

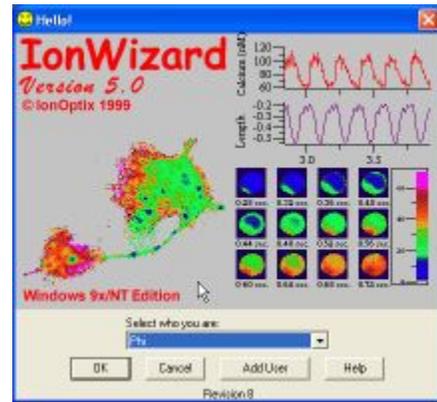
IonWizard in 18 Steps

This tutorial was written to provide a step-by-step guide for how to perform a standard analysis on a datafile with contractility and/or calcium transients. Not all the features of Ionwizard are thoroughly described, just the steps necessary to perform a data averaging procedure and subsequent transient analysis. All the pictures in this tutorial were obtained while analyzing the demo file that can be downloaded from the website as well.

Download the data file “IonWizard DEMO.zpt” from our Product Support page on our website www.ionoptix.nl and save it in a logical place.

1. Start IonWizard.

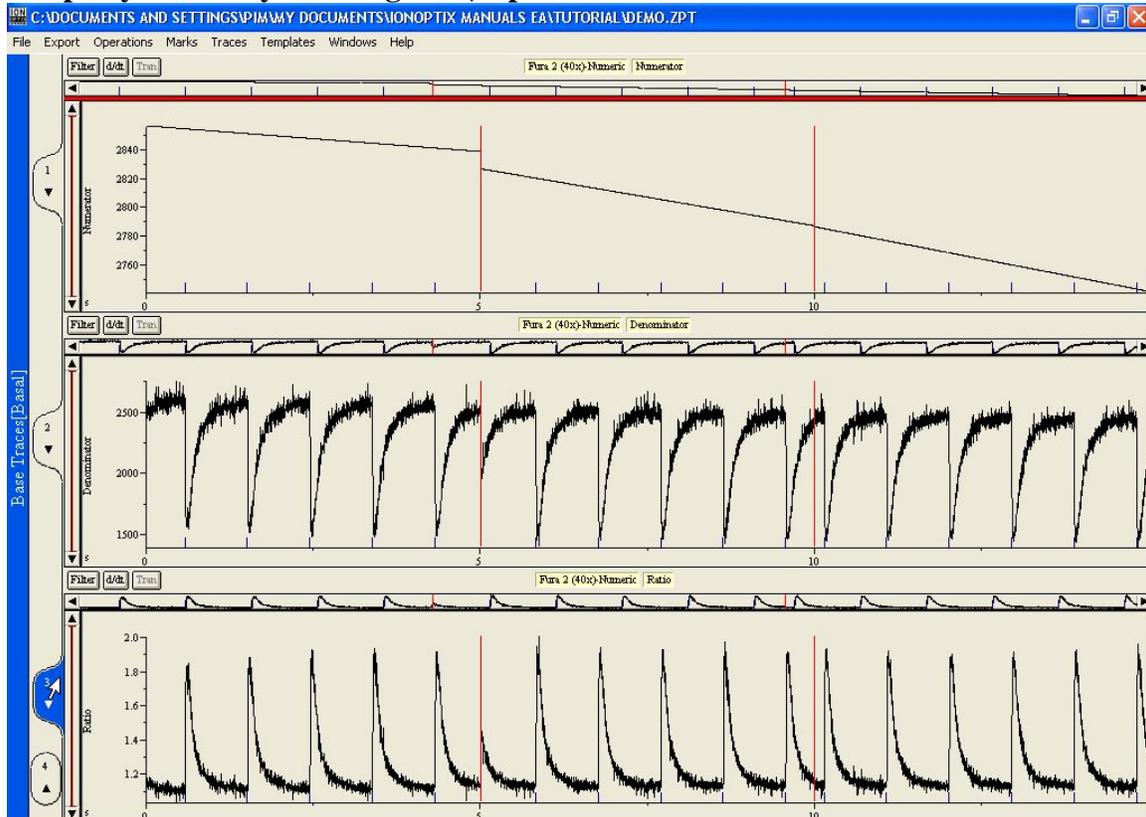
When you start IonWizard for the first time a Template File Notice will pop up, informing about the creation of the template file IONWIZ.prf in the programs directory.



