one or two internal calibrants. These two supporting points do not allow to locally recalibrate all coefficients. However, readjusting the main coefficient corrects almost all deviations that appear from one spectrum to the next. Therefore, **Linear correction** allows using one or a few supporting points, i.e. internal calibrants, to recalibrate the complete spectrum very accurate. All higher coefficients are kept constant, only the new calibrants are used in a least square fit to realign the dominating coefficient.

Note When the mode **cubic enhanced** is chosen the HPC calibration gets lost and a message box containing this information appears. Just confirm this dialog with **OK**, accept the new calibration and save the method with a new name (no HPC-information)!!



: The **Properties** dialog shows the current state of the calibration and some general calibration information. If spectra have been acquired with a flexControl HPC method the check box **High Precision Calibration applied** at the bottom (read-only check box) is activated.

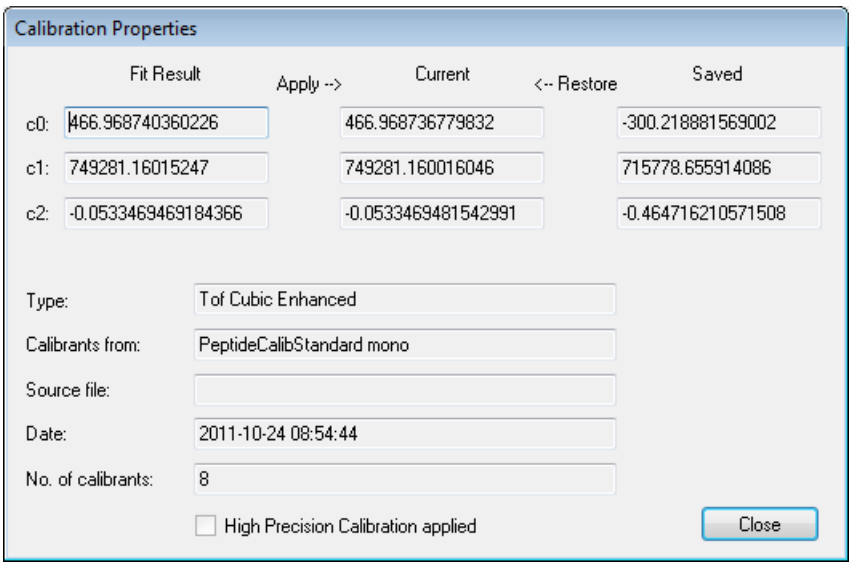
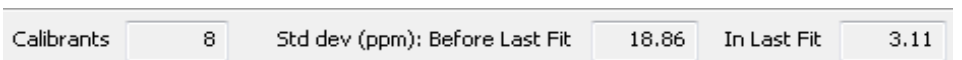


Figure 3-90 Properties dialog (same as in flexAnalysis)

Result Information: Below the calibration list three result fields are shown: the number of used calibrants, the standard deviation before and standard deviation after the last calibration fit.



If no peaks are found during the peak assignment the following message appears.

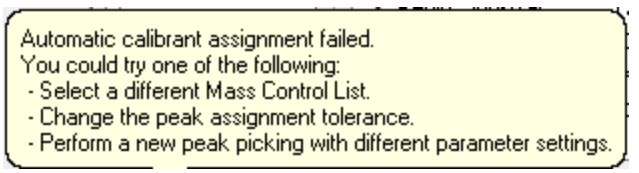
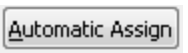


Figure 3-91 Calibration failure

To redo the calibration perform the offered changes or open the processing method and change for example **S/N**. Save and close the method an calibrate again.

►► Short workflow for the Manual Calibration

1. Acquire some spectra containing the masses, which are also calibrants in the selected mass control list and add the spectra to the sum buffer. The peaks must not be saturated!

2. Click the button 

or select a **Zooming Factor** of for example 1% and mark with the left mouse button the first mass from the reference list, which is present in your sample.

   The selected mass is displayed as a red vertical line in the display.

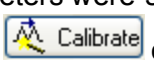
3. Click to the left of the peak that corresponds to the selected mass of the reference mass list.

   The line becomes green and the mass value is labeled at the top of the peak. The **Current Mass** and the **Error** is added to the corresponding column in the mass control list. The corresponding calibration constants **C0** – **C2** are displayed in the **Properties** dialog.

4. Continue with the following masses by using the scroll function of your mouse. This has the advantage that you first may have a look on the actual labeled current mass and scroll then to the next (or the previous) reference mass.

   After assigning a mass, the corresponding row is marked with a check mark.

3.7.7 Automatic MS Recalibration

The automatic MS re-calibration feature performs an automated re-calibration of the current flexControl method. To use this feature, move the sample carrier to a spot with a calibration substance matching the current mass control list, make sure that the parameters were set to reasonable values (see section 3.4.4.7) and click the **Calibrate** button 

on the method selection panel.

The software then performs the automatic MS re-calibration process and displays the results in the autoXecute output window.

►► The automatic MS re-calibration involves the following steps:

1. Acquire a spectrum using the autoXecute method specified in the **Method Calibration Parameters** dialog.

   Optional: Smooth the spectrum.

1. Peak picking using the current processing parameters, some can be overridden by the method calibration parameters.

2. Automatic peak assignment using the mass control list and tolerance from the **Calibration > Property** page.

3. Calculation of new calibration using the algorithm specified on the **Calibration > Property** page.

4. Accept or reject new calibration based on boundary conditions specified in the **Method Calibration Parameters** dialog.

5. Optionally save flexControl method containing new calibration and inform user.

A typical result of the automatic MS re-calibration is shown below (see Figure 3-92).

After the automatic calibration has been done once successfully with a matching selection of parameters (see section 3.4.4.7), these parameters are stored together with the flexControl method. This allows working with different parameters for different flexControl methods, and it is not necessary to adjust them every time again before a new auto-calibration is started.

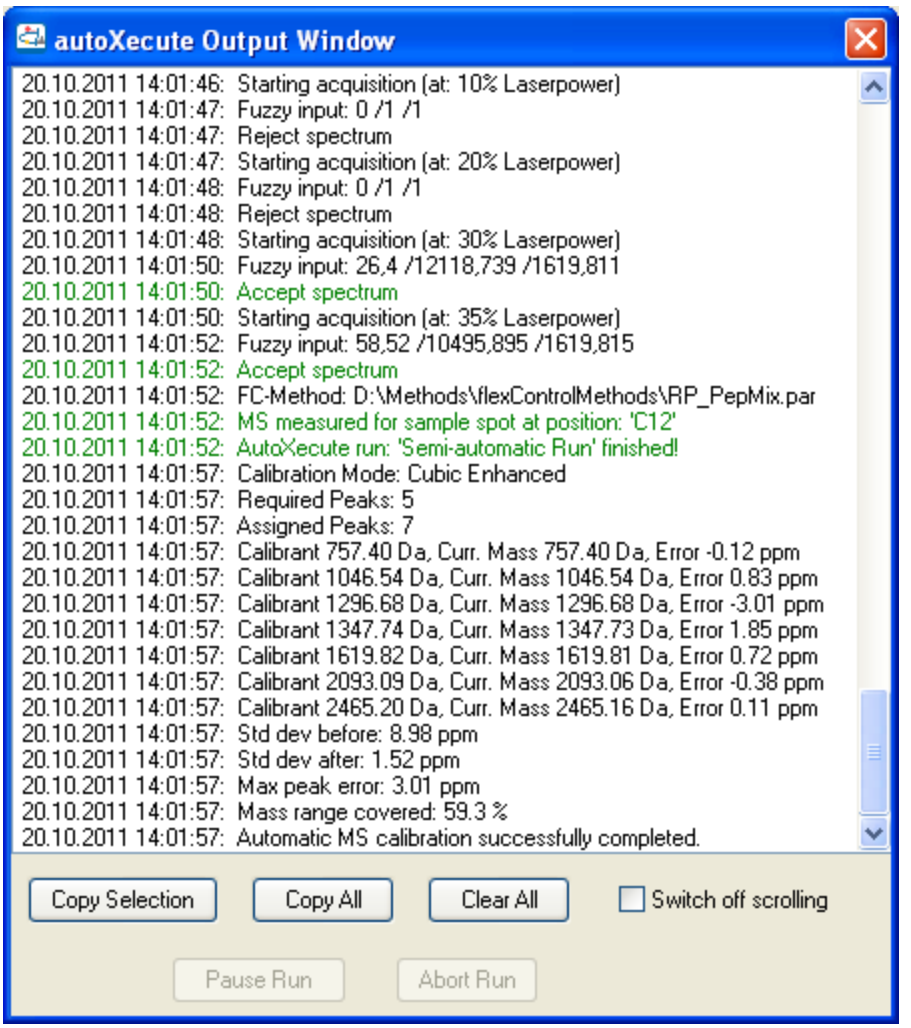


Figure 3-92 Result output for automatic MS re-calibration

3.7.8 Calibration (LIFT Method)

When a LIFT method is loaded the **Calibration** page looks as shown in the following example (see Figure 3-93). Calibration coefficients in LIFT applications are only valid over a small mass range in contrast to coefficients in standard applications, which are valid over a wider mass range. Therefore LIFT applications need several sets of calibration coefficients to cover a wider mass range. It is only necessary to calibrate a LIFT method once, if no changes on the instrument took place. After the calibration it is not possible to change the method, because it is shown in a write protected "valid mode".

Calibration coefficient sets are achieved by calibrating the fragment spectrum of one parent substance, called calibrant in this context. Collecting calibrants together with their associated calibration coefficients is performed by means of the **Calibration** page. This collection is stored in the method file. Thus, each LIFT method gets its particular calibration and spectra acquired with this method are automatically calibrated during the acquisition.

Calibrants additionally carry information concerning the applicability of their calibration coefficients. One part of this information is the mass interval for which this calibrant delivers useful calibration coefficients. The other part determines whether the particular calibrant may currently be used to calibrate a LIFT spectrum.

The most prominent feature of the **Calibration** page is a list either displaying the calibrants of the currently loaded method, or the fragments of a particular calibrant, which are expected to be found in the spectrum. Buttons to handle calibrants are found at the bottom of the **Calibration** page. After one calibrant is selected and set as the LIFT parent mass (double-click or Set As Parent), its name and mass are displayed above the list. On the right of the **Calibration** page two groups of controls are shown. The group box **Select Parent** is activated during the data acquisition and is used to acquire a whole LIFT spectrum of a particular calibrant, and to start calibrating it. The group box **Select Fragment** is used to carry out the calibration process of the LIFT spectrum previously acquired, and is only activated after the data acquisition.

After loading a LIFT method the **Calibration** page appears in the so-called calibrants mode (see Figure 3-93). This mode allows the user to edit the calibrant lists, select a calibrant for calibration, acquire a LIFT spectrum of this calibrant, and begin the calibration process, which will switch this page into the Fragments mode.

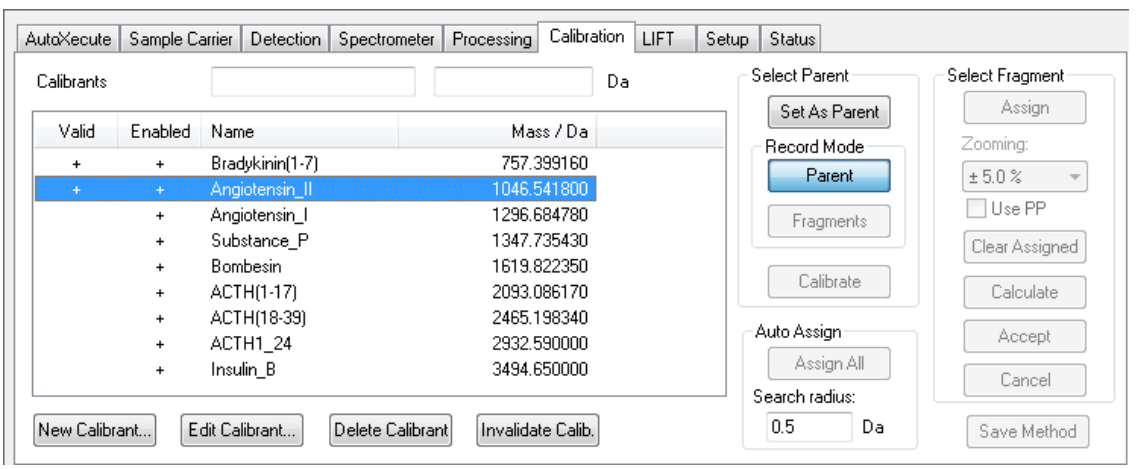


Figure 3-93 LIFT Calibration page in calibrants mode, valid method

In calibrants mode, the columns of the list reflect the properties of a calibrant.

Calibrants: After a calibrant is set as parent mass, its name and mass are displayed here.

Valid: A flag (+) indicates that the calibrant is in a well-calibrated state, i.e., no instrument parameter that influence the LIFT calibration has been changed since the last calibration process of this calibrant.

Enabled: A flag (+) indicates that the operator allows using this calibrant.

Name: This is the name of the calibrant. It does not affect the calibration or the LIFT measurement process.

Mass/Da: This is the mass of the calibrant (parent ion).

The rows are listed in ascending order according to their mass.

When there is a calibrant selected and set as the LIFT parent mass, its name and mass are displayed in the fields above the list.

The four buttons at the bottom of the **Calibration** page (see Figure 3-93) are used for handling calibrants:

New Calibrant... : Opens the **New Calibrant** dialog box (see Figure 3-94) to enter the attributes of a new calibrant, such as name and mass.

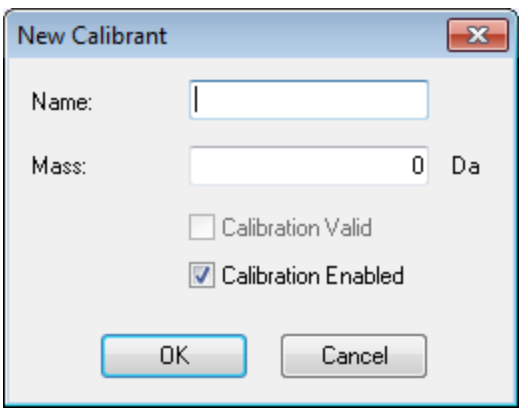


Figure 3-94 New Calibrant dialog

Edit Calibrant... : Opens the **Edit Calibrant** dialog box to edit properties of the selected calibrant (see Figure 3-95).

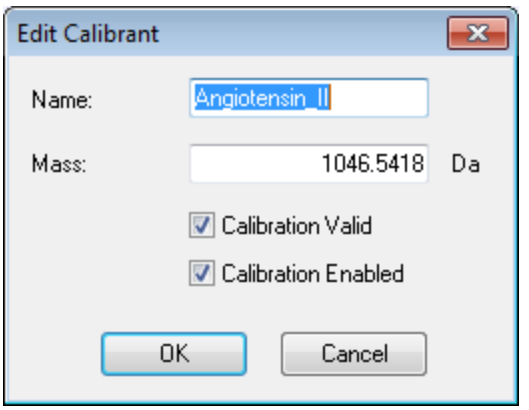


Figure 3-95 Edit Calibrant dialog box

- **Name**: An arbitrary string can be entered.
- **Mass**: No range checking is applied to the mass. The user is responsible for a meaningful value.
- **Calibration Valid**: The user can invalidate a valid calibrant by clearing this check box. In contrast to this, an invalid calibrant cannot be made valid by selecting its **Calibration Valid** check box. It can only be made valid by calibrating it.

Calibration Enabled: Clearing this box temporarily excludes a specific calibrant from a LIFT experiment.

- Delete Calibrant: Deletes the selected calibrant after confirming a warning. Before a calibrant will be deleted, the user must confirm this action in a dialog box (see Figure 3-96).

