Contents

Chapter 1. Introduction

DAx can be used to read and analyse trace files such as the files that are created by the Amersham MegaBACE™ and ABI Genescan® systems.

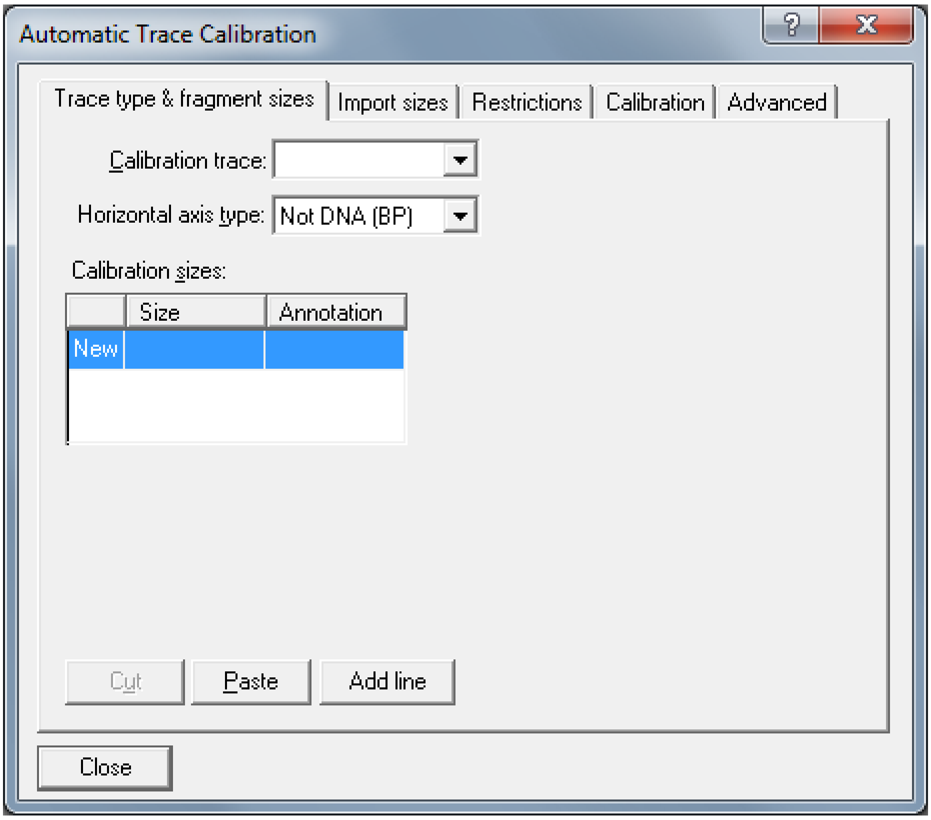
The MegaBACE™ and ABI systems, like other DNA analysis systems, can analyse four or five traces in a single run, using four (five) different (coloured) labels. One way to use this feature is to include a standard sample with known DNA fragment sizes as one of the traces, and apply this standard to the other traces. The standard is used to ascertain the fragment sizes of the components in the unknown samples.

Chapter 2. Defining the Standard

This chapter describes the steps needed to set up the analysis of the sizing standard contained in trace files. Typically, you will go through this process once for a particular sizing standard.
DAx uses a heuristic method called Automatic Trace Calibration to recognise the sizing standard's fragments[1].

Chapter 2.1. Defining the ATC parameters

Use the **Analysis** | **Edit ATC ..** menu option to invoke the Automatic Trace Calibration, or ATC, dialog box. The dialog box looks like this:



No calibration sizes have been entered yet. It is possible to enter these sizes manually.
Taking the MegaBACE ET550-R size standard as an example, you would:

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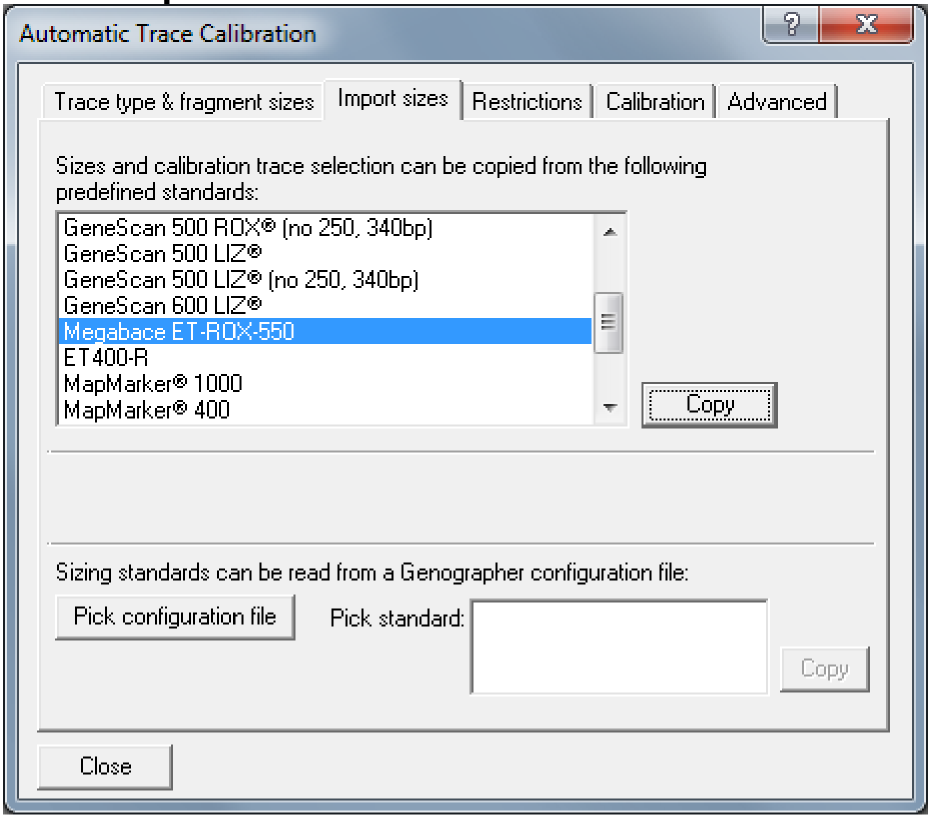
[1] Earlier versions of DAx used a process that recognised the sizing standard's peaks, essentially, by their positions; refer to daxtrace.pdf for an explanation of this procedure.

- Pick ROX as the **Calibration trace**
- Keep the **Horizontal axis type** as **Not DNA** (BP). This means that whenever some data have already been converted to a BP axis, the ATC will not be applied. All other axis types, such as time axes, will have ATC applied, converting them to a BP axis.
- Enter the **Calibration sizes** 60, 75, 100, …, 525 and 550

As an alternative to manually entering the sizing calibration, click on the **Import sizes** tab.



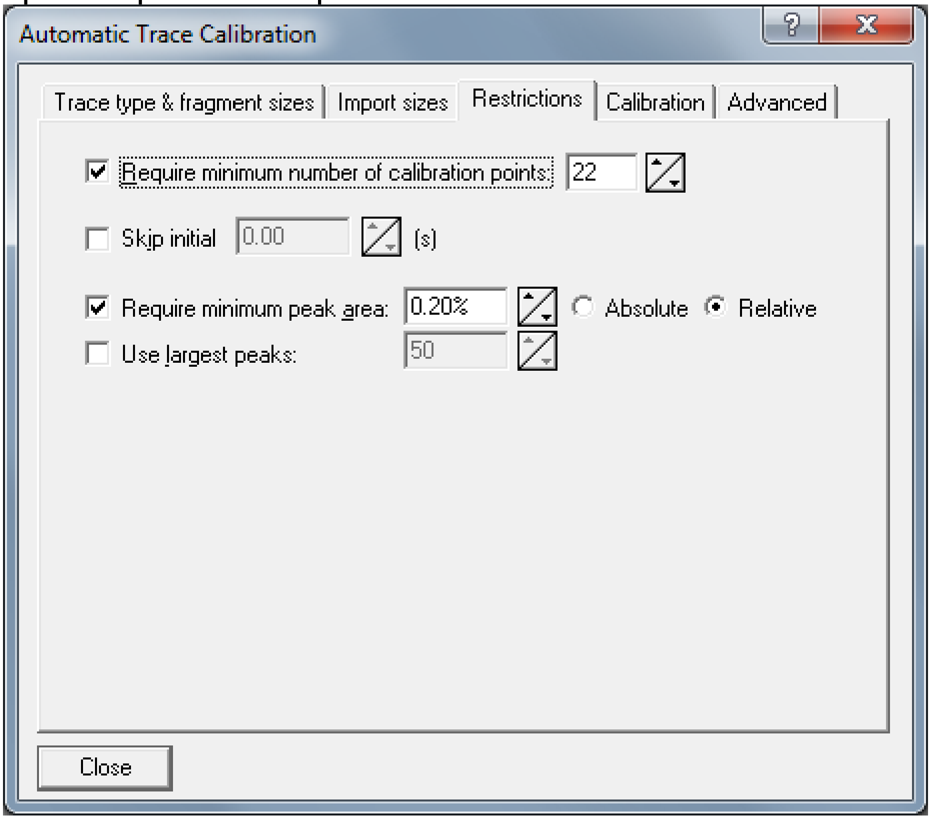
Sizes and calibration trace selection can be copied from the following predefined standards:

GeneScan 500 ROX® (no 250, 340bp)
GeneScan 500 LIZ®
GeneScan 500 LIZ® (no 250, 340bp)
GeneScan 600 LIZ®
Megabace ET-ROX-550
ET400-R
MapMarker® 1000
MapMarker® 400

Sizing standards can be read from a Genographer configuration file:

In the list of predefined standards, select the MegaBACE ET-ROX-550, then click on the **Copy** button. Click on the **Trace type & fragment sizes** tab and confirm that the correct settings are now in place.

In order to set up some restrictions, click on the **Restrictions** tab. These restrictions, while ideally not required, can significantly speed up the ATC process.



☑ Require minimum number of calibration points: 22

☐ Skip initial 0.00 (s)

☑ Require minimum peak area: 0.20% ○ Absolute ● Relative

☐ Use largest peaks: 50

Here, **Require minimum number of calibration points** has been made active, and the total number of sizes in the ET550-R standard, 22, has been entered. Note that if some measurements have been run short, they may not contain all 22 standard sizes. In this case, the minimum number of sizes will have to be lowered.

Require minimum peak area has also been made active, with a minimum peak area of 0.2%. This filters out any really small peaks from consideration as a sizing standard peak.
A similar effect could be achieved by limiting ATC to a number of the largest peaks expressed in the ROX trace. If **n** standard peaks are present, a value of **2n** generally works well.

Some measurements will have very tall peaks early in the measurement, which may bleed through into the ROX trace. When that happens, it can be helpful to exclude an initial time range[2].

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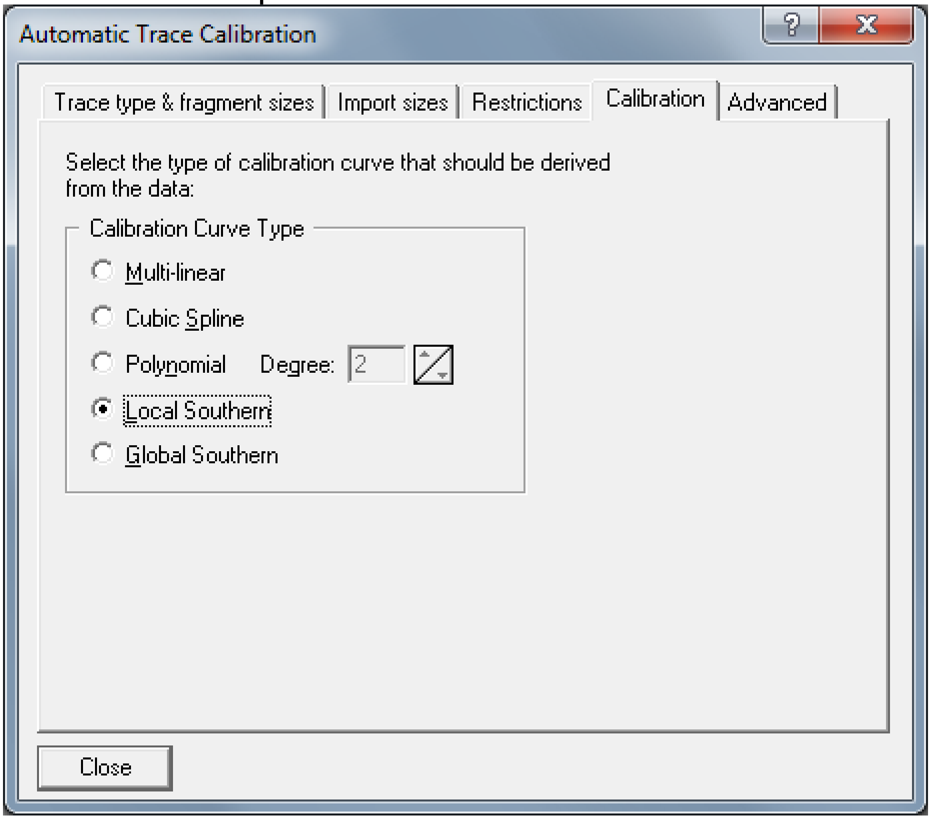
[2] Under these circumstances it is sometimes also necessary to leave out two or three of the initial sizes in the standard from the list of sizes, because those low sizes (60, 75) would have been expressed in the initial time range.

Click on the **Calibration** tab to indicate the type of calibration that should be set up.