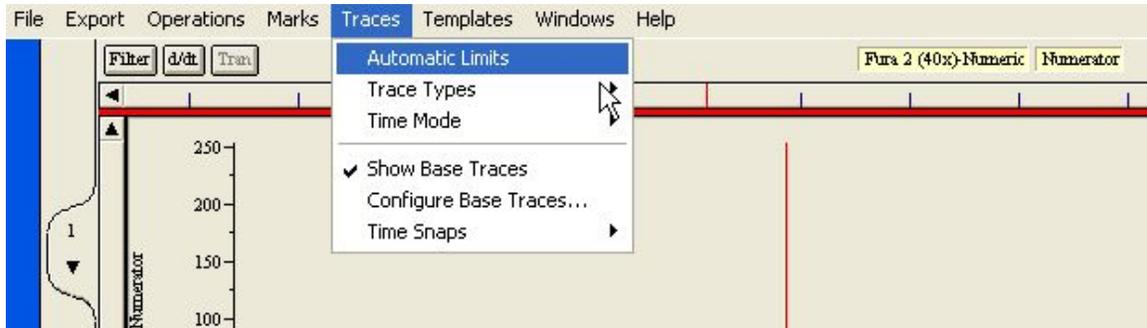
2. Enter a user name and press OK.

IonWizard opens as an empty window.

3. Open your file by selecting File | Open...



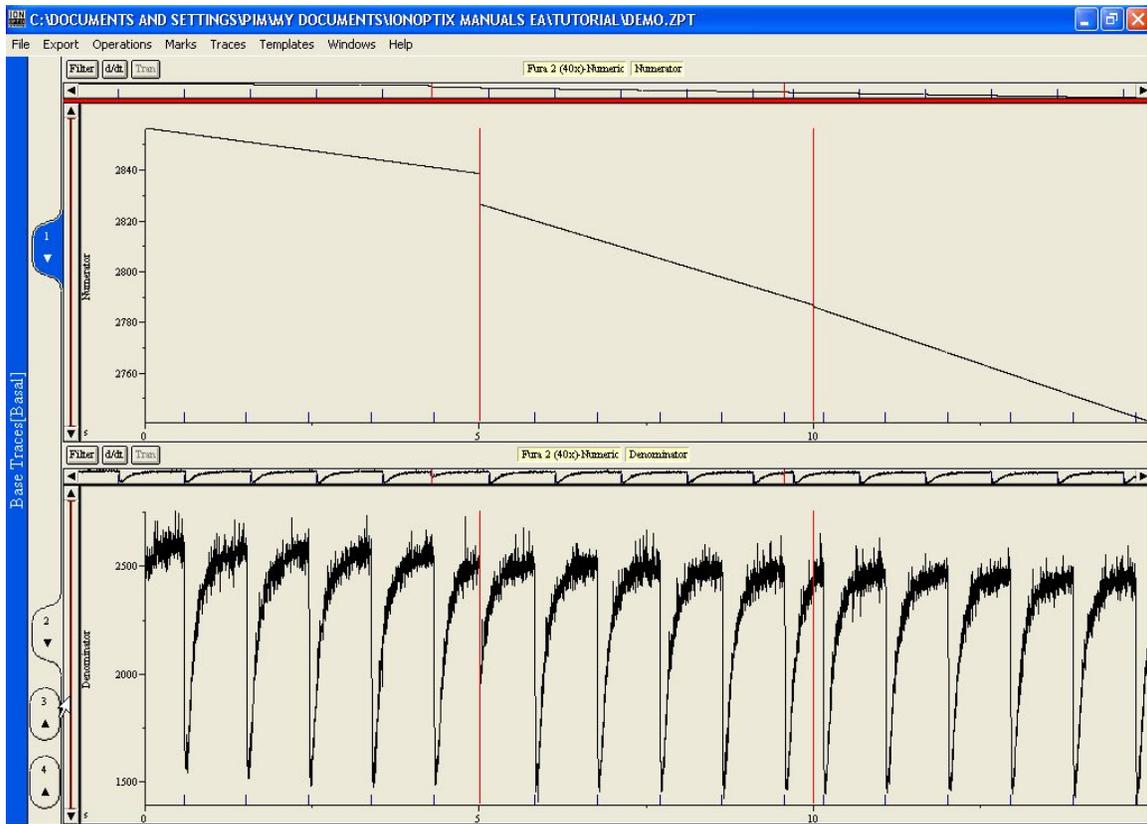
The figure above shows the looks of the IonWizard interface when the demo.zpt file is opened with the default settings of Ionwizard. A window with three visible Base Traces. When the Base Traces appear to be empty, you need to check Traces | Atomic Limits...



