# User Manual of TOCO19

## Introduction

TOCO19 is software for predicting precision of measurements by distinguishing noise and signals (peaks) in a chromatogram. From the former, mathematical properties of uncertainty are extracted and analyzed, and to the latter, limits of detection, quantitation and decision, and precision profiles are given. Users can know these stochastic properties of each peak by inputting a file of a chromatogram into TOCO19. Since the theories and methodologies of ISO 11843 Part 7 and JIS Z 8462 Part 7 underlie the fundamental concepts of TOCO19, repeated measurements of real samples in chromatography can be obviated.

The basic policy of TOCO19 is to provide users with:

- explanations of statistical theories cited in TOCO19 (detection limit, etc.)
- user-friendliness so that TOCO19 can be used without extensive reading of this user manual.

Upon opening the TOCO19 software, one can see instructions in the TOCO main window. We hope that following these instructions will be sufficient to users, but if not, this manual will be of help.

Before TOCO19 was put on the market, its practical suitability had been examined with a variety of chromatographic experiments in collaboration with Prof. Akira Kotani of the Department of Analytical Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences. At present, TOCO19 has been found to be well suited for the following methods: absolute calibration curve method, internal standard method, isocratic elution, and the gradient elution of HPLC. However, there are some cases in which TOCO19 is not effective (see Chapter 3).

We should appreciate it very much if you would kindly let us know of any bugs, if found, in TOCO19 through the "Contact us" of the Help menu. We will make a maximum effort to correct, improve, and further develop TOCO19.

August 5, 2020

## Terms and Abbreviations

| | |
|---|---|
| detection limit | minimum detectable quantity (concentration) |
| precision | standard deviation (SD) or relative standard deviation (RSD) of measurements (responses) or concentration estimates |
| precision profile | plot of measurement RSD against concentration, defined in ISO 11843-7 and JIS Z 8462-7 |
| quantitation limit | minimum concentration for quantitation |
| decision limit | criterion for detection, also called critical value, |
| DL | detection limit |
| FUMI | Function of Mutual Information |
| FUMI theory | theory to predict the precision of measurements from noise and signals in the output of analytical instruments |
| FUMI equation | equation to denote the SD of measurements （$=\sigma(k)$） |
| IS | internal standard |
| PP | precision profile |
| QL | quantitation limit |
| RSD | relative standard deviation or coefficient of variation |
| SD | standard deviation |
| TOCO | Total Optimization of Chemical Operations |

Most of the above terms are specified in views, message boxes, or tool tips of TOCO19.

# Table of Contents

## Chapter 1　　Fundamentals of TOCO19

TOCO19 is an automated system for predicting:

- precision (RSD of measurements) for a peak in a chromatogram;
- detection limit (DL) of the peak.

A data file of a chromatogram containing both noise and signals is the only requirement for performing TOCO19 analyses.

Figure 1 shows the initial window when TOCO19 is launched. TOCO19 is a fully automated software and users are only required to input a data file as follows:

1. click the Import menu (see Fig. 1 and for details, see 2.1);
2. check the input data and analytical results (DL and precision) (see Fig. 2).

After procedure 1, the contents of the file are displayed and automatically detected peaks are numbered in the chart (see Fig. 2). In the view of the main window, measurement RSDs and DLs are shown (see Fig. 2).
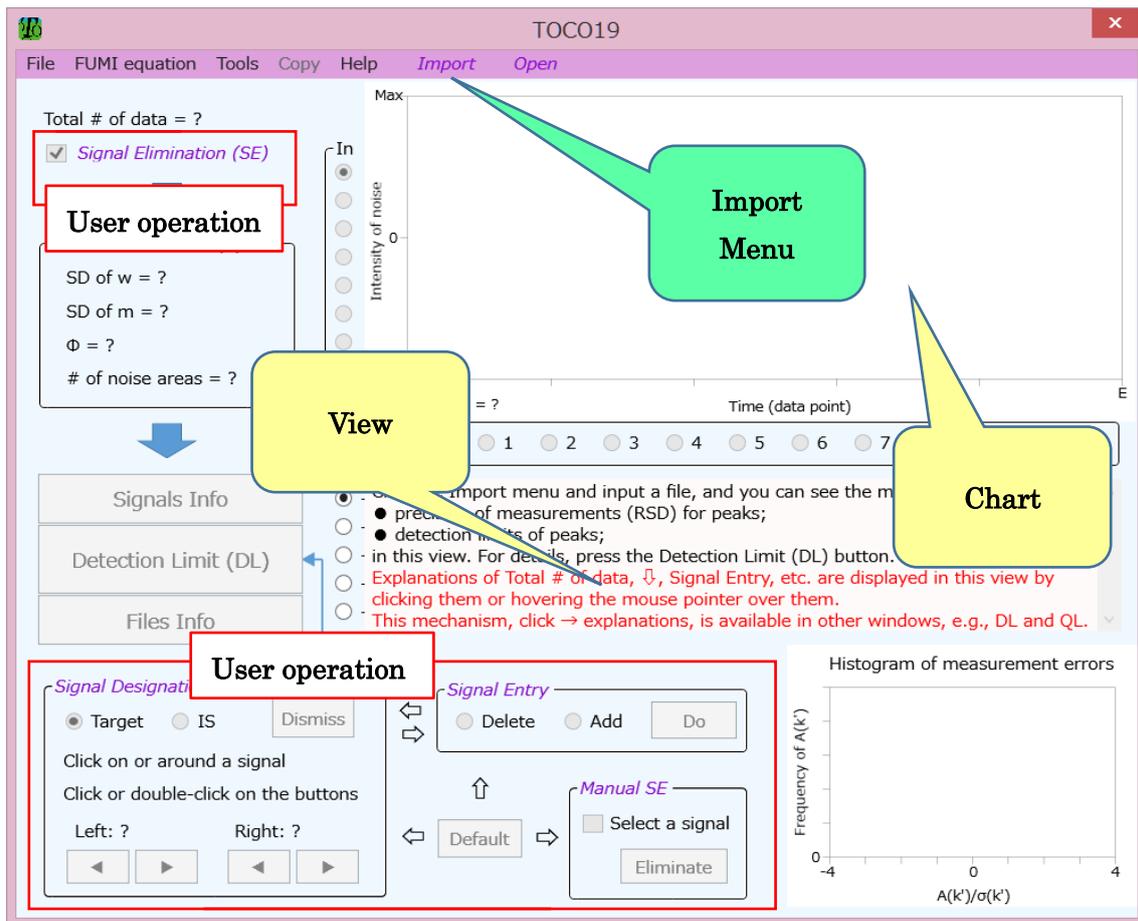


**Figure 1 --- Main window at application startup**
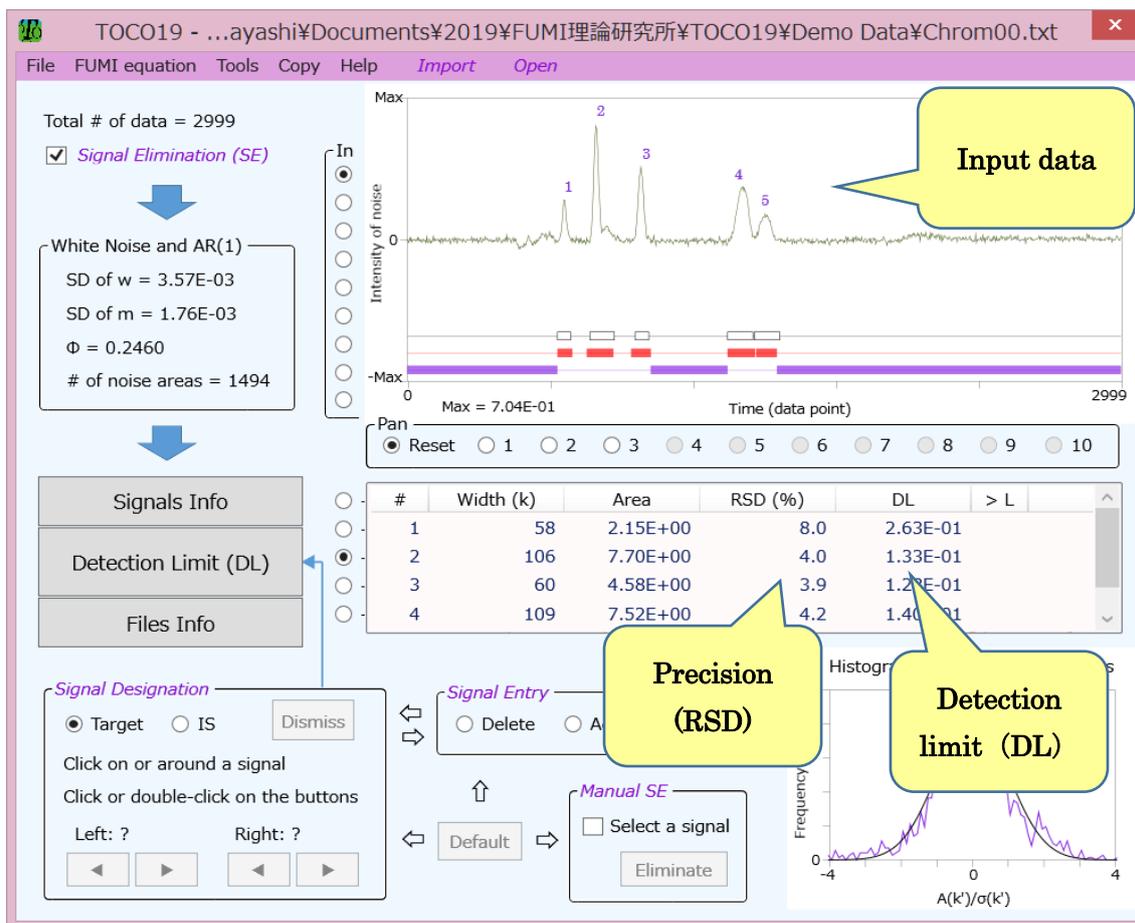
**Figure 2 --- Analytical results of TOCO19 after a data file has been input**

Explanations of almost all of the functions in the main window will appear in the view when you click on them or in the tool tips when you position the mouse pointer over them. Manual operations are denoted by purple labels in the main window (see Fig. 2).

Typical operations after the first default analysis shown in Fig. 2 are:

- Signals Info button → to input or change a name and concentration of a peak in the chart
- Detection Limit (DL) button → detection limit, quantitation limit, decision limit and precision profile (plot of measurement RSD versus concentration)
- Signal Designation group → manual adjustment of peak widths and designation of target and IS peaks.