Select the type of calibration curve that should be derived from the data:

Calibration Curve Type

○ Multi-linear

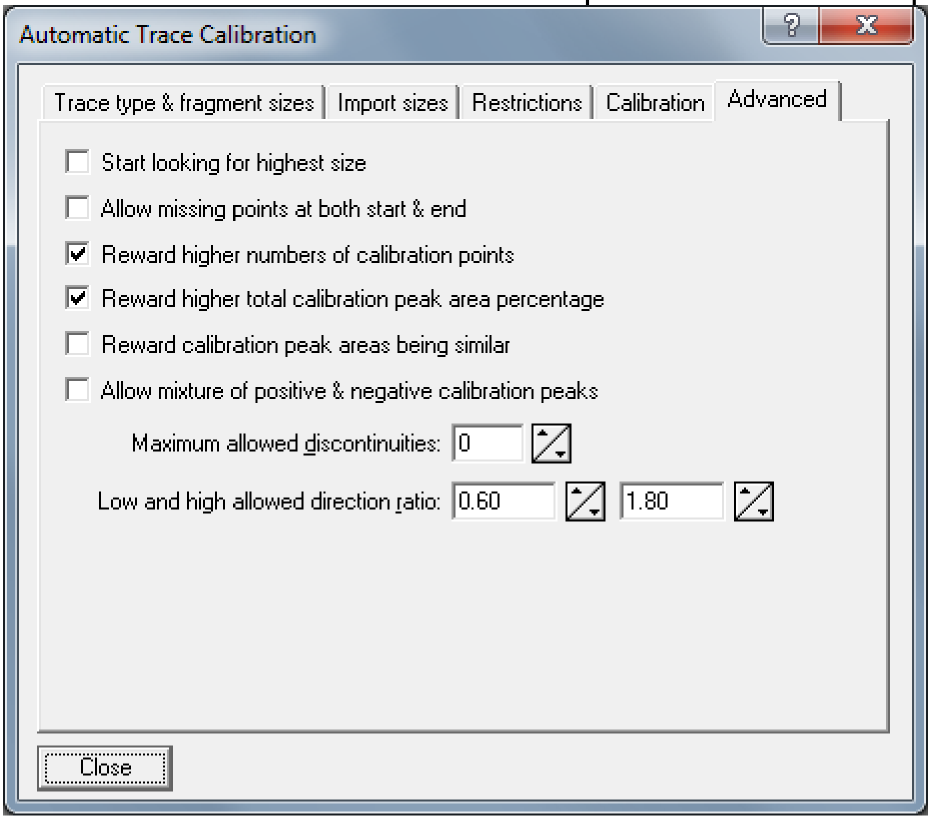
○ Cubic Spline

○ Polynomial Degree: 2

● Local Southern

○ Global Southern

Click on the **Advanced** tab to set up some advanced option.



☐ Start looking for highest size

☐ Allow missing points at both start & end

☑ Reward higher numbers of calibration points

☑ Reward higher total calibration peak area percentage

☐ Reward calibration peak areas being similar

☐ Allow mixture of positive & negative calibration peaks

Maximum allowed discontinuities: 0

Low and high allowed direction ratio: 0.60 1.80

Start looking for highest size runs the ATC algorithm "in reverse", which can sometimes be helpful.

Allow missing points at both start and end allows for low end as well as high end calibration points to be missing.

Reward higher numbers of calibration points tells the ATC algorithm that searches that turn up more calibration points are to be considered better.

Reward higher total calibration peak area percentage tells the ATC algorithm that calibration points are likely to have peaks with significant sizes.

Reward calibration peak areas being similar instructs the ATC algorithm to make use of the fact that calibration peaks probably have similar sizes.

Maximum allowed discontinuities can be used if the measurement is known to contain a discontinuity in the horizontal axis. It is rarely necessary to allow discontinuities.

The **low and high allowed direction ratios** determine how forgiving the ATC algorithm will be. More forgiving settings (further away from 1.0) will result in more inclusive searches, which may take longer to run. Generally, values of 0.7 and 1.5 give excellent results.

Once all fields have been filled, click the **Close** button to close the ATC dialog. You should now use **File | Save Analysis Procedure** to store this setup on disk.

Chapter 2.2.Troubleshooting ATC

In the next chapter, Automatic Trace Calibrations will be used to find the standard peaks in a ROX trace. This is normally a very straightforward process. However, should the procedure run into a snag, there are some steps that should be followed.

The first step is to make sure that the calibration trace being looked at does indeed contain all (or some) expected calibration peaks. Here are some reasons why this might not be the case:

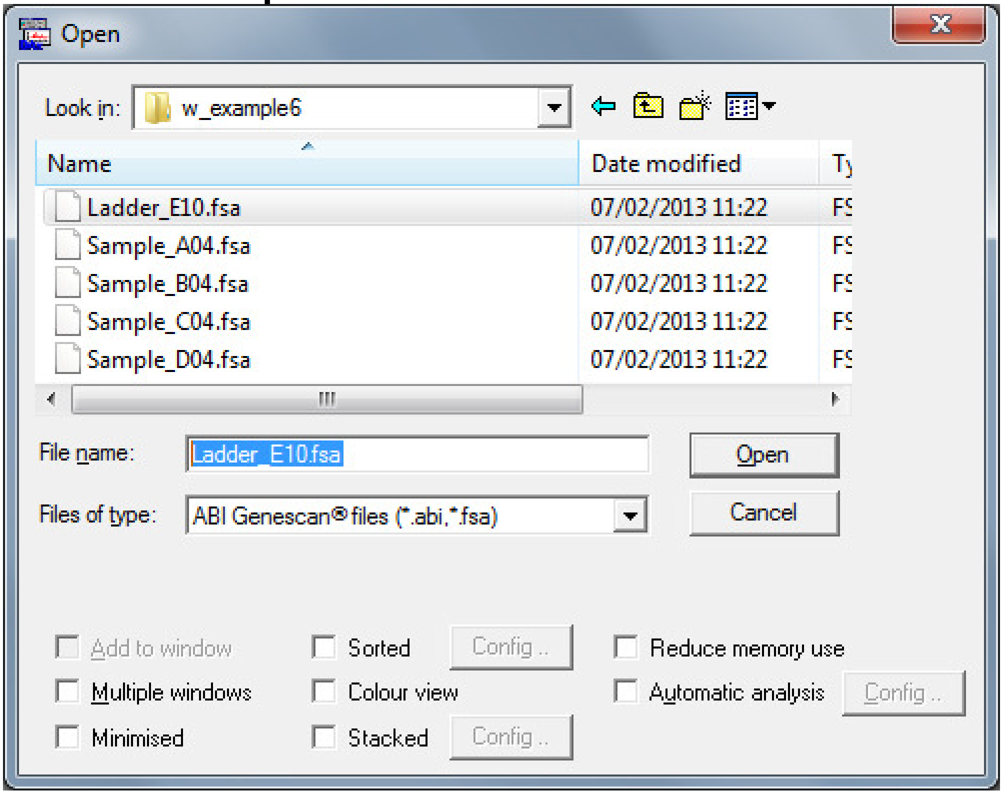
- the most common reason is that too large an interval at the start of the trace is being excluded from peak finding, causing one or more initial calibration peaks to be skipped. Make sure not to skip too large an interval at the start of the trace when finding peaks.
- peak find parameters might have too high a detection limit.
- the trace may actually not contain the calibration peaks, or not all of them. If 10 calibration peaks are expected, but only 8 peaks are present, ATC will fail if the **require minimum number of calibration points** parameter was set higher than 8.

If the calibration peaks are present and found correctly, but ATC still fails, some parameters may have been given values that are too limiting. It is important to understand that most ATC parameters are used to speed up the ATC algorithm, without being strictly necessary for the algorithm to work. Therefore, if the ATC fails to find a calibration, these parameters can be relaxed, advisably in this order:

- if some early sizes are being missed, skip a smaller interval
- if you are sure all sizes are present, and early sizes sometimes get called wrong, use the advanced option **Start looking for highest size**
- require a smaller peak area
- use more peaks
- require fewer points to be present

Chapter 3. Analysis of the Calibration Ladder

Invoke the **File** | **Open** command. Select **Files of Type** ABI Genescan files (or one of the other trace file types: SCF files, MegaBACE files CEQ files), select a file containing the calibration, then click the **Open** button.



Look in: w_example6

Name	Date modified	Ty
Ladder_E10.fsa	07/02/2013 11:22	FS
Sample_A04.fsa	07/02/2013 11:22	FS
Sample_B04.fsa	07/02/2013 11:22	FS
Sample_C04.fsa	07/02/2013 11:22	FS
Sample_D04.fsa	07/02/2013 11:22	FS

File name: Ladder_E10.fsa

Files of type: ABI Genescan® files (*.abi,*.fsa)

☐ Add to window
☐ Multiple windows
☐ Minimised
☐ Sorted
☐ Colour view
☐ Stacked
☐ Reduce memory use
☐ Automatic analysis

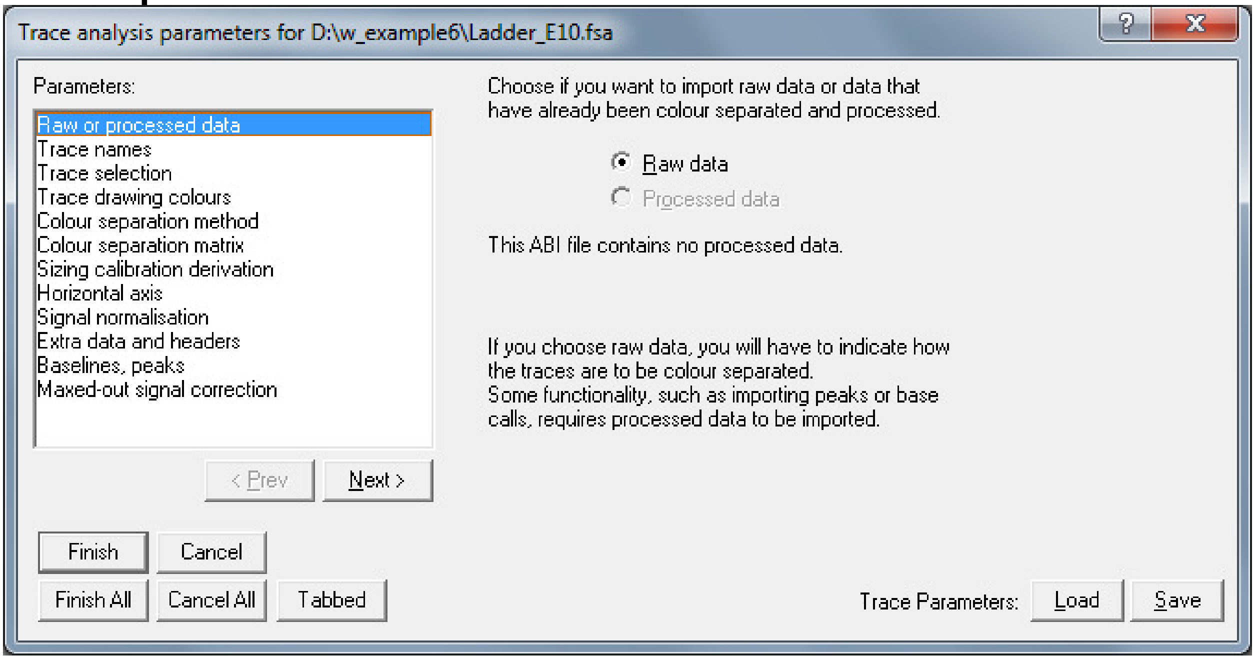
The options at the bottom of the File | Open dialog are discussed in the next chapter.

Note that typically each sample file will contain the calibration ladder, so any sample file can be selected to set up the calibration. Here, one file contains *only* the calibration, so this file is used.

Chapter 3.1. Trace Analysis Parameters (TAP) dialog

When trace files are opened, the Trace Analysis Parameters (TAP) dialog is displayed, so that the way in which the data are to be loaded can be set up. The TAP dialog contains a list of parameters at left, which you should fill out in order[3][4].

Raw or processed data



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose if you want to import raw data or data that have already been colour separated and processed.

● Raw data

○ Processed data

This ABI file contains no processed data.

If you choose raw data, you will have to indicate how the traces are to be colour separated.
Some functionality, such as importing peaks or base calls, requires processed data to be imported.

The example file contains no processed data, so raw data must be loaded.

Click **Next** to display the next page of parameters.

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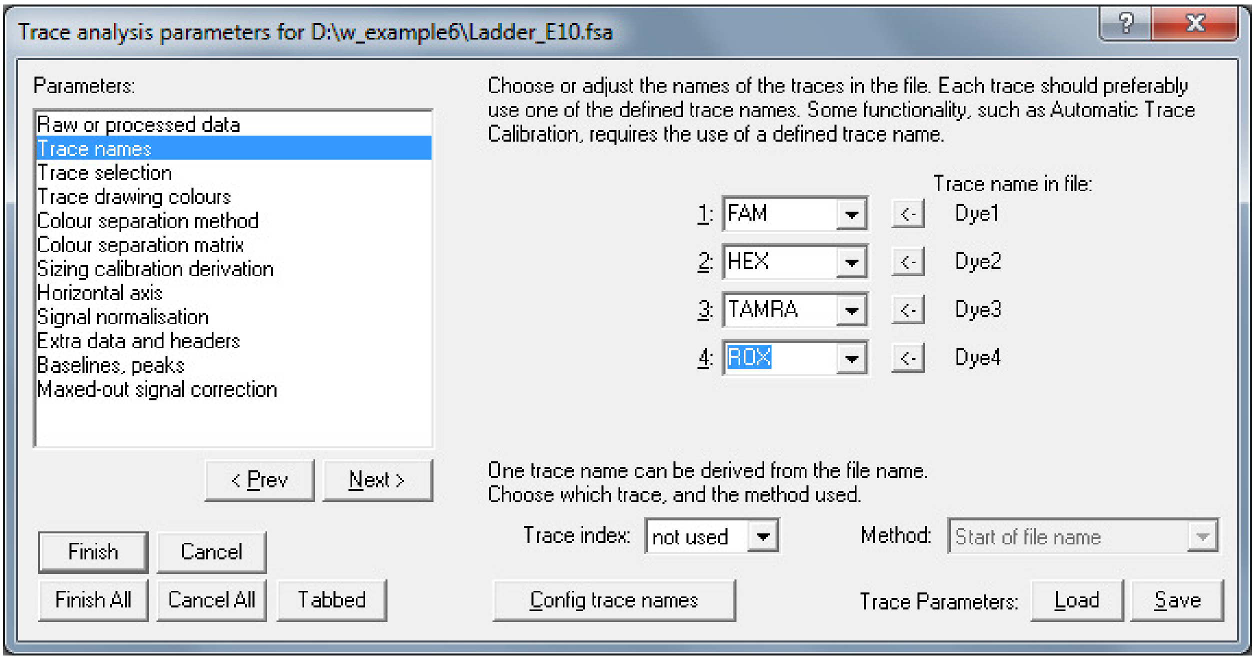
[3] The list changes in accordance with the types of parameters that are needed.

[4] If your TAP dialog does not look like the examples, you should click the **Wizard** button third from bottom left.

Trace names



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Parameters:

- Raw or processed data
- **Trace names**
- Trace selection
- Trace drawing colours
- Colour separation method
- Colour separation matrix
- Sizing calibration derivation
- Horizontal axis
- Signal normalisation
- Extra data and headers
- Baselines, peaks
- Maxed-out signal correction

Choose or adjust the names of the traces in the file. Each trace should preferably use one of the defined trace names. Some functionality, such as Automatic Trace Calibration, requires the use of a defined trace name.

		Trace name in file:
1:	FAM	Dye1
2:	HEX	Dye2
3:	TAMRA	Dye3
4:	ROX	Dye4

One trace name can be derived from the file name.
Choose which trace, and the method used.