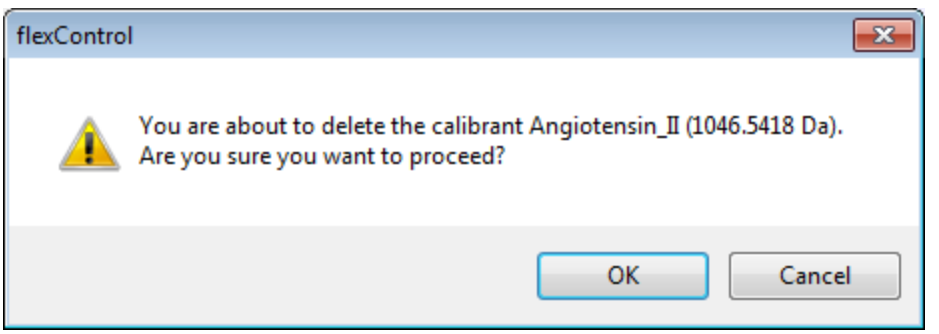


Figure 3-96 Confirming calibrant deletion

When the user confirms deleting, the calibrant will be completely removed. This deletion can only be undone by re-loading the method, discarding any changes since the last load.

- Invalidate Calib. : The state of all calibrants is set to invalid after the user has confirmed this in a dialog box (see Figure 3-97).

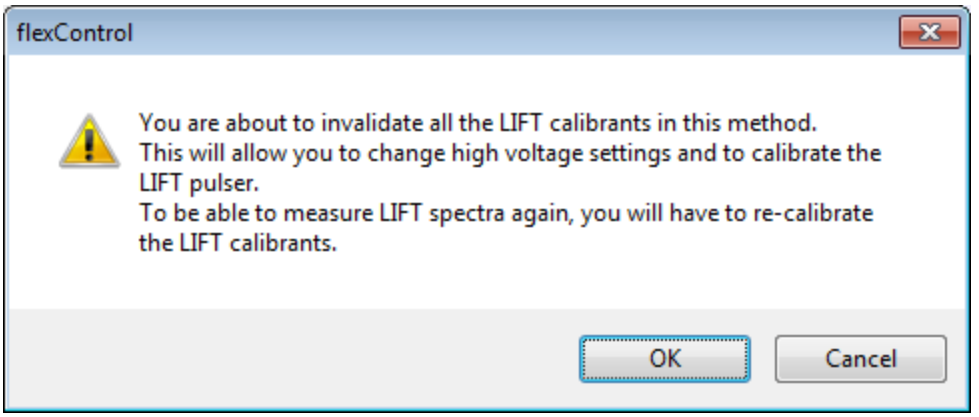


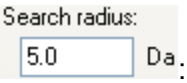
Figure 3-97 Confirming calibrant invalidation

The group box **Select Parent** contains the following features:

- Set As Parent : The mass of the selected calibrant is set as the LIFT parent mass, i.e., the instrument is prepared to acquire a LIFT spectrum of this substance. The same can be achieved by double-clicking an item in the list. This button will only be active if one of the calibrants in the list is selected. When a calibrant has been set as parent, its name and mass will be displayed above the list.
- Parent : This button is active, if a calibrant is set as parent. In this mode the parent spectrum is acquired.
- Fragments : If this button is active the instrument is prepared to measure the fragment spectrum and the LIFT fine calibration is triggered. This button can only be accessed, if a (parent ion) spectrum is present in the sum buffer.
- Calibrate : Clicking this button initiates the actual calibration process by switching the **Calibration** page to Fragments mode. This button is only active, if a complete LIFT spectrum is present in the sum buffer.

- 

: This button can be used after parent and fragment spectra have been summed and you have switched into the calibration mode. All fragments from the LIFT spectrum are assigned at once to the reference masses of the list if they are within the defined search radius.

- 

: Defines the range where the automatic assignment of fragments searches for peaks.

3.7.8.1 Fragments Mode

When the actual calibration process has been initiated, the page will be in Fragments mode (see Figure 3-98). The user can edit the list of fragments, assign peaks in the spectrum displayed to fragment reference masses, calculate a new calibration based on the assigned mass values, and accept or discard the newly calculated calibration, which will switch the page back to calibrants mode.

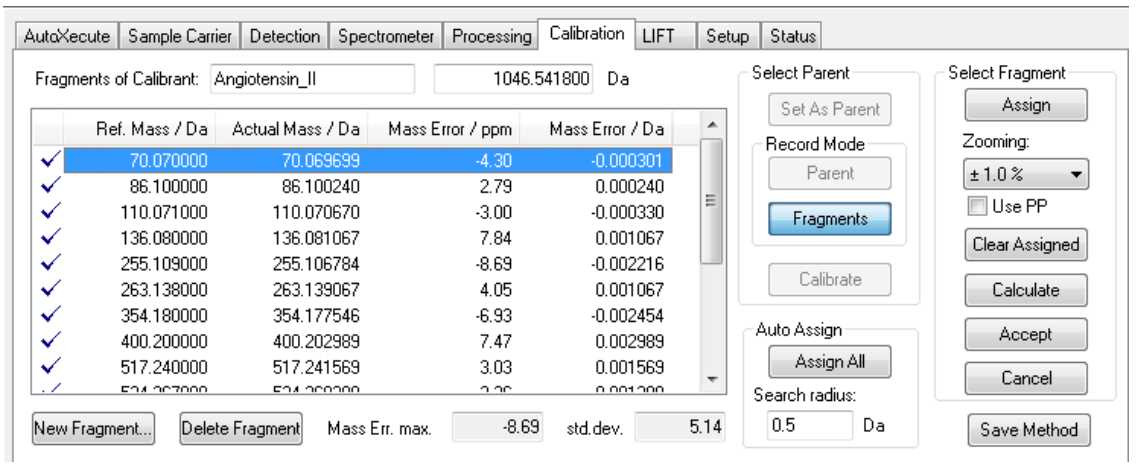


Figure 3-98 LIFT Calibration page in calibrate mode after fragment assignment

- **Fragments of Calibrant**: Contains name and mass of the currently used calibrant to calibrate the instrument.

- **Status**: The symbol left hand side indicates the status of the fragment. A dot means that no actual mass is currently assigned to the particular fragment. An arrowhead indicates that an actual mass is currently being assigned to this

fragment. When a mass assignment exists already for that fragment, the arrowhead is red otherwise it is green. A check mark means that an actual mass is assigned to the fragment.

- **Reference Mass**: This is the literature value of the fragment mass.
- **Actual Mass**: This is the mass of a peak in the spectrum displayed that has been assigned to the fragment.
- **Mass Error**: When a new calibration has been calculated, these columns display the difference between reference mass and actual mass in ppm and Dalton.

Two buttons below the list serve for housekeeping of fragments.

- 

: This button opens a dialog box to enter the mass of a new fragment.
- 

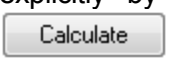
: This button deletes the selected fragment, if the user confirms this action in an appropriate dialog box.

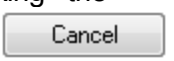
Two fields below the mass list display statistical information regarding the mass error when a new calibration has been calculated. These values help to assess the quality of the calibration.

The group box **Select Fragment** contains control buttons used for the actual calibration process.

- 

: Starts the assign procedure of actual masses to fragments. The button is pressed until assignment is finished. Assignment can either be finished explicitly by clicking the 

button once more, or by clicking 

or 

. When during assignment the end of the list is reached, the procedure is also finished.

Alternatively masses can be assigned to fragments by double-clicking an item in the fragment list.

- **Zooming**: This drop-down list facilitates peak assignment in the Spectrum window. The zooming factor refers either to the respective reference mass, or to absolute mass units. Choosing **off** displays the whole mass range.

- **Use PP**: When this box is selected, click left of a peak in the display to assign it. The peak finder algorithm selected in the processing parameters (see section 3.7.5) is used to calculate the mass. It will appear as **Actual Mass** in the list. When the check box is cleared, the x-axis mass value of the point the user clicked in the spectrum display will be used directly.
- Clear Assigned : Clears all actual mass values and the mass error values. It can be used before starting a new assignment process.
- Calculate : A new calibration (i.e., a set of calibration coefficients) will be performed.
- Accept : Accepts the last calculated calibration and switches the **Calibration** page back to calibrants mode (see Figure 3-93). For storing the updated calibration permanently, the method has to be saved. This can be achieved by clicking the Save Method button.
- Cancel : Any calibration activities are discarded and the **Calibration** page is switched back to calibrants mode.

The button Save Method is activated, when a calibration has been calculated and accepted. This function is the same as that of the command **Save Method** of the **File** menu, or the corresponding feature button on the toolbar.

3.7.8.2 New Fragment

A new fragment can be entered in the **New Fragment** dialog box (see Figure 3-99). No range checking is applied to the entered value. The user is responsible for supplying a meaningful value.

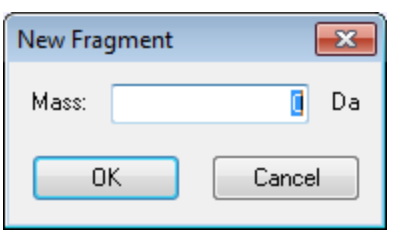


Figure 3-99 New fragment dialog box

3.7.8.3 Delete Fragment

Before a fragment is deleted, the user must confirm this action in a dialog box.

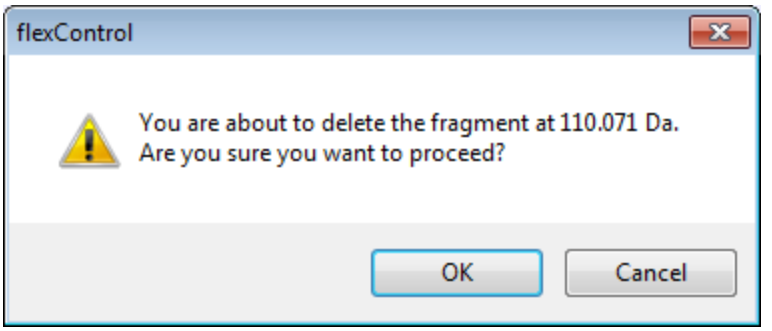


Figure 3-100 Confirm fragment deletion dialog box

When the user confirms deleting, the fragment is removed. This deletion can only be undone by re-loading the method, discarding any changes since the last load.

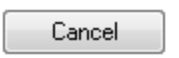
3.7.8.4 Using Buttons and Boxes to Calibrate the Instrument

The aim of this section is to give an idea of the workflow. It cannot supply all the expert knowledge and experience needed to achieve an accurate LIFT calibration over a wide range of parent masses.

Initial conditions

1. A target with the calibrant substance is introduced into the instrument.
2. The calibrants list contains masses of the used substance.
3. The instrument parameters of the loaded LIFT method are suitable to acquire good spectra.

At first, a LIFT spectrum has to be acquired:

1. Switch to the **Calibration** page.
2. If the list contains fragments rather than calibrants, click the 

button in the lower right corner.

3. Choose the first calibrant from the list used for the calibration, and double-click this calibrant in order to set it as LIFT parent mass.

4. Acquire a LIFT spectrum (parent and fragments) of the selected calibrant. The “better” the spectrum (low noise, high peak resolution, etc.), the easier is the calibration.

5. If the spectrum is satisfactory, start the calibration procedure with Calibrate.

6. Click Assign All to assign all fragments at once or Assign to assign the first fragment. The spectrum display will show a red line at the reference mass of the first fragment.

7. To assign the fragments click in the display left of the respective peak. The assigned mass will be entered in the list. Use the mouse wheel to switch to the next fragment. The position of the red line in the spectrum will then be updated to reflect the new fragment mass. If you want you can use peak picking (**Use PP**) for the peak assignment.

8. Repeat this until the end of the list is reached. Assignment will be automatically finished.

9. Click Calculate. Mass errors will be calculated and displayed in the list. The spectrum display will be updated to reflect the modified calibration. Note that at least nine fragments have to be assigned to get access to the button Calculate.

Now it is time for fine-tuning the calibration. For this important part it is possible to delete single fragment assignments with a right-mouse-click that seem to be bad, or with the button Clear Assigned, to remove all actual masses from the list. Select a peak and click Assign to re-assign a different actual mass with it. After finishing click Calculate to update the calibration and the mass error display. If the calibration is acceptable, click Accept and save the updated calibration with Save Method.

3.7.9 LIFT

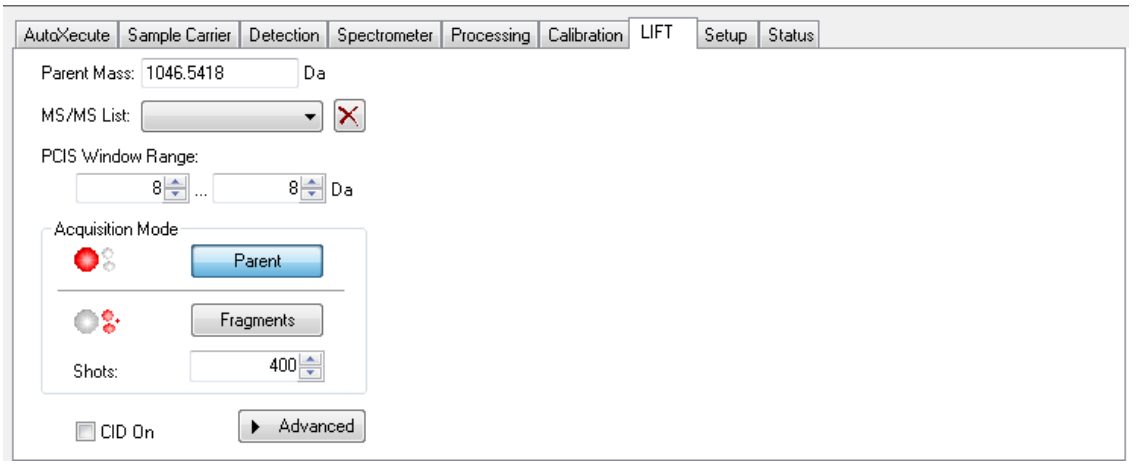


Figure 3-101 Features of the LIFT page

flexControl provides the **LIFT** page (see Figure 3-101) in case of a tandem mass spectrometer and a loaded LIFT method (`<name>.lft`). This page is used to adjust and modify parameters during MS/MS experiments.

Parent Mass: Just as in FAST applications (see section 3.7.10) here the parent mass has to be defined. Default is the mass, which has been entered at end of the last LIFT measurement or during the calibration in the **Calibration** page (see Figure 3-93).

MS/MS List: With this feature it is no longer necessary to type in (or copy and paste) the exact MS masses manually. The MS/MS list (see Figure 3-102) can be filled with masses that have been selected in flexAnalysis from a MS spectrum and added there to the MS/MS list. The complete list can be sent to flexControl (for more details, see the *flexAnalysis User Manual*). Additionally to the mass information the spot information where the MS spectrum has been acquired is shown, since an MS/MS spectrum can only be acquired from the spot where it's MS information comes from. If a mass from this list is selected the respective spot is automatically chosen (the target moves). After the LIFT spectrum is acquired and saved the mass is marked with x (see Figure 3-102) in the list. The list can be deleted by clicking on the cross button.