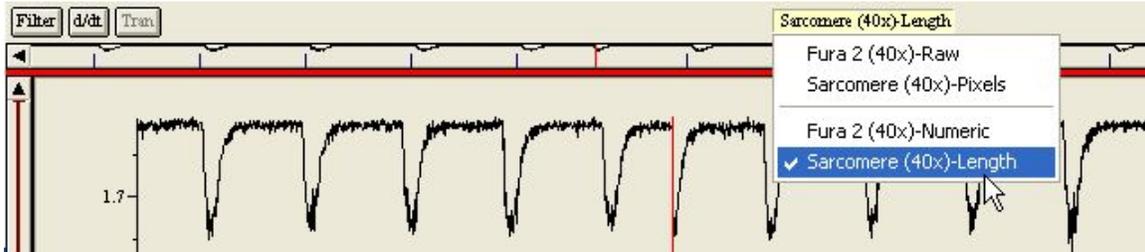
When you open Ionwizard after it has been used by other people, different templates might have been saved and the view could be different.

4. Have viewer 1 and 2 open, close viewer 3 (and maybe 4).

A viewer can be opened or closed by clicking on its tab. In the figure above, 1, 2 and 3 are open and 4 is closed. The cursor of the mouse is pointed at the tab for viewer 3 in the figure above, clicking it will close it (see below).

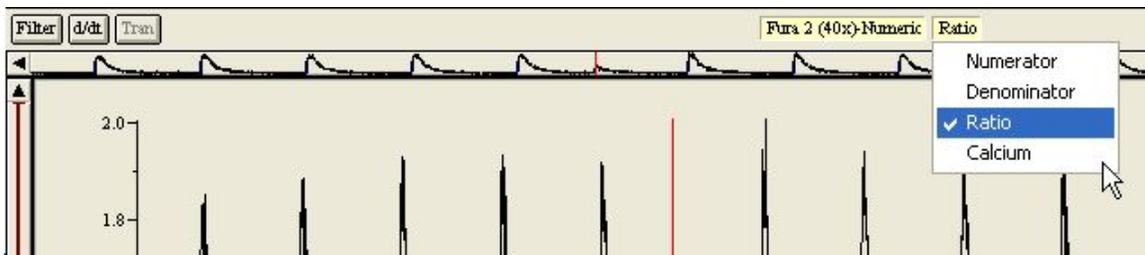


5. Select Sarcomere(40x) – length for the upper trace.



Select the right traces for each viewer by clicking on the yellow box

6. Select Ratio for Fura 2 (40x) – Numeric for the lower trace.



For viewer number 2 first select Fura-Numeric in the (left) yellow box, then ratio from the right yellow box.

The analysis strategy for this file is to first average all contractions for both sarcomere length and fura 2 and subsequently analyse the averaged trace.

