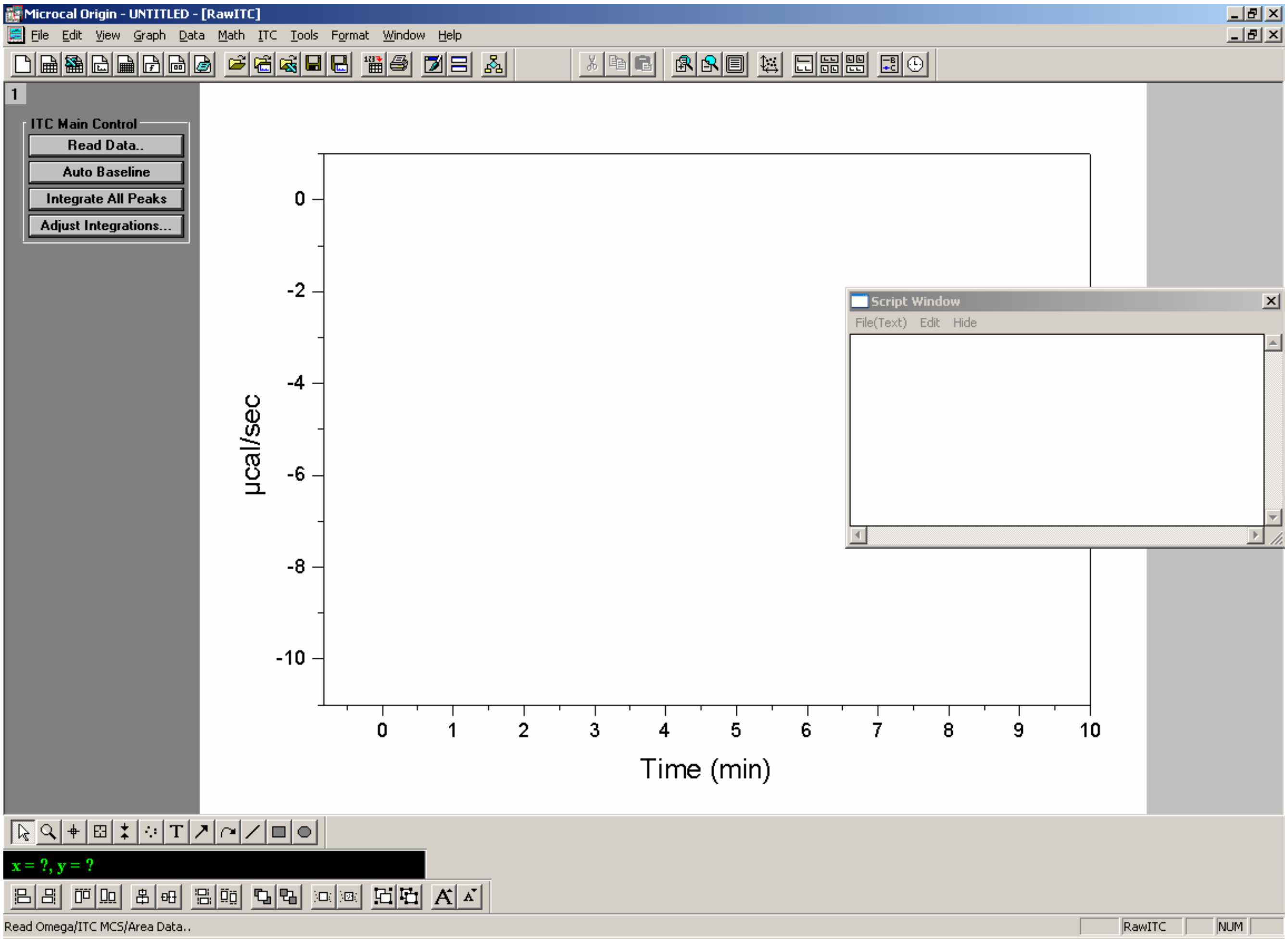
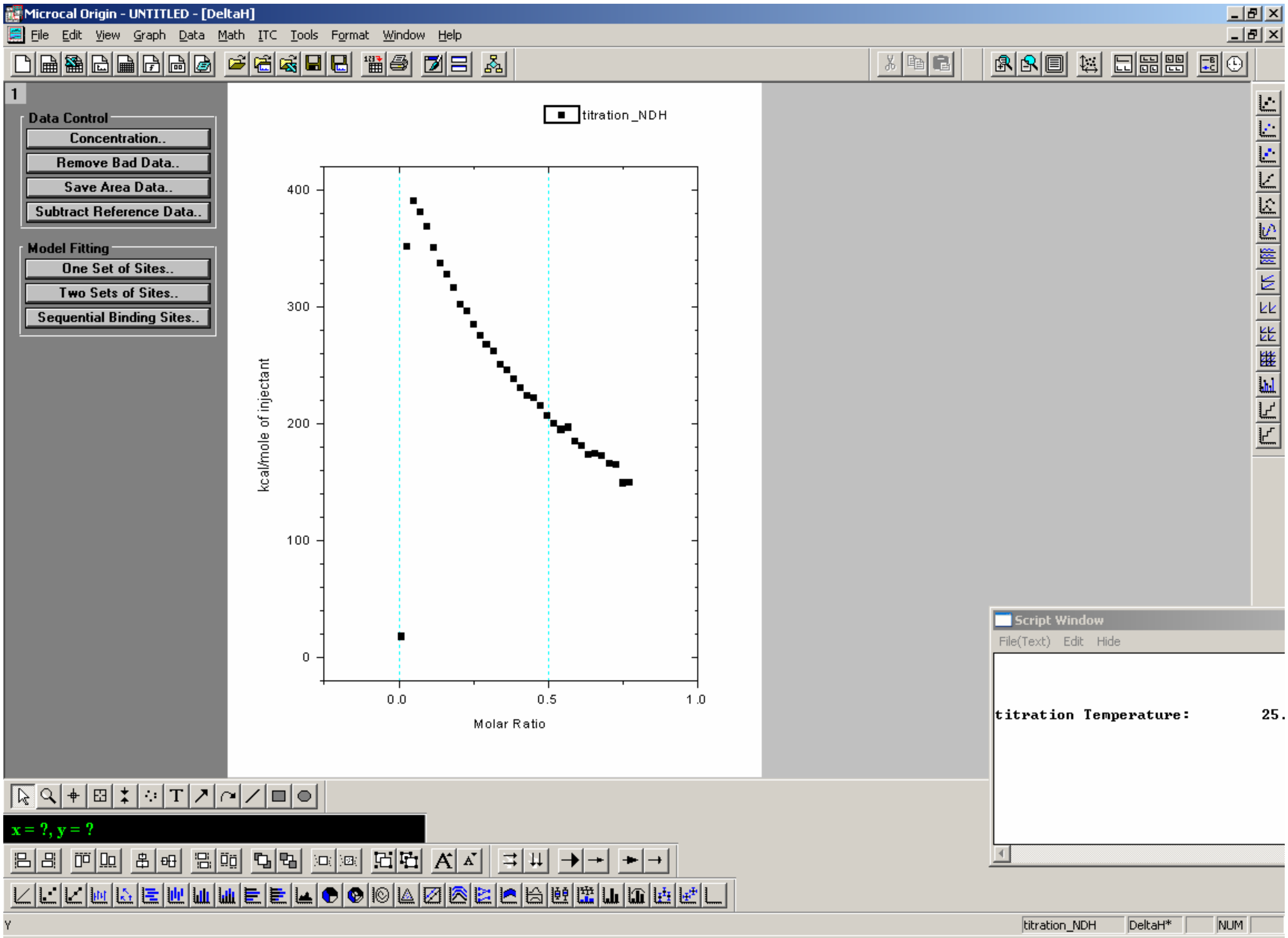
Processing ITC data with point-by-point reference subtraction using ORIGIN 5.0