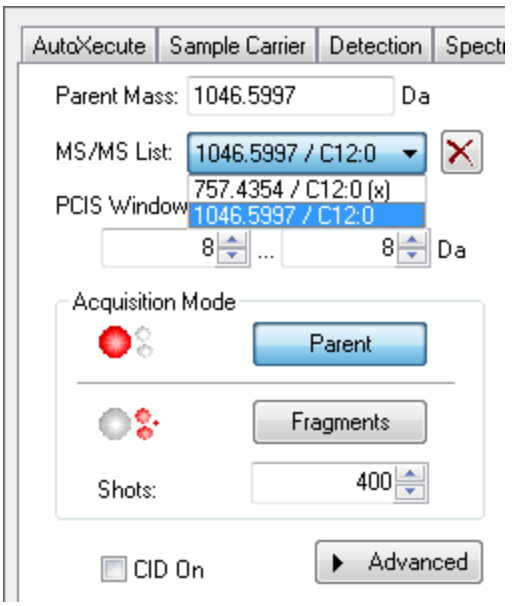
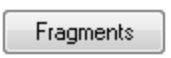


Figure 3-102 List with masses selected for MS/MS measurement

PCIS Window Range: The borders of **PCIS Window Range** insulate the parent mass from other masses. The values preset in **Window Range** depend on the value set in **PCIS Range**. In this example the percentage of ± 0.8% means that the window range of the current precursor mass (1046.54) is ± 8 Da (refer to **PCIS Range** beneath).

Acquisition Mode: On selecting the **LIFT** page the first time the 

button is pressed. This button is the same as that in the **Calibration** page (see Figure 3-93). The instrument is prepared to measure the parent ion spectrum.

The 

button is also the same as that in the **Calibration** page. The instrument is prepared to measure the fragment spectrum and the LIFT fine calibration is triggered.

It is possible to measure **Fragments Only** spectra manually by pressing the 

button. Only in the calibration mode it is necessary to acquire and add a parent ion spectrum to the sum buffer before switching to the fragment mode.

Shots: Enter a number of shots for the fragments measurement mode.

CID On: Checking this box opens a switching valve for argon to flow into the collision cell of the ion source.

CID is an acronym for Collision Induced Dissociation. This term stands for the procedure where molecules get internal energy by collisions with gas molecules during a passage through a particular cell. The internal energy leads to so called metastable fragmentation, what is physically a spontaneous dissociation on a microsecond timescale. CID has proven to be useful to enhance intensities of fragments in the low fragment mass range and to differentiate between isobaric fragments like i/l peptide fragments.

The button 

opens the group box **Advanced Settings**, which contains features to enable and recalibrate LIFT specific features. The accessibility of this group box depends on the Bruker Daltonics UserManagement given rights.

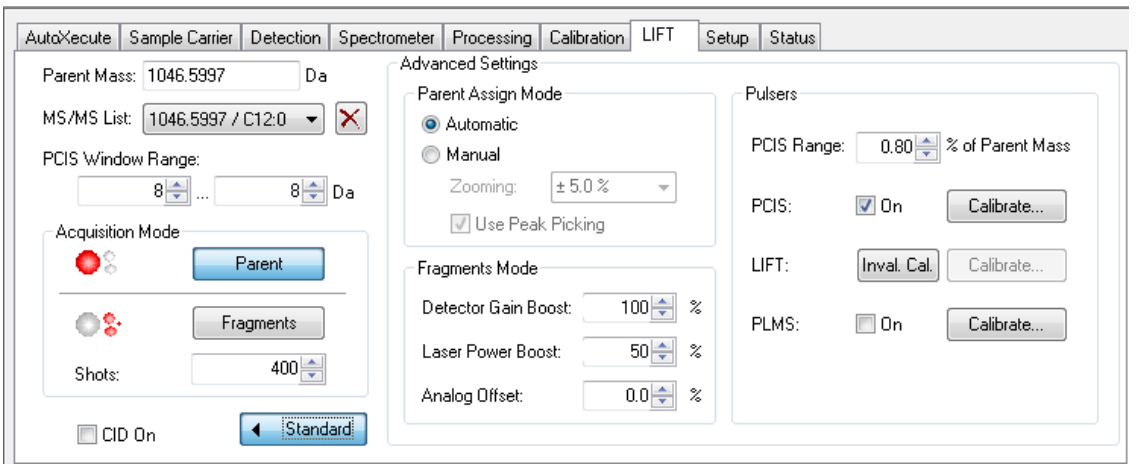
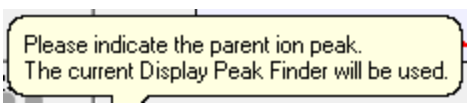


Figure 3-103 LIFT page – advanced mode

Parent Assign Mode: This feature is used to assign the parent mass before switching to the Fragments mode. If it is set to **Automatic** the parent mass is automatically detected, if it is set to **Manual** the parent mass has to be selected in the display (click left of the peak, with **Use Peak Picking** activated). The following dialog appears and must be confirmed with **OK**.



Note This feature applies only to manual LIFT acquisition. During the automatic run the parent is automatically detected, even if **Manual** is selected.

Fragments Mode

- **Detector Gain Boost** is used to enhance the appearance of fragments by increasing the detector gain. Defaults are loaded together with the LIFT method.
- **Laser Power Boost** is also used to enhance the appearance of small fragments now by increasing the laser power. Defaults are loaded together with the LIFT method.
- **Analog Offset** is used to raise the baseline of the digitizer. This may be useful to suppress the background noise of the electronic components, which are involved in guiding signals from the detector to the digitizer. As small fragments may experience a boost in detector gain and laser power, an offset may be set without the risk disappearing in the noise.

PCIS Range: The value entered here specifies the size of the window range around the precursor. The default of 1% should not be changed. But, if this value has been changed enter the precursor value a second time and press the ENTER key. flexControl calculates the new window range.

PCIS: The check box **On** is used to switch the precursor on or off. The button 

opens the dialog **Precursor Ion Selector Calibration** (see Figure 3-104).

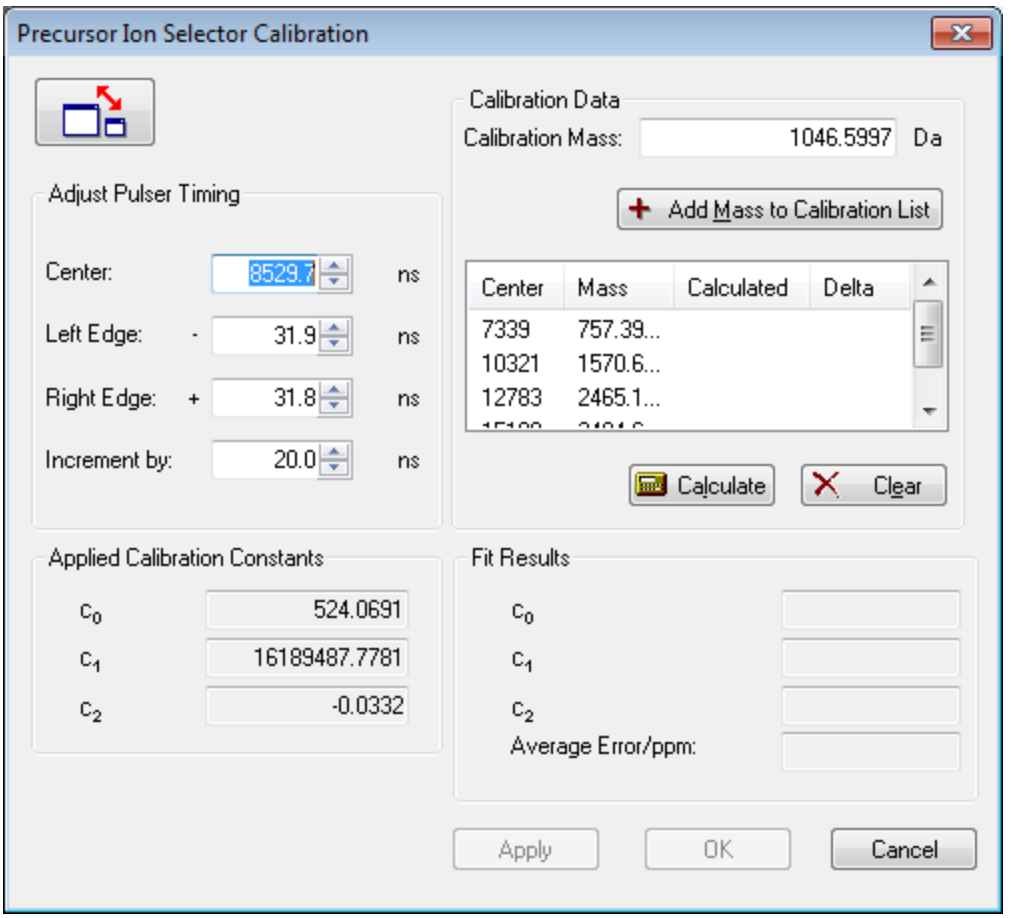
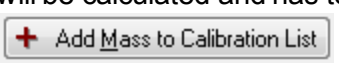
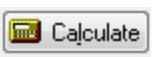
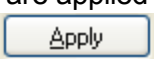


Figure 3-104 Precursor Ion Selector Calibration dialog

If the ion selector has to be calibrated for a specific mass type it in the field **Calibration mass**. The pulser timing will be calculated and has to be adjusted. If a well fitting timing is found press the button 

. The new calibration can now be calculated with the button 

and the constants are displayed in the field **Fit Results**. They are applied and transferred to the field **Applied Calibration Constants** with the button 

.

The button  in the top left of the dialog (see Figure 3-104) is used to reduce the size of this dialog.

LIFT: There is no direct access to a LIFT calibration if you work with a validated LIFT method. The calibration can only be modified after invalidating the current calibration with the button Inval. Cal.. It is recommended to do a new calibration only on the copy of an already calibrated and working method. For acknowledgement press the button **OK** on the corresponding warning message (see Figure 3-105).

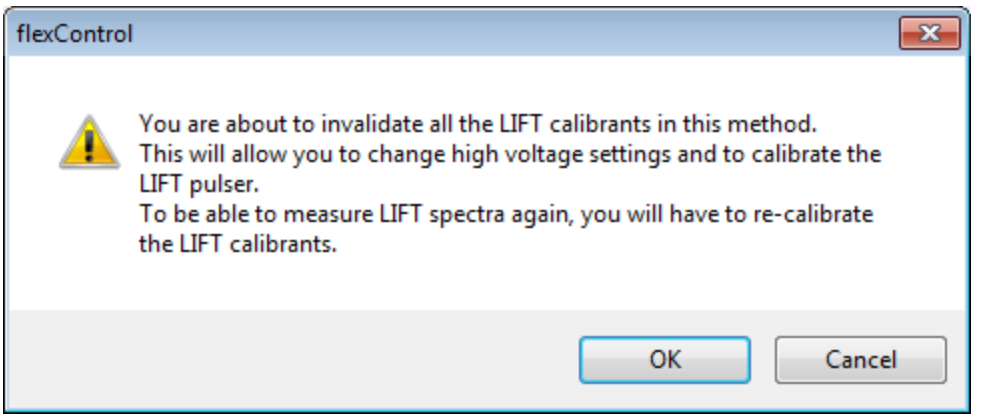


Figure 3-105 Warning before invalidating a LIFT calibration

After that the related button Calibrate... becomes accessible to open the **LIFT Pulser Calibration** dialog (Figure 3-106).

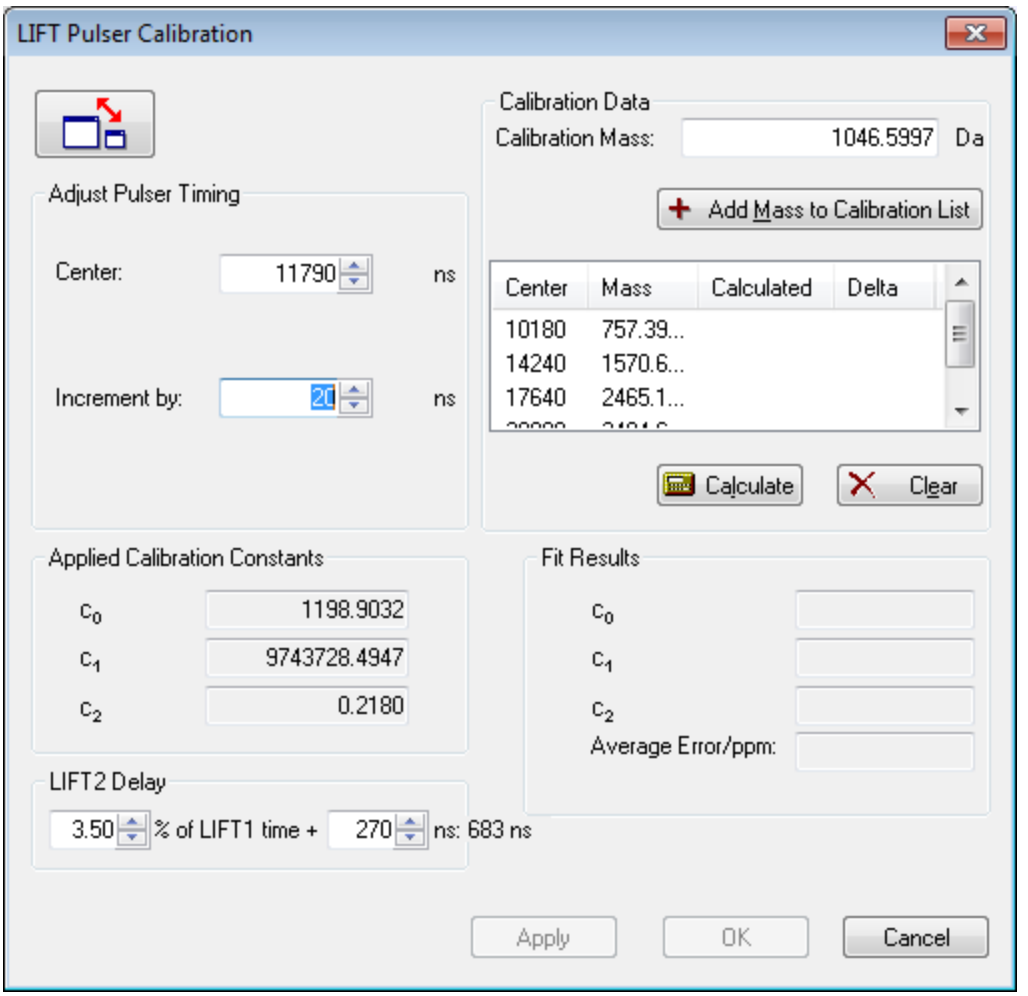


Figure 3-106 LIFT Pulser Calibration dialog

The usage of this dialog corresponds to that of the **Precursor Ion Selector** dialog (Figure 3-104).

PLMS (Post LIFT Metastable Suppressor):
To (de)activate the PLMS function manually use the corresponding check box in the group box **Pulsers** (Figure 3-103). If the check box is selected, PLMS deflects parents formed after LIFT, together with the metastable fragments created after LIFT. As a result only desired fragments are visible. If the check box is cleared, the LID fragments, the huge parent mass and metastable fragments of the parent mass created after LIFT (i.e., Post LIFT Metastable fragment) are additionally visible.

As a consequence MS/MS spectra are recorded in two steps:

1. Check box **PLMS = OFF** together with low laser power: Basically the parent ion is detected, together with only very few LID and post LIFT metastable fragments. The signal recorded in this step is added to the sum buffer. A good resolved and clearly visible parent signal is desired for the later calibration of the MS/MS spectrum.

2. Check box **PLMS = ON**: With increased (boosted) laser power and increased detector gain the fragments are recorded and also added to the sum buffer.

These two steps are automatically performed during the manual and automatic acquisition of LIFT spectra.