## Chapter 2    Operations for different purposes

1. Data file import

　　To import a data file into TOCO19, click the Import menu in the main window, open a target file on the Open dialog box, and operate the Import dialog box as follows (see Fig. 3)

1.　click the header of a column which contains data;
2.　press the OK button.

The first row of valid data is automatically selected by operation 1 and its value is displayed in the text box with yellow background. It is recommended to check its value for confirmation. If the value is not appropriate, click a row you desire.

　　Some points to keep in mind are (see Fig. 3):

● 　Ending row denotes the last row to be imported by default;
● 　The value of the Ending row text box (green box) is changeable, and the data from the Start at (yellow box) to the End row are imported into TOCO19;
● 　The minimum number of data available for TOCO19 is 300.
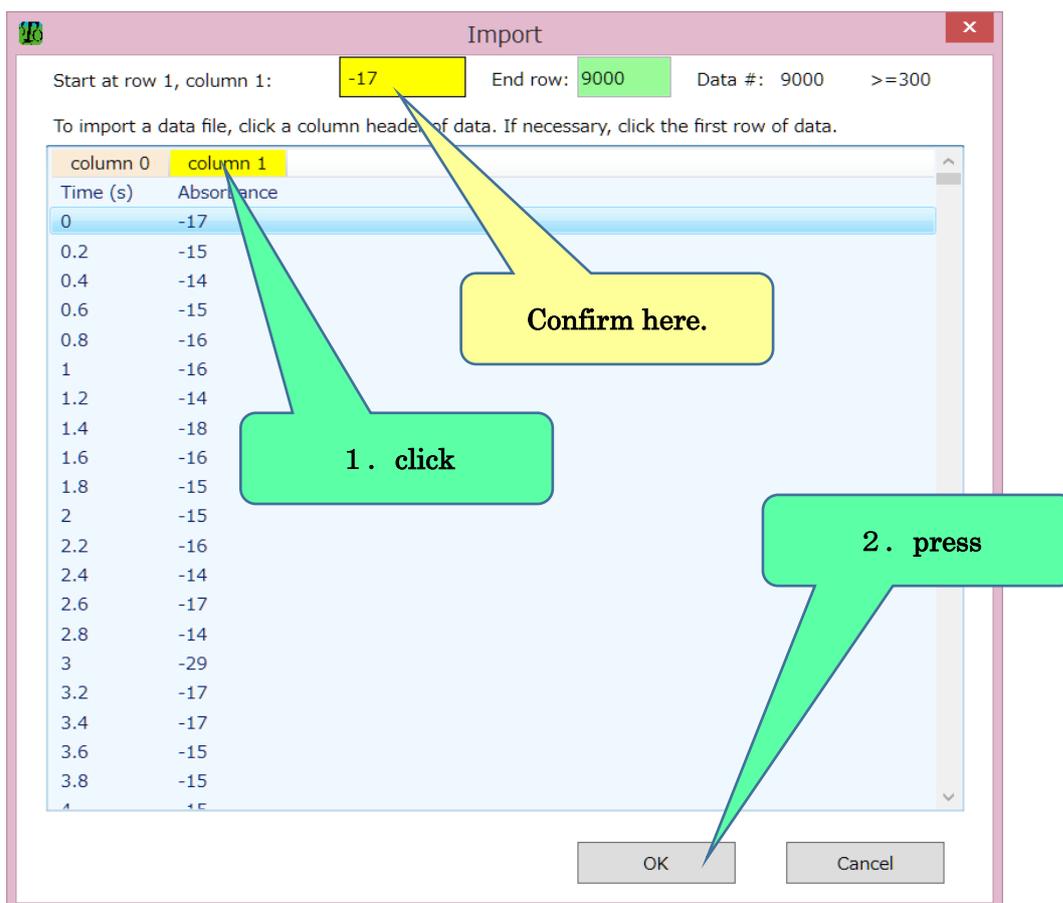


Figure 3 --- Import dialog box

2. Input of peak names and concentrations

The procedures to change a peak name and concentration are:

1. press the Signals Info button after a data file has been imported (see Fig. 4);

2. select a target peak by clicking a row of the view of the Signals Info window (see Fig. 5);

3. click a cell on the Conc column and input a concentration value;

4. type a signal name on the Name column;

5. press the OK button to update the name and concentration and return to the main window.

Other names and concentration values can also be changed by repeating procedures 2 to 4.
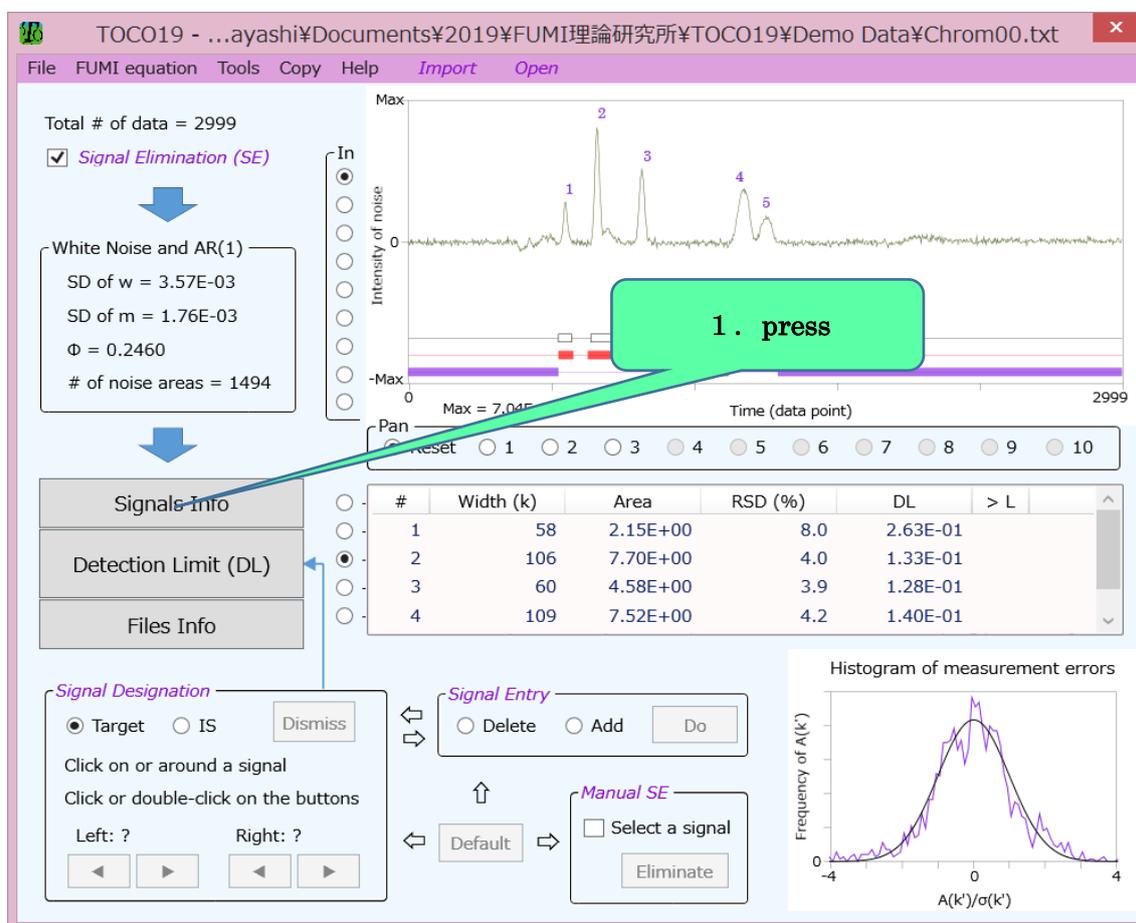


Figure 4 --- Main window
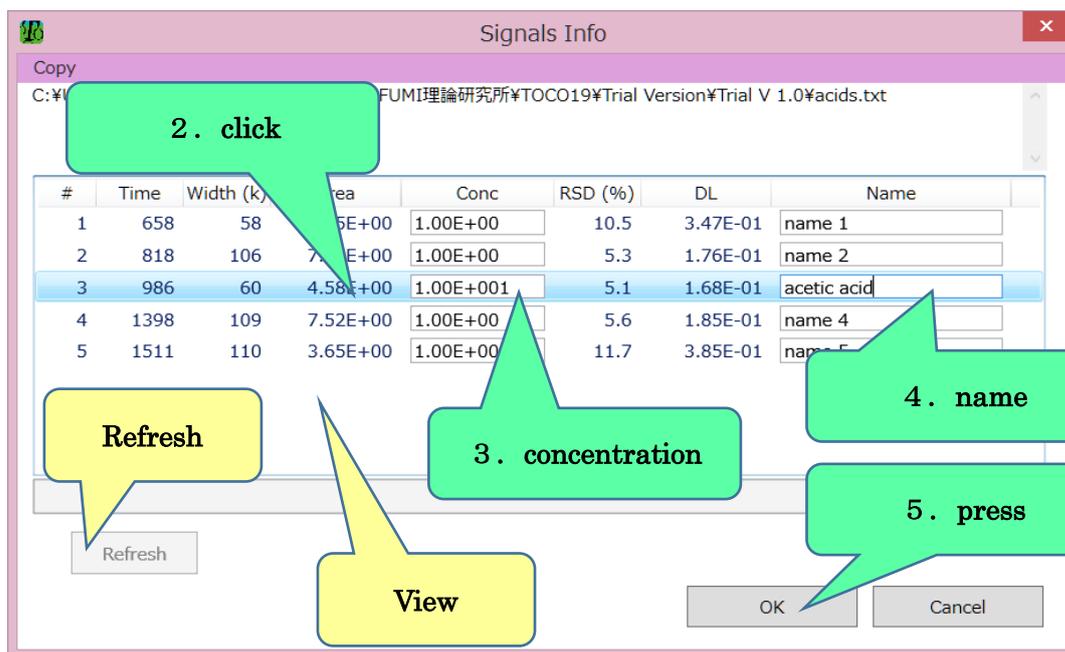
**Figure 5 --- Signals Info window**

Some points to note are:

- The Refresh button is used to confirm DL recalculation due to a change in concentration (see Fig. 5). Since a DL is automatically recalculated when another row in the view is clicked, the Refresh button does not have to be used except for immediate recalculation. If a typed value is illegal, the button will turn red. If not, it will turn green;