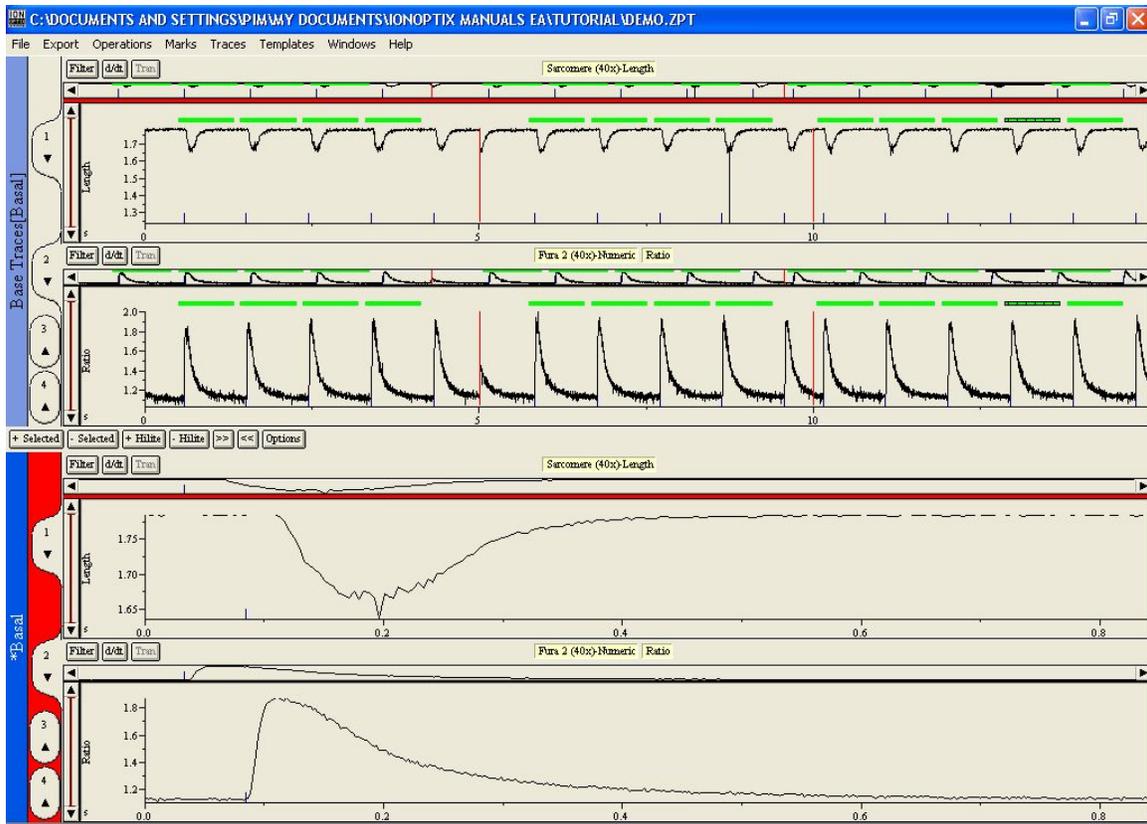
7. Select Operations | Average Events ... from the menu and press OK.

To start, create the average by going to the menu Operations and selecting Average events.

When prompted for a name for the new set of viewers that will contain the averaged traces, just click ok, and the default name of 'basal' will be used.

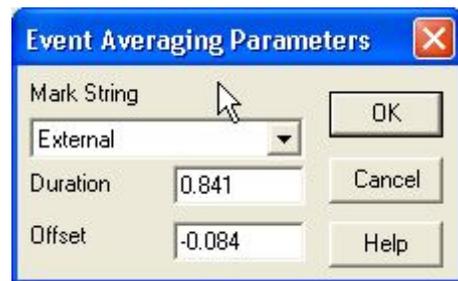
The viewer for the average event is selected by pressing the blue title bar (*Basal) on the left side. All transients that have a green bar above them are included in the average.





8. Press [Options] to check the Duration and Offset.

To change the duration of the green bars, click on the option button which will open the event averaging parameters box. For this file the values shown here are appropriate.