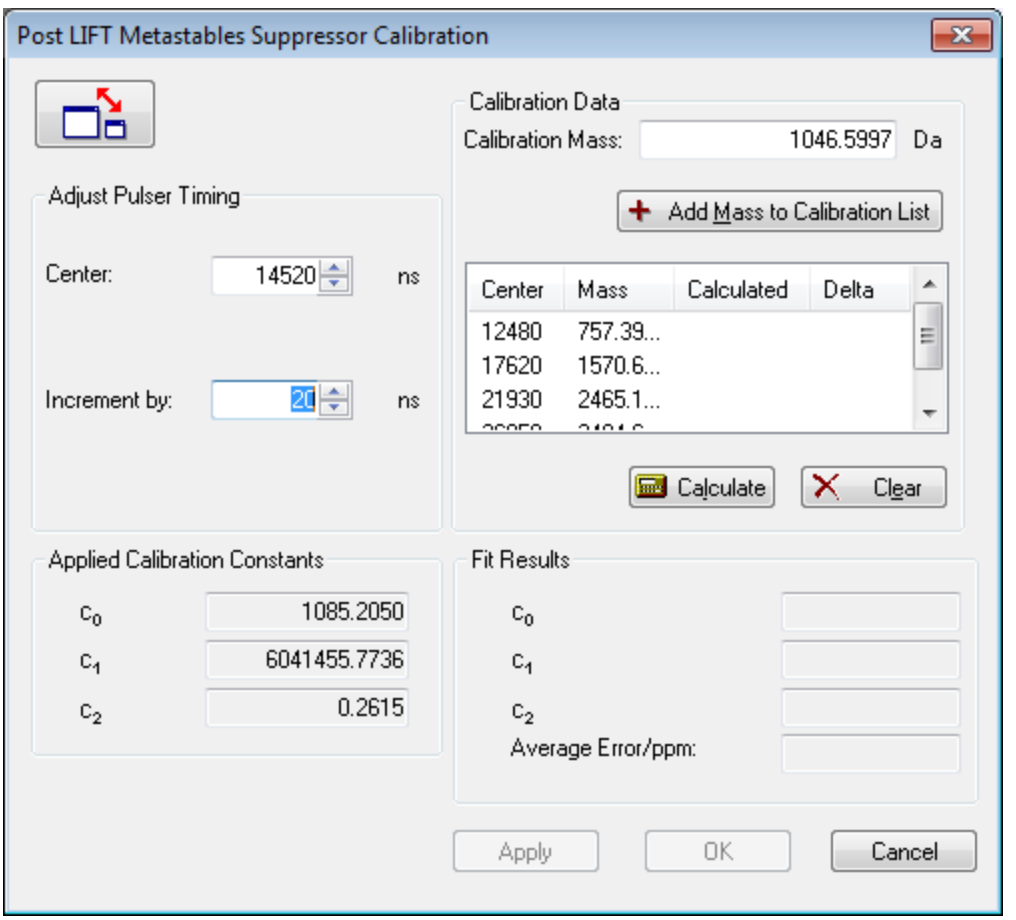


Figure 3-107 Post LIFT Metastables Suppressor Calibration dialog

3.7.10 FAST

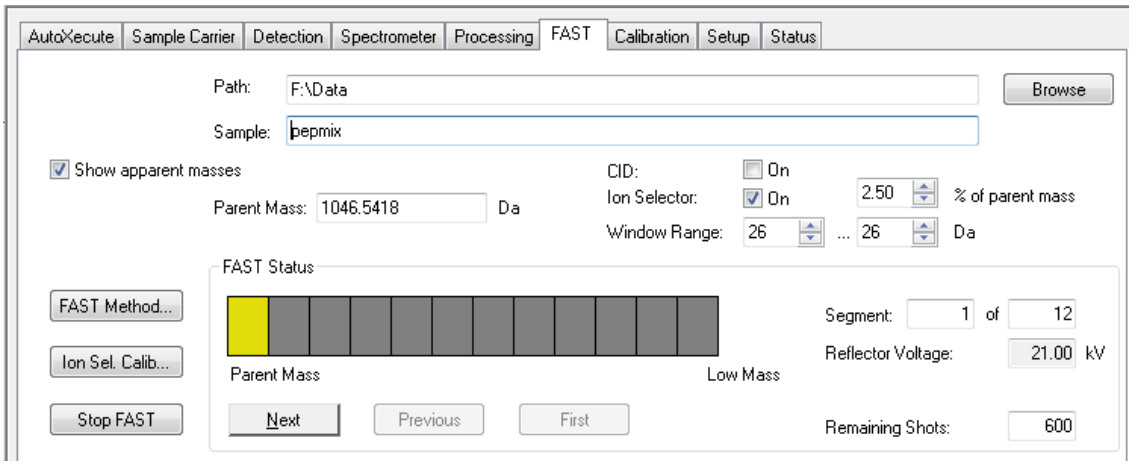


Figure 3-108 Features of the FAST page

Laser-desorbed ions may fragment during the flight in the field-free region. Parent ions and fragments travel with the same velocity side by side through the TOF and would strike the linear detector at the same time. A distinction of parents and fragment ions is not possible. Fragment spectra can only be obtained with a reflector instrument.

flexControl supports four ways to acquire fragment spectra:

1. Manual FAST measurement.
2. Automatic FAST measurement (AutoXecute page).
3. LIFT (**LIFT** page), to record full fragment ion spectra within one single scan.
4. SRM (**SRM** page), to record a specific fragment with reduced reflector potentials.

The FAST (Fragmentation Analysis and Structural TOF) page (see Figure 3-108) appears automatically and only when a FAST method (`<name>.psm`) is loaded. It is used to configure a FAST method for manual and automatic measurements.

During FAST measurements the reflector voltage is stepwise reduced to guide fragments to the reflector detector. Recorded spectra of the fragments can be finally combined to one linearized spectrum.

Compared with a standard acquisition method following parameters are additionally defined in a FAST method:

- Lowest fragment mass (lowest mass to be expected).
- Ion Selector Window settings (window isolating the parent mass).

Each of the following parameters is related to one segment exclusively:

- Total number of laser shots.
- Reflector detector gain.
- FAST high (upper limit of a FAST segment).
- FAST low (lower limit of a FAST segment).
- CID (Collision Induced Dissociation).
- A FAST voltage list (reflector voltage for any FAST segment).

At the top of the FAST page a path and a sample name have to be selected (see Figure 3-108). Choose the path with the **Browse**-button, the sample name has to be typed in. The FAST files are stored in a folder like `<path>\<Sample Name>\spot ID\experiment number\<parent mass>.FAST`. If a folder of the same name already exists, the directory will be extended by a counter, e.g. `experiment number\<parent mass>.FAST_2`.

After a path and a sample name are chosen, the parent mass of the FAST measurement has to be specified. To get only fragments of this ion it has to be isolated by checking the box **Ion Selector**. The mass range of the **Ion Selector** should be small enough to separate the parent mass from all the adjacent ions, which also deliver fragments.

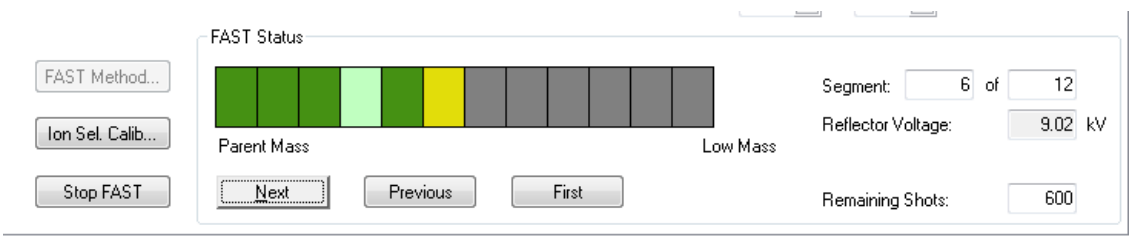


Figure 3-109 Group Box FAST Status

The FAST status indicator displays the current state of the manual FAST measurement. Each rectangle of the indicator represents a FAST segment. The number of the displayed

elements is variable and depends on the *m/z* ratio of the chosen parent mass (on the **FAST** page) and the value of field **Lowest Fragment Mass** in the right upper corner of the **FAST Method Editor** (see Figure 3-110). According to this relation the instrument provides up to 20 FAST segments (maximum).

To distinguish states of the FAST segments they are displayed in different colors. FAST segments, which are not yet processed, are gray. The FAST segment, which is just in progress, is colored yellow. FAST segments having been processed become green if the previously chosen total number of spectra is acquired and stored to the sum buffer.

The user can stop an acquisition at any time. In case of an earlier termination when the pre-selected maximal number of shots is not yet performed the field representing this FAST segment becomes light green in color.

Data storage is performed in a manner that the instrument first saves the shot sequences in the acquisition buffer. The user may add the contents to the sum buffer.

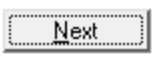
Right of the indicator elements is a group of two fields (view only) showing the number of the (yellow) FAST segment just being measured and the maximum number of elements referring to this measurement. The **Reflector Voltage** field beneath shows the reflector voltage of this active FAST segment. The view only field **Remaining Shots** displays a number of shots still left from the total number of shots previously set in the **Number of Shots for segment** field of the **FAST Method Editor**. Any shot sequence added to the sum buffer reduces the contents of this display by the number of shots associated with this sequence.

The three buttons 

, 

, 

below the FAST segment indicator are used to navigate among the FAST segments for processing.

When applying the button 

the first time the FAST folder mentioned above will be created. The adjacent element (n+1) becomes activated; 

turns to the element processed before (n–1). The button 

activates the leftmost FAST segment. The letter *n* represents the current FAST segment.

Reselecting a FAST segment whose spectra are already stored to disk causes a reload of the data to the sum buffer and displays a spectrum in the related window.

Note Reloading a spectrum sets the instrument in a state specified in the FAST method file! This state might be different to the spectrometer state associated with the reloaded spectrum!

When switching between FAST segments, the spectra in the current segment are saved, the acquisition buffer and sum buffers are cleared, and the parameters specific to the selected segment are loaded.

When the process of the last FAST segment is finished the 

button toggles to 

and the spectrum is automatically saved.

- Check box **Show apparent masses**:

  If this box is selected, the RTD shows the mass spectrum using the MS reflectron calibration. These are sometimes called "apparent" masses, because due to the FAST process, peaks appear at a mass that is different from their actual mass. If this box is not checked, the same data is displayed using the available FAST calibration (and therefore showing correct masses). To see masses of the FAST fragments correctly, leave the box unchecked.

  The "apparent" masses are sometimes useful for alignment or diagnosis, for example, if no valid FAST calibration is available.

Note Due to different calibrations, i.e. conversion from time-of-flight to mass values, the displayed mass range also differs, although the acquired data does not change.

- Check box **CID**:

  Selecting this box opens a valve for guiding collision gas into the collision cell of the ion source.

- Check box **Ion Selector**:

  This box is used to switch the Ion Selector on or off.

3.7.10.1 FAST Method Editor

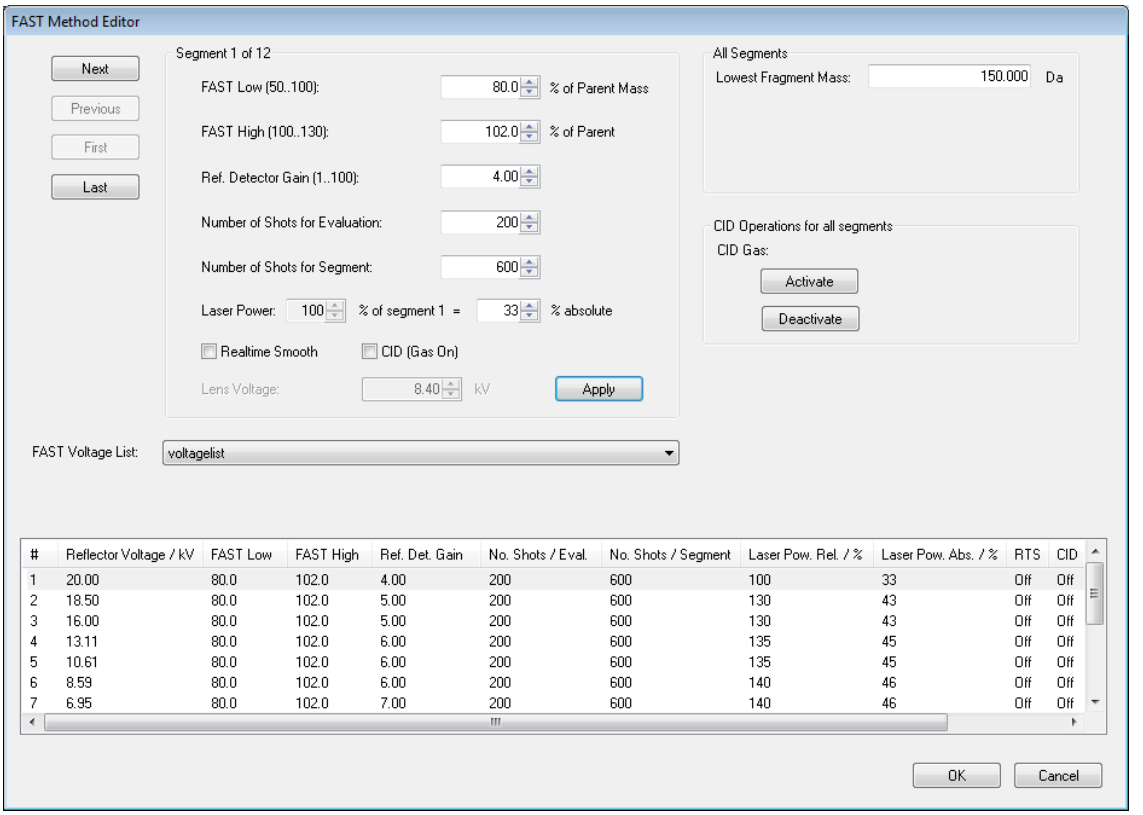


Figure 3-110 Features of the FAST Method Editor

On clicking the button FAST Method... on the **FAST** page (see Figure 3-108) the **FAST Method Editor** window (see Figure 3-110) appears to modify or draft a FAST method.

The four buttons Next, Previous, First, and Last in the left upper corner are used to select rows of the currently loaded method to examine or modify parameters.

Each time on changing a FAST segment, parameters of the previous FAST segment are copied into the list of FAST segment specific parameters. The parameters of the newly activated FAST segment are displayed in the corresponding entry fields. The button Apply is related to the current FAST segment.

The group box **segment *x* of *y*** requires FAST segment specific parameter inputs (The numbers *x* and *y* are variables and vary depending on the *m/z* ratio of parent and fragment masses as explained above).

The fields **FAST Low** and **FAST High** are used to specify the range of a FAST segment where the boundaries should overlap the adjacent segments. For the autoflex defaults are 80% of the parent mass for **FAST Low** and 102% of the parent mass for **FAST High**. Later on the segments can be joined together to a linearized spectrum. The **Ref. Detector Gain** depends on the S/N ratio.

The value in the field **Number of Shots for Evaluation** corresponds to the number of shot per sequence and is updated simultaneously in the field **Shots of the Target Manipulation segment**.

The value in the field **Number of Shots for segment** corresponds to the total number of shots, which are finally the contents of the sum buffer for this specific segment.

flexControl is an universal control software for all the different mass spectrometers of the flex series. Taking into account the different instrument specific hardware, flexControl allows adjusting the total available laser power in proportional steps that is requested for the first segment of a specific instrument.

The function of the check box **Realtime Smooth** corresponds to that one in the **Detection** page (see section 3.7.3). However, in this case it refers to one specific element.

The parameter **Lens Voltage** is the same as that in the **Spectrometer** page. If the **CID** function is selected on the **FAST/LIFT** page the edit field in the dialog becomes activated to set a value different to that previously set on **Spectrometer** page.