- When you press the OK button, although a new value of Conc is not positive or numerical, the left message of Fig. 6 will appear. If a newly typed Name consists of more than 20 characters, the right message of Fig. 6 will appear. You must close the Signals Info window after correcting the illegal input.
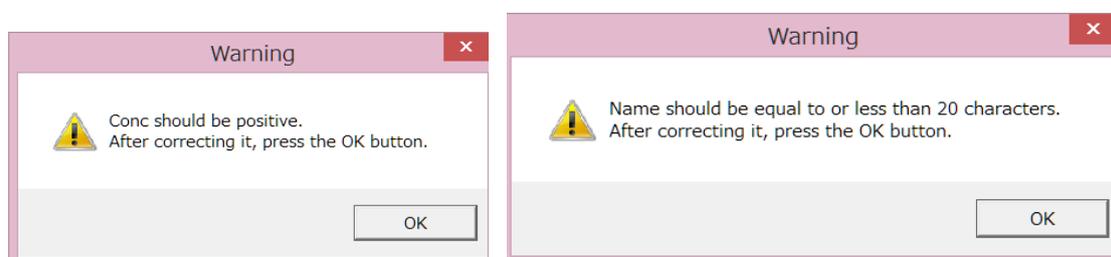


**Figure 6 --- Warning message boxes**

3.  Decision limit, DL and QL for a target peak (1)

TOCO19 analyses are based on the stochastic properties of noise. Decision limits, DLs and QLs are quantities inherent in peaks. TOCO19 provides two operation methods for predicting the precision of a peak from data of noise and signals as follows:

（1）  press the Detection Limit (DL) button and designate a signal as a target peak;

（2）  designate a signal as a target peak and then press the Detection Limit (DL) button (see 2.4).

Both operation methods apply to cases where noise and signals are present in the chart (see Fig. 1), but method (1) is also useful if signals are absent in the chart.

The procedures of method (1) are:

1.  display a file which contains analyzable noise in the chart (analyzable means that either SD of w or SD of m has a positive value);

2.  press the Detection Limit (DL) button in the main window;

3.  select a file (Signal # > 0) from the view of the Target Signal Selection window (see Fig. 7);

4.  select a target peak from the lower-right combo box;

5.  press the OK button to obtain analytical results in the DL & PP······ dialog box (see Fig. 8).

Some points to note are:

●  If the DL & PP …… window (Fig. 8) appears just after procedure 2, see 2.4;

●  The light green background (Fig. 7) indicates a file displayed in the main window;

●  The purple border (Fig. 7) denotes a file without peaks (Signal # = 0);

●  An IS signal is not displayed by procedure 4 (it cannot be a target peak).
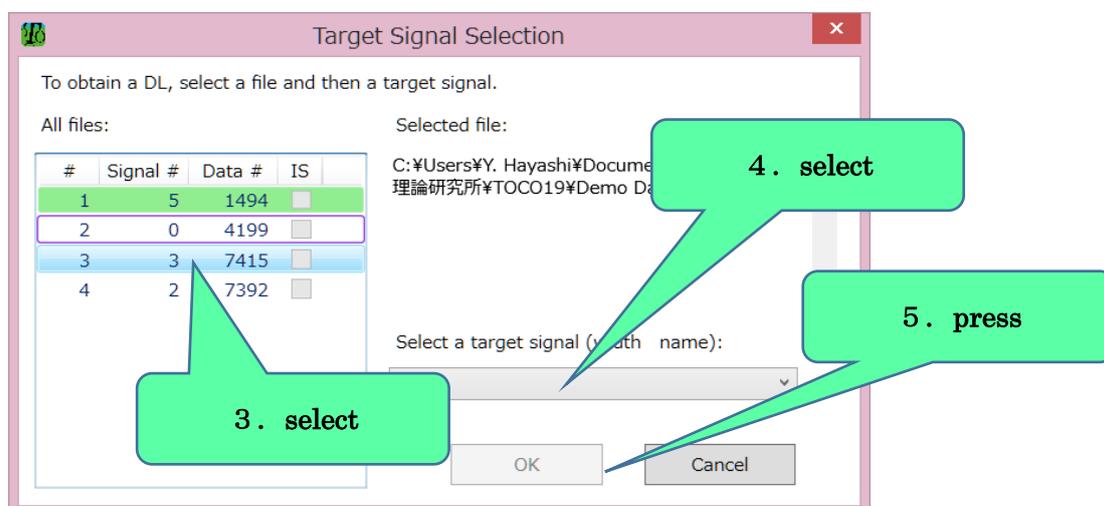


Figure 7 --- Target Signal Selection dialog box

**DL & PP for acetic acid**

Signal Selection    Copy

The target signal has width (k) of  60

area (Y) of  4.58E+00        (time unit × intensity)

at a concentration (X) of   1.00E+01        (conc unit):        Y = aX

☐ Internal standard (IS) method with the IS signal of

width

SD                                        Φ: 0.246.

| Decision limit (conc unit) | Detection limit (DL) (conc unit) | Quantitation limit (QL) (conc unit) |
|---|---|---|
| = 6.38E-01 | = 1.28E+00 | = 3.87E+00 |

RSD (%)  =

30.2

at a concentration of

1.28E+00

(conc unit).

Precision Profile (PP)

RSD of conc (%)

Conc (conc unit)        5.2...00

Assumptions:

RSD of independent error =  0.3    % = [0, 7]%

DL =  3.3   × σ(k) at which RSD (%) ≒ 30.3

QL =  10    × σ(k) at which RSD (%) ≒ 10.0

Default Values

DL Signal            OK

Callouts:
- Width of a target peak (Procedure 4)
- From left: Decision limit Detection (DL) Quantitation limit (QL)
- Target Signal Selection
- precision profile (PP)

Figure 8 --- DL & PP …… window

4.  Decision limit, DL and QL for a target peak (2)

The procedures of operation method (2) of 2.3 are:

1.  confirm that the data in the chart contain both noise (purple blocks) and signals (white blocks) (Fig. 9);

2.  select ◉Target from the Signal Designation group (unless explanations of the next procedures appear in the view, click the uppermost ◉ on the left side of the view);

3.  click on or around a target peak in the chart (Fig. 9) (the designated peak is painted in red and indicated with a T);

4.  press the Detection Limit (DL) button to obtain analytical results in the DL & PP …… window (see Fig. 8).

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**Figure 9 --- Selection of a target peak**

5. Designation and dismissal of target and IS peaks

Analyses from the Detection Limit (DL) button (decision limit, DL, QL, PP, Signal on noise (see Fig. 12 or 2.10)) cannot be obtained until a target peak is designated. For internal standard methods, precision is also predicted with a designated IS peak.

There are two operations for designating and one for dismissing a target peak:

- click ⊙Target in the Signal Designation group of the main window and follow the instructions in the view (unless explanations of the next procedures appear in the view, click the uppermost ⊙ on the left side of the view);

- click the Target Signal Selection menu in the DL & PP …… window (see Fig. 8) and select a target peak (see procedures 3-5 of 2.3);

- press the Dismiss button to release a target peak from its role, when ⊙Target is selected (see Fig. 9).

The procedures for designation and dismissal of an IS peak are the same as those of a target, except that it is selected by the ⊙IS radio button and indicated with an IS.

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6.  Precision of a target peak at an arbitrary concentration (precision profile)

A precision profile means a plot of measurement RSD (%) against concentration, from which is known a measurement RSD (%) of a target peak at an arbitrary concentration. The procedures for this purpose are (see Fig. 10):

1.  press the Detection Limit (DL) button to open DL & PP …… (see 2.3 or 2.4);
2.  input a concentration in the green text box on the left side of Precision Profile (PP);
3.  check the precision in the yellow text box and the RSD pointed by the yellow ◄.

The blue ▼ indicates a DL and red ▼ a QL.

Click the figure of the Precision Profile (PP) to see an explanation of PP.
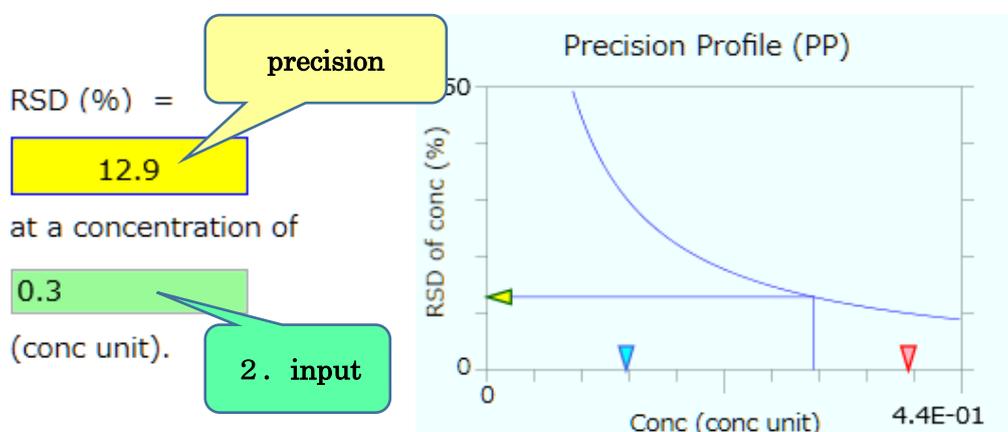


Figure 10 --- A precision profile after a concentration has been input

7.  Set of sample injection errors

In chromatographic analyses, the RSD (%) of sample injection errors needs to be input into the green text box on the right side of "RSD of independent error" (see Fig. 8 or 11).

8.  Explanations of DL, QL and decision limit, and PP

Explanations about limits of decision, detection, and quantitation are displayed by clicking one of the colored large labels (see Figs. 11 and 12). Click the figure of Precision Profile (PP) to see its explanation (see Fig. 11).

9.  Constants for limits of detection and quantitation

DLs and QLs are defined as a constant times an SD of blank sample measurements (= $\sigma(k)$). The default values of the constant are 3.3 and 10 for DLs and QLs, respectively. The latter constant is fixed, but the former is changeable. An arbitrary constant can be assigned to a DL by typing a value in the green text box on the right side of the label "DL" (see Fig. 11). The Default button restores the default values (3.3 and 10).