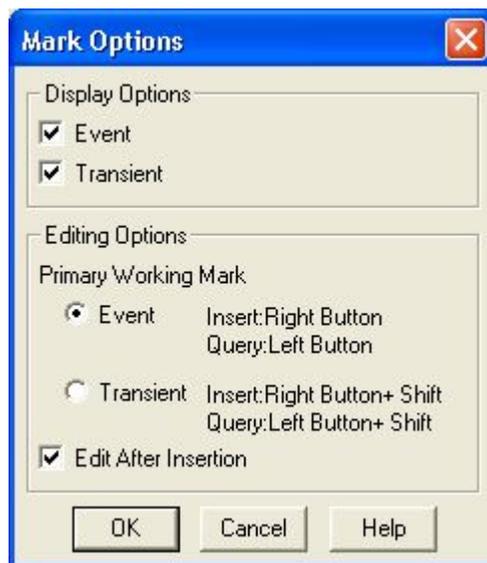


9. Select Marks | Options ... from the menu.

We now have to do a small intermediate check.

This is not something you will have to do always, but it is important that in the remainder of the tutorial we will use the same options and conventions.

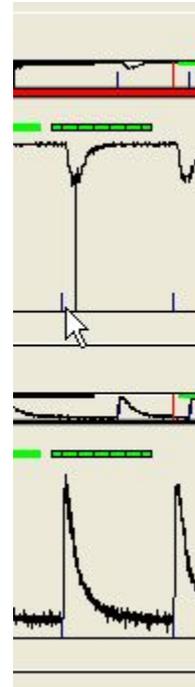
Make sure the settings are as shown in the Mark | Options window on the right.



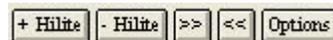
10. Select the 9th contraction in the sarcomere length trace.

The 9th contraction in the sarcomere length trace shows an acquisition error. We will remove this event from the average.

To select events from the base trace, wave the mouse cursor over the corresponding event mark. When selected, the green (or red) bar above it will be shaded.

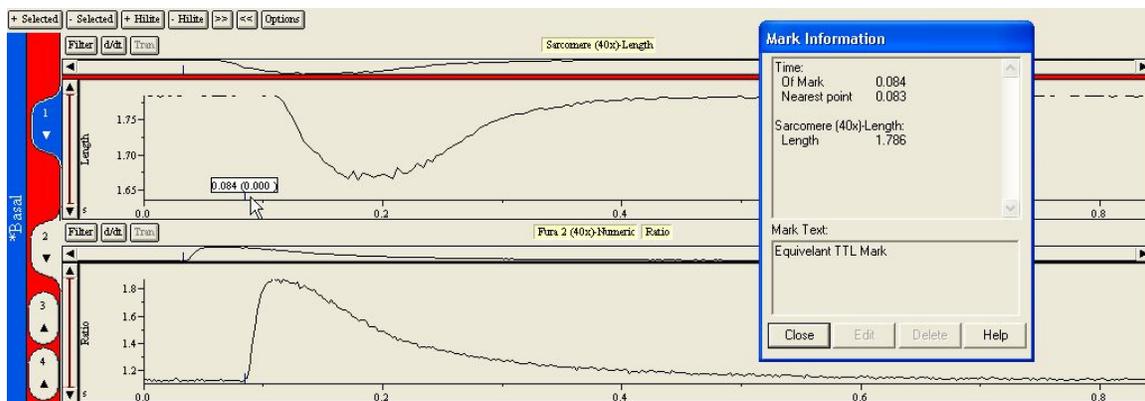


11. Press [- Hilite]



When you press [– Hilite], the selected bar will turn from green to red meaning that it has been removed from the average. You will see that the averaged transient has now changed slightly after removal of the corrupted transient.

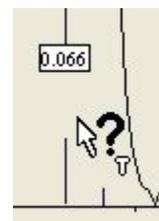
12. Move the mouse over the mark left of the average Sarcomere-Length event and press the left button to open the Mark Information view.