The list contains all the editable parameters of the group box **segment** above and the previously selected voltage list. Any row corresponds to a voltage segment; the columns contain the segment specific adjustments. To experiment with parameters select a row that corresponds to the voltage segment you want to manipulate and edit the fields in the group box **segment**. Any parameter change for a voltage segment takes effect with the button 

. For example, when **Realtime Smooth** is selected the corresponding position in the column **RTS** is set to **ON**.

The group box **All segments** contains parameters with a global character, which are therefore valid for the entire FAST spectrum. The first line is used to specify the **Lowest**

Fragment Mass, whose relation to the parent mass corresponds to the displayed number of FAST segments (see Figure 3-109).

On clicking the OK button the values are buffered and the dialog will be closed. The values are not stored in the FAST method file automatically.

3.7.10.2 Performing a Manual FAST Acquisition

1. Select a suitable FAST method (`*.psm`), if not yet loaded with the button Select Method... on the **System Status** segment of the GUI. The page **FAST** immediately appears in the System Configuration segment.

2. Start the acquisition by applying the button Start on the **Acquisition Control** segment of the GUI (Figure 3-42).

3. Add the acquired spectrum to the sum buffer by applying the Add button.

4. Repeat this action until the number of remaining shots in the corresponding field on the lower right of the **FAST** page has become zero. The Next button becomes green in color.

5. Apply the button Next. The color of the first segment on the page FAST changes from yellow to dark green; the second segment becomes active and yellow in color.

6. Repeat steps 2 to 5 until all segments are processed. If the operator is satisfied with a smaller number of added spectra as defined in the method he may press the button Next between two scans. The current voltage segment becomes light green and the color of following segment turns to yellow: it is now active.

7. When all segments have been processed, they all are colored dark or light green. The color of the button Finish changes from gray to dark green.

The button Stop FAST at the left lower corner of the FAST page becomes active after the first segment is completely processed. It may be used to terminate the FAST measurement in between. Before the measurement is terminated, a confirmation message appears (see Figure 3-111).

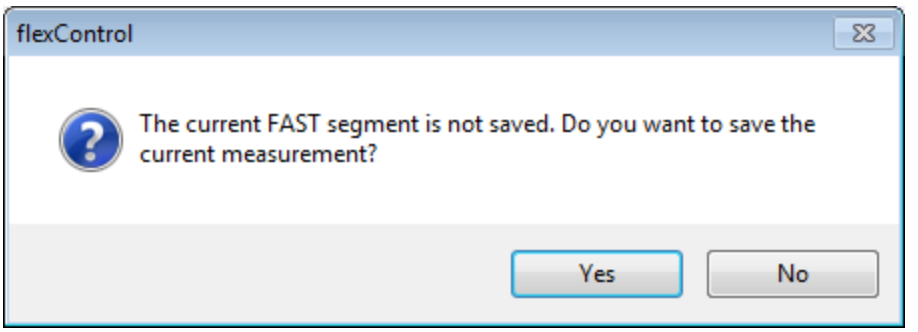


Figure 3-111 Click Yes to terminate

A stop reloads instrument settings referring to the first FAST segment of the run.

3.7.11 SRM

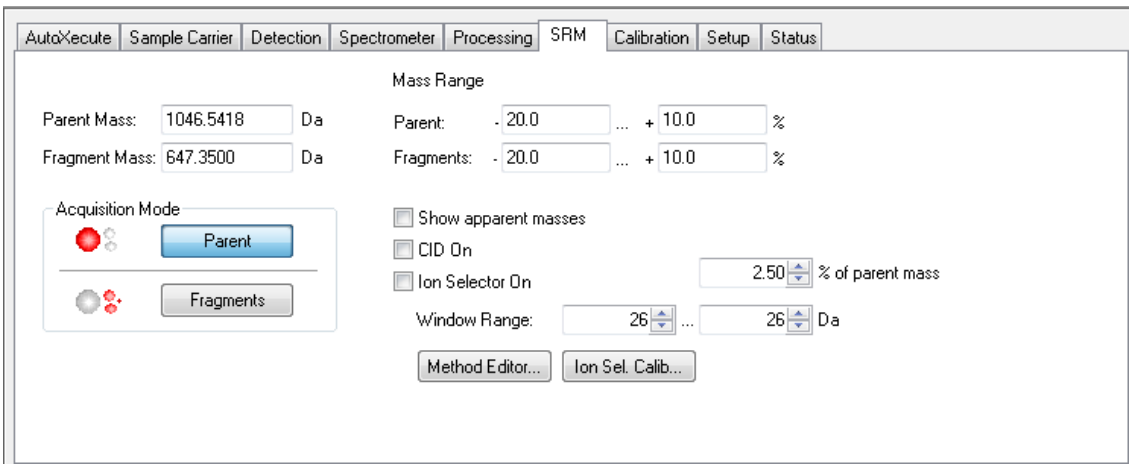


Figure 3-112 Features of the SRM page

The **SRM** (Single Reaction Monitoring) page (see Figure 3-112) appears automatically and only when an SRM method (`<name>.srm`) is loaded. It is used to configure a SRM method for manual and automatic measurements.

During SRM measurements the reflector voltage is reduced to guide fragments to the reflector detector.

Compared with a standard acquisition method following parameters are additionally defined in a SRM method:

- **Fragment mass**.
- **Ion Selector Window** settings (window isolating the parent mass).

Each of the following parameters is related to the fragment exclusively:

- **Total number of laser shots**.
- **Reflector detector gain**.
- **FAST high** (upper limit of a FAST segment).
- **FAST low** (lower limit of a FAST segment).
- **CID** (Collision Induced Dissociation).
- **FAST voltage list** (reflector voltage for any FAST segment).
- **Define Parent Mass**, **Fragment Mass** and the corresponding **Mass Ranges**.

  To get only the fragment of this ion it has to be isolated by checking the box **Ion Selector**. The mass range of the **Ion Selector** should be small enough to separate the parent mass from all the adjacent ions, which also deliver fragments.

- Check box **Show apparent masses** (see Figure 3-112):

  If this box is selected, the RTD shows the mass range and accompanying peaks that are associated with the segment currently in use. Otherwise the entire mass range is displayed.

- Check box **CID**:

  Selecting this box opens a valve for guiding collision gas into the collision cell of the ion source.

- Check box **Ion Selector**:

  This box is used to switch the **Ion Selector** on or off.

- **Window Range** fields: The window range for the **Ion Selector** can be defined with these fields. The borders of **PCIS Window Range** insulate the parent mass from other masses.

3.7.11.1 Method Editor

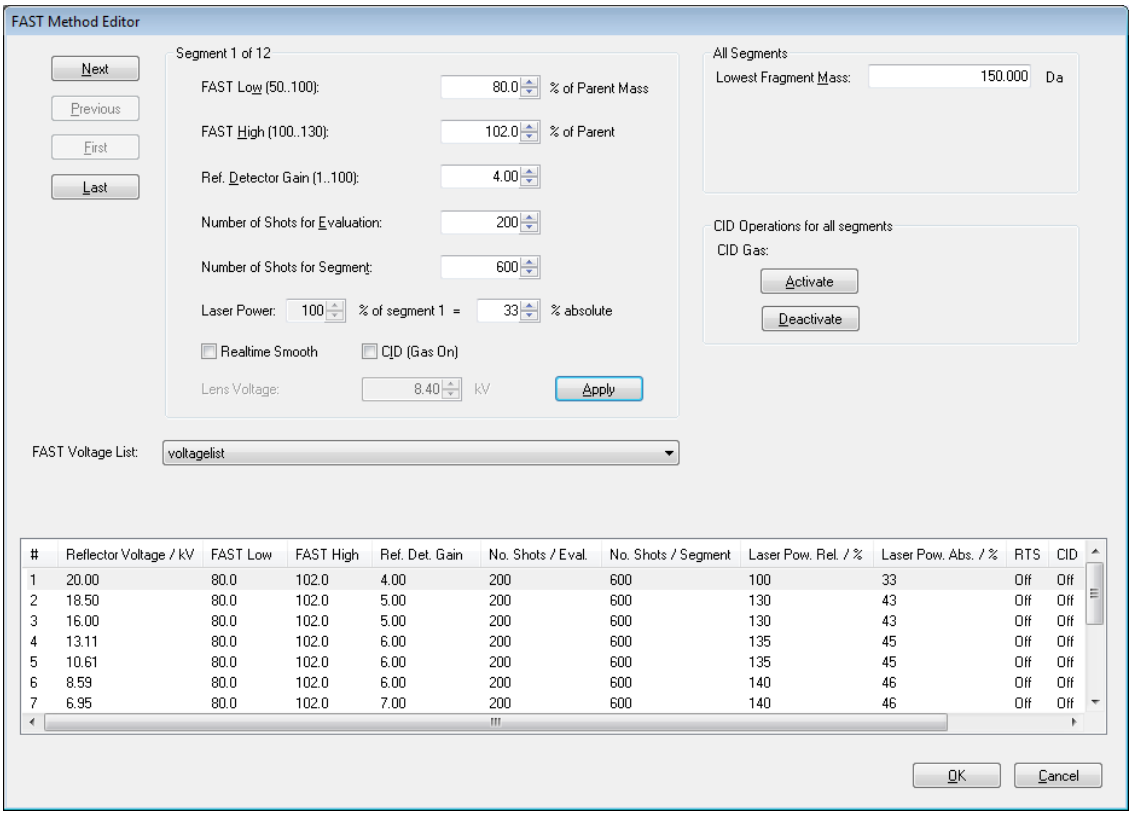


Figure 3-113 Features of the Method Editor

Clicking the button Method Editor... on the **SRM** page (see Figure 3-112) opens the **Method Editor** (see Figure 3-113) for modifying or drafting an SRM method.

The four buttons Next, Previous, First, and Last in the left upper corner are used to select rows of the currently loaded method to examine or modify parameters.

Each time on changing a FAST segment, parameters of the previous FAST segment are copied into the list of FAST segment specific parameters. The parameters of the newly activated FAST segment are displayed in the corresponding entry fields. The button Apply is related to the current FAST segment.

The group box **segment *x* of *y*** requires FAST segment specific parameter inputs (The numbers *x* and *y* are variables and vary depending on the *m/z* ratio of parent and fragment masses).

The fields **FAST Low** and **FAST High** are used to specify the range of a FAST segment where the boundaries should overlap the adjacent segments. For the autoflex defaults are 80% of the parent mass for **FAST Low** and 102% of the parent mass for **FAST High**. Later on the segments can be joined together to a linearized spectrum. The **Ref. Detector Gain** depends on the S/N ratio.

The value in the field **Number of Shots for Evaluation** corresponds to the number of shot per sequence and is updated simultaneously in the field **Shots of the Target Manipulation segment**.

The value edited in the field **Number of Shots for segment** corresponds to the total number of shots, which are finally the contents of the sum buffer for this specific segment.

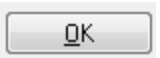
flexControl is an universal control software for all the different mass spectrometers of the flex series. Taking into account the different instrument specific hardware, flexControl allows adjusting the total available laser power in proportional steps that is requested for the first segment of a specific instrument. The function of the check box **Realtime Smooth** corresponds to that one in the **Detection** page (see section 3.7.3). However, in this case it refers to one specific element.

The parameter **Lens Voltage** is the same as that in the **Spectrometer** page. If the **CID** function is selected on the **FAST/LIFT** page the edit field in the dialog becomes activated to set a value different to that previously set on **Spectrometer** page.

The list contains all the editable parameters of the group box **segment** above and the previously selected voltage list. Any row corresponds to a voltage segment; the columns contain the segment specific adjustments. To experiment with parameters select a row that corresponds to the voltage segment you want to manipulate and edit the fields in the group box **segment**. Any parameter change for a voltage segment takes effect with the button 

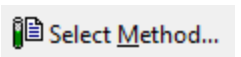
. For example, when **Realtime Smooth** is selected the corresponding position in the column **RTS** is set to **ON**.

The group box **All segments** contains parameters with a global character, which are therefore valid for the entire FAST spectrum. The first line is used to specify the **Lowest Fragment Mass**, whose relation to the parent mass corresponds to the displayed number of FAST segments (see Figure 3-109).

On clicking the 

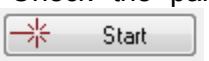
button the values are buffered and the dialog will be closed. The values are not stored in the FAST method file automatically.

3.7.11.2 Performing a Manual SRM Acquisition

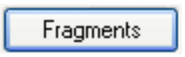
1. Select a suitable SRM method (`*.srm`), if not yet loaded with the button 

on the System Status segment of the GUI. The page **SRM** immediately appears in the System Configuration segment.

2. Enter the parent mass of the ion to be analyzed in the **Parent Mass** field.

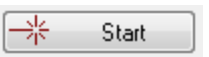
   Optional: Check the parent ion by acquiring a parent spectrum using the 

button on the Acquisition Control segment of the GUI (see Figure 3-42).

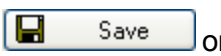
3. Click the 

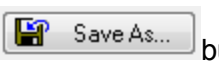
button on the **SRM** page.

4. Enter the fragment mass to be analyzed in the **Fragment Mass** field.

5. Start again the acquisition by clicking the button 

on the **Acquisition Control** segment of the GUI (see Figure 3-42).

6. Save the spectrum with the 

or the 

button.

3.7.12 Setup

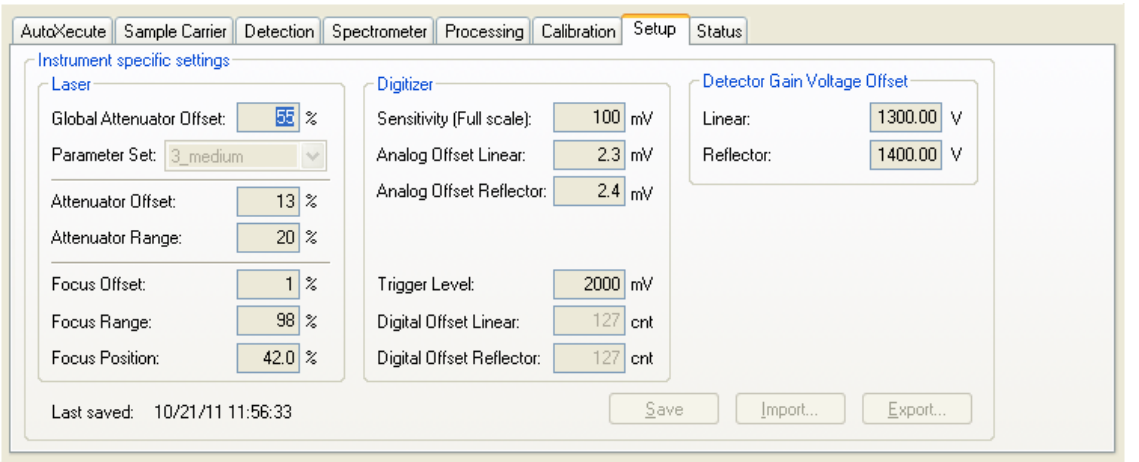


Figure 3-114 Features of the Setup page

The **Setup** page (see Figure 3-114) is used to adjust peripheral equipment, such as detector, laser attenuator and digitizer, etc. Depending on the Bruker Daltonics UserManagement given rights this page is available or unavailable. When available, all controls are read-only on this page. To enable modifications, the service access is required (see section 3.4.6.2).

3.7.12.1 Overview Setup Page

Laser Settings

The offset and range fields define the operating range of the laser attenuator and focus. Normally the values entered in **Offset** and **Range** fix the bounds of a sub range within the range of the total available laser settings. If the system is equipped with a smartbeam laser, multiple parameter sets are available which can have different settings for the attenuator and focus offset and range. The **Parameter Set** drop-down list is used to switch between the settings for the individual sets. Since the **Setup** page is normally deactivated the recommended way to select a different parameter set for a flexControl method is to switch to the **Detection** tab and perform the change there.