## 10.  Signal on noise of limits of decision, detection, and quantitation

The Signal on noise, shown in Fig. 12, is a Gaussian peak which is superimposed on real noise and is useful to confirm a signal-to-noise ratio (S/N) for decision limits, DLs and QLs . The following procedures are necessary for the Signal on noise:

1.  press the DL Signal button in the DL & PP …… window (see Fig. 11) after pressing the Detection Limit (DL) button on the main window;

2.  select one of the three radio buttons (◉) in the Detection Limit Signal on Noise window (see Fig. 12).

An explanation about the Signal on noise will appear by clicking one of the labels of the limits.
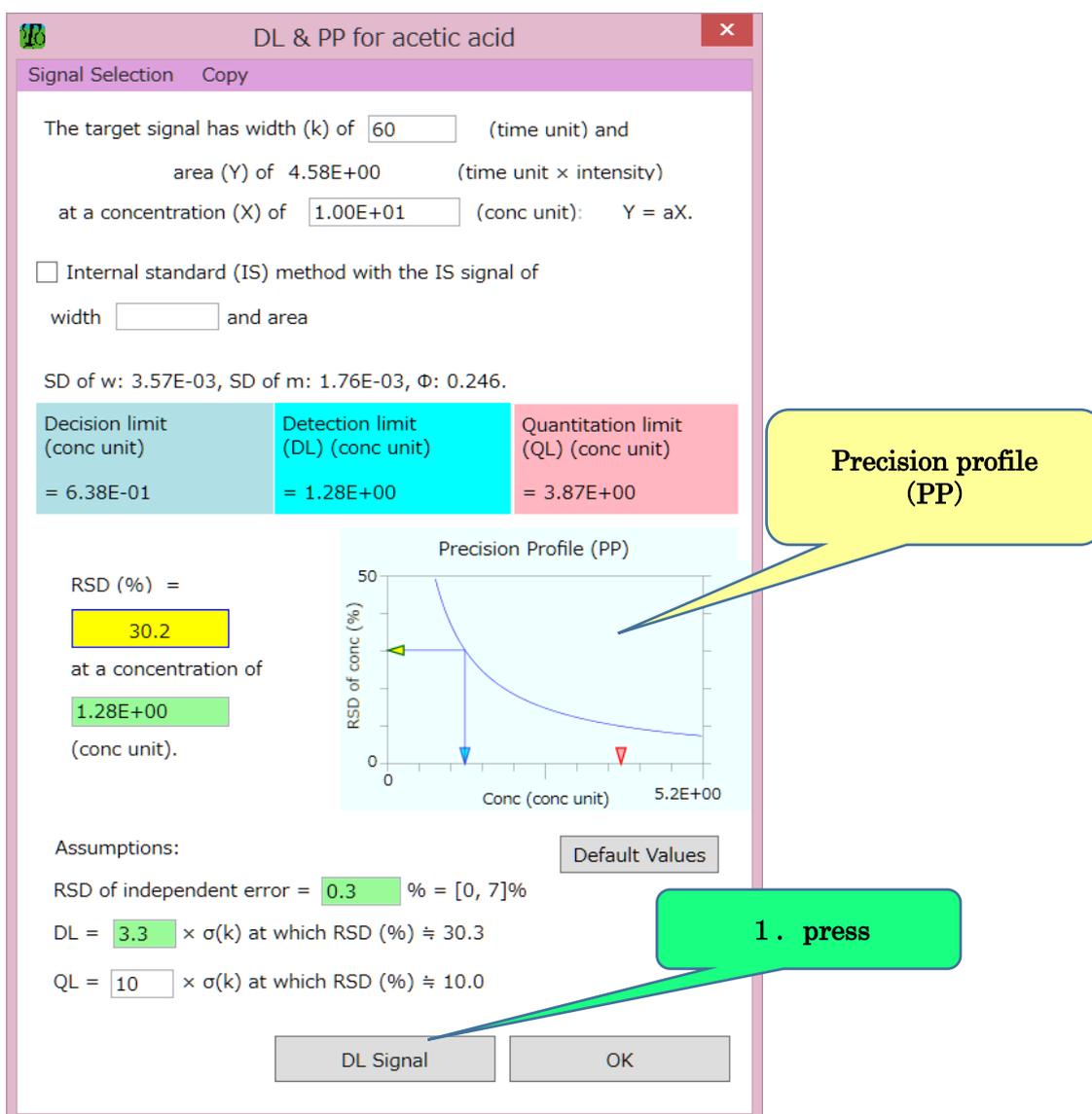


**Figure 11 --- DL & PP …… window**
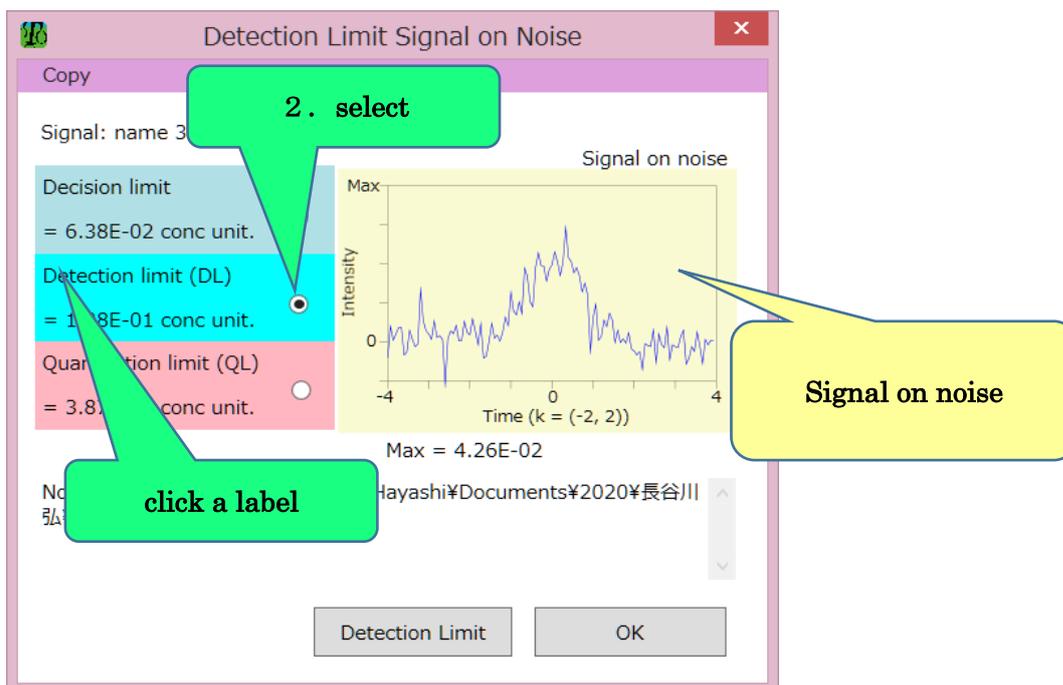
Figure 12 --- Detection Limit Signal on Noise window


11.  Copy and paste of results from TOCO19 to Excel

Clicking the Copy menu leads to the copying of the TOCO19 results to the clip board and you can paste the contents into Excel for further examination. The following TOCO19 results can be copied:

- main window (noise and signal analyses);
- Signals Info window (items in the view);
- DL & PP …… window (decision limit, DL, QL and PP);
- Files Info window (items in the view and control chart);
- Detection Limit Signal on Noise window (selected Signal on noise);
- FUMI Equation window (σ(k)).

These results are useful for technical reports, etc.


12.  Analyses of noise and signals in different files

In cases where noise and signals are in different files, see 2.3.


13.  A chromatogram lacking a sufficient number of noise data points

There often arise situations where enough noise data points are not secured in a data file due to densely populated peaks or narrow noise regions around a peak. In these cases, TOCO19 can also be performed by analyzing noise of another file (see 2.3).

14.  Analyses for internal standard methods

The procedures for analyses of internal standard methods are (see Fig. 13):

1.  select the ◉IS button in the Signal Designation group (see instructions in the view);
2.  click on or around a desired peak in the chart;
3.  follow one of instructions appearing in the view

   ①  DL, QL, decision limit and PP  →  Detection Limit (DL) (see 2.3 or 2.4);
   ②  Detection limit  →  the third or fourth radio button (◉) on the left side of the view (a DL for an IS peak is described as IS, but other peaks are given predicted DLs; meanings of columns appear in the view by clicking one of them).

A point to note is:

● IS peak selection in procedures 1 and 2 should be performed from a file containing both noise and signal. This selection is also possible with a file containing signal alone, but the precision cannot be calculated without noise.