Trace index: not used

Method: Start of file name

Some trace files contain the names of the traces used, but the example does not, instead just specifying Dye1 – Dye4. These should be changed to recognized dye names, as shown.

The **Config trace names** button lets you specify new trace names, and lets you specify standard colours for existing and newly defined trace names[5].

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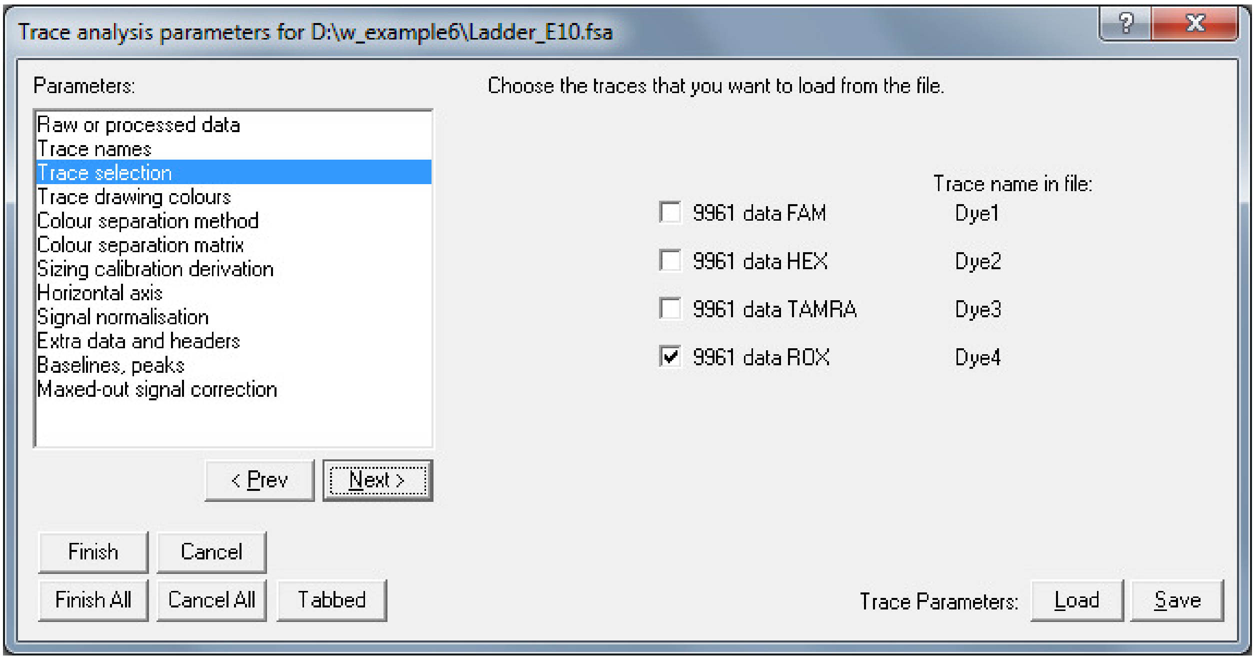
[5] A trace name can also be derived from data file names, which is beyond the scope of this document

Trace selection



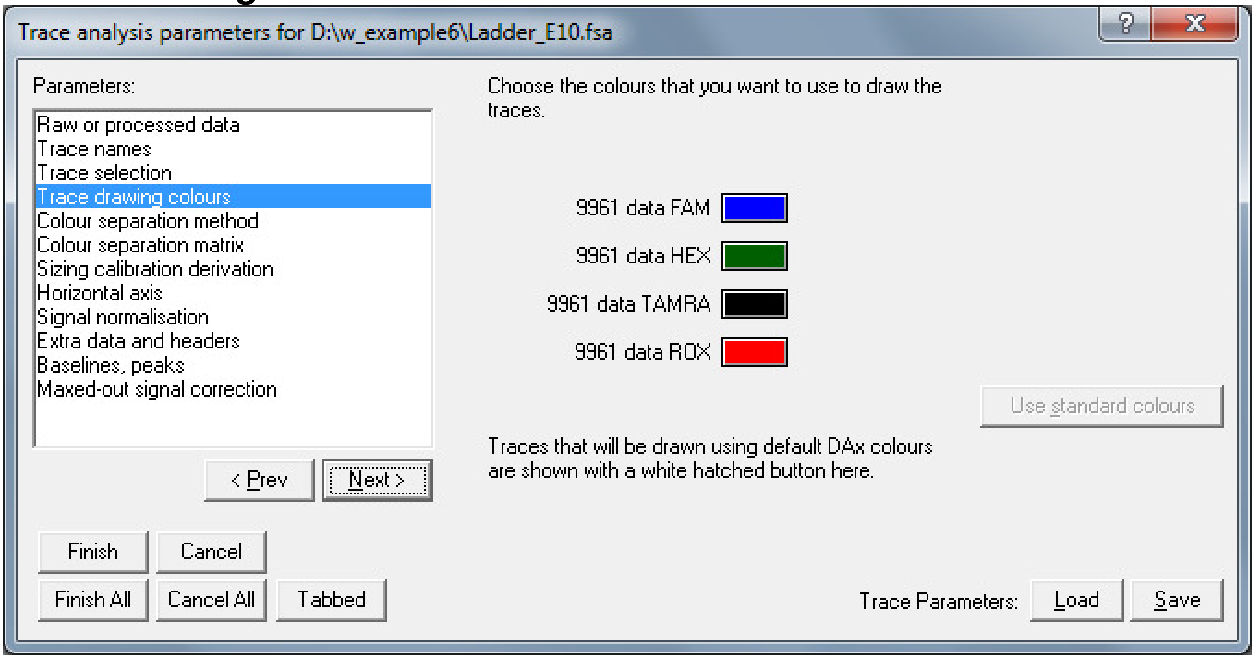
Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose the traces that you want to load from the file.

	Trace name in file:
☐ 9961 data FAM	Dye1
☐ 9961 data HEX	Dye2
☐ 9961 data TAMRA	Dye3
☑ 9961 data ROX	Dye4

Choose which traces to load. In this example, we are only interested in the size calibration ladder, which is measured in the ROX trace, so only ROX is checked.

Trace drawing colours



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

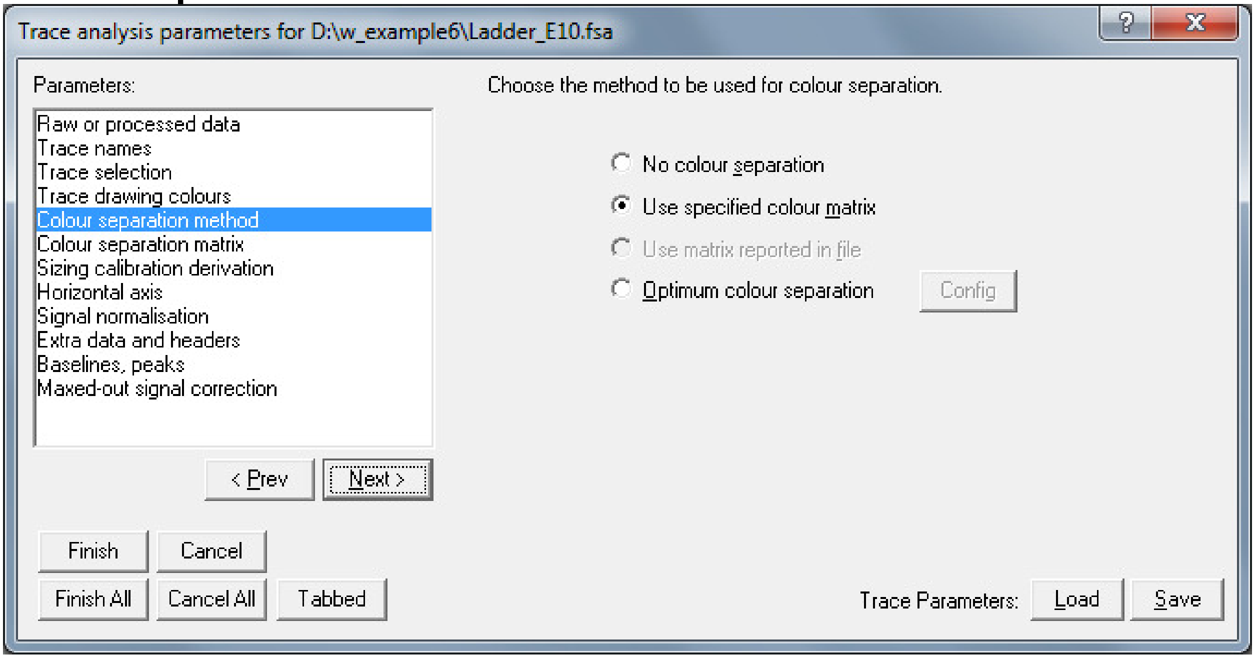
Choose the colours that you want to use to draw the traces.

9961 data FAM 🟦
9961 data HEX 🟩
9961 data TAMRA ⬛
9961 data ROX 🟥

Traces that will be drawn using default DAx colours are shown with a white hatched button here.

The **Use standard colours** button is disabled here because the colours are already the standards for the trace names shown.

Colour separation method



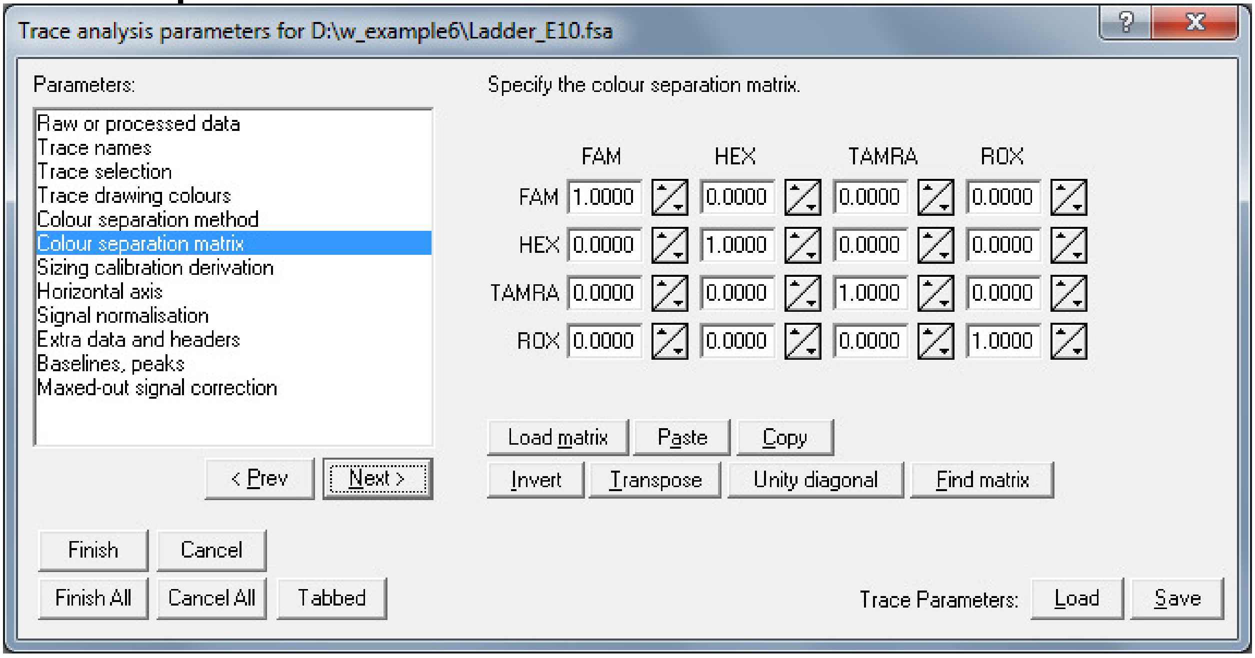
Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose the method to be used for colour separation.

○ No colour separation
● Use specified colour matrix
○ Use matrix reported in file
○ Optimum colour separation

The signals measured for each of the traces are made up of contributions of not just the trace itself, but also contain bleedthrough from the other traces. To correct for that, a colour separation should ideally be used. The best way to do this is to specify a colour separation matrix that has been ascertained by measuring a standard mixture of samples.

Colour separation matrix

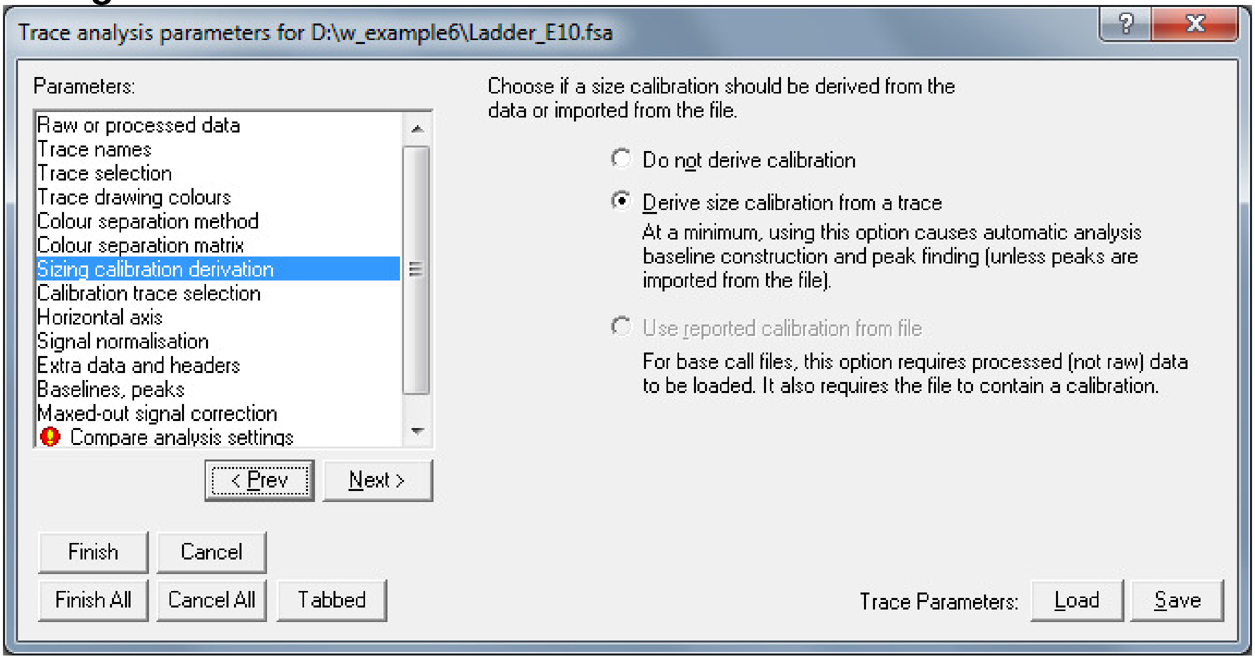


Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Specify the colour separation matrix.