The attenuator offset used is generated by adding the contents of **Global Attenuator Offset** field and the **Attenuator Offset** field. The attenuator offset and range are used to define the operating range of the laser slider on the **Acquisition Control** segment (see

Figure 3-42). This restriction allows a finer adjustment of laser power in this portion. In this example the value entered in **Offset** is the lower bound and initial value within this confined operating range of the slider, which corresponds to 68% of the total available laser power. The entered value in **Range** (20%) corresponds to the upper bound of the slider's operating range, which is 88% of the total available laser power. Within this confined range the (relative) laser power can be varied from 0 % to 100 %.

Laser Focus settings

Depending on the laser type this function is active or not. The values for **Offset** and **Range** define also an operating range and **Value** focuses a lens into the optimal position within this predefined range.

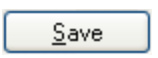
Digitizer Settings

The digitizer is equipped with up to two input channels. One is coupled to the linear detector the second is used for the reflector detector.

The sensitivity for the digitizer inputs is fixed to the value displayed in the **Sensitivity** field. The **Analog Offset** fields are used to adjust the baseline of the acquired spectra.

Detector Gain Voltage Offset Settings

The two reflector voltages correspond to a bias where the amplification factor of each detector is assumed. These values may be adjusted to the experiment. Additionally the voltage for the reflector detector may be changed with the corresponding slider in the **Detection** page (see section 3.7.3).

The **Save** button 

saves the current settings. The software always uses the same file to ensure that all (Windows) users operate with the same instrument settings. Backup copies of the previous setting are automatically created whenever the new settings are saved.

The **Import** button 

can be used to import the settings from a backup copy or from a different file.

The **Export** button 

can be used to save the settings to a different file.

3.7.13 Status

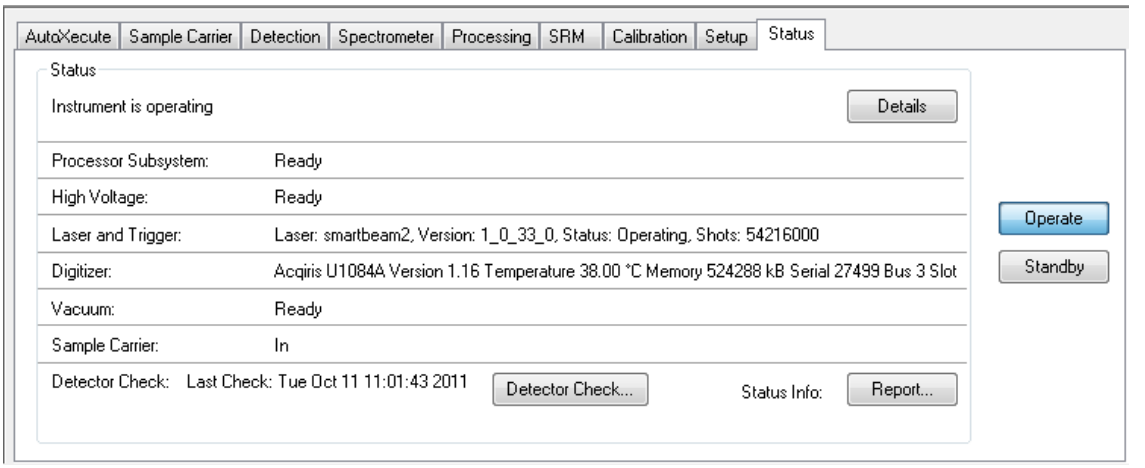


Figure 3-115 Features of the Status page

The **Status** page monitors the mass spectrometer and the attached peripheral equipment, such as the processor subsystem, high voltage, sample carrier, etc. Applying the 

button opens an additional set of pages reflecting detailed instrument states.

Here you can find e.g. the source cleaning maintenance interval bar, which gives you information about when the source of your instrument has to be cleaned.

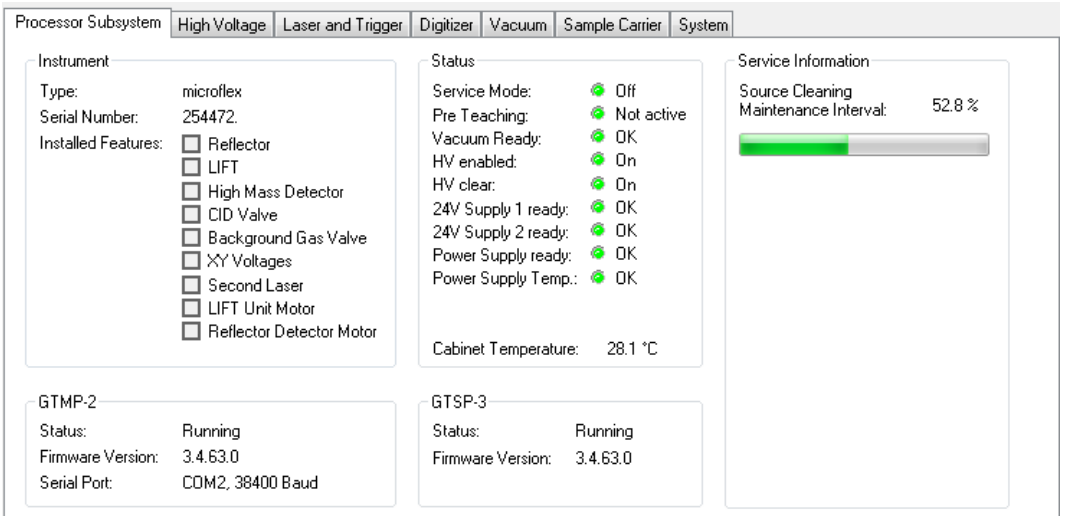


Figure 3-116 Status Details with Source Cleaning Maintenance Interval bar

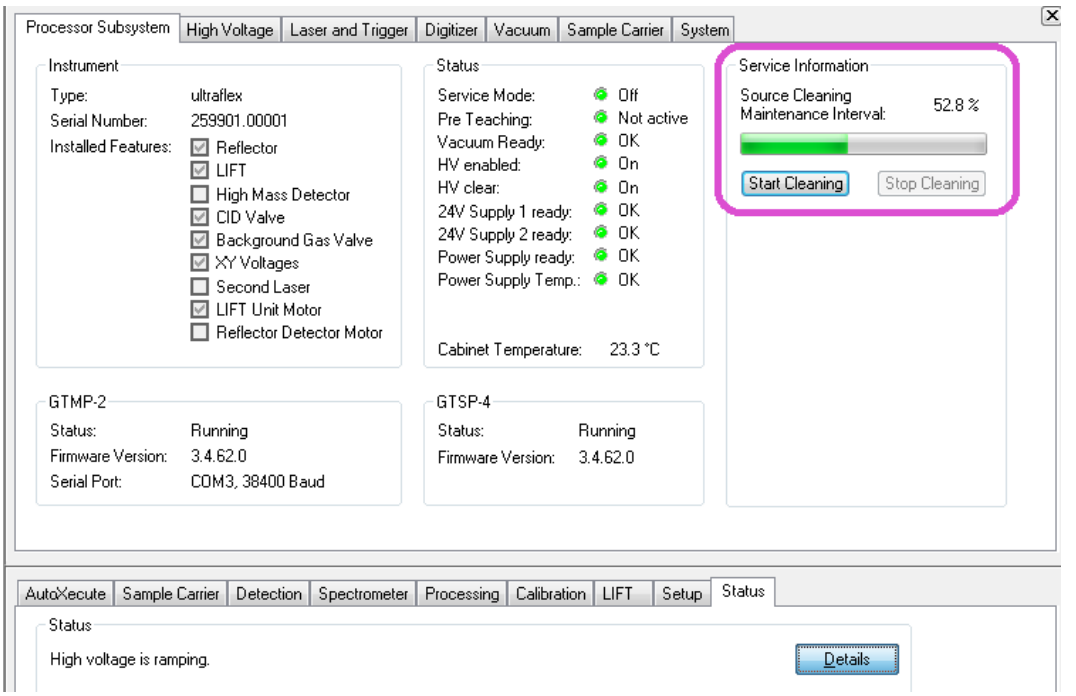


Figure 3-117 Source Cleaning at the ultrafleXtreme

As soon as 80% are reached, you will get a message, which either asks you to clean the source or offers you to start the cleaning procedure. Depending on the connected

instrument, the cleaning of the source must be fulfilled in different ways: For autoflex and ultraflex I-III it is done by using the Source Shower target, a message pops up to remind you that cleaning will be necessary (see Figure 3-118).

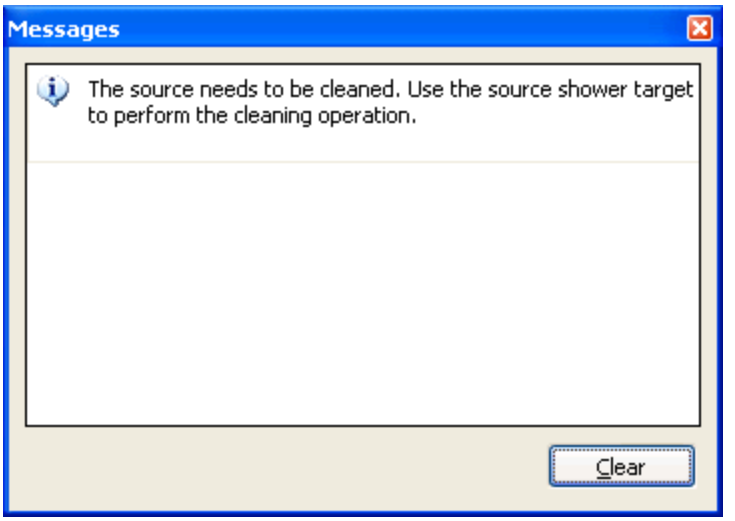


Figure 3-118 Source Cleaning Maintenance Interval >80% (e.g. autoflex)

For further information on using the Source Shower target, please refer to the *Source Shower Target Manual*.

For the ultrafleXtreme and the Autoflex Speed an automatic cleaning procedure is implemented. This procedure can either be started by confirming the cleaning source message or manually by pressing the button Start Cleaning (see Figure 3-117). The procedure takes approximately 20 minutes.

On the right side of the status page, there are the two buttons Operate and Standby to change the status of the mass spectrometer from the operation mode into the stand by mode and vice versa.

Applying the Standby button right hand, the high voltage, the camera, the lamp, the laser, and the digitizer are switched off. Additionally following message appears:

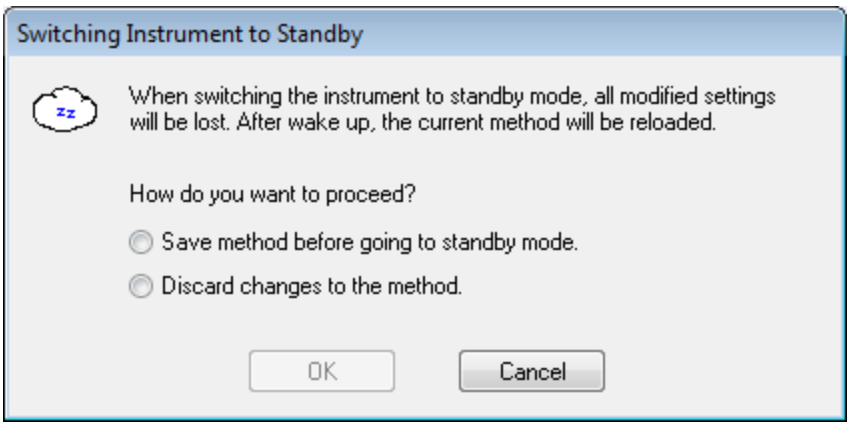


Figure 3-119 Message asking how to continue

The  button opens the **Status Reporter** (see section 3.4.6.3).

The  button opens the **Detector Check** (see section 3.7.14).

3.7.14 Detector Check

DetectorCheck is a software tool used to measure detector gain curves in linear and reflectron mode. The tool also calculates detector voltage values required to obtain certain single ion response values.

Using this tool enables long-term monitoring of detector performance and assists users in compensating for detector aging. This is achieved by readjusting the detector voltage to obtain a constant detector gain in their projects over time.

Clicking **Start** triggers a fully automated five-minute test sequence consisting of:

- loading all instrument settings and methods needed for the test
- optimization of the detector offset and the laser power
- measuring the detector gain in linear and reflectron mode
- restoring the original instrument settings / methods.

It is recommended that the test is run on a monthly schedule and before important projects.

3.7.14.1 DetectorCheck Workflow

3.7.14.1.1 Performing the Test Sequence

1. Insert a MALDI target with prepared HCCA matrix spots into your instrument.

   Use of Bruker Prespotted AnchorChip targets (Part No. # 227463 or # 231968) is recommended for Ultraflex/Autoflex instruments. These targets offer the highest preparation reproducibility and convenience of use because they are precoated with HCCA matrix and therefore no sample preparation is needed to perform this test.

   Note For microflex instruments we recommend to prepare HCCA matrix (Bruker part No. # 201344) saturated in ACN:0.1%TFA (1:2) onto a stainless steel target. A consistent result history can only be achieved when the same matrix preparation is used for all subsequent tests.

2. Teach the sample carrier spot positions and select a fresh matrix spot for the test.

   The teaching procedure is described in flexControl Help (see section 3.4.6). The teaching is considered successful when a selected fresh spot is centered in the cross hairs of the flexControl video window.

3. Start the DetectorCheck test procedure:

   On the flexControl **Setup** tab select **Detector Check** and press the **Start Check** button.

The whole test procedure runs fully automatically. During the measurement (indicated by the progress bar) the detector gain in linear and reflectron mode is measured. You can stop the test at any time by clicking on the **Cancel** button. After approximately five minutes the test is complete and the instrument reloads all previous methods / settings.

It is recommended that the test is run on a monthly schedule and before important projects.

3.7.14.1.2 Displaying the Results

Immediately after the measurement has finished the result is shown on the Detector Check window. The upper graph shows the detector gain in linear and reflector mode, i.e. the response to single ion impact plotted against detector voltage. Due to the nature of the electron amplification process in the detector, the graph in this plot should be straight lines.