Figure 13 --- The main window after an IS peak has been selected

## 15. Analyses for gradient elution

In gradient elution of HPLC, baselines (backgrounds) of chromatograms are not usually horizontal, but oblique. However, TOCO19 can cope with these cases. As is shown in Fig. 14, if the observed histogram (zigzag line) of areas created by the background noise is in good agreement with the theoretical line (smooth line) of a normal distribution, TOCO19 analyses can be considered successful (for details, see 2.25).
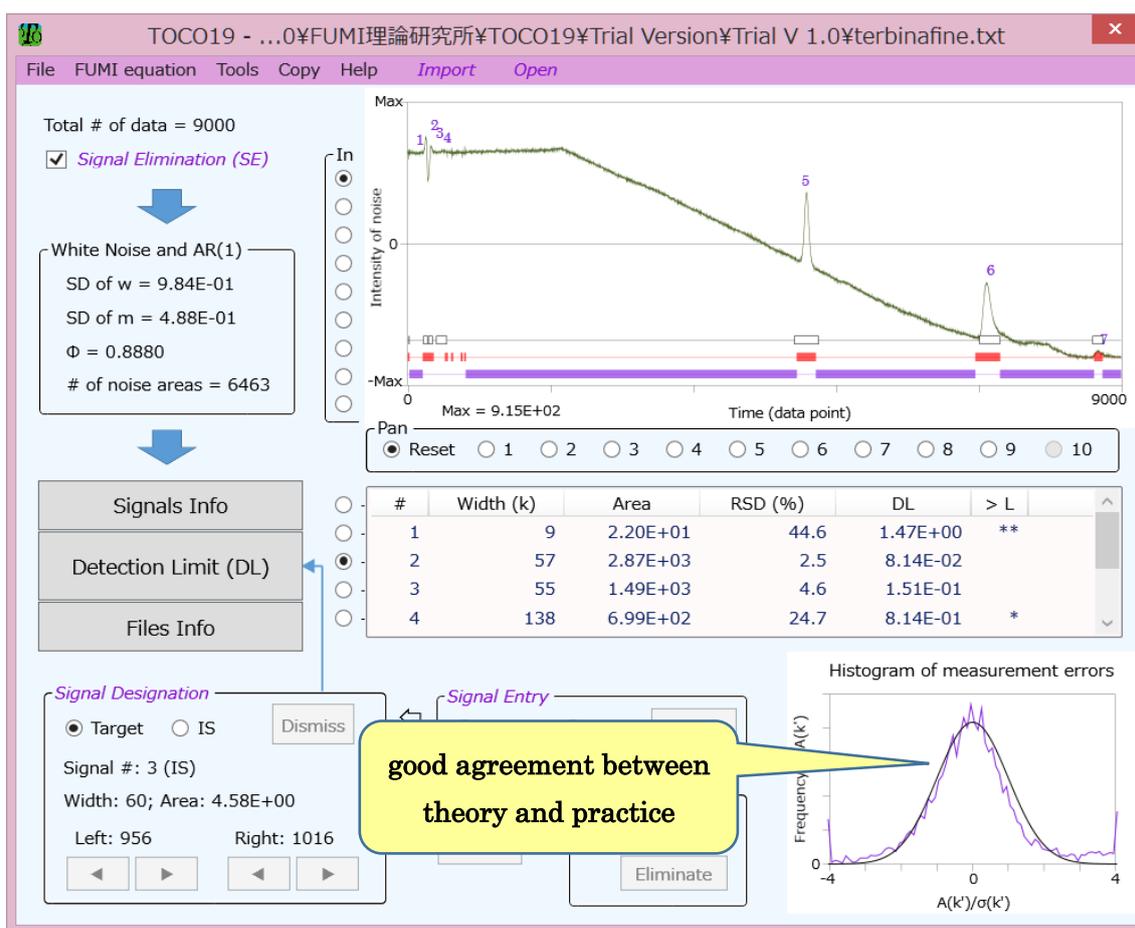


**Figure 14 --- An example of gradient elution analyses**

16. Import of multiple files and display of one

The procedures to import multiple files and display one in the main window are:

1. execute the Import menu repeatedly to import multiple files (see 2.1);

2. press the Files Info button on the left side of the main window (see Fig. 14);

3. select a radio button (◉) of the Sh* column which contains the file you want to show in the main window (see Fig. 15);

4. press the OK button to return to the main window.

    TOCO19 analyses are based on noise and signals in the chart of the main window.


17. Deletion of a file from TOCO19

In the Files Info window (see procedure 2 of 2.16), click a row of the desired file in the view, unselect its radio button (◉) of Sh* and uncheck its check box (☑) of Join, and the selected file will be deleted (see Fig. 15). Deletion will not be executed until the OK button is pressed. Click the Undo button to undo the deletion.
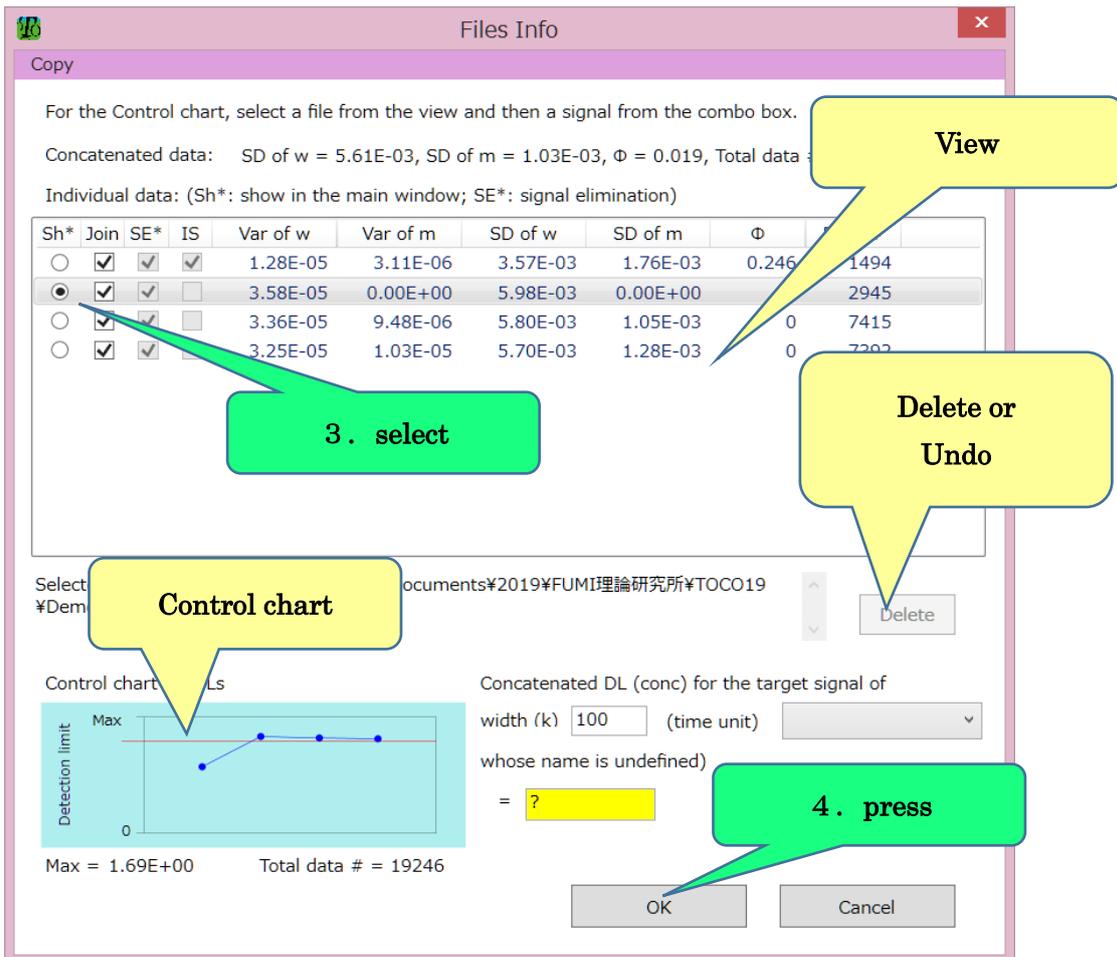


Figure 15 --- Files Info window

## 18. Comparison of data of different files (routine check)

An abnormal condition of an instrument can often be found by a daily check of background noise. TOCO19 provides this function as a control chart. To see a control chart, import multiple files and press the Files Info button.

The procedures to select a specific peak for a control chart are (see Fig. 16):

1. click a row of a file containing a desired peak in the Files Info window;
2. select a desired peak from the combo box on the right side of label "(time unit)";
3. confirm the DL in the yellow text box and also compound name.

Some points to note are:

- DL values are described in terms of absolute calibration curve methods. Even if an IS peak is designated, DL values of IS methods are not shown;
- Files with no peaks are shown by purple borders in the view (see Fig. 16);
- In procedure 2, IS peaks cannot be selected as a peak for the control chart.



Figure 16 --- Files Info window (no signals in file #2 with purple borders)

19.  Manual adjustment of peak widths

Users can manually adjust a width of a peak (a white block in the chart) (see Fig. 17). For this purpose, the Signal Designation group is used as follows:

1.    select a radio button, ◉Target or ◉IS.

Instructions of the next procedure will appear in the view as shown here (see Fig. 17)

2.    click on or around an objective peak in the chart;

3.    click or double-click the ◀ or ▶ button to adjust a width of the peak;

4.    confirm the width, peak area (red or blue) and peak number.

Change in a peak width affects the precision (DL and RSD). The designation of a target or IS peak can be dismissed by the Dismiss button.
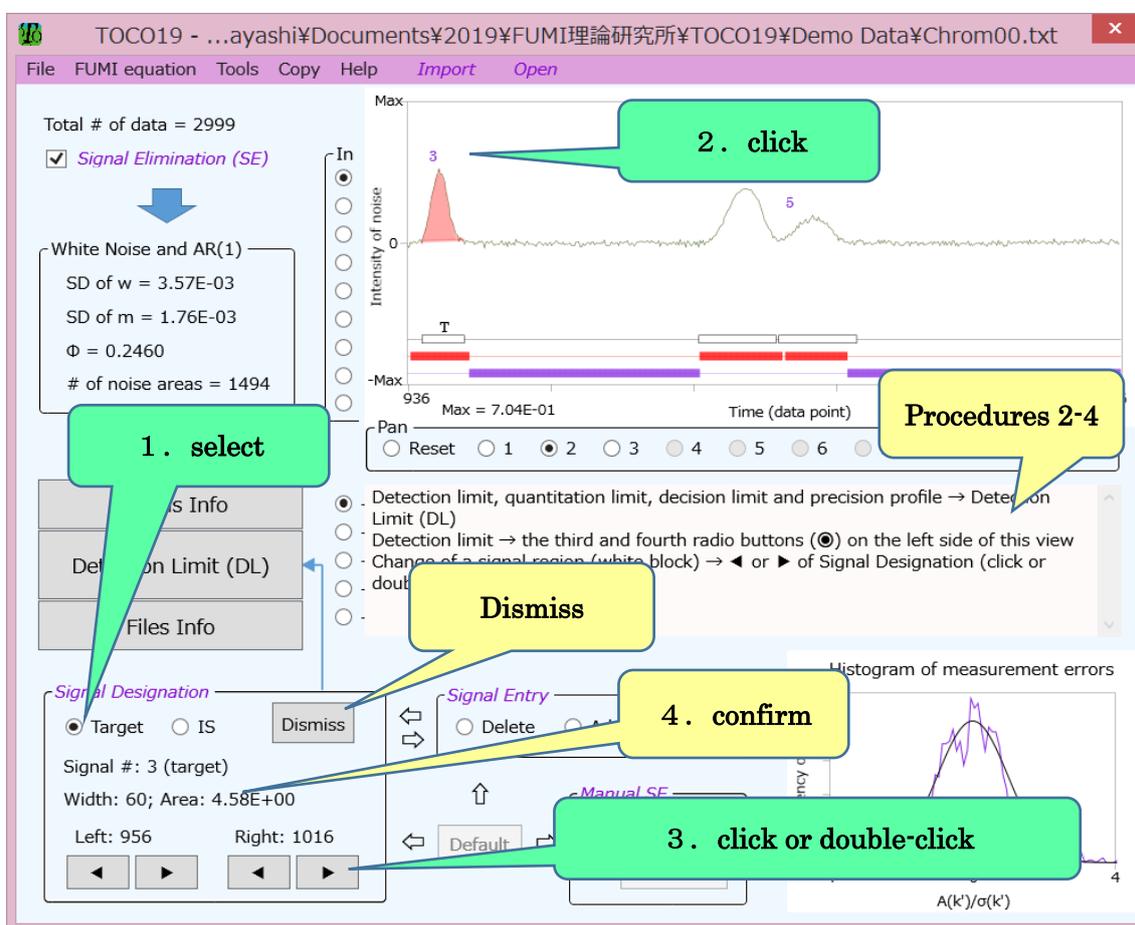


Figure 17 --- Main window

## 20. Deletion of a peak from TOCO19 analyses

If a peak is deleted from TOCO19 analyses, it will no longer have the peak number in the chart or analytical results (precision and DL) in the view. Users can limit the information of peaks by releasing insignificant peaks from analyses. This peak deletion can be done with the Signal Entry group as follows (see Fig. 18):

1. select the ◉Delete button;

Instructions for the next steps will appear in the view as follows:

2. click on or around a peak with a number to select as a candidate (indicated with ▲);
3. press the Do button.