1. Launch the MicroCal processing software (Origin 5.0 with Microcal ITC feature) Microcal Inc. ITC.lnk icon on a desktop.
2. The following window appears:



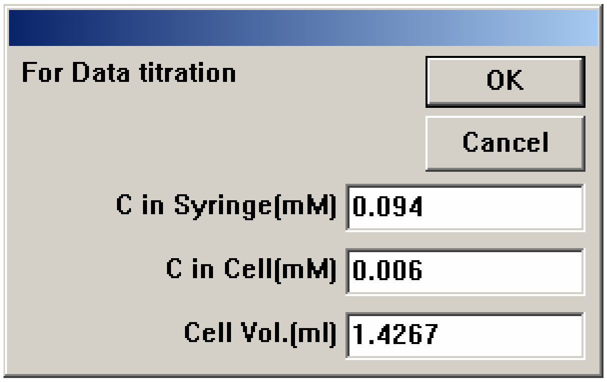
3. Click “Read Data” and open the data file : “*.itc” in this case “titration.itc”

4. Once the data file is loaded, the following window appears:



```
titration Temperature:        25.
```

5. At this point the concentrations could to be adjusted if needed before loading the reference data set by choosing “concentration” button.



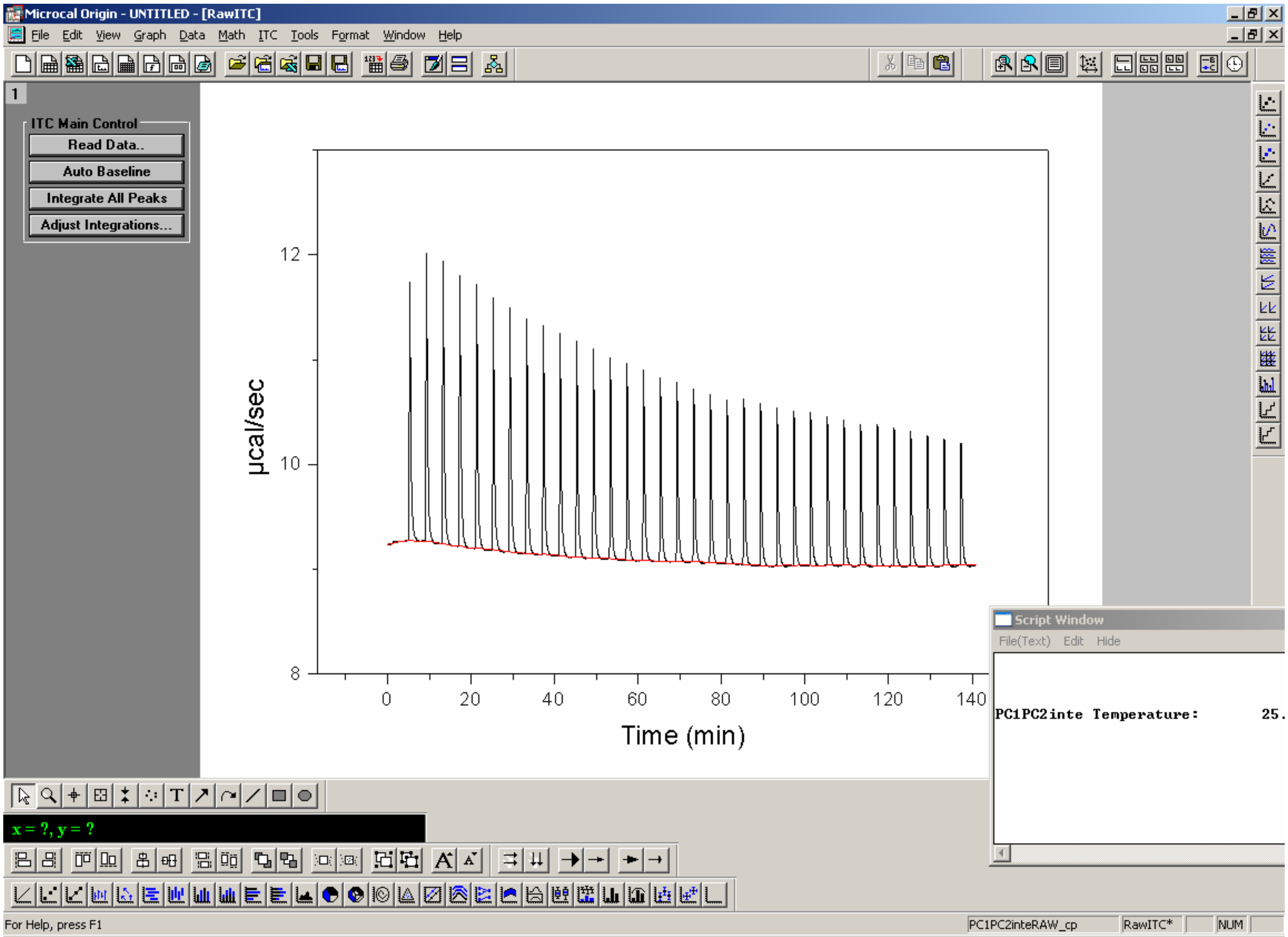
For Data titration

C in Syringe(mM) 0.094

C in Cell(mM) 0.006

Cell Vol.(ml) 1.4267

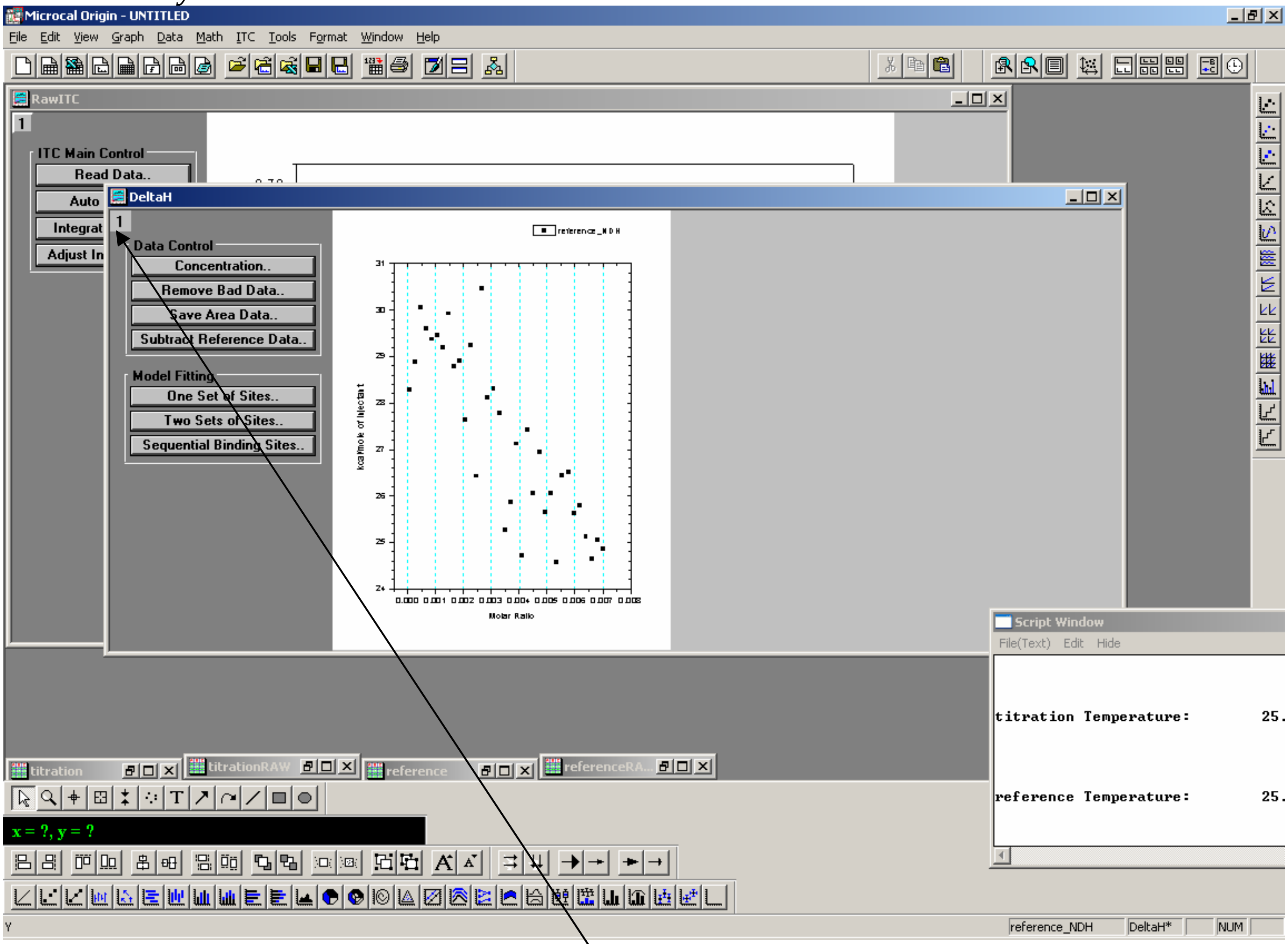
6. If the data file has correct concentration values, go to “window” and choose “raw ITC’, which will lunch the following display:



```
PC1PC2inte Temperature:       25.
```