	FAM	HEX	TAMRA	ROX
FAM	1.0000	0.0000	0.0000	0.0000
HEX	0.0000	1.0000	0.0000	0.0000
TAMRA	0.0000	0.0000	1.0000	0.0000
ROX	0.0000	0.0000	0.0000	1.0000

Sizing calibration derivation


Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Parameters:

Choose if a size calibration should be derived from the data or imported from the file.

○ Do not derive calibration

● Derive size calibration from a trace

At a minimum, using this option causes automatic analysis baseline construction and peak finding (unless peaks are imported from the file).

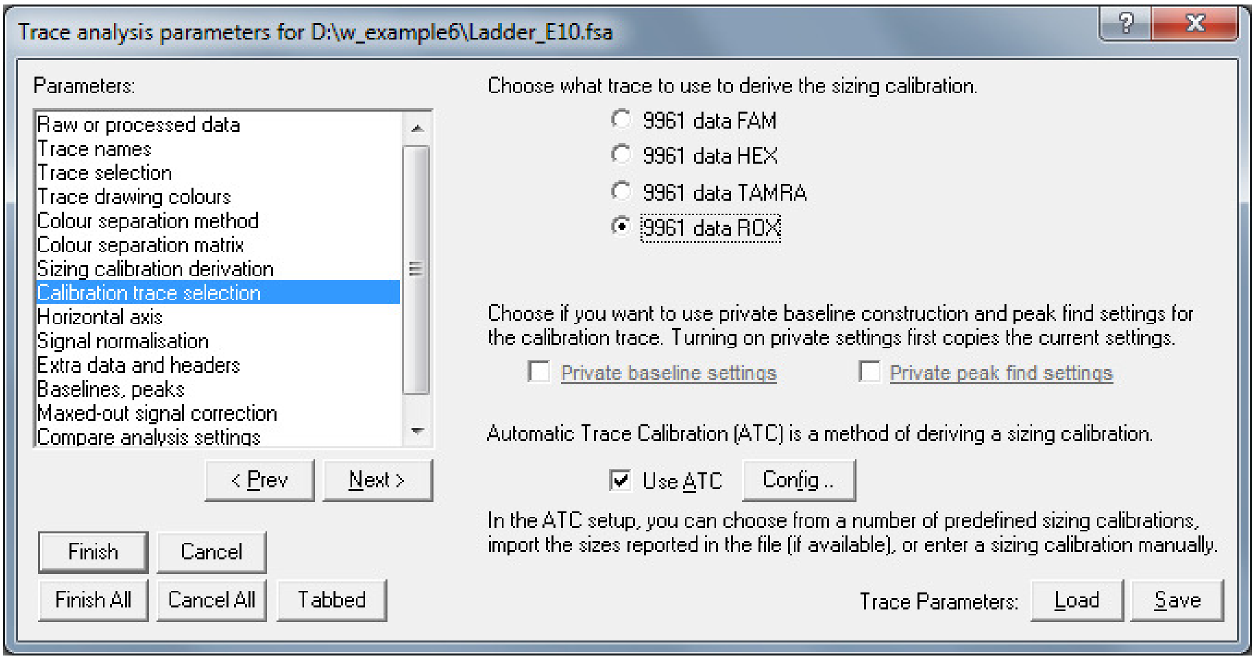
○ Use reported calibration from file

For base call files, this option requires processed (not raw) data to be loaded. It also requires the file to contain a calibration.

In the example, we want to derive the sizing calibration from the ROX trace. Note that the list of parameters at left has changed because the selection on this page was changed from **Do not derive calibration** to **Derive size calibration from a trace**.

The warning symbol displayed next to **Compare analysis settings** is there because DAx has detected an inconsistency. This will be resolved momentarily.

Calibration trace selection



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose what trace to use to derive the sizing calibration.

○ 9961 data FAM
○ 9961 data HEX
○ 9961 data TAMRA
● 9961 data ROX

Choose if you want to use private baseline construction and peak find settings for the calibration trace. Turning on private settings first copies the current settings.

☐ Private baseline settings
☐ Private peak find settings

Automatic Trace Calibration (ATC) is a method of deriving a sizing calibration.

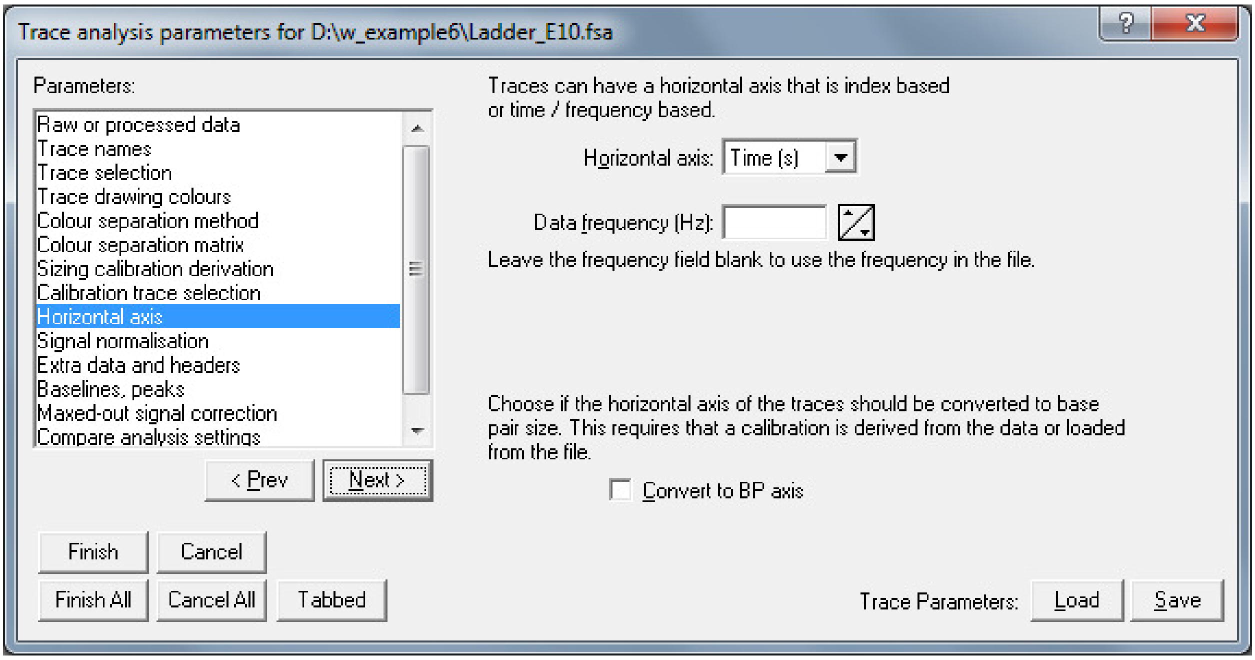
☑ Use ATC

In the ATC setup, you can choose from a number of predefined sizing calibrations, import the sizes reported in the file (if available), or enter a sizing calibration manually.

By changing the calibration trace to ROX, the warning icon next to **Compare analysis settings** is removed. Make sure **Use ATC** is checked, so that the ET 550-R calibration that was set up in Chapter 2 will be applied.

Sometimes it is desirable to analyse the calibration trace with different baseline construction and peak find parameters than the other traces. To do that, check **Private baseline settings** and **Private peak find settings**. When these options are checked, the texts become blue and underlined and can be clicked to set up parameters.

Horizontal axis



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Traces can have a horizontal axis that is index based or time / frequency based.

Horizontal axis: Time (s)

Data frequency (Hz):

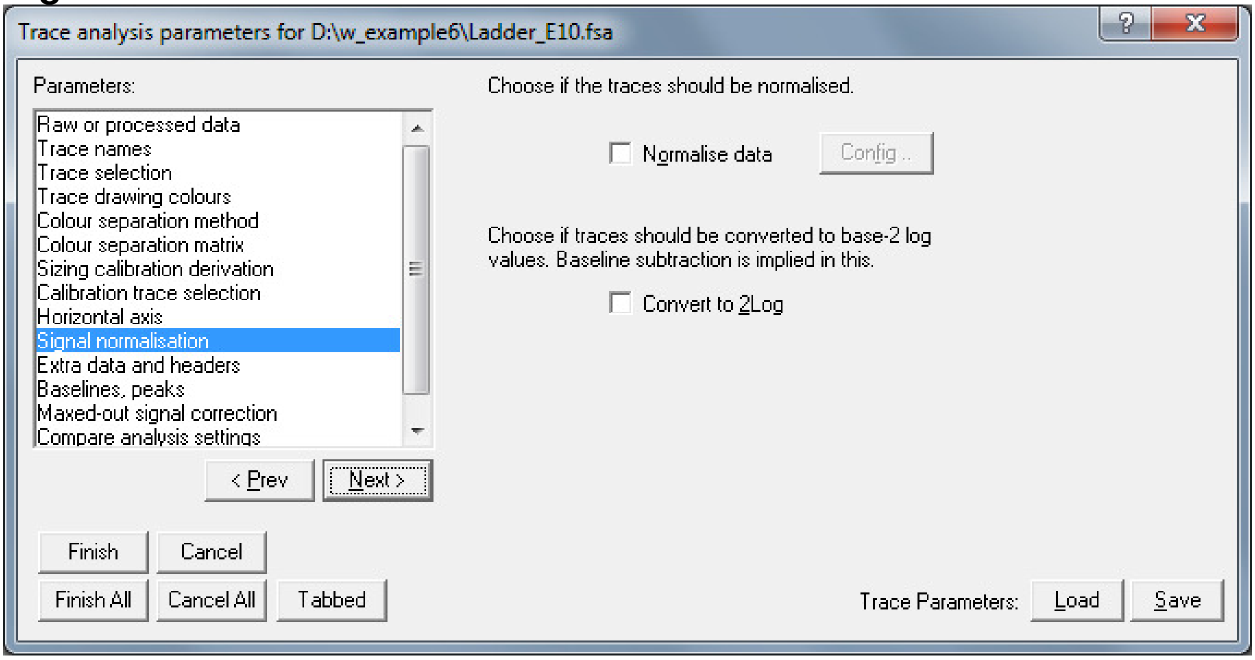
Leave the frequency field blank to use the frequency in the file.

Choose if the horizontal axis of the traces should be converted to base pair size. This requires that a calibration is derived from the data or loaded from the file.

☐ Convert to BP axis

The horizontal axis can be a time axis, or can be converted to a BP axis. When setting up and verifying a calibration, leave the axis as a time axis.

Signal normalisation



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose if the traces should be normalised.

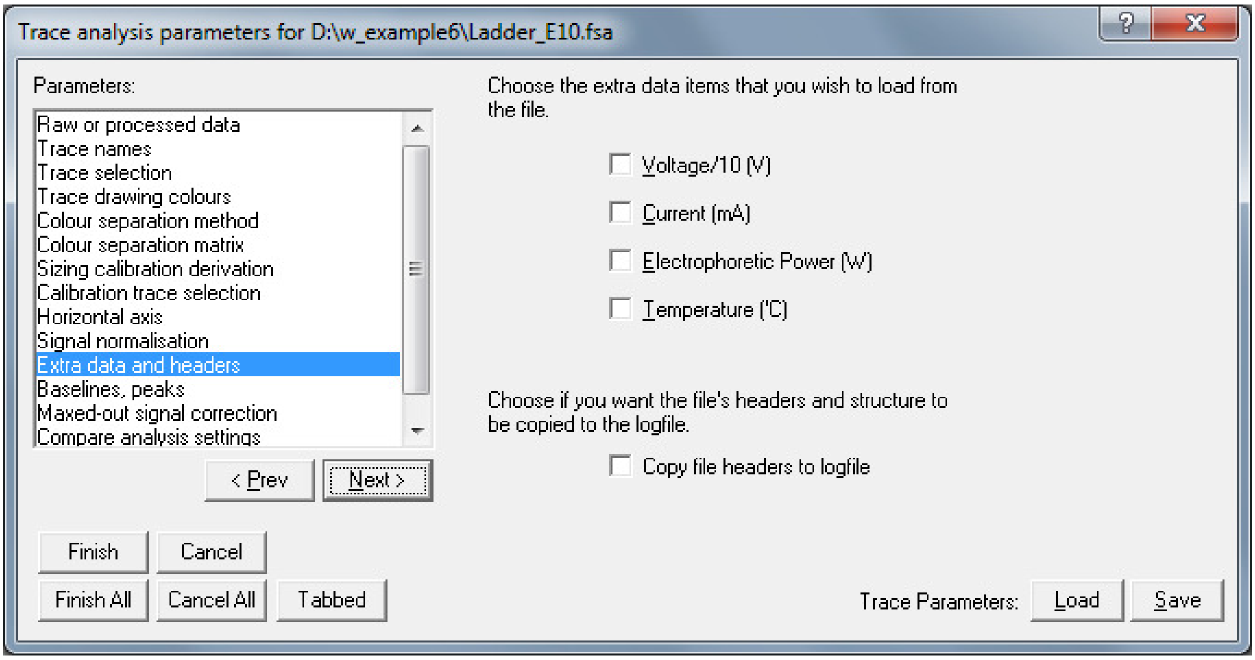
☐ Normalise data

Choose if traces should be converted to base-2 log values. Baseline subtraction is implied in this.

☐ Convert to 2Log

The data will not be normalized.

Extra data and headers



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose the extra data items that you wish to load from the file.