In the updated chart (see Fig. 19, but neglect the green ▼), the red block is retained, but the white block for the selected peak is erased.

A point to note is:

● A peak designated as T or IS cannot be deleted by the above procedures. However, this is feasible, if the designation of T or IS is dismissed by the Dismiss button.
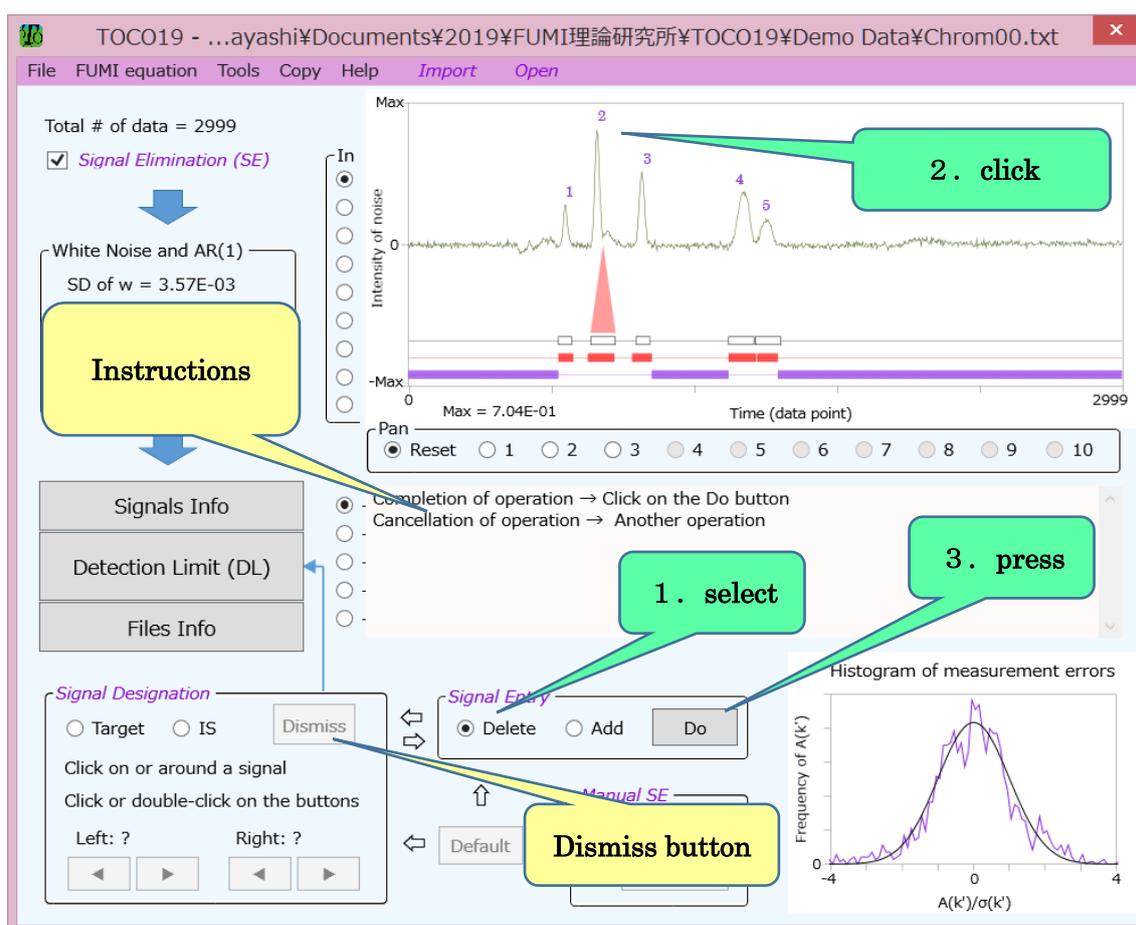


**Figure 18 --- Main window for signal deletion**

21. Entry of a peak in TOCO19 analyses

The procedures to enter a peak in TOCO19 analyses are (see Fig. 19):

1. select the ⊙Add button in the Signal Entry group.

The instructions for the next steps will appear in the view as follows:

2. click on or around a peak without a number to select it as a candidate (indicated with ▼);

3. press the Do button.

In the updated window (see Fig. 18), the red block is retained, but a new white block will be created.

Some points to note are:

● A peak with a non-positive area cannot be analyzed;

● A region outside the red blocks cannot be designated as a new peak to be analyzed. For setting a new signal region (red block), the procedures of 2.22 are useful;

● A peak width (white block) is changeable (see 2.19).
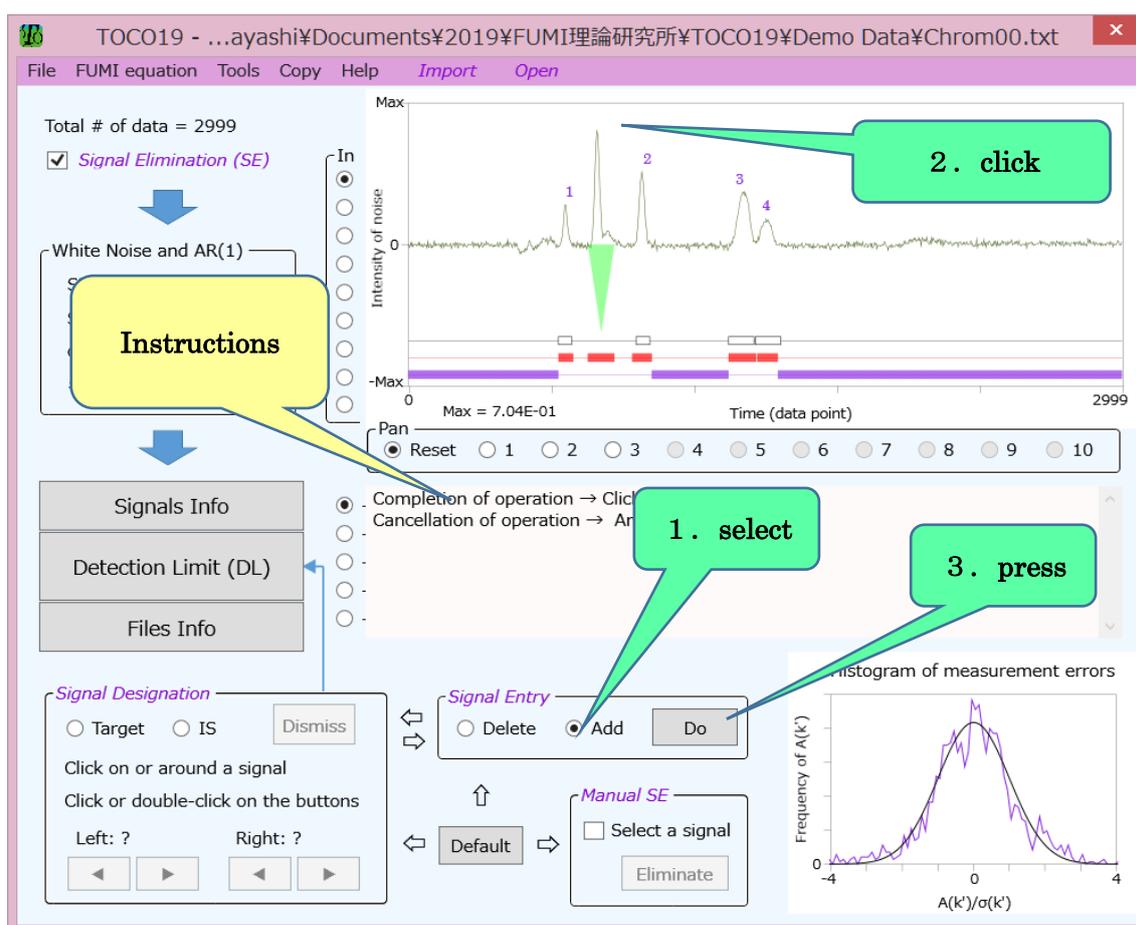


**Figure 19 --- Main window for signal entry**

22. Setup of a new signal region

The Manual SE group is used to set up a new signal region. The chart of Fig. 20 shows an example of data which are all recognized as noise by unchecking the ☑Signal Elimination (SE) check box. If ☑Signal Elimination (SE) is checked, automatic peak detection of TOCO19 is carried out. The procedures to set up a new peak region are

1.  check the ☑Select a signal check box in the Manual SE group;

The instructions for the next steps will appear in the view as follows:

2.  draw a square with the mouse to designate a new region as a signal in the chart;

3.  press the Eliminate button.

Figure 21 shows that the red and white blocks are created after procedure 3. The width (white block) of a peak is changeable (see 2.19).