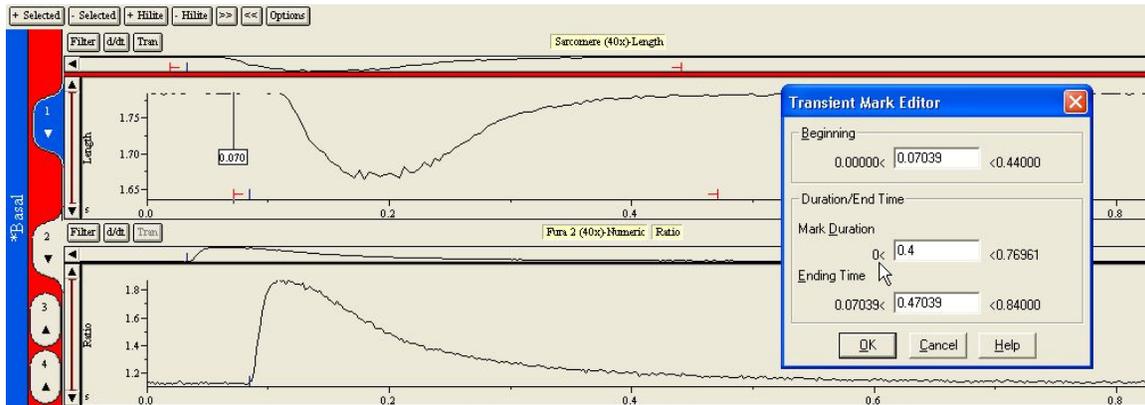
This is just to show you the information associated with the event mark such as the time of the mark in the trace. It is not a step you need to do for the analysis.

13. Keep the shift-key pressed and give a right mouse click left of the event mark of the averaged event.

For the analysis, transient marks need to be put in. This limits the part of the trace on which the computer will perform the analysis. To be able to use the event mark as the zero time of the analysis (a logical time since it represents the electrical stimulus), always open the transient marks to the left of the event mark.



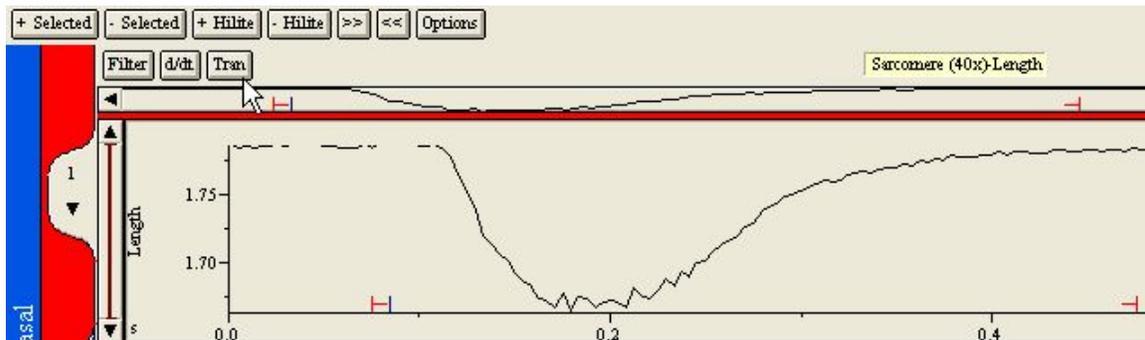
14. Set the mark duration time at 0.4 and Press OK.



For this particular trace, we found that a mark duration of approximately 0.4 seconds works well, so set the mark duration to 0.4 seconds and click 'ok'. Two red marks are now added to the average event (as in the figure above). With the shift-key pressed and moving the mouse over this new transient mark will appear. A left mouse-click will open the Mark Information view, with the possibility to edit this mark.

15. Repeat step 11 to 13 for the average Fura 2-Numeric Ratio event.

16. Press the Tran button for both the average Sarcomere and Fura 2 event



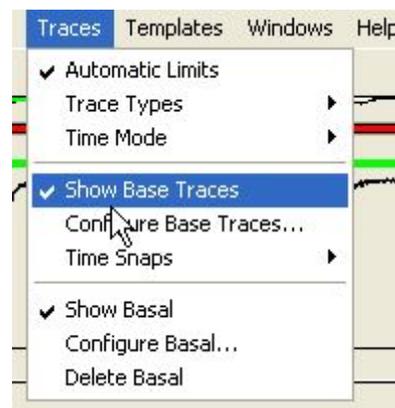
Pressing the 'tran' button will perform the analysis. This will result in the following figure on the next page.