7. At this point a second data set containing the reference titration can be lunched by choosing “read data” button.

8. Load the appropriate reference data:



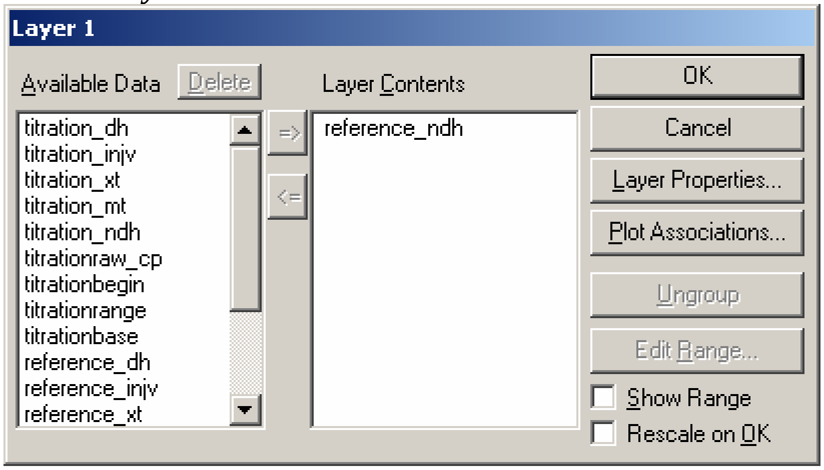
```
titration Temperature:       25.

reference Temperature:       25.
```

9. Make sure the concentrations match the values entered into “titration” data.

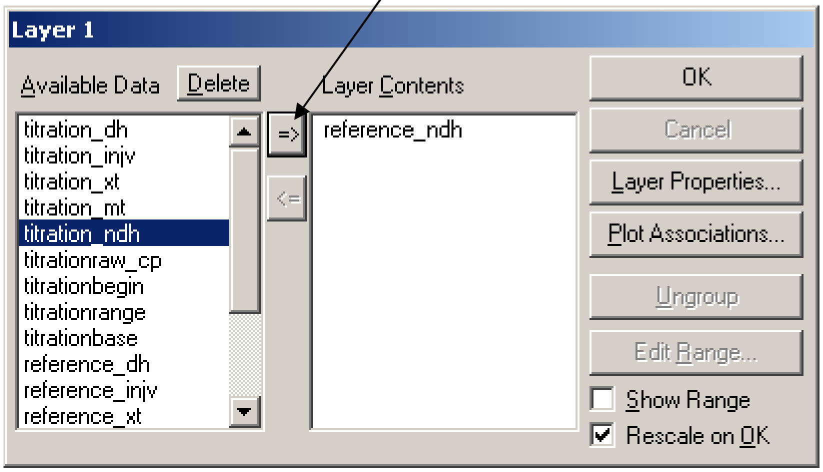
10. At this point one needs to activate windows from the original titration file to be able to work with them. Double click on a small “1” button on the top left corner of the display.