The setup of a new signal region is referred to as Signal Elimination (SE), because signal components in the noise regions (purple blocks) spoil the noise analyses of TOCO19 and elimination of them improves the reliability of the TOCO19 analyses.
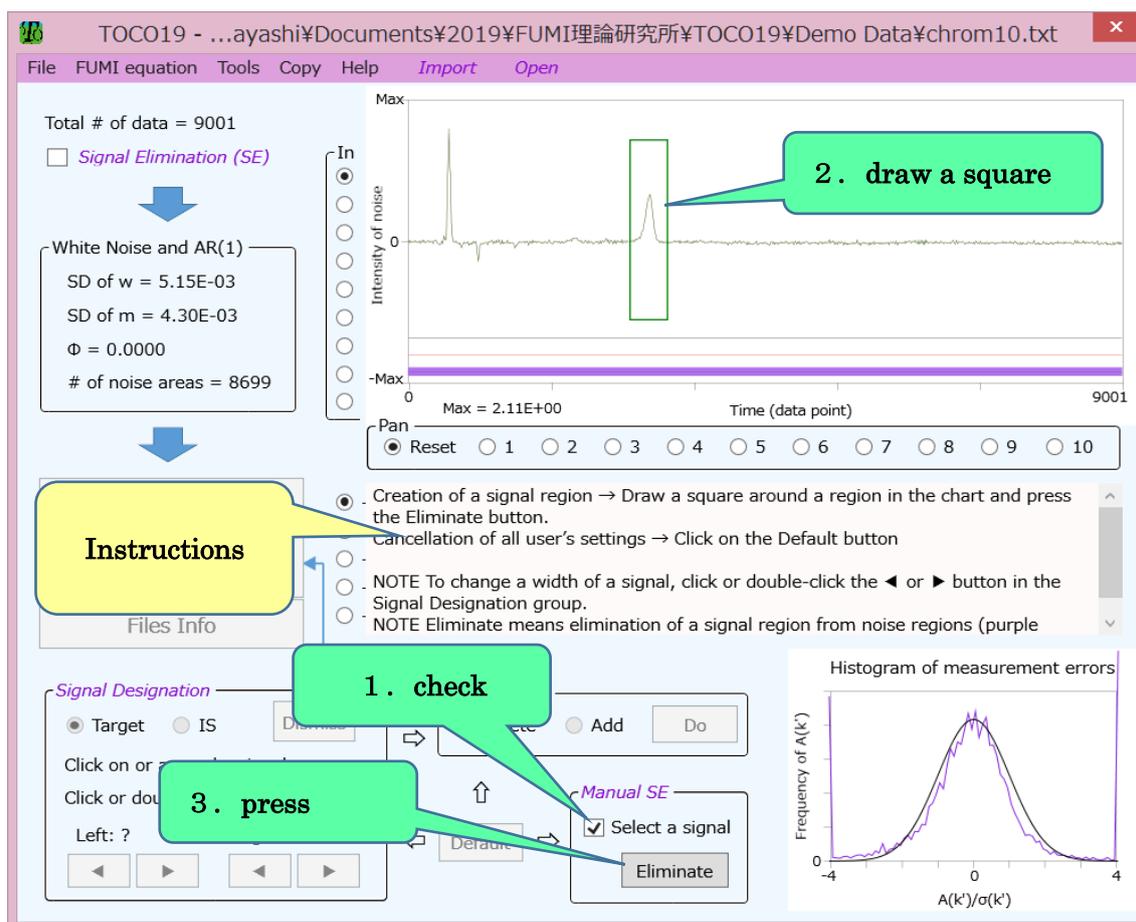


**Figure 20 --- Main window (manual signal elimination)**
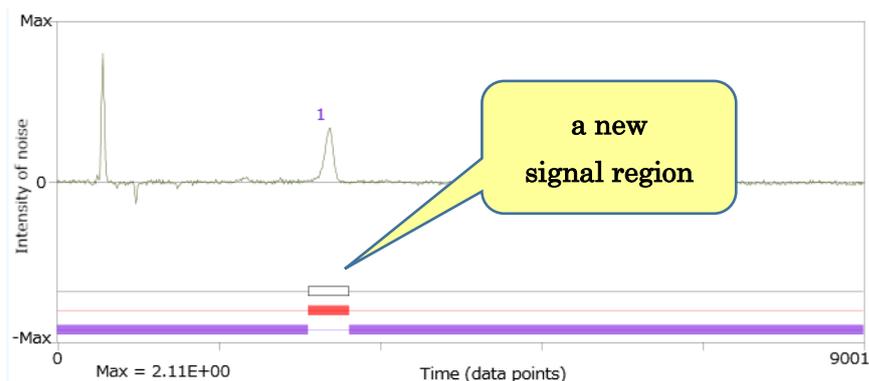
Figure 21 --- Chart after procedure 3

23. Analyses of overlapping peaks

Each overlapping peak can be analyzed by virtually separating them as follows:

1. check ☑Select a signal of the Manual SE group (see Fig. 20);
2. draw a square around one of the overlapping peaks with the mouse (see Fig. 22);
3. press the Eliminate button to create a new signal region (white block) (see Fig. 23, but neglect ▼);
4. select ◉Add of the Signal Entry group and click the other peak to set a new region (see ▼ of Fig. 23);
5. press the Do button to let the new peak participate in analyses; (see Fig. 24).

Instead of procedures 1-3, the ◄ and ▶ buttons of the Signal Designation group can be used to adjust the signal region of the first peak.

Instead of procedures 4 and 5, procedures 1-3 can be used to create another signal region of the second peak (see Fig. 25). If a square drawn by the mouse overlaps with the existing signal region (white block) of the first peak, the latter region will be erased.
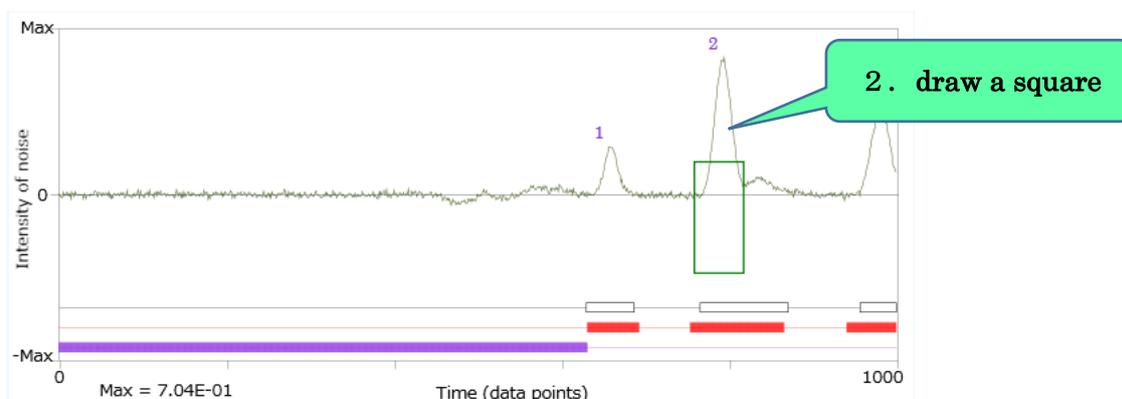


Figure 22 --- Chart (a framed box around one of two peaks)
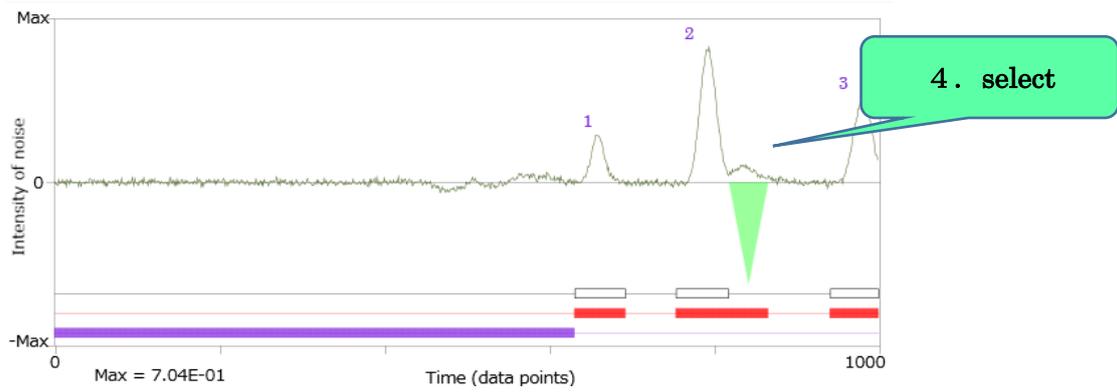
Figure 23 --- Chart (selection of the second peak)
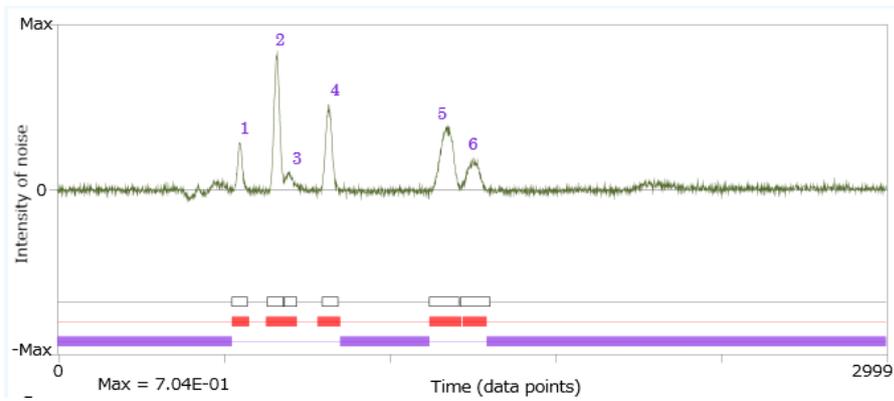


Figure 24 --- Chart (Participation of the second peak)
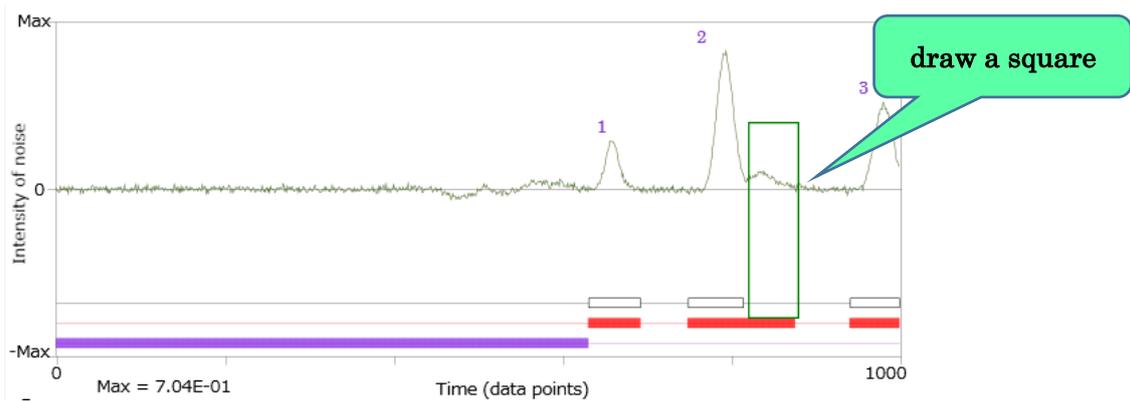


Figure 25 --- Another example of separation of overlapping peaks

24. Complete Manual analyses from scratch

TOCO19 is automatic software for analyzing noise and signals, but it also provides the following complete manual operations from the very beginning which are conducted through the Manual SE group (see Fig. 26):

1. uncheck ☑Signal Elimination (SE), while a data file is displayed on the chart;
2. check ☑Select a signal in the Manual SE group;
3. draw a square with the mouse to designate a signal region in the chart;
4. press the Eliminate button.