☐ Voltage/10 (V)

☐ Current (mA)

☐ Electrophoretic Power (W)

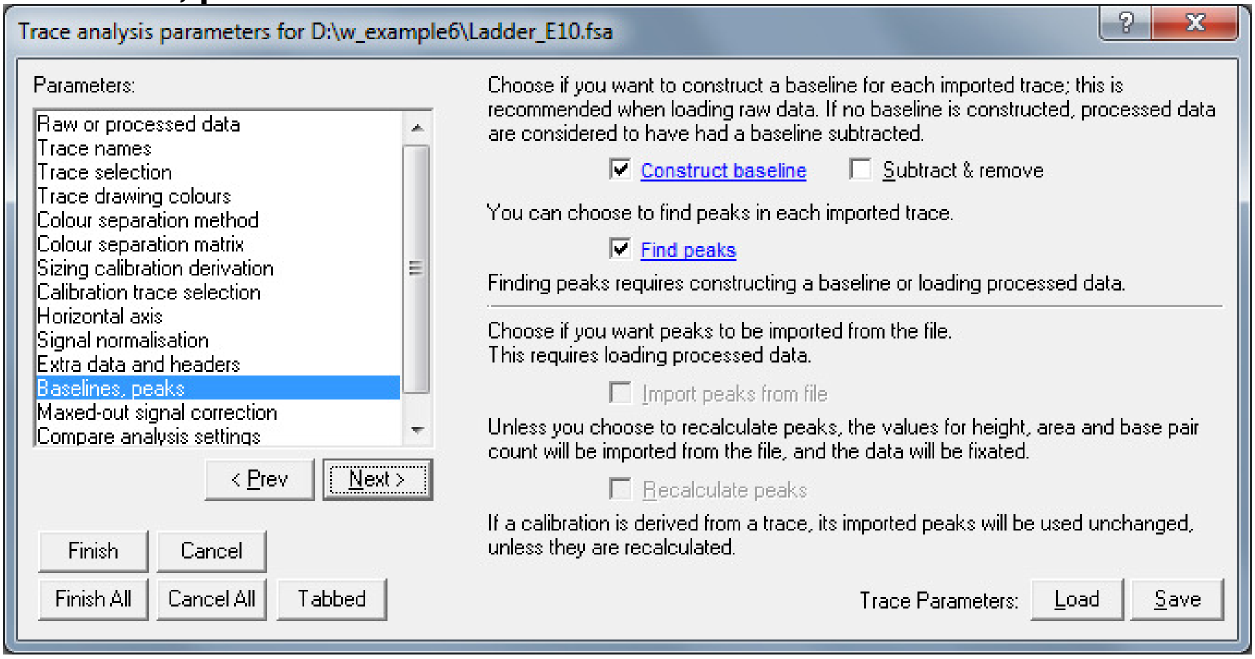
☐ Temperature ('C)

Choose if you want the file's headers and structure to be copied to the logfile.

☐ Copy file headers to logfile

No extra data will be loaded

Baselines, peaks



Trace analysis parameters for D:\w_example6\Ladder_E10.fsa

Choose if you want to construct a baseline for each imported trace; this is recommended when loading raw data. If no baseline is constructed, processed data are considered to have had a baseline subtracted.

☑ Construct baseline ☐ Subtract & remove

You can choose to find peaks in each imported trace.

☑ Find peaks

Finding peaks requires constructing a baseline or loading processed data.

Choose if you want peaks to be imported from the file.
This requires loading processed data.

☐ Import peaks from file

Unless you choose to recalculate peaks, the values for height, area and base pair count will be imported from the file, and the data will be fixated.

☐ Recalculate peaks

If a calibration is derived from a trace, its imported peaks will be used unchanged, unless they are recalculated.

After the data are loaded, baselines will be constructed and peaks found. Click on the blue-underlined items to set up baseline construction and peak find parameters[6].

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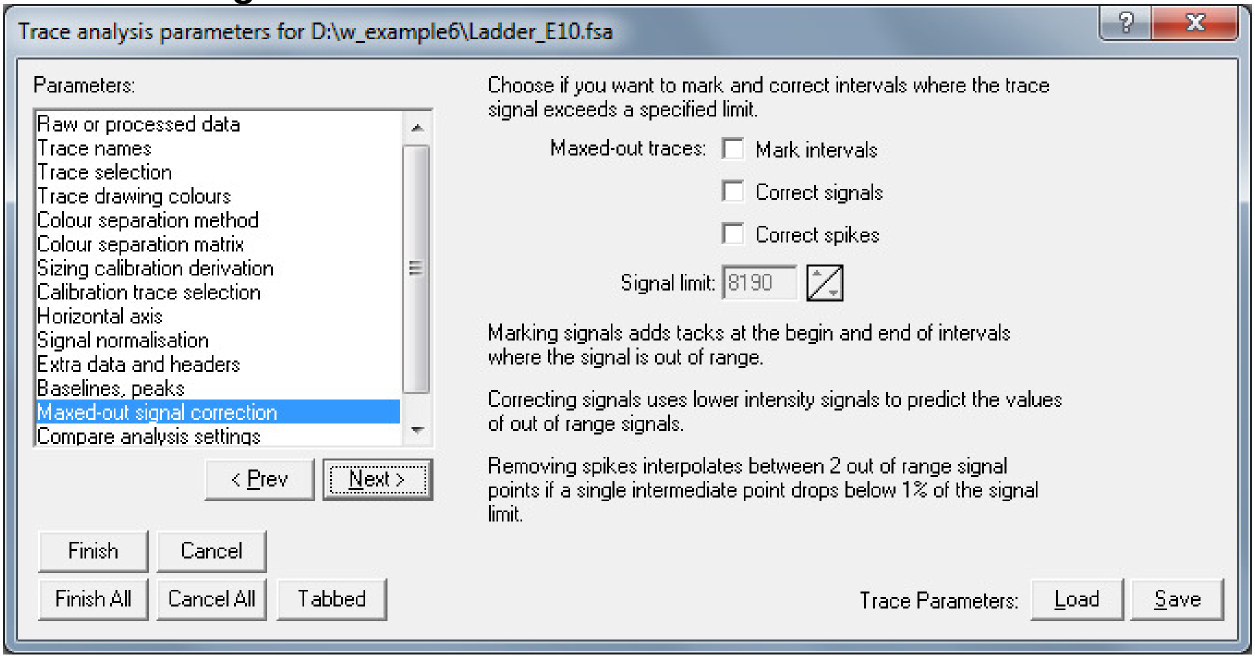
[6] In this example, only the ROX trace is loaded. Since this is the calibration trace, it will have a baseline constructed and peaks found even if the baseline construction and peak find items are not checked here.

Maxed-out signal correction



Choose if you want to mark and correct intervals where the trace signal exceeds a specified limit.

Maxed-out traces: ☐ Mark intervals

☐ Correct signals

☐ Correct spikes

Signal limit: 8190

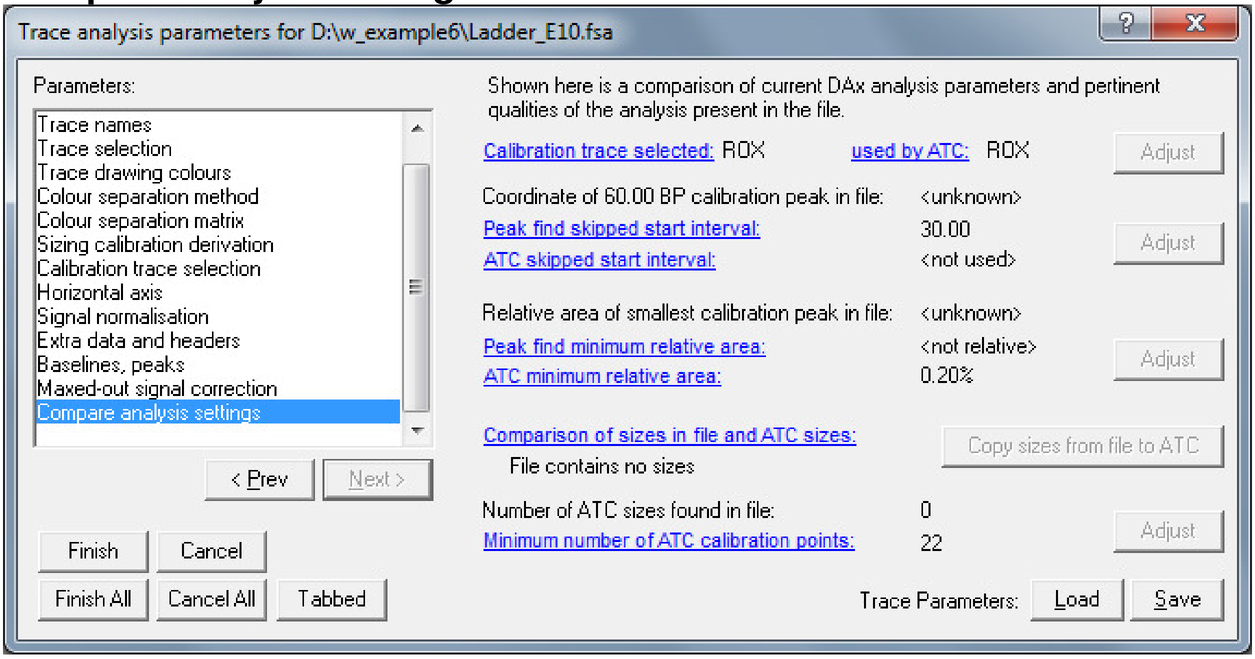
Marking signals adds tacks at the begin and end of intervals where the signal is out of range.

Correcting signals uses lower intensity signals to predict the values of out of range signals.

Removing spikes interpolates between 2 out of range signal points if a single intermediate point drops below 1% of the signal limit.

Sometimes the signal values in a trace file will exceed what the data format can handle. DAx can detect such occurrences and annotate them in the loaded data.

Compare analysis settings



Shown here is a comparison of current DAx analysis parameters and pertinent qualities of the analysis present in the file.

Calibration trace selected: ROX used by ATC: ROX

Coordinate of 60.00 BP calibration peak in file: <unknown>
Peak find skipped start interval: 30.00
ATC skipped start interval: <not used>

Relative area of smallest calibration peak in file: <unknown>
Peak find minimum relative area: <not relative>
ATC minimum relative area: 0.20%

Comparison of sizes in file and ATC sizes:
File contains no sizes

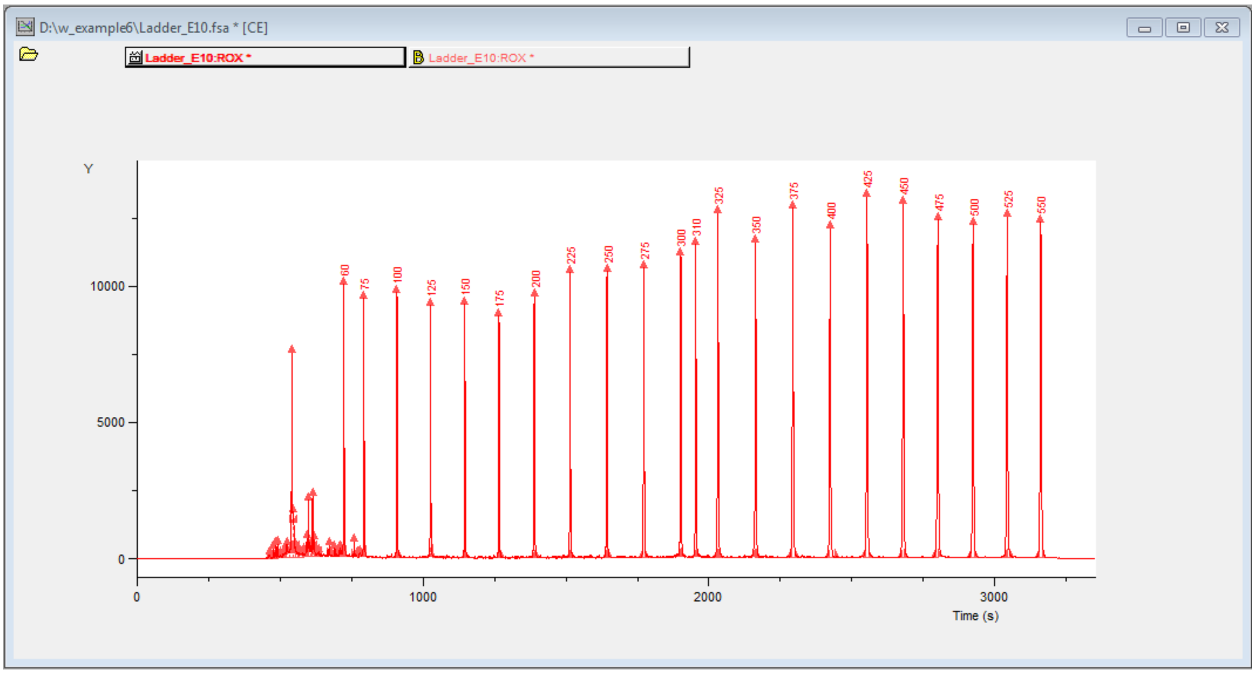
Number of ATC sizes found in file: 0
Minimum number of ATC calibration points: 22

DAx will compare and verify a number of analysis settings, and flag any that do not match. In such instances, clicking the **Adjust** button will remove the conflict.

To finish filling out the TAP dialog, click the **Finish** button.

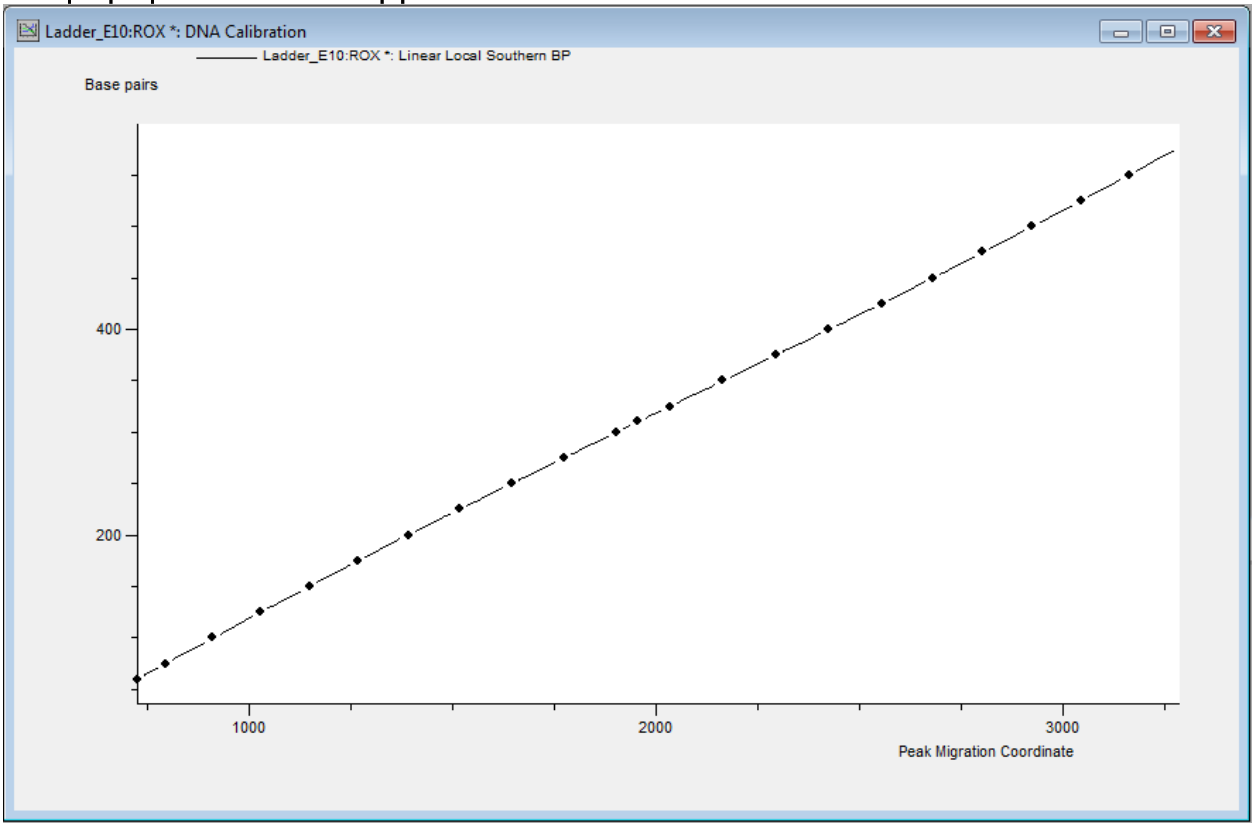
Chapter 3.2.Results

The ROX trace will now be loaded and analysed. The result is shown below.