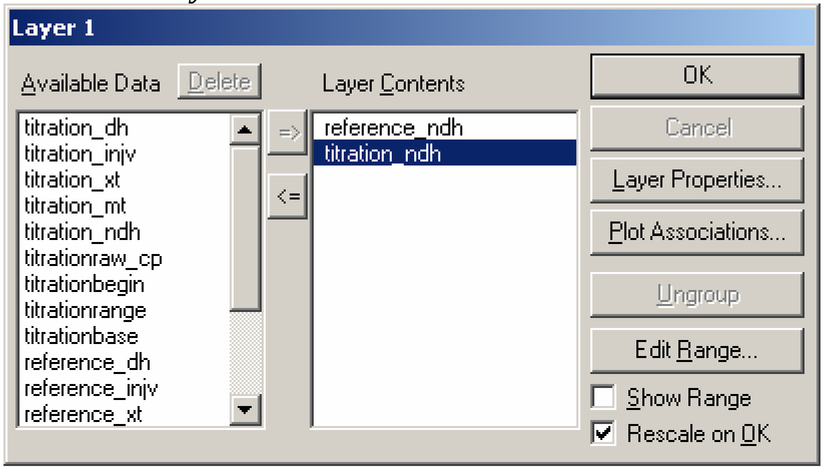
11. The following window will appear:



which lists all available displays and in the right hand corner lists the content of the active window.

12. Move “titration_ndh” into “Layer Contents” window by highlighting the data name and choosing the arrow to move it from left window into the right one:



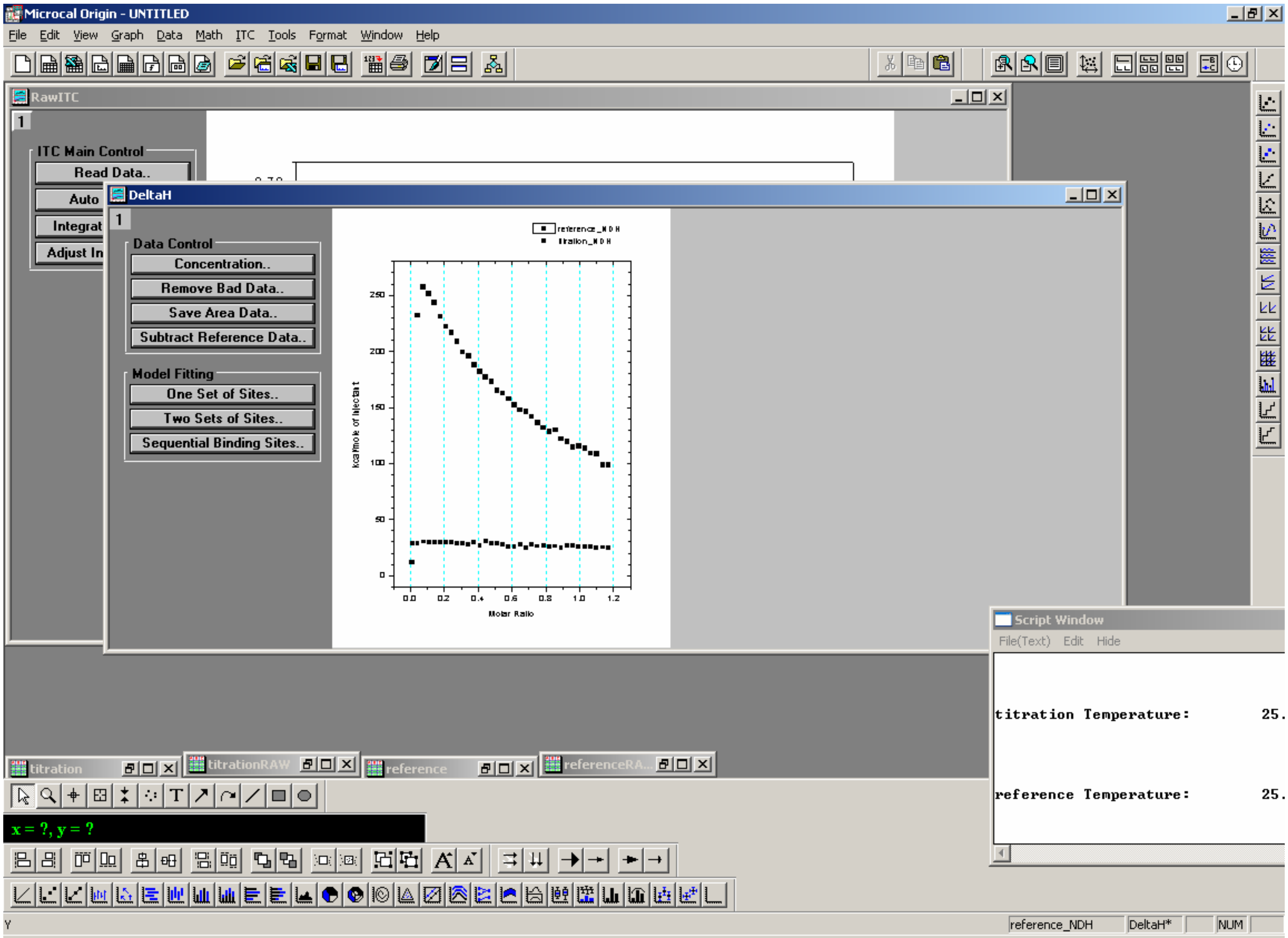


Available Data:
titration_dh
titration_injv
titration_xt
titration_mt
titration_ndh
titrationraw_cp
titrationbegin
titrationrange
titrationbase
reference_dh
reference_injv
reference_xt

Layer Contents:
reference_ndh
titration_ndh

☐ Show Range
☑ Rescale on OK

13. The followin display appear:

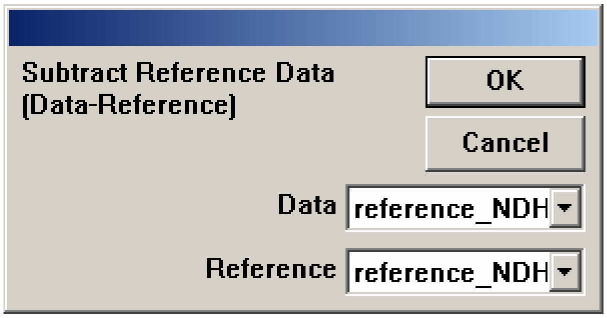


```
titration Temperature:        25.

reference Temperature:        25.
```

14. At this point, having two data sets open, you may choose “subtract reference data” button.

15. The following window appear:

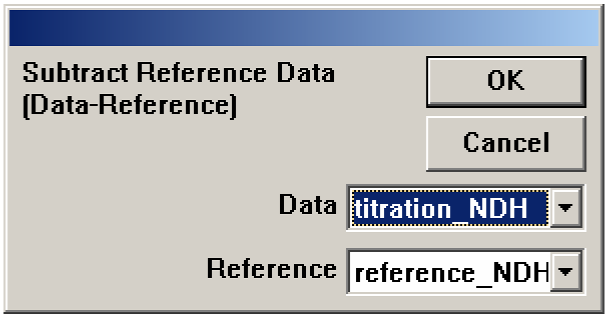


Subtract Reference Data
[Data-Reference]

Data reference_NDH

Reference reference_NDH

16..Choose the name of the set that contains “data” and set the contains “reference” results:



Subtract Reference Data
[Data-Reference]

Data titration_NDH

Reference reference_NDH

17. The display contains now the “titration” data form which the “reference” data had been subtracted.

18. Proceed with fitting using an appropriate model as described in :

http://info.med.yale.edu/wmkeck/biophysics/RNAse_Data_Processing_Origin_5_0_ITC.pdf

following from step#16.