Repeat procedures 2-4 to set more signal regions. If necessary, you can use the ◄ and ► buttons to adjust the signal regions.

Checking ☑Signal Elimination (SE) restores the initial state of file import. The Default button leads to the default state, whether or not ☑Signal Elimination (SE) is checked. For the adequacy of these manual operations, Histogram of measurement errors needs to be checked (see 2.25).
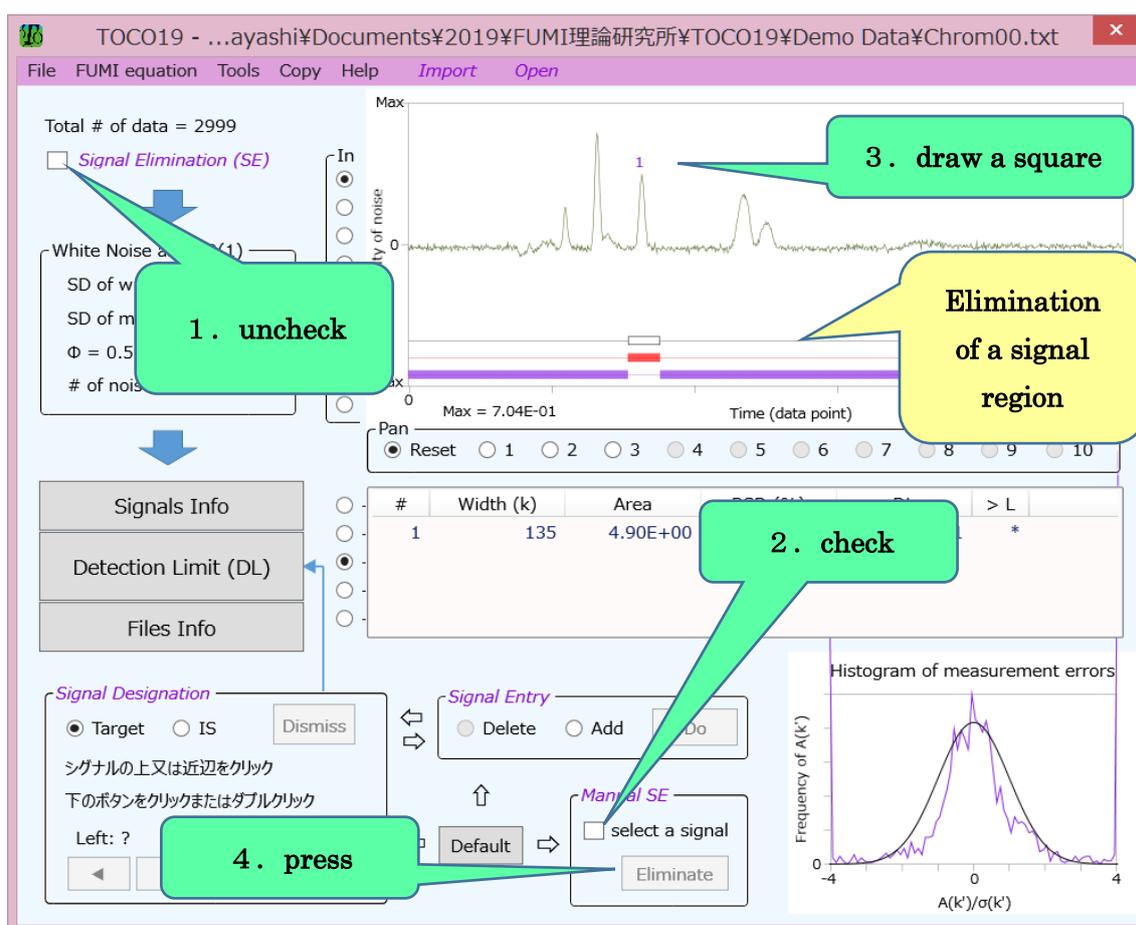


Figure 26 --- Main window (manual operations of a signal region)

25. Statistical reliability of TOCO19 analyses

The FUMI theory approximates a distribution of measurement errors as a normal distribution of areas created by background noise, but sometimes this approximation is not effective. The adequacy of the FUMI theory in a specific problem can be confirmed by the Histogram of measurement errors shown in the lower-right of the main window.

In the histogram of Fig. 27, the normal distribution (smooth line) coincides with the distribution observed from real background noise (zigzag line) and the TOCO19 analyses can be concluded to be successful. Note that the signals (white blocks) are all eliminated from the noise regions (purple blocks). The histogram of Fig. 28 shows a failure of the TOCO19 analyses due to insufficient signal elimination (left figure).
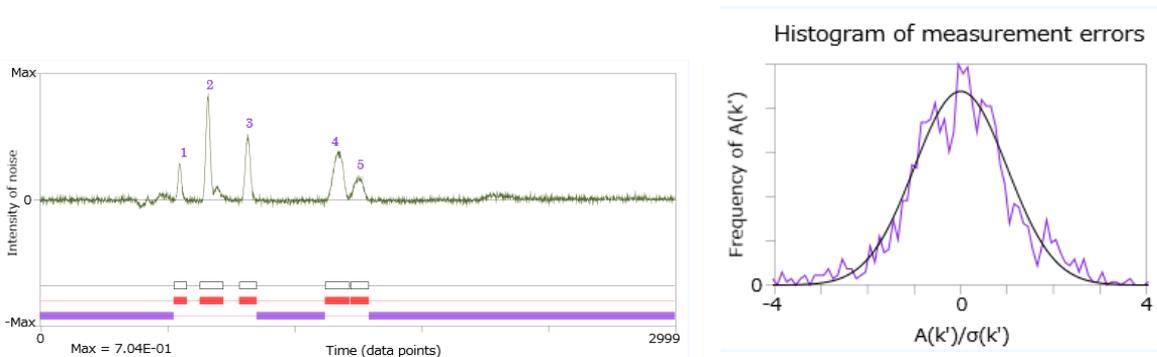


Figure 27 --- Right prediction of measurement precision
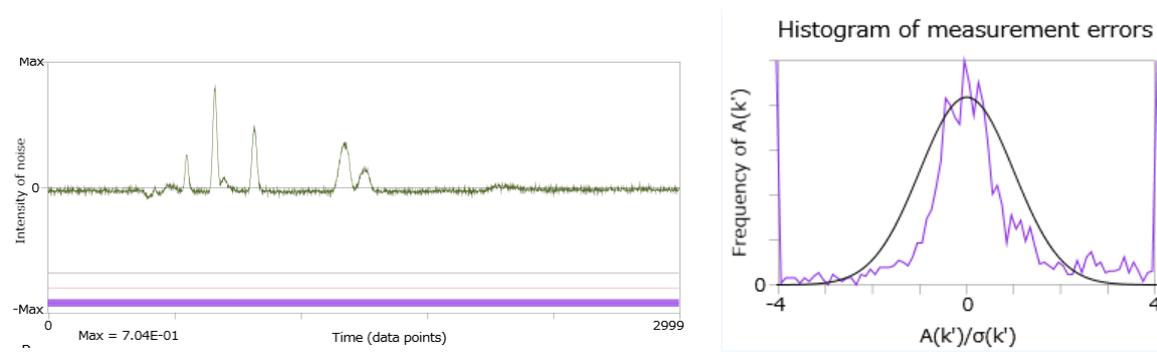


Figure 28 --- Wrong prediction of measurement precision

26. SD of area measurements for a peak in the main window

Click the FUMI equation menu, and you can obtain a theoretical SD ($\sigma(k)$) of a peak designated in the main window. This analysis is based on the noise in the chart.

27. Manual calculation of precision (FUMI equation)

Press the FUMI equation menu and input four parameters (SD of w, SD of m, $\phi$ and peak width (k)), and you can obtain a measurement SD ($\sigma(k)$). The FUMI equation window also gives an explanation of the FUMI theory.

28.  Quick display of file data on the chart

The upper limit of data in TOCO19 is 20,000 points, and the display of data close to the limit is rather slow. In this case, checking Skip Plot in in the Chart of the Tools menu will be able to improve the display time by decreasing the number of data displayed in the chart. The density of the dots in the chart will look thin, but the manner of the analyses is exactly the same.

29.  Explanations of labels and figures in TOCO19

The procedures to see explanations of labels and figures in TOCO19 are:

1.  click labels and figures;
2.  position the mouse pointer over the labels or figures when the Tooltip Assistance of the Tools menu in the main window is checked.

This function applies to the major labels and figures, but not to all of them.

## Chapter 3    Applicability of TOCO19 in theory and practice

The FUMI theory underlying TOCO19 analyses has the following assumptions:

1.  background noise is a major source of measurement errors at low concentrations in instrumental analyses;
2.  background noise is assumed to be approximated by mixed random processes of white noise and AR(1) (first order autoregressive process).

Therefore, analytical methods which violate the above assumptions are beyond the scope of the FUMI theory. If the RSD of other errors than noise is less than 7%, TOCO19 can be considered to be effective.

The following are examples in which TOCO19 fails (based on the above-mentioned assumptions):

- large sampling errors (1);
- large preparation errors (1);
- large injection errors or ionization errors (1);
- too steep a slope of background in the gradient elution (2);
- a large amount of spike noise in background (2).

The applicability of TOCO19 can be confirmed by a histogram of errors (see 2.25).

## Chapter 4    Inquiries and general feedback

Let us know your theoretical or technical questions, comments, and requests about the FUMI theory and TOCO19 through the Contact us menu of Help of TOCO19. We appreciate your cooperation and feedback.