Note that the calibration peaks are lalebelled with their fragment sizes. This is because ATC has named each calibration peak with its fragment size, and peaks are being labelled with their names.

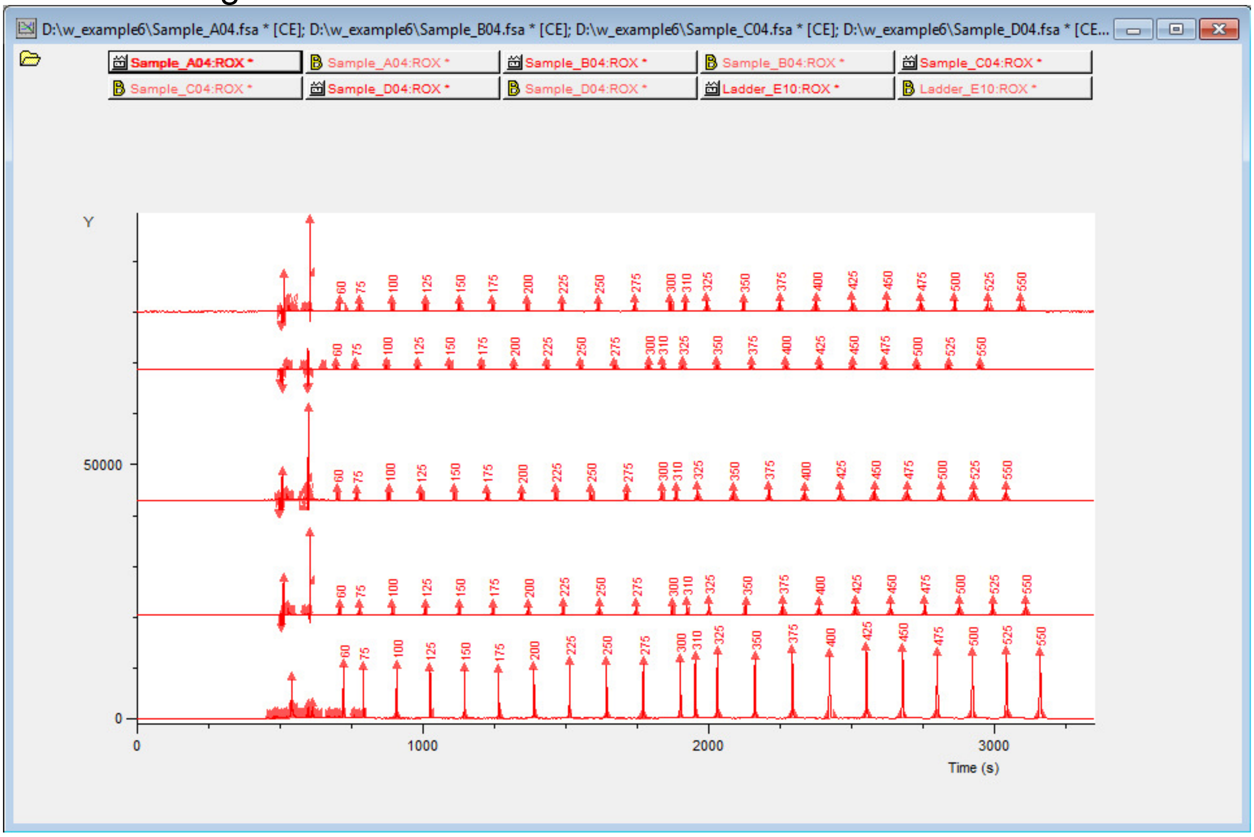
To verify that the calibration is well formed, click on the data tag of the ROX trace, and select the **Calibration** | **Curve** menu item from the popup menu that appears.



For a quick verification of multiple calibrations, try the following:

- Use the File | Open command to once again display the File | Open dialog. Select multiple data files. Check the **Stacked** checkbox at the bottom of the dialog. Click **Open**.
- In the TAP dialog, click **Finish All** without changing any settings.

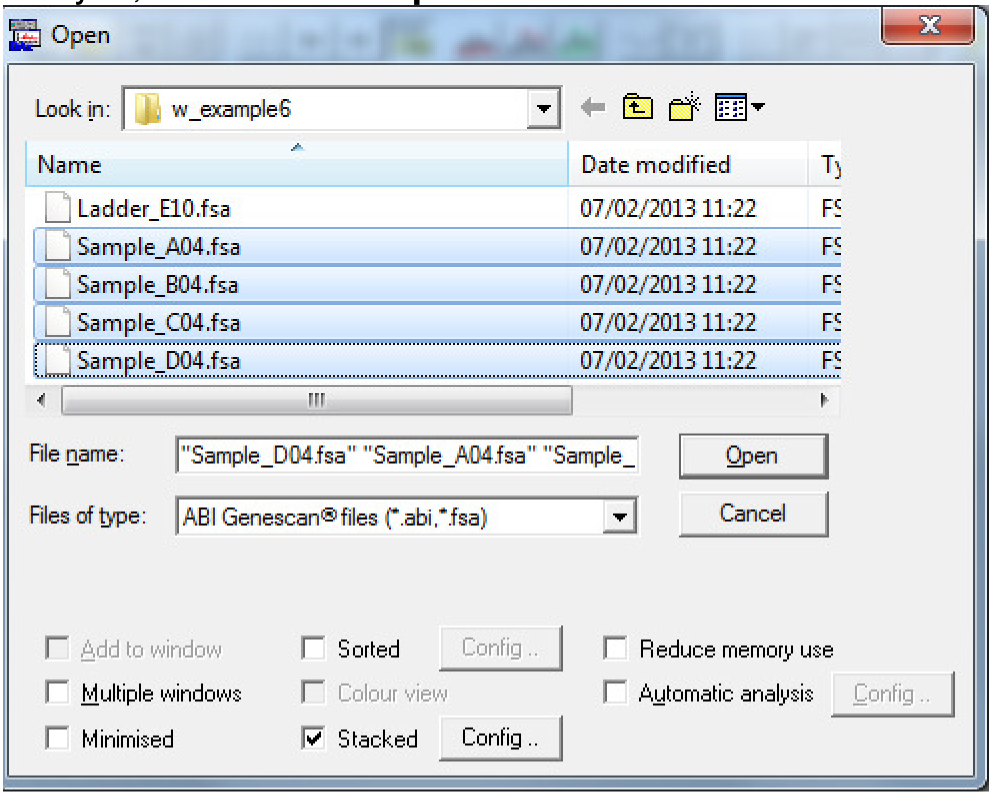
The result might look like this:



Despite running at different times, all 5 calibrations are well recognised.

Chapter 4. Analysis of the Unknowns

Invoke the **File** | **Open** command. Select **Files of Type** ABI Genescan files (or one of the other trace file types: SCF files, MegaBACE files CEQ files), Select the file or files you want to analyse, then click the **Open** button.



Open

Look in: w_example6

Name	Date modified	Ty
Ladder_E10.fsa	07/02/2013 11:22	FS
Sample_A04.fsa	07/02/2013 11:22	FS
Sample_B04.fsa	07/02/2013 11:22	FS
Sample_C04.fsa	07/02/2013 11:22	FS
Sample_D04.fsa	07/02/2013 11:22	FS

File name: "Sample_D04.fsa" "Sample_A04.fsa" "Sample_

Files of type: ABI Genescan® files (*.abi,*.fsa)

☐ Add to window
☐ Multiple windows
☐ Minimised
☐ Sorted
☐ Colour view
☑ Stacked
☐ Reduce memory use
☐ Automatic analysis

Check **Multiple windows** if you want each file to be put in its own window (not typically used for trace analysis).

If you are opening hundreds or thousands of files, you can check **MInimised**. The window into which the files are loaded will be created minimized, so that the traces are not drawn, saving time.

Check **Sorted** and use the **Config** button to indicate how the data will be sorted after loading. For instance, the data can be sorted by well or plate name.

Opening files as a **Colour view** creates a gel type view of the data immediately upon loading.

You can immediately display the data **Stacked**, and use its **Config** button to indicate how to stack.

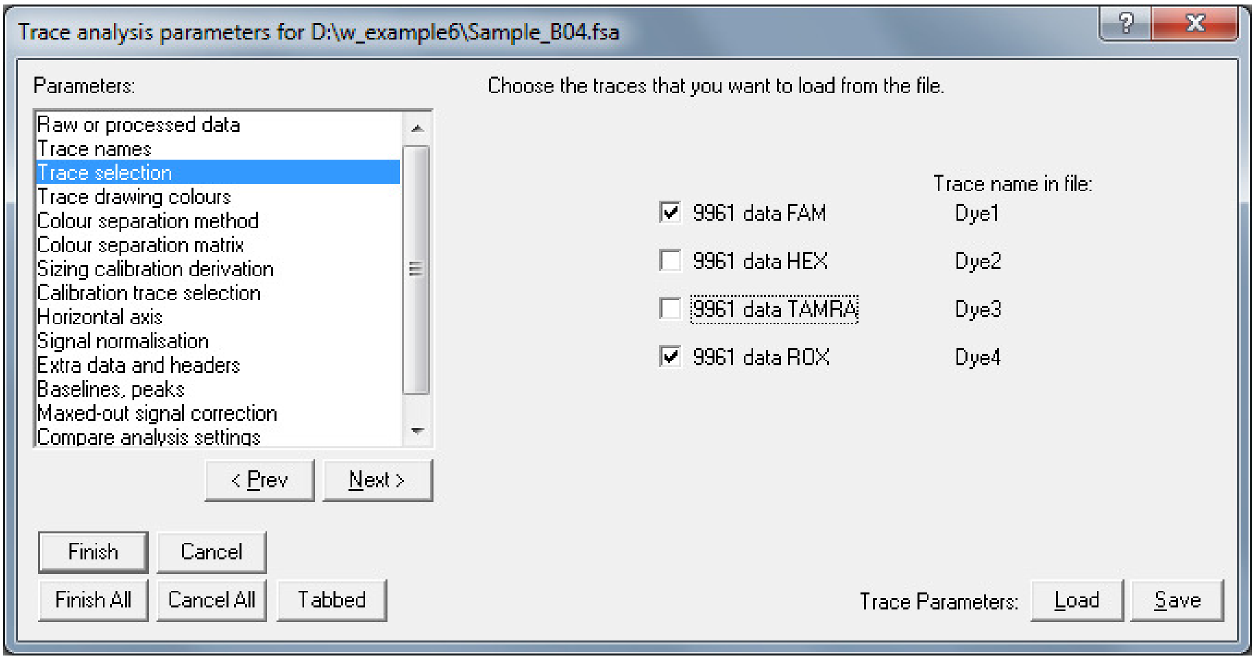
Automatic analysis is used to create baselines and find peaks immediately upon loading. For trace files, using the TAP dialog (see below) is the preferred method, so do not check this option.

Click the **Open** button to start opening all selected sample files.

Chapter 4.1.Trace Analysis Parameters (TAP) dialog

As was mentioned in the previous chapter, when trace files are opened, the Trace Analysis Parameters (TAP) dialog is displayed, so that the way in which the data are to be loaded can be set up. Since we are now analyzing the data, some changes will be made.

Trace selection



Trace analysis parameters for D:\w_example6\Sample_B04.fsa

Parameters:

- Raw or processed data
- Trace names
- Trace selection
- Trace drawing colours
- Colour separation method
- Colour separation matrix
- Sizing calibration derivation
- Calibration trace selection
- Horizontal axis
- Signal normalisation
- Extra data and headers
- Baselines, peaks
- Maxed-out signal correction
- Compare analysis settings

Choose the traces that you want to load from the file.

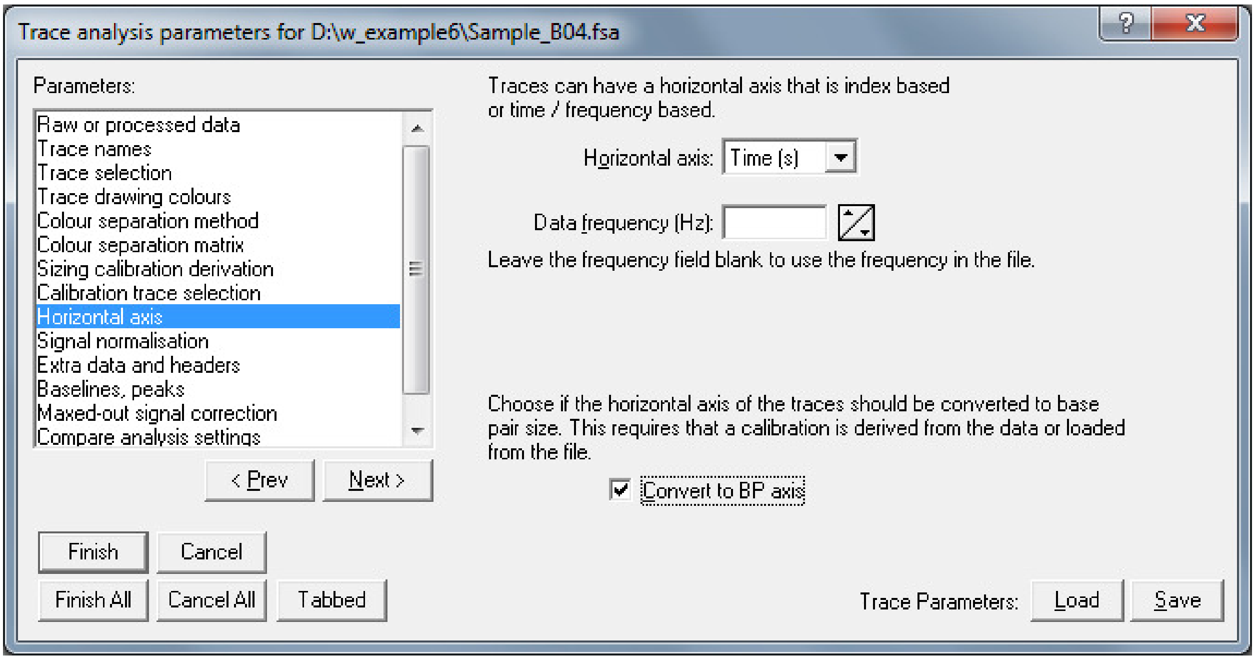
	Trace name in file:
☑ 9961 data FAM	Dye1
☐ 9961 data HEX	Dye2
☐ 9961 data TAMRA	Dye3
☑ 9961 data ROX	Dye4

The example files only contain data in the FAM trace, so only FAM and ROX are checked[7].