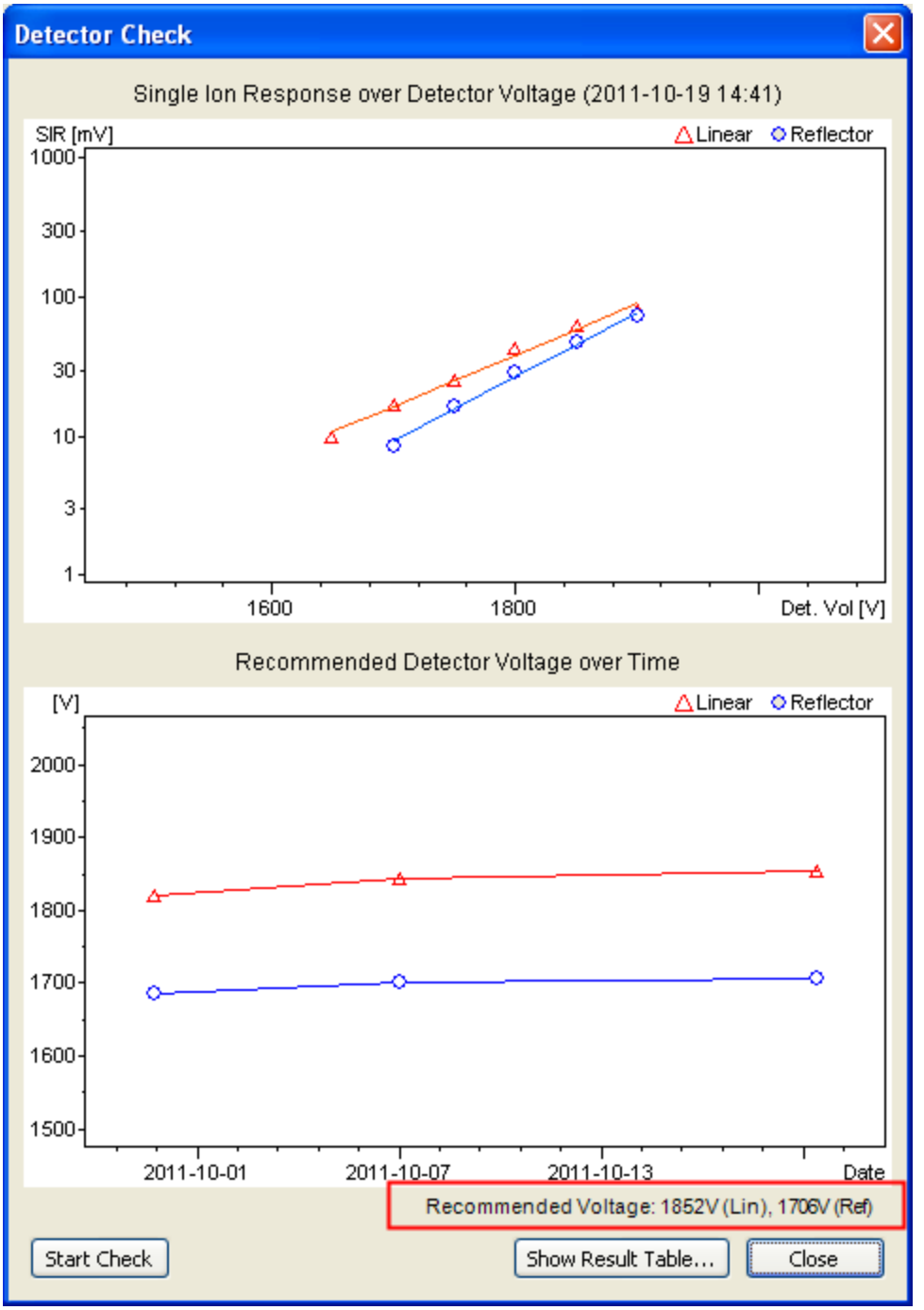


Figure 3-120 Results of the detector check procedure

The result history of all tests run on your instrument is shown in the lower graph. The two curves represent the **recommended detector voltages** to obtain good spectra for small molecules/peptides in reflectron mode and for proteins in linear mode. Detector aging is indicated by a rising curve: When the detector performance decreases, a higher voltage is required for the same response.

The actual recommended voltage values are given below the graph

The result history is also archived in the result table (press **Show Result Table**).

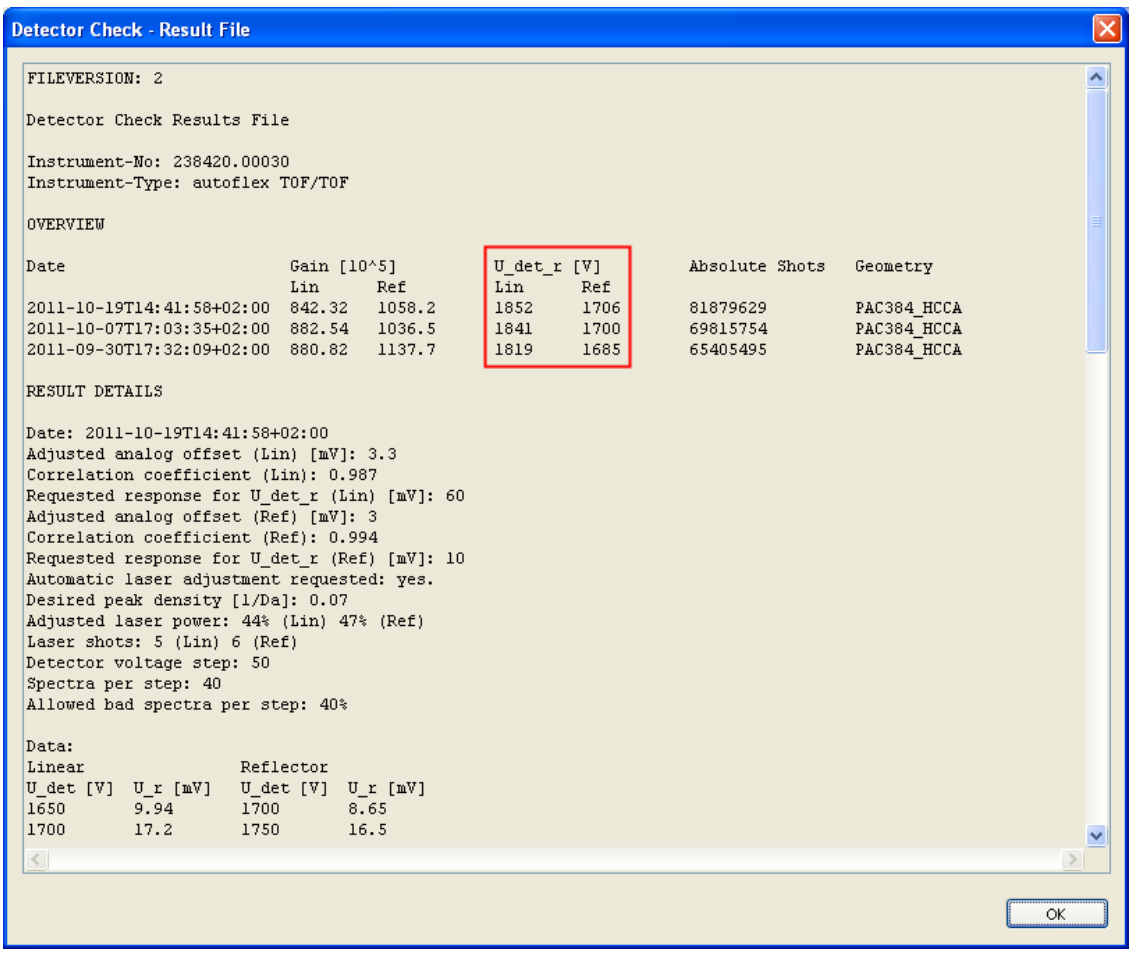


Figure 3-121 Detector check result file

3.7.14.1.3 Achieving Long-Term Detector Response Stability

The main benefit of the Detector Check tool is providing a track record of detector response, enabling users to maintain a stable response even if the detector performance degrades marginally over time.

This long-term stability can easily be achieved by periodically readjusting the detector voltage in your flexControl methods according to Detector Check tool results:

1. Load the flexControl method to be readjusted.
2. Click the **Detection** tab and take note of the actual detector voltage shown on the right side of the detector gain slider.
3. If the actual detector voltage and the recommended detector voltage (as determined by the DetectorCheck tool) differ by more than 30 V, move the **Detector Gain** slider to the recommended value.

   Note If the flexControl method is a linear (reflector) method apply the respective linear (reflector) value of the recommended detector voltage.

4. Save the flexControl method.

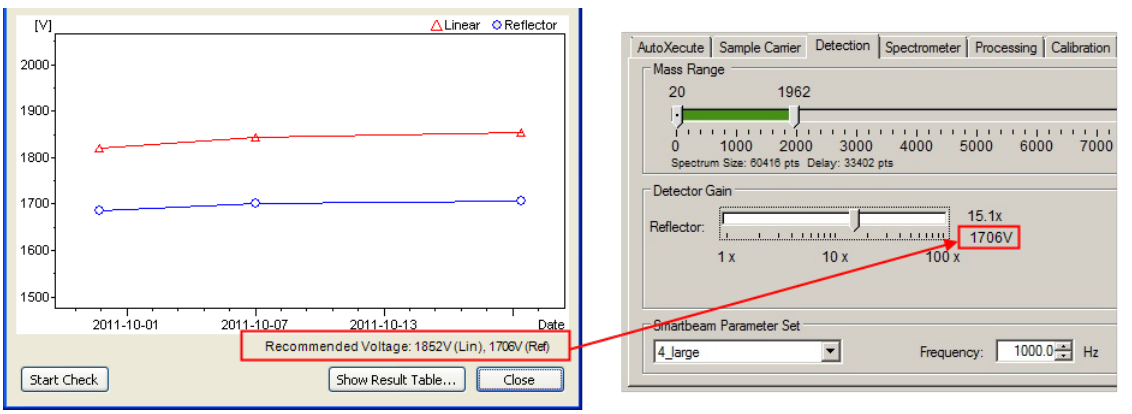


Figure 3-122 Adjusting the Detector Gain

Appendix A — Appendix

A.1 WARP feedback strategy with BioTools

The following workflow gives a short overview of the AutoXecute WARP feedback strategy that is used in combination with BioTools.

1. Select the WARP strategy BioTools in the **AutoXecute settings** dialog (see Figure 3-71) on the **AutoXecute** tab in flexControl and restart flexControl.

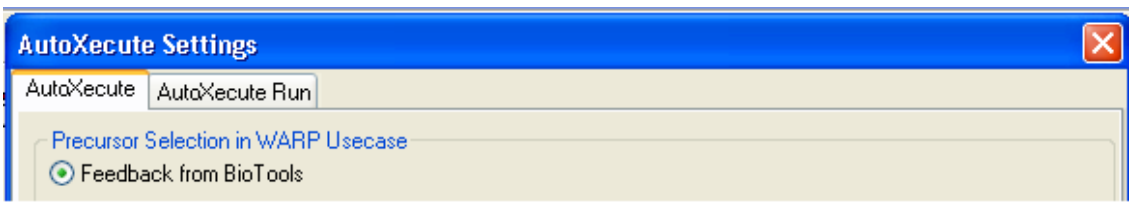


Figure A-1 Feedback from BioTools option

2. Setup an AutoXecute run with MS and MS/MS measurement.
3. Make sure that the MS spectra will be sent to BioTools after post processing in flexAnalysis, i.e. select a BioTools method in the AutoXecute MS method (**Processing** page (see Figure 3-60) or the AutoXecute run (see section 3.7.1.4)).
4. Make sure that **WARP** is selected in the **MS/MS** tab of the AutoXecute MS/MS method (see Figure 3-61). Otherwise the information BioTools adds to the peak list will be ignored!
5. Make sure that the BioTools Feedback Parameters are selected correctly in the used BioTools MS method:

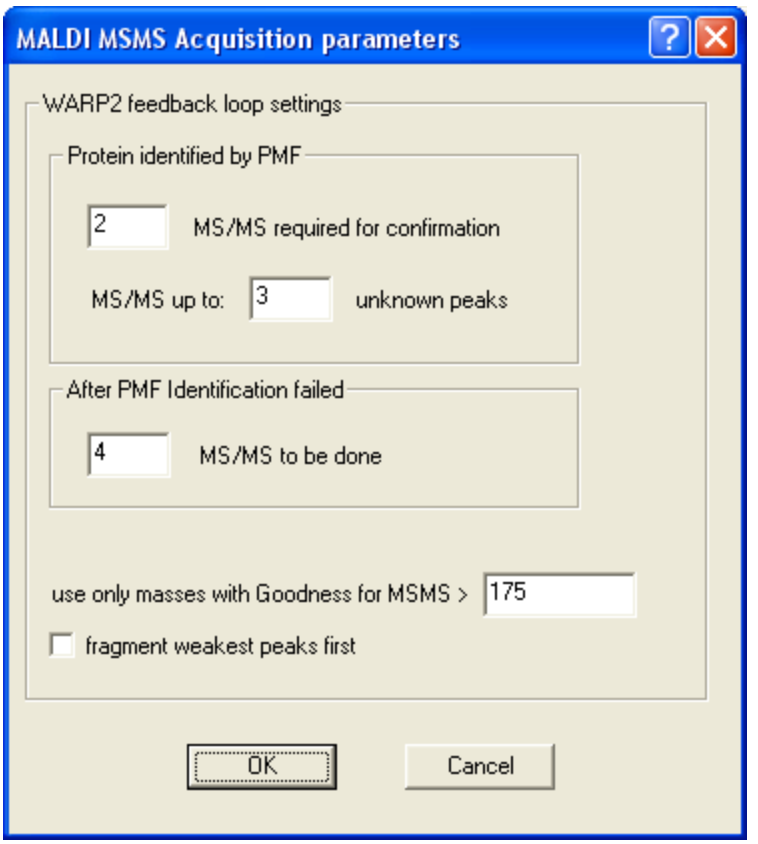


Figure A-2 MS/MS acquisition parameters in BioTools 3.2

A.2 ISD

For a description of the ISD workflow please consult the respective Biotools tutorial.

A.3 T^3-Sequencing

For a description of the T^3-sequencing workflow please consult the respective Biotools tutorial.

A.4 Fuzzy Logic Control Module

What is Fuzzy Logic?

Fuzzy logic tries to resemble human reasoning. Inexact descriptions like “Somebody is small” or “The temperature is high” often lead to questions like “How many centimeters are small?” and “How many centigrade are high?”. For example, the fuzzy or multivalued logic approach allows any value on the temperature axis to represent the quality high to a certain degree. The degree is defined by a weight or truth function, which often falls down on both sides of a maximum and becomes zero outside a certain interval. Other temperature qualities, such as low or medium with overlapping truth functions may be added to build a fuzzy temperature description (see Figure A-3).

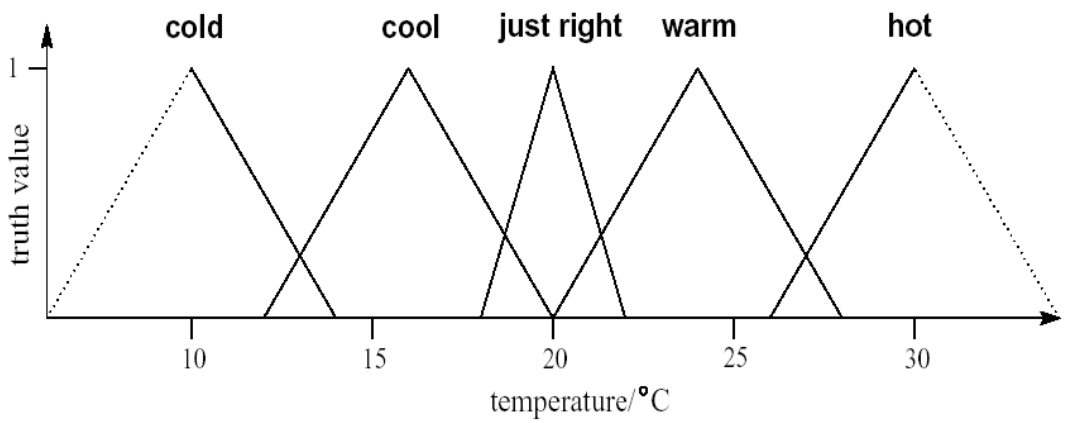


Figure A-3 Overlapping temperature curves

The fuzzy temperature representation between 10°C and 30°C uses five slightly overlapping areas (fuzzy sets) describing five temperature qualities.

The main idea of fuzzy control lies in the usage of these overlapping sets covering the definition interval of each input and output parameter. A certain input value belongs to different input sets to a certain degree (fuzzification). Simple logical rules connect the input sets to appropriate output sets. For example, when the input value is a member of the set I1 to a certain degree than the output value belongs to the set O1 to this degree.

Finally a non-fuzzy output is retrieved from the output sets (defuzzyfication).