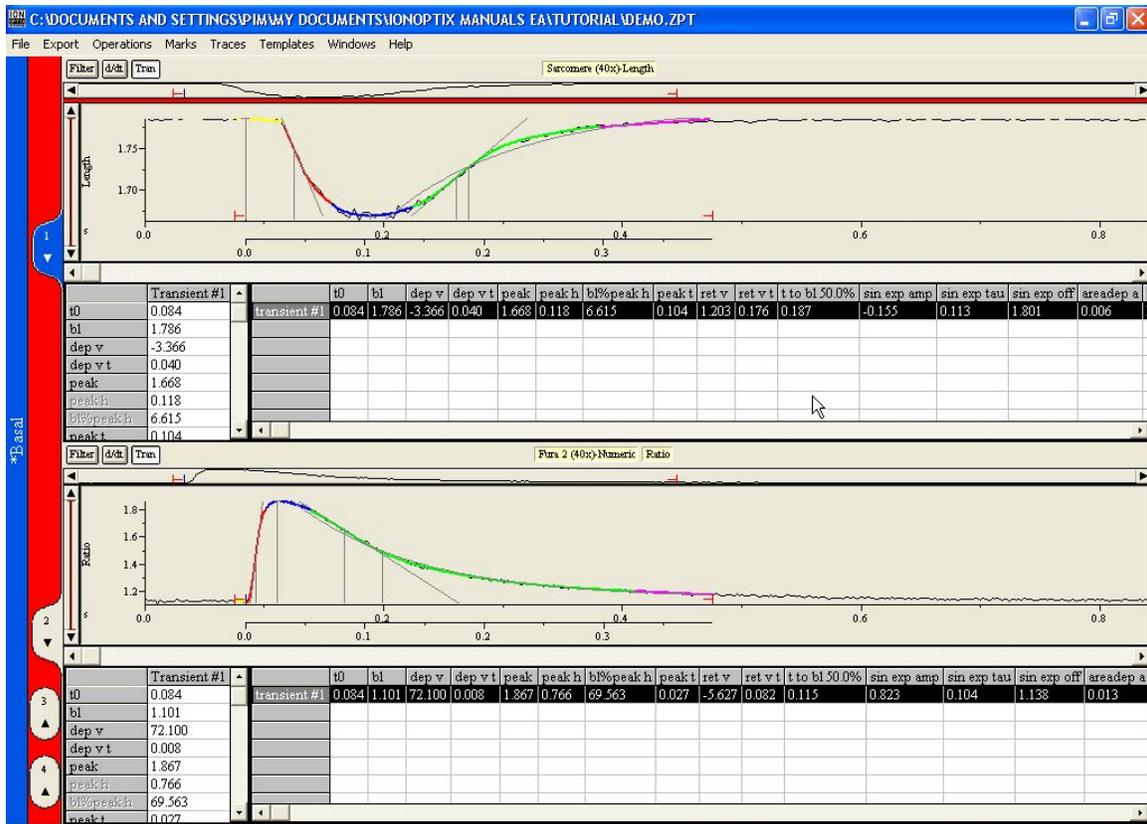
This view is obviously too crowded to make any sense of the analysis, so the next step is to not show the 'base traces' (the original 2 viewers at the top of the screen).

17. Open menu item Traces ... and deselect Show Base Traces.

In the Traces menu there is a check mark in front of both 'show base traces' and the 'show basal'. Select Show Base Traces to remove it (to make it reappear, do it again). See the picture.



The figure on the next page shows the resulting view.



With Operations | Transient Analysis Options ...you can select the output parameters you want to calculate and show.

18. Press Export | Transient Analysis | Clipboard Main ... from the menu to export the output parameters. Open a spreadsheet program to copy the results.

To export the results from the analysis, they can be saved in a file, or copied to the clipboard.