---

[7] It is even possible to check only FAM! The ROX trace will temporarily be loaded to set up the calibration, and will then be discarded.

Horizontal axis


Trace analysis parameters for D:\w_example6\Sample_B04.fsa

Traces can have a horizontal axis that is index based or time / frequency based.

Horizontal axis: Time (s)

Data frequency (Hz):

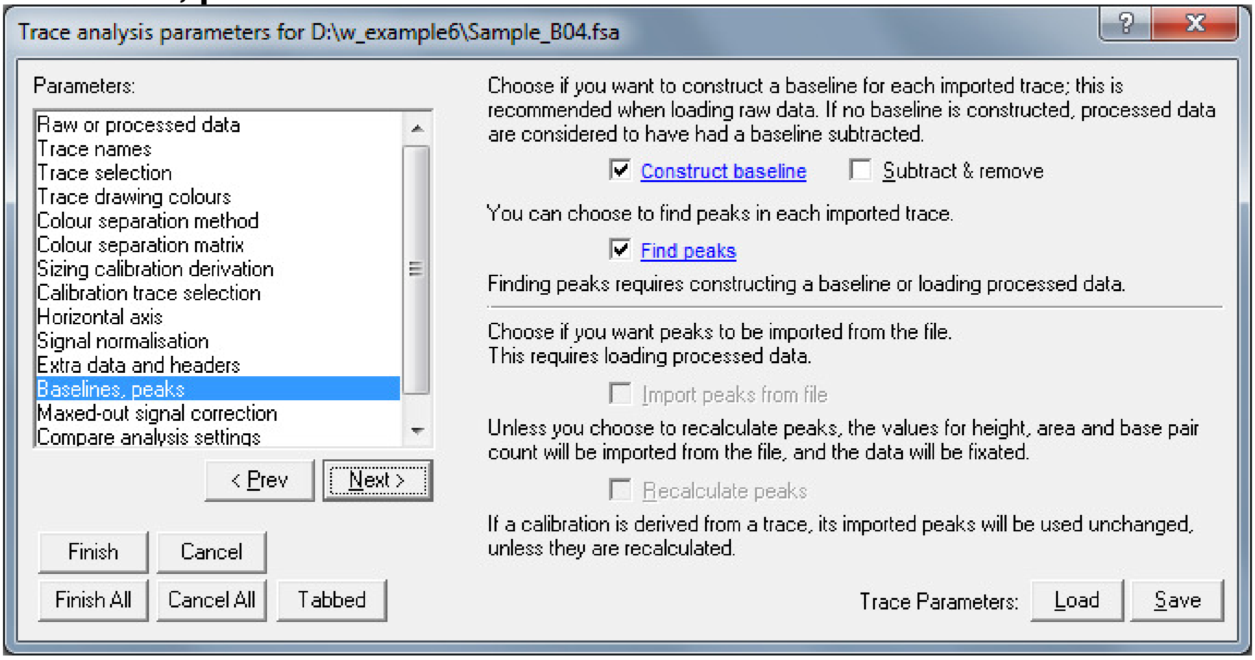
Leave the frequency field blank to use the frequency in the file.

Choose if the horizontal axis of the traces should be converted to base pair size. This requires that a calibration is derived from the data or loaded from the file.

☑ Convert to BP axis

This time, the horizontal axis will immediately be converted to a BP axis.

Baselines, peaks


Trace analysis parameters for D:\w_example6\Sample_B04.fsa

Choose if you want to construct a baseline for each imported trace; this is recommended when loading raw data. If no baseline is constructed, processed data are considered to have had a baseline subtracted.

☑ Construct baseline ☐ Subtract & remove

You can choose to find peaks in each imported trace.

☑ Find peaks

Finding peaks requires constructing a baseline or loading processed data.

Choose if you want peaks to be imported from the file. This requires loading processed data.

☐ Import peaks from file

Unless you choose to recalculate peaks, the values for height, area and base pair count will be imported from the file, and the data will be fixated.

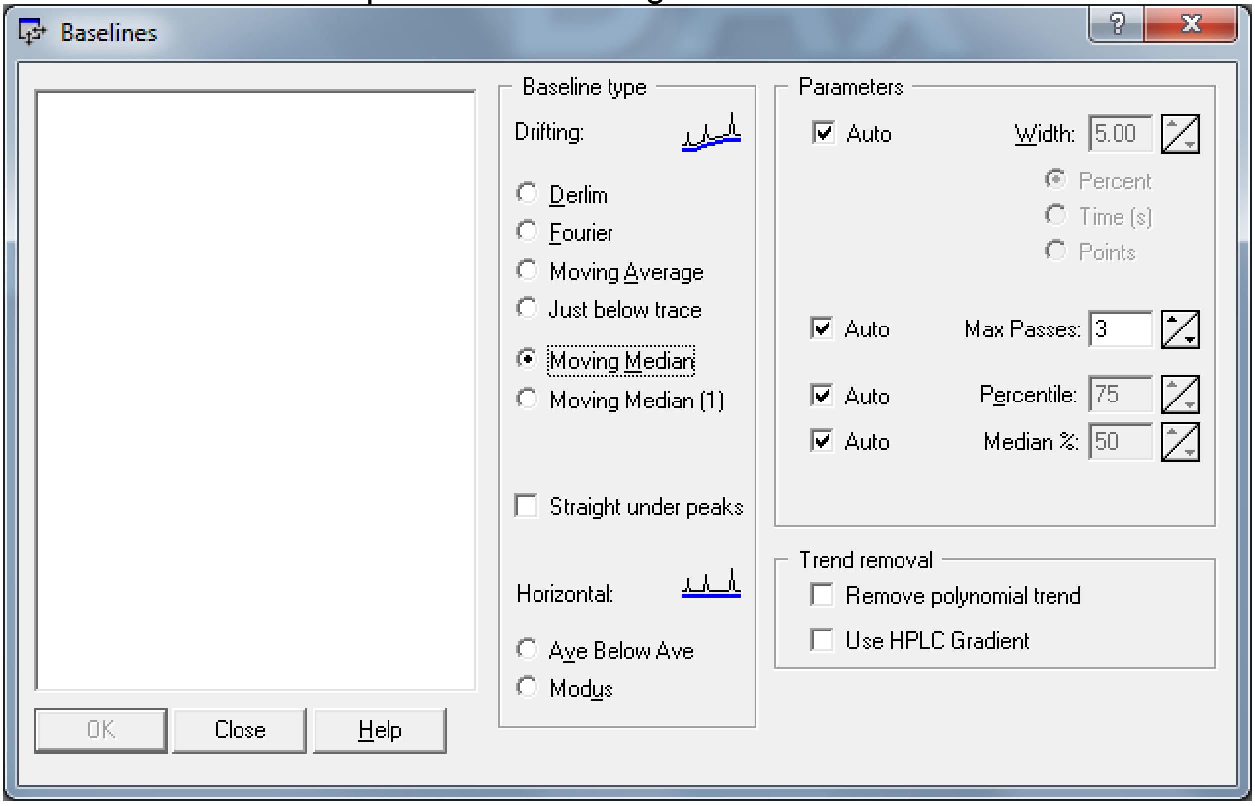
☐ Recalculate peaks

If a calibration is derived from a trace, its imported peaks will be used unchanged, unless they are recalculated.

For both the ROX and FAM traces, a baseline will be constructed and peaks will be found.

Let’s look at the baseline construction and peak find parameters.

Click on the blue underlined **Construct baseline** to display the baseline construction parameters dialog.



Baseline type

Drifting:
○ Derlim
○ Fourier
○ Moving Average
○ Just below trace
● Moving Median
○ Moving Median (1)

☐ Straight under peaks

Horizontal:
○ Ave Below Ave
○ Modus

Parameters

☑ Auto Width: 5.00
● Percent
○ Time (s)
○ Points
☑ Auto Max Passes: 3
☑ Auto Percentile: 75
☑ Auto Median %: 50

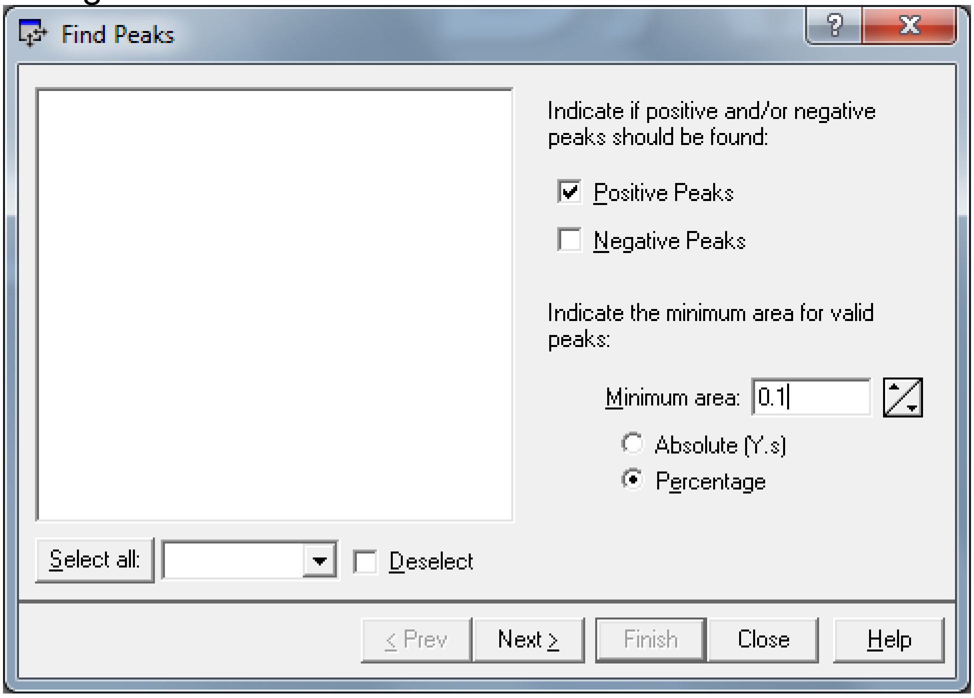
Trend removal

☐ Remove polynomial trend
☐ Use HPLC Gradient

The baseline type has been changed to **Moving Median**, but elsewhere all settings have been left at factory defaults.

When items are checked to use **Auto** values, DAx analyses the data to find values that fit the data being analysed.

Click on the blue underlined **Find peaks** to display the peak find dialog box.



Indicate if positive and/or negative peaks should be found:

☑ Positive Peaks

☐ Negative Peaks

Indicate the minimum area for valid peaks:

Minimum area: 0.1

○ Absolute (Y.s)

● Percentage

Trace files should not contain negative peaks, so they are not being found.

The peak find dialog contains a further 7 pages, dealing with:

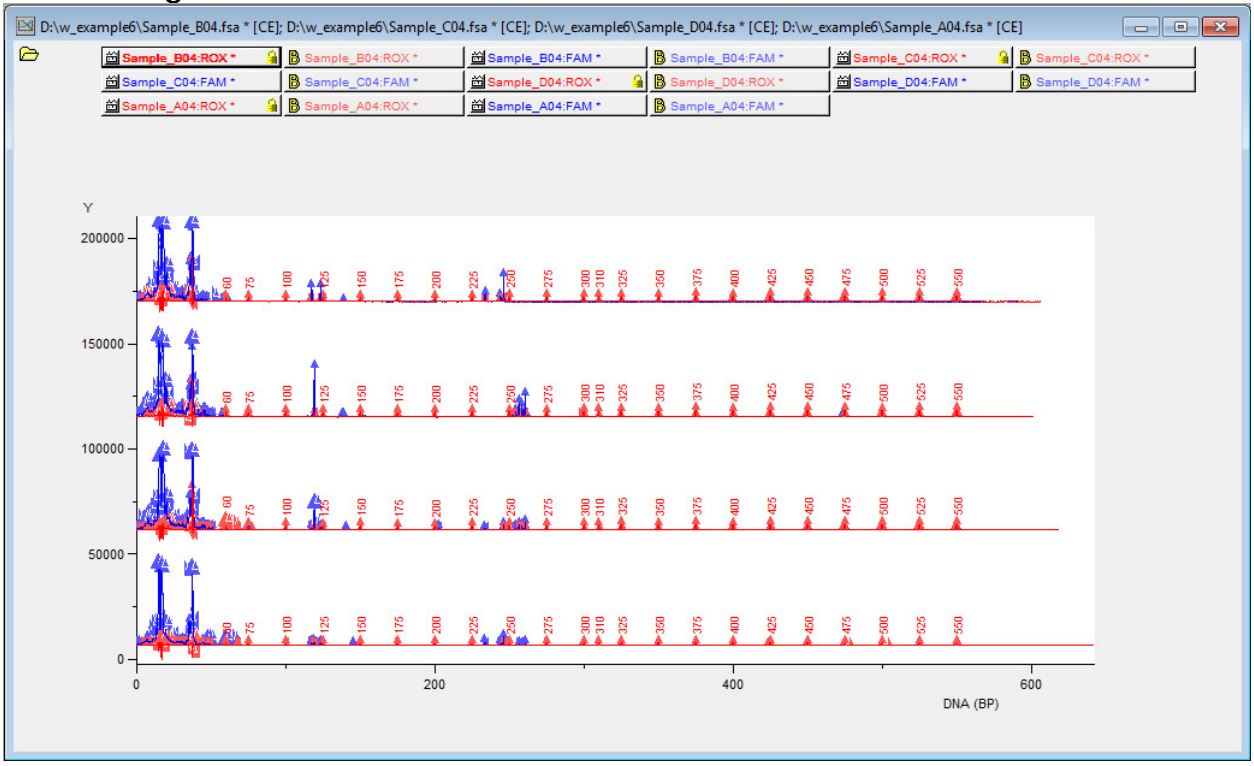
- peak find signal and slope thresholds
- skipping an initial part of the measurement
- detecting shoulder peaks
- normalising peak widths to a set number of base pairs
- removing bleed-through peaks

All these settings can be left at factory defaults in almost all analyses.

To finish filling out the TAP dialog, click the **Finish** button. If more than one data file was selected in the **File | Open** dialog, click **Finish All** to load all data without showing the TAP dialog again.

Chapter 4.2.Results

The FAM and ROX traces will now be loaded and analysed. The results might look like this.



The following has happened in succession.