A simple example:

Suppose the goal is to build up a ventilator control subsystem for an air condition. The only input is the current room temperature. The only output is the relative speed of the ventilator system providing the room with fresh air. To simplify this example the heating of this room may be controlled by a different system. In order to achieve this goal with a fuzzy logic control system the input and output parameter space must be available in a fuzzy representation. For the temperature we can use the fuzzy representation of Figure A-3. For the relative ventilator speed a straightforward approach may also consist of five fuzzy sets covering the speed parameter space (Figure A-4).

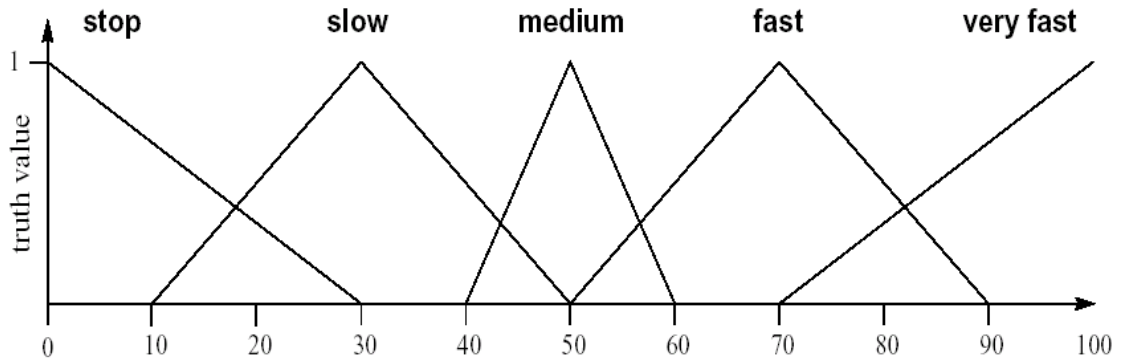


Figure A-4 Relative ventilator speed

The fuzzy representation of the relative ventilator speed uses also five overlapping areas (fuzzy sets) for five different speed qualities. Now the fuzzy sets describing the temperature and the fuzzy sets describing the ventilator speed must be connected. This is done by several logical rules. If the input and output sets are given meaningful names the logical rules will describe the control system in a semantic meaningful way.

In our case we have the following five rules (In general the rules are more complicated employing more complex logical constructs.):

Rule 1: If temperature is cold then speed is stop.

Rule 2: If temperature is cool then speed is slow.

Rule 3: If temperature is just right then speed is medium.

Rule 4: If temperature is warm then speed is fast.

Rule 5: If temperature is hot then speed is very fast.

One evaluation cycle starting with a temperature of 21°C and resulting in a relative ventilator speed of 60.1 is discussed below (Figure A-5).

- Fuzzyfication step: An input temperature of 21°C is presented to the system and is classified as belonging mainly to the temperature quality (fuzzy set) just right but also to a minor degree to warm.
- Implication step: According to the five rules stated above the medium quality is mainly activated (rule3) from the ventilator speed representation but also to a less degree the fast quality (rule4). The fuzzy output sets are scaled according to their activation.
- Aggregation step: All activated areas of the fuzzy ventilator representation are combined into a single fuzzy set in preparation of the final step.
- Defuzzification step: In order to gain just one number describing the new ventilator speed an average value must be somehow extracted from the fuzzy output. In our case a centroiding approach is used to achieve this. The result is 60.1.

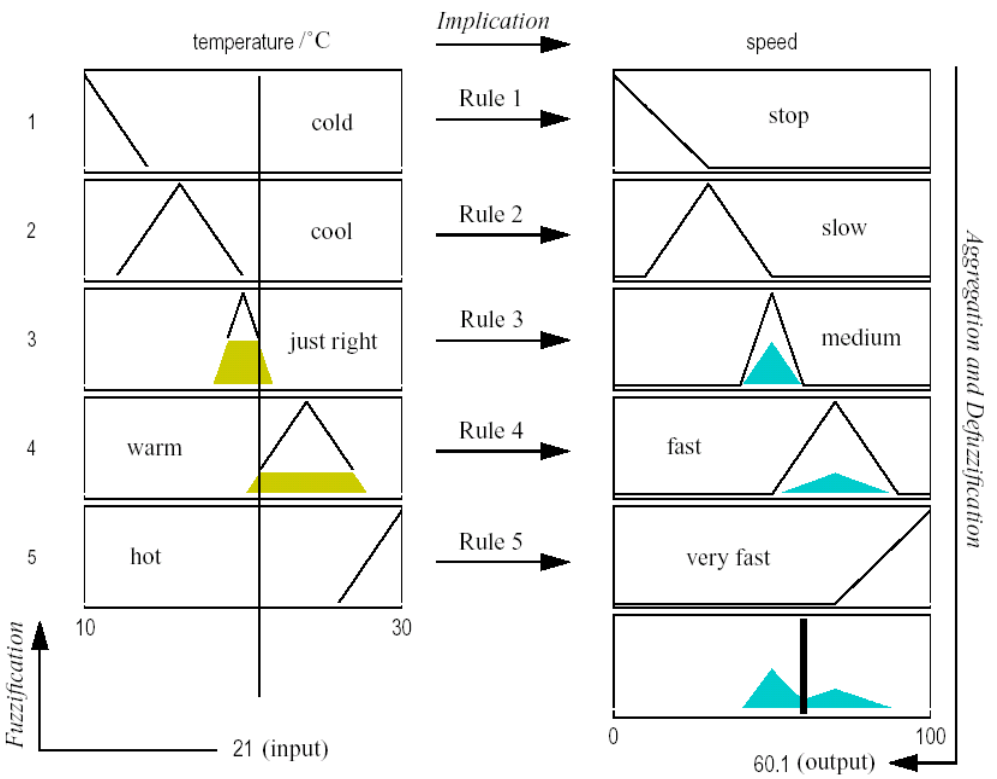


Figure A-5 One evaluation cycle

One evaluation cycle for the fuzzy control logic based ventilator control system. The input value of 21°C is fuzzyfied. According to the specified implication rules some fuzzy output sets are activated to a certain amount. After having aggregated the activated output sets to one fuzzy output an averaging step extracts on specific output value (60.1).

To get an overall impression of the behavior of the controlling system an evaluation may be done for every input value, thus creating a temperature to speed mapping (see Figure A-6). The result is a continuous piecewise linear function. It exhibits a very temperature sensitive behavior in the center part (small temperature changes lead to a large ventilator speed changes), surrounded on both sides by insensitive areas (flat curve parts), which prevent an overshooting of the controlling system. The flat curve parts at both ends of the temperature interval are due to the border effect and can be avoided by a more careful modeling. In order to get a smooth temperature to speed mapping the input fuzzification may be done using Gaussian bell functions instead of triangles.

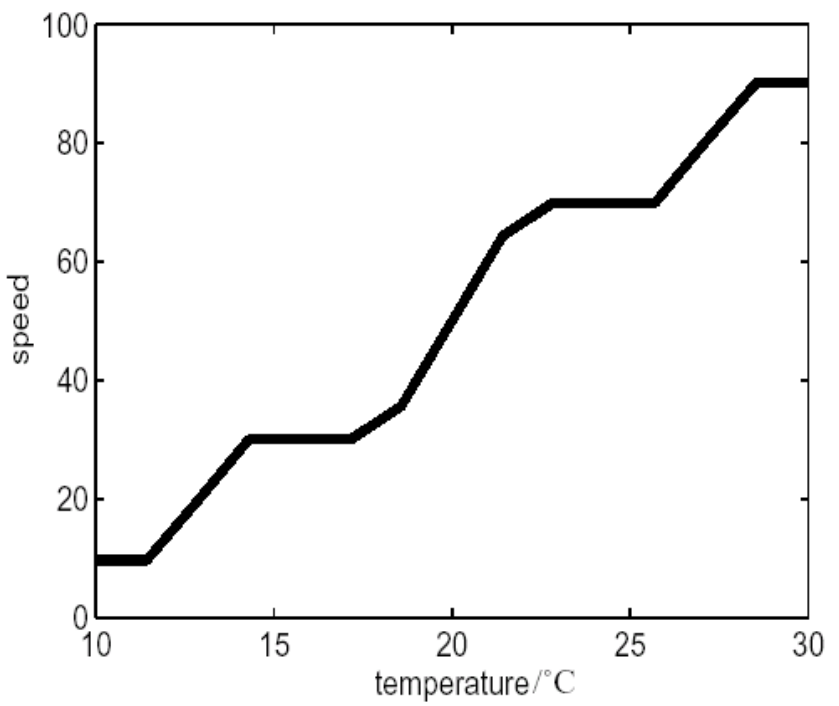


Figure A-6 Temperature to speed mapping

Figure A-6 shows the temperature to speed mapping resulting from the fuzzy logic based ventilator control system. The steep curve part in the center is responsible for a fast reaction on temperature changes around the optimal temperature value.

The following flat curve parts provide insensitivity to small temperature changes there. The steep curve parts at the interval borders create a fast change of ventilator speed in order to leave these areas as soon as possible.

This example shows that even our simple approach to create a fuzzy representation of the input and output space together with five straightforward rules connecting input side to output side led to a well suited controlling system.

The Fuzzy Control of AutoXecute

The fuzzy control module in AutoXecute has two input and two output parameters (see Figure A-7). It judges any acquired spectrum and decides whether the laser power may be changed in order to increase the spectrum quality and if the spectrum is good enough to be added to the sum buffer, which will finally saved to disk. The fuzzy control module in AutoXecute takes intensity and resolution of the acquired spectrum to adapt the laser power and to decide whether data are good enough to be saved.

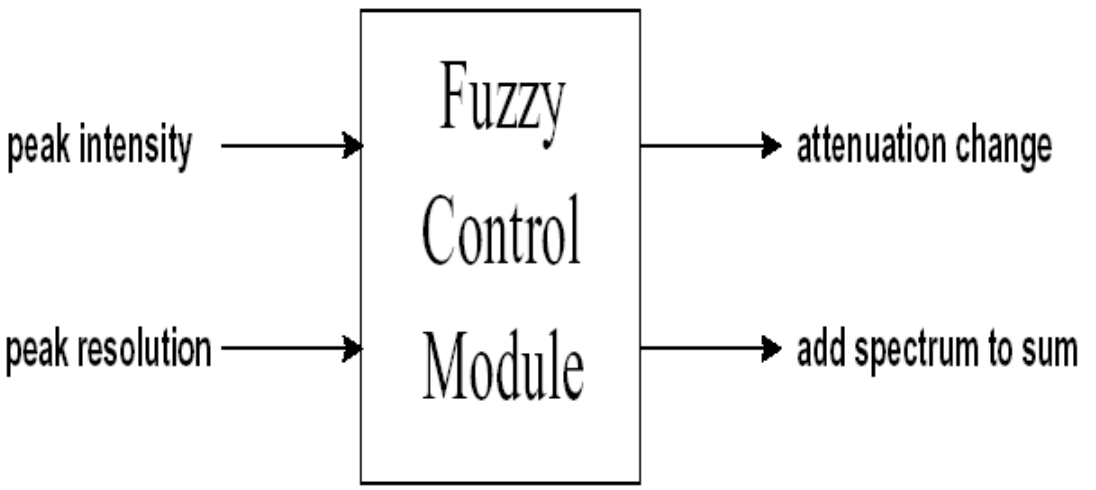


Figure A-7 Scheme of the fuzzy control module

In order to cope with different substance groups and different instrument settings the fuzzy control module may be initialized with different fuzzy control files thus implementing special fuzzy engines, which define the acceptable intensity and resolution values for different

measure processes. Currently are five fuzzy engines available (several more are presently tested and will be released soon).

Finally a 3D-plot (see Figure A-8) showing the rather non-linear connection between the two input parameters (intensity and resolution) and more input parameters (add spectrum to sum) may complete this section.

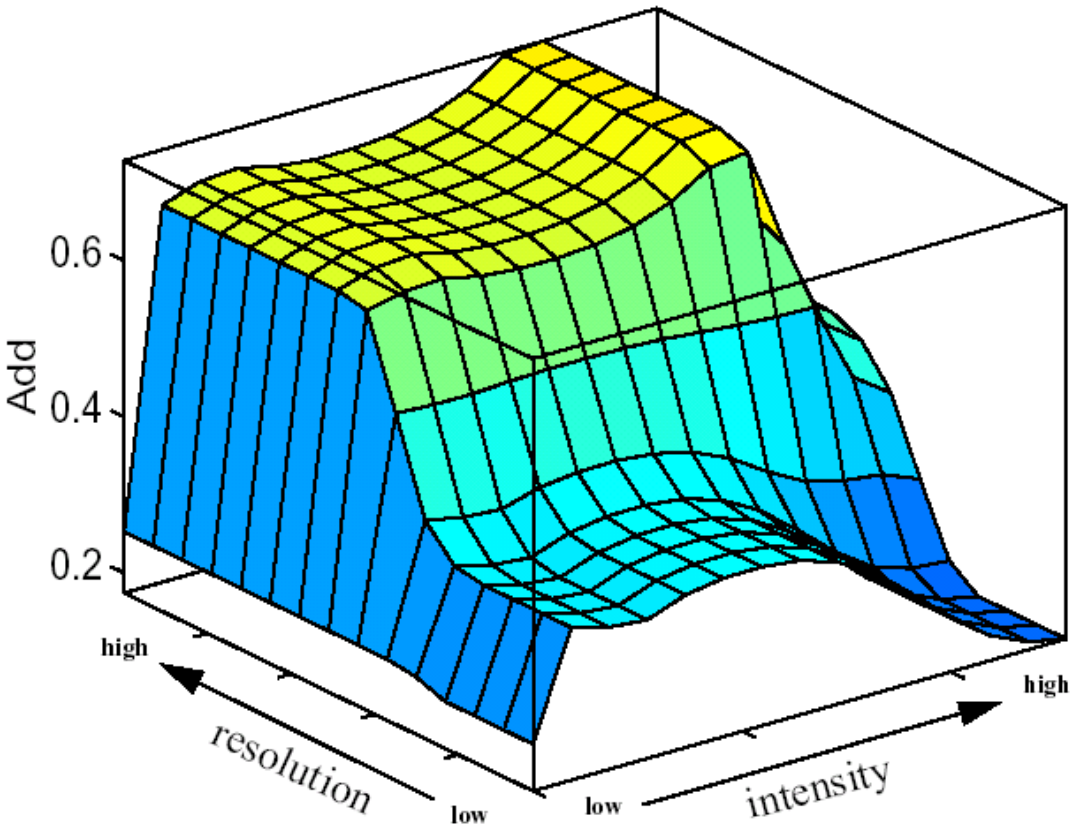


Figure A-8 Dependency of the Add function from resolution and intensity

Only when the value of the adding output is big enough the sum is added to the sum buffer (higher area of the surface). Smaller values lead to a rejection of the spectrum (lower area of the surface).

Table A-1 List of Fuzzy Engines

Fuzzy Engine	Description
peptides	The standard fuzzy engine for peptide measurements. (This fuzzy engine is identical to tof_default)
oligo_resx6	The standard fuzzy engine for oligo measurements. As difference to the peptides fuzzy engine oligo_resx6 accepts very small per shot peak intensity. To avoid the summing of noise peaks, peak resolution 6 times the specified resolution threshold leads to a rejection of the spectrum (resolution too high).
oligo_resx10	This fuzzy engine behaves exactly as oligo_resx6 except that too a high resolution is considered 10 times the specified resolution threshold.
oligo resx3	This fuzzy engine behaves exactly as oligo_resx6 except that too a high resolution is considered 3 times the specified resolution threshold.
oligo resx2	This fuzzy engine behaves exactly as oligo_resx6 except that too a high resolution is considered 2 times the specified resolution threshold.
fast_high_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with relative high substance concentration.
fast_med_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with medium substance concentration.
fast_low_int	This is the fuzzy engine for automated FAST/PSD measurements on sample spots with low substance concentration.

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