- For each file, the ROX trace is loaded. It was analysed using the current settings. Since the Automatic Trace Calibration parameters define ROX as the calibration trace, the sizing standard was recognised, and a calibration relating time coordinates to fragment sizes was derived from the ROX trace.
- the FAM traces were loaded and analysed using the current settings.
- The axis was converted to base pairs using the calibration derived from the ROX trace.

You can now click any of the peak list buttons at the left side of the data tags for the ROX or FAM traces. You will note that the correct fragment sizes are listed in the Base Pairs column.

It is also possible to display the fragment sizes of the peaks that were found in the graphics window. To do this, invoke the **File | Customise** menu option. Select the **Plotting Peaks** tab. Under Peak Labeling, select **Component Name** as the first peak label.

]

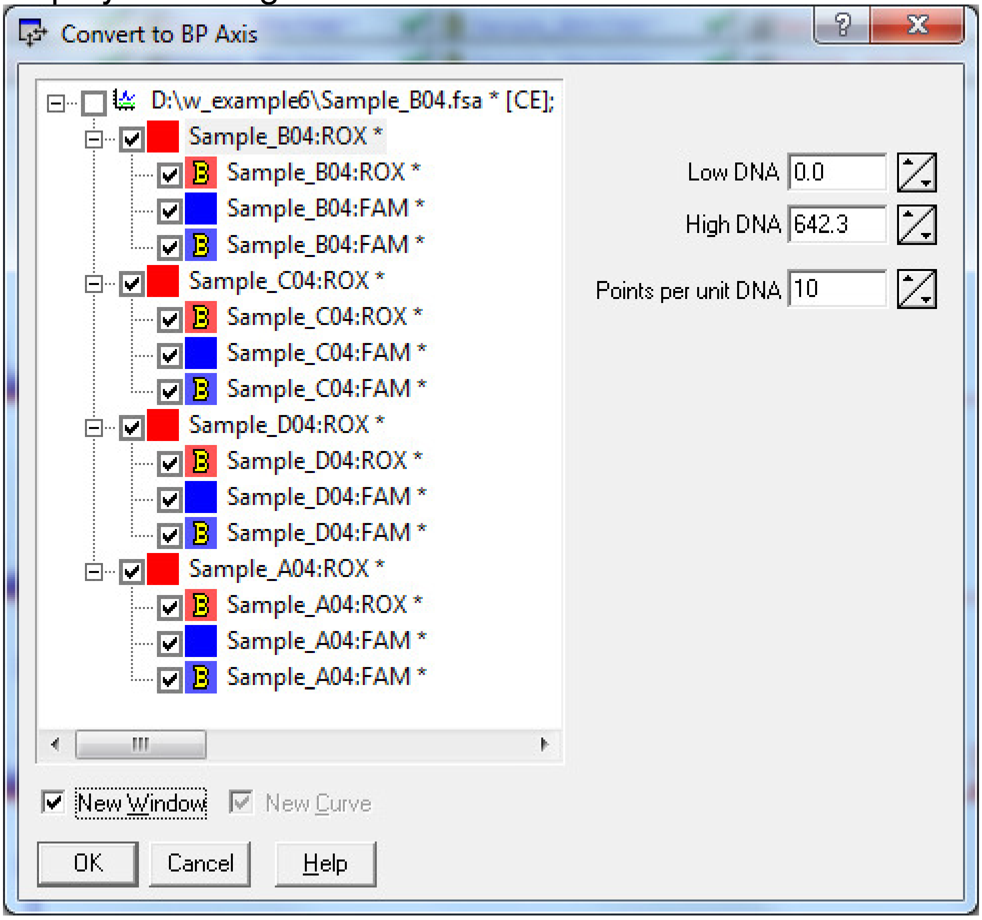
Select **Base Pairs** as the second peak label (it is near the bottom of the list of possible peak labels). Click the **OK** button.

The graphic window will now display base pair counts for all the peaks. You can click and drag the mouse on the graph to zoom in on the peaks, and see more detail.

Chapter 4.3. Converting to a Base Pair axis

In the example above, the horizontal axis was immediately converted to a BP axis, by checking **Convert to BP** axis in the TAP dialog.

If this option had not been checked, the axis can still be converted using the **Calibration | Axis Conversion** menu option. This displays a dialog box.



Low DNA 0.0

High DNA 642.3

Points per unit DNA 10

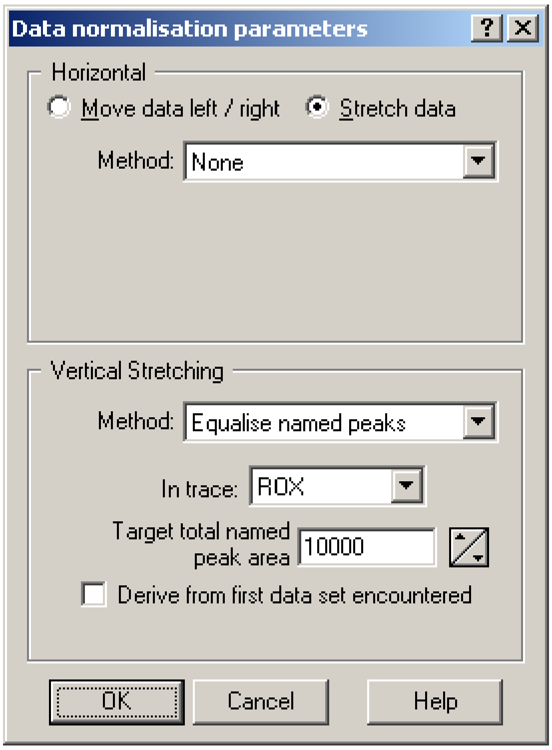
☑ New Window ☑ New Curve

Make sure to check the **New Window** check box, and then make sure that all data are selected. *Note that the dialog box was resized to make all data items visible by dragging its bottom right corner.*

Chapter 4.4. Normalising the data

When loading trace data, it can be desirable to normalise the data after they have been loaded. One common form of normalisation stretches the data vertically in such a way that the total area of the size calibration peaks is equal across several measurements.

Normalisation can be achieved after data have been loaded using the **Data** | **Overlay** menu option. A more convenient way to normalise is to check the **Normalise data** check box on the **Signal normalization** page in the Trace Analysis Parameters dialog box. Click the **Config** button to set up normalisation.



Data normalisation parameters

Horizontal

○ Move data left / right ● Stretch data

Method: None

Vertical Stretching

Method: Equalise named peaks

In trace: ROX

Target total named peak area 10000

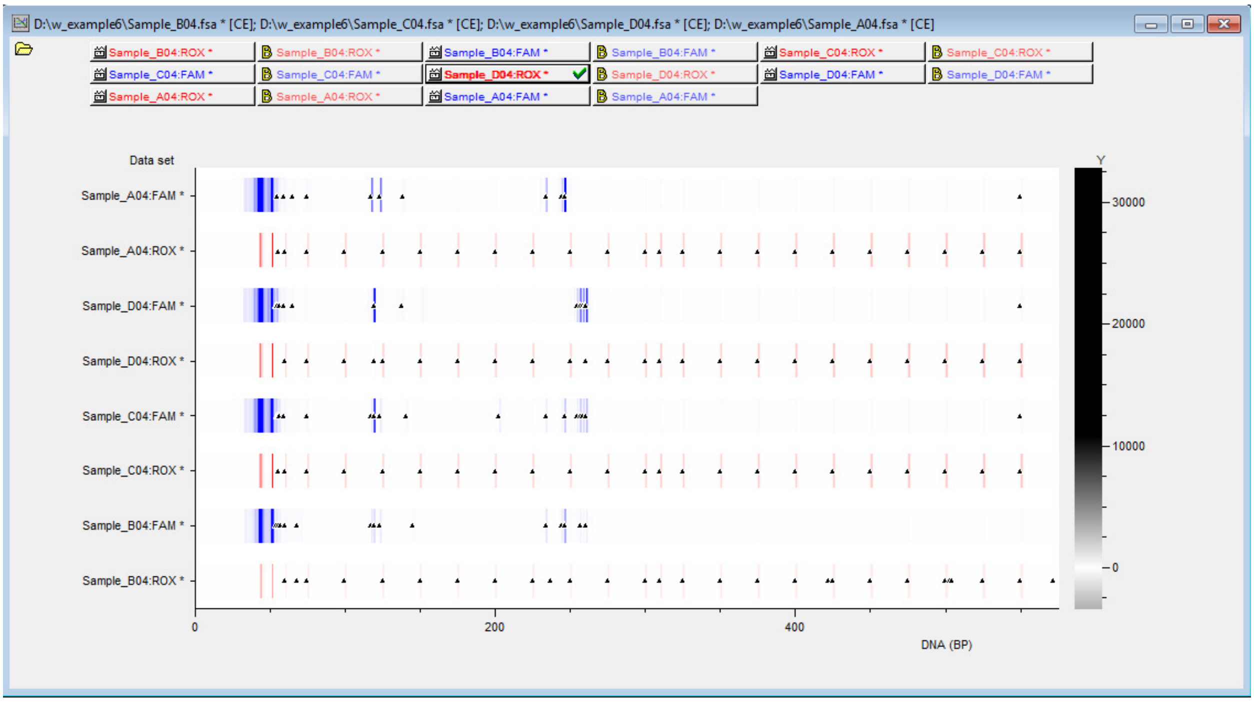
☐ Derive from first data set encountered

In this example, the horizontal axis is not affected.

The vertical axis will be stretched in such a way that the total area of all named peaks in the ROX trace will become 10000. *Named* peaks are used because the named peaks in the ROX trace will be the calibration standard (as called by ATC).
The other trace (FAM) will be stretched an equal amount as the ROX data.

Chapter 4.5. Displaying data as a colour view

Use the **View | Colour view** menu option to display the data as a colour (gel) view.



As always, you can zoom in on the colour view by clicking and dragging the mouse.

The colour view window's **View menu** has a number of options that govern how things are displayed. In the example above, there is **extra space** between lanes, each trace is displayed using its own **trace colour**, colours are displayed **darker** to make them easier to read, the baseline has been **subtracted**, each trace is in a **separate lane** (as opposed to have all traces from a file displayed in a single lane), a **calibrated axis (BP)** is used, and peaks are **marked**.

Chapter 5. Trace Types in DAx

Trace types are used in DAx for a number of purposes.

Their principal use is to distinguish between up to five traces that can be contained in a single measurement file, where obviously the file name alone would be insufficient.

A secondary use is to limit certain operations to specific trace types. These operations include:

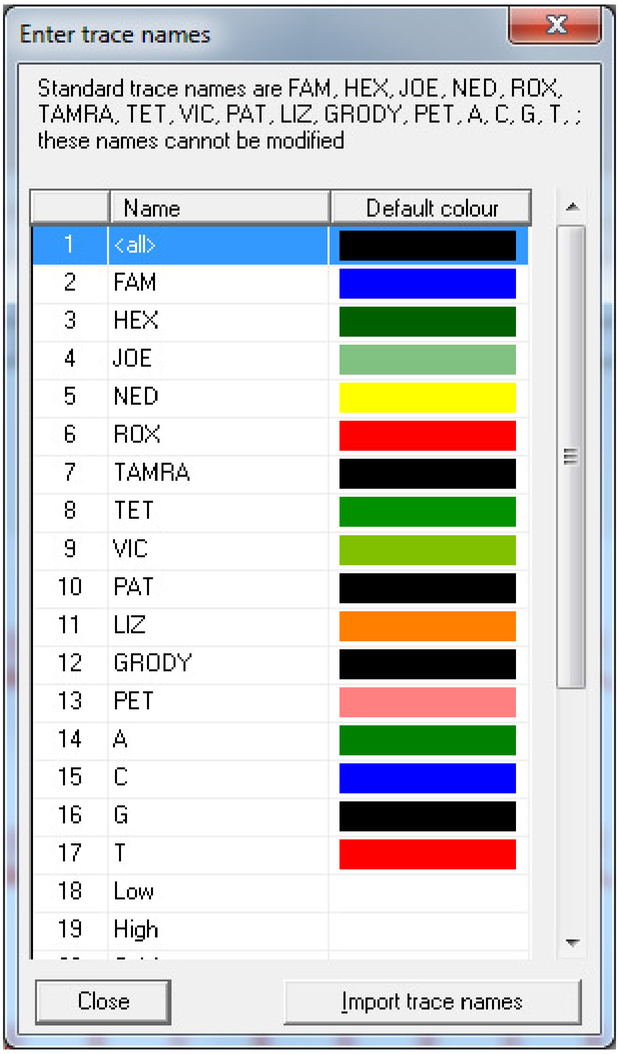
- Automatic Trace Calibrations. The trace type is used to indicate which trace contains the sizing calibration, cf Chapter 2.1.
- Normalise using the total peak area of named peaks of a specified trace type, cf. Chapter 4.4.
- Identification Database and Marker Peak items. The trace type is used to limit the detection of certain components to a specified trace type.

Chapter 5.1. Assigning trace types

DAx attempts to retrieve the trace type of each trace from the measurement file, which often (but not always) contains trace names. The trace names are displayed in the TAP dialog, cf. Chapter 3.1.

The standard trace types that DAx recognises are FAM, HEX, JOE, NED, ROX, TAMRA, TET, VIC, PAT, LIZ, GRODY, and PET. Mainly for use with SCF files, there are also A, C, G and T trace types.

The **Config trace names** button in the TAP dialog allows additional trace types to be added. A dialog box like this one is displayed:



Standard trace names are FAM, HEX, JOE, NED, ROX, TAMRA, TET, VIC, PAT, LIZ, GRODY, PET, A, C, G, T, ; these names cannot be modified

	Name	Default colour
1	<all>	⬛
2	FAM	🟦
3	HEX	🟩
4	JOE	🟩
5	NED	🟨
6	ROX	🟥
7	TAMRA	⬛
8	TET	🟩
9	VIC	🟩
10	PAT	⬛
11	LIZ	🟧
12	GRODY	⬛
13	PET	🟥
14	A	🟩
15	C	🟦
16	G	⬛
17	T	🟥
18	Low	
19	High	

Chapter 6. Conclusion

DAx can be used to perform highly complex analyses of trace files with relatively little effort.

There are a large number of additional features that have not been discussed in this manual. Examples are:

- peak width standardisation. If a sample is known to contain mostly peaks that have a width of 1 fragment, DAx can be told to preferentially assign peak widths of 1 fragment. If a peak is *clearly* wider, its width will not be affected.
- command line analyses. Typically, trace analysers will be used to perform dozens to hundreds of analyses per day. To make it possible to analyse such large quantities of data, DAx can be run from batch files. All the data on an entire hard disk can be analysed with a single command, while highly flexible report files are created.

These features exceed the scope of this manual. Refer to the DAx User's Manual for details.